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Separation of Hydrophobic Ligands From Proteins

INTRODUCTION

Many **high value** proteins have their therapeutic efficacy **derived or enhanced** in their **apoprotein** state (i.e. devoid of associated ligand). Unfortunately, it may be cumbersome or complex to dissociate and purify a ligand from its apoprotein due to their **high affinity**.

Solution: Employ **tangential-flow-filtration (TFF)** to facilitate buffer exchange of protein-ligand complexes into buffers which reduce the ligand-binding affinity such that size-based separations can enable efficient removal of ligand.

Demonstration: Proof-of concept of this approach was demonstrated via the removal of the prosthetic heme group from hemoglobin (Hb) to yield apohemoglobin (apoHb). Under physiological conditions apoHb has an affinity of ~10 pM for heme. ApoHb has utility in biomedical applications such as drug delivery, bioimaging and heme-scavenging.

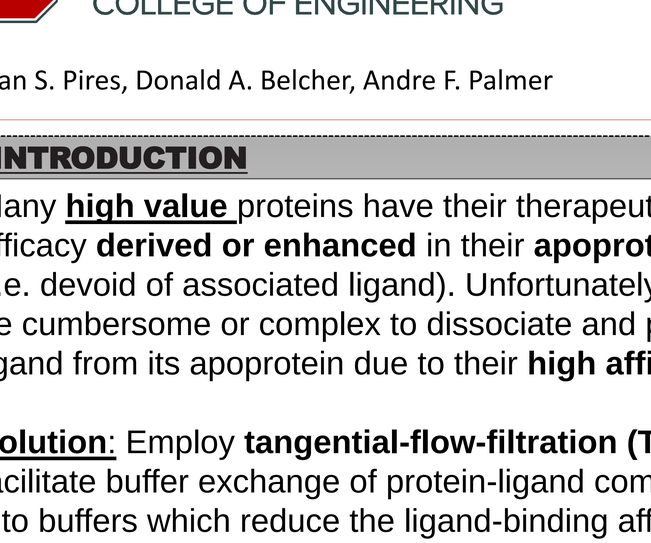

Figure 2: Schematic of common apoHb manufacturing techniques (acetone and MEK) and proposed TFF process.

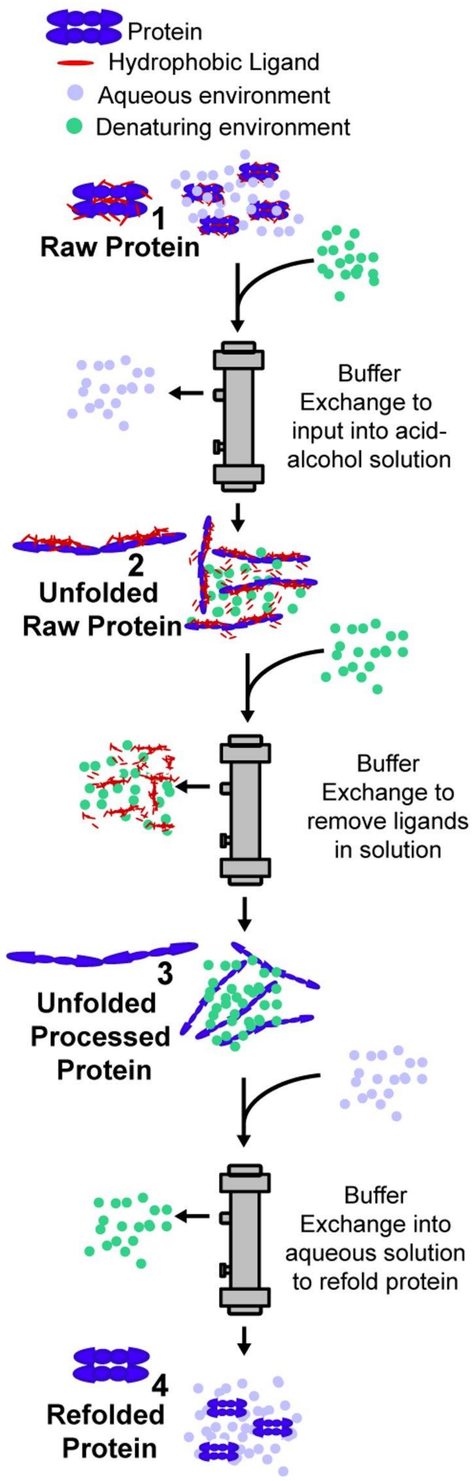

Figure 1: Schematic of general process to remove ligands from proteins via TFF.

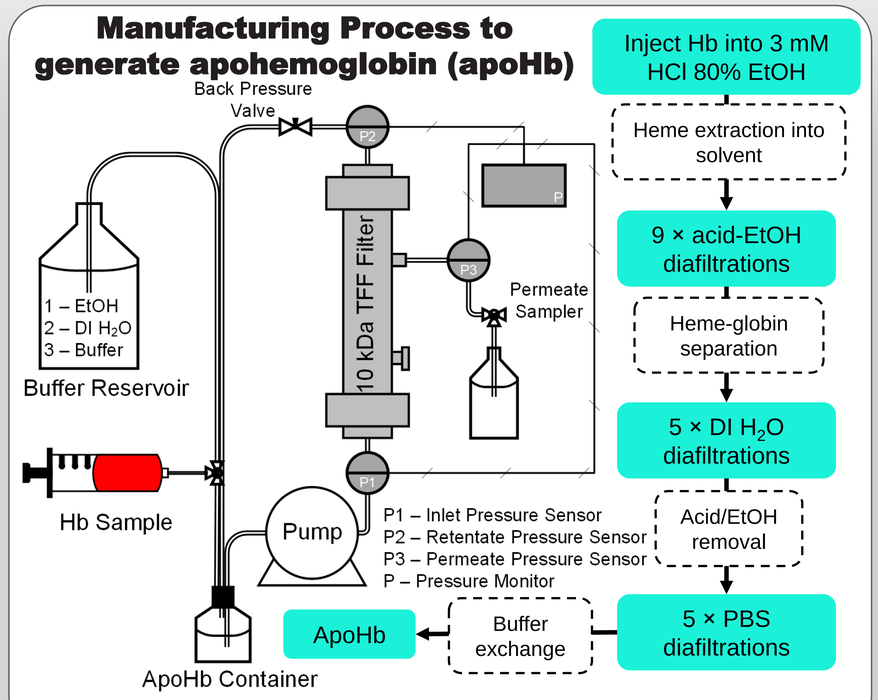

Figure 3: Schematic of process for manufacturing apoHb via TFF.

Table 1: Results from various apoHb production methods and the effect of concentration on the activity of apoHb.

Production Method	Overall Protein Yield	Overall Active ApoHb Yield	N
Acetone	90.5% ± 17.6%	70.3% ± 10.7%[a]	4
MicroKros TFF	96.6% ± 5.5%	83.1% ± 5.4%	6
MiniKros TFF	98.4% ± 11.0%	73.4% ± 5.3%[a]	14

[a] $p < 0.05$ compared with microKros TFF

Active Protein Loss	Total Protein Loss
7.7% ± 1.9%[b]	21.3% ± 8.1%[b]
State	**% Active Protein**
Unconcentrated	62.3% ± 4.8%[c]
Concentrated	69.5% ± 4.0%[c]

[b,c] $p < 0.05$ between pairs with same letter

Other high value apoproteins:

- Lipid-free serum albumin
- Lipid-free apolipoproteins
- Iron-free transferrin
- Metalloproteins

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