Title

Discriminatory Bio-Adhesion Over Nano-Patterned Polymer Brushes

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Abstract

Surfaces functionalized with bio-molecular targeting agents are conventionally used for highly-specific protein and cell adhesion. This thesis explores an alternative approach: Small non-biological adhesive elements are placed on a surface randomly, with the rest of the surface rendered repulsive towards biomolecules and cells. While the adhesive elements themselves, for instance in solution, typically exhibit no selectivity for various compounds within an analyte suspension, selective adhesion of targeted objects or molecules results from their placement on the repulsive surface. The mechanism of selectivity relies on recognition of length scales of the surface distribution of adhesive elements relative to species in the analyte solution, along with the competition between attractions and repulsions between various species in the suspension and different parts of the collecting surface. The resulting binding selectivity can be exquisitely sharp; however, complex mixtures generally require the use of multiple surfaces to isolate the various species: Different components will be adhered, sharply, with changes in collector composition. The key feature of these surface designs is their lack of reliance on biomolecular fragments for specificity, focusing entirely on physicochemical principles at the lengthscales from 1 – 100 nm. This, along with a lack of formal patterning, provides the advantages of simplicity and cost effectiveness.

This PhD thesis demonstrates these principles using a system in which cationic poly-L-lysine (PLL) patches (10 nm) are deposited randomly on a silica substrate and the remaining surface is passivated with a bio-compatible PEG brush. TIRF microscopy revealed that the patches were randomly arranged, not clustered. By precisely controlling the number of patches per unit area, the interfaces provide sharp selectivity for adhesion of proteins and bacterial cells. For instance, it was found that a critical density of patches (on the order of 1000/ m2) was required for fibrinogen adsorption while a greater density comprised the adhesion threshold for albumin. Surface compositions between these two thresholds discriminated binding of the two proteins. The binding behavior of the two proteins from a mixture was well anticipated by the single- protein binding behaviors of the individual proteins.

The mechanism for protein capture was shown to be multivalent: protein adhesion always occurred for averages spacings of the adhesive patches smaller than the dimensions of the protein of interest. For some backfill brush architectures, the spacing between the patches at the threshold for protein capture clearly corresponded to the major dimension of the target protein. For more dense PEG brush backfills however, larger adhesion thresholds were observed, corresponding to greater numbers of patches involved with the adhesion of each protein molecule. . The thesis demonstrates the tuning of the position of the adhesion thresholds, using fibrinogen as a model protein, using variations in brush properties and ionic strength. The directions of the trends indicate that the brushes do indeed exert steric repulsions toward the proteins while the attractions are electrostatic in nature. The surfaces also demonstrated sharp adhesion thresholds for S. Aureus bacteria, at smaller concentrations of adhesive surfaces elements than those needed for the protein capture. The results suggest that bacteria may be captured while proteins

are rejected from these surfaces, and there may be potential to discriminate different bacterial types. Such discrimination from protein-containing bacterial suspensions was investigated briefly in this thesis using S. Aureus and fibrinogen as a model mixture. However, due to binding of fibrinogen to the bacterial surface, the separation did not succeed. It is still expected, however, that these surfaces could be used to selectively capture bacteria in the presence of non-interacting proteins.

The interaction of these brushes with two different cationic species PLL and lysozyme were studied. The thesis documents rapid and complete brush displacement by PLL, highlighting a major limitation of using such brushes in some applications. Also unanticipated, lysozyme, a small cationic protein, was found to adhere to the brushes in increasing amounts with the PEG content of the brush. This finding contradicts current understanding of protein-brush interactions that suggests increases in interfacial PEG content increase biocompatibility.

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