Experimental report

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Proposal: 8-04-801 **Council:** 10/2016

Title: The dynamics of the cyan fluorescent protein Cerulean as function of high hydrostatic pressure

Research area: Biology

This proposal is a new proposal

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Samples: cyan fluorescent protein Cerulean

Instrument	Requested days	Allocated days	From	To
IN16B	2	2	26/01/2017	28/01/2017

Abstract:

The sequencing of green fluorescent protein (GFP) in 1992 allowed for the demonstration of its use as a genetically encoded fluorescent reporter in cells (Chalfie et al., 1994). Since then, it has revolutionized the field of fluorescence imaging. The mutant Cerulean has become a popular reporter, which we studied recently as function of temperature. With the present application, we would like to evaluate the loss of protein flexibility in Cerulean with increasing pressures, as we found hints that pressure-induced structural changes lead to a better stabilization of the chromophore which is correlated with its flexibility.

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The dynamics of the cyan fluorescent protein Cerulean as function of high hydrostatic pressure

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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]. Comparison between structures of ECFP and Cerulean, two CFPs from respectively first and second generation, led to the hypothesis that one constitutive weakness of CFPs is due to the instability of the seventh β-strand out of the 11 ones composing the protein [2]. Because it was shown that this strand is stabilized at 2000 bar, we would therefore like to evaluate the loss of protein flexibility in Cerulean with increasing pressures.

About 100 mg of Cerulean protein was prepared at the ESRF Grenoble. It was lyophilized and the dry protein was then rehydrated from D_2O vapor in order to focus on the protein dynamics during the experiment. The sample was rehydrated and placed in excess of D_2O to guarantee homogeneous pressure transmission. Neutron scattering measures were performed on the spectrometer IN16b, at T = 295K, as a function of pressure (P= 20, 500, 1000, 1500 and 2000 bar). In addition of these measures, correction measurements of the buffer at 30, 1000 and 2000 bar, and of Vanadium were carried out. Because of lack of time, measures on empty cell were not performed. During the experiment, an abnormal pressure decrease was observed : from the second half of measures at P=1000 bar, until the first runs at P=1500 bar. On top of that, at P=2000 bar, there was a leak of sample, and fluorinert, which serves to transmit the hydrostatic pressure, entered in the high-pressure cell.

First, we analysed elastic data and retrieved the Mean-Square Displacement (MSD) (see Fig. 1).

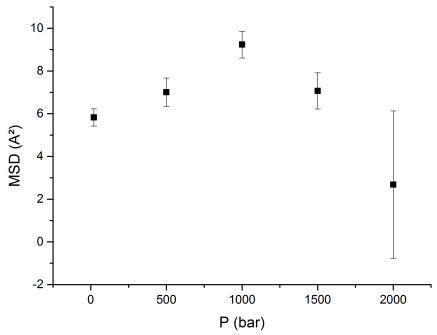
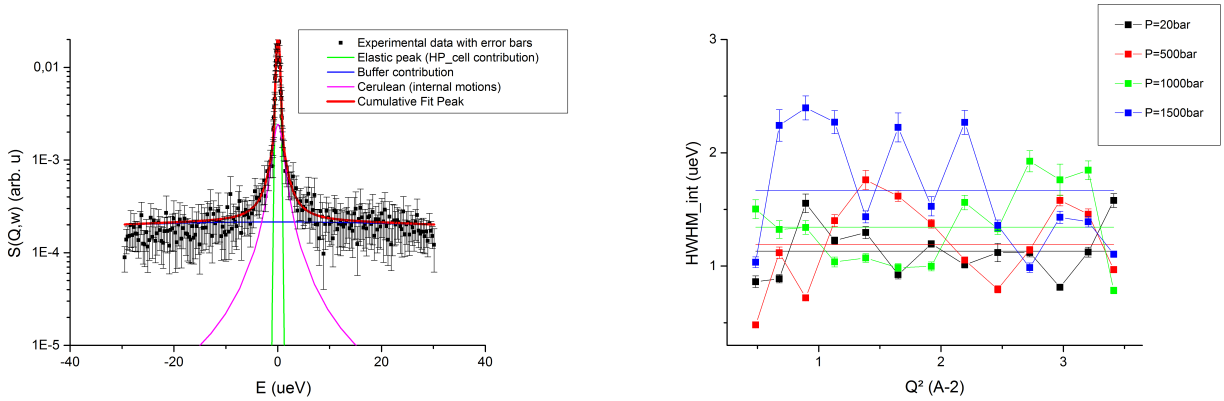


Figure 1: MSD as a function of the pressure P. Elastic data normalized by run time, and corrected by scattered intensities from Vanadium, pure buffer and empty high-pressure cell.

Fig. 1 shows that the MSD increases with the pressure between P=20 bar and P=1000 bar, indicating that flexibility of the sample would increase. After that, we analysed QENS data, but as a consequence of the leak of sample, it was not carried out for P=2000 bar. One Gaussian curve, and two Lorentzian curves (one for the buffer contribution, the other one for Cerulean), convoluted with the instrumental resolution, were used to fit the experimental data (see Fig. 2a). Then, we retrieved the Half-Width at Half-Maximum (HWHM) of the Lorentzian curve interpreted as belonging to Cerulean sample (see Fig. 2b).



(a) (b)

Figure 2: An example of the fit of Cerulean data at Q=1.28Å^{-1} (a), and HWHMs as a function of Q^2 with their mean line for 4 pressures (b).

From that, we plotted the mean value of HWHM for each pressure (see Fig. 3).

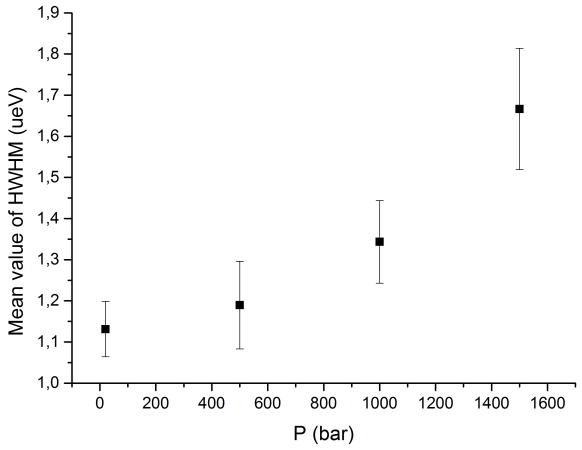


Figure 3: Mean value of HWHMs as a function of P

Similarly to MSD, the variations of HWHM indicates that the Cerulean protein seems to become more flexible with pressure, which is an abnormal behaviour according to Le Chatelier's principle. Unfortunately, due to the experimental problems we had, experiment must be repeated and the results confirmed before publication.

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