Electronic Supplementary Material (ESI) for Soft Matter.
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Supporting Information

Probing the Adhesion Properties of Alginate Hydrogels: A New Approach towards the Preparation of Soft Colloidal Probes for Direct Force Measurments

Nicolas Helfricht,[a] Elena Doblhofer,[b] Vera Bieber,[a] Petra Lommes,[c] Volker Sieber,[c] Thomas Scheibel[b] and Georg Papastavrou[a]*

[a]Physical Chemistry / Physics of Polymers, University of Bayreuth, Universitätsstr. 30, Bayreuth 95440, Germany; E-mail: georg.papastavrou@uni-bayreuth.de

[b]Biomaterials, University of Bayreuth, Universitätsstr. 30, Bayreuth 95440, Germany

[c]Chemistry of Biogenic Resources, Technical University Munich, Schulgasse 16, 94315 Straubing, Germany.

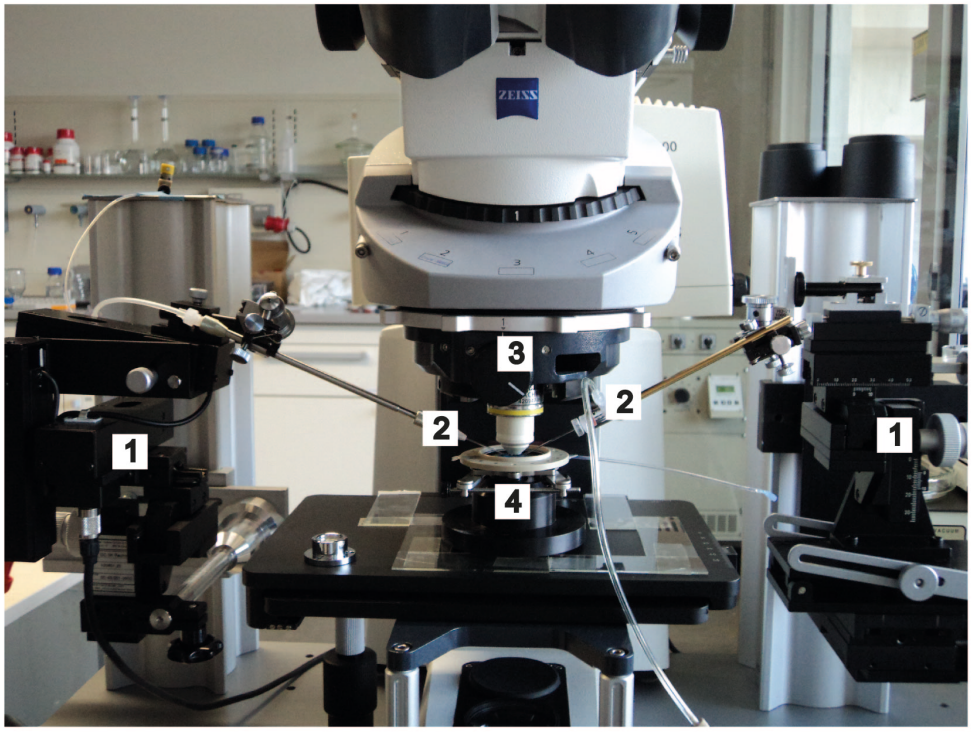
S1. *In-situ* preparation of colloidal probes from alginate beads by micromanipulation (movie)

The provided movie (*In-situ Preparation of Soft Colloidal Probes.m4v*) shows an exemplary and complete sequence for the *in-situ* preparation of a soft alginate hydrogel colloidal probe. The movie is in real-time.

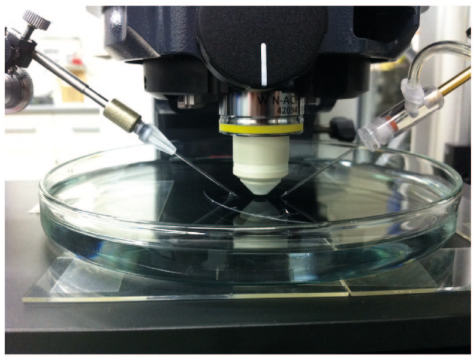
S2. Micromanipulation setup for *in-situ* preparation of alginate probes

Figure S1 (a) shows the experimental set-up used for the *in-situ* preparation of soft hydrogel colloidal probes. Micromanipulators **(1)** allow for a precise positioning and movement of glass micropipettes **(2)** during the preparation procedure. A first micropipette of various opening diameters has been used for injecting a small amount of a particle suspension. The opening diameter was always much larger than the particle diameter. A second micropipette has been used to aspirate a single particle and transfer it to the AFM cantilever. The opening diameter of this second micropipette was significantly smaller compared to the diameter of the alginate beads. The complete procedure can be directly monitored via a fixed stage microscope **(3)** (cf. movie in S1). The presented method allows for the preparation of colloidal probes completely *in-situ*, *i.e.* in aqueous medium. The preparation can be performed in a simple petri dish (cf. Figure S1 b) or directly in an AFM fluid cell (cf. **(4)** and Figure S1 c). In the case that the preparation is performed in a petri dish, it has to be ensured that the prepared soft colloidal probe is transferred rapidly onto a pre-wetted cantilever holder.

(a)



(b)



(c)

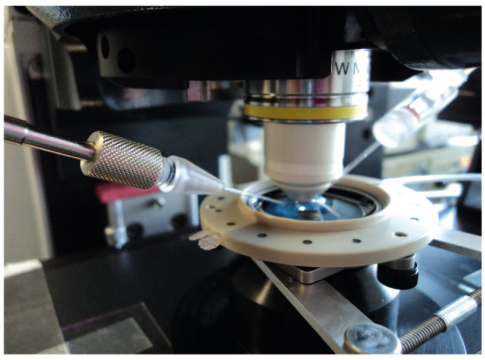


Figure S2-1: (a) Experimental set-up for the *in-situ* preparation of soft hydrogel colloidal probes with: (1) two micromanipulators, (2) glass micropipettes, (3) a fixed stage microscope, and (4) a preparation vessel. The latter is either a petri-dish (b) or an AFM fluid cell (c) with a previously mounted tipless AFM-cantilever. The cell shown in (c) can be sealed completely after preparing the soft colloidal probe.

S3. Apparent spring constant k_{app}

Immobilization of alginate hydrogel beads corresponds to a new effective spring constant for the cantilever as the length of the AFM cantilever L_c is reduced to L_{bead}. According to the lever law this reduction in lever length leads to an apparent stiffening of the used force sensor according to eq. (5).[1,2,3]

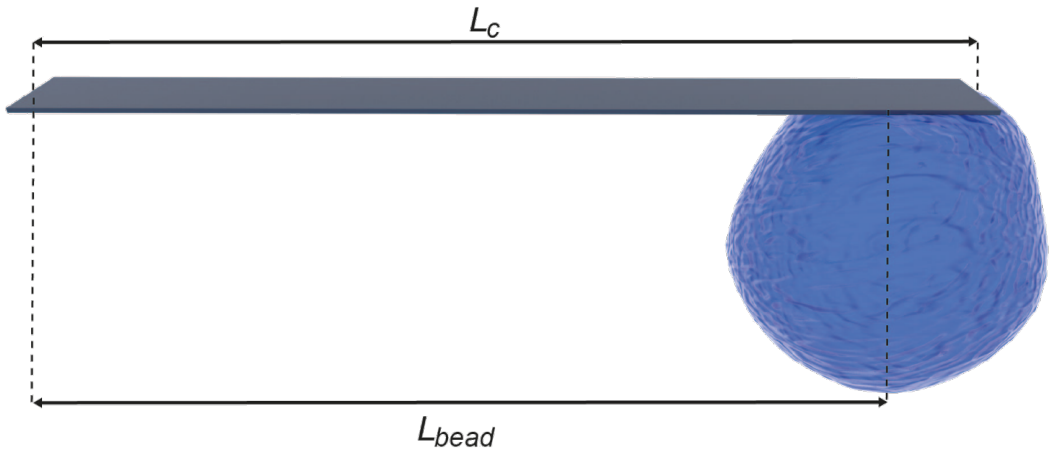


Figure S3-1: Schematic representation of the apparent stiffening of a cantilever with an alginate bead attached due to the reduced effective lever length.

S4 Examples of force vs. distance curves on self-assembled monolayers (Zoom-In)

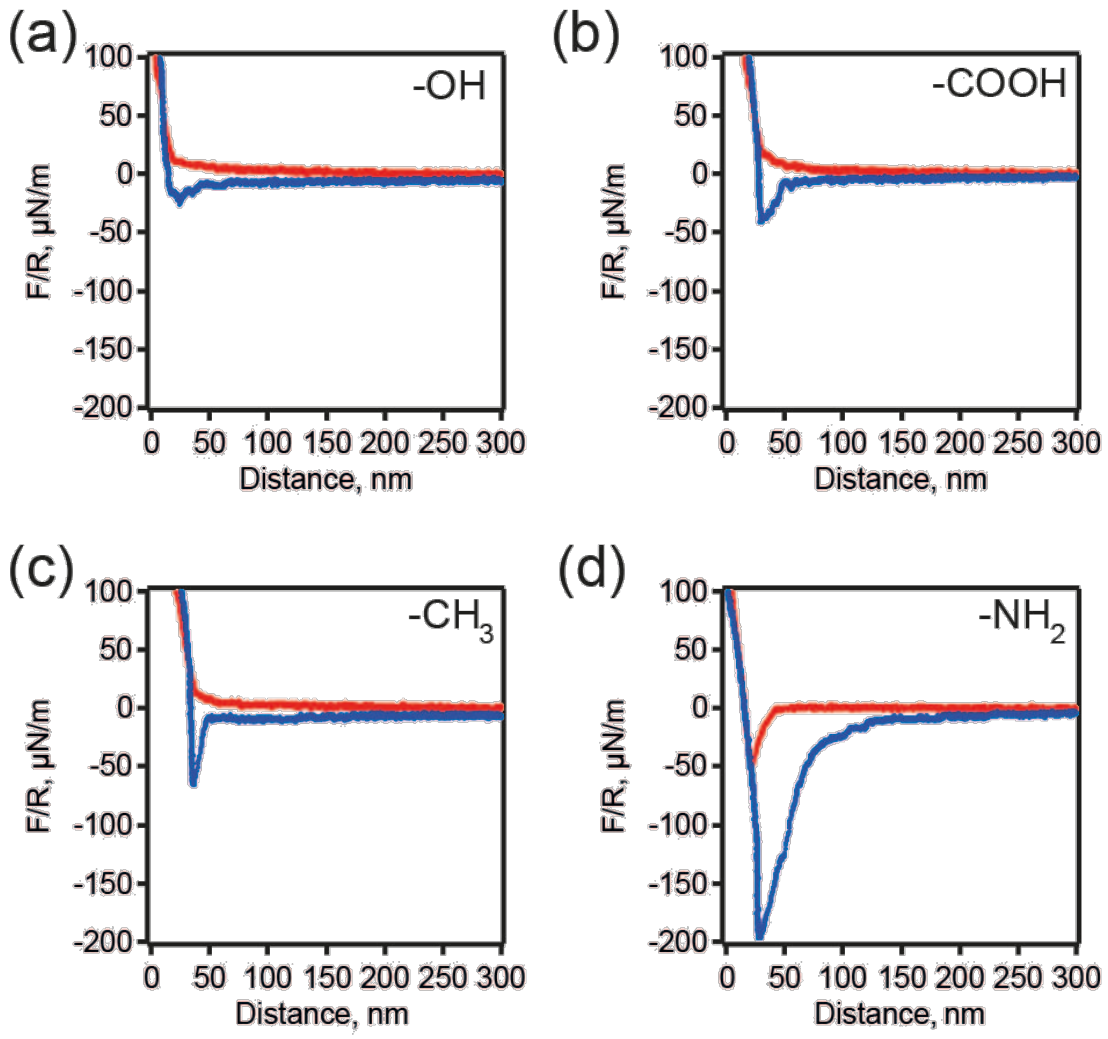


Figure S4-1: Different axis scales for the force profiles as shown in Figure 4. The scaling shows that the adhesion is dominated by the pull-off force for the alginate bead. This force corresponds to the minimum in the retraction part (i.e. blue data) of the force profiles. However, the pull-off is not a sharp transition as for two hard surfaces, as some segments of the alginate bead still adhere to the SAM and are stretched subsequently.

S5. Statistical analysis of the determined adhesion forces

The pull-off forces obtained for an alginate hydrogel colloidal probe were determined as the minimum in the retraction part of the force profile (cf. Figure S4-1). The resulting Gaussian distributions are shown in Figure 5 and the average values and the evaluated standard deviations are given in Table 1. A Student T-test confirms by the differences in the pull-off force forces are significant (p-value < 0.05) for the different SAMs (-OH, -COOH and $–CH_3$). The difference in adhesion behaviour on those SAMs can be additionally visualized by a cumulative distribution plot shown in Figure S5-1.

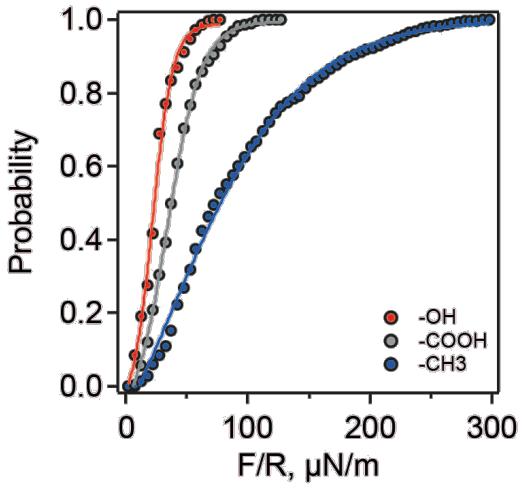


Figure S5-1: Cumulative distribution plot of the pull-off forces between an alginate hydrogel colloidal probe and various SAMs surfaces terminating in different functional groups: -OH (red), -COOH (grey) and $–CH_3$ (blue), respectively.

S6. Force vs. distance curves acquired on NH_2-terminated SAMs

The adhesion behaviour of alginate beads as probed on NH_2-terminated SAMs is more complex than the one observed on the other SAMs as the adhesion is much stronger. We observed that with an increasing number of force vs. distance cycles the adhesion was reduced. This reduction was observed for the pull-off force as well as for the stretching of alginate segments at larger separation distance. Figure S6-1 shows examples for the same alginate bead at two different lateral positions on a NH_2-terminated SAM.

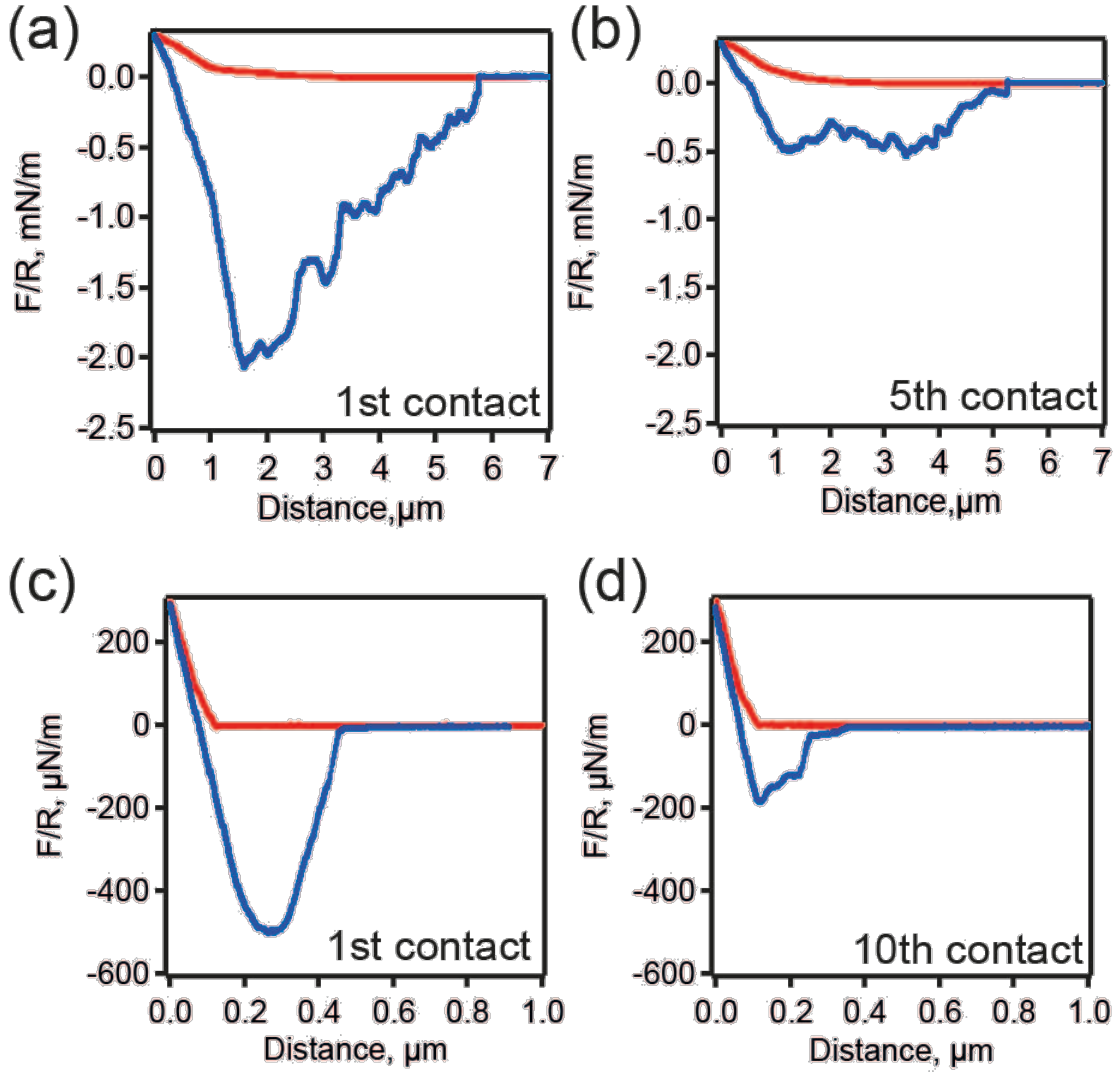


Figure S6-1: Force versus distance curves acquired with an alginate colloidal probe on a NH_2-terminated SAM. (a) and (c) show the normalized force profiles for the initial contact with a "fresh" area of the SAM. The decrease in the adhesion forces after several force versus distance cycles is shown in (b) and (d), respectively. (b) has been acquired after 5 previous force versus distance cycles and (d) after 10 cycles but always at the the same positions as for (a) and (c), respectively.

S7. Adhesion forces on recombinant spider silk protein films

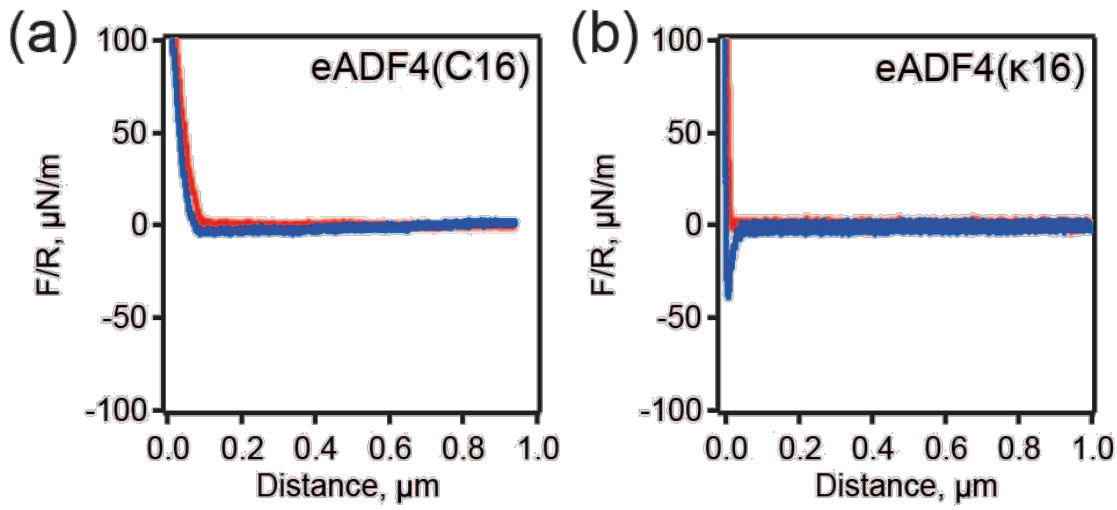


Figure S7-1: Representative force versus distance curves acquired with an alginate hydrogel colloidal probe on two different recombinant spider silk protein films: (a) eADF4(C16) and (b) eADF4(16), respectively. The measurements were performed for at least two probe – protein film combinations.

References

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2 R. Buzio, A. Bosca, S. Krol, D. Marchetto, S. Valeri and U. Valbusa, *Langmuir*, 2007, **23**, 9293–9302.

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