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GUEST EDITORIAL

Highlights on the current state of proteomic detection and characterization with nanopore sensors

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1 | INTRODUCTION

Sensors capable of detecting myriad components in a complex fluid, whether it is clinical or environmental, are a holy grail for biosensing. To be useful in a real-world application, such sensors must be able to operate in the picomolar to nanomolar range and be selective to specific analytes with unambiguous signals in the presence of a veritable soup of confounding molecules. By repurposing the tools of the electrophysiology lab, electrochemical sensors based on analyte-membrane and analyte-pore forming proteins have emerged as a flexible platform for the development of such biosensors.

The quintessential electrophysiological biosensor is a single pore-forming protein, a nanopore, formed in a membrane separating two conductive fluid reservoirs. Electric fields are used to drive ionic current through the nanopore and fluctuations in the pore's conductance—called resistive pulses—are characterized to identify the analyte, monitor its membrane transport properties, detect chemical interactions, and measure the energetics of these interactions [1]. Nanopore biosensors are now routinely used to make measurements fundamental to biological processes (e.g., membrane transport processes) and are an emerging tool for peptide, protein and, with creative application of recognition elements, small molecule characterization. By using biochemical methods, that is, protein engineering, protein nanopores can be created with nearly arbitrary chemistry [2]. The versatility of available protein chemistry is further expanded through solid-state nanopores fabricated in insulating films created with modern nanofabrication techniques offering an ever-expanding menagerie of materials, geometry and chemistry for sensor development [3].

In this special issue, we are pleased to present 10 research and review articles that highlight recent developments in these membrane-based and membrane-supported biosensors with applications towards protein and peptide detection. This collection can be subdivided into three major themes. The first collection of articles focuses on nanopore and membrane-based sensing with targets ranging from the spike S1 protein subunit from SARS-CoV-2 to toxic compounds like perfluorooctanoic acid (PFA). These articles give specific examples of the capabilities now possible with nanopore sensing and detection. They include creative ways to use molecular recognition elements—including using proteins as carriers for the detection of small molecules, along with more traditional biochemical modification of pore elements to enhance detection of specific targets. The second collection of articles includes papers that focus on the fundamental physics and chemistry of these conductance-based sensors. These articles consider voltage-activated complexation, current blockades from DNA–protein complexes in solid-state nanopores, and nanopore-based protein sensing in the frequency domain. Each takes a deeper look at the fundamental underpinnings behind nanopore-based sensing and shows the potential that these systems have for advancing proteomic analysis. Finally, we present several interesting review articles that summarize recent developments in areas ranging from the use of peptide nucleic acid constructs for enhanced DNA detection, to a thorough review of nanopore characterization with various polymer markers.

1.1 | Biosensing applications

Huo et al. [4] investigate engineered aerolysin (AeL) nanopores for the detection of post translational modifications of peptides—specifically acetylation and phosphorylation modifications to Tau proteins that have been implicated in Alzheimer's disease. This is an example of the power of nanopore sensing where distinguishing between modified peptides has proved challenging for more established techniques (i.e., mass spectrometry). By engineering the AeL pore to create electrostatic traps, they can enhance detection by increasing the pore residence time nearly 50-fold for the case of phosphorylated peptides. This article is representative of work that focuses on optimizing nanopore design for peptide sensing with applications in diseases.

In a departure from detecting and characterizing proteins, Liu and co-workers [5] developed a sensing methodology that takes advantage of the therapeutic properties of γ-cyclodextrin (γCD) for the

remediation of PFA contamination. PFA is a pervasive bioaccumulating biotoxin which binds human serum albumin (HSA) in blood. By incubating PFA with HSA and γCD in the presence of a nanopore sensor, the competitive binding reactions were monitored by observing time-resolved γCD-PFA complexes with the nanopore. The resistive pulse method is compared favourably to F^{19}-NMR spectra that are typically more difficult to obtain, thus showing that nanopore methodologies can be a powerful tool for establishing clinical mechanisms for therapeutic treatment.

Xia et al. [6] offer a similar protein mediated nanopore sensor, but with a major change. They employ custom electronics and a nanofabricated solid-state pore to detect bovine serum albumin (BSA). When BSA interacts with two small molecules ibuprofen and sulfamethoxazole which binds to and alters the net charge on the protein. This, and the previous article on PFA detection, are excellent demonstrations of monitoring protein interactions outside the pore by analysis of on-rate kinetics with the pore.

The large number of proteins targeted for biosensing demands the development of a correspondingly large set of nanopore sensors uniquely tuned to the different protein types. In addition to point mutagenesis modifications of commonly used pores, many other groups continue to expand the so-called nanopore "toolbox" by studying the efficacy of a large number of different nanopores. A great example of this effort is highlighted by the submission of Kawano and co-workers [7] who examine the efficacy of a malaria nutrient transport pore, EXP2, for peptide sensing. This pore is a heptameric channel with a diameter of 2.5 nm, and an ability to detect peptides through poly-L-lysine (PLL) generated resistive pulses. They show this new pore is a strong candidate to detect poly-cationic species and it easily distinguishes between large PLL (30 kDa–70 kDa), short PLL (10 kDa) and double-stranded DNA.

The final paper in the biosensing subset is offered by Asandei et al. [8] who take an unconventional approach to biosensor development. They describe a biosensor based on the interaction of a charged analyte with a membrane support using the second harmonic signal of the capacitive current under an externally applied oscillating electric field. The sensor shows sensitivity to the S1 spike protein of SARS-CoV-2, with a detection efficiency in the 10s of nmol/L range. The study points to a low-cost sensor that could be deployed in clinics and biomedical laboratories in the near future. This effort is representative of the numerous reports related to electrochemistry applied to protein detection which is the foundational underpinning of all nanopore sensors.

1.2 | Fundamental underpinnings to nanopore-based sensing

While biosensing applications represent a large percentage of the nanopore community's output, there is still a need to continue exploring the fundamental mechanisms that underlie protein–nanopore interactions. This exploration will help drive further improvements in sensing applications and represents what we believe is an important avenue of research in the nanopore field.

Many studies have modelled polymers in the nanopore environment as they interact with a collection of free energy barriers to transport (i.e., capture, partitioning, and escape) [9–13] and this can help researchers better understand optimization strategies for designing better sensors. Hoogerheide and colleagues [14] extend this theme with a study on voltage dependent transport of α-synuclein and a variety of nanochannels. This study models the free energy profile of the α-synuclein peptides, which yields numerous conclusions about the nature of the polymer–pore interactions. This study is representative of work that focuses on developing better biosensing strategies through first principles calculations of free energy profiles.

In addition to biopore sensors, solid-state pores are an important class of sensor for proteomics. While nanopore sensing with solid state pores shows great promise for protein and peptide detection [15–17], there are difficulties when it comes to better understanding the more complicated current blockades that result in solid-state sensors. To overcome this, researchers typically engineer molecules by adding distinct structural markers, which yield unique current signatures (i.e., DNA barcode labelling [18]), but significant effort is also required to better understand the mechanisms behind the different blockades that appear from a wider variety of protein–DNA constructs.

Carlson and Tabard-Cossa [19] perform a thorough analysis of resistive pulses resulting from proteins and protein–DNA complexes moving through a solid-state nanopore. Their goal is to better understand the origins of the blockade events under different voltages, salts, and ionic strengths in the hopes of improving the sensing capabilities of solid-state pores. Of particular interest, is the discussion on various models used to describe the blockades and how they find considerable success with the so-called "resistors-in-series" (RIS) approach. One example of this success is their ability to associate multi-level blockades with various folding configurations of a biotinylated dsDNA–monovalent streptavidin (MSA) complex inside the pore. This points towards future efforts that could utilize the RIS approach to better understand connections between molecular structure and current blockade fluctuations.

Most nanopore studies focus on the time-dependent kinetics of analyte–pore interactions. This provides a direct connection to the free energy underpinnings of the system. However, in some cases the analyte–nanopore interaction is strong, which leads to long-lived blockade events. This limits the statistical advantage that nanopore sensing can provide (i.e., large number of events for constructing various statistical distributions). One way to address this is considered by Movileanu and co-workers [20], who explore the frequency domain for protein detection. Specifically, they show that the low frequency regime of the power spectra for long-lived events may scale with protein concentration. They find that low concentration detection (below K_d) yields low amplitude white noise, while high concentration detection (above K_d) yields high amplitude 1/f noise. This suggests future efforts could benefit from a multimodal analysis in both frequency and time domains.

1.3 | Nanopore-based protein and peptide sensing reviews

Finally, we are pleased to present two brief reviews covering important topics in nanopore sensing with connections to protein and peptide analysis. The first, by Liu and Nestorovich [21], provides a thorough review of the biophysical processes involved in polymer transport in porins and ion channels. They offer a detailed history of the development and use of synthetic polymers (e.g., polyethylene glycol) for probing the geometry of water-filled proteins. The review primarily focuses on using noise analysis as a powerful tool for investigating polymer–pore interactions, and compares these results to the direct resistive pulse methods more frequently encountered in the literature. The second review, by Luchian and co-workers [22] focuses on the development of hybridization schemes for selective enhancement of short nucleic acids. This review is of particular interest for this special issue because it covers the role of peptide nucleic acids play in improving nucleic acid detection, a relatively new idea with considerable potential in nanopore sensing.

2 | SUMMARY

We believe this entire collection presents a wide overview of topics that are of growing interest to the nanopore sensing community. We have focused this issue on both biosensing applications of nanopore detection and fundamental mechanisms underlying polymer-pore interactions. We have identified a variety of investigators active in these pursuits and we hope that this will help further motivate studies in these areas. We thank each of the authors for their efforts along with the reviewers for making this special issue a reality, and we hope you, the reader, will benefit from the studies reported herein.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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