Experimental Report

Proposal: **8-03-784** **Council:** 10/2012

Title: The effect of confinement on proteins inside silica tubes investigated using SANS

This proposal is a new proposal

Researh Area: Chemistry

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Samples: Hemoglobin
SiO_2

Instrument	Req. Days	All. Days	From	To
D11	2	1	14/03/2013	15/03/2013
D16	3	3	11/03/2013	14/03/2013

Abstract:

Confinement of bio-molecules within porous matrices is of significant interest from the biophysical point of view. These porous matrices mimic the conditions of a living cell and hence it helps in studying the various bio-physical processes taking place inside the cell. The interplay of various parameters such as protein-protein, protein-host and confined solvents significantly influence the protein conformations within the hosts. The changes in structure and conformation of protein can only be vividly accounted using neutron scattering techniques. We would like to study using small angle neutron scattering (SANS) (viz. quaternary) structure of hemoglobin entrapped in silica tubes of varying pore diameters.

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The effect of confinement on proteins inside silica tubes investigated using SANS

The proposal aimed at investigating the structural changes in proteins as a result of its confinement inside silica tubes (SiO_2). Hemoglobin (Hb) was selected as a model protein for these studies. Small angle neutron scattering (SANS) served as a tool to study the change in its shape as well as its size in terms of the radius of gyration (R_g). Confinement results in a change of their functional properties such as melting temperature, redox activity. These consequences are of great significance for various biotechnological applications viz. biosensors where proteins are immobilized on the solid substrates. In this proposal, we had further investigated the effect of Silica tube pore size (20-200 nm) on the Hb structure. The successful confinement of Hb within these Silica tubes was established using confocal laser scanning microscopy (CLSM).

Instrumental configuration used for experiment: The SANS were experiments were performed in the D11 and D16 instruments. In D11, the wavelength was set to 6 Å. The experiments were carried out in the sample to detector distance of 1.2 and 8 m to cover a *q* range of 0.007-0.5 $Å^{-1}$.The scattering intensities were normalized with standard water measurement after the required transmission measurements have been carried out. In D11, the measurements were carried out in quartz cuvettes with 1 mm thickness. In D16, a neutron wavelength of 4.767 Å and a sample to detector distance of 1 m was used covering a momentum transfer range 0.06 – 1.8 $Å^{-1}$. The measurements were carried in cylindrical vanadium sample holder of diameter ~10 mm. This configuration was set in both instruments so as to get considerable overlapping. The scattering contribution of the empty sample holders and the buffer was subtracted from the samples. The scattering intensity was normalized with the standard samples (Cadmium for D11, Vanadium for D16).

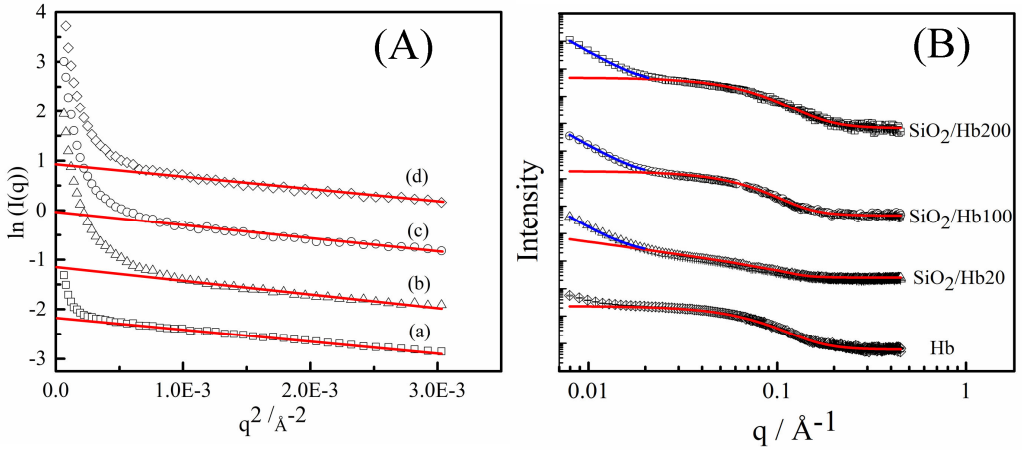

Figure 1: (A) Guinier plots of SANS data. (a) Hb (b) Si-tube-Hb20 (c) Si-tube-Hb100 and (d) Si-tube-Hb200. (B) SANS profiles for Hb and SiO_2-tube-Hb in 0.1 M PBS.

Data analysis and results: The SANS results were analysed by fitting the scattering patterns with a Schultz distribution of spheres. In SiO_2-tube-Hb system, the low q-data was fitted using a

Porod function (q< 0.02) while the higher q region (q> 0.02) was fitted using Schultz distribution for the number distribution of the sphere sizes. Modelling of the 1-D scattering profiles yields useful insight into the 3-D protein structure. The radius of gyration, (R_g) and polydispersity (p) in size distribution was estimated from the SANS profiles shown in figure 1A. The radius of gyration ($R_{g,}$) obtained using the model fitting was compared with that obtained from the Guinier's plot (plot of ln I vs q^2) shown in figure 1B. The comparison of the values obtained from these model fittings and Guinier's plot are shown in the table 1

Sample	**R_g (Schultz sphere)/Å**	**Polydispersity(p)**	**$R_{g,}$ (Guinier analysis)/Å**	**R_g (GNOM)/Å**
Hb	31.2 ±0.1	0.58 ± 0.01	32.2 ± 0.3	30.2 ± 0.2
SiO_2-tube-Hb20	33.8 ±0.2	0.59 ± 0.05	32.6 ± 0.7	33.3 ± 0.6
SiO_2-tube-Hb100	23 ±0.1	0.45 ± 0.02	23.5 ± 0.2	23.1 ± 0.1
SiO_2-tube-Hb200	26.5 ±0.3	0.55 ± 0.06	26.3 ± 0.3	25.29 ± 0.1

The pair-distance distribution functions were also obtained by fitting the scattering data using the program GNOM. This analysis provided additional support to the R_g values obtained from model fitting and Guinier's plot. Maximum diameter of the particle D_{max} was also determined from this pair-distance distribution analysis. The model structure of the free Hb in solution and that confined inside the SiO_2 tubes were constructed using the program DAMMIN and DAMAVER.

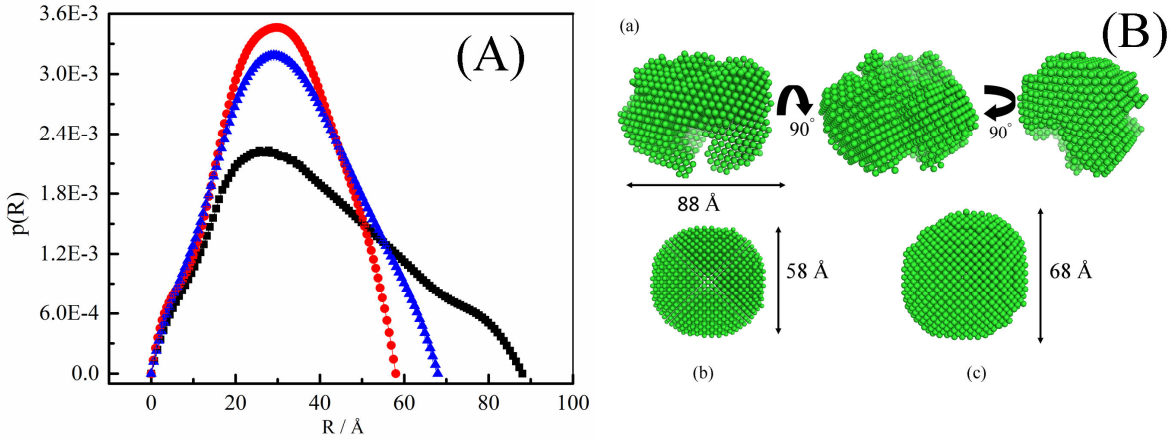

Figure 2: (A) Distance distribution function, p(r) calculated for the samples Hb (black), Si-tube-Hb 100 (red) and Si-tube-Hb200 (blue). (B) Ab initio shape reconstruction from SANS data collected for Hb in solution (top), Hb inside Si-tube of 100 nm diameter (Si-tube-Hb100, bottom left) and Hb inside Si-tube of 200 nm diameter (Si-tube-Hb200, bottom right)

Conclusion: The R_g of Hb inside SiO_2-tubes of 20 nm diameter indicated its significant amount of aggregation. However, for SiO_2-tube with diameters ≥ 100 nm, R_g, of Hb was approximately close to that obtained from the protein data bank (PDB file: 1HHO, R_g of native Hb=23.8 Å). This strongly suggested that the protein has a preference for the more native like non-aggregated state when confined inside tubes of diameter ≥ 100 nm. This fact was further supported by distance distribution function, p(r) and ab-initio models calculated from the SANS patterns. These also suggest that the SiO_2-tube size is a key parameter for the protein stability and structure.