Nucleation of the crystalline phase of proteins in the presence of semidilute non-adsorbing polymer

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Abstract

Starting from a protein solution which is metastable with respect to the crystalline phase, the effect of adding semidilute non-adsorbing polymer is considered. It is found to increase the chemical potential of the protein by a few tenths of kT, which may be enough to lower the barrier to nucleation of the crystalline phase by enough to allow crystallisation. It is also shown that assuming that the polymer induces a pairwise additive attraction leads to qualitatively incorrect results.

1 Introduction

Crystallising proteins from dilute solution is in general rather difficult, yet the crystalline form is required in order to perform X-ray crystallography.\(^1\,^2\) X-ray crystallography is currently the best method of determining the all-important structure of a protein in its native state. This has lead to considerable interest in how proteins crystallise; see Ref. 3 for a recent review from a colloidal physics perspective and Refs. 4–8 for recent work. In a dilute solution there may be a large free energy barrier to nucleation of the crystalline phase,\(^1\,^3\,^9\) which prevents nucleation on accessible time scales. Here, we consider a generic model protein which in the absence of polymer already has coexistence between a dilute solution and a crystalline phase.\(^1\,^3\,^9\) We determine how the barrier to homogeneous nucleation of the crystalline phase changes when non-adsorbing polymer coils which are larger than the protein molecules are added to the protein solution. We find that the polymer lowers the nucleation barrier. This is to be expected as it is well-known that non-adsorbing polymer induces an effective attraction between the protein molecules, and an attraction will favour the dense crystalline phase. However, what is much less common, is that for large polymer coils it is essential to account for the fact that the polymer-induced attraction is strongly non-pairwise additive. A naive calculation using in the crystalline phase, the effective pair potential in dilute phase of protein molecules, greatly overestimates the effect of the polymer.

Protein molecules are typically a few nms across and the root-mean-square end-to-end distance of a flexible polymer can easily be several times this. The protein–protein interaction consists of a repulsive core of diameter \(D\) and an attraction which is sufficiently strong to cause the equilibrium behaviour to be coexistence between a dense crystal and a dilute fluid phase. We do not need to specify the potential explicitly as we will require only the fluid-crystal interfacial tension \(\gamma_0\) and the difference in chemical potential between that of the crystal and that of the fluid, \(\Delta\mu_0\). We use the subscript 0 to indicate quantities before polymer is added. Our results should therefore apply to a very wide range of proteins despite potentially very large variations in the structure and interactions of different proteins.\(^2\)

The polymer is non-adsorbing: the interaction between the protein molecule and a monomer is purely repulsive. There has been extensive work done on systems of small colloidal particles immersed in polymer solutions of large polymer coils which do not adsorb on the particles.\(^10\) Scaling theory for these systems was pioneered by de Gennes,\(^11\) see also Refs. 12–15. Field-theoretic methods have yielded results which are exact for small hard particles and ideal polymers,\(^16\) as well as approximate results for polymers in good solvents.\(^17,18\) Work has also been done using integral equations, see Ref. 19 and references therein. It is well understood that non-adsorbing polymer increases the chemical potential of particles and induces an attraction between these particles which is not pairwise additive: the interaction of three close-by protein molecules is not the sum of three pair potentials.\(^17,20–22\) However, most of this work (exceptions are Refs. 20, 21) only considered a few particles, two or three, whereas a nucleus of a crystalline phase contains 10s of tightly-packed particles. Because of this, the deviations from pairwise additivity observed here are much stronger than found in previous work.

The next section briefly outlines classical nucleation theory for the solution before polymer is added. Section 3 then describes the effect of polymer and section 4 is a conclusion.
2 Classical nucleation theory

The interaction between protein molecules is taken to include a steeply repulsive core and an attraction. This attraction may be isotropic or highly anisotropic or a mixture of the two, if isotropic it may or may not be short-ranged; all we require is that it render a dilute solution of the protein metastable with respect to a crystalline phase of the protein. By metastable we mean that the fluid phase is not the equilibrium phase, it does not correspond to the absolute minimum in the appropriate free energy, but the fluid phase is dynamically stable with respect to crystallisation for times much larger than the characteristic time of the solution. See Ref. 23 for a general introduction to the properties and behaviour of metastable fluids. Classical nucleation theory23–25 assumes that the nucleus of the crystalline phase has a free energy $\Delta F$ which is the sum of a bulk term and surface term. See the book of Debenedetti23 for an excellent introduction to classical nucleation theory. The protein molecules are modeled by spherical particles of diameter $D$. The bulk term is equal to the number of molecules in the nucleus, $n$, times the chemical potential difference $\Delta \mu = \mu_x - \mu$, where $\mu_x$ is the chemical potential of the crystalline phase and $\mu$ is the chemical potential of the fluid phase which contains the nucleus. The fluid phase we are considering is not the true equilibrium phase, nucleation occurs from a fluid which is at a higher chemical potential than the crystal. Thus $\Delta \mu$ is negative. The surface term is the surface area of the nucleus times the surface tension $\gamma$ of the bulk interface between the coexisting crystalline and fluid phases. The surface area of the nucleus is obtained by assuming that the nucleus is a sphere of radius $R$ which is related to $n$ by assuming that the density of spheres within the nucleus is equal to the bulk density of the crystalline phase, taken to be $D^{-3}$; the density of a close-packed simple cubic lattice. Then $n = (4/3)\pi R^3 D^{-3}$. Putting this all together,

$$\Delta F = \frac{4}{3} \pi R^3 D^{-3} \Delta \mu + 4\pi R^2 \gamma$$

(1)

The first term in $\Delta F$ is the bulk term, which is negative and decreases linearly with $n$, and the second term is the surface term which is positive and increases as $n^{2/3}$. Thus $\Delta F$ passes through a maximum, denoted by $\Delta F^*$, at some value of $R$, denoted by $R^*$. This maximum is the top of the free energy barrier to nucleation. The nucleus with radius $R^*$ and $n^*$ spheres is called the critical nucleus. Taking the derivative of Eq. (1) with respect to $R$ and equating to zero,

$$R^* = \frac{2\gamma D^3}{|\Delta \mu|},$$

(2)

and inserting this value of $n$ in Eq. (1)

$$\Delta F^* = \frac{16\pi \gamma^3 D^6}{3|\Delta \mu|^2}.$$  

(3)

Nucleation is an activated process with $\Delta F^*$ as the barrier height and so the nucleation rate varies as $\exp(-\Delta F^*/kT)$, where $k$ and $T$ are Boltzmann’s constant and the temperature $T$, respectively. To increase the rate of nucleation of the crystalline phase of the protein we need to reduce the barrier $\Delta F^*$. In the absence of polymer $\Delta F^*$ and $R^*$ are obtained from Eqs. (2) and (3) by setting $\Delta \mu = \Delta \mu_0$ and $\gamma = \gamma_0$.

3 Nucleation in the presence of semidilute polymer

Now we consider a solution of both protein molecules and flexible polymer molecules. The interaction between a protein molecule and a monomer is taken to be strictly repulsive. Figure 1 is a schematic of the nucleus in a solution of polymer. We remark that this simple model is not adequate for all polymers, for example it is not adequate for PEG (poly(ethylene glycol)). We consider the case where the polymer molecules are larger than the protein molecules and the polymer solution is semidilute. As the protein molecules are only a few nms in diameter this regime is easily accessible in experiment. In the other limit, where the polymer’s radius is smaller than that of the colloidal particles, the polymer induces a short-ranged depletion attraction between the particles. Such attractions are to a good approximation pairwise additive10 and therefore straightforward to treat. See Refs. 22,26–29 for work where the polymer is at most as large as the colloid.

See the book of de Gennes30 for an introduction to semidilute polymer solutions. They are characterised by a correlation length $\xi$ which scales as $c_M^{-3/4}$, where $c_M$ is the monomer concentration. The osmotic pressure exerted by the polymer $\Pi \sim kT \xi^{-3}$.

The crystalline phase of the protein is dense, the gaps between protein molecules available to polymer are around $0.4D$ or less across. Forcing a polymer molecule into a tube of diameter $0.4D$ costs roughly $kT$ in free energy per $0.4D$ of the tube’s length as the polymer collides with the walls of the tube at intervals of roughly its diameter. Thus, the free energy density inside such a crystal, either a bulk crystal or a crystalline nucleus, will be of order $kT$ per $0.06D^3$ volume whereas in solution it is $kT$ per $\xi^3$ volume. For $\xi$ larger than $D$, which we assume throughout, the free energy density inside a crystal is so much higher than outside that to a good approximation there is no polymer inside the crystal. This agrees with calculations using the theory of Ref. 20. So, as the crystalline nucleus is impermeable to polymer it interacts with the polymer, as a hard particle of radius $R$.

The interaction $w$ of a hard spherical particle of diameter $D$ with a semidilute solution of polymer in a good solvent with a correlation length $\xi$ is11–14

$$w \sim kT \left( \frac{D}{\xi} \right)^{4/3} \quad D \ll \xi,$$

(4)

where we took the exponent $\nu$ to have its Flory value of $3/5$.30 $w$ is the work done ($=$ the difference in excess chemical potential) in taking a particle from a pure solvent and
inserting it into the polymer solution. This assumes that
the interaction between a protein and the monomers of a
polymer is purely repulsive and that there is not much more
than one protein molecule per ξ volume. The notation ∼
indicates that we have neglected a coefficient of order unity;
here we derive only the scaling behaviour of the interactions
with respect to the relevant length scales. Equation (4) can
be derived in a couple of ways, see Refs. 11–14. One way is
to note that when D ≪ ξ the interaction between a particle
and polymer must scale as the density of monomers, which
scales as ξ−4/3 when ν = 3/5. As w/kT is dimensionless,
we require that ξ−4/3 appears as the ratio (D/ξ)4/3 which
gives Eq. (4).

Straightaway we can derive an approximation for the
contribution of polymer to the difference in excess chemi-
cal potential ∆µ between the dense crystalline phase of the
protein, and the semidilute polymer solution. It is

\[ \Delta \mu \sim \Delta \mu_0 - kT \left( \frac{D}{\xi} \right)^{4/3} + \Pi D^3, \]  

where the second term on the right hand side is the change in
free energy of the polymer when a single protein molecule
is removed from the semidilute polymer solution and the
last term is the change in free energy of this solution when
it is compressed by the crystalline phase of the protein ex-
expanding by the volume of one protein. The osmotic pres-
sure of a polymer solution Π is related to ξ by Π ∼ kTξ−3.
Thus, the last term is equal to kT(D/ξ)3 and so is smaller
than the second term, indeed it is of order terms we have
dropped and so we drop it and obtain our final expression
for ∆µ in the presence of polymer,

\[ \Delta \mu \sim \Delta \mu_0 - kT \left( \frac{D}{\xi} \right)^{4/3}. \]  

For a large nucleus, radius R ≫ ξ, the width of the
nucleus–polymer-solution interface, ξ, is small and so the capillarity
approximation which underlies classical nucleation
theory still holds: ∆F is still a sum of bulk and inter-
facial terms. The bulk term is given by the ∆µ of Eq.
(6) times the number of protein molecules in the nucleus.
In the presence of semidilute polymer the the interfacial
tension of a flat interface, γ is, 31

\[ \gamma \sim \gamma_0 + \frac{kT}{\xi^2}, \]  

where γ0 is the interfacial tension in the absence of poly-
mer. Bearing in mind that γ0 will be at least of order
kT/D2 we see that the fractional modification to γ0 due to
copolymer is of order (D/ξ)2. For a ∆µ0 of order kT, the
fractional change to ∆µ due to polymer is, see Eq. (6), of
order (D/ξ)4/3. Within our crude scaling theory we only
consider leading order contributions and so neglect the con-
tribution of the polymer to γ, leaving the only contribution
of polymer as the change in ∆µ given by Eq. (6).

In the opposite limit for R, R ≪ ξ, then the contribu-
tion of polymer to the free energy of the nucleus is no
longer the sum of bulk and interfacial terms, i.e., it is not
the sum of terms scaling as R3 and as R2. For R ≪ ξ the
contribution of the polymer to the free energy of formation
of the nucleus ∆F∗ is the sum of two terms: the first is the
number of protein molecules in the nucleus times minus
the increase in chemical potential in solution due to polymer,
Eq. (4), and the second is the free energy cost of inserting
the nucleus into the polymer solution. This second term is
the free energy cost due to the nucleus excluding polymer
from a sphere of radius R. Because the polymer cannot
penetrate into the nucleus it interacts with the polymer as
a single particle, and so this free energy cost is just Eq. (4)
with D replaced by 2R. Adding the two terms together we
have the contribution of polymer to ∆F,

\[ \Delta F = \Delta F_0 - nkT \left( \frac{D}{\xi} \right)^{4/3} + kT \left( \frac{2R}{\xi} \right)^{4/3}, \]  

\[ R < \xi, \]  

where ∆F0 is the free energy of formation of the nucleus be-
fore polymer is added. For R a few times D and D ≪ ξ
the second term in Eq. (8) dominates the last term. So,
we neglect the last term in Eq. (8). Then as the remaining
term is linear in n it is in effect a shift in ∆µ and indeed is
the same shift in ∆µ we found in the opposite limit, R ≫ ξ.
Thus we conclude that as for R ≫ ξ the leading order con-
tribution of semidilute polymer to the nucleation barrier is
simply to shift ∆µ by the amount given in Eq. (6).

So, we have shown that in both limits, R ≫ ξ and
R ≪ ξ, the effect of adding polymer in the semidilute
regime is to shift the chemical potential difference ∆µ by
an amount of order ∆F = ∆F0 − nkT(D/ξ)4/3kT, which will be a few tenths
of kT. Having shown that it holds in both limits we assume
that it also holds for R/ξ = O(1) as well. The shift varies
as ξ−4/3 and so increases linearly with the density of poly-
mer. The size of the critical nucleus also decreases, Eq. (2).
Thus, if decreasing ∆µ by a few tenths of kT is enough to
reduce the nucleation barrier sufficiently to make the nu-
cleation rate significant then adding semidilute polymer is
a viable way of inducing nucleation of the crystalline phase
of small colloidal particles such as proteins and micelles.

3.1 Comparison with assumption of pair-
wise additivity

A pair of particles in a semidilute polymer solution sep-
parated by a distance x of order ξ or less feel a polymer-
induced attraction towards each other, which we denote by
w2. 14, 17 For small particles, D ≪ ξ, and for separations
r much smaller than the correlation length 15, 17

\[ w_2 \sim -kT \left( \frac{D}{\xi} \right)^{4/3} \left( \frac{D}{r} \right)^{4/3}, \]  

\[ r \ll \xi. \]  

Naively it might be thought that the contribution of poly-
mer to the free energy change ∆µ could be estimated using
this pair attraction plus a mean-field approximation, which
would correspond to the approximation

\[ \Delta \mu \sim \Delta \mu_0 + D^{-3} \int_{r > D} d\mu w_2(r) \]  

wrong, (10)
which is just the integral over the number density, \( D^{-3} \) in the crystalline phase, times the potential. As usual with a mean-field approximation, correlations in the positions of particles are neglected and one protein is considered to interact with surrounding particles which are at the mean density. The above integral is \( D^{-3} \) times the contribution of polymer to the second virial coefficient. See Eisenriegler\(^{18}\) for a more accurate treatment of the contribution of polymer to the second virial coefficient. So, inserting Eq. (9), into Eq. (10), we obtain

\[
\Delta \mu - \Delta \mu_0 \sim -kT \left( \frac{\xi}{D} \right)^{1/3} \quad \text{wrong. (11)}
\]

If we compare Eqs. (6) and (11) we see that the assumption of a pairwise additive potential plus the mean-field approximation predicts the wrong scaling with \((D/\xi)\) of the polymer contribution to \(\Delta \mu\). It greatly overestimates, by a factor of order \((\xi/D)^{5/3}\), the effect of adding polymer whose correlation length is large with respect to the protein diameter. Naively using the pair potential between an isolated pair of particles, \(\langle \text{a pair of particles with no others within a distance } \xi \text{ of them} \rangle\), as the pair interaction within a dense, number density \(\gg \xi^{-3}\) (here the crystal), is qualitatively wrong.

4 Conclusion

We have estimated the change in the barrier to nucleation when polymer is added to a metastable dilute protein solution. The polymer is semidilute, and has a correlation length \(\xi\) larger than the diameter of the protein \(D\): the polymer does not adsorb onto the surface of the protein. When polymer is added, the barrier \(\Delta F^*\) is reduced due to the increase in the chemical potential of the protein in solution, possibly by enough to allow nucleation. However, the effect is not large, \(\Delta \mu\) decreases by only a few tenths of \(kT\). Also, a naive use of the polymer induced attraction between a pair of proteins yields results which are qualitatively wrong: they grossly overestimate the effect of adding polymer. This does not mean that an effective potential approach to the effect of polymer cannot reliably estimate the effect of large polymer molecules, just that this effective potential is many-body in nature and cannot simply be approximated by just a pair potential. This conclusion also applies to the phase behaviour of a system of hard-sphere-like colloidal particles and larger polymer molecules.\(^{20}\)

Finally, we note that the second virial coefficient \(B_2\) is often used to measure the strength of the attractions in a protein solution. \(B_2\) is given by

\[
B_2 = B_{2,0} + \frac{1}{2} \int_{r > D} \frac{dw_2(r)}{kT} \quad w_2 \ll kT \quad \text{(12)}
\]

\[
\sim B_{2,0} - D^{8/3} \xi^{1/3}, \quad \text{(13)}
\]

where \(B_{2,0}\) is the second virial coefficient in the absence of polymer and we have assumed not only that \(w_2/kT\) is small but that \(B_{2,0}\) is dominated by interactions with \(\xi \ll \xi\): then the second virial coefficient is the sum of the two terms in Eq. (12). So as the polymer density increases, \(\xi\) decreases and the contribution of polymer to \(B_2\) decreases. But from Eq. (6) we see that as the polymer density increases it has a larger and larger effect on the nucleation barrier. So, the effect of polymer on \(B_2\) is anti-correlated with its effect on \(\Delta F^*\), i.e., when one goes up the other goes down, not correlated as we might naively have expected.

References

[1] N. Chayen and J. Helliwell, Physics World \textbf{11}, 43 (May) (1998).
[2] S. D. Durbin and G. Feher, Ann. Rev. Phys. Chem. \textbf{47}, 171 (1996).
[3] R. Piazza, Curr. Opinion Coll. Int. Sci. \textbf{5}, 38 (2000).
[4] V. Talanquer and D. W. Oxtoby, J. Chem. Phys. \textbf{109}, 223 (1998).
[5] C. Haas and J. Drenth, J. Phys. Chem. B \textbf{104}, 368 (2000).
[6] N. M. Dixit and C. F. Zukoski, J. Coll. Int. Sci. \textbf{228}, 359 (2000).
[7] D. Pini, G. Jialin, A. Parola and L. Reatto, Chem. Phys. Lett. \textbf{327}, 209 (2000).
[8] R. P. Sear, J. Chem. Phys, \textbf{114}, 3170 (2001).
[9] R. P. Sear, cond-mat/9912199.
[10] As emphasised by Evans and coworkers, see for example Refs. 26,27, the interaction is only strictly pairwise additive if the polymer is both ideal and does not interact with a particle more than \(\geq 0.15\) times its radius away. However, if the polymer is larger than this but still small with respect to the protein molecule, the potential will be close to pairwise additive. Then the simultaneous interaction of a polymer molecule with more than two protein molecules (which is the origin of deviations from pairwise additivity) will be weak. In the other limit, which we consider here, where the polymer is much larger than the protein, the polymer molecule can potentially interact with \(\text{many}\) protein molecules, inducing an effective interaction which is very far from being pairwise additive.
[11] P. G. de Gennes, C. R. Acad. Sci. Paris B \textbf{288}, 359 (1979).
[12] T. Odijk, Macromolecules \textbf{29}, 1842 (1996).
[13] T. Odijk, Physica A \textbf{278}, 347 (2000).
[14] R. P. Sear, Phys. Rev. E \textbf{56}, 4463 (1997).
[15] R. P. Sear, Eur. Phys. J. B \textbf{1}, 313 (1998).
Figure 1: A schematic of a crystalline nucleus in a polymer solution. The black discs represent the protein molecules of the nucleus, and the curves are polymer molecules.

[16] E. Eisenriegler, A. Hanke and S. Dietrich, Phys. Rev. E 54, 1134 (1996).
[17] A. Hanke, E. Eisenriegler and S. Dietrich, Phys. Rev. E 59, 6853 (1999).
[18] E. Eisenriegler, J. Chem. Phys. 113, 5091 (2000).
[19] M. Fuchs and K. S. Schweizer, Europhys. Lett. 51, 621 (2000).
[20] R. P. Sear, cond-mat/0012362.
[21] T. Odijk, J. Chem. Phys. 106, 3402 (1997).
[22] E. J. Meijer and D. Frenkel, J. Chem. Phys. 100, 6873 (1994).
[23] P. G. Debenedetti, *Metastable Liquids* (Princeton University Press, Princeton, 1996).
[24] J. D. Gunton, M. San Miguel and P. S. Sahni, in *Phase Transitions* volume 8, edited by C. Domb and J. L. Lebowitz (Academic Press, London, 1983).
[25] P. M. Chaikin and T. C. Lubensky, *Principles of Condensed Matter Physics* (Cambridge University Press, Cambridge, 1995).
[26] M. Dijkstra, J. M. Brader and R. Evans, J. Phys. Cond. Mat. 11, 10079 (1999).
[27] M. Dijkstra, R. van Roij and R. Evans, J. Chem. Phys. 113, 4799 (2000).
[28] A. A. Louis, P. G. Bolhuis, J. P. Hansen, E. J. Meijer, Phys. Rev. Lett. 85, 2522 (2000).
[29] A. A. Louis, cond-mat/0102220.
[30] P. G. de Gennes, *Scaling Concepts in Polymer Physics* (Cornell University Press, Ithaca, 1979).
[31] J. F. Joanny, L. Leibler and P.-G. de Gennes, J. Polymer Sci. Polymer Phys. Ed. 17, 1073 (1979).