Organic LETTERS

Letter

pubs.acs.org/OrgLett

Hydrogen Bonding Directed Self-Assembly of Small-Molecule Amphiphiles in Water

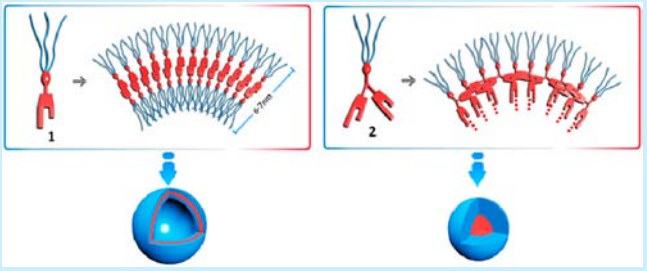
Jiang-Fei Xu,[†,‡] Li-Ya Niu,[†] Yu-Zhe Chen,[†] Li-Zhu Wu,[†] Chen-Ho Tung,[†] and Qing-Zheng Yang*[,†]

[†]Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China

[‡]University of the Chinese Academy of Sciences, Beijing 100049, P. R. China

Supporting Information

ABSTRACT: Compounds comprising one or two quadruply hydrogen bonding units, 2-ureido-4[1*H*]-pyrimidinone (UPy) and tris(tetraethylene glycol monomethyl ether) moieties, were reported to form highly stable hydrogen-bonded assemblies in water. Compound **1**, containing one UPy, assembles into vesicles, and compound **2**, containing two UPy units, forms micelles. The aggregates disassemble reversibly when the solution pH is raised to 9.0 or above. The results demonstrate the utility of hydrogen bonding to direct the self-assembly of small-molecule building blocks in aqueous media.

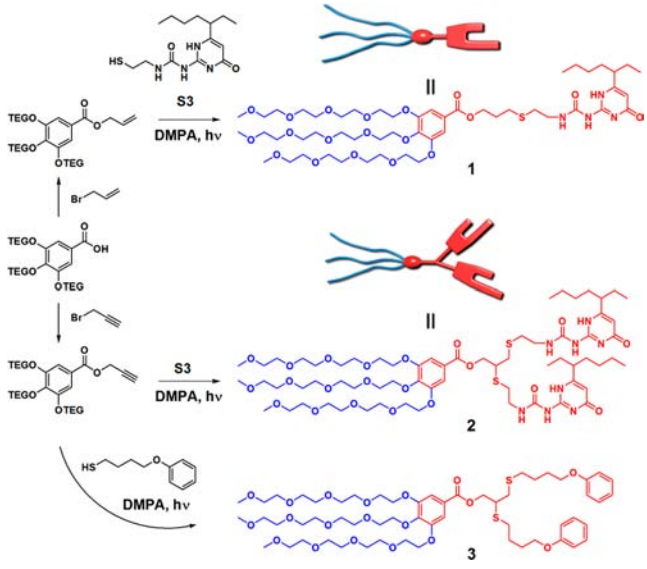


Hydrogen bonding drives the assembly of highly ordered structures in biology and plays a key role in maintaining their function. Examples include the DNA double helix and the 3D structures of proteins and enzymatic reactive centers.[1] Hydrogen bonding is also widely exploited in the preparation of functional materials, such as supramolecular polymers,[2] dendrimers,[3] and foldamers.[4] Using hydrogen bonding to drive self-assembly in aqueous media, however, remains a challenge because polar solvents, such as water, weaken or destroy hydrogen bonds.

A possible strategy to maintain the hydrogen bonding in aqueous media is to exploit multivalent H-bonding motives, such as those provided by 2-ureido-4[1*H*]-pyrimidinone (UPy), in combination with other noncovalent interactions, such as hydrophobic effect.[5] For example, hydrogen bonding in the interiors of water-soluble proteins is protected by the hydrophobic microenvironments of the polypeptides.[1b,c] Water-soluble polymers such as poly(ethylene glycols) (PEGs) end-functionalized with UPy were reported to assemble and manifest dynamic properties in water.[6] In such systems, 3D networks formed by interactions between long PEG chains (M_n 2−35 kDa) were cross-linked by UPy units to form fibrous assemblies and hydrogels. Hydrogen bonding played a modest role in self-assembly of such polymers because of the low weight fraction of the hydrogen-bonding units.

Herein we exploited the quadruple hydrogen bonding of UPy in cooperation with hydrophobic interaction to drive the assembly of small molecules **1** and **2** (Scheme 1) in aqueous media. We used the low molecular weight tetraethylene glycol (TEG) monomethyl ether chains as polar blocks to make molecules highly water soluble. The hydrogen-bonding units in compounds **1** and **2** were shielded by hydrophobic groups to yield stable aggregates.[7] In neutral or acidic aqueous solutions, compound **1** (one UPy per molecule) formed vesicles while compound **2** (two UPy units per molecule) formed micelles. In basic solutions, both the micelles and the vesicles disassembled, releasing guest molecules.

Scheme 1. Synthesis of Compounds 1−3[a]



[a]TEG: tetraethylene glycol monomethyl ether. DMPA: 2, 2-dimethoxy-2-phenylacetophenone. Hydrophilic parts of the molecules are shown in blue, hydrophobic in red.

Received: June 25, 2014
Published: July 18, 2014

dx.doi.org/10.1021/ol501841f | *Org. Lett.* 2014, 16, 4016−4019

Compounds **1** and **2** were synthesized by thiol−ene and thiol−yne reactions in 64% and 92% yields in two steps, respectively (Scheme 1).[8] Compound **3** without hydrogen-bonding units was synthesized as a control. The compounds were fully characterized by NMR spectroscopy and high-resolution ESI-MS (for details of synthesis and characterization, see the Supporting Information). The UPy NH signals in the ^{1}H NMR spectra of **1** and **2** in $CDCl_3$ were shifted downfield (to 10−14 ppm), suggesting the formation of quadruple hydrogen-bonded UPy dimers.

Compounds **1**−**3** are water-soluble, where they self-assemble with the critical aggregation concentration (CAC), determined by the pyrene method,[9] of 22, 1.4, and 120 μM, respectively (Figure S4−S6, Supporting Information). The change in CAC across the series **3** > **1** > **2** is consistent with the important contribution of H bonding to self-assembly of compounds **1**−**3** in water. For example, despite the similar weight fraction of hydrophobic parts of compounds **1** and **3** (w_{HP} = molar mass of the hydrophobic part/molar mass of the whole molecule, $w_{HP\text{-}1}$ = 0.431, $w_{HP\text{-}2}$ = 0.557, $w_{HP\text{-}3}$ = 0.456),[10] the CAC of **1** (1 UPy per molecule) is ∼5.5 times lower than that of **3** (no UPy). Increasing the number of UPy units from one in **1** to two in **2** further lowers the CAC (from 22 to 1.4 μM). The ^{1}H NMR spectrum of **1** in H_2O/D_2O (95/5, v/v) manifested signals at shifts (10 and 14 ppm) typical for hydrogen-bonded protons in UPy dimers and identical to shifts recorded in $CDCl_3$, confirming the formation of hydrogen bonding between molecules of **1** in water (Figure 1). In contrast, the resonances

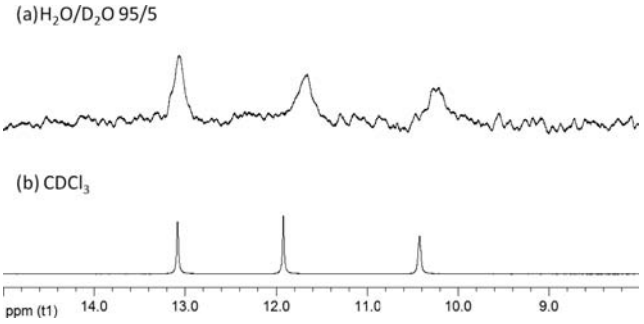


Figure 1. ^{1}H NMR spectra (400 MHz) of **1** in (a) H_2O/D_2O (95/5, v/v, ∼10 mM) and (b) $CDCl_3$.

of the TEG protons of **2** in D_2O were considerably broadened and signals of aliphatic and aromatic protons were absent (Figure S9, Supporting Information). This result suggests the distinct assembly behavior of the two compounds, as discussed below.

The assembly of **1** and **2** in aqueous solution was investigated by transmission electron microscopy (TEM), cryo-TEM, and dynamic light scattering (DLS). The TEM image of the aggregates of **1** revealed spherical vesicles with diameters of 50−150 nm, which showed an obvious color contrast between the peripheries and centers of the spheres (Figure 2a). The average hydrodynamic diameter (D_h) of the vesicles determined by DLS measurements was ∼90 nm (Figure 2d), in accord with the TEM observation. Cryo-TEM images of the vesicles revealed the average thickness of the wall of 6−7 nm (Figure 2b), approximately equal the length of the quadruple hydrogen-bonded dimer of **1**. These results suggest that in H_2O **1** dimerizes through H bonding into a bolaform amphiphile, which self-assembles into vesicles (Scheme 2).[11]

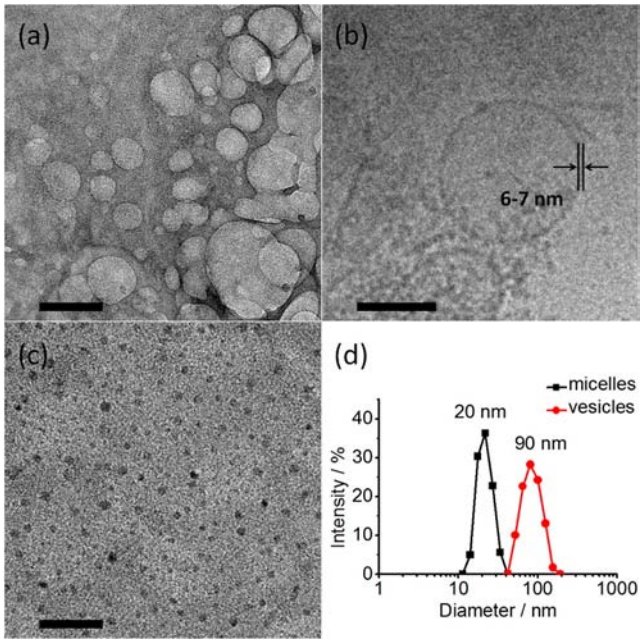
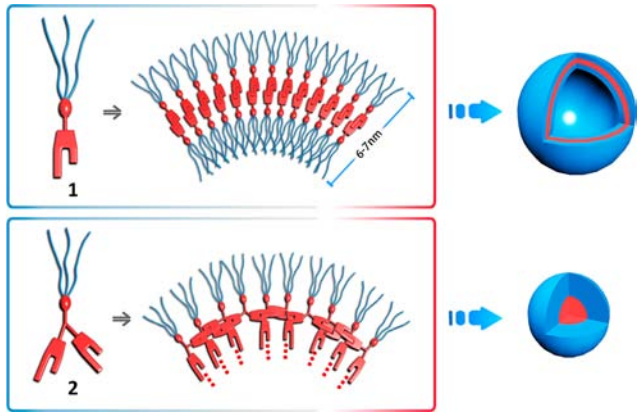


Figure 2. TEM image (a) and cryo-TEM image (b) of the vesicles formed by **1** in water, (c) TEM image of the micelles formed by **2** in water, and (d) DLS measurements of the micelles of **2** and vesicles of **1** in water. Scale bar: 100 nm.

Scheme 2. Tentative Models for the Self-Assembly of Amphiphiles 1 and 2 in Water To Form Vesicles and Micelles



Stained TEM images of dried aqueous solutions of **2** revealed well-dispersed micelles with diameters of 10−15 nm (Figure 2c). DLS measurement gave a mean diameter of 20 nm (Figure 2d). This slightly larger diameter, compared with TEM observations, is consistent with DLS measuring the hydrodynamic diameter of fully hydrated micelles whereas TEM measures dry collapsed micelles.[12] We assume that unlike compound **1**, which tends to dimerize, **2** with its two UPy units forms random-coiled supramolecular polymers, which aggregate into micelles. The UPy units, sequestered into the hydrophobic cores of such micelles, may cross-link with each other via quadruple hydrogen bonds, thus contributing to the compactness and stability of the micelles (Scheme 2). Such a putative structure would be consistent with the ill-defined NMR spectra as it would restrict molecular tumbling.[13] The shape and size of the micelles remained unchanged after the solution was stored for 4 months.

dx.doi.org/10.1021/ol501841f | Org. Lett. 2014, 16, 4016−4019

Because hydrogen bonding is sensitive to pH, as H donor sites can be deprotonated or H acceptor sites can be protonated,[14,6c] we considered that the aggregates of **1** and **2** could be reversibly disassembled by changing the solution pH, thus making them potentially suitable for applications in controlled release. We used Nile Red (NR) as a model guest molecule.[15] NR (1.0 μg/mL), encapsulated into the micelles formed by **2** (0.05 mM), strongly fluoresces at 630 nm, which is the typical emission wavelength of NR in hydrophobic environment. Acidifying the solution with HCl to pH ~2 slightly decreased the fluorescence intensity without changing the wavelength (Figure 3b), suggesting that NR remained

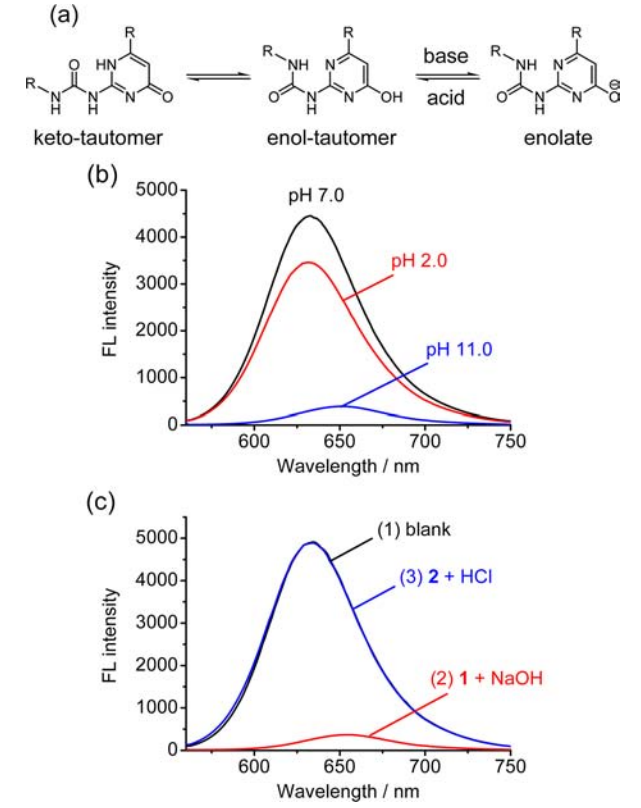


Figure 3. (a) Tautomerizations of UPy unit, (b) fluorescence spectra of Nile Red encapsulated in micelles formed by **2** upon addition of acid or base, and (c) recovery of the fluorescence after the neutralization of the base, λ_{ex} = 550 nm.

encapsulated in the hydrophobic core of micelles. The slight decrease in fluorescence intensity may result from precipitation of larger micelle aggregates as some hydrogen bonds between the TEG chains and water molecules are disrupted.[16] DLS measurement of the micelle solution at pH 3.0 revealed an increased D_h of 40 nm (Figure S14, Supporting Information), which supported the hypothesized micelle aggregation. Similar micelle aggregation phenomenon was also observed upon rising temperature of the solution. At a raised temperature of 343 K, the micelle solution became turbid and the D_h of the aggregates increased to 115 nm (Figure S15, Supporting Information), suggesting the formation of large aggregates. Such aggregation was also related to the change of hydrogen bonds between TEG chains and water molecules which the strength decreased dramatically at raised temperatures.

In contrast, increasing the pH of the solution to ~11 greatly decreased the fluorescence intensity and shifted the emission band to 650 nm, suggesting the disruption of micelles and an almost complete release of encapsulated NR. The collapse of micelles may result from deprotonation of hydroxyl group in the enol tautomer of UPy to the enolate (Figure 3a). The loss of H bonding and electrostatic repulsion between negatively charged enolates may be responsible for the dissolution of the micelles. The dissociation of micelles in basic solutions was also confirmed by DLS measurement, in which a decreased D_h of 3.8 nm was revealed at pH 10.0 (Figure S14, Supporting Information), which was the approximate size of single molecule of compound **2**. The disassembly and assembly of micelles is reversible, as evidenced by the recovery of NR emission upon neutralization of the solution with acid (Figure 3c). It should be mentioned that the speed of release and encapsulate of NR from micelles was very fast; the red color of the encapsulated NR in micelles faded dramatically within ~1−3 s when NaOH was added and recovered in similar speed upon neutralizing the solutions. This result suggested that the kinetics of the reversible disassembly and assembly of micelles were quite fast, which may attributed to the fast reaction rate of deprotonation and protonation of UPy units.

We also studied the controlled release of NR from micelles and from vesicles in buffered solutions (Figures S16 and S17, Supporting Information). In a solution of micelles formed by **2** in glycine−NaOH buffer (pH 9.0), about 66% of the encapsulated NR was released immediately as determined by the changes in fluorescence intensity. At pH 10.0, about 85% of NR released. For the NR encapsulated in vesicles formed by **1**, 82% and 90% of them was released at pH 9.0 and pH 10.0, respectively.

In conclusion, we report highly stable hydrogen-bonded assemblies from small-molecule amphiphiles in aqueous solution. Combination of quadruple hydrogen bonding unit UPy as a hydrophobic part and short TEG chains as a hydrophilic part enables the formation of stable hydrogen bonds. The number of UPy units per molecule influenced the behavior of assembly dramatically. Compound **1** with one UPy unit formed bolaform amphiphilic hydrogen-bonded dimers, which tended to form vesicles, whereas compound **2** formed micellar structures in which building blocks may be cross-linked by intermolecular hydrogen bonding. The correlation between CAC and the number of H-bonding UPy moieties per molecule further suggests that hydrogen bonding is a key contributor to self-assembly of these small-molecule amphiphiles in H_2O. Increasing the solution pH to ~9 disassembled both the micelles and the vesicles and released guest molecules, suggesting potential applications in drug delivery. Subsequently decreasing the pH to <7 leads to reassembly of the structures. Our studies could facilitate the development of new hydrogen-bonded assemblies as novel functional materials.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, synthesis of compounds **1**−**3**, NMR spectra, determination of CACs, fluorescence spectra, DLS measurements, and other materials. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: qzyang@mail.ipc.ac.cn.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the 973 Program (2013CB933800, 2013CB834505) and the National Natural Science Foundation of China (21222210, 91027041, 21272243).

■ REFERENCES

(1) (a) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer: New York, 1984. (b) Creighton, T. E. In *Proteins, Structures and Molecular Properties*; Freeman: New York, 1984. (c) *Prediction of Protein Structure and the Principles of Protein Conformation*; Fasman, G. D.., Ed; Plenum: New York, 1990.

(2) (a) Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071. (b) Fouquey, C.; Lehn, J.-M.; Levelut, A.-M. *Adv. Mater.* **1990**, *2*, 254. (c) Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer, B. J. B.; Hirschberg, J. H. K. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. *Science* **1997**, *278*, 1601. (d) Park, T.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2006**, *128*, 13986. (e) Todd, E. M.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2007**, *129*, 14534. (f) Chen, S.-G.; Yu, Y.; Zhao, X.; Ma, Y.; Jiang, X.-K.; Li, Z.-T. *J. Am. Chem. Soc.* **2011**, *133*, 11124. (g) Shi, Z.-M.; Wu, C.-F.; Zhou, T.-Y.; Zhang, D.-W.; Zhao, X.; Li, Z.-T. *Chem. Commun.* **2013**, *49*, 2673. (h) Yan, X.; Li, S.; Pollock, J. B.; Cook, T. R.; Chen, J.; Zhang, Y.; Ji, X.; Yu, Y.; Huang, F.; Stang, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 15585. (i) Yan, X.; Li, S.; Cook, T. R.; Ji, X.; Yao, Y.; Pollock, J. B.; Shi, Y.; Yu, G.; Li, J.; Huang, F.; Stang, P. J. *J. Am. Chem. Soc.* **2013**, *135*, 14036. (j) Xu, J.-F.; Chen, Y.-Z.; Wu, D.; Wu, L.-Z.; Tung, C.-H.; Yang, Q.-Z. *Angew. Chem., Int. Ed.* **2013**, *52*, 9738. (k) Yan, X.; Jiang, B.; Cook, T. R.; Zhang, Y.; Li, J.; Yu, Y.; Huang, F.; Yang, H.-B.; Stang, P. J. *J. Am. Chem. Soc.* **2013**, *135*, 16813. (l) Yan, X.; Cook, T. R.; Pollock, J. B.; Wei, P.; Zhang, Y.; Yu, Y.; Huang, F.; Stang, P. J. *J. Am. Chem. Soc.* **2014**, *136*, 4460. (m) Peng, H.-Q.; Xu, J.-F.; Chen, Y.-Z.; Wu, L.-Z.; Tung, C.-H.; Yang, Q.-Z. *Chem. Commun.* **2014**, *50*, 1334.

(3) (a) Zimmerman, S. C.; Zeng, F. W.; Reichert, D. E. C.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095. (b) Zeng, F.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681. (c) Frechet, J. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4782. (d) Ma, Y.; Kolotuchin, S. V.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2002**, *124*, 13757. (e) Zhang, H.; Fu, Y.; Wang, D.; Wang, L.; Wang, Z.; Zhang, X. *Langmuir* **2003**, *19*, 8497. (f) Sun, H.; Kaifer, A. E. *Org. Lett.* **2005**, *7*, 3845.

(4) (a) Forgan, R. S.; Sauvage, J.-P.; Stoddart, J. F. *Chem. Rev.* **2011**, *111*, 5434. (b) Zhang, D.-W.; Zhao, X.; Hou, J.-L.; Li, Z.-T. *Chem. Rev.* **2012**, *112*, 5271. (c) Berl, V.; Huc, I.; Khoury, R. G.; Lehn, J.-M. *Chem.—Eur. J.* **2001**, *7*, 2798. (d) Berl, V.; Huc, I.; Khoury, R. G.; Lehn, J.-M. *Chem.—Eur. J.* **2001**, *7*, 2810. (e) Zhu, J.; Lin, J.-B.; Xu, Y.-X.; Shao, X.-B.; Jiang, X.-K.; Li, Z.-T. *J. Am. Chem. Soc.* **2006**, *128*, 12307. (f) Cai, W.; Wang, G.-T.; Du, P.; Wang, R.-X.; Jiang, X.-K.; Li, Z.-T. *J. Am. Chem. Soc.* **2008**, *130*, 13450. (g) Guo, L.; Almeida, A. M.; Zhang, W.; Reidenbach, A. G.; Choi, S. H.; Guzei, I. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2010**, *132*, 7868. (h) Gan, Q.; Ferrand, Y.; Bao, C.; Kauffmann, B.; Grelard, A.; Jiang, H.; Huc, I. *Science* **2011**, *331*, 1172. (i) Xiao, T.; Li, S.-L.; Zhang, Y.; Lin, C.; Hu, B.; Guan, X.; Yu, Y.; Jiang, J.; Wang, L. *Chem. Sci.* **2012**, *3*, 1417.

(5) (a) Brunsveld, L.; Vekemans, J. A. J. M.; Hirschberg, J. H. K. K.; Sijbesma, R. P.; Meijer, E. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4977. (b) Sijbesma, R. P.; Meijer, E. W. *Chem. Commun.* **2003**, *39*, 5. (c) Li, M.; Yamato, K.; Ferguson, J. S.; Gong, B. *J. Am. Chem. Soc.* **2006**, *128*, 12628. (d) Obert, E.; Bellot, M.; Bouteiller, L.; Andrioletti, F.; Lehen-Ferrenbach, C.; Boue, F. *J. Am. Chem. Soc.* **2007**, *129*, 15601. (e) Cafferty, B. J.; Gallego, I.; Chen, M. C.; Farley, K. I.; Eritja, R.; Hud, N. V. *J. Am. Chem. Soc.* **2013**, *135*, 2447. (f) Zhang, K.-D.; Ajami, D.; Rebek, J. *J. Am. Chem. Soc.* **2013**, *135*, 18064.

(6) (a) Dankers, P. Y. W.; Hermans, T. M.; Baughman, T. W.; Kamikawa, Y.; Kieltyka, R. E.; Bastings, M. M. C.; Janssen, H. M.; Sommerdijk, N. A. J. M.; Larsen, A.; van Luyn, M. J. A.; Bosman, A. W.; Popa, E. R.; Fytas, G.; Meijer, E. W. *Adv. Mater.* **2012**, *24*, 2703. (b) Kieltyka, R. E.; Pape, A. C. H.; Albertazzi, L.; Nakano, Y.; Bastings, M. M. C.; Voets, I. K.; Dankers, P. Y. W.; Meijer, E. W. *J. Am. Chem. Soc.* **2013**, *135*, 11159. (c) Bastings, M. M. C.; Koudstaal, S.; Kieltyka, R. E.; Nakano, Y.; Pape, A. C. H.; Feyen, D. A. M.; van Slochteren, F. J.; Doevendans, P. A.; Sluijter, J. P. G.; Meijer, E. W.; Chamuleau, S. A. J.; Dankers, P. Y. W. *Adv. Healthcare Mater.* **2014**, *3*, 70. (d) Guo, M.; Pitet, L. M.; Wyss, H. M.; Vos, M.; Dankers, P. Y. W.; Meijer, E. W. *J. Am. Chem. Soc.* **2014**, *136*, 6969.

(7) (a) Menger, F. M.; Zhang, H. *J. Am. Chem. Soc.* **2006**, *128*, 1414. (b) de Greef, T. F. A.; Nieuwenhuizen, M. M. L.; Stals, P. J. M.; Fitie, C. F. C.; Palmans, A. R. A.; Sijbesma, R. P.; Meijer, E. W. *Chem. Commun.* **2008**, *44*, 4306. (c) de Greef, T. F. A.; Nieuwenhuizen, M. M. L.; Sijbesma, R. P.; Meijer, E. W. *J. Org. Chem.* **2010**, *75*, 598. (d) Wong, C.-H.; Choi, L.-S.; Yim, S.-L.; Lau, K.-N.; Chow, H.-F.; Hui, S.-K.; Sze, K.-H. *Chem.—Asian J.* **2010**, *5*, 2249. (e) Besenius, P.; van den Hout, K. P.; Albers, H. M. H. G.; de Greef, T. F. A.; Olijve, L. L. C.; Hermans, T. M.; de Waals, B. F. M.; Bomans, P. H. H.; Sommerdijk, N. A. J. M.; Portale, G.; Palmans, A. R. A.; van Genderen, M. H. P.; Vekemans, J. A. J. M.; Meijer, E. W. *Chem.—Eur. J.* **2011**, *17*, 5193. (f) Leenders, C. M. A.; Albertazzi, L.; Mes, T.; Koenigs, M. M. E.; Palmans, A. R. A.; Meijer, E. W. *Chem. Commun.* **2013**, *49*, 1963. (g) Pal, A.; Voudouris, P.; Koenigs, M. M. E.; Besenius, P.; Wyss, H. M.; Degirmenci, V.; Sijbesma, R. P. *Soft Matter* **2014**, *10*, 952.

(8) (a) Hoogenboom, R. *Angew. Chem., Int. Ed.* **2010**, *49*, 3415. (b) Peng, H.-Q.; Sun, C.-L.; Xu, J.-F.; Niu, L.-Y.; Chen, Y.-Z.; Wu, L.-Z.; Tung, C.-H.; Yang, Q.-Z. *Chem.—Eur. J.* **2014**, DOI: 10.1002/chem.201402955.

(9) Ding, Y.; Wang, Z.; Zhang, X. *Chem. Commun.* **2013**, *49*, 5580.

(10) Chebotareva, N.; Bomans, P. H. H.; Frederik, P. M.; Sommerdijk, N. A. J. M.; Sijbesma, R. P. *Chem. Commun.* **2005**, *41*, 4967.

(11) (a) Wang, Y.; Xu, H.; Zhang, X. *Adv. Mater.* **2009**, *21*, 2849. (b) Yu, G.; Xue, M.; Zhang, Z.; Li, J.; Han, C.; Huang, F. *J. Am. Chem. Soc.* **2012**, *134*, 13248. (c) Yao, Y.; Xue, M.; Chen, J.; Zhang, M.; Huang, F. *J. Am. Chem. Soc.* **2012**, *134*, 15712.

(12) Zhang, S.; Zhao, Y. *Macromolecules* **2010**, *43*, 4020.

(13) Chen, S.; Ruan, Y.; Brown, J. D.; Gallucci, J.; Maslak, V.; Hadad, C. M.; Badjić, J. D. *J. Am. Chem. Soc.* **2013**, *135*, 14964.

(14) Li, S.-L.; Xiao, T.; Xia, W.; Ding, X.; Yu, Y.; Jiang, J.; Wang, L. *Chem.—Eur. J.* **2011**, *17*, 10716.

(15) (a) Wang, C.; Wang, G.; Wang, Z.; Zhang, X. *Chem.—Eur. J.* **2011**, *17*, 3322. (b) Wang, G.; Wang, C.; Wang, Z.; Zhang, X. *Langmuir* **2011**, *27*, 12375.

(16) (a) Maeda, Y. *Langmuir* **2001**, *17*, 1737. (b) Lutz, J.-F.; Weichenhan, K.; Akdermir, O.; Hoth, A. *Macromolecules* **2007**, *40*, 2503.