Plasmonic Solid State Nanopores for single biomolecule identification

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Sequence identification of peptides and proteins is central to proteomics. Protein sequencing is mainly conducted by insensitive mass spectroscopy because proteins cannot be amplified, which hampers applications such as single-cell proteomics and precision medicine. The commercial success of portable nanopore sequencers for single DNA molecules has inspired extensive research on proteins based on electrical or optical readout. In this regard, a large variety of nanopores, both biological and solid state have been developed. The typical working principle consists in delivering DNA molecules into the pores and detecting the variations of ionic currents caused by the translocation of the molecule (in analogy with Coulter counter). Similarly, methods based on optical readouts have been developed. However, when moving from DNA to proteins some major challenges remain: (1) DNA bases are just 4 against the amino acids which are 20 hence their discrimination only by using electrical current levels or colorimetric readout is extremely difficult; (2) spatial and temporal resolution (sensitivity) to detect single amino acids within the same molecule; and (3) controlling the motion of proteins into the nanopores. In this context, the emergence of label-free optical analysis based on plasmonic enhancement shows great promises to address the first two challenges [1]. In fact, plasmonic nanopores can both confine and amplify the local electromagnetic field into the pore (challenge #2). The confinement improves the spatial resolution while the amplification helps to increase sensitivity. Notably, Raman spectroscopy provides unique molecular fingerprints to discriminate amino acids (challenge #1). Here we show our latest results on plasmonic nanopores combined with Raman Spectroscopy for single-amino-acid identification within single peptides. In fig 1 is reported a sketch representing the concept: a gold plasmonic nanopore is fabricated on silicon nitride membrane (passing through). Molecules in solutions are delivered into the pore by means of electrophoresis and detected by plasmonic enhanced Raman scattering. Notably, the system shows the ability to record and discriminate the twenty amino acids at a single-molecule level [2]. In addition, we discuss the manipulation of molecule translocation and liquid flow in plasmonic nanopores for controlling molecule movement and for enabling high-resolution reading of protein/molecule sequences [3]. We envision that a combination of Raman spectroscopy with plasmonic nanopores can succeed in single-molecule protein sequencing in a label-free way.

References

[1] Jian-An Huang et al., Nature communications, 10 (2019), 1, 1-10.
[2] Yingqi Zhao et al., ACS Photonics, 9 (2022), 3, 730-742.
[3] Yingqi Zhao et al., Nano Lett. 23 (2023), 11, 4830–4836.

Figures

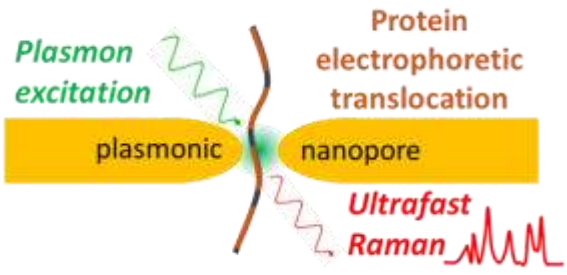

Figure 1: Sketch representing the concept of single molecule identification by means of Raman fingerprint.