Hydrodynamic collective effects of active protein machines in solution and lipid bilayers

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The cytoplasm and biomembranes in biological cells contain large numbers of proteins that cyclically change their shapes. They are molecular machines that can function as molecular motors or carry out various other tasks in the cell. Many enzymes also undergo conformational changes within their turnover cycles. We analyze the advection effects that nonthermal fluctuating hydrodynamic flows induced by active proteins have on other passive molecules in solution or membranes. We show that the diffusion constants of passive particles are enhanced substantially. Furthermore, when gradients of active proteins are present, a chemotaxis-like drift of passive particles takes place. In lipid bilayers, the effects are strongly nonlocal, so that active inclusions in the entire membrane contribute to local diffusion enhancement and the drift. All active proteins in a biological cell or in a membrane contribute to such effects and all passive particles, and the proteins themselves, will be subject to them.

active proteins | collective hydrodynamic effects | nonthermal fluctuation effects | enhanced passive particle diffusion

Protein machines play a fundamental role in biological cells (1, 2). Operating as motors, they are responsible for intracellular transport and force generation. As manipulators, they perform various operations involving other biomolecules, including RNA and DNA. As pumps, they transfer ions across biomembranes. A common feature of protein machines is that they undergo cyclic conformational changes that are induced by ligand binding and product release. Thus, all protein machines are enzymes where substrate binding, catalytic conversion to products, and product release are accompanied by internal mechanochemical motions. Conformational changes within turnover cycles are also characteristic of many other enzymes, which need not function as molecular machines. The results we present in this paper are also applicable to these enzymes.

When a macromolecule cyclically changes its shape, it induces hydrodynamic flows in the surrounding fluid or biomembrane in which it resides. Such pulsating flows can act on any passive particles in solution or lipid bilayers. The aim of the present study is to analyze the collective hydrodynamic effects that active macromolecules have on passive particles in the medium. We shall show that these effects lead to substantial modifications of the diffusion constants of passive particles. Furthermore, directed drift of passive particles can be induced when there are spatial gradients of active macromolecules, a phenomenon that is reminiscent of chemotaxis.

The investigation of hydrodynamic effects in active fluids is an important field of current research (3, 4). Although the hydrodynamics of bacterial motion has been studied often, the active elements may be of inorganic origin and operate through various flow-generation mechanisms (5–13). There has been a considerable amount of work on swimmers that can propel themselves by cyclically changing their shapes (14). Interactions between such swimmers and their collective flows have been analyzed (15–21). Also, interactions between active hydrodynamic dipoles have been investigated theoretically (22, 23) and experimentally (24, 25). Effects of active dipoles on chromatin dynamics in a two-fluid model have been considered (26).

Hydrodynamic effects on individual protein machines have been studied; for example, investigations of simple models of such machines have shown how they propel themselves and behave under a load (27). Also, the effects of hydrodynamic interactions on the internal dynamics have been analyzed (28). Active protein inclusions in lipid bilayers can act as hydrodynamic dipoles (29) and, under certain conditions, such inclusions can behave as active membrane swimmers (30).

The focus of the present study differs in several respects from the work recounted above. We are not interested in the effects of hydrodynamics on the operation of a single machine. Instead, we concentrate on the advection effects that protein machines can have on passive particles in the medium. Although some proteins can indeed behave as motors, we only require that such machines act as cyclic hydrodynamic force dipoles. Consequently, our analysis concerns the statistical effects that populations of incoherently oscillating dipoles can have on passive particles in the system.

In bulk solution, the laws of 3D hydrodynamics need to be applied; however, as already pointed out by Saffmann and Delbrück (31), biological membranes should behave as 2D fluids when lipid flows in a membrane that occur on scales shorter than a micrometer are considered (31–33). Recently, 2D lipid flows were directly observed in mesoscopic simulations of lipid bilayers (34); additionally, 2D diffusion in biomembranes was experimentally investigated (35). It is well known that 2D hydrodynamics is characterized by the presence of ultra-long-ranged logarithmic interactions that make it qualitatively different from the 3D case (33). We study the hydrodynamic effects of active machines in both 3D and 2D systems.

The outline of the paper is as follows. First, we give a brief discussion of how protein machines undergoing random cyclic changes in response to substrate binding and product release under

Significance

Biological cells contain large numbers of active proteins that repeatedly change their conformations and need a supply of ATP or other substrates to maintain their cyclic operation. Whereas these protein machines have a variety of specific functions, acting as motors, ion pumps, or enzymes, they also induce fluctuating hydrodynamic flows in the cytoplasm. Because such fluctuating flows are nonthermal, energy can be extracted from them and work can be performed. We show that these flows can substantially enhance diffusive motions of passive particles. Furthermore, when gradients in concentrations of active proteins or substrate (ATP) are present, a chemotaxis-like drift should take place. Such nonequilibrium effects are universal: They hold for all passive particles and also for the protein machines themselves.

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nonequilibrium conditions can act as force dipoles. A simple model for an active protein is used to illustrate how force dipole effects arise but our general results do not rely on the specific structure of the model. We then show that a cyclically fluctuating hydrodynamic force dipole will induce diffusive motion and directed drift of a passive particle located at some distance from it. When a population of cyclic hydrodynamic force dipoles is randomly distributed in the medium, they will enhance the diffusion of all passive particles in the medium. Moreover, if such dipoles are nonuniformly distributed and concentration gradients in these species are present, directed flows of all passive particles will be induced. Numerical estimates of the magnitudes of the effects are given, and a discussion of the results is presented.

Protein Machines As Force Dipoles

Molecular machines are biomolecules, most often proteins, that undergo structural changes in shape during their operation cycles. These cyclic shape changes, induced by ligand binding and product release, take place under nonequilibrium conditions; therefore, they differ from thermally induced shape fluctuations for which microscopic reversibility holds and the fluctuation-dissipation theorem applies. These molecular machines operate in a viscous environment and their dynamics takes place under low Reynolds number conditions so that inertia does not play a significant role. As a result, if a force is applied to a particle in the fluid, the same force acts on the fluid.

Such protein machines act as stochastic oscillating force dipoles that can influence the motions of other particles in the system. For example, consider a protein with two domains that operates as an enzyme converting substrate into product molecules. The protein domains close in response to binding of ATP or other substrate molecules and open after the reaction and release of a product. We assume that the substrate is continuously supplied and the products are instantaneously removed from the system. When the substrate binds to the protein new bonds are formed and thus the chemical energy, needed to induce conformational changes and cause the domains to close, is supplied. When the product is released, the additional chemical bonds are broken, leading to domain opening, and the protein returns to its initial state. Within one cycle, an active protein consumes the chemical energy whose net value is determined by the difference in internal energies of the substrate and product molecules. If reverse conversion of a product into the substrate is allowed, an active protein can also operate in the opposite direction. Generally, its cycles are driven by the difference in Gibbs potentials of substrate and product; the sign of this difference determines the operation direction of the machine. Because the net force on the protein is zero, the forces that act on the domains are equal in magnitude and opposite in direction, so that a force dipole is created. This oscillating force dipole will act on the surrounding viscous fluid to generate hydrodynamic flows that can induce motions of passive particles in the fluid. A simple dimer model of such an active protein, where the domains in a bidomain protein are represented by beads, is formulated in *SI Text*.

Hydrodynamic Effects

When a force $\mathbf{F}$ is applied to the fluid at a point $\mathbf{r}$, it produces a fluid flow field at $\mathbf{R}$ that advects a particle at this location with velocity

$$\dot{R}_\alpha = G_{\alpha\beta}(\mathbf{R}-\mathbf{r})F_\beta(\mathbf{r}), \qquad [1]$$

where $G_{\alpha\beta}$ is the mobility tensor which, for sufficiently large distances, can be evaluated in the Oseen approximation. (The Einstein summation convention over repeated indices will be used throughout this paper.) For an oscillating dimer of length x with orientation given by the unit vector $\mathbf{e}$ and interaction force magnitude F, we have $\dot{R}_\alpha = [G_{\alpha\beta}(\mathbf{R}-\mathbf{r}-x\mathbf{e}) - G_{\alpha\beta}(\mathbf{R}-\mathbf{r})]e_\beta F$, because the forces on the dimer beads have equal magnitude but are opposite in direction. If the dimer length x is small compared with the distance $|\mathbf{R}-\mathbf{r}|$, we can approximately write

$$\dot{R}_\alpha = \frac{\partial G_{\alpha\beta}}{\partial r_\gamma} e_\beta e_\gamma m, \qquad [2]$$

where $m(t) = x(t)F(t)$ denotes the magnitude of the force dipole. Although we derived Eq. **2** for a specific two-bead model, it is general. If an object immersed in the fluid changes its shape, it generates a hydrodynamic flow that, at large separations from this object, can be described as being produced by an active force dipole, unless special symmetries are present. Below, we shall treat any active protein machine as a nonequilibrium stochastic force dipole.

Consider a collection of such active force dipoles, located at positions $\{\mathbf{R}_i\}$ with orientations $\{\mathbf{e}_i\}$ and subject to fluctuations arising from thermal and active nonthermal effects. The oscillating dipoles collectively create a fluctuating flow field that induces stochastic advection of a passive particle. At low Reynolds numbers, the passive particle will exactly follow the local flow velocity field. The equation of motion of a passive particle at point R_α may then be written as

$$\dot{R}_\alpha = \sum_i \frac{\partial G_{\alpha\beta}(\mathbf{R}-\mathbf{R}_i)}{\partial R_{i\gamma}} e_{i\beta}e_{i\gamma}m_i(t) + f_\alpha(t). \qquad [3]$$

The passive particle is subject to fluctuations from two sources. The random force $f_\alpha(t)$ has zero mean and satisfies the fluctuation-dissipation relation, $\langle f_\alpha(t)f_{\alpha'}(t')\rangle_n = 2k_BT\gamma\delta_{\alpha\alpha'}\delta(t-t')$, where γ is the mobility coefficient of the passive particle. The particle is also subject to thermal and active nonthermal fluctuations of the force dipoles $m_i(t)$ with zero mean, $\langle m(t)\rangle_n = 0$, and correlation function $\langle m_i(t)m_j(0)\rangle_n = \langle m(t)m(0)\rangle_n\delta_{ij} = S(t)\delta_{ij}$, which defines the force dipole correlation function $S(t) = \langle m(t)m(0)\rangle_n$. For simplicity we have assumed that the force dipole correlations of different proteins are independent. We have further assumed that their orientations are randomly distributed. The force dipole tensor $e_{i\beta}e_{i\gamma}m_i(t)$ of a protein is invariant under inversion of the orientation vector $\mathbf{e}_i$ and has properties like the tensor order parameter in nematic liquid crystals. When correlations among active proteins are taken into account it may be possible that nematically ordered active protein states could be found (26, 36). We shall not consider such effects here.

The force dipole correlation function $S(t)$ plays a central role in our study. This correlation function contains effects that arise from both thermal and nonthermal noise. This correlation function may be written as $S(t) = 2S_T\delta(t) + S_A(t)$, where the thermal component $S_T(t)$ is determined by the temperature, whereas the active component $S_A(t)$ results from nonthermal colored noise. In the absence of substrate, or when the system operates under equilibrium conditions, only the delta correlated thermal contribution remains. The integral intensity $S = \int_0^\infty dt\, S(t) \equiv S_T + S_A$ may also be defined. This quantity will enter in the expressions for the diffusion and drift derived below.

Because the conformational transitions that produce the force dipole depend on substrate binding, the dependence on substrate concentration enters the description through the force dipole correlation function $S(t)$ and, thus, the integral intensity S_A of active force dipoles should depend on the substrate concentration c_S. Although the precise form of the dependence $S_A(c_S)$ can only be determined by considering the kinetics of a particular enzymatic molecular system, general comments on its structure may nonetheless be made. The contribution S_A of active force dipoles must vanish in the absence of substrate because only thermal fluctuations are then present and they are accounted for in S_T. At high enough substrate concentrations the enzymes will operate

at their maximum conversion rates and nonthermal effects will saturate. Consequently, $S_A(c_S)$ should display a similar behavior: a linear proportionality at small substrate concentration c_S and saturation at large concentrations. For example, a functional form like that of Michaelis–Menten kinetics, $S_A(c_S) \sim S_0 c_S/(K+c_S)$, where S_0 is the saturation value of S_A and K is a constant, satisfies these criteria, but other forms for $S_A(c_S)$ with similar limiting regimes may well result from a calculation of S_A for a specific molecular system.

If, within the time interval being considered, the displacements in the position $\mathbf{R}(t)$ of a passive particle are small, we can write $R_\alpha(t) = R_{0,\alpha} + \rho_\alpha(t)$ and retain only terms that are of the first order in the displacements $\rho_\alpha(t)$, so that

$$\dot{\rho}_\alpha = \sum_i \left[\frac{\partial G_{\alpha\beta}(\mathbf{R}_0 - \mathbf{R}_i)}{\partial R_{i,\gamma}} + \frac{\partial^2 G_{\alpha\beta}(\mathbf{R}_0 - \mathbf{R}_i)}{\partial R_{i,\gamma} \partial R_{0,\delta}} \rho_\delta \right] \times e_{i,\beta} e_{i,\gamma} m_i(t) + f_\alpha(t). \quad [4]$$

It is convenient to write the first term on the right side of this equation in field-point notation,

$$\dot{\rho}_\alpha = \int d\mathbf{r} \left[\frac{\partial G_{\alpha\beta}(\mathbf{r})}{\partial r_\gamma} - \frac{\partial^2 G_{\alpha\beta}(\mathbf{r})}{\partial r_\gamma \partial r_\delta} \rho_\delta \right] \times \sum_i e_{i,\beta} e_{i,\gamma} m_i(t) \delta(\mathbf{R}_i - \mathbf{R}_0 - \mathbf{r}) + f_\alpha(t), \quad [5]$$

where the term involving the sum over the proteins represents the microscopic density of force dipoles, $e_{i,\beta} e_{i,\gamma} m_i(t)$, at a point $\mathbf{r}$ with origin at the position $\mathbf{R}_0$ of the passive particle. Note that the first and the second derivatives of the Green function correspond to dipole and quadrupole contributions. This equation shows that the instantaneous position of a passive particle evolves with time according to a stochastic differential equation with nonthermal multiplicative noise arising from the collective operation of active force dipoles, in addition to the additive thermal noise.

The diffusion tensor $D_{\alpha\alpha'}$ and the mean drift velocity $\overline{\mathbf{V}}$ of a passive particle can be determined from

$$D_{\alpha\alpha'} = \int_0^\infty dt \, \langle \delta V_\alpha(t) \delta V_{\alpha'}(0) \rangle, \quad \overline{V}_\alpha = \langle V_\alpha \rangle, \quad [6]$$

where $\delta V_\alpha = V_\alpha - \overline{V}_\alpha$ and the angle bracket $\langle \cdots \rangle$ denotes an average over the stochastic fluctuations, both thermal and nonthermal, as well as the orientations and positions of active force dipoles.

The diffusion tensor and mean drift velocity of the passive particle may be obtained by substituting the expression in Eq. **5** for the velocity $V_\alpha = \dot{\rho}_\alpha$ of a particle, retaining only leading terms, into Eq. **6**. As discussed earlier, when computing the average values in Eq. **6**, we assume that the orientations of active force dipoles are not correlated with their positions so that $\langle \sum_i e_\beta e_{\beta'} e_\gamma e_{\gamma'} \delta(\mathbf{R}_i - \mathbf{R}_0 - \mathbf{r}) \rangle = \langle e_\beta e_{\beta'} e_\gamma e_{\gamma'} \rangle c(\mathbf{r}) \equiv \Omega_{\beta\beta'\gamma\gamma'} c(\mathbf{r})$, where $\Omega_{\beta\beta'\gamma\gamma'} = C_d [\delta_{\beta\beta'}\delta_{\gamma\gamma'} + \delta_{\beta\gamma}\delta_{\beta'\gamma'} + \delta_{\beta\gamma'}\delta_{\beta'\gamma}]$, with $C_2 = 1/8$ and $C_3 = 1/15$ for two and three dimensions, respectively, and $c(\mathbf{r}) = \langle \sum_i \delta(\mathbf{R}_i - \mathbf{r}) \rangle$ is the local concentration of active force dipoles at a point $\mathbf{r}$ in the fluid. We find

$$D_{\alpha\alpha'}(\mathbf{R}_0) = D^T_{\alpha\alpha'}(\mathbf{R}_0) + S_A \Omega_{\beta\beta'\gamma\gamma'} \int d\mathbf{r} \, \frac{\partial G_{\alpha\beta}(\mathbf{r})}{\partial r_\gamma} \frac{\partial G_{\alpha'\beta'}(\mathbf{r})}{\partial r_{\gamma'}} c(\mathbf{R}_0 + \mathbf{r}) \equiv D^T_{\alpha\alpha'}(\mathbf{R}_0) + D^A_{\alpha\alpha'}(\mathbf{R}_0) \quad [7]$$

$$\overline{V}_\alpha(\mathbf{R}_0) = -S_A \Omega_{\beta\beta'\gamma\gamma'} \int d\mathbf{r} \, \frac{\partial^2 G_{\alpha\beta}(\mathbf{r})}{\partial r_\gamma \partial r_\delta} \frac{\partial G_{\delta\beta'}(\mathbf{r})}{\partial r_{\gamma'}} c(\mathbf{R}_0 + \mathbf{r}), \quad [8]$$

where $D^T_{\alpha\alpha'}(\mathbf{R}_0)$ is the equilibrium diffusion tensor of the passive particle averaged over protein configurations. It contains effects arising from the thermal contribution S_T as well as the mobility of the individual passive particle. The last line in Eq. **7** defines the contribution of active force dipoles, $D^A_{\alpha\alpha'}(\mathbf{R}_0)$, to the total diffusion tensor.

We shall now analyze Eqs. **7** and **8** separately for 3D and 2D systems.

3D Systems. For applications to protein machines in bulk solution, for example in the cytoplasm of biological cells, the 3D Green function in the Oseen approximation is $G_{\alpha\beta}(\mathbf{r}) = (8\pi\eta r)^{-1} (\delta_{\alpha\beta} + \hat{r}_\alpha \hat{r}_\beta)$, where η is the fluid viscosity and $\hat{\mathbf{r}}$ is the unit vector specifying the direction of $\mathbf{r}$. Suppose that the machines are uniformly distributed in space with constant concentration c_0. In this case, diffusion is isotropic, $D_{\alpha\alpha'} = D\delta_{\alpha\alpha'}$, and Eq. **7** yields

$$D^A = \frac{S_A c_0}{3} \Omega_{\beta\beta'\gamma\gamma'} \int' d\mathbf{r} \, \frac{\partial G_{\alpha\beta}(\mathbf{r})}{\partial r_\gamma} \frac{\partial G_{\alpha\beta'}(\mathbf{r})}{\partial r_{\gamma'}}, \quad [9]$$

for the active force dipole contribution to the diffusion coefficient. Note that whereas the integral (Eq. **9**) diverges as $1/r$ for short distances r, volume exclusion between an enzyme and the passive particle restricts the domain of integration to distances exceeding some cutoff length ℓ_c. The prime on the integral in Eq. **9** indicates this restriction. Introducing dimensionless coordinates $\mathbf{z} = \mathbf{r}/\ell_c$, the nonthermal diffusion coefficient D^A can be estimated as

$$D^A \approx \zeta_3 \frac{S_A c_0}{\ell_c \eta^2}, \quad [10]$$

where the dimensionless factor ζ_3 is given by

$$\zeta_3 = \frac{\Omega_{\beta\beta'\gamma\gamma'}}{192\pi^2} \int' d\mathbf{z} \, \frac{\partial g_{\alpha\beta}(\mathbf{z})}{\partial z_\gamma} \frac{\partial g_{\alpha\beta'}(\mathbf{z})}{\partial z_{\gamma'}} = \frac{1}{60\pi}, \quad [11]$$

and the dimensionless function $g_{\alpha\beta}(\mathbf{z}) = z^{-1}(\delta_{\alpha\beta} + \hat{z}_\alpha \hat{z}_\beta)$.

Turning to Eq. **8**, we notice that the drift velocity vanishes for a uniform distribution of active dipoles. Suppose instead that a constant concentration gradient in the direction $\hat{\mathbf{n}}$, $\nabla c = \hat{\mathbf{n}}(\hat{\mathbf{n}} \cdot \nabla c) = \hat{\mathbf{n}}(\nabla c)$, is present and $c(\mathbf{r}) = c_0 + (\mathbf{r} \cdot \hat{\mathbf{n}}) \nabla c$. Now, the integration in Eq. **8** yields

$$\overline{V}_\alpha = -S_A \Omega_{\beta\beta'\gamma\gamma'} \int' d\mathbf{r} \, \frac{\partial^2 G_{\alpha\beta}(\mathbf{r})}{\partial r_\gamma \partial r_\delta} \frac{\partial G_{\delta\beta'}(\mathbf{r})}{\partial r_{\gamma'}} (\mathbf{r} \cdot \hat{\mathbf{n}}) \nabla c. \quad [12]$$

Again, introducing dimensionless coordinates $\mathbf{z} = \mathbf{r}/\ell_c$, we find

$$\overline{\mathbf{V}} \approx \xi_3 \frac{S_A}{\ell_c \eta^2} \nabla c, \quad [13]$$

where the positive dimensionless factor ξ_3 is

$$\xi_3 = -\frac{\Omega_{\beta\beta'\gamma\gamma'}}{64\pi^2} \int' d\mathbf{z} \, \hat{n}_\alpha \frac{\partial^2 g_{\alpha\beta}(\mathbf{z})}{\partial z_\gamma \partial z_\delta} \frac{\partial g_{\delta\beta'}(\mathbf{z})}{\partial z_{\gamma'}} (\mathbf{z} \cdot \hat{\mathbf{n}}) = \frac{1}{30\pi}. \quad [14]$$

Because ξ_3 is positive the drift is in the direction of the enzyme concentration gradient. We further note for 3D that the dominant contributions to the integrals for the general expressions for the diffusion and drift given in Eqs. **7** and **8** come from short distance separations, so Eqs. **10** and **13** also hold for arbitrary concentration distributions; in this case local concentration values and gradients should be taken to determine the effects at a given point.

2D Systems. As noted by Saffman and Delbrück (31), biological membranes should behave as 2D lipid fluids on submicrometer

length scales. Therefore, the effects of an ensemble of active protein machines on the motion of a passive particle in a lipid bilayer provides an example where a 2D description is appropriate. In the Oseen approximation, the 2D Green function of lipid bilayers is (32)

$$G_{\alpha\beta}(\mathbf{r}) = (4\pi\eta_m)^{-1}\left(-(1+\ln(\kappa r))\delta_{\alpha\beta} + \hat{r}_\alpha \hat{r}_\beta\right). \quad [15]$$

Here, η_m is the 2D viscosity of the lipid bilayer, which is related to its 3D viscosity η_L by $\eta_m = \eta_L h$, where h is the thickness of the bilayer. In contrast to the 3D case, hydrodynamic interactions in 2D are ultra-long-ranged, owing to the logarithmic dependence on the distance r. For biomembranes, one can use the estimate (32) $\kappa^{-1} = \eta_L h/(2\eta)$, where η is the viscosity of the surrounding aqueous medium. Typically, κ^{-1} is of the order of a micrometer. At separations larger than this distance, 3D effects become important for biomembranes. Therefore, our analysis applies only for relatively small membranes of micrometer size; for larger membranes the crossover to 3D hydrodynamics at long length scales needs to be taken into account.

The general expressions in Eqs. **7** and **8** hold in the 2D case as well; however, because of the logarithmic distance dependence in the Green function, hydrodynamic effects are nonlocal. Therefore, it is not possible to obtain precise estimates similar to those in Eqs. **10** and **13** for such systems. Still, some estimates can be made.

Consider a passive particle at the center of a circular membrane with radius ℓ_0 so that 2D behavior applies. If we again consider a uniform distribution of active enzymes, the diffusion coefficient given by Eq. **7** is isotropic at the center of the membrane. Introducing the rescaled dimensionless coordinates $\mathbf{z} = \mathbf{r}/\ell_0$, we obtain

$$D^A \approx \zeta_2 \frac{S_A c_0}{\eta_m^2}, \quad [16]$$

where the dimensionless factor ζ_2 is given by

$$\zeta_2 = \frac{\Omega_{\beta\beta'\gamma\gamma'}}{32\pi^2}\int' d\mathbf{z}\ \frac{\partial q_{\alpha\beta}(\mathbf{z})}{\partial z_\gamma}\frac{\partial q_{\alpha\beta'}(\mathbf{z})}{\partial z_{\gamma'}} = \frac{1}{32\pi}\ln\frac{\ell_o}{\ell_c}. \quad [17]$$

Here $q_{\alpha\beta}(\mathbf{z}) = -\ln(z)\delta_{\alpha\beta} + \hat{z}_\alpha\hat{z}_\beta$. The integral (Eq. **17**) diverges logarithmically at $z = 0$ and a cutoff at $z = \ell_c/\ell_0$ has been introduced.

In a similar manner, the drift velocity in 2D systems can be estimated. Taking $c(\mathbf{r}) = c_0 + (\mathbf{r}\cdot\hat{\mathbf{n}})\nabla c$ in Eq. **8**, and changing variables as indicated above, we find

$$\overline{\mathbf{V}} \approx \zeta_2 \frac{S_A}{\eta_m^2}\nabla c, \quad [18]$$

where

$$\xi_2 = -\frac{\Omega_{\beta\beta'\gamma\gamma'}}{16\pi^2}\int' d\mathbf{z}\ \hat{n}_\alpha \frac{\partial^2 q_{\alpha\beta}(\mathbf{z})}{\partial z_\gamma \partial z_\delta}\frac{\partial q_{\delta\beta'}(\mathbf{z})}{\partial z_{\gamma'}}(\mathbf{z}\cdot\hat{\mathbf{n}}) = \frac{1}{32\pi}\ln\frac{\ell_o}{\ell_c}, \quad [19]$$

so that $\xi_2 = \zeta_2$. Note that because the integrals in Eqs. **17** and **19** diverge logarithmically at large distances, the finite size of the membrane is important. Moreover, as follows from Eq. **19** and similar to the 3D case, passive particles drift toward higher concentrations of active proteins.

If the passive particle is not at the center of the domain or concentration distributions of active protein inclusions are more general than the constant and linear-gradient distributions considered above, the diffusion will no longer be isotropic and the diffusion and drift will depend on the concentration distribution $c(\mathbf{r})$ over the entire membrane, in contrast to the 3D case where only the local structure of the enzyme concentration field in the neighborhood of the passive particle is of importance.

Numerical Estimates

The magnitude of a force dipole m of a protein machine can be roughly estimated as $m \sim F d_P$, where F is the force generated by the machine and d_P is the linear size of the protein. Molecular motors, such as myosin or kinesin, typically generate forces about 1 pN and this can be chosen as the characteristic value for F. Taking the size of a protein to be about 10 nm, the force dipole can be estimated to be about $m = 10^{-20}$ N·m. The correlation time for force-dipole fluctuations can be taken to be the duration t_c of the cycle time in a chemical machine. Although enzyme cycle times vary widely, we choose a time of about $t_c \sim 1$ ms. The parameter S_A can then be evaluated to give $S_A \sim m^2 t_c = 10^{-43}$ N^2·m^2·s. Note that this estimate corresponds to substrate (typically ATP) saturation conditions: The machine binds a new substrate molecule and enters into a new cycle immediately once the previous cycle finishes. If this condition is not satisfied, the protein machine must wait for a new substrate molecule to arrive. During this waiting period, the machine does not act as a force dipole and this will decrease the value of S_A. Obviously, the effects disappear when the substrate is not supplied.

Concentrations of active proteins inside a biological cell can vary over a large range. The highest concentrations of the order of 10^{-4} M are characteristic for the enzymes involved in glycolysis. As a rough estimate, a value of 10^{-6} M can be chosen, corresponding to $c_0 = 10^{21}$m^{-3}, so that the mean distance between the proteins being considered is about 100 nm. Given the protein size, we choose a cutoff length of $\ell_c = 10$ nm. The viscosity of water is about 10^{-3} Pa·s. For the dimensionless numerical factors in Eqs. **11** and **14** we take their order-of-magnitude values $\zeta_3 = 10^{-2}$ and $\xi_3 = 10^{-2}$.

With these values, the contribution (Eq. **10**) to the diffusion coefficient owing to hydrodynamic effects arising from protein machines in bulk 3D solutions is about $D^A \approx 10^{-6}$cm^2/s. This result should be compared with typical diffusion constants in water that can vary from about 10^{-5}cm^2/s for small molecules to 10^{-7}cm^2/s for small proteins in water. If one takes the viscosity of the cytoplasm to be two to four times that of water, the active contribution D^A will decrease by approximately an order of magnitude. However, it is still approximately the same as the thermal Brownian contribution $D^T \approx k_B T\gamma = k_B T/(6\pi\eta R_p)$, where R_p is the radius of the passive particle, under the same viscosity conditions using a Stokes law estimate. To estimate the magnitude of the drift velocity from Eq. **13**, we can take $\nabla c = \Delta c/L$, where Δc is the concentration difference across the cell and $L = 10\ \mu$m is the typical eukaryotic cell size. If we choose $\Delta c \approx 0.1 c_0$, where again $c_0 = 10^{21}$m^{-3}, we obtain a drift velocity magnitude of about $\overline{V} \approx 1\ \mu$m/s.

Proceeding to lipid bilayers, we observe that their 3D viscosity $\eta_L \sim 1$ Pa·s is typically a factor of 10^3 higher than that of water. The 2D viscosity of such bilayers is $\eta_m = \eta_L h$, where $h \sim 1$ nm has been taken as the thickness of the bilayer. The magnitude $S \sim 10^{-43}$ N^2·m^2·s may again be used for the force dipoles. Taking the mean distance between proteins to be ~100 nm, the 2D concentration is about $c_0 = 10^{14}$ m^{-2}. As rough estimates of these factors we again choose their order-of-magnitude values, $\zeta_2 = \xi_2 = 10^{-2}$. A membrane of micrometer size is considered and a cut-off distance of 10 nm is introduced.

Given these numerical values, the hydrodynamic effects of active protein inclusions are predicted to increase the diffusion of passive particles within the membrane by about $D^A \approx 10^{-9}$cm^2/s. For comparison, Brownian diffusion constants for proteins in lipid bilayers are of the order of 10^{-10}cm^2/s and diffusion constants for lipids are about 10^{-8}cm^2/s. The characteristic magnitude of the drift velocity of passive particles in lipid bilayers is estimated to be about $\overline{V} \approx 10^{-2}\ \mu$m/s, assuming that $\Delta c = 0.1 c_0$ and the characteristic length for concentration variation in a membrane is about 1 μm.

These numerical estimates should be used only as rough guide to the possible magnitudes of the effects because many of the parameters may vary significantly from one system to another, or are known only poorly. For example, forces have only been measured for some molecular motors and, for protein machines that are not motors or for enzymes, they may be smaller than 1 pN. However, the concentrations of some proteins that behave as force dipoles may be significantly higher than the value we have assumed. For example, the enzyme phosphoglycerate kinase involved in glycolysis is present in the living cell in the concentrations up to 10^{-4} M, two orders of magnitude larger than our assumed value. Proteins typically contribute about 40% of the mass in biomembranes and their concentrations may well be significantly higher than our 2D values. In addition, the cytoplasm is a very complicated medium that is crowded by a variety of macromolecules, filaments, organelles, and other structures (37). Diffusion in such a crowded environment differs from that in a simple solution. Crowding can also influence the hydrodynamic effects owing to active force dipoles discussed in this paper. Such effects will have to be considered in descriptions of transport in the cell that model the detailed structure of the cytoplasm. Consequently, the uncertainty in the estimates of hydrodynamic effects is high and deviations of up to two orders of magnitude from the numerical estimates given above may well be possible.

The effects considered here depend on the concentration of substrate through $S_A(c_S)$ as discussed earlier. If the substrate concentration varies in a cell or a membrane, the coordinate-dependent dipole intensity S_A should be retained within integrals in Eqs. **7** and **8**. In three dimensions, diffusion is determined by the local dipole force intensity according to Eq. **10**, whereas the drift velocity is determined by the local gradient of $S_A(r)c(r)$, replacing the gradient of concentration in Eq. **13**. In two dimensions, local diffusion and drift are generally dependent on the concentration distribution of active inclusions and substrates over the entire membrane.

Discussion and Conclusions

In a biological cell there are large populations of active proteins, both molecular machines and enzymes, that change their conformations within catalytic cycles. In this paper we showed that when active proteins are present, either in solution or in lipid bilayers, they can substantially modify the diffusion constants of passive particles in the system. These modifications affect all passive particles, and all active proteins, even of different kinds, contribute to the effect provided they are supplied with substrate and remain active. The magnitude of the effect can be comparable to the value of Brownian diffusion constants under physiological conditions.

Furthermore, if protein machines are nonuniformly distributed in a cell or in a biomembrane, directed drift of passive particles, analogous to chemotaxis, can occur. However, the mechanism is completely different: All active proteins contribute toward it and all passive particles experience the drift. Drift velocities of the order of micrometers per second can be realized. The enhancement of diffusion and chemotaxis-like drift should take place for the protein machines (enzymes) themselves as well. Note that the drift velocity is in the same direction as the concentration gradient and therefore the hydrodynamic attraction of incoherent active proteins should occur. Generally, the same proteins would exhibit different interactions depending on whether they are catalytically active or inactive (no ATP is supplied). However, thus far we have not considered collective effects due to hydrodynamic interactions on the populations of active proteins. It may be that orientation alignment leading to nematic order (26, 36) and cycle synchronization also arise.

In three dimensions, hydrodynamic interactions are already long-ranged, with power-law dependence. In two dimensions they become ultra-long-ranged with a logarithmic dependence on distance. Thus, the effects predicted to exist in 3D and 2D systems differ substantially. In solution, the change in the diffusion constant is determined by the local protein machine concentration and the drift velocity is controlled by its local spatial gradient. In contrast, in 2D systems such as lipid bilayers, the effects are essentially nonlocal: The change in the diffusion constant and the drift of passive particles at a given location are determined by the distribution of active inclusions over the entire membrane. Note, however, that only relatively small membranes with micrometer sizes were considered here. In general, diffusion in biomembranes should be anisotropic, reflecting the asymmetry of protein distribution and the membrane shape.

Our description of how active molecular machines, through hydrodynamic interactions, influence the dynamics of passive particles was based on the equations of continuum hydrodynamics. One might question the use of such a continuum description for molecular systems. It is well established (38) that hydrodynamic effects are observable on even very small scales of tens of solvent particle diameters. They persist despite strong fluctuations and their presence is signaled in the long-time tails of velocity correlation functions or even in the transport properties of polymers. Our use of continuum equations is restricted to rather long scales so that the main conclusions of our study should be robust.

Sen and coworkers (39, 40) have shown that catalytically active enzymes have larger diffusion coefficients than their inactive counterparts in the absence of substrate. Recently, chemotaxis-like drift of enzymes in the presence of substrate gradients has been observed and used for sorting of the enzymes (41). Although additional analysis of the experimental data is needed, it may be that such observations can be explained by the effects considered above. Furthermore, in vivo studies have revealed that the diffusion of particles decreases in ATP-depleted biological cells (42). In addition, several studies have proposed specific explanations for the importance of nonthermal random motions in living cells, whose origin lies in the forces generated by the uncorrelated activity of protein machines. (26, 42–44) Such nonthermal fluctuations may also be a consequence of the universal hydrodynamic effects, described here, that arise from active conformational changes in molecular motors and other protein machines powered by ATP.

Our analysis focused on general aspects of the phenomena and is not intended to address the full complexity seen in biological systems. Nevertheless, in accord with the above findings, our results suggest a modified physical picture of kinetic processes in the biological cell. When energy is supplied by ATP or other substrates to active proteins in a cell, such as molecular motors, other protein machines, or enzymes, they cyclically change their shapes in the course of carrying out their various specific functions. In addition to their functions, all such proteins act as oscillating active force dipoles and collectively create a fluctuating hydrodynamic field over the entire cell or a biomembrane. This nonequilibrium flow field can be maintained because a fraction of the energy flux arriving with substrates is diverted through the force-dipole activity to hydrodynamic flows in the cytoplasm. Such fluctuating fields arise from nonequilibrium effects; therefore, in contrast to thermal hydrodynamic fluctuations, the fluctuation-dissipation theorem does not apply to them. Because these fluctuating fields arise from nonthermal noise, it is possible that they can be rectified and work or energy can be extracted from them. Thus, active proteins in a cell not only execute their specific functions but, collectively, they supply power in a distributed way to the system. Such power, originating from substrate supply to active proteins, spans the entire cell.

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