Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Triplex-Directed Covalent Cross-Linking of a DNA Nanostructure

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Experimental Methods

Oligonucleotides and synthesis All oligonucleotides were synthesized on an Applied Biosystems ABI 394 automated DNA/RNA synthesizer on the 0.2 or 1 μmole scale using standard methods. Phosphoramidite monomers and other reagents were purchased from Applied Biosystems or Link Technologies. 4,5′,8-trimethylpsoralen (Link Technologies) was incorporated into the triplex-forming oligonucleotide *via* a C6 linker attaching it the terminal phosphate at the 5′-end of the oligonucleotide. The full sequences of the oligonucleotides used in this study are shown in Fig. S1.

Preparation of the DX assemblies The oligonucleotides of each DX tile (or relevant control complex) were mixed in stoichiometric amounts at an appropriate concentration (0.1-0.3 μM) in TA-Mg buffer (pH 5.0 40 mM Tris-acetate containing 15 mM $Mg(OAc)_2$) and annealed at a rate of 0.5 ˚C min in a thermocycler from 100 ˚C to 5 ˚C. To produce the DX-A_TB_T dimer and the DX-A_CB array, the appropriate DX tiles were then mixed and annealed at a slower rate of 0.25 ˚C min from 50 ˚C to 5 ˚C. To generate the semi-ligated DX-A_CB array, the tiles were annealed in T4 DNA ligase buffer (pH 7.5 50 mM Tris-HCl containing 10 mM $MgCl_2$, 10 mM DTT and 1 mM ATP) before addition of 1 μl (10 units) of T4 DNA ligase (New England Biolabs) for 3 hrs at room temperature. The samples were then mixed with two volumes of TA-Mg buffer to lower the pH (<6.0), a prerequisite for triplex formation. The final concentration of the DX assemblies was kept constant at 0.1 μM.

DNase I protection assay The non-crossover oligonucleotide of the DX-A_C tile (or DS-A_C control) was phosphorylated at its 5′-end with γ-^{32}P[ATP] using T4 polynucleotide kinase (New England Biolabs) and purified by denaturing PAGE. It was then combined with the four remaining unlabelled oligonucleotides and annealed as above. The pso-TFO (0.003-1 μM) was added either before or after the annealing of the DX tile and left to equilibrate for > 8hrs at 4 ˚C. The resulting complexes were mixed with 2 μl of DNase I (typically 0.01 units/ml) dissolved in DNase I buffer (20 mM NaCl, 2 mM $MgCl_2$ and 2 mM $MnCl_2$). The reaction was stopped after 1 min by adding 4 μl DNase I stop solution (80% formamide, 10 mM EDTA, 10 mM NaOH, and 0.1% (w/v) bromophenol blue). Samples were then denatured by heating to 100 °C for 3 min. The products of digestion were

separated on a denaturing 14% polyacrylamide gel, which was fixed, dried and subjected to phosphorimaging.

Cross-linking assay Each DX assembly contained an appropriate oligonucleotide that was phosphorylated at its 5′-end with γ-^{32}P[ATP] using T4 polynucleotide kinase (New England Biolabs), purified by denaturing PAGE and mixed with the remaining unlabelled oligonucleotides. The pso-TFO (1 μM) was added after the annealing of the complexes and left to equilibrate for > 8hrs at 4 ˚C. Samples were then irradiated with UV light at a wavelength of 365 nm at a distance of 2 cm for 1 hr on ice. The products of the reaction were run on an appropriate denaturing polyacrylamide gel, which was fixed, dried and subjected to phosphorimaging.

Atomic force microscopy. After annealing, the DX-A_CB array was incubated with the pso-TFO (1 μM) for > 8hrs at 4 ˚C. An aliquot of this sample was then spotted onto freshly cleaved mica and allowed to adsorb for 5 minutes. Buffer salts were removed by addition of 5-10 drops of ultrapure water from an Elga UHQ-II water purification system with a resistivity of 18 MΩ·cm, the drop was shaken off and the sample dried using compressed air. Imaging was undertaken by tapping mode in air on a Multimode AFM equipped with a Nanoscope III controller using silicon nitride cantilever tips (Windsor Scientific, k = 0.32 N/m).

Full oligonucleotide sequences

DX-A

5′-GATGGCGACATCCTGCCGCTATGATTACACAGCCTGAGCATTGAC

5′-CTGACGCTGGTTGATCGGACGATACTACATGCCAGTTGGACTAACGG

5′-GTAGCGCCGTTAGTGGATGTC

5′-CGACTGCGGTCAATGCTCACCGATCAACCAG

5′-TGTAGTATCGTGGCTGTGTAATCATAGCGGCACCAACTGGCA

DX-B

5′-CGTCAGGCTGCTGTGGTCGTG

5′-GCCATCCGTCGATACGGCACCATGATGCACG

5′-GGTCACTGGTTAGTGGATTGCGTAGTACAACGCCACCGATGC

5′-CGCTACCGTGCATCATGGACTAACCAGTGACCGCATCGGACAGCAGC

5′-CGCAGTCGCACGACCTGGCGTTGTACTACGCAATCCTGCCGTATCGACG

DX-A_C

5′-GATGGCGACATCCTGCCTCTCTTCTTTCTTAGCCTGAGCATTGAC

5′-TGTAGTATCGTGGCTAAGAAAGAAGAGAGGCACCAACTGGCA

DX-A_T

5′-GATGGCGACATCCTGCCGCTATGATTACACAGCCTGATCTCTTCT

5′-CGTAAGAAAGAAGAGATCACCGATCAACCAG

DX-B_T

5′-TTCTTACGCACGACCTGGCGTTGTACTACGCAATCCTGCCGTATCGACG

5′-GCTGCTGTGGTCGTG

5′-CGTCGATACGGCACCATGATGCACG

5′-CGTGCATCATGGACTAACCAGTGACCGCATCGGACAGCAGC

DX-A_G

5′-GATGGCGACATCCTGCCTCTCTTCTTTCTTCGCCTGAGCATTGAC

5′-TGTAGTATCGTGGCGAAGAAAGAAGAGAGGCACCAACTGGCA

DX-A_N

5′-GATGGCGACATCCTGAAGAAAGAAGAGAGGAGCCTGAGCATTGAC

5′-TGTAGTATCGTGGCTCCTCTCTTCTTTCTTCACCAACTGGCA

Pso-TFO

5′-Psoralen-C6-TTCTTTCTTCTCT

Fig. S1 Sequences of the oligonucleotides used in this study. Each DX tile was generated using the appropriate combination of oligonucleotides shown. All oligonucleotides were unmodified except the 13-mer TFO.

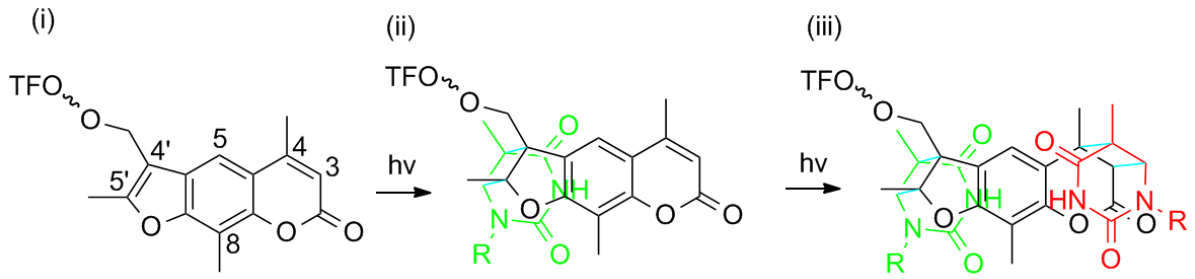

Fig. S2 TFO-directed Psoralen Cross-linking. 4,5′,8-trimethyl psoralen (i) can be attached to a triplex-forming oligonucleotide (TFO) in such a way as to allow the 3,4 and 4′,5′-double bonds to react with one thymine on each strand at a 5′-TpA sequence located at the duplex-triplex junction. Upon intercalation the absorption of a single UV photon of wavelength >310 nm leads to the generation of a monoadduct *via* a cycloaddition reaction at one of these bonds, with the 4′,5′-double bond most susceptible to monoadduct formation (ii). The subsequent absorption of a second photon leads to a second cycloaddition reaction at the 3,4 double bond, and the formation of a bisadduct, with the psoralen-TFO cross-linked to both DNA strands (iii). The psoralen molecule is shown in black and the two thymines within the 5′-TpA step in green and red.

DX tile formation and recognition by the pso-TFO

A DNase I protection assay was carried out on both the fully assembled tile (DX-A_C) and a double-stranded equivalent containing only the top and cyclic crossover strands in both the absence and presence of the pso-TFO. DNase I generates single strand nicks at accessible points in the phosphodiester backbone by cleaving the O3′–P bond and digestion of complexes containing a single ^{32}P-labelled strand with the enzyme generated fragments that were separated by denaturing polyacrylamide gel electrophoresis (PAGE) (Fig. S3A). As expected, analysis of the cleavage patterns for the DS and DX complexes in the absence of the pso-TFO (Lanes 1 and 3) revealed marked differences in cleavage by the enzyme: the DX tile was cut less efficiently than the DS complex, particularly in regions corresponding to the crossover points (marked in bold). This is attributed to the crossovers occluding the binding and cleavage by the enzyme and can be taken as evidence for successful tile formation. In contrast, analysis of the cleavage patterns for each complex in the presence of the pso-TFO (Lanes 2, 4-9) revealed missing bands (a 'footprint') at regions that have undergone triplex formation and that cannot be cut by the enzyme (underlined sequence). Importantly, the footprint is located at the intended sequence and demonstrates only specific binding of the pso-TFO conjugate. Furthermore, the cleavage patterns also provide evidence for intercalation of the psoralen moiety at the 5′-end of the target site, as shown by the enhanced band at this location (marked by asterisk). This hypersensitivity presumably occurs due to distortion of the duplex structure upon intercalation to a form that is more easily accessed by the enzyme. Importantly, the remainder of the cleavage pattern is the same as in the absence of pso-TFO, suggesting that this distortion is not propagated far along the molecule and that the crossovers remain intact. The same result was obtained when the TFO was added before annealing the tile, suggesting that it does not influence tile assembly (Fig. S3B).

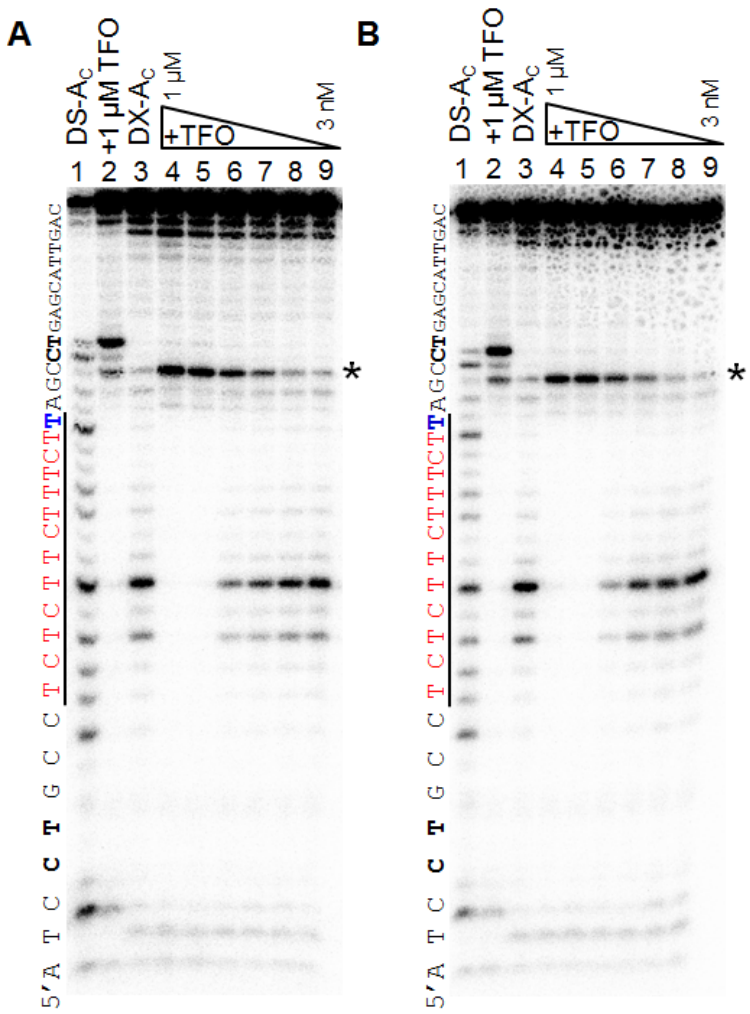

Fig. S3 DNase I cleavage patterns for the DX-A_C tile in the presence and absence of pso-TFO. The TFO was added either before (A) or after (B) the assembly of the tile. The single ^{32}P-labelled non-crossover strand of DX-A_C was annealed with the remaining four unlabelled strands at a final concentration of 0.1 µM in the presence of pso-TFO at a final concentration of 1, 0.3, 0.1, 0.03, 0.01 and 0.003 µM. The complexes were then digested by DNase I (Lanes 3-9) and the products of digestion separated on a denaturing 14% polyacrylamide gel, which was fixed, dried and subjected to phosphorimaging. An additional duplex control containing the ^{32}P-labelled non-crossover strand and its complementary crossover strand was digested in the presence and absence of TFO for comparison (Lanes 1-2). The non-crossover strand of the DX tile is shown on the right of the gel with the TFO target site in red, TpA step in dark blue and crossovers in bold. The underlined region represents the footprint generated upon binding of the TFO and the asterisk the site of DNase I hypersensitivity due to psoralen intercalation.

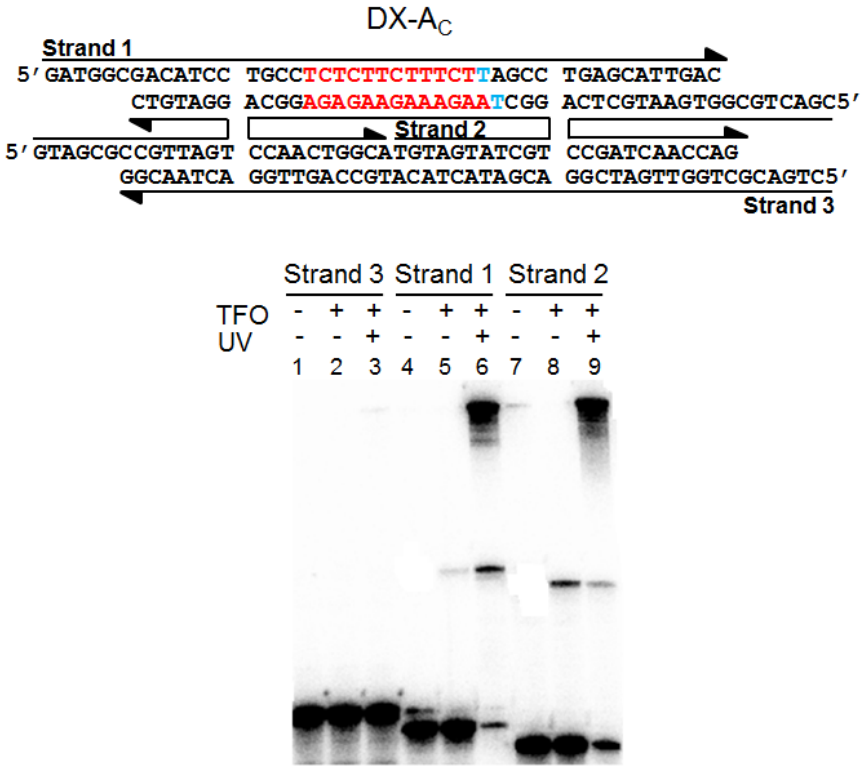

Fig. S4 Cross-linking of the DX-A$_C$ tile by the pso-TFO. Electrophoretic mobility shift assay of the cross-linked products generated from the DX-A$_C$ tile in the absence and presence of pso-TFO. The experiment was undertaken three times with a different ^{32}P-labelled strand within the tile. In each case this was annealed with the remaining four unlabelled strands at a final concentration of 0.1 μM before the pso-TFO was added to a final concentration of 1 μM and left to equilibrate overnight at 4 ˚C. The complexes were then exposed to UV light at a wavelength of 365 nm for 1 hr and the cross-linked products separated on a denaturing 14% polyacrylamide gel, which was fixed, dried and subjected to phosphorimaging. It is clear that the cross-linking reaction was directed to the intended duplex target.

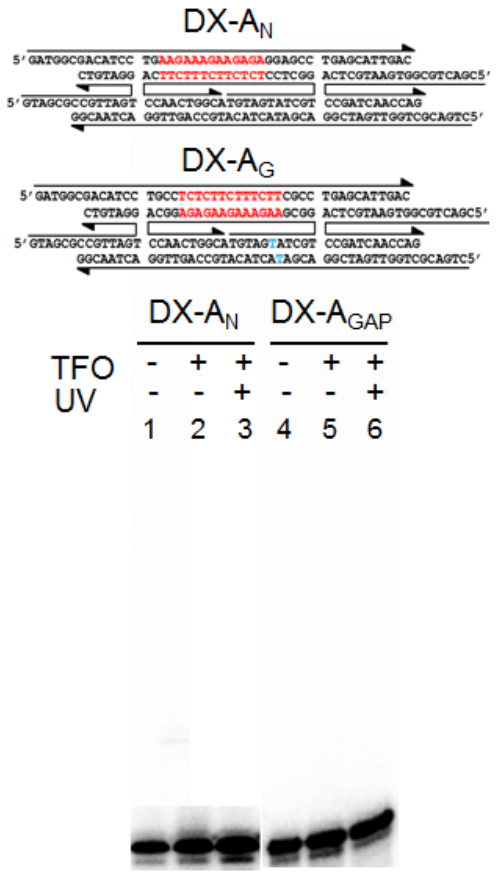

Fig. S5 Cross-linking of DX tiles by the pso-TFO. Electrophoretic mobility shift assay of the cross-linked products generated from the DX-A_N tile, which lacks an adjacent TpA step, and DX-A_G tile, which has a TpA step located across the inter-helical gap on the adjacent helix, in the absence and presence of pso-TFO. The DX-A_N tile carried a ^{32}P-label on the top non-crossover strand, whilst the DX-A_G tile carried a ^{32}P-label on the bottom non-crossover strand. In each case the labelled strand was annealed with the remaining four unlabelled strands at a final concentration of 0.1 μM before the pso-TFO was added to a final concentration of 1 μM and left to equilibrate overnight at 4 ˚C. The complexes were then exposed to UV light at a wavelength of 365 nm for 1 hr and the cross-linked products separated on a denaturing 14% polyacrylamide gel, which was fixed, dried and subjected to phosphorimaging. Neither complex was cross-linked in the presence of the pso-TFO. It is clear that a TpA step adjacent to the TFO target site on the same helix is required for cross-linking.

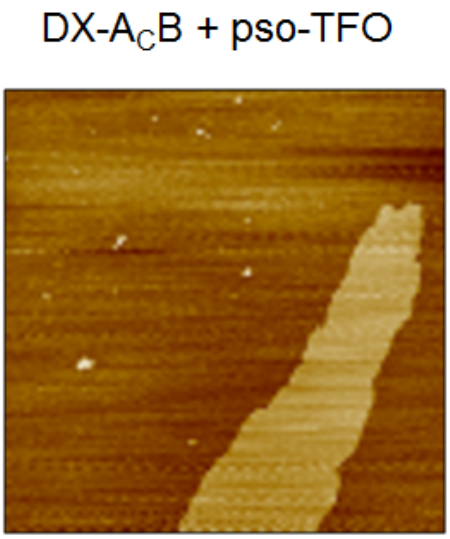

Fig. S6 DX array assembly. 2 x 2 μm AFM image of a mica sample containing the DX-A_CB array in the presence of pso-TFO.