

Supporting Information

**Dual-Input Regulation and Positional Control in Hybrid
Oligonucleotide/Discotic Supramolecular Wires**

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References

Methods and materials

Materials.

All solvents employed were obtained from Biosolve BV. All reagents and solvents were used without further purification. Dibenzocyclooctyne-*N*-hydroxysuccinimidyl ester (NHS-DBCO), triethylamine $\geq 99\%$ (Et₃N), anhydrous dichloromethane (DCM), and minisart filter with pore size 0.2 μm were purchased from Sigma Aldrich. Compound **1** (BiPy-Disc) and **2** (NH₂-BiPy Disc) were provided by dr. Katja Petkau-Milroy (née Petkau).^[1,2] BioBeads™ S-X1 Support 200-400 mesh, *N,N,N',N'*-tetramethyl-ethane-1,2-diamine (TEMED) and Urea sample buffer were purchased from BioRad. Urea PAGE gel was prepared from the kit SequeaGel – UreaGel System and purchased from National Diagnostics. UltraPure™ DNase/RNase-Free Distilled Water and GeneRuler Ultra Low Range DNA Ladder were purchased from Thermo Fisher Scientific. Ammonium persulfate (APS) was ordered from Merck.

All the oligonucleotide sequences were purchased from IDT technology. Cy5 internal modification was commercially available as part of the oligonucleotide backbone. The 3'-Cy3 dye was also commercially available and directly attached to the backbone of the corresponding oligonucleotide. See: <https://eu.idtdna.com/pages/products/custom-dna-rna/oligo-modifications/fluorophores/freedom-dyes>.

Methods

Synthesis of ODN-BiPy Discotic Monomer. DBCO-BiPy-Disc (**3**) was synthesized by the reaction of NH₂-BiPy-Disc (**2**) with NHS-DBCO. Compound **2** (3 mg, 0,91 μmol , 1 eq), NHS-DBCO (1,1 mg, 2,73 μmol , 3 eq) and Et₃N (0,2 μL , 1,37 μmol , 1,5 eq) were dissolved in 0.5 mL of dry DCM. The reaction mixture was stirred at room temperature for two hours and then placed at 4 °C for an additional twelve hours. The crude was purified by size exclusion chromatography using dichloromethane as eluent. The collected fractions were dried under reduced pressure and dissolved in water. The final aqueous solution was lyophilized to give the desired product as a white powder (90% yield). The product (**3**) was characterized by MALDI-TOF.

All oligonucleotides (**a-n**) were dissolved in UltraPure™ DNase/RNase-Free Distilled Water to reach a final concentration of 100 μM . Compounds **a-k** (50 μL , 100 μM) were reacted with an excess of compound **3** (50 μL , 250 μM) at 4 °C with shaking overnight. The crude was saturated with 5 μL of UltraPure™ DNase/RNase-Free NaCl (5 M) solution and dissolved in 300 μL of ice-cold isopropanol. The samples were incubated at -20 °C overnight. The resulting suspension was centrifuged at 4 °C, 14.000 rpm and the supernatant containing unreacted DBCO-BiPy-Disc (**3**) was discarded. The reaction between the DBCO-BiPy-Disc (**3**) and the azide-ODNs (**a-k**) was evaluated by Urea Gel, MS-ES and TLC.

TLC. Analytical thin layer chromatography (TLC) was carried out using Merck pre-coated silica gel using ultraviolet light irradiation at 254 and 365 nm.

Urea PAGE Gel. Urea PAGE gel was prepared fresh using the Mix UreaGel System components. 8 mL of UreaGel Concentrate solution, 1 mL of UreaGel Buffer and 1 mL of UreaGel Diluent were combined in a falcon tube, vortexed and filtered using a 0.2 μm filter. Subsequently, 4 μL of TEMED were added and the mixture was gently swirled to ensure homogeneity. Lastly, 80 μL of freshly prepared 10% (w/v) ammonium persulphate solution was added to the solution. The liquid was inserted in the comb and allowed to polymerize for 2 hours. The gel was pre-run for 60 min using TBE as running buffer. The loading samples were prepared in PCR tubes using UREA sample buffer, heating for 5 minutes at 95°C in a thermal cycler. The ladder used was Thermo Scientific GeneRuler Ultra Low Range DNA Ladder. The gel was run at 150 V for 90 min and washed for 15 minutes with milli-Q water to remove the TBE. The gel was post-stained by using 100 mL of 0.1% Cresyl Violet solution for 10 minutes and washed with water overnight. The gel was imaged using ImageQuant and SYBR Safe filter.

MALDI-ToF. Matrix assisted laser desorption/ionisation time of flight mass spectra (MALDI-TOF-MS) were measured on a PerSeptive Biosystems Voyager-DE Pro spectrometer with a Biospectrometry workstation using α -Cyano-4-hydroxycinnamic acid (CHCA) as matrix material and dichloromethane as solvent.

MS-ES. ESI-MS spectra were recorded using a Quadrupole Electrospray Ionization Mass Spectrometer in negative mode (API-150EX, Applied Biosystems) using 30% isopropanol in water and 0.1% Et₃N as buffer.

UV-Vis. UV-Vis spectra were obtained using a Jasco V-650 spectrophotometer equipped with a Perkin Elmer PTP-1 Peltier temperature control system. The measurements were carried out in quartz cuvettes of 50 μL , with a path length of 0.3 cm. The final concentrations of the ODN-BiPy-Discs were calculated using the extinction coefficient of the Cy3 and Cy5 dyes (136.000 and 250.000 $\text{cm}^{-1}\cdot\text{M}^{-1}$ respectively).

Fluorescence Spectroscopy. Fluorescence emission spectra were recorded in a Varian Cary Eclipse fluorescence spectrophotometer. The excitation wavelength used to excite the discotic molecule, Cy3 and Cy5 derivatives was 345 nm, 545 nm and 635 nm respectively. Subsequently, the emission spectra were recorded at 360 nm, 560 nm and 650 nm.

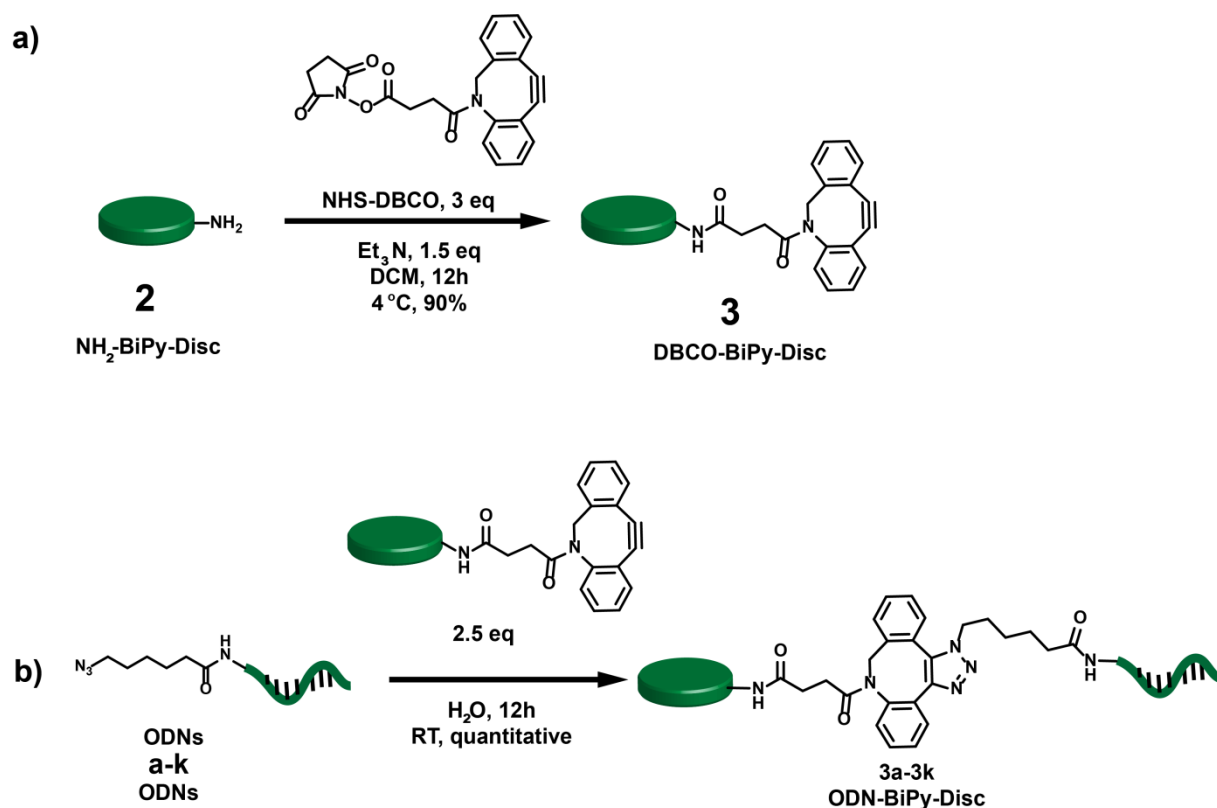


Figure S1. Schematic representation of the synthesis of ODN-BiPy Discs **3a-3k**. (a) Synthesis of the DBCO-BiPy-Disc (**3**) by amide coupling of the NH₂-BiPy-Disc (**2**) with NHS-DBCO. **3** was subsequently reacted with azide-ODNs **a-k** (b) to yield ODN-BiPy Discs (**3a-3k**).

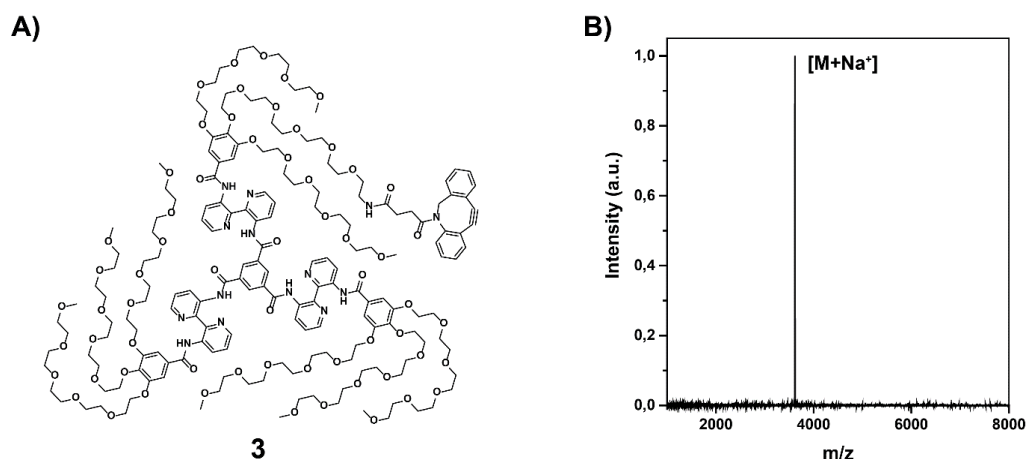


Figure S2. A) Chemical structure of DBCO-BiPy-Disc **3**. B) MALDI-ToF analysis of the DBCO-BiPy-Disc **3** shows a single peak at 3619 Da corresponding to the calculated mass of the $[M+Na^+]$ ion with a mass of 3619 Da.

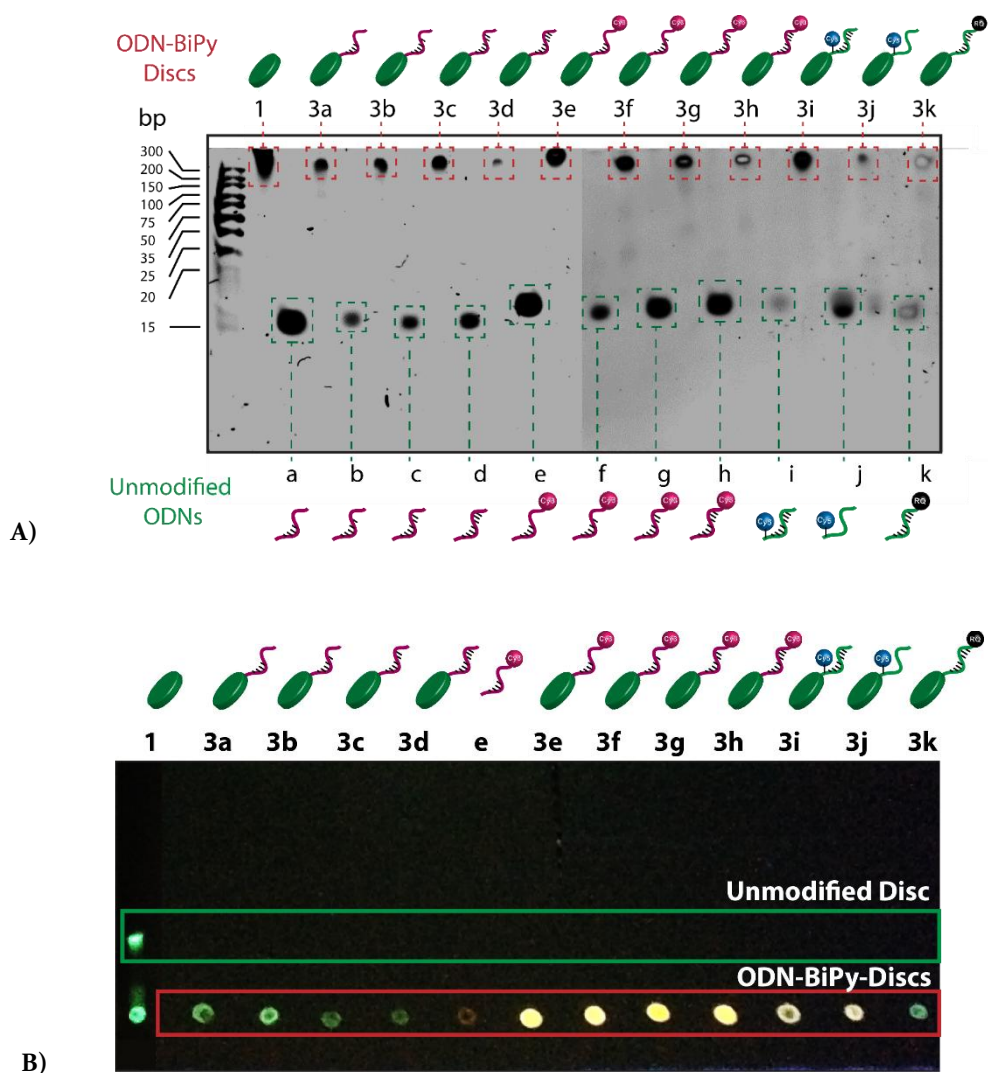


Figure S3. A) Native PAGE Gels (15%Urea) stained with Cresyl violet (0.01 %) of the ODN-BiPy Discs (**3a-3k**). The green dashed-lines indicate the bands corresponding to ODNs **a-k**. In red dashed-lines the bands of ODN-BiPy Discs (**3a-3k**). The number of potential Watson-Crick base pairs are shown for each sequence. B) TLC analysis using DCM:MeOH (1:1) as eluent. Unmodified Disc **1** with $R_f = 0.5$ is compared with ODN-BiPy-Discs (**3a-3k**) and ODN **e**. The green box highlights the absence of unreacted Disc **3** in **3a-3k**. The red box shows the retained ODN-BiPy-Discs, $R_f = 0$.

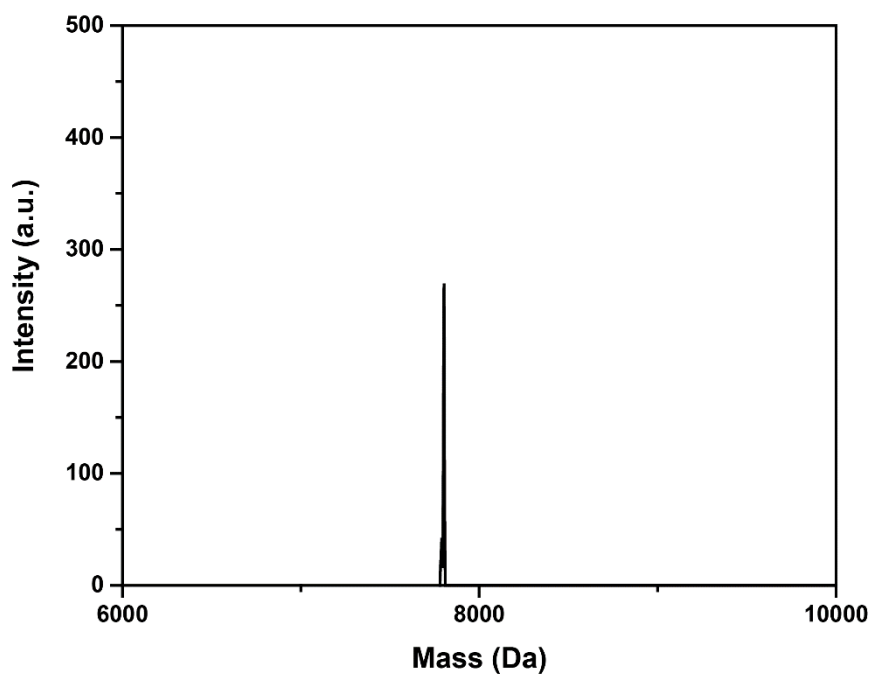


Figure S4. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3a**) measured in negative ion mode. The calculated mass (7800.95 Da) is in agreement with the measured mass (7800.06 Da).

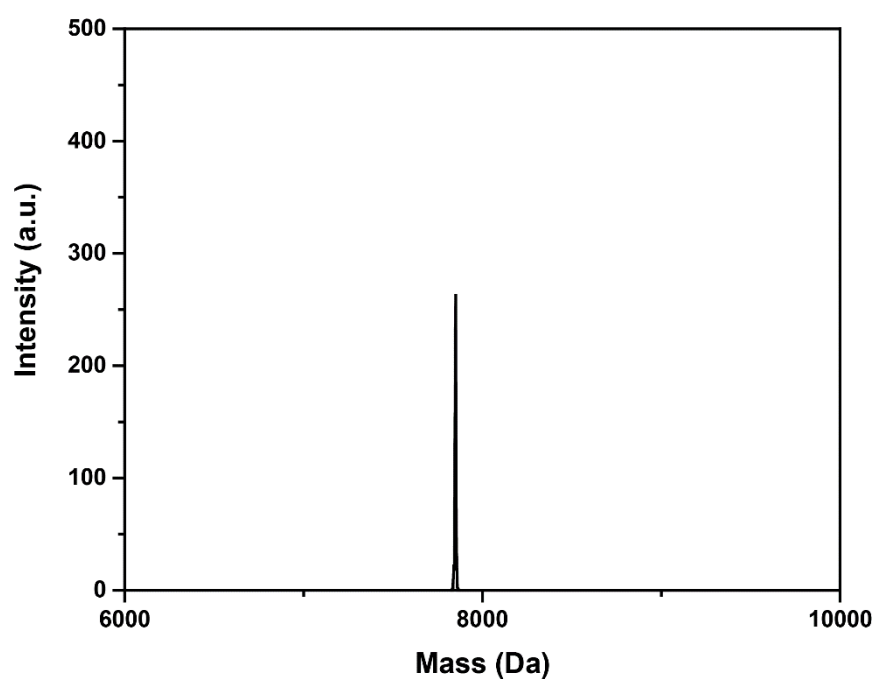


Figure S5. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3b**) measured in negative ion mode. The calculated mass (7827.95 Da) is in agreement with the measured mass (7827.10 Da).

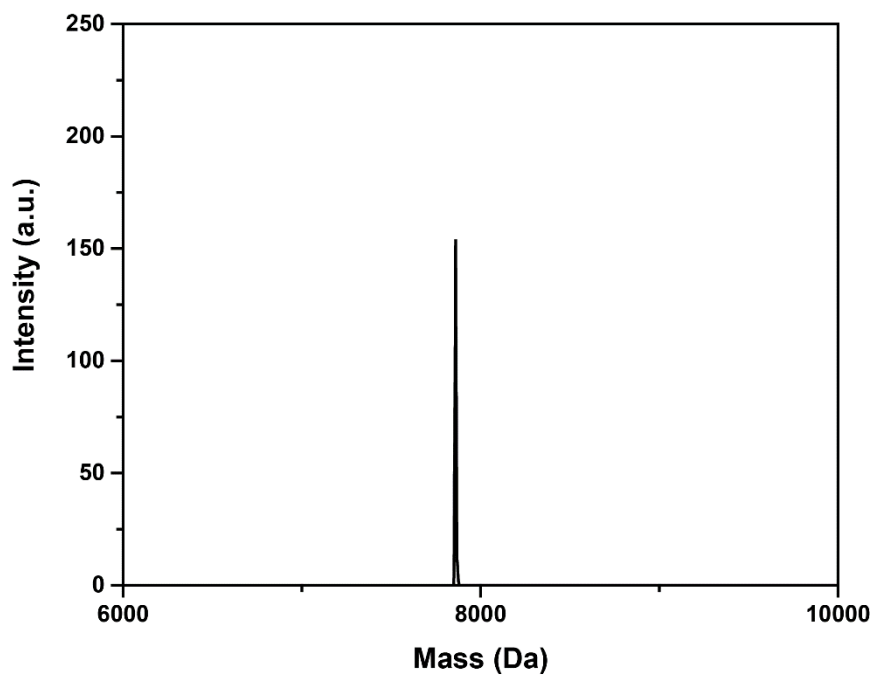


Figure S6. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3c**) measured in negative ion mode. The calculated mass (7855.95 Da) is in agreement with the measured mass (7854.66 Da).

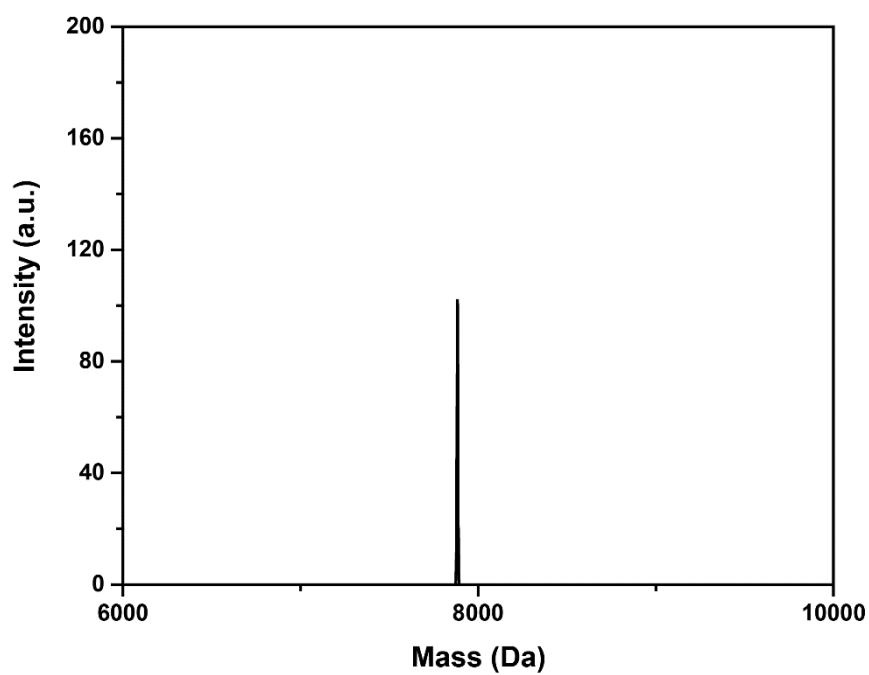


Figure S7. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3d**) measured in negative ion mode. The calculated mass (7880.95 Da) is in agreement with the measured mass (7883.40 Da).

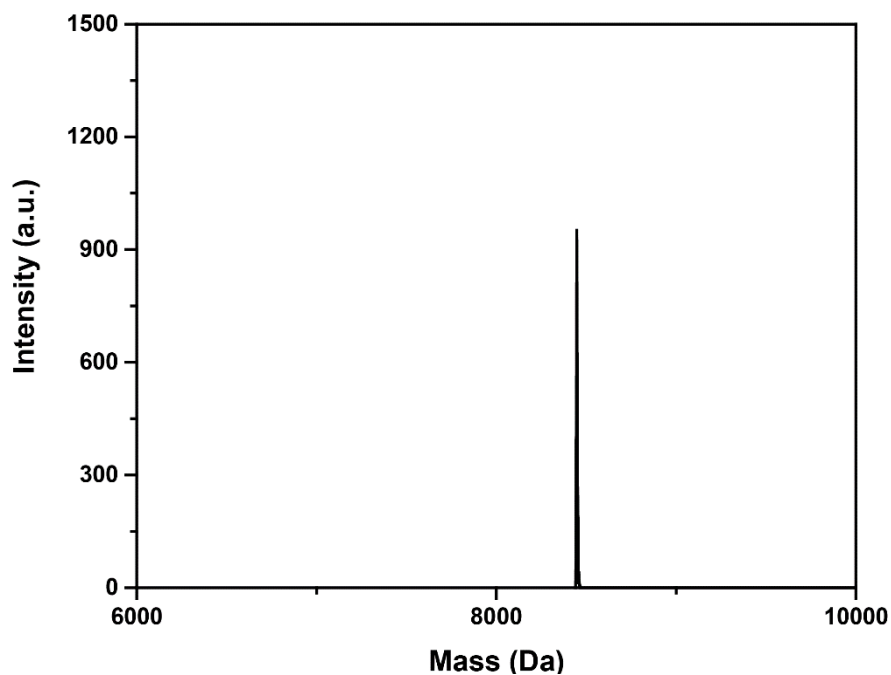


Figure S8. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3e**) measured in negative ion mode. The calculated mass (8446.95 Da) is in agreement with the measured mass (8446.77 Da).

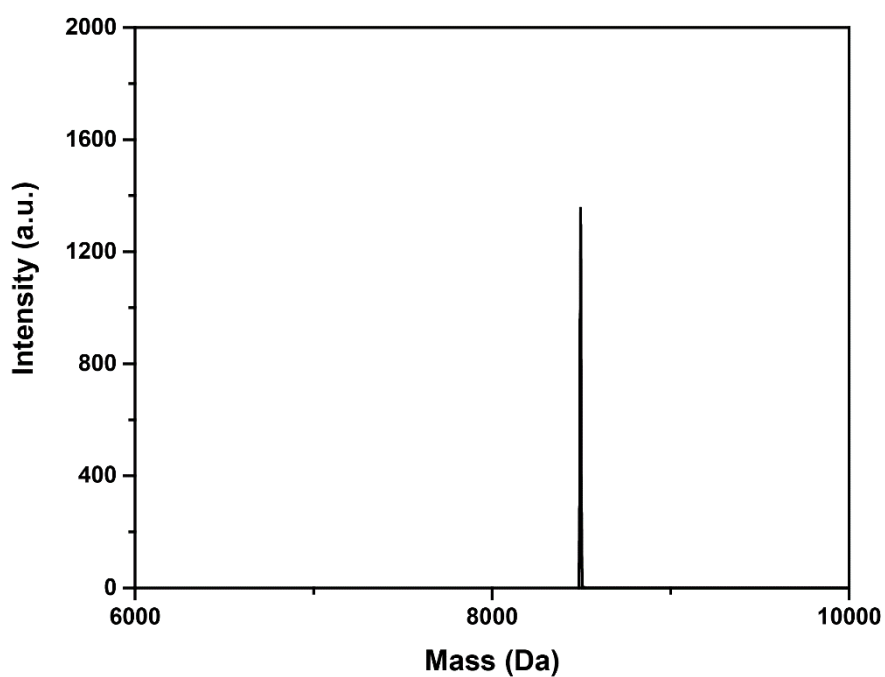


Figure S9. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3f**) measured in negative ion mode. The calculated mass (8493.54 Da) is in agreement with the measured mass (8492.45 Da).

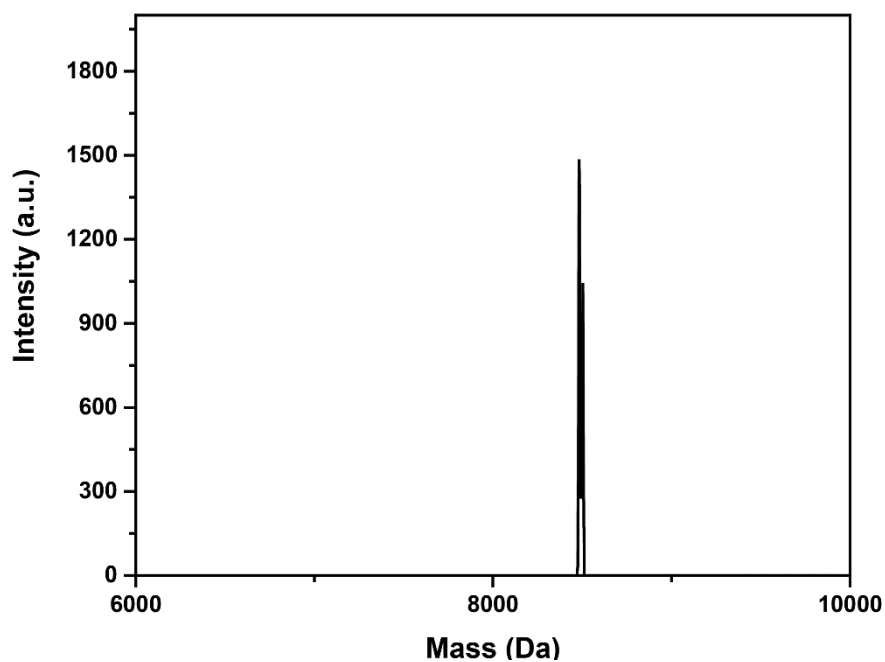


Figure S10. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3g**) measured in negative ion mode. The calculated mass (8492.52 Da) is in agreement with the measured mass (8492.35 Da).

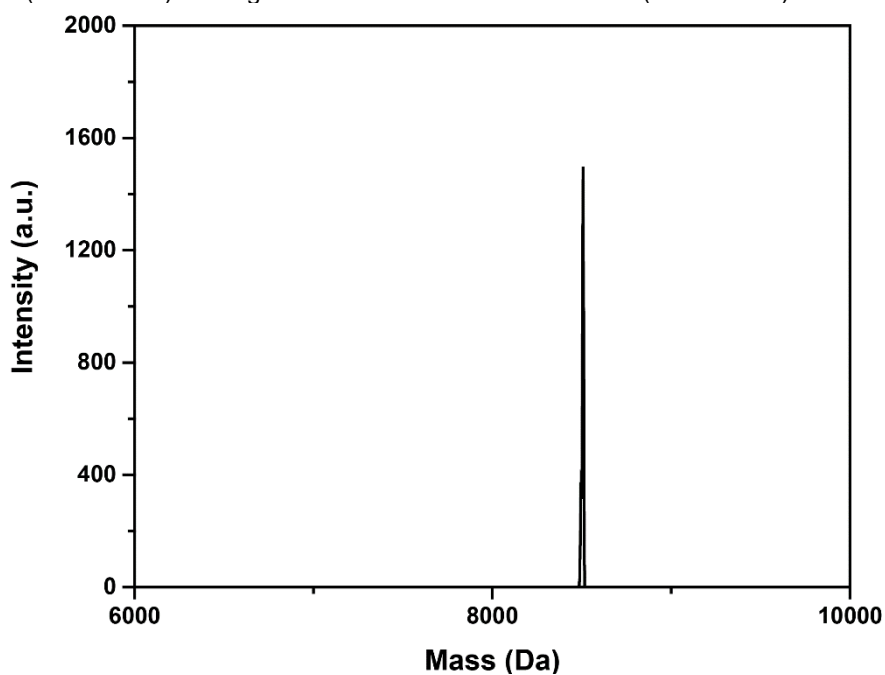


Figure S11. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3h**) measured in negative ion mode. The calculated mass (8506.65 Da) is in agreement with the measured mass (8505.94 Da).

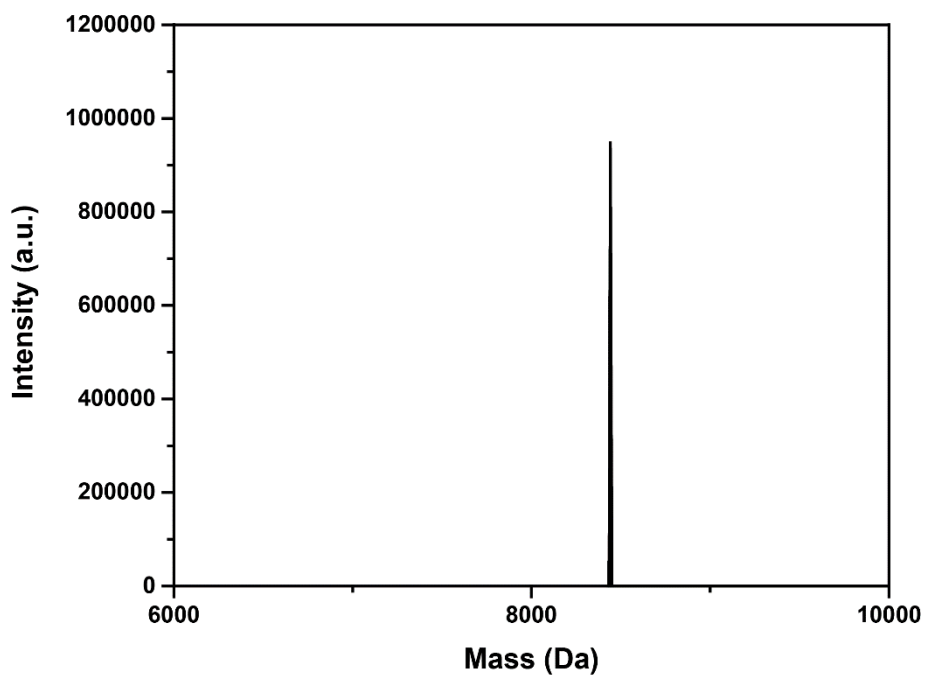


Figure S12. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3i**) measured in negative ion mode. The calculated mass (8445.27 Da) is in agreement with the measured mass (8445.90 Da).

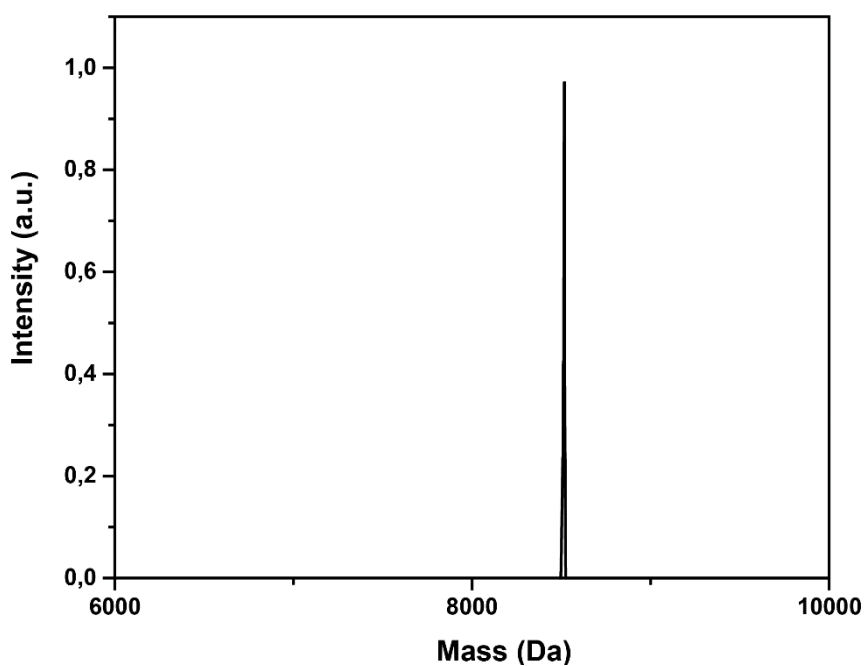


Figure S13. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3j**) measured in negative ion mode. The calculated mass (8406.52Da) is in agreement with the measured mass (8406.70 Da).

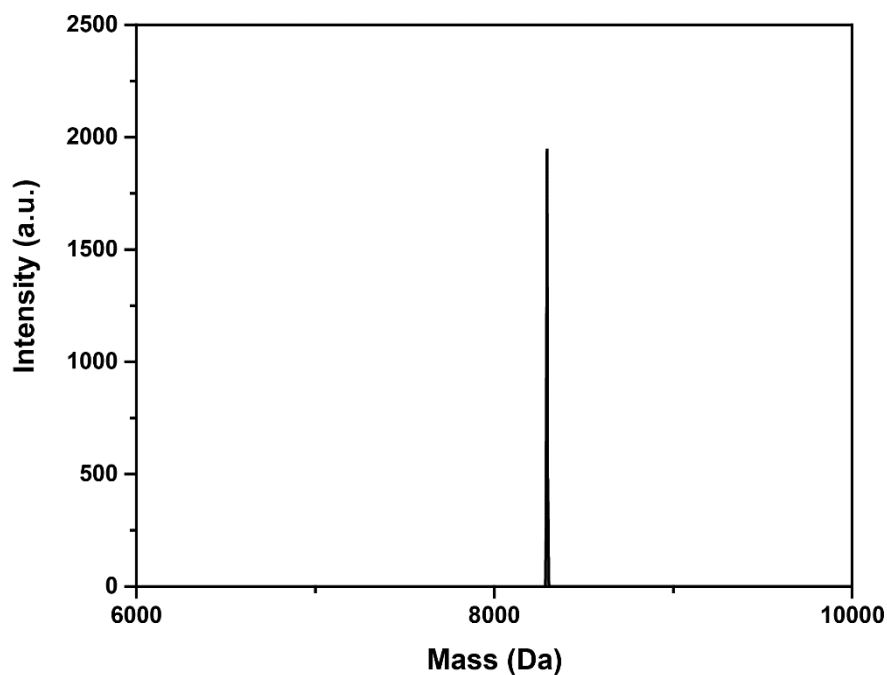


Figure S14. Deconvoluted MS-EI Spectrum of ODN-BiPy-Discs (**3k**) measured in negative ion mode. The calculated mass (8294.92 Da) is in agreement with the measured mass (8294.06 Da).

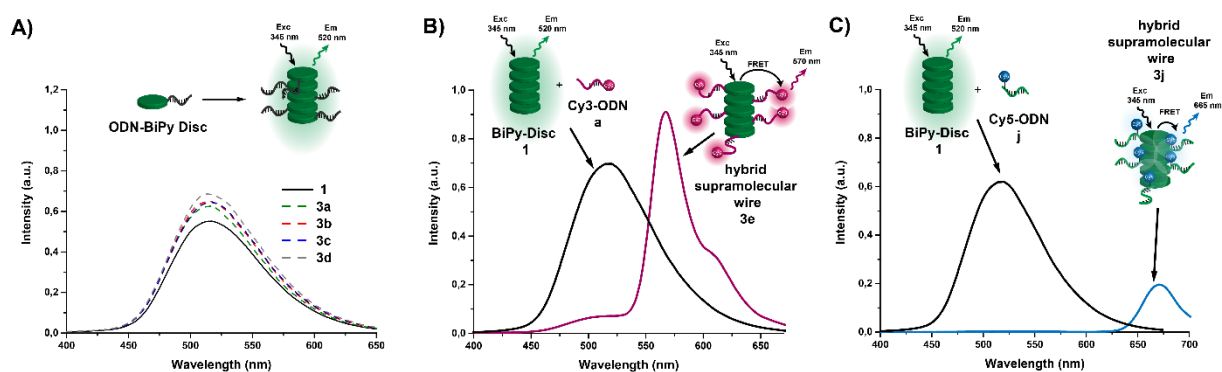


Figure S15. A) BiPy-Disc fluorescence of the self-assembled supramolecular wires with ODNs attached (**3a-3d**) and the unmodified BiPy-Disc (**1**). B) Fluorescence spectra when irradiated at 345 nm of a 1:1 mixture of the BiPy Disc **1** with ODN **a** (black line) and ODN-BiPy Disc **3a** (pink line). C) Fluorescence spectra when irradiating at 345 nm a 1:1 mixture of the BiPy Disc **1** with ODN **j** (black line) and ODN-BiPy Disc **3j** (green line).

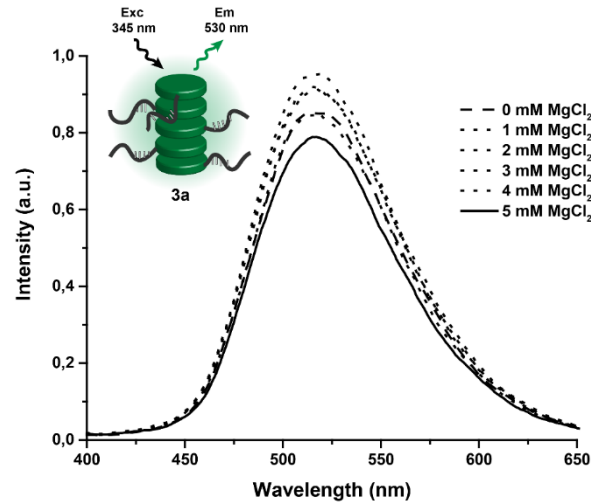


Figure S16. Fluorescence emission spectra of ODN-BiPy Disc **3a** (1 μ M) with increasing concentrations of MgCl_2 (0-5 mM), when irradiated at the absorption band of the BiPy-Disc at 345 nm.

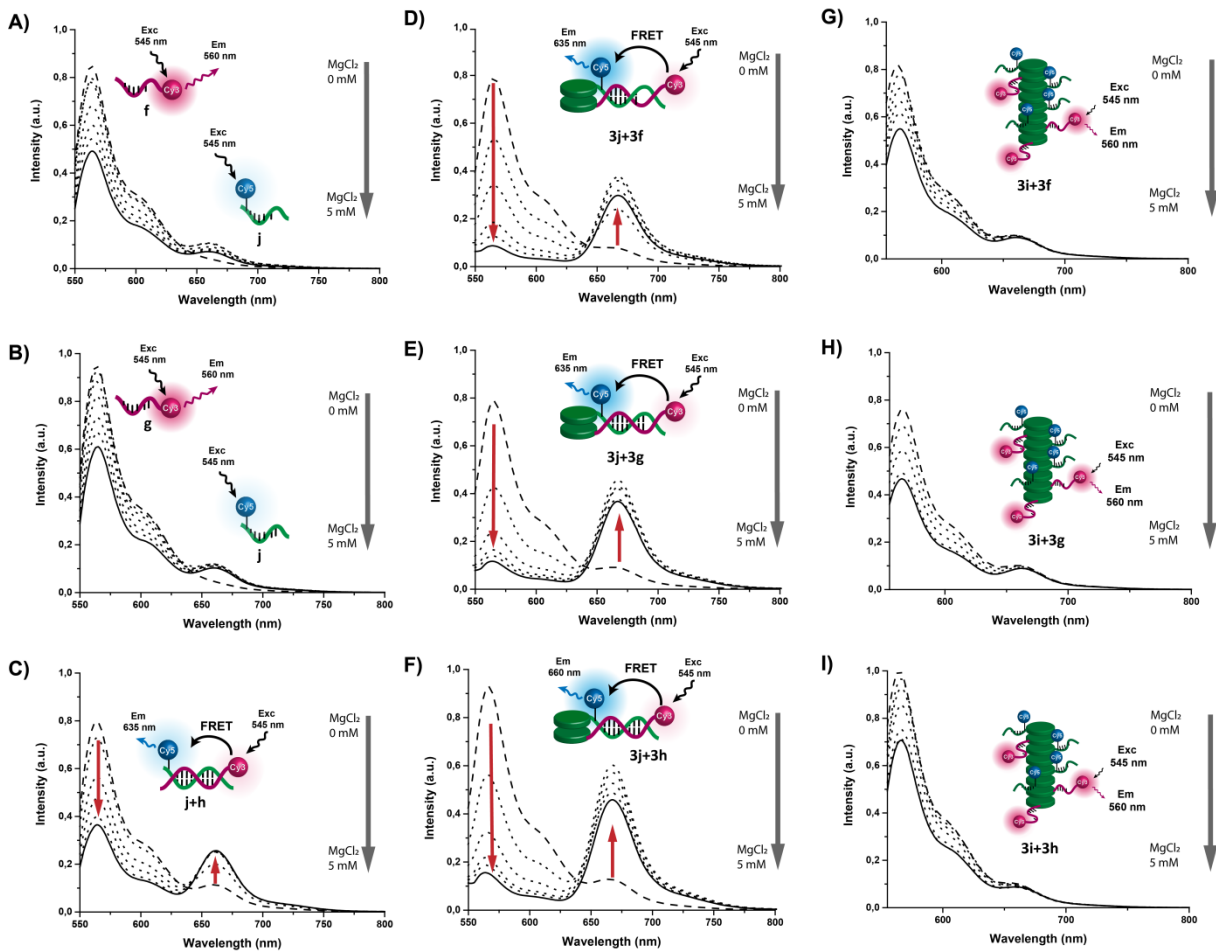


Figure S17. Fluorescence spectra of the energy transfer between Cy3/Cy5 dyes upon duplex formation. All experiments were performed in an equimolar mixture, adding 0.5 μ M of the two components at different concentrations of MgCl_2 (0-5 mM). Background FRET was measured for the different unmodified ODNs with A) five, B) six and C) seven complementary bases. For the evaluation of ODN-BiPy-Discs hybridization, changes in Cy3/Cy5 FRET signals were measured for the systems with E) five, F) six and G) seven complementary bases. Controls with ODN-BiPy-Discs lacking of complementarity G), H) and I) did not show Cy3/Cy5 any FRET signals.

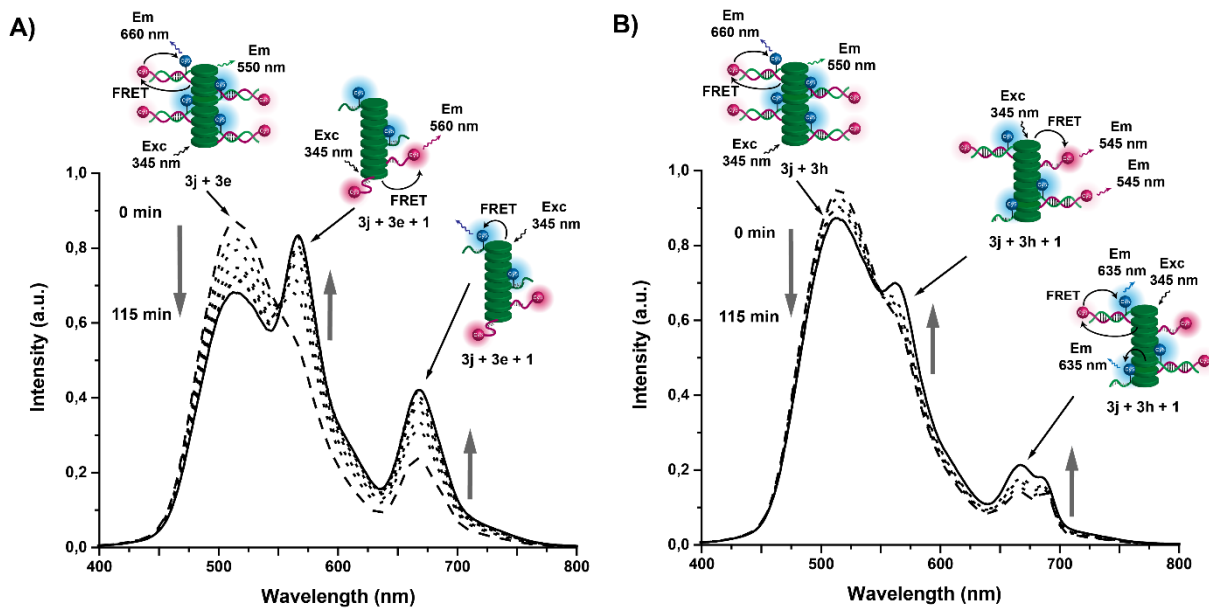


Figure S18. Comparison between the system bearing A) four and B) seven complementary bases. In dashed-lines, fluorescence spectra of an equimolar solution of **3j**, **3h** and **3e** (0.5 μ M, 0.5 μ M) with 0.5 mM of $MgCl_2$ at $t=0$ min with maximum duplex formation (excitation at 345nm). In dotted-lines, evolution over time of the fluorescence of the mixture after the addition of 10 equivalents of BiPy-Disc **1**. In continuous-line, final spectra of the intermixed discs.

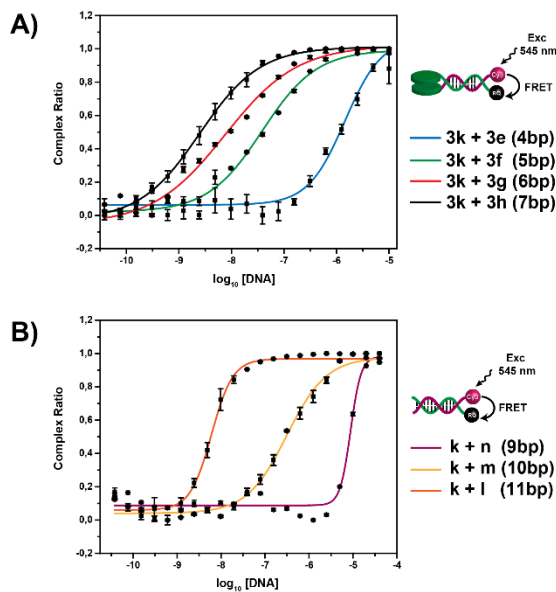


Figure S19. Titration experiment of A) **3k** to **3e**, **3f**, **3g**, **3h**, and B) **k** to **l**, **m** and **n** (5 nM) with 0,5 mM of $MgCl_2$ at 20°C. Representation of the fluorescence of the free Cy3 dye.



Figure S20. ODN sequences of **l**, **m**, **n**.

Calculation of the effective molarity EM.

$$K_{\text{obs}} = K_1 K_2 \text{EM}$$

K_1 : dimerization constant for disc stacking is around $K_1 = 10^8 \text{ M}^{-1}$ for inert discotic^[3] and lowered to around 10^6 M^{-1} depending on the number of appended charges.^[2]

K_2 : DNA duplex formation, which depends on the number of base pairings, and obtained via calculations.

K_{obs} : is the overall association resulting from the combined duplex + disc dimerization and is determined experimentally.

$$\Delta\Delta G = -RT\ln(K_1 K_2 \text{EM}) + RT\ln(K_2) = -RT\ln(K_1 \text{EM}) \sim -6 \text{ kcal/mol}$$

$$K_1 \text{EM} = e^{(-\Delta\Delta G/RT)} = e^{10} = 22000$$

$$\text{With } 10^6 < K_1 < 10^8$$

$$\text{EM} = 0.2\text{-}20 \text{ mM}$$

References

- [1] M. K. Müller, K. Petkau, L. Brunsveld, *Chem Commun* **2011**, 47, 310–312.
- [2] K. Petkau-Milroy, M. H. Sonntag, A. H. A. M. van Onzen, L. Brunsveld, *J. Am. Chem. Soc.* **2012**, 134, 8086–8089.
- [3] L. Brunsveld, B. G. G. Lohmeijer, J. A. J. M. Vekemans, E. W. Meijer, *Chem. Commun.* **2000**, 2305–2306.