Experimental report

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Proposal: 8-04-761 **Council:** 4/2015

Title: Dynamics of a whole family of cyanfluorescent proteins explored by neutron scattering

Research area: Biology

This proposal is a continuation of 8-04-727

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Samples: mTurquoise2
Cerulean
ECFP

Instrument	Requested days	Allocated days	From	To
IN6	3	3	29/06/2015	02/07/2015
IN13	7	0		
IN16B	2	0		

Abstract:

Cyan Fluorescent Proteins (CFPs) are widely used in FRET-based live cell imaging experiments, in which they non-radiatively transfer their excitation energy to a Yellow Fluorescent Protein depending on their vicinity, thus providing a technique to probe protein-protein interactions. The prototypal CFP, Enhanced Cyan Fluorescent Protein (ECFP), suffers from non-optimal fluorescence properties that were not drastically improved in two later-developed CFPs, Cerulean and SCFP3A. Two additional rounds in optimization by structure-based mutagenesis yielded mTurquoise and mTurquoise2, the latter being the monomeric fluorescent protein with the highest fluorescence efficiency (fluorescence quantum yield). A structural analysis by X-ray crystallography revealed a flexibility of one of the beta-strand of the protein, whose interactions with the chromophore could affect its fluorescence properties. In the latest CFPs, this flexibility appeared to have been suppressed. By using neutron scattering, we would like to bring a definitive experimental proof of the mechanism of fluorescence-controlled of the chromophore by its protein matrix by completing the previous experiment.

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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. 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).

After inspection of the indium o-ring and 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. Therefore, we had to repeat the data collection in a new sample holder, especially for the ECFP sample. Weighing before and after the measurements confirmed that we had no loss of hydration this time.

We have analysed the elastic and QENS data of IN6. IN6 was used in the 100 μeV energy resolution configuration; therefore it permits to extract the elastic data and to check whether any QENS contribution from water molecules has to be taken into account for the IN16B QENS data analysis. A sum of two Lorentzian curves, convoluted with the instrumental resolution, was used to fit the experimental curves. As we measured hydrated powder samples, the differences in the QENS curves between the various samples were almost negligible and the linewidths of the Lorentzian curves were mainly constant, as expected (see figure 1).

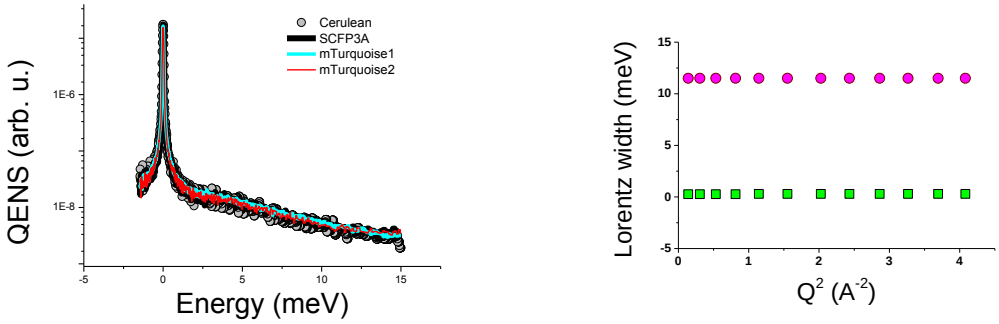

Figure 1: QENS spectra of four different samples collected on IN6 at Q=1.06 $Å^{-1}$ (left) and an example of the linewidths of the two Lorentzians used to fit the data collected for Cerulean (right).

We furthermore extracted the Elastic Incoherent Structure Factor (EISF) for four samples from IN6 data (see figure 2).

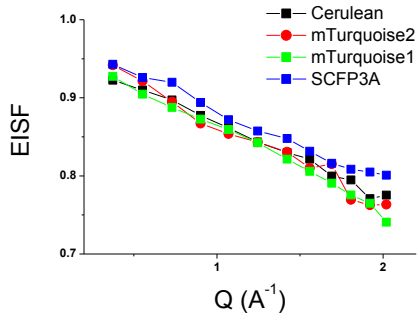

Figure 2: Elastic Incoherent Structure Factor as function of Q for four samples.

The EISF, which is indicative of the geometry of the diffusional process, exhibits a linear behavior in dependence of Q with very similar results for the two mTurquoise samples and Cerulean. As higher it is at high Q values, as more particles do not participate in the diffusional motions. Therefore SCFP3A appears slightly stiffer than the other samples.

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