Experimental report

06/09/2019

Proposal: 8-04-815 **Council:** 4/2017

Title: Protein relaxation dynamics as affected by biocompatible and biodegradable polymer solvation – part IIb.

Research area: Soft condensed matter

This proposal is a continuation of 8-04-778

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Samples: protein polymer
D2O + buffer + Nanoparticles 20 mg/ml + HSA 20 mg/ml
Polystyrene Nonoparticle + PEG in D2O
Polystyrene Nonoparticle + PEG + HSA (commercial) + D2O

Instrument	Requested days	Allocated days	From	To
IN5	2	2	02/10/2018	04/10/2018
IN16B	4	4	21/06/2018	25/06/2018

Abstract:

In our first experiment, we investigate the dynamics of polymer-protein conjugates using new biodegradable and highly water soluble polymers with two different chain lengths in conjugation with Maltose Binding Protein (MBP). An important findings was that 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. In a further experiment (performed on November 2016) we investigated BSA-polymer conjugates at three degree of polymerization (molar ratios of attached polymer chains: BSA-PEEP 1:5, 1:10, 1:20) and in the dry and hydrated stated. 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. First we intend to perform measurement on completely deuterated MBP-polymer conjugated. Then we also wish to use a new type of conjugate with a different secondary structure (Myoglobin-polymer) in order to confirm some last unattended observed results.

From previous measurements we learned that, in the dry state, higher is the number of polymer attached to the protein, larger is the flexibility of the protein itself. Dry mixture polymer/protein and pure polymer show a completely distinct behavior compared to the conjugated. However, the most important results appear in the BSA hydrated samples, included pure BSA, where all T-scan superpose to the dry polymer scans. These results open important questions about the role of the water molecules and how/if/where they can be replaced. Upon hydration, is the water distribution homogeneous in the whole conjugate, and how does it affect the dynamics of both components?

In this continuation proposal, we aim to better understand our last results upon hydration, the enhancement of protein flexibility as a function of polymers attached and the role of hydration water in the (conjugated) protein dynamics. we performed measurement on a new type of conjugate with a different secondary structure (Myoglobin-polymer) in order to confirm our last unattended observed results using another protein.

IN16B. We were allocated 4 days. We were able to run 4 days. In this experiment on In16B, elastic an inelastic (2 ueV) scans of Myoglobin protein-polymer conjugated in dry powders have been investigated. The temperature dependence of Myoglobin protein conjugated with five (5,My 1:5), ten (10, My 1:10) and twenty (20, My 1:20) polymers has been investigated in the range of 20 - 300K. The samples were completely hydrogenated. The elastic measurements lasted about 6 hours/sample. Figure 1 shows an example of summed integrated intensity of elastic scan of dry PEEP polymer 6.4 kDa(green line), My 1:5 (cyan line), My 1:10 (blue line), My 1:20 (pink line), Mixture BSA and polymer (Red line). Figure 2 shows integrated intensity for the *Samples My, PEEP, My1:5 12 KDa.* The MSD were also calculated. Quasi-elastic spectra were also collected at RT for all samples, the HWHM has been extrapolated (Figure 3). Analysis data is completed and ready for publication. A manuscript is in progress.

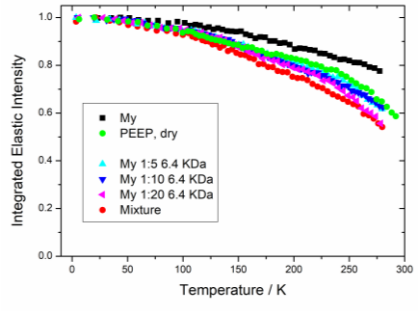


Figure 1.

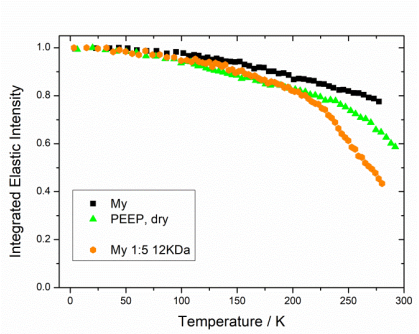


Figure 2.

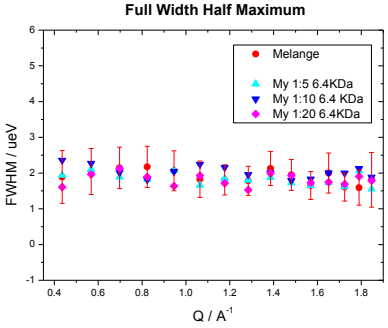
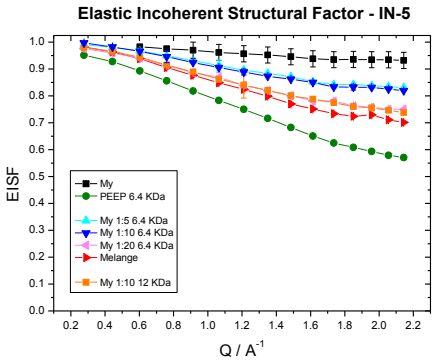
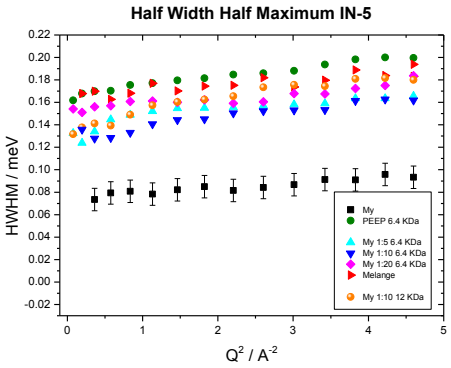


Figure 3

IN5. We were allocated 2 days. We were able to run 2 days. In this experiment on In5, a set of quasi elastic experiment (100 ueV) at 300 and 200K on Myo-PEEP conjugated and BSA-PEEP conjugated. The temperature dependence of both protein conjugated with 5, 10, 20 polymer attached has been investigated. Figure 4 shows the EISF and the HWHM at RT for all samples. Figure 5 a quick comparison of the HWHM between the BSA and Myoglobin conjugates. Analysis data is completed and ready for publication. A manuscript is in progress.

Figure 4





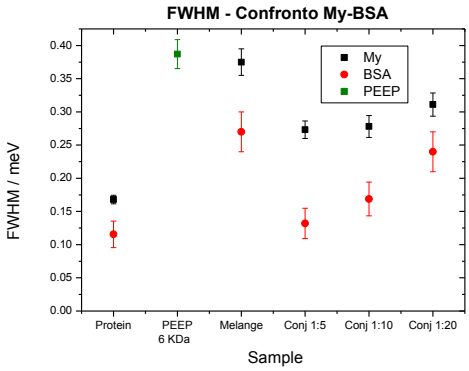


Figure 5