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Title

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Permalink

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Journal

Nature Chemistry, 8(7)

ISSN

1755-4330

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Publication Date

2016-07-01

DOI

10.1038/nchem.2506

Peer reviewed

Imaging Single-Molecule Reaction Intermediates Stabilized by Surface Dissipation and Entropy

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Chemical transformations at the interface between solid/liquid or solid/gaseous phases of matter lie at the heart of key industrial-scale manufacturing processes. A comprehensive study of the molecular energetics and conformational dynamics underlying these transformations is often limited to ensemble averaging analytical techniques. Here we report the detailed investigation of a surface-catalyzed cross-coupling and sequential cyclization cascade of 1,2-bis(2-ethynyl phenyl)ethyne on Ag(100). Using noncontact atomic force microscopy (nc-AFM) we imaged the single-bond-resolved chemical structure of transient metastable intermediates. Theoretical simulations indicate that the kinetic stabilization of experimentally observable intermediates is determined not only by the potential energy landscape, but also by selective energy dissipation to the substrate and entropic changes associated with key transformations along the reaction pathway. The microscopic insights gained here pave the way for rational design and control of complex organic reactions at the surface of heterogeneous catalysts.

Understanding the microscopic mechanisms of surface-catalyzed organic reactions at solid/liquid and solid/gas interfaces is a grand challenge for modern heterogeneous catalysis and its application to industrial-scale chemical processes. Investigation of the underlying reaction mechanisms that transform crude feedstock into complex value-added chemicals at the surface of a heterogeneous catalyst bed is often hampered by competing pathways that lead to numerous intermediates and undesired side-products. Advanced tools such as time-resolved spectroscopy and mass spectrometry provide valuable insight into the product distribution under a variety of reaction conditions. Precise structural identification of transient reaction intermediates and products, however, is limited by their respective concentration in the sample stream, as well as the ability to separate and isolate potentially highly reactive intermediates using chromatographic tools. Crucial transient intermediates that remain adsorbed to the active catalyst during the reaction often escape identification via traditional ensemble-averaging spectroscopic techniques.

Some of these challenges can be overcome by high resolution scanning probe techniques which have been used to study chemical transformations at the single-molecule level.¹⁻¹¹ Unambiguous identification of complex organic molecules by scanning tunneling microscopy (STM), however, generally still requires support from theoretical calculations.^{2,3,7-9} nc-AFM measurements with functionalized tips,^{12,13} on the other hand, allow direct imaging of chemical structure and covalent bonding within organic molecules¹⁴⁻¹⁶ and have even been suggested as a tool to image non-covalent intermolecular contacts.¹⁷⁻²¹ In recent work,¹ nc-AFM-based chemical identification of reaction products has helped to uncover reaction pathways which were subsequently confirmed by *ab initio* calculations. The temporal resolution for these single-molecule imaging techniques, however, remains more than ten orders of magnitude lower than the typical timescale of a chemical reaction. Real-time observations of chemical reaction steps and their associated microscopic behavior are thus beyond the scope of current nc-AFM measurements.

Here we show that it is possible to directly determine complex surface-reaction mechanisms through real-space imaging of reaction intermediates at the single-molecule/single-bond level. We

have successfully stabilized a select series of transient intermediates along a multistep reaction pathway by surface-assisted thermal quenching and entropic effects, and imaged the molecular structures using nc-AFM. This has allowed us to resolve the chemical structure of individual intermediates observed along a reaction pathway involving the bimolecular coupling and intramolecular cyclization cascade of enediyne molecules on a Ag(100) surface. Statistical analysis of large-area scans recorded at sequential time-points during the reaction reveals a distinct correlation of the distribution of reactants, intermediates, and products with a postulated reaction mechanism. Theoretical calculations show that the underlying reaction kinetics (and thus the stabilization of particular intermediate species along the reaction coordinate) are strongly influenced by thermal energy dissipation to the underlying metal substrate, as well as by changes in entropy. These new mechanistic insights into complex reaction cascades involving cross-coupling and cyclization reactions should help in future design/optimization of heterogeneous catalytic systems used in industrial manufacturing processes, as well as the development of novel synthetic tools for carbon-based nanotechnology.^{6,22-25}

Results

The enediyne precursor 1,2-bis(2-ethynyl phenyl)ethyne (**1**) (Fig. 1a) was sublimed in ultrahigh vacuum (UHV) onto a Ag(100) surface held at 290 K. Thermal annealing of the adsorbed molecules on Ag(100) induces a series of chemical reactions leading to monomeric, dimeric and oligomeric structures resulting from a sequence of intermolecular cross-coupling and cyclization cascades (see Fig. 1 and Supplementary Figs. 5-6).^{1,6} To obtain a better understanding of the different reaction pathways observed on the substrate surface, we determined the precise chemical structures of the adsorbates through high-resolution nc-AFM imaging. All cryogenic nc-AFM measurements were performed at 4 K with CO-functionalized tips^{12,13} to resolve the structure of the adsorbates before and after subjecting the sample to a series of annealing steps (Fig. 1f) over a range of temperatures between 290 K and 460 K.

Herein we will focus on the major reaction pathway resulting from the dimerization of **1** on the surface. Strikingly, the observed dimers (Figs. 1b-d) exhibit varying degrees of cyclization (i.e. the number of annulated rings). Uncyclized dimers (i.e. dimers consisting of two uncyclized subunits, e.g. **2b**), half-cyclized dimers (i.e. dimers consisting of one cyclized and one uncyclized monomer subunit, e.g. **3c**), and fully cyclized dimers (i.e. dimers consisting of two cyclized monomer subunits, e.g. **4c**) were found to coexist on the surface. The relative ratio of **2b/3c/4c** gradually shifts towards molecules featuring higher degrees of cyclization, i.e. from uncyclized towards half-cyclized and then to fully cyclized dimers, as temperature is increased with each annealing step (Figs. 1e,f). This observation suggests that partially cyclized structures are transient intermediates along a multistep reaction sequence that eventually leads to fully cyclized dimers (such as **4c**). The total number of molecules within the reaction pathway does not significantly decrease suggesting that desorption, trapping of intermediates at step edges, or side reactions (such as reaction with hydrogen from the residual gas) do not play an important role.

The single-bond-resolved images of Figs. 1a-d provide insight into the mechanistic steps that lead from **1** to the cumulene **4c**. During the first annealing step an intermolecular C–C bond is formed between the terminal alkynes of two 1,2-bis(2-ethynyl phenyl)ethynes (**1**) yielding the eneyne intermediate **2b** (the building block on the left adopts a C_{2h} symmetry while the right half adopts a C_{2v} symmetry (Fig 1b)). A sequence of thermally induced C^1-C^6 , and C^1-C^5 cyclizations of the 1,2-bis(2-ethynyl phenyl)ethyne fragment on the left (C_{2h}), followed by a stepwise hydrogen transfer, leads to the benzo[*a*]fluorene **3c**. A second thermally activated cyclization/hydrogen transfer sequence converts the remaining 1,2-bis(2-ethynyl phenyl)ethyne fragment on the right into a benzo[*b*]fluorene to yield the fully cyclized product **4c**, which features a buta-1,2,3-triene linker.

Intramolecular bonding in the structure of both the partially and fully cyclized intermediates/products can be unambiguously determined despite some distortion of the bond lengths in the nc-AFM images arising from the imaging technique^{6,15,20,26–30} (our structure assignments are consistent with simulated AFM images using the scheme by Hapala et al.,²⁰ see

Supplementary Information Fig. 1). The experimental images provide additional insight into the three-dimensional conformation adopted by the molecules on the surface. For example, the nc-AFM image of **3c** shows that one of the phenyl rings (lower right) in the 1,2-bis(2-ethynyl phenyl)ethyne fragment is twisted out of the plane of the surface. Rotation of this ring around the triple bond linking two phenyl-rings points the terminal alkyne group towards the surface (the terminal alkyne is not resolved in the image) while the opposite edge of the phenyl ring is protruding above the plane of the molecule as shown by the brighter contrast in the nc-AFM image.

Further insight into this reaction sequence is obtained from *ab initio* calculations. We first calculated the reaction energy landscape, i.e. the energies for all transient intermediates along the reaction pathway as well as for the corresponding transition states using density functional-based tight binding (DFTB) theory (Fig. 2). The initial activation barrier (i.e. the energy of the first transition state) for the intermolecular coupling of two 1,2-bis(2-ethynyl phenyl)ethynes (**1**) is 1.38 eV (31.82 kcal mol⁻¹)³¹ and represents the rate determining step. The energies of all subsequent intermediates (i.e., the local minima along the potential energy landscape, marked by filled circles), as well as all transition states (i.e., the local maxima, marked by empty circles), are lower than the energy of the first transition state. Our calculations suggest the existence of seven intermediate states, whereas only two (**2b**, **3c**) were experimentally observed. In order to understand why only two of the seven possible intermediates are observed experimentally, we have solved the temperature-dependent kinetic rate equations for the reaction energy landscape depicted in Fig. 2.³² We find that our data can only be explained by going beyond simple adiabatic and instant thermalization models and by including both selective surface dissipation as well as changes in molecular entropy, as described below.

An adiabatic model of the reaction sequence implies that once the first rate-determining transition state is reached the molecule has sufficient energy to overcome all subsequent reaction barriers along the path to the final product **4c**. The solution of the kinetic rate equations under these conditions (Fig. 3a) is determined by only the first reaction barrier (see Supplementary Information

for additional details). This simple model, however, is incompatible with the experimental observation of metastable intermediates **2b** and **3c** which are not among the predicted chemical species shown in Fig. 3a. A model involving instant thermalization (an approximation commonly used in heterogeneous catalysis^{33–36}) also cannot account for the selectivity of our experimental observations. Instant thermalization implies that energy transfer from the molecule to the surface (which acts as a thermal reservoir) is faster than the subsequent reaction step, thus preferably stabilizing intermediates that are flanked by high activation barriers. However, our *ab initio* calculations (Fig. 2) show that the reaction steps following the experimentally observed intermediates feature comparatively low activation barriers ($\Delta H^\ddagger = 0.7$ eV for **2b** and $\Delta H^\ddagger = 0.5$ eV for **3c**) and so these intermediates are less likely to be stabilized by an instant thermalization model than the intermediates **2a** and **3a**, which have much higher barriers (but which could not be observed experimentally). This is reflected in the solution of our kinetic rate equations under the assumption of instant thermalization (Fig. 3b) which incorrectly predicts stabilization of intermediates **2a** and **3a**, and which also suggests an unreasonably high formation temperature for product **4c**.

Since neither an adiabatic nor an instant thermalization model can explain the experimentally observed reaction kinetics, we are led to consider the dissipation of chemical energy that is released during each reaction step. We have calculated this dissipative energy transfer by using molecular dynamics (MD) simulations within the DFTB framework that allow us to follow the flow of energy from the reactants to the substrate (see computational method section in the Supplementary Information). Fig. 4 shows the resulting atomic kinetic energies (averaged over 1 ps) for the experimentally observed intermediates as well as for the Ag(100) substrate atoms as a function of position (color map). The spatially integrated average kinetic energy for the entire molecule (not including the substrate kinetic energy) and for the entire substrate slab (not including the molecule kinetic energy) are plotted separately beneath each image for a 1 ps period after an energy plateau has been reached (the kinetic energy of the substrate is zero before the reaction). The integrated kinetic energy of the substrate provides a measure for the efficiency of heat transfer

from the molecule to the surface, and arises primarily from phonon coupling between the vibrational modes of the molecule and the substrate.

Energy dissipation to the Ag(100) substrate is an efficient process and proceeds at a rate comparable to the chemical transformations along the reaction pathway (see Supplementary Information Fig. 3). The redistribution of energy from the molecule to the substrate effectively reduces the energy available to overcome subsequent activation barriers. Our calculations show that the efficiency of dissipative processes varies greatly for each reaction step (e.g., it is high for the intermediate **2b** but relatively low for the intermediate **3c**). The effects of selective dissipation are added to our kinetic rate equations through an effective temperature that depends on how much of the released chemical energy remains in the molecule as kinetic energy after dissipative energy transfer to the substrate (as determined by the MD/DFTB calculations). The conversion factor between the remaining molecular internal (kinetic and potential) energy and the molecular effective temperature increase is the molecular heat capacity: $T_{\text{eff}} = T_0 + \Delta E_{\text{molecule}}/C_{V,\text{molecule}}$ (here T_0 is the substrate temperature before the reaction step, $\Delta E_{\text{molecule}}$ is the internal energy remaining in the molecule after dissipation, and $C_{V,\text{molecule}}$ is the molecular heat capacity). In order to solve our kinetic rate equations in the presence of selective dissipation, we utilize an average value of $C_{V,\text{molecule}} = 80 k_B$ obtained by considering the different vibrational degrees of freedom of molecules along the reaction pathway (see Supplementary Information section 9). The addition of selective dissipation to our rate equations in this way significantly lowers the expected onset temperature for the formation of the fully cyclized product **4c** as seen in Fig. 3c, but also suggests a prevalence of the intermediate **3b**, which is not observed experimentally. Since the experimentally observed intermediates **2b** and **3c** are both associated with relatively small activation barriers ($\Delta H^\ddagger = 0.7$ eV for **2b** and $\Delta H^\ddagger = 0.5$ eV for **3c**), even the inclusion of selective dissipation is insufficient to explain why they are the only experimentally observed intermediates along the reaction pathway.

To solve this puzzle, we must go one step further and include the effect of changes in entropy on the reaction kinetics.^{37–40} Classical transition state theory provides a framework for including such

free energy considerations through the Eyring equation:³⁹ $k = \frac{k_B T}{h} e^{\frac{\Delta S^\ddagger}{k_B}} e^{-\frac{\Delta H^\ddagger}{k_B T}}$, where k is the chemical reaction rate constant, k_B is the Boltzmann constant, h is Planck's constant, T is temperature, ΔS^\ddagger is the activation entropy, and ΔH^\ddagger is the activation enthalpy for the reaction step. We implemented this by estimating the activation entropies for the adsorbate intermediates on the Ag(100) substrate within a DFTB framework. This was performed by including vibrational as well as rotational and translational contributions associated with motion of the adsorbates on the surface. The intermediate species **2b** and **3c** are free to undergo rotational and translational motion, but all other intermediates feature carbon-centered radicals that strongly interact with the surface and thus suppress their roto-translational degrees of freedom.

Using a modified Sackur-Tetrode equation⁴¹⁻⁴³ (details in section 6 in the Supplementary Information) and taking into account the degrees of freedom associated with the respective transition states leads to a significant reduction in the activation entropies for the intermediates **2b** and **3c** up to $\Delta S^\ddagger = -2$ meV/K at 573 K (ΔS is not strongly temperature dependent). This large negative activation entropy, which is not present for the other intermediate species, substantially reduces the reaction rates for **2b** and **3c**. As shown in Fig.3d, inclusion of both selective dissipation and entropic effects in our rate equations explains the experimentally observed selective stabilization of transient intermediates **2b** and **3c**. The predicted transformation temperatures depicted in Fig. 3d, however, are higher than the experimentally observed temperatures (Fig. 1e) by about 100 K. This discrepancy is likely caused by a combination of experimental uncertainty in accurately determining the sample surface temperature (± 25 K at the highest measured temperatures) and theoretical uncertainty in determining the transition state reaction barriers and molecular entropy (typical errors in the estimation of the barriers on the order of 0.1 eV will translate to a change in transformation temperatures of ± 70 K; see Supplementary Information for details).

Discussion and conclusion

A key insight gained in this study is the significance of the fact that the experimentally observed structures **2b** and **3c** are the only intermediates that do not exhibit carbon-centered radicals (the reactivity of radicals at surfaces can greatly differ from radicals in solution^{1,6,44}). Importantly, the transition states for intermediates **2b** and **3c** exhibit partial radical character and so have restricted roto-translational degrees of freedom, thus contributing further to the large negative activation entropies for these two particular intermediates. These entropic considerations, along with the effects of selective dissipation, are enough to outweigh the considerably larger activation enthalpies of the other intermediates in order to stabilize species **2b** and **3c** along the pathway from **1** to **4c**.

In conclusion, we have used single-bond-resolved nc-AFM imaging to characterize the thermally induced dimerization/cyclization reaction cascade of 1,2-bis(2-ethynyl phenyl)ethyne on a Ag(100) surface. By imaging the molecules after sequential annealing steps we have identified the structure and relative abundance of transient intermediates and products along the reaction pathway. *Ab initio* calculations indicate that the observation of particular intermediates along the static energy landscape of the reaction can be explained only by considering both the selective efficiency of energy dissipation to the surface and changes in entropy associated with subsequent reaction steps.

Methods

Experimental details: 1,2-bis(2-ethynyl phenyl)ethyne (**1**) was synthesized through iterative Sonogashira cross-coupling reactions. **1** was deposited in ultrahigh vacuum onto a Ag(100) surface held at room temperature (290 K). The sample was subjected to thermal annealing steps (Fig. 1f) in UHV over a range of temperatures between 290 K and 460 K. Cryogenic nc-AFM measurements (T = 4 K) were performed in a qPlus-equipped⁴⁵ commercial Omicron LT-STM/AFM in constant-height mode using CO-functionalized tips^{12,13} (resonance frequency = 29.73 kHz, nominal spring constant = 1800 N/m, Q-value = 90,000, oscillation amplitude = 60 pm, $V_s = 0.05$ V).

Computational details: All calculations were performed using the density functional-based tight binding (DFTB) method as implemented in the DFTB+ code.⁴⁶ Barrier heights along the reaction pathway on the Ag(100) surface were modeled using a periodically repeating 7x7x3 slab fixed at the coordinates derived from the lattice constant of Ag.⁴⁷ Transition states were located by constraining the bonds to be broken/formed in a stepwise fashion while relaxing the other adsorbate degrees of freedom and keeping the Ag slab frozen. The energies of the intermediates, as well as the barrier heights of **2b** and **3c**, were refined by performing structural relaxations of the adsorbates and the upper two Ag layers, and additionally including zero-point energy (ZPE) corrections. The key steps of the reaction (**1**→**2a**, **2b**→**3a**, and **3c**→**4a**) do not involve hydrogen transfer, thus we neglect nuclear quantum effects in the calculations. The accuracy of DFTB is discussed in detail in section 7 of the Supplementary Information.

Dissipative energy transfer to the substrate was calculated through constant-energy (no thermostats) molecular dynamics (MD) simulations that allowed us to monitor the time evolution of the kinetic energy of each subsystem (Fig. 4). The initial configuration was obtained from the optimization of the upper two Ag layers and the adsorbate (the bottom layer was fixed). A bond constraint was used to keep the initial adsorbate geometry slightly beyond its transition state and to prevent a chemical reaction during relaxations. The initial ionic velocities were set to zero to facilitate subsequent analysis of the ionic trajectories.

Vibrational entropies and zero point energies were computed by first performing structural relaxations of the adsorbates and the upper two Ag layers (while keeping the bottom one frozen) followed by a partial normal mode analysis of the adsorbate and the top Ag layer vibrations. For “mobile” non-radical species (**1**, **2b**, **3c**, **4c**) we estimated upper bounds for the roto-translational entropies by assuming free 1D rotations about the surface normal and free 2D translations on the surface. Other activation entropies can be neglected since they only involve radical species with constrained roto-translational degrees of freedom.

Kinetic simulations (Fig. 3) were performed by numerically solving a system of sequentially-coupled rate equations. The reaction rate constants were determined by the Eyring equation.³⁹ Selective dissipation was introduced through an effective absolute temperature T_{eff} that replaces T in the Eyring equation. $T_{eff} = T_0 + \Delta T_{molecule}$, where T_0 is the substrate temperature before the respective reaction step and $\Delta T_{molecule}$ is equal to the difference between the released chemical energy and the dissipative energy transfer to the substrate after 1 ps (i.e., the remaining molecular internal energy) divided by the molecular heat capacity, $C_{V,molecule}$. The internal molecular energy is the sum of the molecular kinetic and potential energy (the potential energy is assumed to be equal to the kinetic energy, which is true for harmonic vibrations). $C_{V,molecule}$ was determined by averaging the vibrational heat capacities of the molecules (calculated in the harmonic approximation) for the respective temperature range .

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Acknowledgements

Research supported by the U.S. Department of Energy Office of Basic Energy Sciences Nanomachine Program under contract no. DE-AC02-05CH11231 (STM and nc-AFM instrumentation development, AFM imaging), the Office of Naval Research BRC Program (molecular synthesis, characterization, and STM imaging), the ERC Advanced Grant DYNamo no. ERC-2010-AdG-267374 (computer resources and support), Spanish Grant no. FIS2013-46159-C3-1-P (MD calculations), and Grupos Consolidados UPV/EHU del Gobierno Vasco no. IT-578-13 (DFTB calculations). A.R. acknowledges fellowship support by the Austrian Science Fund (FWF) no. J3026-N16. A.P.P. acknowledges fellowship support from the Ayuda para la Especialización de Personal Investigador del Vicerrectorado de Investigación de la UPV/EHU-2013. A.Ru. acknowledges fellowship support from the Miller Institute for Basic Research in Science of the University of California at Berkeley (Miller Visiting Research Professor program). We thank Pavel Jelinek and Prokop Hapala for their help with the nc-AFM simulations and Duncan J. Mowbray for useful discussions.

Author contributions

A.R., A.P.P. and S.W. contributed equally to this work. A.R. and S.W. conceived the research and designed the experiments. A.R, S.W. and H.T. performed the nc-AFM experiments. A.R. was

responsible for the kinetic simulations and wrote the first draft of the manuscript. A.P.P conducted the theoretical calculations. A.J.B. and M.M.U. helped with the experiments. D.G.O. helped with the interpretation of the experimental and theoretical results. A. Ru. supervised the theoretical calculations and helped with the interpretation. P.G. and F.F. were responsible for molecular design. P.G. was responsible for synthesis. F.F. helped with the interpretation of the experimental results. M.F.C. supervised the experimental measurements, helped with the design of the study and the interpretation of the results. All authors discussed the results and helped in writing the manuscript.

Competing Financial Interests statement

The authors declare no competing financial interests.

Figure 1| Experimental observation of transient intermediates in a stepwise bimolecular enediyne coupling and cyclization cascade. **a–d**, Constant-height nc-AFM images show intermediates along the reaction pathway from **1** to **4c** (frequency shift ranges are represented by color gradient bars below the images, bright colors correspond to high frequency shift values). The corresponding chemical structures are depicted below the nc-AFM images. **e**, Relative abundance of the experimentally observed intermediates (**2b**, **3c**, **4c**) along the reaction pathway after each successive annealing step (data based on 105 counted molecules; the abundance of **1** is excluded since **1** can also undergo other reaction pathways). **f**, Measured sample temperature for each successive annealing step.

Figure 2| Calculated energy diagram for the stepwise enediyne coupling and cyclization cascade. The graph shows the *ab initio* energy landscape for intermediates and transition state energies along reaction pathway from **1** to **4c**. Chemical structures show metastable intermediates along the reaction coordinate (transition state structures not shown). Calculated activation enthalpies are shown below the reaction arrows. Out of seven theoretically predicted intermediates two are experimentally observed – both structures are followed by comparably low activation enthalpy barriers ($\Delta H^\ddagger = 0.7$ eV for **2b** and $\Delta H^\ddagger = 0.5$ eV for **3c**).

Figure 3| Calculated temperature-dependent relative concentrations of reactant, intermediates and product determined by solving kinetic rate equations for the reaction pathway from 1 to 4c. **a**, Adiabatic model: only reactant **1** and product **4c** are stabilized. **b**, Instant thermalization model: only intermediates that are followed by the highest activation barriers are stabilized (**2a**, **3a**). The product **4c** is predicted to be formed only at high temperature. **c**, Selective dissipation effects: addition of selective dissipation causes **4c** to form at lower temperature and leads to stabilization of **3b**. **d**, Entropic effects: addition of entropic effects results in predominant stabilization of intermediates **2b** and **3c**, in agreement with experimental observations.

Figure 4| Calculated dissipation of the chemical energy at different intermediate reaction steps calculated by DFTB molecular dynamics simulations. The images show spatial maps of the kinetic energy distribution (averaged over 1 ps after the energy plateaus post-reaction) for each atom of the metastable species **2b**, **3c**, and **4c**, as well as the substrate. Relative atom sizes have been adjusted for clarity. Horizontal bars show spatially integrated relative kinetic energy for molecule versus substrate averaged over 1 ps after the energy plateaus post-reaction.

Table of contents summary

The single-bond-resolved chemical structures of transient intermediates in a complex bimolecular reaction cascade were imaged by noncontact atomic force microscopy. Theoretical simulations reveal that the kinetic stabilization of experimentally observable intermediates is governed by selective energy dissipation to the substrate and entropic changes along the reaction pathway.