Vesicle Self-Assembly Hot Paper

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Frame-Guided Assembly of Vesicles with Programmed Geometry and Dimensions**

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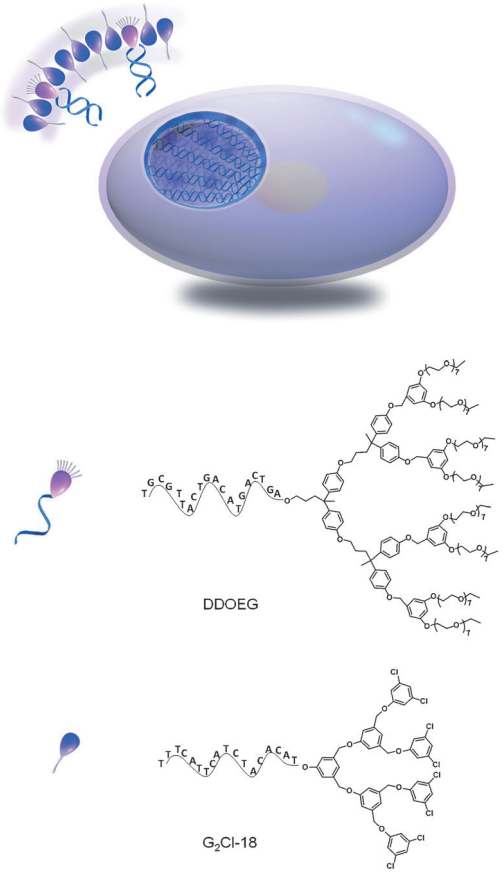
Abstract: *In molecular self-assembly molecules form organized structures or patterns. The control of the self-assembly process is an important and challenging topic. Inspired by the cytoskeletal-membrane protein lipid bilayer system that determines the shape of eukaryotic cells, we developed a frame-guided assembly process as a general strategy to prepare heterovesicles with programmed geometry and dimensions. This method offers greater control over self-assembly which may benefit the understanding of the formation mechanism as well as the functions of the cell membrane.*

Amphiphilic molecules spontaneously assemble into spherical micelles, vesicles, or other symmetric forms in water, with the shape of the assemblies determined by the laws of thermodynamics and their intrinsic molecular properties.[1] Over the past several decades there has been a concerted effort to enhance our understanding of and control over the assembly process.[2] However, obtaining amphiphilic assemblies with customized shapes and sizes still presents a significant challenge in this field.[3] Inspired by the cytoskeletal-membrane protein lipid bilayer system that determines the shape of eukaryotic cells,[4] we developed a frame-guided assembly process as a general strategy to prepare customized heterovesicles. Our method offers greater control over self-assembly which may benefit the understanding of the formation mechanism as well as the functions of the cell membrane.

The principle of frame-guided assembly is illustrated in Scheme 1. Discontinuous, pre-positioned leading hydrophobic groups (LHGs) outline the fringe of the designed structures and guide other amphiphilic molecules to fill in the gaps between LHGs, finally leading to the formation of heterovesicles with designed shape and size. Gold nanoparticles (AuNPs) functionalized with thiolated, 20 nucleotide (20 nt) single-stranded DNA (ssDNA)[5] are used as the foundation of the frame. The LHGs, covalently bound to the complementary 20 nt ssDNA, are directed to the corresponding positions by DNA hybridization. As part of our strategy a given amount of thiolated 6 nt ssDNA was mixed with the 20 nt ssDNA before incubation with the AuNPs; due to



Scheme 1. Illustration of the frame-guided assembly process. The AuNPs are modified with 20 nt and 6 nt ssDNA. DDOEG is anchored to AuNPs by hybridization with DNA. Upon the addition of G_2Cl-18, the LHGs guide G_2Cl-18 to fill in the gaps between LHGs by interactions with the hydrophobic dendron domain.

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electrostatic repulsion the longer ssDNA should be dispersed by the shorter ssDNA. Thus, the LHGs are evenly and discontinuously distributed along the fringe of the frame.[6] Subsequently, other types of amphiphilic molecules are introduced to fill in the gaps between LHGs and ultimately heterovesicles are formed whose shape and size are determined by the frame design.

We considered several factors when selecting LHGs, including molecular structure, size, and hydrophobicity; most importantly, the molecules resulting after connection to DNA must not self-aggregate at the concentration required for hybridization with DNA-modified AuNPs. After systematic optimization we chose the poly(aryl ether) dendron as a model LHG, where one end is covalently bound to the DNA used to anchor the LHG to the AuNPs and the other dendritic end is connected to eight oligo(ethylene glycol) (OEG) tails to suppress self-aggregation.[7] The DNA-Den-OEG complex is referred to as DDOEG, and in our experiments no assemblies of DDOEG in solutions with a concentration of 10 μM is evident by transmission electron microscopy (TEM) and dynamic light scattering (DLS). A second-generation poly(benzyl ether) dendron peripherally functionalized with dichlorophenyl groups and connected to an 18 nt ssDNA (G_2Cl-18) was selected to serve as the amphiphilic molecule to fill in the gaps between LHGs.[8]

In a typical experiment, 13 nm AuNPs (30 nM) were modified with 20 nt and 6 nt ssDNA (details in Table S1 in the Supporting Information) at a ratio of 1:1, and were subsequently incubated with 5 μM DDOEG at 4 °C for 3 h. As shown in Figure 1 a, precipitation was observed and the UV/Vis absorption spectra exhibited a red shift (see Figure S5 in the Supporting Information). Within 10 min of the addition of 5 μM G_2Cl-18 the precipitate disappeared and the solution became clear again as shown in Figure 1 b. Further, the absorption wavelength returned to 518 nm, suggesting that the AuNPs had switched back to the initial monodispersed state.[5] As a control, unmodified AuNPs were incubated with DDOEG under the same conditions with no observed precipitation (Figure 1 c), suggesting that the aggregation is due to the positioning of LHGs by DNA hybridization. Based on these observations we present a possible structure of the sediment seen in Figure 1 a: the interactions between LHGs are amplified by multiple interacting sites[9] which in turn cause aggregation of the frames resulting in precipitation. Here, the frames associate through hydrophobic interactions between LHGs such that the AuNPs between the frames are drawn close enough together to account for the red shift observed in the UV/Vis spectrum. Then, the additional G_2Cl-18 molecules diffuse into the hydrophobic region of the LHGs eliminating the occurrence of multiple interactions among the frames and ultimately resulting in the formation of heterovesicles along the frame (Figure 1 b).

To confirm this mechanism we examined the morphology of the assemblies by transmission electron microscopy (TEM). In Figure 2 a,b there are obvious gaps between the AuNPs in the precipitates that can be attributed to the presence of DNA double helices and LHG aggregates, as illustrated in Figure 1 a. In comparison, aggregates of unmodified AuNPs induced by high salt concentration do not display gaps between particles. This result indicates that the expected frame structures assemble correctly, leading to the observed aggregation. After the addition of G_2Cl-18, only well-dispersed AuNPs were observed; a layer of organic molecules can be seen around the AuNPs where the samples were negatively stained by uranyl acetate (Figure 2 c). These results confirm that an organic vesicle indeed formed as designed. To

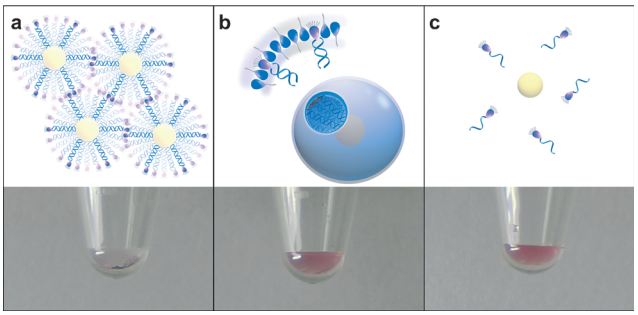

Figure 1. Characterization of the frame-guided assembly process. a) A mixture of 30 nM DNA-functionalized 13 nm AuNPs and 5 μM DDOEG was incubated in buffer (0.5×TBE, 50 mM NaCl) at 4 °C for 3 h and a precipitate formed. b) After removal of the supernatant an amphiphilic molecule, G_2Cl-18, was added to the solution at a final concentration of 5 μM and the precipitate subsequently disappeared. c) Unmodified 13 nm AuNPs were incubated with DDOEG under the same conditions as in (a). The solution remained clear and no precipitate was evident.

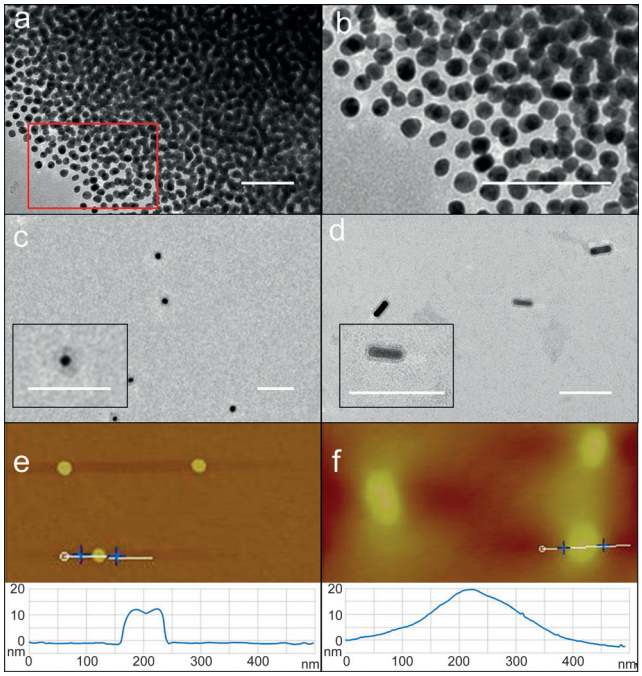

Figure 2. TEM analysis of the assemblies. a) Precipitates (as shown in Figure 1 a), without negative stain. b) Magnification of the area outlined in red in (a). c) Heterovesicles organized by 13 nm spherical frames. d) Heterovesicles organized by rod-shapes frames. e) AFM image of DNA-functionalized 13 nm AuNPs. f) AFM image of the heterovesicles. In (c,d) the samples are negatively stained and the organic molecules appear as coronas around the AuNPs/AuNRs. The scale bars correspond to 100 nm.

further confirm that the organic vesicles are formed from G_2Cl-18 molecules inserting into the gaps between LHGs, 5 nm AuNPs modified with DNA sequences complementary to the DNA domain of the G_2Cl-18 molecules were incubated with the assembled vesicles. These 5 nm AuNPs could specifically attach to the assemblies while unmodified AuNPs do not (see Figure S3 in the Supporting Information). This verifies that G_2Cl-18 molecules are successfully guided by the frame to form the desired heterovesicles. We also employed atomic force microscopy (AFM) to investigate the morphology of the assemblies. As shown in Figure 2e,f, the height of assemblies is considerably greater than that of DNA-modified AuNPs, which further confirms the formation of assemblies.

In order to demonstrate the modularity of the frame-guided assembly process, we changed the components of the frame and studied their assembly by dynamic light scattering (DLS). Firstly, we replaced the 13 nm AuNPs with 30 nm AuNPs and observed similar phenomena, except the size of the assemblies increase accordingly (see Figure S2 in the Supporting Information). Secondly, we changed only the length of double-stranded DNA (dsDNA) in the frame and the results are shown in Figure 3: 1) After the attachment of

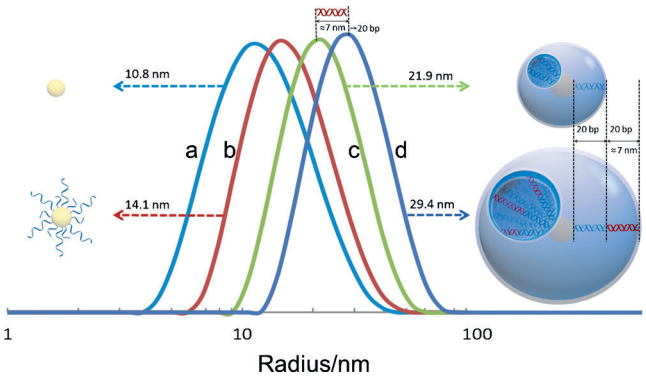

Figure 3. DLS characterization. a) Unmodified 13 nm AuNPs. b) DNA-modified AuNPs with 20 nt ssDNA. c) Heterovesicles with 20 bp dsDNA. d) Extended heterovesicles with 40 bp dsDNA.

20 nt and 6 nt ssDNA to AuNPs at a ratio of 1:1, the radius of the particles increased from 10.8 nm to 14.1 nm. 2) After the formation of heterovesicles, the radius of the assemblies further increased to 21.9 nm. This increase can be attributed to the dsDNA, dendron layer, and the outer ssDNAs in the final assemblies. 3) When the 20 base pair (bp) dsDNA was replaced with a 40 bp dsDNA, the radius increased to 29.4 nm. This 7.5 nm increase matches well with the length of the 20 bp extension.[10] These results demonstrate that the size of vesicles is determined by the frames, with parameters that can be adjusted in the design, and the assemblies are egglike vesicles. According to our strategy, the frame determines not only the size but also the shape of the vesicle. This has been proved by the following experiments: when the AuNPs are changed to gold nanorods (AuNRs), the final assemblies are rod-shaped vesicles (Figure 2d). This observation verifies that the LHG molecules outlining the frame structure determine the final morphology of the heterovesicles, which is analogous to the spatial arrangement of membrane proteins positioned by the cytoskeleton that determines cell shape.[4]

After we had successfully proved our concept, we explored the generality of the method: we performed a series of experiments and found that the density of LHG is variable and the ratio of 20 nt and 6 nt ssDNA can be as low as 1:3, where the calculated coverage of the LHGs is less than 10% based on the relation $A = 4\pi R^2$ (the radius of the AuNPs is about 6.5 nm and the length of the DNA is about 6.8 nm). We also found that this frame-guided assembly method could be applied to different systems to obtain vesicles with programmed geometry and dimensions, for example DNA-PPO/amphiphilic block copolymers and cholesterol/sodium dodecyl sulfate. Collectively, these results validate the adaptability of our method.

In conclusion, we have proposed and demonstrated a general assembly method: the frame-guided assembly, in which LHGs are preanchored to the fringe of frame structures and used to guide amphiphilic molecules to assemble into heterovesicles. This method is modular and can be used to assemble complexes of variable shape and size. We anticipate that owing to the combination of DNA nanotechnology with precisely defined addressable three-dimensional DNA nanostructures utilized as a foundation,[11] our frame-guided assembly method will enable the preparation of monodispersed vesicles, improve the understanding of the fundamental mechanism of self-assembly, and build up more complex and functional assemblies beyond Au scaffolds.

Experimental Section

The AuNPs/AuNRs and the DNA-modified AuNPs/AuNRs were synthesized following the procedure in previous reports.[12] The synthesis of the DDPOG and G_2Cl-18 is based on the solid-phase method developed by our group.[7,8] The detailed description can be found in the Supporting Information. A mixture of 30 nM DNA-AuNPs and 5 μM DDOEG was incubated in 0.5 × TBE and 50 mM NaCl at 4 °C for 3 h with a final volume of 10 μL. After the precipitate had formed, the clear supernatant can be taken out directly. A 10 μL aliquot of 5 μM G_2Cl-18 was dissolved in 0.5 × TBE, and 50 mM NaCl was added to the precipitate for about 10 min. After the mixture had been shaken slightly, it was observed that the precipitate had dissolved. UV/Vis spectroscopy and DLS were applied to characterize the changes in the sample.

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[1] a) M. Antonietti, S. Forster, *Adv. Mater.* **2003**, *15*, 1323 – 1333; b) Y. Mai, A. Eisenberg, *Chem. Soc. Rev.* **2012**, *41*, 5969 – 5985; c) X. Zhang, C. Wang, *Chem. Soc. Rev.* **2011**, *40*, 94 – 101; d) D. Yan, Y. Zhou, J. Hou, *Science* **2004**, *303*, 65 – 67; e) G. M. Whitesides, M. Boncheva, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4769 – 4774.

[2] a) G. Decher, J. D. Hong, J. Schmitt, *Thin Solid Films* **1992**, *210*, 831 – 835; b) F. Caruso, *Adv. Mater.* **2001**, *13*, 11 – 22; c) Z. Yang, W. T. S. Huck, S. M. Clarke, A. R. Tajbakhsh, E. M. Terentjev,

Nat. Mater. **2005**, *4*, 486 – 490; d) T. Chen, M. X. Yang, X. J. Wang, L. H. Tan, H. Y. Chen, *J. Am. Chem. Soc.* **2008**, *130*, 11858 – 11859; e) N. C. Seeman, *Nature* **2003**, *421*, 427 – 431; f) A. V. Pinheiro, D. Han, W. M. Shih, H. Yan, *Nat. Nanotechnol.* **2011**, *6*, 763 – 772.

[3] R. F. Service, *Science* **2005**, *309*, 95.

[4] a) M. S. Bretscher, M. C. Raff, *Nature* **1975**, *258*, 43 – 49; b) V. Bennett, J. Davis, W. E. Fowler, *Nature* **1982**, *299*, 126 – 131; c) T. P. Stossel, J. Condeelis, L. Cooley, J. H. Hartwig, A. Noegel, M. Schleicher, S. S. Shapiro, *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 138 – 145.

[5] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607 – 609.

[6] a) L. M. Demers, C. A. Mirkin, R. C. Mucic, R. A. Reynolds, R. L. Letsinger, R. Elghanian, G. Viswanadham, *Anal. Chem.* **2000**, *72*, 5535 – 5541; b) W. X. Wang, H. J. Liu, D. S. Liu, Y. R. Xu, Y. Yang, D. J. Zhou, *Langmuir* **2007**, *23*, 11956 – 11959.

[7] Y. Sun, H. Liu, L. Xu, L. Wang, Q. H. Fan, D. Liu, *Langmuir* **2010**, *26*, 12496 – 12499.

[8] L. Y. Wang, Y. Feng, Y. W. Sun, Z. B. Li, Z. Q. Yang, Y. M. He, Q. H. Fan, D. S. Liu, *Soft Matter* **2011**, *7*, 7187 – 7190.

[9] a) M. Mammen, S. K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794; b) T. G. Edwardson, K. M. Carneiro, C. K. McLaughlin, C. J. Serpell, H. F. Sleiman, *Nat. Chem.* **2013**, *5*, 868 – 875.

[10] J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737 – 738.

[11] a) M. M. Shih, J. D. Quispe, G. F. Joyce, *Nature* **2004**, *427*, 618 – 621; b) Y. Li, Y. D. Tseng, S. Y. Kwon, L. D'Espaux, J. S. Bunch, P. L. McEuen, D. Luo, *Nat. Mater.* **2004**, *3*, 38 – 42; c) T. Zhou, P. Chen, L. Niu, J. Jin, D. Liang, Z. Li, Z. Yang, D. Liu, *Angew. Chem.* **2012**, *124*, 11433 – 11436; *Angew. Chem. Int. Ed.* **2012**, *51*, 11271 – 11274; d) Y. He, T. Ye, M. Su, C. Zhang, A. E. Ribbe, W. Jiang, C. Mao, *Nature* **2008**, *452*, 198 – 201; e) E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf, J. Kjems, *Nature* **2009**, *459*, 73 – 75; f) H. J. Liu, T. Torring, M. D. Dong, C. B. Rosen, F. Besenbacher, K. V. Gothelf, *J. Am. Chem. Soc.* **2010**, *132*, 18054 – 18056; g) C. K. McLaughlin, G. D. Hamblin, K. D. Hanni, J. W. Conway, M. K. Nayak, K. M. M. Carneiro, H. S. Bazzi, H. F. Sleiman, *J. Am. Chem. Soc.* **2012**, *134*, 4280 – 4286.

[12] a) T. Zhang, Y. Dong, Y. Sun, P. Chen, Y. Yang, C. Zhou, L. Xu, Z. Yang, D. Liu, *Langmuir* **2012**, *28*, 1966 – 1970; b) Z. Chen, X. Lan, Q. Wang, *Small* **2013**, *9*, 3567 – 3571.

