

Supporting Information

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1. Characterization of ene-functionalized polymers

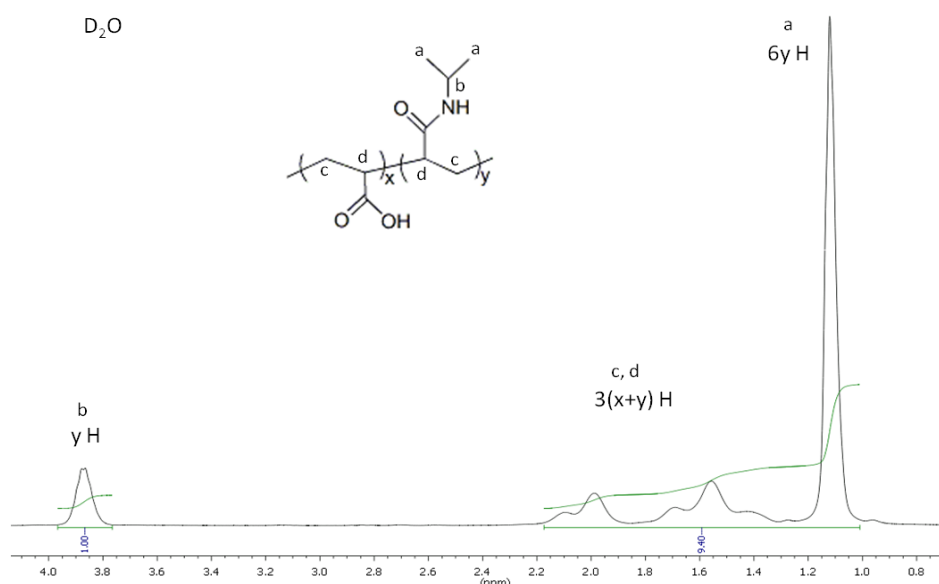


Figure S1. ^1H NMR spectrum of P(AA-co-NIPAM) copolymer synthesized by free radical polymerization. The ratio of acrylic acid in the P(AA-co-NIPAM) chains can be calculated.

The P(NIPAM-co-AA) copolymer is characterized by SEC chromatography and ^1H NMR spectroscopy. SEC provides the weight-average molar mass (M_w) and polymer dispersity index (PDI) of the copolymer, while ^1H NMR gives the ratio of AA in the copolymer. The weight-average molar masses (M_w) obtained are quite close to the expected value. For example, 254 kg/mol and 195 kg/mol are found when 250 kg/mol was targeted. The polymer dispersity index (PDI) of the copolymer is quite low, around 2, and even lower than 2 for copolymers with molar mass higher than 200 kg/mol. The ^1H NMR spectrum shown Figure S1 is an example of spectra obtained with P(NIPAM-co-AA) copolymers in D_2O . The peak at 1.0-1.2 ppm corresponds to the protons on the methyl group (marked as "a"), the peak at 3.8-4.0 ppm to the proton connected to the secondary carbon of the isopropyl group (marked as "b") and the peaks at 1.3-2.2 ppm to the protons connected to the carbon on the main chain (marked as "c" and "d"). The ratio of AA in the polymer can be determined by comparing the integral of the peak. We found here a ratio of AA in the copolymer equal to 9%.

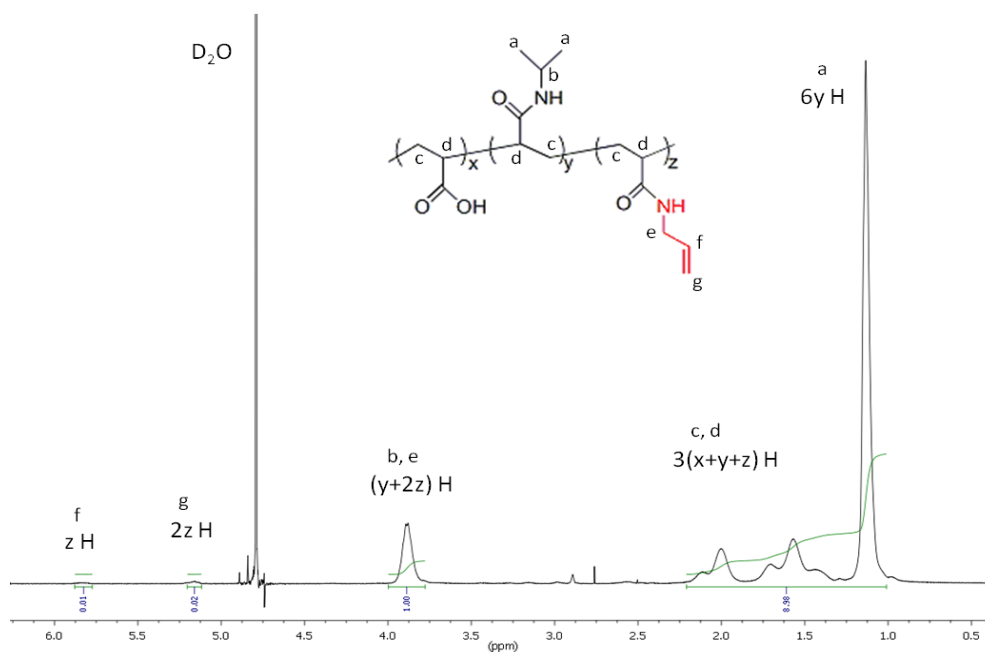


Figure S2. ^1H NMR spectrum of ene-functionalized copolymers obtained by peptide bond using allylamine. The ratio of ene-functionalization can be calculated.

The ^1H NMR spectrum shown Figure S2 is an example of spectra obtained with ene-modified PNIPAM in D_2O . The peak at 1.0-1.2 ppm corresponds to the protons on the methyl group (marked as “a”), the peak at 3.8-4.0 ppm to the proton connected to the secondary carbon of the isopropyl group and the one connected to the primary carbon on the allyl group (marked as “b” and “e” respectively), the peaks at 1.3-2.2 ppm to the protons connected to the carbon on the main chain (marked as “c” and “d”) and the peaks at 5.8 ppm and 5.2 ppm to the protons on the ene group (marked as “f” and “g” respectively). The ratio of ene-functionalization can be determined by comparing the integral of the peak. We found here a ratio of AA in the copolymer equal to 2%. The ratios of ene-groups for most of polymers are equal to 2%.

2. Characterization by neutron reflectivity

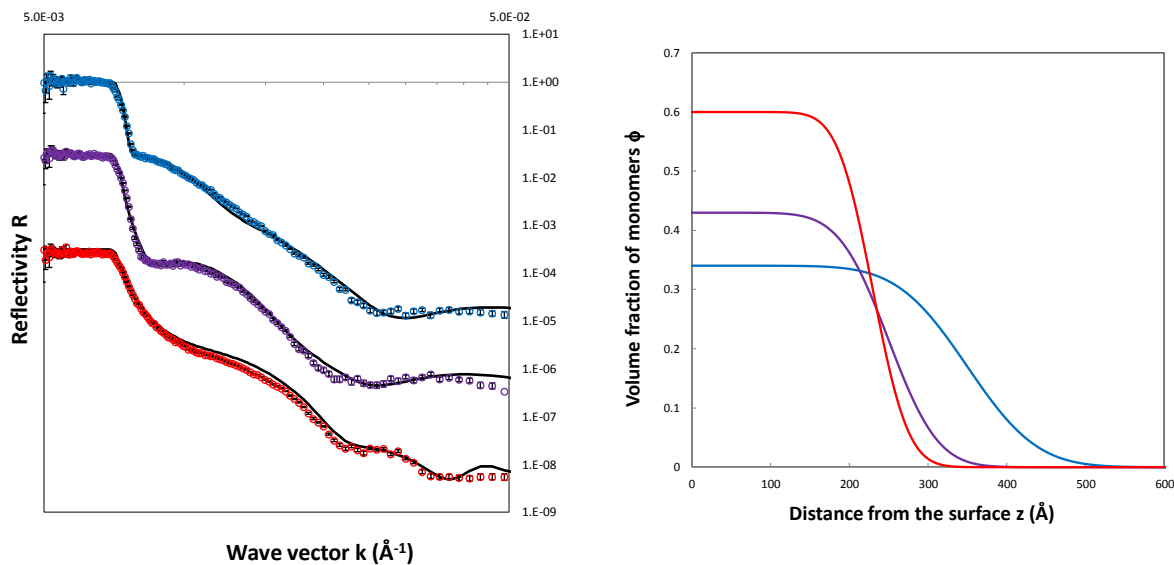


Figure S3. (Left) Neutron reflectivity curves of PNIPAM hydrogel film (with dry thickness equal to 12.5 nm) in water at three temperatures 25°C, 34°C and 40°C. Also are shown the best fitting of the experimental data. Circles are for data and lines for fitting. (Right) Volume fraction profiles corresponding to the best fitting. The same color code as the left figure with reflectivity curves was used: blue for 25°C, purple for 34°C and red for 40°C.

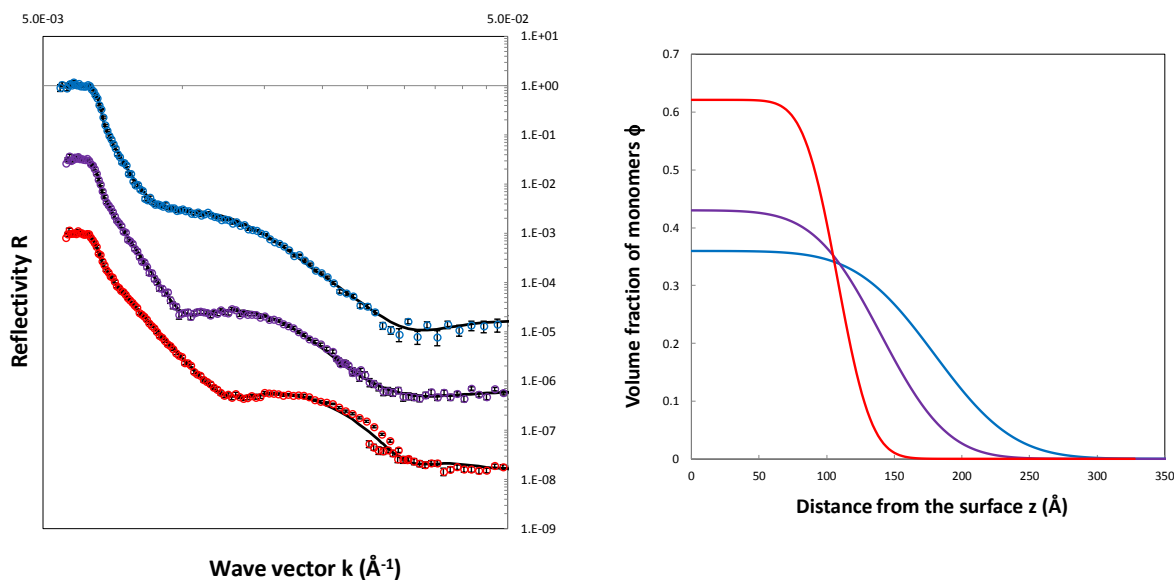


Figure S4. (Left) Neutron reflectivity curves of PNIPAM hydrogel film (with dry thickness equal to 6.7 nm) in water at three temperatures 25°C, 34°C and 40°C. Also are shown the best fitting of the experimental data. Circles are for data and lines for fitting. (Right) Volume fraction profiles corresponding to the best fitting. The same color code as the left figure with reflectivity curves was used: blue for 25°C, purple for 34°C and red for 40°C.

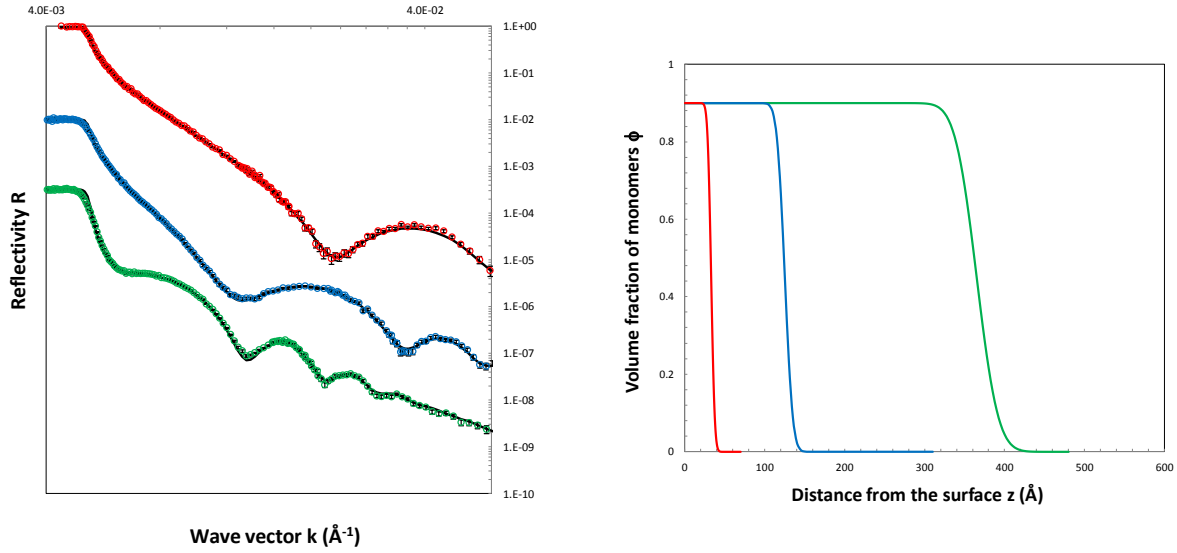


Figure S5. (Left) Neutron reflectivity curves of PNIPAM hydrogel film in air with dry thickness at three temperatures 6.7 nm, 12.5 nm and 36.4 nm. Also are shown the best fitting of the experimental data. Circles are for data and lines for fitting. (Right) Volume fraction profiles corresponding to the best fitting. The same color code as the left figure with reflectivity curves was used: red for 6.7 nm, blue for 12.5 nm and green for 36.4 nm.

Samples	h_a (nm)	h_w (nm)	σ (nm)	Nb (10^{-6} \AA^{-2})	ϕ	h_w/h_a	σ/h
SN7-air	6.7		0.5	0.90			0.07
SN7-25°C	6.7	18.0	6.5	2.36	0.36	2.7	0.36
SN7-34°C	6.7	14.1	5.5	2.02	0.43	2.1	0.39
SN7-40°C	6.7	11.0	2.8	0.96	0.62	1.6	0.25
SN12-air	12.5		1.1	0.90			0.09
SN12-25°C	12.5	35.4	9.9	2.50	0.34	2.8	0.28
SN12-34°C	12.5	25.0	7.0	2.00	0.43	2.0	0.28
SN12-40°C	12.5	22.6	1.1	1.07	0.60	1.8	0.05
SN36-air	36.4		3.0	0.94			0.08
SN36-25°C	36.4	110.7	18.8	2.60	0.32	3.0	0.17
SN36-34°C	36.4	85.6	14.2	2.10	0.41	2.4	0.16
SN36-35°C	36.4	81.2	12.8	2.00	0.43	2.2	0.16
SN36-40°C	36.4	55.5	3.5	1.00	0.61	1.5	0.06

Table S1. Characteristics of the PNIPAM gel films measured by neutron reflectivity in air and in water at different temperatures. The samples are named as SNx-y where x stands for the thickness in air and y for the experimental conditions. Are reported the thickness in water h_w (the thickness in air h_a is reminded), the free interface width σ using erf error function and the scattering length density Nb . The average volume fraction of the layer ϕ is calculated from Nb (assuming that the scattering length density of pure PNIPAM is $1.0 \cdot 10^{-6} \text{ \AA}^{-2}$ and that of silicon equal to $2.1 \cdot 10^{-6} \text{ \AA}^{-2}$). The swelling ratio h_w/h_a (also equal to $1/\phi$) and the relative interface width σ/h are also calculated.

The experimental and best fitted curves are displayed for PNIPAM hydrogel films in water. The samples have thickness in air equal to 6.7 nm and 12.5 nm. Also are given the corresponding density profiles. They are additional to data shown for the sample with thickness equal to 36.4 nm. It is helpful for the discussion of the effect of thickness on the swelling-collapse properties.

In addition, neutron reflectivity data for PNIPAM hydrogel films in air are provided. The values of thickness in air measured in neutron reflectivity and ellipsometry are very similar for all samples with little difference of a few angstroms.

The scattering length density found for PNIPAM hydrogel in air is about $0.9 \times 10^{-6} \text{ \AA}^{-2}$ (from 0.88×10^{-6} to $0.94 \times 10^{-6} \text{ \AA}^{-2}$). From this value, the water content of the PNIPAM gel film in air can be calculated. It was shown by ellipsometry measure that the water content is 10% at the maximum in air (with humidity ratio between 20% and 60%). To get water content of 10%, the scattering length density found for the PNIPAM gel film in air and in dry state (pure PNIPAM without any water) should be equal to $0.90 \times 10^{-6} \text{ \AA}^{-2}$ and $1.05 \times 10^{-6} \text{ \AA}^{-2}$ respectively (as the scattering length density of water is $-0.56 \times 10^{-6} \text{ \AA}^{-2}$).

The results obtained in neutron reflectivity are consistent with those found by ellipsometry even if it is difficult to calculate exactly the scattering length density of pure PNIPAM gel film for many reasons: (i) for the synthesis of PNIPAM gel films, we used linear chains containing 95% of NIPAM and 5% of AA, 2% of AA being ene-functionalized to react with dithiol cross-linkers, (ii) the density of a PNIPAM gel film is not easily determined with precision, Vidyasagar et al. (reference 15) used 1.2 g/cm^2 as the surface density whereas the volume density of PNIPAM chains given by Polymer Handbook is 1.1 g/cm^3 . Vidyasagar et al. (reference 15) estimated the scattering length density to be $0.96 \times 10^{-6} \text{ \AA}^{-2}$ and their PNIPAM gel films were synthesized with a benzophenone UV-irradiated cross linker. In our case, we obtained by calculation for PNIPAM a scattering length density equal to $0.89 \times 10^{-6} \text{ \AA}^{-2}$ (using $\rho = 1.2 \text{ g/cm}^3$ as the density), for polyacrylic acid $1.5 \times 10^{-6} \text{ \AA}^{-2}$ ($\rho = 1.3 \text{ g/cm}^3$) and for poly(sodium acrylate) $2.24 \times 10^{-6} \text{ \AA}^{-2}$ ($\rho = 1.6 \text{ g/cm}^3$). It means that it is not possible to calculate with high precision the scattering length density but $1 \times 10^{-6} \text{ \AA}^{-2}$ could be a reasonably estimated value.

3. Characterization by AFM

Imaging the surface of a hydrogel immersed in water is challenging. Actually, the AFM contact mode leads to a dramatic loss of resolution and the tapping mode is much more difficult to set up in water than in air. Moreover, as the young modulus is very weak (a few kPa), we expect that the surface is deformed instead of the cantilever regardless of the mode used, leading to a misinterpretation of the height signal. This is often ignored in the literature considering that the deformation of the cantilever is only related to the young modulus of the sample and the stiffness of the contact is rarely mentioned. This problem is illustrated by the approach curves shown in Figure S4. The three approach curves are obtained on a PNIPAM film (with a thickness equal to 237nm in the dry state and 950 nm in the swollen state) using cantilevers of different stiffness, $k=0.01$ N/m, $k=0.03$ N/m and $k=0.1$ N/m. The Y-axis of the approach curve represents the deflection of the cantilever. The X-axis represents the height of the cantilever. The reference approach curve is acquired on naked surfaces of silicon substrates. It is infinitely rigid whatever the stiffness of the cantilever with a slope equal to 1. This figure illustrates the difficulty of imaging soft matter. When using a cantilever of very low stiffness ($k=0.01$ N/m), the approach curve is dominated by adhesion forces whereas the curve displays a deep indentation of the cantilever in the gel when using a cantilever of higher stiffness ($k=0.1$ N/m). The magnitude of indentation when using a cantilever of high stiffness is not negligible compared to the thickness of the sample. In both case, the use of a cantilever of very high or very low stiffness can prevent to properly measure the topography of the hydrogel surface in water. In the case of the cantilever of stiffness $k=0.01$ N/m, we interpret the jump by the adhesion of the polymer on the tip when the contact is initiated. The position of the jump is arbitrary put at zero on the X-axis. The interesting point is that in spite of the strong effect of adhesion forces, the slope of the approach curve with $k=0.01$ N/m is close to one, indicating that the cantilever deforms more than the hydrogel during the approach phase. In the QNM (Quantitative Mechanical Measurement) mode, a sinusoidal modulation (1 kHz) is applied on the cantilever in the vicinity of the surface. This excitation frequency should remain ten times below the frequency of the first eigenmode of the cantilever. In these conditions, a QNM experiment can be viewed as a succession of rapid approach-retract curves, each curve being limited by a set-point force. It means that some viscoelastic effects could induce some mistakes in the interpretation of the topography. This preliminary result obtained with approach curves is indicative of the limit of the QNM mode used for our AFM experiments. Considering the Figure S4, a QNM experiment should be carried out with a cantilever of stiffness 0.01 N/m. But the imaging conditions are not perfectly stable and the best compromise when imaging the surface of the hydrogel is to use a tip with stiffness around 0.03 N/m.

In order to confirm the reliability of the topography, QNM images of a PNIPAM film (with thickness equal to 237 nm in dry state and 950 nm in water) were acquired with the same cantilever ($k=0.03$ N/m) with different QNM conditions of set-point (from 80 pN up to 2 nN). The same value of the width of the height distribution was obtained. In another experiment, the same area of a PNIPAM film was imaged with cantilevers of different stiffness (0.01 N/m, 0.1 N/m and 0.5 N/m). The width of the height distribution remained unchanged whatever the value of the stiffness in this range. This experiment proves that, in spite of the strong effect of the adhesion, the QNM conditions are very reliable with respect to parameters such as the set-point and the stiffness of the cantilever in 0.01 N/m – 0.5 N/m range. It guarantees that the height is properly measured.

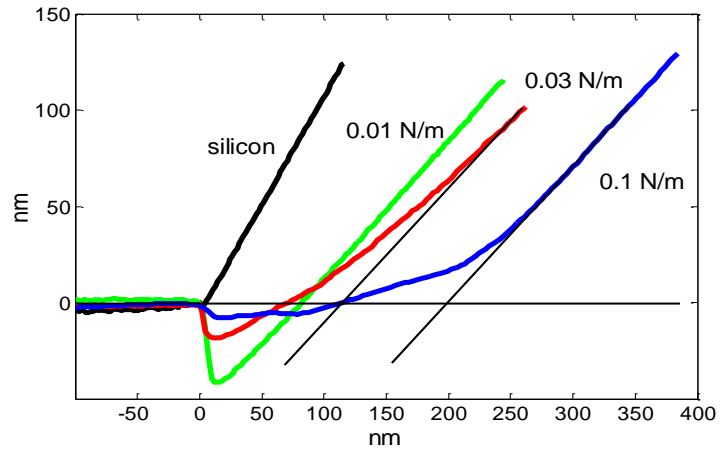


Figure S6. Approach curves obtained at 1 Hz on a PNIPAM film (with thickness equal to 237 nm in the dry state and 950 nm in the swollen state) with cantilevers of different stiffness. The black curve corresponds to the reference approach curve (obtained on silicon substrate) with a slope fixed at 1.