Experimental report

10/09/2024

Proposal: 8-04-955 **Council:** 4/2023

Title: Fast global and internal dynamics of the NIST monoclonal antibody

Research area: Biology

This proposal is a new proposal

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Samples: Deuteriumoxide
NIST monoclonal antibody

Instrument	Requested days	Allocated days	From	To
IN16B	5	4	10/11/2023	14/11/2023

Abstract:

Monoclonal antibodies (mAbs) bind very selectively to different antigens and mAbs are used nowadays as molecularly engineered pharmaceuticals. Successful mAb applications that allow for facile home administration require stable and low viscosity formulations with high mAb concentration, which are often difficult to achieve. The complexity of the system, formed by anisotropic and flexible large molecules that interact through several different intermolecular forces leads to various macroscopic phenomena. In the proposal for IN16B we would like to explore global protein self-diffusion in the short-time limit and internal motions of the NIST mAb using high-resolution quasielastic neutron scattering (QENS). We suggest studying the effect of concentration and temperature on short-time self-diffusion of the NIST mAb with the aim to investigate the fundamental correlation between macroscopic solution viscosity, protein flexibility (domain motions, rotational diffusion) as probed by NSE and their inherent connection to short-time self-diffusion. Furthermore, QENS would allow us to access larger q-vectors and probe faster local internal motions than what is currently available with NSE.

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During the experiment on IN16B we have measured a concentration series of the NIST monoclonal antibody (mAb) with concentrations of around 25, 50, 80, 150, 200 and 270 mg/mL in heavy water buffer at T = 25 °C. Additionally the buffer, the empty Al cell and a vanadium sample for instrumental calibration have been measured. The available neutron beam time has been adapted according to the scattering strength of the mAb samples.

An illustrative QENS spectrum of the NIST mAb solution with concentration of 97 mg/mL including theoretical fits is shown in **Figure 1 (A)**. The narrow component contains information on diffusive motions of the mAb domains and slow diffusive motions within the domains. The broad component is caused by faster internal motions within the mAb fragment and can either be modelled by a single Lorentzian or by a sum of Lorentzians within the model of the overdamped Brownian oscillator. The blue line in Figure 1 (A) is a fit using the overdamped Brownian oscillator. As shown in **Figure 1 (B)** the line-widths of the narrow Lorentzian follow a linear dependance as function of q^2 indicative of Fickian diffusion.

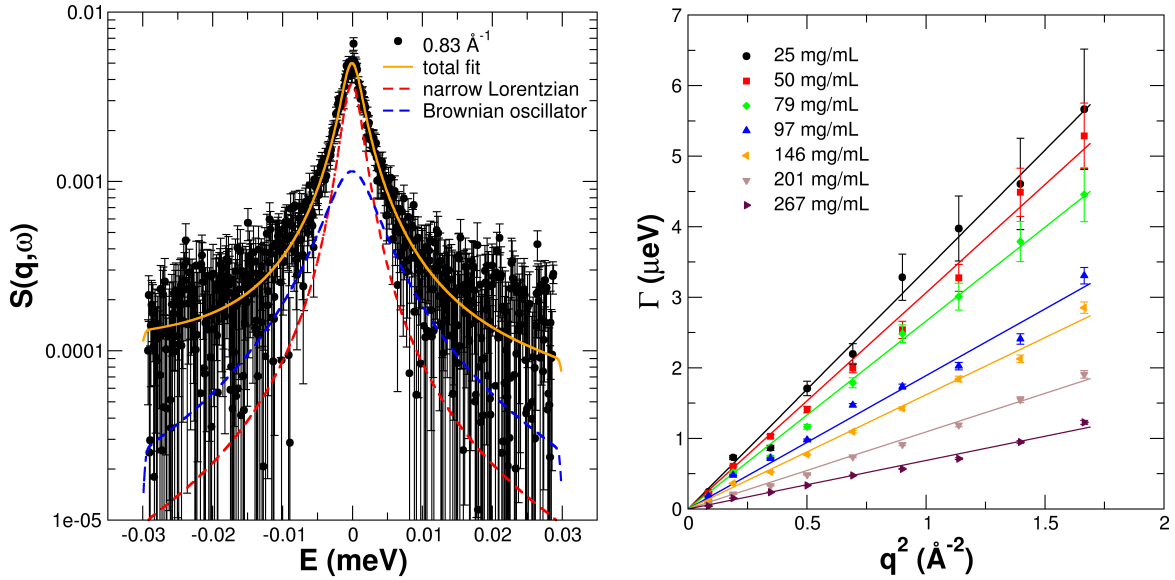

Figure 1. (A) QENS spectrum at q = 0.83 Å^{-1} with theoretical fits. **(B)** Line widths of narrow Lorentzian as function of q^2. Solid lines are linear fits and effective diffusion coefficients have been obtained from the slope of the linear fits.

Figure 2 shows the effective diffusion coefficients as derived from the narrow component. Depending on whether the slow component has been fitted in a global fitting approach or by q-independent fits slightly different values of the D_{eff} are obtained.

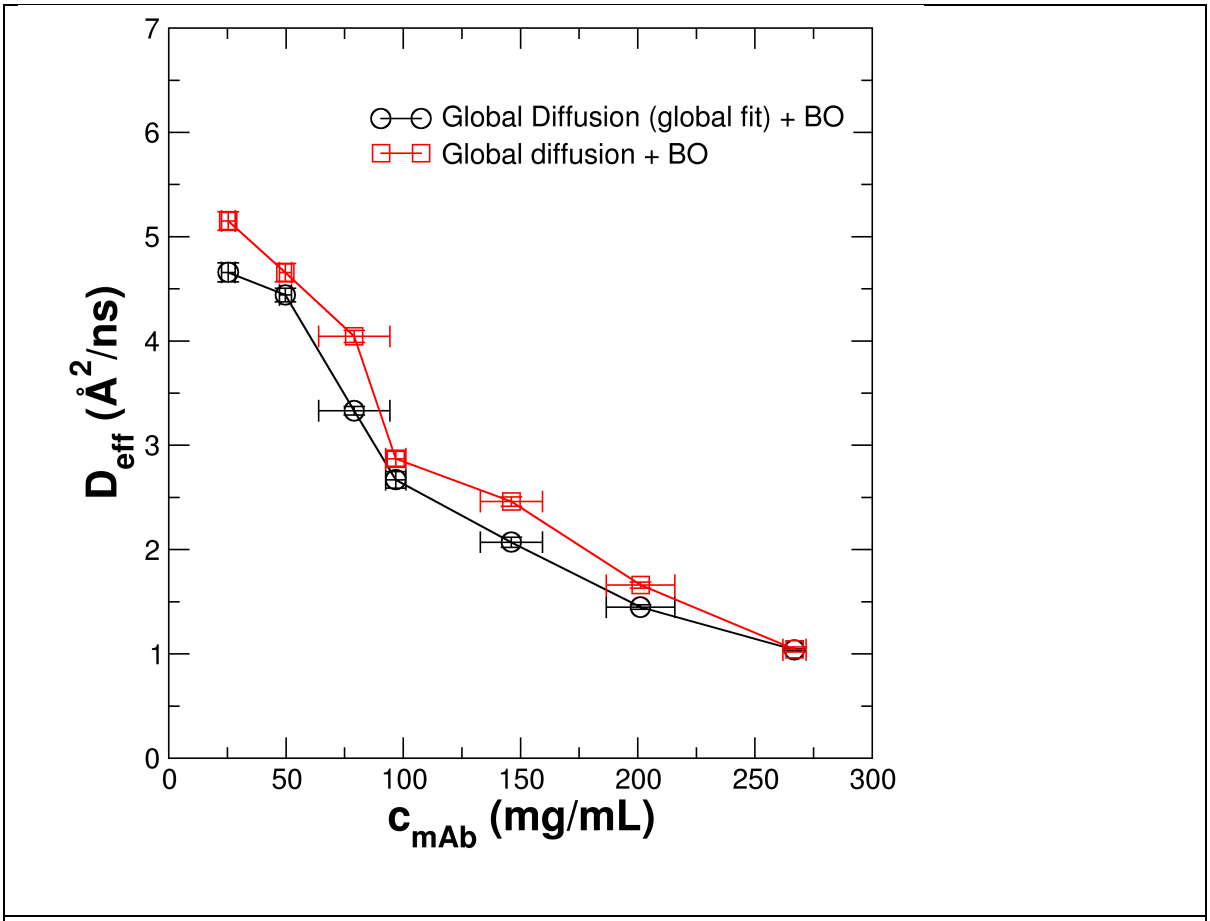

Figure 2. Effective diffusion coefficients that contain information on Fab & Fc domain diffusion, rotation and slow internal domain motion.

The D_{eff} values on slow diffusive dynamics of the NIST mAb are in quantitative agreement with values determined by NSE. To gain more detailed insights into the fast NIST mAb dynamics a second QENS experiment on the IRIS spectrometer at ISIS with a lower energy resolution of 17 µeV and covering an energy transfer range of +/- 0.4 meV has been performed in August 2024. A joint analysis of the IN16B and IRIS data is currently still in progress.

Microsviscosity experiments of the NIST mAb as function of concentration have been performed by the group at Lund University. QENS data will be published in combination with microviscosity data.