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Recent advances in ion-channel probes for nanopore sensing: Insights into the probe architectures

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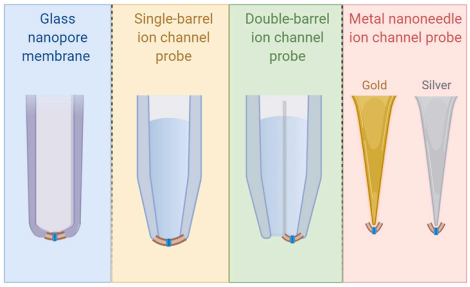
HIGHLIGHTS

- Nanopore sensing using ion channel proteins.
- Using solid probes to support ion channel recordings.
- Different probe architectures for ion channel probes.
- Applications of ion channel probes.

GRAPHICAL ABSTRACT



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ABSTRACT

This review introduces the recent advances in the nanopore sensing platform, ion channel probes (ICPs), with a particular focus on the different probe design (2011–2022). The use of ion channel proteins has emerged in different applications to understand the dynamics of many biological processes and characterize or detect biomolecules. The development of utilizing protein channels in nanopore sensing has led to diverse platforms in which the ion channels, or biological nanopores, can be embedded in a lipid membrane. Ion channel probes, where the ion channels are integrated at the tip of a solid probe, enable higher spatially-resolved detection of small molecules and extend the applications of ion channels to map different surfaces and perform chemical imaging. Different probe materials and designs have been exploited throughout the last decade, which opens the door for multiple probe architecture and applications. We provide more insights into the advances of ICP designs that render them well-suited for further applications.

1. Introduction

The field of nanopore sensing, using ion channel proteins, has received a great emphasis due to the diversity of nanopore applications and platforms coupled with a relatively simple working principle – resistive pulse detection. In nature, ion channels serve as regulatory machines that control molecular transport across the cell membrane and play a vital role in various biological processes [1–3]. The use of ion channel proteins (or biological nanopores) in nanopore sensing offers more opportunities to specifically detect or monitor small molecules with high precision level. As a result, a wide variety of ion channels from different origins have been investigated and characterized in order to

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understand their merits in different applications. Throughout the years, the development of nanopore sensing has brought several platforms to utilize the ion channel proteins in different applications such as single molecule detection, DNA sequencing, chemical mapping, and molecular flux imaging [4–7].

Many published reviews have demonstrated the various types of ion channels, their structural characteristics, the different sensing platforms, and their applications [1,7–11]. We specifically chose to assess the literature related to the nanopore measurement platform, ion channel probes (ICPs), where the ion channel proteins are embedded in a lipid membrane integrated at the end of a solid probe. The ICP platform offers many advantages over the other nanopore sensing platforms, of these, supporting and stabilizing lipid membranes as well as the longevity of ion channel recordings. More interestingly, the ICP design enables the lateral and vertical movement of the probe carrying ion channels, rendering these probes well-suited for different applications. In other words, ICPs can be employed as a mobile nanopore sensor to monitor the activity of various substrates. ICPs can also offer more accurate localized detection of specific molecules at different loci and monitor the ions/molecules flux of at cellular interfaces and thus, cell dynamics measurement can be achievable.

In this review, we introduce an up-to-date report on the ion channel probes with a focus on different probe architectures in the last decade. First, we briefly mention the general concept of nanopore sensing using ion channel proteins, then, we cover the development of ICP design starting from glass nanopore membranes to the recently reported metal nanoneedle probes. Finally, we summarize the main applications for ICPs in the field of sensing and chemical measurements.

2. Principles of nanopore sensing using ion channels

Nanopore sensing is a valuable tool that has been widely applied to detect single molecules such as peptides, DNA, and RNA with high sensitivity, selectivity, and signal-to-noise ratio [2,6,7,11]. Conceptually, nanopore sensing is a resistive pulse sensing technique, an approach that entails the measurement of ionic current passing through a small orifice (or nanopore) in an insulating membrane [12–15]. Nanopores are broadly classified into two main categories: biological (ion channel proteins or pore-forming proteins) and synthetic (solid-state or graphene nanopores) [8,16,17]. Using biological nanopores, where ion channel proteins are embedded in a lipid membrane offered many advantages such as atomically precise structural reproducibility, pore sizes in a scale similar to biologically significant analyte molecules, as well as low electrical noise [8,18].

In a typical set-up, a potential gradient is applied across a lipid bilayer membrane, resulting in the free movement of ions through the nanopore. This ionic current signal readout is dictated by the applied potential and pore resistance according to Ohm's Law (I=V/R, where I is the ionic current through the pore, V is the applied voltage, and R is the pore resistance). When a target molecule passes through the nanopore, it impedes the ions movement, resulting in a current blockade or resistive pulses (see Fig. 1). The frequency of the resistive pulses is proportional to the analyte concentration, while the blockage duration, amplitude, and signature shape of the current-time trace can elucidate the molecule identity [8,18–20]. The current-time response thus allows the detection of different biomolecules of varying size and structure as they pass through the ion channel [5,21,22]. Many research groups have exploited this merit to employ ion channels as a single-molecule identifier for different biomolecules or biomarkers such as miRNA, DNA, and peptides as we discuss later in this review.

3. From glass membranes to metal nanoneedles (probe architectures)

The advancement of ion channel probes stem from their potential merits such as the ability to support and stabilize bilayers, the longevity of ion channel recordings that ICP offers, and the unique feature of probe displacement to scan or hover over surfaces to perform spatially resolved measurement and map the activity of different substrates. Moreover, the ICP design allows for altering probe material and architecture to tune the probe characteristics for different applications. For instance, altering probe geometry enables highly localized detection measurement using ion channels, and altering probe surface modification extends the stability of the supported lipid membranes.

Traditionally, supported bilayers have been formed on a micro-orifice in polytetrafluoroethylene (PTFE) and other hydrophobic materials. The typical diameter for this orifice has ranged from a few microns up to ~150 μm. However, the large orifice dimensions leads to poor mechanical stability and more susceptibility to bilayer rupture due to pressure, temperature, and electrical variations [23–25]. Therefore, many efforts have been devoted to stabilizing and supporting the bilayers on smaller apertures across hydrophobic membranes such as Teflon™, Delrin®, and chemically-modified glass [24,26,27]. More advances to these trials were the development of glass micro-pipettes, as well as metal nanoneedles as ion channel probes suited to enhance the spatial resolution of ion-channel recordings. In order to achieve spatially

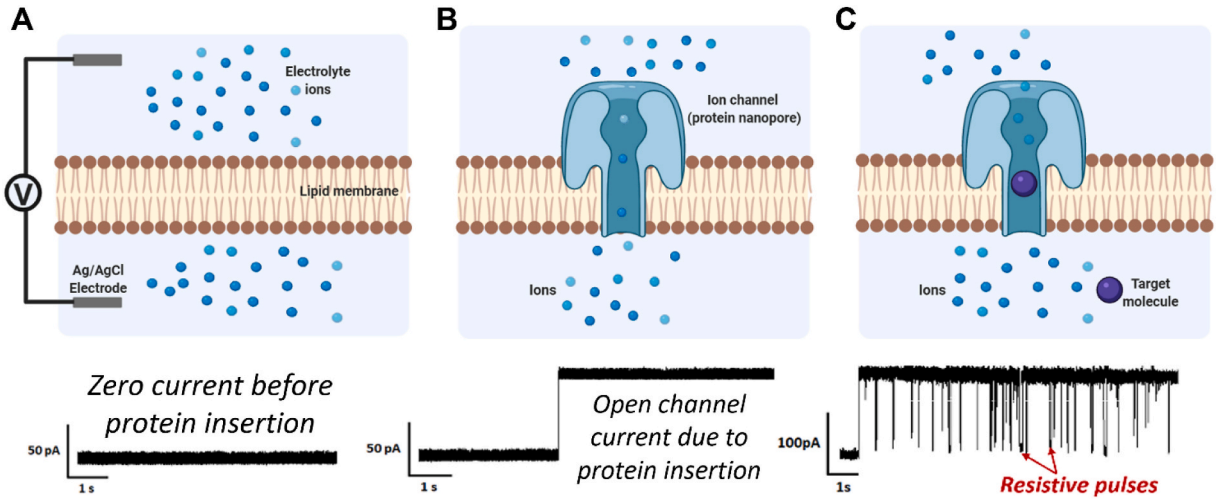

Fig. 1. General concept of using ion channel protein in resistive pulse nanopore sensing. A) Typical set-up for nanopore sensing before insertion of ion channel proteins in the lipid membrane. B) Protein is inserted in the lipid bilayer result in an increase in the ionic current, known as "open channel current". C) When the protein pore is occupied with a target molecule, we observe a quantized decrease in current (or resistive pulses).

resolved ion channel recordings, smaller probe geometry can scan and monitor more loci on the substrate surface. Accordingly, higher localized detection and highly resolved chemical imaging will be more achievable [28–30]. It's noteworthy to mention that using ICP as a sensing platform renders spatially resolved measurements more achievable due to the ability of the probe to move vertically and laterally in addition to the possibility of tuning the probe size. However, other nanopore sensing platforms lack this advantage. In this section, we will discuss in detail the development of ion channel probes starting from the glass nanopore membrane to the most recent probe architectures.

3.1. Glass nanopore membrane ion channel probe

Switching to small pores in glass, first demonstrated using a small conical nanopore in a glass capillary, allows for a more stable, robust platform for ion channel recordings. White et al. [25] pioneered the development of the glass nanopore (GNP) membrane, where in their work, they reported a benchtop method to reproducibly fabricate GNP membranes. In terms of probe architecture, the GNP is simply a small orifice in a 50 μm-thick glass membrane at the end of the glass capillary, templated by an electrochemically sharpened metal (Pt) wire (Fig. 2). After exposing the metal, as a disc electrode, by mechanical polishing, the Pt is etched away to create a conical-shaped pore of radii ranging from 10 nm to several micrometers. In this configuration, the lipid bilayer is suspended over the pore formed at a chemically-modified glass surface. Chemical modification increased the glass hydrophobicity so that the tails of phospholipids are oriented towards the glass surface upon the formation of a lipid monolayer (Fig. 2B). Thus, a stable lipid bilayer was formed by the painting method [31] and consequently, the test-bed protein, α-hemolysin (αHL), was successfully embedded in the lipid membrane for ion channel recordings. Successive reports have followed this work with more variation in the glass nanopore diameter and with controlling the rate of protein insertion by varying applied pressure on the backside of the capillary [32] [–] [34]. The pressure applied to the glass capillary facilitates monitoring αHL activity, where applying a positive pressure induces protein insertion, and a negative pressure results in a protein removal from the lipid membrane.

The glass nanopore membrane design offers many advantages over nanopores designed in PTFE or other polymer membranes; first, the confined size of these glass nanopores allows for ~10^6-fold reduction in the lipid membrane capacitance, which is the major contribution for the noise in the ion-channel measurements [32,34]. A significant reduction in the membrane capacitance results in low noise measurements. Second, controlling the pressure applied in the glass capillary enabled protein insertion and removal from the lipid bilayer. However, there are some challenges encountered in the conical pore design such as the velocity of particles through a conical pore being higher than the cylindrical pores leading to very short dwell time and inaccurate interpretation of the detection experiments results [35].

The glass nanopore membrane has been extensively applied as a nanopore sensing platform for different applications [36–44]. Many of these applications take advantage of the small bilayer area and fast RC time constant. Of particular interest are the studies that exploited the latch zone in α-hemolysin (αHL) pore to differentiate between nucleotides base pairs in DNA molecule when translocated through the protein pore. The latch region is located at the top of αHL channel vestibule (Fig. 2E), which can resolve blocking current changes when dsDNA molecule was temporarily captured in the vestibule [44]. This region in αHL protein channel has been identified in the last decade to investigate DNA mismatch [39,42], single base lesions [43], and variations in DNA sequences [44].

3.2. Single-barrel ion channel probe

Inspired by patch-clamp techniques [45,46], Keyser and co-workers developed a specific implementation of the nanion port-a-patch instrument with the Vesicle Prep Pro setup to form a nanoscale lipid bilayer membrane at the end of glass pipettes [47,48]. In their method, they used the laser puller to form a fine tip (Fig. 3A), where an artificial lipid membrane is attached by inserting the pipettes in a bath solution containing giant unilamellar vesicles (GUVs). With applying a gentle suction, the GUV encounters the pulled pipette tip and breaks leaving a planar bilayer at the tip. This method is characterized by ease of fabrication, rapid formation of lipid bilayers, and production of reusable glass pipettes with tip diameters ranging from 50 nm to several micrometers. On the other hand, patch-clamp pipettes are typically 1–3 μm in diameter and use a natural patch of cell membrane, which suffers from short-time stability [45,49].

An extension to this method was the development of the single-barrel ion channel probe, in which the ion channel proteins are incorporated into a black lipid membrane (BLM) supported at the end of the glass micropipette. Different from the above-mentioned method, where the lipid membrane was ultimately attached at the inner walls of the glass pipette tip (Fig. 3A), the single-barrel ICP configuration indicates the lipid bilayer expands on both inner and outer surfaces, i.e. spans the glass pore (See Fig. 3B). The Baker group first described using an ICP, a pulled, perfluorinated glass pipette with tip dimensions of ~10 μm (internal diameter) and 30 μm (outer diameter), to form the lipid

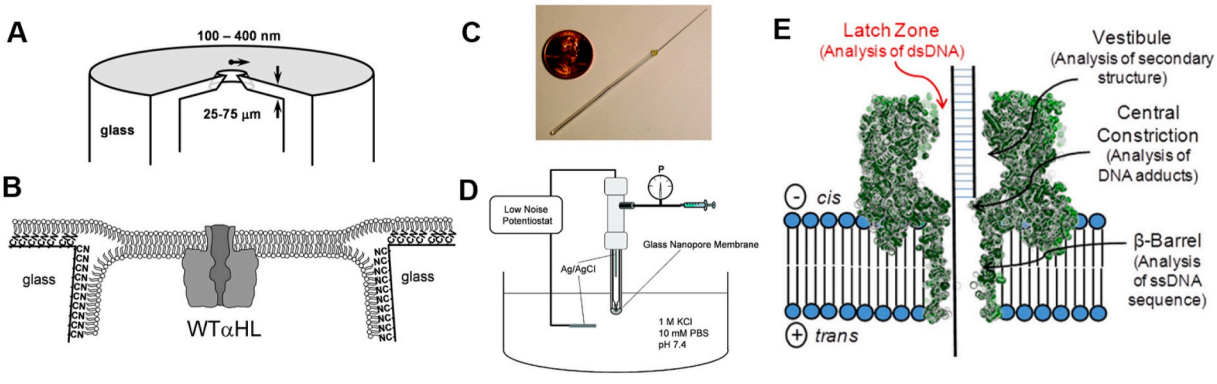

Fig. 2. Representative examples for glass nanopore membrane (GNP). A) Diagram for the first GNP reported (not to scale). B) Schematic illustration of lipid bilayer spanned over the orifice of glass nanopore inner surface and αHL protein pore inserted in the lipid membrane. C) Photographic image of the GNP with the size perspective to a penny. D) Typical experimental set-up to apply GNP as nanopore sensor. A–D reproduced from *White, R. J.; Ervin, E. N.; Yang, T.; Chen, X.; Daniel, S.; Cremer, P. S.; White, H. S. Single Ion-Channel Recordings Using Glass Nanopore Membranes. J. Am. Chem. Soc. 2007, 129 (38), 11766–11775 (ref 25)*. Copyright 2007 American Chemical Society. E) Illustration of DNA-specific sensing regions in the α-hemolysin protein channel showing the location of latch zone at the top of channel vestibule. E reproduced from *Ding, Y.; Fleming, A. M.; White, H. S.; Burrows, C. J. Differentiation of G:C vs A:T and G:C vs G:MC Base Pairs in the Latch Zone of α-Hemolysin. ACS Nano 2015, 9 (11), 11325–11332 (ref 44)*. Copyright 2015 American Chemical Society.

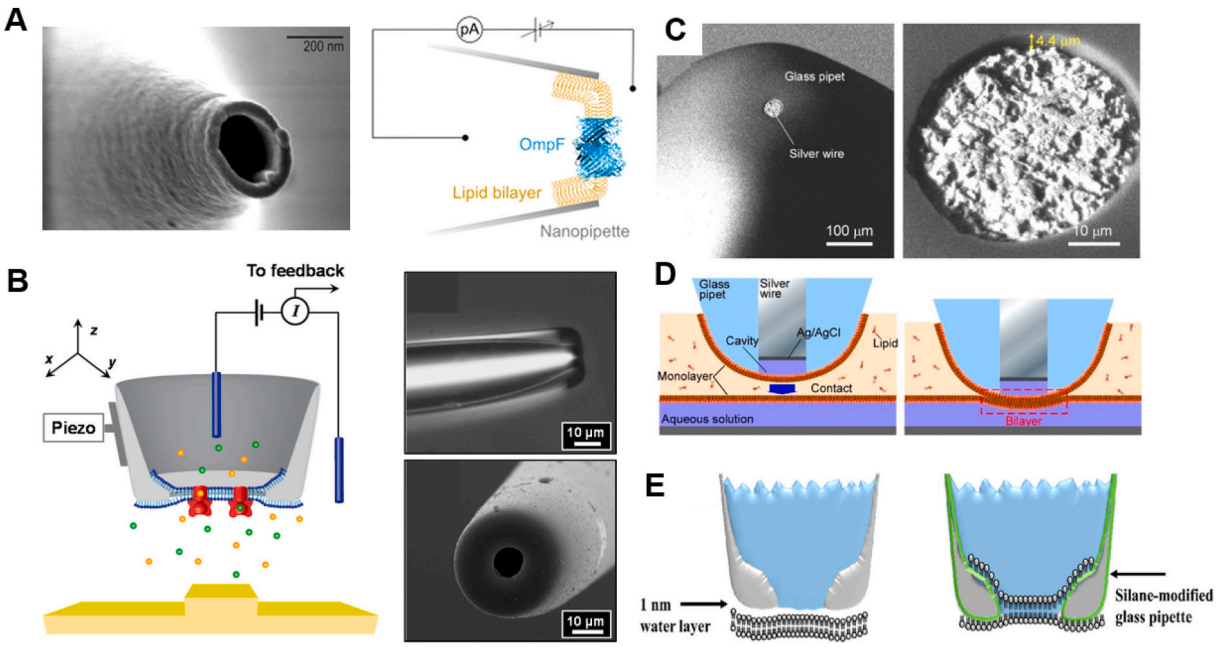

Fig. 3. Different configurations for the single-barrel ICP. A) SEM micrograph and schematic drawing for the glass nanopipette used to attach nano-bilayers at the tip. **A** reproduced from *Gornall, J. L.; Mahendran, K. R.; Pambos, O. J.; Steinbock, L. J.; Otto, O.; Chimerel, C.; Winterhalter, M.; Keyser, U. F. Simple Reconstitution of Protein Pores in Nano Lipid Bilayers. Nano Lett. 2011, 11 (8), 3334–3340* (ref 47). Copyright 2011 American Chemical Society. B) Schematic diagram for the set-up used to employ single-barrel ICP as SICM probe-on the right SEM images showing the size scale of these probes (micrometer size). **B** reproduced from *Zhou, Y.; Bright, L. K.; Shi, W.; Aspinwall, C. A.; Baker, L. A. Ion Channel Probes for Scanning Ion Conductance Microscopy. Langmuir 2014, 30 (50), 15351–15355* (ref 30). Copyright 2014 American Chemical Society. C) SEM micrographs for the tip of the recessed Ag/AgCl microelectrode showing the small cavity on the top of silver wire. D) Schematic illustration of bilayer formation at the tip of the recessed Ag/AgCl microelectrode. **C&D** reproduced from *Shoji, K.; Kawano, R.; White, R. J. Recessed Ag/AgCl Microelectrode-Supported Lipid Bilayer for Nanopore Sensing. Anal. Chem. 2020, 92 (15), 10856–10862* (ref 53). Copyright 2020 American Chemical Society. E) Schematic shows the formation of lipid membranes on unmodified and the silane-modified glass micropipette apertures. **E** reproduced from *Bright, L. K.; Baker, C. A.; Agasid, M. T.; Ma, L.; Aspinwall, C. A. Decreased Aperture Surface Energy Enhances Electrical, Mechanical, and Temporal Stability of Suspended Lipid Membranes. ACS Appl. Mater. Interfaces 2013, 5 (22), 11918–11926* (ref 56). Copyright 2013 American Chemical Society.

membrane via the tip-dip method [30]. Then, αHL protein insertion was observed as a step-like current increase. In the aforementioned work, the ICP was utilized as an imaging probe for scanning ion-conductance microscopy (SICM). SICM is a powerful scanning probe technique employed to study dynamic cellular activities and one of the important applications of ICP as we will discuss later in section 4. Briefly, in SICM, the activity of different substrates can be investigated by monitoring the ionic current feedback when the probe tip approaches the substrate surface [50]. Integration of ion channel proteins in the SICM probes expands the use of SICM to enable molecular detection and chemical imaging of specific target molecules [51]. Fig. 3B shows an SEM image for the single-barrel ICP along with a schematic for SICM set-up using ICP. Subsequently, Macazo and White [52] utilized ion channel probes for simultaneous surface imaging and mapping specific molecular flux across a synthetic porous membrane with sensitivity down to the single-molecule level. The concurrent sensing of β-cyclodextrin (βCD) flux from a single pore in a glass membrane was achievable using an ICP comprised of a glass micropipette with an inner radius of 11 μm and outer radius of 200 μm and αHL protein embedded in the lipid membrane attached at the probe tip. In their report, the average αHL channel current was used to generate the SICM image when the probe is moving across the substrate and the raw channel current – time traces were utilized for spatial localization of βCD. In a similar ICP design, Shoji et al. [53] recently developed recessed in-glass Ag/AgCl microelectrode, where they utilized a glass micropipette with a Ag/AgCl located at the bottom of the glass pipette pore as illustrated in Fig. 3C and D. This arrangement enabled the spanning of lipid bilayer at the glass pore, and thus, supported the ion channel recordings to monitor the translocation of ssDNA molecules through the protein channel.

In an effort to extend the stability of bilayer and single channel recordings, Aspinwall and co-workers have studied the effect of altering probe design, probe surface chemistry, as well as the type of lipids on the physicochemical stability of suspended lipid bilayers and the analytical performance of the designed ICPs [54–58]. They used a silane-modified glass pipette with a micro aperture ranging from 5 μm up to 30 μm, where the BLM was suspended across the aperture. When they compared polymerizable polydienoyl lipids to non-polymerizable lipids, the lifetime in the case of the former was dramatically increased [54,55]. In another report [57], they formed cross-linked polymer scaffold in the glass probe and used a mixture of polymerizable and non-polymerizable lipids, where they found a great enhancement in the lipid membrane stability. Moreover, they studied the effect of the chain length and fluorine composition of the perfluorinated silane compounds that were typically used for surface modifications (Fig. 3E) [56]. In the aforementioned work, they demonstrated that longer chain length and higher fluorine content result in low energy silane compounds with an improved lifetime, electrical, and mechanical stability. More interestingly, they investigated the effect of probe geometry on the temporal resolution of ion channel measurements; using smaller apertures (~3 μm) results in a reduced BLM area, which in turns, minimized the capacitive charging contribution that causes current spikes at short time scales and enabled faster capacitive relaxation (~1 ms), this led to faster measurements and cancellation of most current spikes at short time scales (≤2 ms) [58].

Generally speaking, the design of the above-mentioned ICPs, where the ion channel proteins are integrated at the glass micropipette tip, extends their use as localized detection probes or SICM probes in addition to application in nanopore sensing. This allows for chemical detection of specific target molecules and precise topographic imaging. However, the micrometer-sized ICPs mentioned in the previous studies limit the spatial resolution, when compared to the traditional SICM probes [59,60]. In addition, the number of ion channels is crucial to

generate a suitable ionic current used as feedback in the SICM experiments; a bigger probe size results in an ionic current higher than required. Therefore, smaller probes are needed to enhance the spatial resolution and to incorporate fewer ion channels.

3.3. Dual-barrel ion channel probe

Although single-barrel ICPs showed great promise in applications such as nanopore sensing and chemical imaging when employed for concurrent imaging and sensing, the channel current is utilized as feedback for SICM and at the same time used for nanopore sensing. This feedback coupling may negatively affect the sensing ability of the ion channel probe. To decouple the ion current used as feedback, Baker and co-workers developed a new ICP platform, where they used a dual-barrel or theta pipette with two openings [61,62]. One of these openings was used to attach a membrane patch for ion channel-based nanopore sensing and the other opening was left open for SICM measurements (see Fig. 4). In this configuration, the open barrel was unmodified and used as a SICM barrel to control the probe positioning based on the ion current flowing through it, as in traditional SICM measurements. The second barrel was employed as an ICP, in which a small patch of cell membrane, including its integral ion channel proteins, was excised, and bound in a "sniffer patch" arrangement [49,63]. The dual-barrel probe can be applied to map the flux of ions or molecules signaling from different substrates as shown in the schematic in Fig. 4C. The aforementioned work stated many advantages for the SICM-ICP probe design; first, the probe design used a membrane patch that comprises ion channels, thus, it reduces the number of steps (such as bilayer formation and protein insertion) required for ion channel recordings. Second, it offers the opportunity to apply specific, or ligand-gated ion channels (e.g. NMDA receptors, K^+ ion channel, TRPV1 channel) so that a more selective chemical mapping can be obtained instead of being limited to the well-studied proteins such as αHL [61]. Furthermore, the two barrels have been controlled independently, where the SICM barrel was used only to control the probe-substrate distance and the ICP barrel served for sensing purposes. However, this probe design suffers from some limitations; the probe size is typically within the micrometer range, which hampers spatially resolved measurements. Additionally, the process of attaching membrane patch is time-consuming, it's hard to control the lipid and protein composition in the natural excised patch, and the natural membrane is relatively unstable when compared to the artificial lipid bilayer [49]. Owing to the complexity of using biological cell membranes, using an artificial lipid bilayer provided an alternative route to study ion channels and employ them in different analytical applications. However, forming an artificial lipid bilayer can be sometimes slow and lead to unstable membranes, therefore, there were many attempts to encapsulate ready-made bilayers on solid substrates, polymers, or hydrogels [64,65].

3.4. Gold nanoneedle-based ion channel probe

The need for a durable artificial lipid bilayer motivated many research groups to develop methods to form *in situ* stable lipid membrane. More specifically, for the ICP design, the glass pipettes have been utilized to support an artificial lipid membrane in configurations such as GNP, single-barrel and dual-barrel ICP, where the bilayer spans at the glass pore. Although spanning bilayers were successfully employed for ion channel recordings and SICM applications, the spatial resolution of such measurements is still limited to micrometer-sized glass pipettes. Alternatively, electrode-supported bilayers, where the lipid membrane is supported on a solid electrode rather than spanning over a glass pore, offer advantages such as forming highly stable bilayers and the possibility to control the size of solid support. Additionally, the development of ICP has extended to metal fine electrodes, where the sharp metal tip can support the lipid membrane. For instance, Ide and co-workers developed a method to form an artificial lipid bilayer at the tip of a gold fine electrode (or gold nanoneedle) [66]. In their method, the authors electrochemically etched a 0.25 mm diameter gold wire to fabricate a fine tip, which was then modified with a monolayer of the self-assembled thiol-polyethylene glycol (thiol-PEG). This PEG layer holds a thin aqueous layer around the tip so that the polar heads of phospholipid molecules are attached to this aqueous layer during lipid bilayer formation, thus, a lipid monolayer is successfully self-assembled around the tip as illustrated in Fig. 5A. Then, this monolayer combines

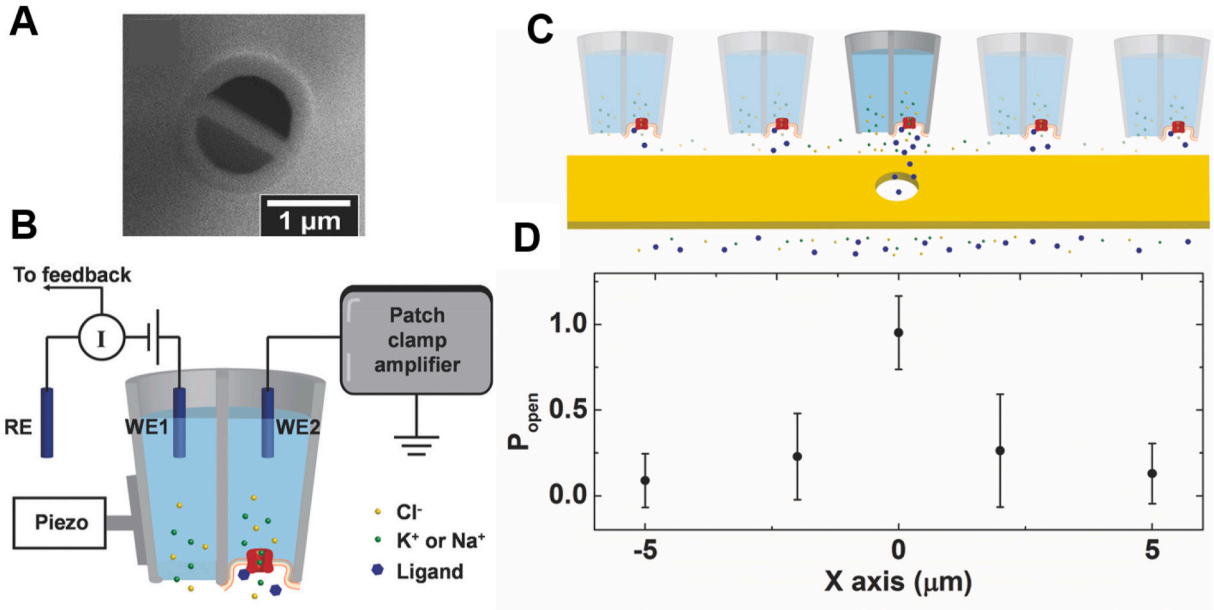

Fig. 4. Design and application of dual-barrel ion channel probe. A) SEM micrograph showing the top view of the theta pipette. B) Schematic illustration of the set-up for the dual-barrel ICP with ICP opening, where a membrane patch with ligand-gated ion channel is attached, and the second opening for SICM. C) Diagram shows the positioning of dual-barrel ICP over a porous substrate to monitor the flux of the ions at different loci. D) Plot of probability of open channel versus the X-displacement of the probe over the porous substrate. **A–D** reproduced from *Shi, W.; Zeng, Y.; Zhu, C.; Xiao, Y.; Cummins, T. R.; Hou, J.; Baker, L. A. Characterization of Membrane Patch-Ion Channel Probes for Scanning Ion Conductance Microscopy. Small 2018, 14 (18), 1–10* (ref 61), with permission from John Wiley and sons.

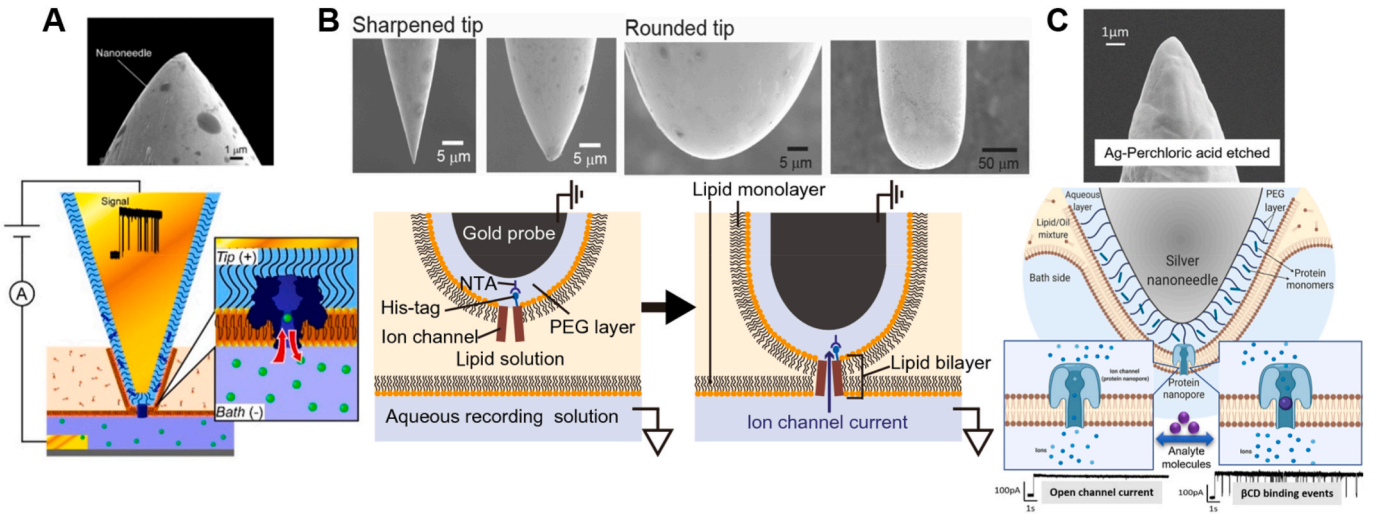

Fig. 5. Representative examples for metal-based ion channel probes. A) (Top) SEM micrograph for the gold nanoneedle prepared by electrochemical etching of gold microwire. (Bottom) Illustrative schematic for the use of gold nanoneedle ICP for nanopore sensing. **A** reproduced from *Shoji, K.; Kawano, R.; White, R. J. Spatially Resolved Chemical Detection with a Nanoneedle-Probe-Supported Biological Nanopore. ACS Nano 2019.* (ref 68). Copyright 2019 American Chemical Society. B) (Top) SEM micrographs for gold probes with different geometrical shape and size. (Bottom) Diagram shows the set-up for using the rounded gold tips for lipid bilayer formation with the ion channel protein immobilized on the gold tip. **B** reproduced from *Hirano, M.; Tomita, M.; Takahashi, C.; Kawashima, N.; Ide, T. Development of an Automated System to Measure Ion Channel Currents Using a Surface-Modified Gold Probe. Sci. Rep. 2021, 11 (1), 1–9.* (ref 72), with permission from Nature Publishing Group, CC BY. C) (Top) SEM micrograph for the silver nanoneedle prepared by electrochemical etching of silver microwire in perchloric acid solution. (Bottom) Schematic diagram for the silver nanoneedle platform for ion channel recordings and single molecule detection. **C** reproduced from *Hussein, E. A.; White, R. J. Silver Nanoneedle Probes Enable Sustained DC Current, Single-Channel Resistive Pulse Nanopore Sensing. Anal. Chem. 2021, 93 (33), 11568–11575* (ref 73). Copyright 2021 American Chemical Society.

with the second monolayer at the oil/water interface when the gold tip is moved downward into the bath chamber (see Fig. 5). Although the bilayer-forming method is similar in the case of single-barrel ICP using glass micropipette, the transition to metal fine electrode rendered the formed bilayer more supported rather than spanning over the pore in the former configuration.

To apply the new nanoneedle platform, Okuno et al. [67] applied a gold tip modified with thiol PEG attached to neutravidin to measure the activity of different ion channel proteins. In their measurement, they used neutravidin-thiol-PEG to immobilize ion channel proteins via the interaction between neutravidin and hexahistidine tag in the protein. In another example, Shoji et al. [68] successfully demonstrated the feasibility of gold nanoneedle for localized, single-molecule detection of βCD with high spatial resolution (Fig. 5A). In their report, they introduced two arrangements for application of the gold nanoneedle platform; first, the tip-side insertion configuration, where the protein molecules were added to the electrolyte aqueous layer supported by thiol-PEG around the gold tip, second, bath-side insertion, where the protein monomers were added to the aqueous compartment in the bath chamber. For each configuration, they investigated the effect of the length of thiol-PEG monolayer on the protein pore conductance and the overall performance of the nanoneedle-based ion channel probe as a nanopore sensor. Their findings stated that short PEG (Mw 282.35 g/mol; length ~ 1.8 nm) negatively affects the channel conductance in the tip-side arrangement, while long PEG (Mw 3000 g/mol; length ~ 24 nm) was the optimum for both tip-side and bath-side configurations. It's important to mention that the channel conductance remains at the reported levels when inserted from the side opposite the electrode (bath side). Compared to similar reports about the ion mobility and conductivity at confined spaces [69–71], we believe the ion conductivity in the gold nanoneedle ICP is not a limiting factor, especially with the bath side configuration or when using surface modification that provides enough space around the gold tip. However, this effect is not totally investigated at long time scales of measurement.

Additionally, the utility of gold nanoneedles to support lipid bilayer and ion channel measurements was harnessed to study the pore-forming mechanisms of different protein channels such as αHL, streptolysin O, alamethicin, and another pore-forming peptide named amyloid β (Aβ), which is believed to have a major contribution in Alzheimer's disease [22]. Very recently, and in attempts to explore how the probe architecture would affect the efficiency of ion-channel measurements, Ide and co-workers investigated the effect of the gold tip shape and the surface modification on the obtained channel current [72]. In detail, they fabricated rounded tips by electropolishing (curvature radii ≥5 μm), and other sharpened tips with radii <5 μm (Fig. 5B). When applying both tips as ion channel probes, they found that rounded tips succeeded to support channel current recording in all trials, however, 74% of the sharpened tips enabled channel current measurements while the rest showed a large current or large background noise. The authors attributed this behavior to the ease of penetration of the bath chamber by the sharper tips without forming bilayer, or, to the rapid bilayer rupture. They further stated that grafting the gold probes, especially the rounded tips, with a hydrophobic layer adjacent to the hydrophilic thiol-PEG layer enabled measuring ion channel current and supporting fragile lipid bilayers made with lipids dissolved in *n*-hexadecane instead of *n*-decane [72]. This could allow the utility of some delicate or fickle proteins as well as studying the activity of human ion channels. Although this report provided a new insight into the effect of probe shape and surface modification layers on the ICP application, the size scale in these gold probes is relatively larger than required to provide sensing or chemical imaging with high spatial resolution.

Overall, the gold needle-based ion channel probe showed a great promise to support lipid bilayer and ion channel recordings. Using gold fine electrodes (or nanoneedles) opens the door to employ these probes for nanopore sensing with enhanced spatial resolution owing to the nanoscale geometry of these probes. More interestingly, this will enable the application of the gold nanoneedles as SICM probes to map different surfaces and monitor cellular activities. However, a major limitation in the gold electrodes is the channel current decay due to the double layer charging at the electrode surface [68]. Equation (1) describes the channel current as a function of time in an RC circuit, where the αHL pore resistance (R_p) and the electric double layer capacitance (C_d) are

linked in series. Accordingly, the channel current drops exponentially with time and the frequency of binding events also decreased, which would affect the overall performance of gold nanoneedles.

$$i(t) = \frac{V}{R} e^{-t/R_p C_d} \quad (1)$$

Although White and co-workers mitigated this decay by using asymmetric salt conditions it's still problematic, especially with long-term measurements. Therefore, the rational design for ion channel probes that mitigate the decay issues associated with gold and keep the nanoscale geometry would offer many ways to enhance the overall performance in different analytical applications.

3.5. Silver nanoneedle-based ion channel probe

In a trial to mitigate current decay observed in gold nanoneedles, we recently developed a silver nanoneedle ICP to obtain a stable open channel current. The silver nanoneedle is fabricated by electrochemical etching of silver microwire in a chloride-containing etchant and applying a potential of 1V. Compared to gold, silver nanoneedle showed a DC stable channel current when employed as an ion channel probe [73]. The resulted DC current was attributed to the formation of silver chloride layer around the silver nanoneedle tip during the etching process, rendering the silver nanoneedle to work as a non-polarizable electrode. The silver nanoneedle ion channel probe was successfully employed as a nanopore sensor for single-molecule detection of β-cyclodextrin (Fig. 5C).

The silver probe ICP platform offers distinct advantages; first, the simple and reproducible fabrication method offers more tuneability for the probe geometry and surface chemistry by controlling the etching conditions. Second, this new probe architecture resulted in a remarkable enhancement of the channel current stability, and thus would extend the application of nanoneedle-based ion channel probe to maintain a single channel recording for a prolonged period. We believe that these merits allow for further analytical applications such as monitoring molecular flux of varying substrates and perform chemical imaging.

4. Applications of ion-channel probes

Generally, ion channel proteins are candidates for diverse applications such as nanopore sensing, DNA sequencing, bio-SICM imaging, and probing the catalytic activity of specific molecules and enzymes. These applications stem from the basic idea of analyzing the ionic current passing through the protein pore with or without capturing small molecules inside the pore. More specifically, using ion channel probes (ICPs), where the ion channel proteins are integrated at the end of a probe, opens the door to enhance the performance of ion channels in different analytical applications and leverages the sensing capabilities of nanopore sensing to achieve highly localized and dynamic measurements. In this section we will discuss in detail the power of employing the ICPs for different purposes with high level of accuracy and precision.

4.1. Spatially resolved nanopore sensing

Ion channel probes have been employed as a nanopore sensor that allowed for spatially resolved measurements. The movement of ICPs at different loci in a substrate or micro-devices can enable the chemical detection of specific molecules released at the substrate surface. For instance, Shoji et al. [68] used gold nanoneedle-based ion channel probe in a microchannel containing different concentrations of βCD. In another report, Schibel and Ervin [37] utilized a 50-nm glass nanopore membrane modified with monoclonal antibody that specifically bind to a target antigen. In their study, they were able to depict the antigen-antibody binding reaction, correlate the binding rate to the antigen concentration, and determine the antigen saturation level. These examples showed the feasibility of applying ICPs for spatially resolved detection of small molecules using ICPs of different materials and designs.

ICPs offer a suitable platform to study biomolecules that could be associated with a specific disease or play a key role in cellular functions such as DNA, RNA, and peptides. For example, alteration in the DNA sequence may result in a significant change in the human genome ending up to different diseases or developmental abnormalities. For this purpose, αHL pore was applied in the GNP platform to detect the DNA sequence variations, where the latch zone in αHL vestibule is unique to detect DNA sequence variations, base pair mismatch, and monitor the base excision repair reaction [41,42,44]. Another example for significant biomolecules is a pore-forming peptide named Amyloid β-42 (Aβ42), associated with Alzheimer's disease. When Aβ42 is embedded in a lipid membrane, it results in an ionic current, which changes during the insertion and de-insertion of Aβ42. The probe deign of gold nanoneedle-based ICP enabled the analysis of the ionic current during Aβ42 insertion and de-insertion controlled via the vertical movement of gold probe [22].

4.2. Scanning ion conductance microscopy (SICM)

SICM is a powerful scanning probe technique that enables noncontact imaging, surface characterization, high-resolution topography scanning, and cell dynamics measurement. Conceptually, SICM technique relies on the measurement of ionic current when a probe approaches a specific substrate [50,74–76]. The traditional SICM set-up consists of pipet electrode (PE), reference electrode (RE), and the surface of interest; the pipet electrode is inserted in an electrolyte-filled glass micro- or nano-pipette, which serves as a scanning probe. The reference electrode and the substrate are bathed in a bulk electrolyte solution (Fig. 6A). By applying a potential bias, the ionic current flows between PE and RE-this ionic current is sensitive to the change in the distance between the probe and the substrate surface. When the scanning probe starts to haver over the substrate surface, the ionic current provides feedback to control the probe-substrate surface. When the probe is far away from the substrate, the ionic current reaches maximum value, however, once the probe comes in proximity to the substrate surface, the current decreased as illustrated in the approach curve in Fig. 6C. The current drop is ascribed to distance-dependent access resistance (R_{acc}) that arises from the impedance of ions flow at the highly confined space when the probe approaches the surface. The ionic current as a function of probe-substrate distance is described mathematically in the following equations:

$$I(d) = \frac{U}{R_T} = \frac{U}{R_p + R_{acc}} \quad (2)$$

$$R_{acc} = \frac{\frac{3}{2}\ln\left(\frac{r_o}{r_i}\right)}{\kappa\, \pi\, d} \quad (3)$$

Equation (2) states the current is dictated by the applied potential (U) and R_T, which is the total resistance of pore resistance (R_p) and access resistance (R_{acc}) in series. In equation (3), solution conductivity (κ) is constant, so, R_{acc} is dictated mainly by the tip-substrate distance (d) as well as the inner and outer radius of glass pipette (r_i) *and* r_o, respectively.

The integration of ion channel proteins at the end of SICM probe, as shown in Fig. 3B, extends the application of SICM to chemical imaging of specific molecules or monitoring the flux of specific ions with high spatial resolution in addition to studying ion channels in a cell membrane such as ligand-gated ion channels [77]. Baker and co-workers demonstrated the feasibility to incorporate ion channel probes in SICM measurements [30,51,62]. They employed an ICP with protein channels inserted at the probe tip to perform current-distance measurements when the ICP approaches the surface of interest. Subsequently, further reports have utilized similar single-barrel ICP design to map the flux of target molecules or employed dual-barrel ICP with ligand-gated ion channels that can respond selectively to specific ions or

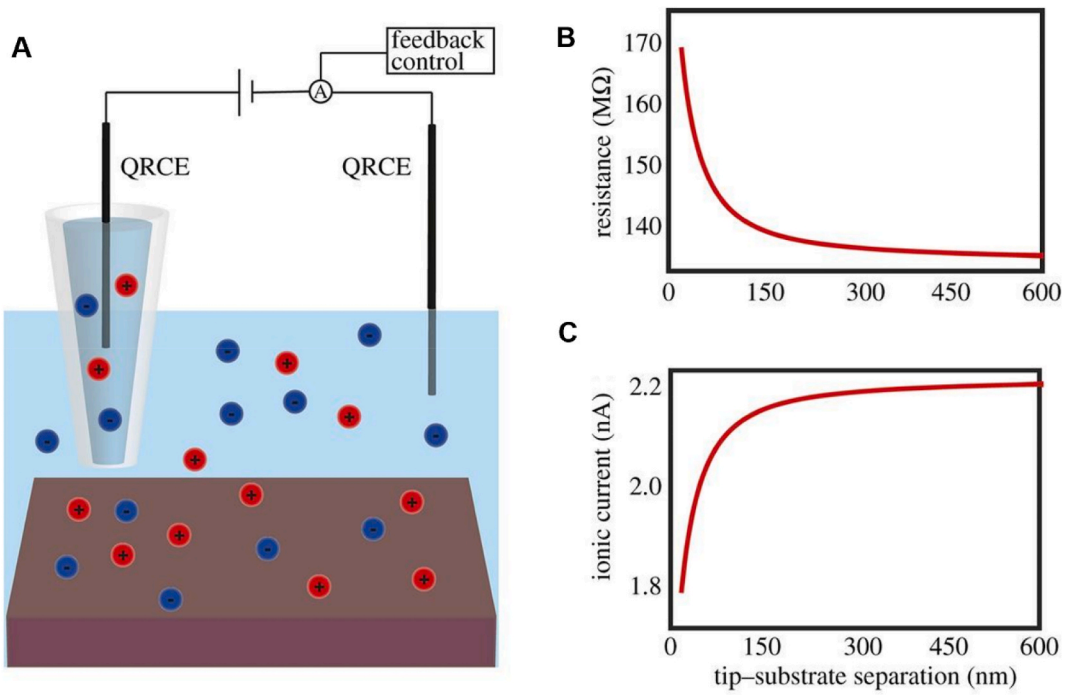

Fig. 6. Design and application of dual-barrel ion channel probe. A) Schematic diagram for the SICM set-up showing an electrolyte-filled nanopipette, with an inserted quasi-reference electrode (QRCE), is used to raster the electrolyte-bathed substrate, where the other QRCE is located. B) Simulated approach curve indicates resistance increases as the separation distance between the nanopipette tip and an insulating substrate decreases. C) Corresponding approach curve of the ionic current as a function of tip-substrate distance. The current dropped as the tip approached the non-conductive surface. **A–C** reproduced from *Page, A.; Perry, D.; Unwin, P. R. Multifunctional Scanning Ion Conductance Microscopy. Proc. R. Soc. A Math. Phys. Eng. Sci. 2017, 473 (2200), 1–34* (ref 74), with permission from The Royal Society (UK).

molecules. The development of SICM-ICP probes open the door to perform topographical measurements and non-destructive chemical mapping of specific substrates and cell surfaces. However, the micrometer-sized probe limits the spatial resolution of SICM measurement using the current reported ICPs. Thus, we believe that developing fine electrodes or nanoneedles as SICM probes will be promising to image subcellular features and activities with high spatial resolution.

Table 1
Summary of different probes reported as ion channel probes (ICPs) for nanopore sensing.

ICP	Probe material/design	Protein channel (reported)	Advantages	Limitations	References
Glass nanoporous membrane ICP	**Glass** Small conical nanopore in a glass capillary	α-hemolysin	Reduced size of the glass nanopore minimizes the membrane capacitance, leading to low noise measurements	Higher particles velocity at the conical pore leads to very short dwell time and inaccurate interpretation of the detection results	[25,34–44]
Single-barrel ICP	**Glass** Pulled glass pipette with tip dimensions of micrometer scale	α-hemolysin Gramicidin A Omp F	Extended application to chemical imaging and scanning ion conductance microscopy	• Micrometer-sized probes limit the spatial resolution • No control over the number of protein insertion in the lipid membrane.	[30,52, 54–58]
Dual-barrel ICP	**Glass** Pulled theta pipette with two openings; one serves for nanopore sensing and the second acts as SICM probe	Fickle ion channels (BK channel and trpv1)	• The two barrels have been controlled independently • Offers the opportunity to utilize specific, or ligand-gated ion channels, thus, more selective measurements can be obtained	• Attaching membrane patch is time-consuming • It's hard to control the lipid and protein composition in the natural excised patch • Probe size limits the spatial resolution	[61,62]
Gold nanoneedle ICP	**Metal- gold** Electrochemically- etched gold wire	α-hemolysin Alamethicin Gramicidin A Streptolysin O amyloid β1-42 KcsA channel	• Forming highly stable bilayers by supporting the lipid membrane on a solid electrode rather than spanning over a glass pore. • Possibility to control the probe size. • Enhanced spatial resolution due to the nanoscale geometry of the probes	• Channel current decay due to the double layer charging at the gold surface.	[22,66–68, 72]
Silver nanoneedle ICP	**Metal- silver** Electrochemically- etched silver wire	α-hemolysin	• Same advantages as gold nanoneedles. • Mitigate the current decay and enable sustained, DC stable channel current.		[73]

Table 1 summarizes different types of ion channel probes applied for various analytical applications. In addition, the table demonstrates different probe designs along with protein channels used in each example.

5. Conclusions and future perspective

The main focus of this review is to highlight one of the nanopore sensing platforms, the ion channel probe (ICP), in which the protein channels are integrated at the end of a solid probe. Many studies in the literature have highlighted the features, types, structures, characteristics, and different applications of ion channel proteins. Apparently, these reports built our understanding about how to implement the protein channels successfully in different platforms to be employed as a sensor element with high level of precision. More specifically, the reported research over the last few years expanded the knowledge of applying protein channels beyond the traditional resistive pulse sensing measurements. A tangible outcome of these studies is the novel designs of nanopore platforms such as ICP, where the nanopore can be moved laterally or vertically to monitor the activity of different loci and chemically map surfaces and substrates. The development of the probe design has provided more insights on enhancing the ICP performance such as extending the stability of lipid membranes and ion channels for prolonged time by using different surface modifications or utilizing different probe materials. In addition, enhancing the spatial resolution can be achieved by minimizing of probe geometry from the micrometer scale to the nanoscale.

In the future, we might expect that further studies for ICP architectures will bring many advantages and mitigate some of the current challenges, for example, can enable precise control over the protein insertion in the lipid bilayer and allow for long-term single channel recordings. This will extend the abilities of ICPs in applications such as chemical imaging and SICM. In addition, designing smaller probes will enhance the special resolution, allow for highly localized detection measurement, and monitor chemical dynamics of live cells. Continuing exploration of new probe materials and designs will provide additional insights to suit the ion channel probes for different applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

This review article reports on previously published data with the appropriate references provided.

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References

[1] J.W.F. Robertson, M.L. Ghimire, J.E. Reiner, Nanopore sensing: a physical-chemical approach, Biochim. Biophys. Acta Biomembr. 1863 (2021), 183644, https://doi.org/10.1016/j.bbamem.2021.183644.
[2] C. Cao, Y.T. Long, Biological nanopores: confined spaces for electrochemical single-molecule analysis, Acc. Chem. Res. 51 (2018) 331–341, https://doi.org/10.1021/acs.accounts.7b00143.
[3] N. Varongchayakul, J. Song, A. Meller, M.W. Grinstaff, Single-molecule protein sensing in a nanopore: a tutorial, Chem. Soc. Rev. 47 (2018) 8512–8524, https://doi.org/10.1039/c8cs00106e.
[4] B.M. Venkatesan, R. Bashir, Nanopore sensors for nucleic acid analysis, Nat. Nanotechnol. 6 (2011) 615–624, https://doi.org/10.1038/nnano.2011.129.
[5] X. Zhang, Y. Wang, B.L. Fricke, L.Q. Gu, Programming nanopore ion flow for encoded multiplex microRNA detection, ACS Nano 8 (2014) 3444–3450, https://doi.org/10.1021/nn406339n.
[6] R.A. Lazenby, F.C. Macazo, R.F. Wormsbecher, R.J. White, Quantitative framework for stochastic nanopore sensors using multiple channels, Anal. Chem. 90 (2018) 903–911, https://doi.org/10.1021/acs.analchem.7b03845.
[7] J.J. Gooding, K. Gaus, Single-molecule sensors: challenges and opportunities for quantitative analysis, Angew. Chem. Int. Ed. 55 (2016) 11354–11366, https://doi.org/10.1002/anie.201600495.
[8] W. Shi, A.K. Friedman, L.A. Baker, Nanopore Sense Anal. Chem. 89 (2017) 157–188, https://doi.org/10.1021/acs.analchem.6b04260.
[9] Y.-L. Ying, C. Cao, Y.-T. Long, Single molecule analysis by biological nanopore sensors, Analyst 139 (2014) 3826–3835, https://doi.org/10.1039/C4AN00706A.
[10] B.J. Berube, J.B. Wardenburg, Staphylococcus aureus α-toxin: nearly a century of intrigue, Toxins 5 (2013) 1140–1166, https://doi.org/10.3390/toxins5061140.
[11] A. Liu, Q. Zhao, X. Guan, Stochastic nanopore sensors for the detection of terrorist agents: current status and challenges, Anal. Chim. Acta 675 (2010) 106–115, https://doi.org/10.1016/j.aca.2010.07.001.
[12] R. Pan, K. Hu, D. Jiang, U. Samuni, M.V. Mirkin, Electrochemical resistive-pulse sensing, J. Am. Chem. Soc. 141 (2020), https://doi.org/10.1021/jacs.9b10329, 19555–19559.
[13] L.T. Sexton, L.P. Horne, S.A. Sherrill, G.W. Bishop, L.A. Baker, C.R. Martin, Resistive-pulse studies of proteins and protein/antibody complexes using a conical nanotube sensor, J. Am. Chem. Soc. 129 (2007) 13144–13152, https://doi.org/10.1021/ja0739943.
[14] H. Bayley, C.R. Martin, Resistive-pulse sensing - from microbes to molecules, Chem. Rev. 100 (2000) 2575–2594, https://doi.org/10.1021/cr980099g.
[15] D. Kozak, W. Anderson, R. Vogel, M. Trau, Advances in resistive pulse sensors: devices bridging the void between molecular and microscopic detection, Nano Today 6 (2011) 531–545, https://doi.org/10.1016/j.nantod.2011.08.012.
[16] H. Tang, H. Wang, D. Zhao, M. Cao, Y. Zhu, Y. Li, Nanopore-based single-entity electrochemistry for the label-free monitoring of single-molecule glycoprotein – boronate A ffi nity interaction and its sensing application, Anal. Chem. 94 (2022) 5715–5722, https://doi.org/10.1021/acs.analchem.2c00860.
[17] H. Tang, H. Wang, C. Yang, D. Zhao, Y. Qian, Y. Li, Nanopore-based strategy for selective detection of single carcinoembryonic antigen (CEA) molecules, Anal. Chem. 92 (2020) 3042–3049, https://doi.org/10.1021/acs.analchem.9b04185.
[18] S.M. Iqbal, R. Bashir, Nanopores : Sensing and Fundamental Biological Interactions, Springer, 2011.
[19] J.K.W. Chui, T.M. Fyles, Ionic conductance of synthetic channels: analysis{,} lessons{,} and recommendations, Chem. Soc. Rev. 41 (2012) 148–175, https://doi.org/10.1039/C1CS15099E.
[20] H. Watanabe, A. Gubbiotti, M. Chinappi, N. Takai, K. Tanaka, K. Tsumoto, R. Kawano, Analysis of pore formation and protein translocation using large biological nanopores, Anal. Chem. 89 (2017) 11269–11277, https://doi.org/10.1021/acs.analchem.7b01550.
[21] M. Hiratani, R. Kawano, DNA logic operation with nanopore decoding to recognize MicroRNA patterns in small cell lung cancer, Anal. Chem. 90 (2018) 8531–8537, https://doi.org/10.1021/acs.analchem.8b01586.
[22] K. Shoji, R. Kawano, R.J. White, Analysis of membrane protein deinsertion-associated currents with nanoneedle-supported bilayers to discover pore formation mechanisms, Langmuir 36 (2020) 10012–10021, https://doi.org/10.1021/acs.langmuir.0c00833.
[23] R.J. White, B. Zhang, S. Daniel, J.M. Tang, E.N. Ervin, P.S. Cremer, H.S. White, Membrane and a Glass Support, vol. 22, 2006. Glass, 10777–10783, http://www.ncbi.nlm.nih.gov/pubmed/17129059.
[24] B. Zhang, J. Galusha, P.G. Shiozawa, G. Wang, A.J. Bergren, R.M. Jones, R. J. White, E.N. Ervin, C.C. Cauley, H.S. White, Bench-top method for fabricating glass-sealed nanodisk electrodes, glass nanopore electrodes, and glass nanopore membranes of controlled size, Anal. Chem. 79 (2007) 4778–4787, https://doi.org/10.1021/ac070609j.
[25] R.J. White, E.N. Ervin, T. Yang, X. Chen, S. Daniel, P.S. Cremer, H.S. White, Single ion-channel recordings using glass nanopore membranes, J. Am. Chem. Soc. 129 (2007) 11766–11775, https://doi.org/10.1021/ja073174q.
[26] M. Mayer, J.K. Kriebel, M.T. Tosteson, G.M. Whitesides, Microfabricated Teflon membranes for low-noise recordings of ion channels in planar lipid bilayers, Biophys. J. 85 (2003) 2684–2695, https://doi.org/10.1016/S0006-3495(03)74691-8.
[27] C.A. Baker, L.K. Bright, C.A. Aspinwall, Photolithographic fabrication of microapertures with well-de fi ned, three-dimensional geometries for suspended lipid membrane studies, Anal. Chem. 85 (2013) 9078–9086.
[28] A. Bhargava, X. Lin, P. Novak, K. Mehta, Y. Korchev, M. Delmar, J. Gorelik, Super-resolution scanning patch clamp reveals clustering of functional ion channels in adult ventricular myocyte, Circ. Res. 112 (2013) 1112–1120, https://doi.org/10.1161/CIRCRESAHA.111.300445.
[29] T. Kaiju, K. Doi, M. Yokota, K. Watanabe, M. Inoue, H. Ando, K. Takahashi, F. Yoshida, M. Hirata, T. Suzuki, High spatiotemporal resolution ECoG recording of somatosensory evoked potentials with flexible micro-electrode arrays, Front. Neural Circ. 11 (2017) 1–13, https://doi.org/10.3389/fncir.2017.00020.
[30] Y. Zhou, L.K. Bright, W. Shi, C.A. Aspinwall, L.A. Baker, Ion channel probes for scanning ion conductance microscopy, Langmuir 30 (2014) 15351–15355, https://doi.org/10.1021/la504097f.
[31] O. Alvarez, in: C. Miller (Ed.), How to Set up a Bilayer System BT - Ion Channel Reconstitution, Springer US, Boston, MA, 1986, pp. 115–130, https://doi.org/10.1007/978-1-4757-1361-9_5.

[32] Q. Chen, J. Liu, A.E.P. Schibel, H.S. White, C. Wu, Translocation dynamics of poly (styrenesulfonic acid) through an α-hemolysin protein nanopore, Macromolecules 43 (2010) 10594–10599, https://doi.org/10.1021/ma1016117.
[33] E.N. Ervin, R. Kawano, R.J. White, H.S. White, Simultaneous alternating and direct current readout of protein ion channel blocking events using glass nanopore membranes, Anal. Chem. 80 (2008) 2069–2076, https://doi.org/10.1021/ac7021103.
[34] A.E.P. Schibel, E.C. Heider, J.M. Harris, H.S. White, Fluorescence microscopy of the pressure-dependent structure of lipid bilayers suspended across conical nanopores, J. Am. Chem. Soc. 133 (2011) 7810–7815, https://doi.org/10.1021/ja1117182.
[35] W.J. Lan, D.A. Holden, J. Liu, H.S. White, Pressure-driven nanoparticle transport across glass membranes containing a conical-shaped nanopore, J. Phys. Chem. C 115 (2011) 18445–18452, https://doi.org/10.1021/jp204839j.
[36] W.J. Lan, H.S. White, Diffusional motion of a particle translocating through a nanopore, ACS Nano 6 (2012) 1757–1765, https://doi.org/10.1021/nn2047636.
[37] A.E.P. Schibel, E.N. Ervin, Antigen detection via the rate of ion current rectification change of the antibody-modified glass nanopore membrane, Langmuir 30 (2014) 11248–11256, https://doi.org/10.1021/la502714b.
[38] R.T. Perera, A.M. Fleming, R.P. Johnson, C.J. Burrows, H.S. White, Detection of benzo[a]pyrene-guanine adducts in single-stranded DNA using the α-hemolysin nanopore, Nanotechnology 26 (2015), 074002, https://doi.org/10.1088/0957-4484/26/7/074002.
[39] R.P. Johnson, A.M. Fleming, R.T. Perera, C.J. Burrows, H.S. White, Dynamics of a DNA mismatch site held in confinement discriminate epigenetic modifications of cytosine, J. Am. Chem. Soc. 139 (2017) 2750–2756, https://doi.org/10.1021/jacs.6b12284.
[40] R.P. Johnson, A.M. Fleming, L.R. Beuth, C.J. Burrows, H.S. White, Base flipping within the α-hemolysin latch allows single-molecule identification of mismatches in DNA, J. Am. Chem. Soc. 138 (2016) 594–603, https://doi.org/10.1021/jacs.5b10710.
[41] Q. Jin, A.M. Fleming, R.P. Johnson, Y. Ding, C.J. Burrows, H.S. White, Base-excision repair activity of uracil-DNA glycosylase monitored using the latch zone of α-hemolysin, J. Am. Chem. Soc. 135 (2013), https://doi.org/10.1021/ja410615d, 19347–19353.
[42] T. Zeng, A.M. Fleming, Y. Ding, H.S. White, C.J. Burrows, Interrogation of base pairing of the spiroiminodihydantoin diastereomers using the α-hemolysin latch, Biochemistry 56 (2017) 1596–1603, https://doi.org/10.1021/acs.biochem.6b01175.
[43] Q. Jin, A.M. Fleming, Y. Ding, C.J. Burrows, H.S. White, Structural destabilization of DNA duplexes containing single-base lesions investigated by nanopore measurements, Biochemistry 52 (2013) 7870–7877, https://doi.org/10.1021/bi4009825.
[44] Y. Ding, A.M. Fleming, H.S. White, C.J. Burrows, Differentiation of G:C vs A:T and G:C vs G:mC base pairs in the latch zone of α-hemolysin, ACS Nano 9 (2015) 11325–11332, https://doi.org/10.1021/acsnano.5b05055.
[45] T.M. Suchyna, V.S. Markin, F. Sachs, Biophysics and structure of the patch and the gigaseal, Biophys. J. 97 (2009) 738–747, https://doi.org/10.1016/j.bpj.2009.05.018.
[46] E. Neher, B. Sakmann, Single-channel currents recorded from membrane of denervated frog muscle fibres, Nature 260 (1976) 799–802, https://doi.org/10.1038/260799a0.
[47] J.L. Gornall, K.R. Mahendran, O.J. Pambos, L.J. Steinbock, O. Otto, C. Chimerel, M. Winterhalter, U.F. Keyser, Simple reconstitution of protein pores in nano lipid bilayers, Nano Lett. 11 (2011) 3334–3340, https://doi.org/10.1021/nl201707d.
[48] K. Göpfrich, C.V. Kulkarni, O.J. Pambos, U.F. Keyser, Lipid nanobilayers to host biological nanopores for DNA translocations, Langmuir 29 (2013) 355–364, https://doi.org/10.1021/la3041506.
[49] R.J. Miller, The ' sniffer-patch ' technique for detection of neurotransmitter release, Trends Neurosci. 6 (1997) 192–197, https://doi.org/10.1016/S0166-2236(96)01039-9.
[50] C. Zhu, K. Huang, N.P. Siepser, L.A. Baker, Scanning ion conductance microscopy, Chem. Rev. 121 (2021) 11726–11768, https://doi.org/10.1021/acs.chemrev.0c00962.
[51] C. Zhu, K. Huang, Y. Wang, K. Alanis, W. Shi, L.A. Baker, Imaging with ion channels, Anal. Chem. 93 (2021) 5355–5359, https://doi.org/10.1021/acs.analchem.1c00224.
[52] F.C. Macazo, R.J. White, Bioinspired protein channel-based scanning ion conductance microscopy (Bio-SICM) for simultaneous conductance and specific molecular imaging, J. Am. Chem. Soc. 138 (2016) 2793–2801, https://doi.org/10.1021/jacs.5b13252.
[53] K. Shoji, R. Kawano, R.J. White, Recessed Ag/AgCl microelectrode-supported lipid bilayer for nanopore sensing, Anal. Chem. 92 (2020) 10856–10862, https://doi.org/10.1021/acs.analchem.0c02720.
[54] B.A. Heitz, J. Xu, H.K. Hall, C.A. Aspinwall, S.S. Saavedra, Enhanced long-term stability for single ion channel recordings using suspended poly(lipid) bilayers, J. Am. Chem. Soc. 131 (2009) 6662–6663, https://doi.org/10.1021/ja901442t.
[55] B.A. Heitz, J. Xu, I.W. Jones, J.P. Keogh, T.J. Comi, H.K. Hall, C.A. Aspinwall, S. S. Saavedra, Polymerized planar suspended lipid bilayers for single ion channel recordings: comparison of several dienoyl lipids, Langmuir 27 (2011) 1882–1890, https://doi.org/10.1021/la1025944.
[56] L.K. Bright, C.A. Baker, M.T. Agasid, L. Ma, C.A. Aspinwall, Decreased aperture surface energy enhances electrical, mechanical, and temporal stability of suspended lipid membranes, ACS Appl. Mater. Interfaces 5 (2013) 11918–11926, https://doi.org/10.1021/am403605h.
[57] L.K. Bright, C.A. Baker, R. Bränström, S.S. Saavedra, C.A. Aspinwall, Methacrylate polymer scaffolding enhances the stability of suspended lipid bilayers for ion channel recordings and biosensor development, ACS Biomater. Sci. Eng. 1 (2015) 955–963, https://doi.org/10.1021/acsbiomaterials.5b00205.
[58] M.T. Agasid, T.J. Comi, S.S. Saavedra, C.A. Aspinwall, Enhanced temporal resolution with ion channel-functionalized sensors using a conductance-based measurement protocol, Anal. Chem. 89 (2017) 1315–1322, https://doi.org/10.1021/acs.analchem.6b04226.
[59] S. Del Linz, E. Willman, M. Caldwell, D. Klenerman, A. Fernández, G. Moss, Contact-free scanning and imaging with the scanning ion conductance microscope, Anal. Chem. 86 (2014) 2353–2360, https://doi.org/10.1021/ac402748j.
[60] S. Zhang, M. Li, B. Su, Y. Shao, Fabrication and use of nanopipettes in chemical analysis, Annu. Rev. Anal. Chem. 11 (2018) 265–286, https://doi.org/10.1146/annurev-anchem-061417-125840.
[61] W. Shi, Y. Zeng, C. Zhu, Y. Xiao, T.R. Cummins, J. Hou, L.A. Baker, Characterization of membrane patch-ion channel probes for scanning ion conductance microscopy, Small 14 (2018) 1–10, https://doi.org/10.1002/smll.201702945.
[62] W. Shi, Y. Zeng, L. Zhou, Y. Xiao, T.R. Cummins, L.A. Baker, Membrane patches as ion channel probes for scanning ion conductance microscopy, Faraday Discuss 193 (2016) 81–97, https://doi.org/10.1039/c6fd00133e.
[63] R.H. Kramer, Patch cramming: monitoring intracellular messengers in intact cells with membrane patches containing detector inn channels, Neuron 4 (1990) 335–341, https://doi.org/10.1016/0896-6273(90)90046-I.
[64] M. T, E. Sackman, Polymer-supported membranes as models of the cell surface, Nature 437 (2005) 656–663, https://doi.org/10.1038/nature04164.
[65] X.F. Kang, S. Cheley, A.C. Rice-Ficht, H. Bayley, A storable encapsulated bilayer chip containing a single protein nanopore, J. Am. Chem. Soc. 129 (2007) 4701–4705, https://doi.org/10.1021/ja068654g.
[66] D. Okuno, M. Hirano, H. Yokota, Y. Onishi, J. Ichinose, T. Ide, A simple method for ion channel recordings using fine gold electrode, Anal. Sci. 32 (2016) 1353–1357, https://doi.org/10.2116/analsci.32.1353.
[67] D. Okuno, M. Hirano, H. Yokota, J. Ichinose, T. Kira, T. Hijiya, C. Uozumi, M. Yamakami, T. Ide, A gold nano-electrode for single ion channel recordings, Nanoscale 10 (2018) 4036–4040, https://doi.org/10.1039/c7nr08098k.
[68] K. Shoji, R. Kawano, R.J. White, Spatially resolved chemical detection with a nanoneedle-probe-supported biological nanopore, ACS Nano 13 (2019) 2606–2614, https://doi.org/10.1021/acsnano.8b09667.
[69] R.J. White, B. Zhang, S. Daniel, J.M. Tang, E.N. Ervin, P.S. Cremer, H.S. White, Ionic conductivity of the aqueous layer separating a lipid bilayer membrane and a glass support, Langmuir 22 (2006) 10777–10783, https://doi.org/10.1021/la061457a.
[70] G. Krishna, J. Schulte, B.A. Cornell, R.J. Pace, P.D. Osman, Tethered bilayer membranes containing ionic reservoirs: selectivity and conductance, Langmuir 19 (2003) 2294–2305, https://doi.org/10.1021/la026238d.
[71] G. Valincius, T. Meškauskas, F. Ivanauskas, Electrochemical impedance spectroscopy of tethered bilayer membranes, Langmuir 28 (2012) 977–990, https://doi.org/10.1021/la204054g.
[72] M. Hirano, M. Tomita, C. Takahashi, N. Kawashima, T. Ide, Development of an automated system to measure ion channel currents using a surface-modified gold probe, Sci. Rep. 11 (2021) 1–9, https://doi.org/10.1038/s41598-021-97237-z.
[73] E.A. Hussein, R.J. White, Silver nanoneedle probes enable sustained DC current, Single-Channel resistive pulse nanopore sensing, Anal. Chem. 93 (2021) 11568–11575, https://doi.org/10.1021/acs.analchem.1c02087.
[74] A. Page, D. Perry, P.R. Unwin, Multifunctional scanning ion conductance microscopy, Proc. R. Soc. A Math. Phys. Eng. Sci. 473 (2017), https://doi.org/10.1098/rspa.2016.0889.
[75] K. Cremin, B.A. Jones, J. Teahan, G.N. Meloni, D. Perry, C. Zerfass, M. Asally, O. S. Soyer, P.R. Unwin, Scanning ion conductance microscopy reveals differences in the ionic environments of gram-positive and negative bacteria, Anal. Chem. 92 (2020) 16024–16032, https://doi.org/10.1021/acs.analchem.0c03653.
[76] J. Zhang, T. Zhu, J. Lang, W. Fu, F. Li, Recent advances of scanning electrochemical microscopy and scanning ion conductance microscopy for single-cell analysis, Curr. Opin. Electrochem. 22 (2020) 178–185, https://doi.org/10.1016/j.coelec.2020.06.001.
[77] Y. Takahashi, A. Kumatani, H. Shiku, T. Matsue, Scanning probe microscopy for nanoscale electrochemical imaging, Anal. Chem. 89 (2017) 342–357, https://doi.org/10.1021/acs.analchem.6b04355.

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