Lise Arleth

A View into the not so Simple Gating Mechanism of the Magnesium Transport Protein CorA

Membrane transport proteins play a central role in securing the necessary and controlled exchange of nutrients and signal molecules across the membrane of the biological cell. The transport of nutrients is required to keep the machinery of the cell alive while the transport of signal molecules is crucial to secure coordination between cells in higher order multicellular organisms. For the same reason, several specific membrane proteins are very efficient targets for pharmaceutical drugs.

To control the molecular transport across the membrane, the membrane proteins rely on mechanisms to open and close their gates. Classical biophysical textbook descriptions often use simple rigid two-state open/closed models to describe the gating. However, as we often see, nature can be much more complicated than this.

About 10-15 years ago my research group started investigating how far small-angle scattering would bring us with respect to structural investigations on membrane proteins. Small-angle scattering using either X-rays (Small-Angle X-ray Scattering, SAXS) or neutrons (Small-Angle Neutron Scattering, SANS) relies on shining a well-collimated beam of monochromatic X-rays or neutrons through a sample. Some of the beam gets elastically scattered, and the obtained scattering pattern is related to the Fourier transform of the sample structure with a resolution ranging from ~10 Å to ~1000 Å (Fig. 1a). Through fitting of a structural model against the experimental scattering data, it is possible to derive the 3D structure of the particles of the sample.

Neutrons have unique properties as compared to X-rays when it comes to investigating biological samples. X-rays interact with the electrons of a sample, and the heavy and electron-rich part of the samples will provide the most contrast. With neutrons, Hydrogen-1, ^{1}H, will stand out as compared to most other isotopes of the elements. So, the light and typically hydrogen-rich part stands out. Further, the contrasts of ^{1}H and ^{2}H (deuterium) are very different. So, through controlled isotope substitution between ^{1}H and ^{2}H in the sample, it becomes possible to systematically either highlight or hide certain parts of the investigated particles. When it comes to investigating membrane proteins, this possibility for contrast variation allows for highlighting the membrane protein, while it sits stable in a membrane-like environment, but without seeing the surrounding membrane environment or the solvent phase (Fig. 1b) [1-3].

Prof. Lise Arleth
Niels Bohr Institute – Faculty of Science
University of Copenhagen
Universitetsparken 5, DK-2100 Copenhagen
Arleth@nbi.ku.dk

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The CorA magnesium transporter is the major magnesium transport system in bacteria. It is a pentameric membrane transporter (Fig. 1c) that serves to control the magnesium concentration inside the bacterial cell. Through X-ray based protein crystallography, a high-resolution structure of the CorA was obtained at high Mg^{2+} concentrations in 2006 [4]. A nearly perfect 5-fold symmetric pentameric conformation was observed, with an inner channel through the membrane bilayer that was clearly too narrow to allow the passage of magnesium. It was concluded that this was the closed conformation. However, the open conformation that should then have a wider channel remained elusive to experiments for many years. Instead, several theoretical hypotheses were developed for its structure. In 2016, Doreen Matthies and co-workers obtained a, still pentameric, but significantly symmetry broken structure though Cryo-Electron Microscopy (Cryo EM) conducted in the absence of Mg^{2+}. Their structure had a resolution 7,1 Å and they proposed that this was the open structure [5].

At about the same time, my own research group had developed a set of neutron invisible carriers for the solubilization of membrane proteins in relation to SANS experiments [1, 3]. We thought, supported by model calculations, that the CorA, with its proposed large structural change between the open and closed conformation, would form a suitable and scientifically interesting model system for testing the limitations of SANS on membrane proteins.

Our experiments showed that we could indeed match out both the nanodisc and the detergent carriers, so that we only saw the membrane protein in our SANS experiments [6]. But to our surprise we also found that the SANS data of CorA measured at low and high concentrations of Mg^{2+} and corresponding to the closed and the open conformation were impossible to distinguish. On top of this, our analysis showed that neither the nearly symmetric CorA structure obtained through protein crystallography nor the symmetry broken structure obtained through CryoEM were in structural agreement with our data of the CorA under solution-like conditions. A range of functional and other experiments were carried out in our attempt to solve this mystery. They all pointed towards that our CorA were in a fine and both functionally and structurally relevant state for our experiments [ibid].

We teamed up with the group of Kresten Lindorff-Larsen, University of Copenhagen, who are experts on Molecular Dynamics and molecular modelling, to derive a structural model that would agree with the obtained SANS data. The analysis with the Lindorff-Larsen group pointed towards that both the low and high Mg^{2+} samples had average structures that were significantly symmetry broken and conformationally disperse, though still pentameric [ibid]. We then teamed up with the group of Guido Pintacuda from ENS Lyon. Pintacuda's group

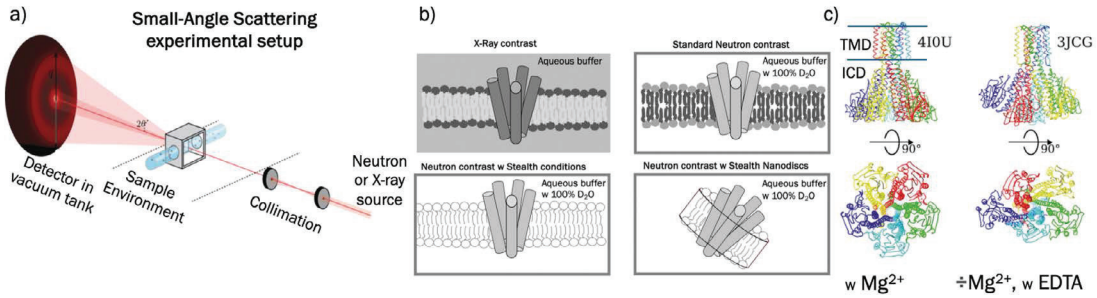

Fig. 1: a) Sketch of the experimental setup for Small-Angle X-ray or Neutron Scattering (SAXS/SANS). b) Illustration of important contrast situations that are obtainable membrane proteins in phospholipid membranes when investigated with, respectively, X-rays (upper left) and neutrons (remaining three illustrations). In the upper-right example the lipid bilayer is produced with standard ^{1}H-containing lipids, while in the two "stealth" cases at the bottom, the lipid bilayer (bottom-left) or the nanodisc (bottom-right) has been made neutron-invisible through systematic exchange from ^{1}H to ^{2}H in the lipids and the surrounding nanodisc belt [1]. This implies that only the membrane proteins are seen in the experiments. c) The CorA membrane protein in two of its observed structural conformations. Left: The nearly pentameric symmetric closed conformation that has been observed by X-ray protein crystallography in presence of Mg^{2+} (pdb code 4IOU [4]) and one of the symmetry-broken conformations that has been observed by Cryo-electron microscopy (pdb code 3JCG [5]) with removed Mg^{2+} that has been proposed to be representative of the open conformation.

are NMR experts and in much the same way as us, they had selected CorA in the expectation that it would be a great model system for investigating and demonstrating membrane proteins by NMR. In accordance with the SANS experiments, they were also surprised to observe minimal structural changes between CorA at low and at high Mg^{2+} in their MAS-NMR, 2D ^{1}H-^{15}N dipolar correlation spectra.

However, a close inspection of their MAS NMR data revealed a small change of the dynamics of the CorA backbone flexibility when removing the Mg^{2+}. The characteristic decay time was significantly shorter without Mg^{2+} than with Mg^{2+} but further, the drop of the decay time was significantly larger for an amino acid sitting in the transmembrane domain than for an amino acid sitting in the intracellular domain [ibid].

Combining the data from the different experiments we propose a gating mechanism between the open and closed CorA that is more subtle than proposed by a simple rigid two-state model with symmetry breaking. To comply with our experimental data, the CorA pentamers must have a broad ensemble of conformations ranging from symmetric to symmetry broken both with and without Mg^{2+}. CorA pentamers can hence be symmetry broken without being open, and we propose that for CorA, and in contrast with the classical text-book explanations, the gating *is not* controlled by the symmetry breaking. Instead, it could be coupled to the observed Mg^{2+} induced change of the backbone flexibility [ibid]. The mechanistic details of how this would come about remain to be fully explored and understood.

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Prof. Dr. Lise Arleth

Lise Arleth is professor of Experimental Biophysics at the Niels Bohr Institute at University of Copenhagen. Since 2020, she has been Vice-dean for research at the Faculty of Science at the same university. Her research specializes on the investigation of biomolecules under solution conditions with Small-Angle X-ray and Neutron scattering. The work of her group has special focus on developing new methods for investigating membrane proteins by also including data from other experiments and computational methods and building on the philosophy and approaches from Integrative Structural Biology.