Experimental report

16/09/2024

Proposal: 8-03-1076 **Council:** 4/2023

Title: SANS of heavy-metal transporting P-type ATPases

Research area: Biology

This proposal is a new proposal

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Samples: CadA
delta MBD CadA
ZntA
delta MBD ZntA

Instrument	Requested days	Allocated days	From	To
D22	2	2	24/11/2023	26/11/2023

Abstract:

Heavy-metal transporting P-type ATPases move toxic heavy metal ions out of cells. A critical question is how this transport is regulated. Cytoplasmic metal-binding domains (MBD¿s) have been attributed a central role in regulation, but structural information remain scarce. We propose to use SANS to describe the position and conformational freedom of regulating MBD¿s for two heavy-metal transporting P-type ATPases ¿ with specificity for cadmium (CadA) and zinc (ZntA), respectively. We will utilize mutant proteins lacking the MBD¿s, match-out deuterated detergent to avoid scattering contributions from the micelles and ligands to promote specific conformational states. We will use SEC-SANS to collect data with protein aggregation minimized, and for additional liganded conditions we will perform measurements in standard cuvette-mode. To achieve the structural refinement, we will use ensemble optimization combined with molecular dynamics simulations. All in all, these experiments will provide direct structural information on the regulation of heavy metal transport by P-type ATPases.

Experimental report for experiment 8-03-1076

This experiment measured size exclusion coupled small angle neutron scattering (SEC-SANS) from two heavy metal transporting P-type ATPases: ZntA and CadA. For both proteins, measurements were made of both the full length proteins (WT) and of a deletion mutant (ΔMBD). In the aim of obtaining high quality data by maximizing the contrast between the protein and the buffer, the experiments were performed using 100% D_2O buffers. Match-out deuterated lipids and nanodiscs were used as membrane mimetics, meaning that the reduced scattering profiles represent the scattering of the membrane protein alone. As this family of proteins have been known to be sensitive to aggregation at high D_2O percentages in the buffer, the exchange to deuterated membrane mimetics was done by an off-line SEC run with H_2O based buffer, and the final exchange to D_2O based buffers was done during the in-line SEC-SANS experiment, using a 3 ml Superdex column.

The ZntA measurements in match-out deuterated detergent (d-LMNG) yielded usable scattering profiles for the full length WT construct, while the deletion mutant showed clear signs of aggregation under a couple of the tested conditions, Figure 1. Four buffer conditions were tested: with zinc present, with zinc absent ensured by presence of the chelating agent EGTA, with the ligand AMP-PcP and zinc present, and the ligand ADP in the absence of zinc with EGTA present. Analysis of the ZntA WT data suggest that the species measured in the experiment was dimeric (Table 1) rather than the monomer expected in corresponding H_2O conditions. ΔMBD ZntA appears to have been dimeric or trimeric in the EGTA containing conditions, and aggregated in the zinc containing conditions. While interpretation of this ZntA WT data is complicated by it likely being from dimers, there were differences in radius of gyration observed between tested buffer variations. The buffers for ZntA in detergent were D_2O, pH 6.8, 20 mM MOPS, 80 mM KCl, 5 mM $MgCl_2$, 1 mM TCEP, 0.01% w/v d-LMNG, one of the following sets of additions: 0.1 mM $ZnCl_2$, 1 mM EGTA, 0.5 mM AMP-PcP with 0.1 mM $ZnCl_2$, and 0.5 mM ADP with 1 mM EGTA.

ZntA WT reconstituted in match-out deuterated nanodiscs were also measured. Two different lipids were tried, deuterated DMPC (d-DMPC) and *E. coli* polar lipids extracted from bacteria grown in heavy water. While scattering profiles were obtained, the reconstitution in d-DMPC resulted in scattering indicative of aggregation, and the reconstitution in *E. coli* polar lipids gave fairly noisy profiles where we think that the protein concentration was insufficient to achieve the desired data quality, Figure 2. The buffers for the ZntA nanodiscs were D_2O, pH 6.8, 20 mM MOPS, 200 mM KCl, 3 mM $MgCl_2$, 1 mM TCEP, with either 1 mM EGTA, 0.1 mM $ZnCl_2$, or 0.5 mM AMP-PcP with 0.1 mM $ZnCl_2$.

The CadA measurement of WT and ΔMBD in nanodiscs yielded high quality scattering profiles, Figure 3. However, a major issue with the nanodisc measurements is that during sample preparation there seems to have been a mistake and at least one nanodisc reconstitution appears to have used hydrogenated membrane scaffolding protein (MSP) rather than match-out deuterated membrane scaffolding protein. Estimations of molecular weight (Table 2) from the scattering profile suggest that the CadA WT nanodiscs may have been the dataset with hydrogenated MSP, while the CadA ΔMBD could be with the match-out deuterated MSP as intended. The buffer for the CadA experiments was D_2O, pH6, 50 mM MES, 200 mM Kcl, 1 mM $MgCl_2$, 1 mM TCEP, and 0.1 mM $CdCl_2$.

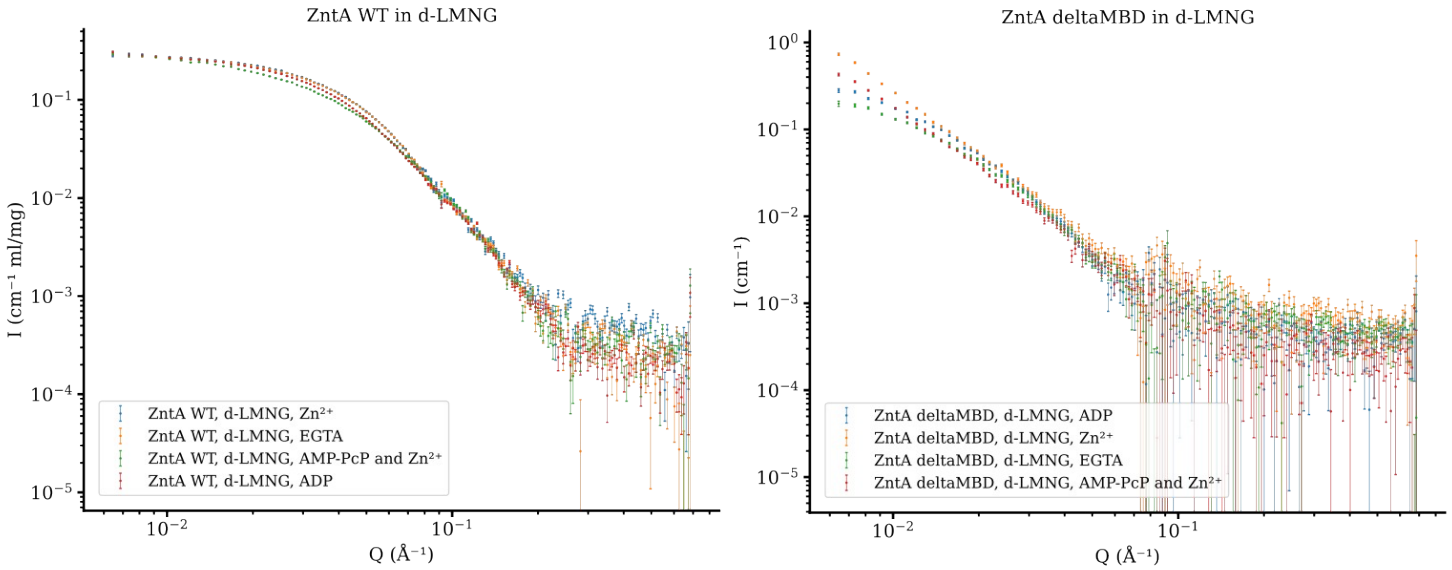

Figure 1: Scattering profiles for ZntA in match-out deuterated LMNG.

Table 1: Expected molecular weight (in kDa) for ZntA WT in d-LMNG, and molecular weights (in Da) calculated from the scattering data using the datmw software.

	ZntA WT			
Expected Mw	77			
	EGTA	**Zn^{2+}**	**AMP-PcP, Zn^{2+}**	**ADP, EGTA**
Mw from Porod volume	146	141	147	154
Mw from Porod invariant	161	153	171	178
Mw from volume of correlation	132	125	133	150
Mw from apparent volume	144	115	148	170
Mw from size and shape	153	140	183	198
Mw from Bayesian inference	147 [134, 151]	138 [121, 142]	147 [134, 151]	170 [151,177]

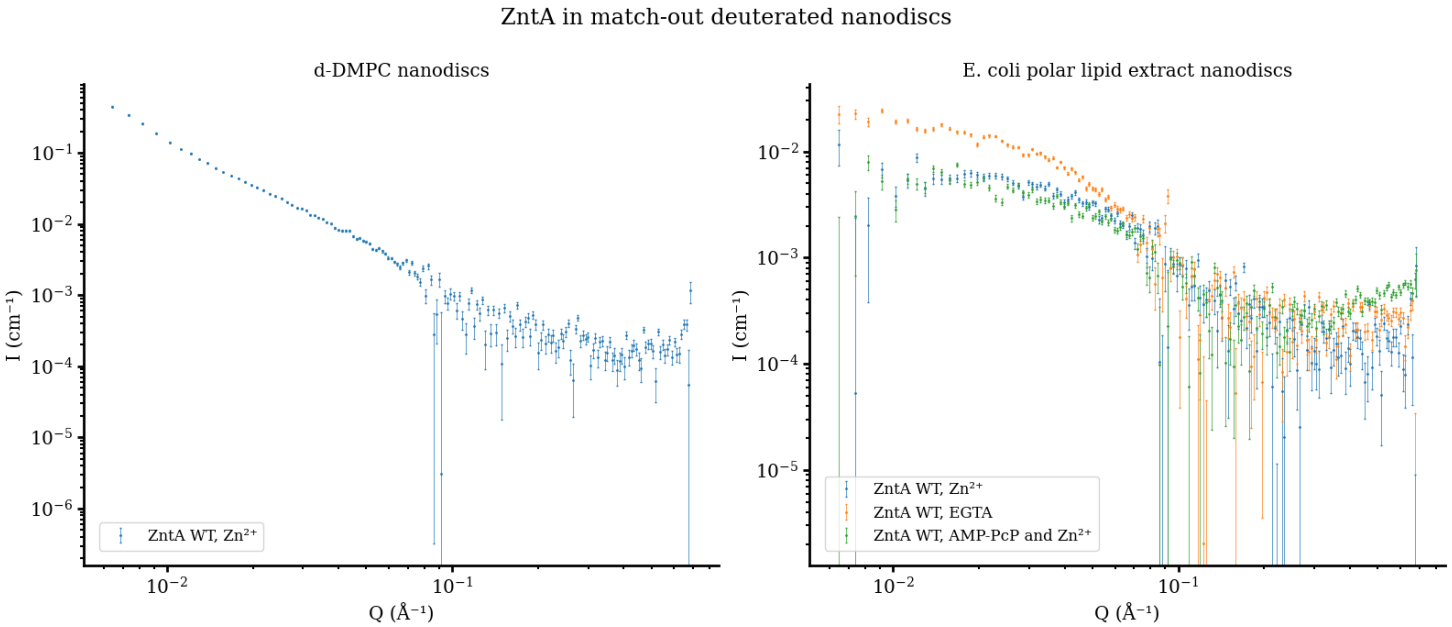

Figure 2: Scattering profiles from ZntA WT in nanodiscs.

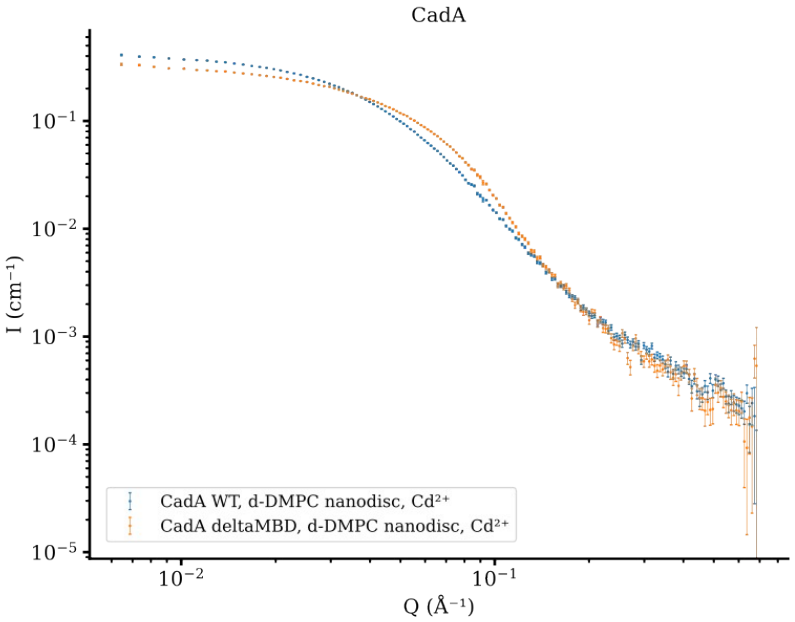

Figure 3: Scattering profile from CadA reconstituted in d-DMPC nanodiscs.

Table 2: Expected molecular weight (in Da) for CadA alone or together with two membrane scaffolding proteins, and molecular weights (in Da) calculated from the scattering data using the datmw software.

	1 CadA:0 MSP	**1 CadA: 2 MSP**	**1 CadA ΔMBD: 0 MSP**	**1 CadA ΔMBD: 2 MSP**
Expected Mw	77089	138632	67820	129363
		CadA WT dataset		**CadA ΔMBD dataset**
Mw from Porod volume		123481		79403
Mw from Porod invariant		144211		92733
Mw from volume of correlation		115421		71957
Mw from apparent volume		122887		92604
Mw from size and shape		154957		84035
Mw from Bayesian inference		124450 [116050,134300]		85650 [81900,89700]