Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Click and photo-release dual-functional nucleic acid nanostructures

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Materials and Methods

Oligonucleotide synthesis

Oligonucleotides were purchased from Integrated DNA Technologies (IDT) with standard desalting. DNA oligonucleotides with photocleavable linkers (PCL) were chemically synthesized at 1.0-μmol scales by solid phase synthesis using an Oligo-800 synthesizer. PCL phosphoramidites were purchased from Glen research and used as 0.1 M solution in acetonitrile. All the other reagents are standard solutions obtained from ChemGenes Corporation. After synthesis, the oligos were cleaved from the solid support and fully deprotected with AMA (ammonium hydroxide:methylamine = 1:1) at 65 °C for 30 min. The amines were removed by Speed-Vac concentrator before purification. The DNA strands were purified by reverse phase HPLC using a Zorbax SB-C18 column at a flow rate of 6 mL/min. Buffer A was 20 mM triethylammonium acetate, pH 7.1; buffer B contains 50% acetonitrile in 20 mM triethylammonium acetate, pH 7.1. A linear gradient from buffer A to 80% buffer B in 25 min was used to elute the oligos. The purified samples were concentrated, desalted and lyophilized to dry before re-dissolving to working buffers.

Copper catalyzed alkyne-azide cycloaddition reaction

2'-O-propargyl modified DNA oligonucleotides (0.38 mM, 200 μl in H_2O) and azido-bipyridine (10 mM, 114 μl, H_2O) were placed in a 1.5 ml vial. In a separate vial, 17 μl CuBr solution (100 mM in DMSO/tBuOH 3:1) and 34 μl CH_3CN solution (100 mM in DMSO/tBuOH 3:1) were mixed and added to the DNA solution. The mixture was shaken at room temperature overnight before being evaporated to near dryness in a speed-vac at 65 °C. Sodium acetate (0.3 M, 100 μl) was then added and the suspension was stirred for 1 h before 1 ml of ethanol was added. The vial was vortexed well and stored in a freezer (-80°C) for 1 h and centrifuged for 15 min at 13000 rpm. The supernatant was carefully removed from the DNA pellet. 70% cold ethanol (-20 °C) was used to wash the pellet three times. Finally, the pellet was left drying on air and dissolved in DI water.

Complex formation

RNA 3WJ

The component strands were annealed in 1:1:1 ratio in 1x TMS buffer (50 mM TRIS pH = 8.0, 100 mM NaCl, 10 mM $MgCl_2$), and annealed by heating to 95 °C and cooling down to room temperature by itself over 2 hours, followed by 4°C for 30 mins.

DNA 3-point-star tile

Strands L, M and S were mixed in 1:3:3 ratio in 1X TA/Mg^{2+} (40 mM Tris base, pH = 8.0; 20 mM acetic acid and 12.5 mM magnesium acetate) at a final concentration of 75 nM. The mixture was annealed slowly from 95°C to 20°C over two days.

DNA tetrahedron

Strands L, M and S were mixed in 1:3:3 ratio in 1X TA/Mg^{2+} at a final concentration of 75 nM. The mixture was annealed slowly from 95°C to 20°C over two days. For the modified DNA tetrahedron formation, strand S was replaced by strand S-t-PCL while keeping all the ratios and concentrations the same.

Non-denaturing PAGE

RNA 3WJ complexes were run on 12% non-denaturing polyacrylamide gel in 1x TBM buffer at 20°C. The DNA tile and tetrahedron were run on non-denaturing 6% and 4% polyacrylamide gels respectively in 1X TA/Mg^{2+} at 4°C. Gels were post-stained with ethidium bromide and imaged using BioRad Chemi Doc. Gels with FAM-complexes were imaged on Amersham (GE) Typhoon 9400 Imager at λ = 488 nm.

UV release

The UV release experiment was conducted by placing the tubes containing the nanostructures in the ice bucket with open tops approximately 3 inches from the light source. The nanostructures were exposed to UV (254 nm) using a UV light source (handheld UV light Spectroline EF 240C with an output of 4 Watts). For release in biological fluids, the nanostructures were mixed to a final of 10% FBS and 10% synthetic urine, followed by exposure to UV for 16 minutes.

Oligonucleotide sequences (written 5' to 3'):

RNA three-way junction

3WJ 1 (18-nt):
UUGCCAUGUGUAUGUGGG

3WJ 2 (20-nt):
CCCACAUACUUUGUUGAUCC

3WJ 3 (16-nt):
GGAUCAAUCAUGGCAA

3WJ 1 PCL (19-nt):
(PU) **(PCL)** UGCCAUGUGUAUGUGGG

DNA 3PST and DNA tetrahedron

Strand L (78-nt):
AGG CAC CAT CGT AGG TTT TTC TTG CCA GGC ACC ATC GTA GGT TTT TCT TGC CAG GCA CCA TCG TAG GTT TTT CTT GCC

Strand M (42-nt):
AGC AAC CTG CCT GGC AAG CCT ACG ATG GAC ACG GTA ACG ACT

Strand S (21-nt):
TTA CCG TGT GGT TGC TAG TCG

Strand S-t-PCL (29-nt):
TTA CCG TGT GGT TGC TAG TCG CCT C **(PCL)** AA **(PG)** A

Strand MB (42-nt):
CGACTAGCAACCTGCCTGGCAAGCCTACGATGGACACGGTAA

Strand SB (21-nt):
AGTCGTTACCGTGTGGTTGCT

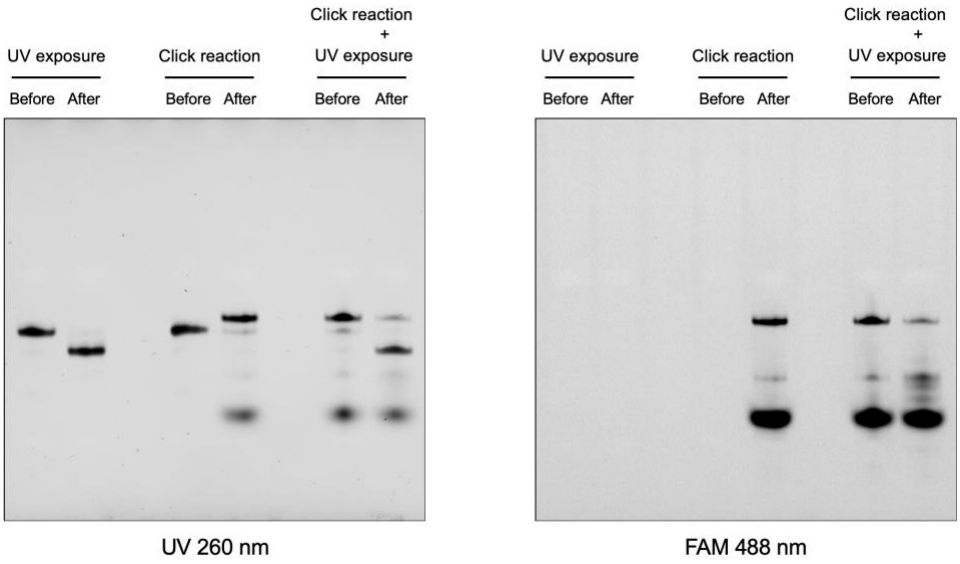

Figure S1. Denaturing PAGE profile of single strand showing incorporation of PCL, FAM (post click reaction), and dual functionality.

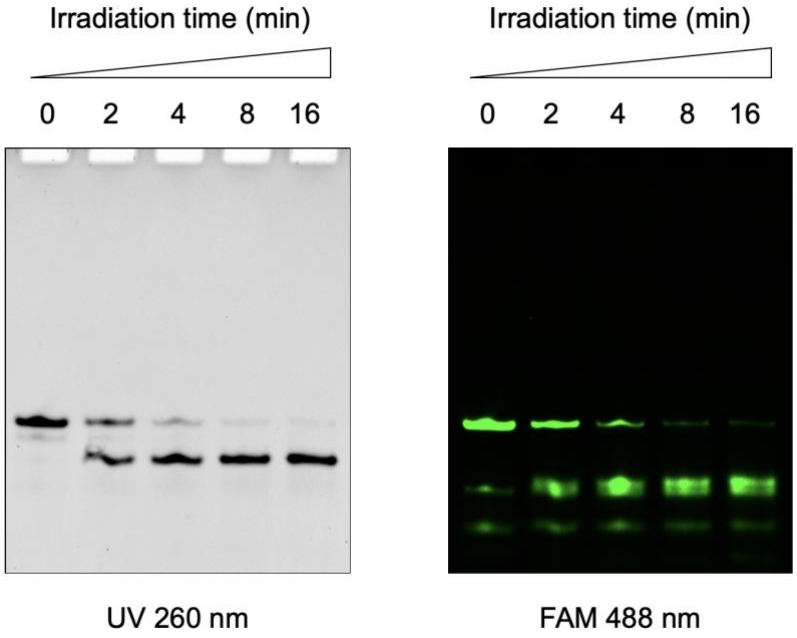

Figure S2. Denaturing PAGE functionalized single strand showing release of FAM over different UV exposure times.

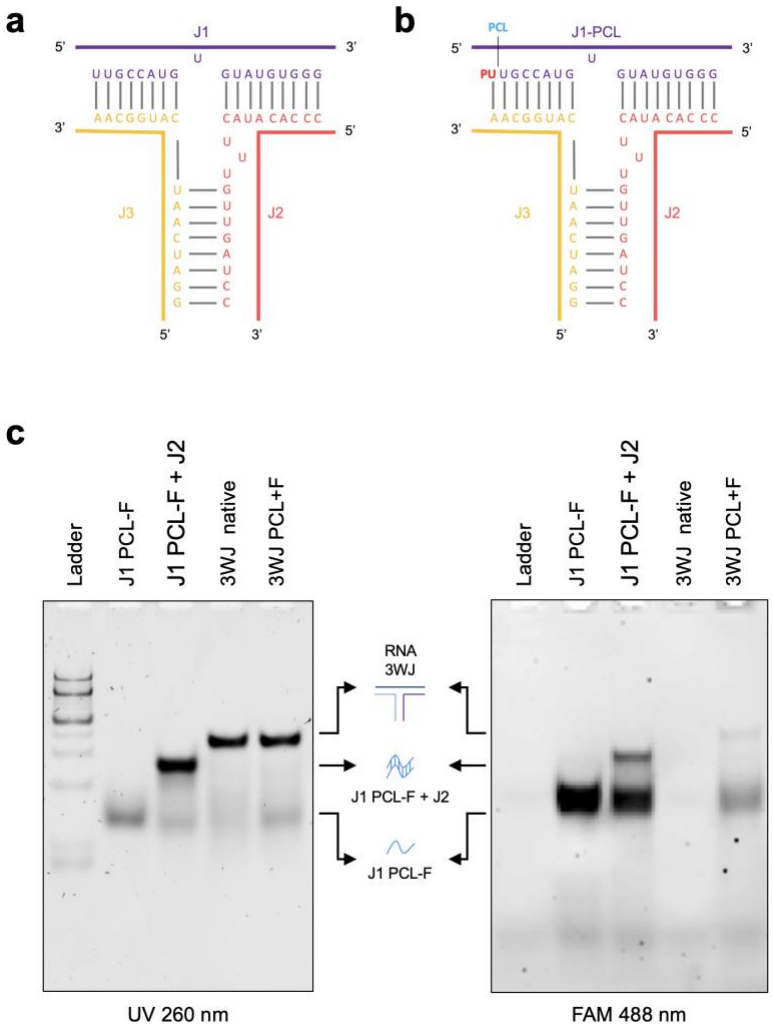

Figure S3. Design and formation of RNA three-way junction. (a) Native RNA 3WJ (b) Dual functional RNA 3WJ (c) RNA 3WJ formation on non-denaturing PAGE (UV image on left and fluorescence image on right).

Lane identities:

- J1 PCL-F: Strand J1 modified with PCL and FAM clicked on
- J1 PCL-F + J2: Strand J1 modified with PCL-FAM + Strand J2 (duplex)
- 3WJ native: 3-way junction native structure without any modifications
- 3WJ PCL+F: 3-way junction containing PCL modification and clicked FAM on strand J1

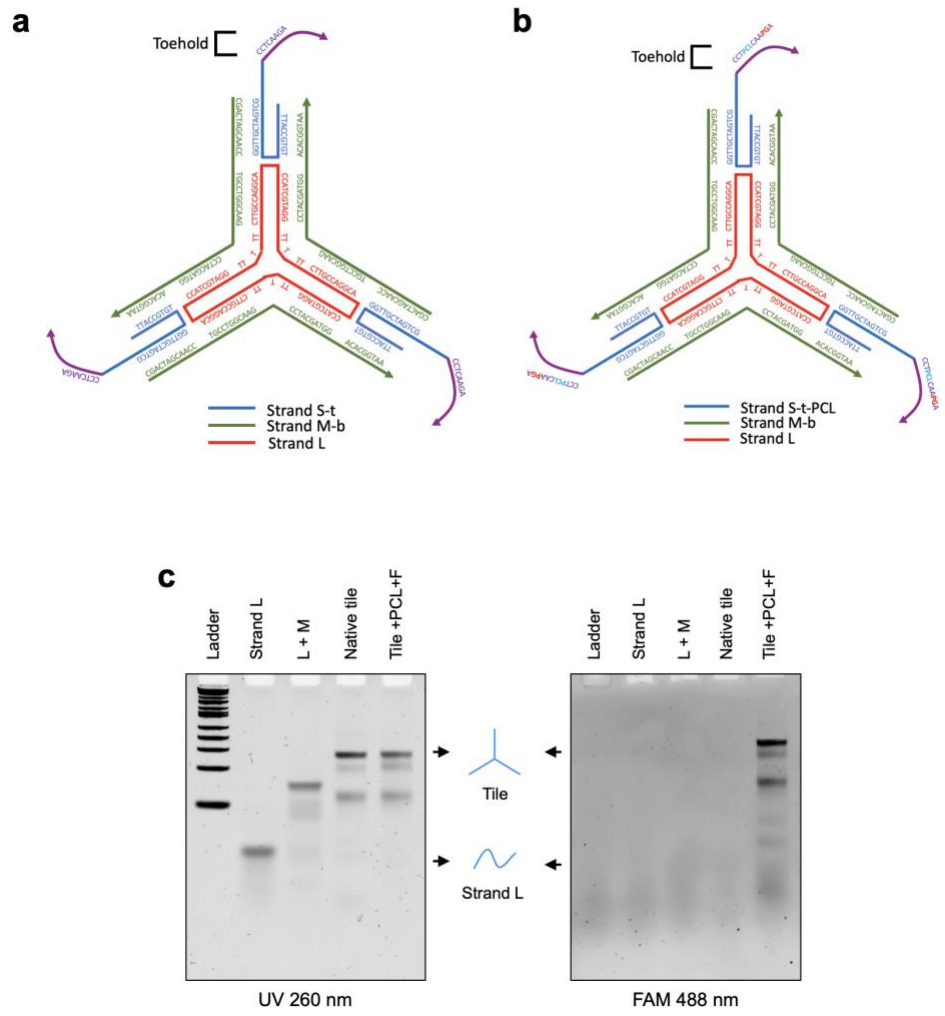

Figure S4. Design and formation of DNA 3-point-star tile. (a) DNA 3-point-star native (b) Dual functional DNA 3-point-star (c) Gel showing formation of DNA 3-point-star (UV image on left and fluorescence image on right).

Lane identities:

- Strand L: Single strand L (shown in red in a and b)
- L+M: Complex of strand L and M in 1:3 ration forming an intermediate structure
- Native tile: 3-point-star tile without any modifications
- Tile +PCL+F: 3-point-star tile containing PCL modification and clicked FAM on strand S-t.

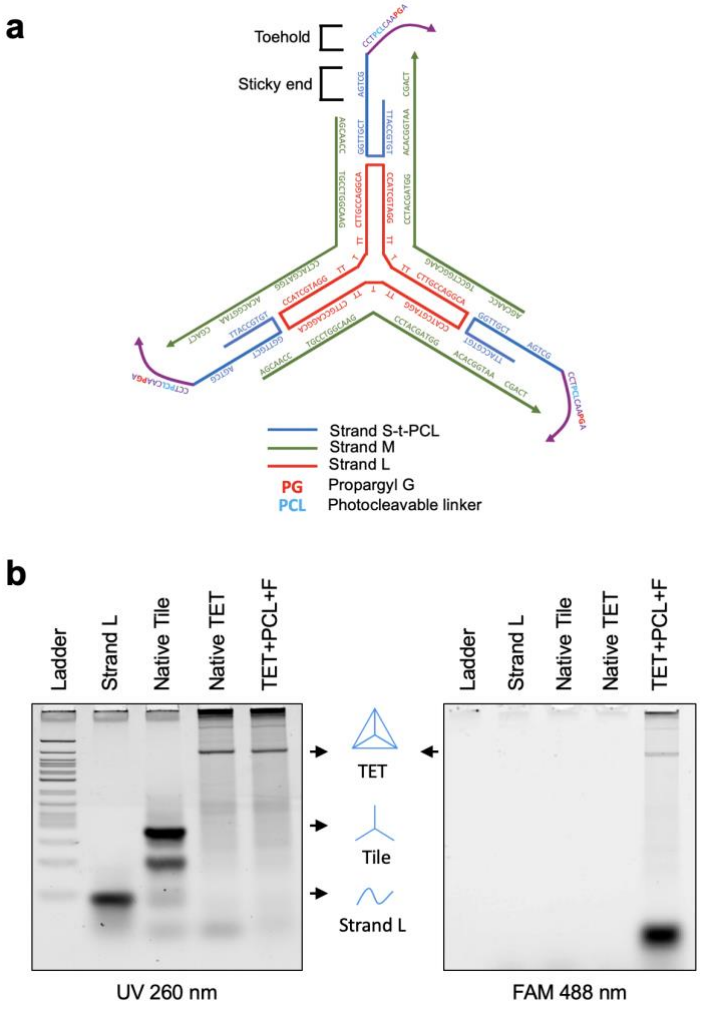

Figure S5. Design and formation of DNA tetrahedron. (a) The 3-point-star tile was modified with sticky ends for hierarchical formation of the tetrahedron. (b) Gels showing formation of DNA tetrahedron (UV image on left and fluorescence image on right).

Lane identities:

- Strand L: Single strand L (shown in red in a and b)
- Native tile: 3-point-star tile without any modifications
- Native TET: Tetrahedron assembled from 3-point-star tile without any modifications
- TET +PCL+F: Tetrahedron assembled from 3-point-star tile containing PCL modification and clicked FAM on strand S-t.

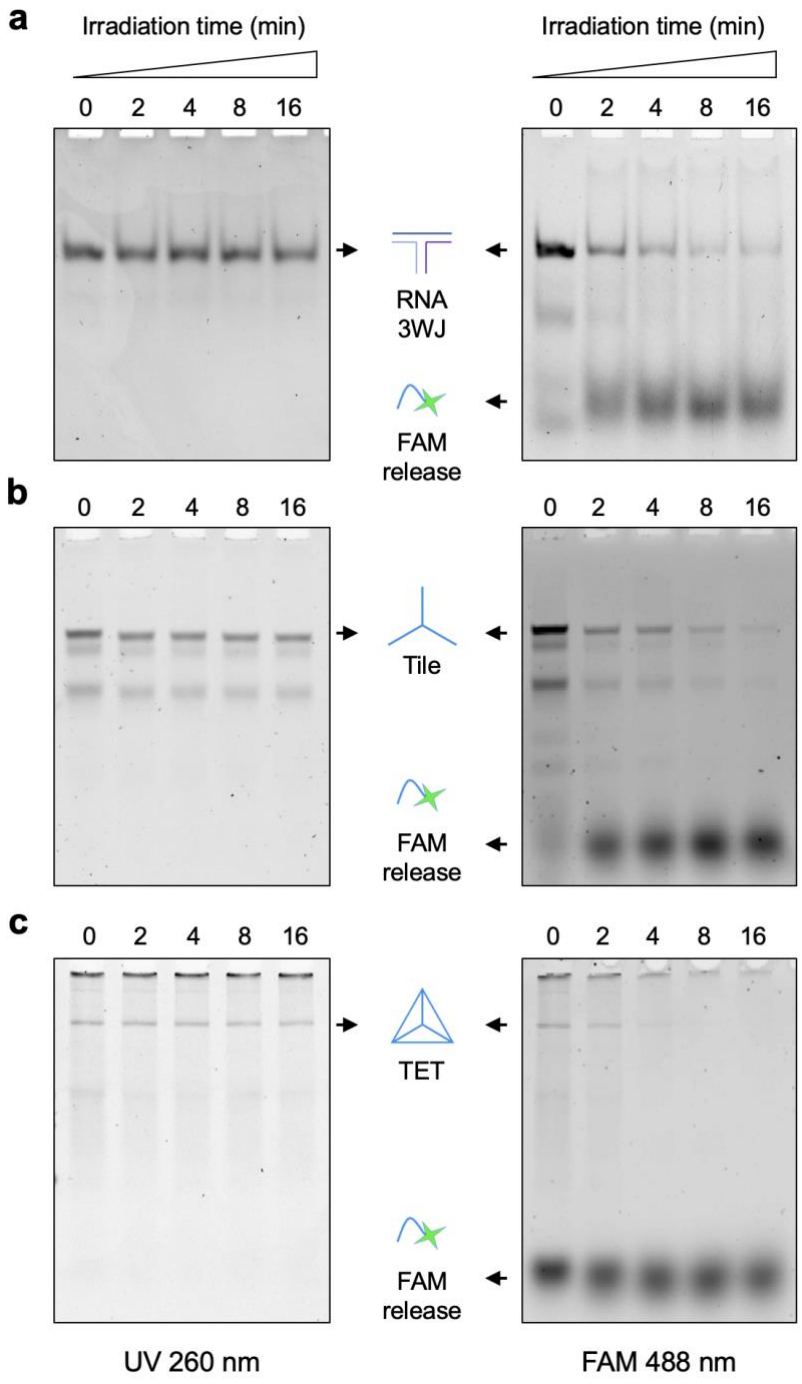

Figure S6. UV release of FAM from different structures. (a) RNA 3WJ (b) DNA 3-point-star (c) DNA tetrahedron (UV image on left and fluorescence image on right).

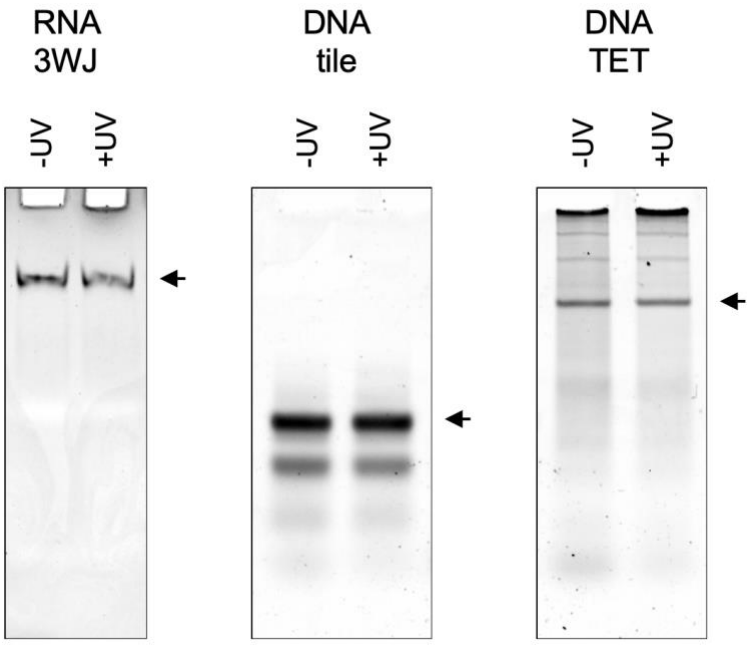

Figure S7. Control experiment for UV irradiation. Native nanostructures exposed to UV for 16 minutes show no noticeable change.

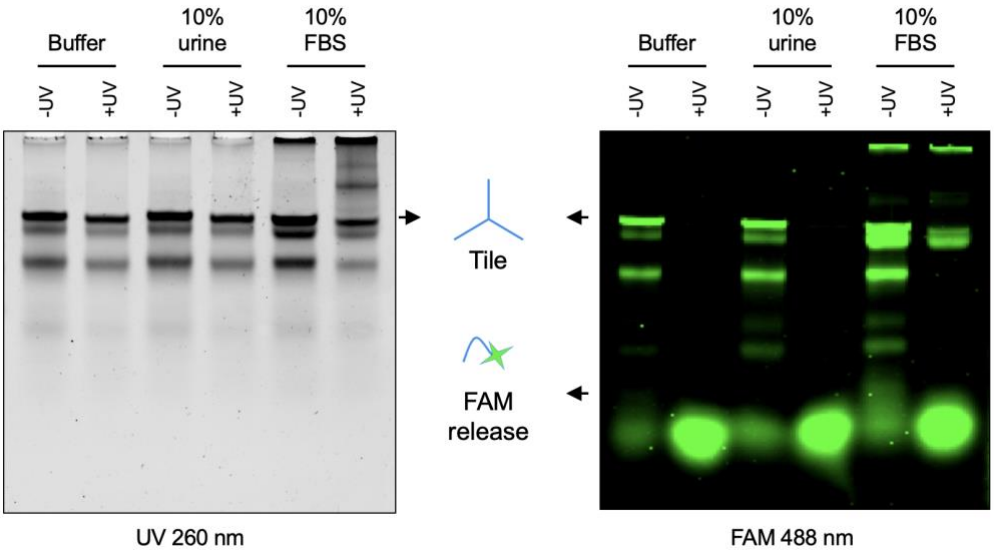

Figure S8. UV release in biofluids. Release of FAM from a DNA tetrahedron in buffer, synthetic urine and 10% FBS.

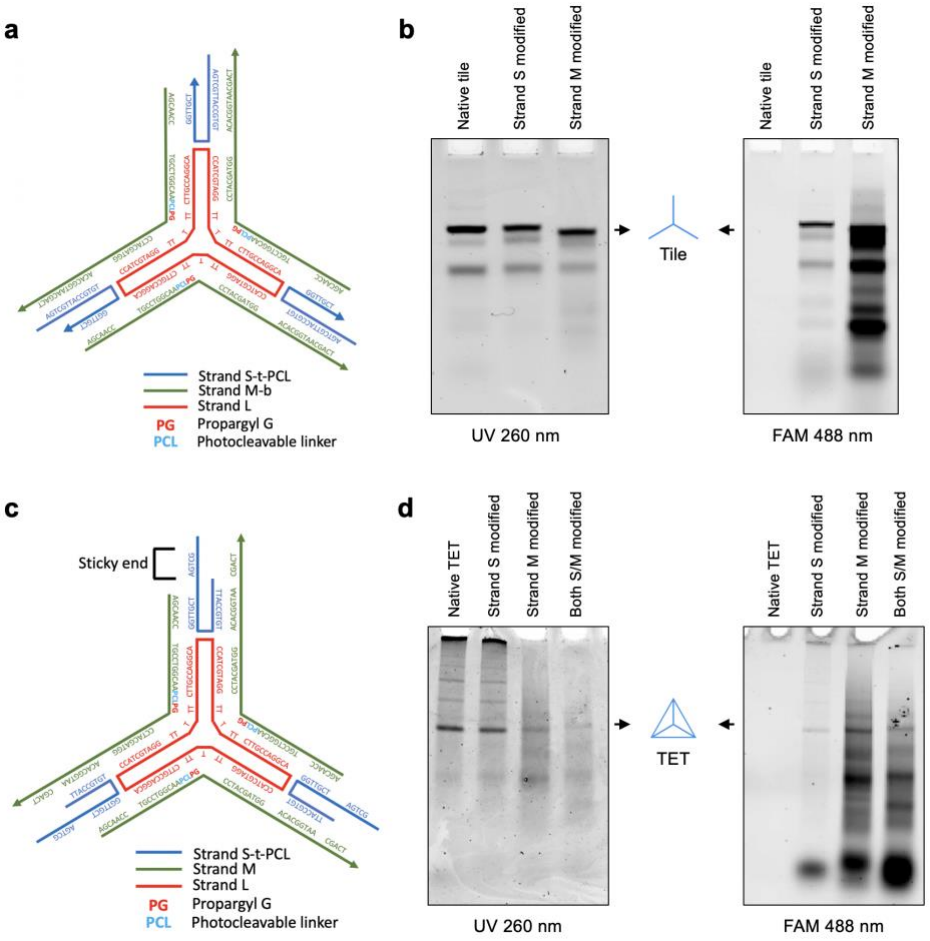

Figure S9. Comparison of external vs internal modifications in the formation of nanostructure. (a) Design and (b) gel analysis of assembly of DNA three-point-star; (c) design and (d) gel analysis of assembly of DNA tetrahedron.

Lane identities:

- Native tile: 3-point-star tile without any modifications
- Native TET: Tetrahedron assembled from 3-point-star tile without any modifications
- Strand S modified: Assembled structures containing PCL modification and clicked FAM on a version of strand S (blue in scheme). This modification is “external” since it is not within the assembled structure.
- Strand M modified: Assembled structures containing PCL modification and clicked FAM on a version of strand M (green in scheme). This modification is “internal” since it is within the assembled structure.
- Both S/M modified: Assembled structures containing PCL modification and clicked FAM on both strands S and M.