Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

for the manuscript entitled

Formation and helicity control of ssDNA templated porphyrin nanoassemblies

Gevorg Sargsyan, Alexandra A. Schatz, Jan Kubelka, and Milan Balaz*

Department of Chemistry, University of Wyoming,
1000 E. University Avenue, Laramie, WY 82071, USA

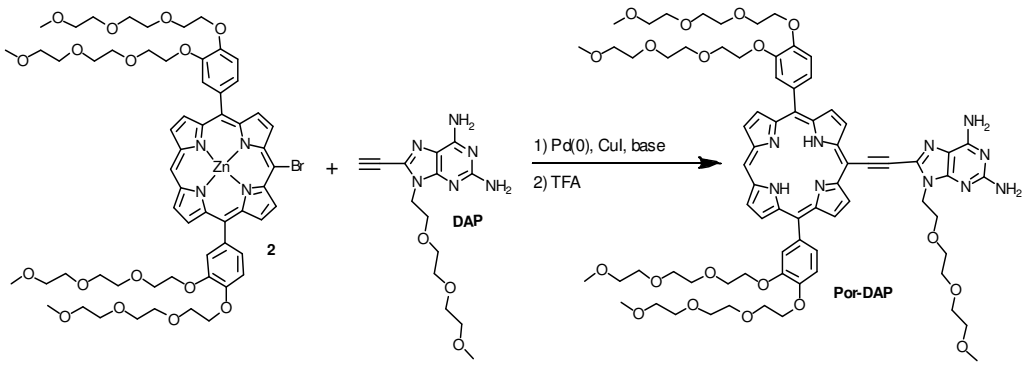
General experimental. All commercially available reagents were used as received without purification. Water was obtained from Milli-Q system with a resistivity of 18.2 MΩ·cm. DNA concentration is reported as base concentration and it was quantified by UV-vis absorption spectroscopy.

Circular Dichroism Spectroscopy. CD spectra were recorded on a Jasco J-815 spectropolarimeter equipped with a Peltier temperature control system. Conditions were as follows: scanning speed 50 nm/min, data pitch 0.5 nm, DIT 2 s, and bandwidth 1 nm. A quartz cuvette with a 1 cm path length was used for all CD experiments.

UV-vis absorption spectroscopy. UV-vis absorption spectra were recorded on a Jasco V-630 double beam spectrophotometer equipped with a single position Peltier temperature control system. A quartz cuvette with a1 cm path length was used for all UV-vis experiments.

Fluorescence emission and Resonance light scattering. Fluorescence emission and resonance light scattering and emission spectra were recorded on the Varian Cary Eclipse fluorescence spectrophotometer equipped with a Peltier temperature control system. RLS spectra were obtained under following conditions: synchronous scan mode, scan rate 600 nm/min, 2.5 nm excitation slit, and 2.5 nm emission slit. Fluorescence spectra were obtained under the following conditions: scan rate 600 nm/min, excitation wavelength 437 nm, with 5.0 nm excitation slit, and 5.0 nm emission slit. A quartz cuvette with a 1 cm path length was used. ^{1}H NMR spectra were obtained with a Bruker DRX-400 instrument (^{1}H: 400 MHz). Chemical shifts are quoted as parts per million (ppm) relative to the solvent residual peak and coupling constants (*J*) are quoted in Hertz (Hz).

Synthesis of Por-DAP.



The monobrominated Zn(II)porphyrin **2** (90.0 mg, 0.072 mmol) was dissolved in freshly distilled DMF (4.0 mL) under nitrogen followed by addition of TEA (4.0 mL). The solution was deoxygenated (vacuum/nitrogen cycle) and triphenylphosphine (18.9 mg, 0.072 mmol) and $Pd_2(dba)_3$ (16.5 mg, 0.018 mmol) were added subsequently. The acetylene-diaminopurine derivative **DAP** (44.8 mg, 0.140 mmol) dissolved in DMF (2.0 mL) and TEA (2.0 mL) was then added. The resulting solution was deoxygenated,

then heated at 40 °C for 10 min under nitrogen. Copper (I) iodide (6.8 mg, 0.036 mmol) was added and reaction was stirred overnight (18 h) at 40 °C. The solvents were removed under reduced pressure, the green oil was dissolved in DCM:MeOH (9:1), loaded onto a silica gel column, and eluted with DCM:MeOH (9:1). Zn(II)porphyrin-diaminopurine was obtained as a green glass (36.5 mg, 34 %).

Zn(II)porphyrin-diaminopurine (15.0 mg, 0.010 mmol) was dissolved in DCM (2.5 mL). The solution was stirred in an ice bath for 15 min then TFA (100 µL) was added dropwise. After 10 min the ice bath was removed and the reaction was stirred at room temperature for 4 h. Then the flask was placed in an ice bath and a saturated solution of $NaHCO_3$ (20.0 mL) was added. The ice bath was removed and solution was stirred at room temperature for 30 min. The organic phase was extracted with DCM (3×20 mL). The solvent was evaporated and the product was purified by column chromatography on silica using DCM:MeOH (9:1) as the eluent. **Por-DAP** was obtained as a green glass (10 mg, 70 %).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: -2.52 (s, 2H, pyrrole), 3.03 (s, 3H, O-CH_3), 3.12-3.15 (m, 2H), 3.19 (s, 3H, O-CH_3), 3.20 (s, 3H, O-CH_3), 3.31-3.38 (m, 6H), 3.42 (s, 6H, O-CH_3), 3.49-3.52 (m, 4H), 3.58-3-64 (m, 10H), 3.74-3.81 (m, 14H), 3.91-3.97 (m, 8H), 4.09-4.11 (m, 4H), 4.19 (t, J = 5.0 Hz, 2H,), 4.32-4.35 (m, 4H), 4.49-4.51 (m, 4H), 4.80 (t, J = 5.5 Hz, 2H), 4.85 (bs, 2H, NH_2), 5.53 (bs, 2H, NH_2), 7.32 (d, J = 8.0 Hz, 2H, Ar), 7.74 (dd, J = 8.0 Hz, 2.0 Hz, 2H, Ar), 7.82 (d, J = 2.0 Hz, 2H, Ar), 8.97 (d, J = 4.5 Hz, 2H, β-H), 9.02 (d, J = 4.5 Hz, 2H, β-H), 9.27 (d, J = 4.5 Hz, 2H, β-H), 9.84 (d, J = 4.5 Hz, 2H, β-H), 10.19 (s, 1H, *meso*-H). **HR-ESI:** $C_{74}H_{96}N_{10}O_{19}$, *m/z* [M-H$^+$]= calc. 1429.6931, found 1429.6920. **UV-vis:** λ_{max} (DMSO) / nm (log ε) 437.0 nm (5.18), 592.0 nm (4.44), 673.0 nm (4.15).

Preparation of Stock solutions:

The **dT40** stock solution (c = 0.546 mM) was prepared in a sodium cacodylate buffer (1 mM, pH 7.0). The concentration of the **dT40** stock solutions was quantified by UV-vis spectroscopy using the extinction coefficient ε = 324,600 M^{-1} cm^{-1} at 260 nm. The concentration is per base. The **Por-DAP** stock solution (c = 1.0 mM) was prepared in DMSO. The concentration of the **Por-DAP** stock solutions was quantified by UV-vis spectroscopy using the extinction coefficient ε = 152,057 M^{-1} cm^{-1} at 437.0 nm.

Preparation of Por-DAP·dT40 assemblies:

Slow cooling without NaCl:

Oligodeoxythymine **dT40** (29.3 µL of 0.546 mM stock solution) was added to a 40% DMSO solution in a sodium cacodylate buffer (1554.7 µL, 1 mM, pH 7.0) at room temperature. The resulting solution was heated to 85 °C followed by addition of **Por-DAP** (16 µL of a 1 mM DMSO stock solution). The final

concentrations of **dT40** and **Por-DAP** were 10 µM each. The resulting solution was kept at 85 °C for 60 min to permit homogenization. The solution was then cooled to 20 °C at 0.5 °C /min with 1 min equilibrating time after each step. Cooling time from 85 °C to 20 °C was 260 min.

Slow cooling with NaCl:

Oligodeoxythymine **dT40** (29.3 µL of 0.546 mM stock solution) and sodium chloride (200 µL of 4.0 M solution in water) were added to a 40% DMSO solution in a sodium cacodylate buffer (1554.7 µL, 1 mM, pH 7.0) at room temperature. The resulting solution was heated to 85 °C followed by addition of **Por-DAP** (16 µL of a 1 mM DMSO stock solution). The final concentrations of **dT40** and **Por-DAP** were 10 µM each. The resulting solution was kept at 85 °C for 60 min to permit homogenization. The solution was then cooled to 20 °C at 0.5 °C /min with 1 min equilibrating time after each step. Cooling time from 85 °C to 20 °C was 260 min.

Fast cooling with NaCl:

Oligodeoxythymine **dT40** (29.3 µL of 0.546 mM stock solution) and sodium chloride (200 µL of 4.0 M solution in water) were added to a 40% DMSO solution in a sodium cacodylate buffer (1554.7 µL, 1 mM, pH 7.0) at room temperature. The resulting solution was heated to 85 °C followed by addition of **Por-DAP** (16 µL of a 1 mM DMSO stock solution). The final concentrations of **dT40** and **Por-DAP** were 10 µM each. The resulting solution was kept at 85 °C for 60 min to permit homogenization. The solution was then quickly transferred into a Peltier cell holder that was pre-equilibrated at 20 °C to achieve fast cooling. Cooling time from 85 °C to 22 °C was 4 min.

Fast cooling without NaCl:

Oligodeoxythymine **dT40** (29.3 µL of 0.546 mM stock solution) was added to a 40% DMSO solution in a sodium cacodylate buffer (1554.7 µL, 1 mM, pH 7.0) at room temperature. The resulting solution was heated to 85 °C followed by addition of **Por-DAP** (16 µL of a 1 mM DMSO stock solution). The final concentrations of **dT40** and **Por-DAP** were 10 µM each. The resulting solution was kept at 85 °C for 60 min to permit homogenization. The solution was then quickly transferred into a Peltier cell holder that was pre-equilibrated at 20 °C to achieve fast cooling. Cooling time from 85 °C to 22 °C was 4 min.

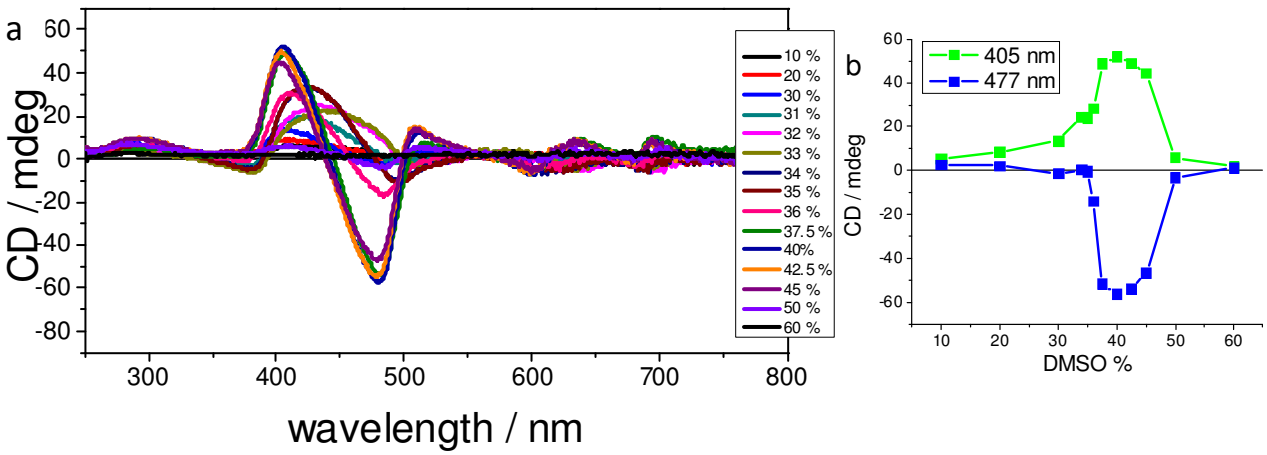


Figure S1. (a) CD spectra of **Por-DAP+dT40** varying the DMSO concentration. Conditions: Slow cooling, [**Por-DAP**] = 10.0 μM, [**dT40**] = 10.0 μM, Na-cacodylate buffer (1.0 mM, pH = 7.0).[1] (b) CD signal intensity depending on DMSO content.

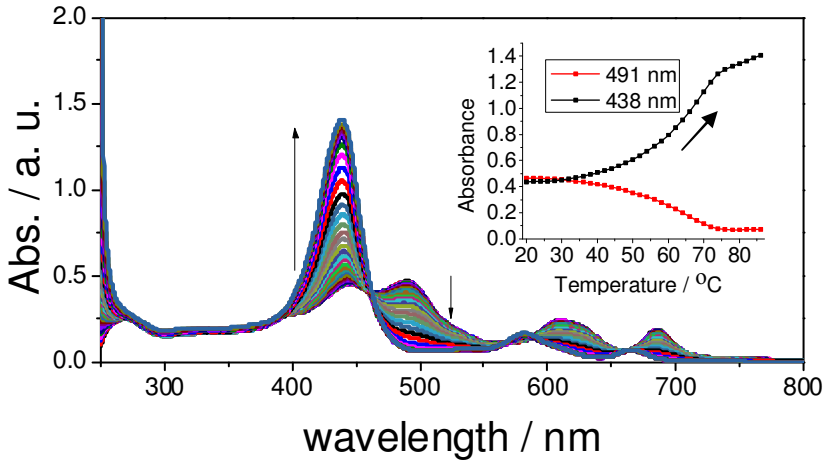


Figure S2. Variable-temperature UV-vis absorption spectra of **Por-DAP·dT40** nanoassemblies[1] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the absorption intensity as a function of temperature.

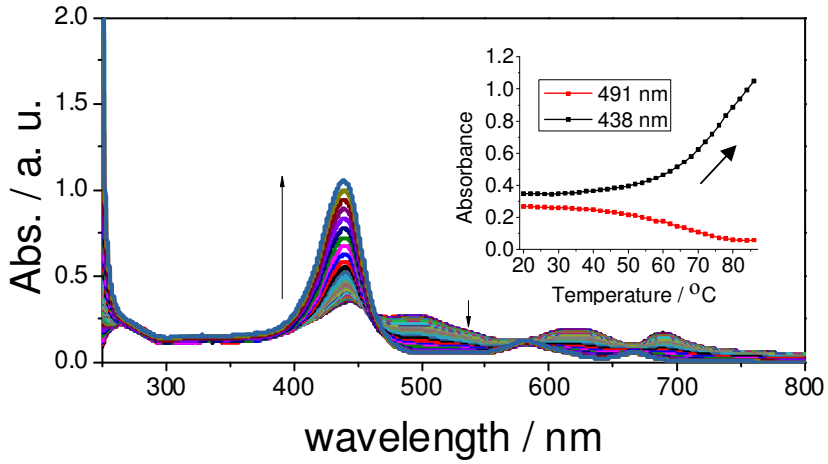


Figure S3. Variable-temperature UV-vis absorption spectra of **Por-DAP·dT40** nanoassemblies[2] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the absorption intensity as a function of temperature.

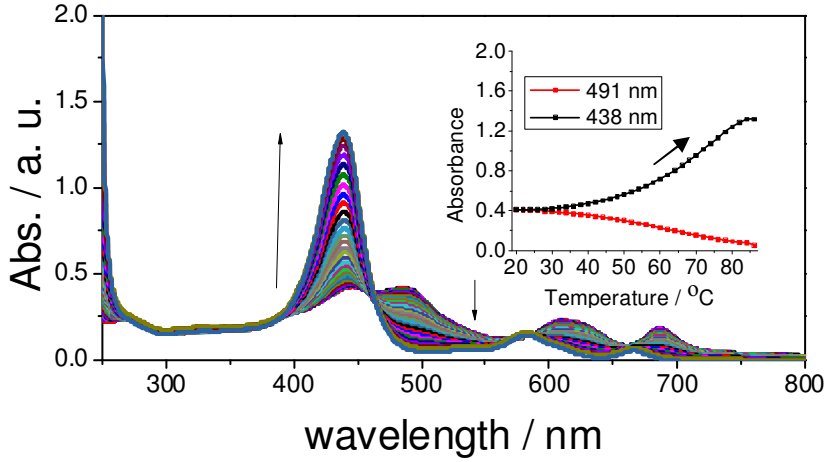


Figure S4. Variable-temperature UV-vis absorption spectra of **Por-DAP·dT40** nanoassemblies[3] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the absorption intensity as a function of temperature.

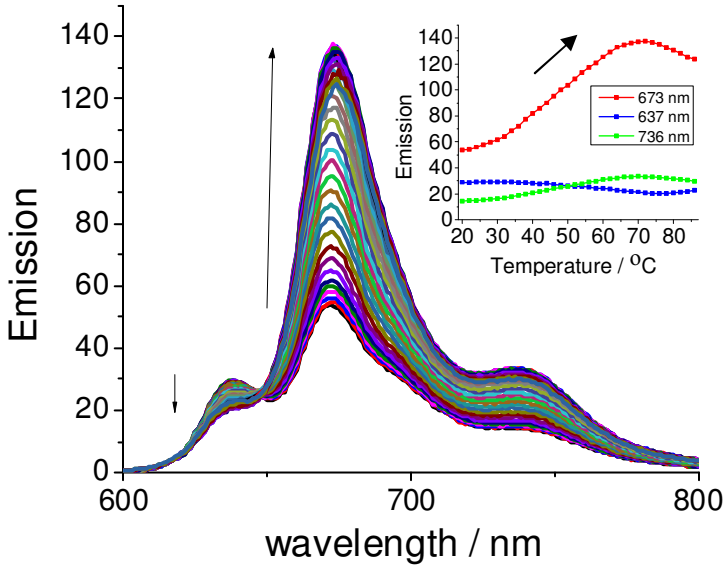


Figure S5. Variable-temperature Fluorescence spectra of **Por-DAP·dT40** nanoassemblies[1] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the emission intensity as a function of temperature.

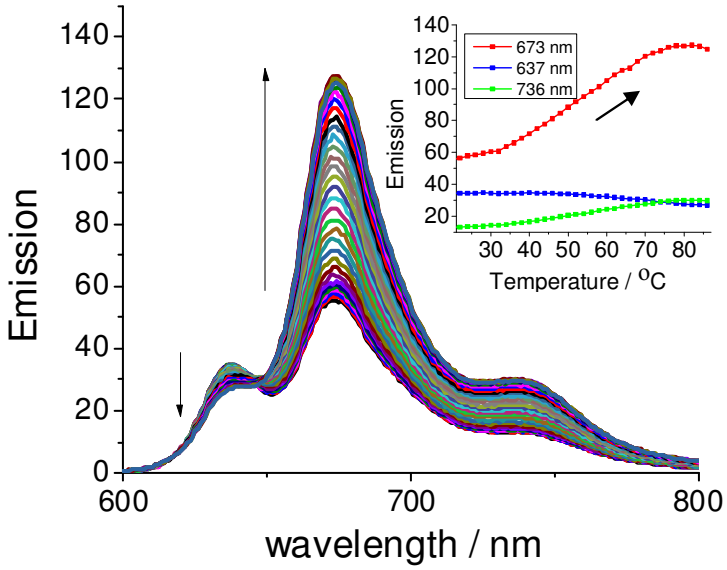


Figure S6. Variable-temperature Fluorescence spectra of **Por-DAP·dT40** nanoassemblies[2] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the emission intensity as a function of temperature.

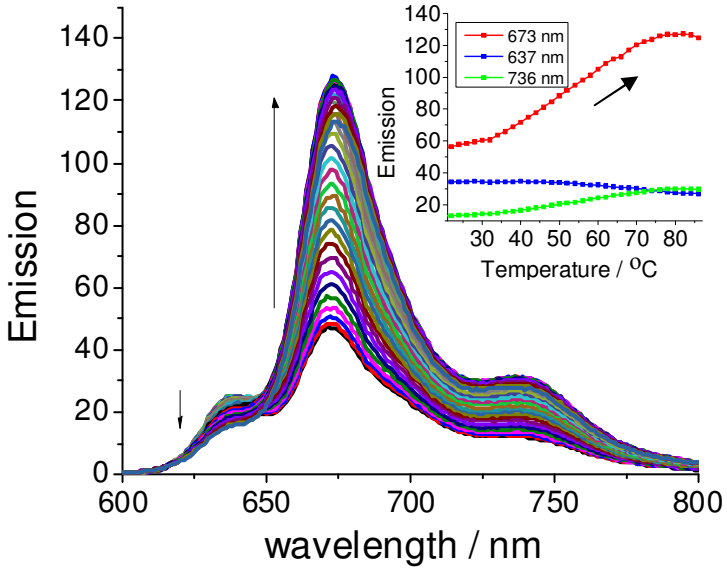


Figure S7. Variable-temperature Fluorescence spectra of **Por-DAP·dT40** nanoassemblies[3] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the emission intensity as a function of temperature.

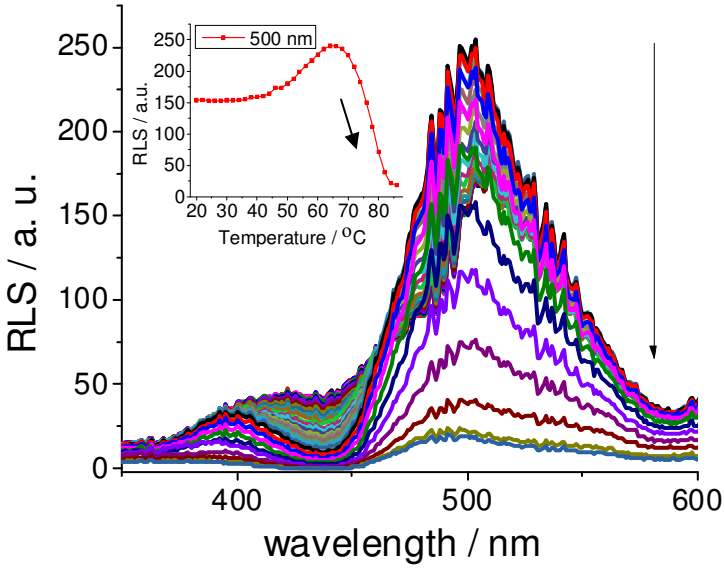


Figure S8. Variable-temperature RLS spectra of **Por-DAP·dT40** nanoassemblies[1] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the RLS intensity as a function of temperature.

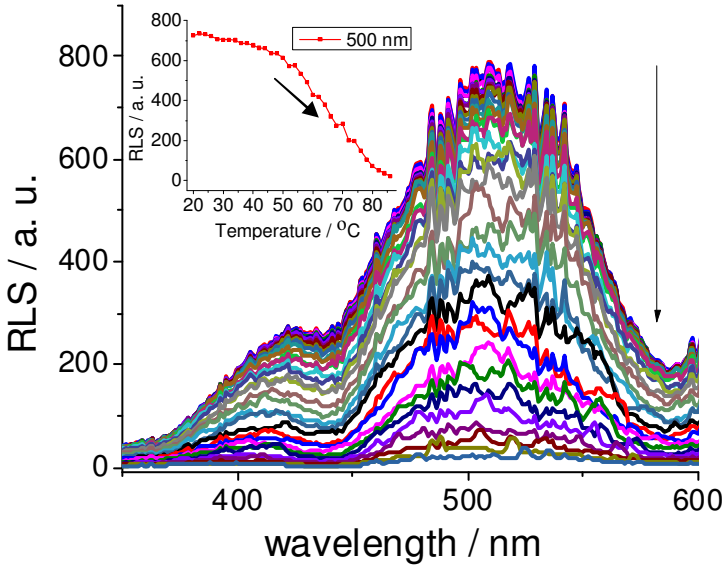


Figure S9. Variable-temperature RLS spectra of **Por-DAP·dT40** nanoassemblies[2] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the RLS intensity as a function of temperature.

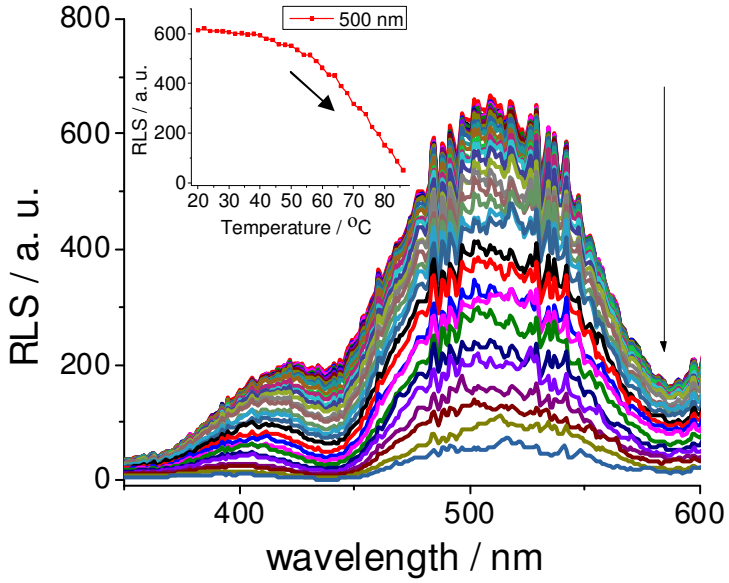


Figure S10. Variable-temperature RLS spectra of **Por-DAP·dT40** nanoassemblies[3] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the RLS intensity as a function of temperature.

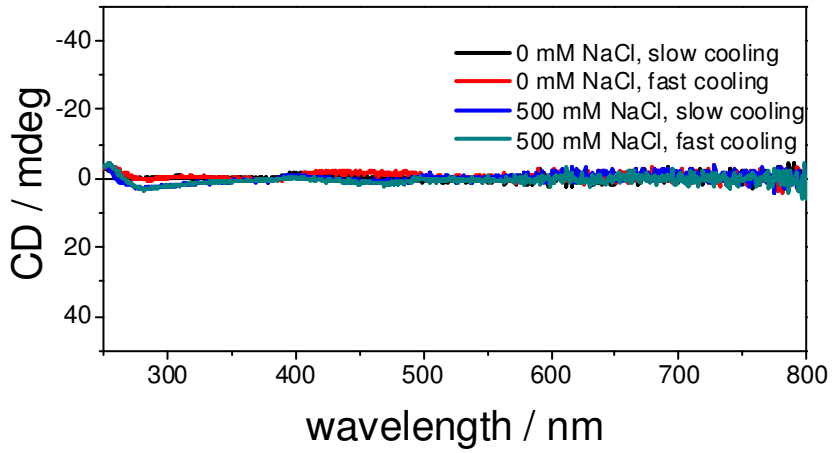


Figure S11. The CD spectra of **Por-DAP** (10 μM) after cooling with a non-complementary **dA40** (10 μM).

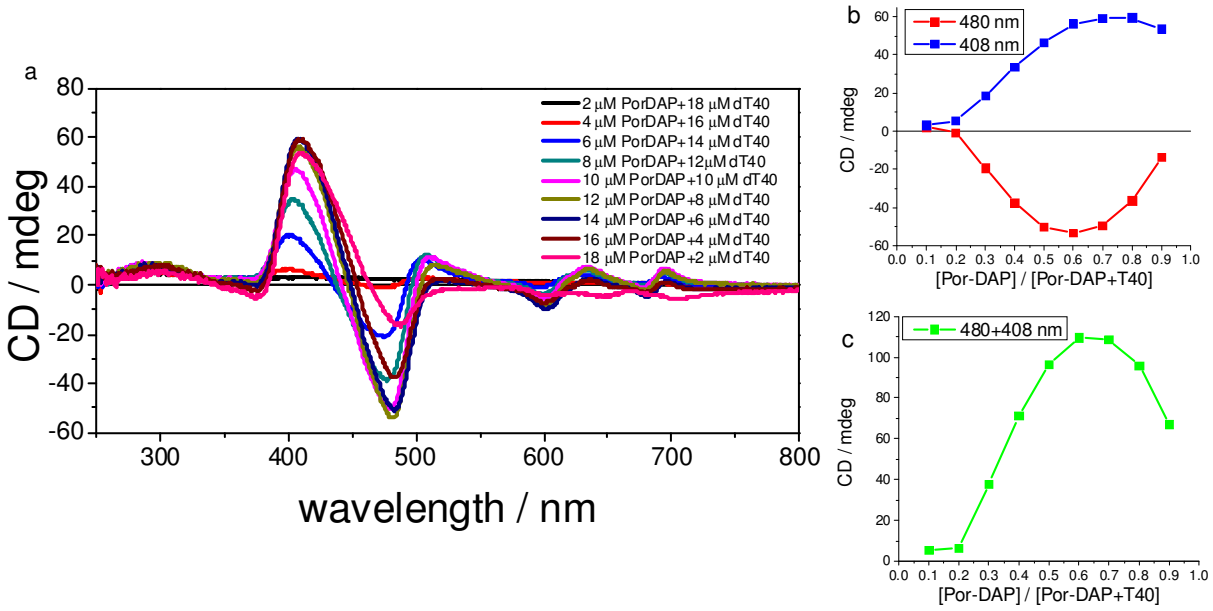


Figure S12. (a) CD spectra with different molar fractions of **Por-DAP** and **dT40** annealed by slow cooling in the absence of NaCl.[1] Job Plot of **Por-DAP·dT40** monitoring the two CD bands at Soret region (b) and the CD signal amplitude (c).

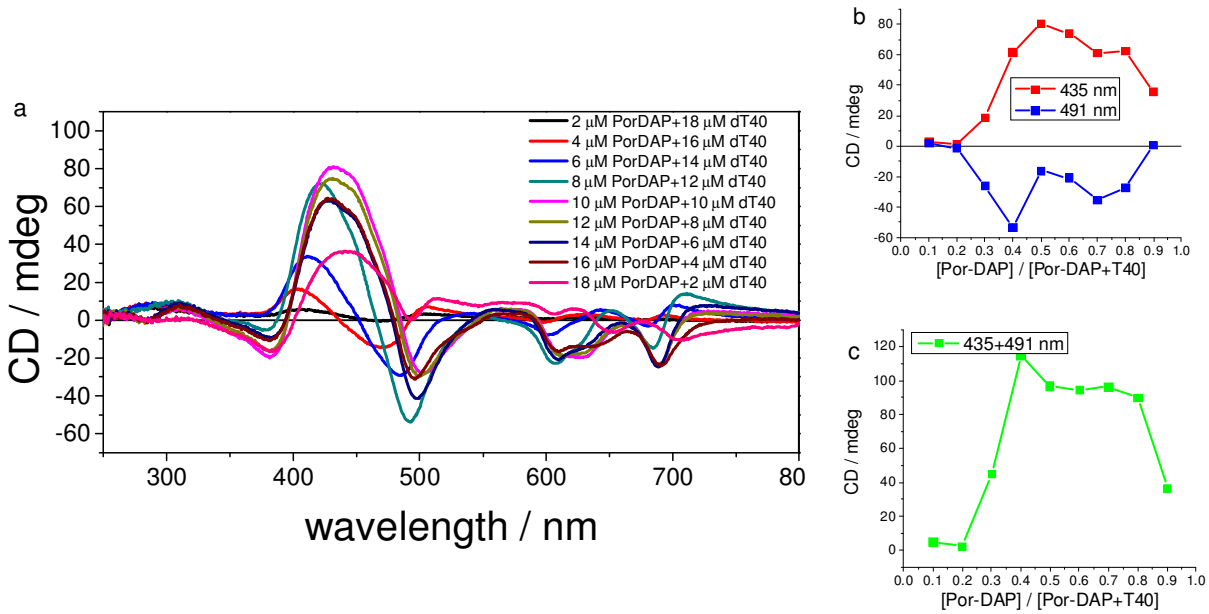


Figure S13. (a) CD spectra with different molar fractions of **Por-DAP** and **dT40** annealed by slow cooling in the presence of NaCl (500 mM).[1] Job Plot of **Por-DAP·dT40** monitoring the two CD bands at Soret region (b) and the CD signal amplitude (c).

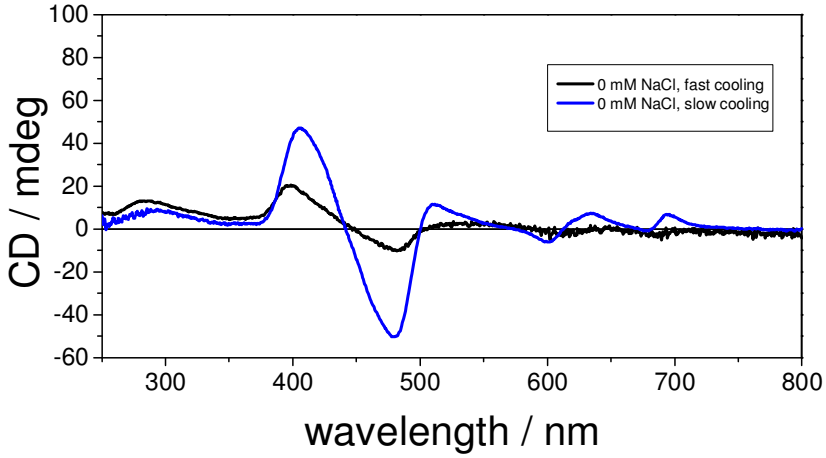


Figure S14. CD spectra of **Por-DAP·dT40** prepared by slow[1] (blue) and fast[4] (black) cooling of the sample in the absence of NaCl.

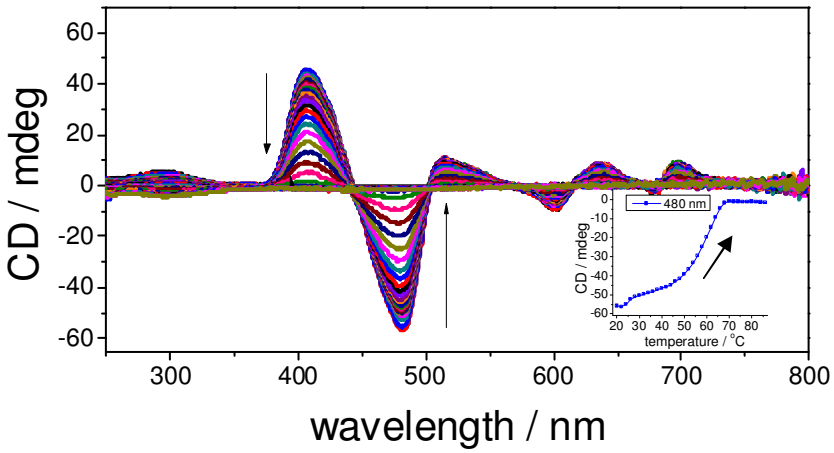


Figure S15. Variable-temperature CD spectra of **Por-DAP·dT40** nanoassemblies[1] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the CD intensity as a function of temperature.

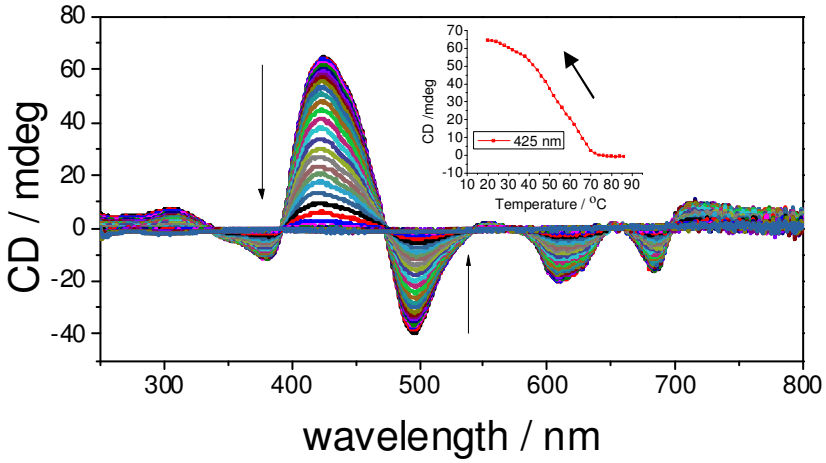


Figure S16. Variable-temperature CD spectra of **Por-DAP·dT40** nanoassemblies[2] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the CD intensity as a function of temperature.

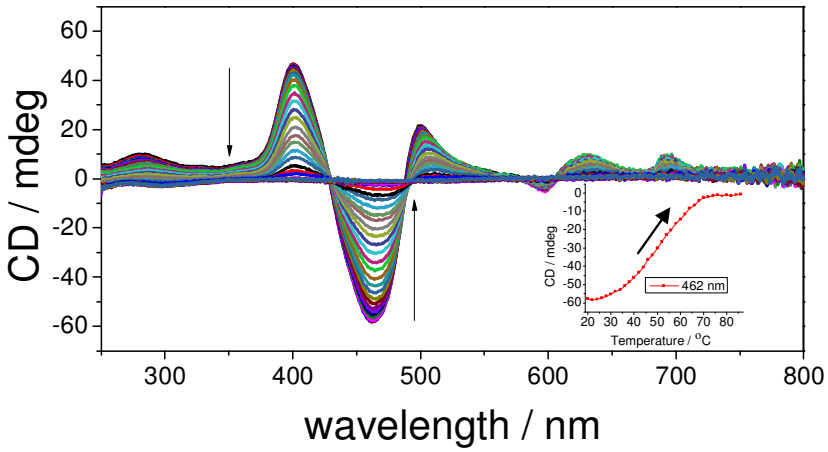


Figure S17. Variable-temperature CD spectra of **Por-DAP·dT40** nanoassemblies[3] from 20 °C to 85 °C at 0.5 °C/min. Spectra were measured every 2 °C. Inset shows the CD intensity as a function of temperature.

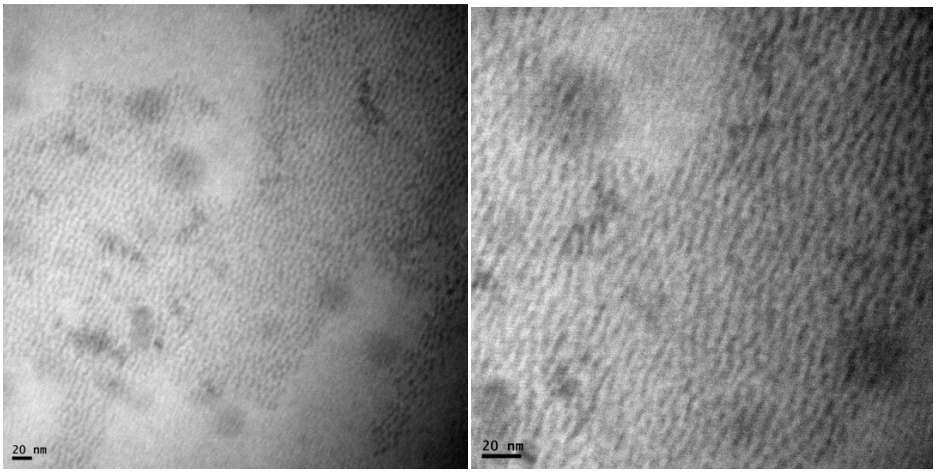


Figure S18. TEM image of **Por-DAP·dT40** prepared by slow cooling.[2] The sample was drop cast on a cupper supported carbon grid and dried for 30 min before obtaining the micrograph.

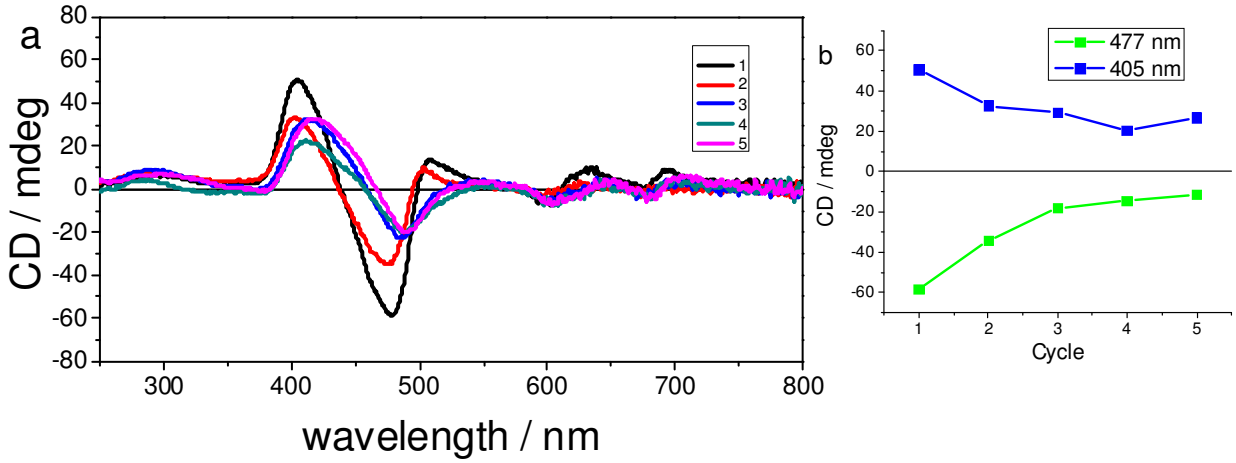


Figure S19. (a) CD spectra of **Por-DAP·dT40** prepared by slow cooling.[1] (b) CD spectral changes of **Por-DAP·dT40** after each heating-cooling cycle.

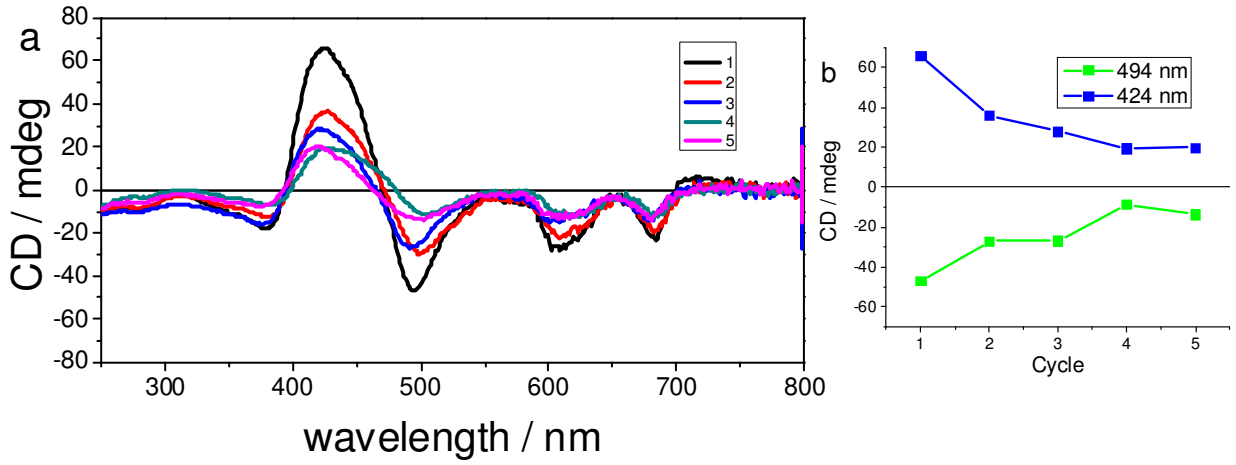


Figure S20. (a) CD spectra of **Por-DAP·dT40** prepared by slow cooling.[2] (b) CD spectral changes of **Por-DAP·dT40** after each heating-cooling cycle.

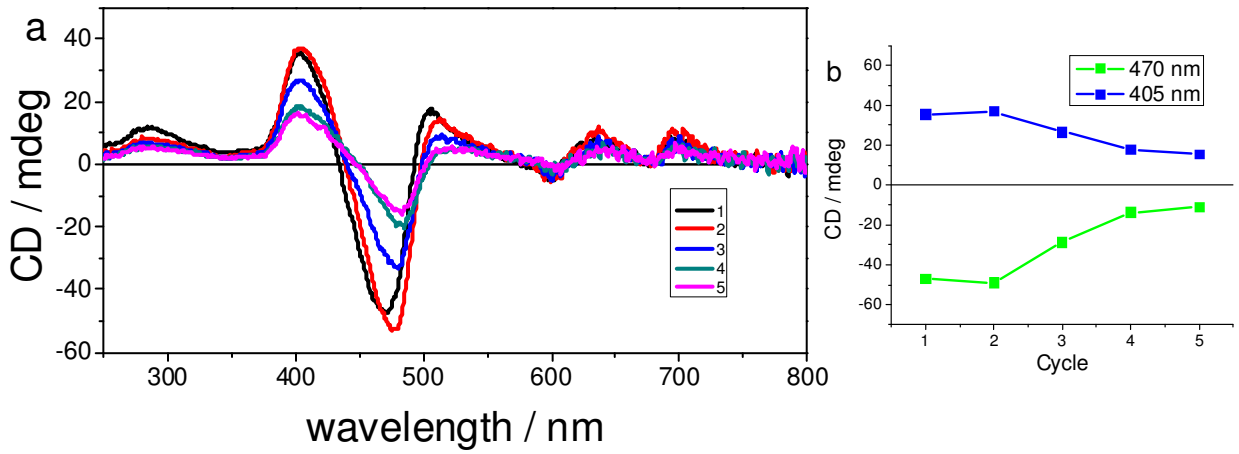


Figure S21. (a) CD spectra of **Por-DAP·dT40** prepared by fast cooling.[3] (b) CD spectral changes of **Por-DAP·dT40** after each heating-cooling cycle.

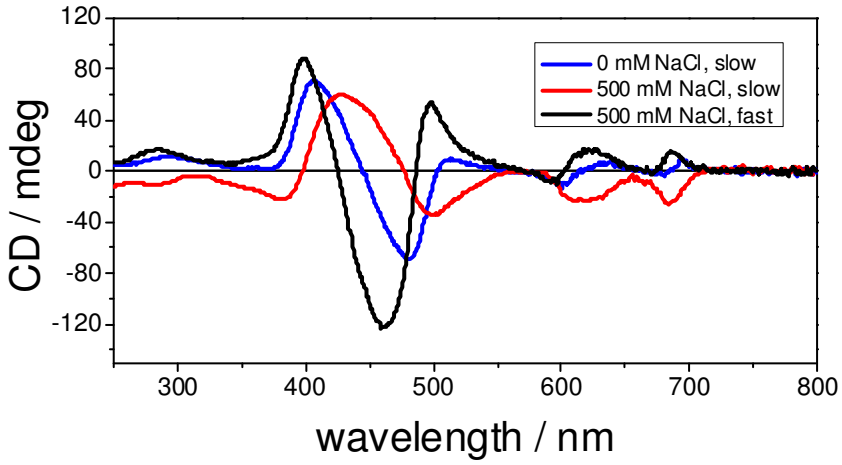


Figure S22. CD spectra of **Por-DAP·dT40** assemblies at increased concentrations of **Por-DAP** and **dT40** (15 μM).

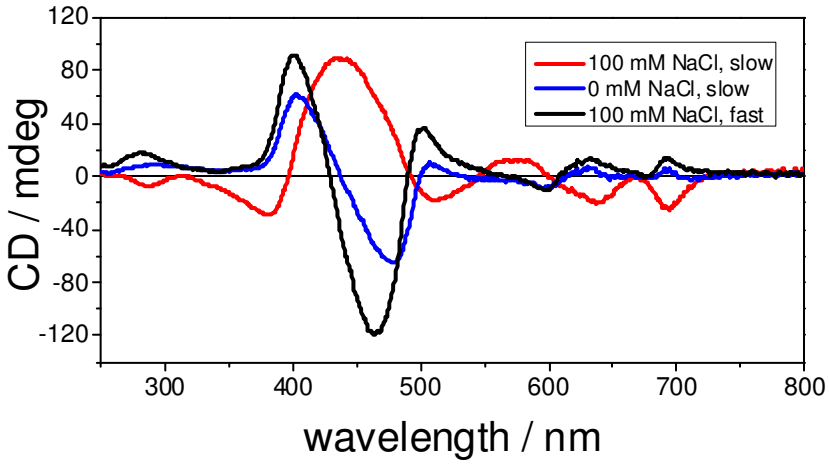


Figure S22. CD spectra of **Por-DAP·dT40** assemblies at increased concentrations of **Por-DAP** and **dT40** (20 μM).

Theoretical simulations of UV/CD spectra:

Theoretical simulations of UV/CD spectra for model porphyrin assemblies were carried out using Gaussian 09 program. First, geometry of a **Por-DAP** monomer was fully optimized at B3LYP/6-31G(d,p) level of theory. Using this optimized structure, the right-handed **Por-DAP** assemblies (P-helix) were constructed by attachment of the individual **Por-DAP** molecules to the poly-**T** DNA strand in a B-form, to form canonical (Watson-Crick) hydrogen bonds. This resulted in the vertical distance and angle between the successive **Por-DAP** units of 3Å, and 34°, respectively. **Por-DAP** oligomers of two to six units were built. The left-handed structures were obtained simply by inverting the angle of the neighboring **Por-DAP** (–34°). The **Por** only structures were obtained from **Por-DAP** by deleting the diaminopurine. Both **Por** and **Por-DAP** model structures for P- and M-helix are illustrated in Figure S23.

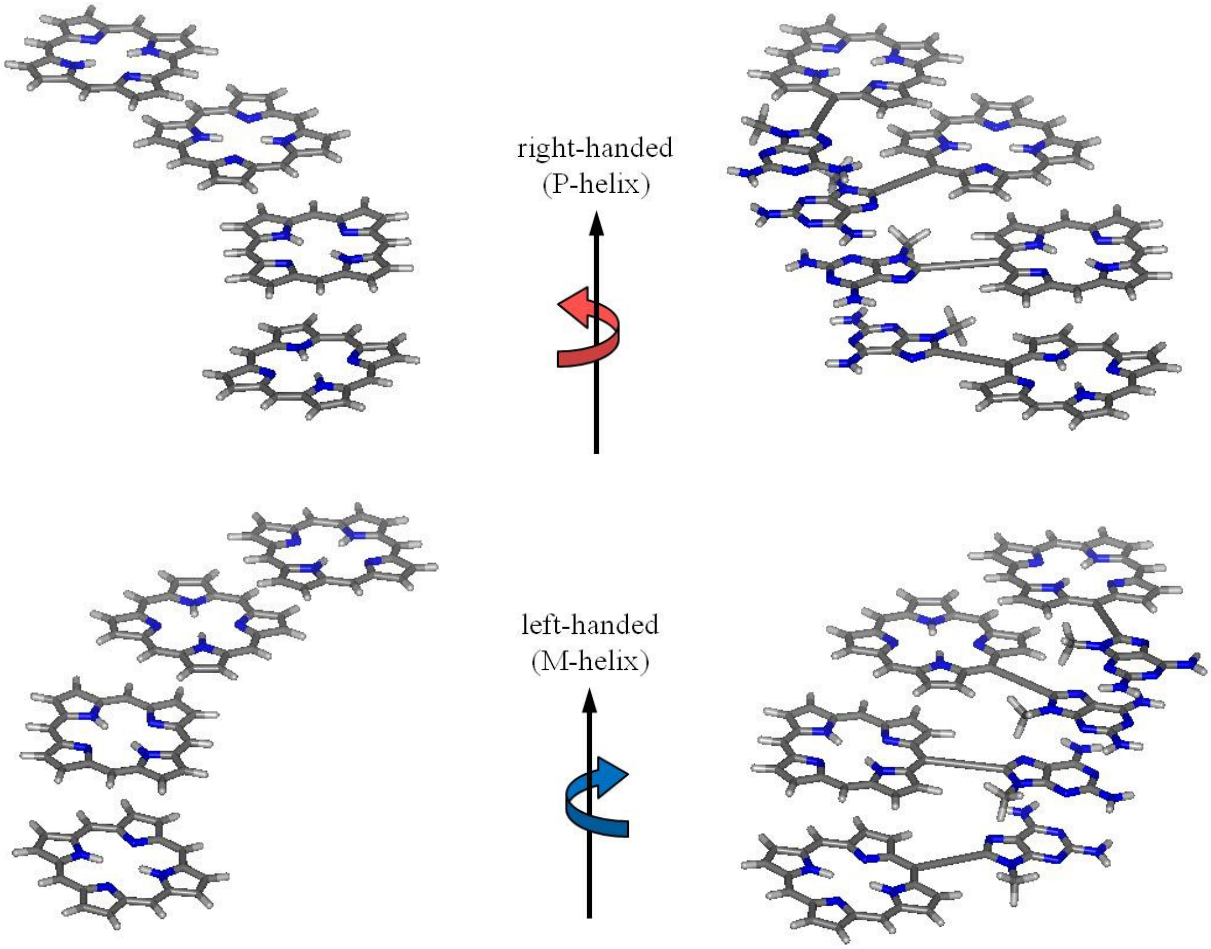


Figure S23. Model structures used for theoretical simulations of CD spectra. Left: **Por** only models, right: **Por-DAP** structures. For both model structures the right-handed (P-helix) arrangements are shown on top, left-handed (M-helix) on the bottom.

The excited state calculations were done using time-dependent (TD) B3LYP/3-21G level of theory. The size of model systems requires relatively small basis set. Calculations with larger basis sets - 6-31G(d,p) and 6-31+G(d,p) - were carried out on **Por-DAP** and **Por** dimers, respectively, to ensure that 3-21G is adequate. Larger basis sets yielded only minor differences in the spectra, but overall qualitative patterns were independent of the basis set size. In addition, the effect of solvent (water) on the computed spectra was tested using Conductor-like Polarized Continuum Model (CPCM) as implemented in Gaussian 09. Again, no significant effects were found.

The dipolar and rotational strengths were computed using both dipole-length and dipole-velocity gauge formalism, and found essentially identical. The results obtained from dipole-velocity formalism are

presented. The obtained rotational strength were converted to molar extinction coefficients and convoluted with Gaussian band shapes of a uniform half-width of 25 nm. The simulated UV and CD spectra for tetramers (**Por**)$_4$ and (**Por-DAP**)$_4$, corresponding to the structures in Figure S23, are shown in Figure S24.

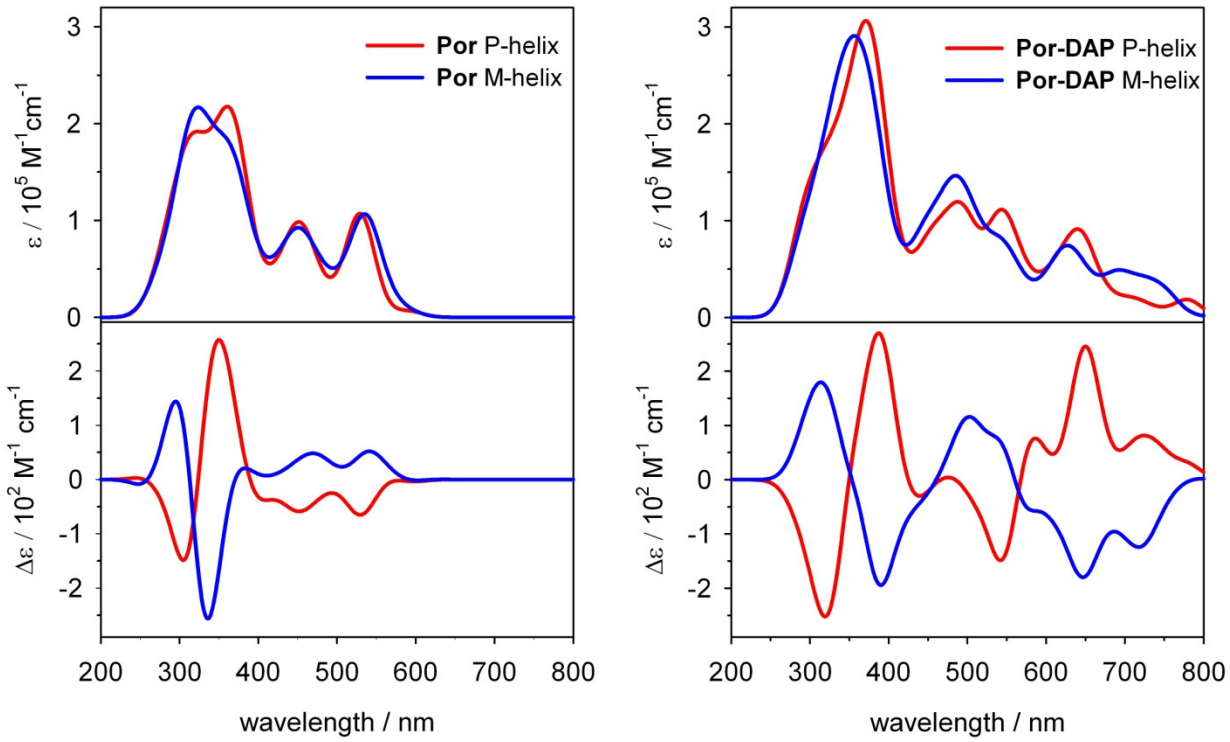


Figure S24. TD-DFT simulations of the UV and CD spectra for model **Por** and **Por-DAP** systems. Left: UV (top) and CD (bottom) spectra simulated for **Por** tetramers in a right-handed (P-helix) arrangement based on the canonical DNA B-form (red) and the corresponding left-handed (M-helix) form (blue). Right: UV (top) and CD (bottom) spectra simulated for **Por-DAP** tetramers, derived from the same model structures.

The predicted CD spectra for the model structures, namely in the Soret region (computed between ~250 - 450 nm) are in a good qualitative agreement with the experimental data (compare to Fig. 3 in the main text). An intense negative couple predicted for the model (M-helix) is seen experimentally. However, essentially an oppositely signed couplet is predicted for the P-helix though with additional negative CD on the short wavelength side, while experimental data show a distinct -/+/- pattern. This suggests that the model structures, in particular of the P-helix, may not be accurate. The inaccuracy of the model structures

is likely also responsible for a too high CD (and UV) signal computed at shorter wavelengths (500-800 nm) in **Por-DAP** models, which are not seen experimentally. Presumably, the close stacking of **DAP** rings (Figure S23) overemphasizes their contributions to the spectra over that of the **Por**. While further work is necessary to determine the definite structural arrangements adopted by these nanoassemblies, the handedness of the M- and P-helices giving rise to the distinct CD patterns is clear.

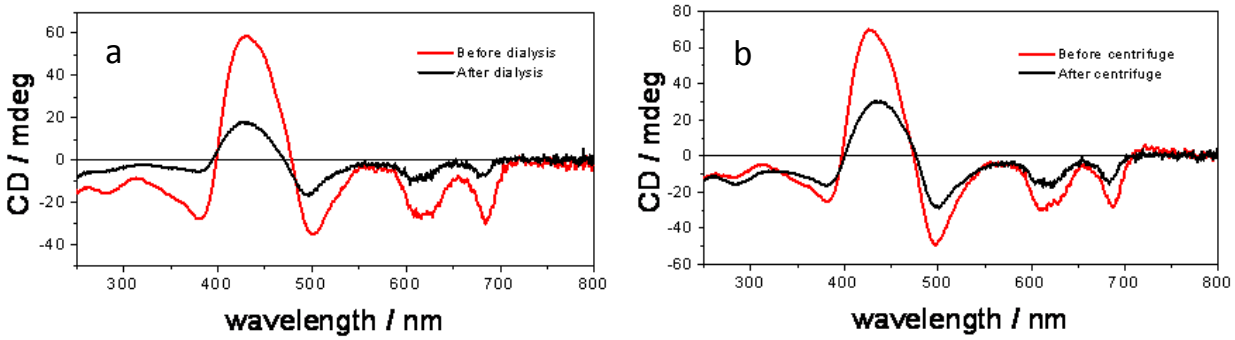


Figure S25. (a) CD spectra **Por-DAP·dT40** before (red curve) and after (black curve) dialysis. (b) CD spectra of the **Por-DAP·dT40** nanoassemblies before (red curve) and after (black curve) centrifugation (black curve).

Dialysis: A **Por-DAP·dT40** sample (1600 µL) prepared by slow cooling in the presence of 500 mM NaCl has been placed in a dialysis cassette (Thermo Scientific, Slide-A-Lyzer, 2K) and submerged in Na-cacodylate buffer (250 mL, 1 mM, pH = 7.0). The buffer solution was stirred together with cassette. After two hours the buffer solution was replaced with a fresh buffer solution (250 mL, 1 mM, pH = 7.0) and stirring continued for additional 16 h. The sample was then removed from cassette, placed into a cuvette and CD spectrum was measured.

Centrifugation: A **Por-DAP·dT40** sample (1600 µL) prepared by slow cooling in the presence of 500 mM NaCl has been centrifuged (14500 rpm, 5 min), the supernatants was removed (1570 µL) and the remaining suspension was redissolved in Na-cacodylate buffer without NaCl (1570 µL, 1 mM, pH = 7.0). The centrifugation-redissolving experiment was repeated twice and CD spectrum was measured.

Notes:

(1) The **Por-DAP·dT40** nanoassemblies were prepared by cooling the solution of **Por-DAP** (10 µM) and **dT40** (10 µM) from 85 °C to 20 °C using Major Science cool-hotter dry bath incubator (cooling rate: 0.5 °C/min+1 min wait). Solvent: 40% DMSO in Na-cacodylate buffer (1 mM, pH=7.0).

(2) The **Por-DAP·dT40** nanoassemblies were prepared by cooling the solution of **Por-DAP** (10 μM) and **dT40** (10 μM) from 85 °C to 20 °C using Major Science cool-hotter dry bath incubator (cooling rate: 0.5 °C/min+1 min wait). Solvent: 40% DMSO, NaCl (500 mM) in Na-cacodylate buffer (1 mM, pH=7.0).

(3) The solution of **Por-DAP** (10 μM) and **dT40** (10 μM) was kept at 85 °C for 60 min. The solution was then quickly transferred into a Peltier cell holder that was pre-equilibrated at 20 °C to achieve fast cooling. Solvent: 40% DMSO, NaCl (500 mM) in Na-cacodylate buffer (1 mM, pH=7.0).

(4) The solution of **Por-DAP** (10 μM) and **dT40** (10 μM) was kept at 85 °C for 60 min. The solution was then quickly transferred into a Peltier cell holder that was pre-equilibrated at 20 °C to achieve fast cooling. Solvent: 40% DMSO, in Na-cacodylate buffer (1 mM, pH=7.0).