Filament networks attached to membranes: cytoskeletal pressure and local bilayer deformation

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Abstract. Several cell types, among them red blood cells, have a cortical, two-dimensional (2D) network of filaments sparsely attached to their lipid bilayer. In many mammalian cells, this 2D polymer network is connected to an underlying 3D, more rigid cytoskeleton. In this paper, we consider the pressure exerted by the thermally fluctuating, cortical network of filaments on the bilayer and predict the bilayer deformations that are induced by this pressure. We treat the filaments as flexible polymers and calculate the pressure that a network of such linear chains exerts on the bilayer; we then minimize the bilayer shape in order to predict the resulting local deformations. We compare our predictions with membrane deformations observed in electron micrographs of red blood cells. The polymer pressure along with the resulting membrane deformation can lead to compartmentalization, regulate in-plane diffusion and may influence protein sorting as well as transmit signals to the polymerization of the underlying 3D cytoskeleton.

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1. Introduction

The cortical cytoskeleton that is grafted to the lipid bilayer of many cells provides the plasma membrane of a cell with significant mechanical stability. The most prominent example of cells with a cortical cytoskeleton are red blood cells that have a two-dimensional (2D) spectrin network connected to their bilayer [1, 2]. Red blood cells can deform considerably without breaking [3]; the lipid bilayer shows large fluctuations [4] while the cells still appear to be stiff when mechanically deformed [5]–[8]. These intriguing elastic properties are modulated in an important manner by the 2D spectrin network; the spectrin network is often modeled as a network of springs, see [9]–[15]. In other mammalian cells, the cortical cytoskeleton is combined with an underlying, 3D cytoskeleton (e.g. an actin network) [16].

Polymers attached to a surface have previously been treated theoretically in investigations of the adsorption of flexible macromolecules to surfaces [17]. For a polymer grafted at one end, the partition function is found to be the product of the partition function of the bulk polymer and a factor that takes into account the grafting of the chain [18]. The correction to the bulk free energy,

$$\Delta F = -k_B T \ln \left( \text{erf} \left( \frac{\sqrt{6}}{N/2} \right) \right),$$

is logarithmic in the chain length, $N$ [19]. In section 3.4, we relate this result to our theory to confirm the magnitude of the bilayer deformation found with our, more detailed model.

In this paper, we consider the pressure exerted by the thermally fluctuating, cortical network of filaments on the bilayer and predict the bilayer deformations that are induced by this pressure. In section 2, we calculate the pressure of a flexible polymer both ends of which are attached to the lipid bilayer (in the case of the red blood cell, the pressure of a spectrin filament on the bilayer). We model the polymer by a Gaussian random walk. The polymer is attached to the membrane at its anchor points, while the entropic fluctuations of the chain lead to a repulsive pressure between polymer and membrane. The pressure field of the filament network can be obtained by superposition of the individual polymer pressure fields. In a first approximation,
we assume the positions of the polymer anchors to be fixed. In section 3, we approximate the pressure field of the filament network by a locally circularly symmetric pressure field. We then minimize the free energy of the bilayer membrane to predict the microscopic deformations of the cell membrane in response to the cytoskeletal pressure. We compare our prediction with localized bilayer deformations observed in electron micrographs.

For a bilayer with bending rigidity, $\kappa \approx 25 k_B T$, and a polymer network with mesh size, $a \approx 100$ nm (i.e. the regular spectrin network of the red blood cell), we predict bilayer deformations with amplitude, $0.1 \text{ nm} \lesssim h \lesssim 1 \text{ nm}$, induced by the cytoskeletal entropic pressure. If there are defects that lead to ‘holes’ in the cytoskeleton of few hundred nanometres in diameter, then we find a lower bound for the deformation amplitude of 1 nm.

2. Pressure of a single polymer bond

To calculate the pressure of a flexible, linear polymer that is attached at its two ends to a bilayer, we evaluate the free energy of a Gaussian random walk that is attached to the bilayer at its endpoints. We assume small membrane deformations (as is justified by our results). This allows us to consider the pressure of a polymer attached to a planar wall instead of having to self-consistently calculate both the membrane shape and the resulting polymer pressure.

Following the previous work on a polymer grafted at one end [22]–[25], we evaluate the path integral that gives the partition function of the polymer, $Z_N$, i.e. we solve Edward’s equation [26, 27],

$$\frac{\partial Z_N(r, r’)}{\partial N} = \frac{a^2}{6} \nabla_r^2 Z_N(r, r’).$$

(1)

$N$ segments are connected beginning at the position $r$ and ending at the position $r’$; $a$ characterizes the monomer size. The partition function of a polymer next to a planar wall located at $z = 0$ is

$$Z_N^{(0)}(r_1, r_2) = \left( \frac{3}{2\pi Na^2} \right)^{3/2} e^{-3(\rho_1 - \rho_2)^2/(2Na^2)} \left( e^{-3(z_1 - z_2)^2/(2Na^2)} - e^{-3(z_1 + z_2)^2/(2Na^2)} \right),$$

(2)

where $r_1$ and $r_2$ are the positions of the polymer endpoints. Defining $r = (\rho, z)$, the vectors, $\rho_1$ and $\rho_2$, are the in-plane coordinates of the two anchor points and $z_1$ and $z_2$ are the heights of the anchor segments. The superscript ‘(0)’ indicates that this is the solution of Edward’s equation for a polymer next to a planar wall (the zeroth-order term in a perturbation expansion, see below).

The fluctuations of the anchor positions in red blood cells have been investigated experimentally in [20]. Root mean squared fluctuations of $\approx 18$ nm have been found, but the time-dependent position data indicate that the fluctuations occur on a longer timescale ($\approx 10$ ms) compared with the Zimm time for the spectrin tetramer ($\approx 1 \mu$s). The authors report that fluctuations were also observed at shorter timescales—these fluctuations most probably had a smaller magnitude. In addition, the motion of the anchor positions is damped by the viscosity of the membrane that hinders the movement of the anchor proteins in the bilayer (typical diffusion coefficient, $D \lesssim 1 \mu m^2 s^{-1}$). We thus expect the dynamics of the polymer conformation changes that give rise to the pressure that we calculated, to vary on time scales, $\tau \gtrsim 100 \mu$s. This justifies the use of the pressure profile of a single chain with fixed end points. We expect the pressure profile to be slightly smeared out and the local pressure to be changed by a factor of the order 1 due to the anchor point fluctuations.
The effect of the membrane shape on the polymer conformations is taken into account by a perturbation expansion of the partition function [23, 24] with respect to the membrane height, \( h \),

\[
Z_N = Z_N^{(0)} + Z_N^{(1)} + Z_N^{(2)} + \cdots,
\]

where the superscript, \((i)\), indicates the order of the perturbation. For the pressure calculation, only the terms up to linear order in the membrane deformation are relevant, \( Z_N^{(0)} \) and \( Z_N^{(1)} \).

The condition that the partition function vanishes at the membrane—the Boltzmann factor \( Z_N \) only the terms up to linear order in the membrane deformation are relevant, \( Z_N^{(0)} \) and \( Z_N^{(1)} \).

\[
0 = Z_N^{(0)}(r_1; \rho, h(\rho)) = Z_N^{(0)}(r_1; \rho, 0) + \frac{\partial Z_N^{(0)}(r_1; \rho, z)}{\partial z} \bigg|_{z=0} h(\rho) + O[h^2]. \tag{3}
\]

The boundary condition for the zeroth order term in \( h(\rho) \) is thus

\[
Z_N^{(0)}(r_1; \rho, 0) = 0, \tag{4}
\]

and the boundary condition for the first-order term in \( h(\rho) \) is

\[
Z_N^{(1)}(r_1; \rho, 0) = -\frac{\partial Z_N^{(0)}(r_1; \rho, z)}{\partial z} \bigg|_{z=0} h(\rho). \tag{5}
\]

The zeroth-order term can be calculated independently and is given by equation (2); the first-order term is then obtained using the coupling boundary condition given by equation (5). For details of the calculation see the appendix.

The free energy difference for a polymer anchored to a deformed membrane compared with a polymer anchored to a planar wall is given by the ratio of the respective partition functions:

\[
\Delta \mathcal{F} = -k_B T \ln \left( \frac{Z_N^{(0)} + Z_N^{(1)}}{Z_N^{(0)}} \right) = k_B T \left[ \frac{Z_N^{(1)}}{Z_N^{(0)}} + O \left( \frac{Z_N^{(1)}}{Z_N^{(0)}} \right)^2 \right]. \tag{6}
\]

As above, we have assumed that the membrane deformation is small and thus \( Z_N^{(1)} / Z_N^{(0)} \ll 1 \). The free energy difference can be written in terms of the product of the membrane deformation and a pressure field,

\[
\Delta \mathcal{F} = \int d\rho \ h(\rho) p(r_1, r_2; \rho), \tag{7}
\]

and this allows us to define the pressure due to the thermally fluctuating polymer.

Comparing equations (6) and (A.5) with equation (7) gives the pressure field of a single polymer that is anchored to a planar membrane at the positions \( \rho_1 \) and \( \rho_2 \):

\[
p(\rho_1, \rho_2; \rho) = \frac{k_B T}{4\pi R^2_g e^{(\rho_1-\rho_2)^2/(4R^2_g)}} e^{-(|\rho_1-\rho_1|^2+|\rho_2-\rho_2|^2)/(4R^2_g)} \frac{|\rho_1 - \rho_1| + |\rho_2 - \rho_2|}{|\rho_1 - \rho|^3|\rho_2 - \rho|^3} \times [||\rho_1 - \rho||\rho_2 - \rho||(|\rho_1 - \rho|+|\rho_2 - \rho|)^2 - 6R^2_g] + 2R^2_g(|\rho_1 - \rho|+|\rho_2 - \rho|)^2. \tag{8}
\]

The polymer pressure depends on the radius of gyration, \( R_g = a\sqrt{N/6} \), and the anchor locations, \( \rho_1 \) and \( \rho_2 \). We assume small anchor sizes so that we can take \( z_1 \to 0 \) and \( z_2 \to 0 \). This means that the anchors lie in the plane of the membrane. A typical pressure field is plotted.
Figure 1. Typical pressure field of a polymer attached by anchors at both ends to a membrane, obtained using equation (8). The high (diverging) pressure at the anchor locations has been cut off in the figure to show the finite pressure along the connection line of the anchors. The sketch in the inset shows the planar wall to which the polymer is anchored, as well as the resulting pressure field.

in figure 1. Our calculation does not take into account the self-avoidance within the polymer chain, self avoidance has been shown to give only a small contribution in the case of a polymer with a single anchor [28]. Our calculation further neglects the finite contour length of the chain\(^6\), \(L\), and only \(R_g\) enters the pressure in equation (8); corrections because of the finite chain length are expected to become relevant for distances between the anchor points that are of the order of the chain length, when the polymer is already considerably stretched.

3. Membrane deformation

In this section, we show how the polymer pressure results in a deformation of the membrane. In subsection 3.1, we write the free energy of the membrane in an external pressure field. The equilibrium membrane shape is given from the minimization of the total free energy and we derive the corresponding Euler–Lagrange equations for the general case of a circularly symmetric pressure field. In subsection 3.2, we define the concept of ‘circular pressure patches’ in order to apply our theory to a cortical network such as the spectrin network of the red blood cell. In subsection 3.3, we use these results to predict the membrane deformation for various pressure profiles. In subsection 3.4, we estimate the microscopic bilayer deformation in the red blood cell membrane caused by the cortical spectrin cytoskeleton.

3.1. Free energy and Euler–Lagrange equation

The free energy of a bilayer in an arbitrary, external pressure field, \(p_e(\rho)\), (due to the fluctuations of the cytoskeletal filaments) is

\[
F[h] = \int dS \left\{ \frac{K}{2} (\nabla^2 h(\rho))^2 - p_e(\rho) h(\rho) \right\},
\]

\(^6\) In particular, our theory allows the distance between the anchor points to be larger than the contour length of the chain, which is clearly unphysical.
where the membrane deformation is parametrized by a height field in Monge gauge, $h(\rho)$, and $\kappa$ is the membrane bending rigidity [29]. We have taken $h = 0$ at the location of the anchor points. For circularly symmetric pressure fields, $p_e(\rho) = p_e(\rho)$, the corresponding Euler–Lagrange equation for the membrane height is

$$\kappa \nabla^2 \rho \nabla^2 h(\rho) - p_e(\rho) = 0,$$

with $\nabla^2 = (1/r) \partial / \partial r + \partial^2 / \partial r^2$. Natural boundary conditions obtained from the minimization are [30]

$$\partial \nabla^2 h(\rho) / \partial \rho = 0,$$

when the membrane height is not fixed at the boundary, and

$$\nabla^2 h(\rho) = 0,$$

if the derivative of the membrane height is not fixed at the boundary. These are supplemented by boundary conditions that fix either the position or the slope of the membrane. Below, we discuss the relevant boundary conditions for our problem.

### 3.2. Circular pressure patches

The cortical cytoskeleton imposes a pressure field on the bilayer that leads to local membrane deformations. In order to estimate these deformations, we define local, circular regions with a circularly symmetric pressure field that we call in the following 'circular pressure patches'. In figure 2, we consider pressure patches with the polymers along the rim. As discussed in section 3.3, the predicted membrane deformation obtained with these pressure patches are a lower bound to the real deformation. However, the patches can be easily generalized to describe defective areas in the polymer network. In figure 3, we consider an alternative set of pressure patches that predict a membrane deformation which is more realistic for regular networks, but cannot be generalized to describe defective areas in the network.

For the pressure patches defined in figure 2, the circumference of the effective pressure patch can be estimated by the sum of the distances between the anchor locations of those polymers that bound the polymer-free membrane region. For simplicity, we model the polymers as being grafted along the rim of the pressure patch, as sketched in figure 2(b). This approximation is accurate for a high density of polymers that are connected along the rim. For the network sketched in figure 2(a), three connection points are on the rim. We assume the pressure to be circularly symmetric inside each pressure patch and write, $\pi_{\text{rim}}(R - r)$, where $R - r$ is the distance from the rim. To estimate the pressure field, we calculate the pressure of a linear chain of polymers with the same anchor distances as those polymers that bound the pressure patch, see figure 2(c). We average the pressure along the direction of the line of polymer anchors to obtain a pressure field as function of the distance from the line of anchor points, $\pi_{\text{rim}}(x)$, see figure 2(d). We then approximate the pressure for the circular patch by the pressure of the linear case and write: $\pi_{\text{rim}}(R - r) = \pi_{\text{line}}(x)$ where we substitute $R - r$ for $x$.

The pressure profile for the pressure patches in figure 2, obtained from equation (8),

$$\pi_{\text{rim}}(R - r) = \int_{y_1}^{y_2} dy \ p((0, y_1), (0, y_2); (r, x_1)) / (y_2 - y_1),$$

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Figure 2. (a) A sketch of a periodic, cortical cytoskeletal network that consists of flexible polymer chains; the connection points are marked by large red dots. For convenience, the polymers are sketched in their stretched state. Black circles mark pressure patches that can be employed to estimate the local membrane deformation. (b) A circular pressure patch with polymers grafted at the edge. If one averages over the angle, the polymer pressure, \( \pi_{\text{rim}}(R - r) \), is only a function of the radial distance from the rim. (c) A line of grafted polymers. (d) The pressure is averaged along the grafting direction of the polymers, i.e. the pressure is only a function of \( x \), \( \pi_{\text{line}}(x) \). We approximate \( \pi_{\text{rim}}(R - r) = \pi_{\text{line}}(x) \).

Figure 3. (a) Regular cortical cytoskeleton as shown in figure 2. Black circles mark an alternative set of circular pressure patches that can be used to estimate the local membrane deformation. (b) A single circular pressure patch with the polymers grafted in the center. The radius of the pressure patch is half the distance between the polymer’s anchors. (c) The radial pressure, \( \pi_{\text{center}}(r) \), is taken to be circularly symmetric and is given in equation (14).

is independent of the size of the pressure patch and only depends on the distance between the anchor locations, \( |y_2 - y_1| \), and the radius of gyration of the polymers, \( R_g \). Since the functional dependence is calculated for the linear geometry in figures 2(c) and 2(d), the approximation for \( \pi_{\text{rim}}(R - r) \) is better the larger the patch size is, compared with \( |y_2 - y_1| \) and \( R_g \).
Figure 4. Pressure profiles and predicted membrane deformations for the pressure patches shown in figure 2. (a) Scaled radial pressure profiles, $\pi_{\text{rim}}(R - r)$, obtained from equation (13). The profiles correspond to different anchor distances, $(y_2 - y_1)/R_g = 2, 4, 6, 8$ and $10$ (from top to bottom). (b) Local membrane deformations for $(y_2 - y_1)/R_g = 2$ (solid) and $(y_2 - y_1)/R_g = 4$ (dashed) and different radii of the pressure patch, $R/R_g = 1, 2.5, 5$ and $30$ (from bottom to top). The variable $d$ gives the position along the diameter of the pressure patch.

For the pressure patches defined in figure 3, the radius of the pressure patch is given by half the distance between the anchors. All polymers are grafted in the center and not attached at the rim of the circular patch, see figure 3(b). Thus, the pressure is largest in the center and decays towards the rim where the polymer density is also small. The pressure is given by an angular average over the polymer pressure field from equation (8),

$$\pi_{\text{center}}(r) = \frac{6 \int_0^{2\pi} d\theta \ p((0, 0), (0, y_2), (r \sin(\theta), r \cos(\theta)))}{2\pi}.$$  

(14)

3.3. Pressure profile and predicted bilayer deformation

Figure 4(a) shows typical radial pressure profiles for different ratios of $(y_2 - y_1)/R_g$, obtained for the pressure patches in figure 2. Due to the diverging pressure at the polymer anchors, $\pi_{\text{rim}}(R - r)$ diverges for $r \to R$. The pressure shows a dramatic decrease when $R - r$ is approximately the polymer size, $r \approx R_g$.

We now calculate the membrane deformations from a numerical solution of equations (10) and (13). The four boundary conditions required to solve the differential equation are: $h'(0) = 0$ to account for the symmetry of the single pressure patch, $h'(R) = 0$ at the rim of the pressure patch to account for the symmetry of the cytoskeletal network and to avoid cusps, $h(R) = 0$ to define the reference height as the height of the polymer anchor locations, and the natural boundary condition from the minimization, given by equation (11), in the center. Figure 4(b) shows the predicted, local membrane deformations for different anchor distances, $(y_2 - y_1)/R_g = 2, 4$, and for several radii of the pressure patches, $R/R_g = 1, 2.5, 5$ and $30$.

The boundary condition at the rim, $h(R) = 0$, is a strong constraint on the membrane compared with several isolated anchor points as sketched in figure 2. Thus, the membrane
deformation may be considerably underestimated in this model, especially when \(|y_2 - y_1| \approx R\). In this regime, the pressure patches defined in figure 3 should give more realistic results.

Figure 5(a) shows typical radial pressure profiles for different ratios of \((y_2 - y_1)/R_g\), obtained for the pressure patches shown in figure 3. Due to the diverging pressure at the polymer anchors, the pressure, \(\pi_{\text{center}}(r)\), diverges for \(r \to 0\). Because of the pressure along the line that connects the polymer’s anchor points, \(\pi_{\text{center}}(r)\) doesn’t decay as strongly as the pressure profile shown in figure 4(a).

We calculate the membrane shape using equations (10) and (14). The four boundary conditions required to solve the differential equation are: \(h'(0) = \phi(0)/\kappa\) obtained by equating the most singular terms at \(r = 0\) (of order \(1/r^3\)) in equation (10), \(h'(R) = 0\) at the rim of the pressure patch to account for the symmetry of the cytoskeletal network, \(h(0) = 0\) to define the reference height as the height of the polymer anchor location, and the natural boundary condition from the minimization, given by equation (11), at the rim. Figure 5(b) shows the local membrane deformation for different anchor distances, \((y_2 - y_1)/R_g = 1, 2, \ldots, 7\) and different radii of the pressure patches \(R/R_g = 0.5, \ldots, 3.5\), respectively.

3.4. Application to red blood cell bilayer and cytoskeleton

In this subsection, we apply our model and its predictions to estimate the deformations of the red blood cell membrane due to thermal fluctuations of the underlying spectrin network.

The linear spectrin tetramers have a contour length of 200 nm and a persistence length of about 5 nm. The spectrin molecules are attached to the bilayer at both ends as well as in the middle [1]; the unattached parts of the molecule may be thus regarded as flexible polymers that can be modeled by Gaussian random walks. The anchor distance is \(|y_2 - y_1| \approx 30\) nm [31], the radius of gyration of spectrin dimers is \(R_g \approx 9\) nm \(^7\), the radius of gyration of the tetramers is \(R_g \approx 13\) nm, and the typical mesh size of an idealized, hexagonal spectrin network is 60–100 nm.

\(^7\) For a contour length of 100 nm and a persistence length in the range 2.5–10 nm, \(R_g \approx 7–13\) nm [14, 32].
Firstly, we use the pressure patches in figure 2. Using $|y_2 - y_1|/R_g \approx 4$, $R/R_g \approx 4$, $R_g \approx 10$ nm and $\kappa = 25 k_B T$ [5], we estimate from figure 4(b) that the expected membrane deformations of the red blood cell bilayer for a regular cytoskeleton are of the order of 0.1 nm. This estimate is a lower bound. As seen in figure 2(a), each vertex connects six polymers. In the vicinity of the vertex, the chains resemble star polymers [28, 33]. Chains that ‘belong’ to adjacent pressure patches contribute to the membrane deformation, which can increase the bilayer deformation by a factor 2–3. Furthermore these pressure patches give a lower bound for the bilayer deformation, because deformations along the line between the anchor points are strongly suppressed by the boundary condition, $h(R) = 0$.

Secondly, we estimate the membrane deformation using the pressure patches defined in figure 3. For this estimate, we neglect the additional anchors in the middle of each spectrin tetramer. Using $|y_2 - y_1|/R_g \approx 6$ (and thus $R/R_g \approx 3$), we estimate from figure 5(b) the deformations to be of the order of 1 nm.

Using the calculation of the free energy difference between a free polymer and a polymer attached with one end to a wall [19], $\Delta F = -k_B T \ln (\text{erf}(\sqrt{6}/N/2))$, we can roughly estimate an upper bound for the expected bilayer deformation. For a spectrin tetramer with $N \approx 40$, the free energy to graft a tetramer with both ends to a wall is approximately twice the energy needed to graft a polymer with $N/2$ bonds: $\Delta F_{\text{spectrin}} \approx 2.5 k_B T$. For a mesh size of 60 nm, the bilayer area per spectrin tetramer is about 1000 nm$^2$, which corresponds to a free energy density, $f \approx 2.5 \times 10^{-3} k_B T$ nm$^{-2}$. This energy density is sufficient to spherically bend a planar membrane with $\kappa = 25 k_B T$ to form a sphere with radius 140 nm. If the circular pressure patches of radius 30 nm are covered by spherical caps with this curvature radius, we expect a maximal deformation of 3 nm.

4. Conclusions

We have calculated the pressure field of a flexible polymer attached at both ends to a lipid bilayer. Our model is appropriate if the distance between the anchor points is much smaller than the contour length of the polymer. We assume the polymer’s anchor positions to be fixed. Superposition of the pressure fields of several polymers can then be used to model the pressure field of a network of flexible polymers that is sparsely attached to a bilayer; this architecture is found in the cortical cytoskeleton of biological cells [1, 16].

We introduce a model of circular pressure patches to predict the deformation of a bilayer membrane caused by the attached network of flexible polymers. We predict the deformation as a function of the radius of gyration and the anchor distance of the polymers, the mesh size of the network, and the bilayer bending rigidity. For a regular cytoskeleton of the red blood cell, our model predicts local bilayer deformations, $0.1 \text{ nm} \lesssim h \lesssim 1 \text{ nm}$. Microscopic deformations of the red blood cell bilayer with amplitudes of the order of $h_{\text{max}} \approx 10$ nm and wavelengths of some hundred nanometres have been observed experimentally in electron micrographs [21]. The discrepancy between the theory and experiment might be explained by defects in the cytoskeleton or by a negative surface tension of the bilayer [9, 34].

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8 The effect of such an additional anchor point is not clear at the first glance. On the one hand the polymer pressure is increased because the chain in closer to the membrane due to the additional anchor, on the other hand the polymer pulls the membrane on this anchor.
Defects in the red blood cell membrane have been suggested by electron microscopy [2]. By increasing the size of the pressure patch with the polymers along the rim (figure 2), our model can be easily extended to defected regions. If spectrin-free areas with radii of a few hundred nanometres are present in the spectrin network of the red blood cell, we predict bilayer deformations of the order of 1 nm. This is estimated by performing the calculations for larger values of $R$ while keeping the other parameters the same. The larger pressure and corresponding larger deformation is due to the fact that in these defect regions there are more polymers along the rim while the curvature energy needed to obtain a certain $\Delta h$ is lower due to the larger radius.

The polymer pressure field and our model of circular pressure patches can be used in future studies of other effects that are due to the polymer–membrane interaction. For example, in addition to the bilayer deformation, the cortical cytoskeleton in cells influences the lateral in-plane diffusion of lipids and proteins [35] and induces compartmentalization [36]. The pressure field, given in section 2, can serve as a starting point for theoretical models of the diffusion. Furthermore, the cytoskeletal pressure may induce aggregation of inclusions within the bilayer (bilayer deformation may lead to aggregation of asymmetric proteins), and may thus be important for ‘protein sorting’ in biological membranes. Protein aggregation in turn may lead to an increased bilayer deformation (for asymmetric proteins) or transmit signals to the underlying, 3D cytoskeleton [37].

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**Appendix. Polymer pressure**

The ‘magic rule’ [38] that connects the solutions of different orders in the Taylor expansion is

$$Z_N^{(i)}(\mathbf{r}_1, \mathbf{r}_2) = \frac{a^2}{6} \int_0^N \mathrm{d}n \int d\rho \frac{\partial G_N^{(0)}}{\partial z}(\mathbf{r}_1; \rho', 0) Z_n^{(i)}(\rho', 0; \mathbf{r}_2),$$  \hspace{1cm} (A.1)

where $G$ denotes the propagator of the polymer and $G_N^{(0)}(\mathbf{r}_1, \mathbf{r}_2) = Z_N^{(0)}(\mathbf{r}_1, \mathbf{r}_2)$, see equation (2). We apply the magic rule to obtain the solution in first order of height deformations from the partition function of the polymer that is grafted to a planar, hard wall. Using equation (2) and (5), the solution of the first order is

$$Z_N^{(1)} = \frac{a^2}{6} \int d\rho' h(\rho') \int_0^N \mathrm{d}n \frac{\partial G_N^{(0)}}{\partial z}(\mathbf{r}_1; \rho', 0) \frac{\partial Z_n^{(0)}}{\partial z}(\rho', 0; \mathbf{r}_2)$$

$$= \frac{a^2}{6} \frac{3^5 \pi^2}{2 \pi^2 a^{10}} \int d\rho' h(\rho') \int_0^N \mathrm{d}n \frac{1}{(N-n)^{5/2}} e^{-k_1^2/(4(N-n))} \frac{1}{n^{5/2}} e^{-k_2^2/(4n)},$$  \hspace{1cm} (A.2)

Such defects in the network may originate from metabolic processes [9].
where $k_1 = \sqrt{6/a} \sqrt{(\rho_1 - \rho)^2 + z^2}$, $k_2 = \sqrt{6/a} \sqrt{(\rho_2 - \rho)^2 + z^2}$.

The integral over the contour length of the polymer is solved using the Laplace transformation [39],

$$Z_N^{(1)} (r_1, r_2) = -\frac{3^4 2^2 z_1 z_2}{\pi^2 a^8} \int d\rho' h(\rho') \mathcal{L}_N^{-1} \left\{ \frac{1}{k_1^2 k_2^3} (k_1 k_2 s + (k_1 + k_2) \sqrt{s} + 1) e^{-(k_1 + k_2) \sqrt{s}} \right\}$$

$$= -\frac{3^4 z_1 z_2}{2 \pi^{3/2} a^8 N^{7/2}} \int d\rho' h(\rho') \frac{k_1 + k_2}{k_1^3 k_2^3} e^{-(k_1 + k_2) (2N + 6) (2N + 2) / (4N)} [k_1 k_2 ((k_1 + k_2)^2 - 6N) + 2N (k_1 + k_2)^2],$$

(A.3)

$s$ is the Laplace transformed variable that corresponds to the chain length, $\mathcal{L}_N^{-1}$ is the operator for the backtransformation to recover $N$.

For small anchor lengths, $z_1$ and $z_2$,

$$Z_N^{(0)} (r, r') = \frac{3^{5/2} z_1 z_2}{2^{1/2} \pi^{3/2} N^{5/2}} e^{-3N (\rho_1 - \rho_2)^2 / (2Na^2)} + O[z^3].$$

(A.4)

and $k_1 = \sqrt{(\rho_1 - \rho)^2 + O[z^2]}$, $k_2 = \sqrt{(\rho_2 - \rho)^2 + O[z^2]}$. The ratio of the first-order term and the zeroth-term is

$$\frac{Z_N^{(1)}}{Z_N^{(0)}} = -\frac{1}{4\pi R_g^2} e^{-(\rho_1 - \rho_2)^2 / (4R_g^2)} \int d\rho' h(\rho') \frac{|\rho_1 - \rho'| + |\rho_2 - \rho'|}{|\rho_1 - \rho'| |\rho_2 - \rho'|^2} e^{-((\rho_1 - \rho')^2 + (\rho_2 - \rho')^2) / (4R_g^2)}$$

$$\times \left[ (|\rho_1 - \rho'|^2 |\rho_2 - \rho'| - |\rho_1 - \rho'| |\rho_2 - \rho'|^2 - 6R_g^2) + 2R_g^2 (|\rho_1 - \rho'| + |\rho_2 - \rho'|)^2 \right].$$

(A.5)

where $R_g = a \sqrt{\pi N / 6}$.

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