Experimental report

18/03/2016

Proposal: 8-04-744 **Council:** 10/2014

Title: Probing dynamics of hemoglobin confined inside silica tubes

Research area: Chemistry

This proposal is a new proposal

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Samples: Hemoglobin
silica

Instrument	Requested days	Allocated days	From	To
IN13	6	5	18/09/2015	23/09/2015

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 using elastic neutron scattering using IN13. Neutron scattering is the only effective characterizing tool to study the effect of the solvation on the protein dynamics.

Preliminary Report on Experiment No. 8-04-744 at IN 13:

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

This experiment involved studying the Elastic Neutron Scattering from Hemoglobin confined inside Silica tubes of different diameters (200 nm and 20 nm). The experiment was a follow up to Quasi Elastic Neutron Scattering measurements done on the same type of samples in IN 5, ILL during the experiment 8-04-726 [1].

Silica tubes were synthesized via a template assisted sol-gel procedure as reported earlier [2]. The diameter of the synthesized silica tubes was 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 50 mg/mL was maintained for optimal ENS signal. The concentration was estimated by monitoring the Tryptophan absorption at 280 nm via UV-Visible spectroscopy.

The protein was loaded in the tubes via solution incubation method which is essentially diffusion driven [3]. The protein loaded tubes were dispersed again in PBS buffer in D_2O for the ENS measurements. Concentration of the protein loaded tubes in buffer was approximately the same as that in solution (≈ 50 mg /mL). The stability of the protein – tube complex over the time of the experimental measurement was verified separately via UV visible spectroscopy.

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

The ENS measurements were done at the IN 13 beam station using flat aluminium sample holder of dimensions 30mm X 40mm X 0.2mm. The holder was sealed with Vanadium wire prior to measurement. Approximately, 2 mL of liquid sample was used during each measurement. The measurements were made at five different temperatures – 288 K, 298 K, 308 K, 318 K, and 328 K respectively. Appropriate corrections were also employed using Vanadium standard and the blank sample holder. The transmission of each sample was recorded prior to measurement. The mass of the sample along with sample holder was also recorded before and after each measurement to check for mass loss under vacuum.

Data for each sample was collected over 7 cycles (30 mins X 7 cycles) to ensure good statistics for the measurement. The samples recorded were:

1. Hemoglobin in D_2O (50 mg/ mL)

2. Hb – Silica 200 nm tubes in D_2O (50 mg/ mL)
3. Silica 200 nm tubes in D_2O (50 mg/ mL)
4. Hb – Silica 20 nm tubes in D_2O (50 mg/ mL)
5. Silica 20 nm tubes in D_2O (50 mg/ mL)
6. Blank D_2O buffer.

The elastic neutron scattering data was treated using the LAMP package [4]. The data was modelled in terms of the Gaussian model [5]:

$$I\,(Q,\omega=0) = I_0 e^{-\frac{\langle \Delta u_{tot}^2 \rangle}{6} Q^2}$$

This model is valid only in the low q range:

$$\sqrt{\frac{\langle \Delta u^2 \rangle Q^2}{2}} \leq 1$$

The force constants were calculated from the earlier report by Zaccai *et. al.* [6].

$$\langle k \rangle = \frac{0.00138}{\frac{d < 3x^2 >}{dt}}$$

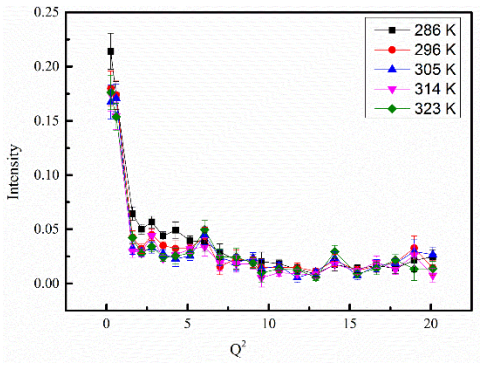

Fig 1: Elastic Neutron Scattering from Hb in D_2O at different temperatures

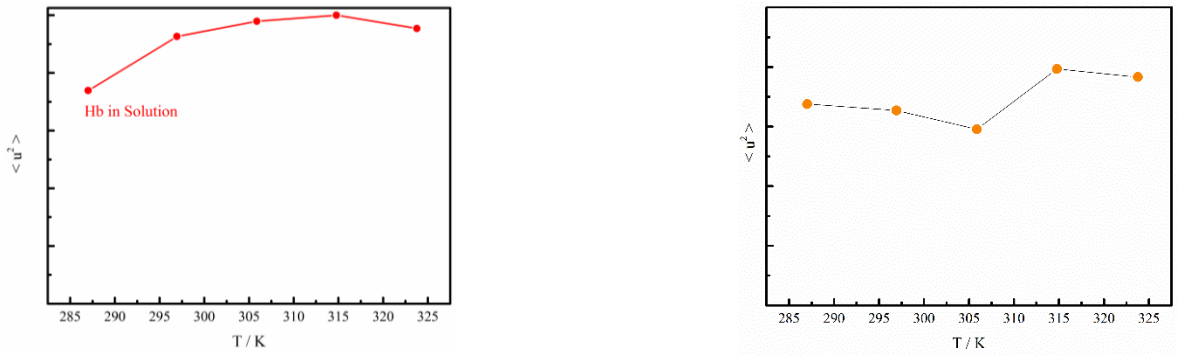

Fig 2: RMSD versus T plots for Hb in solution (red) and Hb – Silica 200 nm (yellow)

The force constants calculated from protein in buffer and protein in tubes are 0.08 N/m and 0.16 N/m respectively.

Preliminary data analysis indicated hardening of the polymer backbone under confinement. The temperature range used for the measurement is beyond the glass transition temperature of the protein. The glass transition of the protein is now being investigated to see if the hardening leads to a higher glass transition temperature.

Once all data is obtained, the results will be communicated to a peer reviewed journal.

References

[1] Samajdar, R. N.; Gope, S.; Ollivier, J.; Natali, F.; Bhattacharyya, A. J. *IN 5 Experimental Report **8-04-726**, ILL, Grenoble.* **2014** (DOI 10.5291/ILL-DATA.8-04-726)

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