Experimental report

13/02/2019

Proposal: 8-03-879 **Council:** 4/2016

Title: SANS studies of clathrin-mediated endocytosis

Research area: Biology

This proposal is a new proposal

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Samples: protein
lipid

Instrument	Requested days	Allocated days	From	To
D22	2	2	09/10/2016	10/10/2016
			21/03/2018	22/03/2018

Abstract:

Clathrin-mediated endocytosis is crucial for the internalization of most eukaryotic cell-surface proteins. Clathrin-coated vesicles (CCV) assemble with their cargo at the plasma membrane then transport these to the early endosome inside the cell. CCV consist of a clathrin scaffold and a lipid vesicle containing the cargo, linked by adaptor proteins that are associated with effectors of CCV assembly, stability and disassembly.

We have shown that a single adaptor protein AP2 is sufficient to initiate and drive clathrin-coated bud formation on appropriate lipid membranes (Kelly et al., Science, 2014). The resultant buds were spherical and uniform in size (approximately 20-nm radius). We are therefore in a position where we can generate clathrin-coated vesicles with known protein and lipid composition. The opportunity to study simpler and more homogeneous CCVs by SANS will allow us to determine the structural effect of factors, like accessory protein FCHO2, that influence CCV morphology.

Experimental report for D22

October 2016 and March 2018

SANS analysis of clathrin-mediated endocytosis

Proposal No. : 8-03-879

Clathrin-mediated endocytosis is crucial for the internalization of most eukaryotic cell-surface proteins. Clathrin-coated vesicles (CCV) assemble with their cargo at the plasma membrane then transport these to the early endosome inside the cell. CCV consist of a clathrin scaffold and a lipid vesicle containing the cargo, linked by adaptor proteins (adaptins) that are associated with effectors of CCV assembly, stability and disassembly.

We have shown that a single adaptin protein is sufficient to initiate and drive clathrin-coated bud formation on appropriate lipid membranes. More, recently we have focused our research on modulators, that by directly interacting with adaptins, influence CCV assembly.

Experimental data collection

The SANS data was collected in October 2016 and May 2018. The final preparation of samples, including the transfer of adaptin complexes into D_2O buffers, was undertaken on-site. Contrast variation SANS data from a total of 24 samples were collected at 2m and at 8m. Data from relevant buffers were also collected with similar data collection times. Unfortunately, a number of other samples were not analysed after short test exposures. The low neutron count suggested their concentrations were too low to obtain meaningful data in a reasonable time.

Adaptin – modulator complex

We collected contrast variation SANS data from hydrogenated adaptin in complex with a deuterated modulator.

In the absence of modulator, the recombinant adaptin was found to be the expected hetero-tetramer (measured/theoretical MW ~ 0.8). Its Rg showed that the adaptin in solution was similar in size to the adaptin present in crystal structures. The SANS I(0) analysis of the unbound modulator determined that this protein was a monomer in solution (measured/theoretical MW ~ 0.8).

The contrast variation SANS analysis of the partially deuterated adaptin-modulator complex was hampered by the low protein concentrations. SANS data were collected at 4 different D_2O concentrations. After adjusting for concentrations, the contrast match point was found to be 47.2%, suggesting that the complex was formed from adaptin bound to a single modulator (theoretical match point 46.7%).

The Sturhmann analysis proved difficult due to the large error associated with the Rg determination. It was however possible to draw important insights about the interaction of adaptin and the modulator, informing our subsequent biochemical studies. In particular, a previous working model of the adaptin-modulator complex is not supported by these experimental data.

Several other proteins involved in clathrin mediated endocytosis were also studied on D22. Their analysis is complete and will form part of future peer-reviewed publications.