THE OHIO STATE UNIVERSITY

Ph.D. Thesis Defense

The William G. Lowrie Department of Chemical and Biomolecular Engineering

"Advances in affinity based methods for downstream process development of monoclonal antibody and recombinant protein therapeutics "

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Date: 11/14/2023
Time: 11:00 AM
Location: CBEC 267
Microsoft Teams meeting ID: 267 424 363 156, Password: fF37tD

Abstract

The biopharmaceutical market has seen tremendous growth over the past few decades as new recombinant proteins, antibody based therapeutics and gene therapies have been commercialized. After production in different mammalian, yeast and microbial expression hosts, each therapeutic product must be concentrated and highly purified before clinical use.

Protein A affinity methods provide a convenient and widely used platform for capturing and purifying monoclonal antibodies (mAbs), Fc-fusion proteins, antibody drug conjugates (ADCs) and bispecific antibodies (BsAbs). The wild-type recombinant Protein A ligand despite having 5 domains, each capable of binding one mAb molecule, has been reported to bind roughly 2-3 mAbs in solution. This has been attributed to the significant steric hindrance present during the binding event. In this work, we initially identified 3 flexible and 2 rigid linkers (12-15 amino acids in size), which have been used previously for increasing protein domain spacing, solubility and stability. Novel Protein A variants comprising of three and four domain C units spaced apart using multiple combinations of the selected linkers were assembled to study the effect of different linkers on the in-solution mAb binding stoichiometry.

Purification strategies based on self-removing and self-precipitating tags have been developed previously for purifying target proteins in a single step. However, these methods utilized pH sensitive contiguous inteins which suffer from premature cleavage, resulting in significant product loss during protein expression. In this work, we evaluated two novel mutants of the *Mtu RecA* ΔI-CM mini-intein obtained through yeast surface display for improved protein purification. When used in fusion with the elastin like-polypeptide (ELP) precipitation tag, the two mutants (ΔI-12 and ΔI-29) resulted in significantly reduced premature cleavage, higher product purities and process yields compared to the original *Mtu RecA* ΔI-CM mini-intein. Further, high cleavage efficiencies were achieved after 5 hours under most conditions.

There is currently no platform technology available for purifying non-mAb protein therapeutics. Protein therapeutics such as single domain antibodies, single chain variable fragments, Fab fragments, interferons, epoetins, growth factors, enzymes etc. have been purified using multiple column steps based on ion exchange, hydrophobic interaction, mixed mode, and ceramic hydroxyapatite chromatography. These multicolumn approaches require significant optimization and often result in low product yields and recoveries. In this work, we present case studies demonstrating the self-removing *i*CapTag™ for highly efficient purification of untagged interferon alpha 2b, the ML39 single chain variable fragment (scFv), and the receptor binding domain (RBD) of SARS-CoV-2 spike protein. These proteins were expressed and secreted by Expi293 cells with the self-removing tag fused to their N-terminus. We were able to obtain highly pure (>99%) tagless protein in a single step with high clearance of process and product related impurities.

Finally, a novel tandem affinity tag is presented that enables the use of cation exchange resins for initial affinity purification, followed by an additional column step for enhanced purity and affinity tag self-removal. In this work, we demonstrate a dual column process in which the tagged protein of interest is first captured from an *E. coli* cell lysate using a cation exchange column via a fused heparin-binding affinity tag. The partially purified protein is then diluted and loaded onto an *i*CapTag™ split-intein column, washed, and then incubated overnight to release the tagless target protein from the bound tag. Case studies demonstrating the purification of diverse tagless proteins with a range of isoelectric points and molecular weights are presented. Overall, the proposed dual column process is shown to be a scalable platform technology capable of accessing both the high dynamic binding capacity of ion exchange resins and the high selectivity of affinity tags for the purification of recombinant proteins.