Experimental report

14/02/2017

Proposal: 8-04-778 **Council:** 4/2016

Title: Protein relaxation dynamics as affected by biocompatible and biodegradable polymer solvation - part II.

Research area: Soft condensed matter

This proposal is a continuation of 8-04-718

Main proposer: Daniela RUSSO

Experimental team: Andrea DE ANGELIS
Marie PLAZANET
Jose TEIXEIRA
Daniela RUSSO

Local contacts: Bernhard FRICK
Jacques OLLIVIER

Samples: Maltose Binding Protein - Poly-methyl-ethylne-phosponate

Instrument	Requested days	Allocated days	From	To
IN5	2	2	21/06/2016	23/06/2016
IN16B	4	4	11/11/2016	15/11/2016

Abstract:

Protein-polymer conjugates combine the biological activities of proteins with the convenient handling and processability of synthetic polymers, and was proven to be an efficient strategy to improve biotechnologies based on protein such as drug delivery. In our first previous experiment, we investigate the dynamics of polymer-protein conjugates using new biodegradable and highly water soluble polymers in conjugation with Maltose binding protein. We were able to show that polymer solvation, in the absence of water, enhances protein fluctuations, but does not enable the protein to undergo the dynamical transition as previously observed in native proteins or in solvent-free liquid proteins. We also observe that the polymer loses its glass properties, providing a signature of a reciprocal and complex influence of both components on their respective dynamics.
In this continuation proposal, we aim to better understand the enhancement of protein flexibility and what is the role of hydration water in the (conjugated) protein dynamics. We wish to highlight why the vitreous properties of the polymer are absent in the conjugates and in which conditions they can be re-established

IN16. We were allocated 4 days. We were able to run 4 days. In this experiment on In16, elastic an inelastic (2 ueV) scans of protein-polymer conjugated in dry and hydrated powders have been investigated. The temperature dependence of BSA protein conjugated with five (5), ten (10) and twenty (20) polymers has been investigated in the range of 20 - 300K. The samples were completely hydrogenated and partially deuterated. The elastic measurements lasted about 6 hours/sample. Figure 1 shows an example of summed integrated intensity of elastic scan of dry polymer (Blue line), hydrated polymer (green line), Mixture BSA and polymer (Red line). Figure 2 shows integrated intensity at Q =1.8A^{-1} for dry BSA (yellow line), BSA-Polymer 1:5, (verde), BSA-Polymer 1:10, (blue), BSA-Polymer 1:20, (red). Quasi-elastic spectra were also collected at RT for the hydrated samples (Figure 3). Analysis data is in progress

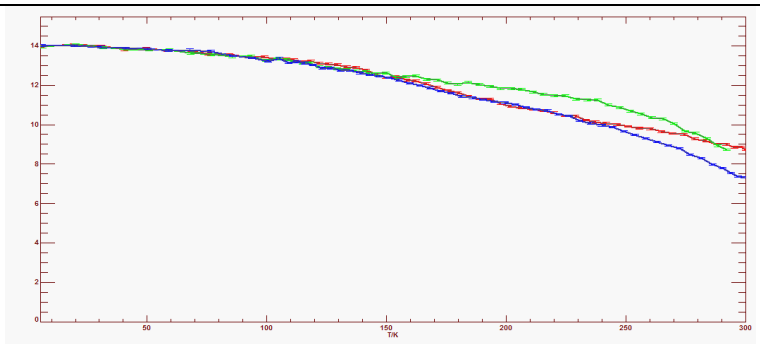

Figure 1.

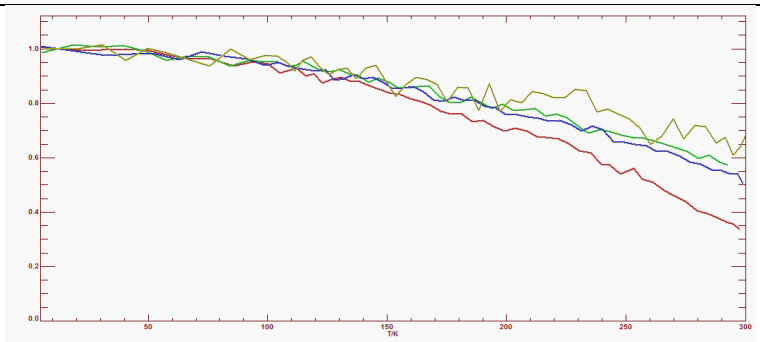

Figure 2.

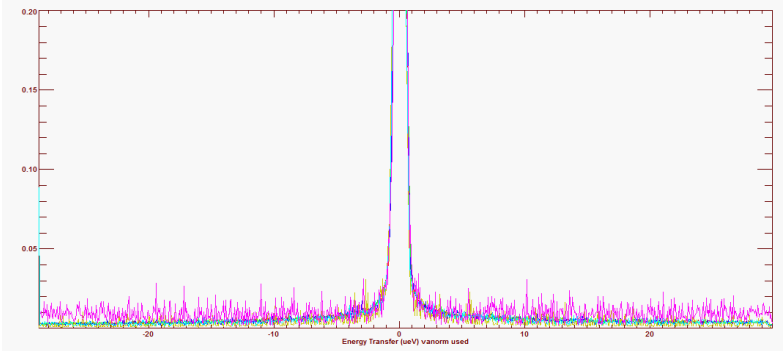

Figure 3.

IN5. We were allocated 2 days. We were able to run 1 days for instrument failure. In this experiment on In5, a set of quasi elastic experiment (100 ueV) at 300 and 200K on MBP-PEEP conjugated in completely hydrogenated and partially deuterated form were performed. The temperature dependence of MBP protein conjugated with high and low molecular weigh polymer

has been investigated. Figure 4 shows data at RT conjugated with 10kDa polymer while Figure 5 with 5kDa polymer. Analysis data is in progress

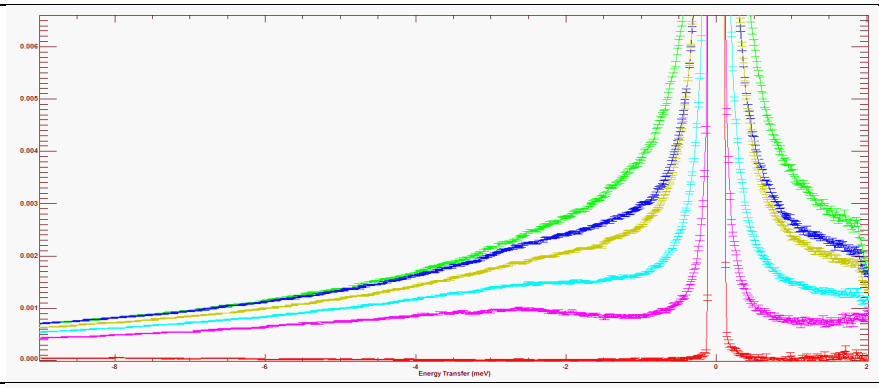

Figure 4
ROUGE= resol;
Verde= Polymer;
Rosa= MBP
Blue = MBP(D)-Pol(H) 10Kda
Giallo= MBP(H)-Pol(D) 10kDa
Cyan = MBP(H)-Pol(H)10kDa

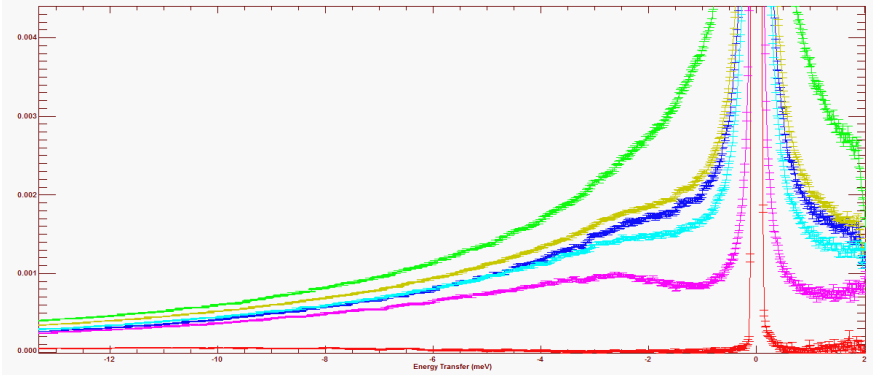

Figure 5
ROUGE= resol;
Verde= Polymer;
Rosa= MBP
Blue = MBP(D)-Pol(H) 5KDa;
Giallo= MBP(H)-Pol(D) 5kDa
Cyan = MBP(H)-Pol(H) 5KDa