Towards single-molecule protein sequencing using nanopore fingerprinting

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Proteins are the major building blocks of life. The protein content of a cell or organism provides key information for the understanding of biological processes and disease. Despite the importance of protein analysis, only a handful of techniques are available to determine protein sequences and these methods face limitations, for example in the sizeable sample volume needed. During the last few decades, nanopore technology has emerged as one of the most promising techniques to sequence DNA. Sequencing proteins at the single-molecule level is a clear next step. This however presents even greater challenges than DNA sequencing, due to the three-dimensional folded structure of proteins and the presence of 20 different amino acids to be identified.

Here, we propose a new scheme for protein sequencing in which we simplify the identification of proteins by using a fingerprinting method. We computationally show that the detection of two amino acids is sufficient for protein identification using an existing database, and we present our first experimental efforts in this direction using nanopore analysis. We use FraC (Fragaceatoxin C) as our nanopore, and explore its narrow constriction (1.2nm) and enhanced electroosmotic flow for peptide analysis. We study different peptides and show that structural differences and specific moieties can be recognized. We use denaturants and analyze the voltage dependency on dwell times to get insight in the nature of the observed populations. Moreover, we demonstrate that chemical side groups can be added and distinguished enabling a fingerprinting scheme. Finally, this principle can be extended to the detection of post-translational modifications, an area of potential major impact.