Vesicle Formation Hot Paper

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Thermally Triggered Frame-Guided Assembly**

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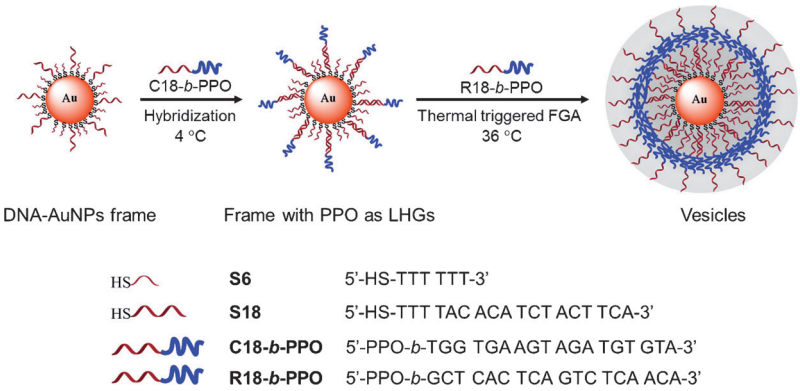
Abstract: *We report a thermally triggered frame-guided assembly (FGA) strategy for the preparation of vesicles. We employ thermally responsive poly(propylene oxide) (PPO) to make the leading hydrophobic groups (LHGs) thermally responsive, so that they are hydrophilic below the low critical solution temperature (LCST) and the frame forms in a homogeneous environment. When the temperature is increased above the LCST, the LHGs become hydrophobic and the assembly process is triggered, which drives DNA-b-PPO to assemble around the LHGs, forming vesicles. This work verified that FGA is a general strategy and can be applied to polymeric systems. The thermally triggered assembly not only provides more controllability over the FGA process but also promotes an in-depth understanding of the FGA strategy and in a broad view, the formation mechanism and functions of cell membrane.*

Recently, a frame-guided assembly (FGA) strategy has been reported[1] and successfully applied to a macromolecular system to realize a structure analogous to the skeleton-membrane protein–lipid bilayers.[2] It is believed that frame-guided assembly would provide a new platform to control self-assembly and even further understand the formation mechanism of cell membranes.[3] To provide more controllability over the frame-guided assembly process, herein we report a thermally triggered FGA strategy: thermally responsive leading hydrophobic groups (LHGs) are employed and anchored on the frame, therefore the assembly process can be triggered through controlling the temperature. The thermally triggered FGA strategy enriches the regulation methods of self-assembly and may benefit the construction of functional and responsive nanostructures.

The thermally triggered FGA strategy is illustrated in Scheme 1. We utilize the thermally responsive poly(propylene oxide) (PPO)[4] as the leading hydrophobic groups. We first modify gold nanoparticles (AuNPs) with short single-stranded DNA (ssDNA) to form the DNA–AuNPs frame.[5] By hybridizing with DNA-*b*-PPO below the low critical solution temperature (LCST), a frame with PPO as the leading hydrophobic groups is formed. PPO is hydrophilic below the LCST and anchored onto the frame in a homogeneous environment, thus avoiding the aggregation of the frame during its formation. As the temperature increases above the LCST, PPO becomes hydrophobic and in situ DNA-*b*-PPO assembles around the frame through hydrophobic interactions, leading to the formation of vesicles.



Scheme 1. The thermally triggered frame-guided assembly (FGA) strategy.

In a typical experiment, 15 nm AuNPs (7.75 nM, 100 μL) were modified with 10 μM, 12 μL thiolated 6-nt ssDNA (S6; nt = nucleotide) and 10 μM, 12 μL thiolated 18-nt ssDNA (S18). The mixed solutions were incubated in 0.5 × TBE (pH 8.0) and 100 mM NaCl at room temperature for 42 h, followed by centrifugation at 14000 rpm for 30 min to remove residual thiolated DNA. PPO with a molecular weight of 2000 g mol^{-1} was used. It is soluble in cold dilute aqueous solution and becomes insoluble above the LCST, 23 °C.[4] The synthesis of DNA-*b*-PPO was based on the established solid-phase method.[6] The characterization can be found in the Supporting Information (Figure S1 and S2). 30 nM DNA modified AuNPs and 10 μM C18-*b*-PPO with a final volume of 20 μL were incubated in 0.5 × TBE (pH 8.0) and 100 mM NaCl overnight at 4 °C, at which temperature PPO is hydrophilic and promotes the complete DNA hybridization, as a result, a frame with PPO as the leading hydrophobic groups is

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formed. The C18-*b*-PPO not attached to AuNPs was removed by centrifugation at 4 °C. Then 40 μL random DNA sequence block PPO hybrids R18-*b*-PPO at a concentration of 10 μM was added at 4 °C for 1 h, followed by incubation at elevated temperature of 36 °C for 30 min, at which temperature the PPO is hydrophobic and plays a role as the leading hydrophobic groups, consequently guiding the excess R18-*b*-PPO in the aqueous phase to assemble through hydrophobic interactions, finally hetero-vesicles are formed.

First, we studied the morphology of the vesicles by transmission electron microscopy (TEM). It was observed that the system contained both vesicles and micelles formed from R18-*b*-PPO in Figure 1 A. To obtain pure vesicles, we

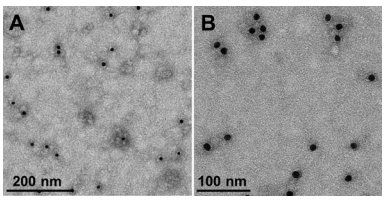

Figure 1. The TEM results. A) The coexistence of vesicles and R18-*b*-PPO micelles. B) The pure vesicles after centrifugation to remove the R18-*b*-PPO micelles.

tried different approaches and found that, by simply centrifuging the mixed system at 14000 rpm for 30 min and subsequently discarding the supernatant containing R18-*b*-PPO spherical micelles, the pure vesicles were obtained (Figure 1 B). These results confirm that thermally triggered FGA indeed induces the formation of vesicles as planned, and the micelles formed from excessive R18-*b*-PPO can be removed by centrifugation.

To further confirm that the vesicles are formed from R18-*b*-PPO, which is thermally triggered to assemble at the periphery of the frame, 5 nm AuNPs (400 nM, 5 μL) modified with DNA sequences complementary to the DNA domain of the R18-*b*-PPO were incubated with the thermally triggered vesicles (10 nM, 20 μL). As shown in Figure 2 A,B, the 5 nm

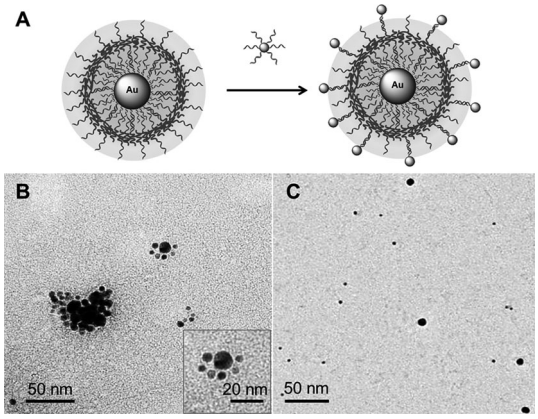

Figure 2. A) Schematic illustration of the hybridization of vesicles with DNA modified 5 nm AuNPs. TEM images of the hybridization of vesicles with 5 nm AuNPs that are modified by B) complementary ssDNA, and C) non-complementary ssDNA, respectively. Inset: enlarged image.

AuNPs were attached to the vesicles along the edge. A control experiment showed that 5 nm AuNPs modified with non-complementary ssDNA did not participate in specific interactions with the vesicles (Figure 2 C) and are just randomly dispersed. These results demonstrate that R18-*b*-PPO is guided and assembled around the frame by the thermally triggered FGA strategy and can hybridize with DNA modified AuNPs, which potentially leads to further functionalization.

Dynamic light scattering (DLS) was used to investigate the size change during the thermally triggered FGA process. As shown in Figure 3, the size of naked AuNPs was 15.69 nm,

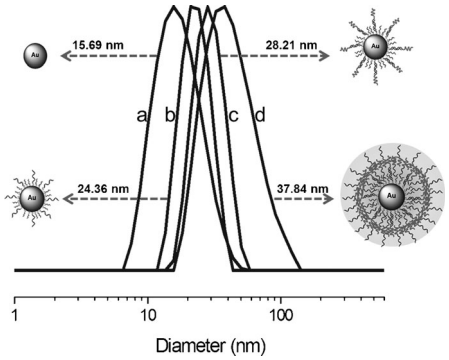

Figure 3. The DLS results. a) Naked 15 nm AuNPs. b) AuNPs modified with S6 and S18. c) The frame with PPO as LHGs. d) The vesicles formed through the thermally triggered FGA strategy.

which is consistent with the result of TEM investigations (see Supporting Information Figure S3). The diameter of the AuNPs modified with S6 and S18 was 24.36 nm, about 8.67 nm larger than the naked AuNPs. This increase is mainly due to the anchoring of S6 and S18. After C18-*b*-PPO anchoring to the DNA-AuNPs frame by hybridization, the size of the frame at 4 °C was about 28.21 nm, which nearly equals to the size of the 15 nm AuNPs plus twice of the size of 18 bp DNA[7] and the expanded flexible PPO segment. Finally, after thermally triggered FGA process by elevating temperature to 36 °C for 30 min and cooling to room temperature overnight, the diameter increased from 28.21 nm to 37.84 nm. The reason for this 9.6 nm increase is that R18-*b*-PPO was induced to assemble at the periphery of the frame by hydrophobic interaction with the PPO domains. These size changes can be well explained and are in a good agreement with our design. Interestingly, when the excess of C18-*b*-PPO was not removed before the in situ assembly by the thermally triggered FGA process, hetero-vesicles can also be obtained. The size change is similar to the R18-*b*-PPO system; the frame with PPO as LHGs is 28.32 nm, the vesicles 38.12 nm.

We also studied the stability of the vesicles, the solution was kept at 4 °C for one day, one week, and two weeks, respectively (see Figure S4). There was little change in size. This phenomenon suggests that the assembled vesicles once formed will stay stable. The stability of the assemblies is probably attributed to entanglement among the PPO chains, which hold the assembled structures together after formation.

Furthermore, to illustrate the size adjustability, we replaced the 15 nm AuNPs with 30 nm AuNPs and observed

a similar trend of size increase by DLS (see Figure S5). Hence, these results validate the adaptability of our method to efficiently control the size of the assemblies.

We studied the optical properties of the AuNPs. It is established that surface plasmon resonance energies decrease as the refractive index of the surrounding medium increases. In buffer solution (pH 8.0, 100 mM NaCl, 0.5 × TBE, $n = 1.33$), naked 15 nm AuNPs showed a characteristic absorbance maximum $\lambda_{max} = 520$ nm (Figure 4, dotted line). Similarly, the

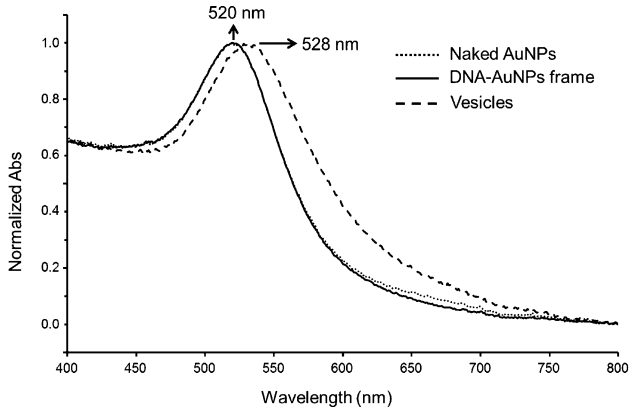

Figure 4. The UV spectra of naked 15 nm AuNPs, DNA-AuNPs frame, and vesicles.

plasma absorption band of DNA-AuNPs frame was also at 520 nm (Figure 4, solid line). However, the higher refractive index of PPO ($n = 1.46$) led to red-shifted absorbance spectra for assembled vesicles ($\lambda_{max} = 528$ nm, Figure 4, broken line). Similar plasmon shifts have been reported for AuNPs within PMMA shells, in which the higher refractive index of PMMA ($n = 1.49$) resulted in higher values of $\lambda_{max} = 540$ nm.[8] These results indicate that there was a DNA-*b*-PPO shell at the periphery of AuNPs, confirming the success of the thermally triggered FGA process.

In conclusion, we have reported a thermally triggered frame-guided assembly (FGA) system: thermally responsive PPO is incorporated onto the frame as the leading hydrophobic groups (LHGs). By changing the temperature, DNA-*b*-PPO assembles around the frame and forms vesicles in situ. This result not only provides further information to understand the principle of the FGA strategy but also offers the chance to manipulate the FGA process by a thermally trigger. It can be anticipated that the development of FGA would play more important roles in molecular self-assembly[9] and enable the construction of more complex and functional nanostructures,[10] such as employing the DNA-AuNPs frame as drug carriers,[11] moreover, by rational design of the DNA sequences, multiple components and functional groups can potentially be introduced to the frame.[12]

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