Observation of small cluster formation in concentrated monoclonal antibody solutions and its implications to solution viscosity

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Estimation of the systematic error of Mw due to the effect of S(Q)

As also shown in the paper,

$$I(Q) = AP(Q)\tilde{S}(Q) + B$$

When estimating Mw in the manuscript, S(Q=0) is considered to be one which will introduce errors to the estimation. When the system is dominating by repulsion, S(Q=0) < 1. The estimated Mw based on the aforementioned method is smaller than the real values. When the system is dominating by very strong attraction interaction, S(Q=0) could be larger than one so that the estimated Mw is larger than the real value. Since we would like to estimate the upper limit of Mw, we thus need to estimate how small the value of S(Q=0) could be. For the worst scenario, we can assume there is no attraction between mAb1 proteins. We therefore estimate S(Q=0) based on the repulsion potential calculated using Debye-Hückel theory and the Ornstein-Zernike (OZ) equation using the hypernetted chain closure (HNC). The calculated S(Q) using the this method is shown in Fig. S1. The estimated S(Q=0) is 0.84. Therefore, based on this simplified calculation, the calculated Mw in the manuscript could be an underestimate by no more than 20%. During the calculation, we have used the ionic strength of the buffer solution and assumed the net charge of a protein to be +17. In fact, a previous study has indicated that the experimental charge number of mAb1 is smaller than the theoretical value.(1) Therefore, the calculated error bars are an overestimate of the uncertainty.

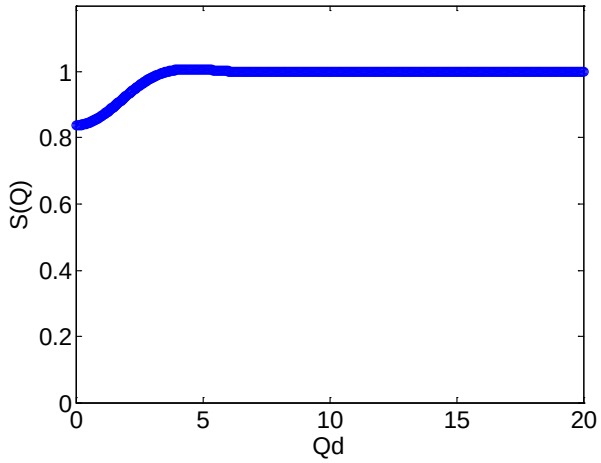

Fig. S1 shows S(Q) calculated from the OZ equation using the HNC closure. d is the diameter of the particle.

Intermediate scattering function measured by neutron spin echo (NSE)

NSE measures the intermediate scattering function, S(Q,t)/S(Q). At even large concentrations, S(Q,t)/S(Q) can be fitted by one single exponential functional form from which we can extract the collective diffusion coefficient, $D_c(Q)$. Fig. S2 shows one example of S(Q,t)/S(Q) measured by NSE at Q=0.21 $Å^{-1}$ for the mAb1 sample at 150 mg/mL.

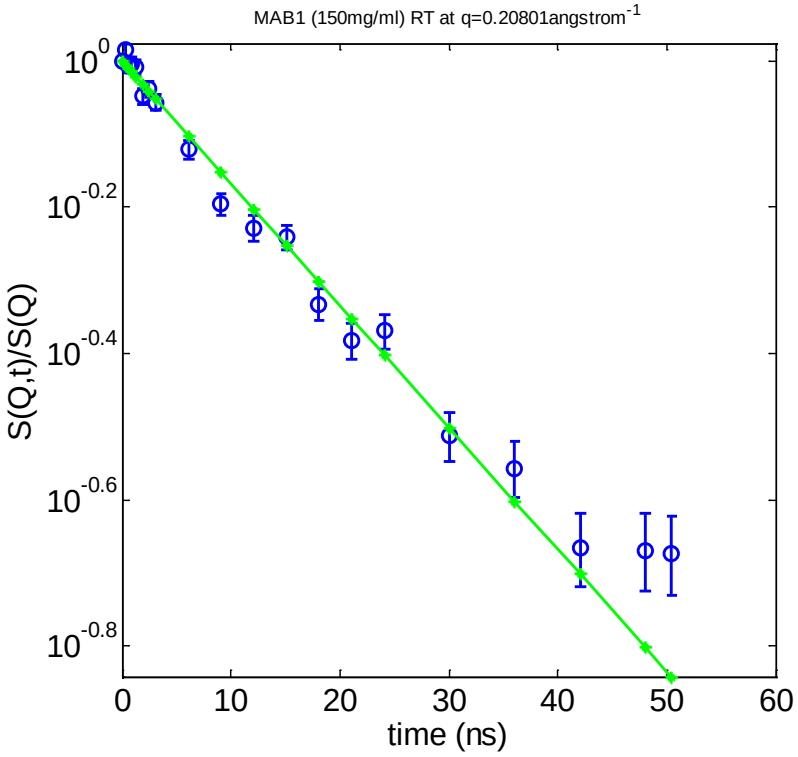

Fig. S2 shows S(Q,t)/S(Q) of 150 mg/ml mAb1 sample measured by NSE at 25 ˚C without adding salts into the buffer. Q=0.21 $Å^{-1}$.

1. Yearley, E. J., I. E. Zarraga, S. J. Shire, T. M. Scherer, Y. Gokarn, N. J. Wagner, and Y. Liu. 2013. Small-angle Neutron Scattering Characterization of Monoclonal Antibody Conformations and Interactions at High Concentrations. Biophys. J. 105:720-731.