Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Characterizing Protein-Protein-Interactions in Highly Concentrated Monoclonal Antibody Systems with the Quartz Crystal Microbalance

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Supporting Information

A) Dependence of k_D and B_{22} on pH.

The main text reports values for the diffusion interaction parameter, k_D, and the second osmotic virial coefficient as determined with self-interaction chromatography, $B_{22,SIC}$. Fig. S1 provides more extensive data. As the figure shows, values depend on the method chosen to some extent. In particular, there are differences between $B_{22,SIC}$ determined with self-interaction chromatography (SIC), on the one hand, and $B_{22,SLS}$ determined by static light scattering (SLS), on the other.

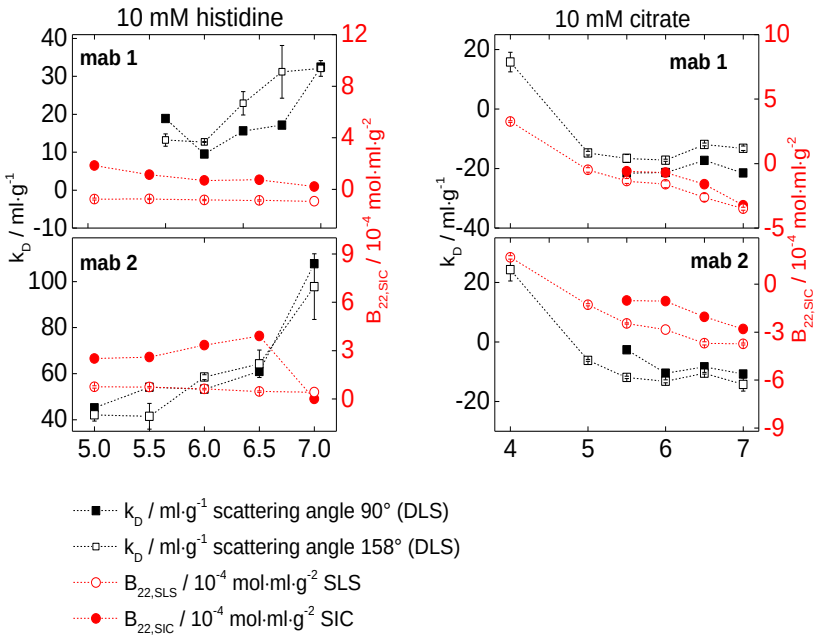

Fig. S1: Values for the diffusion interaction parameter, k_D, and the second osmotic virial coefficient, B_{22}, determined with different techniques.

For the case of mab1 in histidine, $B_{22,SLS}$ as determined with static light scattering is positive but decreases with pH, while k_D increases with pH. Further investigations (not discussed in detail here) suggest that the discrepancy goes back to concentrations being outside the dilute range. At higher concentrations, the "apparent k_D" of mab1 in buffer containing histidine *decreases* with pH (Fig. S2). The "apparent" k_D is obtained by fitting the concentration dependence of the diffusivity with $D = D_0(1 + k_{D,app}\, c)$. $k_{D,app}$ is different from k_D because of the higher concentration. As the concentration increases, short-range attractions become more important and slow down diffusion. Details of this situation are outside the scope. However, these problems highlight the importance of having techniques at hand, which do not require dilution. Higher-order interactions are of much importance.

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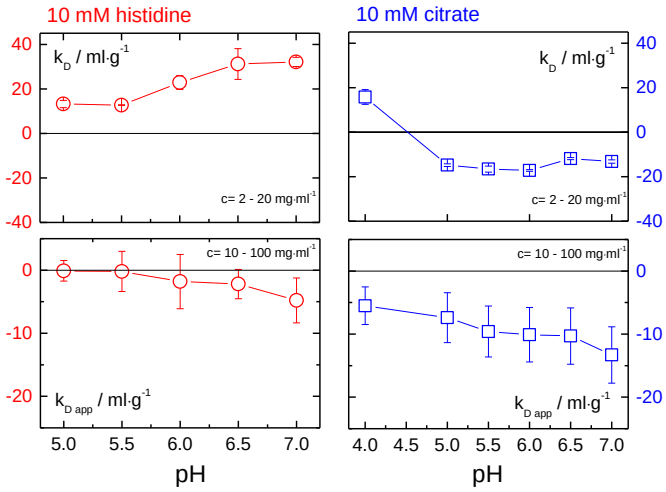

Fig. S2: Apparent k_D of mab1 in histidine and citrate in the range of 2 – 20 mg/ml and 10 – 100 mg/ml. The sign change in the case of histidine is indicative of short-range attraction.

B) Clustering

The main text argues that values of G^* in the kPa range can be explained with clustering, to be distinguished from the formation of a continuous network. Fig. S3 shows apparent hydrodynamic diameters of mab1 as determined with DLS. These data support the notion of clustering, but they do not strictly exclude the existence of a weak gel.

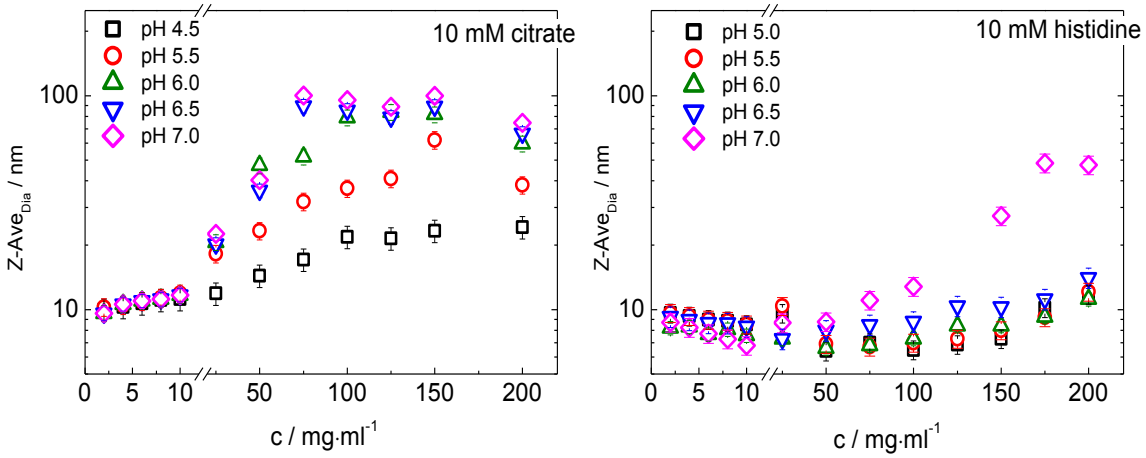

Fig. S3: Apparent hydrodynamic diameters of mab1 as determined with DLS