Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

A Bioinspired Self Assembled Dimeric Porphyrin Pocket that Binds Electron Accepting Ligands

Karl Börjesson, Jakob G. Woller, Elham Parsa, Jerker Mårtensson and Bo Albinsson*

Department of Chemical and Biological Engineering/Physical Chemistry, Chalmers University of Technology, SE-41296 Gothenburg, Sweden

Materials and Methods

The buffer used for all samples was Tris-HCl (25 mM) at pH 8.0 containing 50 mM NaCl. Absorption spectra were recorded on either a Varian Cary 4000 or a Varian Cary 5000 spectrophotometer. Chemicals were purchased from Sigma unless otherwise stated. Synthesis and characterization of porphyrin modified oligonucleotides are described elsewere.[1]

Octanol/water Partitioning Coefficient:
Equal volumes of octanol and saturated aqueous buffered solutions of the various ligands were mixed thoroughly. The partitioning coefficient was taken as the ratio between ligand absorbance in the octanol phase and the buffer phase.

Liposome Preparation:
1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) dissolved in chloroform was purchased from Avanti Polar Lipids. Unilamellar vesicles were prepared using a standard protocol as briefly described here. The dissolved lipids were evaporated in a round-bottom flask using a rotavapor until a thin lipid film was formed on the glass surface. To further remove residual solvent, the round bottom flask was left under higher vacuum for 6 h. The film was suspended in buffer and subsequently subjected to 5 freeze-thaw cycles after which the suspension was extruded 21 times through a 100 nm polycarbonate filter (Avestin) to obtain the liposomes. Vesicle size (~114 nm) and monodispersity (polydispersity ~0.1) were confirmed using dynamic light scattering on a Malvern Zetasizer Nano ZS at a lipid concentration of 20 µM.

Porphyrin-DNA Sample Preparation:
All porphyrin modified single strands were heated to 70 °C for 1 h in presence of a few equivalents of $ZnCl_2$ to ensure that all porphyrin molecules contained zinc. The single stranded DNA concentrations were set by measuring absorption (using $\varepsilon_{545} = 1.4 \bullet 10^4\ M^{-1}\ cm^{-1}$ for the porphyrin strands) and duplexes were made by mixing with complementary strands in a one to one ratio. The strands were annealed by heating to 80 °C followed by a slow cooling to room temperature. Liposome stock was added, reaching a lipid concentration of 150 µM and a DNA duplex concentration of 1 µM for steady state measurements, and 375 µM lipid and 2.5 µM DNA for time resolved measurements. The samples were left overnight at room temperature to allow the porphyrins to bind to the membrane. Ligand concentrations were set by measuring absorbance; py[2] $\varepsilon_{255} = 2.3 \bullet 10^3\ M^{-1}\ cm^{-1}$, apy[3] $\varepsilon_{455} = 2{,}5 \bullet 10^2\ M^{-1}\ cm^{-1}$, dpyane[4] $\varepsilon_{257} = 4.4 \bullet 10^3\ M^{-1}\ cm^{-1}$, dpyene[5] $\varepsilon_{299} = 3{,}3 \bullet 10^4\ M^{-1}\ cm^{-1}$.

Spectroscopic Titration:
The binding constants of the ligands 4-*tert*-butylpyridine, 4,4'-azopyridine, 1,2-di(4-pyridyl)ethylene and 1,2-di(4-pyridyl)ethane were determined using spectroscopic titrations. Steady-state fluorescence was measured on a Spex Fluorolog τ3 spectrofluorimeter (JY Horiba) by exciting the samples at 543 nm. The titrations were performed by adding aliquots of a ligand stock solution. Correction for the volume change was done in the subsequent analysis, and baselines containing only liposome and ligand were subtracted from all spectra.

Time-resolved single photon counting:
Fluorescence decays were acquired through time correlated single photon counting (TCSPC) using pulsed excitation provided by a mode-locked Tsunami Ti:sapphire laser (Spectra-Physics) which in turn was pumped by a Millenium Pro X laser (Specta-Physics). The Tsunami output (750 nm, 80 MHz, ~1 ps pulses) was used to synchronously pump a KTP OPO (GWU, Spectra-Physics), and the

resulting 1080 nm light was frequency doubled to 540 nm, and used for fluorescence excitation. The emitted photons were collected at 640 nm with a spectral bandwidth of 40 nm, and detected using a thermoelectrically cooled microchannel plate photomultiplier tube (R3809U-50, Hamamatsu). The time resolution of the system was about 50 ps (FWHM of IRF), limited by the detector response. The signal was digitalized using a multichannel analyzer with 1024 channels. 10000 counts in the top channel were acquired in order to obtain good statistics, although for apy only 2500 counts were used due to the high degree of quenching, and slow data collection. The decay curves were fitted to three-exponential expressions using the program FluoFit Pro v.4 (PicoQuant).

DNA Sequences

Table S1. DNA sequences used in this study. Sequence 1 and 2 were used to make the bisporphyrin binding pocket and $1_{unmodified}$ and 2 were used to make single modified porphyrin DNA.

Sequence	number
GGCCGCAATCCCAACCAAT^{Porphyrin}CAGCTAGACACACTCAGACG	1
CGTCTGAGTGTGTCTAGCT^{Porphyrin}GATTGGTTGGGATTGCGGCC	2
GGCCGCAATCCCAACCAATCAGCTAGACACACTCAGACG	$1_{unmodified}$

Porphyrin nucleoside structure, $T^{Porphyrin}$

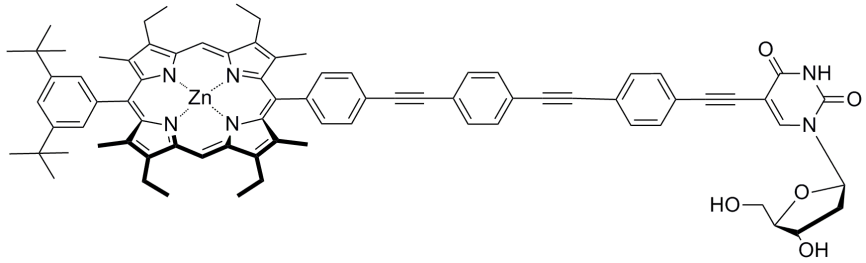


Figure S1. The porphyrin nucleoside used in this study.

Ligand titrations

The binding of the ligands to the single porphyrin and porphyrin pocket was followed by spectrophotometric titration. The change in porphyrin emission with increasing concentration of added ligand was monitored and the acquired data was analysed in accordance to reference 6 using singular value decomposition. In the analysis, the statistical factors (K_σ) associated

with the equilibrium equations were separated from the corresponding chemical binding constants (Table S2).

Table S2. Statistical factors for reactions.

Reaction		Statistical factor (K_σ)
SingleP+py	→SingleP-py	2
SingleP+apy	→SingleP-apy	4
Pocket+py	→Pocket-py	2
Pocket+apy	→Pocket-L	4
Pocket-L_{open}+py	→Pocket-L_2	2
Pocket-L_{open}+apy	→Pocket-L_2	4
Pocket-L_{open}	→Pocket-L_{closed}	2

The statistical factors were calculated using the symmetry number (σ) of the different species present in the equilibrium reactions. The symmetry number is calculated as the product of the external symmetry number (governed by point group of the molecule or the assembly) and the internal symmetry number (governed by intramolecular rotations). The 4-*tert*-butylpyridine ligand and all the bidentate ligands were assigned to the point groups C_s and C_{2h}, respectively. All the porphyrin containing assemblies were assigned to the point group C_1, because of the handedness of the DNA duplex. The symmetry numbers of the species are summarized in Table S3. To more clearly show the influence of the topology of the different assemblies, only allowed two-fold rotations of the zinc porpyrin around the linker have been included in Table S3. The same statistical factors are obtained using the internal symmetry numbers accounting for: the four possible three-fold rotations associated with the 4-*tert*-butyl groups on the porphyrins and the pyridine ligand, the three-fold rotations associated with the eight ethyl and methyl substituents on the porphyrin periphery, and the two-fold rotations of the phenylene units of the linker and the pyridyl groups of the ligands as well as the aryl substituent on the porphyrin.

Table S3. The symmetry factors of the species taking part in the porphyrin-ligand coordination. The porphyrin is assumed to be freely rotating with respect to the DNA backbone.

Species	Point group	σ_{ext}	σ_{int}	σ
py	C_S	1	1	1
dpyane	C_{2h}	2	1	2
dpyene	C_{2h}	2	1	2
apy	C_{2h}	2	1	2
SingleP	C_1	1	2	2
Pocket	C_1	1	4	4
SingleP-L	C_1	1	1	1
Pocket-L_{open}	C_1	1	2	2
Pocket-L_2	C_1	1	1	1
Pocket-L_{closed}	C_1	1	1	1

Binding to single porphyrin

The binding of ligands to the porphyrin in the lipid membrane can be expressed by the equilibrium equation:

$$K \cdot K_\sigma = \frac{PL}{P \cdot L_m} \qquad \text{Eq. S1}$$

where K is the equilibrium constant, P is the concentration of free porphyrin in the membrane, PL is the concentration of coordinated porphyrin in the membrane, and L_m is the concentration of ligands in the membrane. The ligands are located both in the water and lipid phase (the porphyrin is only located in the lipid membrane), and the concentration distribution can be approximated by the octanol/water partitioning coefficient, K_{ow} (Table S4).

$$K_{OW} = \frac{L_m}{L_w} \qquad \text{Eq. S2}$$

where L_w is the concentration of ligands in the water phase.

Table S4. Octanol water portioning coefficient for the different ligands

Ligand	K_{ow}
py	280
dpyane	35
dpyene	90
apy	50

The total amount of free ligand in the system is assumed to be large as compared to the amount of bound ligand (maximum error in ligand concentration using this approximation is less than 10 %). Thus, the concentration of free ligand in the lipid layer can be approximated as in Equation S3.

$$L \approx L_m = L_w \cdot K_{ow} \qquad \text{Eq. S3}$$

The total concentration of porphyrin, P_{tot}, can be expressed as the sum of free and coordinated species,

$$P_{tot} = PL + P \qquad \text{Eq. S4}$$

and can be combined with Equation S1 and S3 to express the fraction coordinated porphyrin (θ) as a function of ligand concentration:

$$\theta = \frac{PL}{P_{tot}} = \frac{K_\sigma \cdot K \cdot L}{1 + K_\sigma \cdot K \cdot L} \qquad \text{Eq. S5}$$

The equilibrium binding constant of ligands to the single porphyrin was analysed using singular value decomposition where Equation S5 was used to relate the recorded porphyrin emission spectra with the extracted ones.

Table S5. Binding of ligands to singleP

Ligand	K (M^{-1})
py	54
dpyane	30
dpyene	40
apy	36

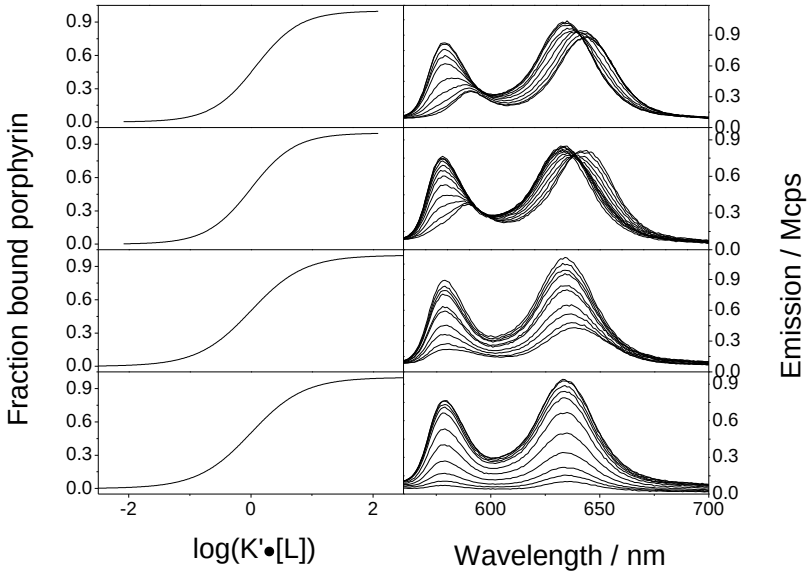
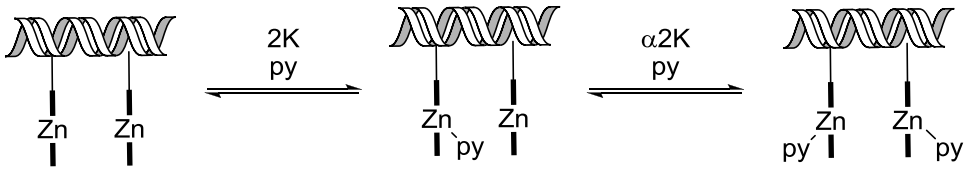


Figure S2. Left: Fraction bound porphyrins as a function of ligand concentration (K'=a×K where a is 2 for the monodentate ligand and 4 for the bidentate ligands; line: fitted amount of coordinated porphyrin). Right: Fluorescence titration data (compensated for dilution). Ligands used from top to bottom are py, dpyane, dpyene and apy.

Binding to dimeric porphyrin pocket

In the two step binding process the first binding constant of the porphyrin pocket-ligand complex was assumed to be the same as for the porphyrin-ligand complex for each respective ligand. The *tert*-pyridine ligand was used to probe the effect of allosteric cooperativity when binding to the porphyrin pocket. The cooperativity factor, α, is defined as the ratio between the first and second ligand binding to the dimeric porphyrin pocket (PP):



$$\alpha = \frac{K_2}{K_1}, PP \overset{L, K_1}{\rightleftharpoons} PP - L \overset{L, K_2}{\rightleftharpoons} PP - L_2 \qquad \text{Eq. S6}$$

The total concentration of porphyrin P_{tot} and concentration of coordinated porphyrin PL in the above equilibrium system are:

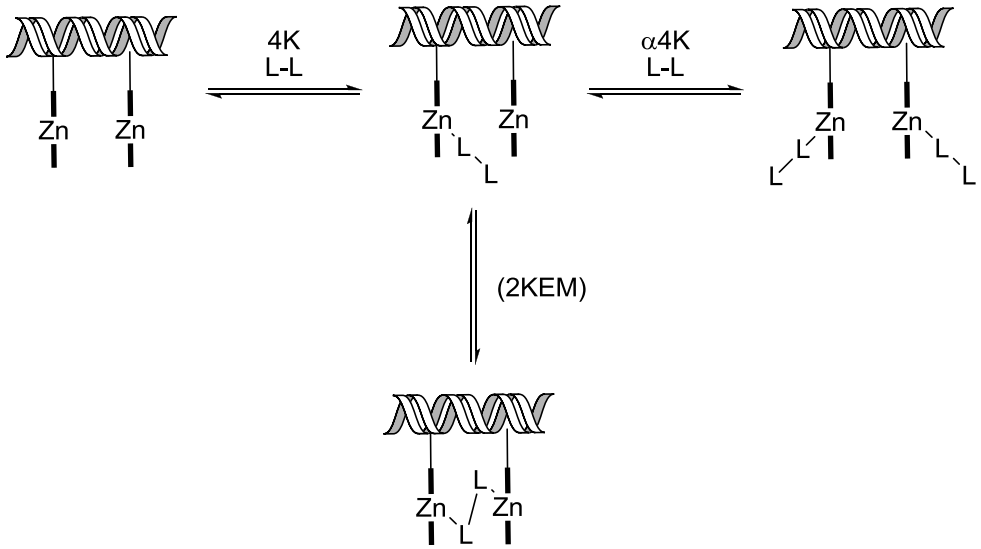
$$PL = (2KL + 2 \times 4K^2\alpha L^2)PP \qquad \text{Eq. S7}$$

$$P_{tot} = 2(1 + 2KL + 4K^2\alpha L^2)PP \qquad \text{Eq. S8}$$

The fraction of coordinated porphyrins can then be calculated according to Eq. S9:

$$\theta = \frac{KL+4K^2\alpha L^2}{1+2KL+4K^2\alpha L^2}$$ Eq. S9

The cooperativity factor was fitted to 0.51 using Equation S9, and this value was assumed to be the same for all the bidentate ligands. For the dimeric porphyrin pocket binding one bidentate ligand there exist two possible binding modes; one where the bidentate ligand only coordinates one of the two porphyrins (Pocket-L_{open}) and another where it coordinates both porphyrins (Pocket-L_{closed}). At high ligand concentrations the two porphyrins will be bound to one ligand each (Pocket-L_2). This gives three possible coordinated porphyrin pocket species for the bidentate ligands and the fraction of coordinated porphyrins can be expressed using equation S10:



$$\theta = \frac{2KL+8K^2EML+16K^2\alpha L^2}{1+4KL+8K^2EML+16K^2\alpha L^2}$$ Eq. S10

where EM is the effective molarity for the intramolecular coordination reaction as defiened in equation S11.[6]

$$2K \times EM = \frac{[Pocket-L_{closed}]}{[Pocket-L_{open}]}$$ Eq. S11

The fitted effective molarities are shown in Table S6 and the fraction of each coordinated specie in solution as a function of ligand concentration is shown in Figure S3.

Table S6. Effective molarities (EM) for intramolecular reactions and the product of binding constant and effective molarity.

Ligand	EM (M)	2K·EM
dpyane	0.029	1.74
dpyene	0.024	1.94
apy	0.045	3.24

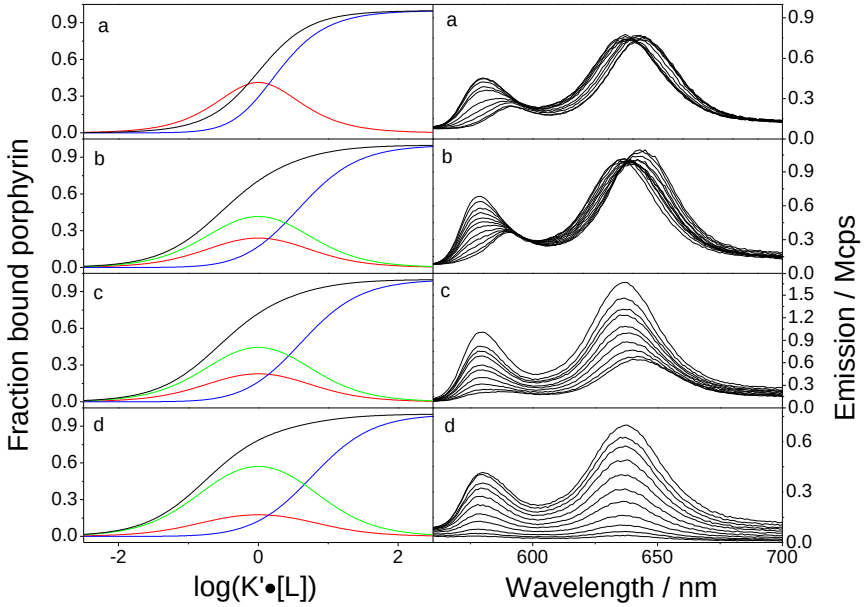


Figure S3. Left: Fraction bound species as a function of ligand concentration (red: Pocket-L_{open}, blue: Pocket-L_2, green: Pocket-L_{closed}, black: total amount of coordinated porphyrin, $K' = a \times K \times \sqrt{\alpha}$ where a is 2 for the monodentate ligand and 4 for the bidentate ligands). Right: Fluorescence titration data (compensated for dilution). Ligands used from top to bottom are py, dpyane, dpyene and apy.

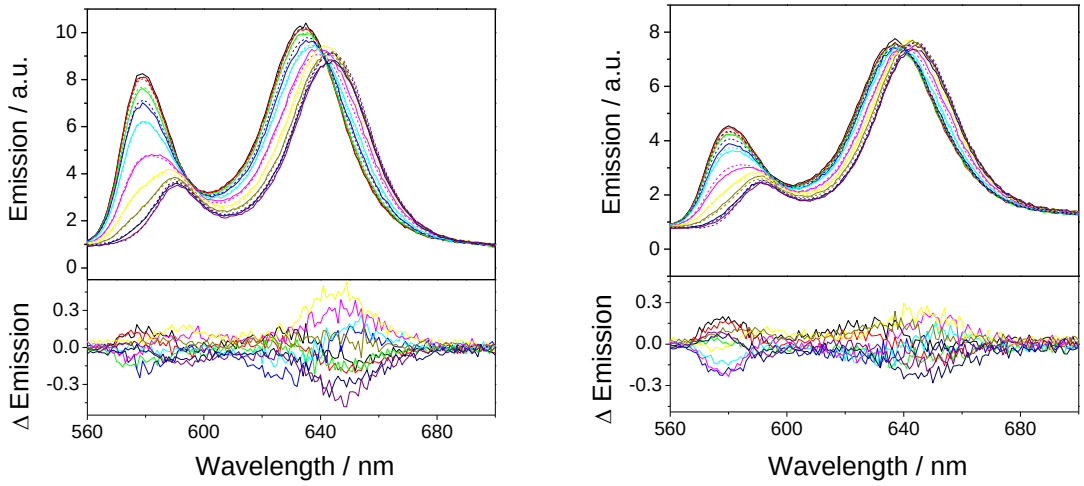


Figure S4. Emission of zinc porphyrin monomer (left) and dimer (right) with increasing amounts of 4-*tert*-butylpyridine. Top: Measured spectra (solid line) and mathematically created spectra using spectral components and the equilibrium equations (dashed line). Bottom: Residual.

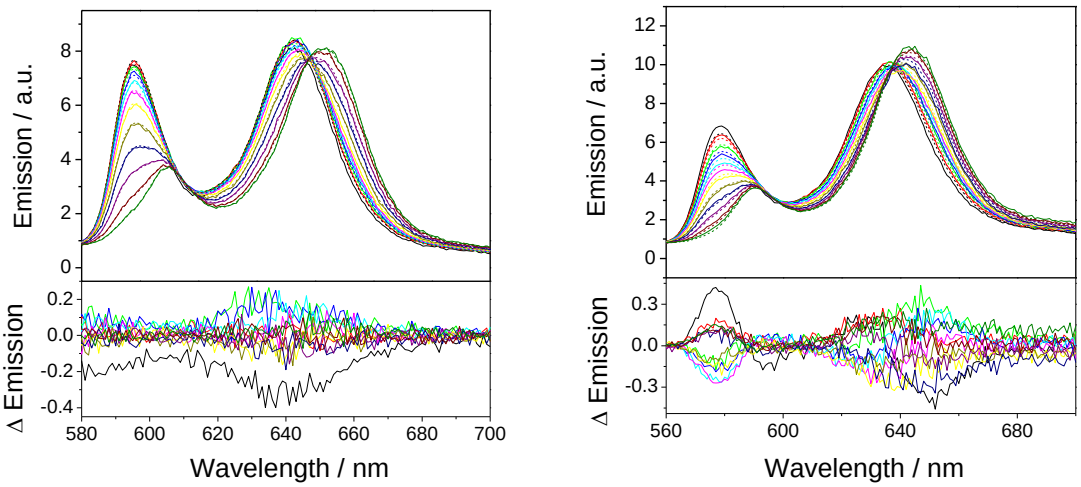


Figure S5. Emission of zinc porphyrin monomer (left) and dimer (right) with increasing amounts of 1,2-di(pyridyl)ethane. Top: Measured spectra (solid line) and mathematically created spectra using spectral components and the equilibrium equations (dashed line). Bottom: Residual.

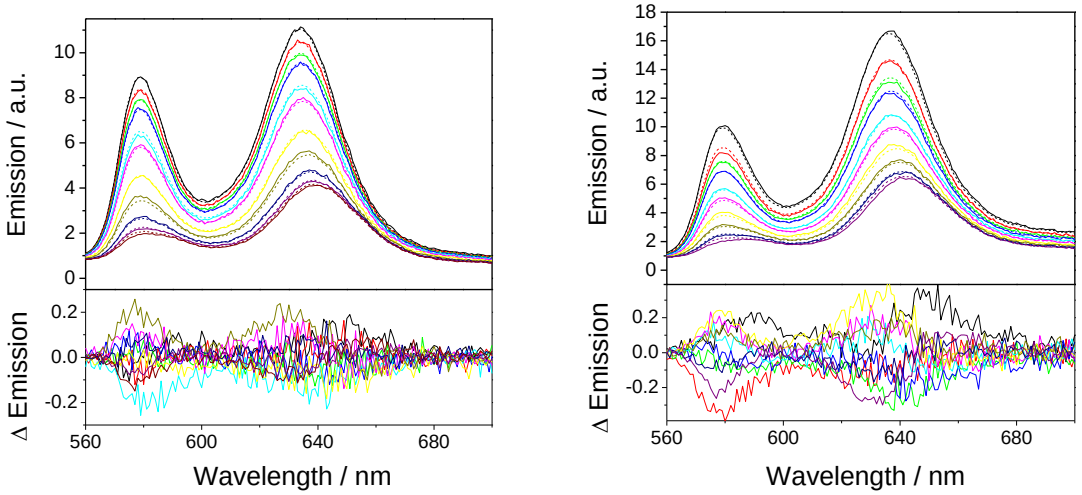


Figure S6. Emission of zinc porphyrin monomer (left) and dimer (right) with increasing amounts of 1,2-di(pyridyl)ethylene. Top: Measured spectra (solid line) and mathematically created spectra using spectral components and the equilibrium equations (dashed line). Bottom: Residual.

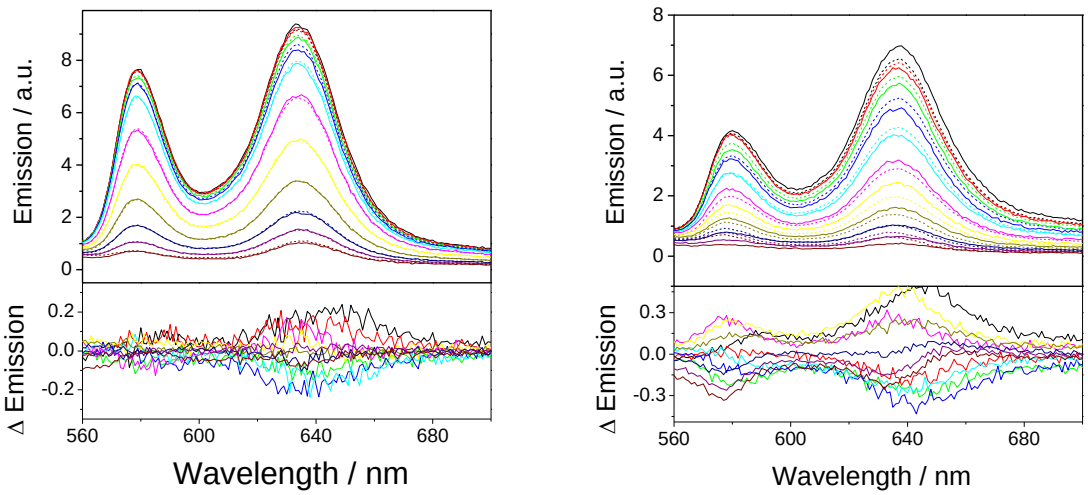


Figure S7. Emission of zinc porphyrin monomer (left) and dimer (right) with increasing amounts of 4,4'-azopyridine. Top: Measured spectra (solid line) and mathematically created spectra using spectral components and the equilibrium equations (dashed line). Bottom: Residual.

Driving Force for Electron Transfer

The driving forces, ΔG^0, for the electron transfer reactions were calculated using Equation S12 (Table S7).

$$\Delta G^0 = e\left(E_{D,ox} - E_{A,red}\right) - E_{00} + \frac{e^2}{4\pi\varepsilon_0 r}\left(\frac{1}{\varepsilon_s} - \frac{1}{\varepsilon_s^{ref}}\right) - \frac{e^2}{4\pi\varepsilon_0\varepsilon_s R_{DA}} \qquad \text{Eq. S12}$$

where ε_s is the dielectric constant of the solvent used in the fluorescence measurements (The dielectric constant of the membrane was approximated by that of an alkane, ε_{octane}=1.94), ε_s^{ref} is the dielectric constant of the solvent used in the electrochemistry measurements, r the average radius of the donor and acceptor (4.5 Å), R_{DA} the donor-acceptor distance (6.5 Å), E_{00} the excitation energy of the donor (2.085 eV), $E_{D,ox}$ the oxidation potential of the donor (0.74 V in dichloromethane),[7] and $E_{A,red}$ the reduction potentials of the acceptors.

Table S7. Reduction potentials of ligands and driving force for charge separation with the zinc porphyrin.

Ligand	Reduction potential vs SHE (V)[a]	ΔG^0 (eV)
py	-2.86[8-9]	1.515
dpyane	-2.86[8-9]	1.515
dpyene	-1.69[9]	0.435
apy	-0.59[3]	-0.755

[a] Measured in *N*,*N*-dimethylformamide.

Time Resolved Fluorescence

Fluorescence decays of the different dimeric porphyrin systems are shown in Figure S8, and fluorescence lifetimes and amplitudes obtained from the data analysis are collected in Table S8. For all systems a three-exponential function was needed to reduce the χ^2 value to an acceptable level. The shortest lifetimes obtained in the analyses are for all systems shorter or of the same order of magnitude as the time resolution of the instrumentation. As such, they represent (a combination of) all processes that decay with a time constant smaller than or comparable to the 50 ps time resolution. Viewing the series of ligands: dpyane (non-quenching), dpyene (partially quenched), apy (fully quenched), the amplitude of the shortest decay component increases with the degree of quenching. Since there are no large differences in the obtained series of three lifetimes for each of these three ligands, the increase in amplitude of the shortest component indicates that the decay constant of the quenched dimeric porphyrins is faster than the time resolution of our instrumentation.The lack of correlation between the time-resolved and steady-state fluorescence measurements might seem surprising at first. However, it could be explained by a situation in which the lifetime of the porphyrin with bound ligand is much shorter than the time-resolution of the TCSPC equipment. This is obviously the case for apy-coordinated porphyrins in which the driving force for electron transfer is large. The very weak residual fluorescence is then presumably due to non-coordinated porphyrin dimers. For the dpyene-coordinated porphyrin, which is only partially quenched even at high ligand concentrations, the residual fluorescence is much stronger (about 30% left) and, furthermore, resembles the fluorescence spectra from a coordinated species. Now, since the fluorescence lifetimes are similar to the unquenched dpyane ligand we must have a distribution of bound species; those with no or very weak quenching leading to lifetimes similar to the unquenched situation and those with complete quenching with lifetimes too short to be resolved by the experiment. This is a likely explanation since the driving force for electron transfer with the dpyene ligand is slightly endergonic (cf. Table S7), thus requiring a high electronic coupling for the quenching to occur.

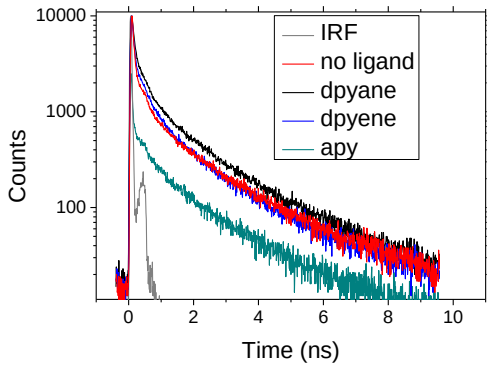


Figure S8. Fluorescence decays of the different ligands coordinated to dimeric porphyrin, and uncoordinated dimeric porphyrin. For the weakly emitting apy complex, only 2500 counts were collected in the top channel.

Table S8. Fluorescence lifetimes, τ_i (ns), and amplitudes α_i obtained from fitting TCSPC data.

Ligand	τ_1	α_1	τ_2	α_2	τ_3	α_3	χ^2
No ligand	0.04	0.88	0.30	0.087	1.85	0.038	1.1
dpyane	0.07	0.77	0.36	0.16	1.86	0.067	1.1
dpyene	0.05	0.84	0.35	0.12	1.77	0.041	1.3
apy[a]	0.02	0.95	0.37	0.033	1.94	0.018	1.1

[a] 2500 counts were collected in the top channel for apy, due to a large degree of quenching.

Optimized Structure of apy Coordinated Dimeric Porphyrin Pocket

The two porphyrin moieties in the dimeric porphyrin binding pocket are located on separate DNA strands. To form the pocket these two complementary strands are hybridised together. A quantum mechanical calculation (at the PM3 level of theory) was performed to examine the possibility of a bidentate ligand bridging the two porphyrins. In the geometry optimized structure in Figure S9 it is clearly shown that an apy ligand indeed can bridge the space between the two porphyrins. The two porphyrins are attached to the DNA strands in such a way that they are in a sandwich position to each other and the ligand is forcing them slightly apart, which is possible due to the quite large flexibility of the linker.

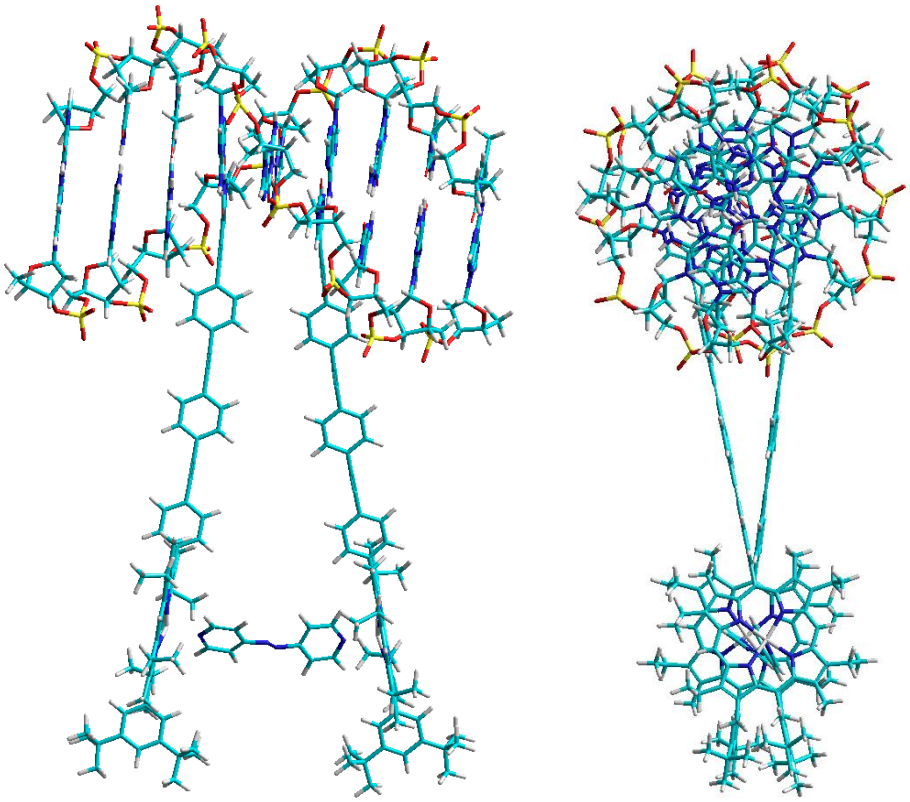


Figure S9. Optimized structure of apy coordinated porphyrin pocket in vacuum at the PM3 level of theory. Left: Front view. Right: side view.

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