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Analytical applications for pore-forming proteins☆

John J. Kasianowicz [a,*], Arvind K. Balijepalli [a], Jessica Ettedgui [a], Jacob H. Forstater [a], Haiyan Wang [a], Huisheng Zhang [b], Joseph W.F. Robertson [a]

[a] NIST, Physical Measurement Laboratory, Gaithersburg, MD 20899, United States
[b] Guangdong Key Laboratory for Biomedical Measurements and Ultrasound Imaging, Dept. of Biomedical Engineering, School of Medicine, Shenzhen University, Shenzhen 518060, China

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ABSTRACT

Proteinaceous nanometer-scale pores are ubiquitous in biology. The canonical ionic channels (e.g., those that transport Na^+, K^+, Ca^{2+}, and Cl^- across cell membranes) play key roles in many cellular processes, including nerve and muscle activity. Another class of channels includes bacterial pore-forming toxins, which disrupt cell function, and can lead to cell death. We describe here the recent development of these toxins for a wide range of biological sensing applications. This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.

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1. Introduction

The study of giant squid axons during the late 1930s through the early 1950s led to the conclusion that there were separate pathways for Na^+ and K^+ transport in nerve fibers [1–6]. A theoretical analysis by Parsegian showed that a water-filled pore provides the lowest energy barrier for ion transport through cell membranes [7], and these portals were only recently shown to be formed by proteins [6,8–10]. Transmembrane protein channels are also the molecular basis of other cellular functions, including water transport across cells [11–13], cell–cell communication [14], and muscle activity [15]. Their malfunction can cause debilitating and deadly diseases [12,16,17], which range from cardiac dysfunction [18] to cystic fibrosis [19]. Channels are nanometer-scale in length and breadth, and their narrow confines, in part, confer ion selectivity to them [20] which is essential to their proper function.

Another class of channels is the family of pore-forming toxins, which are secreted by bacteria [21–23]. Examples of these include *Staphylococcus aureus* alpha-hemolysin (αHL) [24,25], *Mycobacterium smegmatis* MspA [26], *Escherichia coli* OmpF [27], and *Bacillus brevis* gramicidin [28]. The putative structures for these porins are illustrated in Fig. 1. While understanding the mechanisms of action for bacterial pore-forming toxins is vitally important, we discuss here only their development for use in the detection, identification, quantification, and physical characterization of single molecules [29–32].

We will confine most of the discussion below to the channel formed by αHL, because its properties have proven useful for much of the applied nanopore-based sensor research that has been done to date. Briefly, αHL is a pore-forming leukotoxin [25,33] that spontaneously binds to membranes and forms a pore from seven identical subunits [34]. The crystal structure of the channel shows that it contains a conical vestibule, located outside the membrane and a β-barrel segment that spans the membrane [35]. These two components are separated by a narrow constriction, and the nature of the interaction of molecules with the pore can depend on which side of the pore they enter [36,37].

2. Development of nanopores as single molecule sensors

While it might seem unconventional, the use of single channels for sensing applications has a precedent in biology. For example, receptor channels, located at neural synapses, change their conducting state as a function of neurotransmitter concentration. In the late 1980s, several experimental findings made it possible to consider whether these nanometer-scale entities might prove useful for detecting and characterizing individual molecules electronically with high impedance amplifiers (Fig. 2).

2.1. Impediments to channel-based sensor development

The method seems simple at first glance. The entry of an analyte into the pore will alter the ionic current that otherwise flows freely by either

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* Corresponding author.
E-mail address: john.kasianowicz@nist.gov (J.J. Kasianowicz).

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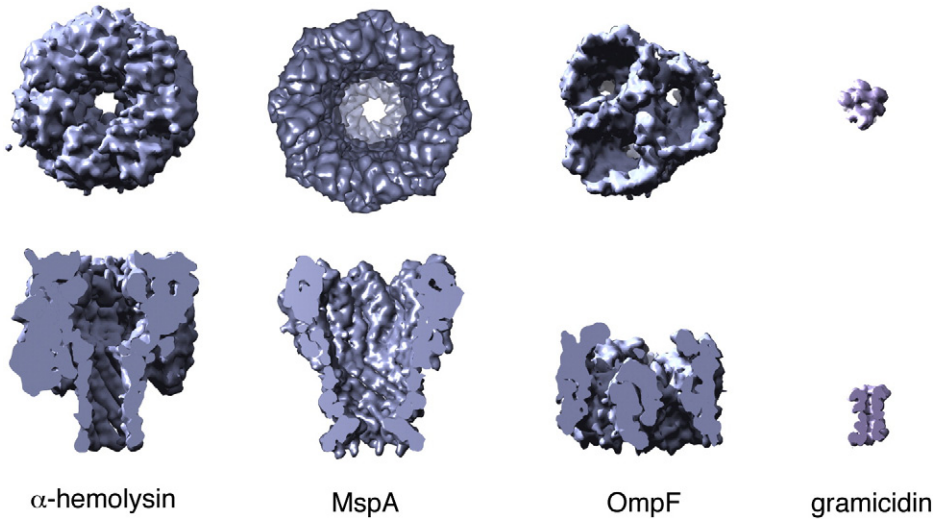

Fig. 1. Putative structures of the pore-forming toxins *Staphylococcus aureus* alpha-hemolysin (αHL) PDB: 7AHL [35], MspA PDB: 1UUN [212], OmpF PDB: 2OMF [213], *Bacillus brevis* gramicidin PDB: 1GRM [214].

altering the electrostatic potential profile across the pore [38,39] (Fig. 3, *left*), or via volume exclusion and adsorption of mobile ions [40, 41](Fig. 3, *center*). In addition, an analyte could inhibit pore formation or decrease the pore conductance, and the target analyte concentration would be inferred from the loss of ionic current [42,43] (Fig. 3, *right*). For the first two schemes, several issues had to be resolved to enable single molecule sensing with nanopores.

First, channels tend to gate spontaneously (i.e., switch between different conducting states), which would obviously confound their use as sensors. Indeed, Gianfranco Menestrina demonstrated that the *S. aureus* αHL channel gates from the fully open to lesser conductance states with relatively slow kinetics (tens of seconds) [25] compared to those for mammalian Na^+ and K^+ channels (milliseconds). Importantly, he also showed that the channel gates more quickly in the presence of divalent and trivalent cations. Serendipitously, we found that other factors could alter the αHL channel gating kinetics, and due to Menestrina's work, we were able to put the results into a useful context. Fig. 4 (*left*) illustrates that the conductance equivalent

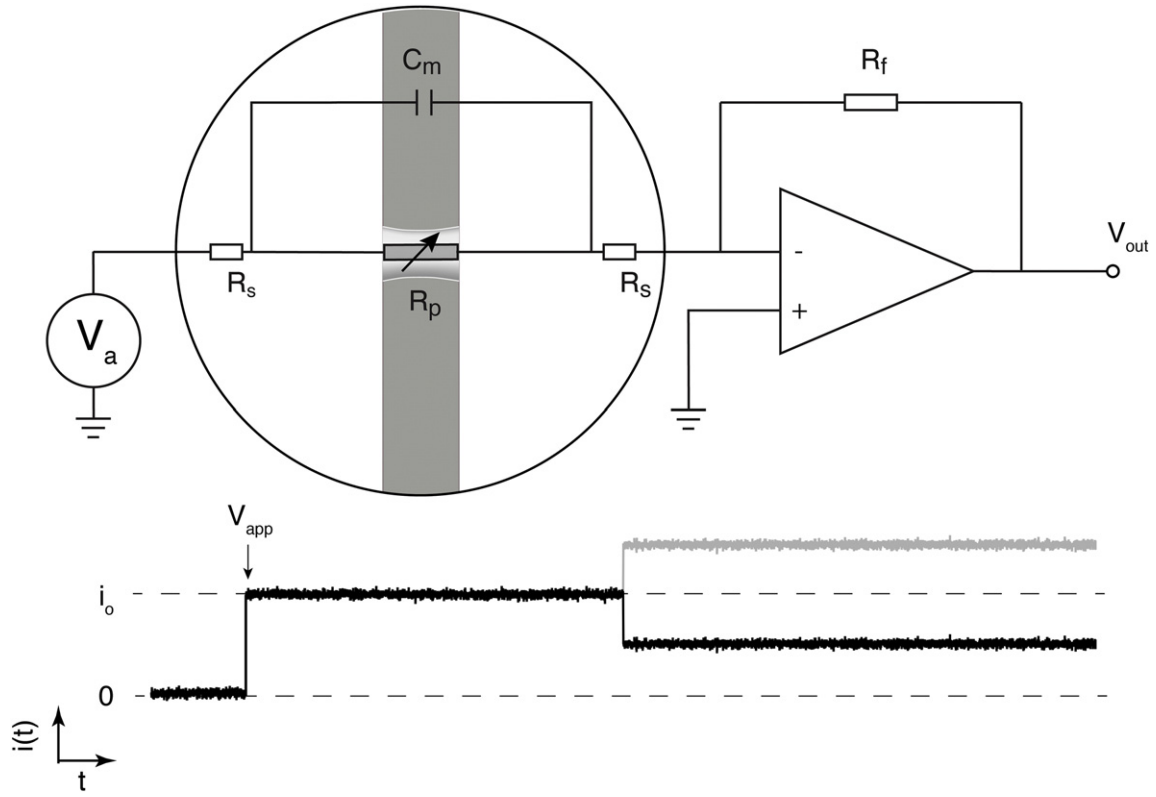

Fig. 2. Single nanopore current measurements. (*Top*) A single nanopore is embedded in a planar lipid bilayer membrane bathed on both sides by an aqueous electrolyte solution. The membrane is represented by a capacitor (C_m), the solutions as resistors (R_s) and the pore as a variable resistor (R_p, which is modulated by analytes). The pore current is measured by applying an electrostatic potential (V_a) via two Ag–AgCl electrodes (*not shown*), and a low-noise, high-impedance amplifier. (*Bottom*) The fully open pore current, i_0, can increase or decrease when an analyte enters the pore.