# Understanding membrane fouling by proteins through molecular dynamics simulations and optical coherence tomography

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**Abstract:**

The use of biocatalysts has proliferated in the production of active pharmaceutical ingredients (API). Membrane-based separation provides for continuous, high-throughput, energy-efficient, green separations, and also allows for the use of the biocatalysts in the free solution rather than immobilized. For practical implementation, membrane fouling is one of the key issues that needs to be addressed.

A challenge with macromolecular foulants like proteins is that macroscopic characterizations, like net electrical charge, may be poorly correlated with membrane fouling. In this study, molecular dynamics simulations have been performed to understand the local interactions between two similar-sized proteins with opposite overall charges (namely, lysozyme and α-lactalbumin) and a negative-charged membrane. Surprisingly, the protein-membrane distances and adsorption probabilities of both proteins are similar. Compared to the positive-charged lysozyme, the negative-charged α-lactalbumin exhibits (i) greater protein-membrane attractive interaction energy due to synergy among adsorption sites; (ii) lower root-mean-squared deviations (RMSD); and (iii) greater number of residues that show low root-mean-squared fluctuations (RMSF). These results highlight the pitfall of using the overall protein characteristics as predictors of membrane fouling.

A parallel experimental study aimed to understand both external and internal membrane fouling by three proteins with different net charges, namely, negative-charged pepsin and bovine serum albumin, and positive-charged lysozyme. The flux decline was the worst for lysozyme, which was attributed by the fouling model to the greatest pore blockage and pore constriction. Between pepsin and BSA, BSA gave worse flux decline despite its more negative net charge. Notably, despite monotonic flux decline with filtration, the optical coherence tomography (OCT) fouling voxel trends show significant fluctuations, which has not been reported before and thus signify the unique behavior of protein foulants. Specifically, the OCT trends indicate the non-uniform protein deposits slipping downwards in the membrane pores as filtration progressed.

**Keywords**: membrane fouling; protein-membrane interaction; molecular dynamics simulation; optical coherence tomography;