
Supporting Text

Characterization of the Ferritin and Apoferritin by Dynamic Light Scattering. Fig. 5 displays the size distributions of the scatterers in solutions of the two proteins resulting from a dynamic light-scattering determination as discussed in detail in ref. 1. We see that the size distributions are narrow, and the two proteins have identical diameters of 13 nm, which is equal to their identical crystallographic diameters (2). Because these sizes are calculated from the set of experimentally determined diffusivities using the Einstein–Stokes law of Brownian motion, the equality to the crystallographic data indicates that the diffusion of both molecules obeys this law (3).

Independence of Solubility on Molecular Mass: Statistical Thermodynamics Arguments. To rationalize the apparently equal solubility of ferritin and apoferritin, we consider the equilibrium between the solution and a crystal, which is equivalent to equilibrium between the states of a molecule in a kink on the crystal surface and in the solution (4-6). At constant temperature and pressure, the activity of a molecule in the crystal does not depend on the concentration of the solute and is equal to the activity of the standard crystal state (7, 8). Then, the equilibrium constant $K_{\text{cryst}}$ can be written as

$$K_{\text{cryst}} = (\gamma C_e)^{-1} = C_e^{-1}, \quad [1]$$

where $\gamma$ is the protein activity coefficient at a protein concentration equal to the solubility, and $C_e$ is the solubility. The activity coefficient depends on the protein concentration and the intermolecular interactions. Hence, we expect equal $\gamma$ values in solutions of ferritin and apoferritin of equal concentration. Determinations of $\gamma$ for apoferritin solutions of concentrations up to 20-fold higher than the solubility have yielded $\gamma \equiv 1$ (9). We expect the same to be true for ferritin, and this is the basis of the second equality above for these two proteins.

From the point of view of statistical thermodynamics, the equilibrium constant for crystallization $K_{\text{cryst}}$ can be written as (10)

$$K_{\text{cryst}} = q_0 \exp(\mu_0 / k_B T), \quad [2]$$

where $q_0$ is the partition function of a molecule in a kink (which is only a function of temperature and pressure), and $\mu_0$ is the standard chemical potential of a molecule in the solution.

To evaluate $q_0$ and $\mu_0$, we assume that the internal molecular vibrations in the solution are the same as in the crystal and are decoupled from the other degrees of freedom. This
assumption allows us to neglect the internal vibrational partition function for both states. Furthermore, we limit ourselves to only translational contributions to the solute partition function, neglecting the rotational contributions, and those stemming from the intermolecular interactions. This limits the validity of the considerations below to molecules similar to the ferritin/apoferritin pair, with symmetry close to spherical and that only exhibits very weak intermolecular interactions and activity coefficient close to 1.

We do not take into account the contribution of the release or binding of the solvent molecules to the free-energy changes of the phase transition. Although previous work has indicated that these contributions may be significant (9), we expect the contributions of the solvent effects to be identical for ferritin and apoferritin. This justifies neglecting them while aiming at comparisons between the two proteins. We also neglect the rotational vibrations in the crystal.

With these assumptions, we can use the expressions for the partition functions from ref. 10 and write

\[ q_0 = q_x q_y q_z \equiv (q_{\text{vib}})^3 \]  

[3]

where \( q_i \ (i = x, y, z) \) is the partition functions for translational vibrations along the respective coordinate. In turn, with \( h \) being the Planck constant, \( \nu \) the vibration frequency, and \( U \) the mean-force potential of a molecule in a kink,

\[ q_{\text{vib}} = \frac{\exp\left(-\frac{h \nu}{k_B T}\right)}{1 - \exp\left(-\frac{h \nu}{k_B T}\right)} = k_B T, \quad \nu = \frac{1}{2\pi} \sqrt{\frac{f}{m}}, \]

where

\[ f = \left( \frac{\partial^2 U}{\partial l^2} \right)_{\text{min}}. \]  

[4]

Combining, we get for \( q_{\text{vib}} \) and \( q_0 \)

\[ q_{\text{vib}} = \frac{2\pi k_B T}{h} \sqrt{\frac{m}{f}}, \]
For $\mu_0$, we have (10)

$$\frac{\mu_0}{k_BT} = \ln \left( \frac{2\pi mk_BT}{h^2} \right)^{\frac{3}{2}}$$

and

$$\exp \left( \frac{\mu_0}{k_BT} \right) = \frac{1}{k_BT} \left( \frac{h^2}{2\pi mk_BT} \right)^{\frac{3}{2}}$$

Comparing the expressions for $q_0$ and $\exp(\mu_0/k_BT)$, we see that the former contains $m^{3/2}$, and the latter has $m^{-3/2}$, i.e., their product $K_{cryst}$ and $C_e$ do not depend on the mass of the molecule.

It is important to reemphasize two issues. (i) The thermodynamic considerations above are simplified, and they only aim to address the issue of the equal solubility of two species that are identical in every respect but their mass. The equations above cannot be used to calculate values of thermodynamic functions. (ii) We did not account for the rotational degrees of freedom of the solute, which limits even this simplified model to molecules with close to spherical symmetry.

**The Flux of Molecules into a Kink.** To calculate the flux $J$ of molecules with concentration $n$ that, driven by a concentration gradient, overcome a barrier to reach the surface, we orient the coordinate $x$ perpendicular to a growing surface and denote the potential relief close to this surface as $U(x)$. From the generalized Fick law, $J = (nD/k_BT)\frac{d\mu}{dx}$, with $\mu(T,x) = \mu_0(T) + k_BT\ln[\gamma n(x)] + U(x)$ and $\gamma = 1$ (9), $J$ is linked to $U(x)$, $n(x)$ and the gradient of $n$ as (11, 12)

$$J = D \left[ \frac{dn(x)}{dx} + n(x) \frac{d(U(x)/k_BT)}{dx} \right], \quad x > 0$$

with $D$ being the Stokes diffusion coefficient of the molecules. In search of a steady $J = const$, we integrate the above equation with two sets of boundary conditions: (i) that at a certain distance from the surface $\delta$, $x \geq \delta$, $U = 0$, and $n = n_\delta$; and (ii) that in the crystal, i.e., at $x \leq 0$, $n = 0$. 

$$q_0 = \left( \frac{2\pi k_BT}{h} \right)^3 \left( \frac{m}{f} \right)^{\frac{3}{2}}.$$
Dividing by \( D \) and multiplying both sides by \( \exp[U(x)/k_BT] \), we get

\[
\frac{J}{D} \exp[U(x)/k_BT] = \frac{d}{dx} \left\{ \exp[U(x)/k_BT] \right\}.
\]  

Integrating from \( x = 0 \) to \( x = \delta \), using the boundary conditions at \( x = 0 \) and \( x \geq \delta \), we get

\[
J = \frac{n_\delta D}{\int_0^\delta \exp[U(x)/k_BT] dx},
\]  

an analogue to equation 9.51 in ref. 13 and the Fuchs expression for coagulation of particles interacting through \( U(x) \).

If \( U(x) \) has a sharp maximum at \( x = 0 \), we can represent it with a symmetric function around the point of the maximum. As shown below, in many cases \( |d^2U/dx^2| < a \), and this justifies the assumption of a sharp maximum. We use only the first two members of its Taylor series: \( U(x) = U_{\text{max}} - \frac{1}{2} |d^2U/dx^2| x^2 \). The minus sign stems from \( d^2U/dx^2 < 0 \) at the maximum. Then, the integral

\[
\delta \int_0^\delta \exp[U(x)/k_BT] dx = \exp \left( U_{\text{max}} \right) \exp \left( -\frac{1}{2} \left| \frac{d^2U}{dx^2} \right|_{x=0} x^2 \right) dx
\]  

\[
\approx \exp \left( U_{\text{max}} \right) \left( -\frac{1}{2} \left| \frac{d^2U}{dx^2} \right|_{x=0} x^2 \right) \]  

\[
= \exp \left( U_{\text{max}} \right) \left( -\frac{1}{2} \left| \frac{d^2U}{dx^2} \right|_{x=0} x^2 \right)^{-1/2}
\]  

The approximate equality above is based on \( \delta \gg \left[ \frac{1}{2} |d^2U/dx^2| \right]^{-1/2} \), the half-width of the Gaussian function in Eq. 10. Finally,

\[
J = D \sqrt{\frac{2}{\pi k_BT}} \left| \frac{\partial^2 U}{\partial x^2} \right|_{\text{max}}^{1/2} \exp \left( -\frac{U_{\text{max}}}{k_BT} \right) n_\delta.
\]  

Note that only half of the flux \( J \) from this equation contributes to growth: On top of the barrier, the force driving the molecules into the crystal is zero, and a molecule has equal
chances of getting incorporated or going back to the solution (14). With this and introducing the parameter Λ as the radius of curvature of \(U(x)/k_BT\) at its maximum,

\[
\Lambda = \left( \frac{1}{2\pi} \left| \frac{\partial^2 (U/k_BT)}{\partial x^2} \right|_{\max} \right)^{-1/2},
\]

the expression for \(J\) becomes

\[
J = \frac{D \exp \left( -\frac{U_{\text{max}}}{k_BT} \right)}{\Lambda} n_\delta.
\]

This last equation is essentially identical to the nucleation-rate expression derived by Zeldovich (12) as a diffusion flux over a potential barrier in the space of cluster sizes.

If \(U_{\text{max}}\) is due to the hydration of the incoming molecule and the site where it attaches, the radius of curvature of \(U(x)\) around \(U_{\text{max}}\) should be the size of a few water molecules, 2–4 Å, and the length \(\Lambda\) should be \(\approx 5–10\) Å. Note that in this evaluation, we apply discreet considerations to a continuous model. Still, we expect the estimate of \(\Lambda\) to be roughly correct.

If all molecules that overcome the barrier are incorporated into a kink, the incoming flux into a kink is \(j_+ = J \Delta S_{\text{kink}} \approx J a^2\), where \(a^2\) is an effective surface area of a kink. If there are no solute transport constraints (kinetic growth regime), \(n_\delta\) is equal to that in the solution bulk \(n\). Furthermore, in equilibrium, when \(n\) equals the solubility, \(n_e\), \(j_+ = j_-\). Because \(j_-\) does not depend on \(n\) in the solution, the step velocity \(v\) is

\[
v = \frac{a}{n_k} (j_+ - j_-) = \frac{a^3}{n_k} \frac{D}{\Lambda} \exp \left( -\frac{U_{\text{max}}}{k_BT} \right) (n - n_e).
\]


