Experimental report

17/02/2020

Proposal: 8-02-851 **Council:** 10/2018

Title: Neutron reflectometry of clathrin-mediated endocytosis

Research area: Biology

This proposal is a continuation of 8-02-804

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Samples: PROTEIN
LIPIDS

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	13/02/2020	15/02/2020
D11	0	1	02/02/2020	03/02/2020

Abstract:

Clathrin-mediated endocytosis is crucial for the internalisation of most eukaryotic cell surface proteins. Clathrin-coated vesicles (CCVs) assemble with their cargo at the plasma membrane then transport these to the early endosome inside the cell. A CCV consists of a clathrin scaffold coating a lipid vesicle, in which the cargo is bound, linked by adaptor proteins, like AP2, that are associated with effectors of CCV assembly, stability and disassembly.
In vivo, AP2 solely interacts with one leaflet of the cellular membrane. Therefore, we have been able to analyse the first stages of CCV assembly by using cargo embedded in a lipid monolayer and Langmuir trough methods. This continuation proposal will now focus the NR studies onto the structure/function relationship of CCV modulators (like EPS15, FCHO2, NECAP and ARF6), which directly interact with AP2 at the cellular membrane.

Experimental report for FIGARO

February 2020

NR analysis of clathrin-mediated endocytosis

Proposal No. : 8-02-851

Clathrin-mediated endocytosis (CME) is the main mechanism by which eukaryotic cells internalize and recycle most membrane proteins (termed cargo). It is driven by different Adaptor Proteins which directly link the clathrin scaffold to cargos, and modulated by accessory proteins. Simplifying the system down to its core elements is enabling us to address a number of important questions.

The focus of this beamtime was on the clathrin adaptor CALM, as well as on the AP2-modulators EPS15 and FCHO2.

Experimental data collection

The Neutron reflectometry data was collected 13th to 15th February 2020, just prior to the proposal deadline (the 17th). The final preparation of proteins and lipids was undertaken on-site. Neutron reflectometry data from a total of five different protein-lipid systems, under different lateral lipid pressures, were each collected at two angles and at three contrast D_2O levels. Data from the relevant lipid monolayers (without proteins) at different pressures were also collected with similar data collection times.

Preliminary analysis

Although binding could be measured by lateral pressure changes in the adsorption trough, no clear differences in the neutron scattering could initially be observed for the N-terminal of the protein CALM. Lateral pressure was therefore reduced to mimic less packed lipids present on curved lipid bilayers, and the experiment repeated. We were then able to measure by NR clear protein binding to the lipid monolayer (thickness ~40-Angstroms, which is compatible with the shortest width of the protein from its structure), while in parallel still observing increase in lipid lateral pressure due to protein' binding.

The binding to a lipid monolayer of both full length and truncated FCHO2 were also investigated by NR. Clear binding by NR could be detected at typical lipid lateral pressure (25 $mN.m^{-1}$, which is the one found in cellular membranes). We were subsequently able to collect NR data after the addition of EPS15 to full length FCHO2. Importantly, EPS15 was not observed to bind truncated FCHO2, as this protein lacks the EPS15 binding site.

As soon as these analyses are complete, these data will form part of future peer-reviewed publications.