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Review

# Membrane transporter research in times of countless structures

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ABSTRACT

Structural biology has advanced our understanding of membrane proteins like no other scientific discipline in the past two decades and the number of high resolution membrane transporter structures solved by X-ray crystallography has increased exponentially over this time period. Currently, single particle cryo-EM is in full swing due to a recent resolution revolution and permits for structural insights of proteins that were refractory to crystallization. It is foreseeable that multiple structures of many human transporters will be solved in the coming five years. Nevertheless, many scientifically important questions remain unanswered despite of available structures, as is illustrated in this article at the example of multidrug efflux pumps and ABC transporters. Structure-function studies likely continue to be a supporting pillar of membrane transporter research. However, there is a trend towards the "integrated structural biologist", whose research focusses on a biological question and who closely collaborates with other research groups specialized in spectroscopy techniques or molecular dynamics simulation. Future membrane protein research requires joint efforts from specialists of various disciplines to finally work towards a molecular understanding of membrane transport in the context of the living cell. 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. Beyond structure-function

The scope of this BBA special issue is to look "Beyond the Structure-Function Horizon of Membrane Proteins". Having dedicated my entire scientific career on structure-function studies of membrane transporters, this is a daunting task. Let me therefore first share my view of structure-function research. Biologists tend to think in pictures. We constantly draw models on a piece of paper or a chalkboard, we are eager to visualize how something works. That's why structural biology satisfies an urgent and often unmet need. A protein structure provides the researcher with a crystal-clear photograph (Fig. 1). Atomic structures, under the condition they are modeled correctly, describe the coordinates of every atom of a protein. A structure is therefore not only beautiful, but is also rich in information. Importantly, an experimentally determined structure of a novel protein class has an enormous value, because *ab initio* structure prediction of a protein of average size is still far too complex. Hence, nothing can compare to a high resolution structure. In addition, homology models profit from the fact that structure is the most conserved trait of a protein. Therefore, homology models based on the coordinates of close homologues are generally astonishingly accurate and informative. A structure - and to some degree a good homology model - is not only a collection of coordinates, but rather a formidable source of inspiration. By seeing how a membrane transporter looks like, mechanistic experiments can be planned on a rational basis. This is why one speaks about the structure-function horizon and not the opposite, namely function-structure.

## 2. The "howevers"

Due to crystal packing, X-ray structures are static *per se*. Cryo-EM structures are static as well, because they are a result of particle classification. Cryo-electron tomography has the capacity to investigate dynamic protein complexes in entire cells, but the resolution is currently in the nanometer-scale [1]. Likewise, high-speed atomic force microscopy permits for the direct visualization of membrane transporters over a time period of several minutes in their membrane context, but only at medium resolution [2]. NMR structures of membrane proteins take dynamics into account, and have revealed novel insights into the molecular mechanism of proton binding of small multidrug transporter EmrE [3]. Unfortunately, NMR studies of larger membrane transporters are technically not feasible at the moment. The bottom line is that atomic membrane protein structures are in general static pictures. **However**, to fulfil their various tasks, proteins - and in particular membrane transporters - need to be dynamic. Other articles within this special issue will discuss techniques suited to study protein dynamics, be it by experimental (EPR, smFRET) or computational (molecular dynamics simulation) means.

Membrane protein structures are usually determined in detergent

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**Fig. 1.** Membrane protein research in times of countless structures. The following structures (PDB entries) are featured (clock wise starting from the bottom left): 3WMG (ABC transporter CmABCB1), 3B9B (*P*-type ATPase SERCA), 4Q4A (ABC transporter TM287/288), 4DX5 (RND transporter AcrB), 2HYD (ABC transporter Sav1866), 4LEP (MFS transporter PepTSo2).

solution. **However**, the natural habitat of membrane proteins is the lipid bilayer. It should be emphasized, that monoolein used for lipidic cubic phase (LCP) crystallization does not form lipid bilayers, but various mesophases [4]. Crystals obtained by the LCP method therefore do not fully reflect the protein's native environment. In contrast, cryo-EM investigation of membrane proteins reconstituted in nanodiscs or liposomes offer unprecedented opportunities to illuminate native structures [5,6].

Despite of these disclaimers, membrane protein structure is king. Structures serve as starting point for the introduction of spin-labelling and FRET pairs, and without them, the field of molecular dynamics would not exist in its current form.

## 3. Structural biology is discovery science

Most membrane protein structures offer exciting and unexpected insights. This is especially the case if the structure depicts a novel transporter fold as for example the - in the meantime famous - LeuT fold [7]. But even structures of a membrane protein solved in a novel conformational state - in a different pose to speak in the language of a photographer - can lead to exciting discoveries. In the following I provide two examples, which I experienced myself as researcher.

The first one is the drug efflux pump AcrB from *E. coli*, the lysozyme of membrane proteins, which loves to crystallize even as minor contaminant [8]. AcrB is a homotrimer and has originally been crystallized in a symmetric conformation, with each monomer depicting the same conformational state [9]. Subsequently, AcrB was crystallized in an asymmetric state, *i.e.* every monomer adopted a different conformation [10,11]. The asymmetric structure of AcrB provided unexpected insights into the transport mechanism of this drug efflux pump. While it was originally believed that drugs are transported through a central pore at center of the trimer, the asymmetric AcrB structure revealed that they are transported through the individual monomers of the trimer by a peristaltic functional rotation mechanism. This exciting discovery could not have been made without the structure.

For the second example, we switch gears and speak about ABC transporters. TM287/288 is a heterodimeric ABC exporter stemming from the thermophilic bacterium *Thermotoga maritima*. Like many of its eukaryotic counterparts, TM287/288 contains asymmetric ATP binding sites, of which one is deficient in ATP hydrolysis and called the degenerate site. Using X-ray crystallography, we solved two closely related inward-facing structures of TM287/288, one with AMP-PNP bound to the degenerate site and the other one in the apostate [12,13]. The first surprise was that the nucleotide binding domains (NBDs) were in molecular contact while the transmembrane domains adopted an inward-facing conformation. At that time, all inward-facing structures had the shape of an inverted V exhibiting fully separated NBDs. Even more surprising was the fact that the so-called D-loops were highly asymmetric. While the degenerate site D-loop was chiefly responsible for mediating cross-NBD contacts, the consensus site D-loop was found to be highly flexible and playing an important role in the allosteric cross-talk between the asymmetric ATP binding sites. Importantly, structural asymmetry of this highly conserved sequence motif was not evident from the protein sequence and it required these two crystal structures to obtain these insights.

With each novel structure there is a considerable chance of making an unexpected discovery. Therefore, structural elucidation of membrane transporters will likely continue even in times of countless structures, driven by the hope of discovering something exciting.

## 4. A tale of many snapshots

A typical transporter cycles through a number of conformational states to fulfil its function. Even before the advent of high resolution membrane protein structures, the various conformational states a membrane transporter cycles through have been described in great detail and accuracy, as for example for the ATP-driven *P*-type ATPases [14]. Conformational changes typically involve domain movements in the range of 5–30 Å. DEER spectroscopy as well as single molecule FRET analyses are ideally suited to monitor these conformational movements [15–18]. Thereby quantitative information is obtained regarding the population of different states, and protein dynamics is detected, which often remains hidden in static structural descriptions. For structural biologists, quantitative information regarding the population of conformational states is in return very useful to identify experimental conditions, which stabilize the protein in a desired state for crystallography and cryo-EM analyses. An important aim of structural biology is to obtain structures of a membrane protein in all its functionally relevant states, because this allows for compiling a morph of the transport cycle. Such detailed movies exist for example for the $Ca^{2+}$- and the $Na^{+}/K^{+}$-ATPase [19,20], the maltose transporter (a type I ABC importer) [21] and the vitamin $B_{12}$ transporter (a type II ABC importer)