Medical & healthcare, Smart device

Single-molecule measurements for single-cell multiomics analyses

Department of Bio-Nanotechnology, SANKEN (The Institute of Scientific and Industrial Science)
Associate Professor Makusu Tsutsui
Researchmap https://researchmap.jp/makusu

Abstract

Rapid advance in single-molecule techniques allowed a sensor platform that enables to address biological information at unprecedented resolution and throughput. This research aims at creating nanosensors that can count the individual proteins and genomes within a cell. The sensor structure is comprised of two nanopore membranes three-dimensionally stacked on a silicon wafer. It can perform cell electrolysis, intracellular molecule extraction, and single molecule detections on-chip by ionic current measurements. The mechanism was tested for *E. coli* for the proof of concept, where it was observed that single-molecule protein and DNA in the bacterial cell were shown to be detectable. This ongoing project currently focuses on identifying the cellular biomolecules by the machine learning-assisted ionic current signal pattern analyses.

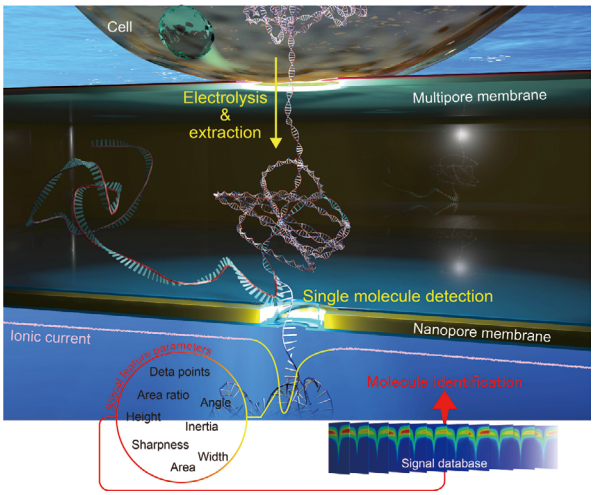
Background & Results

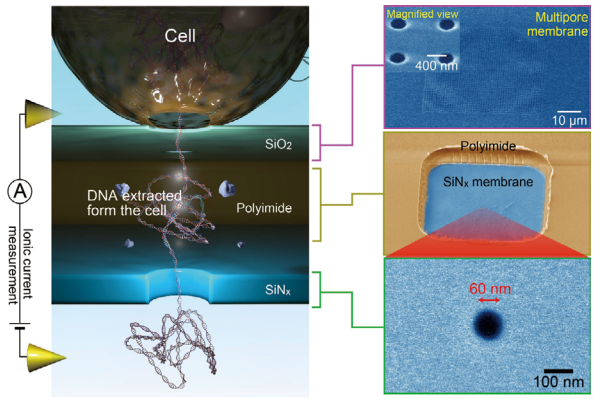
Nanotechnology-based single-molecule measurement techniques have made dramatic progress in the last ten years and started to be applied in omics analysis. The nanopore and the SMRT sequencing technologies are typical examples that can perform DNA and RNA sequence readout at the single-molecule level in real time. Notably, their high throughput and ultralong read length characteristics are already widely practiced in the fields of genomics and transcriptomics. And nowadays there is growing interest beyond the genome sequencing: significant efforts have been devoted in recent years to novel technologies that can count the numerous proteins in a cell by measuring their structure or amino acid sequence at a single-molecule level for the whole proteoform analysis.

This project aims to develop such technology by integrated nanopore sensors. A nanopore is a nanoscale hole formed in a membrane. When a voltage is applied in the electrolyte liquid, ions move inside the nanopore, whose process can be observed via ionic current measurements. When an object such as protein passes through this small hole, the ionic current changes rapidly due to the temporal blockage of the ion transport by its volume thereby allowing a single-molecule sensor of simple mechanism as practiced in the nanopore sequencers. However, it is not straightforward to apply the nanopore approach to proteins since there is no simple method like PCR amplification to amplify the molecules, which makes it difficult to detect rare molecules. Or more simply, if a biological sample contains large particles or aggregates such as amyloid, the nanopore will be easily clogged by them. In order to solve these problems, we devised a structure in which two nanopore membranes are stacked on a silicon chip. A cell can be trapped on nanopores in the upper membrane, and then exposed to the local electric field and Joule heat there for the electrolysis. Subsequently, molecules smaller than the nanopores move toward the second membrane. Here, the distance between the two membranes is made short so that the extracted molecules will swiftly pass through the nanopore in the lower layer for the detection by ionic current measurements. The mechanism was tested for *E. coli* for the proof of concept, where it was observed that single-molecule protein and DNA in the bacterial cell were shown to be detectable. This ongoing project currently focuses on identifying the cellular biomolecules by the machine learning-assisted ionic current signal pattern analyses.

Significance of the research and Future perspective

This technology offers a way for quantitative analyses of protein expression in single cells by simple electrical measurements without special pretreatments, which may contribute to innovation in life sciences and personalized medicine. In addition, its application is not limited to cells; when applied to viruses, for example, it can be a tool for surveillance of infectious strains that can detect a small mutation at a single virion level.





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