Experimental report

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Proposal: 8-04-932

Council: 10/2022

Title: Differences between monoclonal antibodies: Viscosity, diffusion, and transient cluster formation

Research area: Biology

This proposal is a new proposal

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Samples: C6614H10216N1782O2037S42

Instrument	Requested days	Allocated days	From	To
D33	1	0		
IN15	6	4	03/06/2023	07/06/2023
D22	1	1	11/06/2023	12/06/2023

Abstract:

Antibodies are essential in the immune response of mammals and antibody injections are used in various therapies. In pharmaceutical applications, highly concentrated monoclonal antibody (mAb) solutions are required to obtain a significant therapeutic effect. The conflicting requirements of minimizing the injection volumes and of limiting the viscosities for subcutaneous injection make pharmaceutical research on highly concentrated mAb solutions essential.

Importantly, viscosity in mAb solutions can depend sensitively on the type of mAb. Based on neutron spin-echo (NSE) spectroscopy, small angle scattering, and rheology, it has been hypothesized that loosely connected transient clusters are associated with the macroscopic viscosity. So far, the data comparing different mAbs are limited.

Here, we aim to test this hypothesis of loosely connected transient clusters for series of different mAbs provided by Lonza. Their viscosities in solution significantly differ, despite the small structural differences restricted to the mAb Fab (antigen binding fragment) regions. In this way, we aim to obtain a comprehensive quantitative picture of the link between transient clusters and viscosity.

Experimental Report 8-04-932: "Differences between monoclonal antibodies: viscosity, diffusion, and transient cluster formation"

Ilaria Mosca, Christian Beck, Kevin Pounot, Laura Mateo-Minarro, Roody Nasro, Anna Grundel, Christoph Grapentin, Ingo Hoffmann, Olga Matsarskaia, Tilo Seydel, Frank Schreiber

Scientific importance and motivation: Antibody-based biopharmaceuticals span a wide range of therapeutic applications and they are normally administered via intravenous injections. In order to improve patient convenience and compliance, formulations suitable for subcutaneous administration are needed; therefore, highly concentrated monoclonal antibody (mAb) solutions are required to obtain significant therapeutic effects whilst keeping the injected volumes sufficiently small [1]. The conflicting requirements of minimizing both the injection volumes and the tolerable viscosities make pharmaceutical research on dense aqueous antibody solutions both essential and challenging. In practical terms, to minimize solution viscosity [2], protein-protein interactions (PPIs) can be tuned in order to avoid aggregation, e.g., by modifying pH and/or ionic strength, or employing additives. However, the correlation between microscopic mechanisms and macroscopic viscosity is not yet fully understood. It has been demonstrated that self-association of mAbs is one of the main reasons for high solution viscosity [3]. Aqueous mAb solutions have been investigated previously with neutron spin-echo spectroscopy (NSE) [4, 5, 6, 7]. In particular, it was shown that mAbs can form dimers which can "reversibly associate into loosely connected clusters", which has been shown so far for one mAb [6]. Moreover, the formation of these extended dimers might be considered as "a common feature of mAb solutions exhibiting higher viscosities" [6].

Aims of the experiment: In this experiment, we investigated nine different mAbs produced, characterized and provided by the pharmaceutical company Lonza AG/Ltd. in Basel (CH) in the framework of I. Mosca's InnovaXN PhD project. The sequences of these mAbs differ essentially in the Fab (i.e., antigen-binding fragment), which results, for a given concentration, in huge changes in macroscopic viscosity [8, 9]. In order to systematically explore the link between macroscopic viscosity and microscopic cluster formation, we combined NSE and SANS experiments, also aiming to complement existing work where we employed neutron backscattering (NBS), SANS and MD simulations [9]. Several mAbs already investigated in that work show an apparent diffusion coefficient D below the value expected for monomers suggesting the formation of small (few monomers) transient clusters. NSE allows to investigate several diffusive properties at once, e.g. translational and rotational components [10], and to access information on the mAb-specific lobe motions. The accessible short-time collective global diffusion $D_c(q)$ sheds light on the cluster formation. By complementing $D_c(q)$ at different concentrations by SANS measurements, the cluster size can be obtained [11]. Importantly, due to the longer time scales (≈ 100 ns) compared to the previously collected NBS data (≈ 4 ns), the present NSE experiment has closed a critical gap in the observation times between the results from viscometry and NBS and will contribute in understanding how changes on the atomic structure influence the macroscopic viscosity. Moreover, the NSE signal contains information on the internal diffusive modes of the proteins [4, 5, 6, 7, 12].

Samples measured at IN15: mAb3 (AMS-022, IgG1 isotype), mAb5 (AMS-024, IgG1), mAb9 (AMS-038, IgG1), mAb12 (AMS-059, IgG1), mAb15 (AMS-073, IgG1), mAb16 (AMS-074, IgG1), mAb25 (AMS-122, IgG1), mAb4* (AMS-058, IgG4) at $c_p = 50$ mg/mL at $T = 280$, 310 K; mAb19 (AMS-095) at $c_p = 50$ mg/mL at $T = 280$, 310 K (for $T = 280$ K, just one configuration was measured: wavelength $\lambda = 10$ Å, angle $= 3.5°$); Polyclonal IgG (γ-globulin from bovine serum) at $c_p = 50$ mg/mL at $T = 280$, 310 K; 20 mM His-HCl deuterated buffer at pD 6.4 (formulation buffer as background).
Samples measured at D22: mAb3 (AMS-022), mAb5 (AMS-024), mAb9 (AMS-038), mAb12 (AMS-059), mAb15 (AMS-073), mAb16 (AMS-074), mAb19 (AMS-095), mAb25 (AMS-122), mAb4* (AMS-058) at $c_p = 1$, 2, 5, 10, 20, 50 mg/mL at $T = 295$, 310 K; 20 mM His-HCl deuterated buffer at pD 6.4 at $T = 295$, 310 K.

Future Analysis: A preliminary analysis of the NSE data was performed by fitting using a single exponential function with a constant background, but the intermediate scattering functions clearly show deviations from the aforementioned behaviour at lower Fourier times and higher q-values (Fig. 1-left), indicating the presence of interesting features. Although the fit performed is still preliminary, a specific q-dependence of the extracted collective diffusion coefficient $D_c(q)$ is observed in all samples and the mAbs investigated at the very same crowding and temperature display diverse diffusive dynamics (Fig. 1-right). In fact, AMS-024 shows a faster diffusion compared to the other mAbs, especially to AMS-059 (purple squares and cyan triangles in Fig. 1-right, respectively), which is also known to be one of the most viscous in solution. Concerning SANS data, in

Fig. 2 we report the collected curves for different mAbs at the same concentration and temperature (Fig. 2-right plot) and an example of a concentration series measured for mAb9 (AMS-038, Fig. 2-left plot); this plot shows a nicely decreasing intensity at low q at decreasing protein concentration, which is indeed expected for these systems. Please note that SANS profiles are shown without any fitting curves, but at first glance one can already hypothesize about the nature and the intensity of PPIs. In particular, AMS-074 and AMS-059 (cyan and green overlapping curves) display high values of $I(q \to 0)$, clearly suggesting the presence of more attractive PPIs. AMS-024 (blue curve) (Fig. 2-right plot) shows, on the other hand, lower intensity at low q, indicating the presence of more repulsive interactions.
Further analysis, likely including more complex models for NSE and a proper fit function for SANS data, will be performed in the following steps.

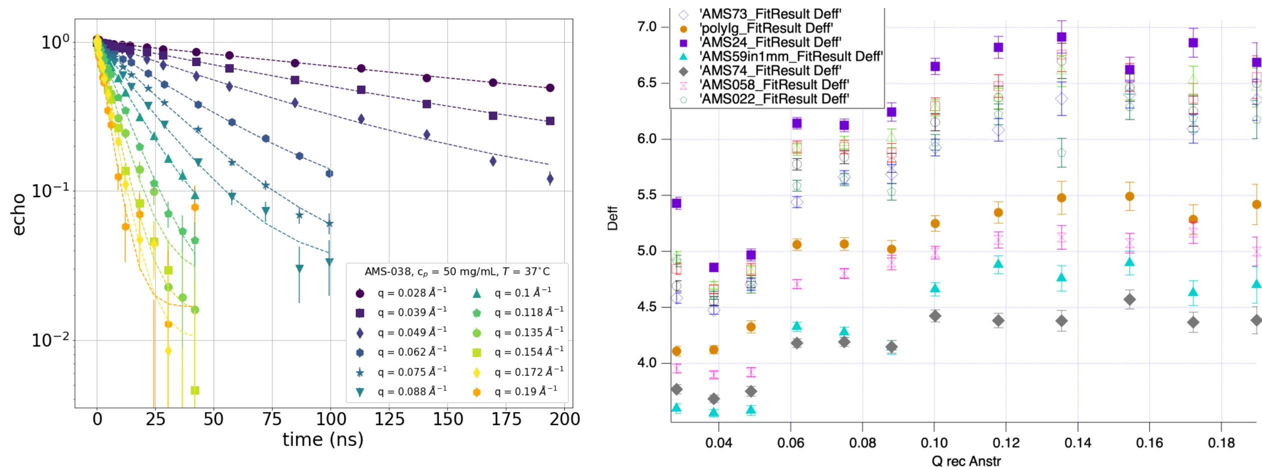

Fig. 1. Left: Intermediate scattering functions $I(q,t)/I(q,0)$ vs Fourier time t collected for mAb9 (AMS-038) at $c_p = 50$ mg/mL and $T = 37°$C, in solution with 20mM His-HCl buffer at pD 6.4. Each series of coloured symbols accounts for a different instrument configuration used (wavelength and diffusion angle) and, hence, a different value of the momentum transfer q, ranging from 0.028 to 0.19 Å^{-1}. **Right:** Collective diffusion coefficient $D_c(q)$ ($\mathrm{D_{eff}}$ in the plot, units: Å^2/ns) for a subset of the antibodies investigated (AMS-073, AMS-024, AMS-059, AMS-074, AMS-058, AMS-022 and polyclonal IgG) at $c_p = 50$ mg/mL and $T = 37°$C as a function of the momentum trasfer q (units: Å^{-1}). Please note that $D_c(q)$ is derived from fitting the intermediate scattering functions $I(q,t)/I(q,0)$ of the samples with just a single exponential, so they constitute very preliminary results.

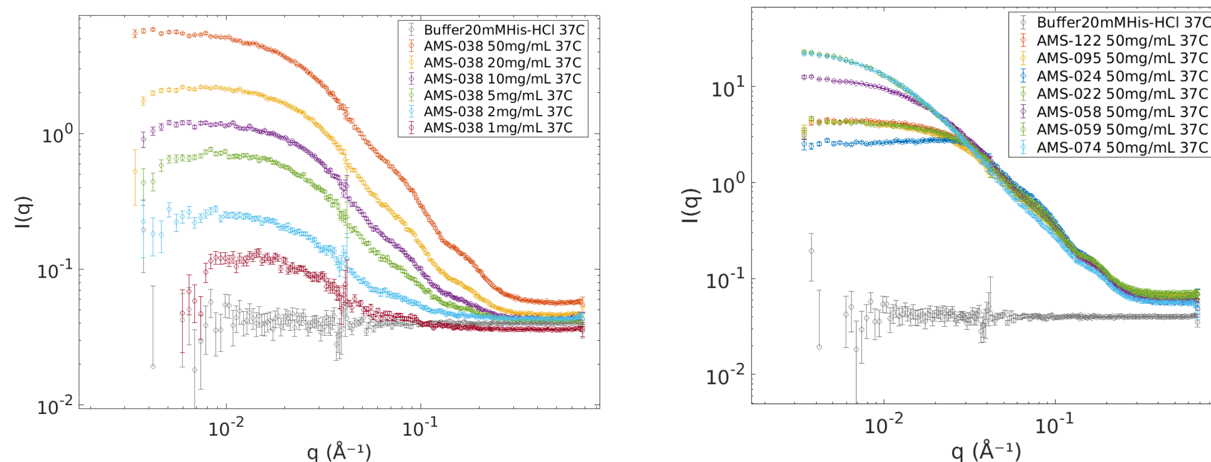

Fig. 2. Left: SANS profiles of mAb9 (AMS-038) dilution series at $c_p = 1, 2, 5, 10, 20, 50$ mg/mL and $T = 37°$C, along with background signal from the 20mM His-HCl formulation buffer at pD 6.4 at the same T. **Right:** SANS curves obtained from different mAbs at $c_p = 50$ mg/mL and $T = 37°$C, with the buffer signal.

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