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Cluster Formation and Entanglement in the Rheology of Antibody Solutions

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Abstract

Antibody solutions deviate from the dynamical and rheological response expected for globular proteins, especially as volume fraction is increased. Experimental evidence shows that antibodies can reversibly bind to each other via F_{ab} and F_c domains, and form larger structures (clusters) of several antibodies. Here we present a microscopic equilibrium model to account for the distribution of cluster sizes. Antibody clusters are modeled as polymers that can grow via reversible bonds either between two F_{ab} domains or between a F_{ab} and a F_c . We propose that the dynamical and rheological behavior is determined by molecular entanglements of the clusters. This entanglement does not occur at low concentrations where antibody-antibody binding contributes to the viscosity by increasing the effective size of the particles. The model explains the observed shear-thinning behavior of antibody solutions.

Graphical Abstract

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Supporting Information

Supporting Information Available: Derivation of accurate counting in the HT partition function. This material is available free of charge via the Internet at <http://pubs.acs.org>

Introduction

Efficient dosing of therapeutic antibodies often requires concentrations in excess of 100 or even 200 mg/ml.¹⁻³ However, many antibodies have a sharp rise in the viscosity that renders production and delivery prohibitive at these concentrations.^{2,4-6} Unfortunately, this problem is only apparent late in the development pipeline when it is not feasible to alter the sequence to reduce viscosity. A better approach would be to choose low viscosity target molecules early in the pipeline so that the problem can be avoided altogether. To achieve this goal it is necessary to understand how minor sequence perturbations within the complementarity determining regions (CDR) contribute to the many-body interactions responsible for the elevated viscosity.

The sharp rise in antibody viscosity has characteristics that are very different from solutions of rigid bodies.⁴ First, the onset of the nonlinear regime occurs at volume fractions on the order of 5–10%, which is much less than the jamming transition for comparably shaped rigid bodies (e.g. 58% for spheres). Secondly, the viscosity of a given molecule correlates well with attractive intermolecular interactions.⁷⁻⁹ While this is intuitively reasonable, the viscosity of a flocculated solution is primarily a function of the solute volume fraction. But, the volume fraction does not change upon aggregation, although entrained water cavities could account for a factor of 2 or 3 increase. To explain these discrepancies, we proposed an alternative model in which transient interactions between antigen binding domains result in long, flexible antibody complexes.¹⁰ These complexes entangle with each other giving the solution viscosity characteristics of a semi-dilute polymer solution. In this paper we expand on this polymer model to explain shear thinning behavior, dilute solution viscosity, and show how the ensemble of complexes depends on the affinity and location of intermolecular interactions.

Antibody cluster morphology depends on the location of binding sites

The large variation in the viscosity of different antibodies requires that the causative interactions involve the variable region. Experiments have shown examples of antibodies where the CDR self-associates or binds to the F_c domain.^{11,12} The type of interaction, and hence the allowed structures, will depend on the specific antibody. The presence of F_{ab} - F_c interactions is expected to have a significant effect on the rheological behavior, as these interactions can lead to branched structures, which cannot relax by the reptation mechanism that dominates in semi-dilute polymer solutions.¹³ For now, we ignore dynamical effects and compute the equilibrium ensemble of complex structures as a function of the binding location.

Head-to-Head binding results in linear aggregates

To begin, we review the simplest case of F_{ab} - F_{ab} interactions, as described in.¹⁰ We refer to this as “head to head” (HH) binding. HH binding results in the formation of linear structures, as shown schematically in Fig. 1

The equilibrium constant for HH association is defined by

$$k = \frac{c_2^{\text{HH}}}{c_1^2} \quad (1)$$

where c_2^{HH} is the concentration of dimers formed by HH binding and c_1 is the monomer concentration. The dimer equilibrium constant provides a valuable connection between dilute solution properties, which can be obtained early in the development pipeline, and the viscosity of concentrated solutions. This connection can also be made using numerical methods or MD simulations.¹⁴⁻¹⁷ Here we present calculations to make this connection analytically.

It follows from Eq. 1 that the concentrations c_i of complexes containing i molecules will be given by

$$c_i = c_1 (k c_1)^{i-1} \quad (2)$$

where we are assuming that the equilibrium constant k is independent of the number of molecules in the i -mer.

Summing over all HH complexes, the grand partition function is given by

$$q_{\text{HH}} = c_1 (1 + (k c_1) + (k c_1)^2 + \dots + (k c_1)^i + \dots) \quad (3)$$

where the monomer concentration plays the role of the fugacity $c_1 = e^{\mu/k_B T}$. We can rewrite the partition function as the following recursion relation

$$q_{\text{HH}} = c_1 (1 + k q_{\text{HH}}) \quad (4)$$

This equation can be physically interpreted as follows: the two terms on the right hand side of Eq. 4 correspond to the two possible states for an antibody head, i.e., one of the F_{ab} domains. The head can be unbound, which terminates the complex and results in the factor of 1, or the head can be bound to another molecule which, in turn, can be bound to another, and so on. In the grand canonical formalism, the number of molecules in this aggregate can range from one to infinity. It follows that the sum of all possible outcomes can be replaced by the factor q_{HH} on the right hand side of Eq. 4. This is depicted schematically as

$$\begin{aligned} Q &= \mathbf{Y} + \mathbf{Y}\mathbf{Y} + \mathbf{Y}\mathbf{Y}\mathbf{Y} + \dots \\ &= \mathbf{Y} \times (1 + \mathbf{Y} + \mathbf{Y}\mathbf{Y} + \dots) \\ &= \mathbf{Y} \times (1 + Q) \end{aligned}$$

Rearranging Eq. 4 we get the following expression for the partition function

$$q_{HH} = \frac{c_1}{1 - k c_1} \quad (5)$$

which can also be obtained by summing the power series in Eq. 3.

Head-to-Tail binding permits the formation of branched clusters

We now consider the self association of antibodies that form bonds between an F_{ab} domain and a F_c , which we refer to as “head to tail” (HT) binding.

Defining s as the association constant for the HT binding, the grand partition function for complexes formed entirely by HT associations can be written as the following recursion relation

$$q_{HT} = c_1(1 + 2 s q_{HT} + s^2 q_{HT}^2) \quad (6)$$

As with Eq. 4, this equation has a physical interpretation: the three terms correspond to the available states for the heads of a single molecule. The first term corresponds to the state where both heads are unbound, the second term corresponds to the case where only one of the heads binds to a tail, and the third term corresponds to the state where each head binds to another molecule. Since each bound molecule can initiate a cluster of any number of molecules, the factor q_{HT} is introduced. Solving the quadratic Eq. 6, and keeping the root with the correct low concentration limit ($q_{HT} \rightarrow c_1$), we obtain an expression for the grand partition function

$$q_{HT} = \frac{1 - 2sc_1 - \sqrt{1 - 4sc_1}}{2s^2c_1} \quad (7)$$

Fig. 2 shows schematically some of the complexes that can be expected from HT association. As in the HH case, we can associate each cluster to a term in the grand partition function (Eq. 3). This can be seen by Taylor expanding Eq. 7

$$q_{HT} = c_1(1 + 2(s c_1) + 5(s c_1)^2 + \dots) \quad (8)$$

The coefficients in Eq. 8 indicate the degeneracy of the relevant i -mer states. For example, the term $5 (s c_1)^2$ corresponds to the five dimer states in Fig. 2. Note that several of these structures are related by rotational symmetry. Therefore, these states should be considered “undistinguished” particles in the sense used in^{18,19} for classical particles. This overcounting can be corrected in an approximate way by dividing the final partition function by two

$$\tilde{q}_{\text{HT}} \simeq \frac{c_1 + q_{\text{HT}}}{2} \quad (9)$$

where $\tilde{q}_{\text{HT}}$ is the corrected partition function and the extra factor of c_1 ensures that the monomer term is accurate. In the Supporting Information we present a more accurate correction and discuss the accuracy of the simple approximation made in Eq. 9.

Comparing HH and HT cases (Eqs. 3 and 8) we notice that in the HH model there is only one possible cluster structure for a given number of monomers, but the HT model allows for many structures for each cluster size. Therefore, for equivalent binding energies, there is an additional entropic factor promoting HT binding.

The average cluster size increases with binding affinity and protein concentration

The cluster concentrations obtained in the previous sections are functions of the monomer concentration c_1 . This quantity is less experimentally accessible than the total protein concentration c . The relationship between c_1 and c can be obtained from the mass conservation law, which requires that the total concentration c must satisfy

$$c = \sum_{i=1}^{\infty} i c_i = c_1 \frac{dq}{dc_1} \quad (10)$$

The average size of the aggregates can be written as

$$\langle n \rangle_{\text{HT}} = c_1 \frac{d(\ln q)}{dc_1} \quad (11)$$

Both equations are valid for both the HH and HT models. Now, the monomer concentration c_1 can be obtained by solving Eq. 10 in terms of the total concentration. This result can be used in Eq. 11 to find the average size. For the HH model the result is¹⁰

$$\langle n \rangle_{\text{HH}} = \frac{2kc}{\sqrt{1+4kc}-1} \quad (12)$$

and for the HT model we have

$$\langle n \rangle_{\text{HT}} = \frac{\sqrt{2} s c}{\sqrt{1+4sc+s^2c^2} - (1+sc)\sqrt{1+6sc+s^2c^2}} \quad (13)$$

We focus now on computing the distribution of aggregates. To do this, we need to compute the concentration, c_i , of all clusters of size i . This can be done by Taylor expanding the

partition function and selecting the term proportional to c_1^i . This procedure is formally given by

$$c_i = \frac{c_1^i d^{i-1} q}{i! d c_1^{i-1}} \bigg|_{c_1=0} \quad (14)$$

In order to analyze the results, we take the approach in¹⁰ and express the binding affinities in terms of the free energy of the binding interaction

$$k = \frac{1}{m} e^{\epsilon_{HH}} \quad (15)$$

$$s = \frac{1}{m} e^{\epsilon_{HT}} \quad (16)$$

where m is the molecular mass, and ϵ_{HH} and ϵ_{HT} are the free energies for HH and HT binding association at the standard concentration of 1M, respectively, in units of $k_B T$.

Fig. 3 compares the average size of cluster $\langle n \rangle$ for both HH and HT models at the same binding energy ϵ . It is clear that increasing concentration or increasing binding energy allows for a larger number of monomers per cluster. Also, at the same ϵ the HT model gives larger structures. This comes from the fact that the HT model allows a larger number of different clusters with the same number of monomers, increasing the probability of populating those states. Finally, we see that the onset of aggregation occurs for binding free energies around 5-6 $k_B T$ in the range of concentrations 100-200 mg/ml.

We now focus on the population of different cluster sizes. Fig. 4 shows the concentration of clusters from monomers to heptamers as a function of the binding free energy for both HH and HT models. The monomer concentration decreases monotonically as a function of binding energy as the monomer pool is depleted to form larger structures. In contrast, the concentration of larger structures shows a non-monotonic dependence. This is because initial increases in the binding energy permit the formation of larger structures, while much larger values shift the weight of the distribution to larger aggregates which deplete the pool of smaller structures.

The viscosity of dilute solutions is determined by the volume occupied by antibody clusters

The equilibrium distribution of antibody cluster sizes can be applied to obtain expressions for the viscosity at low concentrations. For a dilute solution of hard spheres immersed in a solvent, the viscosity is given by the Einstein relation:²⁰

$$\frac{\eta}{\eta_0} = 1 + \frac{5}{2}\phi \quad (17)$$

where η_0 is the solvent viscosity and ϕ is the volume fraction occupied by the spheres. Approximating the antibody complexes as spheres of radius equivalent to radius of gyration R_g and mass $\langle n \rangle$ times that of a monomer, we can approximate the volume fraction as

$$\phi = \frac{4}{3}\pi R_g^3 \frac{c}{\langle n \rangle m} \quad (18)$$

where m is the molecular mass.

The radius of gyration can now be computed by considering the antibody complex as a polymer chain of $3n$ segments of length b^{21}

$$R_g = b \langle 3n \rangle^\nu \quad (19)$$

where $\langle n \rangle$ is the average number of monomers per cluster, ν is the Flory exponent, and the factor of three comes from the number of statistically independent segments (domains) per molecule. Plugging Eq. 19 into Eq. 18, we get an expression for the volume fraction that only depends on the binding energy of a particular molecule,

$$\phi = \frac{4}{3}\pi \frac{c}{m} \tilde{b}^3 \langle n \rangle^{3\nu - 1} \quad (20)$$

where $\tilde{b} \sim 3^\nu b$ is the effective molecular radius. Plugging Eq. 20 into Eq. 17, we obtain an expression for the viscosity of antibodies in dilute solutions

$$\frac{\eta}{\eta_0} = 1 + \frac{5}{2} \frac{c}{\rho} \langle n \rangle^{3\nu - 1} \quad (21)$$

where $\rho = \frac{3}{4\pi} \frac{m}{\tilde{b}^3}$ is the domain density. Note that even though viscosity depends linearly on volume fraction, it does not have a linear dependence on concentration, since $\langle n \rangle$ is non-linear in c .

The HH and HT binding affinities determine the average number of monomers per cluster $\langle n \rangle$. While binding location is not known for most antibodies, we can exploit two systems where Hydrogen-Deuterium exchange experiments have identified the intermolecular interactions as predominantly HH and HT, respectively.^{11,12} In making the latter comparison, we note that Eq. 19, which gives the radius of gyration, is valid only for linear

polymers. Nevertheless, in the low concentration regime, small structures (without branching) dominate, making Eq. 21 an adequate approximation for antibodies with HT interactions.

We can compare this expression to the results obtained in.¹⁰ Using the concentration dependence of $\langle n \rangle$ (Eq. 12) in Eq. 21 we see that viscosity scales like $\eta \sim c^{1.4}$ in the dilute regime, which is a weaker dependence than the semi-dilute result, $\eta \sim c^{3.75}$, found in.¹⁰

The free parameters in the model are the segment length b and the association constants k and s . Since Eq. 21 was developed for low antibody concentrations, we limit the fitting algorithm to the first three data points for each data set (up to 20 mg/mL). We fit b by minimizing the sum of square errors for all cases, and then fit the association constants for each separate case for that value of b . Comparison between the model and the experimental results are shown in Figs. 5A and 5B. Fitting yields an effective segment length $b = 6.1$ nm, or equivalently $\tilde{b} = 11.8$ nm, which is consistent with the domain size and antibody dimension. The fitted association constants are displayed in Table 1.

Ref.¹² also provides a non-rheological test of our theory in the form of dynamic light scattering (DLS) measurements of the radius of hydration of antibody clusters. These measurement are compared to the cluster radius computed from Eq. 19 in table 2, which shows a good agreement between the two quantities.

Eq. 21 gives the zero-shear viscosity of a solution in the dilute regime. This means that it is expected that the model will fail around the overlap concentration c^* that separates the dilute regime from the semi-dilute. The overlap volume fraction is reached when the molecules occupy all the space, i.e. $\phi^* = 1$. This condition is obtained from Eq. 20 and tabulated in Table 1. Inspection of Fig. 5 shows that c^* is predictive of when the dilute model is a good description of the solution viscosity. For concentrations below c^* the fits do an excellent job of describing the measured viscosity. However, above c^* entanglement effects lead to marked deviation. The failure of the dilute model can occur at concentrations as low as 10-20 mg/ml for antibodies with strong attraction.

Due the small complex sizes ($\langle n \rangle < 5$) formed under these conditions (Table 1), these systems are insensitive to the choice of HH or HT models. This is because the lack of branching and the small degeneracy factors make the HH and HT models nearly interchangeable. Because of this, and the lack of experiments that identify the binding location, only the HH model will be used when analyzing the rheological behavior of antibodies in the following sections.

Equilibrium dynamics of antibody solutions

Above the overlap concentration antibodies have a viscous response similar to that of a semi-dilute polymer solution. This is described by the theory of Schmit *et al.* for zero-shear viscosity, which we summarize below. In their theory, each antibody is modeled as a featureless polymer with the polymer ends located at the antigen binding sites.¹⁰ L is a dimensionless polymer length. Since each folded domain is a statistically independent unit $L = 3$. Schmit *et al.* focused on the semidilute regime, since in the range of interest,

concentrations above 100 mg/mL, the center-to-center particle separation is comparable to the molecular size and the antibodies occupy a solution volume on the order of 10%. The reptation mechanism of polymers with entanglements yields a zero-shear viscosity proportional to the product of the shear modulus G and the longest relaxation time τ_{rep} ²²

$$\eta_0 \propto G \tau_{\text{rep}}. \quad (22)$$

In the semidilute regime, the reptation time is given by^{23,24}

$$\tau_{\text{rep}} \sim \frac{\eta_s L^{3\nu} b^3}{k_B T} \left(\frac{c}{c^*} \right)^{\frac{3-3\nu}{3\nu-1}} \quad (23)$$

and the shear modulus can be obtained from scaling calculations to give²²

$$G \sim \frac{c}{L} k_B T \left(\frac{c}{c^*} \right)^{\frac{1}{3\nu-1}} \quad (24)$$

where η_s is the viscosity of the solvent, $\nu \simeq 3/5$ is the Flory exponent, c is the segment concentration, and $c^* \sim L^{1-3\nu} b^{-3}$ is the overlap concentration.²² The reptation model for entangled polymers requires a clear separation of length scales between monomers and polymer chains. However, even after considering self-association of antibodies, the range of polymer lengths is $3 \lesssim L \lesssim 20$, which is still too short to satisfy the above mentioned condition. Nevertheless, the analytic model provides useful qualitative insight and reasonable agreement with measured viscosity.

Eqs. 22-24 yield a zero-shear viscosity of the form

$$\eta_0 \sim c^{\frac{3}{3\nu-1}} L^3 \quad (25)$$

The average length is computed from Eq. 12 which can be included in Eq. 25 to yield

$$\eta_0 = A c^{\frac{3}{3\nu-1}} \left(\frac{2kc}{\sqrt{1+4kc}-1} \right)^3 \quad (26)$$

where A is a constant of proportionality. Eq. 26 predicts that viscosity increases with concentration as $c^{3.75}$ for $\nu \simeq 0.6$. Also, we can see from Eq. 25 that the viscosity depends strongly on polymer length L . This increment in the zero-shear viscosity, proportional to the cube of the length scale, is found for semidilute polymer suspensions, as well as for colloidal suspensions.²⁵

Fitting the model to experiments, the constant was found to be $A = 5.4 \times 10^{-8}$ cP (mg/mL)^{3.75}.¹⁰ Note that the constant A absorbs the factor of 3 difference between the aggregate number and the polymer length, $3 \langle n \rangle = L$.

Shear thinning results from the release of entanglements

According to reptation theory,^{22,24} the viscous response of a polymer solution under shear is the result of two relaxation mechanisms. First, the shear will stretch out the polymers by deforming the entanglements that constrain them. Then the polymer shrinks along the tube generated by the constraints to recover its equilibrium extension. This relaxation process releases entanglements and, since it is not affected by the tube, the relaxation time is given by the Rouse relaxation time τ_R . Second, the polymers undergo reptational diffusion to establish a new conformation with the original number of entanglements points. Therefore, these two processes oppose each other with the chain retraction releasing entanglements and reptation restoring them. It can be checked that Rouse time is always much less than reptation time for any entangled solution where the length of the tube is longer than its diameter, therefore, in the following we treat chain retraction as instantaneous.

Returning to the simple shear case, the shear stress immediately following a step strain is approximately $\sigma(t=0^+) = G d\gamma$, where G is the shear modulus. Following Milner,²⁶ the shear flow with a constant strain rate $\dot{\gamma}$ is approximated as a sequence of step strains $d\gamma$ separated in time by some convenient interval dt , in which the chain relaxes. After a Rouse time, the chain retracts relaxing entanglements and reducing the stress by a factor $h(d\gamma)$

$$\sigma(t = \tau_R) = h(d\gamma) G d\gamma \quad (27)$$

where $h(d\gamma)$ is the nonlinear damping function. Next, the chain relaxes by reptation, which restores the entanglements. For the steady state case, entanglements are released as fast as they are restored. Therefore, the appropriate step strain is $d\gamma = \dot{\gamma} \tau_{rep}$. Since the viscosity is related to the shear stress by $\eta = \sigma / \dot{\gamma}$ we have

$$\eta(\dot{\gamma}) \approx G \tau_{rep} h(\dot{\gamma} \tau_{rep}) \quad (28)$$

where $h(\gamma)$ can be estimated by computing the root-mean-squared tube length increment immediately after the step strain,²² yielding

$$h(\gamma) = (1 + \gamma^2 / 3)^{-1/2} \quad (29)$$

The functional form of $h(\gamma)$ remains an open question and we refer the reader to²⁷ for a discussion of the topic. However, Eq. 29 is sufficient for our purposes here.

We obtain our final expression for the shear rate dependence of viscosity plugging Eq. 29 into Eq. 28 and using Eq. 26 for the product $G \tau_{rep}$

$$\eta = Ac^{\frac{15}{4}} \left(\frac{2kc}{\sqrt{1+4kc}-1} \right)^3 \left(1 + \frac{1}{3} \dot{\gamma}^2 B^2 \left(\frac{2kc}{\sqrt{1+4kc}-1} \right)^6 \right)^{-1/2} \quad (30)$$

In writing Eq. 30 we have introduced a proportionality constant into the expression for the reptation time $\tau_{rep} = B\langle L \rangle^3$.

The free parameters are A , which was obtained in,¹⁰ the equilibrium constant k , which depends on both the molecule and the solution condition, and the reptation prefactor B . The latter parameter contains information about non-specific interactions between antibody molecules. These interactions will depend on both the molecule of interest and the solution conditions. However, to limit the number of free parameters we obtain B from a global fit to all solution conditions for a given molecule.

The binding affinity predicts both the zero shear viscosity and the onset of shear thinning

The model was tested against experimental data on viscosity shear-dependence for two different antibodies under a variety of conditions from Zarraga et al.⁷ and Godfrin et al.²⁸ Fitting Eq. 30 to the data sets, we obtain the prefactor B for the reptation time by minimizing the sum of square errors for both types of antibodies for all system conditions. Next, we obtain the dimerization equilibrium constant k , which can be computed by fitting the viscosity for each individual system condition. The values of B and k are shown in Table 3. Solid lines in Fig. 6 show the fits using the model in Eq. 30.

Eq. 12 shows that the average chain length is determined by k , $\langle n \rangle \propto k^{1/2}$. Both the zero-shear viscosity and the reptation time have a strong dependence on polymer length, (Eq. 26). We have seen that the reptation time captures the onset of the shear-thinning behavior, thus an increment in the polymer length means that η_0 will increase and the onset of shear-thinning behavior will occur for lower values of the shear rate. This correlation between η_0 and the shear thinning is observed in the experimental data and captured by the reptation model. According to the model, this correlation stems from the fact that systems with shorter chains can re-form entanglements faster than systems with longer chains, due to the shorter reptation times.

An inspection of Fig. 6 reveals that the drop in viscosity is noticeably steeper than the $\dot{\gamma}^{-1}$ power law predicted by Eq. 30. This discrepancy is also seen in polymer systems, which typically show shear thinning behavior in the range $\dot{\gamma}^{-1.2}$ to $\dot{\gamma}^{-1.4}$.^{29,30} These exponents are consistent with shear thinning behavior shown in Fig. 6, although the less viscous conditions fall outside of this range, presumably because smaller antibody complexes are less polymer-like. The literature contains many refinements to the reptation model intended to improve the agreement with experiments,^{31,32} however, these fall outside of the scope of the present work which is to demonstrate the utility of the entanglement model in capturing the key features of antibody rheology.

Summary

At the high concentrations used for pharmaceutical formulation, even weak intermolecular interactions result in the formation of antibody clusters. Due to the discrete binding sites on the antibody arms, these clusters will tend to have an elongated morphology. This promotes entanglements, which have a profound effect on the dynamics of the solution. We have shown that a polymer model explains the viscosity, shear thinning behavior of antibody solutions. This model shows that viscosity can be reduced by minimizing entanglements, either by reducing intermolecular interactions or by using antibody constructs that lack the multi-valency required to make extended structures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Cartoon and concentration for the monomer, dimer, and trimer states in the HH model.

Figure 2:

Cartoon of the monomer, dimers, and trimers described by the HT model (Eq. 8). As discussed in the Supporting Information, several of these structures are related by rotational symmetry and, therefore, are over-represented in the partition function q_{HT} . The corrected partition function $\tilde{q}_{\text{HT}}$ removes this over-counting.

Figure 3: Average number of monomers per cluster for the HH model (blue) and the HT model (red) at two different concentrations: 100mg/mL (solid lines) and 200mg/mL (dotted lines) at the same binding energy $\epsilon_{HH} = \epsilon_{HT} = \epsilon$.

Figure 4: Behavior of the relative concentrations for HH model (A) and HT model (B) as a function of binding energy $\epsilon_{HH} = \epsilon_{HT} = \epsilon$ at different concentrations: 50mg/mL (solid lines), 150mg/mL (dotted lines). At low binding energies monomers dominate, but as the binding energy increases the monomer concentration decreases monotonically as larger structures start to form. Clusters larger than the monomer have a non-monotonic concentration because the large clusters formed at high binding energies consume the pool of smaller clusters.

Figure 5:

Comparison of Eq. 21 to the viscosities measured in¹¹ (left), and¹² (right). Binding parameters for both HH and HT models (as noted) are fit to the first three data points ($c \leq 20$ mg/ml). The increasing deviation at higher concentrations, especially in panel A, indicates that the antibodies are exiting the dilute regime and becoming entangled.

Figure 6:

Comparison of Eq. 30 to the shear dependent viscosity of an antibody at A) 200 mg/ml,⁷ B) 150 mg/ml,⁷ and a different molecule at C) 120 mg/ml,²⁸ and D) 146 mg/ml.²⁸ Solutions with lower viscosity deviate more strongly from the theory due to the fact smaller complexes are less polymer-like.

Table 1:

Fitted free energies for the dilute antibody solutions plotted in Fig. 5. Also shown are the overlap concentration, at which Eq. 21 is expected to fail, and the average cluster size at 10 and 10 mg/ml.

	Fig. 5A	$k \left(\frac{\text{ml}}{\text{mg}} \right)$	$c^* \left(\frac{\text{mg}}{\text{ml}} \right)$	$\langle n \rangle$	
				$c = 10 \text{ (mg/ml)}$	$c = 30 \text{ (mg/ml)}$
ref. ¹¹	purple	0.021	27	1.18	1.44
	blue	0.068	22	1.46	2.01
	green	0.136	19	1.77	2.58
	red	0.609	14	3.02	4.80
	Fig. 5B	$s \left(\frac{\text{ml}}{\text{mg}} \right)$	$c^* \left(\frac{\text{mg}}{\text{ml}} \right)$	$\langle n \rangle$	
				$c = 10 \text{ (mg/ml)}$	$c = 30 \text{ (mg/ml)}$
ref. ¹²	purple	0.001	34	1.02	1.06
	blue	0.007	28	1.13	1.36
	green	0.07	16	2.04	3.67
	red	0.11	14	2.53	4.96

Table 2:

Comparison between values of radius of hydration obtained by DLS experiments¹² and radius computed from Eq. 19 using fitted parameters from Table 1.

c_{NaCl} (mM)	R_H (nm)	R_g (nm)
0	17.5	17.9
30	14.1	15.7
60	12.0	11.0
100	10.8	10.4

Table 3:

Parameters extracted from the fits in Fig. 6, including the dimerization equilibrium constant and the global proportionality constant. Also shown is the average cluster size $\langle n \rangle$, which increases with the equilibrium constant.

ref. ⁷	Fig. 6A	k (ml/mg)	$\langle n \rangle$	B (10^{-5} s)
	red	0.094	4.86	1.83
	black	0.074	4.40	
	blue	0.054	3.82	
	green	0.041	3.39	
	Fig. 6B	k (ml/mg)	$\langle n \rangle$	B (10^{-5} s)
	red	0.028	2.61	1.83
	blue	0.019	2.26	
	green	0.012	1.93	
ref. ²⁸	Fig. 6C	k (ml/mg)	$\langle n \rangle$	B (10^{-5} s)
	green	0.214	5.60	0.97
	red	0.091	3.84	
	black	0.067	3.39	
	blue	0.027	2.36	
	Fig. 6D	k (ml/mg)	$\langle n \rangle$	B (10^{-5} s)
	red	0.040	2.97	0.97
	blue	0.011	1.84	