Experimental report

13/02/2019

Proposal: 8-02-829 **Council:** 4/2018

Title: Neutron reflectometry studies of clathrin-mediated endocytosis: effect of modulators FCHO/Eps15

Research area: Biology

This proposal is a continuation of 8-02-795

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Samples: synthetic lipids
Proteins - non-infectious, non-toxic

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	09/10/2018	11/10/2018

Abstract:

Clathrin-mediated endocytosis (CME) is the main mechanism by which eukaryotic cells internalize and recycle most membrane proteins (termed cargo). CME is a fairly well understood process in terms of the molecules involved. It is driven by different Adaptor Proteins (such as AP2), which directly link the clathrin scaffold to cargo, as well by modulators (like accessory proteins FCHO1/2 and EPS15), which temporarily regulate clathrin-coated vesicle (CCV) assembly and dissassembly. Mutations affecting endocytosis, both in the cargo as well as in the modulators, have been directly linked to cancer.
In vivo, AP2 solely interacts with one leaflet of the cellular membrane, therefore, our approach to date has been to build simple, in vitro, experimental models to quantify by Neutron Reflectometry the physical structures formed by AP2 and clathrin on association to planar, cargo-embedded lipid monolayers (article in preparation). This continuation proposal will now focus on the molecular effect of modulators FCHO2 and Eps15 in facilitating conformational activation of AP-2, prior to clathrin binding.

Preliminary Experimental Report for Figaro

October 2018

NR analysis of clathrin-mediated endocytosis

Proposal No. : 8-02-829

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). *In vivo*, AP2 solely interacts with one leaflet of the cellular membrane, therefore, our approach to date has been to build simple, in vitro, experimental models to quantify by Neutron Reflectometry the physical structures formed by AP2 and clathrin on association to planar, cargo-embedded lipid monolayers (article in preparation).

Experimental data collection

The main goal of these experiments were to use SNR to probe the interaction of AP2 and clathrin with the lipid monolayer by obtaining the interfacial structure of the lipid monolayer bound to several AP2 molecules and the clathrin lattice.

Several specular neutron reflectometry datasets were therefore collected in October 2018. The AP2 and clathrin proteins were prepared in Cambridge, prior to being sent to Grenoble for data collection. The final preparation of samples, including the preparation of lipid mixtures, was undertaken on-site.

Contrast variation NR data was collected in ACMW and 100% D_2O buffer. The different Langmuir troughs were prepared with two types of cargo (TGN and CD4 respectively). It is important to note that these two cargo interact differently with AP2 (different binding site, and different behaviour as observed by rheometry).

After observing the binding of AP2 to the lipid monolayer from pressure changes (in the Langmuir trough) and more importantly changes in the SNR profiles, we added clathrin to the trough. A further change in the SNR profile suggests that the clathrin was interacting with the membrane. From our previous work, this interaction is only possible through the cargo-bound AP2.

We are now analysing the SNR profiles obtained to gain insight in the position of clathrin with respect to the lipids and to AP2. These NR data should therefore provided important insights into CCV formation. Importantly, these new NR data should address the influence the clathrin scaffold has on the lipids.