Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting information

Controlled disassembly of a DNA tetrahedron using strand displacement

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Materials and methods

Oligonucleotides

DNA sequences were adapted from previous works [1]. All oligonucleotides were purchased from IDT, Inc. Full sequences (5'-3') are listed below; sticky ends are shown in **bold** and toehold is underlined.

Strands for tetrahedron:

Strand L: 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: AGC AAC CTG CCT GGC AAG CCT ACG ATG GAC ACG GT**A ACG ACT**
Strand S: ACC GTG TGG TTG CT**A GTC GTT** CCT CAA GA

Release strands:

Strand R-8-nt toehold: TCT TGA GGA ACG ACT AGC AAC CAC ACG GT (used for disassembly)
Strand R-6nt toehold: T TGA GGA ACG ACT AGC AAC CAC ACG GT
Strand R-4nt toehold: GA GGA ACG ACT AGC AAC CAC ACG GT
Strand R-2nt toehold: GGA ACG ACT AGC AAC CAC ACG GT
Strand N: TGAGGTAGTAGGTTGTGTGGTT (non-specific)
Strand R-1nt mismatch: TCC TGA GGA ACG ACT AGC AAC CAC ACG GT

Strands for microRNA sensing tetrahedron: (for let7b)

Strand M-7b: AGC AAC CTG CCT GGC AAG CCT ACG ATG GAC ACG GTTAGGTTG
Strand S-7b: ACC GTG T AACCACACAACCTACTACCTCA

MicroRNA sequences (for release):

let-7a: UGAGGUAGUAGGUUGUAUAGUU
let-7b: UGAGGUAGUAGGUUGUGUGGUU
let-7c: UGAGGUAGUAGGUUGUAUGGUU

Formation of tetrahedron

To form TET, DNA strands L, M and S were mixed in 1:3:3 ratio at 30 nM in Tris-Acetic-EDTA-Mg^{2+} (TAE/Mg^{2+}) buffer, which contained 40 mM Tris base (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate. The DNA solution was slowly cooled down from 95°C to room temperature over 48 hours.

Triggered dissociation

The release strand was added to the TET solution at different molar ratios (1:2, 1:5 and 1:10) and incubated at 20°C for different time periods. For testing in FBS and urine, TET was first mixed with FBS or synthetic urine (Surine negative urine control, Millipore Sigma) to a final concentration of 10%, and the release strand was then added to this mixture and incubated for 1 hour at room temperature.

Native polyacrylamide gel electrophoresis (PAGE)

Non-denaturing gels containing 4% polyacrylamide (29:1 acrylamide/bisacrylamide) were run at 4°C (100 V, constant voltage). The running buffer was TAE/Mg^{2+} buffer. After electrophoresis, the gels were stained with GelRed (Sigma) and imaged using Bio-Rad Gel Doc XR+. Gel bands were quantified using ImageJ.

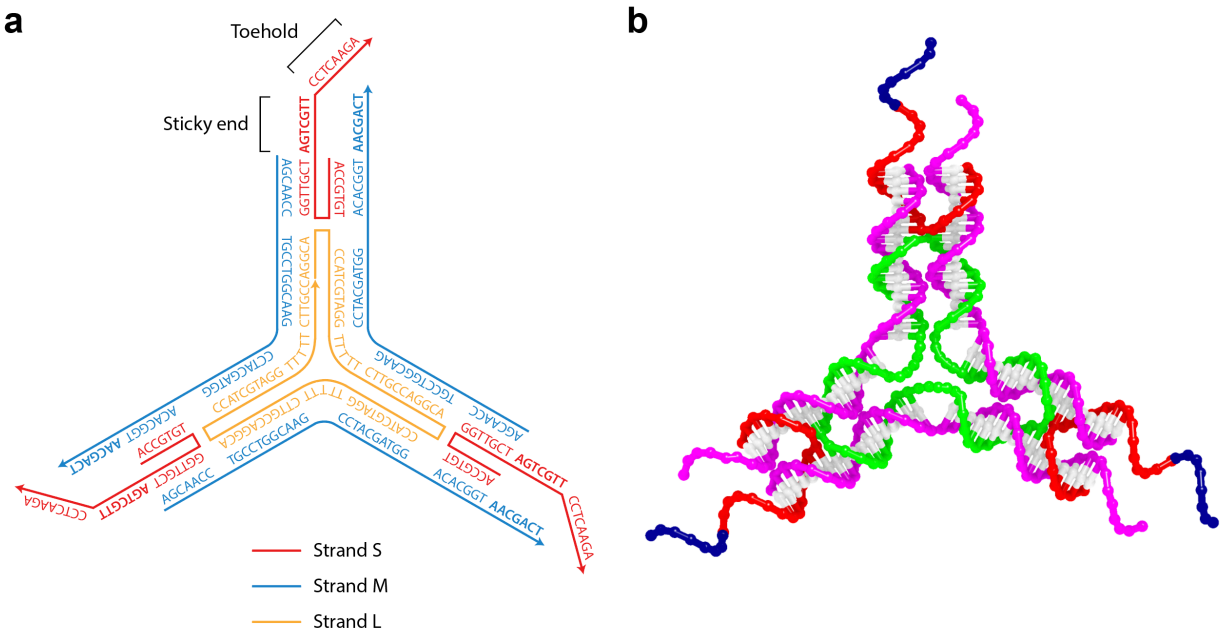

Figure S1. *Design and sequence of the 3-point-star motif*. (a) The 3-point-star motif is three-fold symmetric and consists of strands L, M and S in the ratio 1:3:3. Tiles connect via sticky ends (shown in bold) to form higher order structures. Four such motifs combine to form the DNA tetrahedron. Strand S has a single stranded extension that is not part of the tetrahedron, this acts as the toehold for the strand displacement process. (b) Molecular model of the 3-point-star motif.

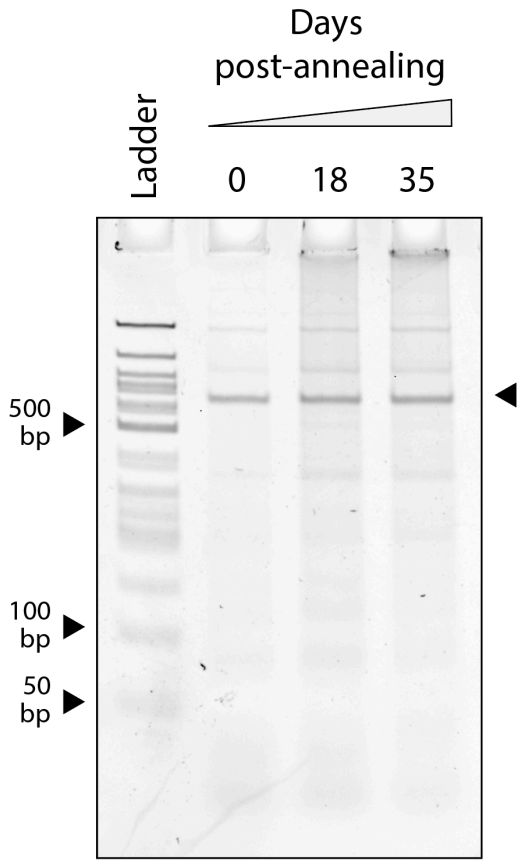

Figure S2. *Tetrahedron stability*. The self-assembled tetrahedron remains stable at room temperature for over a month.

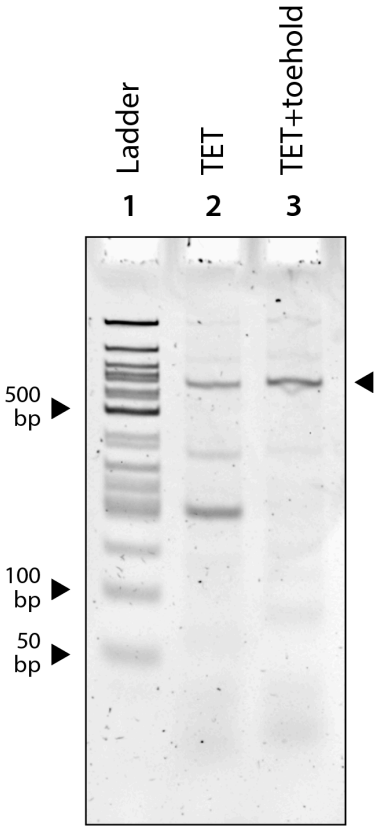

Figure S3. *Toehold-containing tetrahedron*. Non-denaturing polyacrylamide gel showing formation and similar mobilities of the tetrahedra with- and without toeholds.

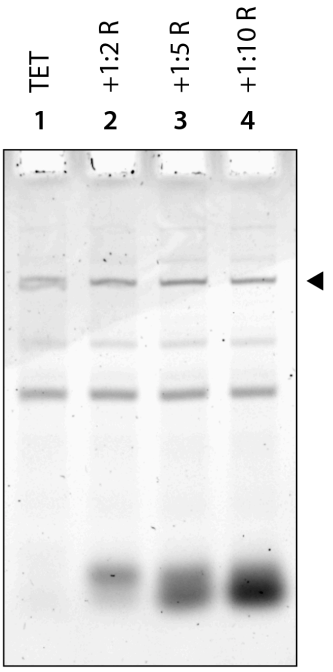

Figure S4. *Negative controls for toehold-based disassembly*. The release strand R was added in molar excess to the tetrahedron without toehold. Non-denaturing polyacrylamide gel shows that the release strands are ineffective in the absence of toeholds.

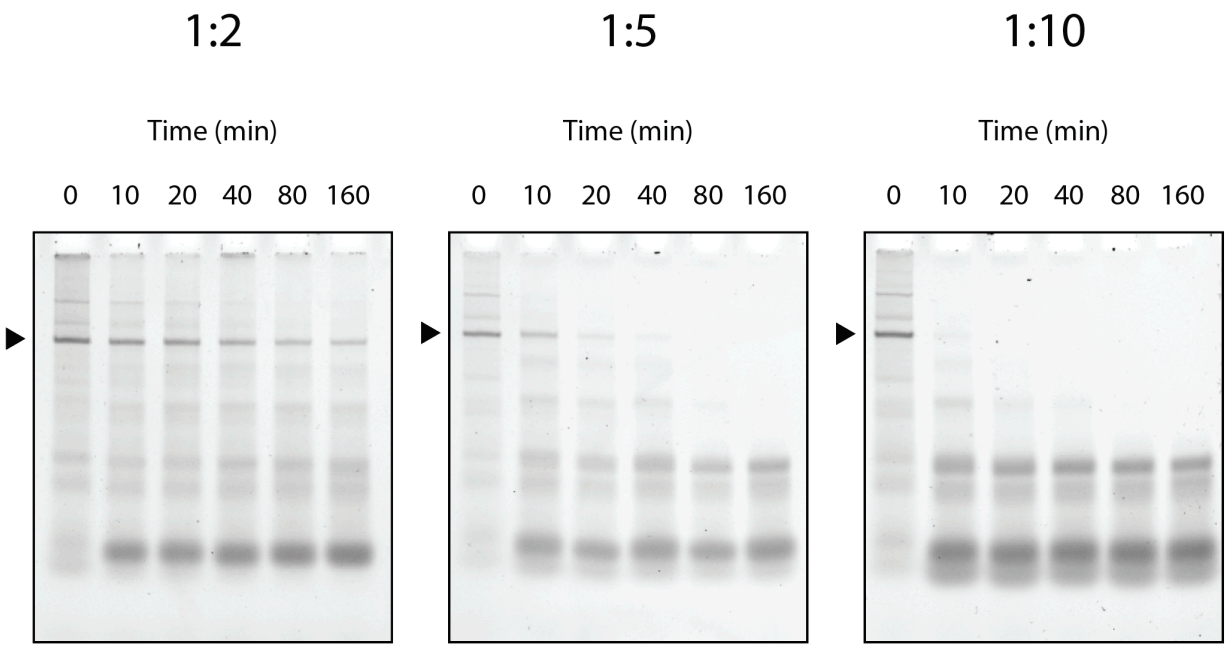

Figure S5. *Displacement kinetics for dissociation of tetrahedron*. Release strand was added to annealed tetrahedron at different molar ratios (1:2, 1:5 and 1:10) and tested at different time points to monitor dissociation over time.

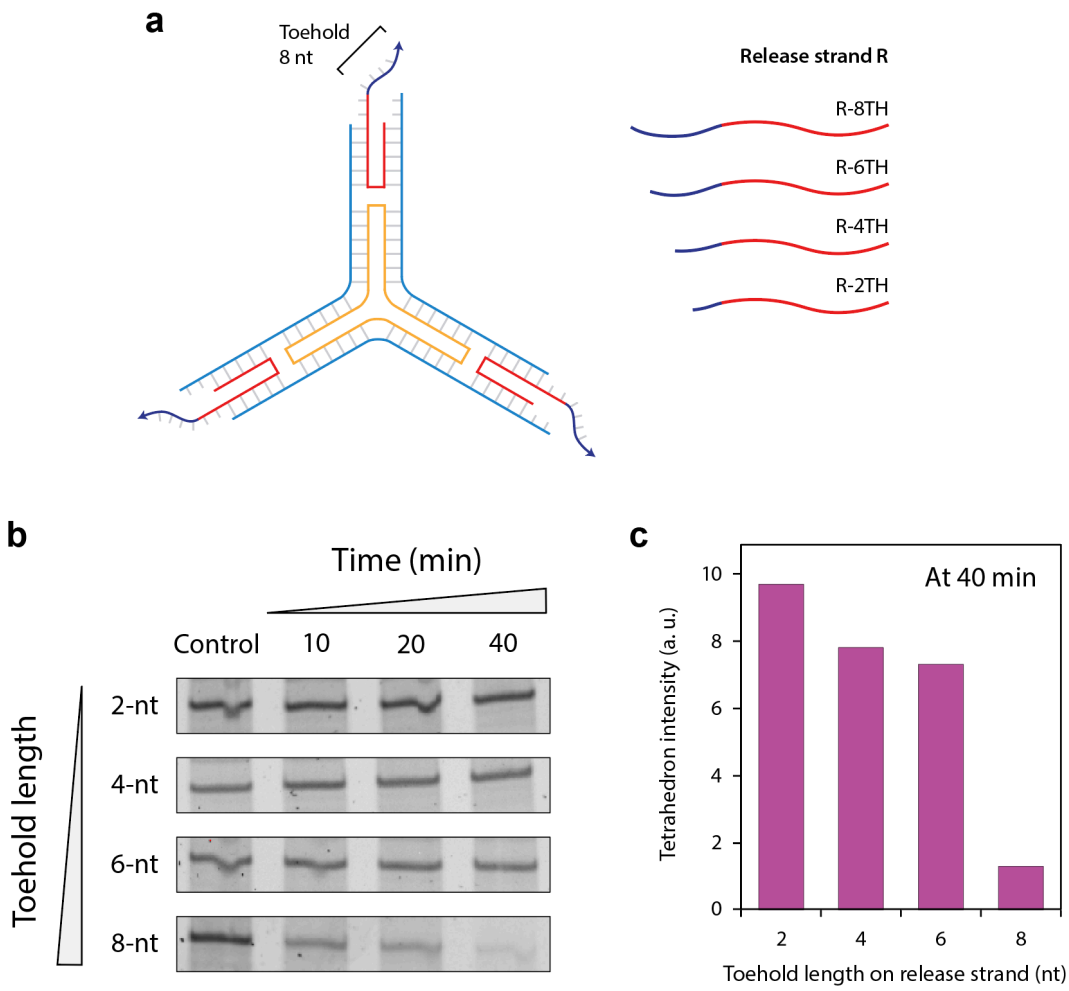

Figure S6. *Effect of toehold length*. (a) Release strands containing different lengths of toeholds were tested for tetrahedron dissociation. (b) Addition of release strands with different toehold lengths, incubated with the tetrahedron for different time periods. (c) At 40 minutes, release strand with toehold length of 8 nucleotides (matching full toehold length one tetrahedron) yielded the highest disassembly.

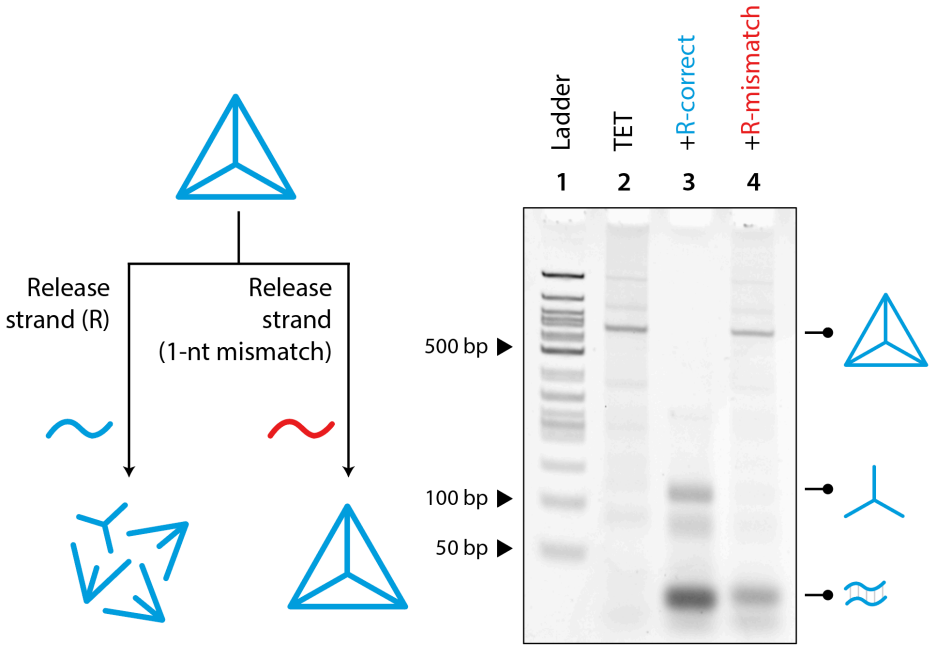

Figure S7. *Specificity of release mechanism*. Polyacrylamide non-denaturing gel showing the disassembly of the tetrahedron only on the addition of the correct release strand (lane 3) and a release strand with even a single nucleotide mismatch has no effect (lane 4).

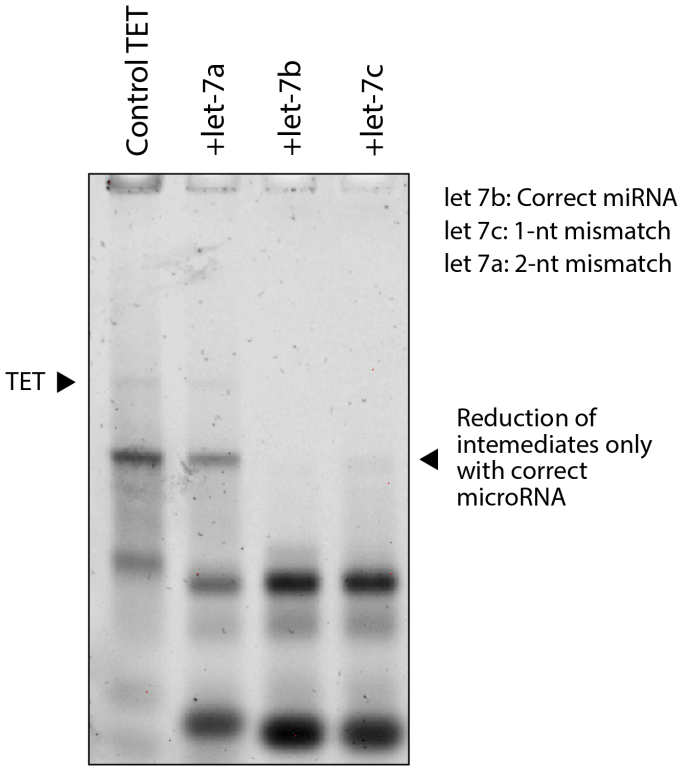

Figure S8. *microRNA-recognizing tetrahedron*. The sequence of the tetrahedron was modified so that strand S is complementary to a microRNA (let 7b). MicroRNA are used as release for this tetrahedron with let-7b being the correct release strand, and let-7c and let-7a with 1- and 2-nt mismatches.