

# The Influence of Substrate Surface Chemistry on Biofilm Formation

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## Introduction

Protein-mediated adsorption of polysaccharides to surfaces is a critical step in the early stages of biofilm formation. This work focuses on the role of the substrate in this process of biofilm nucleation.

Alginate is a linear, charged polysaccharide composed of mannuronic and glucuronic acid subunits. Its relative structural simplicity makes it ideal for exploring the electrostatic interactions that play an important role in polysaccharide/surface interactions. Positively-charged (lysine-rich) proteins such as Mussel Adhesive Protein are known to promote adsorption of alginate, as is the polypeptide poly-L-lysine.<sup>1</sup>

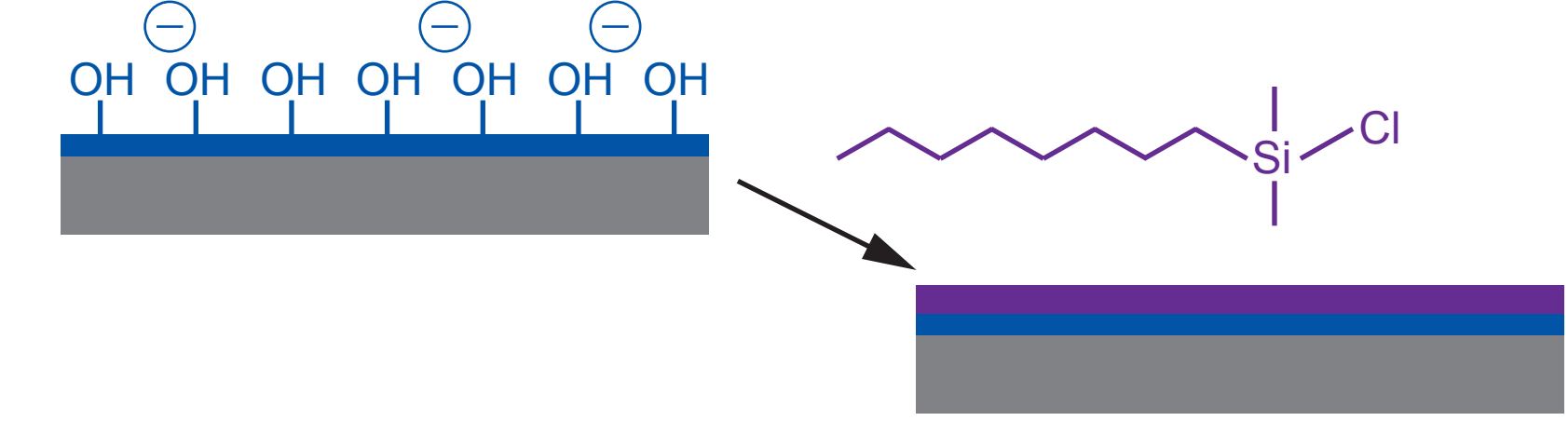
In this work we focus on the role of the surface chemistry of the underlying substrate as it influences poly-L-lysine (PLL) mediated adsorption of alginate. We focus on two representative surfaces: a hydrophobic, electrically neutral surface and a hydrophilic, negatively charged one. The effect of these different surface properties on initial PLL adsorption and on the subsequent adsorption of alginate is investigated with a combination of ellipsometry, dynamic contact angle goniometry and atomic force microscopy.

## Experimental

Hydrophilic silica surfaces were prepared by piranha clean of native oxide-terminated Si(100). Hydrophobic silane layers were prepared by vapor phase silanization of these substrates with octyldimethylchlorosilane (Gelest). PLL (Sigma Aldrich) was adsorbed to the hydrophobic surfaces from pH 11 buffer<sup>2</sup> and to the hydrophilic surfaces from aqueous KBr solutions of varying ionic strength. Alginate (Sigma Aldrich) was adsorbed from aqueous solution. Samples were rinsed with DI water and dried with compressed air prior to measurement.

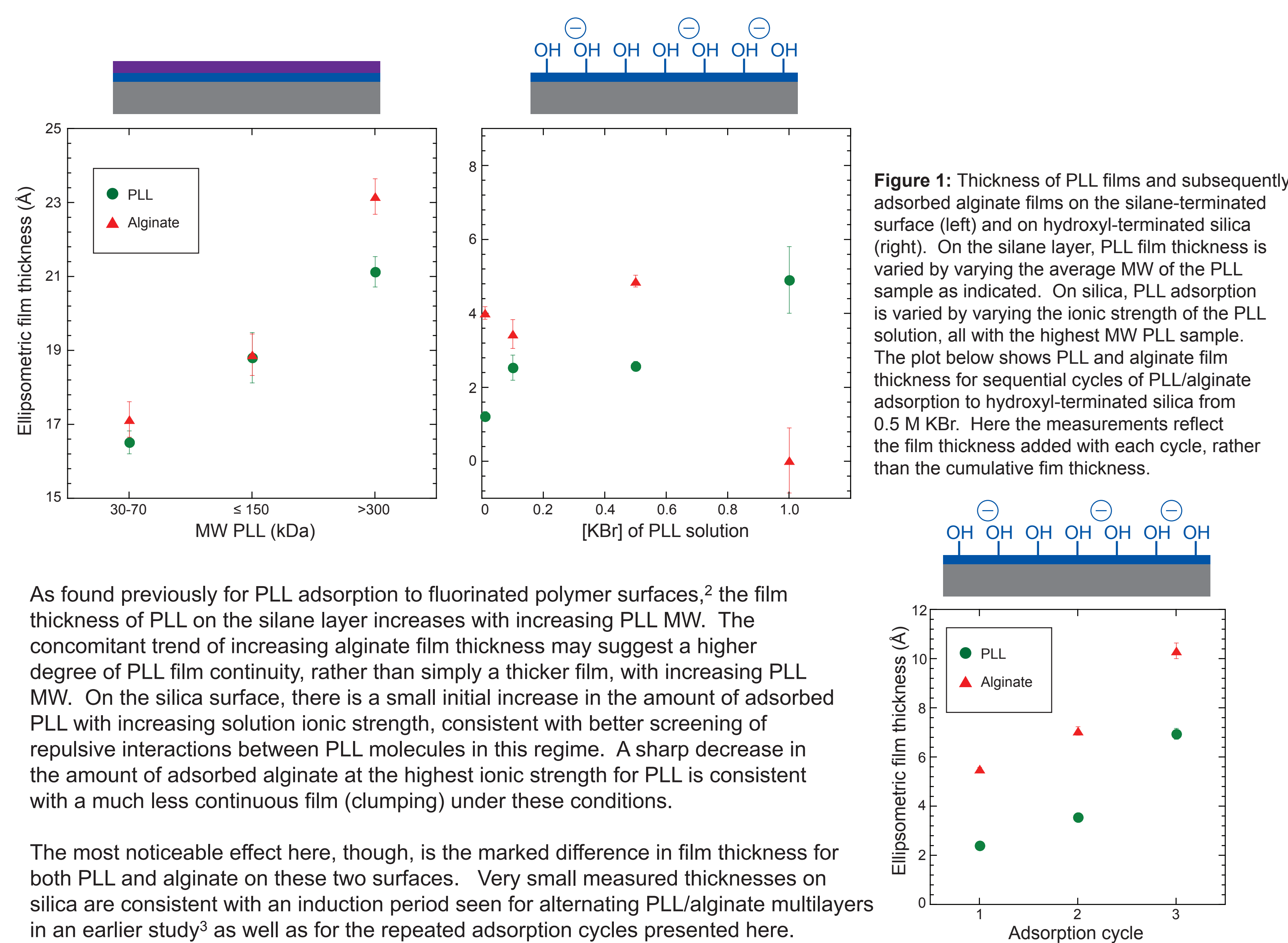
Film thickness was measured with a Gaertner Scientific L116S300 Stokes Ellipsometer at 70°. Advancing and receding contact angles were recorded with a Ramé-Hart manual goniometer. Each sample was measured 3-5 times, and each experiment reported was repeated 4-20 times.

Since multiple measurements were made on each sample, the errors around each estimate were not independent, and the usual way of estimating and determining standard errors is invalid. Instead, estimates and their standard errors (represented by error bars in all graphs) came from a fit of a repeated measures model that includes error terms for both within and across sample errors.



## References

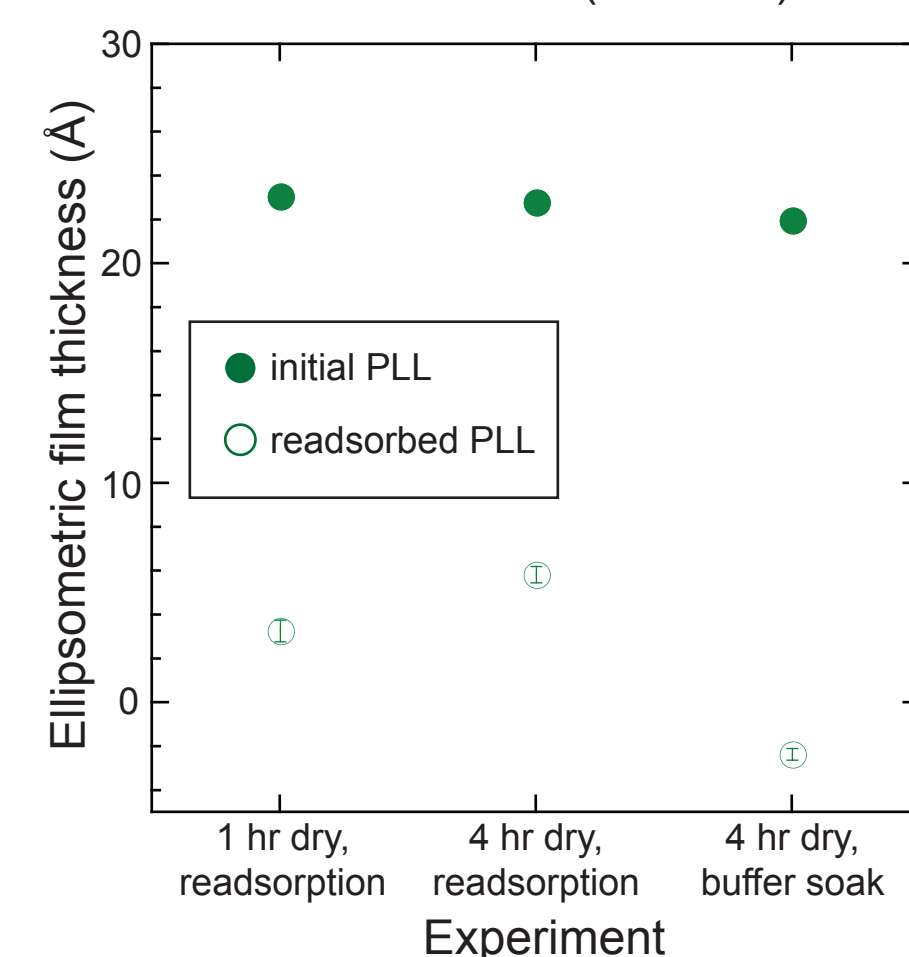
- 1) P. A. Suci and G.G. Geesey, *J. Colloid Interface Sci.* **1995**, 172, 347-357.
- 2) M. S. Shoichet and T. J. McCarthy, *Macromolecules* **1991**, 24, 1441-1442.
- 3) D. L. Elbet, C. B. Herbert and J. A. Hubbell, *Langmuir* **1999**, 15, 5355-5362.
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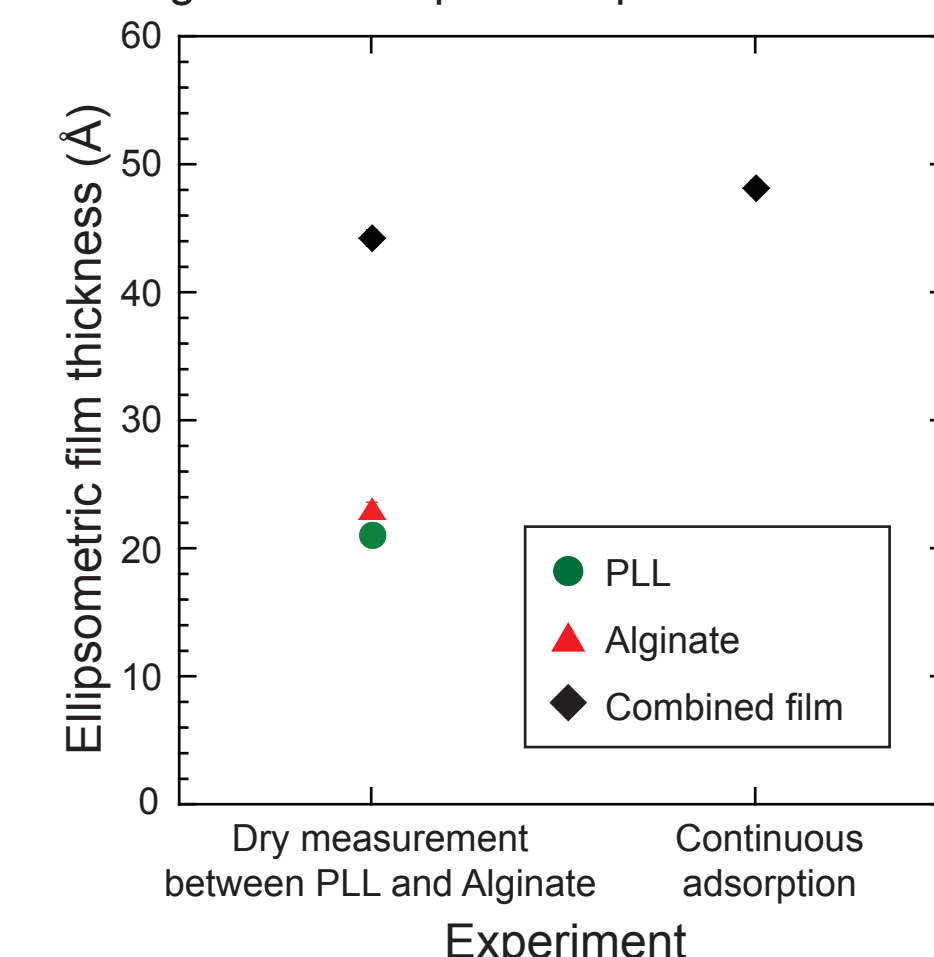
As found previously for PLL adsorption to fluorinated polymer surfaces,<sup>2</sup> the film thickness of PLL on the silane layer increases with increasing PLL MW. The concomitant trend of increasing alginate film thickness may suggest a higher degree of PLL film continuity, rather than simply a thicker film, with increasing PLL MW. On the silica surface, there is a small initial increase in the amount of adsorbed PLL with increasing solution ionic strength, consistent with better screening of repulsive interactions between PLL molecules in this regime. A sharp decrease in the amount of adsorbed alginate at the highest ionic strength for PLL is consistent with a much less continuous film (clumping) under these conditions.

The most noticeable effect here, though, is the marked difference in film thickness for both PLL and alginate on these two surfaces. Very small measured thicknesses on silica are consistent with an induction period seen for alternating PLL/alginate multilayers in an earlier study<sup>3</sup> as well as for the repeated adsorption cycles presented here.

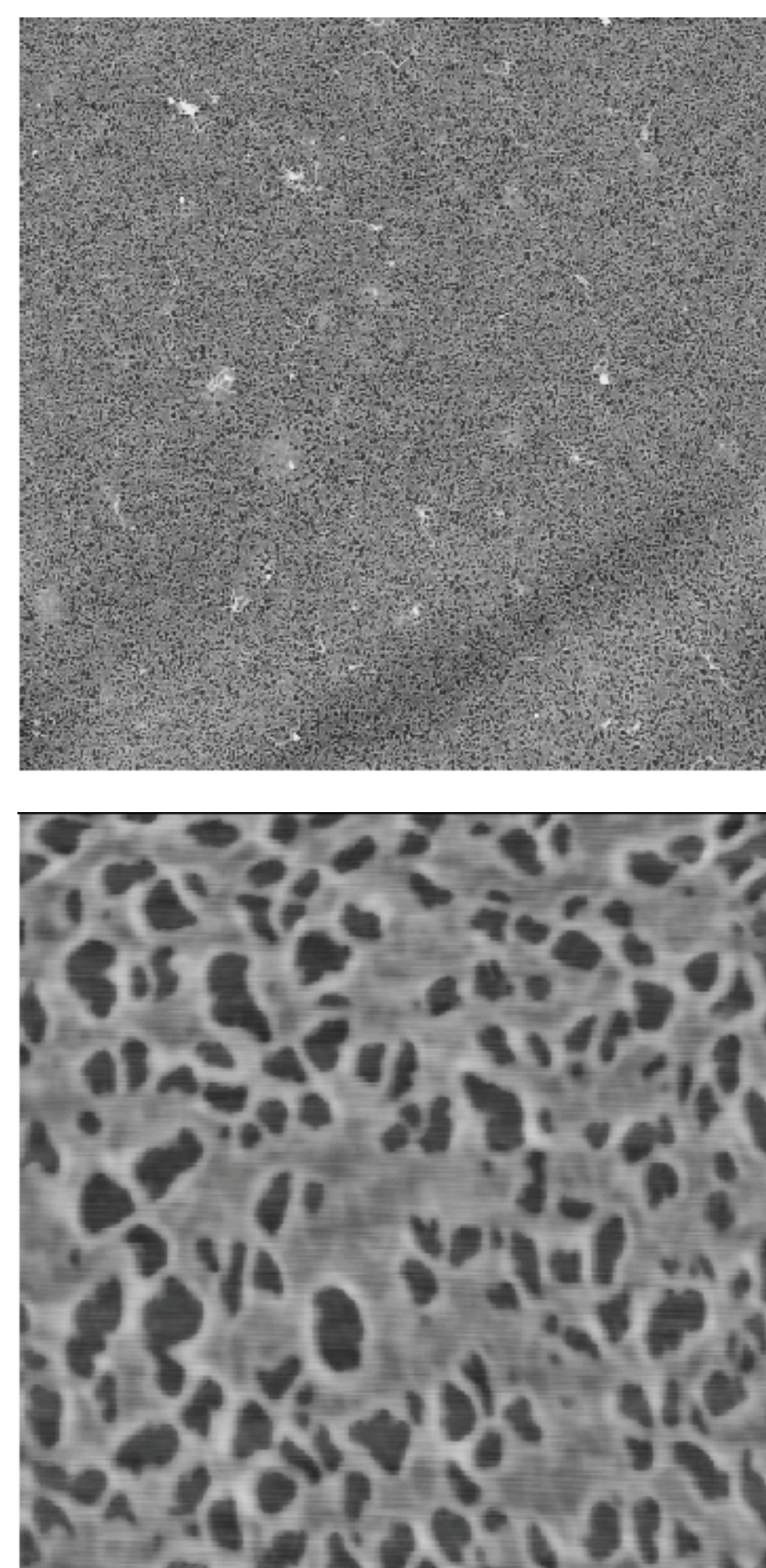
**Figure 2:** Measured film thickness of PLL (highest MW) adsorbed to the silane layer, followed by the indicated drying time in air and re-immersion in PLL (or buffer).



**Figure 3:** Comparison of total film thickness for alginate adsorbed to PLL on the silane layer with and without sample drying between the PLL and alginate adsorption steps.



Measuring PLL and alginate film thickness separately requires drying the sample between adsorption steps. As Figure 2 shows, allowing the PLL film on the silane layer to sit in air causes changes in the film that permit further PLL adsorption upon reimmersion in PLL solution. The data in Figure 3 show a small but significant increase in total (PLL plus alginate) film thickness when this drying step is eliminated. Since alginate adsorption is correlated with the amount of PLL adsorbed on this substrate, this is consistent with PLL films that are slightly more continuous when hydrated.

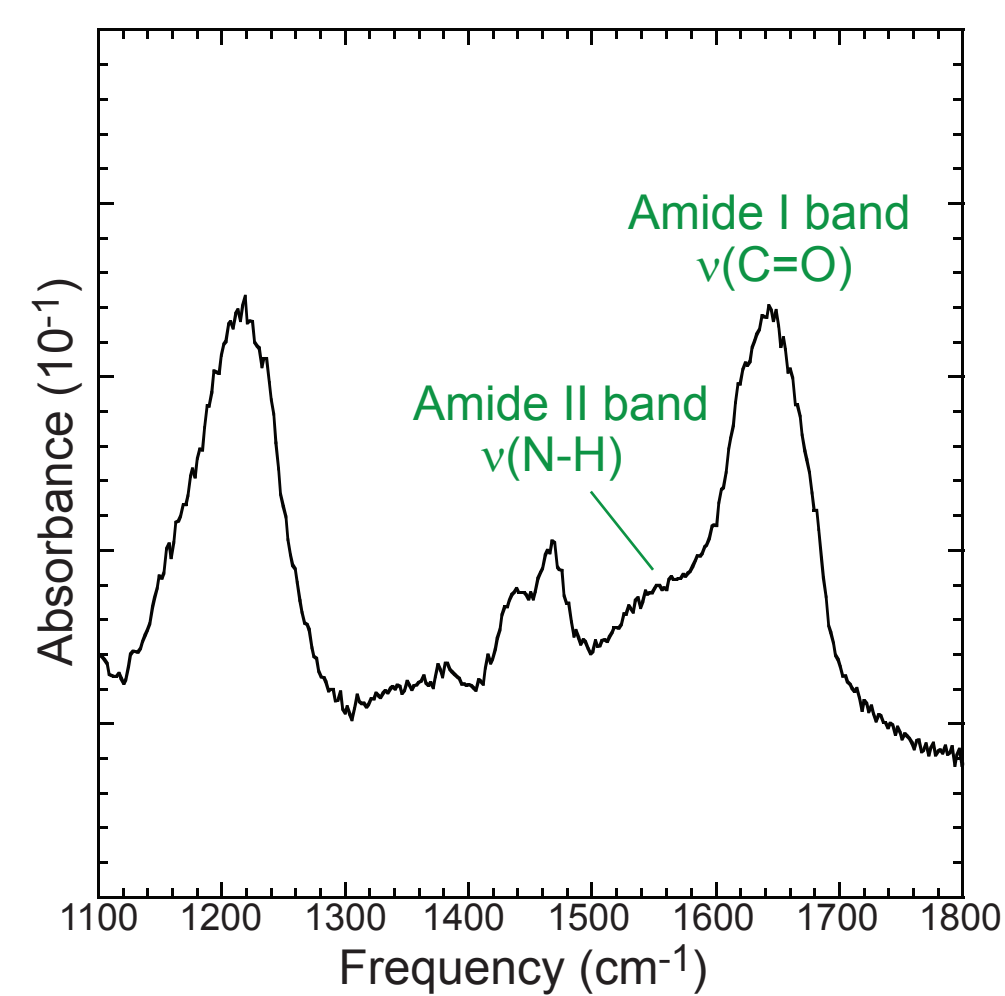
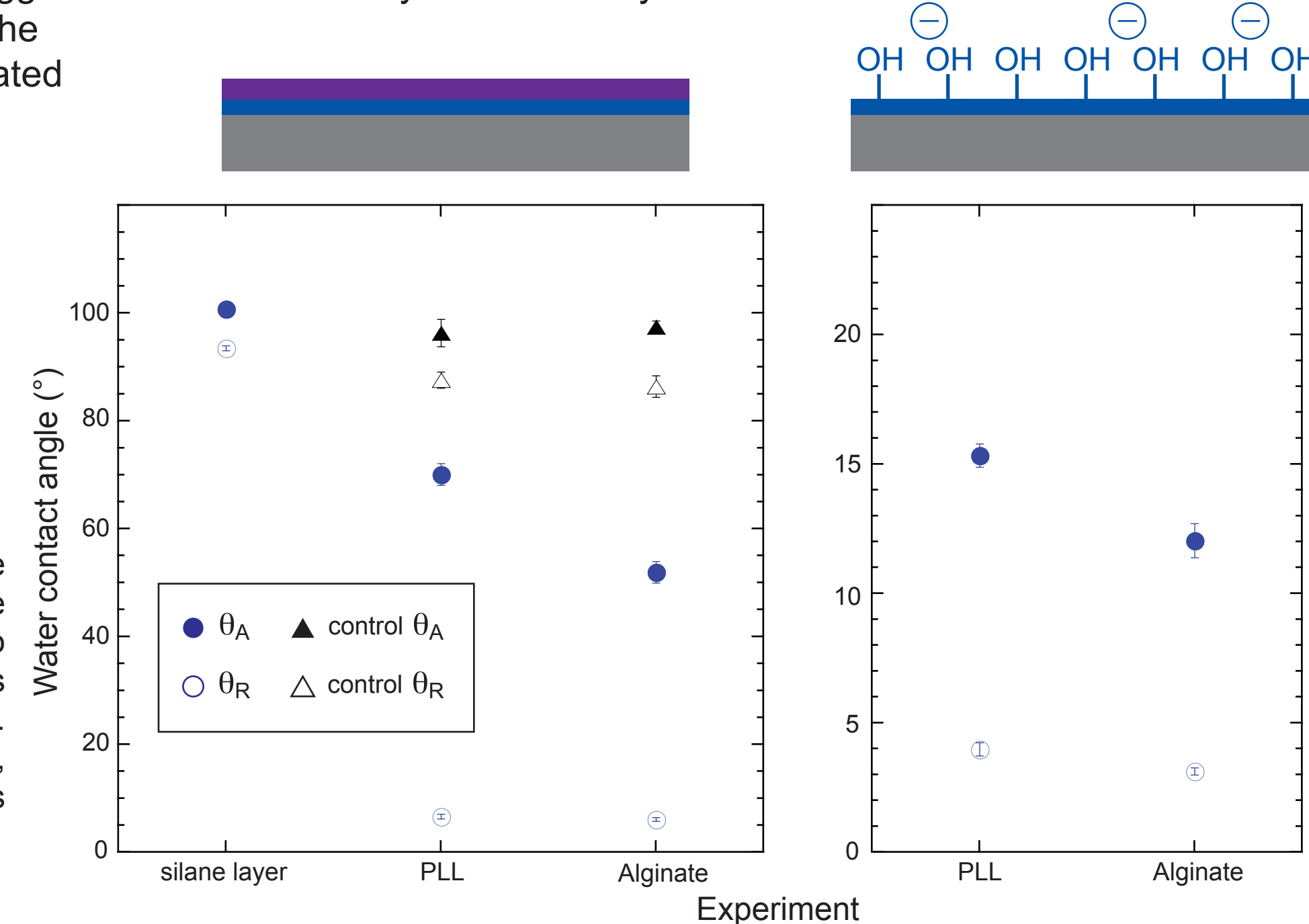


Atomic force microscopy reveals that PLL films on the hydrophobic, silane-terminated surface are indeed discontinuous. As suggested by ellipsometry, this film morphology is at least partly influenced by the film's exposure to air. Dynamic contact angle measurements are consistent with this morphology, with a large contact angle hysteresis on the silane-terminated surface.

Comparison of contact angle data for the hydrophobic and hydrophilic surfaces provide strong evidence that the PLL surface presented to adsorbing alginate is different as the result of differing PLL/substrate interactions. Modeling the advancing contact angle as a combination of the advancing angles of the initial silane layer and of an ideal, "pure" PLL film on that surface, e.g.  $\cos \theta_A = \alpha_{\text{sil}} \cos \theta_{A,\text{sil}} + \alpha_{\text{PLL}} \cos \theta_{A,\text{PLL}}$ ,<sup>4</sup> we use the fractional coverage of PLL determined via analysis of the AFM images at left (~73%) to derive an advancing contact angle for the ideal PLL film of 58°. Substituting this value back into the same equation applied to  $\theta_A$  for PLL on the hydrophilic surface yields an estimated PLL coverage of only 7% on that surface. This value is not consistent with our combined ellipsometry and AFM results for that surface, which suggests that the wettability of the PLL layer on silica is significantly different from the wettability of PLL on the silane-terminated surface.

**Figure 4:** AFM images (tapping mode) of high MW PLL adsorbed on the silane-terminated surface. Top image is 10 μm per side; bottom image is 1 μm per side. Height range is 10 nm for both.

**Figure 5:** Dynamic contact angle measurements of high MW PLL and alginate adsorption to the silane layer (left) and to hydroxyl-terminated silica (right). PLL was adsorbed to the silica surface from 0.5 M KBr. For the control surface on the silane layer, the initial surface was immersed in analogous PLL- and alginate-free solutions.



**Figure 6:** FT-IR plot illustrating the amide region of PLL adsorbed on hydroxy-terminated silica.

Alginate adsorption to PLL films on the hydrophobic surface causes a marked change in the observed film morphology. An increase in film height is consistent with ellipsometry results that suggest alginate adsorbs only to PLL-covered regions of the surface. Restructuring of the film indicates a strong interaction between alginate and the underlying PLL. This type of restructuring is not seen for alginate adsorption to PLL on hydroxyl-terminated silica, where the PLL and substrate are expected to interact more strongly through electrostatic attraction. Studies are underway to monitor changes in the conformation of adsorbed PLL via *in-situ* infrared spectroscopy of these systems.

## Conclusions

Interactions between poly-L-lysine and the underlying substrate govern not only the adsorption of PLL itself but also the subsequent adsorption of alginate to the PLL-coated surfaces in this study. While protein-mediated adsorption of alginate is essential (alginate does not adsorb to either of these surfaces in the absence of pre-adsorbed PLL or another protein conditioning film), this mediation is highly dependent on interactions between PLL and the surface. Strong interactions between PLL and negatively-charged silica result in flat PLL films on that surface. On a silane-terminated surface PLL adsorbs in much thicker films whose morphology appears to be dictated by PLL/PLL interactions and, upon subsequent adsorption, to PLL/alginate interactions. Quantitative comparison of the wettabilities of films on these two surfaces provides information about the PLL surface presented to incoming adsorbates, an effective method for investigating the relative properties of films composed of large, polyfunctional molecules.

## Acknowledgments

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