ACS Macro Letters

Letter

pubs.acs.org/macroletters

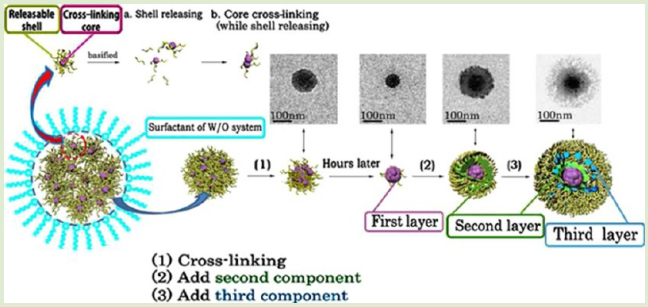
Controlled Formation of Microgels/Nanogels from a Disulfide-Linked Core/Shell Hyperbranched Polymer

Jian Zhang, Fei Yang, Hong Shen, and Decheng Wu*

Beijing National Laboratory for Molecular Sciences, State Key Laboratory of Polymer Physics & Chemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

Supporting Information

ABSTRACT: A general approach to controlled formation of microgels/nanogels is developed for producing hydrogel particles with customizable structures and properties, especially for fabricating multilayered hydrogel particles with flexibly designable structures and properties of each layer. An inverse emulsion technique is adopted to obtain micro- or nanodroplets of a disulfide-linked core/shell hyperbranched polymer. Then pH of the droplets is manipulated to trigger and control in situ core/shell separation of the polymer, dissociation of the shells, and cross-linking of the cores, in the confined space at micro/nanoscales. Loose and compact microgels/nanogels with diverse properties like particle size and swelling capacity are yielded via adjusting the gelation time. Multilayered hydrogel particles with each tailor-made layer are further prepared using the controlled in situ gelation method in association with a seed emulsion technique.



Hydrogel particles, namely, microgels/nanogels, are swollen microsized or nanosized cross-linking networks. They possess high water content, high stability, and desirable mechanical properties, stimulating interest for applications in drug delivery,[1−8] biosensing,[9] bioimaging,[10,11] chemical separation,[12] and catalysis.[13−15] Various approaches have been developed to produce hydrogel particles with diverse structures and properties, e.g., physical self-assembly,[16] chemical in situ polymerization in microemulsions,[17−20] cross-linking of performed polymers,[4−6] template-assisted nanofabrication including photolithographic[21−24] and micromolding,[25] and microfluidic techniques.[26−28] These developed approaches remarkably enrich the production of microgels/nanogels with an enhanced capacity of modulating the structures and properties. However, the design flexibility and complexity of hydrogel particles with the customized structures and properties are still very restricted, and the preparation of tailor-made microgels/nanogels remains a grand challenge.

In the previous work, we reported a facile method to create a "living" in situ gelling system for controlled formation of hydrogels from a hyperbranched polymer (BAP) with disulfide-linked core/shell structures.[29] Herein, we apply the method associated with an inverse emulsion technique to produce hydrogel particles through in situ manipulating gelation of microsized/nanosized droplets of aqueous BAP solution. Furthermore, we recapitulate a general approach to controlled formation of microgels/nanogels, especially for facile fabrication of multilayered hydrogel particles with flexibly designable structures and properties of each layer.

The general approach was summarized in Figure 1. The polymer used is BAP with a poly(amido amine) core and poly(ethylene glycol) shell, as illustrated in Figure 1A. BAP was prepared by α-amino-ω-methoxy-poly(ethylene glycol) sealing terminal vinyl groups of the precursor obtained via addition of a 1-(2-aminoethyl)piperazine and a double-molar *N*,*N*′-bis-(acryloyl)cystamine, and its structure was depicted in Figure S1 (Supporting Information). The mechanism of gelation is a pH-responsive thiol−disulfide exchange reaction. Specifically, basification triggers disulfide reshuffling and the core/shell separation, resulting in the dissociation of shells and the cross-linking of cores as indicated in Figure 1A. If dispersing the BAP solution into micro- or nanodroplets using an inverse emulsion method, we can trigger and control in situ gelation of BAP via manipulating the pH of the droplets in the confined space at micro-/nanoscales. At an early stage, gelation causes the formation of loose hydrogel particles with the particle sizes close to the initial diameters of droplets. When gelation time increases, further dissociation of the hydrophilic shells leads to shrinkage of the loose hydrogel particles into smaller and more compact particles, as shown in Figure 1B. Thus, adjusting initial diameters of the droplets from nano- to microscales and controlling the gelation time, we can achieve controlled formation of hydrogel particles. Through neutralization of the gelation system to trap the intermediate particles at a predetermined time, we can produce customized stable hydrogel particles because neutralization can terminate the

Received: September 21, 2012
Accepted: October 18, 2012
Published: October 19, 2012

dx.doi.org/10.1021/mz300489n | *ACS Macro Lett.* 2012, 1, 1295−1299

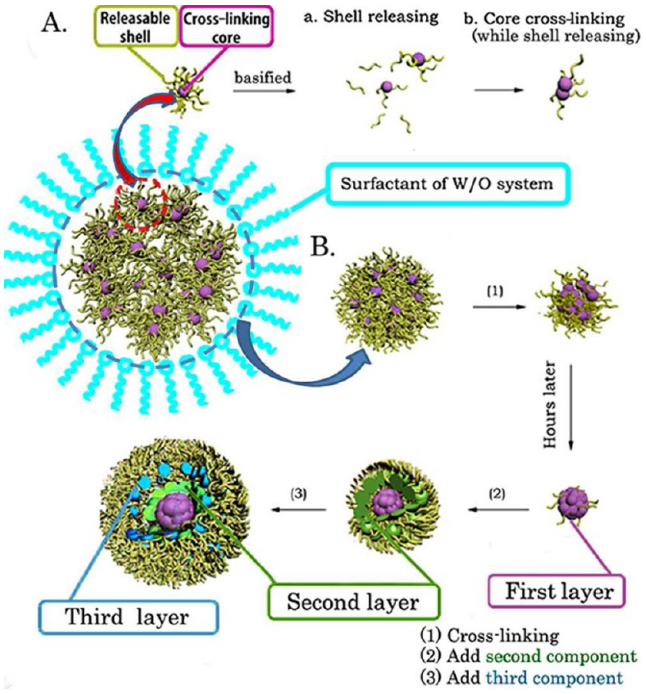


Figure 1. (A) Schematic illustration of the core/shell separation process—dissociation of the shells and cross-linking of the cores—and (B) schematic depiction of the synthetic approach to controlled formation of (multilayered) hydrogel particles.

transition process from the loose to the compact hydrogel particles.

The inverse emulsion method was adopted for producing the micro-/nanodroplets. Span80/tween80 formed a stable inverse miniemulsion of BAP with water in decane, allowing for production of loose and compact nanogels. Transmission electron microscopy (TEM) images in Figure 2a–f demonstrated that the method could prepare the customized uniform nanogels through controlling the gelation time from 1 to 24 h. For example, 190 nm of the loose nanogels was observed after cross-linking of 1 h, but the size significantly decreased to 80 nm after cross-linking of 24 h. The results of atomic force microscopy (AFM) in Figure 2g–l and dynamic light scattering (DLS) in Figure 2m gave a similar variation tendency of the particle sizes as a function of time, as indicated in Figure 2n. However, the sizes obtained from TEM, AFM, and DLS results had an obvious discrepancy, but this discrepancy was reasonable. As known to all, nanogels are swollen nanosized networks that can absorb a lot of water in aqueous solutions. TEM results revealed a "dry state" of the freeze-drying nanogels; AFM performance yielded a "wet state" of the partially swollen nanogels spreading on a substrate through spin-coating of the aqueous droplets; and DLS characterization disclosed a "saturate state" of the fully swollen nanogels at aqueous solutions. So, DLS results were in good agreement with the biggest sizes of the completely swollen particles; AFM performance yielded the big sizes of the partially swollen particles; and TEM images gave the smallest sizes of the completely dry particles. Figure 2n also depicted that the particle sizes of the loose nanogels after short-time cross-linking obtained from different characterization methods had the more significant discrepancy compared to those of the compact nanogels after long-time cross-linking. For example, the sizes of the loose nanogels after cross-linking of 1 h were 190 and 1590

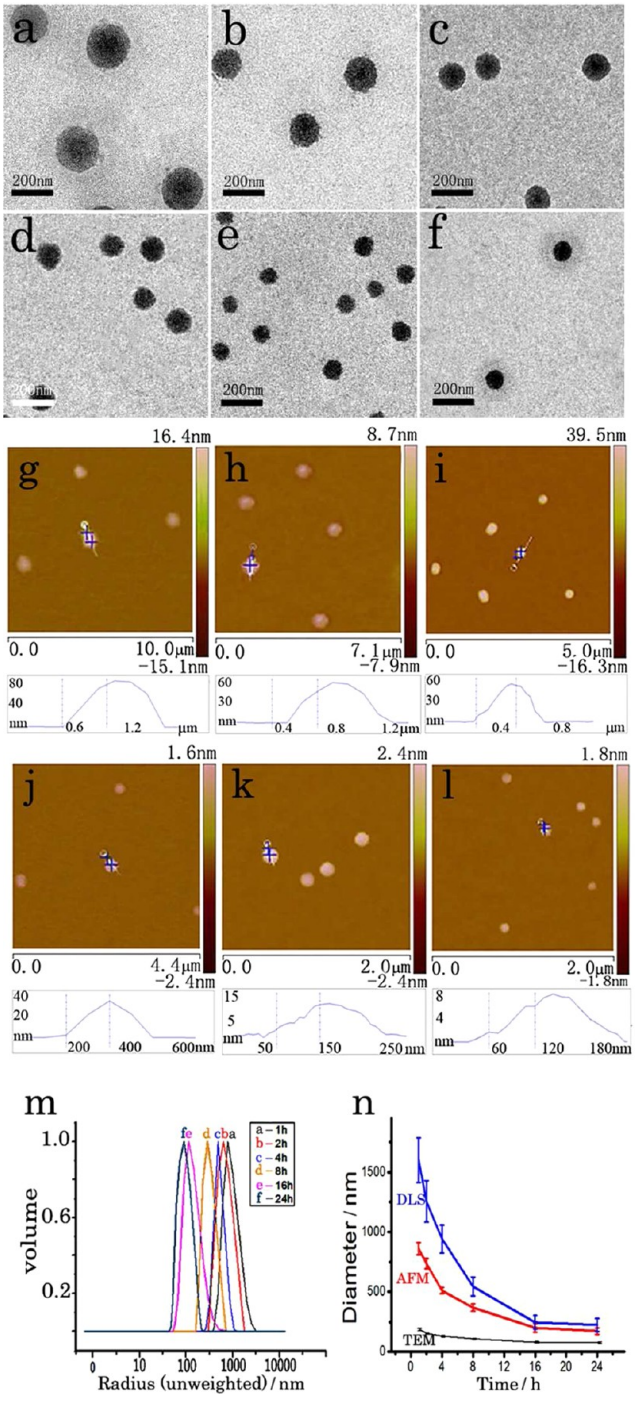


Figure 2. (a–f) TEM images of the nanogels obtained by cross-linking for (a) 1 h, (b) 2 h, (c) 4 h, (d) 8 h, (e) 16 h, and (f) 24 h. (g–l) AFM images of the nanogels obtained by cross-linking for (g) 1 h, (h) 2 h, (i) 4 h, (j) 8 h, (k) 16 h, and (l) 24 h. (m) Size distribution of the nanogels (0.5 mg/mL) in water by DLS. (n) Comparison of the diameter variation of the nanogels as a function of time observed by DLS, AFM, and TEM.

nm from TEM and DLS results, which roughly deduced the full swelling ratio to be 8.3. As a comparison, the sizes of the compact nanogels after cross-linking of 24 h were 80 and 220 nm from TEM and DLS results, and the swelling ratio decreased to be 2.7. The decrement of the swollen ratio was ascribed to the sharp reduction of hydrophilicity of the compact nanogels by losing the most hydrophilic shells. So, these results

dx.doi.org/10.1021/mz300489n | ACS Macro Lett. 2012, 1, 1295–1299

testified the method could easily produce the loose to compact nanogels with tunable properties like particle sizes and swelling capacity.

The previous work has demonstrated that the method produces covalently cross-linking hydrogels without a critical gel concentration, and 2 wt % of a polymer solution can form a loose hydrogel with complete encapsulation of water in the initial solution.[29] Similarly, the resultant nanogels are also covalently cross-linking hydrogel particles, and more than 2 wt % of polymer solution should form loose hydrogel particles with the sizes close to the diameters of the droplet at an early stage. However, after cross-linking for a long time, low polymer concentration facilitates the formation of smaller hydrogel particles in comparison with high polymer concentration since each droplet of low polymer concentration contains less polymer cores. Thus, tuning polymer concentration is also an efficient way for adjusting structures and properties of the hydrogel particles. Figure 3 demonstrated that various polymer

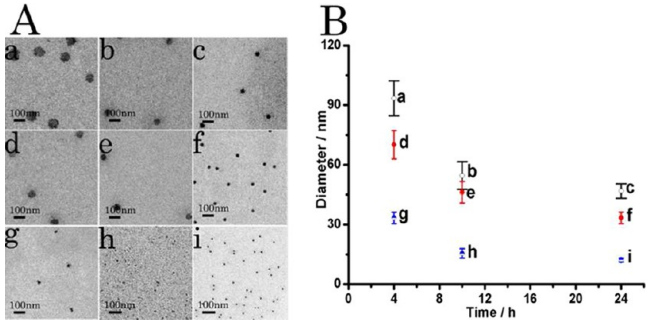


Figure 3. (A) TEM images and (B) comparison of the diameter variation of the nanogels as a function of time and polymer concentration obtained by cross-linking of (a,b,c) 23.5 wt %, (d,e,f) 17.0 wt %, and (g,h,i) 3.7 wt % of polymer solutions for (a,d,g) 4 h, (b,e,h) 10 h, and (c,f,i) 24 h.

solutions could produce diverse nanogels with obviously different sizes after cross-linking of a scheduled time under the same conditions. For instance, after cross-linking of 4 and 24 h, 23.5 wt % of polymer solution yielded 93 and 47 nm of nanogels; in comparison, 3.7 wt % of polymer solution only gave 33 and 12 nm of nanogels.

The sizes of the loose hydrogel particles are close to the initial diameters of the droplets at an early stage. If adopting different experiment conditions to prepare the uniform microdroplets, we could produce the loose and compact microgels with adjustable properties accordingly. Span80/tween80 formed a stable inverse emulsion of BAP with water in cyclohexane, allowing for production of the stable microgels. Scanning electron microscopy (SEM) observation testified that we could yield customizable microgels with tunable particle sizes, such as from 149 μm of the loose microgels after cross-linking of 1 h to 27 μm of the compact microgels after cross-linking of 24 h, as indicated in Figure 4. Through further selecting rational conditions to yield suitable initial diameters of the droplets, the method should be able to realize the controlled formation of the hydrogel particles from nano- to microscales and provide a facile approach to design and prepare several nanometers to hundreds of micrometers of hydrogel particles with regulable structures and properties.

Recently, multilayered hydrogel particles received steadily increasing interest because the appealing multilayered struc-

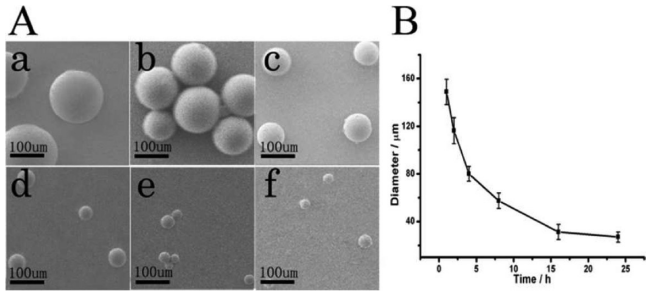


Figure 4. (A) SEM images and (B) comparison of the diameter variation of the microgels obtained by cross-linking of (a) 1 h, (b) 2 h, (c) 4 h, (d) 8 h, (e) 16 h, and (f) 24 h.

tures offer the promising integration of core-based excellent carrier properties and shell-mediated controlled biological interactions.[30−32] Some pioneering works by Lyon's[33−35] and Richtering's[36−38] groups have elaborated various thermoresponsive or multiresponsive "intelligent" core−shell microgels with unique behaviors in that the two domains, core and shell, could be specially designed to be responsive to different external stimuli, respectively. The reported methods are fascinating and robust for building up intelligent core/shell hydrogel particles with tunable structures and properties. However, these methods are still cumbersome for independently adjusting the structures and properties of the respective layer like morphology, cross-linking degree, and particle size. Herein, the controlled in situ gelation method in association with a seed emulsion technique can independently produce each on-demand layer and achieve controlled formation of multilayered hydrogel particles with flexibly designable structures and properties of the respective layer. The detailed approach was briefly illustrated in Figure 1B. First, the hydrogel particles with suitable particle sizes were prepared through cross-linking of a scheduled time according to the aforementioned approach. Then, the pre-prepared hydrogel particles were utilized as the seeds and cores for the heterogeneous growth of subsequent deposition and cross-linking of the shell layer, producing the core/shell hydrogel particles. Further, the fresh solution was added into the core/shell hydrogel particles for the continuously heterogeneous growth of subsequent deposition and cross-linking of the secondary shell layer, yielding triple-layered (core/double shells) hydrogel particles. The structures and properties of the cores and shells could be selectively designed simultaneously for unique behaviors through optimizing suitable cross-linking time or adopting various functional materials with similar disulfide-linked core/shell structures. For simplification purposes, we utilized the same polymer, BAP, but adjusted different cross-linking time for the preparation of the cores and the shells. The results from TEM images in Figure 5a−f demonstrated the method could successfully produce diverse core/shell nanogels with the designable cores and shells, e.g., six typical double-layered nanogels obtained by the cores/shells after cross-linking of from 6/1 and 6/2, to 10/2 and 10/4, to 24/4 and 24/8 h, respectively. Interestingly, the method could even yield triple-layered sophisticated nanogels with customizable structures and properties of each layer, e.g., a triple-layered nanogel obtained by the core/double shells after cross-linking of 24/8/2 h, as shown in Figure 5g,h. Although we demonstrated the formation of multilayered hydrogel particles using a disulfide-linked core/shell polymer, the method should be applicable to some

dx.doi.org/10.1021/mz300489n | ACS Macro Lett. 2012, 1, 1295−1299

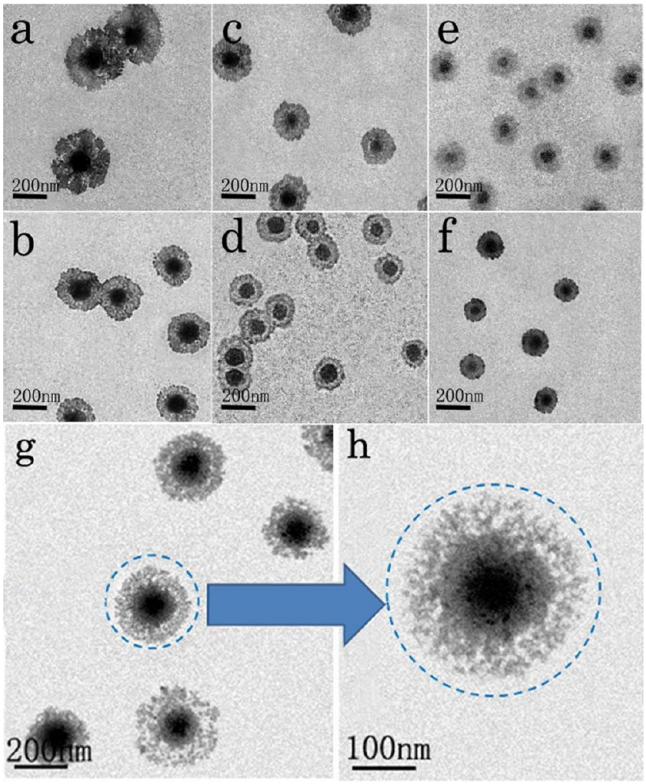


Figure 5. (a–f) TEM images of core/shell double-layered nanogels after different cross-linking time: (a) 6 h/1 h, (b) 6 h/2 h, (c) 10 h/2 h, (d) 10 h/4 h, (e) 24 h/4 h, and (f) 24 h/8 h. (g,h) TEM images of (24h/8h/2h) core/double shells triple-layered nanogels.

materials with similar disulfide-linked core/shell structures, e.g., inorganic nanoparticle cores or stimulus-responsive polymer cores and hydrophilic polymeric shells, for building up high performance or smart layers of hydrogel particles. Thus, the method could provide a facile approach toward various types of the customized multilayered hydrogel particles.

The formation of the hydrogel particles is attributed to the cross-linking of the polymer cores linked by in situ built-up disulfide bonds; therefore, the hydrogel particles are biodegradable since disulfide bonds can be cleaved by glutathione (GSH). GSH is a tripeptide detected within cells at millimolar (mM) concentrations, and its concentration is dependent on the cell types, e.g., ~5 mM for blood cells and ~30 mM for liver cells; however, its concentration outside cells sharply decreases to micromolar (μM).[39,40] Here, we evaluated degradation of three typical nanogels after cross-linking of 1, 8, and 24 h at 1 × PBS (phosphate-buffered saline) containing 10 μM, 5 mM, and 30 mM GSH similar to extracellular, blood cells', and liver cells' environments. The results demonstrated that all three nanogels had good stability at extracellular mediums because no degradation was observed after incubation of two days as shown in Figure S2A (Supporting Information). However, the nanogels were decomposed into small pieces under high GSH concentrations, and the degradation rate was dependent on the concentrations of GSH and the structures of the nanogels. For example, at blood cells' compartments, Figure S2B (Supporting Information) indicated that a plethora of small pieces appeared after incubation of 3, 24, and 48 h for the nanogels after cross-linking of 1, 8, and 24 h, respectively. For liver cells' circumstances with higher GSH concentrations, Figure S2C (Supporting Information) showed that the degradation was accelerated, and a plethora of small pieces emerged at 2, 8, and 24 h for the nanogels after cross-linking of 1, 8, and 24 h, respectively. Similar results were also observed from the core/shell nanogels with the cores after cross-linking of 24 h and the shells after cross-linking of 4 or 8 h as depicted in Figure S3 (Supporting Information). Namely, the core/shell nanogels were also stable at extracellular mediums but decomposable at intracellular compartments. It must be noted that breakaway of the core/shell was faster and easier in comparison with the degradation of each core or shell layer because disulfide linkage of the core/shell interfaces was not stronger than that of each layer inside with higher cross-linking degrees. Figure S3 (Supporting Information) also illustrated that the core/shell structures allowed complete consumption of the shell layers but maintained the relative consolidation of the cores, based on the significant differentiation of degradation behaviors for the compact cores and the loose shells. If in situ coloading drugs into the cores and the shells during formation of the core/shell hydrogel particles, the approach could be further developed for preparing smart drug carriers for the hierarchically controlled delivery of drugs.

In summary, we demonstrated a general approach to controlled formation of the in situ (multilayered) hydrogel particles from a disulfide-linked core/shell hyperbranched polymer, opening up design possibility, flexibility, and complexity of microgels/nanogels with tailor-made structures and properties. The approach outlined here was also applicable to materials with similar disulfide-linked core/shell structures and could produce fine-tunable micro/nanodrug carriers, having broad implications in diagnostics and therapeutic delivery systems.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The detailed experimental procedures, characterization methods, and degradation evaluation of the nanogels. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: dcwu@iccas.ac.cn.

Notes

The authors declare no competing financial interests.

■ ACKNOWLEDGMENTS

The authors appreciate financial support from the National 'Young Thousand Talents Program' and the National Natural Science Foundation of China (21174147 and 51103165).

■ REFERENCES

(1) Kabanov, A. V.; Vinogradov, S. V. *Angew. Chem., Int. Ed.* **2009**, *48*, 5418.
(2) Oh, J. K.; Lee, D. I.; Park, J. M. *Prog. Polym. Sci.* **2009**, *34*, 1261.
(3) Bysell, H.; Mansson, R.; Hansson, P.; Malmsten, M. *Adv. Drug Delivery Rev.* **2011**, *63*, 1172.
(4) Gonzalez-Toro, D. C.; Ryu, J. H.; Chacko, R. T.; Zhuang, J. M.; Thayumanavan, S. *J. Am. Chem. Soc.* **2012**, *134*, 6964.
(5) Ryu, J. H.; Chacko, R. T.; Jiwpanich, S.; Bickerton, S.; Babu, R. P.; Thayumanavan, S. *J. Am. Chem. Soc.* **2010**, *132*, 17227.
(6) Zhuang, J. M.; Jiwpanich, S.; Deepak, V. D.; Thayumanavan, S. *ACS Macro Lett.* **2012**, *1*, 175.
(7) Du, J. Z.; Sun, T. M.; Song, W. J.; Wu, J.; Wang, J. *Angew. Chem., Int. Ed.* **2010**, *49*, 3621.

(8) Shen, X. C.; Zhang, L. Y.; Jiang, X. Q.; Hu, Y.; Guo, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 7104.
(9) Peng, H. S.; Stolwijk, J. A.; Sun, L. N.; Wegener, J.; Wolfbeis, O. S. *Angew. Chem., Int. Ed.* **2010**, *49*, 4246.
(10) Wang, J. Z.; Loh, K. P.; Wang, Z.; Yan, Y. L.; Zhong, Y. L.; Xu, Q. H.; Ho, P. C. *Angew. Chem., Int. Ed.* **2009**, *48*, 6282.
(11) Gota, C.; Okabe, K.; Funatsu, T.; Harada, Y.; Uchiyama, S. *J. Am. Chem. Soc.* **2009**, *131*, 2766.
(12) Nayak, S.; Lyon, L. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 6706.
(13) Terashima, T.; Nomura, A.; Ito, M.; Ouchi, M.; Sawamoto, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 7892.
(14) Lu, Y.; Mei, Y.; Drechsler, M.; Ballauff, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 813.
(15) Yan, M.; Ge, J.; Liu, Z.; Ouyang, P. K. *J. Am. Chem. Soc.* **2006**, *128*, 11008.
(16) Liebert, T.; Hornig, S.; Hesse, S.; Heinze, T. *J. Am. Chem. Soc.* **2005**, *127*, 10484.
(17) McAllister, K.; Sazani, P.; Adam, M.; Cho, M. J.; Rubinstein, M.; Samulski, R. J.; DeSimone, J. M. *J. Am. Chem. Soc.* **2002**, *124*, 15198.
(18) 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.
(19) Sisson, A. L.; Steinhilber, D.; Rossow, T.; Welker, P.; Licha, K.; Haag, R. *Angew. Chem., Int. Ed.* **2009**, *48*, 7540.
(20) Keerl, M.; Pedersen, J. S.; Richtering, W. *J. Am. Chem. Soc.* **2009**, *131*, 3093.
(21) Suh, S. K.; Yuet, K.; Hwang, D. K.; Bong, K. W.; Doyle, P. S.; Hatton, T. A. *J. Am. Chem. Soc.* **2012**, *134*, 7337.
(22) Diao, Y.; Helgeson, M. E.; Myerson, A. S.; Hatton, T. A.; Doyle, P. S.; Trout, B. L. *J. Am. Chem. Soc.* **2011**, *133*, 3756.
(23) Hwang, D. K.; Oakey, J.; Toner, M.; Arthur, J. A.; Anseth, K. S.; Lee, S.; Zeiger, A.; Van Vliet, K. J.; Doyle, P. S. *J. Am. Chem. Soc.* **2009**, *131*, 4499.
(24) Rolland, J. P.; Maynor, B. W.; Euliss, L. E.; Exner, A. E.; Denison, G. M.; DeSimone, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 10096.
(25) Franzesi, G. T.; Ni, B.; Ling, Y. B.; Khademhosseini, A. *J. Am. Chem. Soc.* **2006**, *128*, 15064.
(26) Rossow, T.; Heyman, J. A.; Ehrlicher, A. J.; Langhoff, A.; Weitz, D. A.; Haag, R.; Seiffert, S. *J. Am. Chem. Soc.* **2012**, *134*, 4983.
(27) Seiffert, S.; Thiele, J.; Abate, A. R.; Weitz, D. A. *J. Am. Chem. Soc.* **2010**, *132*, 6606.
(28) Zhang, H.; Tumarkin, E.; Peerani, R.; Nie, Z.; Sullan, R. M. A.; Walker, G. C.; Kumacheva, E. *J. Am. Chem. Soc.* **2006**, *128*, 12205.
(29) Wu, D. C.; Loh, X. J.; Wu, Y. L.; Lay, C. L.; Liu, Y. *J. Am. Chem. Soc.* **2010**, *132*, 15140.
(30) Lu, Y.; Ballauff, M. *Prog. Polym. Sci.* **2011**, *36*, 767.
(31) Xiong, M. H.; Bao, Y.; Yang, X. Z.; Wang, Y. C.; Sun, B.; Wang, J. *J. Am. Chem. Soc.* **2012**, *134*, 4355.
(32) Hu, X. B.; Tong, Z.; Lyon, L. A. *J. Am. Chem. Soc.* **2010**, *132*, 11470.
(33) Jones, C. D.; Lyon, L. A. *Macromolecules* **2000**, *33*, 8301.
(34) Gan, D. J.; Lyon, L. A. *Macromolecules* **2002**, *35*, 9634.
(35) Jones, C. D.; Lyon, L. A. *Macromolecules* **2003**, *36*, 1988.
(36) Berndt, I.; Richtering, W. *Macromolecules* **2003**, *36*, 8780.
(37) Berndt, I.; Pedersen, J. S.; Richtering, W. *J. Am. Chem. Soc.* **2005**, *127*, 9372.
(38) Berndt, I.; Popescu, C.; Wortmann, F. J.; Richtering, W. *Angew. Chem., Int. Ed.* **2006**, *45*, 1081.
(39) Li, C. M.; Madsen, J.; Armes, S. P.; Lewis, A. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 3510.
(40) Carelli, S.; Ceriotti, A.; Cabibbo, A.; Fassina, G.; Ruvo, M.; Sitia, R. *Science* **1997**, *277*, 1681.