

# Supplementary Material for

## Concentration Dependent Effects of Urea

### Binding to Poly-N-isopropylacrylamide Brushes:

### A Combined Experimental and Numerical Study

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## Experimental study

### Ellipsometric brush thickness

The thickness of the polymer brushes was measured by Ellipsometry in dry ( $1.9 \pm 0.1\%$  relative humidity (RH)) and swollen state for different urea concentrations from 0 to 7 mol/L.

The experimental data are reported in Table S1.

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Table S1: Brush thickness measured in dry ( $1.9\pm 0.1\%$  relative humidity (RH)) and swollen state in urea solutions at different concentration, below (288 K) and above (328 K) the critical solution temperature.

$c_u$ [mol/L]	Thickness [nm]	
	288 K	328 K
0	123	45
0.1	126	48
0.2	125	52
0.5	153	77
2	119	51
5	114	53
7	103	47
dry condition	40.5	

## Calculation of refractive index for urea solutions

The calculation of the refractive index for aqueous solutions containing different amounts of urea is based on the following empirical equations:

$$c_u[\text{mol/L}] = 117.66(\Delta n) + 29.753(\Delta n)^2 + 185.56(\Delta n)^3 \quad (\text{S1})$$

where  $\Delta n$  is the difference in refractive index between the denaturant solution and water (or buffer) at the sodium D line. This equation for urea solutions is based on the data of Warren and Gordon<sup>1</sup> and it is an established method to correlate the concentration of urea with the measured refractive index<sup>2</sup>. Table S1 reports the values of the refractive index  $n$  calculated for solution with different urea concentration.

Table S2: Refractive index of aqueous urea solutions containing different concentrations of the solute.

$c_u$ [mol/L]	$n$
0	1.3325
0.1	1.3334
0.2	1.3342
2	1.3495
5	1.3745
7	1.3910

## Calculation of the initiator grafting density

The grafting density of the self-assembled initiator monolayer, 2-bromo-2-methyl-N-3-(triethoxysilyl) propyl propanamide (BTPAm), was calculated from geometrical considerations. In particular, from the molecular structure of BTPAm, the molecular area ( $A_{\text{cyl}}$ ) was computed by using the Software Chem3D Pro (version 12.0.2.1076, CambridgeSoft). From this value and assuming a cylindrical shape, first the radius ( $R_{\text{cir}}$ ) and then the area occupied by the molecule onto the surface ( $A_{\text{cir}}$ ) were estimated, considering the cylinder height equal to 0.7 nm, which is the thickness of the initiator monolayer measured by ellipsometry. Finally, the number of molecules per  $\text{nm}^2$  was calculated assuming a surface coverage of 69%, as reported in literature for the same initiator<sup>3</sup>. The initiator grafting density ( $\sigma$ ) obtained by this approach was equal to 0.40 molecule/ $\text{nm}^2$ .

The experimental data are reported in Table S3.

Table S3: Parameters describing the dimension of a BTPAm molecule and calculated grafting density ( $\sigma$ ).

$A_{\text{cyl}}$ [ $\text{nm}^2$ ]	$R_{\text{cir}}$ [nm]	$A_{\text{cir}}$ [ $\text{nm}^2$ ]	$\sigma$ [ $\text{nm}^{-2}$ ]
6.55	0.73	1.70	0.4

It is worth to mention that the method reported above represents a rough approximation of the molecular dimensions of the surface-grafted initiator molecule. However, the calculated values showed a good agreement with the grafting densities reported in literature<sup>3,4</sup> from experimentally determined molecular weight of the grafted polymer chains.

## Temperature-induced brush collapse of PNIPAM

The percentage of brush collapse was calculated according to the following equation:

$$\text{Collapse} = \frac{d_{\text{sw}}(328\text{K}) - d_{\text{sw}}(288\text{K})}{d_{\text{sw}}(288\text{K})} \cdot 100 [\%] \quad (\text{S2})$$

where  $d_{\text{sw}}(288\text{K})$  and  $d_{\text{sw}}(328\text{K})$  are the thickness of the swollen brush at 288 K and 328 K, respectively. The obtained data are reported in Fig. S1. The reduced brush collapse in urea solutions compared to pure water is the result of two main effects: i) the higher energetic stability of a stretched conformation at low urea concentrations ( $c_{\text{u}} \leq 0.5 \text{ mol/L}$ ), and ii) the direct binding of the osmolyte to the polymer chains at higher urea concentrations ( $c_{\text{u}} \geq 2 \text{ mol/L}$ ), which reduces the water content inside the brush and therefore causes a lower thickness decrease by water extrusion upon the phase transition.

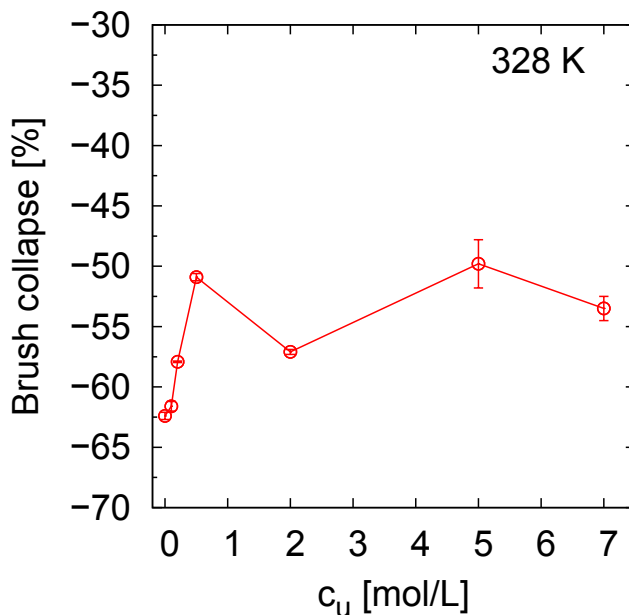


Figure S1: Percentage of brush collapse as a function of urea concentration calculated according to eq. S2.

## Derivation of the relation between $\Delta T_{\text{tr}}$ and $\Delta H_{\text{dehy}}$ of PNIPAM brushes in presence of urea

The process of dehydration of PNIPAM brushes above the critical solution temperature can be considered as a phase transition of water from the brush to the bulk phase.

In absence of urea, the water in the bulk is in equilibrium with the water in the brush at the temperature  $T_{\text{tr}}^0$ , *i.e.* the transition temperature from swollen to collapsed state for PNIPAM brushes in pure water.

By defining the molar ratio of urea as  $\chi_3$ , at  $T=T_{\text{tr}}^0$  and  $\chi_3=0$  (in absence of urea), the chemical potential of water in the brush,  $\mu_{\text{H}_2\text{O,brush}}$ , and in the bulk,  $\mu_{\text{H}_2\text{O,bulk}}$ , can be written as

$$\mu_{\text{H}_2\text{O,brush}} = \mu_{\text{H}_2\text{O,brush}}^\bullet + RT \ln(1 - \chi_2) \quad (\text{S3})$$

$$\mu_{\text{H}_2\text{O,bulk}} = \mu_{\text{H}_2\text{O,bulk}}^\bullet \quad (\text{S4})$$

where  $\mu_{\text{H}_2\text{O,brush}}^\bullet$  and  $\mu_{\text{H}_2\text{O,bulk}}^\bullet$  represent the standard chemical potential of water in the brush and in the bulk state, respectively,  $\chi_2$  is the NIPAM monomer molar fraction,  $R$  the molar gas constant and  $T$  the temperature. Since the two phases (H<sub>2</sub>O-brush and H<sub>2</sub>O-bulk) are in equilibrium at  $T_{\text{tr}}^0$ , the chemical potential of water in each phase will be the same, therefore

$$\Delta(d\mu_{\text{H}_2\text{O}}) = (d\mu_{\text{H}_2\text{O,brush}} - d\mu_{\text{H}_2\text{O,bulk}}) = 0. \quad (\text{S5})$$

Now the addition of urea is considered. Under the assumption that the amount of urea in the brush phase is negligible, it follows that

$$d\mu_{\text{H}_2\text{O,brush}} = R d \ln(1 - \chi_{3,\text{brush}}) \approx 0 \quad (\text{S6})$$

$$d\mu_{\text{H}_2\text{O,bulk}} = R d \ln(1 - \chi_{3,\text{bulk}}) \quad (\text{S7})$$

with  $\chi_3$  the molar fraction of urea. The addition of urea implies that the temperature at which the two phases, H<sub>2</sub>O-brush and H<sub>2</sub>O-bulk, are in equilibrium will be shift at a new transition temperature  $T_{\text{tr}}$ . The change of chemical potential accompanying the change of equilibrium temperature can be written as

$$d \left( \frac{\mu_{\text{H}_2\text{O,brush}}}{T} \right) = \frac{\partial \left( \frac{\mu_{\text{H}_2\text{O,brush}}}{T} \right)}{\partial T} dT \quad (\text{S8})$$

$$d \left( \frac{\mu_{\text{H}_2\text{O,bulk}}}{T} \right) = \frac{\partial \left( \frac{\mu_{\text{H}_2\text{O,bulk}}}{T} \right)}{\partial T} dT \quad (\text{S9})$$

In order to meet the equilibrium criterion of equal chemical potential of water in all phases, both the addition of urea and the temperature shift are considered:

$$\frac{\partial \left( \frac{\mu_{\text{H}_2\text{O,bulk}}}{T} \right)}{\partial T} dT + R d \ln(1 - \chi_{3,\text{bulk}}) = \frac{\partial \left( \frac{\mu_{\text{H}_2\text{O,brush}}}{T} \right)}{\partial T} dT \quad (\text{S10})$$

which can be more conveniently written as

$$\frac{\partial \left( \frac{\mu_{\text{H}_2\text{O,bulk}} - \mu_{\text{H}_2\text{O,brush}}}{T} \right)}{\partial T} dT = -R d \ln(1 - \chi_{3,\text{bulk}}) \quad (\text{S11})$$

The change of chemical potential can be expressed in terms of change of free enthalpy associated to a process, which in this case will be the brush dehydration due to the extrusion of water from the brush to the bulk phase,

$$\mu_{\text{H}_2\text{O,bulk}} - \mu_{\text{H}_2\text{O,brush}} = \Delta G_{\text{dehy}}^0 \quad (\text{S12})$$

By combining eq.S11 and eq.S12,

$$\frac{\partial \left( \frac{\Delta G_{\text{dehy}}^0}{T} \right)}{\partial T} dT = -R d \ln(1 - \chi_{3,\text{bulk}}) \quad (\text{S13})$$

$$-\frac{\Delta H_{\text{dehy}}}{T^2} dT = -R d \ln(1 - \chi_{3,\text{bulk}}) \quad (\text{S14})$$

$$\int_{T_{\text{tr}}^0}^{T_{\text{tr}}} \frac{\Delta H_{\text{dehy}}}{T^2} dT = R \int_0^{\chi_u} d \ln(1 - \chi_{3,\text{bulk}}) \quad (\text{S15})$$

$$\Delta H_{\text{dehy}} \left( -\frac{1}{T_{\text{tr}}} + \frac{1}{T_{\text{tr}}^0} \right) = R \ln(1 - \chi_u) \quad (\text{S16})$$

$$\Delta H_{\text{dehy}} \left( \frac{T_{\text{tr}} - T_{\text{tr}}^0}{T_{\text{tr}} \cdot T_{\text{tr}}^0} \right) = R \ln(1 - \chi_u) \quad (\text{S17})$$

$$\Delta H_{\text{dehy}} \left( \frac{\Delta T_{\text{tr}}}{(T_{\text{tr}}^0)^2} \right) = R \ln(1 - \chi_u) \quad (\text{S18})$$

By approximating  $\ln(1 - \chi_u) \sim -\chi_u$ , the relation between change of transition temperature and dehydration enthalpy is obtained:

$$\Delta T_{\text{tr}} = -\frac{R(T_{\text{tr}}^0)^2}{\Delta H_{\text{dehy}}} \chi_u \quad (\text{S19})$$

## Numerical study

### Hydrogen bond analysis

In order to study the hydration properties, we have evaluated the number of hydrogen bonds of the PNIPAM molecule with water and urea, respectively, and compared them to the values obtained in pure aqueous solution. The corresponding results are shown in Table S4.

Table S4: Hydrogen bonds between PNIPAM and urea  $H_B^u$ , between PNIPAM and water  $H_B^w$  and the total number of hydrogen bonds between PNIPAM and urea and water  $H_B^t$  for different gyration radii ( $R_g$ ), urea concentrations  $c_u$  and temperatures  $T$ . The forward rate constants of hydrogen bonds between urea and PNIPAM ( $k^u$ ) and water and PNIPAM ( $k^w$ ) are plotted in the last two columns.

Concentration	T [K]	$R_g$ [nm]	$H_B^w$	$H_B^u$	$H_B^t$	$k^u$ [ps <sup>-1</sup> ]	$k^w$ [ps <sup>-1</sup> ]
low	288	0.8	$33.87 \pm 0.14$	$0.47 \pm 0.04$	$34.34 \pm 0.18$	0.85	2.43
low	328	0.8	$29.56 \pm 0.14$	$0.38 \pm 0.03$	$29.94 \pm 0.17$	1.06	3.02
low	288	1.4	$38.85 \pm 0.24$	$0.57 \pm 0.05$	$39.42 \pm 0.29$	1.26	1.91
low	328	1.4	$34.13 \pm 0.16$	$0.48 \pm 0.03$	$34.61 \pm 0.19$	0.84	2.40
high	288	0.8	$9.17 \pm 0.09$	$7.45 \pm 0.23$	$16.62 \pm 0.32$	2.26	2.84
high	328	0.8	$6.89 \pm 0.07$	$8.55 \pm 0.12$	$15.44 \pm 0.19$	2.40	3.30
high	288	1.4	$9.37 \pm 0.07$	$7.42 \pm 0.09$	$16.79 \pm 0.16$	3.85	2.84
high	328	1.4	$7.41 \pm 0.09$	$8.25 \pm 0.60$	$15.66 \pm 0.69$	4.11	3.69
pure	288	0.8	$36.25 \pm 0.19$	$0.00 \pm 0.00$	$36.25 \pm 0.19$	—	2.09
pure	328	0.8	$31.62 \pm 0.16$	$0.00 \pm 0.00$	$31.62 \pm 0.16$	—	3.00
pure	288	1.4	$40.93 \pm 0.29$	$0.00 \pm 0.00$	$40.93 \pm 0.29$	—	1.71
pure	328	1.4	$36.87 \pm 0.16$	$0.00 \pm 0.00$	$36.87 \pm 0.16$	—	2.21

## Preferential binding coefficients

The numerical values for the preferential binding coefficient evaluated at  $r = 1.8$  nm are shown in Tab. S5.

## Binding life times

In order to classify the nature of hydrogen bonds with respect to their related energetic contributions according to transition state theory in terms of a Luzar-Chandler approach<sup>5,6</sup>, one can calculate the hydrogen bond life times via

$$\tau \sim \exp(\Delta F^*/k_B T) \tag{S20}$$



Table S5: The preferential binding coefficient  $\nu_{23}$  between urea and the PNIPAM backbone has been evaluated at  $r = 1.8$  nm and calculated according to the equation given in the main article.

Concentration	T [K]	$R_g$ [nm]	$\nu_{23}$	System
low	288	0.8	-1.08	lc288
low	328	0.8	-1.00	lc328
low	288	1.4	-0.64	ls288
low	328	1.4	-0.55	ls328
high	288	0.8	-0.69	hc288
high	328	0.8	5.07	hc328
high	288	1.4	0.25	hs288
high	328	1.4	3.55	hs328

where  $\Delta F^*$  denotes the activation binding free energy. The neglected prefactor can be assumed to be constant and has the unit pico seconds. The corresponding results for the

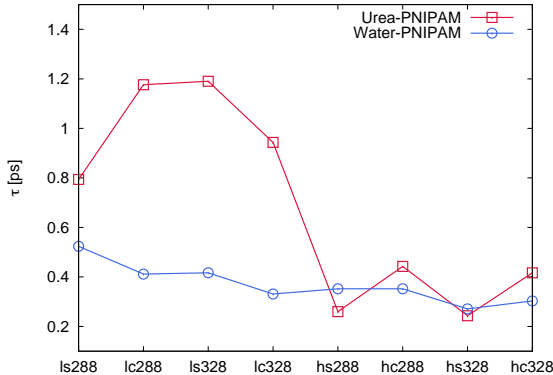


Figure S2: Hydrogen bond lifetimes  $\tau$  for water-PNIPAM hydrogen bonds (red line) and urea-PNIPAM hydrogen bonds (blue line) for the different systems. The abbreviations are given in the main text. The lines are guides for the eyes.

hydrogen bond lifetimes for water-PNIPAM and urea-PNIPAM hydrogen bonds are shown in Fig. S2 and Table S4. Interestingly, the lowest values for the urea hydrogen bond life times can be observed at high molar concentrations and both temperatures (hs288 and hs328) whereas the highest values are given for low molar urea concentrations. It can be seen that in presence of high urea concentrations nearly all water binding activation free energies are

lowered in terms of smaller life times compared to low concentrations of urea. Indeed, the binding of urea to PNIPAM compared to water molecules is energetically more favorable for low temperatures (ls288,lc288,ls328 and lc328). This means that, for energetic reasons, high concentrations of urea mostly result in a weakening of the water interactions. This energetic aspect is in agreement to the experimentally observed shift of  $T_{tr}$  to lower temperatures in presence of any urea concentration: the weakening of PNIPAM-water interaction contributes to the decrease of the temperature required for chain dehydration.

## References

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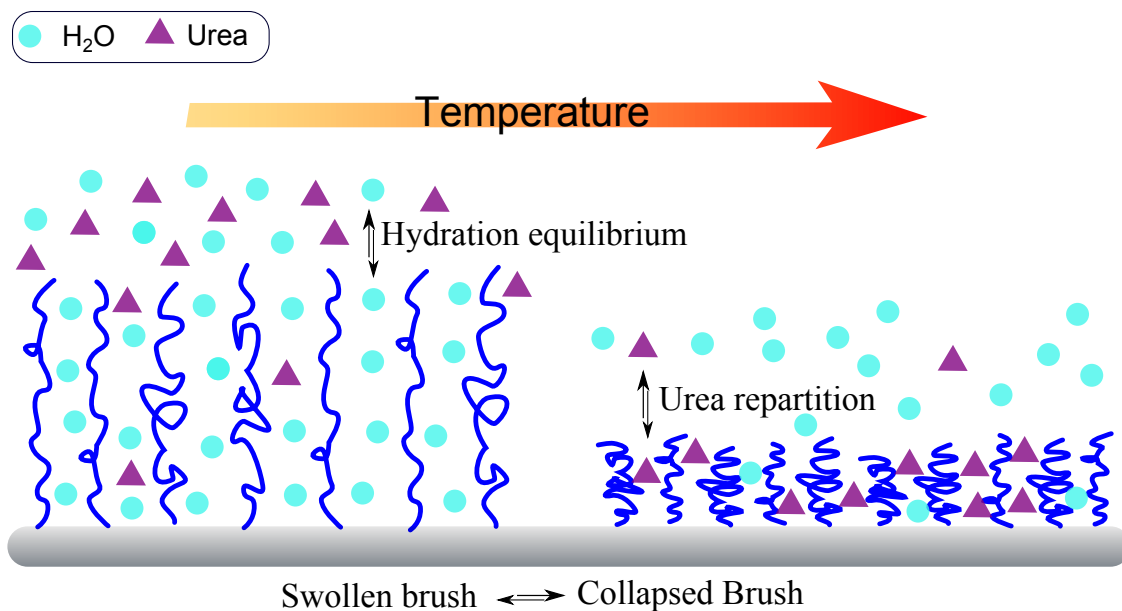


Figure S3: Graphical and textual abstract for the contents pages

The concentration-dependent binding of urea to poly(N-isopropylacrylamide) chains influences the conformational behavior of the macromolecule, which is the result of a subtle interplay between hydration equilibrium and repartition of urea among the polymer chains.