Electronic Supplementary Information

Highly fluorescent water-soluble polyglycerol-dendronized perylene bisimide dyes

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General Methods

^{1}H NMR spectra were recorded on a Bruker ECX 400 spectrometer (400 MHz and 100 MHz for ^{1}H and ^{13}C, respectively) at 25 °C and calibrated against residual solvent peaks as internal standard. The UV/Vis absorption spectra were recorded for different concentrations on a Perkin Elmer Lambda 40P spectrophotometer with cuvettes of 0.1 cm path length (for **G2.0**, **G3.0** and **G4.0** at a concentration of $1\text{x}10^{-4}$ M) and 1.0 cm path length for all other measurements. The steady-state fluorescence spectra were recorded on a PTI QM4-2003 fluorescence spectrometer and corrected against photomultiplier and lamp intensity. The fluorescence spectra were measured with cuvettes of 0.1 cm path length (for **G2.0**, **G3.0** and **G4.0** at a concentration of $1\text{x}10^{-4}$ M) and 1.0 cm path length for all other measurements. Fluorescence quantum yields were calculated from the integrated intensity under the emission band (*I*) using the following equation:

$$\Phi = \Phi_r \frac{I}{I_r} \frac{OD_r}{OD} \frac{n^2}{n_r^2}$$

where *OD* is the optical density of the solution at the excitation wavelength and *n* is the refractive index. The optical density of the solution for the calculation of quantum yields was less than 0.1 at

the excitation wavelength. *N*,*N*'-Di(2,6-diisopropylphenyl)-perylene-3,4:9,10-tetracarboxylic acid bisimide in chloroform was used as reference ($\Phi_r = 1.00$).

ESI-MS spectra were measured on an Agilent 6210 ESI-TOF, Agilent Technologies, Santa Clara, CA, USA. Solvent flow rate was adjusted to 4 μL/min, spray voltage set to 4.000 V. Drying gas flow rate was set up to 15 psi (1 bar). All other parameters were adjusted for a maximum abundance of the relative $[M+H]^+$. The samples for cryogenic transmission electron microscopy (cryo-TEM) were prepared at room temperature by placing a droplet (5 μL) of the solution on a hydrophilized perforated carbon filmed Quantifoil grid (60 s Plasma treatment at 8 W using a BALTEC MED 020 device). The excess fluid was blotted off to create an ultra thin layer of the solution spanning the holes of the carbon film. The grids were immediately vitrified in liquid ethane at its freezing point (-184 °C) using a standard plunging device. Ultra-fast cooling is necessary for an artifact-free thermal fixation (vitrification) of the aqueous solution avoiding crystallization of the solvent or rearrangement of the assemblies. The vitrified samples were transferred under liquid nitrogen into a Philips CM12 transmission electron microscope using the Gatan cryoholder and -stage (Model 626). Microscopy was carried out at -175 °C sample temperature using the microscope's low dose mode. Accelerating voltage was 100 kV and the defocus was chosen to be -1.5 μm up to -2.5 μm.

Materials

All solvents and reagents were purchased from commercial sources and used as received without further purification, unless otherwise stated. The solvents for spectroscopic studies were of spectroscopic grade and used as received. Dialysis was performed in Spectra/Por® Biotech CE dialysis tubing.

Synthesis and Characterization of Compounds 1a-1d

General method for preparation of the *N*,*N*-substituted perylene-3,4:9,10-tetracarboxylic acid bisimides

In a typical experiment, perylene 3,4:9,10-tetracarboxylic acid bisanhydride (PBA, 1.0 eq.), the [G*n*]-NH_2 dendron (2.5 eq.) and imidazole were combined. The mixture was heated to 140 °C under argon for 4 hours and cooled to room temperature. The remaining solid was dissolved in water and dialysed for three days against water to give the desired product.

1a: [G1.0]-NH_2 (74 mg, 0.330 mmol), PBA (52 mg, 0.13 mmol) and 200 mg imidazole, dialysed with MWCO 500, yield 90 mg, 0.108 mmol (83%). ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) = 7.25 (m, 8H, Ar-H), 5.30 (m, 2H, -N-C<u>H</u>-,), 4.40-3.25 (m, 28H, PG-Dendron). MS (ESI-TOF, pos. mode) m/z 857.27 $[M+Na]^+$ (calcd. for $C_{42}H_{46}N_2O_{16}$ 834.2847). UV/Vis (H_2O): λ_{max} (nm) = 501, 536; fluorescence (H_2O): λ_{max} (nm) = 590, 546, Φ_{fl} = 33.1% (c = 10^{-7} M).

1b: [G2.0]-NH_2 (100 mg, 0.186 mmol), PBA (30 mg, 0.078 mmol) and 500 mg imidazole, dialysed with MWCO 500, yield 103 mg, 0.722 mmol (93%). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 7.86 (m, 8H, Ar-H), 5.54 (br. s., 2H, -N-C<u>H</u>-), 4.33-3.65 (m, 68H, PG-Dendron). MS (ESI-TOF, pos. mode) m/z 1449,5722 $[M+Na]^+$; 736.2811 $[M+Na]^{2+}$ (calcd. for $C_{66}H_{94}N_2O_{32}$ 1426.5790). UV/Vis (H_2O): λ_{max} (nm) = 535, 499, 468; fluorescence (H_2O): λ_{max} (nm) = 591, 548, Φ_{fl} = 54.3% (c = 10^{-7} M).

1c: [G3.0]-NH_2 (315 mg, 0.280 mmol), PBA (44 mg, 0.110 mmol) and 300 mg imidazole, dialysed with MWCO 1000, yield 264 mg, 0.101 mmol (92%). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 8.40 (m, Ar-H), 5.63 (br. s, 2H, -N-C<u>H</u>-), 4.37 (br. s, 4H), 4.24 (br. s, 4H), 4.00-3.21 (m, 140H, PG-Dendron). MS (ESI-TOF, pos. mode) m/z 2635.1247 $[M+Na]^+$; 1329.0669 $[M+2Na]^{2+}$ (calcd. for $C_{114}H_{190}N_2O_{64}$ 2611.1674). UV/Vis (H_2O): λ_{max} (nm) = 532, 495, 464; fluorescence (H_2O): λ_{max} (nm) = 584, 543, Φ_{fl} = 74.0% (c = 10^{-7} M).

1d: [G4.0]-NH_2 (190 mg, 0.083 mmol), PBA (13 mg, 0.033 mmol) and 200 mg Imidazole, dialysed with MWCO 3500, yield 118 mg, 0.024 mmol (72%). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 9.00 (m, 8H, Ar-H), 5.70 (br. s, 2H, -N-C<u>H</u>-), 4.37 (br. s, 4H), 4.14 (br. s, 4H), 3.95-3.06 (m, 300H, PG-Dendron). MS (ESI-TOF, pos. mode) m/z 1683.1051 $[M+3Na]^{3+}$; 1268.0764 $[M+4Na]^{4+}$ (calcd. for $C_{210}H_{382}N_2O_{128}$ 4980.3444). UV/Vis (H_2O): λ_{max} (nm) = 532, 495, 46; fluorescence (H_2O): λ_{max} (nm) = 584, 541, Φ_{fl} = 98.9% (c = 10^{-7} M).

Supporting Figures

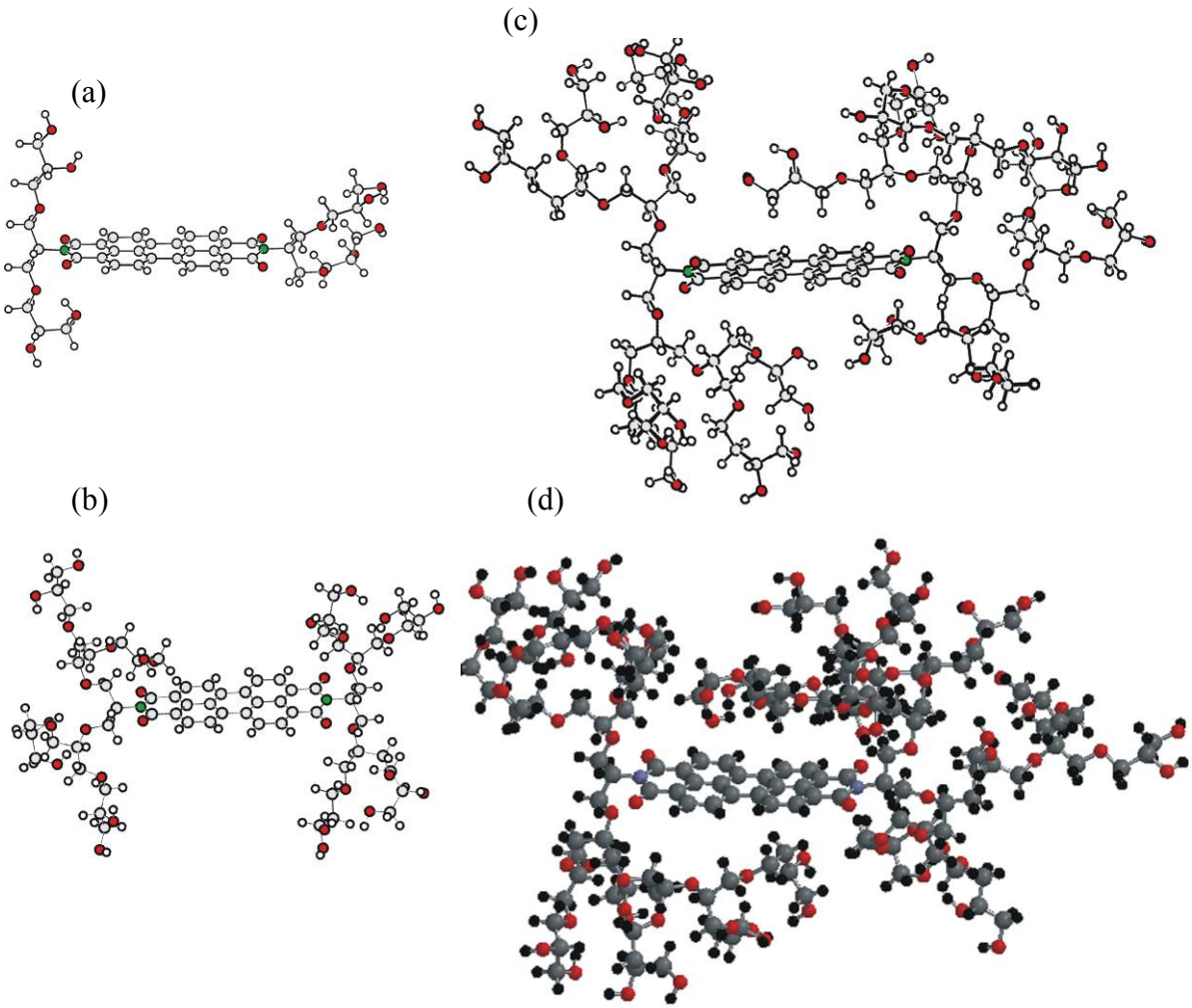

Fig. S1 AM1 modelled structures for (a) **1a,** (b) **1b,** (c) **1c** and (d) **1d**.

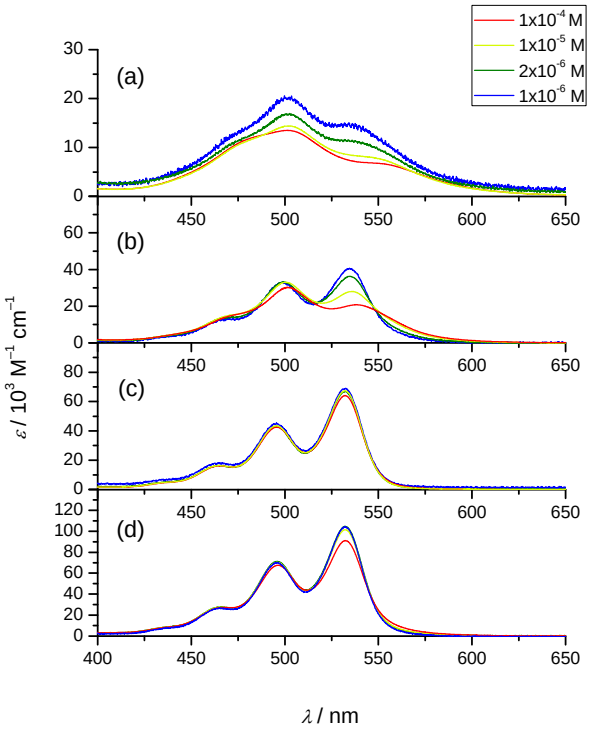

Fig. S2 UV/Vis absorption spectra of aqueous solutions of PBIs (a) **1a**, (b) **1b**, (c) **1c**, (d) **1d** at 25 °C for the concentration range from 10^{-4} to 10^{-6} M.

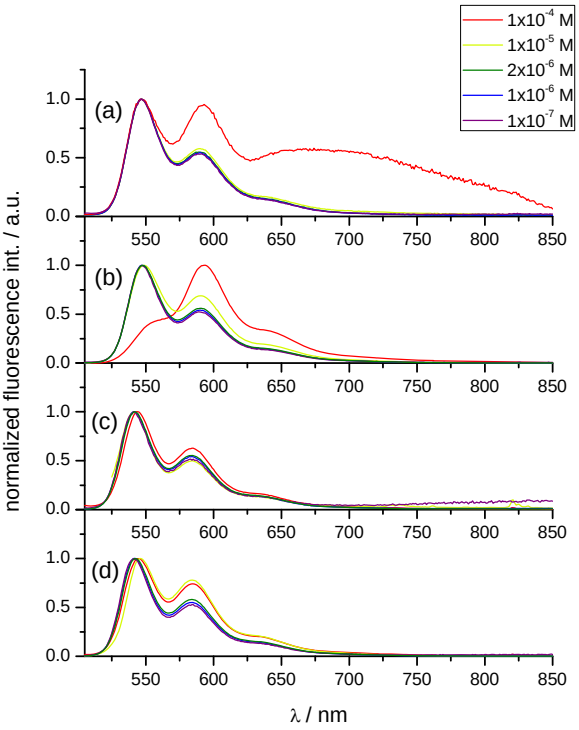

Fig. S3 Normalized fluorescence spectra of aqueous solutions of PBIs (a) **1a**, (b) **1b**, (c) **1c**, (d) **1d** at 25 °C for the concentration range from 10^{-4} to 10^{-7} M, λ_{ex} = 490 nm.

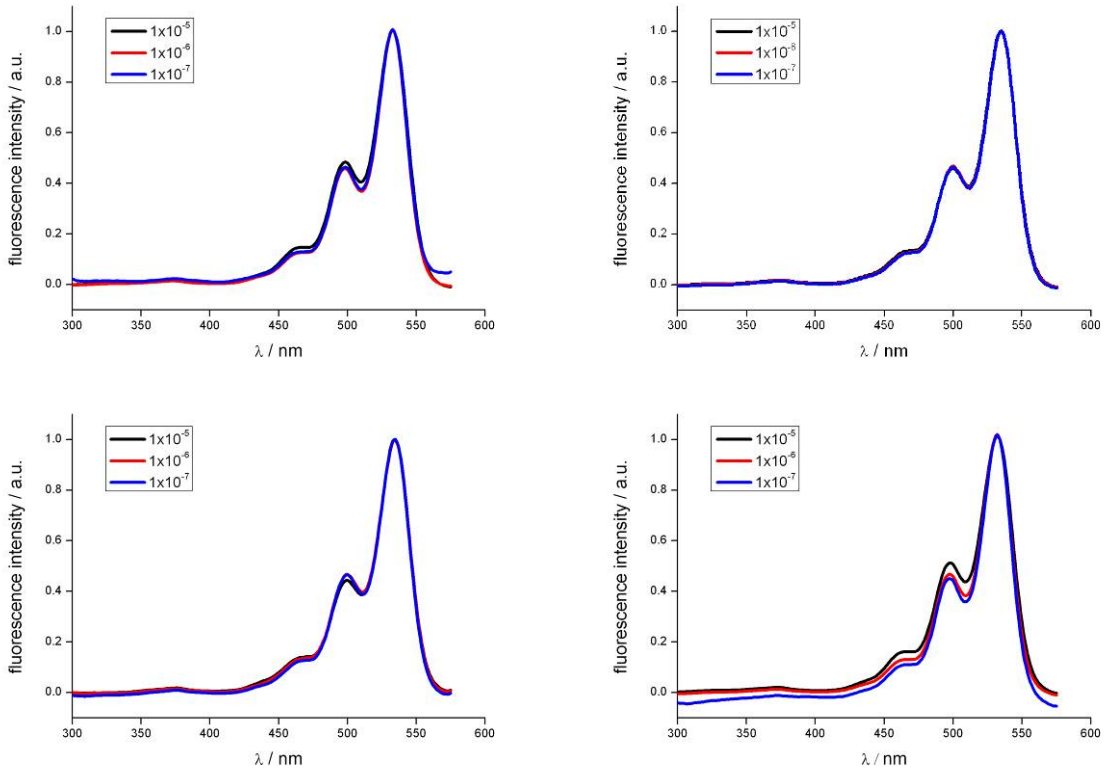

Fig. S4 Excitation spectra (λ_{ex} = 540 nm) of (a) **1a**, (b) **1b**, (c) **1c**, (d) **1d** in aqueous solution at concentrations from 10^{-5} to 10^{-7} M.

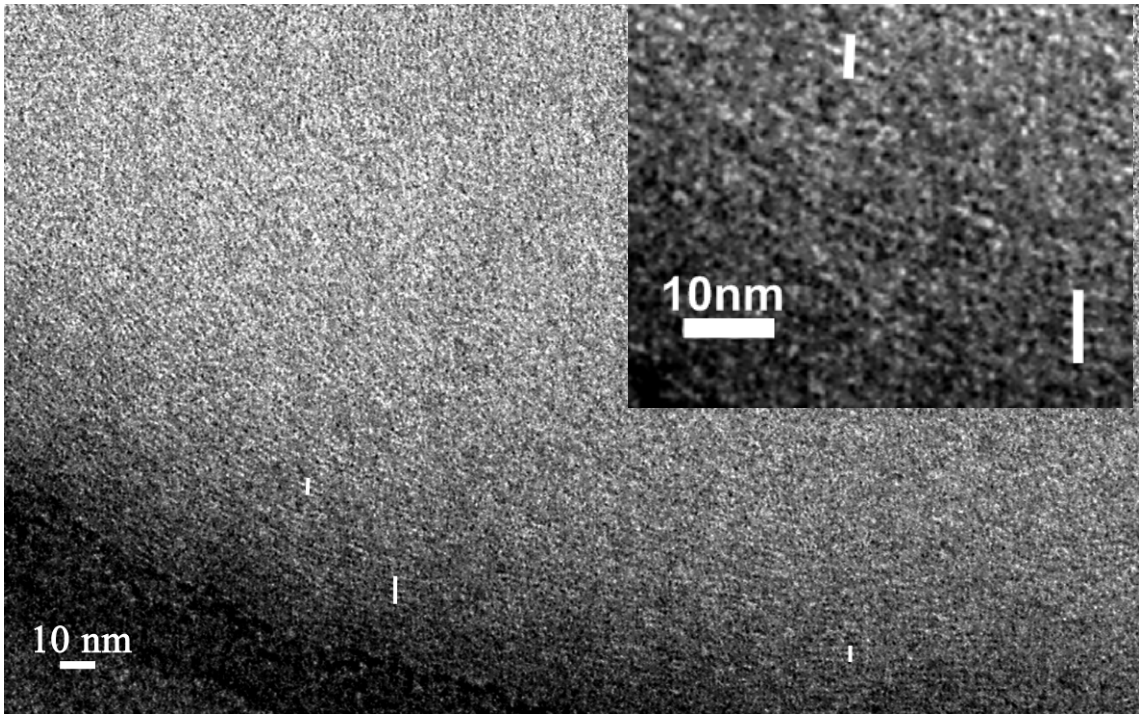

Fig. S5 Cryo-TEM image of **1a** (10 mg/ml H_2O) showing small fibres with a diameter of about 1.6 nm, as measured across the indicated white lines.