UNIVERSITY OF CALIFORNIA,
IRVINE

Catalyst Selectivity and Motion Imaged by Single-Molecule Spectroscopy
and
Curriculum Development for Undergraduate Argument-Driven Inquiry Laboratories

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Chemistry

by

Shannon J. Saluga

Dissertation Committee:
Assistant Professor Joe Patterson, Chair
Teaching Professor Kimberly Edwards
Professor Shane Ardo
Assistant Professor Xiaoyu Shi

2024

DEDICATION

To my late grandmother, Barbara Ragland

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I must acknowledge that my time as a PhD student would have been near impossible without the funding I received from both the U.S. Department of Energy (DE-SC0016467) and the National Science Foundation Graduate Research Fellowship Program (1000302687). I hope that they can continue to fund student's dreams for years to come. The second chapter is adapted with permission from John Whiley and Sons, and adapted from Garcia IV, A.; Saluga, S. J.; Dibble, D. J.; López, P.; Saito, N.; Blum, S. A. Does Molecular Catalyst Selectivity Change with Time? Polymerization Imaged by Single-Molecule Spectroscopy. *Angew. Chem. Int. Ed.* **2021**, *60*, 1550−1555. The coauthors on this publication were Antonio Garcia IV, Pía A. López, D. Josh Dibble, Nozomi Saito and Suzanne A. Blum. The third chapter is adapted with permission from the American Chemical Society and adapted from Saluga, S.J.; Dibble, D. J.; and Blum, S.A. Superresolved Motions of Single Molecular Catalysts during Polymerization Show Wide Distributions. *J. Am. Chem. Soc.* **2022**, *144 (23)*, 10591–10598. The coauthors on this publication were D. Josh Dibble and Suzanne A. Blum. The fifth chapter was adapted with permission from the American Chemical Society and the authors and adapted from Saluga, S. J.; Burns, A. M.; Li, Y.; Nguyen, M. M.; Edwards, K. D. A Specifications-Graded, Spice-Themed, General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach. *J. Chem Ed.* **2023** *100* (10), 3903-3915. The coauthors on this publication were Alyssa M. Burns, Yong Li, Melanie M. Nguyen, and Kimberly D. Edwards.

Thank you, Suzanne A. Blum, for making me ask the hard questions and being invaluable during the first years of my Ph.D. I am the person and scientist that I am today because of the excellent science and professional skills that I learned in your lab. Even though I ended up walking on a different path, thank you for being willing to have hard conversations even when I didn't want to have them. Thank you for inviting me to UCI and thank you for all the skills and experiences I had during my first years.

Thank you, Joe Patterson, for taking a chance on this crazy plan, even though you didn't know me from Adam. Thank you for taking a chance on me, my PhD, and my life goals that I never could get to align before I walked into your office.

Thank you, Renée Link, for being the first person to show me the science of science education. When I first met you, I had no idea where I would end up, and I wouldn't have ended up here without you. Thank you for your mentorship, your belief, and your willingness to teach me how to teach, and then write a paper about it! Thank you for walking me through how to be more than someone trying and start being someone doing.

Thank you, Kim Edwards, for showing me that I could get where I wanted to go even after I felt like my life fell apart. You have been more than a mentor for me, more than a role model. You've been one of my closest friends and confidants and I wish that I could spend the rest of my career working with you! You'll always be one of the first people I go to whenever I have something happen, good or bad. My life is better knowing that you're in my corner.

I must acknowledge that my time as a PhD student would have been near impossible without the funding I received from both the U.S. Department of Energy and the National Science Foundation. I hope that they can continue to fund student's dreams for years to come.

To my first mentor, Tony, I wish nothing but the best. I wouldn't have gotten this far if you weren't willing to put up with my questions and my curiosity. I appreciated every statistics boxing match we had that added hours onto subgroup meeting. Being a co-author with you was the first success of my career, and I have you to thank for getting me started.

To the man, the myth, the legend, Hannah: You're gonna do great in your future career! You've been a wonderful friend and an inspiration to me--I did grad school; you did grad school on hard mode! And it was worth it. Halcy, know your mom is one of the coolest people I've ever met. We entered into this program at the same time and I'm so proud of us for making it all the way to the end. I'm glad we skipped grad orientation together, that first summer.

To the rest of the folks in the Blum lab during my time there: Thanks for a great few years! Pía, I appreciated hiding in the microscope room with you debating everything from refractive index to our latest shows. Martin, Joe, thanks for making the third floor an environment comfortable enough for me to sleep at my desk some days. Erin, Or, thanks for training me when I felt out of my depth.

To Nick, Jordan, and Adam: Thank you for being my informal lab mates when I felt like I no longer had any. I appreciate all the times you three were willing to derail our social plans in order for us to talk shop, and I appreciate every lunch and hang-out that just turned into discussions of statistics and polymers and carbon nanofibers. But truly, I appreciate every lunch that was just us discussing fantasy, rather than reality. The final year of my PhD went by too fast, and I say that only because of our friend group. Sometimes it felt like I counted the year by Tuesdays.

It's no exaggeration that I wouldn't have made it here without the glory that is the made-for-TV movie Rodger and Hammerstein's Cinderella. I'm not sure when this became my all-nighter tradition, but it feels like I started my journey in higher education with it on in the background, and it's only fitting that I'm watching it now at the end.

To Mom, Dad, Brother, Ki, Lorena, Guinevere, Chaela: I did the grad school! And yes, it was just as hard as I made it sound like in all of our video calls. Our talks kept me going. Your support kept me afloat. I would not have made it without all the love I got from the East Coast Time Zone. Here's to me setting my clock back three hours.

And Austin: I made it, love.

VITA

SHANNON J. SALUGA

EDUCATION

UNIVERSITY OF CALIFORNIA, IRVINE — Irvine, CA
Doctorate of Philosophy in Chemistry — Defense Date: May 31, 2024

UNIVERSITY OF CALIFORNIA, IRVINE — Irvine, CA
Master of Science in Chemistry — September 2023

UNIVERSITY OF TOLEDO — Toledo, OH
Bachelor of Science in Chemistry — December 2018
Bachelor of Science in Chemical Engineering

TEACHING EXPERIENCE

UNIVERSITY OF CALIFORNIA, IRVINE — Irvine, CA

Instructor of Record, General Chemistry A (CHEM 1A) — Oct. 2023 – Dec. 2023

- Instructor during Fall Quarter 2023.
- Lower-division lecture course designed for chemistry and non-chemistry majors, with approximately 440 students enrolled. First course in the general chemistry lecture series.
- Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, developing lectures incorporating active learning, teaching assistant management, and providing support to students.

Instructor of Record, Organic Chemistry C (CHEM 51C) — July 2023 – Sept. 2023

- Instructor during Summer Session II in 2023.
- Lower-division lecture course designed for chemistry and non-chemistry majors, with approximately 160 students enrolled. Final course in the organic chemistry lecture series.
- Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, developing lectures incorporating active learning, teaching assistant management, and providing support to students.

Instructor of Record, Organic Chemistry Lab I (CHEM 51LB) — July 2023 – Sept. 2023

- Instructor during Summer Session II in 2023.
- Lower-division lab course designed for non-chemistry majors, with approximately 75 students enrolled. First course in the organic chemistry laboratory series.
- Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, developing lectures incorporating active learning, teaching assistant management, and providing support to students.

Instructor of Record, General Chemistry Lab I (CHEM 1LC) — June 2022 – Aug. 2022

- Instructor during Summer Session I in 2022.
- Lower-division lab course designed for non-chemistry majors, with approximately 115 students enrolled. First course in the general chemistry laboratory series.
- Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, teaching assistant management, and providing support to students.

Instructor of Record, General Chemistry Lab II (CHEM 1LD) June 2022 – Aug. 2022

- Instructor during Summer Session I in 2022.
- Lower-division lab course designed for non-chemistry majors, with approximately 85 students enrolled. Second and final course in the general chemistry laboratory series.
- Performed all duties required of instructor of record at UCI including, but not limited to: designing course curricula, using Canvas, teaching assistant management, and providing support to students.

Head Teaching Assistant, General Chemistry Lab Series (CHEM 1LC/D/E) Jan. 2022 – June 2022

- Administrative TA with Dr. Kimberly Edwards during the 2022 winter and spring quarters.
- Management of the lower-division lab course series designed for non-chemistry majors, with approximately 1,200 undergraduate students enrolled over the two quarters and approximately 60 teaching assistants.
- Performed administrative duties for running a large-scale laboratory class, including but not limited to: preparing and presenting teaching assistant meetings, handling grade uploads and learning management systems, supervising teaching assistants, and providing support to students.

Teaching Assistant, General Chemistry Lab I (CHEM 1LC) Sept. 2021 – Dec. 2021

- Laboratory TA with Dr. Kimberly Edwards during the 2021 fall quarter.
- Taught two four-hour lab sessions, each with approximately 24 students, each week, held weekly office hours, and graded assignments.

Developmental Teaching Assistant, Organic Chemistry Lab Series (CHEM 51LB/C/D) Mar. 2020 – Sept. 2020

- Developmental TA with Dr. Renée Link during the 2020 spring quarter and summer session.
- Designed online and hybrid activities, created quiz and test question banks, transitioned in-person laboratory activities to virtual format. Funded by a fellowship from the Division of Teaching Excellence and Innovation at the University of California, Irvine.

Teaching Assistant, Organic Chemistry Lab I (CHEM 51LB) Jan. 2020 – Mar. 2020

- Laboratory TA with Dr. Renée Link during the 2020 winter quarter.
- Taught two four-hour lab sessions, each with approximately 18 students, each week, held weekly office hours, and graded assignments.

Teaching Assistant, General Chemistry Lab II (CHEM 1LD) Sept. 2019 – Dec. 2019

- Laboratory TA with Dr. Kimberely Edwards during the 2019 fall quarter.
- Taught two four-hour lab sessions, each with approximately 24 students, each week, held weekly office hours, and graded assignments.

RESEARCH EXPERIENCE

UNIVERSITY OF CALIFORNIA, IRVINE Irvine, CA
Graduate Student Researcher, Department of Chemistry Mar. 2023 – June 2024
Advisors: Joe Patterson, Renée D. Link, Kimberly D. Edwards
Sub-discipline: Chemical Education

Research Aim: Development of Argument-Driven Inquiry general and organic chemistry laboratories and performing teaching as research projects exploring the effectiveness of online and hybrid laboratory activities.

UNIVERSITY OF CALIFORNIA, IRVINE Irvine, CA
Graduate Student Researcher, Department of Chemistry Sept. 2019 – Feb. 2023
Advisor: Suzanne A. Blum
Sub-discipline: Organic Chemistry, Fluorescence Microscopy

Research Aim: Investigation of the activity and selectivity of molecular catalysts during active polymerization through the use of subensemble and single-molecule fluorescence microscopy.

UNIVERSITY OF TOLEDO Toledo, OH
Undergraduate Researcher, Department of Chemical Engineering Jan. 2018 – Dec. 2018

Worked with Dr. Ana Alba-Rubio.
Project Title: Development of Hybrid Superacid Catalysts for the Production of 5-Hydroxymethylfurfural

KENT STATE UNIVERSITY Kent State, OH
NSF Research Experience for Undergraduates (REU) Participant June 2018 – Aug. 2018

Worked with Dr. Torsten Hegmann.
Project Title: Silylated Amino-Acid Ligands for the Detection of Toxic Gases

UNIVERSITY OF TOLEDO Toledo, OH
Undergraduate Researcher, Department of Chemistry May 2016 – Aug. 2018

Worked with Dr. Wei Li.
Focused on the synthesis of Ir- and Ru-based photocatalysts.

FLORIDA INTERNATIONAL UNIVERSITY Miami, FL
NSF Research Experience for Undergraduates (REU) Participant May 2017 – July 2017

Worked with Dr. Konstantinos Kavallieratos on rhodium complexation.
Project Title: Complexation of Hg(II) and Cd(II) with Rhodizonate
Resulted in an invited talk during the REU Symposium at the 255th ACS National Conference.

PUBLICATIONS

Saluga, S. J.; Burns, A. M.; Li, Y.; Nguyen, M. M.; Edwards, K. D. "A Specifications-Graded, Spice-Themed, General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach." J. Chem. Ed. 2023, 100, 10, 3903-3915. https://doi.org/10.1021/acs.jchemed.3c00433

Howitz, W. J.; Frey, T. L; Saluga, S. J.; Nguyen, M; Denaro, K.; Edwards, K. D. "A Specifications-Graded, Sports Drink-Themed General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach." J. Chem. Ed. 2023, 100, 2, 672-680. https://doi.org/10.1021/acs.jchemed.2c00860

Saluga, S. J.; Peacock, H.; Seith, D. D.; Boone, C. C. A.; Fazeli, Y.; Huynh, R. M.; Luo, J.; Naghi, Z; Link, R. D. "Inter-Twine-d: Combined Organic Chemistry Laboratory and Choose-Your-Own-Adventure Games." J. Chem. Ed. 2022, 99, 12, 3964-3974. https://doi.org/10.1021/acs.jchemed.2c00481

Saluga, S. J.; Dibble, D. J.; Blum, S. A. "Superresolved Motions of Single Molecular Catalysts During Polymerization Show Wide Distributions." J. Am. Chem. Soc. 2022, 144, 23, 10591-10598. https://doi.org/10.1021/jacs.2c03566

Garcia, A. IV; Saluga, S. J.; Dibble, D.J.; López, P. A.; Saito, N.; Blum, S. A. "Does Selectivity of Molecular Catalysts Change with Time? Polymerization Imaged by Single-Molecule Spectrocopy." Angew. Chem. Int. Ed. 2021, 60, 1550–1555. https://doi.org/10.1002/anie.202010101

Howitz, W. J.; Thane, T. A.; Frey, T. L.; Wang, X. S.; Gonzales, J. C.; Tretbar, C. A.; Seith, D. D.; Saluga, S. J.; Lam, S.; Nguyen, M. M.; Tieu, P.; Link, R. D.; Edwards, K. D. "Online in No Time: Design and Implementation of a Remote Learning First Quarter General Chemistry Laboratory and Second Quarter Organic Chemistry Laboratory." J. Chem. Ed. 2020, 97, 9, 2624-2634. https://doi.org/10.1021/acs.jchemed.0c00895

Alom, N.; Kaur, N.; Wu, F.; Saluga, S.; Li, W. "Catalytic Regio- and Stereoselective Alkene Sulfeonoamination for 1,4-Benzothiazine Synthesis." Chem. Eur. J. 2019, 25, 6902 –6906. https://doi.org/10.1002/chem.201900549

Kaur, Navdeep; Fan, Wu; Alom, Nur-E; Ariyarathna, Jeewani P.; Saluga, S. J.; Li, W. "Intermolecular alkene difunctionalizations for the synthesis of saturated heterocycles." Org. Biomol. Chem., 2019, 17, 1643-1654. https://doi.org/10.1039/C8OB02443J

AWARDS AND HONORS

Graduate Research Fellowship, National Science Foundation, 2020-present

Most Promising Future Faculty, University of California, Irvine, Department of Chemistry, 2022

Division of Teaching Excellence and Inclusion Graduate Scholar Fellowship, University of California, Irvine, 2020

Graduate Award for Lab Team Departmental Service, University of California, Irvine, Department of Chemistry, 2020

College of Engineering Outstanding Senior for Campus and Community Involvement, University of Toledo, 2018

Arthur H. Black Award in Analytical Chemistry, University of Toledo, Department of Chemistry, 2018

Pre-Junior Scholarship and Service Award, University of Toledo, Department of Chemical Engineering, 2017

Physical Chemistry Award, University of Toledo, Department of Chemistry, 2017

Organic Chemistry Award, University of Toledo, Department of Chemistry, 2016

LEADERSHIP AND SERVICE

Pedagogical Liaison, University of California, Irvine, Division of Teaching Excellence and Innovation, TA Professional Development Program, 2023

Graduate Safety Representative, University of California, Irvine, Blum Laboratory, 2020-2023

Peer Mentor, University of California, Irvine, Chemistry Mentorship Program, 2021-2022

Event Writer and Proctor, Orange County Science Olympiad, 2019-2020

Supplemental Instructor, University of Toledo, 2016-2018

PRESENTATIONS

Shannon J. Saluga, Hannah Peacock, Dan D. Seith, Casandra C. A. Boone, Yasmin Fazeli, Rebecca Mai Huynh, Jinyu Luo, Zane Naghi, Renée D. Link. "Inter-Twine-d: Combined Organic Chemistry Laboratory and Choose-Your-Own-Adventure Games." Biannual Conference of Chemical Education 2022, West Lafayette, IN. 2 August 2022. Oral.

Shannon J. Saluga, D. Josh Dibble, Suzanne A. Blum. "Superresolved Motions of Single Molecular Catalysts During Polymerization Show Wide Distributions." American Chemical Society National Conference Spring 2022, San Diego, CA.

22 March 2022. Oral.

Shannon J. Saluga, Ahlam Nemati, Torsten Hegmann. “Silylated Amino-Acid Ligands for the Detection of Toxic Gases.” 15th Annual Northeastern Ohio Symposium for Undergraduate Research, Kent State University, Kent State, OH. 2 August 2018. Poster.

I. Omodolor, S. J. Saluga, S. Kalidindi, M. R. Coleman, A. C. Alba-Rubio. “Multifunctional soluble and reusable catalysts for the production of hydroxymethylfurfural from glucose.” 3rd UT-WSU Graduate Research Symposium, University of Toledo, Toledo, OH. May 2018. Poster.

Shannon J. Saluga, Joseph A. Silverman, Konstantinos Kavallieratos. “Complexation of Hg(II) and Cd(II) with Rhodizonate in the Presence of N-Donor Ligands.” 255th ACS National Conference, New Orleans, LA. Symposium: “Chemistry Students at the Nexus: REU Award Winners.” 20 March 2018. Oral.

ABSTRACT OF THE DISSERTATION

Catalyst Selectivity and Motion Imaged by Single-Molecule Spectroscopy
and
Curriculum Development for Undergraduate Argument-Driven Inquiry Laboratories

by

Shannon J. Saluga

Doctor of Philosophy in Chemistry

University of California, Irvine, 2024

Assistant Professor Joe Patterson, Chair

This dissertation is composed of two main parts. The first half, Chapter 1 through Chapter 3, discusses my efforts to investigate the selectivity and motion of individual molecular catalysts in polymer networks through the use of single-molecule fluorescence microscopy. The second half, Chapter 4 through Chapter 6, discusses my chemical education research for the transition of existing curriculum to Argument-Driven Inquiry.

Chapter 1 focuses on the introduction of single-molecule fluorescence microscopy, specifically towards innovation and elucidation of catalyst dynamics, which is relevant to the first half of this dissertation. Superresolution fluorescence microscopy provides a powerful tool to investigate mechanistic questions and reaction dynamics that would otherwise be resolution- or diffraction-limited, providing insight at the nanometer level. An overview of recent applications of single-molecule and subensemble fluorescence microscopy to synthetic applications and catalytic motion-tracking is included herein, providing context for the scholarship in this dissertation.

Chapter 2 discusses, for the first time, the selectivity of individual molecular catalysts for two different reactions is imaged in real time at the single-catalyst level. This imaging is achieved through fluorescence microscopy paired with spectral probes that produce a "snapshot" of the instantaneous chemoselectivity of a single catalyst for either a single-chain-elongation or a single-chain-termination event during ruthenium-catalyzed polymerization. Superresolution imaging of multiple selectivity events, each at a different single-molecular ruthenium catalyst, indicates that catalyst selectivity may be unexpectedly spatial- and time-variable.

In Chapter 3, the motion of single molecular ruthenium catalysts during and after single turnover events of ring-opening metathesis polymerization is imaged through single-molecule superresolution tracking with positional accuracy of ±32 nm. This tracking is achieved through real-time incorporation of spectrally tagged monomer units into active polymer chains ends during living polymerization; thus, by design, only active-catalyst motion is detected and imaged, without convolution by inactive catalysts. The catalysts show diverse individualistic diffusive behaviors with respect to time that persist for up to 20 s. Such differential motion indicates widely different local catalyst microenvironments during catalytic turnover. These mobility differences are uniquely observable through single-catalyst microscopy and are not measurable through traditional ensemble analytical techniques for characterizing the behavior of molecular catalysts, such as NMR spectroscopy.

Traditional laboratory classes are often administered through "cookbook" style curriculum that does not accurately reflect the scientific inquiry and debate. To reflect this more realistic picture of the scientific process, the traditional curriculum of confirmation labs for the lower division undergraduate labs at University of California, Irvine has been adapted to Argument-Driven Inquiry, a guided inquiry curriculum that allows for debate and revision. Chapter 4

introduces a literature overview of the process of Argument-Driven Inquiry and its use as an alternative style of laboratory curriculum in other institutions.

Chapter 5 describes the creation of a second quarter of a two-quarter sequence of argument-driven-inquiry general chemistry laboratories. The course contains four projects investigating the chemistry of spices (vanilla, cinnamon, spearmint, and cloves) and incorporates a structured review and hands-on applications of fundamental concepts necessary to transition between general and organic chemistry (colligative properties, TLC, synthesis, characterization tests, and unknown determination). The inquiry-based curriculum was designed to give students increasing responsibility and freedom to develop experimental design skills. Specifications grading is used to increase concept iteration and encourage teamwork amongst students. Survey results for student learning style, feelings about chemistry, and perception of the course format are compared for first and second quarter courses. Changes in survey responses show higher average positive responses in many categories for the second quarter course.

Chapter 6 outlines the ongoing effort to design a series of Organic Chemistry experiments to be used in an ADI course, with a focus on designing intentional variation to lead to robust argumentation. These experiments were evaluated by a group of undergraduate beta-testers, who performed the full course as students, including the argumentation sessions. These designed experiments are discussed and analyzed based on student feedback. The argumentation sessions were analyzed by the Assessment of Student Argumentation in the Classroom protocol to quantify the level of discourse achieved by the students. Both results are evaluated to determine the efficacy of the designed curriculum. Future directions and continuing work on the curriculum are outlined.

CHAPTER 1

INTRODUCTION: SINGLE-MOLECULE AND SUB-ENSEMBLE FLOURESCENCE MICROSCOPY FOR THE INVESTIGATION OF CATALYST AND POLYMER DYNAMICS

ABSTRACT: This chapter focuses on the introduction of single-molecule fluorescence microscopy, specifically towards innovation and elucidation of catalyst dynamics, which is relevant to the first half of this dissertation. Superresolution fluorescence microscopy provides a powerful tool to investigate mechanistic questions and reaction dynamics that would otherwise be resolution- or diffraction-limited, providing insight at the nanometer level. An overview of recent applications of single-molecule and subensemble fluorescence microscopy to synthetic applications and catalytic motion-tracking is included herein, providing context for the scholarship in this dissertation.

INTRODUCTION

Single-molecule fluorescence microscopy has been used extensively for biological, biomedical and chemical applications. Since its advent in 2006,[1] scientists have been able to investigate subensemble mechanistic questions on the nanometer scale, allowing insights into the dynamics of individual chemical reactions.

The broadly applicable technique of Single Molecule Localization Spectroscopy (SMLM)[2] incorporates stochastic superresolution, which separates chemical events in time resolve multiple single events underneath the diffraction limit. SMLM is fundamentally based on the concept that individual events, indicated by the Point-Spread Function (PSF) of their respective emitter or fluorophore, can be localized underneath the Abbe diffraction limit provided that their PSFs do not overlap with another event's. SMLM techniques include photoactivated localization microscopy (PALM),[1,3] stochastic optical reconstruction microscopy (STORM),[4,5] and point accumulation in nanoscale topography (PAINT),[6] which is known for the prominence of the specific application towards immobilization via DNA strands, known as DNA-PAINT.[7]

Although SMLM was quickly applied to biological sensing, there was a brief lag before the technique was applied to probe synthetic mechanistic questions. This was likely due to the lack of innate selectivity imparted by enzymes and necessary non-commercial probe design that are complications in synthetic applications.[8] Despite these challenges, in 2006, Hofkens and Roeffaers imaged single-catalytic turnover on single crystals of [Li^{+}-Al^{3+}] layered double hydroxide catalysts, showing face-dependent catalytic activity.[9] Further investigations at the single-molecule, single-turnover level were shown in 2008 by the Blum group, which developed a transition metal-ligand system where fluorescence is prompted by a second ligand,[10,11] and Kiel *et. al.* in 2007 with the imaging of fluorophore quenching via coordination and dissociation of adjacent copper ions.[12]

Single-catalytic turnovers were then imaged by in 2008 Majima for TiO_2 photocatalysts[13,14], followed immediately by Chen in 2009 on gold nanoparticles.[15] In the following years, there have been swift advancements the application of single-molecule fluorescence microscopy to many different catalytic systems.[8,16–18]

As I started my graduate studies at the University of California, Irvine, influential advances were being reported in the literature by the Blum group and other laboratories to probe synthetic mechanistic questions using single-molecule fluorescence microscopy.[19–22] These advances can be exemplified by the reductive transformation of resazurin to resorufin (Figure 1.1a) that has been employed to probe chemical dynamics via SMLM by both the Chen group and the Kisley group to image single-molecule, single-turnover catalysis. Using a combination of electron microscopy and single-molecule fluorescence microscopy, Chen was able to map catalytic activity of Au-Ag nanoparticles at a resolution of ~40 nm in 2018.[23] In 2021, Kisley used this same fluorophore system to observe the corrosion of iron at the single-molecule level, showing spatial heterogeneity in the nanometer regime.[24]

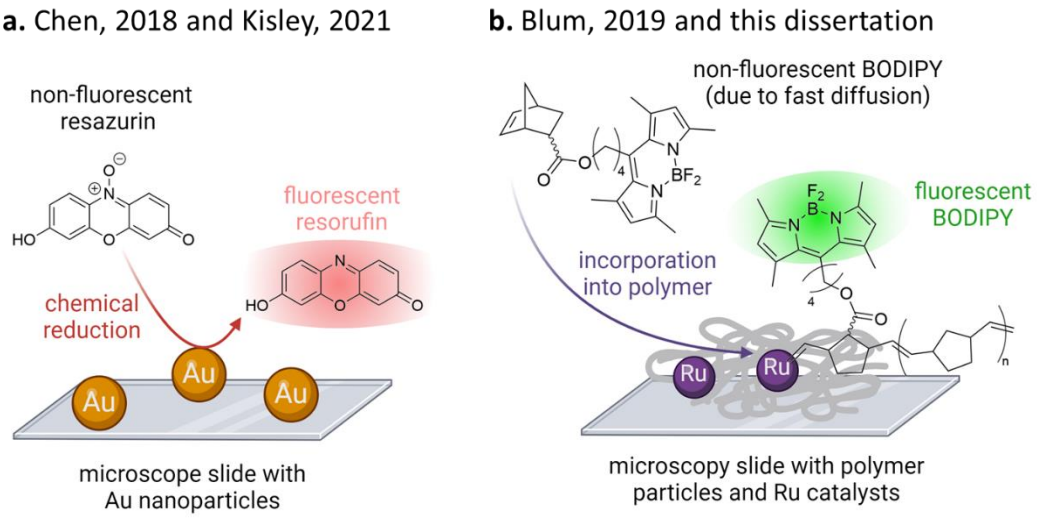


Figure 1.1. Examples of fluorescent probes for single molecule spectroscopy. a) The resazurin/resorufin pair as "participant" probes, used by Chen on gold nanoparticles and Kisley on iron particles. b) Boron dipyrromethene (BODIPY) fluorophore covalently attached to a polymer monomer to be used a "spectator" probe, used by the Blum group to monitor molecular catalysts.

One of the many uses of a high-resolution in situ technique is the ability to visualize individual chemical events without undue influence of the probe or spectroscopic technique. These "spectator probes" allow visualization under reaction-typical conditions and can more accurately mirror an unmonitored reaction. As opposed to "participant" probes, such as the resazurin reduction, spectator probes are designed to have minimal influence over the reaction and do not "turn on" due to a chemical transformation (Figure 1.1b). Instead, their fluorescence is often triggered by diffusion into the focal plane and are therefore excellent for single-molecule motion tracking. Both spectator and participant probes often must be synthetically tailored to report on specific chemical activity. The work described in the first half of this dissertation, and many of the publications published by the Blum group,[17,18,21,25–32] feature the use of spectator boron dipyrromethene (BODIPY) fluorophores (Figure 1.1b).

In addition to uncovering spatiotemporal differences in catalytic activity, subensemble fluorescence microscopy can be used to investigate diffusion and motion of individual particles,

stands, and molecules. Single-molecule tracking has been used to extensively investigate diffusion in nanoporous solids.[33,34] Of particular note to the work discussed in this dissertation is the single-molecule tracking work done by the Wöll and Weckhuysen groups.

The Wöll group has performed a series of studies investigating the diffusion of single molecules in free polymer systems and polymer gels. In 2012, the thermal-initiated polymerization of styrene and methyl methacrylate (MMA) was imaged via a perylene diimide probe, showing significantly more heterogeneities in the MMA polymerization network than the polystyrene network.[35] The Wöll group correlated the lowered diffusion coefficient in the MMA system to its increased amount of heterogeneities, explicitly connecting visualization on the subensemble level to bulk properties. This work was followed in 2014 by the tracking of tagged single clay particles within a polymer-clay composite hydrogel, showing anomalous diffusion at all six different temperatures measured from 24 °C to 38 °C.[36] In 2018, Wöll and Mecking used an air objective and a custom-built heating apparatus to analyze the single-molecule diffusion of both a free molecular probe and polymer strands in block copolymer gels at temperatures up to 100 °C.[37] Interestingly, at the same temperature, some polymer strands exhibited motion that could better be described with 1-dimensional models than 2-dimensional models, showing significant differences in motion within the same system (Figure 1.2a).

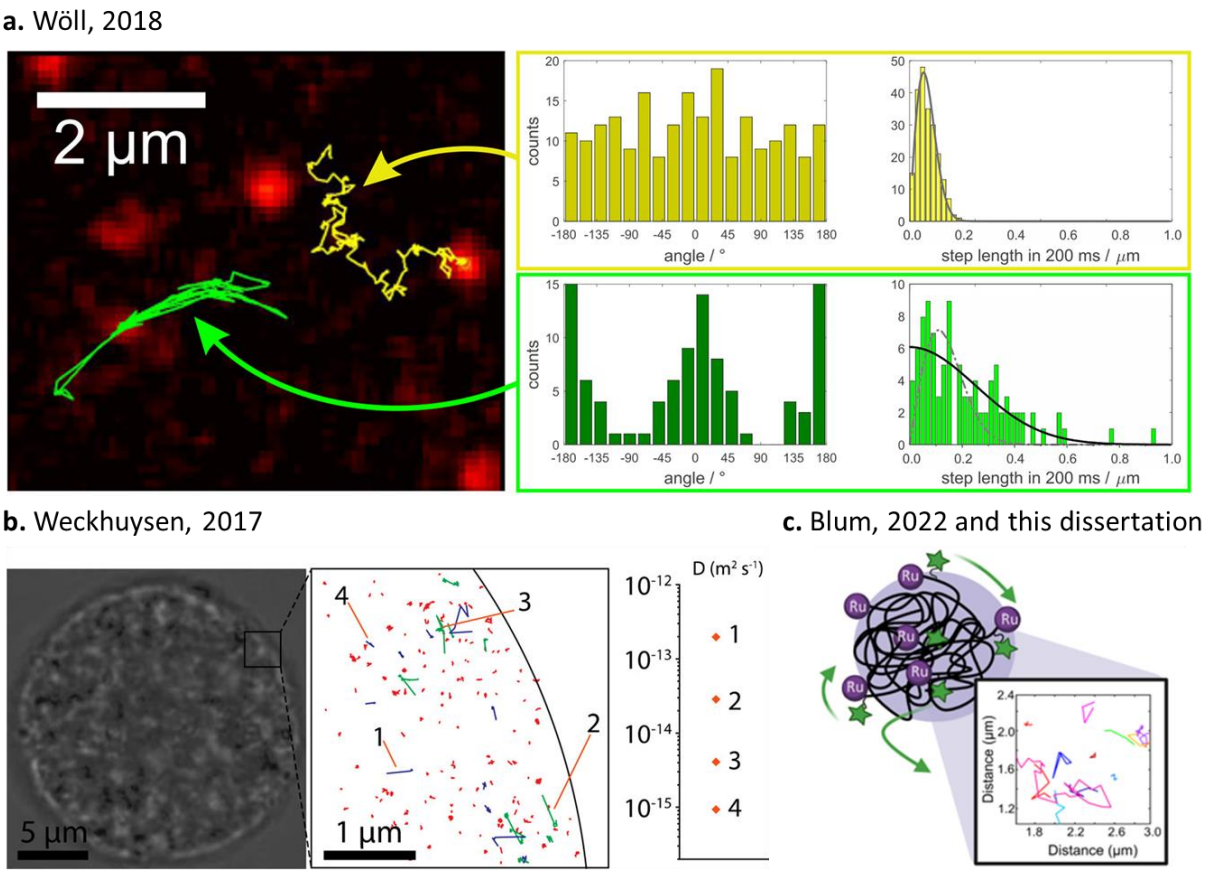


Figure 1.2. a) Trajectories of single molecules in a polymer thin film, showing both 2D (yellow) and 1D (green) motion. Replicate of Figure 7 in Reference 24, copyright held by the American Chemical Society. b) Individual trajectories of a probe within a fluid catalytic cracking particle, showing differences in motion and diffusion constraints. Replicate of the TOC graphic in Reference 25, copyright held by the authors. c) Representation of heterogenous motion shown by molecular catalysts within a polymer particle, as discussed in chapter 3 of this dissertation.

The Weckhuysen group has investigated the spatiotemporal dynamics of catalytic zeolite, as well as the motion tracking of fluorophores throughout the semi-porous zeolite systems.[38,39] In 2017, they investigated the diffusion coefficients of individual molecular probes within a fluid catalytic cracking (FCC) particle.[40] They were able to determine, despite the probe being present throughout the entire diameter of the particle, 88% of the probe molecules were immobile, due to either trapping or physisorption, while 8% were fast-moving and the remaining 4% showed intermediate motion (Figure 1.2b). This study allowed for high-resolution mapping of the catalyst

particle's physiochemical network and was a source of inspiration for the similar work in polymer particles described in chapter 3 (Figure 1.2c).

As exemplified by the Wöll group, single-molecule fluorescence microscopy allows for the elucidation of polymer dynamics. Polymers are a necessary material for many common-use items, such as electronics, clothing, and resins. Due to their usefulness and prevalence in everyday life—in 2017, it was estimated that over 8 billion metric tons of virgin plastics had been produced[41]—polymer science had advanced by leaps in bounds in the past decades, notably due to the precision tunability of living polymerization.[42] Transition metal catalysts enable controlled radical polymerization[43,44] and olefin metathesis polymerization[45,46], but these reactions are often optimized on a bulk scale.

In 2006, Uji-i *et. al.* were able to visualize the rotational diffusion of individual fluorescent probes within poly(methyl acrylate) thin films and observed spatial and temporal heterogeneity through the use of defocused wide-field fluorescence microscopy.[47] In 2008, Wöll *et. al.* imaged evolving heterogeneities in polystyrene networks through the combination of fluorescence correlation spectroscopy and wide-field microscopy.[48] The variable physical properties within these polymers are obscured during bulk characterization, but are observable at the subensemble/single-molecule level. These studies give rise to the question of whether other polymer properties, such as chain growth and strand motion, show unexpected heterogeneity that is averaged out on the ensemble level.

Ring-opening metathesis polymerization (ROMP) is often performed as a living polymerization mediated by a ruthenium catalyst, such as Grubbs 2nd Generation catalyst, Grubbs 3rd Generation catalyst, or the Hoveyda-Grubbs catalyst. As a chain-growth polymerization, individual monomers are added sequentially into the polymer chain, where polymer molecular

weight is dictated by the catalyst to monomer ratio. Escobedo, Coates and Chen evaluated the growth dynamics of the ROMP reaction through the use of magnetic tweezers and discovered that the rate of polymerization is not uniform.[49] Instead, chain growth exhibited wait-and-jump steps, plausibly caused by entanglement of polymer strands. This lack of uniformity recalled the previously mentioned heterogeneity observed in other polymer systems, and the Blum group, led by previous graduate students Easter and Garcia, investigated the polymerization under more reaction-reflective conditions, as well as the associated molecular catalysts. Using single-molecule fluorescence microscopy, the individual polymerization reactions that take place at molecular catalysts could be visualized and elucidate if these individual events can be future mechanistic handles for informed optimization.

Before my tenure in the Blum group, Easter and Blum had shown in 2017 that ROMP could be visualized at the single-molecule level using BODIPY probes covalently attached to a norbornene monomer.[30] This was the first time that polymerization reactions could be imaged at the single-turnover level by fluorescence microscopy. Easter and Blum followed up on this study to distinguish unique kinetic variability that was seen at the single-turnover level with 10^{-6} M – 10^{-12} M concentration of fluorophore that was obscured under typical ensemble measurements such as ^{1}H-NMR.[31] By tracking fluorescence intensity of individual particles over time, the rate of monomer insertion at individual ruthenium catalysts was seen to be dynamic across the particles, but did not arise from a change in overall concentration of reagents or environment.[32] These surprising results were followed up with further investigations of individual polymer particle/molecular catalyst kinetics in the presence of differing crosslinkers, such as dicyclopentadiene and norbornadiene.[29] The presence of the co-monomers had only modest impact on the kinetics, implying that the rigidity imposed by a cross-linker had little effect on the rate of

individual monomer insertion. This research done by the Blum group is consistent with a model where catalyst access is unequal across a polymer particle and is affected by the density of polymer strands in the local microenvironment. I continued this research with Dr. Antonio Garcia in Chapter 2 to investigate these purposed variable microenvironments.

Single-molecule fluorescence microscopy remains a valuable tool for mechanistic insights into biological and chemical systems, and is expanded upon in the first half of this thesis. Chapter 2 reports the investigations into the selectivity of molecular catalysts in polymer strands using two-color fluorescence microscopy, imaging termination and elongation events simultaneously. This instantaneous snapshot of catalytic selectivity showed unexpected space- and time-variability. The motion tracking of individual molecular catalysts is also shown to be variable both within polymer particles and on a particle-to-particle basis, as shown in Chapter 3. These studies represent the heterogeneity visible at the single-molecule level, and provides a novel and unique way to analyze the mechanistic questions that underpin polymer architecture and bulk properties.

REFERENCES

(1) Betzig, E.; Patterson, G. H.; Sougrat, R.; Lindwasser, O. W.; Olenych, S.; Bonifacino, J. S.; Davidson, M. W.; Lippincott-Schwartz, J.; Hess, H. F. Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. *Science* **2006**, *313* (5793), 1642–1645.

(2) Lelek, M.; Gyparaki, M. T.; Beliu, G.; Schueder, F.; Griffié, J.; Manley, S.; Jungmann, R.; Sauer, M.; Lakadamyali, M.; Zimmer, C. Single-Molecule Localization Microscopy. *Nat. Rev. Methods Primer* **2021**, *1* (1), 1–27.

(3) Hess, S. T.; Girirajan, T. P. K.; Mason, M. D. Ultra-High Resolution Imaging by Fluorescence Photoactivation Localization Microscopy. *Biophys. J.* **2006**, *91* (11), 4258–4272.

(4) Heilemann, M.; van de Linde, S.; Schüttpelz, M.; Kasper, R.; Seefeldt, B.; Mukherjee, A.; Tinnefeld, P.; Sauer, M. Subdiffraction-Resolution Fluorescence Imaging with Conventional Fluorescent Probes. *Angew. Chem. Int. Ed.* **2008**, *47* (33), 6172–6176.

(5) Rust, M. J.; Bates, M.; Zhuang, X. Sub-Diffraction-Limit Imaging by Stochastic Optical Reconstruction Microscopy (STORM). *Nat. Methods* **2006**, *3* (10), 793–796.

(6) Sharonov, A.; Hochstrasser, R. M. Wide-Field Subdiffraction Imaging by Accumulated Binding of Diffusing Probes. *Proc. Natl. Acad. Sci.* **2006**, *103* (50), 18911–18916.

(7) Schnitzbauer, J.; Strauss, M. T.; Schlichthaerle, T.; Schueder, F.; Jungmann, R. Super-Resolution Microscopy with DNA-PAINT. *Nat. Protoc.* **2017**, *12* (6), 1198–1228.
(8) Cordes, T.; Blum, S. A. Opportunities and Challenges in Single-Molecule and Single-Particle Fluorescence Microscopy for Mechanistic Studies of Chemical Reactions. *Nat. Chem.* **2013**, *5* (12), 993–999.
(9) Roeffaers, M. B. J.; Sels, B. F.; Uji-i, H.; De Schryver, F. C.; Jacobs, P. A.; De Vos, D. E.; Hofkens, J. Spatially Resolved Observation of Crystal-Face-Dependent Catalysis by Single Turnover Counting. *Nature* **2006**, *439* (7076), 572–575.
(10) Canham, S. M.; Bass, J. Y.; Navarro, O.; Lim, S.-G.; Das, N.; Blum, S. A. Toward the Single-Molecule Investigation of Organometallic Reaction Mechanisms: Single-Molecule Imaging of Fluorophore-Tagged Palladium(II) Complexes. *Organometallics* **2008**, *27* (10), 2172–2175.
(11) Lim, S.-G.; Blum, S. A. A General Fluorescence Resonance Energy Transfer (FRET) Method for Observation and Quantification of Organometallic Complexes under Reaction Conditions. *Organometallics* **2009**, *28* (16), 4643–4645.
(12) Kiel, A.; Kovacs, J.; Mokhir, A.; Krämer, R.; Herten, D.-P. Direct Monitoring of Formation and Dissociation of Individual Metal Complexes by Single-Molecule Fluorescence Spectroscopy. *Angew. Chem. Int. Ed.* **2007**, *46* (18), 3363–3366.
(13) Tachikawa, T.; Majima, T. Single-Molecule, Single-Particle Fluorescence Imaging of TiO2-Based Photocatalytic Reactions. *Chem. Soc. Rev.* **2010**, *39* (12), 4802–4819.
(14) Naito, K.; Tachikawa, T.; Fujitsuka, M.; Majima, T. Real-Time Single-Molecule Imaging of the Spatial and Temporal Distribution of Reactive Oxygen Species with Fluorescent Probes: Applications to TiO2 Photocatalysts. *J. Phys. Chem. C* **2008**, *112* (4), 1048–1059.
(15) Xu, W.; Kong, J. S.; Yeh, Y.-T. E.; Chen, P. Single-Molecule Nanocatalysis Reveals Heterogeneous Reaction Pathways and Catalytic Dynamics. *Nat. Mater.* **2008**, *7* (12), 992–996.
(16) Wang, W. Imaging the Chemical Activity of Single Nanoparticles with Optical Microscopy. *Chem. Soc. Rev.* **2018**, *47* (7), 2485–2508. https://doi.org/10.1039/C7CS00451F.
(17) Eivgi, O.; Blum, S. A. Exploring Chemistry with Single-Molecule and -Particle Fluorescence Microscopy. *Trends Chem.* **2022**, *4* (1), 5–14.
(18) Easter, Q. T.; Blum, S. A. Organic and Organometallic Chemistry at the Single-Molecule, -Particle, and -Molecular-Catalyst-Turnover Level by Fluorescence Microscopy. *Acc. Chem. Res.* **2019**, *52* (8), 2244–2255.
(19) Pujals, S.; Feiner-Gracia, N.; Delcanale, P.; Voets, I.; Albertazzi, L. Super-Resolution Microscopy as a Powerful Tool to Study Complex Synthetic Materials. *Nat. Rev. Chem.* **2019**, *3* (2), 68–84.
(20) Hanada, E. M.; Tagawa, T. K. S.; Kawada, M.; Blum, S. A. Reactivity Differences of Rieke Zinc Arise Primarily from Salts in the Supernatant, Not in the Solids. *J. Am. Chem. Soc.* **2022**, *144* (27), 12081–12091.
(21) Jess, K.; Hanada, E. M.; Peacock, H.; Blum, S. A. Origins of Batch-to-Batch Variation: Organoindium Reagents from Indium Metal. *Organometallics* **2020**, *39* (14), 2575–2579.
(22) Shen, M.; Ding, T.; Hartman, S. T.; Wang, F.; Krucylak, C.; Wang, Z.; Tan, C.; Yin, B.; Mishra, R.; Lew, M. D.; Sadtler, B. Nanoscale Colocalization of Fluorogenic Probes Reveals the Role of Oxygen Vacancies in the Photocatalytic Activity of Tungsten Oxide Nanowires. *ACS Catal.* **2020**, *10* (3), 2088–2099.

(23) Zou, N.; Chen, G.; Mao, X.; Shen, H.; Choudhary, E.; Zhou, X.; Chen, P. Imaging Catalytic Hotspots on Single Plasmonic Nanostructures via Correlated Super-Resolution and Electron Microscopy. *ACS Nano* **2018**, *12* (6), 5570–5579.
(24) Saini, A.; Messenger, H.; Kisley, L. Fluorophores "Turned-On" by Corrosion Reactions Can Be Detected at the Single-Molecule Level. *ACS Appl. Mater. Interfaces* **2021**, *13* (1), 2000–2006.
(25) Peacock, H.; Blum, S. A. Surfactant Micellar and Vesicle Microenvironments and Structures under Synthetic Organic Conditions. *J. Am. Chem. Soc.* **2023**, *145* (13), 7648–7658.
(26) Hanada, E. M.; McShea, P. J.; Blum, S. A. Trimethylsilyl Chloride Aids in Solubilization of Oxidative Addition Intermediates from Zinc Metal. *Angew. Chem. Int. Ed.* **2023**, *62* (43), e202307787.
(27) Peacock, H.; Blum, S. A. Single-Micelle and Single-Zinc-Particle Imaging Provides Insights into the Physical Processes Underpinning Organozinc Reactions in Water. *J. Am. Chem. Soc.* **2022**, *144* (7), 3285–3296.
(28) Eivgi, O.; Ravenscroft, A. C.; Blum, S. A. Imaging Block-Selective Copolymer Solvation. *J. Am. Chem. Soc.* **2023**, *145* (4), 2058–2063.
(29) Easter, Q. T.; Garcia, A. I.; Blum, S. A. Single-Polymer–Particle Growth Kinetics with Molecular Catalyst Speciation and Single-Turnover Imaging. *ACS Catal.* **2019**, *9* (4), 3375–3383.
(30) Easter, Q. T.; Blum, S. A. Single Turnover at Molecular Polymerization Catalysts Reveals Spatiotemporally Resolved Reactions. *Angew. Chem. Int. Ed.* **2017**, *56* (44), 13772–13775.
(31) Easter, Q. T.; Blum, S. A. Kinetics of the Same Reaction Monitored over Nine Orders of Magnitude in Concentration: When Are Unique Subensemble and Single-Turnover Reactivity Displayed? *Angew. Chem. Int. Ed.* **2018**, *57* (37), 12027–12032.
(32) Easter, Q. T.; Blum, S. A. Evidence for Dynamic Chemical Kinetics at Individual Molecular Ruthenium Catalysts. *Angew. Chem. Int. Ed.* **2018**, *57* (6), 1572–1575.
(33) Maris, J. J. E.; Fu, D.; Meirer, F.; Weckhuysen, B. M. Single-Molecule Observation of Diffusion and Catalysis in Nanoporous Solids. *Adsorption* **2021**, *27* (3), 423–452.
(34) Dong, B.; Mansour, N.; Huang, T.-X.; Huang, W.; Fang, N. Single Molecule Fluorescence Imaging of Nanoconfinement in Porous Materials. *Chem. Soc. Rev.* **2021**, *50* (11), 6483–6506.
(35) Stempfle, B.; Dill, M.; Winterhalder, M. J.; Müllen, K.; Wöll, D. Single Molecule Diffusion and Its Heterogeneity during the Bulk Radical Polymerization of Styrene and Methyl Methacrylate. *Polym. Chem.* **2012**, *3* (9), 2456–2463.
(36) Stempfle, B.; Große, A.; Ferse, B.; Arndt, K.-F.; Wöll, D. Anomalous Diffusion in Thermoresponsive Polymer–Clay Composite Hydrogels Probed by Wide-Field Fluorescence Microscopy. *Langmuir* **2014**, *30* (46), 14056–14061.
(37) Baier, M.; Wöll, D.; Mecking, S. Diffusion of Molecular and Macromolecular Polyolefin Probes in Cylindrical Block Copolymer Structures As Observed by High Temperature Single Molecule Fluorescence Microscopy. *Macromolecules* **2018**, *51* (5), 1873–1884.
(38) Ristanović, Z.; Kerssens, M. M.; Kubarev, A. V.; Hendriks, F. C.; Dedecker, P.; Hofkens, J.; Roeffaers, M. B. J.; Weckhuysen, B. M. High-Resolution Single-Molecule Fluorescence Imaging of Zeolite Aggregates within Real-Life Fluid Catalytic Cracking Particles. *Angew. Chem. Int. Ed.* **2015**, *54* (6), 1836–1840.

(39) Ristanović, Z.; Kubarev, A. V.; Hofkens, J.; Roeffaers, M. B. J.; Weckhuysen, B. M. Single Molecule Nanospectroscopy Visualizes Proton-Transfer Processes within a Zeolite Crystal. *J. Am. Chem. Soc.* **2016**, *138* (41), 13586–13596.
(40) Hendriks, F. C.; Meirer, F.; Kubarev, A. V.; Ristanović, Z.; Roeffaers, M. B. J.; Vogt, E. T. C.; Bruijnincx, P. C. A.; Weckhuysen, B. M. Single-Molecule Fluorescence Microscopy Reveals Local Diffusion Coefficients in the Pore Network of an Individual Catalyst Particle. *J. Am. Chem. Soc.* **2017**, *139* (39), 13632–13635.
(41) Geyer, R.; Jambeck, J. R.; Law, K. L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* **2017**, *3* (7), e1700782.
(42) Grubbs, R. B.; Grubbs, R. H. 50th Anniversary Perspective: Living Polymerization—Emphasizing the Molecule in Macromolecules. *Macromolecules* **2017**, *50* (18), 6979–6997.
(43) Ouchi, M.; Terashima, T.; Sawamoto, M. Transition Metal-Catalyzed Living Radical Polymerization: Toward Perfection in Catalysis and Precision Polymer Synthesis. *Chem. Rev.* **2009**, *109* (11), 4963–5050.
(44) di Lena, F.; Matyjaszewski, K. Transition Metal Catalysts for Controlled Radical Polymerization. *Prog. Polym. Sci.* **2010**, *35* (8), 959–1021.
(45) Buchmeiser, M. R. Homogeneous Metathesis Polymerization by Well-Defined Group VI and Group VIII Transition-Metal Alkylidenes: Fundamentals and Applications in the Preparation of Advanced Materials. *Chem. Rev.* **2000**, *100* (4), 1565–1604.
(46) Takeuchi, D. Recent Progress in Olefin Polymerization Catalyzed by Transition Metal Complexes: New Catalysts and New Reactions. *Dalton Trans.* **2010**, *39* (2), 311–328.
(47) Uji-i, H.; Melnikov, S. M.; Deres, A.; Bergamini, G.; De Schryver, F.; Herrmann, A.; Müllen, K.; Enderlein, J.; Hofkens, J. Visualizing Spatial and Temporal Heterogeneity of Single Molecule Rotational Diffusion in a Glassy Polymer by Defocused Wide-Field Imaging. *Polymer* **2006**, *47* (7), 2511–2518.
(48) Wöll, D.; Uji-i, H.; Schnitzler, T.; Hotta, J.; Dedecker, P.; Herrmann, A.; De Schryver, F. C.; Müllen, K.; Hofkens, J. Radical Polymerization Tracked by Single Molecule Spectroscopy. *Angew. Chem. Int. Ed.* **2008**, *47* (4), 783–787.
(49) Liu, C.; Kubo, K.; Wang, E.; Han, K.-S.; Yang, F.; Chen, G.; Escobedo, F. A.; Coates, G. W.; Chen, P. Single Polymer Growth Dynamics. *Science* **2017**, *358* (6361), 352–355.

CHAPTER 2

DOES SELECTIVITY OF MOLECULAR CATALYSTS CHANGE WITH TIME? POLYMERIZATION IMAGED BY SINGLE-MOLECULE SPECTROSCOPY

PREFACE

This project is a collaboration between multiple scientists. The first author, Dr. Antonio Garcia IV, performed most of the physical experiments presented. As a junior student in the laboratory, I assisted and performed preliminary runs during my first year of graduate school that were ultimately optimized for publication. The bulk of my work during this project was the incorporation of the data interpretation (the relativity in time of single events) performed in this project, which I developed during my time in the Blum Laboratory. As my contribution to this project (the creation and implementation of the analysis) is uninterpretable without including Dr. Garcia's experiments, I have included these experiments in their entirety. I also assisted in the implementation of the data analysis, the localization of the particles, analysis of control experiments, and creation and writing of the final publication. Pía A. Lopéz assisted with the analysis of the particles and control experiments, and Drs. D. Josh Dibble and Nozomi Sato helped create the Localizer and Igor Pro scripts used during this project to analyze the raw images. Adapted from Garcia IV, A.; Saluga, S. J.; Dibble, D. J.; López, P.; Saito, N.; Blum, S. A. Does Molecular Catalyst Selectivity Change with Time? Polymerization Imaged by Single-Molecule Spectroscopy. *Angew. Chem. Int. Ed.* **2021**, *60*, 1550−1555.

ABSTRACT

The chemoselectivity of molecular catalysts underpins much of modern synthetic organic chemistry. Yet little is known about the selectivity of individual catalysts because this single-catalyst-level behavior is hidden by the bulk catalytic behavior. Here, for the first time, the selectivity of individual molecular catalysts for two different reactions is imaged in real time at the single-catalyst level. This imaging is achieved through fluorescence microscopy paired with spectral probes that produce a "snapshot" of the instantaneous chemoselectivity of a single catalyst for either a single-chain-elongation or a single-chain-termination event during ruthenium-catalyzed polymerization. Superresolution imaging of multiple selectivity events, each at a different single-molecular ruthenium catalyst, indicates that catalyst selectivity may be unexpectedly spatial- and time-variable.

INTRODUCTION

A long-sought goal of catalytic polymerization reactions is the complete control over the molecular weight distribution, and consequently, over the material properties on the macroscopic scale.[1–3] Despite this, the mechanistic origins of catalyst selectivity on molecular weight distributions remain poorly understood at the fundamental level.[4] Addition of chain terminating agents or functional end groups are a common strategy to target specific chain lengths with resulting narrow molecular weight distributions.[5,6] However, the spatiotemporal selectivity of single molecular catalysts for chain elongation versus chain termination is completely unknown because it is hidden by the ensemble averaging inherent to bulk measurements. This problem is a representative example of a broader set of selectivity questions in chemistry: Does selectivity exhibited by a subset of molecular catalysts change with time or is it constant with time? Do all

the molecular catalysts exhibit the same selectivity trends in time and space or are they different from each other, and if they are different, what are the causes and timescales of these variations? Though the outcome of the reaction on the bulk level, including yield, material properties, and efficiency of the process, all hinge on the ~10^{20} individual selectivity choices made by individual molecular catalysts in a typical bench-scale reaction, there is essentially no direct information on these individual behaviors because there has been no direct way to measure them.

Catalytic polymerization is diverse mechanistically, yields commodity materials (>65 million tons of polyolefins annually[7]), and underpins the discovery of new materials.[8,9] Catalytically active chain ends bearing single molecular catalysts may experience changes in the local environment due to segmental motion, variation in solvation, and chain growth.[10–14] The distributions—and indeed even the presence and timescales—of variable chemoselectivity within these and other catalytic reaction systems are not easily predicted, and to our knowledge have not been observed in any molecular catalyst system due to the ensemble averaging inherent to traditional analytical measurements.

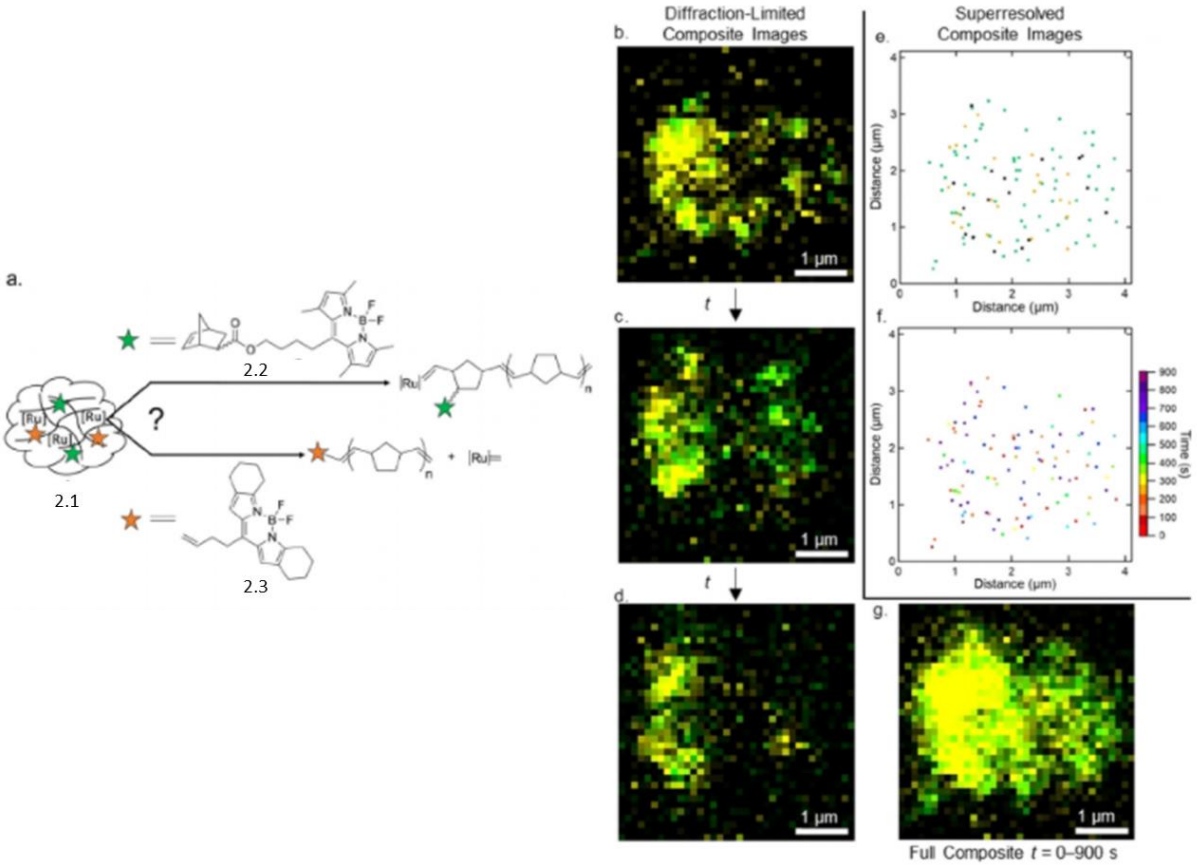


Figure 2.1. (a) Schematic for observing instantaneous chemoselectivity of an individual molecular catalyst for two different reaction pathways. (b–d) Diffraction limited composite images for 100 s observation periods of one polymer aggregate particle **2.1**. (e) Superresolved image of single chain-elongation (green) and -termination (orange) events observed at a single-polymer-particle aggregate, with events not identified with statistical confidence (black). (f) Superresolved image for all single-turnover events, where color of data points indicates reaction time. (g) Diffraction limited composite image for entire observation period.

The Blum Laboratory, including previous graduate student Dr. Antonio Garcia IV and I, became interested whether single-molecule/single-turnover imaging techniques could address these analytical challenges.[15–24] Dr. Garcia started our studies by designing a pair of fluorescent molecules that could differentiate between two different reaction pathways[25] in the living metathesis polymerization reaction at aggregate **2.1** (Figure 2.1a). This aggregate formed upon association of numerous individual living polymer strands, from ruthenium catalyst and untagged (dark) norbornene (5 mM), each strand of which contained potentially one catalyst. After

precipitation of **2.1** onto the microscope coverslip, and a rinse to remove exogenous dark norbornene, **2.2** and **2.3** were added into the sample at low concentration (each at 10^{-14} M). By design, a chain-elongation event with **2.2** produces a single green flash within **2.1**, and a chain-termination event with **2.3** produces a single orange flash. In this competition experiment, each single flash denotes the "instantaneous chemoselectivity" of a single catalyst. The low concentration of imaging agents and the high concentration of living strands within **2.1** means that each flash likely occurs at a different individual catalyst.[16,17,26,27] Further, the high initial concentration of untagged norbornene (prior to precipitation of **2.1** and subsequent rinsing) and the low concentration of imaging agents **2.2** and **2.3** means that most polymer is composed of untagged (dark) norbornene.

We propose two different possibilities for how the two different reaction pathways may be affected by change in catalyst microenvironments: In hypothesis #1, the chemical reagents respond the *same* way to different/changing microenvironments, whereas in hypothesis #2 they respond differently. Reactivity consistent with hypothesis #1 could occur if confinement at the catalyst centers caused by the dynamics of the growing polymers had similar effects on both of the two reactions. Reactivity consistent with hypothesis #2 could occur if these physical dynamics had different effects on each of the two reactions (e.g., accelerating one reaction and decelerating the other by differential pressure or differential diffusion effects at the local level).

RESULTS AND DISCUSSION

To differentiate between hypothesis #1 and #2, the selectivity of catalysts in living strands within individual particles were examined via the doping of imaging agents **2.2** and **2.3** into the reaction. Performed by senior student Garcia, 15-minute time-lapse image was acquired of the reaction, which showed numerous green and orange flashes localized into individual polymer

particles **2.1**. These flashes were absent with control compounds (**S2.4** and **S2.5**), which contained identical boron-dipyrromethene (BODIPY) cores but lacked olefin-metathesis reactive functional groups (see appendix for details). These control experiments rule out adsorption/desorption via physisorption of the reactants as the cause of the flashes. Figure 2.1 shows the behavior of a representative polymer particle during this 15 minute observation period. Figure 2.1b−d displays composite images that show the spatial overlap in the diffraction-limited data of many chemical events over time within a single particle. Figure 2.1g shows the composite of all events in this particle over the full observation time.

Diffraction-limited images showed that the spatial overlap of the fluorescence signals arising from multiple chemical events often prevented precise localization, counting, and determination of the spectral properties associated with individual turnover events. To overcome these limitations, superresolution analysis was employed.[28–33] Figure 2.1e shows the superresolved positions of single elongation events (green) and single termination events (orange). Events that could not be assigned as either are also indicated (black). Assignments are made with 95% confidence in comparison to basis sets of known green and separately known orange reagents (see Appendix for details). In Figure 2.1f, each superresolved single turnover, regardless of selectivity, is assigned a display color on the basis of when it occurred, providing a 2D representation of time-of-reaction. Together, the data in Figure 2.1 show that selectivity at individual catalysts can be spatiotemporally resolved within growing polymer aggregates.

Superresolved one second snapshots of the reaction in progress provided the "instantaneous selectivity" of individual molecular catalysts. Figure 2.2a−c shows individual 1 second frames from time-lapse imaging of an example particle. The top images display diffraction-limited frames

at t = 45, 444, 811 s, and the bottom images display the respective superresolved and spectrally assigned reaction information.

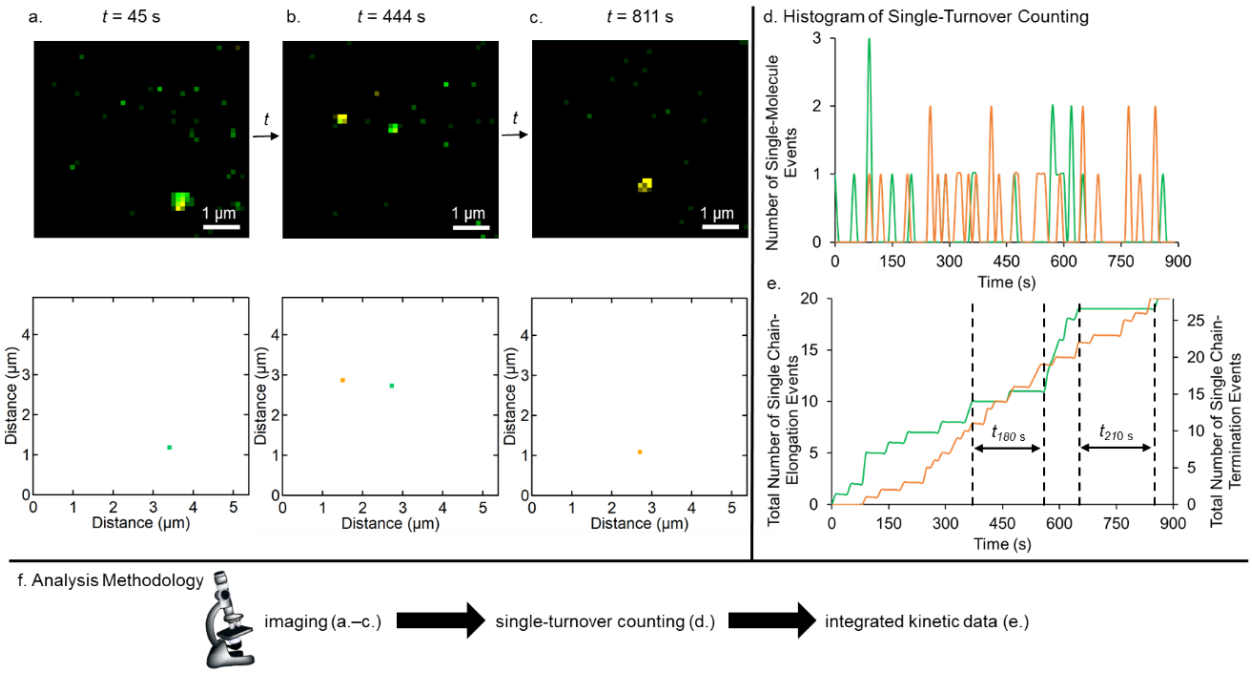


Figure 2.2 (a–c) Comparison of diffraction limited images (top) and superresolved images (bottom) for single frames at different observation times t = 45, 444, 811 s, respectively. (d) Histogram data of single chain-elongation (green) and -termination (orange) events as of function of time. (e.) Kinetics time traces comparing chain-elongation (green) and -termination (orange). (f) Imaging and analysis methodology.

My most substantial contribution to this work is the idea and subsequent implementation to analyze the single-turnover counting data through summation curves. This method of analysis enabled determination of selectivity changes with time within one particle, shown in Figure 2.2d and 2.2e (and later in Figure 2.3a, 2.3b, and 2.3d, and many additional examples in the appendix). The observation of selectivity changes within single particles formed the central narrative of this publication. These graphs, and the subsequent analysis, were initiated during my time in the Blum Laboratory and were essential to the processing and understanding of our data. Figure 2.2d shows histogram data of elongation and termination events within that particle with respect to time, in 10 s bins. The histogram data in Figure 2.2d was then translated to informative kinetics time traces

by summation of the individual selectivity events over time (Figure 2.2e). Comparison of the chain-elongation (green) and chain-termination (orange) kinetics traces showed that selectivity may fluctuate with time. For example, at t = 380 and 660 s, the rate of chain-elongation slows, while the chain-termination rate remains relatively constant. Relative changes in the rates of the two reactions produce selectivity changes.

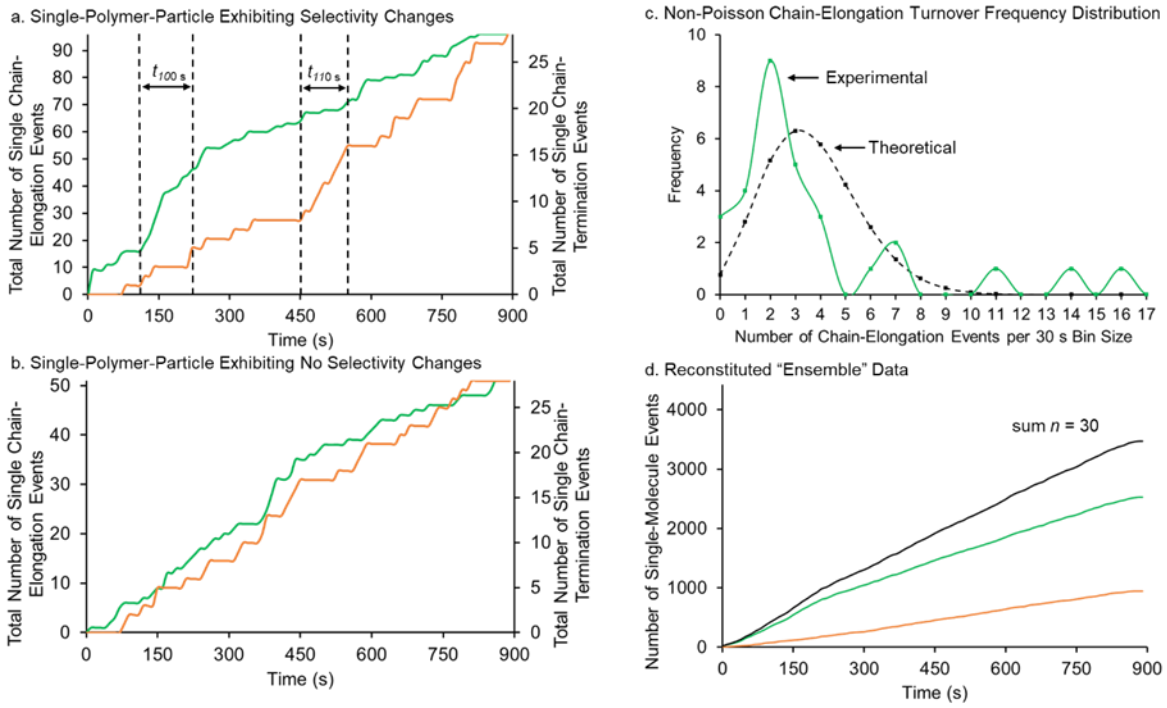


Figure 2.3. Different particles have different behavior: (a) example particle exhibiting a statistically significant time variability, (b) example particle not displaying this behavior, (c) non-Poisson distribution behavior of chain-elongation events, (d) sum of all recorded single-molecule events across 30 particles, showing how reconstituted "ensemble" data obscures the variations observed between and within single particles.

In order to evaluate the statistical significance of differences between the chain-elongation and chain-termination probability with time, I, along with previous graduate student Dr. Garcia and graduate student Pía A. López, analyzed multiple particles across different samples using the analysis methodology that I had originally developed and which had been honed by me and Garcia

after visiting the UCI Center for Statistical Consulting to settle on implementation of the KS test to evaluate our data (Figure 2.2f). Figure 2.3a shows an additional example particle with periods of selectivity changes: most notably a sudden rise in rate of chain-elongation events (green) occurred at t = 110 s and a sudden rise in chain-termination events (orange) occurred at t = 450 s; however, during those time periods, the other reaction rate remained low and relatively constant. Statistically significant variations in selectivity with time were observed in 30% of particles (9 out n = 30) as determined using two statistical tests. The statistical analysis performed herein was the work of myself, previous graduate student Dr. Garcia, and graduate student López. We each analyzed large data files containing thousands of potential single-molecule events spread through tens of particles. Each of us evaluated separate particles for their behavior by processing image files through home-modified superresolution software, signal-fitting, and duplicate removal process, with final chemoselectivity assignments, and statistical evaluation. Particles were only counted as displaying changes in selectivity if their selectivity variations were statistically significant in both tests (e.g., Figures 2.2e and 2.3a):

1) For statistical evaluation of changing selectivity vs. time, data was normalized and evaluated through a nonparametric, Kolmogorov-Smirnov test. This test established that the green and orange rates changed relative to each other ($p < 0.05$) in these 9 particles.

2) A chi-square goodness-of-fit test was used to compare the experimental frequency of single-turnover events to a theoretical Poisson distribution. If selectivity were *not* changing, then the expected single-turnover counting kinetics of both chain-elongation and chain-termination would follow Poisson probability distributions (i.e., the probability of each type of chemical reaction occurring would be constant in time). The two types of reactions may have different probabilities from each other, but they would separately obey constant probabilities if selectivity

were constant. In these nine particles, however, behavior of at least one of the reaction types (elongation or termination) did not fit a Poisson distribution ($p < 0.05$). This changing probability underpins the change in chemoselectivity with time.

The remaining particles (21 out of $n = 30$) did not show statistically significant differences in selectivity (example, Figure 2.3b). This behavior occurred in a subset of particles, but not in all, characterizing the range of time-variable reactivity/selectivity behaviors across the samples, which is typically obscured by ensemble measurements and is uniquely visible to subensemble experiments. Each particle in this subset exhibited different values of t for duration of changes in selectivity, further characterizing the range of reactivity/selectivity behavior.

Comparison between single-molecule and ensemble data: unique data available at single-molecule level. The capabilities of the superresolved, two-color single-molecule spectroscopy to reveal otherwise obscured chemoselectivity information are highlighted by comparison of the single-turnover-counting/single-particle data (shown in Figures 2.1 and 2.2) with reconstituted "ensemble" data in Figure 2.3d as the sum of green, orange, or both (shown in black), based on my method of particle analysis. Notably, the space- and time-variability in chemoselectivity are completely obscured through this reconstituted ensemble data.

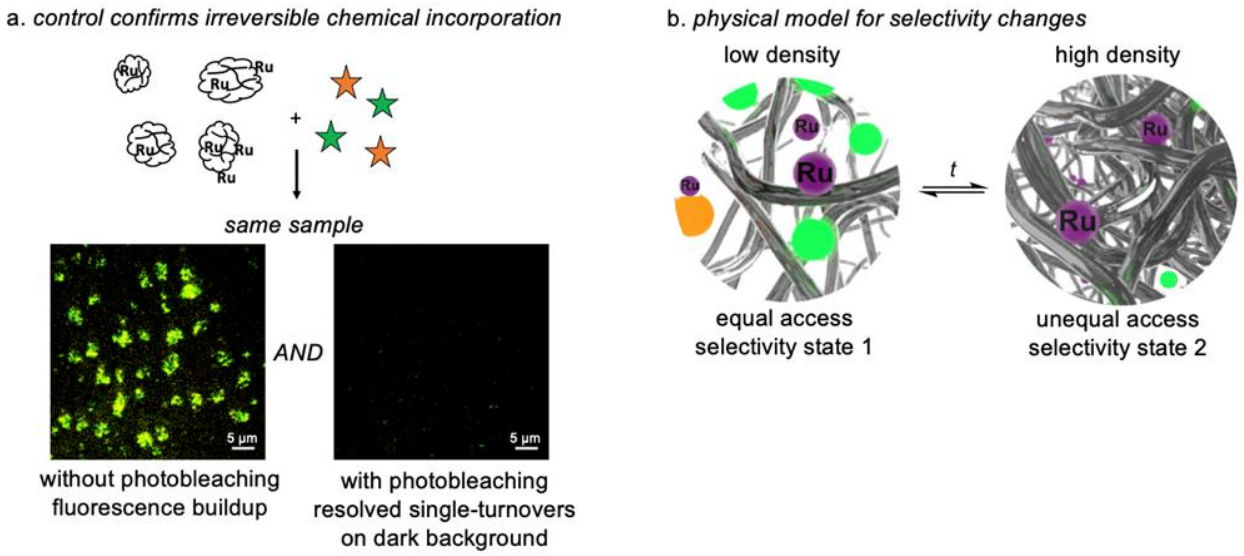


Figure 2.4. (a) Control experiment establishing irreversible incorporation of **2.2** and **2.3**, by fluorescence buildup. (b) Schematic of a physical mechanism consistent with hypothesis #2; consequently, incorporation of imaging agents **2.2** and **2.3** (green and orange) occurs at different rates during different states.

Confirmation that chemical incorporation is irreversible. Experiments described thus far were intentionally performed with high laser power in order to induce photobleaching of fluorophores shortly after incorporation into the polymers. This resulting dark background enabled accurate single-molecule detection, counting, and spectral assignment of subsequent events. Without photobleaching, the fluorescence intensity of each particle increased over time, consistent with irreversible chemical incorporation of **2.2** and **2.3**: A control experiment demonstrated that new (non-photobleached) regions of the same sample, examined after acquiring single-molecule data, showed numerous bright polymer particles (side-by-side comparison, Figure 2.4a). These outcomes are consistent with fluorescence signal arising predominantly from irreversible incorporation of **2.2** and **2.3**. This outcome are inconsistent with fluorescence signal arising predominantly from rapidly reversible on/off complexation/decomplexation of the alkene to the ruthenium center,[34] or to rapidly reversible catalyst quenching, because these alternative processes would not result in fluorescence buildup.

CONCLUSION

These data suggest that behavior consistent with hypothesis #2 could be occurring: that the dynamics of the local catalyst microenvironments might have different effects on each of the two reactions. The reasons for the observed time-variable chemoselectivity are not yet fully understood but may reflect diffusional differences between the two reagents **2.2** and **2.3** and their resulting access to catalysts. For example, hydraulic pressure, which may produce time-variable density changes of growing polymers, has been previously characterized during catalytic polymerization.[35] Such density changes may cause diffusional differences that account for the observed time-variable selectivity in this system. Conformational changes[14] may also produce density changes on a larger length-scale than previously observed. A schematic of this plausible physical mechanism is shown in Figure 2.4b. In this mechanism, increases in local polymer density crowd molecular ruthenium catalysts and restrict access of reagents to the catalyst centers unequally through reduction in effective pore sizes within the polymer. These fluxional processes may then produce differences in concentration of reagents *inside* the particles. Alternative mechanisms, however, cannot currently be ruled out.

In conclusion, these experiments provide spatiotemporally resolved snapshots of individual molecular catalyst selectivity at the single-turnover level, revealing catalytic behavior that is obscured by traditional ensemble measurements. The resulting data reveals that catalysts in neighboring particles exhibit different chemoselectivity trends in time and space from each other. Furthermore, within a single particle, time-variable selectivity may occur. Both these observations are consistent with dynamic catalyst microenvironments that affect chain-elongation and chain-termination reactions unequally.[36]

The outcome of this study raises the question of whether changing chemoselectivity at the single-catalyst level may be a cause of broadened polydispersity in molecular weight compositions or of other macroscale polymer properties. Measurement and correlation of individual particle chemoselectivity with macroscopic properties may thus enable the future improvement of catalyst performance. The bulk characteristics of essentially all molecular catalytic reactions arise from the sum of individual choices at the single-catalyst level. The presence of space- and time-variable selectivity by individual molecular catalysts in other systems is intriguingly unknown, and the plausibility of its wider presence is bolstered by such behavior in the current system.

REFERENCES

(1) R. B. Grubbs, R. H. Grubbs. 50th Anniversery Perspective: Living Polymerization Emphasizing the Molecule in Marcomolecules. *Macromolecules* **2017**, *50*, 6979–6997.

(2) J.-S. Wang, K. Matyjaszewski. Controlled/"Living" Radical Polymerization. Atom Transfer Radical Polymerization in the Prescence of Transition-Metal Complexes. *J. Am. Chem. Soc.* **1995**, *117*, 5614–5615.

(3) D. T. Gentekos, L. N. Dupuis, B. P. Fors. Beyong Dispersity: Deterministic Control of Polymer Molecular Weight Distribution. *J. Am. Chem. Soc.* **2016**, *138*, 1848–1851.

(4) C. J. Hawker, K. L. Wooley. The Convergence of Synthetic Organic and Polymer Chemistries. *Science.* **2005**, *309*, 1200 1205.

(5) J. B. Matson, R. H. Grubbs. ROMP-ATRP Block Copolymers Prepared from Monotelechelic Poly(oxa)norbornenes Using a Difunctional Terminating Agent. *Macromolecules* **2008**, *41*, 5626–5631.

(6) B. R. Elling, Y. Xia. Efficient and Facile End Group Contol of Living Ring-Opening Metathesis Polymers via Single Addition of Functional Cyclopropenes. *ACS Macro Lett.* **2018**, *7*, 656–661.

(7) G. Cecchin, G. Morini, F. Piemontesi. Ziegler-Natta Catalysts. *Kirk-Othmer Encycl. Chem. Technol.* **2003**.

(8) P. S. Chum, K. W. Swogger. Olefin Polymer Technologies—History and Recent Progress at th Dow Chemical Company. *Prog. Polym. Sci.* **2008**, *33*, 797–819.

(9) R. J. Young, P. A. Lowvell, *Introduction to Polymers*, CRC Press, Inc., Boca Raton, FL, USA, **2011**.

(10) S. Farhangi, J. Duhamel. Long Range Polymer Chain Dynamics Studied by Fluorescence Quenching. *Macromolecules* **2016**, *49*, 6149–6162.

(11) C. E. Sing, A. Alexander-Katz. Equilibrium Structure and Dynamics of Self-Associating Single Polymers. *Macromolecules* **2011**, *44*, 6962–6971.

(12) M. Ingratta, J. Duhamel. Correlating Pyrene Excimer Formation with Polymer Chain

Dynamics in Solution. Possibilites and Limitations. *Macromolecules* **2007**, *40*, 6647–6657.
(13) J. Shin, A. G. Cherstvy, R. Metzler. Kinetics of Polymer Looping with Macromolecular Crowding: Effects of Volume Fraction and Crowder Size. *Soft Matter* **2015**, *11*, 472–488.
(14) C. Liu, K. Kubo, E. Wang, K.-S. Han, F. Yang, G. Chen, F. A. Escobedo, G. W. Coates, P. Chen. Single Polymer Growth Dynamics. *Science* **2017**, *358*, 352–355.
(15) Q. T. Easter, S. A. Blum. Single Turnover at Molecular Polymerization Catalysts Reveals Spatiotemporally Resolved Reactions. *Angew. Chem. Int. Ed.* **2017**, *56*, 13772–13775.
(16) Q. T. Easter, S. A. Blum. Organic and Organometallic Chemistry at the Single-Molecule, -Particle, and -Molecular-Catalyst-Turnover Level by Fluorescence Microscopy. *Acc. Chem. Res.* **2019**, *52*, 2244–2255.
(17) Q. T. Easter, S. A. Blum, Kinetics of the Same Reaction Monitored over Nine Different Orders of Magnitude in Concentration: When are Unique Subensemble and Single-Turnover Reactivity Displayed? *Angew. Chemie Int. Ed.* **2018**, *57*, 12027–12032.
(18) M. B. J. Roeffaers, B. F. Sels, H. Uji-i, F. C. De Schryver, P. A. Jacobs, D. E. De Vos, J. Hofkens. Spatially Resolved Observation of Crystal-Face-Dependent Catalysis by Single-Turnover Counting. *Nature* **2006**, *439*, 572–575.
(19) J. B. Sambur, T.-Y. Chen, E. Choudhary, G. Chen, E. J. Nissen, E. M. Thomas, N. Zou, P. Chen. Sub-particle Reaction and Photocurrent Mapping to Optimaize Catalyst Modified Photoanodes. *Nature* **2016**, *530*, 77–80.
(20) T. Tachikawa, T. Majima. Single-Molecule, Single-Particle Fluorescence Imaging of TiO_2-based Photocatalytic Reactions. *Chem. Soc. Rev.* **2010**, *39*, 4802–4819.
(21) A. V. Kubarev, K. P. F. Janssen, M. B. J. Roeffaers. Noninvasive Nanoscopy Uncovers the Impact of the Hierarchical Porous Structure on the Catalytic Activity of Single Dealuminated Mordenite Crystals. *ChemCatChem* **2015**, *7*, 3646–3650.
(22) A. Rybina, C. Lang, M. Wirtz, K. Grussmayer, A. Kurz, F. Maier, A. Schmitt, O. Trapp, G. Jung, D.-P. Herten. Distinguishing Alternative Reaction Pathways by Single-Molecule Fluorescence Microscopy. *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 6322–6325.
(23) E. M. Hensle, S. A. Blum. Phase Separation Polymerization of Dicyclopentadiene Characterized by In Operando Fluorescence Microscopy. *J. Am. Chem. Soc.* **2013**, *135*, 12324–12328.
(24) J. D. Ng, S. P. Upadhyay, A. N. Marquard, K. M. Lupo, D. A. Hinton, N. A. Padilla, D. M. Bates, R. H. Goldsmith. Single-Molecule Investigation of Initiation Dynamics of an Organometallic Catalyst. *J. Am. Chem. Soc.* **2016**, *138*, 3876–3883.
(25) M. Shen, T. Ding, S. T. Hartman, F. Wang, C. Krucylak, Z. Wang, C. Tan, B. Yin, R. Mishra, M. D. Lew, B. Sadtler. Nanoscale Colocalization of Fluorogenic Probes Reveals the Role of Oxygen Vacancies in the Photocatalytic Activity of Tungsten Oxide Nanowires. *ACS Catal.* **2020**, *10*, 2088–2099.
(26) Q. T. Easter, S. A. Blum. Evidence for Dynamic Chemical Kinetics at Individual Molecular Ruthenium Catalysts. *Angew. Chemie Int. Ed.* **2018**, *57*, 1572–1575.
(27) Q. T. Easter, A. Garcia, S. A. Blum. Single-Polymer-Particle Growth Kinetics with Molecular Catalyst Speciation and Single-Turnover Imaging. *ACS Catal.* **2019**, *9*, 3375–3383.
(28) S. W. Hell, J. Wichmann. Breaking the Diffraction Resoltuion Limit by Simulated Emission: Simulated-Emission-Depletion Fluorescence Microscopy. *Opt. Lett.* **1994**, *19*, 780–782.
(29) S. W. Hell. Far-field Optical Nanoscopy. *Science* **2007**, *316*, 1153–1158.

(30) T. A. Klar, S. Jakobs, M. Dyba, A. Egner, S. W. Hell. Fluorescence Microscopy with Diffraction Resolution Barrier Brokem by Simulated Emission. *Proc. Natl. Acad. Sci.* **2000**, *97*, 8206–8210.
(31) E. Betzig, G. H. Patterson, R. Sougrat, O. W. Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz, H. F. Hess. Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. *Science* **2006**, *313*, 1642–1645.
(32) W. E. Moerner, L. Kador. Optical Detection and Spectroscopy of Single Molecules in a Solid. *Phys. Rev. Lett.* **1989**, *62*, 2535–2538.
(33) E. Betzig. Proposed Method for Molecular Optical Imaging. *Opt. Lett.* **1995**, *20*, 237–239.
(34) M. S. Sanford, J. A. Love, R. H. Grubbs. Mechanism and Activity of Ruthenium Olefin Metathesis Catalysts. *J. Am. Chem. Soc.* **2001**, *123*, 6543–6554.
(35) F. Bonini, V. Fraaije, G. Fink. Propylene Polymerization through Supported Metallocene/MAO Catalysts: Kinetic Analysis and Modeling. *J. Polym. Sci. Part A Polym. Chem.* **1995**, *33*, 2393–2402.
(36) Y. Nakamura, T. Ogihara, S. Hatano, M. Abe, S. Yamago. Control of the Termination Mechanism in Radical Polymerization by Viscosity: Selective Disproportionation in Viscous Media. *Chem. A Eur. J.* **2017**, *23*, 1299–1305.

APPENDIX

I. General Information

All reagents and solvents were used as received from commercial sources unless otherwise noted. Analytical thin layer chromatography (TLC) was performed using Merck F250 plates and visualized under UV irradiation at 254 nm. Flash chromatography was conducted using a Teledyne Isco Combiflash® Rf 200 Automatic Flash Chromatography System, and Teledyne Isco Redisep® 35–70 μm silica gel. Spectrophotometric grade heptane (OmniSolv®) was purchased from EMD Millipore and was used for all microscopy studies. Catalyst **S2.6** ("Grubbs Catalyst, 2nd Generation") was purchased from Sigma-Aldrich. Ultra-pure water with >18 MΩ resistivity and total organic content of <5 ppb was obtained from a Milli-Q Gradient A10 water purifier (Millipore, Billerica, MA) using a Q-Gard 2 purification pack and a Quantum EX Ultrapure Organex cartridge. All proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Bruker DRX-500 spectrometer outfitted with a cryoprobe or a Bruker Avance 600 MHz spectrometer. All coupling constants were measured in hertz (Hz).

Chemical shifts were reported in ppm and were referenced to residual protiated solvent peaks (δH = 7.26 ppm for $CDCl_3$; δH = 5.33 ppm for CD_2Cl_2) in ^{1}H NMR spectroscopy experiments.

II. Construction of Reaction Cells and Preparation of Coverslips for Microscopy

Glass coverslips (25 x 25 mm, No. 1.5, VWR Scientific) with a thickness of 0.17 mm were cleaned by sonication in 20 mL of a 0.6% solution of Hellmanex Detergent (Fisher Chemical) in Milli-Q water for 60 min and then rinsed sequentially with Milli-Q water and spectrophotometric grade ethanol six times. The rinsed coverslips were dried with compressed air. Coverslips were either stored in a sealed container or used immediately after drying. Bottomless vials were made by cutting the ends from glass reaction vials (Short Form Style, VWR Scientific). The resulting cylinders were rinsed thoroughly with Milli-Q water and spectrophotometric grade ethanol and dried in an oven at 115 °C overnight before use. To assemble the reaction cells, the cleaned and dried hollow bottomless vials were attached to the cleaned coverslips by applying epoxy (Devcon) to the outside base of the tubes, then the assembled tubes were capped and stored overnight or longer before use in microscopy experiments.

III. Fluorescence Microscopy Parameters

All microscopy imaging was performed with an inverted microscope (IX71, Olympus Corporation) and an oil-immersion, 60x objective with a 1.45 numerical aperture combined with a 1.6x magnification piece engaged. The total magnification was 96x. Samples were imaged with a CMOS Prime 95B camera (Photometrics). Fluorescence microscopy samples were illuminated with the 488 nm line obtained from a solid state laser stack (Intelligent Imaging Innovations) set to 15% power (~6.0 mW measured at the objective). The Backside Illuminated Sensor (95%

quantum efficiency) has an effective 1200 x 1200 array of pixels. The pixel size was 11 μm which with the 96x magnification, resulted in each pixel in the acquired images representing an area of 115 x 115 nm. The focus was changed with a *z*-axis controller (MS-2000, Applied Scientific Instruments, Inc.). All images were acquired in total internal reflection fluorescence (TIRF). An external alternating filter wheel was installed in between the inverted microscope and camera (Sutter Instrument Co). For all time-lapse acquisitions, images were obtained for two different filter channels, 514/30 (green channel) 562/40 (orange channel), with a FF506-Di03-25x35 dichroic beamsplitter (Brightline). The SlideBook 6.0 software (Intelligent Imaging Innovations) was set to acquire images every 1 s with 100 ms exposure to the 488 nm line per frame in each color channel. The time delay for filter wheel alternation was 75 ms. Images were viewed in ImageJ (NIH, available at http://rsbweb.nih.gov/ij/) and analyzed with Igor Pro 8 (WaveMetrics).

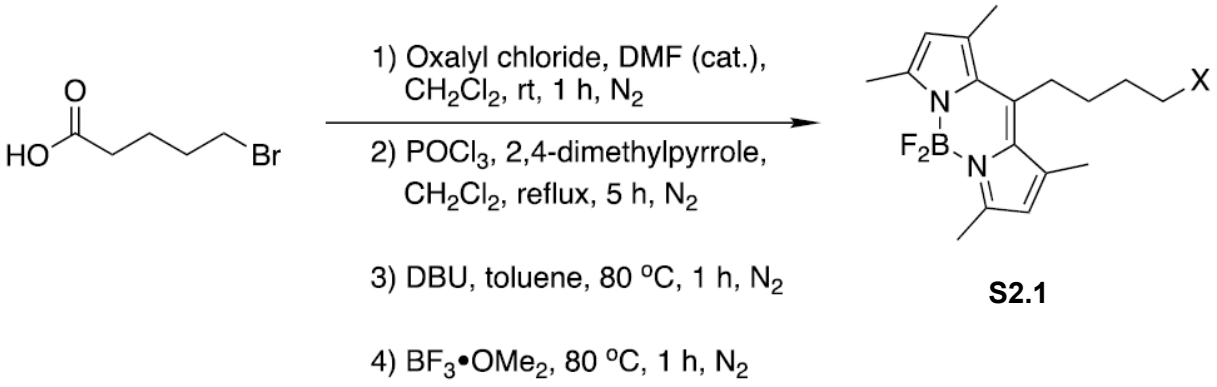
The minimum and maximum intensity values of the videos exported from SlideBook were adjusted in ImageJ and are displayed here at constant min/max for all controls and repetitions of data at a given concentration within a given experiment. When imaging agents **2.2** and **2.3,** and control compounds **S2.4** and **S2.5** were used, the display settings were changed to gamma = 2, min = 100, and max = 145 for the green channel and gamma = 2, min = 100, max = 120 for the orange channel. These display settings are shown in Figures S2.1 and S2.6–S2.13. For the green and orange channel composite images shown in the main text Figure 1, the display settings were set to gamma = 2, min = 150, and max = 1240 for the green channel and gamma = 2, min = 130, and max = 155 for the orange channel. For the green and orange channel composite images shown in the main text Figure 2, the images were denoised using ImageJ Software Non-local Means Denoising with Sigma = 5 and Smoothing = 1. Further, the display

settings were set to gamma = 0.5, min = 130, and max = 140 for the green channel and gamma = 0.5, min = 123, and max = 126 for the orange channel. For the green and orange channel composite images shown in the main text Figure 4, the display settings were set to gamma = 2, min = 130, and max = 140 for the green channel and gamma = 2, min = 115, and max = 123 for the orange channel.

IV. Synthetic Procedures

The work described in this section was performed by senior student Antonio Garcia IV.

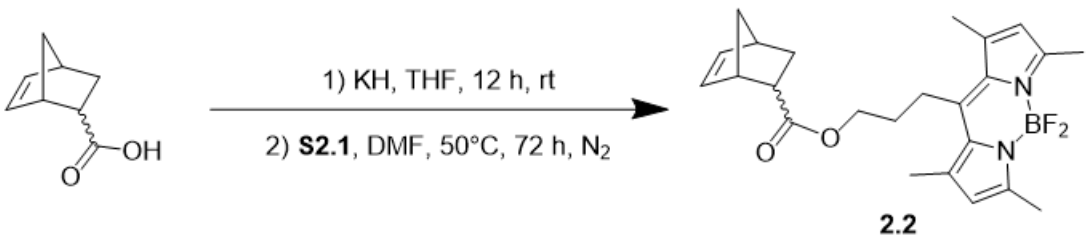
*a. Synthesis of ester norbornene green BODIPY core imaging agent **2.2**.*



S2.1

The Blum group has previously synthesized the functionalized BODIPY compound **S2.1** and imaging agent **2.2** and reported full characterization data.[1] For the experiments reported herein, a revised synthesis was used for step 2 for the synthesis of **S2.1**. This revision replaces the original thionyl chloride with phosphorous oxychloride, which is more readily available. This updated procedure is described below. An oven-dried 100 mL round-bottom flask was charged with 5-bromovaleric acid (2.74 g, 15.1 mmol) and a stir bar, and then placed under a nitrogen atmosphere. Dry CH_2Cl_2 (65 mL) and dry DMF (0.1 mL, 1.30 mmol) were added to dissolve the solid. Oxalyl chloride (1.56 mL, 18.2 mmol) was added via syringe dropwise while

stirring under dynamic nitrogen. The yellow-colored solution was stirred at room temperature for 1 h. The solution was concentrated in vacuo to yield a colorless oil and then put on a high vacuum line for 1 h to remove residual oxalyl chloride. In a separate oven dried 100 mL round-bottom flask, 2,4-dimethylpyrrole (3.88 mL, 37.8 mmol) and phosphorus oxychloride (1.55 mL, 16.6 mmol) were dissolved in dry CH_2Cl_2 (55 mL) and sealed under dynamic nitrogen. The previously prepared acid chloride was dissolved in dry CH_2Cl_2 (10 mL) and added dropwise to the reaction mixture while stirring under nitrogen. The reaction mixture was stirred at reflux for 13 h, cooled to room temperature, and concentrated in vacuo. The resulting black residue was layered with hexanes (70 mL) and the mixture was stored at −35 °C overnight. The hexanes were decanted and the residue was placed under high vacuum for 1 h, then the residue was dissolved in dry toluene (65 mL) and capped with a rubber septum and placed under dynamic nitrogen. The mixture was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (3.38 mL, 22.6 mmol) by adding dropwise. The resulting black solution was stirred for 1 h at 80 °C. Boron trifluoride dimethyl etherate (1.06 mL, 10.1 mmol) was added via syringe and the mixture was stirred at 80 °C for 1 h. The red solution was cooled to room temperature and washed with brine (3 × 50 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo to yield a dark red solid. The solid was purified by flash chromatography (CH_2Cl_2:hexanes (1:1), Rf = 0.3) to yield (856.5 mg, 16.8%) of the product as a dark red solid. This material was subsequently taken to the next synthetic step without characterization.

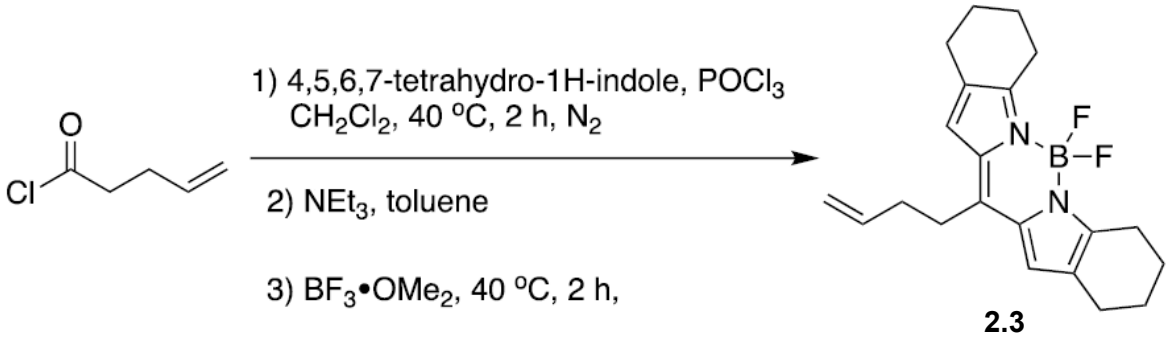


2.2

In an argon-filled glove box, potassium hydride (332 mg, 8.30 mmol) and THF (30 mL) wereadded to a 50 mL round-bottom flask. A stir bar was added, and the solution was capped with a rubber septum and removed from the glove box. In a separate 50 mL round-bottom flask, dry THF (10 mL) was dispensed from the dry solvent system. This flask was capped with a septum and removed from the glove box. From this round bottom flask, 10 mL of THF was taken out via syringe and added to a separate 10 mL round-bottom flask, which had previously been oven dried, capped with a septum, flushed with nitrogen and charged with norbornene-2-carboxylic acid in an endo:exo ratio of 3:1 (0.87, 7.1 mmol). The resulting solution was added dropwise to the separate KH/THF slurry, at which point gas evolution was observed. This solution was stirred overnight. The solution was then filtered (glass frit, medium) to yield an off-white solid. This solid was collected and without washing was immediately transferred by spatula to a second 50 mL round- bottom flask with a stir bar. To the off-white solid was added **S2.1** (0.795 g, 2.35 mmol) and DMF (25 mL). The solution was stirred for 72 h at 50 °C under dynamic nitrogen to prevent protonation of the carboxylic acid. After cooling to room temperature, the solution was diluted with ethyl acetate and washed with water (3 x 50 mL) and brine (3 x 50 mL). The EtOAc/DMF solution was dried over magnesium sulfate, and then concentrated in vacuo to give a dark red solid crude product. The resulting solid was purified by silica gel flash column chromatography using an elution of 50% CH_2Cl_2 in hexanes (Rf = 0.55), which yielded analytically pure bright red-orange solid **2.2** (198.7 g, 17.0%). ^{1}H NMR spectroscopy analysis showed a 3.0:1.0 mixture of endo:exo isomers of ester norbornene BODIPY. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.18 (dd, J = 5.5 Hz, 1H), 6.06 (s, 2H), 5.90 (dd, J = 5.5 Hz, 1H), 4.08 (t, J = 12.5 Hz, 2H), 3.17 (m, 1H), 2.98–2.95 (m, 2H), 2.94–2.89 (m, 2H), 2.52 (s,6H), 2.42 (s, 6H), 1.90–1.87 (m, 1H), 1.82–1.79 (m, 2H), 1.75–1.72 (m, 2H), 1.44–1.37 (m, 2H), 1.28–1.25 (m, 1H). ^{13}C

NMR ($CDCl_3$, 126 MHz): δ 174.8, 154.1, 145.7, 140.2, 138.0, 135.8, 131.9, 121.8, 63.5, 49.7, 45.7, 43.4, 42.6, 29.3, 28.3, 28.0, 16.4, 16.4, 14.5. HRMS (ESI): m/z: $[M+Na]^+$ calcd for $NaC_{25}H_{31}O_2N_2BF_2$ 463.2349, found 463.2337.

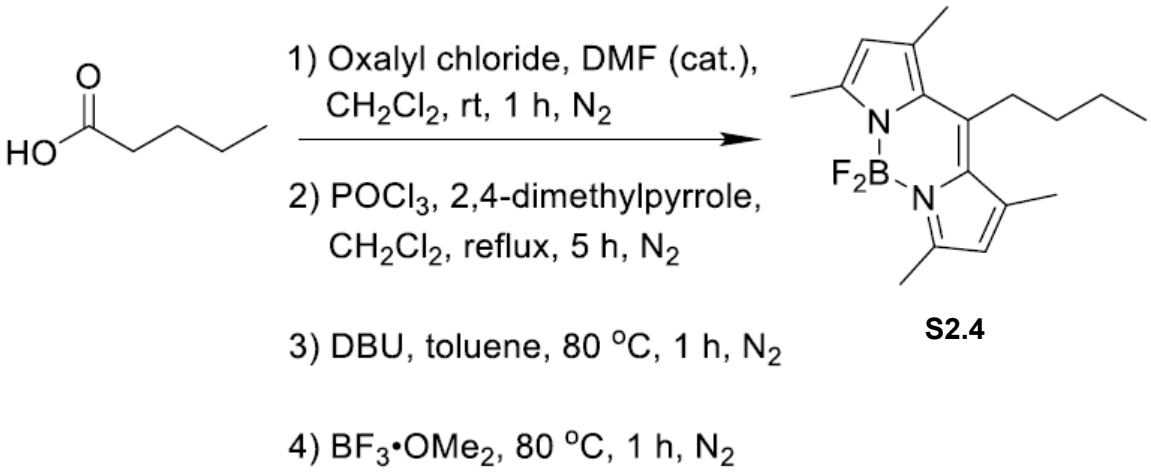
b. Synthesis of terminal olefin orange BODIPY core imaging agent ***2.3***



2.3

The Blum group has previously synthesized terminal olefin orange BODIPY core imaging agent **3**, the characterization[2] of which is reproduced here for convenience and clarity. ^1H NMR (500 MHz, CDCl3) δ 6.79 (s, 2H), 5.86 (ddt, J = 17, 10, 6.7 Hz, 1H), 5.09 (d, J = 17, 1.6 Hz, 1H), 5.03 (appd, J = 10 Hz, 1H), 3.03 (t, J = 6.3 Hz, 4H), 2.81 (t, J = 8.0 Hz, 2H), 2.55 (t, J = 6.2 Hz, 4H), 2.45 (dt, J = 7.5, 7.5 Hz, 2H), 1.87–1.82 (m, 4H), 1.77–1.72 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.6, 142.5, 137.0, 133.8, 128.7, 122.9, 115.8, 37.3, 29.9, 24.6, 23.3, 22.9, 22.4. HRMS (ESI): m/z: $[M+Na]^+$ calcd for $C_{21}H_{25}BF_2N_2$, 377.1981; found, 377.1976.

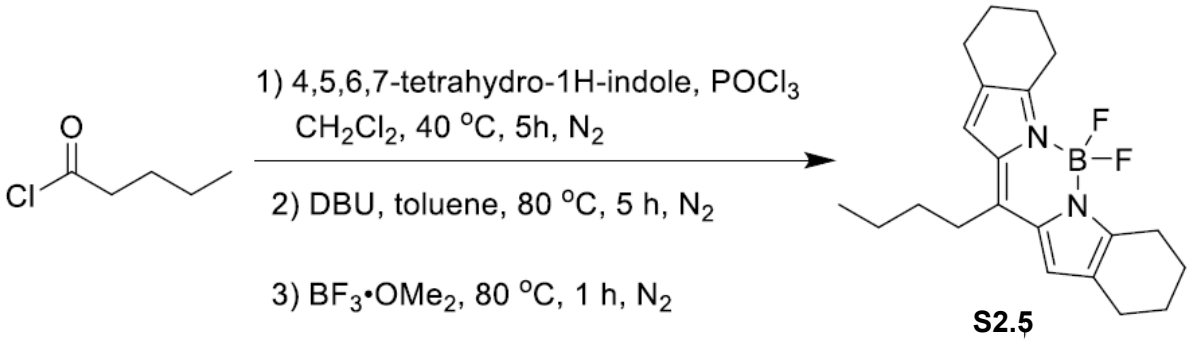
*c. Synthesis of butyl green BODIPY core control compound **S2.4**.*



S2.4

The Blum group has previously synthesized butyl green BODIPY core control compound **S4**, the characterization[3] of which is reproduced here for convenience and clarity. ^{1}H NMR (500 MHz, CDCl3*)* δ: 6.05 (s, 2H), 2.96–2.90 (m, 2H), 2.51 (s, 6H), 1.66–1.58 (m, 2H), 1.52 (sextet, J = 8 Hz, 2H), 0.99 (t, J = 8 Hz, 3H). ^{13}C NMR (126 MHz, CDCl3) δ: 153.8, 146.9, 140.4, 131.6, 121.7, 33.9, 28.2, 23.6, 14.6 (t, $J_{C–F}$ = 3 Hz), 13.9. HRMS (ESI): m/z $[M+Na]^{+}$ calcd for $C_{17}H_{23}N_2BF_2Na$, 327.1823; found, 327.1819.

*d. Synthesis of butyl orange BODIPY core control compound **S2.5**.*



S2.5

A 100-mL oven-dried flask was charged with CH_2Cl_2 (10 mL), 2,4-dimethylpyrrole (0.57g, 4.7 mmol), phosphorus oxychloride (0.20 mL, 2.1 mmol), and a stir bar, placed under nitrogen, and sealed with a rubber septum. The dark-colored solution was brought to 40 °C, and while stirring, valeryl chloride (0.23 mL, 1.9 mmol) was added dropwise under nitrogen. The solution was stirred at reflux under nitrogen for 5 h, cooled to room temperature, and concentrated in vacuo. Hexanes (60 mL) were added and the mixture was stored at −36 °C overnight, producing a dark black residue. The hexanes were decanted and the remaining residue was placed under high vacuum for 1 h, then dissolved in dry toluene (15 mL) and treated with 1,8- diazabicyclo[5.4.0]undec-7-ene (0.43 mL, 2.8 mmol) added dropwise under nitrogen. The solution was stirred for 1 h at 80 °C. Boron trifluoride dimethyl etherate (0.25 mL, 2.7 mmol) was added by syringe and the stirring was continued at 80 °C for 1 h. The resulting dark pink solution was cooled to room temperature and washed with water (3 x 30 mL). The organic layer was dried by passing the solution through a plug of cotton wool and concentrated in vacuo to yield a dark solid. The solid was purified by flash chromatography (CH_2Cl_2:hexanes (1:1), R_f = 0.32) to yield 30.8 mg (0.086 mmol, 4.6%) of a dark red solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.78 (s, 2H), 3.03 (t, J = 6.33, 4H), 2.73 (t, J = 7.86, 2H), 2.55 (t, J = 6.21, 4H), 1.85–1.81 (m, 4H), 1.76–1.73 (m, 4H),1.70–1.67 (m, 2H), 1.45–1.39 (m, 2H), 0.94 (t, J = 7.38, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 156.3, 143.4, 133.9, 128.5, 122.9, 35.8, 30.1, 24.6, 23.3, 23.2, 23.0, 22.5, 13.9. HRMS (TOF MS ES): m/z $[M+Na]^+$ calcd for $C_{21}H_{27}N_2BF_2Na$, 379.2137; found, 379.2136.

V. Preliminary Experiments

I performed preliminary experiments for the initial submission of this manuscript that were ultimately replaced by modified experiments (performed by previous student Dr. Antonio Garcia IV) to address reviewer feedback. I have included my initial data here. Analysis, as discussed in later sections of the SI (Sections XII and XIV), was the same for these particles as it was for these later experiments.

*a. Preparation of a solution containing 1.4 × 10^{-14} M imaging agents **2.2** and **2.3** for two-color fluorescence microscopy.* Imaging agent **2.3** (1.7 mg, 4.8 x 10^{-3} mmol) was weighed into a 1-dram glass vial. Spectrophotometric grade heptane (2.0 mL) was added to the vial via plastic syringe, and the solution was sonicated for 1 h to fully dissolve the compound. This afforded a solution of 2.4 x 10^{-3} M imaging agent **2.3** in heptane, which was orange-pink in appearance. Imaging agent **2.2** (1.0 mg, 2.3 x 10^{-3} mmol) was weighed into a separate 1-dram and dissolved in 1 mL of spectrophotometric grade heptane and 1 mL of the 2.4 x 10^{-3} M imaging agent **2.3** solution. The combined imaging agent solution was sonicated for 1 h to afford a solution containing 1.2 x 10^{-3} M of imaging agents **2.2** and **2.3**. Serial dilution using this 1.2 x 10^{-3} M stock solution, a 10 μL gastight syringe (Hamilton Company), and heptane allowed for the preparation of clear, colorless 1.4 x 10^{-14} M solutions of imaging agents **2.2** and **2.3** in heptane.

*b. Two-color imaging of single chain-elongation and -termination events with imaging agents **2.2** and **2.3** at 1.4 × 10^{-14}* M. A microscopy cell containing a 1.0 mL mixture of Grubbs catalyst 2nd Generation (catalyst **S2.6)** in spectrophotometric grade heptane was imaged to in TIRF mode. This resulted in dark images when surveying regions of the coverslip (Figure S2.1).

In a 20 mL vial, norbornene (28.5 mg, 3.03 x 10^{-4} mol) was weighed out and dissolved in 15.0 mL of spectrophotometric grade heptane, added via plastic syringe. Then, 0.25 mL of the

norbornene solution was added all at once to the mixture of catalyst **S2.6** in heptane (Section VIa), yielding a volume of 1.25 mL. The reaction mixture was imaged in TIRF mode for three minutes, which resulted in dark images and no detection of single-molecules when surveying regions of the coverslip (Figure S2.1).

A 15-minute time-lapse capture was taken in TIRF mode (see Section III for acquisition details). 0.25 mL of imaging agent **2.2** and **2.3** at 1.0 x 10^{-13} M was added immediately before the capture start. This resulted in a reaction mixture containing imaging agent **2.2** and **2.3** at 2.0 x 10^{-14} M and living polynorbornene in solution. The shutter was immediately opened after addition. The imaging "start" moment is referred to as $t = 0$ on all figures and is the moment the capture started. Upon opening the shutter, numerous single-bright flashes were observed on the coverslip surface in both green and orange channels (Figure S2.1). At some regions of the coverslip, the flashes appeared to be localized to particular 2−5 μm areas, indicating areas of high reactivity. These images indicate that flashes are due to the insertion of **2.2** and **2.3** into the growing polymer chains. The flashes appear to be equally numerous and bright in both green and orange channels, corresponding to the expected imaging of both green and orange imaging agents.

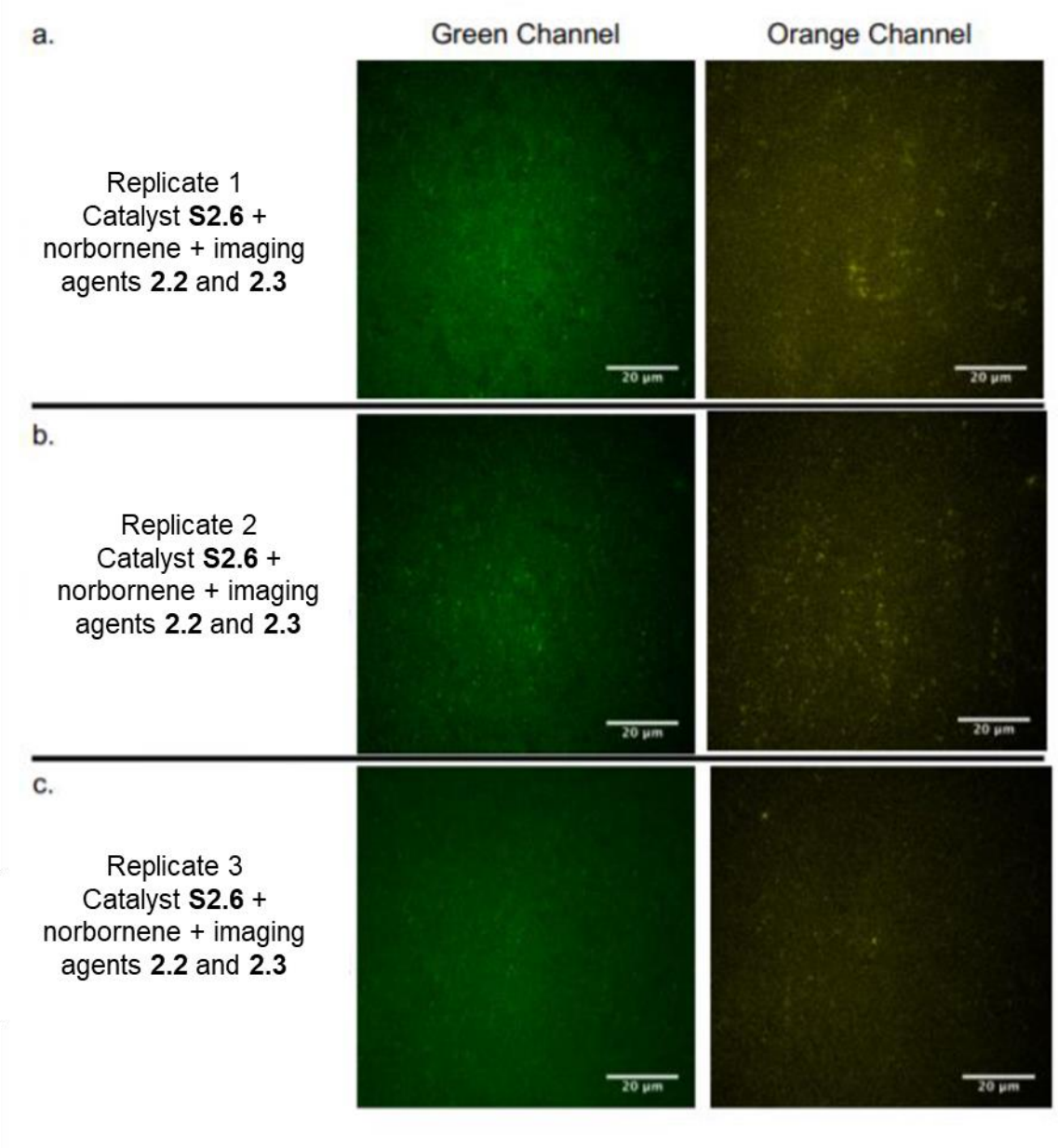


Figure S2.1. Representative single frames from two-color selectivity experiments with imaging agents **2.2** and **2.3** at 1.4 x 10^{-14} M for three replicate samples.

Following the previous time-lapse acquisition, other regions of the coverslip were imaged using the same acquisition parameters (see Section III for acquisition details). This time-lapse capture is an internal control experiment that tests for the buildup of polymeric material and

fluorophore at the coverslip surface and is discussed in detail in Section VIII. This experiment was completed in triplicate.

This procedure was modified (Section VI) to pre-form the polynorbornene particles on the surface of the coverslip and to remove the excess monomer present in solution. Grubbs catalyst (2nd generation) reacts for 5 minutes with the untagged norbornene monomer and then washed 5 times with clean spectroscopic-grade heptane. The removal of excess monomer decreases the likelihood of aggregation events occurring and being misinterpreted as chemical reactions (further details in Section VI).

c. Construction of Superresolved Images

This procedure is the same as the one detailed in Section XII. It is paraphrased here for clarity. The ratios and exact parameters for these preliminary experiments are different from those in the final manuscript, and have been explicitly stated here.

The two-color experiments resulted in one tiff image stack for each color channel. Each tiff file was exported in ImageJ to search for regions where single-molecule events were centralized on single-polymer particle aggregates. To help search for single-polymer particles that were not overlapping, time composite images were created from the tiff image stack. The tiff image stacks were cropped around particles for superresolution analysis in Igor Pro 8 for each color channel. For the analysis of each particle in both color channels, the following filters and conditions were applied; Segmentation Algorithm: GLRT, Particle finding: 8-way adjacency, Particle verification: Remove Overlapping Particles and Gaussian Fitting, Standard Deviation of the PSF (pixels): 1.5, and GLRT Insensitivity: 20. To consolidate identical emitters, the following conditions were applied; Maximum difference in position (in pixels): 3, Maximum allowed gap due to blinking (in frames): 60, Min number of observations for localization error

analysis: -1, Max number of observations for localization error analysis: -1. Using the same method detailed in Section X, the ratios for the single-molecule events were determined for the fitted curves. These ratios were then used to identify the imaging agent with 95% confidence. Taking two standard deviations for the average ratios, green = 2.49 ± 1.74 and orange = 0.31 ± 1.34, the following ranges were used to identify the imaging agents; green (ratio >1.65), orange (ratio < 0.75), not identifiable (0.75–1.65). Once identified, the data was filtered to remove emitters that were localized multiple times within an 8 s time period in 5-pixel area. The resulting data were plotted in two forms; colored data points indicating identity of the emitter and colored data points indicating time a single-molecule event occurred.

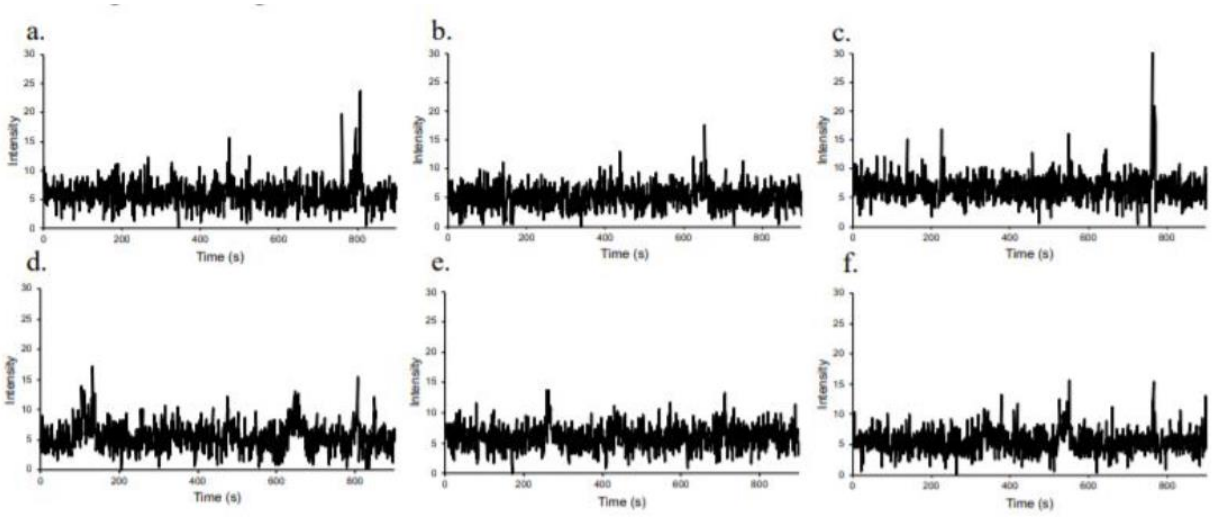


Figure S2.2. Representative intensity versus time traces for chain-elongation (a-c) and -termination (d-f) events for particle I.

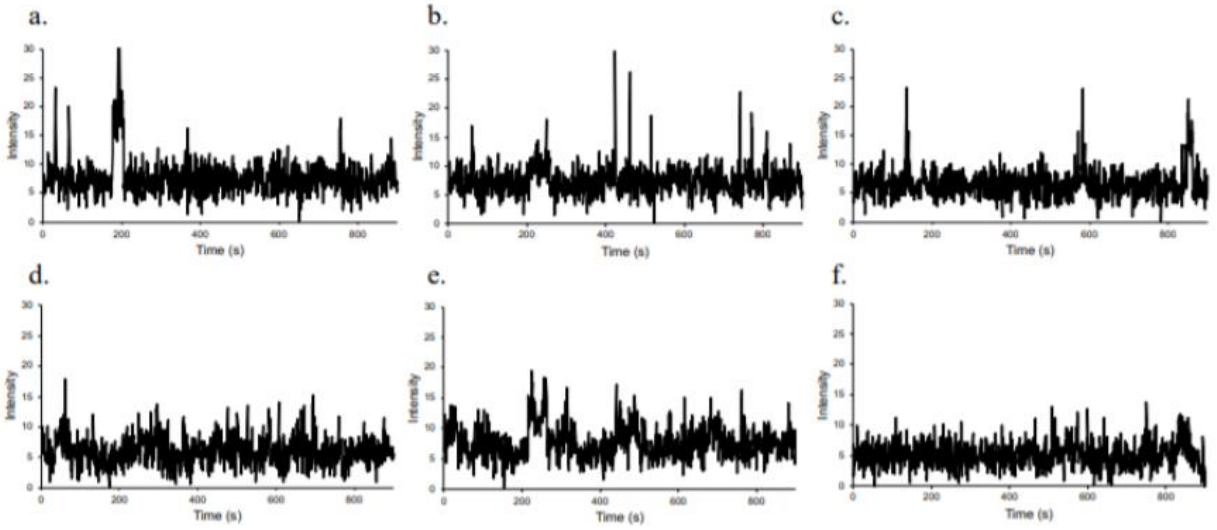


Figure S2.3. Representative intensity versus time traces for chain-elongation (a-c) and -termination (d-f) events for particle II.

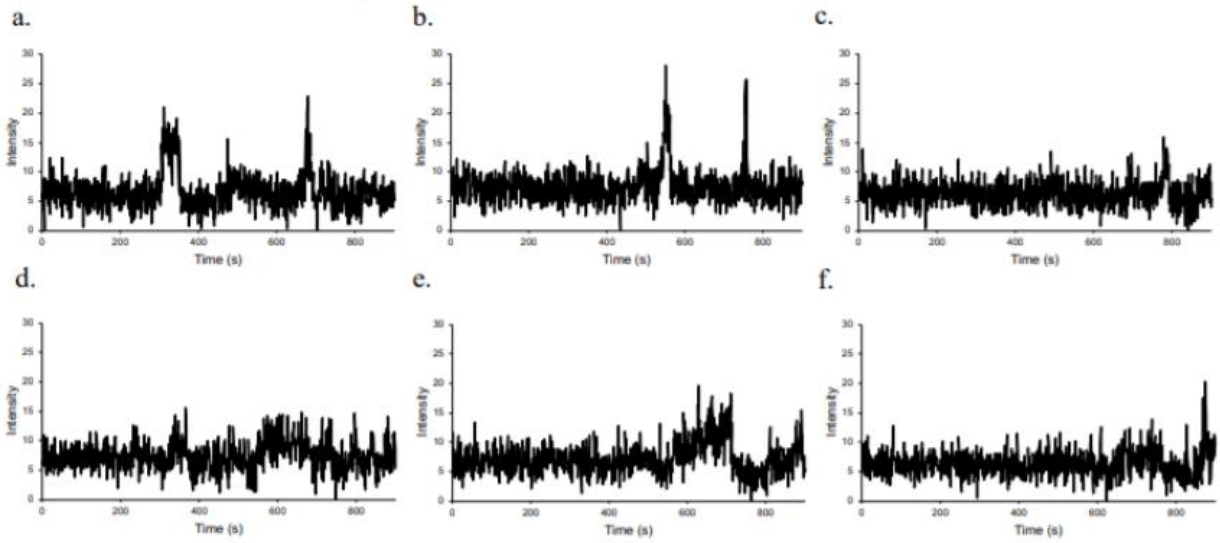
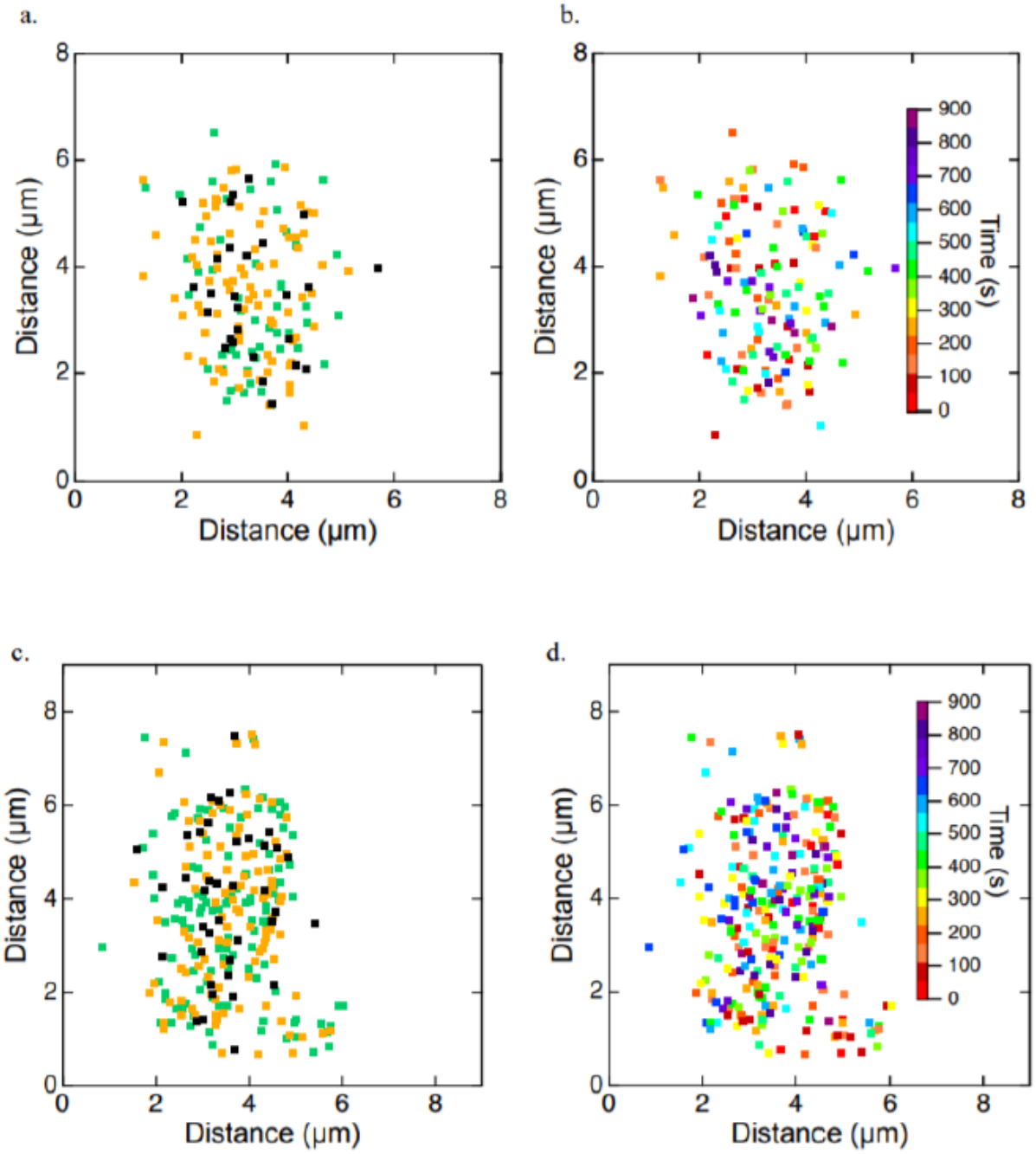


Figure S2.4. Representative intensity versus time traces for chain-elongation (a-c) and -termination (d-f) events for particle III.


a.
b.
c.
d.
Distance (μm)
Distance (μm)
Time (s)
0
100
200
300
400
500
600
700
800
900
0
2
4
6
8

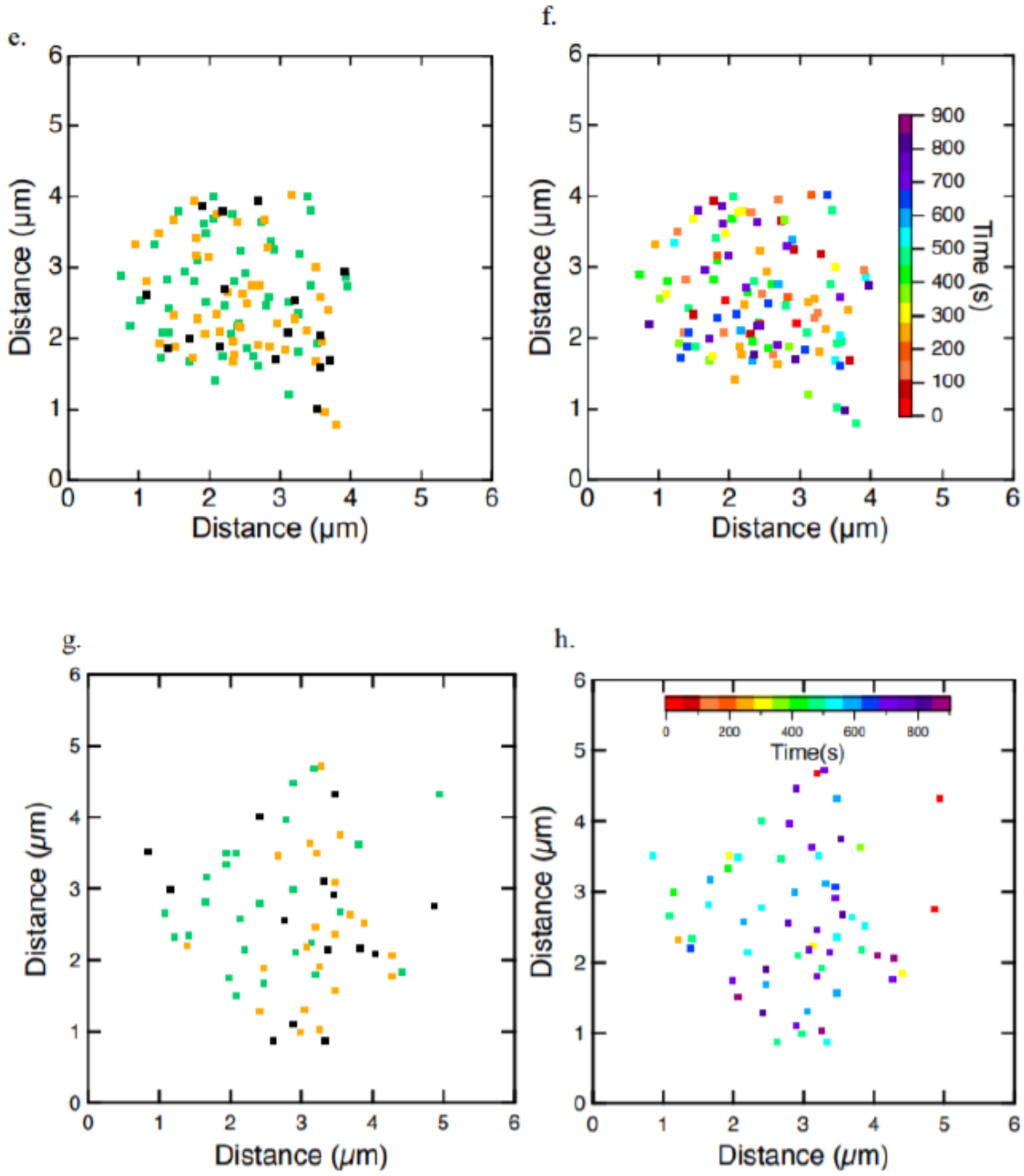


Figure S2.5. (a, c, e, g) Diffraction limited composite images for all single-turnover events for entire observation window, 0–900 s for three different particles in the same reaction. (b, d, f, h) Superresolved images for all single-turnover events, where color of data points indicates reaction time (s) for three different particles in the same reaction.

VI. Sample Preparation

*a. Preparation of catalyst **S2.6** for microscopy*. In a nitrogen-filled glove box, Grubbs second-generation catalyst **S2.6** (1.5 mg, 1.8 x10^{-3} mmol) was weighed into a 1 dram vial, which was then capped and brought out of the box. The cap was removed, and spectrophotometric grade heptane (1.00 mL) was added to the vial via plastic syringe equipped with a 20G needle. The resulting mixture was gently swirled, then transferred via the same syringe to a prepared microscopy reaction vial. The solution was a light pink due to some dissolved catalyst, but much of the catalyst remained insoluble and settled on the bottom of the microscope coverslip inside the reaction vial. The microscopy vial was placed on the microscope, and the top of the coverslip was brought into focus, using room light and catalyst crystals to focus.

*b. Preparation of solution containing 2.0 × 10^{-14} M **2.2** for one-color/one-reagent fluorescence microscopy. One-color/one-reagent microscopy was used to create the basis set to enable robust assignment of fluorophore color during the two-color chemoselectivity experiments.* Imaging agent **2.2** (1.1 mg, 2.4 × 10^{-3} mmol) was weighed into a 1 dram glass vial. Spectrophotometric grade heptane (2.0 mL) was added to the vial via plastic syringe, and the solution was sonicated for 1 h to fully dissolve the compound. This afforded a solution of 1.2 × 10^{-3} M imaging agent **2.2** in heptane, which was green-orange in appearance. Serial dilution using this 1.2 × 10^{-3} M stock solution using a 10 µL gastight syringe (Hamilton Company) and heptane allowed for the preparation of a clear, colorless 1.0 × 10^{-13} M solution of imaging agent **2** in heptane.

*c. Preparation of solution containing 2.0 × 10^{-14} M **2.3** for one-color fluorescence microscopy.One-color/one-reagent microscopy was used to create the basis set to enable robust assignment of fluorophore color during the two-color chemoselectivity experiments.* Imaging

agent **2 . 3** (1.7 mg, 4.8×10^{-3} mmol) was weighed into a 1 dram glass vial. Spectrophotometric grade heptane (4.0 mL) was added to the vial via plastic syringe, and the solution was sonicated for 1 h to fully dissolve the compound. This afforded a solution of 1.2×10^{-3} M imaging agent **2.3** in heptane, which was orange-pink in appearance. Serial dilution using this 2.4×10^{-3} M stock solution, a 10 µL gastight syringe (Hamilton Company), and heptane allowed for the preparation of clear, colorless 1.0×10^{-13} M solutions of imaging agent **2.3** in heptane.

*d. Preparation of a solution containing 2.0×10^{-14} M imaging agents **2.2** and **2.3** for two-color/two-reagent fluorescence microscopy (chemoselectivity experiments).* Two separate solutions of imaging agent **2.2** at 1.0×10^{-11} M and **2.3** at 1.0×10^{-13} M were prepared using the previously stated dilution procedures. To the vial containing imaging agent **2.3** at 1.0×10^{-13} M, 10 µL of imaging agent **2.2** at 1.0×10^{-11} M was added. This allowed for the preparation of a clear, colorless solution containing 1.0×10^{-13} M solution of imaging agents **2.2** and **2.3** in heptane.

*e. Preparation of a solution containing 2.0×10^{-14} M compounds **S2.4** and **S2.5** for fluorescence microscopy control experiment.* Compounds **S2.4** and **S2.5** are control compounds because they contain the identical green and orange BODIPY cores, but lack metathesis-reactive olefins. Control compound **S2.4** (1.5 mg, 4.9×10^{-3} mmol) was weighed into a 1 dram glass vial. Spectrophotometric grade heptane (2.0 mL) was added to the vial via plastic syringe, and the solution was sonicated for 1 h to fully dissolve the compound. This afforded a solution of 2.4×10^{-3} M control compound **S2.5** in heptane, which was green-orange in appearance. Control compound **S2.5** (1.7 mg, 4.8×10^{-3} mmol) was weighed into a separate 1 dram vial and dissolved in 2 mL of spectrophotometric grade heptane, then sonicated for 1 h to afford a 2.4×10^{-3} M of solution. The previous two solutions of 2.4

x 10^{-3} M control compound **S2.4** and **S2.5**, were combined in a 20 mL glass vial to make a 1.2 x 10^{-3} M solution containing control compounds **S2.4** and **S2.5**. Serial dilution using this 1.2 x 10^{-3} M stock solution, a 10 µL gastight syringe (Hamilton Company), and heptane allowed for the preparation of clear, colorless 1.0 x 10^{-13} M solutions of control compounds **S2.4** and **S2.5** in heptane.

VII. Acquisition of Fluorescence Microscopy Data

The images presented in this section were acquired by senior student Antonio Garcia IV.

*a. One-color imaging of single chain-elongation events with imaging agent **2.2** at 2.0 × 10–14 M.* A microscopy cell containing a 1.00 mL of the catalyst **S2.6** mixture (1.5 mg) in spectrophotometric grade heptane was imaged to in TIRF mode. This resulted in dark images when surveying regions of the coverslip (Figure S2.6). In a 20 mL vial, norbornene (28.5 mg, 3.03 x 10^{-4} mol) was weighed out and dissolved in 15.0 mL of spectrophotometric grade heptane, added via plastic syringe. 0.25 mL of the norbornene solution was then added all at once to the mixture of catalyst **S2.6** in heptane (Section VIa), yielding a volume of 1.25 mL. The reaction mixture was imaged in TIRF mode for three minutes, which resulted in dark images and no detection of single molecules when surveying regions of the coverslip (Figure S2.6). At the 5-minute mark after addition of norbornene to the catalyst mixture, the mother liquor was removed leaving behind a layer of liquid (approximately 0.1 mL). The pre-formed polymer material gathered on the surface was rinsed five times with 1 mL of spectrophotometric grade heptane. After the last heptane rinse, the solvent was removed to complete dryness of the microscopy vial. This was done to ensure accurate volume measurements in the next steps. Spectrophotometric grade heptane (1 mL) was added via syringe. 1 mL of heptane was added to the microscopy vial and a 20-minute time-lapse capture was taken in TIRF mode (see Section III

for acquisition details). After 90 seconds into the time-lapse capture, the shutter was closed and 0.25 mL of imaging agent **2 . 2** at 1.0 x 10^{-13} M was added. This resulted in a reaction mixture containing imaging agent **2 . 2** at 2.0 x 10^{-14} M and pre-formed polynorbornene on the coverslip surface. The shutter was immediately opened after addition. The imaging "start" moment is referred to as t = 0 on all figures and is the moment the shutter was re-opened. The stage was not moved after the sample shutter was re-opened. Upon opening the shutter, numerous single-bright flashes were observed on the coverslip surface in both green and orange channels. At some regions of the coverslip, the flashes appeared to be localized to particular 2−5 μm areas, indicating areas of high reactivity. These images indicate that flashes are due to the insertion of **2.2** into the growing polymer chains.[1] The flashes from this green BODIPY probe appear brighter and more numerous in the green channel versus the orange channel, as expected. These observations are consistent with the spectral properties of the green BODIPY core.[3]

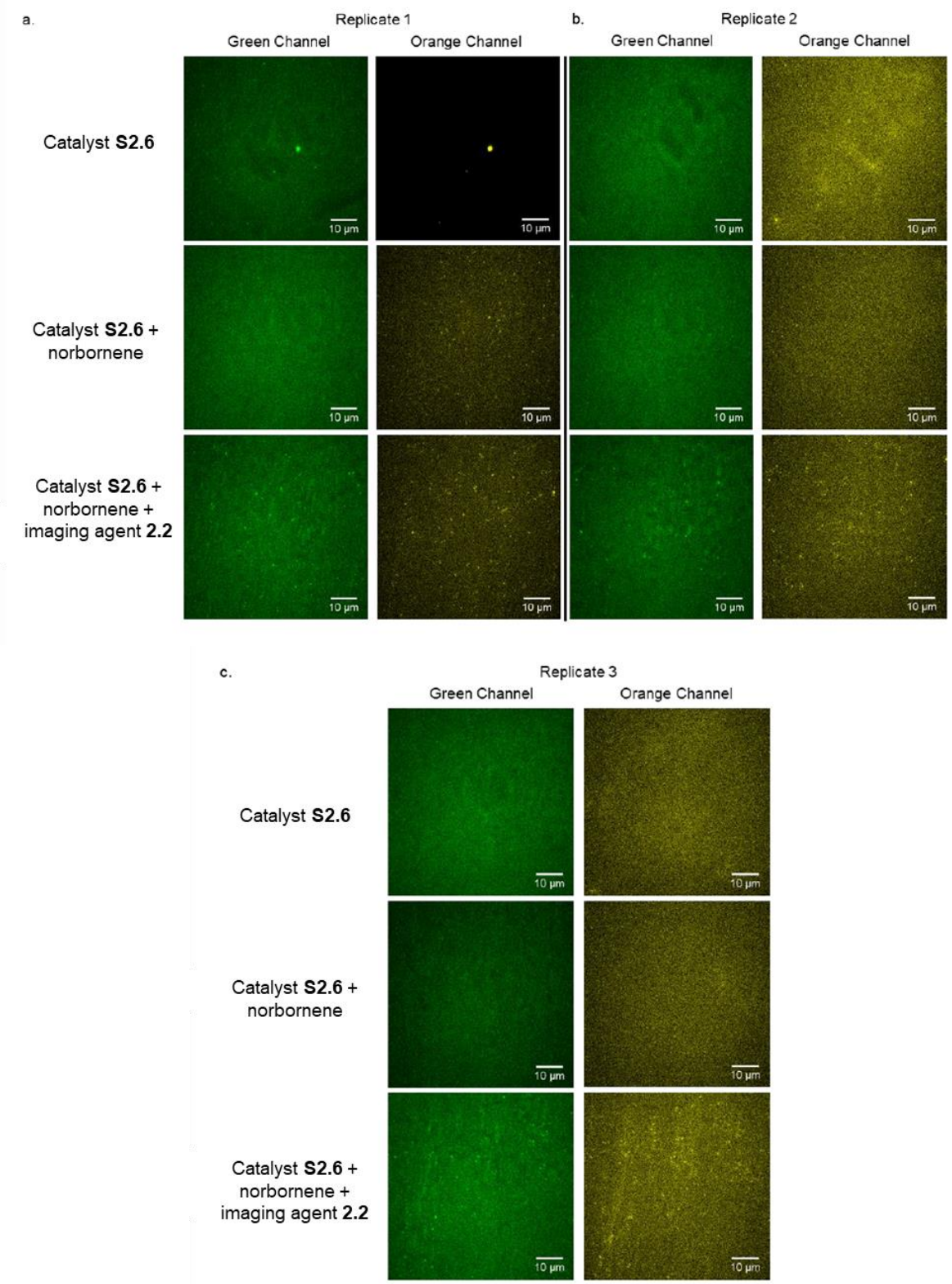


Figure S2.6. Representative single frames from one-color imaging experiments for single chain-elongation events for three replicate samples (a−c). Single-molecule events were observed using imaging agent **2.2** at 2.0×10^{-14} M.

*b. One-color imaging of single chain-termination events with imaging agent **2.3** at 2.0 × 10^{-14} M.* The video acquisition procedures for these experiments are identical to that of section VIIa, with the exception that chain-termination agent **2.3** was used in place of chain-elongation agent **2.2**. Upon addition of imaging agent **2.3** and re-opening the laser shutter, numerous single-bright flashes were observed on the coverslip surface in both, green and orange channels (Figure S2.7). As found with the chain-elongation imaging agent **2.2**, the flashes appeared to be localized to particular 2−5 μm areas, indicating areas of high reactivity. These images indicate that flashes are due to the insertion of **2.3** into the growing polymer chains. The flashes from this orange BODIPY probe are brighter and more numerous in the orange channel versus the green channel, as expected. There were few to none flash events in the green channel in some regions. These observations are consistent with the spectral properties of the orange BODIPY core.[2]

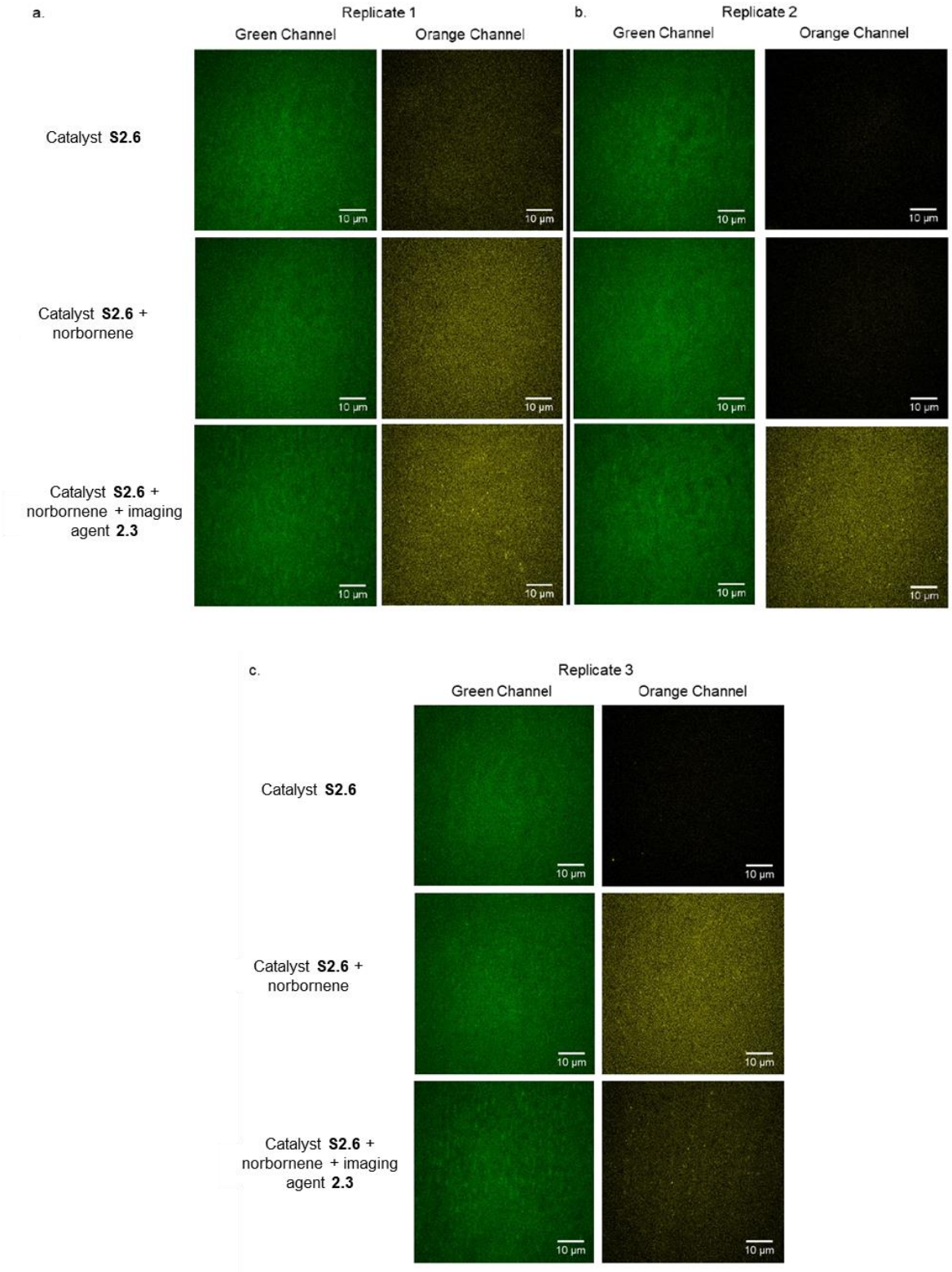


Figure S2.7. Representative single frames from one-color imaging experiments for single chain-termination events for three replicate samples (a–c). Single-molecule events were observed using imaging agent **2.3** at 2.0×10^{-14} M.

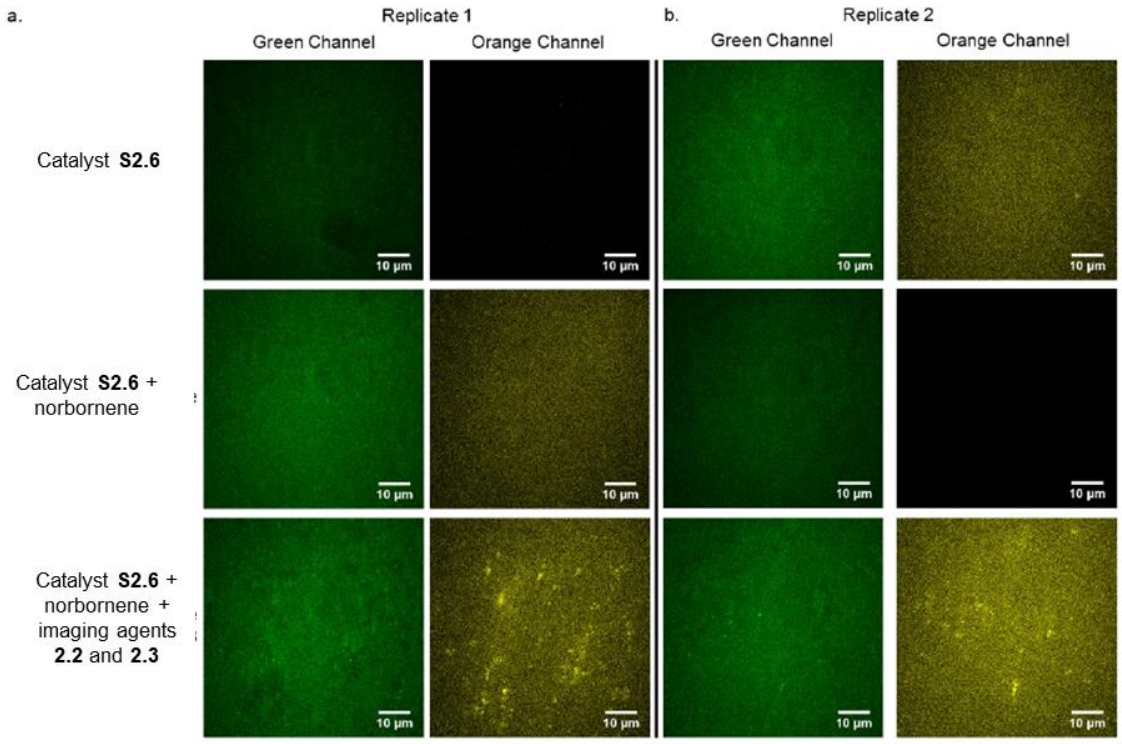
*c. Two-color imaging of single chain-elongation and -termination events with imaging agents **2.2** and **2.3** at 2.0 × 10^{-14}* M. A microscopy cell containing a 1.0 mL mixture of catalyst **S2.6** in spectrophotometric grade heptane was imaged to in TIRF mode. This resulted in dark images when surveying regions of the coverslip (Figure S2.8).

In a 20 mL vial, norbornene (28.5 mg, 3.03 x 10^{-4} mol) was weighed out and dissolved in 15.0 mL of spectrophotometric grade heptane, added via plastic syringe. Then, 0.25 mL of the norbornene solution was added all at once to the mixture of catalyst **S2.6** in heptane (Section VIa), yielding a volume of 1.25 mL. The reaction mixture was imaged in TIRF mode for three minutes, which resulted in dark images and no detection of single-molecules when surveying regions of the coverslip (Figure S2.8).

At the 5-minute mark after addition of norbornene to the catalyst mixture, the mother liquor was removed leaving behind a layer of liquid (approximately 0.1 mL). The pre-formed polymer material gathered on the surface was rinsed five times with 1 mL of spectrophotometric grade heptane. After the last heptane rinse, the solvent was removed to complete dryness of the microscopy vial. This was done to ensure accurate volume measurements in the next steps. Spectrophotometric grade heptane (1 mL) was added via syringe. The microscopy vial was placed on the objective and focused in TIRF mode. Heptane (1 mL) was added to the microscopy vial and a 20-minute time-lapse capture was taken in TIRF mode (see Section III for acquisition details). After 90 seconds into the time-lapse capture, the shutter was closed and 0.25 mL of imaging agent **2.2** and **2.3** at 1.0 x 10^{-13} M was added. This resulted in a reaction mixture containing imaging agent **2.2** and **2.3** at 2.0 x 10^{-14} M and pre-formed polynorbornene on the coverslip surface. The shutter was immediately opened after addition. The imaging "start" moment is referred to as $t = 0$ on all figures and is the moment the shutter was re-opened. The stage was not moved after the

sample shutter was re-opened. Upon opening the shutter, numerous single-bright flashes were observed on the coverslip surface in both green and orange channels (Figure S2.8). At some regions of the coverslip, the flashes appeared to be localized to particular 2−5 μm areas, indicating areas of high reactivity. These images indicate that flashes are due to the insertion of **2.2** and **2.3** into the growing polymer chains. The flashes appear to be equally numerous and bright in both green and orange channels, corresponding to the expected imaging of both green and orange imaging agents.

Following the previous time-lapse acquisition, other regions of the coverslip were imaged using the same acquisition parameters (see Section III for acquisition details). This time-lapse capture is an internal control experiment that tests for the buildup of polymeric material and fluorophore at the coverslip surface and is discussed in detail in Section VIII.



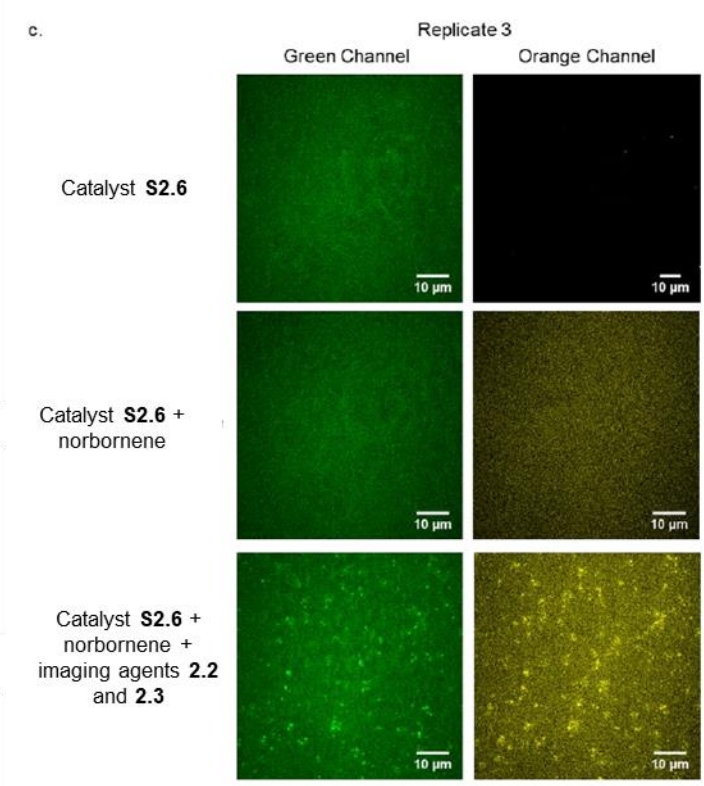


Figure S2.8. Representative single frames from two-color selectivity experiments with imaging agents **2.2** and **2.3** at 2.0×10^{-14} M for three replicate samples (a–c).

VIII. Control Experiments

The work described in this section was performed by senior student Antonio Garcia IV.

a. *Control Experiment for Two-color imaging with control compounds **S2.4** and **S2.5** at* 2.0×10^{-14} M. The video acquisition procedure for this experiment are identical to that of section VIIc, with the exception of using control compound **S2.4** in place of imaging agent **2.2** and control compound **S2.5** in place of **2.3**. Upon initiating video acquisition of the sample and focusing into TIRF, the images remained dark, with little to no single-molecule events observed at some regions of the coverslip surface in both green and orange channels (Figure S2.9). To ensure the correct focal plane for observing single-molecule events was achieved, multiple z-axis planes were focused. This showed dark images for all possible focal planes. These experiments confirm that

the correct metathesis-active functional groups are needed to produce single-molecule images at this concentration range.[5]

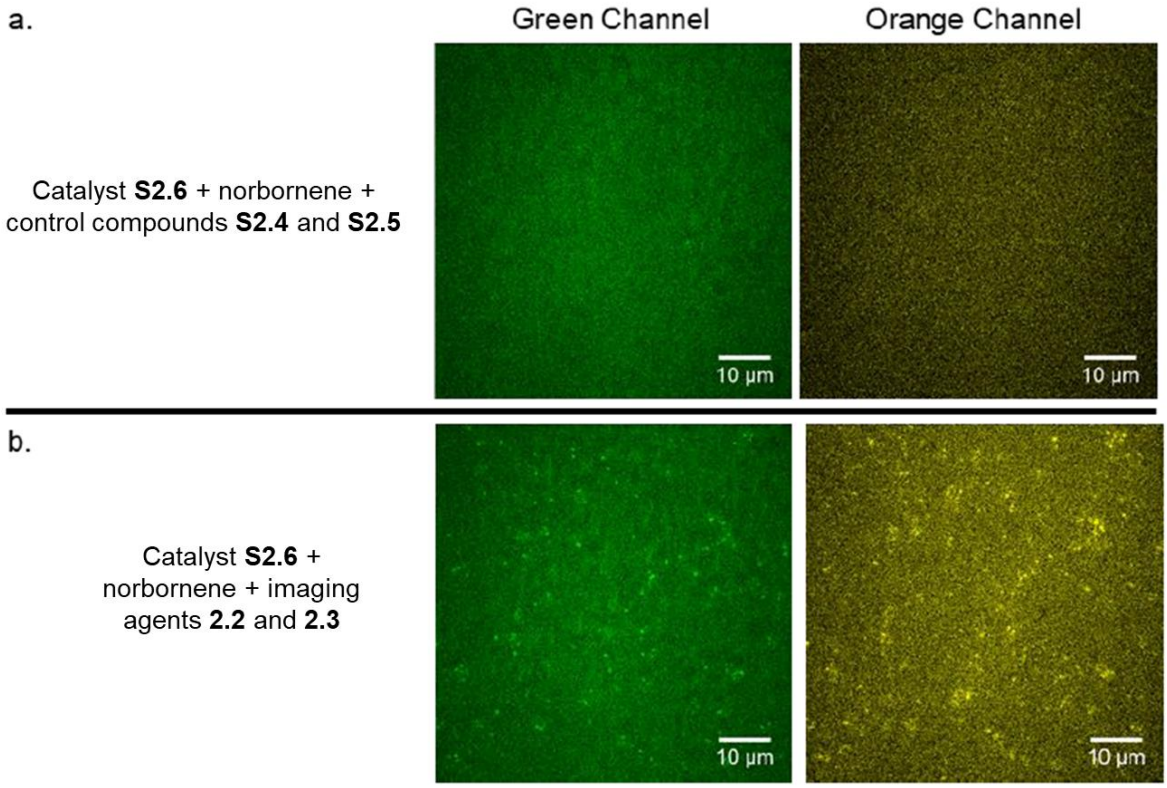


Figure S2.9. Comparison of control and non-control experiments: (a) Representative single frames from two-color control experiment with control compounds **S2.4** and **S2.5** at 2.0×10^{-14} M. (b) Representative single frames from two-color selectivity experiment with imaging agents **2.2** and **2.3** at 2.0×10^{-14} M.

To further compare the imaging of control compounds **S2.4** and **S2.5** with imaging agents **2.2** and **2.3**, composite images were produced. These images are the max intensity values of each pixel over the course of the entire time-lapse video. The composite images were produced separately for each color channel, green and orange. The composite images shown in Figures S2.10 and S2.11 were set to the same brightness and contrast settings. To enable clear comparisons, the

brightness and contrast settings were set to: gamma = 0.5, min = 125, max = 150 for the green channel and gamma = 0.5, min = 120, max = 135 for the orange channel.

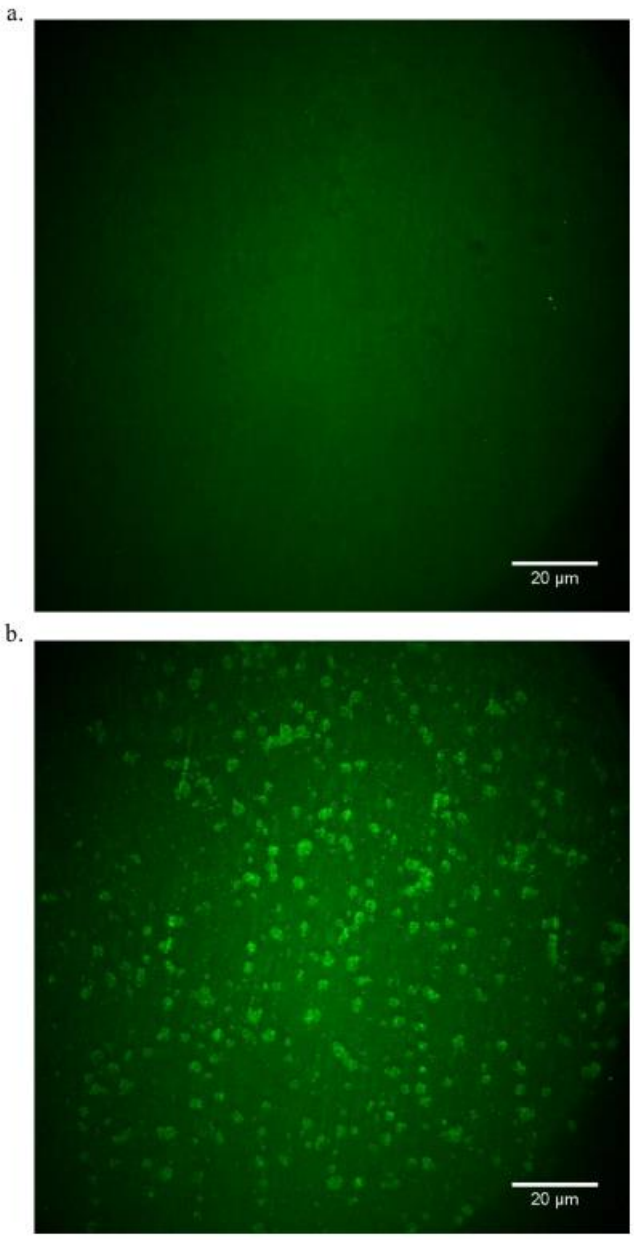


Figure S2.10. Comparison of green channel composite images of (a) control compounds **S2.4** and **S2.5**, with (b) imaging agents **2.2** and **2.3**.

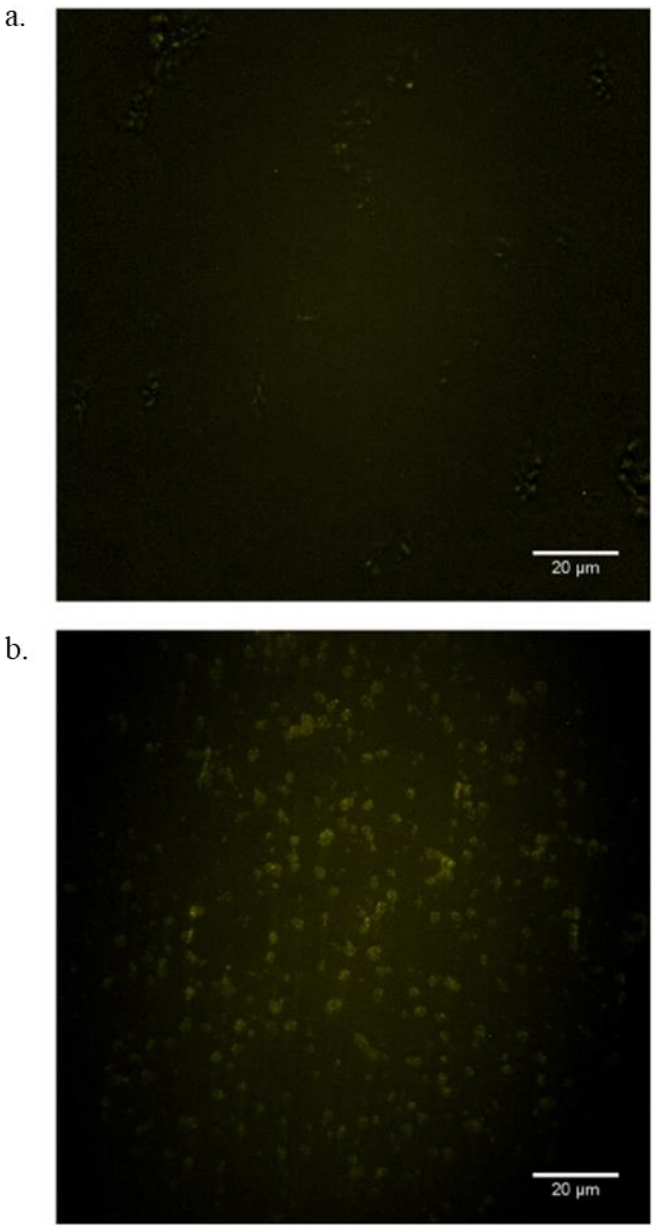


Figure S2.11. Comparison of orange channel composite images of (a) control compounds **S2.4** and **S2.5**, with (b) imaging agents **2.2** and **2.3**.

b. Control experiment for ligand-coordination equilibrium as cause for single-molecule events. The objective for this control experiment is to determine if ligand-coordination equilibrium between the reactive olefin functionalities and the ruthenium catalyst centers is a plausible origin for the observed single-molecule events. To test this hypothesis, other regions of

the coverslip surface were imaged. These other regions of the coverslip were not previously exposed to the photobleaching conditions caused by the laser light. If ligand-coordination equilibrium were the cause of single-molecule events, these regions would appear the same as the regions that were analyzed for a 15-minute period. Conversely, if single-catalyst-turnover events were the cause of the single-molecule signals, these regions unexposed to laser light would show a buildup fluorescence at the polymer-particle aggregates. After completion of each 15-minute imaging acquisition period a new region of the coverslip was imaged for 5–10 s. Figure S2.12 below shows representative single-frame comparisons between the regions of the coverslip that were exposed to laser light for 15 minutes and new unexposed regions.

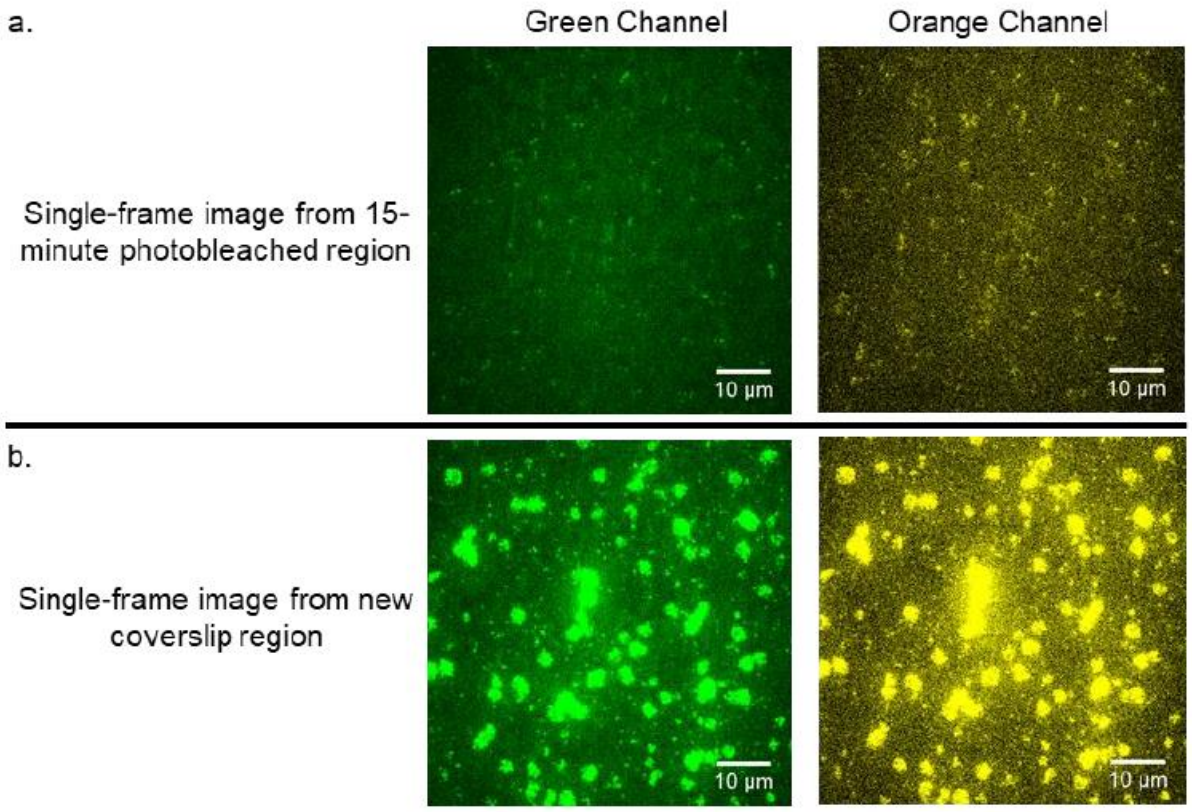


Figure S2.12. Comparison of single-frames images of (a) coverslip region that has been exposed to laser light for 15 minutes and (b) new coverslip region that was not exposed to laser light.

c. Control experiment for build-up of fluorescent imaging agents caused by irreversible incorporation at single-polymer-particle aggregates. The goal for this control experiment is to determine if irreversible incorporation of imaging agents at single-polymer particle aggregates can be directly observed in real-time via fluorescence microscopy. For this experiment, the reaction conditions detailed in Section VIIc were used as described, with the exception to the change in image capture rate. The capture rate was changed from 1 s to 3 min, which effectively reduces of laser light exposure to the sample. The imaging agents **2.2** and **2.3**, were added immediately after the first frame capture.

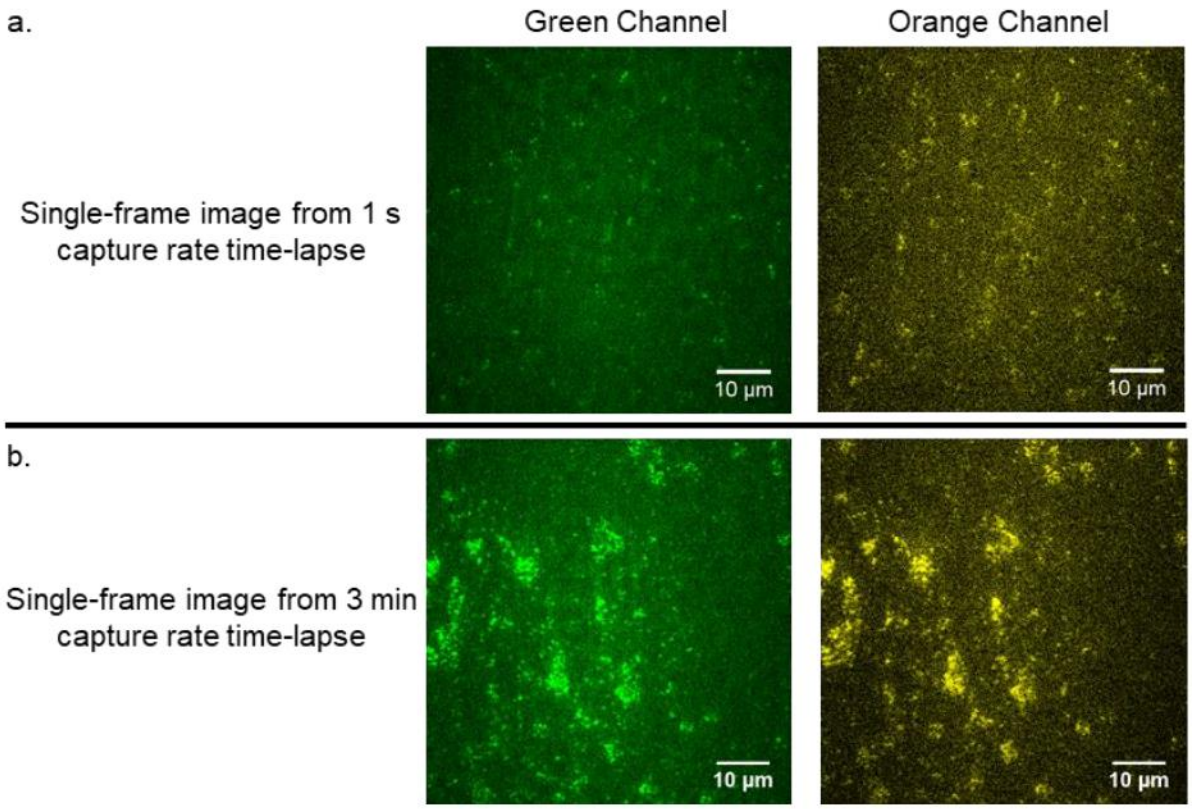


Figure S2.13. Comparison of single-frames images of (a) sample with 1 s capture rate time-lapse and (b) sample with 3 min capture rate time-lapse.

As shown is Figure S2.13b, highly fluorescent polymer-particle aggregates were observed on the coverslip surface. In this sample, fluorescence intensity was measured for five non-

overlapping single-polymer-particle aggregates. For each analyzed single-polymer particle, the intensity was measured for the entirety of the particle. As a control, the background intensity was measured at ~ 3 μm area that did not contain polymer aggregates (dark areas).

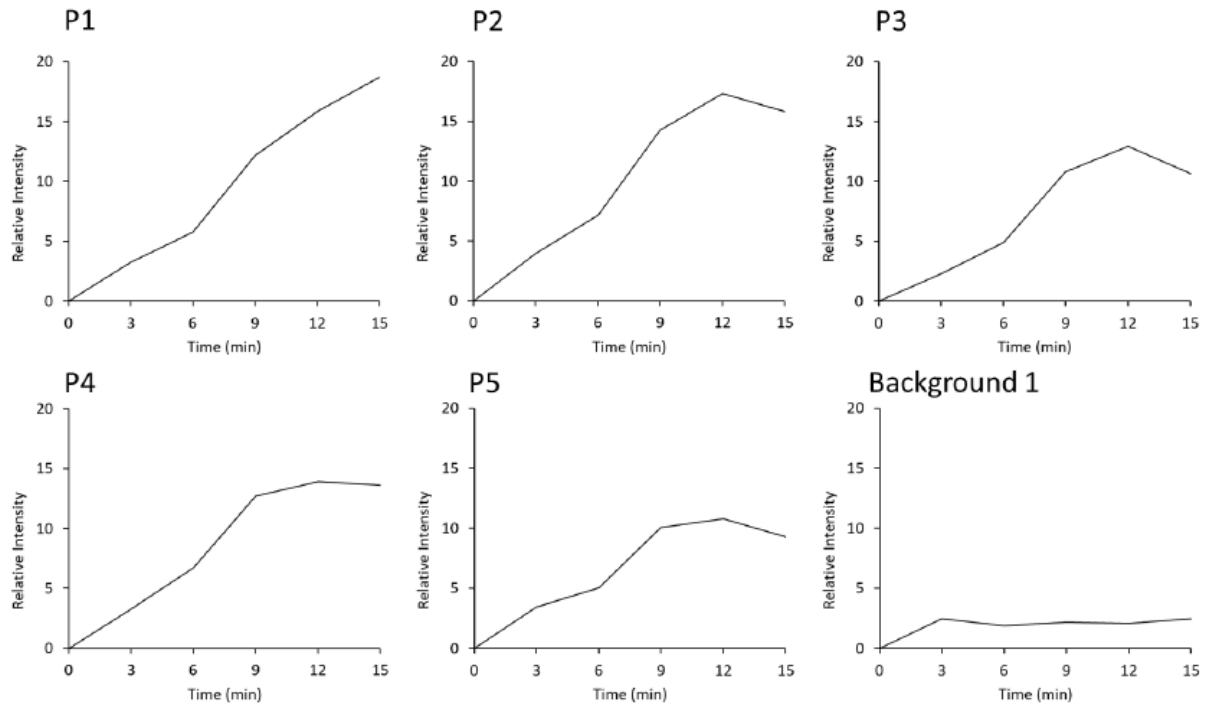


Figure S2.14. Intensity versus time traces for single-polymer-particle aggregates (P1-P5) and for a ~3 μm area that did not contain fluorescent material (Background 1).

As shown in Figure S2.14, the fluorescence intensity signal builds-up upon addition of imaging agents **2.2** and **2.3**. The intensity measured at t = 0 min was taken prior to addition of imaging agents **2.2** and **2.3**. The background intensity increases upon addition of imaging agents **2.2** and **2.3** (t = 3 min), but as shown in the Background 1 intensity time trace, it does not build-up.

In summary, the results from these three control experiments suggest that the observed single-molecule events are predominately caused by single catalytic turnover events between the ruthenium catalyst centers and olefin-functionalized BODIPY imaging probes.

IX. Super-Localization and Identification of Single-Turnover Events

The code described in this section was created by post-doctoral scholar Dr. D.J. Dibble.

Single-molecule activity from the images was quantified using a modified version of Localizer implemented in Igor Pro 8.[6] The resulting TIFF image stacks for each experiment and color channel were exported to Igor Pro 8 for analysis, and the display settings were kept at the default setting. For the basis set experiments, 2.0×10^{-14} M imaging agent **2.2** and 2.0×10^{-14} M imaging agent **2.3**, the images were not cropped and left at the default 1200 × 1200 pixel array. To identify and analyze the single-molecule events, a series of filters and conditions were applied, as here detailed. Segmentation Algorithm: GLRT, Particle finding: 8-way adjacency, Particle verification: Remove Overlapping Particles and Gaussian Fitting, Standard Deviation of the PSF (pixels): 1.5, and GLRT Insensitivity: 50. To consolidate identical emitters, the following conditions were applied. Maximum difference in position (in pixels): 3, Maximum allowed gap due to blinking (in frames): 60, Min number of observations for localization error analysis: -1, Max number of observations for localization error analysis: -1. For the one-color experiment with 7.0×10^{-14} M of imaging agent **2.3**, the orange channel was analyzed with the same settings as previously stated, with the exception of the GLRT Insensitivity setting set to 30. Using these settings numerous emitters were localized for each sample replicate (Table S2.1).

Table S2.1. Number of single molecule events identified by localizer for each one-color sample replicate

Triplicate Data	Number of identified Events
One-color Imaging Agent 2 Replicate 1	1,184
One-color Imaging Agent 2 Replicate 2	1,495
One-color Imaging Agent 2 Replicate 3	342
One-color Imaging Agent 3 Replicate 1	379
One-color Imaging Agent 3 Replicate 2	496
One-color Imaging Agent 3 Replicate 3	757

After determining the position of each emitter, a modification to the default localizer utility was used to filter the individual events down to those corresponding with well-resolved single molecule activity and to curve fit the individual time/intensity traces to determine each event's parameters (https://github.com/dibblda/2ChannelTimeTraceUtility_Localizer). First, each intensity versus time trace was calculated by averaging a 3 x 3 pixel box centered on the floor of the emitter location in pixels, as determined by the Localizer utility. Emitters at the edge of the image were omitted to avoid incomplete averaging. Subsequently, the data was filtered by manual examination, creating a subset of events that corresponded to well-resolved single molecule activity that showed the quantized "step up" and "step down" behavior that is consistent with single molecules (an additional stringency over the events that Localizer fit automatically). In the process of filtering the data, a curve fit was applied to the selected traces to characterize each event. The curve fit algorithm first baseline-adjusted each trace by applying a median-smoothing curve function to the intensity versus time trace over a third of the available points and then subtracted this baseline curve from the unadjusted intensity versus trace.[7] After baseline adjusting the trace,

a boxcar function was fit to the peak in the baseline-adjusted time/intensity curve using a simulated annealing algorithm as implemented in Igor Pro. This fit was repeated ten times and the closest fit to the experimental data was selected for further analysis. The peak height of the event was calculated to be the average value between the onset and fall of the fitted boxcar function. Overall, the modification to localizer allowed for the ready export of the filtered intensity versus time traces and parameters of each event for further analysis.

X. Construction of Histograms of Single-Turnover Event Ratios for One-Color Basis Set Experiments

The work described in this section was performed by myself and senior student Antonio Garcia IV.

To identify green and orange imaging agents in two-color mixing experiments, single-color basis set experiments were performed for each imaging agent in triplicate. These single-color experiments were required due to the known spectral distribution of single molecules (i.e., each individual molecule has a unique spectrum depending on its local environment). Data from these experiments were used to assign "green" and "orange" in the two-color chemoselectivity experiments with >95% confidence. That is, to determine the degree to which the most red-shifted "green" emitters resembled "orange" emitters and vice versa, and thereby to reject emitters that could not be assigned as a specific color with >95% confidence.

a. Histogram ratio analysis for one-color single chain-elongation events with solution containing 2.0 × 10–14 M of imaging agent ***2.2****.* The details for the experimental procedures are found in sections VIb and VIIa. Following the selection and analysis of each single-molecule event, an additional modification to localizer was employed to determine the ratio of intensities between events occurring in different imaging channels on the microscope. After the analysis of

the first channel, the saved curve fit parameters for each event were loaded and the intensity of the event in the corresponding channel was calculated as the mean value of the baseline-adjusted intensity versus time trace from the beginning to the end of the calculated event in the first channel. This allowed for the calculation of intensity ratios between different channels.

For each replicate run, a sub-set of all intensity versus time traces were analyzed (approximately 300 for each sample) resulting in a total of 900 time traces analyzed for the one-color chain-elongation experiments. The determined single-molecule ratio values were binned into a single histogram (bin size = 0.5). Ratio values greater than 6.0 were not included in the histogram, as these values were assigned to green with a high certainty. The histogram was fit to a Gaussian curve (Figure S2.15), with the average ratio found to be 2.2 ± 0.87 (n = 900).

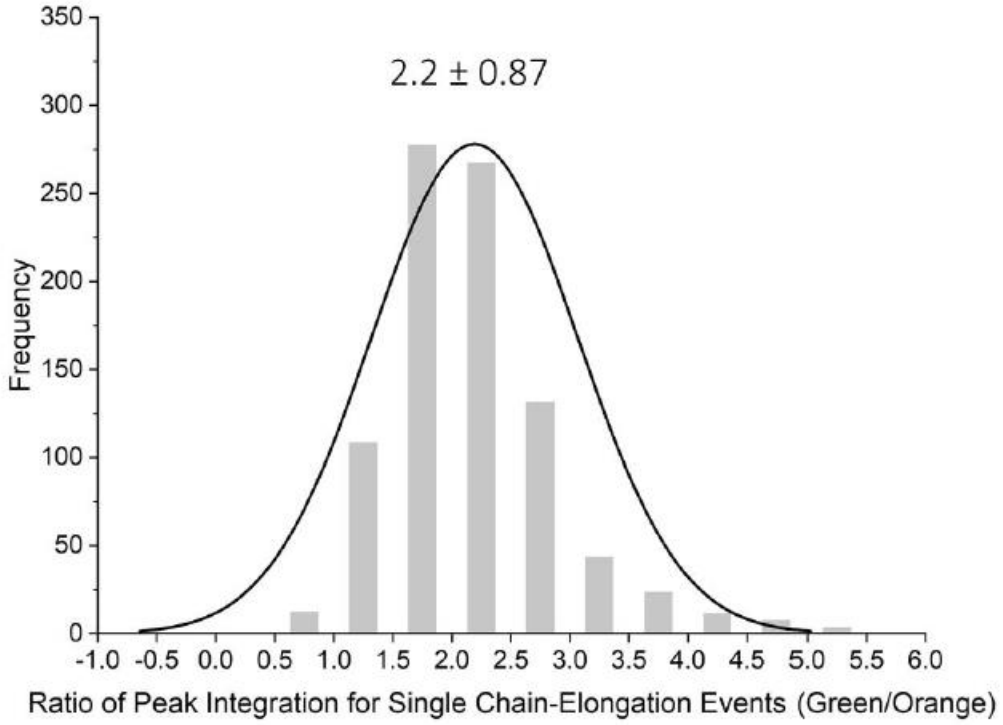


Figure S2.15. Histogram of fluorescent signals for single chain-elongation events in green and orange channels.

*b. Histogram ratio analysis for one-color single chain-termination events with solution containing 2.0 × 10^{-14} M of imaging agent **2.3**.*

The details for the experimental procedures are found in sections VIc and VIIb. To determine the intensity ratio of single chain-termination events for green and orange channels, the square function curve was fit to the single-molecule event in the time trace for both respective channels. The ratio value was calculated by dividing the integration absolute value for the curve in the green channel by the integration absolute value for the curve in the orange channel. For each replicate run, 300 intensity versus time traces were analyzed resulting in a total of 900 time traces analyzed for the one-color chain-termination experiments. The determined single-molecule ratio values were binned into a single histogram (bin size = 0.5). The histogram was fit to a Gaussian curve (Figure S2.16), with the average ratio found to be 0.33 ± 0.40 (n = 900). The Gaussian curve is displayed for clarity of analysis.

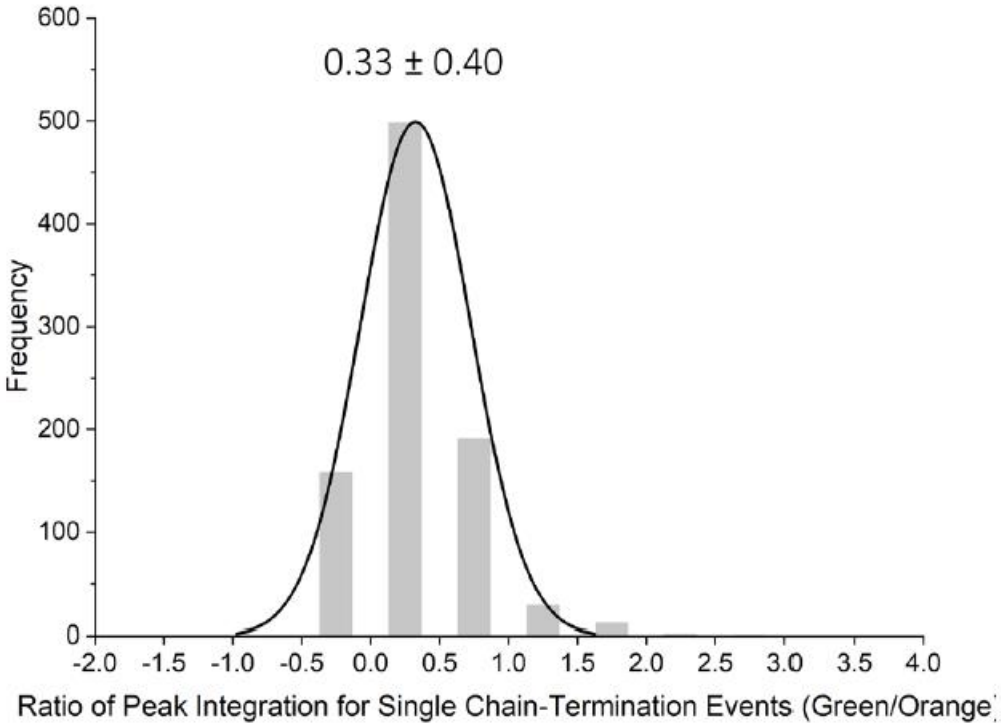


Figure S2.16. Histogram of fluorescent signals for single chain-termination events in green and orange channels.

XI. Limitations of the Method

The analysis method optimized several competing considerations. First: assignment and superlocalization of single chemical reactions. Each potential event was software superlocalized through Gaussian fitting of the point-spread function; this process both removed events that were not clearly single molecules and also removed potential duplicate counting. Further filters were applied to select for the quantized behavior that is a fingerprint of single-molecule events and to limit potential duplicate counting by rejecting a "neighboring" event within 8 s and 570 nm. This neighboring filter accounted for potential motion within growing polymers that may arbitrarily move a reagent after it is incorporated. Such filtering, however, necessarily rejects a subset of chemical reactions. Second: robust assignment of reagent by color. On one hand, high accuracy is

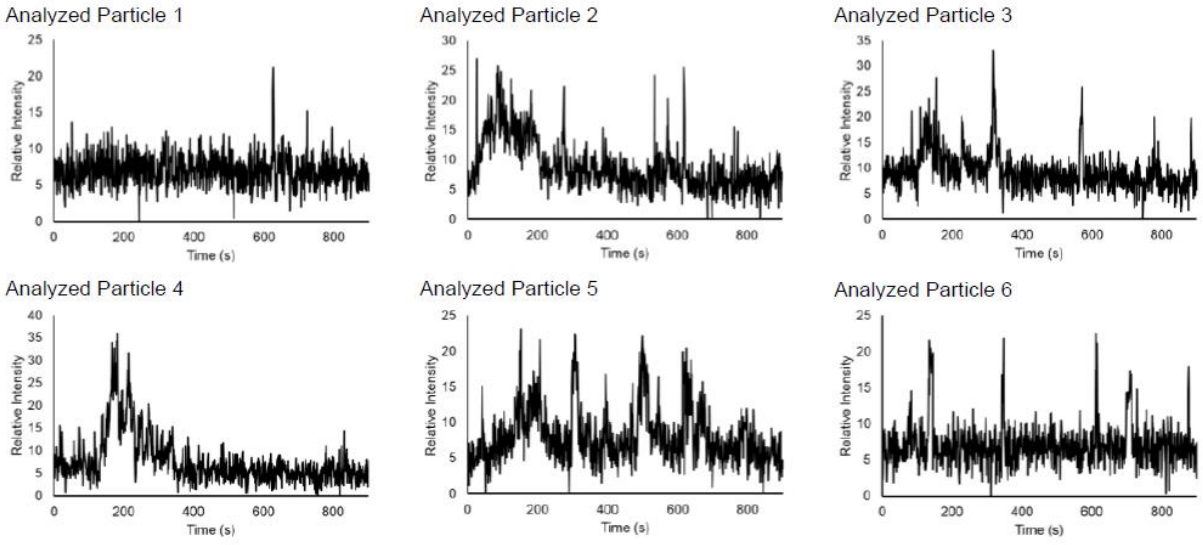
desired for assignment of chain-elongation (green) and chain-termination (orange). Spectral diffusion of single molecules contributes to a subset of events that could not be confidently assigned. In this analysis, we judged accuracy in chemoselectivity assignment (>95% confidence, with rejection of unassigned events) as more important than less-confident assignment of more events. The analyzed particles averaged 14 ± 4% reactions with unassigned chemoselectivity. For particles showing statistically significant time-variable selectivity, the unassigned chemical events did not occur during times in the kinetics data that could negate the differences in chemoselectivity in multiple particles.

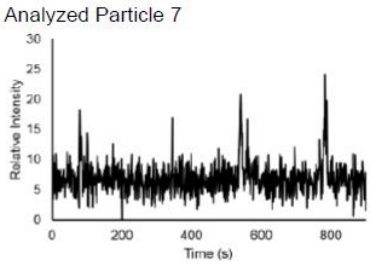
XII. Construction of Superresolved Images

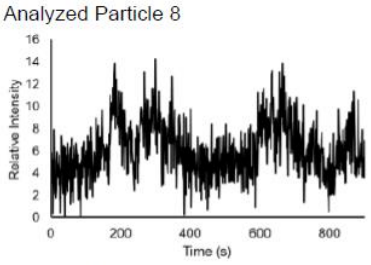
The work described in this section was performed by myself, senior student Antonio Garcia IV and graduate student Pía A. López.

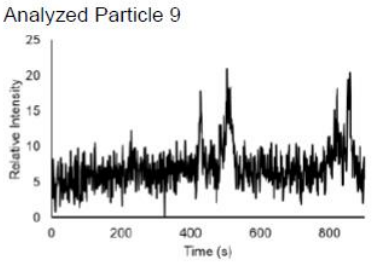
The two-color experiments, detailed in section VIIc, resulted in one TIFF image stack for each color channel. Each TIFF file was exported to ImageJ to search for regions where single-molecule events were centralized on single-polymer particle aggregates. To help search for single-polymer particles that were not overlapping, time composite images were created from the TIFF image stack. The TIFF image stacks were cropped around particles for superresolution analysis in Igor Pro 8 for each color channel. For the analysis of each particle in both color channels, the following filters and conditions were applied; Segmentation Algorithm: GLRT, Particle finding: 8-way adjacency, Particle verification: Remove Overlapping Particles and Gaussian Fitting, Standard Deviation of the PSF (pixels): 1.5, and GLRT Insensitivity: 20. To consolidate identical emitters, the following conditions were applied; Maximum difference in position (in pixels): 3, Maximum allowed gap due to blinking (in frames): 60, Min number of observations for localization error analysis: -1, Max number of observations for localization error analysis: -1.

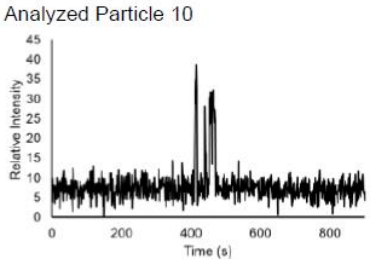
Using the same method detailed in sections IXa and IXb, the ratios for the single-molecule events were determined for the fitted curves. These ratios were then used to identify the imaging agent with 95% confidence. Taking two standard deviations for the average ratios, green = 2.2 ± 1.7 and orange = 0.33 ± 0.8, the following ranges were used to identify the imaging agents; green (ratio >1.1), orange (ratio < 0.46), not identifiable (0.46–1.1). To obtain the mean percentage of unidentified reaction events, the total percentage of unidentified reaction events was calculated for all 30 analyzed particles. The average of the 30 percentages was obtained, along with the corresponding standard deviation, to yield an average of 14 ± 4% unidentified reaction events per analyzed particle. Once identified, the data was filtered to remove emitters that were localized multiple times within an 8 s time period in 5 pixel area. The resulting data were plotted in two forms; colored data points indicating identity of the emitter and colored data points indicating time a single-molecule event occurred.

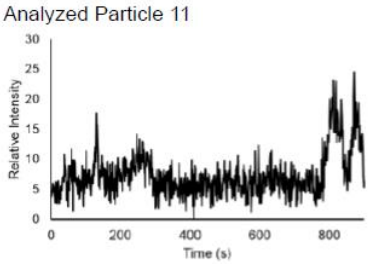


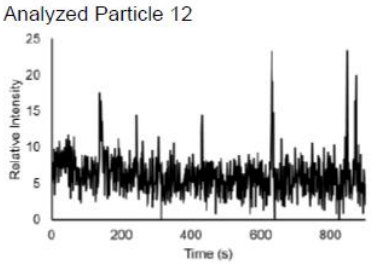

Analyzed Particle 7
Relative Intensity
Time (s)

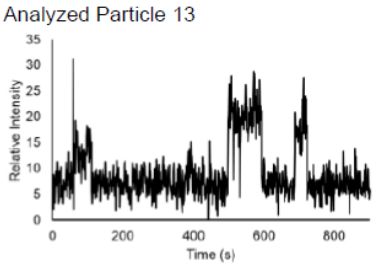

Analyzed Particle 8
Relative Intensity
Time (s)

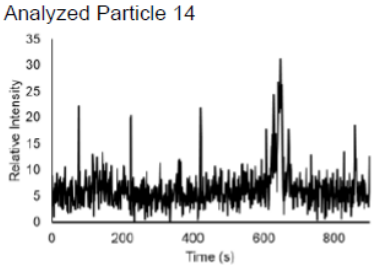

Analyzed Particle 9
Relative Intensity
Time (s)

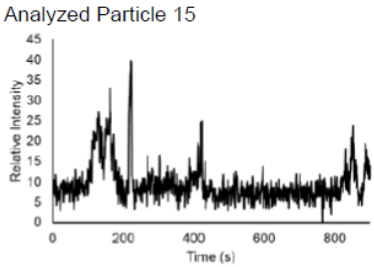

Analyzed Particle 10
Relative Intensity
Time (s)

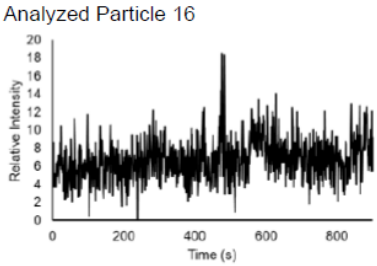

Analyzed Particle 11
Relative Intensity
Time (s)

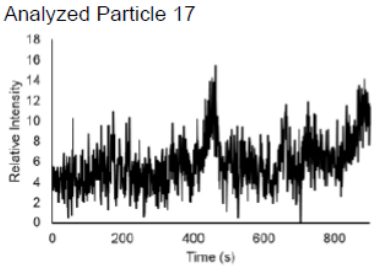

Analyzed Particle 12
Relative Intensity
Time (s)

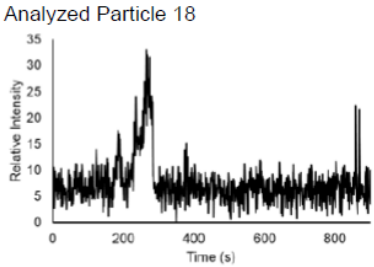

Analyzed Particle 13
Relative Intensity
Time (s)


Analyzed Particle 14
Relative Intensity
Time (s)


Analyzed Particle 15
Relative Intensity
Time (s)


Analyzed Particle 16
Relative Intensity
Time (s)


Analyzed Particle 17
Relative Intensity
Time (s)


Analyzed Particle 18
Relative Intensity
Time (s)

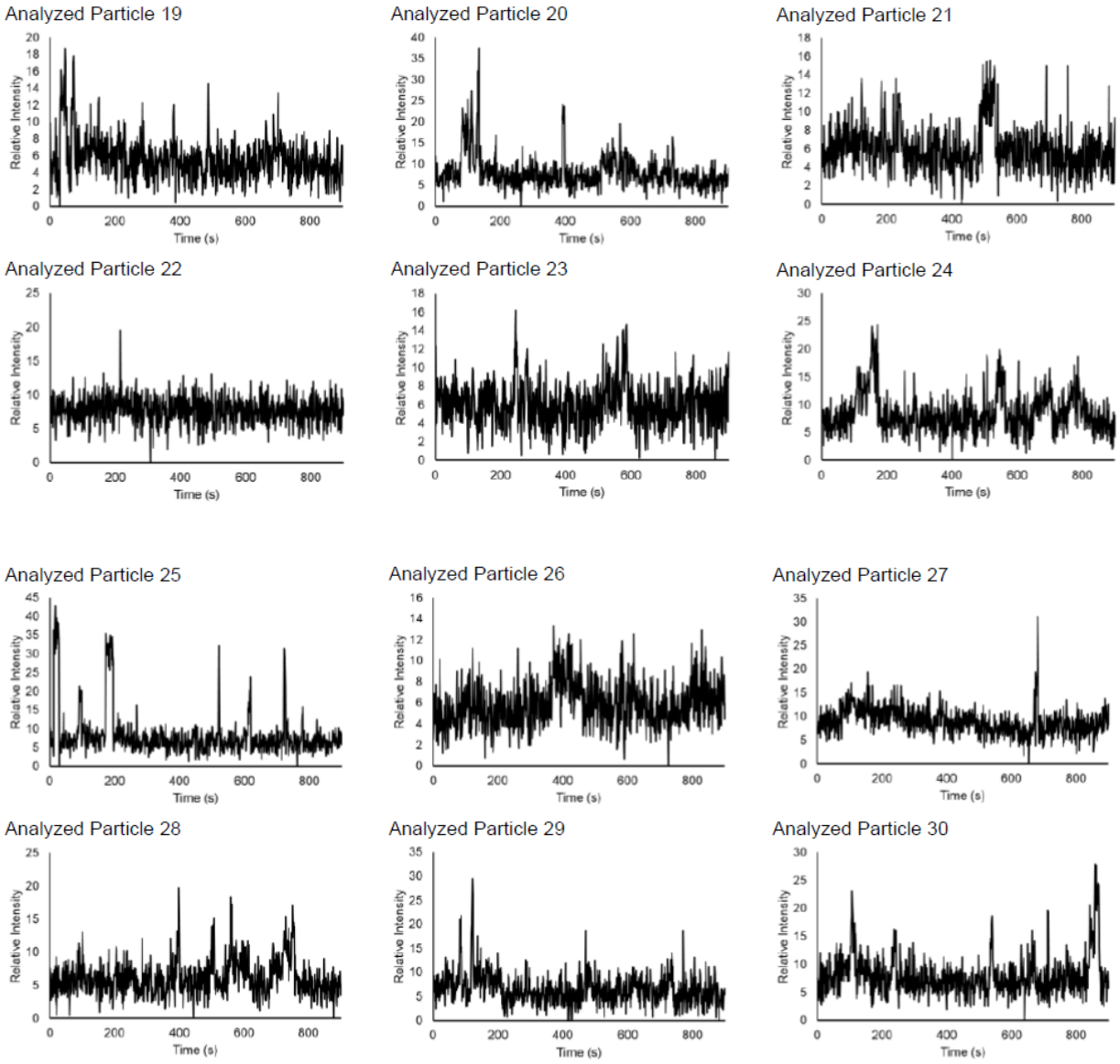


Figure S2.17. Representative intensity versus time traces for each particle showing single chain elongation and -termination events.

XIII. Analysis of Chain Elongation and Termination Selectivity Differences in Time

The work described in this section was performed by myself, senior student Antonio Garcia IV and graduate student Pía A. López.

a. Kolmogorov-Smirnov Test. To determine if the integrated kinetic time traces for chain-elongation (green) and chain-termination (orange) were statistically different, the nonparametric

Kolmogorov-Smirnov test was used in Origin Pro software. The stated null hypothesis was: F(x)=G(y), which states that the two traces are equivalent and are not statistically different. The alternative hypothesis was F(x)≠G(y), which states that the two traces are not equivalent and are statistically different. The probability values (p) were used to determine the degree to which a given hypothesis was true. For example, the smaller the p-value, the greater the probability the null hypothesis is refuted and the alternative hypothesis is true. A p-value ≤ 0.05 refutes the null hypothesis with 95% confidence.

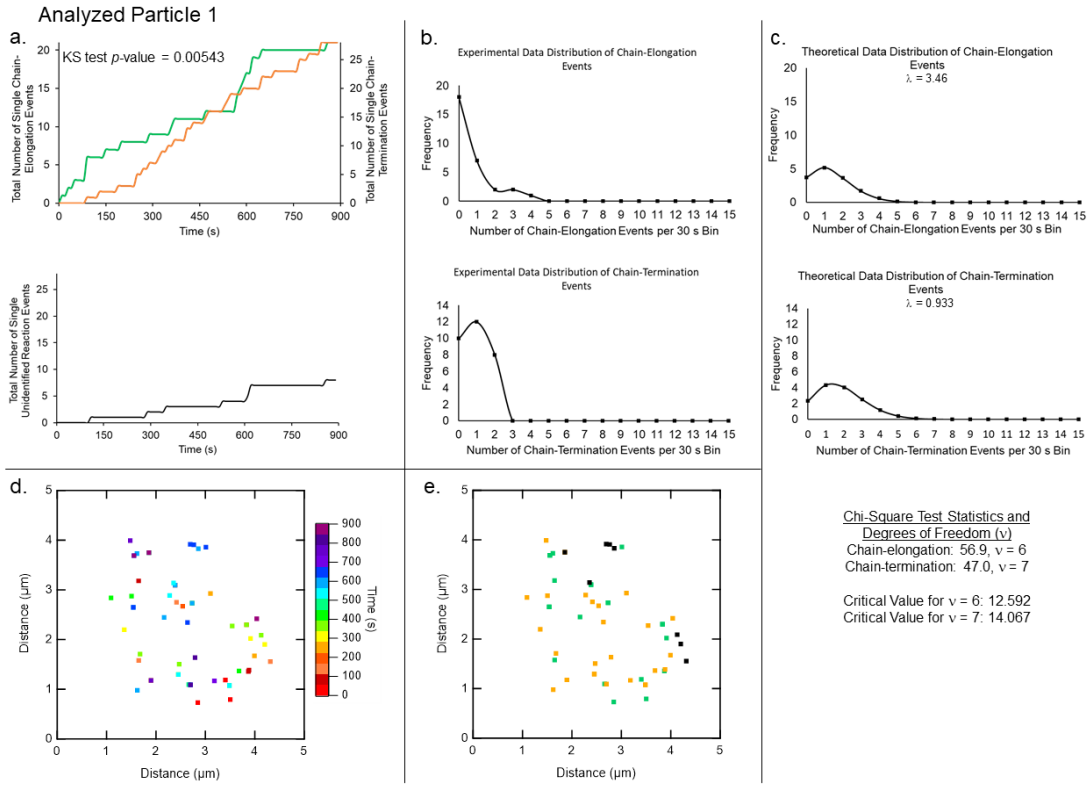
b. Poisson Fitting and Chi-Square Test. A subset of the integrated kinetic time trace data for chain-elongation (green) and chain-termination (orange) show plausible changes in selectivity. Such changes in selectivity are only possible if the turnover frequency rates change differently for chain-elongation and chain-termination as a function of time. For a given data set that fits a Poisson distribution, it is assumed that the frequency of events does not change as a function of time. To determine if the experimental turnover frequency for single chain-elongation and chain-termination events fits a Poisson distribution, a Chi-Square test was performed to test the goodness of fit between the experimental and theoretical distributions. The experimental turnover frequency distribution was constructed for each reaction type, chain-elongation and chain-termination, with a bin size of 30 s. Each particle was analyzed separately and the theoretical Poisson distribution incorporated the average turnover frequency for each reaction type. For the theoretical Poisson distribution, the following equation was used $p(x;\lambda) = \frac{e^{-\lambda}\lambda^{x}}{x!}$ for x=0, 1, 2,…, where x represents the number of events in any given 30 s bin, and λ represents the average turnover rate for a 30 s time period.

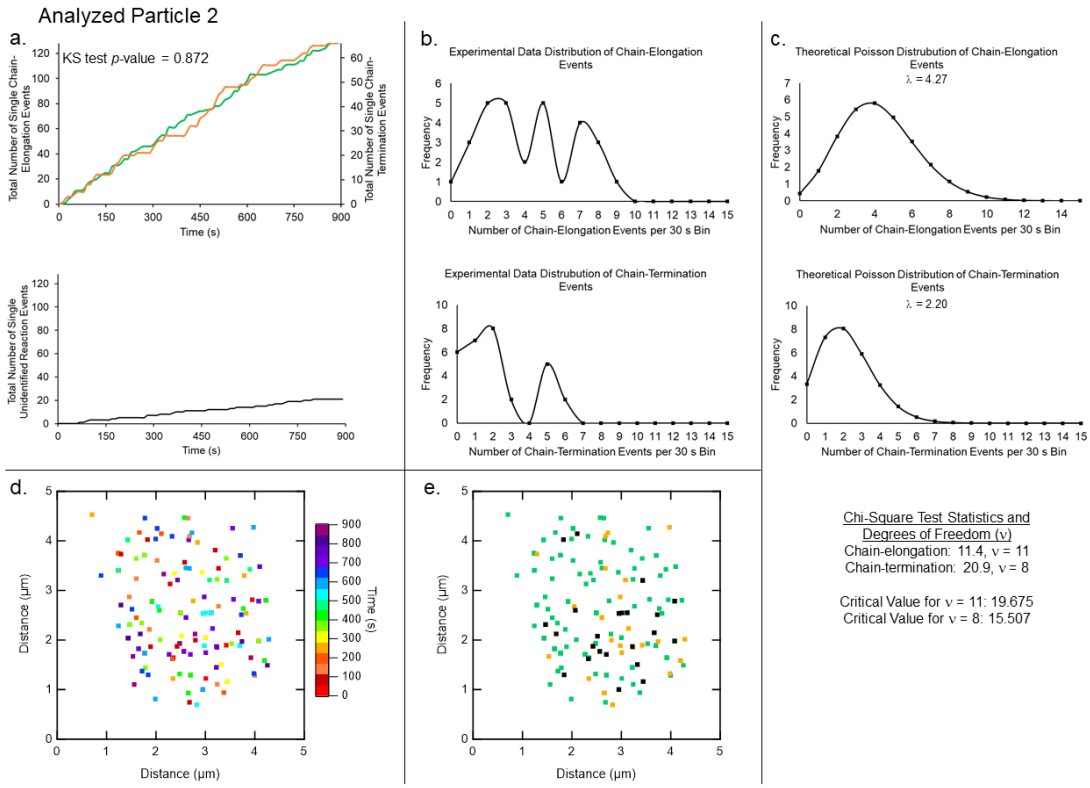
To determine if the experimental turnover frequency fits a theoretical distribution with 95% statistical confidence, a Chi-Square test was performed for each reaction type. The following

equation was used $X^2 = \sum_{i=1}^{k} \frac{(x_i - m_i)^2}{m_i}$. The Williams Correction $q = 1 + \frac{(a^2-1)}{6nv}$ was used given that majority of the frequency data was less than 5.

XIV. 30 Analyzed Particles and Figures

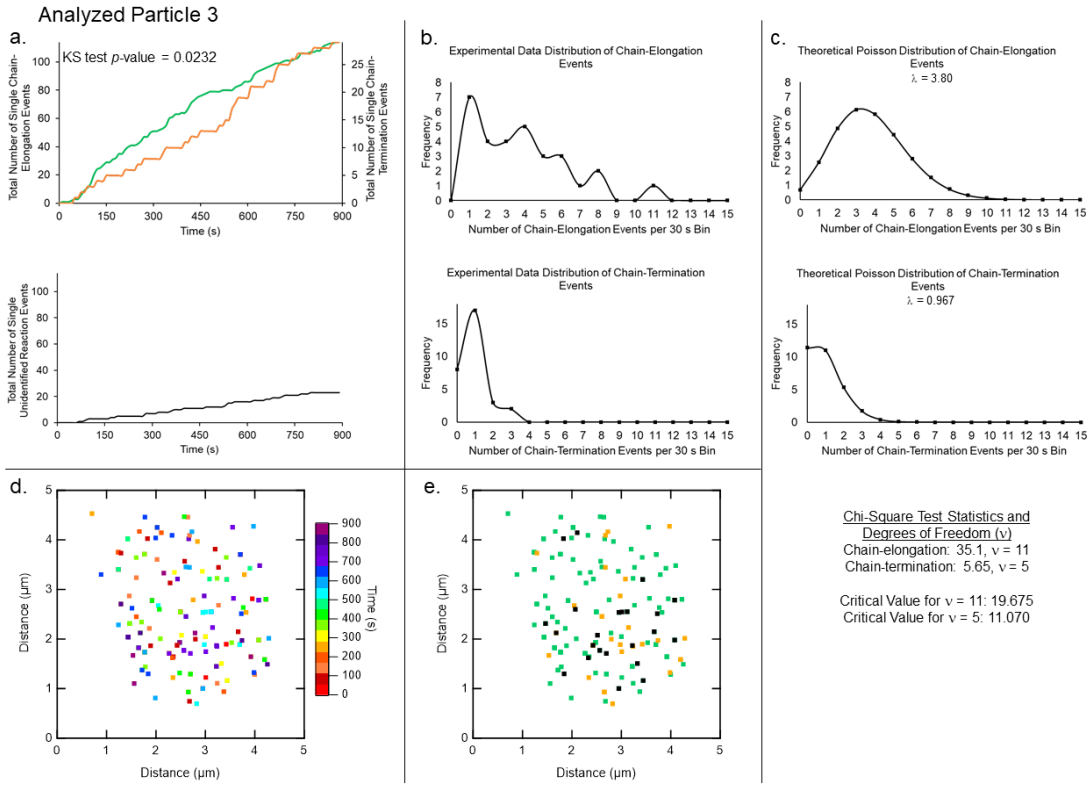
The work described in this section was performed by myself, senior student Antonio Garcia IV and graduate student Pía A. López.





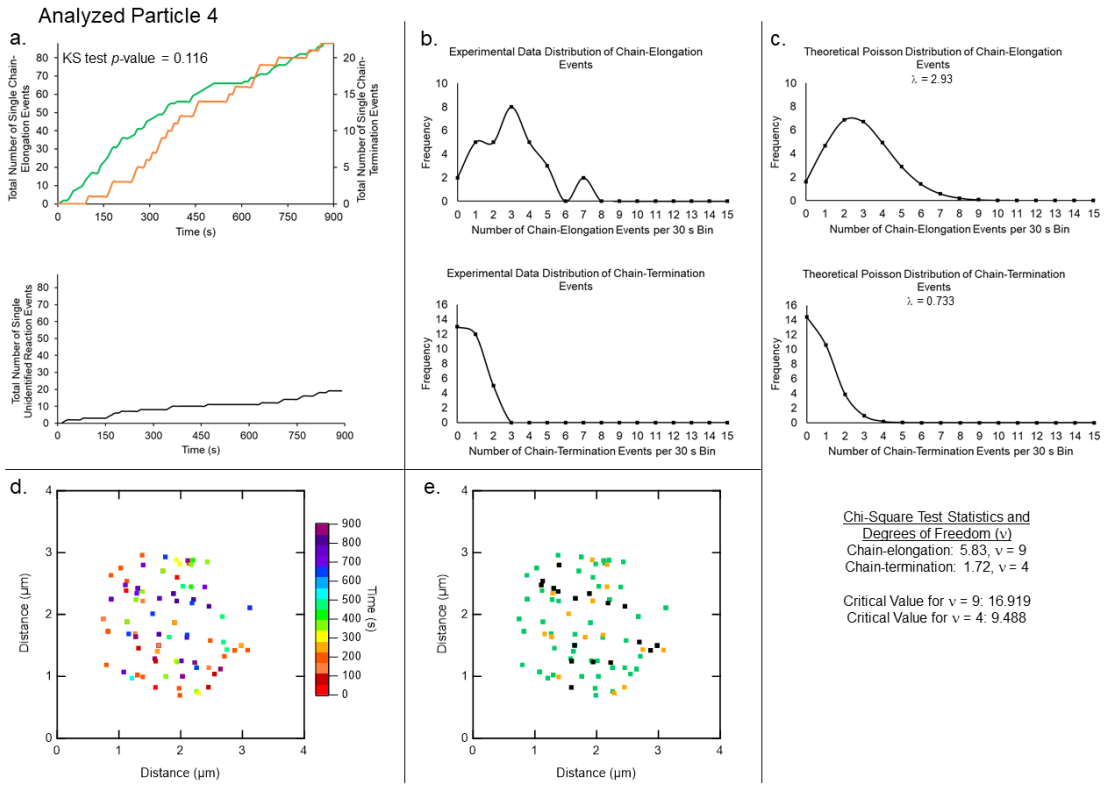
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 11.4, ν = 11
Chain-termination: 20.9, ν = 8

Critical Value for ν = 11: 19.675
Critical Value for ν = 8: 15.507



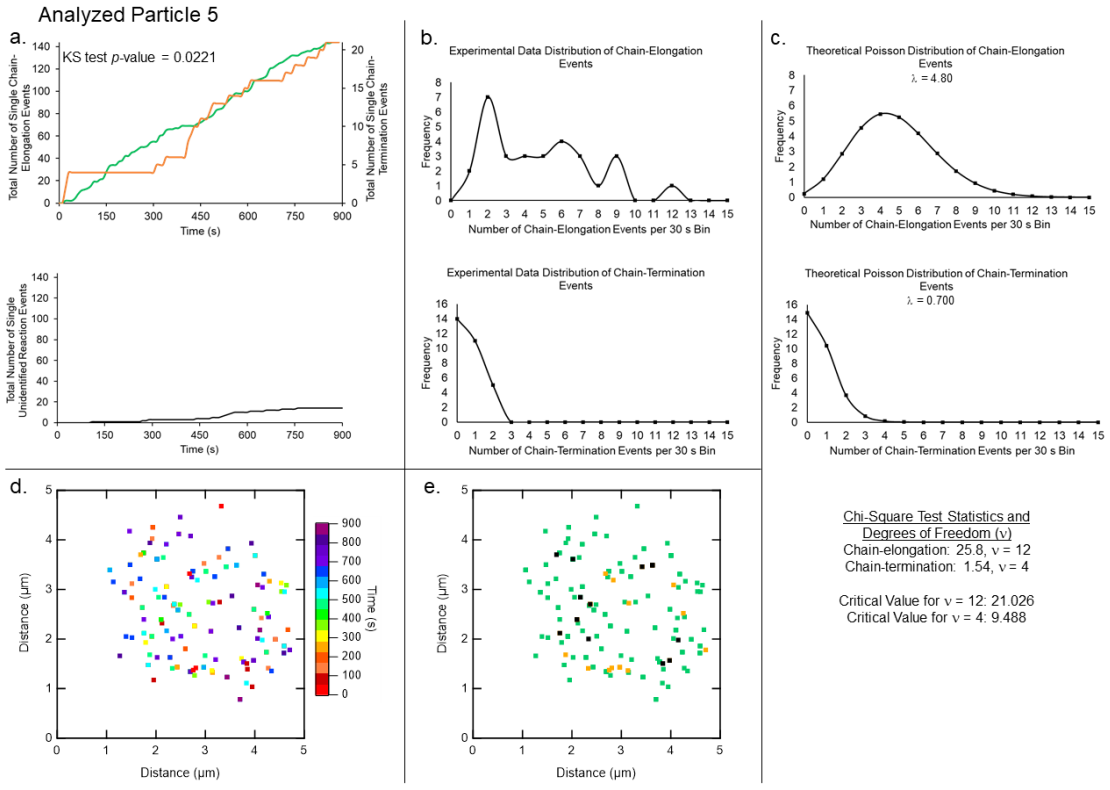
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 35.1, ν = 11
Chain-termination: 5.65, ν = 5

Critical Value for ν = 11: 19.675
Critical Value for ν = 5: 11.070



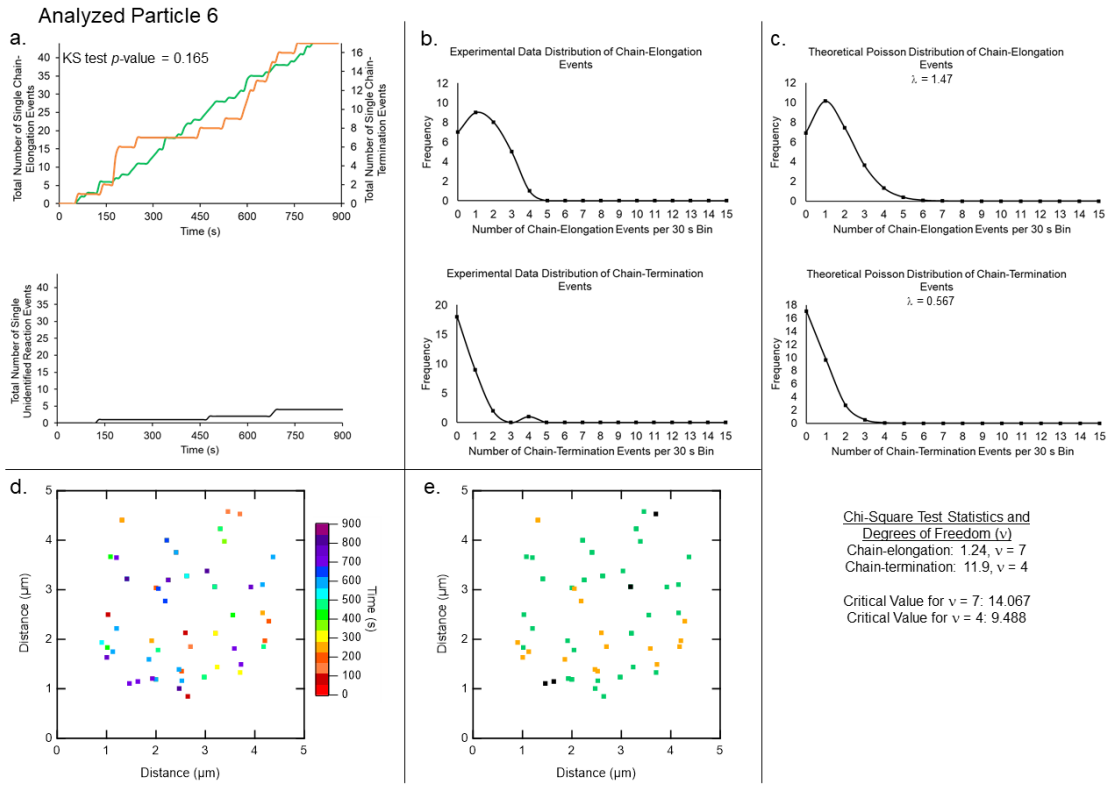
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 5.83, ν = 9
Chain-termination: 1.72, ν = 4

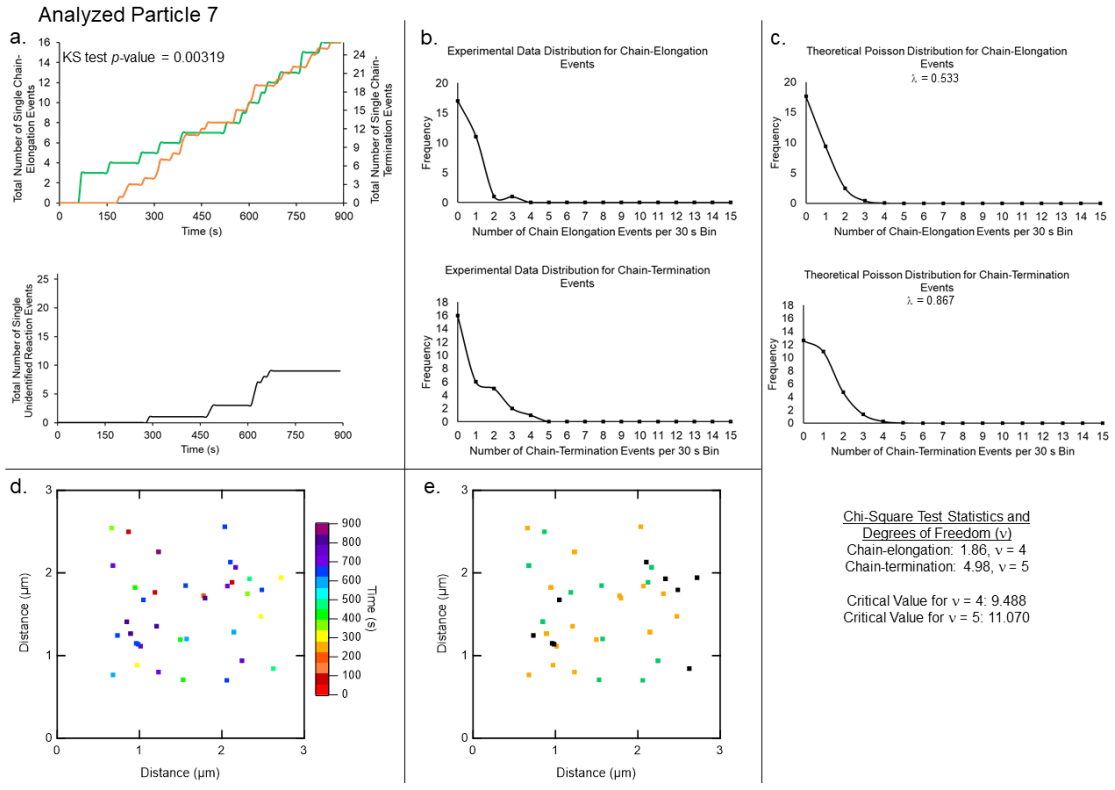
Critical Value for ν = 9: 16.919
Critical Value for ν = 4: 9.488

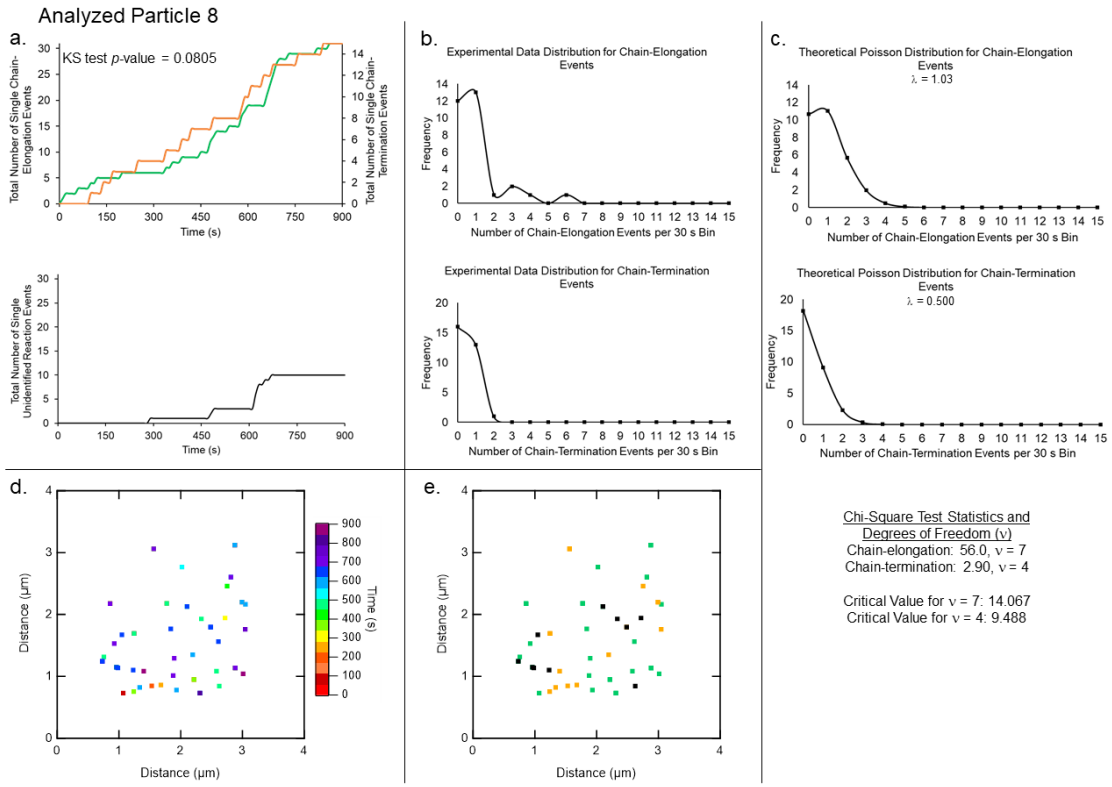


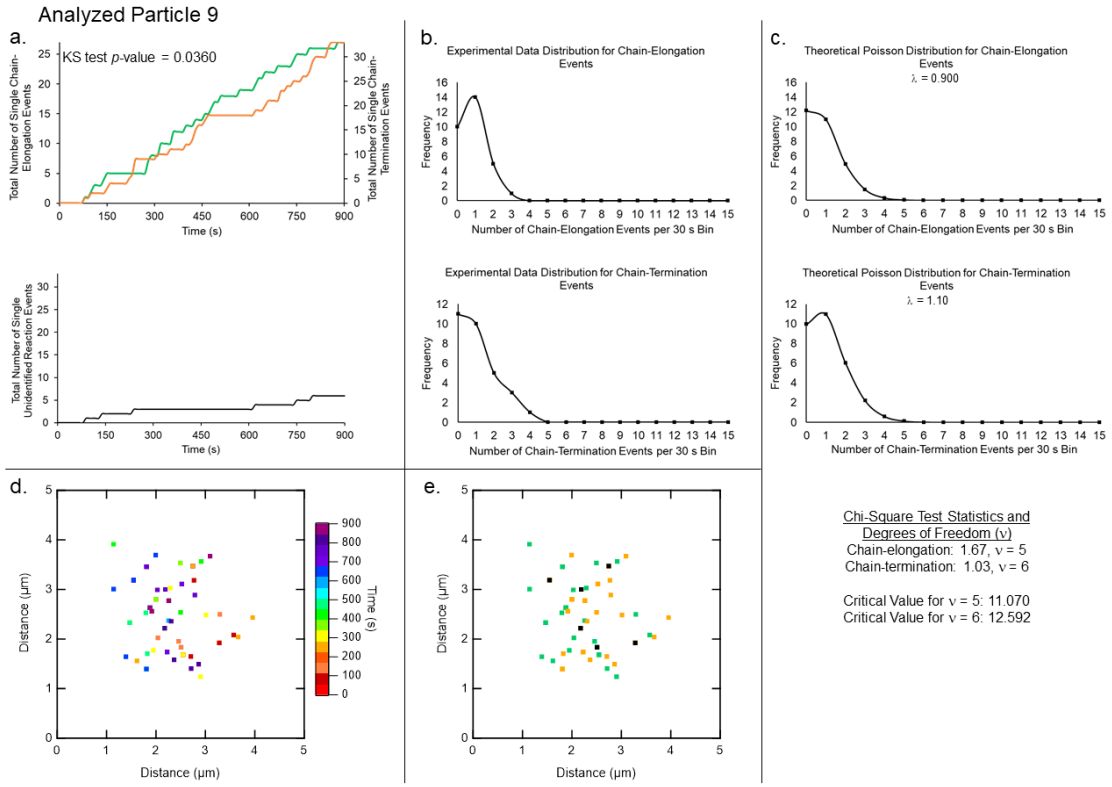
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 25.8, ν = 12
Chain-termination: 1.54, ν = 4

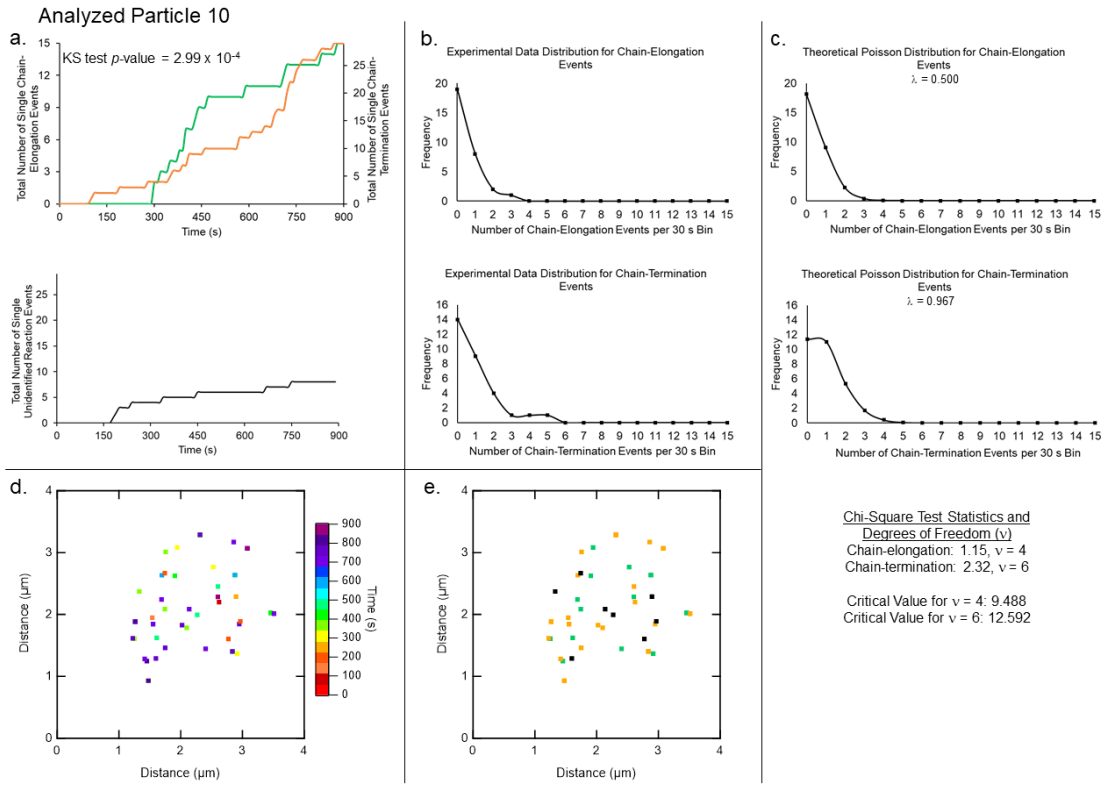
Critical Value for ν = 12: 21.026
Critical Value for ν = 4: 9.488


Analyzed Particle 6
a.
KS test p-value = 0.165
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution of Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution of Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution of Chain-Elongation Events
λ = 1.47
Theoretical Poisson Distribution of Chain-Termination Events
λ = 0.567
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 1.24, ν = 7
Chain-termination: 11.9, ν = 4
Critical Value for ν = 7: 14.067
Critical Value for ν = 4: 9.488


Analyzed Particle 7
a.
KS test p-value = 0.00319
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 0.533
Number of Chain-Elongation Events per 30 s Bin
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.867
Number of Chain-Termination Events per 30 s Bin
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 1.86, ν = 4
Chain-termination: 4.98, ν = 5
Critical Value for ν = 4: 9.488
Critical Value for ν = 5: 11.070

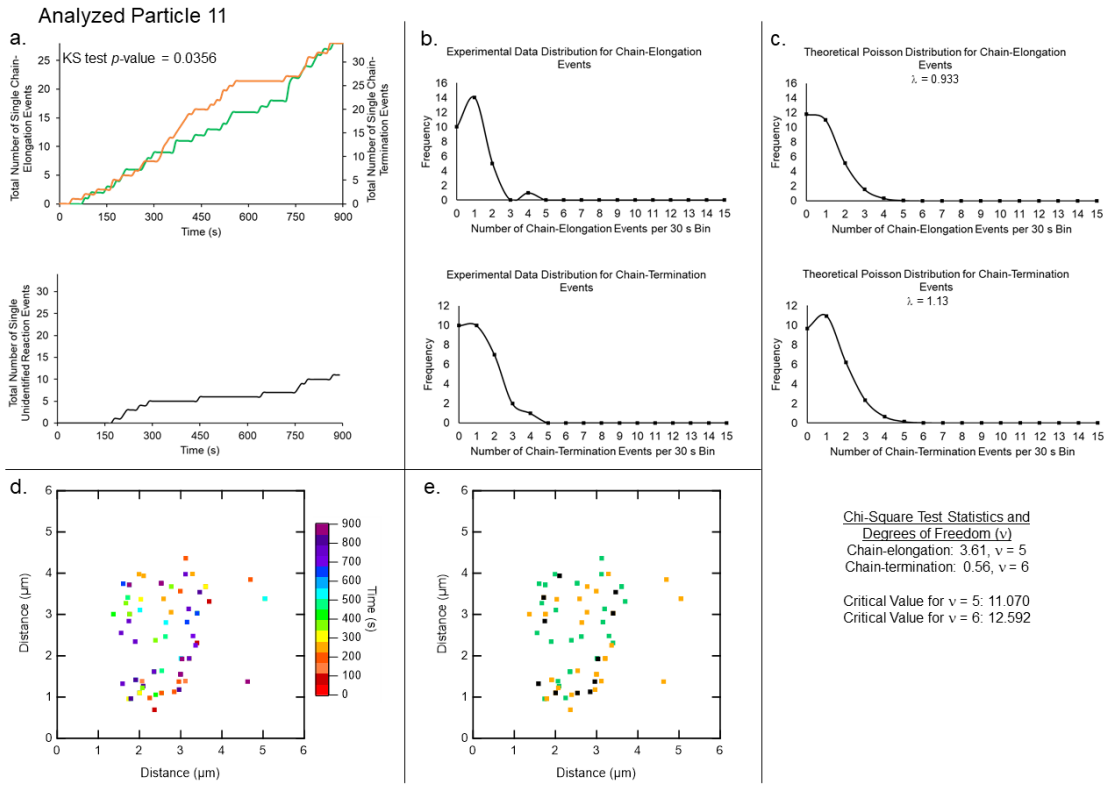

Analyzed Particle 8
a.
KS test p-value = 0.0805
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 1.03
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.500
d.
e.
Distance (μm)
Time (s)
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 56.0, ν = 7
Chain-termination: 2.90, ν = 4
Critical Value for ν = 7: 14.067
Critical Value for ν = 4: 9.488


Analyzed Particle 9
a.
KS test p-value = 0.0360
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 0.900
Theoretical Poisson Distribution for Chain-Termination Events
λ = 1.10
d.
e.
Distance (μm)
Time (s)
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 1.67, ν = 5
Chain-termination: 1.03, ν = 6
Critical Value for ν = 5: 11.070
Critical Value for ν = 6: 12.592



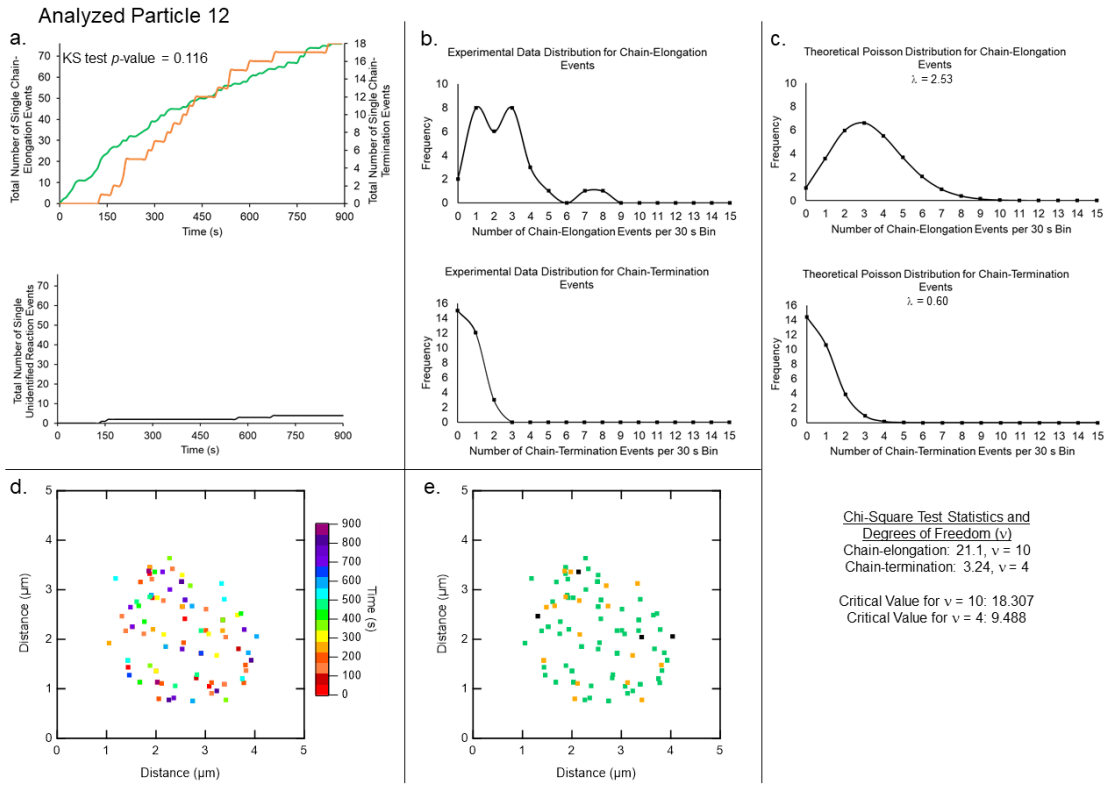
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 1.15, ν = 4
Chain-termination: 2.32, ν = 6

Critical Value for ν = 4: 9.488
Critical Value for ν = 6: 12.592



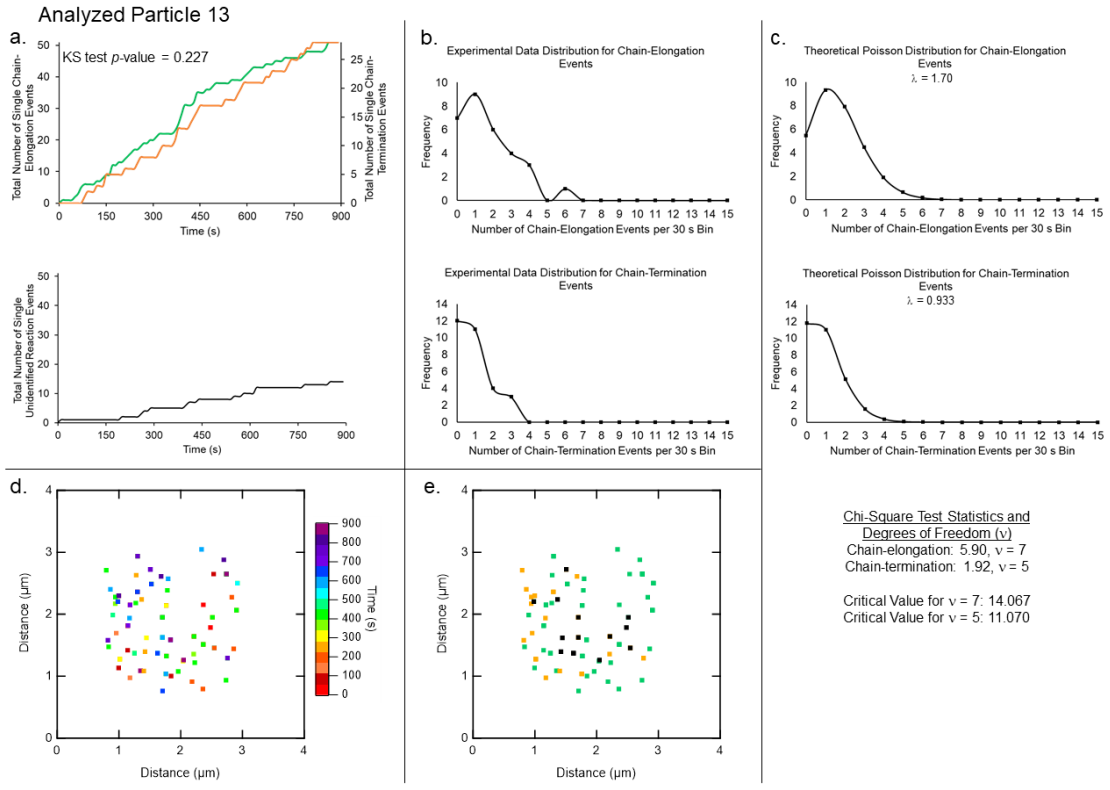
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 3.61, ν = 5
Chain-termination: 0.56, ν = 6

Critical Value for ν = 5: 11.070
Critical Value for ν = 6: 12.592



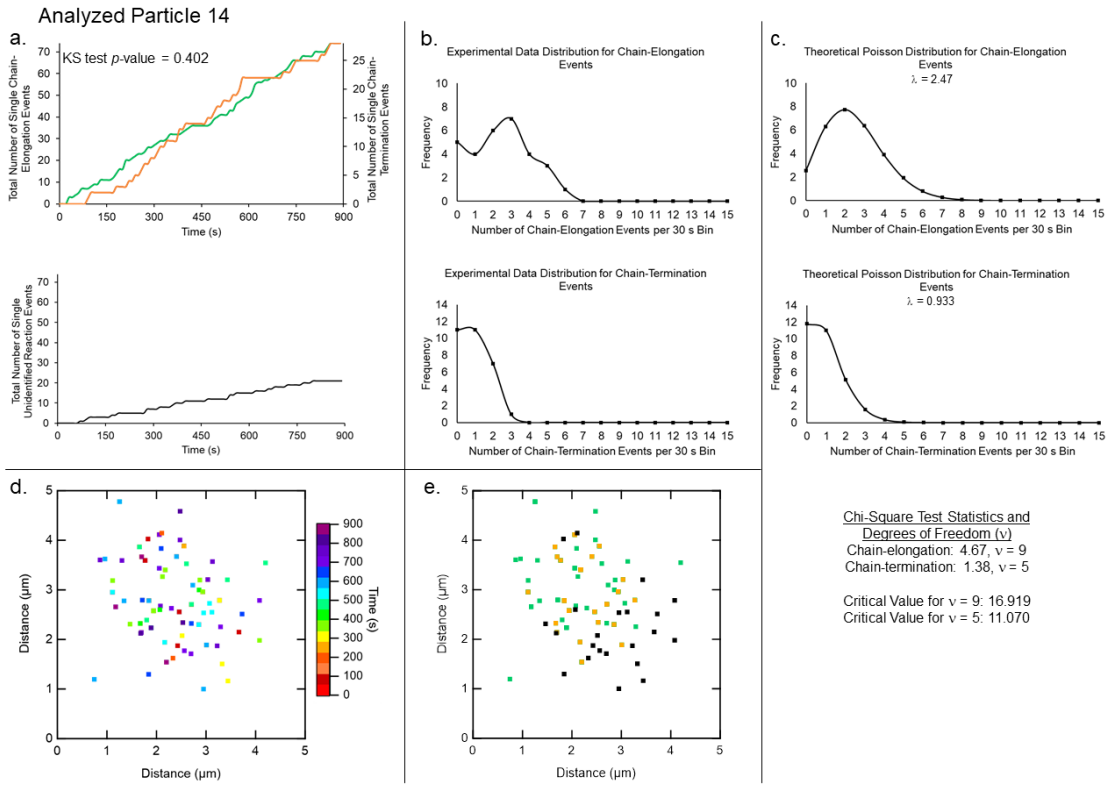
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 21.1, ν = 10
Chain-termination: 3.24, ν = 4

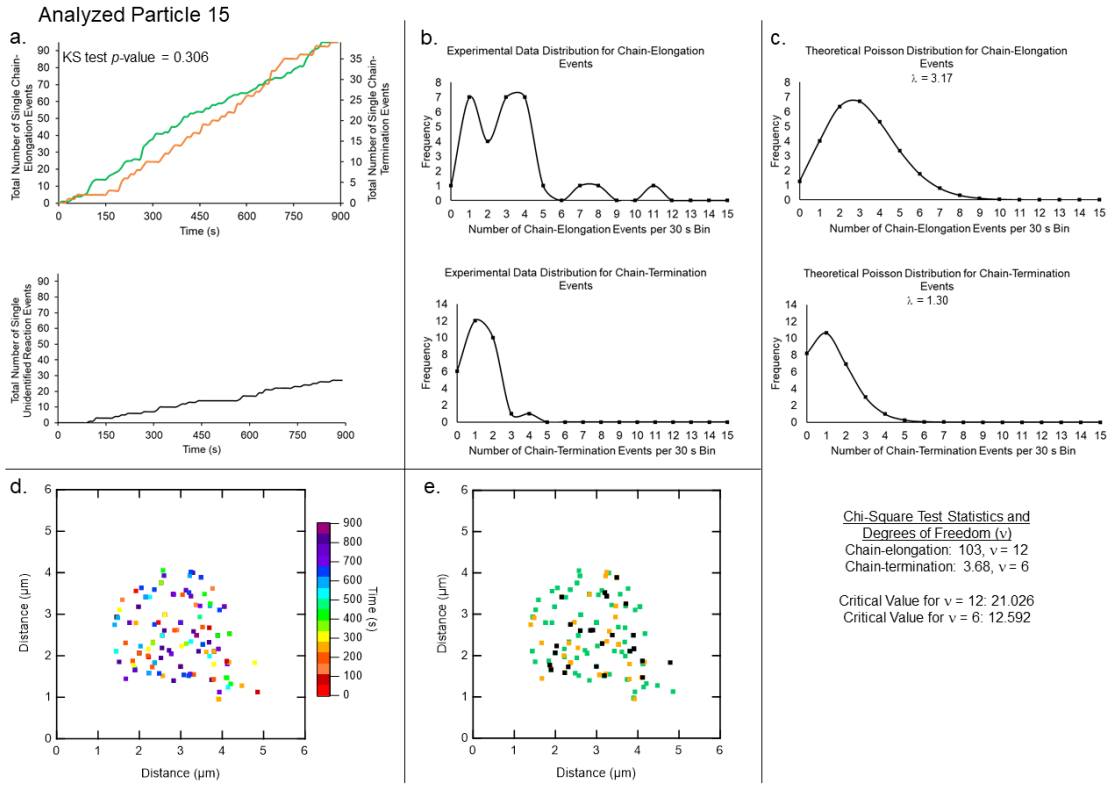
Critical Value for ν = 10: 18.307
Critical Value for ν = 4: 9.488

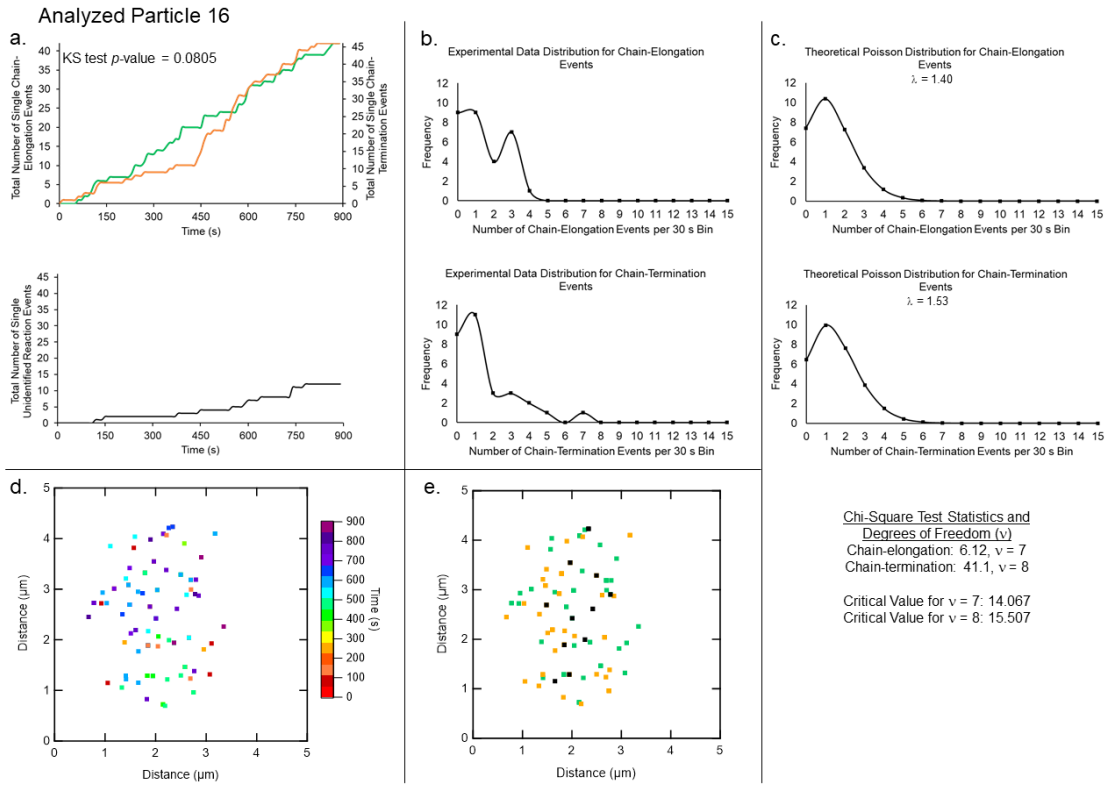


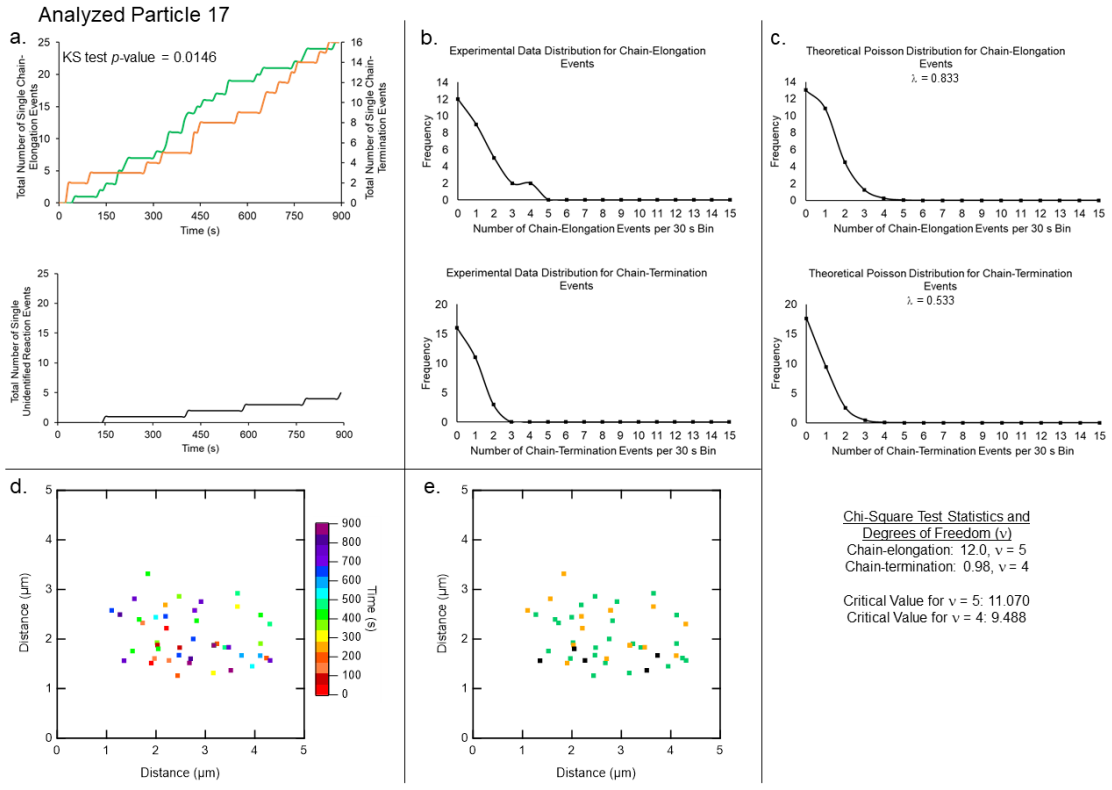
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 5.90, ν = 7
Chain-termination: 1.92, ν = 5

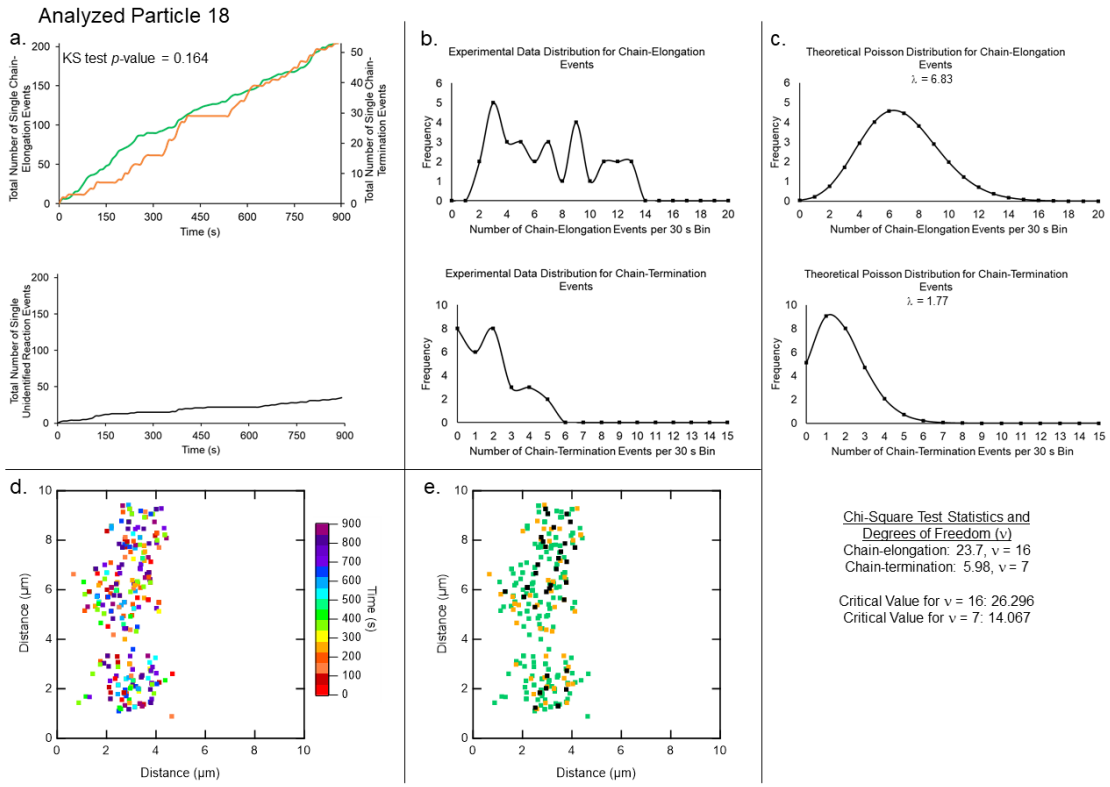
Critical Value for ν = 7: 14.067
Critical Value for ν = 5: 11.070

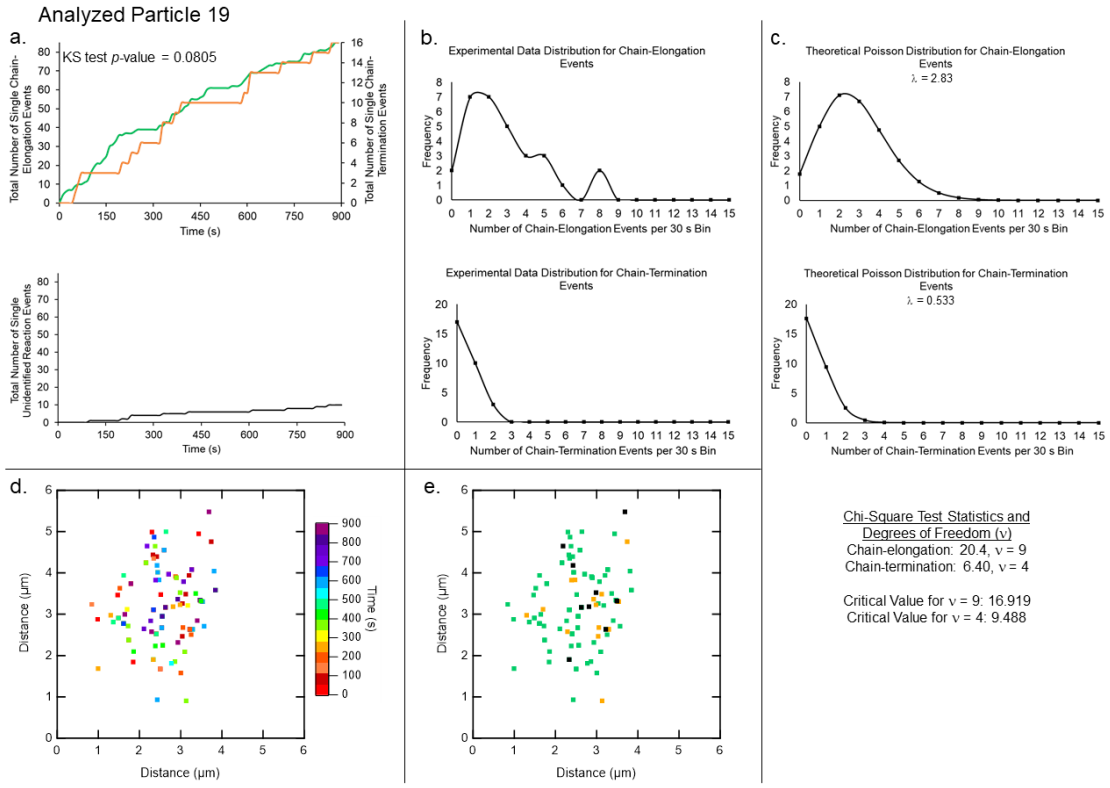

Analyzed Particle 14
a.
KS test p-value = 0.402
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 2.47
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.933
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 4.67, ν = 9
Chain-termination: 1.38, ν = 5
Critical Value for ν = 9: 16.919
Critical Value for ν = 5: 11.070

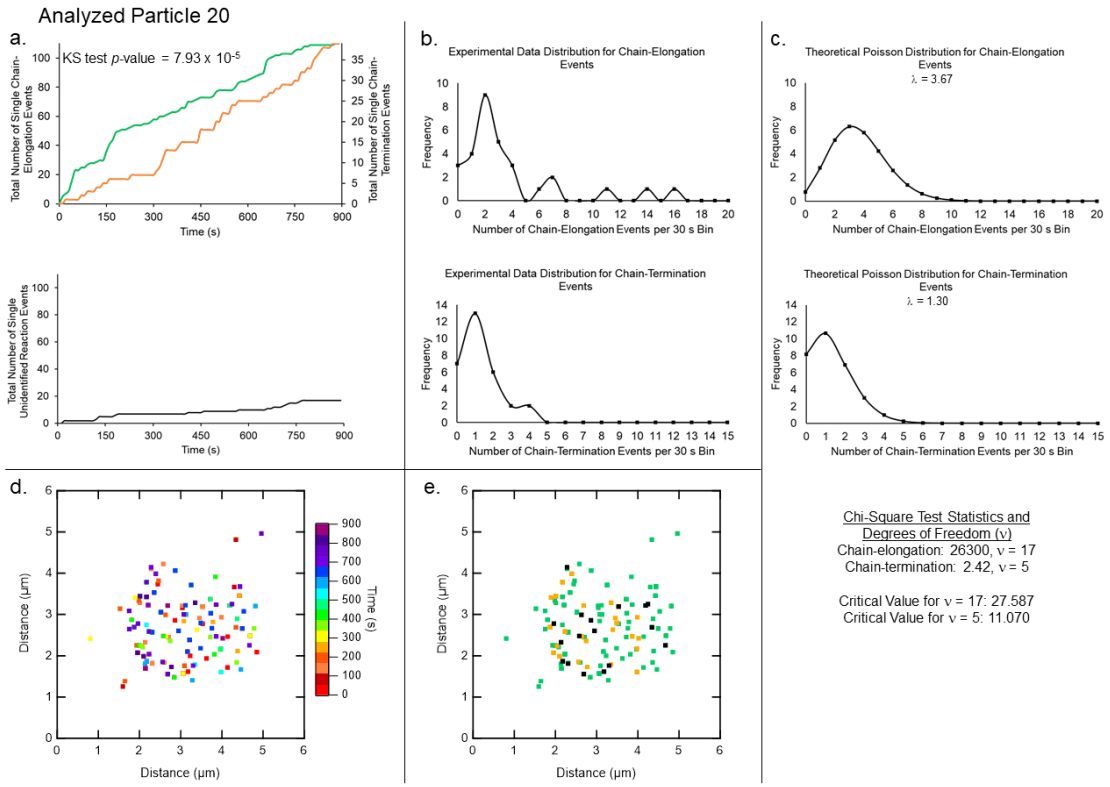

Analyzed Particle 15
a.
KS test p-value = 0.306
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 3.17
Theoretical Poisson Distribution for Chain-Termination Events
λ = 1.30
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 103, ν = 12
Chain-termination: 3.68, ν = 6
Critical Value for ν = 12: 21.026
Critical Value for ν = 6: 12.592

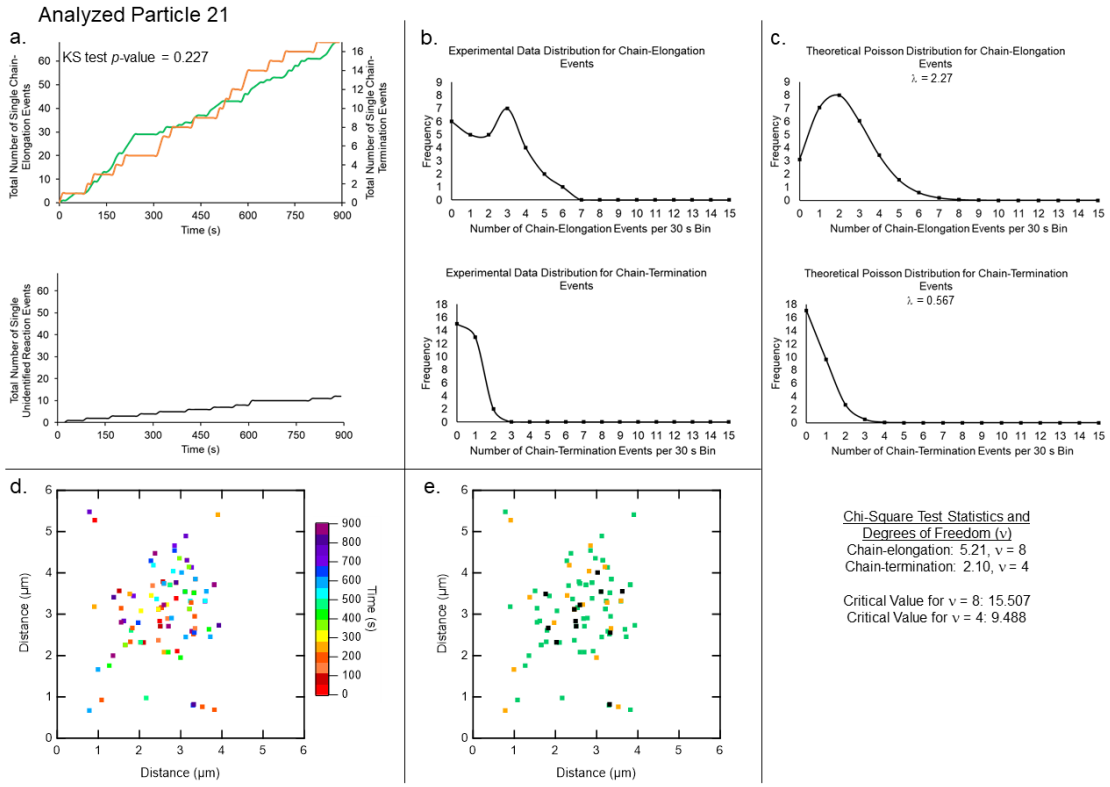

Analyzed Particle 16
a.
KS test p-value = 0.0805
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 1.40
Theoretical Poisson Distribution for Chain-Termination Events
λ = 1.53
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 6.12, ν = 7
Chain-termination: 41.1, ν = 8
Critical Value for ν = 7: 14.067
Critical Value for ν = 8: 15.507

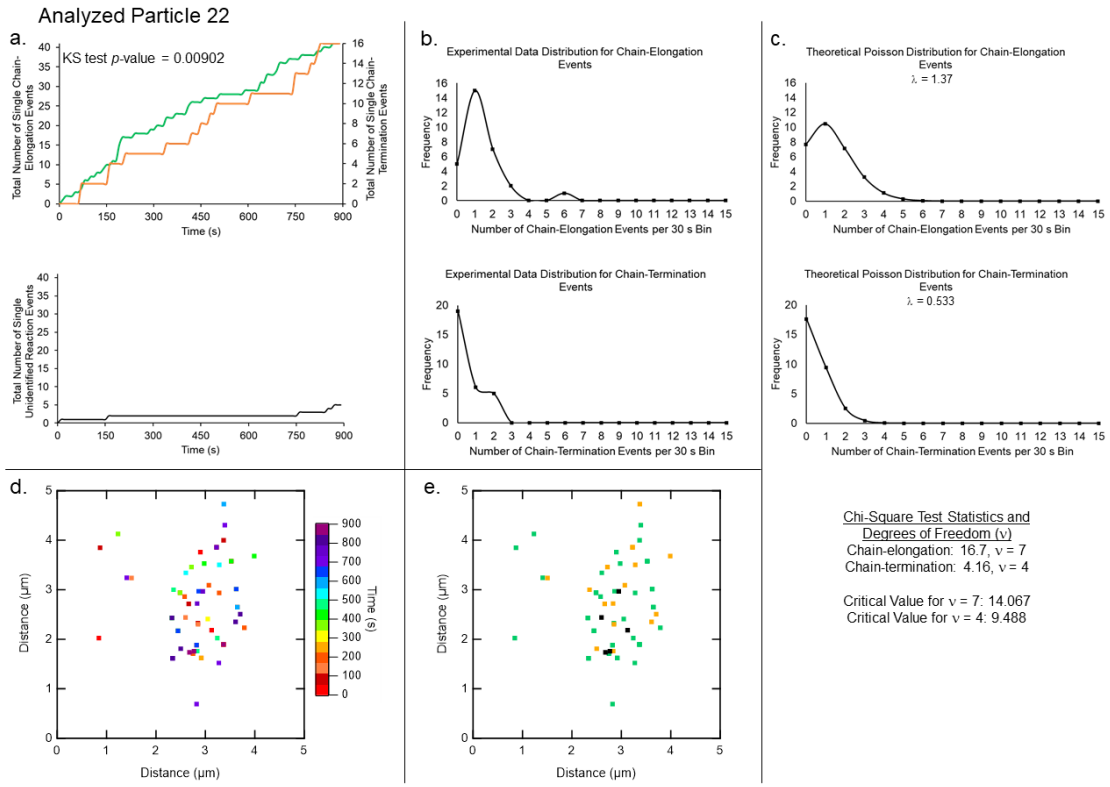

Analyzed Particle 17
a.
KS test p-value = 0.0146
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 0.833
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.533
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 12.0, ν = 5
Chain-termination: 0.98, ν = 4
Critical Value for ν = 5: 11.070
Critical Value for ν = 4: 9.488

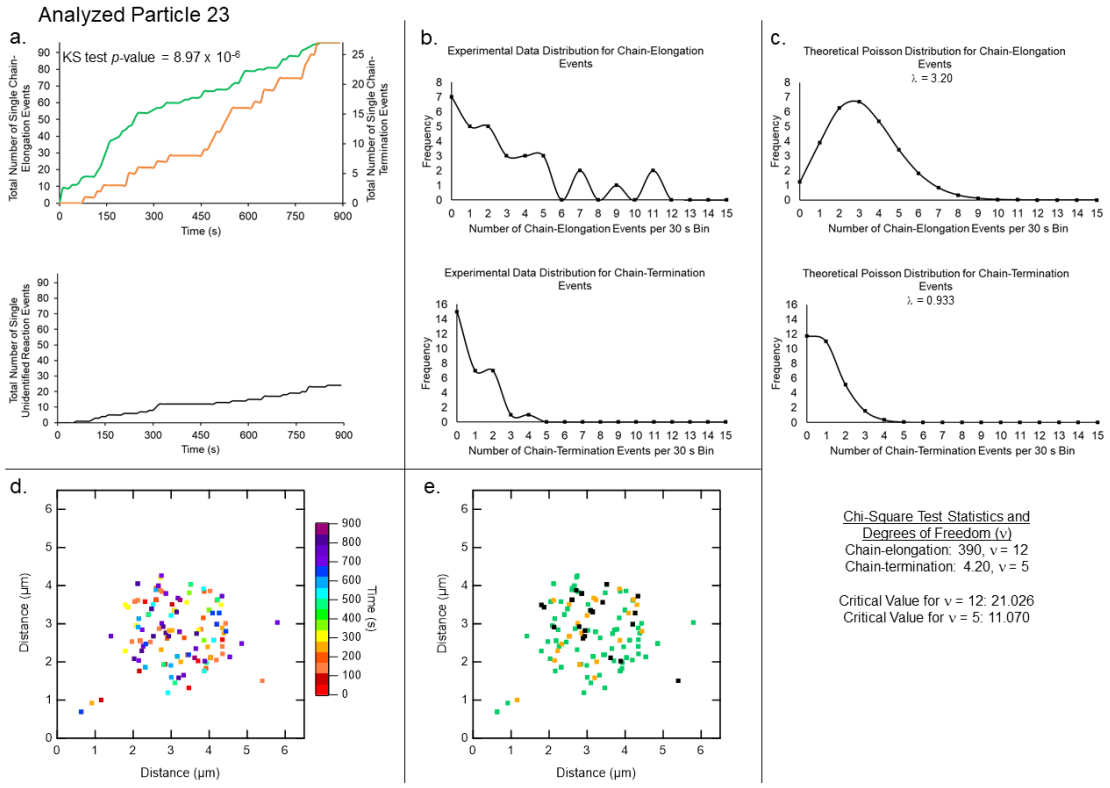

Analyzed Particle 18
a.
KS test p-value = 0.164
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 6.83
Theoretical Poisson Distribution for Chain-Termination Events
λ = 1.77
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 23.7, ν = 16
Chain-termination: 5.98, ν = 7
Critical Value for ν = 16: 26.296
Critical Value for ν = 7: 14.067

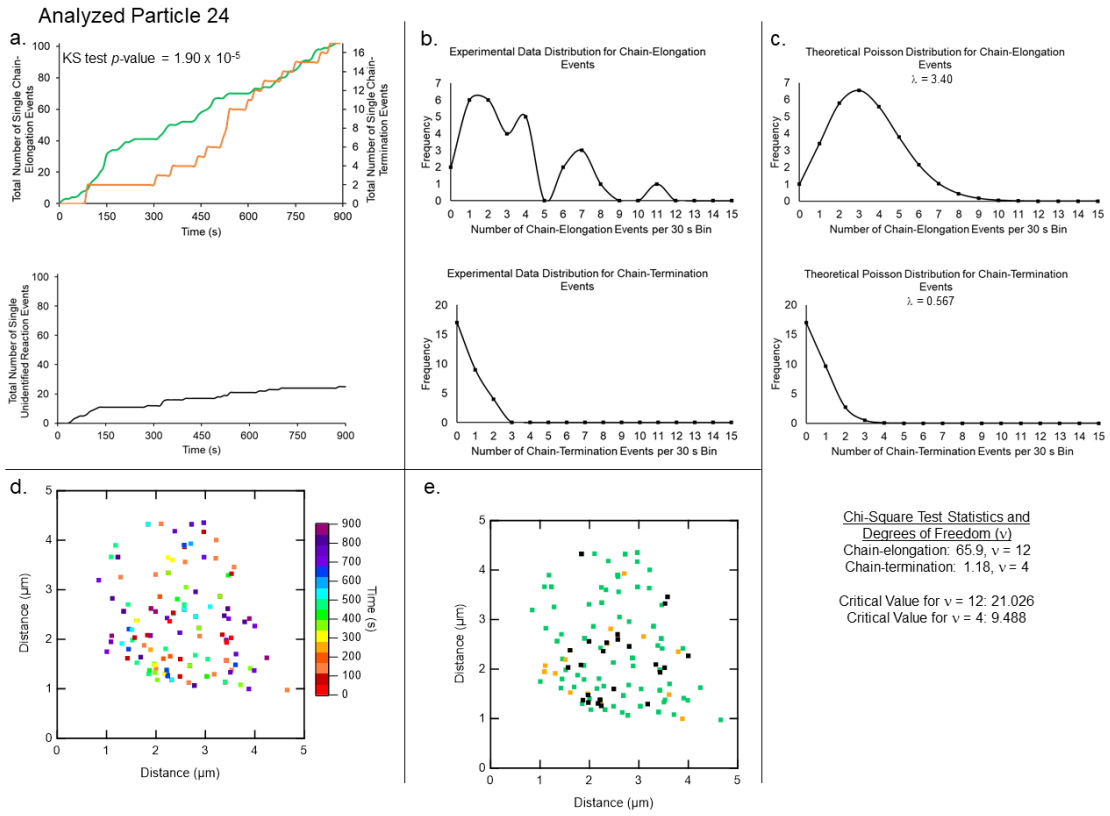

Analyzed Particle 19
a.
KS test p-value = 0.0805
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 2.83
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.533
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 20.4, ν = 9
Chain-termination: 6.40, ν = 4
Critical Value for ν = 9: 16.919
Critical Value for ν = 4: 9.488

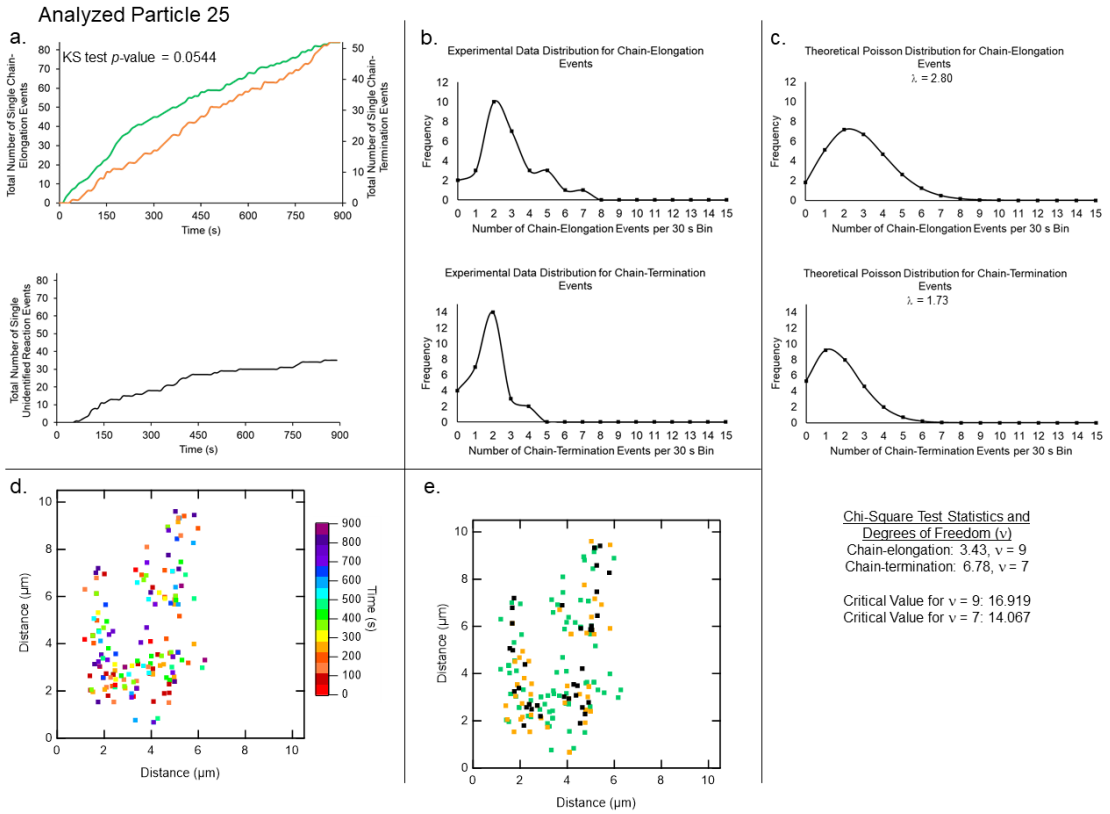

Analyzed Particle 20
a.
KS test p-value = 7.93 x 10-5
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 3.67
Theoretical Poisson Distribution for Chain-Termination Events
λ = 1.30
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 26300, ν = 17
Chain-termination: 2.42, ν = 5
Critical Value for ν = 17: 27.587
Critical Value for ν = 5: 11.070

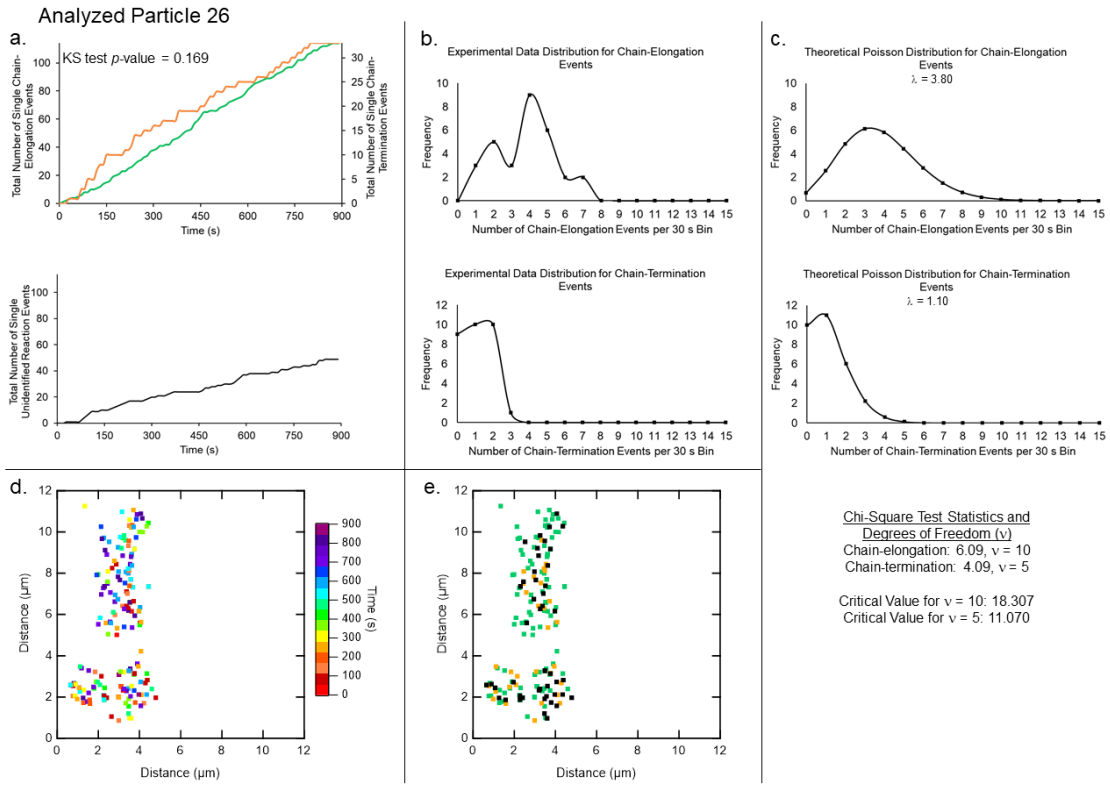

Analyzed Particle 21
a.
KS test p-value = 0.227
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 2.27
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.567
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 5.21, ν = 8
Chain-termination: 2.10, ν = 4
Critical Value for ν = 8: 15.507
Critical Value for ν = 4: 9.488

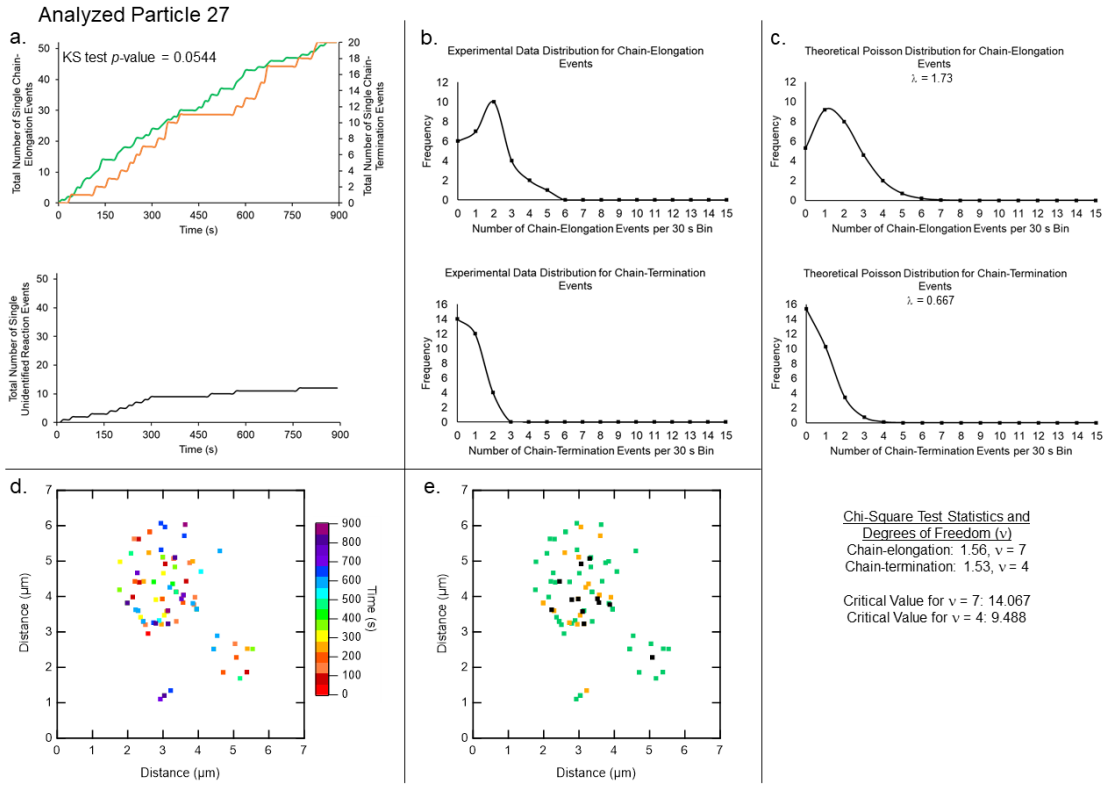

Analyzed Particle 22
a.
KS test p-value = 0.00902
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 1.37
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.533
d.
e.
Distance (μm)
Time (s)
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 16.7, ν = 7
Chain-termination: 4.16, ν = 4
Critical Value for ν = 7: 14.067
Critical Value for ν = 4: 9.488

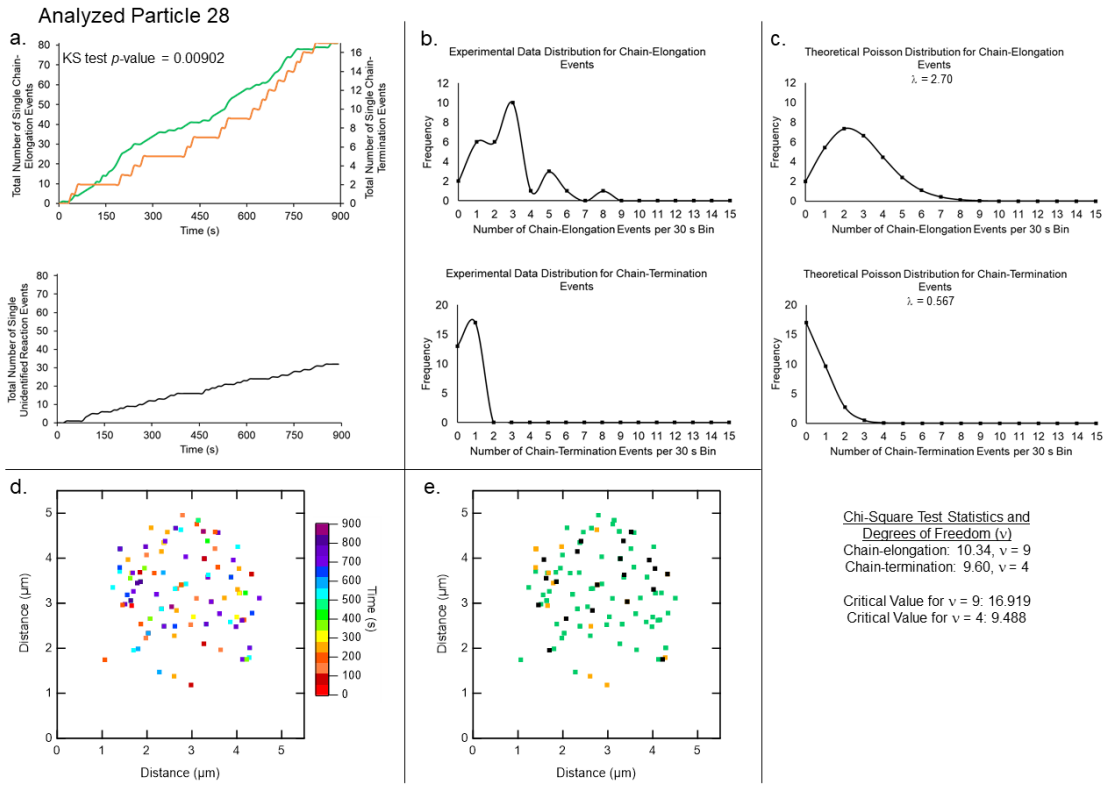

Analyzed Particle 23
a.
KS test p-value = 8.97 x 10-6
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 3.20
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.933
d.
e.
Distance (μm)
Time (s)
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 390, ν = 12
Chain-termination: 4.20, ν = 5
Critical Value for ν = 12: 21.026
Critical Value for ν = 5: 11.070

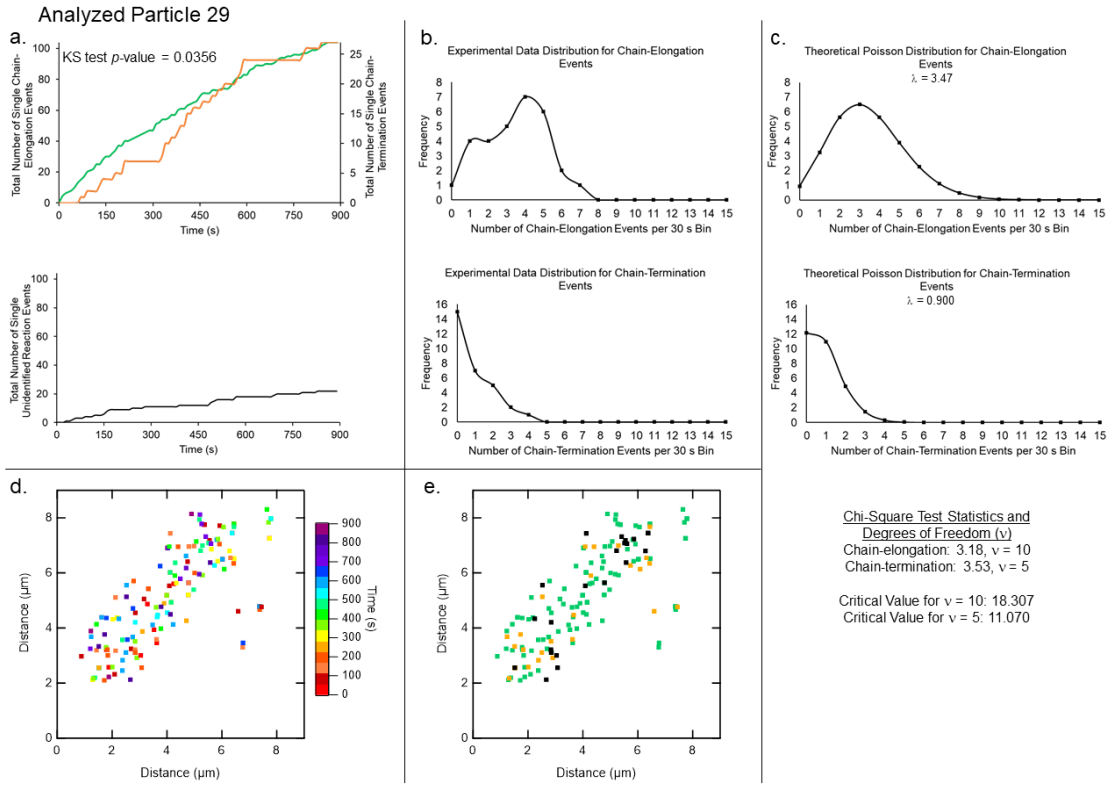

Analyzed Particle 24
a.
KS test p-value = 1.90 x 10-5
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 3.40
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.567
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 65.9, ν = 12
Chain-termination: 1.18, ν = 4
Critical Value for ν = 12: 21.026
Critical Value for ν = 4: 9.488


Analyzed Particle 25
a.
KS test p-value = 0.0544
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 2.80
Theoretical Poisson Distribution for Chain-Termination Events
λ = 1.73
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 3.43, ν = 9
Chain-termination: 6.78, ν = 7
Critical Value for ν = 9: 16.919
Critical Value for ν = 7: 14.067


Analyzed Particle 26
a.
KS test p-value = 0.169
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 3.80
Theoretical Poisson Distribution for Chain-Termination Events
λ = 1.10
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 6.09, ν = 10
Chain-termination: 4.09, ν = 5
Critical Value for ν = 10: 18.307
Critical Value for ν = 5: 11.070


Analyzed Particle 27
a.
KS test p-value = 0.0544
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 1.73
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.667
d.
Distance (μm)
Time (s)
e.
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 1.56, ν = 7
Chain-termination: 1.53, ν = 4
Critical Value for ν = 7: 14.067
Critical Value for ν = 4: 9.488


Analyzed Particle 28
a.
KS test p-value = 0.00902
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 2.70
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.567
d.
e.
Distance (μm)
Time (s)
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 10.34, ν = 9
Chain-termination: 9.60, ν = 4
Critical Value for ν = 9: 16.919
Critical Value for ν = 4: 9.488


Analyzed Particle 29
a.
KS test p-value = 0.0356
Total Number of Single Chain-Elongation Events
Total Number of Single Chain-Termination Events
Time (s)
Total Number of Single Unidentified Reaction Events
b.
Experimental Data Distribution for Chain-Elongation Events
Frequency
Number of Chain-Elongation Events per 30 s Bin
Experimental Data Distribution for Chain-Termination Events
Number of Chain-Termination Events per 30 s Bin
c.
Theoretical Poisson Distribution for Chain-Elongation Events
λ = 3.47
Theoretical Poisson Distribution for Chain-Termination Events
λ = 0.900
d.
e.
Distance (μm)
Time (s)
Chi-Square Test Statistics and Degrees of Freedom (ν)
Chain-elongation: 3.18, ν = 10
Chain-termination: 3.53, ν = 5
Critical Value for ν = 10: 18.307
Critical Value for ν = 5: 11.070

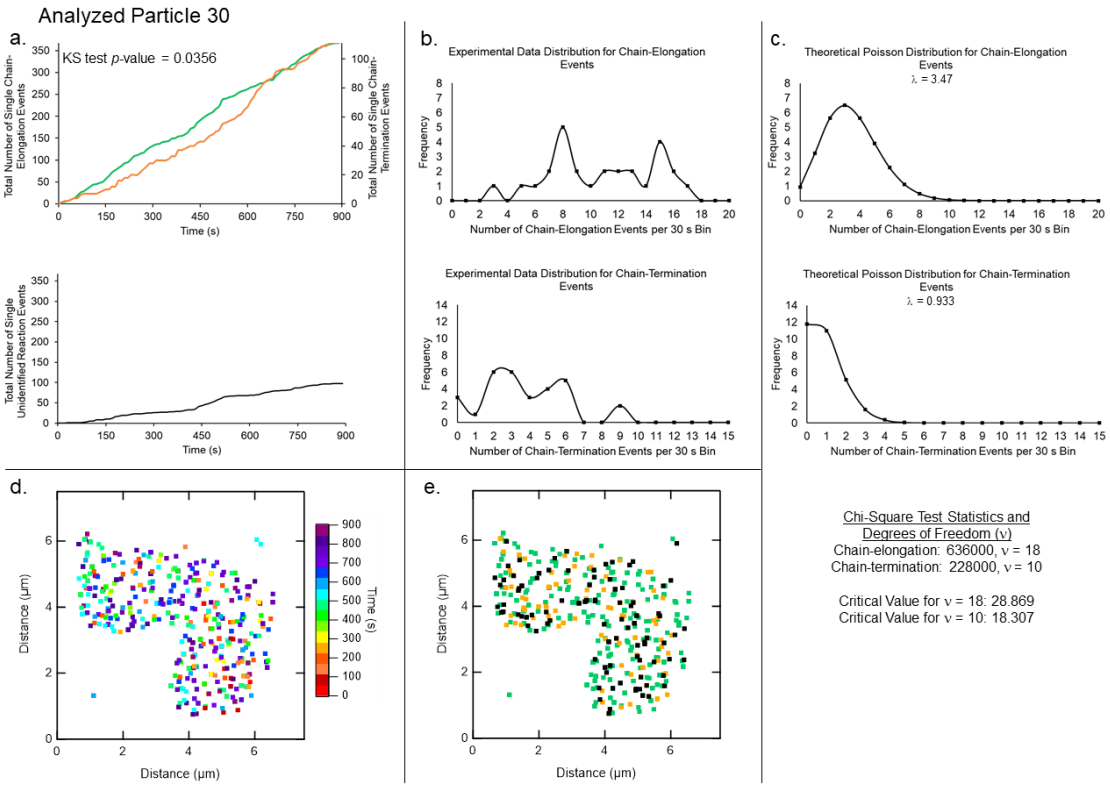


Figure S2.18. List of analyzed particles 1-30 showing; (a) integrated event per time trace for chain-elongation (green) and chain-termination (orange) (top) and integrated event per time trace for unidentified events (bottom), (b) experimental data distribution for chain-elongation (top) and chain-termination (bottom) events, (c) theoretical data distribution for chain-elongation (top) and chain-termination (bottom) events, (d) Superresolved image of single chain-elongation (green) and -termination (orange), with events not identified with statistical confidence (black). (f) Superresolved image for all single-turnover events, where color of data points indicates reaction time.

Table S2.2. List of Analyzed Particles and Statistical Data

Particle ID	Kolmogorov-Smirnov *p*-Value	Green Chi-Square Test Statistic (Degrees of Freedom)	Orange Chi-Square Test Statistic (Degrees of Freedom)	Statistically Significant Selectivity Variation Behavior in Both Tests
P1	0.00543	56.9 (6)	47.0 (7)	Yes
P2	0.8719	11.4 (11)	20.9 (8)	No
P3	0.0232	35.1 (11)	5.65 (5)	Yes
P4	0.11641	5.83 (9)	1.72 (4)	No

P5	0.02206	25.8 (12)	1.54 (4)	Yes
P6	0.16447	1.24 (7)	11.9 (4)	No
P7	0.00319	1.86 (4)	4.98 (5)	No
P8	0.08052	56.0 (7)	2.95 (4)	No
P9	0.03595	1.67 (5)	1.03 (6)	No
P10	2.99E-4	1.15 (4)	2.32 (6)	No
P11	0.00543	3.61 (5)	0.56 (6)	No
P12	0.11641	21.1 (10)	3.24 (4)	No
P13	0.22704	5.90 (7)	1.92 (5)	No
P14	0.40229	4.67 (9)	1.38 (5)	No
P15	0.30603	103 (12)	3.68 (6)	No
P16	0.08052	6.12 (7)	41.1 (8)	No
P17	0.01463	12.0 (5)	0.98 (4)	Yes
P18	0.16447	23.7 (16)	5.98 (7)	No
P19	0.08052	20.4 (9)	0.640 (4)	No
P20	7.93109E-5	26300 (17)	2.42 (5)	Yes
P21	0.22704	5.21 (8)	2.10 (4)	No
P22	0.00902	16.7 (7)	4.16 (4)	Yes
P23	8.96838E-6	390 (12)	4.20 (5)	Yes
P24	1.90191E-5	65.9 (12)	1.18 (4)	Yes
P25	0.05443	3.43 (9)	6.78 (7)	No
P26	0.16906	6.09 (10)	4.09 (5)	No
P27	0.05443	1.56 (7)	1.36 (4)	No
P28	0.00902	10.34 (9)	9.60 (4)	No
P29	0.03595	3.18 (10)	3.53 (5)	No
P30	0.03595	636000 (18)	228000 (10)	Yes

XV. References

(1) Q. T. Easter, S. A. Blum, *Angew. Chemie - Int. Ed.* **2018**, *57*, 1572–1575.
(2) E. M. Hensle, S. A. Blum, *J. Am. Chem. Soc.* **2013**, *135*, 12324–12328.
(3) N. M. Esfandiari, Y. Wang, J. Y. Bass, T. P. Cornell, D. A. L. Otte, M. H. Cheng, J. C. Hemminger, T. M. McIntire, V. A. Mandelshtam, S. A. Blum, *J. Am. Chem. Soc.* **2010**, *132*, 15167–15169.
(4) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, **2006**.
(5) Q. T. Easter, S. A. Blum, *Angew. Chem. Int. Ed.* **2017**, *56*, 13772–13775.
(6) P. Dedecker, S. Duwé, R. K. Neely, J. Zhang, *J. Biomed. Opt.* **2012**, *17*, 126008.
(7) M. S. Friedrichs, *J. Biomol. NMR* **1995**, *5*, 147–153.

CHAPTER 3

SUPERRESOLVED MOTIONS OF SINGLE MOLECULAR CATALYSTS DURING POLYMERIZATION SHOW WIDE DISTRIBUTIONS

PREFACE

This chapter was a collaborative effort between myself and my co-author, Dr. D. Josh Dibble, who was a post-doctoral researcher in the group at the time. Of the work discussed in this chapter, the three motion-tracking experiments with the norbornene-functionalized fluorophore, the three functionalized-coverslip control experiments with stationary fluorophore, the control experiments with the alkyl-functionalized fluorophore, synthesis of the silylated BODIPY fluorophore, and the initial modified Localizer code, including the stationary control Localizer package, were the work of Dr. Dibble. The norbornene- and alkyl-functionalized BODIPYs were made by a previous graduate student in the Blum Laboratory, Dr. Antonio Garcia IV. All other control and characterization experiments were performed by me, as well as all analysis of experimental data, including those experiments run by Dr. Dibble, and writing and creation of the manuscript. Used with permission from Saluga, S.J.; Dibble, D. J.; and Blum, S.A. Superresolved Motions of Single Molecular Catalysts during Polymerization Show Wide Distributions. *J. Am. Chem. Soc.* **2022**, *144 (23)*, 10591–10598.

ABSTRACT

The motion of single molecular ruthenium catalysts during and after single turnover events of ring-opening metathesis polymerization is imaged through single-molecule superresolution tracking with positional accuracy of ±32 nm. This tracking is achieved through real-time incorporation of spectrally tagged monomer units into active polymer chains ends during living polymerization; thus, by design, only active-catalyst motion is detected and imaged, without convolution by inactive catalysts. The catalysts show diverse individualistic diffusive behaviors with respect to time that persist for up to 20 s. Catalysts occupy three mobility populations: quasi-stationary (23%), intermediate (53%, 65 nm), and large (24%, 145 nm) step sizes. Differences in catalyst mobility populations also exist between individual aggregates ($p < 0.001$). Such differential motion indicates widely different local catalyst microenvironments during catalytic turnover. These mobility differences are uniquely observable through single-catalyst microscopy and are not measurable through traditional ensemble analytical techniques for characterizing the behavior of molecular catalysts, such as NMR spectroscopy. The measured distributions of active molecular catalyst motions would not be readily predictable through modeling or first-principles and the range likely impacts individual catalyst turnover rate and selectivity. This range plausibly contributes to property distributions observable in bulk polymers, such as molecular weight polydispersity (e.g., 1.9 in this system), leading to a revised understanding of the mechanistic, microscale origins of macroscale polymer properties.

INTRODUCTION

Little is known experimentally about the distributions of individual-molecule translational motion during chemical reactions, due to limitations in analytical techniques. Notable exceptions have been the substantial characterization of diffusion of small organic reactants and products in

confined pores of catalytic zeolites and of silica core-shell nanoparticle catalysts[1–5] or on surfaces of inorganic nanoparticle-[6-8] or extended-material catalysts[9], where such motion is spatially restricted and easier to measure. Small-molecule molecular catalysts, in contrast, tend to diffuse faster than imaging techniques can measure and have a greater range of potential motion in three dimensions. To our knowledge there is no prior report of measuring motion of molecular chemical catalysts at the single-molecule level during a chemical reaction. Translational kinetic energy can transfer to orbital rotations to impact reaction rate and selectivity,[10,11] and translational motion can serve as a sensitive readout of molecular microenvironment.[3,12–15] Thus, the ability to directly image and characterize motions of molecular catalysts during a chemical reaction would be an enabling technique.

We became interested in if single-molecule superresolution microscopy with single-fluorophore tracking could provide a way to measure the distribution of translational motion of molecular catalysts during turnover. We considered the following questions, uniquely answerable though such a single-molecule tracking technique: 1) *What is the distribution of motion of individual molecular catalysts during turnover?* 2) *Does this distribution indicate different catalyst microenvironments?* 3) *Do differential catalyst motions occur on a timescale sufficiently slow that they are not averaged relative to chemical reaction rates?* Such motion could be the mechanistic cause of observable macroscale outcomes of chemical processes. Catalysts could, for example, move through environments with different local concentrations of reagents, or through different local steric or electronic microenvironments, slow enough to affect reaction outcome.[16]

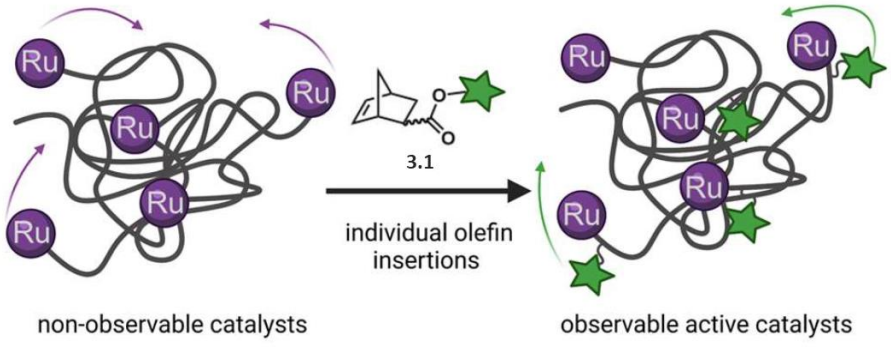


Figure 3.1. Molecular ruthenium catalyst translational motion during turnover, during a living ring-opening metathesis polymerization reaction of norbornene.

Molecular ruthenium catalysts that comprise the reactive ends of polynorbornene strands during a ring-opening metathesis polymerization (ROMP) of norbornene were chosen for initial study (Figure 3.1). Molecular ruthenium polymerization catalysts are described as "well-defined" due to their set ligand coordination environments.[17,18] The impact of the potentially dynamic microenvironment as polymerization proceeds is not well understood,[19] though it has the potential to make the local environment variable and the true reactivity not well-defined. We here develop and demonstrate an imaging approach that enables characterizing the motions of individual catalysts during ROMP to determine the distribution local environments at the level of individual catalysts during the turnover step. The selected system had several attractive features for initial study: 1) Ruthenium-catalyzed ROMP for production of polynorbornene and its derivatives are industrially and scientifically important processes;[20] 2) The entanglement of polymers produced the plausibility of different and dynamic catalyst microenvironments; and 3) We envisioned that attachment to the chain end may slow motions of molecular catalysts sufficiently for tracking. The diffusional motion of single-polymer strands embedded in thin films has been studied,[21–23] as have strands during and after radical polymerization,[24–26] but not during catalysis.

RESULTS

We focused on the polymerization of norbornene with Grubbs 2nd generation catalyst to investigate the motion of active molecular catalysts through single-molecule spectroscopy (Figure 3.2a). Imaging agent **3.1** contained an inert spectator green boron dipyrromethene (BODIPY) fluorophore (represented by the green star) attached through a spacer to a ROMP-reactive norbornene monomer.[16,27] The BODIPY fluorophore is well-suited as a spectator tag due to its lack of chemical reactivity.[28] BODIPY was also selected due to its solubility in nonpolar organic solvents, high quantum yield, and known ability to be functionalized to contain a ROMP-reactive norbornene group. Chemical reaction of **3.1** with the ruthenium carbene results in irreversible covalent attachment of the fluorophore directly to the catalyst (Figure 3.2a). This chemical reaction is imaged in real time at single catalysts. By design, this imaging method produces a signal and then detects motion of only the active catalysts, and precatalyst/noninitiated catalysts remain dark. An alternative method where the catalyst was prelabeled through its ligand sphere prior to reaction has the disadvantage that precatalyst/noninitiated catalyst may make up a substantial portion of the signal, obscuring active catalyst motion.

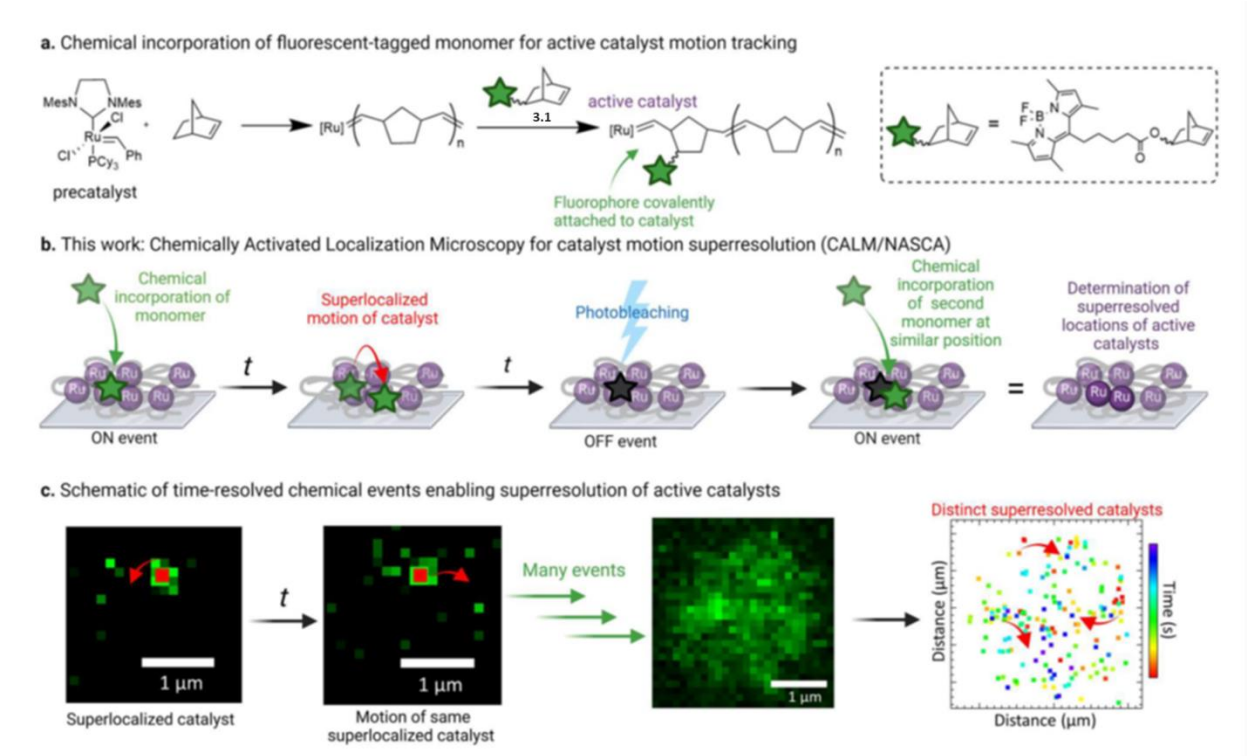


Figure 3.2. (a) Chemical incorporation of fluorescent-tagged monomer allows motion tracking of the active catalysts through a covalent linker. (b) Time-resolved chemical incorporation ON events, following by photobleaching OFF events, empowers the detection and superresolution of active catalyst locations and motions on subdiffraction scale. (c) Imaging schematic of this method, showing simulated data analysis and outcome.

To determine the locations and subsequent motions of single active catalysts within polymer aggregates, we designed and employed a chemically based stochastic superresolution technique that harnessed the ROMP reaction itself (Figure 3.2b).[16] We find it helpful to broadly consider chemical techniques Chemically Activated Localization Microscopy, i.e., CALM, to highlight its analogy to PALM[29,30] (Photoactivated Localization Microscopy), a reference that we anticipate will be understandable by a broad range of scientists. The similar use of chemical ON events to enable superresolution imaging on catalytic nanostructures has been referred to as NASCA (Nanometer Accuracy by Stochastic Chemical (or Catalytic) Reactions) first coined by Hofkens in 2009.[2,31–35] The NASCA acronym highlights the stochastic nature of the chemical

events, akin to STORM (Stochastic Optical Reconstruction Microscopy, by photoactivation).[36] Similar chemical ON events to enable superresolution imaging have also been employed previously by us and others previously without reference to a specific method name.[1,16,37–39] These CALM/NASCA descriptions highlight that the method is chemical-reaction-enabled superresolution in parallel to PALM/STORM—such chemical superresolution techniques can in theory be used for all kinds of stochastic chemical reactions, catalytic or otherwise, for any substrate, at any subdiffraction resolution.

In the case described here, fluorescently-tagged monomers **3.1** are chemically incorporated into the growing polymer network and directly covalently attached to the catalyst (Figure 3.2a). Before reaction, **3.1** was diffusing rapidly in solution and outside the TIRF region, and was therefore not imaged. Catalytic insertion of **3.1** into polymers created green flashes that characterized single catalytic turnovers. This green flash and is the ON event—the moment when a single molecular catalyst becomes imageable in our system (Figure 3.2b). The catalyst position is superloacalized upon appearance. Superlocalization imaging with motion tracking occurs for several seconds, until the fluorophore is either photobleached or diffuses out of the TIRF imaging area, providing the OFF event. This OFF event and its intentional loss of signal are by design and are what enable a subsequent second chemical ON event to be detected and superlocalized at a second catalyst in a similar location (but at a different time) below the diffraction limit. The two active catalysts are then superresolved (from each other). During an experiment, many such catalysts are characterized this way, leading to a superresolution map of active catalyst locations with individual catalyst behaviors (Figure 3.2c).

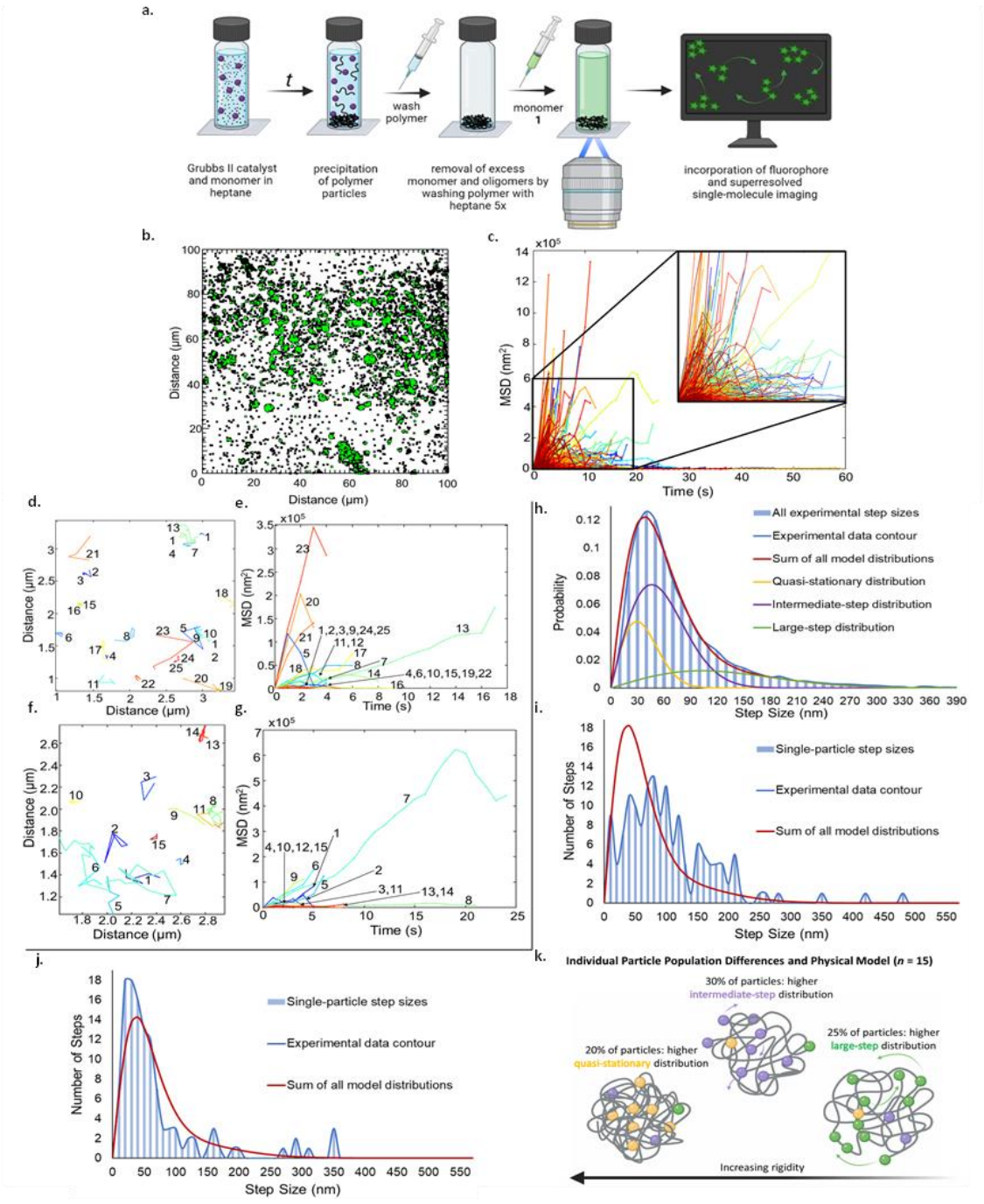


Figure 3.3. (a) Overview of experimental setup. (b) Full-frame: Superresolved single-molecule reaction events (black), motion tracks overlayed (green). Particles of aggregated polynorbornene appear as "hot zone" clusters of black reactivity and overlapping green tracks. (c) MSD of 1644 single catalysts over time, from green motion tracks shown in b. (d–g) Single-particle tracks and corresponding MSD. (h) Step-size histogram of all data, showing three populations of individual-step behavior. (i, j) Representative single-particle step-size histograms. (k) Individualistic behavior of single particles.

To produce imaging conditions, the polymerization reaction with Grubbs 2nd generation catalyst was initiated in heptane (Figure 3.3a). Due to the partially soluble nature of the precatalyst, the ultimate monomer-to-catalyst ratio is unclear; however, even if the fully soluble, not all precatalysts may initiate. Indeed, the identification *active* catalyst speciation, rather than identification of all species containing metal, remains a significant analytical challenge of broad importance in catalysis: here, by using CALM, we were able to identify and learn behavior exclusively of the active catalysts, providing a meaningful step towards solving this analytical challenge.

Aggregates of (dark) oligomers and short-chain polymers of polynorbornene (>1000 M_w) precipitated onto a glass imaging surface. An active chain end containing a ruthenium catalyst was present on up to each strand end. These preinitiated polymers were washed five times with clean heptane to remove the mother liquor that contained residual unreacted norbornene and solution-phase oligomers. These polymer particles, as imaged on the microscope, were stationary for the full duration of image acquisition. The multiple washing steps substantially decreased the likelihood that the subsequent observed fluorescent activity was caused by physical aggregation of oligomers or polymers from solution; however, some continued solution-phase polymerization and aggregation cannot be fully ruled out.

Imaging agent **3.1** was then added and the sample was imaged in total internal reflectance fluorescence (TIRF) mode for 15 min. The reactive monomer is doped at a sufficiently low level (200 fM) to allow for the superresolution of individual catalysts. Each reaction likely arose from a different ruthenium catalyst given the high catalyst loading and low quantity of imaging agent **3.1**. Control experiments with an otherwise similar BODIPY fluorophore without an olefin reactive group did not show similar flashes.[16]

By eye, a range of wiggling and arcing motion of catalysts was clearly visible. This visible subset of motion was greater than the diffraction limit and included distances of up to a couple microns over several seconds, corresponding to traversing roughly half the diameter of some aggregates.

With such a broad range of motion observed, there was the consideration if the motion of a subset of catalysts over distances of microns over several seconds might have been a readout of the motion of the whole-polymer-aggregate rolling/drifting motion. However, unlike the moving active chain ends, the strongly glass-physiosorbed polymer particle aggregates themselves were stationary for the duration of imaging, and did not roll or tumble as particles, as evidenced by the clustered green "hot spots" in Figure 3.3b. Thus, aggregates were fixed in position, and the motions of individual catalysts within the aggregate could be measured independent of any convoluting whole-aggregate motions. The fixed positions of polymer aggregates were further evidenced through transmission light imaging; which showed that no significant whole-aggregate motion was observed over 5 min (see Appendix Section X, Figure S3.7 for details).

We next turned attention to quantification of this motion, including below the diffraction limit. Superresolution imaging and tracking was performed by fitting the point spread function from emitters.[2,3] Diffusion tracks that lasted at least 4 s are overlaid in green in Figure 3.3b (n = 1644 tracks). These diffusion tracks were measured as projections in the x,y plane due to the TIRF experimental setup. In Figure 3.3b, individual polymer aggregate particles appear as clusters of green tracks which correlate to activity "hot zones" of ~2–5 microns in diameter. This experiment was repeated for 3 experimental runs, for a total of n = 3493 tracks.

These tracks revealed a qualitatively wide distribution of mean-squared-displacement (MSD) with time (Figure 3.3c): Linear MSD traces correspond to individual catalysts that

maintained similar motion for several seconds. MSD traces that changed slope correspond to catalysts that changed motions. Figure 3.3c shows that many catalysts maintained their diffusion behavior for several seconds (with some maintaining for longer than 20 s), whereas others changed behavior.

In order to determine the lower limit of our instrumentation and superresolution method to detect motion, control experiments were performed: The positional accuracy of the superlocalization technique on surface-immobilized stationary BODIPY fluorophores was measured separately as ±32 nm. Thus, single catalysts tagged with 1 that moved less than ±32 nm were indistinguishable from stationary. These quasi-stationary catalysts make up 13% of all catalysts in triplicate experiments.

Proceeding with this quantitative analysis characterized MSD values from quasi-stationary to 1.9 x 10^6 nm^2. This data is consistent with the individual catalysts existing in markedly different microenvironments with different mobilities.

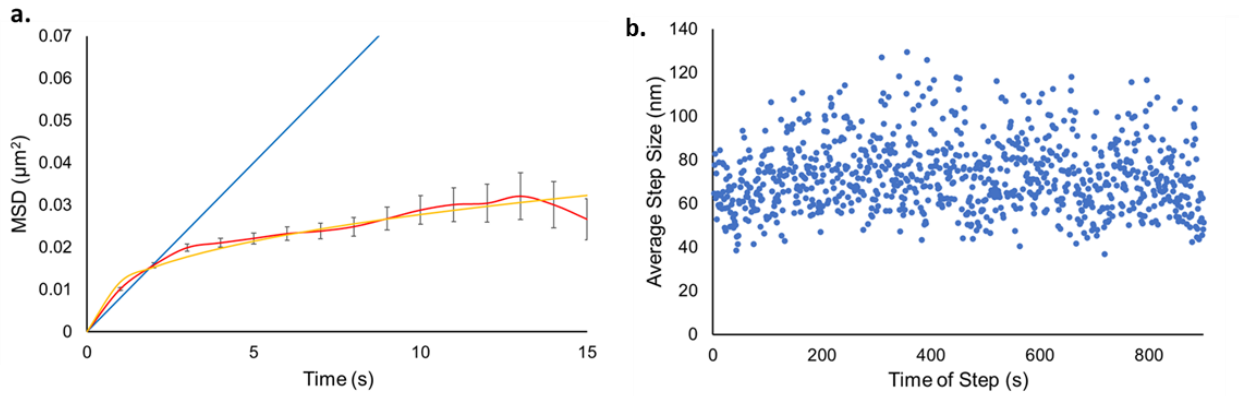


Figure 3.4. a) Diffusion over time for all experimental tracks (red), showing subdiffusion, as opposed to theoretical free diffusion (blue) as derived from random-walk step size distribution data. Orange line shows subdiffusion fit. b) Average step size vs. the time at which the step occurred, showing lack of correlation.

Analyzing the full population of MSD across all three replicates, shown in red in Figure 3.4a, the motion within the polymer aggregate particles deviates significantly from a free diffusion model, matching instead subdiffusion. A theoretical free diffusion model, based on the measured random walk variance from step-size distribution data, is shown in blue in Figure 3.4a. Comparison of the free diffusion (blue) and subdiffusion (red) data is shown to further highlight the disparity between these two options. The observed subdiffusion data is consistent with the polymer aggregate particles having defined boundaries, as observed in transmitted light images (see SI), from with the individual catalysts do not diffuse, and also the rigidity/entanglement within the particles that create additional local confinement possibilities. A diffusion coefficient for active catalysts was measured from all tracks MSD (n = 3493; D = 30. nm^2s^{-1}, or 3.0 x 10^{-15} m^2s^{-1}). This diffusion constant was calculated from the fit of the diffusion data in Figure 3.4a (orange line). This value compared reasonably to simulations for motions,[40] and demonstrated unambiguously that the motions arose from polymer-incorporated **3.1** rather than freely diffusing solution species ($D_{\text{Grubbs2nd (solution)}}$ = 6.5 x 10^{-10} m^2s^{-1})[41].

Limitations of the analysis method include the following: 1) Some insertions of 1 may occur prior to detection upon diffusion into the focal plane. This scenario was quite likely for a minority of events given the long distances traveled by the most rapidly diffusing catalysts. However, such behavior does not change the conclusions herein; 2) Some catalysts may dissociate after insertion of 1, such that tracking continued for a longer duration than the catalyst remained present; however, the catalyst remains attached after ROMP,[42] so this circumstance is not expected for the majority of events. 3) This method is designed to be specific for detecting the motions of catalysts within the precipitated aggregates and does not inform on catalyst motions in homogeneous solution, where behavior is plausibly more uniform. 4) Additional dark monomer

may be incorporated, which may lead to minor extensions at nanoscales that separate the catalyst position from the fluorophore; however, the 5x rinsing steps that remove excess unlabeled monomer that are part of the current experimental procedure minimize the likelihood of this process by significantly limiting the amount of residual dark monomer present and available for incorporation.

The current study is not designed to measure before-and-after reaction effects or to determine if insertion of the monomer impacted the diffusional behavior of the catalyst.[41,43] A plausible scenario is that the measured trajectories report on thermal motion and currents in the sample, which were invisible before turnover, and became visible after turnover.

Tracks and MSD vs time behavior from two different example particles are shown in Figure 3.3d–g (n = 40 tracks total). These tracks show high variation even within single aggregates. Some catalysts exhibited free and rapid motion, such as Catalyst 23 in Figure 3.3d and 3.3e and Catalyst 7 in Figure 3.3f and 3.3g, whereas others exhibited severely restricted motion, such as Catalyst 8 in Figure 3.3f and 3.3g.

Figure 3.3h shows the step size histogram arising from all triplicate data (n = 18608 steps). A step size is the distance between two successive superlocalized positions of the same catalyst. This analysis was performed for all steps in all three replicate experiments and is therefore both cumulative of all experiements and trace-independent. This data was fit using 2D random-walk models, which indicated three populations: stationary/quasi-stationary (23% of population), intermediate- (56%, 65 nm), and large-step sizes (21%, 145 nm). The presence of multiple step-size populations was further consistent with different catalyst microenvironments that imparted different degrees of catalyst mobility. The single-catalyst sensitivity of the experiment enabled measurement of local active catalyst diffusion ability and of the range of diffusions of the active

catalysts in the sample—a difficult distribution to predict or model for given set of polymerization conditions in the absence of measurement data.

We evaluated the hypothesis that catalyst motion might decrease at later imaging times, due to the settling of the strands after the initial agitation from experiment initiation (Figure 3.3a). However, there was no correlation between time of step and average step size at that time (Figure 3.4b). Similarly, the motion of a specific individual catalyst did not slow down significantly with time: 50.4% of the tracks had an overall negative regression (corresponding to slowing of a tracked molecular catalyst) and 49.6% had an overall positive regression (corresponding to increasing speed of a tracked molecular catalyst). These similar values indicate no significant slowing (or speeding) over time of the same single catalyst. Thus, there was no time dependence on the motion of catalysts. Therefore, there was no evidence for strand settling after agitation as the primary causes of the observed motions. Instead, the step-size variations are most consistent with arising from differences in local catalyst environment caused by differences in entanglement of neighboring strands. This single-catalyst tracking thus identifies and characterizes the wide distribution of different local environments around these molecular catalysts caused by secondary environments, despite their "well-defined" primary ligand coordination spheres.

We considered the possibility that individual polymer aggregates may show different particle-wide distributions of motions. For example, a more rigid particle would be expected to display reduced mobility of its component catalysts than a less rigid particle. To compare particle-to-particle behavior, particles (n = 20) that contained 80 or more steps (from 15 or more tracks) were selected.

The distributions of individual step sizes of each catalyst in these 20 particles were compared to the distributions of step sizes from the full experiments. A chi-square goodness of fit

test was performed to evaluate if the step-size distributions in the 20 individual particles were representative of the whole. Of these, 75% (n = 15 particles) exhibited statistically distinct distributions from the whole ($p < 0.001$). Figure 3.3i shows a step size histogram for an example particle with a statistically significant higher catalyst mobility, consistent with a less rigid particle. Figure 3.3j shows an example lower mobility particle. The 15 different particles exhibited all possible permutations, with the majority shifted towards higher populations of quasi-stationary, or intermediate-, or large-step size distributions relative to the full experiments (Figure 3.3k; see Appendix for best fits for each individual particle). These data indicate individualistic single-polymer-particle rigidity, uniquely observable through this subensemble technique.

These different behavior distributions do not clearly correlate to the diameter of the particle (see Appendix for details). Alternative to particle size, these differences may be caused by different degrees of strand entanglement within the particle (e.g., higher entanglement would impart higher rigidity). These particle-to-particle differences are consistent with differences in chemoselectivity previously observed.[16,44–46]

An attempted characterization of the precipitated polymers under similar conditions to those used for microscopy imaging was unsuccessful by both GPC and MALDI-MS, due to insufficient material precipitated on the glass surface. Therefore, to enable characterization, the polymerization reaction was run for a longer time to result in more material (i.e., 20 min for bulk material characterization instead of 5 min for imaging); the resulting characterization therefore provides an upper bound to molecular weights of polymers measured during the imaging experiments. Molecular weight measurements of aggregates precipitated under these longer-reaction times were as follows: M_n = 37,000, M_w = 71,000, PDI = 1.9.

Similar to the observation here, this catalyst is known to produce polynorborenene with broad molecular weight distributions (1.8 – 2.8),[47,48] a feature generally attributed to its established high propagation rate and low initiation rate, along with competing chain-transfer reactions.[49–51] We postulate that the wide distribution of individual catalyst behavior, as characterized by this observed range of active catalyst motion indicating extremely different local environments, is an additional contributing factor to polydispersity in this system (Đ = 1.9).

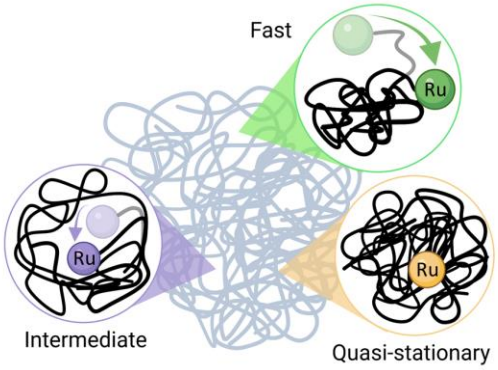


Figure 3.5. Microenvironment physical model consistent with multiple mobility populations, in which ratios of types of microenvironments differ and give rise to a range of molecular catalyst behaviors.

Considering all data described in this manuscript, a microenvironment physical model for the observed distributions of catalyst motion within growing polymers is shown in Figure 3.5. In this model, catalyst motion provides a readout of the differential secondary environment produced by neighboring polymer strands.

CONCLUSIONS

In conclusion, superresolution imaging of the motion of individual molecular catalysts on the reactive chain end of growing polymers has been achieved. This measurement enables returning to the questions posed in the introduction: 1) *What is the range of motion?* The catalysts range in MSD and show diverse individual diffusion behavior with respect to time (Figure 3.2c).

2) *Does distribution of mobility indicate different catalyst microenvironments?* Yes, and individual particles show unique compositions of these microenvironments (Figure 3.2i–k). 3) *Do differential physical motions persist long enough to impact chemical reactivity or are they quickly averaged?* Differential physical motions persist for several seconds, and sometimes for over 20 s (Figure 3.2c–g). This persistence likely mirrors sustained differences in catalyst steric environment, solvation, and accessibility to monomer, which is plausibly a contributing cause of macroscale polymer properties such as molecular weight polydispersity or degree of comonomer incorporation. Measurement of such catalyst diffusion distributions may therefore serve as a handle for future optimization of catalysts to tune bulk polymer properties. To our knowledge, this report is the first method development and demonstration of measuring single molecular catalyst motion during turnover by fluorescence microscopy.

The observations of heterogeneity may be particularly relevant to similar unstirred biphasic polymerization systems, such as for the synthesis of monodispersed polymer particles and the synthesis of porous polymers that form by precipitation polymerization.[52,53] For example, the heterogeneous range of active catalyst motions and microenvironments in these systems—and resulting difference in catalyst accessibility to solvent and monomer—may plausibly be a mechanistic cause of heterogenous molecular weight or other bulk properties of the resulting polymers. In the broader sense, the enabled elucidation of molecular catalyst motion provides a complementary and highly sensitive parameter for understanding and optimizing catalytic processes and the bulk product properties they give rise to.

REFERENCES

(1) Kubarev, A. V.; Janssen, K. P. F.; Roeffaers, M. B. J. Noninvasive Nanoscopy Uncovers the Impact of the Hierarchical Porous Structure on the Catalytic Activity of Single Dealuminated Mordenite Crystals. *ChemCatChem* **2015**, *7* (22), 3646–3650.

(2) Maris, J. J. E.; Fu, D.; Meirer, F.; Weckhuysen, B. M. Single-Molecule Observation of Diffusion and Catalysis in Nanoporous Solids. *Adsorption* **2021**, *27* (3), 423–452.

(3) Hendriks, F. C.; Meirer, F.; Kubarev, A. V.; Ristanović, Z.; Roeffaers, M. B. J.; Vogt, E. T. C.; Bruijnincx, P. C. A.; Weckhuysen, B. M. Single-Molecule Fluorescence Microscopy Reveals Local Diffusion Coefficients in the Pore Network of an Individual Catalyst Particle. *J. Am. Chem. Soc.* **2017**, *139* (39), 13632–13635.

(4) Dong, B.; Mansour, N.; Huang, T.-X.; Huang, W.; Fang, N. Single Molecule Fluorescence Imaging of Nanoconfinement in Porous Materials. *Chem. Soc. Rev.* **2021**, *50* (11), 6483–6506.

(5) Dong, B.; Mansour, N.; Pei, Y.; Wang, Z.; Huang, T.; Filbrun, S. L.; Chen, M.; Cheng, X.; Pruski, M.; Huang, W.; Fang, N. Single Molecule Investigation of Nanoconfinement Hydrophobicity in Heterogeneous Catalysis. *J. Am. Chem. Soc.* **2020**, *142* (31), 13305–13309.

(6) Naito, K.; Tachikawa, T.; Fujitsuka, M.; Majima, T. Single-Molecule Observation of Photocatalytic Reaction in TiO2 Nanotube: Importance of Molecular Transport through Porous Structures. *J. Am. Chem. Soc.* **2009**, *131* (3), 934–936.

(7) Naito, K.; Tachikawa, T.; Fujitsuka, M.; Majima, T. Real-Time Single-Molecule Imaging of the Spatial and Temporal Distribution of Reactive Oxygen Species with Fluorescent Probes: Applications to TiO 2 Photocatalysts. *J. Phys. Chem. C* **2008**, *112* (4), 1048–1059.

(8) Tachikawa, T.; Majima, T. Single-Molecule, Single-Particle Fluorescence Imaging of TiO2-Based Photocatalytic Reactions. *Chem. Soc. Rev.* **2010**, *39* (12), 4802–4819.

(9) Roeffaers, M. B. J.; Sels, B. F.; Uji-i, H.; De Schryver, F. C.; Jacobs, P. A.; De Vos, D. E.; Hofkens, J. Spatially Resolved Observation of Crystal-Face-Dependent Catalysis by Single Turnover Counting. *Nature* **2006**, *439* (7076), 572–575.

(10) Carpenter, B. K. Nonstatistical Dynamics in Thermal Reactions of Polyatomic Molecules. *Annu. Rev. Phys. Chem.* **2005**, *56*, 57–89.

(11) Thomas, J. B.; Waas, J. R.; Harmata, M.; Singleton, D. A. Bifurcating Surface. *J. Am. Chem. Soc.* **2008**, 9, 14544–14555.

(12) Cooper, J. T.; Peterson, E. M.; Harris, J. M. Fluorescence Imaging of Single-Molecule Retention Trajectories in Reversed-Phase Chromatographic Particles. *Anal. Chem.* **2013**, *85* (19), 9363–9370.

(13) Kisley, L.; Landes, C. F. Molecular Approaches to Chromatography Using Single Molecule Spectroscopy. *Anal. Chem.* **2015**, *87* (1), 83–98.

(14) Kulzer, F.; Xia, T.; Orrit, M. Single Molecules as Optical Nanoprobes for Soft and Complex Matter. *Angew. Chemie Int. Ed.* **2010**, *49* (5), 854–866.

(15) Schob, A.; Cichos, F.; Schuster, J.; Von Borczyskowski, C. Reorientation and Translation of Individual Dye Molecules in a Polymer Matrix. *Eur. Polym. J.* **2004**, *40* (5), 1019–1026.

(16) Garcia, A.; Saluga, S. J.; Dibble, D. J.; López, P. A.; Saito, N.; Blum, S. A. Does Selectivity of Molecular Catalysts Change with Time? Polymerization Imaged by Single-Molecule Spectroscopy. *Angew. Chemie. Int. Ed.* **2021**, *60* (3), 1550–1555.

(17) Buchmeiser, M. R. Polymer-Supported Well-Defined Metathesis Catalysts. **2009**, 303–321.
(18) Dias, E. L.; Nguyen, S. B. T.; Grubbs, R. H. Well-Defined Ruthenium Olefin Metathesis Catalysts: Mechanism and Activity. *J. Am. Chem. Soc.* **1997**, *119* (17), 3887–3897.
(19) Liu, C.; Kubo, K.; Wang, E.; Han, K. S.; Yang, F.; Chen, G.; Escobedo, F. A.; Coates, G. W.; Chen, P. Single Polymer Growth Dynamics. *Science* **2017**, *358* (6361), 352–355.
(20) Grela, K. Olefin Metathesis: Theory and Practice, 1st ed.; John Wiley & Sons, Inc.: Hoboken, New Jersey, **2014**; Vol. 1.
(21) Baier, M.; Wöll, D.; Mecking, S. Diffusion of Molecular and Macromolecular Polyolefin Probes in Cylindrical Block Copolymer Structures As Observed by High Temperature Single Molecule Fluorescence Microscopy. *Macromolecules* **2018**, *51* (5), 1873–1884.
(22) Wöll, D.; Flors, C. Super-Resolution Fluorescence Imaging for Materials Science. *Small Methods* **2017**, *1* (10), 1–12.
(23) Flier, B. M. I.; Baier, M. C.; Huber, J.; Müllen, K.; Mecking, S.; Zumbusch, A.; Wöll, D. Heterogeneous Diffusion in Thin Polymer Films as Observed by High-Temperature Single-Molecule Fluorescence Microscopy. *J. Am. Chem. Soc.* **2012**, *134* (1), 480–488.
(24) Wöll, D.; Uji-i, H.; Schnitzler, T.; Hotta, J. I.; Dedecker, P.; Herrmann, A.; De Schryver, F. C.; Müllen, K.; Hofkens, J. Radical Polymerization Tracked by Single Molecule Spectroscopy. *Angew. Chemie. Int. Ed.* **2008**, *47* (4), 783–787.
(25) Stempfle, B.; Dill, M.; Winterhalder, M. J.; Müllen, K.; Wöll, D. Single Molecule Diffusion and Its Heterogeneity during the Bulk Radical Polymerization of Styrene and Methyl Methacrylate. *Polym. Chem.* **2012**, *3* (9), 2456–2463.
(26) Zettl, H.; Zettl, U.; Krausch, G.; Enderlein, J.; Ballauff, M. Direct Observation of Single Molecule Mobility in Semidilute Polymer Solutions. *Phys. Rev. E - Stat. Nonlinear, Soft Matter Phys.* **2007**, *75* (6), 1–6.
(27) Easter, Q. T.; Blum, S. A. Organic and Organometallic Chemistry at the Single-Molecule, -Particle, and -Molecular-Catalyst-Turnover Level by Fluorescence Microscopy. *Acc. Chem. Res.* **2019**, *52* (8), 2244–2255.
(28) Cordes, T.; Blum, S. A. Opportunities and Challenges in Single-Molecule and Single-Particle Fluorescence Microscopy for Mechanistic Studies of Chemical Reactions. *Nat. Chem.* **2013**, *5* (12), 993–999.
(29) Betzig, E.; Patterson, G. H.; Sougrat, R.; Lindwasser, O. W.; Olenych, S.; Bonifacino, J. S.; Davidson, M. W.; Lippincott-Schwartz, J.; Hess, H. F. Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. *Science* **2006**, *313* (5793), 1642–1645.
(30) Kamiyama, D.; Huang, B. Development in the STORM. *Dev. Cell* **2012**, 23 (6), 1103–1110.
(31) Ristanović, Z.; Kerssens, M. M.; Kubarev, A. V.; Hendriks, F. C.; Dedecker, P.; Hofkens, J.; Roeffaers, M. B. J.; Weckhuysen, B. M. High-Resolution Single-Molecule Fluorescence Imaging of Zeolite Aggregates within Real-Life Fluid Catalytic Cracking Particles. *Angew. Chemie. Int. Ed.* **2015**, *54* (6), 1836–1840.
(32) Roeffaers, M. B. J.; De Cremer, G.; Libeert, J.; Ameloot, R.; Dedecker, P.; Bons, A.-J.; Bückins, M.; Martens, J. A.; Sels, B. F.; De Vos, D. E.; Hofkens, J. Super-Resolution Reactivity Mapping of Nanostructured Catalyst Particles. *Angew. Chemie. Int. Ed.* **2009**, *48* (49), 9285–9289.

(33) Ristanović, Z.; Kubarev, A. V.; Hofkens, J.; Roeffaers, M. B. J.; Weckhuysen, B. M. Single Molecule Nanospectroscopy Visualizes Proton-Transfer Processes within a Zeolite Crystal. *J. Am. Chem. Soc.* **2016**, *138* (41), 13586–13596.
(34) Hendriks, F. C.; Mohammadian, S.; Ristanović, Z.; Kalirai, S.; Meirer, F.; Vogt, E. T. C.; Bruijnincx, P. C. A.; Gerritsen, H. C.; Weckhuysen, B. M. Integrated Transmission Electron and Single-Molecule Fluorescence Microscopy Correlates Reactivity with Ultrastructure in a Single Catalyst Particle. *Angew. Chemie. Int. Ed.* **2018**, *57* (1), 257–261.
(35) Chen, T.; Dong, B.; Chen, K.; Zhao, F.; Cheng, X.; Ma, C.; Lee, S.; Zhang, P.; Kang, S. H.; Ha, J. W.; Xu, W.; Fang, N. Optical Super-Resolution Imaging of Surface Reactions. *Chem. Rev.* **2017**, *117* (11), 7510–7537.
(36) Rust, M. J.; Bates, M.; Zhuang, X. Sub-Diffraction-Limit Imaging by Stochastic Optical Reconstruction Microscopy (STORM). *Nat. Methods* **2006**, *3* (10), 793–795.
(37) Han, R.; Ha, J. W.; Xiao, C.; Pei, Y.; Qi, Z.; Dong, B.; Bormann, N. L.; Huang, W.; Fang, N. Geometry-Assisted Three-Dimensional Superlocalization Imaging of Single-Molecule Catalysis on Modular Multilayer Nanocatalysts. *Angew. Chemie. Int. Ed.* **2014**, *53* (47), 12865–12869.
(38) Mao, X.; Liu, C.; Hesari, M.; Zou, N.; Chen, P. Super-Resolution Imaging of Non-Fluorescent Reactions via Competition. *Nat. Chem.* **2019**, *11* (8), 687–694.
(39) Dong, B.; Pei, Y.; Zhao, F.; Goh, T. W.; Qi, Z.; Xiao, C.; Chen, K.; Huang, W.; Fang, N. In Situ Quantitative Single-Molecule Study of Dynamic Catalytic Processes in Nanoconfinement. *Nat. Catal.* **2018**, *1* (2), 135–140.
(40) Saini, A.; Messenger, H.; Kisley, L. Fluorophores "Turned-On" by Corrosion Reactions Can Be Detected at the Single-Molecule Level. *ACS Appl. Mater. Interfaces* **2021**, *13* (1), 2000–2006.
(41) MacDonald, T. S. C.; Price, W. S.; Astumian, R. D.; Beves, J. E. Enhanced Diffusion of Molecular Catalysts Is Due to Convection. *Angew. Chemie. Int. Ed.* **2019**, *58* (52), 18864–18867.
(42) Bielawski, C. W.; Grubbs, R. H. Living Ring-Opening Metathesis Polymerization. *Prog. Polym. Sci.* **2007**, *32* (1), 1–29.
(43) Wang, H.; Park, M.; Dong, R.; Kim, J.; Cho, Y. K.; Tlusty, T.; Granick, S. Boosted Molecular Mobility during Common Chemical Reactions. *Science* **2020**, *369* (6503), 537–541.
(44) Easter, Q. T.; Garcia, A.; Blum, S. A. Single-Polymer-Particle Growth Kinetics with Molecular Catalyst Speciation and Single-Turnover Imaging. *ACS Catal.* **2019**, *9* (4), 3375–3383.
(45) Easter, Q. T.; Blum, S. A. Kinetics of the Same Reaction Monitored over Nine Orders of Magnitude in Concentration: When Are Unique Subensemble and Single-Turnover Reactivity Displayed? *Angew. Chemie. Int. Ed.* **2018**, *57* (37), 12027–12032.
(46) Easter, Q. T.; Blum, S. A. Evidence for Dynamic Chemical Kinetics at Individual Molecular Ruthenium Catalysts. *Angew. Chemie. Int. Ed.* **2018**, *57* (6), 1572–1575.
(47) Al-Hashimi, M.; Bakar, M. D. A.; Elsaid, K.; Bergbreiter, D. E.; Bazzi, H. S. Ring-Opening Metathesis Polymerization Using Polyisobutylene Supported Grubbs Second-Generation Catalyst. *RSC Adv.* **2014**, *4* (82), 43766–43771.
(48) Bielawski, C. W.; Benitez, D.; Morita, T.; Grubbs, R. H. Synthesis of End-Functionalized Poly(Norbornene)s via Ring-Opening Metathesis Polymerization. *Macromolecules,* **2001**, *34* (25), 8610–8618.

(49) Sanford, M. S.; Ulman, M.; Grubbs, R. H. New Insights into the Mechanism of Ruthenium-Catalyzed Olefin Metathesis Reactions. *J. Am. Chem. Soc.* **2001**, *123* (4), 749–750.
(50) Sandford, M. S.; Love, J. A.; Grubbs, R. H. Mechanism and Activity of Ruthenium Olefin Metathesis Catalysts. *J. Am. Chem. Soc.* **2001**, *123* (27), 6543–6554.
(51) Choi, T. L.; Grubbs, R. H. Controlled Living Ring-Opening-Metathesis Polymerization by a Fast-Initiating Ruthenium Catalyst. *Angew. Chemie. Int. Ed.* **2003**, *42* (15), 1743–1746.
(52) Kovačič, S.; Slugovc, C. Ring-Opening Metathesis Polymerisation Derived Poly(Dicyclopentadiene) Based Materials. *Mater. Chem. Front.* **2020**, *4* (8), 2235–2255.
(53) Haehnle, B.; Jathavedan, K. K.; Schuster, P. A.; Karg, M.; Kuehne, A. J. C. Elucidating the Nucleation Event in the C-C Cross-Coupling Step-Growth Dispersion Polymerization. *Macromolecules* **2021**, *54* (13), 6085–6089.

APPENDIX

I. General Information

All reagents and solvents were used as received from commercial sources unless otherwise noted. Analytical thin layer chromatography (TLC) was performed using Merck F_{250} plates and visualized under UV irradiation at 254 nm. Flash chromatography was conducted using a Teledyne Isco Combiflash® Rf 200 Automatic Flash Chromatography System, and Teledyne Isco Redisep® 35–70 μm silica gel. Spectrophotometric grade heptane (OmniSolv®) was purchased from EMD Millipore and was used for all microscopy studies. The ruthenium catalyst ("Grubbs Catalyst, 2nd Generation") was purchased from Sigma–Aldrich. Ultra-pure water with >18 MΩ resistivity and total organic content of <5 ppb was obtained from a Milli-Q Gradient A10 water purifier (Millipore, Billerica, MA) using a Q-Gard 2 purification pack and a Quantum EX Ultrapure Organex cartridge. All proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Bruker DRX-500 spectrometer outfitted with a cryoprobe or a Bruker Avance 600 MHz spectrometer. All coupling constants were measured in Hertz (Hz). Chemical shifts were reported in ppm and were referenced to residual protiated solvent peaks (δ_H = 7.26 ppm for $CDCl_3$; δ_H = 5.33 ppm for CD_2Cl_2) in ^{1}H NMR spectroscopy experiments. GPC was conducted on an

Agilent 1100 GPC-SEC system using a PLGel 5μM MIXED-C Column purchased from Agilent (P/N PL1110-6500) and the molecular weight was determined with respect to polystyrene standards purchased from Aldrich (P/N 81434). Tetrahydrofuran was used as the eluent at a flow rate of 1.0 mL/min with column temperature at 35°C. Graphics in Figures 3.1, 3.2a, 3.2k, and 3.3 were made in Biorender.com.

II. Construction of Reaction Cells and Preparation of Coverslips for Microscopy

Images of our laboratory's reaction vessels have been previously published.[1] Glass coverslips (25 × 25 mm, No. 1.5, VWR Scientific) with a thickness of 0.16–0.19 mm were cleaned by sonication in 20 mL of a 0.6% solution of Hellmanex Detergent (Fisher Chemical) in Milli-Q water for 60 min and then rinsed sequentially with Milli-Q water and spectrophotometric grade ethanol six times. The rinsed coverslips were dried with compressed air. Coverslips were either stored in a sealed container or used immediately after drying. Bottomless vials were made by cutting the ends from glass reaction vials (Short Form Style, VWR Scientific). The resulting cylinders were rinsed thoroughly with Milli-Q water and spectrophotometric grade ethanol and dried in an oven at 115 °C overnight before use. To assemble the reaction cells, the cleaned and dried hollow bottomless vials were attached to the cleaned coverslips by applying epoxy (Devcon) to the outside base of the tubes, then the assembled tubes were capped and stored overnight or longer before use in microscopy experiments.

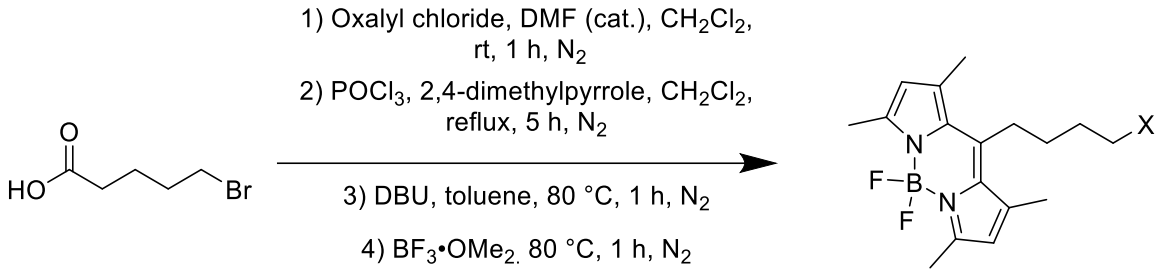
III. Fluorescence Microscopy Parameters

All microscopy imaging was performed with an inverted microscope (IX71, Olympus Corporation) and an oil-immersion, 60× objective with a 1.45 numerical aperture combined with a 1.6× magnification piece engaged. The total magnification was 96×. Samples were imaged with

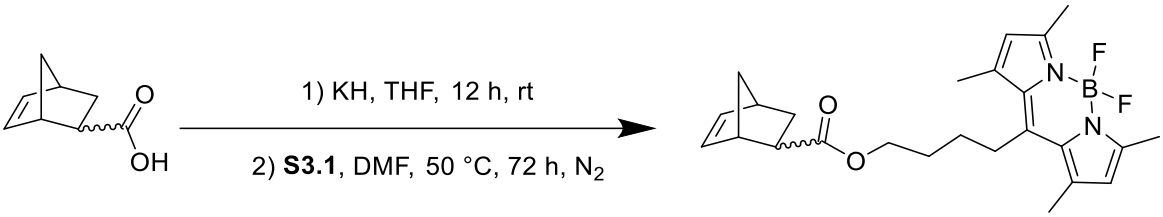
a CMOS Prime 95B camera (Photometrics). Fluorescence microscopy samples were illuminated with the 488 nm line obtained from a solid state laser stack (Intelligent Imaging Innovations) set to 15% power (~6.0 mW measured at the objective). The Backside Illuminated Sensor (95% quantum efficiency) has an effective 1200 × 1200 array of pixels. The pixel size was 11 μm which with the 96× magnification, resulted in each pixel in the acquired images representing an area of 115 × 115 nm. The focus was changed with a *z*-axis controller (MS-2000, Applied Scientific Instruments, Inc.). All images were acquired with the CRISP autofocus engaged for the duration of the experiment. All images were acquired in total internal reflection fluorescence (TIRF). An external alternating filter wheel was installed in between the inverted microscope and camera (Sutter Instrument Co). For all time-lapse acquisitions, images were obtained for the green filter channel 514/30 with a FF506-Di03-25x35 dichroic beamsplitter (Brightline). The SlideBook 6.0 software (Intelligent Imaging Innovations) was set to acquire images every 1 s with 100 ms exposure to the 488 nm line per frame in each color channel. The sample is exposed to the laser every 100 ms during which fluorescence is imaged. During the 900 ms between exposure times, the sample is not exposed to the laser. This exposure followed by non-exposure is achieved by software control. The outcome of this control is extended time tracking and imaging without excess photobleaching. Images were viewed in ImageJ (NIH, available at http://rsbweb.nih.gov/ij/) and analyzed with Igor Pro 8 (WaveMetrics).

IV. Synthetic Procedures

*a. Synthesis of ester norbornene green BODIPY core imaging agent **3.1**.*



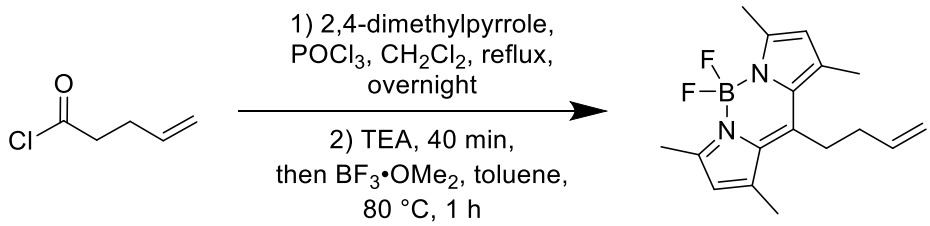
S3.1



3.1

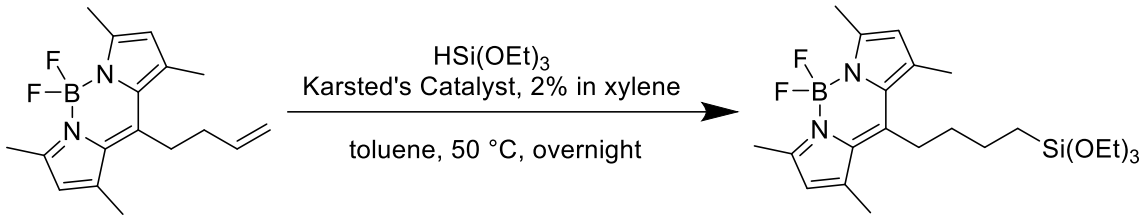
The functionalized BODIPY compound **S3.1** and imaging agent **3.1** were synthesized as previously reported in literature,[2,3] with the following exception: after purification by column chromatography, imaging agent **3.1** was additionally purified by titration with pentanes (60 mL).

*b. Synthesis of terminal olefin green BODIPY **S3.2** and silylated green BODIPY core control compound **S3.3***



S3.2

Synthesis of the terminal olefin green BODIPY core imaging agent **S3.2** followed the procedure reported previously in the literature.[4]

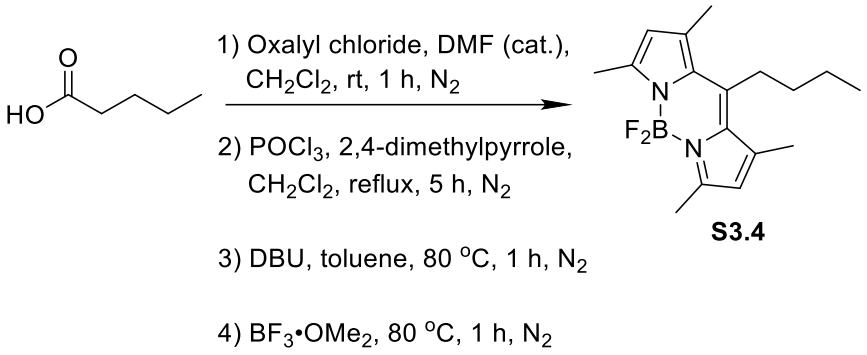


S3.2 **S3.3**

The silylated green BODIPY core control compound was synthesized according to previous literature,[4] with modifications to catalyst loading: An oven-dried flask was charged under N_2 with **S3.2** (0.327 g, 1.08 mmol), dry toluene (3 mL), and a stirbar. Triethoxysilane (0.70 mL, 3.8 mmol) was added followed by platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane (Karstedt's catalyst; 2% Pt in xylenes, 0.022 mL, 0.1 mmol Pt). The suspension was heated to 50 °C. All solids dissolved after a few minutes to afford a dark red-orange solution. The solution was stirred overnight at 50 °C at which point TLC analysis (10% ethyl acetate in hexanes; Rf = 0.3) revealed incomplete conversion of starting material. The solution was stirred for an additional 24 h. The mixture was cooled to room temperature, the excess solvent was evaporated, and pentanes (20 mL) were added to the dried product. This mixture was heated to dissolve most of the solid. This solution was then sonicated for 30 minutes to assist dissolution and placed in a freezer (–35 °C) overnight. The solution was decanted and the solids were then loaded onto a silica gel column and purified by eluting with 0–30% ethyl acetate in hexanes to afford 0.082 g (0.17 mmol, 16%) of **S3.3** as a yellow-orange powder.

Characterization was previously reported[4] and is reproduced here for convenience. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.04 (s, 2H), 3.82 (q, J = 7 Hz, 6H), 2.95–2.92 (m, 2H), 2.51 (s, 6H), 2.41 (s, 6H), 1.71–1.58 (m, 4H), 1.22 (t, J = 7 Hz, 9H), 0.71–0.68 (m, 2H); ^{13}C NMR (126 MHz, CDCl3) δ 153.7, 146.6, 140.4, 131.5, 121.6, 58.5, 34.9, 28.3, 23.8, 18.3, 16.4, (t, $J_{C–F}$ = 2 Hz), 10.4.

*c. Synthesis of butyl green BODIPY core control compound **S3.4**.*



Synthesis of butyl green BODIPY core control compound **S3.4** followed the procedure reported previously in the literature.[1]

V. Sample Preparation

a. Preparation of catalyst for microscopy. In a nitrogen-filled glove box, Grubbs second-generation catalyst (1.5 mg, 1.8×10^{-3} mmol) was weighed into a 1 dram vial, which was then capped and brought out of the box. The cap was removed, and spectrophotometric grade heptane (0.75 mL) was added to the vial via plastic syringe equipped with a 20G needle. The resulting mixture was gently swirled, then transferred via the same syringe to a prepared microscopy reaction vial. The solution was a light pink due to some dissolved catalyst, but much of the catalyst remained insoluble and settled on the bottom of the microscope coverslip inside the reaction vial. The microscopy vial was placed on the microscope, and the top of the coverslip was brought into focus, using room light and catalyst crystals to focus.

*b. Preparation of solution containing 1.0×10^{-12} M **3.1** for fluorescence microscopy.* Imaging agent **3.1** (2.0 mg, 2.3×10^{-3} mmol) was weighed into a 1 dram glass vial. Spectrophotometric grade heptane (4.0 mL) was added to the vial via plastic syringe, and the solution was sonicated for 1 h to fully dissolve the compound. This afforded a solution of 1.2×10^{-3} M imaging agent **3.1**

in heptane, which was green-orange in appearance. Serial dilution using this 1.2×10^{-3} M stock solution using a 10 µL gastight syringe (Hamilton Company) and heptane allowed for the preparation of a clear, colorless 1.0×10^{-12} M solution of imaging agent **3.1** in heptane.

*c. Preparation of solution containing 1.0×10^{-12} M **S3.4** for fluorescence microscopy.* Control probe **S3.4** (1.4 mg, 2.4 x 10^{-3} mmol) was weighed into a 1 dram glass vial. Spectrophotometric grade heptane (4.0 mL) was added to the vial via plastic syringe, and the solution was sonicated for 1 h to fully dissolve the compound. This afforded a solution of 1.2×10^{-3} M control probe **S3.4** in heptane, which was green-orange in appearance. Serial dilution using this 1.2×10^{-3} M stock solution using a 10 µL gastight syringe (Hamilton Company) and heptane allowed for the preparation of a clear, colorless 1.0×10^{-13} M solution of control probe **S3.4** in heptane.

VI. Imaging of Single-Molecule Motion

A microscopy cell containing a 0.75 mL of the catalyst mixture (1.5 mg) in spectrophotometric grade heptane was imaged to in TIRF mode. This resulted in dark images when surveying regions of the coverslip (Figure S3.1). In a 1 dram vial, norbornene (2.4 mg, 2.6 x 10^{-3} mmol) was weighed out and dissolved in 1.0 mL of spectrophotometric grade heptane, added via plastic syringe. 0.25 mL of the norbornene solution was then added all at once to the mixture of catalyst in heptane (Section Va), yielding a volume of 1.00 mL. The reaction mixture was imaged in TIRF mode for three minutes, which resulted in dark images and no detection of single molecules when surveying regions of the coverslip. At the 5-minute mark after addition of norbornene to the catalyst mixture, the mother liquor was removed leaving behind a layer of liquid (approximately 0.1 mL). The pre-formed polymer material gathered on the surface was rinsed five times with 1 mL of spectrophotometric grade heptane. After the last heptane rinse, the solvent was removed to complete dryness of the microscopy vial. This was done to ensure accurate volume measurements

in the next steps. Spectrophotometric grade heptane (1 mL) was added via syringe. 1 mL of heptane was added to the microscopy vial and a 20-minute time-lapse capture was taken in TIRF mode (see Section III for acquisition details). After 90 seconds into the time-lapse capture, the shutter was closed and 0.25 mL of imaging agent **3.1** at 1.0×10^{-12} M was added. This resulted in a reaction mixture containing imaging agent **3.1** at 2.0×10^{-13} M and pre-formed polynorbornene on the coverslip surface. The shutter was immediately opened after addition. The imaging "start" moment is referred to as $t = 0$ on all figures and is the moment the shutter was re-opened. The stage was not moved after the sample shutter was re-opened. Upon opening the shutter, numerous single-bright flashes were observed on the coverslip surface in both green and orange channels. At some regions of the coverslip, the flashes appeared to be localized to particular 2−5 μm areas, indicating areas of high reactivity. These images indicate that flashes are due to the insertion of **3.1** into the growing polymer chains.[5]

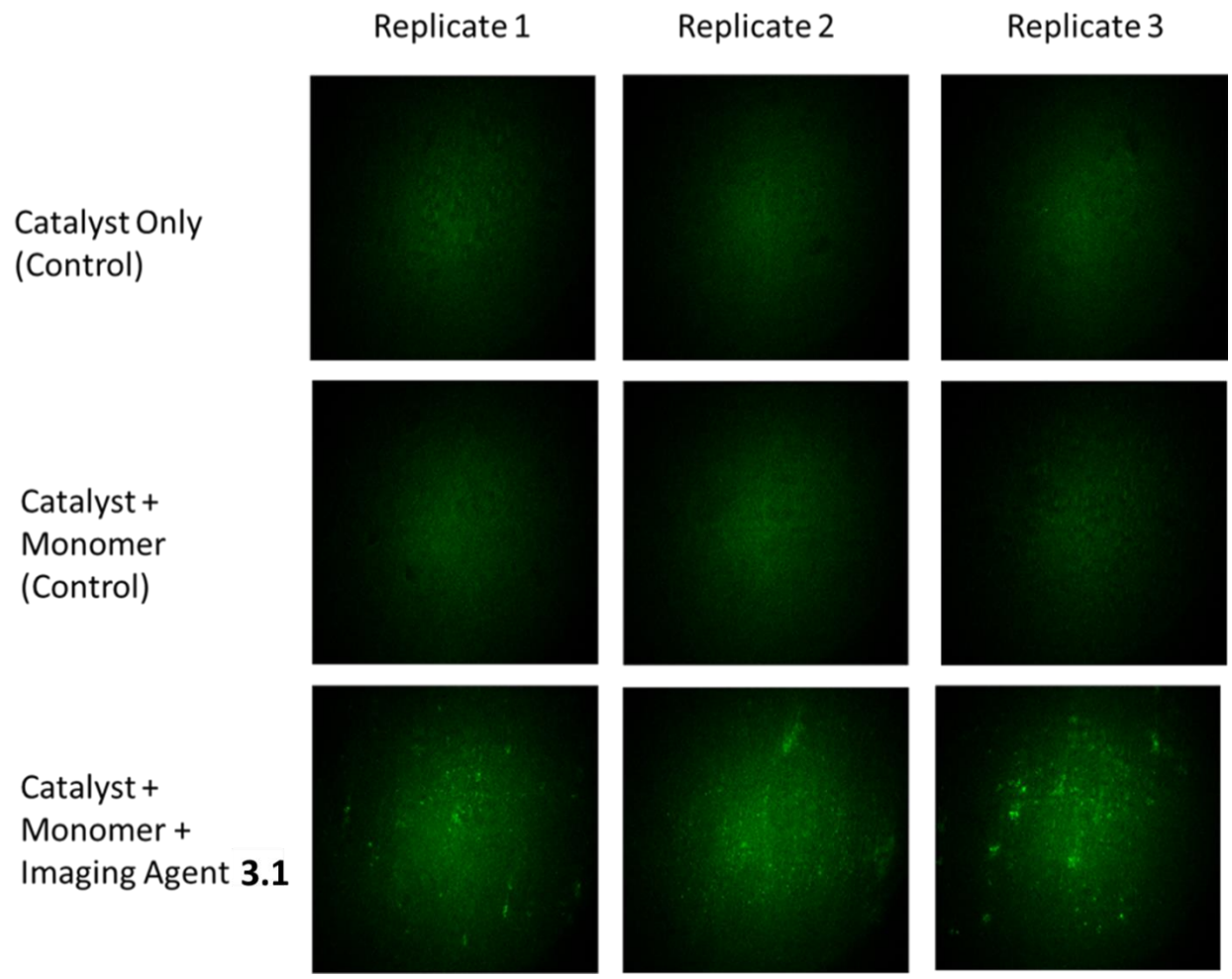


Figure S3.1. Full-frame representative single frames from imaging experiments for single chain-elongation events for three replicate samples. Single-molecule events were observed using imaging agent **3.1** at 2.0×10^{-13} M and not in the control experiments. Images are 1200 pixels x 1200 pixels (137.5 x 137.5 μm^2) and are all displayed at identical brightness/contrast settings.

VII. Control Experiments with Unreactive Imaging Agent

*a. Control Experiment for Imaging with Control Probe **S3.4**.* In order to determine if the green flashes observed were due to irreversible incorporation of fluorophore-tagged monomer, the experiments were repeated under similar conditions with chemically inactive butyl control probe **S3.4**. The video acquisition procedures for these experiments are identical to that of section VIIa, with the exception that control probe **S3.4** was used in place of chain-elongation agent **1** (Figure

S3.2). Upon initiating video acquisition of the sample and focusing into TIRF, the images remained dark, with little to no single-molecule events observed (Figure S3.2). These experiments confirm that the correct metathesis-active functional groups are needed to produce single-molecule images at this concentration range.[6]

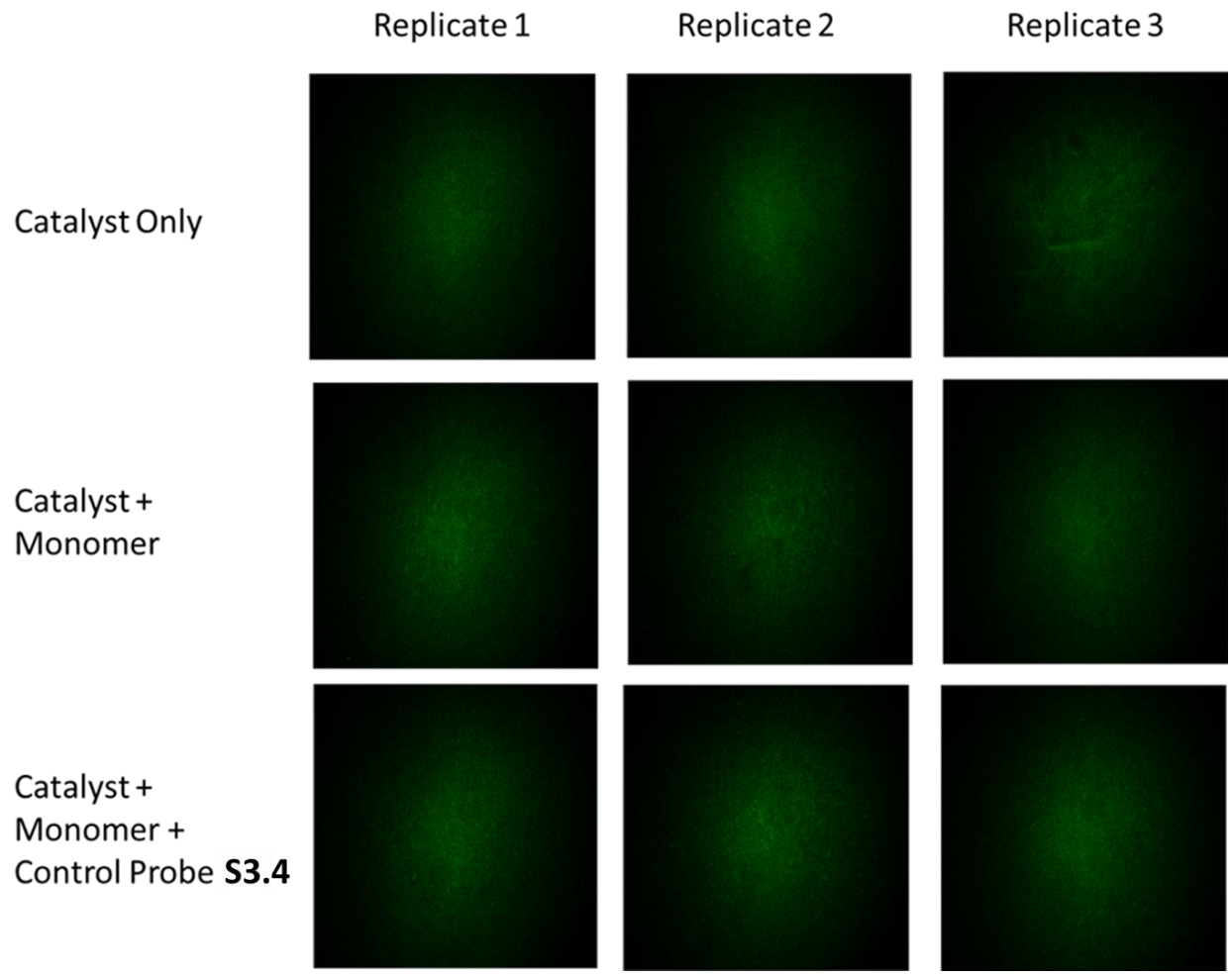


Figure S3.2. Full-frame representative single frames from the control experiments for three replicate samples. Images are 1200 pixels x 1200 pixels (137.5 x 137.5 μm^2) and are all displayed at identical brightness/contrast settings to Figure S3.1.

To further compare the imaging of control compound **S3.4** with imaging agent **3.1**, composite images were produced. These images are the max intensity values of each pixel over the course of the entire time-lapse video. The composite images shown in Figure S3.3 were set to

the same brightness and contrast settings. To enable clear comparisons, the brightness and contrast settings were set to: gamma = 0.5, min = 125, max = 226.

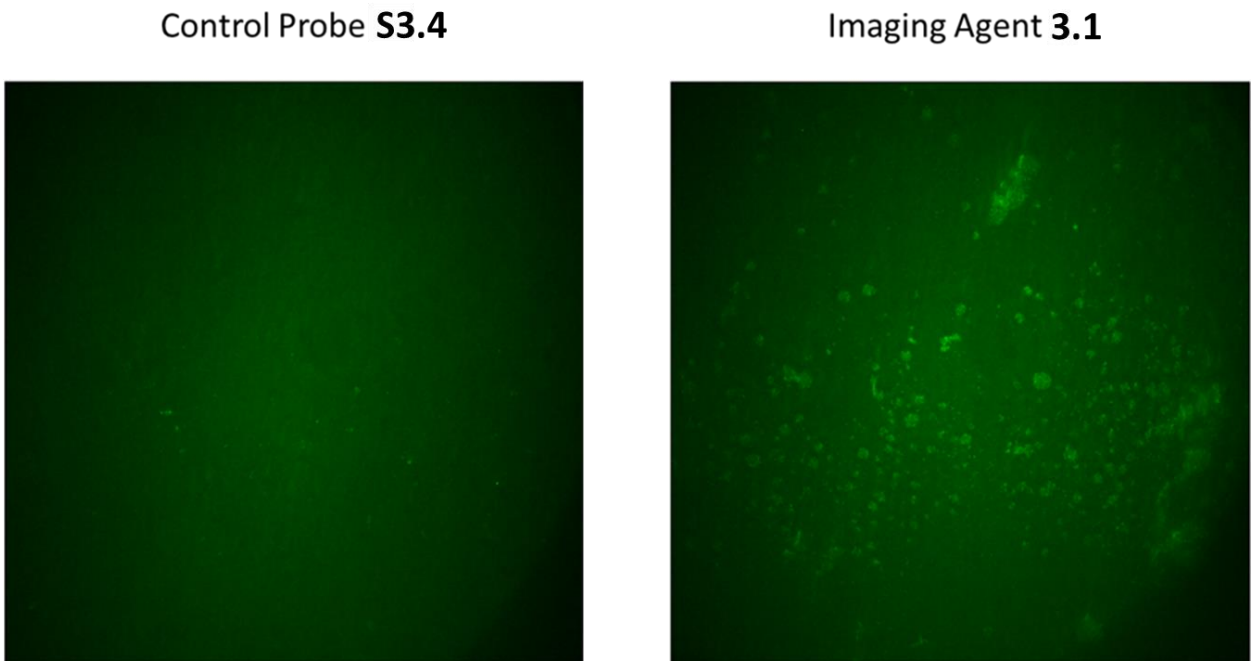
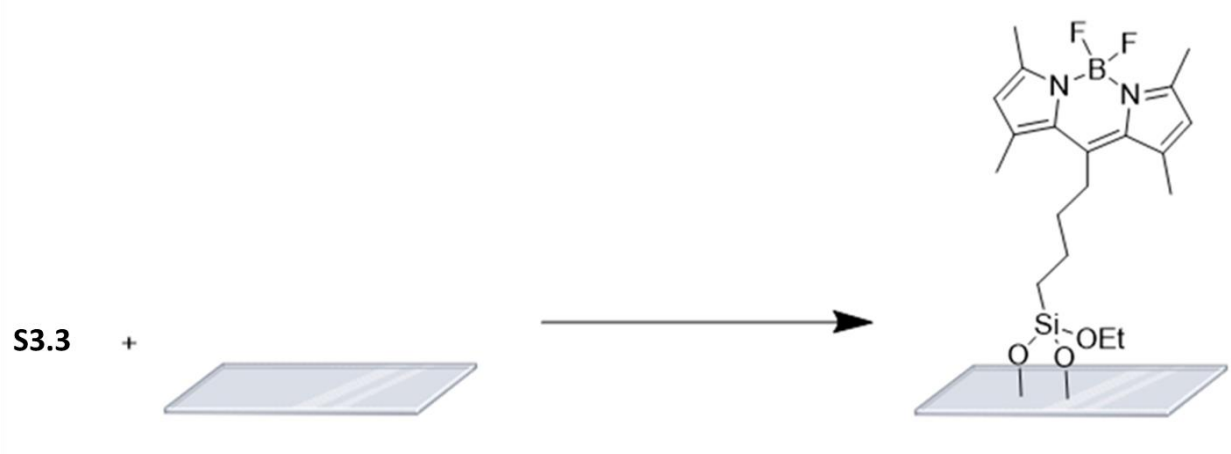


Figure S3.3. Full frame comparison of green channel composite images of (a) control compound **S3.4**, with (b) imaging agent **3.1**. Images are 1200 pixels x 1200 pixels (137.5 x 137.5 μm^2).

b. Control experiment for positional accuracy of stationary emitters. In order to determine the positional accuracy of our technique, controls experiments were performed in which the silylated control probe **S3.3** was covalently bound to the coverslip and therefore immobilized on the surface.

A solution of silylated green BODIPY **S3.3** (4.6 mg, 0.010 mmol) was dissolved in 10 mL spectroscopic grade CH_2Cl_2 in a 20 mL scintillation vial, affording a 0.0010 M solution. An aliquot of this solution (10 μL) was then taken into another 20 mL scintillation vial containing 10 mL of spectroscopic grade CH_2Cl_2 with a glass syringe (Hamilton Company). An aliquot of this solution (1 mL) was taken into a third 20 mL scintillation vial with 19 mL of spectroscopic grade CH_2Cl_2, affording a 5 x 10^{-8} M solution of **S3.3**.



The coverslips were prepared with the surface-bound fluorophore **S3.3** via the following procedure: The coverslips were sonicated for 1 h in Hellmanex™ cleaning solution and then rinsed with ultrapure water. The coverslips were then rinsed with spectroscopic grade ethanol and spin-dried with a stream of air. The coverslips were dried at 130 °C for 1 h before being cooled to ambient temperature and stored in fresh aluminum foil. A glass TLC jar was charged with the previously made 5 x 10^{-8} M solution of **S3.3**. The coverslips were soaked in this solution for 1 h, before being removed and rinsed with ultrapure water 6×, followed by rinsing with spectroscopic grade ethanol 6×. The coverslips were then dried under vacuum in a cold finger for 30 min.

These coverslips were then imaged on the microscope using the same conditions as discussed in Section III. Multiple single-molecule events were seen during the entire length of capture. In order to obtain many data points, every 100 s, the imaging area was moved to a different portion of the coverslip as the functionalized **S3.3** seen in the previous area were significantly photobleached after 100 s (Figure S3.4). Four replicate runs were taken with differing lengths of capture: Control 1 (200 s), Control 2 (600 s), Control 3 (300 s), Control 4 (900 s). For analysis, the frames during the movement of the objective to a new area were excluded post-capture and analyzed in roughly 100 s batches for ease of processing.

In Figure **S3.4**, to enable clear comparisons, the brightness and contrast settings were set to: gamma = 0.5, min = 117, max = 151.

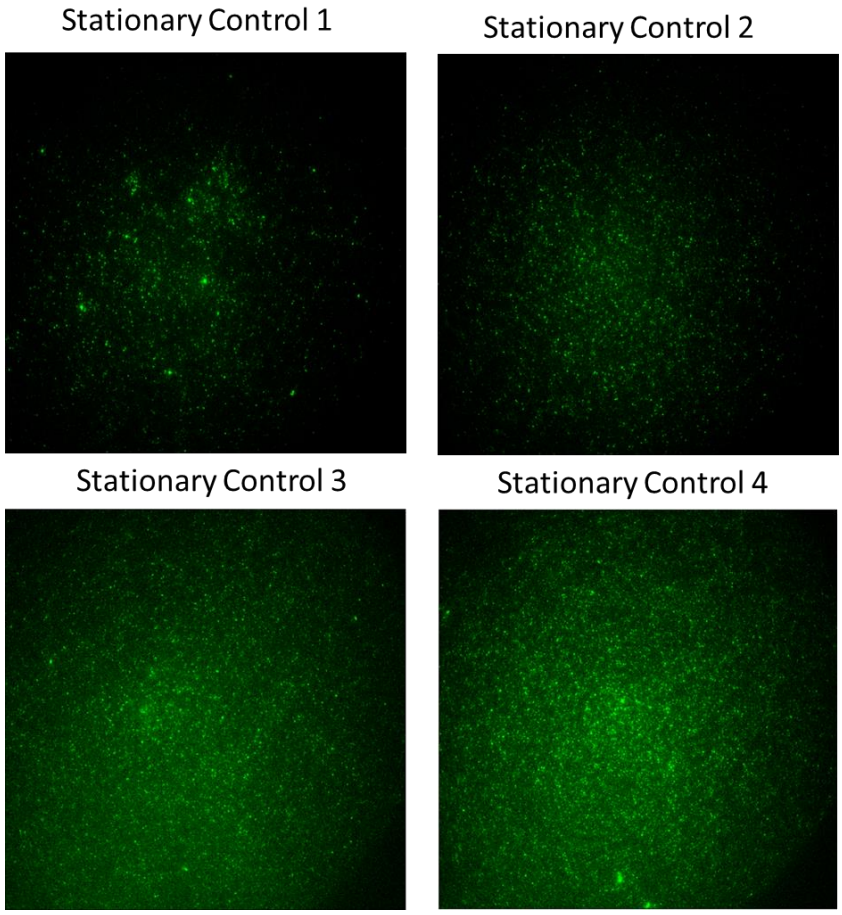


Figure S3.4. Representative single-frame images of each of the stationary control runs of surface-functionalized control probe **S3.3**. Images are 1200 pixels x 1200 pixels (137.5 x 137.5 μm^2).

In order to ensure the single-molecule events seen in Figure S4 were caused by the functionalized **S3.3** and were not caused by contamination of the coverslip, a control batch of coverslips were prepared in parallel simultaneously. These coverslips were prepared with an identical process as detailed above, with the one alternation that they were soaked in a solution of clean, spectroscopic grade CH_2Cl_2 rather than the 5.0 x 10^{-8} M solution of **S3.3**. As seen in Figure S3.5, these blank coverslips prepared similarly do not show the same degree of single molecule

events seen in the functionalized coverslips. In Figure S5, to enable clear comparisons, the brightness and contrast settings were set to: gamma = 0.5, min = 117, max = 151.

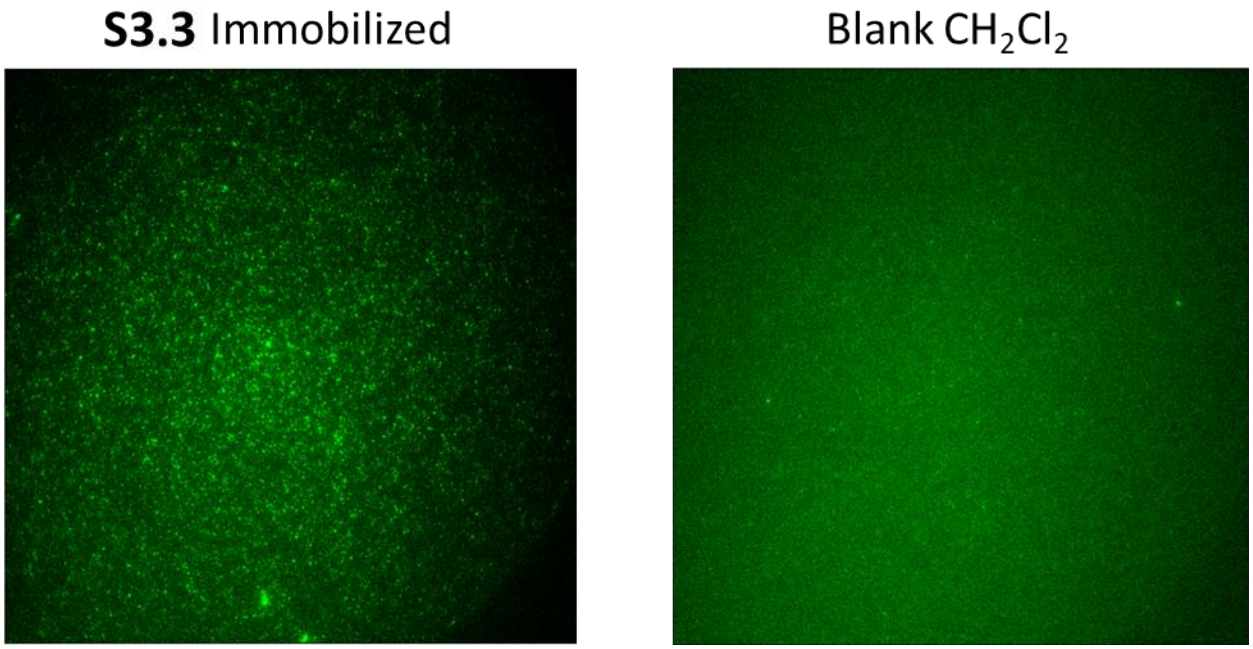


Figure S3.5. Comparison images of the coverslips soaked in the **S3.3** solution (left) and the coverslips soaked in blank CH_2Cl_2 (right). Images are 1200 pixels x 1200 pixels (137.5 x 137.5 μm^2).

VIII. Superlocalization and Identification of Single-Emitter Tracks

Single-molecule activity from the images was quantified using a modified version of Localizer implemented in Igor Pro 8.[7] The resulting TIFF image stacks for each experiment and color channel were exported to Igor Pro 8 for analysis, and the display settings were kept at the default setting. For the processing of all data points, images were not cropped and left at the default 1200 pixel × 1200 pixel array. To identify and analyze the single-molecule events, a series of filters and conditions were applied, as here detailed: Segmentation Algorithm: GLRT; Particle finding: 8-way adjacency; Particle verification: Remove Overlapping Particles and Gaussian Fitting; Standard Deviation of the PSF (pixels): 1.6, and GLRT Insensitivity: 25. To consolidate identical

emitters, the following conditions were applied. Maximum difference in position (in pixels): 5, Maximum allowed gap due to blinking (in frames): 1, Min number of observations for localization error analysis: -1, Max number of observations for localization error analysis: -1.

After the emitters were localized, the emitters were analyzed for motion behavior. Tracks were determined via the following criteria: Maximum distance traveled in a single frame: 5 pixels (570 nm); maximum frames allowed for blinking: 0 frames; Minimum length of track: 4 frames. This method was performed for all replicates with imaging agent **3.1** and for the surface-bound controls with **S3.3** (Table S3.1).

Table S3.1. Summary of Tracks in Experimental and Control Data.

Experiment	Number of Tracks
Replicate 1 with Imaging Agent **3.1**	522
Replicate 2 with Imaging Agent **3.1**	1644
Replicate 3 with Imaging Agent **3.1**	1327
Control 1 with Control Probe **S3.3**	3762
Control 2 with Control Probe **S3.3**	9025
Control 3 with Control Probe **S3.3**	4890
Control 4 with Control Probe **S3.3**	18613

IX. Size of the Polymer Aggregate Particles by GPC

To determine the size of the polymer aggregate particles observed on the microscope, the reaction procedure (Section IV) optimized for the microscope conditions was scaled up 20 times in all factors (i.e., solvent, catalyst, unlabeled norbornene—no fluorophore labeled norbornene was used) with a slightly altered procedure to obtain enough material for processing via gel permeation chromatography (GPC). The modified procedure was as follows:

In two separate 20 mL scintillation vials, 30. mg (0.036 mmol) of Grubbs II catalyst and 12 mg (0.013 mmol) of norbornene monomer were weighed out. Using 10 mL of spectroscopic grade heptane, the norbornene was dissolved and transferred to a third preweighed 20 mL scintillation vial. Another 10 mL of spectroscopic grade heptane was used to suspend the catalyst. The suspended catalyst was transferred into the third vial via a plastic syringe and any unsuspended solids were not transferred. The catalyst and norbornene were allowed to react for 20 min before the mother liquor was removed. The residual polymer particles were washed with spectroscopic grade heptane (5 × 2 mL). The sample was then dried in vacuo and dissolved in THF for GPC analysis.

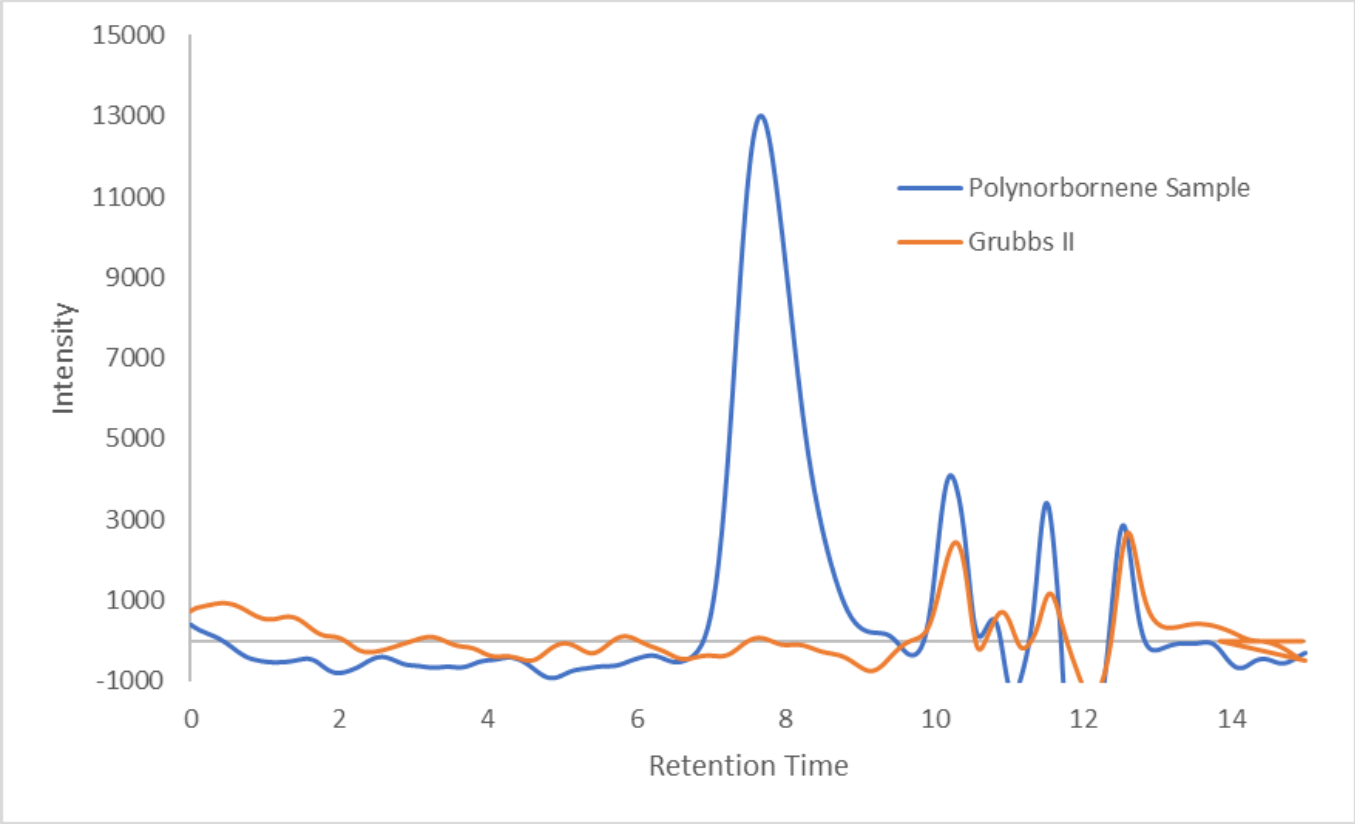


Figure S3.6. GPC trace for the polynorbornene sample (blue) and a blank containing a 1.25 μM solution of Grubbs II catalyst (orange).

The polynorbornene peak at ~7.9 min was identified as having a M_n = 37,000 and a M_w = 71,000 with a polydispersity index of 1.89. As all other major peaks corresponded to the Grubbs II blank, this peak was identified as the majority of polymer within the system.

X. Data Showing that Polymer Aggregate Particles Are Stationary

We considered the hypothesis that the polymer aggregate particles might be moving or "rolling" across the coverslip, adding to the observed motion. In order to assess this possibility, a transmitted light (using a fixed light source flashlight) time-lapse capture of the polymer particles was taken from t = 0 to t = 5 min, in order to determine if whole-aggregate particle motion was present.

To prepare the sample, the polymer particles were precipitated and washed in the same procedure as described previously in Section VI. To this sample was added spectroscopic grade heptane (1 mL) and the sample was placed on the microscope and the microscope was focused. A flashlight held constant at a specific angle was held by a clamp and shined into the sample during the full imaging time. As seen in Figure S3.7, the polymer particles clearly do not move during the 5 min capture. As the timescales of the motion tracking of the individual catalysts are from 4 s (typical) to 60 s (longest), motion of the polymer particle aggregate as a whole does not occur on these time scales and therefore does not contribute to the motion analyzed or the step sizes discussed in the manuscript.

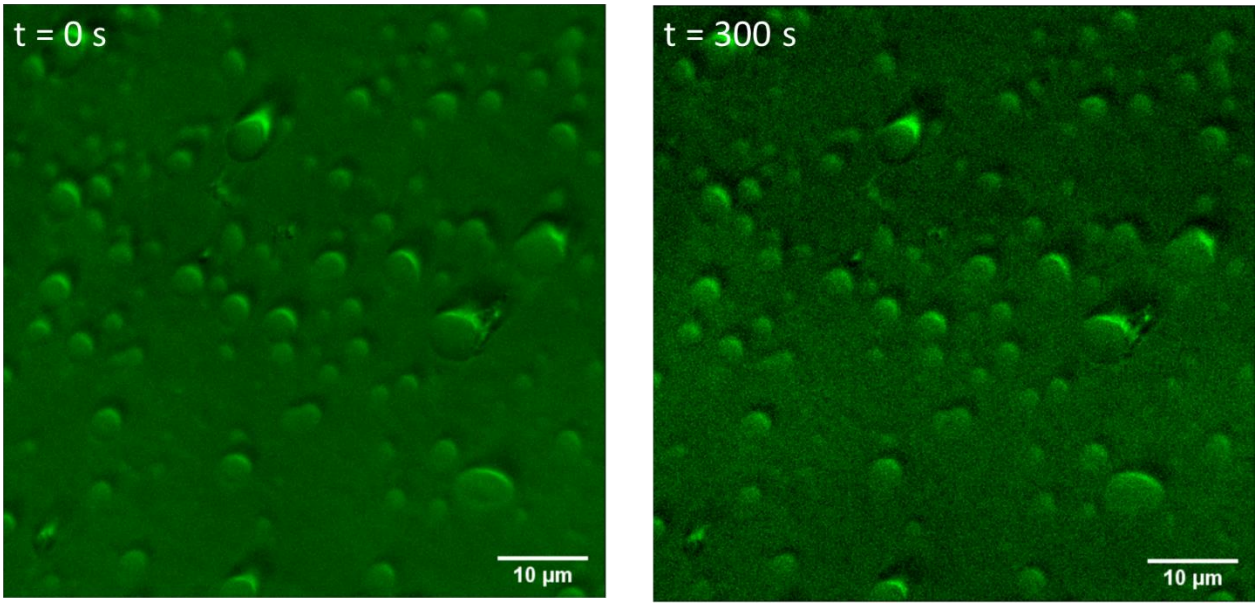


Figure S3.7. Comparison of transmitted light images at t = 0 s and t = 300 s. No motion of the particles is seen during this time.

In Figure S3.7, these images are set to two different brightness-contract settings (right: 581/1497, left: 223/371). Other than this differing brightness–contrast, all of the settings of both the microscope and the software are the same as the procedure discussed previously in Section III.

XI. Limitations of the Method

The analysis method optimized several competing considerations. First: assignment and superlocalization of single chemical reactions. Each potential event was software superlocalized through Gaussian fitting of the point-spread function; this process both removed events that were not clearly single molecules and also removed potential duplicate counting. Further filters were applied to select for the quantized behavior that is a fingerprint of single-molecule events and to limit potential duplicate counting by rejecting a "neighboring" event within 570 nm. Such filtering, however, necessarily rejects a subset of chemical reactions. Second: Adjusting the algorithm to allow for the emitters to blink was tested, but was found to cause tracks to combine in a manner

that was not representative of what was visually observed in the captured images. Therefore, a limitation of the method is that an emitter which blinks or exits for more than a frame and then re-enters the TIRF plane (through movement in *z*) is counted as a different track if it persists for 4 frames after its blink.

XII. Determination of the Positional Accuracy of a Stationary Emitter

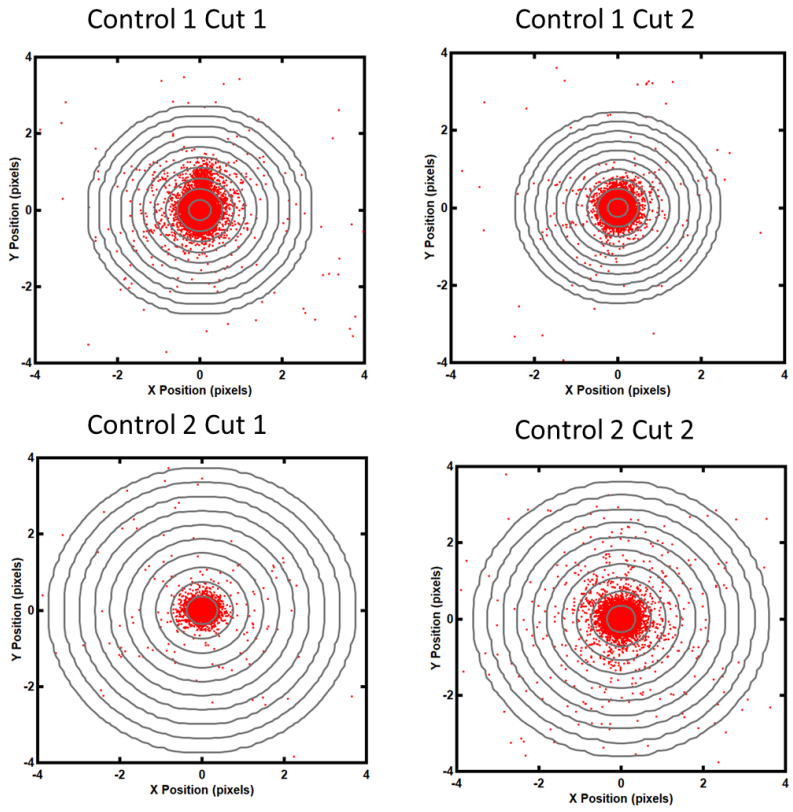
To determine the lower end of our technique's ability to quantify motion, the maximum radius of apparent motion was "traveled" by surface-bound emitters (see section VIIb) was analyzed. While these emitters have no actual motion at all as they are chemically immobilized to the coverslip, the uncertainty of the superlocalization process causes an apparent "motion" of stationary emitters. By analyzing the radius of apparent motion of these controls, the lower limit of the technique to accurately localize positions was quantified.

As previously discussed (see section VIIb), the TIFF stacks were analyzed in 100-second cuts by a modified version of Igor Pro 8 in order to determine the maximum radius of motion. A summary of the 18 different cuts from 4 replicate control experiments are shown below.

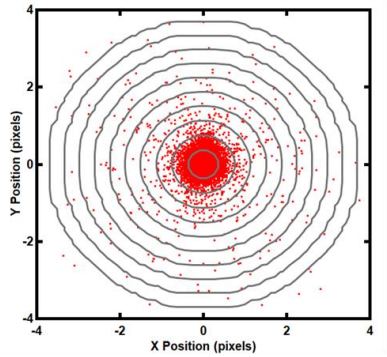
Table S3.2. Summary of the maximum radius of stationary emitters in control experiments.

Experiment	Total Number of Tracks	Standard Deviation from the Center (pixels)	Standard Deviation from the Center (nm)
Control 1 Cut 1	2303	0.27	31
Control 1 Cut 2	1459	0.24	28
Control 2 Cut 1	452	0.37	43
Control 2 Cut 2	1546	0.35	41
Control 2 Cut 3	1694	0.37	42
Control 2 Cut 4	1691	0.45	52

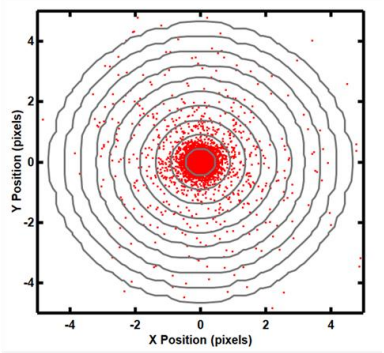
Control 2 Cut 5	1657	0.41	47
Control 2 Cut 6	1985	0.42	49
Control 3 Cut 1	1646	0.25	29
Control 3 Cut 2	1387	0.26	30
Control 3 Cut 3	1857	0.23	26
Control 4 Cut 1	2125	0.23	27
Control 4 Cut 2	2532	0.23	27
Control 4 Cut 3	2737	0.24	28
Control 4 Cut 4	2746	0.23	26
Control 4 Cut 5	2788	0.24	27
Control 4 Cut 6	2536	0.23	26
Control 4 Cut 7	3149	0.23	26



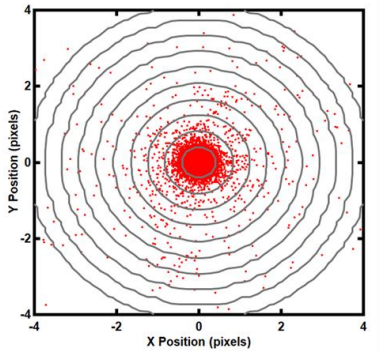
Control 2 Cut 3



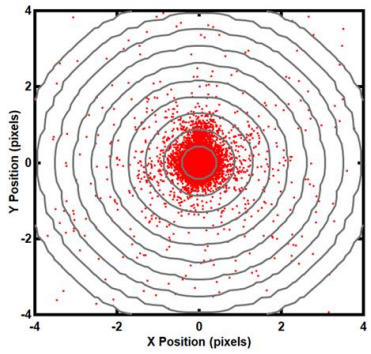
Control 2 Cut 4



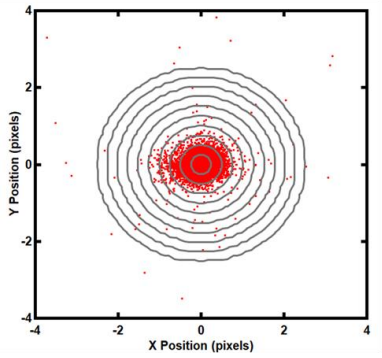
Control 2 Cut 5



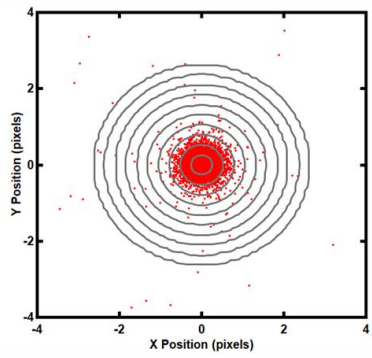
Control 2 Cut 6



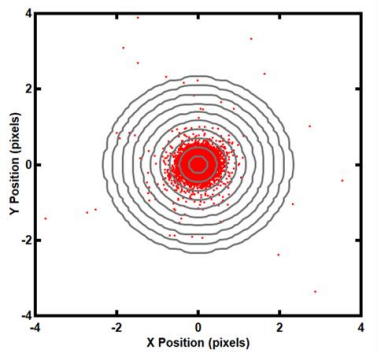
Control 3 Cut 1



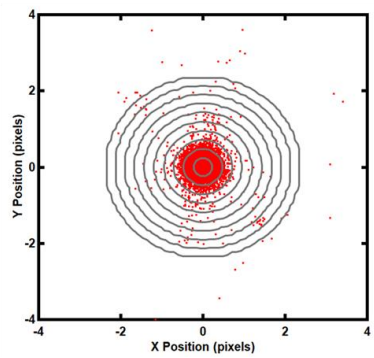
Control 3 Cut 2



Control 3 Cut 3



Control 4 Cut 1



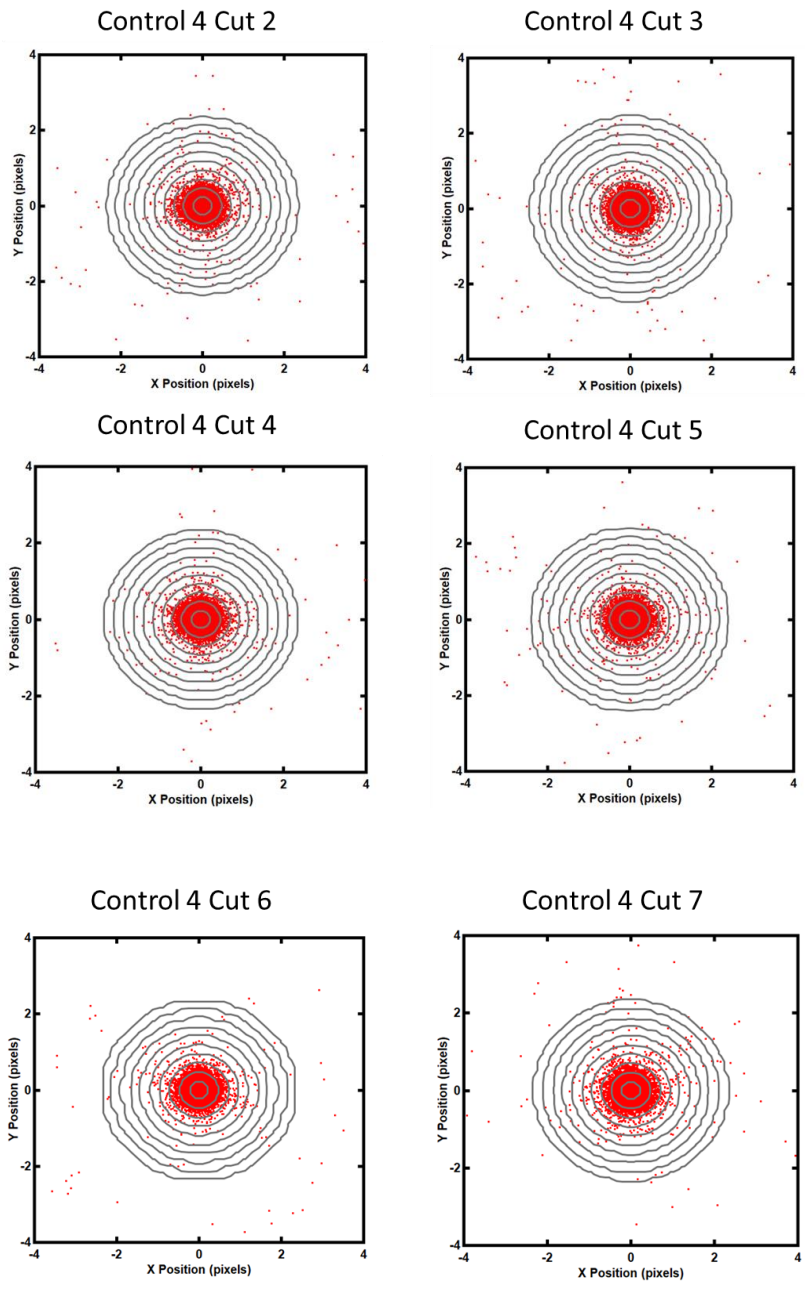


Figure S3.8. Maximum displacement observed by stationary emitters from their position of initial detection. Emitters are represented by red dots, while each degree of standard deviation is represented by a gray ring.

Based on the weighted average of all tracks analyzed in the 4 replicates (36,290 tracks), the perceived motion of a stationary emitter is ±32 nm.

XIII. Discussion of the Prevalence of Blinking within the Tracks

As mentioned previously in Section XI, blinking and diffusion out and in of the imaging plane along the *z* axis in this system may appear similar. For this reason, to evaluate the general prevalence of blinking within this system, a brief analysis was taken of the stationary, immobilized controls, used previously in Section XII. These samples were used as reasonable models for blinking behavior of the BODIPY fluorophore under similar conditions to those of the experiment because they were covalently attached to the coverslip and therefore could not diffuse out of the imaging plane. Thus, blinking could be examined in the absence of convoluting diffusion.

Three cuts of the controls (Control 1 Cut 1, Control 2 Cut 4, and Control 4 Cut 6) were arbitrarily taken and analyzed for the blinking present for the stationary emitters. Using the same parameters for fluorescence microscopy analysis specified in Section VI, the number of individual positions was found for the following two situations: 1) no blinking (each instance of blinking was taken as another individual position), and 2) blinking allowed for 1 s (if a position blinked for 1 s, it was not counted as a separate position). The difference between these amounts of individual positions would give the average blinks per track (or position, in this case, as they are immobilized). Based on this analysis, it was determined that, on average a stationary emitter blinks 1.4 times per track. The data is summarized below in Table S3.3.

Table S3.3. Summary of Blinking Analysis.

Control	Number of Positions (Blinking Not Allowed)	Number of Positions (Blinking Allowed for 1 s)	Number of Blinks per Track
1.1	6486	2989	1.17
2.4	29802	13055	1.28

4.6	49534	19378	1.56
Weighted Average	85822	35422	1.43

Although the amount of blinking indicated from this brief analysis of the stationary controls indicates that it might have a significant presence in the system, it does not have an impact on the conclusions in the manuscript: Because both the MSD curves and step size are analyzed as both time- and track-independent, a track that blinks but is analyzed as a single track and a track that blinks but is analyzed as two separate tracks undergo the same analysis that is not weighted by the time or length of track. Therefore, although blinking occurs in this system, it does not impact the presented conclusions.

XIV. Determination of Step Sizes from Track Data

We considered analyzing step sizes as an intuitive way of looking at motion of individual catalysts. A step size is defined as the distance between two consecutive superlocalized positions of the same catalyst. In order to obtain the step size of individual tracks, the distance between two superlocalized positions was calculated through the following equation: $d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$. The displacements between two points (step sizes) were considered regardless of direction (i.e., "backtracking" or moving back towards the original localized position is allowed and accounted for). These step sizes were calculated in a similar manner for both the surface-bound controls and the triplicate experimental runs, and the data is summarized in the table below. The individual step sizes were then binned into 10 nm bins (0 – 570 nm) for further analysis.

Table S3.4. Summary of Step Sizes for Controls and Experimental Runs

Experiment	Number of Steps	Average Step Size (nm)
Replicate 1	2774	80.
Replicate 2	8920	73
Replicate 3	6933	69
Control 1	24478	35
Control 2	54040	40.
Control 3	30313	40.
Control 4	127830	29

The weighted average step size for an emitter in the polymerization experiments was 73 nm and the weighted average apparent "step size" of a stationary emitter was 41 nm.

XV. Analysis of Step Size vs. Time, Three Analyses

a. Analysis of Average Step Size vs. Reaction Time. To analyze the hypothesis of whether the strands were settling after agitation, the step sizes were averaged by the time at which the step occurred and plotted against the overall time of the reaction (Figure S3.9). No correlation was observed. The number of steps at each time point remained relatively constant, indicating consistent incorporation throughout the full imaging time (Figure S3.9).

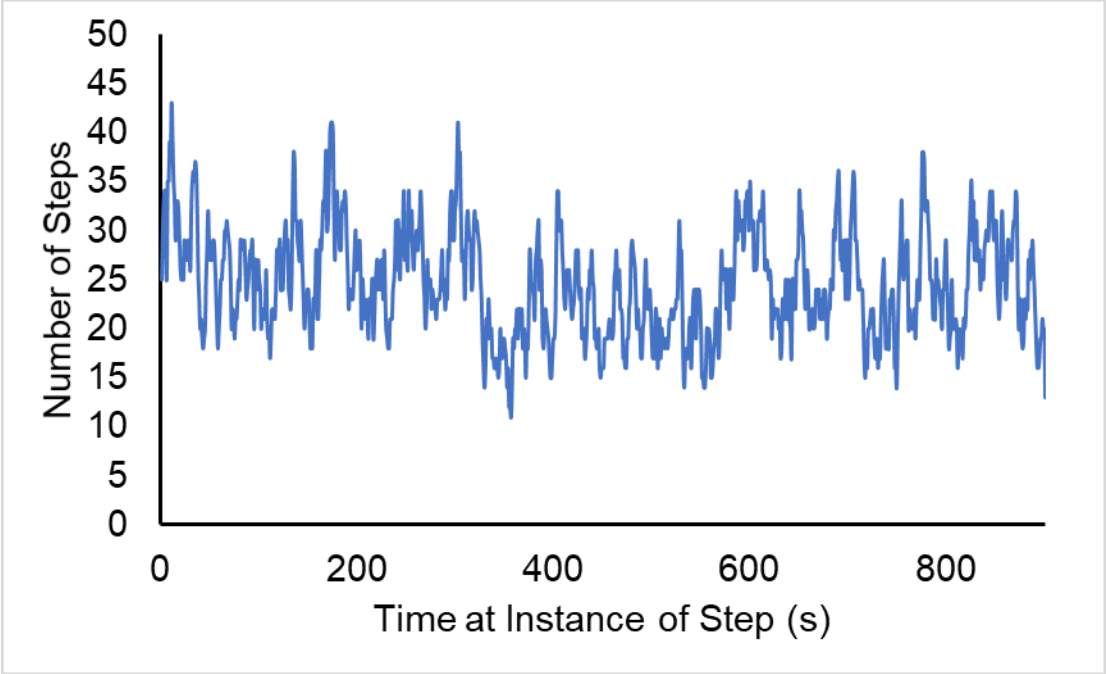


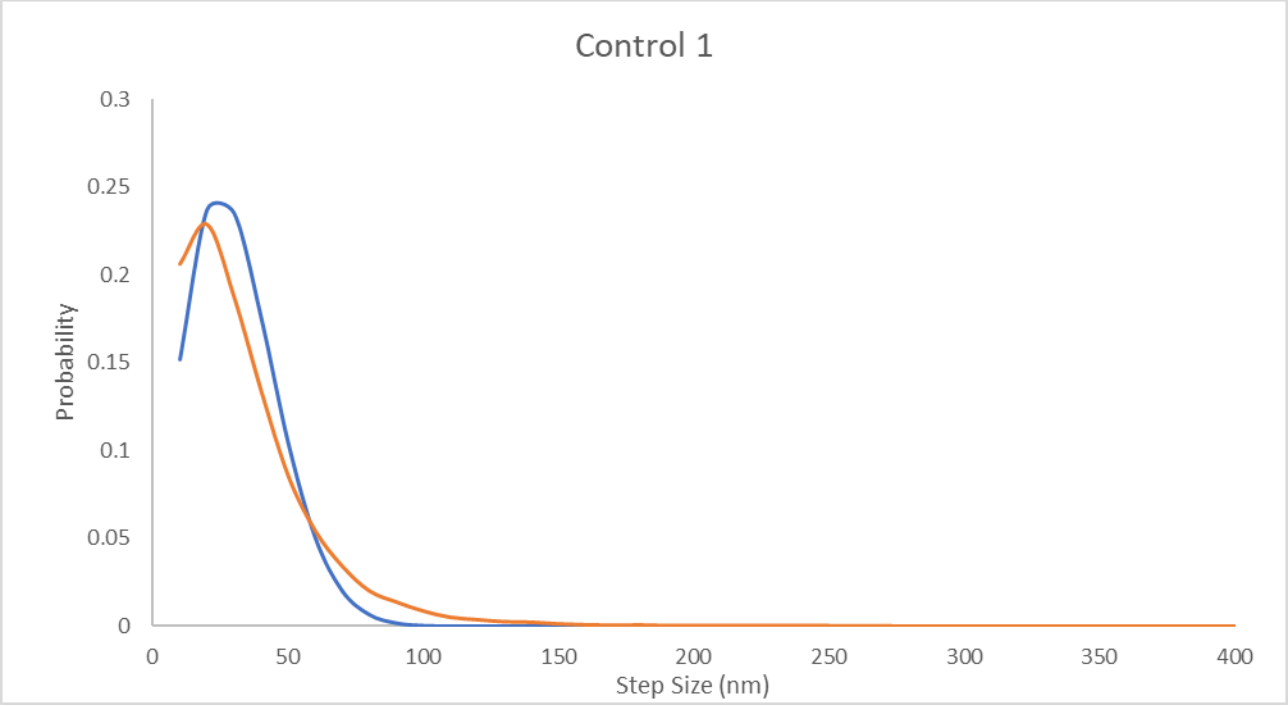
Figure S3.9. The number of steps plotted against the time at which the step was counted, showing a lack of significant correlation. (This graph displays the same data as is in Figure 3.3 in the main text of the chapter, but now displayed with a line instead of points.)

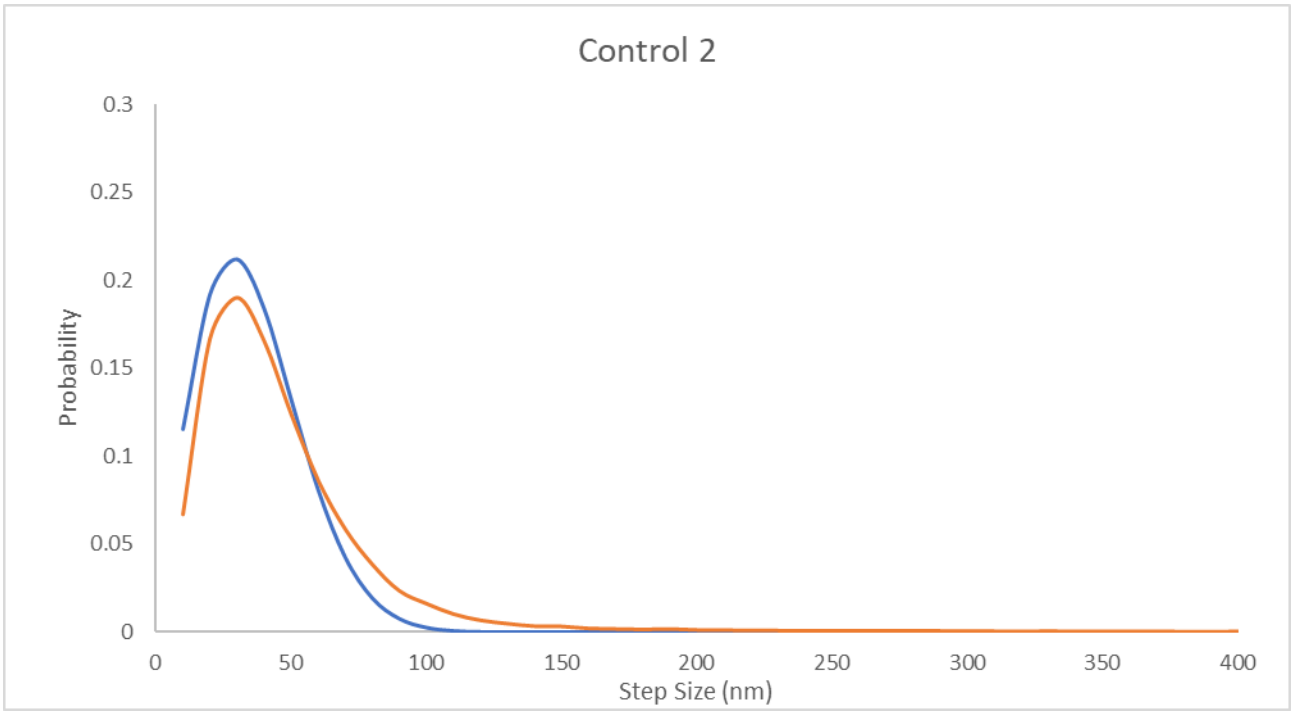
b. Analysis of Average Step Size at Each Time, Trend via Linear Regression for Each Catalyst. To evaluate the hypothesis that the catalysts "slow down" after reaction, a linear regression was placed on each track in order to see if a majority of the tracks had a negative correlation; 2) the difference between the first and final step was taken to see if a majority of the tracks ended with smaller steps sizes at the end of the track than the beginning; and 3) the difference between the first and third step (which all tracks analyzed have, as the minimum track length is 4 frames) to see if the correlation existed regardless of sample variation.

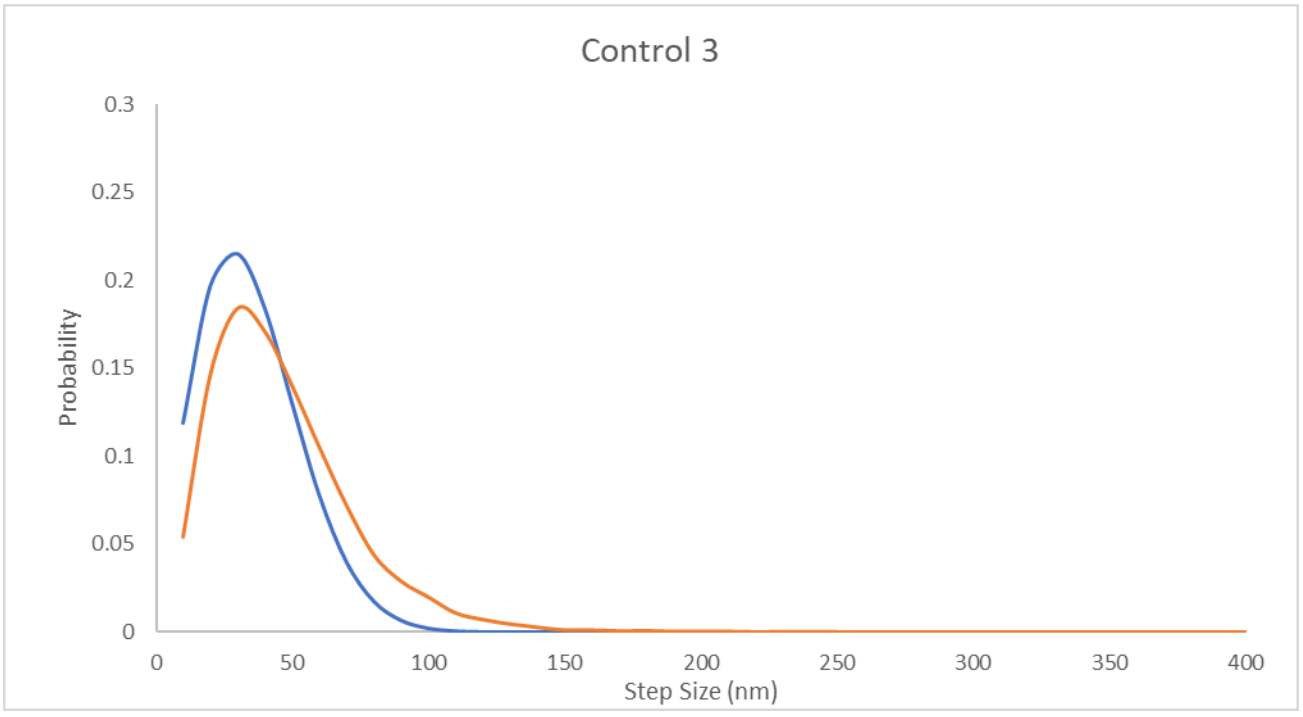
The results indicated that catalysts did not slow down after reaction: 1) 50.4% of the tracks had a negative regression; 2) 50.6% of the tracks had a final step smaller than the first; and 3) 51.8% of the tracks had a third step smaller than the first. As all of these numbers hover around 50%, these results were considered non-significantly different from 50%.

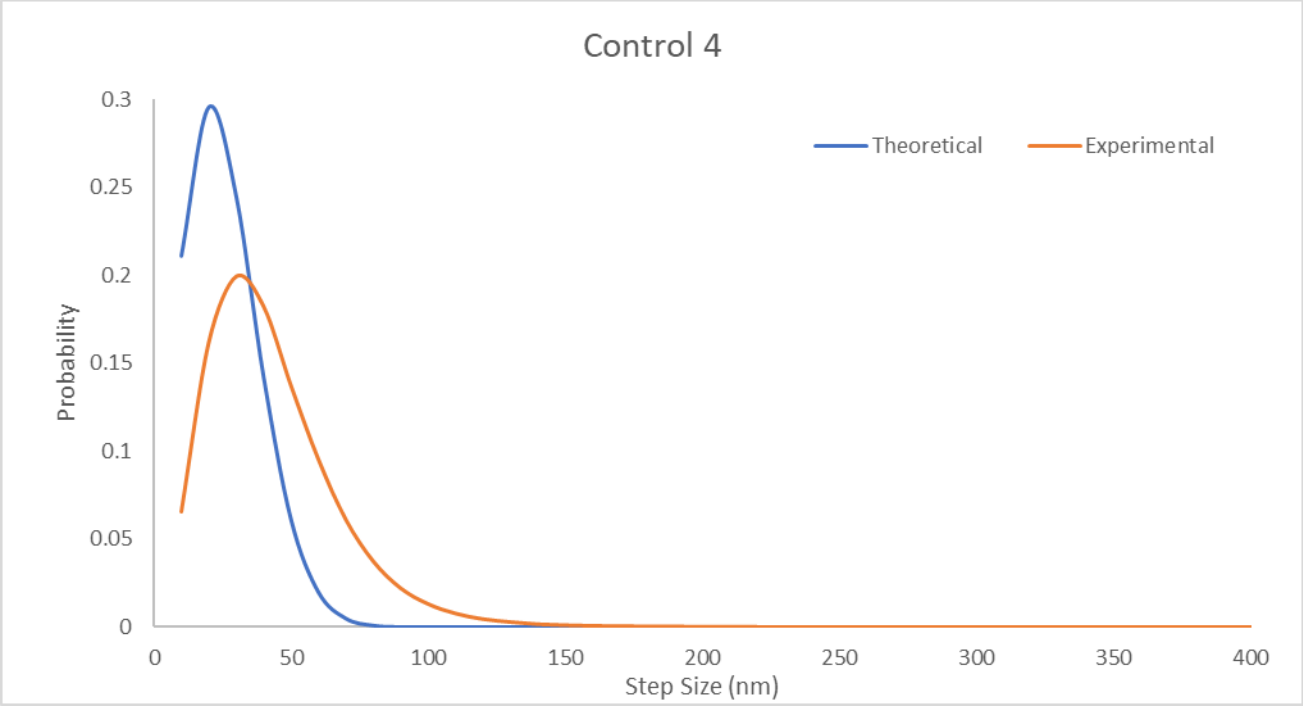
XVI. Construction of Control 2-D Random Walk Distribution

The quasi-stationary distribution was based on the stationary emitters present in the controls. As seen in Figure S3.10, theoretical distribution was created for each of the controls based off of 2-D random walk model, whose equation is as follows: $P(r) = \frac{1}{\pi\langle r^2\rangle} e^{\left(\frac{-r^2}{\langle r^2\rangle}\right)} 2\pi r$.[8] In order to account for the entire probability shown by the experimental data, the 2-D random walk model was scaled by 10.




Control 2
Probability
0.3
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
300
350
400
Step Size (nm)


Control 3
Probability
0.3
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
300
350
400
Step Size (nm)



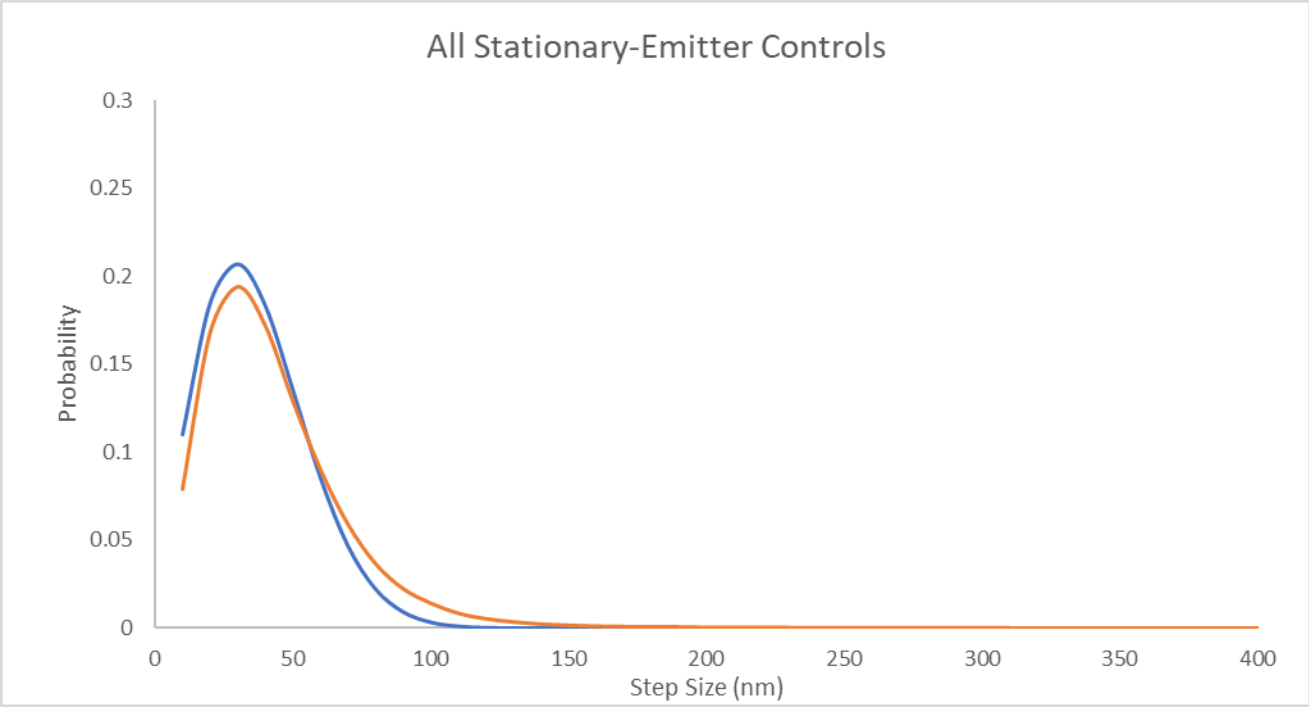


Figure S3.10. Probability graphs for each of the stationary-emitter control runs (1-4) and all stationary controls, showing the experimental probability in blue and the 2-D random walk model shown in orange.

XVII. Construction of the 3-Step Model Distribution

With the quasi-stationary distribution determined, the remaining probability of the experimental data was seen to not closely fit a single "moving" distribution, as seen in Figure S3.11. When fit to a single distribution, the fit does not accurately represent the experimental data. With this in mind, we decided to consider the non-stationary experimental data as likely containing multiple distributions, consisting of at least 2 distributions.

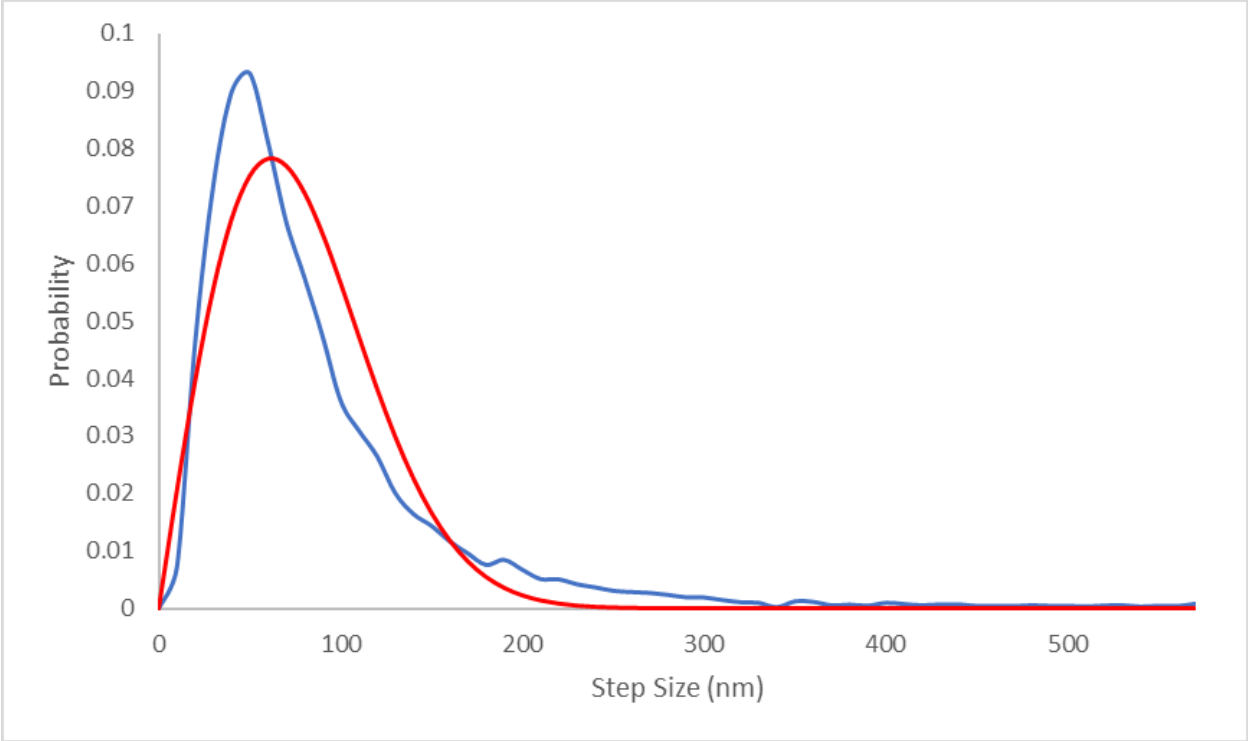


Figure S3.11. Comparison of the experimental probability with a 20% probability of stationary emitters removed (shown in blue) and the single 2-D random walk model for the remaining experimental distribution (shown in red). This model of one distribution does not accurately represent the data.

a. Fitting Intermediate Step Sizes. Multiple different 2-D random walk models were tested in order to determine a distribution of the remaining moving emitters. The control distribution, scaled for this preliminary testing at 20% of all emitters, was removed from the experimental probability

for testing of the different curves. Two-dimensional random walk distributions based on a step size from 65 to 80 nm were tested against this experimental probability (Figure S3.12) in order to determine the best fit for the intermediate-step distribution. Ultimately, the 2-D random walk model with a step size of 65 nm (shown in red in Figure S3.12) was determined to be the best fit.

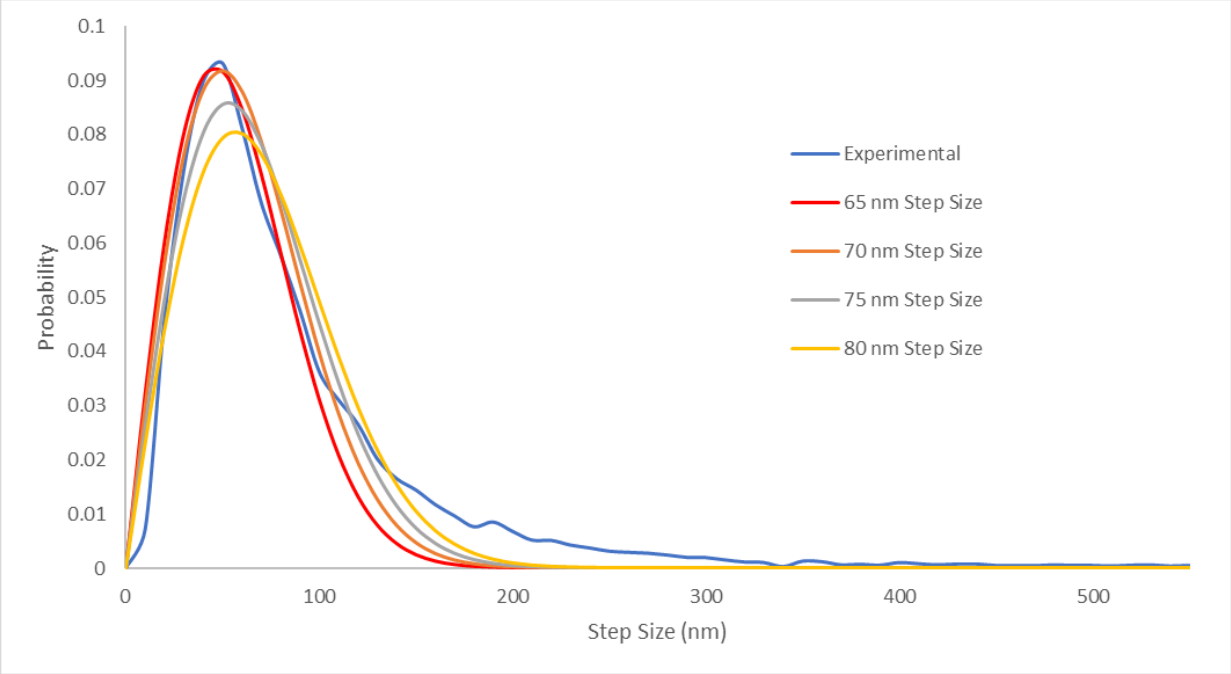


Figure S3.12. Comparison of different 2-D random walk models to the experimental probability with the quasi-stationary step sizes (set at 20%) excluded from analysis. In this graph, the quasi-stationary distribution has been already subtracted from the experimental probability to aid in fitting the second distribution.

b. Fitting Large Step Sizes. A certain amount of larger step sizes was excluded so far from the fit and we next worked on fitting a third distribution to account for the remaining experimental data. In a manner similar to fitting the second random walk model, the quasi-stationary distribution (set to 20% of the full probability for this preliminary analysis) and the intermediate-step distribution (set to 55% of the full probability for this preliminary analysis) was removed from the full experimental probability. Due to the preliminary, unoptimized scaling of the quasi-stationary

and the intermediate-step distributions, the remaining experimental probability has a more jagged appearance in Figure S3.13. Two-dimensional random walk distributions based on a step size from 115 to 180 nm were tested against this experimental probability (Figure S3.13) to determine the best fit for the large-step distribution. Ultimately, the 2-D random walk model with a step size of 145 nm (shown in red in Figure S3.13) was determined to be the best fit.

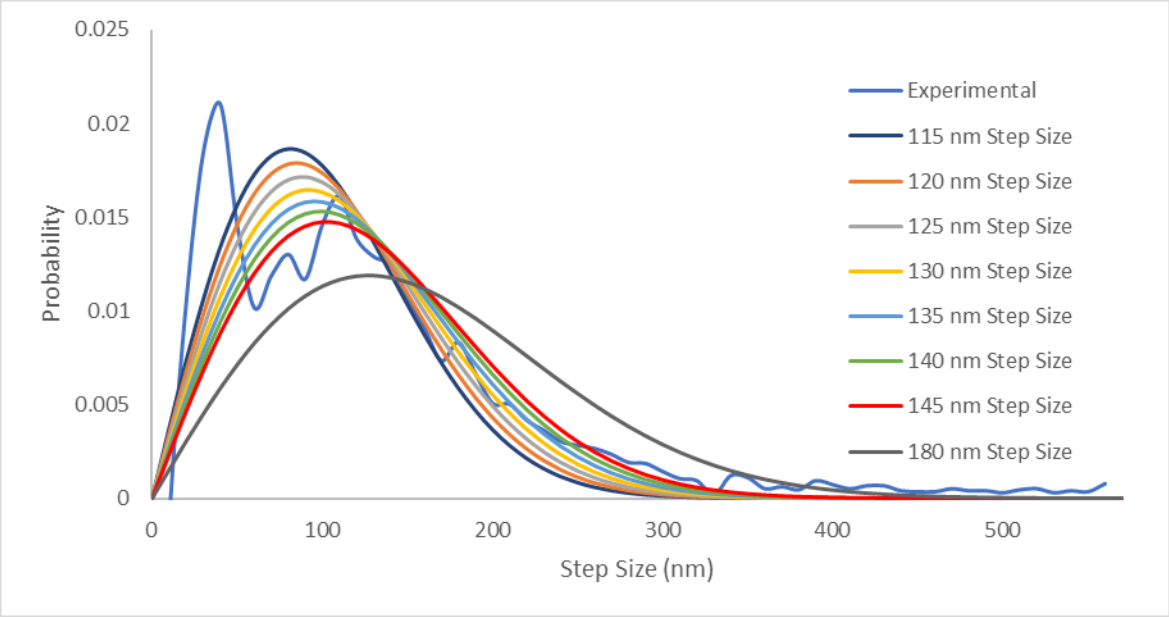


Figure S3.13. Comparison of different 2-D random walk models to the experimental probability with the quasi-stationary step sizes (set at 20%) and the intermediate-step step sizes (set at 55%) excluded from analysis.

With the 3 different approximate distributions accounting for the full experimental distribution, the Solver program in Excel was employed in order to more accurately scale the probabilities (initially set at 20%, 55%, and 25% for the quasi-stationary, intermediate-step, and large-step distributions, respectively) via reducing the residual between the full experimental probability and the theoretical distribution. The sum of model distribution with 23% quasi-stationary steps, 53% intermediate steps, and 24% large steps afforded the best fit. This scaling was used moving forward for analysis.

XVIII. Diffusion Coefficients Determined by Different Methods

The diffusion coefficient listed in the main text of the paper was calculated under the assumption of subdiffusion. For comparison, the diffusion constant calculated under the assumption of free diffusion was significantly smaller (D = 5.3 x 10^{-16} m^2/s).

This determination of the subdiffusion regime is further shown by the similarity of the two diffusion constants measured by two different methods: the one listed in the manuscript body, calculated by direct MSD analysis assuming subdiffusion, and the one listed below, calculated by the variance of the modified random walk distribution shown in Figure 3.3j and detailed above in Section XVII.

The random walk diffusion constant was calculated from the weighted average of diffusion constants from the variances of the three distributions (quasi-stationary, intermediate step, and large step). The diffusion constant was calculated via the random walk variance: $D = \frac{\sigma^2}{4\delta t}$ where σ is the variance of the random walk (the average step size) and δt is the time between each step. The dimensionality is 4 as the imaging and analysis of the motion of the catalysts is in 2 dimensions, although they are actually moving in 3. The diffusion constant of the quasi-stationary random walk distribution calculated in this way is D = 2.6 x 10^{-16} m^2/s. The diffusion constant of the intermediate-step random walk distribution calculated in this way is D = 1.1 x 10^{-15} m^2/s. The diffusion constant of the large-step random walk distribution calculated in this way is D = 5.3 x 10^{-15} m^2/s. The diffusion constant of the weighted sum of the model random walk distributions calculated is D = 1.9 x 10^{-15} m^2/s.

The diffusion constant calculated via the random walk model is lower than the one calculated via the direct MSDs, which lends credence to the conclusion that the active catalyst motion that

occurs in these polymer aggregate particles is operating in a subdiffusion regime, consistent with limitations caused by aggregate size.

XIX. Selection and Analysis of Individual Particles

The polymerization experiment, detailed in section VI, resulted in data output in the form of one TIFF image stack for each experimental run. Each TIFF file was exported to ImageJ.

Regions where single-molecule events were centralized on single-polymer particle aggregates were identified by eye. To help search for single-polymer particles that were not overlapping, time composite images were created from the TIFF image stack. The TIFF image stacks were cropped around particles for superresolution analysis in Igor Pro 8. For the analysis of each particle, the following filters and conditions were applied; Segmentation Algorithm: GLRT, Particle finding: 8-way adjacency, Particle verification: Remove Overlapping Particles and Gaussian Fitting, Standard Deviation of the PSF (pixels): 1.6, and GLRT Insensitivity: 25. To consolidate identical emitters, the following conditions were applied; Maximum difference in position (in pixels): 5, Maximum allowed gap due to blinking (in frames): 1, Min number of observations for localization error analysis: -1, Max number of observations for localization error analysis: -1. . Tracks were determined via the following criteria: Maximum distance traveled in a single frame: 5 pixels (570 nm); maximum frames allowed for blinking: 0 frames; Minimum length of track: 4 frames. Particle were selected for analysis by the following criteria: the particles were not overlapping with another polymer particle, the particles were not located within 20 microns of the edges of the imaging area, and the particles contained at least 15 tracks lasting at least 4 seconds. The 20 particles that fit these criteria were taken for analysis. The individual particles were analyzed for step sizes as discussed previously in section XI.

XX. Size of the Polymer Aggregate Particles by Fluorescence Microscopy

To determine the size of the polymer aggregate particles present during imaging, the diameter of the particles was estimated through the use of the total positions of the superlocalized fluorescent signals of the active catalysts. The particles' diameter was estimated with the following assumptions: a) the particles did not move during the capture (as confirmed by transmitted light microscopy, see Section X for more details), b) the edges of the particle can be reasonably estimated by the bounds of their fluorescent signals, and c) the size of the particle can be reasonably estimated by an ellipse of its furthest fluorescent signals in both x and y. The fluorescent signals were localized in Localizer with the same conditions as listed previously in Section VIII. The approximated sizes of the particles are listed in the table below.

Table S3.5. Approximated sizes of the polymer aggregate particles.

Particle	Diameter in x (μm)	Diameter in y (μm)	Approximated Size (μm^2)
P1	2.21	2.01	3.66
P2	1.96	2.61	4.01
P3	4.57	3.55	12.75
P4	1.66	2.20	2.86
P5	5.05	3.23	12.79
P6	3.50	3.66	10.07
P7	2.40	2.43	4.59
P8	2.72	2.86	6.10
P9	1.95	2.03	3.12
P10	1.31	1.26	1.29
P11	1.65	2.69	3.48
P12	3.84	4.52	13.62

P13	6.36	5.08	25.38
P14	2.27	2.57	4.59
P15	3.56	2.78	7.78
P16	1.96	3.06	4.66
P17	3.39	4.45	11.83
P18	2.55	3.56	7.12
P19	5.69	5.15	23.01
P20	3.52	2.19	6.02

XXI. Correlations between Particle Size and Alternative Variables

a. No Correlation between Average Step Size and Particle Size. To determine if particle size (area) correlated with average step size within a particle, the size of each particle (determined via the approximation in Section XX) was plotted against the average step size of the particles. No significant correlation was present (Figure S3.14).

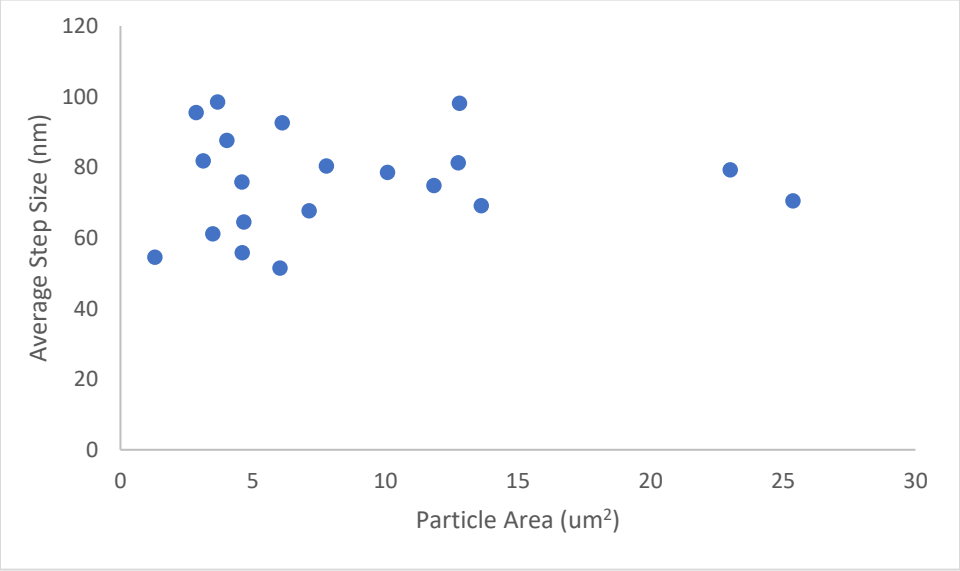


Figure S3.14. Size of each particle plotted against the average step size in that particle.

b. No Correlation between Percentage of Quasi-Stationary Steps and Particle Size. To determine if particle size correlated with percentage of quasi-stationary steps within a particle, the size of each particle (determined via the approximation in Section XX) was plotted against the percentage of quasi-stationary steps present within the particles. A quasi-stationary step is defined, in this analysis, as a step under 30 nm. No significant correlation was present (Figure S3.15).

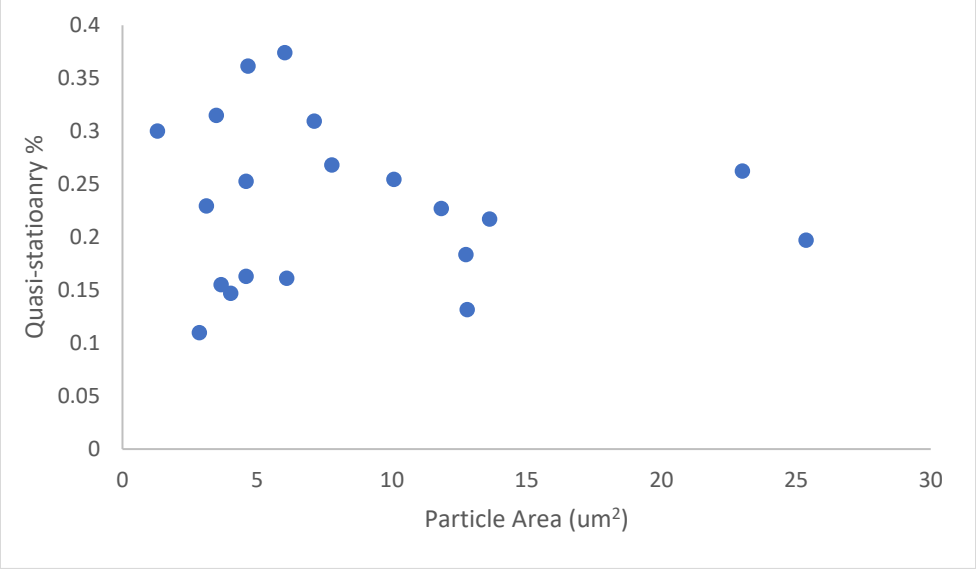


Figure S3.15. Size of each particle plotted against the percentage of quasi-stationary steps in that particle.

c. No Correlation between Percentage of Intermediate Steps and Particle Size. To determine if particle size correlated with percentage of intermediate steps within a particle, the size of each particle (determined via the approximation in Section XX) was plotted against the percentage of intermediate steps present within the particles. An intermediate step is defined, in this analysis, as a step under 100 nm. No significant correlation was present (Figure S3.16).

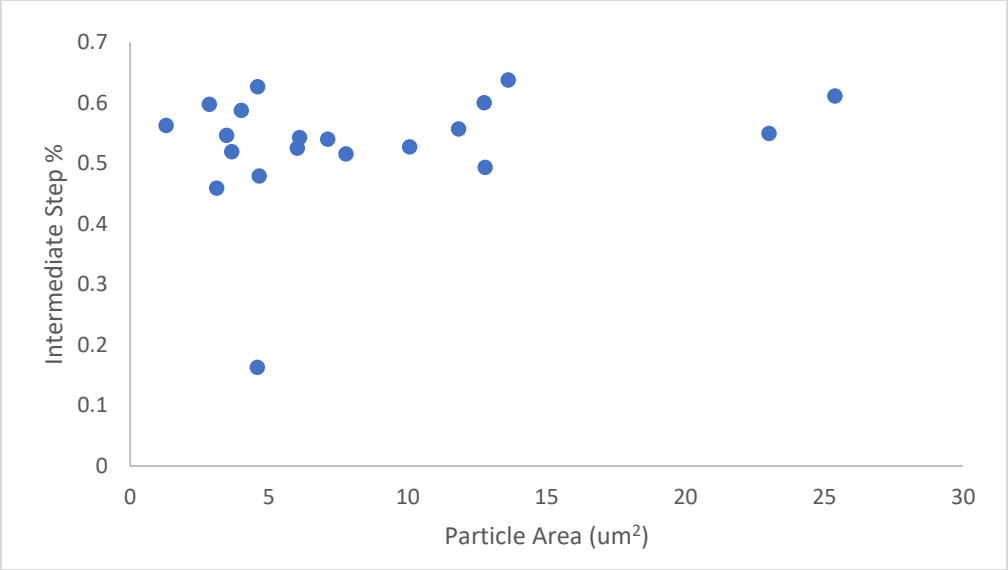


Figure S3.16. Size of each particle plotted against the percentage of intermediate steps in that particle.

d. No Correlation between Percentage of Large Steps and Particle Size. To determine if particle size correlated with percentage of large steps within a particle, the size of each particle (determined via the approximation in Section XX) was plotted against the percentage of large steps present within the particles. A large step is defined, in this analysis, as a step greater than 100 nm. No significant correlation was present (Figure S3.17).

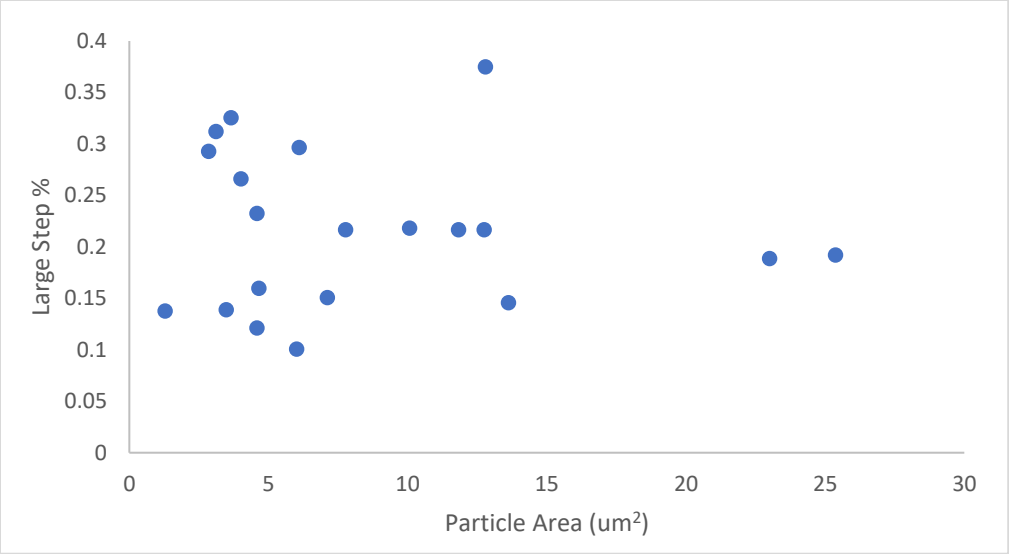


Figure S3.17. Size of each particle plotted against the percentage of large steps in that particle.

e. No Correlation between Residual from Full-Run Experimental Distribution and Particle Size. To determine if particle size correlated with the particle's deviation from the sum of the model distributions (see Section XVII) within a particle, the size of each particle (determined via the approximation in Section XX) was plotted against the residual between the particle's experimental distribution of step sizes and the normalized sum of the model distributions. A stationary step is defined, in this analysis, as a step under 30 nm. No significant correlation was present (Figure S3.18).

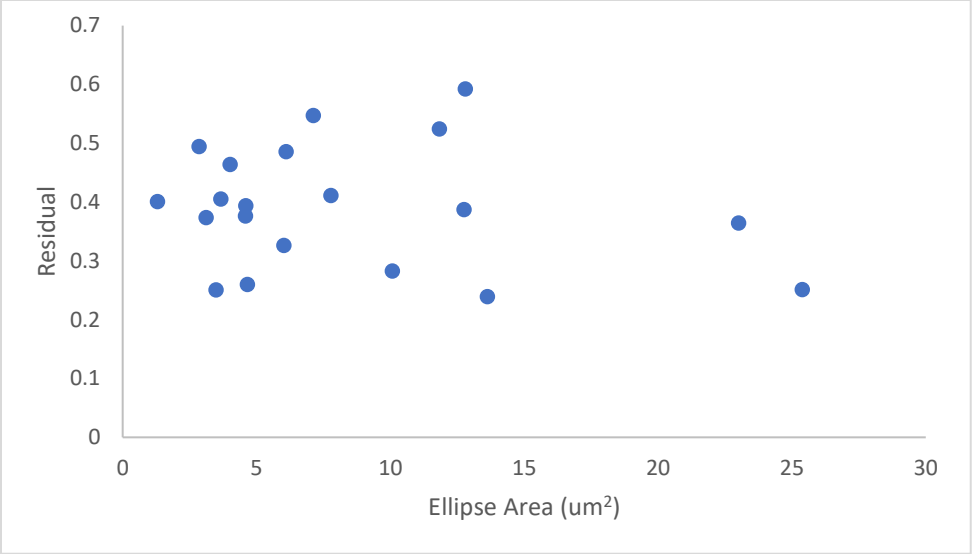


Figure S3.18. Size of each particle plotted against the residual between the experimental distribution in that particle and the normalized sum of the model distributions.

f. Positive Correlation between Particle Size and Number of Tracks. To determine if the size of the particle correlated with activity of the particles, the size of each particle was plotted against the number of tracks present within the particles. A loose positive correlation was present (Figure S3.19). This is expected due to the increased size of the aggregate particles having a higher number of strands and catalysts, and therefore higher likelihood of incorporating tagged monomer.

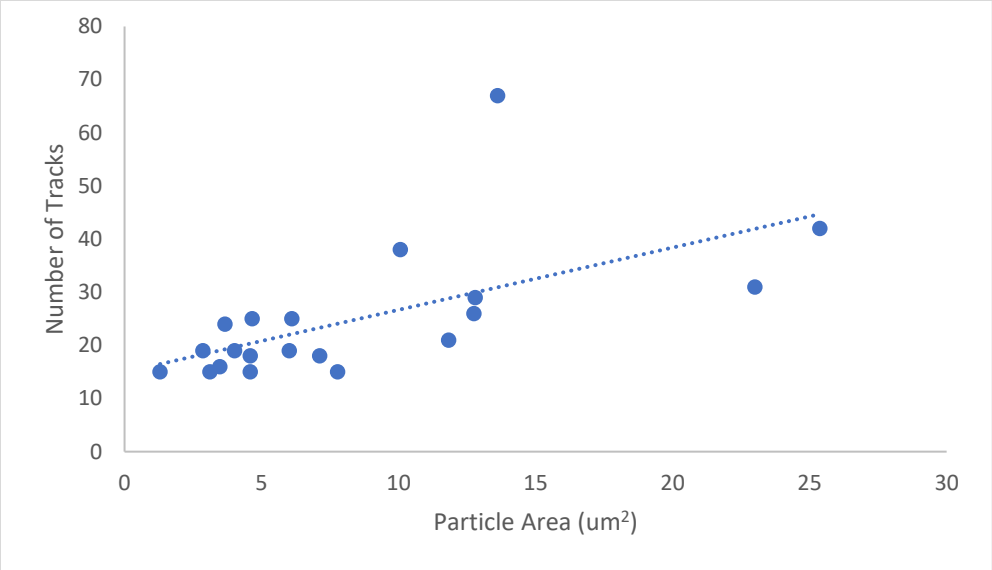


Figure S3.19. Size of each particle plotted against the number of tracks in that particle.

XXII. Correlations between Step Size and Alternative Variables

a. No Correlation between Step Size and Number of Events. To determine if step size correlated with activity of the particles, the average step size of each particle was plotted against the number of tracks present within the particles. No significant correlation was present (Figure S3.20).

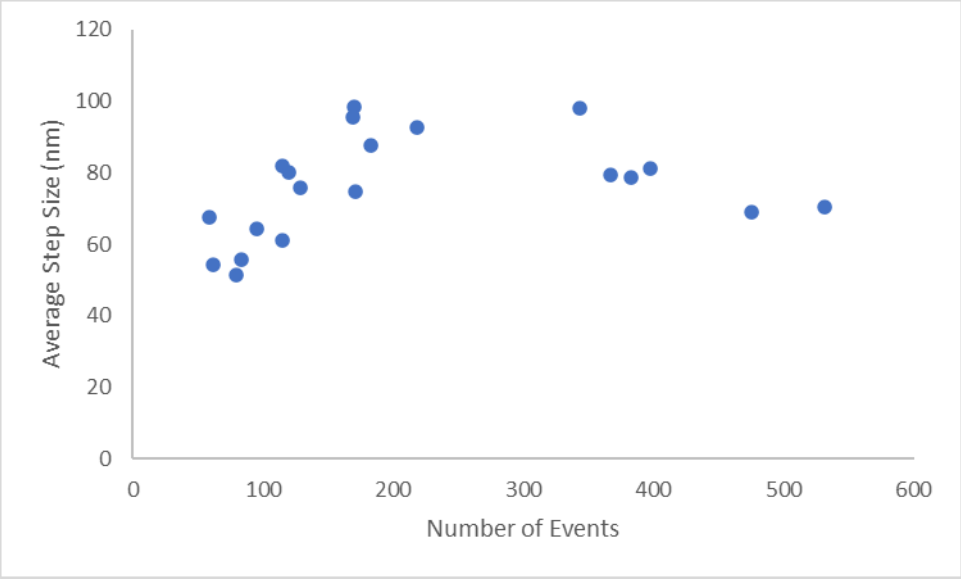


Figure S3.20. Number of events in each particle plotted against the average step size in that particle.

b. No Correlation between Step Size and Number of Tracks. To determine if particles that contained a larger sample size (i.e., contained a larger number of tracks) correlated with step size within that particle, average step size of each particle was plotted against the number of tracks present within the particles. No significant correlation was present (Figure S3.21).

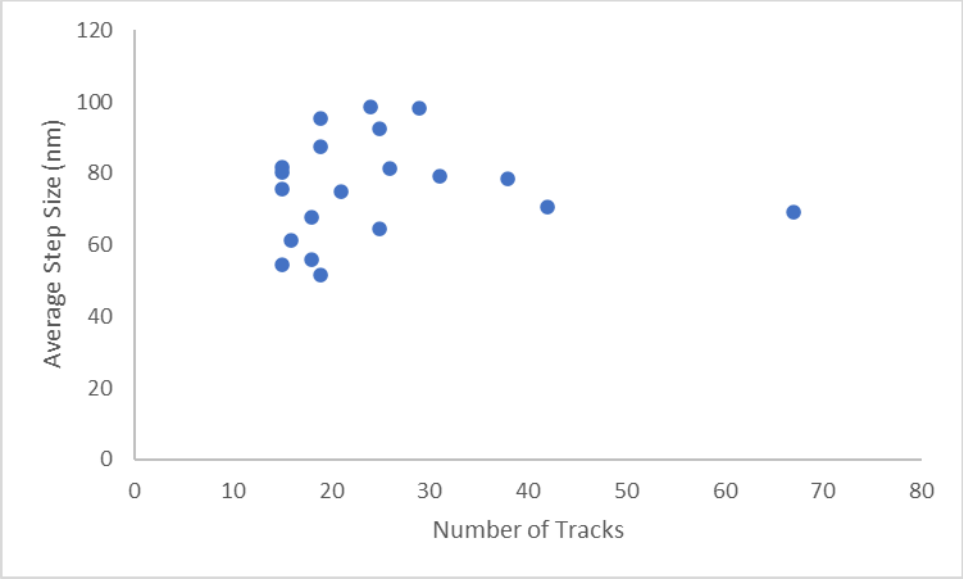


Figure S3.21. Number of tracks in each particle plotted against the average step size in that particle.

XXIII. Analysis of Distribution Differences of Individual Particles

a. Chi-Square Goodness-of-Fit Test. The particles cropped from the data appeared to show a different distribution of step sizes than the entire data set, indicating that particle-to-particle differences might be present within the sample. To determine if the particles fit the sum of the 3-step theoretical distribution (Section XVII) with 95% statistical confidence, a Chi-Square test was performed for each particle. The following equation was used: $X^2 = \sum_{i=1}^{k} \frac{(x_i - m_i)^2}{m_i}$. The theoretical distribution discussed in Section XVII was scaled to the total number of events present in each particle for accurate analysis. The critical value for 56 degrees of freedom (which was used for all particle analysis) is 74.468 and was used for determination of statistical difference with 95% confidence. In order to ensure the outliers past a step size of 400 nm were not overly considered during statistical analysis, a second chi-square goodness-of-fit test was performed without the steps larger than 400 nm. The critical value for 39 degrees of freedom, used for this second set of tests,

is 54.572 and was used for determination of statistical difference with 95% confidence. If a particle showed statistical difference for both of these tests, the particle was considered to have statistically significant differences from the theoretical distribution of the full experiments and therefore exhibiting a different distribution of motions from the full experiment. A summary of the test statistics for each particle and whether they were deemed statistically different from the full experiment theoretical distribution is shown below in Table S3.6.

Table S3.6. Summary of the Chi-Square Test Statistics for All Particles.

Particle	Test Statistic (0-570 nm)	Test Statistic (0-400 nm)	Statistically Different?
P1	321367	93	Yes
P2	12665	84	Yes
P3	7644	64	Yes
P4	525	525	Yes
P5	3852	120	Yes
P6	40206	100.	Yes
P7	122	123	Yes
P8	117065	309	Yes
P9	95	95	Yes
P10	20.	20.	No
P11	43	43	No
P12	9048	92	Yes
P13	442	55	Yes
P14	32	32	No
P15	6987	86	Yes
P16	372	371	Yes
P17	54194	43	No
P18	26200.	88	Yes

P19	185157	67	Yes
P20	37	35	No

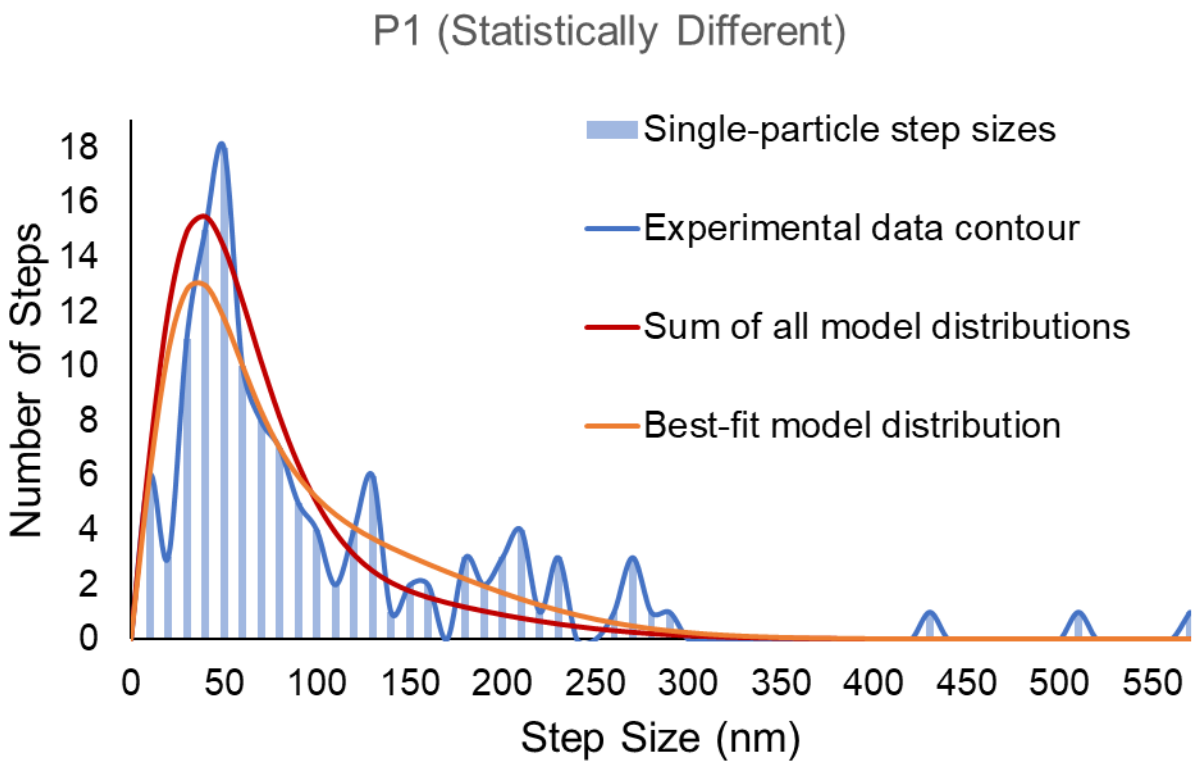
b. Best-fit Curves. For the 15 particles that were statistically different from the sum of the theoretical distributions, residual analysis was used to discover the scaling of the three distributions (see Section XVII) which best described each individual particle. A Solver program was used in Excel (10 iterations) in order to determine the amount of contribution of each distribution towards the particle's best fit. If a particle's best-fit theoretical distributions differed from the full experimental theoretical distribution by a probability of 0.1 (10%) or more, that change was considered to be noteworthy for Figure 3.3k in the manuscript. A summary of the scaling parameters of the quasi-stationary, intermediate-step, and large-step distributions for the best-fit curves for all the statistically different particles in shown in Table S3.7. Distributions which differ from the full-experiment theoretical distribution by 10% or more are highlighted yellow.

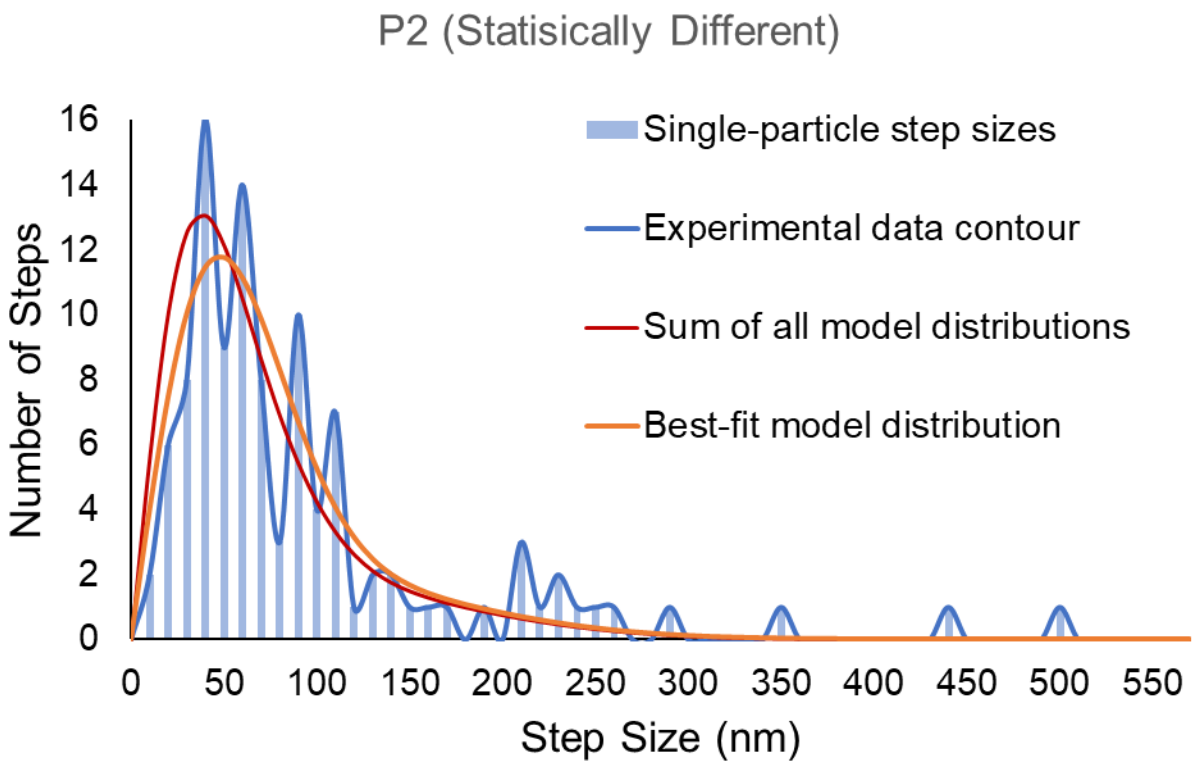
Table S3.7. Scaling parameters for each statistically different particle.

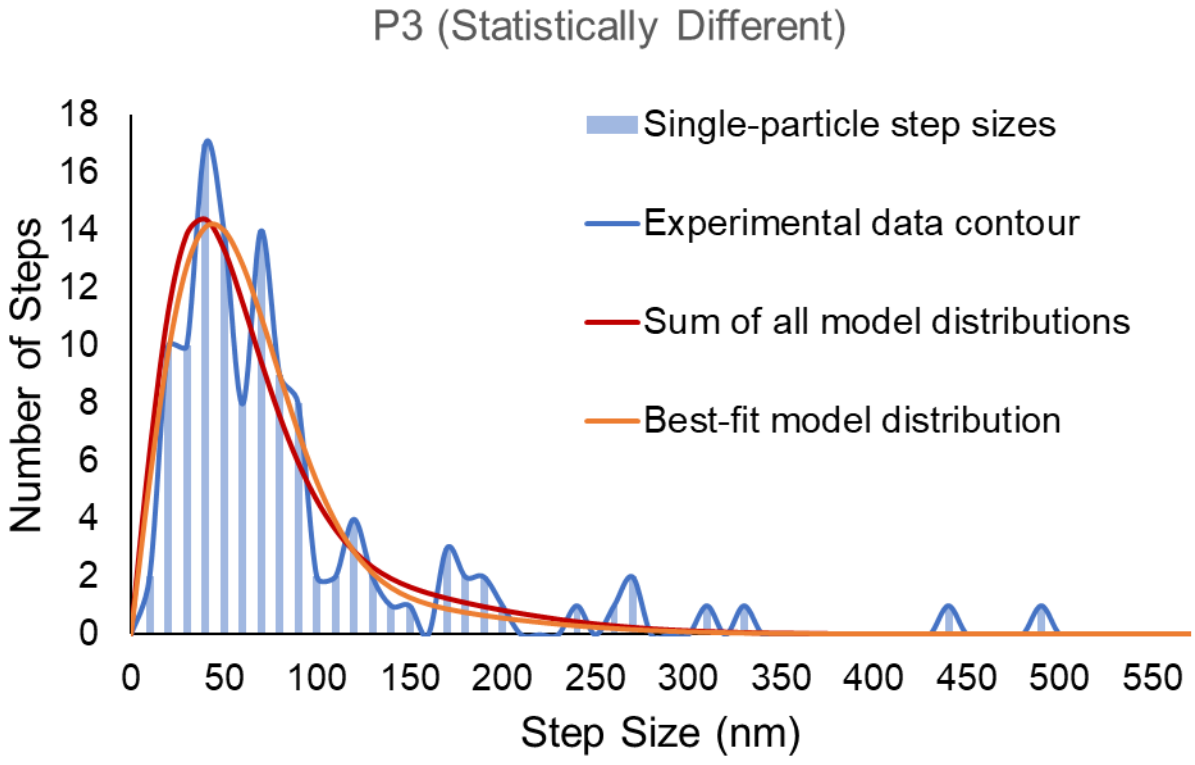
Particle	Quasi-Stationary Scaling Parameter	Intermediate-Step Scaling Parameter	Large-Step Scaling Parameter
P1	2.7028	2.6764	4.6207
P2	0	7.4050	2.5949
P3	0.6361	7.7496	1.6142
P4	0	6.3767	3.6233
P5	0	2.8546	7.1454
P6	4.4017	3.2335	2.3649
P7	0	8.2081	1.7919
P8	1.7917	4.5926	3.6157
P9	2.6102	3.0100	4.3798

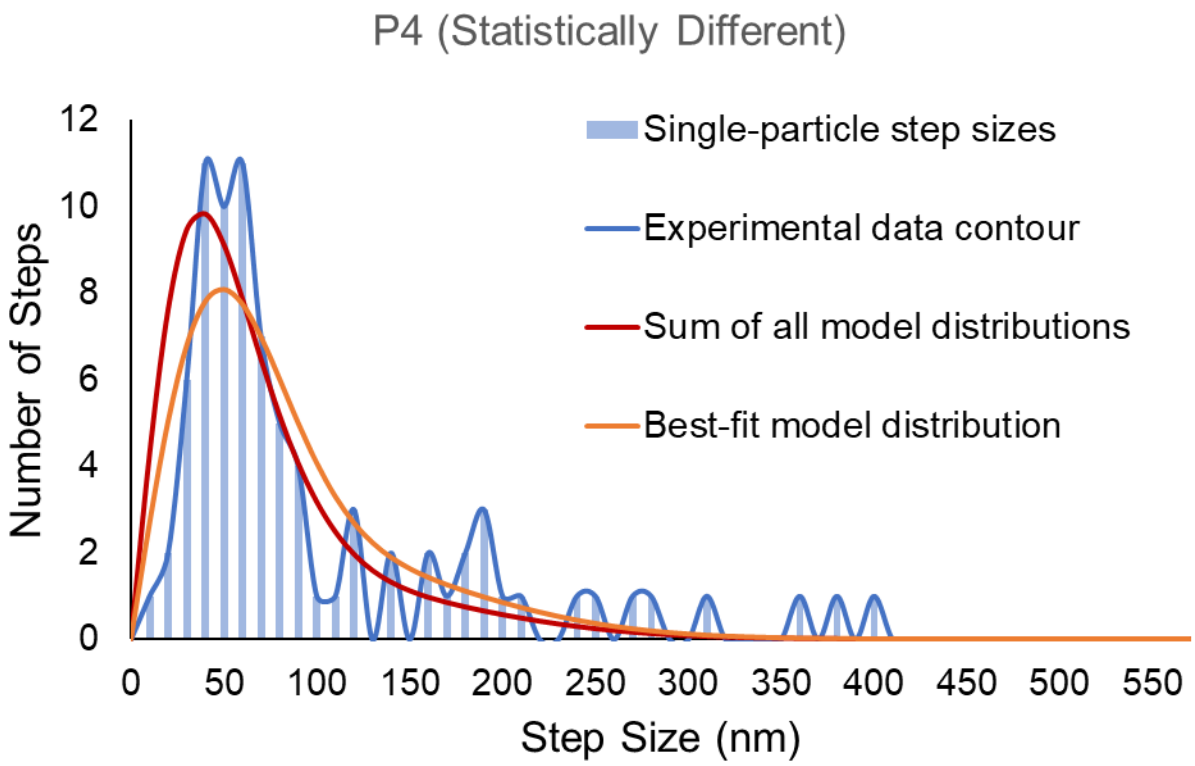
P12	0.3351	8.6233	1.0416
P13	0.8346	7.6404	1.5250
P15	4.6959	4.1736	1.1275
P16	5.9773	1.8841	2.1386
P17	2.1241	5.4763	2.3996
P18	4.7611	5.2389	0
P19	1.8967	5.9261	2.1772

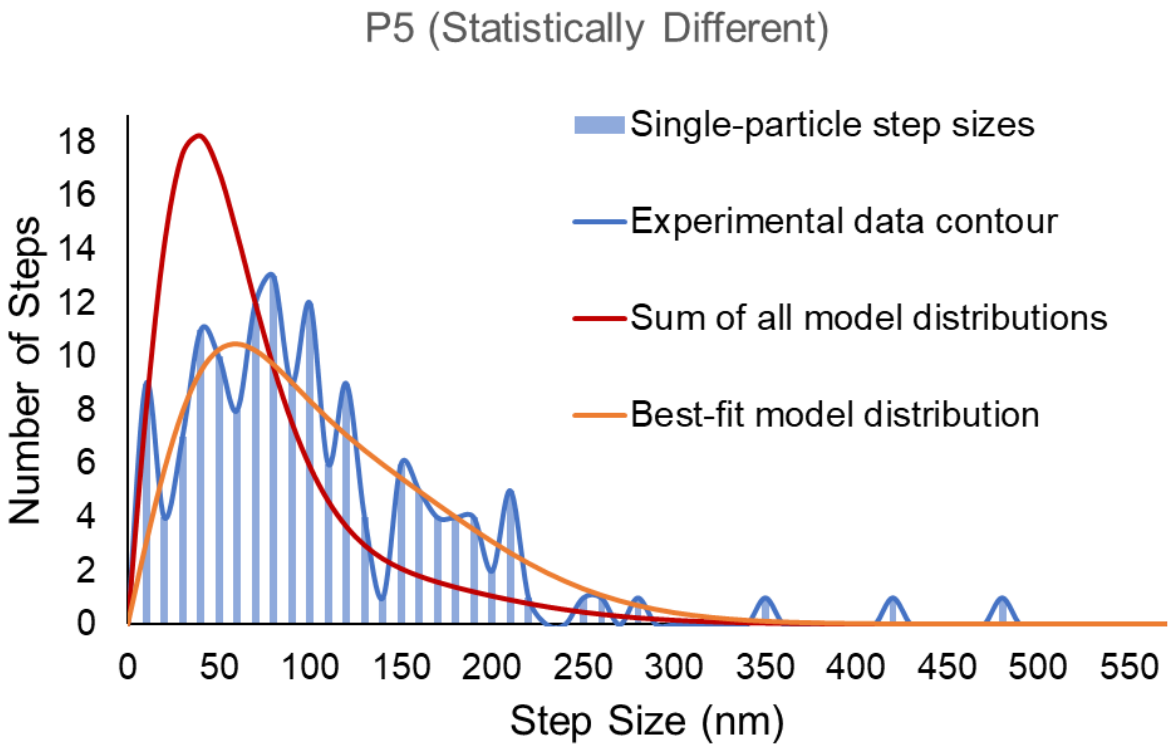
XXIV. 20 Analyzed Particles and Figures

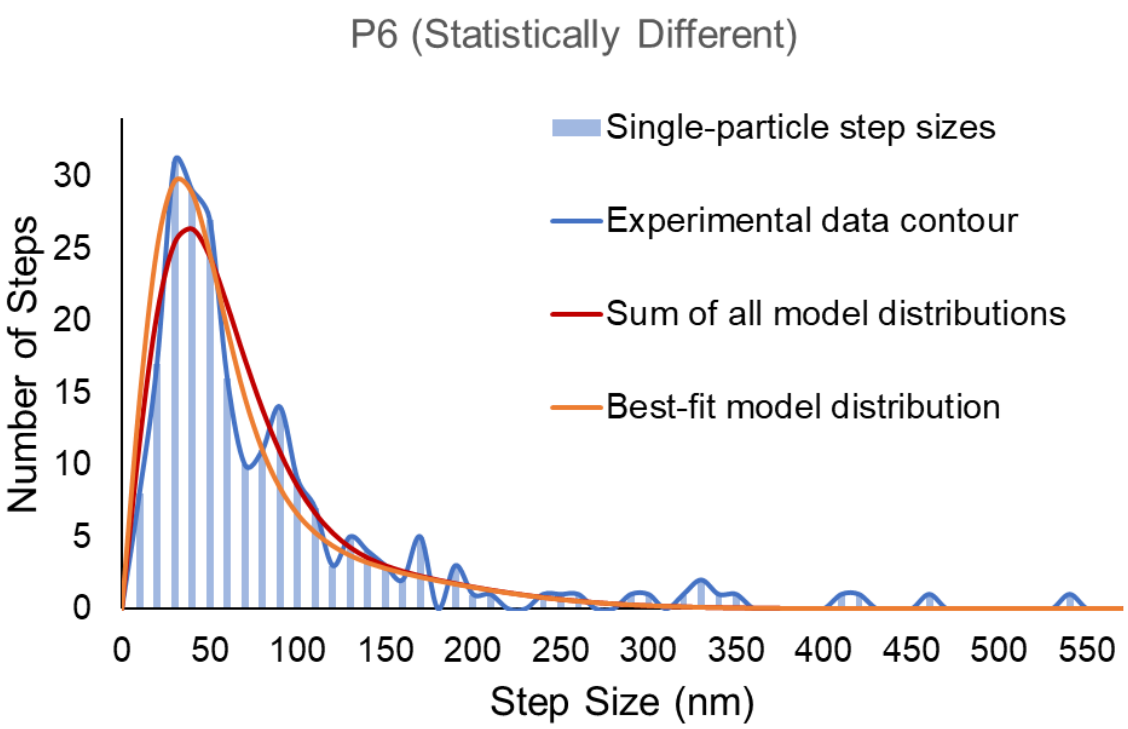


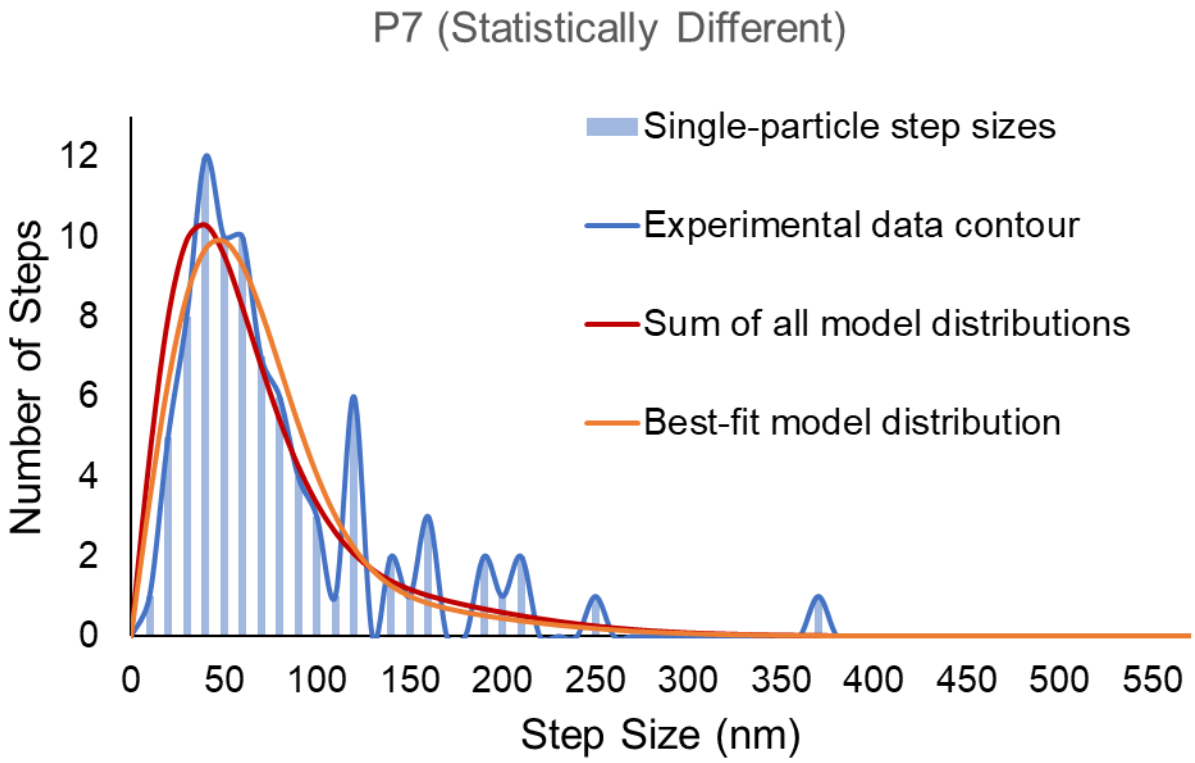

P2 (Statisically Different)
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
2
4
6
8
10
12
14
16
0
50
100
150
200
250
300
350
400
450
500
550

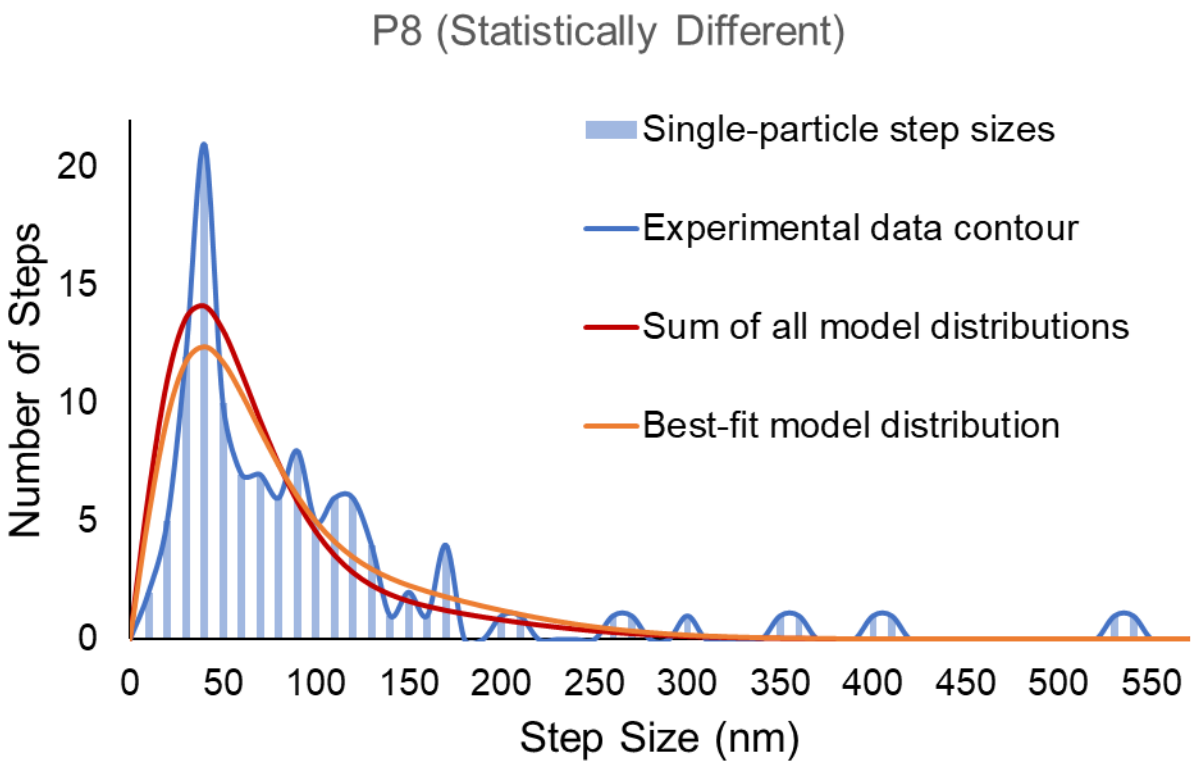

P3 (Statistically Different)
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300
350
400
450
500
550

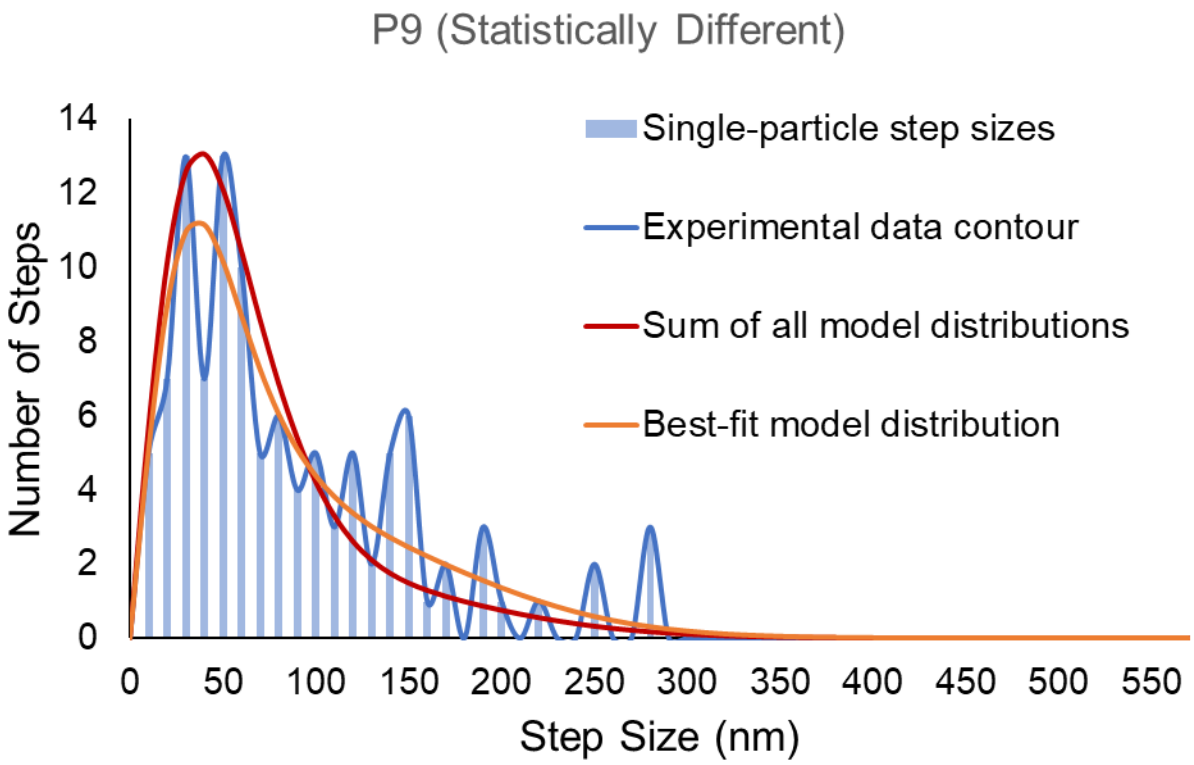

P4 (Statistically Different)
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
2
4
6
8
10
12
0
50
100
150
200
250
300
350
400
450
500
550

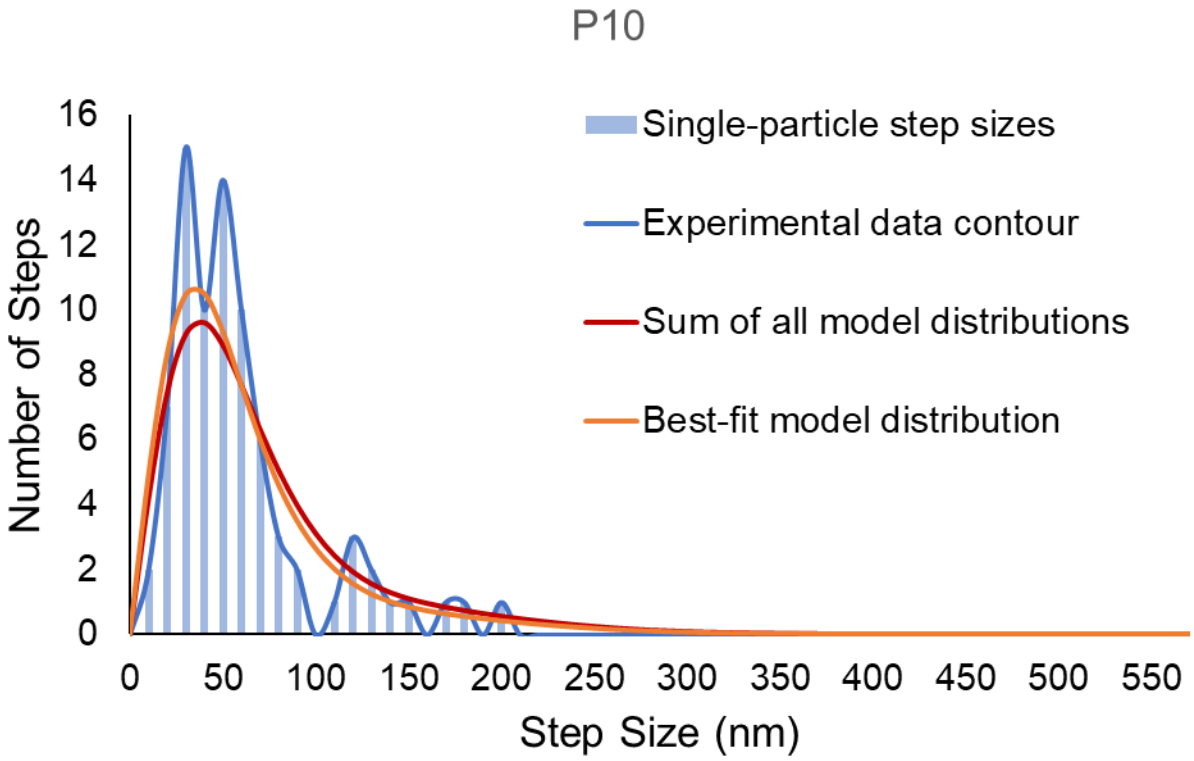

P5 (Statistically Different)
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300
350
400
450
500
550

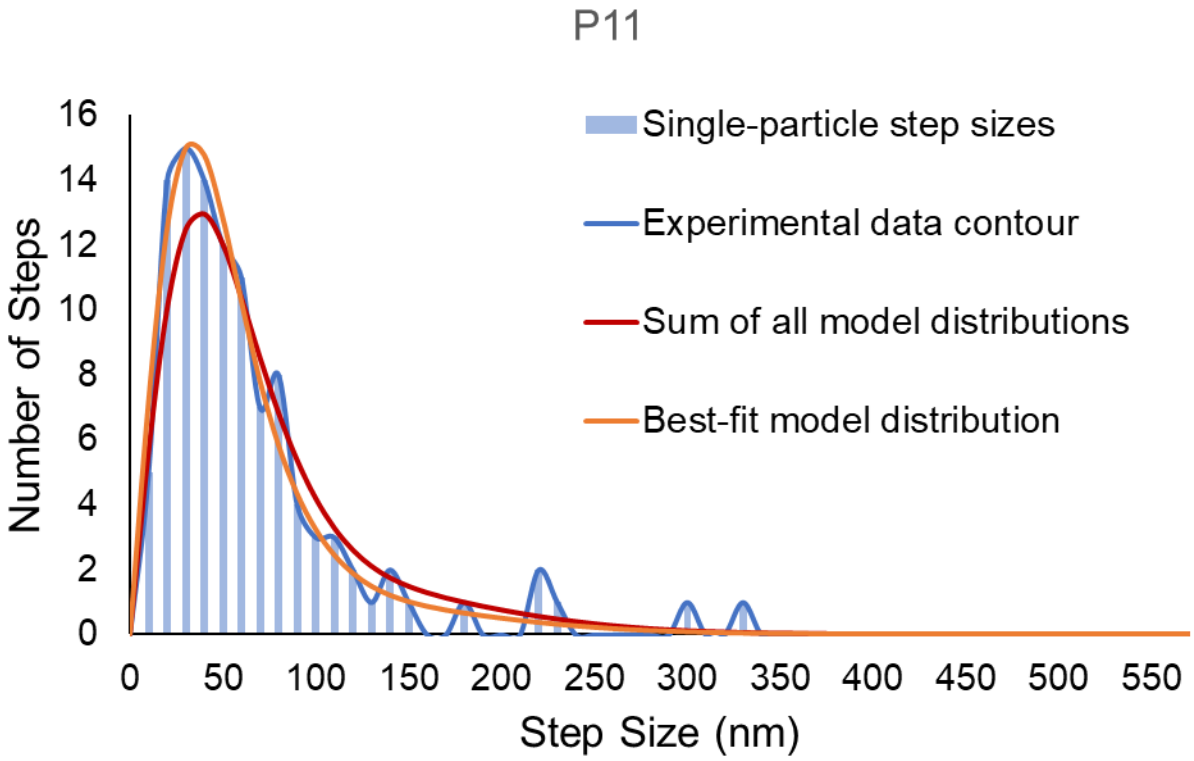

P6 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
5
10
15
20
25
30
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)

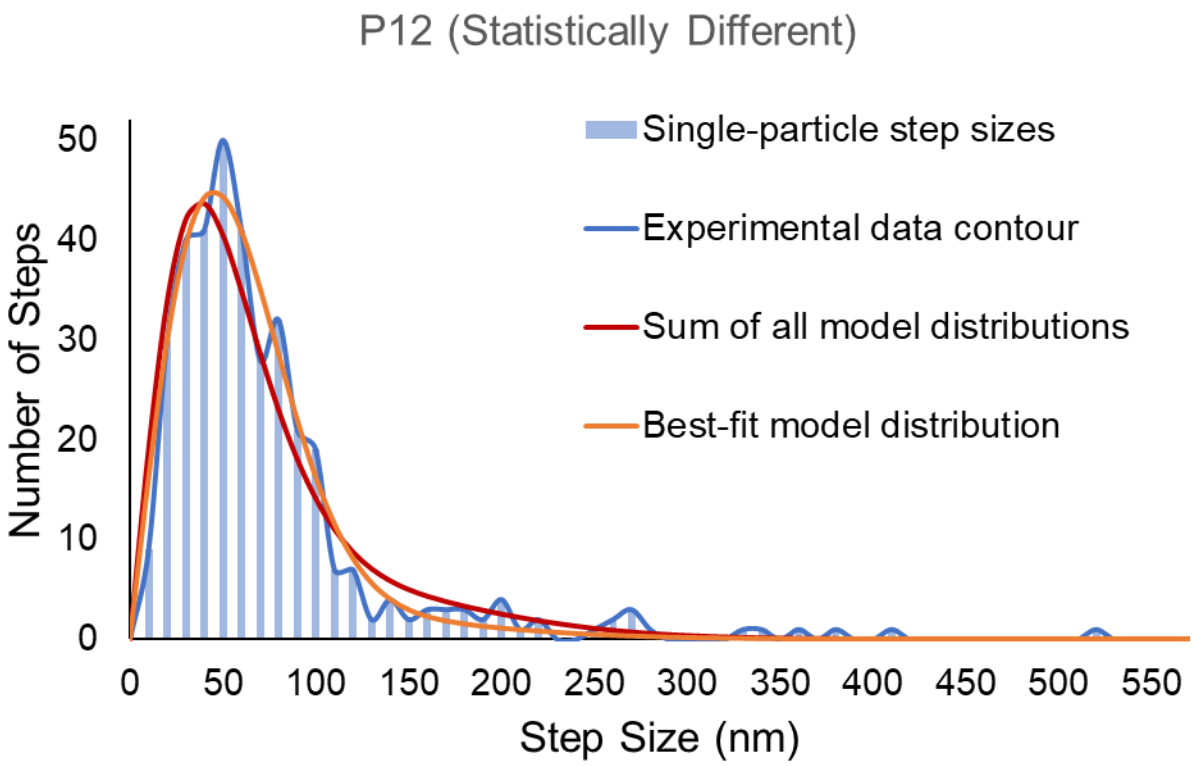

P7 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
2
4
6
8
10
12
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)

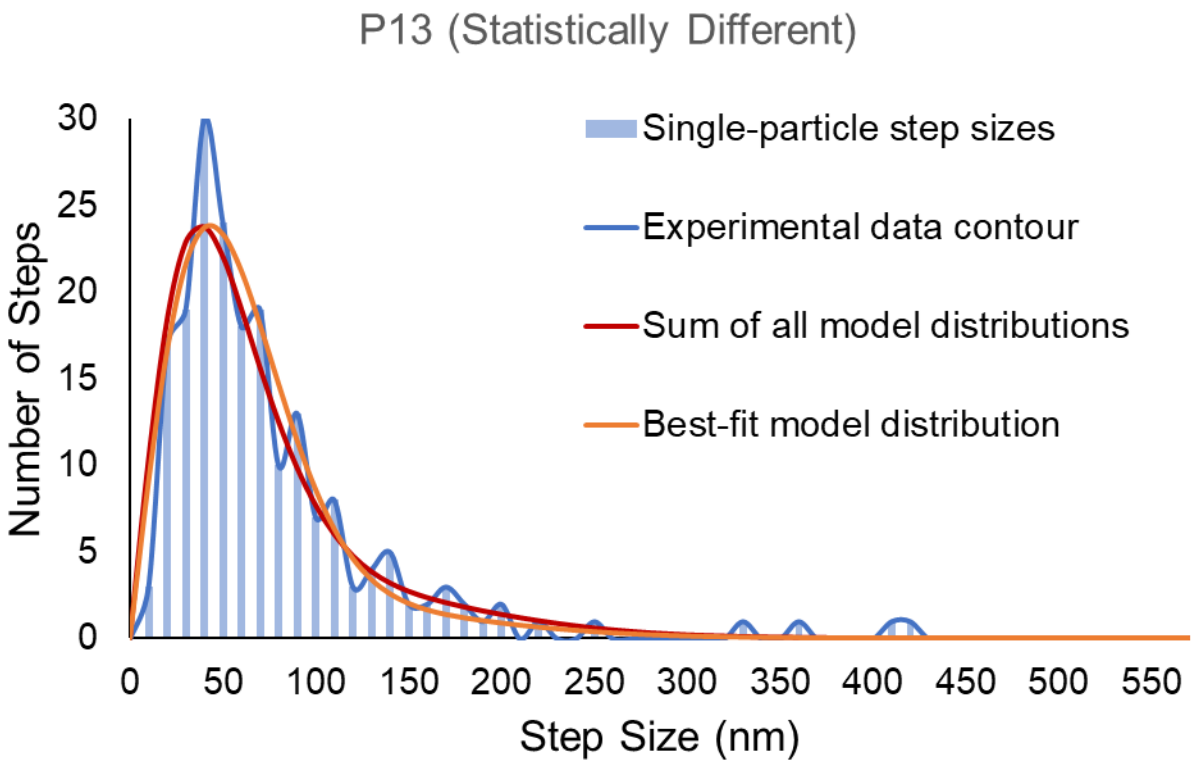

P8 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
5
10
15
20
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)

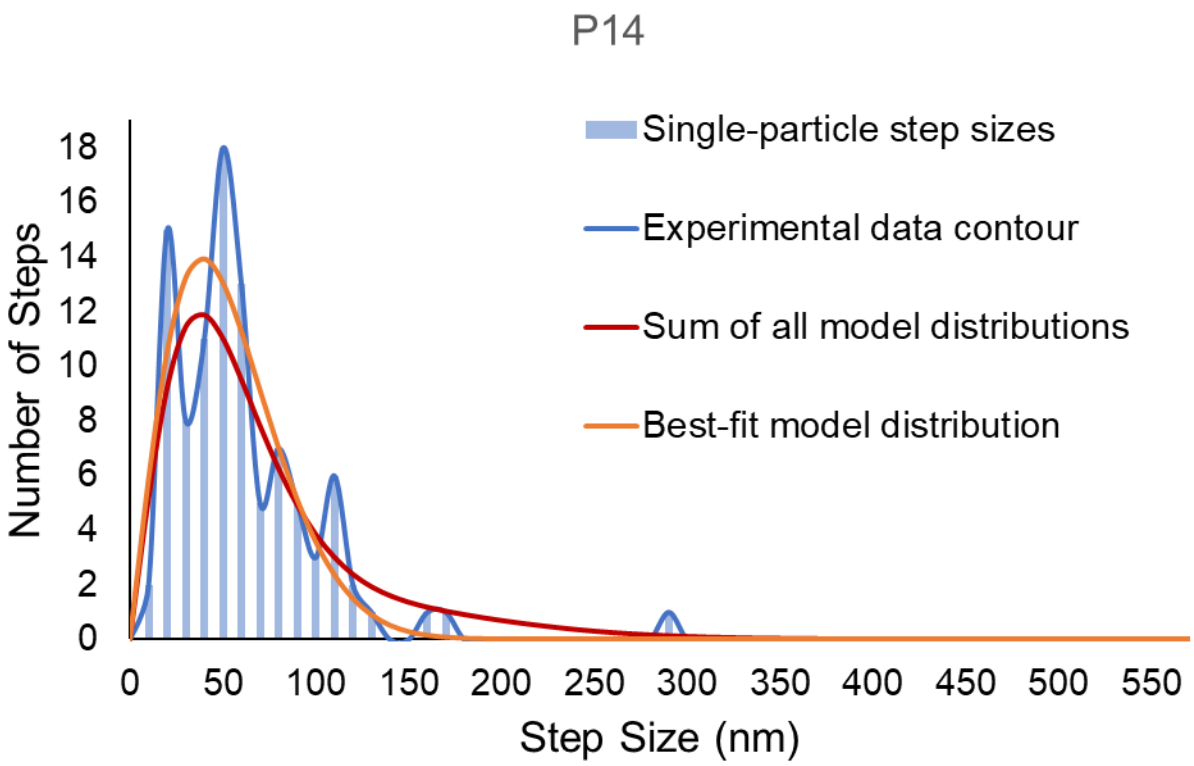

P9 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
2
4
6
8
10
12
14
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)

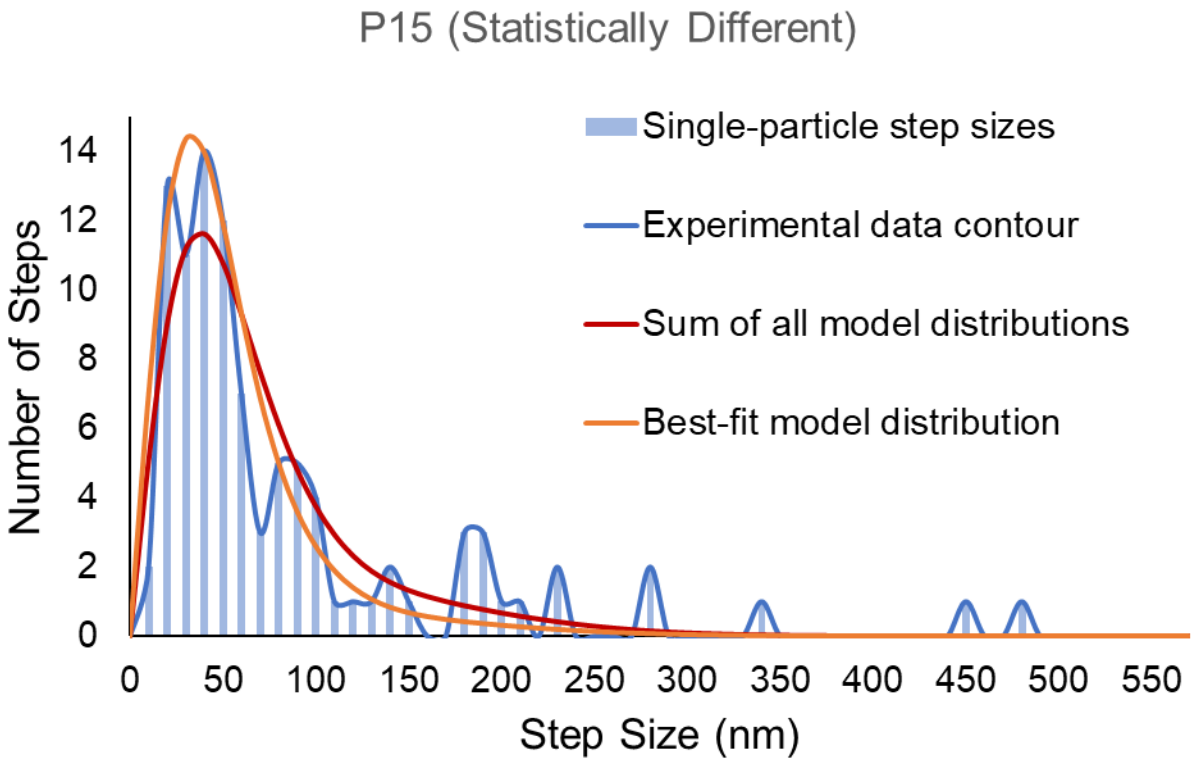

P10
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
2
4
6
8
10
12
14
16
0
50
100
150
200
250
300
350
400
450
500
550

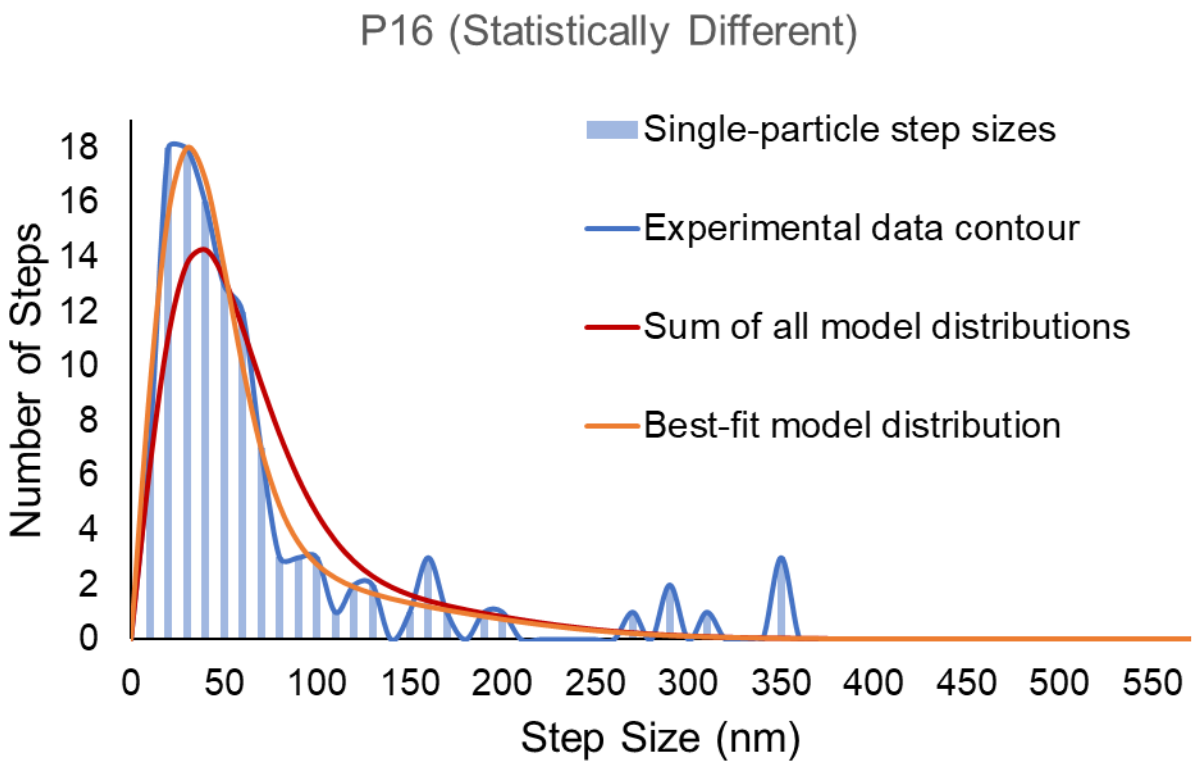

P11
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
2
4
6
8
10
12
14
16
0
50
100
150
200
250
300
350
400
450
500
550

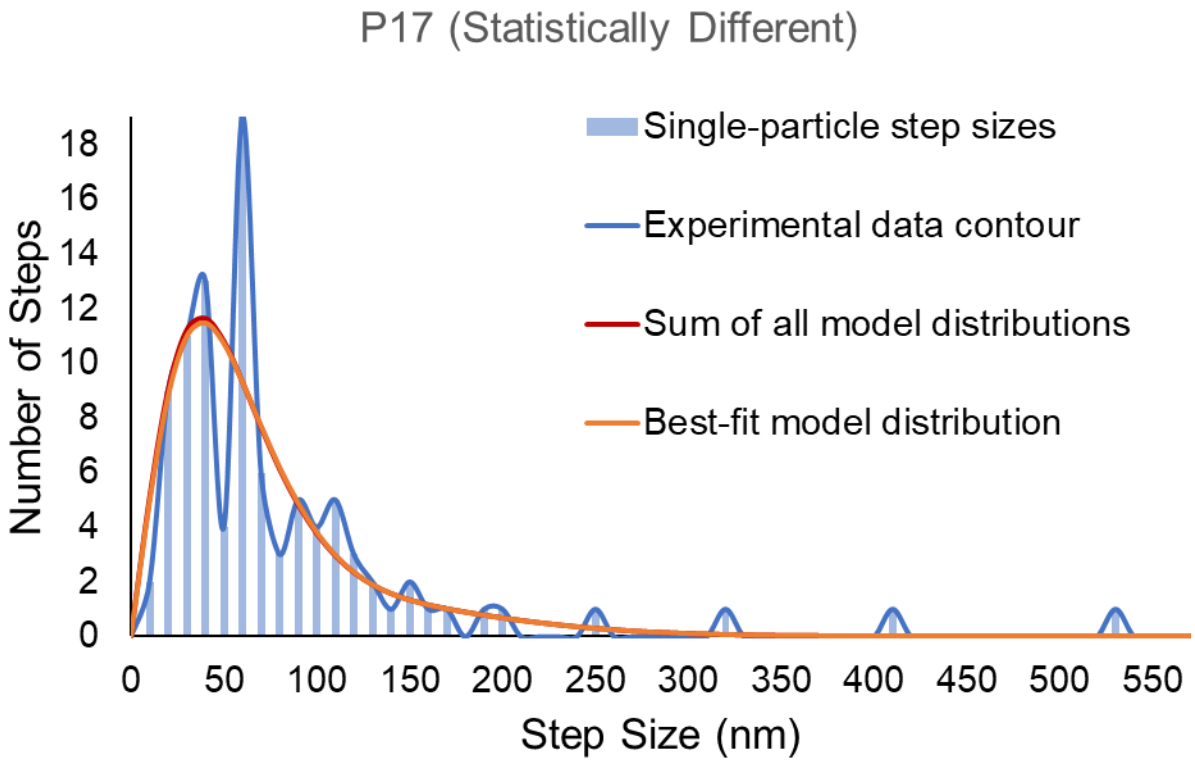

P12 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
10
20
30
40
50
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)

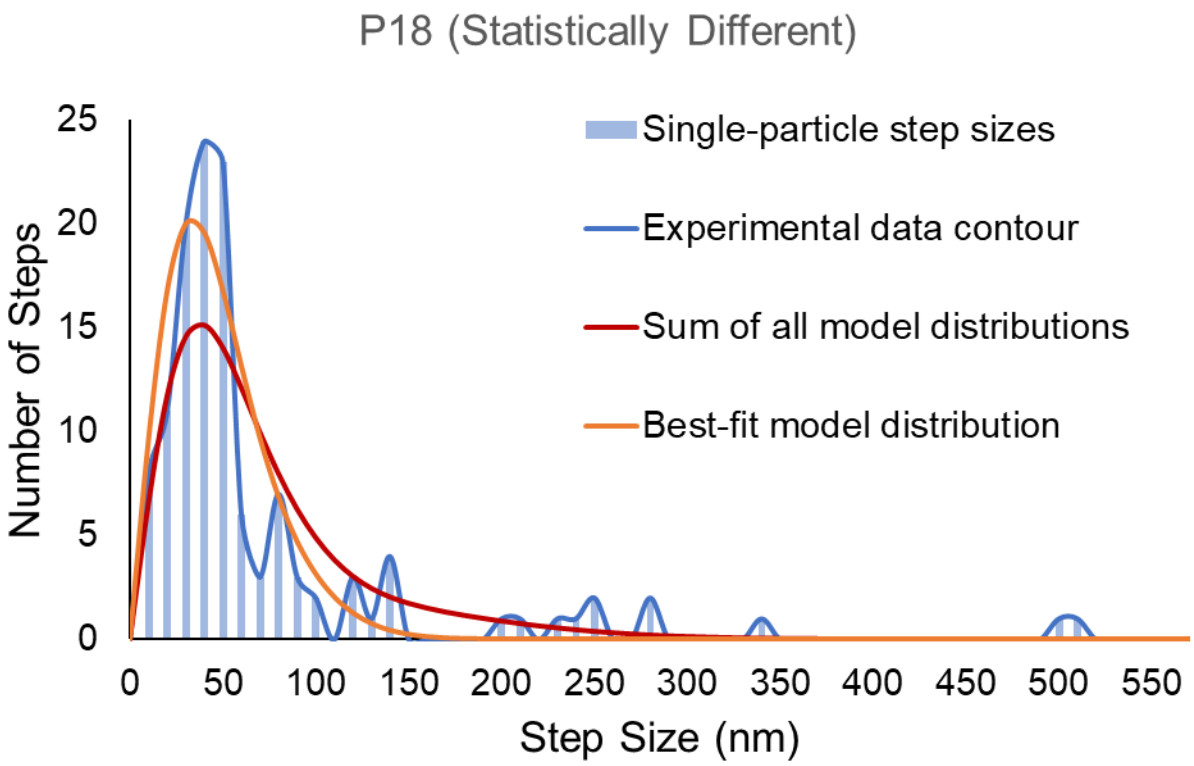

P13 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
5
10
15
20
25
30
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)

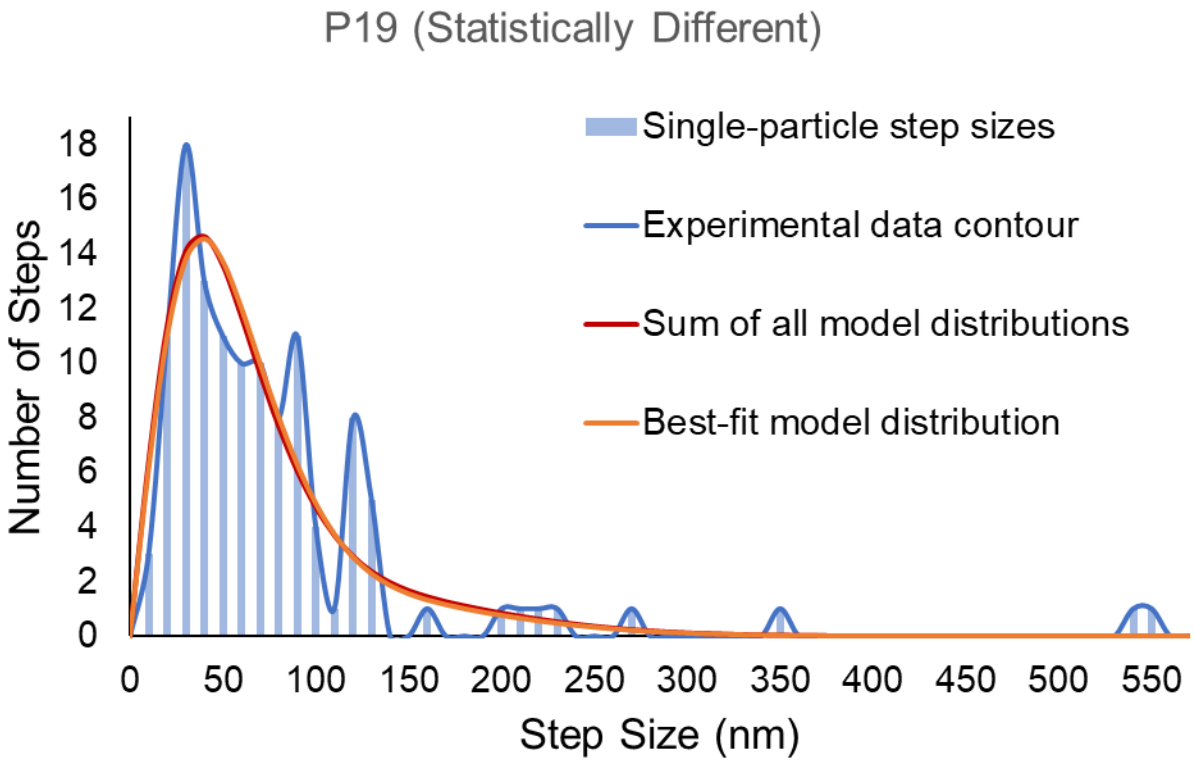

P14
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)


P15 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
2
4
6
8
10
12
14
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)


P16 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)


P17 (Statistically Different)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
Number of Steps
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300
350
400
450
500
550
Step Size (nm)


P18 (Statistically Different)
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
5
10
15
20
25
0
50
100
150
200
250
300
350
400
450
500
550


P19 (Statistically Different)
Number of Steps
Step Size (nm)
Single-particle step sizes
Experimental data contour
Sum of all model distributions
Best-fit model distribution
0
2
4
6
8
10
12
14
16
18
0
50
100
150
200
250
300
350
400
450
500
550

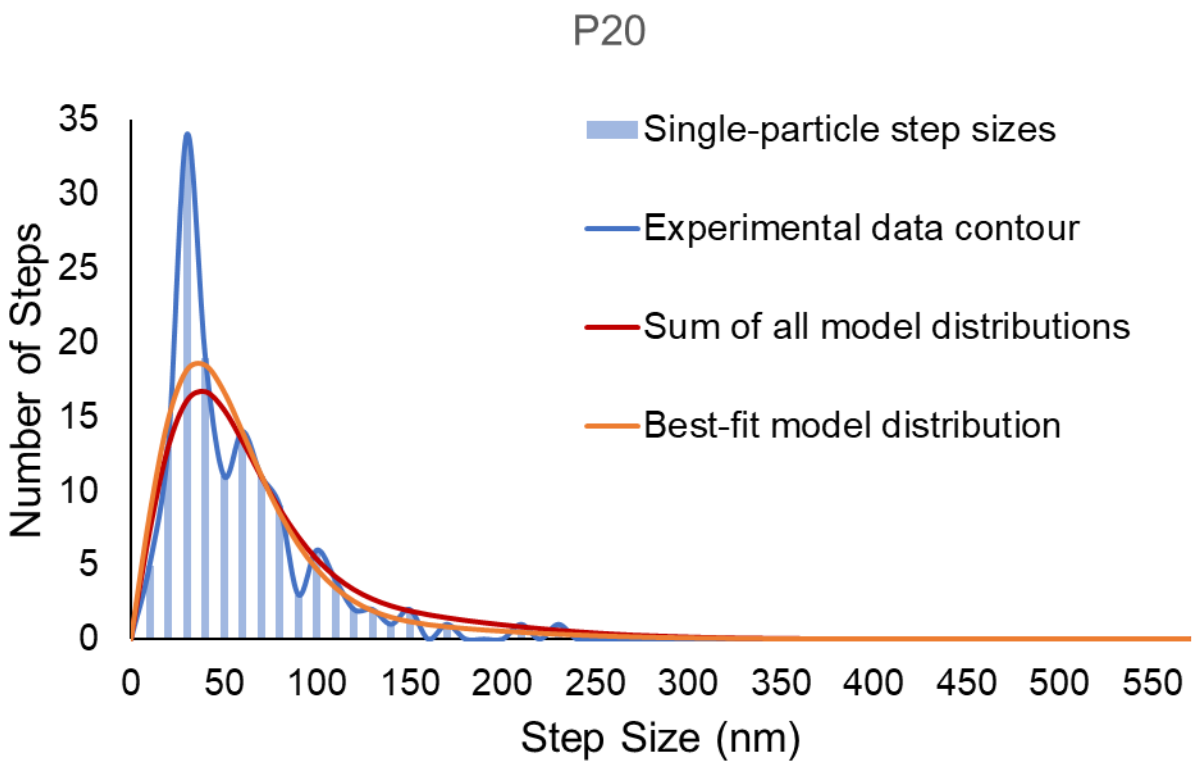


Figure S3.22. List of analyzed particles 1–20.

XXV. References for Appendix

(1) N. M. Esfandiari, Y. Wang, J. Y. Bass, T. P. Cornell, D. A. L. Otte, M. H. Cheng, J. C. Hemminger, T. M. McIntire, V. A. Mandelshtam, S. A. Blum, *J. Am. Chem. Soc.* **2010**, *132*, 15167–15169.

(2) Q. T. Easter, S. A. Blum, *Angew. Chem. Int. Ed.* **2018**, *57*, 12027–12032.

(3) A. Garcia, S. J. Saluga, D. J. Dibble, P. A. López, N. Saito, S. A. Blum, *Angew. Chem. Int. Ed.* **2021**, *60*, 1550–1555.

(4) N. M. Esfandiari, Y. Wang, J. Y. Bass, S. A. Blum, *Inorg. Chem.* **2011**, *50*, 9201–9203.

(5) Q. T. Easter, S. A. Blum, *Angew. Chem. Int. Ed.* **2018**, *57*, 1572–1575.

(6) Q. T. Easter, S. A. Blum, *Angew. Chem. Int. Ed.* **2017**, *56*, 13772–13775.

(7) P. Dedecker, S. Duwé, R. K. Neely, J. Zhang, *J. Biomed. Opt.* **2012**, *17*, 126008.

(8) J. T. Cooper, E. M. Peterson, J. M. Harris, *Anal. Chem.* **2013**, *85*, 9363–9370.

CHAPTER 4

INTRODUCTION: THE FOUNDATION OF ARGUMENT-DRIVEN INQUIRY

ABSTRACT

Traditional laboratory classes are often administered through "cookbook" style curriculum that does not accurate reflect the scientific inquiry and debate. To reflect this more realistic picture of the scientific process, the traditional curriculum of confirmation labs for the lower division undergraduate labs at University of California, Irvine has been adapted to Argument-Driven Inquiry, a guided inquiry curriculum that allows for debate and revision. This chapter introduces a literature overview of the process of Argument-Driven Inquiry and its use as an alternative style of laboratory curriculum in other institutions. Portions of this chapter are adapted with permission from Howitz, W. J.; Frey, T.; Saluga, S. J.; Nguyen, M. M.; Denaro, K.; Edwards, K. D. A Specifications-Graded, Sports Drink-Themed General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach. *J. Chem Ed.* **2023**, *100* (2), 672-680.

INTRODUCTION

Laboratory classes are a necessary and vital part of the undergraduate chemistry education and provides students with the opportunity to gain valuable experience in problem-solving, chemical safety, hands-on technique, and decision-making. These skills are interdisciplinary and are applicable to future careers and experiences in many fields, even if students do not continue on to a traditional chemical education. However, most traditional undergraduate laboratory education is not reflective of the true decision making and problem solving expected of scientists, as they provide students with "recipes" that are confirmed to give reliable positive results.

In the past 30 years, this disconnect between the expectations of post-graduate scientists and the education provided has been noticed and one of the main attempts to bridge the gap is the introduction of guided inquiry. Guided inquiry is a pedagogical style characterized by open-ended questions and student-determined approaches to learning, both in lecture and laboratory settings.[1–4] Based on an evaluation of the same course taught both as a verification course and a guided-inquiry course, there was an increased difficulty for the students but also increased student perseverance and willingness to work through problems,[5] which is one of the most necessary skills for research scientists.

The Science Writing Heuristic (SWH) approach was one of the various guided inquiry styles that were developed over the years, focused on scientific writing and literacy. The main role of the SWH is to encourage students to reflect on and effectively articulate the rationale behind decisions made in the laboratory. Students should pose questions, evaluate evidence, and justify claims made based on data. SWH has been used successfully in the chemistry laboratory in both high school and college settings.[6–8] SWH has also shown significant increases in students' critical thinking and research skills.[9–11] One of the main parts of the SWH curriculum was the explicit

incorporation of a three-part framework to discuss scientific discovery: a claim, which is supported by empirical evidence, and justified by a rationale that pulls on fundamental chemical concepts. The use of "claim, evidence, and justification" as a foundation for scientific argument acted as inspiration for the development of Argument-Driven Inquiry.

Created by Joi Walker and Victor Sampson, Argument-Driven Inquiry (ADI) is a model inspired by SWH and focuses on teaching scientific argumentation in the classroom.[12–14] Adding argumentation — a chance for students to share and constructively critique claims, evidence, and justifications with each other — improves SWH with debate and adds a social aspect. Laboratory experiments incorporating argumentation allow students to propose and defend scientific ideas with peers. Furthermore, the social creation of a scientific argument provides a way for students to determine the argument's veracity by evaluating differing ideas from other individuals or groups.[14–16] Argument-driven inquiry (ADI), as described by Walker, has seven steps:[12]

1. Teams of students are introduced to a task and given a guiding question.
2. Each team designs a procedure to address the research question and collects data following that procedure.
3. Each team analyzes data to find evidence to propose and justify a claim (which answers the research question).
4. Each team then presents their findings to other teams in an argumentation session.
5. Each student writes a report based on the findings.
6. Each student participates in an anonymous peer review of other students' reports.
7. Each student then revises their report to reflect the comments of their peers and submits it to the instructor for final evaluation.

ADI has two main parts: Fundamental Skills and Original Investigation. During Fundamental Skills, each group of 3 to 4 students is given a foundational activity requiring the students to practice new laboratory methods or techniques. Before the end of the Fundamental Skills portion of lab, the team uses knowledge obtained during the foundational activity to plan an experiment aimed at answering a provided research question. During Original Investigation, the team collects and analyzes data to find evidence which answers the guiding question. At the end of this session, each team presents their claim (their answer to the guiding question), evidence, and justification in an academic poster session, referred to as an argumentation session. An example poster is shown below in Figure 4.1 In this student-led argumentation session, members from different teams discuss the validity of evidence and the accuracy of claims with each other.

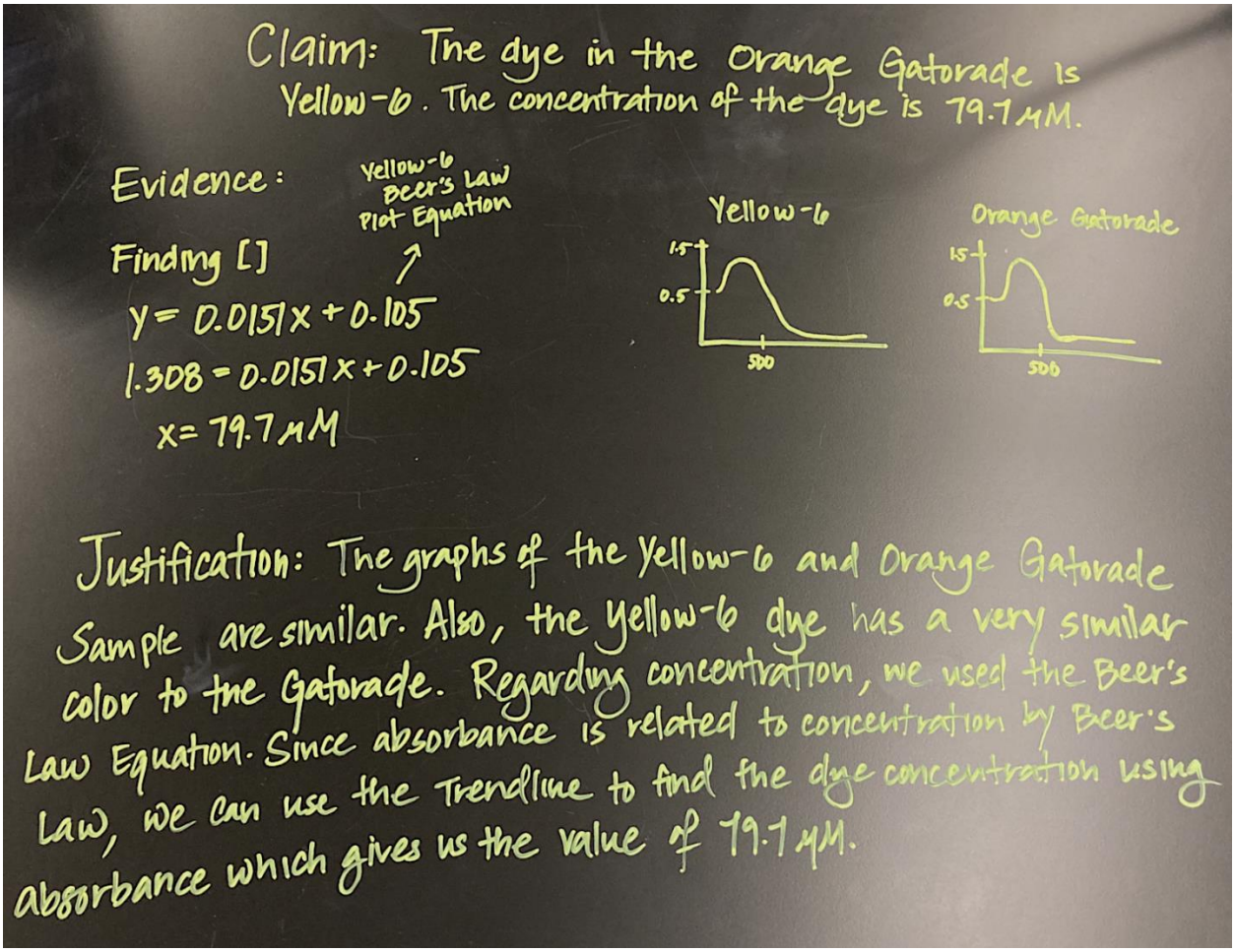


Figure 4.1 Example of a benchtop argumentation poster. Students create these posters to present their claim, evidence, and justification for their Original Investigation. While discussing laboratory results with their classmates, students can adjust their claims. Reproduced from Figure 1 in Reference 22.

While ADI has been investigated heavily as an instructional style, most of these studies focus on middle school or high school students. Few literature examples of ADI experiments exist for chemistry laboratories in higher education. Many ADI experiments are aimed at younger students in middle school and high school, such as Sampson's 22 ADI experiments for Grades 6-8.[17] Of the experiments designed for higher education, Walker presents a full series of experiments for the general chemistry curriculum, focusing on topics such as thermodynamics, hydrate identity, and limiting reagents,[12] as well as density experiment using the ADI model.[18]

During my third year in the graduate program at the University of California, Irvine in 2022, I worked with Prof. Kimberly Edwards and two more senior teaching assistants, Dr. Will Howitz and Dr. Taylor Thane, to address this gap in the literature curriculum. The instructional team was currently working on the implementation of ADI into the general chemistry series, which the three of them had designed during the COVID pandemic in 2020 and 2021. I was brought on as an additional researcher, as well as a graduate student with experience in specifications grading.

One of the unique aspects of the ADI implemented at UCI is the incorporation of specifications grading, an alternative mastery-based graded scheme popularized by Linda Nilson in 2014.[19] Specifications grading moves away from a traditional points-based system, using pass-no pass assignments with specific thresholds for certain letter grades. This system also has a token economy the enables a revise-and-submit model, where students receive a certain number of tokens that can be used to resubmit assignments, make up labs, and turn in assignments. Specifications grading has been implemented here at UCI since 2019 to great success.[20–23] During the transition of the undergraduate general chemistry labs, specifications grading was implemented, synergizing nicely with the revision steps of ADI. Specifications grading supports ADI because it is not a competitive grading system. This fosters collaboration within and between teams. Assignment revision and focus on specific repeated important rubric criteria encourages students to take an iterative approach to course material. With the large enrollment of classes at UCI, we were unable to incorporate the peer review, but found the revise-and-resubmit portion of specifications grading suitable for our purposes.

The first quarter of the general chemistry laboratory series (GCL-I) was implemented in 2021 and 2022 and published in the Journal of Chemical Education in 2023.[22] This was the springboard for my research and development of future ADI courses during my graduate studies.

The transition of the GCL-I was followed by the transition of the second quarter in the series, which is discussed in Chapter 5. As a transitional course between general and organic chemistry, the ADI experiments designed for GCL-II focus on both fundamental techniques such as thin-layer chromatography and introduce reaction-based experiments. As the first quarter was focused on characterization of sports drinks, the experiments of the second quarter of the general chemistry series revolve around the use of spices.

While we have discussed many ADI experiments and curriculum designed for the general chemistry experiments, there are no examples of an ADI curriculum for the organic chemistry serie. My current work, which shall be continued after my defense, is the adaptation of existing organic chemistry curriculum to the ADI model under the guidance of Prof. Renée Link, discussed in Chapter 6.

REFERENCES

(1) Farrell, J. J.; Moog, R. S.; Spencer, J. N. A Guided-Inquiry General Chemistry Course. *J. Chem. Educ.* **1999**, *76* (4), 570.
(2) Schoffstall, A. M.; Gaddis, B. A. Incorporating Guided-Inquiry Learning into the Organic Chemistry Laboratory. *J. Chem. Educ.* **2007**, *84* (5), 848.
(3) Smithenry, D. W. Integrating Guided Inquiry into a Traditional Chemistry Curricular Framework. *Int. J. Sci. Educ.* **2010**, *32* (13), 1689–1714.
(4) Ural, E. The Effect of Guided-Inquiry Laboratory Experiments on Science Education Students' Chemistry Laboratory Attitudes, Anxiety and Achievement. *J. Educ. Train. Stud.* **2016**, *4* (4), 217–227.
(5) Scott, P.; Pentecost, T. C. From Verification to Guided Inquiry: What Happens When a Chemistry Laboratory Curriculum Changes? *J. Coll. Sci. Teach.* **2013**, *42* (3), 82–88.
(6) Hand, B.; Wallace, C. W.; Yang, E. Using a Science Writing Heuristic to Enhance Learning Outcomes from Laboratory Activities in Seventh-grade Science: Quantitative and Qualitative Aspects. *Int. J. Sci. Educ.* **2004**, *26* (2), 131–149.
(7) Greenbowe, T. J.; Poock, J. R.; Burke, K. A.; Hand, B. M. Using the Science Writing Heuristic in the General Chemistry Laboratory To Improve Students' Academic Performance. *J. Chem. Educ.* **2007**, *84* (8), 1371.
(8) Hand, B.; Choi, A. Examining the Impact of Student Use of Multiple Modal Representations in Constructing Arguments in Organic Chemistry Laboratory Classes. *Res. Sci. Educ.* **2010**, *40* (1), 29–44.

(9) Sezen Vekli, G.; Nazli, C. Examining the Effect of Reporting Based on Science Writing Heuristic Approach on Written Argument Quality in General Biology Laboratory. *J. Biol. Educ.* **2022**, *56* (3), 256–270.
(10) S. Stephenson, N.; P. Sadler-McKnight, N. Developing Critical Thinking Skills Using the Science Writing Heuristic in the Chemistry Laboratory. *Chem. Educ. Res. Pract.* **2016**, *17* (1), 72–79.
(11) Gupta, T.; Burke, K. A.; Mehta, A.; Greenbowe, T. J. Impact of Guided-Inquiry-Based Instruction with a Writing and Reflection Emphasis on Chemistry Students' Critical Thinking Abilities. *J. Chem. Educ.* **2015**, *92* (1), 32–38.
(12) Walker, J. P.; Sampson, V.; Zimmerman, C. O. Argument-Driven Inquiry: An Introduction to a New Instructional Model for Use in Undergraduate Chemistry Labs. *J. Chem. Educ.* **2011**, *88* (8), 1048–1056.
(13) Sampson, V.; Gleim, L. Argument-Driven Inquiry To Promote the Understanding of Important Concepts & Practices in Biology. *Am. Biol. Teach.* **2009**, *71* (8), 465–472.
(14) Sampson, V.; Grooms, J.; Walker, J. P. Argument-Driven Inquiry as a Way to Help Students Learn How to Participate in Scientific Argumentation and Craft Written Arguments: An Exploratory Study. *Sci. Educ.* **2011**, *95* (2), 217–257. https://doi.org/10.1002/sce.20421.
(15) Linn, M. C.; Eylon, B.-S. Science Education: Integrating Views of Learning and Instruction. In *Handbook of educational psychology*; Lawrence Erlbaum Associates Publishers: Mahwah, NJ, US, 2006; pp 511–544.
(16) *Taking Science to School: Learning and Teaching Science in Grades K-8*; Duschl, R. A., Schweingruber, H. A., Shouse, A. W., National Research Council (U.S.), National Research Council (U.S.), National Research Council (U.S.), Eds.; National Academies Press: Washington, D.C, 2007.
(17) Grooms, J.; Enderle, P. J.; Hutner, T.; Murphy, A.; Sampson, V. *Argument-Driven Inquiry in Physical Science: Lab Investigations for Grades 6-8*; NSTA Press, 2016.
(18) Walker, J. P.; Wolf, S. F. Getting the Argument Started: A Variation on the Density Investigation. *J. Chem. Educ.* **2017**, *94* (5), 632–635.
(19) Nilson, L. B. *Specifications Grading: Restoring Rigor, Motivating Students, and Saving Faculty Time*; Stylus Publishing, LLC, 2015.
(20) McKnelly, K. J.; Morris, M. A.; Mang, S. A. Redesigning a "Writing for Chemists" Course Using Specifications Grading. *J. Chem. Educ.* **2021**, *98* (4), 1201–1207.
(21) Howitz, W. J.; McKnelly, K. J.; Link, R. D. Developing and Implementing a Specifications Grading System in an Organic Chemistry Laboratory Course. *J. Chem. Educ.* **2021**, *98* (2), 385–394.
(22) Howitz, W. J.; Frey, T.; Saluga, S. J.; Nguyen, M.; Denaro, K.; Edwards, K. D. A Specifications-Graded, Sports Drink-Themed General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach. *J. Chem. Educ.* **2023**, *100* (2), 672–680.
(23) McKnelly, K. J.; Howitz, W. J.; Thane, T. A.; Link, R. D. Specifications Grading at Scale: Improved Letter Grades and Grading-Related Interactions in a Course with over 1,000 Students. *J. Chem. Educ.* **2023**, *100* (9), 3179–3193.

CHAPTER 5

A SPECIFICATIONS-GRADED, SPICE-THEMED, GENERAL CHEMISTRY LABORATORY COURSE USING AN ARGUMENT-DRIVEN INQUIRY APPROACH

PREFACE

This chapter is a collaboration between several scientists. Alyssa Burns and Yong Li were my fellow co-first authors on the publication, each of us contributing to the writing, experimentation, and implementation of the work presented below. Yong Li performed the experiments for Project #1, while Alyssa Burns performed the statistical analysis presented in the survey results, as well as creation of Figures 5.7 and 5.8. The experimental results shown under Projects #2 through #4 were run or collected by me. All three of us assisted in the implementation of the course curriculum, collection of the surveys, and the writing of the manuscript. Melanie Nguyen assisted in class coordination, logistics of the experiments presented herein, and created the graphic shown in Figure 5.1. Used with permission from Saluga, S. J.; Burns, A. M.; Li, Y.; Nguyen, M. M.; Edwards, K. D. A Specifications-Graded, Spice-Themed, General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach. *J. Chem Ed.* **2023** *100* (10), 3903-3915.

ABSTRACT

This chapter describes the creation of a second quarter of a two-quarter sequence of argument-driven-inquiry general chemistry laboratories. The course contains four projects investigating the chemistry of spices (vanilla, cinnamon, spearmint, and cloves) and incorporates a structured review and hands-on applications of fundamental concepts necessary to transition between general and organic chemistry (colligative properties, TLC, synthesis, characterization tests, and unknown determination). The inquiry-based curriculum was designed to give students increasing responsibility and freedom to develop experimental design skills. Specifications grading is used to increase concept iteration and encourage teamwork amongst students. Survey results for student learning style, feelings about chemistry, and perception of the course format are compared for first and second quarter courses. Changes in survey responses show higher average positive responses in many categories for the second quarter course.

INTRODUCTION

Herein, we describe the thematically connected curriculum for the second quarter general chemistry laboratory course (GCL-II) at the University of California, Irvine (UCI). We previously described the creation of the first quarter large-scale general chemistry laboratory course (GCL-I) using the same methodology.[1]

Course Scale and Structure

UCI's non-chemistry-major two-course general chemistry laboratory sequence (GCL-I and GCL-II) spans two 10-week quarters. Weekly four-hour laboratory sessions with 24 students are supervised by one graduate student teaching assistant (GTA). The first quarter of lab (GCL-I) is taken with the last quarter of general chemistry lecture (GC-III) and the second quarter of lab

(GCL-II) is typically taken with the first quarter of organic chemistry lecture (OC-I) (Table 5.1). The high-enrollment (1300+ students) GCL-I course offering occurs each spring, followed by a high-enrollment (1000+ students) GCL-II course offering each fall. Summer session and alternate quarter course offerings for both courses typically have enrollments of approximately 200 with students who either did not initially pass prerequisite lecture courses or completed the course sequence off track. On-sequence high-enrollment GCL-I and GCL-II courses typically require 50 lab sessions and 25 GTAs. Off-sequence low-enrollment courses require 8 lab sessions and 4 GTAs. (Appendix Section X contains GCL-II student demographics).

Table 5.1. Structure of On-Sequence and Off-sequence General Chemistry Courses[1]

Year	Fall Quarter	Winter Quarter	Spring Quarter
First-Year On-Sequence	General Chemistry Lecture I (GC-I) *(No Laboratory Course)*	General Chemistry Lecture II (GC-II) *(No Laboratory Course)*	General Chemistry Lecture III (GC-III) **General Chemistry Laboratory I (GCL-I)**
First-Year Off-Sequence		General Chemistry Lecture I (GC-I) *(No Laboratory Course)*	General Chemistry Lecture II (GC-II) *(No Laboratory Course)*
Second-Year On-Sequence	Organic Chemistry Lecture I (OC-I) **General Chemistry Laboratory II (GCL-II)**		
Second-Year Off-Sequence	General Chemistry Lecture III (GC-III) **General Chemistry Laboratory I (GCL-I)**	No Lecture Course **General Chemistry Laboratory II (GCL-II)**	Organic Chemistry Lecture I (OC-I) *(No Laboratory Course)*

UCI's offset of lower division lab from lecture, specifically the coupling of the large enrollment on-sequence GCL-II with OC-I, permits the incorporation of organic chemistry content in GCL-II, resulting in a transitional course bridging general and organic chemistry. Because GCL-II relies on theories connected to intermolecular forces introduced in GC-II, students in the off-sequence GCL-II can still connect conceptually with course content and benefit from the exposure to introductory organic techniques once enrolled in organic chemistry laboratory.

Originally, the 8 weeks of traditional expository-type experiments in GCL-II addressed diverse topics derived from general chemistry lecture and the corequisite organic chemistry lecture course (e.g., solubility and miscibility, vapor pressure, analysis of a chelated iron salt, aspirin, and chlorophyll). During the laboratory, students worked in pairs to complete procedures outlined in the laboratory manual. After completing the experimentation, each student worked independently to answer post-laboratory questions including calculations with collected data or answering conceptual questions.

Theme

Instead of a broad expository coverage of topics, the new GCL-II course still takes advantage of the corequisite organic lecture (OC-I), but is structured around four spice-themed projects following the argument-driven inquiry (ADI) format developed for GCL-I. Theme-based instruction in general chemistry laboratory courses has been used to contextualize course content for students.[2–8] Thematic connections between experiments provide students a conceptual framework,[9] make course content more relevant,[4,9] and increase student understanding[10,11] and engagement[4,5,9,11,12]. Because of this and the positive student response to the Gatorade theme in GCL-I,[1] we also adopted a theme for GCL-II. Spices were chosen because their organic nature resonates with students concurrently enrolled in OC-I while still utilizing concepts from GC-II to

remain accessible to off-sequence students . Furthermore, their benign nature eliminates most chemical hazards and waste.

In the first project of GCL-II, students use freezing point depression and melting point to determine the identity of an unknown spice compound. In the second project, students identify spice compounds in an essential oil through thin layer chromatography (TLC). The final two projects require students to use techniques learned in GCL-I and the previous projects in the GCL-II to synthesize and determine the product of vanillin oxidation and to synthesize and determine the better sunscreen product from ketone and cinnamaldehyde reactions.

Argument-Driven Inquiry (ADI)

The previous version of GCL-II used confirmation-type experiments which provided detailed procedures and post-laboratory questions. Such an approach encourages students to engage in basic science process skills: observation, measurement, and data interpretation. There is support in the education community for going beyond this type of confirmation style experiments.[13–16]

In comparison, Argument Driven Inquiry (ADI) experiments now used in GCL-II provide general procedural guidance and use the claim-evidence-justification framework.[11] Students engage both in the above skills and in additional science process skills: hypothesis (claim) formulation, experimentation, and communication (through argumentation).[15,17–20] The *inquiry* approach engages students more authentically in experimentation by requiring them to develop their own procedures.[14,21] Furthermore, *argumentation* requires students to defend their claims and critique those of others. By combining inquiry and argumentation, ADI has been shown to increase

student ability to use evidence and reasoning, create a more positive student attitude toward chemistry, and improve performance on summative assessments.[17,20,22,23]

Additionally, the encouraging results of the Laboratory Course Assessment Survey (LCAS) given to GCL-I students led us to continue the ADI approach in GCL-II.[1] The LCAS measures student perception of peer collaboration, knowledge discovery, and iteration (revision and repetition) for course-based undergraduate research experiences (CUREs).[24] We incorporated the survey because it also probes student perception of relevant ADI activities and, hence our course learning outcomes (Table 5.2): planning and conducting investigations (LO2 & LO3), collecting and analyzing data (LO3 & LO4), working with others (LO1 & LO4), and presenting and revising work (LO5).

Table 5.2. GCL-II Learning Outcomes (LOs)

Students will be able to:
LO1: Broadly, engage in scientific inquiry and argumentation with a team of peers.
LO2: Develop fundamental laboratory skills and design experimental procedures. (Skills: recordkeeping, safety/waste disposal, UV-Vis spectroscopy, separations, chromatography, melting and freezing point, and synthesis)
LO3: Collect data, determine and perform data analysis on characterization test results.
LO4: Determine what data are evidence that can be used as justification to support a claim. Defend scientific reasoning to peers.
LO5: Produce an independent report defending their team's claim using scientific reasoning, experimental design, and data analysis. Utilize the revision process to correct misconceptions.
LO6: Demonstrate laboratory skill proficiency and argumentation abilities in the final practical exam.
LO7: Demonstrate a basic understanding of lab safety through safety moments, weekly quizzes, and the safety exam.

METHOD

GCL-II's ADI course structure, like GCL-I, contains 4 two-week projects. The learning outcomes (Table 5.2) are inseparable from the seven-step ADI process (Figure 5.1):

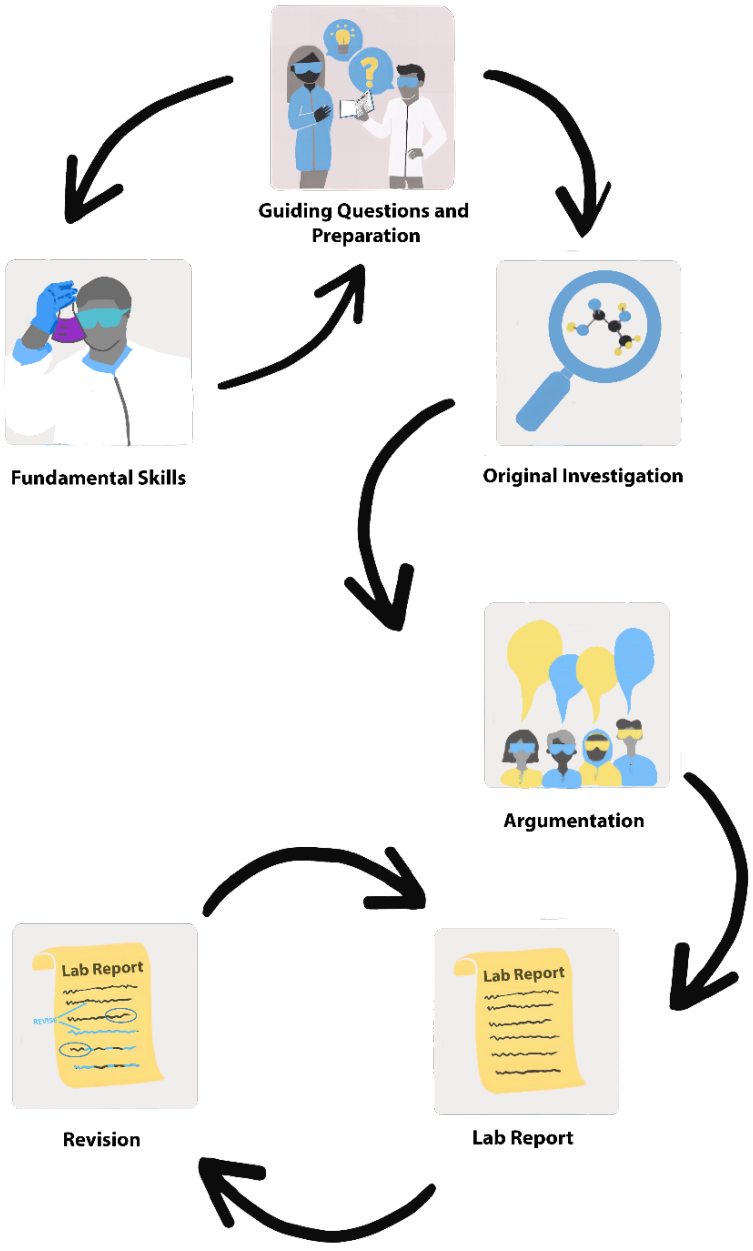


Figure 5.1 ADI Process.

1. **Guiding Question** (LO1)**:** To prepare for a project's first laboratory session (the fundamental skills (FS) session), students are given an initial guiding question (Table 3, second column) and provided general technique information. Before entering the lab, students independently complete a pre-laboratory quiz and prepare their electronic laboratory notebook (ELN) with an objective, safety and chemical tables (LO7), and a draft of procedures to follow in lab.
2. **Fundamental Skills** (LO2 & LO3)**:** During the FS session, a team of 3 to 4 students, (randomly formed during the first course meeting), practice the general technique, collect data, and perform data analysis to answer the guiding question. The team then creates a procedural plan to approach a second guiding question (Table 3, third column) for the original investigation (OI) in the following laboratory session.
3. **Plan for Original Investigation** (LO2)**:** To prepare for a project's second laboratory session (the OI session), students independently complete a pre-laboratory quiz and prepare their ELN as they did for the first lab session.
4. **Original Investigation** (LO3)**:** During the OI session, the team follows their procedural plan to collect more data, performs data analysis, then provides a justification of their claim (the second guiding question's answer).
5. **Argumentation** (LO1 & LO4)**:** Using chalk paint on their benchtop, the team creates a poster with their claim, evidence, and justification. One team member stays with the poster to defend the team's argument to other teams, while the remaining team members travel to other posters and critique their claims.
6. **Laboratory Report** (LO5)**:** After the OI lab session, students individually write a report based on their experimental work, data analysis, and feedback received during the argumentation session.

7. **Revise and Resubmit** (LO5)**:** Students may revise work based on GTA feedback in exchange for tokens earned through the specifications grading system (see Appendix).

Table 5.3. Guiding Questions for GCL-II

Project	**Fundamental Skills (FS)**	**Original Investigation (OI)**
1	What is the average freezing point depression constant for menthol? What is the melting point of the solute? Students measure the freezing point of pure menthol and a menthol solution to determine the freezing point depression constant. Students also measure the solute melting point.	What is the identity of the unknown spice chemical? Students measure the melting point of an unknown and the freezing point of a menthol solution containing a known amount of that unknown. The unknown's molecular mass is determined by freezing point depression.
2	What are the R_f values of the spice compounds? Which heptane:acetone ratio is the best eluent? Students run multiple TLCs of known compounds in various eluent mixtures.	Which spice compounds are present in the essential oil? Students run a TLC of an essential oil with an eluent mixture chosen from the previous work and compare the R_f values to the standards.
3	What is the percent yield of the synthesis if the product is divanillin? If the product is vanillic acid? Students perform an oxidation of vanillin and measure the product's yield.	What is synthesized, divanillin or vanillic acid? Is the product pure? Students use product solubility, melting point, TLC, and UV-Vis absorbance to characterize their product.
4	What are the characteristics of the products observed so far make for a good sunblock? Students synthesize, crystallize, and begin characterization tests.	When combined with **cinnamaldehyde**, which reagent (acetophenone or acetone) makes the best sunblock? Why? Students complete characterization tests on both products.

Our seven ADI steps incorporate scientific inquiry processes: problem identification, making observations, posing questions, collecting data, using scientific concepts to analyze data, and finally, summarizing and communicating results.[25] While most undergraduate laboratory

curriculum contains the above steps, the amount students can control experimentation (the inquiry level) is on a continuum. Experiments can range from confirmation type experiments (in which all experimentation parts are dictated) to authentic inquiry (where the student is responsible for the entire process - from the problem investigated to the conclusion derived from the results). Structured, guided, and open inquiry span the difference between these two extremes (Figure 5.2).[21,26–29]

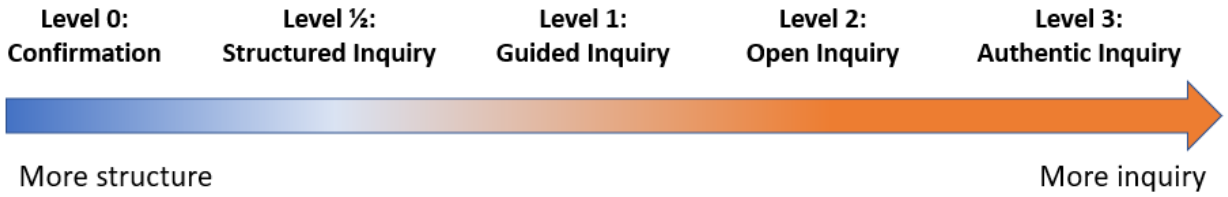


Figure 5.2. Spectrum of Inquiry Instruction[21]

A primary goal in designing the GCL-I and GCL-II curriculum was to increase the trajectory of inquiry during the courses. An iterative approach throughout the course sequence reinforces understanding of laboratory skills and data analysis techniques while providing students a tool chest to use in each progressive experiment. This is especially evident in GCL-II. Students must employ basic laboratory techniques (such as solution preparation, digital balance use, and visible spectroscopy) from GCL-I with little prompting as well as repurpose skills introduced in each progressive GCL-II project to answer the guiding questions. Another design goal was to allow for student result variability to enable robust argumentation (ADI course structure, step 5). The social sense-making of argumentation is short-circuited if all students come to the same conclusion. In conjunction with this, the ability to use scientific reasoning and apply laboratory skills to new problems is more important than finding one right answer.[18,19,30,31]

An analysis of GCL-I and GCL-II experiments using Bruck *et. al.*'s *Rubric to Guide Curriculum Development*[27] is shown in Table 5.4. In the first week of each GCL-I project (the FS session), the question, background, procedures, and data analysis are provided. Therefore, the inquiry level for the FS sessions is structured. The second week of each GCL-I project (the OI session) provides:

- A new guiding question (Table 5.3);
- General instructions about poster creation for argumentation including claim, evidence, and justification;
- Lab report content questions regarding concepts investigated, procedures used, and claim justification. Students have access to a rubric, which is specific to each project and provides the student some direction as they prepare their report. (Rubric examples, Appendix Section IV.)

Because results analysis/interpretation is not provided in the OI sessions, the inquiry level is guided.

Like GCL-I, the first two projects of GCL-II start with structured inquiry FS sessions, followed by guided inquiry OI sessions. The third and fourth projects of GCL-II rely on the techniques learned in GCL-I and the first two projects of GCL-II to characterize products. Students are reminded of the techniques learned thus far and a few experimental directions (potential solvents, dilution factors, and synthesis procedures). This reduces cognitive load and ensures lab work can be completed during the 4 hour time block. Therefore, the inquiry level in the FS sessions has increased to guided inquiry in the last two projects of GCL-II. While the question, background and procedures are given, no indication of how to analyze the data is provided. The OI sessions for these two projects increase inquiry further toward open inquiry by not providing

procedures/design. By project #4, the only information available to provide guidance in answering the OI guiding question is a list of the characterization tests learned during the course sequence with a few experimental details so students can accomplish the work within the allotted laboratory time.

Table 5.4. Level of Inquiry by Laboratory Session.

Characteristic	**GCL-I FS 1-4**	**GCL-I OI 1-4**	**GCL-II FS 1 & 2**	**GCL-II OI 1 & 2**	**GCL-II FS 3 & 4**	**GCL-II OI 3 & 4**
Problem /Question	P[a]	P	P	P	P	P
Theory /Background	P	P	P	P	P	P
Procedures /Design	P	P	P	P	P	NP
Results Analysis	P	NP	P	NP	NP	NP
Results Communication	NP[b]	NP	NP	NP	NP	NP
Conclusion	NP	NP	NP	NP	NP	NP
Level of Inquiry	Structured (0.5)	Guided (1)	Structured (0.5)	Guided (1)	Guided (1)	Open (2)

[a]P = provided
[b]NP = not provided

For an ADI course, content that predictability results in naturally-occurring variability is often chosen. (Note: this is the opposite of confirmation-type curriculum which relies on the students finding one correct answer.)[13,32] Therefore, an experiment that does not consistently provide good data for novices may work well for ADI. Another important aspect of ADI experiment design is the type of guiding question. Choosing a guiding question focusing on distinguishing (or identifying) instead of obtaining mathematical values supports variation in experimental design and data interpretation.[19]

While GCL-II's FS guiding questions often ask students to obtain specific mathematical values (such as melting points, retention factors, and percent yields), the OI guiding questions (which are central to the argumentation process) do not (Table 5.3). Furthermore, while the OI guiding questions of GCL-II's first three projects do have correct, scientifically sound answers, flaws in student extrapolation of FS procedure and data analysis in the OI sessions lead to varied data and result in differing claims between student teams. Finally, the last GCL-II project (#4) starts with a guiding question with only conditional answers. One team's product might be better because it is pure, while another team's product has a higher yield, and so on. Another benefit of this variability is that small experimental details are interchangeable in a way that does not affect project structure or documentation (learning outcomes, the manual, the answer key).

PROJECTS

GCL-II is a transitional course bridging general and organic chemistry with a mixture of concepts and techniques from the two sub-disciplines. The course is designed so major concepts and techniques, such as TLC, reoccur throughout projects. Projects also have enough variability to increase inquiry and foster discussion. TLC eluent, for example, is not dictated, and students can choose characterization tests when determining product identity. Furthermore, some conclusions depend on techniques learned (Projects 1 and 2) while others allow students to choose techniques they wish to perform (Projects 3 and 4).

Project #1: Menthol and Freezing Point Depression

Project 1 focuses on determining an unknown's identity with freezing and melting points. Many freezing-point depression experiments are known;[29,33–37] herein, we use menthol as a solvent and spice compounds as the solute. In the FS session, student teams measure menthol and cinnamic acid melting points using a melting point apparatus. Students also set up, measure, and analyze the

cooling curves for menthol and cinnamic acid-menthol solutions to determine freezing points and calculate their team's K_f and average K_f of menthol for the lab section.

For the OI, students are provided an unknown: cinnamic acid, 4-hydroxybenzaldehyde or vanillin. Students identify the unknown by measuring melting point and finding the molar mass using freezing point depression. Because the collection of the cooling curve requires proper technique (vigorous mixing), freezing point depression data are more varied compared to melting point data (Figure 5.3). As seen in Figure 5.3, the ability to determine the freezing point (based off of the graph's inflection point, or the plateau that follows) is more difficult with incorrect technique.

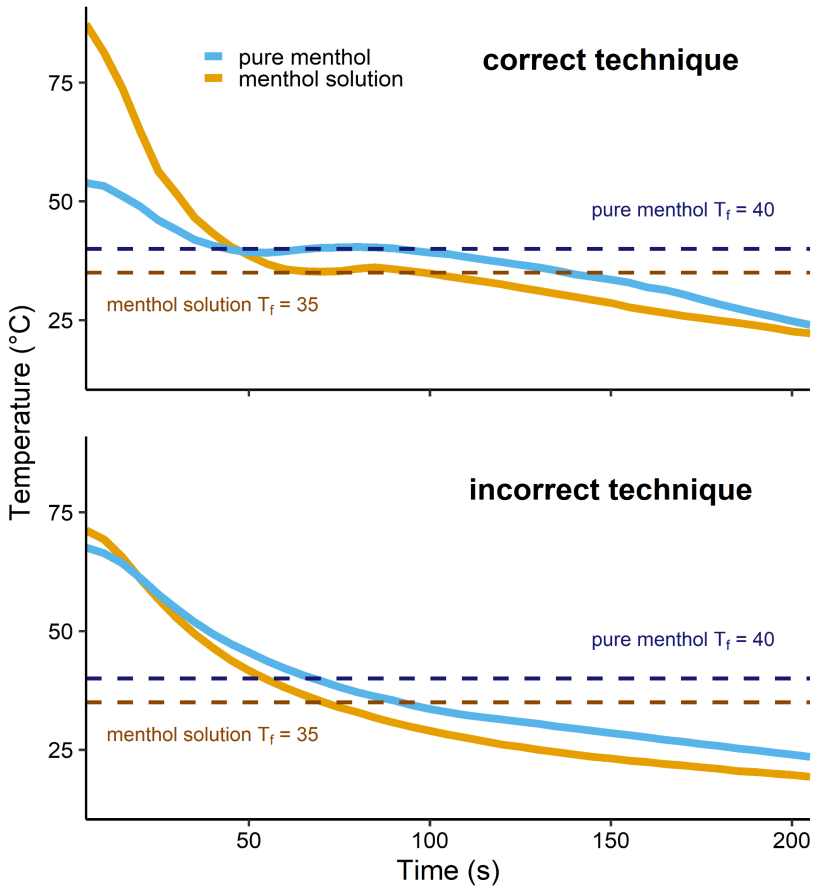


Figure 5.3. Cooling curves of pure menthol and menthol solution acquired by using correct (top) and incorrect (bottom) technique.

As part of argumentation, students must decide if they would use freezing point depression or melting point to characterize an unknown. A majority of students decide freezing point depression is less useful. For future projects, most students choose melting point over freezing point depression.

Project #2: Essential Oils and TLC

Project 2 focuses on thin-layer chromatography (TLC) and serves as a building block for later experiments. TLC is a separation technique widely employed in industry and laboratory courses.[38–40] Food-based analytes such as essential oils have been used for undergraduate TLC experiments since they are common, inexpensive, and often contain a mixture of organics for separation.[41,42] Inquiry-based TLC experiments have also been utilized, though these experiments are generally limited to factors modulating TLC performance.[38,43,44]

In the FS session, students are tasked to determine the best eluent to achieve good TLC separation of the provided standards: carvone, cinnamaldehyde, dihydrocarveol, eugenol, limonene, and vanillin. The eluents are of variable polarity, consisting of 1:1, 1:2, 2:3, or 3:2 heptane:acetone. (This procedure has since been improved; see appendix). Students work within a team, with each student developing TLC plates of all standards for a particular eluent ratio. All standards appear colorless, so UV light and permanganate dip techniques are employed to visualize aromatics/conjugated systems and oxidizable groups respectively. While the various eluent ratios produce varied separation between standards, results are also affected by student technique. Once all plates are developed, teammates compare R_f values to determine the best eluent ratio. (The eluent ratio with the most distinct standard R_f values is 3:2 heptane:acetone, Figure 5.4.) These R_f values are also used in the OI.

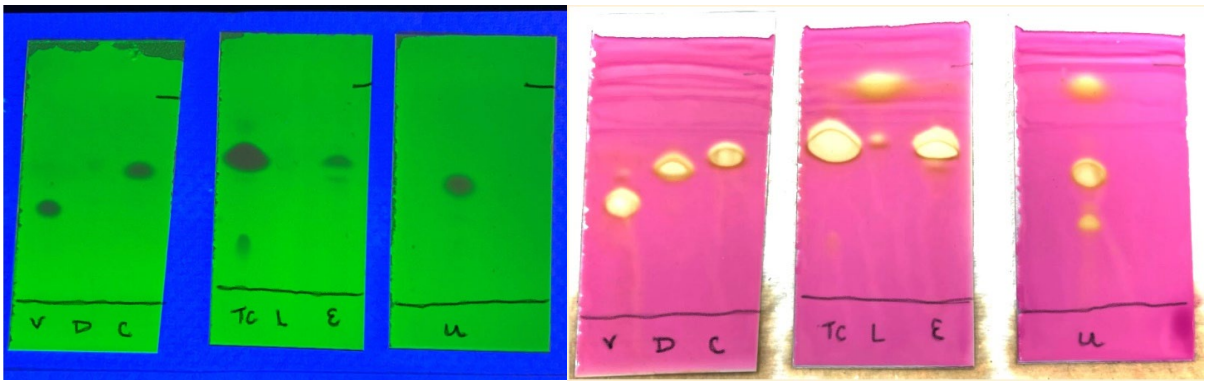


Figure 5.4. TLCs of essential oil standards under UV light and after a permanganate dip (left to right: vanillin (V), dihydrocarveol (D), carvone (C), *trans*-cinnamaldehyde (TC), limonene (L), eugenol (E)) and the unknown spearmint oil (U) (rightmost) in 3:2 heptane:acetone eluent mixture.

In the OI, teammates work together to determine the chemicals present in an unknown essential oil. Ideally, students select the best eluent ratio from the FS session to run TLC on their unknown, then compare R_f values between standard and unknown plates to determine the unknown components. Other approaches may include running multiple plates with different eluent ratios, and/or rerunning standard plates from the FS session. The unknown sample is the same for all students (spearmint oil), and contains only carvone, limonene, and dihydrocarveol. One of the unknown components (carvone) is UV-active, while the other two are visible after the permanganate dip. During argumentation, student results tend to vary both in eluent ratio selected and in the unknown components determined. For example, student claims vary from one to three unknown chemicals. Sample student responses (Table 5.5) and an argumentation poster example (Figure 5.5) are provided and 39% of students sampled (n =157) found 2 of the 3 right unknown compounds, and 56% of students used the best ratio (3:2 heptane:acetone).

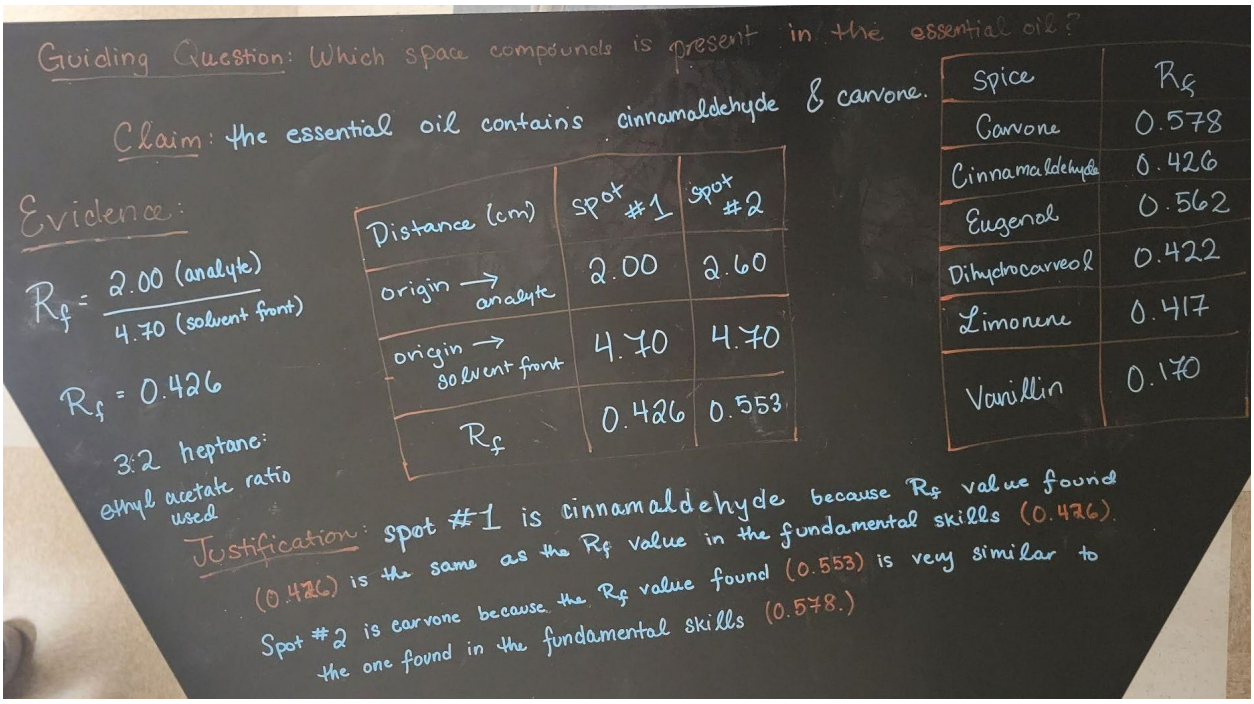


Figure 5.5. Example of a Project #2 Argumentation Poster. Poster has been rewritten from a student poster by the authors for increased legibility.

Table 5.5. Representative Sample of Project #2 Student Claims and the Eluent Ratios they used

Claim: Which essential oils are present in the unknown?	Eluent Ratio (heptane:acetone)
Carvone and limonene	2:3
Carvone and eugenol	2:3
Carvone	3:2
Carvone, dihydrocarveol and limonene	3:2

Project #3: Vanillin Oxidation and Melting Point

Projects 3 and 4 are adapted from literature[45–47] and focus on performing a synthesis during the FS session and then using characterization techniques of the student's choice during the OI session.

In Project 3, vanillin is reacted with hydrogen peroxide in the presence of horseradish peroxidase. The procedure is adapted from Vosburg[45], with one modification: students are

instructed to cool the reaction to room temperature before adding acetic acid and horseradish peroxidase solution (0.05 mg/mL). The FS session is the students' first exposure to organic synthesis and vacuum filtration. During the OI session, students are expected to determine the major product after the synthesis: vanillic acid, divanillin, or recovery of vanillin starting material. While students can choose from characterization techniques they have used before, melting point analysis between product and the provided standards of vanillin and vanillic acid allows for conclusive determination that divanillin is the synthesized product. Other techniques used to determine the identity of the product include solubility, UV-Visible spectroscopy (which students used in GCL-I) and TLC (sample data, Table 5.6). Because students successively build upon concepts learned in the course, no explicit procedure is provided for TLC or UV-Vis spectroscopy.

Table 5.6. Representative Sample of Student Data for Project #3 Chemicals.

	vanillin	**vanillic acid**	**divanillin**
Yield	n/a	n/a	57.8%[a]
Solubility	EtoAc, EtOH, water	EtOH, water	EtOH
Melting Point (°C)	81-83	210	315
R_f (in 7:3 ethyl acetate:heptane)	0.64	0.46	0.15

[a]Averaged from student data, n = 102, after excluding students who calculated above 100% yield

Project #4: Cinnamon Sunscreen and UV-Vis Spectroscopy

Project 4 focuses on the synthesis of two aldol products from cinnamaldehyde and either acetone or acetophenone. At this point in OC-I (typically taken with GCL-II), the students have not learned aldol condensation and do not know the mechanism; therefore, the experimental focus is product characterization (sample data, Table 5.7).

Table 5.7. Representative Sample of Student Data for Project #4 Chemicals.

	acetophenone aldol	**acetone aldol**
Yield	30-70%	40-50%
Solubility	EtOH (partial), EtOAc (partial)	EtOH, EtOAc
Melting Point (°C)	100-102	142-143
R_f (in 3:2 ethyl acetate:heptane)	0.57	0.46

The procedure was adapted from Jaworek-Lopez and Dicks, but with only acetone or acetophenone as the ketone partner to cinnamaldehyde.[46,47] The FS session focuses on the synthesis of either the cinnamaldehyde-acetone or cinnamaldehyde-acetophenone product; students work in teams of four, with each pair performing one synthesis. During the procedure, students are introduced to recrystallization to purify products. The OI session focuses on the student's choice of three previously used characterization techniques to determine which product is the more effective sunscreen. The main technique for determination is designed to be UV-Vis spectroscopy (Figure 5.6), but this characterization test is not required and is performed only at the student's prerogative. A conclusion can be made using whatever characterization tests are performed.

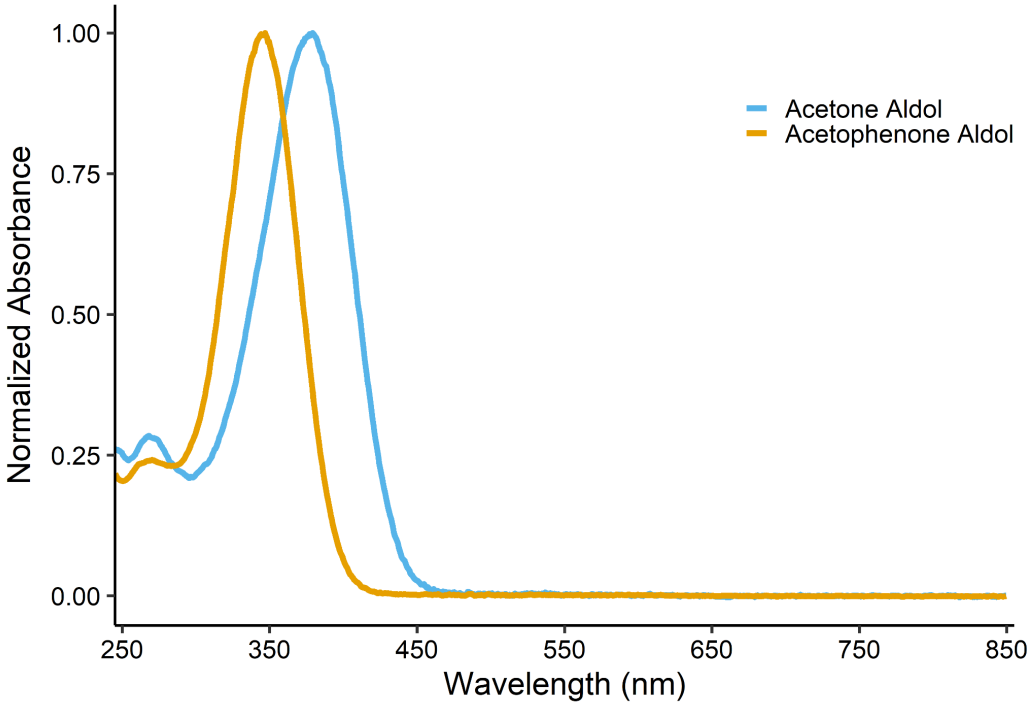


Figure 5.6. UV-Vis Spectra of Acetophenone and Acetone Aldol

Both products could be argued to be the most effective based on a myriad of factors, from the respective absorbances in the UV region to the color of each purified product (Table 5.8).

Table 5.8. Sample Project #4 Student Claims and Justifications

Claim: Which product is a better sunscreen?	**Sample Justification**
Acetophenone Aldol Product	"It is insoluble in water . . . so it will be effective as a water resistant sunscreen. In addition, it had a higher yield, and will therefore be more cost-effective."
Acetone Aldol Product	"[The] acetone [product] is a better sunscreen ingredient because it had a higher melting point than the [acetophenone product] . . and we want a sunscreen that will only melt at high temperatures."
Acetone Aldol Product	"[The acetone product] makes a better sunscreen . . . proven by the broader peak and the higher absorbance [relative to the acetophenone product] of the UV-Vis spectra."
Acetophenone Aldol Product	"The amount of UV light that the [acetophenone aldol product] absorbed was the determining factor. The peak is larger and broader

	than that for the [acetone aldol product]. From this, we can conclude that the acetophenone product absorbs a larger amount of UV light."

Hazards

While many chemicals used (vanillin, cinnamaldehyde, eugenol, carvone, etc.) are flammable, skin and/or eye irritants/sensitizers in concentrated or pure form, all chemicals are used in either small quantities or are provided in dilute forms. The organic solvents used (acetone, heptane, ethanol, and ethyl acetate) have the same hazards, with the addition of central nervous system toxicity. Heptane is used in place of hexane because of its lower volatility. Horseradish peroxidase (project #3) is a respiratory and skin sensitizer. Sodium hydroxide (3M, project #4) is corrosive to the skin and can cause eye damage.

If required, waste is neutralized with citric acid and sodium bicarbonate. Glass TLC spotters are collected and disposed of by the GTAs to minimize contamination and injury.

Required laboratory attire for GCL-II includes safety goggles, lab coats, long thick pants covering ankles, sturdy water-resistant closed toed shoes and nitrile gloves. These steps protect against exposure (to irritants, sensitizers, and corrosives) and cuts from broken glassware. All heating is done with hotplates and water baths to reduce the risk of ignition of flammable substances. Volatile organic solvents are used in the fume hood. No open flame is present in the laboratory.

Student Assessment & Specifications Grading

In specifications grading, assignments or rubric items are combined into bundles and the level to pass each bundle for a particular grade is specified. Each rubric item and bundled assignments are assessed as satisfactory or unsatisfactory. This grading method was first introduced by Linda Nilson in 2014[48]. Examples of specifications grading have been reported for

general chemistry, biochemistry, biology, math, anatomy and physiology, engineering, and physics lectures, as well as scientific writing courses, general, and organic chemistry laboratory.[1,49–62]

The specifications grading structure of GCL-II is the same as GCL-I[1]. For each project, three types (or grading bundles) of assignments are due: (1) the in-laboratory work done during the FS session; (2) the in-laboratory work done during the OI session; and, (3) the lab report completed after the OI session. All assignments have student facing rubrics with each assignment type (bundle) containing the same general rubric item categories (Table 5.9). A final assignment bundle is the laboratory practical consisting of: safety, technique, and argumentation. The practical contains all but two of the same general categories: (1) The safety part of the practical includes the safety rubric item; (2) the technique part includes procedure, observations, and data analysis rubric items; and, (3) the argumentation part includes data analysis, but focuses on argumentation rubric items (claim, justification, and evidence).

Table 5.9. General Rubric Items by Assignment Type

General Rubric Items	**Fundamental Skills (FS)**	**Original Investigation (OI)**	**Lab Reports**	**Final Exam**
Safety	✓	✓		✓
Objective or Purpose	✓	✓	✓	
Concepts	✓		✓	
Procedure	✓	✓	✓	✓
Observations	✓	✓		✓
Data Analysis	✓	✓	✓	✓
Argumentation	✓	✓	✓	✓

The goal of specifications grading is to focus students on the specific skills / knowledge needed to meet course learning outcomes.[48,50,54,63,64] The repetition of general rubric items throughout the courses is central to this effort. To ensure this repetition is obvious to students, rubric items are titled with a general rubric item name and then have a description specific to each assignment (SI Section II). Each rubric item is pass or no pass; no partial credit is given. To further encourage students to meet course learning outcomes,[58] students can revise and resubmit graded work in exchange for tokens earned by completing introductory course assignments, educational study surveys (described below), and mid-quarter GTA student evaluations (Appendix Section VIII). (Note: Completion rate for token earning opportunities (including educational surveys) typically exceeded 98% of course enrollment.)

For a given course grade, students must pass a given number of each of the assignment bundles (Fundamental Skills, Original Investigation, Laboratory Reports) and practical exam sections. Performance on the pre-laboratory quizzes dictates the assignment of + or - to the letter grade. This requirement is the same as published for GCL-I[1] (Appendix Section VIII).

Because specifications grading is a non-competitive grading process, teams work together in a collaborative manner, supporting the ADI process.[50,54,65] Furthermore, token earning and exchange permits revision on 5 of the 12 assignments (4 FS + 4 OI + 4 LR = 12). Token exchange for revision replaces the peer-review step often present in ADI.

RESULTS

Attitude toward the Subject of Chemistry Inventory

Because the **Attitude toward the Subject of Chemistry Inventory** (ASCI(V2)) has shown the impact of a curriculum change, we choose to use the survey to measure students' attitude toward chemistry midway through each quarter of GCL-I and GCL-II.[66–69] In general, students

indicate general chemistry laboratory is hard, tense, challenging work that is beneficial and somewhat interesting and worthwhile regardless of their enrollment in GCL-I or GCL-II. (Figure 5.7).

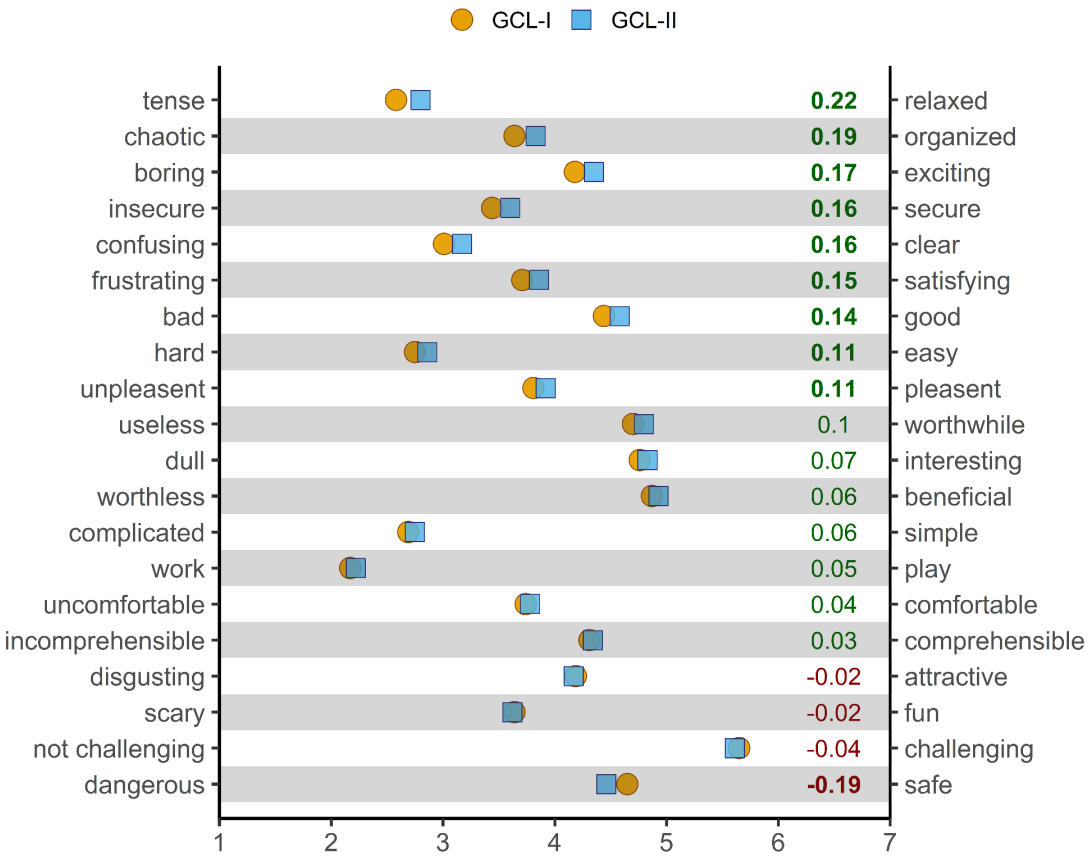


Figure 5.7. Attitude toward the Subject of Chemistry Inventory (ASCI (V2)) Results for GCL-I and GCL-II, Large On-Sequence Courses. Positive adjectives are shown on the right, their corresponding negative values on the left, reported as a continuum from 1-7. Average responses for GCL-I are shown in orange (●) and GCL-II in blue (■). Changes that are statistically significant ($p < 0.05$ with Mann-Whitney U test) are denoted in bolded text. Numerical data can be found in the appendix.

Going from GCL-I to GCL-II, a statistically positive shift occurred in 9 of 20 items surveyed (demonstrated by bold green average shift values, Figure 5.7). While on the negative side of the center value (4), GCL-II is more relaxed, more organized, more secure, and clearer,

than GCL-I. Furthermore, GCL-II is more exciting, with an increase of 0.17. It cannot be discounted, with increased time in the lab, students will become more comfortable, which may influence the positive trends seen.

The only significant negative attitude shift is students perceive GCL-II as less safe than GCL-I, with an average shift of -0.19. The increase in hazards between the GCL-I and GCL-II courses is a potential source. The hazards in the Gatorade themed GCL-I are limited to dilute solutions of acids, bases and bleach. No chemical reactions require heating and no vacuum is used. However, in GCL-II, the organic nature of many of the chemicals require the use of volatile organic solvents (and, therefore, fume hood use), oxidizers, reaction heating, et cetera in combination with safety curriculum covering these hazards.

Laboratory Course Assessment Survey

A modified version of the **Laboratory Course Assessment Survey** (LCAS) was given to GCL-I and GCL-II while students were engaged in the fourth and final project. LCAS, a 17-item survey designed to measure the effectiveness of course-based undergraduate research experiences (CUREs)[24], contains three sections: assessing student perception of peer collaboration, generation of new knowledge, and work revision and repetition. In addition to the above mentioned activities, the LCAS tool measures student perception of course activities central to ADI: experimental design, data collection / analysis and argumentation (Table 5.10). Conclusions from survey data are offered with the caveat that no control group was used for comparison.

Table 5.10. Modified LCAS Results Comparisons between GCL-I and GCL-II.

Course (Enrollment)	GCL-I (1224)		GCL-II (927)	
	Avg	*SD*	Avg	*SD*
Collaboration	22.1	*0.6*	22.2	*0.7*

C1. Discuss elements of my investigation with classmates and instructors	3.8	*0.8*	3.8	*0.6*
C2. Reflect on what I was learning	3.6	*0.8*	3.7	*0.7*
C3. Contribute my ideas and suggestions during class discussions	3.6	*0.7*	3.6	*0.8*
C4. Help other students collect or analyze data	3.7	*0.7*	3.7	*0.7*
C5. Provide constructive criticism and challenge each other's interpretations	3.7	*0.6*	3.6	*0.7*
C6. Share the problems and seek input on how to address them	3.7	*0.7*	3.7	*0.7*
Discovery / Relevance	18.4	*0.9*	19.0	*1.1*
D1. Generate novel results that could be of interest the community	3.0	*1.2*	3.2	*1.2*
D2. Conduct an investigation to find something previously unknown	3.6	*1.1*	3.7	*1.19*
D3. Formulate my own research question or hypothesis to guide an investigation	4.0	*1.0*	4.0	*1.07*
D4. Develop new arguments based on data	4.1	*1.0*	4.2	*0.91*
D5. Explain how my work has resulted in new scientific knowledge	3.8	*0.9*	3.9	*1.14*
Iteration	20.9	*1.0*	22.7	*1.1*
I1. Revise and repeat work to account for errors or fix problems	3.2	*1.3*	3.6	*1.2*
I2. Change methods of investigation if it was not unfolding as predicted	3.1	*0.9*	3.5	*1.2*
I3. Share and compare data with other students	4.0	*1.0*	4.3	*0.9*
I4. Collect and analyze additional data to address new questions	4.1	*1.0*	3.8	*1.2*
I5. Revise and repeat analyses based on	4.1	*1.0*	3.8	*1.2*

feedback				
I6. Revise drafts of papers or presentations based on feedback	3.5	*1.0*	3.8	*1.1*

Collaboration was measured on a four point scale: weekly (4), monthly (3), 1 or 2 times (2) and never (1). Both Discovery / Relevance and Iteration were modified from the six point scale in the earlier work to a five point scale: (5) strongly agree, (4) somewhat agree, (3) neither, (2) somewhat disagree, and (1) strongly disagree. The sum of the averages for each of the three categories is reported in the gray box in the average (Avg) column.

The responses in the Collaboration (C1-C6) section are consistently high (Table 5.10). On average most activities occur almost weekly for both GCL-I and GCL-II. This consistent response indicates the ADI team structure results in the perception of collaboration (C1-C6).

The Discovery/Relevance (D1-D5) section contains survey items closely connected with the inquiry processes of the course: designing experimentation, forming a hypothesis, creating an argument and communicating work. Overall, the averaged response shows small increases in agreement strength from GCL-I to GCL-II, indicating students perceive these inquiry processes are occurring repeatedly throughout the course sequence (Table 5.10). The agreement with the item about new argument creation based on data (D4) is the highest agreement level of all survey items. Furthermore, the agreement to this item increases slightly from GCL-I to GCL-II, suggesting the argumentation sessions (which occur with each project) play a prominent role in the students' perception of the curriculum. Conversely, the "generate novel results… of interest to the community" item (D1) garnered a neutral response in both GCL-I and GCL-II. The theme-based nature of the courses could explain this response. GCL-II's spice theme (like GCL-I's Gatorade

theme) was used to provide students with a familiar connection to the course content, but also reduces the "novelty" of the subject matter.

Multiple survey items showed significant increases in agreement in the Iteration section (I1-I6), specifically in items related to revision (I1 and I2, Table 10). Rather than the period-long techniques in GCL-I, GCL-II provides students with multiple small characterization tests which can be repeated. The time barrier that restricted multiple tries of a single technique is not as prevalent in the GCL-II, which is likely tied to the increase in agreement to I1 and I2. This can also be seen in the previously presented ASCI (V2) survey results, which indicate students in GCL-II felt more organized and more relaxed than in GCL-I.

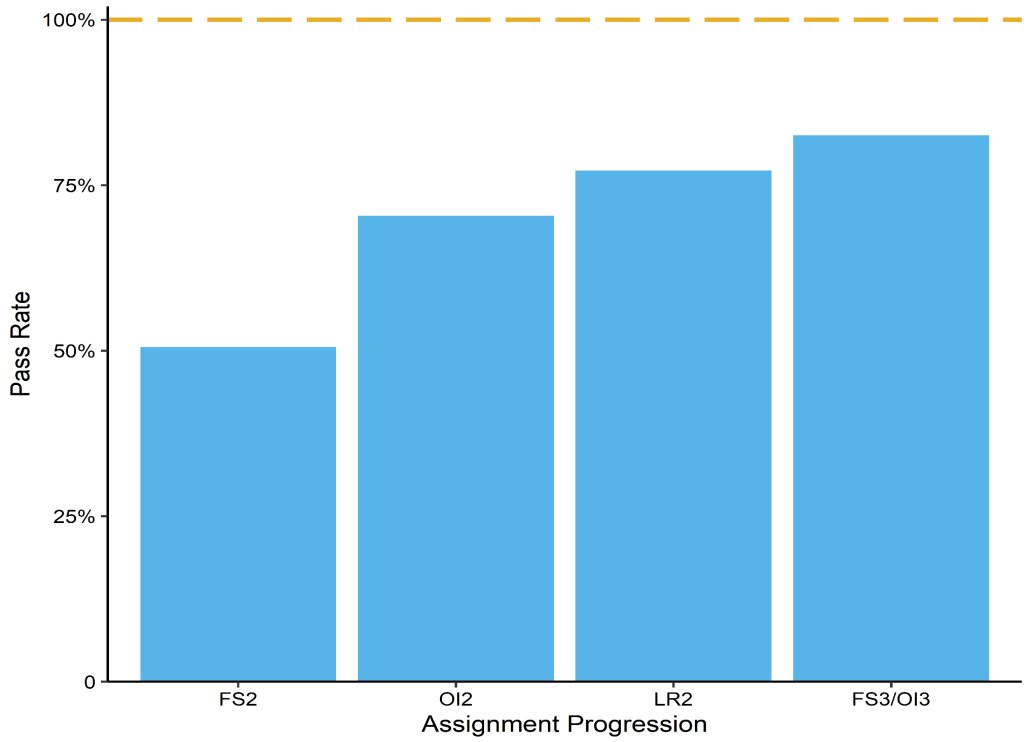


Figure 5.8. Student pass rates on rubric items concerning TLC technique throughout GCL-II.

The effect of iteration is shown in students' mastery of TLC. TLC is repeated throughout GCL-II: in the FS of Project #2, then in the OI and LR of Project #2, and finally in Project #3 with only basic eluent information provided. The pass rate for TLC (Figure 5.8) increases from an initial 50.5% pass rate to a 77.2% pass rate within Project #2, and increases again to a 82.5% pass rate during Project #3, indicating increasing retention of TLC techniques and concepts.

SUMMARY AND FUTURE WORK

Herein, we have presented a theme-based, specifications-graded, ADI-focused lab for GCL-II. The thematic connection was well-received in GCL-I and was continued to connect projects and increase the relevance of the content.[1] Furthermore, the iterative application of methods and skills from previous projects gives student teams increasing responsibility and freedom to collaboratively develop experimental design skills.

The modified LCAS results indicate the GCL-II course results in varying student engagement compared to GCL-I, mostly showing small to significant increases, especially for iteration. The designed repetition of fundamental concepts and techniques results in increased comprehension, as shown in increasing pass rate on related rubric items as the course proceeds. From GCL-I to GCL-II, the ASCI (V2) results show a positive attitude shift, notably with students considering GCL-II to be more relaxed, more organized, and more exciting than GCL-I.

Since the results presented here, adjustments have been made to Project #2 (detailed in the appendix). Future adjustments are being made to the specifications grading tools, such as switching to general rubric items from assignment bundles to ensure a passing score reflects proficiency for course objectives. Technique videos are also being developed and implemented to enhance retention and iteration.

REFERENCES

(1) Howitz, W. J.; Frey, T.; Saluga, S. J.; Nguyen, M.; Denaro, K.; Edwards, K. D. A Specifications-Graded, Sports Drink-Themed General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach. *J. Chem. Educ.* **2023**, *100* (2), 672–680.

(2) Bell, P. Design of a Food Chemistry-Themed Course for Nonscience Majors. *J. Chem. Educ.* **2014**, *91* (10), 1631–1636.

(3) Byrd, H.; Donnell, S. E. O. A General Chemistry Laboratory Theme: Spectroscopic Analysis of Aspirin. *J. Chem. Educ.* **2003**, *80* (2), 174.

(4) Shultz, M. J.; Kelly, M.; Paritsky, L.; Wagner, J. A Theme-Based Course: Hydrogen as the Fuel of the Future. *J. Chem. Educ.* **2009**, *86* (9), 1051.

(5) Logan, J. L.; Rumbaugh, C. E. The Chemistry of Perfume: A Laboratory Course for Nonscience Majors. *J. Chem. Educ.* **2012**, *89* (5), 613–619.

(6) Nivens, D. A.; Padgett, C. W.; Chase, J. M.; Verges, K. J.; Jamieson, D. S. Art, Meet Chemistry; Chemistry, Meet Art: Case Studies, Current Literature, and Instrumental Methods Combined To Create a Hands-On Experience for Nonmajors and Instrumental Analysis Students. *J. Chem. Educ.* **2010**, *87* (10), 1089–1093.

(7) Hopkins, T. A.; Samide, M. Using a Thematic Laboratory-Centered Curriculum To Teach General Chemistry. *J. Chem. Educ.* **2013**, *90* (9), 1162–1166.

(8) Leber, P. A.; Szczerbicki, S. K. Theme-Based Bidisciplinary Chemistry Laboratory Modules. *J. Chem. Educ.* **1996**, *73* (12), 1130.

(9) Chaplin, S. B.; Manske, J. M. A Theme-Based Approach to Teaching Nonmajors Biology: Helping Students Connect Biology to Their Lives. *J. Coll. Sci. Teach.* **2005**, *35* (1), 47.

(10) Tessier, L.; Tessier, J. Theme-Based Courses Foster Student Learning and Promote Comfort with Learning New Material. *J. Learn. Arts* **2015**, *11* (1).

(11) Wingert, J. R.; Wasileski, S. A.; Peterson, K.; Mathews, L. G.; Joy, A.; Clarke, D. Enhancing Integrative Experiences: Evidence of Student Perceptions of Learning Gains from Cross-Course Interactions. *Journal of the Scholarship of Teaching and Learnin*g, **2011**, *11* (3) 34-57.

(12) Mangan, K. *'Fear and Horror'-Themed Courses Help Keep Texas System's Students Engaged*. The Chronicle of Higher Education, 4 March, 2014. https://www.chronicle.com/article/fear-and-horror-themed-courses-help-keep-texas-systems-students-engaged/ (accessed 2023-08-22).

(13) Domin, D. S. A Review of Laboratory Instruction Styles. *J. Chem. Educ.* **1999**, *76* (4), 543.

(14) Abraham, M. R. What Can Be Learned from Laboratory Activities? Revisiting 32 Years of Research. *J. Chem. Educ.* **2011**, *88* (8), 1020–1025.

(15) Hosbein, K. N.; Alvarez-Bell, R.; Callis-Duehl, K. L.; Sampson, V.; Wolf, S. F.; Walker, J. P. Development of the Investigation Design, Explanation, and Argument Assessment for General Chemistry I Laboratory. *J. Chem. Educ.* **2021**, *98* (2), 293–306.

(16) Clark, T. M.; Ricciardo, R.; Weaver, T. Transitioning from Expository Laboratory Experiments to Course-Based Undergraduate Research in General Chemistry. *J. Chem. Educ.* **2016**, *93* (1), 56–63.

(17) Walker, J. P.; Sampson, V. Learning to Argue and Arguing to Learn: Argument-Driven Inquiry as a Way to Help Undergraduate Chemistry Students Learn How to Construct

Arguments and Engage in Argumentation During a Laboratory Course. *J. Res. Sci. Teach.* **2013**, *50* (5), 561–596.
(18) Grooms, J.; Enderle, P.; Sampson, V. Coordinating Scientific Argumentation and the Next Generation Science Standards through Argument Driven Inquiry. **2015**, *24* (1).
(19) Walker, J. P.; Wolf, S. F. Getting the Argument Started: A Variation on the Density Investigation. *J. Chem. Educ.* **2017**, *94* (5), 632–635.
(20) Walker, J. P.; Sampson, V.; Southerland, S.; Enderle, P. J. Using the Laboratory to Engage All Students in Science Practices. *Chem. Educ. Res. Pract.* **2016**, *17* (4), 1098–1113.
(21) Bruck, L. B.; Bretz, S. L.; Towns, M. H. Characterizing the Level of Inquiry in the Undergraduate Laboratory. *Journal of College Science Teaching,* **2008***, 38* (1), 52-58.
(22) Seda Cetin, P.; Eymur, G.; Southerland, S. A.; Walker, J.; Whittington, K. Exploring the Effectiveness of Engagement in a Broad Range of Disciplinary Practices on Learning of Turkish High-School Chemistry Students. *Int. J. Sci. Educ.* **2018**, *40* (5), 473–497.
(23) Walker, J. P.; Sampson, V.; Grooms, J.; Anderson, B.; Zimmerman, C. O. Argument-Driven Inquiry in Undergraduate Chemistry Labs: The Impact on Students' Conceptual Understanding, Argument Skills, and Attitudes Toward Science. *J. Coll. Sci. Teach.* **2012**, *41* (4), 74–81.
(24) Corwin, L. A.; Runyon, C.; Robinson, A.; Dolan, E. L. The Laboratory Course Assessment Survey: A Tool to Measure Three Dimensions of Research-Course Design. *CBE—Life Sci. Educ.* **2015**, *14* (4), ar37.
(25) Walker, J. P.; Sampson, V.; Zimmerman, C. O. Argument-Driven Inquiry: An Introduction to a New Instructional Model for Use in Undergraduate Chemistry Labs. *J. Chem. Educ.* **2011**, *88* (8), 1048–1056.
(26) Bruck, L. B.; Bretz, S. L.; Towns, M. H. A Rubric to Guide Curriculum Development of Undergraduate Chemistry Laboratory: Focus on Inquiry. In *Chemistry Education in the ICT Age*; Gupta-Bhowon, M., Jhaumeer-Laulloo, S., Li Kam Wah, H., Ramasami, P., Eds.; Springer Netherlands: Dordrecht, 2009; pp 75–83.
(27) Banchi, H.; Bell, R. The Many Levels of Inquiry. *Sci. Child.* **2008**, *46* (2), 26–29.
(28) Fay, M. E.; Grove, N. P.; Towns, M. H.; Bretz, S. L. A Rubric to Characterize Inquiry in the Undergraduate Chemistry Laboratory. *Chem. Educ. Res. Pract.* **2007**, *8* (2), 212–219.
(29) Allen, J. B.; Barker, L. N.; Ramsden, J. H. Guided Inquiry Laboratory. *J. Chem. Educ.* **1986**, *63* (6), 533.
(30) Driver, R.; Newton, P.; Osborne, J. Establishing the Norms of Scientific Argumentation in Classrooms. *Sci. Educ.* **2000**, *84* (3), 287–312.
(31) NGSS Lead States. *Next Generation Science Standards: For States, By States;* The National Academies Press: Washington, DC, 2013.
(32) Katchevich, D.; Hofstein, A.; Mamlok-Naaman, R. Argumentation in the Chemistry Laboratory: Inquiry and Confirmatory Experiments. *Res. Sci. Educ.* **2013**, *43* (1), 317–345.
(33) D'Amelia, R. P.; Franks, T.; Nirode, W. F. Introduction of Differential Scanning Calorimetry in a General Chemistry Laboratory Course: Determination of Molar Mass by Freezing Point Depression. *J. Chem. Educ.* **2006**, *83* (10), 1537.
(34) Johnson, T. R.; Shaffer, T. A.; Holland, L. A.; Veltri, L. M.; Lucas, J. A.; Elshamy, Y. S.; Rutto, P. K. A Low-Cost and Simple Demonstration of Freezing Point Depression and Colligative Properties with Common Salts and Ice Cream. *J. Chem. Educ.* **2022**, *99* (10), 3590–3594.

(35) Novo, M.; Reija, B.; Al-Soufi, W. Freezing Point of Milk: A Natural Way To Understand Colligative Properties. *J. Chem. Educ.* **2007**, *84* (10), 1673.
(36) Parker, R. C. Molecular Weight of Volatile Liquids by Freezing Point Depression. *J. Chem. Educ.* **1974**, *51* (7), 492.
(37) McCarthy, S. M.; Gordon-Wylie, S. W. A Greener Approach for Measuring Colligative Properties. *J. Chem. Educ.* **2005**, *82* (1), 116.
(38) Dickson, H.; Kittredge, K. W.; Sarquis, A. M. Thin-Layer Chromatography: The "Eyes" of the Organic Chemist. *J. Chem. Educ.* **2004**, *81* (7), 1023.
(39) Silver, J. Let Us Teach Proper Thin Layer Chromatography Technique! *J. Chem. Educ.* **2020**, *97* (12), 4217–4219.
(40) Brinkman, U. A. Th.; de Vries, G. Chemically Bonded Stationary Phases in (HP)TLC. In *Drug Determination in Therapeutic and Forensic Contexts*; Reid, E., Wilson, I. D., Eds.; Methodological Surveys in Biochemistry and Analysis; Springer US: Boston, MA, 1984; pp 99–100.
(41) Davies, D. R.; Johnson, T. M. Isolation of Three Components from Spearmint Oil: An Exercise in Column and Thin-Layer Chromatography. *J. Chem. Educ.* **2007**, *84* (2), 318.
(42) Pelter, L. S. W.; Amico, A.; Gordon, N.; Martin, C.; Sandifer, D.; Pelter, M. W. Analysis of Peppermint Leaf and Spearmint Leaf Extracts by Thin-Layer Chromatography. *J. Chem. Educ.* **2008**, *85* (1), 133.
(43) Thomson, P. I. T.; Lamie, P. Introducing Elements of Inquiry and Experimental Design in the First Year of an Undergraduate Laboratory Program. *J. Chem. Educ.* **2022**, *99* (12), 4118–4123.
(44) Nash, J. J.; Meyer, J. A.; Everson, B. What Factors Affect the Separation of Substances Using Thin-Layer Chromatography? An Undergraduate Experiment. *J. Chem. Educ.* **2001**, *78* (3), 364.
(45) Nishimura, R. T.; Giammanco, C. H.; Vosburg, D. A. Green, Enzymatic Syntheses of Divanillin and Diapocynin for the Organic, Biochemistry, or Advanced General Chemistry Laboratory. *J. Chem. Educ.* **2010**, *87* (5), 526–527.
(46) Ter Meer Guardia, L. K.; Belli, A. J.; Molta, G. J.; Gordon, P.; Jaworek-Lopes, C. H. Green Crossed Aldol Condensation Reactions Using Trans- Cinnamaldehyde. *Chem. Educ.* **2011**, *16*, 23–25.
(47) Stabile, R. G.; Dicks, A. P. Two-Step Semi-Microscale Preparation of a Cinnamate Ester Sunscreen Analog. *J. Chem. Educ.* **2004**, *81* (10), 1488.
(48) Nilson, L. B. *Specifications Grading: Restoring Rigor, Motivating Students, and Saving Faculty Time*; Stylus Publishing, LLC, 2015.
(49) Noell, S. L.; Rios Buza, M.; Roth, E. B.; Young, J. L.; Drummond, M. J. A Bridge to Specifications Grading in Second Semester General Chemistry. *J. Chem. Educ.* **2023**, *100* (6), 2159–2165.
(50) Donato, J. J.; Marsh, T. C. Specifications Grading Is an Effective Approach to Teaching Biochemistry. *J. Microbiol. Biol. Educ.* **2023**, *0* (0), e00236-22.
(51) Katzman, S. D.; Hurst-Kennedy, J.; Barrera, A.; Talley, J.; Javazon, E.; Diaz, M.; Anzovino, M. E. The Effect of Specifications Grading on Students' Learning and Attitudes in an Undergraduate-Level Cell Biology Course. *J. Microbiol. Biol. Educ.* **2021**, *22* (3), e00200-21.
(52) Evensen, H. Specifications Grading in General Physics and Engineering Physics Courses; Presented at American Society for Engineering Education, 2022.

(53) McKnelly, K. J.; Morris, M. A.; Mang, S. A. Redesigning a "Writing for Chemists" Course Using Specifications Grading. *J. Chem. Educ.* **2021**, *98* (4), 1201–1207.
(54) Howitz, W. J.; McKnelly, K. J.; Link, R. D. Developing and Implementing a Specifications Grading System in an Organic Chemistry Laboratory Course. *J. Chem. Educ.* **2021**, *98* (2), 385–394.
(55) Prasad, P. V. Using Revision and Specifications Grading to Develop Students' Mathematical Habits of Mind. *PRIMUS* **2020**, *30* (8–10), 908–925.
(56) Tsoi, M. Y.; Anzovino, M. E.; Erickson, A. H. L.; Forringer, E. R.; Henary, E.; Lively, A.; Morton, M. S.; Perell-Gerson, K.; Perrine, S.; Villanueva, O.; Whitney, M.; Woodbridge, C. M. Variations in Implementation of Specifications Grading in STEM Courses. **2019**.
(57) Ring, J. ConfChem Conference on Select 2016 BCCE Presentations: Specifications Grading in the Flipped Organic Classroom. *J. Chem. Educ.* **2017**, *94* (12), 2005–2006.
(58) Boesdorfer, S. B.; Baldwin, E.; Lieberum, K. A. Emphasizing Learning: Using Standards-Based Grading in a Large Nonmajors' General Chemistry Survey Course. *J. Chem. Educ.* **2018**, *95* (8), 1291–1300.
(59) Martin, L. J. Introducing Components of Specifications Grading to a General Chemistry I Course. In *ACS Symposium Series*; Kradtap Hartwell, S., Gupta, T., Eds.; American Chemical Society: Washington, DC, 2019; Vol. 1330, pp 105–119.
(60) Mendez, J. Standards-BasedSpecifications Grading in Thermodynamics. Presented at the 2018 ASEE Annual Conference & Exposition; 2018.
(61) Mendez, J. Standards-Based Specifications Grading in a Hybrid Course. Presented at the 2018 ASEE Annual Conference & Exposition; 2018.
(62) Carlisle, S. Simple Specifications Grading. *PRIMUS* **2020**, *30* (8–10), 926–951.
(63) Blackstone, B.; Oldmixon, E. Specifications Grading in Political Science. *J. Polit. Sci. Educ.* **2019**, *15* (2), 191–205.
(64) Toledo, S.; Dubas, J. M. A Learner-Centered Grading Method Focused on Reaching Proficiency with Course Learning Outcomes. *J. Chem. Educ.* **2017**, *94* (8), 1043–1050.
(65) McKnelly, K. J.; Howitz, W. J.; Thane, T. A.; Link, R. D. Specifications Grading at Scale: Improved Letter Grades and Grading-Related Interactions in a Course with over 1,000 Students. ChemRxiv August 18, 2022.
(66) Bauer, C. F. Attitude toward Chemistry: A Semantic Differential Instrument for Assessing Curriculum Impacts. *J. Chem. Educ.* **2008**, *85* (10), 1440.
(67) Mooring, S. R.; Mitchell, C. E.; Burrows, N. L. Evaluation of a Flipped, Large-Enrollment Organic Chemistry Course on Student Attitude and Achievement. *J. Chem. Educ.* **2016**, *93* (12), 1972–1983.
(68) Xu, X.; Lewis, J. E. Refinement of a Chemistry Attitude Measure for College Students. *J. Chem. Educ.* **2011**, *88* (5), 561–568.
(69) Brandriet, A. R.; Xu, X.; Bretz, S. L.; Lewis, J. E. Diagnosing Changes in Attitude in First-Year College Chemistry Students with a Shortened Version of Bauer's Semantic Differential. *Chem. Educ. Res. Pract.* **2011**, *12* (2), 271–278.

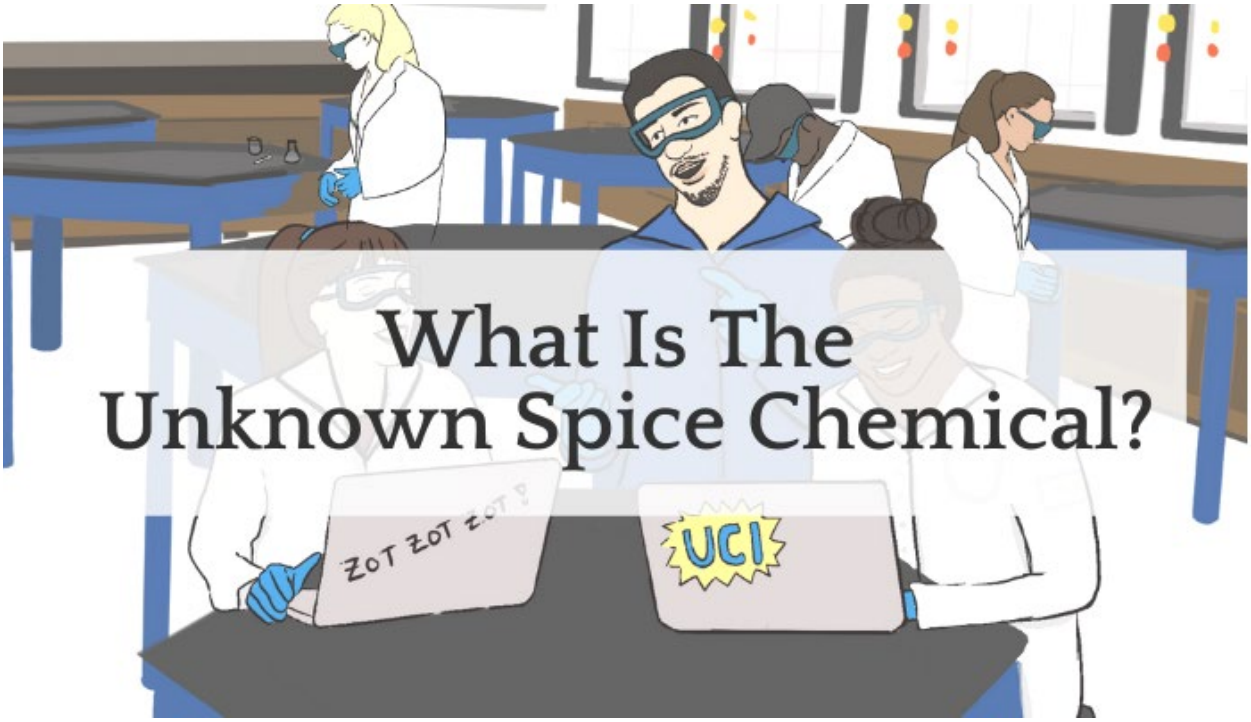
APPENDIX

I. IRB Statement

This study was approved by the University of California, Irvine, Institutional Review Board as exempt (IRB 2018-4661) including FERPA compliance.

II. Student Lab Manuals

a. Project #1 Student Lab Manual



PRE-LAB QUIZ ***Quiz #1 Session #1***

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Sections 2.1.1) and in documents hyperlink within this document.

INTRODUCTION

Argument Driven Inquiry Process

As in Chem 1LC, you will be introduced to ***Fundamental Skills*** (basic skills that will help you complete the ***Original Investigation***) in the first part of each project. You and your team will use what you've learned during the Fundamental Skills section to create a unique plan to tackle an experimental question (the second Guiding Question) and create a "poster" on the benchtop in the lab. The poster should contain the following sections:

- Guiding Question(s): given in each project
- Evidence: neatly and concisely arranged data and/or results tables
- Claim: the answer to the guiding question
- Justification: an explanation using scientific theories or laws that ties together the evidence and the claim

During the ***Argumentation*** (or poster session), one team member serves as the "spokesperson" - staying with the poster to answer questions from your peers. The rest of the team are "travelers" who will visit other posters and engage in discussion to challenge the other teams' reasoning **and** reevaluate their own. The discoveries made during the argumentation process are pivotal to answering these three fundamental questions in your individual (not team) ***Lab Report***:

- What concept(s) were you investigating and how are they related to the guiding question(s)?
- How did you go about your work and why? If you found value in another team's evidence collection or justification, contrast it with the work your team did.
- What is your argument? (In other words, use the evidence you collected to justify your claim.) Indicate if/how your argument was altered by the poster session.

In this first project you will investigate melting and freezing points and see how the formation of a solution affects these physical properties. In the ***Fundamental Skills*** section, you will determine the freezing point depression constant (K_f) for a solution of cinnamic acid dissolved in menthol. In the ***Original Investigation*** section, you will determine the identity of an unknown solute dissolved in menthol. You will then compare and contrast your results with your peers in the ***Argumentation*** section. Before the next session, you will write a ***Lab Report*** about this experiment. Refer to the assignment formatting document for necessary components for all 3 assignment types.

Melting & Freezing Points

What is the difference between a melting point and a freezing point? Put simply, it's whether the temperature is increasing or decreasing toward the temperature in question.

A **melting point** is the temperature at which the kinetic energy of a substance's particles (i.e. molecules) is high enough to slide past each other; it is the temperature when a solid becomes a liquid. A **freezing point** is the temperature at which the kinetic energy of a substance's particles (i.e. molecules) is low enough to become closely packed and vibrate in place; it is the temperature at which a liquid becomes a solid. For a particular substance both the melting and freezing point is the same temperature, what the temperature is called depends on whether the

substance is being heated or cooled. A **cooling (or heating) curve** (Figure 1) demonstrates the connection between the two physical properties. The label T_b (for boiling point) and T_f (for freezing point) are typically used, as they are involved in the colligative properties of freezing point depression (to be discussed below) and boiling point elevation.

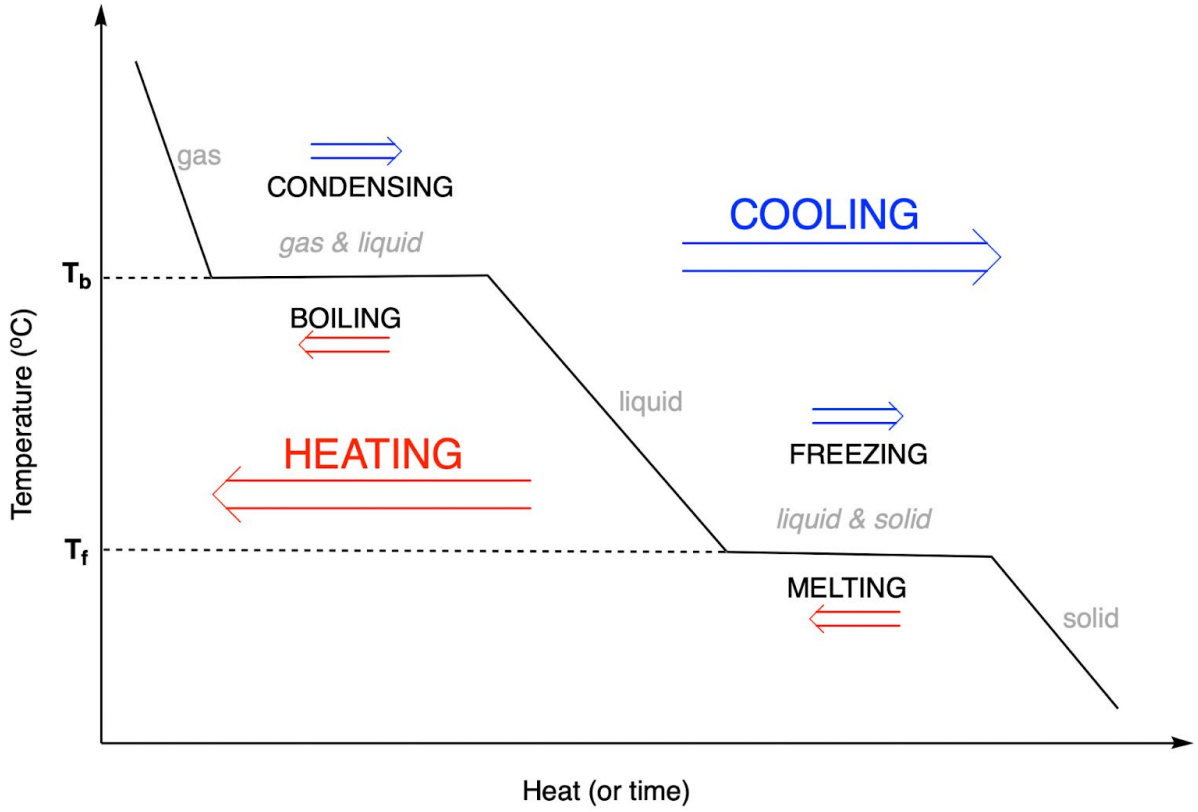


Figure 1. *Cooling / Heating Curve*

The melting (or freezing) point of menthol, one of the main chemicals used in this project, varies from 42-45 °C depending if it is (-)-menthol, the stereoisomer which makes up most of natural menthol, or 36-38 °C if it is a racemic mixture of (-)-menthol and its stereoisomer (+)-menthol.

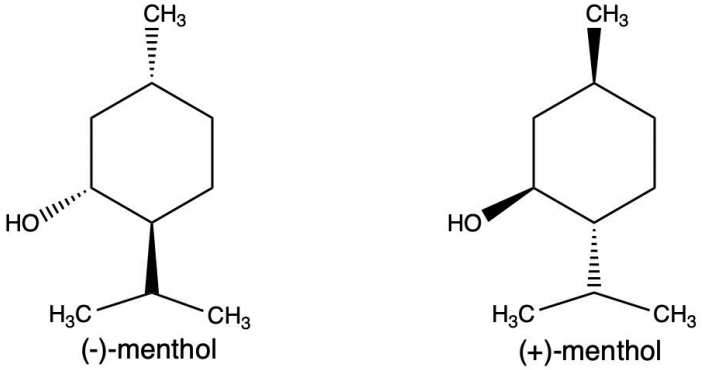


Figure 2. *Common Menthol Stereoisomers*

Freezing Point Depression

When a solute is dissolved in a solvent, the freezing point temperature is lowered in proportion to the number of moles of solute added. This property, known as freezing point depression, is a *colligative property*; it depends on the ratio of solute and solvent particles and not on the identity of the substance itself. The equation for this relationship is:

$$\Delta T = T_{f(solvent)} - T_{f(solution)} = K_f \cdot m$$

where ΔT is the freezing point depression, K_f is the freezing point depression constant specific for a given solvent (menthol in this experiment), and m is the molality of the solution. Molality (m) is used because it is independent of the volume changes that can occur with variations in temperature, unlike molarity (M).

molality (m) = moles of solute / kg of solvent

In this project, the freezing temperature of the pure solvent, menthol, is found first. Next the freezing point of a solution is measured. This will provide the data needed to calculate the freezing point depression constant. Once this value is known, measuring the masses of a solute in menthol, calculating freezing point depression, ΔT, and, the equations above can be used to find the molecular weight of the solute.

molecular weight = mass / moles

Solutions & Solubility

The concept of **solubility** is central to many chemical processes. In Chem 1LC you have already relied on solubility - the ability of a liquid solvent to dissolve a solid solute. Sucrose and chemical dyes were dissolved in the deionized water. In this project menthol, a solid at room temperature, serves as a solvent once it is melted. Then a solid solute will be introduced into this warm liquid. As long as this solute's melting point is above the temperature of the solution, it dissolves, it does not melt.

The idea of solubility has a few layered nuances:

- On a qualitative level, a simple, straightforward indication of solubility is a visual observation of a solute "disappearing" into a solvent.
- Solubility also refers to a quantitative value for a particular solute in a particular solvent at a given temperature. For example, the solubility of sodium chloride is 35.9 g/L in water and 0.65 g/L in ethanol. Adding a solute mass greater than the quantitative solubility will result in the presence of solid in the solution. The presence of solid in this situation does not indicate insolubility, it indicates that the solution is saturated - no more solid will dissolve.

When measuring freezing point depression, solubility must be taken into account. The amount of solute added to solvent should not exceed its solubility.

GUIDING QUESTION(S)

You will be provided a Digi-Melt, menthol, test tubes, a temperature probe and various solutes (cinnamic acid, vanillin and hydroxybenzaldehyde). Using a water bath on a hot plate, determine the melting and freezing points of the pure substances and solutions.

1. *Fundamental Skills*: What is the average freezing point depression constant for the lab section? What is the melting point of the solute?
2. *Original Investigation*: What is the identity of the unknown spice chemical? What is the best method for determining the identity?

Session One

SAFETY MOMENT (WEEK 1):

Emergency Flip Book:

Students will be assigned an emergency scenario from this list. As a group, spend ~5 minutes to discuss the appropriate responses. Select a speaker to present the group's plans to the class. Refer to the UCI Emergency Procedure Flip Chart for reference as you discuss the scenarios. Describe your plans in 2-3 sentences on the *Fundamental Skills* ELN.

FUNDAMENTAL SKILLS

Before your lab section:

- Read the Introduction, Fundamental Skills, and hyperlinked documents in those sections.
- Take the Prelab Quiz on Canvas.
- Complete the Objectives, Chemical, Safety (GHS) tables, and a rough draft of the Procedures in the Fundamental Skills page in your ELN.

At the beginning of the lab section, your TA will:

- Make sure you are dressed appropriately and have personal protective equipment (lab coat, goggles and gloves).

- *Assign you to a team of 3-4 students for the quarter.* Although much of the work in this course is teamwork, you must fill out your own ELN pages and create your own plots. While teamwork will introduce strong similarities between the work of team members, word-for-word copying is a violation of UCI's Academic Integrity policy.
    - *For this experiment, students will be working in separate pairs for the Fundamental Skills portion (parts A-C). The team will regroup for the Original Investigation portion during the second half of the experiment.*
- Briefly introduce the ADI process & grading policies for the course.
- Make sure a team leader is nominated for the project.
- Demonstrate the set up and use of Digi-Melt and temperature probe (stirring and probe removal).
- Send out a link for a shared data spreadsheet which will be used to determine the average K_f for this lab session.

Safety: **Goggles, a lab coat, thick long pants completely covering the ankles, and sturdy water resistant closed toed shoes are required when performing any experiment utilizing chemicals, glassware, and/or lab equipment in a chemistry laboratory. Chemicals in this experiment (**menthol, vanillin and 4-hydroxybenzaldehyde**) can cause eye and skin irritation or damage.**

- Avoid contact with skin and eyes. Wash affected areas immediately if a chemical comes into contact with bare skin. If a large quantity is splashed on you, notify a labmate and/or TA while moving toward the eye wash (by the lab door) or shower (in the hallway). *Contaminated clothing should be removed as soon as possible.*
- Replace gloves if they become contaminated with chemicals, do not continue to wear the gloves and spread the contamination throughout the lab.
- Notify the instructor if any chemical is spilled.

Part A: Melting Points

1. Wear safety goggles and lab coats at all times. **Important:** This lab is "messy". Be sure to clean up any small spills. Keep the lab bench, equipment and balance areas clean.

2. Prepare samples. Obtain a capillary tube and fill it with ~2-3 mm of menthol. Do the same with cinnamic acid. *You can use TLC spotters or the tube tappers to push the solid to the bottom of the capillary tube.*

3. Set up the Digi-Melt by adjusting the start temperature (25 °C), the end temperature (150 °C), and the ramp rate (5-10 °C/min).

1. Connect the temperature probe to Channel 1 of the LabQuest3. In the screen, click on the box that says “Mode:Time Based.” Change the duration to 6 minutes. Change the rate to 6 samples/minute. This will give better results when determining the freezing point of the menthol. Click OK.

2. Set up two water baths using 400 mL beakers.

a. One should be placed on a hot plate and maintained at 60−70 °C.

8. After 6 minutes, data collection will stop.

your notebook. Email the resulting file to your ELN.

Shutdown the LabQuest3 and not simply put it to sleep. To shutdown the LabQuest3: press the home key, select System → Shut Down → OK.

ORIGINAL INVESTIGATION PLAN

Answer the Fundamental Skills Guiding Question and create a plan to answer the Original Investigation Guiding Question:

1. *Fundamental Skills*: What is the average freezing point depression constant for the lab section? What is the melting point of the solute?

As a team, discuss these questions based on the work you have done in the Fundamental Skills section. Answer this question in your own words.

2. *Original Investigation*: What is the identity of your team's unknown spice chemical? What is the best method for determining the identity?

Menthol and unknowns (cinnamic acid, 4-hydoxybenzaldehyde and vanillin) *will be provided next week and all of the laboratory equipment or glassware used in the Fundamental Skills section will be available. Use the Original Investigation planning questions provided in the ELN to help you and your team plan a course of action to answer the question during next week's lab. Before coming to lab next week, perform a Q-test on the class K_f data collected during the Fundamental Skills section to remove any outliers. Use the revised K_f value in your data next week.*

Do not upload the *In-Lab* ELN page. You will turn this page in next week.

Session Two

PRE-LAB QUIZ *Quiz #1 Session #2*

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Section 5.1.2), in the Odyssey program, and in documents hyperlink within this document.

SAFETY MOMENT (WEEK 2)

Flammables:

1. Before Flame Ramp Demonstration Video: Answer the following questions on the Original Proposal ELN page. You may work with your team.
   - What are potential sources of ignition in the lab?

2. Your TA will show you the video.

3. After the Video: Answer the following questions in your ELN with your team. The TA will call on teams to share.
   - What was the source of ignition?
   - What was the flammable in the demonstration?
   - Why do you observe a fire even though the flammable and the source of ignition are not near one another?

ORIGINAL INVESTIGATION

Menthol and unknowns (cinnamic acid, 4-hydoxybenzaldehyde and vanillin) will be provided and all of the laboratory equipment or glassware used in the Fundamental Skills section will be available. As a team, proceed with the plans you outlined last week on your *Original Investigation* page with the unknown assigned to you by your TA. Once your data collection and analysis are complete, create a "poster" on the benchtop containing the following four sections:

- **Guiding Question:** Restate the Original Investigation Guiding Question. Do not feel that you have to write it word-for-word as it appears earlier in this document.
- **Claim:** Answer to the Original Investigation Guiding Question.
- **Evidence:** Use the answer to Guiding Question #1 from the *Fundamental Skills* section and decide, as a team, how the information found in the *Original Investigation* should be presented as concisely as possible.
- **Justification:** How does the evidence you included support your claim? What assumptions made or scientific theories/laws were invoked during data analysis and interpretation?
- **Comments:** The team leader should take down important ideas gained from other teams during the poster presentation.

ARGUMENTATION (POSTER SESSION)

You and your team will then compare and contrast your results with your peers during the poster session. Nominate one team member to be your "spokesperson" who will stay with the poster and answer any questions from your labmates. (Each project will have a different spokesperson so every student will have this role once during the quarter.) The rest of the team will be "travellers" who will go to other posters and engage in discussion about data collection and interpretation. The goal during this session, this *Argumentation*, is to challenge your peers' reasoning and reevaluate your own. Spokespersons and travellers should discuss the claim using the evidence to justify their own point of view. What you discover during this process is pivotal for creating a good *Lab Report*.

The **three boldface questions** below apply to all projects.

- *Purpose & Concepts:* **What concept(s) were you investigating and how are they related to the guiding question(s)?**
- *Procedures & Data Analysis:* **How did you go about your work and why?**
- *Argumentation:* **What is your claim? How does your evidence justify your claim?**

At the end of the poster session, *completely* clean the poster from the lab bench as directed by your TA then your team should hold a short discussion to re-evaluate your claim based on what they have learned during the poster session. If time permits, you can collect more data.

Take a picture of the "poster" and attach it to the *In-Lab* ELN page. Once you have finished all your work on the ELN, review the assignment formatting document then create a PDF with a clearly visible numeric Turnitin similarity score. Upload this PDF to the appropriate Gradescope assignment and assign the Gradescope sections.

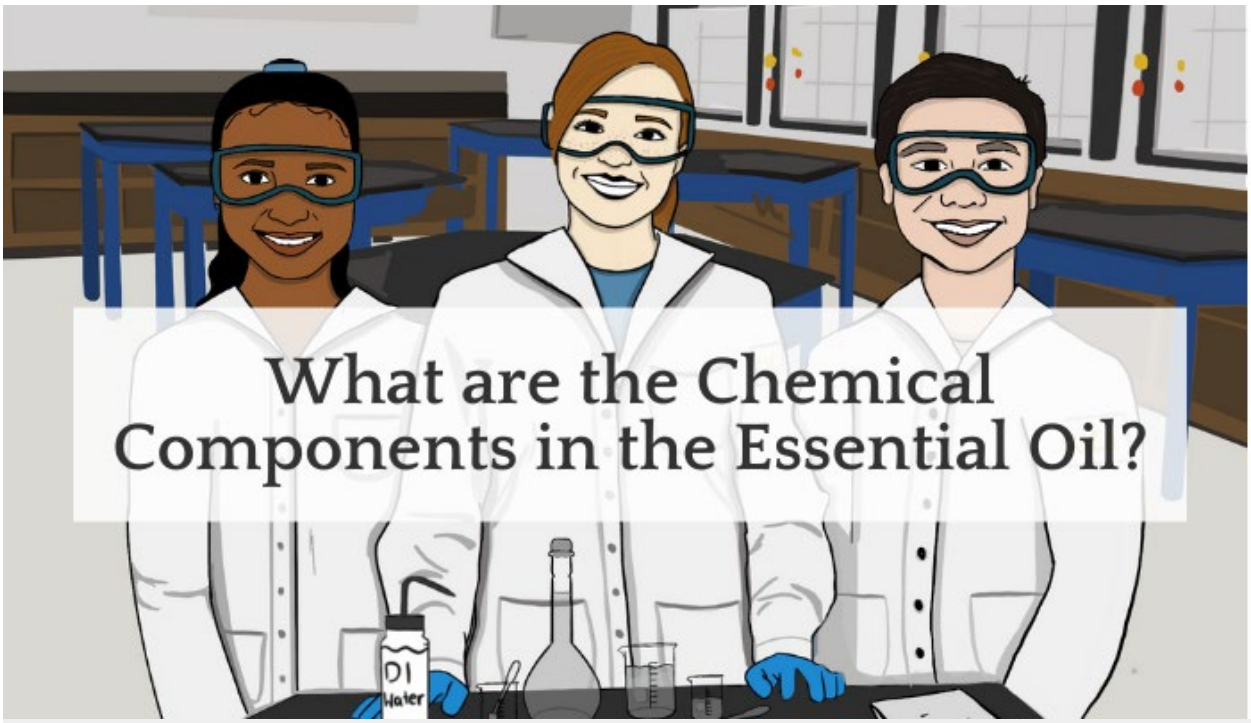
LAB REPORT

The lab report is an individual, not team effort. The content of the report is based on the evidence, claim, and justification your team shared during the argumentation section and the understanding you gained by comparing those ideas against those of other teams. (Be willing to adjust your justification and/or claim in response to what you learn in the argumentation poster session and/or the peer evaluation.)

Use the rubric and the boldfaced questions above to structure your lab report. The first page should answer the first two questions shown above, the second page should answer the third question. However, these are formal reports, not separate responses to the three individual questions - make sure your writing flows and creates a cohesive message. **You should not have individual headers or prompts for each rubric item.**

Lab Report: Review the assignment formatting document before the next step. Create a PDF with a Turnitin similarity score clearly visible of your revised *Lab Report* ELN page and upload it to the appropriate Gradescope assignment. This step must be done by 11:59 pm the day before your week 3 lab period.

b. Project #2 Lab Manual



PRE-LAB QUIZ *Quiz #2 Session #1*

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Sections 7.1.3) and in documents hyperlinked within this document.

INTRODUCTION

In this project, thin layer chromatography (TLC) will be used to determine which spice compounds are present an essential oil. You will be able to use the technique again in future projects and throughout organic chemistry laboratory.

Thin layer chromatography (**TLC**) is frequently to separate components in an analyte mixture to determine the number and identity of components in a mixture, check chemical purity, or follow reaction progress. In this project, we will use it for the first purpose - to find the number and potential identity of the components of an essential oil mixture.

In TLC, the components of the **analyte mixture** are separated by an interplay of intermolecular forces between the **silica stationary phase** and **eluent mobile phase** with the molecules of the analyte mixture.

Silica

The most common TLC plate is typically an inert rigid backing (glass, foil, or plastic) coated with silica powder. **Silica (SiO_2)** is a solid with an extended structure of tetrahedral silica atoms bridged together by bent oxygen atoms. On the surface of the silica particles, the solid terminates in very polar silanol (Si-O-H) groups (Figure 1). Silica is referred to as the **stationary phase** because it does not move when the TLC plate is "developed".

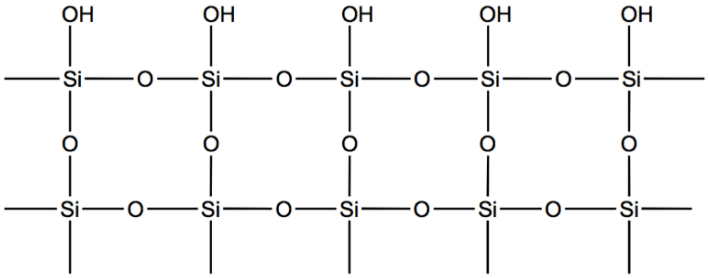


Figure 1. Silica extended structure and surface.

Analyte

The **analyte mixture** is typically dissolved in a minimal amount of solvent to create a very concentrated to saturated solution. Two to three drops of this solution is delivered by *capillary* (a small glass tube with a very small inner diameter (0.5 mm)) to one spot on the *origin line* (drawn in pencil) at the bottom of the silica plate (Figure 2).

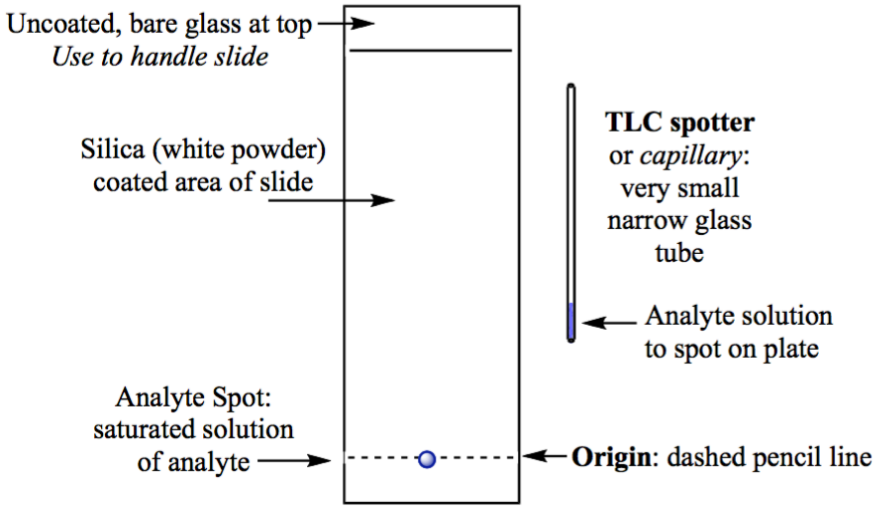


Figure 2. TLC plate.

This plate is then placed into a few milliliters of an organic solvent called *eluent*. This eluent is the **mobile phase** and it travels up the plate via *capillary action*. The line which the eluent moves to is called the *solvent front*.

Eluent & Developing Chamber

Once all solvent has evaporated from the analyte "spot", the TLC plate is placed into a **developing chamber**. To create a TLC developing chamber a small glass jar with a lid just large enough to fit the TLC plate is needed. An **eluent** (an organic solvent or mixture of organic solvents) is added to a height of ~0.5 cm. The level must be below the origin line created on the TLC plate.

Jar	Beaker
	

Figure 3. Developing Chambers

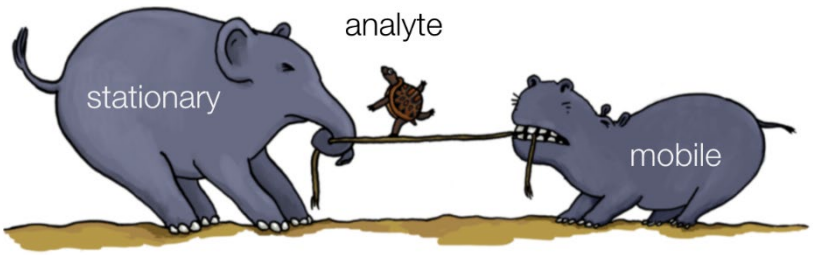
A piece of paper towel (or filter paper) is then fitted around approximately half the interior walls with the bottom edges immersed in the eluent. *Capillary action* will draw the eluent throughout the paper. This wetted paper ensures the vapor of the eluent is uniform throughout the developing jar once it is capped.

When the TLC plate is placed in the jar, the eluent serves as the **mobile phase** – moving up the silica on the plate (also by capillary action). The different compounds in the analyte mixture will be carried in the eluent (up the plate) at different rates depending on polarity. The TLC plate must be removed before the eluent moves beyond the top of the silica powder on the plate. The line which the eluent moves to is called the **solvent front**. The solvent front must be marked with a pencil immediately after removing the plate from the developing jar.

Intermolecular Forces

The components of the analyte mixture are separated by an interplay of intermolecular forces between the silica stationary phase and eluent mobile phase with the molecules of the analyte mixture.

- The **silica stationary phase** is *very polar*, so more polar analyte compounds will *adsorb* more strongly to the silica and are less likely to travel up the plate any significant distance. In fact, if the analyte is too polar it will not move from the origin (the spot where the analyte solution was delivered by the capillary tube).
- The **mobile eluent phase** can vary from *moderately polar to nonpolar* depending on the identity of the organic solvent or mixture of organic solvents. Nonpolar eluents tend to move only nonpolar compounds of the analyte mixture a significant distance up the plate. More polar eluents result in the movement of more of all of the compounds of the analyte mixture up the plate. *A more polar eluent is a better competitor for the analyte molecules versus the polar silica stationary phase.*



You are stuck with the polar silica and the mixture (the analyte molecules) is part of the experimental objective and cannot be altered. You can, however, manipulate the make up of the eluent to separate the mixture of analytes. A common method used to “tune” an eluent polarity is to mix a relatively nonpolar solvent (i.e., heptane) with a solvent of intermediate polarity (i.e., acetone). The overall polarity is determined by the volume to volume ratio of the two solvents. In this project, you want to tune the eluent polarity so that all the analyte molecules travel different distances up the TLC plate - they have different R_f values (explained below).

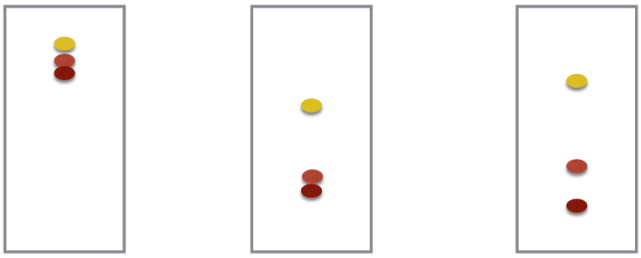


Figure 4. Same Analyte Mixture & Stationary Phase, Different Eluent Mixture

The effect of different eluent mixtures can be seen in Figure 4. Eluent A is too polar, so all the analyte components are easily carried in the mobile phase and separation is not good. On an actual plate, you may not be able to distinguish 3 distinct bands. Eluent B is too nonpolar, two of the analyte components remain adsorbed onto the polar stationary phase and do not separate. Again on an actual plate, you may not be able to see that two different components are in the lower spot. The polarity of Eluent C is well "tuned" separating the three components completely. It would be the eluent to use to meet the objectives of this project.

Retention Factor (R_f)

TLC results can be quantified by calculating the retention factor (R_f). An R_f value is the ratio of the distance the analyte traveled versus distance the eluent travelled.

$$R_f = \frac{\text{distance from origin to analyte} = x_a}{\text{distance from origin to solvent front} \quad x_t}$$

The labeling of x_a and x_t are shown in Figure 5. The distance an analyte travels is dependent on its polarity as well as the identity of the stationary and mobile phases. If the stationary and mobile phases are the same, the distance a particular analyte travels (its R_f value) will always be the same. Keep in mind the significant figure rules when making distance measurements.

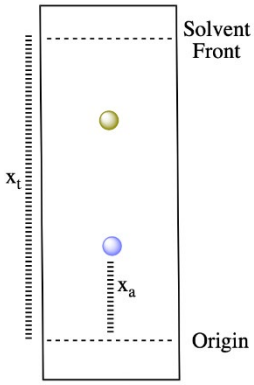


Figure 5. Developed TLC plate.

GUIDING QUESTION(S)

1. *Fundamental Skills*: What are the R_f values of the spice compounds? Which heptane:ethyl acetate ratio is the best eluent?
2. *Original Investigation*: Which spice compounds are present in the essential oil?

Session One

SAFETY MOMENT (WEEK 1):

Fume Hood:

- Read all instructions carefully, once the dry ice is added you will want to move quickly.
- Fill a 1000 mL beaker about halfway with warm water from the sink.
- Add dry ice (~1 cup) to the beaker until a large amount white vapor cloud forms.
- When vapor production declines, pour out the water (not the ice) and refill with warm water. Add dry ice if necessary.

- Take pictures and note your observations for each step.

1. Position the beaker in the back of the hood. How do the fumes move as they evolve from the beaker? Move the beaker to the front of the hood. In what direction do the fumes go?
2. Position the beaker near the edge of the hood. How are the fumes affected as you move your hands over the beaker and in and out of the hood?

3. Open and close the sash (the hood “window”) with the beaker at the front of the hood *(Avoid slamming the sash, it can come off its track.)* How do the fumes react to this movement?
4. Place another beaker behind the water/dry ice beaker. Reach over and pull the empty beaker over the water/dry ice beaker and out of the hood. Were fumes pulled out with the empty beaker?
5. Turn on the emergency exhaust. What do you observe?
6. Open the sash half way, slowly move the dry ice / water beaker from the hood down to the floor. At what point do the fumes stop getting sucked into the fume hood?

FUNDAMENTAL SKILLS

Before your lab section:
- Read the Introduction, Fundamental Skills, and hyperlinked documents in those sections.
- Take the Pre Lab Quiz on Canvas.
- Complete the Objectives, Chemical, Safety (GHS) tables, and a rough draft of the Procedures in the Fundamental Skills page in your ELN.
- Bring a pencil and ruler to lab.

At the beginning of the lab section, your TA will:
- Make sure you are dressed appropriately and have personal protective equipment (lab coat, goggles and gloves).
- Demonstrate how to set up a TLC chamber (both jar and beaker set up) and demonstrate how to spot and develop a TLC plate.

Safety:

Goggles, gloves, a lab coat, thick long pants completely covering the ankles, and sturdy water resistant closed toed shoes are required when performing any experiment utilizing chemicals, glassware, and/or lab equipment in a chemistry laboratory.

Heptane and ethyl acetate are flammable, skin irritants and inhalant hazards. Avoid ignition sources. Wear gloves when handling these chemicals. Cover open containers to reduce evaporation.

Procedures

1. For this project, you will be working in a team with your table group. Using an assigned eluent ratio, each team member is responsible for collecting TLC results for each of the TLC standard solutions.
2. Each team member should collect the following: a TLC developing chamber or developing beaker setup, three TLC plates, and 5.5 cm filter paper.
3. One team member should pick up a vial or a small amount (1-2 mL) of the following solutions from the fumehood: carvone, dihydrocarveol, eugenol, limonene, and vanillin TLC standard solutions.

4. Another team member should obtain no more than 30 mL each of heptane and ethyl acetate in labeled beakers. This amount of solvent should be enough to prepare all four eluent mixtures. Cover the beakers with a watch glass to prevent evaporation. Share the solvent amongst team members.

TLC is being used to show the number of components present in a mixture. Therefore, the best eluent provides the most separation between all the analytes on the developed TLC plate. A common strategy used to "tune" eluent polarity is to mix two miscible solvents of different polarities in different ratios. A plate is run in each system to determine the best eluent system.

5. To determine the best **eluent system**, each team member will be responsible for a *different* eluent heptane volume to ethyl acetate volume ratios: 9:1, 4:1, 3:2 or 2:3. Each eluent ratio will need to be prepared separately, and should be covered when not in use, to prevent evaporation. Aim to create around ~10 mL of total solvent. The volume should be just enough to fill the bottom of each TLC developing chamber to a depth of ≤0.5 cm. Do not make more solutions than needed, as it generates waste.

9. Solvent will move up the plate “wetting” it. Once the ‘solvent front’ is within ~0.5 cm of the top of the plate, remove the plate from the chamber with tweezers and mark the **solvent front line** with a pencil. *Do not let your solvent front travel to the top of the silica on the TLC plate, R_f values cannot be calculated if the solvent front is not visible.*

Organic molecules containing a significant degree of conjugation (alternation of double and single bonds) are often colored and easily seen on TLC plates. Other organic molecules can be visualized with ultraviolet light. The TLC plates contain a fluorescent material that causes the entire plate to appear green under UV light. If the analyte molecules absorb the UV light, their spots will appear dark on the glowing green background.

10. Circle visible spots on the TLC plate with a pencil. Use a UV lamp to visualize colorless/UV active analyte spots. Try both the short and long wave settings. Circle any spots visible under the UV lamp. Take photos of your TLC plate and attach the photos to your ELN.

may appear light brown and may be hard to see against the stained purple plate. If the concentration of the spot is too high, the yellow spot may smear while drying.

4. Allow the TLC plate to dry for 1 minute.

5. For each TLC plate, record the color of each spot, the distance (cm) from the origin line to the spot, and the distance (cm) from the origin line to the solvent front. Calculate the R_f value for each spot and include these values on your Google Sheet.

Session Two

PRE-LAB QUIZ *Quiz #2 Session #2*

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Section 7.2.2), in the Odyssey program, and in documents hyperlink within this document. s

SAFETY MOMENT (WEEK 2)

Glove Permeability
Your TA will set up a demo to illustrate glove permeability against acetone. In your notebook, record the time when your TA adds solvent into the glove. Record any observations until a solvent breakthrough is observed (record the time breakthrough happens). *How does the experimental breakthrough time compare to that reported for the solvent on the SDS? Calculate the % accuracy.*

ORIGINAL INVESTIGATION

Obtain a few drops of essential oil. As a team, proceed with the plans you outlined last week on your *Original Investigation* page. Once your data collection and analysis are complete, create a "poster" on the benchtop containing the following four sections:

- **Guiding Question:** Restate the Original Investigation Guiding Question. Do not feel that you have to write it word-for-word as it appears earlier in this document.
- **Claim:** Answer to the Original Investigation Guiding Question.
- **Evidence:** The TLC information obtained in the *Fundamental Skills* section should be included (i.e. the answer to Guiding Question #1). Decide, as a team, how the information found in the *Original Investigation* should be incorporated as concisely as possible.
- **Justification:** How does the evidence you included support your claim? What assumptions made or scientific theories/laws were invoked during data analysis and interpretation?
- **Comments:** The team leader should take down important ideas gained from other teams during the poster presentation.

ARGUMENTATION (POSTER SESSION)

Compare and contrast your results with your peers during this session. Choose a team member who has not yet been the "spokesperson" to stay with the poster and answer questions. The rest of the team, the "travelers", will go to other posters and challenge your peers' reasoning and reevaluate your own. What you discover during this process is pivotal for creating a good ***Post Lab Report***.

The **three boldface questions** below apply to all projects. A few sample questions specific to this project are provided here to help the spokesperson prepare and travelers initiate in-depth discussion while at each poster.

- *Purpose & Concepts:* **What concept(s) were you investigating and how are they related to the guiding question(s)?**
- *Procedures & Data Analysis:* **How did you go about your work and why?**
- *Argumentation:* **What is your claim? How does your evidence justify your claim?**

At the end of the poster session, your team should hold a short discussion to re-evaluate your claim based on what they have learned during the poster session. If time permits, you can collect more data.

Take a picture of the "poster" and attach it to the *In-Lab* ELN page. Once you have finished all your work on the ELN, review the assignment formatting document then create a PDF with a clearly visible numeric Turnitin similarity score. Upload this PDF to the appropriate Gradescope assignment and assign the Gradescope sections.

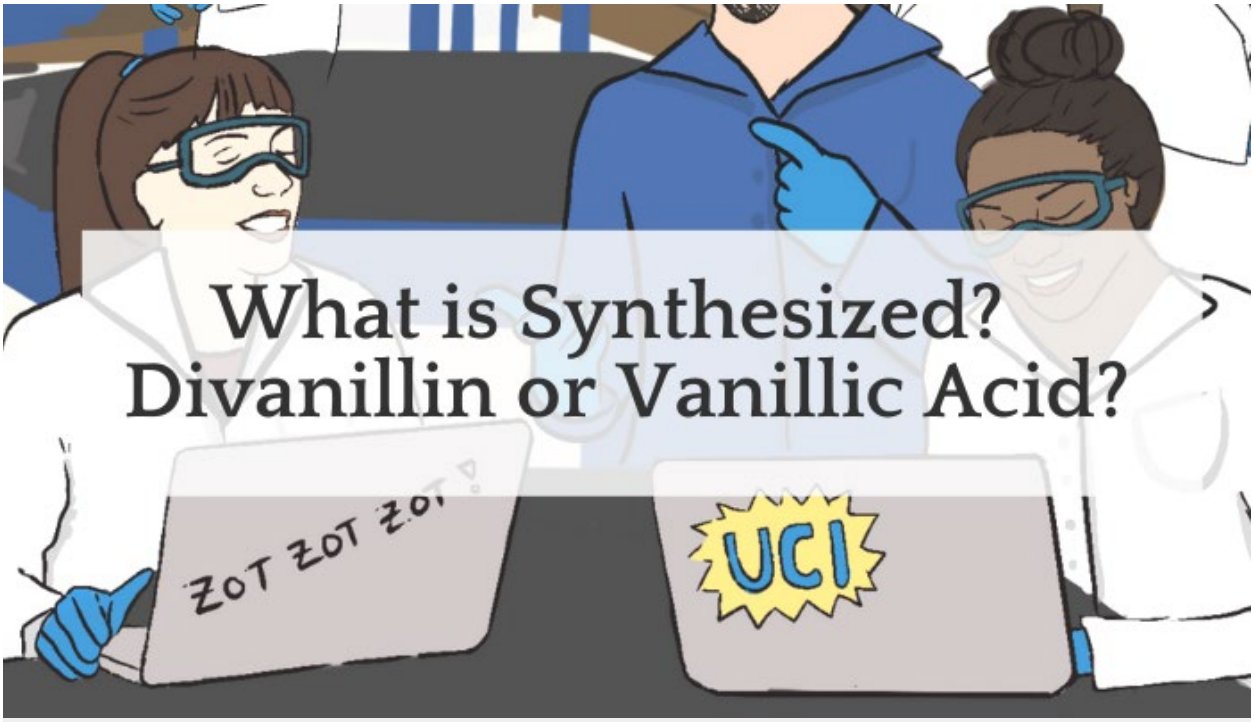
LAB REPORT

The lab report is an individual, not team, effort. The content of the report is based on the evidence, claim, and justification your team shared during the argumentation section and the understanding you gained by comparing those ideas against those of other teams. (Be willing to adjust your justification and/or claim in response to what you learn in the argumentation poster session.)

Use the rubric and the boldfaced questions above to structure your lab report. The first page should answer the first two questions shown above, the second page should answer the third question. However, these are formal reports, not separate responses to the three individual questions - make sure your writing flows and creates a cohesive message.

Report: Review the assignment formatting document before the next step. Create a PDF with a Turnitin similarity score clearly visible of your *Lab Report* ELN page and upload it to the appropriate Gradescope assignment. This step must be done by 11:59 pm the night before your next lab period.

c. Project #3 Lab Manual



PRE-LAB QUIZ *Quiz #3 Session #1*

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Sections 4.1.1 - 4.1.3) and in documents hyperlinked within this document.

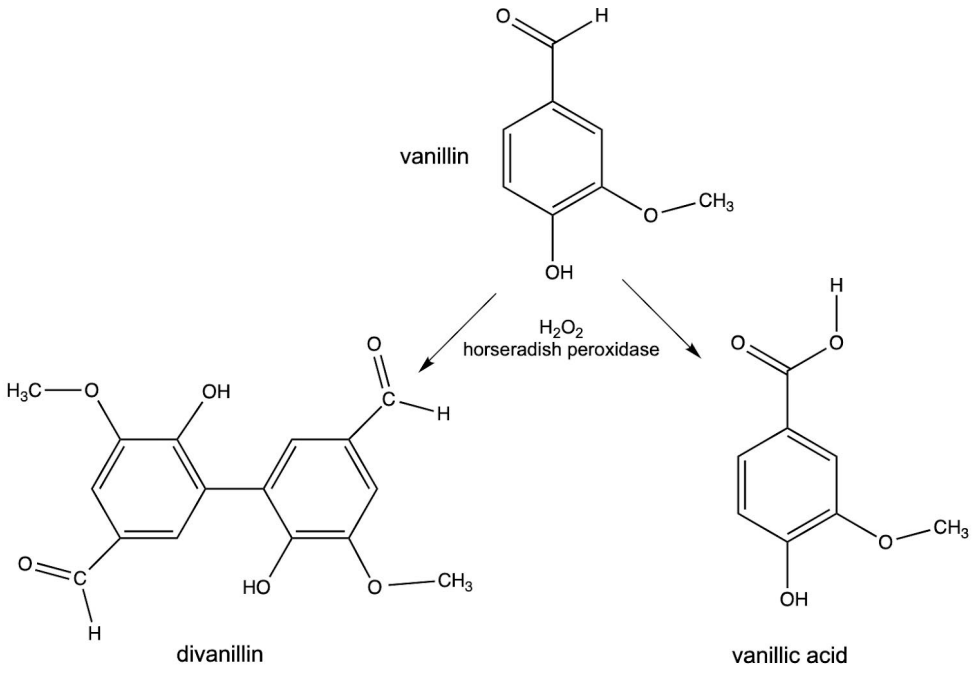
INTRODUCTION

Synthesis

The *Original Proposal* asks you to determine the product of the reaction between vanillin, hydrogen peroxide, and horseradish peroxidase. Hydrogen peroxide is a common oxidizing

agent and horseradish peroxidase is an enzyme (a biological catalyst). Two product options are provided: the dimer divanillin and vanillic acid.

Both possibilities are a result of oxidation: the formation of divanillin occurs through a phenoxy radical coupling (followed by a hydrogen atom rearrangement) *or* the formation of vanillic acid is a more simplistic oxidation of the aldehyde functional group to a carboxylic acid. The techniques introduced in the Fundamental Skills section are a starting point to determine the identity of the product. However, use any skill you have learned in either quarter of general chemistry laboratory.



Stoichiometric Calculations

To perform any synthesis **stoichiometric calculations** determine the amounts of reagents and the theoretical yield. These calculations connect the amounts of reactants and products through *mole ratios* from a balanced chemical equation. The complete dimensional analyses - the calculations showing the unit conversion of one chemical to another - should always be included in your laboratory notebook.

When planning a chemical reaction, two different types of calculations are often needed:

1. **Calculation of reactant amount** from another reactant's amount. The *molar mass* of the first reactant is used to convert mass to moles and then the *mole ratio* between the two reactants is used to calculate the stoichiometric equivalent of the second reactant. (If the pure form of the reagent is a liquid, the liquid can be massed into a tared container or density can be used to convert between volume and mass.)

2. **Calculation of product amount** from a reactant (theoretical yield determination).

a. Theoretical yield is calculated from the limiting reagent. If the limiting reagent is not known, the actual reactant masses measured in lab should each be used to calculate the product mass. This calculation follows the same procedure as the stoichiometric calculations above: the *molar mass* of the reactant is used to convert mass to moles, then the *mole ratio* between the reactant and product is used to calculate the stoichiometric equivalent of the product. Whichever value is smallest is the theoretical yield (and the reactant's whose mass it was calculated from is the limiting reagent).

b. Once the synthesis is complete and the mass of the product (the actual yield) has been recorded, the **percent yield** can be calculated: actual yield/theoretical yield x 100. This value quantifies the efficiency of the chemical reaction.

Solubility

The concept of **solubility** is central to many chemical processes. You've already seen that solubility dictates solution concentration in the first project and it is a central principle of *chromatography* In the second project. After all, the distance a particular analyte travels up a plate in TLC is directly related to its solubility in the eluent versus its attraction to the silica stationary phase.

In this project, the qualitative observation of solubility can be used to characterize that substance. If the solute is visually observed to "disappear into a solvent, that is a simple, straightforward indication of solubility. However, solubility is actually a continuum ranging from complete to incomplete to no solubility. If a solid solute dissolves completely resulting in a transparent solution, the solute has *complete solubility* in that solvent. If a solid solute partially dissolves creating a translucent solution, the solute has *incomplete solubility*. If a solid solute clumps together and the solvent will be clear (and colorless), the solute has *no solubility*.

Solubility also refers to a quantitative value for a particular solute in a particular solvent at a given temperature. For example, the solubility of sodium chloride is 35.9 g/L in water and 0.65 g/L in ethanol. Adding a solute mass greater than the quantitative solubility will result in the presence of solid in the solution. The presence of solid in this situation does not indicate insolubility, it indicates that the solution is saturated - no more solid will dissolve. When using solubility to characterize compounds or perform separations looking up the accepted values of solubility for a solute in a particular solvent can aid in predictions of solubility and the planning of experimental work.

Vacuum Filtration

Solubility is also central to the technique of filtration. A mixture of two compounds with differing solubilities can be separated with relative ease. By dissolving the mixture in a solvent in which only one compound is soluble, the soluble compound can be filtered away from the other insoluble compound (which remains in the solid form).

The solid is separated from the mother liquor (the liquid they form in) by vacuum filtration. Vacuum filtration uses a **Buchner** (pronounced "byook-ner") **funnel** - a flat bottomed,

porous, circular bowl with a short stem. The stem is fitted with a rubber stopper and inserted in the mouth of a side arm filter flask. A thick walled **hose** attaches the side arm of the filter flask to vacuum line connected to a vacuum pump or a vacuum aspirator. Place circular **filter paper**, the same diameter as the funnel's bowl, on the flat bottom and wet with the appropriate solvent to create a seal before turning on the vacuum to start the filtration.

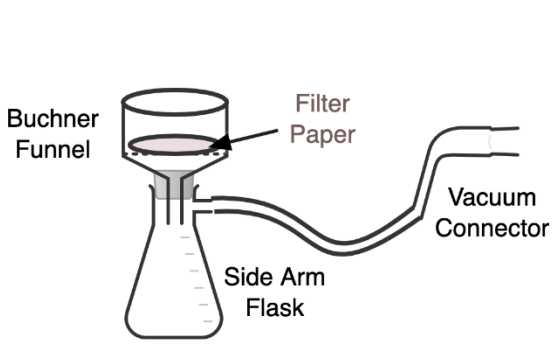


Figure 3a

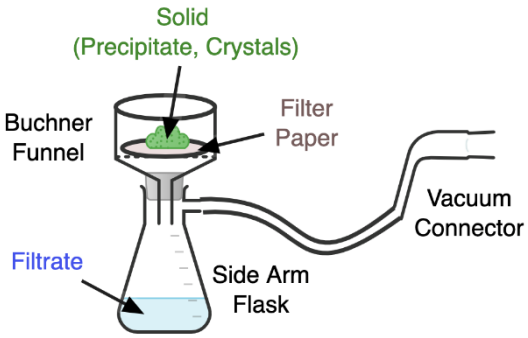
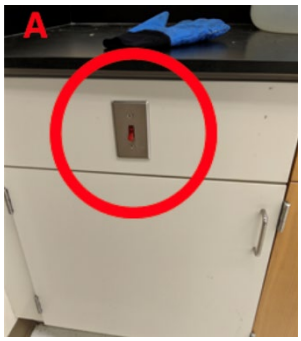


Figure 3b

Figure 3. Vacuum Filtration Set Up.

A vacuum line attaches the vacuum connector in the fume hood to the vacuum pump (Figure 4b). The TA will turn on the pump using switch on the door of the cabinet housing the pump (Figure 4a). Once this is done, pull vacuum by opening the vacuum valve on the fume hood (Figure 4c). *Before adding your sample check for a tight seal. Turn on the vacuum and place a hand on top of the funnel. Suction should be felt. If not, check the hose/side-arm and mouth/funnel connections.*

Pump Power Switch



Pump (cabinet under switch in A)

Vacuum valve & connector (fume hood)

Figure 4. Laboratory Vacuum

GUIDING QUESTION(S)

1. *Fundamental Skills*: What is the percent yield of the synthesis if the product is divanillin? If the product is vanillic acid?
2. *Original Investigation*: What is synthesized, divanillin or vanillic acid? Is the product pure?

Session One

SAFETY MOMENT (WEEK 1):

Poisons

- Your TA will assign each team one of the following questions to answer in your ELN based on the safety video that you will watch as a class during lab this week. You will have 5 minutes to answer the question assigned.
    - What is a poison? Provide a general definition. What is the difference between acute poisoning and chronic poisoning?
    - What type(s) of GHS symbols represent toxicants? What do each of them mean specifically? Hint: There are four you need to know.
    - How is the lethality of a poison measured? What is the name for the metric used and at what doses/concentrations are toxicants considered deadly for ingestion, inhalation, and absorption?
    - Look up the SDS for solid potassium permanganate. What is the LD_{50} for the ingestion of the chemical?
    - What should you do in case of solid potassium permanganate exposure via ingestion? Via absorption?
    - Why isn't potassium permanganate considered a poison? What are 3 key differences between that SDS and the SDS of osmium tetroxide?
- After 5 minutes, your team leader will present (in 1-2 minutes) your team's answer to the class.
- Your TA will then show a video of a poisoning accident from Dartmouth College. Take notes on the video in your ELN and identify at least one thing that could have been done differently to avoid the poisoning.

FUNDAMENTAL SKILLS

Before your lab section:

- Read the Introduction, Fundamental Skills, and hyperlinked documents in those sections.
- Take the Prelab Quiz on Canvas.
- Complete the Objectives, Chemical, Safety (GHS) tables, and a rough draft of the Procedures in the Fundamental Skills page in your ELN.

At the beginning of the lab section, your TA will:

- Make sure you are dressed appropriately and have personal protective equipment (lab coat, goggles and gloves).

Safety:

Goggles, gloves, a lab coat, thick long pants completely covering the ankles, and sturdy water resistant closed toed shoes are required when performing any experiment utilizing chemicals, glassware, and/or lab equipment in a chemistry laboratory. Ethanol, heptane and ethyl acetate are flammable solvents; avoid spark sources.

For this section, teams of four should split into two sets of partners. Each set should perform the synthesis separately.

Synthesis Procedure

The synthesis for a product based on vanillin is described below. For your original investigation, you will follow the synthesis steps and collect your product via vacuum filtration. Once you have your product, you will need to use a minimum of three of the characterization tests from the Fundamentals Skills section to determine which product was synthesized and the percent yield.

1. Clean a 250 mL Erlenmeyer flask with soap and water and then rinse at least 3 times with deionized water.
2. Add ~1.0 g of vanillin to 90 mL of DI water in a 250 mL Erlenmeyer flask.

10. Save your solid product in a labeled scintillation vial in your TA's drawer.

The liquid waste from this part only should be disposed of in the sink.

At least three of the four characterization tests must be done by the team as part of the Original Investigation.

Characterization Test #3: TLC

Use the TLC skills you learned in an earlier project to determine the product's purity. Heptane and ethyl acetate will be available in the lab. A ratio of the two solvents will likely make the best eluent. Create and develop at least 3 TLC plates with different ratios of heptane and ethyl acetate. Present the TLC plate with the best separation (most varied R_f values) between reactants and products.

Characterization Test #4: Absorption Spectrum

1. Add ~1 mg of your substance in about 50 mL of solvent. *Concentration is not important for absorption spectroscopy.* Start with a small amount of solid (~ 1 mg) in a small volume (~5 mL) of solvent. (Solutions should not have any particulates. Use gravity filtration if needed.) Dilute (10-fold) with solvent so that any peaks are smooth and rounded at the top. If there is a straight line or spikes at the top of the spectrum, dilution is needed.
2. Go to the shared spectrometer. Before taking your measurement, you will need to calibrate.
    a. In the main screen of the LabQuest3, click the red box, and then from the dropdown, select Calibrate. A pop-up will appear with the calibration information. Allow the lamp to warm up for 90 seconds.
    b. During this time, prepare a blank by filling an empty cuvette with your chosen solvent.

1. *Fundamental Skills*: What is the percent yield of the synthesis if the product is divanillin? If the product is vanillic acid?

As a team, discuss these questions based on the work you have done in the Fundamental Skills section. Answer this question in your own words.

2. *Original Investigation*: According to the characterization tests learned in this course to which product is synthesized, divanillin or vanillic acid? Is the product pure?

All reagents, instrumentation & equipment necessary for characterization are provided. If you have time, you should start your characterizations during the first session of this project.

Do not upload the *In-Lab* ELN page. You will turn this page in next week.

Session Two

PRE-LAB QUIZ *Quiz #3 Session #2*

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Section 6.1.1), in the Odyssey program, and in documents hyperlinked within this document.

SAFETY MOMENT (WEEK 2)

Risk Assessment:
Your TA will show a video on risk assessment and answer the following questions:
1. Why do you need to be careful while performing a reaction even if it's the 100th time you've done it?
2. How could the scientist in the video have made better judgment regarding monitoring the temperature of the reaction?
3. Is it okay to trust the risk assessment performed by someone other than you? Why or why not? If no, provide reasons why not.

ORIGINAL INVESTIGATION

All reagents, instrumentation & equipment necessary for characterization are provided. As a team, proceed with the plans you outlined last week on your *Original Investigation* page. Once your data collection and analysis are complete, create a "poster" on the benchtop containing the following four sections:

- **Guiding Question:** Restate the Original Investigation Guiding Question. Do not feel that you have to write it word-for-word as it appears earlier in this document.
- **Claim:** Answer to the Original Investigation Guiding Question.
- **Evidence:** Decide, as a team, how the information found in the *Original Investigation* should be incorporated as concisely as possible.
- **Justification:** How does the evidence you included support your claim? What assumptions made or scientific theories/laws were invoked during data analysis and interpretation?
- **Comments:** The team leader should take down important ideas gained from other teams during the poster presentation.

After you are done with the characterization, place your product in the amber product collection bottle in the fume hood.

ARGUMENTATION (POSTER SESSION)

Compare and contrast your results with your peers during this session. Choose a team member who has not yet been the "spokesperson" to stay with the poster and answer questions. The rest of the team, the "travelers", will go to other posters and challenge your peers' reasoning and reevaluate your own. What you discover during this process is pivotal for creating a good *Post Lab Report*.

The **three boldface questions** below apply to all projects. A few sample questions specific to this project are provided here to help the spokesperson prepare and travelers initiate in-depth discussion while at each poster.

- **What concept(s) were you investigating and how are they related to the guiding question(s)?**
- **How did you go about your work and why?**
- **What is your claim? How does your evidence justify your claim?**

At the end of the poster session, your team should hold a short discussion to re-evaluate your claim based on what they have learned during the poster session. If time permits, you can collect more data.

Take a picture of the "poster" and attach it to the *In-Lab* ELN page. Once you have finished all your work on the ELN, review the assignment formatting document then create a PDF with a clearly visible numeric Turnitin similarity score. Upload this PDF to the appropriate Gradescope assignment and assign the Gradescope sections.

LAB REPORT

The lab report is an individual, not team effort. The content of the report is based on the evidence, claim, and justification your team shared during the argumentation section and the understanding you gained by comparing those ideas against those of other teams. (Be willing to adjust your justification and/or claim in response to what you learn in the argumentation poster session and/or the peer evaluation.)

Use the rubric and the boldfaced questions above to structure your lab report. The first page should answer the first two questions shown above, the second page should answer the third question. However, these are formal reports, not separate responses to the three individual questions - make sure your writing flows and creates a cohesive message.

Report: Review the assignment formatting document before the next step. Create a PDF of your *Lab Report* ELN page with a Turnitin similarity score clearly visible and upload it to the appropriate Gradescope assignment. This step must be done by 11:59 pm the night before your next lab period.

d. Project #4 Lab Manual

PRE-LAB QUIZ *Quiz #4 Session #1*

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Sections 6.1.2 & 6.2.1) and in documents hyperlinked within this document.

INTRODUCTION

Synthesis

Cinnamaldehyde contains an aldehyde functional group. Aldehydes can react with ketone functional groups forming new carbon-carbon bonds in a synthesis called **aldol condensation**. Ketones with hydrogen atoms on only one carbon "alpha" to the carbonyl carbon atom undergo aldol condensation in a 1:1 ratio with cinnamaldehyde.

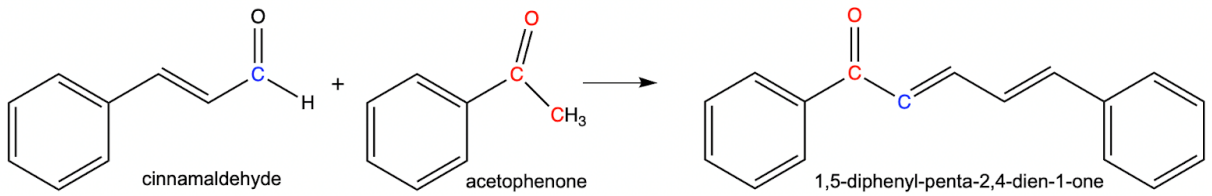


Figure 1. Reaction of cinnamaldehyde with acetophenone. *Hydrogen atoms omitted for clarity.*

Two molecules of cinnamaldehyde can react with acetone (also a ketone), to form dicinnamalacetone, a product that can absorb ultraviolet light. The 2:1 cinnamaldehyde:acetone ratio is due to the presence of hydrogen atoms on both carbons "alpha" (directly bound to) the carbonyl (C=O) carbon atom.

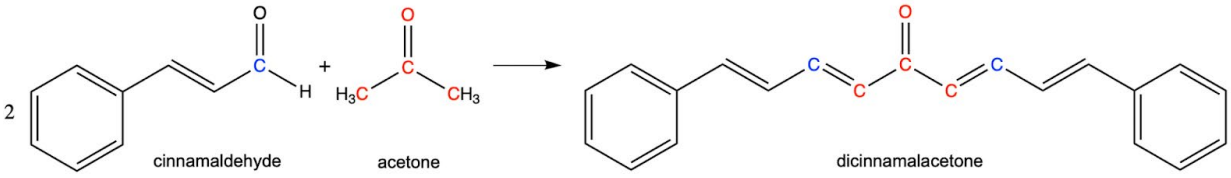


Figure 2. Reaction of cinnamaldehyde with acetone. *Hydrogen atoms omitted for clarity.*

Crystallization

Along with the reaction efficiency, the purity of the product is also important. **Crystallization** (or recrystallization) uses the concept of solubility to purify compounds. During a synthesis reaction, reactions may not go to completion or byproducts may form. When the product is collected, these impurities may be present. The product is first dissolved in a solvent which it is sparingly soluble in. When the solution is heated, the solubility of the product increases and the product fully dissolves in the hot solvent. The solution is cooled to room temperature then cooled further in ice. As the temperature decreases, so does the solubility and the product begins crystallizing out of solution. The impurities remain soluble and the solid product crystals can be collected with vacuum filtration.

Characterization

Assumptions about a chemical's usefulness for a specific application can also be made from the results of the characterization techniques learned in general chemistry lab. For example, chemical sunscreens work by absorbing damaging UV and high energy light. Broadband sunscreens are able to absorb more wavelengths and are considered better sunscreens. Other quantitative or qualitative observations such as melting point, molecular weight, purity, color, consistency, and yield may further aid in evaluating the chemical's usefulness.

GUIDING QUESTION(S)

You will be given cinnamaldehyde and several ketones. You will be performing a synthesis that makes sunscreen from these reagents. You will use several characterization techniques to determine which sunscreen is the best.

1. *Fundamental Skills*: What characteristics of the products observed so far make for a good sunblock?
2. *Original Investigation*: When combined with **cinnamaldehyde**, which reagent (acetophenone or acetone) makes the best sunblock? Why?

Session One

SAFETY MOMENT (WEEK 1):

Managing Risk:
Use the Risk Assessment slide as a guide, and for each event provide a severity score value (1-5), a probability score value (1-5), and a risk level (1-25). Provide a statement for each event on how you can mitigate and/or manage risk.
Attach the Google slide to your ELN (if using ELN) and be prepared to share one of your scenarios with the class.

FUNDAMENTAL SKILLS

Before your lab section:

- Read the Introduction, Fundamental Skills, and hyperlinked documents in those sections.
- Take the Prelab Quiz on Canvas.
- Complete the Objectives, Chemical, Safety (GHS) tables, and a rough draft of the Procedures in the Fundamental Skills page in your ELN.

At the beginning of the lab section, your TA will:

- Make sure you are dressed appropriately and have personal protective equipment (lab coat, goggles and gloves).
- Your TA will demonstrate how to use a Digi-Melt and vacuum filtration.

Safety:

Goggles, gloves, a lab coat, thick long pants completely covering the ankles, and sturdy water resistant closed toed shoes are required when performing any experiment utilizing chemicals, glassware, and/or lab equipment in a chemistry laboratory. All of the ketones are either

flammable, eye irritants, or harmful if swallowed. Avoid ignition sources, wear goggles, replace gloves immediately when they are contaminated, and wash your hands frequently.

For this section, teams of four should split into two sets of partners. Each set should perform a different synthesis (one with acetophenone, the other with acetone). At least four of the six characterization tests must be done by the team.

Synthesis of Cinnamaldehyde Sunscreen

1. Determine the correct volume of acetophenone or acetone for 0.30 mL of cinnamaldehyde. *Use the Canvas quiz as an example.*
2. Using a syringe, add acetophenone or acetone to a scintillation vial. Add 2.0 mL of ethanol and a stir bar, begin stirring.

Complete at least one characterization test described in the Original Investigation Plan below during Fundamental Skills. *Before you leave lab, save your products in vials labelled with the product name, names of your team members, your TA's name, the date (including the year). Your TA will have you store the product in a drawer so you can use them to finish the project next week.*

ORIGINAL INVESTIGATION PLAN

Now it is time to answer the Fundamental Skills Guiding Question and create a plan to answer the Original Investigation Guiding Question:

1. *Fundamental Skills*: What characteristics of the products observed so far make for a good sunblock?

As a team, discuss these questions based on the work you have done in the Fundamental Skills section. Answer this question in your own words.

2. *Original Investigation*: Use the characterization tests learned in this course to answer the following question: When combined with **cinnamaldehyde**, which reagent (acetophenone or acetone) makes the best sunblock? Why?

All reagents, instrumentation & equipment necessary for characterization are provided. If you have time, you should start your characterizations during the first session of this project. You must use at least 4 of the six tests listed below.

- **Percent Yield (see Lab 3 procedure)**
- **Solubility (see Lab 3 procedure)**
- **Melting Point** *MP start and end temperatures are 85 and 150 with a ramp rate of* (5-10 deg/min). **(see Lab 1 procedure)**
- **TLC:** A ratio of heptane and acetone (available in lab) will likely make the best eluent. Present the TLC plate with the best separation (most varied R_f values) between reactants and products. **(see Lab 2 procedure)**
- **Absorption Spectrum:** Concentration is important! Add 0.010 g of your product to a 10 mL volumetric flask. Add solvent that will best dissolve your product. Create more dilute solution by adding 10 drops of the solution just made into a 10 mL volumetric flask. Add ethanol. **(see Lab 3 procedure)**
- **Molecular Weight by Freezing Point Depression:** Perform this characterization test last, as it can require most of the synthesized product. Determine if the product is soluble in menthol by using a small amount of product in less than half a gram of menthol. **(see Lab 1 procedure)**

Clean-Up

- Place capillary tubes and TLC spotters in the broken glass container.
- TLC plates should be placed in the solid waste container in the fume hood.
- Ethanol solutions and leftover TLC solvent should be placed in the organic waste container in the fume hood.
- Put develop jars back uncapped. Return beakers and watch glasses to the shelves.
- Turn in both clearly labeled scintillation vials filled with the two products to your TA.

Do not upload the *In-Lab* ELN page. You will turn this page in next week.

Session Two

PRE-LAB QUIZ *Quiz #4 Session #2*

A Canvas pre-lab quiz is due every week at 11:59 pm the day before your lab section. The information needed to answer these questions can be found in your general chemistry lecture textbook, the Lab Safety for Chemists online text (Section 1.1.2 & 1.2.2), in the Odyssey program, and in documents hyperlinked within this document.

SAFETY MOMENT (WEEK 2)

Green Chemistry
Your TA will place two packing peanuts into two separate jars of water labeled A and B. Take an initial picture of these jars and then a picture every 5 minutes for 30 minutes. Attach the pictures to the Original Proposal ELN page. Before the end of lab the TA will perform another procedural step for this demonstration. Take and attach pictures to your ELN at this point also. Beneath these pictures in your ELN, answer the following: If you have a choice to pick one packing material (A or B) over the other, which would you choose and why (think about which material you would want to wash up on a beach)? How is this demonstration related to green chemistry and how are Chem 1LC and 1LD examples of green chemistry?

ORIGINAL INVESTIGATION

As a team, proceed with the plans you outlined last week on your *Original Investigation* page. Once your data collection and analysis are complete, turn in both products to your TA in properly labeled vials. Then create a "poster" on the benchtop containing the following four sections:

- **Guiding Question:** Restate the Original Investigation Guiding Question. Do not feel that you have to write it word-for-word as it appears earlier in this document.
- **Claim:** Answer to the Original Investigation Guiding Question.
- **Evidence:** Decide, as a team, how the information found in the *Original Investigation* should be incorporated as concisely as possible. Data for both products should be included and appropriately annotated to indicate important characteristics.
- **Justification:** How does the evidence you included support your claim? What assumptions made or scientific theories/laws were invoked during data analysis and interpretation?
- **Comments:** The team leader should take down important ideas gained from other teams during the poster presentation.

After you are done with the characterization, place your products in the appropriate amber product collection bottles in the fume hood. Make sure you are putting each product in the correct bottle.

ARGUMENTATION (POSTER SESSION)

Compare and contrast your results with your peers during this session. Choose a team member who has not yet been the "spokesperson" to stay with the poster and answer questions. The rest of the team, the "travelers", will go to other posters and challenge your peers' reasoning and reevaluate your own. What you discover during this process is pivotal for creating a good *Lab Report*.

The **three boldface questions** below apply to all projects. A few sample questions specific to this project are provided here to help the spokesperson prepare and travellers initiate in-depth discussion while at each poster.

- **What concept(s) were you investigating and how are they related to the guiding question(s)?**
- **How did you go about your work and why?**
- **What is your claim? How does your evidence justify your claim?**

At the end of the poster session, your team should hold a short discussion to re-evaluate your claim based on what they have learned during the poster session. If time permits, you can collect more data.

Take a picture of the "poster" and attach it to the *In-Lab* ELN page. Once you have finished all your work on the ELN, review the assignment formatting document then create a PDF with a clearly visible numeric Turnitin similarity score. Upload this PDF to the appropriate Gradescope assignment and assign the Gradescope sections.

LAB REPORT

The lab report is an individual, not team effort. The content of the report is based on the evidence, claim, and justification your team shared during the argumentation section and the understanding you gained by comparing those ideas against those of other teams. (Be willing to adjust your justification and/or claim in response to what you learn in the argumentation poster session and/or the peer evaluation.)

Use the rubric and the boldfaced questions above to structure your lab report. The first page should answer the first two questions shown above, the second page should answer the third question. However, these are formal reports, not separate responses to the three individual questions - make sure your writing flows and creates a cohesive message.

Fall Quarter (Thanksgiving week 9):
Lab Report: Create a PDF with a Turnitin similarity score clearly visible of your *Lab Report* ELN page and upload it to the appropriate Gradescope assignment. This step must be done by 11:59 pm Monday of week 9.

Winter Quarter:
Lab Report: Review the assignment formatting document before the next step. Create a PDF with a Turnitin similarity score clearly visible of your *Lab Report* ELN page and upload it to the appropriate Gradescope assignment. This step must be done by 11:59 pm the day of your week 9 lab period.

III. In-Lab Assignment Student Rubrics

Table S5.1. In-Lab #1 Rubric.

Rubric Item	Full Marks
ASSIGNMENT WILL NOT BE GRADED WITHOUT THE FOLLOWING:	• Complete procedures are required & they must be in your own words & at a level that a peer could repeat them. • No more than one other section (safety, objectives, chemicals/supplies, observations, data (tables/plots)) is missing. • Time & date stamps for Fundamental Skills section match the day & time that work was done (i.e., no other work was done after lab). • The Turnitin similarity score must be clearly visible on the document.
Format (2 pts)	Mistakes should be crossed through (not deleted). Images should be appropriately sized for their purpose. Clear headings should be used. *These points can also be lost for:* incomplete pre-lab OR late submission OR for inappropriate, improper or unsafe behavior in the lab.
Fundamental Skills	
Objective	Describe the purpose of the experimental work, the general method to be used and the anticipated results. *Objectives longer than 4 sentences will not be graded.*
Procedure: Chemicals/Supplies	Present, completely filled in & no items missing.
Safety: Hazard Table & Moment	*Follow safety rules during lab.* Fill in hazards table and respond to the safety moment. Include safety equipment scavenger hunt picture & photo of the class wearing proper PPE.
Observations: Qualitative Data	Include relevant descriptions of all chemicals & solutions with no more than 1 missing or incomplete description.
Procedure:	Provide detailed procedural steps for melting & freezing point measurements. (A fellow student should be able to follow and achieve your results.)

Observations: Quantitative Data	Provide all measurements with the appropriate number of significant figures & units. Repetitive data must be reported in a table. Only include your data (not that of the entire team)
Data Analysis: Cooling/Freezing Curve(s)	Provide analyzed cooling/freezing curve(s) with appropriate titles & labels. Axes must be scaled so data fills the graph. The image must be visible & legible on the PDF submitted to Gradescope. Data file must be attached & a Google sheet link provided.
Argumentation	Answer the Guiding question for the Fundamental Skills section of the project.
Original Investigation	
Safety: Moment	*Follow safety rules during lab.* Provide answers to the questions in Project #1 document for the Safety Moment.
Objective	Describe the purpose of the experimental work, the general method to be used & the anticipated results in your own words. *Objectives over 4 sentences long will not be graded.*
Observations: Qualitative Data	Include relevant descriptions of all chemicals and solutions.
Procedure:	Provide detailed procedural steps for determining the identity of your unknown.
Observations: Quantitative Data	Provide all measurements/data tables/plots with the appropriate number of significant figures, labeling, scaling, etc.
Data Analysis: Unknown Determination	Connect data to the determination of the unknown identity. Include a relevant data values and properly formatted sample calculation if appropriate.
Argumentation	Attach a legible image of the bench top "poster" to the ELN page. Summarize other teams' critiques of your team's poster & your critique of other teams' posters. Describe how the information exchange in the poster session will affect your lab report's content.

Table S5.2. In-Lab #2 Rubric

Rubric Item	**Full Marks**
ASSIGNMENT WILL NOT BE GRADED WITHOUT THE FOLLOWING:	• Complete procedures are required & they must be in your own words & at a level that a peer could repeat them. • No more than one other section (safety, objectives, chemicals/supplies, observations, data (tables/plots)) is missing. • Time & date stamps for Fundamental Skills section match the day & time that work was done (i.e., no other work was done after lab). • The Turnitin similarity score must be clearly visible on the document.
Format (2 pts)	Mistakes should be crossed through (not deleted). Images should be appropriately sized for their purpose. Clear headings should be used. *These points can also be lost for:* incomplete pre-lab OR late submission OR for inappropriate, improper or unsafe behavior in the lab.
Fundamental Skills	
Objective	Describe the purpose of the experimental work, the general method to be used and the anticipated results. *Objectives longer than 4 sentences will not be graded.*
Procedure: Chemicals/Supplies	Present, completely filled in & no items missing.
Safety: Hazard Table & Moment	*Follow safety rules during lab.* Hazard Table is filled out completely. Provide observation pictures for the safety moment
Qualitative Observations	Include relevant descriptions of all chemicals & solutions with no more than 1 missing or incomplete description.
Procedure:	Provide detailed procedural steps for TLC chamber set up & plate development.
Observations: Developed Plate Appearance	Attach images of TLC plates (visible light, UV & dipped)
Quantitative Observations: R_f Data	Provide TLC plate measurements with the appropriate number of significant figures & units in a table. Only include your data (not that of the entire team).

Data Analysis: R_f calculation	Add calculated R_f values to data table & provide 1 sample R_f calculation.
Argumentation	Answer the Guiding question for the Fundamental Skills section of the project.
Original Investigation	
Safety: Moment	*Follow safety rules during lab.* Provide observations and answer question in Project #2 document for the safety moment.
Objective	Describe the purpose of the experimental work, the general method to be used & the anticipated results in your own words. *Objectives over 4 sentences long will not be graded.*
Observations: Qualitative Data	Describe all chemicals and solutions with no more than 1 missing or incomplete descriptions.
Procedure:	Provide detailed procedural steps for determining the identity of your unknown.
Observations: Data	Provide all measurements/data tables/plots/images with the appropriate number of significant figures, labeling, scaling, etc.
Data Analysis: Unknown Determination	Connect data to the determination of the unknown identity. Include a relevant data values and properly formatted sample calculation if appropriate.
Argumentation	Attach a legible image of the bench top "poster" to the ELN page. Summarize other teams' critiques of your team's poster & your critique of other teams' posters. Describe how the information exchange in the poster session will affect your lab report's content.

Table S5.3. In-Lab #3 Rubric.

Rubric Item	Full Marks
ASSIGNMENT WILL NOT BE GRADED WITHOUT THE FOLLOWING:	• Complete procedures are required & they must be in your own words & at a level that a peer could repeat them. • No more than one other section (safety, objectives, chemicals/supplies, observations, data (tables/plots)) is missing. • Time & date stamps for Fundamental Skills section match the day & time that work was done (i.e., no other work was done after lab).

	• The Turnitin similarity score must be clearly visible on the document.
Format (2 pts)	Mistakes should be crossed through (not deleted). Images should be appropriately sized for their purpose. Clear headings should be used. *These points can also be lost for:* incomplete pre-lab OR late submission OR for inappropriate, improper or unsafe behavior in the lab.
Fundamental Skills	
Objectives	Describe the purpose of the experimental work, the general method to be used and the anticipated results. *Objectives longer than 4 sentences will not be graded.*
Procedure: Chemicals/Supplies	Present, completely filled in & no items missing.
Safety: Hazard Table & Moment	*Follow safety rules during lab.* Hazard Table is filled out completely.
Observations: Qualitative Data	Include relevant descriptions of all chemicals & solutions with no more than 1 missing or incomplete description.
Procedure:	Provide detailed procedural steps for the synthesis and characterization test. (A fellow student should be able to follow and achieve your results.)
Observations: Quantitative Data	Provide all measurements with the appropriate number of significant figures & units. Repetitive data must be reported in a table. Only include your data (not that of the entire team)
Data Analysis: Vanillic Acid Yield	Provides a sample calculation for the percent yield if the product is vanillic acid
Data Analysis: Divanillin Yield	Provides a sample calculation for the percent yield if the product is divanillin
Observations: Characterization Test #1	Provides all data (for standards and product) from the characterization test chosen. If your test has files or pictures, attach them in this section. Quantitative data should be organized in a table.
Argumentation	Answer the Guiding question for the Fundamental Skills section of the project.
Original Investigation	

Safety: Moment	*Follow safety rules during lab.* Provide answers to the questions in Project #3 document for the safety moment.
Objective	Describe the purpose of the experimental work, the general method to be used & the anticipated results in your own words. *Objectives over 4 sentences long will not be graded.*
Observations: Qualitative Data	Describe all chemicals and solutions with no more than 2 missing or incomplete descriptions.
Procedure:	Provide detailed procedural steps for the characterization tests. (A fellow student should be able to follow and achieve your results.)
Observations: Quantitative Data	Provide all measurements with the appropriate number of significant figures & units. Quantitative data must be reported in a table.
Observations: Characterization Test #2	Provide all data from the characterization test chosen. If your test has files or pictures, attach them in this section. Quantitative data should be organized in a table.
Observations: Characterization Test #3	Provide all data from the characterization test chosen. If your test has files or pictures, attach them in this section. Quantitative data should be organized in a table.
Data Analysis: Identification of Product	Identify the synthesis product through analysis of each characterization test result. States whether or not the product is believed to be pure.
Argumentation	Attach a legible image of the bench top "poster" to the ELN page. Summarize other teams' critiques of your team's poster & your critique of other teams' posters. Describe how the information exchange in the poster session will affect your lab report's content.

Table S5.4. In-Lab #4 Rubric.

Rubric Item	**Full Marks**
ASSIGNMENT WILL NOT BE GRADED WITHOUT THE FOLLOWING:	• Complete procedures are required & they must be in your own words & at a level that a peer could repeat them. • No more than one other section (safety, objectives, chemicals/supplies, observations, data (tables/plots)) is missing.

	• Time & date stamps for Fundamental Skills section match the day & time that work was done (i.e., no other work was done after lab). • The Turnitin similarity score must be clearly visible on the document.
Format (2 pts)	Mistakes should be crossed through (not deleted). Images should be appropriately sized for their purpose. Clear headings should be used. *These points can also be lost for:* incomplete pre-lab OR late submission OR for inappropriate, improper or unsafe behavior in the lab.
Fundamental Skills	
Objectives	Describe the purpose of the experimental work, the general method to be used and the anticipated results. *Objectives longer than 4 sentences will not be graded.*
Procedure: Chemicals/Supplies	Present, completely filled in & no items missing.
Safety: Hazard Table & Moment	*Follow safety rules during lab.* Hazard Table is filled out completely.
Observations: Qualitative Data	Include relevant descriptions of all chemicals & solutions with no more than 1 missing or incomplete description.
Procedure:	Provide detailed procedural steps for the synthesis and characterization test. (A fellow student should be able to follow and achieve your results.)
Observations: Quantitative Data	Provide all measurements with the appropriate number of significant figures & units. Repetitive data must be reported in a table. Only include your data (not that of the entire team)
Data Analysis: Characterization Test #1	Provide all data for the characterization test. Attach files or pictures & organize quantitative data in a table.
Argumentation	Answer the Guiding question for the Fundamental Skills section of the project.
Original Investigation	
Safety: Moment	*Follow safety rules during lab.* Provide answers to the questions in Project #1 document for the assigned GHS symbol.

Objective	Describe the purpose of the experimental work, the general method to be used & the anticipated results in your own words. *Objectives over 4 sentences long will not be graded.*
Observations: Qualitative Data	Describe all chemicals and solutions with no more than 1 missing or incomplete descriptions.
Procedure:	Provide detailed procedural steps for the characterization tests. (A fellow student should be able to follow and achieve your results.)
Data Analysis: Characterization Test #2	Provide all data for the characterization test. Attach files or pictures & organize quantitative data in a table.
Data Analysis: Characterization Test #3	Provide all data for the characterization test. Attach files or pictures & organize quantitative data in a table.
Data Analysis: Characterization Test #4	Provide all data for the characterization test. Attach files or pictures & organize quantitative data in a table.
Argumentation	Attach a legible image of the bench top "poster" to the ELN page. Summarize other teams' critiques of your team's poster & your critique of other teams' posters. Describe how the information exchange in the poster session will affect your lab report's content.

IV. Post-Lab Report Student Rubrics

Table S5.5. Lab Report #1 Rubric

Rubric Items	**Full Marks**
REPORTS **WILL NOT BE GRADED** IF THEY DO NOT MEET THE FOLLOWING REQUIREMENTS: • Written directly in the ELN w/ 1.5 line spacing, Arial font, size 16 • Page limit: 4 pages *including figures/plots* (which =~7000 characters across all rich text boxes) • Have 3 broad headings: Purpose & Concepts, Procedures & Data Analysis & Argumentation (consisting of claim, evidence & justification). • Figures/plots must be inserted within the text & unit labels or axes values must be large enough to read. Sample calculations must be typed, not inserted as a picture. • Your name & the Turnitin similarity score must be present & clearly visible at the top of PDF. Margins must be 1 inch on all sides & not overlapped with text/figures/plots/tables.	

• PDF uploaded to Gradescope with: All pages assigned to the "ASSIGN ALL PAGES - Format" question. All other questions assigned to their relevant pages.	
Purpose & Concepts	
Purpose	Describe the main goal of the project & the main techniques used to achieve this goal. *Purposes should be no longer than 4 sentences.*
Concepts (I)	Define freezing point & discuss cooling curves and how they are interpreted.
Concepts (II)	Define freezing point depression, its equation (and variables) & how its used to find a solute's identity. Do not provide numerical values in this section.
Concepts (III)	Define melting point & discuss how it is used to find a pure substance's identity.
Procedure & Data Analysis	
Procedure: FP Measurements	Provide procedure(s) for collecting the freezing point data used to determine the unknown's identity.
Procedure: MP Measurements	Provide procedure(s) for collecting the melting point data used to determine the unknown's identity.
Data Analysis: FP Data Presentation	Provide analyzed freezing point data, sample calculations, and/or relevant properly formatted plots with correct significant figures.
Data Analysis: MP Data Presentation	Provide analyzed melting point data with correct significant figures.
Data Analysis: Comparisons	Report values experimentally found for your unknown and values possible for all possible unknowns for both techniques.
Argumentation	
Claim	State your claim(s).
Evidence (I)	States which unknown value compares the best to the value obtained from the freezing point measurements.
Evidence (II)	States which unknown value compares the best to the value obtained from the melting point measurements.
Evidence (III)	Compare closeness of experimental values to true values for each procedure.

Justification (I)	Discuss why freezing point depression can be used to determine the identity.
Justification (II)	Discuss why melting point can be used to determine the identity.
Justification (III)	Discuss the accuracy of the techniques for determining the unknown identity.
Peer to Peer Argumentation	Discuss how your understanding of your claim, interpretation of your evidence or your justification was deepened or changed during the poster session. *This section should be 2-3 sentences.*

Table S5.6. Lab Report #2 Rubric.

Rubric Items	**Full Marks**
REPORTS **WILL NOT BE GRADED** IF THEY DO NOT MEET THE FOLLOWING REQUIREMENTS: • Written directly in the ELN w/ 1.5 line spacing, Arial font, size 16 • Page limit: 4 pages *including figures/plots* (which =~7000 characters across all rich text boxes) • Have 3 broad headings: Purpose & Concepts, Procedures & Data Analysis & Argumentation (consisting of claim, evidence & justification). • Figures/plots must be inserted within the text & unit labels or axes values must be large enough to read. Sample calculations must be typed, not inserted as a picture. • Your name & the Turnitin similarity score must be present & clearly visible at the top of PDF. Margins must be 1 inch on all sides & not overlapped with text/figures/plots/tables. • PDF uploaded to Gradescope with: All pages assigned to the "ASSIGN ALL PAGES - Format" question. All other questions assigned to their relevant pages.	
Purpose & Concepts	
Purpose	Describe the main goal of the project & the main techniques used to achieve this goal. *Purposes should be no longer than 4 sentences.*
Concepts (I)	Describe the identity and polarity of analytes and the mobile and stationary phase.
Concepts (II)	Discuss how analytes are separated on a TLC plate.
Concepts (III)	Discuss the use of ultraviolet light and permanganate solution to visualize spots on a TLC plate

Procedure & Data Analysis	
Procedure	Provide procedure(s) for collecting the TLC data used to determine the unknown's identity.
Data Analysis: Standards	Provide all measurements/data tables/plots/images with the appropriate number of significant figures, labeling, scaling, etc for standard TLCs.
Data Analysis: Unknown	Provide all measurements/data tables/plots/images with the appropriate number of significant figures, labeling, scaling, etc for unknown TLC(s).
Data Analysis: Comparisons	Provide standard and unknown R_fs with proper format.
Argumentation	
Claim	State your claim.
Evidence (I)	List the evidence *associated with the unknown essential oil* that allowed the determination of its components
Evidence (II)	Connect the evidence *associated with the standards* and *the unknown* that allows for the determination of unknown components.
Justification (I)	Explain why standards have different R_fs
Justification (II)	Explain the difference between spots identified by UV light and spots identified by permanganate dip.
Peer to Peer Argumentation	Discuss how your understanding of your claim, interpretation of your evidence or your justification was deepened or changed during the poster session. *This section should be 2-3 sentences.*

Table S5.7. Lab Report #3 Rubric.

Rubric Items	**Full Marks**
REPORTS **WILL NOT BE GRADED** IF THEY DO NOT MEET THE FOLLOWING REQUIREMENTS: • Written directly in the ELN w/ 1.5 line spacing, Arial font, size 16 • Page limit: 4 pages *including figures/plots* (which =~7000 characters across all rich text boxes) • Have 3 broad headings: Purpose & Concepts, Procedures & Data Analysis & Argumentation (consisting of claim, evidence & justification). • Figures/plots must be inserted within the text & unit labels or axes values must be large enough to read. Sample calculations must be typed, not inserted as a picture.	

• Your name & the Turnitin similarity score must be present & clearly visible at the top of PDF. Margins must be 1 inch on all sides & not overlapped with text/figures/plots/tables. • PDF uploaded to Gradescope with: All pages assigned to the "ASSIGN ALL PAGES - Format" question. All other questions assigned to their relevant pages.	
Purpose & Concepts	
Purpose	Describe the main goal of the project & the main techniques used to achieve this goal. *Purposes should be no longer than 4 sentences.*
Concepts (I)	Describe the two potential reaction pathways & products.
Concepts (II)	Describe the purpose of filtration.
Concepts (III)	Describe how the product identity will be determined. Define standards and describe how they are used in characterization tests.
Procedure & Data Analysis	
Procedure: Vanillin Synthesis	Articulate the synthesis performed, including all relevant equipment, chemicals, and/or concentrations. This section should be approximately 2 sentences, but this point will not be removed if it is longer.
Data Analysis 1: (1 out of the 4 characterizations)	Provide & interpret data analysis 1.
Data Analysis 2: (1 out of the 4 characterizations)	Provide & interpret data analysis 2.
Data Analysis 3: (1 out of the 4 characterizations)	Provide & interpret data analysis 3.
Argumentation	
Claim	State your claim.
Evidence (I)	Provides 3 pieces of evidence that allowed for the determination of the product's identity.
Evidence (II)	Provides 1 piece of evidence that allowed for the determination of product purity.

Justification (I)	Explain how comparison of product to standard data is used to determine product identity.
Justification (II)	Explain how product data is used to determine purity.
Peer to Peer Argumentation	Discuss how your understanding of your claim, interpretation of your evidence or your justification was deepened or changed during the poster session. *This section should be 2-3 sentences.*

Table S5.8. Lab Report #4 Rubric.

Rubric Items	**Full Marks**
REPORTS **WILL NOT BE GRADED** IF THEY DO NOT MEET THE FOLLOWING REQUIREMENTS: • Written directly in the ELN w/ 1.5 line spacing, Arial font, size 16 • Page limit: 4 pages *including figures/plots* (which =~7000 characters across all rich text boxes) • Have 3 broad headings: Purpose & Concepts, Procedures & Data Analysis & Argumentation (consisting of claim, evidence & justification). • Figures/plots must be inserted within the text & unit labels or axes values must be large enough to read. Sample calculations must be typed, not inserted as a picture. • Your name & the Turnitin similarity score must be present & clearly visible at the top of PDF. Margins must be 1 inch on all sides & not overlapped with text/figures/plots/tables. • PDF uploaded to Gradescope with: All pages assigned to the "ASSIGN ALL PAGES - Format" question. All other questions assigned to their relevant pages.	
Purpose & Concepts	
Purpose	Describe the main goal of the project & the main techniques used to achieve this goal. *Purposes should be no longer than 4 sentences.*
Concepts (I)	Describe the two reaction pathways & their products
Concepts (II)	Conceptually describe the process and purpose of recrystallization.
Concepts (III)	Describe the chemical qualities of a good sunscreen.
Procedure & Data Analysis	
Procedure: Cinnamaldehyde-Ketone Synthesis	Articulate the synthesis performed, including all relevant equipment, chemicals, and/or concentrations. This section should be approximately 2 sentences, but this point will not be removed if it is longer.

Data Analysis: Characterization Test #1	Provide & interpret characterization test data. Data must be discussed in the text, and *if applicable* presented as a • properly labeled & formatted image or plot • combined table with the other characterization tests
Data Analysis: Characterization Test #2	Provide & interpret characterization test data. Data must be discussed in the text, and *if applicable* presented as a • properly labeled & formatted image or plot • combined table with the other characterization tests
Data Analysis: Characterization Test #3	Provide & interpret characterization test data. Data must be discussed in the text, and *if applicable* presented as a • properly labeled & formatted image or plot • combined table with the other characterization tests
Data Analysis: Characterization Test #4	Provide & interpret characterization test data. Data must be discussed in the text, and *if applicable* presented as a • properly labeled & formatted image or plot • combined table with the other characterization tests
Argumentation	
Claim	State your claim.
Evidence (I)	Describe characterization test results that support the claim.
Evidence (II)	Describe characterization test results that do not support (or strongly support) the claim.
Justification (I)	Discuss which characterization tests contributed to the decision that the chosen sunscreen is the preferred sunscreen, and why
Justification (II)	Discuss which characterization tests did not contribute to the decision that the chosen sunscreen is the preferred sunscreen, and why
Justification (III)	Analyze possible sources of error that could have affected the data and the decision of the preferred sunscreen

Peer to Peer Argumentation	Discuss how your understanding of your claim, interpretation of your evidence or your justification was deepened or changed during the poster session. *This section should be 2-3 sentences.*

Tables S5.9 and S5.10 give approximate time frames for activities conducted in the fundamental skills and original investigation laboratory sessions.

Table S5.9. Approximate times for Fundamental Skills Session Activities

Activities	Time (mins)
Safety Moment	15
TA pre-lab talk/ demonstration	15
Experimentation	170
Analysis of data and draft of procedure outline	30

Table S5.10. Approximate times for Original Investigation Activities

Activities	Time (mins)
Safety Moment	15
TA pre-lab talk/ demonstration	15
Experimentation	140
Analysis of data and creation of poster	30
Argumentation session	30

The times provided above are the maximum times for each activity, however, frequently these times are variable depending on students' speed.

V. Chemicals and Sample Preparations

a. Project #1 additional chemicals and equipment

Additional Equipment:

- Melting point apparatus
- Melting point capillary tubes
- Temperature probes
- Large test tube
- Ring stand and clamp
- Hot plate

Chemicals Needed:

- L-menthol
- Vanillin
- Cinnamic acid
- 4-hydroxybenzaldehyde

Waste Containers:

- Contaminated glass waste

b. Project #2 additional equipment and chemicals

Additional Equipment:

- TLC plates
- TLC spotters, taped to each vials of standards
- TLC developing jars w/caps
- 3 TLC lamps setup per lab

- 1 watch glass per student
- 1 small forcep at each permanganate dip station

Chemicals Needed:

- Heptane
- Ethyl acetate
- KNO_4 solution
- ~ 20 mL of the following:
    - 1:75 spearmint oil:acetone (essential oil for Original Investigation)
    - 1:50 limonene:acetone
    - 1:50 dihydrocarveol:acetone
    - 1:100 carvone:acetone
    - 1:50 cinnamaldehyde:acetone
    - 1:50 eugenol:acetone
    - 1:50 vanillin: acetone

Waste Containers:

- Organic waste container
- TLC plate waste container

c. Project #3 additional chemicals and equipment.

Additional Equipment:

- pH paper
- Hot plate
- Buchner funnel (vacuum filtration)

- Test tubes
- Melting point apparatus
- Melting point capillary tubes
- TLC plates
- TLC spotters
- UV-Vis spectrophotometer
- Cuvettes (for absorbance)
- Micro stir bar

Chemicals Needed:

- Vanillin
- 0.010 M acetic acid
- 0.05 mg/mL horseradish peroxidase
- 3% hydrogen peroxide
- 95% ethanol
- Ethyl acetate

Waste Containers:

- Organic waste container
- TLC waste container
- Contaminated glass container

d. Project #4 additional equipment and chemicals.

Additional Equipment:

- Scintillation vials

- Melting point apparatus
- Melting point capillary tubes
- TLC plates
- TLC spotters
- Ice baths
- UV-Vis spectrophotometer
- Cuvettes (for absorbance)
- Micro stir bar

Chemicals Needed:

- 95% ethanol
- Acetone
- 0.2M NaOH in ethanol
- Cinnamaldehyde
- Acetophenone

Waste Containers:

- Organic waste container
- TLC waste container
- Contaminated glass container

VI. Survey Questions and Data

Significance ($p < 0.05$) of the changes between the GCL-I and GCL-II responses was determined via a Mann-Whitney U Test.

Table S5.11. Mid-Quarter Survey Results: Attitude toward the Subject of Chemistry Inventory (ASCI (V2))

Field	GCL-I (1119)	GCL-II (930)	*p*-value	Significance
easy:hard	5.25	5.14	0.02	No
worthless:beneficial	4.87	4.93	0.5	No
exciting:boring	3.82	3.65	0.005	Yes
complicated:simple	2.69	2.75	0.1	No
confusing:clear	3.01	3.17	0.002	Yes
good:bad	3.56	3.42	0.01	Yes
satisfying:frustrating	4.29	4.14	0.04	Yes
scary:fun	3.64	3.62	0.7	No
comprehensible:incomprehensible	3.69	3.66	0.7	No
challenging:not challenging	2.35	2.39	0.2	No
pleasant:unpleasant	4.19	4.08	0.04	Yes
interesting:dull	3.24	3.17	0.3	No
disgusting:attractive	4.19	4.17	0.3	No
comfortable:uncomfortable	4.26	4.22	0.5	No
worthwhile:useless	3.30	3.20	0.07	No
work:play	2.17	2.22	0.4	No
chaotic:organized	3.64	3.83	0.004	Yes
safe:dangerous	3.35	3.54	0.004	Yes
tense:relaxed	2.58	2.80	0.0001	Yes
insecure:secure	3.44	3.60	0.02	Yes

Scale: Range 1-7 from the first adjective listed to the second adjective.

The end of quarter student survey was used verbatim except a five-point Likert scale was applied instead of a six-point scale. This survey was adapted from Corwin, L.A.; Runyon, C.; Robinson, A.; Dolan, E. The Laboratory Course Assessment Survey: A Tool to Measure Three Dimensions of Research-Course Design. *CBE-Life Sci. Educ.* **2015**, (14), 1-11.

1. Collaboration. In this course, I was encouraged to… *Weekly, Monthly, 1 or 2 times, Never*

a. Discuss elements of my investigation with classmates and instructors.
b. Reflect on what I was learning.
c. Contribute my ideas and suggestions during class discussions.
d. Help other students collect or analyze data.
e. Provide constructive criticism to classmates and challenge each other's interpretations.
f. Share the problems I encountered during my investigation and seek input on how to address them.

2. Discovery/Relevance. In this course, I was expected to… *Strongly Disagree, Somewhat disagree, Neither agree nor disagree, Somewhat agree, Strongly agree*
    a. Generate novel results that are unknown to the instructor and the could be of interest to the broader scientific communityor others outside of class
    b. Conduct an investigation to find something previously unknown to myself, other students, and the instructor.
    c. Formulate my own research question or hypothesis to guide an investigation.
    d. Develop new arguments based on data.
    e. Explain how my work has resulted in new scientific knowledge.
3. Iteration. In this course, I had time to… *Strongly Disagree, Somewhat disagree, Neither agree nor disagree, Somewhat agree, Strongly agree*
    a. Revise and repeat work to account for errors or fix problems.
    b. Change the methods of investigation if it was not unfolding as predicted.
    c. Share and compare data with other students.

d. Collect and analyze additional data to address new question or further test hypotheses that arose during the investigation.

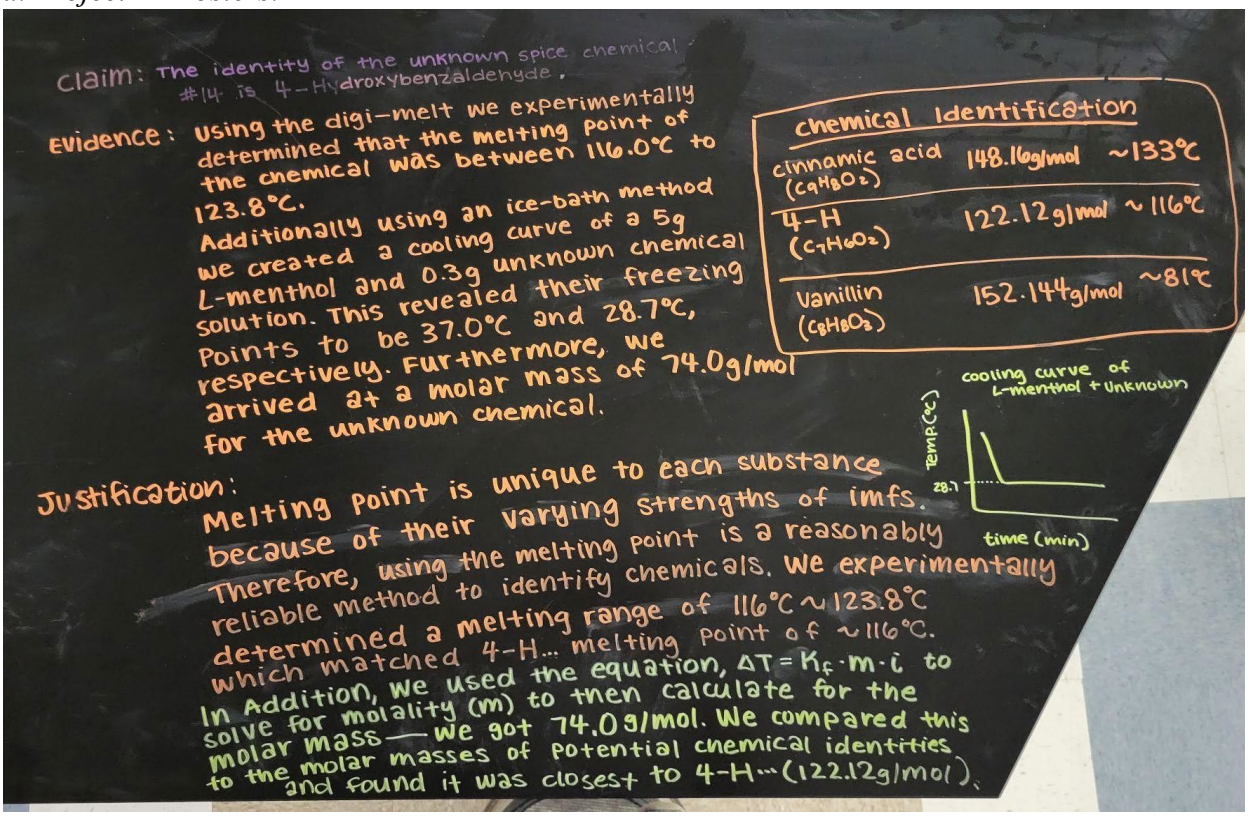
e. Revise and repeat analyses based on feedback.

f. Revise drafts of papers or presentations about my investigation based on feedback.

VII. Poster Examples

All poster examples shown below have been rewritten by the authors from student data for legibility.

a. Project #1 Posters.



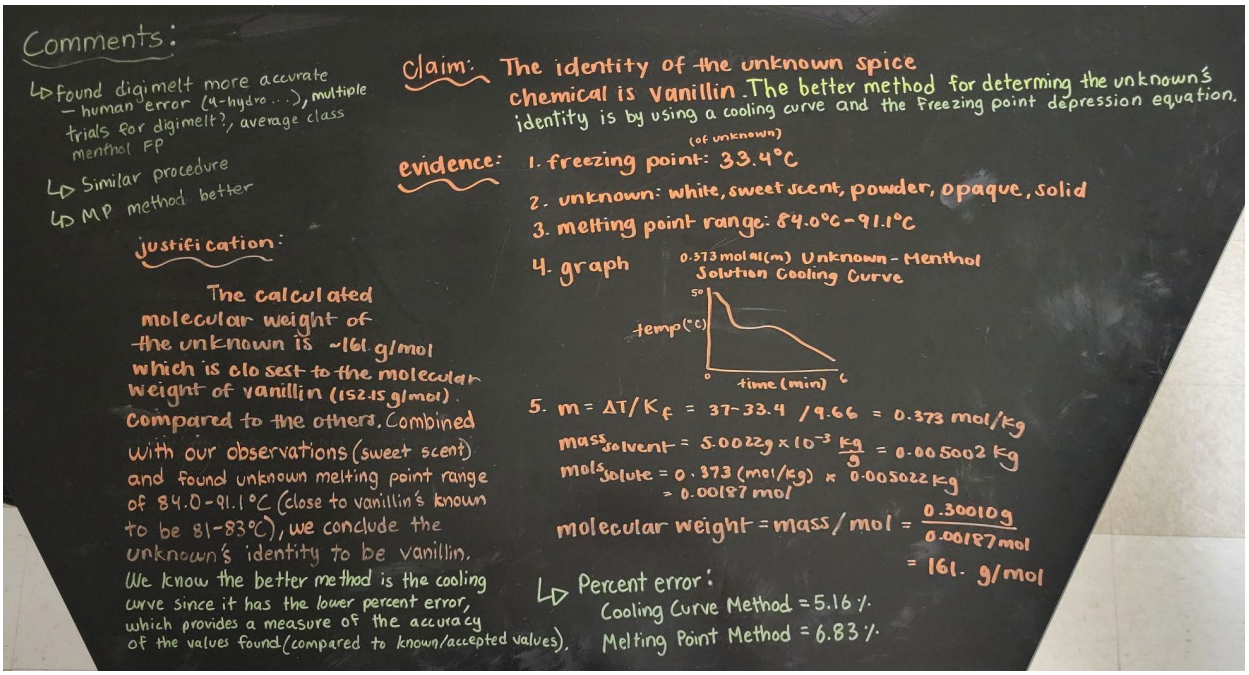
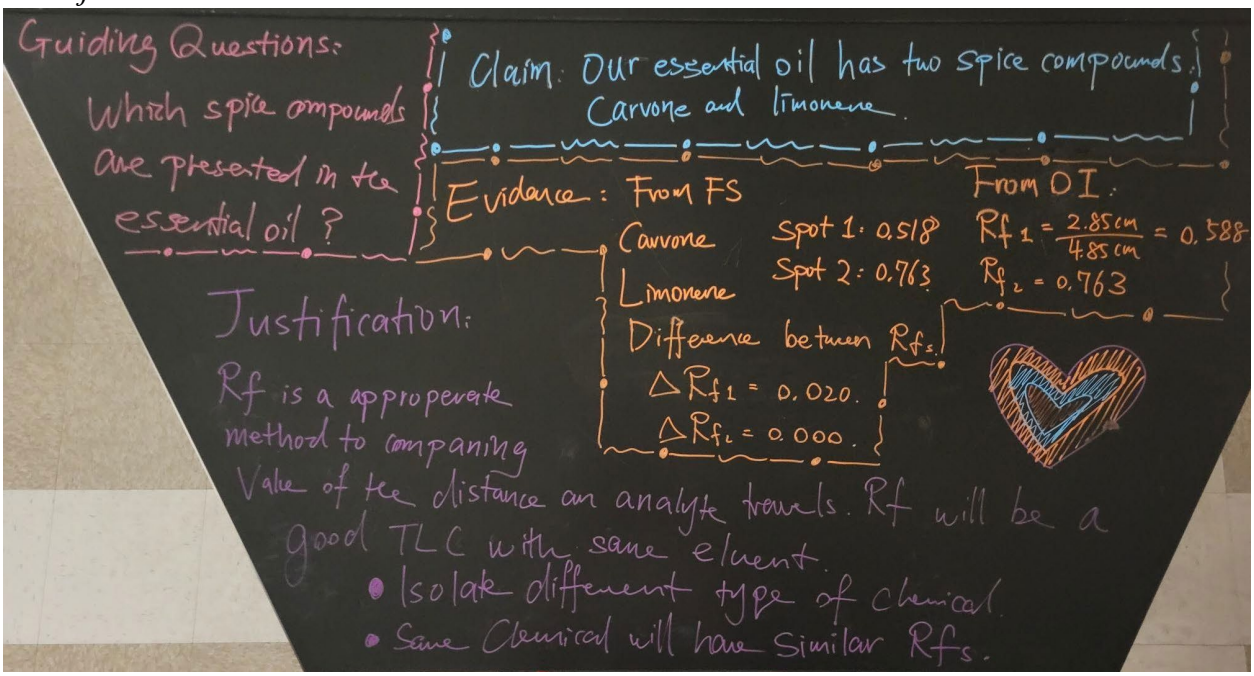


Figure S5.1. Poster Examples for Project 1.

b. Project #2 Posters.



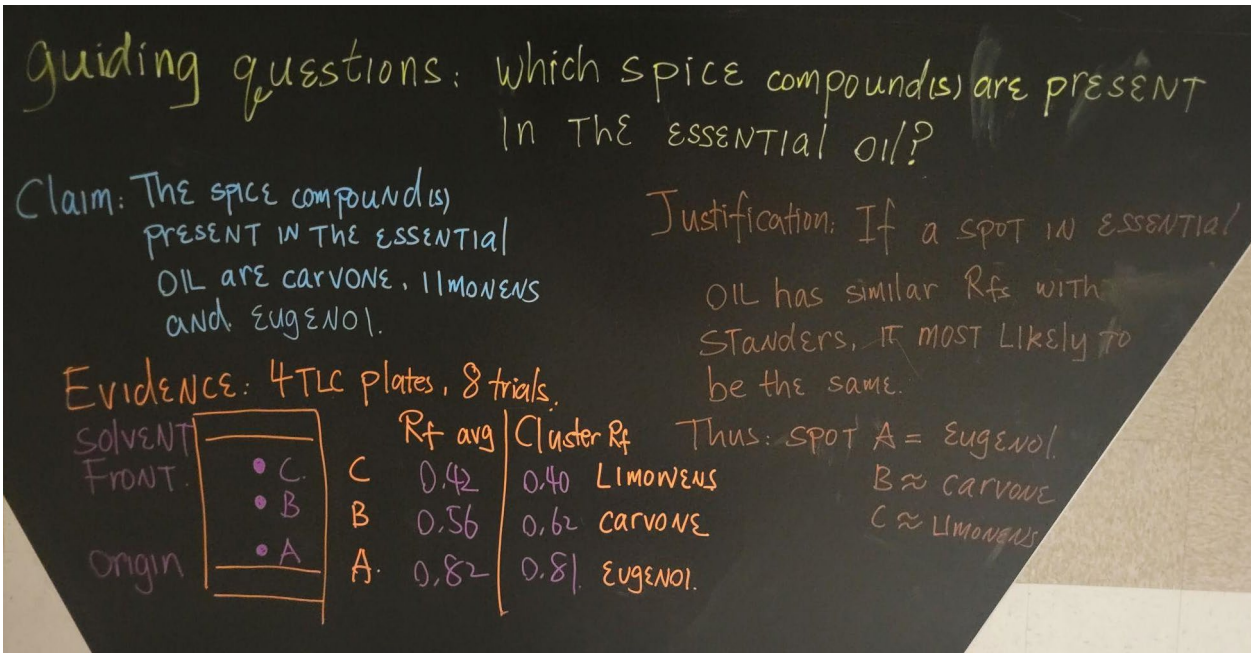
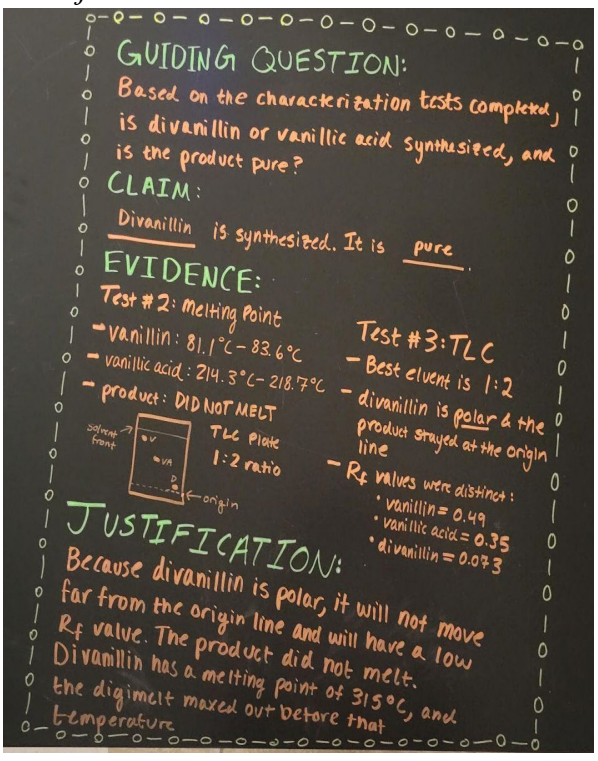


Figure S5.2. Poster examples for Project 2.

c. Project #3 Poster.



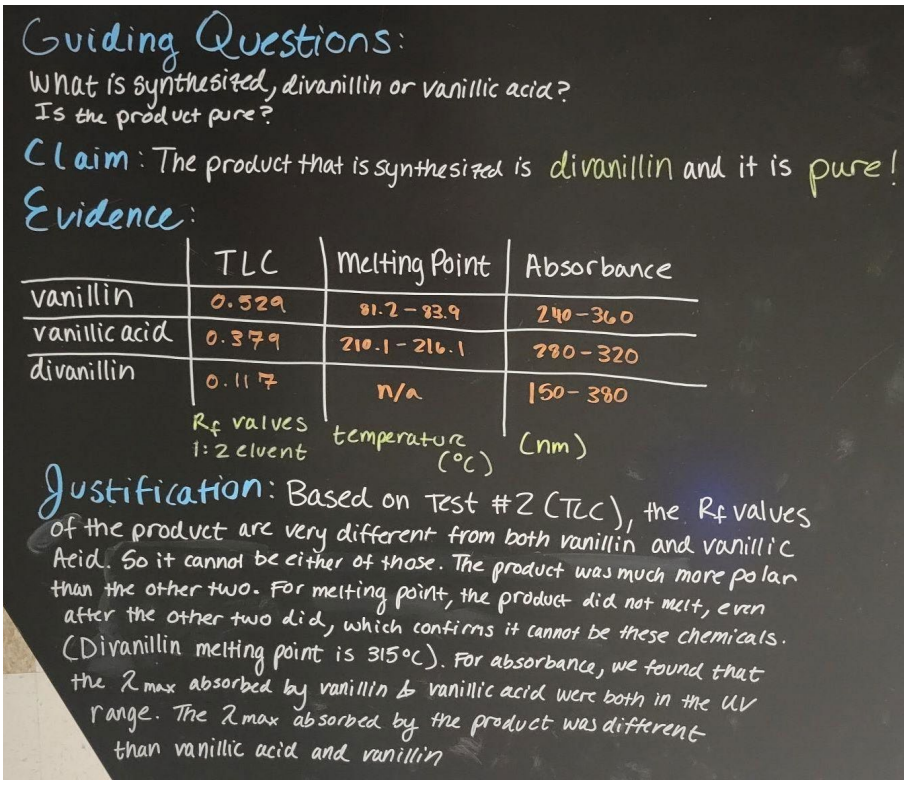


Figure S5.3. Poster Examples for Project 3.

d. Project #4 Poster.

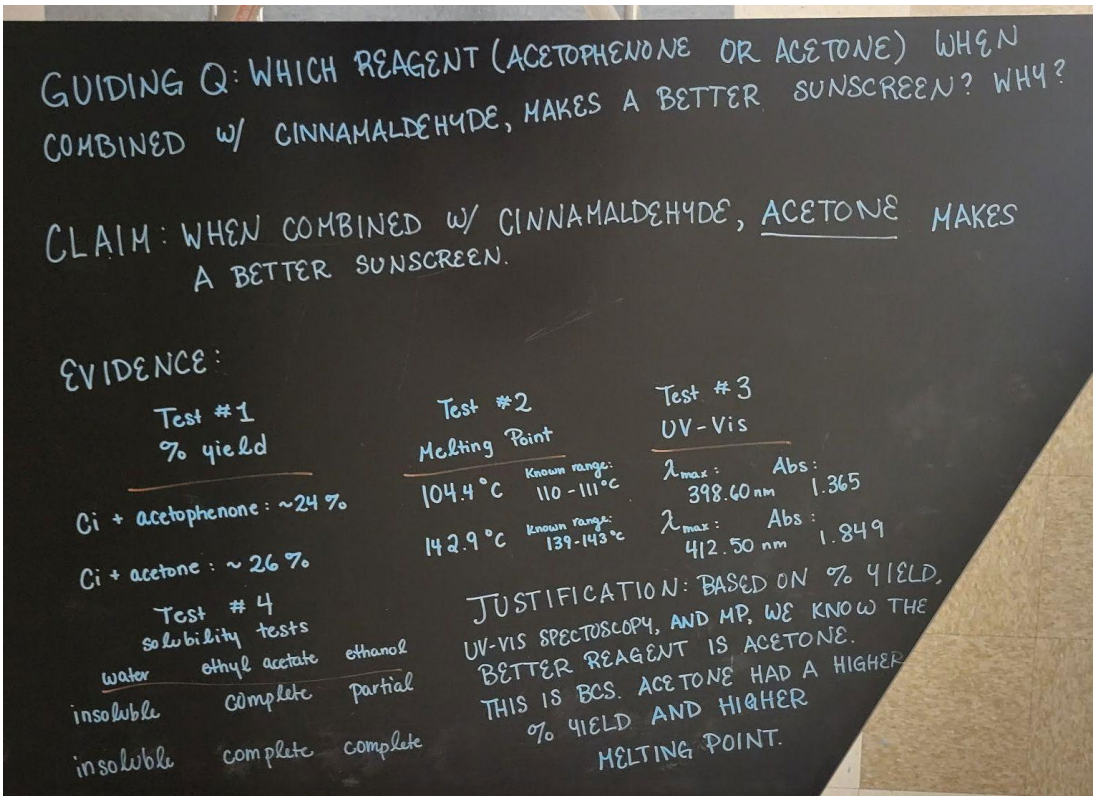


Figure S5.4. Poster Examples for Project 4.

VIII. Grading

This course was graded using specifications grading, which requires passing a certain number of each assignment type in order to achieve certain letter grades in the course (Table S5.12).

Table S5.12. Letter Grade Requirements

Assessment	Minimum to Earn D[a,b]	Minimum to Earn C[b]	Minimum to Earn B[b]	Minimum to Earn A[b,c]
Fundamental Skills Laboratory Notebook Assignments	Pass 2	Pass 3	Pass 3	Pass 4
Original Investigation Laboratory Notebook Assignments	Pass 2	Pass 2	Pass 3	Pass 4
Postlaboratory Reports	High Pass 1 & Low Pass 1 OR	High Pass 1 & Low Pass 2 OR	High Pass 2 & Low Pass 1 OR	High Pass 3 OR High Pass 2

	Low Pass 3	Low Pass 4	High Pass 1 & Low Pass 3	& Low Pass 2
Final Exam Components: Safety Knowledge, Technique, Argumentation	Pass 1	Pass 2	Pass 2	Pass 3

[a]Students who do not meet the minimum criteria for D grade earn an F in the course.

[b]Our institution does use plus and minus grades, and our course has set criteria for students to achieve these grades. To earn a plus grade, students must meet the criteria for the letter grade above and also earn at least 80% on prelaboratory quizzes. To earn a minus grade, students must meet the criteria for the letter grade above and also earn less than 65% on prelaboratory quizzes.

[c]To earn an A+ requires earning a High Pass on all four laboratory reports and earning at least 95% on prelaboratory quizzes.

All assignments were graded using a pass/no-pass format for in-lab assignments (Fundamental Skills (FS) Laboratory Notebook Assignments and Original Investigation (OI) Laboratory Notebook Assignments) and on a high-pass/low-pass/no-pass basis for Post-Laboratory Reports. The passing thresholds for each category are set at approximately 80% for the FS and OI Laboratory Notebook Assignments (Table S5.13). For Lab Reports (LR), the high-pass threshold is set to approximately 85%, while the low pass is set to around 60% (Table S5.14).

Tokens can be earned and exchanged as follows:

<u>Token Earning Opportunities:</u>

- 2 Tokens are earned for completing the work with 70% or higher in the first Canvas module.
- 3 Tokens can be earned for completing all course surveys.

Tokens Trade-Ins:

- 1 Token:
    - a resubmission (within 72 hours from the time your TA sends an announcement) of an "incomplete" Fundamental Skills or Original Investigation assignment (as long as you made a significant attempt on the assignment) or a "low pass" Lab Report
    - make-up lab session (only 1 allowed per quarter). *Please note:* The availability of this option depends on the day & time of your lab section and the timeliness of your request. Make-ups can only be made during the week of the particular assignment.
- 2 Tokens:
    - a resubmission of a Lab Report (as long as you made a significant attempt on the assignment) within 72 hours
    - data set if you cannot attend lab (only 1 data set OR make up lab allowed per quarter - in other words, you can only miss one lab)
- 3 Tokens:
    - a pass on one section of the practical (can only be used once). Tokens left over at the end of the quarter will automatically be applied to this criteria.

Specific pass requirements are shown below:

Table S5.13. Pass Thresholds for FS and OI Assignments

Assignment	Pass Threshold
Fundamental Skills #1	11/14
Original Investigation #1	8/10
Fundamental Skills #2	8/11
Original Investigation #2	8/10

Fundamental Skills #3	10/12
Original Investigation #3	8/11
Fundamental Skills #4	8/10
Original Investigation #4	8/11

Table S5.14. Pass Thresholds for LR Assignments

Assignment	Low Pass Threshold	High Pass Threshold
Lab Report #1	8/14	12/14
Lab Report #2	9/15	13/15
Lab Report #3	9/15	13/15
Lab Report #4	9/15	13/15

IX. Modifications to Project 2: TLC

After collection of the data presented in this chapter, modifications were made to Project 2 to improve the quality of data collected by students. The TLC shown in the main paper was performed with 4 ratios of heptane:acetone; this has been optimized to improve the separation of the spice compound standards for clearer identification of the unknown.

We recommend using a mixture of heptane and ethyl acetate as the TLC solvent. The four ratios used in the current iteration of this experiment are: 9:1, 4:1, 3:2 and 2:3, as we found making the TLC solvent more nonpolar achieved better separation of the spots.

The ideal ratio is 9:1 heptane:ethyl acetate, as shown below in Figure S5.5.

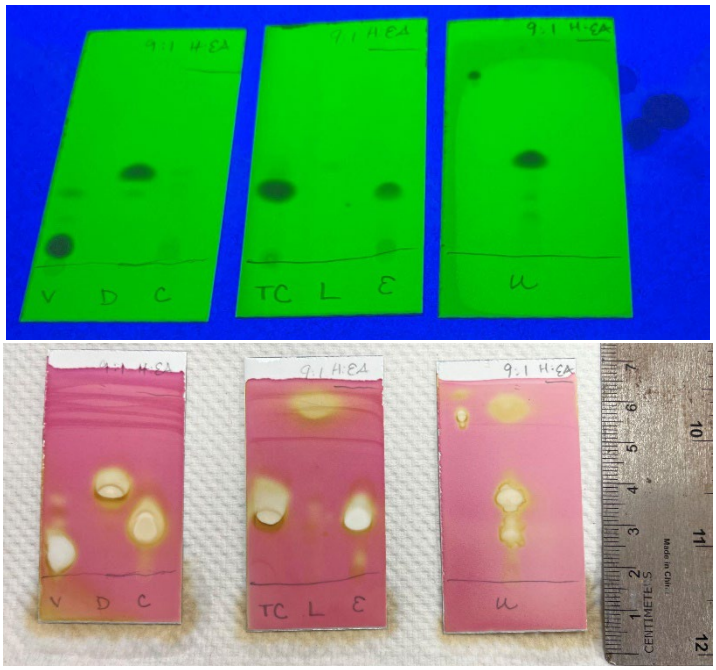


Figure S5.5. TLC of standards and unknown in 9:1 heptane:ethyl acetate under UV light (top) and after KNO_4 stain. From left to right: vanillin, dihydrocarveol, carvone, *trans*-cinnamaldehyde, limonene, eugenol, and unknown (containing carvone, dihydrocarveol, and limonene).

X. Demographic Information

Table S5.15. GCL-II Student Demographics

Quarter	**Fall On-Sequence**	**Winter Off-Sequence**
Enrollment	945	154
Number of G-TAs	22	4
Biological Sciences Majors	64.2%	48.1%
Undeclared/Unaffiliated Students	4.6%	7.1%
First Generation College Students	32%	47%
Low income Students	30%	45%
International Students	7%	7%

CHAPTER 6

ON-GOING PROJECT: DEVELOPMENT OF ARGUMENT-DRIVEN INQUIRY LABORATORIES FOR ORGANIC CHEMISTRY

ABSTRACT

Argument-Driven Inquiry (ADI) laboratory experiments are one of the many advances to move away from confirmatory, "cookbook" style of laboratory curriculum, among other advances for more guided inquiry in the teaching laboratory. With student-led argumentation and revision of claims based on evidence and discussion, ADI is an attractive alternative to incorporate guided inquiry into the laboratory in a structured framework. While there are multiple examples of ADI General Chemistry laboratories, there are few literature examples of ADI experiments designed for a second-year Organic Chemistry course. This chapter outlines the ongoing effort to design a series of Organic Chemistry experiments to be used in an ADI course, with a focus on designing intentional variation to lead to robust argumentation. These experiments were evaluated by a group of undergraduate beta-testers, who performed the full course as students, including the argumentation sessions. These designed experiments are discussed and analyzed based on student feedback. The argumentation sessions were analyzed by the Assessment of Student Argumentation in the Classroom protocol to quantify the level of discourse achieved by the students. Both results are evaluated to determine the efficacy of the designed curriculum. Future directions and continuing work on the curriculum are outlined.

INTRODUCTION

The following on-going project builds on the materials discussed in Chapter 4 and Chapter 5, to expand the Argument-Driven Inquiry-style laboratories to Organic Chemistry, which, to our knowledge, has never been reported. Argument-Driven Inquiry (ADI) requires the students to design their own experimental procedures during the second half of each project (Original Investigation, or OI), which can provide significant logistic and safety challenges during organic chemistry experiments. ADI, as well, requires variability in results for students to discuss and debate their results. An experiment that gives reproducible results with no variation in the hands of beginners will not provide sources for argumentation, and is therefore unsuited to ADI. However, these reliable results are found in most published undergraduate experiments. To address this gap, I have worked with the instructional team at University of California, Irvine (UCI) to develop and design new ADI curriculum.

Herein, we describe preliminary attempts to transition existing curriculum to the ADI format, building upon the previously reported success of UCI's ADI General Chemistry Laboratories. Consistency across lower-division laboratories is an additional benefit for both the general chemistry series and organic chemistry series to adopt the ADI process.

Organic Chemistry Laboratory I (OCL-I) at UCI is a 10-week course with course meetings once a week for 4 hours, with a week being used for final examinations and a floating week to account for holidays, leaving 8 course meetings for experiments. The ADI curriculum moves away from independent single-week experiments to add projects spanning at least two weeks, with one week for Fundamental Skills (straight-forward experiments with procedures provided by the instructor) and Original Investigation (open-ended experiments with student-written procedures). With these time constraints and the multiweek nature of ADI, we focused on fundamental organic

chemistry techniques and concepts such as competition between reactions rather than attempting to incorporate as many reactions as possible. As seen in the Guiding Questions table below (Table 6.1), the Fundamental Skills questions focus on the understanding of a particular technique and reaction, while the Original Investigation questions focus on the *application* of techniques and reactions to answer a conceptual question.

Table 6.1. Guiding Questions for OCL-I

	Fundamental Skills (FS)	Original Investigation (OI)
Project #1	(1) How can you determine the identity of each pure substance through the use of ^{1}H NMR and IR spectroscopy? How can you recognize that a given sample is a mixture containing more than one substance using ^{1}H NMR and IR spectroscopy? (2) How can you determine the identity of each mixture through the use of melting point and TLC?	What is the identity of the compounds in the unknown mixture? How did you conclusively determine the identity?
Project #2	What is the ratio of substitution to elimination products for the reactions performed?	What reagents favor substitution and/or elimination, and how could you conclusively determine this?
Project #3	(1) What recrystallization procedure was most effective for purifying trans-stilbene and why was this the most effective procedure? (2) What is the major component of your crude product and how were you able to confirm this?	Which recrystallization technique was the best for isolating your major compound? Why was this technique the most effective?

As the laboratory sequence builds on each other, we wanted to increase the level of inquiry, as defined by Bruck *et. al.*,[1] during the Organic Chemistry series, compared to the previously reported General Chemistry series.[2,3] As seen in Table 6.2, nearly all of the experiments have increased inquiry, especially during the Original Investigation of each project. With consistent open inquiry during the last week of each project, we want to move the curriculum closer and closer to reflecting authentic research.

Table 6.2. Level of Inquiry by Laboratory Session.

Characteristic	Fundamental Skills	Original Investigation
Problem /Question	P	P
Theory /Background	P	P
Procedures /Design	P	NP
Results Analysis	NP	NP
Results Communication	NP	NP
Conclusion	NP	NP
Level of Inquiry	Guided (1)	Open (2)

[a]P = provided
[b]NP = not provided

When designing the experiments for OCL-I, we had to consider the logistics of the course. During on-sequence quarters, OCL-I can have up to 1500 students enrolled; the off-sequence quarter has approximately 300 students. The course is taught by graduate teaching assistants, many of which have not taught the course before, even in its previous iteration. The experiments designed must be able to be prepped on large scale and give results that are consistent (to prevent excessive troubleshooting) but variable (to foster robust argumentation). A further consideration is the lack of available equipment: lower-division teaching laboratories do not have access to rotary evaporators and NMR spectra cannot be provided the same day as the experiment due to scheduling conflicts. We also account for hazards and cost when choosing materials and reactions, and have adapted the curriculum to greener, safer reagents that require no special waste disposal.

All of the projects presented here take and account for these restraints and are therefore readily adaptable to many universities and colleges in different situations. We present three projects for the first-quarter or -semester organic chemistry lab. Students work in groups of 3 to 4 to answer the guiding questions for each project, with each student working in tandem to complete the techniques and reactions. In Fundamental Skills, they learn procedures for techniques and reactions, followed by a modified procedure designed by the group in the Original Investigation.

PROJECTS

Project #1: Identity and Purity of Pharmaceutical Mixtures

Project #1 is adapted from the current curriculum used at UCI, developed by Prof. Will Howitz, a previous graduate student. This project focuses on the fundamental techniques that are essential to undergraduate organic chemistry laboratory experiments: thin-layer chromatography (TLC), melting point analysis, infrared spectroscopy (FT-IR), and interpretation of ^{1}H NMR spectra. The students use the four techniques listed to determine the composition of a mixture of two unknown pharmaceutical compounds. This is a three-week project, with two weeks of Fundamental Skills to learn the individual techniques, followed by one week of Original Investigation, where students attempt to determine the composition of an unknown mixture of two pharmaceutical compounds.

The Fundamental Skills portion of this project lasts for two weeks. In these two weeks, the students are presented with an unknown mixture made up of two of four pharmaceutical compounds: acetaminophen, acetylsalicylic acid, naproxen, and sorbitol (Figure 6.1a). Standards of each of the four compounds are also provided. The first week is focused on the acquisition and interpretation of FT-IR spectra and the interpretation of instructor-provided ^{1}H-NMR spectra for both the standards and the unknown mixture. The provided NMR spectra is fully integrated but

not assigned for both the standards and the unknown mixtures. In the second week, they perform the analysis of their same unknown mixture with TLC and melting point analysis. In order to have a valid conclusion, they must compare the TLC and melting point results of their mixture to the standards, which must be run in tandem by the students. As this is the Fundamental Skills portion of the project, and this is the first time some students will be performing these techniques, an explicit procedure is provided for each of the four techniques. For future projects, and for the Original Investigation, step-by-step procedures for these techniques are not provided.

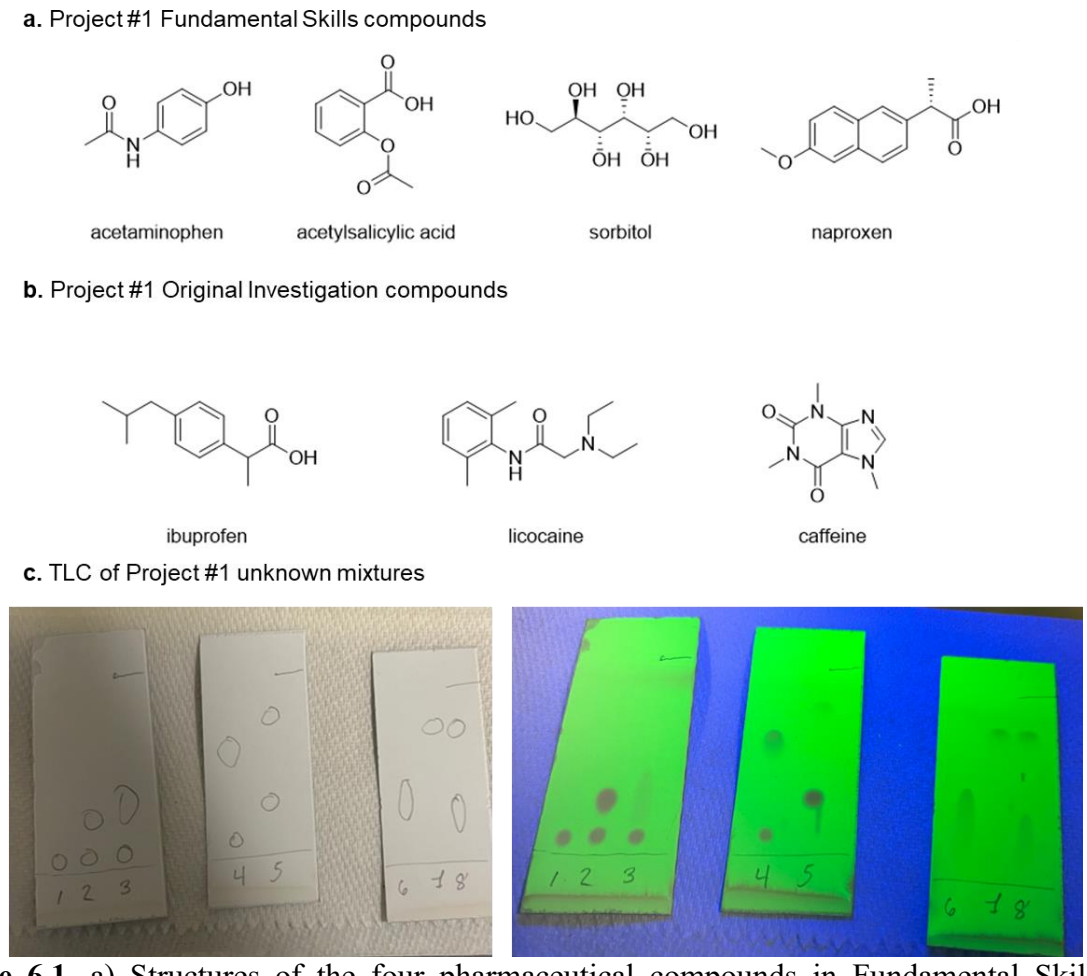


Figure 6.1. a) Structures of the four pharmaceutical compounds in Fundamental Skills and Original Investigation. b) Structures of the three additional pharmaceutical compounds in Original Investigation. c) TLCs of the 8 unknown mixtures for Original Investigation. For both pictures, from left to right: 1. sorbitol:caffeine (1:1), 2. acetaminophen:caffeine (1:1), 3. acetylsalicylic acid:caffeine (1:1), 4. naproxen:caffeine (1:1), 5. acetaminophen:ibuprofen (1:3), 6. sorbitol:lidocaine (1:3), 7. sorbitol:ibuprofen (1:3), 8. acetylsalicylic acid:ibuprofen (1:3).

Within the Fundamental Skills portion of lab, students should discover that some techniques were better suited to determining the identity of some compounds, rather than others. Melting point can not be used to identify a mixture, and sorbitol does not appear under UV light for TLC. The NMR, particularly for the aromatic region, can be difficult for students to interpret for mixtures including naproxen and acetylsalicylic acid.

The third week of this project is the Original Investigation, each group of 4 students is given a new mixture composed of one of the previous four pharmaceutical compounds (acetaminophen, acetylsalicylic acid, naproxen, sorbitol) and one of three new compounds (ibuprofen, lidocaine, and naproxen) (Figure 6.2b). Standards of the new compounds were provided as well, and no procedure is given other than the guideline that at least three different techniques must be performed. In an ideal procedure, students characterize both the unknown mixture provided to them and the three standards using any of the four techniques to properly compare and contrast data. The eight unknown mixtures in Original Investigation, and the eight mixtures in Fundamental Skills (see appendix for list), were chosen because of their ability to provide confirmation of identities through TLC or IR in addition to ^{1}H-NMR, as students should have multiple pieces of evidence to support their claim.

Project #2: Nucleophile and Electrophile Choice for Substitution and Elimination Reactions

Project #2 focuses on the competition between substitution and elimination reactions, and is adapted from a previously published paper on guided inquiry experiments by Wharry.[4] The experiments presented in this publication appealed to us for use in ADI due to the variable results between the difference nucleophiles (sodium ethoxide, sodium methoxide, or potassium tert-butoxide) and electrophiles (1-bromopentane, 2-bromopentane, and 2-bromo-2-methylbutane). When the students design their own procedures for the Original Investigation, they can choose

between these different nucleophiles and electrophiles to attempt to achieve either more substitution or elimination, ideally designing experiments based on their conceptual understanding of how steric interactions influence the ratio between substitution and elimination products. This is a two-week project, with the first week being devoted to the Fundamental Skills section, and the second week for Original Investigation.

In Fundamental Skills, students perform a standard reaction of 2-bromopentane with sodium methoxide in methanol, which was reported to give a roughly equal mixture of substitution and elimination product. We have found that it gives slightly more substitution product (Figure 6.2) but is nevertheless a good starting point for conceptual understanding, with a secondary alkyl halide and a "medium-sized" nucleophile. This reaction, which is performed at reflux for 1 hour, is then extracted with methyl tert-butyl ether and saturated ammonium chloride (to remove the precipitated sodium bromide salt). The organic layer of the extraction is taken directly to the GC and analyzed.

During the reaction time, the students perform a brief extraction activity in order to learn the technique in a low-stakes environment. The students are provided a known mixture of sodium benzoate and acetanilide, where sodium benzoate is extracted into the aqueous layer and acetanilide into the organic. The solvent system of MTBE and water reflects the extraction solvents used in the reaction, and is confirmed by the students' choice of either TLC or melting point.

In the Original Investigation, students are asked to modify the previous reaction by changing the nucleophile, electrophile, or both, to favor either substitution or elimination. Students are allowed to change experimental parameters such as time and temperature as well, but must confirm these changes with the laboratory instructor before proceeding. Students are only requires

to perform one additional reaction, but groups can perform more than one, provided that they budget their time efficiently.

For legibility of the gas chromatograph, the MTBE/alcohol peak, which consistently appears between the olefin and ether product, is included from the calculated ratios. In all cases, the alkyl halide starting material is the last significant peak to elute from the column, as confirmed by GC-MS and standards. (See section VIII in the appendix.)

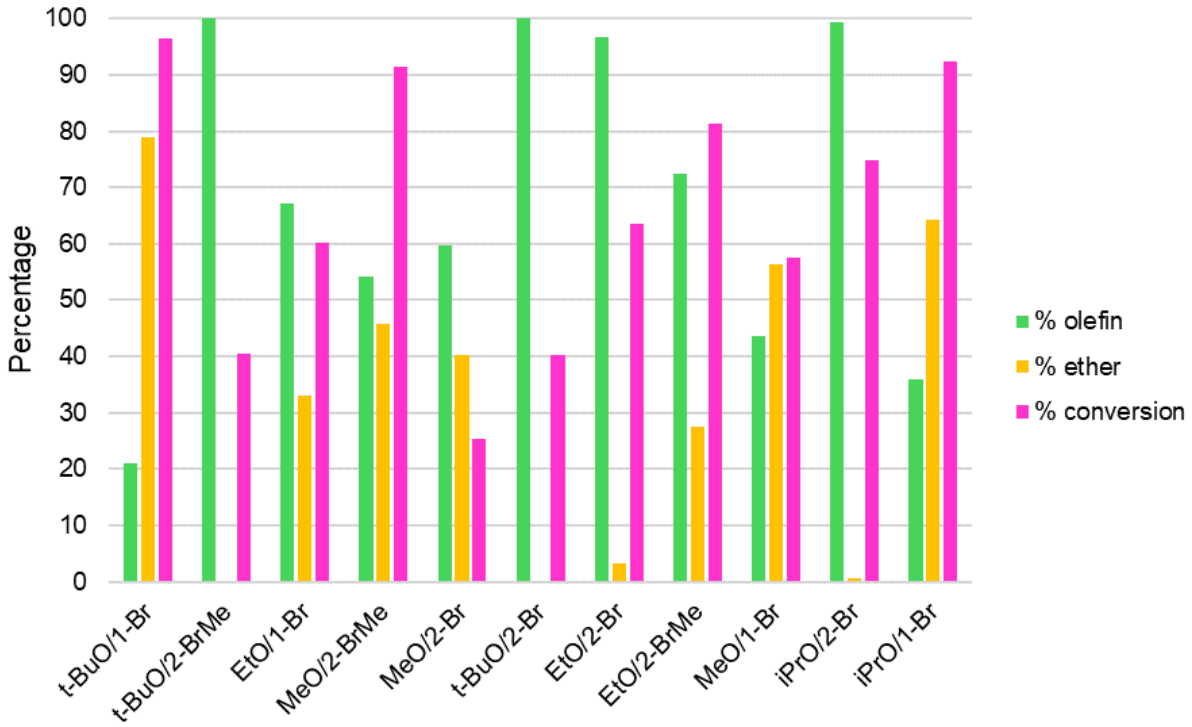


Figure 6.2. The percentages of olefin and ether products and percent conversion for the Project #2 reactions, calculated via GC. Abbreviations: t-BuO = sodium t-butoxide/t-butanol, MeO = sodium methoxide/methanol, EtO = sodium methoxide/methanol, iPrO = sodium isopropoxide/isopropanol, 1-Br = 1-bromopentane, 2-Br = 2-bromopentane and 2-BrMe = 2-bromo-2-methylbutane.

When attempting to replicate the results presented by Wharry,[4] we discovered differences with our experiments that were relevant to the design of the course. We found that, generally, the amounts of olefin were higher than previously reported, particularly noticeable in the reactions with low conversion. This is likely due to the high temperature and short reaction time, which will

favor the elimination product. This deviation from the expected results can foster discussion amongst the students, but instructors should be aware of the increased difficulty of shifting the product ratio towards substitution rather than elimination.

In addition, we found that the 2-methyl-2-bromobutane provided significant impurities that hindered interpretation of the data. As confirmed by gas chromatography, the 2-bromo-2-methylbutane used in these experiments matched its reported 95% purity, with 1-bromopentane (90%) and 2-bromopentane (98%) having similar purity. The impurities in the case of 2-bromo-2-methylbutane did not elute with the solvent and instead convoluted the GC spectra to the point where the students were unable to determine the substitution product and starting material peaks, even when provided with standard GC spectra. We considered the hypothesis that the 2-bromo-2-methylbutane was breaking down inside the column or at the injection site due to the heat, but similar results were obtained even with a room temperature injection port and minimal heating.

Isopropoxide, as seen in Figure 6.2, has been incorporated into this project as a fourth nucleophile, outside the scope of the Wharry paper.[4] This, along with the continued optimization of Project #2 to find a reliable third electrophile for the Original Investigation to replace the 2-bromo-2-methylbutane, adds to the choices the students can make when designing their own unique procedure.

Project #3: Recrystallization of an Epoxidation Product

The third project focuses on recrystallization, a technique not introduced yet into the course. We have found, anecdotally, that the concepts that underly recrystallization are often lost due to the complexity of the technique itself. This project spans three weeks, with one Fundamental Skills week being devoted to a recrystallization workshop. The second week of Fundamental Skills focuses on the epoxidation of trans-stilbene to achieve and impure product and characterizing that

crude product. The Original Investigation allows the student to design their own recrystallization procedure based on the ones presented in the recrystallization workshop, or try a new procedure with any combination of common organic solvents.

The recrystallization workshop in the first week of Project #3 presents each group of students a known impure mixture where the major component is trans-stilbene. Each group of 4 students will attempt three different recrystallization techniques, which differ by solvent: 1) single solvent with isopropanol; 2) dual solvent with toluene/ethanol; and 3) dual solvent with ethanol/water. The expected results of these three recrystallization should favor the ethanol/water system; isopropanol alone gives a lower percent recovery (due to difficulties in determining the saturation point) and toluene/ethanol gives a high percent recovery, but an impure product (due to the similar solubility of both components in toluene).

The recrystallization workshop has been tested with the minor compound as either biphenyl or cis-stilbene and works well. We recommend biphenyl as the minor components as the mixture of cis- and trans-stilbene results in a rather "goopy" mixture with a melting point very close to room temperature. After recrystallization with any of three options, a more solid product is obtained, but this can make the technique more difficult for some students. In addition, biphenyl is significantly cheaper than cis-stilbene, and therefore might be preferred for classes with large enrollments.

The second week of Fundamental Skills focuses on the students obtaining the crude epoxidation product. Trans-stilbene can be easily epoxidized through the use of mCPBA but we shied away from this route due to the dangers of this highly reactive oxidant.[5] When looking for alternative green procedures, we came across the Course-based Undergraduate Research Experience by Wilczek *et. al.*[6], adapting the procedure reported by Limnios and Kokotos.[7] This

procedure reports a 70% conversion for unactivated alkenes such as trans-stilbene through the use of hydrogen peroxide and 2,2,2-trifluoroacetophenone as a catalyst, and this was ideal for our purposes. As the Original Investigation focuses on recrystallizing an impure product, we sought a procedure that results in incomplete conversion, resulting in a crude product that is a mix of trans-stilbene and trans-stilbene oxide. During this experiment, extraction must be performed as part of the workup, and TLC and melting point are used to characterize the crude product, incorporating the previously learned techniques.

The Original Investigation results in students designing their own recrystallization procedure for their crude product. The only guidance given in the Original Investigation is that each group of 4 must try at least two different recrystallizations, in order to answer the guiding question to determine what recrystallization procedure is preferred and why. Many of the students in the beta-testing course used the solvent systems presented in the recrystallization workshop. The toluene/ethanol solvent system is particularly unsuited to the crude product, as it has high solubility at both low and high temperatures. Students also have the option to try different solvent systems or use different temperatures instead of the procedures given; we anticipate this will be less common than using those presented in the recrystallization workshop, but students should be made aware that it is an option.

Although the green oxidation procedure with 2,2,2-trifluoroacetophenone as a catalyst was designed to give an impure product, the beta-testing class had difficulty obtaining the epoxide as the major product, which resulted in the students obtaining a "major product" of their starting material and recrystallizing trans-stilbene during their Original Investigation. We decided to transition to a different green procedure presented by Broshears *et. al.* using the salt Oxone as the oxidizing agent,[8] which we hope will give the students a major product of trans-stilbene oxide.

ARGUMENTATION ANALYSIS

At the end of each Original Investigation, there is an argumentation session, where the students discuss and debate their results in a "poster session." Engaging in active debate and discussion is a necessary part of science and is an essential part of the ADI curriculum. In order to evaluate our new curriculum to see if it met the standards of ADI, we evaluated the argumentation through the use of the Assessment of Scientific Argumentation in the Classroom (ASAC) protocol.

The ASAC protocol was first introduced by Sampson *et. al.* in 2012 to address the lack of a protocol targeted specifically at the cognitive, epistemic, and social aspects of scientific argumentation.[9] This observation protocol has been adapted to analyze argumentation sessions in ADI classrooms in several studies.[10–13] This tool, being designed to analyze the nature and quality of student argumentation, was uniquely suited to assist us in determining the level of argumentation achieved by the beta-testing class. For analysis, we used a modified version of the ASAC protocol, used by Hosbein *et. al.*,[12] with 18 possible points in each of the categories (cognitive, epistemic and social) for a total of 54 possible points (see appendix section IV for the completely protocol).

The beta-testers were a group of 12 undergraduates who participated in a mock course and provided feedback on assignments and experiments. They performed the course as written and participated in the argumentation session, which was recorded and then transcribed. The beta-testing students were in their third and fourth year, have all taken the organic chemistry laboratory series and were all STEM majors. Therefore, the argumentation data comes from a more experienced group of students than would be present in a classroom and is skewed by this. As well, due to the small size of the beta-testing class, there is not a significant sample size to draw any statistical conclusion from this data. However, in these preliminary results, we can compare to the

existing studies that have used the ASAC protocol to evaluate argumentation and determine if the argumentation demonstrated by the beta-testing class is comparable to other undergraduate scores.

Sampson *et. al.* and Hosbein *et. al.* reported a cumulative ASAC score between 18 to 35 points for their undergraduate participants, fluctuating over the course of a semester.[9,12] As seen in Figure 6.3, the ASAC scores achieved by the beta-testing course easily match this standard set by previous studies.

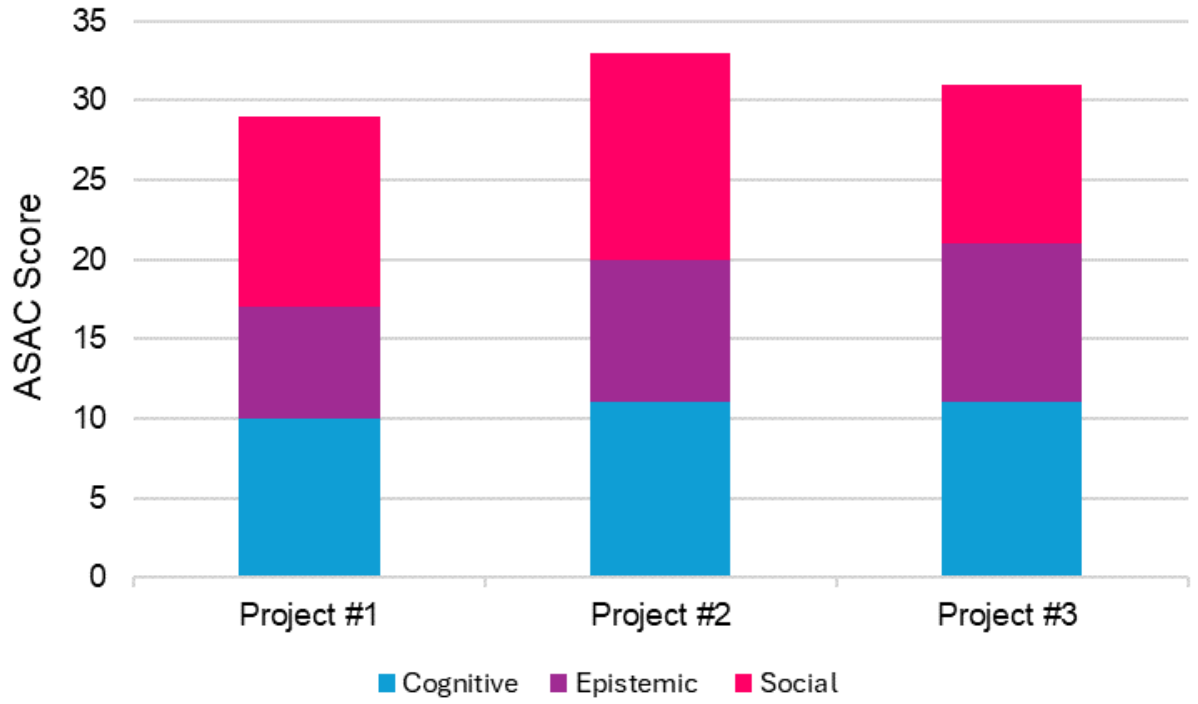


Figure 6.3. ASAC observation scores for the argumentation sessions in the beta-testing course.

There are a few caveats for this comparison, as mentioned previously. The students are more experienced than the normal demographic of second-year organic chemistry students, and the two studies cited for comparison are based on general chemistry students, who are often freshman and have little lab experience. Nevertheless, we consider these preliminary results promising, as it indicates the experiments have enough intentional variability to support robust argumentation. When these experiments are implemented into an active course, we hope to analyze

the argumentation sessions of the students to obtain statistically significant data to guide future curriculum.

CONCLUSIONS AND FUTURE DIRECTIONS

These experiments consist of the first Argument-Driven Inquiry experiments designed for the organic chemistry laboratory series, to our knowledge. These experiments incorporate the main techniques of organic chemistry, as well as introducing introductory chemical concepts such as competition between substitution and elimination. These three projects were designed to give variable results that would allow for robust discussion. The beta-testing course demonstrated that the argumentation was comparable to other undergraduate ADI courses presented in the literature. We hope that this curriculum will be used at both UCI and other institutions to bring authentic inquiry into the undergraduate organic laboratory.

These experiments are still being tweaked before their first implementation at UCI in the summer of 2024, with small procedural changes such as those mentioned in the Projects section. The second ADI Organic Chemistry laboratory, OCL-II, is also in development, and will be implemented in Fall 2024. During these courses, the argumentation will be recorded and analyzed as it was presented herein, and student surveys will be gathered in order to evaluate student response to the new curriculum.

REFERENCES

(1) Bruck, L. B.; Bretz, S. L.; Towns, M. H. Characterizing the Level of Inquiry in the Undergraduate Laboratory. *Res. Teach.*

(2) Howitz, W. J.; Frey, T.; Saluga, S. J.; Nguyen, M.; Denaro, K.; Edwards, K. D. A Specifications-Graded, Sports Drink-Themed General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach. *J. Chem. Educ.* **2023**, *100* (2), 672–680.

(3) Saluga, S. J.; Burns, A. M.; Li, Y.; Nguyen, M. M.; Edwards, K. D. A Specifications-Graded, Spice-Themed, General Chemistry Laboratory Course Using an Argument-Driven Inquiry Approach. *J. Chem. Educ.* **2023**, *100* (10), 3903–3915.

(4) Wharry, D. L. The Study of Substitution and Elimination Reactions Using Gas Chromatography: An Examination of the Effects of Alkane and Base Structure on Product Distributions. *J. Chem. Educ.* **2011**, *88* (12), 1720–1723.
(5) Kim, C.; Traylor, T. G.; Perrin, C. L. MCPBA Epoxidation of Alkenes: Reinvestigation of Correlation between Rate and Ionization Potential. *J. Am. Chem. Soc.* **1998**, *120* (37), 9513–9516.
(6) Wilczek, L. A.; Clarke, A. J.; Guerrero Martinez, M. del C.; Morin, J. B. Catalyzing the Development of Self-Efficacy and Science Identity: A Green Organic Chemistry CURE. *J. Chem. Educ.* **2022**, *99* (12), 3878–3887.
(7) Limnios, D.; Kokotos, C. G. 2,2,2-Trifluoroacetophenone: An Organocatalyst for an Environmentally Friendly Epoxidation of Alkenes. *J. Org. Chem.* **2014**, *79* (10), 4270–4276.
(8) Broshears, W. C.; Esteb, J. J.; Richter, J.; Wilson, A. M. Simple Epoxide Formation for the Organic Laboratory Using Oxone. *J. Chem. Educ.* **2004**, *81* (7), 1018.
(9) Sampson, V.; Enderle, P. J.; Walker, J. P. The Development and Validation of the Assessment of Scientific Argumentation in the Classroom (ASAC) Observation Protocol: A Tool for Evaluating How Students Participate in Scientific Argumentation. In *Perspectives on Scientific Argumentation: Theory, Practice and Research*; Khine, M. S., Ed.; Springer Netherlands: Dordrecht, 2012; pp 235–264.
(10) Clevenger, L.; Teshera-Levye, J.; Walker, J. P.; Vance-Chalcraft, H. D. Using an Observation Protocol To Evaluate Student Argumentation Skills in Introductory Biology Laboratories. *J. Microbiol. Biol. Educ.* **2023**, *24* (2), e00209-22.
(11) Walker, J. P.; Sampson, V. Learning to Argue and Arguing to Learn: Argument-Driven Inquiry as a Way to Help Undergraduate Chemistry Students Learn How to Construct Arguments and Engage in Argumentation During a Laboratory Course. *J. Res. Sci. Teach.* **2013**, *50* (5), 561–596.
(12) Hosbein, K. N.; Lower, M. A.; Walker, J. P. Tracking Student Argumentation Skills across General Chemistry through Argument-Driven Inquiry Using the Assessment of Scientific Argumentation in the Classroom Observation Protocol. *J. Chem. Educ.* **2021**, *98* (6), 1875–1887.
(13) Walker, J. P.; Van Duzor, A. G.; Lower, M. A. Facilitating Argumentation in the Laboratory: The Challenges of Claim Change and Justification by Theory. *J. Chem. Educ.* **2019**, *96* (3), 435–444.

APPENDIX

I. IRB Statement

This study was approved by the University of California, Irvine, Institutional Review Board as exempt (IRB 2024-4482) including FERPA compliance.

II. Student Lab Manuals

a. Project #1 Student Lab Manual

Project 1: Identity and Purity of Pharmaceutical Mixtures

WHO USED THIS CHEMISTRY?

In the 1950s and 1960s, there was a famous and controversial "miracle cure" for cancer: Krebiozen. Created by Stevan Durovic and Andrew C. Ivy, they distilled this white powder from the blood serum of horses who had spontaneously recovered from tumors.

Although there were claims of curing patients of cancer in 20 out of 22 cases, the scientific community looked at it with skepticism: no double-blind trials had been performed and most importantly, no one truly knew *what* Krebiozen was.

This is where **Alma Levant Hayden** and her team came in. Hayden was the one of the first Black female scientists to be employed at the National Institutes of Heath and then the Food and Drug Administration, and in 1963, Hayden was asked to discover what Krebiozen actually was. She acquired a tiny vial of the mystery substance – so little that federal researchers were concerned it couldn't be tested.

By taking a microgram amount of Krebiozen in a potassium bromide solution, Hayden ran a series of infrared spectroscopy experiments of both Krebiozen and standard compounds, comparing the fingerprint data of the unknown compound to the standards.

The "miracle cure" for cancer ended up being creatine, an amino acid derivative readily available in a diet that includes meat, and a compound that could be made cheaply by chemical retailers.

By using identification techniques, such as the ones that you will be performing in this experiment, Hayden was able to discover the identity of an unknown substance and determine that the proposed cure was fraudulent.

INTRODUCTION TO ARGUMENT-DRIVEN INQUIRY

In CHEM 51LB, you and your classmates will use Argument-Driven Inquiry to answer a series of Guiding Questions in multi-week projects. Each project will have two parts: Fundamental Skills and Original Investigation.

You will be introduced to Fundamental Skills (basic skills that will help you complete the Original Investigation) in the first part of each project. During the second part of each project, you and your team will use what you've learned during the Fundamental Skills section to create a unique plan to tackle an experimental question (Original Investigation). At the end of the Original Investigation, you and your team will create a "poster" on the benchtop in the lab. The poster should contain the following sections:

Guiding Question(s): given in each project
Claim: the answer to the guiding question
Evidence: neatly and concisely arranged data and/or results tables
Justification: an explanation using scientific theories or laws that ties together the evidence and the claim

During the Argumentation (or poster session), one team member serves as the "spokesperson" - staying with the poster to answer questions from your peers. The rest of the team are "travelers" who will visit other posters and engage in discussion to challenge the other teams' reasoning and reevaluate their own. The discoveries made during the argumentation process are pivotal to answering these three fundamental questions in your Lab Report:

1) What concept(s) were you investigating and how are they related to the guiding question(s)?
2) How did you go about your work and why? If you found value in another team's evidence collection or justification, contrast it with the work your team did.
3) What is your argument? (In other words, use the evidence you collected to justify your claim.) Indicate if/how your argument was altered by the poster session.

WEEK 1

Fundamental Skills 1: Infrared and NMR Spectroscopy

Adapted from Prof. William Howitz, Georgia Institute of Technology

EXPERIMENT LEARNING OBJECTIVES:

After completing this experiment, you should be able to:

- Identify key signals present in IR spectra and assign them to specific functional groups present in the sample compound(s).

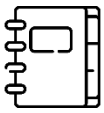
- Identify key signals absent in IR spectra and assign them to specific functional groups not present in the sample compound(s).
- Use chemical shift, integration, and splitting (multiplicity) information in ^{1}H NMR spectra to identify fragments of the structure of the sample compound(s).
- Use fragments of structures identified from ^{1}H NMR spectra data to construct the structure(s) of the sample compound(s).
- Identify whether a sample contains one or more than one compound based on ^{1}H NMR data.
- Use collected IR and ^{1}H NMR spectra data as evidence to support claims about the structure(s) of the sample compound(s).

REACTIONS: None
TECHNIQUES: IR Spectroscopy, ^{1}H NMR Spectroscopy

Infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy are two of the most powerful tools chemists use to determine the structures of compounds. In this experiment you will determine the structures of the unknowns (may be mixtures or pure substances) using IR and NMR spectra. We will revisit the same unknowns in the second part of this project later in your next lab period.

READING ASSIGNMENT:

- NEW MATERIAL
  - NMR spectroscopy of mixtures - Don't worry much about the exact ratios of compounds. Just focus on clues that a spectrum might contain more than one compound.
  - Sections 1.2.1 and 5.3.7 from *Laboratory Safety for Chemistry Students*
- REVIEW (these are topics you have seen before but might need to review) Spectroscopy Introduction, Infrared Spectroscopy, and NMR
  - IR Spectroscopy Overview
  - How and FTIR Spectrometer Operates
  - Identifying the Presence of Particular Groups
  - IR: Application
  - IR: Interpretation
  - IR: Theory
  - **Interpreting IR Spectra**
  - IR Spectroscopy Background
  - NMR: Overview
  - NMR: Background and Physics
  - NMR: Introduction
  - **NMR: Structural Assignment**
  - NMR: Experimental
  - NMR: Theory
- Supplementary info can be found in Janice Gorzynski Smith (3rd, 4th ed, or 5th ed), Ch 13.5-13.8, Ch 14
- Watch lecture videos on Canvas!

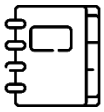


PRE-LAB ASSIGNMENT:

- Complete all portions of pre-lab ELN work according to guidelines
    - Objective
    - GHS table
- Locate the SDS files for the following compounds and add them to the SDS folder in your notebook.
    - naproxen
    - acetaminophen
    - acetylsalicylic acid
    - sorbitol
- Add the chemical structures of the following compounds to your notebook.
    - naproxen
    - acetaminophen
    - acetylsalicylic acid
    - sorbitol
- Look at the literature IR and NMR spectra for naproxen, acetaminophen, acetylsalicylic acid, and sorbitol. You can find the spectra in a folder labeled "Literature IR and NMR spectra" in your ELN.
- Video Quiz(zes) on Canvas

IMPORTANT SAFETY INFORMATION

- **Naproxen, acetaminophen, acetylsalicylic acid, and sorbitol** are all nontoxic
- **Dichloromethane** is toxic, a carcinogen, an irritant, can be absorbed through the skin, and harmful if swallowed. Wear gloves and wash your hands thoroughly after handling it. Avoid contact with skin and eyes. Dichloromethane is a suspected carcinogen when inhaled in large quantities.



IN-LAB ASSIGNMENT:

Jamboard

At the beginning of your lab period, work with your partner(s) to complete the Jamboard Activity assignment linked in your LabArchives (ELN) page (also linked on the Canvas Week 1 Roadmap page). Insert screenshots of your team's answers where indicated.

GUIDING QUESTIONS

For this experiment, you will be looking to answer the following question, and it should guide your experiments:

Fundamental Skills 1 Guiding Question(s): How can you determine the identity of each pure substance through the use of ^{1}H NMR and IR spectroscopy? How can you recognize that a given sample is a mixture containing more than one substance using ^{1}H NMR and IR spectroscopy?

EXPERIMENTAL PROCEDURE

Unknown Groups

You will work in a team of 4 during this lab period. Your TA will help coordinate unknown teams in your section. Your team will be tasked with identifying the identities of the compounds present in each of 3 unknown solid samples. In this lab period, you will use ^{1}H NMR and IR spectroscopy to accomplish your goal. Pure compound IR and ^{1}H NMR spectra will be provided. ^{1}H NMR spectra of the unknowns will be provided. Your team will take IR spectra.

Compound/Mixture	**IR Spectra**	**NMR Spectra**
Unknown mixtures	Students perform	GIVEN
Pure compound standards	GIVEN - See ELN	GIVEN - See ELN

1. Become an expert on one of the pure compound's ^{1}H NMR and IR spectra.

   In your unknowns team, decide which compound each teammate will be responsible for.

Teammate	**Expert Compound**
	acetaminophen
	naproxen
	acetylsalicylic acid
	sorbitol

   Find the experts in <u>your</u> compound from each of the other unknowns teams in your section. Work with your fellow experts (~30 minutes) to analyze the provided ^{1}H NMR and IR spectra for your pure compound. Fill in the "expert known compound" tables in the IR & NMR Worksheets (see ELN). Your goal is to know the spectra for your expert compound well enough to be able to explain it to the other members of your unknowns team and help identify the compound if it appears as one of your unknowns.

2. Obtain IR Spectra for your Unknown Compounds

Return to your unknowns team. Your team will receive a set of Eppendorf tubes containing a small amount of each of your unknowns. Each team member will obtain an infrared spectrum for an unknown, and make copies for your teammates. Your TA will help you with the IR instrument. How might you identify the compounds in your unknown by IR spectroscopy? By NMR spectroscopy?

General Steps for Obtaining an IR Spectrum

(See Instrument Instructions in Lab Archives or on Canvas for an idea of how the program works. Your TA will help you!)

- Ensure the ATR stage is clean prior to use
- If not clean, squirt a small amount of the provided methanol (in the squirt bottle) onto a KimWipe and gently wipe the stage clean
- Follow the instructions provided on instrument use
    - Starting by taking a blank
    - Then collect a spectrum of your sample
- Clean the sample off the stage using a dry KimWipe
- Remove any remaining chemical residue by squirting some methanol on a KimWipe and then gently wipe the stage down thoroughly

3. Identify the Functional Groups in Your Unknowns:

With your unknowns team, determine the wavenumber of important absorption bands for each unknown from its spectrum. Keep in mind that each unknown is a mixture of two compounds. Interpret the infrared spectra to determine what kind of bond or group is responsible for each significant IR band. Use the IR Functional Groups and Frequencies Chart from your lecture textbook and/or the abbreviated table in the pre-lab video to make these determinations.

Fill in the IR portion of your worksheet for each of your unknowns. Narrow down the possibilities for compounds that could be in the unknown as much as possible. Remember that each unknown is a mixture of two standard compounds.

REMEMBER TO PUT YOUR UNKNOWN NUMBER AND LETTER ON YOUR WORKSHEET.

4. Use NMR Spectra to Identify Pieces of Your Unknown:

For each unknown, fill out the NMR table in the worksheet provided in your ELN. Note that although there is a ^{13}C column, there will be no ^{13}C spectra provided in this lab so you may ignore that column in the worksheet. That column is there, however, if you want to use the worksheet as you practice NMR problems in the future as you will be expected to be able to interpret ^{13}C NMR for the lab practical at the end of the quarter.

IMPORTANT NOTES TO CONSIDER WHEN ANALYZING REAL NMR SPECTRUM:

- *Broad carboxylic acid peaks:* Hydrogen-containing groups that can participate in hydrogen bonding (such as carboxylic acids, amines, and alcohols) can appear as very broad peaks

in the NMR spectrum. Sometimes these peaks are so broad that they don't even look like "peaks" anymore, but if you integrate underneath them, you can still tell that there is a hydrogen there.

- *$CDCl_3$ vs. DMSO-d_6:* The most common solvent to use for NMR samples is deuterated chloroform ($CDCl_3$), but another common solvent is deuterated dimethyl sulfoxide (DMSO-d_6). In $CDCl_3$, hydrogens on heteroatoms (e.g., -OH, -NH_2, etc.) do *not* participate in splitting (they almost always appear as singlets). However, in DMSO-d_6, hydrogen atoms on heteroatoms *can* split and be split by their neighbors.

REMEMBER TO PUT YOUR UNKNOWN NUMBER AND LETTER ON YOUR WORKSHEET.

4. Use IR and NMR to Determine Unknown Identity

Use the information from your IR and NMR tables to determine the identity of the compound(s) in each of your unknowns. Draw the structures of the two compounds. Fill out your justification. Include any key evidence you used to determine whether you unknown contains one or two compounds and what key features in the IR and NMR spectra support your claims.

TURNING IN YOUR ELN ASSIGNMENT

- This assignment is due the day following your lab section day.
- Instructions and a rubric for your ELN assignment can be found on Canvas. See the Weekly Roadmap for this week.
- Include your IR and NMR worksheets with your ELN assignment on Gradescope. Save as a pdf and submit. They do not have to be 100% complete and correct yet. We will be looking for clear good faith efforts to complete them. Mistakes and/or notes about what you've narrowed down so far are fine.

WEEK 2

Fundamental Skills 2: Melting Range and TLC Analysis

Adapted from Prof. William Howitz, Georgia Institute of Technology

EXPERIMENT LEARNING OBJECTIVES:

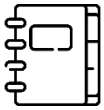
After completing this experiment, you should be able to:

- Use melting range data to determine whether a sample is a pure substance or a mixture, within the inherent limits of the technique.

- Experimentally determine an appropriate eluting solvent mixture for TLC of a set of samples.
- Use TLC data to determine whether a sample is a pure substance or a mixture, within the inherent limits of the technique.
- Experimentally identify at least one scenario in which melting range and TLC could provide conflicting experimental evidence about whether a sample is a pure substance or a mixture and explain this conflict.
- Use TLC data to determine the identity of the component(s) of a sample, within the inherent limits of the technique.
- Explain when data from each technique (melting range and TLC) can be used to identify the component(s) of a sample conclusively and when the data cannot be used conclusively.

READING ASSIGNMENT:

- Organic Chemistry Lab Techniques textbook (Nichols) for TLC and melting point
- *Laboratory Safety for Chemistry Students*
  - Sections 4.1.3, 7.3.1 (focus on heating and chromatography sections), 8.1.1, 8.2.2

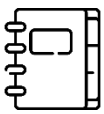


PRE-LAB ASSIGNMENT:

1. Complete video quizzes on Canvas
2. Achieve assignment
3. Complete all portions of pre-lab notebook work according to guidelines

IMPORTANT SAFETY INFORMATION:

- **Hexanes** is volatile, flammable, and a neurotoxin. Avoid contact with skin and eyes. Use in the fume hood only. Avoid breathing fumes.
- **Ethyl acetate, methanol, and acetone** are flammable. Inhalation of vapors can be toxic. Work in the fume hood and keep away from sparks or flames.
- **Naproxen, acetaminophen, acetylsalicylic acid, and sorbitol** are all nontoxic.



IN-LAB ASSIGNMENT:

Jamboard

At the beginning of your lab period, work with your partner(s) to complete the Jamboard Activity assignment linked in your LabArchives (ELN) page (also linked on the Canvas Week 2 Roadmap page). Insert screenshots of your team's answers where indicated.

GUIDING QUESTION

For this experiment, you will be looking to answer the following question, and it should guide your experiments:

Fundamental Skills 2 Guiding Question: How can you determine the identity of each mixture through the use of melting point and TLC?

EXPERIMENTAL PROCEDURE:

Note: Your TA will assign you to either perform Melting Point Analysis or Thin Layer Chromatography first. Halfway through the lab period, each group will swap and perform the other technique.

Melting Point Analysis:

Note that you will be performing this procedure for each of your unknowns A, B, and C.

Take a piece of weigh paper and fold it in half along the diagonal. Place the folded weigh paper onto the analytical balance and tare the balance. What does it mean to "tare" the balance? Measure out ~50 mg of your unknown A onto the weigh paper. The exact amount does not matter, but be sure to record the exact reading from the balance. Make sure you label which sample is which on your piece of weigh paper!

Take a melting point capillary tube and press the open end into your mixture on the weigh paper. You are aiming to get enough mixture into the tube to fill it ~3 mm high. When you think you have enough, invert the tube and place it into the tube tapper slot on the Digimelt. Press and hold the tube tapper button until the sample falls to the bottom of the capillary tube.

Repeat the above procedure for unknown B and C.

Place the capillary tube containing A into the leftmost opening at the top of the Digimelt, then place B in the middle and C on the right. Set the start temperature on the Digimelt to 115 °C, the end temperature to 175 °C, and the ramp rate to 2 °C/min. Why is this an appropriate ramp rate? What would happen to the observed melting range if you increased the ramp rate? **Follow the instructions on the front of the Digimelt** (or the Quick Start Guide on Canvas) to begin

preheating the instrument and ramping the temperature at the ramp rate. Observe the samples carefully during the ramp and record the temperature at which the first bit of liquid appears in each tube and the temperature at which the solid in each tube has completely become liquid.

Thin Layer Chromatography:

Note that you will be performing this procedure for each of your unknowns A, B, and C.

Preparing your TLC samples

Transfer ~30 mg of your unknown A to an appropriately labeled test tube. Add 1 mL of methanol to the test tube to dissolve the sample. Repeat for unknowns B and C, making sure each test tube is clearly labeled. Prepare 10 mL of a 1:1 hexanes:ethyl acetate solvent mixture by measuring 5 mL of hexanes and mixing it with 5 mL of ethyl acetate. Add the solvent mixture to a TLC chamber. Place a piece of filter paper inside the chamber against the side and let the solvent mixture contact the filter paper. What is the purpose of the filter paper? Cover the chamber. Why is it important to cover the chamber? Prepare your TLC plate by drawing a horizontal line (baseline) in pencil above the bottom of the plate that is higher than the height of the solvent in the TLC chamber. Why must the solvent be below the baseline? Make three tick marks along the baseline where you will spot your unknowns A, B, and C. Label the marks.

Preparing and analyzing your first TLC plate

Dip a TLC spotter into your sample A, then spot it on the left tick mark by gently touching the end of the spotter to the TLC plate. Repeat for your samples B (middle tick mark) and C (right tick mark), using a clean spotter each time. *To clean a TLC spotter, dip it into a small amount of acetone, then "spot" on a paper towel until the liquid is gone. Repeat two more times.* Why should you clean the spotter between each sample? After spotting all three mixtures, but **before** putting the TLC plate into the chamber, check your TLC plate under the UV lamp. If you don't see anything on the baseline for a particular spot, it may mean that your sample is too dilute and you need to re-spot it.

Once your TLC plate is prepared, gently lower it into the TLC chamber with the baseline end down using tweezers. Cover the chamber. Allow the plate to develop until the solvent front is about 1 cm from the top (or until it stops moving up the plate). Once the plate has finished developing, carefully remove the TLC plate from the chamber. Quickly mark the solvent front, then look at the TLC plate under a UV lamp. Why do you need to mark the solvent front quickly? Circle any spots you see.

To calculate the retention factor (R_f), for each spot on the TLC plate, measure the distance between the baseline and the center of the spot and divide this by the distance between the baseline and the solvent front. Take a picture of your TLC plate and add it to your ELN.

If the R_f value for any of your samples is very high or very low (i.e., the spot is very close to the bottom or top of the plate), you are probably not able to observe clear separation between compounds with different R_fs in a mixture. Determine whether the R_f values of the spots in any of the lanes on this TLC plate fall within the range where you can accurately determine differences

in R_f. How does changing the solvent mixture affect the R_f values of the compounds on the TLC plate? Create a new solvent mixture based on your previous results.

Preparing and analyzing a co-spotted TLC plate

Determine which of the lanes (A, B, or C) from your first TLC plate would most benefit from being re-run in this new and improved solvent system. You are going to prepare a new TLC plate with what is called a "co-spot." Prepare a TLC plate with three tick marks as before. Label the left lane with the unknown letter you are re-running, label the middle lane "CS" (for "co-spot"), and label the right lane "H" (for "hypothesis"). Spot the unknown that you are re-running in *both* the left and middle lanes. Make a hypothesis as to the identity of this unknown. Make a TLC sample of your hypothesis (if you hypothesize that this unknown is a mixture, make a mixture using a small amount of the two pure components). Using a clean TLC spotter, spot your hypothesis sample in *both* the middle and right lanes. Run and analyze the TLC plate as before. After analyzing this new TLC plate, you should be able to answer the following question: What is a "co-spot," and what is it used for?

If you would like to re-run any of your unknowns in a new solvent system, you may follow the above instructions in order to do so.

Clean-Up:

1. Non-halogenated organic waste
    a. Hexanes
    b. Ethyl acetate
    c. Acetone
    d. Methanol
    e. Mixture samples
2. Solid waste
    a. TLC plates
3. Broken glass container
    a. Used glass pipettes
    b. Used TLC spotters
    c. Used melting point capillary tubes
4. Trash can
    a. Used paper towels
    b. Weigh paper that is free of solid chemicals
    c. Used filter paper (the residual solvent is minimal and will evaporate rapidly)

Clean the glassware following the procedure below:

- Rinse the glassware with a small amount of acetone and dispose of it in the non-halogenated organic waste container.
- Wash the glassware with soap and water using a glassware cleaning brush.
- Rinse the glassware using DI water.
- Rinse the glassware with acetone to dry it and dispose of the acetone rinse in the non-halogenated organic waste container.

FINALIZE YOUR PLAN FOR ORIGINAL INVESTIGATION

Next week will be the Original Investigation, where you will need to answer the Original Investigation Guiding Question through the skills you've learned in the first two weeks of this project.

In the Original Investigation, you will be provided an unknown mixture or pure substance that could contain one of the four chemicals you have previously analyzed in Fundamental Skills (**naproxen, acetylsalicylic acid, sorbitol, acetaminophen**) or three new pharmaceutical compounds (**ibuprofen, caffeine, lidocaine**).

For this project, you will need at least 3 different pieces of evidence to reach a conclusion. You can use any of the four techniques in Fundamental Skills (TLC, melting point, IR or H NMR*) to answer the question.

*H NMR of the pure substances and mixtures will be provided by your TA if asked.

With your group, write a detailed procedure for how you will answer the following Guiding Question:

Original Investigation Guiding Question: What is the identity of each compound in the unknown substance or mixture? How did you conclusively determine their identity or identities?

WEEK 3

Original Investigation: Determination of an Unknown Pharmaceutical Mixture

In the Original Investigation, you will use the skills you've previously learned to answer the Guiding Question. No procedure is explicitly provided, but you should have created one with your group.

PRE-LAB ASSIGNMENT:

1. Write a procedure for your Original Investigation with your group.

2. In addition to the four previous pharmaceutical compounds, make sure you include the SDS and safety information for the three other possible unknown compounds:
    - Ibuprofen
    - Caffeine
    - Lidocaine
3. Complete all pre-lab portions of your lab notebook according to guidelines.

PRELIMINARY DATA COLLECTION

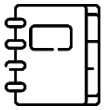
You will be provided an unknown mixture that could contain one of the four chemicals you have previously analyzed in Fundamental Skills (**naproxen, acetylsalicylic acid, sorbitol, acetaminophen**) or three new pharmaceutical compounds (**ibuprofen, caffeine, lidocaine**).

For this Original Investigation, you will need at least 3 different pieces of evidence to reach a conclusion. You can use any of the four techniques in Fundamental Skills (TLC, melting point, IR or H NMR*) to answer the question.

*H NMR of the pure substances and mixtures will be provided by your TA if asked.

As a team, proceed with the plan you've previously created with your group to answer the Original Investigation Guiding Question.

Original Investigation Guiding Question: What is the identity of the compounds in the unknown mixture? How did you conclusively determine the identity?



ARGUMENTATION (POSTER SESSION)

Once your preliminary data collection and analysis are complete, create a "poster" on the benchtop containing the following four sections:

- **Guiding Question:** Restate the Original Investigation Guiding Question. Do not feel that you have to write it word-for-word as it appears earlier in this document.
- **Claim:** Answer to the Original Investigation Guiding Question.
- **Evidence:** Use the answer to Guiding Questions from the *Fundamental Skills* section and decide, as a team, how the information found in the *Original Investigation* should be presented as concisely as possible.
- **Justification:** How does the evidence you included support your claim? What assumptions made or scientific theories/laws were invoked during data analysis and interpretation?
- **Comments:** The team leader should take down important ideas gained from other teams during the poster presentation.

You and your team will then compare and contrast your results with your peers during the poster session. Nominate one team member to be your "spokesperson" who will stay with the poster and

answer any questions from your labmates. The rest of the team will be "travelers" who will go to other posters and engage in discussion about data collection and interpretation. The goal during this session, this argumentation, is to challenge your peers' reasoning and reevaluate your own. Spokespersons and travelers should discuss the claim using the evidence to justify their own point of view, and adjust their conclusions as necessary.

As you discuss with other groups, make sure to fill out the Argumentation worksheet and attach it to your ELN!

At the end of the poster session, your team should hold a short discussion to re-evaluate your claim based on what they have learned during the poster session. **You can change or adjust your claim after the poster session.**

CONFIRMATORY DATA COLLECTION

After argumentation, collect another piece of evidence that supports your claim (i.e. an additional TLC, an additional mixed melting point, etc.).

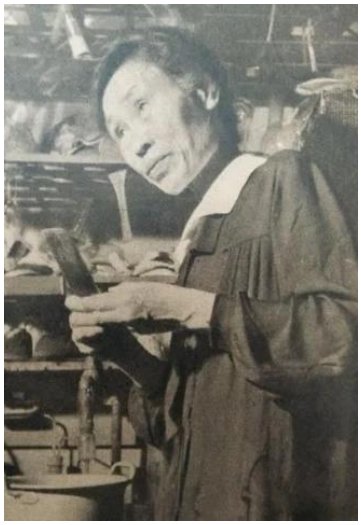
If your claim changed, obtain an additional piece of evidence that supports your new claim based on the discussions that you have in the argumentation session.

If your claim did not change, obtain an additional piece of evidence that you did not obtain previously (such as a new TLC eluent ratio or an additional mixed melting point) that supports your original claim based on the discussions that you had in the argumentation session.

b. Project #2 Student Lab Manual

Project 2: Nucleophile Choice and Solvent Effects for Substitution and Elimination Reactions

WHO USED THIS CHEMISTRY?



One of the main focuses for this project is the use of *extraction* to isolate and purify products. You'll need to use extraction in this project to purify your products to be able to be analyzed via gas chromatography—a necessary step for accurate interpretation of results.

As a foundational skill in organic chemistry, extraction has been an essential technique for many scientists and **Chika Kuroda**, the first woman in Japan to receive a Bachelor of Science in 1916, is no different. Later going on to receive a doctorate in chemistry for her research in carthamin (a pigment of safflower plants), Kuroda was a professor at Tohoku Imperial University, Tokyo Women's Higher Normal School, and Ochanomizu University for many years.

Kuroda's work focused on the extraction of natural pigments from plants, like carthamin, and the synthesis of them in the lab. Her work at Ochanomizu University in 1949 resulted in the extraction and isolation of quercetin crystals from onion skin. This discovery led to the creation of Kerutin C, an antihypertensive drug. This was a solid-liquid extraction, a different kind of extraction to the liquid-liquid extraction you'll be performing in this experiment, but you'll be following the same chemical principles as Kuroda and many other scientists like her.

WEEK 1

Fundamental Skills: Extraction and Initial Reaction

Adapted from Prof. William Howitz, Georgia Institute of Technology

EXPERIMENT LEARNING OBJECTIVES:

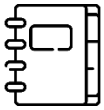
After completing this experiment, you should be able to:

- Use liquid-liquid extraction to isolate product(s)
- Prep and perform a reaction under reflux conditions
- Understand the mechanistic underpinnings of elimination and substitution reactions
- Analyze product ratios through the use of gas chromatography

REACTIONS: None
TECHNIQUES: Extraction, Reaction Setup, Gas Chromatography

READING ASSIGNMENT:

[To be added later]

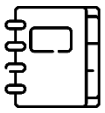


PRE-LAB ASSIGNMENT:

- Complete all portions of pre-lab ELN work according to guidelines
    - Objective
    - GHS table
- Locate the SDS files for the following compounds and add them to the SDS folder in your notebook.
    - 2-bromopentane
    - 2-bromo-2-methylbutane
    - sodium methoxide/methanol solution
    - methyl tert-butyl ether (MTBE)
    - ammonium chloride solution
    - magnesium sulfate anhydrous
- Add the chemical structures of the following compounds to your notebook.
    - 2-bromopentane
- Write out the mechanisms for BOTH alkyl halides undergoing BOTH substitution and elimination, for 4 mechanisms total. (Note: please write the *most plausible* substitution or elimination mechanism for each)
- Video Quiz(zes) on Canvas

IMPORTANT SAFETY INFORMATION

- **Sodium methoxide** is a strong base and is corrosive. Handle with gloves and wear safety goggles at all times.
- **2-bromopentane** is a serious skin and eye irritant. Hand with gloves and wear safety goggles at all times. It is also highly flammable, so keep away from open flames and sources of ignition.
- **Methanol** and **methyl tert-butyl ether** are volatile, flammable organic solvents. Avoid breathing in fumes and handle in the fume hood.
- **Acetanilide** and **sodium benzoate** are eye irritants. Handle with gloves and wear safety goggles at all times.



IN-LAB ASSIGNMENT:

In this experiment, you will be working in your same group of 4 to perform two reactions, both with 2-bromopentane. Each reaction will be performed by a pair at the same time, and analyzed by gas chromatography. MAKE SURE TO PERFORM YOUR REACTIONS IN DUPLCATE.

During the heating time of the experiment, you will be performing a short extraction workshop to get you familiarized with the technique.

GUIDING QUESTIONS

For this experiment, you will be looking to answer the following question, and it should guide your experiments:

Fundamental Skills Guiding Question(s): What is the ratio of substitution to elimination products for the reactions performed?

EXPERIMENTAL PROCEDURE

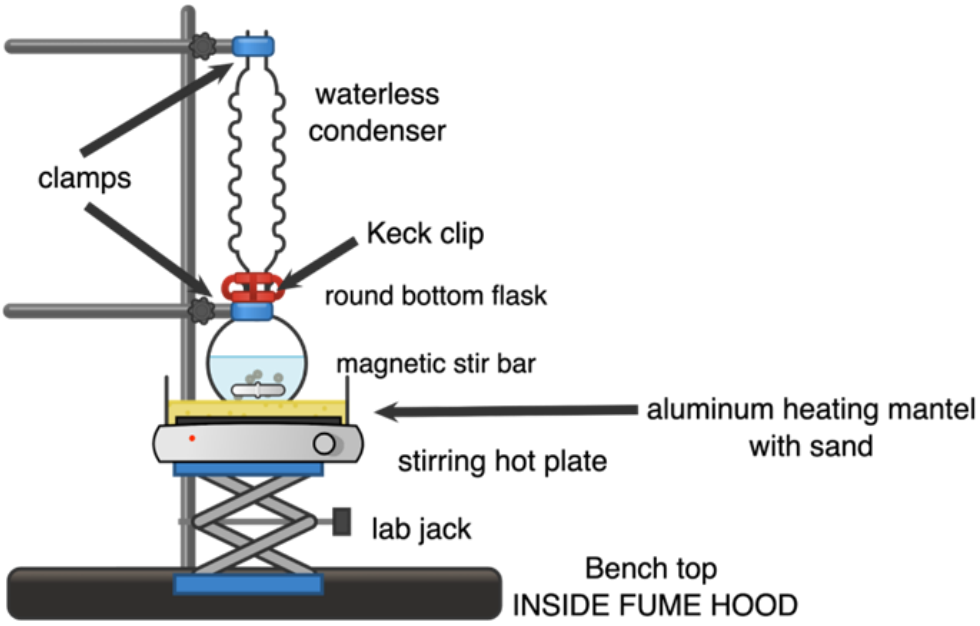
Reaction procedure:

Before you begin, obtain a 50 mL round bottom flask, a stir bar, and a waterless condenser from the stockroom. Ensure that all glassware is completely dry to minimize water contamination.

Set up the apparatus: a hot plate on a jack, a boiler plate with sand on top of the hot plate, and two clamps that can hold the round bottom flask and waterless condenser.

In the dry 50 mL round bottom flask, add 1.2 mL of the 1.5 M sodium methoxide solution (or 0.6 mL of the 25% w/w sodium methoxide in methanol and 0.6 mL of methanol) and a stirbar. Add 0.5 mL of the alkyl halide to the flask.

Lightly grease the joint of the condenser and connect the condenser to the round bottom flask. Set the whole apparatus on the hot plate, start the stirring (the stir should be set to around 2, so that it stirs without making a vortex), and heat to reflux (start the heat around 3-4 and adjust as needed). Reflux occurs when you can see the solvent condensing on the sides of the flask and the condenser, but should not be a vigorous boil.



This reaction should reflux for 1 hr.

Extraction Workshop:

During the reflux, perform a small extraction activity. Gather a 60 mL separatory funnel and 3 100 mL beakers. Secure a clamp or a ring stand that can hold that separatory funnel comfortably.

Place approximately 0.5 g (record this value, but it doesn't need to be exact!) of the 1:1 acetanilide:sodium benzoate mixture into one of the beakers. Add 10 mL of MTBE (methyl t-butyl ether) and 10 mL of saturated ammonium chloride solution to the beaker. Stir lightly to dissolve the solids, and then transfer the whole mixture to a *closed* separatory funnel. Make sure the stopper is well fitted on the top of the sep. funnel and gently shake the funnel to ensure mixing of the two layers. Vent every 30 seconds to avoid dangerous pressure build up! Secure the funnel back on the ring stand or clamps and remove the stopper. Allow the two layers to separate and carefully drain the bottom layer into a clean beaker. Which layer is organic? Which is aqueous? What compounds are currently in each layer? Wash the organic layer that is in the sep. funnel with ~10 mL of saturated ammonium chloride. What does "wash" mean in the context of extraction? What is the purpose of washing the organic layer? After the layers have separated, drain the bottom layer into the same flask containing the aqueous layer from the previous extraction.

Drain the top layer into a separate beaker.

Use one of the two ways to analyze the organic layer:

1. **TLC**: Run a TLC of the sodium benzoate and acetanilide standards and the organic layer using a 1:1 hexanes:ethyl acetate mixture.

   Analyze your TLC - what compound did you isolate?

2. **Melting point**: Evaporate off the organic solvent by running a **light** stream of air over it. It should evaporate in 5-10 minutes, leaving behind crystals. Gather the crystals and place in a melting point tube. Run a melting point experiment and compare to literature values of sodium benzoate and acetanilide.

Analyze your melting point data - what compound did you isolate?

Work-up Procedure:

After 1 hr, turn off the heat and stirring for the hot plate. Allow the reaction flask to cool down to room temperature (around 10 minutes). After 10 minutes, remove the condenser from the reaction flask and pull the magnetic stir bar out of the flask with a magnet.

Add 10 mL of MTBE to the flask. Pour the entire contents of the flask into a *closed* 60 mL separatory funnel. There will be solids at the bottom of the flask - it's okay if you're not able to get all of the solids into the funnel. What are likely the solids at the bottom? Is it your product? Add 10 mL of saturated ammonium chloride to the separatory funnel and mix, inverting and shaking the funnel to mix the two layers. Make sure to vent the funnel regularly.

Drain the bottom layer into a beaker, leaving the top layer in the funnel. Add another 10 mL of saturated ammonium chloride to the funnel. The two layers should reform. Repeat the mixing process and drain the bottom layer into the same beaker with the original bottom layer.

Repeat this washing procedure once more with 10 mL of water.

Drain the organic layer into a separate beaker. Add magnesium sulfate anhydrous until the solid flows freely in the beaker when swirled (the "snowglobe effect"). Decant off the liquid with a glass pipette, making sure not to catch any solid in the pipette, and into a scintillation vial. Label the vial with your initials and clean up before taking your GC spectra.

Clean Up:

1. Non-halogenated Liquid Waste
    a. Water
    b. Acetone
    c. Methanol
    d. Sodium methoxide in methanol
    e. 2-bromopentane/2-bromo-2-methylbutane
    f. MTBE (methyl tert-butyl ether)
2. Aqueous Waste
    a. saturated ammonium chloride
    b. aqueous layers
3. Glass Waste
    a. Used glass pipettes
4. Trash Can

a. Used paper towels
b. Used gloves
c. Used disposable plastic pipettes (expel any liquid into liquid waste first)

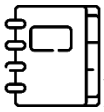
Clean the glassware following the procedure below:

- Rinse the glassware with a small amount of acetone and dispose of it in the non-halogenated organic waste container.
- Wash the glassware with soap and water using a glassware cleaning brush.
- Rinse the glassware using DI water.
- Rinse the glassware with acetone to dry it and dispose of the acetone rinse in the non-halogenated organic waste container.

GC Analysis:

Take your organic layer to the GC in a scintillation vial. Your TA will run a GC method on your product. What compounds come out first? Where is your solvent peak? Make sure to email your spectra to yourself for analysis.

GC spectra of starting materials and solvents will be provided, along with a list of boiling point.



FINALIZE YOUR PLAN FOR ORIGINAL INVESTIGATION

Next week will be the Original Investigation, where you will need to answer the Original Investigation Guiding Question through the skills you've learned in the first two weeks of this project.

In the Original Investigation, you will be adjusting the reaction you performed last week in the Fundamental Skills portion to achieve a higher yield of either the substitution or the elimination product by adjusting the reagents used. You can change either the alkylbromide, the alkoxide, or both, using the lists below.

Options for the alkoxide: sodium methoxide/methanol, sodium ethoxide/ethanol, sodium isopropoxide/isopropanol or potassium tert-butoxide/t-butanol

Options for the alkylbromide: 2-bromopentane, 1-bromopentane, or 2-bromo-2-methylpentane

You can follow the same reaction procedure for the reaction as in Fundamental Skills. If you would like to make a modification to the reaction other than changing the alkylbromide or alkoxide, clear the modification with your TA.

Before you perform the reaction, you should have a <u>hypothesis</u> of whether you are adjusting the reaction towards substitution or elimination, and this should be mentioned in your procedure and Answer to the Guiding Question.

With your group, write a detailed procedure for how you will answer the following Guiding Question:

Original Investigation Guiding Question: What reagents favor substitution and/or elimination, and how could you conclusively determine this?

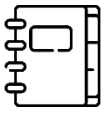
TURNING IN YOUR ELN ASSIGNMENT

- This assignment is due the day following your lab section day.
- Instructions and a rubric for your ELN assignment can be found on Canvas. See the Weekly Roadmap for this week

WEEK 2

Original Investigation: Adjusting the Original Reaction

In the Original Investigation, you will use the skills you've previously learned to answer the Guiding Question. No procedure is explicitly provided, but you should have created one with your group.



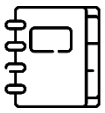
PRE-LAB ASSIGNMENT:

1. Write a procedure for your Original Investigation with your group.
2. Complete all pre-lab portions of your lab notebook according to guidelines.
3. Add in the SDS and chemical structures for the chemicals you decided to use in your Original Investigation experiment.

PRELIMINARY DATA COLLECTION

As a team, proceed with the plan you've previously created with your group to answer the Original Investigation Guiding Question.

Original Investigation Guiding Question: Based on the reactions ran in Fundamentals Skill and your Original Investigation, which reagents favor substitution and/or elimination and how did you conclusively determine this?



ARGUMENTATION (POSTER SESSION)

Once your preliminary data collection and analysis are complete, create a "poster" on the benchtop containing the following four sections:

- **Guiding Question:** Restate the Original Investigation Guiding Question. Do not feel that you have to write it word-for-word as it appears earlier in this document.
- **Claim:** Answer to the Original Investigation Guiding Question.
- **Evidence:** Use the answer to Guiding Questions from the *Fundamental Skills* section and decide, as a team, how the information found in the *Original Investigation* should be presented as concisely as possible.
- **Justification:** How does the evidence you included support your claim? What assumptions made or scientific theories/laws were invoked during data analysis and interpretation?
- **Comments:** The team leader should take down important ideas gained from other teams during the poster presentation.

You and your team will then compare and contrast your results with your peers during the poster session. Nominate one team member to be your "spokesperson" who will stay with the poster and answer any questions from your labmates. The rest of the team will be "travelers" who will go to other posters and engage in discussion about data collection and interpretation. The goal during this session, this argumentation, is to challenge your peers' reasoning and reevaluate your own. Spokespersons and travelers should discuss the claim using the evidence to justify their own point of view, and adjust their conclusions as necessary.

At the end of the poster session, your team should hold a short discussion to re-evaluate your claim based on what they have learned during the poster session. **You can change or adjust your claim after the poster session.**

CONFIRMATORY DATA COLLECTION

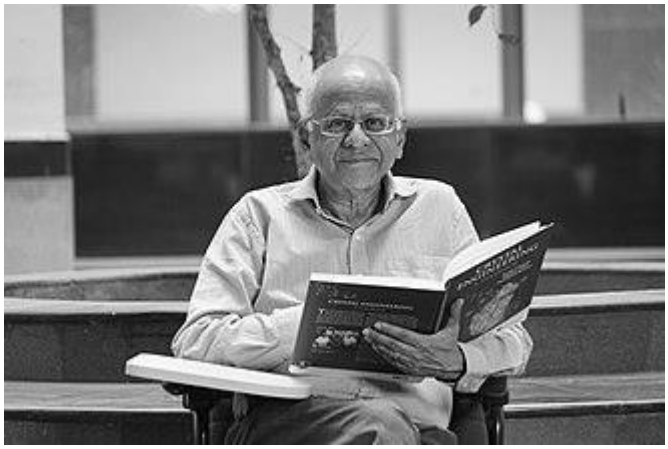
After argumentation, collect another piece of evidence that supports your claim. *For this experiment, you will NOT be able to run an additional reaction.* You can, however, obtain and analyze the GC spectra from another group, and add its evidence to your own. *Your group must perform its OWN analysis on the additional evidence, and make sure to record the additional evidence as* ***another group's, not your own.***

If your claim changed, obtain an additional piece of evidence that supports your new claim based on the discussions that you have in the argumentation session.

If your claim did not change, obtain an additional piece of evidence that you did not obtain previously that supports your original claim based on the discussions that you had in the argumentation session.

Project 3: Recrystallization of an Epoxidation Product

WHO USED THIS CHEMISTRY?



This experiment focuses on understanding the underlying principles of recrystallization, the process in which a compound is purified by dissolving the impure substance in a low-solubility solvent and then precipitating out a more pure substance after cooling. Very few understand the properties of crystals better than **Gautum R. Desiraju**, who is known as “the father of crystal engineering.”

Crystal engineering is a field that bridges physics, architecture and supramolecular chemistry. Desiraju himself defined the field as “the understanding of intermolecular interactions in the context of crystal packing and the utilization of such understanding in the design of new solids with desired physical and chemical properties." Born in 1952, Desiraju earned his PhD at the age of 24 from the University of Illinois, Urbana-Champaign. The major problem in crystal engineering is that the prediction of a crystal structure from a molecular structure is very difficult and not easily derivable from functional groups. Identification of supramolecular synthons, Desiraju’s main research focus, simplifies this otherwise intractable problem, by identified a repeatable unit that is an approximate of the crystal.

He is one of the most cited scientists in the world and currently works as a Professor Emeritus at the Indian Institute of Science to this day.

WEEK 1

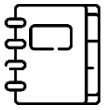
Fundamental Skills 1: Recrystallization Workshop

EXPERIMENT LEARNING OBJECTIVES:

After completing this experiment, you should be able to:

- recrystallize and impure mixture with both one-solvent and dual-solvent mixtures
- determine the purity of your recovered solid through TLC and melting point
- calculate percent recovery
- determine the best recrystallization method amongst the three recrystallizations performed

REACTIONS: None
TECHNIQUES: Recrystallization, Melting Point, TLC

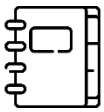


PRE-LAB ASSIGNMENT:

- Complete all portions of pre-lab ELN work according to guidelines
    - Objective
    - GHS table
- Locate the SDS files for the following compounds and add them to the SDS folder in your notebook.
    - trans-stilbene
    - biphenyl
    - cis-stilbene
- Add the chemical structures of the following compounds to your notebook.
    - trans-stilbene
    - biphenyl
    - cis-stilbene
- Video Quiz(zes) on Canvas

IMPORTANT SAFETY INFORMATION

- **Ethanol** and **isopropanol** are volatile, toxic and flammable. Use in the fume hood.
- **Hexanes** is volatile, flammable, and a neurotoxin. Avoid contact with skin and eyes. Use in fume hood only. Avoid breathing fumes.
- **Toluene** is volatile, flammable, and a carcinogen. Avoid contact with skin and eyes. Use in fume hood only. Avoid breathing fumes.
- **Trans-stilbene** and **biphenyl** are irritants. Avoid contact with skin and eyes.



IN-LAB ASSIGNMENT:

GUIDING QUESTIONS

For this experiment, you will be looking to answer the following question, and it should guide your experiments:

Fundamental Skills 1 Guiding Question(s): What recrystallization procedure was most effective for purifying trans-stilbene and why was this the most effective procedure?

EXPERIMENTAL PROCEDURE

In this experiment, you will be testing different recrystallization techniques in order to determine the best procedure for purification. You will be working in pairs for this experiment, but you should confer with your group to answer the Fundamental Skills Guiding Question.

Before beginning the recrystallization, obtain a small plastic vial of impure trans-stilbene from your TA. This vial contains 75% trans-stilbene and 25% of a minor component (either biphenyl or cis-stilbene). Your goal during each of the recrystallization procedures will be to remove the minor component.

You will attempt to purify the trans-stilbene by each of the three recrystallization techniques. **With this in mind, please divide the provided sample into 4 equal parts of approximately 0.3 g each.** Weigh and record the mass of each portion. (The 4th part is in case you would like to retry a recrystallization.)

Before you begin the recrystallizations, make sure you have the following equipment set up: a hot plate, a Buchner funnel with a filtration flask, and an ice bath in a 500 mL beaker.

RECRYSTALLIZATION #1: SINGLE SOLVENT (ISOPROPANOL)

Obtain around 20 mL of isopropanol. Recrystallize your crude product from ethanol by placing your product into an appropriately sized Erlenmeyer flask and slowly adding portions of the isopropanol just until the solid is completely dissolved. You can assist in this process by heating the isopropanol/product mixture on a hot plate, swirling constantly (it should NOT boil, and you should be able to handle the flask with your gloved hands). Be careful not to add too much solvent! What will happen if you add too much solvent? What can you do if you add too much solvent?

Place the flask into an ice bath to complete recrystallization. Cool a small beaker of water in ice while you wait for crystallization to occur.

Vacuum filter the solid and rinse with ice-cold water. Why is it important to rinse the crystals? Why is it important that the water is ice-cold? Allow the solid to dry in the filter funnel while pulling air through the sample for at least 15 minutes. Record the mass of the dried product.

Use the original mass and the mass of your dried product to calculate the percent recovery of your recrystallization.

RECRYSTALLIZATION #2: DUAL SOLVENT (TOLUENE/ETHANOL)

Heat approximately 20 mL of toluene and 20 mL of ethanol in separate flasks or beakers, but on the same hot plate. Adjust the temperature of the hot plate so that the toluene is hot but not boiling. Recrystallize your crude product from toluene/ethanol by placing your product into an appropriately sized Erlenmeyer flask and slowly adding portions of the hot toluene just until the solid is completely dissolved. You will need to stir or swirl the flask containing your product solution and keep it warm during this process. Why is it important to keep the solution warm? Be careful not to add too much solvent! Add ethanol dropwise and swirl your recrystallization flask just until a slight cloudiness persists (be aware for this particular recrystallization, the cloudiness may go away on its own, or appear similar to snowflakes before disappearing). Add hot toluene again dropwise until the cloudiness disappears. Allow the solution to cool to room temperature and then place the flask into an ice bath to complete recrystallization. Why don't we just put the solution directly into the ice bath without cooling to room temperature first? Cool a small beaker of water in ice while you wait for crystallization to occur. (Note that this is the general procedure for recrystallizing from mixed solvent when the exact solvent ratios needed are not known. You will be expected to remember this general procedure in the future.)

Vacuum filter the solid and rinse with ice-cold water. Why is it important that the water is ice-cold? Allow the solid to dry in the filter funnel while pulling air through the sample for at least 15 minutes. Record the mass of the dried product.

Use the original mass and the mass of your dried product to calculate the percent recovery of your recrystallization.

RECRYSTALLIZATION #3: DUAL SOLVENT (ETHANOL/WATER)

Heat approximately 20 mL of ethanol and 20 mL of water in separate flasks or beakers, but on the same hot plate. Adjust the temperature of the hot plate so that the ethanol is hot but not boiling. Recrystallize your crude product from ethanol/water by placing your product into an appropriately sized Erlenmeyer flask and slowly adding portions of the hot ethanol just until the solid is completely dissolved. You will need to stir or swirl the flask containing your product solution and keep it warm during this process. Why is it important to keep the solution warm? Be careful not to add too much solvent! Add water dropwise and swirl your recrystallization flask just until a slight cloudiness persists (be aware for this particular recrystallization, the cloudiness may go away on its own, or appear similar to snowflakes before disappearing). Add hot ethanol again dropwise until the cloudiness disappears. Allow the solution to cool to room temperature and then place the flask into an ice bath to complete recrystallization. Why don't we just put the solution directly into the ice bath without cooling to room temperature first? Cool a small beaker of water in ice while you wait for crystallization to occur. (Note that this is the general procedure for recrystallizing from mixed solvent when the exact solvent ratios needed are not known. You will be expected to remember this general procedure in the future.)

Vacuum filter the solid and rinse with ice-cold water. Allow the solid to dry in the filter funnel while pulling air through the sample for at least 15 minutes. Record the mass of the dried product.

Use the original mass and the mass of your dried product to calculate the percent recovery of your recrystallization.

TLC:

Obtain a TLC for each of the recrystallized products (each on a separate TLC plate).
Draw three lanes on each TLC plate: one for the standard (trans-stilbene), one for a co-spot, and one for the recrystallization product. Develop the TLC plates in an eluent mixture of 100% hexanes. Remove your TLC from the chamber when the solvent has gone up ¾ of the plate and mark the solvent line. After your TLC plate has dried, use UV light to determine the spots present on your plate.

Melting Range:

Obtain a melting range for each of the recrystallized products and your crude mixture. Why are we interested in the melting range of both crude and recrystallized solid? When you have narrowed down which sample is pure trans-stilbene, perform a mixed melting range experiment(s) using your pure product with the trans-stilbene standard provided. What does a mixed melting range experiment tell us? If the melting range of your recrystallized product is low, try drying it again for a longer period of time.

Clean-Up:

1. Non-halogenated organic waste
    a. Ethanol
    b. Isopropanol
    c. Toluene
    d. hexanes
2. Solid waste
    a. TLC plates
3. Broken glass container
    a. Used melting point capillary tubes
    b. TLC spotters
4. Trash can
    a. Used paper towels
    b. Weigh paper that is free of solid chemicals
    c. Used filter paper (the residual solvent is minimal and will evaporate rapidly)

Before you leave lab, make sure you have data from all 3 recrystallizations to answer the Fundamental Skills 1 Guiding Question(s).

TURNING IN YOUR ELN ASSIGNMENT

- This assignment is due the day following your lab section day.

- Instructions and a rubric for your ELN assignment can be found on Canvas. See the Weekly Roadmap for this week.
- Save as a pdf and submit. They do not have to be 100% complete and correct yet. We will be looking for clear good faith efforts to complete them. Mistakes and/or notes about what you've narrowed down so far are fine

WEEK 2

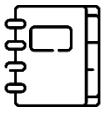
Fundamental Skills 2: Epoxidation Reaction

EXPERIMENT LEARNING OBJECTIVES:

After completing this experiment, you should be able to:

- perform an epoxidation reaction of trans-stilbene to stilbene oxide
- isolate the impure product through the use of extraction
- use melting point and TLC to determine your product(s) and its purity
- calculate percent yield
- determine the major component of your crude product

REACTIONS: Epoxidation of Stilbene
TECHNIQUES: Extraction, Melting Point, TLC

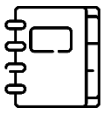


PRE-LAB ASSIGNMENT:

1. Complete video quizzes on Canvas
2. Achieve assignment
3. Complete all portions of pre-lab notebook work according to guidelines

IMPORTANT SAFETY INFORMATION:

- **Ethanol** and **ethyl acetate** are volatile, toxic and flammable. Use in the fume hood.
- **Hexanes** is volatile, flammable, and a neurotoxin. Avoid contact with skin and eyes. Use in fume hood only. Avoid breathing fumes.
- **pH 11 buffer** is a strong base. Avoid contact with skin and eyes. Neutralize before disposal.
- **30% Hydrogen peroxide** is very corrosive and should only be handled by your TA.



IN-LAB ASSIGNMENT:

GUIDING QUESTION

For this experiment, you will be looking to answer the following question, and it should guide your experiments:

Fundamental Skills 2 Guiding Question: What is the major component of your crude product and how were you able to confirm this?

EXPERIMENTAL PROCEDURE:

You will be performing this reaction in *pairs*, so each group should be running two reactions, and obtain two sets of your product (you'll need this for your original investigation!)

Reaction Set-Up:

Before you begin, obtain the following:

- a 50 mL round bottom flask
- a jack and a hot plate
- two clamps
- a thermometer
- a 250 mL beaker filled halfway with water
- a heating block and a sand
- a stir bar

Arrange your reaction apparatus: place the hot plate on top of the jack and adjust to a height where the clamp could reasonably hold the neck of your round bottom flask. Place the 250 mL beaker filled with water on the hot plate. Clamp the thermometer so that it sits inside the beaker of water without touching the sides or the bottom.

Weight out 270 mg (0.270 g) of trans-stilbene and record the mass. Transfer the solid to the 50-mL round bottom flask with a stir bar. Add 3 mL of the pH 11 buffer, and then 3 mL of the tu-butanol/catalyst mixture, using the syringes provided. Then add 0.35 mL of acetonitrile, measured out in the syringe provided. Place your round bottom flask on the heating block and start the stirring so that the solution is well-mixed, but a vortex is not formed. There will be undissolved solids at the bottom of your flask.

Begin to heat your reaction, keeping a watch on the thermometer in the water bath. You're looking to heat your reaction to 60 degrees Celsius.

After your reaction is stirring and heating, call your TA over. They will add 0.70 mL of 30% hydrogen peroxide to your flask.

Heat your reaction at 60 degrees Celsius for 1 hour.

If your mixture starts to boil, it is TOO HOT. Be vigilant during these 60 minutes, and make sure that the thermometer reads between 50 and 70 degrees Celsius. The reaction may start to reflux on the sides of the flask, but if it starts to steam, it is too hot. What does it mean to reflux?

Reaction Work-Up:

While the reaction is cooling, weigh an empty scintillation vial (with cap) and record its mass.

After your reaction is completed and cooled, remove the stir bar with a magnetic rod. Transfer the content of the flash, including the solids, into a 60 mL separatory funnel. Rinse the flask with 10 mL of 10% aqueous sodium thiosulfate and transfer this into the separatory funnel. Rinse the flask with 10 mL of ethyl acetate and transfer this into the separatory funnel.

Cap and invert the funnel 2-3 times, venting for gas every 30 seconds or so. Swirl the funnel. Before separating the layers, ensure that all the solids are dissolved. If the solids persist, add 1 mL of ethyl acetate at a time (no more than 5 mL) to the separatory funnel and mix again.

Drain the bottom layer into a beaker. What is the bottom layer? Drain the top layer into a different beaker. What is the top layer? Add sodium sulfate to the organic layer until you observe the "snow globe" effect and the sodium sulfate moves around the flask when swirled.

Decant the liquid into your weighed scintillation vial, making sure to not carry over any solids.

Blow off the organic solvent with a gentle stream of air. This will take around half an hour, so use jacks or clamps to hold the scintillation vial under the air stream while you and your partner cleans up the workstation. Run a TLC while the organic solvent evaporates. Use your time wisely!

Melting Range and TLC

Obtain a TLC for the crude product. Draw three lanes on the TLC plate: one for the starting material (trans-stilbene), one for a co-spot, and one for the crude product. Develop the TLC plates in an eluent mixture of 100% hexanes. Remove your TLC from the chamber when the solvent has gone up ¾ of the plate and mark the solvent line. After your TLC plate has dried, use UV light to determine the spots present on your plate.

Obtain a melting range for your crude mixture.

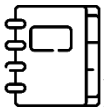
You'll be using this melting range and TLC as a starting point to analyze your purification during the Original Investigation.

Clean-Up

1. Aqueous waste
    a. Sodium sulfate
    b. Aqueous layers after extraction
2. Organic Waste
    a. TLC eluent
3. Solid waste
    a. Used TLC plates
4. Broken glass container
    a. Used glass pipettes
    b. Melting point capillary tubes
    c. TLC spotters
5. Trash can
    a. Used paper towels
    b. Weigh paper that is free of solid chemicals
    c. Used filter paper (the residual solvent is minimal and will evaporate rapidly)

Clean the glassware following the procedure below:

- Rinse the glassware with a small amount of acetone and dispose of it in the non-halogenated organic waste container.
- Wash the glassware with soap and water using a glassware cleaning brush.
- Rinse the glassware using DI water.
- Rinse the glassware with acetone to dry it and dispose of the acetone rinse in the non-halogenated organic waste container.



FINALIZE YOUR PLAN FOR ORIGINAL INVESTIGATION

Next week will be the Original Investigation, where you will need to answer the Original Investigation Guiding Question through the skills you've learned in the first two weeks of this project.

For a complete original investigation, your group will need to do at least **two** recrystallization techniques of your isolated crude product, so plan accordingly.

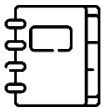
With your group, write a detailed procedure for how you will answer the following Guiding Question:

Original Investigation Guiding Question: Which recrystallization technique was the best for isolating your major compound? Why was this technique the most effective?

WEEK 3

Original Investigation

In the Original Investigation, you will use the skills you've previously learned to answer the Guiding Question. No procedure is explicitly provided, but you should have created one with your group.



PRE-LAB ASSIGNMENT:

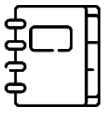
1. Write a procedure for your Original Investigation with your group.
2. In addition to the previous compounds, make sure you include the SDS and safety information for the compounds you intend to use in
3. Complete all pre-lab portions of your lab notebook according to guidelines.

PRELIMINARY DATA COLLECTION

As a team, proceed with the plan you've previously created with your group to answer the Original Investigation Guiding Question.

For a complete original investigation, your group will need to do at least **two** recrystallization techniques of your isolated crude product, so plan accordingly.

Original Investigation Guiding Question: Which recrystallization technique was the best for isolating your major compound? Why was this technique the most effective?



ARGUMENTATION (POSTER SESSION)

Once your preliminary data collection and analysis are complete, create a "poster" on the benchtop containing the following four sections:

- **Guiding Question:** Restate the Original Investigation Guiding Question. Do not feel that you have to write it word-for-word as it appears earlier in this document.
- **Claim:** Answer to the Original Investigation Guiding Question.
- **Evidence:** Use the answer to Guiding Questions from the ***Fundamental Skills*** section and decide, as a team, how the information found in the ***Original Investigation*** should be presented as concisely as possible.

- **Justification:** How does the evidence you included support your claim? What assumptions made or scientific theories/laws were invoked during data analysis and interpretation?
- **Comments:** The team leader should take down important ideas gained from other teams during the poster presentation.

You and your team will then compare and contrast your results with your peers during the poster session. Nominate one team member to be your "spokesperson" who will stay with the poster and answer any questions from your labmates. The rest of the team will be "travelers" who will go to other posters and engage in discussion about data collection and interpretation. The goal during this session, this argumentation, is to challenge your peers' reasoning and reevaluate your own. Spokespersons and travelers should discuss the claim using the evidence to justify their own point of view, and adjust their conclusions as necessary.

As you discuss with other groups, make sure to fill out the Argumentation worksheet and attach it to your ELN!

At the end of the poster session, your team should hold a short discussion to re-evaluate your claim based on what they have learned during the poster session. **You can change or adjust your claim after the poster session.**

CONFIRMATORY DATA COLLECTION

After argumentation, collect another piece of evidence that supports your claim (i.e. an additional TLC, an additional mixed melting point, etc.).

If your claim changed, obtain an additional piece of evidence that supports your new claim based on the discussions that you have in the argumentation session.

If your claim did not change, obtain an additional piece of evidence that you did not obtain previously (such as a new TLC eluent ratio or an additional mixed melting point) that supports your original claim based on the discussions that you had in the argumentation session.

III. Lab Report Rubrics

The following are the lab report scaffolds and rubrics that will be provided to the students and were provided to the undergraduate beta-testers. The scaffold for each project provides the individual Experiment-Specific Questions (ESQ) that correlate to the rubric items. The associated ESQs are listed in the "rubric item" column in each lab report rubric.

a. Project #1 Lab Report Scaffold

Chem 51LB Project #1 Report Scaffold

Identification of Pharmaceutical Mixtures

Instructions:

Report scaffolds are provided to help you learn how to write about the experiments you conduct. For this first experiment, we will start small. You will only be discussing how you interpreted your IR and NMR data to identify the unknowns provided because talking about IR and NMR data in a written format is challenging. Be aware that this information would normally be included as part of the results and discussion sections of a full lab report. For this assignment, answer the **specific** questions provided using complete sentences in the third person passive voice, but keep the general questions in mind as a guide.

Each question in this scaffold is numbered. In a separate document, number your responses to the *Experiment-Specific Questions* only. Do not write the specific questions in your separate document as these will only take up the space you need for your responses to those questions. Also, do not include the general questions or answers to the general questions.

You will need to repeat answering the set of questions below six times, one set for each of the six unknowns. Label your responses with a lowercase letter next to each number to distinguish the different unknowns. For example, you should label your responses as 1a, 2a, etc. for unknown A and then 1b, 2b, etc. for unknown B. The document containing your typed responses to these questions should not exceed a total of six pages (one page per unknown compound).

Post-Lab Report Format: *MUST BE TYPED IN WORD OR A SIMILAR PROGRAM, NOT AN ELN PAGE!* Times New Roman; 12 pt. font; double spaced; 1" margins; no more than 6 pages; use 3rd person passive voice only (*For example, "We dissolved the white solid in 10 mL of hot water," should be written as, "The white solid was dissolved in 10 mL of hot water."*). Include your name, student ID number, and lab course code. This format is **NOT OPTIONAL** and TA's will remove points for failure to follow these instructions.

Submitting Your Assignment

Attach the worksheets for each unknown at the very end of the document. Submit the completed assignment document (answers to these questions and the worksheets) as a single pdf file on Canvas. If you need help creating a single pdf, check out this resource.

Introduction

1. *What was the purpose of this experiment? How did we plan to accomplish this? The purpose of an experiment is not simply to introduce a student to a technique or reaction! The purpose*

corresponds to the objective you wrote for your in-lab notebook pages so if you got full credit for it previously, just copy and paste it into the introduction section of your post-lab report. If you did not previously earn full credit for them, carefully review your TA's feedback to help you improve it.

Theory

Experiment-Specific Questions:

2. *What does IR spectroscopy measure? How was it used to determine the identity of the unknowns in this experiment?*
3. *What do the peaks on a ^{1}H NMR spectrum represent? What are the **three** primary components of a ^{1}H NMR peak, and what does each component tell you about what the peak represents? How was this information used to determine the identity of the unknowns in this experiment?*
4. *How does the melting range of a compound change if it becomes contaminated with one or more impurities? Explain. Refer back to the melting point analysis technique video and/or safety readings for more information. How was melting point analysis used to determine the identity of the unknowns in this experiment?*
5. *Specifically explain how and why polarity allows compounds to separate on a TLC plate. This section should focus on the compounds being separated and on the stationary phase.*
6. *Specifically explain how the eluting solvent impacts the movement of compounds on a TLC plate. For example, what happens if your solvent system has more hexanes than ethyl acetate or vice versa? What makes a particular solvent mixture the best choice for analyzing a compound or mixture of compounds by TLC? Refer back to the TLC technique video and/or safety readings for more information.*
7. *How was TLC used to determine the identity of the unknowns in this experiment?*

Results

General Questions:

What important data were obtained in this experiment? Do not explain your results yet. Just provide them in an organized format. Don't forget to include any assigned unknown number! You should include a table to organize your data in addition to sentences.

Experiment-Specific Questions:

None. The rubric is already very specific. Follow the rubric.

Note: All of the necessary data needs to be present in table format, but you can combine and organize the tables in an efficient way to save space as long as the tables are clear enough to be interpreted by the reader. Tables may be single spaced.

Discussion

General questions:

How do you know the identity of the product and/or unknown? What conclusions can you draw from the data? What conclusions can you NOT draw from the data?

Experiment Specific Questions:

8. *Answer the Original Investigation Guiding Question: What is the identity of each compound in the unknown substance or mixture? How did you conclusively determine their identity or identities?*

9. *What analytical techniques did you perform in your original investigation? Why did you choose these techniques to answer the guiding question? What safety considerations informed the choices made in the Original Investigation? If you choose a technique over a different method, explain your rationale.*

10. *How were you able to conclusively determine the identity of the Original Investigation unknown? What specific evidence from the technique was used to determine the identity of the unknown? Was it possible to identify a mixture or a pure substance via the technique? If this technique alone was not enough to determine the identity of the unknown, what other evidence was necessary?*

ESQ 10 should be repeated for each technique performed in the original investigation.

11. *What are the limitations of each of the techniques that were used? Did any information from one analytical technique seem to contradict another? (For example, did one technique seem to indicate a pure compound, but a different technique indicated a mixture? Did one technique seem to indicate one molecule, but a different technique indicated a different molecule?) If information appeared to be contradictory, what decisions were made about which evidence was most important?*

12. *What discussions from argumentation shaped your conclusions? What information was obtained from your post-argumentation confirmatory experiment? Did the data support or refute your original claim?*

Conclusion

13. *How would you summarize your results and analysis in the context of the objective in 2-3 sentences? In other words, what is it that you want the reader to remember after having read your report? Make sure you remind the reader briefly what the objective(s) was/were before indicating whether the data obtained was sufficient to meet the objective(s).*

b. Project #1 Lab Report Rubric

Table S6.1. Project #1 Lab Report Rubric

Rubric item	Item description
Introduction (ESQ1)	Clearly states the overall goal of the experiment, including specific compounds and techniques used.
Theory 1 (ESQ2) IR Spectroscopy Theory	Clearly explains what is being measured in IR spectroscopy and how IR spectroscopy is being used in the context of the experiment.
Theory 2 (ESQ3) NMR Spectroscopy Theory	Clearly explains what signals represent in a 1H NMR spectrum. Describes the three primary components of a 1H NMR signal and clearly explains what information can be gathered from each. Explains how 1H NMR was used in the context of the experiment.
Theory 3 (ESQ4) Melting Point Analysis Theory	Clearly explains how the melting range of a pure compound differs from that of a mixture. Explains how melting point analysis was used in the context of the experiment.
Theory 4a (ESQ5) TLC Separation Theory	Clearly explains how and why polarity allows for compounds to separate on a TLC plate. Explanation should focus on compounds being separated and stationary phase. Mobile phase (eluting solvent) does NOT need to be included for this item.
Theory 4b (ESQ6) TLC Solvent Mixture Theory	Clearly explains how the ratio of solvents in the eluent mixture affects Rf and compound separation by TLC.
Theory 4c (ESQ7) TLC Unknown Identification Theory	Clearly explains how TLC was used in the context of the experiment.
Results 1 Original Investigation Unknown Labels & Identity Table (BODY)	All of the following data are presented in well-organized table(s): 1) unknown number/letter 2) unknown identity. *May be merged with IR data and NMR data
Results 2a Original Investigation IR Data Table	**If IR was performed during the OI, this table MUST be included. If it was not, you do not need this rubric item.** All of the following data are presented in well-organized table(s):

(BODY)	values for relevant peaks present from the IR spectrum for *your original investigation unknown* and its functional groups. *Standards must be clearly labeled *May be merged with unknown labels and NMR data
Results 2b Fundamental Skills + Original Investigation (Standards) IR Data Table (BODY)	**If IR was performed during the OI, this table MUST be included. If it was not, you do not need this rubric item.** All of the following data are presented in well-organized table(s): values for relevant peaks present from the IR spectrum for each standard *relevant to your original investigation* and their functional groups. *Standards must be clearly labeled *May be merged with NMR data
Results 3a Original Investigation NMR Data Table (BODY)	**If NMR was performed during the OI, this table MUST be included. If it was not, you do not need this rubric item.** All of the following data for each standard *relevant to your original investigation* are presented in well-organized table(s): for each peak in the 1H NMR spectrum, the 1) chemical shift, 2) integration, 3) splitting, and 4) proton assignment 5) letter (a, b, c, etc.) corresponding to a group of equivalent hydrogens in the ChemDraw structures in Results 4 *Standards must be clearly labeled *May be merged with IR data
Results 3b Fundamental Skills + Original Investigation (Standards) NMR Data Table (BODY)	**If NMR was performed during OI, this table MUST be included. If it was not, you do not need this rubric item.** All of the following data for each standard *relevant to your original investigation* are presented in well-organized table(s): for each peak in the 1H NMR spectrum, the 1) chemical shift, 2) integration, 3) splitting, and 4) proton assignment 5) letter (a, b, c, etc.) corresponding to a group of equivalent hydrogens in the ChemDraw structures in Results 4 *Standards must be clearly labeled *May be merged with IR data
Results 4 Labeled ChemDraw Structures (APPENDIX)	1) ChemDraw structures of the compounds in of each standard (2) Each group of equivalent hydrogens *in your original investigation unknown(s)* is clearly labeled with a letter (a, b, c, etc.)

	corresponding to an NMR signal in the NMR table(s) in Results 3. If you did not include Results 3, please correlate to Results 2. (in Appendix)
Results 5 Melting Range (Standards + Unknowns) Data Table (BODY)	**If melting range was performed during the OI, this table MUST be included. If it was not, you do not need this rubric item.** All of the following items are present in well-organized table(s): 1) unknown sample numbers/letters 2) melting ranges for each unknown sample *(from both OI and FS)*, and 3) literature melting ranges for standards *(from both OI and FS)*. *May be merged with Rf data
Results 6 Rf (Standards + Unknowns) Data Table (BODY)	**If TLC was performed during the OI, this table MUST be included. If it was not, you do not need this rubric item.** All of the following items are present in well-organized table(s): 1) unknown sample numbers/letters, 2) Rf values for each unknown sample *(from both OI and FS)*, and 3) Rf values for each relevant standard *(from both OI and FS)*. *May be merged with melting range data
Results 7 Rf Sample Calculation (APPENDIX)	Clearly and correctly provides sample calculations for Rf values. One sample calculation is sufficient.
Discussion 1a (ESQ 8 & 9) Answer to OI Guiding Question (1)	States the identification of the unknown mixture for the Original Investigation, and which analytical techniques were performed to answer the Original Investigation Guiding Question.
Discussion 1b (ESQ 8 & 9) Answer to OI Guiding Question (2)	Clearly explains the rationale for choosing the analytical techniques that were performed during the Original Investigation, including which chemicals were investigated.
Discussion 1c (ESQ 9) Safety Concerns	Explains specific safety considerations and how these considerations informed choices made during the Original Investigation.
Discussion 2a (ESQ 10) Technique 1	Clearly explains which potential unknown identity/identities are supported by the first technique performed and makes an argument for which possible structures should be included and excluded based on this evidence. Evidence must be an accurate interpretation of data and support the claims made.
Discussion 2b (ESQ 10)	Clearly explains which potential unknown identity/identities are supported by the second technique performed and makes an

Technique 2	argument for which possible structures should be included and excluded based on this evidence. Evidence must be an accurate interpretation of data and support the claims made.
Discussion 2c (ESQ 10) Technique 3	Clearly explains which potential unknown identity/identities are supported by the third technique performed and makes an argument for which possible structures should be included and excluded based on this evidence. Evidence must be an accurate interpretation of data and support the claims made.
Discussion 3 (ESQ 10) Pure Substance or Mixture	Clearly explains if your Original Investigation unknown was a pure substance or a mixture and includes evidence from multiple techniques.
Discussion 4a (ESQ 11) Limitations of Technique	Clearly explains the practical limitations of the techniques used in the Original Investigation, in the context of using evidence for determination of unknown identity.
Discussion 4b (ESQ 11) Contradictory Results	Clearly explains whether or not any data obtained through different techniques in Original Investigation was contradictory. If any data is contradictory, provides a reasonable explanation for the contradiction and which piece of evidence was used to make the conclusion. If no data is contradictory, give an example of a scenario where data would be contradictory and explain how it would appear and how you would identify the contradiction.
Discussion 5a (ESQ 12) Argumentation (1)	Discusses the claim of at least one other group, and whether or not that claim supports or refutes your own claim. If the claim was changed during argumentation, discuss the rationale behind changing the claim.
Discussion 5b (ESQ 12) Argumentation (2)	Clearly explains whether the post-argumentation confirmatory data supported or refuted your post-argumentation claim.
Conclusion (ESQ13)	(1) Clearly summarizes the results in the context of the objective(s). (2) Clearly states whether the objective(s) was/were met or not, and includes 1-2 sentences that support that statement.

c. Project #2 Lab Report Scaffold

Chem 51LB Project #2 Report Scaffold

Instructions:

Report scaffolds are provided to help you learn how to write about the experiments you conduct. Each question in this scaffold is numbered. In a separate document, number your responses to the *Experiment-Specific Questions* only. Do not write the specific questions in your separate document as these will only take up the space you need for your responses to those questions. Also, do not include the general questions or answers to the general questions.

Post-Lab Report Format: *MUST BE TYPED IN WORD OR A SIMILAR PROGRAM, NOT AN ELN PAGE!* Times New Roman; 12 pt. font; double spaced; 1" margins; no more than 6 pages; use 3rd person passive voice only (*For example, "We dissolved the white solid in 10 mL of hot water," should be written as, "The white solid was dissolved in 10 mL of hot water."*). Include your name, student ID number, and lab course code. This format is **NOT OPTIONAL** and TA's will remove points for failure to follow these instructions.

Submitting Your Assignment

Submit the completed assignment document (answers to these questions and the worksheets) as a single pdf file on Canvas. If you need help creating a single pdf, check out this resource.

Introduction

1. *What was the purpose of this experiment? How did we plan to accomplish this? The purpose of an experiment is not simply to introduce a student to a technique or reaction! The purpose corresponds to the objective you wrote for your in-lab notebook pages so if you got full credit for it previously, just copy and paste it into the introduction section of your post-lab report. If you did not previously earn full credit for them, carefully review your TA's feedback to help you improve it.*

Theory

Experiment-Specific Questions:

2. *What are the mechanistic differences between substitution and elimination mechanisms? What are the different types of substitution and what mechanistically defines each type? What are the different types of elimination and what mechanistically defines each type?*
3. *What conditions favor elimination? What conditions favor substitution? Explain why.*
4. *In the Fundamental Skills portion of the lab, what was the nucleophile? What was the electrophile?*
5. *Compared to the reaction performed in Fundamental Skills, what did you anticipate your products to be in your Original Investigation? State the hypothesis you had when designing your Original Investigation.*

6. *Specifically explain how gas chromatography allows you to determine the ratio of substitution and elimination product. What allows you to determine which peak elutes first?*

Results

General Questions:

What important data were obtained in this experiment? Do not explain your results yet. Just provide them in an organized format. Don't forget to include any assigned unknown number! You should include a table to organize your data in addition to sentences.

Experiment-Specific Questions:

None. The rubric is already very specific. Follow the rubric.

Note: All of the necessary data needs to be present in table format, but you can combine and organize the tables in an efficient way to save space as long as the tables are clear enough to be interpreted by the reader. Tables may be single spaced.

Discussion

General questions:

How do you know the identity of the product and/or unknown? What conclusions can you draw from the data? What conclusions can you NOT draw from the data?

Experiment Specific Questions:

7. *Answer the Original Investigation Guiding Question: What reagents favor substitution and/or elimination and how could you conclusively determine this? Even if you only hypothesized for one of the two (elimination or substitution), answer both parts of the Guiding Question.*
8. *Why did you choose the particular combination of reagents? Explain your rationale for choosing these reagents over the other options. What safety concerns were considered when writing your Original Investigation procedure?*
9. *How did your data differ between the Fundamental Skills and Original Investigation? What is the conceptual reasoning for this? Do you believe that this evidence supports your hypothesis?*
10. *How were you able to conclusively determine the ratio of the Original Investigation reaction? What specific evidence was used to determine the ratio? If this reaction alone was not enough to reach a conclusion, what other evidence was necessary?*
11. *What are the limitations of the technique or reaction? Did any information from one reaction seem to contradict another? (For example, did one reaction seem to indicate a substitution*

product, but a different technique indicated the same substitution product? Did one reaction seem to indicate one conclusion, but a different reaction indicated a different conclusion?) If information appeared to be contradictory, what decisions were made about which evidence was most important?

12. *What discussions from argumentation shaped your conclusions? What information was obtained from your post-argumentation confirmatory experiment? Did the data support or refute your original claim?*

Conclusion

13. *How would you summarize your results and analysis in the context of the objective in 2-3 sentences? In other words, what is it that you want the reader to remember after having read your report? Make sure you remind the reader briefly what the objective(s) was/were before indicating whether the data obtained was sufficient to meet the objective(s).*

d. Project #2 Lab Report Rubric

Table S6.2. Project #2 Lab Report Rubric

Rubric item	Item description
Introduction (ESQ1)	Clearly states the overall goal of the experiment, including specific compounds and techniques used.
Theory 1 (ESQ 2) Substitution vs. Elimination	Clearly explains the difference between elimination and substitution reactions mechanistically, including necessary components of the reaction.
Theory 2a (ESQ 2 & 3) Substitution Reactions	Explains the two types of substitution reactions and the differences between the two of them mechanistically. Discusses which conditions favor substitution reactions and why.
Theory 2b (ESQ 2 & 3) Elimination Reactions	Explains the two types of eliminations reactions and the differences between the two of them mechanistically. Discusses which conditions favor elimination reactions and why.
Theory 3 (ESQ 4) FS Reaction	Identifies the nucleophile and electrophile of the reactions ran in the Fundamental Skills.
Theory 4 (ESQ 5)	Identifies the nucleophile and electrophile of the reaction(s) ran in Original Investigation and whether or not this reaction was hypothesized to favor

OI Reaction + Hypothesis	substitution or elimination. Explains the conceptual reasons for this hypothesis.
Theory 5a (ESQ 6) GC Theory	Clearly explains how gas chromatography separates out compounds from a mixture. Discusses the order in which compounds elute from the column.
Theory 5b (ESQ 6) GC Ratio	Clearly explains how gas chromatography is used to determine the ratio of substitution or elimination products.
Results 1 (BODY) Fundamental Skills Data	Gas Chromatography data from the Fundamental Skills reactions is presented in a clearly organized table with the area of the substitution product(s), elimination product(s), and starting material(s), if applicable. *can be combined with the OI tables
Results 2 (BODY) Original Investigation Data	Gas Chromatography data from the Fundamental Skills reactions is presented in a clearly organized table with the area of the substitution product(s), elimination product(s), and starting material(s), if applicable. *can be combined with the FS tables
Results 3 (APPENDIX) Fundamental Skills Mechanism	Full and complete mechanism of the major product(s) of the two reactions ran in Fundamental Skills with no more than two mistakes (which include incorrect formal charges, arrows, or structures).
Results 4 (APPENDIX) Original Investigation Mechanism	Full and complete mechanism of the major product(s) of the reaction(s) ran in Original Investigation with no more than two mistakes (which include incorrect formal charges, arrows, or structures).
Discussion 1a (ESQ 7) Answer to OI Guiding Question (1)	States the reagents which favor substitution and elimination in this experiment to answer the Original Investigation Guiding Question.
Discussion 1b (ESQ 7 & 8) Answer to OI Guiding Question (2)	Explains whether the particular reactions performed during Fundamental Skills and Original Investigation favored substitution or elimination and how this was determined. Discusses the choice of particular reagents and why they were chosen.

Discussion 1c (ESQ 8) Safety Concerns	Explains specific safety considerations and how these considerations informed choices made during the Original Investigation.
Discussion 2a (ESQ 9 & 10) FS + OI Differences	Discusses the differences in the data between the reactions run in Fundamental Skills and Original Investigation and how this data assisted in answering the Original Investigation Guiding Question.
Discussion 2b (ESQ 9) Hypothesis	Clearly states whether or not the hypothesis at the beginning of Original Investigation was confirmed or refuted and what evidence led to this conclusion.
Discussion 3a (ESQ 11) Limitations of Technique	Clearly explains the practical limitations of the techniques and reactions used in the Original Investigation, in the context of using evidence for determination of substitution and/or elimination products.
Discussion 3b (ESQ 11) Contradictory Results	Clearly explains whether or not any data obtained in Original Investigation was contradictory. If any data is contradictory, provides a reasonable explanation for the contradiction and which piece of evidence was used to make the conclusion. If no data is contradictory, give an example of a scenario where data would be contradictory and explain how it would appear and how you would identify the contradiction.
Discussion 4a (ESQ 12) Argumentation (1)	Discusses the claim of at least one other group, and whether or not that claim supports or refutes your own claim. If the claim was changed during argumentation, discuss the rationale behind changing the claim.
Discussion 4b (ESQ 12) Argumentation (2)	Clearly explains whether the post-argumentation confirmatory data supported or refuted your post-argumentation claim.
Conclusion (ESQ13)	(1) Clearly summarizes the results in the context of the objective(s). (2) Clearly states whether the objective(s) was/were met or not, and includes 1-2 sentences that support that statement.

e. Project #3 Lab Report Scaffold

Chem 51LB Project #3 Report Scaffold

Recrystallization of an Epoxidation Product

Instructions:

Report scaffolds are provided to help you learn how to write about the experiments you conduct. For this assignment, answer the **specific** questions provided using complete sentences in the third person passive voice, but keep the general questions in mind as a guide.

Each question in this scaffold is numbered. In a separate document, number your responses to the *Experiment-Specific Questions* only. Do not write the specific questions in your separate document as these will only take up the space you need for your responses to those questions. Also, do not include the general questions or answers to the general questions.

Post-Lab Report Format: *MUST BE TYPED IN WORD OR A SIMILAR PROGRAM, NOT AN ELN PAGE!* Times New Roman; 12 pt. font; double spaced; 1" margins; no more than 6 pages; use 3rd person passive voice only (*For example, "We dissolved the white solid in 10 mL of hot water," should be written as, "The white solid was dissolved in 10 mL of hot water."*). Include your name, student ID number, and lab course code. This format is **NOT OPTIONAL** and TA's will remove points for failure to follow these instructions.

Submitting Your Assignment

Submit the completed assignment document (answers to these questions and the worksheets) as a single pdf file on Canvas. If you need help creating a single pdf, check out this resource.

Introduction

1. *What was the purpose of this experiment? How did we plan to accomplish this? The purpose of an experiment is not simply to introduce a student to a technique or reaction! The purpose corresponds to the objective you wrote for your in-lab notebook pages so if you got full credit for it previously, just copy and paste it into the introduction section of your post-lab report. If you did not previously earn full credit for them, carefully review your TA's feedback to help you improve it.*

Theory

Experiment-Specific Questions:

2. *What type of reaction are we doing over the course of this project? What is the starting material and the anticipated product?*
3. *Why did we perform extraction in this experiment? What were we extracting?*
4. *How is recrystallization being used in this experiment? Why are we using it? What would someone need to know about how recrystallization works to understand how and why it is being used and to understand the data and results?*
5. *Explain the concepts between the different types of recrystallizations as performed in the first week of Fundamental Skills. You're not discussing your results here, but rather discussing the differences between the procedures and why it would result in a difference in the results.*

6. *How is melting point being used in this experiment? Why are we using it? What would someone need to know about how melting point works to understand how and why it is being used and to understand the data and results? Don't include any results here. You are providing the proper background for the reader to be able to interpret your results later.*
7. *How is thin layer chromatography (TLC) being used in this experiment? Why are we using it? What would someone need to know about how thin layer chromatography works to understand how and why it is being used and to understand the data and results?*

Results

General Questions:

What important data were obtained in this experiment? Do not explain your results yet. Just provide them in an organized format. Don't forget to include any assigned unknown number! You should include a table to organize your data in addition to sentences.

Experiment-Specific Questions:

None. The rubric is already very specific. Follow the rubric.

Note: All of the necessary data needs to be present in table format, but you can combine and organize the tables in an efficient way to save space as long as the tables are clear enough to be interpreted by the reader. Tables may be single spaced.

Discussion

General questions:

How do you know the identity of the product and/or unknown? What conclusions can you draw from the data? What conclusions can you NOT draw from the data?

Experiment Specific Questions:

8. *Answer the Fundamental Skills 2 Guiding Question: What product did you obtain, and how did you decide that was the product? Use your data to build an argument for your conclusion.*
9. *Based on the evidence from your Fundamental Skills, what was the efficacy of your reaction? Did your reaction appear to go to completion? Do you believe your major product was the expected product?*
10. *Answer the Original Investigation Guiding Question: Which recrystallization technique was the best for isolating your major compound? Why was this technique the most effective?*

11. *Why did you choose the particular solvent or solvents? Explain your rationale for choosing this solvent system over the other options. What safety concerns were considered when writing your Original Investigation procedure?*
12. *How did your chosen recrystallization data differ from other recrystallizations performed in the Fundamental Skills and Original Investigation? What is the conceptual reasoning for this? Do you believe that this evidence supports your hypothesis?*
13. *How were you able to conclusively determine the best recrystallization out of the Original Investigation? What specific evidence was used to determine this? If this evidence alone was not enough to reach a conclusion, what other evidence was necessary?*
14. *What are the limitations of the technique or reaction? Did any information from one reaction seem to contradict another? (For example, did a recrystallization work well in Fundamental Skills and then not work effectively in the Original Investigation?) If information appeared to be contradictory, what decisions were made about which evidence was most important?*
15. *What discussions from argumentation shaped your conclusions? What information was obtained from your post-argumentation confirmatory experiment? Did the data support or refute your original claim?*

Conclusion

16. *How would you summarize your results and analysis in the context of the objective in 2-3 sentences? In other words, what is it that you want the reader to remember after having read your report? Make sure you remind the reader briefly what the objective(s) was/were before indicating whether the data obtained was sufficient to meet the objective(s).*

f. Project #3 Lab Report Rubric

Table S6.3 Project #3 Lab Report Rubric

Rubric item	Item description
Introduction (ESQ1)	Clearly states the overall goal of the experiment, including specific compounds and techniques used.
Theory 1 (ESQ 2) Reaction	Describes the type of reaction performed in this project, including reagents and anticipated product.
Theory 2 (ESQ 3) Extraction	Clearly explains the purpose of extraction in this experiment, including the solvents used and what is being extracted.
Theory 3a (ESQ 4) Recrystallization Purpose	Clearly explains the purpose and use of the technique of recrystallization and provides a conceptual rationale for the procedure used to recrystallize.

Theory 3b (ESQ 5) Recrystallization Differences	Clearly articulates the differences between the three different recrystallizations performed in the Fundamental Skills portion in the lab. Connects the difference in procedure to anticipated differences in results, conceptually (this may be different than your experimental data)
Theory 4 (ESQ 6) Melting Point	Clearly explains the purpose of melting point in this experiment and how the data is being interpreted, including mixed melting point (if performed).
Theory 5 (ESQ 7) TLC	Clearly explains the purpose of TLC in this experiment and how the data is being interpreted.
Results 1 (BODY) Percent Recovery	Provides a well-organized table that presents the percent recovery for all recrystallizations performed in both Fundamental Skills and Original Investigation *may be combined with the melting point and Rf value tables
Results 2 (BODY) Melting Point Values	Provides a well-organized table that presents the melting point for the standard (trans-stilbene), the crude product from the reaction, and the recovered product for all recrystallizations performed in the Original Investigation ONLY. You do not need to include the Fundamental Skills data in this table. *may be combined with the percent recovery and Rf value tables
Results 3 (BODY) Rf Values	Provides a well-organized table that presents the Rfs for the standard (trans-stilbene), the crude product from the reaction, and the recovered product for all recrystallizations performed in the Original Investigation ONLY. You do not need to include the Fundamental Skills data in this table. *may be combined with the percent recovery and melting point tables
Results 4 (APPENDIX) Sample Calculation - Percent Recovery	Provides a full and complete sample calculation for percent recovery
Results 5 (APPENDIX) Sample Calculation - Rf	Provides a full and complete sample calculation for Rf

Discussion 1 (ESQ 8) Answer to FS Guiding Question	States the major product that was obtained from the epoxidation reaction and how it was determined by citing evidence that supports the claim.
Discussion 2 (ESQ 9) FS Reaction Efficacy	Explains whether the particular reaction performed during Fundamental Skills was an effective reaction, based on the evidence. States whether or not this was the expected product.
Discussion 3a (ESQ 10) Answer to OI Guiding Question (1)	States the answer to the Original Investigation Guiding Question, giving an overview of the evidence that allowed this conclusion to be reached.
Discussion 3b (ESQ 10 & 11) Answer to OI Guiding Question (2)	Discusses the solvent choice for recrystallization and why these choice(s) were made to answer the Original Investigation Guiding Question.
Discussion 3c (ESQ 11) Safety Concerns	Explains specific safety considerations and how these considerations informed choices made during the Original Investigation.
Discussion 4a (ESQ 12) FS + OI Differences	Discusses the differences in the data between the crystallizations run in Fundamental Skills and Original Investigation and how this data assisted in answering the Original Investigation Guiding Question.
Discussion 4b (ESQ 13) Best Recrystallization	Explains how you determined the best recrystallization between the two (or more) recrystallizations performed in your Original Investigation
Discussion 5a (ESQ 14) Limitations of Technique	Clearly explains the practical limitations of the techniques and reactions used in the Original Investigation, in the context of using evidence for determination of substitution and/or elimination products.
Discussion 5b (ESQ 14) Contradictory Results	Clearly explains whether or not any data obtained in Original Investigation was contradictory. If any data is contradictory, provides a reasonable explanation for the contradiction and which piece of evidence was used to make the conclusion. If no data is contradictory, give an example of a scenario where data would be contradictory and explain how it would appear and how you would identify the contradiction.

Discussion 6a (ESQ 15) Argumentation (1)	Discusses the claim of at least one other group, and whether or not that claim supports or refutes your own claim. If the claim was changed during argumentation, discuss the rationale behind changing the claim.
Discussion 6b (ESQ 15) Argumentation (2)	Clearly explains whether the post-argumentation confirmatory data supported or refuted your post-argumentation claim.
Conclusion (ESQ16)	(1) Clearly summarizes the results in the context of the objective(s). (2) Clearly states whether the objective(s) was/were met or not, and includes 1-2 sentences that support that statement.

IV. ASAC Protocol

The following is the adjusted ASAC protocol used to evaluate the argumentation for the beta-testing of the OCL-II projects. This protocol rubric was used both by the graduate student researcher (myself) and the 4 undergraduate student researchers. It is included in its entirety below.

Assessment of Scientific Argumentation in the Classroom Observation Protocol

CONCEPTUAL AND COGNITIVE ASPECTS OF SCIENTIFIC ARGUMENTATION

HOW THE GROUP ATTEMPTS TO NEGOTIATE MEANING OR DEVELOP A BETTER UNDERSTANDING

(THESE ITEMS TARGET HOW THE GROUP ATTEMPTS TO MAKE SENSE OF WHAT IS GOING ON)

C1. The talk of the group was focused on solving a problem or advancing understanding.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: The emphasis on advancing understanding indicates that there were some significant claims or explanations at the heart of discussion. Groups that score high on this item maintain the focus of their talk and efforts on understanding or solving the problem rather than the best way to finish their work quickly or with the least amount of effort. *Note:* Groups that stay on topic but never go engage in an in-depth discussion about what is happening should be scored low on this item.				
	0	**1**	**2**	**3**

C2. The participants sought out and discussed alternative claims or explanations.	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: Divergent thinking is an important part of argumentation. A group that meets this criterion would talk about more than one claim, explanation, or solution. Individuals that valued alternative modes of thinking would respect and actively solicit new or alternative claims, explanations, or solutions from the other participants. *Note:* Groups that discuss multiple types of grounds or support for a claim, explanation, or solution but only one claim, explanation, or solution should be scored low on this item.				
C3. The participants modified their explanation or claim when they noticed an inconsistency or discovered anomalous information.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: Inconsistencies between claims or explanation and the phenomenon under investigation are common. A group that modified their claim or explanation when they noticed inconsistencies or anomalies would not ignore "things that do not fit" or attempt to discount them once they are noticed by one of the participants. Groups that score high on this item try to modify their claim or explanation (not just their reasons) in order to account for an inconsistency or an anomaly rather than attempting to "explain them away."				
C4. The participants were skeptical of ideas and information.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: During scientific argumentation, allowing a variety of ideas to be presented, but insisting that challenge and negotiation also occur would indicate that group members were skeptical. Accepting ideas without accompanying reasons would result in a low score because it is a sign of credulous thinking. In other words, students must be willing to ask, "how do you know?" or "Are you sure?" Groups that respond to the ideas of others with comments such as "ok", "that sounds good to me", or "whatever you think is right" would score low on this item.				
C5. The participants provided reasons when supporting or challenging an idea.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: Providing reasons to support or challenge a claim, conclusion, or explanation is a crucial characteristic of argumentation. Claims must have some support provided for them beyond simply restating the claim itself. Making claims without support would result				

in a low score on this item and including any reason like "that's what I think", "it doesn't make sense", "the data suggests…" or "but that doesn't fit with…" would result in a higher score. *Note:* Personal or past experiences count as a reason for this item.				
C6. The participants attempted to evaluate the merits of each alternative claim or explanation in a systematic manner.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: This addresses the tentative or responsive nature of science. The idea that there is often more than one way to interpret data or evidence and that only through careful analysis can an idea be accepted or eliminated. This gets at the "gut" response factor. Conclusions are not based on opinion or inference.				

COGNITIVE SUBSCORE: _____/18

EPISTEMIC ASPECTS OF SCIENTIFIC ARGUMENTATION

HOW CONSISTENT THE PROCESS IS WITH THE CULTURE OF SCIENCE

(THESE ITEMS TARGET HOW THE GROUP DETERMINES WHAT COUNTS AS VALID OR ACCEPTABLE)

E7. The participants used evidence to support and challenge ideas or to make sense of the phenomenon under investigation.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: A goal of scientific argumentation is the use of data that has been collected, analyzed, and interpreted as evidence to defend a claim, conclusion, or explanation. This item implies that students were attempting to use evidence in their arguments. This should more than an opinion; they must discuss data that was collected, how the data was analyzed, or what the results of an analysis means. Statements like "that's what I think" or "it doesn't make sense" would result in a low score. Statements like "the data we found suggests that …" or "our evidence indicates…" would result in a higher score.				
E8. The participants examined the relevance, coherence, and sufficiency of the evidence.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: This item draws attention to the amount and kinds of evidence used to support a claim or explanation. Groups that attempt to (a) determine the value of a piece of evidences (e.g., "does that matter?"), (b) look at links or the relationship between multiple				

pieces of evidence (e.g., "This supports X and Y but this only supports X"), or (c) attempt to determine if there is enough evidence to support an idea (e.g., "We do not have any evidence to support that") would score higher on this item.				
E9. The participants evaluated how the data was gathered, analyzed or interpreted.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: The evidence provided for a claim or explanation should be evaluated based on how well the data was gathered, analyzed, and interpreted. A question such as "Why is that evidence included?" or "How did they gather their data?" or "Where did that data come from?" indicates that the participants are assessing methods or an interpretation of data and would result in a higher score.				
E10. The participants used scientific theories, laws, or models to support and challenge ideas or to help make sense of the phenomenon under investigation.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: Science is theory-laden. In other words, scientists rely on broad, well-supported organizing ideas to frame their arguments and claims. Students should also employ these paradigmatic ideas in providing warrants for the evidence and claims they make or use to refute others' claims. Explicit reference to these "big ideas" will result in a higher score on this item.				
E11. The participants made distinctions and connections between inferences and observations explicit to others.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: The structure of scientific arguments includes evidence involving both empirical (such as quantitative measurements and systematic observations) and inferential (noting of trends and logical connections among observations) aspects. Making these distinctions and their connections explicit to others enhances the quality of the argumentation and thus results in a higher score.				
E12. The participants used the language of science to communicate ideas.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: This item stresses the importance of the accurate use of scientific language by students. The adoption and use appropriate terms (e.g., condensation, force, etc.), phrases				

(e.g., “it supports” rather than “it proves”) or ways of describing information is a characteristic of argumentation that is scientific. *Note:* Ideas may be explicated before being labeled with the correct terminology.

EPISTEMIC SUBSCORE: _____/18

SOCIAL ASPECTS OF SCIENTIFIC ARGUMENTATION

HOW THE PARTICIPANTS INTERACT WITH EACH OTHER

(THESE ITEMS TARGET GROUP DYNAMICS)

S13. The participants were reflective about what they know and how they know.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: It is important for members of the group to agree on what they know and to be specific about how they know. Statements such as, “do we all agree?” or “is there anything else we need to figure out?” or “can we be sure?” indicate that participants are monitoring their progress and have an end goal in mind.				
S14. The participants respected what each other had to say.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: Respecting what others have to say is more than listening politely or giving tacit agreement. Respect also indicates that what others had to say was actually heard and considered (e.g., “that is a good point”, interesting idea”, or “I hadn’t thought of that”). A group that scored high on this would allow everyone to present their ideas and express their opinions without censure or ridicule.				
S15. The participants discussed an idea when it was introduced into the conversation.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: To be a participating and contributing member of the group, it is important to feel valued. Ideas and opinions need to be critically acknowledged. This means they are considered and given weight by the group. Groups that ignore ideas when they are proposed (results in the same idea being mentioned over and over) would earn a low score on this item.				

S16. The participants encouraged or invited others to share or critique ideas.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: Good argumentation comes from considering and comparing competing ideas from multiple individuals to construct the most robust explanation of the phenomenon under study. Groups that consist of individuals that invite others to share (e.g., "what do you think"), critique (e.g., "do you agree" or "it is ok to disagree with me"), or discuss an idea (e.g., "let's talk about this some more") would score higher that a group with an alienating leader that dominates the conversation and the work of the group.				
S17. The participants restated or summarized comments and asked each other to clarify or elaborate on their comments.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: The depth of discussion will be enhanced by not making implicit judgments or assumptions about another person's ideas or views, and it demonstrates that their point of view is valued and is furthering the discussion. Communication provides students with opportunities to identify the strengths and weaknesses of their understanding.				
S18. There was equal participation from all members of the group.	**0**	**1**	**2**	**3**
	Not at all	One or Two Instances	Three or Four Instances	Often (> 4 Instances)
Description: The degree to which in member contributed to the argumentation impacts the depth and breadth of the discourse. Also, one or two high performers may result in a high score on some items, but not be representative of the actual argumentation event. Groups where some members are not engaged would score low on this item.				

SOCIAL SUBSCORE: _____/18

TOTAL SCORE: _____/54

V. Project #1 Unknown List

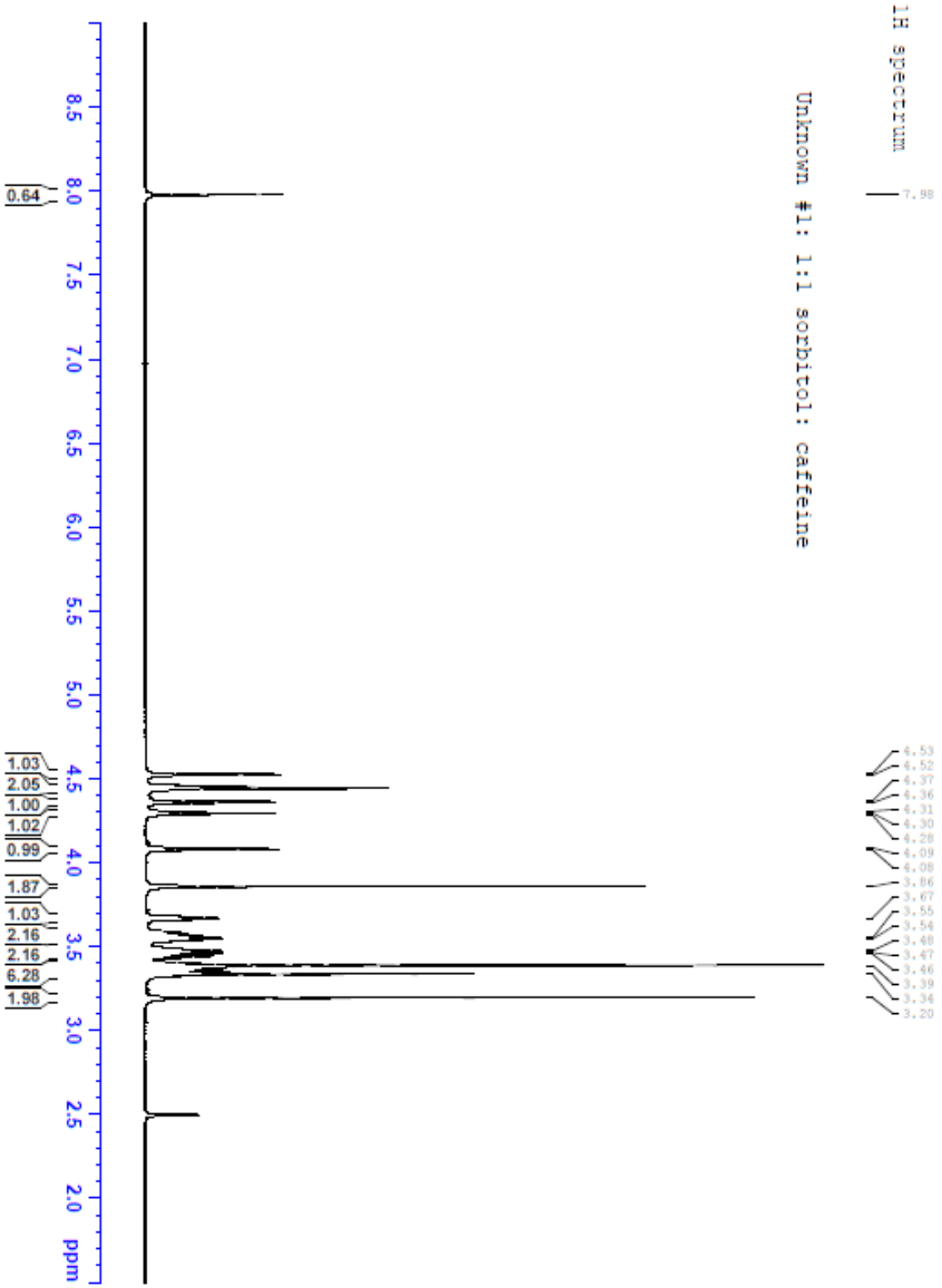
Fundamental Skills Unknowns:

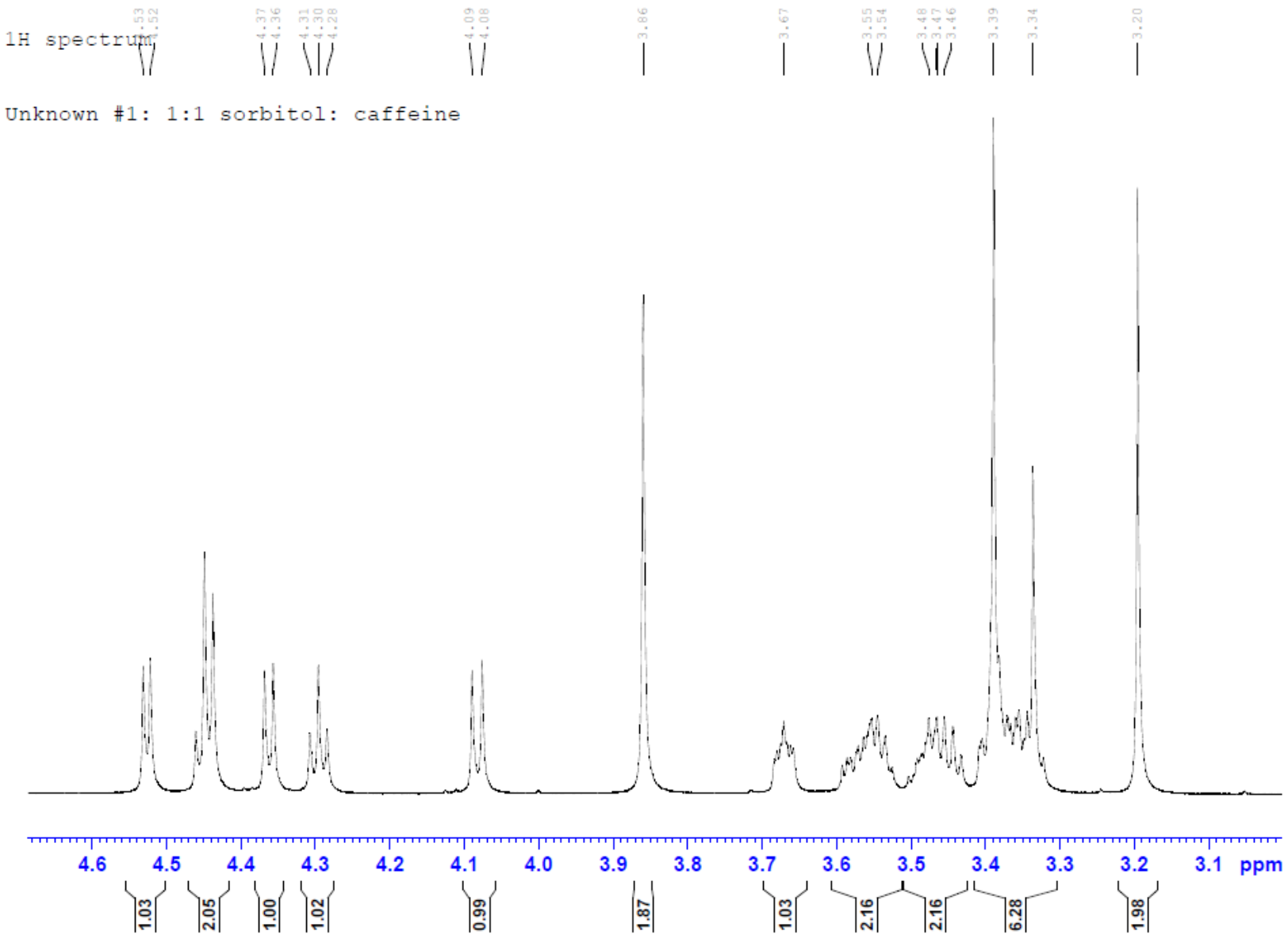
1. 1:3 acetylsalicylic acid:acetaminophen
2. 1:1 acetylsalicylic acid:sorbitol
3. 1:1 acetylsalicylic acid:naproxen
4. 1:3 naproxen:acetaminophen
5. 1:3 acetaminophen:acetylsalicylic acid
6. 1:3 acetaminophen:naproxen
7. 1:1 naproxen:sorbitol
8. 1:3 naproxen:acetaminophen

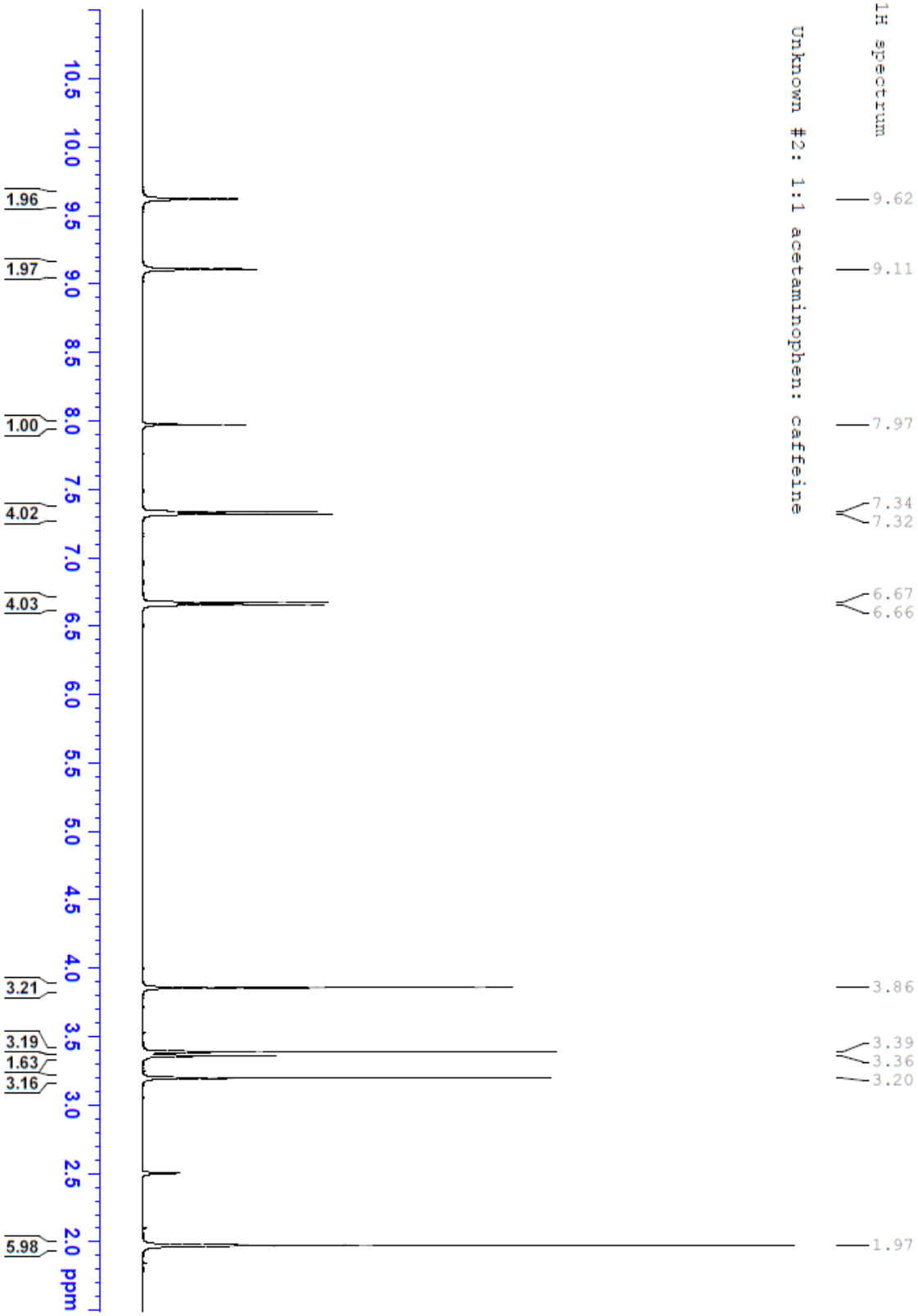
Original Investigation Unknowns:

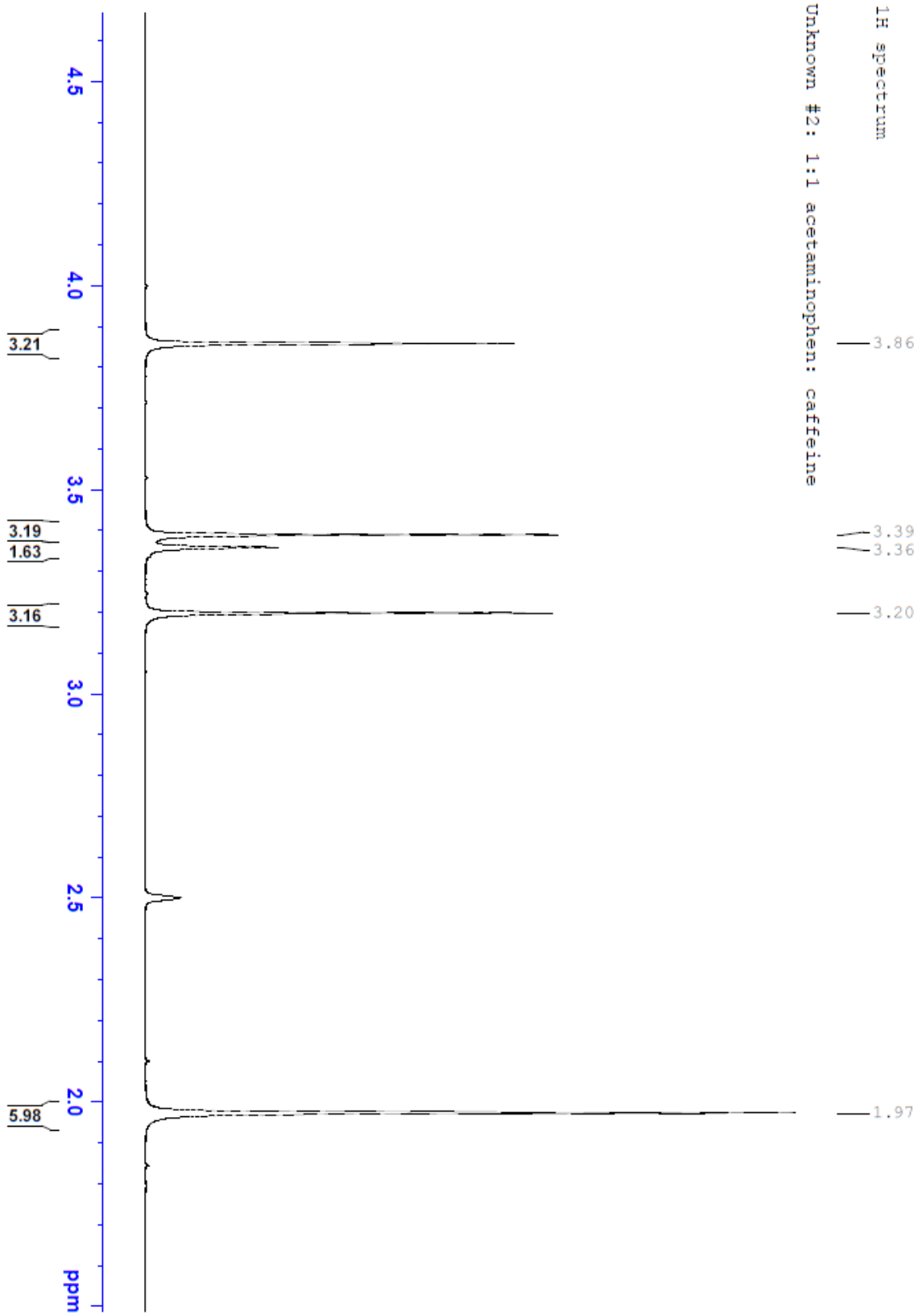
1. 1:1 Sorbitol: Caffeine
2. 1:1 Acetaminophen: Caffeine
3. 1:1 Acetylsalicylic Acid: Caffeine
4. 1:1 Naproxen: Caffeine
5. 1:3 Acetaminophen: Ibuprofen
6. 1:3 Sorbitol: Lidocaine
7. 1:3 Sorbitol: Ibuprofen
8. 1:3 Acetylsalicylic Acid: Ibuprofen

VI. NMR Spectra for Project #1




1H spectrum
Unknown #1: 1:1 sorbitol: caffeine
4.53
4.52
4.37
4.36
4.31
4.30
4.28
4.09
4.08
3.86
3.67
3.55
3.54
3.48
3.47
3.46
3.39
3.34
3.20
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
ppm
1.03
2.05
1.00
1.02
0.99
1.87
1.03
2.16
2.16
6.28
1.98

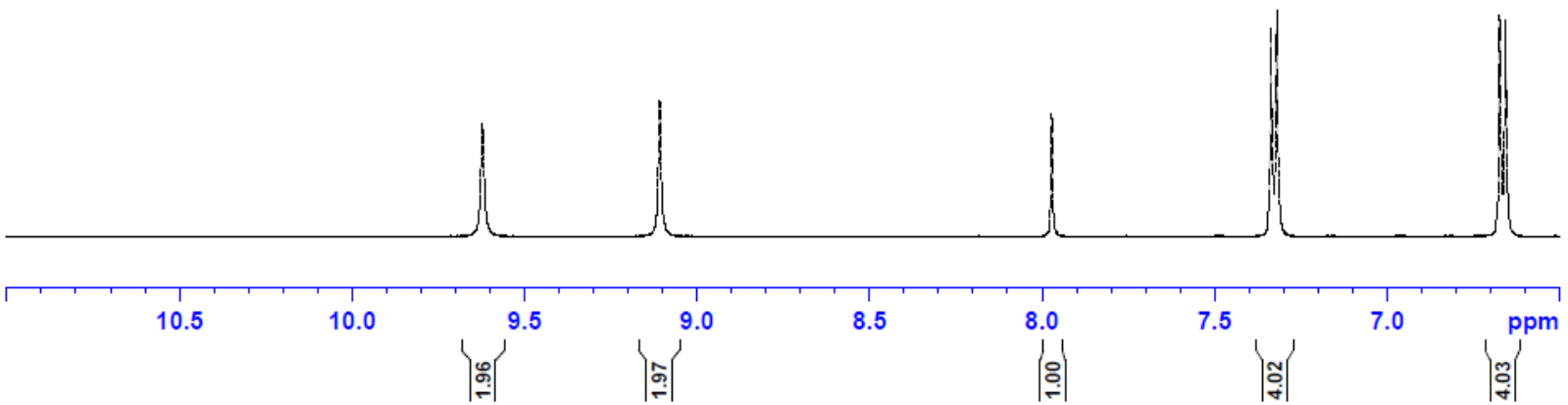

1H spectrum
Unknown #2: 1:1 acetaminophen: caffeine
9.62
9.11
7.97
7.34
7.32
6.67
6.66
3.86
3.39
3.36
3.20
1.97
1.96
1.97
1.00
4.02
4.03
3.21
3.19
1.63
3.16
5.98
ppm

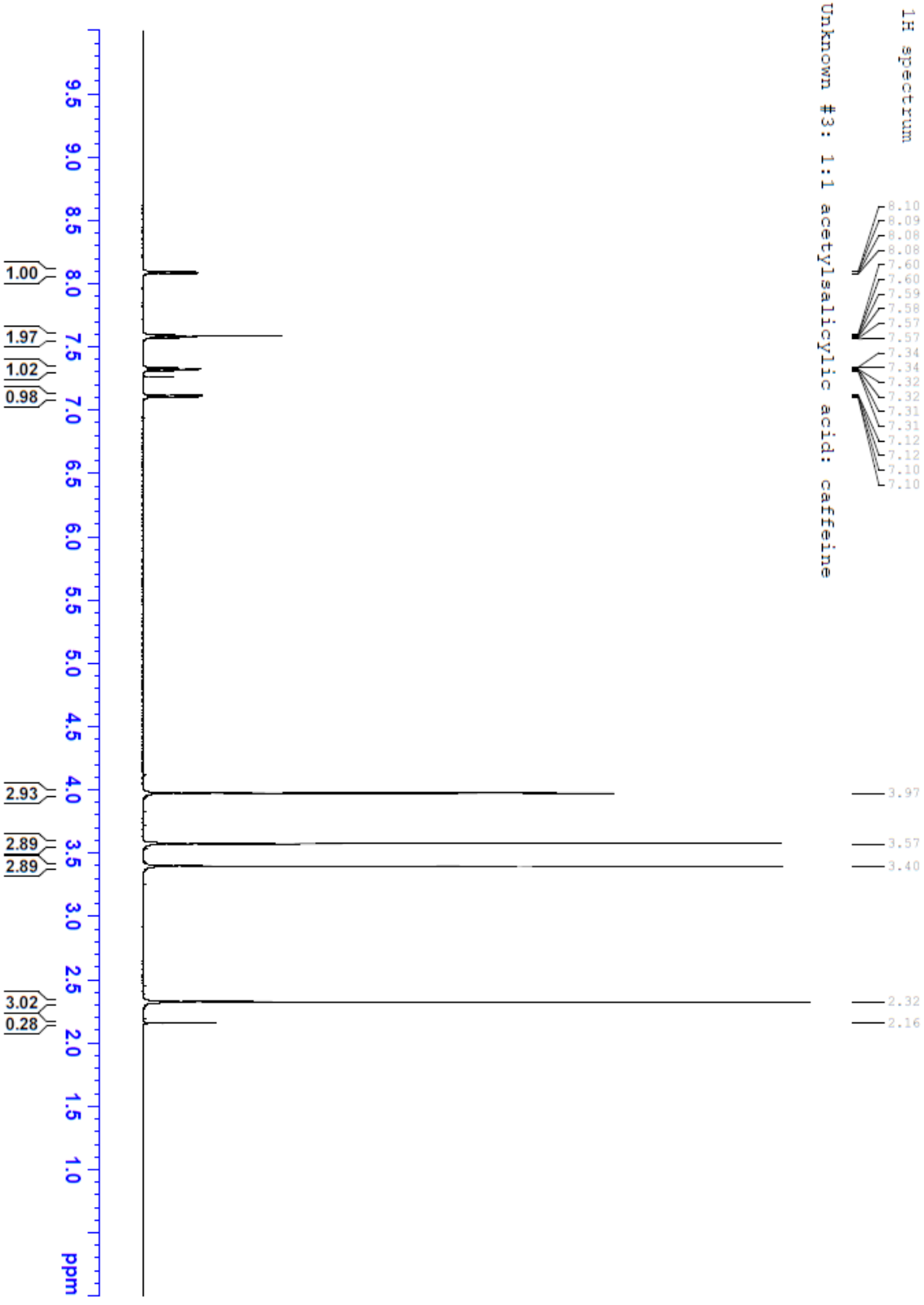

1H spectrum
Unknown #2: 1:1 acetaminophen: caffeine
3.86
3.39
3.36
3.20
1.97
3.21
3.19
1.63
3.16
5.98
4.5
4.0
3.5
3.0
2.5
2.0
ppm

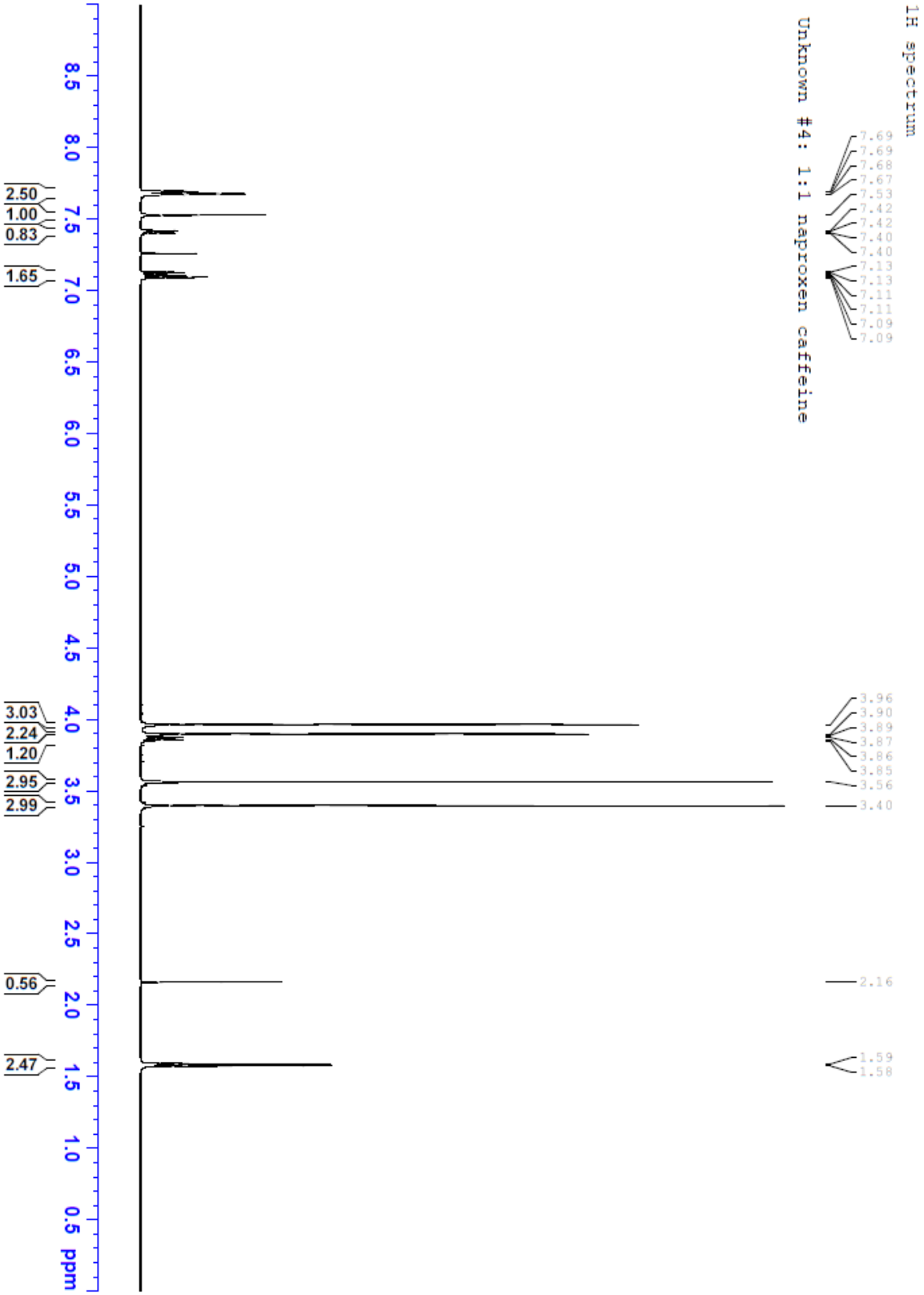
1H spectrum

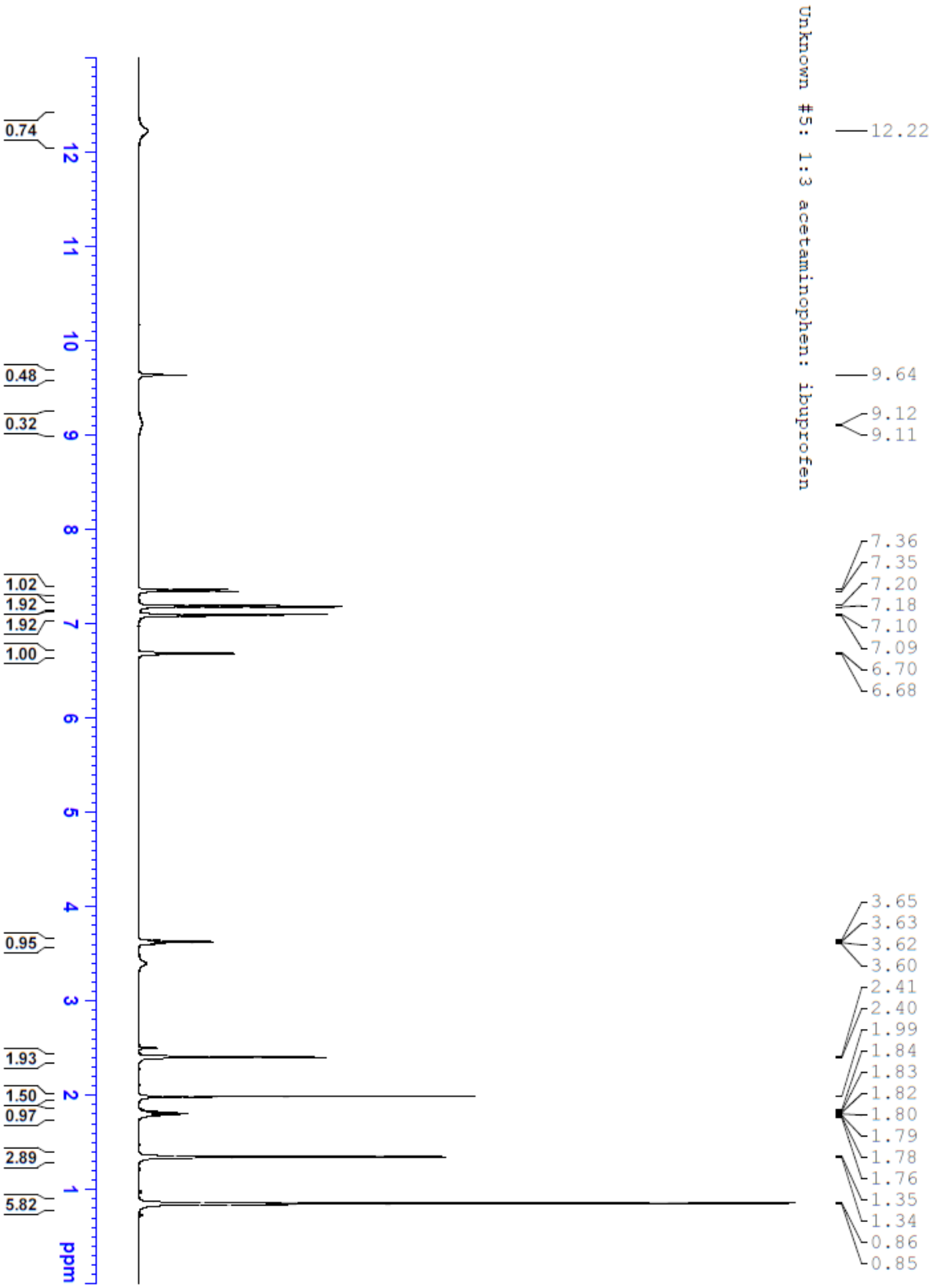
9.62
9.11
7.97
7.34
7.32
6.67
6.66

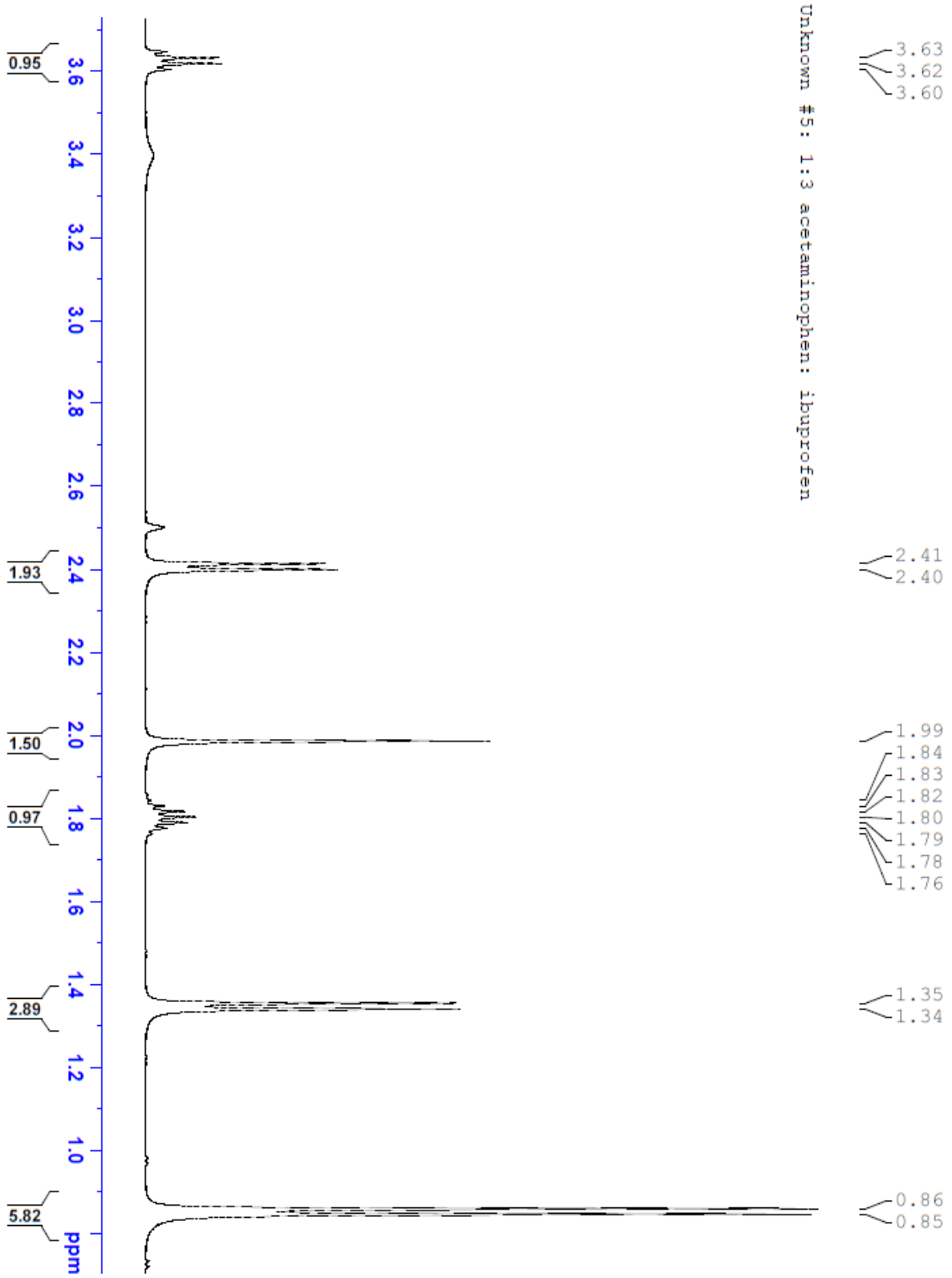
Unknown #2: 1:1 acetaminophen: caffeine

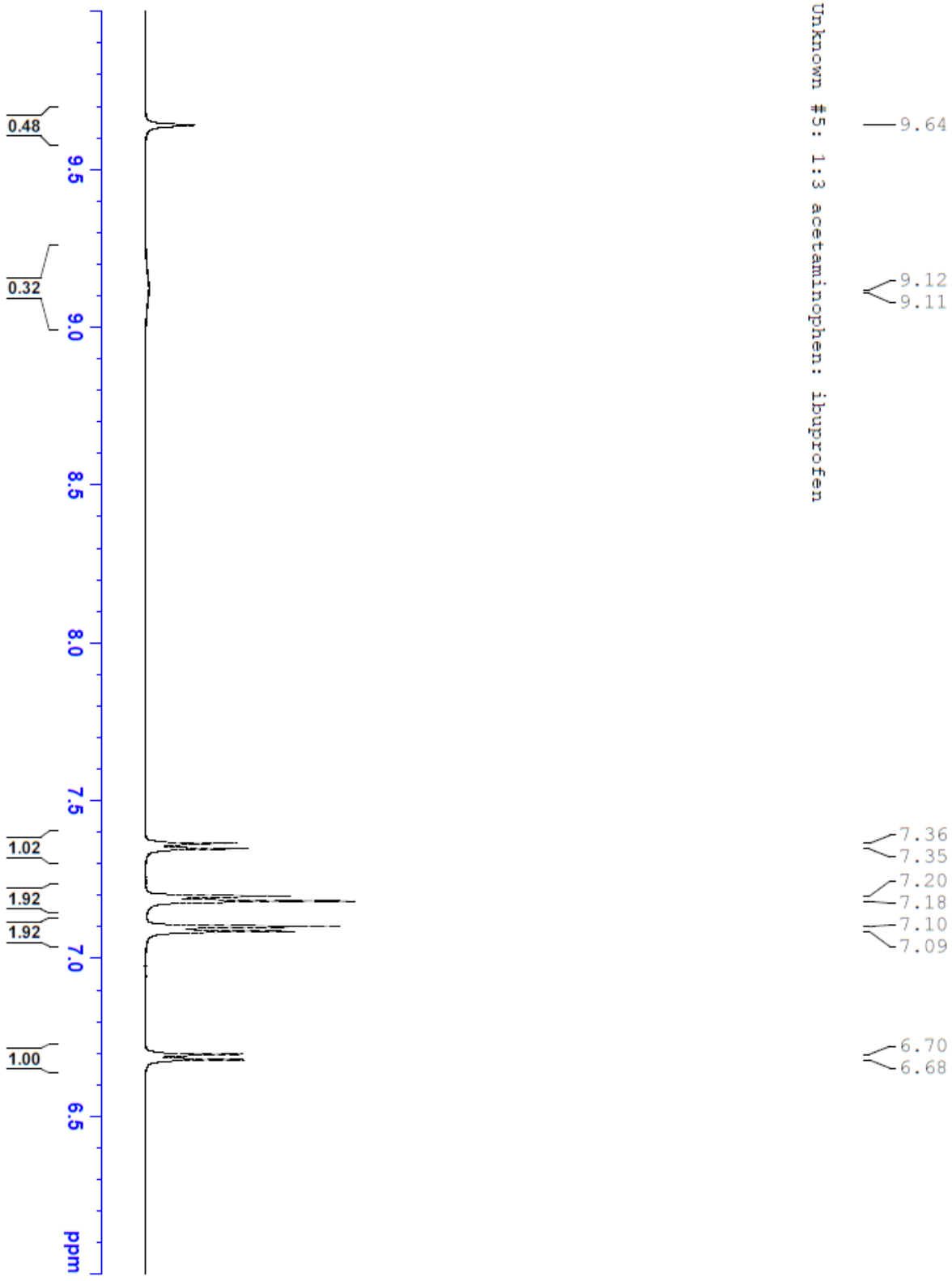


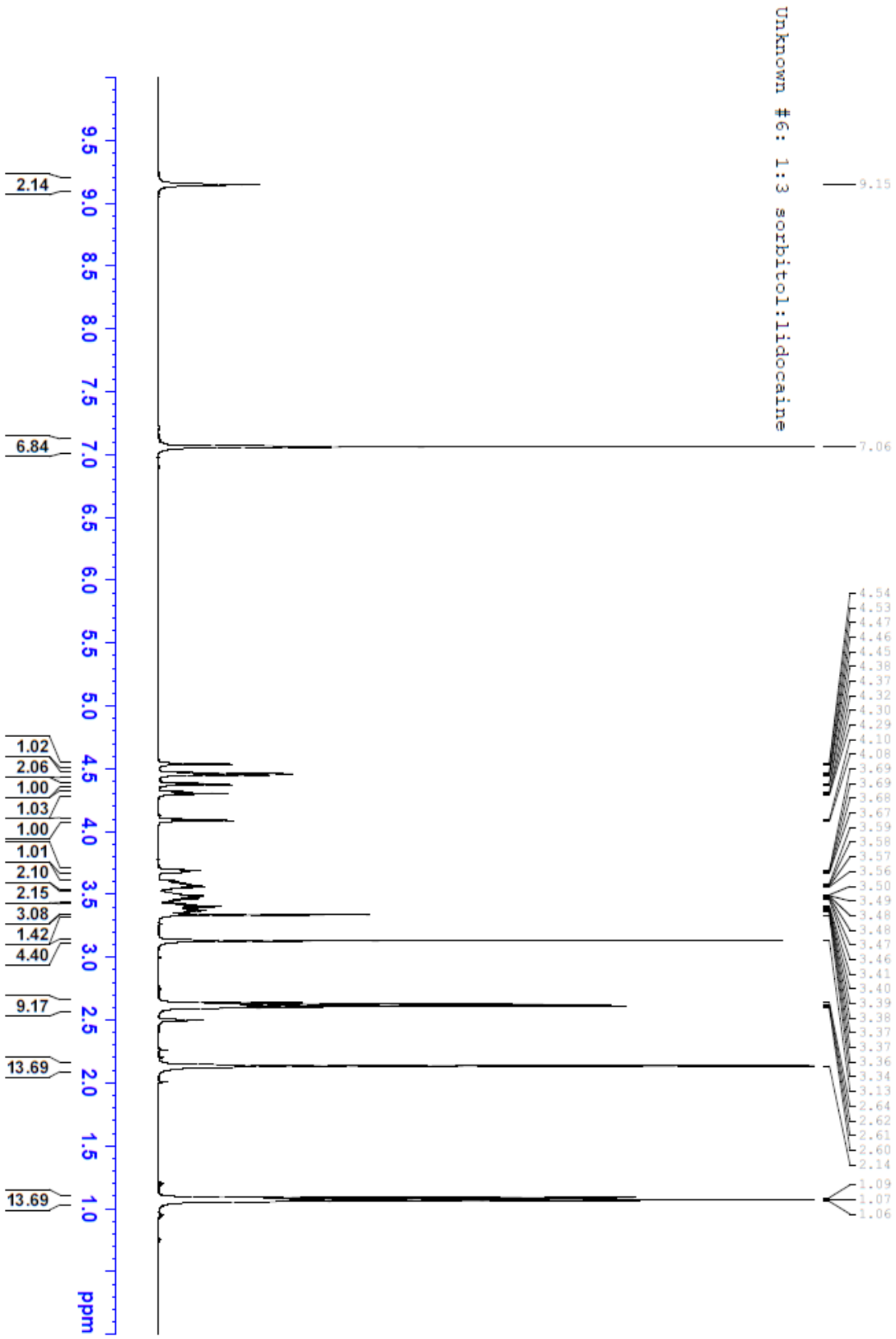

1H spectrum
Unknown #3: 1:1 acetylsalicylic acid: caffeine
8.10
8.09
8.08
8.08
7.60
7.60
7.59
7.58
7.57
7.57
7.34
7.34
7.32
7.32
7.31
7.31
7.12
7.12
7.10
7.10
3.97
3.57
3.40
2.32
2.16
1.00
1.97
1.02
0.98
2.93
2.89
2.89
3.02
0.28
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

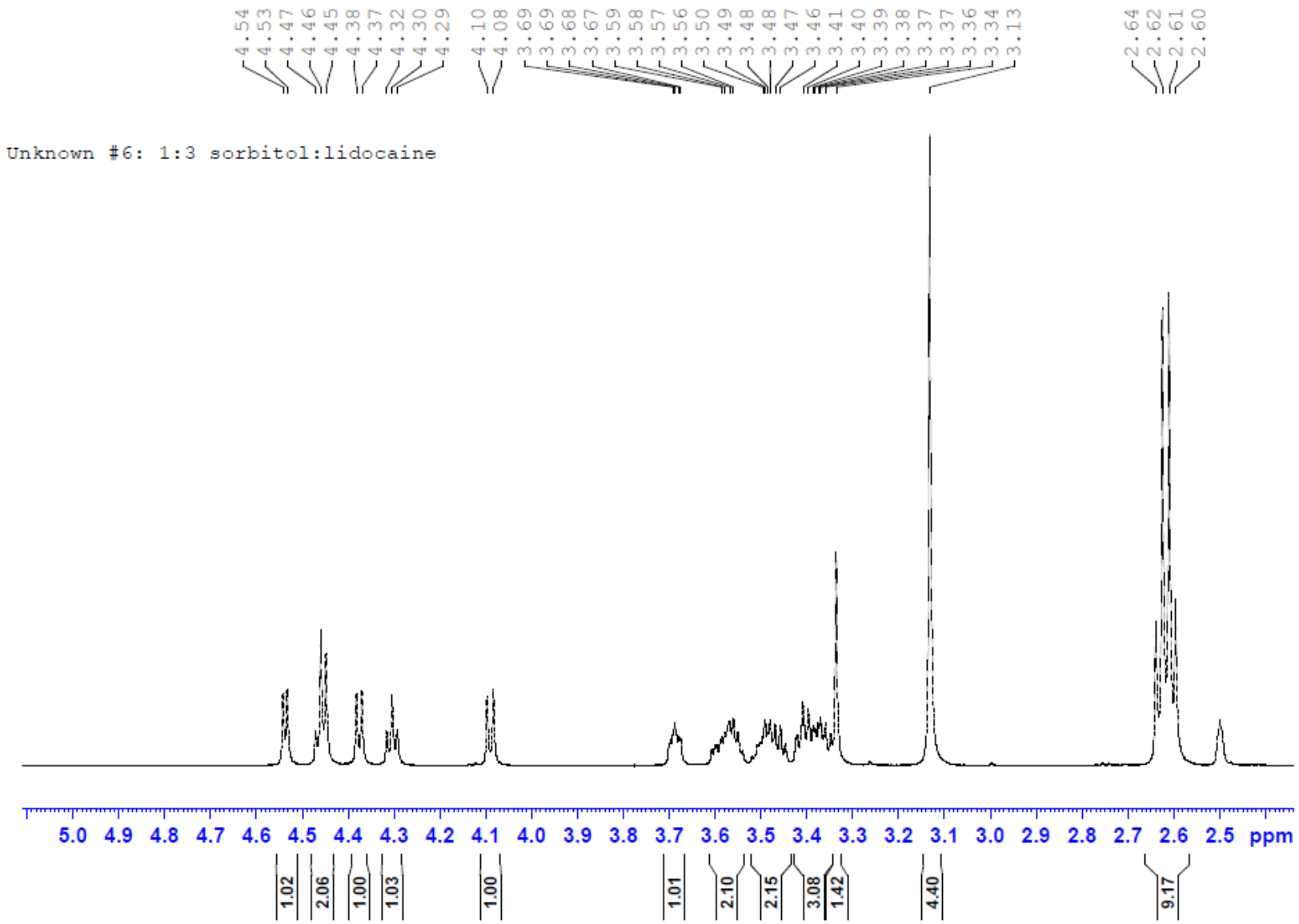

1H spectrum
Unknown #4: 1:1 naproxen caffeine
7.69
7.69
7.68
7.67
7.53
7.42
7.42
7.40
7.40
7.13
7.13
7.11
7.11
7.09
7.09
3.96
3.90
3.89
3.87
3.86
3.85
3.56
3.40
2.16
1.59
1.58
2.50
1.00
0.83
1.65
3.03
2.24
1.20
2.95
2.99
0.56
2.47
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

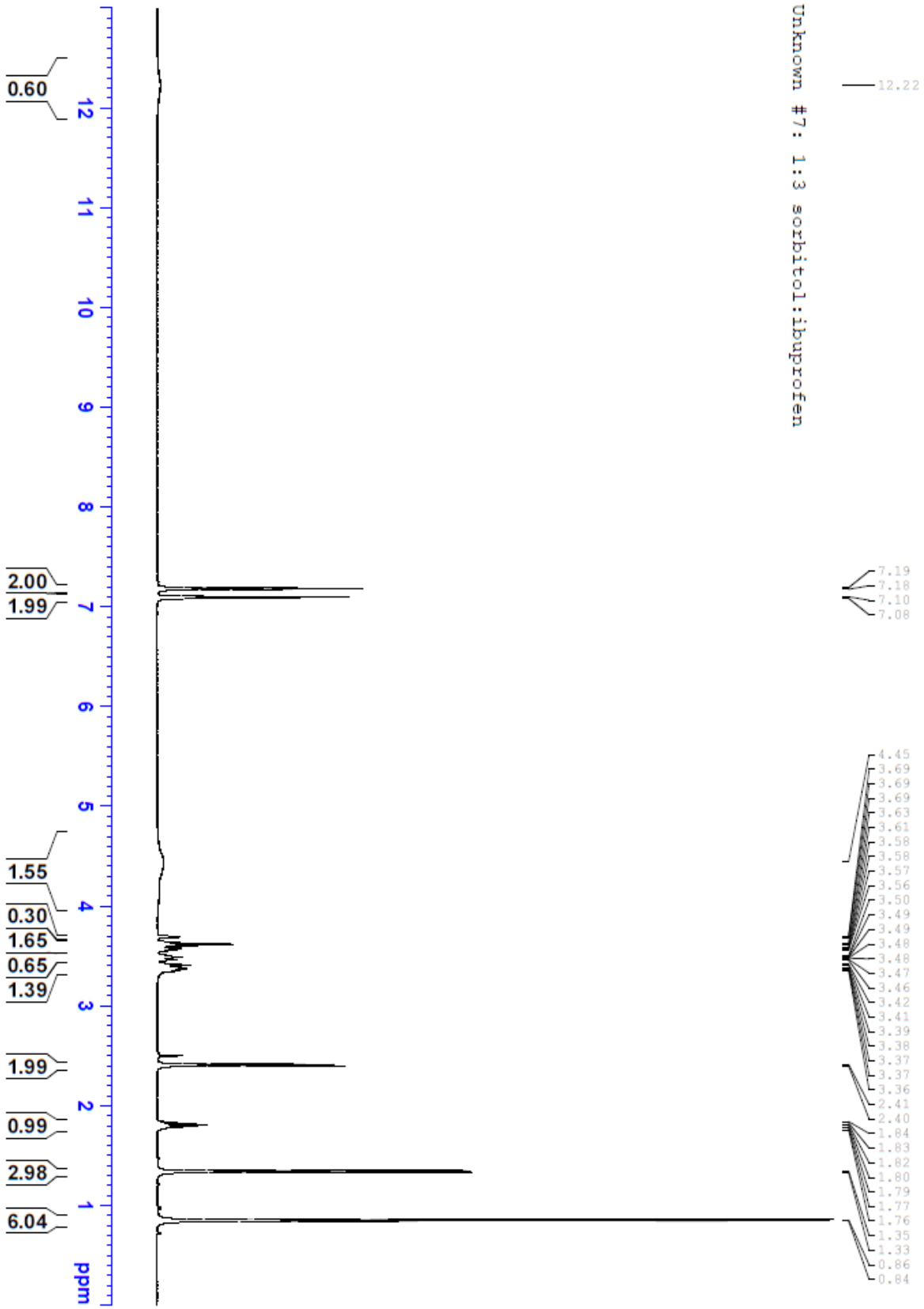

Unknown #5: 1:3 acetaminophen: ibuprofen
12.22
9.64
9.12
9.11
7.36
7.35
7.20
7.18
7.10
7.09
6.70
6.68
3.65
3.63
3.62
3.60
2.41
2.40
1.99
1.84
1.83
1.82
1.80
1.79
1.78
1.76
1.35
1.34
0.86
0.85
0.74
0.48
0.32
1.02
1.92
1.92
1.00
0.95
1.93
1.50
0.97
2.89
5.82
ppm

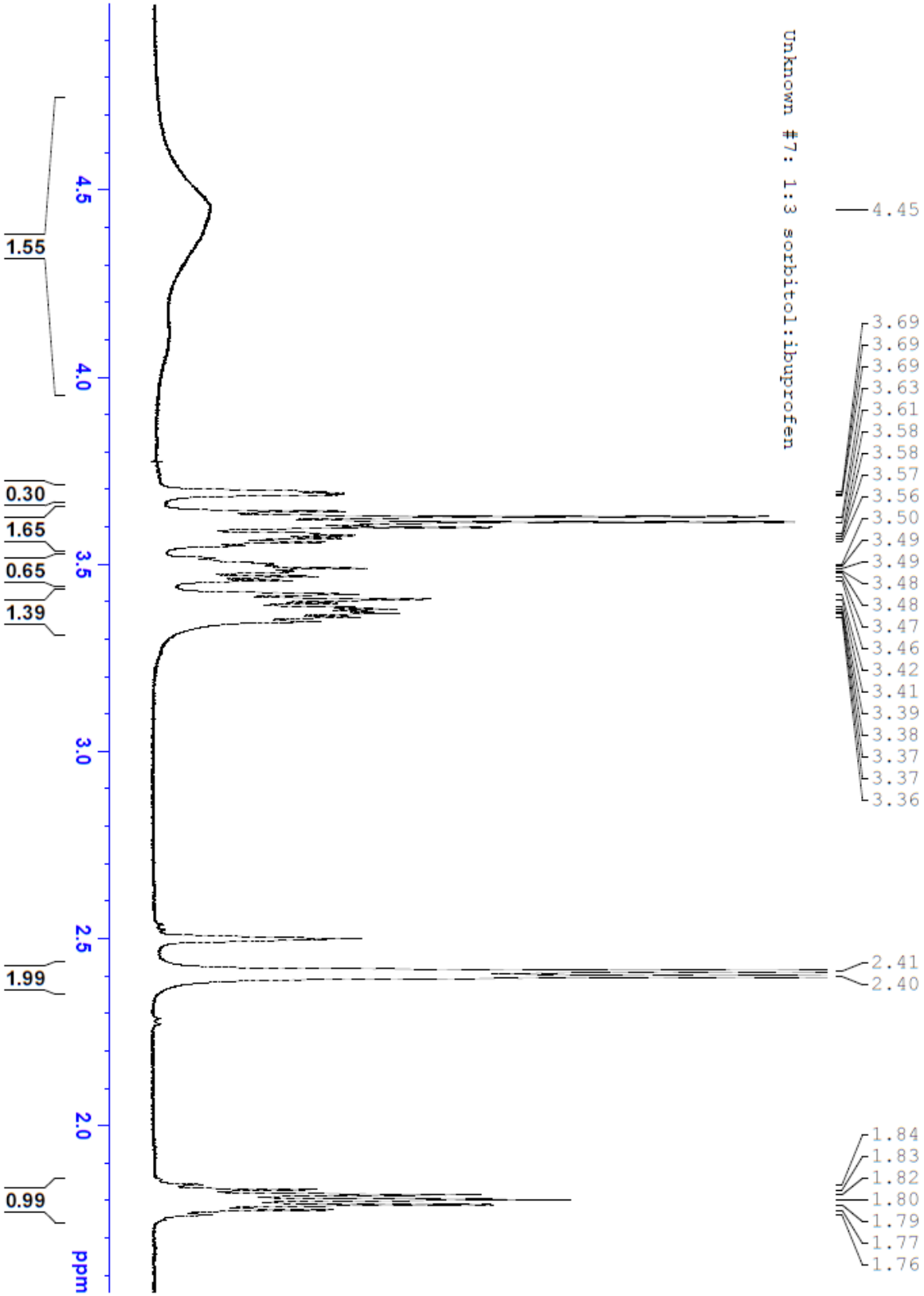

Unknown #5: 1:3 acetaminophen: ibuprofen
3.63
3.62
3.60
2.41
2.40
1.99
1.84
1.83
1.82
1.80
1.79
1.78
1.76
1.35
1.34
0.86
0.85
0.95
1.93
1.50
0.97
2.89
5.82
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
ppm

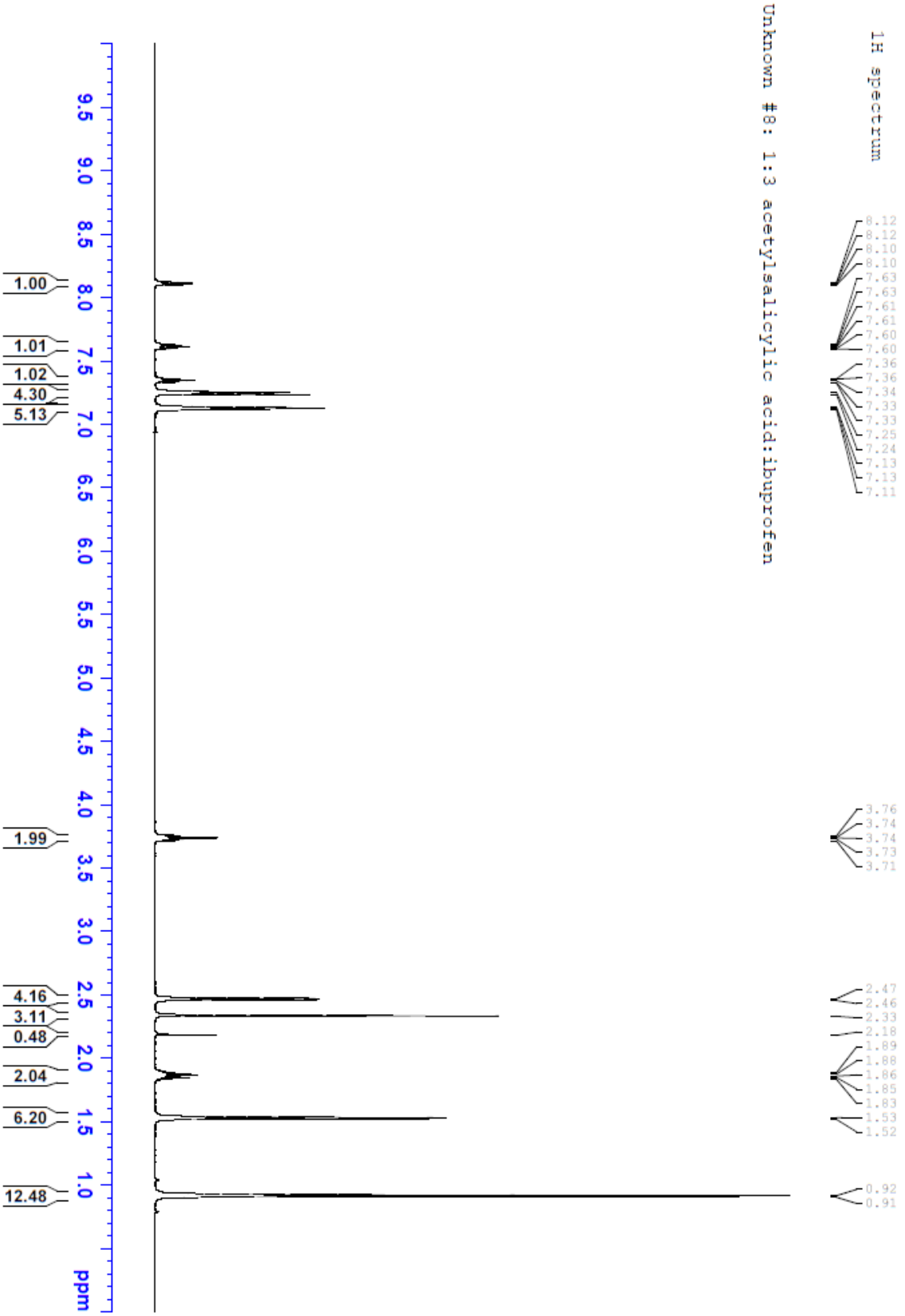

Unknown #5: 1:3 acetaminophen: ibuprofen
9.64
9.12
9.11
7.36
7.35
7.20
7.18
7.10
7.09
6.70
6.68
0.48
0.32
1.02
1.92
1.92
1.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
ppm


Unknown #6: 1:3 sorbitol:lidocaine
2.14
6.84
1.02
2.06
1.00
1.03
1.00
1.01
2.10
2.15
3.08
1.42
4.40
9.17
13.69
13.69
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
9.15
7.06
4.54
4.53
4.47
4.46
4.45
4.38
4.37
4.32
4.30
4.29
4.10
4.08
3.69
3.69
3.68
3.67
3.59
3.58
3.57
3.56
3.50
3.49
3.48
3.48
3.47
3.46
3.41
3.40
3.39
3.38
3.37
3.37
3.36
3.34
3.13
2.64
2.62
2.61
2.60
2.14
1.09
1.07
1.06


4.54
4.53
4.47
4.46
4.45
4.38
4.37
4.32
4.30
4.29
4.10
4.08
3.69
3.69
3.68
3.67
3.59
3.58
3.57
3.56
3.50
3.49
3.48
3.48
3.47
3.46
3.41
3.40
3.39
3.38
3.37
3.37
3.36
3.34
3.13
2.64
2.62
2.61
2.60
Unknown #6: 1:3 sorbitol:lidocaine
5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 ppm
1.02
2.06
1.00
1.03
1.00
1.01
2.10
2.15
3.08
1.42
4.40
9.17

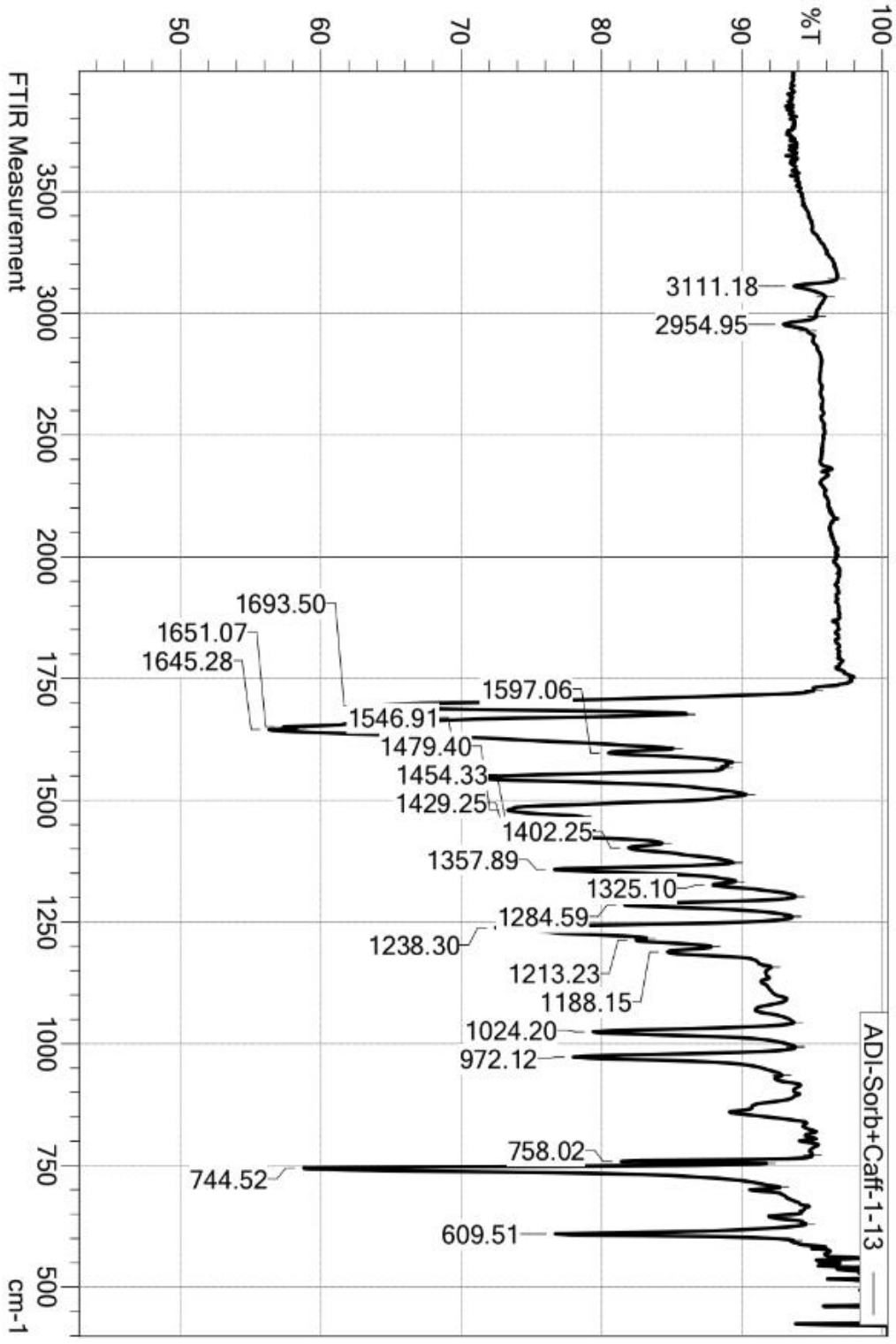

Unknown #7: 1:3 sorbitol:ibuprofen
0.60
2.00
1.99
1.55
0.30
1.65
0.65
1.39
1.99
0.99
2.98
6.04
ppm
12.22
7.19
7.18
7.10
7.08
4.45
3.69
3.69
3.69
3.63
3.61
3.58
3.58
3.57
3.56
3.50
3.49
3.49
3.48
3.48
3.47
3.46
3.42
3.41
3.39
3.38
3.37
3.37
3.36
2.41
2.40
1.84
1.83
1.82
1.80
1.79
1.77
1.76
1.35
1.33
0.86
0.84


Unknown #7: 1:3 sorbitol:ibuprofen
4.45
3.69
3.69
3.69
3.63
3.61
3.58
3.58
3.57
3.56
3.50
3.49
3.49
3.48
3.48
3.47
3.46
3.42
3.41
3.39
3.38
3.37
3.37
3.36
2.41
2.40
1.84
1.83
1.82
1.80
1.79
1.77
1.76
1.55
0.30
1.65
0.65
1.39
1.99
0.99
4.5
4.0
3.5
3.0
2.5
2.0
ppm

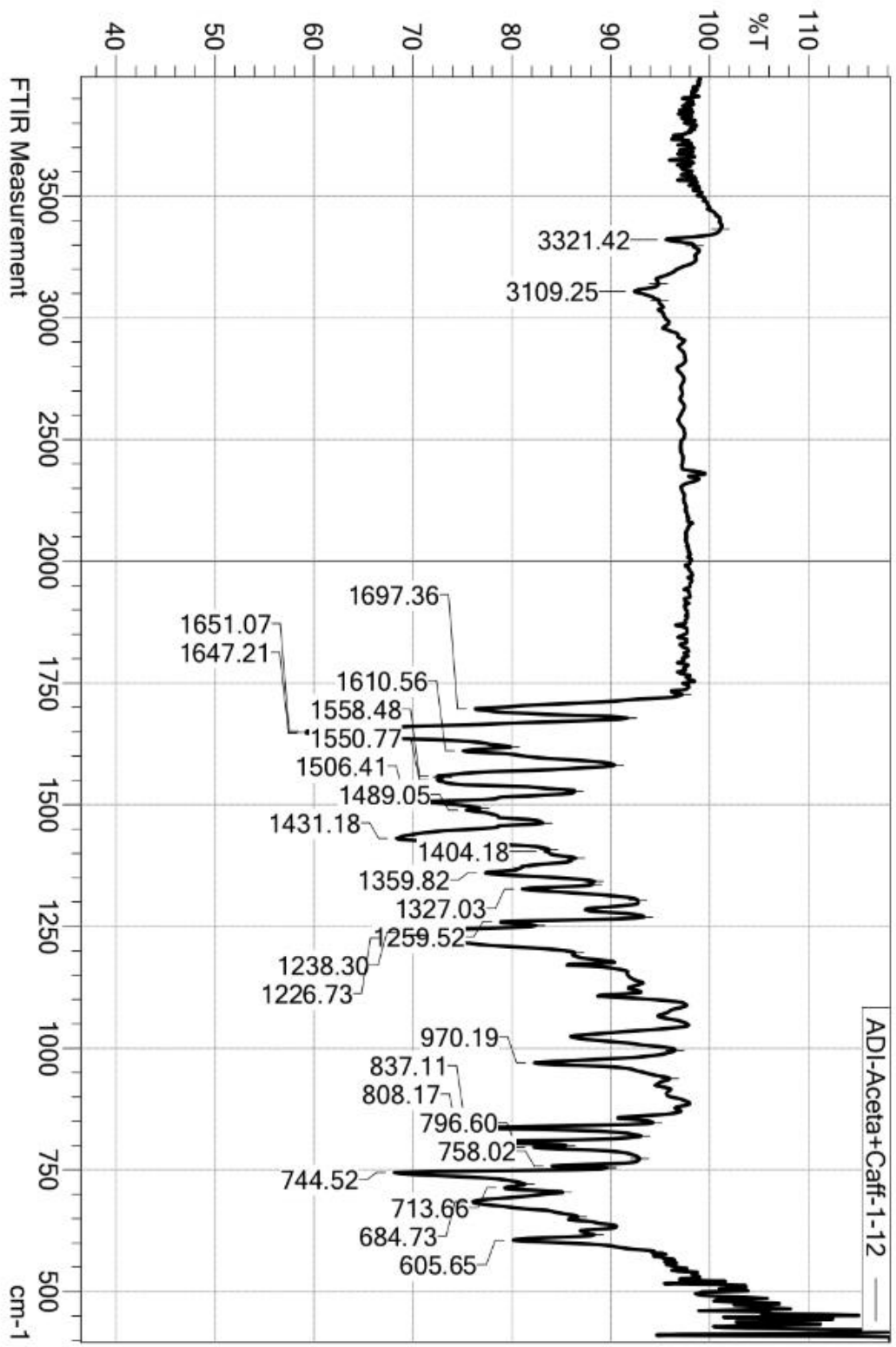

1H spectrum
Unknown #8: 1:3 acetylsalicylic acid:ibuprofen
8.12
8.12
8.10
8.10
7.63
7.63
7.61
7.61
7.60
7.60
7.36
7.36
7.34
7.33
7.33
7.25
7.24
7.13
7.13
7.11
3.76
3.74
3.74
3.73
3.71
2.47
2.46
2.33
2.18
1.89
1.88
1.86
1.85
1.83
1.53
1.52
0.92
0.91
1.00
1.01
1.02
4.30
5.13
1.99
4.16
3.11
0.48
2.04
6.20
12.48
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

VII. IR Spectra for Project #1

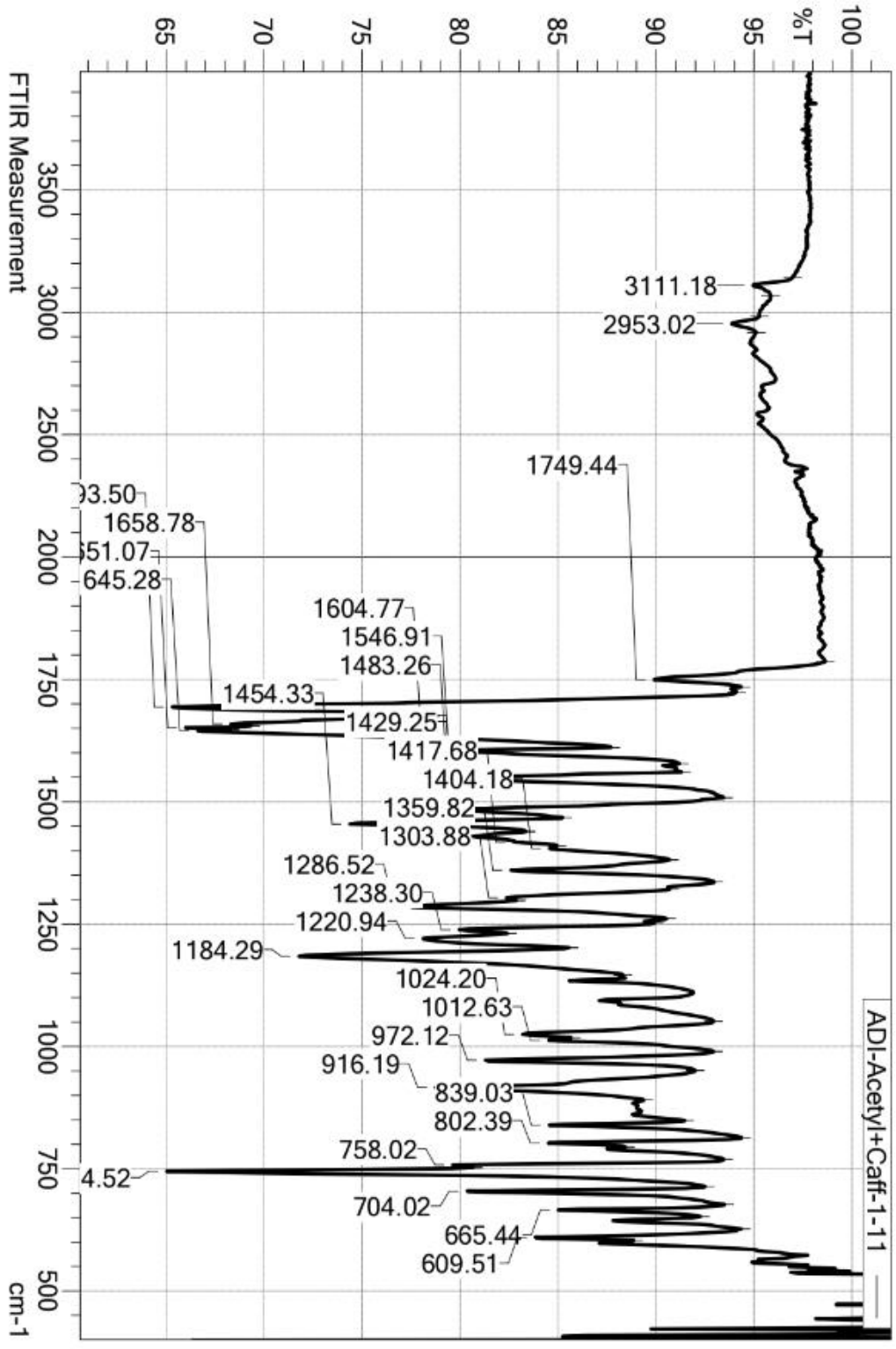
a. Sorbitol: Caffeine 1:1 (Unknown 1)



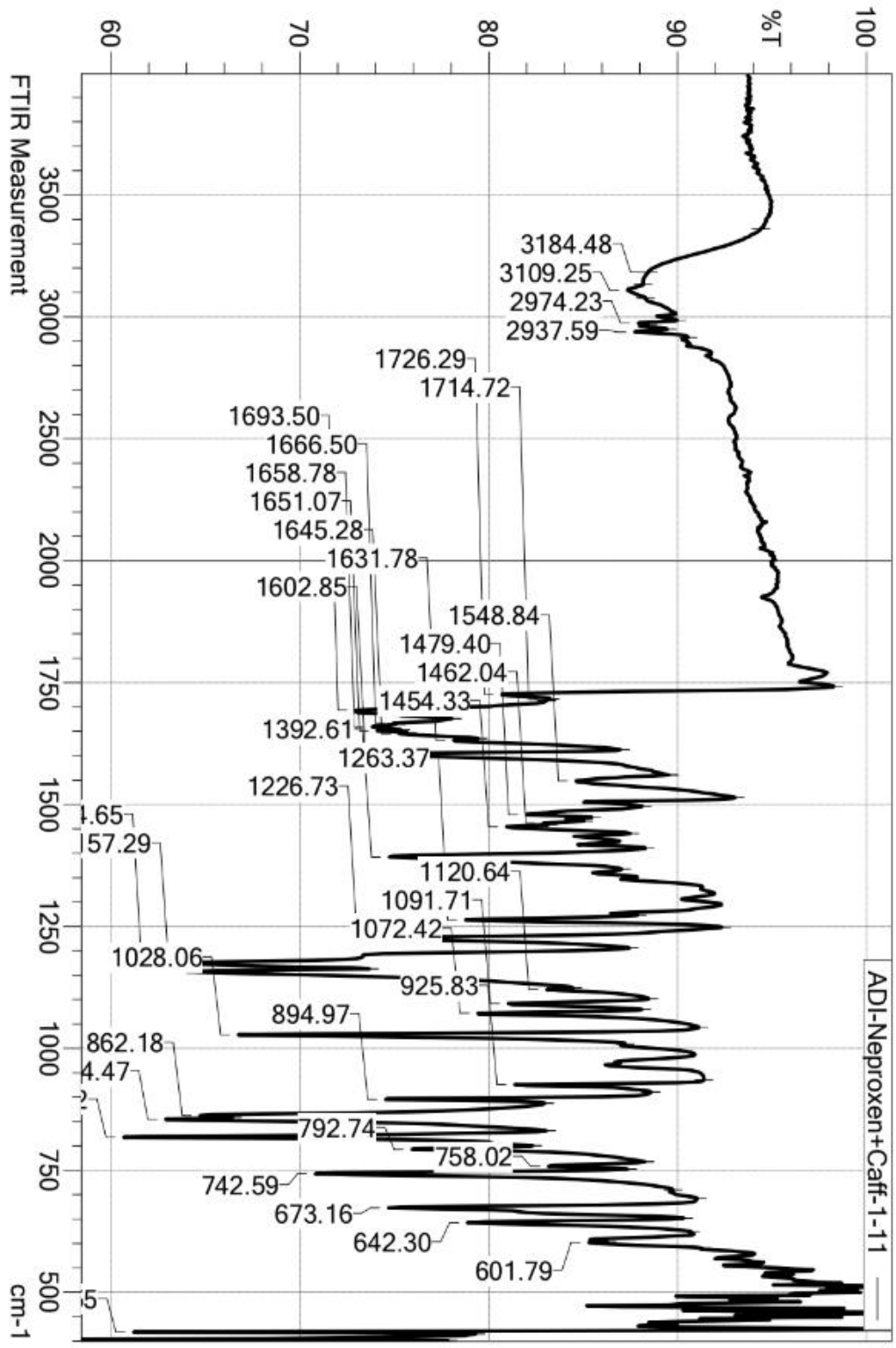
b. Acetaminophen: Caffeine, 1:1 (Unknown 2)



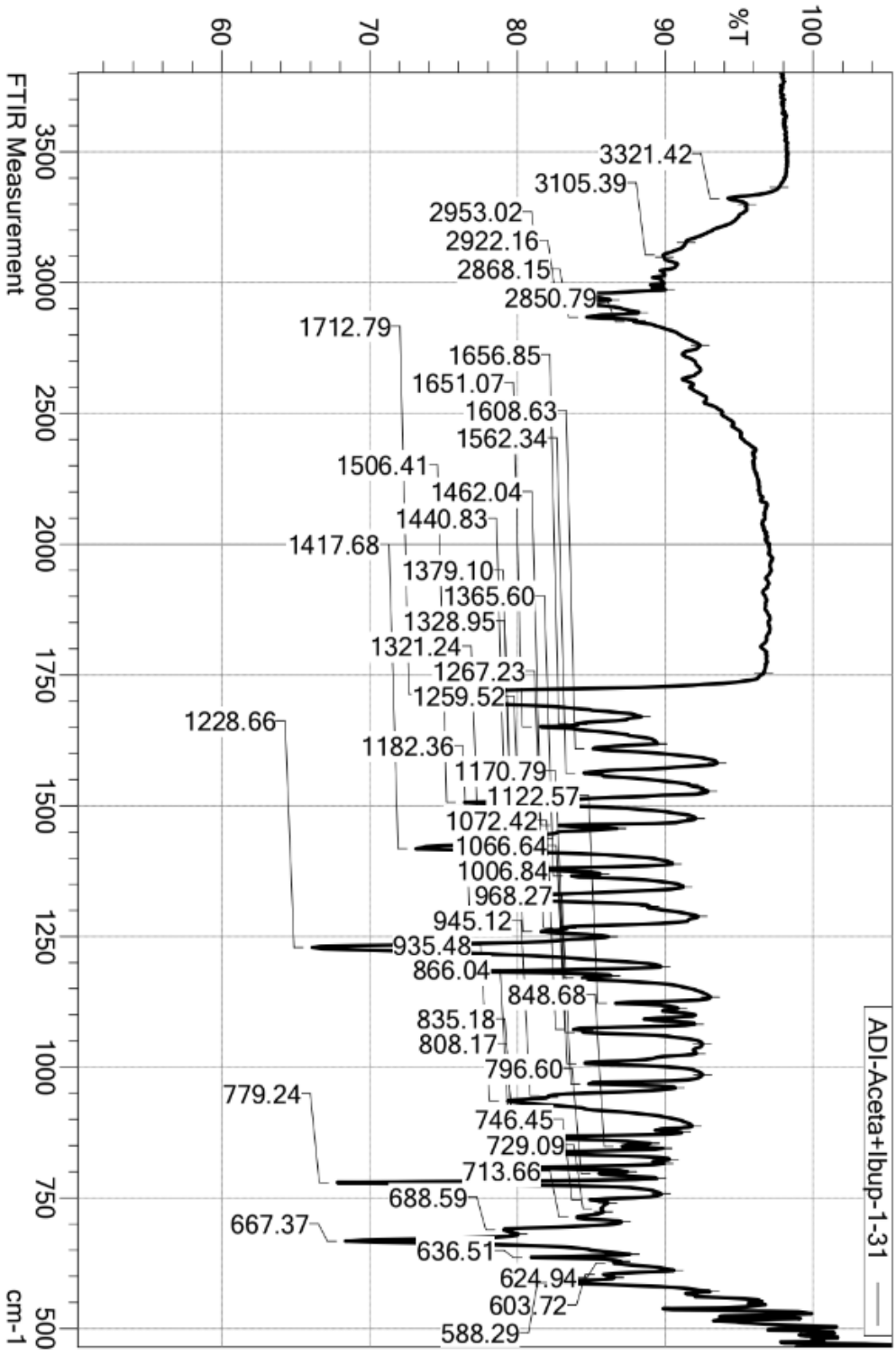
c. Acetylsalicylic Acid: Caffeine 1:1 (Unknown 3)



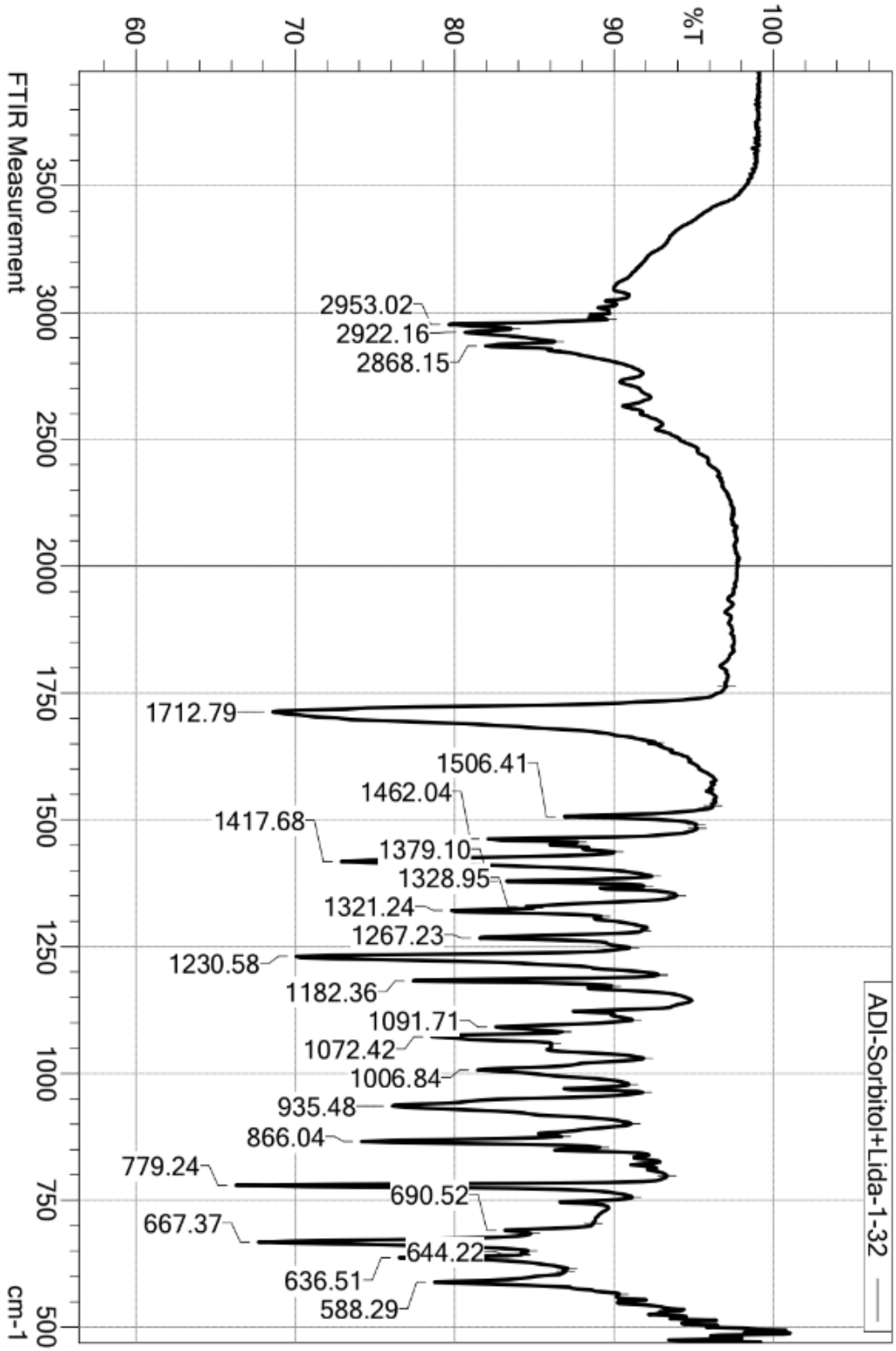
d. Naproxen: Caffeine 1:1 (Unknown 4)



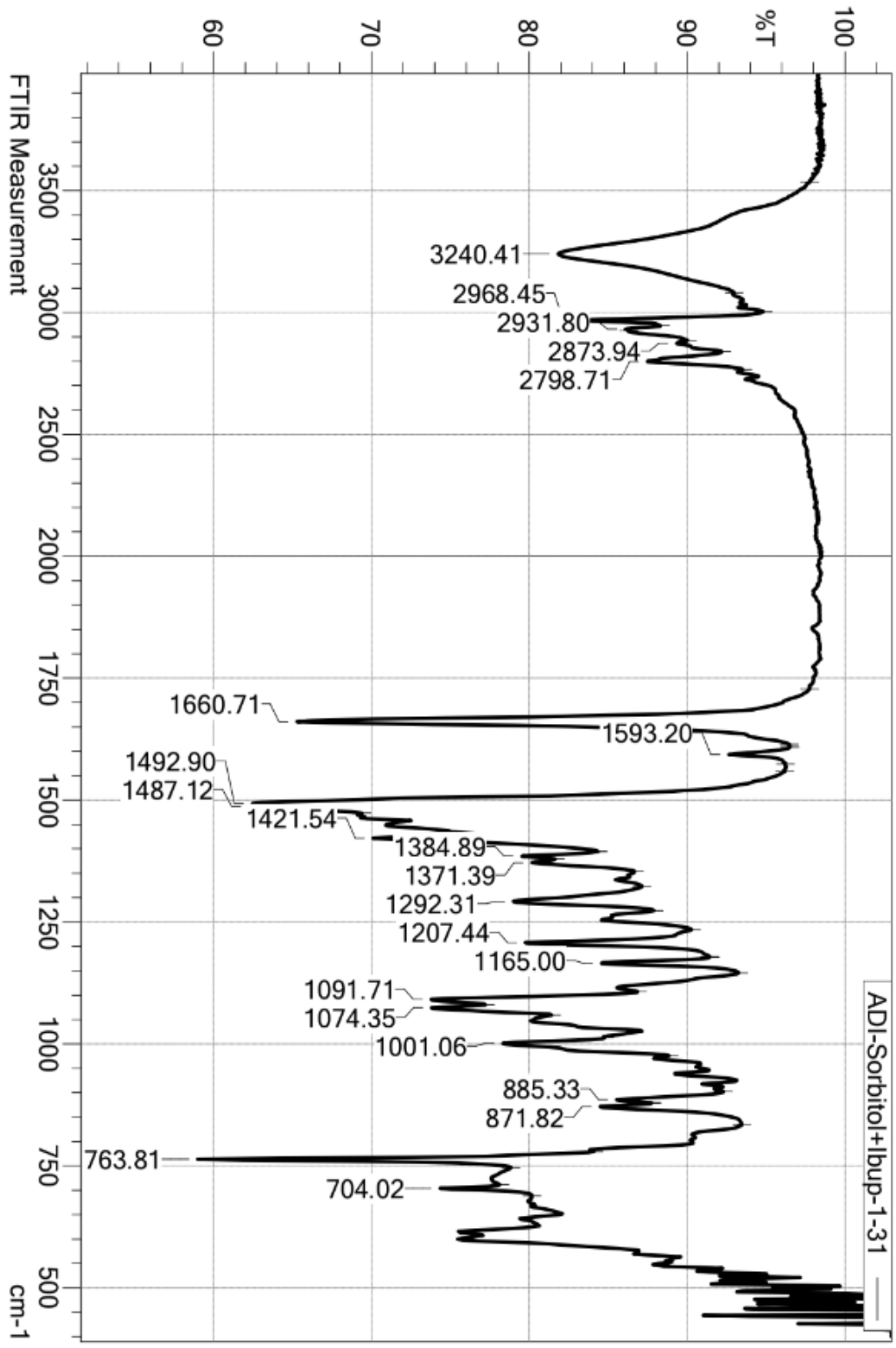
e. Acetaminophen: Ibuprofen 1:3 (Unknown 5)



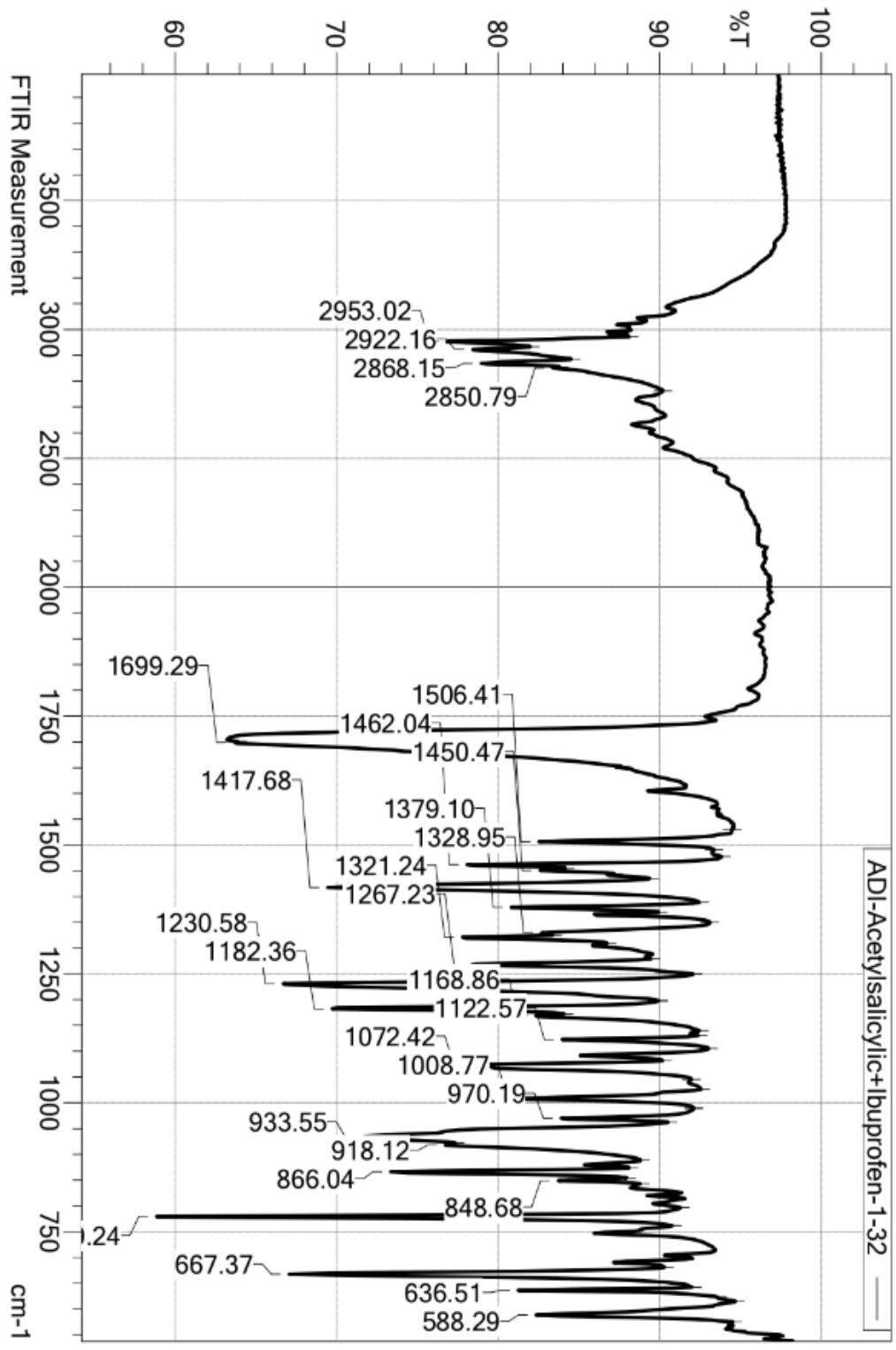
f. Sorbitol: Lidocaine 1:3 (Unknown 6)



g. Sorbitol: Ibuprofen 1:3 (Unknown 7)

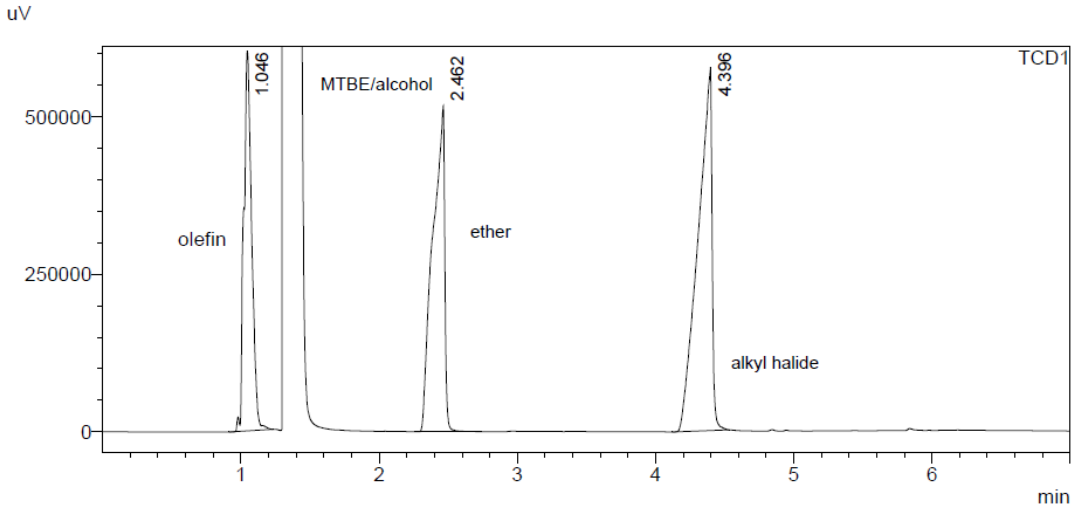


h. Acetylsalicylic Acid: Ibuprofen 1:3 (Unknown 8)



VIII. GC Spectra for Project #2

a. methoxide + 1-bromopentane

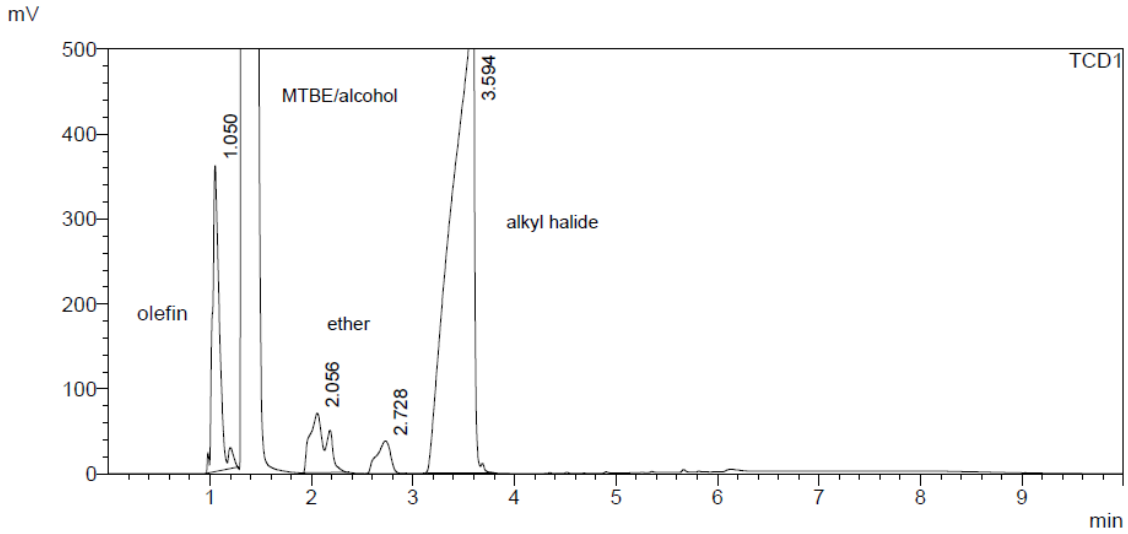


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.046	2389255	596445	25.131		M	
2	2.462	3082234	512698	32.420		M	
3	4.396	4035751	570621	42.449		M	
Total		9507240	1679764				

b. methoxide + 2-bromopentante

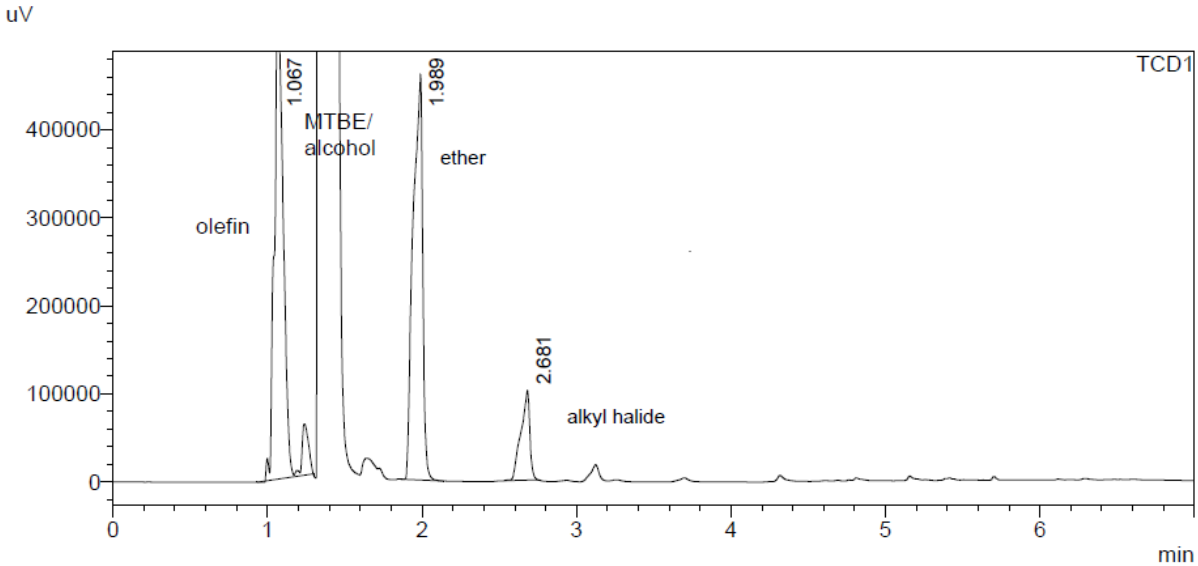


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.050	1715145	358552	15.104		M	
2	2.056	793976	69892	6.992		M	
3	2.728	358930	38318	3.161		M	
4	3.594	8487686	553125	74.744		M	
Total		11355738	1019887				

c. methoxide + 2-methyl-2-bromobutane

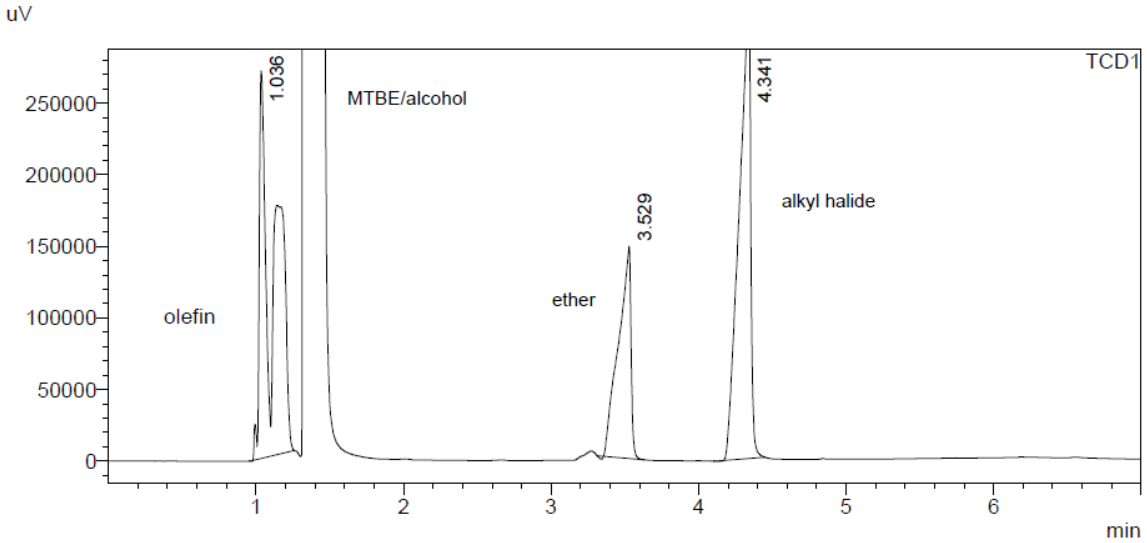


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.067	2342098	554566	49.505		M	
2	1.989	1980405	458242	41.860		M	
3	2.681	408514	101686	8.635		M	
Total		4731017	1114493				

d. ethoxide + 1-bromopentane

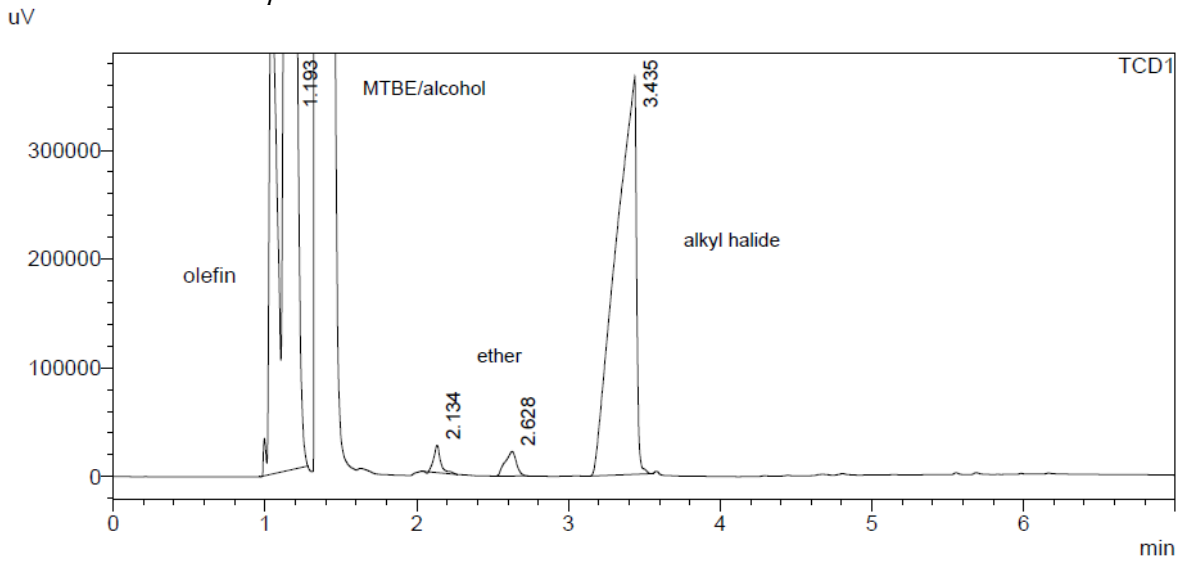


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.036	1776054	268664	40.323		M	
2	3.529	871994	145943	19.797		M	
3	4.341	1756571	333646	39.880		M	
Total		4404618	748253				

e. ethoxide + 2-bromopentante

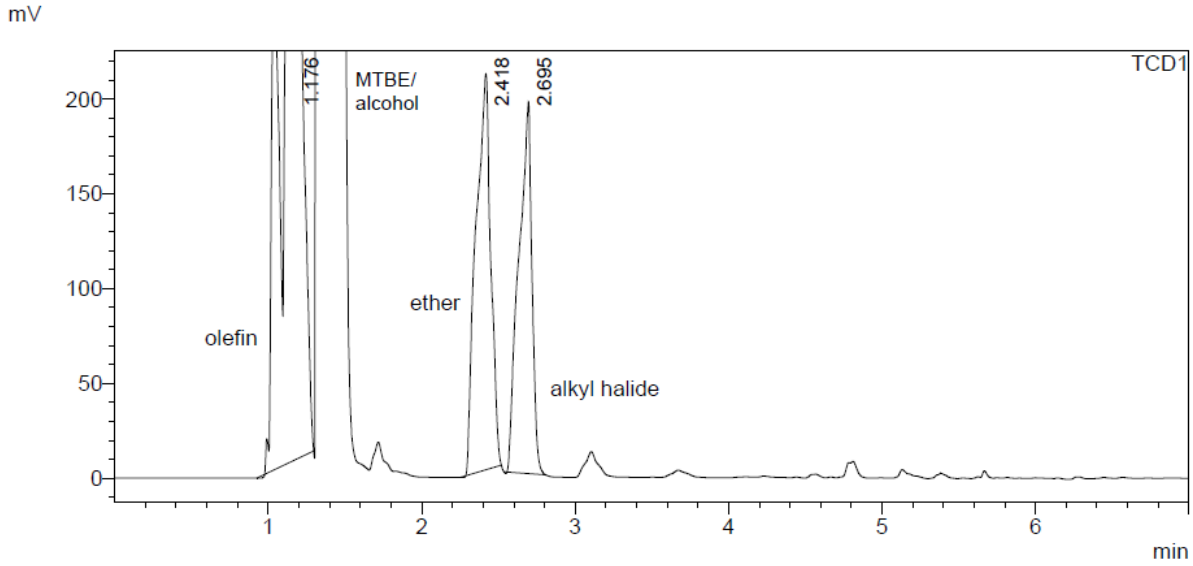


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.193	5782245	722411	61.480		M	
2	2.134	79587	24745	0.846		M	
3	2.628	116883	22453	1.243		M	
4	3.435	3426416	364556	36.431		M	
Total		9405131	1134165				

f. ethoxide + 2-methyl-2-bromobutane

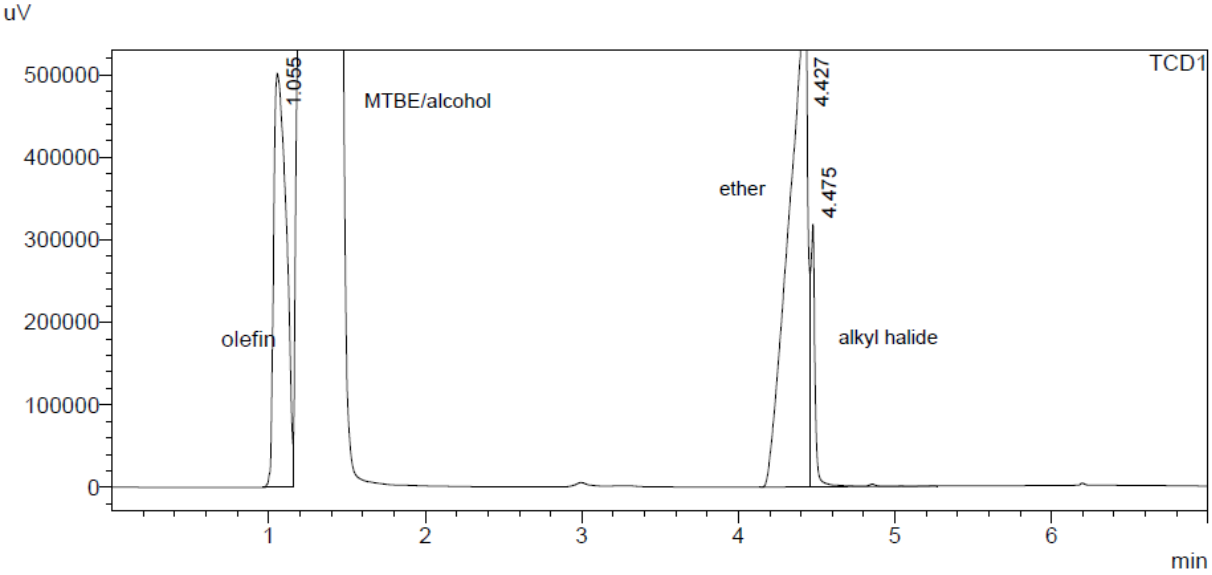


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.176	3671874	364294	58.850		M	
2	2.418	1398690	207494	22.417		M	
3	2.695	1168796	195785	18.733		M	
Total		6239360	767573				

g. isopropoxide + 1-bromopentane

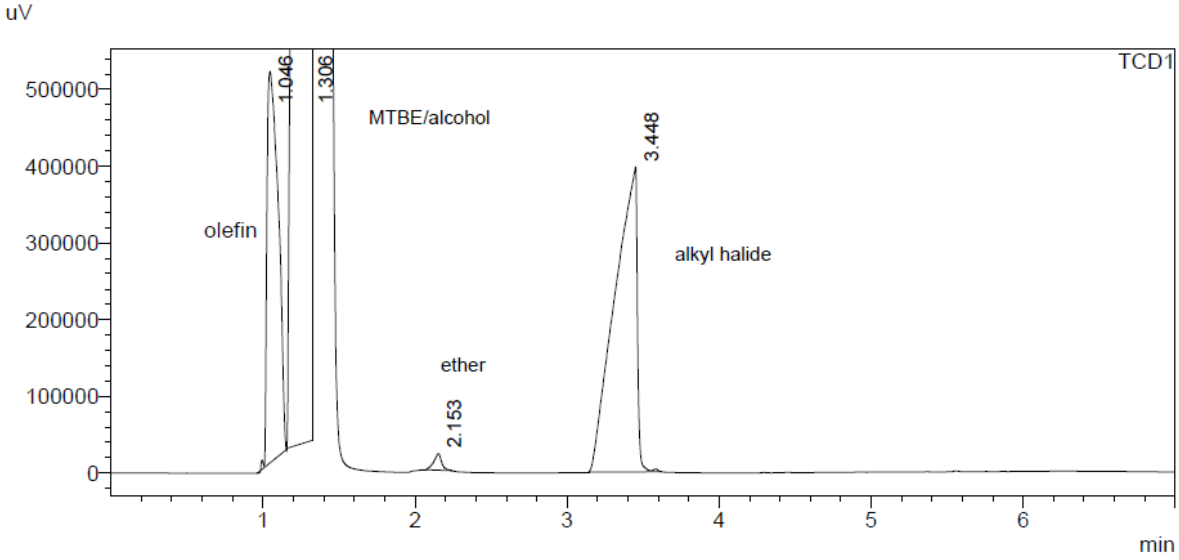


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.055	2794856	500819	33.123			
2	4.427	5004320	638720	59.309			
3	4.475	638528	311193	7.568		SV	
Total		8437704	1450732				

h. isopropoxide + 2-bromopentane

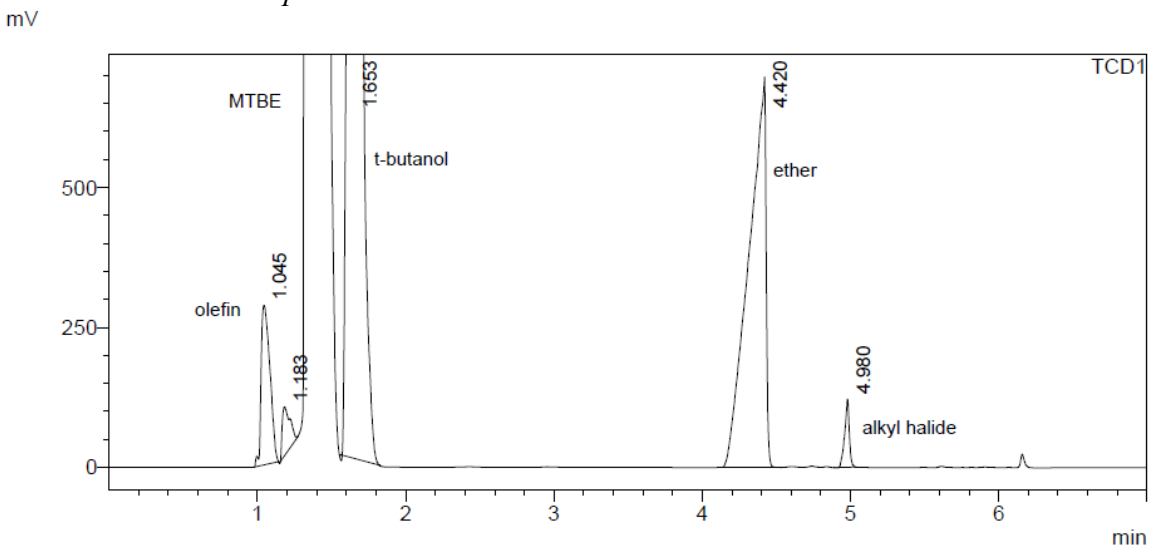


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.046	2618776	508962	16.294		M	
2	1.306	9320664	1616432	57.992		M	
3	2.153	77459	21433	0.482		M	
4	3.448	4055291	394871	25.232		M	
Total		16072190	2541698				

i. t-butoxide + 1-bromopentane

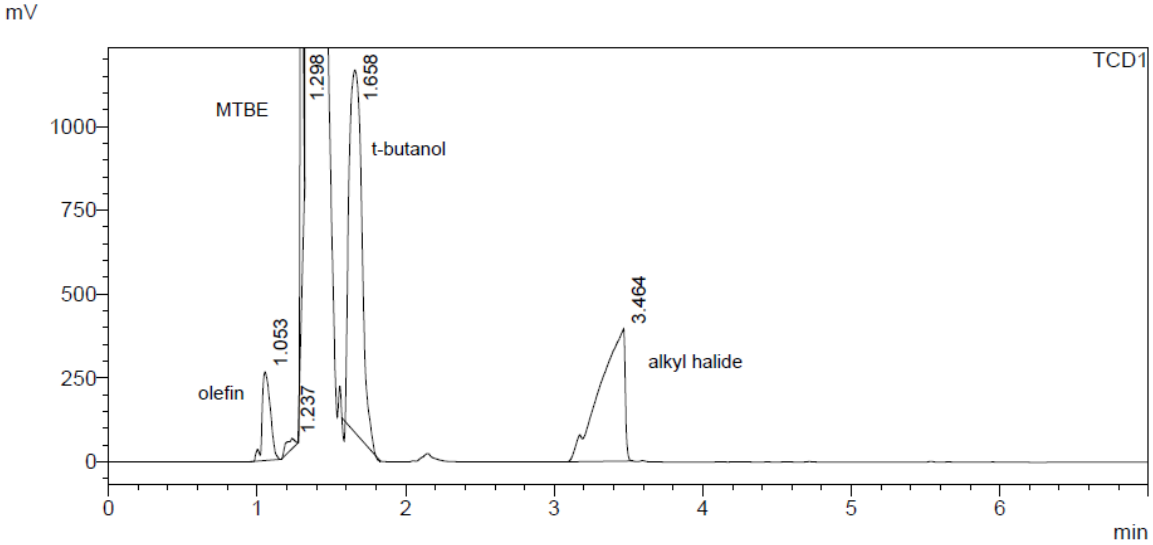


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.045	1177036	284589	6.385		M	
2	1.183	310870	86248	1.686		M	
3	1.653	11091949	1557367	60.171		M	
4	4.420	5593629	685986	30.344		M	
5	4.980	260583	116729	1.414		M	
Total		18434068	2730920				

j. t-butoxide + 2-bromopentane

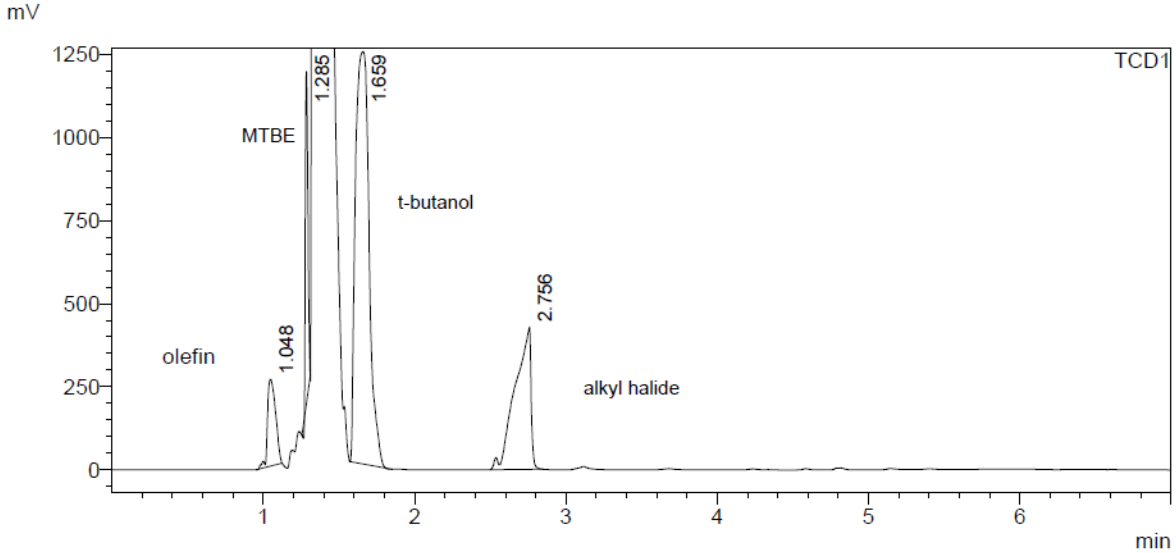


<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.053	1015436	263394	7.199		M	
2	1.237	141467	28164	1.003		M	
3	1.298	1927700	1540298	13.667		M	
4	1.658	6451355	1078892	45.737		M	
5	3.464	4569266	392841	32.394		M	
Total		14105225	3303589				

k. t-butoxide + 2-methyl-2-bromobutane



<Peak Table>

TCD1

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	1.048	969284	260384	7.436		M	
2	1.285	1162611	970524	8.919		M	
3	1.659	7784385	1241115	59.718		M	
4	2.756	3118866	423768	23.927		M	
Total		13035146	2895791				