Probing cucurbit[8]uril-mediated supramolecular block copolymer assembly in water using diffusion NMR

Jameel M. Zayed, Frank Biedermann, Urs Rauwald, and Oren A. Scherman*

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW. Fax: +44 (0)1223 334866; Tel: +44 (0)1223 334370

E-mail: oas23@cam.ac.uk

Experimental

Materials and general methods. All starting materials were purchased from Alfa Aesar and Sigma Aldrich and used as received unless stated otherwise. AIBN was recrystallized twice from methanol. *N*-isopropylacrylamide (NIPAAM) was recrystallized from toluene/hexane (3:2). 3-Benzylsulfanylthiocarbonylsulfanyl propionic acid was synthesized according to the literature procedure.¹ Commercial PEGs were obtained from Fluka. **MVdimer** and CB[8] were prepared as documented previously.^{2,3}



Synthesis of 2-Np-NHCOO-PEG-OMe **P1**. A solution of 2-naphthoyl azide⁴ (1.2 g, 6.0 mmol) in 30 mL anhydrous *o*-dichlorobenzene was heated to 145 °C for 2 h under a nitrogen atmosphere

^{*}To whom correspondence should be addressed

in a 100 mL two-necked RBF equipped with a reflux condenser. Upon cooling the mixture down to room temperature, polyethylene glycol monomethyl ether (0.6 mmol) dissolved in 10 mL anhydrous DCM was added at once and the mixture was stirred for 48 h 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 diethyl ether (2x). Suction filtration yielded **P1** (0.5 mmol, 85%) as a white solid. ¹H NMR (500 MHz, D₂O): 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.



Synthesis of chain transfer agent **1**. A 50 mL RBF was charged with 3-benzylsulfanylthiocarbonylsulfanyl propionic acid (0.54 g, 2 mmol), N,N'-dicyclohexylcarbodiimide (1.03 g, 5 mmol), 3-amino-2-methoxydibenzofuran (0.36 g, 1.67 mmol) and dry dichloromethane (25 mL). The reaction mixture was then stirred at room temperature overnight. A white precipitate formed which was filtered off and discarded. The solvent was then removed under reduced pressure and the product was then isolated by column chromatography (silica gel, 95:5 chloroform/hexane) as a yellow powder (0.27 g, 29%). ¹H NMR (500 MHz, CDCl₃): δ = 8.75 (s, 1H), 8.11 (s, 1H), 7.88 (d, 1H), 7.57 (d, 1H), 7.4-7.3 (m, 8H), 4.66 (s, 2H), 4.05 (s, 3H), 3.82 (t, 2H), 2.93 (t, 2H) ppm; ¹³C NMR (125 MHz, CDCl₃): δ = 223.7, 168.4, 156.7, 150.8, 144.6, 134.8, 129.3, 128.7, 127.8, 126.3, 124.5, 122.5, 119.8, 118.5, 111.7, 103.5, 101.0, 56.4, 41.5, 36.5, 31.9 ppm. Anal. Calcd for C₂₄H₂₁NO₃S₃: C, 61.64; H, 4.53; N, 3.00. Found: C, 61.42; H, 4.48; N, 3.16.



Synthesis of functional poly(*N*-isopropylacrylamide) **P2**. A dry vial was charged with chain transfer agent **1** (24.5 mg, 0.05 mmol), NIPAAM (1 g, 8.84 mmol), AIBN (1.3 mg, 0.008 mmol), dioxane (4.5mL) and a stir bar. The solution was degassed thoroughly using three freeze-pump-thaw cycles, back-filled with nitrogen and then stirred in an oil bath preheated to 70 °C. After 4 h the polymerization mixture was quenched in liquid nitrogen and **P2** was isolated by precipitating the mixture twice into cold ether. M_n (g.mol⁻¹): 19,000, PDI: 1.09. ¹H NMR (CDCl₃): δ = 8.73 (s, 1H), 8.14 (s, 1H), 7.88 (d, 1H), 7.58 (d, 1H), 7.4-7.1 (m, 8H), 6.43 (broad, n*1H), 4.04 (s, 3H), 4.02 (s, n*1H), 3.8 (broad, 2H), 2.9 (broad, 2H), 2.3-1.5 (broad, n*3H), 1.16 (broad, n*6H) ppm.

Characterisation

NMR measurements

NMR was conducted on a Bruker Avance 500 MHz Ultrashield equipped with a 5 mm BBO ATM probe with a *z*-gradient. For diffusion measurements, a WATERGATE, HDO-suppressing stimulated echo pulse sequence stebpgp1s19 using bipolar gradient pulses and 1 spoil gradient was used. The machine was calibrated against the HDO peak in a D₂O reference at 25 °C, and the 0.8 mM samples were not spun during measurement. δ was varied between 4-9 ms, and Δ was kept constant at 0.1 s. A typical experiment involved 64 scans over 16 steps up to a maximum gradient of 32.03 G cm⁻¹. Diffusion coefficients were then evaluated using Topspin's T1/T2 relaxation module, where a vargrad fitting was applied to the sigmoidal plots of I/I₀ vs. G.



Figure S1: Partial 1-dimensional WATERGATE ¹H-NMR stack of **MVdimer** and **P1** before CB[8] addition (top), and after (below), showing the line-broadening and complexation-induced shifts upfield of **MVdimer**_{bound}'s H_{α} and H_{β} protons. D₂O, 20 °C.



Figure S2: 1-Dimensional WATERGATE ¹H-NMR stack of **MVdimer** and **P2** before CB[8] addition (top), and after (below), showing the line-broadening and complexation-induced shifts upfield of **MVdimer**_{bound}'s H_{α} and H_{β} protons. D₂O, 20 °C.

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Figure S3: Variation of D_{MVdimer} in the presence of increasing molecular weight of added PEG. 27 °C, $\delta = 2-2.16$ ms.

Determination of solution binding constants by ITC

Titration experiments were carried out on a VPITC from Microcal Inc., at 25°C in 10 mM sodium phosphate buffer (pH = 7), prepared from 1.560 g NaH₂PO₄·2 H₂O and 1.110 g Na₂HPO₄·2 H₂O in 1 L deionised water (Millipore, 18.2 M Ω ·cm). Fresh analyte solutions were dissolved by sonication and with heating up to 60°C. All compounds were weighed within an accuracy of at least two significant figures. The solutions were degassed prior to titration. The binding equilibria were studied using a cellular **MVdimer** · 2 CB[8] concentration of typically 0.07 mM to which the 1.5-2.0 mM polymer guest solution was titrated. Typically 20-30 consecutive injections of 10 μ L each were used, whereby the first injection was chosen to be 2 μ L in all cases. Thus the first data point was removed from the data set prior to curve fitting. Heats of dilution were checked by titration into the buffer solution and were found to be negligible. The data was analyzed with Origin 7.0 software, using the *one set of sites* model. Table S1: Binding constants for the complexation of **MVdimer** \cdot 2 CB8 with guests **G** as determined by ITC in buffered aquesous solutions at 298K

| Guest (G) | Ka | ΔG_{aq} |
|---------------------------------------|------------------|-----------------|
| | (M^{-1}) | (kJ/mol) |
| 2,6-dihydroxynaphthalene ² | $3.0 \cdot 10^5$ | -31.25 |
| Np-PEG-OMe $(1.1K)^2$ | $3.4 \cdot 10^4$ | -25.85 |
| Np-PEG-OMe (2K) | $2.6 \cdot 10^4$ | -25.18 |
| Np-PEG-OMe (5K) | $1.6 \cdot 10^4$ | -23.87 |

 2 Sigmoidal curve shape allowed for confirmation of 2:1 binding stoichiometry of polymer guest with **MVdimer** \cdot 2 CB8 .



Figure S4: ITC for the complexation of $MVdimer \cdot 2CB[8]$ with 2,6-dihydroxynaphthalene as determined by ITC in buffered aqueous solutions at 298K



Figure S 5: ITC for the complexation of **MVdimer** \cdot 2CB[8] with Np-PEG-OMe (M_n = 1100 g mol⁻¹) as determined by ITC in buffered aqueous solutions at 298K



Figure S6: ITC for the complexation of **MVdimer** \cdot 2CB[8] with Np-PEG-OMe (M_n = 2000 g mol⁻¹) as determined by ITC in buffered aqueous solutions at 298K



Figure S7: ITC for the complexation of **MVdimer** \cdot 2CB[8] with Np-PEG-OMe (M_n = 5000 g mol⁻¹) as determined by ITC in buffered aqueous solutions at 298K

Effect of CB[8] on PEG viscosities

Solution viscosities were measured using glass, Schott-Geräte Ubbelohde microviscometers with a suspended level bulb using a PVS1 measuring device, and the microviscometers were thermostated in a PV15 water bath at 30.00 (0.01) °C using a DLK10 thermostat unit (all manufactured by Lauda). Measured solutions were in H₂O at concentrations between 5-8.05 mg mL⁻¹ and filtered through a 0.45 μ m PVDF syringe filter prior to measurement.

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Figure S8: The decrease in reduced viscosity (η_{red}) observed by incremental CB[8] addition to commercial PEGs of different molecular weights ($M_n = 750 \text{ g mol}^{-1}$, red upward triangles. $M_n = 5000 \text{ g mol}^{-1}$, blue downward triangles).

References

- (1) Skey, J.; O'Reilly, R. K. Chem. Commun. 2008, 35, 4183-4185.
- (2) Deroo, S.; Rauwald, U.; Robinson, C. V.; Scherman, O. A. Chem. Commun. 2009, 644-646.
- (3) Huang, W.-H.; Liu, S.; Isaacs, L. Personal communication.
- (4) Joong-Gon, K.; Doo Ok, J. Synlett 2008, 13, 2072–2074.