Cytoskeleton confinement of red blood cell membrane fluctuations

N. Gov, A.G. Zilman and S. Safran
Department of Materials and Interfaces,
The Weizmann Institute of Science,
P.O.B. 26, Rehovot, Israel 76100

We analyze both the static and dynamic fluctuation spectrum of the red-blood cell in a unified manner, using a simple model of the composite membrane. In this model, the two-dimensional spectrin network that forms the cytoskeleton is treated as a rigid shell which is located at some constant average separation from the lipid bilayer. The cytoskeleton thereby confines both the static and dynamic fluctuations of the lipid bilayer. The predictions of the model account for the wavevector and frequency dependence of the experimental data. The observed amplitude of the thermal fluctuations is related to effects of ATP-driven fluctuations.

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cytoskeleton \([\xi]\), here treated as a separated, infinitely rigid shell, that does not participate in the thermal fluctuations. The discrete contacts that maintain the constant average separation are not specifically described in this continuum model; in a coarse-grained picture, these contacts are the physical origin of the constraint (potential) that determines the average membrane-spectrin network separation.

The attachment of the cytoskeleton to the bilayer also causes stretching and undulations of the bilayer \([\xi]\), partly due to steric repulsion between the spectrin and the bilayer around the point of attachment \([\xi]\). Balancing the spectrin stretching with a local curvature of the bilayer, results in a membrane with undulations of wavelength \([L]\sim\sqrt{\kappa/\mu}\sim100-200\text{nm}\) and amplitude \(\sim10\text{nm}\) (\([\xi]\)). In our confining-shell model this length-scale is related to the potential-induced persistence length of the bilayer \(\xi_0=(\kappa/\gamma)^{1/4}\sim L\) \([\xi]\), i.e. the wavelength below which the bilayer is freely fluctuating.

We now calculate the spatial correlations for a two-dimensional, flat bilayer, since for all but the largest wavelengths \(\lambda\), the surface of the RBC is relatively flat: \(50\text{nm}<\lambda<1\mu m<\lambda\sim4\mu m\). We determine the values of \(\sigma\) and \(\gamma\) by fitting to the experimental data. From Eq. \([8]\) the equal-time (static) correlations of the normal deflections of the bilayer can be written \([8, 14]\)

\[
\langle h_q h_{-q}\rangle = \frac{k_BT}{\kappa q^4}, \quad \kappa_q = \kappa + \sigma q^2 + \gamma q^4
\]  

(2)

In the inset of Fig.1 we plot the measured value of \(\kappa_q\) \([8]\) in the form \((\kappa_q/\kappa-1)^{-1}\) as a function of the normalized wavevector \((qd)\) \((\text{where } d \text{ is determined by fitting the data to obtain } \gamma \propto d^{-4})\). From the linear slope in the limit of \(q \to 0\) we find the values of the parameter \(\gamma = 7.5, 1.0 \times 10^{-7}\text{J/m}^4\), for the two cells measured. These values correspond to mean amplitudes \(d \sim 200, 350\text{A}\) and \(\xi_0 = (\kappa/\gamma)^{1/4} = 130, 220\text{nm}\) respectively. At larger values of \(q\) there is a noticeable deviation from a straight line, which arises from the effective surface tension \(\sigma \sim 7.7, 2.8 \times 10^{-7}\text{J/m}^2\) for the two cells. Note that surface tension alone, without the confining effect of the cytoskeleton (i.e. \(\gamma = 0\)), does not fit the data (dash-dot line, Fig.1 inset). In addition, there is a rather abrupt change at the crossover wavevector \(q_0 = 1/\xi_0\) (indicated by the vertical dashed lines in Fig.1, above which the data are better described using \(\sigma \sim 0.4 \times 10^{-9}\text{J/m}^2\) (solid lines in Fig.1).

The measured surface tension is consistent with: \(\sigma \sim \kappa/\xi_0^2\) (order \(\sim \mu/10\)). This expression gives the effect of bilayer shape constraint due to the static undulations of lateral size \(\xi_0 \times \xi_0\), described above. Indeed at length-scales shorter then \(\xi_0\), there is no stretching of the cytoskeleton (Fig.1). Note that the effective surface tension of a closed bilayer is a very sensitive function of the excess area of the bilayer \([10, 14]\), which is affected by the induced undulations. The spread in the measured parameters may be due to natural variations in the cytoskeleton network of normal RBC cells.

There is a qualitative difference in power-law of the wavevector dependence of \(\kappa_q\) for RBC and empty vesicles \([16]\). The vesicles are well described (Fig.2) by equations \([12]\) with \(\gamma = 0\), and an effective surface tension: \(\sigma_{\text{vesicle}} \sim \kappa/R^2 \sim 2 \times 10^{-10}\text{J/m}^2\), where \(R \sim 27\mu m\) and \(\kappa \sim 1.3 \times 10^{-19}\text{J}\) are the vesicle radius and bending modulus respectively. Both the RBC's and vesicles data collapse when the wavelength is scaled by the r.m.s amplitude \(d\) (Fig.2). For the vesicles of diameter \(\sim 50\mu m\) \([15]\) the r.m.s. thermal amplitudes are \(d \sim 1 - 1.5\mu m\) (note that here \(d\) is not related to confinement). The good scaling of the data indicates that there is indeed a single important length-scale in the problem, namely the persistence length \(\xi_0\), that determines all the parameters appearing in the free energy (\(\gamma\) and \(\sigma\)) and the r.m.s amplitude \(d\).

We now use the same simple model of spectrin confinement of the bilayer to describe the temporal correlations of the membrane fluctuations. The shape fluctuations of the RBC membrane are driven by both thermal and metabolic energies. The active fluctuations have a frequency spectrum that is confined to the range 0.3-1Hz \([24]\). For higher frequencies, our analysis shows that the active processes can be accounted for by an increase in the effective temperature of the fluctuations \([21, 22]\). The temporal height-height correlation function \([8, 22]\) for a flat bilayer at a distance \(D_o\) from a rigid wall, is

\[
\langle h_q(t) h_{-q}(0) \rangle = \frac{k_BT}{\kappa q^4} e^{-\omega(q)t}
\]  

(3)

where \(\kappa_q\) is given in \([2]\). The hydrodynamic interaction (Oseen interaction kernel \([23]\)) has a modified form: \(\Lambda(q) = (1/4\eta q) [1 - (1 + 2qD_o)e^{-2qD_o}]\) \((\Lambda_f(q) = 1/4\eta q\) for a free bilayer), so that the relaxation frequency \(\omega(q)\) is

\[
\omega(q) = \left[\frac{1}{4\eta} \left(\kappa q^3 + \sigma q + \frac{\gamma}{q}\right)\right] \left[1 - (1 + 2qD_o)e^{-2qD_o}\right]
\]  

(4)

where \(\eta \sim 3\eta_{\text{water}}\) is some average viscosity of the cytoplasm and external solution. In the limit of short wavelengths \((q \to \infty)\) we recover the free bilayer frequency:

\[
\omega(q) \sim \kappa q^3/4\eta.
\]

The mean square amplitude of the normal fluctuations, as a function of frequency \(\omega\), is the Fourier transform

\[
d(\omega)^2 = \frac{1}{(2\pi)^2} \int dq \int \langle h_q(t) h_{-q}(0) \rangle e^{-i\omega t} dt
\]

\[
= \frac{1}{(2\pi)^2} \int \langle h_q(0) h_{-q}(0) \rangle \frac{\omega(q)}{\omega(q)^2 + \omega^2} dq
\]  

(5)

For a free bilayer this expression \([8]\) gives an anomalous frequency dependence \([22]\): \(d(\omega)^2 \sim \omega^{-5/3}\). We integrate the expression \([8]\) numerically in the range \(\pi/R < q < \pi/a\) \((a \sim 50\text{A})\), and compare with the experimental data \([2]\). In the inset of Fig.3 we plot \(d(\omega)\)
using the parameters of the two cells of Fig.1, and both the high and low values of the effective surface tension ($\sigma$ and $\sigma_0$ respectively). We find a reasonable agreement between the calculation and the measurements, when taking $D_\omega \approx 0.4d$. The similar magnitude of the bilayer rigid shell separation from both static and dynamic experiments, shows the overall consistency of our confinement model.

In the limit of high frequencies, the earlier result of Brochard et.al. [7], gave $d(\omega)^2 \propto \omega^{-4/3}$. Since $qD_\omega \ll 1$ in the measured range, we find in this limit [1]: $\omega(q) \sim kq^2 D_\omega^2 / 4 \eta$, leading to $d(\omega)^2 \propto \omega^{-7/5}$. It is difficult to distinguish between these two values using the newer data [7]. Our calculation has the advantage of consistently describing both the static (spatial) and dynamic (temporal) fluctuation data. Note that the case of a pure bilayer, with large effective surface tension $\sigma$, and without the effect of the rigid wall, is in complete disagreement with the data (dotted line, Fig.3 inset).

In Fig.3 we show that the normal RBC, ATP depleted RBC and RBC ghost, are all well described by the same expression [7] (using the softer cell from Fig.1, i.e. the smaller $\gamma$ and $\sigma$), differing by an effective temperature factor of up to $\sim 3$. This is similar to the amplitude enhancement factor of $\sim 2.5$ found in a previous study [2].

The largest effect of the rigid shell is to increase the effective viscosity of the water near the bilayer, by constraining its flow. Defining an effective viscosity by: $\omega = kq^2 / \eta_{eff}$, we get from [4]: $\eta_{eff} / \eta = \alpha q^2 / (\omega^2 (\gamma + \eta q^2))$. At the crossover wavevector $q_0$ this function has its peak: $\eta_{eff} / \eta \approx 45 - 30$, depending on the value of $\sigma$. These values are in close agreement with the value $\eta_{eff} / \eta \approx 50$ found from the relaxation times of an electrodeformed RBC [8, 9]. In these experiments, the cytoplasm flows through the cytoskeleton mesh, setting up a flow field at the crossover wavevector $q_0$. Thus, a rigid cytoskeletal wall, separated by a fixed distance from the bilayer, accounts for the larger effective viscosity required to fit these dynamical experiments.

While this model accounts for the wavevector dependence of the statics and the frequency dependence of the dynamics, the absolute amplitude of the fluctuations and the different values observed in active and ATP-depleted cells, must still be explained. One possibility is that ATP driven fluctuations completely determine the amplitude of the largest wavelength fluctuations [21] through the process of spectrin-actin disconnections and reconstructions [11, 21]. These ATP-driven conformational changes give rise to defects in the triangular spectrin network, resulting in nodes with more or less than 6 spectrin bonds. The local curvature of the cytoskeleton may change at the site of a defect, from being locally flat (6 bonds) to having a $\sim 50$nm deviation out of the plane of the flat cytoskeleton (5-fold node). The effect of this random buckling is to increase the mean bilayer-rigid shell separation by a factor of $\sim 4$. According to our model, this will increase the amplitude of the $q \rightarrow 0$ modes by a factor of $\sim 4^4$, as measured [4].

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FIG. 1: The calculated (Eq. 2) wavevector dependence of the bending modulus $\kappa/\kappa_q$ of the RBC (solid lines, taking $\sigma = \sigma_0$) compared with the experimental data for the RBC [9](o,x). The crossover wavevector $q_0$ is indicated by the vertical dashed lines. Inset: A plot of $(\kappa_q - \kappa)^{-1}$ as a function of the normalized wavevector $(qd)^4$ for small wavevectors. The linear slope in the limit of $q \to 0$ is indicated by the dotted line. The deviation from linear behavior is well described by an effective surface tension $\sigma \simeq \kappa/\xi_0^2 \sim 2.8 \times 10^{-7} J/m^2$ for the two cells (solid line). Note that surface tension alone, without confining wall ($\gamma = 0$), does not describe the data (dash-dot line).

the bilayer thermal fluctuations per area with the areal modulus of the cytoskeleton: $\mu \simeq (k_B T)^2/\kappa d^2 \Rightarrow d \sim 10 nm \sim w$ [see J.O. Radler, T.J. Feder, H.H. Strey and E. Sackmann, Phys. Rev. E. 51 (1995) 4526].

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FIG. 2: A plot of the the measured effective modulus $\kappa/\kappa_q$ of RBC (o,x) and empty giant vesicles (x) as a function of the normalized wavevector $qd$. The calculations are given by the solid and dashed line respectively. Inset: A plot of $(\kappa_q - \kappa)^{-1}$ as a function of the normalized wavevector $(qd)^4$ for small wavevectors. The linear slope in the limit of $q \to 0$ for the RBC is indicated by the dotted line. The calculation for the vesicle is given by the solid line.
FIG. 3: Frequency dependence of the mean-square amplitude $d(\omega)^2$ of the RBC (o), showing the reduction in the amplitude due to partial ATP depletion (x) and complete absence (RBC ghost)(∗). The lines of the calculation (Eq. 5) differ by the effective temperature enhancement factor of ∼3 (solid lines: $\sigma_0$, dash-dot lines: $\sigma$). Inset: A normalized Log-Log plot showing the powerlaw dependence ($\omega_0 = 1$Hz). The calculation is done using the parameters of the two cells of Fig.1 (solid lines: $\sigma_0$, dash-dot lines: $\sigma$). The dashed line shows the free bilayer behavior $d(\omega)^2 \propto \omega^{-5/3}$. The case of a free bilayer with large effective surface tension $\sigma$, but without the effect of the rigid wall, is in complete disagreement with the data (dotted line).