# Mobility Measurements Probe Conformational Changes in Membrane Proteins due to Tension

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The function of membrane-embedded proteins such as ion channels depends crucially on their conformation. We demonstrate how conformational changes in asymmetric membrane proteins may be inferred from measurements of their diffusion. Such proteins cause local deformations in the membrane, which induce an extra hydrodynamic drag on the protein. Using membrane tension to control the magnitude of the deformations, and hence the drag, measurements of diffusivity can be used to infer—via an elastic model of the protein—how conformation is changed by tension. Motivated by recent experimental results [Quemeneur *et al.*, Proc. Natl. Acad. Sci. U.S.A. **111**, 5083 (2014)], we focus on KvAP, a voltage-gated potassium channel from *Aeropyrum pernix*. The conformation of KvAP is found to change considerably due to tension, with its "walls," where the protein meets the membrane, undergoing significant angular strains. The torsional stiffness is determined to be $26.8k_BT$ per radian at room temperature. This has implications for both the structure and the function of such proteins in the environment of a tension-bearing membrane.

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Recently, Quemeneur *et al.* [1] measured how the diffusion of KvAP, a voltage-gated potassium channel from *Aeropyrum pernix*, was affected by membrane tension. KvAP is an example of a protein that is found to have an affinity for curved membranes [2], implying an asymmetric, truncated cone shape. The protein induces a localized deformation, or "dimple," in the membrane, the magnitude (and extent) of which decreases as the applied tension is increased. To investigate the effect of shape on dynamics, the authors of Ref. [1] traced the motion of KvAP at different membrane tensions and compared the corresponding diffusion constant to the reference, or control, values exhibited by a cylindrically shaped protein (of equivalent radius), which can be related to the theory of Saffman and Delbrück [3]. At high tensions the corrections due to the shape of KvAP were very small (∼5%), while at lower tensions the corrections (∼40%) were much more pronounced.

In order to explain these results, the authors of Ref. [1] invoked a polaronlike theory [4–6]. This involves adding an extra term to the Hamiltonian of the membrane, which is coupled locally to membrane curvature and gives rise to a dimple consistent with the protein's shape. An Oseen approximation is then used to calculate an additional drag, which arises because a moving dimple must displace the surrounding viscous fluid. The corresponding reduction to the diffusion constant is then found by using the Stokes-Einstein relation. However, the approach neglects (i) the fact that membranes are themselves incompressible fluids, satisfying a two-dimensional form of the Stokes equation, and (ii) that the movement of the protein imposes particular boundary conditions on the membrane flow (and the membrane flow, in turn, imposes conditions on the surrounding fluid flow). Moreover, the additional drag calculated in Ref. [1] was found to be too small to explain the experimental data, leading the authors to explore additional dissipative mechanisms. These were traced to membrane shear flows, or to the assumption that a protein might drag a large island of immobilized lipids through the membrane. However, the effects of these modifications were calculated within the same Oseen approximation, and they cannot be expected to reliably describe any properties related to membrane flows for the reasons given: such flows must satisfy the equations of two-dimensional incompressible Stokes flow, and they are subject to appropriate physical boundary conditions near the moving object. It is for these reasons that the results of Saffman and Delbrück do not emerge in the appropriate limit of zero curvature in Ref. [1].

Here, we instead seek a classical hydrodynamic explanation for the additional drag, and hence the reduced diffusion, of curvature-inducing proteins. In order to take account of the geometry of the membrane, we employ a covariant formulation of low Reynolds number hydrodynamics in two dimensions [7–10]. In doing so, we neglect both membrane fluctuations and any chemical interactions occurring between the protein and the amphiphilic molecules that make up the membrane [11,12]. By treating the membrane hydrodynamics in this way, we find that no additional dissipative mechanisms are required.

If the shape of the protein is fixed, our calculations predict an *increased* hydrodynamic drag at high tensions. The reason is that the induced dimple in the membrane becomes localized and sharp, increasing the Gaussian curvature of the membrane in the vicinity of the protein and introducing additional hydrodynamic shear stresses (see, for example, Ref. [9]). Such an effect is not apparent in the data, which suggests that, for sufficiently high tensions, the Brownian motion of KvAP should be

 

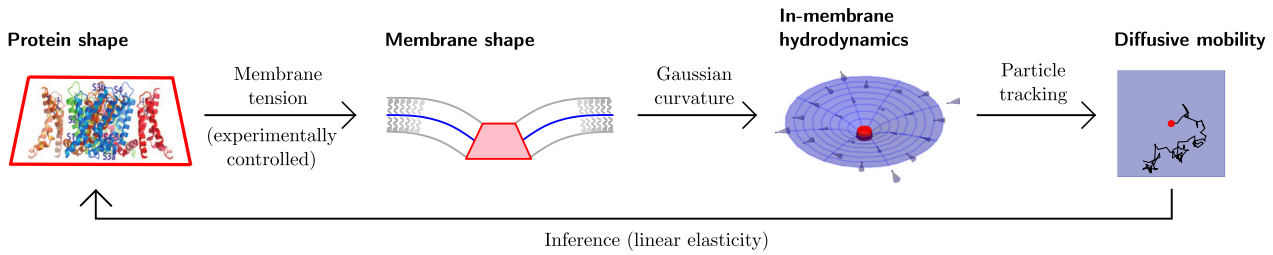


FIG. 1 (color online). Flow diagram. The shape of KvAP induces a local deformation in the membrane, resulting in nonzero Gaussian curvature in the vicinity of the protein. As tension is applied to the membrane, the deformation becomes more localized, increasing Gaussian curvature. A covariant formulation of low Reynolds number hydrodynamics demonstrates that Gaussian curvature increases the drag on the protein, therefore reducing diffusion. As a result, measurements of particle trajectories, such as those in Ref. [1], can be used alongside a simple elastic model of protein deformation to infer how protein shape is changed by applied tension.

indistinguishable from a cylindrically shaped protein of the same radius (such as the aquaporin AQP0, used as a control by Quemeneur *et al.*). This is evidence that the conformation, or shape, of the protein is changed by the torque exerted on the "walls" where it meets the membrane [13]. Combining our hydrodynamic theory with linear elastic response yields an excellent fit to the data [1] and predicts the relevant torsional stiffness of KvAP. A flowchart representing our approach is shown in Fig. 1.

To develop a theory for the hydrodynamics associated with the motion of KvAP, the induced shape of the membrane must first be calculated. Taking the midplane of the bilayer to be a smooth Riemannian manifold $\mathcal{S}$, each point on $\mathcal{S}$ is attributed a Helfrich-like free energy per unit area [14,15]. The lipids are assumed to remain well ordered everywhere, and therefore the bilayer has a bending energy of $2\kappa H^2$, where $\kappa$ is a constant and $H$ is the mean curvature. The spontaneous curvature is zero, while the contribution from the Gaussian curvature is neglected due to the Gauss-Bonnet theorem. The membrane is also under lateral tension $\sigma$. In the experiments of Ref. [1], this is controlled by the pressure difference between the interior and exterior of a giant unilamellar vesicle. Neglecting fluctuations, the shape of the membrane at equilibrium is then found by minimizing the total free energy

$$E = \int_{\mathcal{S}} (2\kappa H^2 + \sigma) dA, \tag{1}$$

where $dA$ is used as a shorthand for the volume 2-form, vol[2], associated with $\mathcal{S}$. Using a small angle approximation, the solution can be characterized by an axisymmetric height field $\alpha h(r)$, $\forall r \in [a, \infty)$, where $a$ is the radius of the protein and $\alpha$ is the contact angle subtended at the walls of the protein (see Fig. 2). Up to a constant factor, the variational procedure yields an order-0 modified Bessel function of the second kind [see Ref. [16] and the Supplemental Material (SM) [17]]:

$$h(r) = l K_0(r/l)/K_1(a/l), \tag{2}$$

where $l = \sqrt{\kappa/\sigma}$ is the membrane correlation length. Notice that increasing the surface tension leads to an increasingly localized membrane deformation, or dimple (see Fig. 1 of the SM [17]).

The effect of the induced shape (2) on protein diffusion may be calculated by first computing the hydrodynamic drag, $\lambda$, on a protein moving with constant velocity, and then relating this to the diffusion constant via the fluctuation-dissipation theorem [18]. We consider the protein moving laterally (i.e., perpendicular to the $z$ axis of Fig. 2) with a velocity whose magnitude $V$ is sufficiently small that $h(r)$ remains a good approximation to the membrane shape [19] and the hydrodynamics remains at a low Reynolds number [20]. The force balance condition for this motion is then $F = -\lambda V$, where $F$ is the hydrodynamic stress integrated over the walls of the protein [18] and the sign signifies that drag forces act opposite to the direction of motion.

This otherwise straightforward calculation is greatly complicated by the shape of the membrane, and it requires the use of differential geometry. For the uninitiated, a summary of both notation and relevant results is given in the SM [17]. In brief, at each point on the manifold, the components $\Pi^{ij}$ $(i, j = 1, 2)$ of the rank-(2,0) Cauchy stress

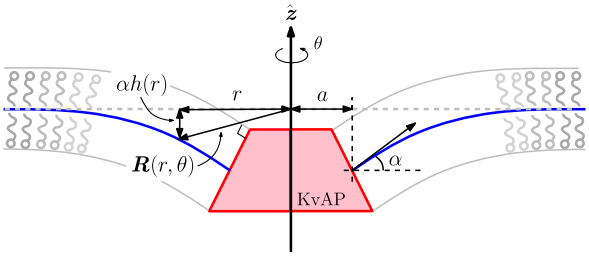


FIG. 2 (color online). Sketch. The embedded membrane protein KvAP induces a local curvature in an otherwise planar membrane. The midplane of the membrane is characterized by a cylindrically symmetric height $h(r)$, $\forall r \in [a, \infty)$ and is further proportional to the contact angle $\alpha$, which also serves as the small parameter in our perturbation theory for the hydrodynamic drag acting on KvAP.

tensor are defined with respect to a non-normalized basis $\boldsymbol{e}_i$, which spans the tangent plane to $\mathcal{S}$ at that point. In order to calculate such stresses, both the hydrostatic pressure $p$ and components of the fluid velocity field $v^i$ are required, i.e.,

$$\Pi^{ij} = -pg^{ij} + \eta(v^{i;j} + v^{j;i}), \tag{3}$$

where the constant $\eta$ is a two-dimensional viscosity and $g^{ij}$ are the components of the inverse metric. Here, a comma and a semicolon placed before a lower index represent partial and covariant differentiation, respectively, while upper indices may be lowered and lower indices raised by contraction with the metric and its inverse, respectively (i.e., $v^i = v_j g^{ij}$ and $v^{i;j} = v^i{}_{;k} g^{kj}$, etc.). If the direction of motion of the inclusion is assumed (without loss of generality) to be in the $x$ direction, the net force $F$ becomes

$$F = \int_{\partial\mathcal{S}} (\hat{\boldsymbol{\imath}} \cdot \boldsymbol{e}_i)\Pi^{ij} dl_j = -\lambda V, \tag{4}$$

where $\partial\mathcal{S}$ is the boundary between the surface and the protein, and $dl_j$ is shorthand for the appropriate line 1-form(s). Under steady state conditions, the hydrostatic pressure and fluid velocity fields satisfy the *covariant* form of Stokes's equation [8–10]:

$$\eta(v^i{}_{;j}{}^{;j} + Kv^i) - p^{;i} = 0. \tag{5}$$

Here, the crucial difference from standard (Euclidean) hydrodynamics is that, if the membrane has a nonzero Gaussian curvature $K$, the shear stresses exerted by the fluid are modified.

In principle, the two equations (5) can be solved, subject to boundary conditions, when combined with the constraint of incompressibility, $v^i{}_{;i} = 0$. In practice, it is often easier to solve for a scalar stream function $\psi$ by writing

$$v^i = \frac{1}{\sqrt{|g|}} \varepsilon^{ij} \psi_{,j}, \tag{6}$$

where $\varepsilon^{ij}$ is a two-dimensional antisymmetric Levi-Civita symbol and $|g|$ is the determinant of the metric $g_{ij}$. Consigning the cumbersome derivation to the SM [17], we present the result in index-free notation using angle brackets $\langle \cdot, \cdot \rangle$ to indicate an inner product taken with respect to the metric

$$\left(\frac{1}{2}\Delta + K\right)\Delta\psi + \langle \nabla K, \nabla\psi \rangle = 0. \tag{7}$$

Here, $\nabla$ is the gradient operator, extended to apply on a smooth manifold, and $\Delta$ is the Laplace-Beltrami operator. Equation (7) is a fourth order partial differential equation which encapsulates incompressible Stokes flow on a two-dimensional smooth manifold (surface) in one single equation. Notice that if the manifold is planar, i.e., the Gaussian curvature is zero, then the usual biharmonic equation, $\Delta^2\psi = 0$, is recovered.

Unfortunately, for most nontrivial geometries, finding a closed-form solution to Eq. (7) is problematic. However, approximate solutions may be constructed by considering the equation perturbatively. In our case, both the Laplace-Beltrami operator and the Gaussian curvature may be expanded as power series in terms of the small angle $\alpha$. We further postulate (and later verify) that $\psi$ can be expanded in the same way, i.e., $\psi = \psi^{(0)} + \alpha\psi^{(1)} + \alpha^2\psi^{(2)} + O(\alpha^3)$. Equation (7) can now be solved order by order, subject to boundary conditions. We impose a no-slip condition at the interface between the protein and the membrane, while as $r \to \infty$, we follow Ref. [21] and match with the leading term, in $r$, of a different velocity field, found by solving a Stokes equation that incorporates the extra drag from the embedding fluid. At both boundaries, these conditions are satisfied at lowest order, leading to the following results.

At lowest order, $\psi^{(0)}$ satisfies the biharmonic equation and the results of Saffman [21] are reproduced by design. The resulting drag is $\lambda^{(0)} = 4\pi\eta/\mathcal{C}$, where $\mathcal{C} = \log(\eta/a\mu) - \gamma$, and $\gamma$ is Euler's constant.

At first order, $\psi^{(1)}$ also satisfies the biharmonic equation. However, applying the boundary conditions gives $\psi^{(1)} = 0$, implying that $\lambda^{(1)} = 0$ [22]. This is a natural consequence of the up or down symmetry of the membrane: corrections to the drag coefficient $\lambda$ must be invariant under $\alpha \to -\alpha$.

At second order, $\psi^{(2)}$ satisfies an *inhomogeneous* biharmonic equation. The general solution can be constructed by combining the solution to the homogeneous equation with a particular solution that can be calculated via an appropriate Green's function; see the SM [17] for details. The resulting integrals must be calculated numerically [23] and there is, therefore, no closed-form solution for $\lambda^{(2)}$. Nevertheless, our result may still be compared with experiments [1] by invoking the Stokes-Einstein relation

$$D = D^{(0)}[1 - \alpha^2(\lambda^{(2)}/\lambda^{(0)})] + O(\alpha^3), \tag{8}$$

where $D^{(0)} = k_B T/\lambda^{(0)} = k_B T\mathcal{C}/4\pi\eta$ is the diffusion coefficient of a cylindrical protein moving in a planar membrane [21]. Here, $\lambda^{(2)}$ depends implicitly on $\sigma$ through the shape of the membrane, and hence the metric. Figure 3 shows this result as a function of applied tension (the green dotted curve). By kind permission of the authors, our results are shown against the original data from Ref. [1]. We see that rigid proteins, assumed to have a constant contact angle $\alpha$, would experience a *reduction* in their diffusion constant at high tensions. The reason is that the dimple induced in the membrane becomes an increasingly localized region of high Gaussian curvature, resulting in extra shear stresses in the fluid, and hence extra drag on the

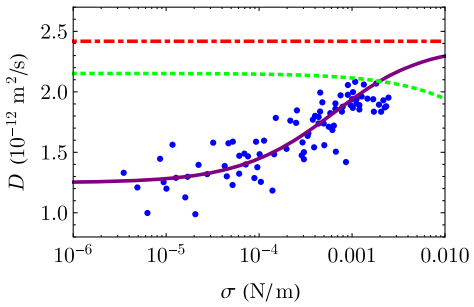


FIG. 3 (color online). Tension-dependent diffusion. Log-linear plot of membrane tension against diffusion constant for KvAP. The blue points represent experimental data from Ref. [1]. The red dot-dashed line is the tension independent diffusion constant of a cylindrical inclusion of equivalent radius [21]. This emerges at zeroth order in our perturbative scheme for KvAP. The next lowest nonzero corrections must be calculated numerically and are of order $\alpha^2$. For proteins with a completely rigid conformation (constant contact angle $\alpha = 0.16$ rad, irrespective of tension) the hydrodynamic picture is not compatible with the data (the green dotted line). However, if the protein is permitted to deform elastically in response to the torque it experiences on its walls, we obtain an excellent single-parameter fit (the solid purple line). In all cases, the protein radius $a = 5$ nm, the membrane and solvent viscosities are $\eta = 6 \times 10^{-10}$ kg s$^{-1}$ and $\mu = 10^{-3}$ kg m$^{-1}$ s$^{-1}$, respectively, and the membrane rigidity is $\kappa = 20 k_B T$ at room temperature.

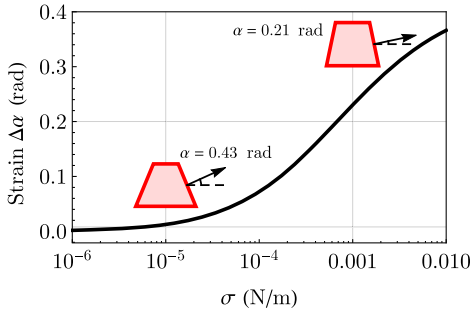


FIG. 4 (color online). Protein shape changes. Log-linear plot of angular strain against membrane tension. In the physiological range investigated by Ref. [1], i.e., $10^{-5}$–$10^{-3}$ N/m, we predict an angular variation of around 0.22 rad, roughly equivalent to a material strain of about 20%.

protein. This indicates that, regardless of the tension, a completely rigid conical protein (otherwise resembling KvAP) will never diffuse like a cylindrical one, such as AQP0.

We therefore propose that the shape of the protein changes with tension, and we invoke linear torsional response $\tau = \tau_r + k(\alpha - \alpha_r)$. The torque $\tau$ exerted on the walls of the protein can be found from the boundary terms in the earlier variational analysis,

$$\tau = 2\pi a \sigma h(a) \alpha. \tag{9}$$

The subscript $r$ denotes "reference," where $\tau_r$ is calculated by identifying the tension $\sigma_r$ at which the green dotted line of Fig. 3 intersects the data, and then substituting both $\sigma = \sigma_r$ and $\alpha = \alpha_r = 0.16$ rad (i.e., the angle used in Ref. [1]) into Eq. (9). The result is a tension-dependent expression for the angle $\alpha(\sigma)$, which depends on the torsional stiffness $k$. Using a least-squares procedure, a single-parameter fit for $k$ gives excellent agreement with the data (the solid purple line in Fig. 2) yielding a value of $k = 26.8 k_B T$ at room temperature. Reassuringly, this is entirely consistent with the energies required for voltage activation [24]. Moreover, we predict non-negligible angular strains $\Delta\alpha = \alpha_0 - \alpha(\sigma)$, where $\alpha_0 = \lim_{\sigma \to 0} \alpha(\sigma) = 0.44$ rad; for the range of tensions investigated in Ref. [1], see Fig. 4.

In the context of our evidence for significant structural strains at physiological tensions, a reassessment of the function and structure of membrane proteins under tension may be required. Our results are especially pertinent since the highly specialized functions of membrane-embedded proteins are currently thought to require precise spatial positioning of at least the key functional residues [25,26]. We therefore welcome further work in this area.

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