The accurate nanopore analysis by the electrochemically confined effects

With the supports by the National Natural Science Foundation of China, the research team led by Prof. Long YiTao (龙亿涛) from the East China University of Science and Technology demonstrated the construction of an aerolysin nanopore with the electrochemically confined effects for single molecule detection, which was published in *Nature Protocols* (2017, 12(9): 1901—1911) and *Nature Nanotechnology* (2016, 11(8): 713—716).

Nanopores provide a promising opportunity for exploring a variety of dynamic processes in a confined space at the single molecule level. Since 2007, Long's group has been making continuous efforts in the nanopore-based single molecule detection, which includes the detection of aggregation peptides, monitoring the conformational changes of single molecules and constructing the light responsive nanopore. To achieve the high sensitivity of nanopores, the pore-forming materials require a suitable lumen that constructs a confinement for electrochemical accommodation of a single molecule. Long's group found that the aerolysin nanopore provides extremely confined structure in a wide range of pH conditions and could directly distinguish one-base difference of oligonucleotides. Owing to the positively charged amino acid residues and the confined diameter of the aerolysin nanopore down to 1 nm, there are strong aerolysin-oligonucleotides interactions defined as electrochemically confined effects to significantly enhance the temporal resolution and the current resolution of the single molecule detection. Therefore, the subtle structural differences between oligonucleotides of different lengths can be discriminated by characterizing current levels.

Moreover, nanopores are expected to provide more dynamic information for the structure-activity relationships of a single molecule, which is beyond the discrimination of its structure, conformation and charges. For example, the instinct electron transferring behavior of the redox enzyme should be detected and linked to its dynamic structural features. Consequently, Long's group developed new mechanisms in nanopore sensing which confines the electrochemical redox process inside a metal coated nanopore. In this novel strategy, the coated metal layer plays as a "wireless" nanoelectrode which owns both the cathode and anode owing to the bipolar electrochemistry phenomenon (*Small*, 2017, 13(25): 1700234; *Analytical Chemistry*, 2017, 89(14): 7382—7387). Therefore, the confined electrochemical processes could efficiently convert the redox behavior of single molecules redox into the ionic pattern. This new kind of nanopore electrode holds the potential to observe the redox characteristic on a single molecule, to study the structure-function relationship of single molecules, and to manipulate the electron transfer from one molecule to another in the electric confinement.

These proposed electrochemically confined effects have important implications in the development of novel nanopore sensors to motivate exciting discoveries in the nanopore-based analysis of biological processes and chemical reactions in confined spaces.

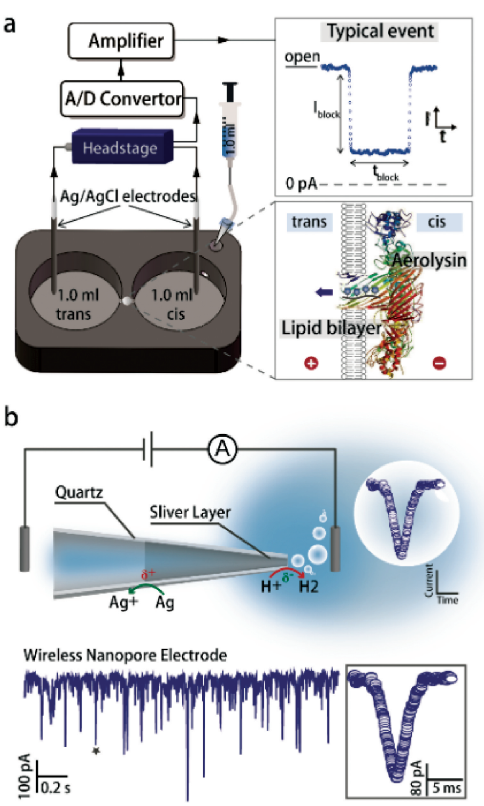

Figure Electrochemical confined nanopore for (a) discriminating one-base difference of a oligonucleotide; (b) analyzing the redox process at single moleculelevel.