Supporting Information

Moisture changes inside hydrogel particles during their drying process investigated with fluorescence lifetime imaging

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S1 Confocal FLIM images and fit values at different drying times.

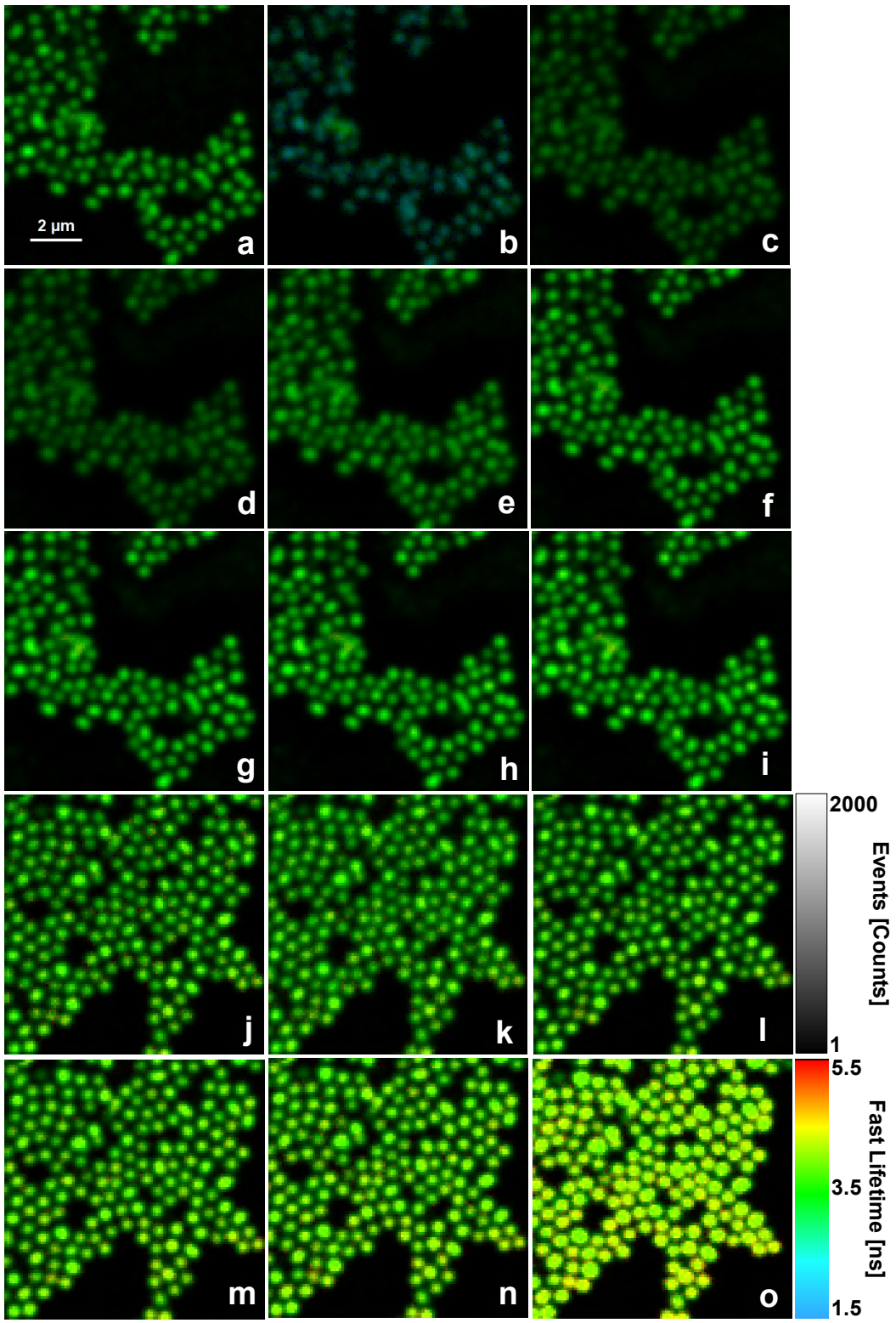

Figure S1 Confocal FLIM images of ATTO 655 labelled P(NIPAM-co-APMH) microgels measured at different drying times at open atmospheric pressure. a to o; spin coated microgel on plasma cleaned glass coverslip, 2, 3, 5, 8, 10, 12, 14, 16, 18, 30, 60, 120, 180, and 720 minutes after addition of water, respectively.

Figure S1 presents an extended series of FLIM images. The corresponding TCSPC curves were fitted with the following biexponential model:

$$\frac{I}{I_0} = a_1 \cdot \exp\left(-\frac{t}{\tau_1}\right) + a_2 \cdot \exp\left(-\frac{t}{\tau_2}\right) \tag{1}$$

with the fluorescence intensity I normalized to its initial value I_0 at $t = 0$, the relative amplitudes a_1 and a_2 and the corresponding fluorescence lifetimes τ_1 and τ_2.

The amplitude-weighted average fluorescence lifetime was calculated as follows:

$$\langle\tau\rangle = a_1 \cdot \tau_1 + a_2 \cdot \tau_2 \tag{2}$$

Table S1 Fitted TCSPC data obtained from recorded FLIM images at different drying times of the spin-coated microgel after adding water. The TCSPC decay profiles were best fitted using a bi-exponential decay equation, where τ_1 and τ_2 are two lifetimes with corresponding components a_1 and a_2, respectively. $\langle\tau\rangle$ is the amplitude-weighted average fluorescence lifetime. The χ^2 represents the quality of the fitting. All lifetime values have an uncertainty of 0.02 ns. The corresponding equilibrium RH values were calculated based on the calibration curve of $\langle\tau\rangle$ in Figure 4.

Drying time / min	τ_1 / ns	τ_2 / ns	a_1 / %	a_2 / %	$\langle\tau\rangle$ / ns	χ^2	calculated RH / %
spin coated μG	4.00	2.10	61	39	3.27	1.16	70
+10 μL H_2O	2.89	1.82	56	44	2.42	1.14	-
2	3.01	2.00	57	43	2.45	0.99	-
3	3.88	2.00	63	37	3.19	1.13	73
5	3.93	2.16	62	38	3.26	0.99	70
8	3.98	2.21	59	41	3.25	1.06	70
10	4.09	2.23	63	37	3.40	1.12	63
12	4.14	2.28	63	37	3.44	1.14	61
14	4.19	2.34	63	37	3.50	1.14	59
16	4.21	2.37	64	36	3.55	1.19	56
18	4.36	2.44	66	34	3.70	1.08	49
30	4.38	2.53	64	36	3.72	1.07	48
60	4.41	2.52	65	35	3.76	1.17	46
120	4.45	2.57	65	35	3.80	1.11	44
180	4.48	2.65	64	36	3.83	1.18	43
360	4.49	2.69	66	34	3.88	1.11	40
720	4.57	2.83	65	35	3.96	1.10	36

S2 Fitted TCSPC curves and residuals

Figure S2 shows the fits of TCSPC curves presented in Figure 2 of the main paper. The corresponding residuals are presented in Figure S3

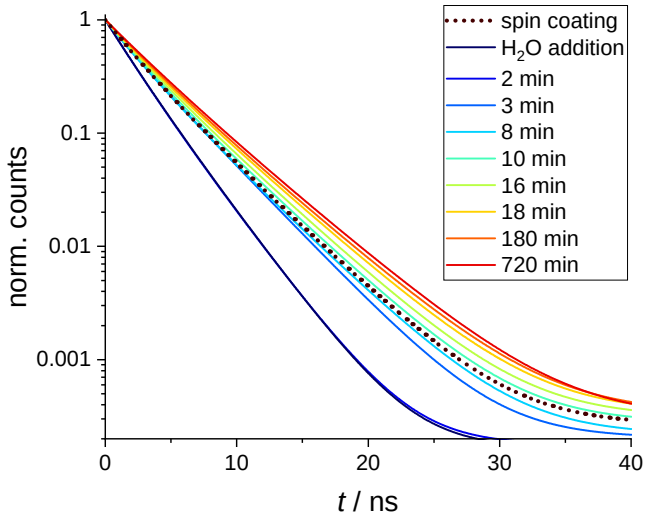

Figure S2 Normalized TCSPC bi-exponential fits curves.

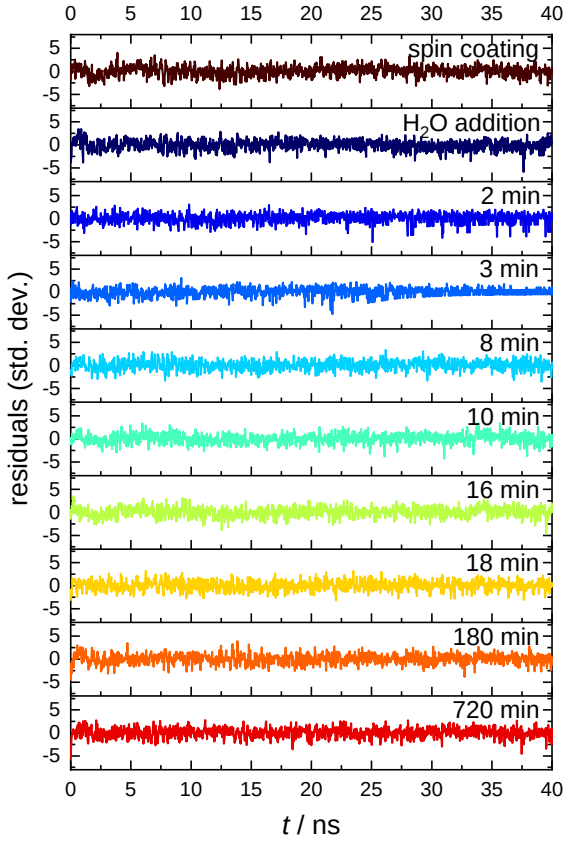

Figure S3 Residuals of normalized TCSPC bi-exponential fits.

S3 TCSPC curves and their fits obtained from ATTO 655 labeled microgels at different air humidities

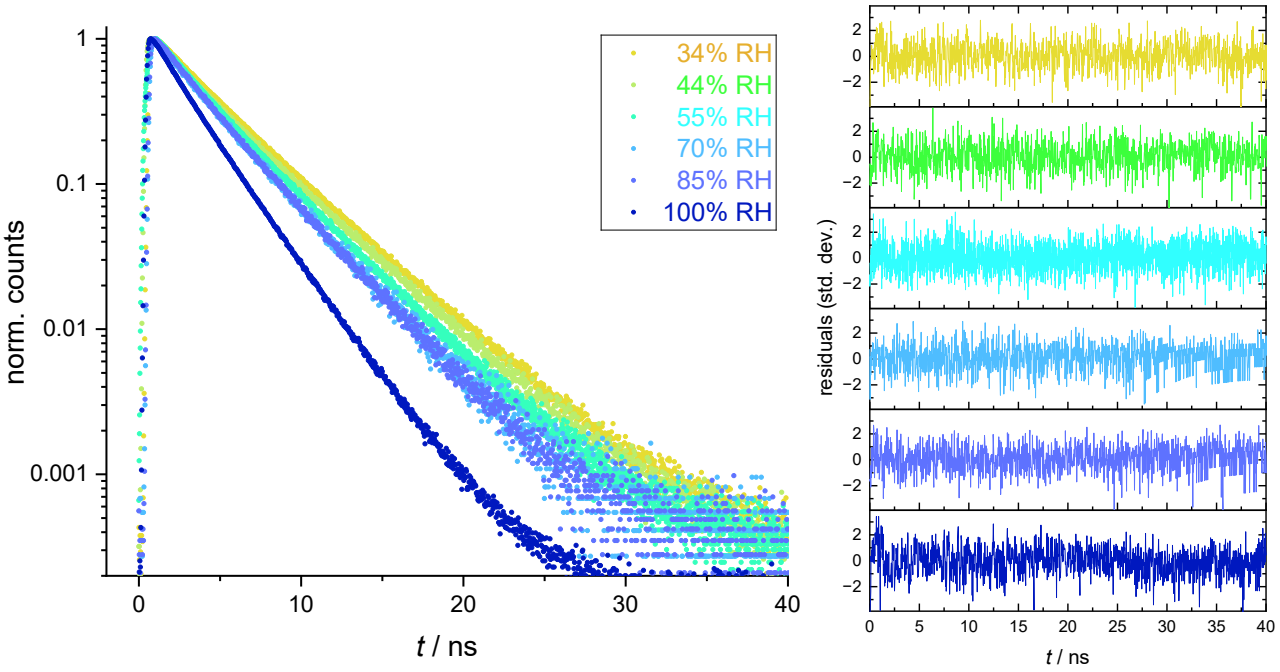

Figure S4 (Left) Normalized TCSPC curves of ATTO 655 labeled microgels at different air humidities and (right) the residuals of the corresponding biexponential fits. The fit values are presented in Table S2 (see below).

Table S2 Fitted TCSPC data obtained from FLIM images of the spin-coated P(NIPAM-co-APMH) microgels equilibrated at different relative humidities (RH). The 100% RH value was recorded for microgel covered with water. The TCSPC decay profiles were best fitted using a bi-exponential decay equation, where τ_1 and τ_2 are two lifetimes with corresponding relative amplitudes a_1 and a_2, respectively. $\langle\tau\rangle$ is the amplitude-weighted average fluorescence lifetime. The χ^2 represents the quality of the fitting. All lifetime values have an uncertainty of 0.02 ns.

RH	τ_1 / ns	τ_2 / ns	a_1 / %	a_2 / %	$\langle\tau\rangle$ / ns	χ^2
100%	2.89	1.82	56	44	2.42	1.14
85%	3.71	2.10	70	30	3.23	1.02
70%	3.79	2.20	68	32	3.30	1.04
55%	4.11	2.33	65	35	3.49	1.01
44%	4.47	2.80	62	38	3.84	1.13
34%	4.57	2.83	65	35	3.96	1.10

S4 Chemical structures of monomers and dye derivative

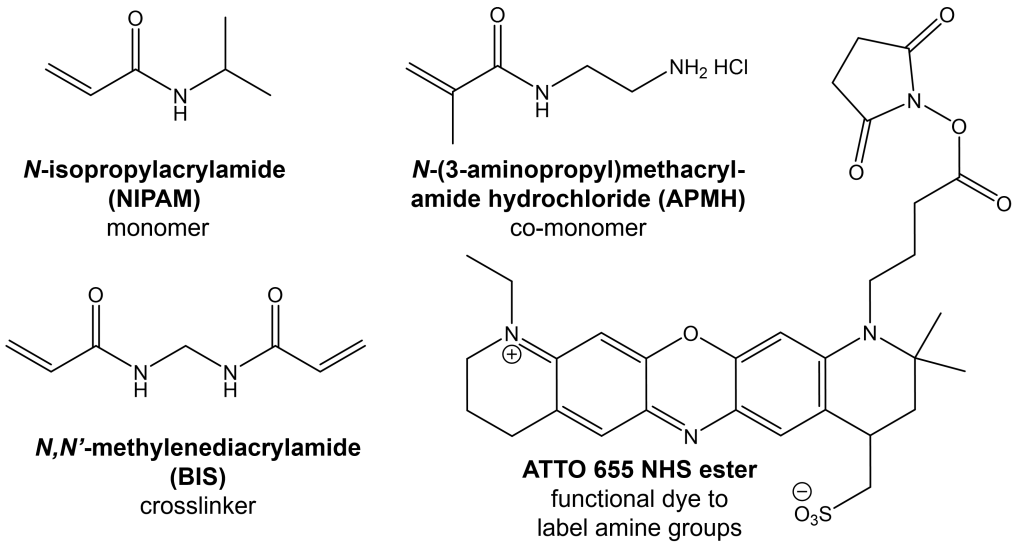

Figure S5 Chemical structures of monomer, co-monomer, crosslinker, and ATTO 655 dye.

S5 AFM images and analyses of swollen and collapsed microgels

All AFM measurements were conducted using the Bruke JPK NanoWizard 4 NanoOptics AFM. For all measurements, the microgel sample in pure water was spin coated (4000 rpm for 40 seconds followed by 2000 rpm for 1.5 seconds at room temperature) on a Hellmanex III-washed, plasma-cleaned coverslip.

S5.1 AFM measurements of dry microgels

For the measurements in dry state after spin-coating, a OTESPA Micro Cantilever from OPUS by MikroMasch was used in tapping mode. The cantilever had a nominal frequency of 300 kHz, a nominal spring constant of 26 N m^{-1}, a rectangular geometry with a nominal tip radius of 7 nm. The backside of the cantilever was coated with reflective aluminum. The alignment of the cantilever and the laser was done using an Olympus IX83 inverted microscope equipped with an Olympus Plan N Air 20$\times$ objective with a Numerical Aperture (NA) of 0.4. For calibration, the laser was located on the backside of the cantilever and the detector position was optimized to achieve the highest voltage. The resonance frequency, 260.4 kHz, and the spring constant, 39.8 N m^{-1} were measured via the thermal noise method. The drive frequency was manually set to approximately 80% of the amplitude maximum. The following parameters were applied: Image size 5 $\times$ 5 μm^2, pixel size 9.8 nm, line rate 0.5 Hz, setpoint 10 nm, gain 600, sample temperature 22 °C. The obtained AFM images were processed using the open source software Gwyddion by leveling the data, applying basic filters, correcting horizontal scars and by shifting the minimum data value to zero.

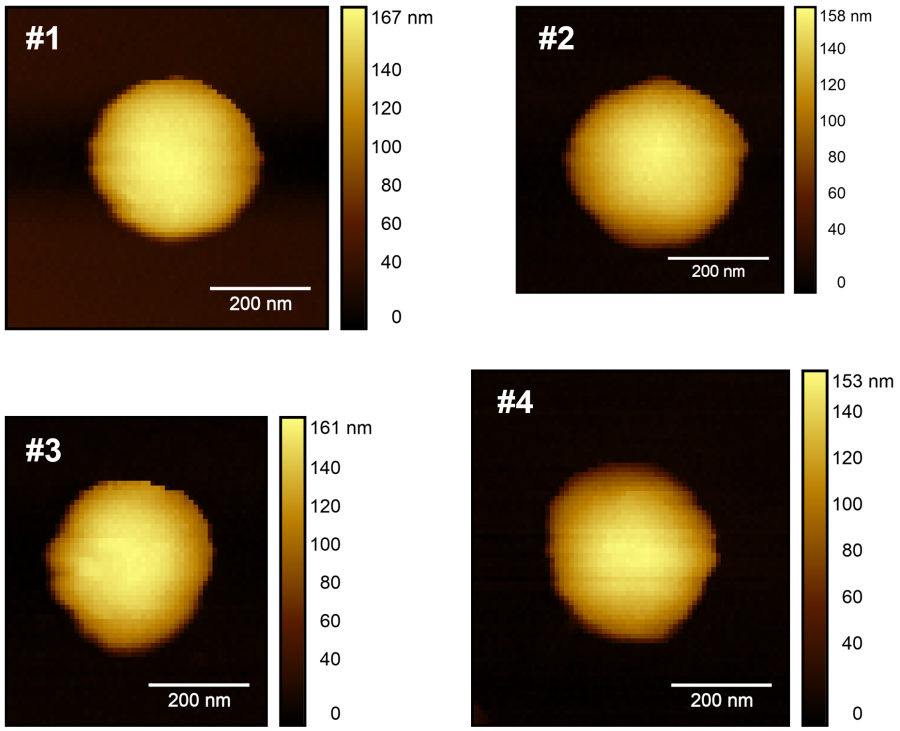

Figure S6 AFM images of four representative microgels in the dry state after spin-coating.

S5.2 AFM measurements of swollen microgels

For the measurements in water, water was added to the spin-coated microgels in a customized liquid cell. For these quantitative imaging (QI) measurements in liquid, a Bruker MSNL-10-E cantilever was used, featuring a nominal tip radius of 2 nm, a nominal resonance frequency of 38 kHz, and a nominal spring constant of 0.1 N/m. With a contact-based calibration, a sensitivity of 14.1 nm/V and a spring constant of 0.113 N/m were obtained. The measurements were performed with a setpoint of 1.000 nN, a cantilever speed of 10 μm/s, and a z-length of 1 μm, resulting in a curve duration of 100 ms and a pixel time of 220 ms.

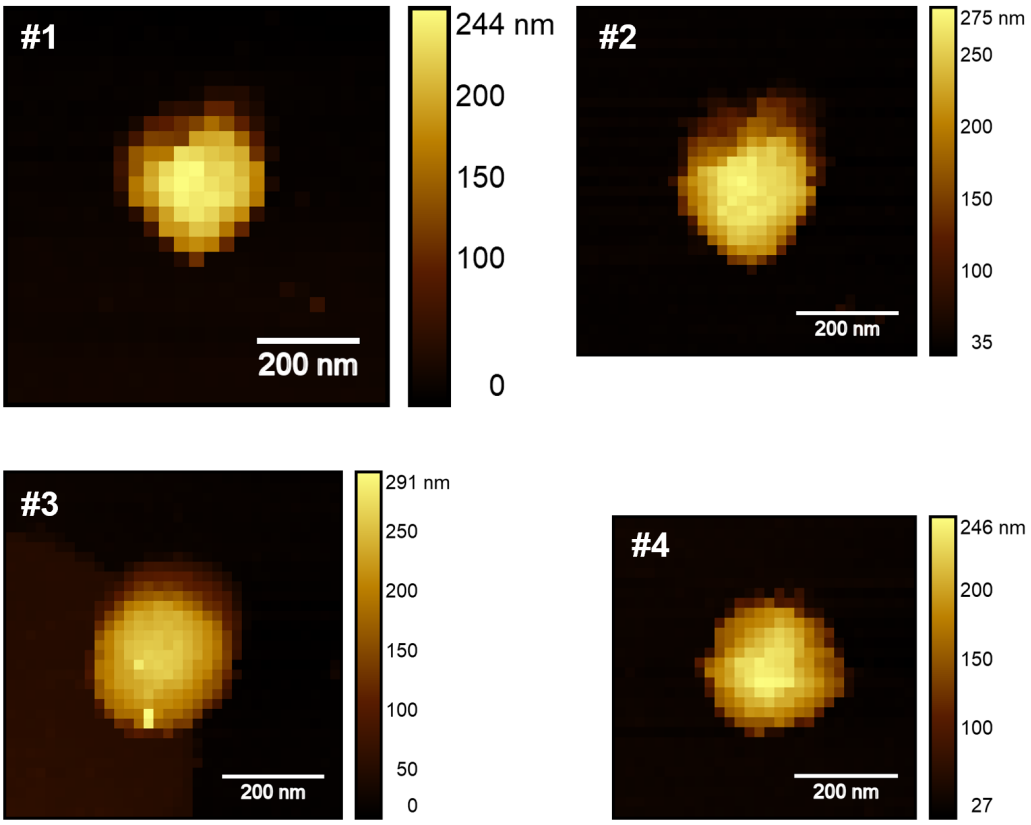

Figure S7 AFM images of four representative microgels in the swollen state after spin-coating and reswelling the sample with water.

S5.3 Comparison of the height profiles of swollen and collapsed microgels

As shown in Figure S8, the microgels are close to spherical in the swollen state. In the dry state they shrink by approximately 40% in z-direction whereas they expand slightly in lateral dimensions.

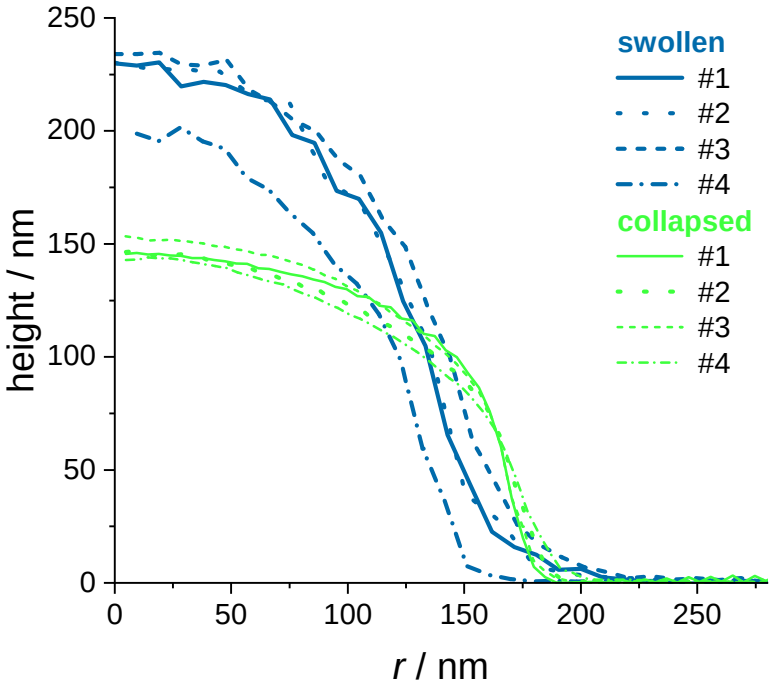

Figure S8 Radial height profiles of the microgels presented above for the wet (blue colour) and the spin-coated (green colour) sample. The profiles were determined using the open source software Gwyddion.