Electronic control of H\textsuperscript{+} current in a bioprotonic device with Gramicidin A and Alamethicin

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In biological systems, intercellular communication is mediated by membrane proteins and ion channels that regulate traffic of ions and small molecules across cell membranes. A bioelectronic device with ion channels that control ionic flow across a supported lipid bilayer (SLB) should therefore be ideal for interfacing with biological systems. Here, we demonstrate a biotic–abiotic bioprotonic device with Pd contacts that regulates proton (H\textsuperscript{+}) flow across an SLB incorporating the ion channels Gramicidin A (gA) and Alamethicin (ALM). We model the device characteristics using the Goldman–Hodgkin–Katz (GHK) solution to the Nernst–Planck equation for transport across the membrane. We derive the permeability for an SLB integrating gA and ALM and demonstrate pH control as a function of applied voltage and membrane permeability. This work opens the door to integrating more complex H\textsuperscript{+} channels at the Pd contact interface to produce responsive biotic–abiotic devices with increased functionality.
From electroceuticals to wearable devices, and from electronic plants to edible electronics, interfacing electronic devices with biological systems promises new therapies and device functionalities beyond silicon. In biological systems, most of the communication between cells is mediated by membrane proteins and ion channels that passively allow or actively control the flow of ions and small molecules across the cell membrane. In this fashion, complex functions such as muscle contraction, neuronal signalling and metabolism are achieved. Membrane proteins are studied using patch clamps, micropore arrays and electrode-supported lipid bilayers, and passive transmembrane ionic transport is controlled by local electrical and chemical potential gradients according to the Nernst–Planck equation. While most common ions are Na⁺, K⁺ and Cl⁻, proton (H⁺) currents and concentration [H⁺] gradients play important physiological roles. Examples include oxidative phosphorylation in mitochondria, light-activated H⁺ pumping by archaeal bacteriorhodopsins, H⁺-activated bioluminescence in dinoflagellates, flagellar propulsion in bacteria, voltage gated H⁺ channels and antibiotic action by polypeptides such as Gramicidin.

Ionic currents and membrane proteins are the most intimate interface for electronics to communicate with cells and biological systems. However, conventional electronics typically use electrons as charge carriers instead of ions. To address this issue, gramicidin and bacteriorhodopsin have been integrated into the Pd to form PdH gates and allows H⁺ to diffuse into Pd and form a hydride (PdH). This device architecture and allows H⁺ to diffuse into Pd and form a hydride (PdH) gate and allows H⁺ to diffuse into Pd and form a hydride (PdH). A reduction current at the Pd contact is measured as current density (J) where oxidation of H corresponds to a positive i and reduction of H⁺ corresponds to a negative i. We apply a potential difference between the Pd contact and the Ag/AgCl electrode (V) and measure the resulting H/H⁺ current density (i) where oxidation of H corresponds to a positive i and reduction of H⁺ corresponds to a negative i. In short, by measuring i, we effectively monitor the flow of H⁺ between the solution and the Pd contact mediated by H⁺ + e⁻ ↔ H, and subsequent absorption of H into Pd to form PdHx or desorption of H from the contact to form H⁺ (refs 37,44).

To electrically isolate the Pd contact from the solution and provide a template for ion channel insertion, we deposit a 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC, Sigma-Aldrich Lipids) SLB onto the Pd contact using vesicle fusion. Force rupture measurements by Atomic Force Microscopy (AFM) show that the thickness of the SLB membrane is

![Figure 1 | Schematic depiction of the ion channel bioprototic device.](image-url)

(Left) A bioprototic device with integrated Gramicidin (gA) supports the flow of H⁺ across the SLB and when applied to the Pd contact, when H⁺ reach the surface of the Pd contact, they are reduced to H by an incoming electron and diffuse into Pd to form a hydride (PdH). A reduction current at the Pd contact is measured as current density (i). (Right) A bioprototic device with integrated Alamethicin (ALM), applying a negative or positive voltage (V) above a threshold value at the Pd contact opens the gate and allows H⁺ flow across the SLB, turning the device ON. At V = 0 mV, no H⁺ flows across ALM and the device switches OFF.
4.8 ± 0.7 nm (n = 20, for a device with dimension of 2 × 50 μm), which is close to the expected value of 5.5 nm for a DOPC bilayer. In essence, the SLB mimics a cell membrane that electrically insulates the Pd contact (ρ ~ 3 GΩ cm⁻¹; see Supplementary Fig. 2 and Supplementary Note 1). In contrast, the bioprotonic device with gA and subjected to V = −200 mV experiences a large i_H+ that continually increases to a maximum of i_H+ = −4.5 mA cm⁻² at t = 10 min (Fig. 2b,d), because the gA inserted within the SLB create pathways for H⁺ flow. The dependence of i_H+ as a function of time will be discussed in the modelling section. Setting V = 0 mV after 10 min causes H⁺ to transfer from the Pd/H⁺ to the solution with a maximum i_H+ = −0.5 mA cm⁻² (Fig. 2d, green trace). The presence of a large oxidation peak for H⁺ at 50 mV in the I–V sweep confirms that this transfer is indeed occurring (Supplementary Fig. 4). To verify that gA was responsible for H⁺ flow across the SLB, we added 1 mM Ca²⁺ to block H⁺ transfer across the gA channel (Fig. 2c). Under these conditions, for V = −200 mV we measure i_H⁺ = −0.7 mA cm⁻² and subsequently measure i_H⁺ = −0.5 mA cm⁻² when setting V = 0 mV after 10 min (Fig. 2d, blue trace). These values of i_H⁺ are comparable to those measured when no gA is incorporated in SLB and are consistent with Ca²⁺ blocking gA channels.

**Voltage control of H⁺ flow with Gramicidin A.** Gramicidin A (gA) is a short helical polypeptide from *Bacillus brevis* that dimerizes in lipid bilayers to form a transmembrane channel that allows the passage of small cations (including H⁺) while remaining impermeable to anions. To control the flow of H⁺ as a function of V, we integrate gA in the SLB of our devices. In the absence of gA and at an applied V = −200 mV, we measure i_H⁺ = −1.2 mA cm⁻² (Fig. 2a,d). This small i_H⁺ indicates that few H⁺ diffuse across the bilayer to become reduced at the Pd surface. To confirm this result, we set V = 0 mV after applying V = −200 mV for 10 min. If any H⁺ flow were to occur across the SLB during the V = −200 mV step, a significant amount of Pd/H⁺ should form at the Pd/solution interface. This Pd/H⁺ has a higher protocchemical potential (μ_H⁺) than the solution of pH = 7.0 at V = 0 mV, leading to H⁺ oxidation at the Pd/H⁺ contact, H⁺ flow from the Pd/H⁺ into the IL, and giving rise to a measurable positive i_H⁺ (ref. 34). The black trace in Fig. 2d (right hand side panel) shows that this is clearly not the case.

**Alamethicin voltage-gated H⁺ switches.** To demonstrate that our device architecture can be used to create a voltage-dependent switch that turns the H⁺ flow on and off between bulk solution and isolation layer (Fig. 3), we integrate alamethicin (ALM) into the SLB. Alamethicin is a 20-amino-acids long peptide from the fungus *Trichoderma viride*. ALM undergoes spontaneous insertion into lipid bilayers and forms a voltage-gated channel when 4- to 6-molecules associate to form an α-helical bundle. An asymmetric threshold voltage above ~60 mV induces reorientation of the helices, thereby opening the channel to selective transport of cations, including H⁺, in the

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**Figure 2 | Schematics of bioprotonic gA devices.** (a) Pd contact coated with a SLB. The SLB inhibits the flux of H⁺ from the bulk solution to the Pd/solution interface. (b) Pd contact with SLB incorporating gA is semipermeable to H⁺, with gA channels facilitating the rapid flow of H⁺ to the Pd/solution interface. (c) Addition of 1 mM Ca²⁺ to the bulk solution, blocks gA and prevents the flow of H⁺ to the Pd/solution interface. (d) i_H⁺ versus time plot for V = −200 mV and V = 0 mV. Black trace SLB, green trace SLB + gA, blue trace SLB + gA blocked by Ca²⁺. (The data are collected from 3 different devices with different dimensions: Pd / SLB: 3 different devices of 2 × 50 μm, Pd / SLB + gA: 2 × 20 μm, 2 × 50 μm, 2 × 70 μm, Pd / SLB + gA + Ca²⁺: 2 different devices of 2 × 50 μm and 1 device of 2 × 70 μm. The error bars are the root mean square of the displacement of the data from the average value).
the case with the gA device, the ALM is responsible for the bioprotonic device behaviour. Moreover, as is expected, the ALM can only oxidize H under a small positive voltage of V = 100 mV which is also required to open ALM channels and allow flow of H+ from the PdHx contact to bulk solution. (d) iH, versus time plot for V = −200, 0 and 100 mV, SLB black trace and SLB integrating ALM red trace. (The data is collected from 3 different devices with the different dimensions, Pd/SLB: 3 different devices of 2 × 50 µm, Pd/SLB + ALM: 2 × 20 µm, 2 × 40 µm, 2 × 50 µm. The error bars are the root mean square of the displacement of the data from the average value).

Figure 3 | Schematics of bioprotonic voltage-gated devices. (a) Pd contact with SLB and ALM. At V = −200 mV, ALM channels open and H+ flows to the Pd/solution interface. (b) PdHx contact with SLB and ALM remains closed under V = 0 mV and there is no H+ flow. (c) PdHx contact coated with SLB and ALM can only oxidize H under a small positive voltage of V = 100 mV which is also required to open ALM channels and allow flow of H+ from the PdHx contact to bulk solution. (d) iH, versus time plot for V = −200, 0 and 100 mV, SLB black trace and SLB integrating ALM red trace. (The data is collected from 3 different devices with the different dimensions, Pd/SLB: 3 different devices of 2 × 50 µm, Pd/SLB + ALM: 2 × 20 µm, 2 × 40 µm, 2 × 50 µm. The error bars are the root mean square of the displacement of the data from the average value).

direction of V (ref. 57). For V values inferior to this threshold, ALM is closed and there is no H+ flow across the membrane in spite of differences in [H+]i, which typically creates a driving force for H+ flow (Fig. 3).

Similar to the gA device, we measure $i_{H+i} = -5.5 \text{ mA cm}^{-2}$ at $V = -200 \text{ mV}$ for an ALM integrated bioprotonic device, at $V = -200 \text{ mV}$ the ALM channel is open, allowing flow of H+ (Fig. 3a,d). When the voltage is set at $V = 0 \text{ mV}$, $i_{H+i} = 0 \text{ mA cm}^{-2}$. This is because the ALM channel is closed, and no H+ flow occurs between IL and B, even after PdHx formation. For $V = 100 \text{ mV}$, the ALM channel opens and we measure $i_{H+i} = 1.9 \text{ mA cm}^{-2}$, indicating that H+ flows from the PdHx contact and across the SLB. As a control, we added 40 mM urea to the bulk solution to irreversibly disrupt the ALM channel function. Similar to the gA device, we measure $i_{H+i} = 1.2 \text{ mA cm}^{-2}$ for $V = -200 \text{ mV}$ and $i_{H+i} = 0 \text{ mA cm}^{-2}$ for both $V = 0 \text{ mV}$ and $V = 100 \text{ mV}$ (Supplementary Fig. 5), confirming that ALM is responsible for the bioprotonic device behaviour. Moreover, as is the case with the gA device, the H+ oxidation peak at 50 mV dominates the I–V sweep. However, for $V > 0 \text{ mV}$, $i_{H+i}$ in the ALM device has a rectifying diode-like behaviour (Supplementary Fig. 6). We conclude that the voltage-gated functionality of the ALM channel is maintained on its integration into the device’s SLB.

To summarize, the device switches ON when ALM voltage-gated channels are open, allowing H+ flow across the membrane and in the direction of V. The device is OFF when $V = 0 \text{ mV}$, a voltage below the threshold value needed for ALM opening. Thus, an ALM device can be used to modulate H+ flow and [H+]i variation between IL and B, much like cells control [H+]i differences between cytosol and extracellular space.

A model for H+ transfer across gA and ALM channels. To better understand the dynamics of H+ flow in our biotic–abiotic devices, we modelled H+ transport characteristics using the Nernst–Planck equation to fit our device current density data, $i_{H+i}$ (Fig. 4). To this end, we divide the device into four distinct layers: the bulk solution (B); a Supported Lipid Bilayer membrane (SLB) with variable permeability ($P_{SBL}$) that encompasses the SLB with integrated gA or ALM; the isolation layer (IL); and the Pd/PdHx contact (Fig. 4a). When a negative voltage $V$ is applied to the Pd contact, H+ flow from B across the SLB and into the IL. We quantify this transport as the H+ current density across membrane, $j_{H+i}$. A portion of the H+ from $j_{H+i}$ contribute to an increase in [H+]i in the IL, [H+]i, while the remaining H+ are reduced to H at the Pd contact and form PdHx. We assume that $i_{H+i}$ measured between the Pd contact and the Ag/AgCl electrode arises entirely from electrons participating in H+ oxidation (positive $i_{H+i}$) or reduction processes (negative $i_{H+i}$), and we quantify it as current density, $i_{H+i}$.

From conservation of mass (equation 1)\textsuperscript{59}

$$i_{H+i} = -j_{H+i} + ed[H^+]_{IL}$$

(1)

where, $e$ is elementary charge $= 1.6 \times 10^{-19}$ C, and $d[H^+]_{IL}$ is the incremental change in molar concentration of H+ in the IL in moles m$^{-3}$. $j_{H+i}$ is obtained by inserting the Goldman–Hodgkin–Katz (GHK) solution\textsuperscript{50} into the Nernst–Planck equation (equation 2)\textsuperscript{59}:

$$j_{H+i} = -P_{SBL}FV_m[H^+]_{IL} - [H^+]_a \exp(-V_m)$$

$$1 - \exp(-V_m)$$

(2)

where $P_{SBL}$ is the permeability of H+ through the SLB, $F$ is Faraday’s constant, $f$ is F/RT, or 38.6 V$^{-1}$, $V_m$ is the potential difference across the membrane and [H+]i is the H+
between Ag/AgCl electrode and Pd contact for H⁺ equations 2 and 3, respectively. A rise in membrane and Pd contact. (equation 2) was used to describe the behaviours of membrane and Pd contact, an SLB incorporating ALM (red trace) bioprotonic device. A combination solution interface for SLB (black trace), SLB incorporating gA (green trace), and SLB, SLB permeability; [H⁺]IL increases the d[H⁺] over time. These results can be rationalized as follows: at the Pd/PdH⁺ interface (Fig. 4c), is six orders of magnitude higher than the resistance of B (Fig. 2 and 3), is six orders of magnitude higher using a modified version of the Tafel equation61 (equation 4):

\[
\text{d}[H^+]_{IL} = 10^{-i_{H^+}} dt / dH_N \beta
\]

We assume that [H⁺]IL remains constant and calculate the change in [H⁺]IL from the change of pH in the IL, dPdHIL, assuming a buffering capacity for the solution, β (Supplementary Note 2)92:

\[
dPdHIL = (i_{H^+} + j_{H^+}) dt / dH_N \beta
\]

To fit i_{H^+} to the experimental data, we programed an iterative solution to the above model using the membrane permeability, P_{slb}, as a fitting parameter. For V = −200 mV and D_{IL} = 0.5 nm the model fits the experimental data well using membrane permeabilities of P_{SLB} = 0.006 s⁻¹ (unmodified SLB), P_{SLB+gA} = 0.58 s⁻¹ (SLB with integrated gA) and P_{SLB+ALM} = 0.74 s⁻¹ (SLB with integrated ALM). Consistent with our expectation, insertion of either gA or ALM into the SLB increases membrane permeabilities by three orders of magnitude. We did not control or accurately measure the density of the channels and these permeabilities are characteristic of the device and do not represent the absolute conductivities of individual gA or ALM.

We next calculate the change in pH in the isolation layer. The resulting d[H⁺]IL is (ref. 59):

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contacts that measures and controls the flow of $H^+$ across a lipid bilayer with the ion channels gramicidin A (gA) and alamethicin (ALM). We use gA to increase membrane permeability and control the $H^+$ flow across the lipid bilayer with a voltage applied on the Pd/PdH contact. We also demonstrate bidirectional voltage gated switching of the $H^+$ flow across the SLB with ALM. Finally, we model $H^+$ transport in this system using the Goldman solution and the Nernst equation and use the model to derive the permeability parameters of the lipid bilayer with integrated gA and ALM ion channels and to predict change in pH of the solution at the lipid bilayer/Pd contact interface. Our results indicate that ion channels increase SLB permeability by three orders of magnitude. This is the first time that $H^+$ transport at the Pd contact interface. Our results indicate that ion channels increase SLB permeability by three orders of magnitude. This is the first time that $H^+$ conducting channels have been integrated with Pd/PdH $H^+$-conducting contacts and that the $H^+$ current flowing through these channels has been directly measured and controlled. Future integration of more complex proteins may require Pd contact functionalization with a self-assembled monolayer or cushioning lipopolymer to passivate the Pd surface and overcome protein denaturation. To this end, we demonstrate that passivation of the Pd contact with self-assembled 3-aminopropyl-triethoxy-silane retains garamicidin A device functionality (Supplementary Figs 7–9). On the other hand, passivating the Pd contact with poly (ethylene glycol) insulates the Pd surface and hampers $H^+$ transfer at the Pd contact solution interface. Alternatively, larger proteins such as H. turkmenica detarhodopsin can be engineered to bind to the Pd contact surface without experiencing denaturation by making use of fused Pd-binding peptides. This work opens the door to integrating more complex active $H^+$ channels at the Pd contact interface to produce biotic–abiotic devices with increased functionality.

The resulting bilayers were characterized by AFM, with rupture depth measurements showing a thickness of ca. 4.8 $\pm$ 0.7 nm (Supplementary Fig. 2 and Supplementary Note 13). Home-built systems were used to conduct cyclic voltammetry experiments in the presence of a redox probe and I–V measurements in the presence of buffer solution. Throughout this process, the pH was monitored using a calibrated pH metre.

**Ion channel incorporation into supported lipid bilayers.** Gramicidin A (5 mg ml$^{-1}$) in 200-proof ethanol was mixed with DOPC in chloroform followed by solvent evaporation with $N_2$. The rehydration with aqueous buffer solution. DOPC/gramicidin vesicles at a molar ratio of 290:1 were formed by tip sonication and vesicle size determined by dynamic light scattering ($d_{100}$ = 105 $\pm$ 26 nm, n = 3). These vesicles were then fused onto Pd microfluidic devices. ALM was incorporated into bilayers by incubating 5 mg ml$^{-1}$ solution of ALM peptide for 30 min on freshly deposited Pd-bilayer structures followed by rinsing with K-PBS buffer solution.

**Electrical measurements.** All electrical measurements were performed using a Signature S probe station with a custom built environmental chamber. The probe station was connected to an Agilent 4155C semiconductor parameter analyser.

**Simulations.** Iterative simulations were executed in the MATLAB software package. Pd contact Tafel parameters $i_0$ and $z$ were measured with low-voltage $I–V$ sweeps at a scan rate of 5 mV s$^{-1}$ (Supplementary Fig. 10). The permeability of the three membranes were estimated using a composite model of the permeability of individual cationic channels and the permeability of the DOPC matrix, normalized to their respective occupied area. The length of the isolation layer (ca. 1 nm) was estimated using AFM force versus displacement measurements.

**Code availability.** MATLAB code for simulations is available at https://dx.doi.org/10.6084/m9.figshare.3509846.v1.

**Data availability.** The data that support the findings of this study are available from the corresponding author on request.

**Methods**

**Materials.** 1,2-di-oleoyl-sn-glycero-3-phosphocholine (DOPC; Sigma-Aldrich), Bacillus brevis Gramicidin A (90% purity; BioChemika), Trichoderma viride Alamethicin (90% purity; Sigma-Aldrich) were used as received. The heterofunctional PEG, 1,2-distearyl-sn-glycerol-3-phosphoethanolamine-N-poly (ethylene glycol)-2000 N- (3-(2-(pyridylidithio)) propionate) (DSPE-PEG-PDP) and lipids, 1,2-distearoyl-sn-glycérol-3-phosphoethanolamine-N-poly (ethylene glycol) were purchased from Avanti Polar Lipids (Alabaster, AL). 3-aminopropyl-triethoxy-silane was purchased from, Sigma-Aldrich. Potassium ferrocyanide (K$_4$(Fe(CN)$_6$), 99.9% purity) K$_2$HPO$_4$, H$_2$PO$_4$, Polar Lipids (Alabaster, AL). 3-aminopropyl-triethoxy-silane retains garamicidin A device functionality (Supplementary Figs 7–9). On the other hand, passivating the Pd contact with poly (ethylene glycol) insulates the Pd surface and hampers $H^+$ transfer at the Pd contact solution interface. Alternatively, larger proteins such as H. turkmenica detarhodopsin can be engineered to bind to the Pd contact surface without experiencing denaturation by making use of fused Pd-binding peptides. This work opens the door to integrating more complex active $H^+$ channels at the Pd contact interface to produce biotic–abiotic devices with increased functionality.

**Device fabrication and characterization.** Bioprototypic devices were fabricated with conventional soft- and photo-lithography on a 100 nm thick layer of silicon dioxide. Each device has a microfluidic SU-8 channel of 4 mm thick. The Pd contacts have varied contact area of 10 mm$^2$ ($\times$ 5 mm), 20 mm$^2$ ($\times$ 10 mm), 40 mm$^2$ ($\times$ 20 mm), 80 mm$^2$ ($\times$ 40 mm), 160 mm$^2$ ($\times$ 50 mm), 120 mm$^2$ ($\times$ 60 mm), 140 mm$^2$ ($\times$ 70 mm), 160 mm$^2$ ($\times$ 80 mm), 180 mm$^2$ ($\times$ 90 mm) and 200 mm$^2$ ($\times$ 100 mm) with a thickness of 50 nm. Pd is deposited on top of 5 nm Cr adhesion layer. Each device has an electrical probe pad out of the microfluidic channel that provides the electrical contact. The microfluidic channel confines the flow of liquid to the top of the Pd contact, while a polydimethylsiloxane well on top of the channel provides space to insert the counter electrode (Supplementary Fig. 1).

**SLB formation and characterization.** DOPC vesicles ($d_{100}$ = 100 $\pm$ 25 nm, n = 3) were prepared by tip sonication of a solution of 0.5 mg ml$^{-1}$ DOPC, in K-PBS buffer solution (pH = 7.0). Dynamic light scattering was used to characterize vesicle size. Before deposition, the Pd surface was hydrophilized by oxygen plasma. The vesicle solution was dispensed in the microfluidic channel and the device was gently agitated for 12h at 100% relative humidity to ensure vesicle fusion, followed by rinsing with K-PBS buffer solution to wash away unused DOPC vesicles.

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Author contributions
M.R. conceived the research. M.R. and Z.H. designed the experiments. Z.H. and S.K. performed the experiments. S.K. designed and implemented the simulations.
M.R., Z.H. and S.K. analysed the data. E.J. and Z.H. fabricated the devices. J.R., C.A. and F.B. helped with SLB device integration. Z.H. and M.R. wrote the manuscript with input from all authors. All authors revised the manuscript.

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