Experimental report

28/08/2022

Proposal: EASY-669 **Council:** 4/2020

Title: Deciphering the role of solvent viscosity for the dynamical properties of folded and intrinsically disordered proteins

Research area: Biology

This proposal is a new proposal

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Experimental team:

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Samples: myoglobin

Instrument	Requested days	Allocated days	From	To
IN16B	24	24	26/03/2021	27/03/2021

Abstract:

The protein COR15A is intrinsically disordered in D2O, but strongly gains helical structure in 70% glycerol buffer. In a previous experiment on IN16B BATS (8-04-830) we measured QENS of COR15A in 0% and 70% deuterated glycerol buffer. Global protein diffusion in 70% glyc. was found to be reduced by a factor of 23 as compared to the value obtained in D2O. This is in agreement with the change of solvent viscosity between 70% glyc. and D2O (reduction of factor 24). Concerning internal protein dynamics, however, we found a 16 times smaller value of the internal diffusion coefficient in the folded protein in 70% glyc. than in the unfolded protein in D2O. We currently cannot differentiate between the effects of changed solvent viscosity and the specific role of protein folding for the reduced internal diffusivity in folded COR15A in 70% glyc. as compared to the unfolded protein in D2O. To complete our experimental QENS data, we suggest to measure a fully folded protein in D2O buffer and 70% glyc. as reference, which would allow us to distinguish between both effects. We would need 1 day on IN16B BATS mode to complete our study (6h per sample: 2 protein solutions and 2 buffers = 1 day).

Deciphering the role of solvent viscosity for the dynamical properties of folded and intrinsically disordered proteins

The protein COR15A is intrinsically disordered in D2O, but strongly gains helical structure in 70% glycerol buffer. In a previous experiment on IN16B BATS (8-04-830) we measured QENS of COR15A in 0% and 70% deuterated glycerol buffer. Global protein diffusion in 70% glyc. was found to be reduced by a factor of 23 as compared to the value obtained in D2O. This is in agreement with the change of solvent viscosity between 70% glyc. and D2O (reduction of factor 24). Concerning internal protein dynamics, however, we found a 16 times smaller value of the internal diffusion coefficient in the folded protein in 70% glyc. than in the unfolded protein in D2O. We could not differentiate between the effects of changed solvent viscosity and the specific role of protein folding for the reduced internal diffusivity in folded COR15A in 70% glyc. as compared to the unfolded protein in D2O. To complete our experimental QENS data, we suggested to measure the fully folded myoglobin (Mb) in D2O buffer and 70% glyc. as reference, which would allow us to distinguish between both effects.

The missing QENS data of Mb in D2O and 70% deut. glycerol as well as the corresponding buffers have been measured on IN16B BATS mode and we are very grateful for the IN16B team who has performed the remote experiment. Unfortunately, we could not come for the experiment to the ILL because of travel restrictions in 2020 and 2021.

In total we have now a complete QENS data set of COR15A and myoglobin (Mb) in D2O buffer and in 70% deuterated glycerol. High resolution QENS was measured on the high-resolution backscattering spectrometers EMU at ANSTO of COR15A and Mb was measured on IN16B with the 0.7 μeV resolution in June 2021. Additionally, we could measure medium resolution backscattering with 3.5 μeV on IN16B BATS of both COR15A and Mb in D2O and 70% deuterated glycerol. Time-of-flight (TOF) spectroscopy was only measured of COR15A in D2O and 70% deuterated glycerol.

TOF data of Mb in D2O and 70% deut. glycerol are, however, still missing. The original plan was to measure TOF of Mb on TOFTOF and the TOFTOF proposal has been accepted in spring 2020 but could not be measured during the well-known problems

of the FRM2 reactor. We aim to publish the data now without the still missing TOFTOF data set in the next future.

The so far existing QENS data has been analysed using a model free approach that is based on fitting the QENS data with 2 Lorentzians for global diffusion and internal dynamics as well as by using the mathematical model of the overdamped Brownian oscillator. Depending on the exact choice of the model for internal dynamics slightly different physical quantities are obtained.

Currently still missing is a data analysis approach that considers the fractional diffusion of the internal dynamics. For this purpose it is planned to model the internal dynamics using Mittag Leffler functions or alternatively just by stretched exponentials in the time-domain.

Overall, we found using high resolution backscattering that global protein diffusion of COR15A and Mb change by factors of 22.8 and 27.2, respectively, between D2O and 70% deut. Glycerol. This observation is in agreement with the result that translational diffusion of D2O and 70% deut. glycerol varies by a factor of 24.2.

Concerning the change of internal dynamics of COR15A and Mb when going from D2O to 70% deut. glycerol, we found a particularly strong variation in the internal diffusion coefficient of COR15A (factor of 16), while for Mb a significantly smaller change in the internal diffusivity has been found (factor between 3 and 6 depending on the model).

The internal diffusivity of the folded state of COR15A and Mb in 70% deut. glycerol is approximately the same, while the fully disordered COR15A in D2O is around 2.5 to 3 more mobile than the fully folded Mb. This points out that COR15A behaves dynamically like a normal folded protein in 70% deut. Glycerol.

Interestingly, when comparing COR15A in 70% deut. glycerol with Mb in D2O we find a ratio of the mobility of 5 to 7. Within the errors this corresponds to the change of Mb between 70% deut. Glycerol with Mb in D2O and corroborates the observation that COR15A is dynamically changed to a folded protein by the structural collapse in 70% deut. glycerol.

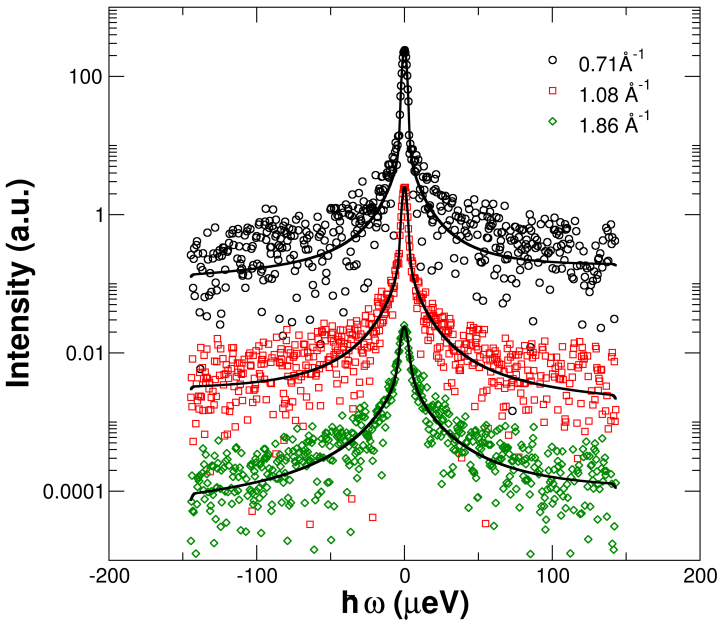

Figure 1: QENS data of Mb in 70% deut. glycerol and theoretical fits using the model of the overdamped Brownian oscillator for internal dynamics.

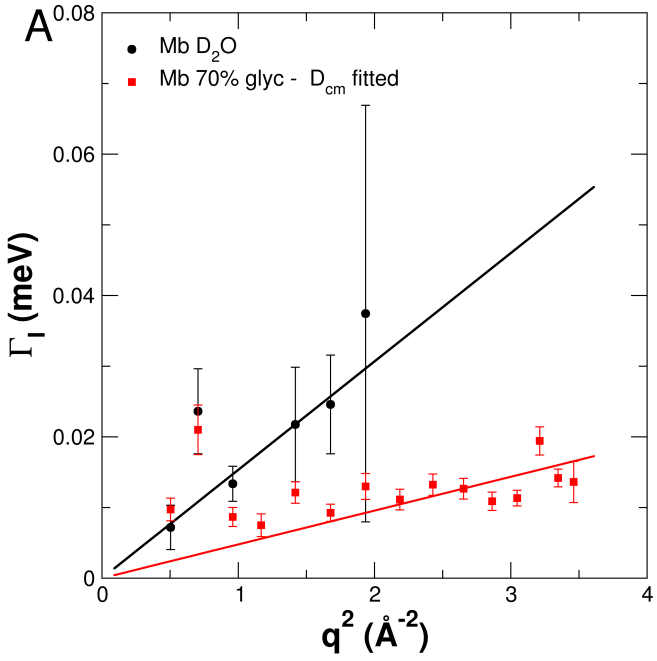

Figure 2: Line-widths of internal dynamics of Mb in D2O and 70%. deut. glycerol. Linear fits were used to extract the internal diffusion coefficients.