Experimental report

03/05/2017

Proposal: 9-13-599 **Council:** 4/2015

Title: Neutron scattering analysis of the effect of RTILs on the equilibrium properties and fibrillation kinetics of amyloidogenic proteins

Research area: Biology

This proposal is a new proposal

Main proposer: **Antonio BENEDETTO**

Experimental team: Antonio BENEDETTO

Local contacts: Miguel Angel GONZALEZ

Samples: Hydrated Protein: Lys/H2O/(RTILs) and Lys/D2O/(RTILs)

Instrument	Requested days	Allocated days	From	To
IN16B	10	4	24/05/2016	28/05/2016

Abstract:

Supra-molecular protein structures known as amyloid fibres play a major role in neurological conditions such as Parkinson and Alzheimer, but also represent promising new materials for nanotechnology. Recent bio-chemical investigations report that the self-assembly of lysozyme into amyloid fibrils is greatly affected by the addition of room temperature ionic liquids (RTILs) to the proteins' solution, The full exploitation of these observations in pharmacology and in the nanotechnology, however, requires a better understanding of the mechanisms of action of these compounds. I propose to use Elastic and Quasi elastic neutron scattering to investigate how RTILs change the ps-to-ns dynamics of the protein and of its hydration water, thus affecting the fibrillation kinetics. Measurements will be carried out using the backscattering spectrometer IN16B. The results will enhance our understanding of protein-RTIL interactions, and clarify the role of the solvent (water) in the evolution of the system structure and stability upon the RTIL addition. Furthermore, the dynamical information provided by experiments will allow us to check/validate standard force fields used in computer simulations.

Experimental reports n. 8-04-757 and 9-13-599

Experimental team: Antonio Benedetto (antonio.benedetto@ucd.ie)

Local contacts: Miguel Gonzalez, and Bernhard Frick

Both the two experiments, 8-04-757 and 9-13-599, are focused on the interaction between ionic liquids and biomolecules, with the aim to determine the microscopic mechanisms at the origin of the ability of ionic liquids to, respectively, prevent the denaturation of DNA at ambient temperature, and trigger the protein amyloidogenesys. To study these microscopic mechanisms, computer simulations have been also used *in tandem* with neutron experiments.

Experiment n. 8-04-757 focuses on DNA, whereas experiment n. 9-13-599 on the model amyloidogenic protein lysozyme. We have asked for a total of 9 and 10 days, respectively, on the high resolution spectrometer IN16B, and we got 4+4 days in total. The two experiments have been scheduled one after the other. As a result, we have decided to focus on project n. 9-13-599, and to re-submit the project 8-04-757 in the future (we will do so in one of the following cycles). We have opted to focus on the interaction between proteins and ionic liquids since our simulations on this subject were in an advanced stage at that time and were showing promising results, and also because the higher interest on this topic expressed by the international scientific community.

The highest energy resolution set-up of IN16B has been used, i.e. 0.3 μeV. We have opted for this specific set-up to study slow motions, and actually this was one of the reason we asked for beam time on IN16B.

First, we have measured the neat protein (i.e. without ionic liquid) hydrated both in H_2O and D_2O, and then the protein hydrated in a water solution of ionic liquid, again using both H_2O and D_2O. Infrared, done before the experiments, confirmed that there is no H-D exchange between the heavy water and the ionic liquid.

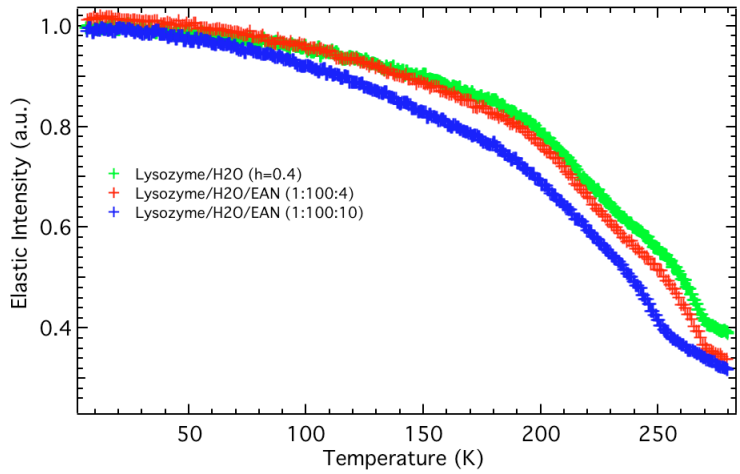

Fig. 1 – Elastic scattering intensity of lysozyme hydrated in pure water (green), and water solution of ethylammonium nitrate at two different low-concentrations (red and blue).

By comparing the spectra in H_2O with the one in D_2O we were able to disentangle the protein dynamic from the solvent dynamics, and so we were able to study the effects of ionic liquids on both the protein and its hydration water. This has been also eased by the high resolution set-up that we have opted for.

We have considered two concentrations of ionic liquid in water (i.e. 0.5 M and 5M), whereas the final hydration level of the protein was h=0.4 in all cases. Even if the amount of ionic liquids per protein was negligible (i.e. between 4 to 10 ionic liquid molecules per protein), it was possible to measure its "non-negligible" effect on the protein dynamics (Fig. 1).

We measured also the protein in pure ionic liquids, and also in a water solution of ionic liquids and a natural bio-protectant.

As reported above, during the experiments we have measured the neat lysozyme hydrated in water, i.e. without ionic liquids. By comparing the lysozyme hydrated in H_2O with the one hydrated in D_2O, and thanking to the high resolution of the spectrometer, we were able to see a clear de-coupling between the dynamics of the protein and the dynamics of its hydration water. This is the second main result of the experiments, with no-negligible implication in the well-accepted *protein dynamical transition* scenario.