

MONTE CARLO SIMULATIONS OF VESICLES AND FLUID MEMBRANES TRANSFORMATIONS

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Abstract. The appearance of compartmentalization is recognized as a key step in biogenesis. The study of the dynamical behaviour of amphiphilic close membranes at equilibrium or under some external stress (osmotic pressure or dehydration process) can be useful in order to better elucidate the role of vesicles in the origin of life and to get insight into the molecular and membrane properties that bring to a spontaneous vesicle division. A Monte Carlo approach to simulate the evolution of close membranes under an external stress will be presented. This approach is mainly based on the accepted surface energy model introduced by Helfrich (1973) and Seifert (1997a). Some preliminary results will be also illustrated and possible developments and limits of this method discussed.

Keywords: Monte Carlo, origin of life, simulation, vesicle

1. Introduction

The appearance of compartmentalization has been claimed by different authors (Deamer and Oro', 1980; Goldrath, 1958; Morowitz *et al.*, 1988), as a key step in biogenesis: the continuum of chemical processes that led from an essentially random mixture of organic and inorganic molecules to the first assemblies recognizable as living systems. The presence of a boundary that separates the inside from the outside appears to be per se fundamental to individuate a living organism (Fleischhaker, 1990) since all known life forms are cellular and each cell is separated from the essentially aqueous outside environment by a thin bilayer water-insoluble membrane composed of both lipid and protein (Singer and Nicolson, 1972). Then, from the knowledge of contemporary living organisms it seems only reasonable to include the self-assembly of amphiphilic molecules into bilayer membranes as an essential step in the evolution of life on earth.

In most experimental conditions, amphiphilic molecules can self-assemble spontaneously into spherical closed bilayered shells with only a small amount of external work (for instance mild shaking) (Small, 1986). These aggregates are called vesicles and they consist of a water pool encapsulated into a bilayer membrane of lipids. They have flexible surfaces and can show a huge variety of morphologies and topologies depending on the material properties and on the



environment physicochemical parameters. Possible routes that bring from simple molecules to the synthesis of amphiphiles able to form vesicles have been hypothesized (Ourisson and Nakatani, 1999) and experimental works that show evolutionary autocatalytic pathways from surfactant precursor molecules to this aggregates in presumable prebiotic environments have also been published by Luisi and coworkers (Bachmann, 1992; Blöchliger, 1998; Wick, 1995). Dehydration-hydration cycles have been also proposed (Deamer and Barchfeld, 1982) as mechanisms for the encapsulation of macromolecules or ions inside vesicle: during drying vesicles flatten and fuse to form lamellar structures and then, when rehydration occurs, lamellae swell and disperse into large vesicles. Finally, experimental conditions where a vesicle ancestor can split into two daughter aggregates with the same topology have been reported (Döbereiner *et al.*, 1993; Wick *et al.*, 1995). In this framework, the study of the dynamical behaviour of amphiphilic membranes at equilibrium or under some external stress (osmotic pressure increase due to a dehydration process) can be useful in order to better elucidate the role of membranes in the origin of life and to get insights into the molecular and membrane properties that bring to a spontaneous vesicle fission, i.e. without involving complex proteins as in modern cells.

2. Surface Energy of Lipidic Membranes

Helfrich was the first to point out the role of curvature in the description of vesicle conformation (Helfrich, 1973) by expressing the surface energy of a flexible membrane in terms of the mean curvature (H), and Gaussian curvature (K):

$$E \equiv \frac{\kappa}{2} \oint_{\text{surface}} (2H - c_0)^2 dA + \kappa_G \oint_{\text{surface}} K dA, \quad (1)$$

where A is the surface area, c_0 is the spontaneous curvature and κ and κ_G are the bending rigidity and the Gaussian bending rigidity respectively. The mean curvature and the Gaussian curvature at a point on a surface are the average and the product of the principal curvatures at that point, the principal curvatures being the minimum and maximum curvatures of curves on the surface lying in planes including the tangent vector at the given point. A spontaneous curvature c_0 can arise either from a different chemical composition of the two membrane layers, or from different environments on both sides of the bilayer.

The surface energy together with constraints on the total area (assuming a fixed number of lipids) and the enclosed volume (assuming no osmotic stress) defines the spontaneous-curvature (SC) model. Therefore, neglecting effects due to convection and thermal fluctuations, vesicles will acquire the shape that minimises the surface energy at given constraints.

Seifert extended the theoretical work of Helfrich introducing the difference between inner and outer layer areas (Döbereiner *et al.*, 1997; Seifert, 1997a, b).

During years, several works have been then published where Euler–Lagrange equations were analytically solved to find the best shape in quasi-equilibrium vesicular systems under specific constrains (Kralj-Iglic *et al.*, 1999; Seifert, 1997a; Seifert and Wintz, 1996). One limitation of this approach is that it cannot handle systems under strong stressing conditions, such as high osmotic pressure or long-range deformation and, furthermore, the temporal evolution of membranes cannot be addressed, but only minimum energy membrane conformations can be determined.

To follow the time evolution of many-particle systems, Molecular Dynamics (MD) is normally used. This method is based on Newton dynamics and semi-empirical force-fields that specify inter-molecular force laws. Although this approach can provide atomic detailed descriptions of lipid membranes and it can reproduces local bilayer properties (Goetz and Lipowsky, 1998; Lindahl and Edholm, 2000; Tieleman *et al.*, 1997), it is computationally very expensive to be feasible for studying a vesicle as a whole (Ben-Shaul, 1996; Tieleman *et al.*, 1997) since a very large amount of interacting molecules has to be taken into account. Another problem is that MD can permit the investigation of the system time evolution in the range of nanoseconds (Tieleman *et al.*, 1997) that is a very short time scale compared to vesicle conformation changes.

Recently, another possibility for studying the conformations of lipid vesicles has been developed. By using a triangularized surface approach, the lipidic membrane is represented at a mesoscopic level as a collection of vertices of connected triangles and the curvature energy is minimized numerically by the gradient descent method (Brakke, 1992) or by using a Monte Carlo approach (Brakke, 1992; Koibuchi and Yamada, 2000a, b). Koibuchi and Yamada adopted a simplified Polyakov’s rigid string model (Polyakov, 1986) that worked out to be very efficient in analyzing the dynamics of flexible membranes (Brakke, 1992; Koibuchi and Yamada, 2000a, b). Furthermore, these authors introduced an approximate formula to estimate the energy of triangularized surfaces that is computationally less expensive than Equation (1). The surface energy was split into two contributions:

$$E = c_1 E_1 + c_2 E_2, \quad (2)$$

where the first term, called the area energy, depends on the size of the surface, whereas the second one estimates the bending energy, c_1 and c_2 being two adjustable parameters. Two different pairs of expressions have then been proposed and tested to estimate these two energetic contributions: the first based on the triangle sides (*edge*):

$$E_1 = \sum_{\text{surface}} \text{edge}^2 \quad (3)$$

$$E_2 = \sum_{\text{edge}} (1 - \cos \vartheta_{\text{edge}}) \quad (4)$$

and the second one, based on the triangle areas (A_{tr}):

$$E_1 = \sum_{\text{surface}} A_{tr} \quad (5)$$

$$E_2 = \sum_{\text{surface}} \frac{1}{A_{tr}} \sum_{i=1}^3 \text{edge}^2 (1 - \cos \vartheta_{\text{edge}}) \quad (6)$$

θ being the angle between the normal lines of two adjacent triangles. It is possible to demonstrate that the three formulae of the surface energy, Equations (1) and (2) along with the definitions (3)–(6), are equivalent for an implemented triangularization (Koibuchi and Yamada, 2000a).

Following this idea, we have developed a computer program based on a Monte Carlo algorithm to simulate the time evolution of a close membrane that undergoes conformation changes in order to minimise the surface energy under physical perturbations. In the next section, this program and the parameters needed to run *in silico* experiments of vesicle transformations will be presented.

3. Setting Up *In Silico* Experiments

The use of a triangular mesh rather than a smooth and continuous surface permits to reduce the calculation of geometrical properties, as for instance volume and surface, into an easy and automatic task (Piotto, 2000). A triangulated surface (*trSurface*) can be considered a suitable representation if surface integrals, analytically calculated on the continuous surface, are equal to summations over all triangles within the expected accuracy. It should be clear that increasing the number of used triangles can improve the calculation accuracy at the cost of increasing the computational time. Working on a triangularized mesh Gaussian (K) and mean (H) curvature can be also easily estimated (Piotto, 2000) and consequently the bilayer surface energy can be evaluated and numerically minimised accordingly Helfrich's (Helfrich, 1973) or the Koibuchi's (Koibuchi and Yamada, 2000b). Hamiltonians, as reported in Equations (1) and (2)–(6), respectively.

The iterative MC procedure used to find the minimal energy conformation of a given structure is the traditional *Metropolis Method* (Metropolis *et al.*, 1953). It simply consists in randomly changing the state of the system, i.e. the position of some vertices of the triangularized surface, and storing the new conformation if its energy (E_2) is less than before (E_1). When the energy results are to be higher, the new conformation will be accepted or rejected if an acceptance probability law ($P = \exp[-(E_2 - E_1)/k_B T]$) is randomly satisfied, where T is temperature and k_B the Boltzman's constant. The acceptance condition is verified if generating a pseudo-random number u , uniformly distributed between 0–1, will result $u < P$. Whereas a Monte Carlo procedure can be computationally very expensive to converge to the minimal energy conformation, an obvious advantage is its robustness

and the wide range of membrane transformations that can encompass. Given a *trSurface* oscillating around the surface energy minimum conformation, an external perturbation can be simulated opportunely modifying the expression of the surface energy. In the case of an external osmotic stress an additive energetic contribution E_V has to be considered in the definition of energy:

$$E \equiv \frac{\kappa}{2} \oint_{\text{surface}} (2H - c_0)^2 dA + \kappa_G \oint_{\text{surface}} K dA + E_v \quad (7)$$

or

$$E = c_1 E_1 + c_2 E_2 + E_V . \quad (8)$$

According to Seifert (Seifert, 1997a), this osmotic energetic term can be approximated as follows:

$$E_v \approx \frac{RTcV_0}{2} \left(\frac{V}{V_0} - 1 \right)^2 , \quad (9)$$

where R is the gas constant, V is the volume of the vesicle and c is the concentration of molecules caught within the vesicle. Subscript 0 indicates the equilibrium value.

It is worthwhile to mention that the second term on the right side of Equation (7) is constant for a given topology and then it can be neglected for any vesicle transformation at constant genus (Seifert, 1997a).

The osmotic perturbation can be introduced by suddenly setting $V_0 \ll V$ (osmotic shock) or more smoothly imposing a linear decrease of V_0 with time (slow dehydration). The energy minimization can be then performed setting different constrains, for instance keeping the vesicle surface A constant along with the spontaneous curvature c_0 and/or the reduced volume that is the ratio between the vesicle volume and the volume of a sphere of equal surface. In Figure 1 a diagram of some possible *in silico* experiments of vesicular evolution at constant topology is illustrated.

To perform all these kind of simulations we developed a C++ object-oriented computer program called *EM-solv*. This program has been compiled and linked to the AVS/Express graphical libraries that supply all the necessary graphic tools for the real time visualization of the simulations. Triangularized surfaces of any topology can be easily imported from input data files or automatically generated by using a friendly graphical user interface. A wide variety of geometrical surface properties can also be calculated and it is possible to set parameters to simulate stress conditions and analyse the MC evolution of the system in real time. Moreover, *EM-solv* can export simulation outcomes in the most common graphic data file formats and produce compressed videos as well. All the algorithms and the data structures implemented in *EM-solv* have been designed and optimized for the simulation of flexible membranes (Piotto and Mavelli, 2003). *EM-solv* has been

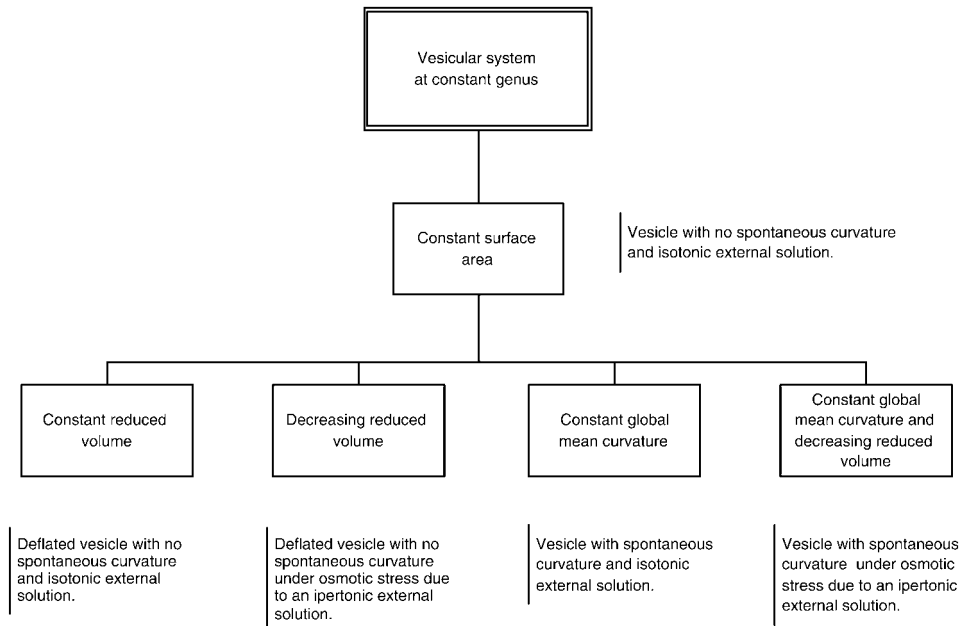


Figure 1. Scheme of the *in silico* experiments: different simulation types reported along with the required constrains and physical meanings.

firstly tested on a set of transformations with fixed topology in order to validate the method and to evaluate the best set of parameters to investigate dynamics and division of vesicles in prebiotic conditions. All the parameters needed to start a simulation are listed in Table I. It should be stressed that running *in silico* experiments requires the same attentions of test-tube experiments, since only an accurate choice of parameters can lead to successful results. It is worth to mention that the symmetry of the initial triangularized vesicle is critical in determining the convergence time of the algorithm. Whereas a nonsymmetrical vesicle converge to the conformation corresponding to a minimum of energy in tens of iterations, a high symmetrical surface (sphere like) does not converge even after thousands of iterations.

4. Results

Herein the attention will be focused on a spherical vesicle that undergoes an osmotic perturbation, i.e. a fixed topology transformation with the only constrains of constant surface. The adopted surface energy will be the Koibuchi and Yamada's formula defined by Equations (8), (5), (6), and (9). Simulation results obtained using Equation (7) will be not illustrated, since they are substantially in agreement with those shown. The target systems are vesicles of POPC (palmitoyl-oleoyl-

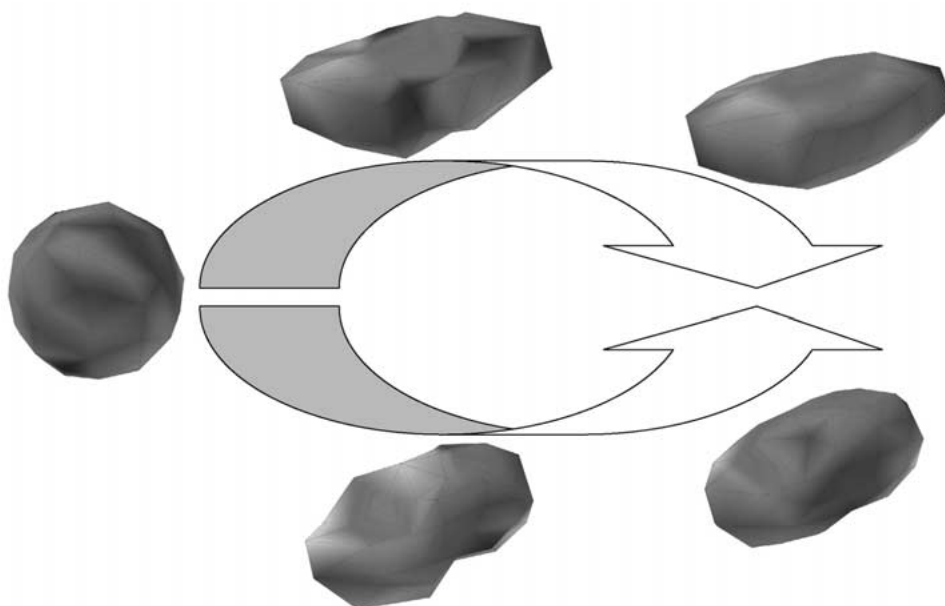


Figure 2. Relaxation of a 200 nm diameter vesicle undergoing an external osmotic stress. A smooth variation of the external aqueous ionic strength corresponds to a smooth deflation of the triangular vesicle, on top. The case of an osmotic shock is shown on bottom. The colour map of the surface mean curvature shows the roughness difference between the two cases.

phosphatidylcholine), and oleic acid/oleate, since they have been extensively studied (Blöchliger, 1998; Wick *et al.*, 1995) as prototypes of minimal cells. The most remarkable aspect of the simulation is that the time evolution of triangularized vesicles accurately represents the experimentally observed dynamics of real vesicular systems such as oleate/oleic acid giant vesicles (Blöchliger, 1998; Wick *et al.*, 1995). In fact, spheres or ellipsoids can only roughly approximate real vesicles because, even if these shapes correspond to the optimal equilibrium structures, thermal fluctuations and convective motions continuously perturb them. Moreover, the osmotic energy term, Equation (9), can strongly perturb the vesicle geometry because of the different energy scale of the osmotic and the surface contributions (Seifert, 1997a). When the osmotic term E_v in Equation (8) vanishes the energy area E_1 and the bending energy E_2 are responsible for the smoothness of the membrane, vice versa in presence of an osmotic stress a variety of morphologies can appear since, in some cases, E_v can overcome of some orders of magnitude the first two contributions. Consequently the smoothness of the membrane is not guaranteed and geometrically highly stressed conformations can appear. Figure 2 shows two relaxation paths of a vesicle of approximately 200 nm in diameter undergoing an osmotic shock (far from equilibrium process), on bottom, or a gently osmotic stress (quasi-equilibrium process), that simulates a slow dehydration process, on top.

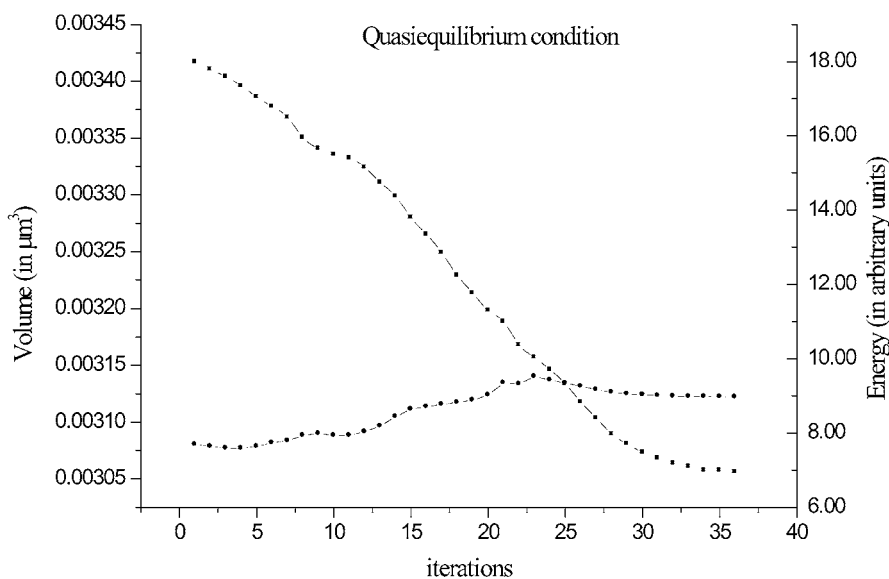


Figure 3a. Surface Energy and Volume time evolution of a 200 nm diameter vesicle undergoing to a gently osmotic stress: quasi-equilibrium process.

The surface energy and volume diagrams versus time are reported in Figures 3a, b.

When the osmotic energy is comparable with the area and the bending energy the vesicle remains always in a quasi equilibrium state: its surface energy increases slowly as a consequence of the osmotic stress and conversely its volume decreases regularly up to the equilibrium value, see Figure 3a. Throughout the time evolution the vesicle does not show high curvature regions. These results confirm the supposed large molecule encapsulation mechanism (Deamer and Barchfeld, 1982) based on dehydration-hydration cycles showing as during a slow dehydration vesicles tend to flatten.

On the other hand, if the osmotic energy is much larger than the area and the bending energy (osmotic shock), the vesicle deflates rapidly (see Figure 3b) and the membrane appears rough and inhomogeneous. In this region, conformations with high bending energy appear, and in some cases higher genus structures can be formed. In these conditions, the vesicle fission process can occur. Moreover, if the osmotic perturbation exceeds the mechanical limits of the membrane, the vesicle can collapse.

5. Conclusions

Monte Carlo simulations of triangulated vesicles worked out to be an extremely powerful tool to investigate vesicle transformations. The main achievement of this

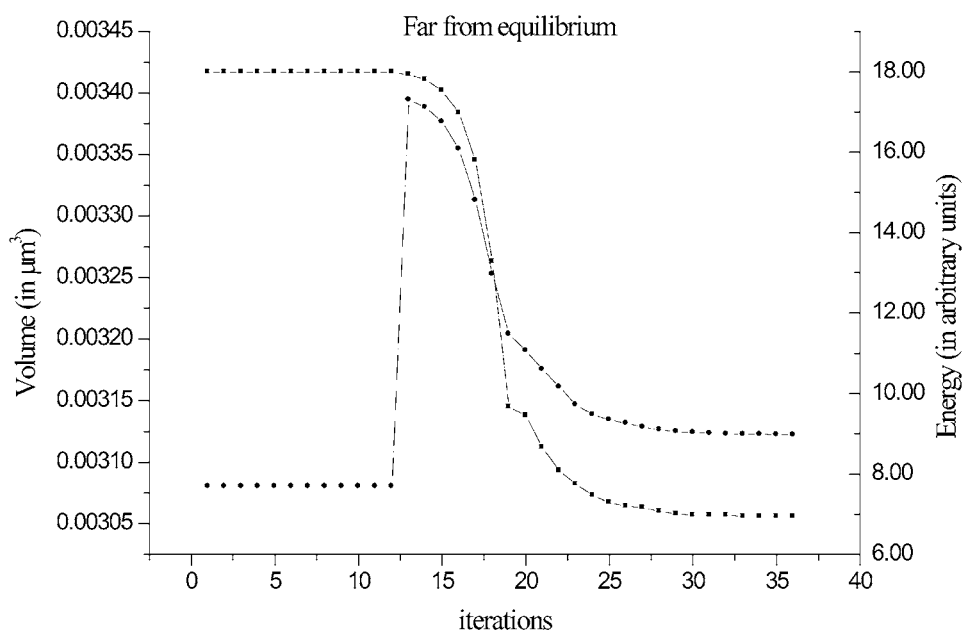


Figure 3b. Surface Energy and Volume time evolution of a 200 nm diameter vesicle undergoing an osmotic shock: far from equilibrium process.

approach is to account for the hugely different behaviour of vesicles in quasi-equilibrium states or in far from equilibrium conditions. In the first case vesicle dynamics consists in fluctuations around a minimal energy conformation and the simulation results are in good qualitative agreement with the analytical approach due to Helfrich and Seifert. On the other hand, far from equilibrium processes can be also studied by means of MC simulations, and the formation of high bending energy structures can be also elucidated as a consequence of a strong perturbation. In the present work we illustrated a simulation of lipid vesicles undergoing an osmotic stress due to a slow or a quick evaporation, in order to simulate the behaviour of vesicles in a prebiotic water pool. The method can be extended to analyse transformations of membranes with varying spontaneous curvature c_0 and vesicular aggregates with different topology.

Furthermore, the knowledge of surfactant molecular properties can be used to parameterize triangulated membranes, and consequently to help the design of vesicular systems composed of mixed amphiphiles capable to show complex cellular behaviours, like divisions, channels formation, and invaginations, triggered by simple chemical-physical perturbations.

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