Experimental Report

Proposal: **9-13-482** **Council:** 10/2012

Title: Identifying the geometry of pico-second motion in lyophilised proteins

This proposal is a new proposal

Researh Area: Soft condensed matter

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Samples:
apoferritin
insulin
superoxide dismutase
green fluorescent protein

Instrument	Req. Days	All. Days	From	To
IN13	4	5	02/04/2013	09/04/2013

Abstract:

We recently used IN16 to study ps-ns dynamics in lyophilized proteins (apoferritin, green fluorescent protein, superoxide dismutase and insulin) By operating IN16 in elastic fixed window mode (EFWS) we showed that while only CH3 activation is apparent in Apo, a more enhanced dynamic environment is evident in Ins, SOD and GfP; a second, possibly structure dependent, dynamic contribution being required to describe the data The origin of this extra component is unclear Due to the limited Q range afforded by IN16 no information about the geometry of motion associated with the second component could be inferred from the elastic incoherent structure factor, EISF. Indeed, the EISF of the second process in all three proteins showed a weak Q dependence which could be modelled by either the response predicted for protons undergoing 2-site jumps or for protons diffusing on a sphere. To distinguish between these two models we now ask for time on IN13 to perform complementary EFWS measurements on Apo, SOD, Ins and GfP The greatly extended Q range on IN13 will allow us to ascertain which motion is applicable; deviation from either prediction perhaps suggesting an alternative diffusive response

Identifying the geometry of pico-second motion in lyophilised proteins

Experiment No: 9-13-482 / IN13 Dates: 02/04/2013 To : 09/04/2013

Atomic fluctuations in the pico- and nano-second (ps, ns) time scale in proteins facilitate the slower and larger length-scale dynamics involved in protein folding and in biological functions such as enzyme catalysis or ligand binding [1] As a result, detailed appreciation of structure-dynamics-function relationships at these scales requires analysis based on models that realize the full complexity of biomacromolecular material. Neutron spectroscopy, in particular the backscattering technique, provides temporal information on such a scale and is an ideal tool with which to aid development of such models; parameters extracted from experimental studies validating molecular dynamic (MD) simulations [2]

Results of previous work

To investigate ps-ns dynamics in proteinaceous material, we previously collated data using the backscattering instruments IN16 (ILL), OSIRIS (ISIS) and IRIS (ISIS) to investigate the hydration dependence of the dynamical transition in apoferritin (Apo, the iron-depleted form of the iron storage protein, ferritin). While a transition was observed in the mean squared displacement (msd) parameter in the hydrated state, an inflection was also seen at 100K in both hydrated and lyophilized (reference) material [3]**.** By modeling the data using theory developed for glassy polymers we showed that the 100K inflexion is due to the onset of CH_3 dynamics; a distribution of CH_3 activation energies ($E_{a,ave}$=17kJmol^{-1}) of width (σ) 4kJmol^{-1} [4] being determined

To advance this work, we very recently used IN16 to investigate low-temperature dynamics in other lyophilized proteins; namely green fluorescent protein (GfP), superoxide dismutase (SOD) and insulin (Ins). This work was in part to investigate the influence of helicity on methyl group activation; GfP being a beta-sheet protein where as insulin comprises of a helical secondary structure. Like Apo, GfP, SOD and Ins have approx. 25% of all protons associated with methyls.

By operating IN16 in first elastic fixed window, EFWS (Fig.1), and then inelastic fixed window, IFWS (Fig. 2.) [5]**,** mode we showed [6] that while only CH_3 activation is apparent in our time window in Apo, a more enhanced dynamic environment is evident in Ins, SOD and GfP. We find that a second dynamic contribution, with a $E_{a,ave}$ distribution comparable to that of methyl species, is required to fully describe the experimental data. It is worth commenting that despite the limited number of proteins studied, the increase in IFWS intensity (Fig. 2) correlates with α-helix and β-sheet composition; higher quasi-elastic intensities being measured from β-sheet rich species.

The IN13 experiment

The origin of this additional component is unclear. While our IN16 results are in line with:

i) early NMR studies which suggest that CH_3 group reorientations can account for just 60 – 90 % of observable proton relaxation [7] and
ii) the fact that non-methyl side-chain motions (e.g., the phenolic ring) can contribute to an EFWS intensity decrease / IFWS intensity increase by 10 – 20 % [8]**.**

they are, surprisingly, inconsistent with those published from lyophilized bacteriorhodopsin [9] and lysosyme [10]; systems for which only CH_3 dynamics are reported. Such a discrepancy could be due to the fact that, unlike previous works, no H/D exchange of the labile protons was performed during dialysis of our materials. As a result, our measurements are possibly sensitive to relaxation from both exchangeable AND non-exchangeable protons i.e. reorientations of side groups

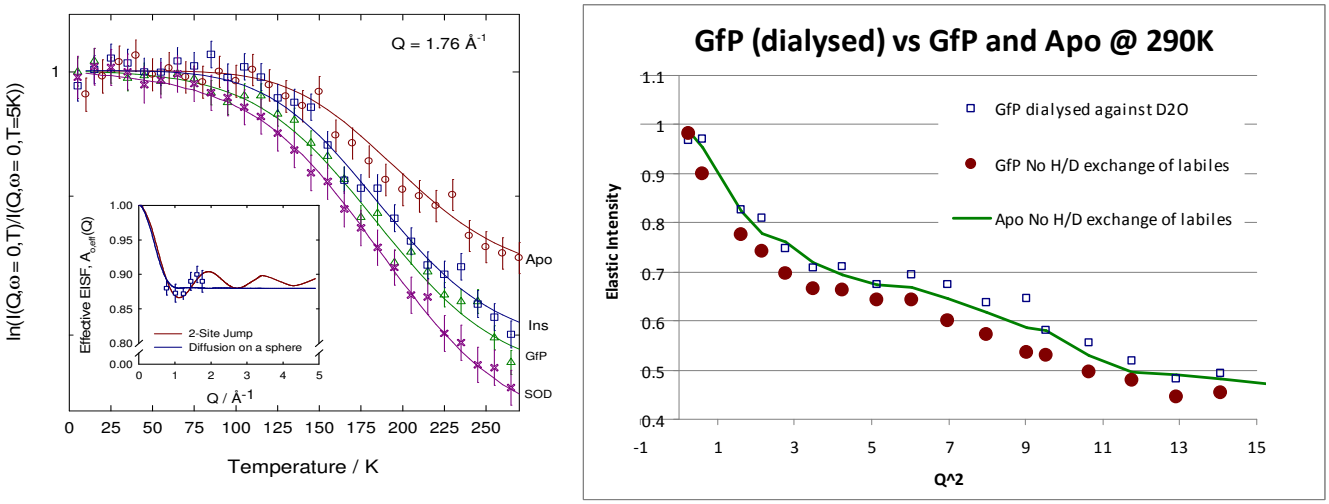

Fig. 1: Normalised EFWS from Apo, GfP, Ins and SOD ( ) at Q = 1.76 modelled as described in [6]. Inset : The effective EISF from fitting the Ins EFWS data. A similar EISF response was seen from SOD and GfP. The solid lines are fits using EISF predictions for a 2-site jump and diffusion on a sphere extended to the Q range accessible on IN13; d and r = 4A. Fig 2, I(Q,T=290K) for h-apo, h-gfp and d-gfp

containing un-bound protons (e.g., NH2, OH on amino groups). Recent computer simulations of the dynamic neutron scattering susceptibility (1-1000 GHz) expected from lyophilized lysosyme (Hong *et al* [11]) suggest that the protein atoms should undergo three classes of motion - rotation, jumps and localized diffusion [12]. It should be possible to glean information about the localized geometry of the additional component via the Q dependence of the elastic incoherent structure factor, EISF ($A_{o,eff}(Q)$). Unfortunately, over the Q-range accessible on IN16 (0.2–1.9Å^{-1}) $A_{o,eff}(Q)$ associated with the second process shows only weak Q dependence (Fig 1). As a result, and considering the predictions of Hong *et al* , we can broadly described $A_{o,eff}(Q)$ using either the theoretical EISF response predicted for i) protons undergoing 2-site jumps of distance, *d*, i.e. $A_{o,jump}(Q) = 0.5[1+j_o(Qd)]$, or ii) protons diffusing on a sphere of radius, *r*, i.e. $A_{o,diff}(Q) = |(3j_1(Qr))/Qr|^2$. Little geometric information can be inferred from the IN16 data available alone

Our preliminary IN13 EFWS results, however, show very subtle differences between all four proteins over a much extended Q range (0.2 – 4.9 A^{-1}), However, two results, highlighted in (Fig 2), stand out. First there was a marked difference between the GfP sample that had been lyophilised against D2O (d-GfP, i.e. all labile protons had been removed from the scattering intensity) and that lyophilised in H2O (h-GfP). We hypothesize that the extra loss of elastic scattering intensity in h-GfP arises from reorientations of side groups containing un-bound protons. Second, the response from h-Apo was comparable to d-GfP adding weight to our hypothesis that in un-deuterated Apo (h-Apo) it is CH3 species alone that dominate the scattering response; at least on the experimental time scale of the IN13 instrument

To conclude this work we plan to perform complementary measurements on d-SOD, d-Ins, d-Lys and d-Apo to see: a) if a similar differences in the elastic scattering is observed in all but the apoferritin samples and b) if said differences correlate with protein structure / our previous IN16 results. In addition, the results will be modeled according to the theory given in [6] and compared to previously reported data published by ourselves and other authors. In addition C-lab effort will be sought to help corroborate our experimental findings. The partnership between simulation and experiment is imperative for a clearer understanding of the dynamic landscape in such complex systems.This data will contribute greatly to the ILL Neutron Dynamics Data Bank

1 K. A. Henzler-Wildman et all, *Nature*, 2007, **450**, 913-U927. 2 .L. Meinhold, et al , *PNAS*, 2007, **104**, 17261-17265. 3. M. T. F. Telling, et al *JPCB*, 2008, **112**, 10873-10878. 4. M. T. F. Telling, C. Neylon, L. Clifton, S. Howells, L. van Eijck and V. Garcia Sakai, *Soft Matter*, 2011, **7**, 5. B. Frick, J. Combet and L. van Eijck, *Nuclear Instruments & Methods* 2012, **669**, 7-13. 6. M. T. F. Telling, L. Clifton, J. Combet, B. Frick, S. Howells and V. G. Sakai, *Soft Matter*, 2012. 7. E. R. Andrew, D. N. Bone, D. J. Bryant, E. M. Cashell, R. Gaspar and Q. A. Meng, *Pure and Applied Chemistry*, 1982, **54**, 585-594.8. G Schiro, et al , *J. Am. Chem. Soc.*, 2010, **132**, 1371-1376. 9.K. Wood, D. J. Tobias, B. Kessler, F. Gabel, D. Oesterhelt, F. A. A. Mulder, G. Zaccai and M. Weik, *J. Am. Chem. Soc.*, 2010, **132**, 4990. 10.J. H. Roh, et al , *PRL*, 2005, **95**, 038101. 11.L. Hong, et al *PRL*, 2011, **107**, 148102. 12. L Hong, X. Cheng, D. C. Glass and J. C. Smith, *PRL*, 2012, **108**, 238102.