Multi-modal and Chemically Selective Displacement Chromatography for Protein Purification: Displacer Design, Mechanism Elucidation and Implementation

by

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ABSTRACT

The effective purification of proteins plays a vital role for applications ranging from the production of biopharmaceuticals to the fractionation of complex biological mixtures for bioanalytical applications. This thesis focuses on the development of novel selective displacement systems for protein purification. These new modes of displacement chromatography introduce orthogonal degrees of selectivity into the process, potentially reducing the number of required separation steps for the purification of biopharmaceuticals and creating unique windows of selectivity unattainable using traditional modes of operation.

The first phase of the thesis has been aimed at the elucidation of the underlying mechanisms of chemically selective displacement. Robotic high-throughput screens, using multiple protein systems and large libraries of displacers, have given valuable insight into the design of a chemically selective displacer on a molecular level. These screens have also shown that the design of a chemically selective displacer is an intricate balance between the displacer's affinity for the resin and protein. Different separations are then possible using chemically selective displacers, namely selective vs. exclusive displacement. Analytical techniques, such as surface plasmon resonance and saturation transfer difference NMR, have shown that a selective binding event between the displacer and protein is the mechanism by which chemically selective displacement occurs. For this particular class of displacers the binding event has also been shown to be hydrophobic in nature, corroborating trends previously seen in the high-throughput screens. By using the underlying protein binding mechanism in displacer design, whole new classes of chemically selective displacers can be developed using affinity moieties already present in the literature. These analytical results have also been verified using molecular dynamic simulations (in collaboration with the Grade group), which give deeper insight into the actual displacer–protein binding process. Finally, in collaboration with the Moore group, fluorescent selective displacers have been designed and implemented to provide the ability to monitor a displacement separation online. This approach provides a valuable tool for industrial applications, where a separation process must be monitored in an online fashion.

The second phase of the thesis has focused on the development of selective displacement processes in multi-modal chromatographic systems, specifically hydroxyapatite. Fundamental studies were first carried out to elucidate mechanisms of protein binding in hydroxyapatite chromatography, revealing the importance of multi-interaction synergistic binding on the angstrom lengthscale, a previously undefined mode of protein binding in hydroxyapatite. With this knowledge, robotic high-throughput screening has been used to examine selective displacement in hydroxyapatite using model protein systems. These screens have given valuable insight into the design of displacers for hydroxyapatite and determined the effect of mobile phase modifiers on the selective separations. Column separations achieved baseline resolution of previously unresolved protein pairs, establishing a new class of separations which combine the selectivities of multi-modal resins, displacers/eluents, and mobile phase modifiers to create unique selectivity windows unattainable using traditional modes of operation. Finally, this technique was implemented for the purification of monomeric monoclonal antibody from associated aggregates and other post-protein A step impurities. Initial results from batch screening experiments identified optimal separation regimes which were then used to guide column separations, achieving baseline separation of monomeric monoclonal antibody from associated aggregates and impurities. This work suggests a new platform paradigm for the purification of monoclonal antibodies, starting with a protein A capture step followed by a single polishing step using selective displacement/elution on hydroxyapatite.