Fine-tuning the transition temperature of a stimuli-responsive polymer by a simple blending procedure – supplementary information

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General Procedures. NMR spectra were recorded using a Varian Inova 500 spectrometer at 499.87 (^{1}H) and 125.67 MHz (^{13}C) (^{1}H decoupled at 500 MHz) or using a Bruker Avance 400 at 400.13 MHz (^{1}H). NMR spectra were analyzed using the MestreC® software.

Aqueous SEC was performed using a triple detection method (with angular correction) and measurements were performed on a Viscotek TDA 301 triple detection SEC fitted with two (300 x 7.5 mm) GMPWxl methacrylate-based mixed bed columns with an exclusion limit of $5x10^7$ g·mol^{-1}, having refractive index, viscometer and RALLS detectors. The eluent used was a buffered aqueous solution containing 0.2 M $NaNO_2$ and 0.01 M NaH_2PO_4, at a flow rate of 1.0 mL·min^{-1} and at a constant temperature of 30 °C. Calibration (for detector response) was achieved using a single narrow PEO standard (Polymer Labs) of 82,500 g·mol^{-1} and a dn/dc value of 0.133 mL·g^{-1}. Molecular weights were determined using Omnisec 4.0 software for Windows with a dn/dc value of 0.121 mL·g^{-1} for poly(MA-VPGVG) (determined online using the RI detector response from a number of different, volumetrically measured, concentrations of the pure polymer). The theoretical number average degree of polymeriation ($DP_{n,th}$) values of polymers prepared were calculated according to the following

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formula: $DP_{n,th} = x * ([M]_0/[RAFT]_0)$, where x = fractional conversion, $[M]_0$ = initial monomer concentration and $[RAFT]_0$ = initial RAFT agent concentration.

Turbidimetry experiments were carried out using a Varian CaryBio-100 UV-Vis spectrophotometer, equipped with multicell, thermoelectric temperature controller. Phosphate buffered aqueous solutions at different pH and concentration of the polymer were prepared in glass cuvettes, sealed with a Teflon stopper and heated at a rate of 1 ºC/min. The measurements were carried out at a fixed wavelength of 480 nm. All the plots represent an average of at least two measurements. In order to calculate the phase transition temperature, all the experimental data were normalized to 1 using Microcal™ Origin 6.0® software for Windows and the transition temperature was considered to be that corresponding to an absorption of 0.5.

Reagents. Fmoc-Gly-"Wang" resin (Novabiochem, 0.55-0.77 mmol/g), H-Gly-2-chlorotrityl resin (Novabiochem, 0.59-1.00 mmol/g), 9-fluorenylmethoxy carbamate-protected valine (Fmoc-Val-OH) (Novabiochem, >99%), Fmoc-glycine (Fmoc-Gly-OH) (Novabiochem, >99%), Fmoc-proline (Fmoc-Pro-OH) (Novabiochem, >99%), 2-isocyanatoethyl methacrylate (Aldrich, 98%), N,N-diisopropylcarbodiimide (DIPCDI) (Aldrich, 98%), 1-hydroxybenzotriazole hydrate (HOBt) (Fluka, 98%), trifluoroacetic acid (TFA) (Aldrich, 98%), DMSO-d6 (Aldrich, 99.9%), 2,2,2-trifluoroethanol (TFE) (Aldrich, 99%) and 4,4'-azobis(4-cyanopentanoic acid) (Fluka, >98 %) were all used as received. (4-Cyanopentanoic acid)-4-dithiobenzoate was synthesized according to a literature procedure.[1]

Synthesis of Methacrylate-functionalized VPGVG (MA-VPGVG). Methacrylate-functionalized VPGVG was synthesized by standard solid-phase methods using a "Wang" or "Cl-Trityl" resin as described previously.[2] A representative procedure is given: 1 g of the Fmoc-Gly functionalized resin (loading 0.55 $mmol \cdot g^{-1}$) was swollen and filtered three times in 9 mL of DMF. Next, 10 mL of DMF

containing 20% v/v piperidine was added to remove the Fmoc group. A positive Kaiser test indicated completeness of this reaction.[3] The next amino acid was coupled by adding a mixture of 0.571 g (1.65 mmol, 3 eq) of Fmoc-Val-OH, in 10 mL of DMF, with 0.232 g (1.82 mmol) of DIPCDI and 0.303 g (1.98 mmol) of HOBt. The mixture was shaken for 30 min., after which it was washed thoroughly with DMF. A negative Kaiser test indicated the completeness of the reaction. This procedure was repeated with the following three amino acids: Fmoc-Gly-OH (0.502 g, 1.65 mmol, 3 eq), Fmoc-Pro-OH (0.568 g, 1.65 mmol, 3 eq), and Fmoc-Val-OH (0.571 g, 1.65 mmol, 3 eq). While still on the resin, the Fmoc protecting group on the terminal valine was removed, and the free amine was subsequently coupled with 0.290 g (1.65 mmol, 3 eq) of 2-isocyanatoethyl methacrylate in DCM. After one hour the resin was thoroughly washed with DCM and Et_2O. The resin was allowed to dry and the methacrylate-functionalized VPGVG was cleaved from the resin using 90% TFA/water solution in the case of "Wang" Resin or DCM/TFE/AcOH (3:1:1) in the case of "Cl-Trityl" Resin. The obtained monomer was precipitated into Et_2O and freeze-dried from aqueous acetic acid (10%). From 10 g of Fmoc-Gly functionalized resin, ~ 0.296 g (92%) of peptide was obtained.

IR (KBr) ν/cm^{-1}: 3318 (NH str); 2964 (CH str); 1718 (C═O str); 1644 (C═C str); 1555 (NH bend); 1173 (C-N str).

^{1}H NMR (400.13 MHz, DMSO): δ 0.8-0.9 (12H, m, CH_3-*i*Pr); 1.8-2.1 (9H, m, CH-*i*Pr, CH_2-Pro and CH_3-MA); 3.3-3.4 (8H, m, H_2O + CH_2-Gly); 3.5-3.6 (1H, m, CH-Val); 3.7-3.8 (4H, m, CH_2-Et); 4.04 (2H, t, J = 5.4 Hz, CH_2-Pro); 4.1-4.2 (2H, m, CH_2-Gly); 4.31 (1H, dd, J = 4.3, 8.9 Hz, CH-Val); 5.68 (1H, s, CH-MA), 6.05 (1H, s, CH-MA); 6.1-6.2 (2H, m, NH); 7.61 (1H, d, J = 8.9 Hz, NH); 8.17 (1H, t, J = 5.7 Hz, NH); 8.31 (1H, t, J = 5.9 Hz, NH).

LS-MS: *m/e* 583 (MH^+); 605 (M^+-H + Na); 627 (M^+-2H + 2Na).

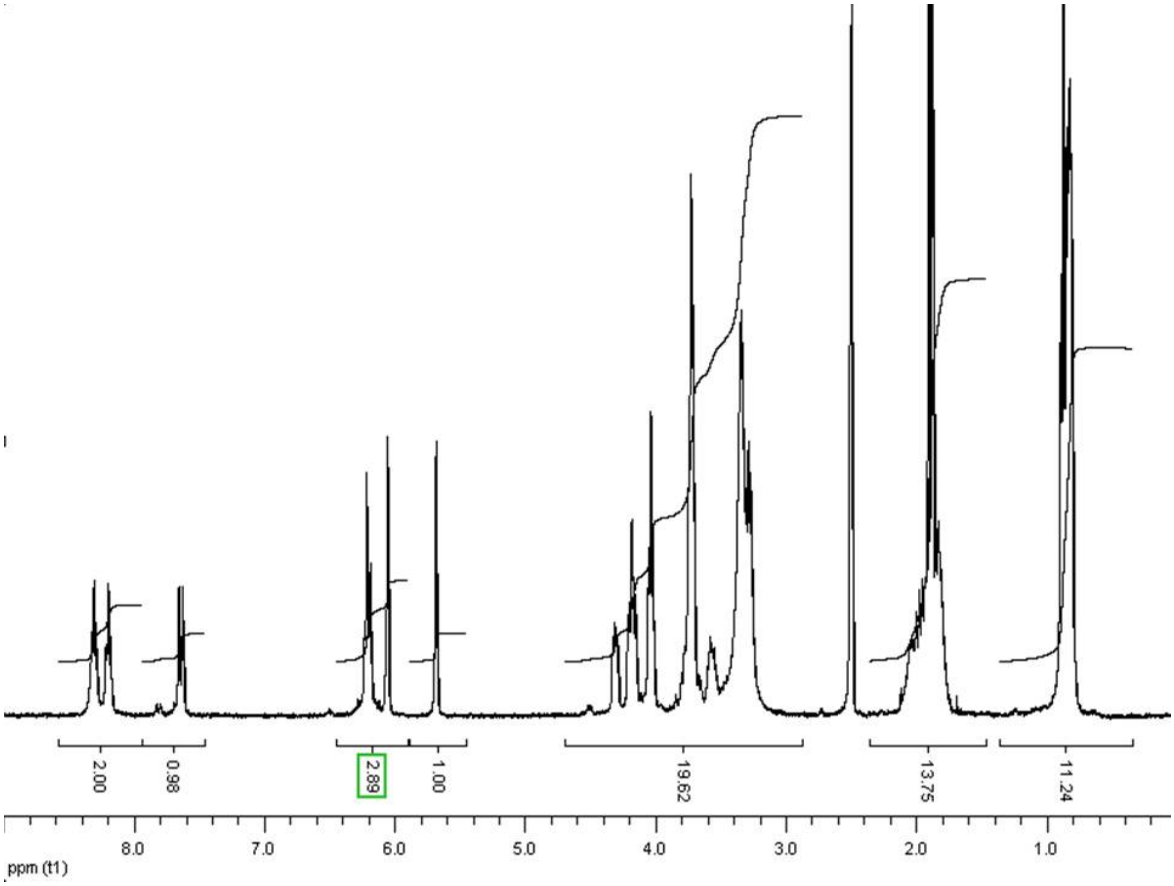


Figure S1. ^{1}H NMR spectrum (d_6-DMSO) of methacrylate-functionalized VPGVG (MA-VPGVG).

RAFT Polymerization. Synthesis of poly(MA-VPGVG) dithiobenzoate: Polymerizations were carried out using 4-cyanopentanoic acid dithiobenzoate (**1**) as RAFT agent and commercially available 4,4'-azobis(4-cyanopentanoic acid) (**V-501**) as the radical source. All experiments were conducted in Schlenk tubes sealed with a Young's tap. An NMR spectrum was recorded at the beginning of the experiment to calculate the conversion. The polymerization solutions were degassed with 3–4 freeze–evacuate–thaw cycles and transferred to an oil bath pre-heated to 70 ºC. For the kinetic experiments, the reaction was run under the same conditions in a dry NMR tube fitted with a Young's tap, spectra being acquired every 14 minutes. After reaction, the solution was quenched by cooling in ice–water for 10 s and another NMR spectrum was recorded for conversion purposes. The polymer was recovered by precipitation in excess THF followed by centrifugation and freeze–drying from water.

In a typical experiment, to a solution of MA-VPGVG (433 mg, 0.80 mmol) in 2.20 mL of DMSO-*d6* were added 0.41 mL of a 0.06 M solution of **1** (6.6 mg, 0.02 mmol) and 0.42 mL of a 0.02 M solution of **V-501** (2.73 mg, 0.01 mmol) both prepared in DMSO-*d6*. After quenching the reaction and purification, the title compound was recovered as a pink–white powder by freeze–drying from water (dark, 2 days). Yield: 210 mg, 57%. M_n (SEC) 25,100; PDI (SEC) 1.03; UV (MeOH): λmax = 504 nm.

^{1}H NMR (299.95 MHz, DMSO): δ 0.7-0.9 (12H, m, CH_3-*i*Pr); 1.6-2.0 (10H, m, CH-*i*Pr, CH_2-Pro, CH_3-MA and CH_2-MA backbone); 3.1-4.4 (15H, m, H_2O + CH_2-Gly, CH-Val, CH_2-Et, CH_2-Pro, CH_2-Gly); 6.1-6.3 (2H, m, NH); 7.5-7.7 (1H, m, NH); 8.0-8.4 (2H, m, NH).

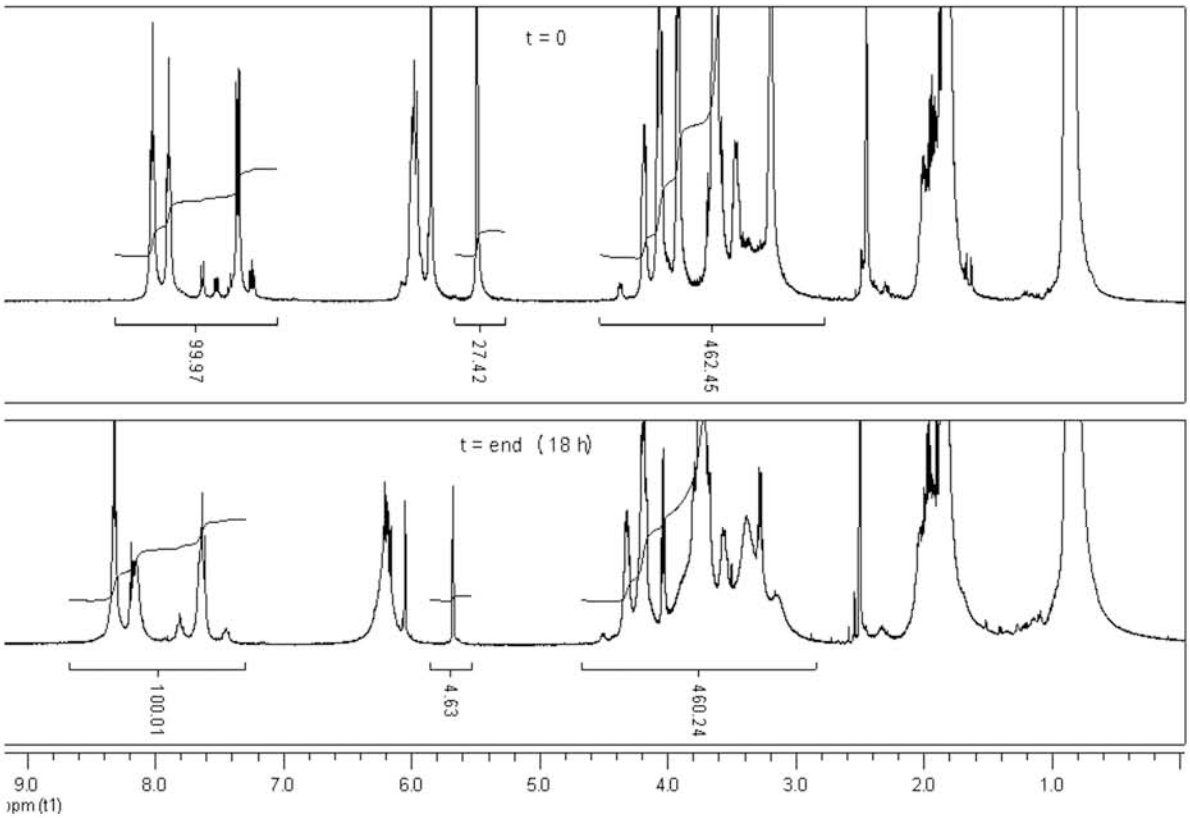
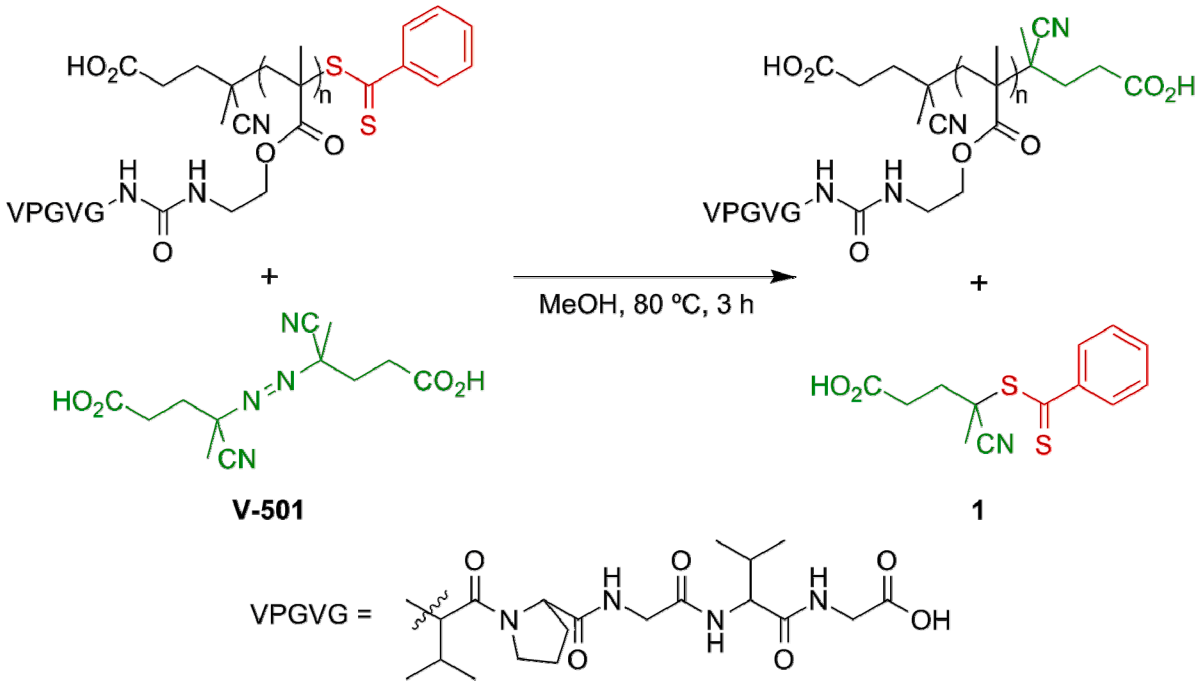


Figure S2. ^{1}H NMR spectra (d_6-DMSO) of the RAFT polymerisation of methacrylate-functionalized VPGVG (MA-VPGVG). Top: initial polymerization mixture (major peaks from MA-VPGVG); bottom: reaction mixture after 18 hours (methacrylate peaks from residual monomer are visible at δ 5.6 and 6.0)

End Group Modification. Synthesis of poly(MA-VPGVG):



The dithioester moiety was removed using the procedure described by Perrier et al.[4] In a typical experiment, poly(MA-VPGVG) dithiobenzoate (210 mg, 0.01 mmol) and **V-501** (98 mg, 0.34 mmol) were dissolved in 5 mL of MeOH. The polymerization solutions were degassed with 3 freeze–evacuate–thaw cycles and transferred to an oil bath pre-heated to 80 ºC. After reacting for 3 h, the solution was quenched by cooling in ice–water for 10 s and the polymer was recovered as a white solid by precipitation in excess THF followed by centrifugation and freeze–drying from water. Yield: 172 mg, 82%. M_n (SEC) 23,500; PDI (SEC) 1.05.

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