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Lipid-Coated Solid-State Nanopores for Characterization of Single Proteins

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The importance and diversity of proteins in the human body cannot be overstated; proteins are pivotal in every life process, from DNA replication to the immune response. Increased understanding of these molecules, especially their structure and function is necessary for improved diagnostics and treatment of disease.

Solid-state nanopores provide a means to characterize proteins on the single-molecule level in their native, un-labeled state. Unfortunately, proteins are prone to non-specifically interact with the walls of synthetic nanopores, causing blockages and clogging. A solution to this problem consists in the application of fluid lipid coatings to the wall of a synthetic nanopore, providing a non-stick surface with the possibility to anchor specific proteins of interest to mobile lipid anchors in the membrane.

The characterization and comparison of fluid coatings from a wide range of lipids represent one important aspect of this work. Specifically, I present an investigation into the optimal lipid coating with the goal of further improving the utility of synthetic nanopore sensing with regard to protein characterization. Various lipid compositions were examined and evaluated based on the following criteria: baseline noise and stability of the electrical current through lipid coated nanopores, viscosity of the lipid coating, and ease of preparing a lipid coating of high quality. In this work, we tested bolaamphiphilic, membrane-spanning lipids inspired by the lipids in thermophile Archaea and compared them to compositions containing only bilayer-forming lipids that are typical for Eukaryotes. We developed a model for determining charge by expanding the biased first passage time equation to allow for multiple populations of dwell times. This extension enabled charge determination from experiments performed with nanopores coated by especially viscous lipid membranes. The best coatings among 15 lipid compositions tested were those composed of either 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) with 10-20% cholesterol for their low noise qualities during recording or Archaea-inspired lipids with 10-20% POPC, as they provided the longest dwell times of lipid anchored proteins translocating through the nanopore.

Using lipid coatings to anchor proteins slows their translocation and increases measured dwell times, allowing for the resolution of the full duration and magnitude of the resistive pulse from protein translocation. It also provided significantly more data points than from un-anchored protein translocations for the determination of analysis-intensive characteristics such as charge, volume, shape, dipole moment, and rotational diffusion coefficient. This thesis presents the process and results of the application of this analysis to ten different proteins. We also used this multiparametric characterization to distinguish between a single protein and the protein in complex with an antibody.

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