

Supramolecular Peptide Amphiphile Vesicles through Host–Guest Complexation**

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Single-tail peptide amphiphiles, have been explored as a new class of biomaterials in many fields including nanotechnology and tissue engineering.^[1–3] A typical peptide amphiphile molecule is linked through a covalent amide bond between a hydrophilic peptide sequence and a hydrophobic lipid of variable length. In an aqueous environment, these peptide amphiphiles undergo self-assembly into structures such as vesicles, both spherical and cylindrical micelles or nanotubes, and have been successfully applied in the biomedical sciences for biomaterial conjugation^[4] and as bioactive scaffolds for tissue engineering.^[5,6] Although covalent attachment of two components to form peptide amphiphiles has been extremely successful,^[7,8] the synthetic versatility and the ability to respond to external triggers remains limited. A supramolecular approach to form the peptide amphiphile by connecting two building blocks through a non-covalent interaction would represent a major advance, especially in designing stimuli-responsive systems capable of being targeted by specific triggers.^[9–15]

Cucurbit[*n*]urils (CB[*n*]) are a family of macrocyclic hosts known to form inclusion complexes with selectivity and high binding affinity in aqueous media.^[16,17] One of the larger macrocycles in this family, CB[8], can be used as a “molecular handcuff” to join two molecules together in a non-covalent fashion,^[18,19] and has been applied to form biomaterials such as polymer–protein conjugation and protein dimerization.^[20–24] Additionally, CB[*n*] hosts have found great utility in “switch on/switch off” fluorescence assays by supramolecular complexation with various fluorescent guests.^[25–28]

Pyrene and its derivatives have been widely used as fluorescence probes in a large number of complex systems, on account of their high fluorescence quantum yields, long excited state lifetimes and the ability to form excimers.^[29] Herein, we utilize a functional pyrene bearing an imidazolium group both as a fluorescence sensor and as a guest for

CB[8]^[30] and linked it to a simple peptide sequence (1). Pyrene-functionalized peptide 1 is able to form the supramolecular peptide amphiphile complex 3 with viologen lipid 2 through CB[8] conjugation, as shown in Figure 1 a. During the

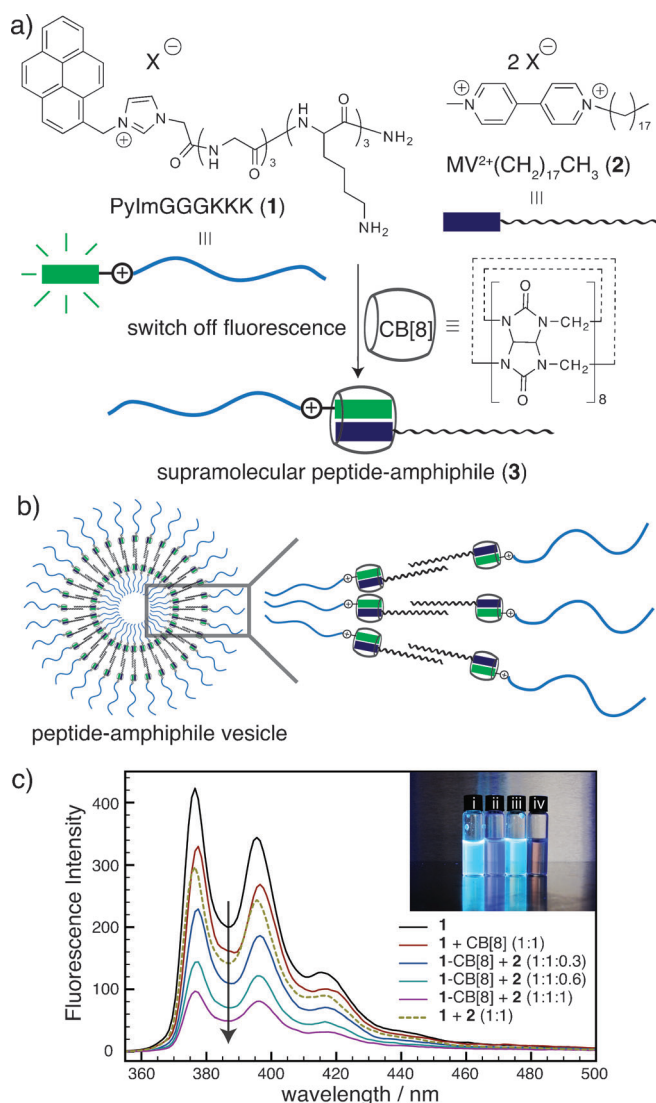


Figure 1. a) Chemical structures of pyrene imidazolium-labeled peptide and viologen-functionalized lipid, and their formation of ternary complex with CB[8]. b) Supramolecular peptide amphiphile vesicle with “switching off” of the fluorescence by the formation of ternary complexes. c) Emission spectra of 1 (0.05 mM, excited at 303 nm) with addition of CB[8] and 2 in different molecular ratio. Inset shows the pictures of vials containing aqueous solution of i) 1, ii) 1 + CB[8] (1:1), iii) 1 + 2 (1:1), iv) 1 + CB[8] + 2 (1:1:1) (0.5 mM) upon UV irradiation (350 nm).

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formation of the pyrene–viologen–CB[8] ternary complex, a marked “switch off” of the fluorescence is observed as shown in Figure 1c.

Once the non-covalent peptide amphiphiles are formed in water, they readily undergo a subsequent self-assembly step into vesicles (Figure 1b).^[9] We explored these vesicles as potential peptide delivery agents into living (HeLa) cells, the release of which can be stimulated with different triggers thereby producing both a “switch on” of the fluorescence as well as inducing cytotoxicity in cells (Figure 2).

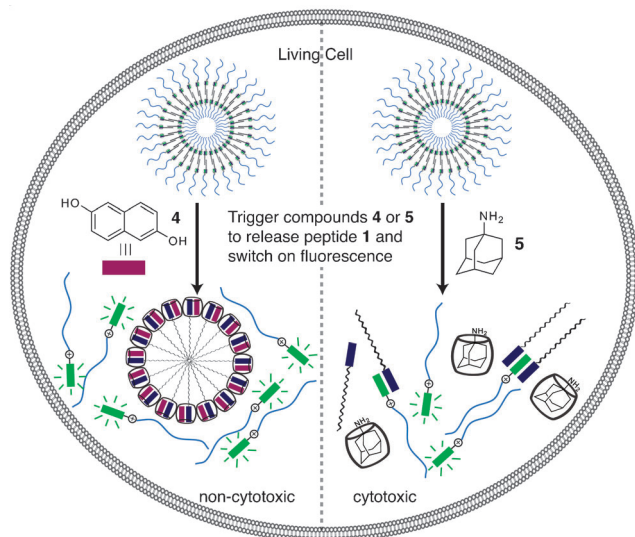


Figure 2. Peptide release with the “switching on” of the fluorescence through dissociation of host–guest complexes with external trigger 4 or 5.

The size of the pyrene group in arylimidazolium peptide **1** allowed for 1:1 complexation to occur with CB[8] (but not with the smaller homologue CB[7])^[30,31] with a slight reduction in the fluorescence intensity upon host–guest binding (Figure 1c). As CB[8] is large enough to encapsulate two molecules at the same time,^[16,17,32] addition of the electron-poor viologen lipid led to ternary complexation inside CB[8] resulting in a significant effect on the optical properties of the pyrene residue. Emission spectra (Figure 1c) indicated the formation of the supramolecular peptide amphiphile as a significant fluorescence quenching was observed from the electron acceptor–donor interactions between the pyrene and viologen moieties inside the CB[8] cavity.^[33,34] Even in the absence of CB[8], a weak interaction between the pyrene and viologen moieties was observed, which resulted in a slight reduction of fluorescence intensity as illustrated in Figure 1c (dashed line).

Complexation of the peptide amphiphile through CB[8] binding was confirmed by ¹H NMR spectroscopy carried out in D₂O as shown in a series of spectra in Figure 3a–c. When one equivalent of CB[8] was added into a solution of peptide **1**, the pyrene proton peaks (labeled with triangles) became broad with a concomitant upfield shift, indicating encapsulation of the pyrene moiety inside the hydrophobic CB[8] cavity. Simultaneously, the imidazolium proton peaks (la-

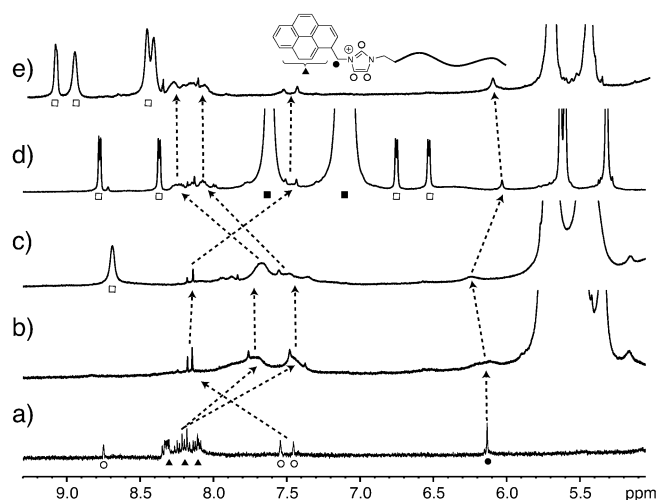


Figure 3. ¹H NMR spectra in D₂O at 25 °C of a) peptide **1**, b) **1** + CB[8] (1:1), c) **1**-CB[8] + **2** (1:1), d) **3** + 2,6-dihydroxynaphthalene **4** (1:12), e) **3** + 1-adamantylamine **5** (1:2). Protons of the pyrene, CH₂ linker and imidazolium moieties are labeled with triangles, black and white dots, respectively. Protons of viologen and **4** are depicted with white and black squares, respectively.

beled with white dots) clearly shifted downfield and remained sharp, suggesting that ion–dipole interactions existed between the cationic imidazolium ring and the carbonyl groups located at the CB[8] portals.^[31,35,36] When viologen lipid **2** was added into an aqueous solution of the **1**-CB[8] complex, the proton peaks for both the pyrene group on **1** and the viologen group on **2** exhibited upfield shifts in the ¹H NMR spectrum as illustrated in Figure 3c. These observations indicated that hydrophobic lipid **2** was dissolved in water through the formation of a ternary inclusion complex with peptide **1** and CB[8].

In order to investigate the binding affinity of the supramolecular peptide amphiphiles, isothermal titration calorimetry (ITC) was carried out to measure the solution binding constant (*K*_a) of approximately 10¹¹ M^{−2} for the ternary complex (see Supporting Information Figures S6 and S7). The ¹H NMR studies, emission spectra and ITC measurements together offer compelling evidence that a supramolecular peptide amphiphile **3** is indeed formed by the ternary complexation of peptide **1**, CB[8], and lipid **2** in water as depicted in Figure 1a.

On account of the amphiphilic nature of complex **3**, double-layer vesicles were observed in water (0.05 mM), as illustrated in Figure 1b. In order to investigate the self-assembly behavior of these unique supramolecular peptide amphiphiles, transmission electron microscopy (TEM), dynamic light scattering (DLS) measurements, and dilution studies were carried out. In Figure 4a, vesicles were observed after uranyl acetate staining with diameters ranging from 100 to 200 nm (calculated from the TEM images), which is in good agreement with the average diameter obtained from DLS, 200 ± 60 nm (see Figure S11). The effect of dilution on vesicle assembly was studied by investigating the steady-state excitation spectra at 376 nm for the labeled pyrene probe

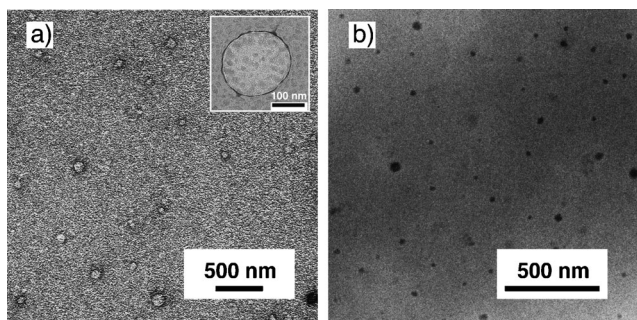


Figure 4. TEM images of a) the formation of peptide amphiphile vesicles and b) decomplexation of the peptide amphiphile structure with 12 equiv of trigger **4**. The inset of (a) is a single vesicle.

(see Supporting Information).^[37] The observed critical concentration of approximately $1\ \mu\text{M}$ corresponds to the dissociation of the vesicles through decomplexation of the supramolecular peptide amphiphile building blocks into molecular fragments consisting of lipid **2**, peptide **1**, and CB[8].

The vesicles prepared from the supramolecular peptide amphiphiles are stimuli-responsive to a number of different external triggers, such as competitive guests 2,6-dihydroxynaphthalene (**4**) and 1-adamantylamine (**5**), leading to the release of pyrene peptide **1** into the surrounding environment and simultaneously a “switch on” of the fluorescence. In the ^1H NMR spectra in Figure 3d, the proton peaks of pyrene experiences a downfield shift back to an “unbound” state with addition of guest **4** (in excess). This observation indicated that the guest exchange readily occurred, leading to the removal of

the peptide **1** from the CB[8] cavity. Meanwhile, viologen proton peaks remained in a “bound” position through the formation of a new **2–4–CB[8]** ternary complex. On account of separating the hydrophilic peptides from lipid **2**, insoluble aggregates of **2–CB[8]–4** complexes were formed as illustrated in the TEM image (Figure 4b).

Since the second guest exchange equilibria of CB[8] ternary complexes with viologen derivatives are dynamic and rapid,^[38,39] the pyrene fluorescence is immediately “switched on” with addition of suitable competitive guests. As illustrated in Figure 5b, the fluorescence intensity of the pyrene probe was enhanced with increasing concentration of **4**, enabling a continuous fluorescence assay for the decomplexation of the supramolecular peptide amphiphiles. Thus, the utility of the fluorescence probe is able to detect and quantify the amount of peptide released from the vesicles.

A different response of the supramolecular peptide amphiphile vesicles was observed with addition of **5** as a stimulus. Instead of replacing one guest as was the case with addition of **4**, both pyrene and viologen moieties were replaced from the host cavity upon the addition of **5**. ^1H NMR spectrum (Figure 3e) was the first insight to prove the dissociation of the **1–CB[8]–2** ternary complexes, in that the proton peaks of both pyrene and viologen shifted downfield to “unbound” positions. However, the fluorescence was also enhanced by titration of **5** into the solution of **1–CB[8]–2** complexes (Figure 5b); the intensity of the fluorescence could not be fully “switched on”, as depicted in the inset of Figure 5b. Experimentally, even over the guest exchange equilibria (more than 1.2 equivalent of **5**), the complexation

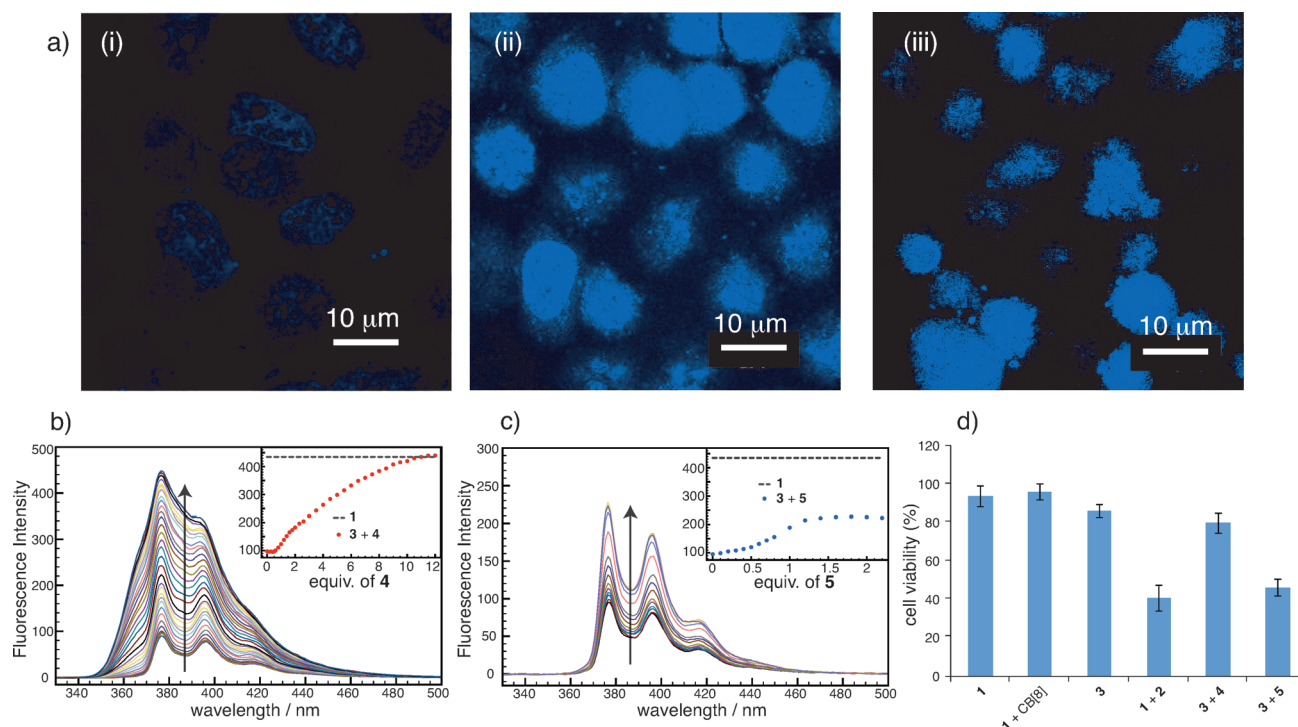


Figure 5. a) CLSM images of HeLa cells with addition of i) **3**, ii) **3 + 4**, and iii) **3 + 5**. b, c) Emission spectra of **3** (0.05 mM, excited at 303 nm) with addition of b) 2,6-dihydroxynaphthalene (**4**) and c) 1-adamantylamine (**5**). Insets show the direct comparison of the increase in intensity (at 376 nm) with competitive guests **4** and **5**, respectively. d) Cytotoxicity of **3**, and triggered with **4** and **5**, measured by MTT assay after 24 h incubation.

between peptide **1** and lipid **2** remained in the aqueous solution. When the CB[8] cavity was occupied by **5**, the weak charge transfer interactions between viologen and pyrene moieties (without CB[8] hosts) became dominant, and resulted in quenching fluorescence as discussed previously in Figure 1c (dashed line).

To demonstrate that the peptide amphiphile vesicles could be used as delivery vehicles, confocal laser scanning microscopy (CLSM) was employed to monitor uptake of the vesicles by HeLa cells. As shown in Figure 5a, after a 24 h incubation of the HeLa cells with the supramolecular peptide-amphiphile vesicles, the fluorescence of the cell nuclei remained weak. Turning on the fluorescence in the sample was readily accomplished by addition of either competitive guest **4** or **5** as illustrated in Figure 5a, which resulted in an intense blue fluorescence from released **1**.

The tunable cytotoxicity of **1**–CB[8]–**2** ternary complexes with an external triggering stimuli of **4** or **5** against HeLa cells was studied as illustrated in Figure 5d. With addition of triggers **4** or **5**, peptide **1** was released and simultaneously turned on the fluorescence. Electron-rich guest **4** was able to form ternary complexes with viologen lipid **2** inside the CB[8] cavity as previously depicted, and this resulted in a relatively non-cytotoxic entity. On the other hand, when using trigger **5**, both pyrene and viologen moieties were displaced from the CB[8] cavity, leading to a similar cytotoxic effect as mixing **1** and **2** without any CB[8] host (Figure 5d). In this latter case, upon the addition of **5**, when the samples were subsequently incubated for a longer period of time (a further 24 h), the HeLa cells died on account of the release of viologen derivative **2** into the environment.^[40] A continuous fluorescence assay could be created from the disassociation of the peptide amphiphile vesicles, thus quantitative detection of the amount of released peptide could be accomplished and was calculated by measuring the intensity of the pyrene fluorescence (see Figure S12). Therefore, the controlled release of peptide **1** alone or of both peptide **1** and viologen lipid **2** to alter the toxicity is possible on account of the supramolecular peptide amphiphile structure.

In summary, we exploited a supramolecular approach to form peptide amphiphiles in water through specific host–guest complexation with CB[8]. Pyrene imidazolium derivatives were utilized as both a reporting fluorophore and as a suitable CB[8] guest to label a peptide sequence. A “switch on/off” fluorescence assay was created by the controlled association and dissociation of supramolecular peptide amphiphile **3**. Additionally, the self-assembled vesicles containing peptide amphiphile **3** were readily taken up by HeLa cells and responded to multiple external triggers (**4** or **5**), which could modulate the toxicity and non-toxicity of the supramolecular system. The exploitation of host–guest chemistry to trigger the activation of a therapeutic system by competitive complexation allows for the construction of synthetic host–guest supramolecular systems that can present dynamic and responsive behavior within living cells. Multi-triggered systems may allow for orthogonally responsive drug delivery and activation of a therapeutic system that achieves site-specific toxicity and reduces damage to healthy systems. We envision that this class of supramolecular peptide

amphiphiles, which displays fluorescence switching, is responsive to multiple triggers in a controlled and reversible manner and could lead to a new generation of hierarchical, stimuli-responsive vehicles for drug-delivery, peptide therapeutics, and supramolecular biomaterial conjugation with a wide variety of biological sensing applications.

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- [1] J. D. Hartgerink, E. Beniash, S. Stupp, *Science* **2001**, 294, 1684–1688.
- [2] G. Silva, C. Czeisler, K. Niece, E. Beniash, D. Harrington, J. Kessler, S. Stupp, *Science* **2004**, 303, 1352–1355.
- [3] S. Paramonov, H. Jun, J. Hartgerink, *J. Am. Chem. Soc.* **2006**, 128, 7291–7298.
- [4] D. Spencer, T. Wandless, S. Schreiber, G. Crabtree, *Science* **1993**, 262, 1019–1024.
- [5] P. Dankers, M. Harmsen, L. Brouwer, M. V. Luyn, E. Meijer, *Nat. Mater.* **2005**, 4, 568–574.
- [6] L. Zhang, F. Rakotonradany, A. J. Myles, H. Fenniri, T. J. Webster, *Biomaterials* **2009**, 30, 1309–1320.
- [7] L. C. Palmer, S. Stupp, *Acc. Chem. Res.* **2008**, 41, 1674–1684.
- [8] H.-W. Jun, S. E. Paramonov, H. Dong, N. Forraz, C. McGuckin, J. D. Hartgerink, *J. Biomater. Sci. Polym. Ed.* **2008**, 19, 665–676.
- [9] X. Zhang, C. Wang, *Chem. Soc. Rev.* **2011**, 40, 94–101.
- [10] K. Liu, C. Wang, Z. B. Li, X. Zhang, *Angew. Chem.* **2011**, 123, 5054–5058; *Angew. Chem. Int. Ed.* **2011**, 50, 4952–4956.
- [11] C. Wang, Q. S. Chen, Z. Q. Wang, X. Zhang, *Angew. Chem.* **2010**, 122, 8794–8797; *Angew. Chem. Int. Ed.* **2010**, 49, 8612–8615.
- [12] C. Kim, S. S. Agasti, Z. Zhu, L. Isaacs, V. M. Rotello, *Nat. Chem.* **2010**, 2, 962–966.
- [13] X. Zhang, S. Rehm, M. M. Safont-Sempere, F. Wuerthner, *Nat. Chem.* **2009**, 1, 623–629.
- [14] Y. P. Wang, N. Ma, Z. Q. Wang, X. Zhang, *Angew. Chem.* **2007**, 119, 2881–2884; *Angew. Chem. Int. Ed.* **2007**, 46, 2823–2826.
- [15] C. Wang, Z. Q. Wang, X. Zhang, *Acc. Chem. Res.* **2012**, 45, 608–618.
- [16] J. Lee, S. Samal, N. Selvapalam, H. Kim, K. Kim, *Acc. Chem. Res.* **2003**, 36, 621–630.
- [17] J. Lagona, P. Mukhopadhyay, S. Chakrabarti, L. Isaacs, *Angew. Chem.* **2005**, 117, 4922–4949; *Angew. Chem. Int. Ed.* **2005**, 44, 4844–4870.
- [18] H. Kim, J. Heo, W. Jeon, E. Lee, J. Kim, S. Sakamoto, K. Yamaguchi, K. Kim, *Angew. Chem.* **2001**, 113, 1574–1577; *Angew. Chem. Int. Ed.* **2001**, 40, 1526–1529.
- [19] U. Rauwald, O. A. Scherman, *Angew. Chem.* **2008**, 120, 4014–4017; *Angew. Chem. Int. Ed.* **2008**, 47, 3950–3953.
- [20] H. D. Nguyen, D. T. Dang, J. L. J. van Dongen, L. Brunsveld, *Angew. Chem.* **2010**, 122, 907–910; *Angew. Chem. Int. Ed.* **2010**, 49, 895–898.
- [21] F. Biedermann, U. Rauwald, J. M. Zayed, O. A. Scherman, *Chem. Sci.* **2011**, 2, 279–286.
- [22] J. J. Reczek, A. A. Kennedy, B. T. Halbert, A. R. Urbach, *J. Am. Chem. Soc.* **2009**, 131, 2408–2415.
- [23] J. M. Chinai, A. B. Taylor, L. M. Ryno, N. D. Hargreaves, C. A. Morris, P. J. Hart, A. R. Urbach, *J. Am. Chem. Soc.* **2011**, 133, 8810–8813.
- [24] J. Zhang, R. J. Coulston, S. T. Jones, J. Geng, O. A. Scherman, C. Abell, *Science* **2012**, 335, 690–694.

- [25] A. Hennig, H. Bakirci, W. M. Nau, *Nat. Methods* **2007**, *4*, 629–632.
- [26] A. Bhasikuttan, J. Mohanty, W. Nau, H. Pal, *Angew. Chem.* **2007**, *119*, 4198–4200; *Angew. Chem. Int. Ed.* **2007**, *46*, 4120–4122.
- [27] G. Ghale, V. Ramalingam, A. R. Urbach, W. M. Nau, *J. Am. Chem. Soc.* **2011**, *133*, 7528–7535.
- [28] R. N. Dsouza, U. Pischel, W. M. Nau, *Chem. Rev.* **2011**, *111*, 7941–7980.
- [29] G. Pereira, A. S. Abreu, E. M. S. Castanheira, P. J. G. Coutinho, P. M. T. Ferreira, M.-J. R. P. Queiroz, *Eur. J. Org. Chem.* **2009**, 3906–3916.
- [30] D. Jiao, F. Biedermann, F. Tian, O. A. Scherman, *J. Am. Chem. Soc.* **2010**, *132*, 15734–15743.
- [31] D. Jiao, F. Biedermann, O. A. Scherman, *Org. Lett.* **2011**, *13*, 3044–3047.
- [32] M. Bush, N. Bouley, A. R. Urbach, *J. Am. Chem. Soc.* **2005**, *127*, 14511–14517.
- [33] W. Jeon, E. Kim, Y. Ko, I. Hwang, J. Lee, S. Kim, H. Kim, K. Kim, *Angew. Chem.* **2005**, *117*, 89–93; *Angew. Chem. Int. Ed.* **2005**, *44*, 87–91.
- [34] C. Wang, Y. Guo, Y. Wang, H. Xu, R. Wang, X. Zhang, *Angew. Chem.* **2009**, *121*, 9124–9127; *Angew. Chem. Int. Ed.* **2009**, *48*, 8962–8965.
- [35] Z. Miskolczy, L. Biczok, M. Megyesi, I. Jablonkai, *J. Phys. Chem. B* **2009**, *113*, 1645–1651.
- [36] N. Zhao, L. Liu, F. Biedermann, O. A. Scherman, *Chem. Asian J.* **2010**, *5*, 530–537.
- [37] X. J. Loh, Z.-X. Zhang, Y.-L. Wu, T. S. Lee, J. Li, *Macromolecules* **2009**, *42*, 194–202.
- [38] W. Jeon, H. Kim, C. Lee, K. Kim, *Chem. Commun.* **2002**, 1828–1829.
- [39] Y. H. Ko, E. Kim, I. Hwang, K. Kim, *Chem. Commun.* **2007**, 1305–1315.
- [40] J. Bus, S. Aust, J. Gibson, *Environ. Health Perspect.* **1976**, *16*, 139–146.