Experimental Report

Proposal: **8-04-726** **Council:** 4/2014

Title: Probing dynamics of the hemoglobinconfined inside silica tubes

This proposal is a new proposal

Researh Area: Chemistry

Main proposer: **BHATTACHARYYA Aninda Jiban**

Experimental Team: BHATTACHARYYA Aninda Jiban
SAMAJDAR Rudra Narayan
GOPE Subhra

Local Contact: OLLIVIER Jacques
NATALI Francesca

Samples: Hemoglobin
Silica

Instrument	Req. Days	All. Days	From	To
IN5	3	3	04/11/2014	07/11/2014

Abstract:

Artificial matrices such as inorganic porous media are often employed to mimic a living cell. Unlike the in vivo environment inside a cell, artificial matrices can be suitably constructed to impart beneficial physical properties to confined biological molecules such as proteins. The vivid manifestation of biological molecules confined inside inorganic hosts can be observed in the fields of sensing, catalysis, sustained delivery. We have recently shown enhanced electrochemical response and structural stability of heme proteins confined inside various organic and inorganic hosts compared to the proteins in solution. The observed changes were attributed to the differences in protein configurations between unconfined and confined proteins. The protein configurations and dynamics are often correlated to the solvation dynamics. We would like to investigate the unconfined and confined protein dynamics

Preliminary Report on Experiment 8-04-726 at IN 5:

Exploring the dynamics of Hemoglobin confined inside Silica tubes by Quasi Elastic Neutron Scattering.

This experiment involved studying the Quasi Elastic Neutron Scattering from Hemoglobin confined inside Silica tubes of different diameters (200 nm and 20 nm).

Silica tubes were prepared via a template assisted sol-gel synthesis.[1] The diameter of the synthesized silica tubes were controlled by the pore size of the templates (Anodisc Aluminium Oxide, Whatman®) – 20 nm and 200 nm respectively. The protein solutions were prepared in PBS buffer in D_2O (Sigma Aldrich). A concentration of 20 mg/mL was maintained for optimal QENS signal. The concentration was estimated via Nanodrop spectrometer, Thermo scientific.

The protein was loaded in the tubes via the incubation method as described in our earlier work.[2] The protein loaded tubes were dispersed again in PBS buffer in D_2O for the QENS measurements. Concentration of the protein loaded tubes in buffer was again kept at 20 mg/mL for optimal QENS signal.

Initial characterization of the protein loaded in silica tubes using UV Visible Spectroscopy and Circular Dichroism indicated the retention of the secondary and tertiary structural integrity of the protein even after being loaded inside the silica tubes.

The QENS measurements were done at the IN 5 beam station using a cylindrical aluminium sample holder. 2.2 mL liquid sample was used during each measurement. Data was collected at $5A^o$ and $10A^o$. The measurements were made at three different temperatures – 293 K, 313 K and 328 K respectively. Appropriate corrections were also employed using Vanadium standard and the blank sample holder before starting the measurements for the actual samples.

Each measurement was done over 120 minutes (12 cycles of 10 minutes each). Before each temperature change, a buffer time of 30 minutes was given to equilibrate the temperature distribution over the entire sample.

Data was collected for the following samples:

1. Blank buffer with 20 nm tubes
2. Blank buffer with 200 nm tubes
3. Protein in buffer
4. 200 nm tube, loaded with protein, dispersed in buffer.
5. 20 nm tube, loaded with protein, dispersed in buffer.

The analysis of the QENS data is being done using the LAMP package available at the ILL.[3] The QENS spectra binned over the accessible Q range shows a distinct quasi elastic component.

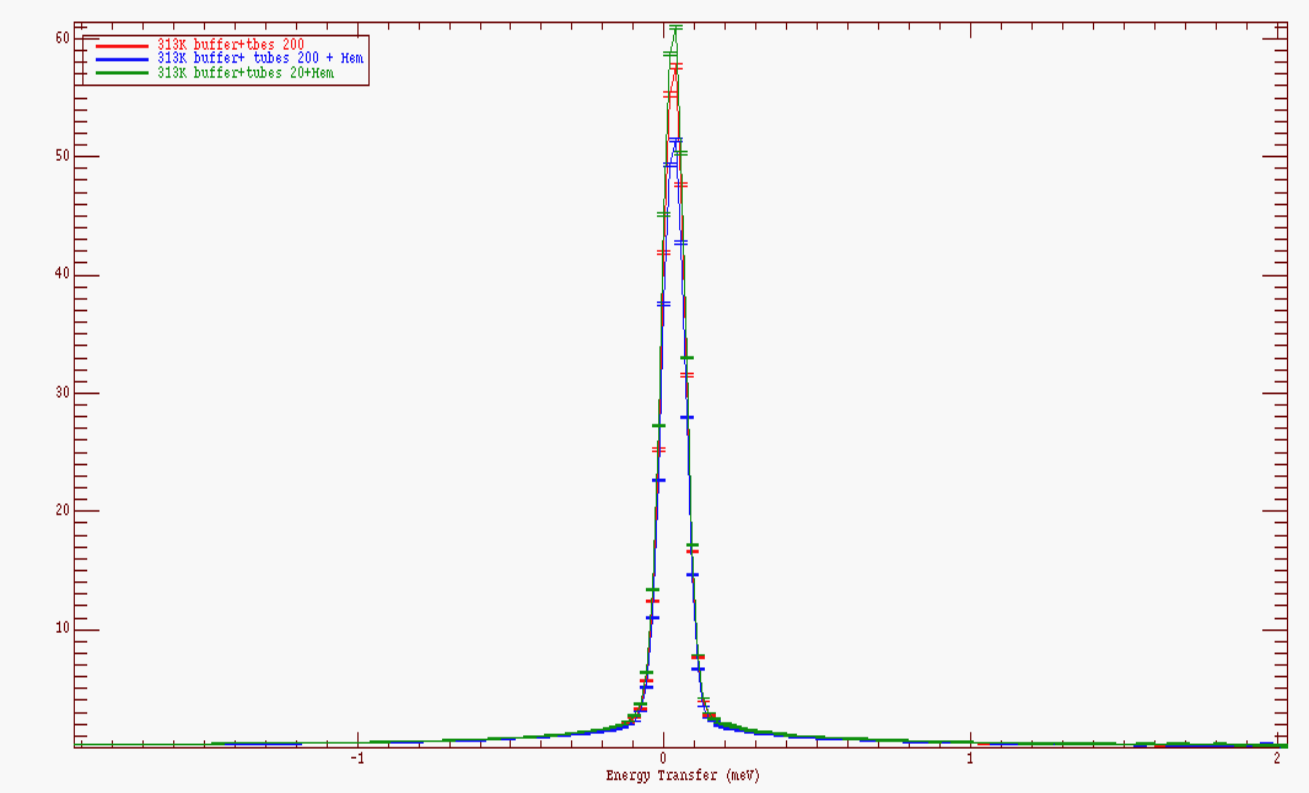

Fig 1: QENS spectra of protein in 200 nm tube (blue), protein in 20 nm tubes (green) and blank 200 nm tubes in buffer (red) all recorded at 313 K. The spectra have been binned over entire accessible Q range.

This Q averaged QENS structure function has been assumed to have the form[4]:

$$< S(Q,w) >_Q = < e^{-\frac{<u^2>}{6}Q^2} >_Q \; [A_0\delta(\omega) + (1 - A_0)L(\omega)]R(\omega) + C$$

Here A_0 = Elastic Incoherent Structure Factor, L(ω) = Lorentzian quasi elastic component, R(ω) = experimental resolution function and C = flat background term for correcting inelastic contribution in the QENS energy region.

The Q dependence of the Lorentzian broadening and the Elastic Incoherent Structure Factor are now being studied to understand the diffusion behaviour of the confined protein. It is expected that the motion of the mobile protons will follow the model of restricted diffusion within a sphere or rotation coupled with diffusion as the system essentially involves protein under confinement. [5]

Further analysis of the data is required before a clearer picture can be presented about the system.

Once the analysis is complete, the results will be communicated to a peer reviewed journal.

References

[1] Kovtyukhova, N. I.; Mallouk, T. E.; Mayer, T. S. *Adv. Mater.* **2003**, *15*, 780-785

[2] Kapoor, S.; Mandal, S. S.; Bhattacharyya, A. J. *J. Phys. Chem. B* **2009**, *113*, 14189-14195

[3] Richard, D.; Ferrand, M.; Kearley, G. J. *J. Neutron Research* **1996**, *4*, 33-39

[4] Caronna, C.; Natali, F.; Cupane, A. *Biophys. Chem.* **2005**, *116*, 219-225

[5] Volino, F.; Dianoux, A. J. *Mol. Phys.* **1980**, *41*, 271-289.