DESIGN, SYNTHESIS, AND SCREENING OF LIGANDS FOR THE CHROMATOGRAPHIC SEPARATION OF PROTEINS

by

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ABSTRACT

One significant aspect of biotechnology research is to engineer proteins with optimized properties for use as biopharmaceuticals. It is important to isolate and purify proteins of interest and study their conformations, substrate specificities, and specific activities for the selection, design, and engineering of better therapeutics. The development of techniques and methods for protein purification has, therefore, been an essential pre-requisite for the improved production of protein-based drugs. Chromatographic techniques offer high specificity and efficiency and are widely used in biotechnology as they bring a protein-based drug from the initial identification stage to the stage of a becoming a marketed product. To date, a major part of research in the field of chromatography has relied on the development of selective separation media. However, it has proved challenging to obtain a clear understanding of the nature of interactions between proteins and chromatographic media. This thesis work has focused on understanding the fundamental physics underlying protein chromatographic processes and designing novel ligands for the chromatographic separation of proteins.

We used a model system based on surface plasmon resonance (SPR) spectroscopy and self-assembled monolayers (SAMs) to understand the characteristics of surfaces that promote the adsorption of proteins at high ionic strengths. We have synthesized SAMs presenting different multimodal ligands, and determined the influence of surface composition, solution composition, and the nature of the protein on the extent of protein adsorption onto the SAMs. Our results confirm that hydrophobic interactions can contribute significantly to protein adsorption under high-salt conditions. We demonstrated an affinity-based strategy for designing selective protein displacers for the chromatographic separation of proteins. To design a displacer that is selective for a target protein, we attached a component with affinity for the target protein to a resin-binding component; we then tested the ability of such displacers to selectively retain the target protein on a resin relative to another protein having a similar retention time.

Finally, we have developed a high-throughput screening technique that combines the use of SPR spectroscopy and SAMs for the rapid identification of ligands, designed to improve the performance of multimodal, displacement and affinity chromatographic processes. We generated a series of model surfaces presenting commercially available

organic amines for the screen. The extent of adsorption of lysozyme and cytochrome *c* onto such multimodal surfaces was used to identify molecules that could function as affinity ligands and displacers.