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Supporting Information.

Tuning the Lower Critical Solution Temperature of Thermoresponsive Polymers by Biospecific Recognition

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Materials

Chemicals used. 2-(2-Methoxyethoxy) ethyl methacrylate (Aldrich, 95%) and oligo(ethylene glycol) methyl diethylether methacrylate (OEGMA, Aldrich, M_n 475 g·mol^{-1}) were passed through a basic alumina column before use to remove inhibitors. 2-2'-Azobisisobutyronitrile (AIBN, Acros, 98%) was recrystallized from methanol before use. 3-Aminopropyl methacrylate (Polyscience Inc.), D-biotin (Iris Biotec, 99%), N-hydroxysuccinimid (NHS, Fluka, 97%), N,N'-dicyclohexylcarbodiimide (DCC, Fluka, 99%), pyridine (dry, 99,8%), phosphate buffered saline (PBS, Fluka), 4'-hydroxyazobenzene-2-carboxylic acid (HABA = 2-(4'-hydroxyphenylazo)benzoic acid, ABCR, 98%) and avidin (bnl food, Luxembourg) were used as received.

Synthesis of biotinyl-3-aminopropyl methacrylamide.
(a) Synthesis of biotinyl-N-hydroxysuccinimide ester (Biotin-NHS) [1]. Biotin (2.00 g, 8.19 mmol) and N-hydroxy succinimide (0.94 g, 8.19 mmol) were dissolved in dry DMF (60 mL) at 60°C. After adding DCC (2.21 g, 10.71 mmol), the solution was stirred overnight at room temperature. Then, the precipitated solid (of the dicyclohexylurea formed) was filtered off and washed with DMF. The combined filtrates were concentrated under reduced pressure and precipitated into diethylether. The white solid was recrystallized from isopropanol and dried in vacuo to yield the product. White powde, yield 2.2 g (79%).
^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 1.34 -1.74 (m, 6H, (C$\underline{H_2}$)$_3$-COO), 2.54-2.89 (m, 8H, -C$\underline{H_2}$S-, -C(=O)C$\underline{H_2}$C$\underline{H_2}$C(=O)- and -C$\underline{H_2}$COO-), 3.06-3.16 (m, 1H, -C$\underline{H}$S-), 4.10-4.12 and 4.27-4.37 (m + m, 1H + 1H, twice -C$\underline{H}$-N-CO-N-), 6.36-6.42 (m, 2H, -N$\underline{H}$CON$\underline{H}$).
^{13}C NMR (300 MHz, DMSO-d6) δ (ppm): 24.7 (-$\underline{C}$H$_2$CH$_2$COO-), 25.9 (-$\underline{C}$H$_2$CON-), 28.0 (-$\underline{C}$H$_2$CH$_2$CH$_2$COO-), 28.3 ($\underline{C}$H$_2$CH$_2$CH2CH2COO), 30.5 ($\underline{C}$H$_2$S), 40.4 (-$\underline{C}$H$_2$COO-), 55.7 (-$\underline{C}$HS-), 59.6 and 61.5 (-$\underline{C}$HNHCO-), 163.1 (-NH$\underline{C}$ONH-), 169.4 (-$\underline{C}$OO), 170.7 (-$\underline{C}$(=O)N-$\underline{C}$(=O)-.
(b) Synthesis of biotinyl-3-aminopropyl methacrylamide in analogy to [1]. 3-Aminopropyl methacrylate hydrochloride (379 mg, 2.12 mmol), biotin-NHS (713 mg, 2.09 mmol) and hydroquinone (21 mg, 0.19 mmol) were dissolved in DMF (15 mL) and triethylamine (0.6 mL, 4.32 mmol) was added. The mixture was stirred over night at room temperature. The resulting white precipitate was filtered off, the filtrate was concentrated under reduced pressure and precipitated into a large excess of diethylether. The obtained white solid was washed with cold isopropanol and dried in vacuo. White powder, yield 725 mg (90%).

^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 1.25-1.76 (m, 8H, -CH(C$\underline{H_2}$)$_3$- and -C$\underline{H_2}$(CH$_2$NHCO)$_2$), 1.85 (s, 3H, -CH$_2$CC$\underline{H_3}$), 2.06 (t, 2H, J=7.3 Hz, -C$\underline{H_2}$CONH-), 2.54--3.15 (m, 7H, -C$\underline{H_2}$S-, -C$\underline{H}$S-, twice -CONHC$\underline{H_2}$-), 4.08-4.18 and 4.26-4.36 (m+m, 1H+1H, twice -C$\underline{H}$NCON-), 5.31 and 5.64 (s+s, 1H+1H, C$\underline{H_2}$=C-CO-), 6.35-6.41 (m, 2H, -N$\underline{H}$CON$\underline{H}$-), 7.70-8.02 (m, 2H, -CON$\underline{H}$-).
^{13}C NMR (300 MHz, DMSO-d6) δ (ppm): 18.6 (C$\underline{H_3}$-), 25.3 (-C$\underline{H_2}$CH$_2$CON-), 28.0 (-C$\underline{H_2}$CH$_2$CH$_2$CON-), 28.2 (-NHCH$_2$-C$\underline{H_2}$-CH$_2$NH-), 29.3 (-C$\underline{H_2}$CH$_2$CH$_2$CH$_2$CON-), 35.3 (-C$\underline{H_2}$S-), 35.8 (-C$\underline{H_2}$CONH-), 36.7 and 36.6 (-C$\underline{H_2}$NHCO-), 55.9 (-C$\underline{H}$S-), 59.2 and 61.0 (C$\underline{H}$HNHCO), 118.8 (C$\underline{H_2}$=), 140.0 (=C-CO), 162.7 (-C$\underline{O}$NH-), 167.4 (=C-C$\underline{O}$NH-), 172.1 (-CH$_2$-C$\underline{O}$NH-).

Copolymerisation. Biotinyl-3-aminopropyl methacrylamide (BAPMA, 20 equivalents), 2-(2-methoxyethoxy) ethyl methacrylate (MEO$_2$MA, 68 equivalents) and oligo(ethylene glycol) methyl diethylether methacrylate (OEGMA, 12 equivalents) were dissolved in DMF at 60°C (10 wt.-% monomers in DMF). Then the solution was cooled to room temperature before adding AIBN (1 equivalent). The flask was sealed with a rubber septum, the mixture purged with argon for about 30 min, and then heated to 60°C for 20 h in an oil bath. Then the reaction was stopped and the mixture diluted with deionized water and dialysed against deionized water (Roth, ZelluTrans membrane, molecular weight cut off: 4000-6000). The thus purified polymer was isolated by freeze drying, to give to a colourless glue. Yield 85%. $M_{n,\ GPC}$ = 27700 g·mol^{-1}, PDI_{GPC} = 3.5 (relative to polystyrene standards).
The copolymer was analyzed by ^{1}H-NMR spectroscopy (Figure S1). Copolymer composition was calculated from the intensity ratio of characteristic signals of the various comonomers. (BAPMA:MEO$_2$MA:OEGMA = 18:68:14)

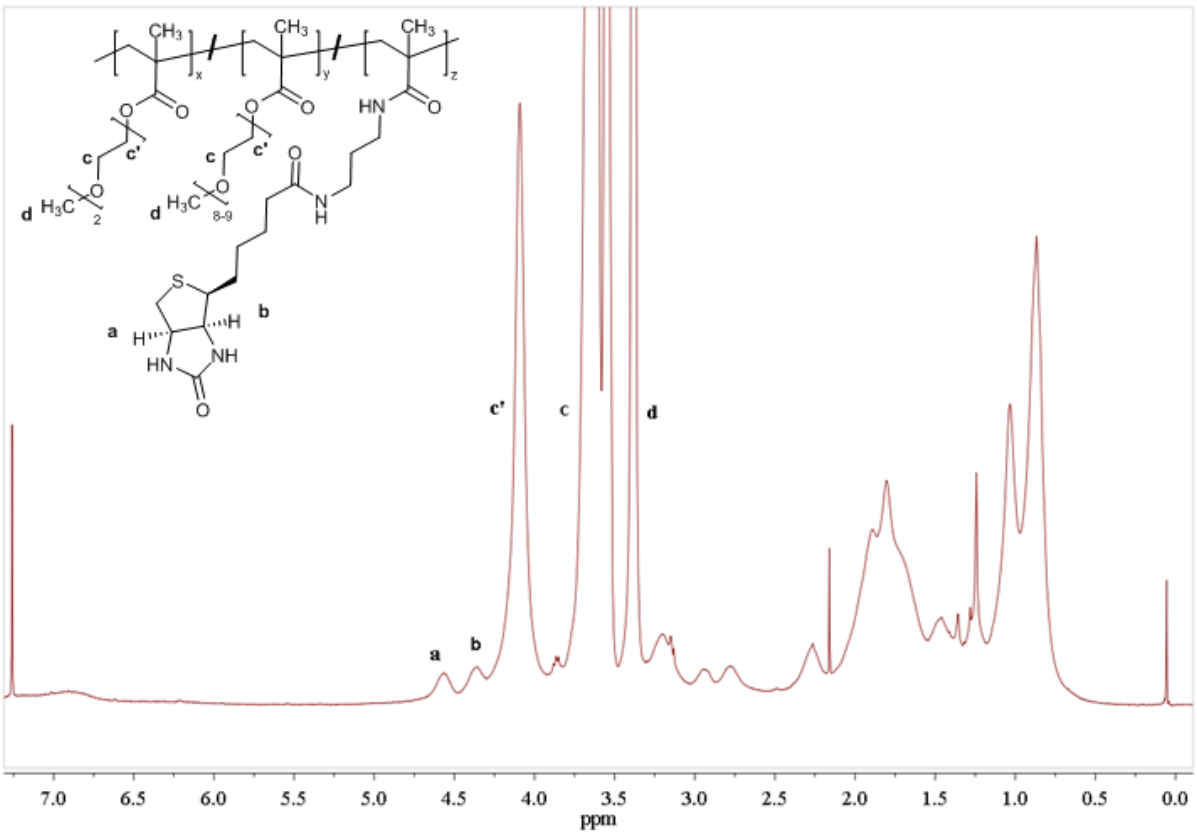

Figure S1 ^{1}H-NMR of a biotin containing copolymer in $CDCl_3$. Copolymer composition was calculated by comparing the respective integrals of signals a, (b + c') and (c + d).

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Methods

Characterization. Cloud points of the polymer solutions were measured in 2.0 $g \cdot L^{-1}$ phosphate buffered saline solutions on a Tepper TP1 photometer (Mainz, Germany). Transmittance of these polymer at 670 nm was monitored as a function of temperature (with heating/cooling cycle at rates of 1°C·min^{-1}, cell path length 12 mm). ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d6 with a Bruker Avance 300 spectrometer. UV–vis spectra were recorded with a spectrophotometer Cary-1 (Varian) in quartz cuvettes (d = 1 cm). SEC was run at 50°C in DMF (flow rate 1mL/ min) using a Spectra Physics Instruments apparatus equipped with a UV-detector SEC-3010 and a refractive index detector SEC 3010 from WGE Dr. Bures (Columns: Guard (7.5 x 75 mm), PolarGel-M (7.5 x 300 mm)), calibration with linear polystyrene standards (PSS, Germany).

HABA/avidin binding assay. This procedure follows a technical support provided by Interchim Inc. (France) based on [2]. 24.2 mg HABA were dissolved in 9.9 mL of ultrapure water and 100 µL 1N NaOH. After filtration 600 µL of this solution were mixed with 5.0 mg of avidin and completed to 10 mL of PBS. Subsequently 1.8 mL were filled in a cuvette and mixed with 0.2 mL of a 0.5 g/L solution of biotin containing polymer in PBS. Absorbance of the solution with and without biotinylated sample was measured at 500 nm.
The concentration of accessible biotin c_{Biotin} was calculated from the difference of absorbance of the assay before and after the addition of the biotin containing copolymer:

$$\Delta A_{500} = 0.9 \cdot A_{1,500} - A_{2,500}$$
$$c_{Biotin} = \Delta A_{500} \cdot 10 / \varepsilon_{500}$$

$A_{1,500}$ = Absorbance of pure HABA/avidin assay at 500 nm
$A_{2,500}$ = Absorbance after addition of biotin containing copolymer
0.9 = dilution factor of avidin/HABA with sample
10 = dilution factor of biotin containing copolymer
ε_{500} = molar extinction coefficient of HABA/avidin at 500 nm = 34000 $L \cdot mol^{-1} \cdot cm^{-1}$

References

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[2] N. M. Green, Avidin. In *Advances in Protein Chemistry, Vol. Volume 29*, Academic Press, **1975**, pp. 85.