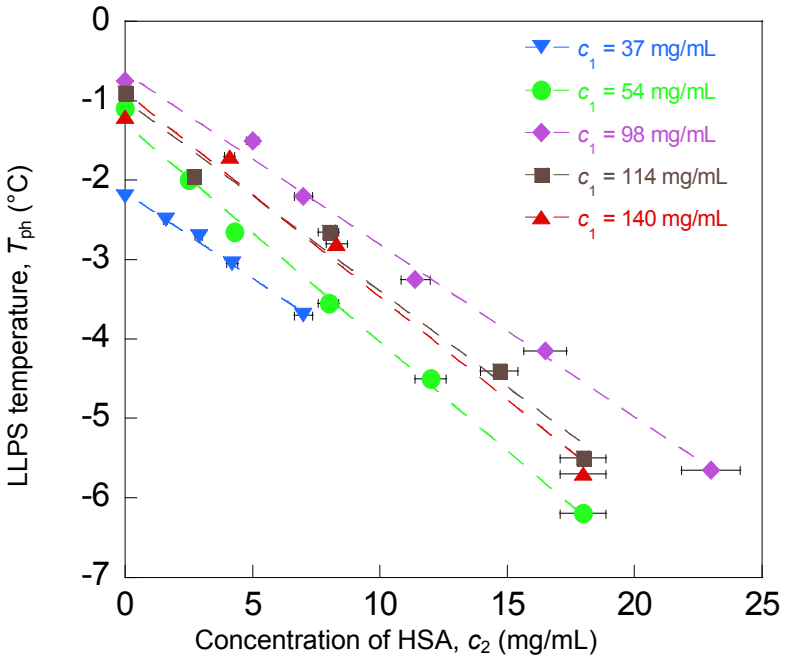


Fig. S1. The LLPS temperature, $T_{ph}$, versus the HSA concentration, $c_2$, at fixed MAb concentration, $c_1$. Linear fitting of $T_{ph}$ vs $c_2$ at each $c_1$ is shown by dashed lines.

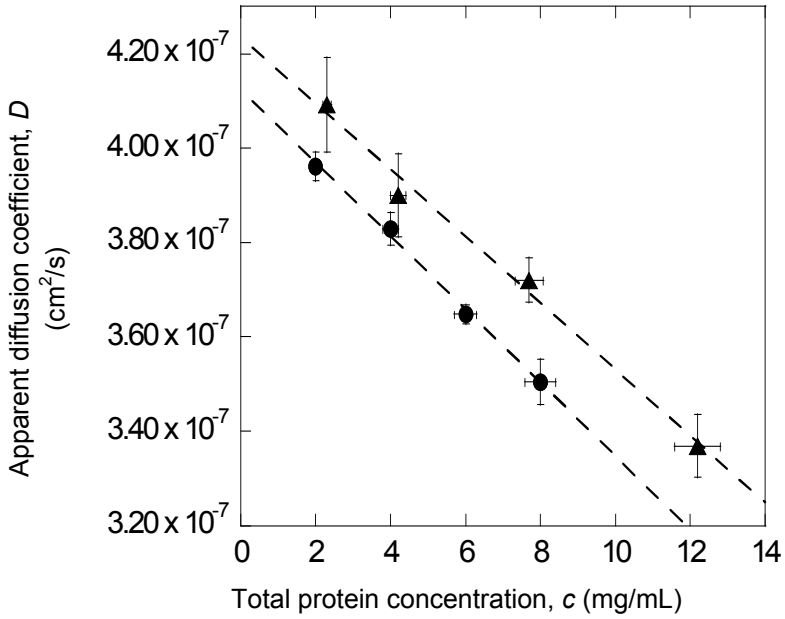


Fig. S2. Apparent diffusion coefficients, $D$, of pure MAb solutions (circles) and 30% (w/w HSA) MAb-HSA mixture solutions (triangles) as a function of total protein concentration, $c$, in 0.1 M Tris·HCl buffer at pH 7.4 measured by QLS. The dashed lines are linear fits given by: $D = (1-0.020c)\cdot 4.12\times 10^{-7}$ for pure MAb solutions; $D = D_0\,(1-0.017c)\cdot 4.23\times 10^{-7}$ the mixture solutions.

***Partitioning of $_{QpE}$MAb and $_{pEpE}$MAb:***

Our IgG2-A samples were in fact a mixture of two species: $_{QpE}$MAb, antibody with partially cyclized heavy chain N-termini (i.e., with glutamine (Q) at one of the Fab domains and pyroglutamate (pE) at another); and $_{pEpE}$MAb, antibody with pyroglutamates at N-termini of both Fab domains. The CEX retention time difference between $_{QpE}$MAb and $_{pEpE}$MAb results from the abolition of a single negative charge in $_{QpE}$MAb (see Fig.S3). The ratio of the concentrations of $_{QpE}$MAb to $_{pEpE}$MAb in original samples is 0.302 ± 0.001. Table S1 below shows that, in the partitioning measurements at −4.2 °C, $_{QpE}$MAb preferentially partitions into the protein-rich phase as compared to $_{pEpE}$MAb.

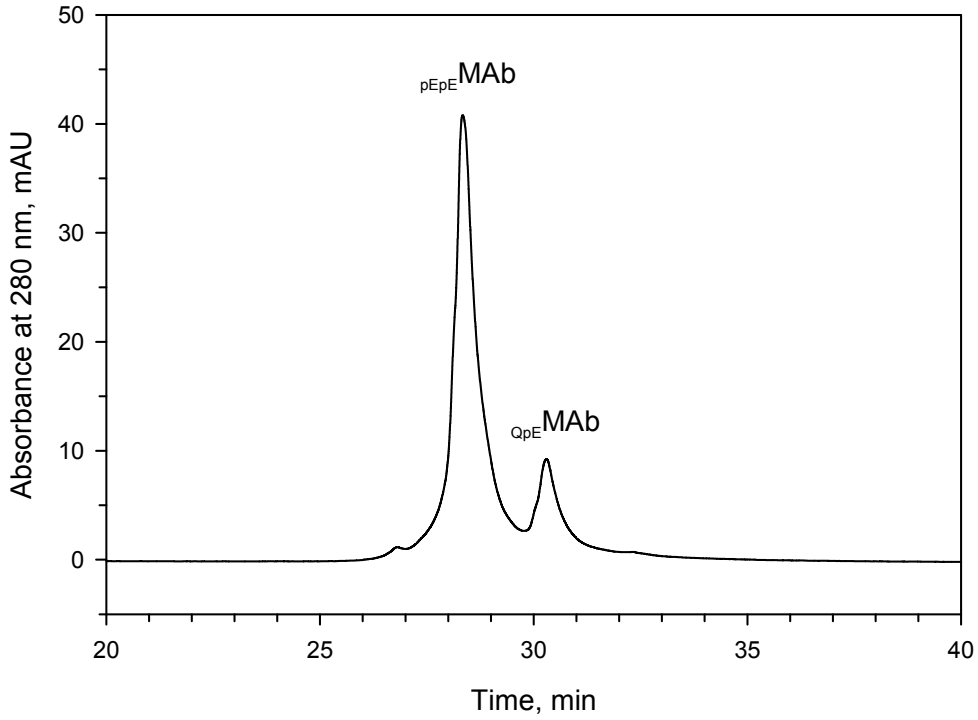


Fig. S3. A representative of CEX chromatogram of IgG2-A. The abolition of a negative charge in $_{QpE}$MAb results in its late elution compared to $_{pEpE}$MAb.

| Sample | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| Phase | I | II | I | II | I | II |
| HSA (mg/mL) | 0 | 0 | 7.9 | 11.1 | 9.2 | 12.5 |
| MAb (mg/mL) | 30 | 234 | 42 | 205 | 44 | 192 |
| $_{QpE}$MAb : $_{pEpE}$MAb | 0.276 | 0.307 | 0.289 | 0.313 | 0.279 | 0.311 |

Table S1. Concentrations of total MAb and HSA and the ratio of $_{QpE}$MAb:$_{pEpE}$MAb in protein-poor (I) and protein-rich (II) phases in pure MAb solutions and in two MAb/HSA mixtures at $T_{ph}$ = −4.2 °C.

***Thermodynamic expression of the change in phase separation temperature upon addition of small molar fraction of a second solution component:***

In this section, we will derive a general expression for the change in phase separation temperature $\Delta T_{ph}\left(\phi_1\right)$ at a fixed volume fraction of primary solute 1, $\phi_1$, upon addition of small amount of solute 2. In our experiments MAb is solute 1 and HSA is solute 2. In a two-solute mixture, if the second component mole fraction is small: i.e. $x=N_2/N_1<<1$, the chemical potential of solute 1 is given by a general formula (1): $\mu_1 = \mu_1^0\left(\Pi, T\right) - kTx$. For a solution of pure solute 1, the equilibrium condition of LLPS is: $\mu_1^{0I}\left(\Pi, T_{ph}\right) = \mu_1^{0II}\left(\Pi, T_{ph}\right)$. In the presence of solute 2, a new equilibrium is reached at $\Pi+\Delta\Pi$ and $T_{\rm ph}+\Delta T_{\rm ph}$, and thus $\mu_1^{0I}\left(\Pi+\Delta\Pi, T_{ph}+\Delta T_{ph}\right) - kTx^I = \mu_1^{0II}\left(\Pi+\Delta\Pi, T_{ph}+\Delta T_{ph}\right) - kTx^{II}$. Expanding $\mu_1^{0I}$ and $\mu_1^{0II}$ in this equation with respect to $\Pi$ and $T_{\rm ph}$ and applying the condition: $\mu_1^{0I}\left(\Pi, T_{ph}\right) = \mu_1^{0II}\left(\Pi, T_{ph}\right)$, we obtain:

$$\left(\mathrm{v}^I - \mathrm{v}^{II}\right)\Delta\Pi - \left(s^I - s^{II}\right)\Delta T_{ph} - kT_{ph}\left(x^I - x^{II}\right) = 0 \qquad [S1]$$

Here, $\mathrm{v} = V / N_1 = \partial\mu_1^0 / \partial\Pi$ is the solution volume per molecule 1; $s = S / N_1 = -\partial\mu_1^0 / \partial T$ is the entropy per molecule 1; and $x=N_2/N_1$ is the molar fraction of solute 2. Eq.[S1] describes how addition of solute 2 is related to changes in the phase transition temperature and pressure. In the absence of solute 2, $x$=0, Eq.[S1] reduces to the Clausius-Clapeyron equation for the variation of the pressure with temperature along the coexistence curve:

$$d\Pi / dT_{ph} = \left(s^I - s^{II}\right) / \left(\mathrm{v}^I - \mathrm{v}^{II}\right) \qquad [S2]$$

Here, we are interested in the change in $T_{\mathrm{ph}}$ at a constant $\phi_1$. Noting that pressure can generally be considered a function of $\phi_1$, $x$ and $T$, we can write the change of pressure as: $\Delta\Pi = \left(\partial\Pi/\partial\phi_1\right)\Delta\phi_1 + \left(\partial\Pi/\partial T\right)\Delta T + \left(\partial\Pi/\partial x\right)\Delta x$. Under the conditions we want to apply in Eq.[S1], $\Delta\phi_1 = 0$, $\Delta T = \Delta T_{ph}$ and $\Delta x = x$ where $x$ is either $x^{\mathrm{I}}$ or $x^{\mathrm{II}}$ and $\Delta T_{ph}$ is either $\Delta T_{ph}\left(\phi_1^{I}\right)$ or $\Delta T_{ph}\left(\phi_1^{II}\right)$ depending on which phase is considered. Under these conditions: $\Delta\Pi = \left(\partial\Pi/\partial T\right)\Delta T_{ph} + \left(\partial\Pi/\partial x\right)x$. If we now substitute this expression for $\Delta\Pi$ into Eq.[S1], we obtain:

$$\left[\frac{s^{I} - s^{II}}{\mathrm{v}^{I} - \mathrm{v}^{II}} - \frac{\partial\Pi}{\partial T}\right]\Delta T_{ph} = -kT_{ph}\frac{x^{I} - x^{II}}{\mathrm{v}^{I} - \mathrm{v}^{II}} + \frac{\partial\Pi}{\partial x}x \qquad \text{[S3]}$$

Using Eq.[S2], the bracketed term in Eq.[S3] becomes: $d\Pi/dT_{ph} - \partial\Pi/\partial T$. The change of the osmotic pressure with temperature along the coexistence curve can be written as: $d\Pi/dT_{ph} = \partial\Pi/\partial T + \left(\partial\Pi/\partial\phi_1\right)/\left(dT_{ph}/d\phi_1\right)$. Thus, the bracketed term in Eq.[S3] is equal to $\left(\partial\Pi/\partial\phi_1\right)/\left(dT_{ph}/d\phi_1\right)$. On the right side of Eq.[S3], $x = N_2/N_1 = \left(\phi_2/\phi_1\right)\left(\Omega_1/\Omega_2\right)$ and $\mathrm{v} = V/N_1 = \Omega_1/\phi_1$, where $\Omega_1$ and $\Omega_2$ are the molecular volume of solutes 1 and 2 respectively. Therefore, Eq.[S2] becomes:

$$\Delta T_{ph} = \frac{dT_{ph}/d\phi_1}{\partial\Pi/\partial\phi_1}\left[-\frac{kT_{ph}\left(\phi_2^{I}/\phi_1^{I} - \phi_2^{II}/\phi_1^{II}\right)}{\Omega_2\left(1/\phi_1^{I} - 1/\phi_1^{II}\right)} + \frac{\partial\Pi}{\partial\phi_2}\phi_2\right] \qquad \text{[S4]}$$

This is the result given as Eq.[1] in the main text. It connects $\Delta T_{ph}$ to the properties of pure solute 1 system (the term before the brackets) and the effect of solute 2 (the term in the brackets). In this equation, we can see that the effect of solute 2 consists of two components: the partitioning part (the first term in the brackets) reflects the reaction of the system to the perturbation in the balance of chemical potentials $\mu_1$ in the coexisting phases upon addition of solute 2; the incompressibility part (the second term in the brackets) reflects the perturbation in the balance of osmotic pressures $\Pi$.

It is interesting to consider two limiting cases when the effect of addition of solute 2 is straightforward. In the first case, let us consider non-interacting point-like solute 2. We do not expect any changes in the coexistence curve in this case. Indeed, this ideal solute 2 will partition equally into the volumes accessible to it in each phase, $V_{\mathrm{eff}} = V - b\Omega_1 N_1$, where $b\Omega_1$ is the effective excluded volume per molecule 1. That is to say: $\phi_2/\left(1 - b\phi_1\right)$ is the same in both phases, and consequently the first bracketed term in Eq.[S4] becomes equal to $-kT_{ph}\phi_2/\left(1 - b\phi_1\right)\Omega_2$. Furthermore, since solute 2 is an ideal solute in $V_{\mathrm{eff}}$, its partial pressure is $kT_{ph}N_2/V_{eff}$ and thus

the second bracketed term in Eq.[S4] cancels the first term. Therefore, $\left(\Delta T_{ph}\right)_{\phi_1} = 0$ as expected. In the second case, let us consider solute 2 being essentially identical to solute 1. The effect of addition of such solute should be simple replacement of $T_{ph}\left(\phi_1\right)$ with $T_{ph}\left(\phi_1+\phi_2\right)$. In the limit of small $\phi_2$, $\left(\Delta T_{ph}\right)_{\phi_1} = T_{ph}\left(\phi_1+\phi_2\right) - T_{ph}\left(\phi_1\right) = \left(\partial T_{ph}/\partial\phi_1\right)\phi_2$. Indeed, in this case $\phi_2/\phi_1$ must be the same in both phases and the partitioning term in Eq.[S4] is zero. The osmotic incompressibilities $\partial\Pi/\partial\phi_1$ and $\partial\Pi/\partial\phi_2$ are the same and cancel each other. Consequently, $\left(\Delta T_{ph}\right)_{\phi_1} = \left(\partial T_{ph}/\partial\phi_1\right)\phi_2$, as expected.

***Monte Carlo simulation on the free volume of HSA in a solution of MAb:***

To evaluate the volume, $V_{\text{eff}}=\alpha V$, accessible to an HSA molecule in a solution of MAb, we use a simple three-sphere model for the Y-shaped MAb molecule. In this model, the $F_c$ domain and the two $F_{ab}$ domains are represented by three spheres, whose radii and centers are chosen so as to reasonably represent the geometry of MAb molecules insofar as their excluded volume effects are concerned. The HSA molecule is modeled as a single sphere. These models are shown in Fig.S4.

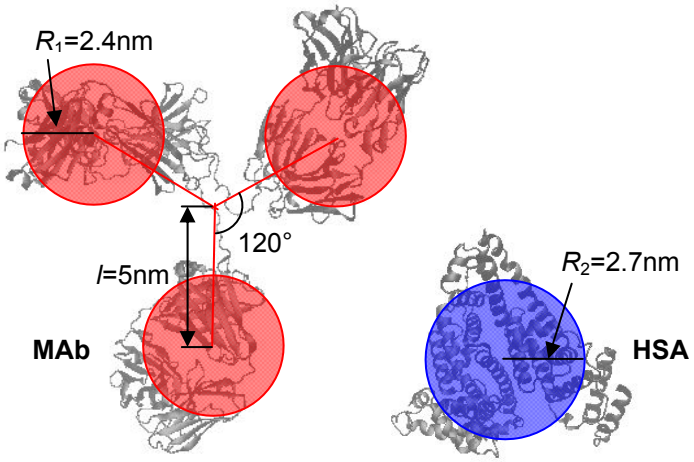


Fig. S4. The three-sphere model for a MAb molecule and the single sphere model for an HSA molecule superimposed on the X-ray structures of an IgG (DOI: 10.2210/pdb1IGT/pdb) and HSA (DOI: 10.2210/pdb1E7B/pdb), respectively. The radii of spheres in the MAb model, $R_1$ = 2.4 nm, and that in the HSA model, $R_2$ = 2.7 nm, are calculated using their molecular weights $M_1$ = 150875 g/moL and $M_2$ = 66472 g/moL, as well as the specific volume for proteins, $v_{sp}$ = 0.71 mL/g. In this three-sphere model of MAb, the distance between the center of each sphere and the center of the molecule is assigned to be 5 nm according to the X-ray structure of IgG2-A.

Using these simple models for MAb and HSA, we have conducted Monte-Carlo simulations to calculate the free volume fraction, $\alpha$, for a HSA molecule in a MAb solution as a function of MAb volume fraction, $\phi_1$. The simulations were conducted in a high temperature approximation, i.e. only excluded volume effects were taken into account and energetic interactions were ignored. Briefly, the system consisting of 2000 model MAb molecules at a desired volume fraction $\phi_1$ was equilibrated over $10^6$ Monte Carlo steps, and then the probability, i.e. $\alpha$, of a successful placement of a model HSA molecule at a random location was measured using $10^6$ attempts to place a HSA molecule. The result is presented in Fig.S5 for $\phi_1$ from 0 to 0.142 (corresponding to a MAb concentration from 0 to 200 mg/mL). This simulation result can be fitted using a quadratic equation: $\alpha = 1 + A\phi_1 + B\phi_1^2$, where $A = -7.60$ and $B = 15.6$. The value of coefficient $A$ reflects both the core volume and the "depletion layer" around each MAb molecule inaccessible to the center of a HSA molecule. The positive value of the coefficient $B$ of the second order term of $\phi_1$ takes into account the overlap of the depletion layers as $\phi_1$ increases.

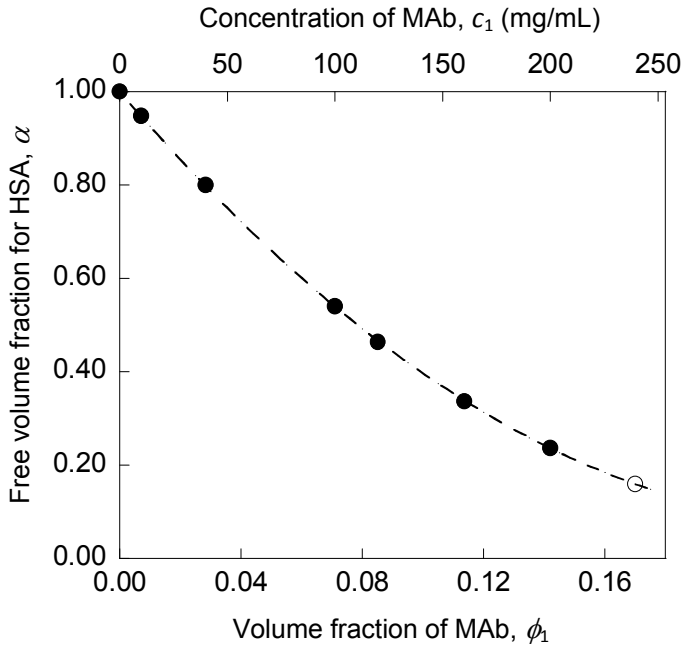


Fig. S5. Free volume fraction $\alpha$ for a HSA molecule as a function of volume fraction of MAb, $\phi_1$. The solid circles are the data points from Monte Carlo simulation which were carried out in the range of concentrations used in experiments. The dashed line is the quadratic fitting of the simulation data. The open circle is the extrapolation to our highest experimental concentration of MAb using the quadratic fitting equation. The values of $\alpha$ at high MAb concentration, $c_1$ > 200mg/mL, are difficult to determine by simulation due to the long equilibration time in the "gel-like" solution.

***Evaluation of the energy of MAb-HSA interaction by considering the reduction of phase separation temperature upon addition of HSA:***

As shown in the discussion section, Eq.[1], the non-ideal contribution of HSA to the osmotic incompressibility $\partial\left(E_{12}/kT_{ph}-\ln\alpha\right)/\partial\phi_1$, must be negative at $\phi_1<\phi_c$, and positive at $\phi_1>\phi_c$, and therefore must be zero in the vicinity of the critical point. Using the $\alpha(\phi_1)$ determined by simulation, we calculated $\partial\left(-\ln\alpha\right)/\partial\phi_1$, and found it to be equal to 10 at the critical volume fraction, $\phi_c$=0.063. Thus, in the vicinity of the critical point, $\partial\left(E_{12}/kT_{ph}\right)/\partial\phi_1=-10$. In the mean field approximation, $E_{12}\left(\phi_1\right)=\varepsilon_{12}\phi_1$, and thereby $\varepsilon_{12}/kT_{ph}=-10$. In Fig. S6, we plot both $\partial\left(-\ln\alpha\right)/\partial\phi_1$ and the $\varepsilon_{12}/kT_{ph}+\partial\left(-\ln\alpha\right)/\partial\phi_1$, which is consistent with experimentally observed downward shift of the whole coexistence curve, as a function of $\phi_1$.

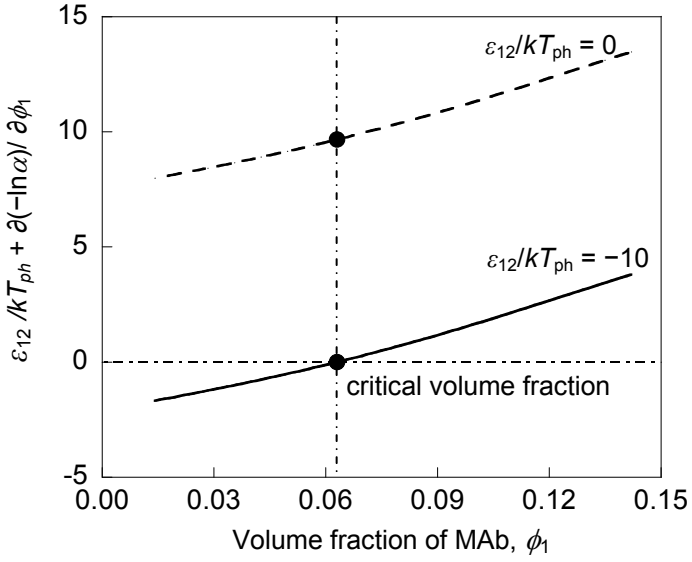


Fig. S6. The non-ideal contribution of HSA to the osmotic incompressibility, $\partial\left(E_{12}/kT_{ph}-\ln\alpha\right)/\partial\phi_1$, as a function of $\phi_1$ in the mean field approximation with $\varepsilon_{12}/kT_{ph}$ equal to 0 and −10. The quantity $\partial\left(-\ln\alpha\right)/\partial\phi_1$ is evaluated using Monte Carlo simulation. The circles mark the position of critical volume fraction.

*Reference:*

S1. Landau LD, Lifshitz EM, & Pitaevskii LP (1980) *Statistical physics* (Pergamon Press, Oxford ; New York) 3d rev. and enl. Ed.