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Probing the Self-assembly Process of Amphiphilic Tetrahedral DNA Frameworks

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Experiment Procedures

Materials and Reagents. Oligonucleotides used in this work were custom-synthesized, labeled and purified by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of these oligonucleotides are listed in Table S1. All the chemicals needed for the reactions were purchased from commercial sources and used as received without further purification. 10×TAE (Tris-acetate-EDTA) were purchased from Sangon Biotechnology Inc. GelRed nucleic acid stain (10000× in DMSO) was purchased from Invitrogen (Grand Island, NY, USA). All other reagents used in this work were of analytical grade and directly used without further purification. All the other chemicals, including PBS buffers, were obtained from Sigma Aldrich.

Instrumentation. Ultraviolet visible (UV-vis) absorption spectra of the samples were analyzed by UV-vis spectroscopy (Perkin-Elmer, Lamda 850). The fluorescence emission spectra were recorded by a microplate reader (BioTek Instrument, Winooski, VT, USA) using a black 384-well microplate (Fluotrac 200, Greiner, Germany). The gels were scanned with a BioRad Molecular imager. MALDI-MS analysis were performed using MALDI-imaging mass spectrometry (MALDI TOFTOF 7090, Shimadzu, Germany). The thermal hysteresis data were recorded by a Circular dichroic spectrometer (Jasco-1500, Japan). TEM image was captured under a transmission electron microscope (Talo L120C G2, USA).

1. Preparation of am-TDF. To prepare am-TDF, four DNA single strands (S1, S2, S3, S4, with S1, S2, and S3 modified with Chol, 57 bp in length, Table S1) were mixed in an equimolar ratio in PBS buffer (10 mM PBS, 2 mM Mg^{2+}, pH 8.0). The solution was heated to 95 °C for 10 min and then quickly cooled to 4 °C, and kept at 4 °C for at least 2 h. The number of Chol on TDF can be quantitatively controlled by changing relative number ratio of ssDNA-Chol to other ssDNAs. For one Chol labelled TDF (TDF-Chol1), two Chols labelled TDF (TDF-Chol2), and three Chols labelled TDF (TDF-Chol3), one, two and three Chols were labelled at the left vertices on the same plane, respectively.

2. Determination of the CMC of the Chol-modified ssDNA. Determination of the CMC of the Chol-modified ssDNA was performed following a previously reported protocol[1]. A stock solution of Nile Red 0.1 mM in acetone was used for all experiments. Series dilutions of DNA sample (in the range 10 μM to 10 pM) were made up to a final volume of 150 μL, with a concentration of 2.5 μM Nile Red in 1xTAMg in a 96-well top-read microplate. The samples were left to incubate at room temperature for 4 h. The plate was read using a Biotek Synergy well-plate fluorimeter. Excitation was at 535 nm, with a slit width of 9 nm and emission was monitored between 560 nm and 750 nm.

3. Polyacrylamide gel electrophoresis (PAGE) analysis of am-TDFs. Gels of 20% PAGE was used for the characterization of ssDNA and am-TDFs. Gels of 8% PAGE was used for the characterization of the prepared am-TDFs. A 5 μL sample was loaded in the gel and electrophoresis separation was performed in electrophoretic buffer 1xTAE (containing 12.5 mM Mg^{2+}). Electrophoresis separation was performed with a Bio-Rad electrophoresis system at 100 V for 2 h. After that, the gel was stained with 1× GelRed solution for 10 min and washed with H2O, and then gels were imaged with a BioRad Molecular imager.

4. Atomic force microscope (AFM) characterization. AFM was used to image the am-TDF. First, freshly cleaved mica surface was dropped with 5‰ APTES for 30 sec, then washed off by Milli-Q water and dried by nitrogen. Second, Samples were diluted to 5 nM using TM buffer (20 mM Tris, 10 mM Mg^{2+}, pH 8.0). 10 μL samples (5 nM) were incubated on treated mica for 5 min and additional TM buffer was added to a total volume of 50 μL. Third, a multimode 8 (Bruker) operated in peak force mode was employed to scan the sample.

5. Transmission electron microscopy (TEM) characterization. 10 μL of DNA samples were adsorbed onto glow discharged, carbon-film-coated copper grids for 3 min. The sample drop was wicked from the copper grids with a filter paper

(making sure the copper grids were not dried completely) and then the sample were stained for 40 seconds using a 0.75% aqueous uranyl formate solution. After this, the excess solution was removed with a filter paper and the copper grids were allowed to dry at 25 °C. Imaging was performed using a Talos L120c G2 operated at 120 kV.

6. Thermal hysteresis (TH) profiles of am-TDF. The TH experiment was performed following a previously reported protocol with minor revision[2].The first step of the TH method is using different temperature scan rates to measure sets of thermal melting and annealing spectrophotometric profiles. Samples contained 1 μM Chol-S1, Chol-S2, Chol-S3, S4 in 1xPBS buffer. The absorbance signals for annealing and melting were monitored at 260 nm over the 0 °C to 95 °C temperature range at different scan rates (0.2, 0.5, 1, 2, 3, and 4°C min^{-1}). Samples were maintained at 95°C and 4°C for 10 min before annealing and melting, respectively. A rubber seal was applied on top of the sample solution to minimize evaporation. A temperature probe is inserted into the solution through the rubber seal to directly measure the actual temperature of the solution. Gently stirring was supplied to the sample chamber to prevent condensation on the cuvette.

7. Model-free analysis of TH profiles. The second step of TH method is using each trace to estimate the fraction of subunits, which are dissociated monomers (θ_U), as a function of temperature and scan rate. Because the instrument directly measured the actual temperature of the solution, temperature correction was not required. Briefly, the fraction unfolded (θ_U) were converted from TH profiles according to Equation 1.

$$\theta_U(T) = \frac{A(T) - A_F(T)}{A_U(T) - A_F(T)} \quad (1)$$

In Equation 1, A(T) refers to the absorbance intensity of real-time. $A_F(T)$ refers to the absorbance intensity when all ssDNAs are assembled into products at low temperature. $A_U(T)$ refers to the absorbance intensity when samples are in the form of ssDNA at high temperature. At low and high temperatures $\theta_U(T)$ takes limiting values of 0 and 1, respectively, corresponding to the completely assembled or completely monomeric states.

The total concentration of ssDNA in the experimental cuvette (C_T) is related to the concentrations of the monomeric [M] and assembled [F] states at each temperature by Equation 2.

$$C_T = [M](T) + N[F](T) \quad (2)$$

In Equation 2, N accounts for the number of monomers that reside within a folded assembly. The concentration of free monomers at each scan rate were calculated from the fraction unfolded assuming

$$[M](T) = \theta_U(T)C_T \quad (3)$$

The slopes of the monomer concentration with respect to temperature d/dT[M](T) were calculated numerically using rolling window regression. The rates of change of the monomer concentration were then obtained from the slopes and the scan rate:

$$\frac{d}{dt}[M](T) = \frac{dT}{dt}\frac{d}{dT}[M](T) \quad (4)$$

The sets of scan rate dependent d/dT[M](T) and [M](T) from the annealing and melting portions of the experiment provide access to the temperature/reaction rate supramolecular assembly maps. The surfaces are sliced with respect to temperature and a log-log analysis is performed according to Equations 5-8. The intercepts correspond to effective rate constants for assembly and disassembly.

The third step is calculating the release or consumption rate of monomer (d[M]/dt) according to Equation 5.

$$\frac{d[M]}{dt} = C_T \frac{d\theta_U dT}{dT\ dt} \quad (5)$$

In Equation 5, $(d\theta_U/dT)$ and (dT/dt) refer to the slopes of the curves and the temperature scan rate, respectively. The last step is calculating the effective reaction orders according to Equation 6.

$$\frac{d[M]}{dt} = -k_{on}[M]^n + k_{off}N\left(\frac{C_T - [M]}{N}\right)^m \quad (6)$$

In Equation 6, n and m refer to effective reaction orders of the assembly and disassembly process, respectively. k_{on} and k_{off} are reaction rate constants of the assembly and disassembly, respectively.

To simplify Equation 6, the degree of hysteresis can be enhanced by reducing ionic strength, improving scan rates, or decreasing the total concentration of subunits[2,3].

When the degree of hysteresis is large, the first term dominates during the annealing scan and Equation 6 can be simplified to Equation 7. Therefore, a plot of $\log(-d[M]/dt)$ vs. $\log([M])$ is linear with a slope of n and y-intercept of $\log(k_{on})$.

$$\frac{d[M]}{dt} \approx -k_{on}[M]^n \quad (7)$$

When the degree of hysteresis is large, the second term dominates during the melting scan and Equation 6 can be simplified to Equation 8. Therefore, a plot of $\log(d[M]/dt)$ vs. $\log(C_T - [M])$ is linear with a slope of m and y-intercept of $(1 - m)\log(N) + \log(k_{off})$.

$$\frac{d[M]}{dt} \approx k_{off}N\left(\frac{C_T - [M]}{N}\right)^m \quad (8)$$

Table S1. **Sequence information for oligonucleotides used in this study**

Name	Sequence (5′-3′)
S1	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACTT
S1-C_{36}	C_{36}-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACTT
S1-Chol	Chol-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATACTT
S2	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTT
S2-C_{36}	C_{36}-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTT
S2-Chol	Chol-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTCTT
S3	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTT
S3-C_{36}	C_{36}-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTT
S3-Chol	Chol-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCATTT
S4	TTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
S4-C_{36}	C_{36}-TTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
S4-Chol	Chol-TTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA

Supporting Figures

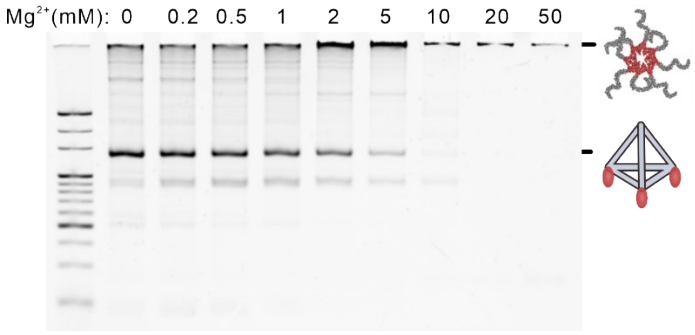

Fig. S1. PAGE characterization of the effect of Mg^{2+} concentration on the yield of am-TDF.

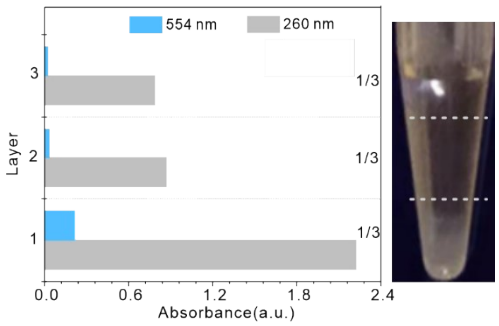

Fig. S2. Absorbance of DNA at different layers of 100 μL reaction system. Inset: represented bright field image of the sample. Stained by Nile red. The solution was diluted for 3 times before the measurement.

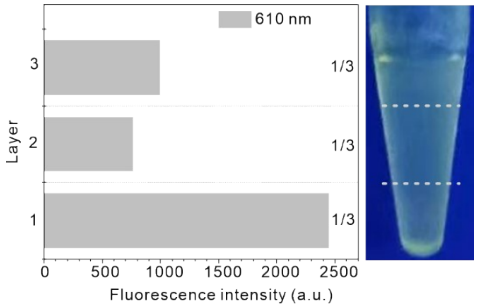

Fig. S3. Fluorescence intensity of Nile red stained micelles at different layers of 100 μL reaction system. Inset: represented bright field image of the sample. Excited at 530 nm. The solution was diluted for 3 times before the measurement.

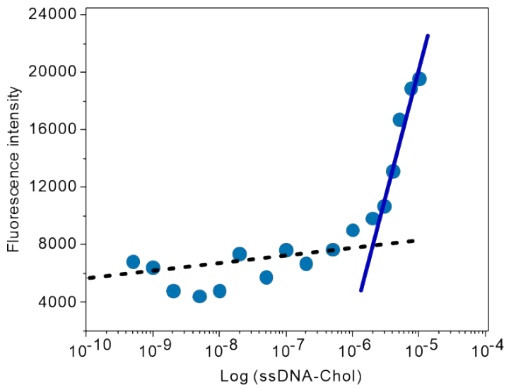

Fig. S4. Measurement of CMC value of Chol-labeled single-stranded DNA in PBS solution.

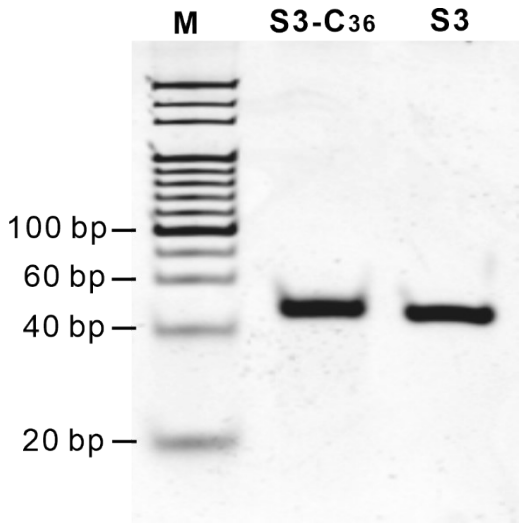

Fig. S5 PAGE analysis of single-stranded DNA and C_{36}-labelled single-stranded DNA.

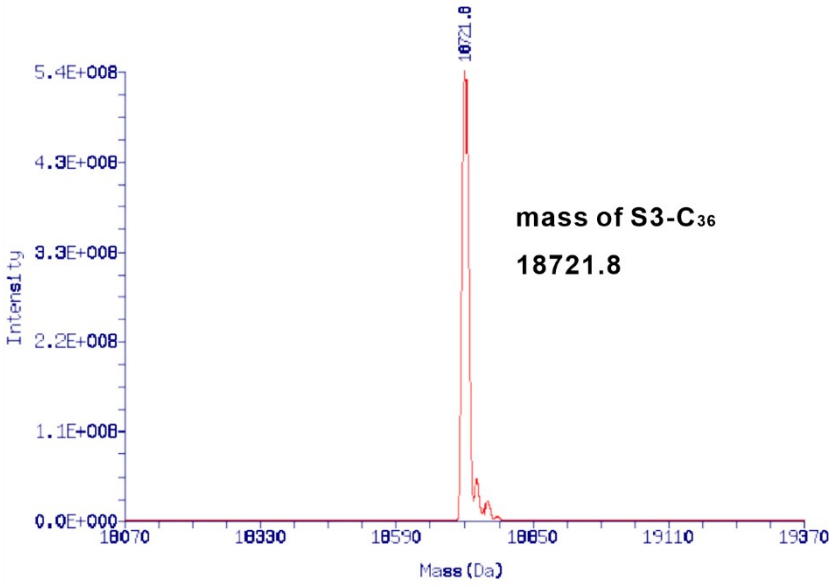

Fig. S6 Mass spectra of C_{36}-labeled single-stranded DNA.

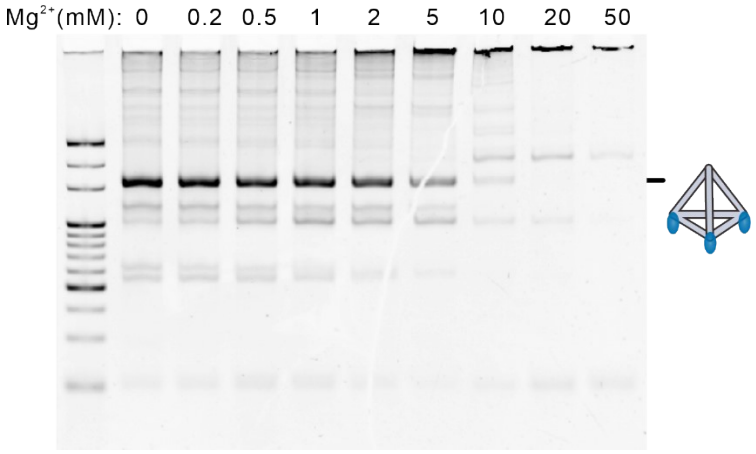

Fig. S7. PAGE characterization of the effect of Mg^{2+} concentration on the yield of TDF-C_{36}.

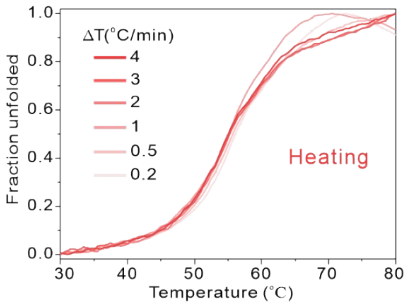

Fig. S8. Multi-scan rate fraction unfolded TH profiles for TDF-Chol3 disassembly as a function of temperature scan rate.

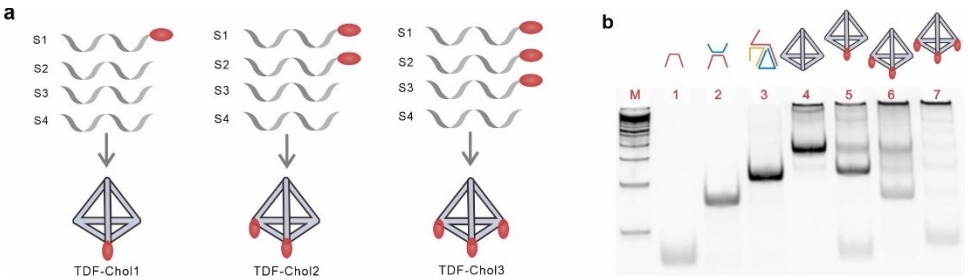

Fig. S9. Assembly of am-TDF structures modified with different valence of Chols (termed TDF-Chol0, TDF-Chol1, TDF-Chol2, and TDF-Chol3, respectively). a) Schematic illustration of the assembly of TDF-Chol probes with different number of Chol on the vertices. B) PAGE assay of different DNA samples. From lanes 1 to 8: S1; S1+S2; S1+S2+S3; S1+S2+S3+S4; S1-Chol+S2+S3+S4; S1-Chol+S2-Chol+S3+S4; S1-Chol+S2-Chol+S3-Chol+S4.

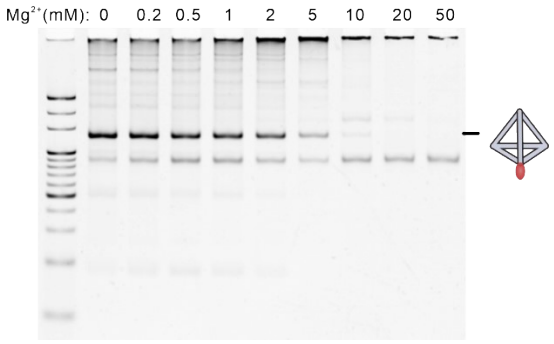

Fig. S10. PAGE characterization of the effect of Mg^{2+} concentration on the yield of TDF-Chol1.

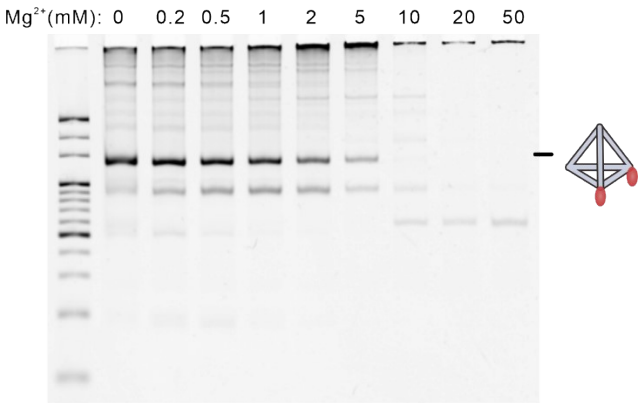

Fig. S11. PAGE characterization of the effect of Mg^{2+} concentration on the yield of TDF-Chol2.

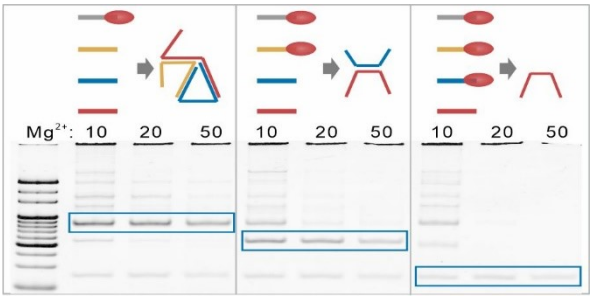

Fig. S12. PAGE assay of am-TDF with different valence of Chol at high Mg^{2+} concentration (10 mM).

Reference:

1. T. G. Edwardson, K. M. Carneiro, C. K. McLaughlin, C. J. Serpell and H. F. Sleiman, *Nat. Chem.*, 2013, **5**, 868-875.
2. V. R. Harkness, N. Avakyan, H. F. Sleiman and A. K. Mittermaier, *Nat. commun.*, 2018, **9**, 3152.
3. J. L. Mergny and L. Lacroix, *Oligonucleotides*, 2003, 13, 515-537.