Experimental report

01/06/2022

Proposal: EASY-861 **Council:** 10/2020

Title: Additional buffer condition for a SEC-SANS study of the ion channel DeCLIC

Research area: Biology

This proposal is a new proposal

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Experimental team:

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Samples: DeCLIC

Instrument	Requested days	Allocated days	From	To
D22	5	5	23/06/2021	24/06/2021

Abstract:

Pentameric ligand-gated ion channels play key roles in converting chemical to electrical signals in the nervous system. Bacterial homologs are valuable structure-function model systems in this complex superfamily. We previously collected SEC-SANS data for the bacterial channel DeCLIC in deuterated detergent. This gave us data from monodisperse protein with a hidden micelle, revealing subtle effects of the modulator calcium on the conformational ensemble. We recently discovered a new open state of DeCLIC, favored below pH 7. We therefore seek to complete our characterization by collecting additional SEC-SANS data at low pH. Based on previous runs (see workflow https://tinyurl.com/yndea6um), one 5-hour session would be sufficient to collect the most critical sample and buffer reference; if another 5-hour slot were available, we would also collect another control with calcium. These results would enable comparative modeling of DeCLIC modulation by two physiologically relevant stimuli, calcium and pH; and would provide validation for our new cryo-electron microscopy structures, possibly representing unexplored regions of the gating landscape.

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Introduction

We measured SEC-SANS from the ion channel DeCLIC, pausing the flow at the peak max to extend the measuring time. DeCLIC is a bacterial member of the pentameric ligand gated ion channels, and is of interest given that it has N-terminal domains not commonly seen in this protein family, and a large difference between the closed and open conformation. Functional studies initially revealed that calcium depletion leads to the channel opening, and we have previously (experiment 8-03-1002) measured SANS from DeCLIC at neutral pH with 10 mM Ca^{2+} (resting conditions) and with EDTA (inhibitor depleted condition). Since those measurements further functional studies have revealed that low pH activates the channel, so in this experiment we collected SANS data at pH 5 with 10 mM Ca^{2+} (activating condition with inhibitor) and with EDTA (activating condition).

Experiment

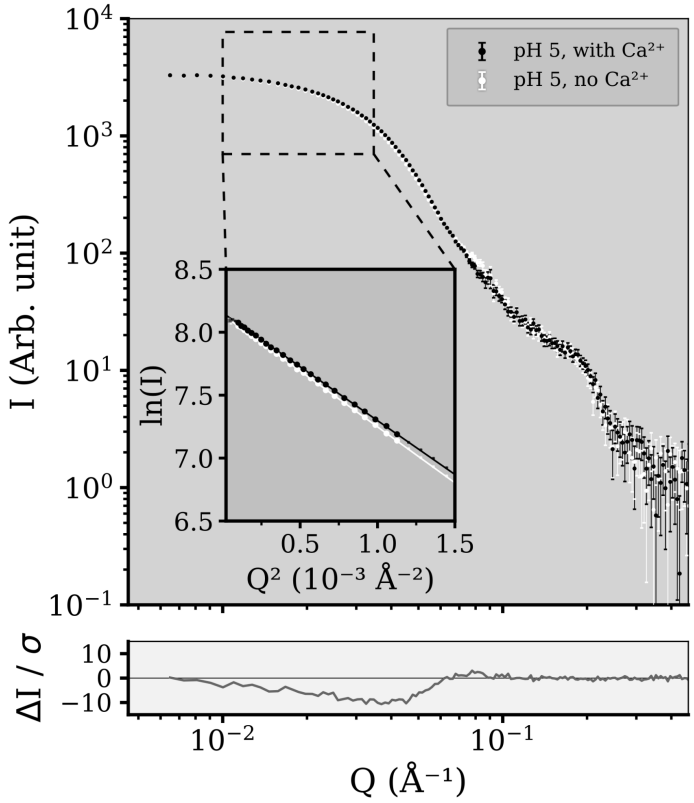

Figure 1: *SANS profiles from DeCLIC in the presence (black) and absence (white) of calcium. Dashed box shows data-points used for Guinier analysis, the insert shows the Guinier plot with the fit shown as lines. The lower panel shows the error weighted residual between the two profiles.*

The collected scattering profiles (Figure 1) were of high quality, and shaped in line with what was expected from DeCLIC. Overall the curves were similar to each other, except for Q-values between 0.06 and 0.1 $Å^{-1}$ where a slight difference was observed. This difference is likely indicative of a shift in the conformational population between the with and without calcium condition. Guinier analysis revealed a slight difference in radius of gyration, 50.7 Å in the presence of calcium, and 51.6 Å in its absence. Comparing the pair-distance distributions of the pH 5 data-sets here collected with the corresponding data-sets previously collected at pH 7, and open and closed models from our cryo-EM work, it can be seen that the distance distribution shifts with buffer condition (Figure 2).

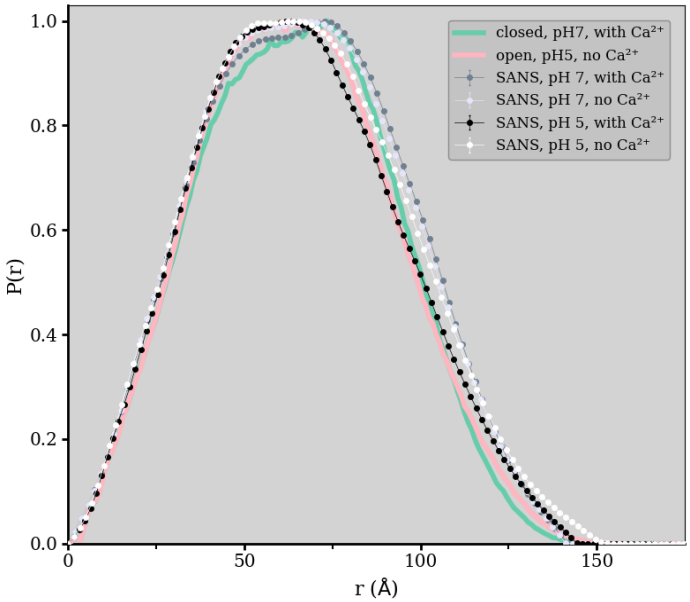

Figure 2: *Pair distance distributions from SANS of DeCLIC at pH 5 with (black) and without (white) calcium, at pH 7 with (dark gray) and without (light gray) calcium. Solid lines show distance distributions calculated from closed (aquamarine) and open (pink) cryo-EM structures.*

Concluding remarks

With this set of measurements we have SANS measurements from the complete suite of experimental conditions currently known to be relevant for DeCLIC, matching the conditions at which we have collected single particle cryo-electron microscopy data.

In this experiment we utilized the recent detector upgrade at D22, which successfully allowed us to measure the full Q-range of interest with a single detector setting and a single SEC-SANS run per sample. We manually paused the flow at the peak max to allow for a longer measuring time, beyond this we could program the experiment in NOMAD to the point that no other manual intervention was required during the execution of the experiment. Here we would like to highlight the utility in the SEC-SANS NOMAD module which allows the flow to be changed when a chosen UV-vis absorbance is reached, which worked like a charm for slowing the flow at the beginning of the protein peak. While a live computational detection of the peak max is unlikely to be feasible due to the normal slight fluctuations of the UV-vis absorbance, it may be useful to expand on the “change flow at absorbance”-functionality to allow for multiple

thresholds. Multiple thresholds would allow an experiment to be programed to hold one flow up to the protein peak, then change to a slow flow for part of the peak up to a higher absorbance at which the flow could be changed to a very slow flow, followed by the flow ramping up through the same steps during the latter half of the peak.