Experimental report

18/05/2016

Proposal: INTER-300 **Council:** 10/2014

Title: Internal time on IN13

Research area:

This proposal is a new proposal

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Experimental team:

Local contacts: Judith PETERS

Samples: Cyan fluorescent proteins

Instrument	Requested days	Allocated days	From	To
IN13	7	7	22/06/2015	29/06/2015

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 IN13 and 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.

On IN13 we repeated elastic scans as function of temperature of three samples: ECFP, Cerulean and SCFP3A over the temperature range 20 – 310 K and analysed the data (see figure 1) to get elastic intensities summed over all available scattering angles and atomic mean square displacements (MSD).

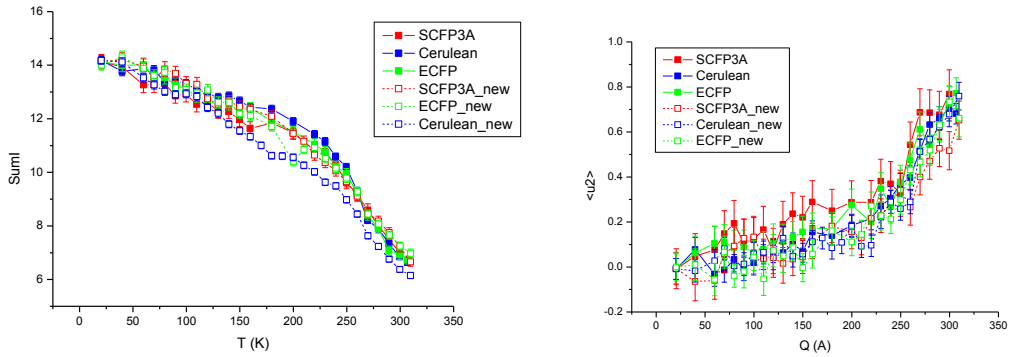

Figure 1: Elastic Intensities summed over all available scattering angles (left) and MSD extracted for the three samples (right).

The MSD measured on IN13 are rather noisy what makes it difficult to conclude, but the summed intensities have smaller error bars. The results are in good agreement for SCFP3A and ECFP, but there might still be a little difference in hydration for Cerulean, what must be checked with data collected on IN6 and IN16B.

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