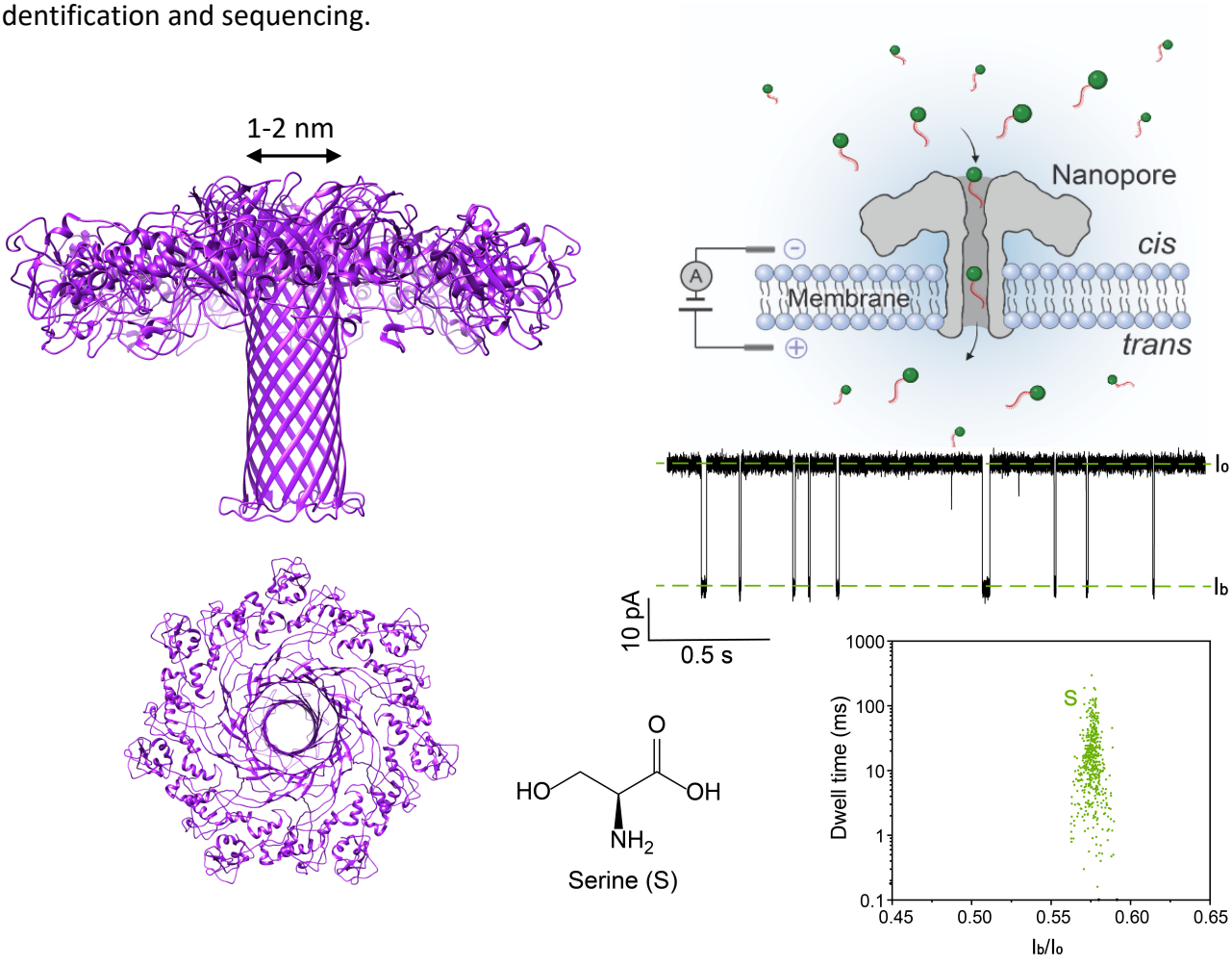
Identification of Twenty Proteinogenic Amino Acids using an Engineered Aerolysin Nanopore

Arianna Fuggetti, Yun Zhang, Chan Cao

Department of Analytical and Inorganic Chemistry, School of Chemistry and Biochemistry, University of Geneva, 30 Quai Ernest-Ansermet, Geneva, Switzerland
Arianna.Fuggetti@etu.unige.ch

Proteins drive most cellular functions and are central to human diseases, yet proteomics still lags behind genomics because of the vast complexity, diversity, and dynamic nature of proteoforms. Recent advances in nanopore single-molecule methods are transforming the field of protein analysis[1]. Here, we investigate the ability of an engineered aerolysin nanopore to discriminate the twenty proteinogenic amino acids[2] by labeling each of them with a molecular tag, to prolong its interaction time with the aerolysin nanopore, thereby improving the resolution for amino acid recognition[3]. First, we optimized the chemical conjugation conditions to improve coupling efficiency between the amino acids (AAs) and the tag. Then, we characterized the current signatures for both individual single AA–tag conjugates and their mixtures under different applied voltages using different aerolysin mutants. Our results demonstrated that the distinct current blockades generated from each AA-tag conjugate can be used to identify corresponding natural amino acids. These findings may pave the way for nanopore-based protein identification and sequencing.



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[2] H. Ouldali, S. Kumar, T. Ensslen, *Nature Biotechnology*, **2020**, *38*, 176–181.
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