# Experimental report

05/09/2022

**Proposal:** 8-04-908 **Council:** 10/2020

**Title:** Dynamic cluster formation, viscosity, and diffusion in monoclonal antibody solutions depending on antibody type and crowding

**Research area:** Biology

**This proposal is a new proposal**


**Main proposer:** Louise COLIN

**Experimental team:** Tilo SEYDEL

**Local contacts:** Tilo SEYDEL


**Samples:** recombinant monoclonal antibody proteins (mAbs) (up to 300mg/mL) in aqueous (D2O) solution, buffered with 20mM Histine-HCl
reference sample: polyclonal antibodies from bovine serum in aqueous (D2O) solution (commercially available)

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| IN16B | 5 | 5 | 23/05/2021 | 28/05/2021 |


## Abstract:

Within an InnovaXN EU MSCA COFUND PhD project (www.innovaxn.eu) in collaboration with the pharmaceutical company Lonza AG (Basel, Switzerland), we wish to explore different kinds of medically important IgG1-type monoclonal antibodies (mAbs) in aqueous (D2O) solutions. The aim of this research is to unambiguously determine the size of possibly transient clusters via their short-time self-diffusion accessible by neutron backscattering spectroscopy, depending on the kind of mAb.

Previous research by Lonza AG has established that the 5 different kinds of mAbs proposed as samples here drastically differ in their macroscopic viscosity, although the structural difference of these mAbs is minimal.

Theoretical work establishes that the macroscopic viscosity, and the nanometer length scale diffusion properties and possible transient cluster formation are fundamentally linked by so-called Generalized Stokes-Einstein (GSE) relations.

Here we aim to provide crucial data required to experimentally test the GSE and to ultimately be able to predict the macroscopic viscosity based on molecular properties and, hence, to improve mAb drug formulations for which viscosity is a limiting factor.

**Experimental report - 8-04-908 (IN16B, 23-28/05/2021)**

***Dynamic cluster formation, viscosity, and diffusion in monoclonal antibody solutions depending on antibody type and crowding***

**Scientific background.** Antibody proteins are essential in the immune response of mammals and are, hence, generally important for therapeutics. Antibody injections are used in various therapies. For pharmaceutical applications, highly concentrated monoclonal antibody (mAb) solutions (up to 200 mg/ml) are required to obtain a significant therapeutic effect whilst keeping the injected volumes sufficiently small. The phase behavior and viscosity of highly concentrated mAb solutions is influenced by protein-protein-interaction and, thus, by mAb structure and physicochemical properties, as well as solution conditions (pH, buffer, ionic strength), and temperature. mAb solutions with viscosities > 20 mPa*s are considered difficult to inject (due to the high injection forces required) [1], limiting their market access and potential therapeutic use. The conflicting requirements of minimizing the injection volumes and limiting injectable viscosities render pharmaceutical research on dense aqueous antibody solutions essential. On the microscopic level, reversible cluster formation has been found to be at the origin of the large viscosities of some monoclonal antibody solutions. To minimize the viscosity of mAb solutions, the interactions between the mAbs can be modified, e.g. by employing different additives and/or by changing the storage/transport temperatures. Here, we investigate highly concentrated solutions of therapeutically relevant mAbs provided by our industrial partner Lonza AG using QENS at different temperatures, in order to understand how temperature affects their clustering behavior and how the antibody type influences the inter-molecular interactions from a microscopic point of view and, eventually, the viscosity from a macroscopic one.

Rheology measurements performed on aqueous solutions of these Lonza mAbs showed that their viscosity dramatically depends on mAb type (Fig. 1), even if the aminoacidic sequences of the different antibodies have an overlapping of more than 90%. In fact, their structures are basically identical in the Fc (constant fragment) region, while show differences in the extremal part of their lobes, specifically in the Fab (antigen-binding fragment) (see Fig. 2).

This behavior suggests that viscosity is somehow related to inter-molecular interactions restricted in the lobe-region and thus to faster dynamics.

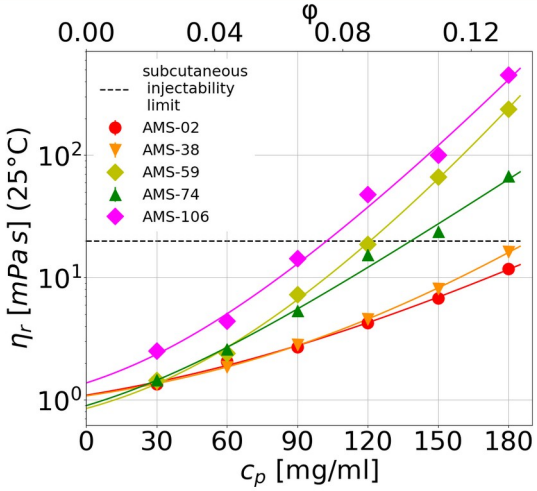


***Figure 1:*** *Viscosities (log scale) of Lonza mAbs versus their concentration $c_p$ and volume fraction φ in solution (T=298 K).*

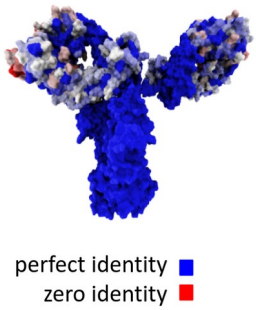


***Figure 2:*** *Sequence identity of the different studied mAbs by using a color code ranging from blue for perfect identity to red for zero identity.*

**Experimental results. QENS.**

Spectra from solutions of five different Lonza antibodies in D2O were collected at 280, 295 and 310 K. Samples were prepared at different concentrations (also taking into account the availability of the initial antibodies). Polyclonal Ig solutions at three different concentrations were also measured as a reference. The total of measured samples has been reported in the following table.

| Antibody type | Concentrations (mg/ml) | Temperatures (K) |
|---|---|---|
| AMS-02 | 68.01 | 280 |
| AMS-38 | 63.36 | 280, 310 |
| AMS-38 | 85.7 | 280, 295, 310 |
| AMS-38 | 146 | 280, 295, 310 |
| AMS-59 | 105 | 280, 310 |
| AMS-74 | 62 | 280, 310 |
| AMS-106 | 167 | 280, 295, 310 |
| polyclonal Ig | 60, 140, 180 | 280 |

Data have been analysed by following the usual framework used in previous works on protein dynamics from our group [2, 3, 4, 5]. Spectra have been fitted using a three-Lorentzian model (Fig. 3) and the contributions respectively refer to the deuterated buffer dynamics, the global dynamics (center of mass motion) and the internal one (side-chain and lobe motions).
From the different fitting contributions, diffusion coefficients for each motion have been obtained. The apparent diffusion coefficient, coming from the global dynamics and referring to the center of mass motion, has been investigated in a colloid physics framework. In order to gain information about the geometry of the motions under study, visualizing the *q*-dependence of the elastic incoherent structure factor (EISF) $A_0(q)$ has also been useful (Fig. 4).

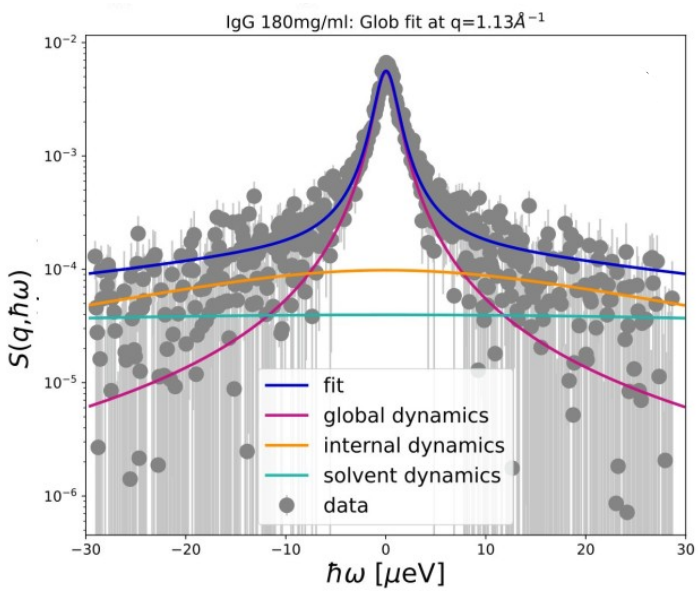


***Figure 3:*** *Global fit at q=1.13 Å$^{-1}$ of the QENS spectrum obtained from polyclonal Ig at 180 mg/ml at 280 K. Grey symbols with errorbars are experimental points, while solid lines are the global fit and its different contributions coming from different motions: global fit (dark blue), solvent dynamics (aquamarine), global motions (magenta) and internal ones (orange).*

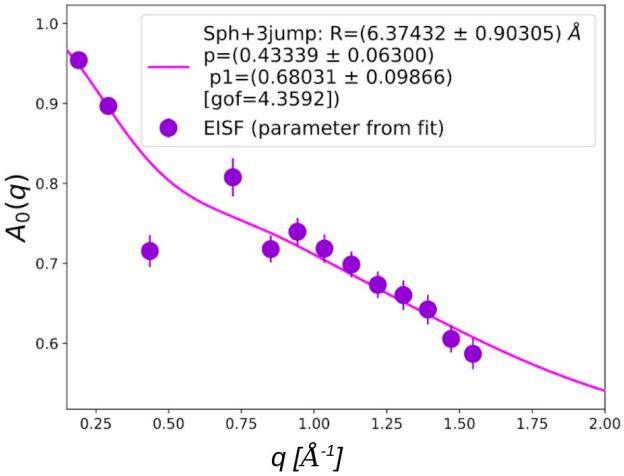


***Figure 4:*** *Values for each q-value of the elastic incoherent structure factor (EISF) obtained after the fitting of the measured spectrum of AMS-38 at 146 mg/ml at 280K. The q-dependence of this parameter can be fitted using one of the existing theoretical models; the model that best fits with the data points (in purple) is a combination between a motion in a spherical volume and a jump-diffusion among three-sites (magenta line).*

**Conclusions.** QENS allows to separate different contributions of the system dynamics and to determine the hydrodynamic size of the aggregates forming in mAb solutions, thus giving us understanding on the clustering. By determining the diffusion coefficients of the different motions, one can also establish which antibody type forms solutions which are more or less diffusive and link this information to macroscopic viscosity of the systems.

**References**

[1] S. Jolles and J. Sleasman. Advances in Therapy 28.7 (2011).
[2] M. Heinen, F. Zanini, F. Roosen-Runge, D. Fedunova, F. Zhang, M. Hennig, T. Seydel, R. Schweins, M. Sztucki, M. Antalık, F. Schreiber and G. Nägele, *Viscosity and diffusion: crowding and salt effects in protein solution*, Soft Matter 8, 1404 (2012).
[3] M. Grimaldo, F. Roosen-Runge, F. Zhang, F. Schreiber and T. Seydel, *Dynamics of proteins in solution*, Quart. Rev. Biophys. 2019, 52, e7 (2019).
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