Supplementary Information

Influence of cyclodextrin size on fluorescence quenching in conjugated polyrotaxanes by methyl viologen in aqueous solution

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Experimental

General. All manipulations of air- or water-sensitive compounds were performed using standard high-vacuum techniques. Light petroleum refers to the fraction of boiling point 40–60°C.

Ultrafiltration was carried out using a polysulfone membrane (5 kDa nominal molecular weight limit) at a pressure of 4 bar in a Amicon 8200 ultrafiltration stirred cell.

NMR spectra were recorded at ambient probe temperature using the following instruments: Bruker DPX400, AV400, AV500 and AV500 with cryoprobe. The 500 MHz spectra were recorded by Dr. B. Odell. Chemical shifts (δ_H and δ_C) are reported in parts per million (ppm) relative to the residual solvent peak. Coupling constants (*J*) are reported in Hz. Mass spectra were obtained using electrospray ionization (ES) or electron impact (EI). Elemental analysis was carried out by the Inorganic Chemistry Laboratory analytical service, Oxford. UV-visible spectra were recorded using a Perkin-Elmer Lambda 20. Fluorescence spectra were recorded using a J-Y Horiba Fluoromax-2. Solution absorption and emission spectra were acquired using a silica fluorescence cuvette with 10 mm path length.

HPLC analyses were carried out on an HP 1100 series workstation using a Zorbax Eclipse XDB C8 analytical column

(4.6 x 15 mm, 5 micron). The analytes were eluted using a timetabled two-solvent gradient system and monitored using a diode array detector using ‘Method 1’, as shown in Figure S1. [Solvent A = 0.25% *w/v* dicyclohexylammonium phosphate (CHAPS) in ultra-pure water, Solvent B = MeOH]. Semipreparative HPLC was carried out using a Zorbax Eclipse XDB C8 semi-preparative column (9.4 x 250 mm, 5 micron) using ‘Method 2’ (Figure S1) [Solvent A = 0.25% w/v dicyclohexylammonium phosphate (CHAPS) in ultra-pure water, Solvent B = MeCN].

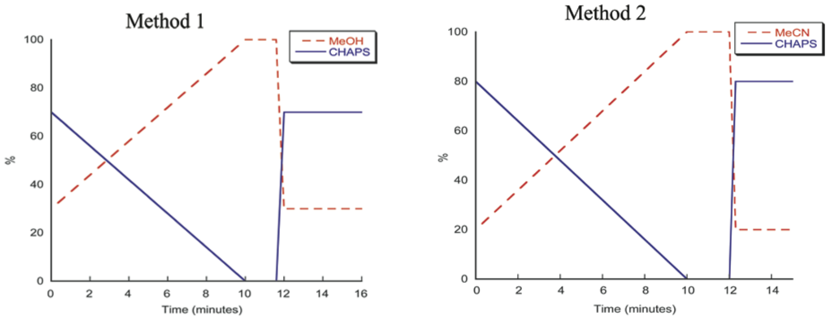

Figure S1. HPLC gradients for reaction analysis. ‘Method 1’ uses a flow rate of 1 mL min^{-1}, while ‘Method 2’ uses a flow rate of 4 mL min^{-1}.

Stern-Volmer quenching experiments in solution were carried out in the dark by micro-titration in a fluorescence cuvette. For each titration, 2.60 mL of polyrotaxane solution, at an absorbance of 0.095, was placed in the cuvette, and the emission spectrum recorded in the absence of quencher at room temperature. The emission spectra were repeatedly acquired after adding microlitre aliquots of a solution containing the polyrotaxane at the same concentration, and the quencher at a known concentration. A 3 mm × 3 mm magnetic sphere was used to stir each solution in the cell for 2 min after addition of quencher.

Time-resolved data were taken for **1**⊂α-CD, **1**⊂β-CD, **1**⊂γ-CD, and **1** in buffered solutions (11.6 mM NaOH/20 mM KH_2PO_4) at a concentration of the conjugated portion of 5 × 10^{-2} mg/mL, A_{max} = 0.095. The solutions were investigated in a Spectrosil® fluorescence cuvette (d = 10 mm). Their concentrations were determined using UV-Vis absorption spectroscopy (Agilent 8543). The same spectrometer Agilent 8543 was used to collect the UV-Vis absorption spectra. The 1,1'-dimethyl-4,4'-bipyridinium dichloride (methyl viologen, **Hazard: Toxic!**) solution of concentration 1 × 10^{-5} mol/mL was prepared in the same solvent. Time-resolved measurements were performed by exciting with a ps-pulsed diode laser (Edinburgh Instruments EPL-375; 40 ps FWHM; E_{exc}=3.3 eV) and the luminescence detected with a photomultiplier tube coupled with a monochromator and time-correlated single-photon counting unit (Edinburgh Instruments F-900, Instrument time response ~150 ps). All measurements were performed at room temperature and all spectra were corrected for the instrumental spectral response.

Uninsulated poly(4,4'-diphenylenevinylene) (1).

Under an atmosphere of nitrogen, degassed water (10 mL) was added to a mixture of iodoterphenylene dicarboxylic acid **3** (15.0 mg, 32.8 µmol), *trans*-1,2-bis(4-(1,3,2-dioxaborolan-2-yl)phenyl)ethene **4** (64.5 mg, 0.202 mmol), 4,4'-diiodostilbene-2,2'-disulfonate sodium salt **5** (116 mg, 0.164 mmol), palladium(II) acetate (3.7 mg, 16 µmol) and lithium carbonate (77.6 mg, 1.05 mmol). The mixture was heated to 85 °C for 18 h, and then diluted with deionised water (25 mL), cooled and filtered through paper. The filtrate was subsequently filtered through a 0.2 µm membrane with mechanical stirring. Sodium cyanide (**Caution: Toxic!**) (50 mg, 1.0 mmol) was added and the solution stirred at room temperature for

2 h. Ultrafiltration (8.0 L water) was followed by addition of lithium carbonate (56 mL, 90 mM). The solution was re-ultrafiltered (3.0 L water), and subsequently freeze-dried to yield **1** as a yellow solid (123 mg, 90%); found C 49.8, H 4.7%, $C_{334}H_{212}O_{68}S_{20}Li_{24}\cdot 85H_2O$ requires C 52.4, H 5.0%; λ_{max} (phosphate buffer pH 7) 402 nm; δ_H (500 MHz, DMSO-d_6) 7.1–8.6.

α-Cyclodextrin polyrotaxane (1$\subset$α-CD).

Under an atmosphere of nitrogen, degassed water (10 mL) was added to a mixture of iodoterphenylene dicarboxylic acid **3** (15.0 mg, 32.8 μmol), *trans*-1,2-bis(4-(1,3,2-dioxaborolan-2-yl)phenyl)ethene **4** (64.5 mg, 0.202 mmol), 4,4'-diiodostilbene-2,2'-disulfonate sodium salt **5** (116 mg, 0.164 mmol), palladium(II) acetate (3.7 mg, 16 μmol), α-cyclodextrin (798 mg, 0.820 mmol) and lithium carbonate (77.6 mg, 1.05 mmol). The mixture was heated at 85 °C for 18 h, and then diluted with deionised water (25 mL), cooled and filtered through paper. The filtrate was subsequently filtered through a 0.2 μm nylon membrane with mechanical stirring. Sodium cyanide (CAUTION: TOXIC!) (50 mg, 1.0 mmol) was added and the solution stirred at room temperature for 2 h. Ultrafiltration (8.0 L water) was followed by adding lithium carbonate (56 mL, 90 mM). The solution was re-ultrafiltered (3.0 L water), and subsequently freeze-dried to yield **1**$\subset\alpha$-CD as a yellow solid (95 mg, 38%). Integration of the ^{1}H NMR spectrum of this polymer indicated a threading ratio of 0.54 α-CD units per unsubstituted stilbene unit; found C 47.1, H 6.0%, $C_{548}H_{568}O_{246}S_{20}Li_{24}\cdot 110H_2O$ requires C 47.2, H 5.8%; λ_{max} (phosphate buffer pH 7) 399 nm; δ_H (500 MHz, DMSO-d_6) 3.5–4.1 (214 H, m), 4.3–4.6 (br s), 4.7–5.0 (36 H, br s), 5.2–6.0 (br s), 6.8–8.5 (212 H, m).

β-Cyclodextrin polyrotaxane (1$\subset$β-CD).

Under an atmosphere of nitrogen, degassed water (10.25 mL) was added to a mixture iodoterphenylene dicarboxylic acid **3** (15.0 mg, 32.8 μmol), *trans*-1,2-bis(4-(1,3,2-dioxaborolan-2-yl)phenyl)ethene **4** (64.5 mg, 0.202 mmol), 4,4'-diiodostilbene-2,2'-disulfonate sodium salt **5** (116 mg, 0.164 mmol), palladium(II) acetate (3.7 mg, 16 μmol), β-cyclodextrin (470 mg, 0.41 mmol) and lithium carbonate (77.6 mg, 1.05 mmol). The mixture was heated at 85 °C for 18 h, then diluted with deionised water (25 mL), cooled and filtered through paper. The filtrate was subsequently filtered through a 0.2 μm nylon membrane with mechanical stirring. Sodium cyanide (CAUTION: TOXIC!) (50 mg, 1.0 mmol) was added and the solution stirred at room temperature for 2 h. Ultrafiltration (4.0 L water) was followed by adding lithium carbonate (56 mL, 90 mM). The solution was re-ultrafiltered (4.0 L water), and subsequently freeze-dried to yield **1**$\subset\beta$-CD as a yellow solid (180 mg, 65%). Integration of the ^{1}H NMR spectrum of this polymer indicated a threading ratio of 0.79 β-CD units per unsubstituted stilbene unit; found C 47.60, H 5.78%, $C_{699}H_{820}O_{372}S_{20}Li_{24}\cdot 88H_2O$ requires C 47.6, H 5.8%; λ_{max} (phosphate buffer pH 7) 396 nm; δ_H (500 MHz, DMSO-d_6) 3.5–3.8 (365 H, m), 4.5–4.6 (br s), 4.8–5.0 (61 H, br s), 5.8–5.9 (br s), 7.2–8.3 (212 H, m).

γ-Cyclodextrin polyrotaxane (1$\subset$γ-CD).

Under an atmosphere of nitrogen, degassed water (10 mL) was added to a mixture of iodoterphenylene dicarboxylic acid **3** (15.0 mg, 32.8 μmol), *trans*-1,2-bis(4-(1,3,2-dioxaborolan-2-yl)phenyl)ethene **4** (64.5 mg, 0.202 mmol), 4,4'-diiodostilbene-2,2'-disulfonate sodium salt **5** (116 mg, 0.164 mmol), palladium(II) acetate (3.7 mg, 16 μmol), γ-cyclodextrin (1.06 g, 0.820 mmol) and lithium carbonate (77.6 mg, 1.05 mmol). The mixture was heated at 85 °C for 18 h, and then diluted with deionised water (25 mL), cooled and filtered through paper. The filtrate was subsequently filtered through a 0.2 μm nylon membrane with mechanical stirring. Sodium cyanide (CAUTION: TOXIC!) (50 mg, 1.02 mmol) was added and the solution stirred at room temperature for 2 h. Ultrafiltration (8.0 L water) was followed by adding lithium carbonate (56 mL, 90 mM). The solution was re-ultrafiltered (3.0 L water), and subsequently freeze-dried to yield **1**⊂*γ*-CD as a yellow solid (149 mg, 63%). Integration of the ^{1}H NMR spectrum of this polymer indicated a threading ratio of 0.33 *γ*-CD units per unsubstituted stilbene unit; found C 49.6, H 5.5%, $C_{508}H_{502}O_{213}S_{20}Li_{24}\cdot 57H_2O$ requires C 50.0, H 5.4%; λ_{max} (phosphate buffer pH 7) 389 nm; δ_H (500 MHz, DMSO-d_6) 3.5–3.7 (174 H m), 4.5 (br s), 4.9 (29 H, br s), 5.8–6.0 (m), 7.2–8.5 (212 H, m); δ_C (125 MHz, DMSO-d_6) 60.3, 72.5, 72.8, 73.2, 81.2, 102.0, 125.0–130.2, 134.4–138.7, 141.1, 141.8, 144.5, 144.8, 145.4, 172.2.

α-Cyclodextrin [2]rotaxane (2⊂α-CD).

Under a nitrogen atmosphere, degassed water (10 mL) was added to a mixture of *trans*-1,2-bis(4-(1,3,2-dioxaborolan-2-yl)phenyl)ethene **4** (36 mg, 0.11 mmol), iodoterphenylene dicarboxylic acid **3** (100 mg, 0.25 mmol), α-cyclodextrin (652 mg, 0.67 mmol), and potassium carbonate (0.30 g, 2.0 mmol). The mixture was heated to 45 °C and palladium(II) acetate (25 μL, 1.11 μmol) was added from a stock solution (45 mM in DMSO). The mixture was stirred under nitrogen at 45 °C for 6 h, then cooled, diluted with deionised water (15 mL), and filtered through paper. Hydrochloric acid (25 mL, 1.0 M) was added and the resulting suspension was centrifuged. The supernatant was removed and the solid was washed twice with water (40 mL). The solid was then dissolved in DMSO (2 mL) and isolated by semipreparative HPLC, using Method 2 (see Figure S1). The fractions were collected and concentrated in vacuo, until a suspension was observed. The suspension was collected by centrifugation, dried to yield **2**⊂α-CD as a white solid (21% conversion by HPLC; 23 mg, 11% isolated) λ_{max} (phosphate buffer pH 11.4) 347 nm (ε = 53,000); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.25 (d, *J* = 12.0 Hz, 6H), 3.36 (d, *J* = 11.0 Hz, 6H), 3.40-3.44 (m, 6H), 3.60 (d, *J* = 10.0 Hz, 6H), 3.67-3.75 (m, 12H), 4.77 (s, 6H), 7.00 (d, *J* = 16.0 Hz, 1H), 7.23 (d, *J* =16.0 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 2H), 7.81 (d, *J* = 7.5 Hz, 2H), 7.92 (d, *J* = 7.5 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 2H), 8.00-8.08 (m, 22H); *m/z* (ESI-) 446 $[M-4H]^{4-}$ (10%), 594 $[M-3H]^{3-}$ (100%), 892 $[M-2H]^{2-}$ (30%).

β-Cyclodextrin [2]rotaxane (2⊂β-CD).

Under a nitrogen atmosphere, degassed water (10 mL) was added to a mixture of *trans*-1,2-bis(4-(1,3,2-dioxaborolan-2-yl)phenyl)ethene **4** (36 mg, 0.11 mmol), iodoterphenylene dicarboxylic acid **3** (100 mg, 0.25 mmol), β-cyclodextrin (761 mg, 0.67 mmol), potassium carbonate (300 mg, 2.0 mmol). The mixture was heated to 45 °C and palladium(II) acetate (25 μL, 1.11 μmol) was added from a stock solution (45 mM in DMSO). The mixture was stirred under nitrogen at 40 °C for 6 h, then cooled, diluted with deionised water (15 mL), and filtered through paper. Hydrochloric acid (25 mL, 1.0 M) was added and the resulting suspension was centrifuged. The supernatant was removed and the solid was washed three times with 40 mL water. The solid was subsequently dissolved in DMSO (2 mL) and isolated by semipreparative HPLC using Method 2 (see Figure S1). The fractions were collected and concentrated in vacuo. The resulting suspension was collected by centrifugation, dried in vacuo to yield **2**⊂β-CD as a white solid (38% conversion by HPLC; 15 mg, 7% isolated) λ_{max} (phosphate buffer pH 11.4) 350 nm (ε = 48,000); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.28 (d, *J* = 9.0 Hz, 7H), 3.34 (tr, *J* = 9.0 Hz, 7H), 3.39-3.47 (m, 14H), 3.56-3.62 (m, 14H), 4.77 (s, 7H), 7.09 (d, *J* = 16.0 Hz, 1H), 7.16 (d, *J* = 16.0 Hz, 1H), 7.57 (d, *J* = 7.0 Hz, 2H), 7.71 (d, *J* = 7.0 Hz, 2H), 7.79-7.83 (m, 4H), 7.93-8.08 (m, 22H); *m/z* (ESI-) 486 $[M-4H]^{4-}$ (20%), 648 $[M-3H]^{3-}$ (100%), 973 $[M-2H]^{2-}$ (20%).

The synthesis of compounds **2** and **2**⊂β-CD have been reported previously (E. J. F. Klotz, T. D. W. Claridge and H. L. Anderson, *J. Am. Chem. Soc.*, 2006, **128**, 15374-11575).

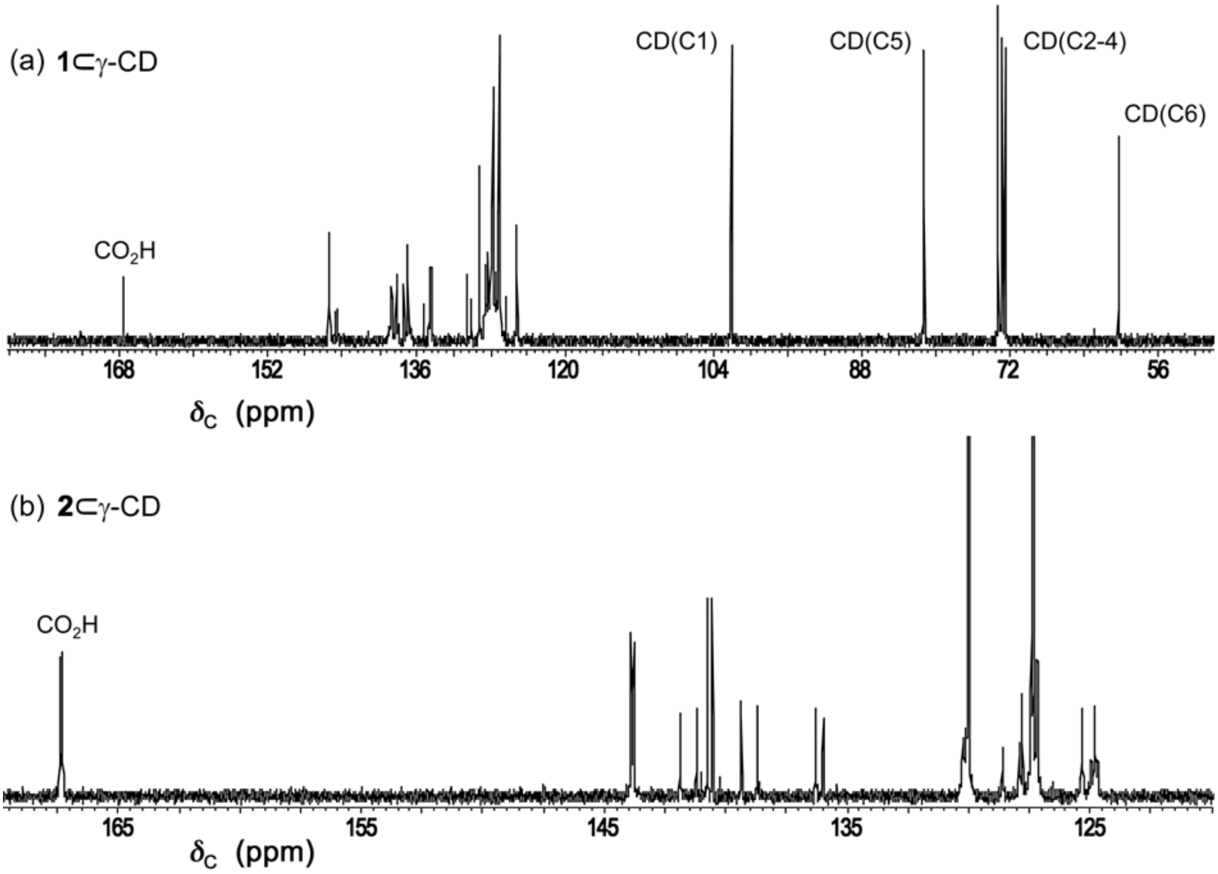

Figure S2. ^{13}C NMR spectra of (a) polyrotaxane **1**⊂*γ*-CD and (b) [2]rotaxane **2**⊂*γ*-CD in DMSO-*d*$_6$ (125 MHz, inverse-gated, with cryoprobe, relaxation delay: *D1* = 20s, at 298 K). The solution of **1**⊂*γ*-CD was acidified with concentrated hydrochloric acid (2.0 M, 50 µL) so as to be able to observe the carboxylic acid carbons of the end groups; note that this carbon resonance is split in the [2]rotaxane due to the asymmetry of the cyclodextrin.

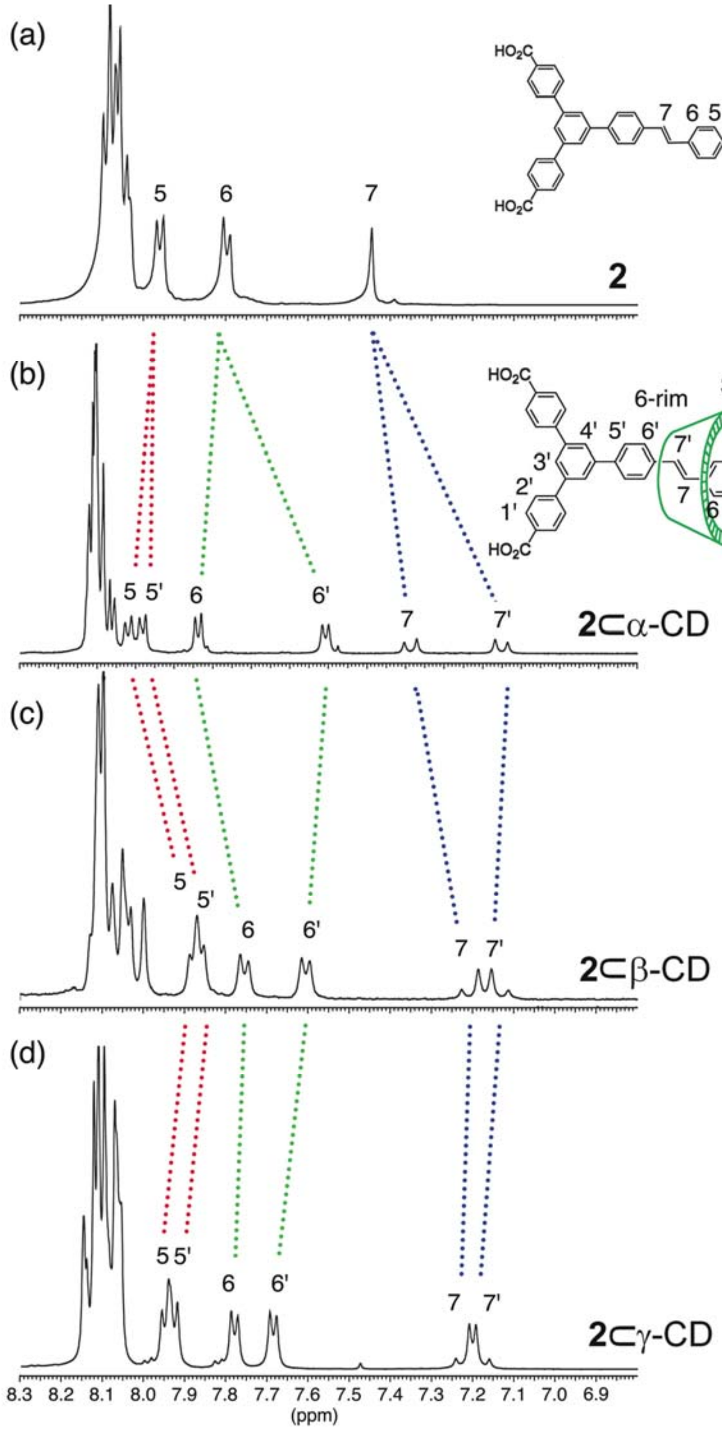

Figure S3. ^{1}H NMR spectra (500 MHz, 298 K, in DMSO-d_6) of (a) dumbbell **2**, (b) [2]rotaxane **2**$\subset\alpha$-CD, (c) [2]rotaxane **2**$\subset\beta$-CD and (d) [2]rotaxane **2**$\subset\gamma$-CD. The resonances were assigned from NOESY and COSY 2D NMR spectra. The stilbene resonances 5, 6 and 7 are split in the rotaxanes, due to the asymmetry of the cyclodextrin, and the extent of this splitting decreases as the diameter of the cyclodextrin increases. The other aromatic signals 1–4 give rise to the unresolved multiplet at 8.05–8.15 ppm.