Experimental Report

Proposal: **8-04-727** **Council:** 4/2014

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

This proposal is a new proposal

Researh Area: Biology

Main proposer: **ROYANT Antoine**

Experimental Team: MARTINEZ Nicolas
PETERS Judith
ROYANT Antoine
LAFAYE Celine
GOTTHARD Guillaume
VON STETTEN David
CLAVEL Damien

Local Contact: PETERS Judith
FRICK Bernhard
SEYDEL Tilo

Samples: mTurquoise2
Super Cyan Fluorescent Protein 3A
Cerulean
mTurquoise2
Enhanced Cyan Fluorescent Protein

Instrument	Req. Days	All. Days	From	To
IN13	11	7	06/10/2014	13/10/2014
IN16B	2	2	15/09/2014	17/09/2014
IN6	5	5	07/11/2014	12/11/2014

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.

Exp. Report 8-04-727

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

Judith Peters, Institut de Biologie Structurale, Université Joseph Fourier, Institut Laue Langevin
Antoine Royant, Institut de Biologie Structurale, European Synchrotron Radiation Facility
Damien Clavel, Virginia Guillon, Céline Lafaye, Institut de Biologie Structurale
David von Stetten, Guillaume Gotthard, European Synchrotron Radiation Facility
Nicolas Martinez, Bernhard Frick, Marek Koza, Institut Laue Langevin

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).

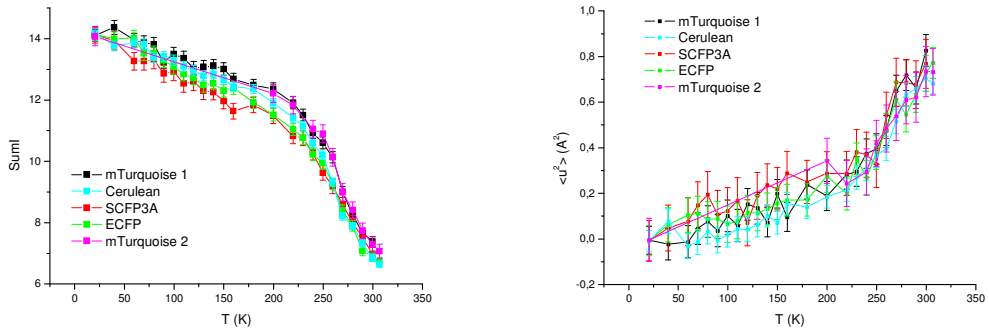

Figure 1: (left) Intensities summed over the available Q-range and (right) mean square displacements extracted from elastic data taken on IN13.

Experimental results reveal that evolutions of summed intensities and mean square displacements (MSDs) with temperature are very similar for each sample (see for

example IN13 data on **Figure 1**). In particular, MSDs cannot be differentiated since differences are within experimental errors. The magnitude of protein movements can be tentatively hierarchized as follows:

IN6	mTurquoise > SCFP3A > Cerulean > mTurquoise2 > ECFP
IN13	mTurquoise > SCFP3A > ECFP > mTurquoise2 > Cerulean
IN16B	Cerulean > mTurquoise > SCFP3A

What is striking is that all mutants obey to the same hierarchy except for Cerulean which is in the middle of the pack on the 10 ps time scale, the more rigid on the 100 ps time scale and the less rigid on the ns timescale (**Figure 2**). It should be noted that molecular dynamics simulation predict large scale movement of one particular beta-strand for Cerulean [2]. The onset of increased flexibility on the ns time window for Cerulean is in accordance with this result. However, this experiment needs to be repeated with the same Cerulean sample on all spectrometers to confirm these findings.

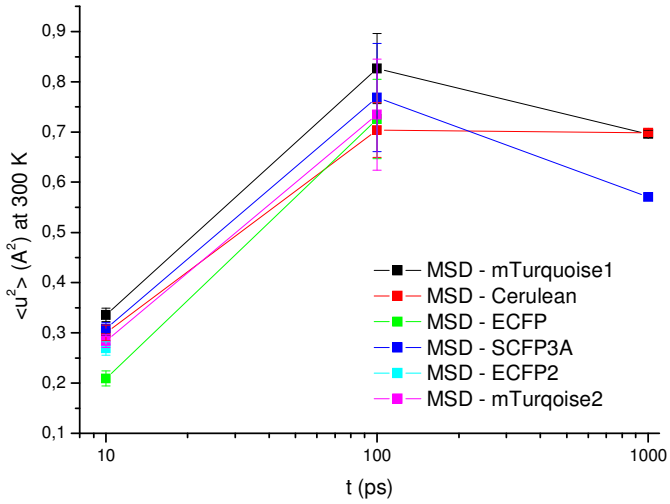

Figure 2: Mean square displacements at 300 K (as interpolated from our data) according to the time window of each spectrometer.

In summary, CFPs appear to exhibit similar protein dynamics on the sub-nanosecond timescale, while the particular mutant Cerulean, known to have the largest magnitude of atom movements, appears to be the more flexible on the nanosecond time window. These are promising results that need to be completed (for IN16B) and confirmed (for Cerulean data on all spectrometers). In addition, we expect ECFP to be more flexible on IN6. We will thus resubmit a proposal on all spectrometers for the next round.

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