Supporting information

Proximity-induced H-aggregation of cyanine dyes on DNA-duplexes

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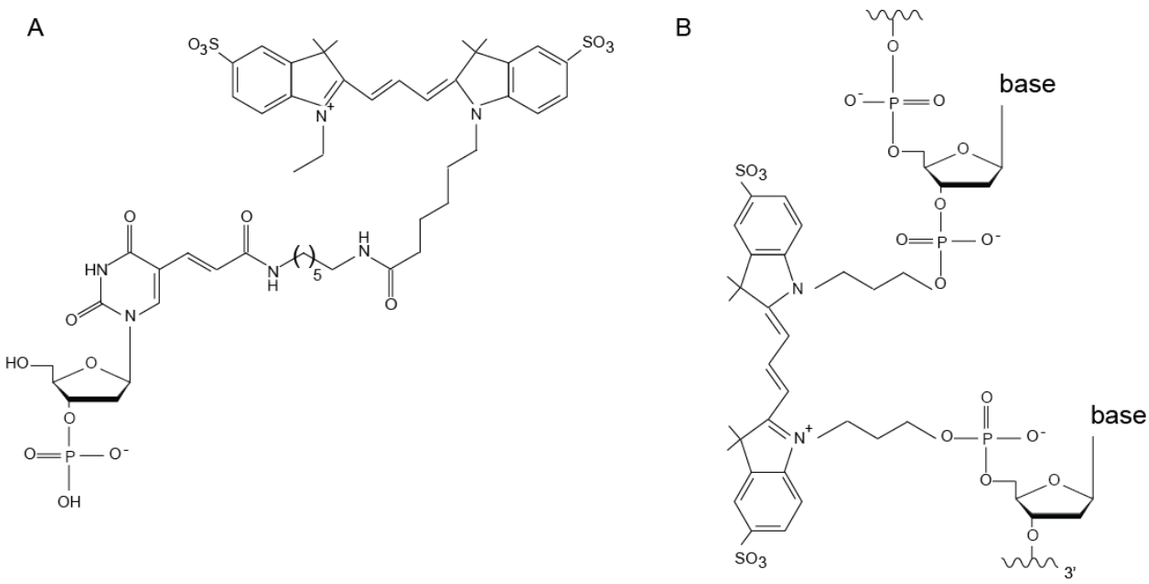
To better understand the constructs used in this work, we show below the functionalization schemes for linking Cy3 to DNA. In the case of Cy3-monomer, Cy3-G-Cy3 and Cy3-Cy3 dimer the dye is linked to thymine bases of the DNA strand by NHS-ester reaction (Scheme 1.a). In this case Cy3 has only one anchor point to the DNA, consisting of a 6-CH linker chain. In the dimer N=0 and N=6 constructs the dyes are linked to the DNA backbone. Both CH terminal groups of Cy3 are linked to an oxygen atom of the DNA phosphate group (Scheme 1.b). In this case Cy3 is anchored in two points along the DNA strand, effectively connecting two shorter oligos to form a longer chain. This Cy3-modified oligo can be seen as a DNA strand with a sugar-base complex missing which is "substituted" by the dye molecule.



Scheme 1 : Functionalization strategies used in the constructs. A) NHS coupling scheme where a Cy3 molecule is attached to a thymine base via single linker. B) Doubly labeled Cy3 attached to a ssDNA through two linkers.

The absorbance of free Cy3 dissolved in aceton nitrile (MeCN) was measured as a reference spectrum for the monomeric state of Cy3. MeCN was used in this case to prevent any possible, partial aggregation of Cy3 in a polar solvent, such as water.

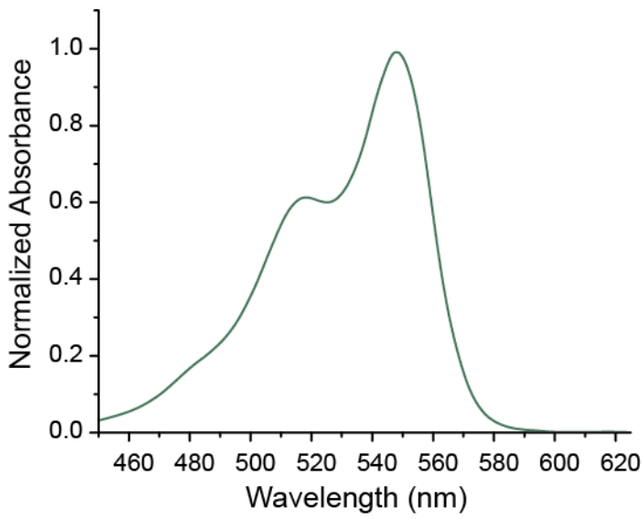


Figure S1: Absorbance spectrum of free Cy3 in solvent (MeCN).

The Cy3-modified DNA oligos were annealed with their complementary strand and then photographed. The difference in color between the monomer and dimer is evident. The latter is seen by bare eyes as a “darker”, bluer solution.

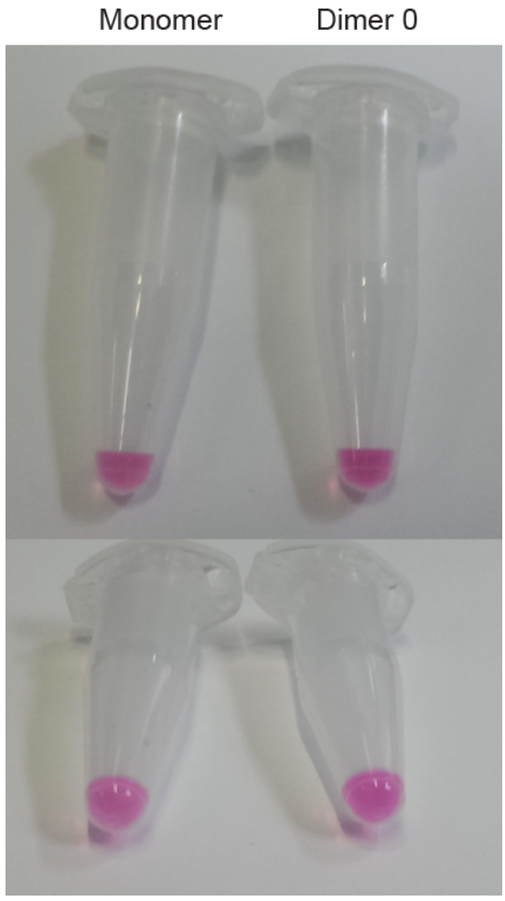


Figure S2: Photo of the Cy3 monomer on dsDNA and Cy3-Cy3 dimer 0 on dsDNA. The different color, lighter and darker pink, for monomer and dimer respectively, indicates a change in the absorption spectrum.

In order to prove that the dimerization of Cy3 is not caused by the high concentration of dye, we performed absorbance measurements at different concentrations of the construct. The concentration value refers to the dye, i.e. the DNA concentration is half or equal in case of Cy3 dimer or monomer, respectively. The buffer conditions are kept constant in all the measurements. The DNA strands were diluted from a 100 µM stock to the desired concentration in 10 mM Tris, 1 mM EDTA and 150 mM NaCl. In case of double stranded constructs, the two complementary strands were mixed in a 1:1 ratio, the solution, heated to 65°C fot 5 min and cooled to room temperature over the course of 2 hours.

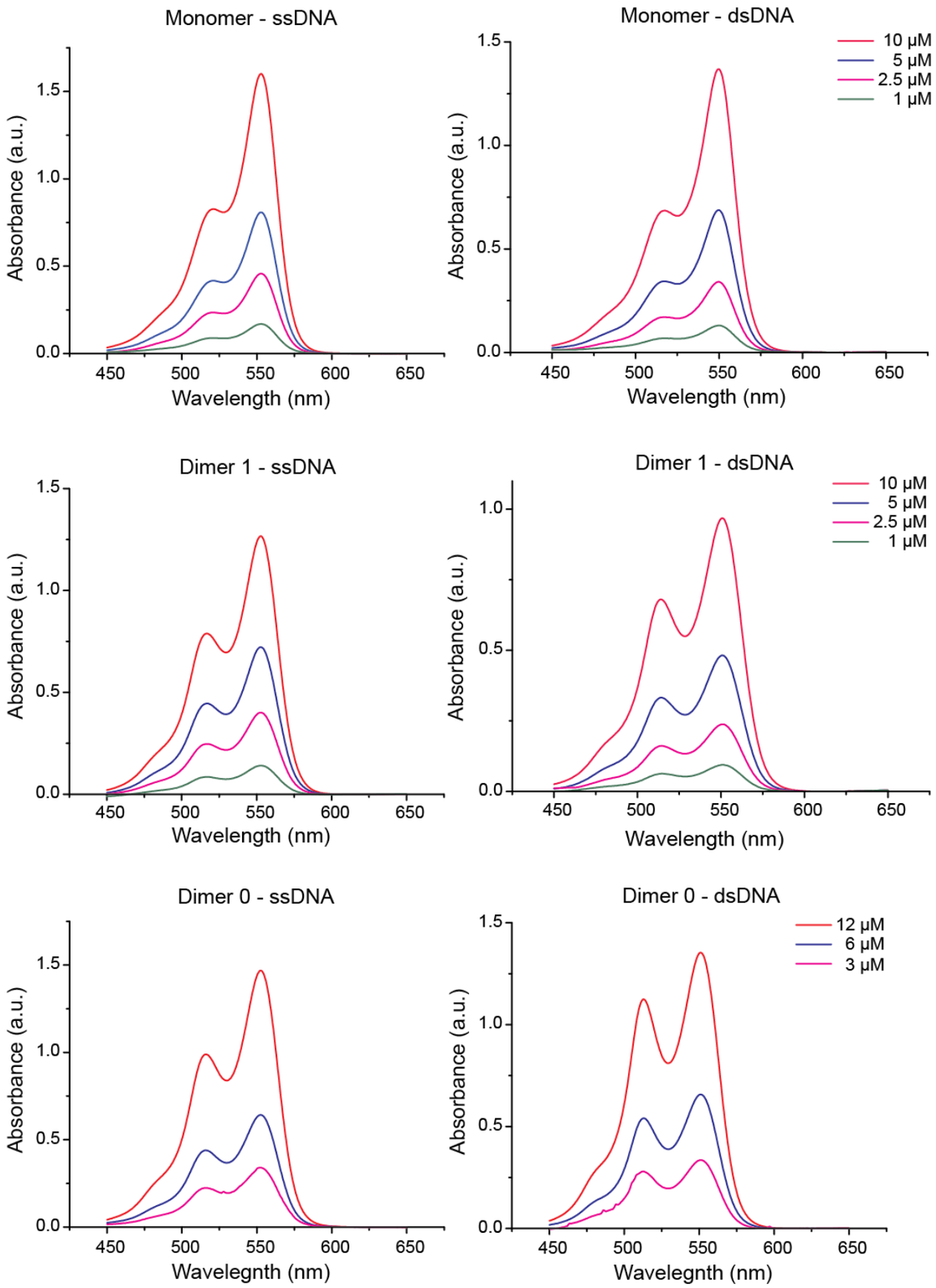


Figure S3: Absorbance measurements of all DNA constructs in 10 mM Tris, 1 mM EDTA and 150 mM NaCl buffer at different concentrations. All the graphs on the left, right, refer to ssDNA, dsDNA, constructs respectively. The overall shape of the spectra does not change with concentration, but the difference is given by the type of DNA-dye construct.

Since it is known that cyanine dyes can intercalate, as aggregates, in the minor groove of DNA, we performed a control experiment where we mixed free dye with dsDNA at different ratios. No significant change in the spectrum could be observed, so we can safely assume that the H-band appearing in the absorption spectrum of the DNA constructs is not caused by groove intercalation.

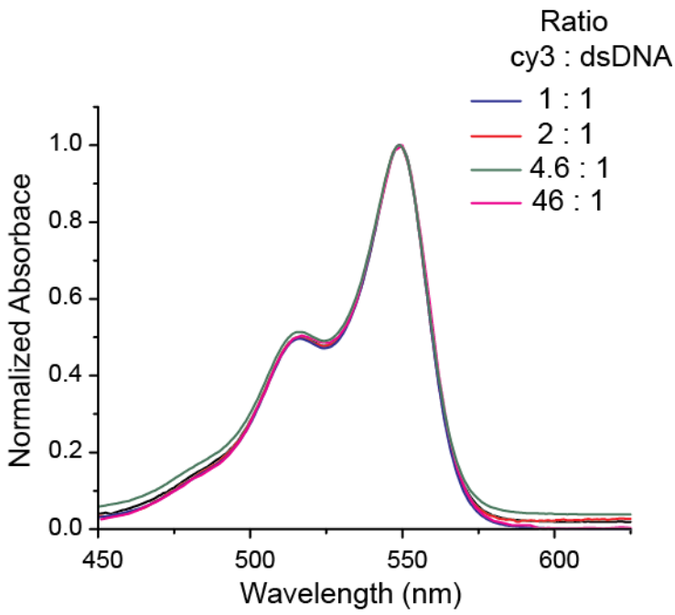


Figure S4: Absorbance measurements of a mixture of free Cy3 molecules and dsDNA in 10 mM Tris, 1 mM EDTA and 150 mM NaCl. The ratio between fluorophore and DNA is changed from 1:1 to 46:1 Cy3 to DNA so that the possible intercalation of Cy3 in the DNA groove[1] *could be excluded from what is observed with our constructs.*

We also obtained Cy3 dimerization using a different DNA functionalization strategy. Here, the Cy3 molecule acted as a substitute for the sugar-base complex. This is achieved by conjugating the 3' and 5' ends of two shorter strands to the two free hydroxy groups of the dye (Scheme S1.b). When only a single Cy3 molecule is present or alternatively, the two dyes are separated by ~2 nm along the axis of the dsDNA scaffold (6 bases apart, Dimer N=6), is similar to the monomer spectrum. Contrary, when two Cy3 molecules are directly adjacent on opposite strands (Dimer N=0), the hypsochromic peak appears at 516 nm, indicating the formation of an H-dimer of higher energy (Figure S5). Since the dyes possess a double anchor to the DNA, their movement is expected to be limited, which is consistent with the behavior displayed with increasing scaffold rigidity in the constructs where Cy3 is attached to the thymine bases.

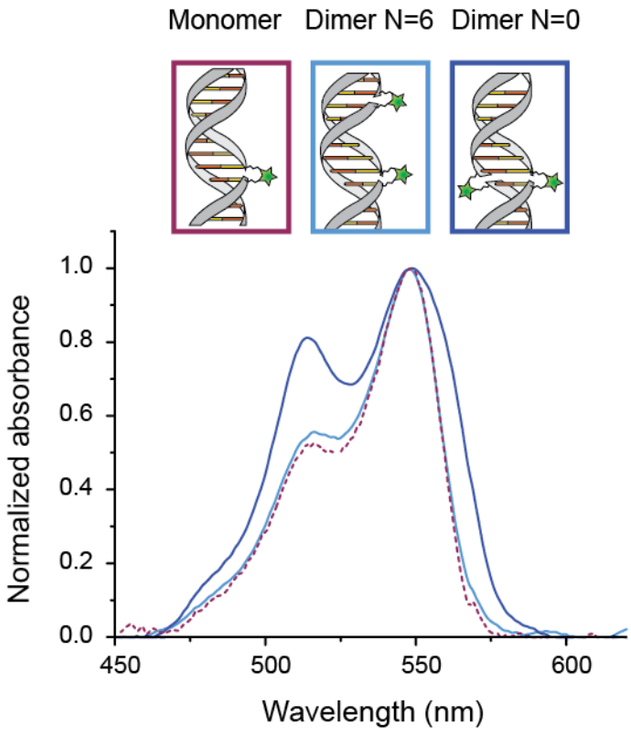


Figure S5: Absorption measurements of the doubly labeled cy3-DNA constructs. Dimer N=0 (N=6) corresponds to one doubly labeled cy3 on each ssDNA of the duplex with a relative distance between fluorophores of 0 bases (6 bases). In the monomer, only one of the duplex strands carries a doubly labeled Cy3.

We chose a model system where, we substituted the $C(CH_3)_2$ groups in the five membered rings with sulfur atoms, in order to obtain a planar monomer and therefore simplify dimerization. This substitution leads to no significant shift of the signal (551 nm in water),[2] and to no change in the overall spectral shape compared to a regular Cy3 monomer. We optimized minimum energy structures for the ground state and for the excited states S_1 and S_4 of the monomer and dimer respectively The method used in our calculations is TD-DFT/B3LYP/6-31G(d) in water (PCM), including Grimme's GD3 dispersion correction. Ground state minimum energy structures are shown in Figure S6. Note that the rotation of the monomers by 180 degrees to form the dimer is not of importance here, as the transition dipole moment is located along the molecular axis and therefore the coupling picture according to Molecular Exciton Theory is unchanged. Note further that the frontier orbitals (Figure S7) of the dimer are linear combinations of the frontier orbitals of the monomer.

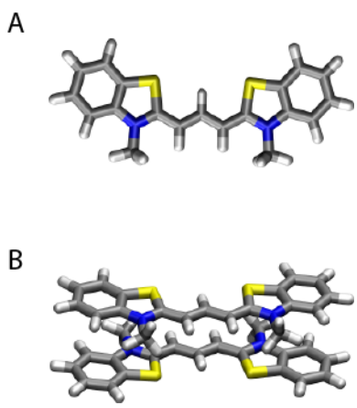


Figure S6: Optimized molecular structures of the model system for Cy3 with sulfur atoms instead of $C(CH_3)_2$ groups in the ground state. Optimized with DFT/B3LYP/6-31G(d) and PCM (water). A) Monomer. B) Dimer consisting of two monomers rotated by 180 degrees at a distance of 3.40 Å. Here, the dispersion correction GD3 from Grimme[3] was also used.

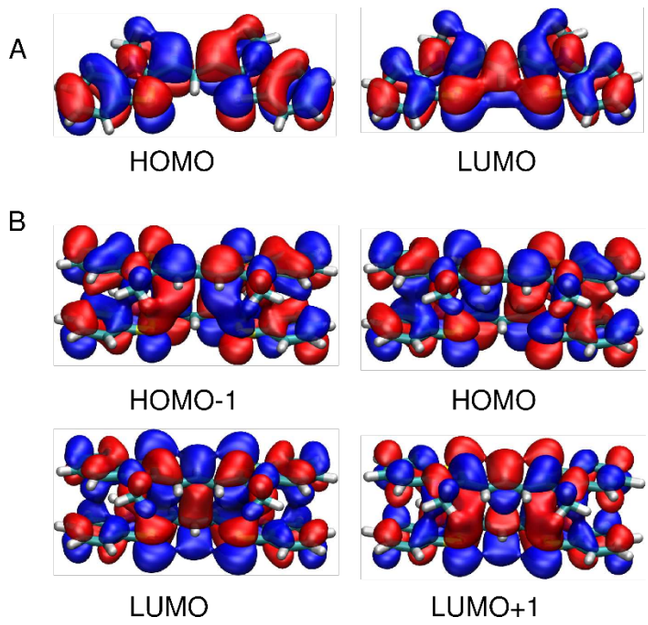


Figure S7: Molecular orbitals for the Cy3 with sulfur atoms (DFT/B3LYP/6-31G(d)). A) Monomer. B) Dimer.

References

(1) Hannah, K. C.; Armitage, B. A. DNA-Templated Assembly of Helical Cyanine Dye Aggregates : A Supramolecular Chain Polymerization. *Acc. Chem. Res.* **2004**, 845–853.

(2) Sims, P. J.; Waggoner, A. S.; Wang, C.-H.; Hoffman, J. F. Mechanism by Which Cyanine Dyes Measure Membrane Potential in Red Blood Cells and Phosphatidylcholine Vesicles. *Biochemistry* **1974**, *13*, 3315–3330.

(3) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A Consistent and Accurate Ab Initio Parametrization of Density Functional Dispersion Correction (DFT-D) for the 94 Elements H-Pu. *J. Chem. Phys.* **2010**, *132* (15), 154104.