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Submitted date: 17/06/2019 • Posted date: 18/06/2019

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Citation information: Li, Po-Nan; Herrmann, Jonathan; Wakatsuki, Soichi; van den Bedem, Henry (2019): Transport Properties of Nanoporous, Chemically Forced Biological Lattices. ChemRxiv. Preprint.

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Transport properties of nanoporous, chemically forced biological lattices.

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Abstract

Permselective nanochannels are ubiquitous in biological systems, controlling ion transport and maintaining a potential difference across a cell surface. Surface layers (S-layers) are proteinaceous, generally charged lattices punctuated with nano-scale pores that form the outermost cell envelope component of virtually all archaea and many bacteria. Ammonia Oxidizing Archaea (AOA) obtain their energy exclusively from oxidizing ammonia directly below the S-layer lattice, but how the charged surfaces and nanochannels affects availability of NH_4^+ at the reaction site is unknown. Here, we examine the electrochemical properties of negatively charged S-layers for asymmetrically forced ion transport governed by Michaelis-Menten kinetics at ultra-low concentrations. Our 3-dimensional electro-diffusion reaction simulations revealed that a negatively charged S-layer can invert the potential across the nanochannel to favor chemically forced NH_4^+ transport, analogous to polarity switching in nanofluidic field effect transistors. Polarity switching was not observed when only the interior of the nanochannels was charged. We found that S-layer charge, nanochannel geometry and enzymatic turnover rate are finely tuned to elevate NH_4^+ concentration at the active site, potentially enabling AOA to occupy nutrient-poor ecological niches. Strikingly, and in contrast to voltage-biased systems, magnitudes of the co- and counter-ion currents in the charged nanochannels were nearly equal and amplified disproportionately to the NH_4^+ current. Our simulations suggest that engineered arrays of crystalline proteinaceous membranes could find unique applications in industrial energy conversion or separation processes.

keywords nanochannels, chemically forced transport, surface layers, proteinaceous membrane

Virtually all living cells control electrolyte concentrations within their volume by selectively exchanging charged ion species with the environment through nano-scale ion channels and pumps.¹ These macromolecular nanochan-

nels enforce biochemical selectivity, can control ion transport direction across the lipid membrane and respond to external stimuli.

Engineering these signature properties of biological nanochannels into synthetic, nanofluidic

Figure 1: (A) Heavy-metal stained (1% uranyl acetate) transmission electron micrograph of an *Candidatus Nitrosoarchaeum limnia* strain SFB1 cell and its S-layer. (B) A side view of the AOA S-layer stained with 2% ammonium molybdate illustrates the geometry of S-layer proteins (SLPs). (C) Schematic of the molecular organization of the AOA cell-surface. SLPs are shown in blue. The membrane (grey) associated AMO trimer is shown in yellow, with the AMO active sites in orange. (D) Schematic of the cylindrical simulation domain together with the three ion species. An S-layer (blue) with a pore is placed in the center of the domain. Negative charges are uniformly distributed on the surface of the S-layer and in the nanochannel. A reaction site (ion sink) is placed at the center axis of the simulation domain, below the nanochannel. The potential difference across the nanochannel is measured between the active site, and a site equidistant from the S-layer in extracellular space. Shaded wedge indicates the actual simulation domain, after taking domain symmetries into account. Inset: a nanochannel of 1.3 nm diameter connects the upper reservoir and the pseudo-periplasmic space (PPS).

devices can have important industrial applications,^[2-5] for example in bioanalytics,^[6,7] battery design,^[8,9] or harvesting energy from salinity gradients.^[10] Transport properties of synthetic nanochannels are typically studied under electrical forcing, by an externally applied bias potential.^[11,12] In those scenarios, charged nanochannels can give rise to unusual non-linear electrokinetic effects, for example diode-like current rectification, current limiting or overlimiting behavior.^[13-15] However, despite the promise of nanofluidic devices, interactions of solutes with charged surfaces at the nanoscale remain poorly understood, both theoretically and experimentally.^[16] In particular, characterizing electrokinetic behavior in response to coupling to other driving forces, e.g., pressure-driven transport^[17] remains largely unexplored.

In biological systems, an enzymatic reaction which converts its substrate into product in nano-porous confinement is an example of chemically forced transport. Dissipation of concentration and charge by an ion sink acting on a solute induces an electro-chemical potential, forcing ions across the nano-channel. Ammonia Oxidizing Archaea (AOA)^[19,20] are among the most abundant and ubiquitous organisms in the biosphere,^[21,22] and play a major role in global nitrogen cycling. AOA are chemolithoautotrophs; their metabolism depends exclusively on ammonia oxidation by an ammonia monooxygenase (AMO) metallo-enzyme complex and a copper-based electron transport system.^[19,23] AOA have an extraordinarily high affinity for their substrate, with cultures of *Nitrosopumilus maritimus* strain SCM1 showing exponential growth at substrate con-

centrations (measured as $\text{NH}_3 + \text{NH}_4^+$) below 10 nM. The precise cellular location of the archaeal AMO active site is unknown, but the bacterial homologue indicates a location within the pseudo-periplasmic space (PPS), above the inner membrane and underneath a protein coat known as the surface layer (S-layer)^[23,24] (Figure 1(A-C)).

S-layers are crystalline lattices punctuated with nanoscale pores that form the outermost cell envelope component of virtually all archaea and many bacteria.^[24,26] Although functionally diverse, the S-layer structure acts as a particle sieve to protect the cell.^[27,28] Nano-scale lattices affect the diffusion behavior of low-concentration solutes, even if they are significantly smaller than the pore size.^[29] S-layer proteins are highly charged, and it is believed that a charged S-layer surface mitigates diffusion limitations imposed by a physical permeability barrier.^[18,30] We previously established that S-layer charges can elevate NH_4^+ concentration throughout PPS, using a 2-D electro-diffusion reaction simulation and a linear approximation to the Michaelis-Menten kinetics.^[30]

Here, we focus on transport characteristics of the S-layer nanochannels in response to an electro-chemical potential applied by an ion sink acting on a low-concentration cationic electrolyte in solution. Importantly, although the ionic solution is composed of three ion species, Na^+ , Cl^- , and NH_4^+ , the sink acts asymmetrically on the electrolytes; only one of the charged species, NH_4^+ , is reacted away. To distinguish such a system from traditional nanochannels under external voltage bias which affects all ion species, we will refer to it as an 'asymmetric, chemically forced nanochannel'. To the best of our knowledge, chemically forced nanochannels at steady state have not been studied in the literature before.

To determine the molecular organization of the cellular envelope of AOA for our simulations, we obtained transmission electron micrographs of *Candidatus Nitrosoarchaeum limnia* strain SFB1 cells (Figure 1(A,B)).^[31] We quantitatively modeled the cellular envelope of AOA with a 3-dimensional Poisson-Nernst-Planck (PNP) electro-diffusion reaction trans-

port model to probe how the environment, a nanoporous S-layer, AMO, its substrate, and co- and counter-ions interact to modulate electrolyte concentrations and transport properties (Figure 1(C,D), Methods).

Our system shares characteristics with a nanofluidic ion field-effect transistor (IFET).^[32,33] The S-layer surface charges govern nanochannel ionic selectivity and conductance, analogous to an IFET's gate electrode with potential V_g . The catalytic turnover rate of the ion sink modulates the 'source-to-drain' potential V_{ds} . Our non-equilibrium simulations suggest that a charged S-layer elevates NH_4^+ concentration at a theoretical pseudo-periplasmic active site at low bulk concentrations. Remarkably, and in contrast to a nanochannel in an external electrical field, the electro-chemical potentials are dominated by the S-layer charges leading to electrolyte gradients perpendicular to the surfaces. The S-layer charge density, nanochannel dimensions and enzymatic rate are finely tuned to elevate substrate availability at the active site at low bulk concentrations. Near neutral S-layer surface charge densities lead to an NH_4^+ diffusion barrier in the nanochannel. However, at sufficiently negative charge densities, chemical forcing of the electrolyte generates a favorable potential difference across the nanochannel.

These simulations can help us understand how the molecular organization of the AOA cell envelope contributes to concentrating the microbe's sole energy source at the ammonia-oxidation site of the cell, enabling them to occupy ecological niches. Spatially resolved images of the microbial components together with mesoscale molecular dynamics simulations can provide important additional details on specific molecular interactions, towards a structural systems biology understanding of these ubiquitous and important organisms, and eventual rational design and bioengineering synthetic systems.

Results and Discussion

We imaged *Nitrosoarchaeum limnia* cells^[31] stained with either 1% uranyl acetate or 2% ammonium molybdate using transmission electron microscopy (Figure 1(A,B)).^[34] The images revealed a crystalline outer cell envelope of S-layer proteins (SLPs) organized in hexagonal patterns, characteristic of similar archaeal cells.^{[25][35]} Each hexagonal unit measures 22 nm in diameter, and connects extracellular space to PPS with nano-scale channels Figure 1(C)).^[30] The distance from the membrane to the center of the S-layer is 20nm (Figure 1(D)). Thermodynamic characterization of the S-layer of *Nitrosopumilus maritimus*^[18] and the measured zeta potential of *N. Limnia*^[30] revealed that cellular envelopes of AOA are highly charged. Ion exchange between extracellular space and PPS takes place through the charged nano-channel.

We capitalized on the symmetries of the system to formulate a pseudo three-dimensional simulation. We approximate the hexagonal cell

Figure 2: Concentration profile of NH_4^+ at steady state (A) with a neutral S-layer, (B) with a negatively charged S-layer. Surface charges on the S-layer lead to the formation of an EDL ((B), dark red) and create a concentration gradient near the surface layer. Note that the EDLs in the nano-channel overlap. The white circle marks the location of the ion sink. Bulk concentrations equal $c_{i=\text{NH}_4^+}^{\text{bulk}} = 10 \text{ nM}$, $c_{i=\text{Cl}^-}^{\text{bulk}} = 0.14 \text{ M}$, and $c_{i=\text{Na}^+}^{\text{bulk}} = (0.14 - 10^{-8}) \text{ M}$. The S-layer surface charge density is $\rho = 0.02 e \text{ \AA}^{-2}$. (C) NH_4^+ , Cl^- and Na^+ ion concentrations across the nanochannel. Gray: with a neutral S-layer; orange, blue, red: with a negatively charged S-layer.

with a cylindrical simulation domain 11 nm in radius and 40 nm in height (Figure 1(D)). A 4.5 nm thick, impenetrable S-layer is located at the center ($z = 20 \text{ nm}$) of the simulation domain, with a nanochannel centered at the z -axis. The diameter of the nanochannel is set to 1.3 nm,^[30] unless stated otherwise. The bulk ion concentrations c_i^{bulk} in our simulations match those of the marine habitat of AOA (Table 2): $c_{i=\text{NH}_4^+}^{\text{bulk}} = 10 \text{ nM}$, $c_{i=\text{Cl}^-}^{\text{bulk}} = 0.14 \text{ M}$, and $c_{i=\text{Na}^+}^{\text{bulk}} = (0.14 - 10^{-8}) \text{ M}$ to maintain bulk electro-neutrality. The S-layer surface charge density equals $\rho = 0.02 e \text{ \AA}^{-2}$.

S-layer surface charge elevates steady-state NH_4^+ concentrations in pseudo-periplasmic space

The S-layer surface charges lead to the formation of an electric double layer (EDL), which extends approximately 0.8 nm on both sides of the S-layer (Figure 2(A,B)).

At steady state, NH_4^+ and Na^+ concentrations were elevated by an order of magnitude near the S-layer, while the Cl^- concentration is similarly reduced compared to bulk (Figure 2(C)). Consequently, at the boundaries of the EDL, above and below the S-layer, we observe a strong ion concentration gradient (Figure 2(C)). In the nanochannel, cation concentrations were enriched compared to the neutral channel ($c_{i=\text{Na}^+}^{\text{bulk}} = 2.46 \text{ M}$ vs. 0.14 M), while the anion concentration was sharply reduced ($c_{i=\text{Cl}^-}^{\text{bulk}} = 0.008 \text{ M}$ vs. 0.14 M).

The active site itself is positioned too far from the S-layer to experience the sharp concentration gains of the EDL (Figure 2(B)). However, at steady state the NH_4^+ concentration in pseudo-periplasmic space was elevated nearly uniformly by 29.4% for the charged S-layer compared to the neutral S-layer, in reasonable agreement with our previous 2-D simulation and linear kinetics.^[30]

Figure 3: Steady state electro-chemical potential for (A) neutral and (B) charged nanochannel. In the neutral case, the electro-chemical potential of NH_4^+ and co- and counter-ions shows regularly spaced equipotential surfaces, perpendicular to the pore walls. In the charged case, the electrical and chemical potentials are parallel to the pore walls, in stark contrast to a nanochannel under external voltage bias.

Electro-chemical potentials across the nanochannel

At steady state in the neutral case, the direction of the NH_4^+ electro-chemical gradient $\nabla E_{ec,\text{NH}_4^+}$ across the pore was towards PPS (Figure 3). The pore was mildly resistive for NH_4^+ , indicated by an electrical potential that was $31.5\mu\text{V}$ higher in PPS than in extracellular space, i.e., a weak electric field $\mathbf{E}$ was oriented outward through the nanochannel (Methods). E_{ec,NH_4^+} was dominated by its chemical component, which was eight orders of magnitude larger than the electrical component. Na^+ and Cl^- presumably responded to the NH_4^+ -driven charge redistribution, indicated by chemical potentials that compensated for NH_4^+ concentrations. For the Na^+ and Cl^- ion species, the electro-chemical potential was eight orders of magnitude smaller than E_{ec,NH_4^+} . Their chemical potential was of the same order as the electrical potential, reflecting the difference in bulk concentration between NH_4^+ and the co- and counter-ion. Cl^- experienced a marginally conductive nanochannel, while Na^+ was marginally resistive. The electrical potentials in the nanochannel were highly symmetric across the three ion species.

In the charged case, the system entered a dramatically different regime. Strikingly, the polarity of the nanochannel inverted with a

charged ($\rho = 0.02 \text{ e}^- \text{ \AA}^{-2}$) S-layer. The electrical potential was $95.2\mu\text{V}$ higher in extracellular space than in the PPS: $\mathbf{E}$ was oriented inward. Unlike the neutral case, or the case with an externally applied voltage bias acting symmetrically on ion species,⁴⁴ electro-chemical equipotential surfaces were no longer perpendicular to the nano-pore surface (Figure 3(B)). Instead, electric and chemical components of the ion potentials were dominated by the surface charges leading to electro-chemical potentials parallel to the nano-pore surfaces. The electrical and chemical components of the potentials were on the same order of magnitude for all ion species. In the neutral case E_{ec,Na^+} and E_{ec,Cl^-} responded to NH_4^+ -driven charge redistribution, but in the charged case E_{ec,Na^+} was indistinguishable from E_{ec,NH_4^+} while E_{ec,Cl^-} was inverted from E_{ec,NH_4^+} .

Ion currents

The lack of a strong inward directed electro-chemical gradient across the pore (Figure 3B) raises the question how a charged S-layer modulates influx of NH_4^+ into PPS. In the neutral case, we observed a 'funnel'-like NH_4^+ ion flux near the entrance and exit of the nanochannel (Figure 4(A) top left panel). In the charged case, our simulations indicated strong NH_4^+ cur-

Figure 4: (A) Current magnitude and direction of the ion species with a neutral (upper panel) and charged (lower panel) S-layer with surface charge density $0.02 \text{ e}^- \text{ \AA}^{-2}$. The saturation of color indicates the magnitude of the ion current and the hue indicates the direction of the ion current. Scale of magnitude in charged Cl^- and Na^+ (lower middle and right) is 20,000 times greater than in other panels. (B) Current profiles for Na^+ , Cl^- , and NH_4^+ along the central axis in a neutral (upper) and charged (lower) setting.

rents in the EDL parallel to the S-layer and, surprisingly, parallel to its electrical and chemical equipotential surfaces (Figure 4(A) bottom left panel). NH_4^+ currents flowed towards the nanochannel entrance along the top (extracellular) surface of the S-layer, and away from the nanochannel exit along the bottom (enzyme facing) surface. In the nanochannel, NH_4^+ currents were directed also along the surface. This suggests that the charged S-layer acts as a NH_4^+ reservoir. At the enzyme facing side of the S-layer, NH_4^+ ions diffuse out of the EDL towards the active site (Figure S2). These ions are replaced by transport along the slipping plane at the extracellular and nanochannel surface of the S-layer (Figure 4(A)).

In both the neutral and charged cases co-

and counter-ions revealed no significant current along the top or bottom S-layer surfaces (Figure 2A).

In the neutral case, the current carried by NH_4^+ was $I_{\text{NH}_4^+} = 8.31 \times 10^{-20} \text{ A}$ (Figure 4(B) top panel; Table 1). Co- and counter-ions had near equal current magnitude Figure 4(B) top panel), jointly compensating for $I_{\text{NH}_4^+}$: $I_{\text{Na}^+ + \text{Cl}^-} = -8.31 \times 10^{-20} \text{ A}$. In the charged case, the NH_4^+ current increased by 28% to $I_{\text{NH}_4^+} = 1.06 \times 10^{-19} \text{ A}$ Figure 4(B) top panel). Strikingly, while Na^+ and Cl^- currents still neutralized the total current ($I_{\text{Na}^+ + \text{Cl}^-} = -1.06 \times 10^{-19} \text{ A}$), each was amplified by four orders of magnitude compared to the neutral case (Table 1). The current of Cl^- had reversed sign. Even more surprising, co- and counter-ion cur-

Table 1: Current, potential and conductance data in a neutral and charged nanochannel with surface charge density $0.02e^-/\text{\AA}^2$. The potential is measured as depicted in Figure 1(B)

Neutral			
Species	NH_4^+	Cl^-	Na^+
Current	-8.31E-20 A	4.41E-20 A	3.89E-20 A
Potential	-3.15E-05 V		
Conductance	-2.63E-15 S	1.40E-15 S	1.23E-15 S
Charged			
Species	NH_4^+	Cl^-	Na^+
Current	1.06E-19 A	1.40E-15 A	-1.40E-15 A
Potential	9.52E-05 V		
Conductance	1.11E-15 S	1.47E-11 S	-1.47E-11 S

rents did not reflect cation selectivity despite a concentration difference between Na^+ and Cl^- in the nanochannel exceeding two orders of magnitude (Figure 2(B)). Instead, co- and counter-ion current magnitudes were near equal ((Figure 4(B) bottom panel). By contrast, in external-voltage biased nanochannels with negative surface charges, anionic (Cl^-) currents are reduced with respect to Na^+ owing to lower concentrations in the nanochannel. We postulate that the current response in a chemically forced nanochannel is limited by the least conductive ion species. Thus, an NH_4^+ -driven potential difference solicits a current response in Na^+ and Cl^- which is symmetric to enforce electro-neutrality but limited by the ability of the Cl^- ion concentration to carry current.

The simulations indicated a sharp increase in co- and counter-ion currents with a charged S-layer compared to a neutral S-layer (Table 1). In both cases $I_{\text{Na}^+ + \text{Cl}^-}$ compensated $I_{\text{NH}_4^+}$, but it remains unclear why I_{Na^+} and I_{Cl^-} in the charged case are amplified by four orders of magnitude compared to the neutral case. We did not detect co- and counter-ion current amplification in our 2D simulation.³⁰ To examine the origin of current amplification, we approximated the 2D simulation by omitting the $J_{r,i}/r$ term from Equation 11 in our 3D implementation. Simulations with this model reduced the co- and counter-ion current magnitudes comparable to those observed with the 2D model (Table S1), suggesting that current amplification is due to a 'concentration-focusing' effect

at the radial axis of the nanochannel in the 3D model.

Catalytic turnover rate and surface charges finely tuned to maximize gain

The low NH_4^+ bulk concentration suggests that the reaction rate will be diffusion limited. To identify a range of operational reaction rates, we determined how NH_4^+ concentrations at the active site depend on the catalytic turnover rate k_c and the surface charge density of the S-layer (Figure 5(A)). For all surface charges, including neutral, the concentration depended non-linearly on the value of k_c . Unsurprisingly, increasing k_c significantly lowered the steady-state concentration at the active site. For a fixed value of k_c , higher surface charge densities partially compensated for the faster NH_4^+ turnover rate, maintaining a higher concentration at the active site. However, we detected no further gain when surface charge densities exceeded $0.05e^-/\text{\AA}^2$ (Figure 5(A,B)). The enzymatic rate reached a limiting point at $k_c \sim 65s^{-1}$, when ions near the active site were depleted completely independent of surface charge. Thus, the NH_4^+ turnover rate at the active site and S-layer charges are delicately balanced, and together define a narrow operating regime.

Figure 5: (A) Steady state NH_4^+ concentrations as a function of reaction constant k_c . Data points of different colors represent different surface charge density. (B) Concentration gain of NH_4^+ at the active site for different values of the S-layer surface charge. The gain sharply increased near $k_c \sim 65 \text{ s}^{-1}$, where NH_4^+ at the active site was depleted with a neutral S-layer, but maintained a finite concentration with a charged S-layer.

Current-voltage characteristics of a chemically forced nanochannel

To determine the current-voltage characteristics of a chemically forced system, we measured the potential difference across the S-layer and the ion current in the nanochannel over a range of catalytic turnover rates k_c and surface charge densities.

We detected a strong non-linear current-voltage response to variations in surface charge densities (Figure 6A). At fixed turnover rates, we observed a shift towards more negative potential differences across the channel and a concomitant gain in current as the surface charge densities increased from neutral up to $\sim 0.005 \text{ e}^- \text{\AA}^{-2}$. In other words, NH_4^+ overcomes a larger (negative) potential difference across the nanochannel but can maintain a higher current, manifested as decreasing (more negative) cationic conductivity (Figure 6B).

After reaching a critical surface charge density between 0.002 and $0.005 \text{ e}^- \text{\AA}^{-2}$ depending on turnover rate (Figure 6A,B; dashed yellow line at $0.005 \text{ e}^- \text{\AA}^{-2}$), further increasing surface charge densities reduced the potential difference that NH_4^+ needs to overcome across the nanochannel. We again observed a simultaneous gain in current, albeit generally

smaller than that in the regime before the critical surface charge density. Thus, a substantially smaller adverse potential difference with a small gain in current suggests the nanochannel is turning more conductive for cations in this regime. Eventually, the potential (and conductance) across the nanochannel turned positive for all surface charge densities, except at the highest turnover rates. The marginal gain in current diminishes rapidly beyond $0.01 \text{ e}^- \text{\AA}^{-2}$ surface charge density, until no further gain is observed at $0.05 \text{ e}^- \text{\AA}^{-2}$ (cf. Figure 5(A,B)). We observed peak conductance at $\rho = 0.05 \text{ e}^- \text{\AA}^{-2}$ and $k_c = 30 \text{ s}^{-1}$ (Figure 6B). The turning point in conductance with respect to surface charge densities is reached at the cusp of the curves in Figure 6A. The surface layer requires a turnover rate-dependent minimum negative surface charge density to enter a favorable cationic conductance regime (Figure 6B).

Simultaneously, we observed a shift towards unfavorable current-voltage characteristics at higher turnover rates independent of surface charge densities (Figure 6A). For example, at a rate of 10 s^{-1} , the potential across the nanochannel turned positive, i.e. favorable for cations, for surface charge densities between 0.005 and $0.01 \text{ e}^- \text{\AA}^{-2}$. However, for turnover

Figure 6: Current-voltage characteristics. (A) Each line corresponds to the potential difference across the S-layer and the NH_4^+ current response in the nanochannel for a fixed k_c at different surface charge densities. From left to right, circles indicate surface charge densities at neutral, 0.001, 0.002, 0.005, 0.01, 0.02, 0.03, 0.04 and $0.05 e^-/\text{\AA}^2$. The polarity across the nanochannel inverts at 0V. Positive values correspond to a favorable potential (conductive) across the pore for NH_4^+ . The slope of the lines is a measure of conductivity, as explained in the text. For a fixed surface charge, the reaction rate k_c modulates the current response (dotted lines) (B) Conductance $G = \text{sgn}(V)I/V$ of the data points in panel (A) as a function of k_c , color-coded by surface charge density. Yellow and pink dotted lines in panel (A) correspond to 0.005 and $0.05 e^-/\text{\AA}^2$ in panel (B).

rates $50 s^{-1}$ the system operated in a favorable potential difference only for surface charges densities $0.03 e^-/\text{\AA}^2$ and higher. These results again indicate finely balanced turnover rates and surface charge densities.

These observations suggest that weak S-layer surface charge densities enrich NH_4^+ concentrations in the channel insufficiently to support chemical forcing of NH_4^+ at high turnover rates. The effect of S-layer charge is similar to the effect of the gating potential in nanofluidic field effect transistors, which controls cation depletion or enrichment.^[36] In conventional, voltage biased nanofluidic systems an increase in voltage bias will lead to ion depletion on the anodic side of the nanochannel, resulting in a non-ohmic response.^{[13][15][37][38]} By contrast, for a chemically forced nanochannel, increasing the reaction rate k_c limits the channel conductance, leading to ion depletion at the ion sink (cathode) before current limiting behavior can be observed.

Redistributed electrolyte charge densities drive concentration gain

Next, to understand how a nanoporous S-layer strikes a balance between diffusion limitations imposed by a physical barrier and particle siev-

ing, we examined the effect of nanochannel diameter on NH_4^+ concentrations at the active site and nanochannel currents.

For all bulk concentrations, we observed a non-linear dependence on the nanochannel radius (Figure 7). Unsurprisingly, the gain was highest for small diameters, but decreased rapidly for larger diameters.

The concentration gain at the active site was sharply reduced at higher NH_4^+ bulk concentrations, independent of the nanochannel diameter. The gain was highest at a NH_4^+ bulk concentration of 1 nM, and became negligibly small at bulk concentrations exceeding 1 μM . The small marginal gain between 10 and 1 nM (Figure 7(A)) suggests that the maximum gain was limited by a minimum NH_4^+ concentration required at the active site to maintain the reaction. Likewise, at 10 μM bulk concentration, the active site turnover rate was well below the diffusion-limited regime even with a neutral S-layer, explaining the low total gain.

The remarkable ability of S-layer charges to elevate NH_4^+ concentrations at the active site is exemplified by comparing ion currents through the nanochannel. For the smallest nanochannel diameter, we observed NH_4^+ currents up to two orders of magnitude larger with a charged S-layer compared to a neutral S-layer (Fig-

Figure 7: (A) Gain of NH_4^+ concentration at the active site for different pore diameters and bulk concentration of NH_4^+ . The gain is the ratio of concentrations between surface charge $0.02 e^- \text{Å}^{-2}$ and neutral. (B) Current magnitudes in the nanochannel for each ion species as a function of diameter (NH_4^+ bulk concentration equals 10 nM). Solid lines: surface charge $0.02 e^- \text{Å}^{-2}$. Dash-dot lines: neutral S-layer.

ure 7(B)). The differences were smaller towards larger diameter. Notably, NH_4^+ currents remained in a narrow band as the nanochannel diameter increased, suggesting near perfect compensation by S-layer charges for reduced NH_4^+ nanochannel diffusion to enable particle sieving.

The magnitude of the co- and counter-ion currents, i.e., Na^+ and Cl^- , were amplified by four orders of magnitude (Figure 7(B)) in the case of a charged S-layer. In the neutral case, co- and counterion currents had magnitudes smaller than NH_4^+ currents. In the charged case, the co- and counterion currents were many orders of magnitude larger due to their elevated concentration and thus larger magnitude of flux. Furthermore, the co- and counter-ion currents increased by more than two orders of magnitude as the channel diameter increased from 1 to 15 Å. We reason that the nanochannel becomes less cation-selective, elevating the Cl^- concentration and current in the nanochannel. The Na^+ current reacts accordingly to neutralize the nanochannel.

To tease apart the roles of horizontal and vertical surface charges, we examined NH_4^+ fluxes in the nanochannel and active site concentrations for a neutral S-layer with a charged nanochannel and a charged S-layer with a neutral nanochannel (Figure 8(A)). Our simulations indicated that both surface only or channel only charges elevated the NH_4^+ concentrations at the active site. The contribution of surface only charges to elevating active site

concentrations was stronger than that of channel only charges (Figure 8(A,B)). Remarkably, nanochannel charges slightly countered the effect of surface charges on the NH_4^+ fluxes in the channel (Figure 8(A,B)). Importantly, the electric field inverted ($\mathbf{E}$ directed towards PPS) in the case of surface only charges, but not in the case of nanochannel only charges. These results suggest that the fixed surface charges redistribute the charge densities of the electrolyte to create a favorable electric field through the channel.

Conclusion

We analyzed transport properties of NH_4^+ ions across a nanoporous lattice under chemical forcing. The NH_4^+ ion charge and concentration dissipation at the active site does not directly act on the co- and counter-ions Na^+ and Cl^- . Instead, NH_4^+ ion dissipation promotes a charge redistribution in the simulation domain, which, in turn, solicits a redistribution of the co- and counter-ions Na^+ and Cl^- . We observed a starkly different transport regime with a charged S-layer compared to a neutral S-layer or compared to a voltage biased nanochannel. The charged S-layer and nanochannel showed electrochemical characteristics similar to those of a nanofluidic ion field effect transistor. Our simulations revealed that the negative charges on the S-layer can invert the polarity across

Figure 8: (A) NH_4^+ concentration at the active site with different setting of nanochannel diameter and charge configuration. Inset: gain, defined as active site NH_4^+ concentration of the charged case over the neutral case, as a function of pore diameter. (B) NH_4^+ flux across the nanochannel with different diameter and charge configurations. Inset: percentage gain, defined as the flux in the charged case over the neutral case, as a function of channel diameter.

the S-layer to favor NH_4^+ transport, amplify the counter-ions Na^+ and Cl^- currents by several orders of magnitude, and significantly elevate the NH_4^+ concentration at an underlying enzyme active site to up to 7-fold compared with a neutral one.

Higher enzymatic turnover rates required stronger negative surface charges to enter the regime with potential favorable for NH_4^+ . While the current carried by NH_4^+ increased concomitantly, ultimately turnover at the ion sink was diffusion limited at $k_c \sim 65\text{s}^{-1}$ independent of surface charge. At the same time, for surface charge densities we measured in the laboratory, near $0.02\text{e}\text{\AA}^{-2}$, our simulations found that conductance across the pore reached a maximum at $k_c \sim 20\text{s}^{-1}$, remarkably close to the measured turnover ($\sim 10\text{s}^{-1}$).

Our simulations necessarily simplified the geometry of the AOA molecular envelope and its components. The role of the fluid flow field was also ignored.^[45] Treating ions as point masses in the PNP formalism can artefactually increase ion concentrations near highly charged surfaces. Finite element numerical simulations could account for finite ion size,^[46] and allow more complex geometries of the simulation domain than our finite difference approach. While the precise atomic structure of the AOA S-layer and nanochannels remain unknown, those of other organisms suggest the pores are conically shaped.^[25,26] Geometric asymmetries^[48] and non-

uniform distribution of surface charge in the nanochannel^[14] can lead to distinctive electrokinetic effects like current rectification, which we did not detect in our simulations. Atomically detailed molecular dynamics simulations^[47] will be required to fully reveal the mechanisms and electrokinetics of chemically forced ion transport across nanoporous bio-lattices. Such insights can help design and engineer synthetic nanotechnology.

Methods

Poisson-Nernst-Planck equations

We modeled ion transport using Poisson-Nernst-Planck mean-field continuum theory. Ion transport in narrow nanochannels with diameters in the low (1-5) nm range is dominated by electro-phoresis,^[14] and we omitted the contribution of fluid flow.^[39] We replaced the Poisson equation by a displacement current equation.^[40,41] At time t , the electric field $\mathbf{E}$ at $\mathbf{r}$ is governed by

$$\frac{\partial \mathbf{E}}{\partial t}(\mathbf{r}, t) = - \sum_{i=1}^m \frac{Fz_i}{\epsilon} \mathbf{J}_i(\mathbf{r}, t), \quad (1)$$

Table 2: Definitions of constants and values

variable	value	variable	value
$c_{i=\text{NH}_4^+}^{\text{bulk}}$	10 nM	$c_{i=\text{Cl}^-}^{\text{bulk}}$	0.14M
$c_{i=\text{Na}^+}^{\text{bulk}}$	$0.14 - 10^{-8}$ M	N_A	6×10^{23}
$z_{\text{NH}_4^+}$	1	e	1.6×10^{-19}
z_{Cl^-}	-1	F	$N_A e$
z_{Na^+}	1	R	8.314 J/mole
$D_{\text{NH}_4^+}$	1.64 m ² /s	T	300 K
D_{Cl^-}	2.03 m ² /s	$\epsilon_{\text{solvent}}$	80 ϵ_0
D_{Na^+}	1.33 m ² /s	$\epsilon_{\text{S-layer}}$	8 ϵ_0

where index i denotes the ion species. The ion flux is governed by

$$\mathbf{J}_i(\mathbf{r}, t) = -D_i \nabla c_i(\mathbf{r}, t) + \frac{F}{RT} D_i z_i c_i(\mathbf{r}, t) \mathbf{E}(\mathbf{r}, t), \quad (2)$$

and finally the concentration is governed by

$$\frac{\partial c_i}{\partial t}(\mathbf{r}, t) = -\nabla \cdot \mathbf{J}_i(\mathbf{r}, t). \quad (3)$$

Equations (1) and (3) are known as the Poisson-Nernst-Planck model for electrodiffusion. Table 2 details the values used in this work.

Reaction term

We modeled the enzymatic reaction with Michaelis-Menten kinetics. We first define a pseudo-first-order rate constant^[42]

$$k = k_c c_\Sigma / K_M, \quad (4)$$

where k_c is the reaction (turnover) rate, c_Σ is the effective concentration in the voxel that corresponds to the active site, defined as

$$c_\Sigma = \frac{1}{N_A \pi (0.5 \Delta r)^2 \Delta z} \quad (5)$$

and K_M is the Michaelis constant. Table 3 summarizes the values of the constants. The reaction term is defined by

$$R_i(\mathbf{r}, t) = \begin{cases} \frac{k c_i(\mathbf{r}, t)}{1 + \frac{c_i(\mathbf{r}, t)}{K_M}} \delta(\mathbf{r}_{\text{rec}}), & i = \text{NH}_4^+ \\ 0, & \text{otherwise} \end{cases} \quad (6)$$

where $c_{i=\text{NH}_4^+}$ is the concentration of the substrate, $\delta(\mathbf{r})$ is a delta function of space and $\mathbf{r}_{\text{rec}}$ is the position of the active site in cylindrical coordinates. Adding the reaction term to Equation (3), we obtain

$$\frac{\partial c_i}{\partial t}(\mathbf{r}, t) = -\nabla \cdot \mathbf{J}_i(\mathbf{r}, t) - R_i(\mathbf{r}, t). \quad (7)$$

Note that the reaction terms acts on NH_4^+ only.

3D numerical implementation in cylindrical coordinates

Space discretization We discretized the simulation domain using a cylindrical coordinate system with grid cell dimensions in r and z equal to $\Delta r = \Delta z = 0.1$ nm. These dimensions are nearly an order of magnitude smaller than the Debye length, ensuring that boundary effects near the charged surface are adequately resolved. We don't explicitly define the angular grid size $\Delta\phi$ as the system is rotationally symmetric. Owing to the symmetry of the model, we only simulated a wedge of the cylindrical simulation domain (Figure 1(D)). We placed the active site, i.e., the NH_4^+ ion sink defined in (7) at position ($r = 0, z = 30$ nm).

Update equations The PNP model in cylindrical coordinates is given by Equations (1), (2) and

Table 3: Definitions of variables and default values

Variable	definition	value	unit	ref.
k_c	catalytic rate	10	1/s	30
c_Σ	effective concentration at sink	2.12×10^6	M	
K_M	Michaelis constant	133	nM	43

$$\begin{aligned}
 \frac{\partial c_i}{\partial t}(r, \phi, z, t) = & \\
 & - \left[\frac{1}{r} \frac{\partial (r J_{r,i})}{\partial r}(r, \phi, z, t) \right. \\
 & + \frac{1}{r} \frac{\partial J_{\phi,i}}{\partial \phi}(r, \phi, z, t) \\
 & \left. + \frac{\partial J_{z,i}}{\partial z}(r, \phi, z, t) \right] - R_i(r, \phi, z, t).
 \end{aligned} \tag{8}$$

The angular components $E_{\phi,i}$ and $J_{\phi,i}$ vanish everywhere as the system is rotationally symmetric. The discretized equations are given by:

$$\begin{aligned}
 E_{r,i}(u + \frac{1}{2}, v, w)^{(t+1)} = & \\
 E_{r,i}(u + \frac{1}{2}, v, w)^{(t)} - \Delta t \sum_{i=1}^m \frac{F z_i}{\epsilon} J_{r,i}(u + \frac{1}{2}, v, w)^{(t)} & \\
 E_{z,i}(u, v, w - \frac{1}{2})^{(t+1)} = & \\
 E_{z,i}(u, v, w - \frac{1}{2})^{(t)} - \Delta t \sum_{i=1}^m \frac{F z_i}{\epsilon} J_{z,i}(u, v, w - \frac{1}{2})^{(t)}, &
 \end{aligned} \tag{9}$$

$$\begin{aligned}
 J_{r,i}(u + \frac{1}{2}, v, w)^{(t+1)} = & \\
 - D_i \frac{\partial c_i^{(t)}}{\partial r} + \frac{F}{RT} D_i z_i c_i^{(t)} E_{r,i}(u + \frac{1}{2}, v, w)^{(t+1)} & \\
 J_{z,i}(u, v, w - \frac{1}{2})^{(t+1)} = & \\
 - D_i \frac{\partial c_i^{(t)}}{\partial z} + \frac{F}{RT} D_i z_i c_i^{(t)} E_{z,i}(u, v, w - \frac{1}{2})^{(t+1)}, &
 \end{aligned} \tag{10}$$

and

$$\begin{aligned}
 c_i(u, v, w)^{(t+1)} = c_i(u, v, w)^{(t)} - & \\
 \Delta t \left[\frac{\partial}{\partial r} J_{r,i}^{(t+1)} + \frac{1}{r} J_{r,i}^{(t+1)} + \frac{\partial}{\partial z} J_{z,i}^{(t+1)} + R_i(u, v, w)^{(t)} \right]. &
 \end{aligned} \tag{11}$$

Furthermore, $\lim_{r \downarrow 0} 1/r = \infty$ and by continuity of flux at $r = 0$, $\lim_{r \downarrow 0} J_{r,i} = 0$. By l'Hopital's

rule $\lim_{r \rightarrow 0} \frac{1}{r} J_{r,i} = \frac{\partial J_{r,i}}{\partial r}$, and therefore a modified update equation at $r = 0$ reads

$$\begin{aligned}
 c_i(0, v, w)^{(t+1)} = c_i(0, v, w)^{(t)} - & \\
 \Delta t \left[2 \frac{\partial}{\partial r} J_{r,i}^{(t+1)} + \frac{\partial}{\partial z} J_{z,i}^{(t+1)} + R_i(0, v, w)^{(t)} \right]. &
 \end{aligned} \tag{12}$$

The concentration profiles are defined at grid points (u, v, w) whereas the $J_{r,i}$ are at the middle of two consecutive u points, namely $(u + \frac{1}{2}, v, w)$ and $J_{z,i}$'s are located at $(u, v, w - \frac{1}{2})$.

Boundary and initial conditions Starting profiles for the concentrations and electric field were calculated analytically from equilibrium solutions to the Poisson-Boltzmann equations in the absence of a nanochannel.[30](#) We assumed no current or ion flux at $t = 0$. We applied a diffusive boundary condition (BC) to the top domain boundary ($z = 0$) to allow ion influx or efflux from the simulation domain, i.e. the flux at the top domain boundary is determined by

$$J_{z,i}(u, v, -\frac{1}{2}) = -\frac{\Delta t}{\Delta z} D_i [c_i(u, v, 0) - c_{i,\text{bulk}}] \tag{13}$$

and Neumann BCs of $\partial c / \partial u = 0$, i.e., 'wall' conditions, to the bottom domain ($z = 40$ nm) and the S-layer boundaries to prevent diffusion. The remaining boundaries are periodic.

We calculated the Debye length $l_D = \kappa^{-1}$ and the Gouy-Chapman length l_{GC} at equilibrium given a surface charge density $\rho = 0.02 \text{ e}^- \text{ \AA}^{-2}$ and a bulk NaCl concentration $c_{\text{NaCl}\pm, \text{bulk}}$ of 0.14 M as $l_D = [\epsilon k_B T / (8 \pi e^2 n_0)]^{1/2} = 8.15 \text{ \AA}$, and $l_{GC} = \epsilon T / (2 \pi e |\sigma|) = 1.14 \text{ \AA}$, where k_B denotes the Boltzmann constant, and c_0 is the bulk concentration in moles per m^3 .

Simulations

The numerical model was evolved in time by a Poisson-Nernst-Planck equation solver written

Figure 9: (A) NH_4^+ concentration at the active site vs. simulation time. Time step size is 1×10^{-12} s. (B) Current of each ion in the nanochannel vs. simulation time in logarithmic scale. Dash-dot (solid) lines represent neutral (charged) S-layer. The reaction is turned on at $t = 10 \mu\text{s}$.

in C++. Each simulation was equilibrated for $10 \mu\text{s}$ with the reaction term switched off. We monitored ion concentrations at the sink and ion fluxes over the nanochannel to ensure the system had reached equilibrium (Figure 9). We then switched on the reaction term, and simulated the system for another $80 \mu\text{s}$. The time step size $\Delta t = 1 \times 10^{-12}$ s was selected to ensure numerical stability while maximizing simulation speed.

Numerical convergence

To determine if the simulations had converged to steady state, we monitored the concentration of NH_4^+ at the active site in the simulation with a neutral S-layer and a charged S-layer. Simulations with a charged S-layer converged within $5 \mu\text{s}$ after switching on the reaction (Figure 9(A)). By contrast, the neutral S-layer simulations reached a steady state only $50 \mu\text{s}$ after switching on the reaction. Slow convergence in the presence of an uncharged membrane is generally attributed to slow co-diffusion of oppositely charged ion species.⁴⁴

Electrical properties

We calculated potentials across the pore by integrating $\mathbf{E}_z$ with respect to z across the pore from the upper reservoir to the sink ($z = [10.0, 30.0]$ nm) and averaging over the area of the pore ($r = [0, 7]$ nm). The chemi-

cal potential for ion species i is calculated as $E_{c,i} = k_B T \ln(c_i/c_{Bi})$, where c_{Bi} is the bulk concentration of species i ; the electrical potential is calculated as $E_{e,i} = z_i e V$. The electrical current for ion species i is calculated by $I_i = 2z_i N_A e \Delta r \pi \sum_r (J_{i,\text{avg}}(r, z) r_i)$, where $J_{i,\text{avg}}(r, z)$ is obtained by averaging $J_i(r, z)$ over the depth of the pore ($[17.8, 22.4]$ nm) and r_i is the distance of the i -th voxel from the $r = 0$ axis. The sign of $G = I/V$ follows the convention that flux of negative charge toward positive potential V is positive conductance.

Acknowledgements

JH was supported in part by the National Science Foundation Graduate Research Fellowship Program (NSF-GRFP) and the US Department of Energy Office of Science Graduate Student Research Program (DOE-SCGSR). HvdB is supported by NIH GM123159. The authors gratefully acknowledge Christopher A. Francis and Bradley B. Tolar for providing *N. limnia* strain SFB1 cells, and A. Majumdar for stimulating discussions. Simulations were partly performed at the Stanford Research Computing Center and the Linac Coherent Light Source (LCLS).

Supporting Information Available: A table comparing ion currents observed from a 2D approximation of our 3D model with a true 2D model. A figure of the ion flux profiles in the

nanochannel, and a figure of the NH_4^+ flux in PPS. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Supplemental Information to:

Transport properties of nanoporous, chemically forced biological lattices.

Po-Nan Li, Jonathan Herrmann, Soichi Wakatsuki, and Henry van den Bedem

Table S1: Ion currents in the charged case (surface charge density $\rho = 0.02e \text{ \AA}^{-2}$) observed using a 2D approximation of the 3D model in this manuscript vs. the 2D model³⁰

2D approximation with 3D model			
Species	NH_4^+	Cl^-	Na^+
Current	1.04E-18 A	1.57E-20 A	-1.02E-18 A

2D model ³⁰			
Species	NH_4^+	Cl^-	Na^+
Current	3.30E-20 A	1.60E-20 A	-1.60E-20 A

Figure S1: Current profiles in a charged nanochannel with surface charge density $0.02e^{-}\text{\AA}^{-2}$

Figure S2: Quiver plot showing the direction of NH_4^+ fluxes near the active site, marked as a gray circle. The contour lines show the concentration gradient of NH_4^+ near the surface layer.

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