Experimental report

29/08/2022

Proposal: 8-04-931 **Council:** 4/2021

Title: antibody domain dynamics

Research area: Biology

This proposal is a new proposal

Main proposer: Ralf BIEHL

Experimental team:

Local contacts: Ingo HOFFMANN
Orsolya CZAKKEL

Samples: protein in D2O phosphate buffer

Instrument	Requested days	Allocated days	From	To
IN15	6	6	01/10/2021	07/10/2021

Abstract:

We want to examine the influence of common excipients onto the domain/internal dynamics of monoclonal antibodies at lower concentrations and to identify relevant motional patterns. We will use different excipients modulating the free volume (crowding), the surface charge (zwitterionic molecules) and solvent quality which are used in common mAb drug formulations.

Experimental Report : antibody domain dynamics

Proposal 8-04-931
R. Biehl, M. Kruteva, A. Stadler, D. Richter
Forschungszentrum Jülich, Germany

Experimental conditions were slightly changed to allow remote experiments (Corona). We used an 15mM acetic buffer (pH 5) because the planned phosphate buffers show a pH jump during freezing (sample transport on dry ice). These conditions were used for all samples in the LINXS project.
All experiments went quite well with excellent performance of the IN15 instrument. The instrument responsible thaw the samples and sent the samples back after experiments for later measurements.

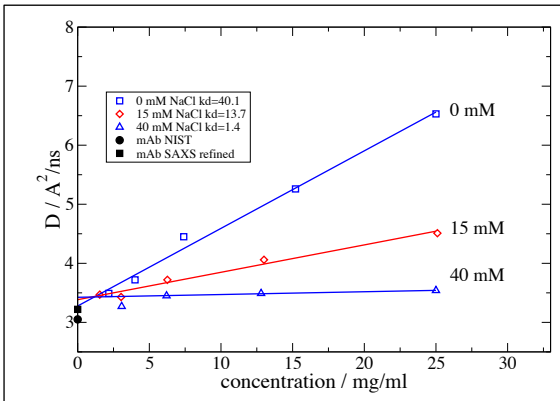

Figure 1:Effective diffusion constant D measured by DLS.

We describe here evaluation of one set of data exemplary for the whole measurements including complementary data.
We evaluated the series comparing different NaCl concentrations. Figure 1 shows complementary DLS measurements for a concentration series of the 3 different NaCl concentrations. With increasing NaCl concentration the slope decreases showing the transition of a strong repulsive to a weakly attractive interaction. Later SAXS data were used for a structural refinement of mAb to result in a new PDB structure. HYDROPRO(1) was used to calculate the corresponding diffusion coefficient. Compared to the artificial NIST PDB structure the refined structure fits excellent to the extrapolated DLS value with a faster diffusion indicating a more compact structure. Differences in the structures found in SAXS cannot be observed in DLS.
SAXS measurements were used to extract the mAb structure factor and respective formfactors as presented in Figure 2. Compared to the NIST PDB structure the respective structures are more compact as already found in DLS. The NIST PDB structure was used as a starting point to generate new structures (using a differential evolution fit) by scaling of the central linker region and bending of the Fab and Fc domain in the center of mass plane and out of it. Resulting formfactors are shown in Figure 2 (black lines) with corresponding structures of the mAb. The small differences in the SAXS data indicate a more open configuration for low NaCl concentration. The radius of gyration is 5.0 nm in comparison to the NIST PDB structure with 5.6 nm.
NSE measurements were done in a Q range from 0.03-0.13 nm^{-1} for times up to 300ns. Data were evaluated using a model assuming fast domain movements and decoupled slow diffusion with contributions of translational/rotational diffusion and slow domain motions. Fast domain motions with a relaxation time of 12±3 ns and rmsd amplitudes of 1±0.1 nm was observed that fit to stretching movements of the domains away from the center of mass. The slow diffusion coefficients are presented in Figure 3 together with model calculations according to(2)

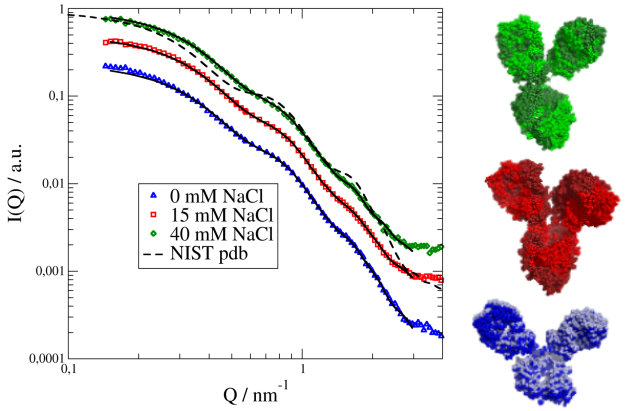

Figure 2:Left: Formfactors extracted from SAXS measurements for different NaCl concentrations at 35°C. Original NIST PDB as black dashed line. Right: Resulting mAb structures in corresponding colors. Each contains light and darker color for 15°C and 35°C.

$$D(Q) = D_t(Q)\frac{H(Q)}{S(Q)} + \sum_l S_l(Q) l\,(l+1) H_r D_r + \frac{d^2/\lambda}{Q^2 P(Q)} \hat{F}_a(Q)$$

With trans/rot diffusion coefficients D_t and D_r, corresponding hydrodynamic functions H(Q) and H_r, structure factor S(Q) and partial amplitudes $S_l(q)$. P(Q) is the mAb formfactor and $\hat{F}_a(Q)$ are mode formfactors that describe changes of the effective diffusion (in the initial slope) according to normal modes of msd amplitude d^2 and relaxation time λ. The low Q behavior is well described by the H(Q)/S(Q) correction for translational diffusion (dotted lines) using the measured structure factor and calculated H(Q) (δγ expansion)(3). H(Q→∞) was fixed by respective viscosity measurements. Adding the rotational contribution (solid lines) we achieve near matching of the 40mM NaCl data. To match all data we need to add a domain motion component (bending motions) with increasing rmsd amplitude for decreasing NaCl concentration assuming a relaxation time of 100 ns (dashed lines).

We conclude that the fast motions are independent on the domain interaction that are screened by the increasing salt concentration while the slow domain bending motions show larger amplitudes with less screening.

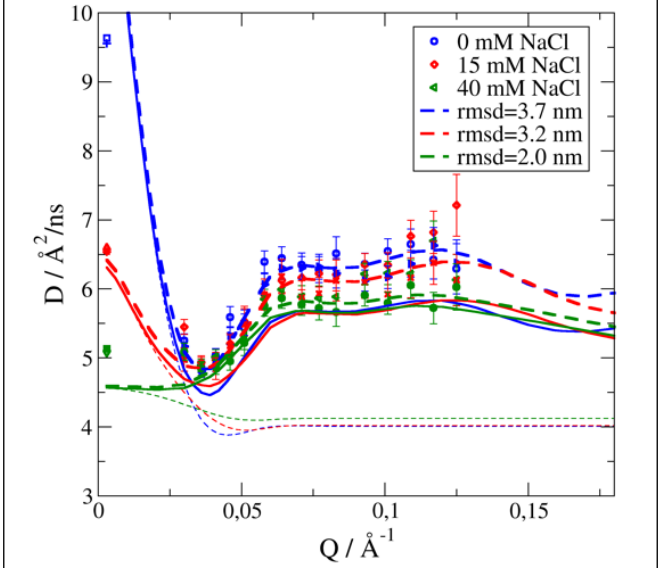

Figure 3:Effective diffusion measured by NSE for 25 mg/ml mAb. Lines show model calculation as described in the text. Points at low Q represent DLS measurements at same concentration.

1. de la Torre JG, Huertas ML, Carrasco B, de la Torre J. Calculation of hydrodynamic properties of globular proteins from their atomic-level structure. Biophys J [Internet]. 2000 Feb;78(2):719–30. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1300675&tool=pmcentrez&rendertype=abstract
2. Monkenbusch M, Stadler AM, Biehl R, Ollivier J, Zamponi M, Richter D. Fast internal dynamics in alcohol dehydrogenase. J Chem Phys [Internet]. 2015 Aug 21 [cited 2015 Nov 13];143(7):075101. Available from: http://scitation.aip.org/content/aip/journal/jcp/143/7/10.1063/1.4928512
3. Beenakker CWJ, Mazur P. Self-diffusion of spheres in a concentrated suspension. Phys A Stat Mech its Appl [Internet]. 1983;120(3):388–410. Available from: http://www.sciencedirect.com/science/article/pii/0378437183900614