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Development of an Exceptionally Large Biological Nanopore for Characterization of Native Folded Proteins

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Biological nanopore technologies have become increasingly important analytical tools as they allow the characterization of unlabeled synthetic and biological molecules such as polymers, DNA, proteins, peptides, and small molecular analytes. The diameters of typical biological nanopores typically range from 1 to 4 nm, and the small ones among these have been used for DNA sequencing. Nonetheless, the detection of folded proteins using biological nanopores still remains challenging since the nanopore diameter is too small to accommodate protein translocation through their lumens. Recently, a two-component pleurotolysin (PlyAB) toxin was used as the largest biological nanopore with an internal diameter of 5.5 nm and length of 10 nm to detect proteins. The PlyAB nanopore enables the detection of hemoglobin with a molecular weight of approximately 65 kDa. However, the lumen of the PlyAB pore needs to be engineered to allow proteins to pass through. In this dissertation, I explore the development of a new biological nanopore and its application towards single molecule detection of native folded proteins.

I highlight the recent advancements in the utilization of the complement component 9 (C9) from the membrane attack complex protein family to develop robust nanopore sensing devices. This protein oligomerizes in a complex of 22 C9 monomers (polyC9) on the membranes of pathogens and forms nanopores. I present the use of polymeric surfactants, such as Amphipol that improve stability and facilitate the insertion of polyC9 nanopores in phospholipid membranes. Using a combination of transmission electron microscopy, SDS-PAGE, and mass photometry, I characterized the inner diameter (11.2 nm) and approximate molecular weight (1.6 MDa) of polyC9 in our experimental conditions. I demonstrate the capability of polyC9 nanopores to translocate folded proteins to determine their shape and volume. Furthermore, I discuss the selective control of protein translocation probability using polyC9 nanopores by modifying the applied electric field and pH of the electrolyte solution. Lastly, we developed a data analysis algorithm to accurately determine analyte volume and shape within biological nanopores. In addition to this, we developed a new algorithm to predict the spheroidal shape of proteins from crystallography structures.

In the following study, I tested the possibility of using C9 pores to resolve two conformations of the enzyme adenylate kinase (ADK). The obtained preliminary results are promising but require further validation. C9 pores can resolve the open and closed conformation of ADK in their unlabeled natively folded state using the probability distribution of current blockades and the residence time of the open (substrate-free) and closed (inhibitor-bound) conformation in the nanopore lumen.

In summary, the research presented in this thesis demonstrates a new biological nanopore with the largest diameter described to date. Furthermore, this work is the first to demonstrate the use of biological nanopores to determine the volume and shape of single, unmodified proteins with tunable translocation frequency by choice of pH values between 5.5 and 8.5. In addition, polyC9 demonstrates that resistive pulse analysis makes it possible to distinguish the open from the closed conformation of ADK on a single molecule level. I believe that the polyC9-based nanopore has exciting potential in the field of label-free single molecule detection of analytes since it enables effective size and shape estimation of folded proteins.

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