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Supplementary Information

Synthesis and Characterization of Porphyrin-DNA Constructs for the Self-Assembly of Modular Energy Transfer Arrays

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1. Experimental details

1.1 General methods. HPLC was conducted at 50 °C using a Dikmatech Inspire C18 RP 250 x 4.6 mm (Dikmatech) column on a Shimadzu Prominence Series instrument, which is equipped with a diode array detector, with a gradient of acetonitrile (HPLC grade) in 200 mM aqueous triethylammonium acetate (TEAA) solution. 1 x TAE/Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, 10 mM Mg^{2+}, pH 7.6) was used for the self-assembly of DNA-templated EnT arrays. Concentration of DNA oligonucleotides was determined by using NanoDrop ND-1000 spectrophotometer. Molecular modeling was completed using Molecular Operating Environment (MOE) version 2013.08. Molecular linker regions were energy minimized using an MMFF94x force field and DNA was modeled with a helicity of 10.5 base pairs per turn.

1.2 Materials. Solvents, ACS Reagent grade or better, were purchased from J. T. Baker and used as received. Copper sulfate (Fisher Scientific), sodium ascorbate (Aldrich), bathophenanthroline disulfonic acid (bathophen, Alfa Aesar), acetic acid (Amresco), triethylamine (Fisher), 5nm AuNPs (Ted Pella), and UC-A on holey 400-mesh Cu TEM grids (Ted Pella) were used as received. The 5'-azide modified, 3'-**A546** modified, 5'-thiol modified, and unmodified oligonucleotides were purchased from IDT DNA and diluted in nuclease free water. 5,10,15,20-Tetra(4-phenylethynyl)porphyrin (**ZnTPEP**) was synthesized *via* the Lindsey method,[1] metalated with Zn(II), and deprotected according to literature methods.[2]

1.3 CuAAC reaction. 6.8 mM aqueous $CuSO_4$ (1 mL) and 0.34 mM bathophen in N-methyl-2-pyrrolidone (NMP) (4 mL) were mixed and 81.6 nmol (60 uL) of the resulting solution was added freshly prepared 0.6 M aqueous sodium ascorbate (12 uL) and shaken briefly. To this was added a 3.9 mM solution of **ZnTPEP** (24 uL) in NMP and 1 mM aqueous **azide-DNA** (48 uL) and 108 uL of NMP for a final solvent ratio of 3:1 NMP:water. The headspace was purged with nitrogen and the reaction was shaken at 37 °C for 5 hours. After quenching with a solution of 250 mM EDTA (10 uL), the reaction was directly injected into a C18 RP-HPLC column with a gradient of acetonitrile from 2-100%

with 200 mM TEAA. The ssDNA-**ZnTPEP** adducts were collected in the order of increasing hydrophobicity and the including fractions were dried under reduced pressure and kept at -20 °C. Near quantitative yield was observed via denaturing PAGE with 60% recovery after purification.

1.5 Assembly and TEM visualization of AuNP-DNA-porphyrin arrays. 1 mM Thiolated DNA (2.5 uL) was reduced with 0.1 M dithiothreitol (DTT, 1mL) in water while mixing for 1 hour at room temperature. DTT was then removed using a NAP-5 column (GE Healthcare Life Sciences) yielding a nearly quantitative amount of reduced thiol DNA, which was then incubated with AuNPs (100 pmol) overnight in 1x PBS buffer at room temperature with shaking. Excess DNA was removed through 3 rounds of centrifugation at 21,000 rcf with the pellet being resuspended in 1x PBS. Stoichiometric amounts of DNA-porphyrin constructs were added and the mixture was thermally annealed from 45 to 4 °C over 65 minutes and deposited on the TEM grids followed by two rinses with Milli-Q water. Samples were visualized with a JEOL 2011 TEM at 200kV with an XR280 camera with 350ms exposure time and a magnification of 1,500,00x.

1.6 Assembly of porphyrin/DNA EnT arrays. A mixture of each arm product was added to an equimolar solution of complementary **A546**-labeled strand (**A546-T** or **A546-S**). The mixture was allowed to cool from 45 to 4 °C over 65 minutes in 1 x TAE/Mg^{2+} buffer.

1.7 Photophysical Methods. Each photophysical spectrum was taken in aqueous 1x TAE unless otherwise noted. Electronic absorption spectra were taken on a Perkin-Elmer Lambda 950 spectrometer or a NanoDrop ND-1000 spectrometer. Steady-state fluorescence and quantum yield measurements were taken on a HORIBA Jobin Yvon FluoroLog3 spectrofluorometer using a right-angle detection method. Quantum yield measurements were carried out using the comparative method[3] with optical densities below 0.09 past the excitation wavelength of 537 nm to minimize inner filter effects. Standards used were Zn-octaethylporphyrin in toluene (0.045),[4] Zn-tetraphenylporphyrin in toluene (0.033),[5] and Rhodamine-B in ethanol (0.5).[6] Each sample was prepared in matched 10mm optical path length quartz fluorescence cuvettes and purged with nitrogen. A blank was recorded for both absorbance and fluorescence prior to the addition of the sample compounds. Every fluorescence spectrum was corrected for both instrument and lamp variations. Refractive index of each solvent has been taken into account. Wavelengths were kept in nanometers and emission peaks were completely integrated using Orgin 8 Pro. SigmaPlot was used to graph absorbance vs. integrated fluorescence to obtain the gradients. Each sample was performed in triplicate with R^2 values greater than 0.99.

Table S1. Overlap integrals ($J(\lambda)$), calculated using equation S1, and predicted Förster distances (R_o) of the set of arrays using the **A546** donor and **ZnTPEP** acceptor.

	4-Arm	**3-Arm**	***cis*-2-Arm**	***trans*-2-Arm**	**1-Arm**	**A546**
J(λ) ***($M^{-1}cm^{-1}nm^4$)***	7.4×10^{14}	7.5×10^{14}	6.13×10^{14}	6.9×10^{14}	6.4×10^{14}	3.4×10^{15}
R_o ***(nm)***	4.4	4.4	4.3	4.4	4.3	5.7

$$J(\lambda) = \int_0^\infty \varepsilon_A(\lambda)\lambda^4 F_D(\lambda)d\lambda \qquad \text{(Eq. S1)}$$

where $\boldsymbol{\varepsilon}_A$ is the extinction coefficient spectrum of the acceptor in units of $M^{-1}cm^{-1}$, λ is wavelength in units of nm, and F_D is the normalized donor emission spectrum.

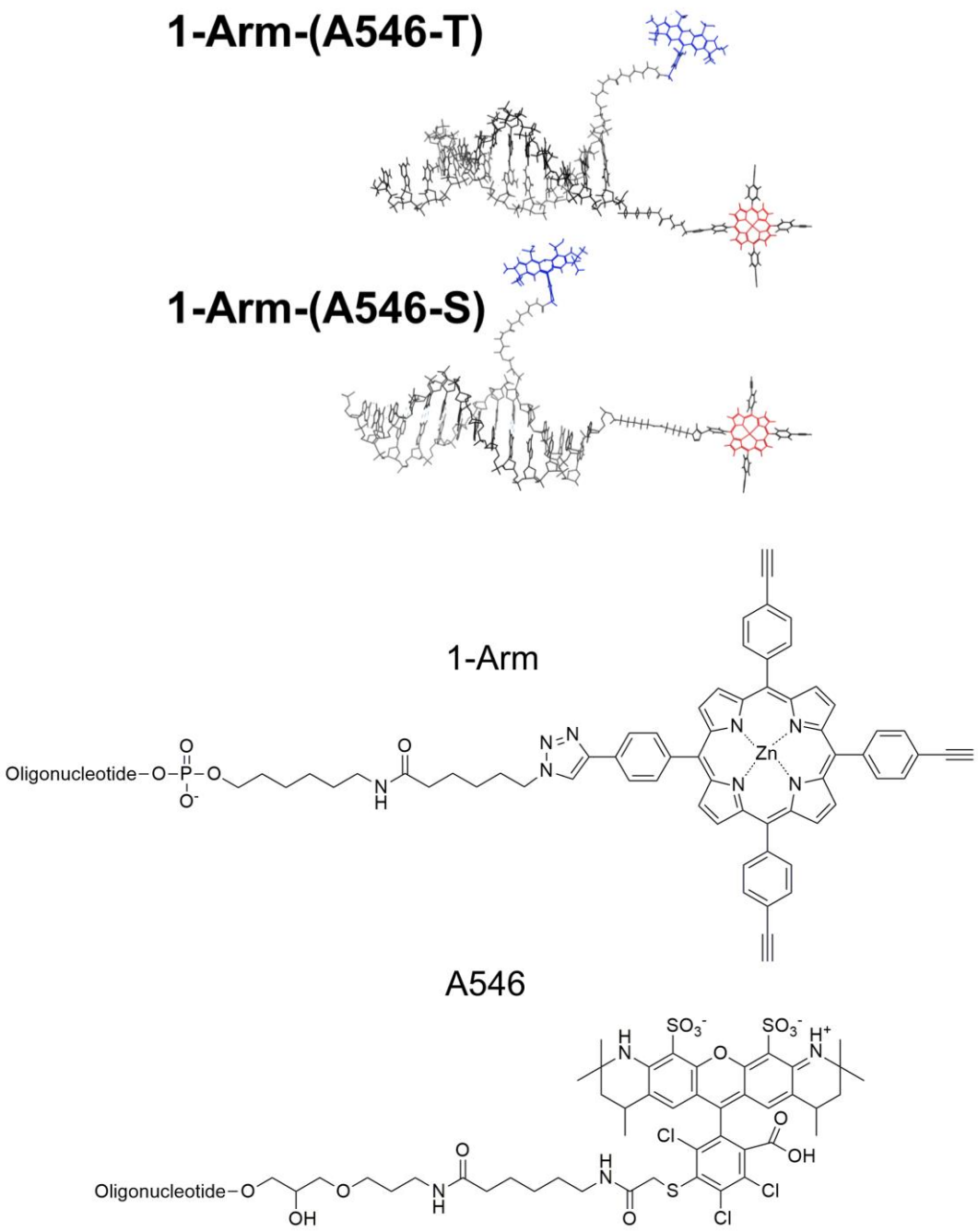

Figure S1. Top: Structures of **1-Arm** scaffold assembled with either **A546-T** or **A546-S**. The point-to-point distance between A546 donor and porphyrin acceptor is 3.6 nm, 75% E_{EnT} (**A546-T**) or 4.9 nm, 32% E_{EnT} (**A546-S**) which closely match the experimental values displayed in Table 2. Bottom: Structures of the oligonucleotide dyes 1-Arm and A546 showing the alkyl linker regions in more detail. **ZnTPEP** has been covalently attached to the 5' end of **N_3-DNA** while **A546-T** and **A546-S** have been purchased covalently attached to the 3' end of the oligo.

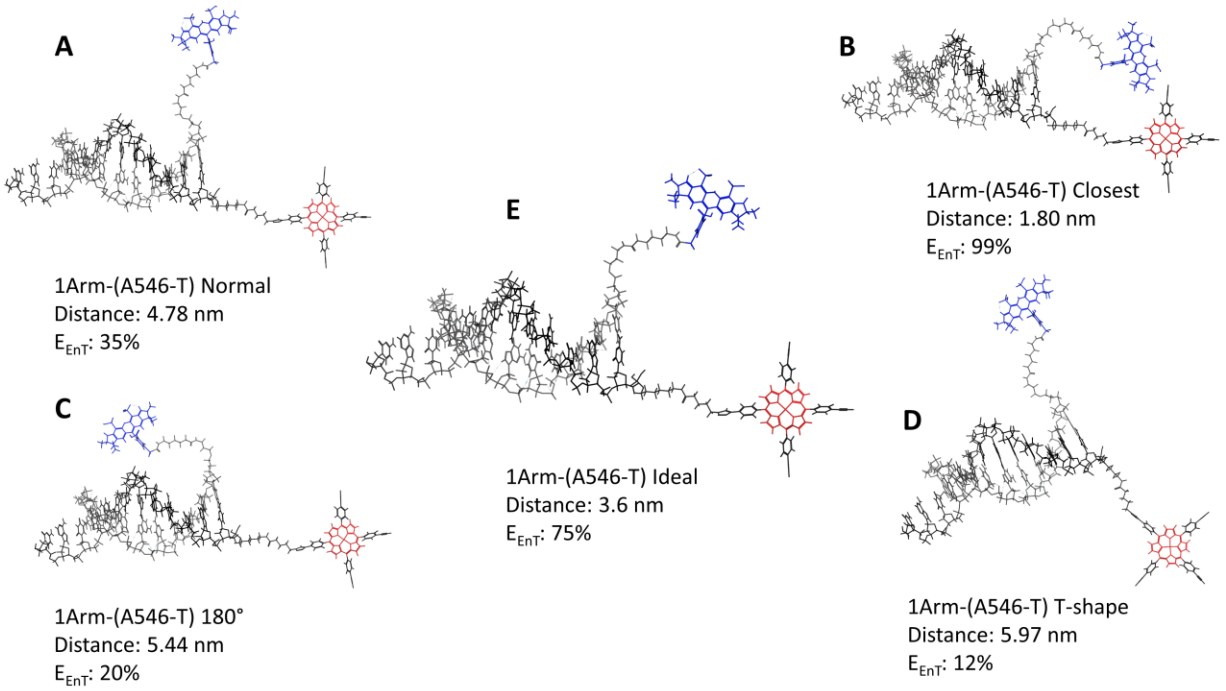

Figure S2. Potential positions for **A546** and **ZnTPEP** in **1-Arm-(A546-T)**. Dyes and linker regions have been geometry optimized using an MMFF94x force field in MOE but the structures were otherwise manually manipulated to form each combination of positions. A. **A546** and **ZnTPEP** positioned at a 90° angle with **A546** perpendicular to the DNA helix. B. The dyes positioned as near to each other as the linkers allow. C. **A546** positioned laying down on the DNA axis near the maximal distance possible between the dye pair. D. Both dyes normal to the DNA axis in a T-shape formation with the largest inter-dye distance possible with these linkers. E. The position in which the distance between dyes leads to an identical value of E_{EnT} as calculated through experiment.

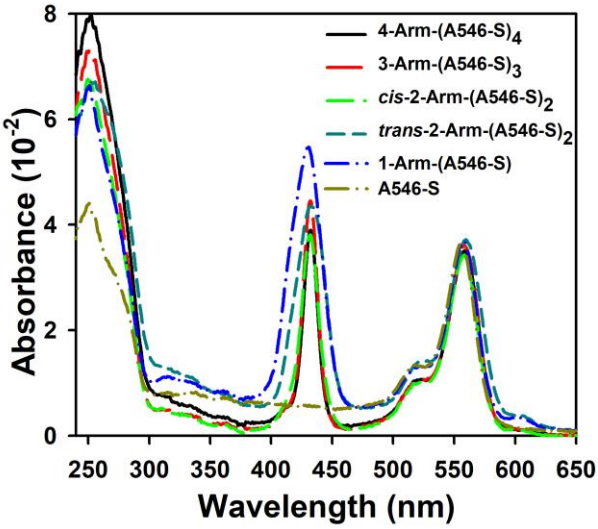

Figure S3. Absorption spectra of fully assembled FRET arrays with equimolar amounts of **A546-S**.

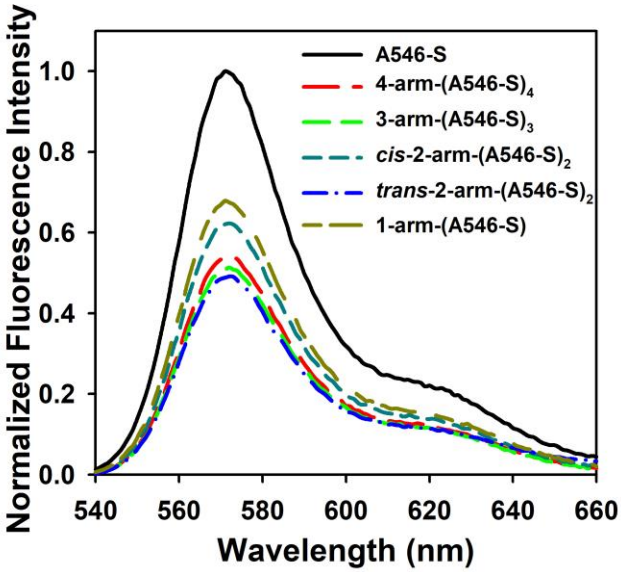

Figure S4. Emission spectra of fully assembled FRET arrays with equimolar solutions of **A546-S** excited at 520nm. Spectra have been normalized to **A546-S** donor emission. Quenching of A546-S emission can be attributed to FRET efficiency proportional to the ratio of donors:acceptors.

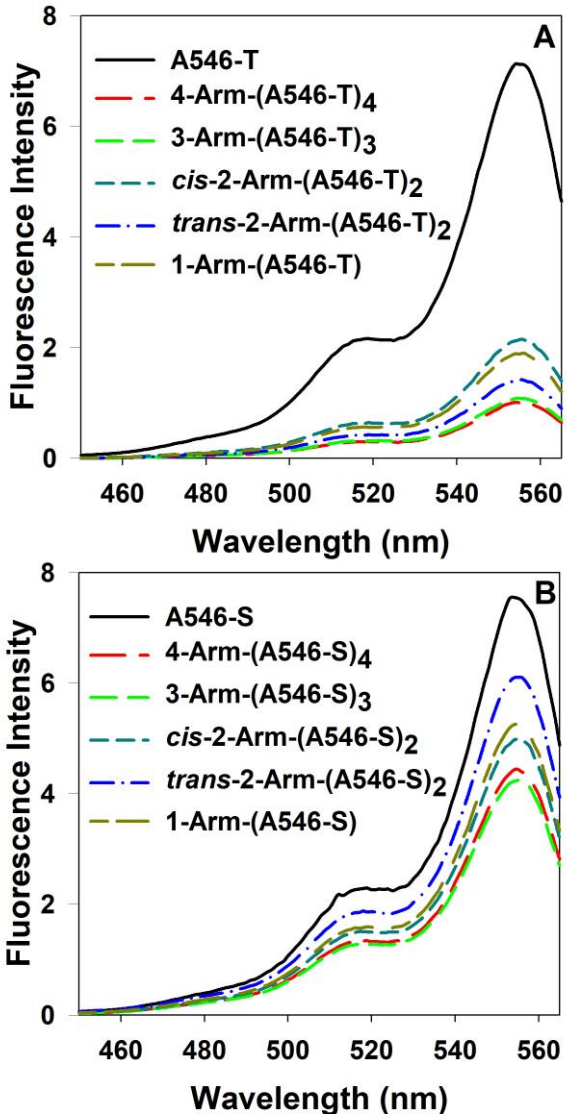

Figure S5. Excitation scans with emission at the peak of **A546**, 571 nm. Similar levels of quenching can be seen as compared to the related emission spectra in Figs 6 and S4.

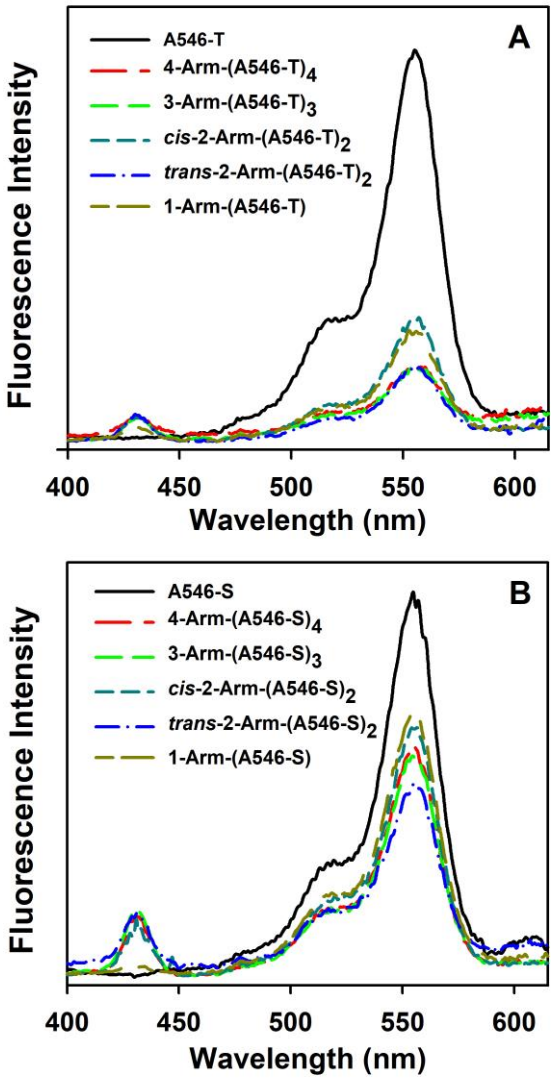

Figure S6. Excitation scans with emission at 665 nm. At this wavelength, there is both **ZnTPEP** and **A546** emission present due to the large overlap of the two spectra. Porphyrin Soret band excitation can be seen between 427 and 431 nm as well as Q-band excitation at 605 nm.

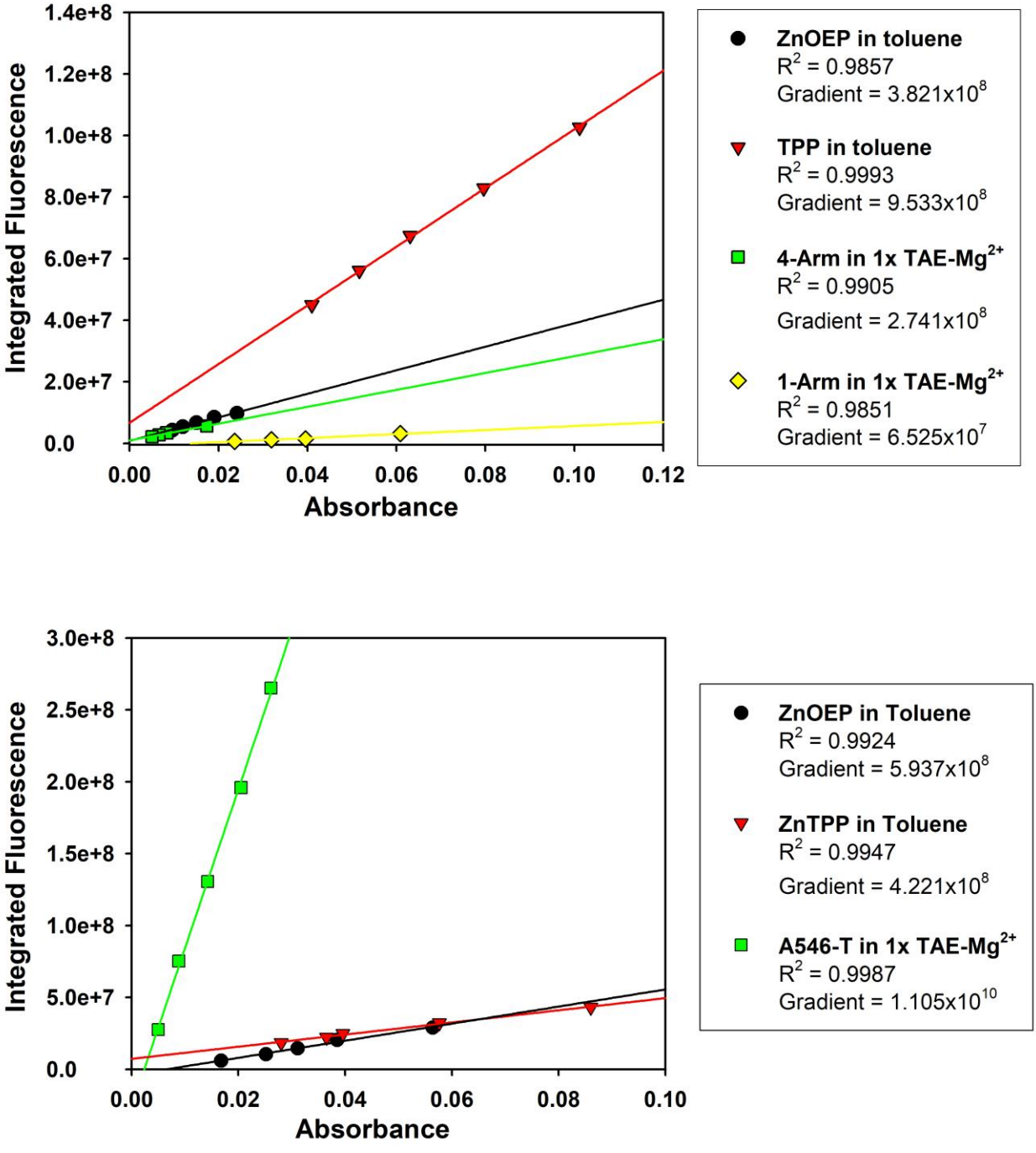

Figure S7. Top: Quantum yield data for **1-Arm** (Φ = 0.0074% ± 0.000075) and **4-Arm** (Φ = 0.031 ± 0.00031) using ZnOEP (Φ = 0.045)[4] and TPP (Φ = 0.11)[8] as standards. Bottom: Quantum yield data for **A546-T** (Φ = 0.83 ± 0.013) using ZnOEP and ZnTPP (Φ = 0.033)[5] as standards. Quantum yields were determined using the comparative method as outlined by Jobin Yvon Horiba in reference 3.

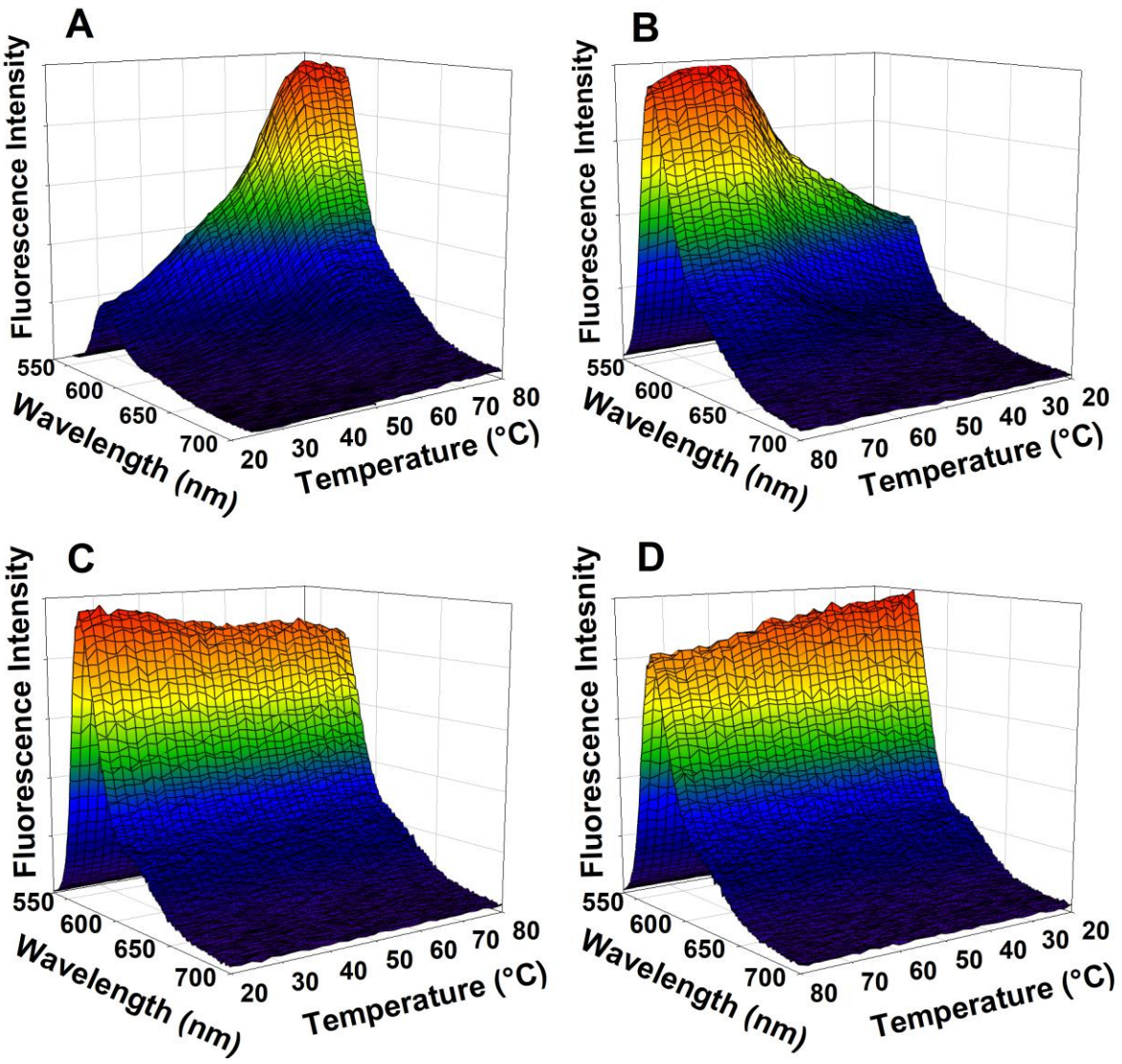

Figure S8. Emission of **4-Arm-(A546-T)$_4$** with temperatures ranging from 20 to 80 °C (A) with **A546-T** emission increasing due to EnT decreasing as the complex melts and the dyes are separated. Emission quenching returns as the DNA complex is reannealed while cooling from 80 to 20 °C (B). **A546-T** emission decreases inversely to temperature ranging from 20 to 80 °C (C) due to an increase in collisional quenching[9]. Emission can be seen increasing again as temperature decreases from 80 to 20 °C (D).

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