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Actuating Biology via Protonics

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14. ABSTRACT

While several technologies have been proposed for bioactuation, one of the more promising ones is protonics, the recapitulation of electronic devices by combining biocompatible polymers that carry protons with Pd electrodes that can inject protons. Our efforts in this field have established a sound theoretical and experimental approach to understanding the proton carrier materials and new results on actuating biological materials. We have gained greater understanding of how protons move in different media and how to interface protonic devices with patterned beds of cells. Lastly we have developed tools that allow us to exchange ionic and electronic signals, demonstrated within this program for protein layers and laying the groundwork for more complex conversation between the fields.

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EXECUTIVE SUMMARY

A central challenge for the 21st century is establishing meaningful two-way communication between biology and the powerful electronic systems developed over the past 50 years. While transducing biological signals into electronic ones, or biosensing, has been extensively developed, the other half of the feedback loop, transduction of electronic signals into biological ones, or bioactuation, demands more attention. The subset of bioactuation that uses an electrical bias to stimulate voltage responsive cells has advanced rapidly, showing how powerful well-developed communication can be. It has also highlighted the paucity of available techniques to properly actuate cells that do not respond to voltage, which includes most of nature. Actuating these cells, and even improving the actuation of neurons, requires a sophisticated system that can mimic the native cell stimuli, emitting biochemical signals with a spatial and temporal resolution that matches those of the target biological system. Such a system would advance the Navy's mission, addressing current challenges such as superior prosthetics that enable proprioception for amputees and future challenges such as synthetic immune systems that fight diseases that the body cannot, and the harnessing of highly evolved biological systems to new ends.

The requirements to advance biological actuation are straightforward: the voltage pulses currently used for nerve stimulation must be replaced by spatio-temporally controlled release of chemical signals to stimulate cells. Chemical signals contain more information than voltage pulses, can actuate essentially any cell from fungi to mammalian cells (and not just muscle and nerve cells), and should be less damaging to the cells than voltage pulses, which can degrade the cells as they do in cochlear implants. Secondly, the chemical signals need to be introduced with high spatial and temporal resolution. The required resolution depends on the application at hand, but one can say generally that they should at least address the length scale of a mammalian cell (~10 μm) and generate a signal within a minute. Microfabrication will be essential to achieving such resolution. Finally, the signal must be released such that it can diffuse to the target. The convective transport provided by current microfluidic techniques causes problems when it washes away all existing signaling molecules in order to deliver the desired molecule. Taken together, the goal is to generate an interface that mimics the native interface to these cells.

While several technologies have been proposed for bioactuation, one of the more promising ones is protonics, the recapitulation of electronic devices by combining biocompatible polymers that carry protons with Pd electrodes that can inject protons. Devices constructed from these materials can direct the accumulation of protons in a channel, generating protons that can either directly drive biochemical reactions or that can be translated into other chemical signals. Our initial efforts in this field have established a sound theoretical and experimental approach to understanding the proton carrier materials and new results on actuating biological materials. We have gained greater understanding of how protons move in different media and how to interface protonic devices with patterned beds of cells. Lastly we have developed tools that allow us to exchange ionic and electronic signals, demonstrated within this program for protein layers and laying the groundwork for more complex conversation between the fields.

This report presents research conducted by Stanislav Tsoi (6177), Gloria Bazargan (6189), Brian Eddie (6910), Sean Fischer (6189), Sarah Glaven (6910), David Kidwell (6177), Woo Kyung Lee (6177), Bradley Lusk (6177), Jeremy Robinson (6876), Thomas O'Shaughnessy (6352), Matthew Yates (6910) and Shawn Mulvaney (6177).

PROTONICS FOR BIOLOGICAL ACTUATION

1. INTRODUCTION

1.1. Objective

Biology and modern electronics have in common the transfer and manipulation of enormous quantities of information via feedback loops. The profound difference between those feedback loops stems from biology speaking ions while electronics speaks…well…electrons. A major challenge for science in the 21st century will be understanding and then implementing true two-way communication between these two information systems. Electronics has already achieved much for listening to biological feedback loops. While such biosensing is important, without actuation one cannot participate fully in biological feedback loops. Some progress has been made using direct capacitive coupling to cell types such as neurons or heart muscles that respond directly to voltages, yet this is a very small subset of cell types. Even there, the standard methods used such as patch clamp disrupt cell function and cannot be scaled to access multiple feedback loops.

We seek a deeper understanding of techniques to translate between the languages of electronics and biology. Fortuitously, new methods for exchanging electrons into ions in a biocompatible way have emerged: Simon et al. built a FET with a PEDOT polymer channel to release glutamate ions, a neurotransmitter [1]. Implanting this into the ear of an anesthetized guinea pig, they could observe changes in the receptor dynamics of its auditory cells. Separately, Rolandi demonstrated biocompatible protonic structures in which the conversion of electrons into protons occurs in a manner analogous to conventional solid state electronics, enabling them to produce transistors that show gain or diodes that rectify [2]. This approach is particularly interesting in that it uses hydrogen gas to provide an endless supply of protons to the structure as opposed to Berggren's approach which had limited reservoirs [3]. These two examples demonstrate the possibility of actuating cells using electronic structures and ultimately to harmonize biological and electronic feedback loops.

This program undertaken by researchers at NRL aims to build the tools to close the feedback loop with biology. We have built protonic structures that can release ions, the native language of biology, and thereby establish meaningful, two-way conversations.

1.2. Motivation

Proton gradients are the primordial source of power for all cells and so are the most critical flow of ions for life [4]. They directly drive a wide range of biological processes from energy production by mitochondria to the rotation of bacterial flagella. As one progresses to more complicated life forms such as eukaryotes and multicellular organisms, other ion and small molecule concentration gradients grow in importance for communication and respiration. For instance, nearly a third of all energy generated by the mitochondria in our cells is used to maintain a gradient in Na^+ and K^+ across the cell membrane [5]. Similar to the evolutionary path taken by nature, we began by establishing electronic control over the nanoscale flow of protons and then built in complexity to control the flow of other ions and signaling molecules.

These new bioprotonic conversion techniques will be the foundation for understanding cellular actuation, therefore they merit a brief description. As an example, Figure 1 depicts a protonic field effect transistor (FET). The basic components are a Pd "protode" that can absorb hydrogen gas, H_2, and then inject protons, H^+, under a potential bias. These protons may be transported through a proton conductive material, or PCM, to the drain where they accept electrons and are absorbed to complete the circuit.

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Critically, a backgate can enhance or deplete the proton concentration within the channel and thus modulate conductivity. We emphasize that this structure applies an electronic voltage to establish a protonic gradient. We will use this effect to enhance the acidity of the channel and ultimately to release ions to actuate biological structures.

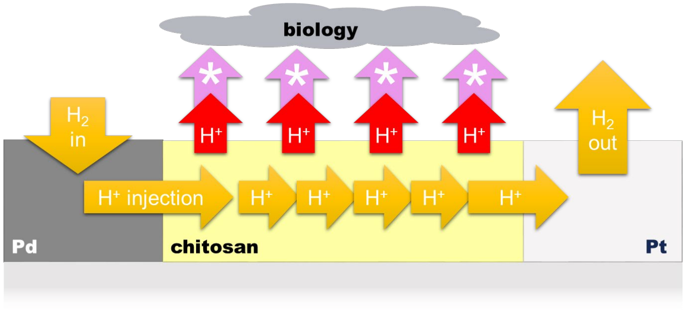

Figure 1. Cartoon depicting a protonic FET device. Hydrogen gas loads into a Pd "protode." An applied potential, which can be modulated by the back gate, causes the protons to move through the chitosan – a proton conductive material – and the circuit is complete when the Pt electrode converts the signal back into electrons.

2. APPROACH

This program seeks to demonstrate a generic method to actuate biological processes using protonics. For proper implementation, we needed to understand the basic nanoscale processes that control the performance of the protonic structure, demonstrate that they actuate biological processes, and ultimately interface them with cells. In some ways we recapitulated evolution in pursuing ever greater complexity but all powered by proton gradients.

2.1. Understanding the operation and physics of protonic devices

Protonic devices are the equivalent of electronic devices except they move protons instead of electrons. Prior to this program, the field of protonics was new. The phenomena being reported and the devices were one-off designs that were built or rigged by researchers with lab-based techniques. NRL's protonics program brought the power of our nanoscience institute and its bevy of fabrication tools to bring rigor to the design and construction of protonic devices. Using mask sets and lithography techniques, 6 inch wafers with hundreds of protonic devices per wafer were fabricated. Protonic devices in a number of configurations, including 4 probe, 2 probe, transmission line, and interdigitated electrodes were built. Using these devices, statistically relevant numbers of data points could be generated to learn about the reliability and performance of protonic devices. NRL's expertise in electrochemistry was also brought to bear and a fundamental understanding of the electrochemistry at every interface of a protonics device was achieved. We have published a seminal paper in the field connecting experimental and theoretical work [6].

2.2. Ordering of the protonic conductive material (PCM)

An essential piece of the protonic FET device is the proton conductive material (PCM) present in the gate area. Chitosan is a biocompatible polymer and in some forms is known to transport protons when in a hydrated state. We sought to improve the performance of PCMs in general, and specifically chitosan, through both theoretical and experimental means.

NRL developed theory and simulations that modeled the movement of protons in what are termed “water wires.” Using density functional theory calculations and Monte Carlo simulations, the movement of protons was studied and compared with classical ideas reported in the literature. First, calculations showed the proton movement in a water wire was not as straight forward as first thought, and second, that random walk steps were common to the process. Next the presence of side groups and functionalization on the chitosan chain were studied for the interactions with water chains. This work resulted in two publications and provided greater understanding of how protonic devices operated [7,8]. Experimentally, we tried to confirm that better ordering in the polymer backbone would enhance proton movement in a PCM. Methods to order the backbone included atomic force microscopy both for deposition and post-deposition alignment by heat and shear force. In addition, we explored the use of electrospinning to deposit materials that were aligned. Success was achieved with the electrospinning approach, showing that ordering in individual wires led, in part, to higher conductivity [9]. This work also demonstrated the power of the nanoscience institute and tools available. A helium ion microscope was used to isolate and prepare individual fibers for analysis. This provided a way to create miniature protonic devices with controlled PCMs.

2.3. Interfacing with biology

Protonic devices offer the promise to hold meaningful, two-way conversations between biology and electronics. In addition to understanding the performance and operation of protonic devices, it was necessary to determine a biocompatible interface between the two worlds. The protonic devices fabricated must be properly insulated from the aqueous media supporting live cells. Various materials solutions were explored, including the classic cell biology technique of over coating everything with PDL/laminin. While this was sufficient for initial experiments, the need to pattern biological materials onto areas of interest was an essential skill to be developed. A large number of patterning techniques exist, with the use of 3-D printing methods becoming popular in view of their control over size scales useful to biological interactions. OrmoComp, an acrylate-based polymer, is a popular choice for these types of applications and was reported to be biocompatible with very robust cell lines. However, when we used it with neurons, the cells would either not adhere or would be killed by the surface. Using Michael’s addition, we have transformed the remaining, unreacted acrylate groups of OrmoComp into pendent amine groups by reacting the polymer with diamines. This transformation makes 3-D printed OrmoComp structures appear as though they have the same surface chemistry as PDL/laminin surfaces. Importantly, the application of our surface chemistry renders surfaces amenable to orthogonal deposition meaning that we can collect cells in the areas of the device we determine and those cells can thrive [10].

3. EXPERIMENTS

The following sub-sections provide an overview of specific experimental efforts that led to peer-reviewed journal publications. Further details of each effort can be found within the referenced published papers. These efforts advance theoretical understanding of protonic movement in materials, the movement of protons in electrospun wires, and the movement of protons in the biological entity *Desulfobulbaceae*, also known as cable bacteria.

3.1. Enhancing protonic movement in meta-polyaniline thin films by chemical functionalization [11]

We investigate proton diffusion in chemically functionalized meta-polyaniline thin films by ab initio molecular dynamics (AIMD) simulations. Our results show that protonic movement in unfunctionalized meta-polyaniline is hindered by proton migration to the polymer amine sites. Functionalization of the meta-polyaniline backbone with small chemical groups enhances protonic movement through the thin film water channels by reducing protonation of the amines. Additionally, the path of proton diffusion is influenced by the choice of chemical group. Proton-accepting groups facilitate diffusion near the polymer backbone while hydrogen-bond-accepting groups enable movement through the center of the water channels.

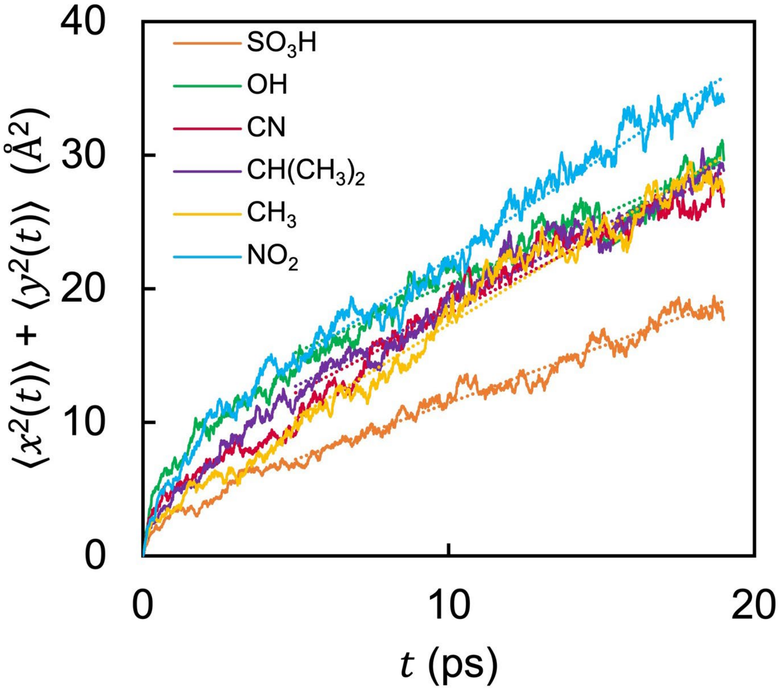

Figure 2. Proton mean-squared displacement $\langle x^2(t)\rangle+\langle y^2(t)\rangle$ vs. time t in m-PANI-R where R is SO_3H, OH, CN, CH_3, $CH(CH_3)_2$, or NO_2. These curves were used to calculate the proton diffusion coefficient D_{H+} in each thin film according to Eq. (2) of the reference, by fitting the region between 5 and 19 ps to a line. Dashes indicate the line of best fit in this region.

3.2. Quantifying proton diffusion in acid-functionalized chitosan membranes [12]

We explore proton diffusion in hydrated, maleic-acid-functionalized chitosan membranes using ab initio molecular dynamics (AIMD) simulations. Our simulations show that more frequent proton hopping between water molecules leads to an increase in the proton diffusion coefficient at higher water content in membranes based on 50 and 100% de-acetylated maleic chitosan. Moreover, mobile protons interact with the oxygen atoms of the 50% de-acetylated polymer's acetyl groups, making them susceptible to protonation. The maleic acid group's three oxygen atoms hydrogen-bond to water molecules in the membrane channels and are protonated less frequently than the acetyl groups.

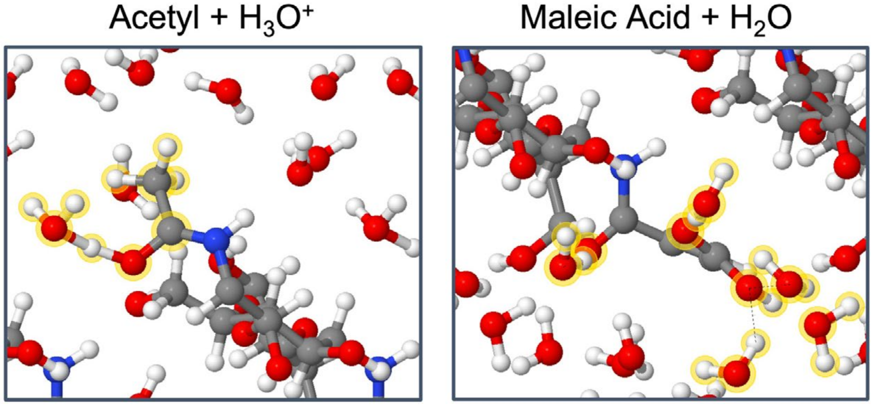

Figure 3. Snapshot of the partially de-acetylated maleic chitosan membrane (χ = 0.5) at a hydration level of 38 water wt% (16 waters per dimer). The acetyl oxygen is protonated by a hydronium ion and the maleic acid oxygens hydrogen-bond to water molecules within the channels.

3.3. Effect of structure and hydration level on water diffusion in chitosan membranes [13]

Bioprotonic devices show promise for medicinal/therapeutic treatments because of their ability to deliver a controlled flow of small molecules and ions to living organisms. These devices rely on biocompatible, conductive polymer membranes to facilitate molecular/ionic transport. Herein, ab initio molecular dynamics simulations are used to probe the effects structure and hydration level on water diffusion in chitosan-based polymer membranes. The diffusion coefficient of water is shown to be highest in membranes with low overall densities and weak non-covalent interactions. Insight from these results may aid in optimizing the molecular/ionic transport properties of polymer membranes for bioprotonic applications.

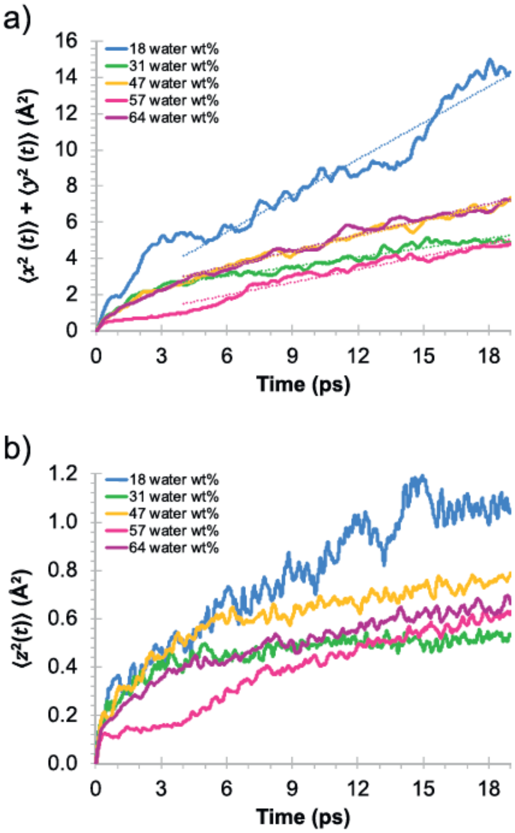

Figure 4. Mean-squared displacement of water molecules as a function of time a) within the xy-plane, and b) along the z-direction, for hydrated chitosan membranes with water channels perpendicular to the planes of chitosan's pyranose rings. Each curve represents the average over 40 trajectories. Dashes represent lines of best fit for the region of each curve between 4 and 19 ps.

3.4. Enhanced protonic conductivity and IFET behavior in individual proton-doped electrospun chitosan fibers [14]

A major challenge for biomedical science is developing materials and processing methods to enable creation of devices that can meaningfully translate signals between biology and electronics. Protonics-based devices-devices analogous to electronic devices but which use protons as charge carriers instead of electrons-are a promising strategy for such translation. Proton-conductive materials (PCMs)-the media through which protons are transported in such devices-are an element ripe for improvement, since they dominate the performance of protonic devices. We investigate protonic devices comprising sub-micrometer diameter, chitosan-fiber PCMs and palladium hydride (PdHx) protodes, the proton-injecting contact, to analyze how proton transport depends on the chemistry and ordering of the PCM. Current-voltage (I-V) measurements of single fiber-based devices under hydrogen atmospheres show that fibers electrospun from trifluoroacetic acid (TFA) solutions feature substantially higher proton conductivity, up to two orders of magnitude, compared to chitosan PCM films cast from acetic acid solutions. We further used digital simulation of the I-V data to elucidate the electrochemical and electrical processes that control device operation. The hydrogen oxidation reaction kinetics of the protode interfaces with the electrospun chitosan fibers agree well with those reported previously. Using X-ray photoelectron spectroscopy (XPS), we observed that single chitosan fibers spun from TFA solutions are more highly proton-doped than chitosan cast from acetic acid. Furthermore, I-V measurements on electrospun chitosan fibers vs. spin-cast chitosan films, both derived from TFA solutions, reveal that electrospun chitosan fibers yield similar to 10-fold higher proton conductivity, suggesting that local polymer ordering within the electrospun fibers further enhances proton transport for chitosan PCMs. Finally, devices fabricated from single doped chitosan fibers behave as ionic field effect transistors (IFETs) when gated locally with a conducting AFM tip. Transfer characteristics indicate that proton conduction in a single fiber increases by over an order of magnitude under a negative gate. The switchable ion currents and enhanced conductivity of the chitosan fiber-based PCMs comprise a means of establishing spatiotemporal control over ionic communication between protonic devices and adjacent biological cells and membranes.

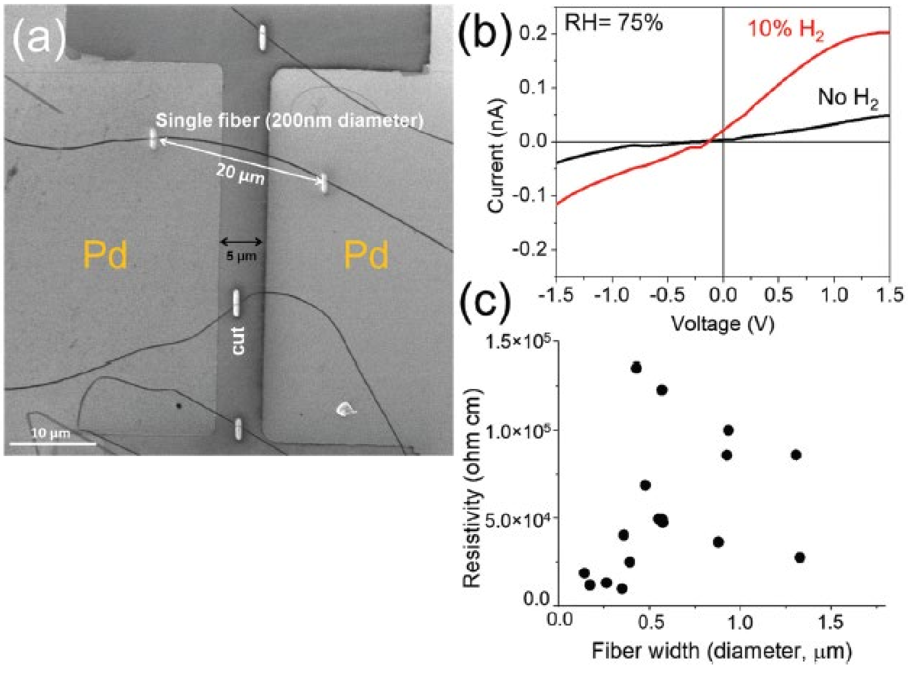

Figure 5. Characterization of protonic conductivity of a single chitosan fiber: (a) single chitosan fiber between Pd protodes isolated by cutting with focused helium ion beam using the HIM; (b) current–voltage curve of a single chitosan fiber under 75% RH N2 atmosphere in H2-free conditions (black curve) and with 10% H2 (red curve); (c) calculated resistivities of single chitosan fibers from measured dimensions (diameter and channel length). The R-squared value from a linear regression was 0.356, suggesting that there is no clear dependence between resistivity and fiber dimensions.

3.5. Analysis of correlated dynamics in the Grotthuss mechanism of proton diffusion [15]

Using a large set of ab initio molecular dynamics trajectories we demonstrate that the mechanistic details of aqueous proton diffusion are insensitive to finite size effects. Furthermore, we show how correlation in the proton hopping direction is related to the presolvation of the hydronium ion. Specifically, we observe a dependence of the probability for the excess proton to return to its previous hydronium ion on whether that hydronium ion was accepting a hydrogen bond from a fourth water molecule at the time the excess proton left. The dynamics of this fourth water molecule was previously linked to the net displacement of the proton, and our analysis shows that this connection is due to the changes in the hopping probability that we calculate. Additionally, we show how our simulated dynamics with correlations that imply a faster time scale are compatible with recent spectroscopy results that point to a slower hopping time scale by looking closely at which proton transitions are being taken into consideration. Finally, we show that the correlation in proton hopping directions is not strongly influenced by interactions among hydronium ions.

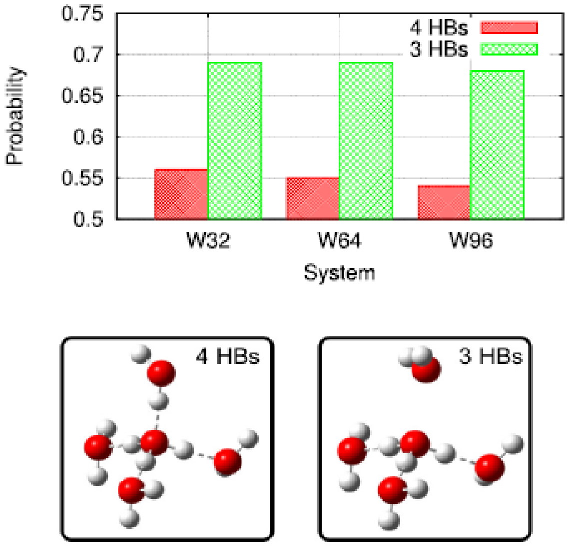

Figure 6. Probability for the excess proton to hop backward, dependent on whether there was a fourth water molecule donating a hydrogen bond to the hydronium ion at the time of the initial hop. Standard errors of the mean were all less than 0.005. The bottom panel shows schematically when a fourth water molecule is donating a hydrogen bond versus not donating.

3.6. Correlated dynamics in aqueous proton diffusion [16]

The aqueous proton displays an anomalously large diffusion coefficient that is up to 7 times that of similarly sized cations. There is general consensus that the proton achieves its high diffusion through the Grotthuss mechanism, whereby protons hop from one molecule to the next. A main assumption concerning the extraction of the timescale of the Grotthuss mechanism from experimental results has been that, on average, there is an equal probability for the proton to hop to any of its neighboring water molecules. Herein, we present ab initio simulations that show this assumption is not generally valid. Specifically, we observe that there is an increased probability for the proton to revert back to its previous location. These correlations indicate that the interpretation of the experimental results need to be re-examined and suggest that the timescale of the Grotthuss mechanism is significantly shorter than was previously thought.

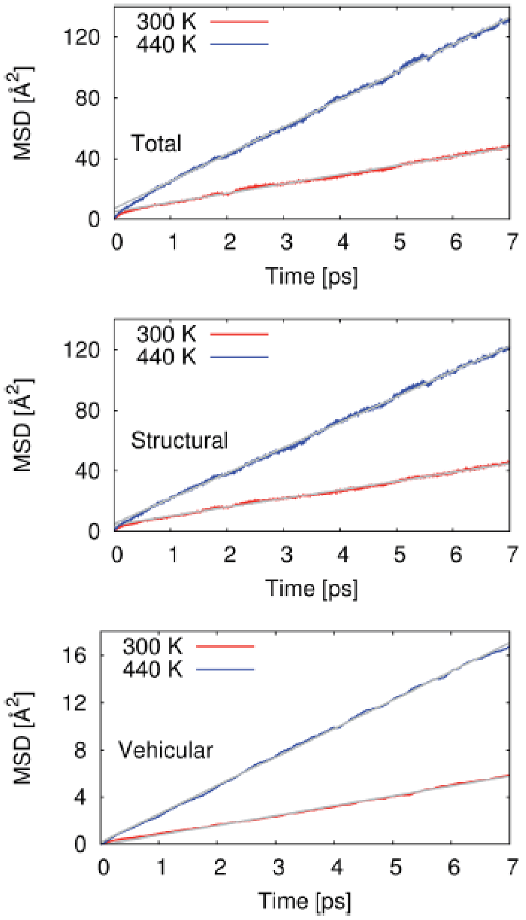

Figure 7. Mean-squared displacements (MSD) as functions of time for the proton at 300 and 440 K. The gray lines represent the linear regression used for extraction of the diffusion coefficients. The linear regression was performed on the data between 1 and 7 ps. The top panel gives the total MSD, while the middle and bottom panels show the structural and vehicular components, respectively.

3.7. Transport through hydrated chitosan-based polymer membranes under electric fields [17]

Proton transport is essential in many areas of chemistry and biology and is especially important in the fields of proton exchange membrane fuel cells and biocompatible, protonic semiconductors. These devices make use of membranes to control the flow of protons for either the generation of energy or to more closely couple electronics and biology. In the present study, we make use of ab initio molecular dynamics simulations, including the effect of applied electric fields, to gain atomistic insight into the intrinsic conductivity of chitosan-based polymers and demonstrate that chitosan does not act as a significant source of friction for the transport of protons while increasing the number of free ions.

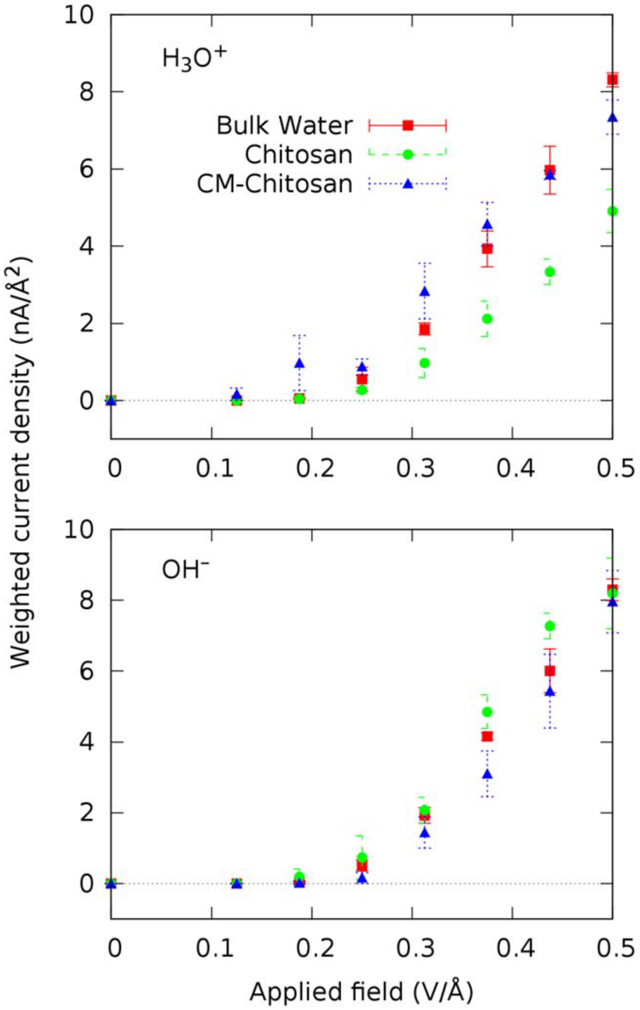

Figure 8. Current density as a function of applied electric field for proton (top) and hydroxide (bottom) conduction. The current densities were weighted by the inverse of the mass fraction of water in the simulation cell. The threshold for significant field-induced dissociation of water molecules is approximately 0.3 V/A˚.

3.8. Hydrated cable bacteria exhibit protonic conductivity over long distances [18]

This study presents the first direct measurement of proton transport along filamentous *Desulfobulbaceae*, or cable bacteria. Cable bacteria are multicellular microorganisms that grow in filamentous interconnected groups and have garnered much interest due to their ability to serve as electrical conduits, transporting electrons over several millimeters. Our results indicate that cable bacteria can also function as protonic conduits since they are able to transport protons at distances > 100 µm. We find that protonic conductivity (σ_P) along cable bacteria varies between samples and is measured as high as 8.1 ± 2.0 mS cm^{-1} at 25 °C and 70% relative humidity. For cable bacteria, the protonic conductance (G_P) and σ_P are dependent upon the relative humidity, increasing by as much as 30 fold between 60% and 80% humidity. This implies that proton transport occurs via the Grotthuss mechanism along water associated with cable bacteria, forming proton wires. In order to determine σ_P and G_P along cable bacteria, we implemented a new protocol using a modified transfer-printing technique to deposit either palladium interdigitated protodes or gold interdigitated electrodes on top of cable bacteria. Due to the relatively mild nature of the transfer-printing technique, this method should be applicable to a broad array of biological samples and curved materials. The observation of protonic conductivity in cable bacteria presents new possibilities to build biotic or biomimetic scaffolds to interface with materials via proton mediated gateways or channels.

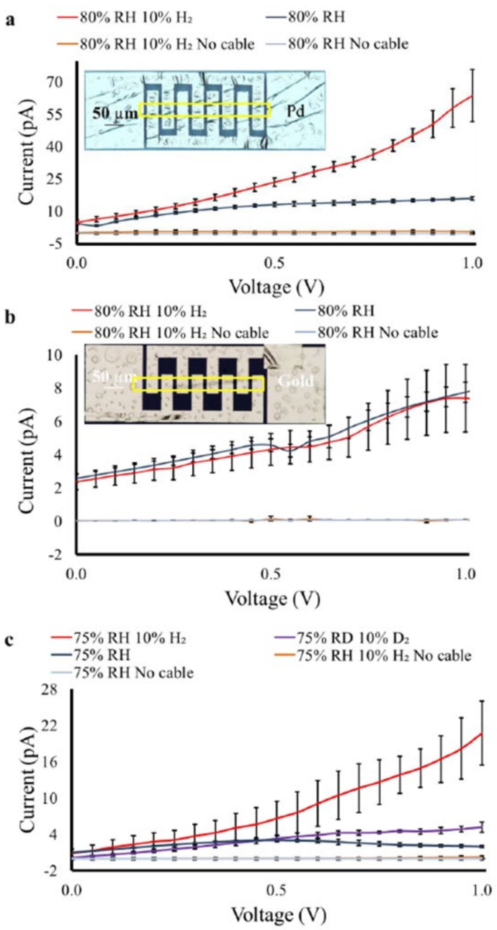

Figure 9. I-V curves of inter-digitated electrode (IDE) devices with and without cable bacteria and in the presence and absence of H_2 and D_2. a) I-V curve for a cable bacterium spanning six ~10 μm gaps on a Pd IDE. A picture of the IDE is inlaid with cable bacteria highlighted by the yellow box. b) I-V curve for a cable bacterium spanning six ~10 μm gaps on a Au IDE. A picture of the IDE is inlaid with cable bacteria highlighted by the yellow box. c) I-V curve for cable bacterium spanning nine ~10 μm gaps on a Pd IDE.

4. Conclusions

This program has built rigorous tools for pursuing the field of protonics and establish two-way communication between electronics and biology. The understanding of protonic device performance and the materials from which they are constructed is informing the design and construction of a more capable protonics testing devices. The added understanding for how to interface these devices with biology is leading to higher order experiments where neuron beds can be probed by releasing ions and observing the frequency of the firing rates. Protonics promises future devices that can release signals from neurotransmitters to food to antibiotics with the requisite bandwidth would be a game changer, enabling multiple new technologies to evolve:

- Advanced neural prostheses that use native signaling
- Wound healing—why is repairing tissue so much harder than the original growth
- Artificial organs—imprinting chemical information

- Artificial immune system—biosensors could trigger the release of the antidote before the immune system responds
- New biosurveillance paradigms where cells are interrogated and not just “listened” to

These types of advancements would advance the ONR framework goals of Operational Endurance, Warfighter Supremacy, and Force Health Protection.

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