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Mesoporous Silica Nanoparticles and Films for Cargo Delivery

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of
Philosophy in Chemistry

by

Tania Maria Guardado Alvarez

2014

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ABSTRACT OF THE DISSERTATION

Mesoporous Silica Nanoparticles and Films for Cargo Delivery

by

Tania Maria Guardado Alvarez

Doctor of Philosophy in Chemistry

University of California, Los Angeles, 2014

Professor Jeffrey I. Zink, Chair

Mesoporous silica materials are well known materials that can range from films to nanoparticles. Mesoporous silica nanoparticles (MSNs) and mesoporous silica films have been of increasing interest among the scientific community for its use in cargo delivery. Silica provides ease of functionalization, a robust support and biocompatibility.

Several methods have been used in order to give the mesoporous silica nanomaterials different qualities that render them a useful material with different characteristics. Among these methods is surface modification by taking advantage of the OH groups on the surface. When a molecule attached to the surface can act as a molecular machine it transforms the nanomaterial to act as delivery system that can be activated upon command. The work covered in this thesis focuses on the development and synthesis of different mesoporous silica materials for the purpose of trapping and releasing cargo molecules. Chapter 2 focuses in the photoactivation of “snap-top” stoppers over the pore openings of mesoporous silica nanoparticles that releases intact cargo molecules from the pores. The on-command release can be stimulated by either one UV photon or two coherent near-IR photons. Two-photon activation is particularly desirable for use in biological systems because it enables good tissue penetration and precise spatial control. Chapter 3 focuses on the design and synthesis of a nano-container consisting of mesoporous silica nanoparticles with the pore openings covered by “snap-top” caps that are opened by near-IR light. A photo transducer molecule that is a reducing agent in an excited electronic state is covalently attached to the system. Near IR two-photon excitation causes intermolecular electron transfer that reduces a disulfide bond holding the cap in place, thus allowing the cargo molecules to escape. The operation of the “snap-top” release mechanism by both one- and two photon is described. This system presents a proof of concept of a near-IR photoredox-induced nanoparticle delivery system that may lead to a new type of photodynamic drug release therapy. Chapter 4 focuses on the attachment of a photoacid molecule on the surface of silica nanoparticles. Upon light irradiation the pKa of the photoacid molecules decreases causing the dissociation of the proton and the acidification of the nanoparticle surface. The local nanoparticle surface

acidification was probed using a pH sensitive nanovalve that was attached to MSNs next to a photoacid. The particles were loaded with a fluorescent dye that was contained by the nanovalve and released upon acidification of the surrounding environment. The amount of the dye release was measured continuously by detecting its fluorescence. Chapter 5 focuses on the synthesis of materials that utilize the micropatterned structure of a mesoporous silica film to successfully load and release cargo using a thermal sensitive polymer. Films with pore sizes of ~ 2 and ~ 5 nm aligned in the pulling direction were synthesized using evaporation induced self-assembly techniques. The pores are exposed using a new method of stamping micropatterns without the use of hydrofluoric acid. A well-studied temperature dependent polymer [poly(N-isopropylacrylamide-co-acrylamide)] was grafted onto the surface of these films to act as a temperature activated gatekeeper. Below the lower critical solution temperature (LCST) the polymer is erect and can block the pore openings, trapping cargo inside the pores. When the temperature is above the LCST the polymer collapses and unblocks the pores, allowing cargo to escape. The loading capacities as well as the reusability of these films were studied.

The dissertation of Tania Maria Guardado Alvarez is approved.

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Jeffrey I. Zink, Committee Chair

University of California, Los Angeles

2014

This dissertation is dedicated to my most wonderful grandparents, Hilario and Lilia Alvarez, my loving parents, Javier Guardado and Lourdes Alvarez de Guardado, my brother Javier Guardado, and Ira Staehle.

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Chapter 1

Functionalized Mesoporous Silica by the Sol-Gel Process

1.1 The Sol-Gel Process

Materials prepared by the sol-gel process have become the source of an important field of research in materials science. Since the 1970's, sol-gel chemistry has been researched extensively and has presented a variety of inorganic networks.¹ The sol-gel process can be defined as a set of reactions that convert an aqueous metal alkoxide, that has a molecular formula of $M(OR)_n$ into different types of inorganic networks.^{1, 2} This process has the advantage of being able to yield high purity inorganic oxide glasses. Furthermore, this process can be conducted at room temperature, which is much lower compared to the high temperatures needed by standard glass manufacturing processes. Another advantage is its versatility in creating different types of materials that include, thin films,³⁻⁵ spun fibers,^{6, 7} particles,⁸⁻¹¹ aerogels,^{12, 13} and xerogels.^{14, 15} As the name implies the sol-gel process is the conversion of a sol to a gel.^{2, 16} A sol is defined as a colloid formed by small particles that are well dispersed in a liquid. A gel is defined as a continuous network that simultaneously contains a phase of continuous liquid.¹⁶⁻¹⁸ The sol-gel process is therefore a series of hydrolysis and condensation reactions of the inorganic alkoxide monomers that forms colloidal particles (sol) and converts them into a continuous network (gel). Tetraethylorthosilicate (TEOS) has been widely used for the manufacture of silica gels.^{3, 10, 11, 19} The hydrolysis step of TEOS can be represented as the Equation: $Si(OEt)_4 + H_2O \rightarrow HO-Si(OEt)_3 + EtOH$. The first step in this equation is the generation of a silanol group (Si-OH) and the corresponding alcohol. The second step is the condensation of the silanol group. This step can occur in two different ways and is represented by the equation $HO-Si(OEt)_3 + HO-Si(OEt)_3 \rightarrow (EtO)_3-Si-O-Si(OEt)_3 + H_2O$ and $HO-Si(OEt)_3 + Si(OEt)_4 \rightarrow (EtO)_3-Si-O-Si(OEt)_3 + EtOH$.

The process continues as the silanol groups condense with other silanol groups or with an alkoxide. If the silanol groups condense with an alkoxide they make siloxane bonds (Si-O-Si) that have water or an alcohol as a byproduct. By repeating these steps many times a gel or a solid material is generated. The hydrolysis reaction can be greatly influenced by the presence of an acid or a base.^{16-18, 20} If it is conducted in the presence of an acid catalyst the hydrolysis step involves the electrophilic attack from the proton on the alkoxide oxygen atom. This results in the oxygen having a positive charge. This causes the bond between the silicon center and the oxygen that was attacked to become more polarized and facilitates the departure of the alcohol.²¹ The alcohol leaves forming the silanol bond. In the case of a base catalyzed reaction, the hydroxyl attacks the silicon atom. After this, the alcohol group leaves to form HO-Si(OEt)₃. This results in the hydrolysis reaction being much faster under acidic condition than under basic.

The pH of the solution also affects the condensation rates of the reaction which happens in two steps.²² The first step is the electrophilic attack of the proton on the oxygen of the silanol group. The second step is the formation of the siloxane bond after the loss of the hydronium cation. Similarly under basic conditions the condensation reaction also has two steps.^{2, 16} The hydrogen on the silanol group gets deprotonated by the hydroxide ion leaving a negatively charged oxygen on the silanol. This results in the formation of the siloxane bonds through an S_N2 reaction. The condensation rate under basic conditions is faster than that under acidic conditions.

Because the hydrolysis and condensation rates differ depending on the catalyst used, the structures can also be controlled by using the right catalyst. For acid catalysis the hydrolysis reaction is faster than the condensation reaction. As a consequence a network with less siloxane

bonds and more silanol groups yields more linearly branched polymeric species. This makes this catalyst the preferred method for the formation of thin films. On the other hand, the condensation reaction is much faster using base as a catalyst. Therefore, there are fewer silanol groups in the network. The network then consists of highly branched clusters, which yields denser materials. This is the method used for the formation of particles.

1.2 Materials Made of Mesoporous Silica and Their Functionalization

Mesoporous silica was first produced in 1970 but was forgotten for several years.²³ Then in 1972 Mobil Corporation developed a method to convert methanol into gasoline using the catalyst zeolite ZSM-5. Because of this there was an interest in the synthesis of mesoporous materials with larger pores.⁹ Then in 1992 Mobil Corporation Laboratories produced MCM-41 materials.²⁴ In 2001 MCM-41 materials were first proposed as a drug delivery system.⁹ That same year a university in Beijing reported the first synthesis of MCM-41 silica nanoparticles.^{25, 26} After this MCM-41 silica nanoparticles have been heavily researched because of their many attractive characteristics such as ease of functionalization, stability, biocompatibility and large surface area. These materials are made using a surfactant as a templating agent, TEOS as the silica source and a base catalyst. After the formation of the materials the surfactant can be removed leaving empty nanopores incorporated into the silica framework. One of the many advantages of using the sol-gel process for the synthesis of materials is the silanol groups present on the surface.^{27, 28} Taking advantage of these OH group the materials can be further functionalized by the attachment of functional groups on the

surface. The most common way to functionalize the mesoporous materials is through silylation²⁸. Silylation happens on the surface silanol groups and allows the mesoporous structure to be maintained after the modification. Surface functionalized mesoporous silica materials are of particular interest because of their potential use in a variety of applications.¹ One in particular is the growing attention in trapping guest molecules inside the pores for use in drug delivery applications.^{3, 9, 29, 30}

This thesis describes the synthesis, characterization and study of mesoporous silica for the trapping and releasing of guest molecules from the nanopores. Chapter 2 focuses on the design, synthesize and operation of a photo activated “snap-top” stopper over the pore opening of mesoporous silica nanoparticles. The “snap-top” releases cargo molecules from the pores when stimulated by either one UV photon or two coherent near-IR photons. Two-photon activation using near IR light is particularly desirable for use in biological systems because it enables good tissue penetration and precise spatial control. Stoppers were assembled by first binding photolabile coumarin-based molecules to the nanoparticle surface. Then, after loading the particles with cargo, bulky β -cyclodextrin molecules were noncovalently associated with the substituted coumarin molecule, blocking the pores and preventing the cargo from escaping. One-photon excitation at 376 nm or two-photon excitation at 800 nm cleaves the bond holding the coumarin to the nanopore, releasing both the cyclodextrin cap and the cargo. The dynamics of both the cleavage of the cap and the cargo releasing was monitored using fluorescence spectroscopy. This system traps intact cargo molecules without the necessity of chemical modification, releases them with tissue penetrating near-IR light and has possible applications in photo-stimulated drug delivery.

Chapter 3 discusses the synthesis of a nano-container consisting of mesoporous silica nanoparticles with the pore openings covered by “snap-top” caps that are opened by near-IR light. A photo transducer molecule that is a reducing agent in an excited electronic state is covalently attached to the system. Coherent two-photon near IR absorption causes inter-molecular electron transfer and reduces a disulfide bond that holds the cap in place, thus allowing the cargo molecules to escape. The operation of the “snap-top” release mechanism by both one- and two-photon activation is described. This system presents a proof of concept of a near-IR photoredox-induced nanoparticle delivery system that may lead to a new type of photodynamic drug release therapy.

Chapter 4 focuses on the synthesis and characterization of a MSN nano-container to hold and release cargo using either a pH or light stimuli. A previously published pH active nanovalve was reconfigured for light activation. The nanovalve consists of an aniline based stalk that was covalently attached to the surface of the nanoparticles. The bulky molecule α -cyclodextrin was used as a stopper that associates to the stalk due to hydrophobicity. A photoacid was also covalently attached to the surface of the nanoparticles and upon 408 nm light excitation the pKa of the photoacid increases, dissociating a proton that acidifies the surface of the nanoparticles opening the nanovalve and allowing the cargo to escape.

Chapter 5 centers on mesostructured sol-gel thin films formed by evaporation induced self-assembly (EISA).^{2, 31} The films had pore sizes of ~ 2 and ~ 5 nm aligned in the pulling direction and were synthesized using evaporation induced self-assembly techniques. The pores are exposed using a new method of stamping micropatterns without the use hydrofluoric acid. A well-studied temperature dependent polymer [poly(N-isopropylacrylamide-co-acrylamide)] was

grafted onto the surface of these films to act as a temperature activated gatekeeper. Below the lower critical solution temperature (LCST) the polymer is erect and can block the pore openings, trapping cargo inside the pores. When the temperature is above the LCST the polymer collapses and unblocks the pores, allowing cargo to escape. The loading capacities as well as the reusability of these films were studied.

1.3 References

1. Al-Othman, Z. A., A Review: Fundamental Aspects of Silicate Mesoporous Materials. *Materials* 2012, 5, 2874-2902.
2. Brinker, C. J.; Scherer, G. W., *Sol-gel science: the physics and chemistry of sol-gel processing*. Academic Pr: 1990.
3. Klichko, Y.; Khashab, N. M.; Yang, Y.-W.; Angelos, S.; Stoddart, J. F.; Zink, J. I., Improving pore exposure in mesoporous silica films for mechanized control of the pores. *Microporous and Mesoporous Materials* 2010, 132, 435-441.
4. Johansson, E.; Zink, J. I., Nanostructured Silica Thin Films Self-Assembled with Electron Donors and Acceptors to Measure Electron Tunneling. *Journal of the American Chemical Society* 2007, 129, 14437-14443.
5. Koganti, V. R.; Rankin, S. E., Synthesis of surfactant-templated silica films with orthogonally aligned hexagonal mesophase. *The Journal of Physical Chemistry B* 2005, 109, 3279-3283.
6. Lee, S. W.; Kim, Y. U.; Choi, S.-S.; Park, T. Y.; Joo, Y. L.; Lee, S. G., Preparation of SiO₂/TiO₂ composite fibers by sol-gel reaction and electrospinning. *Materials Letters* 2007, 61, 889-893.
7. Choi, S.-S.; Lee, S. G.; Im, S. S.; Kim, S. H.; Joo, Y. L., Silica nanofibers from electrospinning/sol-gel process. *Journal of materials science letters* 2003, 22, 891-893.
8. Wu, S.-H.; Mou, C.-Y.; Lin, H.-P., Synthesis of mesoporous silica nanoparticles. *Chemical Society Reviews* 2013.

9. Vallet-Regí, M., Mesoporous Silica Nanoparticles: Their Projection in Nanomedicine. *ISRN Materials Science* 2012, 2012.
10. Li, Z.; Barnes, J. C.; Bosoy, A.; Stoddart, J. F.; Zink, J. I., Mesoporous silica nanoparticles in biomedical applications. *Chemical Society Reviews* 2012, 41, 2590-2605.
11. Ambrogio, M. W.; Thomas, C. R.; Zhao, Y.-L.; Zink, J. I.; Stoddart, J. F., Mechanized silica nanoparticles: a new frontier in theranostic nanomedicine. *Accounts of chemical research* 2011, 44, 903-913.
12. Hüsing, N.; Schubert, U., Aerogels—airy materials: chemistry, structure, and properties. *Angewandte Chemie International Edition* 1998, 37, 22-45.
13. Pierre, A. C.; Pajonk, G. M., Chemistry of aerogels and their applications. *Chemical Reviews-Columbus* 2002, 102, 4243-4266.
14. Fields, S. M., Silica xerogel as a continuous column support for high-performance liquid chromatography. *Analytical chemistry* 1996, 68, 2709-2712.
15. Huang, H. Y.; Yang, R. T.; Chinn, D.; Munson, C. L., Amine-grafted MCM-48 and silica xerogel as superior sorbents for acidic gas removal from natural gas. *Industrial & engineering chemistry research* 2003, 42, 2427-2433.
16. Brinker, C. J., Sol-gel processing of silica. *Advances in Chemistry Series* 1994, 234, 361-361.
17. Ng, L. V.; Thompson, P.; Sanchez, J.; Macosko, C. W.; McCormick, A. V., Formation of cage-like intermediates from nonrandom cyclization during acid-catalyzed sol-gel polymerization of tetraethyl orthosilicate. *Macromolecules* 1995, 28, 6471-6476.

18. Wen, J.; Wilkes, G. L., Organic/inorganic hybrid network materials by the sol-gel approach. *Chemistry of Materials* 1996, 8, 1667-1681.
19. Klein, L., Sol-gel processing of silicates. *Annual Review of Materials Science* 1985, 15, 227-248.
20. Alberius, P. C. A.; Frindell, K. L.; Hayward, R. C.; Kramer, E. J.; Stucky, G. D.; Chmelka, B. F., General Predictive Syntheses of Cubic, Hexagonal, and Lamellar Silica and Titania Mesostructured Thin Films §. *Chemistry of Materials* 2002, 14, 3284-3294.
21. Schmidt, H.; Scholze, H.; Kaiser, A., Principles of hydrolysis and condensation reaction of alkoxysilanes. *Journal of Non-Crystalline Solids* 1984, 63, 1-11.
22. Hench, L. L.; West, J. K., The sol-gel process. *Chemical Reviews* 1990, 90, 33-72.
23. Chiola, V., Process for producing low-bulk density silica. Google Patents: 1971.
24. Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S., Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. *nature* 1992, 359, 710-712.
25. Grun, M. Lauer, I. Unger, K. K.; The synthesis of micrometer- and submicrometer size spheres of ordered mesoporous oxide MCM-41. *Advanced Materials* 1997, 9, 254-257
26. Cai, Q.; Luo, Z.-S.; Pang, W.-Q.; Fan, Y.-W.; Chen, X.-H.; Cui, F.-Z., Dilute solution routes to various controllable morphologies of MCM-41 silica with a basic medium. *Chemistry of materials* 2001, 13, 258-263.
27. Brinker, C., Hydrolysis and condensation of silicates: effects on structure. *Journal of Non-Crystalline Solids* 1988, 100, 31-50.

28. Anwander, R., SOMC@ PMS. Surface organometallic chemistry at periodic mesoporous silica. *Chemistry of materials* 2001, 13, 4419-4438.
29. Rieter, W. J.; Taylor, K. M.; Lin, W., Surface modification and functionalization of nanoscale metal-organic frameworks for controlled release and luminescence sensing. *Journal of the American Chemical Society* 2007, 129, 9852-9853.
30. Wang, Y.; Price, A. D.; Caruso, F., Nanoporous colloids: building blocks for a new generation of structured materials. *Journal of Materials Chemistry* 2009, 19, 6451-6464.
31. Klichko, Y.; Liong, M.; Choi, E.; Angelos, S.; Nel, A. E.; Stoddart, J. F.; Tamanoi, F.; Zink, J. I., Mesostructured silica for optical functionality, nanomachines, and drug delivery. *Journal of the American Ceramic Society* 2008, 92, S2-S10.

Chapter 2

Activation of Snap-Top Capped Mesoporous Silica Nano Containers Using Two Near-Infrared Photons

Adopted from recently published TM. Guardado-Alvarez; L. Sudha Devi; M. Russell; BJ. Schwartz; JI. Zink, Activation of Snap-top Capped Mesoporous Silica Nano Containers Using Two Near-Infrared *Journal of the American Chemical Society* 2013, 135, 14000–14003

2.1 Introduction

A fast evolving area of nano-medicine is the delivery of drugs using on-command stimuli responsive nanoparticle carriers. These nanoparticles would deliver localized high concentrations of drugs without any premature leakage. One of the preferred delivery methods is via light activation because it offers the possibility of precise spatial localization¹ of the release and also of the timing of the drug delivery.¹⁻⁸ One of the limitations faced by the use of light activated systems is the depth of tissue penetration of the light because the best transmission occurs with near-IR light at wavelengths of 750-800 nm.^{4, 8, 9} Since most large amplitude motion that is light activated occurs in the UV, researchers are increasingly focusing attention on coherent two-photon excitation to achieve excitation energies to activate the release.^{4-8, 10} A highly researched subject for light activated drug delivery is in developing prodrug motifs in which a drug molecule is chemically bonded to a carrier molecule or particle and is photochemically cleaved from the carrier.¹¹ However for this approach to work the chemical modification of the original drug is required in order to attach it to a photolabile group. This becomes problematic for the reason that the prodrug needs to be biocompatible and it must give the active drug as a result of the cleavage. Because of this designing a prodrug is a lengthy and costly process that requires both screening of the prodrug for toxicity and the development of new synthetic technique for each drug that is used.^{11, 12} Designs that have a prodrug grafted on silica nanoparticles have been reported,¹² even though these examples still have the same limitations as any other prodrug.

Mesoporous silica nanoparticles (MSNs) have increased in popularity over the past decade primarily when used for drug delivery purposes in part because intact drug molecules can be stored in the pores. MSNs also have been shown to be nontoxic in multiple *in vivo* studies and have been proven to hold a wide variety of drugs.¹⁴ MSNs have other advantages for example; ease of functionalization, a robust framework, and are biocompatible.^{3, 11-17} Frequently used functionalizations include polymers attached to the outer surface,^{11, 18} incorporation of molecules into the framework,^{3, 7, 11, 19} and attachment of molecules including nanomachines around the pore openings.^{12-14, 20-22} The last derivatized particles can deliver drugs using a number of different stimuli.¹¹ There are many examples of photo-activated^{2-4, 6, 7, 23-33} drug release from MSNs as well as snap-top^{12, 22, 34-36} caps that have been reported. Some of these systems include: a system that uses coumarin dimerization to block the MSN pores and acts as a gate keeper;³² dissociation β -cyclodextrin that is caused by the photoisomerization of an azobenzene and upon light excitation uncaps the mesopores;³ and photocleavage of the N-C bond of an *o*-methoxybenzylamine-based gatekeeper.³⁶ However, all of the previous systems use high energy UV light that is not biocompatible.

In this chapter a system that utilizes MSNs of the MCM-41 type and a 7-hydroxy-4-(hydroxymethyl)-2H-chromen-2-one as the basis for a “snap-top” that can be released with either one- or two-photon activation for use in drug delivery is described. Because the MSN pores are chemically inert, drug molecules can be stored in the pores without chemical modification, and the release can be performed with near-IR light that is at the optimal wavelength for tissue penetration. The system is based on the photocleavage of the C-O bond shown in Figure 2.1. This photolabile protecting group was reported to have a two-photon cross-section of ~ 1.07 GM

at 740 nm and 0.13 GM at 800 nm³³ (measured for **1**); when excited by one- or two-photons, the C-O bond is cleaved, creating a carbocation that is subsequently hydrolyzed. The mechanism for the photocleavage was described by Furuta *et al.* for **2**, and a similar mechanism likely operates for the one- and two-photon cleavage of **4**.³⁷

2.2 Experimental Section

2.2.1 General Methods

All reagents including tetraethyl orthosilicate, cetyl trimethylammonium bromide, sodium hydroxide, hydrogen chloride, β -cyclodextrin, 7-hydroxy-4-(chloromethyl)-2H-chromen-2-one and 3-(triethoxysilyl)propyl isocyanate are commercially available and were used without further purification. Nuclear magnetic resonance (NMR) spectra were recorded in a Bruker DSX 300 at room temperature. Powder X-ray diffraction (XRD) measurements were carried out using a Panalytical X'Pert Pro powder diffractometer. The radiation source was copper ($K\alpha_1$ and $K\alpha_2 = 1.5418 \text{ \AA}$). FT-IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 500 spectrometer. The transmission electron microscope (TEM) images of the silica nanoparticles were collected on a JEOL1200-EX instrument in the California NanoSystems Institute. Microfilms for TEM imaging were made by placing a drop of the particle suspension in methanol onto a 200-mesh copper TEM grid (Ted Pella, Inc., Redding, CA) and dried at room temperature. N_2 adsorption-desorption isotherms were obtained at 77 K on a Quandrochrome Surface Area and Pore size analyzer. The continuous monitoring of fluorescence release profiles were obtained using an

Acton SpectraPro 2300i monochromator connected to a CCD was used for detection and CUBE 448, CUBE 375 and CUBE 408 (Coherent Inc., Santa Clara, CA, USA) diode lasers were used as the excitation source. UV-Vis spectra were collected on a Cary 5000 UV-Vis-NIP spectrophotometer

2.2.2 Synthesis of MSNs

The MSNs used in this work were synthesized as previously reported in the literature.^{11, 38-40} The pore structure of the nanoparticles was confirmed using powder X-ray diffraction (PXRD) and (TEM) (Figure 2.2). From the TEM studies the pore diameter was calculated to be about 2.5 nm and the particle size of about 100 nm. From the PXRD the higher order peaks observed can be indexed as the (1 0 0), (1 1 0) and (2 0 0) planes with a lattice spacing of 4 nm. The N₂ absorption-desorption isotherms showed a specific surface of 1044 m² / g.

2.2.3 Synthesis of and Attachment of Snap-Top

The 7-hydroxy-4-(chloromethyl)-2H-chromen-2-one was commercially obtained and refluxed in DI water for 48 hours to yield **3**^{2,3}. Next, .025 g of **3** was dried and reacted with 31.6 μ L of 3-(triethoxysilyl)propyl isocyanate in 10 mL dry toluene under an N₂ atmosphere and refluxed for 24 hours to generate **4** (Figure 2.3) which was then condensed on the surface of the silica nanoparticles. The nanoparticles were washed to remove any excess **4** using a centrifugation method. The attachment of **4** on the surface of the nanoparticles was confirmed using IR, UV-Vis and solid-state NMR spectroscopy. The IR spectrum showed a peak at around 1600 cm⁻¹ indicating the presence of a carbonyl stretch attributed to the lactone functional group

(Figure 2.4). The ^{13}C solid-state NMR shows chemical shifts at 160 ppm for the carbonyl carbon as well as the peaks corresponding to the aromatic region around 110 ppm (Figure 2.5). The ^{29}Si solid-state NMR showed chemical shifts at -91, -101, -110 ppm corresponding to the silica framework and -66 ppm corresponding to the functionalization shift (Figure 2.6). The UV-Vis of the particles in solution showed the absorption peak of **4** at ~ 320 nm (Figure 2.7).

2.2.4 Loading and Capping of the MSNs

After the attachment, 100 mg of the functionalized nanoparticles in 10 mL of a 1 mM solution of Rhodamine B, which serves as the “cargo” and incorporates into the pores by diffusion. The mixture was stirred at room temperature for 24 hours after which 1 g of β -cyclodextrin was added. β -cyclodextrin associates with **4** due to hydrophobic interactions. This mixture was then left stirring for an additional 48 hours and at that point the particles were recovered and washed thoroughly to remove any Rhodamine B adhered to the surface using a centrifugation method. Finally the fully assembled system was air dried for 4 days. Due to its size, β -cyclodextrin blocks the pore openings and thus traps the chemically unmodified Rhodamine B cargo molecules inside the pores. Previous *in vitro* studies show that β -cyclodextrin hydrophobic associations are not affected by biomolecules.¹⁴

2.3 Results and Discussions

2.3.1 One-Photon Experiments

2.3.1.1 *Experimental Set-up*

The operation of the light-activated snap-top system was monitored by measuring the amount of Rhodamine B cargo released from the particles into solution using fluorescence spectroscopy. The nanoparticles were placed into one corner of a cuvette to which deionized water was carefully added. The opposite corner contained a small stir bar operated at a low speed to disperse released molecules with minimal disturbance to the particles (Figure 2.8). The concentration of the released molecules in the solution above the particles was measured at one-second time intervals by fluorescence spectroscopy using 408 nm laser excitation. The fluorescence intensity of the released Rhodamine B cargo at 580 nm and that of the dissociated cap, which contains fragment **3**, at 490 nm was simultaneously monitored. The flat baseline measured at both wavelengths before the system was irradiated shows that there is insignificant leakage from the capped pores. The fluorescence intensity increased after activation of the snap-top because the molecules left the particles and diffused into solution.

2.3.1.2 *One-Photon Activation of the System*

The results of the release measurements are shown in Figure 2.9. The fluorescence intensity remained within the background when the pump laser was off; showing that the cap remains bonded to the particles and that there is no leakage of the cargo. After verifying that there was no change in fluorescence intensity for two hours, a 376 nm excitation light was

applied to the sample. This has the effect of cleaving the bond indicated by the arrow in Figure 2.1, which in turn uncaps the pores, allowing both the cap and the cargo to escape into solution. Immediately after activation, an increase in fluorescence intensity of both the capped and the Rhodamine B cargo was observed. As expected, the release of the cap was observed to occur at a faster rate than that of the cargo because diffusion of the cargo out of the 2.5 nm pores is a slower process, particularly given the favorable electrostatic interaction of Rhodamine B with the walls of the silica pores. The released amount of Rhodamine B cargo after 14 hours of irradiation was calculated to be 1.6 weight percent. To quantify the total amount of Rhodamine B released by irradiation, the solution was stirred for an extra 16 hours in the dark to allow any leftover Rhodamine B to diffuse out into solution. Then the UV-Vis absorbance was measured, which yielded a released amount of Rhodamine B cargo of 2.3 weight percent.

2.3.1.3 Control Experiments

To prove that the cleavage and cargo release resulted from a photochemical reaction and not from a thermal process caused by local laser heating, two control experiments were conducted. First, the same release detection measurements while externally heating the sample without any laser excitation was carried out. In this experiment, the emission was measured for two hours to verify that no cap dissociation occurred. Then the solution was heated to 70 °C and no increase in fluorescence intensity was found, verifying that the capping system remained stable. Finally, after about 4.5 hours, the 376-nm pump laser (0.025 W/cm²) was turned on and the fluorescence intensity of the cap immediately increased, showing that the system retained its photo-triggered capability (Figure 2.10).

In a separate experiment, the particles were irradiated with a high laser power (0.125 W/cm^2) at 514 nm where the photocleavable group does not absorb. No release of the cargo was observed in this experiment (Figure 2.11), verifying that absorption of a photon with a wavelength that can initiate cleavage of the stopper is necessary for the cargo release. Together, these two experiments prove that the activation of the snap-top and the on-command release of the cargo are caused by a photochemical reaction and not by a thermal process.

2.3.2 Two-Photon Experiments

2.3.2.1 *Experimental Set-up*

Then the activation of the snap-top by two-photon excitation was investigated, which was done using the output of an amplified Ti:Sapphire laser, which consisted of 40 fs, 60 μJ pulses centered around 800 nm at a repetition rate of 1 kHz. The 800-nm light is also not absorbed by the photocleavable system, so a two- (or greater) photon process is required for this choice of photolysis wavelength. The beam was focused to a 2.5 mm spot size at 0.2 W/cm^2 . Since the ultrafast laser system was not compatible with *in situ* fluorescence monitoring, the way the cargo release measurement was performed was slightly modified for these experiments. The cuvette was set up in the same manner as for the one-photon experiments, but then irradiated the samples with the 800-nm light for a series of fixed time intervals. After each interval, aliquots of the supernatant containing the released cargo and cap molecules were taken, and the fluorescence intensity was measured, and then the aliquots were returned to the cuvette prior to further irradiation (Figure 2.12).

2.3.2.2 Two Photon Activation of the System

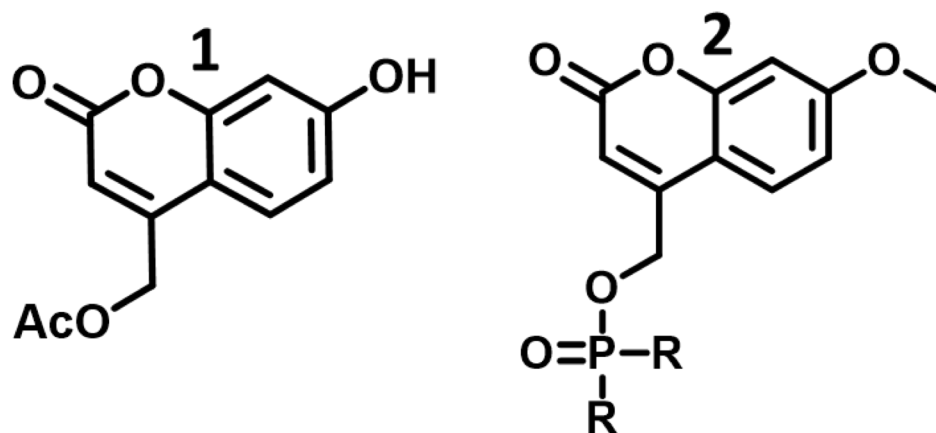
The release profiles shown in Figure 2.13 are plots of the fluorescence intensities of the released cap at 490 nm and Rhodamine B at 580 nm at one-hour intervals instead of the one-second intervals used in the one-photon experiments. The release profiles for two-photon activation shown in Figure 2.13 verify that the snap-top can be successfully activated by near-IR light. As before, in the absence of the pump laser excitation, the flat baseline (no fluorescence intensity increase over time) shows that no leakage occurs. The 800-nm excitation light was turned on after five hours, producing an immediate increase in the fluorescence of both the cap and the Rhodamine B cargo. The release efficiency during these two-photon experiments appeared similar to that in the one-photon experiments. Unfortunately, the weight percent of cargo released in these experiments was not calculated because the laser in the two-photon experiments was focused to a spot that was smaller than the size of the aggregated MSN's, and only a fraction of the particles was excited at a given moment. Figure 2.13 shows that after 500 minutes, the cleavage of the cap was nearing completion, but when the laser beam was moved to a different spot, the release rate started increasing again. Thus, since the release rate is similar to the one-photon case, if all of the particles were two-photon irradiated simultaneously, the same weight percent release of cargo would be anticipated as in the one-photon case.

2.4 Conclusion

In summary a photocleavable snap-top based on molecule **3** that can be activated using either one or two photon absorption was synthesized. The two-photon capabilities of this photocleavable snap-top are highly beneficial for biological purposes. The near-IR light will be able to achieve deeper tissue penetration, activation will cause little to no tissue damage, the method has the additional benefit of precise focal control, all of which make this system ideal for photo-activated cancer therapy without the need to chemically modify a drug.

2.5 Figures and Tables

Scheme 2-1 Photocleavable Coumarin Derivatives



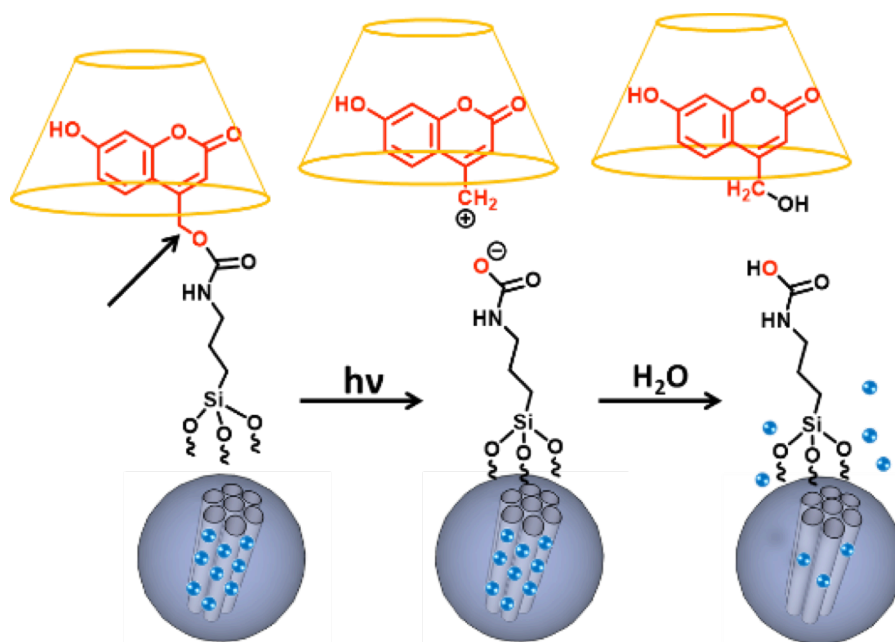


Figure 2-1 Photocleavage mechanism of the snap-top cap. Final products are shown on the right.

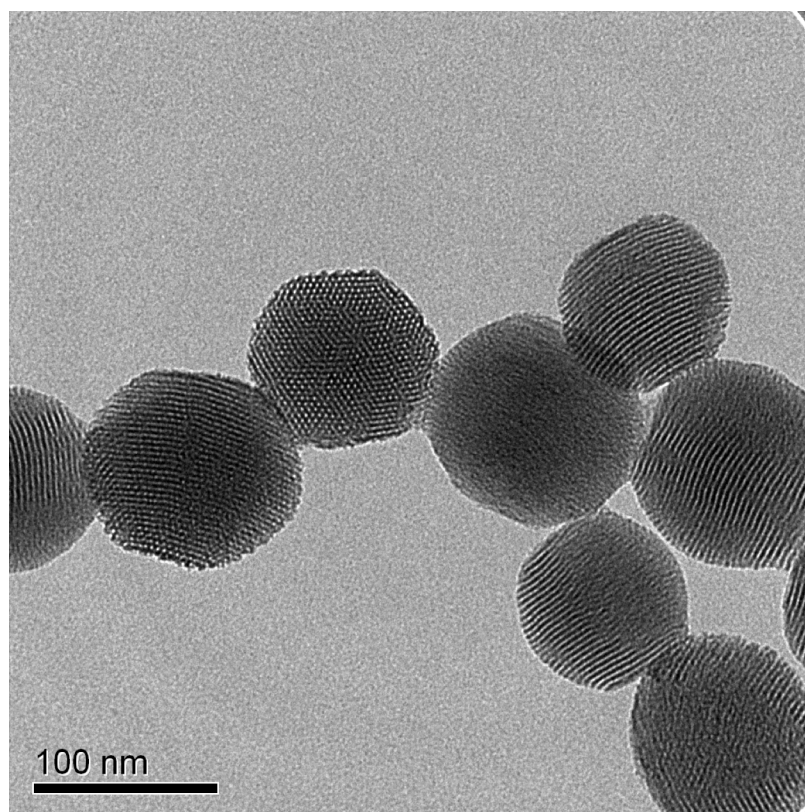


Figure 2-2 TEM image of MSNs after surfactant extraction. Scale bar is 100 nm.

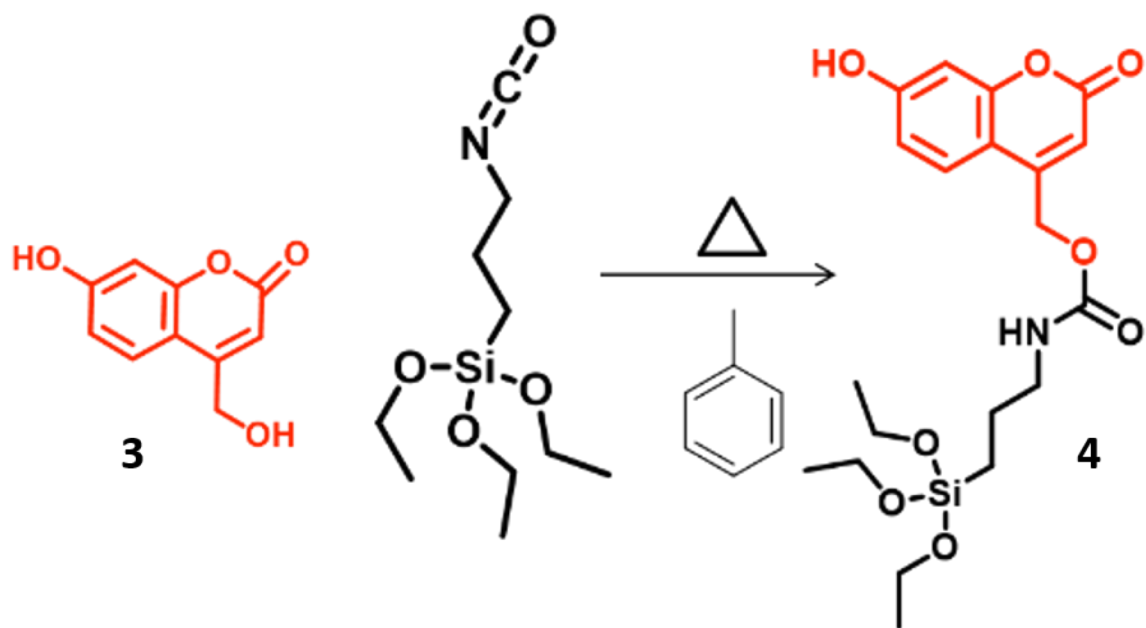


Figure 2-3 Synthesis of the silane modified coumarin molecule

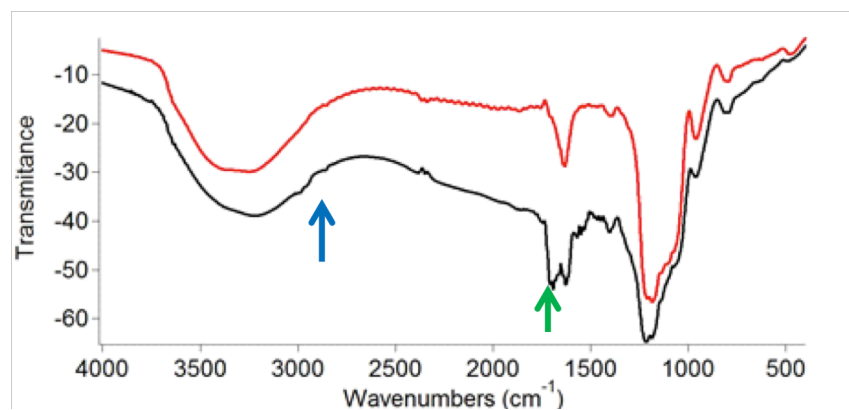


Figure 2-4 The IR spectrum of the snap-top modified nanoparticles (black) shows a peak around 1600 cm^{-1} (blue arrow) indicating the presence of a carbonyl stretch attributed to the lactone functional group. The band around 3000 cm^{-1} (green arrow) can be attributed to the C-H stretch. The unmodified MSNs spectrum (in red) shows the absence of the previously mentioned stretches. Both samples were prepared as KBr pellets.

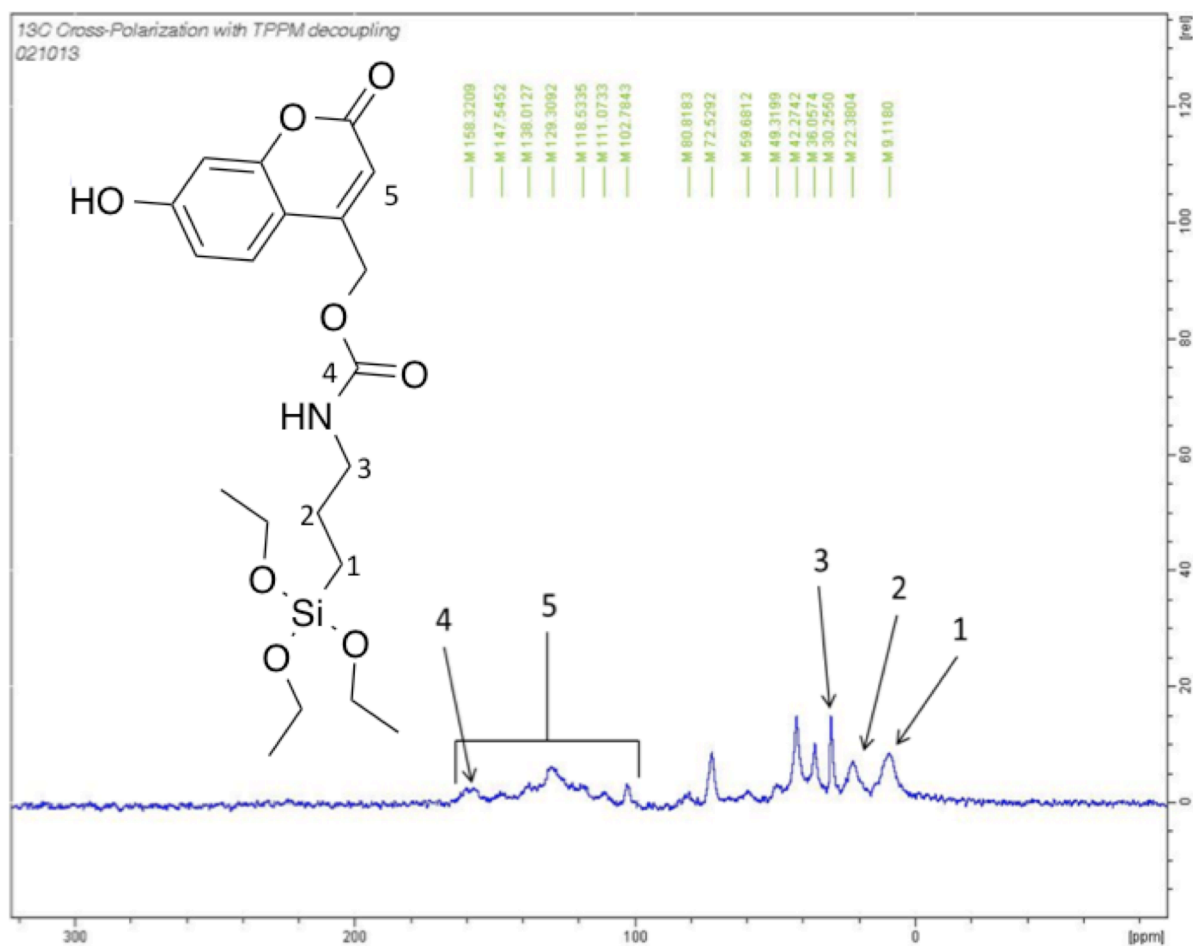


Figure 2-5 The ^{13}C solid-state NMR of the functionalized MSNs representing the chemical shift associated with the molecule represented on the left.

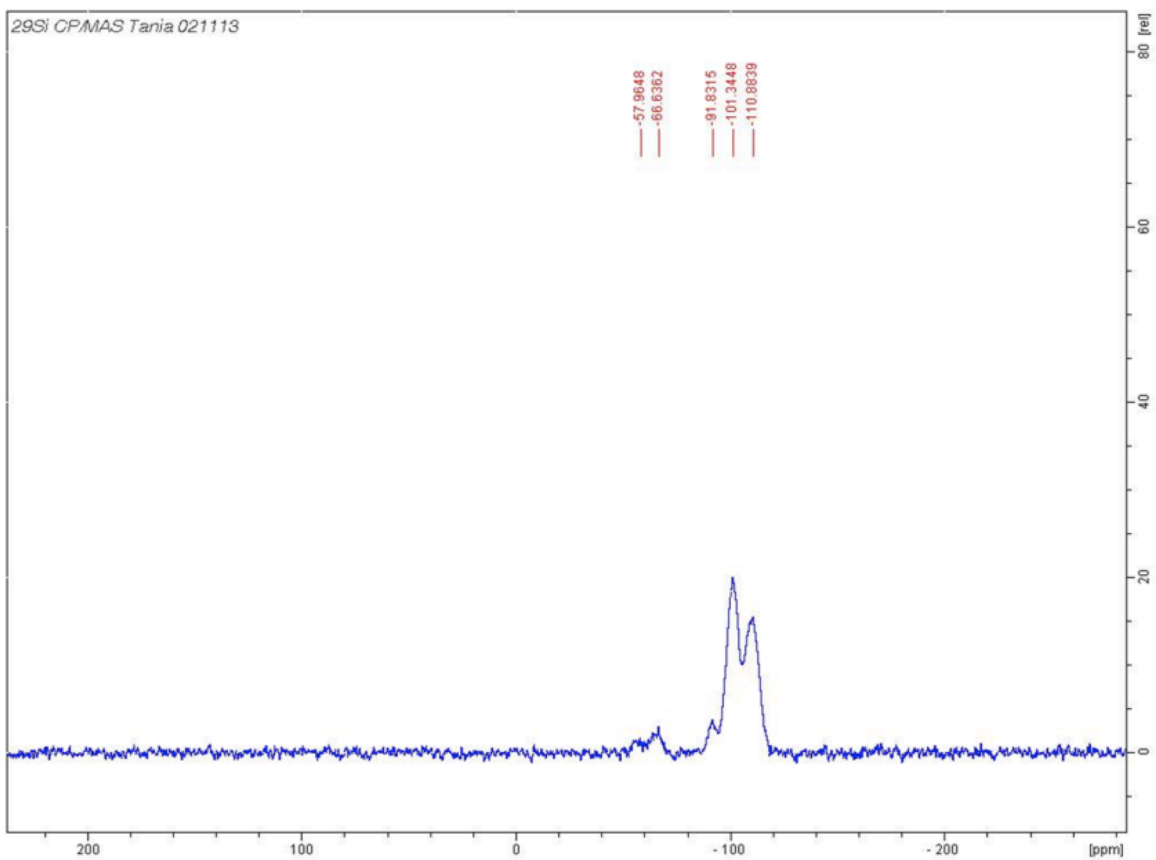


Figure 2-6 The ^{29}Si solid-state NMR spectrum of the functionalized MSNs that shows a peak at 66 ppm corresponding to the functionalized silica and at 91 ppm, 101 ppm and 110 ppm corresponding to the silica framework.

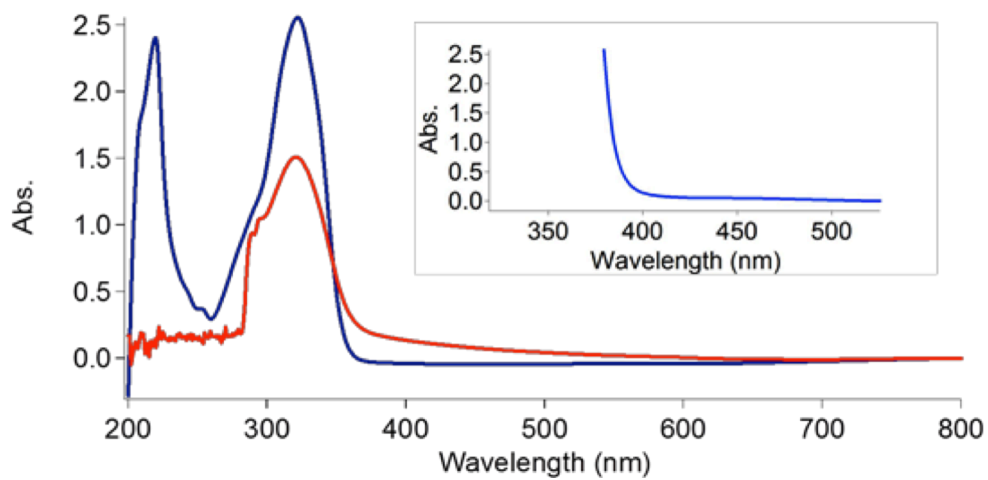


Figure 2-7 The UV-vis NIR spectra of free 7-hydroxy-4-(hydroxymethyl)-2H-chromen-2-one (blue) and the functionalized MSNs (red). The inset is the expanded absorbance of a saturated 7-hydroxy-4-(hydroxymethyl)-2H-chromen-2-one solution showing that the absorbance at 376 nm is nonzero.

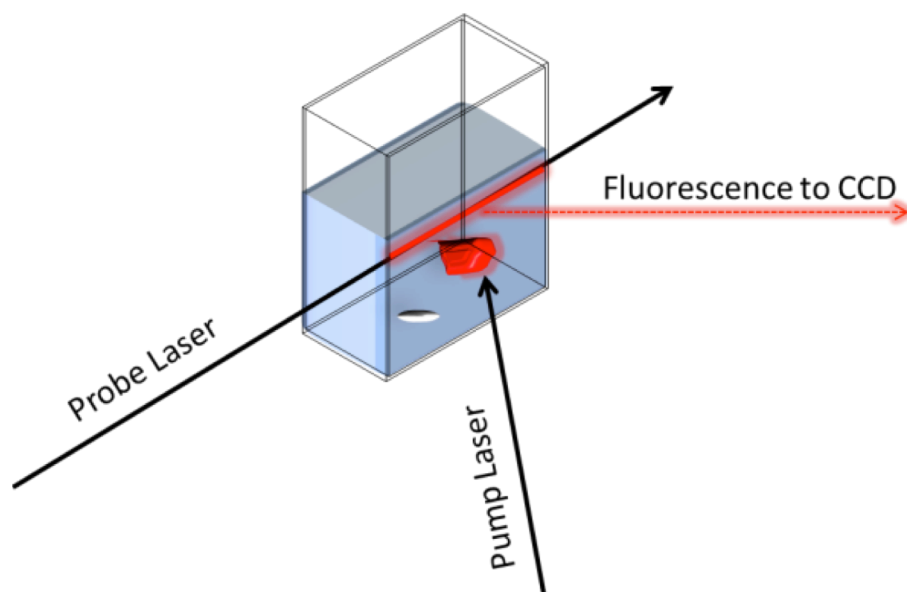


Figure 2-8 Detection set up for the continuous fluorescence monitoring during one photon experiments

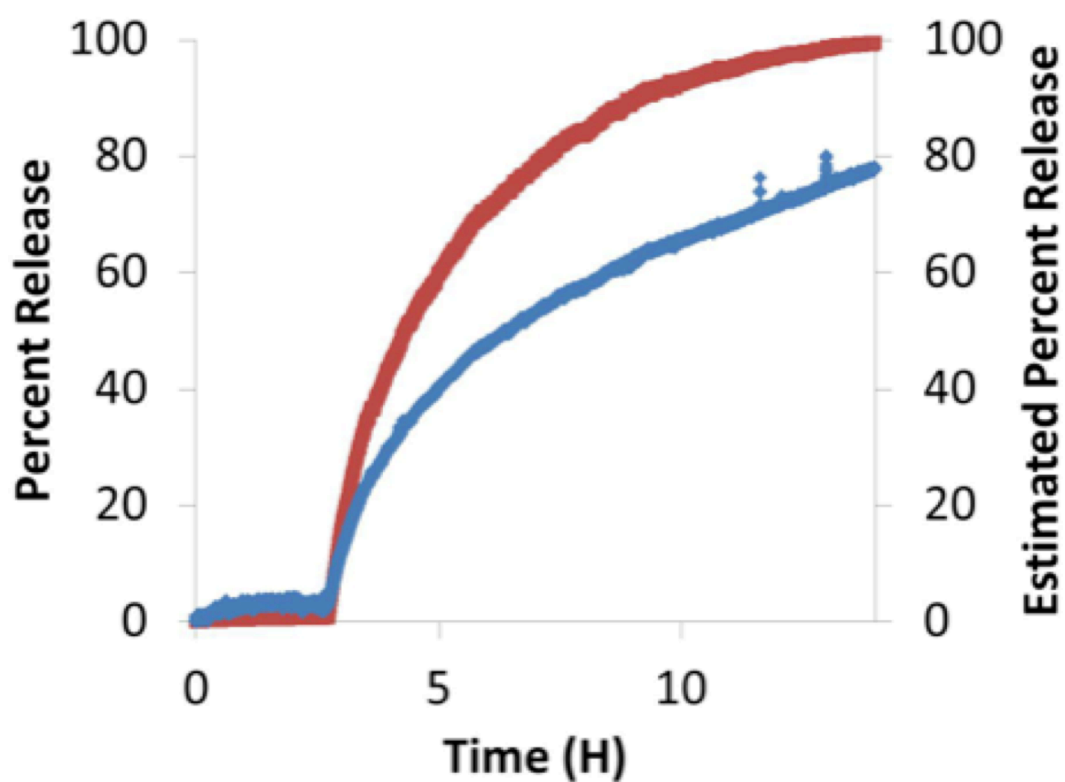


Figure 2-9 Release profiles of cap (bottom) and Rhodamine B cargo (top). The probe laser is on continuously. The 376 nm pump laser is turned on after 2.5 hours.

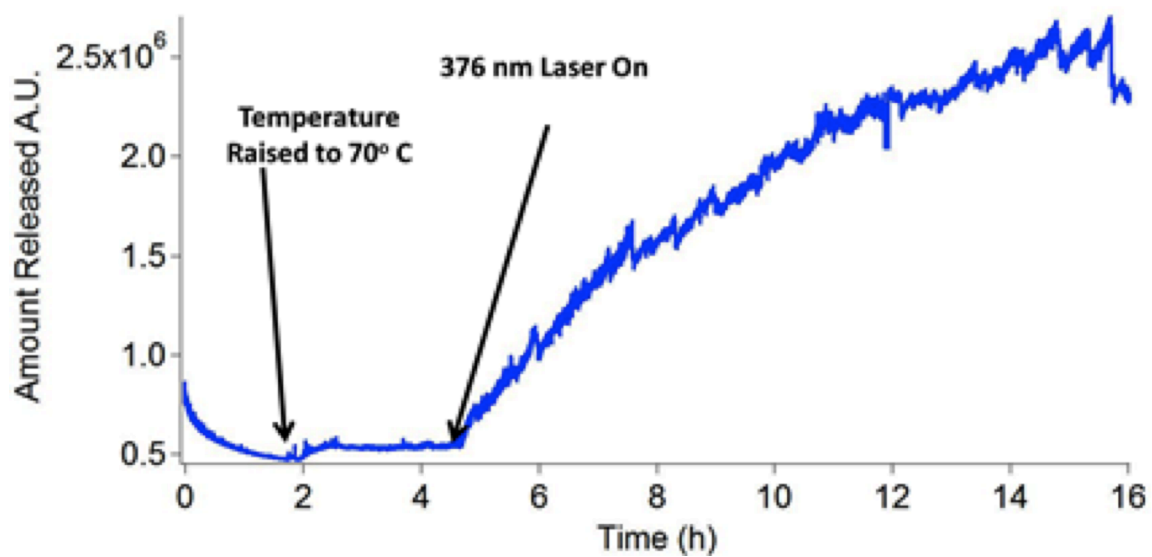


Figure 2-10 Release profile of snap-top on MSNs using external heating at 2 hours and 377 at 5 hours.

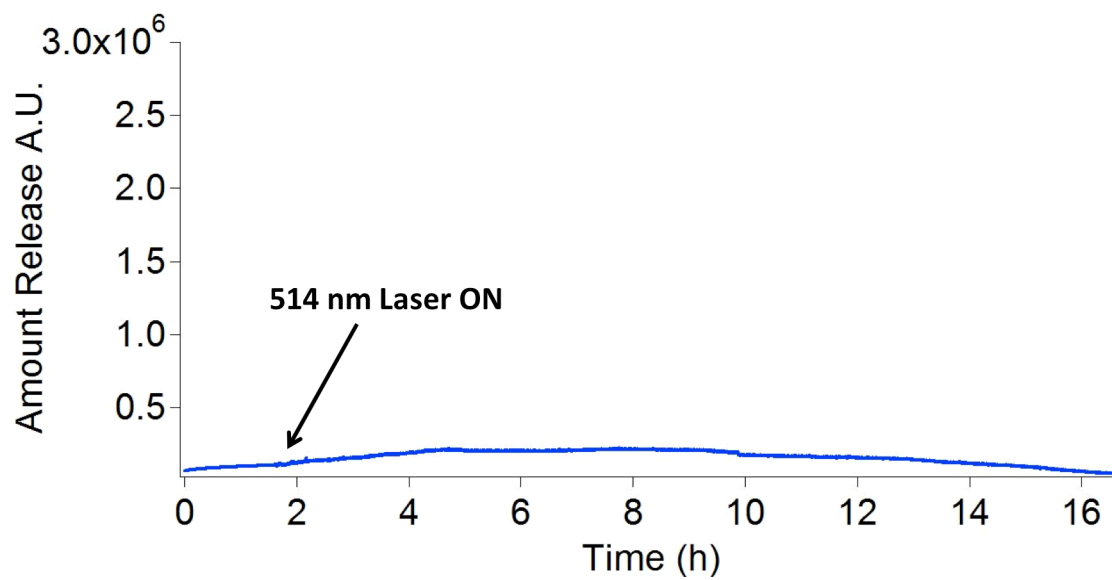


Figure 2-11 Release profile of the loaded snap-top on MSNs with 514 nm. probe laser.

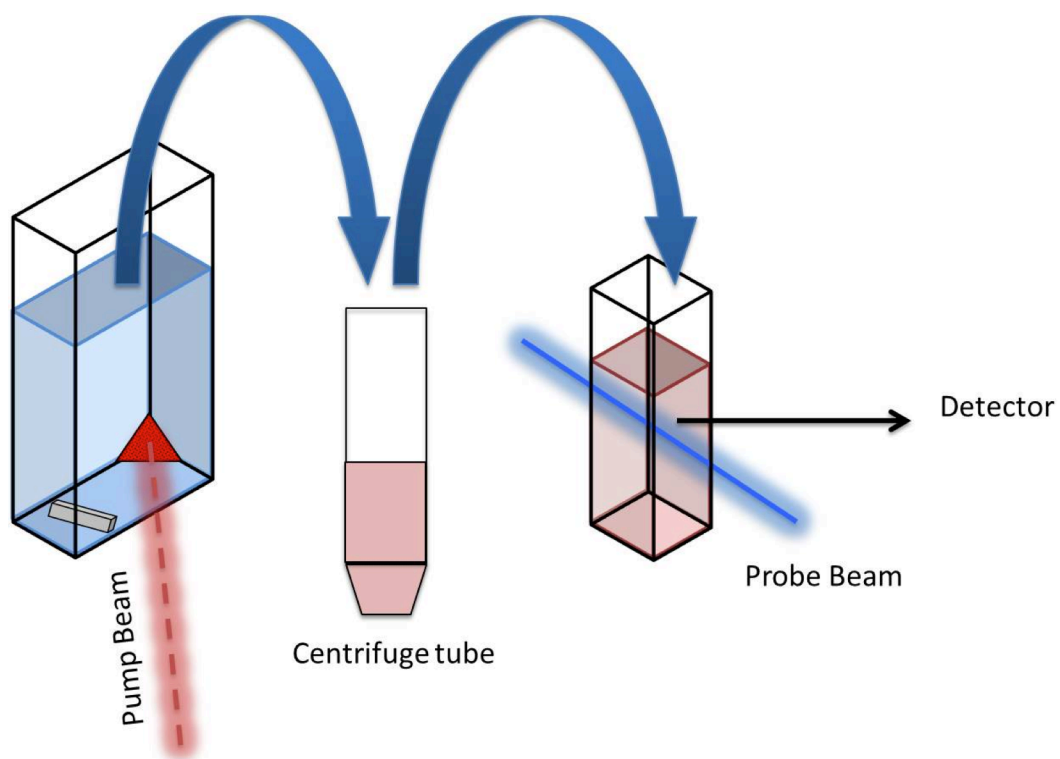


Figure 2-12 Detection set up for the continuous fluorescence monitoring

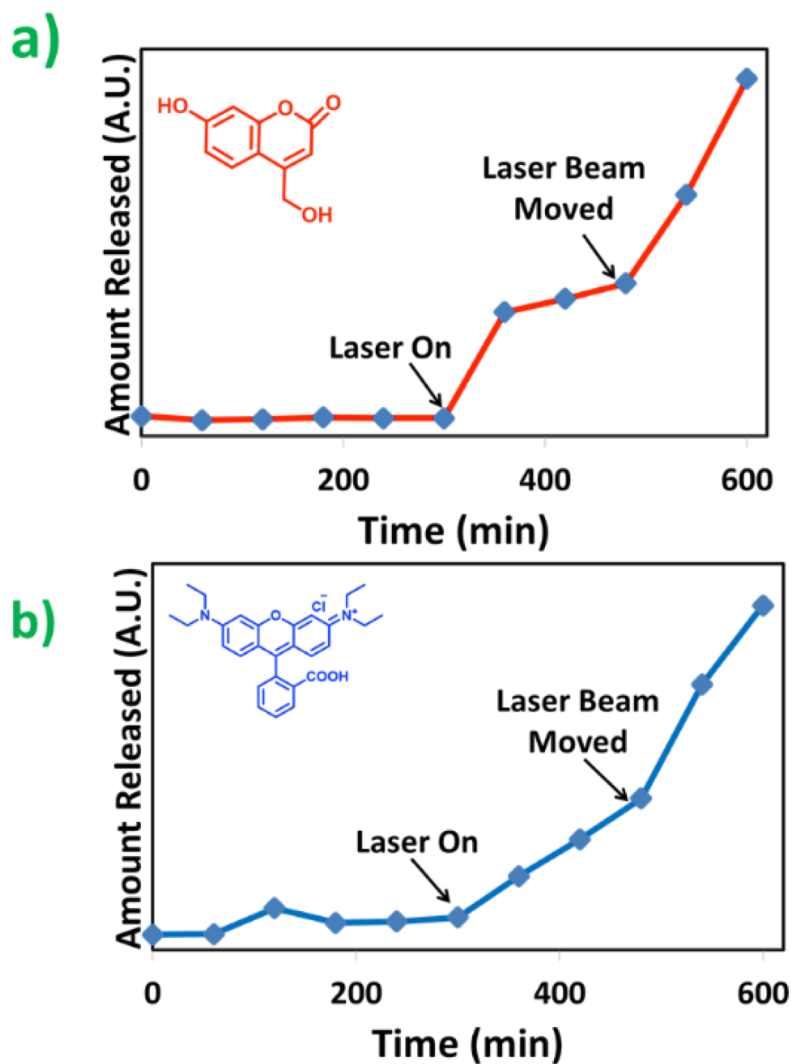


Figure 2-13 a) Release profile showing the fluorescence intensity increase of the cap. b) Release profile showing the fluorescence intensity increase of the Rhodamine B cargo. In both release profiles excitation with 800 nm femtosecond laser pulses were used. The fluorescence intensity of the aliquots was taken every hour.

2.6 References

1. Alvarez-Lorenzo, C.; Bromberg, L.; Concheiro, A., Light-sensitive Intelligent Drug Delivery Systems†. *Photochemistry and photobiology* 2009, 85, 848-860.
2. Azagarsamy, M. A.; Alge, D. L.; Radhakrishnan, S. J.; Tibbitt, M. W.; Anseth, K. S., Photocontrolled Nanoparticles for On-Demand Release of Proteins. *Biomacromolecules* 2012.
3. Ferris, D. P.; Zhao, Y. L.; Khashab, N. M.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I., Light-operated mechanized nanoparticles. *Journal of the American Chemical Society* 2009, 131, 1686-1688.
4. Fomina, N.; McFearin, C.; Sermsakdi, M.; Edigin, O.; Almutairi, A., UV and near-IR triggered release from polymeric nanoparticles. *Journal of the American Chemical Society* 2010, 132, 9540-9542.
5. Bhawalkar, J. D.; Kumar, N. D.; Zhao, C. F.; Prasad, P. N., Two-photon photodynamic therapy. *Journal of clinical laser medicine & surgery* 1997, 15, 201-204.
6. Chang, Y. T.; Liao, P. Y.; Sheu, H. S.; Tseng, Y. J.; Cheng, F. Y.; Yeh, C. S., Near-Infrared Light-Responsive Intracellular Drug and siRNA Release Using Au Nanoensembles with Oligonucleotide-Capped Silica Shell. *Advanced Materials* 2012.
7. Choi, S. K.; Thomas, T.; Li, M. H.; Kotlyar, A.; Desai, A.; Baker Jr, J. R., Light-controlled release of caged doxorubicin from folate receptor-targeting PAMAM dendrimer nanoconjugate. *Chemical Communications* 2010, 46, 2632-2634.
8. Kim, S.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Pandey, R. K.; Prasad, P. N., Organically modified silica nanoparticles co-encapsulating photosensitizing drug and aggregation-enhanced

two-photon absorbing fluorescent dye aggregates for two-photon photodynamic therapy. *Journal of the American Chemical Society* 2007, 129, 2669-2675.

9. Starkey, J. R.; Rebane, A. K.; Drobizhev, M. A.; Meng, F.; Gong, A.; Elliott, A.; McInnerney, K.; Spangler, C. W., New two-photon activated photodynamic therapy sensitizers induce xenograft tumor regressions after near-IR laser treatment through the body of the host mouse. *Clinical Cancer Research* 2008, 14, 6564-6573.

10. König, K., Multiphoton microscopy in life sciences. *Journal of Microscopy* 2001, 200, 83-104.

11. Li, Z.; Barnes, J. C.; Bosoy, A.; Stoddart, J. F.; Zink, J. I., Mesoporous silica nanoparticles in biomedical applications. *Chemical Society Reviews* 2012, 41, 2590-2605.

12. Ambrogio, M. W.; Pecorelli, T. A.; Patel, K.; Khashab, N. M.; Trabolsi, A.; Khatib, H. A.; Botros, Y. Y.; Zink, J. I.; Stoddart, J. F., Snap-top nanocarriers. *Organic Letters* 2010, 12, 3304-3307.

13. Angelos, S.; Yang, Y. W.; Patel, K.; Stoddart, J. F.; Zink, J. I., pH-Responsive Supramolecular Nanovalves Based on Cucurbit [6] uril Pseudorotaxanes. *Angewandte Chemie* 2008, 120, 2254-2258.

14. Angelos, S.; Khashab, N. M.; Yang, Y. W.; Trabolsi, A.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I., pH clock-operated mechanized nanoparticles. *Journal of the American Chemical Society* 2009, 131, 12912-12914.

15. Casasús, R.; Climent, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruiz, E., Dual aperture control on pH-and anion-driven supramolecular

nanoscopic hybrid gate-like ensembles. *Journal of the American Chemical Society* 2008, 130, 1903-1917.

16. DeMuth, P.; Hurley, M.; Wu, C.; Galanie, S.; Zachariah, M. R.; DeShong, P., Mesoscale porous silica as drug delivery vehicles: Synthesis, characterization, and pH-sensitive release profiles. *Microporous and Mesoporous Materials* 2010.

17. Thomas, C. R.; Ferris, D. P.; Lee, J. H.; Choi, E.; Cho, M. H.; Kim, E. S.; Stoddart, J. F.; Shin, J. S.; Cheon, J.; Zink, J. I., Noninvasive remote-controlled release of drug molecules in vitro using magnetic actuation of mechanized nanoparticles. *Journal of the American Chemical Society* 2010, 132, 10623-10625.

18. Zhang, J.; Misra, R. D. K., Magnetic drug-targeting carrier encapsulated with thermosensitive smart polymer: core-shell nanoparticle carrier and drug release response. *Acta Biomaterialia* 2007, 3, 838-850.

19. Lin, Y. S.; Tsai, C. P.; Huang, H. Y.; Kuo, C. T.; Hung, Y.; Huang, D. M.; Chen, Y. C.; Mou, C. Y., Well-ordered mesoporous silica nanoparticles as cell markers. *Chemistry of Materials* 2005, 17, 4570-4573.

20. Johansson, E.; Choi, E.; Angelos, S.; Liong, M.; Zink, J. I., Light-activated functional mesostructured silica. *Journal of Sol-Gel Science and Technology* 2008, 46, 313-322.

21. Klichko, Y.; Liong, M.; Choi, E.; Angelos, S.; Nel, A. E.; Stoddart, J. F.; Tamanoi, F.; Zink, J. I., Mesostructured silica for optical functionality, nanomachines, and drug delivery. *Journal of the American Ceramic Society* 2008, 92, S2-S10.

22. Patel, K.; Angelos, S.; Dichtel, W. R.; Coskun, A.; Yang, Y. W.; Zink, J. I.; Stoddart, J. F., Enzyme-responsive snap-top covered silica nanocontainers. *Journal of the American Chemical Society* 2008, 130, 2382-2383.
23. Kumar, S.; Allard, J.-F.; Morris, D.; Dory, Y. L.; Lepage, M.; Zhao, Y., Near-infrared light sensitive polypeptide block copolymer micelles for drug delivery. *Journal of Materials Chemistry* 2012, 22, 7252-7257.
24. Lin, Q.; Huang, Q.; Li, C.; Bao, C.; Liu, Z.; Li, F.; Zhu, L., Anticancer drug release from a mesoporous silica based nanophotocage regulated by either a one-or two-photon process. *Journal of the American Chemical Society* 2010, 132, 10645-10647.
25. Lu, J.; Choi, E.; Tamanoi, F.; Zink, J. I., Light-Activated Nanoimpeller-Controlled Drug Release in Cancer Cells. *Small* 2008, 4, 421-426.
26. Park, C.; Lee, K.; Kim, C., Photoresponsive Cyclodextrin-Covered Nanocontainers and Their Sol-Gel Transition Induced by Molecular Recognition. *Angewandte Chemie* 2009, 121, 1301-1304.
27. Park, C.; Oh, K.; Lee, S. C.; Kim, C., Controlled Release of Guest Molecules from Mesoporous Silica Particles Based on a pH-Responsive Polypseudorotaxane Motif. *Angewandte Chemie International Edition* 2007, 46, 1455-1457.
28. Schloßbauer, A.; Sauer, A. M.; Cauda, V.; Schmidt, A.; Engelke, H.; Rothbauer, U.; Zolghadr, K.; Leonhardt, H.; Bräuchle, C.; Bein, T., Cascaded Photoinduced Drug Delivery to Cells from Multifunctional Core–Shell Mesoporous Silica. *Advanced Healthcare Materials* 2011.

29. Shamay, Y.; Adar, L.; Ashkenasy, G.; David, A., Light induced drug delivery into cancer cells. *Biomaterials* 2011, 32, 1377-1386.
30. Sun, L.; Yang, Y.; Dong, C. M.; Wei, Y., Two-Photon-Sensitive and Sugar-Targeted Nanocarriers from Degradable and Dendritic Amphiphiles. *Small* 2011, 7, 401-406.
31. Yang, Y.; Song, W.; Wang, A.; Zhu, P.; Fei, J.; Li, J., Lipid coated mesoporous silica nanoparticles as photosensitive drug carriers. *Physical Chemistry Chemical Physics* 2010, 12, 4418-4422.
32. Mal, N. K.; Fujiwara, M.; Tanaka, Y., Photocontrolled reversible release of guest molecules from coumarin-modified mesoporous silica. *Nature* 2003, 421, 350-353.
33. Suzuki, A. Z.; Watanabe, T.; Kawamoto, M.; Nishiyama, K.; Yamashita, H.; Ishii, M.; Iwamura, M.; Furuta, T., Coumarin-4-ylmethoxycarbonyls as phototriggers for alcohols and phenols. *Organic Letters* 2003, 5, 4867-4870.
34. Vivero-Escoto, J. L.; Slowing, I. I.; Wu, C. W.; Lin, V. S. Y., Photoinduced intracellular controlled release drug delivery in human cells by gold-capped mesoporous silica nanosphere. *Journal of the American Chemical Society* 2009, 131, 3462-3463.
35. Lai, C.-Y.; Trewyn, B. G.; Jeftinija, D. M.; Jeftinija, K.; Xu, S.; Jeftinija, S.; Lin, V. S. Y., A mesoporous silica nanosphere-based carrier system with chemically removable CdS nanoparticle caps for stimuli-responsive controlled release of neurotransmitters and drug molecules. *Journal of the American Chemical Society* 2003, 125, 4451-4459.
36. Agostini, A.; Sancenón, F.; Martínez-Máñez, R.; Marcos, M. D.; Soto, J.; Amorós, P., A Photoactivated Molecular Gate. *Chemistry-A European Journal* 2012, 18, 12218-12221.

37. Furuta, T.; Iwamura, M., [3] New caged groups: 7-substituted coumarinylmethyl phosphate esters. *Methods in enzymology* 1998, 291, 50-63.
38. Meng, H.; Xue, M.; Xia, T.; Ji, Z.; Tarn, D. Y.; Zink, J. I.; Nel, A. E., Use of size and a copolymer design feature to improve the biodistribution and the enhanced permeability and retention effect of doxorubicin-loaded mesoporous silica nanoparticles in a murine xenograft tumor model. *Acs Nano* 2011, 5, 4131-4144.
39. Liong, M.; Lu, J.; Kovichich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I., Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *Acs Nano* 2008, 2, 889-896.
40. Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S., Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. *nature* 1992, 359, 710-712.

Chapter 3

Photo-Redox Activated Drug Delivery Systems Using Two Near-IR

Photons

Adopted from recently published TM. Guardado-Alvarez; L. Sudha Devi; J. Vabre; T. Pecorelli; BJ. Schwartz; J. Durand; O. Mongin; M. Blanchard-Desce; JI. Zink, Photo-Redox Activated Drug Delivery Systems Operating Under Two Photon Excitation in the Near-IR *Nanoscale* **2014**, 6, 4652-4658

3.1 Introduction

Photodynamic therapy is an established method for treating several medical indications such as lung and oesophageal cancer. Although the most common form of phototherapy uses nontoxic compounds that become toxic upon light irradiation (e.g. singlet oxygen formation from an FDA-approved porphyrin containing drug),¹ there is a need for more general treatment methods, especially delivery of apoptosis-inducing anticancer drugs. It is particularly desirable to take advantage of light activated release of desired intact cargo molecule because it offers the benefit of both temporal and spatial control²⁻¹¹ over cargo delivery. A platform that is under active investigation for drug delivery is mesoporous silica nanoparticles (MSNs). Silica provides ease of functionalization, a robust support and little to no biotoxicity^{10, 12-22} As previously mentioned several methods have been used in order to give the silica nanoparticles different material qualities that render them useful for drug delivery. One such method is surface modification, which is done by taking advantage of the chemistry of the surface silanol groups.^{15, 17, 18, 22-26} This chemistry is used to attach molecular machines to the nanoparticle surface, allowing the particles to act as delivery system that can be activated upon command. Several examples of photodynamic activation of delivery systems in MSNs have been reported, including a supramolecular system that involves a cyclodextrin threaded onto an azobenzene-based molecule grafted onto the surface of MSNs that functions as a nanocarrier and is activated using ultraviolet (UV) light.¹⁰ Multiple examples of azobenzene derivatives attached to the interiors of pores that are static in the dark and hold cargo molecules in the pores but act as impellers when irradiated and release the cargo are also known.^{27, 28} Another variation involves

direct photocleavage of a bulky group blocking the pore openings, leading to the release of cargo.^{8, 23}

A major drawback of the photo-activated systems mentioned above is the need for a high energy (frequently UV) light source to break a chemical bond to initiate delivery; such light has limited tissue penetration and thus these systems have limited applicability for internal drug delivery. The optimal wavelengths for tissue penetration are between 750 to 800 nm²⁹⁻³¹ but the excited states of the photo-activatable groups do not absorb at these wavelengths. As mentioned in the previous chapter one way of using near-IR wavelengths for activating systems that require higher-energy photons is via coherent two-photon excitation. The two-photon excitation process is nonlinear, depending on the square of the intensity of the light, and involves selection rules different from those for one-photon absorption.³² Two-photon activation can be highly advantageous in biological systems due to better focal control, deeper tissue penetration and reduced tissue damage.³¹ Unfortunately the two-photon absorption cross-section of most light-responsive delivery systems is too small to be useful. By combining two-photon-absorbing transducers with nanomachines on MSNs, many existing drug delivery therapeutic systems could be modified to function using tissue penetrating near-IR light.

In this chapter, a proof-of-concept a nanomachine system stimulated by chemical reduction that has been reconfigured for two-photon light activation (Figure 3.1) is described. The system takes advantage of the two-photon activated photo-transducer N¹-(4-((1E,3E)-4-(4-(dipropylamino)phenyl)buta-1,3-dien-1-yl)phenyl)-N¹-propylethane-1,2-diamine (2PNT), whose chemical structure is shown in Figure 3.2, to reduce a disulfide bond and release cargo from MSNs. Direct photolysis of similar disulfide-based systems has been investigated previously,

but the photolysis typically requires short wavelength light sources.¹⁵ To the best of our knowledge, photocleavage of a disulfide bond has not been used for drug delivery purposes, and this work constitutes the first example of the use NIR photo-induced disulfide bond breaking to release cargo from MSNs carriers.

Prior work on a molecule similar to 2PNT has shown that electrons are generated through a reversible photo-oxidation of the neutral molecule to the dication.³³ This is because the 2PNT molecule is a conjugated donor- π -donor system bearing electronegative end groups that, upon excitation, becomes a photo-reducing agent that operates at low oxidation potentials.^{33, 34} Thus, when photo-excited, the 2PNT transducers will transfer electrons to the delivery system (snap-top), cleaving the disulfide bond, which in turn uncaps the pore and allows the cargo to be released as shown in Figure 3.3. For this system, a disulfide snap-top based (3-(adamantan-1-yl)disulfanyl)propyl)triethoxysilane associated to β -cyclodextrin (due to hydrophobicity) to act as a gatekeeper for the nanopores is used. To use the 2PNT on the surface of MSNs for transferring an electron to the disulfide bond, it is necessary to inhibit back electron transfer, and therefore the use of a sacrificial agent (EDTA) that donates an electron to the oxidized 2PNT is also needed. The combination of the 2PNT transducer, sacrificial EDTA electron donor and releasable snap-top allows the system to be activated on command via either one UV photon or two near- IR photons. The activation can release a wide variety of cargo molecules, and the photo-transducer is generalizable to a wide variety of reduction processes.

3.2 Experimental Section

3.2.1 General Methods

Transmission electron microscopy(TEM) images were collected on a JEM1200-EX (JEOL) instrument in the California NanoSystem Institute. Microfilms for TEM imaging were made by placing a drop of the particle suspension in methanol onto a 200-mesh copper TEM grid (Ted Pella, Inc., Redding, CA) and dried at room temperature. Powder X-ray diffraction (XRD) patterns were collected using a Philips X'Pert Pro diffractometer equipped with Cu KR radiation. UV-vis spectra were collected using a Cary 5000 UV-vis-NIR spectrophotometer. The photo-continuous fluorescence spectroscopy was done using a monochromator connected to an Acton SpectraPro 2300i CCD and a coherent cube laser. A femtosecond Ti:Sapphire amplifier (Coherent, Legend Elite) seeded with a broadband Ti:Sapphire oscillator (Coherent, Mantis) was used for the two photon excitation. The amplifier output consisting of 40 fs, 60 μ J pulses centered on 800 nm (at 1 kHz repetition rate) was focused to a 2.5 mm spot size. Nuclear magnetic resonance (NMR) spectra were recorded by a Bruker DSX 300 at room temperature. N₂ adsorption-desorption isotherms were obtained at 77 K on a Quandrchrome Surface Area and Pore size analyzer. The emission spectra were measured on an Edinburgh FLS-920 spectrophotometer.

3.2.2 Synthesis of Two-Photon Nanotrigger

(E,E)-1,4-Bis(4-nitrophenyl)-1,3-butadiene (**1**); In a 1 L round bottom flask, trans-4-nitrocinnamaldehyde (6.0 g, 33.9 mmol) and (4-nitrobenzyl)triphenylphosphonium bromide (16.2 g, 33.9 mmol) were mixed in absolute EtOH (68 mL). The solution was purged with argon before dropwise addition of t-BuOK (11.4 g, 102.0 mmol) in absolute EtOH (205 mL). The mixture was stirred at room temperature for 12 h. Distilled water (200 mL) was added and the precipitate was filtrated, washed with water/EtOH (40:60) and dried at 50 °C. The resulting powder was dissolved in 230 mL of THF and solution of (0.032 g, 0.127 mmol) iodine in THF (27 mL) was then added. The solution was stirred for 48 h under irradiation (75 W tungsten lamp). The solution was then treated with a saturated Na₂S₂O₃ solution. The precipitate was filtered, washed with water, and dried at 50°C to afford **1** (9.04 g, 90%).

4,4'-(1E,3E)-1,3-Butadiene-1,4-diylbis(benzenamine) (**2**); In a 1 L round bottom flask, a solution of **1** (9.20 g, 31.1 mmol) in EtOH (123 mL) was purged with argon. Anhydrous tin (II) chloride (59.0 g, 311 mmol) was added and the mixture was stirred for 14 h at 70 °C. Aqueous NaOH was added until pH 8. The mixture was then filtered and the filtrate was extracted with AcOEt. After drying and evaporation under reduced pressure, the resulting solid was refluxed with AcOEt (750 mL) for 12 h under vigorous stirring. The mixture was filtered and rinsed with hot AcOEt. The solvent was evaporated and the residue was dried to afford **2** (6.61 g, 90%).

Carbamate **3**; A solution of **2** (3.10 g, 13.1 mmol) in THF (136 mL) was cooled to 0 °C and Et₃N (2.2 mL, 16.3 mmol) was added. A solution of di-tert-butyl dicarbonate (2.86 g, 13.1 mmol) in THF (50 mL) was added drop wise (10 mL/h) at 65 °C for 5 h. THF was evaporated

under reduced pressure and AcOEt and water were added. The organic layer was dried (MgSO_4), filtered and evaporated. The residue was purified by flash chromatography on silica gel (AcOEt/heptane 1:3) to afford compound **3** (2.58 g, 58%).

Compound **4**; To a solution of **3** (1.09 g, 3.24 mmol) in anhydrous 1,2-dichloroethane (20 mL) under argon, was added a solution of propanol (0.555 mL) in anhydrous 1,2-dichloroethane (5 mL). The mixture was stirred at room temperature for 3 h. Sodium triacetoxyborohydride (1.58 g, 7.45 mmol) was added and the mixture was stirred at room temperature for 12 h. A saturated sodium bicarbonate solution was then added until pH 7-8. After evaporation of 1,2-dichloroethane, the aqueous layer was extracted with AcOEt. The organic phase was then washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (AcOEt/heptane/ Et_3N 30:70:0.1) to afford **4** (0.98 g, 72%).

Compound **5**; To a solution of **4** (0.84 g, 2.0 mmol) in anhydrous 1,2-dichloroethane (25 mL), was added dropwise 85 % phosphoric acid (3.3 mL). The mixture was then stirred for 15 h at room temperature. Saturated sodium bicarbonate was added (100 mL) until pH 7-8. Dichloroethane was evaporated, and the mixture was extracted with AcOEt. The organic layer was washed with water and brine and dried over Na_2SO_4 . After evaporation of the solvents under reduced pressure, the residue was purified by flash chromatography (AcOEt/heptane/ Et_3N 30:70:0.2) to afford **5** (0.582 g, 91%).

Compound **6**; To a solution of **5** (0.54 g, 1.68 mmol) in anhydrous 1,2-dichloroethane (11 mL) under argon, was added N-Boc-2-aminoacetaldehyde (0.32 g, 2.02 mmol). The reaction was stirred at room temperature for 3 h. Sodium triacetoxyborohydride (0.46 g, 2.18 mmol) was then

added and the mixture stirred for 5 h. A saturated sodium bicarbonate solution was then added until pH 7-8. After evaporation of 1,2-dichloroethane, the aqueous layer was extracted with AcOEt. The organic phase was then washed with water and brine, dried over Na₂SO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography (AcOEt/heptane/Et₃N 30:70:0.1) to afford **6** (0.76 g, 1.63 mmol, 97%).

Compound 7; To a solution of **6** (0.76 g, 1.63 mmol) in anhydrous 1,2-dichloroethane (10 mL) under argon, was added a solution of propanal (0.13 mL, 1.8 mmol) in anhydrous 1,2-dichloroethane (1.2 mL). The mixture was stirred at room temperature for 3 h. Sodium triacetoxyborohydride (0.42 g, 1.96 mmol) was added and the mixture was stirred at room temperature for 12 h. A saturated sodium bicarbonate solution was then added until pH 7-8. After evaporation of 1,2-dichloroethane, the aqueous layer was extracted with AcOEt. The organic phase was then washed with water and brine, dried over Na₂SO₄ and evaporated under reduced pressure. The residue was purified by flash chromatography (AcOEt/heptane/Et₃N 10:90:0.1) to afford **7**.

2PNT; To a solution of **7** (0.82 g, 1.63 mmol) in anhydrous 1,2-dichloroethane (20 mL), was added dropwise 85 % phosphoric acid (2.8 mL). The mixture was then stirred for 3 h at room temperature. Saturated sodium bicarbonate was added until pH 7-8. Dichloroethane was evaporated, and the mixture was extracted with AcOEt. The organic layer was washed with water and brine and dried over Na₂SO₄. The solvents were evaporated under reduced pressure to afford **2PNT** (0.63 g, 96%) as shown in Scheme 3.1.

3.2.3 Characterizations of Two-Photon nanotrigger

The characterizations for the synthesis of the 2PNT were done using NMR spectroscopy. For compound **1**; ^1H NMR (DMSO- d_6 , 300.13 MHz): δ 6.99 and 7.41 (AA'BB', 4H), 7.63 and 8.24 (AA'XX', JAX = 8.9 Hz, 8H); ^{13}C NMR (DMSO- d_6 , 75.46 MHz): δ 124.1, 127.5, 133.0, 133.5, 143.4, 146.4. For compound **2**; ^1H NMR (DMSO- d_6 , 300.13 MHz): δ 5.23 (s, 4H), δ 6.37 and 6.65 (AA'BB', 4H), 6.50 and 7.11 (AA'XX', JAX = 8.5 Hz, 8H); ^{13}C NMR (DMSO- d_6 , 75.46 MHz): δ 114.4, 125.4, 125.8, 127.5, 131.1, 148.7. For compound **3**; ^1H NMR (DMSO- d_6 , 300.13 MHz): δ 1.47 (s, 9H), 5.30 (s, 2H), 6.45-6.83 (m, 4H), 6.53 and 7.14 (AA'XX', JAX = 8.5 Hz, 4H), 7.34 and 7.38 (AA'BB', 4H), 9.38 (s, 1H); ^{13}C NMR (DMSO- d_6 , 75.46 MHz): δ 28.1, 79.1, 113.9, 118.2, 124.2, 124.9, 126.3, 127.4, 128.4, 129.3, 131.5, 132.8, 138.5, 148.6, 152.7. For compound **4**; ^1H NMR (DMSO- d_6 , 300.13 MHz): δ 0.87 (t, J = 7.3 Hz, 6H), 1.52 (m, 4H), 1.48 (s, 9H), 3.23 (t, J = 7.3 Hz, 4H), 6.46-6.96 (m, 4H), 6.59 and 7.26 (AA'XX', JAX = 8.4 Hz, 4H), 7.33 and 7.40 (AA'BB', JAB = 8.4 Hz, 4H), 9.40 (s, 1H); ^{13}C NMR (DMSO- d_6 , 75.46 MHz) δ 11.1, 20.0, 28.1, 51.8, 79.0, 111.4, 118.1, 124.0, 124.3, 126.3, 127.5, 128.4, 129.3, 131.5, 132.4, 138.5, 147.4, 152.6. For compound **5**; ^1H NMR (DMSO- d_6 , 300.13 MHz): δ 0.87 (t, J = 7.3 Hz, 6H), 1.51 (sext, J = 7.5 Hz, 4H), 3.22 (t, J = 7.3 Hz, 4H), 5.26 (s, 2H), 6.53 and 6.68 (AA'BB', 4H), 6.50 and 7.12 (AA'XX', JAX = 8.5 Hz, 4H), 6.57 and 7.22 (AA'XX', JAX = 8.9 Hz, 4H). For Compound **6**; ^1H NMR (DMSO- d_6 , 300.13 MHz) : δ 0.87 (t, J = 7.3 Hz, 6H), 1.37 (s, 9H), 1.51 (sext, J = 7.5 Hz, 4H), 3.06 (m, 4H), 3.22 (t, J = 7.3 Hz, 4H), 5.83 (br s, 1H), 6.42 and 6.70 (AA'BB', 4H), 6.91 (br s, 1H), 6.52 and 7.19 (AA'XX', JAX = 8.6 Hz, 4H), 6.57 and 7.23 (AA'XX', JAX = 8.9 Hz, 4H); ^{13}C NMR (DMSO- d_6 , 75.46 MHz) δ 11.3, 20.1, 28.3, 30.7, 42.7,

52.0, 77.8, 111.5, 112.0, 124.5, 125.1, 125.5, 127.2, 127.3, 130.4, 130.6, 147.1, 148.1, 155.8. For compound **7**; ^1H NMR (DMSO- d_6 , 300.13 MHz): d 0.87 (t, $J = 7.3$ Hz, 9H), 1.37 (s, 9H), 1.51 (sext, $J = 7.5$ Hz, 6H), 3.03 (m, 2H), 3.22 (t, $J = 7.3$ Hz, 6H), 3.31 (m, 2H), 6.44 and 6.72 (AA'BB', 4H), 6.58 and 7.23 (AA'XX', JAX = 8.7 Hz, 4H), 6.72 and 7.23 (AA'XX', JAX = 8.7 Hz, 4H), 6.94 (t, $J = 6.0$ Hz, 1H). For the 2PNT; ^1H NMR (DMSO- d_6 , 300.13 MHz): d 0.87 (t, $J = 7.3$ Hz, 9H), 1.51 (sext, $J = 7.5$ Hz, 6H), 2.67 (br s, 2H), 3.31-3.23 (m, 10H), 6.43 and 6.71 (AA'BB', 4H), 6.58 and 7.23 (AA'XX', JAX = 8.9 Hz, 4H), 6.62 and 7.23 (AA'XX', JAX = 8.9 Hz, 4H); ^{13}C NMR (DMSO- d_6 , 75.46 MHz) d 11.1, 19.9, 20.0, 30.6, 51.8, 52.0, 111.3, 111.4, 124.3, 124.6, 124.9, 125.1, 127.0, 127.1, 130.1, 130.3, 147.0, 147.1; HRMS (ES+) calculated for $\text{C}_{27}\text{H}_{39}\text{N}_3$ (M^{+}) m/z 405.3144, found 405.3135.

3.2.4 Attachment of the Disulfide Snap-top to MSN Surface

The MCM-41 nanoparticles were synthesized using a base-catalyzed sol-gel procedure previously described in literature.^{6, 14, 15, 35-37} In a separate round bottom flask, the 2PNT (5×10^{-5} mol, 20.3 mg) was dissolved in 2 mL of ethanol. After the 2PNT was completely dissolved, 3-isocyanatopropyltriethoxysilane (5×10^{-5} mol, 12.4 μL) was added to the round bottom flask and left stirring overnight at room temperature under an inert nitrogen atmosphere. In a separate round bottom flask, 10 ml of dry toluene were mixed with 100 mg of surfactant extracted MSNs. The solution was added to the nanoparticle solution so the 2PNT would condense on the surface. The nanoparticle-2PNT mix was heated to reflux ($\sim 110^\circ\text{C}$) and stirred overnight under an inert

nitrogen atmosphere. The next day the particles were washed with methanol and water to afford 2PNT-linked mesoporous silica nanoparticles (2PNT-linked MSNs).

The disulfide snap-top was attached to the surface of the 2PNT-linked MSNs. In a round bottom flask 100 mg of the 2PNT-linked MSNs were dissolved in 15 mL of dry toluene and 3-(triethoxysilyl)-1-propanethiol (5×10^{-5} mol, 12.1 μ L) and the mixture was heated to reflux and stirred overnight under an inert nitrogen atmosphere. The next day the product was washed with methanol and toluene to remove any excess 3-(triethoxysilyl)-1-propanethiol adsorbed on the surface. Separately in a container lead(II) thiocyanate was combined with bromine in 10 mL of chloroform to yield lead (II) bromide and thiocyanogen. 1-Adamantanethiol (5×10^{-5} mol, .0085 mg) was added to this toluene mixture. The thiocyanogen was removed from the reaction as a yellow liquid and slowly added to the 2PNT-linked MSNs toluene solution while the reaction was stirred at 4°C under a nitrogen atmosphere for four days. The product was then washed thoroughly to remove molecules adsorbed on the surface of the MSNs. After the disulfide snap-top was fully assembled on the surface of the MSNs, the particles were soaked in a concentrated dye solution for 24 hr. to allow the dye molecules to diffuse into the pores of the MSNs. Then β -cyclodextrin was added and the solution stirred for an additional 24 hr. to allow β -cyclodextrin to associate with the adamantane molecule due to hydrophobicity. The bulky β -cyclodextrin acts as a pore cap preventing the cargo from escaping (Figure 3.3). Once the β -cyclodextrin is associated to the adamantane the MSNs were washed thoroughly to remove any dye that was adsorbed on the surface.

3.2.5 Characterization of Disulfide Snap-top Functionalized MSNs

The pore structure of MCM-41 nanoparticles was confirmed using powder X-ray Diffraction (PXRD) and transmission electron microscopy (TEM). From the TEM images the pore diameter was calculated at about 2.5 nm and the particle size about 100 nm (Figure 3.4a). From the PXRD the higher order peaks observed can be indexed as the (1 0 0), (1 1 0) and (2 0 0) planes with a lattice spacing of 4 nm (Figure 3.4b). The N₂ absorption-desorption isotherms showed a specific surface of 1044 m²/g.

The solid state NMR (ssNMR) of the snap-top-MSNs confirmed the condensation of the disulfide snap-top on the surface of the nanoparticles. The ¹³C ssNMR shows the peaks between 20 to 30 ppm from the propyl carbons attached to the surface of the MSNs. A separate ¹³C ssNMR was taken of the 2PNT-MSNs showing peaks in the aromatic region corresponding to the 2PNT molecule. Furthermore, the ²⁹Si ssNMR of both samples confirmed the functionalization of the silica showing peaks at -60 ppm corresponding to the silica functionalization and at around -100 ppm corresponding to the silica framework

The attachment of the 2PNT was also confirmed using excitation emission spectra showing peaks at around 350 nm for the excitation and around 480 nm for the emission spectra which match the emission-excitation peaks of the 2PNT in solution (Figure 3.4c). The emission spectrum of the cap and Rhodamine B loaded nanoparticles was taken showing an emission peak at around 580 nm (Figure 4.4d).

3.3 Results and Discussions

The operation of the snap-top was monitored by measuring the cargo released from the particles into the solution using continuous monitoring by fluorescence spectroscopy. The dye-loaded snap top- 2PNT-linked MSNs were placed in one corner of a two-by-one glass cuvette. Distilled water (pH~7) was carefully added to the cuvette in order to prevent the particles from mixing into the solution. In the opposite corner of the cuvette, a stirring magnet was placed to gently mix the released dye in to solution. The cuvette was then placed in front of monochromator to measure the fluorescence intensity.

3.3.1 Chemical snap-top operation

The activation of the snap-top container is based on the cleavage of disulfide bonds when they are reduced.^{38, 39} Mercaptoethanol causes chemical cleavage by the mechanism shown in Scheme 3.2.^{15, 40, 41} The reagent needs to be added in excess to drive the reaction equilibrium to the desired side. The modified particles loaded with pinacyanol iodide cargo were placed in front of a monochromator and the release of the dye monitored using 448 nm excitation laser. A baseline was taken for 80 minutes to check for any unwanted leakage of the dye. No increase in fluorescence intensity was observed during this time proving that the snap-top system successfully contains the cargo without any premature leakage. Then 2-mercaptoethanol was added in order to chemically reduce the disulfide bond. The cleavage of the disulfide bond releases the cap, unblocks the pores and allows the cargo to escape in to solution. As a result, the

fluorescence intensity immediately increases as shown in Figure 3.5. This experiment confirmed that the system is functional upon the attachment of the 2PNT on the surface of the nanoparticles.

3.3.2 One-photon stimulated release experiments

3.3.2.1 Release Experiments in the Presence of EDTA

The snap-top system was then tested for light activation. In this process, the 2PNT transfers a photo-excited electron to the disulfide bond, causing cleavage of the disulfide bond. For this experiment, the pinacyanol iodide-loaded particles were set up in a similar fashion as previously described for the chemical reduction. EDTA was added to the water solution as a sacrificial agent that donates an electron back into the 2PNT and reduces back electron transfer. A baseline was taken for 95 minutes to verify that there was no unwanted leakage of the dye. Then the 408-nm pump laser was directed into the particles precipitate and was turned on. The laser excited the 2PNT and induced electron transfer to the disulfide bond. The disulfide bond was cleaved, which removed the cap from the nanopores and allowed the cargo to escape into solution. The release profile was measured by the increase in fluorescence intensity, which plateaued after 8 hours, indicating the release was complete (Figure 3.6).

3.3.2.2 Release Experiments in the Absence of EDTA

A control experiment was carried out to study the system in the absence of a sacrificial electron donating agent. To verify that the system is in fact activated by an electron transfer from the nanotrigger to the disulfide bond, the EDTA was removed to increase the rate of back electron transfer and decrease/stop the activation of the system. A baseline was taken for 140

minutes with no premature leakage of the dye, after which the 408-nm pump laser was turned on (Figure 3.7). No increase in fluorescence intensity of the dye was observed, showing that no cargo escaped because the disulfide bond was not cleaved. After 150 minutes, EDTA was added to the cuvette and the fluorescence intensity increased. This experiment shows that the disulfide bond is cleaved only after the 2PNT is able to transfer an electron to the disulfide bond and the back electron transfer is suppressed. It is important to note that this experiment also shows that electron transfer not thermal heating is the major factor in the activation of the system, since the presence of transparent EDTA cannot change the amount of heat generated by absorption of the laser light.

3.3.3 Two-photon stimulated release experiments

To investigate the activation of the snap-top by two-photon excitation of the sensitizer, an ultrafast laser that emits 800 nm light was used as the excitation source. Since the UV-Vis absorption spectrum of the sensitizer shows no absorption at 800 nm (Figure 3.8), a two- (or more) photon process is required for photo-activation. The laser system used for the two-photon activation studies was not located near the *in situ* continuous fluorescence monitoring set-up that was used in the case of chemical and one-photon activation, therefore the way the release measurements for the two-photon case were performed had to be slightly modified. The cuvette was set up in a similar manner to the one-photon experiment, and the sample was irradiated with 800-nm fs light pulses for a fixed interval of time (60 minutes). Aliquots of the supernatant that contained the released cargo were taken after each interval and the fluorescence intensity was measured externally. After the measurements, the aliquots were returned carefully to the cuvette

prior to subsequent irradiation. The release profiles for two-photon activation of the functionalized and loaded MSNs were measured using Rhodamine B instead of pinacyanol iodide as the cargo due to its photostability and high fluorescence quantum yield. As in the one-photon experiments, EDTA was added to the water solution in the cuvette to act as a sacrificial electron-donating agent. The fluorescence intensity of several aliquots were measured prior to the 800 nm irradiation to verify the absence of premature cargo leakage (the flat baseline indicates no increase of fluorescence intensity over time). Once the baseline was established for 135 minutes, the 800-nm femtosecond pulsed laser was turned on. An immediate increase of the fluorescence intensity of the dye was observed. Aliquots were taken out for fluorescence measurement every sixty minutes for a total of four hours. During this time, the fluorescence intensity consistently continued to increase caused by the release of the dye in to the solution. The release profile shown in Figure 3.9 verifies that two-photon excitation of the sensitizer followed by electron transfer to the disulfide bond cleaves the bond holding the cap in place and allows the cargo to escape.

The femtosecond pulsed laser used for this study can produce high peak powers that might be able to break the disulfide bond directly by two-photon photodissociation. Thus, in order to prove that the system was activated by electron transfer, a control experiment was ran where no EDTA was added to the solution. A baseline was established once more to verify that there was no unwanted dye leakage. Excitation by the femtosecond pulsed laser was initiated after 180 m. As expected in the absence of EDTA, the back electron transfer was not suppressed and the disulfide bond was not cleaved. Thus, this experiment confirms the two-photon electron transfer

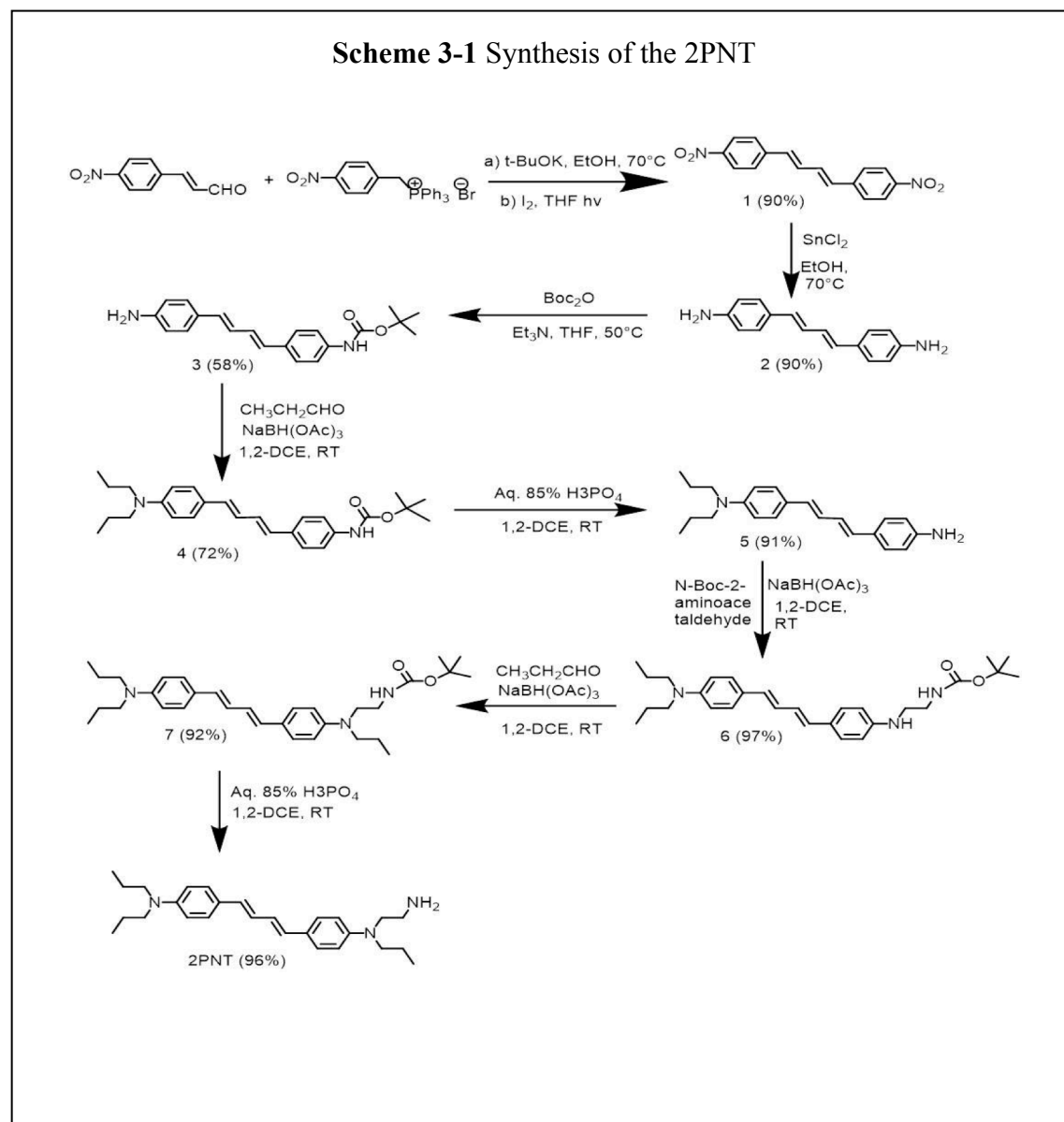
mechanism of activation (Figure 3.10).The operation of the system was monitored using fluorescence spectroscopy.

3.4 Conclusions

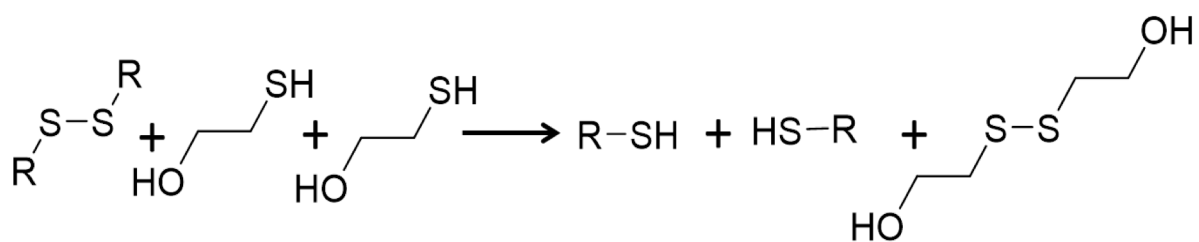
A highly versatile disulfide-based snap-top on MSNs has been synthesized and the activation of cargo release via chemical reduction or photo-excitation either by one UV photon or two near-IR coherent photons successfully demonstrated. Direct chemical reduction with 2-mercaptoethanol clearly shows that despite the attachment of the bulky 2PNT to the surface of the MSNs, the disulfide still retained its capacity to hold cargo inside the nanopores and release it when the bond is broken. The one-photon experiments prove that the system can be successfully activated with a 408-nm light source that causes electron transfer from 2PNT to the disulfide bond. Finally, it was also proven that 2PNT is able to transfer an electron and reduce the disulfide bond with two coherent near-IR photons. To our knowledge, this is the first example of a snap-top disulfide nanovalve activated by two Near-IR photons. The fact that we can use two-photon activation is especially important for use in biological environments owing to higher tissue penetration, greater focal control and the lack of tissue damage with the use of near-IR wavelengths. Thus, this system offers a new method for generating photoactivated drug delivery systems that would offer the possibilities of controlled cargo release of a wide variety of drugs.

3.5 Figures and Tables

Scheme 3-1 Synthesis of the 2PNT



Scheme 3-2 Disulfide chemical reduction mechanism



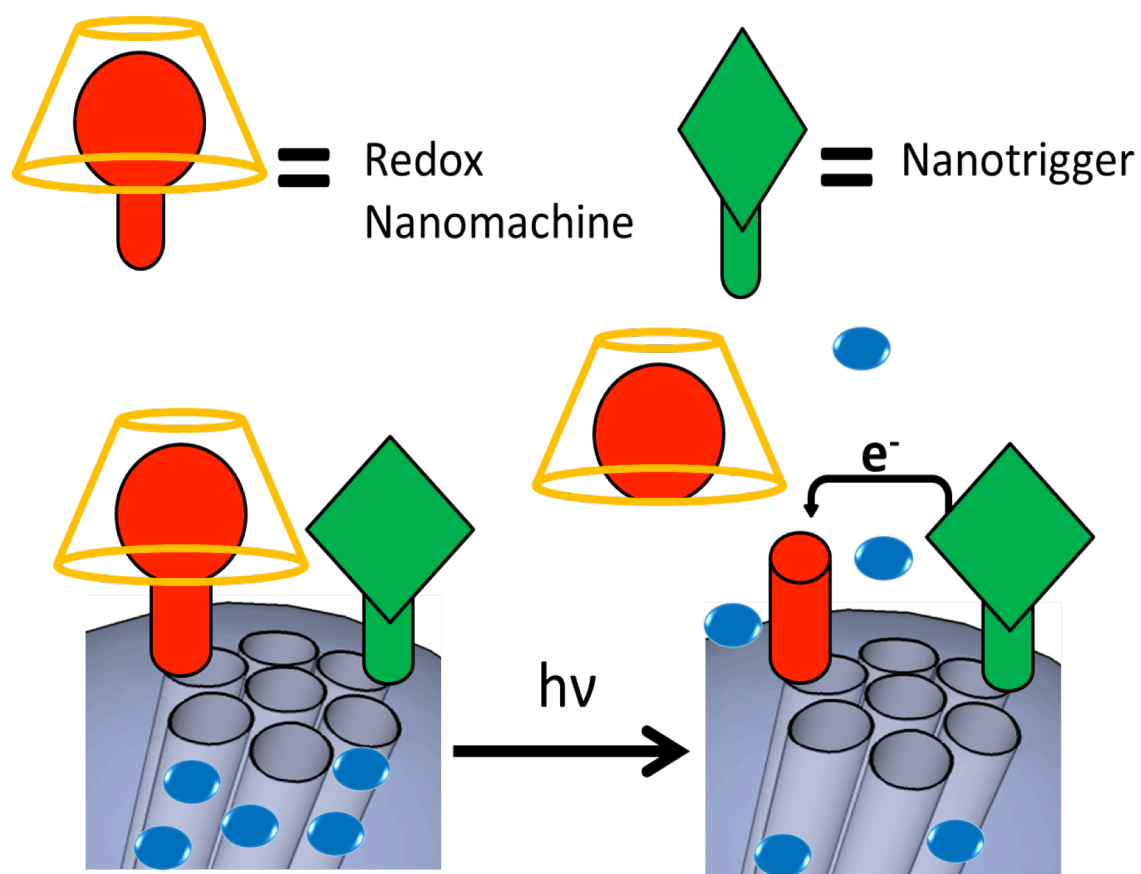


Figure 3-1 Schematic representation showing the components of the two photon activated redox "snap top".

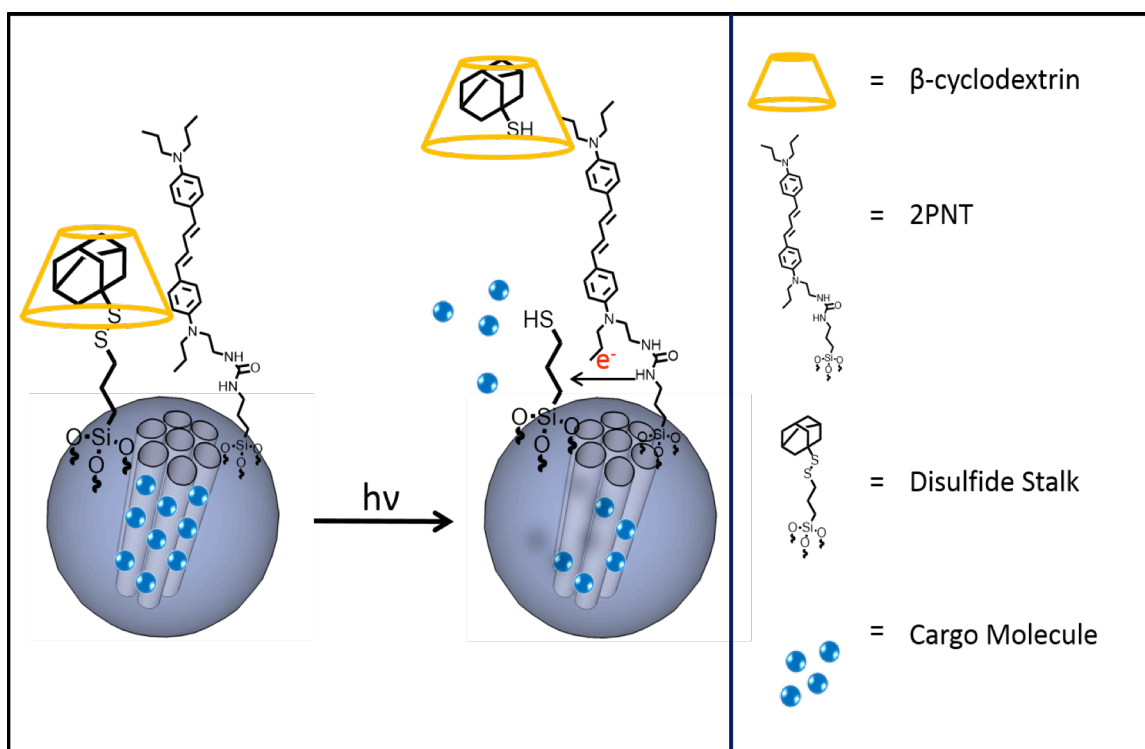


Figure 3-2 Disulfide snap-top opening mechanism. Photo-excitation of the photosensitizer causes an electron transfer to the snap-top cleaving the disulfide bond and opening the nanopores.

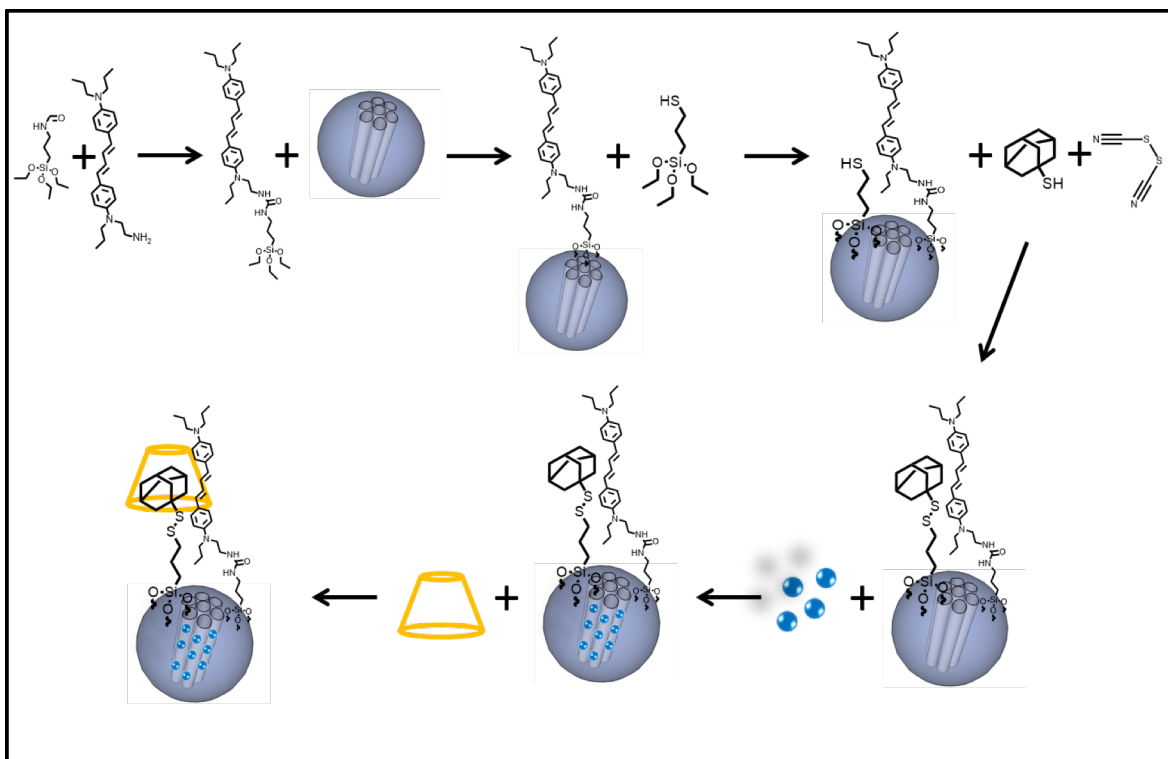


Figure 3-3 Schematic representation of the synthesis of fully assembled snap-top with 2PNT system.

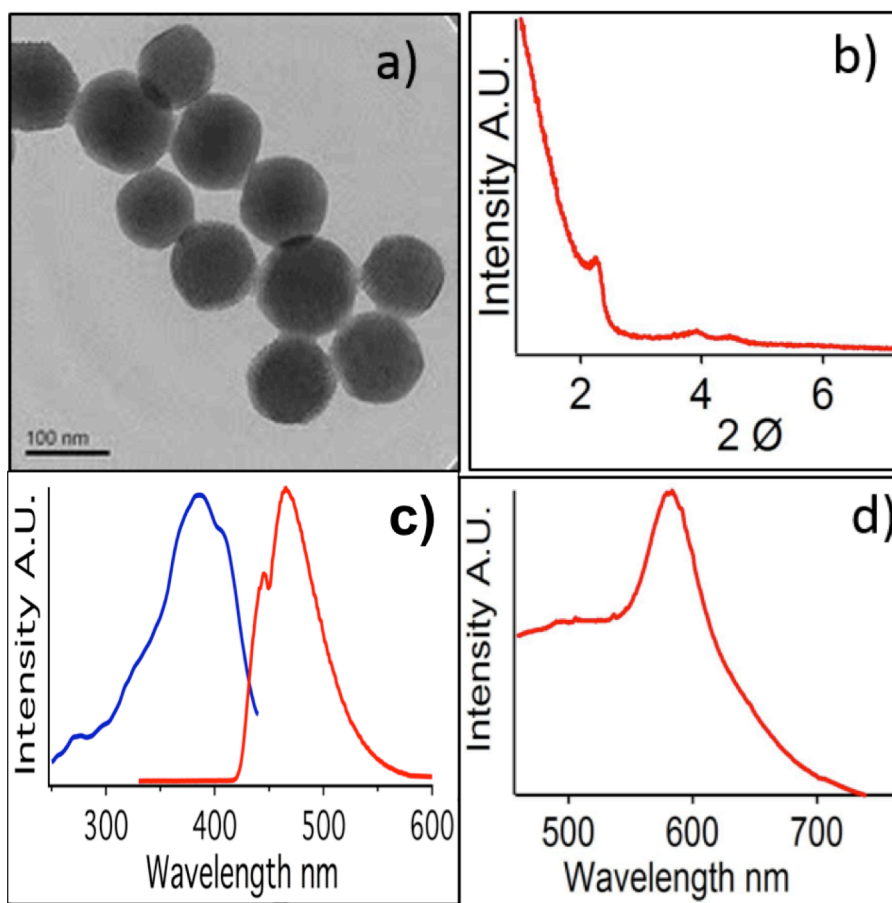


Figure 3-4 a) Transmission electron microscope image of surfactant extracted MCM-41 silica nanoparticles showing the hexagonal pore structure. b) Powder XRD of the surfactant extracted MCM-41 silica nanoparticles. c) Excitation spectrum of the bare MSNs (red, left), emission spectrum of the bare MSNs (purple, right), excitation spectrum of the 2PNT-MSNs (blue) and, emission spectrum of the 2PNT-MSNs (green). d) Emission of the Rhodamine B loaded and capped nanoparticles.

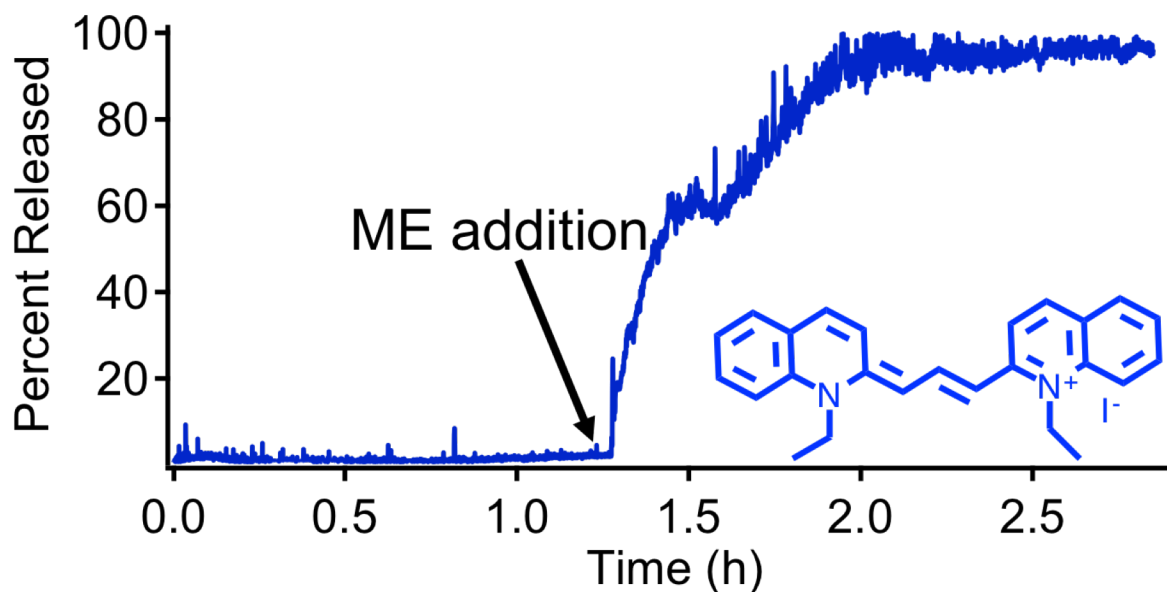


Figure 3-5 Release profile of pinacyanol iodide dye from the nanopores by chemical reduction of the disulfide snap-top. The arrow points to the addition of mercapto ethanol to the solution after 1.5 hours.

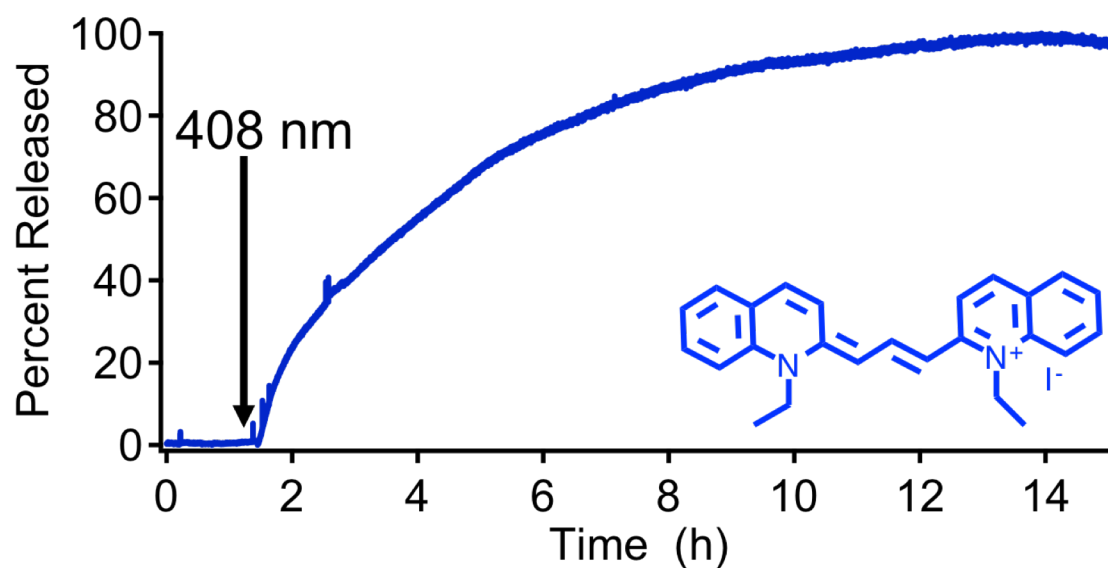


Figure 3-6 Release profile of pinacyanol iodide (EDTA was added to the solution) by the light induced electron transfer from the 2PNT when excited with a 408 nm laser source. The arrow points to the activation of the 408 nm pump laser. EDTA acts as a sacrificial agent that donates an electron to the 2PNT reducing back electron transfer

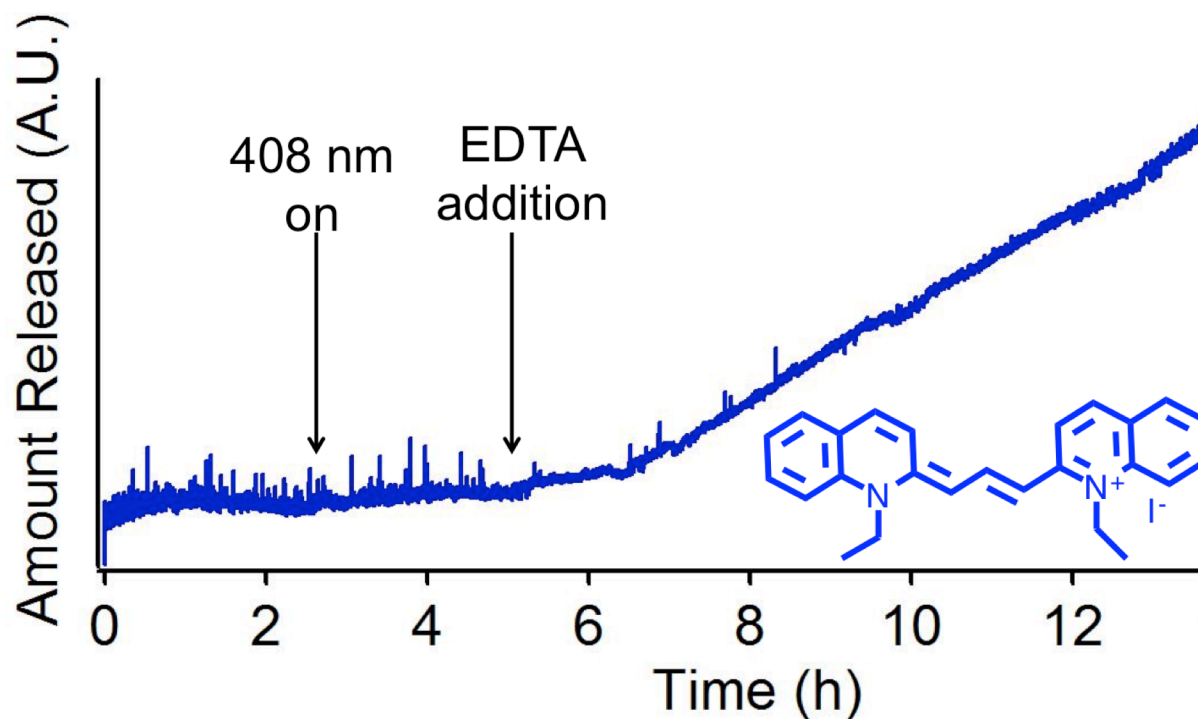


Figure 3-7 Release profile of pynocianol iodide in a solution without EDTA. The first arrow indicates when the 408 nm pump laser was turned on and no increase in fluorescence is observed. The second arrow points to the addition of EDTA and the fluorescence of the dye starts increasing. This proves that the opening of the snap top only occurs when the sacrificial agent is present and the back electron transfer to the 2PNT is minimized.

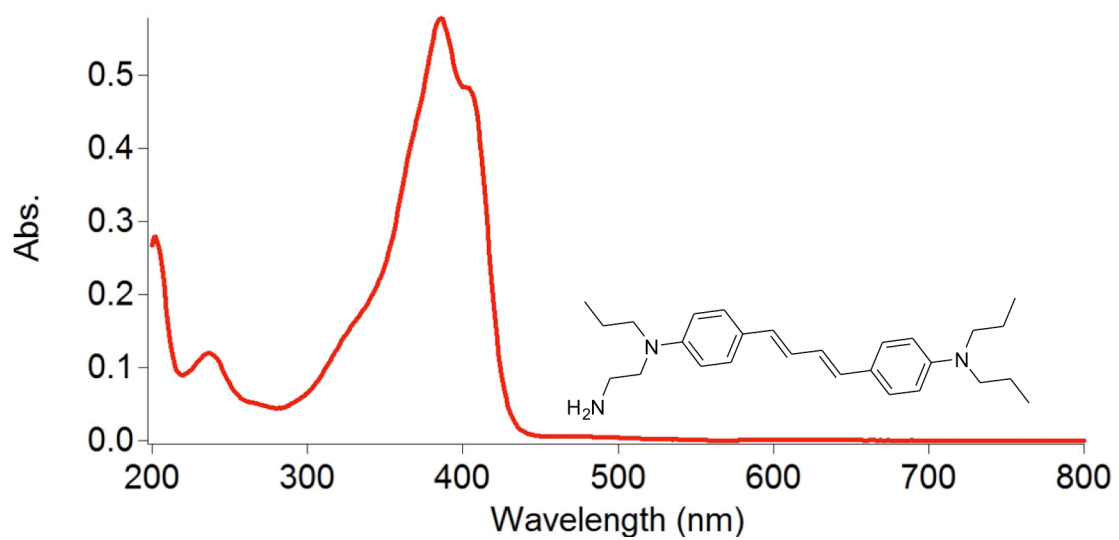


Figure 3-8 UV-Vis spectra of the 2PNT in methanol.

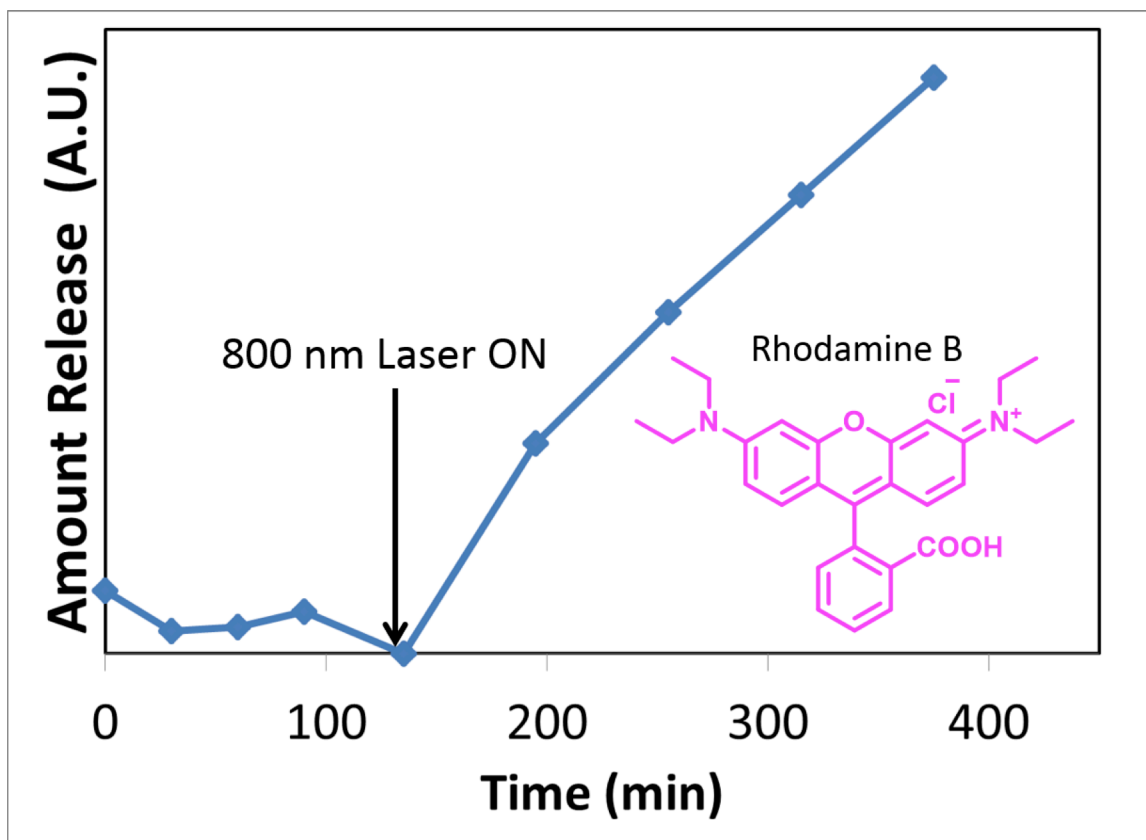


Figure 3-9 Release profile of Rhodamine B before and after the 800 nm pump laser has been turned on (shown by the arrow) with EDTA present in the aqueous solution. EDTA acts as a sacrificial agent that donates an electron to the 2PNT reducing back electron transfer

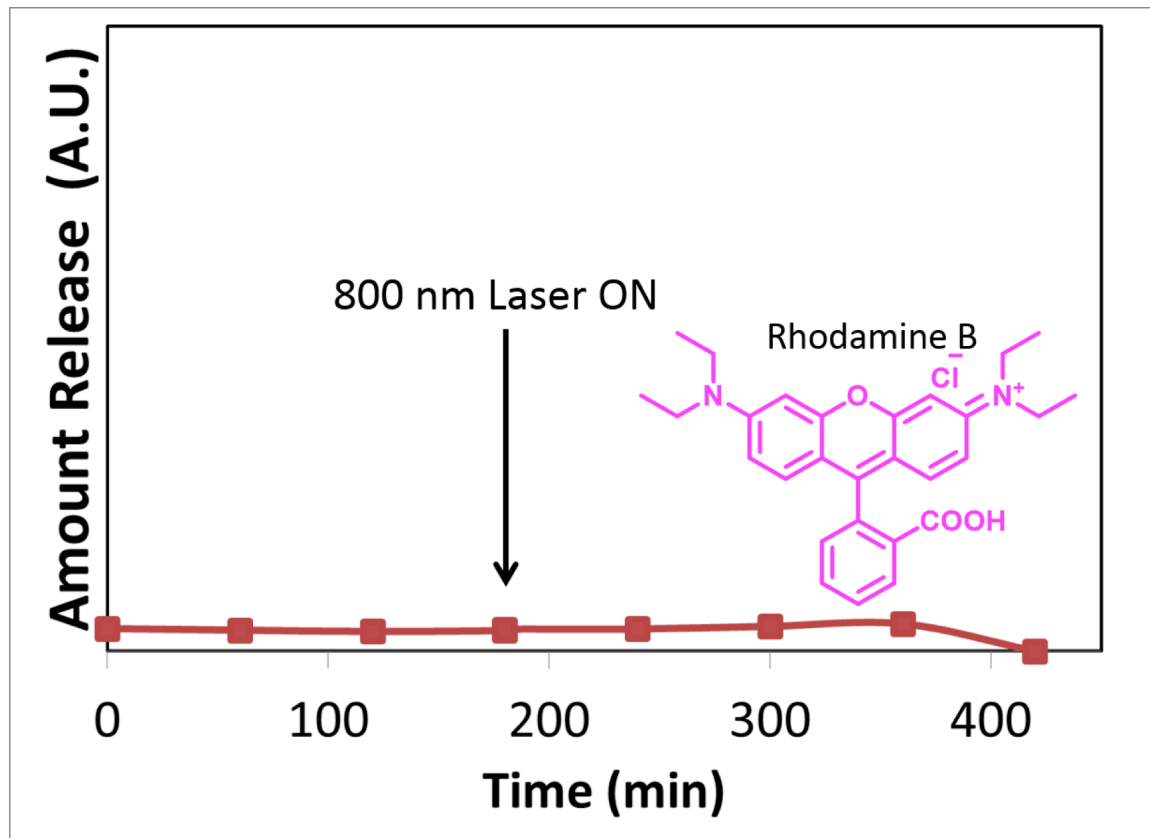


Figure 3-10 Release profile of Rhodamine B before and after the 800 nm pump laser has been turned on (shown by the arrow) without EDTA present in the solution.

3.6 References

1. Dolmans, D. E.; Fukumura, D.; Jain, R. K., Photodynamic therapy for cancer. *Nature Reviews Cancer* 2003, 3, 380-387.
2. Yoo, J.-O.; Ha, K.-S., 4 New Insights into the Mechanisms for Photodynamic Therapy-Induced Cancer Cell Death. *International Review of Cell and Molecular Biology* 2012, 295, 139.
3. Chatterjee, D. K.; Fong, L. S.; Zhang, Y., Nanoparticles in photodynamic therapy: an emerging paradigm. *Advanced drug delivery reviews* 2008, 60, 1627-1637.
4. Konan, Y. N.; Gurny, R.; Allémann, E., State of the art in the delivery of photosensitizers for photodynamic therapy. *Journal of Photochemistry and Photobiology B: Biology* 2002, 66, 89-106.
5. Lin, Y. S.; Wu, S. H.; Hung, Y.; Chou, Y. H.; Chang, C.; Lin, M. L.; Tsai, C. P.; Mou, C. Y., Multifunctional composite nanoparticles: magnetic, luminescent, and mesoporous. *Chemistry of materials* 2006, 18, 5170-5172.
6. Liong, M.; Lu, J.; Kovichich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I., Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *Acs Nano* 2008, 2, 889-896.
7. Park, H.; Yang, J.; Seo, S.; Kim, K.; Suh, J.; Kim, D.; Haam, S.; Yoo, K. H., Multifunctional nanoparticles for photothermally controlled drug delivery and magnetic resonance imaging enhancement. *Small* 2008, 4, 192-196.

8. Guardado-Alvarez, T. M.; Sudha Devi, L.; Russell, M. M.-L.; Schwartz, B. J.; Zink, J. I., Activation of Snap-Top Capped Mesoporous Silica Nano Containers Using Two Near-Infrared Photons. *Journal of the American Chemical Society* 2013.
9. Lin, Q.; Huang, Q.; Li, C.; Bao, C.; Liu, Z.; Li, F.; Zhu, L., Anticancer drug release from a mesoporous silica based nanophotocage regulated by either a one-or two-photon process. *Journal of the American Chemical Society* 2010, 132, 10645-10647.
10. Ferris, D. P.; Zhao, Y. L.; Khashab, N. M.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I., Light-operated mechanized nanoparticles. *Journal of the American Chemical Society* 2009, 131, 1686-1688.
11. Angelos, S.; Liong, M.; Choi, E.; Zink, J. I., Mesoporous silicate materials as substrates for molecular machines and drug delivery. *Chemical Engineering Journal* 2008, 137, 4-13.
12. Lau, Y. A.; Ferris, D. P.; Zink, J. I. In *Photo-driven nano-impellers and nanovalves for on-command drug release*, Proceedings of SPIE, 2010; p 75740P.
13. Thomas, C. R.; Ferris, D. P.; Lee, J. H.; Choi, E.; Cho, M. H.; Kim, E. S.; Stoddart, J. F.; Shin, J. S.; Cheon, J.; Zink, J. I., Noninvasive remote-controlled release of drug molecules in vitro using magnetic actuation of mechanized nanoparticles. *Journal of the American Chemical Society* 2010, 132, 10623-10625.
14. Li, Z.; Barnes, J. C.; Bosoy, A.; Stoddart, J. F.; Zink, J. I., Mesoporous silica nanoparticles in biomedical applications. *Chemical Society Reviews* 2012, 41, 2590-2605.
15. Ambrogio, M. W.; Pecorelli, T. A.; Patel, K.; Khashab, N. M.; Trabolsi, A.; Khatib, H. A.; Botros, Y. Y.; Zink, J. I.; Stoddart, J. F., Snap-top nanocarriers. *Organic Letters* 2010, 12, 3304-3307.

16. Ambrogio, M. W.; Thomas, C. R.; Zhao, Y.-L.; Zink, J. I.; Stoddart, J. F., Mechanized silica nanoparticles: a new frontier in theranostic nanomedicine. *Accounts of chemical research* 2011, 44, 903-913.
17. Angelos, S.; Yang, Y. W.; Patel, K.; Stoddart, J. F.; Zink, J. I., pH-Responsive Supramolecular Nanovalves Based on Cucurbit [6] uril Pseudorotaxanes. *Angewandte Chemie* 2008, 120, 2254-2258.
18. Angelos, S.; Khashab, N. M.; Yang, Y. W.; Trabolsi, A.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I., pH clock-operated mechanized nanoparticles. *Journal of the American Chemical Society* 2009, 131, 12912-12914.
19. Casasús, R.; Climent, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruiz, E., Dual aperture control on pH-and anion-driven supramolecular nanoscopic hybrid gate-like ensembles. *Journal of the American Chemical Society* 2008, 130, 1903-1917.
20. DeMuth, P.; Hurley, M.; Wu, C.; Galanie, S.; Zachariah, M. R.; DeShong, P., Mesoscale porous silica as drug delivery vehicles: Synthesis, characterization, and pH-sensitive release profiles. *Microporous and Mesoporous Materials* 2010.
21. Zhang, X.; Yang, P.; Dai, Y.; Ma, P. a.; Li, X.; Cheng, Z.; Hou, Z.; Kang, X.; Li, C.; Lin, J., Multifunctional Up-Converting Nanocomposites with Smart Polymer Brushes Gated Mesopores for Cell Imaging and Thermo/pH Dual-Responsive Drug Controlled Release. *Advanced Functional Materials* 2013.

22. Klichko, Y.; Liong, M.; Choi, E.; Angelos, S.; Nel, A. E.; Stoddart, J. F.; Tamanoi, F.; Zink, J. I., Mesoporous silica for optical functionality, nanomachines, and drug delivery. *Journal of the American Ceramic Society* 2008, 92, S2-S10.
23. Agostini, A.; Sancenón, F.; Martínez-Mañez, R.; Marcos, M. D.; Soto, J.; Amorós, P., A Photoactivated Molecular Gate. *Chemistry-A European Journal* 2012, 18, 12218-12221.
24. Zhao, Y.-L.; Li, Z.; Kabehie, S.; Botros, Y. Y.; Stoddart, J. F.; Zink, J. I., pH-operated nanopistons on the surfaces of mesoporous silica nanoparticles. *Journal of the American Chemical Society* 2010, 132, 13016-13025.
25. Wang, C.; Li, Z.; Cao, D.; Zhao, Y. L.; Gaines, J. W.; Bozdemir, O. A.; Ambrogio, M. W.; Frasconi, M.; Botros, Y. Y.; Zink, J. I., Stimulated Release of Size-Selected Cargos in Succession from Mesoporous Silica Nanoparticles. *Angewandte Chemie International Edition* 2012, 51, 5460-5465.
26. Patel, K.; Angelos, S.; Dichtel, W. R.; Coskun, A.; Yang, Y. W.; Zink, J. I.; Stoddart, J. F., Enzyme-responsive snap-top covered silica nanocontainers. *Journal of the American Chemical Society* 2008, 130, 2382-2383.
27. Lu, J.; Choi, E.; Tamanoi, F.; Zink, J. I., Light-Activated Nanoimpeller-Controlled Drug Release in Cancer Cells. *Small* 2008, 4, 421-426.
28. Johansson, E.; Choi, E.; Angelos, S.; Liong, M.; Zink, J. I., Light-activated functional mesoporous silica. *Journal of Sol-Gel Science and Technology* 2008, 46, 313-322.
29. Fomina, N.; McFearn, C.; Sermsakdi, M.; Edigin, O.; Almutairi, A., UV and near-IR triggered release from polymeric nanoparticles. *Journal of the American Chemical Society* 2010, 132, 9540-9542.

30. Kim, S.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Pandey, R. K.; Prasad, P. N., Organically modified silica nanoparticles co-encapsulating photosensitizing drug and aggregation-enhanced two-photon absorbing fluorescent dye aggregates for two-photon photodynamic therapy. *Journal of the American Chemical Society* 2007, 129, 2669-2675.
31. Starkey, J. R.; Rebane, A. K.; Drobizhev, M. A.; Meng, F.; Gong, A.; Elliott, A.; McInnerney, K.; Spangler, C. W., New two-photon activated photodynamic therapy sensitizers induce xenograft tumor regressions after near-IR laser treatment through the body of the host mouse. *Clinical Cancer Research* 2008, 14, 6564-6573.
32. Pawlicki, M.; Collins, H. A.; Denning, R. G.; Anderson, H. L., Two-Photon Absorption and the Design of Two-Photon Dyes. *Angewandte Chemie International Edition* 2009, 48, 3244-3266.
33. Porrès, L.; Alain, V.; Thouin, L.; Hapiot, P.; Blanchard-Desce, M., Optical and electrochemical properties of soluble donor- π -donor compounds as potential molecular wires and electrochemically-triggered optical switches. *Physical Chemistry Chemical Physics* 2003, 5, 4576-4582.
34. Robin, A.-C.; Gmouh, S.; Mongin, O.; Jouikov, V.; Werts, M. H. V.; Gautier, C.; Slama-Schwok, A.; Blanchard-Desce, M., A NADPH substitute for selective photo-initiation of reductive bioprocesses via two-photon induced electron transfer. *Chemical Communications* 2007, 1334-1336.
35. Meng, H.; Xue, M.; Xia, T.; Ji, Z.; Tarn, D. Y.; Zink, J. I.; Nel, A. E., Use of size and a copolymer design feature to improve the biodistribution and the enhanced permeability and

retention effect of doxorubicin-loaded mesoporous silica nanoparticles in a murine xenograft tumor model. *Acs Nano* 2011, 5, 4131-4144.

36. Beck, J. S.; Vartuli, J. C.; Roth, W. J.; Leonowicz, M. E.; Kresge, C. T.; Schmitt, K. D.; Chu, C. T. W.; Olson, D. H.; Sheppard, E. W., A new family of mesoporous molecular sieves prepared with liquid crystal templates. *Journal of the American Chemical Society* 1992, 114, 10834-10843.

37. Kresge, C. T.; Leonowicz, M. E.; Roth, W. J.; Vartuli, J. C.; Beck, J. S., Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. *nature* 1992, 359, 710-712.

38. Anfinsen, C. B.; Haber, E., Studies on the reduction and re-formation of protein disulfide bonds. *J Biol Chem* 1961, 236, 1361-1363.

39. Lu, C.; Bucher, G.; Sander, W., Steady-State and Time-Resolved Studies on Photoinduced Disulfide Bond Cleavage Using Aniline as an Electron Donor. *ChemPhysChem* 2004, 5, 399-402.

40. Lai, C. Y.; Trewyn, B. G.; Jeftinija, D. M.; Jeftinija, K.; Xu, S.; Jeftinija, S.; Lin, V. S. Y., A mesoporous silica nanosphere-based carrier system with chemically removable CdS nanoparticle caps for stimuli-responsive controlled release of neurotransmitters and drug molecules. *Journal of the American Chemical Society* 2003, 125, 4451-4459.

41. Vivero-Escoto, J. L.; Slowing, I. I.; Wu, C. W.; Lin, V. S. Y., Photoinduced intracellular controlled release drug delivery in human cells by gold-capped mesoporous silica nanosphere. *Journal of the American Chemical Society* 2009, 131, 3462-3463.

Chapter 4

Acidification of the Surfaces of Mesoporous Silica Nanoparticles by a Photoacid Activates an Acid Nanovalve

4.1 Introduction

Over the past decades photoacids have had a major role in the study of proton transfer. They are aromatic molecules that have an excited state pK_a much lower than that of the ground electronic state. Photoacids are organic molecules that become more acidic upon electronic excitation.^{1, 2} The change in acidity causes the proton to dissociate in aqueous solutions within its excited state lifetime. Some photoacids exhibit adiabatic acid-base equilibrium in their excited state. Other than their transient state as photoacids, they completely resemble ground state protic acids.^{2, 3} They have been used for several application such as lithography,⁴ and sensors.⁵ However to our knowledge photoacids have never been used with mesoporous silica nanoparticles (MSNs) to deliver cargo. Functionalized MSNs have gained increased popularity for their use in cargo delivery applications. The most popular technique involves trapping molecules inside the mesopores,⁶⁻⁹ which is mainly achieved by functionalizing the MSN surface in order to block the entrance to the nanopores.^{10, 11} This allows the trapping and releasing of host molecules on command by using a variety of stimuli such as changes in redox potential,^{12, 13} light,^{8, 13-17} and pH,^{6, 18} as well as the use of enzymes.¹⁹ Nanovalves that are pH responsive have been extensively researched over the last decade.^{18, 20, 21} Du *et. al.* reported the use of an acid nanovalve over the pore opening of MSNs.⁶ Nanovalves that are light activated offer the advantage of spatial and temporal control.^{8, 13, 14, 16} This approach typically requires the use of a light responsive molecule to be used as the molecular machine.^{8, 14, 15, 17, 22, 23} Other examples include photocleavable caps,^{17, 22} pseudorotaxanes^{13, 14} among others.

In this chapter a photoacid was covalently attached to the surface of MSNs next to a previously published acid nanovalve (Figure 4.1).⁶ The acid responsive nanovalve was used as a probe to determine whether the photoacid could cause local acidification on the nanoparticle surface without changing the pH in the bulk solution. The proton transfer was indirectly tracked through the release of the fluorescent cargo molecule. For the photoacid to be able to activate an acid nanovalve the proton dissociation and recombination needs to be fast compared to the excited state lifetime. Pyrene based photoacids are particularly convenient for this purpose. It has been shown that these molecules have a geminate recombination that is reversible. This is the case of 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS). HPTS is known to have a ground state pKa of 8.1 that changes to 1.6 in its excited state.² 6,8-Dihydroxy-1,3-pyrenedisulfonic acid disodium salt (DHDS) was used instead of HPTS because it has an extra OH that was used as a reactive group for the attachment to silica. DHDS has been previously reported to act very similar to HPTS when one of the OH groups has been functionalized.²⁴ The nanovalve was chosen to have a pKa of ~6 which will allow the system to remain closed in a neutral environment, while being sensitive enough to the photoacid acidification of the MSNs surface. The acid nanovalve consists of an aniline based molecule (stalk) that forms a pH-dependent complex with α -cyclodextrin (α -CD). The α -CD functions as a bulky group that is able to cap to nanopores preventing the cargo from escaping. Adjacent to the stalk, DHDS was chemically bound to the surface of the MSNs. Upon light excitation DHDS protonates the stalk which causes the dethreading of α -CD uncapping the pores and allowing cargo to escape. This system demonstrates the ability of the photoacid to cause local acidification of the MSNs and

also provides an alternative method using light activation on previously existing pH sensitive systems in MSNs.

4.2 Experimental

4.2.1 General Methods

All reagents including tetraethyl orthosilicate, cetyl trimethylammonium bromide, sodium hydroxide, hydrogen chloride, α -cyclodextrin, triethylamine, 3-iodopropyltrimethoxysilane, p-anisidine, propidium iodide, 6,8-Dihydroxy-1,3-pyrenedisulfonic acid disodium salt and 3-(triethoxysilyl)propyl isocyanate are commercially available and were used without further purification. Powder X-ray diffraction measurements were carried out using a Panalytical X'Pert Pro powder diffractometer. The radiation source was copper ($K\alpha_1$ and $K\alpha_2 = 1.5418 \text{ \AA}$). FT-IR spectra were recorded on a Perkin-Elmer FT-IR Paragon 500 spectrometer. The transmission electron microscope (TEM) images of the silica nanoparticles were collected on a JEOL1200-EX instrument in the California NanoSystems Institute. Microfilms for TEM imaging were made by placing a drop of the particle suspension in methanol onto a 200-mesh copper TEM grid (Ted Pella, Inc., Redding, CA) and dried at room temperature. N₂ adsorption-desorption isotherms were obtained at 77 K on a Quandrchrome Surface Area and Pore size analyzer. The continuous monitoring of fluorescence release profiles were obtained using an Acton SpectraPro 2300i monochromator connected to a CCD for detection and CUBE 445, CUBE 375 and CUBE 408 (Coherent Inc., Santa Clara, CA, USA) diode lasers were used as the excitation source. UV-Vis spectra were collected on a Cary 5000 UV-Vis-NIP spectrophotometer.

4.2.2 Synthesis of Photoacid-Nanovalve-MSNs

The MSNs of the MCM-41 type were synthesized using a sol-gel method as previously published.^{11, 12, 31, 35, 36} The attachment of the stalk was accomplished by reacting MSNs (100 mg) with 3-iodopropyltrimethoxysilane (0.1 mmol) in 10 ml of dry toluene under N₂ atmosphere and reflux for 24 hours. After 24 hours the MSNs were washed with toluene and methanol and dried in a vacuum oven. Once the particles were dried, *p*-anisidine (1.0 mmol) and triethylamine (3.0 mmol, as catalyst) were added to the functionalized particles in a toluene solution, and refluxed under an inert N₂ atmosphere for 48 hours. The stalk-MSNs were then collected using centrifugation, followed by washing with toluene and methanol, prior to drying in a vacuum oven. DHDS (0.01 mmol) was reacted in dry toluene under an inert N₂ atmosphere with 3-isocyanatopropyltriethoxysilane (ICPES) (0.01 mmol) in order to condense onto the MSNs surface (Scheme 4.1). After that, the stalk-MSNs were added to the flask and allowed to react for an additional 24 hours. The DHDS-stalk-MSNs were recovered via centrifugation and washed with methanol.

4.2.3 Characterization of Photoacid-Nanovalve-MSNs

The MSNs were characterized using powder X-ray diffraction (pXRD) and transmission electron microscopy. From the TEM studies, the pore diameter was found to be around 2.5 nm and the particle size between 50 to 100 nm (Figure 4.2). From the pXRD the higher order peaks observed can be indexed as the (1 0 0), (1 1 0) and (2 0 0) planes with a lattice spacing of 4 nm (Figure 4.3). The N₂ absorption-desorption isotherms showed a specific surface of 1044 m² / g. The

Attachment of the DHDS was confirmed by fluorescence spectroscopy that was taken of the DHDS-MSNs showing an emission peak at around 450 nm which is consistent with the DHDS molecule emission when in solution (Figure 4.4). An infrared spectrum of the DHDS-MSNs was also taken showing a stretch at around 1300 cm^{-1} corresponding to the asymmetric sulfonate stretch (Figure 4.5). The attachment of the stalk was confirmed using UV-Vis which showed an absorption peak at around 300 nm consistent with the analine absorption (Figure 4.6). DHDS to valve ratios were found to be approximately 1:1 using UV-Vis and fluorescence spectroscopy.

4.2.4 Loading and Capping of Photoacid-Nanovalve-MSNs

The particles were then soaked in a concentrated solution of propidium iodide (PI) for 24 hours. Due to diffusion, PI enters the nanopores thus loading the particles with cargo. α -CD (100 mg) was then added to the solution so that it threads on the stalk in order to block the pores and prevent the cargo from escaping. Upon irradiation at 408 nm (which is within the absorption band of DHDS) the photoacid protonates the stalk causing the α -CD to dethread and allowing the release of the PI cargo.

4.3 Results and Discussions

4.3.1 One Photon Stimulated Release Experiments

The system was tested using continuous monitoring of the fluorescence of the cargo released. The particles were placed in a corner of a one by two centimeter glass cuvette. Deionized water

was then carefully added to the cuvette without disturbing the particle precipitate. On the opposite side of the cuvette, a small stirring magnet was placed to aid in the diffusion of PI. The cuvette was then placed in front of a detector and the fluorescence intensity was monitored over time. A 448 nm probe laser was used to excite PI (emission maximum at 617 nm) in the supernatant of the cuvette. In order to excite the photoacid a 408 nm (10 mW) pump laser was directed to the particles in the corner of the cuvette. The experimental results showed that the system can remain closed under aqueous conditions (pH ~7). Figure 4.7 shows that the fluorescence intensity does not increase for the first 45 minutes. Later, the 408 nm pump laser was turned on causing the photoacid to protonate the stalk dissociating the α -CD. Immediately after, an increase in fluorescence intensity is observed, indicating the release of PI from inside the MSNs nanopores and into solution showing the activation of the nanovalve via the photoacid.

4.3.2 Control Experiments

4.3.2.1 One Photon Stimulated Release in a TRIS Buffer

As a control experiment the system was tested in a tris(hydroxymethyl)aminomethane (TRIS) buffer solution (pH of 8.03) using the experimental set up previously described. The particles were excited using a 408 nm (10 mW) pump laser. As expected, the results showed (Figure 4.8a) that when the solvent is a buffer the proton dissociation from the photoacid is unable to significantly change the local pH on the surface of the MSNs, preventing the nanovalve from opening. The TRIS buffer trapped the proton that would have been transferred to the stalk. This suggests that even though there is no overall detectable pH change in solution, the local pH on the surface of the MSNs becomes acidic under aqueous conditions.

4.3.2.2 On and Off One Photon Stimulated Release Experiment

To test the on-command activation of the system an experiment was conducted where the pump laser was switched on and off. Without continuous laser excitation of the system, the proton returns to the DHDS and the pH on the MSNs surface becomes neutral. In this experiment the pump laser was first kept off to establish a baseline. At 30 minutes the 408 nm pump laser was turned on and an immediate increase in fluorescence intensity from the release of PI was observed. After 90 minutes, the pump laser was turned off again. The rate of release immediately decreased suggesting that the local pH returned to neutral and no more stalks were being protonated which stopped the uncapping of the remaining nanovalves as shown in Figure 4.9. The fluorescence intensity was still slightly increasing over time due to the slow diffusion of PI out of the pores that were already uncapped. This was repeated and the same overall trend was observed. This suggests that the protonation of the valve occurs only when exposed to the adequate light source that excites the DHDS allowing the protonation of the stalk and the uncapping of the nanopores.

4.3.2.3 Non-Absorbing 514 nm Wavelength Release Experiment

A separate control experiment was done to eliminate local heating by the pump laser as the cause for the dissociation of α -CD from the stalk. The wavelength was changed to 514 nm where the DHDS shows no absorption and thus is unable reach the excited state and transfer a proton to the stalk. The power used was 50 mW, which is five times higher the one previously use with the 408 nm (10 mW) pump laser experiments. In this experiment, as previously described, the pump laser was left off to establish a base line. After 20 minutes the 514 nm pump laser was turned on. No noticeable change in fluorescence intensity was observed. Then, in order to prove that the

system was still functional when the right excitation wavelength was used, the 514 nm laser was turned off and the 408 nm laser was turned on. Immediately after this, an increase in the fluorescence intensity of the PI was detected (Figure 4.10).

4.3.2.4 Acid Stimulated Release Experiment

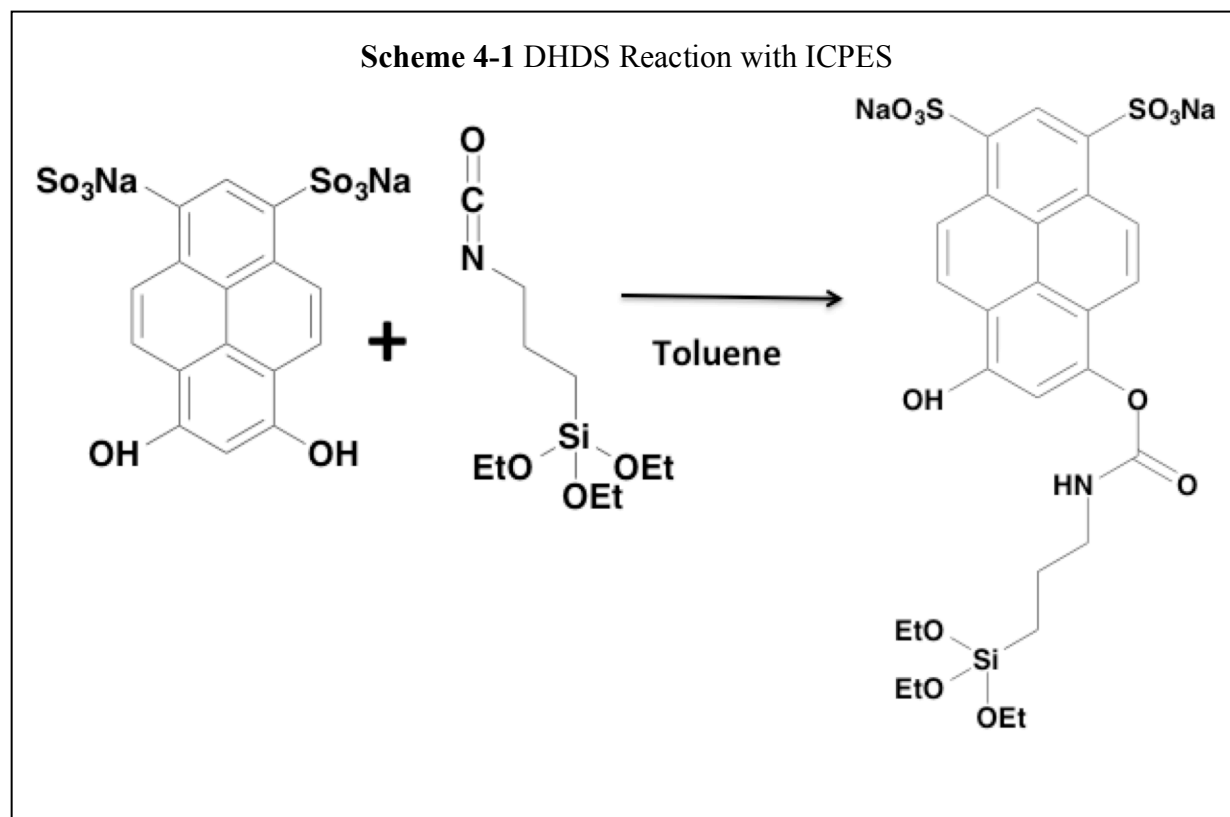
This system can alternatively be activated through a pH change in the surrounding solution similar to the original acid nanovalve. Setting up the experiment as previously described, the pump laser was left off for 20 minutes to establish a baseline. The 408 nm pump laser was then turned on and the fluorescence intensity showed an immediate increase. About 2 hours later the 408 nm laser was turned off and the solution was chemically acidified by the addition of concentrated HCl lowering the solution pH to 5. The fluorescence continued to increase in the same manner as by using the 408 nm pump laser as shown in Figure 4.11. This suggests that the local pH change in the MSN caused by the photoacid as a similar effect on the nanovalve to the acidification of the bulk solution to pH 5. The total amount of dye released during this experiment was calculated to be 2.78 w%.

4.4 Conclusion

A system has been synthesized by attaching DHDS next to an acid nanovalve on MSNs. By loading the MSNs with a fluorescent dye the acid nanovalve was used as a probe to detect local acidification on the MSN surface by the photoacid. Several control experiments were conducted that lead to the conclusion that the system is activated by the protonation of the acid nanovalve and not by local heating caused by the laser. This system is to our knowledge the first example of

a photoacid used with MSNs for cargo release. It also provides a method to probe chemical reaction on the surface of nanoparticles.

4.5 Figures and Tables



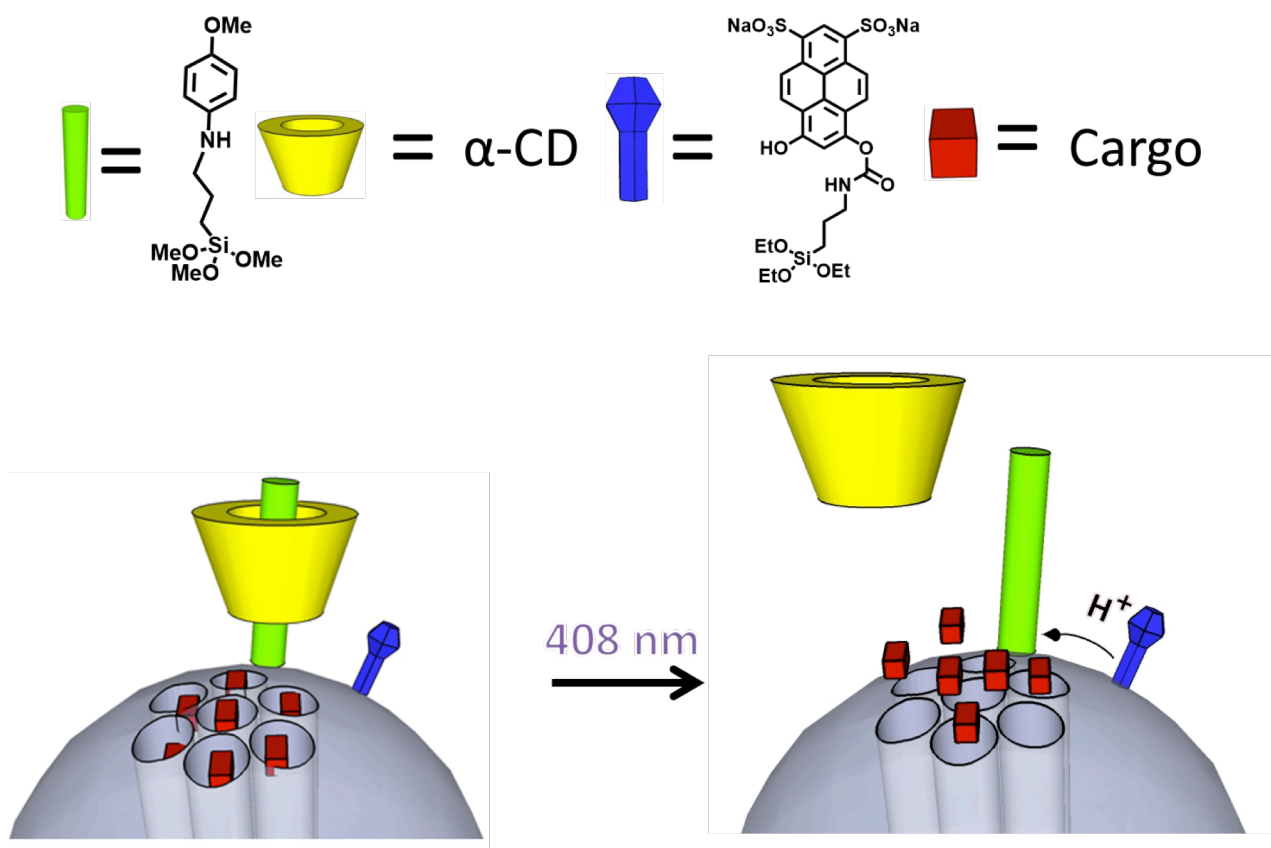


Figure 4-1 Mechanism for proton transfer from the photoacid to the acid nanovalve causes the dissociation of α -CD

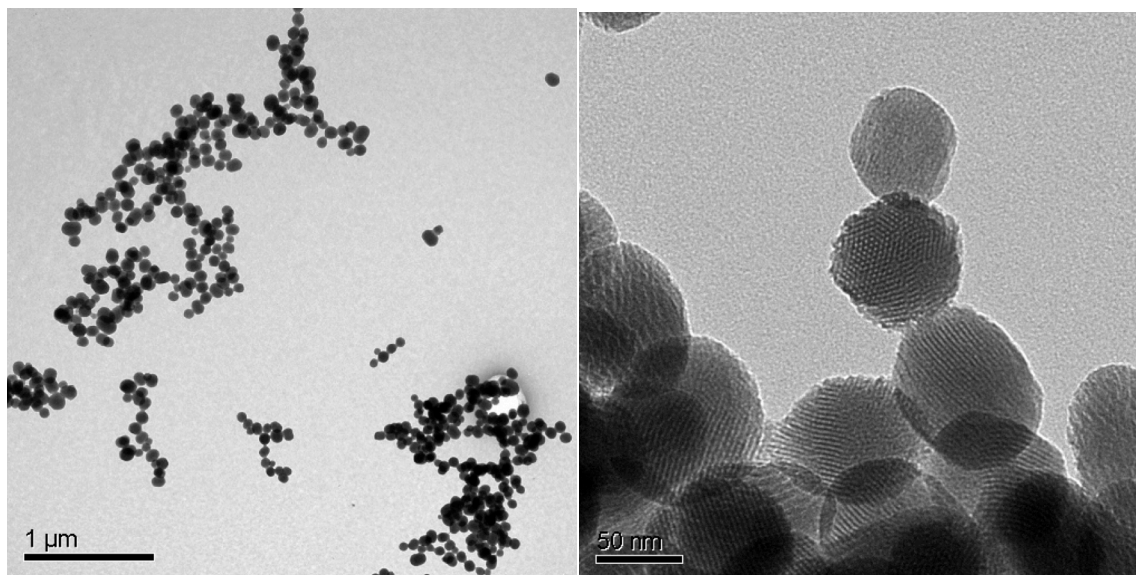


Figure 4-2 TEM images of the surfactant-extracted silica nanoparticles showing a hexagonal pore structure and a particles size of ~100 nm.

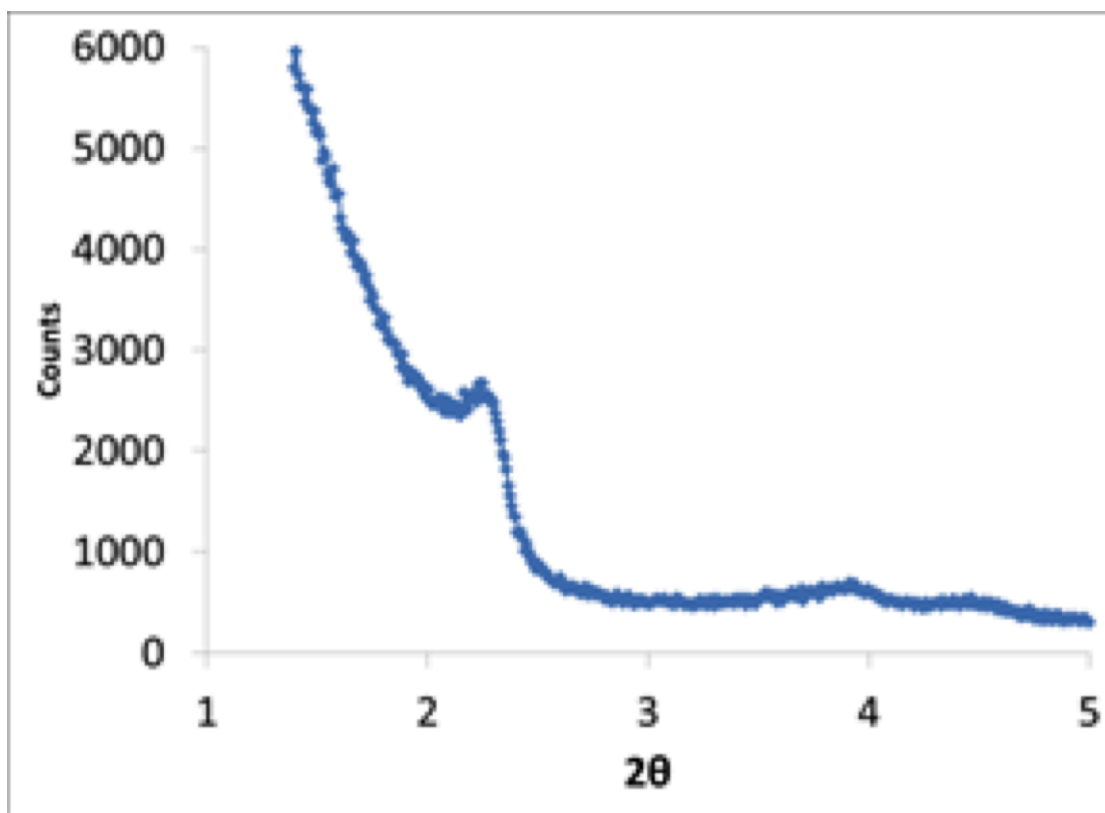


Figure 4-3 Powder XRD of the surfactant extracted MCM-41 silica nanoparticles.

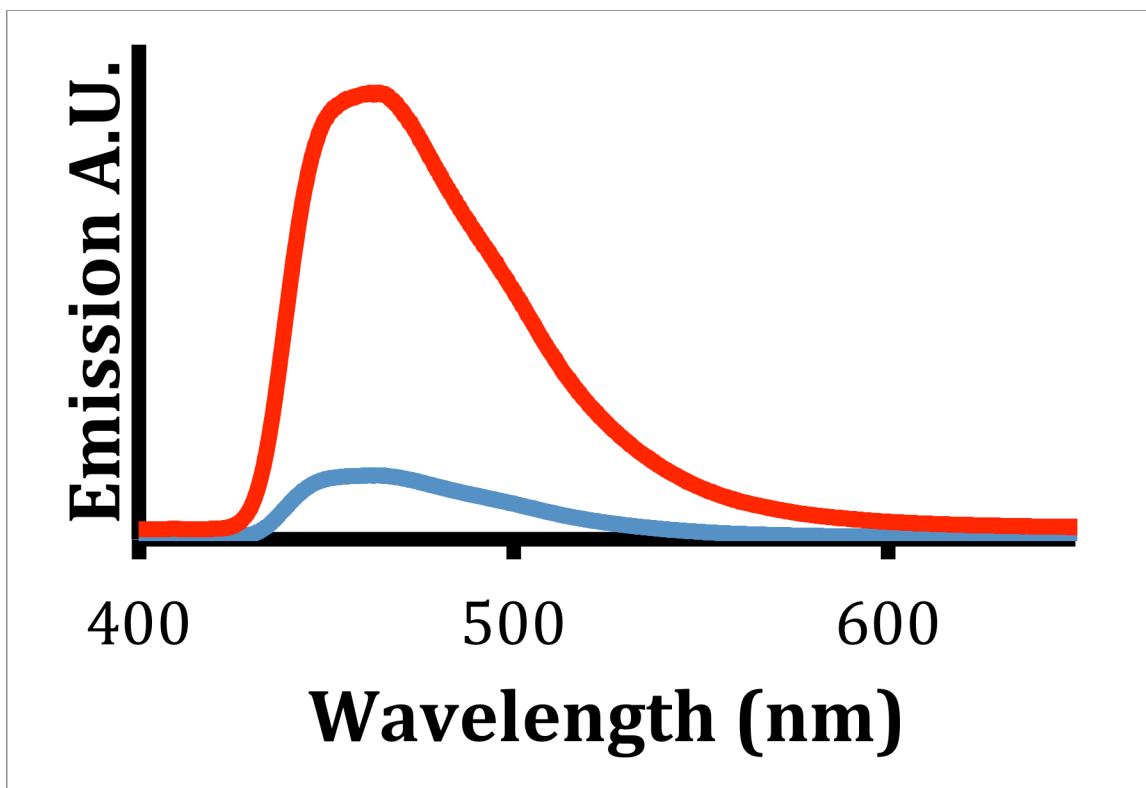


Figure 4-4 Emission spectrum of photoacid attached to particles (blue) and of the photoacid in solution (red).

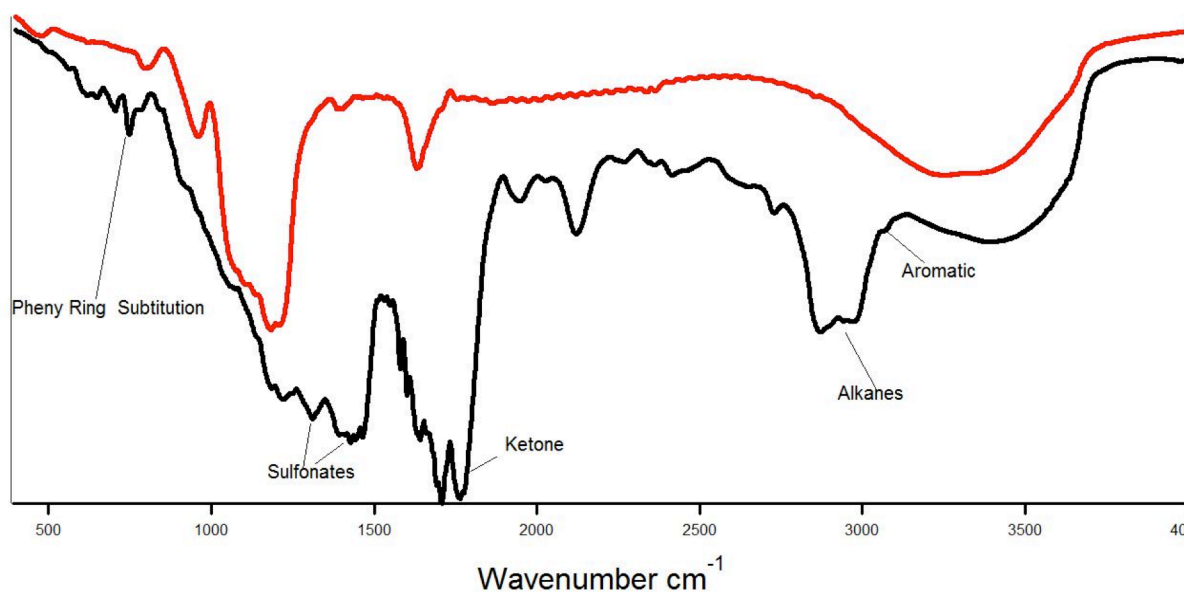


Figure 4-5 In the IR we can observe some of the distinctive peaks associated with the photoacid molecule. In the red the plain surfactant extracted MSNs, and in black the DHDS functionalized MSNs.

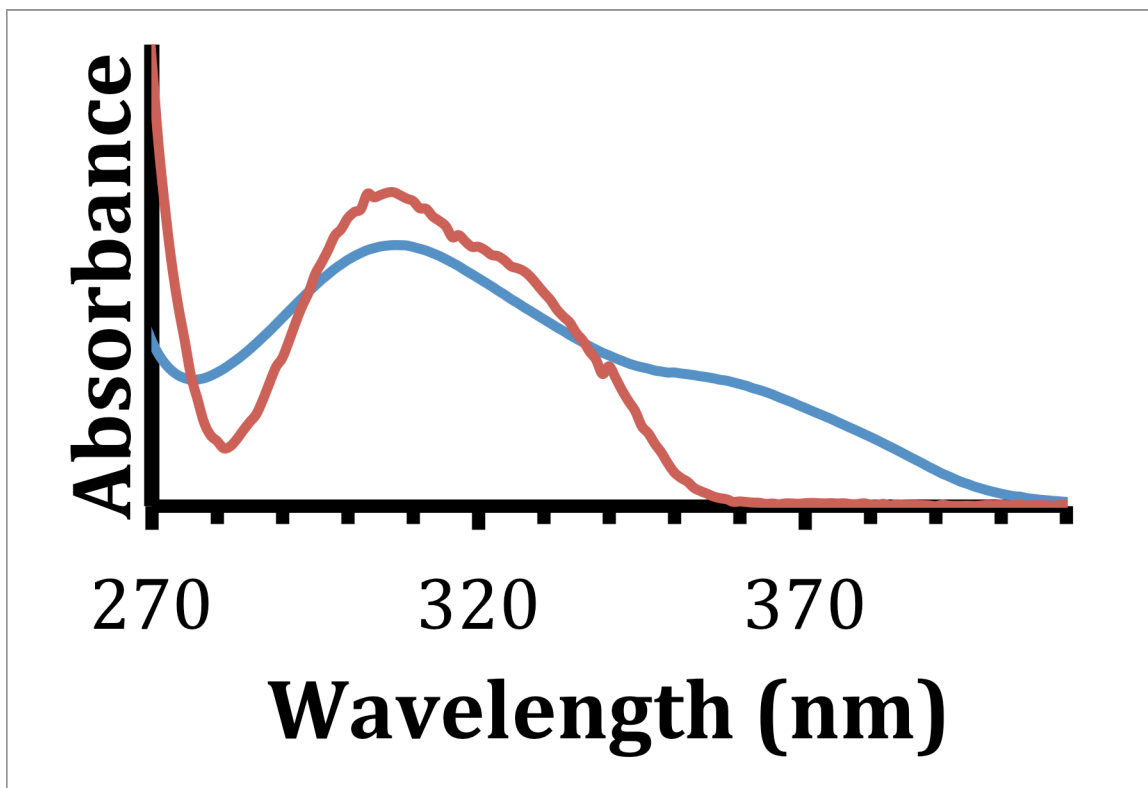


Figure 4-6 Blue line shows the absorbance of a standard solution of anisidine and the Red line represents a solution of the particles modified with the anisidine acid valve

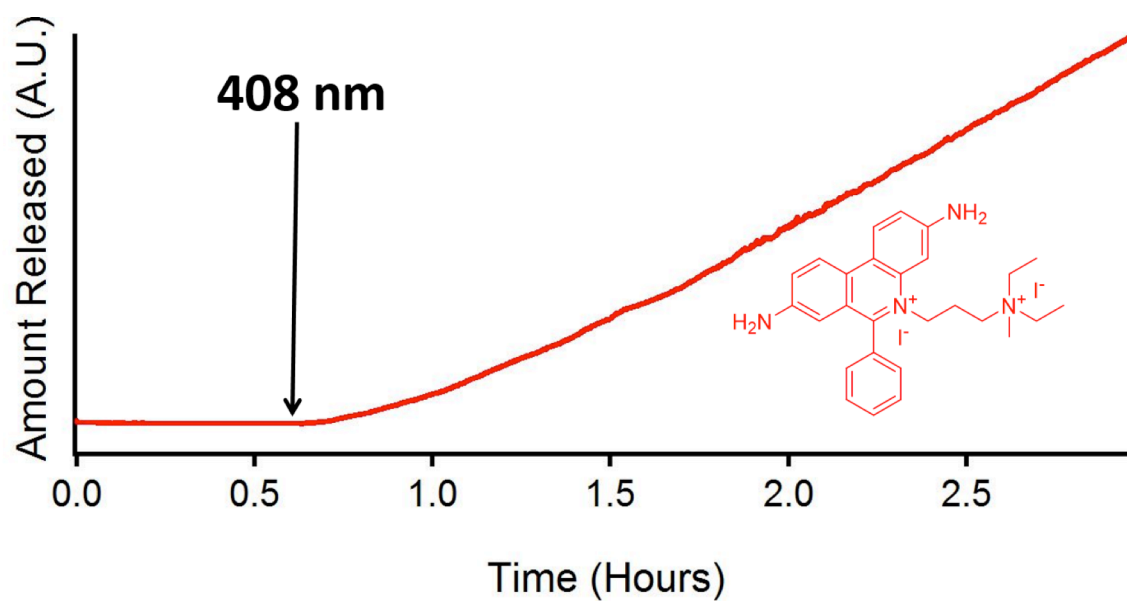


Figure 4-7 DHDS-stalk-MSNs in an aqueous solution. An increase in fluorescence intensity can be observed after the activation of the 408 nm pump laser

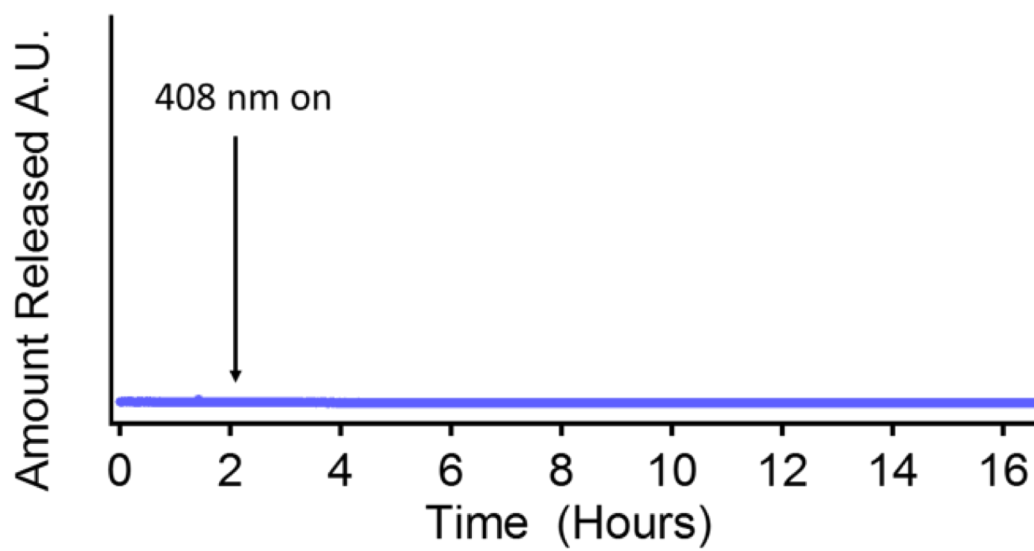


Figure 4-8 DHDS-stalk-MSNs in a TRIS buffer solution. Arrow indicates activation of the 408 nm (10 mW) pump laser

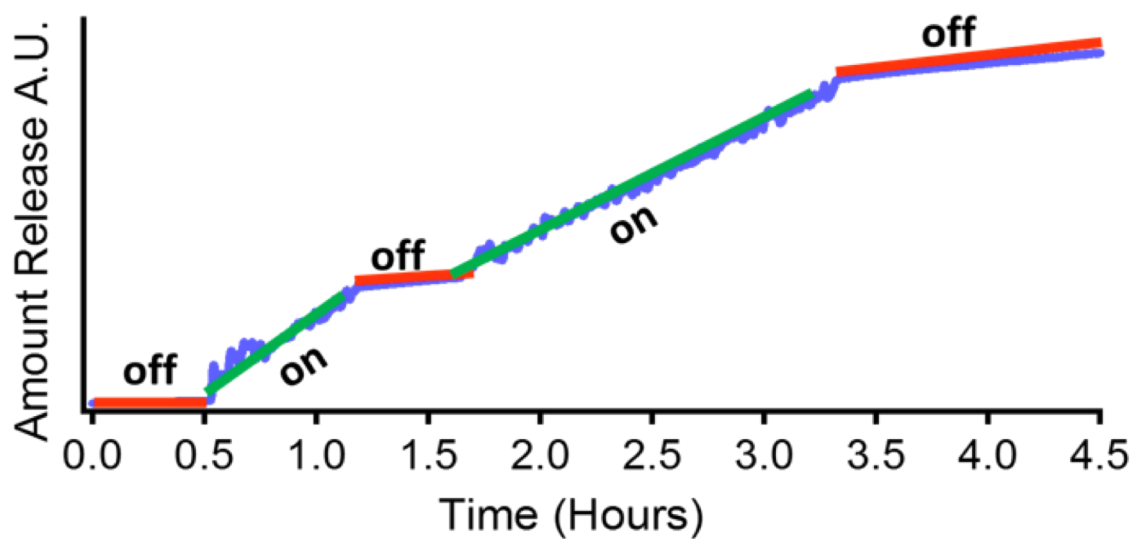


Figure 4-9 DHDS-stalk-MSNs in an aqueous solution, 408 nm (10 mW) pump laser was turned on and off showing that activation only occurs when the pump laser was on.

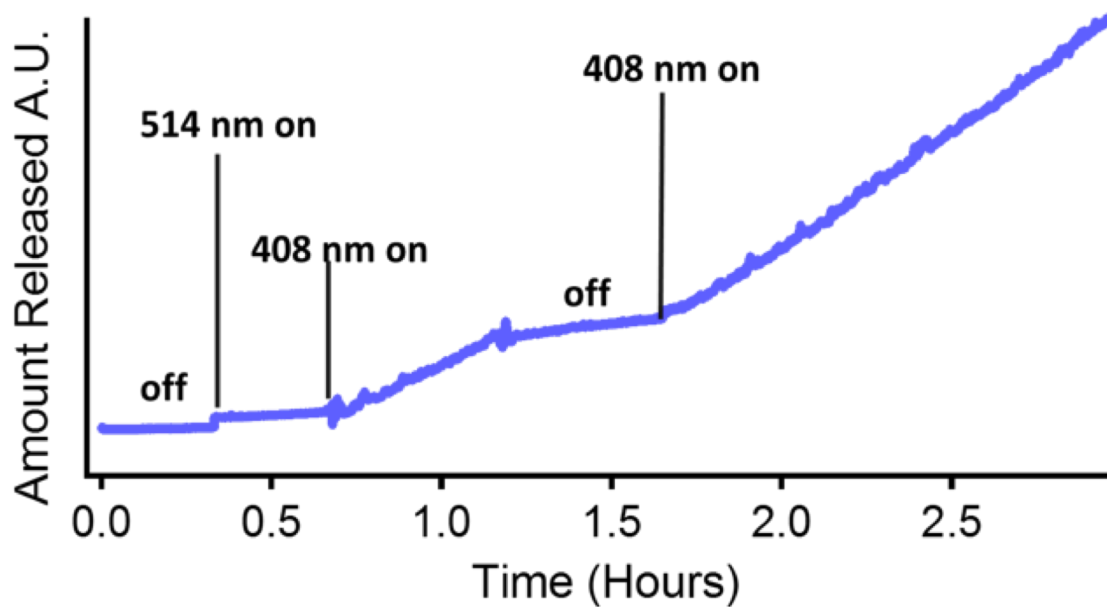


Figure 4-10 DHDS-stalk-MSNs in an aqueous solution. At 0.4 hours the 514 nm (50 mW) pump laser was turned on then at 0.75 hours the 514 nm (50 mW) pump laser was turned off and the 408 nm (10 mW) pump laser was turned on

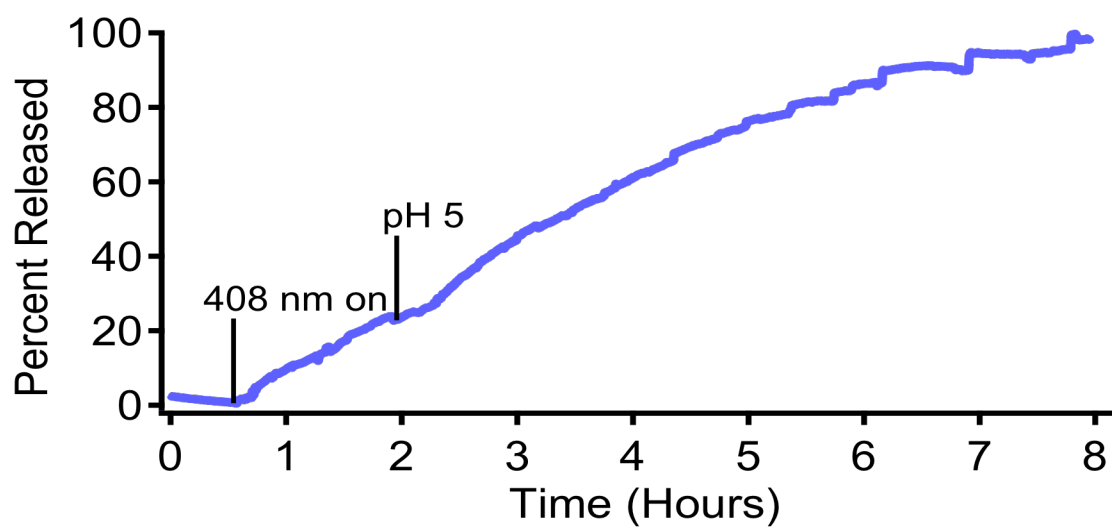


Figure 4-11 DHDS-stalk-MSNs in aqueous solution. First arrow points to the activation of the 408 nm (10 mW) pump laser second arrow indicates 408 nm pump laser off and solution acidification to pH 5 .

4.6 References

1. Ireland, J.-F.; Wyatt, P., Acid-base properties of electronically excited states of organic molecules. *Advances in physical organic chemistry* 1976, 12, 131-221.
2. Goldberg, S. Y.; Pines, E.; Huppert, D., Proton scavenging in photoacid geminate recombination processes. *Chemical physics letters* 1992, 192, 77-81.
3. Huang, M. H.; Soye, H. M.; Dunn, B. S.; Zink, J. I., In situ fluorescence probing of molecular mobility and chemical changes during formation of dip-coated sol-gel silica thin films. *Chemistry of Materials* 2000, 12, 231-235.
4. Nakano, K.; Maeda, K.; Iwasa, S.; Ohfuji, T.; Hasegawa, E. In *Positive chemically amplified resist for ArF excimer laser lithography composed of a novel transparent photoacid generator and an alicyclic terpolymer*, SPIE's 1995 Symposium on Microlithography, International Society for Optics and Photonics: 1995; pp 433-444.
5. Wu, H.; Gonsalves, K., Preparation of a photoacid generating monomer and its application in lithography. *Advanced Functional Materials* 2001, 11, 271-276.
6. Tokita, S.; Watanabe, F.; Hashimoto, K.; Tachikawa, T., Application of Photo Acid Generators for gamma Rays Detective Materials. *Journal of Photopolymer Science and Technology* 2001, 14, 221-224.
7. Du, L.; Liao, S.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I., Controlled-access hollow mechanized silica nanocontainers. *Journal of the American Chemical Society* 2009, 131, 15136.

8. Angelos, S.; Liong, M.; Choi, E.; Zink, J. I., Mesoporous silicate materials as substrates for molecular machines and drug delivery. *Chemical Engineering Journal* 2008, 137, 4-13.
9. Johansson, E.; Choi, E.; Angelos, S.; Liong, M.; Zink, J. I., Light-activated functional mesostructured silica. *Journal of Sol-Gel Science and Technology* 2008, 46, 313-322.
10. Klichko, Y.; Liong, M.; Choi, E.; Angelos, S.; Nel, A. E.; Stoddart, J. F.; Tamanoi, F.; Zink, J. I., Mesostructured silica for optical functionality, nanomachines, and drug delivery. *Journal of the American Ceramic Society* 2008, 92, S2-S10.
11. Liong, M.; Lu, J.; Kovichich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I., Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *Acs Nano* 2008, 2, 889-896.
12. Li, Z.; Barnes, J. C.; Bosoy, A.; Stoddart, J. F.; Zink, J. I., Mesoporous silica nanoparticles in biomedical applications. *Chemical Society Reviews* 2012, 41, 2590-2605.
13. Chen, Y.; Chen, H.; Shi, J., In Vivo Bio-Safety Evaluations and Diagnostic/Therapeutic Applications of Chemically Designed Mesoporous Silica Nanoparticles. *Advanced Materials* 2013.
14. Ambrogio, M. W.; Pecorelli, T. A.; Patel, K.; Khashab, N. M.; Trabolsi, A.; Khatib, H. A.; Botros, Y. Y.; Zink, J. I.; Stoddart, J. F., Snap-top nanocarriers. *Organic Letters* 2010, 12, 3304-3307.
15. Nguyen, T. D.; Leung, K. F.; Liong, M.; Liu, Y.; Stoddart, J. F.; Zink, J. I., Versatile supramolecular nanovalves reconfigured for light activation. *Advanced Functional Materials* 2007, 17, 2101-2110.

16. Ferris, D. P.; Zhao, Y. L.; Khashab, N. M.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I., Light-operated mechanized nanoparticles. *Journal of the American Chemical Society* 2009, 131, 1686-1688.
17. Lau, Y. A.; Ferris, D. P.; Zink, J. I. In *Photo-driven nano-impellers and nanovalves for on-command drug release*, Proceedings of SPIE, 2010; p 75740P.
18. Alvarez-Lorenzo, C.; Bromberg, L.; Concheiro, A., Light-sensitive Intelligent Drug Delivery Systems†. *Photochemistry and photobiology* 2009, 85, 848-860.
19. Banghart, M.; Borges, K.; Isacoff, E.; Trauner, D.; Kramer, R. H., Light-activated ion channels for remote control of neuronal firing. *Nature neuroscience* 2004, 7, 1381-1386.
20. Choi, S. K.; Thomas, T.; Li, M. H.; Kotlyar, A.; Desai, A.; Baker Jr, J. R., Light-controlled release of caged doxorubicin from folate receptor-targeting PAMAM dendrimer nanoconjugate. *Chemical Communications* 2010, 46, 2632-2634.
21. Lu, J.; Choi, E.; Tamanoi, F.; Zink, J. I., Light-Activated Nanoimpeller-Controlled Drug Release in Cancer Cells. *Small* 2008, 4, 421-426.
22. Shamay, Y.; Adar, L.; Ashkenasy, G.; David, A., Light induced drug delivery into cancer cells. *Biomaterials* 2011, 32, 1377-1386.
23. Guardado-Alvarez, T. M.; Sudha Devi, L.; Russell, M. M.-L.; Schwartz, B. J.; Zink, J. I., Activation of Snap-Top Capped Mesoporous Silica Nano Containers Using Two Near-Infrared Photons. *Journal of the American Chemical Society* 2013.
24. Angelos, S.; Yang, Y. W.; Patel, K.; Stoddart, J. F.; Zink, J. I., pH-Responsive Supramolecular Nanovalves Based on Cucurbit [6] uril Pseudorotaxanes. *Angewandte Chemie* 2008, 120, 2254-2258.

25. Angelos, S.; Khashab, N. M.; Yang, Y. W.; Trabolsi, A.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I., pH clock-operated mechanized nanoparticles. *Journal of the American Chemical Society* 2009, 131, 12912-12914.
26. Patel, K.; Angelos, S.; Dichtel, W. R.; Coskun, A.; Yang, Y. W.; Zink, J. I.; Stoddart, J. F., Enzyme-responsive snap-top covered silica nanocontainers. *Journal of the American Chemical Society* 2008, 130, 2382-2383.
27. Casasús, R.; Climent, E.; Marcos, M. D.; Martínez-Máñez, R.; Sancenón, F.; Soto, J.; Amorós, P.; Cano, J.; Ruiz, E., Dual aperture control on pH-and anion-driven supramolecular nanoscopic hybrid gate-like ensembles. *Journal of the American Chemical Society* 2008, 130, 1903-1917.
28. DeMuth, P.; Hurley, M.; Wu, C.; Galanie, S.; Zachariah, M. R.; DeShong, P., Mesoscale porous silica as drug delivery vehicles: Synthesis, characterization, and pH-sensitive release profiles. *Microporous and Mesoporous Materials* 2010.
29. Park, C.; Oh, K.; Lee, S. C.; Kim, C., Controlled Release of Guest Molecules from Mesoporous Silica Particles Based on a pH-Responsive Polypseudorotaxane Motif. *Angewandte Chemie International Edition* 2007, 46, 1455-1457.
30. Zhao, Y.-L.; Li, Z.; Kabehie, S.; Botros, Y. Y.; Stoddart, J. F.; Zink, J. I., pH-operated nanopistons on the surfaces of mesoporous silica nanoparticles. *Journal of the American Chemical Society* 2010, 132, 13016-13025.
31. Meng, H.; Xue, M.; Xia, T.; Zhao, Y.-L.; Tamanoi, F.; Stoddart, J. F.; Zink, J. I.; Nel, A. E., Autonomous in vitro anticancer drug release from mesoporous silica nanoparticles by pH-sensitive nanovalves. *Journal of the American Chemical Society* 2010, 132, 12690-12697.

32. Agostini, A.; Sancenón, F.; Martínez-Máñez, R.; Marcos, M. D.; Soto, J.; Amorós, P., A Photoactivated Molecular Gate. *Chemistry-A European Journal* 2012, 18, 12218-12221.
33. Mal, N. K.; Fujiwara, M.; Tanaka, Y., Photocontrolled reversible release of guest molecules from coumarin-modified mesoporous silica. *Nature* 2003, 421, 350-353.
34. Kermis, H. R.; Kostov, Y.; Rao, G., Rapid method for the preparation of a robust optical pH sensor. *Analyst* 2003, 128, 1181-1186.
35. Ambrogio, M. W.; Thomas, C. R.; Zhao, Y.-L.; Zink, J. I.; Stoddart, J. F., Mechanized silica nanoparticles: a new frontier in theranostic nanomedicine. *Accounts of chemical research* 2011, 44, 903-913.
36. Yang, Y.; Song, W.; Wang, A.; Zhu, P.; Fei, J.; Li, J., Lipid coated mesoporous silica nanoparticles as photosensitive drug carriers. *Physical Chemistry Chemical Physics* 2010, 12, 4418-4422.

Chapter 5

Temperature Controlled Poly(NIPAAm-co-AAm) Modified Mesoporous Thin Films For Pore Control

Adopted from recently published MM. Russell; L. Raboin; TM. Guardado-Alvarez; JI. Zink,
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5.1 Introduction

Mesoporous silica materials, prepared by sol–gel methods, are of great interest because of their many attractive features such as stable mesoporous structures, large surface areas, tunable pore sizes, and the simplicity in modifying the inside pores with organic groups.¹⁻⁵ The nanopores exhibit narrow pore size distributions and can store a wide variety of molecules.^{6, 7} Accordingly, these materials have been studied for many applications including catalysis,⁸⁻¹⁰ medical drug delivery,^{6, 11, 12} or separation technology.^{2, 13, 14} Mesostructured sol–gel thin films formed by evaporation induced self-assembly (EISA) during dip- or spincoating are an important class of materials.¹⁵⁻²¹ A sol containing a silica precursor and a template agent is deposited as a thin liquid layer onto a suitable substrate. The evaporation of the solvent drives the formation of surfactant micelles, which further assemble into a liquid crystal. At the same time the silica condenses around the micelles. By choosing a specific composition of the sol, environmental conditions, and the method of deposition, mesostructured films with highly-ordered hexagonal, lamellar, or cubic structures can be produced.^{20, 22} The surfactant molecules can be removed from the pores of the film by solvent extraction or calcination, thus making it possible to fill the empty pores with nano-sized cargos. The controllable release of stored molecules from the nanopores is attracting increasing interest. Because a macrosubstrate can be more easily handled and manipulated than nanospheres, thin films containing mesopores would be very convenient if the openings of the nanopores were accessible to molecules outside of the films.

Many efforts have been made to prepare films in which the pore openings are oriented towards the surface of the films.²³⁻²⁷ Unfortunately such procedures remain complex, time-consuming and difficult, and the types of templating surfactants or polymers that can be used are.²⁸ An alternative approach consists of preparing a film with a well-known structure such as a 2D-hexagonal structure in which arrays of tubes in highly arranged stacks are aligned parallel to the upper surface of the films, then etching away selectively narrow regions of the film that are perpendicular to the nanopore orientation. This procedure allows for the creation and the exposure of pore openings. An example of such a film, with a thickness of ~300 nm and a pore diameter of 2.5 nm, has been reported.²⁹ Based on these patterned films, a molecular storage and on-demand release system was realized. In this chapter, an improved material that allows more cargo to be stored inside the pores is presented. By changing the surfactant from CTAB to F127 the pore size changes from ~2nm to ~5nm. This material could be useful in biomedical applications to deliver larger doses of drugs as well as the potential to deliver larger cargo molecules. In order to test the cargo trapping capacity of this material, a well-studied synthetic responsive co-polymer, poly(N-isopropylacrylamide-co-Acrylamide) (poly(NIPAAm-co-AAm)), which undergoes a sharp coil-globule transition in water at 41 °C, changing from a hydrophilic state below this temperature, to a hydrophobic state above it was used.³⁰⁻³³ The temperature at which this occurs is called the lower critical solution temperature (LCST). While this polymer has been used on silica to release cargo, the results have been either leaky or releases at room temperature.³⁴⁻³⁶ The grafted polymer acts as a gate that controls the pore opening. At room temperature the polymer stands erect in front of the pores, entrapping the cargo inside. Above the LCST the polymer collapses allowing the cargo to flow freely out of the pores. Since the

polymer is also covalently attached to the surface of the film, the system is not leaky at room temperature and after an initial release, the films can be re-loaded with cargo to perform a second release. The overall system is depicted in Figure 5.1.

5.2 Experimental Section

5.2.1 Preparation of Mesoporous Silica Films

Three porous silica films were synthesized with different pore diameters using different silica templates. Large pore films (pore size 5.5 nm) were prepared using triblock copolymers as templates, while small pore films (pore size 2.5 nm) were produced by surfactant templating. Additional microporous films (pore size < nm) were created using no template.

Mesostructured silica films were synthesized according to a two-step process as previously reported.^{22, 37} First, a stock solution was prepared by mixing TEOS [$\text{Si}(\text{OC}_2\text{H}_5)_4$], absolute ethanol, water and HCl (mole ratios 1 : 3.8 : 1 : 5×10^{-5}), and heating at 60°C for 1.5 hrs. Then, a 7.5 mL sample of this initial solution was cooled to room temperature and mixed to 1 mL of 0.07 M HCl and 0.35 mL of water. After stirring for 15 min, the mixture was allowed to age for 15 min. without stirring, followed by dilution with two equivalents of absolute ethanol. Finally, after the sol was aged for 3 days, triblock copolymer poly(ethyleneoxide₁₀₆-propyleneoxide₇₀-ethyleneoxide₁₀₆) (Pluronic F127) or cetyltrimethylammonium bromide (CTAB) was added. The surfactant/TEOS mole ratios were 0.007 for Pluronic F127 and 0.1 for CTAB, respectively. An additional mixture was prepared without adding surfactant into the silica sol.

The as-prepared mixtures were dip-coated on silicon or glass substrates at a constant speed of 3 mm s^{-1} to yield the mesostructured films. Before deposition, the substrates were soaked in a 1M HNO_3 solution overnight, then rinsed with water, ethanol and acetone, to remove contaminates. The entire film-pulling apparatus was placed inside a controlled-humidity chamber. The relative humidity was fixed to $35 \pm 5\%$ when using Pluronic F127, and $55 \pm 5\%$ when using CTAB. This results in several hundred nanometer thick crack-free films. Only one side of the substrate consists of the mesostructured film as the other side was cleaned using dilute HF.

5.2.1.1 Stamp Preparation and Micropatterning

An AFM calibration grating (Mikromasch TGZ04) was used as a source of the pattern. The pattern consists in lines $1.5 \text{ }\mu\text{m}$ wide, $1 \text{ }\mu\text{m}$ high, with $3 \text{ }\mu\text{m}$ pitch. A poly(dimethylsiloxane) (PDMS, Sylgard 184) mixture was poured onto the grating and heated at $100 \text{ }^\circ\text{C}$ until it solidified. The PDMS stamp was then removed from the grating surface, rinsed with ethanol and dried in air. The micropatterned films were prepared by gently pressing the PDMS stamp onto the surface of the as-synthesized films right after the dip-coating. The films covered with the stamp were heated at $100 \text{ }^\circ\text{C}$ for 30 min then allowed to cool at room temperature. The stamp was delicately peeled off using tweezers. A successful pattern transfer leaves a visible indication as a diffraction effect is observed at the film surface. The micropatterned mesostructured films were heated at $100 \text{ }^\circ\text{C}$ overnight to consolidate the silica network. Finally, the films were

calcined at 500 °C for 5 hrs. in air to remove the template, and stored in a vacuum sealed desiccator. The mesoporosity of the films was verified by XRD and TEM.

5.2.1.2 MEH-PPV Incorporation

First, the inner pore surface of the patterned films was made hydrophobic to aid in incorporation of the fluorescent polymer (MEH-PPV). The films were treated with a solution of dimethyldichlorosilane in dry toluene in presence of triethylamine as an activating base. After reaction, the films were washed with ethanol and water, and cured at 100°C for 1 hr. To incorporate the polymer MEH-PPV (average $M_n = 40\,000 - 70\,000$ Da) into the pores, the hydrophobic films were soaked in a 1% solution of MEH-PPV in chlorobenzene for 48 hrs. at 80 °C. The samples were extensively washed with chlorobenzene and chloroform, dried in air, and stored in a dark nitrogen atmosphere.

5.2.2 Synthesis of Thermoresponsive Silica Films

5.2.2.1 Polymerization of Poly(NIPAAm-co-AAm)

NIPAAm (3.66 g), AAm (0.26 g), N,N,N',N',N'-pentamethyldiethylenetriamine (PMDETA) (35 μ l), 2-bromo-2-methyl propionic acid (0.01 g), deionized water (36 ml) and methanol (24 ml) were mixed in a Schlenk flask and degassed by freeze-pump-thaw cycles. While the mixture was frozen, CuBr (0.01 g) was added. The flask was then filled with argon and the mixture was left to melt at room temperature. The reaction solution was magnetically

stirred overnight at room temperature. After evaporation of the solvent, the crude product was dissolved in water and purified by dialysis to yield poly(NIPAAm-co-AAm) with an LCST close to 41 °C.

5.2.2.2 Surface Modification of Films

The films were soaked in a 0.01 M solution of 3-aminopropyltriethoxysilane (APTES) in dry toluene under nitrogen and stirred at reflux overnight. Finally the surface were extensively rinsed with dry toluene and dried in vacuum. 50 mg of poly(NIPAAm-co-AAm) is dissolved in 10 ml of deionized water. 50 mg of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) was added and the amine modified silica film was immediately added afterwards. The solution was stirred overnight at room temperature. After the reaction, the films were removed from the solution, extensively rinsed with deionized water and dried in vacuum. The surface modification was confirmed from by FTIR and AFM.

5.2.2.3 Dye Loading and Release Studies

Poly(NIPAAm-co-AAm) modified patterned films were loaded with propidium iodide as a fluorescent dye. The film was submerged in a 2 mM propidium iodide solution (10 mL), heated to 50 °C (> LCST), and left stirring for 24 hrs. Afterwards, the loaded film was removed from the solution, thoroughly rinsed with room temperature water, and used for release studies right away to avoid propidium iodide degradation.

The operation of the thermoresponsive films was monitored using fluorescence spectroscopy. A small 1 cm x 1 cm film (size of pattern 0.8 cm x 0.8 cm) was placed on the bottom of a cuvette. 6 mL of deionized water and a small stir-bar were added. The sealed cuvette was placed on a temperature-controlled plate. The emission of propidium iodide in the solution above the film was measured as a function of time by using a 377 nm excitation beam (10 mW) to excite the dye molecules as they are released from the film. The emission spectrum was recorded as a function of time at 1 second intervals. The release profiles were obtained by plotting luminescence intensities of propidium iodide at the emission maximum (650 nm) as a function of time. The release of the cargo dye was activated by increasing the aqueous media temperature from room temperature (< LCST) to 45 °C (> LCST).

5.2.3 Characterization Methods

The structure of mesostructured silica films were investigated by X-ray diffraction (XRD) experiments in Bragg-Brentano geometry (θ - 2θ) on a Philips X'Pert Powder Diffractometer operated at 40 kV, 40 mA using CuK α radiation ($\lambda=1.54$ Å). For the observation of the mesoporous films with transmission electron microscopy (TEM), the films were detached from the substrates and dispersed in ethanol, then deposited and dried on a carbon Cu grid. Micrographs were recorded on a JEM1200-EX (JEOL) electron microscope operating at 50 kV. Infrared (FTIR) measurements were acquired on a Jasco Model 420 spectrometer after the samples were dehydrated under vacuum for 5 hrs. at room temperature. The thickness and the surface morphology of the patterned films were determined by atomic force microscopy (AFM)

on a Dimension 5000 instrument in contact mode. For fluorescence depolarization measurements, mesoporous films treated with MEH-PPV polymer were illuminated using the 407 nm line of a Coherent I302C krypton ion laser (10 mW). A polarizer was used to select out parallel and perpendicular emission components. A 420 nm filter was placed in front of the polarizer to block stray laser light. The photoluminescence spectra of the samples were collected throughout the visible region using an Acton 2300i monochromator and a Princeton Instruments CCD. Fluorescence microscopy images of poly(NIPAAm-co-AAm) derivatized patterned films were acquired on a Leica confocal SP5 MP-STED microscope. The films were loaded with Rhodamine B, then the pores were closed, sealing the dye inside the pores. The excess dye on the surface was washed off.

The LCST was measured spectrophotometrically with a polymer solution being heated 1°C every three minutes. When the light transmittance is 90% of the original transmittance (at 450nm), is defined as the LCST. The average molecular weight (M_n) and polydispersity index (PDI) of the polymer was measured by Gel Permeation Chromatograph (GPC) using a Waters 515 Differential Refractometer with Waters 410 HPLC pump and two styragel HR 5E columns in THF (0.1 mg/L) as an eluent at 42 °C, calibrated with polystyrene standards.

5.3 Results and Discussion

5.3.1 Micropatterned Nanoporous Silica Films

A simple dry-stamping method is used to prepare mesoporous films with a significant accessibility to the pore openings. While the nanopore orientation can easily be controlled in the plane of the film,³⁸⁻⁴⁰ it is difficult to synthesize films in which the pores are running perpendicular to the film surface. Only few reports show a successful orientation of the pore openings toward the upper surface,²³⁻²⁶ The procedure remains difficult and a limited number of surfactants can be handled, that restricts the range of pore sizes that can be studied. Thus, to cause accessibility to the interior of the films *via* the nanopores, a simple dry-stamping procedure that selectively removes narrow regions of a film that are perpendicular to the pore orientation was implemented. The films preferentially consist of highly arranged arrays of aligned tubes in the plane of the films.

The first step consists in preparing organized nanostructured films with various pore sizes. The films studied were prepared through evaporation-induced self-assembly (EISA).^{21, 22} Ordered mesostructured films were obtained using F127 and CTAB as templates. Dip-coating was used to deposit the films rather than spin-coating to have a preferential orientation of the pores along the direction of the pull. The XRD patterns of the as-synthesized films are consistent with a 2D-hexagonal mesostructure with lattice spacings of 114 Å and 39.4 Å for F127 and CTAB, respectively (Figure 5.2). This is confirmed by TEM characterization (Figure 5.3). The micrographs show straight mesochannels regularly arrayed parallel to the substrate with a wall-

to-wall distance estimated to around 10 nm and 6 nm for F127 and CTAB, respectively. Additional films were prepared using no template. No structure was recorded by XRD and no pores were observed by TEM. The porosity of such films is expected to be sub-nanometric.^{41, 42}

The second step consists in micro-patterning the as-prepared films. Molecular transport is extremely limited in the 2D-hexagonal structure as the nanopore openings are only present at defects or at each end of the films. To create pore accessibility, a PDMS stamp is pressed on the continuous films, as previously described, thus allowing the creation of surface defects according to a known pattern. This procedure has to be done immediately after the deposition while the silica network is soft. As the stamp is placed on the silica material, the film regions that are in physical contact with the highest parts of the patterned stamp are constricted. After 30 min at 100 °C to consolidate the framework, the stamp is removed. The pattern leaves strips of silica material 1.5 μm wide and 600 nm high, that approximately corresponds to the thickness of the films. Profiles remain similar regardless pore sizes. The stamp is pressed in a way that the strips run perpendicular to the nanopore orientation. Therefore the nanopores that are inside the strips are exposed and open along the vertical sides of these strips.

The patterned films contain template molecules that are removed by calcination at 500°C. The silica films conserve its 2D-hexagonal mesostructure, as shown by XRD and TEM. After calcination, the pore diameters were estimated to around 5.5 ± 1.0 nm and 2.5 ± 0.5 nm for F127 and CTAB, respectively. For the remainder of this chapter, the resulting mesoporous patterned films will be referred to as large pore films, small pore films and micropore films, using F127, CTAB, and no template, respectively.

Our method has been suggested as an alternative to usual printing techniques, that are soft lithography⁴³⁻⁴⁵ and reactive wet-stamping methods (r-WETS).^{46, 47} Our procedure is a one-step technique based on a mechanical compression that does not require surface preparation, layer deposition and/or use of reactives, which makes it easy-to-use and cheap. In addition, it should be noted that reactive wet-stamping techniques could not be used for our films. Attempts were made to etch a pattern into films by having them come into a contact with a micro-patterned hydrogel stamp. The stamp was soaked in a buffered hydrofluoric acid (BHF) solution, as described by Klichko *et al.*²⁹ Although this method was successful for CTAB-templated films (pore size 2.5 nm), it systematically failed for F127-templated films (pore size 5.5 nm) as BHF drastically infiltrates the porosity and quickly diffuses into the silica framework to destroy the film.

5.3.2 Nanopore Alignment and Accessibility

To explore the ability for external molecules to enter the porosity of the films, fluorescence polarization of a long-chain linear polymer (MEH-PPV) incorporated into the pores of a patterned film were investigated. In addition, a film infiltrated with a small dye molecule, Rhodamine B, and was characterized by confocal microscopy.

5.3.2.1 *Polymer Incorporation and Characterization*

A fluorescent polymer MEH-PPV was infiltrated into the pores of a large pore film. It is known that the conformation of the polymer chains controls the polymer photophysics.⁴⁸ Emission polarization, as optical properties, can be affected by the orientation, the degree of aggregation and the surrounding environment of the polymer chains.⁴⁹⁻⁵¹ In an homogeneous solution, polymer strands are randomly oriented and free to move, which leads to a non-polarized fluorescence. On the other hand, when polymers are excited with a given polarization of light they show a preferential orientation. Photoluminescence is expected to be stronger in the direction parallel to the excited polarization than at 90° to the excitation. Inside the pores of a patterned film, the polymer chains are forced to stretch out and be aligned in the direction of the nanopores. This preferential alignment gives rise to polarized fluorescence. The polymer that is not in the pore has random orientation, which gives non-polarized fluorescence.

Fluorescence emission spectrum of infiltrated materials are shown in Figure 5.4a. Both the polarization of the emitted light and the peak shifts in the polymer emission are examined. As the sample is not washed, three main peaks are observed at 492 nm, 568 nm, and 625 nm. A difference in the parallel and perpendicular intensities is observed and is particularly pronounced for the peak at 492 nm. This peak corresponds to polymer chains aligned preferentially inside the nanopores while the other peaks correspond to polymers at the surface. In fact, the polarization effect is the greatest at 492 nm, which would correspond to the behavior of aligned chains in the direction of the nanopores. In contrast, the emission peaks of the polymer on the outside of the film are only slightly polarized, probably caused by the tail of the 492 nm polarized band. In addition, a polymer chain that is surrounded only by silica or silane that is confined in a

nanopore is expected to result in a blue shift in emission, compared to polymer chains surrounded by other polymers.^{51, 52} This effect has been observed by Klichko and coworkers for mesoporous CTAB-templated silica films infiltrated by MEH-PPV.²⁹ Finally, as the sample is extensively washed (Figure 5.4a), only the peak at 492 nm remains. That is a supplementary evidence that the peak at 492 nm corresponds to polymers that infiltrated the nanopores. Once the sample is washed, aggregated polymer chains are removed from the surface while polymer strands are kept inside the pores.

A patterned mesoporous large pore film was derivatized with poly(NIPAAm-co-AAm), as described below, and loaded with Rhodamine B. The polymer chains blocked the pore openings, sealing in the dye inside the pores, and the excess dye on the surface was washed off. A fluorescence confocal microscopy revealed the presence of the dye inside the material (Figure 5.4b). The patterned film surface appears as fluorescent parallel lines, while regions where the silica was constricted are dark. This is an evidence that molecules of Rhodamine B size are able to enter the pores and to be retained inside by the polymer.

5.3.3 Thermoresponsive Poly(NIPAAm-co-AAm) modified Films

The synthesis of poly(NIPAAm-co-AAm) is depicted in Figure 5.5.³² 2-Bromo-2-methylpropionic acid reacts with N-isopropyl acrylamide and acrylamide in the presence of copper bromide to form long polymer chains ($M_n = 102,000$ g/mol, $M_n/M_w = 1.42$ through atom transfer radical polymerization. The LCST of the polymer was determined to be 41 °C by plotting the percent transmittance vs. temperature at 450 nm (Figure 5.6).

The patterned silica films are submerged in dry toluene upon which APTES is added to the solution and then refluxed for 24 hrs. under nitrogen (Figure 5.7). Afterwards, the films are thoroughly rinsed and then left to dry. Poly(NIPAAm-co-AAm) is dissolved in water and immediately after adding EDAC the amine modified films are carefully placed in the solution. After 24 hrs. the long polymer chains are grafted onto the stamped silica surface. Figure 5.8 is an AFM image of the film before and after the polymer grafting. The channels of the pattern are well defined before the polymer is grafted. Afterwards, the polymer fills the insides of the channels as well as the top surface of the film.

5.3.4 Release Studies

As stated in the introduction, Figure 5.1 is a schematic of how the polymer coated film works. Below 41 °C, the polymer strands stand erect due to hydrophilicity and block the pores. To load the film, the film is placed in a solution containing the desired cargo molecules and heated above 41 °C. The polymer changes from hydrophilic to hydrophobic, collapsing the polymer and exposing the pore openings. The solution is then cooled to room temperature allowing the polymer to once again block the pores. The film is then washed to remove excess dye. When the film is heated above 41 °C a second time, the cargo molecules are allowed to exit the unblocked pores, again due to the polymer changing to a hydrophobic state.

The polymer coated large, small, and microporous films were submerged in 2 mM propidium iodide solution. The solution was heated to 50 °C and was left stirring for 24 hrs. The films were then thoroughly washed.

The operation of the thermoresponsive films was monitored in an aqueous solution using luminescence spectroscopy. Figure 5.9 and Figure 5.10 depict the release profiles of propidium iodide for polymer coated films and non-polymer coated films, respectively. The emission intensity was monitored for 1 hr. at room temperature before the solution was heated to 45°C. The decrease in the baselines indicates the degradation of propidium iodide that was absorbed onto the silica film.

In order to confirm that the large pore film can contain more cargo than the small pore film release studies were conducted using six different films; large pore, small pore, and microporous films with and without polymer. Figure 5.9 shows the release profiles for the films with polymer. The large pore film's intensity increases the most and the microporous film's intensity increases the least. The small pore film released only half as much propidium iodide than the large pore film. This indicates that more cargo can be contained due to the larger sized pores, which was expected. The small release on the microporous film is caused by dye trapped solely by the polymer, which is roughly 15% of the amount the large pore can release. Therefore, 85% of dye is being released from the pores of the large pore film. The data implies that the larger the pore the more dye or cargo can potentially be trapped and released.

Looking at Figure 5.10, the same trend can be seen. The large pore non-polymer coated film has a slightly higher intensity compared to the small pored non-polymer coated film, and the microporous non-polymer coated film has a negligible increase. The reason there is an increase in intensity is due to some dye being trapped inside the pores even after several washings. Heat causes the dye that is trapped or absorbed onto the surface to leave the films and thus the increase is observed.

Comparing the two figures, the data shows that, in all three cases, the films with the polymer coating traps and releases more dye than the films without polymer coating. For the large and small pore film, this suggests that the polymer is blocking the pore opening and is successfully trapping the dye inside. The release from the polymer coated microporous film suggests that the polymer is trapping some dye onto the surface.

To test the reusability of the films, a large pored film was used. The film was loaded with propidium iodide using the same method as before. After the first release experiment, the film was reloaded with propidium iodide and a second release experiment was conducted. Figure 5.11 shows the results of the experiment. The reloaded film does not hold as much dye as a fresh film, as seen from the data, but the sharp increase in intensity indicates that the polymer on the film is still able to trap and release dye. The data shows that the films can be reused, however it has been observed that after the second release the film degrades and you can no longer see the pattern on the film.

To ensure that the polymer keeps the dye trapped in the pores until the temperature is past 41 °C, temperature studies were done. Figure 5.12 shows the temperature release dependence on a large pore film. The initial temperature was set at 35 °C and then ramped to 45 °C and then 55 °C. No increase in intensity was observed at 35 °C. This implies that the polymer is still blocking the pore openings. At 45 °C the intensity starts to increase and at 55 °C the intensity increases only slightly faster. The data shows that the release is caused by the collapse of the polymer chains once the LCST is reached.

5.4 Conclusion

Mesoporous silica films with pore diameters of ~ 2 and ~ 5 nm aligned in the pulling direction were synthesized. The thickness of the films were determined to be ~ 600 nm and the patterned features were $1.5\ \mu\text{m}$ wide strips oriented perpendicular to the direction of the nanopores which are separated from each other by $1.5\ \mu\text{m}$ gaps. The patterned features were obtained using a PDMS stamp without the need for hydrofluoric acid. Loading the films with a fluorescent polymer and measuring the emission and polarization of the polymer inside the pores confirmed the nanopores accessibility and orientation. Poly(NIPAAm-co-AAm) was grafted onto the surface of the films. The films with the polymer grafted on the surface were able to trap more dye than the films with no polymer.

The large-pored film contained more dye than both the smaller pored and the micropored films. Release of the dye was only observed when the temperature reached the polymers LCST. While less dye was released, the films proved that when the pattern stays intact the film can be reloaded with dye and a second release can be obtained. These smart materials utilize the micropatterned structure to successfully load and release cargo using a thermal sensitive polymer under external thermal.

5.5 Figures and Tables

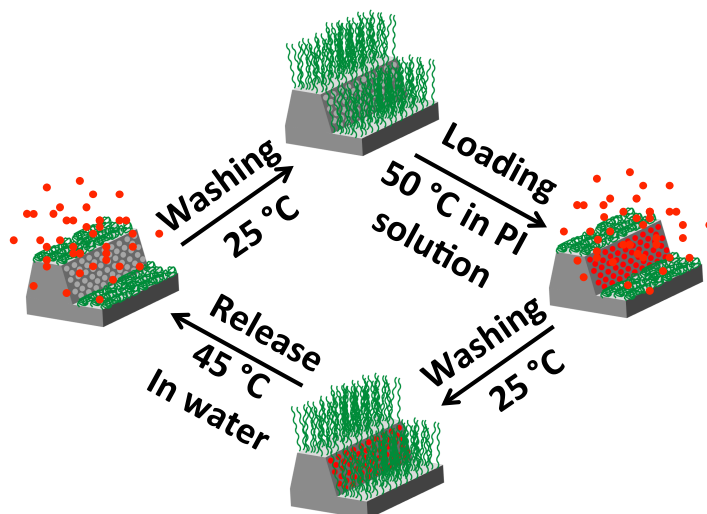


Figure 5-1 The operation of the temperature-activated opening of the nanopores. A side view of one of the stamped channels is illustrated. When heated past the polymer's LCST, the polymer chains collapse allowing cargo molecules to enter the pores. Washing with cool water below the LCST causes the chains to go back to their extended conformation, blocking the pore openings. Heating the water again causes the polymer to shrink, releasing the cargo. This process can be repeated.

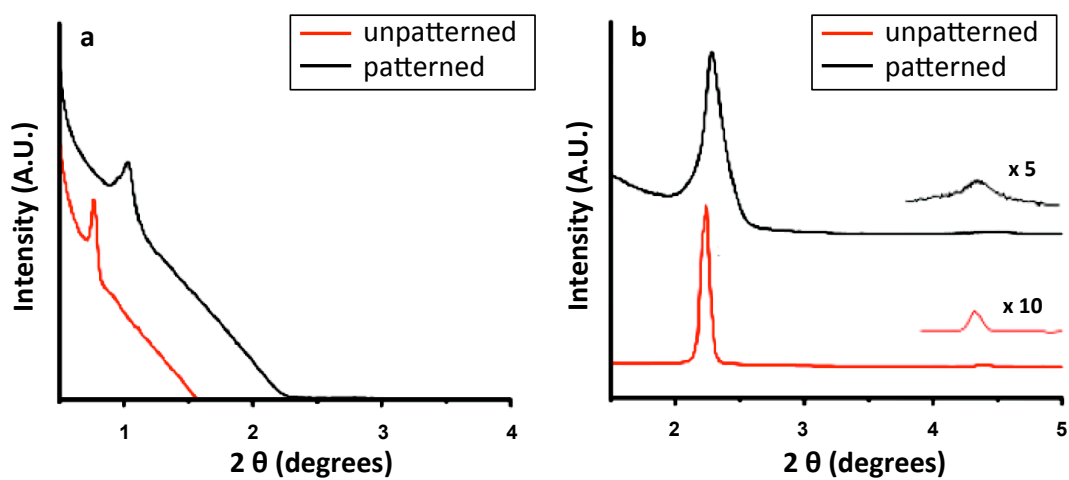


Figure 5-2 X-ray diffraction spectra of mesostructured (red line) and patterned mesoporous (black line) silica films prepared using (a) Pluronic F127 and (b) CTAB.

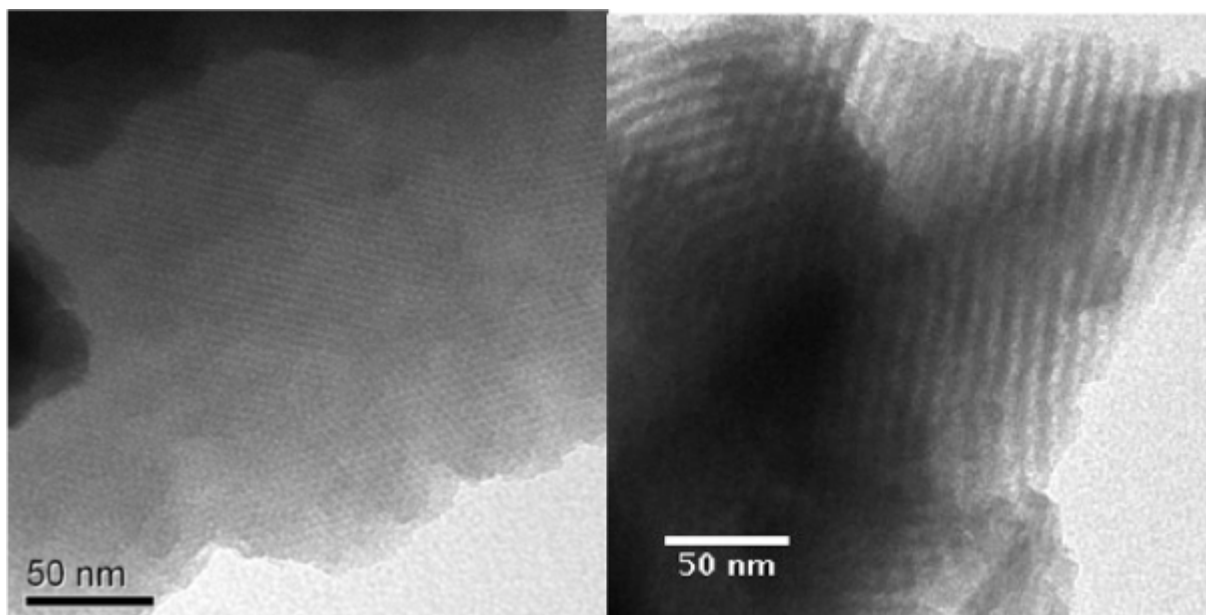


Figure 5-3 TEM images of mesostructured silica films prepared using CTAB (left) and Pluronic F127 (right).

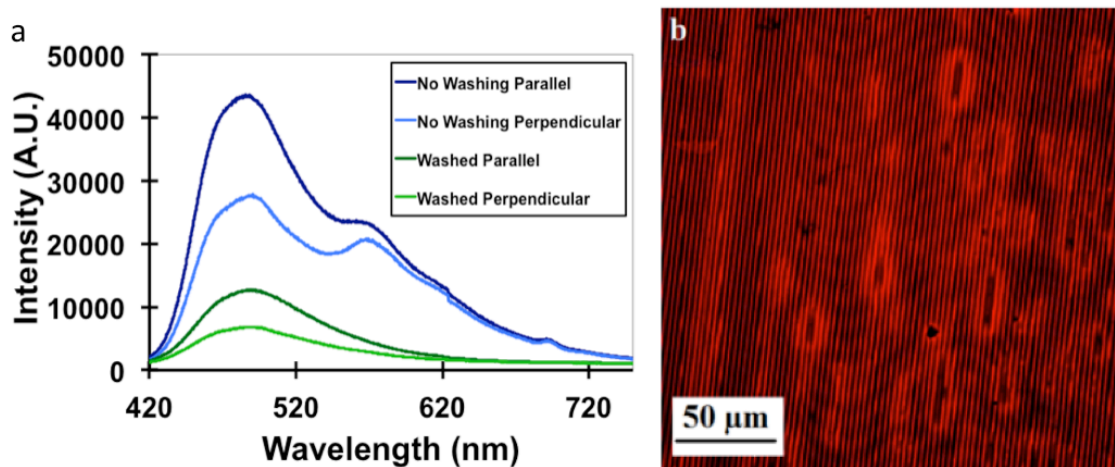


Figure 5-4 (a) Fluorescence emission spectrum of MEH-PPV incorporated into a patterned large pore film. As the sample is not washed (blue lines), multiple peaks are observed which correspond to various environments. As the sample is extensively washed (black lines), the peak at 492 nm corresponds to polymer chains aligned preferentially inside the nanopores. The emission intensity is greater in the plane parallel to the orientation of the pores (solid) compared to perpendicular (dashed). (b) Fluorescence confocal microscopy images of Rhodamine B dye doped patterned F127-templated mesoporous silica films after derivatization of poly(NIPAAm-co-AAm) polymer using (a) Pluronic F127 and (b) CTAB.

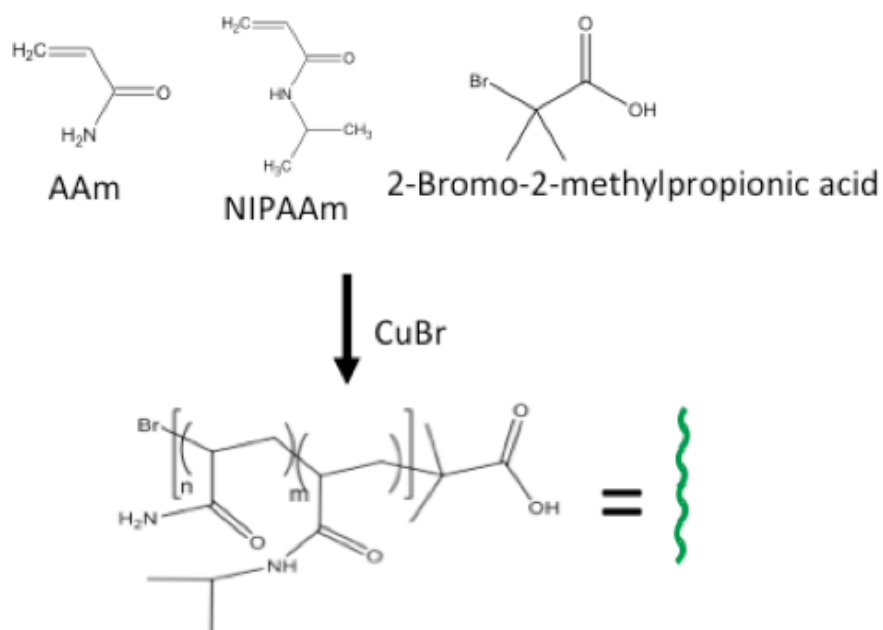


Figure 5-5 Atom-transfer radical polymerization of NIPAAm and AAm monomers (at a molar ratio of $m=7n$) as initiated by 2-bromo-2-methylpropionic acid in the presence of a Cu(I) catalyst.

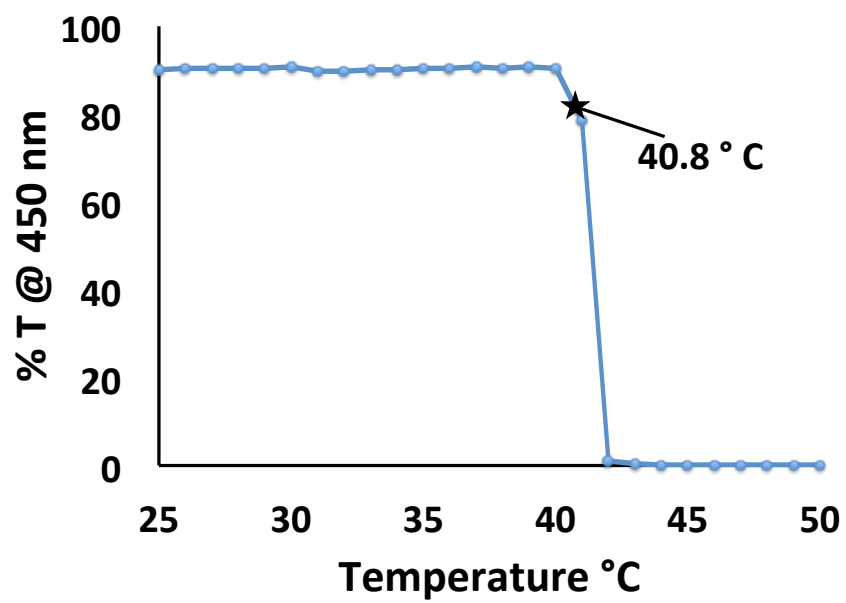


Figure 5-6 Percent transmittance vs. temperature at 450 nm.

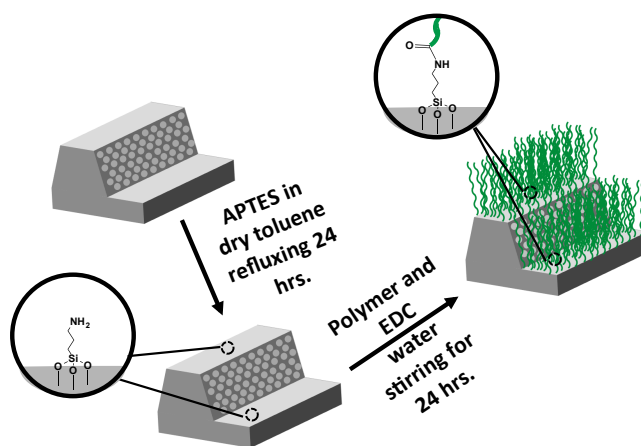


Figure 5-7 Attachment of the thermal polymer onto the stamped silicon film. In dry toluene, APTES is added to the film refluxing for 24 hours. The polymer can then be attached using EDC in water stirring for 24 hours at room temperature.

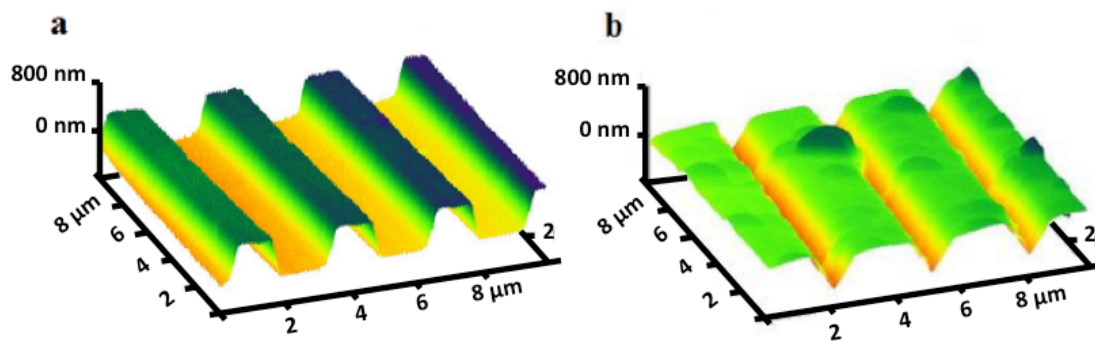


Figure 5-8 AFM images of a patterned mesoporous silica film prepared using Pluronic F127 (a) before and (b) after derivatization with chain poly(NIPAAm-co-AAm) polymer. The scanned area is 10 x 10 μm . The patterned features are strips 1.5 μm wide, 500 nm high, with 3 μm pitch.

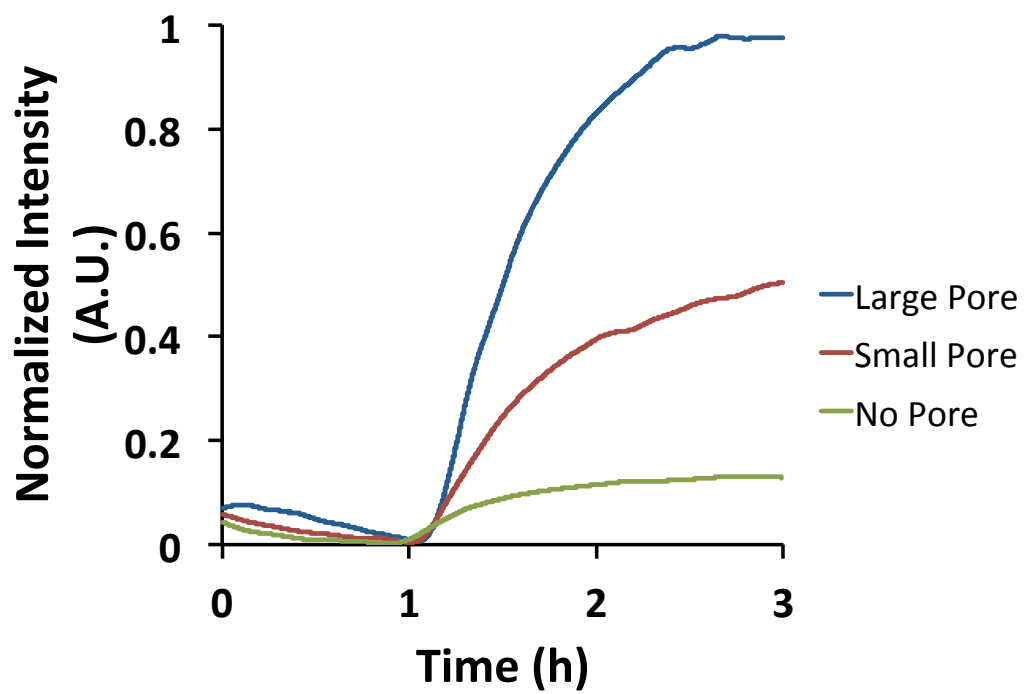


Figure 5-9 Release of propidium iodide from a poly(NIPAAm-co-AAm) modified large/small/no pore films.

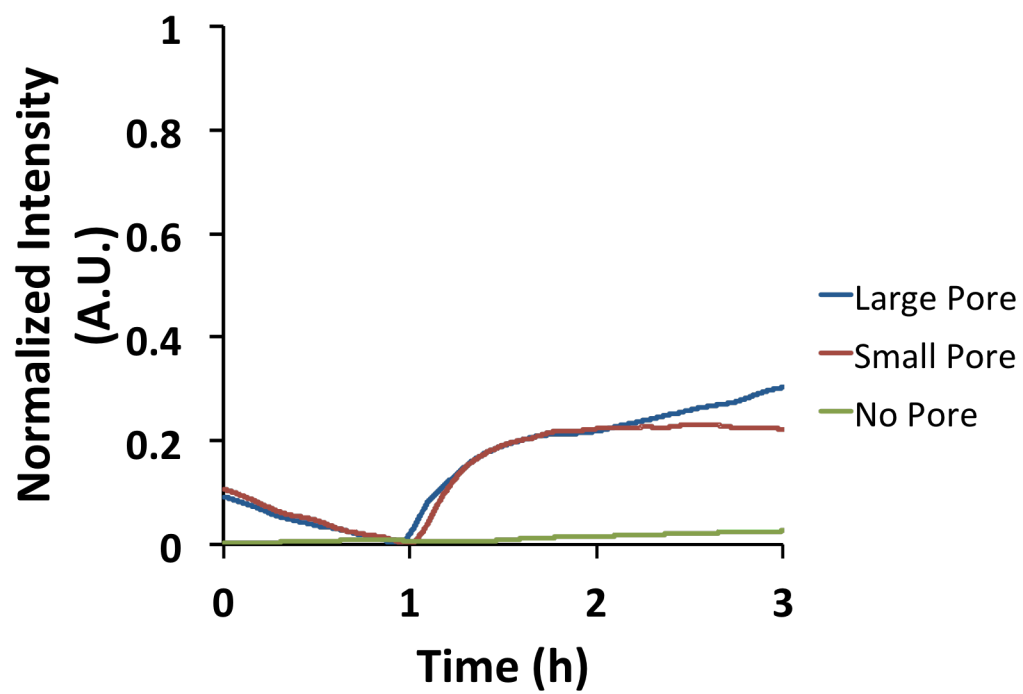


Figure 5-10 Release of unmodified large/small/no pore films.

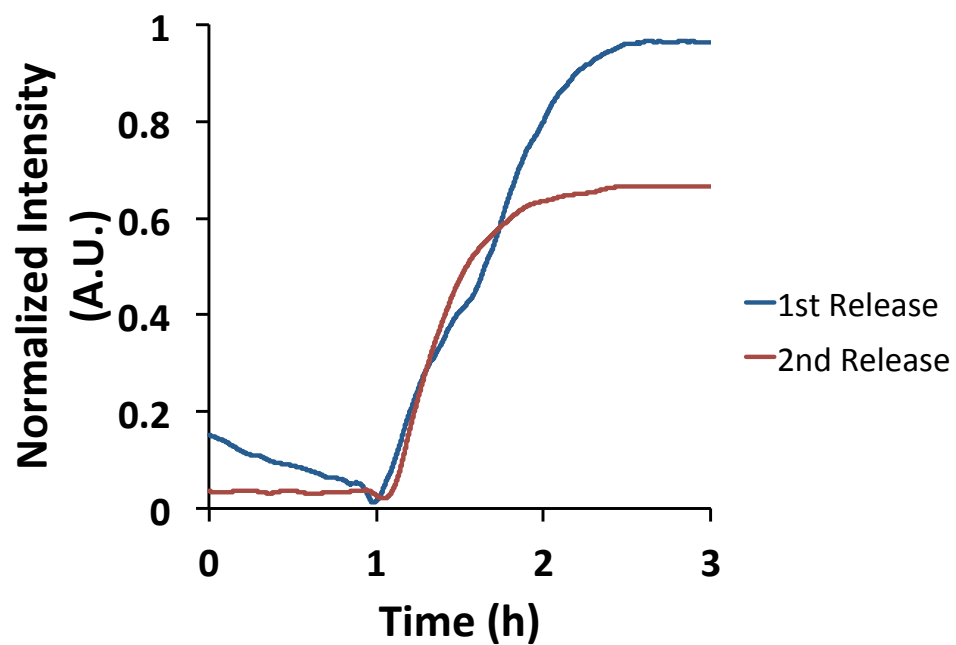


Figure 5-11 Release profile of a large pore film (blue). Release profile of the same large pore film re-loaded with cargo after the first release (red).

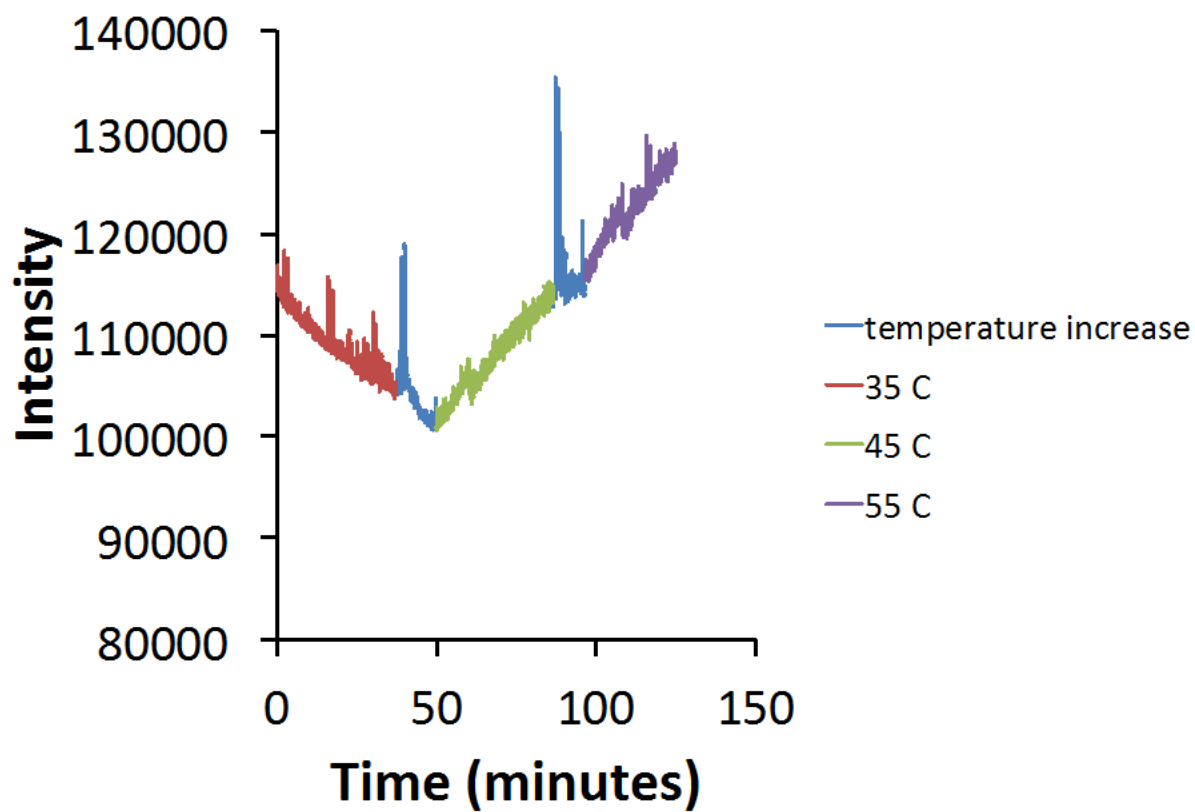


Figure 5-12 Release profile of a large pore film with the baseline set at 35 °C. The temperature was then ramped to 45 °C and then 55 °C.

5.6 References

1. Beck, J. S.; Vartuli, J. C.; Roth, W. J.; Leonowicz, M. E.; Kresge, C. T.; Schmitt, K. D.; Chu, C. T. W.; Olson, D. H.; Sheppard, E. W., A new family of mesoporous molecular sieves prepared with liquid crystal templates. *Journal of the American Chemical Society* 1992, 114, 10834-10843.
2. Stein, A.; Melde, B. J.; Schrodin, R. C., Hybrid inorganic–organic mesoporous silicates—nanoscopic reactors coming of age. *Advanced Materials* 2000, 12, 1403-1419.
3. Liong, M.; Lu, J.; Kovichich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I., Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery. *Acs Nano* 2008, 2, 889-896.
4. Cai, Q.; Luo, Z.-S.; Pang, W.-Q.; Fan, Y.-W.; Chen, X.-H.; Cui, F.-Z., Dilute solution routes to various controllable morphologies of MCM-41 silica with a basic medium. *Chemistry of materials* 2001, 13, 258-263.
5. Huh, S.; Wiench, J. W.; Yoo, J.-C.; Pruski, M.; Lin, V. S. Y., Organic functionalization and morphology control of mesoporous silicas via a co-condensation synthesis method. *Chemistry of materials* 2003, 15, 4247-4256.
6. Vallet-Regí, M.; Balas, F.; Arcos, D., Mesoporous materials for drug delivery. *Angewandte Chemie International Edition* 2007, 46, 7548-7558.
7. Han, Y. J.; Stucky, G. D.; Butler, A., Mesoporous silicate sequestration and release of proteins. *Journal of the American Chemical Society* 1999, 121, 9897-9898.

8. Rác, B.; Molnár, Á.; Forgo, P.; Mohai, M.; Bertóti, I., A comparative study of solid sulfonic acid catalysts based on various ordered mesoporous silica materials. *Journal of Molecular Catalysis A: Chemical* 2006, 244, 46-57.
9. Dufaud, V.; Beauchesne, F.; Bonneviot, L., Organometallic chemistry inside the pore walls of mesostructured silica materials. *Angewandte Chemie* 2005, 117, 3541-3543.
10. Van Rhijn, W.; De Vos, D.; Sels, B.; Bossaert, W., Sulfonic acid functionalised ordered mesoporous materials as catalysts for condensation and esterification reactions. *Chemical communications* 1998, 317-318.
11. Lu, J.; Liong, M.; Li, Z.; Zink, J. I.; Tamanoi, F., Biocompatibility, Biodistribution, and Drug-Delivery Efficiency of Mesoporous Silica Nanoparticles for Cancer Therapy in Animals. *Small* 2010, 6, 1794-1805.
12. Lu, J.; Liong, M.; Zink, J. I.; Tamanoi, F., Mesoporous silica nanoparticles as a delivery system for hydrophobic anticancer drugs. *Small* 2007, 3, 1341-1346.
13. Feng, X.; Fryxell, G. E.; Wang, L. Q.; Kim, A. Y.; Liu, J.; Kemner, K. M., Functionalized monolayers on ordered mesoporous supports. *Science* 1997, 276, 923-926.
14. Mercier, L.; Pinnavaia, T. J., Access in mesoporous materials: Advantages of a uniform pore structure in the design of a heavy metal ion adsorbent for environmental remediation. *Advanced Materials* 1997, 9, 500-503.
15. Alberius, P. C. A.; Frindell, K. L.; Hayward, R. C.; Kramer, E. J.; Stucky, G. D.; Chmelka, B. F., General Predictive Syntheses of Cubic, Hexagonal, and Lamellar Silica and Titania Mesostructured Thin Films. *Chemistry of Materials* 2002, 14, 3284-3294.

16. Zhao, D.; Yang, P.; Margolese, D. I.; Stucky, G. D., Synthesis of continuous mesoporous silica thin films with three-dimensional accessible pore structures. *Chemical Communications* 1998, 2499-2500.
17. Zhao, D.; Yang, P.; Melosh, N.; Feng, J.; Chmelka, B. F.; Stucky, G. D., Continuous mesoporous silica films with highly ordered large pore structures. *Advanced Materials* 1998, 10, 1380-1385.
18. Hatton, B. D.; Landskron, K.; Whitnall, W.; Perovic, D. D.; Ozin, G. A., Spin-Coated Periodic Mesoporous Organosilica Thin Films—Towards a New Generation of Low-Dielectric-Constant Materials. *Advanced Functional Materials* 2005, 15, 823-829.
19. Ogawa, M., A simple sol–gel route for the preparation of silica–surfactant mesostructured materials. *Chemical Communications* 1996, 1149-1150.
20. Ogawa, M.; Masukawa, N., Preparation of transparent thin films of lamellar, hexagonal and cubic silica-surfactant mesostructured materials by rapid solvent evaporation methods. *Microporous and mesoporous materials* 2000, 38, 35-41.
21. Brinker, C. J.; Lu, Y.; Sellinger, A.; Fan, H., Evaporation-induced self-assembly: nanostructures made easy. *Advanced Materials* 1999, 11, 579-585.
22. Lu, Y.; Ganguli, R.; Drewien, C. A.; Anderson, M. T.; Brinker, C. J.; Gong, W.; Guo, Y.; Soye, H.; Dunn, B.; Huang, M. H., Continuous formation of supported cubic and hexagonal mesoporous films by sol–gel dip-coating. *Nature* 1997, 389, 364-368.
23. Richman, E. K.; Brezesinski, T.; Tolbert, S. H., Vertically oriented hexagonal mesoporous films formed through nanometre-scale epitaxy. *Nature materials* 2008, 7, 712-717.

24. Koganti, V. R.; Rankin, S. E., Synthesis of surfactant-templated silica films with orthogonally aligned hexagonal mesophase. *The Journal of Physical Chemistry B* 2005, 109, 3279-3283.
25. Koganti, V. R.; Dunphy, D.; Gowrishankar, V.; McGehee, M. D.; Li, X.; Wang, J.; Rankin, S. E., Generalized coating route to silica and titania films with orthogonally tilted cylindrical nanopore arrays. *Nano letters* 2006, 6, 2567-2570.
26. Freer, E. M.; Krupp, L. E.; Hinsberg, W. D.; Rice, P. M.; Hedrick, J. L.; Cha, J. N.; Miller, R. D.; Kim, H.-C., Oriented mesoporous organosilicate thin films. *Nano letters* 2005, 5, 2014-2018.
27. Shan, F.; Lu, X.; Zhang, Q.; Wu, J.; Wang, Y.; Bian, F.; Lu, Q.; Fei, Z.; Dyson, P. J., A Facile Approach for Controlling the Orientation of One-Dimensional Mesochannels in Mesoporous Titania Films. *Journal of the American Chemical Society* 2012, 134, 20238-20241.
28. Teng, Z.; Zheng, G.; Dou, Y.; Li, W.; Mou, C. Y.; Zhang, X.; Asiri, A. M.; Zhao, D., Highly Ordered Mesoporous Silica Films with Perpendicular Mesochannels by a Simple Stöber-Solution Growth Approach. *Angewandte Chemie International Edition* 2012, 51, 2173-2177.
29. Klichko, Y.; Khashab, N. M.; Yang, Y.-W.; Angelos, S.; Stoddart, J. F.; Zink, J. I., Improving pore exposure in mesoporous silica films for mechanized control of the pores. *Microporous and Mesoporous Materials* 2010, 132, 435-441.
30. de las Heras Alarcón, C.; Pennadam, S.; Alexander, C., Stimuli responsive polymers for biomedical applications. *Chemical Society Reviews* 2005, 34, 276-285.

31. Stuart, M. A. C.; Huck, W. T. S.; Genzer, J.; Müller, M.; Ober, C.; Stamm, M.; Sukhorukov, G. B.; Szleifer, I.; Tsukruk, V. V.; Urban, M., Emerging applications of stimuli-responsive polymer materials. *Nature materials* 2010, 9, 101-113.
32. Yavuz, M. S.; Cheng, Y.; Chen, J.; Cobley, C. M.; Zhang, Q.; Rycenga, M.; Xie, J.; Kim, C.; Song, K. H.; Schwartz, A. G., Gold nanocages covered by smart polymers for controlled release with near-infrared light. *Nature materials* 2009, 8, 935-939.
33. Hoffman, A. S., Stimuli-responsive Polymers: Biomedical Applications and Challenges for Clinical Translation. *Advanced drug delivery reviews* 2012.
34. You, Y.-Z.; Kalebaila, K. K.; Brock, S. L., Temperature-controlled uptake and release in PNIPAM-modified porous silica nanoparticles. *Chemistry of Materials* 2008, 20, 3354-3359.
35. Fu, Q.; Rama Rao, G. V.; Ward, T. L.; Lu, Y.; Lopez, G. P., Thermoresponsive transport through ordered mesoporous silica/PNIPAAm copolymer membranes and microspheres. *Langmuir* 2007, 23, 170-174.
36. Zhang, X.; Yang, P.; Dai, Y.; Ma, P. a.; Li, X.; Cheng, Z.; Hou, Z.; Kang, X.; Li, C.; Lin, J., Multifunctional Up-Converting Nanocomposites with Smart Polymer Brushes Gated Mesopores for Cell Imaging and Thermo/pH Dual-Responsive Drug Controlled Release. *Advanced Functional Materials* 2013.
37. Johansson, E.; Zink, J. I., Nanostructured Silica Thin Films Self-Assembled with Electron Donors and Acceptors to Measure Electron Tunneling. *Journal of the American Chemical Society* 2007, 129, 14437-14443.

38. Miyata, H.; Kuroda, K., Preferred alignment of mesochannels in a mesoporous silica film grown on a silicon (110) surface. *Journal of the American Chemical Society* 1999, 121, 7618-7624.
39. Miyata, H.; Suzuki, T.; Fukuoka, A.; Sawada, T.; Watanabe, M.; Noma, T.; Takada, K.; Mukaide, T.; Kuroda, K., Silica films with a single-crystalline mesoporous structure. *Nature materials* 2004, 3, 651-656.
40. Tolbert, S. H.; Firouzi, A.; Stucky, G. D.; Chmelka, B. F., Magnetic field alignment of ordered silicate-surfactant composites and mesoporous silica. *Science* 1997, 278, 264-268.
41. Brinker, C. J.; Scherer, G. W., *Sol-gel science: the physics and chemistry of sol-gel processing*. Academic Pr: 1990.
42. Bourgeois, A.; Brunet-bruneau, A.; Fisson, S.; Rivory, J.; Matheron, M.; Gacoin, T.; Boilot, J.-p., Adsorption and Desorption Isotherms at Ambient Temperature Obtained by Ellipsometric Porosimetry to Probe Micropores in Ordered Mesoporous Silica Films. *Adsorption* 2005, 11, 195-199.
43. Xia, Y.; Whitesides, G. M., Soft Lithography. 1998, 37, 24.
44. Quake, S. R.; Scherer, A., From micro-to nanofabrication with soft materials. *Science* 2000, 290, 1536-1540.
45. Rogers, J. A.; Nuzzo, R. G., Recent progress in soft lithography. *Materials today* 2005, 8, 50-56.
46. Campbell, C. J.; Smoukov, S. K.; Bishop, K. J. M.; Grzybowski, B. A., Reactive surface micropatterning by wet stamping. *Langmuir* 2005, 21, 2637-2640.

47. Smoukov, S. K.; Bishop, K. J. M.; Klajn, R.; Campbell, C. J.; Grzybowski, B. A., Cutting into solids with micropatterned gels. *Advanced Materials* 2005, 17, 1361-1365.
48. Schwartz, B. J., Conjugated polymers as molecular materials: How chain conformation and film morphology influence energy transfer and interchain interactions. *Annual review of physical chemistry* 2003, 54, 141-172.
49. Lin, H.-N.; Lin, H.-L.; Wang, S.-S.; Yu, L.-S.; Perng, G.-Y.; Chen, S.-A.; Chen, S.-H., Nanoscale charge transport in an electroluminescent polymer investigated by conducting atomic force microscopy. *Applied physics letters* 2002, 81, 2572-2574.
50. Schaller, R. D.; Lee, L. F.; Johnson, J. C.; Haber, L. H.; Saykally, R. J.; Viece, J.; Benjamin, I.; Nguyen, T.-Q.; Schwartz, B. J., The nature of interchain excitations in conjugated polymers: Spatially-varying interfacial solvatochromism of annealed MEH-PPV films studied by near-field scanning optical microscopy (NSOM). *The Journal of Physical Chemistry B* 2002, 106, 9496-9506.
51. Cadby, A. J.; Tolbert, S. H., Controlling optical properties and interchain interactions in semiconducting polymers by encapsulation in periodic nanoporous silicas with different pore sizes. *The Journal of Physical Chemistry B* 2005, 109, 17879-17886.
52. Greenfield, S. R.; Svec, W. A.; Gosztola, D.; Wasielewski, M. R., Multistep photochemical charge separation in rod-like molecules based on aromatic imides and diimides. *Journal of the American Chemical Society* 1996, 118, 6767-6777.