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Review

Permeating disciplines: Overcoming barriers between molecular simulations and classical structure-function approaches in biological ion transport☆

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ABSTRACT

Ion translocation across biological barriers is a fundamental requirement for life. In many cases, controlling this process—for example with neuroactive drugs—demands an understanding of rapid and reversible structural changes in membrane-embedded proteins, including ion channels and transporters. Classical approaches to electrophysiology and structural biology have provided valuable insights into several such proteins over macroscopic, often discontinuous scales of space and time. Integrating these observations into meaningful mechanistic models now relies increasingly on computational methods, particularly molecular dynamics simulations, while surfacing important challenges in data management and conceptual alignment. Here, we seek to provide contemporary context, concrete examples, and a look to the future for bridging disciplinary gaps in biological ion transport. This article is part of a Special Issue entitled: Beyond the Structure-Function Horizon of Membrane Proteins edited by Ute Hellmich, Rupak Doshi and Benjamin McIlwain.

1. Introduction

Regulated ion transport across biomembranes is crucial to a wide range of processes including cell motility [1], photosynthesis [2], and neurotransmission [3]. For biophysicists, ion transport has been a key area of research for decades, due in part to the unique intersection of biological, chemical, and physical principles it represents. Among other things, the ability to directly measure ion channel activity via transmembrane electrical properties enabled some of the first measurements of protein function at the single-molecule level [4].

Despite these and other advances, our molecular understanding of biological ion transport remains limited, due in large part to its reliance on multipass membrane proteins including channels and transporters. Alongside inherent challenges these molecules pose to classical biochemistry and structure determination [5], ion channels and transporters can undergo critical conformational changes in the course of routine function such that, even at high resolution, a single structure reveals only one chapter in a complex mechanistic story [6]. Computational methods have proved crucial in the interpretation of both electrophysiological and structural data in this field [7,8]; however, given the wide scales of time and space involved, no single approach presently provides a comprehensive understanding.

Ion transport research has benefitted substantially in recent years from advances both in structure-function methods (e.g. increased accessibility of atomic-resolution structures [9] and throughput of electrophysiological recordings [10]) and in simulation tools (e.g. processing power and force field accuracy [11]). Accordingly, this field has offered increasing opportunities for interdisciplinary collaboration—accompanied by occasional clashes of technical terminology, conceptual paradigms, and data access. Even the terminology used to describe these intuitively distinct disciplines can prove problematic under close inspection: research carried out at the biochemistry or electrophysiology bench is often described as *experimental,* in contrast to the *computational* work involved in generating and analyzing molecular simulations; yet *experiments* can be performed with keystrokes as well as pipettes, and *computation* contributes to numerous scientific

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activities beyond the particular realm of molecular dynamics under discussion here.

Whereas comprehensive summaries of relevant techniques have been admirably provided by other recent reviews [12,13], we seek here to detail a few key research questions currently bridging in vitro and in silico approaches (Section 2), and to identify some critical challenges for integrating structure-function and simulation techniques in this and related fields (Section 3). Remaining paragraphs of this introduction (Section 1) aim to provide relevant opening context to readers with more limited expertise in either laboratory or computational methods, particularly highlighting capabilities and limitations of a few landmark structure-function and molecular simulations approaches relevant to the research questions to follow. Case studies and recommendations in this work are based in part on presentations and discussions at the 2017 workshop of the Centre Européen de Calcul Atomique et Moléculaire (CECAM), *Ion Transport from Physics to Physiology: the Missing Rungs in the Ladder*.

1.1. Structural studies of purified membrane proteins

Structural biology has been instrumental in populating multi-state molecular mechanisms of ion transport, as reviewed in detail elsewhere [e.g. 14–16]. However, ion channels and transporters can pose particular challenges to classical structure methods such as X-ray diffraction, cryo-electron microscopy (cryo-EM), and solution-phase spectroscopy, particularly in their demand for purified, concentrated membrane proteins. Indeed, membrane proteins currently represent < 2% of structures in the Protein Data Bank (PDB), despite constituting up to 30% of the human protein-coding genome [9]. Here, we briefly review preparation considerations and key approaches to structure determination (Fig. 1, green), with a focus on features and limitations for biological ion transport.

1.1.1. Membrane protein preparation poses special challenges to structural biology

Due in part to their amphiphilic surfaces and need for post-translational processing, overproduction of membrane proteins has often required extensive screening across expression hosts (e.g. bacteria, yeast, insect or mammalian cells) and vectors (e.g. engineering with viral promoters, untranslated regions, or fusion partners) [17]. When full-length pharmacological targets prove inaccessible, researchers frequently take advantage of simplified homologs from bacteria or archaea, or selectively alter flexible domains or processing sites. In the absence of—or sometimes complementing—full-length protein structures, useful information may also be obtained from isolated domains in a “divide and conquer” approach to identify structural consequences of

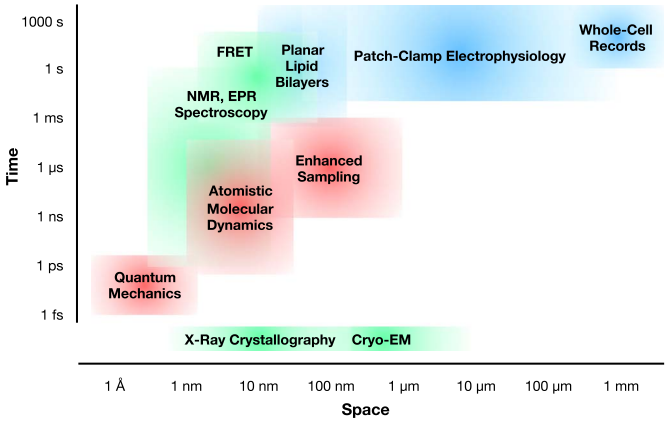

Fig. 1. Comparative space and time scales accessed by representative experimental methods in ion transport research, including structural/spectroscopic (green), electrophysiology (blue), and molecular simulations (red) approaches.

mutations or ligands [18,19]. Once a membrane protein is produced, it must be further purified and reconstituted in solution, generally by replacing most or all of the lipid bilayer with a suitable detergent [20]. Finding a purification and solubilization scheme that preserves structural and functional integrity can be challenging [21], and may require specialized activity assays: substrate binding may be used as a proxy for integrity in membrane transporters, whereas ion flux (e.g. in proteoliposomes) should be verified for purified ion channels [22,23].

1.1.2. X-ray methods capture well-behaved proteins up to atomic detail

For several decades, the capacity of macromolecules to scatter X-rays has provided crucial tools for structure determination, covered at the textbook level in biochemistry curricula [24]. Briefly, whereas solution-phase scattering can provide low-resolution information about macromolecular size and shape [25], the distinctive diffraction pattern of a cryoprotected protein crystal can yield a three-dimensional electron density up to subatomic resolution [26]. X-ray methods are well suited to characterizing macromolecules on the scale of ion transport proteins, as well as small molecule agonists, modulators, and even ions [27]. Indeed, from the first glimpses of potassium-selective and mechanosensitive channels in detergent micelles [28,29] to high-resolution views of transporters in lipid bilayers [30], crystallography has contributed substantially to our understanding of ion transport. On the other hand, crystallization of ion transport proteins can require prohibitively large quantities of pure protein; extensive screening of crystallization conditions; and packing of target molecules into dense lattices, potentially trapping acutely nonphysiological states [31]. With some exceptions, X-ray crystallography provides a snapshot of a single, crystallographically accessible state, potentially to high resolution but with limited dynamic or physiological content.

1.1.3. Cryo-EM provides new opportunities for large complexes

Although recognized by the 1970s as a structural tool for membrane proteins [32], it was almost five decades before single-particle cryo-EM successfully determined a protein structure at atomic resolution—notably, that of an ion channel [33]. Cryo-EM does not generally require the large scales of pure, crystallized protein demanded by X-ray methods. Instead, a few microliters of dilute sample are typically applied to a carbon film grid, then vitrified in liquid ethane to hydrate and protect the protein from radiation damage. After imaging the transmission of an electron beam through the grid, tens of thousands of individual molecules (“particles”) are binned into class averages, then interpolated into a three-dimensional structure. Depending on microscope access and resources, millions of individual particles may be merged into each class, although for well characterized samples (e.g. ribosomes) tens of thousands of particles may suffice [34]. The new prominence of cryo-EM owes in great part to the development of direct electron detectors, which accumulate images as movies (i.e. with a time component) with enhanced sensitivity and motion correction [35], and to recent innovations such as phase plates to enhance sample contrast [36].

Advances in cryo-EM have shed light on some historically inaccessible targets, including human ion channels [37]; however, several limitations remain. The technique has been mostly restricted thus far to larger molecules (> 100 kD) and lower resolutions (> 3 Å), with some recent exceptions [38]. For membrane proteins, detergent solubilization remains standard, though alternative preparations such as lipid nanodiscs offer more native-like conditions [39]. Grid preparation and vitrification may introduce further conformation and/or orientation bias, undersampling certain orientations and reducing the quality and rigor of particle classification. In the process of structure refinement, less populated classes are further discarded to improve resolution, such that the final structure(s) may represent only a subset of available conformations [35]. The full potential of cryo-EM for high-resolution structure determination likely remains to be realized.