TWJ-Screen: an isothermal screening assay to assess ligand/DNA junction interactions *in vitro*.

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Supplementary Data

Part 1. Oligonucleotides.

Sequences (from 5' to 3'), extinction coefficient (ε) values expressed in $M^{-1}.cm^{-1}$

Unlabeled DNA:

ds26 (ε: 235200): CAATCGGATCGAATTCGATCCGATTG (*self-complementary sequence*)

TG5T (ε: 67900): TGGGGGT

PAGE TWJ-S1 (ε: 131900): CGGAACGGCACTCG

PAGE TWJ-S2 (ε: 135200): CGAGTGCAGCGTGG

PAGE TWJ-S3 (ε: 118700): CCACGCTCGTTCCG

Labeled DNA:

fam*-TWJ-*tamra (ε: 435500): *fam*-ACTCTTCTCG-TTTTTT-CGAGAGCGAC-TTTTTT-GTCGCAGAGT-*tamra*

Preparation of the oligonucleotide stock solutions. The lyophilized strands are firstly diluted in deionized water (18.2 MΩ.cm resistivity) at 500µM for monomolecular and bimolecular DNA constitutive strands and at 1000µM for trimolecular and tetramolecular DNA constitutive strands. The actual concentration of stock solutions is determined by a dilution to 1µM theoretical concentration and a UV-Vis spectra analysis at 260nm (after 5min at 90°C) using the molar extinction coefficient values provided by the manufacturer (*vide supra*).

Preparation of the DNA structures. All DNA structures are prepared in a Caco.K buffer, comprised of 10mM lithium cacodylate buffer (pH 7.2) plus 10mM KCl/90mM LiCl. Monomolecular structures are prepared by mixing the constitutive strand (40µL at 500µM) with lithium cacodylate buffer solution (8µL, 100mM, pH 7.2), plus KCl/LiCl solution (8µL, 100mM/900mM) and water (24µL). Bimolecular structures are prepared by mixing each of the two constitutive strands (40µL, 500µM) with lithium cacodylate buffer solution (16µL, 100mM, pH 7.2), plus KCl/LiCl solution (16µL, 100mM/900mM) and water (48µL). Tetramolecular structures are prepared by mixing each of the four constitutive strands

(20µL, 1000µM) with lithium cacodylate buffer solution (32µL, 100mM, pH 7.2), plus KCl/LiCl solution (32µL, 100mM/900mM) and water (96µL). The final concentrations are theoretically of 250, 125 and 83.3µM for mono-, bi- and tetra-molecular DNA structures respectively. The actual concentration of each DNA is determined via a dilution to 1µM theoretical concentration (expressed in motif concentration) for monomolecular structure (i.e. 4µL in 996µL water), at 1µM for bimolecular structure (i.e. 8µL in 992µL water) and at 0.2µM for tetramolecular structure (i.e. 2.4µL in 997.6µL water), via UV-Vis spectra analysis at 260nm (after 5min at 90°C, baseline correction at 390nm), using the molar extinction coefficient values indicated above.

The high-order DNA structures were folded according to two distinct procedures: i- for the intramolecular architectures, the solutions are heated at 90°C for 5min, cooled in ice for 7h, and then stored at least overnight at 4°C; ii- for the intermolecular structures, the solutions are heated at 90°C for 5min, cooled at 80°C for 10min, 60°C for 30min, 40°C for 30min, 25°C for 4h and then stored at least overnight at 4°C.

Part 2. Gel electrophoresis.

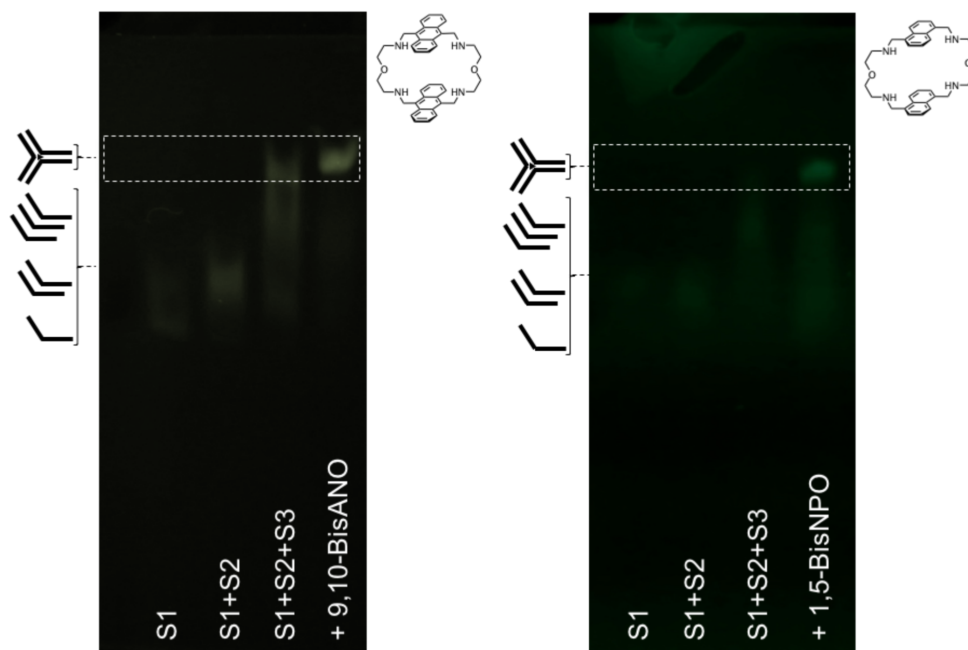

Figure S1. Native PAGE analysis (20% polyacrylamide, 1xTBE, 4°C, 1.5hrs, fluorescent visualization (λ_{ex} = 254nm) after post-staining (10min, 25°C) with SYBR® Safe) of the three-way junction assembling properties of a panel of 9,10-BisAN-O (left panel) and 1,5-BisNP-O (right panel).

Nondenaturing polyacrylamide gel electrophoresis was carried out with 20% polyacrylamide (AA)-bisacrylamide (BAA) (29:1) gel (including a 6% AA-BAA stacking gel). The “running” gel was prepared as follows: 1.89mL H_2O, 2.5mL AA-BAA (40%), 0.5mL TB 10X, 50µL $MgCl_2$ followed by 50µL APS

(100mg/mL) and 15µL TEMED prior to use (5mL final volume); the “stacking” gel was prepared as follows: 1.45mL H_2O, 300µL AA-BAA (40%), 0.2mL TB 10X, 20µL $MgCl_2$ followed by 20µL APS (100mg/mL) and 15µL TEMED prior to use (2mL final volume). DNA samples (TWJ-S1, TWJ-S2 and TWJ-S3) and ligands (9,10-BisAN-O and 1,5-BisNP-O) were prepared as 25µM solutions; DNA/ligand mixtures were prepared prior to use in 20µL (final volume) of 1xTB buffer + 10mM $MgCl_2$ as follows: TWJ-S1 (4µL) + buffer (16µL); TWJ-S1 (4µL) + TWJ-S2 (4µL) + buffer (12µL); TWJ-S1 (4µL) + TWJ-S2 (4µL) + TWJ-S3 (4µL) + buffer (8µL); TWJ-S1 (4µL) + TWJ-S2 (4µL) + TWJ-S3 (4µL) + ligand (4µL) + buffer (4µL). Mixtures were gently steered for 1h30 at room temperature before being loaded on the gel: samples were prepared mixing 15µL of the previously prepared DNA samples (±ligands) and 3µL of sucrose (15% in H_2O; w/v). Prior to the migration, gel is submitted to a pre-treatment at 180V for 1.5hr at 4°C. After loading, the electrophoretic migration was performed in 1×TB (tris-borate buffer), pH 8.3, for 1.5hr at 4°C (180V). After the migration, gels were analyzed after a post-staining step (SYBR® Safe solution, 1:10000, 10mn, 25°C under gentle agitation) with a UVP MultiDoc-It® imaging system (λ_{ex} = 254nm).

Part 3. FRET-melting experiments.

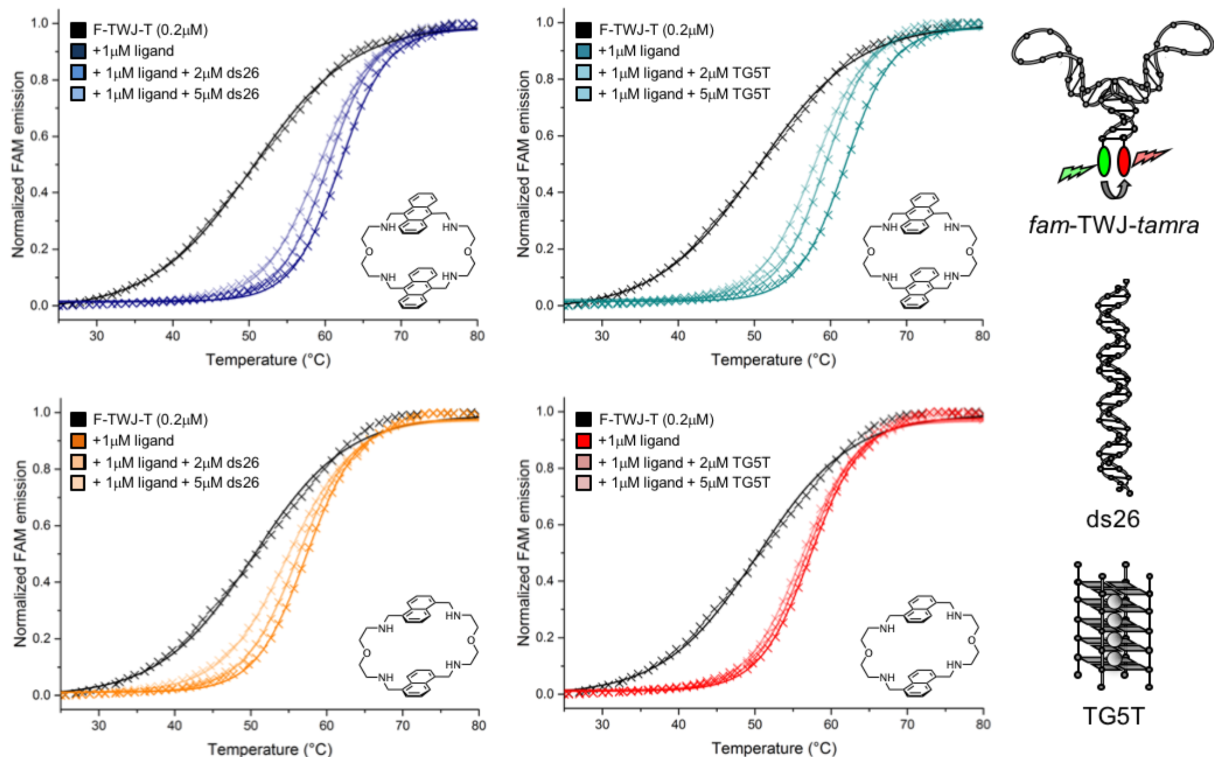

Figure S2. FRET-melting experiments performed with 9,10-BisAN-O (upper panels) and 1,5-BisNP-O (lower panels) with *fam*-TWJ-*tamra* (F-TWJ-T), in absence of presence of competitors, either ds26 (left panels) or TG5T (right panels).

Experiments were performed in a 96-well format using a Mx3005P qPCR machine (Agilent) equipped with a FAM filter (λ_{ex} = 492nm; λ_{em} = 516nm). Experiments were carried out in 100µL (final volume) of

10mM lithium cacodylate buffer (pH 7.2) + 99mM LiCl/1mM KCl, with 0.2µM of labeled DNA and 1.0µM (5equiv.) of ligands (9,10-BisAN-O and 1,5-BisNP-O). Competitive experiments were carried out in 100µL (final volume) of 10mM lithium cacodylate buffer (pH 7.2) + 99mM LiCl/1mM KCl, with labeled DNA (0.2µM), ligands (1.0µM) and increasing amounts (0, 10 and 25equiv.) of unlabeled competitors (ds26 or TG5T). After a first equilibration step (25°C, 30s), a stepwise increase of 1°C every 30s for 65 cycles to reach 90°C was performed, and measurements were made after each cycle. Final data were analyzed with Excel (Microsoft Corp.) and OriginPro®8 (OriginLab Corp.). The emission of *fam* was normalized (0 to 1), and $T_{1/2}$ was defined as the temperature for which the normalized emission is 0.5; $\Delta T_{1/2}$ values are means of 2 to 4 experiments.

		ds26 competitor				TG5T competitor			
	1µM	+2µM	^{FRET}S	+5µM	^{FRET}S	+2µM	^{FRET}S	+5µM	^{FRET}S
9,10-BisAN-O	11.3°C	9.7°C	0.86	8.5°C	0.75	8.6°C	0.76	7.2°C	0.64
1,5-BisNP-O	7.0°C	6.0°C	0.85	4.6°C	0.66	6.5°C	0.93	5.9°C	0.84

Table S1. FRET-melting results (expressed as $\Delta T_{1/2}$ values, in °C) of experiments performed with 9,10-BisAN-O and 1,5-BisNP-O with *fam*-TWJ-*tamra* (F-TWJ-T), in absence of presence of competitors, either ds26 or TG5T. ^{FRET}S is calculated as follows: $^{FRET}S = \Delta T_{1/2[+ligand+competitor]} / \Delta T_{1/2[+ligand]}$