Supporting Information

A supramolecular route for reversible protein-polymer conjugation

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1 Materials and methods

All starting materials were purchased from Alfa Aesar and Sigma Aldrich and used as received unless stated otherwise. Bovine serum albumin was purchased from Fisher Scientific. CB[8][*], DPPA[†], *p*-Maleimidobenzoyl azide[‡] and, MV-C3-SH[§] and Np-TEG-OH[¶] were prepared according to literature procedure. ^{1}H and ^{13}C NMR spectra were recorded on an Avance 500 BB-ATM (500 MHz) spectrometer, UV/visible spectra on a Varian Cary 4000 UV-Vis spectrophotometer and dynamic light scattering measurements on a Malvern Zetasizer Nano S.

1.1 Synthesis of Np-BSA1 Np-BSA2 and MV-BSA3

Synthesis of Np-TEG-Mal: A solution of triphenyl phosphine (0.94 g, 3 mmol) under nitrogen was cooled to -78 $^{\circ}$C. Diisopropyl azodicarboxylate (0.6 mL, 3 mmol) was added slowly and the solution was stirred for 10 min. Np-TEG-OH (0.91 g, 3.3 mmol) was added and the solution was stirred for another 5 min before maleimide (0.29 g, 3 mmol) was added. The reaction mixture was allowed to cool to room temperature and stirred for 2 days. The solution was concentrated in vacuo and the resulting oil was purified by chromatography on silica (1:2:2 diethyl ether:hexane:ethyl acetate). The title compound was obtained as a yellowish oil. (0.59 g, 1.5 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.70-7.64 (m, 3H), 7.35 (t, 1H), 7.26 (t, 1H), 7.11-7.07 (m, 2H), (m, 2H), 6.58 (s, 2H), 4.18 (t, 2H), 3.82 (t, 2H), 3.66-3.58 (m, 8H) ppm. UV/vis ($CDCl_3$): 243 nm (4.28), 264 nm (4.04), 273 nm (4.07), 284 nm (3.88), 314 nm (3.60), 328 nm (3.64). HR-MS: m/z calcd for $[M+H]^+$ 356.1498, found 356.1501; calcd for $[M+Na]^+$ 378.1317, found 378.1319.

Synthesis of **Np-BSA1**: To a suspension of 2-Np-TEG-Mal (4.1 mg, 0.012 mmol) in 100 mL of 100 mM phosphate buffer (pH = 7) was added bovine serum albumin (0.17 g, 0.0026 mmol) and the mixture was stirred at room temperature for 24 hours. The solution was filtered, concentrated to ca 20 mL by lyophilisation and transferred into a dialysis membrane (MW cutoff 10,000 Daltons) and dialysed against deionised water (Millipore, 18.2 MΩ cm) for 3 days. **Np-BSA1** was obtained as a white, fluffy solid after lyophilisation (0.17 g, >99%). The purity of **Np-BSA1** was confirmed by aqueous gel permeation chromatography (GPC).

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Synthesis of 2-Np-TEG-Benz-Mal: A solution of *p*-maleimidobenzoyl azide (0.29 g, 1.2 mmol) in 10 mL anhydrous toluene was refluxed for 2 h under nitrogen atmosphere. After cooling to room temperature 2-Np-TEG-OH (0.30 g, 1.1 mmol) was added and the solution was stirred over night. The solution was concentrated in vacuo and the resulting oil was purified by chromatography on silica (3:1 diethyl ether:ethyl acetate). The title compound was obtained as a yellowish, waxy solid (0.5 g, 1.0 mmol, 83%). ^{1}H NMR (400 MHz, d^{6}-DMSO): 9.93 (s, 1H), 7.80 (q, 3H), 7.55 (d, 2H), 7.44 (t, 1H), 7.35-7.31 (m, 2H), 7.22-7.20 (m, 2H), 7.17-7.14 (m, 3H), 4.21 (t, 2H), 3.82 (t, 2H), 3.68-3.58 (m, 8H) ppm; ^{13}C NMR (100 MHz, d^{6}-DMSO) δ = 170.6, 156.8, 153.9, 139.2, 135.1, 134.7, 129.8, 128.9, 128.0, 127.9, 127.1, 126.8, 126.1, 124.0, 119.2 118.8, 107.1, 70.4, 70.2, 69.4, 69.1, 67.6, 64.1 ppm. UV/vis ($CDCl_3$): 246 nm (4.36), 273 nm (3.74), 314 nm (3.17), 328 (3.27). HR-MS: m/z calcd for $[M+H]^+$ 491.1818, found 491.1823; calcd for $[M+Na]^+$ 513.1638, found 513.1641.

Synthesis of **Np-BSA2**: Following the procedure described above, **Np-BSA2** was synthesized from MV-C2-Benz-Mal (6.1 mg, 0.012mmol) and bovine serum albumin (0.17 g, 0.0026 mmol) and obtained as a white, fluffy solid (0.17 g, 0.0026 mmol, > 99%).The purity of **Np-BSA2** was confirmed by aqueous gel permeation chromatography (GPC).

Synthesis of MV-C2-Benz-Mal: A solution of *p*-Maleimidobenzoyl azide (0.10 g, 0.41 mmol) in 10 mL anhydrous DMF was heated to 145 $^{\circ}$C for 2 h under nitrogen atmosphere in a 50 mL two-neck RBF, equipped with a reflux condenser. After cooling to room temperature MV-C_2H_4OH $2PF_6$ (0.16 g, 0.32 mmol) was added and the solution was stirred over night. The solution was concentrated to a few millilitres in vacuo and diethylether was added. The precipitate was collected by suction filtration, redissolved in 10 mL of acetone and filtered. A solution of tetrabutylammonium chloride (0.33 g, 1.2 mmol) in 5 mL of acetone was added and the solution was allowed to stand overnight in the fridge. The resulting precipitate was collected by suction filtration, washed with cold acetone, to yield the title compound (0.10 g, 60%) as a yellowish solid. ^{1}H NMR (400 MHz, d^{6}-DMSO): δ = 9.89 (s, 1H), 9.41 (d, 2H), 9.28 (d, 2H), 8.83 (d, 2H), 8.76 (d, 2H), 7.46 (d, 2H), 7.22 (d, 2H), 7.16 (s, 2H), 5.05 (t, 2H), 4.71 (t, 2H), 4.15 (m, 1H), 4.41 (s, 3H) ppm; ^{13}C NMR (100 MHz, d^{6}-DMSO) δ = 170.4, 153.1, 149.5, 148.33, 147.0, 146.8, 138.44, 135.0, 133.3, 127.9, 126.7, 126.5, 118.9, 105.7, 63.2, 60.5, 48.4 ppm. UV/vis (H_2O): 248 nm (4.05). HR-MS: m/z calcd for $[M]^{2+}$ 215.0821, found 215.0821.

Synthesis of **MV-BSA3**: Following the procedure described above, **MV-BSA3** was synthesized from MV-C2-Benz-Mal (5.7 mg, 0.012mmol) and bovine serum albumin (0.17 g, 0.0026 mmol) and obtained as a white, fluffy solid (0.17 g, 0.0026 mmol, >99%). The purity of **MV-BSA3** was confirmed by aqueous gel permeation chromatography (GPC).

Synthesis of Mal-Benz-5k-PEG-OMe: A solution of *p*-Maleimidobenzoyl azide (1.2 g, 5.0 mmol) in 30 mL anhydrous *o*-dichlorobenzene was heated to 145 $^{\circ}$C for 2 h under nitrogen atmosphere in a 100 mL two-neck RBF, equipped with a reflux condenser. After the mixture was cooled down to room temperature, polyethylene glycol 5000 monomethyl ether (3.0 g, 0.6 mmol) dissolved in 10 mL anhydrous DCM was added at once and the mixture was stirred for 48 hours at room temperature. The product was obtained by precipitation from cold diethylether.The yellowish solid was redissolved in ca 30 mL DCM, filtered and reprecipitated in cold diethylether (2x). Suction filtration yielded the title compound (2.7 g, 0.5 mmol, 83%) as a yellowish solid. ^{1}H NMR (500 MHz, D_2O): δ = 7.51 (d, 2H), 7.28 (d, 2H), 6.97 (s, 2H), 4.30 (t, 2H), 3.67 (m, PEG backbone), 3.34 (s, 3H) ppm.

Synthesis of **MV-PEG4**: To a solution of Mal-Benz-5k-PEG-OMe (0.52 g, 0.1 mmol) in 15 mL H_2O was added MV-C3-SH (0.16 g, 0.30 mmol) and the solution was stirred for 24 h at room temperature. The solution was filtered and transferred into a dialysis membrane (MW cutoff 2,000 Daltons) and dialysed against a solution of sodium chloride in water (3g in 2L) to exchange for chloride counter

ions for 1 day and then against deionised water (Millipore, 18.2 MΩ cm) for 3 days. **MV-PEG4** was obtained as a off-white, fluffy solid after lyophilisation (0.4 g, 0.07 mmol, 70%). ^{1}H NMR (500 MHz, D_2O): δ = 9.10 (d, 2H), 9.00 (d, 2H), 8.48 (m, 4H), 7.49 (d, 2H), 7.18 (d, 2H), 4.88 (t, 2H), 4.45 (s, 3H), 4.15 (m, 1H), 3.67 (m, PEG backbone), 3.34 (s, 3H), 2.88 (2H, m), 2.48 (2H, m) ppm; ^{13}C NMR (100 MHz, D_2O) δ = 178.9, 177.5, 165.9, 155.2, 150.1, 146.2, 145.6, 138.8, 132.5, 127.6, 126.5, 126.0, 122.2, 121.9, 119.9, 82.7, 70.9 ppm and PEG backbone signals.

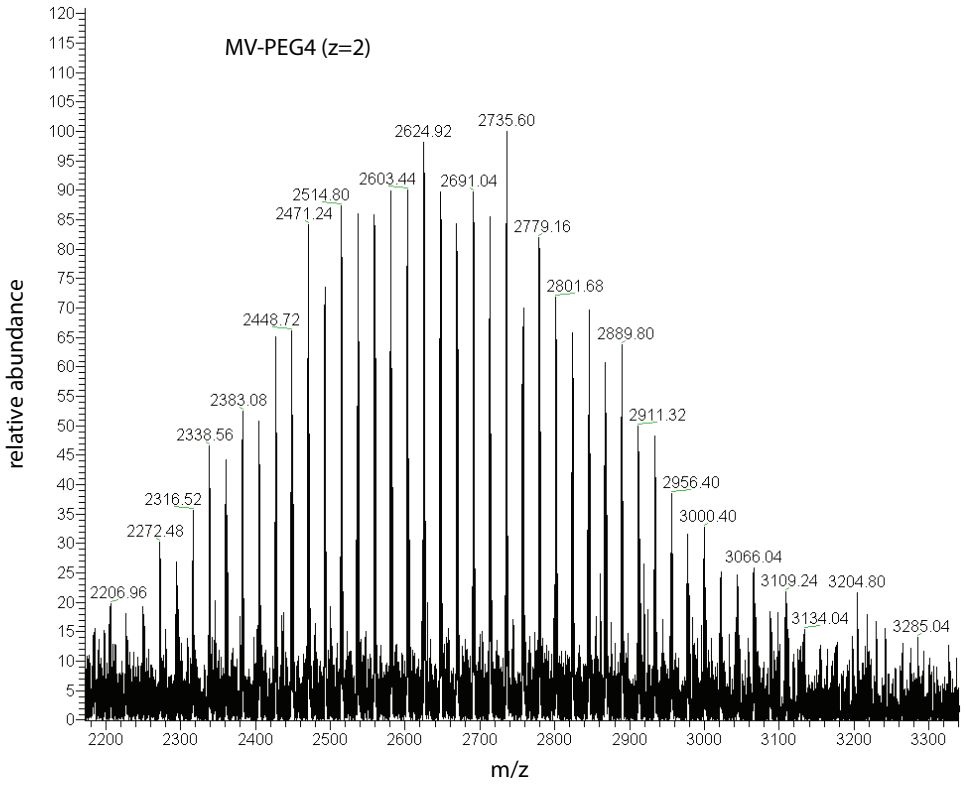

Figure S1: ESI-MS of MV-PEG4.

Synthesis of 2-Naphthoyl azide: 2-Naphthoyl azide was prepared in analogy to literature procedures. To a stirred suspension of 2-naphthoic acid (3.4 g, 20 mmol) in 150 mL dry toluene was added triethylamine (3.1 mL, 22 mmol) and DPPA (4.7 mL, 22 mmol). The solution was stirred at room temperature for 48 h. The solvent was evaporated in vacuum and the purple residue was purified by chromatography on silica with DCM to yield the title compound (2.8 g, 16 mmol, 81%) as a white, waxy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.50 (s, 1H), 7.92 (dd, 1H), 7.85 (d, 1H), 7.80-7.78 (m, 2H), 7.54-7.45 (m, 2H) ppm.

Synthesis of **Np-PEG5**: A solution of 2-naphthoyl azide (1.2 g, 6.0 mmol) in 30 mL anhydrous *o*-dichlorobenzene was heated to 145 $^{\circ}$C for 2 h under nitrogen atmosphere in a 100 mL two-neck RBF, equipped with a reflux condenser. After the mixture was cooled down to room temperature, polyethylene glycol 5000 monomethyl ether (3.0 g, 0.6 mmol) dissolved in 10 mL anhydrous DCM was added at once and the mixture was stirred for 48 hours at room temperature. The product was obtained by precipitation from cold diethylether. The yellowish solid was redissolved in ca 30 mL DCM, filtered and reprecipitated in cold diethylether (2x). Suction filtration yielded **Np-PEG5** (2.6 g, 0.5 mmol, 85%) as a white solid. ^{1}H NMR (500 MHz, D_2O): δ = 7.94 (s, 1H), 7.88 (m, 3H), 7.50 (m, 3H), 4.33

(t, 2H), 3.67 (m, PEG backbone), 3.34 (s, 3H) ppm; ^{13}C NMR (100 MHz, D_2O) δ = 155.4, 135.4, 133.6, 127.6, 127.3, 126.9, 126.5, 125.2, 124.2, 119.1, 76.1 ppm and PEG backbone signals. The purity of **Np-PEG5** was confirmed by THF-GPC.

Synthesis of **Ant-PEG6**: **Ant-PEG6** was synthesized in analogy to the procedure for **Np-PEG5** from the corresponding 2-anthracylisocyanate to yield **Ant-PEG6** (0.5 g, 0.1 mmol, 83%) as a yellowish solid. ^{1}H NMR (500 MHz, d^6-DMSO): δ = 10.03 (s, 1H), 8.45 (s, 1H), 8.38 (s, 1H), 8.18 (s, 1H), 8.02-7.99 (m, 3H), 7.54-7.44 (m, 3H) 4.26 (t, 2H), 3.67 (m, PEG backbone), 3.22 (s, 3H) ppm; ^{13}C NMR (100 MHz, d^6-DMSO) δ = 153.7, 136.3, 131.9, 131.8, 130.3, 128.9, 128.3, 128.1, 127.7, 125.9, 125.7, 124.9, 124.7, 120.6, 112.2, 76.5 ppm and PEG backbone signals. The purity of **Ant-PEG6** was confirmed by THF-GPC.

1.2 Determination of solution binding constants by ITC

Titration experiments were carried out on a ITC200 from Microcal Inc., at 25 °C in 10 mM sodium phosphate buffer (pH = 7), prepared from 1.560 g $NaH_2PO_4{\cdot}2\ H_2O$ and 1.110 g $Na_2HPO_4{\cdot}2\ H_2O$ in 1 L deionised water (Millipore, 18.2 MΩ·cm). The binding equilibria were studied using typically a cellular protein concentration of typically of 0.04 mM to which the 0.5-0.7 mM polymer solution was titrated. Typically 20-30 consecutive injections of 2.4 μL each were used. The first data point was removed from the data set prior to curve fitting. The data was analyzed with Origin 7.0 software, using the *one set of sites* model.

1.3 NMR

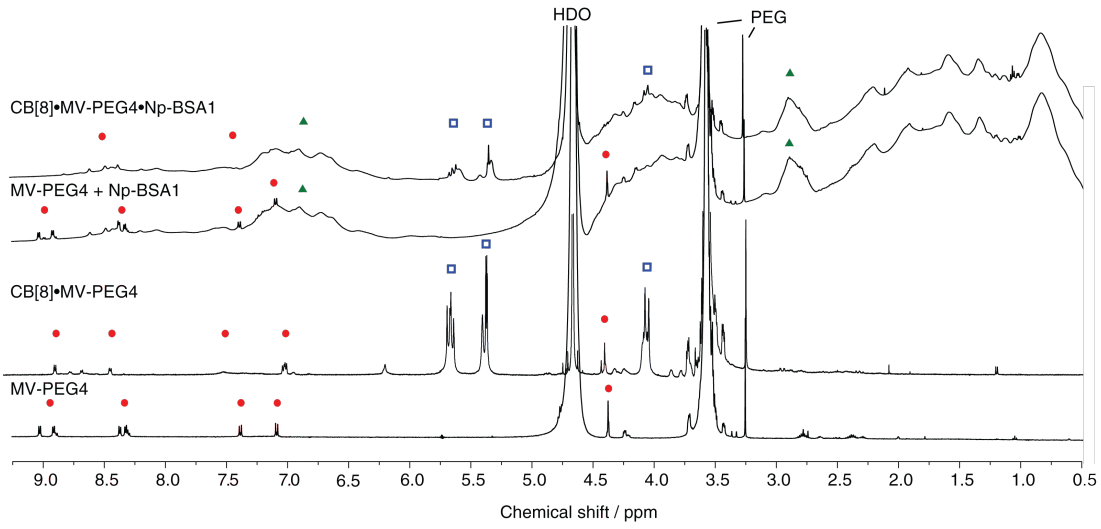

Figure S2: ^{1}H NMR analysis (500 MHz, D_2O) of the CB[8]·**MV-PEG4**·**Np-BSA1** system, indicating a supramolecular protein-polymer entity, as viologen (9.0-8.0 ppm) and N-methyl end-group (4.45 ppm) protons of **MV-PEG4** and the CB[8] protons are substantially broadened and shifted.

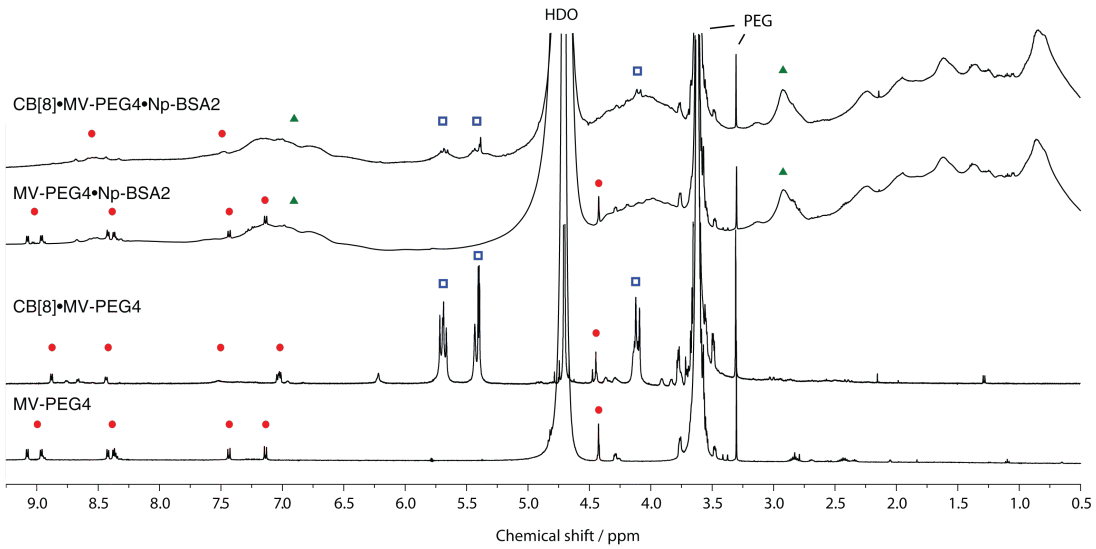

Figure S3: ^{1}H NMR analysis (500 MHz, D_2O) of the CB[8]·**MV-PEG4**·**Np-BSA2** system, indicating a supramolecular protein-polymer entity, as viologen (9.0-8.0 ppm) and N-methyl end-group (4.45 ppm) protons of **MV-PEG4** and the CB[8] protons are substantially broadened and shifted.

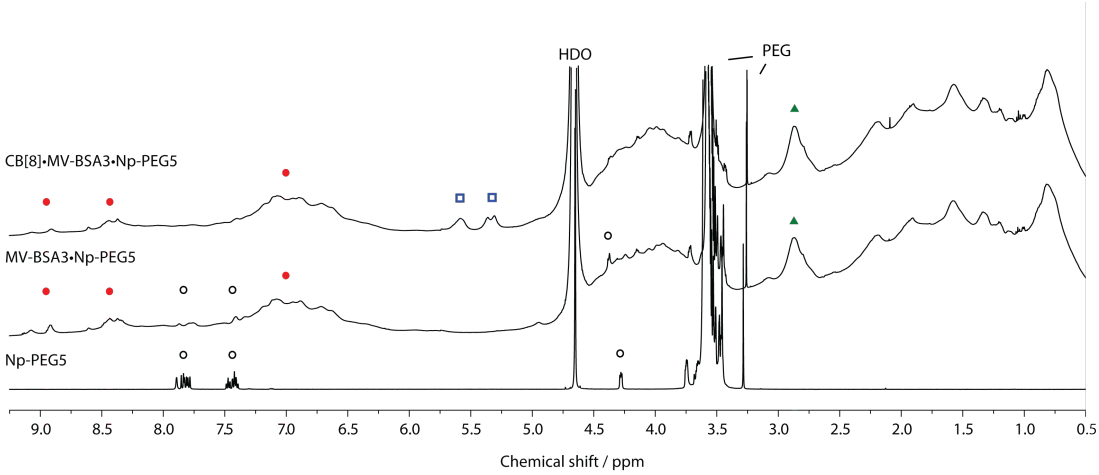

Figure S4: ^{1}H NMR analysis (500 MHz, D_2O) of the CB[8]·**MV-BSA**·**Np-PEG** system, indicating a supramolecular protein-polymer entity, as naphthalene (8.0-7.3 ppm) and methylene end-group (4.33 ppm) protons of **Np-PEG** and the CB[8] protons are substantially broadened and shifted. However, even in the absence of CB[8], an interaction between the polymer **Np-PEG** and protein **MV-BSA** is apparent.

1.4 DOSY-NMR

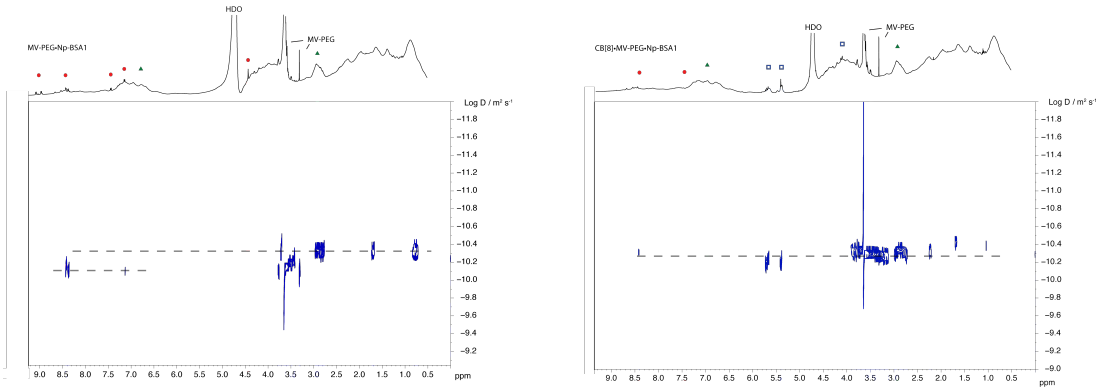

Figure S5: DOSY NMR spectra for equimolar mixtures of **MV-PEG4** and **Np-BSA1** (a), CB[8], **MV-PEG4** and native BSA (b) and CB[8], **MV-PEG4** and **Np-BSA1** (c) at 0.05 mM concentration in D_2O. The aromatic protons of **MV-PEG4** (red circle) and the CB[8] protons (blue square) are highlighted.

1.5 DLS

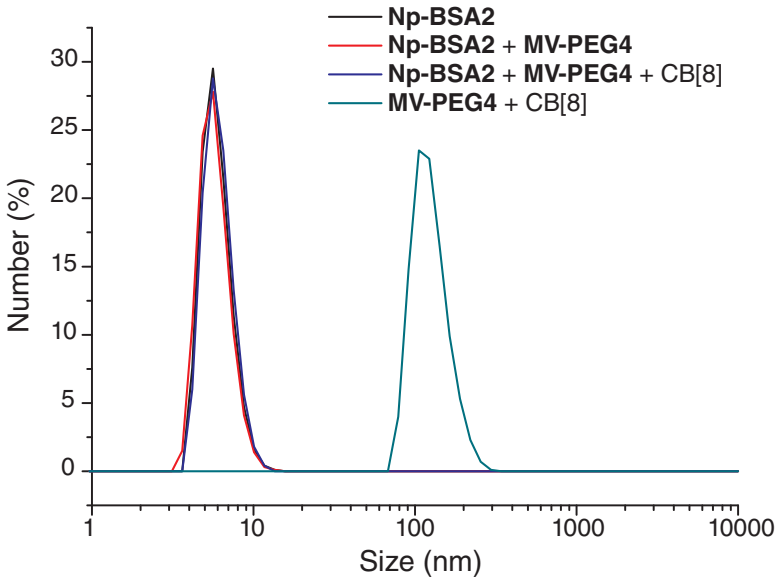

Figure S6: **MV-PEG4**·CB[8] aggregates into particles with average diameters (D_{avg}) = 105 nm in aqueous solution (0.05 mM at 25 °C) as measured by dynamic light scattering. Upon addition of **Np-BSA2**, the ternary complex forms and the measured D_{avg} significantly decreases to 6 nm, which is roughly the same as for **Np-BSA2** alone or the binary solution mixture of **Np-BSA2** and **MV-PEG4**.

1.6 UV/vis

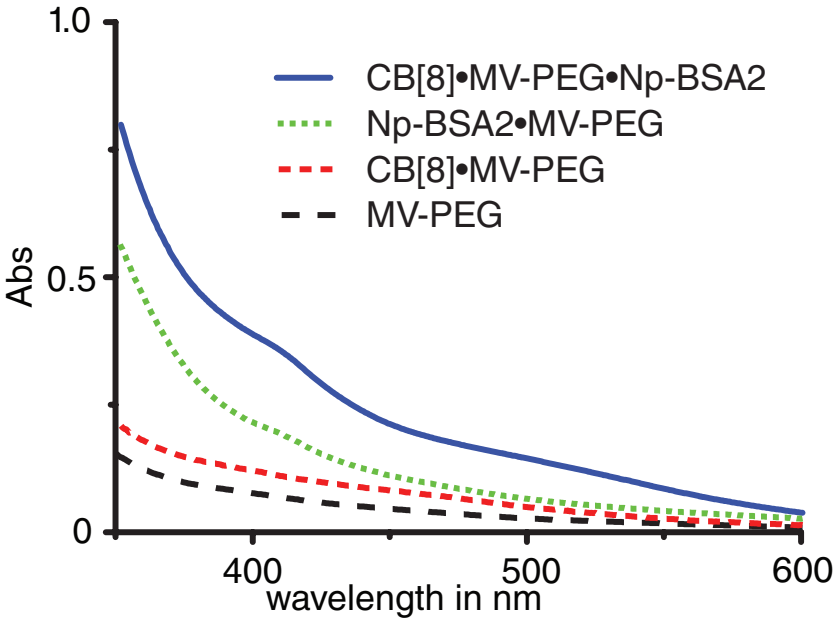

Figure S7: In the presence of 1 eq. CB[8] there is a charge transfer type interaction between the electron-deficient viologen functionalised **MV-PEG4** and the electron-rich naphthol-functionalised **Np-BSA2**, indicative of ternary complex formation. All components at 0.5 mM in aqueous solutions.

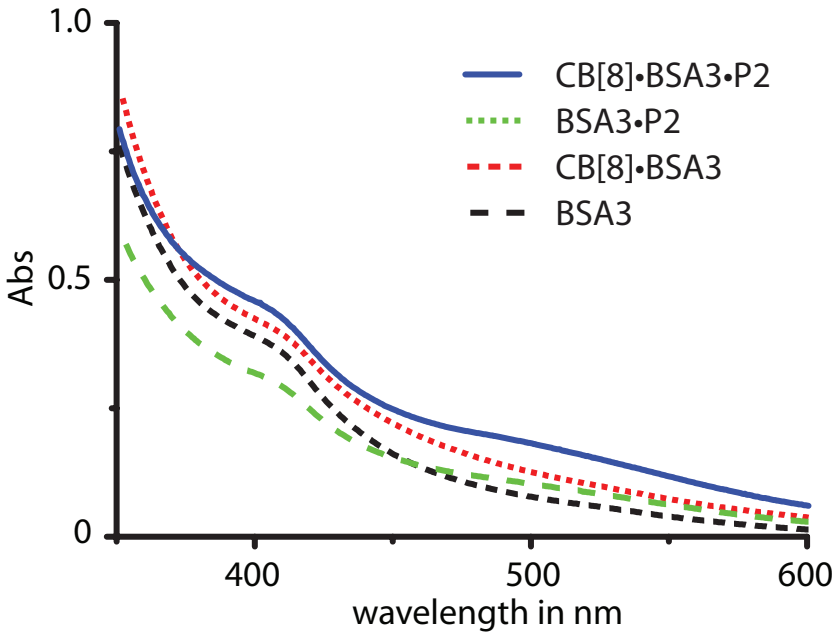

Figure S8: In the presence of 1 eq. CB[8] there is a charge transfer type interaction between the electron-deficient viologen functionalised **MV-BSA3** and the electron-rich naphthol-functionalized **Np-PEG5**, indicative of ternary complex formation. All components at 0.5 mM in aqueous solutions.

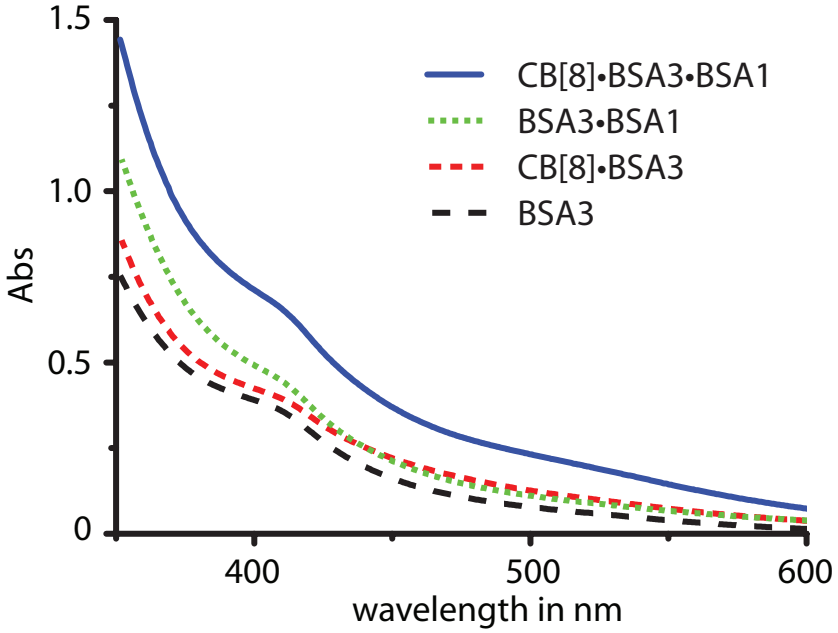

Figure S9: In the presence of 1 eq. CB[8] there is a charge transfer type interaction between the electron-deficient viologen functionalised **MV-BSA3** and the electron-rich naphthol-functionalized **Np-BSA2**, indicative of ternary complex formation. All experiments at 0.5 mM total concentration of protein in aqueous solutions.

1.7 Fluorescence

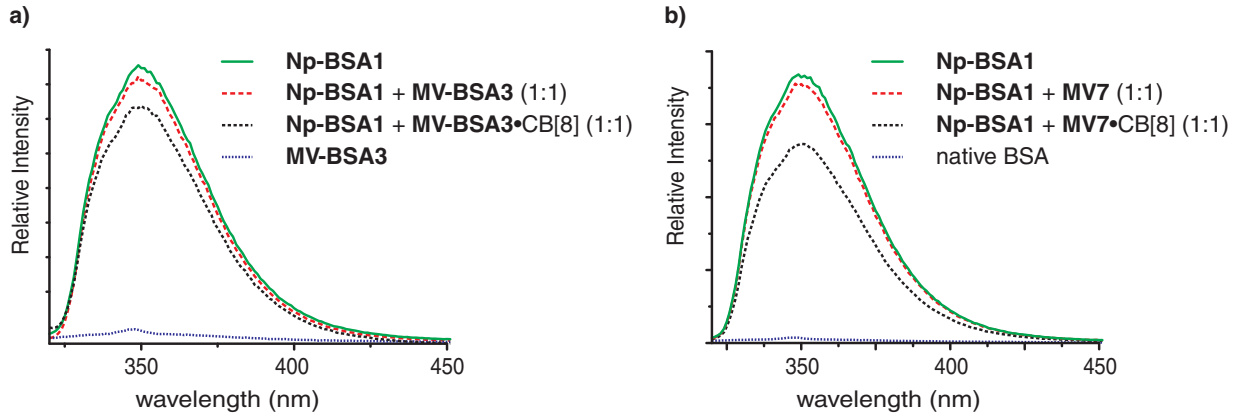

Figure S10: Fluorescence spectra of **Np-BSA1** with **MV-BSA3** (a) and **MV7** (b) and CB[8] at 2 μM concentration and 310 nm excitation.

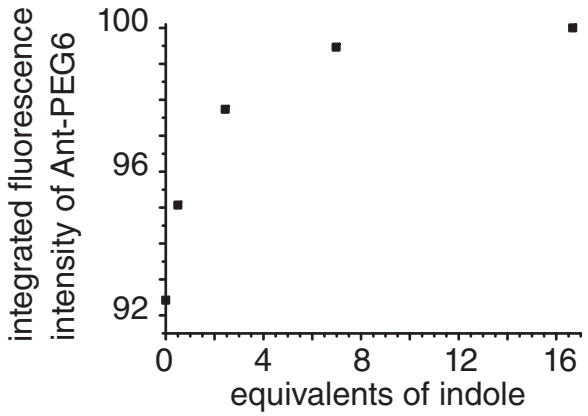

Figure S11: The partly quenched fluorescence intensity of **Ant-PEG6** in the presence of 1:1 equivalents **MV-BSA3** and CB[8] (at 2 μM concentration and 360 nm excitation) recovers when the competitive second guest indole is added.

1.8 ITC

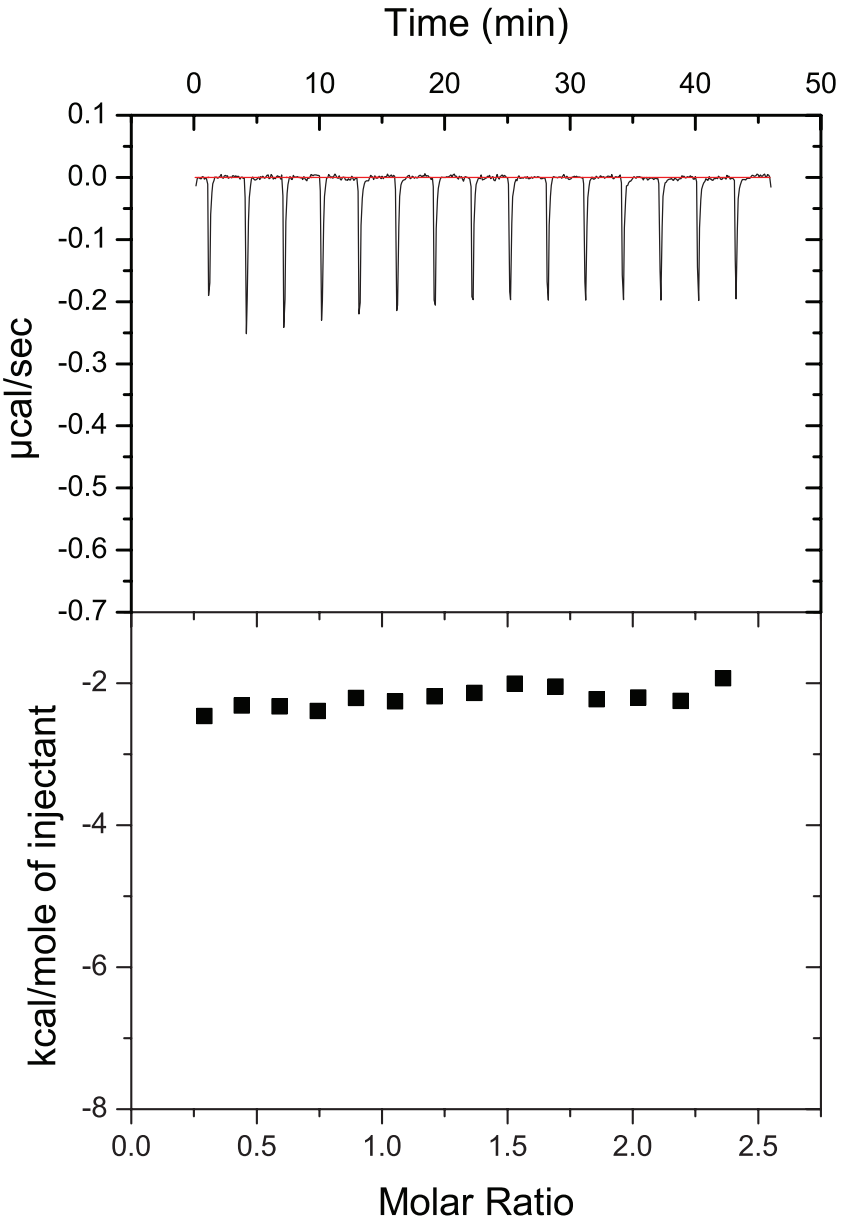

Figure S12: In the absence of the supramolecular host CB[8] there is only some rather weak exothermic interaction between Np-PEG and MV-BSA.

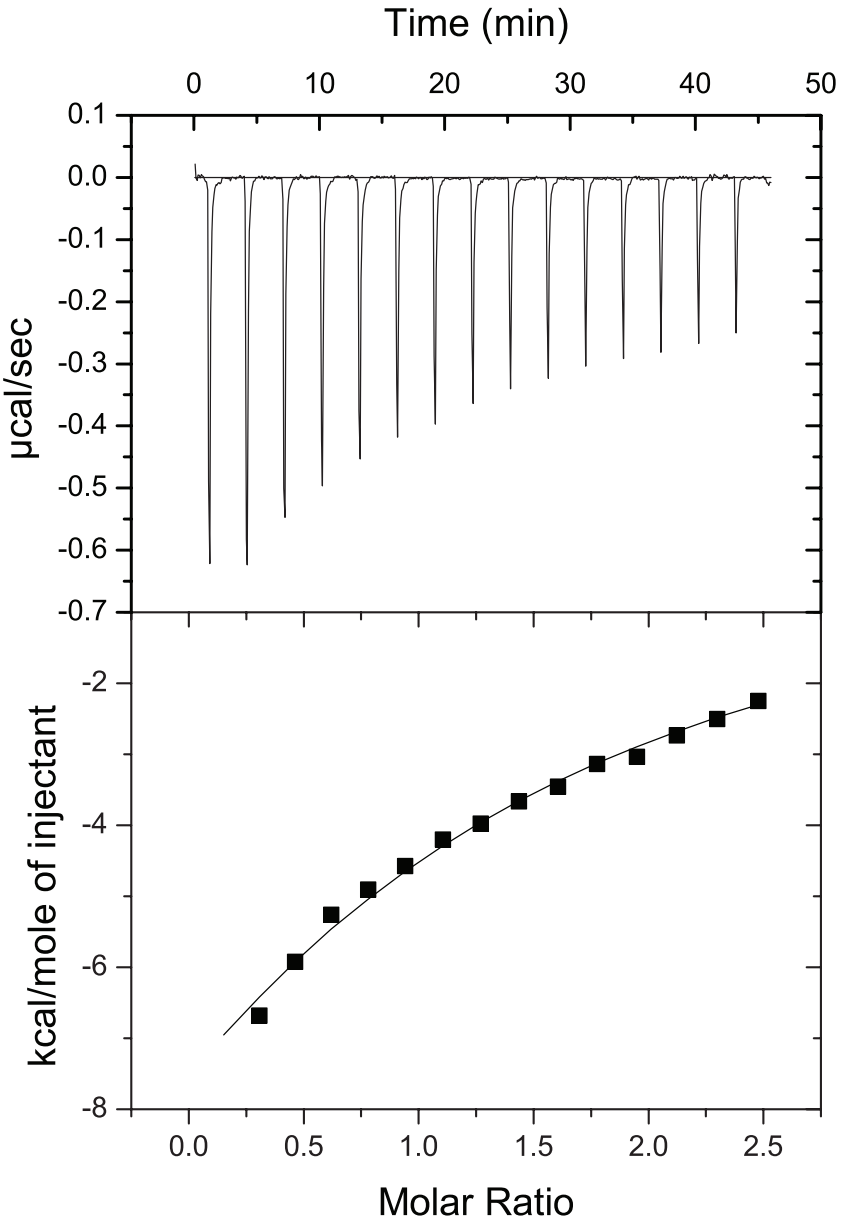

Figure S13: **Np-PEG5** binds exothermically to **MV-BSA3** in the presence of one equivalent CB[8] with a binding constant of (1.0±0.5) x 10^4 M^{-1} in buffered aqueous solution at 298 K.

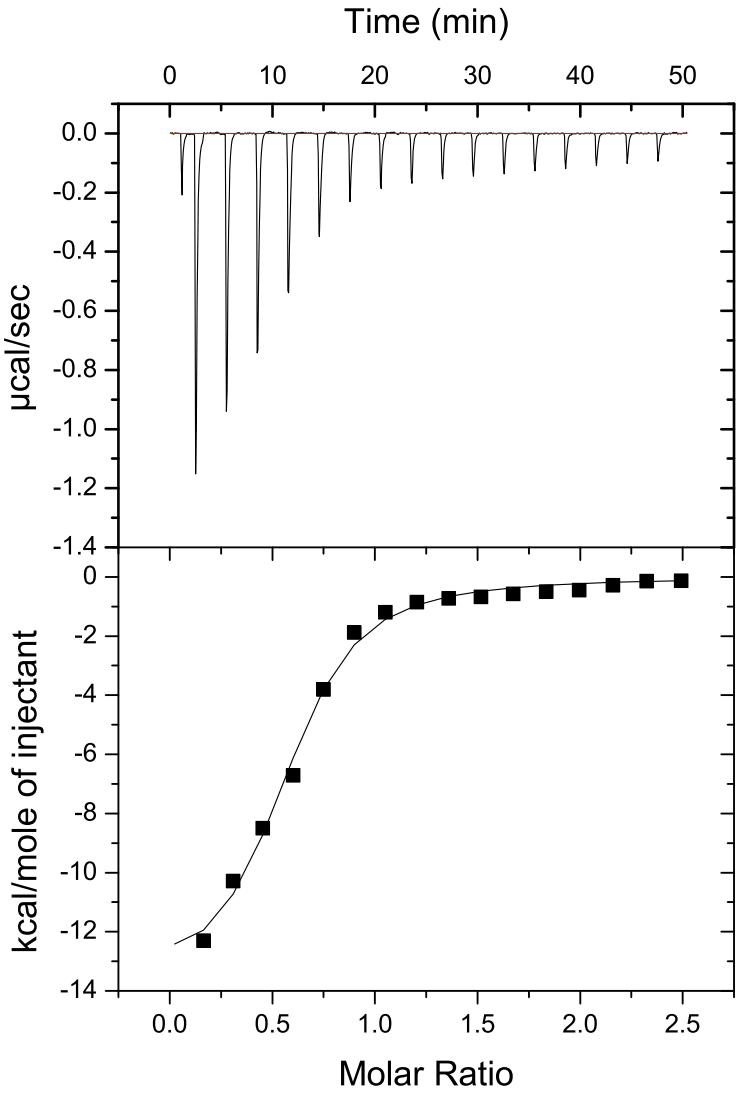

Figure S14: The first equilibrium between **MV-PEG4** and CB[8] possesses a association constant of (3.8±1.0) x 10^4 M^{-1} in buffered aqueous solution at 298 K.

1.9 CD

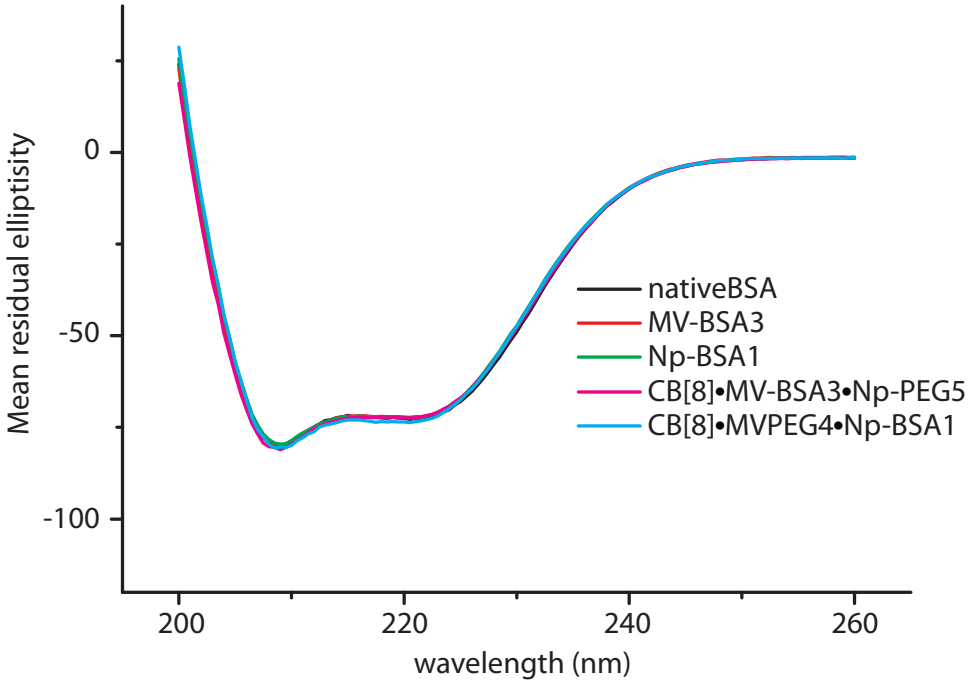

Figure S15: CD spectra of functionalised BSA proteins in the presence and absence of the complementary polymer and CB[8] at 0.067 mM concentration in phosphate buffer.