Experimental report

04/02/2016

Proposal: 8-02-754 **Council:** 4/2015

Title: Real-time monitoring of the change in lipid vesicle structure upon amyloid fibril formation

Research area: Biology

This proposal is a new proposal

Main proposer: Celine GALVAGNION

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Local contacts: Anne MARTEL

Samples: aqueous solution of protein and lipids

Instrument	Requested days	Allocated days	From	To
D22	2	2	11/09/2015	13/09/2015

Abstract:

We are aiming to apply small angle neutron scattering in order to study whether or not the nucleation of alpha-synclein amyloid fibrils in presence of lipid SUVs leads to the formation of lipid-protein co-aggregates. In particular, using contrast matching of the protein and by following the time evolution of the form factor of the lipids, we focus on the question whether the lipid molecules remain quantitatively within spherical vesicles or whether, at which stage and in which manner they are partly incorporated into the growing fibrils.
Co-aggregation of lipids and amyloid fibrils can lead to disruptions of the lipid membrane and could hence be at the origin of the toxicity induced by the aggregation of alpha-synclein that ultimately leads to neuronal death. The proposed experiment will thus tackle a very basic question of protein science, with potential outreach for a better understanding of neurodegenerative diseases.

Report for proposal 8-02-754

Researchers involved: Dr. Celine Galvagnion, Dr. Felix Roosen-Runge, Dr. Alexander Buell and Prof. Christopher Dobson

Scientific contacts at ILL: Dr. Anne Martel, Dr. Lionel Porcar

Title of the proposal: Real time monitoring of the change in lipid vesicle structure upon amyloid fibril formation

Aim of the study:

We had previously shown that the binding of the intrinsically disordered protein α-synuclein to vesicles made from negatively charged lipids can dramatically accelerate the rate of amyloid fibril formation of this protein (1). Certain features of the aggregation process in the presence of lipids (distinct morphology, quantity of fibrils proportional to initial lipid concentration, rather than initial protein concentration) led us to suspect that the lipids are incorporated into the protein aggregates. The aim of this proposal was to study the aggregation process by means of neutron scattering in order to find out whether or not the lipids are indeed incorporated into the protein aggregates.

Experimental strategy:

We had planned to perform experiments with two different types of lipids, DMPS and DLPS. In both cases, we had shown that the incubation of α-synuclein with the lipids leads to aggregate formation, with the kinetics in the presence of DLPS being much faster than that in the presence of DMPS (2). We intended to contrast-match the protein component (via adjustment of the D_2O content of the solution) in order to selectively monitor the lipids, and then follow the aggregation reaction in real time by neutron scattering.

Results:

1) We determined the protein match point to correspond to 40% D_2O (Fig. 1A)
2) We found that while the pure DLPS vesicles gave a scattering intensity as a function of scattering vector in very good agreement with a shell structure (Fig. 1B), the DMPS lipid yielded a signal indicative of a less well-defined and more polydisperse structure (Fig. 2A, black trace)

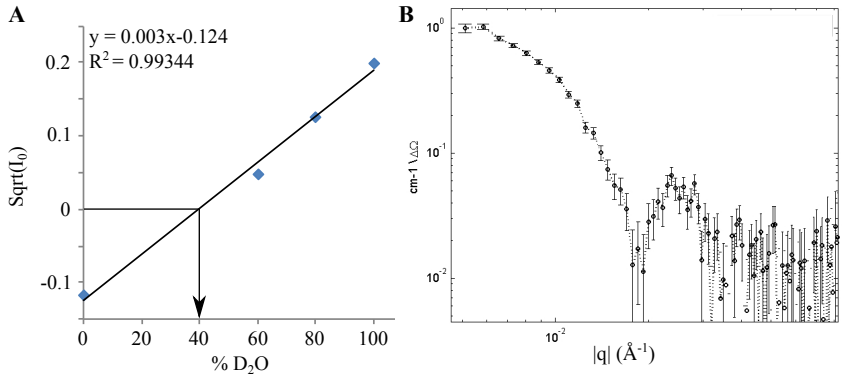

Figure 1: Match point of the protein and scattering function of 1 mM DLPS at the match point conditions of the protein (40% D_2O).

3) Interestingly, and highly unexpectedly, upon addition of the α-synuclein, the scattering intensity at low q values drastically decreased, for both DMPS and DLPS, on a timescale of seconds to minutes (Fig. 2A and B, respectively). For the DLPS, this decrease in intensity at low q was also accompanied by the loss of the undulations characteristic of a shell structure.

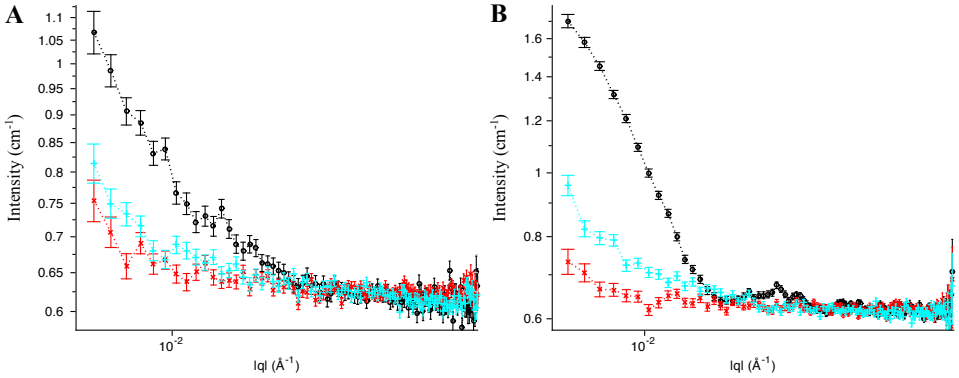

Figure 2: Scattering function of 1 mM DMPS (A) or DLPS (B) in the absence (black) and in the presence (red and cyan) of 200 uM α-synuclein, respectively (40% D_2O).

4) This initial decrease was, in the case of DLPS, followed by a slow increase in scattering intensity at low q (Fig 3A), on a timescale of hours, consistent with the emergence of Thioflavin T fluorescence in bulk aggregation assays (Fig 3B). Therefore, we attribute this increase in scattering intensity to the formation of fibrillar structures. The kinetics in the case of DMPS were found to be too slow to lead to a significant change in scattering amplitude during the time scale of the neutron scattering experiments.

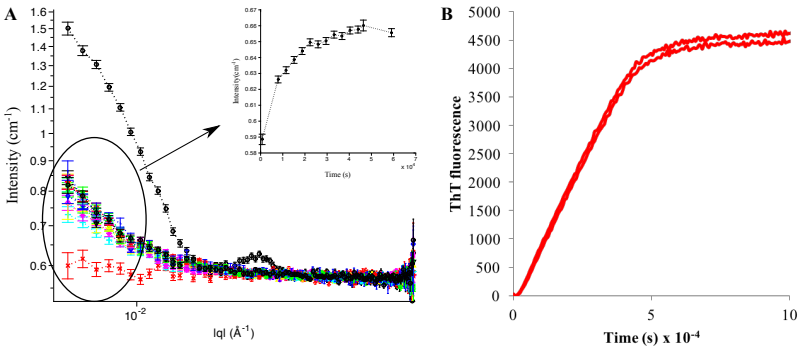

Figure 3: A. Scattering function of 1 mM DLPS in the absence (black) and in the presence (multicolor) of 200 uM α-synuclein (40% D_2O) (Inset: evolution of the scattering intensity at low q with time). B. Change in the ThT fluorescence when 100 uM DLPS is incubated in the presence of 20 uM α-synuclein at 30C.

5) We also performed experiments in pure D_2O (for higher scattering intensity), where we added the protein in several small aliquots to the lipid vesicles, in order to see whether the initial collapse of scattering intensity could be resolved into smaller steps (Fig 4). This was indeed the case as we found that the scattering intensity could be decreased stepwise by adding more and more α-synuclein up to the point where the lipid vesicles were completely saturated.

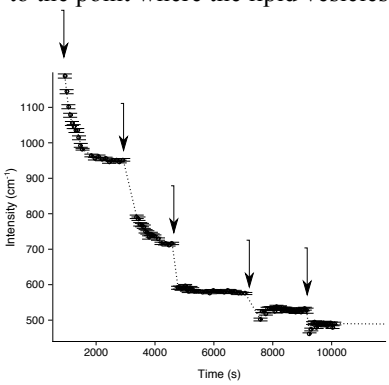

Figure 4: Scattering intensity at low q measured for 1 mM DLPS in the absence (red) and after adding 5 uM of α-synuclein (black arrows).

Conclusions:

The experiments we have been able to carry out have radically changed our picture of the protein-lipid system that we are investigating. Previously, we assumed that the lipid vesicles, upon incubation with α-synuclein, are covered by a layer of the protein, while largely staying intact. However, our data suggests that the binding of the protein to the lipid vesicles induces the collapse of the latter, leading to the formation of mixed aggregates, from which eventually protein fibrils can emerge.

Thanks to these intriguing results, and thanks to the outstanding scientific support and feedback we have received from our local contacts and instrument supervisors, we are now able to propose a range of follow up experiments that should allow us to obtain a more detailed insight into the structural rearrangements induced by the binding of the protein to the lipids. We would like to focus on the DLPS system, due to its rapid time course of aggregation and we propose to extend the previous study by using deuterated lipids, as well as deuterated protein, in order to be able to selectively study both components in separate time course experiments. We believe that while these experiments involve simple model lipids, they reveal a phenomenon of potentially very general importance, namely structural changes in lipid membranes induced by protein binding and therefore we hope that we will be allocated additional beam time to finish this project and obtain the missing data necessary for a publication.

References:

1. Galvagnion C, Buell AK, Meisl G, Michaels TC, Vendruscolo M, Knowles TP, Dobson CM. Lipid vesicles trigger α-synuclein aggregation by stimulating primary nucleation. Nat Chem Biol. 2015 Mar;11(3):229-34.

2. Galvagnion C, Brown J, Ouberai MM, Flagmeier P, Vendruscolo M, Buell AK, Sparr E, Dobson CM. The chemical properties of lipids strongly affect the kinetics of the membrane-induced aggregation of α-synuclein. Submitted to PNAS.