Single-Molecule Protein Encapsulation in a Rigid DNA Cage

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Oligonucleotide sequences
All oligonucleotides were supplied by Integrated DNA Technologies. Sequences were designed using NANEV.\[^{[5]}\]

s1:
TCACCAGGCAAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTA

s1 was supplied with a 5’ C\(_6\)-amino modification, used for conjugation to the protein. The sequence given here leads to a protein attachment at the 5\(^{th}\) nucleotide along the edge formed by hybridization of s1 and s4 (see main text). Other versions of s1 with different protein attachment points are obtained by transferring nucleotides from the 5’ to the 3’ end.

s2:
CTTGCTACGATCAGATTTAGGGAATGTCGACATGCGAGGGTCCAATACCGACGATTACAG

s3:
GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG

s4:
CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC

Conjugation of cytochrome \(c\) to oligonucleotide s1
Holo-cytochrome \(c\) from equine heart was supplied by Sigma. The protein was conjugated to the amino-modified oligonucleotide s1 by means of two heterobifunctional cross-linkers, sulfosuccinimidyl 4-(\(N\)-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) and \(N\)-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) supplied by Pierce Biotechnology.

Amino-modified oligos s1 were ethanol precipitated and re-suspended in phosphate buffer (100 mM Na\(_2\)HPO\(_4\), 100 mM NaCl, pH 7.3) to 1 mM. The DNA solution was combined 1:2 with a saturated sulfo-SMCC solution (2.9 mg/mL) in phosphate buffer and incubated for 1 hour. Cytochrome \(c\) was dissolved in phosphate buffer to 1 mM, combined 25:2 with SPDP solution (5 mg/ml in DMSO) and incubated for 1 hour. Bio-Rad Micro Bio-Spin P-6 columns were used to remove excess cross-linkers from both solutions. Tris(2-carboxyethyl)phosphine hydrochloride (Sigma) was dissolved in phosphate buffer (10 mg/mL) and added 1:10 by volume to the cytochrome \(c\) solution to reduce the S-S bond in SPDP. After 30 minutes the DNA and cytochrome \(c\) solutions were combined (~5:4 by volume) and incubated overnight at 4°C. \(N\)-ethylmaleimide (Sigma) was dissolved in phosphate buffer (10 mg/mL) and added 1:25 by volume to the DNA-protein conjugate solution to bind any unreacted thiol groups. After 30 minutes a Bio-Rad Micro Bio-Spin P-6 column was used to transfer the conjugates into TM buffer (see below).

The conjugates were gel-purified (see below) to remove proteins bound to more than one oligonucleotide.

Self-assembly of tetrahedra
Equal amounts of s1-cytochrome \(c\) conjugate and oligonucleotides s2 - s4 were combined to a final concentration of 250 nM of each oligonucleotide in Tris buffer with 5 mM of divalent cations (either TM buffer: 10 mM Tris-HCl (pH 8), 5 mM Mg\(_2\)Cl\(_2\) or TC buffer: 20 mM Tris-HCl (pH 8), 5 mM CaCl\(_2\)). Annealing was performed by holding the mixture at 54°C for 3 minutes followed by cooling to 4°C over approximately 30 seconds. Tetrahedra can also be formed by incubation at room temperature, although the yield of the correctly self-assembled structure is reduced (see Figure S3).

Polyacrylamide gel electrophoresis
Native gels were run in TAE buffer. Figure 1c, Figure 2a, Figure S2a and Figure S3: 8% acrylamide; Figure S1a: 10% acrylamide (29:1 acrylamide:bisacrylamide ratio).

The SDS denaturing gel in Figure S1b has a 5% stacking and 12% separating layer.\[^{[2]}\]

Urea-SDS denaturing gels (Figure 2b and Figure S2b): Samples were combined 1:1 with a loading buffer containing 7 M urea and 3% SDS, held at 95 degrees for 5 minutes, then run on a denaturing gel containing 2.5 M urea and 0.1% SDS with stacking and separating layers containing 5% and 12% acrylamide respectively.
Gel purification

The s1-protein conjugates were gel purified on native PAGE gels with 5% stacking and 12% separating layers (29:1 acrylamide:biacrylamide ratio) in Tris-glycine buffer. The desired bands were cut out of the gel and the DNA recovered: gel slices were crushed, covered with buffer, soaked overnight and spun on Millipore Ultrafree-MC HV centrifugal filters (0.45µm) to remove the crushed gel. Tetrahedra and tetrahedra-protein conjugates were gel purified on 8% native polyacrylamide gels in TAE buffer.

Ligation

Oligonucleotides s2 - s4 to be ligated were purchased with 5’ phosphorylation (Integrated DNA Technologies) and ligated by incubating overnight with T4 DNA ligase (New England Biolabs) at room temperature.

Figure S1. Confirmation of the identity of the s1-protein conjugate. a) Native polyacrylamide gel showing products of incubation with proteinase K. b) SDS denaturing polyacrylamide gel showing products of incubation with exonuclease I. P: protein (cytochrome c), s1: oligonucleotide s1, c: s1-protein conjugate. Only the band that we have identified as the conjugate is digested by both enzymes, as expected.

Figure S2. Products of ligation of unmodified tetrahedra. a) Native PAGE of gel-purified unmodified tetrahedra with 0 - 4 edges ligated (labeled 0L – 4L). b) Denaturing PAGE of the samples shown in a). Control: linear oligonucleotide s4.
Figure S3. Assembly of DNA tetrahedra with cytochrome c attached at nucleotide 13 at different temperatures. Lanes marked 34º - 94º: Oligonucleotides were combined and held at the stated temperature (in ºC) for 3 minutes followed by fast cooling to 4ºC. The yield of tetrahedron formation does not increase strongly above 54ºC. The conjugate is stable up to 74ºC. Lanes marked 94ºS and 54ºS: Oligonucleotides were combined and heated to the stated temperature (in ºC) and then cooled slowly (~10 minutes) to room temperature. Lanes marked R0.5 and R2: Oligonucleotides were combined and held at room temperature for ~0.5 and 2 hours respectively. DNA tetrahedra are formed, although with a lower yield of the correctly assembled structure; residual DNA and DNA-protein conjugates that have not been incorporated into tetrahedra can be seen in lane R0.5.

Images
Figure 1b was rendered using PYMOL v.99© (Delano Scientific).

References