

Advances in Nanopore Technology for Single-Nucleotide Polymorphism Detection: From Principles to Translational Precision Medicine

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Abstract:

Single-nucleotide polymorphisms (SNPs) are the most common type of variation in the human genome and crucial biomarkers for assessing susceptibility to disease, response to pharmacological intervention. Conventional methods of SNP detection, including Sanger sequencing, polymerase chain reaction (PCR), and microarray analysis, have been widely used; however, these are limited with regards to throughput, scalability, and point-of-care compatibility. Alternatively, nanopore-based sequencing technologies are new platforms, enabling real-time, label-free analysis at the single-molecule level. This review outlines the recent developments in the application of biological, solid-state, and hybrid nanopores as devices for SNP detection, focusing on their fundamental principles, highlights of notable platforms, and technological developments. Key milestones include advancements in sample preparation and translocation control, signal resolution optimization through geometric pore design and the use of ultrathin membranes, and the development of probe engineering strategies targeting allele-specific discrimination as well as minimizing background noise. These advances aim to improve the accuracy and clinical relevance of nanopore-mediated SNP detection, and the translational significance of precision medicine.

Keywords: Nanopore Technology; Single-Nucleotide Polymorphism Detection; Precision Medicine.

1. Introduction

Single-nucleotide polymorphisms (SNPs) referring to a single-base change at a specific position in DNA,

it is commonly found in the human genome, with millions of variations scattered across the genome. While most gene mutations have little or no effect on organisms due to gene degeneracy and conservatism,

some mutations can alter protein function or gene regulation, making them key biomarkers for determining disease susceptibility, drug response, and other phenotypic characteristics. Evolution of personalized medicine has made SNP critical biomarkers for disease diagnosis, pharmacogenomic use, and clinical risk analysis. Current methods for identifying SNP, including Sanger sequencing, PCR, and microarray hybridization, are widely applied and have proved effective; however, they face challenges in terms of throughput, sensitivity, and adaptability to point-of-care settings. Such challenges have hindered effective and rapid diagnosis, particularly in time-sensitive or resource-limited settings.

In contrast, nanopore sequencing technology, as the leader among third-generation sequencing platforms, offers a promising solution to overcome these limitations. Nanopore sequencing technology is based on the measurement of changes in ionic current when DNA or RNA strands pass through nanoscale pores, consequently facilitating the direct sequencing of nucleic acid sequences. Its core advantages—real-time data analysis, long read lengths, and exceptional portability—directly address the need for rapid, high-throughput genetic analysis that can be performed on-site and have outstanding performance monitoring single molecules in real-time without amplification [1]. The nanopore devices are mostly classified under three classes depending on the structural and material properties: biological nanopores, solid-state nanopores, and hybrid nanopores. They have different advantages and disadvantages regarding resolution, stability, and integration prospects in the clinic.

Although nanopore sequencing technology shows great potential for SNP detection, existing studies have mostly focused on optimizing the performance of a single type of nanopore or validating specific applications, with a lack of systematic comparative studies. Different nanopore platforms exhibit significant differences in pore size, surface chemical properties, and noise characteristics, which directly impact their sensitivity and accuracy in single-base resolution. However, there is currently a lack of comprehensive comparative analyses under standardized experimental conditions evaluating the performance of these platforms in SNP detection, including signal-to-noise ratio, error rate, read length stability, and detection limit. This knowledge gap not only hinders the optimization of the clinical translation pathway for this technology but also makes it difficult for users to quickly select the most suitable nanopore system based on specific application scenarios. Therefore, systematically evaluating and comparing the performance of mainstream nanopore platforms in SNP detection is of great significance for advancing this technology toward standardized and reproducible clinical

applications.

2. Representative Nanopore Platforms for SNP Detection

2.1 Biological Nanopores

Biological nanopores are protein nanopores embedded inside lipid bilayers, having the capability to recognize nucleic acid sequences by measuring ionic current interruptions caused by DNA or RNA transit. Some of the most well-researched nanopores include α -HL and MspA. α -HL from *Staphylococcus aureus* forms a heptameric structure defined by a pore size of approximately 1.4 nm and a β -barrel channel length approaching 5 nm, and therefore, is classified as the first nanopore utilized for DNA sequencing [2]. However, its resolution is limited to the single base level, since several nucleotides can simultaneously reside in the sensing region. Early work presented its ability to detect base mismatches and small structure changes within DNA duplexes; however, accurate SNP discrimination still remained a serious problem. MspA is a mycobacterium *smegmatis* porin, has a short, narrow funnel-like constriction about 0.5 nm in length and has an octameric symmetric structure. The particular organizational structure allows for superior single-nucleotide discrimination when compared to α -HL [3]. When coupled to molecular motors, MspA enables controlled single-base progress during sequencing, significantly enhancing both the signal-to-noise and base-calling accuracy.

Additionally, biological nanopores have been engineered through changes to engineering design to enable the integration of sophisticated approaches to detection. For example, specifically engineered pores have been used to monitor protein-DNA interaction or changes to nucleic acid conformation on a single-molecule level. These approaches not only increase the possible applications for biological nanopores but also enable the development and integration of SNP analysis into broader biomolecular sensing platforms.

2.2 Solid-State Nanopores

Solid-state nanopores are nanoscopic pores that are engineered from thin dielectric materials, the most common material includes silicon nitride (Si_3N_4), aluminum oxide (Al_2O_3), or hafnium oxide (HfO_2). They often have thicker membrane and limited sensing area; its atomic resolution is generally inferior to that of biological nanopores. This enables greater efficiency over a wider range of experimental conditions including higher voltages and varied chemical conditions. By nanofabrication methods, it is

possible to precisely control the size of the pores, their surface properties, and overall dimensions, producing pores that enjoy greater mechanical stability and electronic device compatibility. Modern systems employ molecular probes that permit the conversion of base alterations into discernible electrical current signatures. Examples include DNA carriers that incorporate mutation-selective overhangs or chemical tags specifically designed to produce differential blockade intensities upon undergoing translocation [4].

Advances in pore geometry engineering, such as the creation of conical nanopores and ultrathin membranes, have greatly improved the spatial resolution of ionic current measurements. Such optimizations increase the electric field concentration in the sensing area, thus allowing the recognition of features that are only a few nanometers apart [5]. This possibility opens the way to high-density molecular barcoding and the multiplexed detection of single nucleotide polymorphisms with portable devices.

2.3 Hybrid Nanopores

Hybrid nanopores attempt to balance between mechanical stability and high resolution using the beneficial aspects of solid-state and biological nanopores. One of the common implementations is the inclusion of a protein nanopore, within a polymer membrane to create a sensing channel that is both atomically accurate and mechanically stable. This utilizes the inherent baseline detectability in biological pores, boosted by the stability and flexibility of solid substrates.

Hybrid nanopores can be functionalized through molecular adapters or probes to maximize their sensing capability. In particular, the binding of specific DNA or RNA probes on the protein pore allows for distinctive sequence binding, which is then converted to distinctive ionic current features resulting from the binding events themselves. Such a strategy has been explored for SNP genotyping via integrated multiplexed biomarker analysis, where a single system is able to perform multi-omics analysis on the single-molecule scale.

Previous research demonstrated the use of biotin–streptavidin complexes attached to DNA carriers for highly specific SNP detection. The presence or absence of the protein tag altered the ionic current blockade, enabling single-molecule discrimination without sequencing [6]. This probe-based detection method combines the robustness of solid-state platforms with the high resolution of biological platforms.

Hybrid nanopore development offers a flexible platform for precise genetic analysis. Merging the atomic-scale precision found in biological pores and the scale ability

and robustness found in solid-state materials places these systems well for expanding clinical SNP genotyping, most notably concerning portable and point-of-care diagnostic devices.

3. Methodological Innovations in Nanopore SNP Detection

3.1 Sample Preparation and Translocation Control

Adequate sample preparation and control over nucleic acid translocation are still the necessary *a priori* requirements for reproducible detection of SNPs through nanopores. Largely inherent in the initial challenge is the inherently fast translocation of DNA strands through the pore, which reduces the available time for base identification. Remedies towards this are the employment of molecular motors, e.g., DNA polymerases, DNA helicases, capable of ratcheting single strand nucleotide by nucleotide through the pore, increasing temporal resolution. Chemical surface modification has been integrated into solid-state nanopores for management of DNA-pore interactions to regulate translocation velocity with maintenance of structural integrity [7]. Designs of DNA carriers are another promising avenue, where SNP-specific overhangs or hybridized probes are attached to double-stranded scaffolds. The carriers stabilize the molecule through the translocation process and also produce discernible patterns of current blockade enabling single-base substitutions to be easily recognized. Together, improvement in sample preparation protocols and translocation control significantly increase the accuracy of nanopore-based genotyping of SNPs.

3.2 Signal Resolution and Base Calling Accuracy

The high-fidelity detection of SNP in nanopore technologies is dependent upon the physical resolution of the ionic current signal go pass through the pore. An important parameter affecting this process is the pore geometry: biological nanopores, such as MspA, defined by their short constriction length (~ 0.5 nm) and narrow funnel shape, increase the electric field in a reduced sensing area, thus allowing better discrimination between single nucleotides in comparison to α -HL [8].

In the field of solid-state nanopores, advances in ultrathin membrane materials, such as graphene and MoS₂, have greatly enhanced the resolution of observed signals. These atomically thin membranes reduce the sensing volume down to about one base, thus lowering the averaging of the signal over a quantity of nucleotides [7]. In a similar

manner, controlled variations in surface chemistry and pore diameter have been shown to diminish stochastic noise and stabilize DNA-pore interactions, improving the precision of the single-base current signature.

Another crucial aspect relates to the reduced electrical noise achieved through the use of low-capacitance substrates and the ionic conditions optimized. This enables the detection of small variations in the current due to single-base mismatches. In conclusion, developments on the nanoscale hole design and material science significantly enhanced the accuracy of the ionic current measurement such that an empirical groundwork was laid for the consistent base identification towards the discovery of SNPs.

3.3 Probe Engineering for SNP Discrimination

The development of probe engineering is a core component in enhancing the discrimination of SNPs in nanopore-based assays. Progress in the field suggests a shift in strategies from purely pore-intrinsic recognition techniques towards a combination of chemical probes, aptamers, and encoded carriers.

Another important aspect of current research involves the use of coded DNA molecular probes as sequence-specific identifiers. It was found through recent research that the intentional introduction of aptamers and matching oligonucleotides into defined locations on double-stranded DNA scaffolds creates position-coded signals that may be enumerated in the resulting nanopore data to provide a direct correlation to the detection of single-nucleotide differences within viral RNA.

The technique allows for the simultaneous detection of multiple locations and has the ability to detect lineage-defining mutations such as D614G and Y144del without the need for whole-genome sequencing [9]. By ensuring that the specificity constraints are confined to the engineered probes, the system uses nanopore sensing as a universal electrical readout mechanism with a high selectivity level. Latest developments in solid-state nanopore sensors reveal the essential contribution of surface functionalization in improving the detection resolution of SNPs. Chemical modification of Al_2O_3 nanopores through complementary oligonucleotides or responsive molecular adapters enables temporary hybridization of the target strand, providing characteristic blockade signatures separating matched and mismatched bases. Such a setup not only slows down the translocation but also sets up energetically distinct probe-target interactions, enhancing the contrast in the allele pairs differing in a single nucleotide base.

4. Conclusion

Nanopore technology is gradually moving from theoretic

cal exploration to practical application and shows significant potential for transformation. Originally considered to be biophysical phenomena for the evaluation of ionic currents at the single-molecule level, nanopores have been continuously optimized with advances in pore design, signal sensing, and probe fabrication that have increasingly overcome the long-standing hurdles to their clinical use.

Biological nanopores have established the benchmark for single-nucleotide resolution due to their sharply confined constrictions. In contrast, solid-state nanopores have proven invaluable for their robustness, tunability, and integration with electronics. The emergence of hybrid systems—where biological pores are stabilized within solid-state membranes—represents a natural convergence that combines selectivity with scalability. This convergence is especially important for SNP genotyping, where single-base sensitivity must be paired with device durability for point-of-care or high-throughput applications.

Nanopore SNP detection is becoming increasingly relevant in a variety of clinical applications. For example, portable nanopore sequencers have been used to identify key variant sites during the SARS-CoV-2 outbreak, directly supporting epidemiological monitoring. Similarly, SNPs in pharmacogenomics can be rapidly identified on point-of-care testing platforms, enabling personalized drug dose adjustments without reliance on centralized sequencing facilities. In oncology, early studies suggest that nanopore-based SNP genotyping may aid intraoperative decision-making by revealing somatic mutations associated with cancer risk or drug resistance. Although most of these applications remain in the early stages of translation, they collectively indicate that nanopore SNP detection has the potential to become a core technology in precision medicine.

Despite the significant progress made, there are several challenges that will need to be overcome before nanopore SNP detection can be implemented on a large scale in clinical settings. Foremost is the urgent need for increased reproducibility between laboratories and platforms, which will require the establishment of standardized protocols for pore channel fabrication, probe preparation, and data analysis. In addition, there is a need to increase throughput and multiplexing capabilities to meet the demands of population-scale genotyping programs. Finally, regulatory systems need to be further developed to ensure the safety and accuracy of nanopore diagnostics in clinical settings. The future application of nanopore sensing with microfluidics, machine learning-driven analysis, and modular probe design has the potential to accelerate translation, ultimately leading to the creation of next-generation diagnostic platforms that can enable rapid, low-cost, and accurate SNP detection at either the point-of-care or in primary

care clinics.

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