

Supporting Information

Electrochemical control of a non-covalent binding between ferrocene and beta-cyclodextrin

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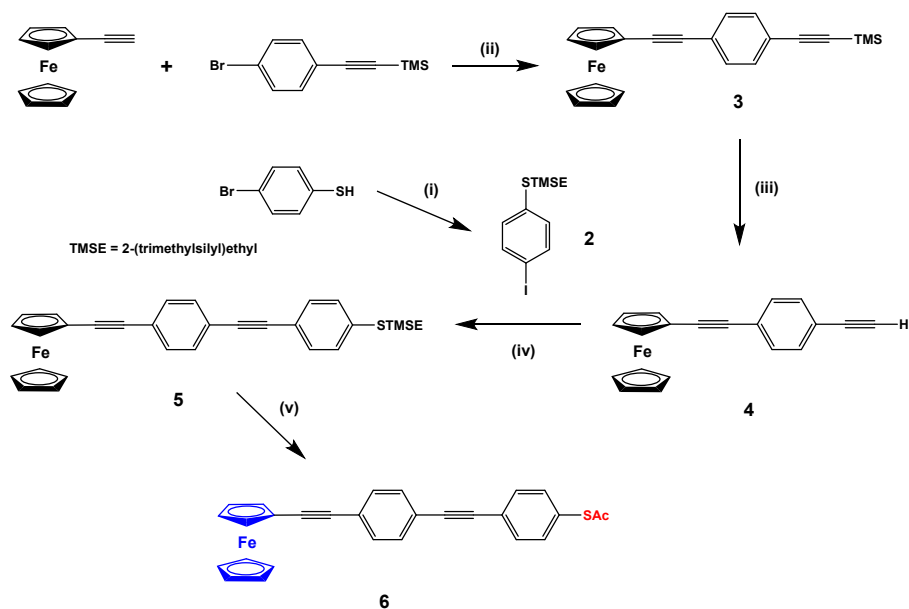
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1. Synthetic strategy for the preparation of compounds 1-8

Our synthetic strategy used for the preparation of the Fc-OPE-SAc rod-like molecule **6** (Scheme S1) is based on a stepwise assembly of building blocks via Sonogashira cross-coupling reaction starting from the commercially available ethynylferrocene and (4-bromophenylethynyl)trimethylsilane. This stepwise methodology afforded the title Fc-OPE compound **5** terminated with 2-(trimethylsilyl)ethylsulfanyl group. Final trans-protection of the thiol was successfully performed using tetrabutylammonium fluoride (TBAF) and acetyl chloride (AcCl) to obtain the desired thioacetate **6** in the overall yield of 23% after six reaction steps. The presence of terminal thioacetate group allows the Fc-OPE rod to be bound to a gold surface. The acetyl works as a labile thiol protecting group and can be mildly and efficiently cleaved by 10 % (v/v) triethylamine (Et₃N) in dry ethanol (reaction not shown) prior to the physical investigations or in-situ upon binding to the gold surface.



Scheme S1. Synthesis of the Fc-OPE-SAc rod-like molecule **6**. Reagents and conditions: (i) a) vinyltrimethylsilane, azobisisobutyronitrile (AIBN), 89%, b) *tert*-BuLi, THF; c) I₂, THF, 90%; (ii) PdCl₂(PPh₃)₂, CuI, Et₃N, 50-80 °C, 40%; (iii) TBAF, CH₂Cl₂, 98%; (iv) **2**, PdCl₂(PPh₃)₂, CuI, Et₃N, 40 °C, 80%; (v) a) TBAF, THF, b) AcCl, 90%.

Per-6-sulfanyl- β -cyclodextrin **8** was prepared via iodination of β -cyclodextrin and subsequent thiolation of per-6-iodo- β -cyclodextrin **7** according to published procedure.^{1,2}

All compounds were fully characterized by conventional analytical and spectroscopic techniques like ^1H and ^{13}C NMR spectroscopy, mass spectrometry, and IR spectrometry, as well as by elemental analysis.

2. Experimental procedures and data for compounds 1-8

Materials. All starting materials and reagents were obtained from commercial suppliers and used without further purification. TLC was performed on Silica gel 60 F₂₅₄ plates (Merck), spots were detected by fluorescence quenching under UV light at 254 nm, and/or staining with appropriate solutions (anisaldehyde, phosphomolybdic acid, KMnO_4). Column chromatography was performed on Silica gel 60 (0.040-0.063 mm, Merck). All experimental manipulations with anhydrous solvents were carried out in flame-dried glassware under inert atmosphere of argon. Degassed solvents were obtained by three cycles of the freeze-pump-thaw. Tetrahydrofuran and diethyl ether were dried and distilled from sodium/benzophenone under the argon atmosphere. Dichloromethane and triethylamine were dried and distilled from CaH_2 under the argon atmosphere.

Equipment and Measurements. NMR spectra were recorded on a Bruker Avance 500 spectrometer at 25 °C in CDCl_3 , CD_2Cl_2 or acetone- d_6 . ^1H NMR (500.16 MHz) spectra were referenced to TMS as internal standard ($\delta_{\text{H}} = 0$ ppm) or to the solvent residual proton signal (CDCl_3 , $\delta_{\text{H}} = 7.24$ ppm; CD_2Cl_2 , $\delta_{\text{H}} = 5.32$ ppm; d_6 -DMSO, $\delta_{\text{H}} = 2.50$ ppm). ^{13}C NMR (125.78 MHz) spectra with total decoupling of protons were referenced to the solvent (CDCl_3 , $\delta_{\text{C}} = 77.23$ ppm, CD_2Cl_2 , $\delta_{\text{C}} = 54.00$ ppm, d_6 -DMSO, $\delta_{\text{C}} = 39.51$ ppm). For a correct assignment of both ^1H and ^{13}C NMR spectra, the ^1H - ^1H COSY, ^{13}C DEPT-135, HSQC and HMBS experiments were performed. EI MS spectra were recorded with a Thermo ISQ

GC/MS instrument (samples were introduced directly using direct injection probes DIP, DEP) and m/z values are given along with their relative intensities (%) at the ionizing voltage of 70 eV. HR ESIMS spectra were obtained with a Bruker microTOF QII mass spectrometer. IR spectra were recorded with a Perkin-Elmer GX FTIR spectrometer in KBr pellets. Analytical samples were dried at 40 - 100 °C under reduced pressure (10^{-2} mbar). Melting points were measured with a Büchi Melting point B-540 apparatus and were not corrected. Elemental analyses were obtained using a Vario Micro Cube CHNS analyzer.

4-Bromo-1-[2-(trimethylsilyl)ethylsulfanyl]benzene (1).³ A 50 mL pressure tube was charged with 4-bromothiophenol (14.3 g, 75.5 mmol), vinyltrimethylsilane (8.9 g, 13 mL, 88.8 mmol) and azobisisobutyronitrile (AIBN) (657 mg, 4 mmol) under argon. The tube was sealed and stirred at 100 °C for 16 h. After cooling to room temperature, the solution was diluted with hexane (300 mL), passed through a short pad of silica gel (60 g, hexane) and evaporated. The residue was purified by column chromatography on silica gel (800 g) in pure hexane to provide product **1** (19.3 g) as a pale yellow oil in 89% yield (R_f = 0.26, hexane). ¹H NMR (500 MHz, CD₂Cl₂) δ ppm: 0.05 (s, 9H, CH₃), 0.92 (m, 2H, CH₂Si), 2.96 (m, 2H, SCH₂), 7.17 (m, 2H, C^{2,6}H), 7.40 (m, 2H, C^{3,5}H). ¹³C NMR (125.8 MHz, CD₂Cl₂) δ ppm: -1.57 (CH₃), 17.23 (CH₂Si), 29.97 (SCH₂), 119.57 (C⁴), 130.72 (C^{2,6}H), 130.31 (C^{3,5}H), 137.45 (C¹). Anal. calcd. for C₁₁H₁₇BrSSi (289.31): C, 45.67; H, 5.92. Found: C, 45.79; H, 6.01.

4-Iodo-1-[2-(trimethylsilyl)ethylsulfanyl]benzene (2).^{3,4} Compound **1** (45 g, 13.8 mmol) was dissolved in anhydrous THF (100 mL) in a 500 mL Schlenk flask under argon, cooled to -78 °C and degassed. Afterwards *tert*-BuLi (17.9 mL, 30.4 mmol, 1.7 M in pentane) was added dropwise over 15 min and the resulting yellow solution was stirred at -78 °C for 45 min. Iodine (5.1 g, 20 mmol) was dissolved in THF (60 mL) in the second 100 mL Schlenk flask under argon and cooled to -78 °C. The iodine solution was cannulated

dropwise into the flask containing lithiated species. The dark solution was stirred at -78 °C for 30 min, then allowed to warm to room temperature and stirred for additional 2 h. The reaction mixture was quenched with Na₂SO₃ (100 mL, 10% in water). The aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic layer was subsequently washed with brine (100 mL) and dried over magnesium sulfate. After filtration over a short pad of silica gel (60 g, diethyl ether) and evaporation, the residue was purified using column chromatography on silica gel (1000 g) in pure hexane to provide product **2** (4.2 g) in 90% yield as a pale yellow oil (*R*_f = 0.28, hexane). ¹H NMR (500 MHz, CDCl₃) δ ppm: 0.02 (s, 9H, CH₃), 0.89 (m, 2H, CH₂Si), 2.91 (m, 2H, SCH₂), 7.00 (m, 2H, C^{2,6}H), 7.56 (m, 2H, C^{3,5}H). ¹³C NMR (125.8 MHz, CD₂Cl₂) δ ppm: -1.55 (CH₃), 16.93 (CH₂Si), 29.61 (SCH₂), 90.39 (C⁴), 130.66 (C^{2,6}H), 137.63 (C¹), 137.92 (C^{3,5}H). IR (KBr) ν cm⁻¹ 3070 (vw, ν(=CH)), 2951 (s, ν_{as}(CH₂, CH₃)), 2916, 2894 (w, ν_s(CH₂, CH₃)), 1568 (w, ν(CC), Ph), 1471 (s), 1417 (m), 1382 (m), 1248 (s, δ_s(CH₃), TMS), 1163 (m), 1092 (s), 1003 (s), 858 (vs) and 840 (vs, δ_{as}(CH₃), TMS), 802 (s), 753 (m), 721 (w), 693 (m, ν_{as}(SiC₃), TMS). Anal. calcd. for C₁₁H₁₇ISSi (336.31): C, 39.28; H, 5.10. Found: C, 39.51; H, 5.26.

({4-[(Trimethylsilyl)ethynyl]phenyl}ethynyl)ferrocene (3).⁵ A 150 mL Schlenk flask was charged with ethynylferrocene (0.5 g, 2.38 mmol), PdCl₂(PPh₃)₂ (84 mg, 0.12 mmol), CuI (46 mg, 0.24 mmol) under argon, and dry triethylamine (60 mL) was added. Then (4-bromophenylethynyl)trimethylsilane (0.72 g, 2.86 mmol) was added and the mixture was stirred at 50 °C for 4 h and at 80 °C for 16 h. After cooling to room temperature, the reaction mixture was treated with diethyl ether (100 mL), the precipitate was filtered through a pad of silica gel (20 g, diethyl ether), and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (250 g, hexane:CH₂Cl₂ equal to 8:1) to afford the title compound **3** (365 mg) as a yellow solid in 40% yield (*R*_f = 0.37, hexane:CH₂Cl₂ equal to 5:1). ¹H NMR (500 MHz, CD₂Cl₂) δ ppm: 0.25 (s, 9H, CH₃), 4.24

(s, 5H, Cp, Fc), 4.27 (t, $J = 2$ Hz, 2H, C^{3,4}H, Fc), 4.50 (t, $J = 2$ Hz, 2H, C^{2,5}H, Fc), 7.40 (s, 4H, C^{2^{II},3^{II},5^{II},6^{II}}H). ¹³C NMR (125.8 MHz, CD₂Cl₂) δ ppm: 0.13 (CH₃), 65.35 (C^I, Fc), 69.68 (C^{3,4}H, Fc), 70.57 (C^{Cp}H, Fc), 72.05 (C^{2,5}H, Fc), 85.73 (C^{2^I}), 91.28 (C^{1^I}), 96.51 (C^{2^{III}}), 105.14 (C^{1^{III}}), 122.78 (C^{4^{II}}), 124.76 (C^{1^{II}}), 131.67 (C^{3^{II},5^{II}}H), 132.33 (C^{2^{II},6^{II}}H). Anal. calcd. for C₂₃H₂₂FeSi (382.35): C, 72.25; H, 5.80. Found: C, 72.31; H, 5.77.

[(4-Ethynylphenyl)ethynyl]ferrocene (4).⁵ The silylated acetylene **3** (180 mg, 0.47 mmol) was dissolved in CH₂Cl₂ (40 mL) in a 100 mL flask. Then a solution of TBAF (0.7 mL, 0.7 mmol, 1 M in THF) was added, and the mixture was vigorously stirred at the room temperature for 1 h. The reaction mixture was quenched with water (50 mL), extracted with dichloromethane (3 $\times$ 100 mL), and dried with MgSO₄. After evaporation under reduced pressure, the crude product was purified by column chromatography on silica gel (100 g, hexane:CH₂Cl₂ equal to 6:1). After evaporation and drying under vacuo at the room temperature, the title compound **4** (144 mg) was isolated as a dark red solid in 98% yield (R_f = 0.33, Hex:CH₂Cl₂ = 5:1). ¹H NMR (500 MHz, CDCl₃) δ ppm: 3.14 (s, 1H, $\equiv$ CH), 4.23 (s, 5H, Cp, Fc), 4.25 (t, $J = 2$ Hz, 2H, C^{3,4}H, Fc), 4.49 (t, $J = 2$ Hz, 2H, C^{2,5}H, Fc), 7.38-7.44 (m, 4H, C^{2^{II},3^{II},5^{II},6^{II}}H). ¹³C NMR (125.8 MHz, CDCl₃) δ ppm: 65.10 (C^I, Fc), 69.30 (C^{3,4}H, Fc), 70.29 (C^{Cp}H, Fc), 71.74 (C^{2,5}H, Fc), 78.81 (C^{2^{III}}H), 83.67 (C^{1^{III}}), 85.51 (C^{2^I}), 90.95 (C^{1^I}), 121.34 (C^{4^{II}}), 124.72 (C^{1^{II}}), 131.43 (C^{3^{II},5^{II}}H), 132.23 (C^{2^{II},6^{II}}H). Anal. calcd. for C₂₀H₁₄Fe (310.17): C, 77.45; H, 4.55. Found: C, 77.31; H, 4.62.

{[4-({4-[(2-Trimethylsilyl)ethylsulfanyl]phenyl}ethynyl)phenyl]ethynyl}ferrocene (5). A 50 mL Schlenk flask was charged with **4** (140 mg, 0.45 mmol), **2** (170 mg, 0.51 mmol), PdCl₂(PPh₃)₂ (18 mg, 25 μ mol), CuI (10 mg, 50 μ mol) under argon, and dry triethylamine (20 mL) was added. The reaction mixture was stirred at 40 °C for 12 h under argon. After cooling to room temperature, the reaction mixture was treated with diethyl ether

(50 mL), the precipitate was filtered through a pad of silica gel (10 g, diethyl ether), and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (200 g, hexane:CH₂Cl₂ equal to 7:1) to afford the title compound **5** (187 mg) as an orange solid in 80% yield (*R*_f = 0.20, Hex:CH₂Cl₂ = 5:1). Mp: 88-89 °C. ¹H NMR (500 MHz, CDCl₃) δ ppm: 0.04 (s, 9H, CH₃), 0.93 (m, 2H, CH₂Si), 2.97 (m, 2H, SCH₂), 4.23 (s, 5H, Cp, Fc), 4.24 (t, *J* = 2 Hz, 2H, C^{3,4}H, Fc), 4.49 (t, *J* = 2 Hz, 2H, C^{2,5}H, Fc), 7.22 (m, 2H, C^{3^{IV},5^{IV}}H), 7.41 (m, 2H, C^{2^{IV},6^{IV}}H), 7.41-7.45 (m, 4H, C^{2^{II},3^{II},5^{II},6^{II}}H). ¹³C NMR (125.8 MHz, CDCl₃) δ ppm: -1.53 (CH₃), 16.85 (CH₂Si), 29.14 (SCH₂), 65.15 (C^I, Fc), 69.20 (C^{3,4}H, Fc), 70.22 (C^{Cp}H, Fc), 71.68 (C^{2,5}H, Fc), 85.77 (C^{2^I}), 89.75 (C^{1^{III}}), 90.76 (C^{2^{III}}), 91.05 (C^{1^I}), 120.08 (C^{1^{IV}}), 122.60 (C^{4^{II}}), 123.98 (C^{1^{II}}), 128.03 (C^{3^{IV},5^{IV}}H), 131.50 (C^{3^{II},5^{II}}H), 131.63 (C^{2^{II},6^{II}}H), 132.07 (C^{2^{IV},6^{IV}}H), 138.76 (C^{4^{IV}}). IR (KBr) ν cm⁻¹ 3107 (w), 3089 (w, ν(=CH)), 2952 (m) and 2918 (m, ν_{as}(CH₃)), 2880 (m, ν_s(CH₃)), 2208 (w), 2147 (w, ν(C≡C)), 1643 (w), 1589 (m, ν(CC), Ph), 1397 (m), 1270 (m), 1245 (s, δ_s(CH₃), TMS), 1167 (m), 1106 (m), 1085 (s), 1011 (m), 895 (m), 822 (bs, Fc, δ_{as}(CH₃), TMS), 758 (m), 730 (m), 692 (m, ν_{as}(SiC₃), TMS), 539 (m), 485 (s) and 459 (m, Fc). EI MS *m/z* (%) 518 (2, M⁺), 418 (75, [M-TMSE]), 390 (32), 121 (13), 104 (26), 73 (100). Anal. calcd. for C₃₁H₃₀FeSSi (518.57): C, 71.80; H, 5.83. Found: C, 72.01; H, 5.70.

[(4-{[4-(Acetylsulfanyl)phenyl]ethynyl}phenyl)ethynyl]ferrocene (6). Compound **5** (150 mg, 0.29 mmol) was dissolved in dry THF (20 mL) in a 50 mL Schlenk flask under argon, and the solution was cooled to 0 °C under argon. Then tetrabutylammonium fluoride (TBAF) (1.5 mL, 1.5 mmol, 1 M in THF) was dropwise added at 0 °C and the resulting solution was stirred at 0 °C for 30 min and at ambient temperature for 20 min. After cooling to 0 °C, acetylchloride (144 mg, 130 μL, 1.83 mmol) was dropwise added. After 30 min of stirring at 0 °C, the reaction mixture was quenched with water (50 mL), followed by addition of CH₂Cl₂ (50 mL) and saturated aqueous solution of NaHCO₃ (3 mL). The aqueous layer

was extracted with CH₂Cl₂ (3 × 60 mL). The combined organic layer was subsequently washed with brine (80 mL), dried over magnesium sulfate, and filtered. Volatiles were removed under reduced pressure, and the residue was purified by column chromatography on silica gel (150 g) in hexane:CH₂Cl₂ (4:1) to afford 120 mg of the title product **6** (R_f = 0.17, Hex: CH₂Cl₂ = 2:1) as an orange solid in 90%. Mp: 198-199 °C. ¹H NMR (500 MHz, CDCl₃) δ ppm: 2.42 (s, 3H, CH₃), 4.23 (s, 5H, Cp, Fc), 4.24 (t, J = 2 Hz, 2H, C^{3,4}H, Fc), 4.49 (t, J = 2 Hz, 2H, C^{2,5}H, Fc), 7.38 (m, 2H, C^{3^{IV},5^{IV}}H), 7.42-7.48 (m, 4H, C^{2^{II},3^{II},5^{II},6^{II}}H), 7.54 (m, 2H, C^{2^{IV},6^{IV}}H). ¹³C NMR (125.8 MHz, CDCl₃) δ ppm: 30.52 (CH₃), 65.09 (C^I, Fc), 69.23 (C^{3,4}H, Fc), 70.23 (C^{Cp}H, Fc), 71.70 (C^{2,5}H, Fc), 85.72 (C^{2^I}), 90.39 (C^{2^{III}}), 91.01 (C^{1^{III}}), 91.16 (C^{1^I}), 122.17 (C^{4^{II}}), 124.40 (C^{1^{II}}), 124.61 (C^{1^{IV}}), 128.40 (C^{4^{IV}}), 131.52 (C^{2^{II},6^{II}}H), 131.79 (C^{3^{II},5^{II}}H), 132.37 (C^{2^{IV},6^{IV}}H), 134.46 (C^{3^{IV},5^{IV}}H). IR (KBr) ν cm⁻¹ 3079 (w, ν(=CH)), 2952 (w) and 2913 (w, ν_{as}(CH₃)), 2203 (m, ν(C≡C)), 1697 (vs, ν(CO)), 1587 (w, ν(CC), Ph), 1514 (m), 1397 (m), 1354 (m), 1298 (w), 1265 (w), 1164 (w), 1134 (m), 1119 (s), 1106 (s), 1031 (m), 1005 (m), 951 (s), 924 (m), 829 (vs) and 813 (s, Fc), 736 (w), 623 (s), 541 (m), 500 (s), 489 (m) and 472 (m, Fc). EI MS *m/z* (%) 460 (75, M⁺), 418 (32, [M-Ac]), 121 (14), 84 (100), 49 (92). Anal. calcd. for C₂₈H₂₀FeOS (460.37): C, 73.05; H, 4.38. Found: C, 73.29; H, 4.47.

Per-6-iodo-β-cyclodextrin (7). Per-6-iodo-β-cyclodextrin was prepared according to a published procedure¹ from β-cyclodextrin (11.6 g, 10.2 mmol), PPh₃ (40.1 g, 153 mmol), and iodine (40.5 g, 160 mmol) in anhydrous DMF (150 mL), to obtain the desired product **7** (15.8 g) as a white powder in 81% yield. ¹H NMR (500 MHz, *d*₆-DMSO) δ ppm: 3.28 (t, J = 9 Hz, 7H, C⁴H), 3.37 (m, 7H, C²H), 3.44 (m, 7H, C^{6a}H), 3.59 (t, J = 9 Hz, 7H, C⁵H), 3.64 (t, J = 9 Hz, 7H, C³H), 3.80 (bd, 7H, C^{6b}H), 4.98 (d, J = 3 Hz, 7H, C¹H), 5.93 (d, J = 2 Hz, 7H, C^{3OH}), 6.03 (d, J = 7 Hz, 7H, C^{2OH}). ¹³C NMR (125.8 MHz, *d*₆-DMSO) δ ppm: 9.47 (C^{6H₂}), 70.92 (C⁵H), 71.90 (C³H), 72.15 (C²H), 85.93 (C⁴H), 102.11 (C¹H). IR (KBr) ν cm⁻¹ 3390 (bs, ν(OH)), 2916 (s, ν_{as}(CH₂)), 1626 (bm), 1417 (s), 1371 (s), 1329 (s), 1222 (s), 1148

(s), 1062 (bs), 944 (bs), 751 (m), 590 (m), 445 (m). ESI(-) HRMS calcd. for $C_{42}H_{63}I_7O_{28}Na$ ($[M+Na]^+$, 1926.6711). Found: m/z 1926.6748. Anal. calcd. for $C_{42}H_{63}O_{28}I_7$ (1904.27): C, 26.49; H, 3.33. Found: C, 25.10.; H, 3.67.

Per-6-sulfanyl- β -cyclodextrin (8). Per-6-sulfanyl- β -cyclodextrin was prepared according to a published procedure^{2,6} from per-6-iodo- β -cyclodextrin **7** (1.5 g, 0.79 mmol), and thiourea (480 mg, 6.30 mmol) in anhydrous DMF (15 mL), to afford the desired product **8** (765 mg) as a white powder in 78% yield. 1H NMR (500 MHz, d_6 -DMSO) δ ppm: 2.12 (t, J = 8 Hz, 7H, SH), 2.75 (m, 7H, $C^{6a}H$), 3.19 (m, 7H, $C^{6b}H$), 3.30-3.45 (m, 14H, $C^{2,4}H$), 3.60 (t, J = 9 Hz, 7H, C^5H), 3.67 (t, J = 9 Hz, 7H, C^3H), 4.92 (d, J = 2 Hz, 7H, C^1H), 5.82 (bd, J = 2 Hz, 7H, C^3OH), 5.93 (d, J = 7 Hz, 7H, C^2OH). ^{13}C NMR (125.8 MHz, d_6 -DMSO) δ ppm: 26.03 (C^6H_2), 70.09 (C^5H), 72.34 (C^3H), 72.60 (C^2H), 85.00 (C^4H), 102.25 (C^1H). IR (KBr) ν cm^{-1} 3371 (bs, $\nu(OH)$), 2930 (m, $\nu_{as}(CH_2)$), 1634 (bw), 1416 (m), 1368 (m), 1332 (m), 1154 (s), 1069 (s), 1043 (vs), 945 (w), 753 (w), 588 (m). ESI(-) HRMS Calcd. for $C_{42}H_{70}S_7O_{28}Na$ ($[M+Na]^+$, 1269.1991). Found: m/z 1269.2083. Anal. calcd. for $C_{42}H_{70}O_{28}S_7$ (1247.45): C, 40.44; H, 5.66. Found: C, 38.97.; H, 5.85.

3. ^1H and ^{13}C NMR spectra of compounds 1-8

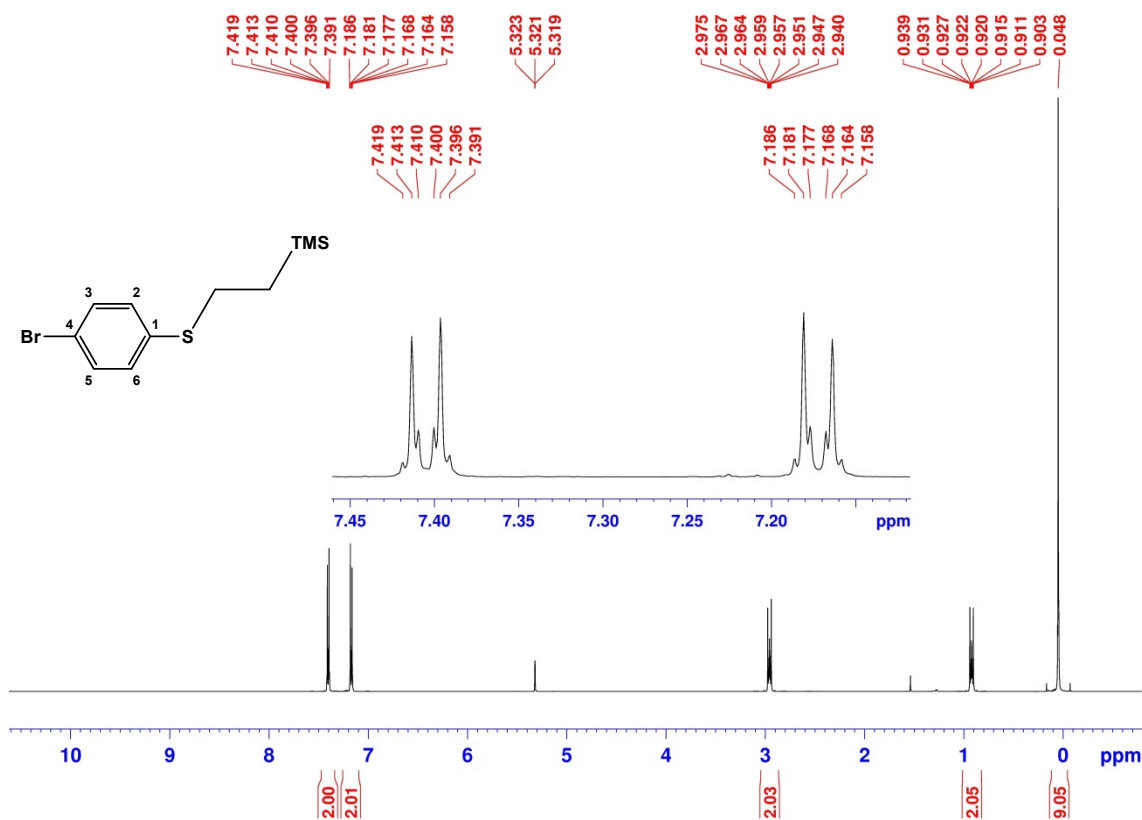


Figure S1. ^1H NMR spectrum (500 MHz, CD_2Cl_2) of 1

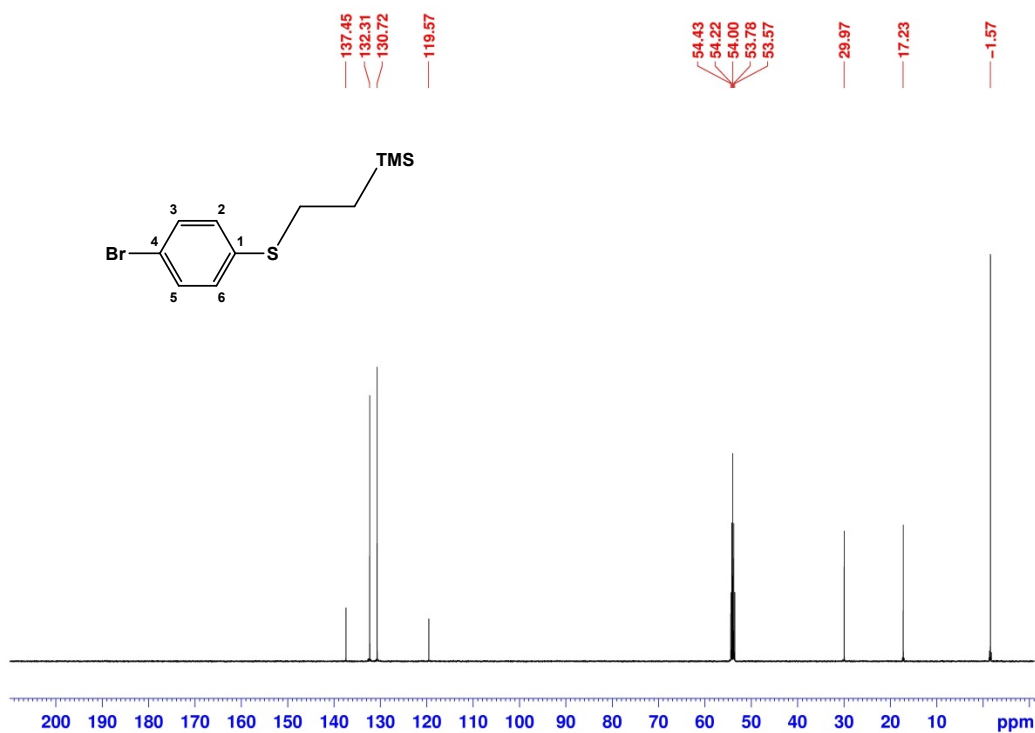


Figure S2. ^{13}C NMR spectrum (125.8 MHz, CD_2Cl_2) of 1

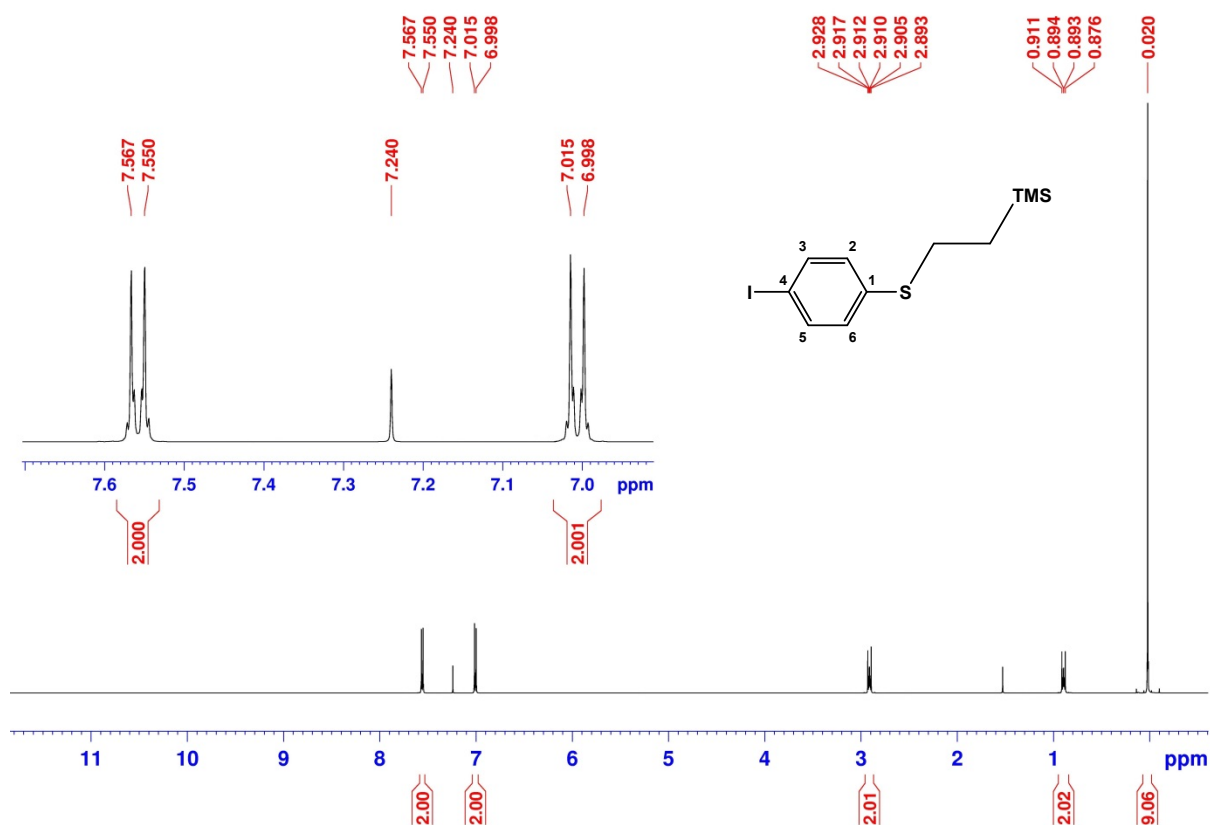


Figure S3. ¹H NMR spectrum (500 MHz, CDCl₃) of 2

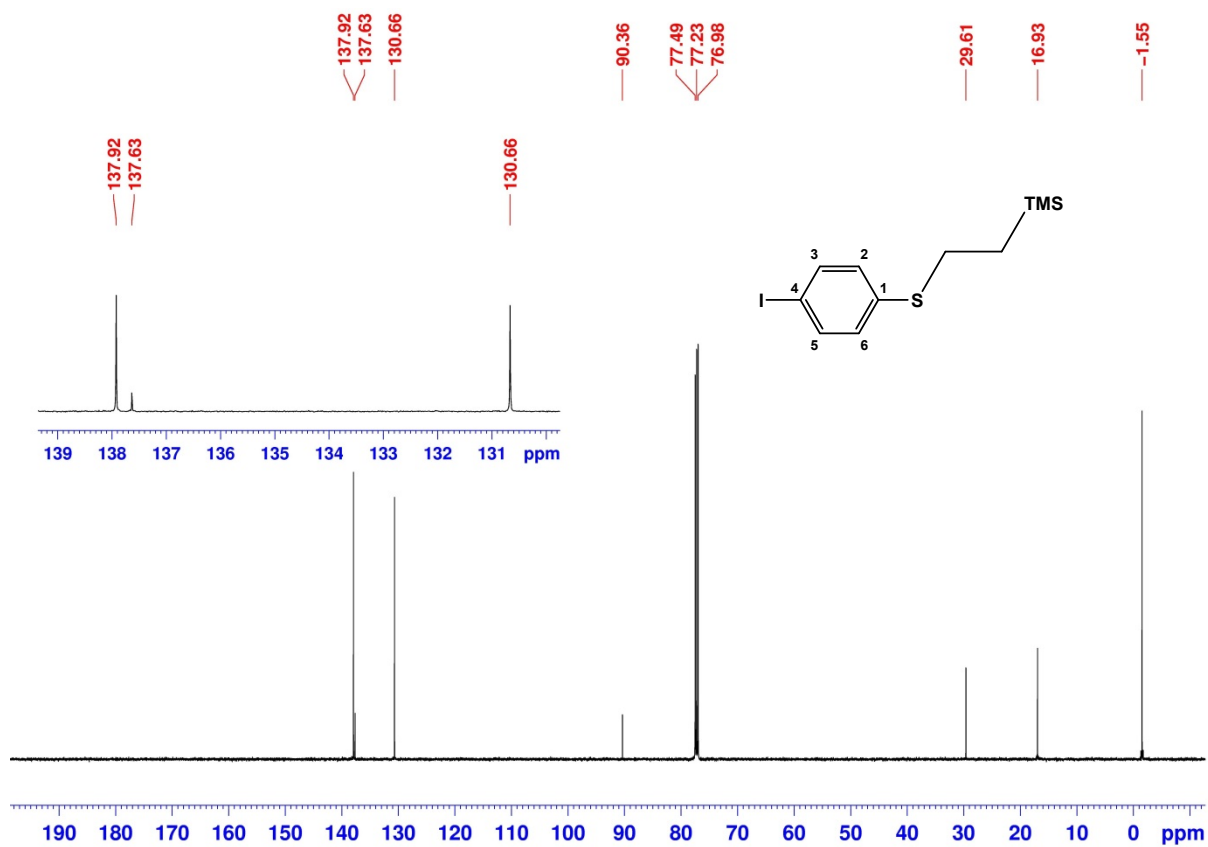


Figure S4. ¹³C NMR spectrum (125.8 MHz, CDCl₃) of 2

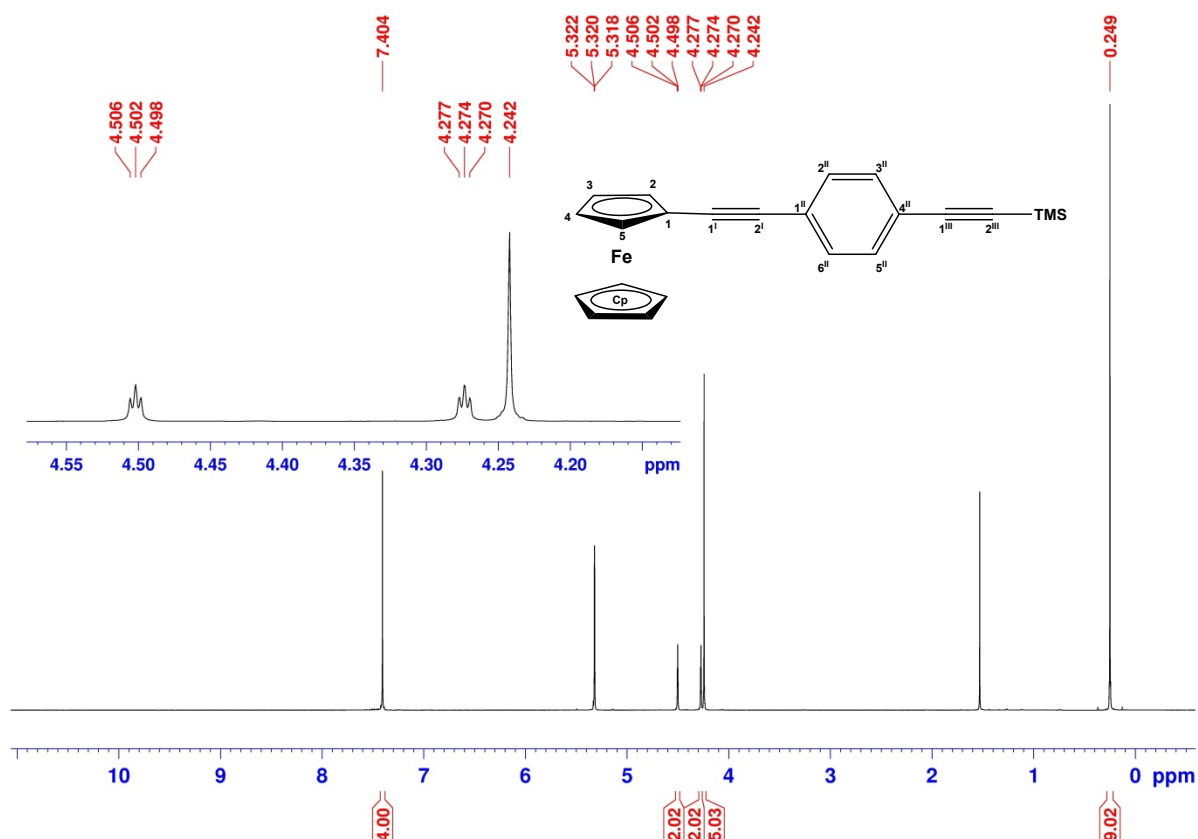


Figure S5. ^1H NMR spectrum (500 MHz, CD_2Cl_2) of **3**

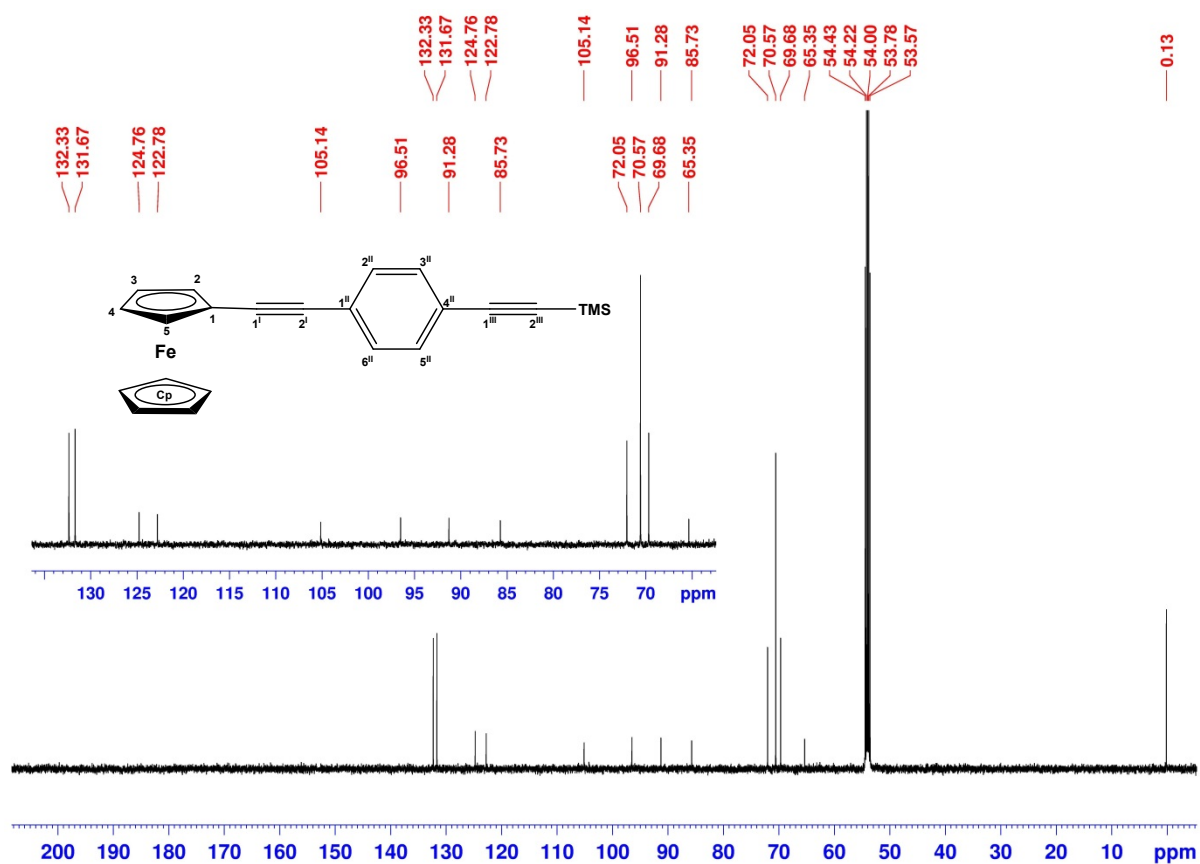


Figure S6. ^{13}C NMR spectrum (125.8 MHz, CD_2Cl_2) of **3**

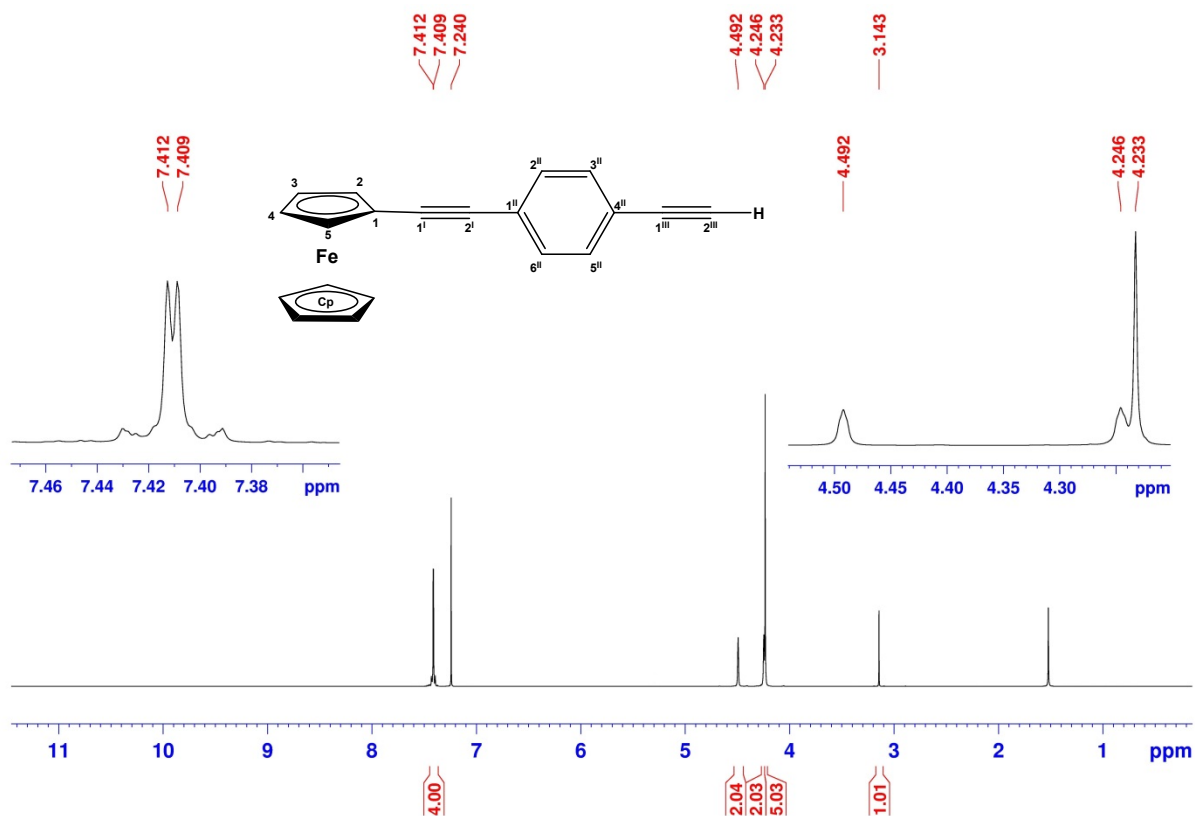


Figure S7. ¹H NMR spectrum (500 MHz, CDCl₃) of **4**

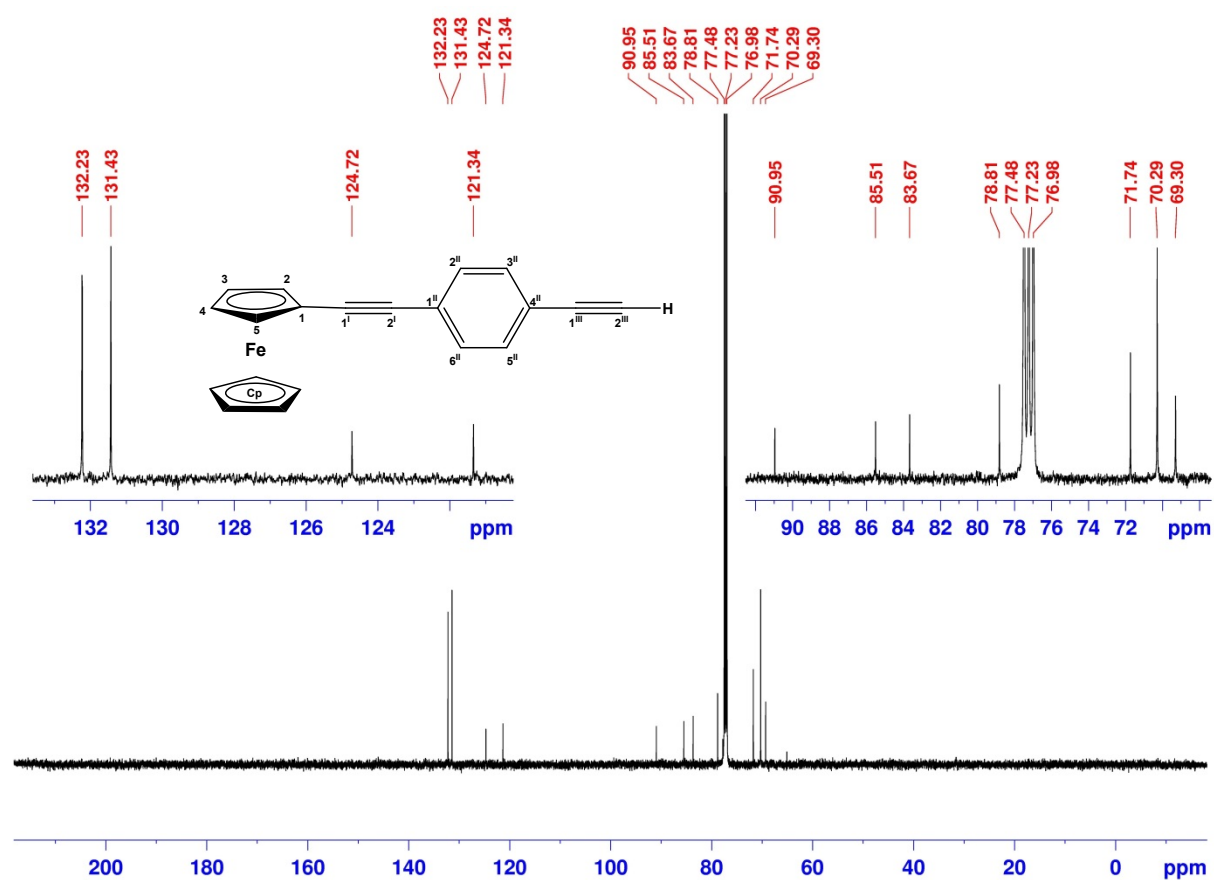


Figure S8. ¹³C NMR spectrum (125.8 MHz, CDCl₃) of **4**

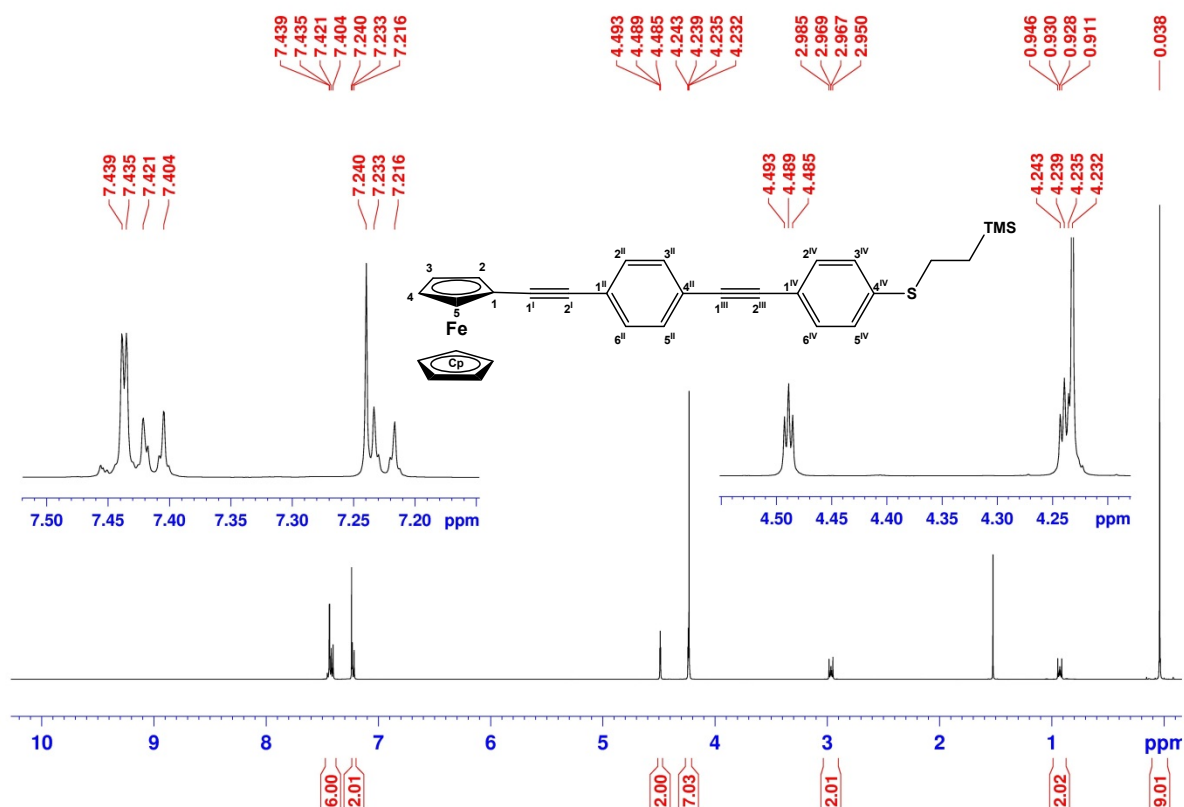


Figure S9. ¹H NMR spectrum (500 MHz, CDCl₃) of **5**

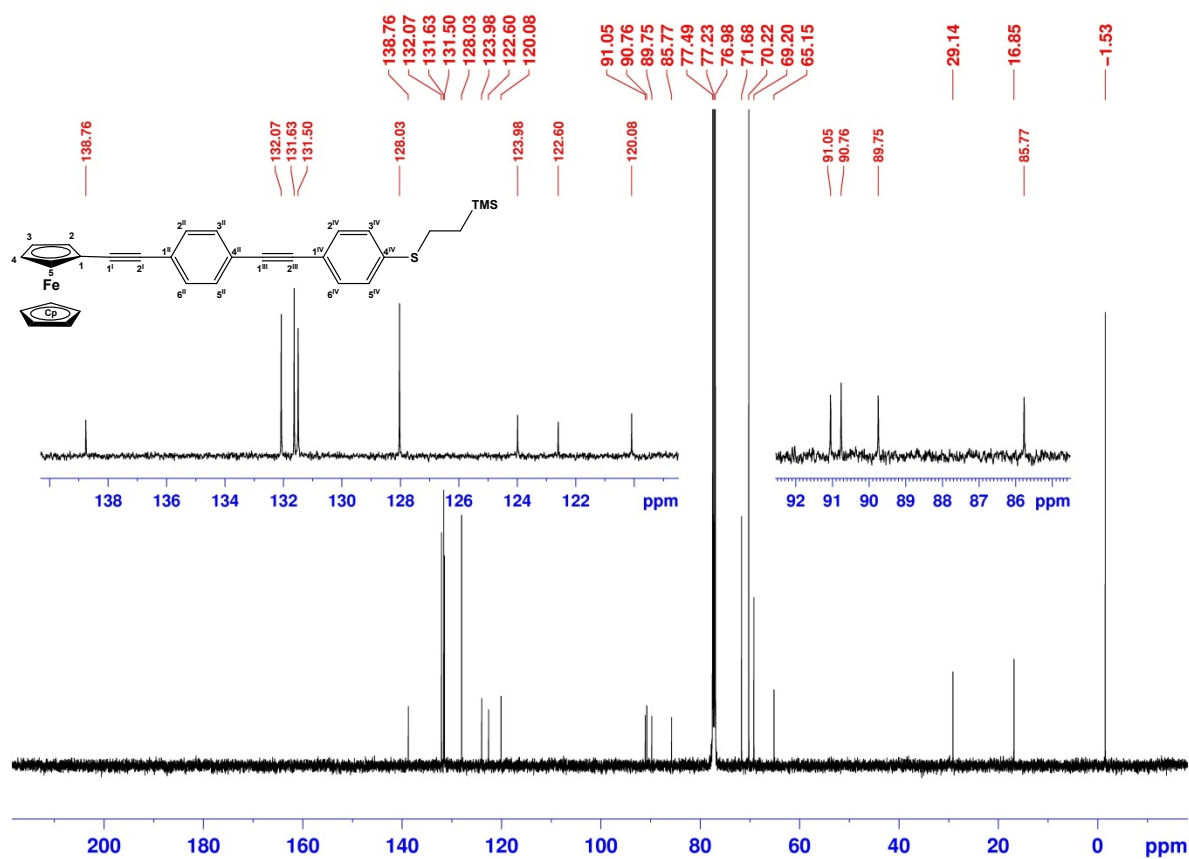


Figure S10. ¹³C NMR spectrum (125.8 MHz, CDCl₃) of **5**

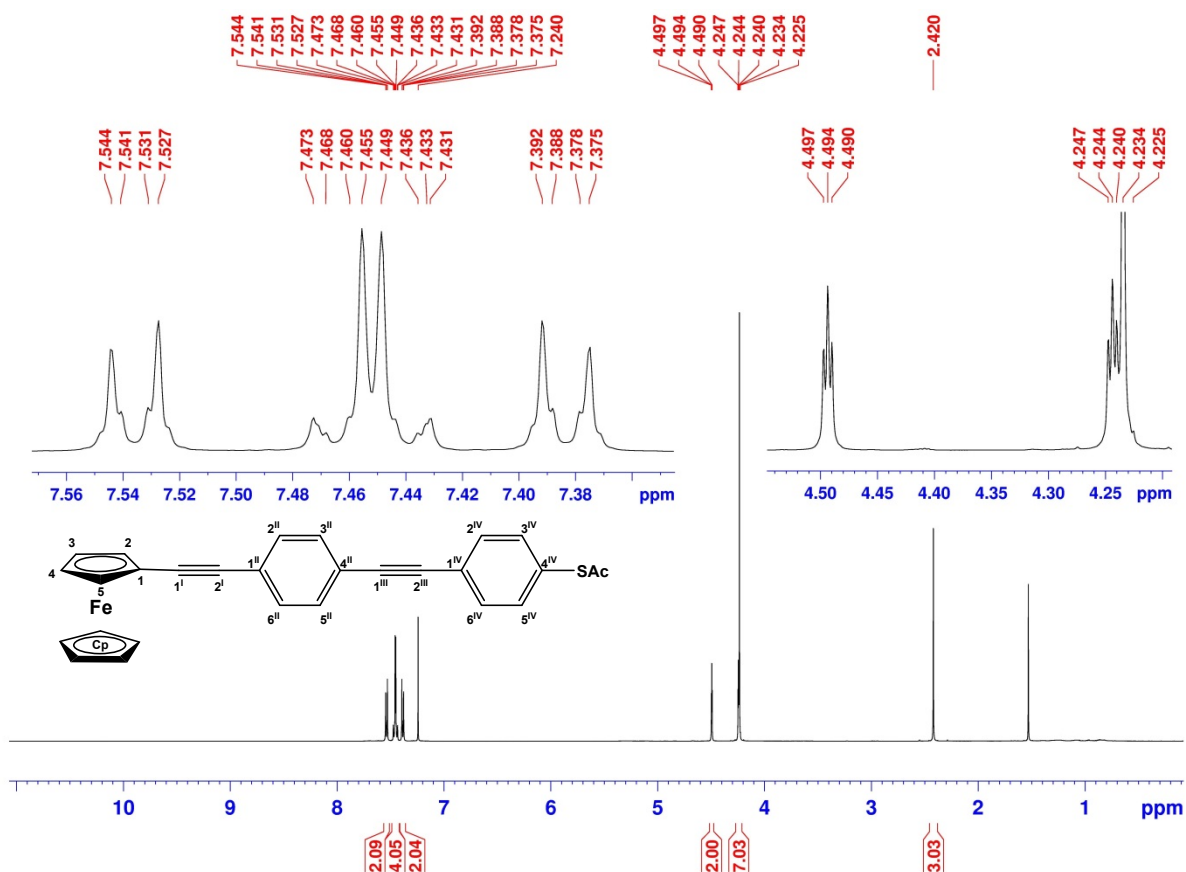


Figure S11. ¹H NMR spectrum (500 MHz, CDCl₃) of 6

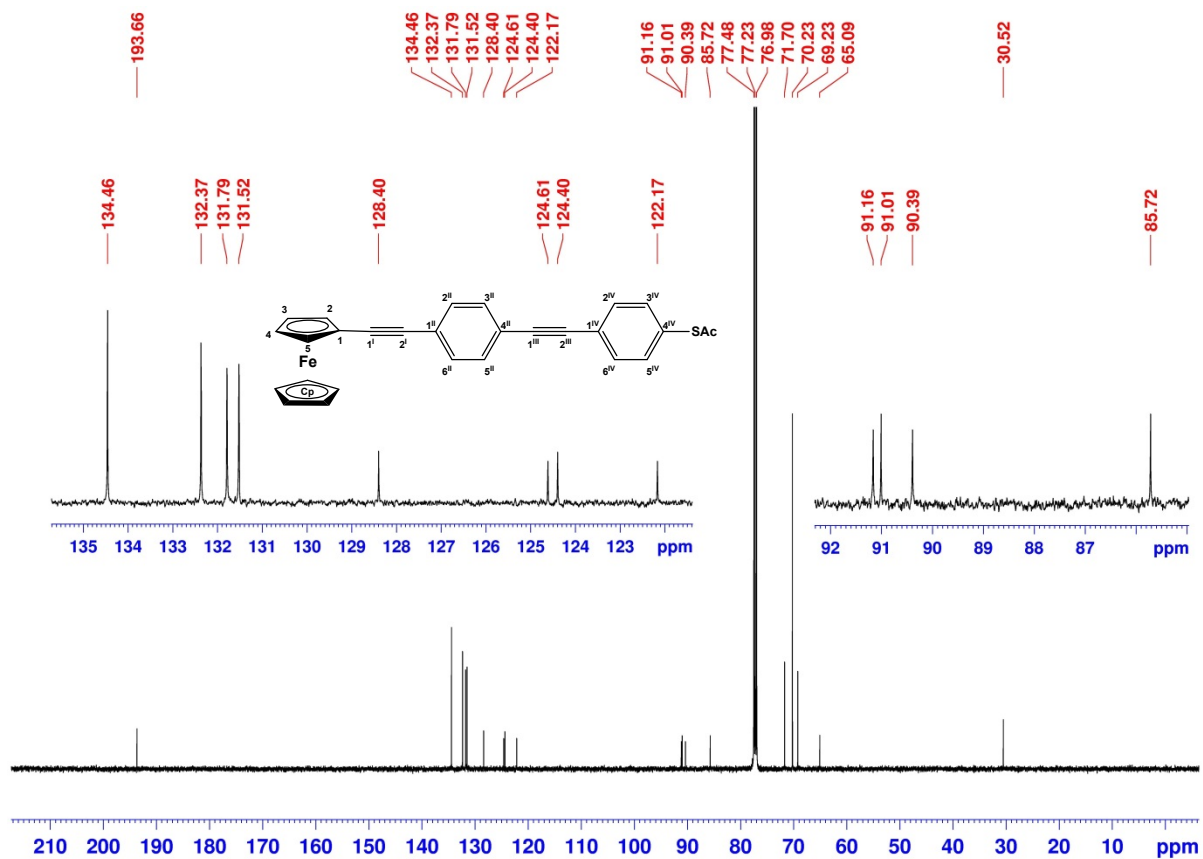


Figure S12. ¹³C NMR spectrum (125.8 MHz, CDCl₃) of 6

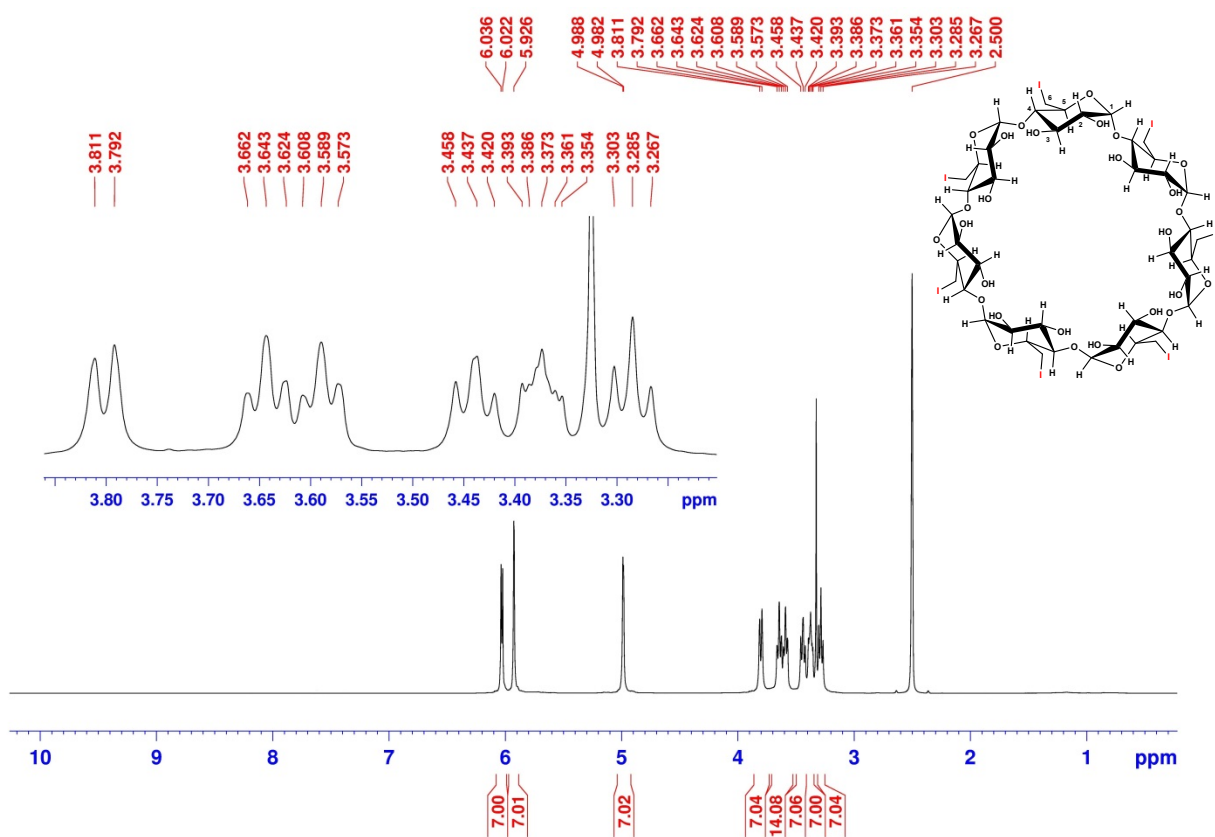


Figure S13. ^1H NMR spectrum (500 MHz, d_6 -DMSO) of **7**

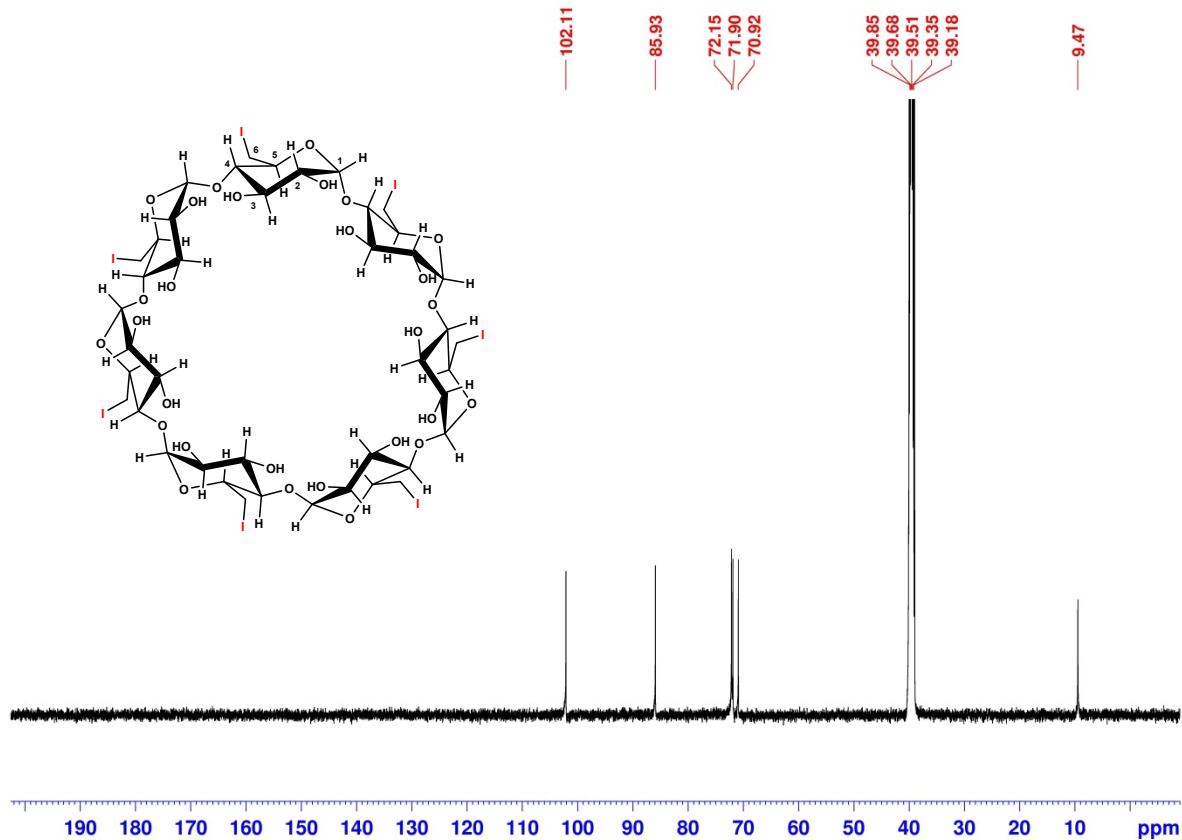


Figure S14. ^{13}C NMR spectrum (125.8 MHz, d_6 -DMSO) of **7**

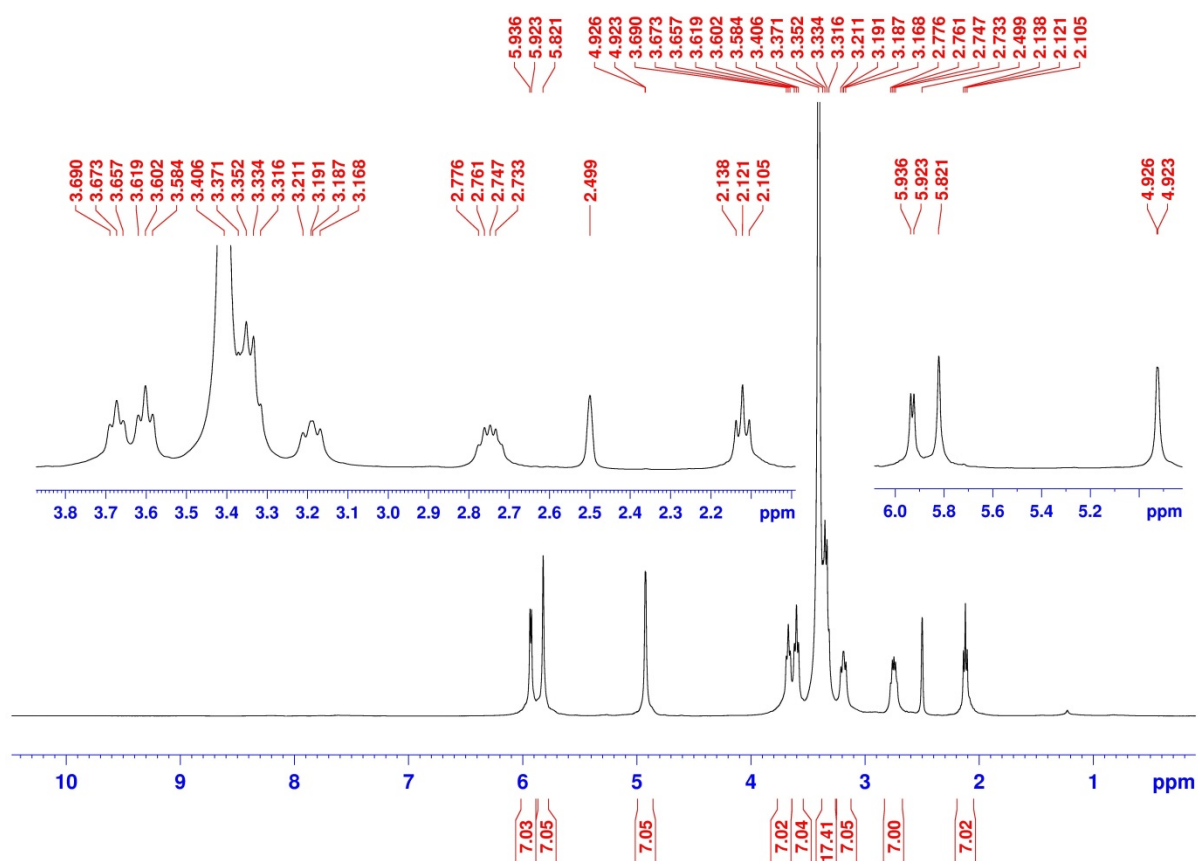


Figure S15. ^1H NMR spectrum (500 MHz, d_6 -DMSO) of **8**

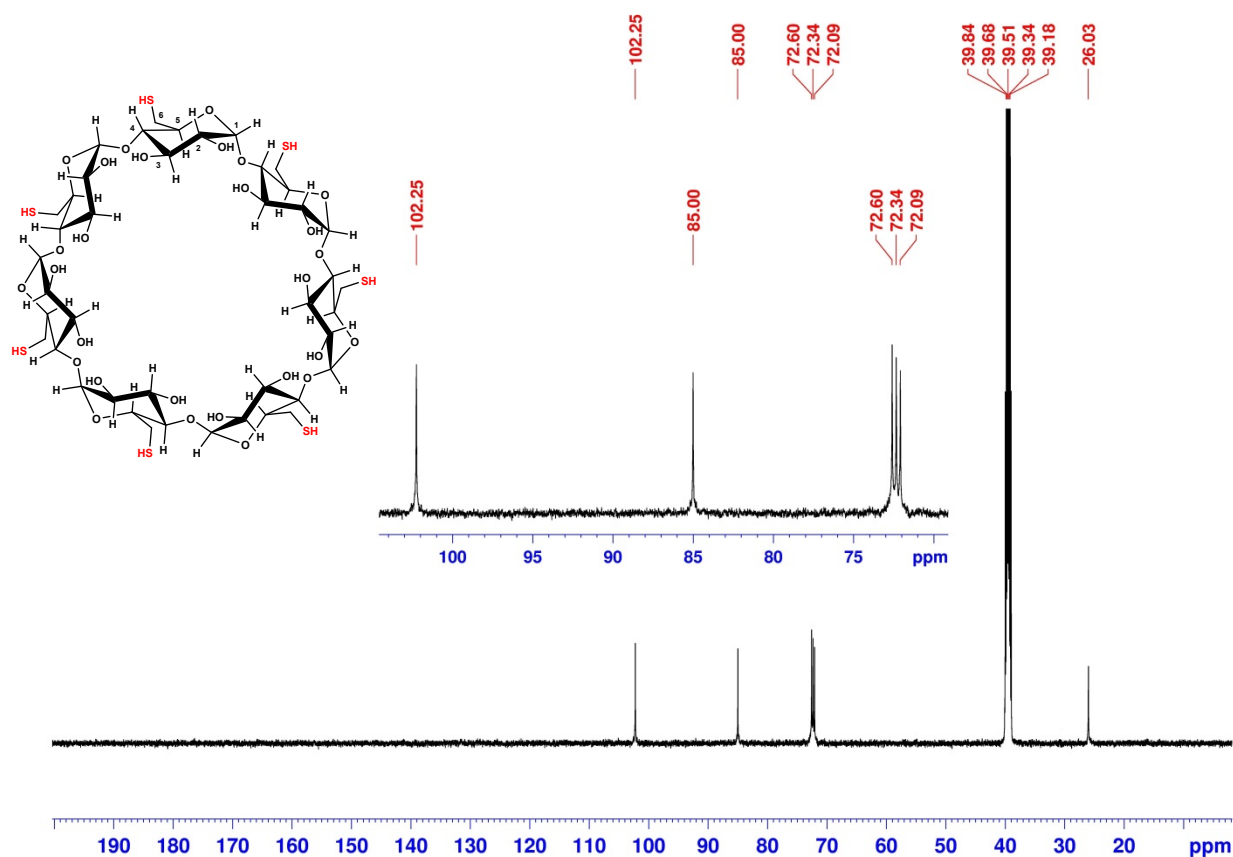


Figure S16. ^{13}C NMR spectrum (125.8 MHz, d_6 -DMSO) of **8**

4. General procedures for electrochemical and AFM experiments

Water. Freshly prepared Milli-Q water (18.2 MΩ cm, 2 ppb TOC, Millipore Corporation) was used in all experiments.

Inert atmosphere. Argon gas (Carbagas, Alphagaz 99.999%) was employed to deaerate solutions and electrolytes during the molecular self-assembly, during electrochemical (EC) characterization and atomic force microscopy (AFM) force spectroscopy measurements.

Electrolytes. Perchloric acid (70%, Suprapure) and sodium perchlorate (Suprapure, 99.99 %) were purchased from Merck.

Organic solvents and agents. Absolute ethanol (for analysis, 99.8%) and dimethylsulfoxide (ACS, 99.9%) were purchased from Merck. Triethylamine (>99%) was purchased from Sigma-Aldrich.

Alkanethiols. Pentane-1-thiol ($C_5H_{11}SH$, 98%), hexane-1-thiol ($C_6H_{13}SH$, 95%), octane-1-thiol ($C_8H_{17}SH$, 98.5%) and decane-1-thiol ($C_{10}H_{21}SH$, 96%) were purchased from Sigma-Aldrich.

Tool cleaning procedure. All glassware (electrochemical cells for cyclic voltammetric (CV) experiments, volumetric flasks for the preparation of electrolytes, vials for storage of organic solvents and solutions, gas bubblers, beakers etc.) as well as all polytetrafluoroethylene (PTFE) parts (stoppers and joints, electrode holders, self-assembly chambers etc.) used for electrochemical characterization of self-assembled molecular adlayers were cleaned by boiling in 25% aqueous HNO_3 for at least one hour. Upon cooling to the room temperature, all tools were repeatedly boiled in Milli-Q water, dried in an oven and either immediately used or stored in a dust-free and dry environment.

A home-made PTFE AFM cell, together with an O-ring (Kalrez® 4079, DuPont) and PTFE tweezers used to manipulate the former two items were cleaned by an overnight immersion into carboxic acid (96% H₂SO₄ added dropwise to 30% H₂O₂ in 3:1 ratio (v/v) while cooling the mixture with ice). The items were then repeatedly boiled in Milli-Q water, dried in the oven and immediately employed in the EC-AFM based force spectroscopy (FS) measurements.

5. Samples for electrochemical and AFM experiments

Fabrication of bead electrodes. A gold wire (0.50 mm in diameter, 99,999%, Goodfellow) was first cleaned by immersion into the *aqua regia* mixture for ≈ 10 s. The latter was prepared by mixing 65% aqueous HNO₃ (Sigma Aldrich) and 35% aqueous HCl (Sigma Aldrich) in the ratio of 1:3 (v/v) in a fume hood while cooling the mixture with ice. Afterwards, the wire was rinsed with Milli-Q water and vertically positioned by the aid of a lab clamp. The lower part of the gold wire was melted by a hydrogen flame, forming a progressively growing gold bead. The melting of the wire was terminated when the bead size reached approximately 2 mm in diameter. Subsequently, the bead was heated up and melted again. After letting it solidify without cooling down completely, bead was quickly immersed into the *aqua regia* mixture for 10 s and copiously rinsed by Millipore water. This melting/immersing/rinsing cycle is known to gradually remove impurities driven to the surface of the gold bead. The cycles were repeated until no impurities could be observed on the surface upon heating the bead in the flame. Thus prepared gold bead was melted again and left to solidify very slowly using the hydrogen flame from a nozzle positioned on a slowly moving lab jack. A resulting gold bead electrode (Fig. S17A) was immediately placed in a glass test-tube to avoid contamination. The bead electrode was further processed either (i) to form a half-bead Au(111) electrode (for hanging meniscus-type of measurements, Fig. S17B) or alternatively (ii) was fixed on a gold sheet (10 mm x 10 mm x 1.0 mm, 99.95%,

Goodfellow, Fig. S17C) equipped with a 0.50 mm (diameter) gold wire for electrical connection. The half-bead Au(111) electrode (Fig. S17B) was used for electrochemical characterization of the molecular adlayers. The gold “bead-on-a-sheet” electrode (Fig. S17C) was fabricated so that one of the (111) facets (naturally grown on the bead and having an area of several μm^2) pointed upwards (Fig. S17D). The facet was used as a substrate for AFM imaging and force spectroscopy.

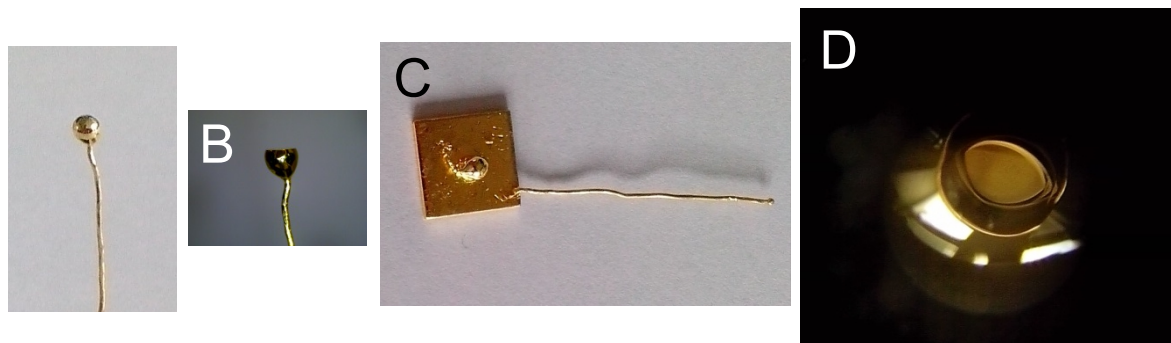


Figure S17. Image of a bead (A) and a half-bead electrode (B), a bead-on-a-sheet AFM substrate (C) (all three real size images) and a (111) facet (D) of the latter, as imaged by a reflection optical microscopy with 40x magnification.

Fabrication of half-bead (hanging-meniscus type) Au(111) electrodes. A gold wire attached to a bead electrode (Fig. S17A) was glued to an aluminum cylinder. The bead was oriented so that one of the naturally grown (111) facets was parallel to the cylinder surface. A proper alignment was ensured by inspecting the direction of a laser beam reflected off the (111) facet. The aluminum cylinder with the bead electrode was placed into a steel drum of a matching size. Quickly solidifying two-component dispersion (Technowitz 4004, Heraeus Kulzer GmbH) was poured on the gold bead in the steel drum until the gold bead was entirely immersed. The assembly was left hardened overnight. The entire assembly was then abraded by an emery paper (coarseness P100) in the direction parallel to the electrode surface until approximately half of the bead was removed. The newly exposed surface was subsequently polished by a series of emery papers with decreasing coarseness (P100 to P1200) and then with an aqueous colloidal dispersion of alumina with gradually decreasing particle sizes

(from 1 μm through 0.3 μm to 50 nm). Finally, the Technowitz dispersion was removed by dissolving by a repetitive boiling in acetone. The released half-bead electrode (Fig. S17B) was subsequently cleaned by an immersion into the concentrated H_2SO_4 (Sigma Aldrich) overnight, to remove the Technowitz dispersion and alumina residues. The electrode was then extensively rinsed with Milli-Q water, tempered in an electromagnetic induction oven at orange heat ($\approx 850^\circ\text{C}$) in an argon atmosphere overnight, cooled down to room temperature and immediately placed in a clean glass test-tube under the argon atmosphere for storage. The electrode surface was inspected by cyclic voltammetry in the aqueous 0.1 M H_2SO_4 (Merck, Suprapure). Only electrodes showing characteristic features of the Au(111) surface were employed further in this study.

Cleaning of Au(111) half-bead and bead-on-a-sheet electrodes. Hanging-meniscus type electrodes (employed for electrochemical characterization of self-assembled monolayers, Fig. S17B) as well as bead-on-a-sheet substrates (employed for EC-AFM based force spectroscopy, Fig. S17C) were typically stored in clean glass test-tubes in an argon atmosphere or in concentrated H_2SO_4 . Prior to usage, an electrode was rinsed with plenty of Milli-Q water and electrochemically cleaned by polarizing at +5.0 V against a flag gold counter electrode for 5 - 10 s in 1.0 M aqueous solution of H_2SO_4 . Upon this procedure, the surface gold oxide is known to be generated. The electrode was then rinsed with Milli-Q water, immersed in a 1.0 M aqueous solution of HCl (Sigma-Aldrich) for 1 min to remove the surface gold oxide and again washed with Milli-Q water. This oxidation and surface oxide removal cycle was repeated three times. Afterwards, the electrode was extensively rinsed with Milli-Q water to remove adsorbed chloride ions, dried in an argon atmosphere, annealed by a butane flame for 2 min on a clean Ceran plate and subjected to an argon flow for 5 min to cool it to room temperature. Subsequently, the electrode was immediately immersed into a deaerated solution of a compound to be characterized.

6. Assembly of mixed FcOPE₂SH/C_nH_{2n+1}SH adlayers

Preparation of FcOPE₂SH/C_nH_{2n+1}SH (n = 6, 8 or 10) adlayers. Adlayers of diluted FcOPE₂SH molecules in alkanethiol (C₆H₁₃SH, C₈H₁₇SH or C₁₀H₂₁SH) self-assembled monolayers were formed by a partial displacement of the alkanethiol monolayers as follows. A freshly cleaned gold electrode was immersed into a freshly prepared 1.0 mM solution of the respective alkanethiol in absolute ethanol in an argon atmosphere inside a dedicated PTFE gas-tight chamber. The latter was subsequently tempered at 60 °C for 16 hours. The modified electrode was extensively rinsed by absolute ethanol to remove an excess of physisorbed alkanethiol molecules, dried in an argon stream and immersed into a deaerated solution of FcOPE₂SAc (compound **6**) in absolute ethanol containing 10 % (v/v) of triethylamine. The latter served as a deprotection agent cleaving the Ac-S bond, i.e. to convert FcOPE₂SAc into FcOPE₂SH. The partial displacement of alkanethiol molecules by those of FcOPE₂SH was performed at 60 °C in the deaerated PTFE chamber.

Optimization of assembly for FcOPE₂SH/C_nH_{2n+1}SH (n = 6, 8 or 10) adlayers.

For all three alkanethiols, the assembly conditions (FcOPE₂SAc concentration and displacement time) were optimized. The aim was to obtain molecular adlayers with the coverage of FcOPE₂SH, θ , ranging between 5 and 20 % and thus to get the Fc moieties well separated at the electrode/electrolyte interface for subsequent EC-AFM-FS measurements. The coverage values were calculated with respect to the experimentally obtained value of the maximum FcOPE₂SH surface concentration (2.5×10^{-10} mol·cm⁻²) obtained for a complete FcOPE₂SH monolayer. The latter was prepared by immersing a freshly annealed Au(111) electrode (i.e. without any alkanethiol molecules pre-assembled) into 0.2 mM ethanolic solution of FcOPE₂SAc containing 10 % triethylamine (v/v) at 60 °C for 16 hours.

Every monolayer assembled at particular conditions on a Au(111) hanging-meniscus type electrode was scrutinized by cyclic voltammetry (CV) in 0.1 M NaClO₄ + 0.5 mM HClO₄ in an all-glass cell employing a lab-built potentiostat⁷. The surface concentration of FcOPE₂SH was calculated by integrating the CV current after baseline subtraction from the charging current. Surface coverage θ was determined as described above. As an example, Fig. S18A shows a CV of a diluted FcOPE₂SH monolayer ($\theta = 7\%$) in the C₈H₁₇SH matrix formed by exposing an Au(111) electrode covered by a monolayer of C₈H₁₇SH to a 0.2 mM solution of FcOPE₂SAC in ethanol containing 10 % (v/v) of triethylamine, for 30 min.

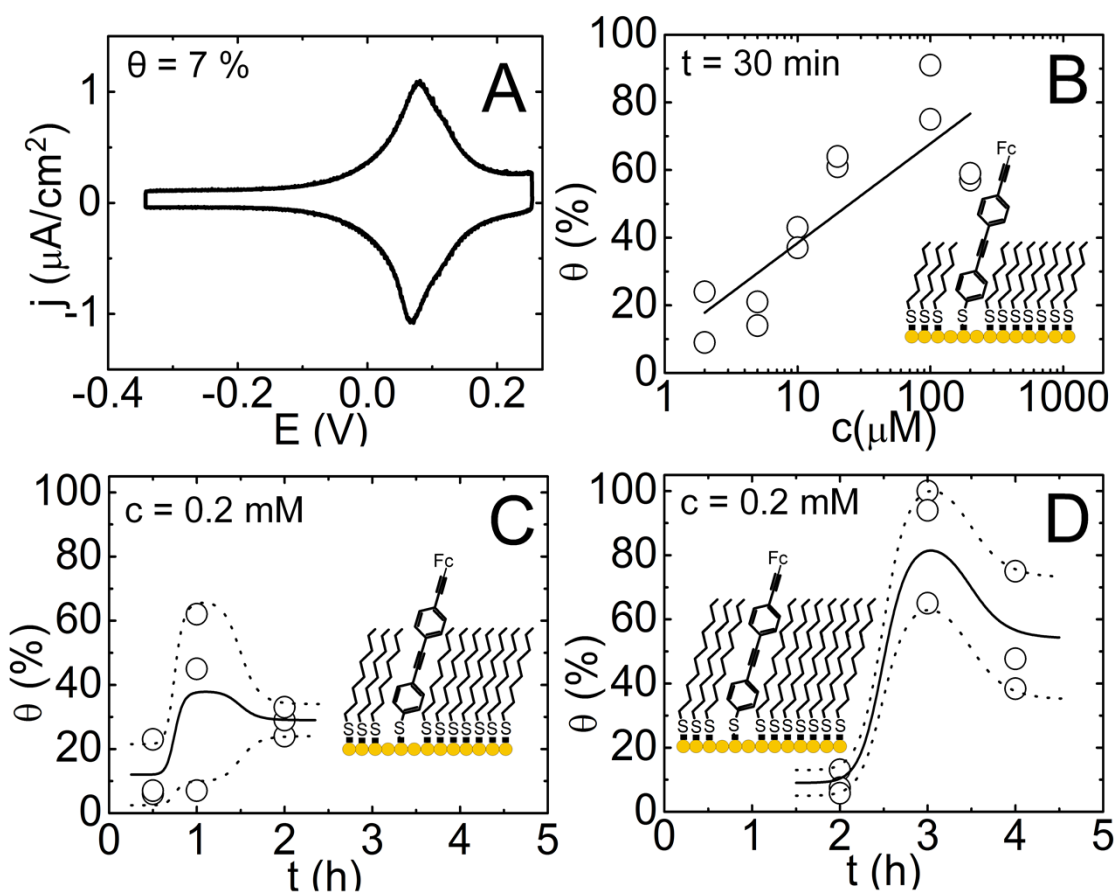


Figure S18. (A) Cyclic voltammogram of FcOPE₂SH/C₈H₁₇SH adlayer on Au(111) in 0.1 M NaClO₄ + 0.5 mM HClO₄ supporting electrolyte, sweep rate 50 mV/s. (B) Surface coverage θ of ferrocene groups in the FcOPE₂SH/C₆H₁₃SH adlayer as a function of FcOPE₂SAC assembly concentration. Surface coverage θ of ferrocene groups in FcOPE₂SH/C₈H₁₇SH (C) and FcOPE₂SH/C₁₀H₂₁SH (D) adlayers as a function of displacement time t .

In the assembly protocol employing $C_8H_{17}SH$ and $C_{10}H_{21}SH$ self-assembled monolayers as the supporting matrices, the concentration of $FcOPE_2SAc$ was kept constant at 0.2 mM, while the exposure time was varied. In the case of the $C_{10}H_{21}SH$ matrix, it was found that the optimal exposure time equals to two hours (Fig. S18D). Coverage values higher than 20% were obtained at longer exposure times (Fig. S18D). However, the same exposure time (2 hours) was too long for the $C_8H_{17}SH$ supporting matrix (Fig. S18C). This can be easily rationalized by the difference in the degree of the organization between $C_8H_{17}SH$ and $C_{10}H_{21}SH$ self-assembled monolayers. Alkanethiols composed of long molecules are generally known to form more ordered self-assembled monolayers than the short ones, due to a higher number of $-CH_2-$ groups that show hydrophobic intermolecular interactions with the neighbouring molecules, supporting the ordered state of the monolayer. Therefore, the $FcOPE_2SH$ molecules can easier displace molecules in the $C_8H_{17}SH$ matrix rather than those in the $C_{10}H_{21}SH$ matrix, leading to a different $FcOPE_2SH$ coverage under otherwise the same conditions (compare Fig. S18C and D). In the $C_8H_{17}SH$ matrix, desired $FcOPE_2SH$ coverage was achieved upon decreasing the exposure time to 30 min (Fig. S18C). The same conditions ($c(FcOPE_2SAc) = 0.2$ mM, $t = 30$ min) led to a higher coverage in the $C_6H_{13}SH$ monolayer (roughly 60 %, Fig. S18B), which was most probably related to an even higher disorder within the latter compared to the $C_8H_{17}SH$ adlayer. In the case of $C_6H_{13}SH$, we decided to decrease the $c(FcOPE_2SAc)$ while keeping a constant assembly time 30 min. The desired $FcOPE_2SH$ coverage was achieved upon decreasing the $c(FcOPE_2SAc)$ value to 2 μM (Fig. S18B). Optimal assembly conditions for adlayers with diluted $FcOPE_2SH$ molecules were thus found for all three alkanethiol monolayers, allowing us to perform subsequent EC-AFM-FS measurements with well separated Fc moieties. The molecular adlayers for the latter measurements were prepared on gold bead-on-a-sheet substrates (Fig. S17C) with exposed

(111) facets (Fig. S17D). Thus modified Au(111) electrodes were used in the EC-AFM-FS measurements.

7. Assembly of mixed β CD(SH)₇/C₅H₁₁SH adlayers

Preparation of β CD(SH)₇/C₅H₁₁SH adlayers. As mentioned in the main text, a cavity of β CD was chosen as the host component of the molecular switch under study. A heptathioloated derivative of β CD, β CD(SH)₇ (compound **8**), was synthesized to assure its covalent bonding to a gold electrode (either a hanging-meniscus type Au(111) electrode for the EC characterization or a gold-coated AFM cantilever for FS measurements in the EC-AFM configuration). Compound **8** readily forms chemical bonds with gold surface, forming a molecular adlayer (Fig. S19B), which was employed previously as a sensor to detect small non-polar organic molecules such as ferrocene by Domi et al⁶. We applied a similar assembly strategy (Fig. S19A-E). Additionally, the entire procedure including preparation of all solutions was carried out in glovebox at the room temperature and in the argon atmosphere in order to prevent the oxidation of the –SH groups of **8**.

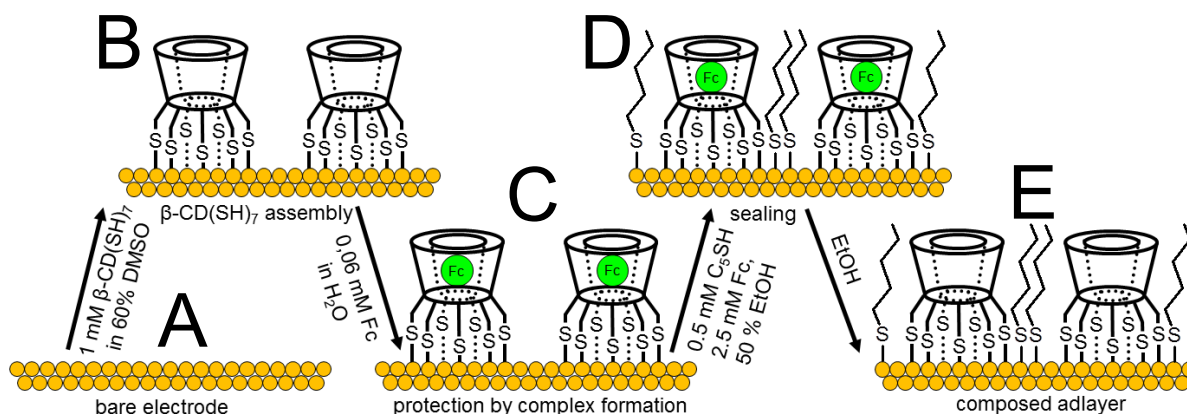


Figure S19. A sequential strategy to form a mixed β CD(SH)₇/C₅H₁₁SH adlayer.

A freshly cleaned hanging-meniscus type Au(111) (Fig. S19A) was immersed into a 1.0 mM solution of β CD(SH)₇ in 60% aqueous DMSO (v/v) for 48 hours, which led to the formation of the molecular adlayer (Fig. S19B). The electrode was then taken out from the

assembly solution and rinsed with 60% aqueous DMSO (v/v). To cover the electrode area that was left free after the assembly of $\beta\text{CD}(\text{SH})_7$ molecules, we further “sealed” the electrode by $\text{C}_5\text{H}_{11}\text{SH}$ molecules. This allowed us to exclude non-specific interactions of ferrocene (Fc) moiety with the bare electrode surface in FS experiments. In order to prevent the βCD cavities to be also filled by the $\text{C}_5\text{H}_{11}\text{SH}$ molecules, the $\beta\text{CD}(\text{SH})_7$ modified electrode was first immersed into the 0.06 mM (saturated) aqueous solution of Fc for 1 hour. This procedure created surface-bound Fc- βCD complexes, thus protecting the βCD cavities (Fig. S19C). After the protection step, the electrode was taken out from the Fc solution and quickly immersed into 50% (v/v) aqueous ethanol solution containing 0.5 mM $\text{C}_5\text{H}_{11}\text{SH}$ and 2.5 mM Fc for 16 hours. Molecules of $\text{C}_5\text{H}_{11}\text{SH}$ sealed bare electrode areas while the Fc kept the βCD cavities blocked. Thus we created a mixed $\beta\text{CD}(\text{SH})_7/\text{C}_5\text{H}_{11}\text{SH}$ adlayer, in which the βCD cavities pointed towards the electrolyte and the rest of the electrode surface was sealed by inactive $\text{C}_5\text{H}_{11}\text{SH}$ molecules. The electrode modified by the Fc- $\beta\text{CD}(\text{SH})_7/\text{C}_5\text{H}_{11}\text{SH}$ adlayer (Fig. S19D) was then extensively rinsed by absolute ethanol to remove any excess of physically adsorbed $\text{C}_5\text{H}_{11}\text{SH}$ molecules from the surface and the Fc molecules from the βCD cavities (Fig. S19E), and dried in an argon flow. The electrode was further stored in an argon-filled clean glass test-tube.

Characterization of $\beta\text{CD}(\text{SH})_7/\text{C}_5\text{H}_{11}\text{SH}$ adlayers. The $\beta\text{CD}(\text{SH})_7/\text{C}_5\text{H}_{11}\text{SH}$ adlayer on Au(111) electrode was subsequently inspected by cyclic voltammetry in 0.1 M NaClO_4 + 0.5 mM HClO_4 . As expected, βCD was not found to be electroactive within the accessible potential window (Fig. S20B, grey dashed line). This prevented us to determine the βCD surface concentration by means of the direct current integration. However, a non-covalent host-guest interaction of βCD and Fc allowed us to determine the βCD surface concentration indirectly, upon adding Fc into the electrolyte bulk (Fig. S20A). A clear electroactive response related to the oxidation and reduction of Fc could be observed upon its addition to

the electrolyte bulk, as shown in Fig. S20B (solid lines). The magnitude of the Fc response was found to grow upon further increasing its bulk concentration.

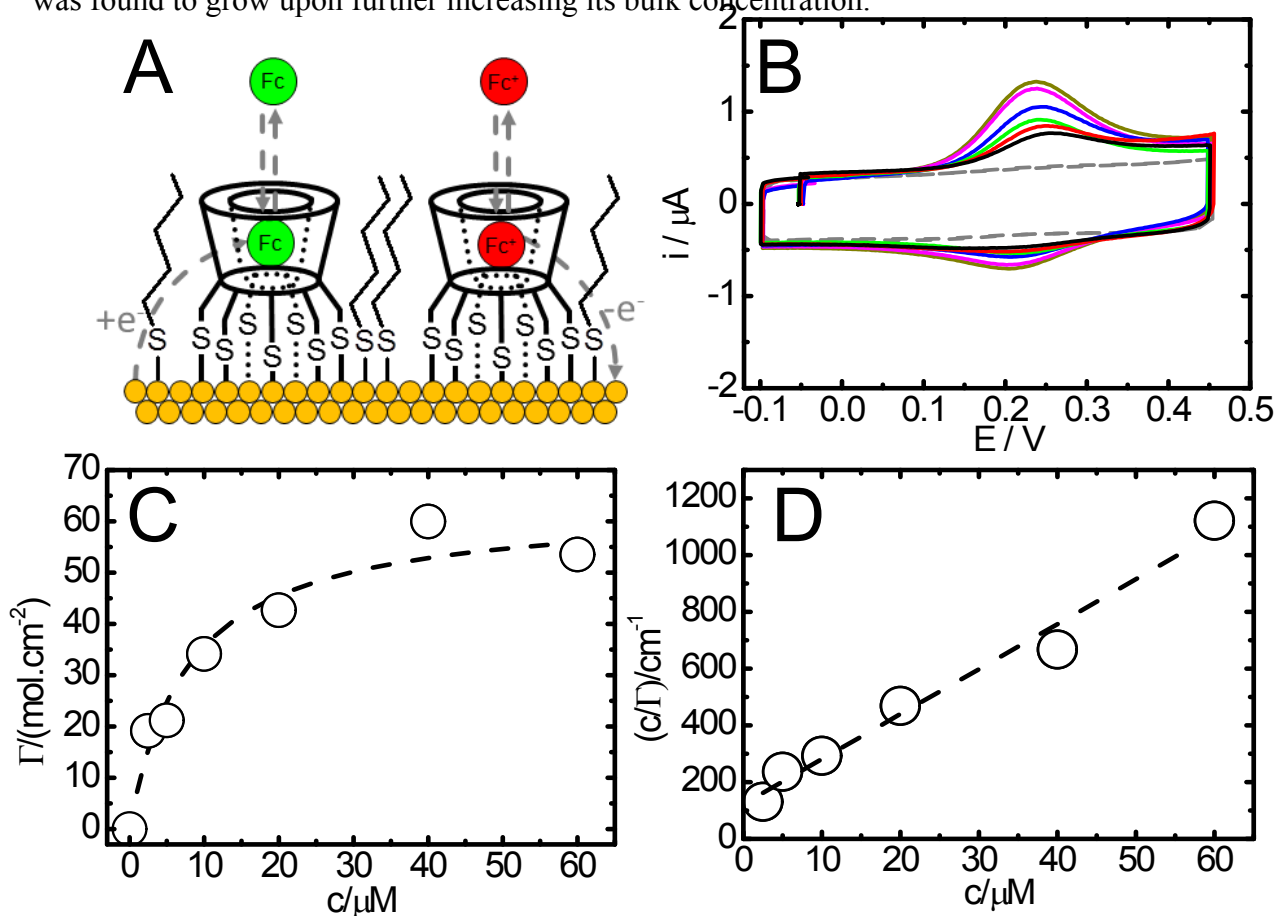


Figure S20. (A) Scheme of the electrode/electrolyte interface. (B) Cyclic voltammetric response of β CD(SH)₇/C₅H₁₁SH adlayer on Au(111) surface in 0.0 (grey dashed line), 2.5 (black), 5.0 (red), 10 (green), 20 (blue), 40 (dark yellow) and 60 μM (magenta) aqueous solution of Fc in 0.1 M NaClO₄ + 0.5 mM HClO₄, scan rate 0.5 V/s. (C) Γ_{FcCD} as a function of c_{Fc} . (D) Regression plot taken to extract the values of Γ_{CD} and K .

Cyclic voltammetry at different scan rates was used to determine whether the observed CV response originates from the surface-bound Fc molecules or those from the solution bulk. Since the magnitude of the faradaic current was linearly proportional to the scan rate value (data not shown), we demonstrated that the electron transfer takes place to/from the surface-confined species. In order to further prove that the observed Fc response originated from the molecules bound to the β CD cavities and was not related to (non-specifically) adsorbed Fc molecules on the C₅H₁₁SH part of the β CD(SH)₇/C₅H₁₁SH adlayer, the following control experiment was performed. Upon the electrochemical characterization

of $\beta\text{CD}(\text{SH})_7/\text{C}_5\text{H}_{11}\text{SH}$ adlayer in the aqueous electrolyte containing 60 μM Fc, the adlayer was removed by means of flame annealing and immersion into caroic acid. The cleaned electrode was further modified solely by $\text{C}_5\text{H}_{11}\text{SH}$ molecules and investigated in the same aqueous electrolyte containing 60 μM Fc. The obtained CV showed no oxidation-reduction features. This proves that the observed faradaic response found in the Fc- $\beta\text{CD}(\text{SH})_7/\text{C}_5\text{H}_{11}\text{SH}$ adlayer was indeed related to the presence of Fc bound in the βCD cavities.

We would like to further emphasize that the electroactive response originating from the presence of ferrocene bound to βCD cavities was observed in both directions of the electrode polarization, confirming that both Fc and Fc^+ are bound by the βCD cavities. However, it should be mentioned that the magnitude of the cathodic response was found to be lower than the anodic one, pointing to the fact that Fc^+ ion within the βCD cavity is less stable than its neutral counterpart and tends to partially dissociate from the cavity at electrode potentials more positive to the Fc/ Fc^+ redox potential (+0.22 V, Fig. S22B). Importantly, both Fc and Fc^+ were found in their “surface-bound” form, being decisive for the subsequent EC-AFM-FS measurements.

Fig. S20C shows the dependence of the surface concentration of neutral Fc bound within the βCD cavities (Γ_{FcCD}) on the bulk Fc concentration. Γ_{FcCD} was obtained by the integration of the anodic faradaic response of the CVs shown in Fig. S20B. In order to determine the surface concentration of βCD (Γ_{CD}) as well as the complexation constant of the Fc- βCD pair (K) an analysis of the CV data was performed. The constant K is defined as

$$K = \frac{\Gamma_{\text{FcCD}}}{\Gamma_{\text{CD, free}} c_{\text{Fc}}}$$

where Γ_{FcCD} , $\Gamma_{\text{CD, free}}$ and c_{Fc} denote the surface concentration of Fc- βCD complex, empty βCD cavities and ferrocene bulk concentration. Assuming that $\Gamma_{\text{FcCD}} + \Gamma_{\text{CD, free}} = \Gamma_{\text{CD}}$ and

eliminating the variable $\Gamma_{CD,free}$ one obtains following expression upon mathematical rearrangement

$$\Gamma_{FcCD} = \Gamma_{CD} \frac{Kc_{Fc}}{1 + Kc_{Fc}}$$

This relation is analogous to the Langmuir-type of isotherm, which yields after further rearrangement values of Γ_{CD} and K

$$\frac{c_{Fc}}{\Gamma_{FcCD}} = \frac{c_{Fc}}{\Gamma_{CD}} + \frac{1}{K\Gamma_{CD}}$$

Plotting c_{Fc}/Γ_{FcCD} against c_{Fc} allows one to obtain the values of Γ_{CD} and K from the slope ($1/\Gamma_{CD}$) and the intercept ($1/K\Gamma_{CD}$) (Fig. S20D). Thus obtained values amount to $\Gamma_{CD} = 62.9 \text{ pmol.cm}^{-2}$ and $K = 1.29 \times 10^5 \text{ M}^{-1}$ (the dashed lines in Figs. S20C and D represent fits employing these two values). While the value of Γ_{CD} is very close to the one reported in the literature⁶ ($68.0 \text{ pmol.cm}^{-2}$), the obtained value of K is slightly higher than that reported⁶ ($0.76 \times 10^5 \text{ M}^{-1}$). A slight discrepancy is probably related either to a difference in the environment (pH value was not reported in the literature, whereas $\text{pH} = 3.30$ in this manuscript) or to a different substrate (Au(111) against Au(poly) in the literature).

In summary, the surface-immobilized β CD cavities bind Fc molecules with a very high affinity, as apparent from a high magnitude of the complexation constant. Therefore, the same assembly protocol was also applied to modify gold-coated cantilevers for the EC-AFM based FS experiments. The cantilevers were first cleaned by a repeated immersion into ethanol, dried in a stream of argon and cleaned in a UV-ozone chamber for 1 hour (Digital UV Ozone Lamp, PSD Series, NOVASCAN). Subsequently, they were modified by the very same procedure as described above, and stored inside a desiccator filled with argon.

8. AFM measurements

Preparation of the measurement setup. All EC-AFM measurements were carried out with the PicoSPM 5000 (Agilent) setup, equipped with a 10 μm multi-mode AFM scanner and a contact mode nose cone. PicoView 1.14.3 was employed as the controlling software. The parts of the AFM scanner, which were in contact with the electrolyte during the measurement (the nose-cone, gasket and a spring clamp), were copiously rinsed with Milli-Q water and dried in the argon stream. A $\beta\text{CD}(\text{SH})_7/\text{C}_5\text{H}_{11}\text{SH}$ modified cantilever was then placed into the nose-cone. The AFM substrate was prepared as follows: a gold bead-on-a-sheet substrate freshly modified by the $\text{FcOPE}_2\text{SH}/\text{C}_n\text{H}_{2n+1}\text{SH}$ ($n = 6, 8$ or 10) adlayer with the FcOPE_2SH coverage ranging between 5 and 20% was placed on the sample plate. A freshly cleaned and dried O-ring (Kalrez®, DuPont) as well as a home-made PTFE AFM cell were immediately mounted on the gold substrate. Freshly annealed gold and platinum wire was then placed into the AFM cell and served as counter and reference electrode, respectively. The sample plate with the entire assembly was then mounted to the microscope stage and positioned into an environmental chamber filled by argon. The electrolyte (aqueous $0.1\text{ M NaClO}_4 + 0.5\text{ mM HClO}_4$, $\text{pH} = 3.30$) was then injected to the AFM cell under electrochemical control ($E = -0.30\text{ V}$ vs. Pt-wire reference electrode). The scanner with the cantilever were placed to the microscope and the cantilever was positioned above the (111) facet of the gold bead with the aid of an optical camera. The environmental chamber was then purged with water-saturated argon for 1 hour, thus providing inert atmosphere and at the same time suppressing the electrolyte evaporation. The cantilever was then coarsely approached towards the facet, at the rate of 100 nm/s .

Force-distance spectroscopy measurements. The force-distance spectroscopy measurements (approach and retraction cycles) were carried out at the rate of 50 nm/s . To

avoid damaging of the molecular adlayer, the approach of the AFM cantilever was always stopped and reversed upon detecting the contact with the substrate, in particular when a repulsion force of 500 pN was detected. For each value of the electrode potential inspected, 500 measurement cycles were performed. Typically, 200 to 400 of them showed at least one plateau related to the Fc- β CD complex rupture. A force difference of the last plateau was evaluated and data were presented as force histograms.

Characteristic force histograms. Representative force histograms obtained under various conditions are shown in panels A to F of Fig. S21. Importantly, all force histograms obtained in this work showed at least one very well defined probability maximum, from which a value of the most probable rupture force was extracted. As practically no data density was observed below this feature, this value was also assigned to the single-complex (fundamental) rupture force. In some of histograms (such as those shown in panels A and B), this feature was the only one noticed. On the other hand, other histograms (examples shown in panels C and D) also exhibited additional features, appearing as low (sometimes very tiny) satellite peaks, with the probability maxima being not far from twofold value of the fundamental rupture force value, extracted from the first (the left) feature. The presence of the secondary features confirms that the first maximum is indeed related to the rupture of a single Fc- β CD complex. Interestingly, still other (typically broad) features were additionally found in some of the histograms (examples shown in panels E and F), with the probability maxima being greater than the twofold value of the fundamental rupture force value. Nonetheless, the position of these features was found to be relatively undefined and they were not considered in the further data analysis. Only the probability maxima extracted from the first (leftmost) feature was considered and discussed further in the manuscript as well as in the Supplementary information.

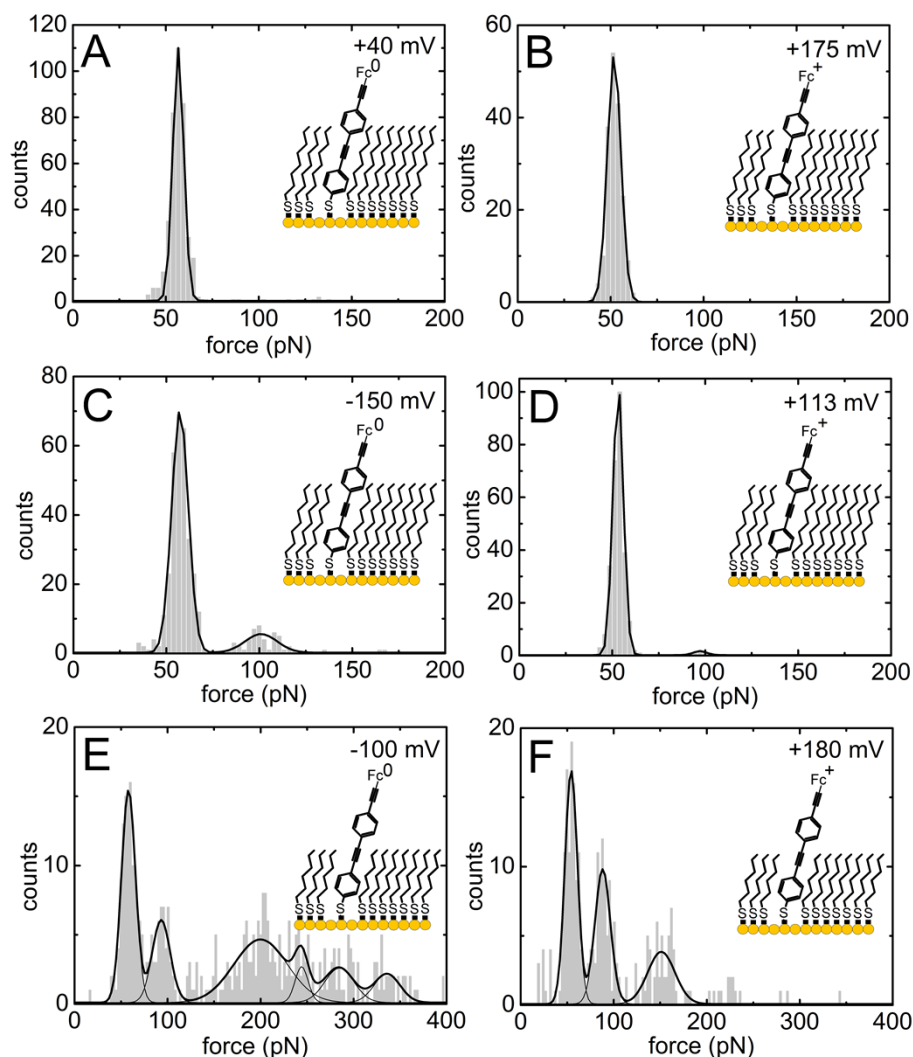


Figure S21. Typical force histograms showing one (A, B), two (C, D) and at least three (E, F) maxima. Histograms were constructed from datasets obtained at potentials pertaining to the neutral (A, C, E) and oxidized (B, D, F) ferrocene in the $C_8H_{17}SH$ (A – D) and $C_6H_{13}SH$ (E, F) matrix.

Calibration of cantilevers. Gold-coated n^+ -silicon soft cantilevers (type PPP-CONTAu-10, final radius ca 70 nm, resistivity 0.01 – 0.02 $\Omega\cdot\text{cm}$) with the nominal spring constant of 0.2 N/m and resonant frequency of 15 kHz were purchased from Nanosensors. The actual values of the spring constant and of the resonant frequency were determined by the thermal noise measurement technique combined with determining of the deflection sensitivity. Typically, three measurements were carried out and the obtained data were averaged. The found values of the force constant and resonance frequency were found to be in a very good agreement with the specification provided by Nanosensors.

9. Comparison of single-complex rupture forces in $C_6H_{13}SH$, $C_8H_{17}SH$ and $C_{10}H_{21}SH$ matrices.

Figure S22 shows the average single-complex rupture forces of single Fc- β CD complexes in $C_{10}H_{21}SH$ (panel A, squares), $C_8H_{17}SH$ (panel B, circles) and $C_6H_{13}SH$ (panel B, asterisks) matrices as a function of the electrode potential. The single complex rupture values were obtained from positions of Gaussian fits to the first (leftmost) probability maxima in force histograms, with representative examples shown in Fig. S21. The complex rupture forces obtained in $C_6H_{13}SH$ and $C_8H_{17}SH$ matrices show a clear dependence on the electrode potential (Fig. S22B), whereas those obtained in the $C_{10}H_{21}SH$ matrix show no potential dependence and generally lower values (Fig. S22A). Furthermore, the rupture force values obtained in the $C_{10}H_{21}SH$ matrix appear to scatter to a higher extend than those in the shorter two matrices (compare the error bars). An increased data scattering in the $C_{10}H_{21}SH$ may be related to the presence of non-specific repulsion forces acting between the β CD cavity and the top of the alkanethiol layer.

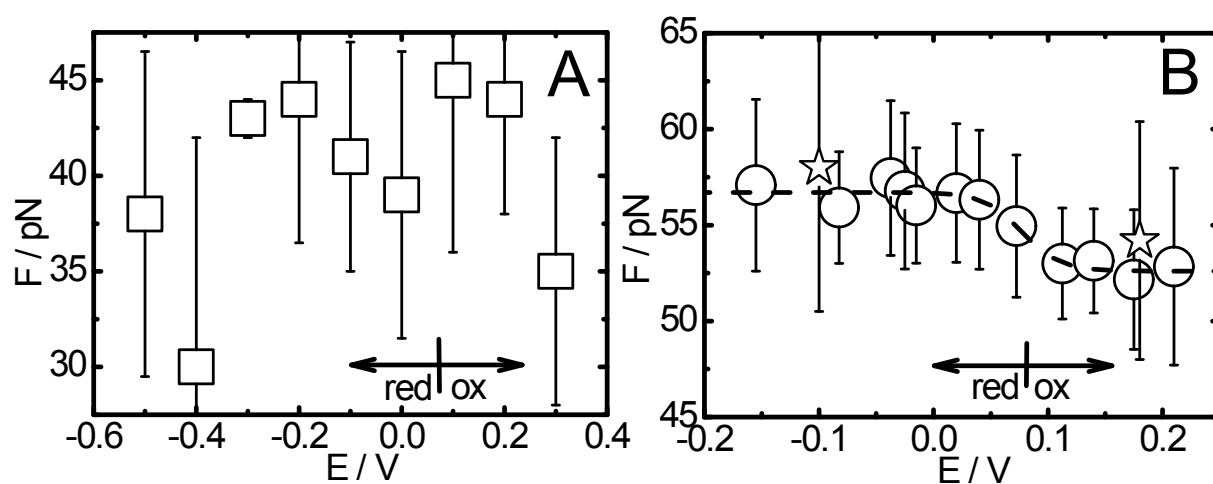


Figure S22. The rupture force of single Fc- β CD complex as a function of the electrode potential for the FcOPE₂SH in $C_{10}H_{21}SH$ (A, squares), $C_8H_{17}SH$ (B, circles) and $C_6H_{13}SH$ (B, asterisk) matrices.

10. DFT calculations

In order to elucidate the structure of the $C_nH_{2n+1}SH/FcOPE_2SH$ adlayers, we optimized the geometry and determined the length of $FcOPE_2SH$ and of the three alkanethiol molecules employing the B3LYP DFT functional within the SPARTAN '08 V1.2.0 package⁸. The distance between the sulfur atom and the top-most carbon atom of the alkanethiol molecules was measured. For the $FcOPE_2SH$ molecule, the carbon atom of the OPE backbone closest to the Fc group was taken as the upper boundary. Assuming a tilting angle of 30° between the molecular backbone and a normal direction to the electrode surface, we estimated the vertical projection of the molecular length i.e. the theoretical adlayer thickness. Fig. S23 shows obtained results. While the two shorter alkanethiol molecules form monolayers with a thickness that is considerably shorter (6.77 Å and 9.04 Å) than the vertical projection of the OPE_2 backbone (12.24 Å), the $C_{10}H_{21}SH$ molecule (11.27 Å) closely approaches the latter, imposing a steric hindrance for the Fc- β CD complex formation. This can rationalize the experimentally found differences in the rupture forces of Fc- β CD complex in the $C_{10}H_{21}SH$ (Fig. S22A) and $C_8H_{17}SH$ or $C_6H_{13}SH$ (Fig. S22B) adlayers.

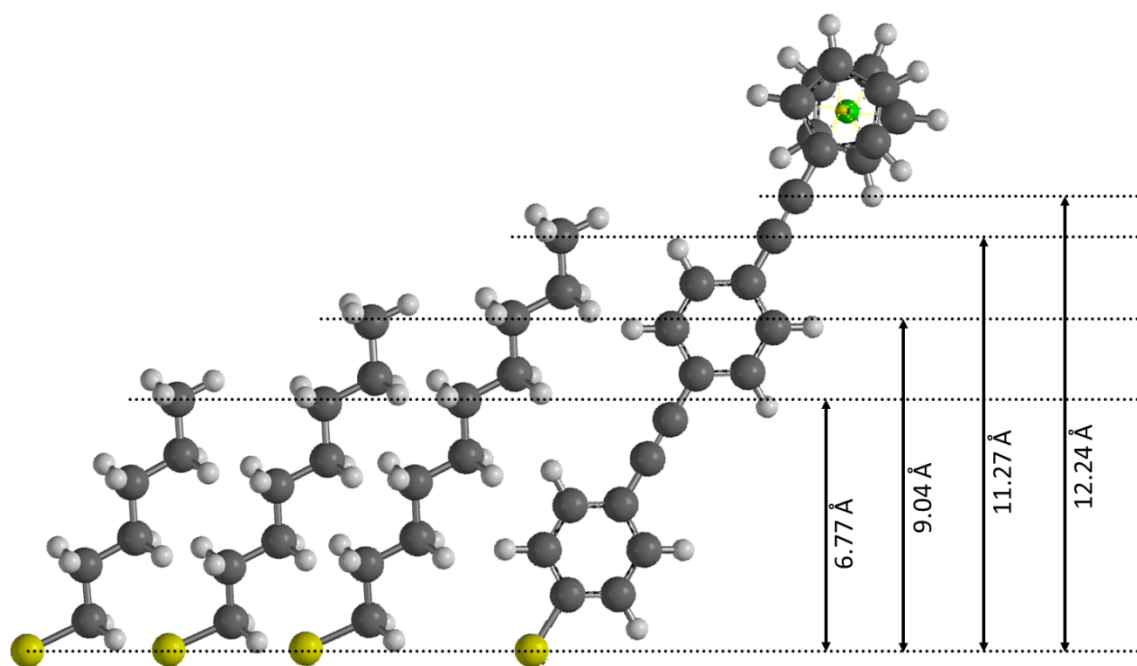


Figure S23. Optimized geometry of $C_6H_{13}SH$, $C_8H_{17}SH$, $C_{10}H_{21}SH$ and $FcOPE_2SH$ molecules in their ground state in a vacuum, as found by the DFT technique employing the B3LYP functional with the 6-31G* basis set. Singlet state and no charge within the molecules were assumed. The arrows denote the vertical component of the S-C(topmost) atom-atom distance, assuming 30 deg tilting angle.

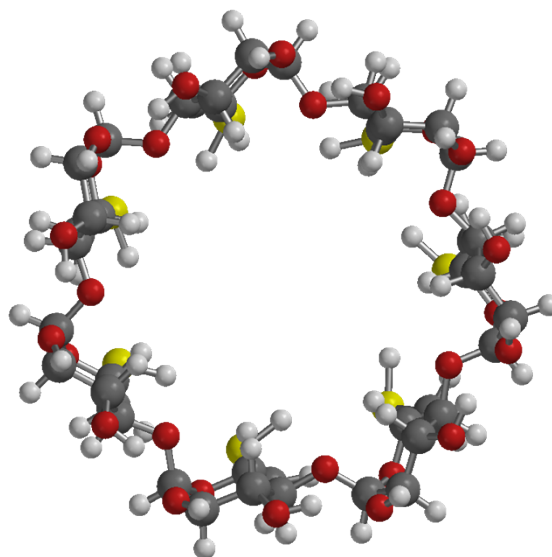


Figure S24. Optimized geometry of $\beta CD(SH)_7$ (upper view) in its ground state in a vacuum, as found by the DFT technique employing the B3LYP functional with the 6-31G* basis set. Singlet state and no charge within the molecule were assumed.

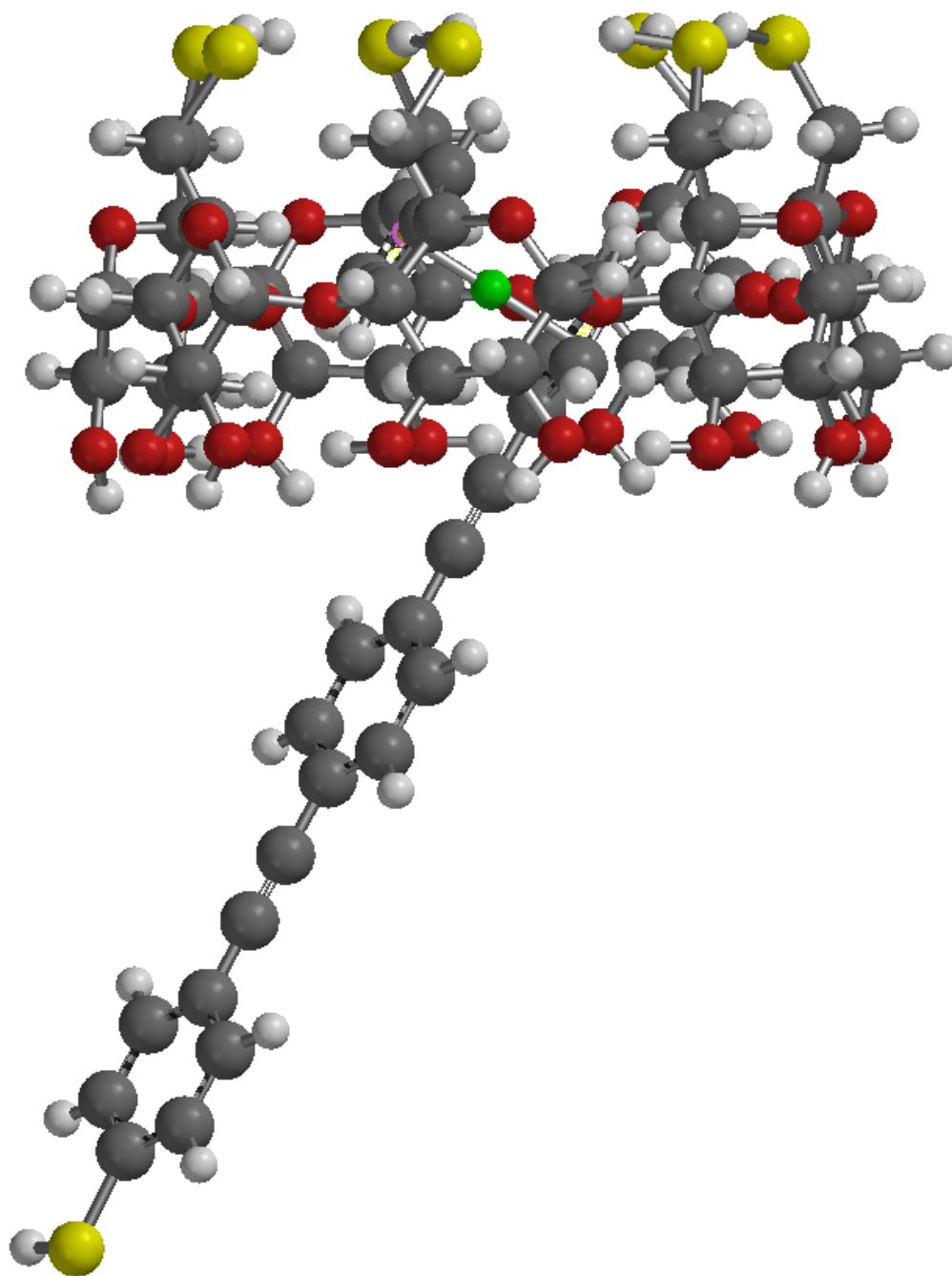


Figure S25. Optimized geometries of FcOPE₂SH (also shown in Fig. S23) and β CD(SH)₇ (also shown in Fig. S24), as obtained separately by B3LYP DFT. The geometry of the two molecules upon their assembly could not be optimized due to memory limitations of our computer.

Fig. S24 shows the optimized geometry of β CD(SH)₇ as obtained employing the B3LYP DFT functional. Importantly, the hydrophobic carbon atoms of the saccharide rings as well as the bridging etheric oxygen atoms are located rather in the interior of the β CD(SH)₇ molecule, forming thus a hydrophobic cavity allowing the binding of Fc.

Fig. S25 shows separately-obtained optimized geometries of FcOPE₂SH (Fig. S23) and β CD(SH)₇ (Fig. S24) brought together. Unfortunately, due to memory limitations of our computer, the geometry of the molecular assembly (FcOPE₂SH + β CD(SH)₇) could not be successfully optimized. We resorted ourselves to position the Fc moiety of the FcOPE₂SH molecule within the β CD cavity so that the iron atom (green sphere in Figs. S23 and S25) was in the center of the regular heptagon formed by the seven etheric oxygen atoms between the oligosaccharide rings. In this configuration, the fourteen OH- groups located at the larger rim of the β CD ring screen the carbon atom of the OPE backbone that is closest to the Fc group. This justifies the discussion on the relation between projected lengths of alkanethiol and FcOPE₂SH molecules (see Fig. S23 and the corresponding text).

11. References

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