Experimental report

27/07/2021

Proposal: 8-03-1002 **Council:** 10/2019

Title: Size exclusion chromatography coupled SANS to elucidate the solution structure of three prokaryotic ligand-gated ion channels

Research area: Biology

This proposal is a new proposal

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Samples: DeCLIC
ELIC
DeCLIC, Ca2+ insensitive mutant
sTeLIC
DeCLIC, N-terminal domain truncated

Instrument	Requested days	Allocated days	From	To
D22	2	2	21/08/2020	22/08/2020

Abstract:

Pentameric ligand-gated ion channels mediate fast signal transduction in a variety of excitable cells, and are important drug targets. These channels are also found in bacteria, where their function and dynamics remain largely unexplored. The prokaryotic channels are valuable model systems and may offer insights into states that are difficult to capture in the eukaryotic homologs, especially the conducting state. Structures of bacterial channels in this family have been solved with pore sizes ranging from wide to narrow, but uncertainty remain in the field as to how these states correspond to functional states. We propose to probe this question by using SANS to explore the variability in pore dimensions among the related bacterial channels ELIC, sTeLIC and DeCLIC. Use of deuterated detergent and the SEC-SANS capabilities of D22 will give structural data free from crystal artefacts and detergent signal. This investigation promises to demonstrate the value of SANS in annotating functional states of membrane proteins under room-temperature solution conditions, and to provide insights about the ion pores of the wide but poorly characterized family of bacterial ligand-gated channels.

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Introduction

We measured SEC-SANS on two ion channels using a “pseudo-cuvette” approach where the SEC run was paused when the sample peak was in the neutron beam, allowing for data collection at two detector distances during a single run of sample.

Experiments

The pseudo-cuvette SEC-SANS approach

For a protein where the conformational changes are moderate to small it is often necessary to measure SANS at two detector distances to capture both the Guinier region and the high Q-region. When employing SEC-SANS to prevent aggregation this has necessitated splitting the sample over two separate runs. As more protein gives a stronger signal and that the amount of protein available often is limited, it is often desirable to run it as one sample rather than as two. To do so and cover the desired Q-range (0.006-0.45 $Å^{-1}$) we chose to pause the SEC flow when the peak max was in the neuron beam and measure our second detector distance (8 m) while the flow was paused, mimicking a cuvette-mode measurement. We also collected at the first detector distance (2.8 m) under these conditions, albeit for a shorter time, to complement the data collected with a slow flow speed during the SEC-SANS phase of the approach. Comparison of data previously collected for one of our samples using cuvette-mode SANS and the normal SEC-SANS approach show that SEC-SANS with pseudo-cuvette measurements reduced aggregation compared to cuvette-mode, but not as much as true SEC-SANS (Figure 1).

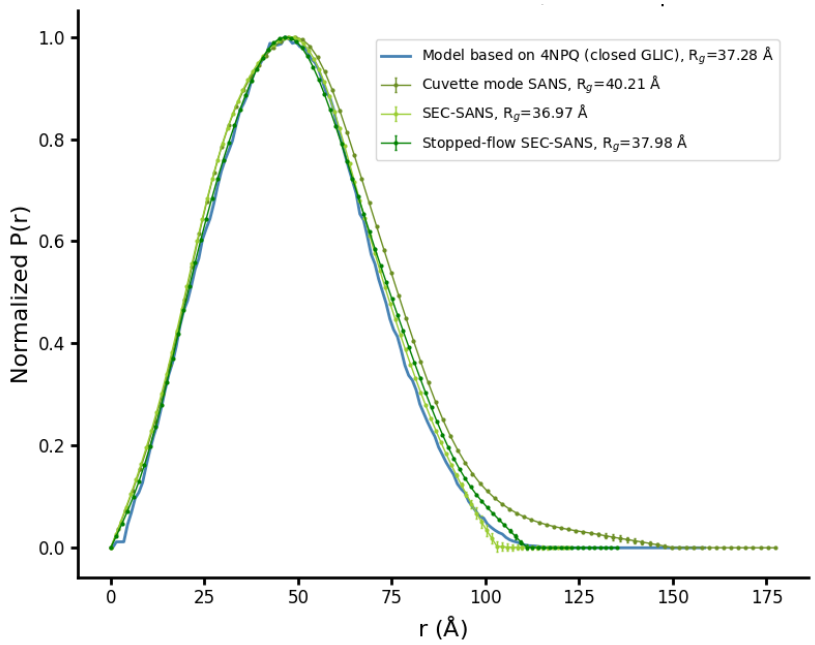

Figure 1: Pair-distance distribution for wild type GLIC from cuvette-mode SANS, SEC-SANS, pseudo-cuvette SANS, and from an all residue model based on the crystal structure 4NPQ.

GLIC

GLIC is a pH activated ion channel which is used as a model system for the receptor family of pentameric ligand-gated ion channels. While structures of GLIC have been determined using X-ray crystallography and cryo-electron microscopy, the solution structure of GLIC has remained an open question. To gain insight into this, and to determine if it is possible to with SANS resolve the subtle differences between open and closed GLIC, we measured wild type (WT) GLIC and the F238L mutant of GLIC at both resting (pH 7.5) and activating (pH 3) conditions. GLIC WT yielded high quality SANS data; in a Guinier analysis both dataset yielded a radius of gyration and molecular weight from I(0) in good agreement with expectations, and at higher Q (~0.1 $Å^{-1}$) a slight difference between buffer conditions was evident (Figure 2). The F238L mutant was measured as this mutation promotes the open state, however it proved too prone to aggregation to yield usable SANS data.

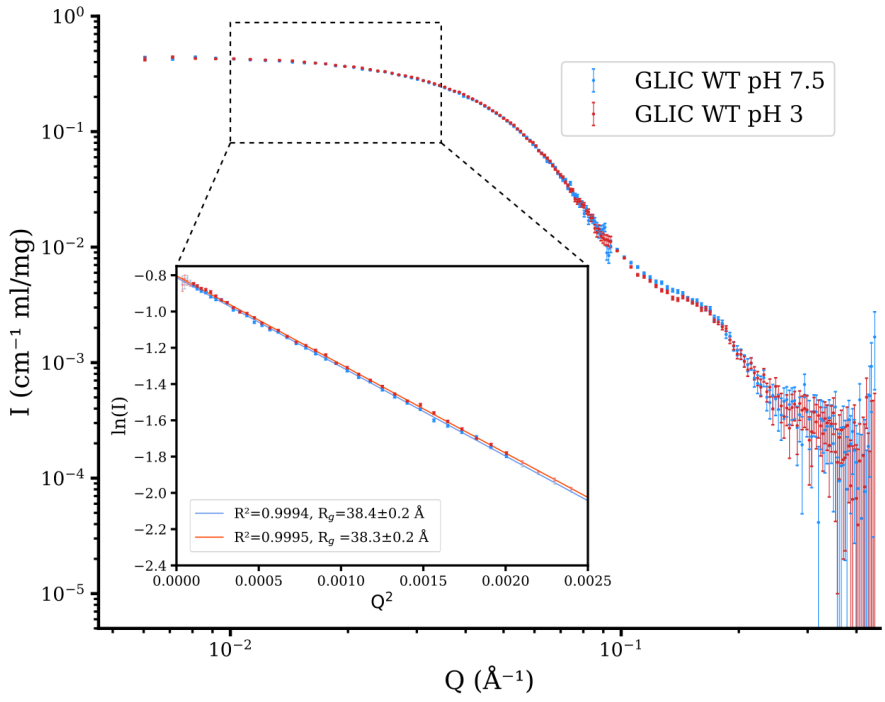

Figure 2: SANS scattering profiles form GLIC WT with the Guinier plot as an insert.

DeCLIC

DeCLIC is a less studied channel in the same protein family as GLIC. DeCLIC makes a suitable target for SANS by virtue of having a large difference between the open and closed conformations, and by having additional N-terminal domains increasing the size of the protein. At the time of this experiment it had been determined that DeCLIC opens upon depletion of Ca^{2+}, so we collected SANS data from DeCLIC at pH 7.5 with 10 mM Ca^{2+} (resting conditions) and with EDTA (activating conditions). The measurements yielded high quality SANS data (Figure 3) with differences between the two buffer conditions at low and high Q. The difference at low Q means that further review of steps like the buffer subtraction and concentration normalization is

warranted to ensure that it is a real difference. As it is, Guinier analysis yields a similar radius of gyration for both conditions, while the molecular weight estimation from I(0) is as expected for the Ca^{2+} containing condition and underestimated for the EDTA containing condition. Fits of the open and closed crystal structures reveal that while the closed structure gives a better fit for both conditions, neither fully explains the solution structure of DeCLIC. This SANS study has thus revealed that the existing structures of DeCLIC are insufficient for describing the conformations available to this channel.

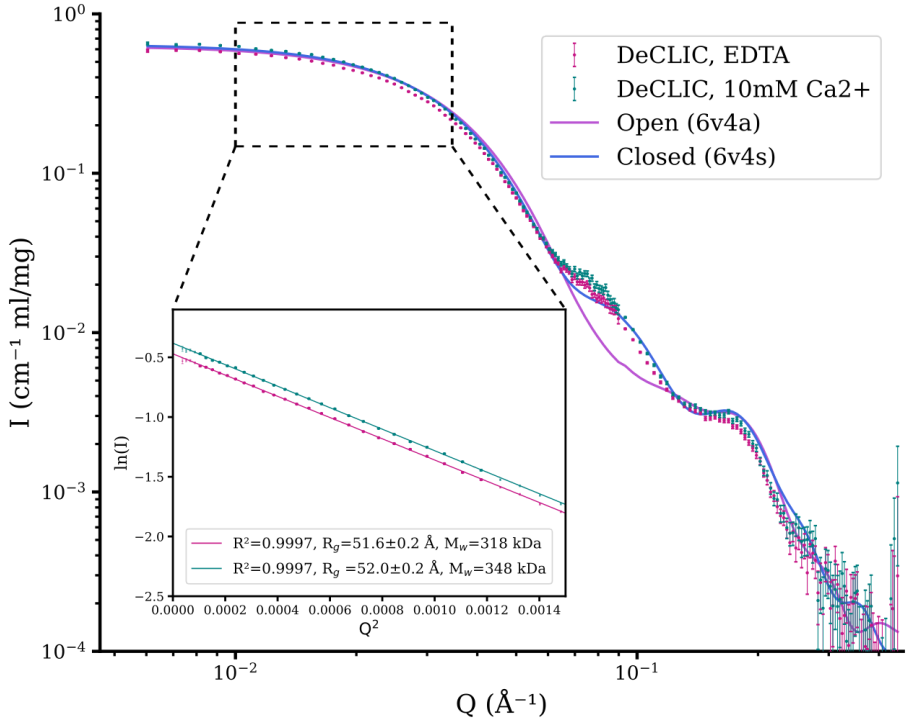

Figure 3: SANS scattering profiles from DeCLIC with the DeCLIC crystal structures fitted to the Ca^{2+} data, and with the Guinier plot as an insert.

Concluding remarks

With this experiment we have all the measurements we need for the GLIC project - for which the manuscript is now submitted - and all measurements at pH 7.5 needed for wild type DeCLIC.

The pseudo-cuvette SEC-SANS measurements provided a good balance between the benefits of cuvette-mode SANS and SEC-SANS, but is labour intensive to execute and dependent on manual decisions from the experimenter as to when to pause the flow. While success was achieved with this approach, the detector upgrade on D22 - which has been installed in the time which has elapsed - allows for collecting at two detector distances at once. This is of great benefit as the full Q-range needed for an experiment on proteins like these then can be collected during a single true SEC-SANS run, or with a paused flow approach to extend the measuring time. With the upgraded detectors the execution of the paused flow approach will be simpler as it will not be necessary to change the detector distances during the experiment.