Experimental report

25/05/2020

Proposal: 8-03-987 **Council:** 4/2019

Title: Probing structural states of pentameric ligand-gated ion channels in match-out deuterated detergent

Research area: Biology

This proposal is a resubmission of 8-03-963

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Samples: GLIC
DeCLIC

Instrument	Requested days	Allocated days	From	To
D22	1	1	20/01/2020	21/01/2020

Abstract:

Pentameric ligand-gated ion channels mediate electrochemical signal transduction in neuronal and other cellular systems, and are critical targets of disease mutations and drugs. However, the structural changes associated with gating and modulation in this family remain unclear. The bacterial protein GLIC has provided a valuable model system, but has raised recent questions as to the functional states of known crystal structures, particularly the existence of an additional, more expanded open state. We propose to answer these questions by small-angle neutron scattering experiments, taking advantage of deuterated match-out detergents, and the high-flux and state-of-the-art detector at D22 to collect structural data free from crystal artefacts or detergent signal. Resolving between conflicting models of GLIC open and closed states in this way will provide critical templates for biophysical characterization and drug design.

Introduction

We measured kinetic SANS of lipid exchange between nanodiscs, and static SANS from three ion channels; GLIC, DeCLIC, and CorA. To hide the scattering signal from the detergent micelle around the transmembrane domains of the ion channels we utilized the detergent n-dodecyl β-D-maltoside (DDM) deuterated to match out in 100% D_2O [1].

Static SANS experiments

Magnesium transport protein CorA

Magnesium transport protein CorA (CorA) is the main uptake system for magnesium ions in prokaryotes. CorA is gated by intracellular magnesium depletion, with large conformational changes expected between the resting and active state. Finally, it is believed to possess periplasmic high-affinity sites (K_d of µM) and intracellular regulatory low-affinity sites (K_d of mM) . To study the solution structure of CorA under different levels of bound Mg^{2+}, we measured it at three conditions: 40 mM Mg^{2+}, 1 µM Mg^{2+}, and magnesium free (Figure 1).

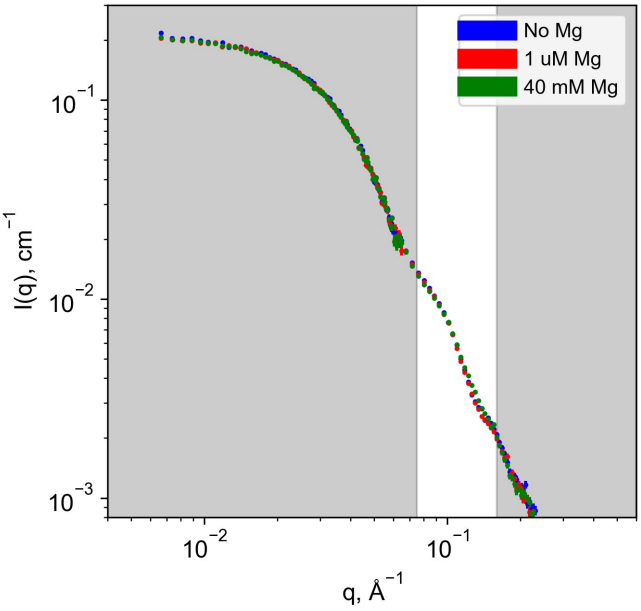

Figure 1: *Scattering curves from CorA at different magnesium concentrations, highlighting the Q-range where the curves display slight differences.*

***Gloeobacter violaceus* ligand-gated ion channel**

Gloeobacter violaceus ligand-gated ion channel (GLIC) is a bacterial ligand-gated ion channel that is used as a model system for eukaryotic channels of the same family. An ongoing question in the field is what constitutes an open state. High-resolution structures of GLIC in several distinct conformations have been determined, and other conformations have been seen in the structures of related channels. However, there are still questions regarding the functional relevance of these states and how representative they are of the channels at room temperature. To investigate this we are probing the solution structure of GLIC through SANS.

We measured GLIC under two buffer conditions; a resting condition (pH 7.5), and an activating condition (pH 3). To maximize the possibility of capturing the open state, we measured both wild type GLIC (GLIC-WT), and the F238L GLIC mutant (GLIC-F238L), which has increased proton sensitivity. In a previous experiment [2] we measured GLIC-WT at pH 7.5

and GLIC-F238L at pH3. In this experiment we measured GLIC-WT at pH 3 and GLIC-F238L at pH 7.6, which overlaid with the previous data reveals that the activating condition shifts the curve in accordance with expectations (Figure 2).

Minor aggregation was present in the samples, so in our upcoming experiment 8-03-1002 we will measure the worst affected samples again using SEC-SANS, which in our experience with these samples completely removes aggregates. However, SEC-SANS data tends to be noisier than cuvette SANS data, and the differences between states that we are aiming to capture are slight. Currently we are using two detector distances (2m and 11.2m) to get good coverage of both the Guinier region and the high Q region, which means that two SEC-SANS runs are needed for each sample. With a two detector set-up that can capture both high and low Q at once it would be possible to use the full amount of protein in a single SEC-SANS run rather than splitting it over two, and thereby increase the signal and thus the quality of the data. For us this would especially improve our data collected with SEC-SANS, but the reduced time needed per sample would also apply to cuvette mode SANS.

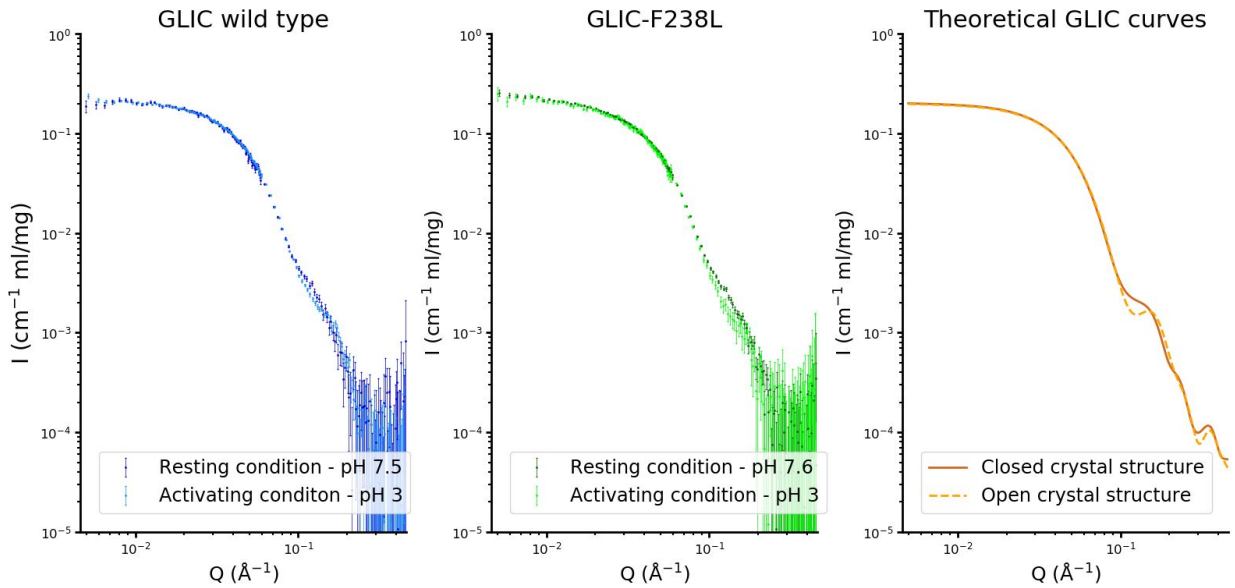

Figure 2: *SANS curves from GLIC, from left to right: GLIC wild type experimental curves at pH 7.5 and pH 3, GLIC-F238L experimental curves at pH 7.6 and pH 3, theoretical curves of GLIC calculated from the closed state crystal structure 4NPQ and the open state crystal structure 4HFI.*

Desulfofustis sp. ligand-gated ion channel

Desulfofustis sp. ligand-gated ion channel (DeCLIC) is a bacterial pentameric ligand gated ion channel for which structures have recently been determined through X-ray crystallography, revealing both a closed and a wide open state. To reveal if the states from the crystal structures are consistent with the conformations assumed when not in a crystal packing, we are applying single particle cryogenic electron microscopy and SANS. The DeCLIC sample for this beamtime was contaminated by H_2O, giving a flat scattering curve with a high intensity.

Kinetic SANS experiments

To investigate the exchange of lipids between nanodiscs of different composition and size we performed kinetic SANS measurements at temperatures between 30 and 37ºC. At time 0 a sample with hydrogenated lipids was mixed with a sample with deuterated lipids, the low Q intensity was then monitored over time until the nanodiscs reached contrast match with the buffer (50% D_2O), which is also the point at which full exchange has been reached. From these experiments the exchange rate of lipids between the nanodiscs can be determined (Figure 3).

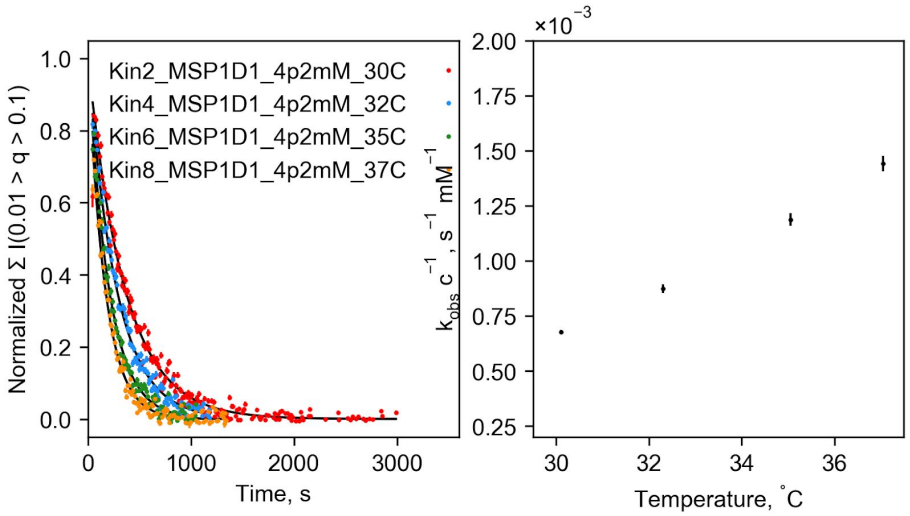

Figure 3: *Kinetic SANS measurements from a membrane scaffolding protein nanodisc at several temperatures, from left to right: Decay of intensity over time at different temperatures, observed rate constants plotted versus temperature.*

Concluding remarks

Our experience of D22 is overall very positive, from the instrument performance, to the format of the resulting data, and wonderful staff support from Anne Martel and Lionel Porcar. For instrument upgrades that would further improve the data we collect at D22, we suggest a two detector set-up that can simultaneously collect at low and high Q. This would especially benefit SEC-SANS collections as samples wouldn't need to be split to cover the full Q-range. We greatly appreciate the SEC-SANS set-up at D22, and as we often need both cuvette-mode SANS and SEC-SANS data from our samples we would love to see the set-up improved even further.

References

[1] Midtgaard SR, Darwish TA, Pedersen MC, Huda P, Larsen AH, Jensen GV, Kynde SA, Skar‐Gislinge N, Nielsen AJ, Olesen C, Blaise M. Invisible detergents for structure determination of membrane proteins by small‐angle neutron scattering. The FEBS journal. 2018 Jan;285(2):357-71.

[2] Arleth L, Blemmer S, Johansen NT, Kassem N, Larsen AH; Lycksell M, Martel A, Orioli S, Porcar L, Tidemand FG. SEC-SANS for investigation of three challenging and biologically relevant membrane proteins. Institut Laue-Langevin (ILL). 2019. doi:10.5291/ILL-DATA.8-03-959