ISSN: 2157-7439
Journal of Nanomedicine & Nanotechnology

OPEN ACCESS Freely available online

Research Article

# DNA Sequencing: Characterization of Proteins with Nanopores

**Iqbal Arfa***

*Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, Netherlands*

**ABSTRACT**

Inspired by the biological processes of molecular recognition and transportation across membranes, nanopore techniques have evolved in recent decades as ultrasensitive analytical tools for individual molecules. In particular, nanopore-based single-molecule DNA/RNA sequencing has advanced genomic and transcriptomic research due to the portability, lower costs and long reads of these methods. Nanopore applications, however, extend far beyond nucleic acid sequencing. In this Review, we present an overview of the broad applications of nanopores in molecular sensing and sequencing, chemical catalysis and biophysical characterization. We highlight the prospects of applying nanopores for single-protein analysis and sequencing, single-molecule covalent chemistry, clinical sensing applications for single-molecule liquid biopsy, and the use of synthetic biomimetic nanopores as experimental models for natural systems. We suggest that nanopore technologies will continue to be explored to address a number of scientific challenges as control over pore design improves.

## INRODUCTION

Nanopores as single-molecule biosensors were initially developed for ultrasensitive DNA sequencing and other label-free biomolecular sensing techniques. They register geometrically confined single molecules that bind within or translocate through their interior volumes to allow label-free sensing. In a typical nanopore measurement, individual analytes enter the nanopore under an applied potential, which alters the flow of ions through the nanopore and is reflected in a time-dependent current recording. By analysing the modulation of the ionic current in terms of the blockade amplitude, duration and frequency, nanopores have been applied to the stochastic sensing and characterization of DNA, RNA, peptides, proteins, metabolites and protein–DNA complexes at the single-molecule level. In particular, the success of nanopore-based DNA/RNA sequencing has stimulated many potential applications in a relatively simple, high-throughput and label-free format [1].

Ideally, the nanopore dimensions should be comparable to those of the analyte for the presence of the analyte to produce a measurable change in the ionic current amplitude above the noise level. Nanopores can be formed in several ways, with a wide range of pore diameters [2]. Biological nanopores are formed by the self-assembly of protein subunits, peptides or even DNA scaffolds in lipid bilayers or block copolymer membranes. Solid-state nanopores are crafted in thin inorganic or plastic membranes, which allow the nanopores to have extended diameters of up to hundreds of nanometres, permitting the entry or analysis of large biomolecules and complexes. The tools for fabricating solid-state nanopores, which include electron/ion milling, laser-based optical etching and the dielectric breakdown of ultrathin solid membranes, can be used to manipulate nanopore size at the nanometre scale, but allow only limited control over the surface structure at the atomic level in contrast to biological nanopores [3]. The chemical modification and genetic engineering of biological nanopores, or the introduction of biomolecules to functionalize solid-state nanopores, can further enhance the interactions between a nanopore and analytes, improving the overall sensitivity and selectivity of the device. This feature allows nanopores to controllably capture, identify and transport a wide variety of molecules and ions from bulk solution.

Nanopore technology was initially developed for the practicable stochastic sensing of ions and small molecules. Subsequently, many developmental efforts were focused on DNA sequencing. Now, however, nanopore applications extend well beyond sequencing, as the methodology has been adapted to analyse molecular heterogeneities and stochastic processes in many different biochemical systems [4]. First, a key advantage of nanopores lies in their ability to successively capture many single molecules one after the other at a relatively high rate, which allows nanopores to explore large populations of molecules at the single-molecule level in reasonable timeframes. Second, nanopores essentially convert the structural and chemical properties of the analytes into a measurable ionic current signal, even achieving enantiomer discrimination. The technology can be used to report on multiple

***Correspondence to**: Iqbal Arfa, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, Netherlands; E-mail: Arfa863@wsu.com

**Received:** 03-Feb-2023, Manuscript No. JNMNT-23-19908; **Editor assigned:** 06-Feb-2023, Pre QC No. JNMNT-23-19908 (PQ); **Reviewed:** 20-Feb-2023, QC No. JNMNT-23-19908; **Revised:** 22-Feb-2023, Manuscript No. JNMNT-23-19908 (R); **Published:** 28-Feb-2023, DOI: 10.35248/2157-7439.23.14.662.

**Citation:** Arfa I (2023) Nanotechnology: Role Development Impact of Nanomedicine on Covid-19 Pandemic Management. J Nanomed Nanotech. 14: 662.

molecular features while circumventing the need for labelling chemistries, which may complicate the overall analysis process and affect the molecular structures [5]. For example, nanopores can discriminate nearly 13 different amino acids in a label-free manner, including some with minute structural differences. An important aspect is the ability of nanopores to identify species that lack suitable labels for signal amplification or whose information is hidden in the noise of analytical devices. Consequently, nanopores may serve well in molecular diagnostic applications required for precision medicine, which achieves the identification of nucleic acid, protein or metabolite analytes and other biomarkers. Third, nanopores provide a well-defined scaffold for controllably designing and constructing biomimetic systems, which involve a complex network of biomolecular interactions [6]. These nanopore systems track the binding dynamics of transported biomolecules as they interact with nanopore surfaces, hence serving as a platform for unraveling complex biological processes. Fourth, chemical groups can be spatially aligned within a protein nanopore, providing a confined chemical environment for site-selective or regioselective covalent chemistry. This strategy has been used to engineer protein nonreactors to monitor bond-breaking and bond-making events.

### Single molecule chemistry within biological nanopores

Single-molecule sensing generally involves non-covalent interactions. Advances in this area suggest that covalent chemistry may be examined in a similar manner, and indeed the bond-making and bond-breaking events of individual molecules attached to the interior wall of a nanopore can be analysed on the basis of their modulation of the ionic current. Biological nanopores engineered to contain reactive sites are referred to as protein nanoreactors [7].

The strengths and weaknesses of the nanoreactor approach with regard to single-molecule covalent chemistry must be considered. On the plus side, no tagging of reactants is required. As the pores formed by bacterial proteins are generally highly stable, a wide range of pH values, salt concentrations and temperatures can be used. However, so far, only aqueous chemistry has been examined [8]. Both irreversible and reversible chemistry have been explored, and because there are two compartments in a bilayer set-up, incompatible spatially separated reactants can be used. Attachment to the wall of the lumen is required to prevent diffusion out of a pore during a reaction sequence and to prevent kinetic complications, such as the dimerization of intermediates. If repeated turnover at a defined site is considered to be catalysis, examples have been observed, but further progress in the use of nanopores to alter the course and rate of reactions is expected. Computer analysis of the frequency and lifetime of current states produces reaction schemes and kinetic constants for covalent chemistry with time resolutions that can reach the 100-μs range. While the nanoreactor approach provides a single-molecule reaction trajectory in which all steps are visible whether or not they are rate-limiting, the molecular identification of intermediates can be problematic, as in any single-molecule approach [9].

### Synthetic nanopores for mimicking biological systems

While nanopores understandably attract the most attention for their use in sequencing and bioanalytical applications, they also offer exciting opportunities to study questions that arise in cell biology [10]. Cells feature a wide variety of nanometre-sized pores within their membranes that act as gateways for molecular transport between compartments. For example, the flow of ions and small molecules is regulated by ion channels and transporters, with crucial roles in homoeostasis, energy production, cellular communication and sensory transduction. Larger pores, such as the mitochondrial translocase and the nuclear pore complex (NPC), are responsible for regulating the transport of proteins and RNAs between cellular compartments [11].

## CONCLUSION

This Review has outlined diverse nanopore research directions and applications beyond DNA sequencing. Tremendous progress has been made over the past two decades. Nanopores have become an essential single-molecule tool in multiple disciplines, including chemistry, biophysics and nanoscience. However, there are still challenges to overcome before the full potential of nanopore technology can be attained. Therefore, nanopores will most probably require tailoring, as the volume of the sensing region should be of comparable size to a single unit of the biopolymer. More importantly, the nanopore should be optimally sensitive to the chemical or physical properties of the building blocks, producing distinguishable ionic current signatures for each unit. This could be achieved by carefully functionalizing a pore's inner surfaces to manipulate the interactions between the biopolymer and the nanopore, providing the required sensitivity, selectivity and capture efficiency. Interesting directions to explore are the de novo design of nanopores and the synthesis of DNA origami scaffolds, which will allow the size and shape of nanopores to be tailored beyond the abilities of current engineering methods. The use of non-natural amino acids may expand the diverse chemical functionalities of biological nanopores to facilitate the study of covalent and non-covalent reactions under nanopore confinement. While the functionalization of solid-state nanopores with natural elements has proven fruitful, the incorporation of new modalities, such as optically, magnetically and electrochemically sensitive chemical groups and materials, at the pore interface is worth exploring to allow spectrometric readouts and facilitate the active control of the detection process.

The ability to design nanopores with bespoke structures, shapes and chemical properties will provide a well-defined environment for the precise control of single-molecule catalysis. By taking advantage of nanopores designed at the molecular scale, catalytic sites might be introduced into a protein nanopore lumen; then, reactant molecules captured inside the nanopore can be catalyzed to form a product that is further released and translocated through the nanopore. This would provide a bottom-up approach for the production of customized chemicals. Nanopores have also been increasingly used as force transducers, allowing the controlled localization, trapping and orientation of a diverse range of biomolecules for single-molecule biophysics studies. Finally, nanopore-based biomedical applications have developed beyond DNA sequencing and epigenetic modification analyses, and are now used to sense molecular biomarkers in biofluids and other biological specimens. Given the fast growth rate of nanopore applications, it is likely that nanopore technology will become a prominent technique in single-molecule in vitro diagnostics.

In parallel with advances in nanopore design, portable nanopore devices consisting of millions of individual pores on a chip could produce enormous amounts of sensing data at high speeds. Similar devices could be used for the retrieval of various forms of data stored in DNA or other polymers.

## REFERENCES

1. Magnuson BA, Jonaitis TS, Card JW. A brief review of the occurrence, use, and safety of food-related nanomaterials. J Food Sci. 2011; 76(6):126-133.
2. Hayat T, Qayyum S, Imtiaz M, Alsaedi A. Impact of Cattaneo-Christov Heat Flux in Jeffrey Fluid Flow with Homogeneous-Heterogeneous Reactions. PLoS One. 2016; 11(2):0148662.
3. Chang XL, Yang ST, Xing G. Molecular toxicity of nanomaterials. J Biomed Nanotechnol. 2014; 10(10):28-51.
4. Alsaadi FE, Hayat T, Khan SA, Alsaadi FE, Khan MI. Investigation of physical aspects of cubic autocatalytic chemically reactive flow of second grade nanomaterial with entropy optimization. Comput Methods Programs Biomed. 2020; 183: 105061.
5. Lai DY. Approach to using mechanism-based structure activity relationship (SAR) analysis to assess human health hazard potential of nanomaterials. Food Chem Toxicol. 2015; 85:120-126.
6. Rhee JW, Wu JC. Advances in nanotechnology for the management of coronary artery disease. Trends Cardiovasc Med. 2013; 23(2):39-45.
7. Balkany TJ, Whitley M, Shapira Y, Angeli SI, Brown K, Eter E, et al. The temporalis pocket technique for cochlear implantation: an anatomic and clinical study. Otol Neurotol. 2009; 30(7):903-907.
8. Karimi M, Zare H, Bakhshian Nik A, Yazdani N, Hamrang M Nanotechnology in diagnosis and treatment of coronary artery disease. Nanomedicine Lond. 2016; 11(5):513-30.
9. Martins RL, Rabelo ÉM, Tomazi R, Santos LL, Brandão LB Development of nano-emulsions based on Ayapana triplinervis essential oil for the control of Aedes aegypti larvae. PLoS One. 2021; 16(7):254-325.
10. Ziemys A, Yokoi K, Kai M, Liu YT, Kojic M, Simic V, et al. Progression-dependent transport heterogeneity of breast cancer liver metastases as a factor in therapeutic resistance. J Control Release. 2018; 291:99-105.
11. Yokoi K, Tanei T, Godin B, van de Ven AL, Hanibuchi M, Matsunoki A, et al. Serum biomarkers for personalization of nanotherapeutics-based therapy in different tumor and organ microenvironments. Cancer Lett. 2014; 345(1):48-55.