

Supporting Information

Conformational Mechanics, Adsorption and Normal Force Interactions of Lubricin and Hyaluronic Acid on Model Surfaces

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Methods and Materials

Adsorption Measurements using Surface Plasmon Resonance (SPR)

The output of a typical SPR adsorption experiment (Biacore AB, Uppsala, Sweden) is shown in Figure S1. The SPR output signal is reported in response units (RU) and is related to the angular change in the minimum of the reflected light intensity. The reflected angle change results here from a combination of lubricant adsorption onto the SAM layer, conformational changes of the molecules on the SAM surface, and changes in the refractive index of the solution layer in the supernatant. The RU value measured before the initial injection of the solution of interest is chosen as the reference value. The difference between the value at the equilibrium steady state (after PBS rinse) and the reference value thus reflects the change in adsorbed amount and surface conformation on the SAM surface.

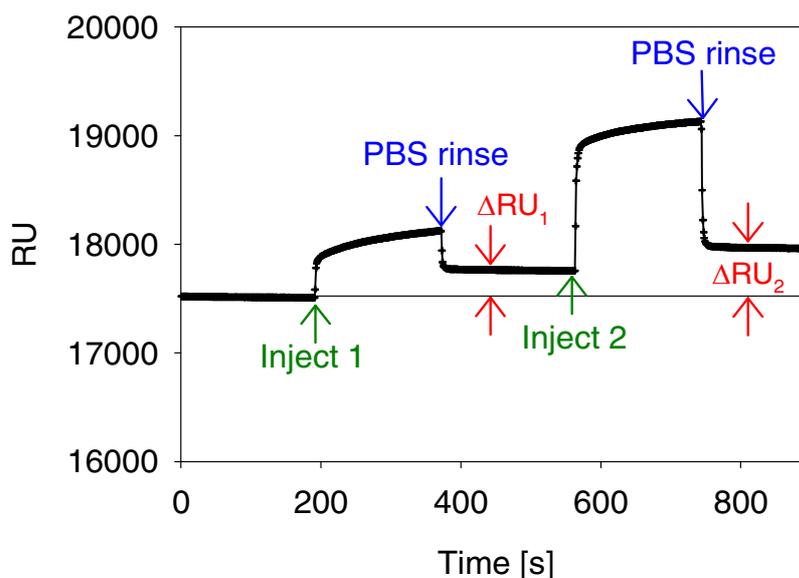


Figure S1 – Typical Surface Plasmon Resonance (SPR) Data.

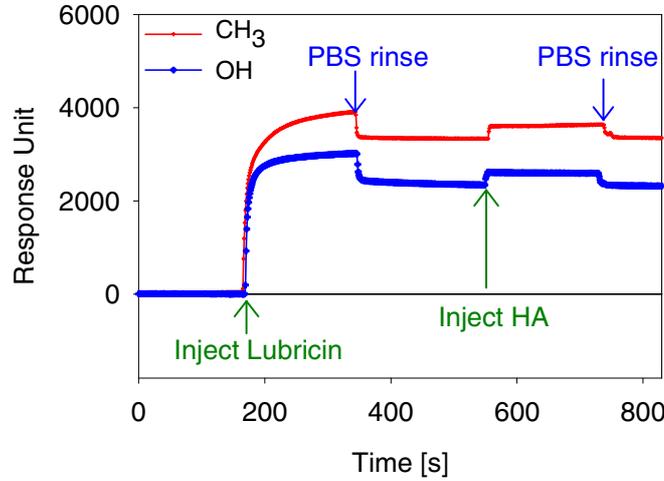


Figure S2. SPR result showing sequential injection of 200 $\mu\text{g/ml}$ lubricin followed by 3.34 mg/ml HA on CH₃ (red line) and OH (blue line) SAM surfaces.

To further investigate the possibility of interaction between lubricin and HA, we injected a HA solution (3.34mg/ml) on a pre-adsorbed lubricin layer (200 $\mu\text{g/ml}$). The HA injection did not lead to an increase in the adsorbed amount beyond that of the lubricin already present on the surface (Fig. S2).

Steric Forces

To better interpret the steric repulsive force between two lubricin coated surfaces, we chose to model the interaction with the polymer brush theory by Alexander and de Gennes (AdG).¹ This simple model describes the steric repulsive force that arises from the confinement of two polymer brush layers; *i.e.*, when two polymer brush layers are closer than twice the polymer brush thickness ($2L$) to each other, the thermal movement of the polymer chains is limited and the entropic energy is decreased, leading to a repulsive osmotic pressure.² This repulsive pressure between two parallel brushes, separated by a distance D is

$$P(D) \approx \frac{kT}{s^3} \left[\left(\frac{2L}{D} \right)^{9/4} - \left(\frac{D}{2L} \right)^{3/4} \right], \quad (\text{S1})$$

where L is the equilibrium thickness of one brush layer, s is the average distance between grafting sites (*i.e.*, a measure of grafting density), k_b is Boltzmann's constant and T is the absolute temperature. The steric repulsive force between a flat and spherical surface bearing polymer brushes, can be determined using the Derjaguin approximation,^{3,4}

$$F(D) = 2\pi RW(D) = 2\pi R \int P(D) dD = \left(\frac{16\pi R k T L}{35 s^3} \right) \left[7 \left(\frac{2L}{D} \right)^{5/4} + 5 \left(\frac{D}{2L} \right)^{7/4} - 12 \right]. \quad (\text{S2})$$

In equation S2, the AdG theory invokes a step profile for the polymer segment density distribution. A more realistic description of the polymer segment density distribution is the parabolic profile provided by the Milner-Witten-Cates (MWC) model,^{5,6}

$$F(D) = 4\pi R P_o \left[\frac{2L}{D} + \left(\frac{D}{2L} \right)^2 - \frac{1}{5} \left(\frac{D}{2L} \right)^5 - \frac{9}{5} \right] \quad (\text{S3})$$

with

$$P_o = \frac{k T n}{2} \left(\frac{\pi^2 l^4}{12 s^{10}} \right)^{1/3},$$

where n is the number of monomers, and l is the segment length.

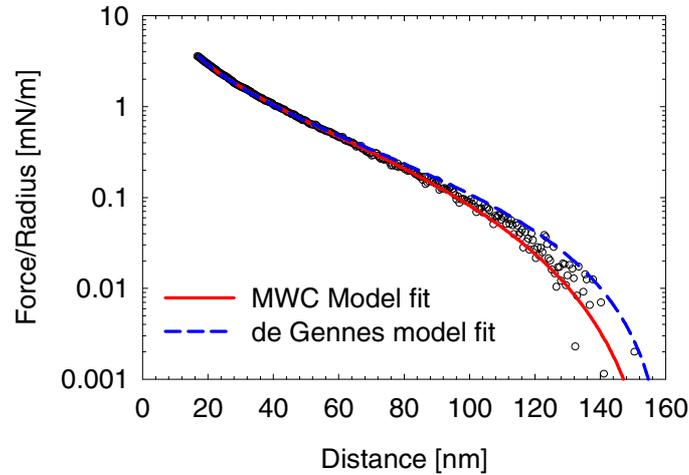


Figure S3. Force-distance curve upon approach between a –OH terminated colloidal probe and a –OH terminated flat surface in a 100 $\mu\text{g/ml}$ solution of lubricin. At these solution concentrations, lubricin adsorbs readily onto the –OH surface (approaching monolayer coverage). The measured force profile (circles) is fitted by the AdG theory (dashed line) and the MWC model (solid line).

Figure S3 shows that repulsive interactions between the polymer surfaces can be approximated reasonably well with both theories; however, the brush thickness predicted by the MWC model is slightly smaller than that predicted by the AdG model. Although the MWC

model showed a slightly better fit at large separation distance than the AdG model, the MWC model required one extra fitting parameter. To minimize the degrees of uncertainty, all steric forces measurements in the paper have been fitted with the Alexander-deGennes model.

Additional Results

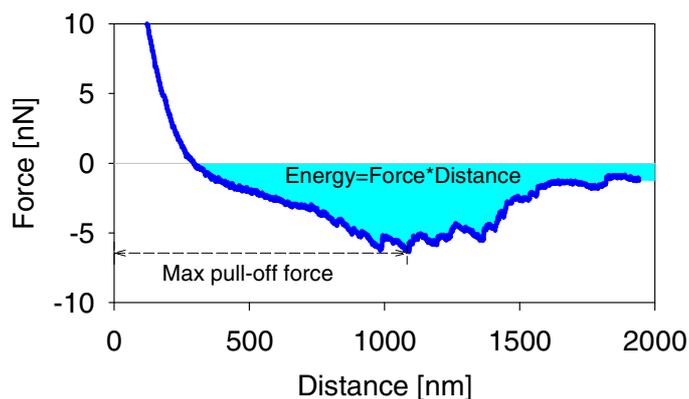


Figure S4. Typical force versus distance curve showing the maximum pull-off force and distance and the total energy expended upon retraction (shaded area under the curve).

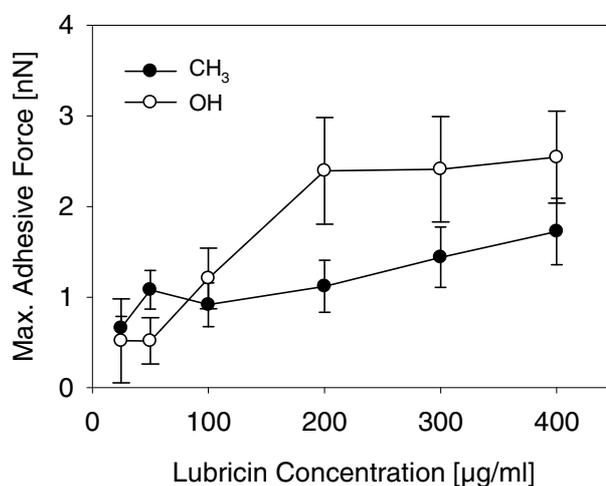


Figure S5. Average of the max adhesive force measured upon retraction as a function of lubricin concentration. Each adhesion force was averaged from at least 50 force curves. The error bars represent the standard deviation of mean.

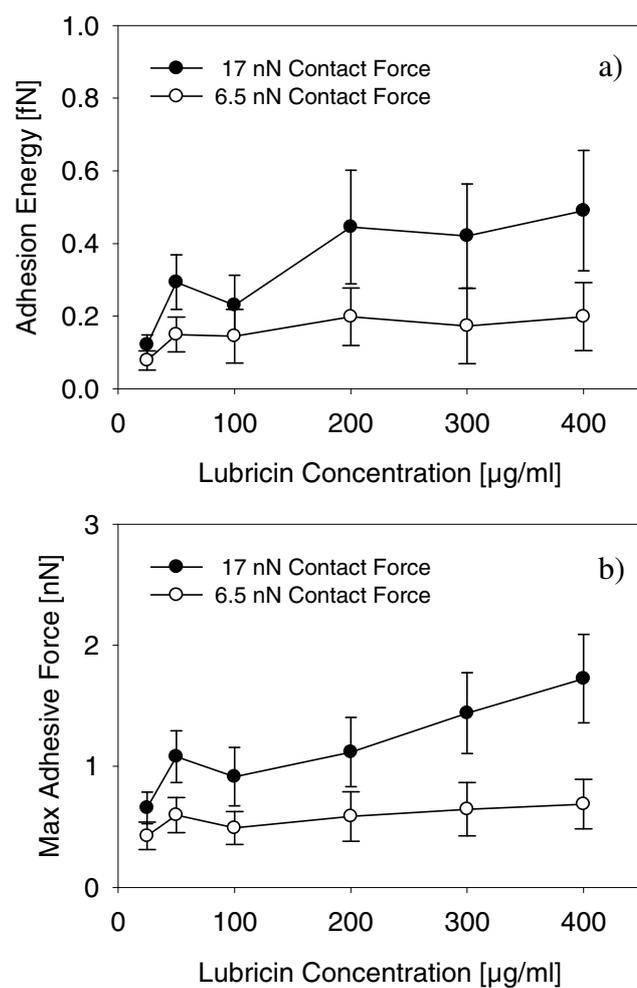


Figure S6. Average a) adhesion energy and b) max adhesion force measured upon retraction on a $-\text{CH}_3$ terminated SAM surface as a function of lubricin concentration. Each data point was averaged from at least 50 force curves. The error bars represent the standard deviation of mean.

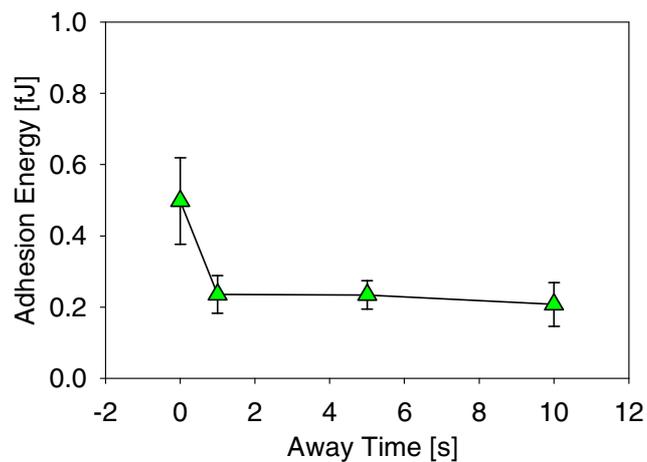


Figure S7 Average adhesion energy as a function of wait time out of contact at large separation between $-\text{CH}_3$ SAM surfaces in 200 $\mu\text{g/ml}$ lubricin solution. The error bar represents the standard deviation of the mean.

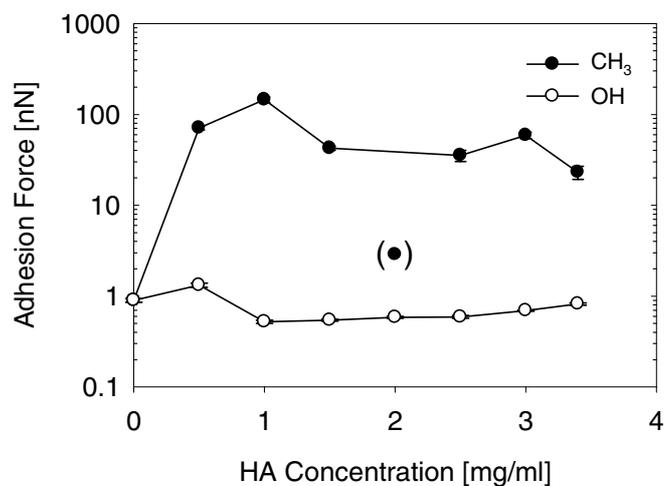


Figure S8. The adhesion force (pull-off force) measured for HA on hydroxyl ($-\text{OH}$) and methyl ($-\text{CH}_3$) surfaces. Each adhesion force represents an average from at least 50 force curves. (Note: log scale)

References

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- (6) Milner, S. T.; Witten, T. A.; Cates, M. E. *Macromolecules* **1988**, *21*, 2610-2619.