Experimental Report

15/09/2014

Proposal: **8-02-679** **Council:** 10/2012

Title: Cell Membrane destabilisation by IAPP fragments

This proposal is a new proposal

Researh Area: Biology

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Samples:
DPhPC
POPC
POPS
H-Amylin 1-37
H-Amylin 1-20
H-Amylin 8-20
H-Amylin 8-37
H-Amylin 23-37
DMSO

Instrument	Req. Days	All. Days	From	To
D17	3	3	02/07/2013	05/07/2013

Abstract:

IAPP is an amyloid peptide which causes diabetes by killing beta-pancreatic cells. However, its mechanism of cell destruction is still under debate. It is not clear which structural species is responsible of cell disruption, whether it is the final amyloid fibril, a fibrillation-intermediary polymer or a stable oligomer. Here, we intend to characterize the mechanism of IAPP interaction with a model membrane by studying how different fragments of this peptide adsorbs, inserts and possibly desorbs, once in an oligomeric form, in order to map IAPP sequence for membrane disruption capabilities. The results of this functional study will bring direct information about the role of each part of IAPP in the bilayer disruption mechanism that will have tremendous implication in the development of therapeutics against amyloid diseases.

A large number of diseases (Alzheimer's disease, Diabetes, Creuztfeld-Jacob's diseases…) are associated with amyloidosis, ie the deposition of large and insoluble aggregates of beta-sheet rich protein outside cells. This phenomenon is correlated to important cell depletion in key organs (brain, pancreas…). Although each disease is related to the amyloidosis of a particular peptide killing a specific type of cell, a common mechanism of action is suspected: misprocessing of a peptide leads to its aggregation and misfolding into antiparallel beta-sheet structures forming micrometer-long fibers.
We are involved in understanding how cytotoxicity and amyloid aggregation are related and how the behavior of these amyloid peptides is encoded in their sequence. For this purpose, the Islet Amyloid Polypeptide (IAPP), an amyloid peptide responsible for diabetes, is used as a model. The full length peptide (1-37) and several fragments of it (1-20, 8-20, 8-37 and 23-37, named after the position of their first and last aminoacids in the full-length sequence) have been studied by electrophysiology to measure their propensity to destabilise lipid bilayers, making them permeable to ions. These fragments are pictured in Figure 1, together with literature data about the role of certain regions of the full length peptide in its folding and cytotoxicity.

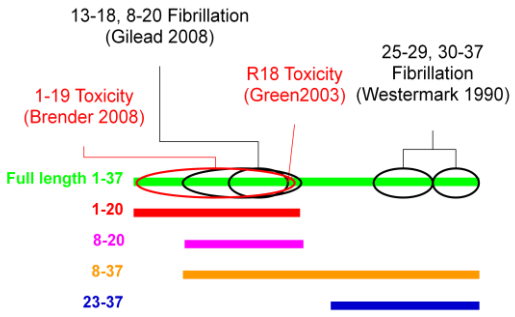
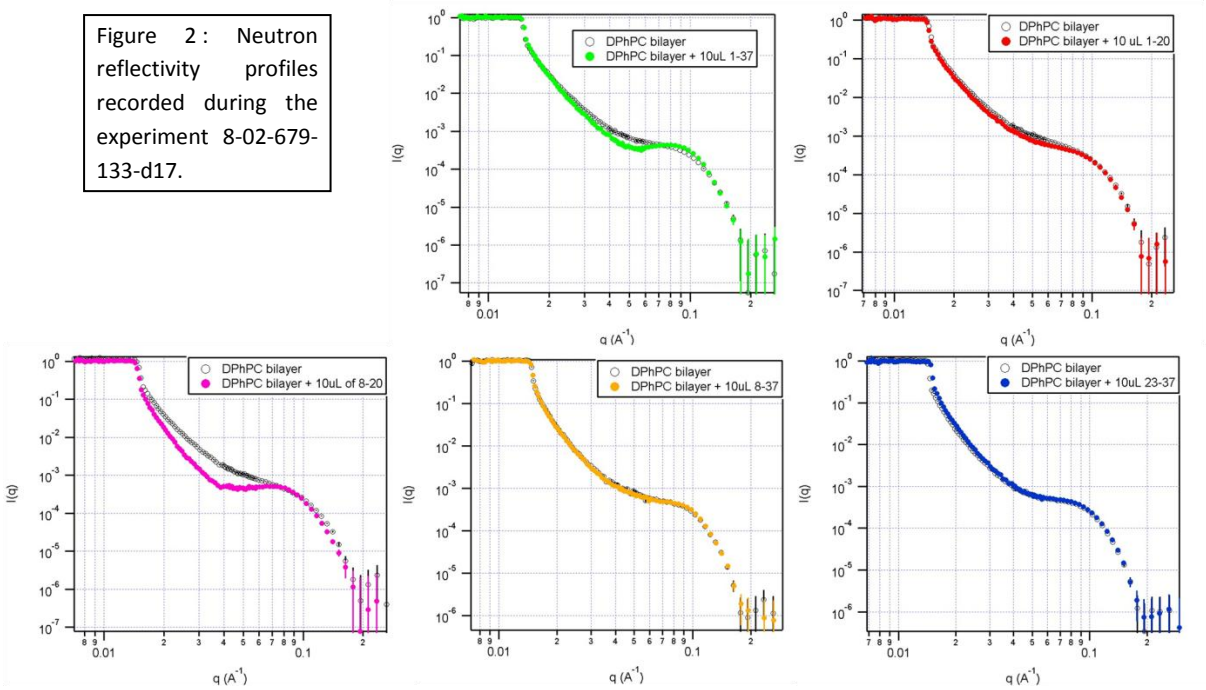


Figure 1: List of IAPP fragments studied and literature data about different regions of the sequence.

Following our Neutron Reflectivity (NR) proposal 8-02-679 we have been granted 3 days of beamtime on d17. We intended to investigate the behavior of each peptide fragments in contact with a lipid bilayer in order to quantify and determine the mechanism of peptide insertion and its conformation within the bilayer. We planned to investigate bilayers of different lipid composition: DPhPC, POPC, POPS and mixtures of these lipids POPC:POPS (9:1, 7:3, 5:5) to mimic membrane of islet cells and to obtain further insight in the importance of the electrostatic interaction on the peptide insertion mechanism.

As first time NR users, we underestimated a lot the time necessary for such a wide study. Our official beamtime 8-02-679 enabled us to learn a lot about the conditions to prepare lipid bilayers, which are variable from a lipid to another, to test the effect of the full length peptide on both DPhPC and POPC bilayers, and provided a few results about the fragments, which are presented Figure 2.

Figure 2 : Neutron reflectivity profiles recorded during the experiment 8-02-679-133-d17.



Although some peptides definitely trigger modifications of the bilayer reflectivity profile, issues concerning the reproducibility and robustness of the bilayer itself kept us skeptical about these results.

The 35 MW cycle of the reactor, just after the long shut-down, was dedicated to test the instruments and some beamtime was attributed to us upon proposals TEST-2358 (on D17) and TEST-2335 (on Figaro). This time, we took advantage of the PSCM Quartz Microbalance to set up robust protocols to deposit reproducibly bilayers of our different lipids with an extremely good coverage. Fortunately, both instruments worked very well in spite of the long shut down and, except for a few minor issues concerning the pump which distribute the samples to the different crystal chambers, we had a very successful beamtime. We recorded full contrast series on the full length peptide in contact of 3 bilayers of different composition: DPhPC, tail-deuterated POPC (d_{31}POPC) and natural perdeuterated lipid mix extracted from yeast (Figure 3). Furthermore, we recorded contrast series of the N-terminal and C-terminal fragments: 1-20 and 23-37 of the peptides, the most interesting ones, as from our electrophysiological studies, they have a completely opposite behavior: 23-37, the most amyloidogenic part of IAPP seems to have no effect on the bilayer permeability while 1-20 would be even more "aggressive" than the full length peptide. As a control, we recorded as well the signal of the rat full length peptide (r1-37) which is not cytotoxic (Figure 4). These data, presented on figure 3 are of fittable quality, and will undoubtedly lead to publication.

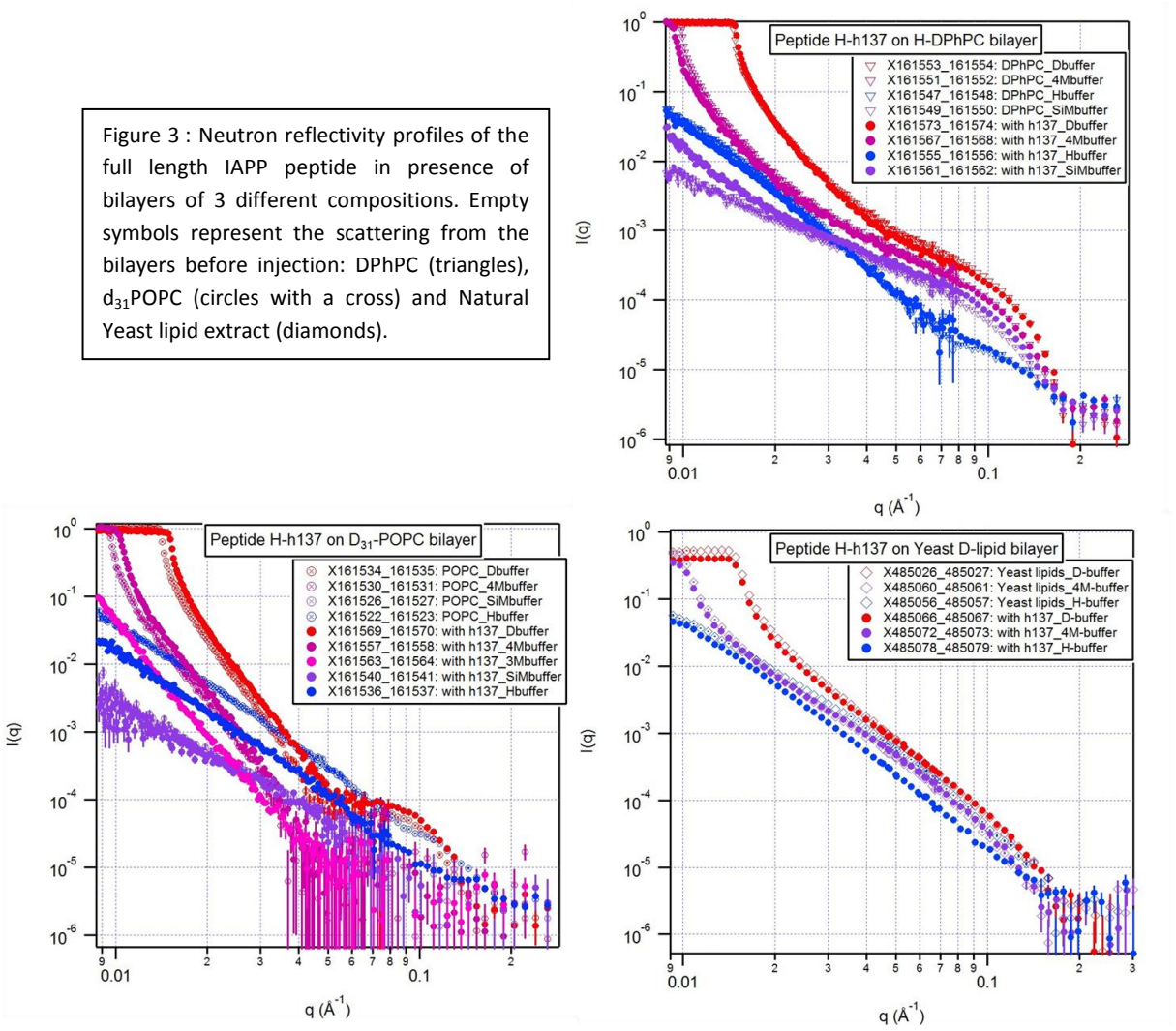


Figure 3 : Neutron reflectivity profiles of the full length IAPP peptide in presence of bilayers of 3 different compositions. Empty symbols represent the scattering from the bilayers before injection: DPhPC (triangles), d_{31}POPC (circles with a cross) and Natural Yeast lipid extract (diamonds).

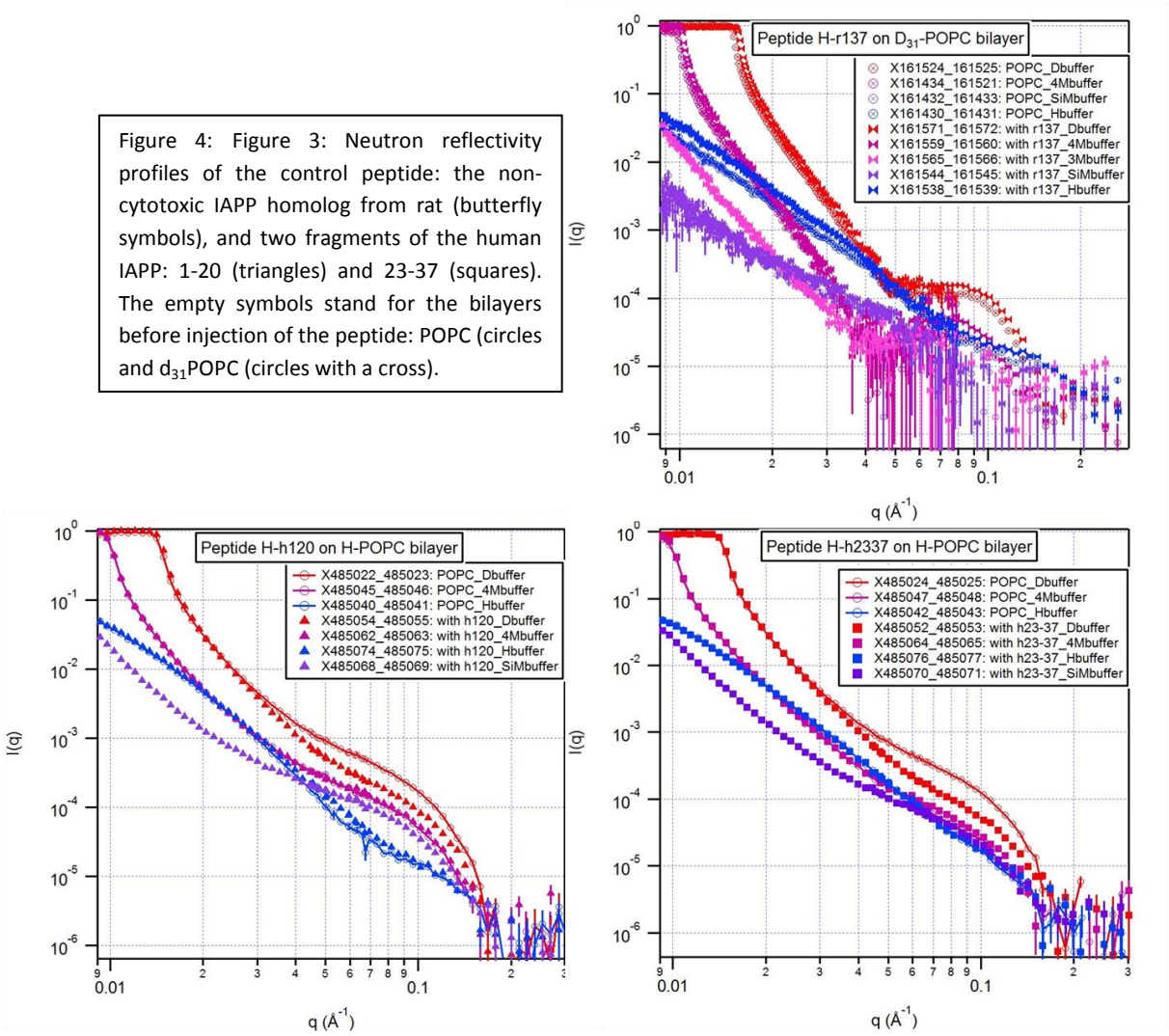


Figure 4: Figure 3: Neutron reflectivity profiles of the control peptide: the non-cytotoxic IAPP homolog from rat (butterfly symbols), and two fragments of the human IAPP: 1-20 (triangles) and 23-37 (squares). The empty symbols stand for the bilayers before injection of the peptide: POPC (circles and d_{31}POPC (circles with a cross).

These data are currently being fitted and will be compared to scattering density profiles calculated from dynamic simulations of IAPP in contact with lipid bilayers in order to determine plausible conformations while in contact with a lipid bilayer. This work will hopefully lead to a mechanistic explanation of their degrees of cytotoxicity.