Shape anisotropy and Voids

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Abstract

Numerical simulations on a 2-dimensional model system showed that voids are induced primarily due to shape anisotropy in binary mixtures of interacting disks. The results of such a simple model account for the key features seen in a variety of flux experiments using liposomes and biological membranes [1].

PACS numbers: 87.16.Ac, 87.15.Aa, 05.65.+b, 87.16.Dg, 87.68.+z
A variety of lipid molecules contribute to the structure and barrier function of biological membranes. Part of each molecule is hydrophilic (head) and part hydrophobic (tail). This amphipathic nature, in the presence of water, leads to a bilayer structure in which hydrophilic head groups have maximum contact with water and hydrophobic tails have minimum contact with water. The process of membrane formation is one of minimizing the free energy and maximizing the stability of the structure [2].

A major question has been whether the bilayer is to be viewed as an isotropic homogeneous phase or as a heterogeneous phase [3]. If osmotic contraction of the bilayer vesicles leads to an altered hydraulic conductivity (water flux coefficient), one obviously favors a heterogeneous membrane model. Otherwise a homogeneous, isotropic phase model would be adequate, obviating the need to look for a fine structure within the bilayer. Using the erythrocyte as an experimental system (in which the area of the biconcave cell does not change when it is osmotically expanded to a spherical shape), it was concluded that hydraulic conductivity was stretch independent, i.e., in support of the isotropic model [4]. An alternative way to assess hydraulic conductivity is to use hydrogen peroxide as an analog of water: since many experimental systems have catalase (an enzyme that degrades hydrogen peroxide to molecular oxygen and water) within the vesicle/cell, an assay of this occluded catalase directly permits one to measure the conductivity to exogenous hydrogen peroxide. Under equilibrium conditions of assay, the rate of degradation would be same as the rate of permeation of the peroxide into the vesicle. Thus, one can directly assess the stretch sensitivity of the membrane by osmotic titrations with osmolites, using non-electrolytes like hydrogen peroxide as probes of flux. In the course of these experiments it was found that [1]: (i) among all the lipid combinations tested, the phosphatidylcholine (PC) vesicles and intact erythrocytes, both, did not show a decrease in occluded catalase activity on osmotic compression of the membrane (ii) on the other hand, all other membrane systems, such as peroxisomes, E.coli, macrophages showed stretch (osmotic) sensitivity (iii) so did liposomes made from these cells and organelles (iv) further, when binary mixtures were investigated, only cardiolipin and cerebrosides when added to PC (5 to 10% of PC) conferred stretch sensitivity in liposomes (v) these binary mixtures also exhibited enhanced activation volume(osmotic sensitivity) and diminished activation energy for hydrogen peroxide flux (vi) further, glucose was readily permeable across these membranes of binary mixtures (vii) addition of cholesterol, which is abundantly found in erythrocytes, inhibited the stretch
sensitivity to peroxide permeation (viii) evidence was also seen that this diffusion increases with decrease in temperature, i.e., the process has a negative temperature coefficient.

These studies on biological membranes and liposomes, in which composition as well as dynamics considerably vary, prompted us to question as to what constitutes a minimal description to account for variable permeability induced by doping across a liposomal membrane. For instance, cardiolipin enhanced permeation of hydrogen peroxide and molecules as large as glucose. Though possible descriptors could be many, (composition, structure, dynamics in terms of inter and intra molecular potentials), we adopted a bare-bone approach to resolve this complex issue to arrive at a minimal description adequate to account for the observations.

Structural changes in the membrane are best identified by non-interactive molecules and therefore leaks across bilayers are commonly studied using non-electrolytes. The diagnostic for non-specific permeation is size dependence such that, these hydrated solutes intercalate, penetrate and navigate through such interstices, spaces or voids, stochastically or in files to reach the other side of the membrane. In order to capture the diverse features in a parsimonious manner, we restrict to a two dimensional cross-section of a three dimensional system. Such a restriction is reasonable since the probe particle permeating across the membrane at any instant of time experiences the effective cross section rather than the three dimensional obstruction. The permeation across the membrane depends primarily on the availability of free space or voids. Thus the problem reduces to the study of packing of 2-dimensional objects at the first instance. Then one needs to determine which factor(s) determine the appearance and size-distribution of voids in such a 2-dimensional system.

The configuration space of this model system (membrane) is a 2-dimensional box with toroidal boundary conditions. The constituents of this two dimensional box are the circular disks (and/or the rigid combinations of the circular disks as dopants) of unit radii. A circular disk simply represents the hard core scattering cross section, seen by the passing particle (a non-electrolyte which acts as a probe), across the thickness spanned by two lipid molecules, viewed somewhat as cylinders. A typical dopant is two or more circular disks rigidly joined in a prespecified geometry. These constituents are identified by the position coordinates of their centers, the angle made by the major axes with the side of the box (in case of dopants) and the radii of the circular disks. It is reasonable that the disks (which represent molecules, with long range attractive interaction and hard core repulsion near center, contained within
a structure) interact pairwise via Lennard-Jones potential (a measure of the interaction energy), which has the form

\[ V_{LJ}(r_{ij}) = 4\epsilon \sum_{i=1}^{N} \sum_{j=i+1}^{N} \left( \left( \frac{\sigma}{r_{ij}} \right)^{12} - \left( \frac{\sigma}{r_{ij}} \right)^{6} \right) \]

where, \( r_{ij} \) is the distance between the centers of the \( i^{th} \) and \( j^{th} \) disks, \( \sigma \) determines the range of hard core part in the potential and the \( \epsilon \) signifies the depth of attractive part.

While studying the binary mixtures, we consider different shape anisotropic combinations (impurities or dopants) of \( \kappa \) number of circular disks. We treat these combinations as one unit. e.g. \( \text{rod}_n \) denotes a single dopant made of \( n \) unit circular disks rigidly joined one after another in a straight line. The impurities interact with constituent circular disks via potential,

\[ V(r_{ij}) = \sum_{\alpha=1}^{\kappa} V_{LJ}(r_{\alpha ij}) \]

where, \( r_{\alpha ij} \) is the distance between the centers of \( \alpha^{th} \) disk in \( i^{th} \) impurity and the \( j^{th} \) circular disk, and among themselves interact via

\[ V(r_{ij}) = \sum_{\alpha=1}^{\kappa_1} \sum_{\beta=1}^{\kappa_2} V_{LJ}(r_{\alpha ij\beta}) \]

where, \( r_{\alpha ij\beta} \) is the distance between the centers of \( \alpha^{th} \) disk in \( i^{th} \) impurity and the \( \beta^{th} \) disk in \( j^{th} \) impurity.

An \( r \)-void is defined as a closed area in a membrane devoid of disks or impurities, and sufficient to accommodate a circular disk of radius \( r \). Clearly, larger voids also accommodate smaller probes, i.e., an \( r \)-void is also an \( r' \)-void if \( r' < r \). Similarly, the voids for the particle of size zero are the voids defined in the conventional sense, i.e., a measure of the net space unoccupied by the disks.

Equilibrium configurations of the model system are obtained by a Monte Carlo method (using the Metropolis algorithm) starting with a random placement of the disks. The box was filled with disks such that they occupy 70% area of the box, i.e., loosely packed to facilitate the formation of voids. The temperature parameter, \( T \), was so chosen that the quantity \( k_BT < 4\epsilon \). This ensured an approximate hexagonal arrangement of the disks and the presence of very few large voids in the absence of dopants. (Fig. 1a; far too less \( r \)-voids for \( r \geq 0.5 \)). Similar numerical simulations are performed on the model system with
dopants. The number of dopants is chosen to be 10% of the number of circular disks with a constraint that the total occupied area of the box is still 70% as the focus was on the redistribution of void sizes. Fig. 1b illustrates the formation of larger voids in the vicinity of the rod₂ impurities.

The variation in the number of r-voids as a function of the size of the permeating particle (using the digitization algorithm described in [4]) is shown in Fig. 2. When only circular disks are present (dotted curve), hardly any large r-voids are seen. When mixed with the anisotropic impurities, say, rod₂, a distinct increase in the number of large r-voids is seen with appropriate redistribution of smaller r-voids (solid curve). This result is consistent with the unexpected permeation of large molecules such as glucose through the doped membrane, observed experimentally [1]. The difference curve showed the formation of a significant extent (≈ 30%) of r-voids of size 0.5 and above.

Is the induction of large voids due to the anisotropy in potential of the impurities and, should the large voids form around the rods, the centers of anisotropy? Firstly, we carried out simulations with large circular disks in place of rod₂ as impurities. The radius of large disk was chosen in such a way that the area occupied by each of the large disk is same as that of a rod₂. Fig. 3 shows the result of such simulations. The curve (a) in Fig. 3 represents the difference curve of r-void distribution of pure membrane and that of membrane doped with rod₂ impurity. The curve (b) represents the same when the membrane is doped with large circular disks. It can be clearly seen that the number of larger r-voids is always less in the latter case, thus confirming the role of shape anisotropy in the induction of large r-voids. Further, simulations are carried out with rod₂ of smaller size and rod₄ type impurities. The curves (c), (d) in Fig. 3 respectively shows the corresponding difference curves. It shows an interesting feature that the peak of the difference curve shifts with the change in the type of anisotropy. This suggests a possible way of constructing membrane with selective permeability properties. The simulation regime adopted here, limited the exploration of ternary mixtures in yielding statistically significant results on transport. However, by using rod₂ of 0.5 size (Fig.3, curve (c)) (an oval approximation of the small dopant cholesterol), we could demonstrate a shift in void sizes to left in binary mixtures, consistent with our experimental results in ternary mixtures with cholesterol [1].

Further, we considered rodₙ type impurities. Fig. 4 represents the relation between the length dimension of rodₙ and the number of r-voids (for r=0.55). The anisotropy in the
potential of rod$_n$ increases with $n$, such that the number of large $r$-voids should increase with $n$. Fig. 4 indeed shows a jump when the rod$_2$ impurities are added and afterwards, it shows a slow and almost linear increase with increase in $n$.

Since dopants induced voids, their influence is most likely to be seen in their own vicinity, enhancing the “local transport”. As the dopants exhibit different potential in different directions, certain positions of the constituents are preferred from the point of view of energy minimization, eventually giving rise to voids in the vicinity of impurities. To verify this, we calculate the local permeation probability for particle of size $r$, which is a ratio of the area of $r$-voids and the area of the local neighborhood. Fig. 5 shows the local permeation probability around ten randomly chosen impurities and ten randomly chosen circular disks. The higher local permeation probability is seen to be associated with rod$_2$.

The model is realistic in that, one can compute elastic properties (surface tension like attribute) by stretching the membrane from one side and computing the energy change, which yielded a change of $\approx 13$ dyn/cm which is of the same order as the observed surface tension and changes there of in bilayers [7]. The model is limited in relation to an investigation of temperature effects which requires incorporation of multiple time scales. The model is really general because it not restricted by the parameter space of the components and therefore it is extendible to a variety of phenomena including transport in weakly bound granular media, voids in polymers, modeling of zeolites which may act like a sponge absorbing only desired species.

In summary, we proposed a two dimensional computational model system comprising a mixture of objects interacting via Lennard-Jones potential to explain anomalous permeation seen in bilayers. The significance of these observations on permeation, in what is essentially a granular medium (with long range attraction as an added feature), relates to development of large voids seeded by impurities. Unlike shape anisotropy, the change in composition (via a change in $\sigma$), lateral pressure/density, and presumably temperature (though dynamic simulations would need to be done) would all simply produce voids within the bounds of a hexagonal array. Even among various dopants in shapes, X, L, Y, Z, T symmetrical or otherwise, all other factors being equal, size per size, rod$_n$ produce the largest voids [4].

Thus the largest $r$-voids induced by anisotropy yield biological observations of interest, without manifesting as ordered geometric structures. In this sense these $r$-voids differ from the conventional results obtained in studies on granular media (which also do not usually
incorporate long range interactions). It is increasingly becoming clear that voids play a pivotal role in relating the dynamics of biopolymers to specific functional states.

We thank C. N. Madhavarao for discussions. The author (GRP) is grateful to CSIR (India) for fellowship and (ADG) is grateful to DBT (India) for research grant.

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[10] The equilibrium configurations thus obtained are further confirmed by simulated annealing [3].

[11] It may be recalled that glucose offers approximately half the radius of the PC cross section, yielding a relevant definition for a larger void of interest.
FIGURE CAPTIONS

Fig. 1 Typical equilibrium configurations for interacting disks. The parameter, $\sigma$ in Lennard-Jones potential is 2 units. The size of the box is $50 \times 50$ (a) A pure membrane with 556 unit circular disks. (b) A doped membrane with 464 circular disks and 46 rod$_2$ (shown in gray).

Fig. 2 Distribution of $r$-voids in two different configurations. The main graph shows the number of $r$-voids as a function of the relative size ($r$) of the probe particle. The vertical bars represent the error margins at the corresponding points. The dotted curve gives the distribution in pure membrane while the solid curve shows the same in a membrane doped with rod$_2$ (10:1). Difference curve clearly demonstrates the presence of large voids in the doped membrane.

Fig. 3 Difference curves of distribution of $r$-voids. Curves are obtained by treating the void distribution for pure membrane as the base. Difference curves for membranes (a) doped with rod$_2$ (b) doped with large circular disks (of radii $\sqrt{2}$) which occupy the same area as that of rod$_2$. These induce smaller number of large voids as compared to rod$_2$. (c) doped with small rods. Irrespective of their small size, they induce large voids, but the peak shifts towards left. (d) doped with rod$_4$. Significantly large voids are induced. The peak shifts towards the right.

Fig. 4 Dependence of the number of $r$-voids on the length of the rod-shaped impurities. The graph shows a steady increase in the number of $r$-voids (for $r = 0.55$) with $n$. The first expected large jump in the number of voids because of the shape anisotropy is seen clearly when the configuration consists of molecules in the shape of circular disks and rod$_2$.

Fig. 5 Local permeation probability in a doped model system. The points show the local permeation probability around ten randomly chosen unit disks and ten randomly chosen rod$_2$s. Further, as a guide line, averages are shown by the heights of the boxes, clearly indicating significantly more permeation in the neighborhood of rod$_2$. 
(a)  

(b)  

Fig. 1

(GRP et al.)
Fig. 2

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Fig. 3

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Fig. 4

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Fig. 5

(Local permeation probability)

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