Erythrocyte Flickering

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In this work we will study the fluctuations of the red blood cell membrane where either bending or surface tension energy is present. This work will be based on a previously proposed phase field model for cellular membranes [1, 2] but including a thermal-driven noise. We simulated the fluctuations generated by this thermal noise in a membrane and compared them with recent experimental data for two morphologies of RBC cells of a discocyte and a spherocyte cell shapes. We derived the correlation function for the phase field order parameter and computed the correlation function of our numerical simulations and then we compared our results with the experimental data.

I. INTRODUCTION

Nowadays cellular membranes are a subject of interest and relevance in biophysical research [3–6]. The case of red blood cell (RBC) membranes is of utmost interest because is the principal control of the morphology and mechanics of the RBC. These thermal fluctuations on the RBC membrane were first observed over a hundred years ago and are usually called the flickering of the RBC [7]. A good understanding of the nature of the fluctuations of cellular membranes could be used to understand and classify, not only the different membranes present through the totality of cells, but could also be key to diagnose and identify many diseases such as malaria [8–10] or sickle cell anemia [11] that affect directly the properties of the membrane of the RBC.

The role of oxygen transport in the human body is carried by RBC, thus they are essential for the metabolism of our bodies. These cells could be roughly described as sacks of hemoglobin, a protein with an iron core that by diffusion absorbs oxygen directly from the air. The RBCs have to go through very small veins, sometimes even smaller than the cell itself, which has a typical diameter of $8\,\mu m$ and thickness $2\,\mu m$. Therefore the RBC membrane have to be very flexible and elastic.

Cell membranes are complex systems made of a thin lipid bilayer with a fluid-like behaviour that also hosts thousands of proteins attached to it and in some cases moving freely through its surface. This component of the cell defines the extracellular world from the intracellular one and whose modulus, properties and energies will define the shape and deformability of the cell. It is the RBC membrane what gives this cell its typical shape known as biconcave discocyte. The RBC membrane is not only made of the lipid bilayer since this bilayer is tethered to an elastic 2D spectrin network, which conforms another layer made of spring-like elements that conform a approximately hexagonal lattice via junctional complexes.

Because of the complexity, the small scale of the membrane (5 nm width), and the fact that bending has a big relevance, it is interesting to be able to measure its bending modulus. This modulus is extremely small with a value of $\kappa = 5.5 \pm 1.7\,\mathrm{K}_B\mathrm{T}$ [4] and has been measured by different methods. The typical experimental procedure would consist of using micropipette aspiration [12], but recently new methods have been developed to obtain this κ as studying the fluctuations of membrane [6], or the correlation function of its fluctuations [4].

Here we study an already existing phase field model for cellular membranes and use it to simulate the dynamics of the RBC flickering phenomenon. Phase field models have been used extensively in mathematical modelling of solid-liquid transitions and they have been recently used for modeling cellular membranes [1, 2, 13, 14]. Therefore, we will use this model and take it to a field which still has to be studied, the thermal-driven fluctuations of a membrane.

In the presence of a thermal bath the membrane will suffer collisions from the particles that conform the liquid leading to macroscopic displacements of the membrane from its equilibrium position. From this fluctuations of the thermal displacements of the membrane it is possible to obtain several macroscopic properties such as the bending modulus, the shear modulus or the area modulus [4]. The membrane seems to have an active nature to enhance its fluctuations which several researchers are trying to prove [3, 5], therefore the study of these fluctuations is the key to prove this active nature of the RBC membrane flickering. Quoting Turlier (2016) "*However, although equilibrium fluctuation models have been used to infer mechanical properties of RBCs since the seminal work of Brochard and Lennon, the fundamental question whether membrane fluctuations are driven by an active process or simply by thermal agitation remains controversial*". Even while saying that this active nature seems to have been finally proven true by Turlier [5], trough an analysis of the fluctuations under the Fluctuation Dissipation Theorem and its violation on certain ranges. Furthermore it has been observed long ago, that when a RBC is depleted of ATP (adenosine triphosphate), which is the molecule used as energy resource by the cells, the membrane displacement fluctuations drops.

II. THEORETICAL APPROACH

To get an insight into the stochastic fluctuations properties it is necessary a mathematical tool to understand the information of these random displacements. To analyze the thermal fluctuations in a typical experiment of membrane flickering, a correlation function is defined [7]. Being the perpendicular displacement of the membrane Δh at a point r and a time t a stochastic variable of the system, the correlation function is written as

$$\mathcal{C}(x',t) = \langle \Delta h(x,t) \cdot \Delta h(x+x',t) \rangle_x. \tag{1}$$

This Δh is the displacement of the membrane from its equilibrium point $\langle h \rangle_t$. This correlation function was already studied for the case of RBC membrane by Brochard and Lennon in the 70s [7] and recently by Park *et al.* in the several stages of morphology of RBCs [4]. Therefore there is plenty of experimental data and analysis to look into, and we will use this data for a qualitative evaluation of our results.

The most interesting information that we can get of this function will be in the Fourier space, therefore we will have to apply a Fourier transformation, defined as

$$\bar{\mathcal{C}}(x', f_j) = \sum_{i=0}^{N-1} \frac{\langle \Delta h(x,t_i)\, \Delta h(x+x',t_i) \rangle}{N} e^{-i2\pi\, t_i\, f_j}, \tag{2}$$

where the frequencies $f_j = j/(\Delta t\, N)$ are defined as fractions of the total time elapsed $t_i = i\,\Delta$, and Δt refers to the constant time separating the data i and $i+1$. Note that before computing the correlation function we already know some characteristics that will have to fulfill. First that will have its maximum at $x' \to 0$ because neighboring points will be affected by the same fluctuations and with similar intensities, while at $x' \to \infty$ the function equals zero because the fluctuations within these two points will be independent. Secondly, for an stationary case there will not be any correlation between two separated enough times t_1 and t_2. Then for the longer frequencies there will be no correlation between displacements giving $\bar{C}(x', f_j) = 0$.

Phase field Model

From Landau theory Canham [15] and Helfrich [16] derived a curvature-dependant energy for a 2D surface

$$F = \int_S \frac{\kappa}{2}(H - H_0)^2 + \bar{\kappa} K, \tag{3}$$

where H is the mean curvature, K the mean Gaussian curvature, H_0 a possible spontaneous mean curvature and κ the bending modulus for each curvature. Taking into account that the mean Gaussian curvature is a topological invariant for our case this contribution will be only a constant that we will not take into account during the formulation.

This phase field model consists on defining a order parameter which we will call ϕ that represents to which phase the point (x,y,t) belongs to, either extracellular ($\phi = -1$) or intracellular ($\phi = +1$). Therefore the transition surface between bulks where ϕ takes values in the range $\phi = (-1,+1)$ will be our diffuse membrane which position will be defined by the position where $\phi = 0$. This model reproduces very accurately membrane dynamics and morphology and will be the tool that we will use for our simulations.

Solving the Canham-Helfrich bending free energy for a given membrane written in function of our order parameter ϕ as done by Campelo and Hernández-Machado [1] leads to the following free energy

$$F_m[\phi] = \frac{\kappa}{2} \int \Big(\big[-\phi + \phi^2 - \epsilon^2 \nabla^2 \phi \big]^2 + \sigma_A \epsilon^2 (\nabla \phi)^2 \Big) dV, \tag{4}$$

where σ_A is a Lagrange multiplier that ensures the area conservation. Now here we are including a white noise $\xi(x,t)$ for the order parameter value $\phi(x,t)$ different for each point of the space and every time, from which we get a dynamic equation as follows

$$\frac{\partial \phi(x,y,t)}{\partial t} = \nabla^2 M \Big((3\phi^2 - 1)\Phi - \epsilon^2 \nabla^2 \Phi + \epsilon^2 \sigma_A \nabla^2 \phi + \xi(x,y,t) \Big). \tag{5}$$

Where $\Phi = -\phi + \phi^3 - \epsilon^2 \nabla^2 \phi$. The noise ξ intensity will be related to the temperature simulated and will have a Gaussian distribution with a mean equal to zero and a standard deviation σ. Following this equation the system will conserve its volume and area.

We will also be using the well-known Cahn-Hilliard Hamiltonian, with a surface tension energy, to compare with our results, which has the following dynamic equation

$$\frac{\partial \phi(x,y,t)}{\partial t} = \nabla^2 M \Big((-\phi + \phi^3) - \epsilon^2 \nabla \phi + \xi(x,y,t) \Big). \tag{6}$$

Correlation function for the Phase field Model

We now will be able to simulate a cell in a thermal bath, which will lead to membrane fluctuations of this cell. To study this fluctuations we will use and define a correlation function of this fluctuations for the phase field model and study its behaviour. Instead of height displacements (Δh) our correlation function will analyze the ϕ variations over the space, resulting

$$C(x',y',t) = \Big\langle \big(\phi(x,y,t) - \langle \phi(x,y) \rangle_t\big) \big(\phi(x+x',y+y',t) - \langle \phi(x+x',y+y') \rangle_t\big) \Big\rangle_{x,y}. \tag{7}$$

The term $\langle \phi(x,y) \rangle_t$ is the mean value of ϕ in that point averaged over time, which can be approximated as the

equilibrium value of the order parameter ϕ. And we can define the ϕ displacement as $\Delta\phi(x,y,t) = \phi(x,y,t) - \langle\phi(x,y)\rangle$. For the Fourier space, for a discretized data sample we define our discrete Fourier transform as following

$$\bar{C}(x',y',f_j) = \sum_{i=0}^{N-1} \frac{C(x',y',t_i)\, e^{-i\,2\pi\, f_j\, t_i}}{N}. \qquad (8)$$

III. NUMERICAL STUDY

For our simulations we have defined a spatial 2D lattice divided in elements of length $\Delta x = \Delta y$. We compute the evolution of the order parameter $\phi(x,y,t)$ between each time t and the successive time $t + \Delta t$ by a numerical integration of eq. 5 or eq. 6.

We have simulated a number of discocytes (Fig.1) and spherocytes (Fig.2) by solving eq. (5). Since we intended to study the bending model also with a spheroidal morphology (spherocyte) we also computed simulations of the well-known model that gives this spherocyte solution, the Cahn-Hilliard model, to compare with our results. This model writes a surface tension free energy for a membrane. Our simulations consisted on a transversal 2D section of the cell with one type of free energy, either bending as Campelo and Hernández-Machado or surface tension as the Cahn-Hilliard model. Each free energy is expressed by a different dynamic equation. In the surface tension energy case the equation tries to minimize the total area of the membrane thus leading always to a spherocyte solution.

The reason why we find two different shaped solutions for discocyte and spherocyte under bending energy, being dominated both by the same Hamiltonian and dynamic equation, is that the initial conditions (an ellipse or a circle) have a different volume to area quotient. The difference in this quotient, given that the bending case conserves the area and volume of the system, gives us different-shaped solutions under the same dynamic equation.

Positions of intracellular or extracellular volume that are far from the membrane have a constant ϕ value along the simulation. This means that the volume does not contribute to the correlation function and all the contributions will only come from the fluctuations of the membrane itself. Consequently, even computing the correlation function over all the space will get a function characteristic to the membrane and its displacements from the equilibrium position.

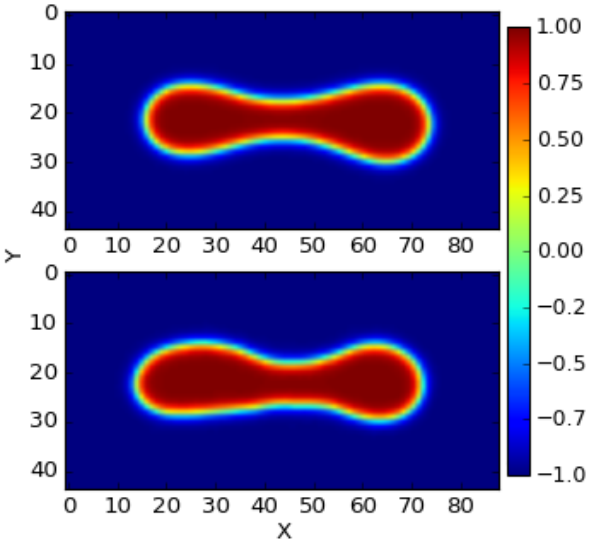

FIG. 1: Plot for a given time of the simulation of a discocyte under thermal noise. Colour indicates the value of the order parameter $\phi(x,y,t)$. Top: represented the whole lattice for a simulation with mild noise, of a deviation $\sigma = 0.0003$. Bottom: representation of a simulation with a big fluctuation and a very intense noise with a standard deviation of $\sigma = 0.001$.

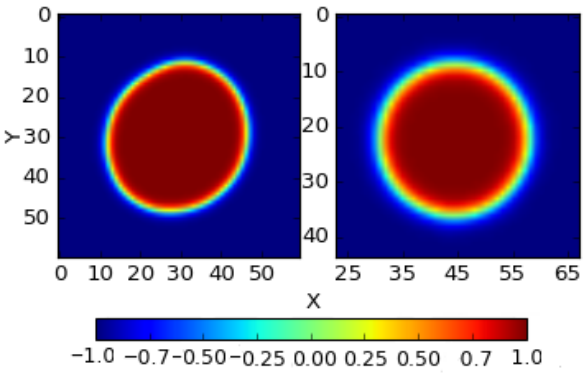

FIG. 2: Left: simulation of a spherocyte with bending energy under thermal-driven noise with a deviation of ξ of $\sigma = 0.0002$. Colour indicates the value of the order parameter $\phi(x,y,t)$. Has been plotted the whole simulated lattice. Right: plot of a spherocyte under surface tension energy and thermal-driven noise with a deviation $\sigma = 0.0005$. We have not plotted the whole simulated lattice of 44×88.

Discocyte shape

The discocyte is a shape that can only be obtained with a bending energy. From an initial condition of an ellipsoidal body without thermal-driven noise ξ we obtain this discocyte shape. We use this solution as initial condition for simulations that include ξ as it reduces considerably the computational time spent in evolving from ellipsoid to discocyte. We perform several simulations which give results similar to Fig.1, with different standard deviation σ for ξ.

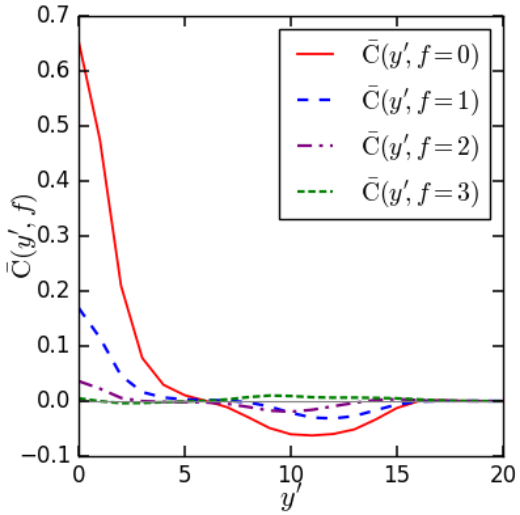

FIG. 3: Correlation function $\bar{C}(y', f)$ for a discocyte with bending energy. The y' axis is the perpendicular to the cell axis (not a superficial one) and the correlations we see are mostly between a face of the membrane and the face on the other side of the cell.

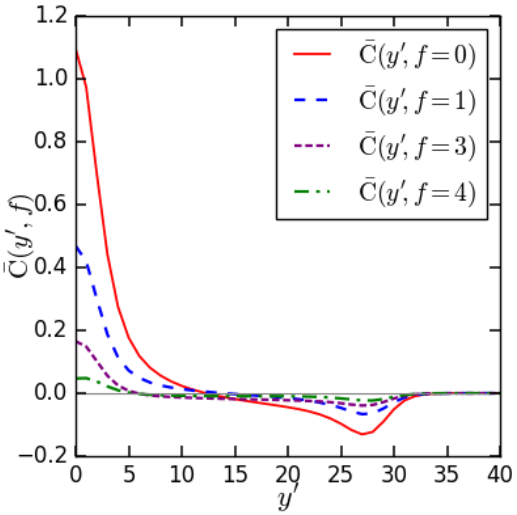

FIG. 4: Correlation function $\bar{C}(y', f)$ for a spherocyte with surface tension energy. The y' axis is the perpendicular to the cell axis (not a superficial one) and the correlations we see are mostly between a face of the membrane and the face on the other side of the cell.

Spherocyte shape

For the spherocyte morphology we have two simulation cases which converge to this shape, (i) having already spheroidal initial conditions and a bending energy, and (ii) any initial conditions and a surface tension energy for the membrane. Therefore we have two cases with two different dynamic equations. The main two differences are that the case of surface tension energy is much more robust against thermal noise than the bending energy case, because the energies involved in the Cahn-Hilliard Hamiltonian (surface tension case) are much higher than the Canham-Helfrich bending Hamiltonian (4). The surface tension case also tries to minimize its area, unlike the bending case which tries to minimize its curvature maintaining a constant area and volume.

IV. RESULTS AND DISCUSSION

As we can see in the bottom picture of Fig.1, the left side of the discocyte membrane is having a fluctuation from the equilibrium discocyte shape (top picture), we have obtained noise-driven fluctuations of the membrane. We can see the same kind of fluctuation for the spherocyte case in the left Fig.2. To study the fluctuations, its characteristics and their respective differences, we use the correlation function that we defined in eq. (7). The value of this function is computed from the obtained data of $\phi(x, y, t)$ during the simulation. The eq. (7) depends on the difference of the $\Delta\phi$ between two points separated by a distance y', where $\Delta\phi = \phi(x, y, t) - \langle\phi(x, y)\rangle$, and is the ϕ displacement from the equilibrium value $\langle\phi\rangle$.

In Fig.3 and Fig.4 the correlation function $\bar{C}(y', f)$ decays by increasing the value of distance y'. This indicates that increasing the distance between two points the correlation between them decreases.

In Fig.3 and Fig.4 we observe an anti-correlation for large values of y', this could be understood in Fig.5. For two points (x, y) and $(x, y + y')$, if their displacement has the same sign (Fig.5.a), there will be a peak in the correlation function. On the other hand, if the sign is different (Fig.5.b) the correlation function will take a negative value. In Fig.3 we have an anti-correlation at a distance $y' = 12$, which is the value of the mean width of the discocyte as can be observed in Fig.1. A similar anti-correlation can be seen in Fig.4 for a value of y' that takes the mean width of the simulated spherocyte $y' = 27$ as can be seen in the right Fig.2. The distance y' is taking these values because $\bar{C}(f, y')$ is the correlation through the y axis, and therefore is comparing the behaviour between the upper face of the cell and the bottom face of the cell.

Finally, we compare our results with the experimental data of RBC flickering obtained by Park *et al.* [4] represented in Fig.6. In both cases, when the frequency f increases, the correlation function decreases. The reason is that the longer time we take between comparing two points the less probability we have to find that they share a fluctuation or have any other kind of correlation.

In comparison with the obtained by Park *et al.* we can observe the active nature mentioned in the introduction. In Fig.6.A and Fig.6.B is represented how after extracting the ATP from a RBC the membrane fluctuations drop and therefore so does the correlation function of these fluctuations. In Fig.6.C they present results for a spherocyte where the bending modulus κ raises and

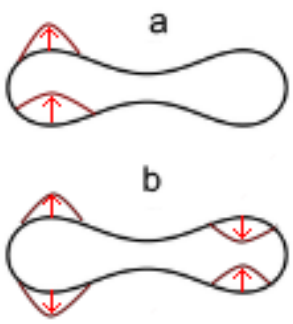

FIG. 5: Scheme of the two possibilities that give a positive (a) or a negative peak (b) in the correlation function plot.

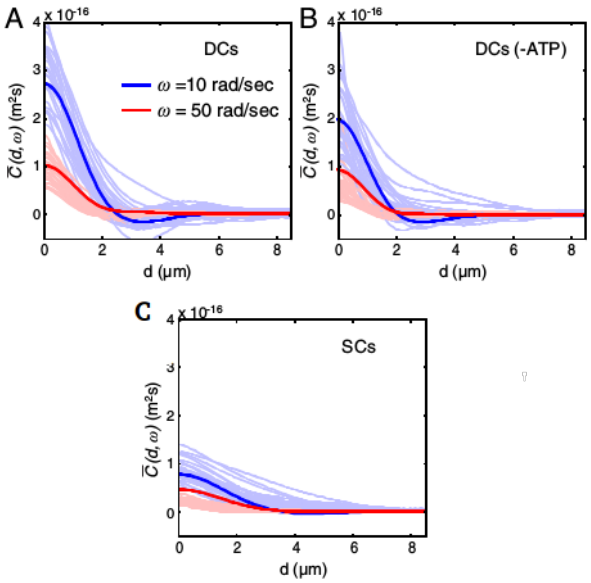

FIG. 6: Correlation function from experimental data of a healthy RBC (A), of a healthy RBC with its ATP depleted (B) and of a spherocyte with a higher rigidity (C). In vivid color and thicker lines the mean value computed for the different experiments. Figures from [4] by Park *et al.*

have a decrease in the fluctuations. We obtain a qualitative agreement when taking into account the reduction of the noise ξ when increasing κ for the equations (4) and (5). The data for these correlation functions have been obtained with a microscope measuring the perpendicular displacement on the membrane. In our case we have not computed the superficial membrane axis and we have worked with a defined lattice (x, y), therefore we are representing different correlation functions from Fig.6.

V. CONCLUSIONS AND FUTURE WORK

We have proven qualitatively the membrane phase field model for the Canham-Helfrich energy is robust under thermal equilibrium. We obtained a good correlation function which can qualitatively be compared with Park *et al.* and have seen that the correlation decreases with distance and can develop anti-correlations. We can see from our equations that when increasing the rigidity the fluctuations will drop as for the experimental data.

The most limiting factor may be the size of the lattice, which limits the resolution. Decreasing the resolution of the system does not have a huge impact on the results but limits the minimum noise ξ necessary for being able to observe fluctuations. Then the bigger the number of elements Δx that compose the simulated cell the smaller membrane fluctuations will be possible to compute. As the membrane must be at least several Δx width to give a stable solution, the size of the system will also limit the membrane width range available to simulate.

There could be work to do with the quantitative corroboration of the bending modulus behaviour in this model. It is also possible to develop simulations that mimic the out-of-equilibrium nature of the RBC with modifications of the noise distribution. Research on the validity of the Fluctuation Dissipation Theorem on this phase field model could be pursued by studying the system subjected to a periodic ϕ displacement. This would be a major prove the goodness of this phase field model under a thermal regime.

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