# Depletion Theory of Protein Transport in Semi-Dilute Polymer Solutions

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ABSTRACT We consider the effect of polymer depletion on the transport (diffusion and electrophoresis) of small proteins through semi-dilute solutions of a flexible polymer. A self-consistent field theory may be set up in the important case of quasi-ideal interactions when the protein is small enough. Dynamic depletion, the reorganization of the depletion layer as the protein diffuses, is computed within a free-draining approximation. The transport of the dressed particle (protein + depletion layer) is tackled by extending Ogston's analysis of probe diffusion through fibrous networks to the case of a probe diffusing through a semi-dilute polymer inhomogeneous on the scale of the polymer correlation length. The resulting exponential retardation agrees almost quantitatively with that found in recent electrophoresis experiments of small proteins in polymer solutions that have been ascertained to be semi-dilute (S. P. Radko and A. Chrambach, *Electrophoresis,* 17:1094–1102, 1996; *Biopolymers,* 4:183–189, 1997).

## INTRODUCTION

The transport of particles through concentrated polymer solutions and gels is still incompletely understood despite many investigations over several decades. In particular, major unsolved problems are the diffusion and electrophoresis of proteins in congested solutions. These are important both with regard to the characterization of the proteins themselves and the mechanism of their transport through biopolymer suspensions (synthetic and in tissues). Here, the effects of polymer depletion on these transport problems will be addressed, for they appear not to have been dealt with before. I concentrate especially on the practical case of small proteins migrating through a semi-dilute solution of flexible polymer chains. The protein radius may then be substantially smaller than the correlation length of the suspension leading to a considerable simplification of the depletion theory.

I first summarize the transport properties of various protein and other small probes determined experimentally. An attempt will be made to stick to the requirements: 1) $a \ll \xi$ i.e., the protein radius (or its equivalent when the protein is not exactly spherical in shape) is much smaller than the correlation length of the polymer solution (or gel for illustrative purposes). It is recalled that the static correlation length $\xi$ has several interpretations in polymer scaling theory (de Gennes 1979b). In the context of this paper, it is well to realize that $\xi$ determines the screening of the excluded-volume effect: the average interaction between two segments is effectively zero when their separation is greater than $\xi$. ($\xi \sim c_0^{-3/4}$ for a polymer of concentration $c_0$ in a good solvent). 2) The solutions are really semi-dilute, i.e., the molar mass of the flexible polymer must be high enough and the concentration well beyond that of the overlap concentration $c^*$, although the volume fraction must remain smaller than unity. 3) The probe particle ought to be mesoscopic in size, i.e., $a \gg A$, the protein should be larger than $A$, the length of a Kuhn segment of the polymer. 4) Ideally, the interaction between the protein and the polymer should be hard or purely entropic. It is, of course, difficult to judge how well these conditions have been met. Diffusion, sedimentation and electrophoresis coefficients have all been measured, but I will simply group these in terms of a retardation factor $R$. A local Stokes–Einstein relation may or may not hold. In some experiments, the concentration dependence of the respective retardation factors for sedimentation and diffusion have turned out to be identical (see Ogston et al., 1973, who investigated the proteins ovalbumin, serum albumin, and γ-globulin in sulphated proteoglycan). The general form of $R$ has often been found to be a stretched exponential in the polymer concentration $c_0$.

$$R = \exp(Ka^{\mu}c_0^{\nu}) . \quad (1)$$

Here, $K$ is a generalized retardation coefficient and $\mu$ and $\nu$ are exponents determined by fitting the data to the exponential forms imposed. ($R$ equals the respective retardation factors for sedimentation $S_0/S$, diffusion $D_0/D$ or electrophoresis $E_0/E$ where the index 0 signifies the transport coefficient of the protein in pure water).

I have collected the exponents $\mu$ and $\nu$ from a variety of experiments in Table 1. There is no pretense to completeness; the data are representative, although I have included especially those measurements where the authors are concerned with defining the semi-dilute regime. It is obvious that there is no clear consensus with regard to the values for $\mu$ and $\nu$. Unfortunately, the complete data concerning the range of polymer concentrations are not always presented; incorporating any data within the dilute regime will markedly affect the exponent $\nu$. The scatter in the data also implies the necessity for more theoretical work on the complicated phenomena involved in the hindered transport

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**TABLE 1 The exponents from Eq. 1 measured for various probe particles in semi-dilute polymer solutions and gels**

| Experimental Technique | Nanoparticle | Radius (nm) | Polymer | $\mu$ | $\nu$ |
|---|---|---|---|---|---|
| Capillary electrophoresis | Human serum albumin | 2.7 | Polyacrylamide solution | 1.0 | 1.0 |
| Radko and Chrambach (1996) | Polystyrene carboxylate | 7.0 | | | |
| | Polystyrene sulfate | 9.5 | | | |
| Electrophoresis | a-Lactalbumin | 1.4 | Polyethylene glycol solution | | 1.0 |
| Radko and Chrambach (1997) | Carbonicanhydrase | 1.85 | | | 1.1 |
| | Various proteins | >2.2 | | 0.69 | 0.69 |
| Diffusion by holographic interferometry | Bovine serum albumin (BSA) | 3.55 | Dextran solution | | 0.5–1.0 |
| Kosar and Phillips (1995)* | | | | | |
| Tracer diffusion | BSA | 3.55 | DNA solution | | 1.0 |
| Wattenberger et al (1992) | | | | | |
| Diffusion by light scattering | BSA | 3.55 | Polyethylene oxide (PEO) solution | | 0.6–0.76 |
| Phillies et al (1985) | | | | | |
| Sedimentation | BSA | 3.55 | PEO solution | | 0.70 |
| Langevin and Rondelez (1978) | BSA | 3.55 | PEO solution | | 0.70 |
| Diffusion and sedimentation | Ovalbumin | 2.8 | Sulphated proteoglycan solution | | 0.5 |
| Ogston et al (1973) | BSA | 3.55 | | | |
| | $\gamma$-Globulin | 5.6 | | | |
| Diffusion | $\gamma$-Crystallin | 2.35 | Hyaluronic acid solution | | 1.0 |
| Laurent et al (1963)† | | | | | |
| Sedimentation | BSA | 3.55 | Hyaluronic acid solution | | 1.0 |
| Laurent and Pietruszkiewics (1961)† | | | | | |
| Electrophoresis | Various proteins and dyes | 0.51–5.81 | Polyacrylamide gel | | 1.0 |
| Rodbard and Chrambach (1971a) | | | | | |
| Electrophoresis | BSA | 3.55 | Polyacrylamide gel | | 1.0 |
| Rodbard and Chrambach (1971b) | | | | | |
| Diffusion | Various small molecules | Average one nm | Polyacrylamide gel | 1.0 | 0.75 |
| Tokita et al (1996) | | | | | |

*My estimates for $\nu$ from their Fig. 7.

†My estimates for $\nu$ for their data in the semi-dilute regime.

of probe particles. Also included in Table 1 are several gel experiments for the sake of comparison. If the cross-linking density of the gel is relatively low, the restricted transport of proteins ought to be similar to that in a polymer solution.

A variety of theories has been put forward to explain Eq. 1. Ogston introduced the notion of relating the volume accessible to a probe within a fibrous network to the diffusion of the particle (Ogston, 1958; Ogston et al., 1973). (Note that this idea was also used independently in percolative transport theories of electrons in disordered media [see Balberg, 1987; Isichenko, 1992]). If the volume excluded to a probe by one fiber is $v$ the pertinent accessible probability is $1 - (v/V)$ where $V$ is the volume of the system. For n fibers interacting with the probe independently, the total accessible volume must be

$$V_a = V(1-(v/V))^n \rightarrow V\exp(-nv/V) \text{as} \quad n \rightarrow \infty .$$

Ogston's assumption in its simplest form (diffusion proportional to accessible volume) then implies $\nu = 1$ for the exponent in Eq. 1. This line of reasoning has been corroborated by computer simulations (Johansson and Löfroth, 1993) on the diffusion of spheres in networks of slender fibers. The Ogston ansatz has also been tested by others (Slater and Guo, 1995, 1996), though on porous media that are not necessarily always semi-dilute. For concentrated systems, the assumption of independent probabilities must clearly break down. Ogston et al. (1973) also tried to explain why the exponent $\nu$ in Eq. 1 might deviate from unity.

A second class of theories deals with the screening of the hydrodynamic flow induced by the diffusing probe. The surrounding fibrous or polymeric network forms an obstruction because the fluid sticks to its convoluted surface. Such argumentation leads to a form given by Eq. 1 in view of Brinkman screening (Brinkman, 1947; Cukier, 1984). The concentration dependence of the diffusion is then given in terms of the hydrodynamic screening length $\xi_H$

$$D \simeq D_0 e^{-a/\xi_H} . \tag{2}$$

It is often thought that $\xi_H$ should be identical to the correlation length $\xi$ for a flexible polymer in a good solvent (de Gennes, 1976, i.e., $\xi_H \sim c_0^{-3/4}$. Originally, the proposal for $\xi_H$ was $\xi_H \sim c_0^{-1/2}$ (the Freed–Edwards theory (see Freed, 1978). More recently, detailed hydrodynamic theories have been developed for a sphere diffusing through a network of fibers (Phillips et al., 1989, 1990; Clague and Phillips, 1996). The fibrous obstruction causes an exponential-like dependence of the diffusion on the fiber concentration.

The segment distribution surrounding a protein in a semi-dilute polymer is depleted. The density tends to zero at the surface of the probe (de Gennes, 1979b). There are thus two

types of effects missing from the theories quoted above. First is the rearrangement of the depletion layer as the probe diffuses through the polymeric network. Second, the segment density fluctuates strongly so the particle is hindered by an inhomogeneous medium. As we shall see, these difficulties become manageable theoretically when the probe is small compared with the polymer correlation length. It is first well to recall the equilibrium depletion theory in this precise limit. A small sphere immersed in a semi-dilute polymer has a depletion layer surrounding it of volume $\varphi$ ($a^3$) where $a$ is the radius of the sphere (de Gennes, 1979a; Odijk, 1996). Hence, the number of depleted segments should be proportional to $c_0a^3$ and so the work $w_d$ of inserting the sphere into the solution must also be proportional to $c_0$. Accordingly, we have (de Gennes, 1979a).

$$w_d \simeq \left(\frac{a}{\xi}\right)^{4/3} k_BT \tag{3}$$

valid for a polymer in a very good solvent (where $k_B$ is the Boltzmann's constant and $T$ is temperature). For polymers and proteins in aqueous solution, there is often an important intermediate regime that may be termed quasi-ideal (Odijk, 1996, 1997a, 2000). For water-soluble polymers, one usually has $\beta \ll A^3$. The solvent in that case is "fairly good" rather than "very good" or "excellent"; for small proteins, we often have the condition $a < A^4/\beta$ where $\beta$ is the excluded volume between two Kuhn segments, so the protein displaces an effectively almost ideal sequence of polymer segments (see Odijk, 1996, 2000). Self-consistent field arguments for depletion are then valid. Because the depletion volume is very small, only entropic effects need to be accounted for. The work of insertion is then (Odijk 1997a,b, 2000)

$$\begin{aligned} w_d &= \frac{1}{6}A^2k_BT\int d\vec{r}\left(\frac{\partial c^{1/2}(\vec{r})}{\partial \vec{r}}\right)^2 \\ &= \frac{2\pi}{3}A^2ac_0k_BT. \end{aligned} \tag{4}$$

This is the usual expression for the entropic contribution to the free energy (Lifshitz et al., 1978) where $A$ is the Kuhn segment length and $c(\vec{r})$ is the segment density at position $\vec{r}$. For depletion around a small sphere situated at the origin, we have $c(\vec{r}) = c_0\,[1 - (a/r)]^2$ (Odijk, 1996, 1997b). The fact that $w_d$ should be proportional to the polymer concentration has been recently verified in experiments concerning the phase separation of protein–polymer solutions (S. Wang, J. van Dijk, J. Smit, T. Odijk, manuscript in preparation).

Small proteins are several nanometers in diameter. At volume fractions of aqueous polymer $<\sim 0.1$, the correlation length $\xi$ is generally greater than $\sim 10$ nm, so the asymptotic limit $\alpha \ll \xi$ is perfectly realizable. The semi-dilute solution may be viewed as a strongly fluctuating background (de Gennes, 1979b) in which a protein is diffusing. The typical length scale of the polymer inhomogeneity is $\xi$, and the cooperative diffusion coefficient of the polymeric gel is $k_BT/6\pi\,\eta\,\xi$, where $\eta$ is the viscosity of water, (de Gennes, 1976, 1979b). Hence, in this, effectively nondraining, approximation, the characteristic time of decay of the polymeric background inhomogeneities is about $\tau_b \approx \eta\,\xi^3/k_BT$. In contrast, within the depletion layer surrounding the translating protein, the polymer segments must reorganize themselves on a much faster time scale. In effect, the number of segments associated with the depletion layer is of order $a^3c_o$: a very small number, because we require $\xi \gg a$. A section of depleted polymer contains $(a/A)^2$ segments (Odijk, 1996, 2000), so the time scale associated with such a section diffusing out of the depletion layer should be of order $\tau_s \approx \eta\,a^5/A^2k_BT$, which is considerably shorter than $\tau_b$. In summary, the diffusive transport of the protein may be split into two parts. One involves the very local friction exerted by the probe on the polymer, an effect that may be termed dynamic depletion. Second, this "dressed" particle (protein together with dynamic depletion layer) diffuses through the inhomogeneous polymer solution on much longer time scales. In the next section, I compute the local effect of dynamic depletion in a free-draining approximation. Few segments are involved in this process and most of the polymeric stress turns out to be restricted to a region close to the moving protein. The diffusion of the dressed probe will be dealt with by extending Ogston's argumentation to semi-dilute polymers.

## DYNAMIC DEPLETION

The velocity of a segment in the polymer surrounding the protein is given by a balance of forces exerted on the segment (Yamakawa, 1971)

$$\vec{v}_s = \vec{u} + m\vec{f}. \tag{5}$$

Here, $\vec{u}(\vec{r})$ is the velocity of the solvent, $m$ is the mobility of a segment, and $f = -\partial\,\mu/\partial\vec{r}$ is the force on the particular segment by the surrounding swarm of segments in terms of the chemical potential $\mu$. Because the Stokesian approximation to the hydrodynamics applies, the velocity of the solvent is a superposition of a background velocity $\vec{u}_0$, the original velocity of the fluid in the absence of the polymer, and the velocity $\vec{u}_{in}$, induced by the force $f$, exerted by the polymer on the fluid. The latter velocity would involve a screened Oseen tensor in a Freed–Edwards description (Freed, 1978) with a screening length $\xi_H$ as introduced above, but it is neglected in the free-draining approximation used here. In fact, the velocity $\vec{u}_o$ leading to convective diffusion may also be disregarded, a supposition proven below.

Next, we need the segment chemical potential. Assuming the nonequilibrium-free energy is now given by Eq. 4, we

compute the potential as a functional derivative in terms of the more convenient variable $\Psi(\Psi^2 \equiv c/c_0)$

$$\mu \equiv \frac{\delta w_d}{\delta c} = \frac{\gamma w}{2c_0 \Psi \delta \Psi} = -\frac{A^2 k_B T \Delta \Psi}{6\Psi}, \tag{6}$$

where $\Psi = \Psi(\vec{r}, t)$; $t$ = time; $\Delta$ = Laplacian. Accordingly, the continuity equation for the segment density leads to a nonlinear diffusion equation

$$\begin{aligned} \frac{\partial c}{\partial t} &= -\frac{\partial}{\partial \vec{r}} \cdot (\vec{v}_s c) \\ &= -\frac{1}{6} m A^2 c_0 k_B T \frac{\partial}{\partial \vec{r}} \left( \Psi^2 \frac{\partial (\Psi^{-1} \Delta \Psi)}{\partial \vec{r}} \right). \end{aligned} \tag{7}$$

The form of this expression is not new: de Gennes (1980) discussed a transport theory of dense polymer chains at very long times on the order of the reptation time using a free energy whose entropic part was similar to Eq. 4. Here, the energetic term is negligible for small probes (Odijk, 1996, 1997a). It is convenient to let the protein particle be fixed at the origin of our Cartesian coordinate system. At great distances from the probe, the polymer segments have a uniform velocity $\vec{w}$ in the $z$ direction and a uniform density $\Psi^2 = 1$. At the surface of the spherical probe ($r = a$), we have $\Psi = 0$, the segment density must tend to zero. Moreover, the segments cannot penetrate the protein, so the radial flux must also vanish at the surface.

$$J_r = c v_{s,r} = 0 \quad (r = a). \tag{8}$$

We have introduced spherical coordinates ($r$, $\theta$, $\varphi$) defined with respect to the $z$ axis.

At low velocities of the probe, it is possible to solve Eq. 7 perturbatively. We seek a stationary solution: $\partial c/\partial t = 0$. We introduce $\Psi = \Psi_0 + \epsilon$ into Eq. 7, letting $\epsilon(\vec{r})$ be a relatively small variable. The zeroth-order distribution, $\Psi_0$, is the solution to a Laplace equation (Odijk, 1997b, 2000)

$$\Delta \Psi_0 = 0 \quad \Psi_0 = 1 - \frac{a}{r}. \tag{9}$$

Retaining terms of order $\epsilon$ and using Eq. 9, we obtain a biharmonic equation for the perturbation $\epsilon$. Concurrently, it is expedient to introduce the new variable, $\alpha(\vec{r}) \equiv \Delta\epsilon$, satisfying a Laplace equation,

$$\Delta \Delta \epsilon = 0, \tag{10}$$

$$\Delta \alpha = 0. \tag{11}$$

It so happens that the polymeric drag on the protein may be evaluated solely in terms of $\alpha$.

We next rewrite the segment velocity in terms of $\alpha$ and $\Psi_0$ using Eqs. 5 and 6.

$$\vec{v}_s = \frac{1}{6} m k_B T A^2 \frac{\partial \alpha \Psi_0^{-1}}{\partial \vec{r}}, \tag{12}$$

with

$$\vec{v}_s \to \vec{w} \quad \text{as} \quad r \to \infty. \tag{13}$$

Note that our perturbative expansion seems to break down for segments near the protein surface. The difficulty is that $\Psi_0$ tends to zero there. Nevertheless, the boundary condition at the surface does not relate to the velocity but rather to the flux (see Eq. 8), which is a well-defined quantity throughout. The solution to Eq. 11 may be expressed in terms of Legendre polynomials (Jackson, 1975)

$$\alpha = \sum_\ell \left[ B_\ell r^\ell + C_\ell r^{-\ell-1} \right] P_\ell^-(\cos\theta). \tag{14}$$

Thus, the outer boundary condition (Eq. 13) leads to coefficients $B_0$ and $B_\ell$ ($\ell \geq 2$) equaling zero because $\alpha$ must have the asymptotic behavior,

$$\alpha \to \alpha_\infty \equiv \left( \frac{6w}{m k_B T A^2} \right) r \cos\theta \quad \text{as} \quad r \to \infty. \tag{15}$$

At the same time, we have the flux requirement (Eq. 8) so that Eq. 14 must reduce to

$$\alpha = \frac{6w}{m k_B T A^2} \left( r - \frac{a^3}{r^2} \right) \cos\theta. \tag{16}$$

We may now compute the polymeric drag on the protein. There is a macroscopically large virtual force on the polymer suspension in the absence of the protein, arising from the fact that we let all the segments have a uniform velocity $\vec{w}$. Upon positioning the fixed probe at the origin, the velocity of the segments changes by virtue of the impenetrability of its surface. The difference between the two forces in the respective cases gives us the polymer contribution to the drag,

$$\begin{aligned} \vec{F}_p &= -\int d\vec{r} c(\vec{r}) \left[ \frac{\partial \mu}{\partial \vec{r}} - \frac{\partial \mu}{\partial \vec{r}} \bigg|_{r \to \infty} \right] \\ &= -2\pi \int_{-1}^{1} d(\cos\theta) \int_a^\infty dr\, r^2 c_0 \Psi_0^2(r) \left( \frac{A^2 k_B T}{6} \right) \\ &\quad \times \left[ \cos\theta \left( \frac{\partial \alpha \Psi_0^{-1}}{\partial r} - \frac{\partial \alpha_\infty \Psi_0^{-1}}{\partial r} \right) \right. \\ &\quad \left. + \frac{\sin^2\theta}{\cos\theta} \left( \frac{\alpha \Psi_0^{-1}}{r} - \frac{\alpha_\infty \Psi_0^{-1}}{r} \right) \right] \frac{\vec{w}}{w} \\ &= -\frac{4\pi}{3} (c_0 a^3) \frac{\vec{w}}{m}. \end{aligned} \tag{17}$$

This expression is interpreted as follows. Approximately $c_0 a^3$ segments are depleted from the vicinity of the moving probe. Yet the number density $c_0(1 - a/r)^2$ is not identical

to zero, so the number of segments remaining in its neighborhood is also about $\Phi$ ($c_0a^3$). In a free-draining approximation, the total polymeric drag is simply additive. Note that the drag is a finite quantity despite the long range of the segment density (this sometimes leads to pathological divergences in the computation of certain variables; these are illusory because the depletion interactions are always screened beyond $\xi$). I also point out that the polymeric stress acting on the protein is greatest at the protein surface. Although the segment density vanishes there, the velocity (Eq. 12) increases without bound.

Next, we still have to ascertain whether or not the effect of convective diffusion is negligible. Because the fluid is incompressible, we express the convective term missing from Eq. 7 as

$$\vec{u}_0 \cdot \frac{\partial c}{\partial \vec{r}} = w \cos\theta \left[1 - \frac{3}{2}\left(\frac{a}{r}\right) + \frac{1}{2}\left(\frac{a}{r}\right)^3\right] \frac{2ac_0}{r^2}\left(1 - \frac{a}{r}\right). \qquad (18)$$

Here, we have used the radial component of Stokes's velocity about a sphere moving uniformly through the pure solvent (Landau and Lifshitz, 1959). This should be compared with the radial component of the entropic term in Eq. 7,

$$m \frac{\partial}{\partial \vec{r}} \cdot (\vec{f}c)|_{\mathrm{r}} = w \cos\theta \left[1 - 3\left(\frac{a}{r}\right)^2 + 4\left(\frac{a}{r}\right)^3\right] \frac{2ac_0}{r^2}. \qquad (19)$$

The ratio of Eq. 18 to Eq. 19 is generally much smaller than unity, and, at most, $<0.1$ within the entire depletion layer ($a \leq r \lesssim 2a$). The convective term is effectively dominated by the low value of the distribution, $\Psi_0 = (1 - a/r)$.

## DIFFUSION THROUGH THE INHOMOGENEOUS POLYMERIC NETWORK

On time scales considerably longer than the reorganization time $\tau_s$ of segments within the dynamically evolving depletion layer, the protein diffuses as one dressed particle (protein + depletion layer) through the polymer network. The latter is quite inhomogeneous because it fluctuates strongly as discussed earlier. We would now like to compute the volume $V_a$ accessible to the protein in a manner similar to Ogston's analysis of the same quantity for a sphere in a fibrous network (Ogston, 1958). His straight rigid fibers are, however, fixed entities, whereas the semi-dilute polymer is not, an issue we deal with in what follows.

The polymer solution is enclosed in a container of volume $V$, which is hypothetically split up into cubic boxes each of size $\lambda^3$. The scale $\lambda$ is chosen such that $a \ll \lambda \ll \xi$. Thus, a protein in a certain box $i$ sees an essentially homogeneous polymer solution on the scale of the box given one particular realization out of an ensemble of polymer configurations. On a scale $\lambda$, we may neglect details concerning the dressed particle (protein + depletion layer) and fluctuations of the semi-dilute network on scales of order $\xi$.

As was discussed above, the number of segments depleted by a small protein is proportional to the concentration, so the depletion energy also scales with the concentration. A particular realization of the polymer is defined by the function $c(\vec{r}_i)$, which denotes the (effectively constant) polymer density in each box situated at $\vec{r}_i$ and labeled $i$. Hence, the work of depletion may be written as

$$w_i = kc(\vec{r}_i)k_BT, \qquad (20)$$

where $k$ is a constant. Eq. 20 is valid only whenever $a \ll \xi$.

We next need the excluded volume between the protein and the polymer enclosed solely within box $i$. This is simply the cross virial coefficient

$$\begin{aligned} B_i &= \frac{P_i}{V} \int d\vec{r}_{\mathrm{protein}} \int d\vec{r}_{\mathrm{blob}} \left[1 - \exp(-w_b/k_BT)\right] \\ &= \frac{\lambda^3 w_i}{k_BT}. \end{aligned} \qquad (21)$$

In this statistical calculation, the protein samples the entire volume $V$ but the semi-dilute polymer remains confined to box $i$. The polymer segments do sample the volume of this box at fixed concentration $c(\vec{r}_i)$. The protein interacts with a "blob" of polymer of size $a$; this contains $a^2/A^2$ segments in the quasi-ideal case (this number would be different were the solvent to be very good; for a full discussion, see Odijk, 1996). There are $p_i$ blobs in box $i$ interacting independently with the probe. This work of depletion $w_b$ is smaller than $k_BT$, which allows the Boltzmann factor to be linearized ($w_i = p_i w_b$). Note that Eq. 21 is asymptotically exact in the limit $a \ll \xi$.

The fraction of volume accessible to the protein owing to the polymer in box $i$ is simply $(1 - (B_i/V))$. Accordingly, the total accessible volume is

$$\begin{aligned} V_a/V &= \left\langle \prod_i \left(1 - \frac{B_i}{V}\right) \right\rangle \\ &= \left\langle \prod_i \left[e^{-B_i/V} + \varphi\left(\frac{B_i^2}{V^2}\right)\right] \right\rangle \\ &= \left\langle \exp - \sum_i \frac{B_i}{V} \right\rangle \end{aligned} \qquad (22)$$

We have averaged over all realizations of the polymer with regard to the hypothetical segregation into boxes. The last line follows from adopting the thermodynamic limit,

$$N \to \infty, \quad V \to \infty, \quad \langle B_i^2 \rangle = \text{finite}, \quad c_0 = \text{constant}.$$

Since $c(\vec{r}_i)$ is the number of segments within box $N_i$ divided by the volume $\lambda^3$, the summation may be carried out independent of the distribution of polymer into the respective boxes. We finally end up with an expression for the accessible volume in terms of the depletion energy, $w_d = kc_0k_BT$

(where $c_0$ is the bulk concentration),

$$V_a = V\exp(-w_d/k_BT)\,. \tag{23}$$

For a semi-dilute suspension of fibers, Ogston argued that the accessible volume ought to be proportional to the diffusion coefficient of a probe through such a sparse network (Ogston, 1958; Ogston et al., 1973). This point of view was verified by computer calculations (Johansson and Löfroth, 1993). Ogston's argument is expected to become suspect for more concentrated (or nonsemi-dilute) suspensions. Probabilities are independently factorized in his analysis, which must break down for heavily congested media. Indeed, regions totally inaccessible to the probe may exist in that case. Here, the validity of expressing the diffusion coefficient as

$$D = \frac{D_0V_a}{v} = D_0\exp(-w_d/k_BT) \tag{24}$$

also hinges on the polymer network being sparse or semi-dilute. A second virial description is used and probabilities have again been factorized. There is another reason Ogston's reasoning should apply. The protein effectively interacts with a chain section of size $a^2/A^2$, and so it interacts with a linear sequence of such sections (Odijk, 1996). Despite the small size of the Kuhn segments, the interaction between a small sphere and polymer chain is not dissimilar from that between a sphere and a rod.

## DISCUSSION

We have focused on two effects that have been analyzed independently here: local dynamic depletion and diffusion of the probe at long time scales. They should be compared with the retardation by hydrodynamic screening (see Eq. 2). An ideally consistent theory would include all three effects at the same time but is a formidable undertaking for the following reasons. In the hydrodynamic screening theories, all polymeric detail is smeared out on scales less than the screening length $\xi_H$ (Freed, 1978; de Gennes, 1976). Such a smoothing would be incompatible with the existence of a dynamic depletion layer of size $a$ of the protein. Next, inhomogeneity of the polymeric network is an essential phenomenon in trying to understand the diffusion of the probe. Hence, even within a self-consistent field scheme, hydrodynamic screening and the fluctuating polymeric drag on the protein must be dealt with and derived on the same level. If the solvent is very good, fluctuations in the polymer density are so great that we should turn to renormalization theory. Setting up dynamic versions of current renormalization analyses of equilibrium depletion about small particles (Eisenriegler et al., 1996; Eisenriegler, 1997; Hanke et al., 1999) is clearly no mean task.

The polymeric drag on the protein has been estimated within a free-draining approximation. It is difficult to assign a definite value to the segment mobility because it depends on the chemical detail of a segment. If one were to insist on the segments interacting with each other in a fully non-draining limit, one would have a local drag (i.e., for a dressed probe = protein + its depletion layer, in the absence of long-range hydrodynamic screening),

$$F_{non} = k_n a\eta w\,.$$

We know that only those segments remaining in the depletion layer are involved in dynamic depletion. In the absence of any draining, the dressed particle should then behave like a solid sphere with a radius larger than that of the bare particle. The coefficient $k_n$ is larger that the Stokes value $6\pi$. An estimate for the so-called draining parameter analogous to the one introduced in the Kirkwood–Riseman theory for the dynamics of a single polymer chain (Yamakawa, 1971), is of order $c_0a^2m\eta^{-1}$ multiplied by a small numerical coefficient. It thus would appear that the draining parameter is much smaller than unity in our case. Hence, the free-draining approximation for local dynamic depletion is reasonable. Nevertheless, it is difficult to judge the precise impact of Eq. 17 on the dynamics of the probe. We still lack a quantitative theory of the polymeric hydrodynamics on scales on the order of $\xi_H$. The dressed particle exerts a long-range hydrodynamic force on the surrounding polymer solution.

If the three effects discussed above contribute in principle to the impediment of a diffusing protein, it may explain the variety of (effective) values for the exponents $\mu$ and $\nu$ compiled in Table 1. One difficulty of interpretation is the lack of quantitative precision in the hydrodynamics as stressed above. Recent electrophoresis experiments on small probes (Radko and Chrambach, 1996, 1997) in well-defined semi-dilute polymer solutions suggest that $\mu$ and $\nu$ should be equal to unity. It is thus of interest to test the prediction, Eq. 24 as such, quantitatively. Radko and Chrambach (1996, 1997) used Ferguson plots in which the logarithm (base 10) of the electrophoretic mobility of the protein was plotted against the concentration $c_0$ of the polymer in g/ml. The depletion theory (Eqs. 4 and 24) predicts a retardation coefficient $K_{10}$ (ml/g) (see Eq. 1)

$$K_{10} = \left(\frac{2400\pi}{^e\log 10}\right)\frac{aR_g^2}{M}\,, \tag{25}$$

in terms of the protein radius $a$ (nm) and the polymer radius of gyration $R_g$ (nm) in the theta state and polymer molar mass $M$ (g/mol). It is often difficult to reach theta states for polymers soluble in pure water. The next best thing is to use a suitable aqueous solution. For polyacrylamide in a mixture of water and methanol, François et al., (1980) determined $R_g^2/M$ to be 0.00152 nm$^2$ mol/g. Eq. 25 then yields $K_{10}/a = 5.0$ ml/g nm compared with a value 4.2 ml/g nm evident from Fig. 1 of Radko and Chrambach (1996). In the case of polyethylene oxide (or polyethylene glycol),

Kawaguchi et al., (1997) established a theta state in 0.45 M aqueous $K_2SO_4$ with $R_g^2/M = 0.00111$ nm$^2$ mol/g. Eq. 25 then predicts a retardation coefficient $K_{10} = 5.1$ ml/g for a-lactalbumin in good agreement with $K_{10} = 5.3$ ml/g from Fig. 2 of Radko and Chambrach (1997). Thus, straightforward depletion theory offers a good explanation for these recent data. The implication seems to be that polymeric friction and hydrodynamic screening are too weak to be seen in these experiments, at least for small probes.

Given the variety of retardation exponents measured in the past (Table 1), the discussion of the previous paragraph must be regarded as preliminary. One conclusion of the present work is that several regimes for probe transport may exist depending on the probe size and the properties of the polymer. The particular exponents (unity) found by Radko and Chrambach (1996, 1997) may well stem from the fact that 1) the protein radii $a$ are actually considerably smaller that the polymer correlation lengths $\xi$; 2) care has been exercised in establishing the concentration regimes are really semi-dilute; 3) the interaction between probe and polymer is quasi-ideal (see Eq. 4). Their retardation exponents deviated from unity for larger proteins. We have recently determined the partitioning of small proteins between the two isotropic phases resulting from the phase separation of protein–polysaccharide solutions (S. Wang, J. van Dijk, J. Smit and T. Odijk, manuscript in preparation). Eqs. 4 and 23 are well satisfied when the polymer solutions are semidilute. There is therefore strong evidence for the empirical validity of an Ogston-like argument leading to Eq. 24. A rigorous analytical proof for the proportionality of the diffusion coefficient of a probe in a semidilute system to its accessible volume is lacking.

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