Supporting Information

Stabilisation of Self-Assembled DNA Crystals by Triplex-Directed Photo-Cross-Linking

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Experimental Procedures

Oligonucleotides and synthesis: Oligonucleotides were synthesized on an Applied Biosystems ABI 394 automated DNA/RNA synthesizer on the 0.2 μmole scale using standard procedures or purchased from Integrated DNA Technologies (Coralville, IA). Phosphoramidite monomers and other reagents were obtained from Applied Biosystems, Link Technologies and Glen Research. All oligonucleotides were purified by 15-20% denaturing polyacrylamide gel electrophoresis (19:1 acrylamide/bisacrylamide). 4,5′,8-trimethylpsoralen was attached to the triplex-forming oligonucleotide (TFO) via a C6 linker to the 5′-terminal phosphate. Oligonucleotide sequences are shown on page 3.

Tile assembly: Oligonucleotides for each tile were mixed stoichiometrically in pH 5 TA-Mg buffer (40 mM Tris-acetate containing 15 mM magnesium acetate) and annealed using the following protocol: 90 °C for 5 minutes, 65 °C for 20 minutes, 45 °C for 20 minutes, 37 °C for 30 minutes and 20 °C for 30 minutes. The TFO was added after tile assembly and left to equilibrate at 4 °C for >8 hrs. Unless otherwise stated the final concentration of the tile was 4 μM, whilst the final concentration of the TFO was 4 and 12 μM for the 3TA and 3TS tile systems, respectively (i.e., a 1:1 ratio of TFO:TFO binding sites per tile).

Non-denaturing polyacrylamide gel electrophoresis (PAGE): TFO binding was examined by subjecting the tiles to an electrophoretic mobility shift assay (EMSA). Complexes were run on a non-denaturing 8% polyacrylamide gel in pH 5 TA-Mg buffer at 4 °C and visualised by post-staining with Stains-All (Sigma Aldrich).

Psoralen photo-cross-linking: Cross-linking was initiated by irradiating the tiles or crystals with UV light at a wavelength of 365 nm at a distance of 2 cm for one hour on ice. Crystals were also washed three times by transferring them consecutively between three drops of mother liquor placed on a glass slide.

Denaturing polyacrylamide gel electrophoresis (PAGE): Cross-linking was examined by subjecting the products of the reaction to denaturing PAGE. The complexes were heated to 95 °C and then run on a denaturing 20% polyacrylamide gel containing 8.3 M urea at 55 °C and post-stained with Stains-All dye (Sigma Aldrich). Running buffer consisted of 89 mM Tris, 89 mM Boric acid, and 2 mM EDTA (TBE). Sample buffer consisted of 10 mM NaOH, 1 mM EDTA, containing 0.1% of Bromophenol Blue and Xylene Cyanol FF tracking dye.

Ultraviolet (UV) melting: Thermal stability of the tiles was determined by measuring their UV absorbance at 260 nm as a function of temperature with a Cary 100 Bio UV-Visible Spectrophotometer. Samples were heated at a rate of 0.15 °C/min. The final concentration of the tile and TFO was 0.5 and 1.5 μM, respectively. Melting temperatures (T_m) were determined from the first derivatives of the melting profiles using the software provided with the machine and differed by less than 1 °C between experiments. Hysteresis was not observed between melting and annealing profiles.
**Crystallisation:** Crystals were grown from 5 μl hanging drops containing pH 5 TA-Mg buffer and equilibrated against a 600 μl reservoir of 1.75 M ammonium sulfate. The whole set-up was kept at 4 °C until crystals were obtained (typically 3-5 days).

**Crystal stability:** The influence of cross-linking on crystal stability was examined by heating the crystals using a Warner TC-324b in-line heater and observing crystal morphology as a function of temperature using an optical microscope. The temperature at which the crystals were no longer visible was considered the crystal melting temperature.
Oligonucleotide sequences (5'- to 3'-)

3-turn symmetric (3TS)-intra
3TS-1: TCTGATGTGGCTGC (14 nts)
3TS-2: GAGCAGCCTAAGAAAGAGAGGAGCATCA (31 nts)
3TS-3: TCTTTCTTACCTCTCTTTTTCTTACCTCTCTTCTCTTTCCTCTT (51 nts)

3-turn asymmetric (3TA):
3TA-1: TCTGATGTGGTAGG (14 nts)
3TA-2: GTCCTACCTGGTGAGTACGTTCAGGACT (31 nts)
3TA-3: ACAGTCTGAGGAT (14 nts)
3TA-4: CAGATACCTGATAATAGGTTGACCGACTACG (31 nts)
3TA-5: TGCGTAGTGGTGCTC (14 nts)
3TA-6: GAGCGACCTGCTCTGGTGACATCA (31 nts)
3TA-7: CTATTATCCTGAACGTACTCAACCCCTCTTCTCTTTTCTACAGTAC (51 nts)

3TA-intra (with 3TA1, 3TA2, 3TA3, 3TA4, and 3TA5)
3TA-6-1: GAGCGACCTAAGAAAGAGAGGACATCA (31 nts)
3TA-7-1: CTATTATCCTGAACGTACTCAACCCCTCTTCTCTTTTCTACAGTAC (51 nts)

3TA-inter (with 3TA2, 3TA3 and 3TA4)
3TAJ-1: TATGATGTGGTAGG (14 nts)
3TAJ-5: TGCGTAGTCTTTCTC (14 nts)
3TAJ-6: TAAGAAAGAGAGGACGTCTGGTGACATCA (31 nts)
3TAJ-7: CTATTATCCTGAACGTACTCAACCCCTCTTCTCTTTTCTACAGTAC (51 nts)

Triplex forming oligonucleotides (TFO):
TFO: TTCTTTTTCTCTCTCTCTCT (13 nts)
pso-TFO: Pso-TTCTTTTTCTCTCTCTCTCTCT (13 nts) (where Pso is psoralen)

Figure S1. Sequences of the oligonucleotides used in this study. All oligonucleotides were unmodified except the psoralen-modified TFO that contained 4,5',8-trimethylpsoralen appended via a C6-linker to the 5'-end of the oligonucleotide.
**Figure S2.** Non-denaturing PAGE demonstrating the binding of the unmodified TFO and psoralen-modified TFO to the 3TA-intra, 3TS-intra and 3TA-inter triangles. Tiles were annealed in pH 5 TA-Mg buffer before addition of the TFO and the complexes left to equilibrate at 4 °C for > 8 hours. The band shifts upon binding of the TFO to the 3TA-intra and 3TA-inter triangles are not large on account of the relatively small differences in molecular weight of the two species (186-nt triangle versus 199-nt TFO-bound triangle). Nevertheless, these small shifts were reproducible and demonstrate the successful interaction of the TFO with the triangles. The shift for the TFO with the 3TS-intra triangle is much larger due to the presence of three TFO binding sites and a greater difference in molecular weight of the two species (186-nt triangle versus 225-nt TFO-bound triangle).
Figure S3. Ferguson analysis was used to examine the electrophoretic mobility of the cross-linked species (ii) and (iii) shown in Figure 2. (a) Products of the UV cross-linking reaction were separated on 14, 16, 18 and 20% polyacrylamide gels alongside a linear single-stranded DNA marker. (b) Ferguson plot. Blue squares correspond to the proposed 94-nt cross-linked species (ii) (bis-adduct) shown by the blue arrows in (a) and the blue diamonds the 100-nt single-stranded marker of similar size. Red circles correspond to the proposed 64-nt cross-linked species (i) (mono-adduct) shown by the red arrows in (a) and the red triangles the 60-nt single-stranded marker of similar size. Comparing the slopes of these plots suggest the bis-adduct exhibits roughly twice the frictional drag of the single-stranded 100-nt linear control (i.e., twice the slope), whilst the mono-adduct exhibits roughly one and half times the frictional drag of the single-stranded 60-nt linear control (i.e., one and half times the slope).
Figure S4. Cross-linking of the 3TA-intra tile with a 1:1, 2:1, 5:1 and 10:1 concentration of pso-TFO to binding sites (a) and 1, 2, 3 or 5 hr UV exposure (b). Tiles were annealed in pH 5 TA-Mg buffer before addition of the TFO and the complexes left to equilibrate at 4 °C for > 8 hours. The complexes were then exposed to UV light at 4 °C and the products separated on a 20% denaturing polyacrylamide gel. Increasing both TFO concentration and UV exposure enhances the efficiency of cross-linking.
Figure S5. Triplex-directed cross-linking of the 3TS-intra tile in solution. Tiles were annealed to 20 °C before addition of the TFO. The complexes were then exposed to UV light at 4 °C for one hour and the products separated on a 20% denaturing polyacrylamide gel. The cartoon shows each of the denatured strands of the tile and the potential products of the cross-linking reaction. Strand colourings are the same as those shown in Figure 1.
Figure S6. The thermal stability of cross-linked tensegrity triangles was examined by UV melting. Tiles were annealed to 20 °C before addition of the TFO. The complexes were then exposed to UV light at 4 °C for one hour and UV melting and annealing profiles recorded with a rate of heating of 0.1 °C/min. It can be seen that these profiles did not exhibit any hysteresis. Melting curves for all of the complexes exhibit two transitions: a transition at low temperature attributable to the dissociation of the shorter crossover strands located at the corners of the triangle, followed by a transition at higher temperature for the dissociation of the central and helical strands that contain the TFO binding site. It can be seen that psoralen cross-linking at the TFO binding site led to an increase in these higher transitions, and was greater for the 3TS-intra complex with three cross-links within the triangle.
**Figure S7.** Cross-linking of the 3TA-intra crystal with a 1:1, 2:1, 5:1 and 10:1 concentration of pso-TFO to binding sites (a) or 1, 2, 3 and 5 hr UV exposure (b). Tiles were annealed in pH 5 TA-Mg buffer before addition of the TFO and the complexes left to equilibrate at 4 °C for > 8 hours. The complexes were then exposed to UV light at 4 °C and the products separated on a 20% denaturing polyacrylamide gel. Increasing both TFO concentration and UV exposure enhances the efficiency of cross-linking.
Figure S8. Optical images of the 3TA-intra crystals at different temperatures. For each image the drop on the right contains the cross-linked crystals and on the left the uncross-linked crystals. Tiles were annealed to 20 °C before addition of the TFO and the setup left to equilibrate at 4 °C until crystals were observed (3-5 days). The drops were heated using an in-line heating probe. The image was taken in the absence of polarizer.
**Figure S9.** Optical images of the 3TS-intra crystals at different temperatures. For each image the drop on the right contains the cross-linked crystals and on the left the uncross-linked crystals. Tiles were annealed to 20 °C before addition of the TFO and the setup left to equilibrate at 4 °C until crystals were observed (3-5 days). The drops were heated using an in-line heating probe. The image was taken in the absence of polarizer.
Figure S10. Optical images of the 3TA-inter crystals at different temperatures. For each image the drop on the right contains the cross-linked crystals and on the left the uncross-linked crystals. Tiles were annealed to 20 °C before addition of the TFO and the setup left to equilibrate at 4 °C until crystals were observed (3-5 days). The drops were heated using an in-line heating probe. The image was taken in the absence of polarizer.