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# SINGLE MOLECULE DETECTION USING NANOPORE TECHNOLOGY

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## Editorial

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## EDITORIAL

The growing need for cheaper and faster detection tools for health and environmental monitoring has prompted the development of new technologies that surpass conventional methods in terms of speed and cost. In recent years, Nanopore-based sensing technology has emerged as one of the promising tools for rapid, reliable and cost effective approach for single molecular level detection nucleic acid and protein biomarkers. A nanopore is a very small opening (typically nanometer in diameter) in an insulating membrane that connects two tanks filled with conducting electrolytes eg. KCl. Application of voltage across the nanopore allows the flow of ionic current through it, just like the process of electrophoresis. Movement of highly charged molecules in the electrolytes through the nanopores is known as translocation. The architecture of nanopore make it so sensitive that it can accurately measures the change in ionic current (passing though it) produced by a single molecule. The nanoporous systems work on the pricople of Coulter-counter technique and have been largely explored to detect DNA (DNA sequencing). Based on their origin, nanopores are typically divided into two categories: 1. Biological nanopores, 2. Solid state nanopores. Biological nanopores present into lipid bilayers offer several advantages for single-molecular detection. Certain specific features of biological nanopores are as:1. Large numbers of biological nanopores with an atomic level of precision (and pore size with angstrom length scale) can be produced by cell, 2. Physical and chemical properties of the biological nanopore can be tailored by genetic manupulations for eg. Site-directed mutagenesis,3. Biological nanopores are remarkable heterogeneneous in terms of size and composition. Along with DNA sequencing the biological nanopores have tremendous potential for application in molecular diagnositcs and DNA finger printings. Regardless of the heterogeneity and incredible sensitivity of biological nanopores, they do suffer with some inherent disadvantages like: 1. Lipid bilayer that supports the nanopore are mechanically unstable, 2. Biological nanopores are sensitive of experimental setting such as pH, temperature and concentration of salt, 3. Integrating biological systems into large-scale arrays is very challenging.

To overcome the associated problems with biological nanopores, solid state nanopores technology i.e. nanopores from solid-state materials have been introduced. The solid state nanopres due to their very high stability, better control of diameter and channel length, adjustable surface properties and the potential for integration into devices and arrays have shown obvious advantages over their biological counterpart. Etching a tiny hole into an insulating layers to create a pore is one of the way among various routes have been explored to meet the challenge of fabricating pores with true nanometer dimensions. Narrow opening angle of ~1° of such created pores make these non-ideal for translocation of polynucleotides. Ion beam sculpting is another approach to create single nanopores on SiO2 and SiN with true nanometers control which provided a starting point for DNA translocation measurements. Further, electron-beam lithography coupled with etching have made possible to create pore with 20 nm over Si, SiN, or SiO2 membrane. Graphene membrane has also been used to create nanopore with high hope for DNA sequencing. Some unanswered fundamental questions about sequencing with graphene nanopores are like: 1. How single-nucleotide resolution is possible in the presence of thermodynamic fluctuations and electrical noise? 2. Will the identification of individual nucleotides using ionic current with the chemical and structural similarity of the purines (A and G) and the pyrimidines (C and T)?

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Hybrid biological–solid A state nanopores have been initiated to synergistically employ the useful features of both type of nanopores. Selective hybrid nanopores (unlike solid state nanopores) have been develpoed by using specific recognition sequences and receptors to uniquely identify nucleotides in sequencing applications, or to differentiate and quantify target proteins in diagnostic applications.

The potential of nanopores suggest that they are likely to have an increasing role in medical diagnostics and DNA sequencing (at single molecular level) in coming years in a strong competition with number of other techniques. Biological nanopores have helped in resolving many challenges (the high translocation velocity and the lack of nucleotide specificity) in sequencing. Likewise, the progress with solid-state nanopores, sequence a molecule containing one million bases in less than 20 minutes would be possible by reduced translocation velocity to a single nucleotide (which is ~3 Å long) per millisecond, and specific identification of single nucleotides with an unique electronic signature (need to be explore as a prime objective),. Moreover, sequencing of an entire human genome with 50-fold coverage in less than one hour is possible by this technology if it could be scaled to an array of 100,000 individually addressed nanopores operating in parallel. A right combination of both biological and solid state nanopores will provide an efficient and rapid personalized DNA sequencing tool as well as nanopore based ultarsensitive sensors for detection of disease biomarkers at single molecular level.