Self-Assembly of Responsive Multi-Layered DNA Nanocages

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Supporting information

Materials and methods

Oligonucleotides

DNA sequences were adapted from previous works, which were originally designed by the SEQUIN computer program [(Seeman, N. C. De novo design of sequences for nucleic-acid structural engineering. *J. Biomol. Struct. Dyn.* **8**, 573-581 (1990)]. All oligonucleotides were purchased from IDT and purified by denaturing PAGE.

Strands: (the underlined sequences are the tails for inter-layer linkages)

Y-L: 5'- AGG CAC CAT CGT AGG TTT AAC TTG CCA GGC ACC ATC GTA GGT TTA ACT TGC CAG GCA CCA TCG TAG GTT TAA CTT GCC -3'

Y-M^1: 5'- AGC AAC CTG CCT GGC AAG CCT ACG ATG GAC ACG GTA ACG ACT -3'

Y-M^2: 5'- TAG CAA CCT GCC TGG CAA GCC TAC GAT GGA CAC GGT AAC GCC -3'

Y-S^1: 5'- ACC GTG TGG TTG CTA GTC GTT <u>TTC CTC AAG A</u> -3'

Y-S^2: 5'- TTA CCG TGT GGT TGC TAG TTA -3'

Y-S^3: 5'- CCG TGT GGT TGC TAG TTA -3'

Y-S^{Atp}: 5'- ACC GTG TGG TTG CTA GTC GTT <u>TTC AGG TTC TC</u> -3'

DX-L: 5'- TAG CAA CCT GCC TGG CAA GCC TAC GAT GGA CAC GGT AAT AAC -3'

DX-M: 5'- GCC AGG CAC CAT CGT AGG CTT <u>TTT CTT GAG G</u> -3'

DX-M^{Atp}: 5'- GCC AGG CAC CAT CGT AGG CTT <u>TTG AGA ACC TGG GGG AGT ATT GCG GAG GAA GGT</u> -3'

DX-S^1: 5'- TTA CCG TGT GGT TGC TAG GCG -3'

DX-S^2: 5'- TTA CCG TGT GGT TGC TAG GCG TTA <u>TTT TGT TCG TAT CG</u>-3'

eDX-L: 5'- TAG CAA CCT GCC TGG CAA GCC TAC GAT GGA CGA ACA GCT CAG ATA GCG CCT GCC TGG CAA GCC TAC GAT GGA CAC GGT AAT AAC-3'

eDX-M: 5'- GCC AGG CAC CAT CGT AGG CTT GCC AGG CAC CAT CGT AGG CTT

TTT TCG ATA CGA AC-3'
eDX-M': 5'- TCT GAG CTG TTC GTG GCG CTA TCT GAG CTG TTC GTG GCG CTA -3'

Motifs:
Y^1: Y-L + Y-M^1 + Y-S^1 (1 : 3 : 3)
Y^{1Atp}: Y-L + Y-M^1 + Y-S^{Atp} (1 : 3 : 3)
Y^2: Y-L + Y-M^2 + Y-S^2 (1 : 3 : 3)
DX^2: DX-L + DX-M + DX-S^1 (1 : 2 : 2)
DX^{2Atp}: DX-L + DX-M^{Atp} + DX-S^1 (1 : 2 : 2)
Y^3: Y-L + Y-M^2 + Y-S^2 (1 : 3 : 3)
eDX^2: eDX-L + eDX-M + eDX-M' + DX-S^1 (2 : 2 : 1 : 2)

Nanocages:
TET^1: Y^1
TET^{1Atp}: Y^{1Atp} (for assembly of ATP-responsive double-layered TET)
TET^2: Y2 + DX^2 (4:6)
dTET: Y^1 + Y^2 + DX^2 (4 : 4 : 6)
tTET: Y^1 + Y^2 + Y^3 + DX^2 + eDX^3 (4 : 4 : 4 : 6 : 6)
$dTET^{Atp}$: Y^{1Atp} + Y^2 + DX^{2Atp} (4 : 4 : 6) (ATP-responsive double-layered TET)

Assembly of multi-layered DNA tetrahedron

Step-wised method was used for the assembly of multi-layered DNA tetrahedron. Take double-layered tetrahedron (dTET) for example, the overall assembly process consisted of three main steps. Step 1: individually assemble TET^1 (100 nM in terms of motif concentration) as well as all other DNA motifs [DX^2 (500 nM) and Y^2 (500 nM)] via slow cooling from 95 °C to 4 °C over 16 hours in 1×TAE/Mg^{2+} buffer (40 mM Tris base, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate). Step 2: incubate the products of the former step at 37 °C for one hour, followed by adding DX^2 and then Y^2 motifs sequentially (one type of motif at a time with 30 min interval) to the solution of TET^1. Step 3: the resulted mixture (with concentration around 15 nM in terms of dTET particles) in step 2 was further incubated at 37 °C for another hour, then slowly cooled down to room temperature over 12 hours to assemble double-layered tetrahedron. For the assembly of triple-layered tetrahedron (tTET), pre-assembled dTET (15 nM), Y^3 (500 nM) and eDX^3 (500nM) motifs were separately incubated 37 °C for one hour. Y^3 (500 nM) and eDX^3 (500nM) motifs were then sequentially added to the solution of dTET. The resulted mixture (with concentration around 10 nM in terms of tTET particles) was further incubated at 37 °C for another hour, then slowly cooled down to room temperature over 12 hours to assemble triple-layered tetrahedron.

ATP-activated layer-separation

The ATP-responsive dTET ($dTET^{Atp}$) was first assembled following the step-wised method. Step 1: individually assemble TET^{1Atp} (100 nM in terms of motif concentration) as well as all other DNA motifs [DX^{2Atp} (500 nM) and Y^2 (500 nM)] via slow cooling from 95 °C to 4 °C over 16 hours in 1×TAE/Mg^{2+} buffer (40 mM Tris base, 20 mM acetic acid, 2 mM EDTA

and 12.5 mM magnesium acetate). Step 2: incubate the products of the former step at 37 °C for one hour, followed by adding DX^{2Atp} and then Y^2 motifs sequentially (one type of motif at a time with 30 min interval) to the solution of TET^{1Atp}. Step 3: the resulted mixture (with concentration around 15 nM in terms of $dTET^{Atp}$ particles) in step 2 was further incubated at 37 °C for another hour, then slowly cooled down to room temperature over 12 hours to assemble double-layered tetrahedron. 3 μl ATP solution (100 mM) was then added to the DNA sample (300 μl, 15 nM) at room temperature, making the ATP concentration of the mixture to be 1 mM. The resulted solution was incubated under room temperature over 12 hours before further analysis with native PAGE.

Native polyacrylamide gel electrophoresis (PAGE)

Native PAGE containing 2.5 % polyacrylamide (19:1acrylamide/bisacrylamide) was run on a FB-VE10-1 electrophoresis unit (FisherBiotech) at 4 °C (90 V, constant voltage). The running buffer and the buffer in the gel were TAE/Mg^{2+} buffer (40 mM Tris base, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate). After electrophoresis, the gels were stained with Stains-All (Sigma) and scanned with a HP Scanjet 4070 Photosmart scanner. The yields were estimated based on the relative intensities of the bands via an ImageJ [Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 671-675 (2012)].

Atomic force microscopy (AFM) imaging

5 μL of DNA sample was deposited onto freshly cleaved mica for 2 min, washed with 30 μL of water and then dried with compressed air. A MultiMode 8 AFM (Bruker) was used to image the samples under ScanAsyst-Air mode, using a ScanAsyst-air probe (Bruker).

Cryogenic electron microcopy (CryoEM) imaging

DNA samples were concentrated to ~ 1 μM of particles with Micron-100 (100 kDa) centrifugal filters. 3 μL of sample were spread on a Quantifoil grid (Electron Microscopy Sciences, Q250-CR2-400) for 30 s at room temperature (the grid was glow discharged on negative mode for 14 s before loading the sample), blotted with the filter paper (blotting time was 1.1 s, humidity was 100 %), and flash frozen in the ethane flurry (cooled by liquid nitrogen). The images were taken on FEI CM200 TEM with accelerating voltage of 200 kV under low-dose condition to minimize radiation damages to the samples. Images were recorded by films with under-focus in the range of 2-4 μm. The calibrated magnification used was ×51,040, resulting in a pixel size of 3.732 Å.

Single particle reconstruction

The single-particle reconstruction was carried out using "EMAN2" software [Tang, G., Peng, L., Baldwin, P. R., Mann, D. S., Jiang, W., Rees, I. & Ludtke, S. J., EMAN2: an extensible image processing suite for electron microscopy, J. Struct. Biol. 157, 38-46 (2007)]. In total, 454 particles were used for single-particle reconstruction. The tetrahedral symmetry was imposed during the initial model building and reconstruction. The refinement was carried out with a 2 degree angel interval. A projection matching algorithm was applied for the determination of the center and orientation of raw particles in the interactive refinements. The

resolution of the density map was determined by using Fourier shell correlation (0.5 threshold criterion) of two 3D map separately built from even and odd halves of the datasets. The final 3D map was visualized using "UCSF Chimera" software [Goddard, T. D., Huang, C. C. & Ferrin, T. E. Visualizing density maps with UCSF chimera. J. Struct. Biol. 157, 281-287 (2007).]

Figure S1. A double-layered DNA tetrahedron (dTET): an enlarged view of its raw cryoEM image, which is the same as Figure 3a in the main text.

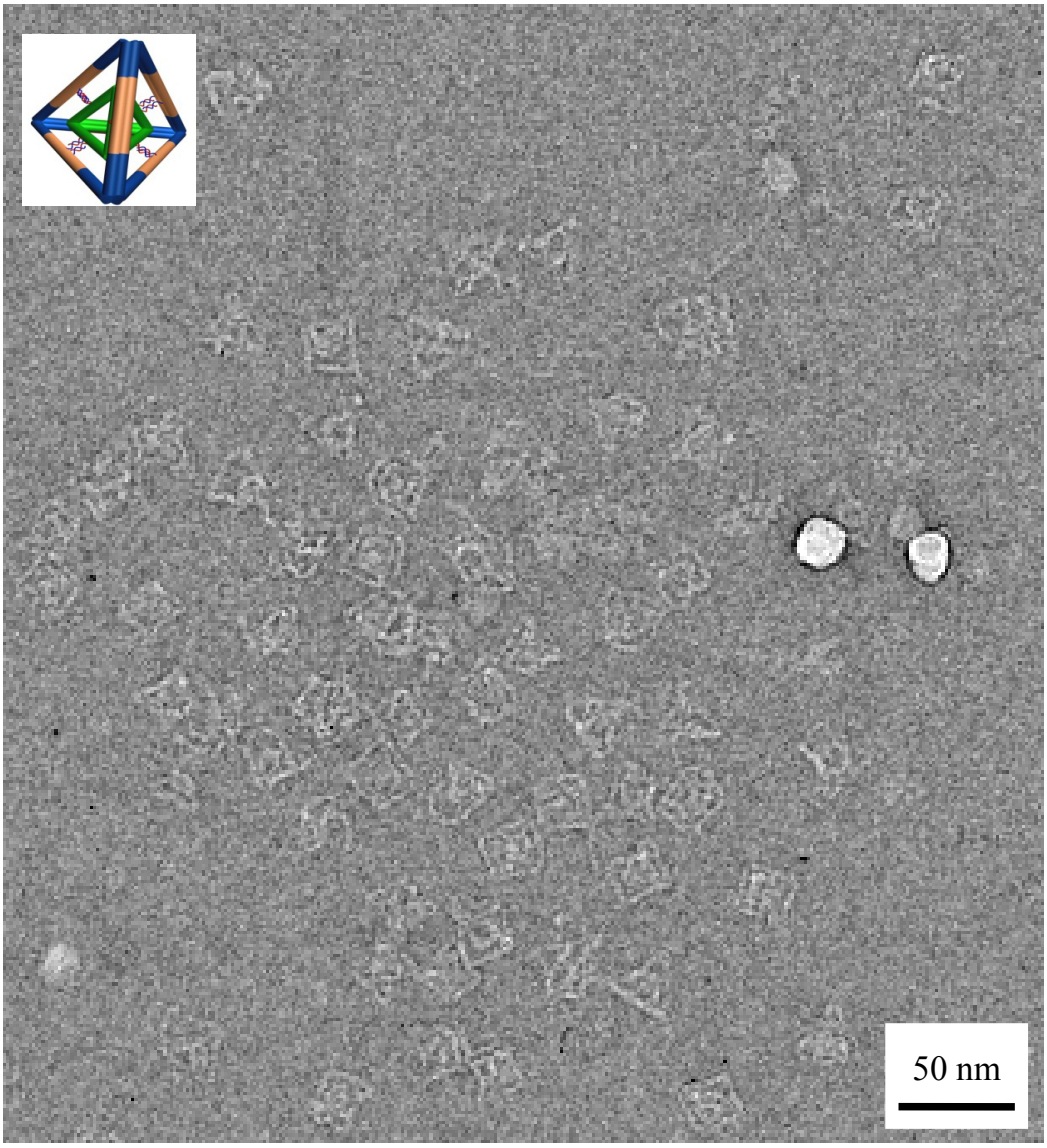

Figure S2. A double-layered DNA tetrahedron (dTET): comparison of its class averaged images with the corresponding projections of the structural model. For each pair, the class average is shown on the top and the projection is shown on the bottom.

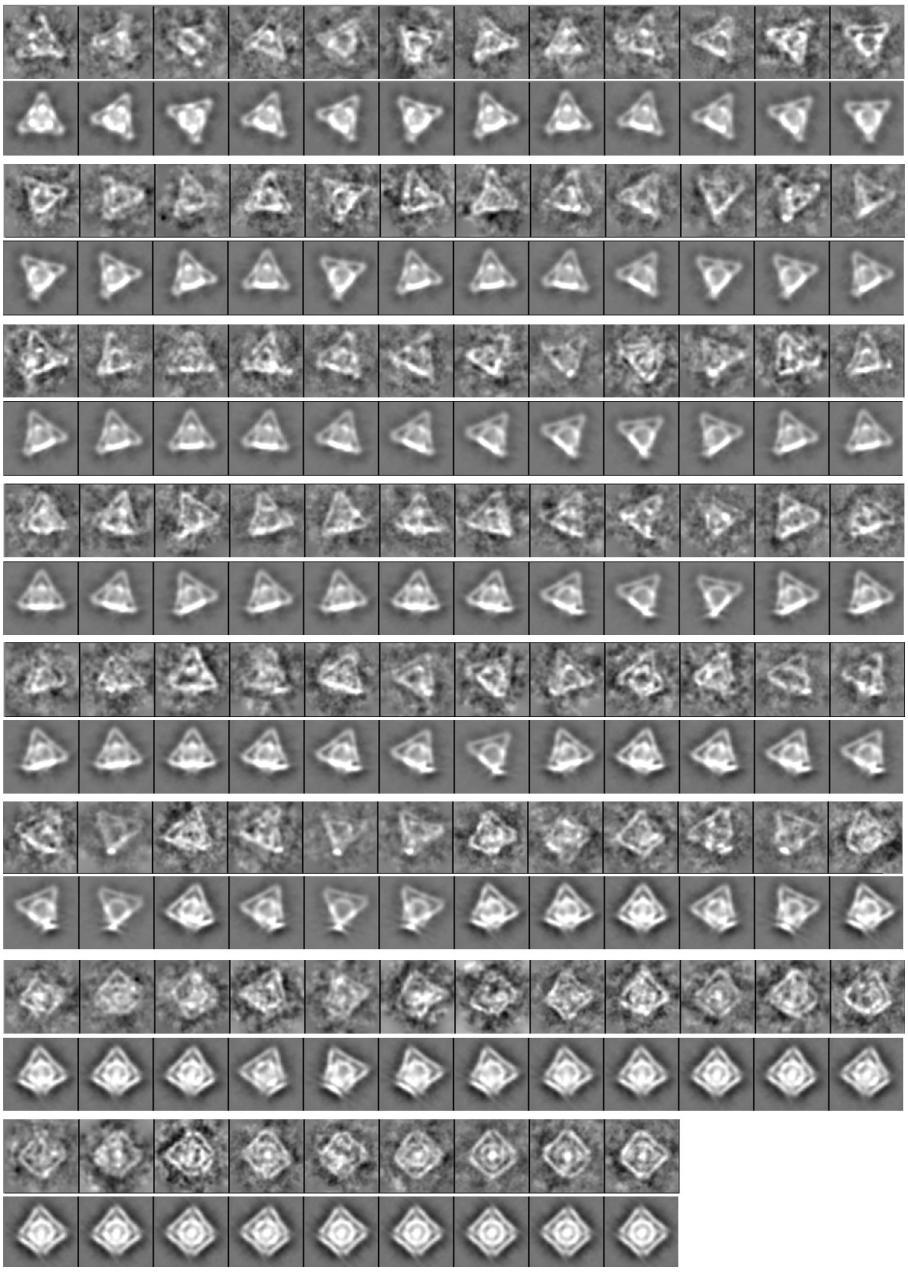

Figure S3. A triple-layered DNA tetrahedron (tTET): an enlarged view of its raw cryoEM image, which is the same as Figure 4c in the main text.

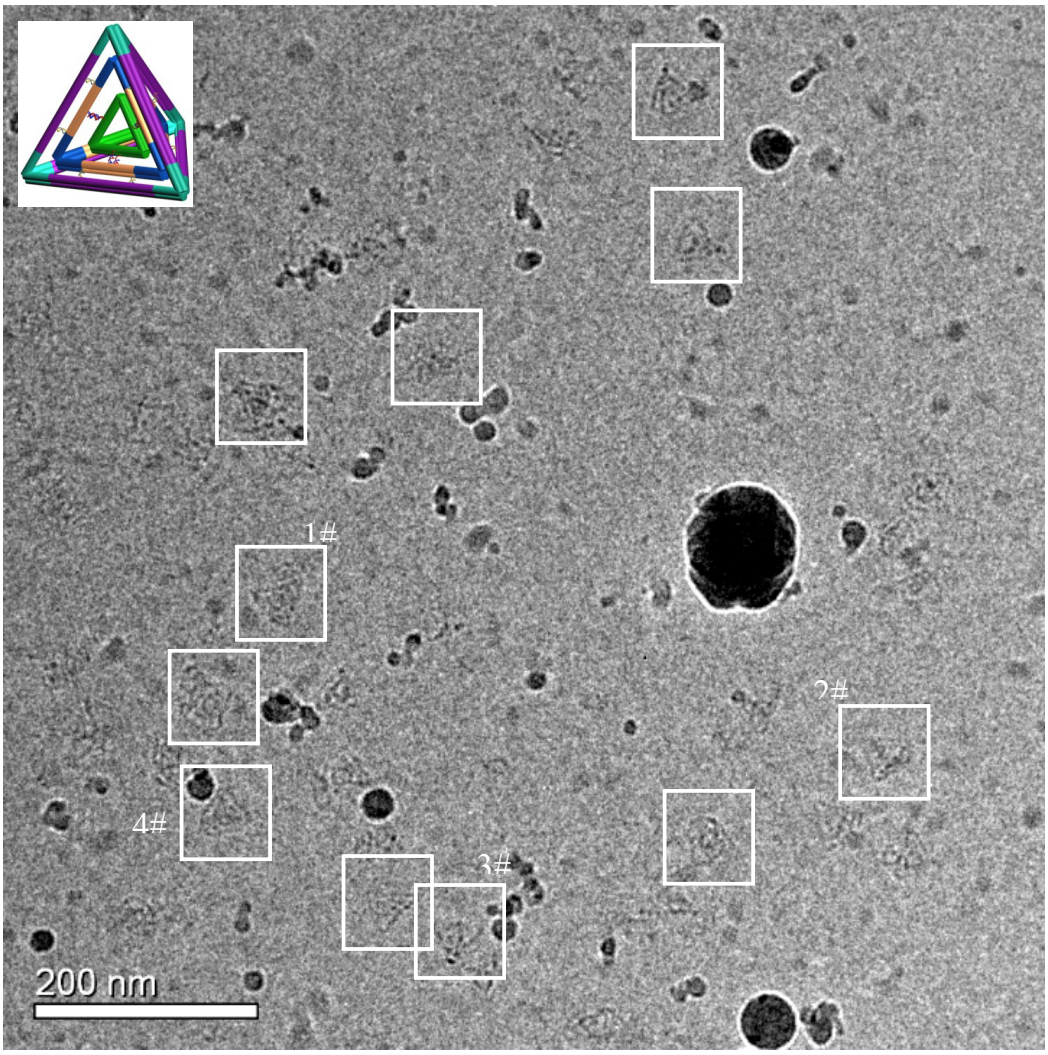

Figure S4. Mechanism of ATP-activated layer-separation designed for DNA double-layered tetrahedron (dTET). Sequence of the ATP-responsive region is labeled within the scheme.