

Supporting Information

Upper Critical Solution Temperature Thermo-Responsive Polymer Brushes and a Mechanism for Controlled Cell Attachment

Xuan Xue, Lalitha Thiagarajan, Shwana Braim, Brian R Saunders, Kevin M Shakesheff* and Cameron Alexander*

Methods

Solution phase synthesis of co-polymers

In a typical procedure, NAGAm, PhAm, Me₆TREN, and CuCl₂ were dissolved in DMSO/D₂O at room temperature in a 25 mL two-necked round-bottom flask, and degassed with dry nitrogen gas for 30 min. Detailed amount of materials used are summarized in Table S1. In another flask 2-Chloropropionamide stock solution (19 mg CPA in 10 mL of H₂O) was prepared separately. CuCl was then added into the monomer solution, and degassed for another 15 min. Thereafter 1 ml of CPA solution was added to the reaction system. The CRP of NAGAm and PhAm was then initiated by heating the contents to 45 °C. After 4 h, the reactions were quenched by exposure to air. Then, the polymers were precipitated from a 10-fold excess volume of methanol and recovered by centrifugation (10 min, 8000 rpm). The centrifugation–wash cycles were repeated three times. Finally, the polymers were dried in a vacuum oven at 70 °C for 24 h.

Table S1. Reagents and quantities for example solution phase synthesis of polymers (PNAGAm-PNPhAm).

Reagent	M _w (g/mol)	Mass (mg)	Volume (mL)	Concentration (mmol)	Feed ratio
2-Chloropropionamide	107.5	1.9	1	0.017	1
NAGAm	128.1	400.0		3.12	180
PhAm	147.2	51.6		0.35	20
CuCl ₂	134.5	2.3		0.02	1
Me ₆ TREN	230.4	8.2	0.0096	0.03	2
CuCl	99.0	1.8		0.02	1
DMSO/H ₂ O			4.0		

Table S2 – Properties of polymers synthesised

Polymer	Feed ratio (NAGA:NPhAm)	Found ratio (NMR) (NAGA:NPhAm)	M _n (NMR)	M _n (GPC)	Đ	Cloud point (°C)
1	200:0	200:0	11000	8448	1.055	22
2	190:10	178:22	10600	7953	1.069	28
3	180:20	163:37	11400	8205	1.046	34

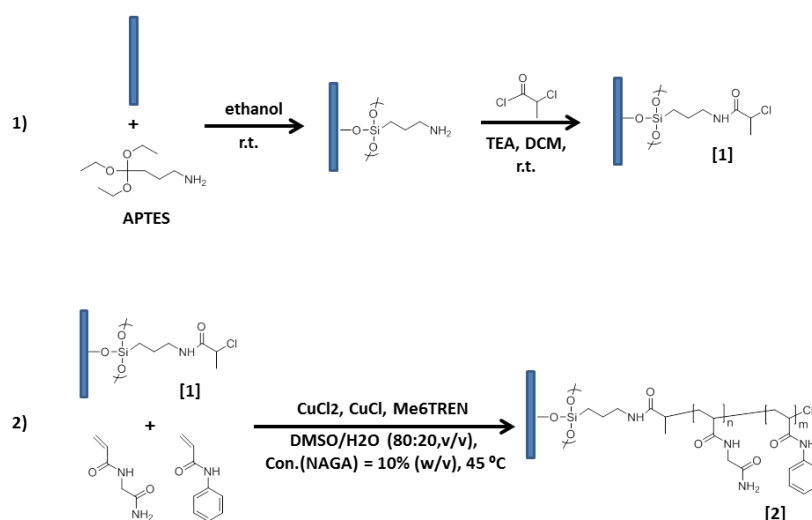
Temperature dependent turbidity measurements

The UCST of the polymers was inferred from the turbidity of the polymer solutions in PBS. 1% (w/v) polymer solution were placed in a UV/Visible spectrophotometer (Beckman Coulter DU 800) with a equipped with thermostat. The temperature was raised at a rate of 1 °C min⁻¹ over a temperature range of 15–50 °C. Absorbances of the samples were measured at a wavelength of 550 nm. The

UCST was defined as the onset temperature of turbidity at 550 nm. The inflection point of the transmittance curve was considered as the cloud point.

Gel permeation chromatography (GPC)

Gel permeation chromatography was used to determine the weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity (\mathcal{D}) of the synthesized free polymers. The instrument (Shimadzu Prominence UPLC) fitted with fitted with SIL-20A autosampler, CTO-20A column oven and RID-10A refractive index detector. The sample solution was filtered (0.2 μm filter) before injection. The calibration and analyses were performed at 40°C and at a flow rate of 1mL/min.



Scheme S1. Preparation of UCST-type thermoresponsive polymer brushes by SI-CRP

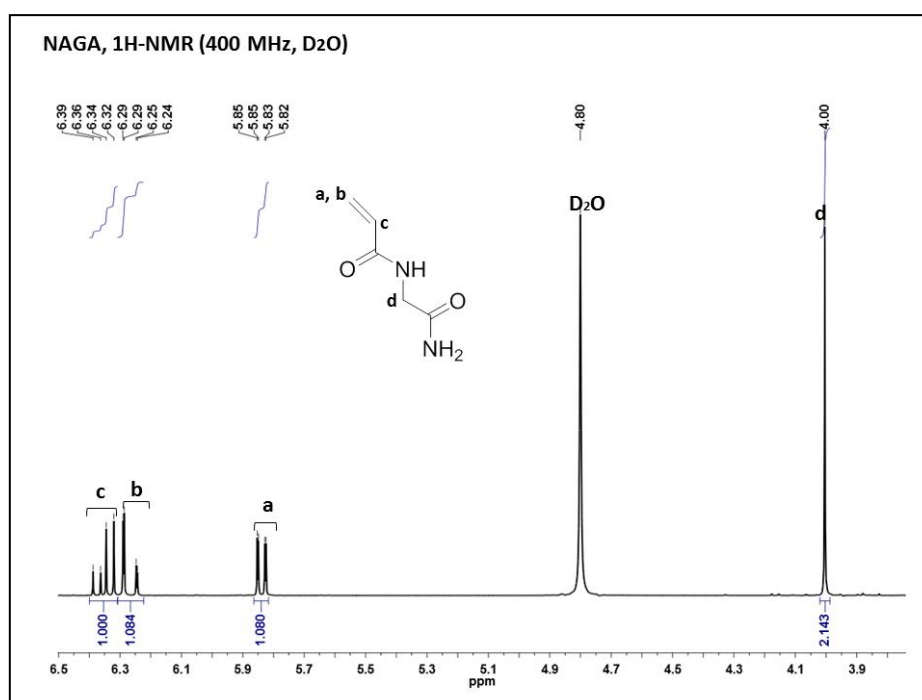


Figure S1. ^1H -NMR (400 MHz) spectrum of synthesized *N*-acryloyl glycineamide (NAGAm) monomer in D_2O at room temperature.

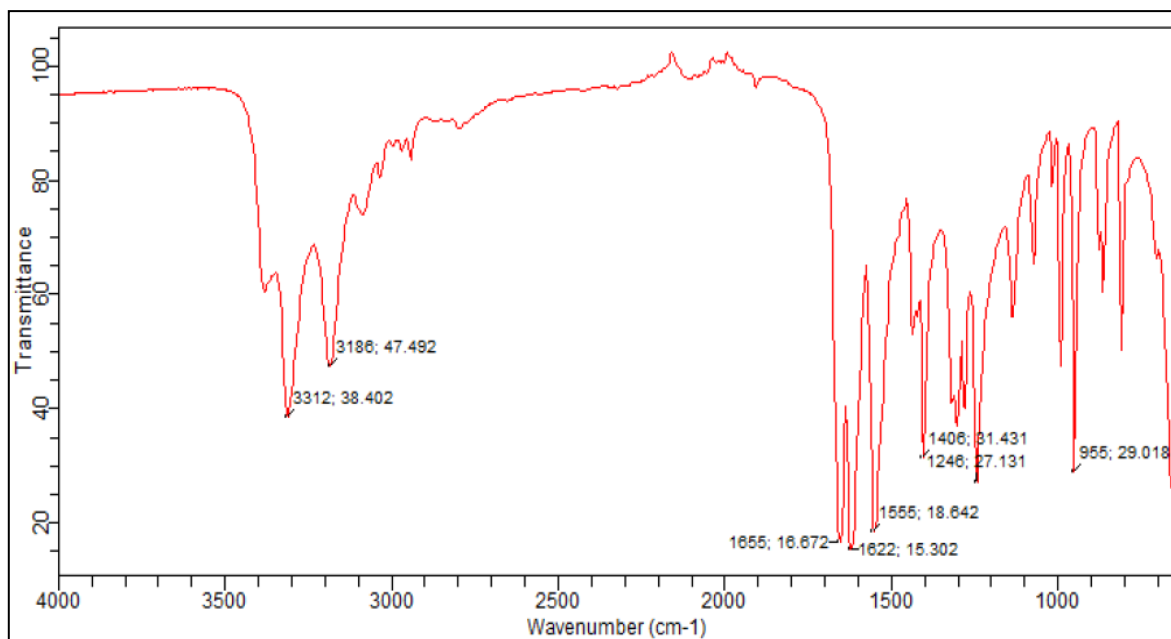


Figure S2. FT-IR spectrum of synthesized *N*-acryloyl glycineamide (NAGAm) monomer

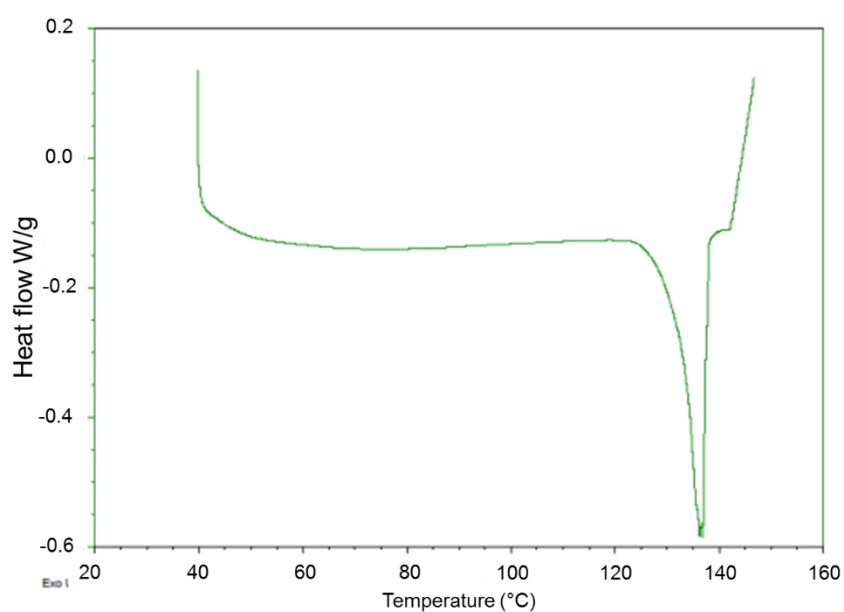


Figure S3. DSC data for synthesized *N*-acryloyl glycineamide (NAGAm) monomer showing its melting point at 137 °C.

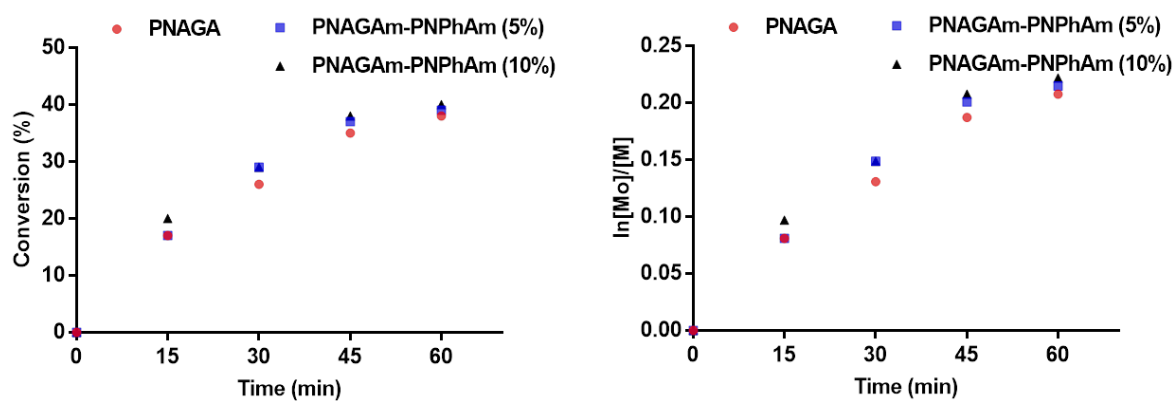


Figure S4. Kinetic study of copolymerization of NAGAm and PhAm under the reaction condition: [CPA]:[NAGA]:[PhAm]:[CuCl₂]:[CuCl]:[Me₆TREN] (molar ratio) = 1:180:20:1:1:2, DMSO/H₂O (80:20, v/v), Con.(NAGA) = 10% (w/v), 45 °C.

Surface chemical analysis

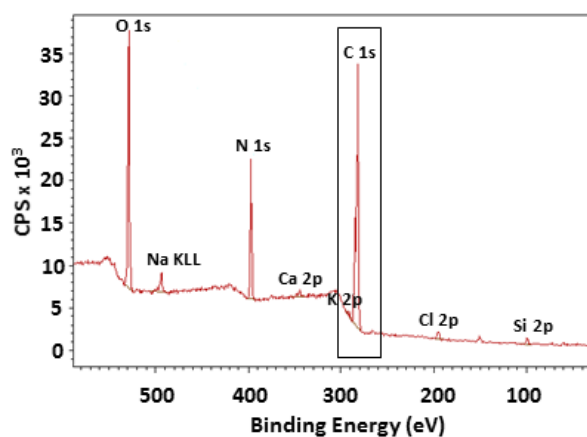


Figure S5. XPS survey spectrum for polymer brush PNAGAm-PPhAm (Brush 3) (In addition to the three main elements O 1s, N 1s and C 1s, traces of elements from the glass substrate were also detected indicating penetration of the photoelectrons into the underlying surface)

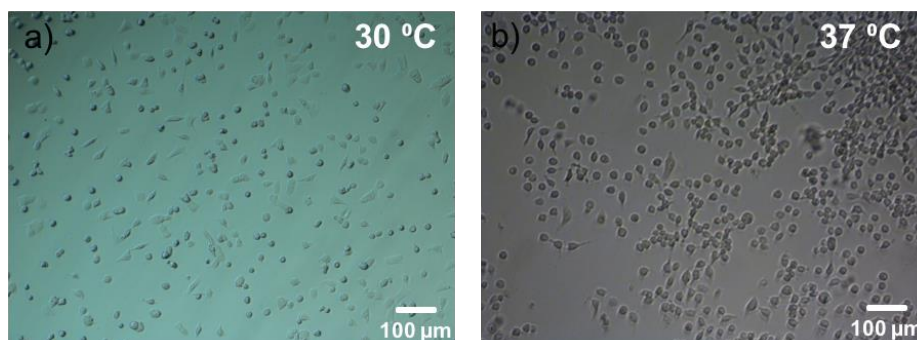


Figure S6. Microscopy images of a) cell attachment on unmodified glass coverslips after culture at 30 °C for 20 h, and b) no significant cell release was observed after switching to 37 °C for 24 h.

Table S3. Elemental composition of polymer brush PNAGAm-PPhAm (Brush **3**) measured from XPS survey spectrum (Figure S5).

Name	Pos.	At%
Cl 2p	195	0.57
Si 2p	99	1.28
Na KLL	493.5	1.20
O 1s	528.5	17.41
N 1s	397	13.17
C 1s	282	65.92
K 2p	290	0.20
Ca 2p	344	0.25

Surface energy calculation – Three components methods of van Oss *et al.*^[1]

$$\gamma_L (1 + \cos \theta) = 2 (\sqrt{\gamma_S^{LW} \gamma_L^{LW}} + \sqrt{\gamma_S^+ \gamma_L^-} + \sqrt{\gamma_S^- \gamma_L^+}) \quad (1)$$

$$\gamma_S = \gamma_S^{LW} + 2 \sqrt{\gamma_S^+ \gamma_S^-} \quad (2)$$

θ : contact angle; γ_S : (total) surface energy of a solid; γ^{LW} : the Liftshitz/van der Waals component; γ^+ : Lewis-acid component; γ^- : Lewis-base component; γ_L : surface tension of the liquid; mJ/cm² (or mN/m); S = solid, L = liquid

Table S4. Surface tension (γ_L) of three liquids measured at three different temperatures.

Liquid	25 °C	37 °C	50 °C
Double Deionized Water (DDW)	70.8 ± 0.09	66.4 ± 0.09	66.1 ± 0.13
Ethylene Glycol (EG)	47.8 ± 0.23	44.6 ± 0.16	42.5 ± 0.18
Diiodomethane (DIM)	48.6 ± 0.14	47.7 ± 0.21	45.7 ± 0.11

The values of γ_L^{LW} , γ_L^+ and γ_L^- are known, and θ and γ_L are measured with three different liquids at each determined temperature. Therefore, γ_S^{LW} , γ_S^+ and γ_S^- can be calculated based on Equation 1, and also γ_S can be obtained based on Equation 2.

References

- [1] C. J. Van Oss, M. K. Chaudhury, R. J. Good, *Chemical Reviews* **1988**, 88, 927-941.