COMMENTARY

Biophysical Reviews' "Meet the Councilor Series"—a profile of Peter Pohl

Peter Pohl[1] ![ORCID]

Abstract

It is my pleasure to write a few words to introduce myself to the readers of *Biophysical Reviews* as part of the "Meet the Councilor Series." Currently, I am serving the second period as IUPAB councilor after having been elected first in 2017. Initially, I studied Biophysics in Moscow (Russia) and later Medicine in Halle (Germany). My scientific carrier took me from the Medical School of the Martin Luther University of Halle-Wittenberg, via the Leibniz Institute for Molecular Pharmacology (Berlin) and the Institute for Biology at the Humboldt University (Berlin) to the Physics Department of the Johannes Kepler University in Linz (Austria). My key research interests lie in the molecular mechanisms of transport phenomena occurring at the lipid membrane, including (i) spontaneous and facilitated transport of water and other small molecules across membranes in reconstituted systems, (ii) proton migration along the membrane surface, (iii) protein translocation, and (iv) bilayer mechanics. Training of undergraduate, graduate, and postdoctoral researchers from diverse academic disciplines has been—and shall remain—a consistent part of my work.

Training

My scientific carrier started in East Germany, at the Martin Luther University of Halle-Wittenberg. I had only been working there for a month when the Berlin Wall fell. The subsequent unification of Germany had far-reaching consequences for science. The new federal state of Saxony-Anhalt took over the hundreds of years old Martin Luther University. However, its government declined to do the same with the employed professors. As a consequence, 80 out of 83 professors of the Faculty of Medicine had to step down. It took many years until their positions were filled again. I was lucky to attract extramural funding from the Deutsche Forschungsgemeinschaft (DFG). Only about a year after receiving my MD, I was awarded a grant that enabled me to pursue my research. Equally important was the continuous support from my wife, Elena Pohl, who initially worked with me in the same research department (see, e.g., Pohl et al. 2000).

With DFG's unceasing support, I built up my research team at the Martin Luther University. However, the abrupt closure of my "scientific home," the Institute of Medical Physics and Biophysics, posed a new challenge. Again, the DFG paved a way out of the dilemma. Endowed with DFG's Heisenberg Fellowship, I moved to the Leibniz Institute of Pharmacology in Berlin with some of my group members. Its director Walter Rosenthal warmly welcomed me, and the scientists working there, especially Burkhard Wiesner, supported and encouraged me. Subsequently, I accepted an interim position as Professor for Biophysics at the Institute for Biology of the Humboldt University of Berlin. In 2004,

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I moved on to Linz (Austria), where I was appointed full professor for Biophysics in the Physics Department of the Johannes Kepler University Linz. Under my leadership, the Institute of Biophysics has grown considerably and has become one of the largest institutes at the Johannes Kepler University. It now has two departments, whose heads take turns leading the institute.

In addition to my work in the IUPAB's council, I am serving as a member of the editorial boards of Biophysical Reviews, Biomolecules, and Scientific Reports. I was the founding chair of the youngest of the three sections of the German Biophysical Society. In this capacity, I have organized the first section meetings. Since then, I have organized and helped organizing further scientific conferences and workshops, such as the 2021 meeting of the European Biophysical Societies' Association (EBSA) in Vienna.

Spontaneous membrane transport of small molecules

I have long been interested in the spontaneous diffusion of small molecules across membranes (for a review, see Hannesschlaeger et al. (2019)). Despite its apparent simplicity, the spontaneous transport of small molecules is still a matter of debate (Saparov et al. 2006; Missner et al. 2008a; Boron et al. 2011). With Yuri N. Antonenko from the Moscow Lomonosov University, I have introduced scanning electrochemical microscopy to quantify small molecule transport through planar lipid bilayers (Antonenko et al. 1993; Pohl et al. 1993). This technique exploits the fact that the anionic form of weak acids gets preferably protonated upon membrane partitioning. As a consequence, pH increases in the aqueous layers adjacent to the membrane. Proton release from buffer molecules and the ensuing buffer diffusion counteract the pH change. Eventually, a steady-state pH profile develops in the immediate membrane vicinity. Recording the proton concentration as a function of the distance to the membrane by scanning pH-sensitive microelectrodes allows quantification of the transmembrane acid flux. The same approach works at the acid receiving side of the membrane or for the permeability assessment of weak bases (Antonenko et al. 1997). The determination of the intrinsic membrane permeabilities of H_2S (Mathai et al. 2009), CO_2 (Missner et al. 2008b), or vitamin C (Hannesschlaeger and Pohl 2018) represents valuable applications. The method can easily be adapted to permeability measurements on cell monolayers (Zocher et al. 2012) or the characterization of near-membrane unstirred layers (Pohl et al. 1998).

Membrane transport of water

Together with my friend and colleague Sapar Saparov, we advanced scanning electrochemical microscopy to assess transmembrane water flow (Pohl et al. 1997). The approach is similar to that described in the previous paragraph. Instead of pH sensitivity, we relied on cation-selective microelectrodes. Using these, we detected the dilution of specific ions in the near-membrane water layers induced by transmembrane water flux (Fig. 1A). Such measurements of ion concentration as a function of the distance to the membrane allow for the accurate determination of membrane permeability to water. Notably, this technique works equally well with profiles recorded from within both the water-donating and water-receiving compartment of the planar bilayer.

The approach enabled demonstrating the excellent selectivity of aquaporins. In collaboration with 2003's Nobel Prize winner Peter Agre, we showed that no more than one in 10^9 transport events facilitated by orthodox aquaporins is electrogenic (Saparov et al. 2001; Pohl et al. 2001). Later, we also characterized aquaglyceroporins which, despite the larger cross-section of their lumen, also do not conduct ions (Saparov et al. 2005).

In a related study, we focused on water transport through ion channels. We found that the bacterial potassium-selective channel KcsA conducts water well (Saparov and Pohl 2004). The channel's slow C-type inactivated state precludes only ion conduction, i.e., the inactivated channel represents a selective water channel (Hoomann et al. 2013).

In the latter report (Hoomann et al. 2013), we departed from counting channels by electrophysiology. Instead, we exploited fluorescence correlation spectroscopy. The optical technique allows counting both activated and inactivated channels in reconstituted bilayers. It also afforded me and my younger colleague, Andreas Horner, an exact count of the number of aquaporins reconstituted into large unilamellar vesicles (Horner et al. 2015) or giant unilamellar vesicles (Boytsov 2020) Fig. 1B. Eventually, this step was crucial for identifying the primary determinant of unitary water channel permeability (Horner et al. 2015): the number of H-bonds that permeating water molecules may form with residues in the channel walls. That is, the water permeability of pores so narrow that water molecules cannot overtake each other is not determined by geometrical factors (channel length and diameter), which govern the flux through micrometer wide pores (Horner and Pohl 2018b). The subject is gaining importance as single-file channels may afford perfect selectivity for water (Horner and Pohl 2018a). Thus, they appeal as tools for water purification and desalinization. The interested reader
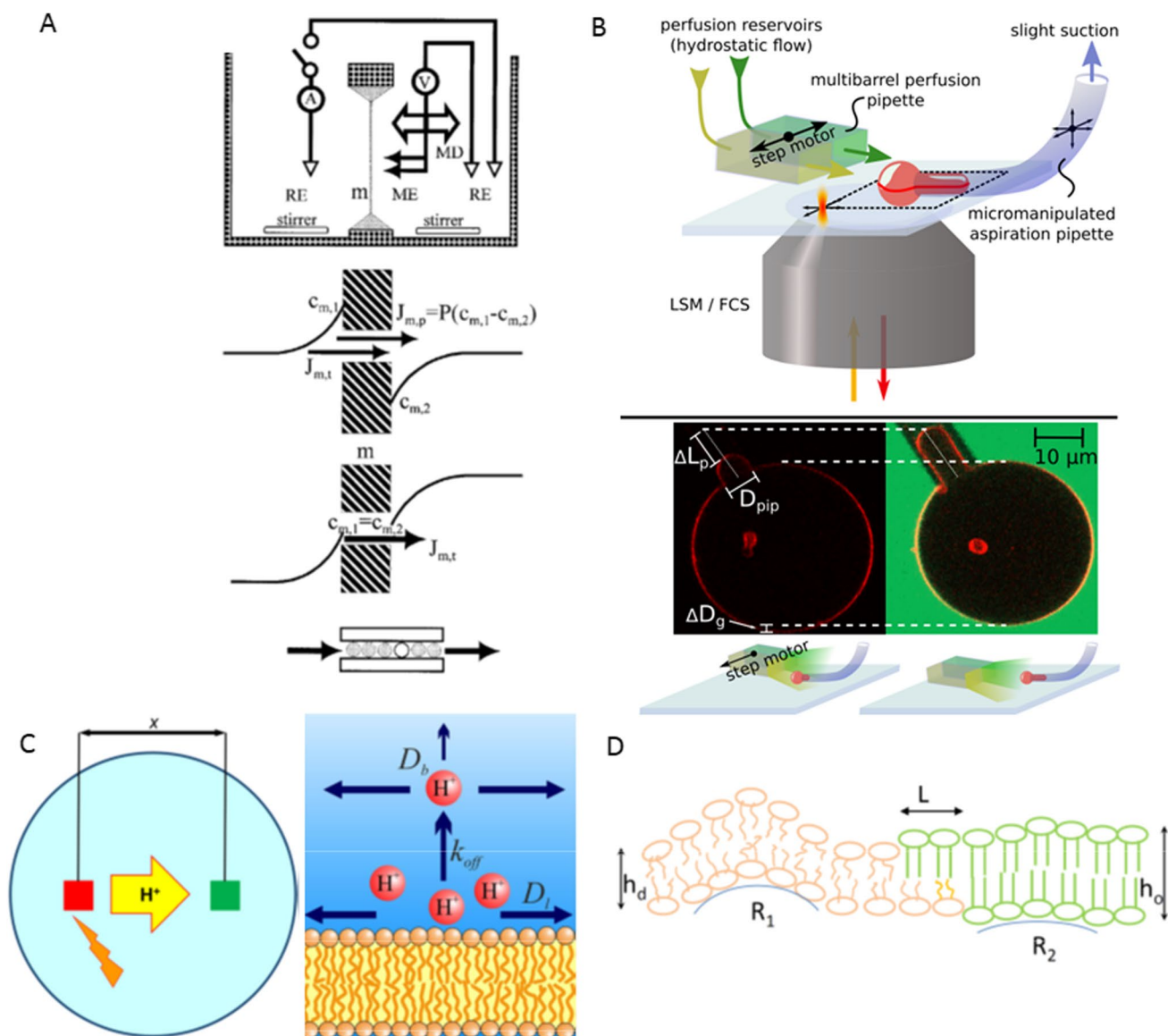
Fig. 1 Different research areas. **A** Scanning electrochemical microscopy for water flux measurements. A picoampermeter measures the current across the cation permeable membrane using reference electrodes (RE). Osmotic water flow up-concentrates both membrane permeable and impermeable cations on one side of the membrane and depletes them on the other side (near-membrane concentrations, $c_{m,1}$ and $c_{m,2}$). To monitor these changes, a hydraulic microdrive (MD) moves a double-barreled microelectrode (ME) perpendicular to a planar lipid bilayer (m). A transmembrane difference in the permeable cation's bulk concentrations served to level the concentrations at the two membrane/water interfaces, thereby rendering possible the quantitative assessment of true solvent drag. That is, water molecules push the cations (flux component J_t) through the roughly one-water molecule wide channel, while no net flux due to an ion concentration gradient (flux component $J_{m,p}$) occurs. The concentration distribution of the impermeable ion allows the calculation of the total water flux. Reprinted from Pohl and Saparov (2000) with permission from Elsevier. **B** Measurements of unitary water permeability by micropipette aspiration. An inverse confocal laser scanning microscope allows the observation of fluorescently labeled aspirated giant unilamellar vesicles (GUV). GUV's protrusion length changes when the perfusion pipette switches position to expose the adjacent GUV to a hyperosmotic, carboxyfluorescein-containing buffer solution. Counting the reconstituted water channels by fluorescence correlations spectroscopy allows obtaining the unitary water permeability. Reprinted from Boytsov et al. (2020). **C** The left panel shows the experimental settings. We monitor proton surface diffusion by photo-releasing caged protons from a membrane spot (red square) and monitoring their arrival in a distant patch (green square) as a change in fluorescence intensity of a lipid-anchored pH sensor. The right panel outlines our nonequilibrium model for proton migration along the membrane surface. Proton diffusion within the confinement of the membrane hydration layers does not involve titratable residues on the surface. Proton surface-to-bulk release is irreversible. Both panels are from Weichselbaum et al. (2017). **D** Schematic of the mechanisms leading to the registration of lipid domains from the two membrane leaflets. Ordered lipids (green lipids) phase separate from disordered lipids (orange). Both (i) the line tension around ordered domains and (ii) membrane undulations induce leaflet cross-talk. The line tension occurs as a result of the hydrophobic mismatch between the lipids in the thicker (bilayer thickness h_o) ordered domain and the lipids in the thinner (bilayer thickness h_d) disordered domain. Deformations of border lipids minimize the access of water to the hydrophobic acyl chains. A two-step transition from h_o to h_d, i.e., a small shift L between the edges of the ordered domains in the two leaflets saves deformational energy (Galimzyanov et al. 2015). Softer, disordered lipids and stiffer ordered domains populate areas with different monolayer curvatures (R_1, R_2). Reprinted from Friedman et al. (2018)

may find a scientific letter about the energetics of single-file water transport enclosed in the current issue of *Biophysical Reviews* (Pfeffermann et al. 2021b).

Proton migration along the membrane surface

Narrow water-filled pores may represent railways for transmembrane proton movement as protons may easily hop along water wires. Channels formed by the pentadecapeptide gramicidin provide an example (Saparov et al. 2000). However, aquaporins can exclude protons by providing barriers in the form of positively charged residues and helical dipoles pointing toward the center of the channel (Saparov et al. 2005; Pohl et al. 2001). In the past, we have been interested in monitoring proton movement along interfacial water molecules (Serowy et al. 2003; Zhang et al. 2012) Fig. 1C. The latter endow protons with a high affinity to the membrane surface (Weichselbaum et al. 2017) and further ensure protons' high interfacial hopping rate, which exceeds the release rate from titratable residues by orders of magnitude (Springer et al. 2011). The combination of high affinity and high mobility may aid the vital efficiency of proton exchange between proton pumps and ATPases.

Protein translocation across membranes

The transmembrane proton gradient also plays a role in protein translocation through the bacterial plasma membrane facilitated by the heterotrimeric translocon SecYEG. We established that the closed/resting translocon is impermeable to small ions and water by studying the purified channel reconstituted into planar lipid bilayers (Saparov et al. 2007). What is more, our reductionistic experimental system allowed identifying structural elements crucial for sealing the channel (Saparov et al. 2007). We also found that the channel is voltage-sensitive (Knyazev et al. 2020). This property affects the (undesired) leak of ions and protons during protein translocation (Knyazev et al. 2014). It will be key to understanding the protein translocation mechanisms through SecYEG.

Membrane mechanics

Driven by the realization that the function of membrane proteins frequently depends on constituents of the embedding medium, i.e., lipids, bilayer mechanics became more and more the focus of my research activities. We set out

by exploring the coupling between the two membrane leaflets. Contrary to a wide-spread hypothesis, we found a negligible contribution of interdigitation to interleaflet coupling by observing the friction at the membrane midplane of both short- and long-chain fluorescent lipid analogs (Horner et al. 2013). Instead, lipid undulations exert a noticeable effect on interleaflet coupling (Horner et al. 2009). Stiffer regions from both leaflets attract each other simply because their bending rigidities exclude them from highly bent membrane regions (Galimzyanov et al. 2017). The contribution due to undulations scales with the area of the lipid domain and may thus be insufficient to stabilize nanoscopic lipid domains (lipid rafts). In their case, the line tension between thinner disordered domains and thicker ordered domains provides a significant driving force for registration. The elastic energy stored within the border between domains reaches a minimum when domains from opposing leaflets are in register (Fig. 1D). Both undulations and line tension work hand-in-hand (Saitov et al. 2020).

Lipids with light-sensitive conformations allow switching between lipid-ordered and lipid-disordered domains (Saitov et al. 2020). Concerning mechanosensitive channels, photoswitchable lipids pave the way for gating ion channels by light, which we recently demonstrated on the model channel gramicidin A (Pfeffermann et al. 2021a).

Declarations

Conflict of interest The author declares no competing interests.

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