

## Protein–Macromolecule Interactions

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Although the interactions between proteins and various types of macromolecules are of considerable technological interest,<sup>1</sup> little theoretical work has been done that makes use of the fact that proteins are often comparatively small particles. Thus, our interest is in a regime opposite to that focused on in the usual depletion theories.<sup>2</sup> Here, a protein will be viewed, perhaps naively, as a small hard sphere whose dielectric permittivity is negligible compared to that of water. Its interaction otherwise with some macromolecular segment will be inert. Some time ago, de Gennes already presented several, not so widely known, preliminary calculations of a spherical particle interacting with a semidilute solution of polymers.<sup>3</sup> One supposition he made concerning the irrelevance of a certain scale, will be proved here. My aim is to present a scaling analysis of the interaction between a small sphere and a macromolecule, particularly in dilute solution. Though obviously of restricted validity, the expressions derived may prove helpful in qualitatively understanding phase separation phenomena occurring in nondilute suspensions.

We first consider a protein sphere of radius  $a$  immersed in an aqueous solution containing a semidilute polymer which is well soluble. Its Kuhn length is  $A_K$ , and the excluded volume between two segments is  $\beta = A_K^3$ . In a self-consistent field approximation, the polymer segment density  $\psi^2(\vec{r})$  at position  $\vec{r}$  is given by<sup>2</sup>

$$-\frac{1}{6}A_K^2\Delta\psi + \beta\psi^3 = \epsilon\psi \quad (1)$$

where the origin is at the center of the sphere,  $\epsilon$  is an eigenvalue, and  $\psi$  must tend to zero at the protein surface ( $r = a$ ). Without solving eq 1, I wish to investigate the nature of the depletion layer surrounding the protein. Far from the sphere, the concentration  $\psi^2$  asymptotes toward a constant  $c_0$ , the bulk concentration of Kuhn segments, so we conveniently introduce  $\psi \equiv c_0^{1/2}f$  and eq 1 becomes

$$\Delta f - \frac{2}{\xi^2}f^3 + \frac{2}{\xi^2}f = 0 \quad (2)$$

Here, the correlation length  $\xi \equiv A_K(3\beta c_0)^{-1/2}$  is supposed to be larger than the radius  $a$ , a condition easily realizable in practice. Assuming spherical symmetry and setting  $r \equiv aR$ , one is faced with finding the solution  $f = f(R, a/\xi)$  to

$$\frac{1}{R^2} \frac{d}{dR} \left( R^2 \frac{df}{dR} \right) - \frac{2a^2}{\xi^2} f^3 + \frac{2a^2}{\xi^2} f = 0 \quad (3)$$

with boundary conditions  $f = 0$  at  $R = 1$  and  $f = 1$  at  $R = \infty$ . For an infinitesimally small sphere, we have simply

$$f(R, 0) = 1 - R^{-1} \quad (4)$$

which implies the depletion layer around the sphere is

approximately of size  $a$ . Moreover, the second and third terms in eq 3 are straightforward *regular* perturbations for  $a \ll \xi$ . Hence, eq 4 remains valid within a zero-order approximation, even when  $a > 0$  provided  $a \ll \xi$ . We conclude that the scale of the depletion layer for a small sphere is given solely by its radius  $a$  and does not involve  $\xi$  at all, at least to the leading order. The irrelevancy of  $\xi$  (assumed earlier<sup>3</sup>) is nontrivial for it is circumstantial. Note that an improved theory—a hybrid approach combining scaling and self-consistent arguments as in the theory of polymer adsorption<sup>4</sup>—would not alter this conclusion. One would require a correlation length  $\xi \sim c_0^{-3/4}$  from scaling theory instead of  $\xi \sim c_0^{-1/2}$  and hence replace  $\beta$  in eq 3 by  $\beta^4$ .

We now rederive a previous result<sup>3</sup> by way of illustration. We wish to compute the work  $w$  expended by inserting a protein sphere in a semidilute solution. Since  $a$  and  $\xi = A_K^{-5/4}c_0^{-3/4}$  are the only relevant scales, we have  $w/k_B T = h(a/\xi)$  with  $h$  a dimensionless function,  $k_B$  Boltzmann's constant, and  $T$  the temperature. As shown above, a volume of order  $a^3$  surrounding the protein, is depleted of  $a^3 c_0$  segments. Therefore, the number of protein–segment interactions involved in the work  $w$  must be proportional to  $c_0$  or in other words  $\xi^{-4/3}$ . Assuming  $h(x)$  is a simple power law, we then have<sup>3</sup>

$$\frac{w}{k_B T} \approx \left( \frac{a}{\xi} \right)^{4/3} \quad (\xi > a) \quad (5)$$

Next, the same argument may be used for a small sphere enclosed within a chain of radius<sup>5</sup>  $R \approx N_K^{3/5} A_K$  consisting of  $N_K$  Kuhn segments:  $a^3 c$  segments are depleted from the vicinity of the protein; the average segment concentration in the coil is  $c \approx N_K R^{-3}$ . Hence we obtain

$$\frac{w}{k_B T} \approx \left( \frac{a}{R} \right)^{4/3} \quad (R > a) \quad (6)$$

for  $a$  and  $R$  are the relevant scales in this case. The interaction  $w$  as such is not so interesting but rather the cross second virial coefficient

$$\bar{B} \equiv B_{2,pm} \equiv \frac{1}{2V} \int d\vec{r}_p \int d\vec{r}_m (1 - e^{-w(\vec{r}_p, \vec{r}_m)/k_B T}) \quad (7)$$

given in terms of the system volume  $V$  and the potential of mean force  $w(\vec{r}_p, \vec{r}_m)$  between the protein centered at  $\vec{r}_p$  and the macromolecule with center of mass fixed at  $\vec{r}_m$  and with all configurations integrated out. Since  $w(\vec{r}_p, \vec{r}_m) = \mathcal{A}(w)$  and  $w \ll k_B T$ , eq 7 reduces to

$$\bar{B} \approx \frac{1}{V} \int_{R^3} d\vec{r}_p \int_V d\vec{r}_m \frac{w}{k_B T} \approx R^{5/3} a^{4/3} \quad (R > a) \quad (8)$$

Accordingly, the cross coefficient is quite small and proportional to  $N_K$  which is plausible in retrospect: the interaction between a small, inert object and a long chain is expected to be extensive. Equation 8 should be compared to the analogous coefficient between a short and a long chain discussed by Witten and Prentis.<sup>6</sup> The same reasoning applied to a small sphere interacting with an ideal Gaussian coil of radius  $R_0 \approx N_K^{1/2} A_K$  would lead to

$$\frac{w}{k_B T} \approx \frac{a}{R_0} \quad (9)$$

$$\bar{B} \approx R_0^2 a \quad (R_0 > a) \quad (10)$$

Equation 10 agrees with that derived by Jansons and Phillips.<sup>7</sup>

Next, it is of some interest to compute the cross coefficient in case water is no longer a very good solvent for the polymer (i.e.  $\beta < A_K^3$ ). There is now a subtlety connected with the excluded-volume effect arising from the relatively small size of the protein. If the excluded-volume parameter  $Z_N = N_K^{1/2} \beta A_K^{-3}$  pertaining to the whole chain, is much greater than unity, we have the usual Flory law<sup>5</sup>  $R \approx N_K^{3/5} \beta^{1/5} A_K^{2/5}$ . Accordingly, we expect to regain eq 8 since  $\beta$  is irrelevant

$$\bar{B} \approx R^{5/3} a^{4/3} \approx N_K \beta^{1/3} A_K^{2/3} a^{4/3} \quad (a > A_K^4 \beta^{-1}) \quad (11)$$

Note, however, that there is now a new stringent condition on  $a$ .

Again, a depletion volume of order  $a^3$  excludes a chain section consisting of  $g$  segments with  $a \approx g^{3/5} \beta^{1/5} A_K^{2/5}$ . But now we insist that the pertinent excluded-volume parameter  $z_g = g^{1/2} \beta A_K^{-3}$  be greater than unity, if the excluded-volume effect is to be fully exerted within the section expelled from the neighborhood of the sphere. In the adjoining regime ( $z_g < 1$ ), the interaction between the segments of the displaced section is effectively ideal, so we actually attain the ideal chain case given by eq 10, even though the whole chain itself is expanded by the excluded-volume effect.

$$\bar{B} \approx N_K A_K^2 a \quad (L > a > P; a < A_K^4 \beta^{-1}) \quad (12)$$

This indeed crosses over to eq 11 when we set  $a = A_K^4 \beta^{-1}$ , as it must. It is inferred that the interaction between a protein and a flexible chain is peculiarly sensitive to the chain stiffness and the quality of the solvent.

Next, it is straightforward to apply the same argument when the macromolecule is a semiflexible chain of length  $L$ , persistence length  $P = 1/2 A_K$ , and diameter  $D$ , for we know<sup>8</sup> that  $\beta = P^2 D$ .

$$\bar{B} \approx L P^{1/3} D^{1/3} a^{4/3} \quad (L > P > D; a > P^2 D^{-1}) \quad (13)$$

$$\bar{B} \approx L P a \quad (L > a > P > D; a < P^2 D^{-1}) \quad (14)$$

Often, the chain may be so stiff that the inequality  $P > a > D$  is valid, implying that we view the small sphere as interacting with a thin curve that is effectively straight on the scale of  $a$ . Hence, the following simple relation is valid

$$\bar{B} \approx L a^2 \quad (L > P > a > D) \quad (15)$$

which crosses over to eq 12 at  $a = P$ , as it should. Finally, biofilaments exist for which  $P > D > a$  in which case we may write

$$\bar{B} \approx L D^2 \quad (L > P > D > a) \quad (16)$$

It is also a matter of quadrature to extend these expressions to the case of a protein interacting with a highly charged polyelectrolyte in excess salt. The correlation length  $\xi$  is given in terms of the total persistence length  $P_t$ , the Debye length  $\kappa^{-1}$ , and the

monomer concentration  $c_A$ , each monomer of length  $A$  bearing one elementary charge.<sup>9</sup>

$$\xi \approx (\kappa/P_t)^{1/4} (A c_A)^{-3/4} \quad (17)$$

Therefore, the ionic-strength dependence of the protein–polyelectrolyte interaction can be expressed by

$$\frac{W}{k_B T} \sim (P_t/\kappa)^{1/3} \sim n_s^{-1/2} \quad \text{at low salt} \quad (18)$$

where  $n_s$  is the 1:1 electrolyte concentration and  $P_t \sim \kappa^{-2} \sim n_s^{-1}$  at low salt.<sup>9</sup> In dilute solution, the polyion radius is<sup>9</sup>

$$R \approx L^{3/5} (P_t/\kappa)^{1/5} \quad (19)$$

so there are four regimes analogous to those for semiflexible chains discussed above

$$\bar{B} \approx L (P_t/\kappa)^{1/3} a^{4/3} \quad (L > P_t > \kappa^{-1}; a > P_t^2 \kappa) \quad (20)$$

$$\bar{B} \approx L P_t a \quad (L > a > P_t > \kappa^{-1}; a < P_t^2 \kappa) \quad (21)$$

$$\bar{B} \approx L a^2 \quad (L > P_t > a > \kappa^{-1}) \quad (22)$$

$$\bar{B} \approx L \kappa^{-2} \quad (L > P_t > \kappa^{-1} > a) \quad (23)$$

In the last expression, the exclusion radius is the Debye screening length  $\kappa^{-1}$  because the protein is repelled by the chain via the formation of image charges.<sup>10</sup>

**Note Added in Proof:** Recently, Wills et al.<sup>11</sup> performed gel chromatography and sedimentation experiments on poly(ethylene glycol) and a substantial number of globular proteins so as to determine the cross coefficient  $\bar{B}$  as a function of the protein radius  $a$ . The expression derived by Jansons and Phillips,<sup>7</sup> valid even for large radii, appears to agree with the experimental curve fairly well except for a deviation possibly attributable to eq 11. The peculiar ionic-strength dependence of  $\bar{B}$  described by eqs 20–23 stems from the OSF theory of the persistence length. Note that entropic fluctuations<sup>12</sup> will not perturb the scaling nature of these expressions.

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