Utrafiltration to Concentrate Membrane Proteins without Concentrating Detergents

Membrane proteins (MPs) are of rapidly growing interest in the design of pharmaceutical products, novel sensors and synthetic membranes (Kim et al. 2012; Kowalczyk et al. ; Shen et al. 2014). The use of MPs in engineered systems such as biomimetic membranes will require purification scales well beyond what is currently available. The hydrophobic exterior of MPs necessitates their stabilization in detergent micelles. Ultrafiltration using commercially available centrifugal concentrators is typically employed for concentration of low-yield MPs, but its use is accompanied by a concomitant increase in concentration of detergent micelles. The resulting high detergent concentrations can destabilize MPs, interfere with subsequent structural and biophysical characterization of MPs, and present challenges in applications. We will present a detailed analysis of the hydrodynamic processes that control detergent passage during ultrafiltration of MPs and propose methods to optimize detergent passage during protein concentration. Experiments were conducted using nonionic detergents, octyl–β-D glucoside (OG) and decyl-β-D maltoside (DM) with the bacterial water channel protein, Aquaporin Z (AqpZ) and the light driven chloride pump, Halorhodopsin (HR), respectively. The observed sieving coefficient (S_o), a measure of detergent passage, was evaluated in the stirred cell and centrifugal systems. The sieving coefficient for detergent was much greater in the stirred cell systems than in the commercial filters (**Figure 1**). Similar effects

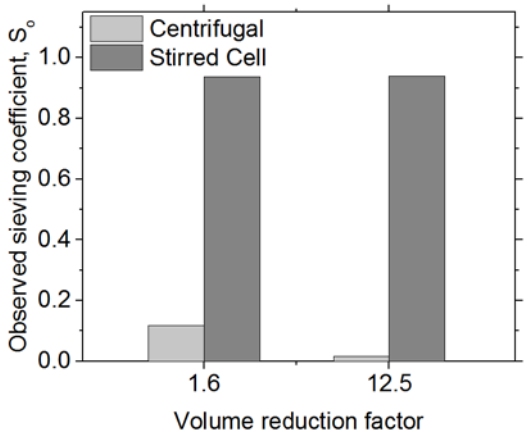


Figure 1. Concentration polarization promotes passage of detergent, DM during ultrafiltration. DM shows higher transmission in stirred cell versus centrifugal filter under equivalent conditions of transmembrane pressure of 11 psi (75.9 kPa) and 14 psi (96.6 kPa) and concentration factors of 1.6 and 12.5 respectively.

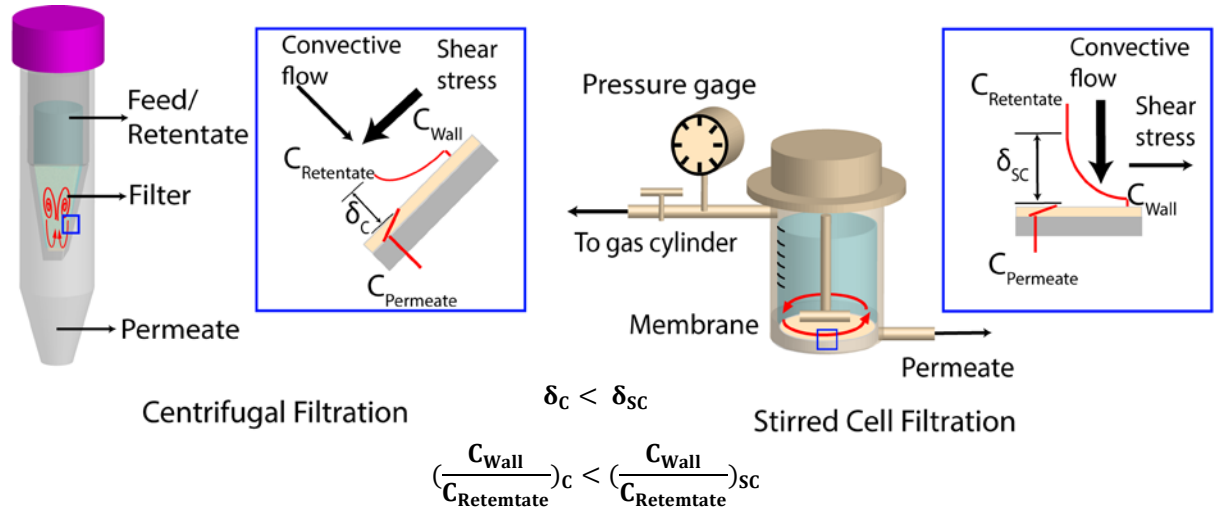


Figure 2. Protein concentration using centrifugal filtration is designed to minimize concentration polarization by maximizing shear stress. This is achieved by inducing circulatory flow by using a specific geometry of the filter. In pressure- driven filtration using a stirred cell, the shear rates can be controlled and thus the extent of concentration polarization can be tuned to study the effect of concentration polarization on detergent passage.

were observed during filtration of protein-detergent micelles. Thus, almost complete transmission of detergent can be achieved by operating under conditions that encourage concentration polarization. This is in complete contrast to current approaches in soluble protein concentration systems, including centrifugal filters, which are designed to maximize flux by minimizing concentration polarization (**Figure 2**). The results in this study clearly indicate the importance of CP in achieving greater detergent passage during ultrafiltration of MP-detergent systems, which has important implications for downstream processing of MPs.

References

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