The Nuclear Pore Complex Mystery and Anomalous Diffusion in Reversible Gels

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ABSTRACT The exchange of macromolecules between the cytoplasm and the nucleus of eukaryotic cells takes place through the nuclear pore complex (NPC), which contains a selective permeability barrier. Experiments on the physical properties of this barrier appear to be in conflict with current physical understanding of the rheology of reversible gels. This paper proposes that the NPC gel is anomalous and characterized by *connectivity fluctuations*. It develops a simplified model to demonstrate the possibility of enhanced diffusion constants of macromolecules trapped in such a gel.

THE NUCLEAR PORE COMPLEX

Introduction

The nuclear pore complex (NPC) is among the largest of the molecular machines that are being probed by the methods of single-molecule biophysics (Elbaum, 2001; Salman et al., 2001). It plays the crucial role of regulating the import traffic of proteins from the cytoplasm across the nuclear envelope into the nucleus and the export traffic of gene transcription RNA strands from the nucleus into the cytoplasm (Panté and Aebi, 1996; Allen et al., 2000; Wente, 2000). The NPC has remarkable transport capabilities with two distinct modes: *passive* and *facilitated*. Passive transport is nonspecific and takes place by ordinary diffusion. Colloidal gold particles with radii up to 4 nm, and generic proteins up to 50 kDa in size, pass efficiently through the NPC in this way (Paine et al., 1975). Facilitated transport is highly specific and can proceed against concentration gradients (Dingwall et al., 1982). Macromolecules with sizes exceeding 1 MDa can be transported this way (Panté and Kann, 2002), while the mass-flow rate can reach the amazing level of 100 MDa/s with as many as 10^3 macromolecules passing an individual NPC each second (Ribbeck and Görlich, 2001; Smith et al., 2002).

Because facilitated NPC transport requires a free energy source, it would seem that understanding the surprising transport properties of the NPC demands a full analysis of the intricate molecular machinery. As discussed below, the NPC is an "affinity switch" that relies on the concentration difference of another molecular species to act as the free energy source for essentially diffusive transport. The singular physical properties of a gel-like plug in the central core of the NPC—the transporter—is the key determining factor for the specificity of facilitated transport, with the additional biochemical machinery responsible for the tagging of cargo molecules and the maintenance of the concentration differences. The present article will discuss the fact that the properties of the transporter appear to be in conflict with what is known about the rheology of gels and propose a solution, illustrated by a simple, analytical soluble model. We will start with a brief review of the basic structure of the NPC.

The NPC is a self-assembled, eightfold symmetric ring-like structure consisting of 30–50 different proteins with a total mass of ~125 MDa (Rout et al., 2000; Ryan and Wente, 2000), connecting the inner and outer nuclear membranes. On the inner (nuclear) side eight fibrils are connected to two rings, forming a basket structure with a size of ~100 nm. On the outer (cytoplasmic) side, another eight 100-nm fibrils also are connected to a ring. The coaxial rings themselves are attached on opposite sides of a spoke-complex with, at the center, a porous core called the transporter forming a selective permeability barrier. The free energy source that permits facilitated transport against a concentration gradient is the hydrolysis of guanosine triphosphate (GTP) molecules. First, a short peptide tag ("NLS") identifies cargo macromolecules for nuclear import. Tagged cargo macromolecules then form a complex with β importin, a transporter protein (Allen et al., 2000). Following import, the cargo/importin complex breaks apart when a GTP-associating protein, known as Ran (Azuma and Dasso, 2000), forms a complex with the importin. The Ran/GTP/importin complex is then exported through the NPC. Following GTP hydrolysis the importin is released, after which it is ready for another import cycle. The actual transport of the cargo/transportin complex across the NPC does not require GTP hydrolysis: no mechanical forces are exerted by the NPC during macromolecular traffic (Schwoebel et al., 1998; Nachury and Weis, 1999). The concentration difference of Ran/GTP and Ran/GDP across the nuclear envelope is the free energy source that compensates for unfavorable cargo molecule concentration differences. GTP hydrolysis is necessary only to maintain the high concentration of Ran/GTP inside the nucleus.

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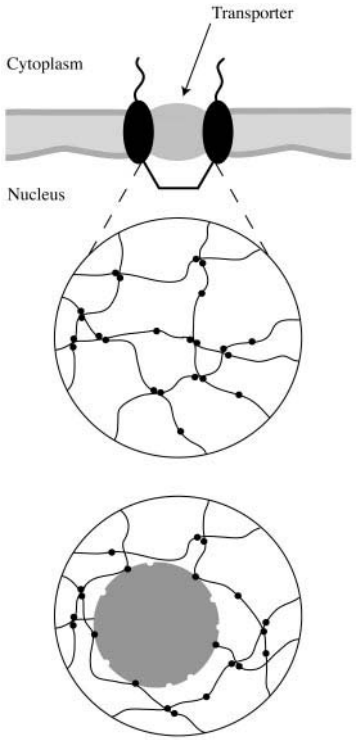

FIGURE 1 Selective phase model of Ribbeck and Görlich for the transporter. The transporter is filled with long diblock copolymers, rich in hydrophobic phenylalanine-glycine repeat units. These units can form weak bonds, which leads to formation of a reversible gel. Particles smaller than the mesh size of the network can diffuse freely through the pore. Generic macromolecules larger than the mesh size cannot pass the transporter, but macromolecules with chemical affinity for the linker units are incorporated into the network. Adapted from Ribbeck and Görlich, 2001.

Recent experiments (Ribbeck and Görlich, 2001; Quimby et al., 2001; Smith et al., 2002) on the translocation of transporter proteins across the NPC in fact demonstrate that the traffic can be described as conventional permeation. However, compared to passive transport, the NPC permeability for transporter proteins is extremely high, about a quarter of the permeability of an equivalently sized water-filled cylindrical channel. In other words, the permeability barrier is *selectively transparent* for transporter proteins. The NPC transporter can switch between different levels of permeability, depending on whether or not it "recognizes" the macromolecule.

The Ribbeck-Görlich (RG) Model

Ribbeck and Görlich (2001) proposed the model for the permeability of NPC switching shown in Fig. 1. The permeable core of an NPC has a length of ~40 nm and a comparable diameter. It contains a 12-MDa low-density peptide "plug" consisting of long diblock copolymers rich in hydrophobic phenylalanine-glycine repeat units separated by hydrophilic spacers (Bayliss et al., 1999; Rout et al., 2000). These hydrophobic repeat units attract each other with a low binding energy (of order k_BT). The NPC loses its selectivity when this plug is removed, in which case the transport kinetics of the NPC reduces to that of a water-filled pore. In the RG model, the plug is treated as a homogeneous polymer network with a mesh size in the range of 3 nm, as deduced from the fact that there are $\sim 10^3$ hydrophobic units per NPC. Generic proteins (or gold particles) smaller than this mesh size can pass easily while generic proteins with larger size are increasingly blocked. Transporter proteins have a selective affinity for the hydrophobic repeat units, through surface residues such as tryptophane, which causes them to be incorporated into the bonding network. The recognition of the transporter protein by the gel allows it to enter the permeability barrier. The opening/closing kinetics of the weak bonds then produces efficient diffusive transport of the transporter protein across the barrier.

From the viewpoint of conventional polymer rheology, the RG model cannot work. The proposed structure of the NPC core corresponds to what is known as a reversible gel in the polymer physics literature (Tanaka and Edwards, 1992; Semenov et al., 1995; Semenov and Rubinstein, 1998). The diffusion constant D_0 of a sphere with no recognition groups, moving through a reversible gel having a mesh size small compared to the radius r, equals $k_BT/(6\pi\eta r)$ with $\eta = G\tau$ the gel viscosity, G the plateau modulus, and τ the stress relaxation time (de Gennes, 1979). If reversible recognition groups are placed on the macromolecule surface, connecting it to the gel, then the diffusion constant should be *reduced* by a factor $P = D/D_0$ equal to the probability of the sphere to be "free" (Leibler et al., 1991). A macromolecule that is recognized by a reversible gel should have a lower, not a higher, mobility. The interaction does indeed allow a sphere with linker groups to enter the reversible gel more easily, but in Appendix A we show that the net permeability for a sphere to pass a cylindrical pore containing a reversible gel remains highest when the sphere does not carry any recognition groups. This is in direct contradiction with the in vitro experiments on NPCs, so either the RG model for facilitated NPC transport is incorrect or the permeability barrier is not a conventional reversible gel, the option pursued in this paper.

Reversible gels in poor solvent and force fluctuations

We would like to propose that the RG model still may work provided the permeability plug is not a conventional gel, but a *confined reversible gel in poor solvent* (de Gennes, 1979). Consider a reversible gel in aqueous solvent with the polymers constituting the gel consisting of hydrophobic groups separated by hydrophilic spacers, as in the RG model. Let a plug of the gel be confined to the interior of a hollow cylinder with the chain ends grafted to the cylinder surface.

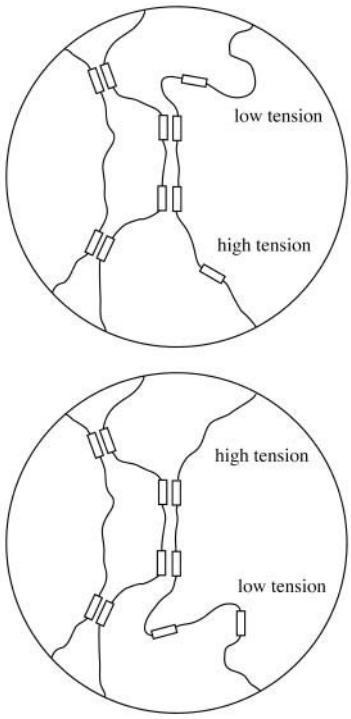

FIGURE 2 Connectivity fluctuations. If a link connecting a pair of hydrophobic groups opens because of a thermal fluctuation, then the tensions of the polymers in the local neighborhood are rearranged.

It is well known that under bulk conditions, a reversible gel undergoes a collapse transition upon reduction of the "solvent quality" (the average solubility per monomer) (Rubinstein and Dobrynin, 1999). In a confined geometry with the polymers pinned to the container walls, a collapse transition is not possible. Instead, the polymer chains are placed under tension at this point: by stretching selective chains, a larger number of paired hydrophobic groups can be achieved. It should be emphasized at this point that the stability of such stretched structure requires the chain extremities to be held at a fixed position. Indeed, releasing this constraint would lead to a collapse of the gel to its desired density into a cylindrical ring.

We claim that this arrangement is characterized by strong thermal fluctuations in terms of the network connectivity, somewhat in the nature of a spin glass. There are in general many different arrangements, with comparable free energies, to pair-off the hydrophobic groups. If one link connecting two groups would fail, due to a thermal fluctuation, or if a new pair forms, then the network connectivity would locally change with a corresponding rearrangement of the tensions in the connecting spacer groups (see Fig. 2). A tense, reversible gel of this type thus would be characterized as well by *force fluctuations*.

Now assume that a macromolecule contains reversible linker groups allowing weak links with the gel. The force exerted by the fluctuating network on the macromolecule is the sum of the tension forces exerted by polymers attached to its surface. Rearrangements of the tension field due to the thermal configuration fluctuations would produce a time-dependent fluctuating force on the macromolecule. In the neighborhood of a given local minimum of the position of the macromolecule, there would be neighboring minima characterized by local changes in the gel connectivity. Unlike a generic macromolecule, a macromolecule with recognition groups could move from local minimum to local minimum.

The remainder of the paper is dedicated to the analysis of a simple "toy" model of a reversible gel, introduced in the next section, with just two minima for an embedded plate-like macromolecule. The aim of the model is to demonstrate that the form of transport outlined above, with hopping between different local minima, can produce enhanced diffusion constants. The model is certainly too simple to describe the complex properties of confined gels in poor solvent outlined above, but it has the virtue that it can be examined on an analytical basis. The model demonstrates that enhanced diffusion is possible, but only by a careful tuning of the system parameters, such as the linker binding energy. The model was inspired by a recent experimental and numerical study of the forces between two surfaces covered by an array of tethered ligands and receptors and in principle could be studied experimentally or numerically (Jeppesen et al., 2001).

The Kinetics section discusses the model in terms of the "chemical noise" generated by the opening/closing kinetics of the ligand-receptor pairs. In the subsequent section we apply the model to estimate the anomalous diffusion constant of a plate embedded in a tense reversible gel. We find that, as a function of the binding energy and the degree of stretching of the network polymers, there indeed is an intermediate range where the diffusion coefficient is significantly enhanced.

THE PLATE MODEL: EQUILIBRIUM PROPERTIES

We represent the macromolecule as a flat plate of area $\mathcal{A}$ confined between two parallel walls with spacing $2L$ (see Fig. 3). The position X of the plate is defined with respect to the middle of the gap, and is allowed to vary between $-L$ and $+L$. Each side of the plate has a surface concentration $\sigma = n/\mathcal{A}$ of receptor groups. Ideal polymers with radius of gyration R are grafted on both walls with the same surface coverage. The free end of each grafted polymer contains a ligand group that can bind to a receptor group on the plate with a binding energy ϵ.

The ligand-receptor statistical mechanics will be described by treating the polymer population as a two-state system with polymers either paired $(+)$ or unpaired $(-)$. Let $w_+(y)$ and $w_-(y)$ be the free energies of polymers belonging to the $(+)$, respectively, $(-)$ population with y equal to $L - X$ on the right side of the plate and equal to $L + X$ on the left

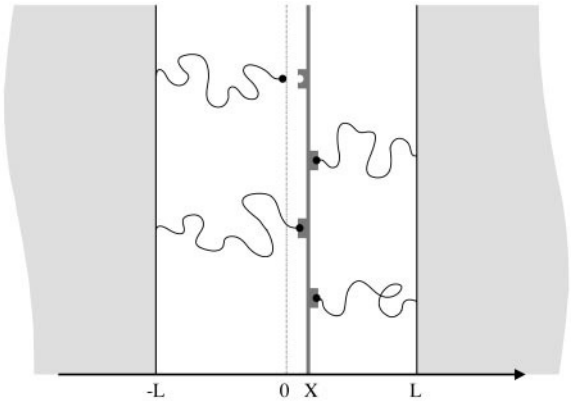

FIGURE 3 Plate model. The plate can move freely between two walls, each covered with n grafted polymers. The free end of each chain can adsorb onto the plate with binding energy ϵ. The equilibrium position of the plate is determined by the balance between compression of the two polymer layers and the stretching of bound chains.

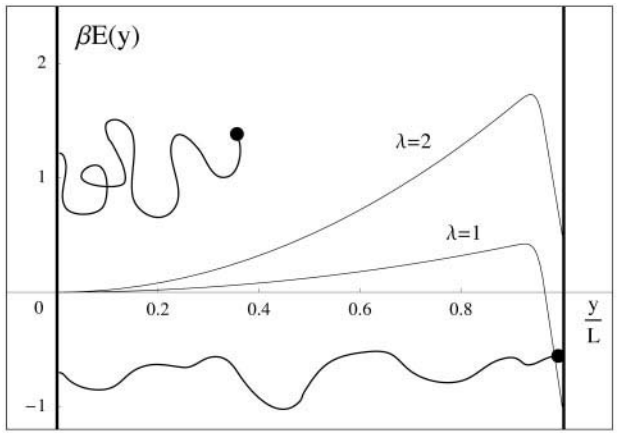

FIGURE 4 Two-state model for the polymer chains. Unpaired, relaxed polymers (*top*) correspond to zero on our energy scale. If a polymer is stretched (*bottom*), then the free energy increases initially due to the entropic elasticity of the chain until the endpoint can make a bond with the plate where the polymer gains a binding energy ϵ. Depending on the degree of stretching λ (ratio of the end-to-end length and the unstretched radius of gyration), binding may or may not lower the free energy of the polymer.

side. In particular, $w_+(y)$ is the configurational free energy of an ideal polymer whose endgroups are attached to two plates separated by a distance y, while $w_-(y)$ is the configurational free energy with only one of the two ends fixed. In the small y limit (i.e., $y \ll R$) $w_+(y) \approx w_-(y) \approx k_BT(y/R)^{-2}$, while for $y > R$, $w_+(y) \approx k_BT(y/R)^2$ and $w_-(y) \approx 0$ (de Gennes, 1979). The two-state free energy per polymer $g(y)$ is then given by

$$g(y) = -k_BT \ln(e^{-\beta(w_+(y)-\epsilon)} + e^{-\beta w_-(y)}), \quad (1)$$

with $\beta = 1/(k_BT)$. A reasonable interpolation formula for $g(y)$ valid for general y is then $g(y) \approx u(y) - k_BT \ln(1 + e^{-\beta(v(y)-\epsilon)})$, where $u(y) = \frac{1}{2}k_BT(y/R)^{-2}$ and $v(y) = \frac{1}{2}k_BT(y/R)^2$. Adding the contributions coming from polymers on both sides of the plate, we find for the total free energy $V(X) = n\{g(L - X) + g(L + X)\}$

$$V(X) = n\{u(L - X) - k_BT \ln(1 + e^{-\beta E(L-X)}) + [X \to -X]\}, \quad (2)$$

where $E(y) = -\epsilon + v(y)$ is shown in Fig. 4. We can consider $V(X)$ as the "potential of mean force" for the plate. It is an even function of X, diverging at $X = \pm L$, whose form depends sensitively on the binding energy ϵ.

Single-minimum regime

In the regimes of ϵ/k_BT large or small compared to one, $V(X)$ adopts the following limiting forms:

$$V(X) = n\{-2\epsilon + u(L - X) + u(L + X) + v(L - X) + v(L + X)\} \quad (3)$$

for $\epsilon/k_BT \gg 1$, and

$$V(X) = n\{u(L - X) - k_BT \ln(1 + e^{-\beta v(L-X)}) + [X \to -X]\} \quad (4)$$

for $\epsilon/k_BT \ll 1$. Because both $u(y)$ and $v(y)$ are convex functions of y, $V(X)$ is a convex function as well in these two limits. Because it is even, $V(X)$ has a single minimum at $X = 0$. For the case $\epsilon/k_BT \gg 1$, the free energy minimum is located at $X = 0$ because that maximizes the number of links, while for $\epsilon/k_BT \ll 1$, the minimum is located at $X = 0$ because that minimizes the configurational free energy of the two confined polymer brushes. It also can be shown from Eq. 2 that the even, convex form for $V(X)$ is encountered for general values of ϵ/k_BT in the "low-tension" limit $\lambda = L/R < \lambda_c = \sqrt{3}$, where the stretching energy of a paired polymer chain is small compared to the thermal energy. There are no multiple minima in the free energy landscape of the plate in these regimes and the equilibrium position of the plate is at $X = 0$ with no anomalous kinetic properties. In the following, we will assume that ϵ/k_BT is of order one and $\lambda > \lambda_c$.

Multiple-minima regime

Expand the free energy $V(X)$ to fourth-order in the dimensionless plate displacement $x = X/L$

$$V(X) = V_0 + nk_BT\left\{a_2[\lambda, \epsilon]\frac{x^2}{2} + a_4[\lambda, \epsilon]\frac{x^4}{4}\right\} + \mathbb{O}(x^6), \quad (5)$$

where $a_2[\lambda, \epsilon]$ and $a_4[\lambda, \epsilon]$ are dimensionless functions of ϵ and λ. For ϵ/k_BT either large or small compared to one, a_2

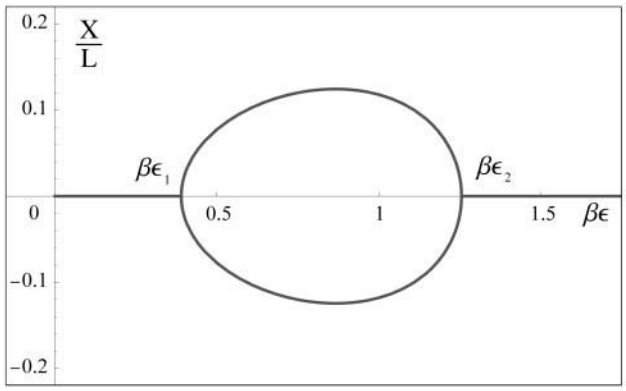

FIGURE 5 Plate positions minimizing the free energy as a function of the adsorption energy ϵ (in units of k_BT) for $\lambda = 1.75$. Two degenerate minima appear in the range $\epsilon_1 < \epsilon < \epsilon_2$, where ϵ_1 and ϵ_2 are functions of the parameter λ given in the text.

is positive. Because a_4 is always positive, it follows that $V(X)$ again has a single minimum at $X = 0$ in these regimes, as noted earlier. Over an intermediate energy interval $\epsilon_1 < \epsilon < \epsilon_2$, the coefficient a_2 is negative: it follows that the function $V(x)$ has two symmetrically placed minima $X \approx \pm\sqrt{|a_2|/a_4}$, separated by a maximum at $X = 0$. Fig. 5 shows the position of these minima as a function of ϵ. The location of the two bifurcation points $\epsilon = \epsilon_{1,2}$ is given by

$$\frac{\epsilon_{1,2}}{k_BT} = \frac{\lambda^2}{2} + \ln\left\{\frac{\lambda^6 - \lambda^4 - 6 \pm \sqrt{\lambda^6 - 2\lambda^4 + \lambda^2 - 12}}{2(3 + \lambda^4)}\right\}. \tag{6}$$

The two bifurcations correspond to continuous transitions where the symmetry between $+X$ and $-X$ is broken spontaneously. In this intermediate regime, the plate chooses to associate itself with either of the two boundaries, relieving the tension on one side of the plate at the cost of losing the pairing energy on the other side of the plate.

KINETICS

The plate is exposed to two types of noise: the usual thermal fluctuations of the surrounding solvent and the *chemical* noise of the on-off fluctuations of the linker groups. To describe these tension-sensitive fluctuations, we assume that the plate position $X(t)$ can be treated as an adiabatic, collective degree of freedom (Risken, 1996) with a separation in time scales between the *microscopic* chain degrees of freedom—treated again as a dynamical two-level system—and the *macroscopic* plate position.

Chemical noise

Let $p(y, t)$ be the fraction of chains in the paired state and $1 - p(y, t)$ the unpaired fraction. If $k(y)$ is the "off-rate" for the break-up of a paired state and $r(y)$ the "on-rate" for the formation of the paired state, then the stochastic equation of motion for $p(y, t)$ is

$$\frac{dp}{dt} = -k(y)p + r(y)(1 - p) + \mu_y(t). \tag{7}$$

Here, $\mu_y(t)$ is a zero-average Gaussian noise source

$$\langle \mu_y(t)\mu_y(t')\rangle = \gamma(y)\delta(t - t'), \tag{8}$$

which describes the chemical noise whose intensity $\gamma(y)$ remains to be determined. The on- and off-rates depend on the collective coordinate $X(t)$ through the variable $y(t)$, which equals $L + X(t)$ or $L - X(t)$, depending which side of the plate the chain is located on. The on-rate is

$$r(y) = \omega e^{-\beta v(y)}, \tag{9}$$

where ω is the attempt frequency that will be identified with the inverse of the Rouse relaxation time τ_R of the polymer chains (proportional to N^2 with N the number of Kuhn lengths). The off-rate then follows from the condition that the steady-state solution of Eq. 7, $\langle p\rangle_y = r(y)/(k(y) + r(y))$, must be consistent with the pairing probability as predicted by the Boltzmann distribution

$$\langle p\rangle_y = \frac{e^{-\beta E(y)}}{1 + e^{-\beta E(y)}}. \tag{10}$$

From this condition it follows that the relaxation time $\tau(y) = \{r(y) + k(y)\}^{-1}$ equals

$$\tau(y) = \frac{e^{-\beta\epsilon}}{1 + e^{-\beta E(y)}}\,\tau_R. \tag{11}$$

We can determine the autocorrelation function for fluctuations in the population of bound and free chains by integrating the equation of motion Eq. 7

$$\langle \delta p(t)\delta p(t')\rangle_y = \tfrac{1}{2}\,\gamma(y)\tau(y)e^{-|t-t'|/\tau(y)}, \tag{12}$$

where $\delta p(t) = p(t) - \langle p\rangle_y$. Finally, the noise intensity $\gamma(y)$ in Eq. 7 is given by the fluctuation-dissipation theorem

$$\gamma(y) = \frac{2}{n\tau(y)}\,\langle p\rangle_y(1 - \langle p\rangle_y). \tag{13}$$

Force fluctuations

We now turn to the macroscopic equation of motion for the plate and the force fluctuations exerted by the binding and unbinding of the polymers. We will assume that solvent and

chemical noise operate in an additive way on the plate. The equation of motion of the plate then takes the form

$$\zeta(X)\frac{dX}{dt} = -\frac{dU}{dX} + F(X, t) + \nu(t). \quad (14)$$

On the left side, $\zeta(X)$ is the effective friction coefficient of the plate in the presence of the chemical noise. In the first term on the right-hand side, the potential $U(X)$ equals $n\{u(L - X) + u(L + X)\}$ and describes the compression of the polymer layers. The second term $F(X, t)$ is the stochastic tension force exerted by the chains on the plate

$$F(X, t) = n\{p(L - X, t)f(L - X) - p(L + X, t)f(L + X)\}. \quad (15)$$

Here, $f(y) = -dv/dy$ is the restoring force of a stretched polymer chain. The last term of Eq. 14 represents the conventional hydrodynamic noise

$$\langle \nu(t)\nu(t')\rangle = 2k_BT\zeta_0\delta(t - t'), \quad (16)$$

where the hydrodynamic friction coefficient ζ_0 of the plate moving through chain-free solvent is assumed to be known.

Separate the tension force $F(X, t) = \langle F(X)\rangle + \delta F_X(t)$ into an average and a random force of zero mean, then the Langevin equation simplifies to

$$\zeta(X)\frac{dX}{dt} = -\frac{dV}{dX} + \delta F_X(t) + \nu(t), \quad (17)$$

with $V(X)$ the equilibrium potential energy determined earlier. The autocorrelation function of the random force $\delta F_X(t)$ follows from the autocorrelation function (Eq. 12) for the ligand-receptor fluctuations

$$\langle \delta F_X(t)\delta F_X(t')\rangle = n\{f(L - X)^2\langle p\rangle_{L-X}(1 - \langle p\rangle_{L-X}) \cdot e^{-|t-t'|/\tau(L-X)} + [X \to -X]\}. \quad (18)$$

The RMS of the chemical noise force exerted on the plate is thus of order $f(L)n^{1/2}$, as is reasonable on intuitive grounds. By assumption, the kinetics of the plate is slow compared to that of the chains, which means that we can approximate the force autocorrelation function by a delta function

$$\langle \delta F_X(t)\delta F_X(t')\rangle \approx 2n\{f(L - X)^2\langle p\rangle_{L-X}(1 - \langle p\rangle_{L-X}) \cdot \tau(L - X) + [X \to -X]\}\delta(t - t'). \quad (19)$$

To compare the chemical and hydrodynamic noise levels in Eq. 17, let r be the size of the macromolecule. The solvent contribution to the friction is then of order $\zeta_0 \sim r\eta_0$, with η_0 the solvent viscosity. The intensity of hydrodynamic force fluctuations is then of order $k_BTr\eta_0$ (see Eq. 16). However, the intensity of the on-off force fluctuations is, according to Eq. 19, of order $nf^2\tau$. The relaxation time τ is estimated as the Rouse time $\tau_R = \beta\eta_0N^2a^3$ of the polymers of the gel, where a is the Kuhn segment length of the polymer. The typical tension level in the polymer chains is $\beta f \sim L/(Na^2)$. Assuming, moreover, that the area density of binding sites on the surface of the macromolecule is matched to the gel mesh size (i.e., that n is of order $(r/L)^2$), the ratio of chemical over hydrodynamic noise intensities is of order $\sim r/a$. For the experiments on NPCs described in the Introduction, this is of order of 10^1–10^2, so we expect the chemical fluctuations to dominate.

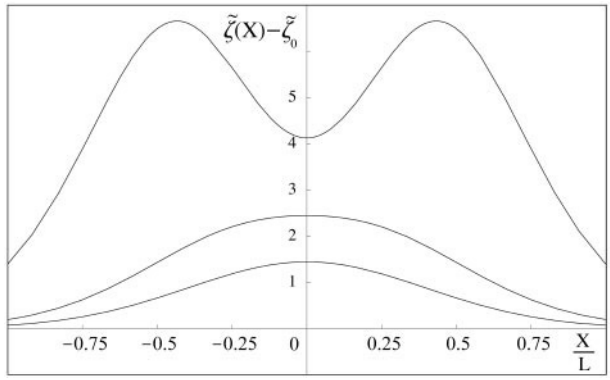

FIGURE 6 The dimensionless contribution to the friction coefficient due to reversible binding of the polymers to the plate, shown as a function of plate position, for chain extension $\lambda = 1.75$ and binding energies $\epsilon = 0.5$, 1, and 2 k_BT (from *bottom* to *top*). The plots are shown in dimensionless units, $\tilde{\zeta} = \zeta\beta L^2/(n\tau_R)$, with τ_R the Rouse relaxation time of the chain.

DIFFUSION BY FORCE FLUCTUATIONS

Friction coefficient and transition rate

The stochastic differential equation (Eq. 17) for $X(t)$ combined with the noise correlation function (Eq. 19) represents a Brownian walk in a potential $V(X)$ with a position-dependent Gaussian noise-source (van Kampen, 1992; Risken, 1996). Using well-established methods (see Appendix B), it can now be shown that the solution of the Fokker-Planck equation for the probability distribution of the stochastic variable $X(t)$ only leads to the Boltzmann distribution in steady state, provided the friction coefficient has the form

$$\zeta(X) = \zeta_0 + \frac{n}{k_BT}\{f(L - X)^2\langle p\rangle_{L-X}(1 - \langle p\rangle_{L-X}) \cdot \tau(L - X) + [X \to -X]\}. \quad (20)$$

The friction coefficient is a non-monotonic function of X, as shown in Fig. 6. Using this expression for the friction coefficient, we now can treat the kinetics of the plate.

Recall that if $a_2 < 0$, $V(X)$ has two minima $X \approx \pm\sqrt{|a_2|}/a_4$. The chemical noise fluctuations will cause a transitions between the two minima. The crossing rate Γ can be com-

puted by solving the Fokker-Planck equation using the Kramers method (see Appendix B)

$$\Gamma \sim \frac{\sqrt{|V''(X_{\max})|V''(X_{\min})}}{\zeta(X=0)} \exp(-\beta\Delta V), \qquad (21)$$

where $\Delta V = V(0) - V(X_{\min})$. The reason that only the friction coefficient at $X = 0$ appears in Eq. 21 is that, during a successful transition between the two wells, the system spends more time at the "transition state" $X = 0$ separating the two wells than elsewhere.

Diffusion coefficient

In the first section we proposed that the diffusion constant of a macromolecule embedded in a confined, reversible gel under poor solvent conditions could be anomalously high and that it took place by random "hopping" between adjacent minima of an effective, three-dimensional potential. We will now apply the plate model, in the bifurcation regime, to estimate the diffusion coefficient as $D \propto \Gamma\langle X\rangle^2$, with $\langle X\rangle$ the typical spacing between the two adjacent minima. Using Eqs. 5 and 21, this leads to

$$D[\lambda, \epsilon] = \frac{n^2 k_B T|a_2[\lambda, \epsilon]|^3}{\zeta(0)a_4[\lambda, \epsilon]} \exp\left(-\frac{n|a_2[\lambda, \epsilon]|^2}{4a_4[\lambda, \epsilon]}\right), \qquad (22)$$

with the friction coefficient given by Eq. 20.

According to Eq. 22, the diffusion coefficient has a non-monotonic dependence on the number of linker units. The diffusion coefficient vanishes exponentially in the large n limit, because the energy barrier separating the two minima is proportional to n. However, unlike conventional rheology, D *increases* with n for small n. In the regime where friction is dominated by the chemical noise, i.e., for ϵ in the range $\epsilon_1 < \epsilon < \epsilon_2$, maximizing Eq. 22 with respect to n gives an optimal choice for the number of linkers $n = 4a_4/|a_2|^2$, *independent of the size of the macromolecule*. This diffusion "magnification" disappears at the boundaries of the bifurcation regime. As shown in Fig. 7, the specific diffusion coefficient is highly sensitive to small variations of the binding energy ϵ and the extension rate λ.

CONCLUSIONS

The results obtained from the plate model indicate that enhanced diffusion coefficients for embedded macromolecules are, at least in principle, possible. It must be clear though that our results on the plate model do not constitute a proof of the proposed mechanism. We have not demonstrated that the "energy landscape" of the plate model is realistic for real reversible gels in poor solvents. The description for such gels offered in the first section bears similarity with the physics of spin glasses, which are characterized by complex energy landscapes (Kumar and Douglas, 2001). Whether transport is determined by a "typical" energy barrier—as assumed implicitly in the present paper—is far from obvious. A second important point concerns the fact that in the plate model, unbinding events of different linkers are correlated only in a mean-field sense through the position X of the plane. Actually, the unbinding of a ligand group should affect its neighbors over a distance of the order of a certain correlation length ξ, that may diverge at a stress percolation transition. Because the plate model is tractable, however, it would be very interesting if it could be realized experimentally, for instance by trapping a colloid between two plates that have grafted layers of polymers with recognition groups at their extremities. The mobility of the colloid, both in the plane of the plates and in the perpendicular direction, could be measured as a function of the plate spacing to control the tension of the polymers.

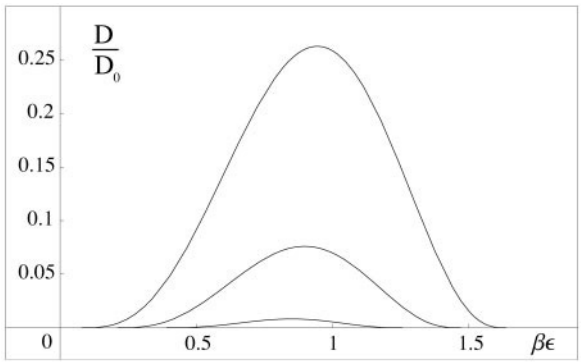

FIGURE 7 Specific diffusion coefficient as a function of ϵ (in units of k_BT) for dimensionless chain extensions λ of 1.75, 1.77, and 1.79 (from *bottom* to *top*). We have fixed $n = 5$ and the ratio $\tau_R D_0/L^2 = 10^{-2}$, where D_0 is the diffusion coefficient in pure solvent.

APPENDIX A: PERMEABILITY OF A REVERSIBLE GEL

In this Appendix we apply conventional polymer rheology to compute the permeability of a cylindrical plug, consisting of reversible gel material, for a diffusing macromolecule that has chemical affinity for the gel.

Permeability

Consider a reversible gel confined in a cylindrical pore of length L and cross-section A. If the medium is homogeneous, the concentration $\phi(x)$ of diffusing species inside the pore only depends on the position x along the cylinder, with $\phi_L = \phi(x = 0)$ and $\phi_R = \phi(x = L)$ the concentrations at the two ends of the cylinder. Outside the pore, there is a reservoir of particles with fixed concentrations c_L (respectively c_R) on the left (respectively right) side, with $\Delta c = c_L - c_R > 0$. The current I of particles, i.e., the number of particle crossing the cylinder area A each second, is given by

$$I = -D\frac{d\phi}{dx}A = D\left(\frac{\phi_L - \phi_R}{L}\right)A. \qquad (A1)$$

We want to evaluate the permeability P of the pore defined by $I = P\Delta c$. Let k_{in} and k_{out} be the rates at which the particles enter or leave the pore. The currents at the entry (left) and at the exit (right) of the pore are then

$$I = \{k_{in}c_L - k_{out}\phi_L\}A\delta \tag{A2a}$$

$$I = \{k_{out}\phi_R - k_{in}c_R\}A\delta \tag{A2b}$$

with δ a microscopic length corresponding to the width of the transition region, which we assume to be of the order of the size of the diffusing species. Combining these equations, we get the following expression for the permeability

$$P = \frac{k_{in}A\delta}{2 + k_{out}\delta L/D}. \tag{A3}$$

Diffusion coefficient

The diffusion constant D_{gel} of a sphere with no recognition groups moving through a reversible gel with a mesh size small compared to the sphere radius r equals $k_BT/(6\pi\eta r)$ with $\eta = G\tau$ the gel viscosity, G the plateau modulus, and τ the stress relaxation time. If n specific reversible recognition groups are placed on the surface of the sphere, with binding energy ϵ, then the mobility of the sphere—and hence the diffusion constant—are reduced by a factor proportional to the probability to be free

$$D = D_{gel}\left(\frac{1}{1 + e^{\beta\epsilon}}\right)^n \approx D_{gel}e^{-\beta n\epsilon}. \tag{A4}$$

The diffusion coefficient decreases exponentially with the number of stickers or with increasing binding energy (Leibler et al., 1991).

The permeability of the pore also depends on the free energy cost (or gain) $\Delta F = \pi v - n\epsilon$ of inserting the sphere into the gel. Here, v is the volume of the sphere and π the osmotic pressure inside the gel. Detailed balance requires the in- and out-rates to be related through $k_{in}/k_{out} = e^{-\beta\Delta F}$. We will discuss separately the two cases that ΔF is positive, respectively, negative.

$\Delta F > 0$: low-affinity regime

If the chemical affinity is low, then the particle has to overcome an energy barrier $\Delta F > 0$ to enter the gel. The entrance rate k_{in} follows an Arrhenius Law

$$k_{in} \approx \frac{D}{\delta^2}e^{-\beta\Delta F}, \tag{A5}$$

where we assume that the viscosity of the gel is much higher than that of the surrounding medium. The escape rate k_{out} follows from detailed balance condition. The permeability is

$$P = P_0 = D_{gel}\frac{A}{L}e^{-\beta\pi v}, \tag{A6}$$

which does not depend on the number of linkers.

$\Delta F < 0$: high-affinity regime

Assume that the particle interacts sufficiently strongly so $\Delta F < 0$. It has now to overcome the energy barrier $-\Delta F$ to *leave* the gel, and

$$k_{out} \approx \frac{D}{\delta^2}e^{+\beta\Delta F}. \tag{A7}$$

The in-rate follows from detailed balance, and the permeability is

$$P = \frac{P_0}{1 + 2\ \delta/L\ e^{-\beta\Delta F}}. \tag{A8}$$

For lower affinity $(\delta/L)e^{-\beta\Delta F} \ll 1$, we recover Eq. A6, whereas in the very strong affinity limit $n \to \infty$ or $\epsilon \to \infty$, the permeability decreases exponentially

$$P = P_0\frac{L}{2\delta}e^{-\beta n\epsilon}. \tag{A9}$$

Cross-over between weak and strong affinity occurs when $(\delta/L)e^{-\beta\Delta F} \approx 1$. We see that the permeability decreases monotonically with the number of linker groups n, so that increasing the chemical affinity of a sphere with a conventional reversible gel should not increase the permeability.

APPENDIX B: DIFFUSION WITH INHOMOGENEOUS NOISE

Consider a stochastic process described by the following Langevin equation for the random variable $\xi(t)$

$$\zeta(\xi)\frac{d\xi}{dt} = -\frac{dV}{d\xi} + \eta(\xi, t). \tag{B1}$$

We assume that η obeys a Gaussian distribution characterized by the first two moments $\langle\eta(\xi, t)\rangle = 0$ and $\langle\eta(\xi, t)\eta(\xi, t')\rangle = 2\gamma(\xi)\delta(t - t')$, where the noise amplitude $\gamma(\xi)$ depends on position. We wish to obtain the local friction coefficient $\zeta(\xi)$ such that the probability distribution for ξ approaches the Boltzmann distribution in the long-time limit (van Kampen, 1992; Risken, 1996). The corresponding Fokker-Planck equation for the probability density $P(x, t)$ (probability that the stochastic variable ξ equals x at time t) for this inhomogeneous problem can be obtained by standard methods (Reguera and Rubi, 2001)

$$\frac{\partial P}{\partial t}(x, t) = \frac{\partial}{\partial x}\left\{\frac{1}{\zeta(x)}\frac{dV}{dx}P + D(x)\frac{\partial P}{\partial x}\right\}, \tag{B2}$$

where the position-dependent diffusion coefficient $D(x)$ satisfies a local Einstein relation

$$D(x) = \frac{k_BT}{\zeta(x)}. \tag{B3}$$

We now apply Eq. B2 to the escape of a particle from a potential well. Starting from a local minimum of the potential $V(x)$ at $x = x_0$, we want to know the typical time needed for the particle to cross the barrier $\Delta V = V(x_1) - V(x_0)$ located at $x = x_1$. Following the Kramers method (Risken, 1996), we must solve the stationary problem for the probability density current J, together with adsorbing boundary conditions at $x = x_{max} > x_1$. When the particle reaches $x = x_{max}$, it is re-introduced in the well so that the probability density is always normalized to unity. The mean first passage time is then simply the inverse of the probability current: $\tau = 1/J$.

The Fokker-Planck equation can be written as $\partial P/\partial t = \mathcal{L}_{FP}P$, where the Fokker-Planck operator $\mathcal{L}_{FP}$ is defined as

$$\mathcal{L}_{FP} = \frac{\partial}{\partial x} D(x)e^{-\beta V(x)} \frac{\partial}{\partial x} e^{\beta V(x)}. \quad \text{(B4)}$$

Within this operator formalism, the stationary problem $\partial P/\partial t = 0$ reduces to

$$-D(x)e^{-\beta V(x)} \frac{\partial}{\partial x} e^{\beta V(x)} P(x) = J, \quad \text{(B5)}$$

with J the constant current of probability. Equation B5 can be integrated between x and $x = x_{max}$. Using the adsorbing boundary condition $P(x_{max}) = 0$, we obtain the stationary probability density

$$P(x) = \beta J e^{-\beta V(x)} \int_x^{x_{max}} dx'' \zeta(x'') e^{\beta V(x'')}. \quad \text{(B6)}$$

Imposing conservation of the probability $\int_{-\infty}^{x_{max}} dx' P(x') = 1$, we find the following expression for the mean first passage time

$$\tau = \beta \int_{-\infty}^{x_{max}} dx' e^{-\beta V(x')} \int_{x'}^{x_{max}} dx'' \zeta(x'') e^{\beta V(x'')}. \quad \text{(B7)}$$

We note that in the limit of high barrier $\Delta V \gg k_B T$, the first integral is dominated by the minimum of the potential at $x = x_0$, whereas the second integral is dominated by the maximum of the potential at $x = x_1$. We then evaluate τ using the saddle-point method at these two points. Assuming that $\zeta(x)$ varies smoothly around x_1, the mean first passage time is given, in the limit of high barriers and high friction, by

$$\tau \sim \frac{\zeta(x_1) e^{\beta \Delta V}}{\sqrt{V''(x_0)|V''(x_1)|}}. \quad \text{(B8)}$$

The passage time is governed by the friction coefficient at the top of the barrier. The corresponding crossing rate is $\Gamma = 1/\tau$.

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