Experimental report

17/05/2016

Proposal: **INTER-303** **Council:** 10/2014

Title: Internal time on IN16B

Research area:

This proposal is a new proposal

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Experimental team: Judith PETERS

Local contacts: Tilo SEYDEL

Samples: Cyan fluorescent proteins

Instrument	Requested days	Allocated days	From	To
IN16B	1	2	24/06/2015	25/06/2015

Abstract:

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Dynamics of a whole family of cyan fluorescent proteins explored by neutron scattering

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The influence of protein dynamics on the fluorescence properties of proteins homologous to Green Fluorescent Protein (GFP) from the jellyfish *Aequorea victoria* is largely unknown, and is restricted to the influence of a few residues in the vicinity of the fluorescent chromophore, a tripeptide that is autocatalytically cyclized during protein folding. We have extensively studied a family of such proteins, Cyan Fluorescent Proteins (CFPs) by ways of X-crystallography and molecular dynamics simulations [1, 2]. This family comprises the following mutants with increased fluorescence efficiency (QY: fluorescence quantum yield) ECFP (QY = 30%), Cerulean (44%), SCFP3A (56%), mTurquoise (84%) and mTurquoise2 (93%). Because fluorescence lifetimes of these mutants range from 2 to 4 ns, we were eager to probe differences in protein dynamics on the ps to ns timescale, and see if they could correlate with fluorescence efficiency.

We have overexpressed, purified and dried 5 samples of these mutants consisting of 82 to 167 mg of dry protein, which were re-hydrated with D_2O before the experiments. Because of time constraints, we could only measure Cerulean, SCFP3A and mTurquoise on IN16B. All other samples could be measured on IN6 and IN13. Unfortunately, it was realized that the ECFP sample lost mass both on the IN6 and IN13 experiments, even after re-hydration in-between experiments, what made the data on IN16B also questionable. Besides, the Cerulean sample had to be re-prepared in-between the IN16B and IN13/IN6 experiments since the first preparation was too light (62 mg of protein). Therefore, we had to repeat the data collection, especially for the Cerulean and ECFP samples, and asked one day of internal beam time for that. Weighing before and after the measurements confirmed that we had no loss of hydration this time for Cerulean, but again for ECFP. After inspection of the sample holder, we finally realized that the drying of the sample was due to a manufacturing fault of the sample holder whose pit for the indium wire was too deep. Unfortunately, we will thus have to repeat the scan for ECFP a third time.

We analyzed now the elastic and QENS data of IN16B after having checked on IN6 data [3] that there is no significant water motions' contribution, what was expected as we measured hydrated powders. One Lorentzian curve, convoluted with the instrumental resolution, was used to fit the experimental curves (see figure 1).

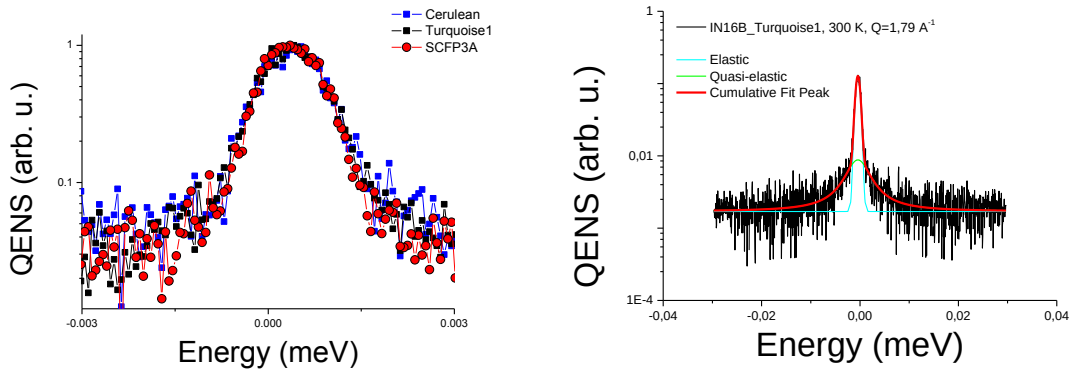

Figure 1: QENS spectra summed over all accessible scattering angles of three different samples collected on IN16B (left) and an example of the fit of mTurquoise1 data at Q = 1.79 Å^{-1} (right).

We furthermore extracted the Elastic Incoherent Structure Factor (EISF), which is indicative of the geometry of the diffusional process, for three samples from IN16B data (see figure 2).

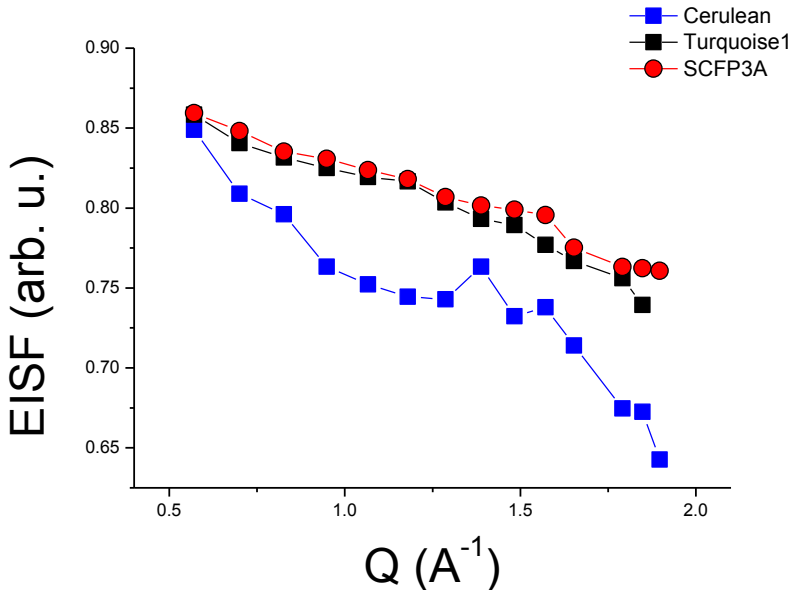

Figure 2: Elastic Incoherent Structure Factor as function of Q for three samples.

Cerulean appears to be significantly more flexible as its EISF is about 15 % lower at high Q values. This might be a hint for an onset of dynamics which correlates with the lower fluorescence efficiency of Cerulean compared to those of the other samples.

[1] J. Goedhart et al., Nat. Commun. 2012, 3, 751.
[2] M. Lelimousin et al., Biochemistry 2009, 48, 10038-10046.
[3] ILL report 8-04-761.