Electronic Supporting Information

for the manuscript entitled

Functional Nanoassemblies with Mirror-Image Chiroptical Properties Templated by a Single Homochiral DNA Strand

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**Materials**

All commercially available reagents were used as received without purification. Water was obtained from Milli-Q system with a resistivity of 18.2 MΩ·cm. The DNA was purchased and used as received from Alpha DNA. DNA concentration is reported as base concentration (c = 0.546 mM). Using the extinction coefficient $\varepsilon = 324,600$ M$^{-1}$ cm$^{-1}$ at 260.0 nm, the concentration (per base) of the dT40 stock solutions was quantified by UV-vis spectroscopy. The concentration of the DZnPD stock solutions (c = 1.0 mM) was quantified by UV-vis spectroscopy using the extinction coefficient $\varepsilon = 230,000$ M$^{-1}$ cm$^{-1}$ at 465.0 nm.

**Synthesis of DZnPD:** The bis-bromo zinc porphyrin$^1$ (150.0 mg, 0.112 mmol) was dissolved in freshly distilled DMF (10.0 mL) under nitrogen followed by addition of TEA (5.0 mL). The solution was deoxygenated (3× vacuum/nitrogen cycle) and triphenylphosphine (29.3 mg, 0.112 mmol) and Pd$_2$(dba)$_3$ (25.6 mg, 0.028 mmol) were added subsequently. Next, acetylene-diaminopurine (78.8 mg, 0.246 mmol) was added in DMF (3.0 mL). The resulting mixture was deoxygenated (3× vacuum/nitrogen cycle), then heated to 40 °C for 10 min. CuI (10.6 mg, 0.056 mmol) was added and the reaction was stirred at 40 °C overnight (15 h), at which point TLC (85:15, DCM:MeOH) confirmed completion. The mixture was cooled and the solvents removed. Porphyrin-diaminopurine DZnPD was obtained as a green glass (160.0 mg, 76%) by flash silica chromatography using DCM:MeOH:Py (85:15:1).

$^1$H NMR (CDCl$_3$, 400 MHz) δ: 3.06 (s, 6H, O-CH$_3$), 3.17-3.18 (m, 4H), 3.20 (s, 6H, O-CH$_3$), 3.34-3.39 (m, 14H), 3.49-3.51 (m, 4H), 3.56-3.61 (m, 12H), 3.70-3.78 (m, 16H), 3.87-3.89 (m, 4H), 3.91-3.93 (m, 4H), 4.06 (dd, J = 5.5 Hz, 5.0 Hz, 4H), 4.18 (t, J = 5.5 Hz, 4H), 4.30-4.32 (m, 4H), 4.46 (dd, J = 5.5 Hz, 5.0 Hz, 4H), 4.78 (t, J = 5.5 Hz, 4H), 4.89 (bs, 4H, NH$_2$), 5.62 (bs, 4H, NH$_2$), 7.26 (d, J = 8.0 Hz, 2H, Ar), 7.64 (dd, J = 8.0 Hz, 2H, Ar), 7.75 (d, J = 2.0 Hz, 2H, Ar), 8.88 (d, J = 4.5 Hz, 4H, β-H), 9.72 (d, J = 4.5 Hz, 4H, β-H). MALDI/TOF: C$_{88}$H$_{112}$N$_{16}$O$_{22}$Zn, m/z [M-H]$^-$ = calc. 1809.7506, found 1809.7202. UV-vis $\lambda_{max}$ (DMSO) / nm (log $\varepsilon$) 475.4 nm (5.36), 696.2 nm (4.98).

**Methods and Instrumentation**

**UV-vis absorption spectroscopy:** UV-vis absorption spectra were collected at 25 °C using Jasco V-630 and Jasco V-650 UV-vis double beam spectrophotometers equipped with a single position Peltier temperature control system. A quartz cuvette with a 1 cm path length was used.

**Emission:** Emission spectra were collected at 25 °C using a Jasco FP-8300 spectrometer. The conditions were as follows: scanning speed 500 nm/min, excitation slit 5 nm, emission slit 5 nm, sensitivity medium. A quartz cuvette with a 1 cm path length was used.

**CD:** CD spectra were recorded at 20 °C using a Jasco J-815 spectropolarimeter equipped with a Peltier temperature control system. All spectra were background subtracted using manufacturer provided software. The conditions were as follows: scanning speed 100 nm/min, data pitch 0.5 nm, DIT 2 s, and bandwidth 1 nm. Quartz cuvettes with a 1 cm or 0.1 cm path lengths were used for CD experiments.
**HR TEM:** Imaging was performed on an FEI Tecnai G2 F20 scanning transmission electron microscope (STEM) operating at 200 kV. Samples for TEM were prepared by self-assembly of porphyrins onto ssDNA strands controlled by different cooling methods. DNA-porphyrin assemblies (5 µl) were deposited onto carbon-coated copper grids and air dried.

**Confocal laser scanning microscopy (CLSM) and polarized light microscopy (PLM):** Samples of dialyzed/annealed nanoassemblies were dried on glass slides. These were examined by CLSM on a Zeiss LSM 510 Meta CLSM system controlled by LSM 510 V. 4.2 SP1 software, using Zeiss 10X/0.3 NA EC Plan-Neofluar and 20X/0.8 NA Plan-Apochromat objective lenses, excitation at 514 nm (30 mW argon multiline laser, 6.1 A tube current, 5.0% transmission), and emission collected from 703-799 nm using the Meta (spectral) detector with pinhole set to 1 Airy unit. Slides were also examined by PLM on a Leica DM750 P PLM system running Leica Application Suite (LAS) X software, using Leica 10X/0.25 NA and 20X/0.40 NA Hi Plan Pol Achromat objective lenses, crossed polarizers, and a Leica EC3 digital color camera (3.1 MPx).

**SC** assembly preparation: Oligodeoxythymine dT40 (0.546 mM stock solution in cacodylate buffer) was added to a solution of 700 mM NaCl, 18% DMSO in sodium cacodylate buffer (1 mM, pH 7.0) at room temperature. This solution was heated to 85 °C followed by addition of DZnPD. The final concentrations of dT40 and DZnPD were 20 µM and 10 µM respectively. The resulting solution was kept at 85 °C for 60 min. The solution was then cooled to 20 °C at the rate of 0.5 °C /min with 1 min equilibrating time after each 1 min step. Cooling time from 85 °C to 20 °C was 260 min.

**FC** assembly preparation: Oligodeoxythymine dT40 (0.546 mM stock solution in cacodylate buffer) was added to a solution of 26% DMSO in sodium cacodylate buffer (1 mM, pH 7.0) at room temperature. The resulting solution was heated to 85 °C followed by addition of DZnPD (1 mM DMSO stock solution). The final concentrations of dT40 and DPD were 20 µM and 10 µM each. The resulting solution was kept at 85 °C for 60 min. The solution was then quickly transferred into a holder that was pre-equilibrated at 4 °C to achieve fast cooling. Cooling time from 85 °C to 20 °C was < 5 min.

**Dialysis:** The dT40-DZnPD solution was transferred to a Slide-A-Lyzer™ Dialysis Cassette (2,000 MWCO). The cassette was submerged in DI water (250 mL) and gently stirred. The dT40-DZnPD solution was transferred to a new cassette every 4 h as well as stirred in fresh DI water (250 mL) for up to 12 h.

**Thermal Annealing:** The dialyzed dT40-DZnPD solution (SC and FC respectively) was heated to 85 °C and kept there/homogenized for 20 min. It was then allowed to cool to 20 °C to yield SC* and FC* assemblies.

**Photooxidation:** NaI was added to the dialyzed and thermally annealed SC* assembly. The solution (500 mM NaI and SC*) was irradiated with 532 nm light, and an absorption spectrum was recorded every 5 minutes for up to 60 min.
Figure S1. CD spectra of crude mixtures of T40 templated SC porphyrin nanoassemblies prepared under different DMSO concentration (700 mM NaCl, slow cooling). Inset: Soret band CD signal as a function of DMSO concentration for 700 mM, 500 mM and 200 mM NaCl.

Figure S2. CD spectra of crude mixtures of T40 templated porphyrin nanoassemblies at 0 mM NaCl and 26% DMSO concentration prepared under regular fast cooling (blue curve) and under “switched” slow cooling (orange curve) conditions.

Figure S3. Comparison of CD spectra of nanoassemblies prepared by fast cooling (left) and slow cooling (right) of DZnPD with the non-self-complementary dT40 and the non-complementary dA40 templates.
Figure S4. CD spectra showing the time stability of SC* and FC* assemblies.

Figure S5. CD spectra showing the pH stability of FC* nanoassemblies over pH range 2.0-12.0.

Figure S6. CD spectra showing the pH stability of SC* nanoassemblies over pH range 2.0-12.0.
Figure S7. Normalized Soret band UV-vis absorption spectra of DZnPD in MeOH at RT (purple curve) and in buffer/DMSO at 85 °C (red curve).

Figure S8. Normalized emission spectra (\(\lambda_{\text{exc}} = 450 \text{ nm} \)) of crude FC and dialyzed FC chiral nanoassemblies.
Figure S9. Molecular dynamics simulations of the 2 DNA–40 DPD system. Snapshots of the 2 DNA–40 DPD system are shown in two different views before the simulation was initiated (a), after minimization (b), as well as at t = 90 ns (c), 150 ns (d), and 200 ns (e). **DPD** molecules 9-24, part of the assembly that has been selected for the CD spectra calculations, are highlighted in black.

Table S1. Details of molecular dynamics simulation system.

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<table>
<thead>
<tr>
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<tr>
<td><strong>Number of atoms</strong></td>
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Figure S10: Calculated UV-vis absorption (top) and CD (bottom) spectra of the DPD trimer by full TDDFT B3LYP/6-31G(d) (left) and approximate sTDDFT (right).

Figure S11: Calculated sTDDFT UV-vis absorption (top) and CD (bottom) spectra of the DPD hexamer (left) and decamer (right).

Table S2 compares the theoretical $g$ (Kuhn dissymmetry) values, which were obtained in two ways: first according to the general formula $g = 4R/D$, where $R$ is the rotational strength and $D$ the dipolar strength, by summing all the positive and negative contributions to $R$ for each band. Second, to mimic experimental estimation, $g$ values were computed as $\Delta A/A$ at the wavelengths of the maximum (+) and minimum (−) CD for the two couplets. The values for the Q band are in reasonable agreement, while those for the Soret band are significantly smaller using the latter method, reflecting a large amount of +/- cancellation, which is apparent from the spectra (Figures S7-8). Although the simulated $g$ values keep increasing with length (Table S2), for the largest model - (DPD)$_{10}$ - the agreement with the experimental values (Table 1) is very good (it should be noted that the enantiomeric excess of the experimental samples is unknown, making the
precise comparison difficult). This shows that not just the spectral shapes, but also the calculated CD intensities agree well with the experiment, lending further support to the proposed structural models.

**Table S2:** CD anisotropy factors \((g)\) from theoretical calculations of the UV-VIS and CD spectra for the model structures.

<table>
<thead>
<tr>
<th></th>
<th>((\text{DPD}_3))</th>
<th>((\text{DPD}_6))</th>
<th>((\text{DPD}_{10}))</th>
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<tr>
<td></td>
<td>4R/D spectral contour</td>
<td>4R/D spectral contour</td>
<td>4R/D spectral contour</td>
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<td>(g (- \text{CD Q band}))</td>
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