Fluctuation-Driven Molecular Transport Through an Asymmetric Membrane Channel

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Channel proteins that selectively conduct molecules across cell membranes often exhibit an asymmetric structure. By means of a stochastic model, we argue that channel asymmetry in the presence of nonequilibrium fluctuations, fueled by the cell's metabolism as observed recently, can dramatically influence the transport through such channels by a ratchetlike mechanism. For an aquaglyceroporin that conducts water and glycerol, we show that a previously determined asymmetric glycerol potential leads to enhanced inward transport of glycerol, but for unfavorably high glycerol concentrations also to enhanced outward transport that protects a cell against poisoning.

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Living cells interact with their extracellular environment through the cell membrane, which acts as a protective permeability barrier for preserving the internal integrity of the cell. However, cell metabolism requires controlled molecular transport across the cell membrane, a function that is fulfilled by a wide variety of transmembrane proteins, acting as passive and active transporters [1]. Channel proteins as passive transporters facilitate the diffusion of specific molecules across the membrane down a free energy gradient. Active transporters conduct molecules along or against the free energy gradient consuming for that purpose external energy. However, the plasma membrane of living cells is subject to a variety of nonequilibrium, i.e., nonthermal processes, e.g., interaction with the cytoskeleton and with active membrane proteins [2,3]. In this Letter we want to argue that in an active plasma membrane, even passive channel proteins can act as active transporters by consuming energy from nonequilibrium fluctuations fueled by cell metabolism [4].

The *Escherichia coli* glycerol uptake facilitator (GlpF) is an aquaglycerol channel protein, which transports both water and glycerol molecules, but excludes charged solutes, e.g., protons [5]. The recently determined 3D structure of GlpF at atomic resolution [6] (Fig. 1) has provided much insight into the underlying microscopic mechanism of molecular transport and selectivity through GlpF [7]. In particular, molecular dynamics (MD) studies [8–11] established that water and glycerol diffusion through GlpF is single file, and for biologically relevant periplasmic glycerol concentrations, correlation effects between consecutive glycerol molecules are negligible due to their large spatial separation. The corresponding *potential of mean force* (PMF) [8] that guides the transport of glycerol through the channel is highly asymmetric reflecting the atomic structure of GlpF (see Fig. 1), with a prominent potential well at the external (periplasmic) side and a constriction region with several pronounced potential barriers towards the internal (cytoplasmic) side of the channel [6,12]. Besides GlpF, there are several other channels that exhibit similar spatial asymmetry [13–15], and in spite of recent efforts, no biological function could be attributed to the asymmetry [16]. Here we propose and demonstrate that under realistic physiological conditions, the asymmetry of GlpF furnishes active glycerol transport through a *ratchetlike* mechanism. In general, the ratchet effect refers to the generation of directed motion of Brownian particles in a spatially *periodic* and *asymmetric* (ratchet) potential in the presence of *nonequilibrium* fluctuations and/or externally applied time-periodic force with zero mean [17–19]. Since cell membranes are subject to nonequilibrium fluctuations, which span a wide range of time and length scales [2,4], one expects a ratchet effect contribution to the transport through asymmetric channel proteins, such as GlpF, even if the PMF is nonperiodic. Assuming simplified, heuristic models, the ratchet effect has been invoked before to explain the functioning of active biomolecules, e.g., motor proteins and ATP hydrolysis driven ion pumps [17,20]. To our knowledge, this Letter uses for the first time a realistic, microscopically determined PMF to in-

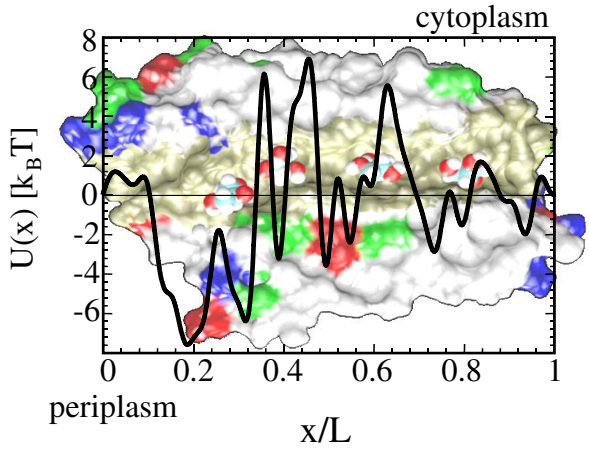

FIG. 1 (color online). Section through the glycerol conduction pathway in GlpF and the corresponding asymmetric PMF (solid curve) reported in [8].

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vestigate the precise role of nonequilibrium fluctuations in facilitated transport. We find that as a result of channel asymmetry, glycerol uptake driven by a concentration gradient is enhanced significantly in the presence of nonequilibrium fluctuations. Furthermore, the ratchet effect-caused enhancement is larger for the outward, i.e., from the cytoplasm to the periplasm, flux than for the inward one, suggesting that nonequilibrium fluctuations also play an important role in protecting the interior of the cell against excess uptake of glycerol.

Glycerol transport through GlpF can be modeled in terms of overdamped Brownian motion along the axis of the channel as a result of the concentration gradient established at the ends of the channel. The interaction of a diffusing glycerol molecule with the protein, solvent, lipid, and other glycerol molecules is taken into account through the PMF, $U(x)$, as determined from steered molecular dynamics simulations by employing the Jarzynski equality [8]. The motion of a glycerol molecule inside GlpF and in the presence of an external force $F(t)$ in the strong friction limit is described by the Langevin equation

$$\gamma\dot{x} = f(x) + \xi(t) + F(t), \tag{1}$$

where γ is the friction coefficient, $f(x) = -U'(x)$ is the deterministic force derived from the PMF, and $\xi(t)$ is the Langevin force due to the equilibrium thermal fluctuations. As usual, $\xi(t)$ is a Gaussian white noise with $\langle\xi(t)\rangle = 0$ and $\langle\xi(t)\xi(0)\rangle = 2D\gamma^2\delta(t)$, where $\delta(t)$ is the Dirac-delta function, and D is the effective diffusion coefficient of a glycerol molecule inside GlpF. According to the *fluctuation-dissipation* theorem, D and γ are related through the Einstein relation $D = k_BT/\gamma$. We assume that $F(t)$ is time-dependent, but homogeneous. It describes either an externally applied force, or some intrinsic nonequilibrium fluctuations of the system (see below). Because of the single file nature of the glycerol transport through GlpF, a force applied at either end of the channel will be transmitted along the file without significant loss in intensity (*incompressibility* of the single file), which justifies our assumption that $F(t)$ is homogeneous along the channel. For a periodic $f(x)$, Eq. (1) describes what is often referred to as a fluctuating force (tilting) ratchet [17].

At this point, we introduce dimensionless units that will be employed throughout this Letter, unless otherwise stated. All other units can be expressed in terms of the following three: length of GlpF $\mathcal{L} = L \approx 4.8$ nm, diffusion time $\mathcal{T} = \tau_D = L^2/D \approx 10^{-7}$ s, and thermal energy $\mathcal{E} = k_BT \approx 4.28 \times 10^{-21}$ J; here k_B is the Boltzmann constant, $T = 310$ K is the physiological temperature, and $D \approx 2.2 \times 10^{-10}$ m^2/s is the effective diffusion coefficient of glycerol inside GlpF [16]. Thus, the force unit is $\mathcal{F} = \gamma D/L = k_BT/L \approx 0.9$ pN. In the new units, the Fokker-Planck equation (FPE) corresponding to Eq. (1) reads

$$\partial_t p(x, t) = -\partial_x J(x, t), \tag{2a}$$

$$J(x, t) = -\partial_x p(x, t) + [f(x) + F(t)]p(x, t), \tag{2b}$$

where $p(x, t)dx$ is the (unnormalized) probability of finding a glycerol molecule in $(x, x + dx)$ (see Fig. 1), and $J(x, t)$ is the local, instantaneous flux of glycerol through the channel. The probability density $p(x)$ is related to the local concentration $C(x)$ by $p(x) = S(x)C(x)$, with $S(x)$ the area of the channel cross section. From the crystal structure, one finds that the opening area at both ends of GlpF is $S_0 \equiv S(0) \approx S(1) \approx 100$ Å^2 [6].

GlpF can be regarded as a nanopore, which connects two reservoirs of glycerol molecules located at $x = 0$ (periplasm) and $x = 1$ (cytoplasm), respectively, (Fig. 1). The glycerol uptake is driven by a concentration gradient: glycerol concentration (and therefore $p_0 \equiv p(0)$) is finite in the periplasm and vanishingly small in the cytoplasm ($p_1 \equiv p(1) \approx 0$). Indeed, once glycerol enters the cytoplasm it gets phosphorylated by glycerol kinase and, as a charged particle, the product glycerol phosphate cannot leave the cell [21]. However, excessive accumulation of glycerol phosphate may result in cell poisoning and death. To avoid this, the enzyme glycerol kinase is genetically turned off, preventing further glycerol phosphorylation [21]. Since the glycerol concentration gradient across the cell membrane persists, one expects that glycerol uptake should continue in spite of its potential damaging effect on the cell until this gradient vanishes (i.e., $p_1 = p_0$). Below we demonstrate that channel asymmetry, combined with nonequilibrium fluctuations, can stop glycerol uptake against the persistent concentration gradient, keeping the cytoplasmic glycerol at a level $p_1 < p_0$. To this end, we calculate the steady glycerol flux through GlpF in four distinct cases corresponding to suitable choices of $F(t)$.

Transport driven by concentration gradient [$F(t) = 0$].— In the steady state, the flux is constant throughout the channel [c.f. Eq. (2a)] and, in the absence of the external force $F(t)$, is given by

$$J(p_0, p_1) = A_0p_0 - A_1p_1, \tag{3a}$$

$$A_i = e^{U_i}\left[\int_0^1 e^{U(x)}dx\right]^{-1}, \qquad i = 0, 1. \tag{3b}$$

Since the PMF $U(x)$ vanishes on both sides of the channel, i.e., $U_0 \equiv U(0) = U(1) \equiv U_1 = 0$ (see Fig. 1), one has $A_0 = A_1$, and the flux is proportional to the glycerol concentration difference, i.e., $J(p_0, p_1) = A_0(p_0 - p_1) = A_0S_0(C_0 - C_1)$. Hence, the flux vanishes in the absence of a concentration gradient, and the flux is insensitive to the asymmetry of the channel. In order to produce directed transport, one needs to drive the system out of equilibrium, e.g., by applying an external force or by subjecting the system to nonequilibrium fluctuations.

Transport driven by potential gradient [$F(t) = F_0 =$ const].— The dependence of the flux on the asymmetry of the channel is manifest when $U_0 \neq U_1$,

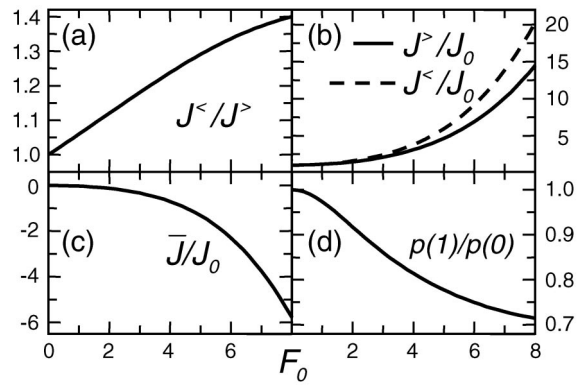

FIG. 2. (a) Ratio of outward and inward glycerol fluxes in GlpF as function of a constant force F_0. (b)–(c) Transport induced by a square-wave force in GlpF. Shown are the inward $[p_1 = 0]$, outward $[p_0 = 0]$, and equal concentration $[p_1 = p_0]$ fluxes vs force amplitude F_0. (d) Ratio of the inner to outer glycerol concentrations vs F_0 for vanishing flux.

i.e., in the presence of a potential gradient. The constant external force leads to an effective, tilted PMF, $U_{eff}(x) = U(x) - F_0 x$, and according to Eqs. (3) the stationary flux reads

$$J(F_0|p_0, p_1) = A_0(F_0)p_0 - A_1(F_0)p_1 \tag{4a}$$

$$A_0(F_0) = \left[\int_0^1 e^{U_{\rm eff}(x)}dx\right]^{-1}, \quad A_1(F_0) = A_0(F_0)e^{-F_0}. \tag{4b}$$

For a symmetric PMF, i.e., $U(1-x) = U(x)$, follows $A_1(-F_0) = A_0(F_0)$, implying that the (magnitude of the) flux is also symmetric $|J(F_0|p_0, p_1)| = |J(-F_0|p_1, p_0)|$. In general, for a generic asymmetric PMF, the inward and outward fluxes will be different. Under normal conditions when $p_1 = 0$, the inward flux is $J^> = A_0(F_0)p_0$. The same flux through an inverted channel, i.e., $(p_0, p_1) \to (0, p_0)$ and $F_0 \to -F_0$, would be $J^< = A_1(-F_0)p_0$. It can be readily checked that $J^{\gtrless}/J_0 > 1$, i.e., the constant driving force enhances the flux when applied along the concentration gradient. Furthermore, according to Fig. 2(a), under identical conditions, the flux through the inverted channel is always larger than the flux through the normally oriented GlpF, the ratio of the two increasing monotonically with F_0.

Transport driven by an external periodic driving force.— Next, we consider an external force $F(t)$ that switches periodically between $\pm F_0$, $F_0 =$ const (*square-wave* force). Although the time average of $F(t)$ is zero, this force induces a finite flux through GlpF even in the absence of a concentration gradient. Indeed, assuming that one can neglect the transient in the instantaneous flux after switching $F(t)$, the mean flux $\bar{J}$ through the channel can be expressed as $\bar{J}(F_0|p_0, p_1) = [J(F_0|p_0, p_1) + J(-F_0|p_0, p_1)]/2$, where $J(F_0|p_0, p_1)$ is given by Eq. (4a). Then, for $p_1 = p_0$, $\bar{J}/J_0 \equiv \bar{J}(F_0|p_0, p_0)/\bar{J}(0|p_0, 0)$ is negative, and decreases monotonically with F_0, as shown in Fig. 2(c). In this case, too, $\bar{J} < 0$ implies that for GlpF the outward flux $J^< = -J(-F_0|0, p_0)$ is bigger than the inward flux $J^> = J(F_0|p_0, 0)$ as shown in Fig. 2(b), and both fluxes $J^{\gtrless}$ are bigger than J_0, the flux in the absence of the external force. Furthermore, the concentration ratio p_1/p_0 at which the flux through the channel vanishes (current reversal), i.e., $\bar{J}(F_0|p_0, p_1) = 0$, is plotted as a function of F_0 in Fig. 2(d). The values $p_1/p_0 < 1$ found are expected and consistent with the fact that for the same force level and concentration gradient, the outward flux is larger than the inward flux.

Transport driven by nonequilibrium fluctuations.— Finally, we consider the effect of nonequilibrium fluctuations of the cell membrane on the glycerol transport through GlpF. We model such fluctuations by a *random telegraph force* (RTF), i.e., a homogeneous *dichotomous* force $F(t)$, which switches between two states $\pm F_0$ with

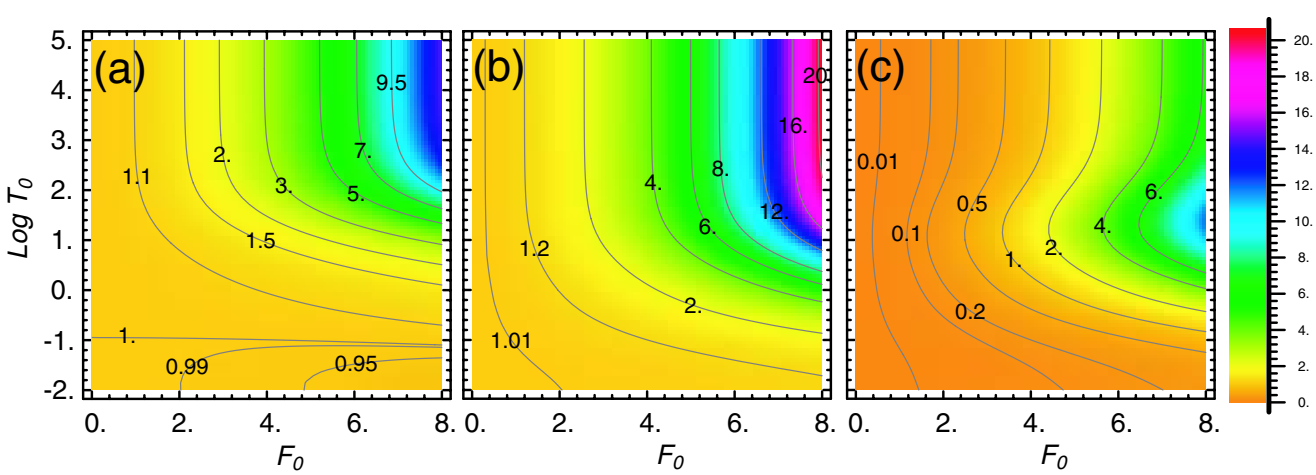

FIG. 3 (color online). Contour density plots of the numerically evaluated relative (a) inward $J(F_0, T_0|p_0, 0)/J_0$, (b) outward $-J(F_0, T_0|0, p_0)/J_0$, and (c) equal concentration $-J(F_0, T_0|p_0, p_0)/J_0$ glycerol fluxes in GlpF as a function of the amplitude F_0 and mean switching time T_0 of the RTF; $J_0 \equiv J(0, T_0|p_0, 0)$ is the inward flux in the absence of the RTF.

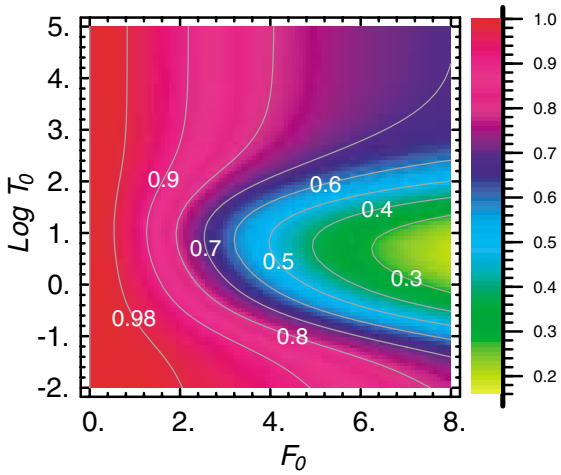

FIG. 4 (color online). Contour density plot of the concentration ratio $p_1/p_0 = \mathcal{A}_0/\mathcal{A}_1$ at which current reversal ($J = 0$) takes place in GlpF subjected to RTF.

switching times that obey a Poisson distribution. For the RTF holds $\langle F(t)\rangle = 0$, and $\langle F(t)F(0)\rangle = F_0^2 e^{-2t/T_0}$, where T_0 is the mean switching time. The stationary Fokker-Planck equation in this case consists of two coupled equations, $-\partial_x^2 p_\pm(x) + \partial_x[f(x)p_\pm(x)] \pm F_0 p_\pm(x) - p_\pm(x)/T_0 + p_\mp(x)/T_0 = 0$, where $p_\pm(x)$ is the conditional probability density that the RTF is in the $\pm F_0$ state. The corresponding flux is $J = -p'(x) + f(x)p(x) + F_0\Delta p(x) =$ const, where $p = p_+ + p_-$ is the total probability density, and $\Delta p = p_+ - p_-$. For the feature-rich GlpF PMF (Fig. 1), the flux $J(F_0, T_0|p_0, p_1) = \mathcal{A}_0(F_0, T_0)p_0 - \mathcal{A}_1(F_0, T_0)p_1$ needs to be computed numerically, e.g., by employing a matrix continued-fraction method. The computed flux is shown in Fig. 3 as two-dimensional density plots for $F_0 \in [0, 8]$ and $T_0 \in [10^{-2}, 10^5]$ (logarithmic scale). In SI units, these values correspond to $F_0 \in [0, 7.2]$ pN and $T_0 \in [10^{-9}, 10^{-2}]$ s, respectively. The conclusions drawn from Fig. 3 are consistent with our previous findings for externally applied deterministic forces. First, the RTF-induced asymmetry between the inward and outward fluxes is manifest [Figs. 3(a) and 3(b)], with bigger outward flux for the same F_0, T_0, and concentration gradient. Second, the RTF-induced flux enhancement is more pronounced for slower fluctuations and for larger F_0. Third, the flux in the absence of a concentration gradient across the membrane is always outward. Thus, the ratio of the inner to outer glycerol concentrations at which glycerol uptake ceases is always less than 1, as shown in Fig. 4.

Our calculations have demonstrated that nonequilibrium force fluctuations acting on glycerol in GlpF can have an important effect on the glycerol uptake by a cell. On the one hand, slow, large amplitude fluctuations enhance the concentration gradient-driven glycerol uptake, which may be vital for the cell under poor nutrient conditions. On the other hand, when glycerol is abundant, fluctuations provide an effective protection mechanism to the cell by stopping glycerol uptake well before the cytoplasmic concentration reaches the periplasmic one. The mechanism underlying this behavior is related to the ratchet effect and depends sensitively on the asymmetric shape of the PMF characterizing glycerol conduction in GlpF. The effects described should be testable experimentally. In fact, recent experiments have demonstrated that cell membranes are subject to slow, kHz frequency ($\log T_0 \sim 10^5$) nonequilibrium fluctuations that may be related to pumping mechanisms by which cells supplement the passive diffusion of nutrients [4].

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[1] B. Alberts *et al.*, *Molecular Biology of the Cell* (Garland Science, New York, 2002), 4th ed.
[2] S. Levin and R. Korenstein, Biophys. J. **60**, 733 (1991); L. Mittelman, S. Levin, and R. Korenstein, FEBS Lett. **293**, 207 (1991).
[3] S. Ramaswamy, J. Toner, and J. Prost, Phys. Rev. Lett. **84**, 3494 (2000).
[4] A. E. Pelling *et al.*, Science **305**, 1147 (2004).
[5] M. Borgnia, S. Nielsen, A. Engel, and P. Agre, Annu. Rev. Biochem. **68**, 425 (1999).
[6] D. Fu *et al.*, Science **290**, 481 (2000); P. Nollert *et al.*, FEBS Lett. **504**, 112 (2001).
[7] E. Tajkhorshid *et al.*, Science **296**, 525 (2002); B. Roux and K. Schulten, Structure **12**, 1 (2004), and references therein.
[8] M. Ø. Jensen, S. Park, E. Tajkhorshid, and K. Schulten, Proc. Natl. Acad. Sci. U.S.A. **99**, 6731 (2002).
[9] B. L. de Groot and H. Grubmüller, Science **294**, 2353 (2001).
[10] M. Ø. Jensen, E. Tajkhorshid, and K. Schulten, Structure **9**, 1083 (2001).
[11] P. Grayson, E. Tajkhorshid, and K. Schulten, Biophys. J. **85**, 36 (2003).
[12] M. S. P. Sansom and R. J. Law, Curr. Biol. **11**, R71 (2001).
[13] S. W. Cowan *et al.*, Nature (London) **358**, 727 (1992).
[14] Y. Wang *et al.*, J. Mol. Biol. **272**, 56 (1997).
[15] D. Forst, W. Welte, T. Wacker, and K. Diederichs, Nat. Struct. Biol. **5**, 37 (1998).
[16] D. Lu, P. Grayson, and K. Schulten, Biophys. J. **85**, 2977 (2003).
[17] P. Reimann, Phys. Rep. **361**, 57 (2002).
[18] R. D. Astumian, Science **276**, 917 (1997).
[19] M. O. Magnasco, Phys. Rev. Lett. **71**, 1477 (1993).
[20] T. Y. Tsong and T. D. Xie, Appl. Phys. A **75**, 345 (2002).
[21] R. T. Voegele, G. D. Sweet, and W. Boos, J. Bacteriol. **175**, 1087 (1993).