Supplementary Material (ESI) for Chemical Communications

Molecular Engineering of Photoresponsive Three-dimensional DNA Nanostructures

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Supporting information

Chemicals and Regents

The chemicals for synthesis of the phosphoramidite monomer and reagents for DNA modification were purchased from ChemGene (MA). The CPG columns for DNA synthesis were purchased from Glen Research (VA). The reagents for BIDBE, azobenzene phosphoramidite synthesis and gold nanoparticle synthesis were purchased from Sigma-Aldrich (MO).

Instruments

For azobenzene phosphoramidite synthesis, the chemical compounds at different steps were purified by glass column silica gel chromatography and identified by thin layer chromatography (TLC) plate (silica gel 60F254; Merck) and NMR spectrometry (Mercury 300). All the DNA-related syntheses were completed on an ABI3400 DNA/RNA synthesizer (Applied Biosystems), and sequences were purified on a

ProStar HPLC system equipped with a gradient unit (Varian) using a C18 column (Econosil, 5U, 250 x 4.6 mm) (Alltech Associates). The measurements of all DNA concentrations were performed with a Cary Bio-300UV spectrometer (Varian) using the absorbance of DNA at 260 nm. A Horiba Fluomax-4 Spectrofluorometer with a temperature controller (Jobin Yvon) was used for all steady-state fluorescence measurements. The light source in the fluorometer was also used for the illumination. The transmission electron microscopy (TEM) imaging system was provided by Hitachi (Japan). AFM experiments were carried out on a Nanoscope IIIa (Veeco, Santa Barbara, CA).

Oligonucleotides

The DNA sequences used for tetrahedron assembly were similar to those described by Goodman et al. The only difference was an increase in base number on the stem of hairpin structure from 4 bps to 5 bps in order to have better structural change efficiency in response to UV and visible irradiation. Strand 1: GGT GAT AAA ACG TGT AGC AAG CTG TAA TCG ACT CTA Dabcyl GGC GGA AGA ACC CAC AAC CGC C FAM CGC TCA CTA CTA TGG CG. Strand 2: 5' AGG CAG TTG AGA CGA ACA TTC CTA AGT CTG AAA TTT ATC ACC CGC CAT AGT AGA CGT ATC ACC. Strand 3: CTT GCT ACA CGA TTC AGA CTT AGG AAT GTT CGA CAT GCG AGG GTC CAA TAC CGA CGA TTA CAG. Strand 4: TAG AGA CGG TAT TGG ACC CTC GCA TGA CTC AAC TGC CTG GTG ATA CGA GAG CG. To obtain the maximum fluorescence signal, strand S5 cDNA was used to totally extend the hairpin structure: GCG GTT GTG GGT TCT TCC GC. To obtain the best photon-controllable results, four different azo-cDNA sequences were synthesized ("X" represents azobenzene moieties). S5-7azo: GXC GGX TTG XTG GXG TTX CTT XCC GXC. S5-9azo: GCX GGX TTX GTX GGX GTX TCX TTX CCX GC. S5-10azo: GXC GXG TXT GXT GXG GXT TXC TXT CXC GXC. S5-17azo: GCX GXG XTX TXG XTX GXG XGX TXT XCX TXT XCX CXG C.

Synthesis of DNA sequences with modifications

Synthesis of azobenzene phosphoramidite: Azobenzene phosphoramidite was synthesized according to a previously reported paper.[S1,S2]

Synthesis of BF-PS-DNA: For the synthesis of BF-PS-DNA, the method reported by Lee et al.[S3] was used. First, phosphorothioate-modified DNA (PS-DNA) was synthesized at a specific position on DNA strand 1 using sulfuration reagent (Glen Research). The ligand BIDBE was then synthesized by using the protocol reported by Luduena et al. [S4] DNA, BIDBE solution, and Tris-HCl buffer were incubated at 50°C for 5-6h to form BIDBE-DNA. The best ratio for BIDBE and DNA phosphorothioate sites was around 200:1. Then, to reduce the disulfide bond, 100uL 10uM BIDBE-DNA solution, 1uL 50mM acetate buffer and 10uL 1mM TECP were mixed and incubated at room temperature for 2 hours. Gel-filtration was used to remove the

impurities.

Assembly of DNA nanostructures

Assembly of contracted DNA tetrahedra: 10uL 10uM DNA strand S1, 10uL 10uM DNA strand S2, 10uL 10uM DNA strand S3, 10uL 10uM DNA strand S4, 10uL 150mM Tris-HCl (pH=7.5), 10uL 150mM $MgCl_2$ and 40uL DNA water were mixed and annealed at 95°C for 5min and cooled to room temperature in approximately 5 minutes.

Assembly of extended DNA tetrahedra (no azobenzene incorporated): 10uL 10uM DNA strand S1, 10uL 10uM DNA strand S2, 10uL 10uM DNA strand S3, 10uL 10uM DNA strand S4, 10uL S5-cDNA (no azobenzenes), 10uL 150mM Tris-HCl (pH=7.5), 10uL 150mM $MgCl_2$ and 40uL DNA water were mixed and annealed at 95°C for 5 min and cooled to room temperature in approximately 5 minutes.

Assembly of azobenzene-incorporated DNA tetrahedra: 10uL 10uM DNA strand S1, 10uL 10uM DNA strand S2, 10uL 10uM DNA strand S3, 10uL 10uM DNA strand S4, 10uL DNA strand S5, 10uL 150mM Tris-HCl, 10uL 150mM $MgCl_2$ and 30uL DNA water were mixed and annealed at 95°C for 5min and cooled to room temperature in approximately 5 minutes. Tetrahedra were purified using PAGE gel.

Native polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide solution (40%, 750uL) was diluted to 6% by adding 4.25mL 10mM TAE buffer containing 15mM $MgCl_2$. Fifty uL APS and 5uL TEMED were added to the polyacrylamide solution to polymerize it. After loading 10uL 1uM DNA sample solutions, the gels were run on an electrophoresis unit (Biorad) at 4 ^{o}C using a constant voltage of 80 V for 90 minutes. After electrophoresis, the gels were stained with Stains-All for 30 min and imaged using a digital camera.

Phosphorothioate DNA tetrahedra assembled with gold nanoparticles

It has been reported that AuNPs are more stable when capped with phosphine reagent[S5]. Therefore, in this step, 10mL 60nM gold nanoparticle solution was mixed with 2mg bis(para-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (Strem Chemicals, Newburyport, MA) and shaken at 25 ^{o}C overnight. After precipitation and centrifugation, the supernatant was removed, and the AuNP precipitate was redispersed into water. After stabilization, AuNPs were assembled with the DNA tetrahedron to visualize the shape change of structures. Ten uL 100nM DNA tetrahedron solution was mixed with 50uL 60nM AuNPs and incubated overnight at room temperature. The best TEM images were obtained from the mixture solution containing the tetrahedra and AuNPs at a ratio of 1:3.

FRET measurement of structures in response to UV and Visible irradiation

After assembly, DNA tetrahedral structures were excited at λexc=488nm at constant temperature. After that, the light source in the fluorometer was used to irradiate the solution at 350nm for 3 min, and the fluorescence spectrum was obtained again immediately after the UV irradiation. Next, visible light (450nm) was applied to the structures for another 3 min, and fluorescence spectrum of the solution was measured. Additional cycles with alternate UV and Visible irradiation were performed, and the fluorescence spectra were recorded.

AFM measurements and TEM measurements

AFM experiments were carried out on a Nanoscope IIIa (Veeco, Santa Barbara, CA) using tapping mode in ambient air. The radius of curvature of silicone tip was about 10 nm. All topographic images were obtained with 512 x 512 pixels^2 at a scan rate of 1.5 Hz. After annealing strands S1, S2, S3, S4 and S5-10Azo under visible light, the structures were scanned by using AFM (Figure S-1). The tetrahedra were expected to have one 10.5nm edge and five 7nm edges for the extended state, and based on the AFM image, the sizes and heights were consistent with the calculated values.

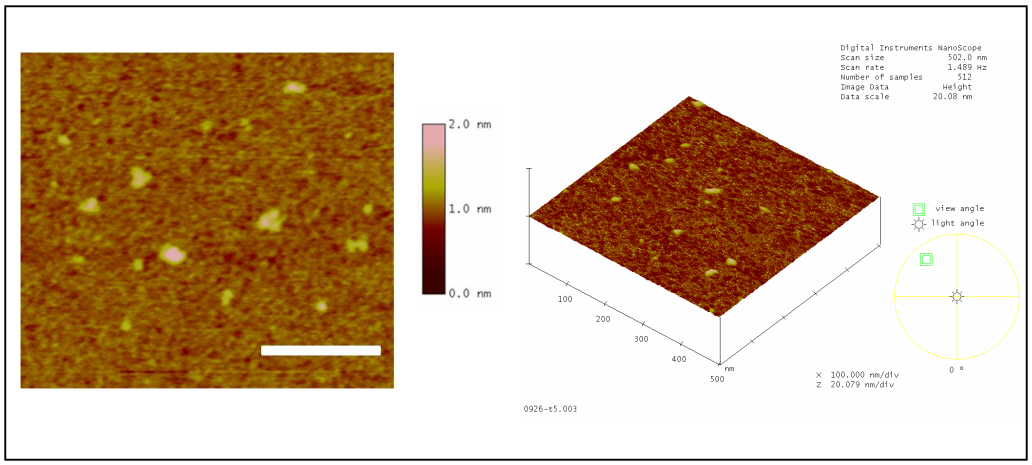

FIGURE S-1. AFM image of T-10Azo structures recorded with 10nm tips. (bar length: 100nm)

Transmission electron microscopy (TEM) images were obtained on a Hitachi H-7000 NAR transmission electron microscope. For the extended structures, samples were dried on copper film under visible light. Then a parallel sample was irradiated at 350nm until it was completely dry on the copper film. Afterwards, the samples were imaged by using TEM at a working voltage of 100 kV.

Light-responsive fluorescence measurements on fraction of DNA tetrahedral structures

To prove our concept, experiments were performed on the reconfigurable edge of the DNA tetrahedra. Three DNA strands whose sequences form parts of tetrahedra were assembled with Azo-cDNA to prepare the structures in the scheme (Figure S-2). To obtain the background and maximum signals, closed structures with 1, 2, 3 and open structures with 1, 2, 3 and cDNA (no azobenzenes) were also assembled. When UV was applied to the azo-incorporated structures, azo-cDNA could dehybridize and trigger the formation of the hairpin structure. The fluorescence data shown in Figure S-3 clearly show that the structures moved upon UV irradiation by the decrease in the fluorescence signal as a result of the proximity of the fluorophore and quencher.

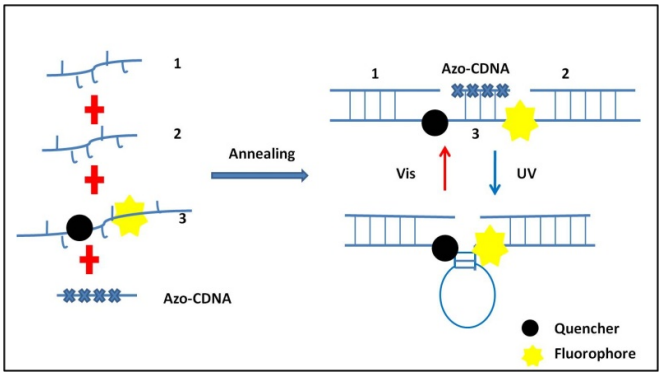

FIGURE S-2. Working scheme for illumination of the formed fraction of DNA tetrahedra.

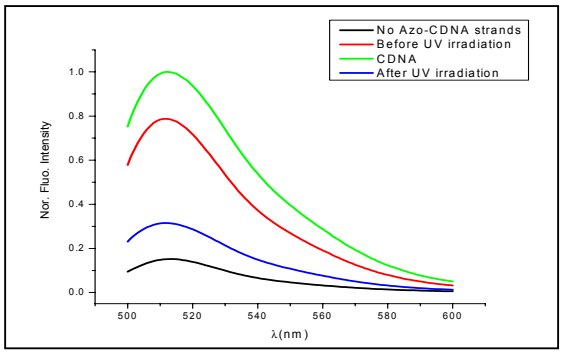

FIGURE S-3. FRET measurement of the reconfigurable component of tetrahedron structures.

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