**Protons at bio-interfaces**

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Proton reactions and pH sensing at bio-interfaces are fundamental for cell physiology and human disease. Membrane-bound proteins and protein complexes that transfer protons across membranes generate and maintain proton gradients –used, for example, by the ATP-synthase to generate Adenosine Triphosphate (ATP) in the mitochondria –a process that is disregulated when the cytosolic pH becomes more alkaline in cancer [1]. Acidic external pH may characterize aggressive tumors, and peptides that interact with membranes in a pH-dependent manner are of interest for developing strategies to detect regions with altered pH [2]. Synthetic peptides that form pH-dependent macromolecular pores are of major interest to deliver cargo to low-pH cells and cell compartments [3].

This topical issue includes 16 original research and review articles on broad topics pertaining to reactions at membrane interfaces.

Among the best-studied membrane-bound proton transporters are the proton-transporting microbial rhodopsins –seven-helical membrane proteins that couple photo-isomerization of the covalently bound retinal chromophore with ion-transporting reaction cycles. The comprehensive *review article by Brown* [4] summarizes experimental data on proton transport by microbial rhodopsins with focus on more recently discovered rhodopsin variants. The discussion of the consensus picture of the reaction mechanism of bacteriorhodopsin, whose three-dimensional structure was solved with cryo-Electron Microscopy (cry-EM) back in 1975 [5], provides the foundation to present general models of outward and inward light-driven proton transport by microbial rhodopsins, passive transport (proton leakage), mechanisms for vectoriality of proton pumping and sequence-structure-function relationships, and challenges in identifying proton donor and acceptor groups in new rhodopsins such as Antartic rhodopsin, AntR [6].

The light-driven reaction cycles of microbial rhodopsins involve passage of the protein through a series of intermediates distinguished by the absorption maximum and isomeric state of the retinal chromophore, protonation states of a few internal titratable sidechains, and the conformation of the protein [7-10]. Description of the properties of the intermediate states of microbial rhodopsins is required to understand how these proteins work and, importantly, is of direct interest to engineer rhodopsin variants suitable for applications in optogenetics to control neuronal activity –such as, for example, the channelrhodopsin-2 variants proposed for long-term depolarization [11], or WiChR, the potassium-selective channelrhodopsin recently proposed as an optogenetic inhibitor [12].

The two articles by *Kouyama and Ihara* [13, 14] report comprehensive experimental data on the late O intermediate of two microbial proton-pump rhodopsins, bacteriorhodopsin [14] and archaeorhodopsin-2 [13]. Both proteins are reported to have two O substates, denoted as O1 and O2. The O1 intermediate of bacteriorhodopsin is in a pH-dependent equilibrium with the preceding intermediate N and it has a distorted 13-*cis* retinal; the decay rate of O2, which has all-*trans* retinal, decreases when the pH becomes acidic [14]. Due to differences in the pKa values of a key internal carboxylic group, archaeorhodopsin-2 appears suitable to study the O1 intermediate over a wider range of pH values than in the case of bacteriorhodopsin [13].

Schizorhodopsins are inward proton-pumping rhodopsins that have only one Asp counterion on helix G, as compared with the two Asp on helices C and G in the outward proton-pumping bacteriorhodopsin [4, 15]; spectroscopic properties of schizorhodopsin-3 fom Asgardarchaeota were suggested to make this protein potentially suitable for optogenetics studied on fast-firing neurons [15]. Here, *Kawasaki and colleagues* [16] use spectroscopy measurements to characterize the reaction cycles of Asgard schizorhodopsin-1 and of two schizorhodopsins from thermophile archaea, *Mt*SzR and *Ms*SzR. Observations from kinetic isotope effect and temperature dependence measurements indicate that, in schizorhodopsin-1 and *MtSzR*, proton transfers at the retinal Schiff base associate with entropic barriers arising from rearrangements within the protein-water H-bond network; the larger temperature dependence of *MsSzR* is indicative of a protein structure optimized for thermostability [16].

As reviewed by Brown for the proton-pumping microbial rhodopsins [4], conserved structural elements, or motifs, at the proton-binding sites of proton-coupled membrane proteins –and variations of such motifs– are central to formulating hypotheses about reaction mechanisms. *Lazaratos and colleagues* [17] present a computational methodology to identify H-bond motifs in large datasets of static protein structures of membrane transporters from various families. Static protein structures were assembled in two datasets according to the resolution at which they have been solved, and subjected to H-bond network computations using a graph-based algorithm [18]; from these H-bond graphs, ten distinct H-bond motifs were searched. Carboxyl-hydroxyl H-bonds are found in many protein structures of the dataset and, importantly, may be part of H-bond paths that could potentially help couple the carboxyl’s protonation state with protein dynamics [17].

Synthetic peptides and other molecules that interact with membranes in a pH-dependent manner are of direct interest for bio-medical applications, particularly to modulate lipid bilayer properties and to deliver drugs to cells and cell compartments with acidic pH. Biochemical and biophysical characterization of the lipid interactions of such molecules is essential, as it may help guide the design and synthesis of compounds with activities suitable for specific bio-medical applications. Several contributions to this topical collection discuss synthetic membrane-binding peptides, pores and transporters with pH-dependent functionalities.

The pH low insertion peptide (pHLIPs) was designed back in 1997 using as a template helix C of bacteriorhodopsin [19]. The original 36-residues peptide contained the two Asp groups of bacteriorhodopsin helix C, and had a Glu-to-Gln substitution at its N-terminus [19]. The Asp groups made the peptide pH sensitive, such that at neutral pH the peptide was unstructured and located at the membrane periphery, whereas at pH <6 one of the Asp group protonated and the peptide was membrane-inserted as an -helix [19]. *Ataka and colleagues* [20] apply Surface Enhanced Infrared Absorption (SEIRA) spectroscopy [21] to characterize the insertion pathway of a pHLIP peptide into a lipid bilayer casted on a gold surface; this pathway is found to include a new intermediate state that is populated at pH values between 5.3 and 7. Moreover, the spectroscopy data identify two carboxylic groups implicated in the pH-dependent conformational dynamics of the peptide [20].

*Howe and colleagues* present experimental data on synthetic pH-switchable anion transporters based on phenylthiosemicarbazones, PTSC [22]. At pH 7.2 the PTSC molecule is neutral and has a *trans* conformation in which an intra-molecular H-bond makes the chloride-binding site inaccessible. At low pH, PTSC binds a proton at its imine nitrogen, isomerizes to a *cis* conformation, and binds anions [22]. The paper reports on the synthesis and characterization, using X-ray diffraction and membrane transport assays, of nine PTSC-based transporters, and demonstrates that transporters have high selectivity for chloride over nitrate –such that nitrate transport is rate-limiting and inhibited at neutral pH [22].

*Salnikov and colleagues* [23] use solid-state Nuclear Magnetic Resonance (NMR) spectroscopy to characterize the pH-dependent topology change of the LAH4 peptide in lipid membranes distinguished by the headgroup and alkyl chains of the lipid molecules. LAH4 peptides, designed based cationic microbial peptides, are of interest as antimicrobial peptides and to deliver cargo to cells [24]. The peptides are amphipatic, with a hydrophobic core, one Lys group at each terminus, and four His groups located on the same side of the helix. The four His groups make the membrane interactions of LAH4 pH sensitive, such that the peptides prefer the membrane interface at pH <5.5, and insert in POPC at pH > 7. Experimental data presented by Salnikov and colleagues demonstrate that the transition of LH4 from the in-plane (interfacial) to the transmembrane orientation depend on the lipid saturation [23]. In the accompanying paper, *Salnikov and Bechinger* [25] discuss exhaustive solid-state NMR studies that decipher the effect of the lipid alkyl chain saturation on the structural properties of six polypeptide sequences –magainin-2 and PGLa, the synthetic LAH4 peptide, alamethicin, and the transmembrane helical anchoring domains of the MHC II receptor, DQA1 and DQA2. Intriguingly, at pH 5.3 LAH4 prefers a transmembrane orientation in 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC, has two saturated C14 acyl chains), and a surface orientation in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC, has the C16 acyl chain saturated, and the C18 chain, unsaturated) [25].

*Ramos-Martín and colleagues* [26] combine NMR spectroscopy and molecular dynamics simulations to characterize the structure and dynamics of Cecropin D (CecD), an antibacterial peptide of interest due to its potential activity against two particular lines of esophageal cancer. The Authors report that CecD tends to favor -helical conformations when in aqueous solution, and it becomes more structured in the presence of a biomimetic membrane model [26]. The molecular dynamics simulations reveal specific amino acid residue sidechains that can mediate CecD-lipid membrane interactions, and how CecD-membrane interactions depend on the lipid membrane composition [26].

The molecular picture of membrane pores formed by pore-forming peptides is essential to understand the inter-molecular interactions that govern pore dynamics. Three-dimensional structures of oligomeric membrane pores solved with structural biology provide data essential for deciphering how pores may interact with membranes. The *review article by Mondal and colleagues* [27] presents a detailed molecular picture of the mechanism of pore formation by bacterial pore-forming toxins with focus on recent developments in cryo-EM structure solving of oligomeric pores. The systematic discussion of bacterial pore-forming toxins pore formation solved with cryo-EM includes anthrax protective antigen (PA) and *Vibrio cholerae* cytolysin (VCC), and challenges that remain in describing the mechanism for pore formation for bacterial pore-forming toxins –such as the existence of multiple assembly states for membrane-bound VCC, membrane interactions, and the importance of solving pore structures at high resolution [27].

The Islet Amyloid PolyPeptide, IAPP, also known as amylin, is the main component of the amyloid deposits found in the pancreatic islets of the majority of patients suffering from type 2 diabetes [28, 29]. The *review article by Guillemain and colleagues* [29] focuses on the physiology and pathology of human IAPP (hIAPP), a relatively small peptide of 37 amino acid residues expressed as a longer pre-peptide that is first cleaved in the endoplasmic reticulum, and then again in the trans-Golgi network via pH-dependent reactions [29]. Essential properties of hIAPP are discussed in the wider context of other amyloid forming proteins, including amyloid-beta, tau, -synuclein, and prion, together with a perspective on preventing the toxic effects associated with hIAPP fibril formation [29].

Detergents that allow native protein structures to be maintained during the process of extraction and purification are essential for structural biology and other biophysics techniques. *Urner and colleagues* [30] report on synthesis and characterization of an asymmetric hybrid non-ionic detergent designed for studies of membrane proteins, and evaluate how the detergent concentration and properties of the detergent micelles impact delipidation of membrane proteins. The hybrid detergent contains fragments of two detergents with distinct delipidating properties: the maltose headgroup (Mal) of n-dodecyl--D-maltoside (DDM, mildly delipidating), and the tetraethylene glycol head (E4) of tetraethylene glycol monooctyl ether (C8E4, strongly delipidating); Mal and E4 are connected by a spacer, and the lipophilic tail is bound to Mal [30]. The data demonstrate that the hybrid detergent is valuable for the analysis of membrane proteins [30].

Photo-switchable lipid molecules are synthetic lipids in which an alkyl chain was replaced by a light-absorbing moiety that changes its isomeric state once it has absorbed light of the appropriate wavelength. Photo-switchable lipids that have an azobenzene moiety allowed, for example, control of the lateral membrane pressure to open and close mechanosensitive ion channels [31]. The *cis*-azobenzene has more favorable water interactions than the *trans* conformation, which would associate with the *cis* photo-switchable lipid or fatty acid molecule locating closer to the membrane polar interface [32].

In this topical collection, *Korn and colleagues* [33] report on extensive experimental measurements to compare specific properties of two photo-reactive lipids, azide-modified PC (AzidoPC) and diazirine-modified PC (DiazPC), and on studies of a transmembrane model peptide in the presence of mixtures of either photo-reactive lipid and DMPC. Both photo-reactive lipids are found suitable to evaluate interactions between lipids and peptides or proteins, however more by- and degradation products are identified in the case of AzidoPC than DiazPC [33].

To bind protons at bulk-exposed interfaces, proton-binding membrane proteins are thought to use clusters of closely spaced carboxylic and histidine sidechains, or proton-collecting antennas, which collectively capture a proton and increase the dwell time of the proton at the protein surface, and may transfer a proton to an internal protein site [34-36]. Bondar [37] presents atomistic simulations and graph-based analyses of the protonation-coupled dynamics of the PsbO subunit of the large membrane-embedded photosystem II complex. PsbO, which is has a carboxylic cluster thought to function as a proton antenna [38], is an excellent model system to study dynamic water bridging to carboxylic clusters [39], because it is a relatively small soluble protein with a rather rigid -barrel domain. The computations presented here demonstrate that, dynamic water-mediated H-bond networks transiently give rise to extended H-bond networks. However, persistent H-bond bridging tends to be rather local, and carboxylic groups of local H-bond clusters typically anchor to basic sidechains; H-bond bridging within a local H-bond cluster couples to the protonation state [37].

The extensive *review by Nachliel and Gutman* [40] summarizes some >40 years of experimental and computational research performed in their laboratory –including pioneering research on proton transfer dynamics in aqueous solution, the theory of proton transfer within the Coulomb-cage and the chemical kinetic formalism developed to analyze intra-Coulomb-cage proton transfer, the role of water molecules, the binding of sodium and calcium ions at protein surfaces, the passage of ions through ion channels, and ion collecting antennas. The concepts presented in the review are of general interest to proton-binding bio-molecular systems.

We anticipate that the exciting developments reported in the original and review articles of this topical collection will serve as valuable reference for the broad field of experimental and computational research on membrane reactions and protons at bio-interfaces.

**CRediT authorship contribution statement**

A-NB wrote the manuscript with input from MB.

**Declaration of competing interest**

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

**Acknowledgements**

We thank the Authors for their valuable contributions that made possible this topical collection, and the Editors of Biochimica Biophysica Acta – Biomembranes for hosting the issue. We are grateful to Mr. Selvam Balasubramaniam, from the BBA-Biomembranes Editorial Office, for assistance throughout the entire process This work was supported by funds from the European Union’s Horizon 2020 Research and Innovation Program under the Marie Sklodowska-Curie grant agreement No H2020-MSCA-ITN-2019-860592, Innovative Training Network “Proton and proton-coupled transport”.

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