Precisely Controlled 2D Free-Floating Nanosheets of Amphiphilic Molecules through Frame-Guided Assembly

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Amphiphilic molecules normally assemble into 3D symmetric closed structures such as micelles/vesicles or 1D fibers/tubes in aqueous solution to minimize the free energy.[1] 2D self-assembly of amphiphilic molecules largely occurs at the solid/liquid, liquid/air, or liquid/liquid interfaces, forming monolayer or bilayer films through a spontaneous process, such as Langmuir–Blodgett films,[2] solid supported lipid bilayers,[3] and self-assembled monolayers,[4] which are achieved through strong adsorption or even covalent bonding between the amphiphilic molecules and solid substrates. Few examples of preparing free-floating 2D sheet-like assemblies rely on the specific structure and/or interactions of the building blocks, and the amphiphilic sheets normally grow infinitely and ultimately form microsized nanosheets without size and/or shape control.[5] Recently, a frame-guided assembly strategy has been used to control the assembly process of amphiphilic molecules in which the structure of the assemblies is mainly determined by the inside frame rather than the intrinsic properties of the amphiphilic molecules.[6] However, the currently reported examples are all limited to the preparation of 3D vesicles. Here, we report a DNA origami-based, frame-guided assembly that can overcome the obstacle of the 2D assembly of amphiphiles in aqueous solution. Moreover, the prepared free-floating 2D amphiphilic assemblies could be readily tailored to a custom size and shape, and the assembly parameters could be programmed.

Our strategy is illustrated in **Figure 1**. DNA origami is formed by folding a long scaffold DNA using a set of short staple strands.[7] Employing different sequence design of staple strands, fully addressable DNA origami with various shapes could be prepared. Certain arrangements of anchor strands extended from staple strands on the same surface are designed. An amphiphilic molecule named DDOEG (Figure 1b), consisting of a hydrophobic poly(aryl ether) dendron molecule and eight oligo(ethylene glycol) tails, was covalently conjugated to a 15 nt piece of single-stranded DNA. Through sequence-specific DNA hybridization, the DDOEG molecules could be rationally positioned onto a DNA origami, following the arrangement of anchor strands. The DDOEG molecules anchored on origami via DNA hybridization act as the leading hydrophobic groups (LHGs) and together form a 2D frame domain above the DNA origami. The local concentration of hydrophobic groups within the frame domain is much higher than that dispersed in the solution. Driven by hydrophobic–hydrophobic interactions, additional DDOEG or other amphiphilic molecules would be absorbed into this domain to decrease the total free energy and gradually form a homogeneous or heterogeneous nanosheet above the DNA origami. Thus, the 2D self-assembly of amphiphilic molecules occurred without the direct involvement of any interface. According to this mechanism, the size and shape of the formed nanosheets would not exceed the range of the frame and could be readily tailored by the arrangement of the LHGs on the DNA origami.

As a model system, we first employed single-layer rectangular DNA origami (≈100 nm × 70 nm) as the foundation of the frame to prepare the homogeneous amphiphilic molecular nanosheets. In a typical experiment, a DNA origami structure containing 59 anchor strands arranged (details in Figure S1, Supporting Information) on one surface was designed and prepared according to a well-established strategy[7a] and purified by molecular weight cut-off filtration. Two nanomolar purified DNA origami were incubated with 10×10^{-6} M DDOEG at room temperature for 3 d, and atomic force microscopy (AFM) was employed to investigate the formation of assembled structures. As shown in **Figure 2**a, rectangular nanosheets with a total height of ≈5 nm (Figure 2a,ii) could be observed with dimensions slightly smaller than the bare DNA origami structure (Figure 2a,i), which agrees with the frame size defined by the LHGs. As a control, the DNA origami solely incubated with DNA strands complementary to the anchor strands without any DDOEG molecule modifications did not show such features (Figure S3, Supporting Information).

To further confirm that the nanosheets are formed by the guided assembly of DDOEG and verify that the size of the assemblies could be tailored by the frame, we altered the design of the DNA origami to allow only half of the area to position LHGs at the same density. As shown in Figure 2b, the assembled structure only forms on the half of the origami with anchor strands for LHGs and has a half-sized rectangular shape. The half-naked DNA origami structure is an excellent internal reference to the formed nanosheets, and the height profile indicates

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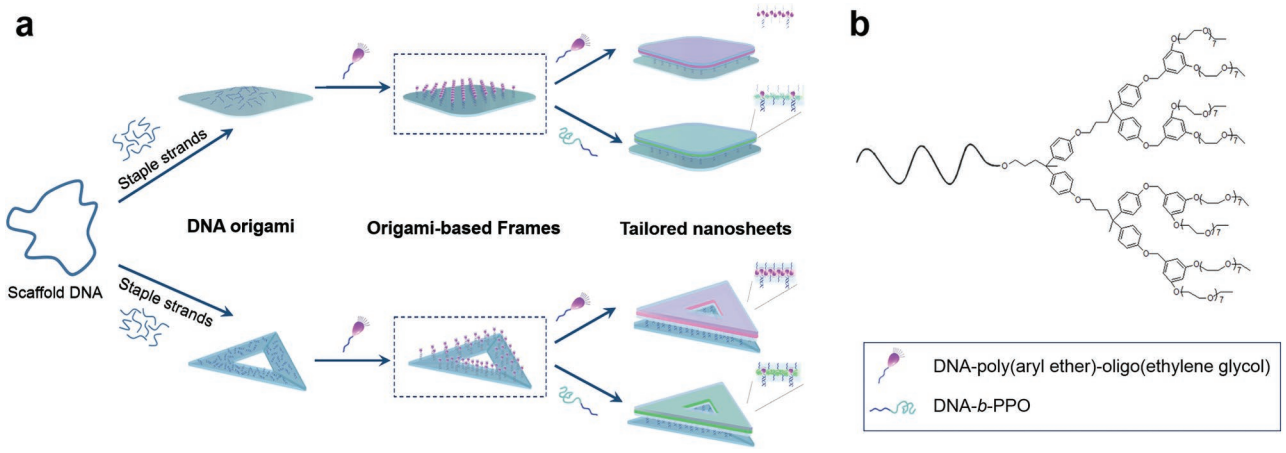

Figure 1. Illustration of the 2D assembly of amphiphilic molecules via a frame-guided process. a) First, a 2D DNA origami structure with anchor strands on the same surface is assembled. Then, amphiphilic molecules modified by DNA strands complementary to the anchor strands are added. The amphiphilic molecules are then hybridized on the surface of the DNA origami, which makes the DNA origami surface an area with a high local concentration of hydrophobic molecules. This surface then acts as a frame to guide the free hydrophobic molecules in the aqueous solution to assemble via hydrophobic effects, ultimately forming continuous 2D nanosheets above the DNA origami. b) The scheme of DDOEG molecule.

that the height difference is ≈3 nm. Considering that the height difference includes the thickness of the nanosheet as well as the space between the nanosheet and DNA origami structure supported by anchor strands, this implies that such amphiphilic molecular nanosheets are monolayer. Based on the principle of amphiphilic self-assembly, we infer that the nanosheets are constructed with the hydrophilic DNA strands on the outside and the hydrophobic dendron groups protected inside, forming a sandwich-like structure of "DNA–dendron–DNA" as shown in Figure 2.

To verify this hypothesis, we prepared 5 nm gold nanoparticles (AuNPs) modified by DNA strands that were complementary to the sequences of DDOEG and then incubated them with the prepared nanosheets. As shown in the TEM image (Figure 2a,iii), the AuNPs are concentrated on the negative-stained area of the nanosheets. Conversely, the AuNPs modified by a random DNA sequence are randomly dispersed on the TEM grid after the same treatment (Figure 2a,iv). These results not only corroborate the inferred structure of the amphiphilic molecular nanosheets but also imply the possibility of further assembling other entities through the DNA strands exposed on the outside of the structure.

For a successful guided assembly, the local concentration of DDOEG and concentration in solution need to work in coordination, and their coeffects are studied. The local concentration of LHGs is determined by the density of anchor strands in the frame domain, which could be easily tailored by changing the number and arrangement of anchor strands. Our results (Figure S4, Supporting Information) show that the assembly of nanosheets requires a relatively high local concentration (≈200 × 10^{-6} M) and a relatively close distance between adjacent LHGs (≈10 nm), which explains why the assembly could be strictly restrained in a finite 2D area and means the nanosheets could be fabricated with nanometer precision. Meanwhile, the lowest limit of free DDOEGs in solution was also explored. We observed some discontinuous lines or dots when the concentration is 2 × 10^{-6} M while the fully covered assembly layers could be easily observed only when it increased to 10 × 10^{-6} M (for detailed information, see Supporting Information, Figure S4). Based on these results, it is conceivable that the nanosheets follow the frame-guided process via a nucleation-growth process. The nucleation is determined by the density of the LHGs, and the growth process primarily depends on the concentration of the free amphiphilic molecules in solution.

We have demonstrated that the assembly of nanosheets can be restrained in a finite 2D area and will not assemble beyond the fringe of the frames. However, it is still unclear whether the strategy is suitable for fabricating complex assemblies, especially hollow structures. To address this question, we attempted to prepare triangular nanosheets containing inner cavities. The frame is based on a triangular DNA origami structure formed by three trapezoidal domains (**Figure 3**a) with a central triangular cavity (each side length is ≈50 nm) inside.[7a] The density of LHGs needed to form the frame above the origami structure is similar to that of the rectangular frame (detailed design in Figure S2). If the frame is only allowed to form above one of the three domains, assembly only occurs on the domain in which a trapezoidal structure is observed (Figure 3b). When formation is allowed in two domains, the assembled nanosheets on these two domains form a continuous layer with a sharp corner (Figure 3c). When all three domains are decorated, triangular nanosheets can form, and their central cavities are well maintained (Figure 3d). The corners of these triangular cavities are clear and sharp, suggesting high precision of the frame-guided 2D assembly. This result confirms that the frame not only serves as the nucleation site of the guided assembly but also plays a crucial role in restricting the assembly to within the designed shape. The assemblies cannot grow beyond the frame because the LHGs are anchored firmly within the designed frame via DNA hybridization.

To explore the generality of this strategy, we attempted to prepare hetero-molecular nanosheets formed by DNA-*b*-PPO

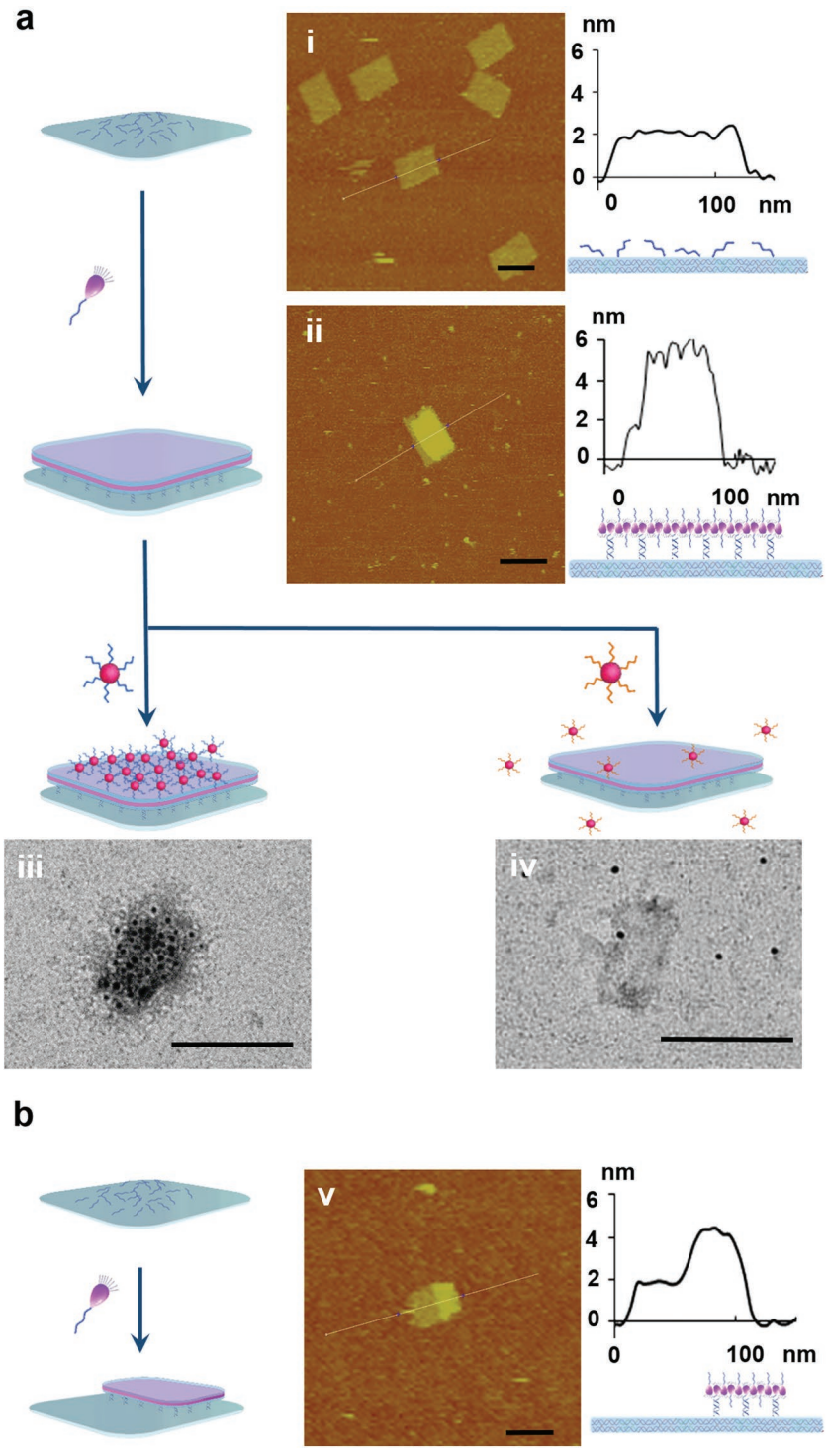

Figure 2. Characterization of assembled 2D rectangular nanosheets. a) Formation of rectangular nanosheets and further modification by AuNPs. i) AFM image of assembled rectangular DNA origami structures with anchor strands. ii) Magnified AFM image of a single piece of nanosheet. iii) TEM image of a nanosheet with concentrated AuNPs on it. The AuNPs are modified by DNA complementary to DDOEG. iv) TEM image of a nanosheet with randomly dispersed AuNPs. The AuNPs are modified by random DNA sequence. b) Half-size nanosheet assembled on DNA origami with only half anchor strands. The right columns provide schematic illustrations of each structure and a height profile analysis. Scale bar: 100 nm.

(poly(p-propylene oxide)) and guided by DDOEG as a demonstration. PPO is a type of thermo-responsive linear polymer, which is quite different from the dendron molecules that we used as LHGs. To ensure the assembly DNA-*b*-PPO is guided by the LHGs of the DDOEG molecules, different DNA sequence is used for DNA-*b*-PPO to exclude its hybridization with anchor strands. First, we incubated the DNA origami structure with 1×10^{-6} M DDOEG molecules to form the frame (using the 59 anchors arrangement). Then, 10×10^{-6} M of DNA-*b*-PPO was added to the system at 4 °C, followed by incubation at 37 °C for 3 d. We observed the formation of nanosheets on the DNA origami frame as shown in **Figure 4**a.

DDOEG at 1×10^{-6} M is not high enough to form stable nanosheets as mentioned above (Figure S4, Supporting Information), and DNA-*b*-PPO would not hybridize with the anchor strands to form frame. Therefore, we reasoned that these nanosheets could only be guided by the frame formed by the DDOEGs, but their main bodies are formed by DNA-*b*-PPO, which was confirmed by the gold nanoparticle-dressing experiments observed by TEM (Figure S6, Supporting Information). Similar to the DDOEG nanosheets, the heterogeneous nanosheets also have a thickness of ≈3 nm, and both rectangular and triangular nanosheets were prepared (Figure 4). These results demonstrate that the 2D frame-guided assembly is a general method, and the shape as well as size of the final assemblies is determined by the designed frame.

We have reported an approach to efficiently prepare free-floating, monodispersed, size- and shape-defined, and homogeneous (or heterogeneous) nanosheets from amphiphilic molecules. Unlike traditional self-assembled amphiphilic layers formed at interfaces, the nanosheets prepared here are separated from the soft DNA origami structure through discrete DNA duplexes, which could minimize the influence of the substrates while retaining the fluidity and impermeability of the amphiphilic layers.[8] Moreover, the distance between the nanosheets and the DNA origami could be easily tailored by adjusting the length of the DNA anchor strands, which can impart important flexibility to create extra space for introducing entities for further functionalization.[9] The nanosheets above the DNA origami cannot only mimic the hydrophobic environment of biological membrane but

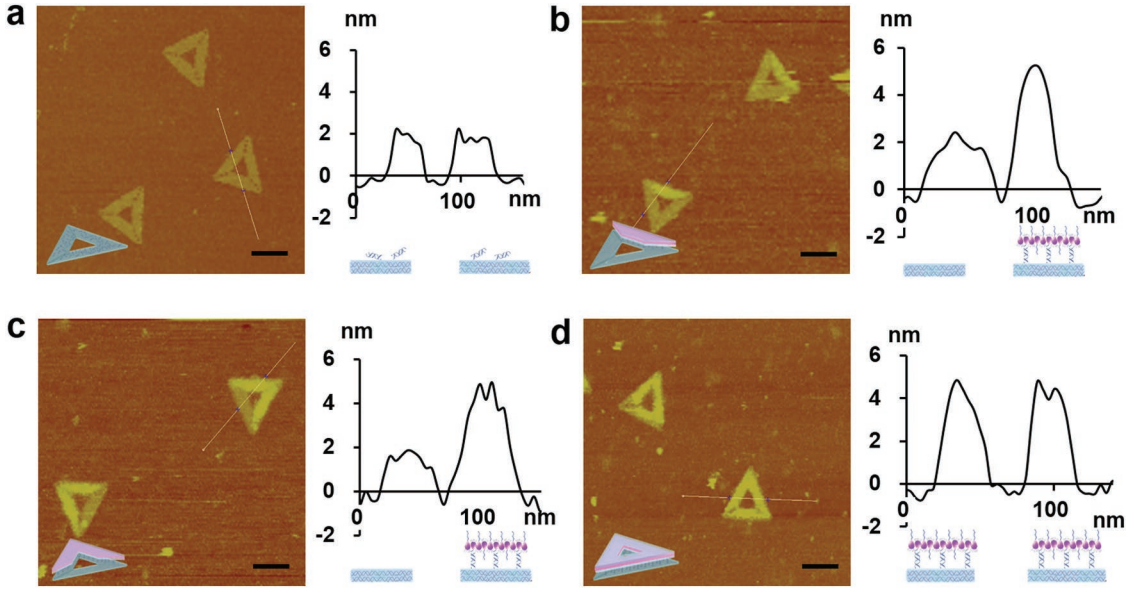

Figure 3. AFM images and schematics of triangular nanosheet assembly. a) AFM image and schematics of triangular DNA origami. The triangular DNA origami structure is formed by three trapezoidal domains. b–d) Nanosheets assembled on one (b), two (c), and three (d) domains of DNA origami by controlling the arrangements of the anchor strands. Scale bar: 100 nm.

also provide the addressability for further implantation and study of the properties of biological entities, e.g., membrane proteins.[3a,10]

The approach here is applicable to DNA-based amphiphilic molecules,[11] which have already been widely used as building blocks to prepare versatile assemblies.[12] The diversity of such molecules provides the possibility to build various nanosheets with designated properties. With the development of DNA-conjugation technology,[13] we believe that this strategy could even be used to prepare other 2D materials, e.g., inorganic nanosheets or polymers.[14]

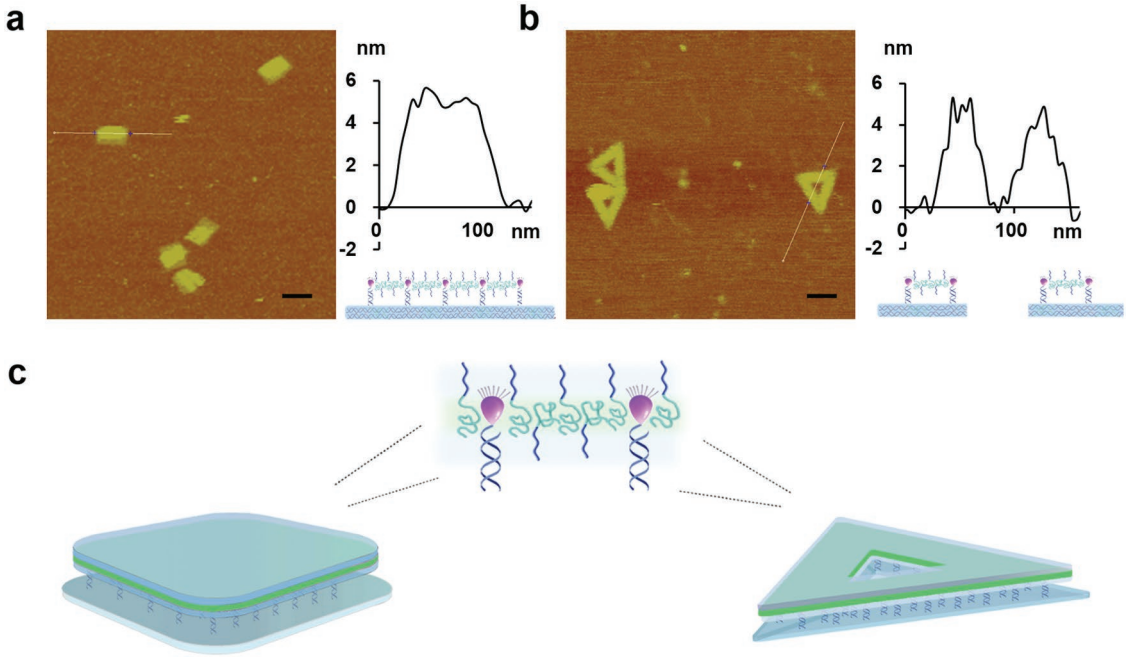

Figure 4. Assembly of heterogeneous nanosheets with tailored shapes. a) AFM image and profile analysis of rectangular heterogeneous nanosheets. b) AFM image and profile analysis of triangular heterogeneous nanosheets. c) Schematic of the heterogeneous nanosheet guided by DDOEG on DNA origami through DNA hybridization and mainly formed by the assembled DNA-*b*-PPO via hydrophobic effects. Scale bar: 100 nm.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] a) M. Antonietti, S. Forster, *Adv. Mater.* **2003**, *15*, 1323; b) C. Wang, Z. Q. Wang, X. Zhang, *Acc. Chem. Res.* **2012**, *45*, 608; c) Y. Mai, A. Eisenberg, *Chem. Soc. Rev.* **2012**, *41*, 5969; d) S. D. Zhang, H. J. Sun, A. D. Hughes, R. O. Moussodia, A. Bertin, Y. C. Chen, D. J. Pochan, P. A. Heiney, M. L. Klein, V. Percec, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 9058; e) H. Qiu, Z. M. Hudson, M. A. Winnik, I. Manners, *Science* **2015**, *347*, 1329; f) M. J. Huang, C. H. Hsu, J. Wang, S. Mei, X. H. Dong, Y. W. Li, M. X. Li, H. Liu, W. Zhang, T. Aida, W. B. Zhang, K. Yue, S. Z. D. Chen, *Science* **2015**, *348*, 424.

[2] J. A. Zasadzinski, R. Viswanathan, L. Madsen, J. Garnaes, D. K. Schwartz, *Science* **1994**, *263*, 1726.

[3] a) E. Sackmann, *Science* **1996**, *271*, 43; b) E. T. Castel-lana, P. S. Cremer, *Surf. Sci. Rep.* **2006**, *61*, 429.

[4] a) A. Ulman, *Chem. Rev.* **1996**, *96*, 1533; b) J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo, G. M. Whitesides, *Chem. Rev.* **2005**, *105*, 1103; c) L. Ding, Y. Fang, *Chem. Soc. Rev.* **2010**, *39*, 4258.

[5] a) E. D. Gomez, T. J. Rappl, V. Agarwal, A. Bose, M. Schmutz, C. M. Marques, N. P. Balsara, *Macromolecules* **2005**, *38*, 3567; b) E. Lee, J. K. Kim, M. Lee, *Angew. Chem., Int. Ed.* **2009**, *48*, 3657; c) K. T. Nam, S. A. Shelby, P. H. Choi, A. B. Marciel, R. Chen, L. Tan, T. K. Chu, R. A. Mesch, B. C. Lee, M. D. Connolly, C. Kisielowski, R. N. Zuckermann, *Nat. Mater.* **2010**, *9*, 454; d) Z. M. Hudson, C. E. Boott, M. E. Robinson, P. A. Rupar, M. A. Winnik, I. Manners, *Nat. Chem.* **2014**, *6*, 893.

[6] a) Y. Dong, Y. Sun, L. Wang, D. Wang, T. Zhou, Z. Yang, Z. Chen, Q. Wang, Q. Fan, D. Liu, Z. Yang, Z. Chen, Q. Wang, Q. Fan, D. Liu, *Angew. Chem., Int. Ed.* **2014**, *53*, 2607; b) Z. Zhao, C. Chen, Y. Dong, Z. Yang, Q. Fan, D. Liu, *Angew. Chem., Int. Ed.* **2014**, *53*, 13468; c) Y. Dong, Z. Yang, D. Liu, *Small* **2015**, *11*, 3768; c) S. D. Perrault, W. M. Shih, *ACS Nano* **2014**, *8*, 5132; d) Y. Yang, J. Wang, H. Shigematsu, W. Xu, W. M. Shih, J. E. Rothman, C. Lin, *Nat. Chem.* **2016**, *8*, 476.

[7] a) P. W. K. Rothemund, *Nature* **2006**, *440*, 297; b) S. M. Douglas, H. Dietz, T. Liedl, B. Hogberg, F. Graf, W. M. Shih, *Nature* **2009**, *459*, 414; c) D. R. Han, S. Pal, J. Nangreave, Z. T. Deng, Y. Liu, H. Yan, *Science* **2011**, *332*, 342; d) R. Iinuma, Y. G. Ke, R. Jungmann, T. Schlichthaerle, J. B. Woehrstein, P. Yin, *Science* **2014**, *344*, 65.

[8] L. Wang, J. S. Roth, X. Han, S. D. Evans, *Small* **2015**, *11*, 3306.

[9] a) B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, R. J. Pace, *Nature* **1997**, *387*, 580; b) B. Raguse, V. Braach-Maksvytis, B. A. Cornell, L. G. King, P. D. J. Osman, R. J. Pace, L. Wieczorek, *Langmuir* **1998**, *14*, 648.

[10] Y. H. M. Chan, S. G. Boxer, *Curr. Opin. Chem. Biol.* **2007**, *11*, 581.

[11] M. Kwak, A. Herrmann, *Chem. Soc. Rev.* **2011**, *40*, 5745.

[12] a) F. E. Alemdaroglu, N. C. Alemdaroglu, P. Langguth, A. Herrmann, *Adv. Mater.* **2008**, *20*, 899; b) C. J. Serpell, T. G. W. Edwardson, P. Chidchob, K. M. M. Carneiro, H. F. Sleiman, *J. Am. Chem. Soc.* **2014**, *136*, 15767; c) J. List, M. Weber, F. C. Simmel, *Angew. Chem., Int. Ed.* **2014**, *53*, 4236; d) C. Zhou, D. Wang, Y. Dong, L. Xin, Y. Sun, Z. Yang, D. Liu, *Small* **2015**, *11*, 1161; e) T. R. Wilks, J. Bath, J. W. de Vries, J. E. Raymond, A. Herrmann, A. J. Turberfield, R. K. O'Reilly, *ACS Nano* **2013**, *7*, 8561; f) L. Wang, Y. Feng, Y. Sun, Z. Li, Z. Yang, Y. He, Q. Fan, D. Liu, *Soft Matter* **2011**, *7*, 7187; g) P. Chidchob, T. G. W. Edwardson, C. J. Serpell, H. F. Sleiman, *J. Am. Chem. Soc.* **2016**, *138*, 4416.

[13] Y. Dong, D. Liu, Z. Yang, *Methods* **2014**, *67*, 116.

[14] a) Y. Liu, Y. Liu, J. J. Yin, Z. Nie, *Macromol. Rapid Commun.* **2015**, *8*, 711; b) S. Srivastava, D. Nykypanchuk, M. Fukuto, J. D. Halverson, A. V. Tkachenko, K. G. Yager, O. Gang, *J. Am. Chem. Soc.* **2014**, *136*, 8323; c) J. B. Knudsen, L. Liu, A. L. B. Kodal, M. Madsen, Q. Li, J. Song, J. B. Woehrstein, S. F. J. Wickham, M. T. Strauss, F. Schueder, J. Vinther, A. Krissanaprasit, D. Gudnason, A. A. A. Smith, R. Ogaki, A. N. Zelikin, F. Besenbachor, V. Birkedal, P. Yin, W. M. Shih, R. Jungmann, M. Dong, K. V. Gothelf, *Nat. Nanotechnol.* **2015**, *10*, 892.