Self-Assembled Systems

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Dual Stimuli-Responsive Self-Assembled Supramolecular Nanoparticles**

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***Abstract:** Supramolecular nanoparticles (SNPs) encompass multiple copies of different building blocks brought together by specific noncovalent interactions. The inherently multivalent nature of these systems allows control of their size as well as their assembly and disassembly, thus promising potential as biomedical delivery vehicles. Here, dual responsive SNPs have been based on the ternary host–guest complexation between cucurbit[8]uril (CB[8]), a methyl viologen (MV) polymer, and mono- and multivalent azobenzene (Azo) functionalized molecules. UV switching of the Azo groups led to fast disruption of the ternary complexes, but to a relatively slow disintegration of the SNPs. Alternating UV and Vis photoisomerization of the Azo groups led to fully reversible SNP disassembly and reassembly. SNPs were only formed with the Azo moieties in the trans and the MV units in the oxidized states, respectively, thus constituting a supramolecular AND logic gate.*

The self-assembly of molecules into higher ordered supramolecular structures constitutes a powerful approach for the design of novel materials such as nanoparticles,[1] hydrogels,[2] and polymers.[3] These aggregates are held together by noncovalent weak interactions such as van der Waals, hydrophobic, and electrostatic interactions. More robust host–guest interactions, for example, the inclusion of hydrophobic guests in cyclodextrins (CDs)[4] or the inclusion of amines and hydrophobic guest molecules into cucurbit[*n*]urils (CB[*n*]),[5] have been studied extensively in the last decade.

Among these systems, supramolecular nanoparticles (SNPs) based on host–guest interactions currently receive high interest.[1] The self-assembly of small building blocks offers size control and colloidal stability through an interplay of multivalent and monovalent interactions. Their drug-encapsulation properties and the easy implementation of targeting ligands make these aggregates promising candidates as site-selective biomedical delivery vectors.[1a,c]

Although a variety of external controls have been used for the triggered release of proteins and cells from surfaces,[6] RNA from micelles,[7] and DNA from hydrogels[8] and electrostatic delivery vectors,[9] only chemical reduction[10] and a magnetic field[11] have been used as specific triggers for the release of drugs encapsulated in SNPs.

Azobenzene (Azo) is known to bind to α- and β-CD in the stable *trans* form. Upon irradiation with light at a wavelength shorter than 360 nm, it undergoes a conversion into the unstable, bulkier *cis* form, which is released from the CD cavity. This phenomenon has been used to build photoresponsive supramolecular vesicles,[12] mesoporous silica nanoparticles,[13] and hydrogels,[14] which can bind and release DNA, drugs, and proteins, respectively. In contrast, thermal *trans–cis* isomerization has been used to fabricate an amine-displacement assay driven by the complexation of Azo by cucurbit[7]uril.[15] Recently, the formation of a ternary light-responsive host–guest complex between Azo, methyl viologen (MV), and cucurbit[8]uril CB[8] was reported.[16] Upon irradiation of the system with UV light, the *cis*-Azo was formed, thus disrupting the ternary complex formed between *trans*-Azo, MV, and CB[8]. By using this photoinduced mechanism, changes in the surface wettability[16a] as well as the polymerization of supramolecular building blocks[16b] could be triggered using light. This system holds the, so far uninvestigated, promise to function as a reversible supramolecular glue for the controlled assembly and disassembly of supramolecular nanoparticles.

Recently, we showed by employing a ternary charge-transfer complex between CB[8], MV, and naphthol-functionalized components that SNPs could be formed with controllable particle sizes.[17] Here we report a fully reversible and dual-responsive SNP system with Azo building blocks, in which SNP assembly and disassembly can be switched reversibly in multiple cycles by the photoswitching of Azo, and irreversibly by chemical reduction of the MV units. SNPs based on the ternary host–guest generated from CB[8], MV-polymer, and mono- and multivalent Azo-functionalized guest molecules have been formed with controllable particle sizes. As shown schematically in Figure 1, disintegration of the formed SNPs is observed after photochemical conversion from *trans*- to *cis*-Azo on irradiation with UV light ($\lambda <$ 400 nm). Isomerization back to *trans*-Azo is induced by visible light, which leads to full restoration of the SNPs. Additionally, the SNPs have been fully disassembled by chemical reduction.

To this end, Azo-terminated poly(amidoamine) dendrimer (PAMAM) generation 1 (Azo-PAMAM$_8$) was synthesized (see Figure S1 in the Supporting information) to act as the multivalent photoresponsive supramolecular cross-linker

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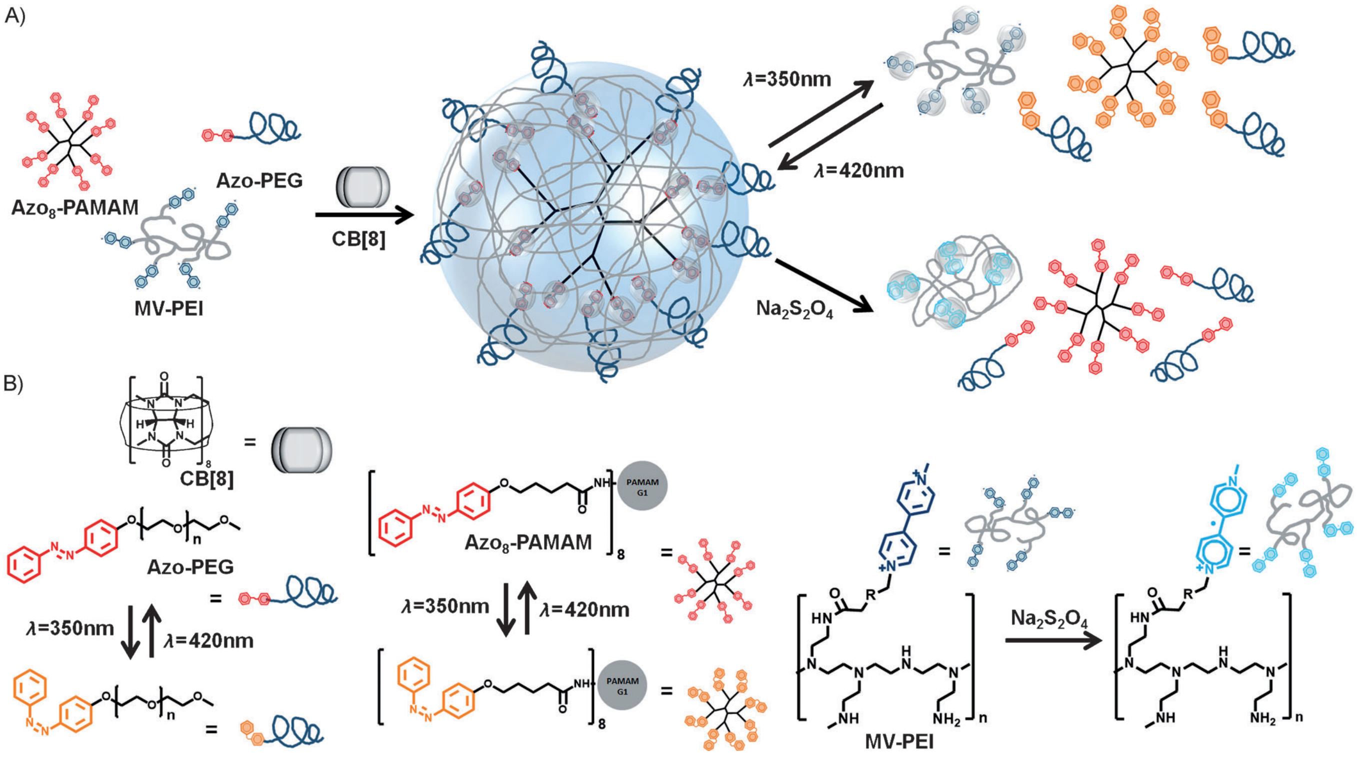

Figure 1. A) Schematic presentation of the supramolecular nanoparticle (SNP) self-assembly and triggered disassembly mediated by formation and disruption of the ternary complex formed between cucurbit[8]uril (CB[8]), methyl viologen (MV), and azobenzene (Azo) moieties. B) The supramolecular host (CB[8]) and responsive guest molecules involved in SNP formation: azobenzene-functionalized poly(ethylene glycol) (Azo-PEG), methyl viologen functionalized poly(ethylene imine) (MV-PEI), and Azo$_8$-poly(amidoamine) (Azo$_8$-PAMAM).

in the core of the particles. In the presence of the previously reported MV-substituted poly(ethyleneimine) (MV-PEI, degree of substitution: 4.5 MV units per polymer chain)[17] and CB[8], this Azo-substituted dendrimer forms multiple ternary complexes in aqueous solution. Monovalent, Azo-functionalized poly(ethylene glycol) (Azo-PEG, $M_W = 5000\,\mathrm{g\,mol^{-1}}$) was also prepared (see Figure S4 in the Supporting Information) to provide colloidal stability and size control of the formed SNPs. As described by us[17] for a similar, although nonswitchable, ternary complex motif, the monovalent Azo-PEG competes with the multivalent Azo$_8$-PAMAM for binding to CB[8], thereby leading to termination of the aggregates and resulting in the size-controlled formation of SNPs. The Azo-PEG also facilitates the water solubility of the assembled SNPs as the PEG chains are exposed at the outside of the SNPs.

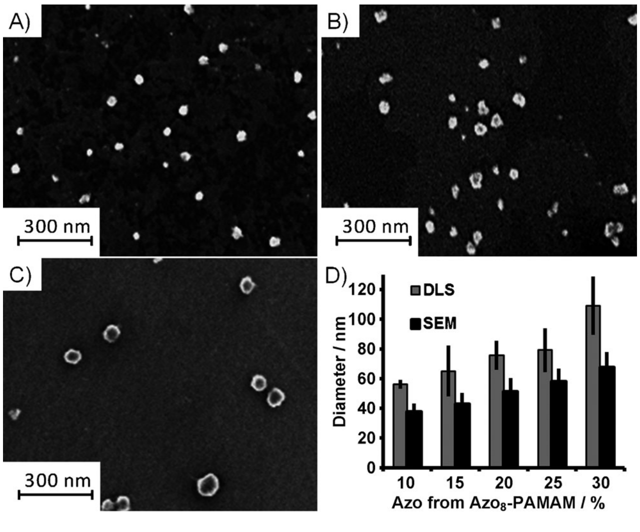

Figure 2. Size determinations of SNPs prepared with different formulations: A)–C) SEM images of the resulting SNPs as a function of the Azo fraction derived from Azo$_8$-PAMAM dendrimers (A: 10% B: 20% C: 30%) upon formation of the assembly using Azo/MV/CB[8] = 1:1:1. D) SNP diameters as measured by DLS (gray bars) and SEM (black bars).

Size-controlled self-assembly of the particles was observed by mixing in various ratios the four supramolecular blocks, that is, mono- and multivalent Azo, multivalent MV-PEI, and CB[8], involved in the formation of the ternary complex. A solution of MV-PEI was added to a mixture of Azo$_8$-PAMAM dissolved in DMSO, aqueous CB[8], and aqueous Azo-PEG. Successful size tuning by varying the ratio of the two Azo compounds, keeping the overall stoichiometry of Azo/MV/CB[8] = 1:1:1 (Figure 2), was observed on analyzing the mixtures after two days by scanning electron microscopy (SEM) and dynamic light scattering (DLS). As shown in Figure 2, the observed SNP size depends strongly on the fraction of Azo$_8$-PAMAM used during particle self-assembly. By increasing the amount of Azo derived from Azo$_8$-PAMAM from 10% to 30%, while decreasing the amount of Azo-PEG correspondingly, the recorded particle size was found to increase from 55 nm to 110 nm by DLS and from 35 nm to 70 nm by SEM. The commonly observed discrepancy between the results from DLS and SEM can be attributed to DLS reporting hydrodynamic diameters and to the loss of water during SEM sample preparation. Overall, the size increased monotonously and was approximately linearly dependent on the amount of Azo$_8$-PAMAM up to 30 %. DLS analysis further revealed that addition beyond 35 % Azo$_8$-PAMAM led to uncontrolled particle growth, which yielded large aggregates with a high polydispersity (see Figure S7 in the Supporting Information). This observation can be ascribed to the supramolecular competition between the multivalent dendrimers that reside in the core of the SNPs, while the monovalent Azo-PEG assembles at the exterior of the SNPs. An increasing amount of the multivalent component allows an extended growth of the SNPs, although a minimum amount of monovalent supramolecular stopper is apparently required for the formation of stable SNPs. To prove the supramolecular specificity of the SNP self-assembly, CB[7] instead of CB[8] was used during the assembly of the SNPs; for steric reasons the smaller CB[7] does not allow the formation of the ternary complexes. In the presence of MV-PEI, Azo-PEG, and Azo$_8$-PAMAM (20 % Azo from Azo$_8$-PAMAM), DLS analysis revealed, as expected, no distinct particle formation when CB[7] was used (see Figure S9 in the Supporting Information). This observation shows that CB[8] is essential for SNP formation through the generation of multiple ternary complexes.

As a next step, the light-induced disassembly and reassembly of the particles was investigated by making use of the photoresponsive ternary complex by conversion of *trans*-Azo into *cis*-Azo. UV/Vis spectroscopy of the complex formed between CB[8], methyl viologen, and Azo-PEG as well as ^{1}H NMR spectroscopy of Azo-PEG in D_2O showed that 80–90 % of the *trans*-Azo-PEG was converted into *cis*-Azo-PEG within 1 minute after irradiating the aqueous solution with UV light ($\lambda < 400$ nm; Figure 3 and see Figure S12 in the Supporting Information). Time-dependent UV/Vis measurements showed that the absorption band at 345 nm was reinstalled within 20 min after exposing the sample to ambient light (Figure 3), which is indicative of *cis–trans* isomerization and re-formation of the ternary complex.

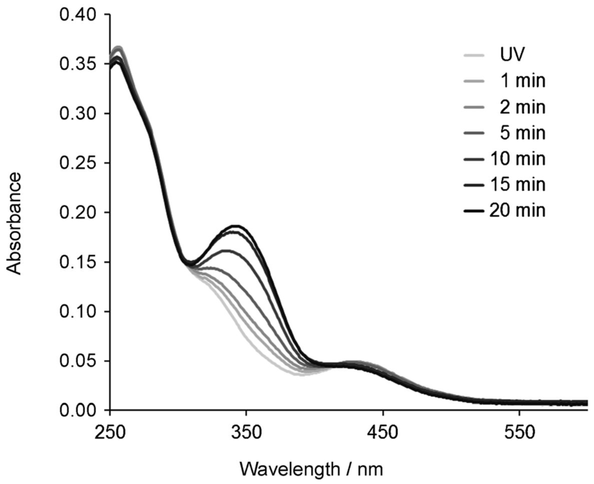

Figure 3. UV/Vis absorbance of Azo-PEG/methyl viologen/CB[8] (1:1:0.95) before and after 1 min irradiation with UV light as well as the time-dependent interconversion of *cis*- to *trans*-Azo-PEG in ambient light.

Subsequently we irradiated previously prepared solutions of the SNPs with UV light ($\lambda < 400$ nm) and followed the change in the SNP size by DLS (Figure 4 A). After an initial UV exposure time of 1 h, aggregated particles with a high polydispersity were observed (Figure 4 A). In contrast, full disintegration of the SNPs was observed after prolonging the irradiation time to 14 h. This particle disassembly process is relatively slow in comparison to the 1 min switching time of Azo in the individual ternary complex as described above. This difference can be explained by 1) an incomplete *trans*-to-*cis* isomerization of Azo and 2) a slow rearrangement of multivalently interacting building blocks. It has been reported that the photostationary state of the UV irradiation of Azo contains up to 20 % *trans*-Azo.[12a] Presumably, upon UV illumination, the *cis*-Azo-PEG is immediately expelled from the SNP shell, which leads to exposure of the core. The photostationary state ensures at the same time that the multivalent Azo$_8$-PAMAM will initially remain interacting through a few moieties inside the core, thus leading to significant clustering of the nanoparticles. Since the assembly of SNPs is slow, as has been observed before,[17] because of rearrangement of the multivalently interacting components, the disintegration of the remaining building blocks only takes place over the course of hours (Figure 4 A).

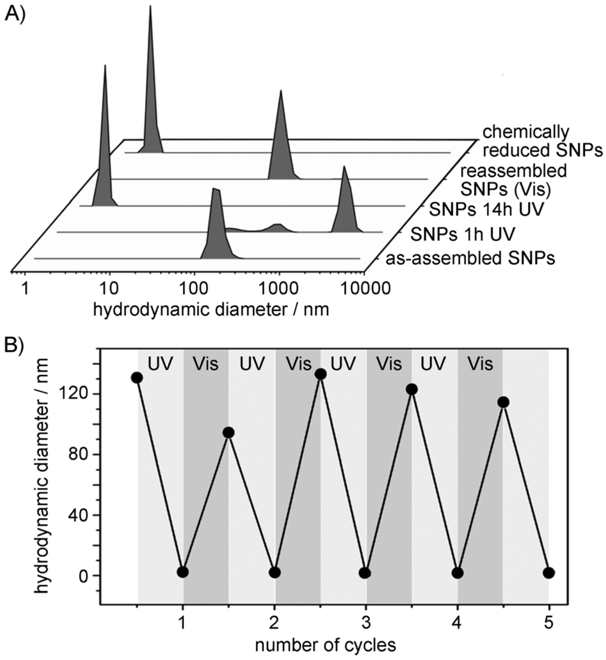

Figure 4. A) DLS size determination of as-prepared SNPs, and subsequently after 1 h UV light, after 14 h UV light, after 8 h ambient light, and after chemical reduction of MV by $Na_2S_2O_4$. B) UV/Vis light-induced switching to trigger SNP assembly and disassembly for four consecutive cycles.

To verify the reversibility of the *cis*-to-*trans* switching that leads to the reassembly of the individual components to nanoparticles, the disassembled SNP samples were kept in ambient light to induce the reformation of *trans*-Azo, thus enabling the re-formation of the ternary complex with MV and CB[8]. Complete SNP reassembly was observed after keeping the samples for 8 h in visible light (Figure 4 A). In contrast, no distinct particle reformation was observed for SNP samples kept in the dark for 6–14 h after UV irradiation (see Figure S14 in the Supporting Information). The SNP assembly/disassembly process was conducted several times by switching between UV and visible light. Figure 4 B shows the alternate switching for four complete cycles, and in all states a complete re-formation (ca. 116 nm ± 16 nm) or rupture (ca. 2–4 nm) of the SNPs was observed. These results confirm the successful development of a reversible, light-responsive supramolecular nanoparticle system.

The constitutive ternary supramolecular complex containing MV also results in the SNPs being susceptible to changes induced by chemical reduction of the MV moieties. Chemically triggered particle degradation was observed after the addition of the reducing agent $Na_2S_2O_4$. DLS analysis demonstrates the disappearance of particles with a size of about 110 nm and formation of small aggregates of about 2–4 nm (Figure 4 A) after 12 h. This observation is in agreement with the supramolecular mechanism reported before. The addition of a chemical reductant leads to formation of the MV^{+} radical cation, which is known to from a stable homoternary complex with CB[8],[18] thus causing the release of the Azo guest unit (Figure 1) and concomitant disassembly of the SNPs.[16a]

To prove that particle reassembly requires both the *trans*-Azo as well as the dicationic MV species, the two particle disassembly processes were carried out consecutively. The formed SNPs were disassembled first by chemical reduction with $Na_2S_2O_4$, followed by UV irradiation. DLS studies (see Figure S17 in the Supporting Information) show that the initially formed SNPs were neither observed after chemical reduction nor after UV light irradiation (Figure 5). Furthermore, SNP reassembly was not observed after subsequent treatment with visible light, which can be ascribed to the still-present ternary complexes formed between MV^{+} radical cations and CB[8].

In logic terms, the SNP system described here functions as an AND operator. Only when the Azo moiety is in the *trans* form and the MV moiety in the dicationic form are SNPs formed (Figure 5). Switching either of them leads to inhibition of the particle formation process. Molecular systems representing logic operators have been shown and reviewed before.[19] To our knowledge, this is the first example in which the result of the operation is visualized by the formation of supramolecular particles.

In conclusion, we have displayed a supramolecular strategy for the self-assembly of size-controlled supramolecular nanoparticles based on the ternary interaction between a MV polymer, mono- and multivalent Azo building blocks, and CB[8]. The ternary complex enables the particles to be assembled and disassembled, the latter triggered either by UV irradiation or by a chemical reductant. Reversible disassembly and reassembly can be induced by illumination by UV and visible light, respectively, whereas irreversible disassembly occurs following chemical reduction. The dual responsive character and its reversibility make this SNP system a promising candidate for advanced material engineering.

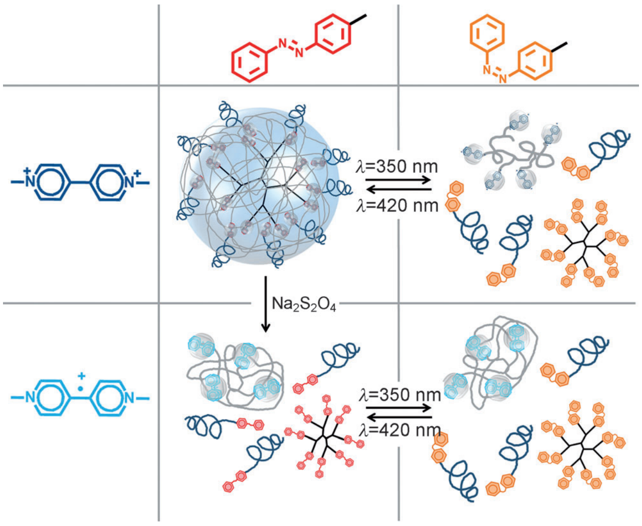

Figure 5. Molecular AND logic operator in dual-responsive SNPs: SNPs are only formed with *trans*-Azo and the MV dication; with *cis*-Azo (by photoirradiation) and/or MV in the radical cation form (by chemical reduction), only small building blocks are observed.

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[1] a) M. E. Davis, J. E. Zuckerman, C. H. J. Choi, D. Seligson, A. Tolcher, C. A. Alabi, Y. Yen, J. D. Heidel, A. Ribas, *Nature* **2010**, *464*, 1067 – 1070; b) H. Wang, S. Wang, H. Su, K.-J. Chen, A. L. Armijo, W.-Y. Lin, Y. Wang, J. Sun, K.-i. Kamei, J. Czernin, C. G. Radu, H.-R. Tseng, *Angew. Chem.* **2009**, *121*, 4408 – 4412; *Angew. Chem. Int. Ed.* **2009**, *48*, 4344 – 4348; c) L. Y. Liu, B. He, Y. Li, G. Wang, S. Chang, Z. Gu, *Int. J. Nanomed.* **2012**, *7*, 5249 – 5258.

[2] a) T. Kakuta, Y. Takashima, M. Nakahata, M. Otsubo, H. Yamaguchi, A. Harada, *Adv. Mater.* **2013**, *25*, 2849 – 2853; b) K. L. Liu, Z. Zhang, J. Li, *Soft Matter* **2011**, *7*, 11290 – 11297; c) E. A. Appel, X. J. Loh, S. T. Jones, F. Biedermann, C. A. Dreiss, O. A. Scherman, *J. Am. Chem. Soc.* **2012**, *134*, 11767 – 11773.

[3] a) G. Gröger, W. Meyer-Zaika, C. Böttcher, F. Gröhn, C. Ruthard, C. Schmuck, *J. Am. Chem. Soc.* **2011**, *133*, 8961 – 8971; b) P. A. Korevaar, S. J. George, A. J. Markvoort, M. M. J. Smulders, P. A. J. Hilbers, A. P. H. J. Schenning, T. F. A. De Greef, E. W. Meijer, *Nature* **2012**, *481*, 492 – 496; c) Y. Liu, Z. Huang, X. Tan, Z. Wang, X. Zhang, *Chem. Commun.* **2013**, *49*, 5766 – 5768.

[4] a) J. Voskuhl, M. Waller, S. Bandaru, B. A. Tkachenko, C. Fregonese, B. Wibbeling, P. R. Schreiner, B. J. Ravoo, *Org.*

Biomol. Chem. **2012**, *10*, 4524–4530; b) M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875–1918.

[5] J. Lagona, P. Mukhopadhyay, S. Chakrabarti, L. Isaacs, *Angew. Chem.* **2005**, *117*, 4922–4949; *Angew. Chem. Int. Ed.* **2005**, *44*, 4844–4870.

[6] a) A. González-Campo, M. Brasch, D. A. Uhlenheuer, A. Gómez-Casado, L. Yang, L. Brunsveld, J. Huskens, P. Jonkheijm, *Langmuir* **2012**, *28*, 16364–16371; b) Q. An, J. Brinkmann, J. Huskens, S. Krabbenborg, J. de Boer, P. Jonkheijm, *Angew. Chem.* **2012**, *124*, 12399–12403; *Angew. Chem. Int. Ed.* **2012**, *51*, 12233–12237.

[7] Q. Hu, W. Li, X. Hu, Q. Hu, J. Shen, X. Jin, J. Zhou, G. Tang, P. K. Chu, *Biomaterials* **2012**, *33*, 6580–6591.

[8] H. Izawa, K. Kawakami, M. Sumita, Y. Tateyama, J. P. Hill, K. Ariga, *J. Mater. Chem. B* **2013**, *1*, 2155–2161.

[9] A. Kulkarni, W. Deng, S.-h. Hyun, D. H. Thompson, *Bioconjugate Chem.* **2012**, *23*, 933–940.

[10] Y. Ping, Q. Hu, G. Tang, J. Li, *Biomaterials* **2013**, *34*, 6482–6494.

[11] J.-H. Lee, K.-J. Chen, S.-H. Noh, M. A. Garcia, H. Wang, W.-Y. Lin, H. Jeong, B. J. Kong, D. B. Stout, J. Cheon, H.-R. Tseng, *Angew. Chem.* **2013**, *125*, 4480–4484; *Angew. Chem. Int. Ed.* **2013**, *52*, 4384–4388.

[12] S. K. M. Nalluri, J. Voskuhl, J. B. Bultema, E. J. Boekema, B. J. Ravoo, *Angew. Chem.* **2011**, *123*, 9921–9925; *Angew. Chem. Int. Ed.* **2011**, *50*, 9747–9751.

[13] a) S. Angelos, Y.-W. Yang, N. M. Khashab, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2009**, *131*, 11344–11346; H. Yan, C. Teh, S. Sreejith, L. Zhu, A. Kwok, W. Fang, X. Ma, K. T. Nguyen, V. Korzh, Y. Zhao, *Angew. Chem.* **2012**, *124*, 8498–8502; *Angew. Chem. Int. Ed.* **2012**, *51*, 8373–8377.

[14] K. Peng, I. Tomatsu, A. Kros, *Chem. Commun.* **2010**, *46*, 4094–4096.

[15] J. Wu, L. Isaacs, *Chem. Eur. J.* **2009**, *15*, 11675–11680.

[16] a) F. Tian, D. Jiao, F. Biedermann, O. A. Scherman, *Nat. Commun.* **2012**, *3*, 1207; b) J. del Barrio, P. N. Horton, D. Lairez, G. O. Lloyd, C. Toprakcioglu, O. A. Scherman, *J. Am. Chem. Soc.* **2013**, *135*, 11760–11763.

[17] C. Stoffelen, J. Huskens, *Chem. Commun.* **2013**, *49*, 6740–6742.

[18] W. S. Jeon, H.-J. Kim, C. Lee, K. Kim, *Chem. Commun.* **2002**, 1828–1829.

[19] U. Pischel, J. Andréasson, D. Gust, V. F. Pais, *ChemPhysChem* **2013**, *14*, 28–46.