Experimental report

23/05/2022

Proposal: 8-05-468 **Council:** 4/2021

Title: Using polarization analysis to separate the coherent and incoherent scattering from protonated and deuterated protein samples

Research area: Biology

This proposal is a new proposal

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Samples: p-GFP
d - DFP

Instrument	Requested days	Allocated days	From	To
IN12	4	4	11/06/2021	16/06/2021

Abstract:

We want to use polarization analysis to separate experimentally the coherent and spin-incoherent nuclear static scattering functions, from a set of protonated and deuterated samples of green fluorescent protein. We studied the same two samples by elastic and inelastic neutron scattering on IN5 as function of temperature to validate a recent formalism proposed by G. Kneller in the aim to determine whether quantum effects can be probed by neutron scattering. Data analysis gave already encouraging results, but we need to be sure about the contributions from coherent and incoherent scattering to separate the all effects. For that, polarisation analysis is ideal and can be employed on IN12.

IN12 and D7 polarisation analysis to extract the incoherent part of room temperature EISF of deuterated and protonated GFP hydrated powders.

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11.06.21-15.06.21 (IN12, 8-05-468); 07.10.21 (D7, EASY-1064)

Introduction

Biological systems are mostly studied with classical approaches, but to what extent can quantum effects govern the dynamics of such complex systems? Our approach uses G.Kneller's theory (Kneller [2018]) to explain proteins' dynamics with a quantum development of scattering functions, leading to a different qualitative understanding of neutron quasi-elastic scattering.

An experiment carried out on IN5 in October 2019 (DIR-173, cycle 192) provided us the Elastic "Incoherent" Scattering Factor (EISF) evaluated for a hundred temperatures in the $[2K, 300K]$ range for deuterated (d) and protonated (p) GFP hydrated powder samples of 100mg of proteins, 40% gD_2O/gGFP.
Interesting mass-dependent behaviour of MSDs motivated us into investigating to what extent coherent scattering intensity contributes to total scattering intensity at room temperature. Furthermore, the dGFP elastic scattering profile tends to be less smooth at higher temperatures, displaying evidence of coherent contribution to scattering.
A first trial to decipher the importance of coherent scattering for both proteins consisted in the evaluation of the coherent and incoherent fractions of scattering using a CRYSON generated Small Angle Neutron Scattering profile with pGFP and dGFP structures (pGFP : 10.2210/pdb6KL1/pdb, dGFP : 10.2210/pdb6KKZ/pdb).
It appeared that incoherent scattering is clearly dominant for pGFP above $Q = 0.3\mathring{A}^{-1}$, however dGFP fraction of coherent scattering averages 30% of total scattering over all Q-range and reaches 50% about $Q = 1.5\mathring{A}^{-1}$, corresponding to the β barrel secondary structure. This is consistent with dGFP atomic formula ([D=1848,C=1204,O=320,N=364,S=7]) consisting of atoms with dominant coherent cross-sections. Therefore, experimental separation of both contributions had to be carried out (Gaspar et al. [2010]).

Experiments

For both experiments on IN12 and D7 we studied per-deuterated GFP and protonated GFP powder samples, of respectively 137.5mg and 173mg after 43.5% and 37.4% hydration with D_20. Our samples were kept in the same 0.5mm thick flat sample holder for both experiments.
We carried out uniaxial polarisation analysis at room temperature on IN12 TAS with incoming wavelength $3\mathring{A}$ in June 2021 (6 days, exp 8-05-468 cycle 212). Upon difficult correction of data with Vanadium we submitted an Easy Proposal for uniaxial polarisation analysis with D7 in diffraction mode for incoming wavelength $4.8meV$ (intensity integrated from $-k_bT$ to 3.5 meV) in October 2021 (1 day, exp EASY1064 cycle 213) which provided us better resolution on the Q-axis.

IN12 experiment :
We have acquired the spin flip and non spin flip intensities of dGFP and pGFP, of the 0.5mm thick flat holder, of a 1.5mm thick Cadmium slab and a 1mm thick Vanadium slab for corrections. The samples/slabs/container scattering intensities were acquired for the same period of time (about 21 hours, 650s scan per Q value). We probed a Q range from $0.05\mathring{A}^{-1}$ to $3\mathring{A}^{-1}$. The wavelength of the incoming beam is equal to $3\mathring{A}$. Intensities are normalized to a monitor arbitrary number of counts. We used a vacuum box to suppress multiple scattering at low Q values that made it possible to go down to $Q = 0.1\mathring{A}^{-1}$.
Reduction of data was carried out with Mathematica software.

D7 experiment :
Polarisation analysis was carried out on D7 diffuse scattering spectrometer, with incoming beam of 4.8 corresponding to a Q range between 0.3 and 2.5, and an incident energy of $3.55meV$. We calibrated the acquisition windows and the flipper magnetic field, and acquired 30 minutes of scattering data with Quartz, Empty Cell, Cadmium and Vanadium for reduction of data. We also calculated their transmission as well as each sample's transmission. Then we acquired 1h30 of neutron scattering events for each protein sample.
Data was reduced with Lamp software and Mathematica software.

Analysis :

Polarisation analysis on both instruments provides us spin flip and non spin flip intensities, that make it possible to retrieve coherent and incoherent scattering intensities of the sample. There were about 100 times more events probed per Q value with the IN12 experiment compared to the D7 experiment.
By calculating the fraction of incoherent scattering :

$$\frac{S_{inc}}{S_{coh}} = \frac{1}{\frac{I_{coh}(Q)}{I_{inc}(Q)} + 1} \tag{1}$$

One has access to the incoherent fraction of scattering to correct IN5 elastic data at room temperature (300K) and to deduce the impact of coherent scattering on the Elastic Scattering with $EISF_{corrected} = (1-\alpha)EISF_{IN5}$.
Figure 1 displays fractions of scattering obtained with both instruments. Both curves display the same shapes. It appears that even for the dGFP sample, the coherent fraction of scattering never exceeds 30% of total scattering. This is less than our former estimation. However the relative difference of coherent scattering fraction obtained with both instruments averages 30% which is non negligible, and it might be due to different calibrations of the instruments. Statistics should not have an impact on this difference ($\frac{I_{inc/coh}(Q)}{\Delta I_{inc/coh}(Q)}$ have the same order of magnitude).

Table 1 displays the MSDs $< u^2_{corrected} >$ calculated with the Gaussian approximation $EISF_{corrected} = exp(-\frac{Q^2 * < u^2_{corrected} >}{3})$ with data acquired from $Q = 0.5\mathring{A}$ to $Q = 2.5\mathring{A}$. MSD values seem to be under-estimated with IN5 experiment due to coherent scattering contribution, and are systematically lower for D7 experiment than for IN12 experiment. It appears that coherent scattering does not contribute more than to 10% in the MSD values at room temperatures.

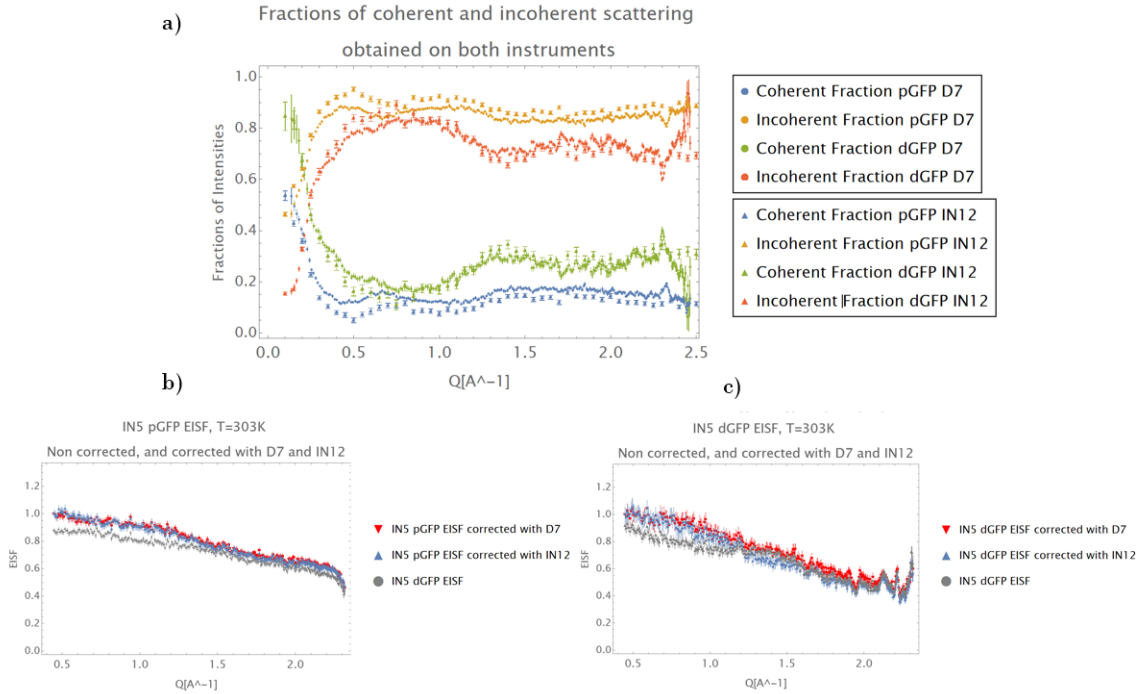

Figure 1: a) Fractions of coherent and incoherent scattering intensities obtained with D7 and IN12 polarisation experiments. b) pGFP IN5 elastic data at $T = 300K$ corrected with both instruments. c) dGFP IN5 elastic data at $T = 300K$ corrected with both instruments.

pGFP			**dGFP**		
MSDs IN5	MSDs corrected with D7	MSDs corrected with IN12	MSDs IN5	MSDs corrected with D7	MSDs corrected with IN12
0.309 ± 0.004	0.33754	0.34888	0.474 ± 0.009	0.494147	0.559769

Table 1: MSDs obtained for both protein samples with incoherent scattering fraction correction of EISF data, from $Q = 0.5\mathring{A}^{-1}$ to $Q = 2.3\mathring{A}^{-1}$.

References

Gerald R. Kneller. Franck–condon picture of incoherent neutron scattering. *Proceedings of the National Academy of Sciences*, 115(38):9450–9455, August 2018. doi: 10.1073/pnas.1718720115. URL `https://doi.org/10.1073/pnas.1718720115`.

Ana M. Gaspar, Sebastian Busch, Marie-Sousai Appavou, Wolfgang Haeussler, Robert Georgii, Yixi Su, and Wolfgang Doster. Using polarization analysis to separate the coherent and incoherent scattering from protein samples. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1804(1):76–82, January 2010. doi: 10.1016/j.bbapap.2009.06.024. URL `https://doi.org/10.1016/j.bbapap.2009.06.024`.