THE OHIO STATE UNIVERSITY
COLLEGE OF ENGINEERING

William G. Lowrie Department of Chemical and Biomolecular Engineering

Andre Palmer Laboratory for Artificial Blood Research
https://people.engineering.osu.edu/people/palmer.351

Platform for Purification of Protein Complexes

Ivan S. Pires, Andre F. Palmer

INTRODUCTION

Proteins are used in various research and biomedical applications. However, purification of a specific target protein from a complex mixture requires costly process development. Furthermore, current strategies rely on cumbersome or non-scalable purification techniques.

Solution: Employ **tangential-flow-filtration (TFF)** to define the molecular weight distribution of proteins in a complex mixture, then add a protein complexing agent to increase the size of the target protein beyond that of other proteins such that size-based separations allows for purification of the complex.

Demonstration: Proof-of concept of this approach was demonstrated via the isolation of the haptoglobin-hemoglobin (Hp-Hb) complex from Cohn Fraction IV paste and the complex between an anti-human serum albumin (HSA) immunoglobulin-G and HSA from serum.

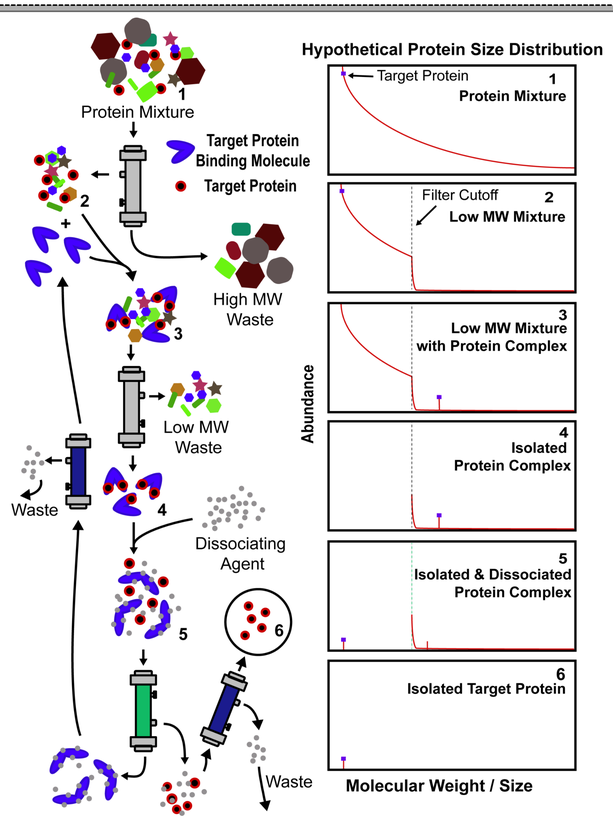

Figure 1: General schematic for purification of a target protein.

Separation fundamentals allows for simple models with predictive capability

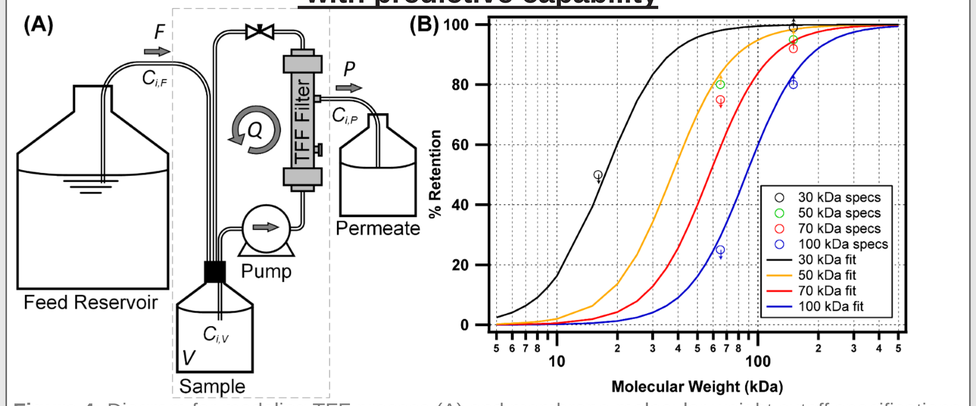

Figure 4: Diagram for modeling TFF process (A) and membrane molecular weight cutoff specifications (B).

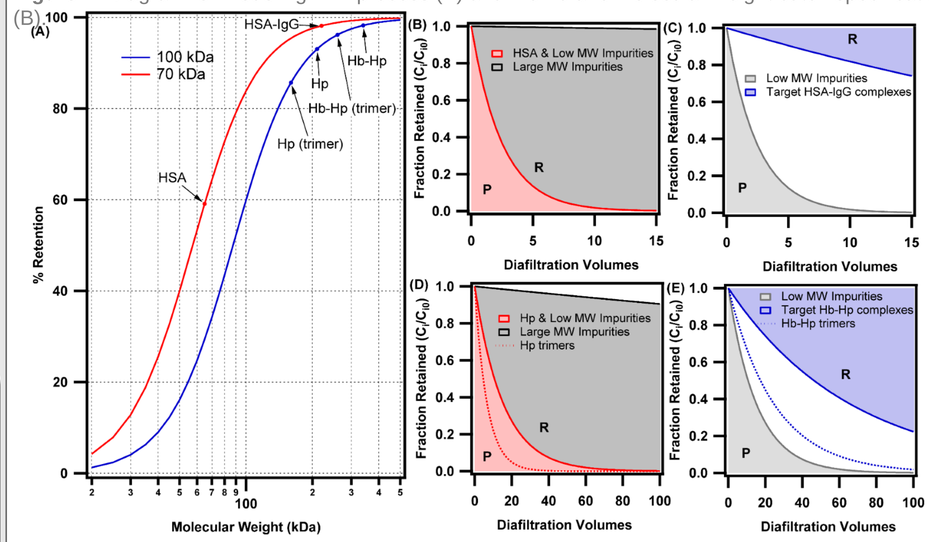

Figure 5: Example model results. (A) Estimated retention curves and the expected retention for the target species. (B) Separation of HSA and low MW species from large MW impurities using a 70 kDa TFF module. (C) Separation of HSA-IgG complexes from low MW species using a 70 kDa TFF module. (D) Separation of Hp and low MW species from large MW impurities using a 100 kDa TFF module. (E) Separation of Hp-Hb complexes from low MW impurities using a 100 kDa TFF module. Shading indicates the range of curves that could comprise the permeate (P) and retentate (R).

Results

Experimental results match the expected progression during purification and demonstrate feasibility of isolating a purified protein complex (Hb-Hp).

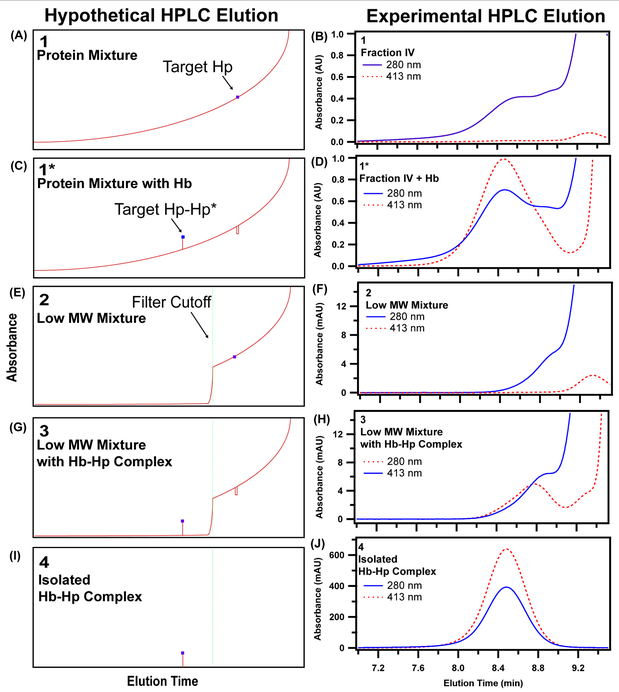

Figure 2: Theoretical HPLC-SEC curves (left) and experimentally obtained curves (right) for purification of the Hp-Hb complex.

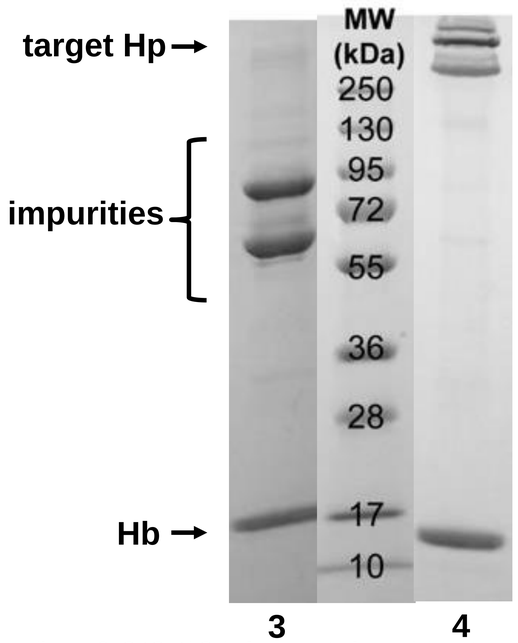

Figure 3: SDS-PAGE of permeate from FIV paste with added Hb (left) and the isolated Hp-Hb complex (right).

Benefits:

- Scalable
- TFF routinely used for concentration or buffer exchange
- Applicable to any target protein

BIBLIOGRAPHY

(1) Pires, I. S., Palmer, A.F. *Journal of Membrane Science* 618 (2021)

FUNDING SOURCES AND ACKNOWLEDGEMNTS

This work was supported by National Institutes of Health grants R56HL123015, R01HL126945, R01EB021926, and R01HL138116, The Ohio State University Office of Undergraduate Research & Creative Inquiry Summer Research Fellowship, and the Pelotonia Fellowship Program.