Experimental report

21/07/2022

Proposal: 8-05-469 **Council:** 4/2021

Title: Density of states and EINS of protonated and deuterated GFP in view of an analysis including quantum effects

Research area: Biology

This proposal is a new proposal

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Samples: p-GFP
d-GFP

Instrument	Requested days	Allocated days	From	To
IN1 LAG	1	0		
PANTHER	2	2	25/08/2021	27/08/2021

Abstract:

There is a growing number of indications that quantum mechanics might play a role for the functioning of certain biomolecules and within living cells, as for instance through tunnelling effects or quantum entanglement. However, not much is reported in the literature about experimental evidence, certainly because not much is known about their identification. As quantum effects depend on the mass of the scatterer, their signature should be detectable when comparing a protonated protein with its per-deuterated counterpart. We propose therefore to study Green fluorescent protein (GFP) in the two versions by recording the density of states at low temperature over a broad energy domain to permit data analysis within a newly developed approach taking into account quantum effects [1].

Density of states and EINS of protonated and deuterated GFP in view of an analysis including quantum effects, 8-05-469, cycle 213.

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1 Purpose of the experiments

The functioning of biological systems has always been described with classical physics, since biomolecules are complex heavy molecules functioning at physiological temperatures. However litterature testifies the impact of purely quantum mechanisms explaining the efficiency or speed of some biological processes, as for tunneling effects in enzymatic activities for instance (Brookes [2017]). Our purpose is to investigate quantum effects with neutron scattering techniques, using full deuteration of proteins in order to investigate isotope effects in the dynamics of the hydrogen nuclei of the proteins.
We study the green fluorescent protein (GFP), a 27kDa protein which is rather rigid since it is composed of a 11-strand β barrel $42\mathring{A}$ long and $24\mathring{A}$ wide, and a few α helices holding the chromophore of the protein inside the barrel. About 150mg of pure proteins were prepared for both versions, a fully protonated sample was produced by P.Oger in INSA Lyon, and a fully deuterated one by the D-lab at ILL by M.Moulin and M.Haertlein.
These experiments were performed in order to look for isotope effects with a fully deuterated (dGFP) and a fully protonated green fluorescent protein (pGFP). It is assumed that incoherent scattering is most important for the hydrogen atom in both samples. The search for isotopic effects is done through calculations of the generalized density of states for both proteins.

The densities of states are defined as $g(\omega) = \sum_\lambda \delta(\omega - \omega_\lambda)$ for all λ modes, with ω the energy transfer and ω_λ the energy transfer of the given λ mode in the protein, which are continuous in the case of such a complex biomolecule. However, generalized densities of states (GDOS) are defined as $G(\omega) = \sum_{\lambda,l} \frac{b_l^2 |C_l^\lambda|^2}{m_l} \delta(\omega - \omega_\lambda)$ with b_l the scattering length of atom l, m_l its mass, and $|C_l^\lambda|$ the norm of displacement vector for atom l and mode λ. Smith et al. [1986]
The latter is the quantity that one can access through Panther's experimental dynamic structure factor $S(Q,\omega)$ at the low Q limit : $G(\omega) = \lim_{q\to 0} \frac{6\omega}{\hbar q^2}(exp(\frac{\hbar\omega}{k_B T} - 1) S(Q,\omega)$ with k_b Boltzman's constant and T the temperature probed by the experiment. However in hydrogen-rich protonated proteins both $G(\omega)$ and $g(\omega)$ behave the same at low frequencies as observed with Normal mode analysis experiments (Smith [1991]). This statement is although unclear for our dGFP sample which scatters mostly coherently (contributions stem from both the protein's and the solvent's deuterium).
In Gainaru et al. [2014] authors also use the generalized densities of states to extract the MSDs of the sample and calculate the impact of zero point fluctuations at the glass transition temperature of heavy and light water.

2 Methods

This experiment was performed with two $40\% g_{D_2O}/g_{protein}$ hydrated powder samples of green fluorescent protein and fully deuterated green fluorescent protein. Both samples were prepared in an aluminium flat sample holder of 0.55 mm width. The exact same samples have been used during the beamtime on IN1 EASY-881 (inelastic scattering experiments at high incident energies) and D7 EASY-1064 (polarisation analysis diffraction experiment). Those samples where used on IN5 with $12\mu eV$ and a $80\mu eV$ resolutions at similar temperatures (10K, 150K, 310K) in the purpose of a QENS study in October 2019 (DIR-173) and lyophylised/hydrated before this round of experiments.

The experiment was performed following the scheme introduced in figure 1. Both samples were exposed at three different temperatures (1.5K, 150K, 300K) for 30 minutes or 1 hour for three different reference incident energies (19meV, 76meV, 171meV). A temperature scan was performed between 1.5K and 150K, and 150K and 310K, at incident energy 19meV. Each scan was measured for 5 minutes. This procedure was also performed for the empty cell on shorter times.

Data is reduced with MANTID using routines DirectILLCorrectData to correct for the flat background, monitor normalisation and the elastic peak. DirectILLReduction is used to change the data from $S(\theta, TOF)$ (Time-of-Flight in time units) to $S(Q,\omega)$. Then the data is cropped to the energy and Q ranges of interest and exported in the CSV format. The routine ComputeIncoherentDos from Mantid is used to calculate the generalized densities of states for preliminary

results before further investigation. This approximation holds for coherent scattering by neglecting the correlations between atoms and neglecting the phase term. The large temperature, energy and Q ranges will probably require to make multiphonon corrections and more rigourous data analysis.

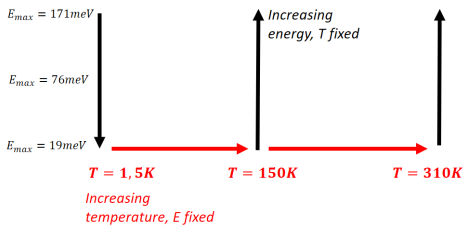

Figure 1: Scheme of the planning of the experiment.

3 Preliminary results

Below 20meV, study of $S(Q,\omega)$ provides important information to our QENS data on IN5 (dir 173, PETERS Judith et al. [2019]) It is paramount to evaluate the impact of collective vibrations at 150K and 300K since the model we apply in time on the intermediate scattering function (adapted from Hassani et al. [2022]) is really sensitive to small fluctuations at high energies (corresponding to low "t"). This "Boson Peak" extending from about 2meV to 6meV is especially important for the protonated GFP, see figure 4 b). Below 20meV, the GDOS are rather similar for both proteins within the resolution probed by PANTHER. However, important differences of the GDOS below 76meV between both samples are reflected by both PANTHER and IN1 experiments which yield very close results (see figure 2). A peak visible for pGFP around 30meV is absent for dGFP, and is not sustained at 150K, and doesn't seem to have its equivalent in the GDOS of the dGFP. It also appears that the amplitudes of librations are more important for dGFP than for pGFP as observed on figure 3, which is consistent with QENS studies that highlight that D_2O contribution is important in the dGFP sample.

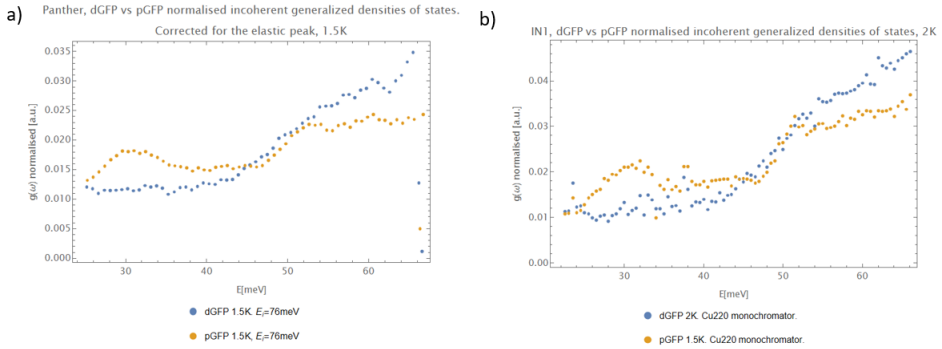

Figure 2: a) Generalized Density of States (GDOS) obtained with PANTHER for T=1.5K, b) GDOS obtained with IN1 Lagrange for T=2K. a) Was calculated using equation [1] considering a unity Debye Waller factor using MANTID, b) was obtained multiplying the raw $S(Q,\omega)$ by the energy exchange vector. Both curves were then normalized on the same ω range (E in [22,62] meV).

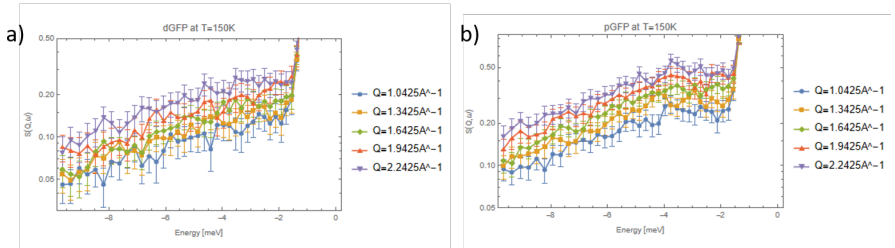

Figure 3: Comparison of pGFP and dGFP vibrational densities of states for $E_{max} = 171meV$, for T=1.5K (a) and for T=300K (b).

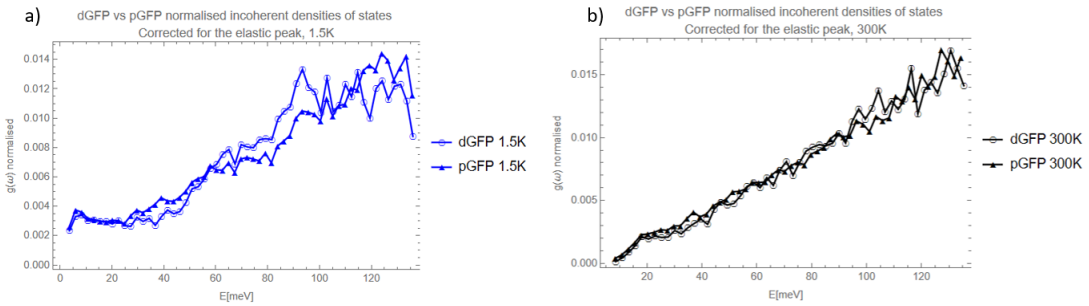

Figure 4: Collective vibrations occuring at T=150K between 2meV and 6meV for different Q values, measured on PANTHER for incoming energy 19meV. a) dGFP, b)pGFP.

4 Data analysis to be performed

First of all it is paramount to evaluate the impact of multiphonon scattering in our data since we reach high energy (up to 170 meV) and high temperature (300K). The calculation of the GDOS at higher energies will require caution about the impact of coherent scattering from the dGFP sample (30% of total scattering, increasing at higher Q values) as well as the inclusion of multiphonon scattering. Most litterature on proteins cover the low frequency ranges (below $100meV$). As observed with QENS data, the impact of the solvent will be non negligeable for dGFP.

References

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