Supplementary Material for: Mixing salts and polyethylene glycol into protein solutions: The effects of diffusion across semipermeable membranes and of convection

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Further details of how the physical mechanisms for mixing depend on the dimensional numbers

This section has three parts. The first part is a brief discussion of the effect of stirring a solution at speed u. This puts the effect of convection, which also induces flow, in context. The second and third parts are brief examples of how we can use the dimensionless numbers to predict how mixing occurs. These are for free-interface diffusion, and for a system with a semipermeable membrane, respectively.

Table S1: Table of six key dimensionless numbers, typical values, and a brief comment on the implication of each value. The top two values are for a stirred volume, the bottom four are for convection. The values are for a cubic volume $L = 5$ mm across (125 µl), with a gradient of NaCl concentration from 0.5 to 0 M. The parameter values used for the salt solution are a viscosity $\eta = 0.9 \times 10^{-3}$Pa s,[1] mass densities of $\rho = 997$ kg m^{-3} for pure water, and 1017 kg m^{-3} for 0.5 M NaCl solution,[2] and a diffusion constant for salt of $D_{\text{coop}} = 1.6 \times 10^{-9}$ m^{2}s^{-1} at 298 K.[3-5] The value for Bi$_\text{S}$ assumes a diffusion constant inside the membrane $D_\text{M} = D_{\text{coop}}/30$, and a semipermeable-membrane width of $w = 40$ µm.

number	value for 125 µl NaCl solution	comment
Stirring at speed $u = 1$ mm s^{-1}		
Péclet Pe	5000	flow across volume faster than diffusion
Reynolds Re	5	flow not quite laminar, can mix
Convection		
Rayleigh Ra$_\text{S}$	10^7	convection in liquid
Sherwood Sh	30	convection accelerates mixing by a factor of ~ 30
Grashof Gr$_\text{S}$	10^4	flow not laminar so mixes even independently of diffusion
Biot Bi$_\text{S}$	0.1	rate of transport through membrane 10 % of rate through solution volume

Mixing in systems stirred at a stirring speed u

For a volume L across, if the solutions are flowing at a speed u, then the timescale for flow to cross the volume is

$$\tau_{\text{Xu}} = \frac{L}{u} \tag{1}$$

If this timescale is much larger than τ_{MIXD} then mixing is via diffusion alone, but this is rare. The ratio $\tau_{\text{MIXD}}/\tau_{\text{Xu}}$ is so common and useful that it has a name: the Péclet number

$$\text{Pe} = \frac{\tau_{\text{MIXD}}}{\tau_{\text{Xu}}} = \frac{uL}{D_{\text{coop}}} \tag{2}$$

Mixing is a problem in fluid mechanics, and as with many problems in fluid mechanics, to work out what is going on, we need to estimate the values of some dimensionless numbers. The Péclet number is first of these numbers we will be considering here.

Mixing is only solely due to diffusion when Pe $\ll 1$, and this is typically hard to achieve. Consider a μl volume, which is ~ 1 mm across. Then a Péclet number much smaller than one requires any flow to be at speeds of less than 1 μm s^{-1}. This is why mixing is rarely due to diffusion alone, even for volumes of a microlitre and smaller, and why even modest amounts of stirring of small volumes, accelerate mixing.[6–8] The small volumes typically used in protein crystallisation are comparable to those seen in microfluidics, so the review of Squires and Quake[6] of microfluidics, is a very relevant here.

Flows can combine with diffusion to cause mixing at a rate faster than with diffusion alone.[6] However, slow flows, often called Stokes or laminar flows, cannot mix by themselves, without cooperating with diffusion.[6–8] Here, a slow flow is one at small values of another dimensionless number, the Reynolds number

$$\mathrm{Re} = \frac{\rho u L}{\eta} \tag{3}$$

for ρ the mass density of the liquid ($\approx 10^3$kg m^{-3} for water) and η is the viscosity ($\approx 10^{-3}$Pa s for water). Flow at large values of the Reynolds number will mix directly, even in the absence of diffusion.

For flows due to convection, the analog of the Reynolds number is the Grashof number.[6] The Grashof number is defined by

$$\mathrm{Gr_S} = \frac{\Delta \rho g L^3}{\rho \nu^2} \tag{4}$$

When the Grashof number is less than one, the flow is laminar, due to inertial effects being negligible. This has the consequence that without diffusion there is no mixing, i.e., laminar flow and diffusion can mix faster than diffusion alone, but laminar flow itself cannot mix on its own. By contrast, even without diffusion, flows with $\mathrm{Gr_S} > 1$ can mix, and there is turbulence at large values of $\mathrm{Gr_S}$.

Mixing of NaCl in a free-interface diffusion experiment with volume 125 μl volume

To see how the dimensionless numbers discussed in the main text can be used in practice, we consider a cubic volume $L = 5$ mm across, filled with a solution which has a NaCl concentration of 0.5 M NaCl solution at one end, which decreases to zero (pure water) at the other end. This is at room temperature. Here the relevant numbers are the Rayleigh, Sherwood and Grashof numbers. Their values are given in Table S1.

The Rayleigh number is far above the 1000 threshold for convection to start in vertical concentration gradients, so there is strong convection. Also, both the Sherwood and Grashof numbers are greater than one, so mixing is significantly accelerated by convection, and this convection is strong enough to mix on its own.

The distribution of salt during mixing by diffusion alone, is a simple gradient, but when convection is present, the distribution will be much more complex, due to the convective flows. This complex and heterogeneous distribution of the precipitant salt, means that the supersaturation will vary by large amounts and in a complex way with space and time. Both nucleation[9–12] and growth rates[13,14] are very sensitive to supersaturation. Therefore, at any one time during mixing, nucleation and growth rates presumably vary by orders of magnitude across the flow streams, and a growing crystal will experience a highly variable supersaturation as it grows.

It is worth noting that although we considered a volume of 125 μl, convection occurs even in much smaller volumes. It has been studied in microfluidics experiments in systems of heights of only 100 μm.[15,16] In such small systems convection is slower but is still present.

Mixing of NaCl in an experiment with a semipermeable membrane and with a volume 125 μl

We return to the example above of a 125 μl volume, but now the volume is separated by a

semipermeable membrane from a large reservoir of 0.5 M NaCl solution. We assume that transport in the large volume is fast enough to keep the concentration on that side near the assumed fixed bulk concentration. Then, if we, for example, assume a semipermeable membrane of width $w = 40$ μm, and that the diffusion constant for NaCl inside the semipermeable membrane is 30 times smaller than in the bulk, i.e., $D_\mathrm{M} = D/30$, we obtain the Biot number in Table S1. This is $\mathrm{Bi_S} = 0.1$. For this system the mixing time is mostly determined by diffusion across the semipermeable membrane, because it is about ten times slower than mixing inside the chamber.
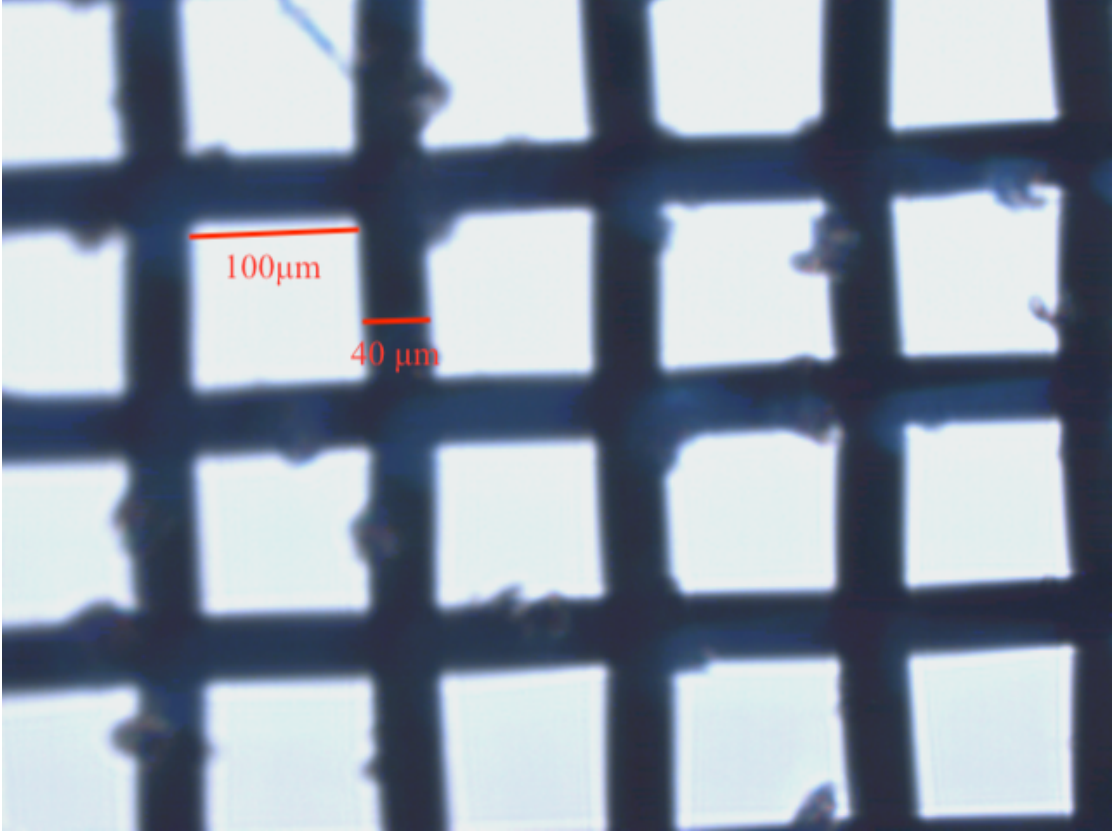

Figure S1: Microscope image of a grid of known dimensions used to calibrate our images. The crossbars are 40 μm across and the gaps are 100 μm across. We make 20 measurements, using the Fiji distribution of ImageJ (https://fiji.sc/),[17,18] on this image and as a result determine that one pixel is 0.9 ± 0.04 μm.
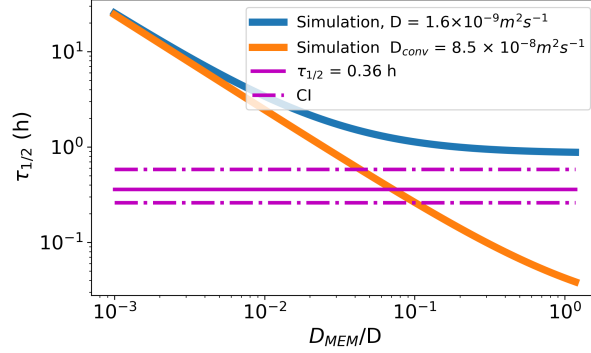
Figure S2: The equilibration time for 3 M NaCl, $\tau_{1/2}$, as a function of the ratio D_{M}/D. The blue curve is for diffusion only (no convection), and the orange curve is for an effective diffusion constant $D = 8/5 \times 10^{-8} \mathrm{m^2\ s^{-1}}$, to account for a speed up by a factor of Sh = 53 due to for convection. The purple lines are at the experimental values for $\tau_{1/2}$. The solid line is at the mean of the three best-fit values, and the dashed lines bound the 95% CI. The behaviour is similar to that with 0.5 M NaCl (Figure 6 of main text). The experimental $\tau_{1/2}$ is a little lower and the Sherwood number is about twice as big, but these are the only differences.

Estimating the overlap concentration of polymers c^* using measurements of the intrinsic viscosity

In polymer solutions, the boundary between the dilute regime and unentangled semidilute regime is at the overlap concentration, c^*.[19,20] We estimate c^* using measurements of the intrinsic viscosity.[19] The intrinsic viscosity for PEG in water is defined[19] by the ratio

$$[\eta] = \frac{\eta(c) - \eta_{\mathrm{W}}}{\eta_{\mathrm{W}} c} \qquad c \to 0 \tag{5}$$

i.e., it is defined in the limit of low polymer concentration, c. Here $\eta(c)$ is the viscosity of the polymer solution, and η_{W} is the viscosity of pure water. For PEG solutions in water, measurements have been used to obtain an expression for the intrinsic viscosity as a function of MW,[21]

$$[\eta] = 2.0 + 0.016 \mathrm{MW}^{0.76} \quad \mathrm{ml\ g^{-1}} \tag{6}$$

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Then we define the overlap concentration as one over the intrinsic viscosity (which has units of inverse concentration not viscosity)

$$c^* = \frac{1}{[\eta]} \tag{7}$$

We use % w/v = g/100 ml not g/ml so we simply convert to a c^* in our units by multiplying by 100. The values for c^* for the three MWs we study are in Table 2 of the main text.

Concentration dependence of the bulk mixing time τ_{MIXDC}

Here we look at how varying the concentration of a salt or of PEG, affects mixing in the bulk. This is in the regime where mixing is due to a combination of diffusion and convection, and so the relevant timescale is τ_{MIXDC}. Within our simple model, the concentration dependence of τ_{MIXDC} comes from both the cooperative diffusion constant and the Sherwood number

$$\tau_{\mathrm{MIXDC}} \propto 1/(D_{\mathrm{coop}}\mathrm{Sh}) \tag{8}$$

Below we first consider salts then polymer solutions.

Bulk mixing time τ_{MIXDC} for salts

For salts the cooperative diffusion constant varies only weakly with concentration, for NaCl the cooperative diffusion constant varies only by of order 10 % from very low to molar concentrations.[5] Thus in the absence of convection, mixing times for salts are almost independent of the concentration.

If there is convection, then the mixing time will depend on the Rayleigh number. The viscosity varies only weakly with concentration,[2] while the mass density difference varies approximately linearly with salt concentration. So the Rayleigh number varies essentially linearly with salt concentration, due to the variation of the mass density difference. As our

formula for the Sherwood number scales as $\mathrm{Ra_S^{1/3}}$, the mixing time in the bulk scales with salt concentration approximately as

$$\tau_{\mathrm{MIXDC}} \sim 1/(D_{\mathrm{coop}}\mathrm{Sh}) \sim c^{-1/3} \tag{9}$$

This inverse cube root dependence is a weakly decreasing function of increasing salt concentration. Thus we predict that on increasing salt concentration, the amount of convection slowly increases, and so mixing times slowly decrease.

Bulk mixing time τ_{MIXDC} for polymer solutions

As for salt solutions, the mass density difference to pure water varies approximately linearly with PEG concentration.[22] The mass density is approximately independent of MW. In the dilute regime $(c < c^*)$, where the PEG polymer coils do not overlap, the diffusion constant is approximately constant at its Stokes-Einstein value, and the viscosity is close to that of the solvent (water). Thus, in the dilute regime, diffusion varies only weakly with concentration, but convection will increase, just as it does for salts, see eq (9).

However, in the semidilute regime where the polymer coils overlap, both the cooperative diffusion constant and the viscosity vary with polymer concentration. For the PEG MWs we consider (of order 1000s) there are only two regimes: dilute $(c < c^*)$ and unentangled semidilute $(c^* < c < c_e)$,[23] as discussed in the main text.

Dynamics of unentangled semidilute polymer solutions

The Rouse/de Gennes model[20,23–25] is widely used for unentangled semi-dilute polymers $(c^* < c < c_e)$. Here we will restrict ourselves to just looking at how the timescale for mixing scales with concentration,[20] i.e., if in the semidilute regime we expect mixing to speed up or slow down.

We will also switch from concentration c to volume fractions $\phi = \rho a^3$, where ρ is the

number density of monomers and a^3 is the volume of a monomer. Polymer concentration c and ϕ are proportional to each other. The overlap and entanglement volume fractions are ϕ^* and ϕ_e, respectively. We use the number of monomers N, related to the MW by $N = \text{MW}/\text{MW}_0$, for MW_0 the molecular weight of a monomer.

The de Gennes model treats the polymers in an unentangled semidilute polymer solution as composed of a chain of blobs ξ across. Within blobs a polymer's configuration is close to that in the dilute solution, while over distances greater than ξ interactions between different polymer molecules dominate. Over those distances hydrodynamic interactions are screened and so Rouse dynamics is approximately correct.[24]

The de Gennes scaling prediction for the blob-size/correlation-length ξ, is $\xi = a\phi^{-3/4} \propto c^{-3/4}$. Here and below we use Flory's value $\nu = 3/5$ for the scaling of the polymer radius of gyration with the number of monomers.[20] The number of monomers in a blob is $g = \phi^{-5/4}$.

For the cooperative or collective diffusion coefficient, the Rouse/de Gennes prediction is that collective diffusion is approximately that of quasi-ideal (i.e., Stokes-Einstein) blobs of the correlation size ξ[20,23]

$$D_{\text{coop}} \sim \frac{kT}{6\pi\eta_s\xi} \propto \text{MW}^0\phi^{3/4} \quad \text{for} \quad \phi^* < \phi < \phi_e \tag{10}$$

this is Eq. VII.24 of de Gennes.[20] Here η_s is the viscosity of the solvent, Note that in this regime, the collective diffusion constant does not depend on MW, and increases with polymer concentration c as $c^{3/4}$.

The contribution of the polymer to the viscosity (at low shear rates) is approximately a long-time modulus, G, times the longest relaxation time, τ_{PC}, which is that of the polymer chain, i.e.,

$$\eta_{\text{ROUSE}} \sim G\tau_{\text{PC}} \tag{11}$$

Here, and below we follow chapter eight of Rubinstein and Colby.[24] Within a Rouse model

the modulus is just kT times the number density of chains

$$G = kT \frac{\phi}{Na^3} \tag{12}$$

and in the Rouse model the longest relaxation time is just that for a polymer chain of radius R to diffuse its own radius, i.e.,

$$\tau_{\mathrm{PC}} = \frac{R^2}{D_{\mathrm{self}}} \tag{13}$$

where D_{self} is the self (not collective) diffusion constant of the polymer. Note that relaxation of the stress requires diffusion of one polymer molecules relative to the surrounding polymer molecules, so the required diffusion constant is the self-diffusion constant.

In semidilute polymer solutions, the polymers are approximately ideal at lengthscales over ξ, so the radius R is

$$R = \xi N_\xi^{1/2} = a\phi^{-1/8}N^{1/2} \tag{14}$$

where $N_\xi = N/g$ is the number of blobs per polymer chain. In the Rouse model the drag on each monomer is additive, so the self-diffusion coefficient is that for a blob divided by N_ξ

$$D_{\mathrm{self}} = \frac{kT}{6\pi\eta_s\xi N_\xi} = \frac{kT}{6\pi\eta_s N\phi^{1/2}} \tag{15}$$

Note that this decreases with increasing polymer concentration, unlike the cooperative diffusion constant that increases.

Finally, using eq (13), we have

$$\eta_{\mathrm{ROUSE}} = 6\pi\eta_s N^2\phi^{5/4} \tag{16}$$

the contribution of unentangled semidilute polymer to the viscosity increases as $c^{5/4}$.

Rayleigh and Sherwood numbers in unentangled semidilute polymer solutions

The concentration of a salt or PEG affects the Rayligh number in three ways, by changing: the mass density, the viscosity, and the collective diffusion constant. The Rayleigh number is

$$\text{Ra}_\text{S} = \frac{\Delta\rho L^3 g}{D_\text{coop}\eta} \sim \frac{c}{c^{3/4}c^{5/4}} \sim c^{-1} \quad \text{for} \quad c^* < c < c_e \tag{17}$$

where we used a linear scaling for $\Delta\rho$, eq (10) for D_coop, and eq (16) for the viscosity. In the semidilute, non-entangled regime, Ra decreases as one over the concentration. This is because the increasing mass density difference is more than outweighed by the increasing viscosity and collective diffusion constant. This in contrast to the dilute regime where only the mass density difference strongly depends on cocentration and so convection increases with increasing concentration.

The Schmidt number is

$$\text{Sc} = \frac{\nu}{D_\text{coop}} = \frac{\eta_{Rouse}}{D_\text{coop}\rho} \sim \frac{c^{5/4}}{c^{3/4}} \sim c^{1/2} \quad \text{for} \quad c^* < c < c_e \tag{18}$$

Then substituting eqs (17) and (18) into the simple empirical formula of Globe and Dropkin[26,27] (eq (8) of the main text), we have that the Sherwood number is

$$\text{Sh} \sim \left(c^{-1}\right)^{1/3}\left(c^{1/2}\right)^{0.074} \sim c^{-0.30} \quad \text{for} \quad c^* < c < c_e \tag{19}$$

And finally, we have the prediction that the timescale for bulk mixing scales with PEG concentration as

$$\tau_\text{MIXDC} \sim 1/(D_\text{coop}\text{Sh}) \sim c^{-0.45} \quad \text{for} \quad c^* < c < c_e. \tag{20}$$

In the unentangled semidilute regime, the bulk mixing time decreases slowly with increasing concentration. As $c_e \sim 10c^*$,[23,24] this holds over a range of concentrations of about an order of magnitude.

If we compare salt solutions and semidilute (but unentangled) polymer solutions, we

see that in both cases the mixing times in the bulk are predicted to slowly decrease with increasing concentration. However, the mechanisms for this decrease are very different in salt and polymer solutions. For salt solutions, increasing concentrations strengthens convection (only) while for polymer solutions the opposite occurs, convection weakens but despite this, mixing becomes faster as cooperative diffusion becomes much faster.

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