JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

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Near Infrared Light Triggered Release of Biomacromolecules from Hydrogels Loaded with Upconversion Nanoparticles

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Supporting Information

ABSTRACT: Using a photosensitive hybrid hydrogel loaded with upconversion nanoparticles (UCNPs), we show that continuous-wave near-infrared (NIR) light (980 nm) can be used to induce the gel−sol transition and release large, inactive biomacromolecules (protein and enzyme) entrapped in the hydrogel into aqueous solution "on demand", where their bioactivity is recovered. This study is a new demonstration and development in harnessing the unique multiphoton effect of UCNPs for photosensitive materials of biomedical interest.

Among the many biomedical applications of polymer hydrogels, their use in encapsulating and releasing large biomacromolecules (e.g., proteins or enzymes) in response to stimuli such as changes in pH[1] or temperature;[2] the presence of redox species,[3] biomolecules,[4] or ions;[5] or electric fields[6] or light[7] has the great potential to improve how we treat disease and study complex biochemical processes. Also noticeable are hydrogels using aptamers and complementary oligonucleotides to achieve controlled release of multiple drugs.[8] In this context, a hydrogel can act as a "cage" and hide biomacromolecules to prevent them from interacting with other species until desired. Once the stimulating signal triggers a structural disruption of the hydrogel through a process such as a gel−sol transition or gel volume expansion/contraction, the entrapped biomacromolecules are released into the bulk solution, where their bioactivity is recovered.

Light offers many distinct advantages over other stimuli for controlling this disruption process. It can be tuned (color and intensity) and used for remote activation of a wide range of materials at a specific time and location with relatively high precision. However, the examples of light-responsive hydrogels developed in recent years suffer from a serious drawback that hinders their potential use in biomedical applications: typically, their photochemical reactions all require the use of high-energy UV or visible light, neither of which can penetrate deeply into tissues and both of which can cause detrimental side effects to healthy cells. The most common way to overcome this problem is the use of photoresponsive systems that can absorb two photons of near-infrared (NIR) light to induce the same reactions that are induced by UV or visible light. However, the usefulness of this approach is limited because the low two-photon absorption cross-section of typical chromophores[9] makes the process very inefficient even when femtosecond-pulse lasers are used. An alternative strategy to harness the characteristics of NIR light takes advantage of NIR-absorbing nanostructures, such as gold nanoparticles (NPs),[10] carbon nanotubes[11] and graphene oxide NPs,[12] by incorporating them into thermoresponsive hydrogel matrices. In these cases, the photothermal effect is used to convert NIR light into heat, which triggers a disruption of the hydrogel that results in the release of entrapped molecules. However, the photothermal-effect-based approach has constraints: (1) generally it is applicable only to thermosensitive polymers having a hydration−dehydration phase transition temperature and (2) photoinduced disruption of the hydrogel cannot be retained after the NIR irradiation is turned off because the heating effect disappears and the initial solution temperature recovers.

In this report, we demonstrate an alternative use of NPs to convert NIR light into UV light, enabling UV-light-induced photoreactions to be used to affect the structure of hydrogels. Our example is the first of its kind. We use core−shell lanthanide-doped upconverting NPs (UCNPs) to induce a gel−sol transition when they are irradiated with 980 nm NIR light, consequently triggering the release of entrapped biomacromolecules. This is feasible because these particular NPs convert as many as five NIR photons into a UV photon, which is emitted back into the system to trigger the photochemical reactions of active groups within the polymer matrix.[13] The UCNPs are convenient for this application because they are also nontoxic and do not "blink".[14]

We previously demonstrated that UCNPs can be loaded within block-copolymer micelles and act as internal UV and visible-light sources upon NIR irradiation, with the emitted UV or visible-light photons activating a photoreaction leading to micelle disassembly.[15] This and other representative examples highlight the use of UCNPs in NIR-light-controllable biomaterials and imaging systems.[16] The present study is a novel demonstration of how this universal and robust strategy allows photosensitive biomaterials to be activated by continuous-wave (CW) NIR light using hydrogels. In this report, we show how we can "hide" large biomacromolecules inside a polymer hydrogel, effectively "shutting down" their bioactivity, and then release them "on demand" using NIR light, restoring their bioactivity.

Our approach is illustrated in Figure 1, which shows a schematic representation of the NIR-light-degradable hydrogel (black lines for polymer chains and red triangles for photocleavable cross-links) loaded with UCNPs (green spheres) and biomacromolecules (yellow rods). The figure also shows the

Received: September 6, 2012
Published: September 26, 2012

   dx.doi.org/10.1021/ja308876j | J. Am. Chem. Soc. 2012, 134, 16558−16561

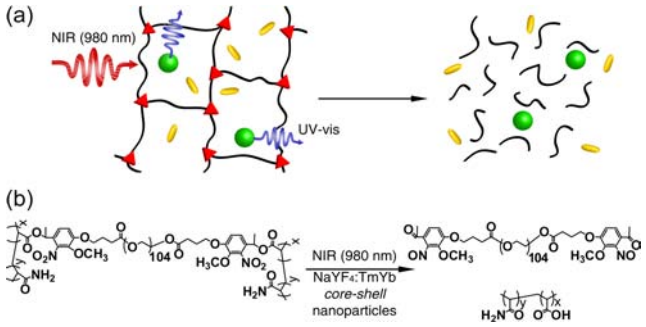

Figure 1. (a) Schematic illustration of the NIR-light-triggered degradation of a photosensitive hydrogel using the UV light generated by encapsulated UCNPs. The polymeric components are depicted as black lines, the photocleavable cross-links as red triangles, the UCNPs as green spheres, and the trapped biomacromolecules as yellow rods. (b) Chemical structure of the hydrogel containing photocleavable *o*-nitrobenzyl moieties in the cross-linker and the NIR-light-induced photoreaction of the hydrogel via UV light emitted by loaded NaYF4:TmYb core–shell UCNPs. The number of monomer units per PEG cross-linker was 104, and the molar ratio between the acrylamide monomer units and the PEG cross-linker, y/x, was ~50 (as determined from the ^{1}H NMR spectrum of a fully UV-degraded gel sample in $CDCl_3$).

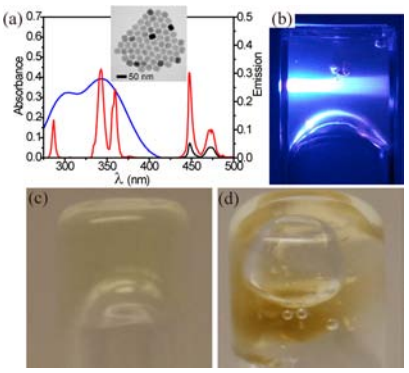

Figure 2. (a) Absorption spectrum of the hydrogel dispersed in water (blue line) and emission spectra of the neat UCNPs in aqueous solution (red line) and the UCNP-loaded hydrogel (black line) upon 980 nm excitation. The inset is a transmission electron microscopy image of neat UCNPs. (b) Photograph of a UCNP-loaded hydrogel under a 980 nm CW diode laser exposure (3.1 W). (c, d) Photographs of a UCNP-loaded hydrogel (~0.08 mL) (c) before and (d) after irradiation with 980 nm light (5 W, 195 min), showing the NIR-light-induced gel–sol transition of the hydrogel.

chemical structure of the hydrogel, which has a cross-linked hybrid polyacrylamide–poly(ethylene glycol) (PEG) structure held together by photoresponsive *o*-nitrobenzyl groups. NIR irradiation of the UCNPs within this cross-linked system generates the UV light needed to cleave the *o*-nitrobenzyl groups in their typical photooxidation process. This results in the breakdown of the entire gel (gel–sol transition), releasing the trapped components. Details of materials synthesis and characterization as well as experimental procedures for loading of UCNPs in the hydrogel, simultaneous loading of proteins or enzymes, and NIR-light-triggered release are provided in the Supporting Information (SI). The photocleavable PEG cross-linker was synthesized using a literature method[7a−d] with some modifications. The core–shell $NaYF_4$:TmYb NPs (core = $NaYF_4$:0.5 mol % Tm^{3+}:30 mol % Yb^{3+}; shell = $NaYF_4$), which had a uniform hexagonal prism shape with an average length of 36.0 ± 1.1 nm and width of 32.0 ± 1.5 nm, were synthesized using a literature method.[17] For incorporation of the UCNPs into the hydrogel, a water-compatible system is required (the UCNPs are prepared in an organic-soluble form coated with oleate ligands). This was achieved by a ligand exchange process that replaced the hydrophobic oleate ligands with water-compatible polyvinylpyrrolidone.[18] The UCNPs and biomacromolecules were conveniently loaded into the photosensitive hydrogel by polymerizing an aqueous mixture of the acrylamide monomer and the photocleavable PEG cross-linker by a redox-initiated radical polymerization process.

As designed, the emission spectrum of the UCNPs changes when the NPs are trapped within the hydrogel as a result of the overlap of specific bands with the absorption band of the photoreactive component holding the hydrogel together (Figure 2a). When irradiated with 980 nm light, the NPs emit at several wavelengths within the UV–vis region of the spectrum. The ones relevant to the current study are those appearing between 250 and 400 nm, since these wavelengths are absorbed by the hydrogel and induce photoreactions of the *o*-nitrobenzyl groups. This phenomenon was clearly revealed by the fact that the UV emissions from the UCNPs could no longer be observed when the UCNPs were trapped within the hydrogel. In contrast, the emission peaks in the visible region (430–500 nm), a spectral region where the hydrogel has no absorptions, clearly remained. As the laser beam traveled through the UCNP-loaded hydrogel, visible photoluminescence (PL) and scattered blue light were visible (Figure 2b). Figure 2c,d shows photographs of a hydrogel formed inside a 4.7 mm diameter capillary tube before and after NIR irradiation using a diode laser, respectively. The NIR-light-induced degradation (gel–sol transition) is indicated by the flow of the hydrogel after the NIR irradiation. We note that a relatively high NIR laser power and a long irradiation time were used to allow the visualization of the flow of the hydrogel confined in the tube, which occurred in the late stage of the hydrogel disruption.

Our first demonstration of NIR-light-triggered release of proteins from the hydrogel used fluorescein isothiocyanate bovine serum albumin (FITC-BSA) as a general model and proof-of-concept. The hydrogel-trapped protein was prepared by adding a phosphate-buffered saline (PBS) solution of the protein (pH 7.0, 100 mM PBS, protein concentration 1 mg/mL) to an aqueous solution containing the UCNPs, the acrylamide monomer, the PEG cross-linker, and an initiator (a redox pair of ammonium persulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine). After the polymerization was complete, the hydrogel loaded with both FITC-BSA and the UCNPs was washed with fresh PBS solution to remove any nontrapped protein. The release experiments were carried out by exposing a sample of the hydrogel (~100 mg) resting on the bottom of a cuvette to 980 nm light while monitoring the increase in absorption and emission of the liquid phase above the sample due to the photoreaction and subsequent release of the nitrobenzaldehyde cross-linker and the fluorescently labeled protein from the gel. The results of these experiments are summarized in Figure 3. The experimental setup is illustrated in Figure 3a. The changes in the UV–vis absorption spectrum of a hydrogel sample containing the UCNPs but no protein (Figure 3b) demonstrated the success of the multiphoton effect and the conversion of the photosensitive *o*-nitrobenzyl groups to their nitrosobenzaldehyde products, which diffused from the gel into the aqueous phase only when NIR light was used. Prior to NIR light exposure, only very minor changes in the absorption spectra were detected even

dx.doi.org/10.1021/ja308876j | *J. Am. Chem. Soc.* 2012, 134, 16558–16561

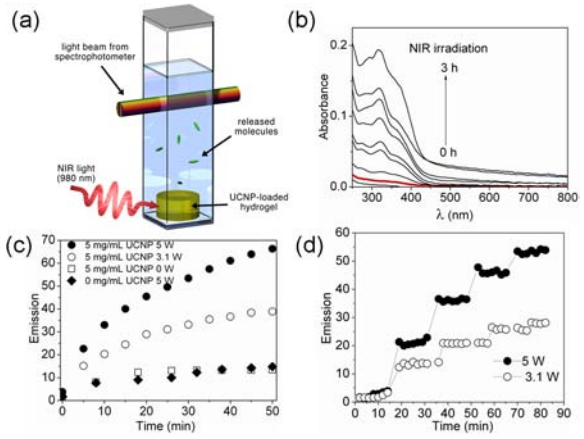

Figure 3. (a) Setup used to detect molecular species diffusing from the hydrogel into the aqueous solution as a result of 980 nm excitation. (b) Changes in the UV−vis absorption spectrum of an aqueous solution containing a hydrogel exposed to NIR light (5 W) for different times (0−3 h). For comparison, the absorption spectrum of the hydrogel immersed in the solution for 12 h without NIR irradiation is shown in red. (c) Plots of fluorescence emission intensity (λ_{em} = 514 nm, λ_{ex} = 494 nm) detected from FITC-BSA loaded in the hydrogel vs NIR irradiation time, showing NIR-light-triggered release of the protein from the hydrogel. The results of two control tests are also shown for comparison. (d) Temporal control of the protein release by turning the NIR diode laser on (5 min) and off.

12 h after immersion of the hydrogel in the solution. Irradiation of the same hydrogel with 980 nm light (~0.1 mm diameter beam size) resulted in the appearance of absorption bands at 300−400 nm characteristic of the nitrosobenzaldehydes produced from the photocleavage reaction. The intensity of these absorption bands increased as long as the gel was exposed to NIR light, indicating a continuous photoreaction.

Figure 3c illustrates release of trapped protein from our hydrogel using NIR light. In this case, the amount of FITC-BSA released into the solution was monitored by the increase in the PL intensity at the emission maximum wavelength. Only a small observable amount of the protein was released from the hydrogel containing the NPs in the absence of NIR light, as expected on the basis of several cases reported in the literature.[1a,3a,b] Similar amounts of released protein were observed for samples of the hydrogel containing only the protein upon exposure to 980 nm light (5 W). The amount of protein released from the hybrid system increased significantly when the hydrogel loaded with both UCNPs and protein was exposed to the NIR laser (3−5 W), clearly demonstrating the NIR-light-triggered release of the large biomacromolecules as a result of hydrogel degradation. As expected, the release was power-dependent, slowing when the laser intensity was reduced. On the basis of the emission intensity of the same amount of FITC-BSA in solution without the hydrogel, the release of the protein entrapped in the UCNP-loaded hydrogel after 50 min of NIR irradiation was ~67% at a laser power of 5 W and ~38% at 3.1 W (Figure S6 in the SI). The latter experiment was repeated twice, and the release of protein was found to be ~40 and ~33%, respectively, suggesting quite good reproducibility. The on-demand release of protein from the hybrid hydrogel was best demonstrated by alternating between "light" and "dark" conditions (Figure 3d). As designed, release events occurred only when the NIR light was on (3−5 W), as illustrated by the increase in fluorescence intensity. Each time the light was turned off, there was no change in the emission intensity because the protein molecules remained entrapped in the hydrogel until the release was triggered again by turning on the laser.

Control over the bioactivity due to NIR-triggered release from our hybrid hydrogel was readily demonstrated by monitoring the activity of trypsin (an enzyme that cleaves peptide bonds in proteins) on a fluorogenic substrate, (CBZ-Arg)2-R110. The results are shown in Figure 4. The enzyme was loaded into our

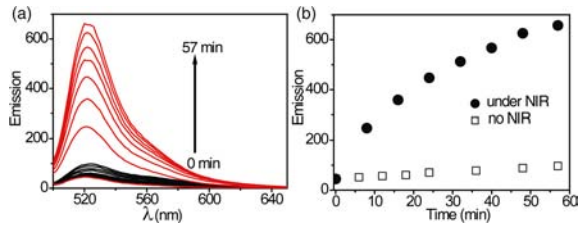

Figure 4. Increase in the emission intensity revealing the enzymatic activity of trypsin on a fluorogenic substrate (λ_{ex} = 498 nm) recorded with a hydrogel loaded with the enzyme and UCNPs in aqueous solution (using the setup shown in Figure 3a): (a) in the dark (black curves) and upon 5 W 980 nm NIR irradiation from 0 to 57 min (red curves); (b) plots of fluorescence emission intensity at the 521 nm peak vs time with (●) and without (□) NIR light irradiation.

hydrogel as described above. After washing and removal of nontrapped enzyme molecules, the hydrogel was immersed in water containing (CBZ-Arg)2-R110. There was little increase in fluorescence due to the enzymatic conversion of the substrate into its emissive product (rhodamine 110) in the absence of NIR light even after 57 min, indicating that the enzyme was effectively trapped within the hydrogel, which suppressed its activity. The observed weak activity was likely caused by a very small amount of trypsin that diffused from the gel (as was shown previously for BSA) and/or diffusion of (CBZ-Arg)2-R110 into the hydrogel, where it could be converted into its fluorescent product within the gel matrix before diffusing into the whole solution. Exposing the hydrogel to 980 nm light produced an immediate increase in the fluorescence corresponding to rhodamine 110, indicating successful release of trypsin and recovery of its enzymatic activity. Again, according to a control test using the same amount of trypsin dissolved in solution, ~72% of the enzyme was released after 57 min of NIR irradiation at a laser power of 5 W (Figure S6).

Before concluding, we mention that several in-depth studies have examined the long-term toxicity of $NaYF_4$-based UCNPs using both cell and small-animal models;[19] the results suggest that these UCNPs possess little or no toxicity over extended exposure times. For future studies of NIR-light-sensitive UCNP-loaded hydrogels, it would be of interest to apply the same design to other biocompatible hydrogels and use photocleavable moieties that may be more suitable for biomaterials (e.g., coumarin).[20] In view of the on-demand release activated by NIR light, this type of hydrogel would be better suited for applications where a temporally controllable, stepwise release is desired, rather than applications requiring prolonged release (e.g., drug release from implants).

In conclusion, our hybrid UCNP−hydrogel system represents the first demonstration of the use of the multiphoton effect of UCNPs to trigger structural changes in photosensitive hydrogels. It allows the use of CW NIR light to induce the gel−sol transition and release large, inactive biomacromolecules on demand, where their bioactivity can be recovered. This study is an important new

dx.doi.org/10.1021/ja308876j | J. Am. Chem. Soc. 2012, 134, 16558−16561

development in harnessing the unique properties of UCNPs for photosensitive materials of biological and biomedical interest.

■ ASSOCIATED CONTENT

Supporting Information

Details of materials synthesis and characterization and experimental procedures for loading of UCNPs in the hydrogel, simultaneous loading with proteins or enzymes, and NIR-light-triggered release. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC), le Fonds Québécois de la Recherche sur la Nature et les Technologies of Québec (FQRNT), the Canada Research Chairs Program, and Simon Fraser University through the Community Trust Endowment Fund. Y.Z. is a member of FQRNT-funded CSACS, CQMF, and FRQS-funded Centre de Recherche Clinique Étienne-Le Bel. J.-C.B. thanks the Michael Smith Foundation for Health Research for support. B.Y. thanks the China Scholarship Council for scholarship support.

■ REFERENCES

(1) (a) Murthy, N.; Thng, Y. X.; Schuck, S.; Xu, M. C.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 12398. (b) Zhao, B.; Moore, J. S. *Langmuir* **2001**, *17*, 4758. (c) Wu, J.; Sailor, M. J. *Adv. Funct. Mater.* **2009**, *19*, 733. (d) Yoshikawa, H. Y.; Rossetti, F. F.; Kaufmann, S.; Kaindl, T.; Madsen, J.; Engel, U.; Lewis, A. L.; Armes, S. P.; Tanaka, M. *J. Am. Chem. Soc.* **2011**, *133*, 1367. (e) Petka, W. A.; Harden, J. L.; McGrath, K. P.; Wirtz, D.; Tirrell, D. A. *Science* **1998**, *281*, 389.

(2) (a) Wang, C.; Stewart, R. J.; Kopeček, J. *Nature* **1999**, *397*, 417. (b) Shim, W. S.; Yoo, J. S.; Bae, Y. H.; Lee, D. S. *Biomacromolecules* **2005**, *6*, 2930. (c) Tai, H.; Howard, D.; Takae, S.; Wang, W.; Vermonden, T.; Hennink, W. E.; Stayton, P. S.; Hoffman, A. S.; Endruweit, A.; Alexander, C.; Howdle, S. M.; Shakesheff, K. M. *Biomacromolecules* **2009**, *10*, 2895. (d) Lutz, J.-F. *Adv. Mater.* **2011**, *23*, 2237.

(3) (a) Verheyen, E.; van der Wal, S.; Deschout, H.; Braeckmans, K.; de Smedt, S.; Barendregt, A.; Hennink, W. E.; van Nostrum, C. F. *J. Controlled Release* **2011**, *156*, 329. (b) Choh, S.-Y.; Cross, D.; Wang, C. *Biomacromolecules* **2011**, *12*, 1126. (c) Dunn, S. S.; Tian, S.; Blake, S.; Wang, J.; Galloway, A. L.; Murphy, A.; Pohlhaus, P. D.; Rolland, J. P.; Napier, M. E.; DeSimone, J. M. *J. Am. Chem. Soc.* **2012**, *134*, 7423. (d) González-Toro, D. C.; Ryu, J.-H.; Chacko, R. T.; Zhuang, J.; Thayumanavan, S. *J. Am. Chem. Soc.* **2012**, *134*, 6964. (e) Oh, J. K.; Siegwart, D. J.; Lee, H.-i.; Sherwood, G.; Peteanu, L.; Hollinger, J. O.; Kataoka, K.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2007**, *129*, 5939.

(4) (a) Miyata, T.; Asami, N.; Uragami, T. *Nature* **1999**, *399*, 766. (b) Xiong, M.-H.; Bao, Y.; Yang, X.-Z.; Wang, Y.-C.; Sun, B.; Wang, J. *J. Am. Chem. Soc.* **2012**, *134*, 4355. (c) Toledano, S.; Williams, R. J.; Jayawarna, V.; Ulijn, R. V. *J. Am. Chem. Soc.* **2006**, *128*, 1070.

(5) Ehrick, J. D.; Deo, S. K.; Browning, T. W.; Bachas, L. G.; Madou, M. J.; Daunert, S. *Nat. Mater.* **2005**, *4*, 298.

(6) Ge, J.; Neofytou, E.; Cahill, T. J.; Beygui, R. E.; Zare, R. N. *ACS Nano* **2012**, *6*, 227.

(7) (a) Kloxin, A. M.; Tibbitt, M. W.; Anseth, K. S. *Nat. Protoc.* **2010**, *5*, 1867. (b) Kloxin, A. M.; Tibbitt, M. W.; Kasko, A. M.; Fairbairn, J. A.; Anseth, K. S. *Adv. Mater.* **2010**, *22*, 61. (c) DeForest, C. A.; Anseth, K. S. *Nat. Chem.* **2011**, *3*, 925. (d) DeForest, C. A.; Anseth, K. S. *Angew. Chem.* **2012**, *124*, 1852. (e) Griffin, D. R.; Kasko, A. M. *J. Am. Chem. Soc.* **2012**, *134*, 13103. (f) Kloxin, A. M.; Kasko, A. M.; Salinas, C. N.; Anseth, K. S. *Science* **2009**, *324*, 59. (g) Peng, K.; Tomatsu, I.; van den Broek, B.; Cui, C.; Korobko, A. V.; van Noort, J.; Meijer, A. H.; Spaink, H. P.; Kros, A. *Soft Matter* **2011**, *7*, 4881. (h) Luo, Y.; Shoichet, M. S. *Nat. Mater.* **2004**, *3*, 249. (i) Muraoka, T.; Cui, H.; Stupp, S. I. *J. Am. Chem. Soc.* **2008**, *130*, 2946. (j) Ramanan, V. V.; Katz, J. S.; Guvendiren, M.; Cohen, E. R.; Marklein, R. A.; Burdick, J. A. *J. Mater. Chem.* **2010**, *20*, 8920. (k) Woodcock, J. W.; Wright, R. A. E.; Jiang, X.; O'Lenick, T. G.; Zhao, B. *Soft Matter* **2010**, *6*, 3325.

(8) (a) Yang, H.; Liu, H.; Kang, H.; Tan, W. *J. Am. Chem. Soc.* **2008**, *130*, 6320. (b) Zhu, Z.; Wu, C.; Liu, H.; Zou, Y.; Zhang, X.; Kang, H.; Yang, C. J.; Tan, W. *Angew. Chem.* **2010**, *122*, 1070. (c) Wei, B.; Cheng, I.; Luo, K. Q.; Mi, Y. *Angew. Chem., Int. Ed.* **2008**, *47*, 331. (d) Battig, M. R.; Soontornworajit, B.; Wang, Y. *J. Am. Chem. Soc.* **2012**, *134*, 12410.

(9) (a) Pawlicki, M.; Collins, H. A.; Denning, R. G.; Anderson, H. L. *Angew. Chem., Int. Ed.* **2009**, *48*, 3244. (b) Ellis-Davies, G. C. R. *Nat. Methods* **2007**, *4*, 619. (c) He, G. S.; Tan, L.-S.; Zheng, Q.; Prasad, P. N. *Chem. Rev.* **2008**, *108*, 1245. (d) Fomina, N.; Sankaranarayanan, J.; Almutairi, A. *Adv. Drug Delivery Rev.* **2012**, *64*, 1005.

(10) (a) Gorelikov, I.; Field, L. M.; Kumacheva, E. *J. Am. Chem. Soc.* **2004**, *126*, 15938. (b) Shiotani, A.; Mori, T.; Niidome, T.; Niidome, Y.; Katayama, Y. *Langmuir* **2007**, *23*, 4012. (c) Charati, M. B.; Lee, I.; Hribar, K. C.; Burdick, J. A. *Small* **2010**, *6*, 1608.

(11) (a) Samanta, S. K.; Pal, A.; Bhattacharya, S.; Rao, C. N. R. *J. Mater. Chem.* **2010**, *20*, 6881. (b) Zhang, X.; Pint, C. L.; Lee, M. H.; Schubert, B. E.; Jamshidi, A.; Takei, K.; Ko, H.; Gillies, A.; Bardhan, R.; Urban, J. J.; Wu, M.; Fearing, R.; Javey, A. *Nano Lett.* **2011**, *11*, 3239.

(12) Zhu, C.-H.; Lu, Y.; Peng, J.; Chen, J.-F.; Yu, S.-H. *Adv. Funct. Mater.* **2012**, DOI: 10.1002/adfm.201201020.

(13) Wu, S.; Han, G.; Milliron, D. J.; Aloni, S.; Altoe, V.; Talapin, D. V.; Cohen, B. E.; Schuck, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10917.

(14) (a) Chatterjee, D. K.; Yong, Z. *Nanomedicine* **2008**, *3*, 73. (b) Cheng, L.; Yang, K.; Shao, M.; Lu, X.; Liu, Z. *Nanomedicine* **2011**, *6*, 1327. (c) Mader, H. S.; Kele, P.; Saleh, S. M.; Wolfbeis, O. S. *Curr. Opin. Chem. Biol.* **2010**, *14*, 582.

(15) Yan, B.; Boyer, J.-C.; Branda, N. R.; Zhao, Y. *J. Am. Chem. Soc.* **2011**, *133*, 19714.

(16) (a) Carling, C.-J.; Nourmohammadian, F.; Boyer, J.-C.; Branda, N. R. *Angew. Chem., Int. Ed.* **2010**, *49*, 3782. (b) Yang, Y.; Shao, Q.; Deng, R.; Wang, C.; Teng, X.; Cheng, K.; Cheng, Z.; Huang, L.; Liu, Z.; Liu, X.; Xing, B. *Angew. Chem., Int. Ed.* **2012**, *51*, 3125. (c) Zhou, H.-P.; Xu, C.-H.; Sun, W.; Yan, C.-H. *Adv. Funct. Mater.* **2009**, *19*, 3892. (d) Boyer, J.-C.; Carling, C.-J.; Gates, B. D.; Branda, N. R. *J. Am. Chem. Soc.* **2010**, *132*, 15766. (e) Carling, C.-J.; Boyer, J.-C.; Branda, N. R. *J. Am. Chem. Soc.* **2009**, *131*, 10838. (f) Liu, Q.; Yang, T.; Feng, W.; Li, F. *J. Am. Chem. Soc.* **2012**, *134*, 5390. (g) Jayakumar, M. K. G.; Idris, N. M.; Zhang, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 8483. (h) Garcia, J. V.; Yang, J.; Shen, D.; Yao, C.; Li, X.; Wang, R.; Stucky, G. D.; Zhao, D.; Ford, P. C.; Zhang, F. *Small* **2012**, DOI: 10.1002/smll.201201213. (i) Vetrone, F.; Naccache, R.; Juarranz de la Fuente, A.; Sanz-Rodriguez, F.; Blazquez-Castro, A.; Rodriguez, E. M.; Jaque, D.; Sole, J. G.; Capobianco, J. A. *Nanoscale* **2010**, *2*, 495. (j) Wu, W.; Yao, L.; Yang, T.; Yin, R.; Li, F.; Yu, Y. *J. Am. Chem. Soc.* **2011**, *133*, 15810. (k) Boyer, J.-C.; Carling, C.-J.; Chua, S. Y.; Wilson, D.; Johnsen, B.; Baillie, D.; Branda, N. R. *Chem.—Eur. J.* **2012**, *18*, 3122. (l) Wang, F.; Liu, X. *Chem. Soc. Rev.* **2009**, *38*, 976.

(17) Qian, H.-S.; Zhang, Y. *Langmuir* **2008**, *24*, 12123.

(18) Dong, A.; Ye, X.; Chen, J.; Kang, Y.; Gordon, T.; Kikkawa, J. M.; Murray, C. B. *J. Am. Chem. Soc.* **2010**, *133*, 998.

(19) (a) Jalil, R. A.; Zhang, Y. *Biomaterials* **2008**, *29*, 4122. (b) Xiong, L. Q.; Yang, T. S.; Yang, Y.; Xu, C. J.; Li, F. Y. *Biomaterials* **2010**, *31*, 7078. (c) Zhou, J.; Zhu, X.; Chen, M.; Sun, Y.; Li, F. Y. *Biomaterials* **2012**, *33*, 6201.

(20) Zhao, Y. *Macromolecules* **2012**, *45*, 3647.