ESRF	**Experiment title:** Investigation of the collective dynamics in a protein solution	**Experiment number:** SC10-20
Beamline: ID28	**Date of experiment:** from: Dec 14, 2002 to: Dec 19, 2002	**Date of report:** July 17, 2003
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Report:

The purpose of the proposed experiment was to investigate the collective dynamics of a globular protein, namely trypsin, as a function its concentration in an aqueous buffer solution. In order to reduce the electronic density contrast that generates the small angle scattering signal in the powder systems, we had proposed to focus onto solution systems. IXS spectra of trypsin solutions with different concentration levels were achieved in a first shot of the experiment. This study revealed that even for the highest protein concentrations, meaning as soon as the water content dominates the mixture, the solutions spectra cannot be distinguished from those of the aqueous buffer, which consists of a 1mmol HCl solution in H_2O (Figure 1). The parameters of the inelastic excitations (energy position and energy widths) were found to be identical in both cases, therefore compromising the initial goal of our proposal.

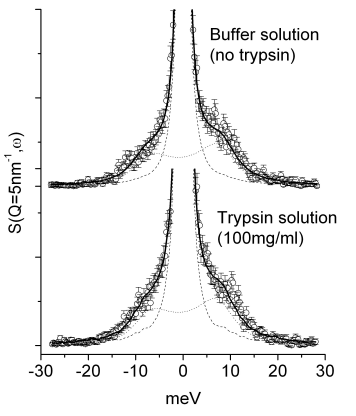

Figure 1: S(Q=5nm^{-1},ω) of a trypsin solution (bottom graph) and that of its buffer (top graph)

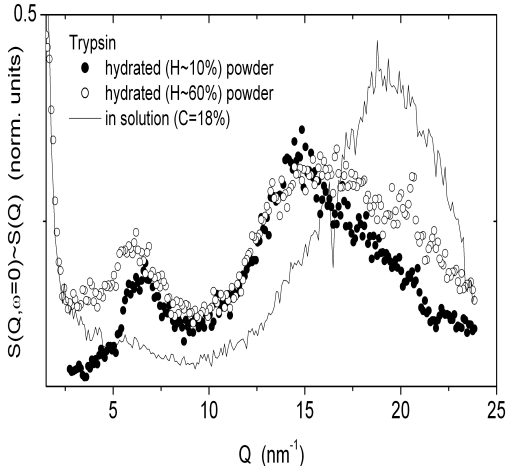

Figure 2 : Elastic structure factor of hydrated trypsin powders (circles) and solution (line)

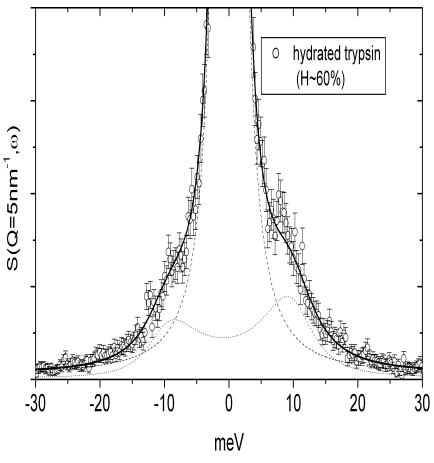

Figure 3 : S(Q=5 nm^{-1},ω) of a hydrated trypsin powders

Considering this result, we turned back on the study of hydrated powders, using an experimental setup that allowed the sample to be maintained in contact with water vapor while under beam. The static structure factors of several powder samples hydrated at different levels are displayed in Figure 2. These curves consist of the main diffraction peak ($\sim$ 15 nm^{-1}) and of a prepeak culminating around $\sim$ 7 nm^{-1}. The origin of this prepeak is still unclear; it may arise from internal domain structures of the protein [1]. One sees that upon increasing hydration level, the curves increasingly feature the contribution of water, whose main peak rises around 20 nm^{-1}. As a good surprise, the low Q parts of the trypsin curves show a rather weak prepeak, in comparison with previously investigated protein powders (exp. LS727, M. Belissent-Funel *et al*, ID16). This favorable situation was confirmed in the inelastic scans (Figure 3) where a clear inelastic excitation could be observed. This excitation mostly reflect hydration water, which is expected to behave differently from bulk water. Once again, analyzing the inelastic excitations on these differently hydrated samples did not reveal any appreciable change in the linear regimes of the corresponding dispersion curves (neither in the Q-dependence of the excitation energy widths).

On the basis of these results, we attempted to characterize the temperature dependence of the inelastic signal observed on the hydrated samples. Several studies on hydrated protein powders have unveiled the existence of a dynamical transition around 200 K [2], analogous to the glass transition in glass-forming liquids, whose origin is still debated. Since the glass transition has been shown to reflect onto the temperature dependence of the IXS signal [3], we expected that the protein dynamical transition could also be evidenced, in a similar way to glasses. For this experiment (performed in a second slot of beamtime in Dec. 2002), we used another batch of trypsin, purchased from the same supplier. Unexpectedly, this new trypsin sample did not feature exactly the same $S(Q)$ as that shown in Figure 2. In particular, the scattered intensity in the prepeak region (where the IXS signal could be detected in the previous case) was increased by more than a factor 2, therefore killing the already low inelastic/elastic contrast. This unexpected situation, that we ascribe to a poor quality of the second protein sample set, did not allow us to perform the temperature measurements. Newly purchased samples are currently under diffraction tests to assess their favorable weak prepeaks. We hope to be able to renew this experiment shortly.

References

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[2] W. Doster *et al*, Nature, **337** 754 (1989); B.F. Rasmussen *et al*, Nature, **357** 423 (1992).

[3] C. Masciovecchio *et al*, Phys. Rev. Lett., **80**, 544 (1998); A. Mermet *et al*, Phys. Rev. E, **66**, 0031510 (2002)