

The Potential of Hybrid and Biocompatible Nanopores in Biomedical Field

Xiaowei Du

*Ulink College of Shanghai, Shanghai, China
Xiaowei.Du@ulink.cn*

Abstract: Nanopore sensing has emerged as a transformative technology for single-molecule detection, offering high resolution and multimolecular compatibility. While traditional biological and solid-state nanopores face limitations in stability, selectivity, and signal-to-noise ratio, hybrid nanopore which integrate biological components with synthetic materials like graphene or carbon nanotubes. It combines the precision of biological pores with the robustness of solid-state systems. This review explores the applications of hybrid nanopores in biomedical fields, focusing on three key areas. The first one is targeted drug delivery and stimulus-responsive systems, where nanoporous materials like anodic aluminum oxide enable controlled drug release and biocompatible implants. The second one is protein sensing, addressing challenges such as weak charge and structural complexity through innovations like silicon nitride-carbon nanotube hybrids that decelerate translocation for high-fidelity amino acid discrimination and the last one is DNA detection, where DNA origami-graphene nanopores achieve base-specific recognition by leveraging hydrogen bonding and slowed translocation dynamics. By analyzing these applications, the review highlights the advantages of hybrid nanopores compared to the pure nanopores and traditional technologies which include enhanced stability, tunable pore chemistry, and improved spatiotemporal resolution, while acknowledging challenges in fabrication scalability and material compatibility. This review also underscores the potential of hybrid nanopores to advance precision diagnostics, therapeutics, and sequencing, providing a roadmap for future research to overcome existing barriers and unlock their clinical potential.

Keywords: Hybrid nanopore, biomedical application, sequencing, drug delivery

1. Introduction

Nanopore sensing is a crucial tool for single molecule detection, its high resolution and Multimolecular compatibility make it more competitive over other technologies. As continuous progress in nanopore technology, the application of it has become a hot spot for scientists to research intensely. However, the traditional biological and some solid-state materials both have their own outstanding advantages and drawbacks that limit the range of practical usage. So, hybrid nanopore has developed which embeds biological nanopores in solid films (such as graphene) so that it takes both advantages of the two nanopore. Hybrid nanopores combine the high selectivity of biological nanopores with the mechanical stability of solid-state materials [1]. This integration enhances sensitivity and accuracy, making hybrid nanopores highly promising for biomedical applications such as targeted drug delivery, single-molecule sequencing, and antimicrobial uses. It has already offered

previously unheard-of capacities for DNA sequencing and analysis [2], precise detection of disease biomarkers and control of drug delivery. Those applications made the hybrid nanopore have crucial significance in the medical field. Recently, early, and ongoing detection of disease prevention is one of the most important health challenges in society with an aging population [3]. Because the elderly have a decline in function of physiological and immune system, developing sensitive point-of-care tests and some medical technology by using the nanopore is necessary [3]. Properties like high sensitivity and stability make the nanopore one of the most suitable tools for it. By the early and accurate detection of the biomarker, doctors can intervene at the early stage of the disease and treat it which can reduce the death rate. However, hybrid nanopore still faces some issues and challenges that should be solved and the applications of it are still in the searching stage. Unlike the two traditional nanopore, the hybrid nanopore is novel so that there are not many scientists doing research for it. This review is based on papers written by other scientists. It focuses on the applications in the medical field that show exciting potential in the future and discusses the advantages and drawbacks of hybrid nanopore compared to other technologies.

2. Application

The remarkable properties of nanostructured materials have enabled groundbreaking applications across biomedical fields. This section highlights three key applications: (1) targeted drug delivery and stimulus-responsive systems for precision therapy, (2) advanced protein sensing technologies for molecular diagnostics, and (3) hybrid nanopore platforms for high-accuracy DNA detection. These innovations demonstrate how nanotechnology bridges laboratory research with clinical solutions.

3. Application cases 1: (target delivery and stimulus response system)

Because conventional drug therapies have problems like limited drug solubility, poor biodistribution and lack of selectivity [4], the development of nanomedicine has brought enormous potential for the development of new therapeutic possibilities. For instance, the Nanoporous anodic aluminum oxide (AAO) and nanotubular titania (TiO₂) that are manufactured by electrochemistry. The structure of AAO can be controlled by changing the anodic oxidation condition[4], It can also modify the structure and surface, to manufacture complex hole structures with different hole geometry and internal structure so that it can meet the application needs such as drug delivery[4], It is worth mentioning that the surface of this kind of material can also be modified, so it has good biocompatibility. That leads to the material does not cause serious immune or inflammatory reactions when interacting with cells and tissues. So that it can be safely used in implantable drug delivery systems to ensure the safety and effectiveness of the treatment process [4]. Therefore, it provides innovative solutions for precision medical treatment. The following examples highlight their targeted applications in drug delivery, ranging from implantable devices to stimuli-responsive drug delivery systems which can illustrate the versatility of these hybrid nanopore.

4. Preparation of coronary artery stent implants

For example, by investigating the interaction of nanoporous AAO with osteoblasts and blood components, it can be utilized in preparation coronary artery stent implants: The controlled release of drug from the polymer membrane serves to suppress inflammatory responses and inhibit proliferation, thereby addressing post-implantation inflammatory complications associated with stent implants [4].

5. Fabrication of biocapsules for immunoisolation

These semi-permeable membranes can allow the small molecules (e.g., glucose, insulin) to pass through, while selectively blocking the passage of macromolecules [5] (e.g., immunoglobulins), so that make the cell transplantation treatment for diseases (e.g., diabetes) to be a therapy.

6. Development of stimuli-responsive systems

Use nanotubes that are sensitive to certain conditions (e.g. PH, temperature) to achieve the stimuli-triggered drug release. For example, the Platinum-based anticancer drugs which is used for treatment of various cancers. While it's heavily restricted by their severe toxicity and drug resistance/cross resistance. Stimuli-responsive nanocarrier drug delivery systems are one of the most promising strategies that is developed for overcoming these limitations [6]. In these systems, a variety of triggers including endogenous and extracorporeal stimuli have been employed. For example, Endogenous stimuli like pH-, thermo-, enzyme- and Extracorporeal stimuli include light-, magnetic field-responsive nanocarriers etc. The application of this system can inhibit drug resistance and enhance the solubility of drugs. Plus, it can extend the half-life of drugs and provide a method to maintain and stimulate the reactive drugs to release and deliver two or more drugs simultaneously. Therefore, it can maximize the therapeutic effectiveness of Pt-based cancer drugs and reduce side effects [6].

7. Application cases 2: Protein sensing (molecular diagnosis and pharmaceutical development)

Proteins serve as fundamental biomolecules that govern critical cellular processes and biological functions, including structural maintenance, enzymatic catalysis, and metabolic regulation. A comprehensive understanding of protein sensing is therefore essential for elucidating complex biological mechanisms, with significant implications for molecular diagnosis and pharmaceutical development [7]. Nanopore technology has emerged as a powerful tool for single-molecule sensing, especially the solid-state nanopores, so that it offers promise for protein characterization. However, the pure solid-state nanopores have some limitations due to the material that made the nanopores. The reproducibility of the pore size and the complex nature of the inner surface are highly dependent on nanomanufacturing techniques and the intrinsic properties of the pore carrier material [8]. Plus, protein sensing has challenges compared to nucleic acid detection, due to proteins' weak charge, uneven distribution, and greater compositional complexity [7].

Given proteins' biological significance, there is a pressing need to develop new advanced nanopore capable of overcoming these limitations. To overcome these problems, researchers tried to combine biofilm membrane with solid-state nanopores [8]. Recent innovations in hybrid nanopore design which integrate multiple material, have demonstrated enhanced protein capture and detection capabilities. These engineered systems show considerable potential for advancing protein sequencing in biomedical applications.

Traditional nanopores and other conventional technologies face a fundamental limitation wherein proteins translocate at excessively high velocities. This rapid translocation kinetics results in severely compromised current signal resolution, rendering the discrimination of individual amino acids particularly challenging. A study has found that the velocity of unfolded peptide translocating through a hybrid nanopore composed of silicon nitride membrane and carbon nanotube is much slower compared to bare silicon nitride nanopore due to the significant interaction between amino acids and the surface of carbon nanotubes [7]. So, they combine silicon nitrate with carbon nanotubes which build a new hybrid nanopore system. The silicon nitrate membrane provides a stable base and signal detection interface.

The strong van der Waals force interactions between the CNT surface and amino acids significantly prolong the translocation duration to tens of nanoseconds, which is sufficient for acquiring high-resolution current signals. This enables the high-resolution current signal acquisition while simultaneously addressing multiple challenges in protein sequencing. The initiative-taking control of protein translocation is achieved by using the strong interaction and charge adjustability of CNT. Plus, the competition and combination of the electrophoretic and electroosmotic flows through the nanopores could be controlled by introducing variations in the charge states at the boundaries of carbon nanotube nanopores [7].

By employing charge regulation technology, the surface charge density at the CNT boundary can control the competition between electroosmotic flow (EOF) and electrophoretic force. Thereby, EOF's influence on protein translocation can be investigated. There are experimental results demonstrated that positive surface charges enhanced protein capture efficiency but resulted in faster translocation velocities. Conversely, negative surface charges generated EOF opposing the electrophoretic direction, significantly decelerating translocation (albeit requiring higher driving voltages). This confirms that charge density optimization can further improve deceleration effects, with minimal translocation velocities achieved at low positive charge densities. The slowed protein translocation produced prolonged current blockade signals (Fig. 1 vs Fig. 2e), whose amplitude and duration directly correlated with amino acid types. Furthermore, proteins exhibited intermittent pausing behavior during CNT pore translocation (Fig. 3c-d), enabling multi-timepoint signal acquisition and consequent signal-to-noise ratio enhancement [7]. Furthermore, this approach offers exceptional environmental parameter flexibility by achieving efficient deceleration at physiologically compatible voltages (1-1.4V) and ion concentrations (2 M KCl), unlike conventional methods requiring harsh conditions, while maintaining technical compatibility with downstream analysis techniques like mass spectrometry. The combination of these effects - including intermittent pausing behavior during translocation for multi-timepoint signal acquisition - collectively enhances signal-to-noise ratios and detection fidelity, demonstrating how the strategic integration of CNT interactions, charge regulation, and flow competition creates a robust platform that overcomes fundamental limitations in protein sequencing.

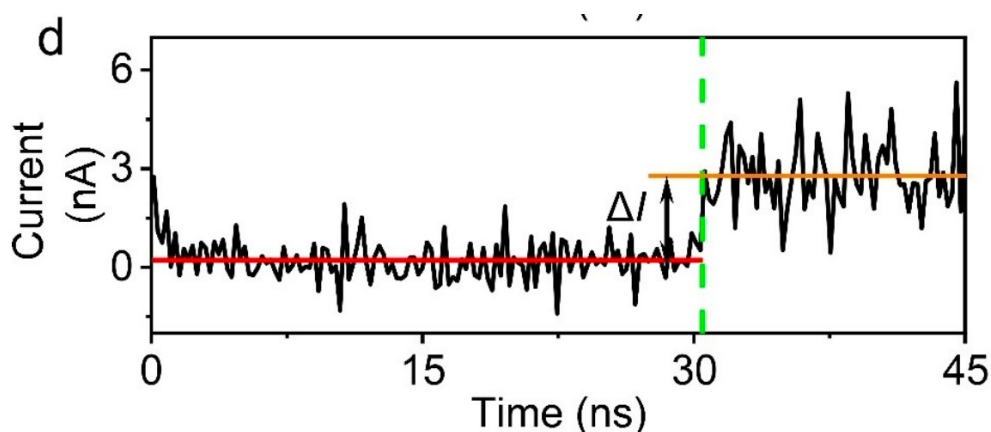


Figure 1: MD simulation of a peptide translocation through a 1.4 nm Si₃N₄ nanopore.

Ionic current trace versus simulation time during the entire translocation process of peptide through the Si₃N₄ nanopore. The currents were sampled at 2.4 ps intervals and blocks averaged in 0.24 ns blocks. The red and orange solid lines indicated the mean residual current and mean open pore current, respectively. The green dashed line indicates the time that the peptide just completely translocated through the Si₃N₄ nanopore [7].

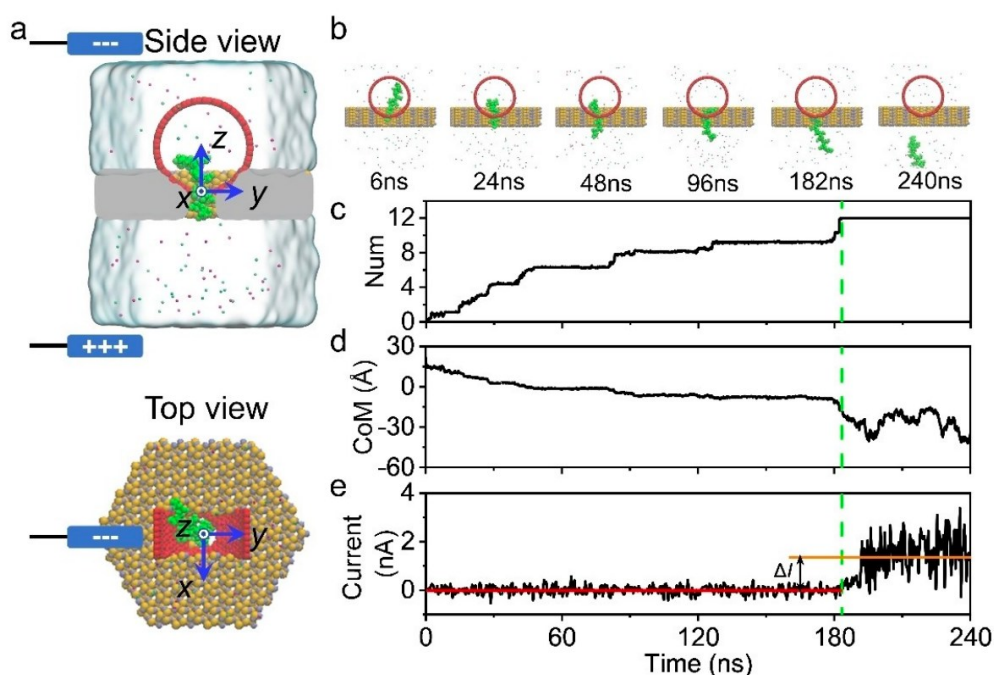


Figure 2: MD simulation of a peptide translocation through the hybrid nanopore composed of a silicon nitride membrane and carbon nanotube.

(a) Side view (top) and top view (bottom) of the simulation system setup. The carbon atoms of carbon nanotube are shown as red spheres, and the rest is the same as the representation already shown in Fig. 1. (b) A sequence of microscopic configurations of the peptide electrophoretically moving from the cis side to trans side of the hybrid nanopore. (c) Number of protein residues permeated through the hybrid nanopore versus simulation time (d) Similar to panel c but for the CoM of the peptide. The number of protein residues and CoM of the peptide was sampled at 2.4 ps intervals and block averaged in 0.048 ns blocks. (e) Ionic current trace versus simulation time during the entire translocation process of peptide through the hybrid nanopore. The currents were sampled at 2.4 ps intervals and block averaged in 0.48 ns blocks. The red and orange solid lines indicate the mean residual current and mean open pore current, respectively. The green dashed line indicates the time that the peptide just completely translocated through the hybrid nanopore [7].

8. Application cases 3: DNA detection (use DNA origami-graphene hybrid nanopore)

In nanopore devices, the ionic current is modulated during DNA translocation to reveal the nucleotide type. While the detection signal will be masked by the noise in the electric current readout. Plus, the traditional nanopores have limitations. For example, biological nanopores have poor mechanical stability because proteins are susceptible to degeneration or degradation under non-physiological conditions. Also, the thickness of it is larger so that it causes high noise in the signal (the signals will overlap when multiple bases pass at the same time) And it cannot be functionalized due to the native structure. The solid nanopores have problems in lack of selectivity so that it can't recognize the bases (e.g. the current signals of A/T/C/G is almost the same in the graphite nanopores) And the DNA translocations in graphene nanopores are extremely fast that causes the difficulty to analyze the signals. To overcome these problems, scientists has developed some practical solutions including using thin 2D material membranes, slowing down DNA translocation, employing flexible biological nanopores, pore functionalization. Among these, hybrid nanopores as the newly developing

technology, it has immense potential in DNA sequencing because they offered the label-free and fast single molecule detection [9].

By utilizing the experimental technology, the DNA origami was inserted into a silicon nitride nanopore and used for detection of λ -DNA. Or the DNA origami nanoplate with some single-stranded DNA (ssDNA) overhangs attached to the pore mouth were deposited on the surface of a solid-state nanopore [9]. This hybrid nanopore have been used in the selective detection of protein. The DNA origami-graphene hybrid nanopore combined the benefits of the biological and solid materials. Through programming design, unpaired T-bases are introduced at the edge of the hole to form hydrogen bonds with complementary A-bases to realize base-specific recognition. So that the size of the nanopores and the chemical environment can be precisely controlled (e.g. Adjust the number and type of bases) [9]. Moreover, the graphite offered the supporting role which can improve the mechanical stability to prevent DNA origami structures from deforming or detaching in an electric field. In addition, the Ultra-thin thickness (0.34 nm) reduces signal crosstalk [9]. Plus, this nanopore can also reduce the translocation velocity because the frictional action of DNA grease significantly slows down translocation (e.g., it takes 26 ns for 30 bases in a double-layer structure, but only 5 ns for graphene pores) [9]. Thereby, it has longer dwell time to allow more precise collection of current signals. What is more, it can combine the current and dwell time, so that the signal-to-noise ratio and differentiation have been improved significantly. Not like the traditional technologies, the hybrid nanopore does not rely on the single current signals, it has more dwell time signals like the A base gene has the longest hydrogen bonding residence time (3 ns) and the G base has the shortest bonding time (0.64 ns) [9]. Therefore, it can distinguish the bases easily. The key to this technology is that it compensates for the inherent defects of traditional nanopores through bio-solid-state hybrid design and provides a new path for low-cost, high-precision single-molecule sequencing.

9. Discussion (advantages and challenges)

The hybrid nanopore technology demonstrates significant potential in the biomedical field since it combines the durability of a solid-state nanopore with the precise structure of a biological nanopore. Apart from this, it has a lot of benefits due to the properties of the materials it uses and also it can be combined with other technologies to further increase its application potential. As is mentioned above, it can be used in target drug delivery and Stimulus response system; Protein sensing which can be further used in molecular diagnosis and pharmaceutical development and the nanopore sequencing that used for DNA detection.

In this review, the following advantages are mentioned in these applications:

1) Higher stability and durability: Traditional nanowires like pure biological nanopores have poor stability that they are vulnerable to pH, temperature, or chemical environment. (e.g., Alpha-hemolysin). Pure solid-state nanopores have high mechanical strength but may lack biocompatibility. The hybrid nanopores combined the benefits of the two so that they not only retain the high sensitivity of the biological hole, but also enhance the stability, suitable for long-term or harsh conditions.

2) Enhance the accuracy of molecular detection: The time resolution advantage of this technique lies in its ability to achieve extended translocation times, enabling more precise measurements and analysis. For example, in protein sequencing, when using the pure solid-state nanopores (e.g., pure Si_3N_4), the protein translocation speed is too fast (microsecond level), resulting in fuzzy current signal. The Si_3N_4 membrane combined with CNT extend the translocation time 10-100 times due to the Strong van der Waals forces (vdW) or electrostatic interactions between carbon nanotubes and biomolecules [7]. Plus, traditional mass spectrometry cannot directly measure the single molecule translocation dynamics, but the hybrid hole realizes the single molecule time trajectory.

The spatial resolution advantage can enhance nanopore's analytical and diagnostic capabilities. The surface of traditional solid holes is rough, resulting in molecular friction or non-specific

adsorption and high noise. While graphene has atomic smoothness on the surface so that it can reduce molecular friction and increase current signal signal-to-noise ratio (SNR) by 5-10 times. For instance, in the pure Si_3N_4 nanopore, the coefficient of variation (CV) of the DNA translocation signal was 30%, while in the mixed hole it dropped to <10% (due to no surface defects).

3) Adjustable aperture and surface chemistry: The size of the biological nanopore is fixed, and the size of solid state nanopores can be adjusted by processing, but the surface chemical modification is complex. While by designing hybrid interfaces (such as solid holes modified with DNA origami), the pore size and surface chemistry can be flexibly adjusted to meet the detection needs of different molecules (such as DNA, proteins, small molecules) [10].

4) Expanded application scenario: For traditional uses, biological holes are mainly used for sequencing, and solid holes are used for physical sensing. By combining the two, the hybrid nanopores can achieve several purposes at the simultaneously. For example, carry out high-precision DNA sequencing, protein structure analysis, virus detection, and even single-molecule chemical reaction monitoring at the same time.

However, there are several challenges remaining in practical applications:

1) Hard manufacture and has excessive cost. Because hard manufacture requires precise integration of biological nanopores with solid-state materials and needs high-precision nano fabrication techniques.

2) It has limitations of functionalization. Although functional modifications can improve selectivity, the process may affect the nanopore's structure and performance.

3) It is hard to have production. At present, applications are still mainly laboratory production. How to reduce costs and improve production efficiency are the main challenges in commercialization.

4) Material compatibility problems exist. Because the physical and chemical properties of varied materials may not be completely compatible.

10. Conclusion

Hybrid nanopores have emerged as a transformative technology in single-molecule sensing, combining the precision of biological components with the robustness of engineered nanomaterials. This review highlights their biomedical potential in three key areas: targeted drug delivery, protein sequencing, and DNA detection. By integrating biocompatible materials (e.g., DNA origami, graphene, CNTs) with solid-state materials, hybrid nanopores overcome critical limitations of conventional systems—offering enhanced stability, adjustable sensing properties, and improved resolution.

In drug delivery, stimuli-responsive hybrid nanopores enable controlled release with reduced off-target effects. For protein analysis, Si_3N_4 -CNT hybrids leverage charge modulation to slow translocation, allowing better amino acid discrimination. Plus, DNA origami-graphene nanopores achieved single-base recognition through programmable interactions which overcoming the speed and noise limitations of traditional sequencing. These advances demonstrate hybrid nanopores' unique ability to bridge fundamental research and clinical applications.

However, challenges remain in fabrication scalability and signal standardization. Future efforts can focus on optimizing manufacturing techniques for consistent nanopore integration, exploring novel 2D materials (e.g., MoS_2 , hBN) to further enhance performance. Expanding applications toward theragnostic and multi-omics integration could unlock their full potential in precision medicine.

In conclusion, hybrid nanopores represent a versatile platform poised to revolutionize molecular diagnostics and therapeutics. As interdisciplinary innovations advance, they will play an increasingly vital role in enabling high-precision, low-cost biomedical technologies.

Reference

- [1] Qin, F., Sheng, M., Li, R., Liu, D., Ren, M., Tian, R., ... & Liang, L. (2024). Construction and applications of hybrid nanochannels with helical foldamers and solid-state nanopores. *Chemical Engineering Journal*, 489, 151006.
- [2] Sharma, P., Sana, T., Khatoon, S., Naikoo, U. M., Malhotra, N., Hasnain, M. S., ... & Narang, J. (2025). Nanopores for DNA and biomolecule analysis: Diagnostic, genomic insights, applications in energy conversion and catalysis. *Analytical Biochemistry*, 115791.
- [3] Stierlen, A., Greive, S. J., Bacri, L., Manivet, P., Cressiot, B., & Pelta, J. (2023). Nanopore discrimination of coagulation biomarker derivatives and characterization of a post-translational modification. *Biophysical Journal*, 122(3), 156a.
- [4] Losic, D., & Simovic, S. (2009). Self-ordered nanopore and nanotube platforms for drug delivery applications. *Expert opinion on drug delivery*, 6(12), 1363–1381. <https://doi.org/10.1517/17425240903300857>
- [5] Desai, T. A., Chu, W. H., Tu, J. K., Beattie, G. M., Hayek, A., & Ferrari, M. (1998). Microfabricated immunisolating biocapsules. *Biotechnology and bioengineering*, 57(1), 118–120. [https://doi.org/10.1002/\(sici\)1097-0290\(19980105\)57:1<118::aid-bit14>3.0.co;2-g](https://doi.org/10.1002/(sici)1097-0290(19980105)57:1<118::aid-bit14>3.0.co;2-g)
- [6] Wang, T., Wu, C., Hu, Y., Zhang, Y., & Ma, J. (2023). Stimuli-responsive nanocarrier delivery systems for Pt-based antitumor complexes: a review. *RSC advances*, 13(24), 16488–16511. <https://doi.org/10.1039/d3ra00866e>
- [7] Si, W., Zhang, Z., Chen, J., Wu, G., Zhang, Y., & Sha, J. (2024). Protein Deceleration and Sequencing Using Si3N4-CNT Hybrid Nanopores. *Chemphyschem : a European journal of chemical physics and physical chemistry*, 25(7), e202300866. <https://doi.org/10.1002/cphc.202300866>
- [8] He, S., Liu, Y., Fang, S., Li, Y., Weng, T., Tian, R., ... & Wang, D. (2024). Solid-State nanopore DNA Sequencing: Advances, challenges and prospects. *Coordination Chemistry Reviews*, 510, 215816.
- [9] Barati Farimani, A., Dibaeinia, P., & Aluru, N. R. (2017). DNA Origami-Graphene Hybrid Nanopore for DNA Detection. *ACS applied materials & interfaces*, 9(1), 92–100. <https://doi.org/10.1021/acsami.6b11001>
- [10] Nehra, A., Ahlawat, S., & Singh, K. PP..(2019). A biosensing expedition of nanopore: a review. *Sensors and Actuators B: Chemical*.