

Supplementary Materials for
**In situ imaging of the three-dimensional shape of soft responsive particles at
fluid interfaces by atomic force microscopy**

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Sci. Adv. **8**, eabq2019 (2022)
DOI: 10.1126/sciadv.abq2019

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1 Supplementary Experimental Data

Table 1: Microgels SLS (D_{sls}) and hydrodynamic diameter (D_h) in aqueous solution

Microgel	D_{sls} at 25°C [nm]	D_h at 22°C [nm]	D_h at 40°C [nm]	Volumetric swelling ratio
5 mol % BIS	800 ± 20	1150 ± 27	525 ± 3	10.6 ± 0.3
1 mol % BIS	564 ± 26	786 ± 13	319 ± 1	15.0 ± 0.3
"inverse"	712 ± 22	903 ± 16	324 ± 0.4	21.6 ± 0.4

Table 2: Microgels interfacial dimensions^a at 25°C and volumetric swelling ratio

Microgel	D_i [nm]	h_w [nm]	h_o [nm]	Interfacial volumetric swelling ratio ^b
5 mol % BIS	2088 ± 250	488 ± 34	71 ± 5	3.0 ± 0.19
1 mol % BIS	1556 ± 180	295 ± 15	21 ± 1	1.86 ± 0.06
"inverse"	2160 ± 270	396 ± 37	12 ± 2	144 ± 50

^a D_i is the particle diameter at the interface as measured by AFM height images from the water side. h_w and h_o are the maximum particle height in water and oil, respectively. ^b Swelling ratio of microgels adsorbed at the fluid interface, calculated as the volumetric ratio between the volume occupied by the particles in water at 25°C and 40°C.

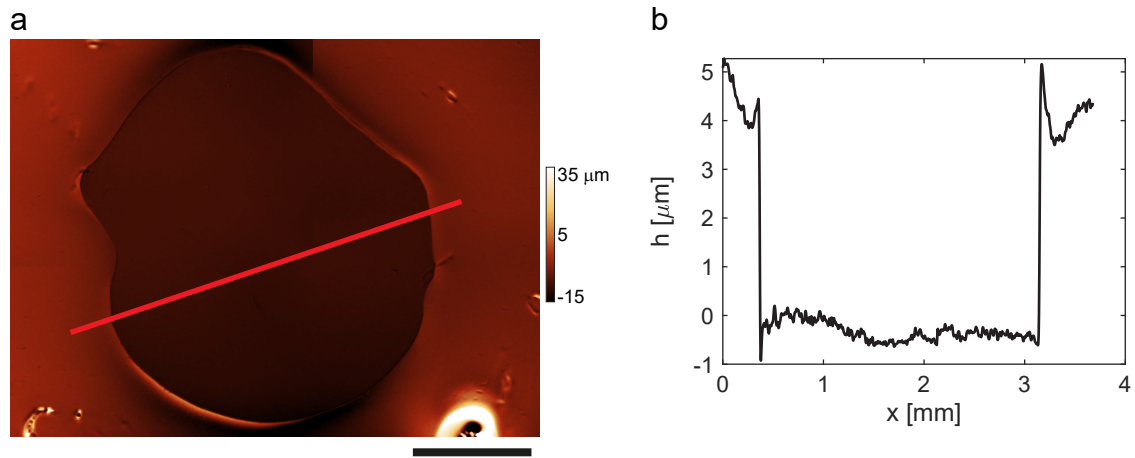


Figure S1: **3D image of the experimental cell** a) Optical profilometer image and b) height profile along the red line in (a) of a reservoir made of a thin ring of UV-curable glue on a silicon wafer. Scale bar: 1 mm.

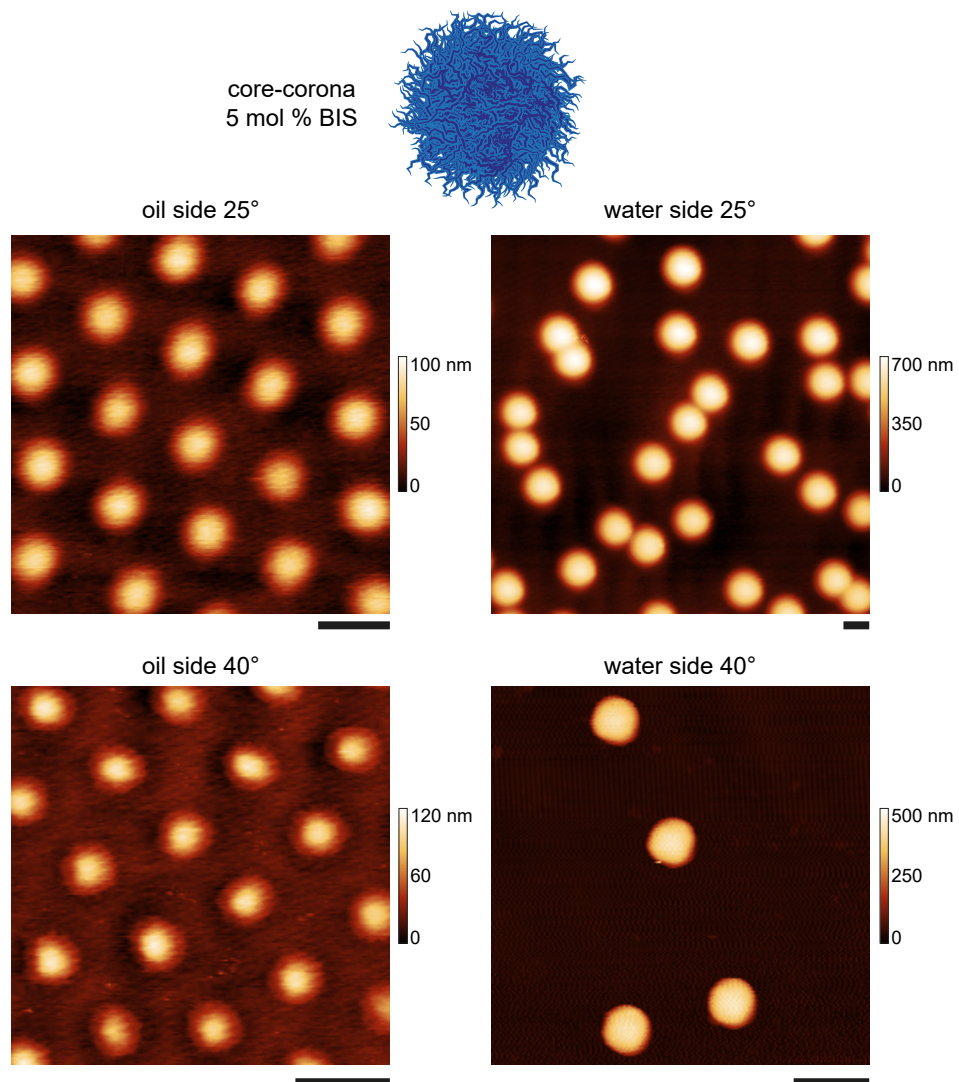


Figure S2: **AFM profiles of CC5 microgels at the hexadecane-water interface** AFM height images taken at the fluid interface, at 25 (top row) and 40°C (bottom row), with tip in the oil (left column) and water phase (right column). Scale bars: 1 μm .

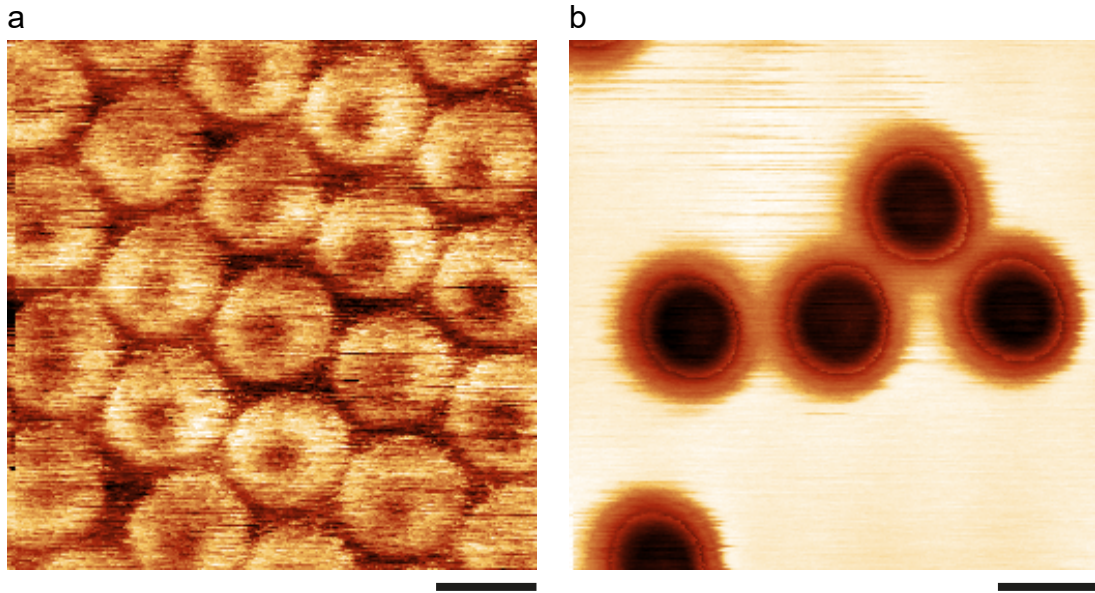


Figure S3: AFM adhesion images at the hexadecane-water interface. AFM adhesion images captured from the oil (a) and water (b) side, for *CC5* microgels. The image in (a) corresponds to the height image in Fig. 2a; the image in (b) to the height image in Fig. 2b. Scale bars: 1 μm .

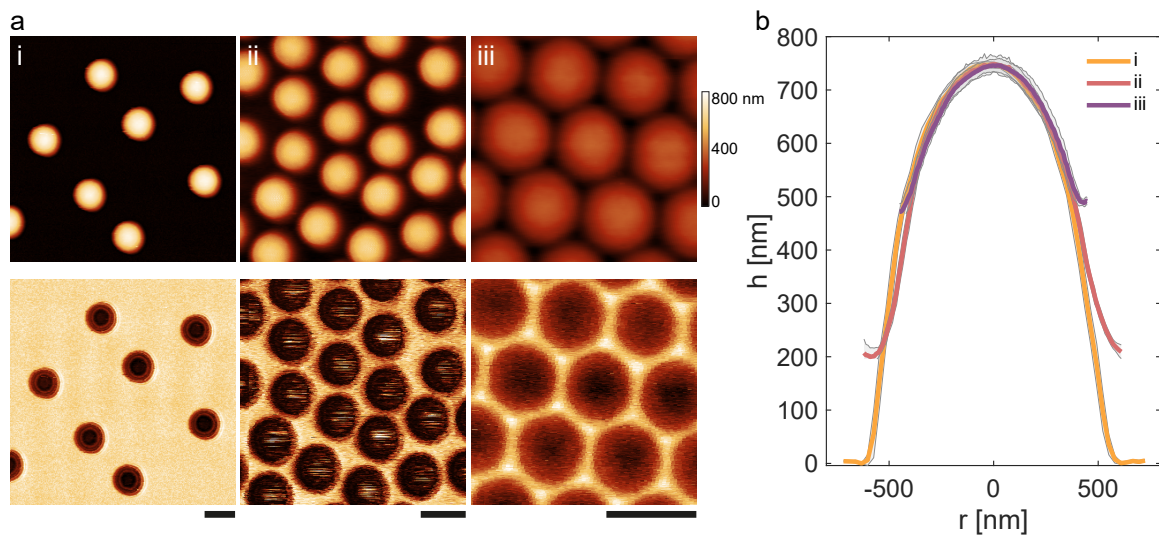


Figure S4: **AFM images at the 1-decanol-water interface as a function of microgel density.** (a) AFM height (top) and adhesion (bottom) images of *CC5* microgels imaged from the 1-decanol side. The microgel interfacial concentration is increased from image (i) to (iii). For images (ii) and (iii), $h = 0$ is the lower height measured by the AFM tip, and does not correspond to the fluid interface. Scale bars: $1 \mu\text{m}$. (b) Averaged height profiles of the microgels in (a). The height profiles are shifted on the y-axis to have matching maximum heights.

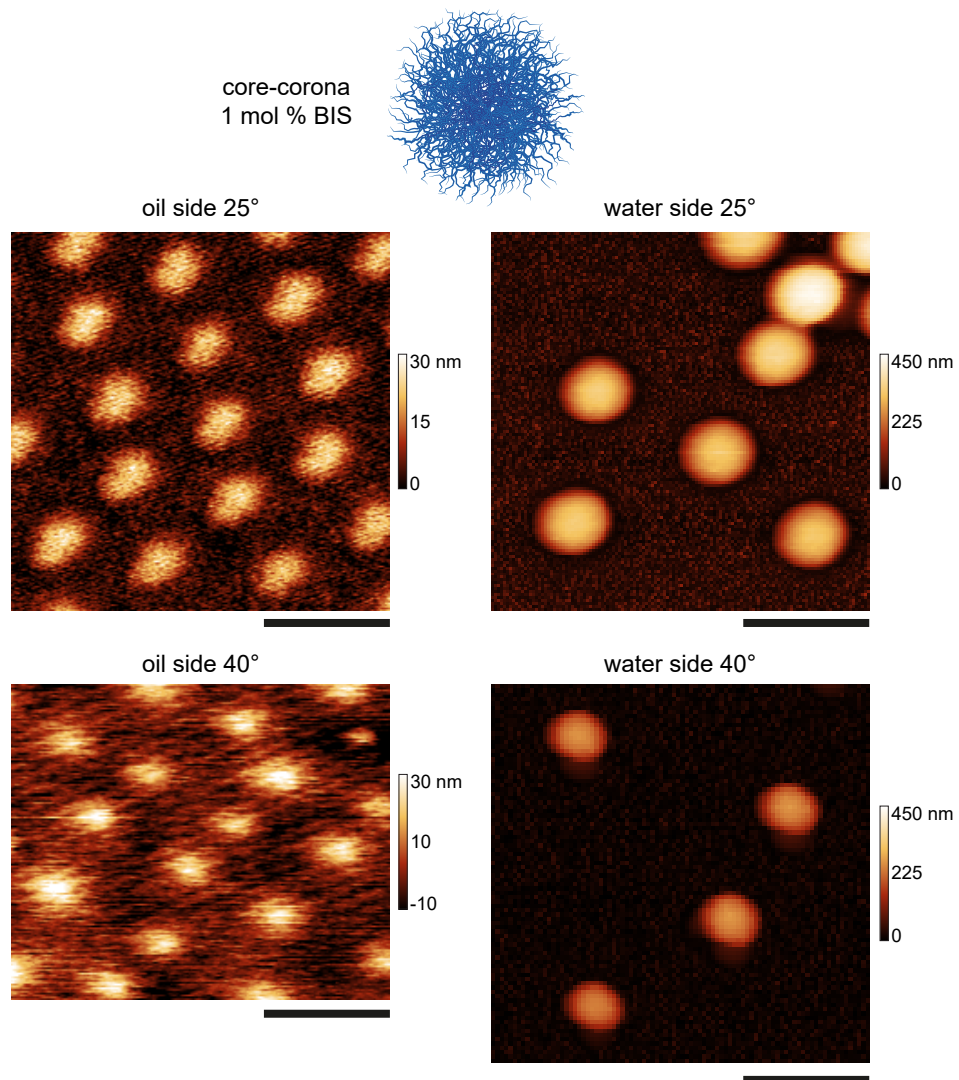


Figure S5: **AFM profiles of *CCI* microgels at the hexadecane-water interface** AFM height images taken at the fluid interface, at 25 (top row) and 40°C (bottom row), with tip in the oil (left column) and water phase (right column). Scale bars: 1 μm .

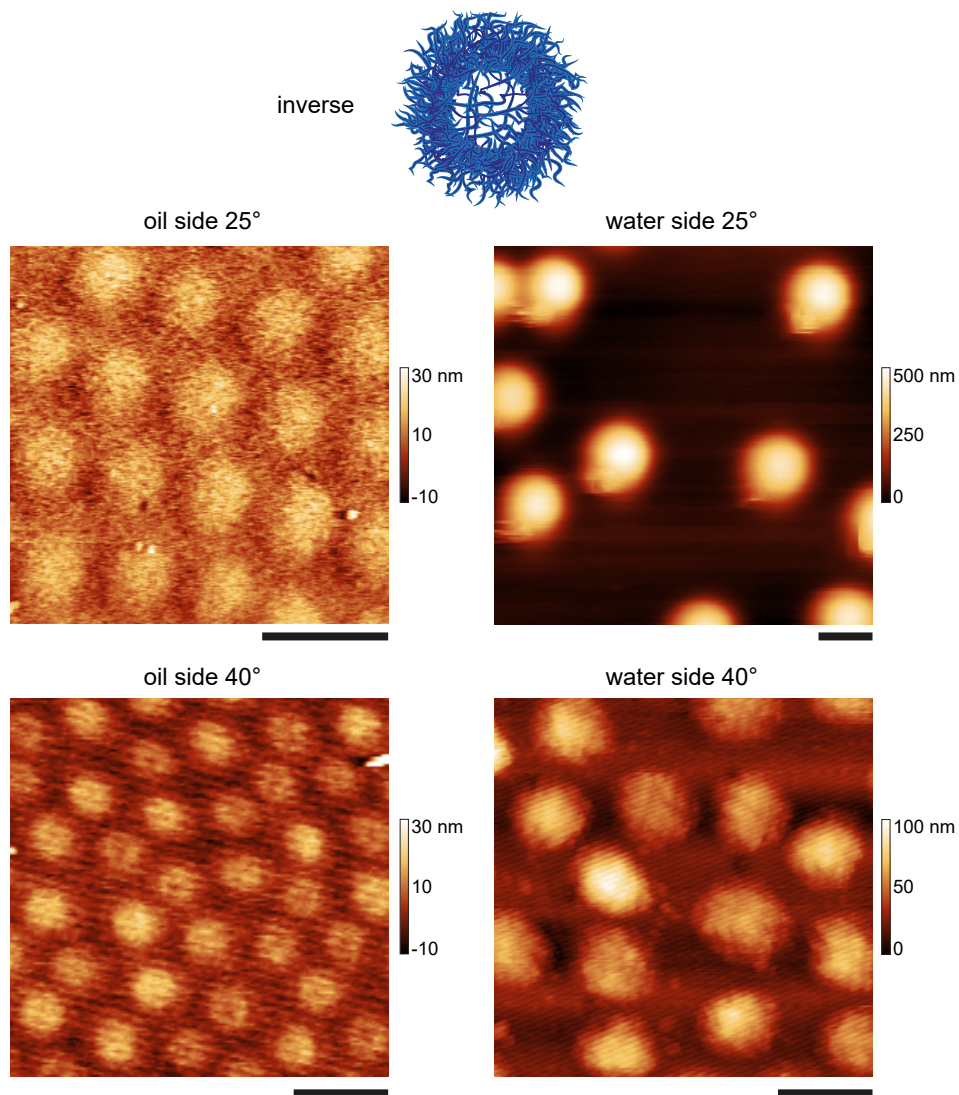


Figure S6: **AFM profiles of *INV* microgels at the hexadecane-water interface** AFM height images taken at the fluid interface, at 25 (top row) and 40°C (bottom row), with tip in the oil (left column) and water phase (right column). Scale bars: 1 μm .

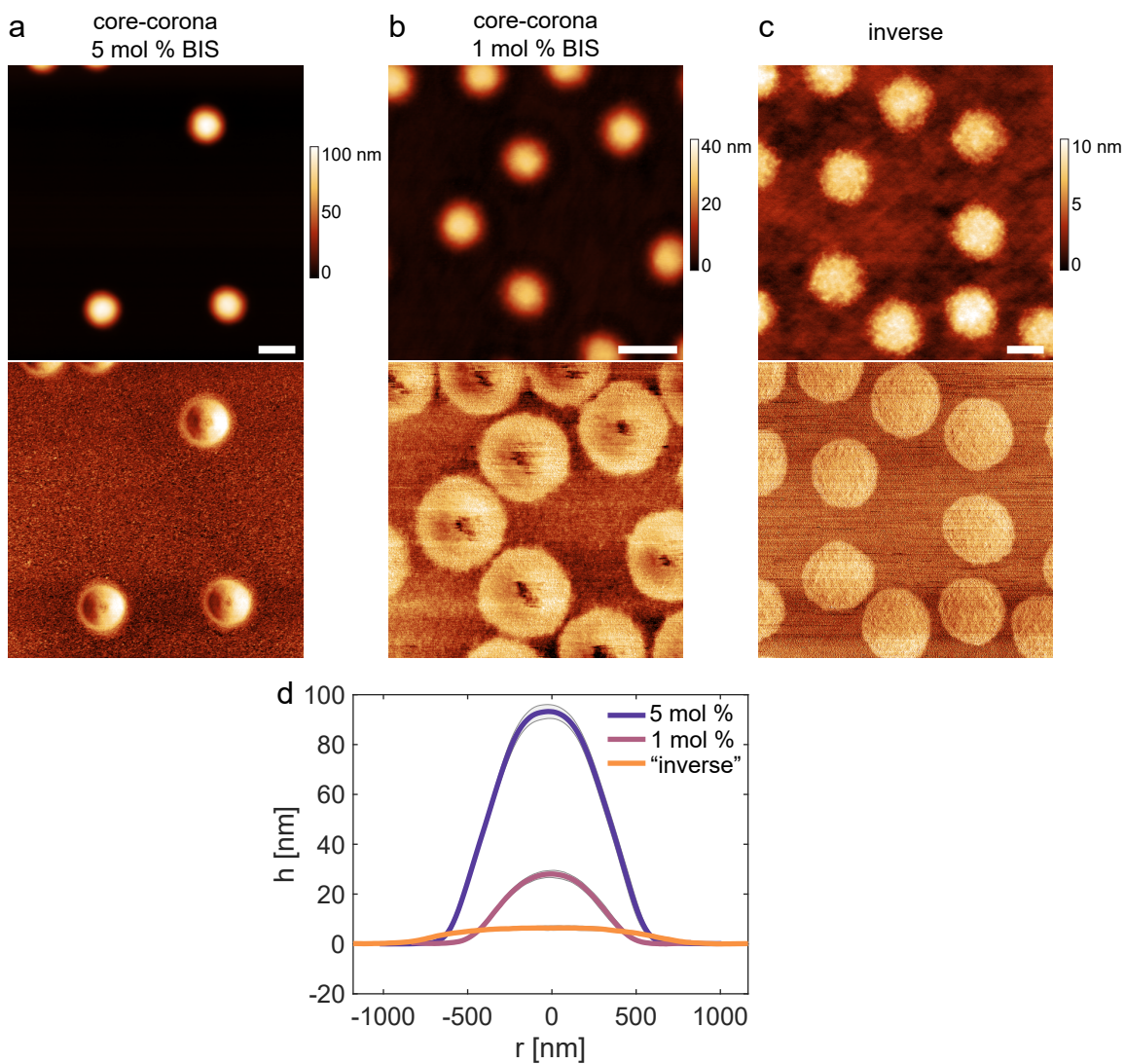


Figure S7: AFM profiles of dried microgels (a-c) Representative AFM height (top) and phase (bottom) images of microgels transferred from the hexane-water interface onto a silicon wafer, and imaged in dry condition. Scale bar: 1 μm . d) Experimental height profiles in dry condition. The shaded regions correspond to the standard deviations of the height profiles calculated on around 20 particles.

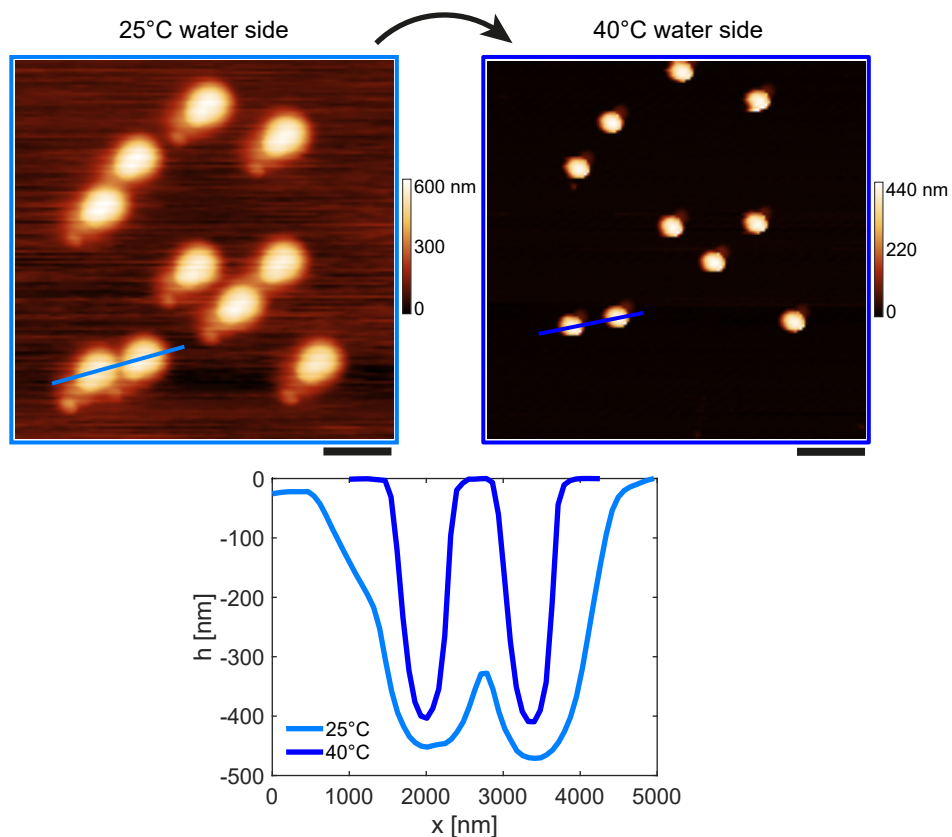


Figure S8: AFM profiles of two microgels in contact imaged from the water side at 25 and 40°C. Top: AFM height images taken from the water side of the same *CC5* particles at the hexadecane-water interface, at two different temperatures. The “protrusion” visible in the bottom right of each particle in the image at 25°C, is an artifact due to tip imaging. Scale bar: 2 μm . Bottom: height profiles extracted from the images on the left, along the indicated lines, at 25°C (light blue) and 40°C (dark blue).

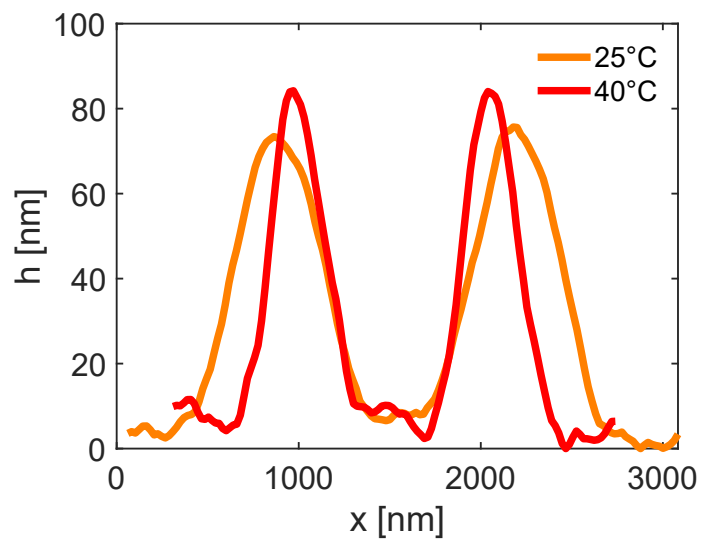


Figure S9: **AFM profiles of two microgels in contact imaged from the hexadecane side at 25 and 40°C.** The height profiles are extracted from images in Figure S2, oil side, at 25°C (orange) and 40°C (red).

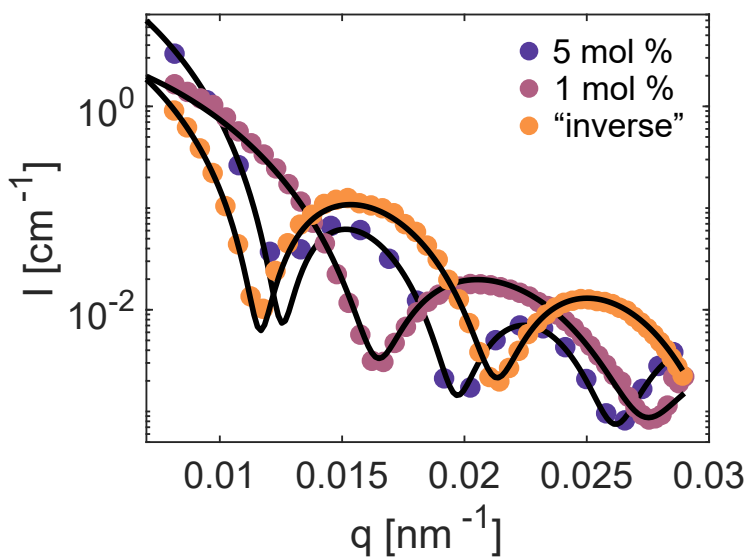


Figure S10: **Microgel form factors in aqueous solution.** Experimental form factors obtained from SLS experiments at 25°C. Black lines are fits (see Methods).

1.1 Force curves at the fluid interface

The PeakForce tapping technique enables the possibility of simultaneously acquiring force *vs* distance curves along with the topographical images. By knowing the spring constant of the cantilever and the deflection sensitivity values, one can obtain the force as a function of the tip-to-sample separation. Figure S11 shows the extracted force *vs* separation curves from the PeakForce topographical image obtained for the sample *CC5* at the hexadecane-water interface.

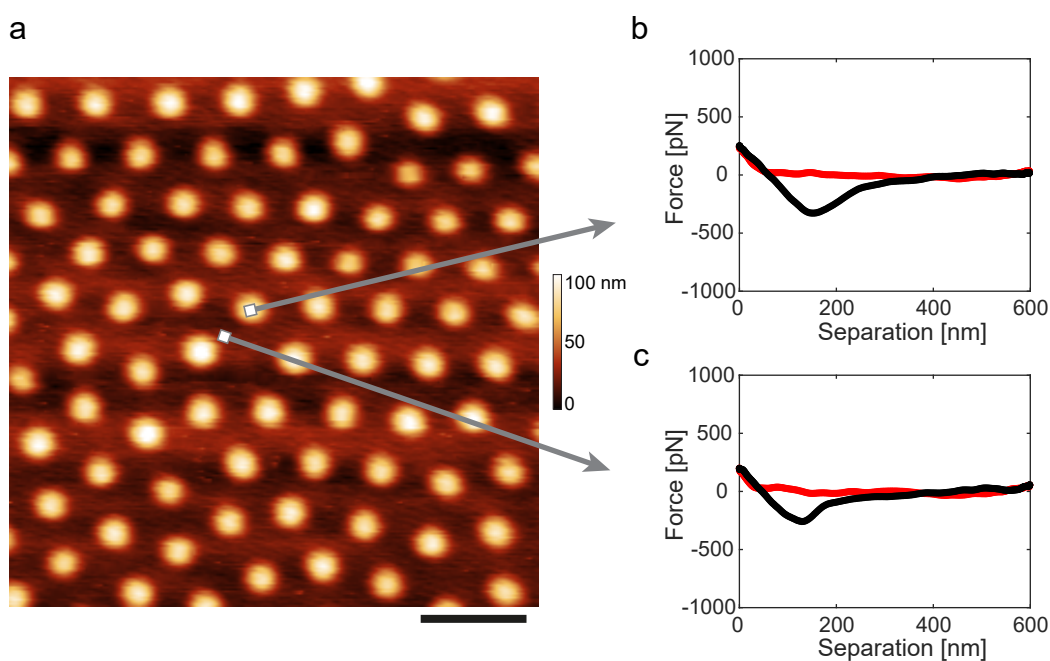


Figure S11: **PeakForce curves at the fluid interface** a) PeakForce image of microgel *CC5* at the hexadecane-water interface, imaged from the oil side. b-c) Approach (red) and retract (black) force curves from the image in (a), measured on a microgel (b) and in-between two microgels (c). The slope from the retraction curve for b) and c) was found to be 4.3 and 4.5 mN/m, respectively. Scale bar: 2 μm .

The slope of the force *vs* separation curves taken at the hexadecane-water interface can be used to estimate its interfacial tension, which is found to be of around 4 mN/m. Comparable estimations, around 15 mN/m, were reported for the octane-water interface, by using a similar AFM imaging technique (41). Such low values of the measured interfacial stiffness with respect to typical values obtained when imaging a solid substrate, can be used as a direct confirmation

that the images are taken at a fluid interface, and that there was no influence of the underlying substrate during the PeakForce imaging.

1.2 PeakForce set point dependency of measured height profiles in the water phase

We investigated the influence of the PeakForce set point (applied load during the AFM imaging) on the height of adsorbed microgels in two cases: i) individual and ii) closely packed microgels.

In the case of individual, separated microgels (Figure S12), the PeakForce set point does not affect significantly the particles height profiles in the range of applied forces (~ 100 pN) typically used in this study. When microgels are in contact on the water side, however, the

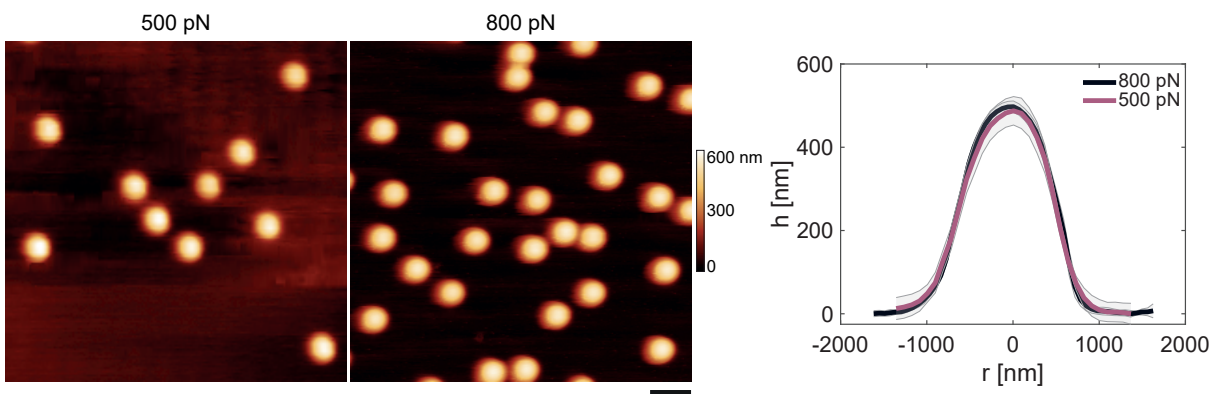


Figure S12: **Set point dependency for individual microgels.** AFM height images, and corresponding height profiles, of *CC5* microgels at the hexadecane-water interface imaged from the water side at 25°C, as a function of the PeakForce set point. Scale bar: 2 μm

swelling of the polymer network in water prevents accessing the fluid interface and the space in-between the particles, precluding the quantification of the complete 3D shape. In such a case, the interconnected, swollen polymer corona of the microgels is highly deformable, and an increase in the PeakForce set point causes an increased deformation of the particles that is more pronounced in the contact region, as evidenced by Figure S13.

The extent of the deformation caused by the approaching AFM tip on the microgels can be

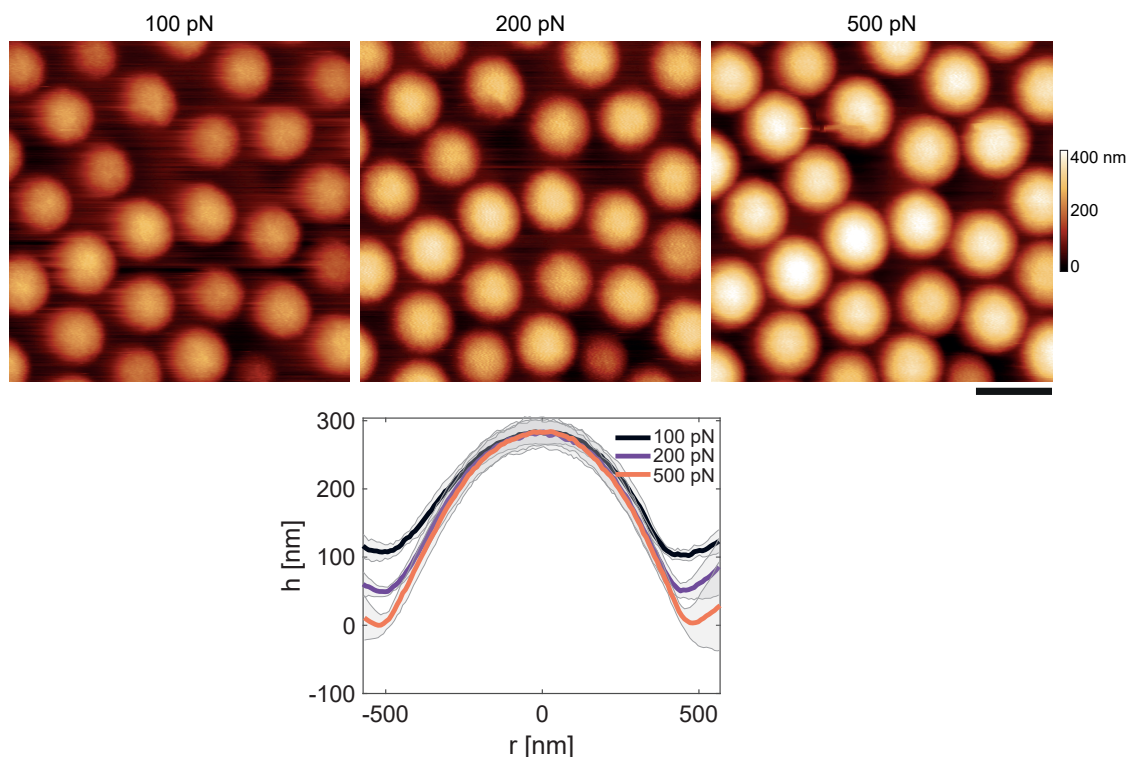


Figure S13: **Set point dependency for closely packed microgels** AFM height images, and corresponding height profiles, of *CC5* microgels in a closely packed monolayer at the hexadecane-water interface imaged from the water side at 25°C, as a function of the PeakForce set point. Note that $h = 0$ does not correspond to the bare interface, which is covered by the polymer network. The height profiles are shifted on the y-axis to have matching maximum heights. Scale bar: 1 μm .

calculated based on the Hertz equation for contact. In water, for the force range we applied in our experiment, the deformation values obtained is in the range of 60 nm - 80 nm (assuming a tip radius of 20 nm, and for a sample modulus of 100 kPa, as measured in (35)). This range is comparable with error bars reported throughout the manuscript (for example in Figure 1f), which results from an averaging over at least 10 particles. Therefore, polydispersity in particle size is comparable with possible deformations coming from the applied force.