ELSEVIER

Biophysical Chemistry 50 (1994) 139–145

Biophysical Chemistry

Diffusion and chemical reactions in phase-separated membranes

Winchil L.C. Vaz

Unidade de Ciências Exactas e Humanas, Universidade do Algarve, P-8000 Faro, Portugal

(Received 5 January 1994)

Abstract

The biological membrane may be viewed as a two-dimensional solvent system, the lipid bilayer, in which the membrane components are either dissolved (intrinsic) or to the surface of which they are adsorbed (extrinsic). The solvent bilayer is made up of a large number of lipid chemical species derived from a few lipid classes. Experience with model systems has shown that in mixed lipid bilayers immiscibility of components is the rule rather than the exception. This suggests that the bilayer in a biological membrane is not a homogenous two-dimensional fluid but rather a heterogenous system consisting of a mosaic of co-existing phase domains in which the phases differ both chemically and physically from each other. A consequence of this is the physical separation of membrane components, including proteins, based on their phase solubility. The percolation in such a phase-separated system then determines the range over which free lateral diffusion is possible and bimolecular reactions can occur. Phase percolation and long-range translational diffusion have been studied in model systems using the fluorescence recovery after photobleaching (FRAP) technique, and theoretical work shows that bimolecular reaction yields can be seriously reduced in phase-separated membranes. Transitions between percolating and non-percolating states in biomembranes is proposed as a potential trigger mechanism in the control of membrane physiology.

Key words: Membranes; Lipid mixtures; Phase separation; Diffusion; Percolation; Reaction yields

1. Introduction

The "fluid mosaic" model for membrane structure and dynamics [1] stimulated and provided the orientation for a very large body of research literature on this important biological structure. A large body of evidence accumulated over the past two decades has confirmed the validity of the three most important theses of the model: (1) the basic structure of the membrane is a lipid bilayer, a quasi-two-dimensional fluid sheet, 50–100 Å thick and extending infinitely in its plane; (2) the chemical components of the membrane (protein and lipid) are either dissolved in (intrinsic or integral) or adsorbed onto one of the two surfaces (external or extrinsic) of the fluid sheet; (3) translational diffusion of the membrane components is permitted in the plane of the sheet and forbidden in a direction perpendicular to it whereas rotational motions are permitted around an axis perpendicular to the plane of the membrane and impeded around the other axes. However, certain doubts have also arisen whether the model as proposed in 1972 provides an all-englobing description of the complexity of the structure, dynamics and physiology of the biological membrane. One of the more important questions relates to the implicit assumption of the

SSDI 0301-4622(94)00015-C

fluid mosaic model that the lipid bilayer of a biological membrane satisfies the physico-chemical criteria of a homogenous fluid system. Much evidence argues against this.

2. The biological membrane is a heterogenous chemical system

Several studies (for reviews, see refs. [2,3]) on cells in living tissues or in *in vitro* cultures show that the external or "plasma" membrane of cells in general is macroscopically heterogenous. Thus, for example, the chemical compositions (protein and lipid) of apical and basolateral parts of the plasma membrane of cells in culture with adhesion to the sustrate on which they grow are known to be quite distinct. There are also various examples of similar heterogeneities in cells within living tissues, one of the best documented cases being that of hepatocytes which have a different type of membrane on sides that are exposed to the blood vessels, bile ducts or other hepatocytes. Another well-documented case of membrane heterogeneity is the case of the sperm cell in which certain regions of the cell membrane are clearly not even fluid. Some membranes long thought to be homogenous in plane, such as the erythrocyte membrane, have been recently demonstrated to be microscopically heterogenous [4]. All this evidence violates an implicit assumption of the fluid mosaic model that the lipid bilayer is a continuous homogenous fluid sheet. Still another well-documented aspect of membrane heterogeneity,

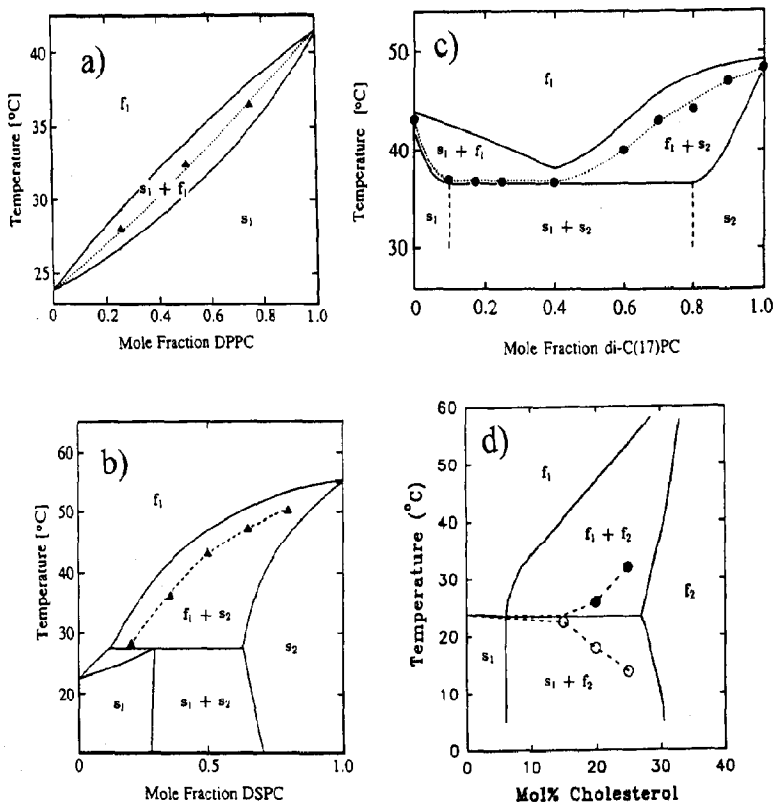

Fig. 1. Temperature-composition phase diagrammes for four binary lipid mixtures in excess water and at constant pressure: (a) an isomorphous mixture of di-$C_{(14:0)}$PC and di-$C_{(16:0)}$PC; (b) a peritectic mixture of di-$C_{(14:0)}$PC and di-$C_{(18:0)}$PC, (c) an eutectic mixture of di-$C_{(17:0)}$PC and $C_{(22:0)}C_{(12:0)}$PC; (d) a monotectic mixture of di-$C_{(16:0)}$PC and cholesterol. The percolation threshold as a function of temperature and composition, experimentally determined using the FRAP technique, is demonstrated in each case.

although of less importance for the purposes of this article, is the transbilayer asymmetry of most natural membranes which appears to be maintained at the cost of cellular energy (for a review, see ref. [5]). We shall try here to understand some of the principles underlying membrane heterogeneity in general and examine some of its consequences.

Chemical analyses [6] show that the lipid bilayers of cellular membranes are composed of a complex mixture of some classes of polar lipids, generally phospholipids. For a chemist this fact immediately raises the suspicion that the system may be a heterogenous one whose behaviour at equilibrium, at least in its fundamental aspects, obeys the laws enunciated by Gibbs over a century ago [7]. Specifically, the possibility exists that the system can exist in any one or more of various phases depending upon temperature, pressure (lateral pressure is particularly important for membranes), mechanical and electrical stress, ionic conditions of the aqueous phase in which the membrane encounters itself, and on the number of chemical constituents of the system. It becomes highly probable that more than one phase co-exist simultaneously in the same membrane.

Phase diagrams of lipid bilayers composed of a single chemical component show that these bilayers can exist in an ordered phase (popularly known as the gel phase) or in a highly fluid and somewhat disordered phase (popularly known as the liquid crystalline or fluid phase), besides other phases which exist under conditions of less relevance to the biological system. Transitions between these phases are provoked by variations in temperature, pressure and degree of hydration. Thus, these simple systems show a mesomorphism which may be thermotropic, barotropic or lyotropic. In the case of lipids that have a net charge in the polar part of the molecule, the ionic strength and pH of the aqueous medium is also important for the mesomorphism of the system.

In bilayers formed from mixtures of lipids, the mesomorphism becomes considerably more complex, often demonstrating immiscibility of co-existent phases. Fig. 1 shows temperature-composition phase diagrams for some fully hydrated binary lipid mixtures. It is clear that very small structural differences between the components of the binary mixture may lead to phase immiscibilities. Thus, for example, the binary mixture of ditetradecanoylphosphatidylcholine (di-$C_{(14:0)}$PC) and dihexadecanoylphosphatidylcholine (di-$C_{(16:0)}$PC), different from each other only in the fact that the latter has two carbon atoms more per acyl chain, is an isomorphous mixture in which the two components are completely miscible in both fluid and gel phases (Fig. 1a), whereas a mixture of di-$C_{(14:0)}$PC and dioctadecanoylphosphatidylcholine (di-$C_{(18:0)}$PC), where the acyl chains in the two lipids differ by four carbon atoms, is a peritectic mixture with clearly immiscible solid phases (Fig. 1b), although the solid phases ($P_{\beta'}$ in both cases) have very similar crystallographic characteristics. The same is seen in the eutectic mixture (Fig. 1c) of diheptadecanoylphosphatidylcholine (di-$C_{(17:0)}$PC) and 1-docosanoyl-2-dodecanoylphosphatidylcholine ($C_{(22:0)}C_{(12:0)}$PC) where the sum of carbon atoms in the acyl chains of the two lipids is identical but their distribution is asymmetric in one of the lipids. A very important binary mixture from the point of view of the biological membrane is the monotectic mixture of (di-$C_{(16:0)}$PC) and cholesterol (Fig. 1d). Here there is an immiscibility of two fluid phases, one more ordered than the other, which differ from each other merely in the relative amount of cholesterol in each phase. In one region of this phase diagram the two fluid phases co-exist side by side in the same bilayer.

3. Origins of phase separations and formation of domains in membranes

The fundamental reason for phase separation in a chemical mixture at equilibrium is thermodynamic: the intermolecular interaction energy between two molecules in a mixture leads to an association or repulsion between them, while entropy tends to take the system to a homogenous state. When a system at equilibrium is perturbed, for example by the addition of new molecules to it or by other processes, it tends to relax to a new equilibrium state at a rate which is a complex

function of several processes some of which may be extremely slow. Thus, a system in a non-equilibrium state may also exihibit phase separations determined by kinetic reasons. Since a biological membrane does not necessarily function at equilibrium, being continuously perturbed by several factors including the addition of new molecules to it, kinetics arguments become important in a consideration of phase separations in this complex system.

When there is a phase separation in a three-dimensional system such as, for example, in a mixture of oil and water, the surface tension at the interface between domains leads to a coalescence of initially dispersed domains of the same phase thereby reducing the interfacial area between the two co-existing phases. The result is a bulk separation of phases with a minimum of interfacial area between them. Reduction of the surface tension, in the case under consideration by the addition of surfactants to the system, reduces the tendency to coalescence of the dispersed domains upto the point of resulting in relatively stable emulsions (for example, mayonnaise). In two-dimensional systems, such as lipid bilayers, the reduction of dimensionality (from 3 to 2) of the system reduces the forces (now a line tension at the interface between domains) which impel the system to a coalesced state. It thus becomes more probable to have a dispersion of domains as a stable state of such a system. The experience in my laboratory with bilayers formed from binary lipid mixtures indicates that microdispersion of domains is a stable state of such systems over at least three months, a time sufficiently long for biological relevance.

4. Percolation and translational diffusion in phase-separated membranes

The existence of phase separations and microdispersion of domains in a membrane makes the connectivity between the domains of the same phase and phase percolation an important physical characteristic of the membrane. To understand the phenomenon of percolation let us consider a membrane in which two phases co-exist, one of them (the major phase, called phase A here) is continuous and the other (the minor phase, called phase B here) is microdispersed as domains within phase A (Fig. 2). Let us further assume that the mass fraction of phase B can be increased at the expense of phase A by changing one or more of the degrees of freedom (temperature, pressure, etc.) of the system. This may occur through increase in the size of pre-existing phase B domains or by nucleation of new phase B domains, or both (see Fig. 2). Eventually a critical mass fraction of phase B is achieved at which there is a continuous path over domains of this phase and the previously continuous phase A now becomes discontinuous. This critical point is the *percolation threshold* of the system. A given phase is defined as being percolative at the point when it attains the minimum mass fraction which permits it to become continuous.

Now let us examine the translational diffusion of a particle which is soluble only in phase A. As long as this phase is continuous, our test particle can access any point within phase A in the system by Brownian diffusion. The domains of the dispersed phase B only act as obstacles to the free diffusion of the particle but it can go around

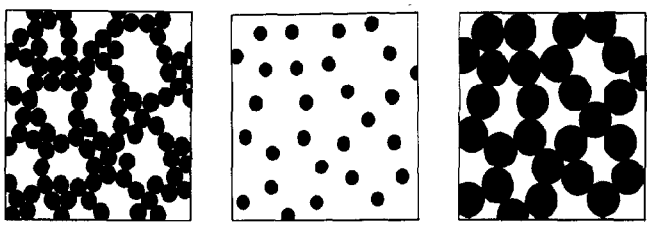

Fig. 2. The concept of the percolation threshold. Beginning from the same state (central panel), the percolation threshold may be achieved by increasing the size of the minor phase domains without increasing their number (right-hand panel), or by increasing the number of domains without increasing their size (left-hand panel), or by a combination of the two processes (not shown).

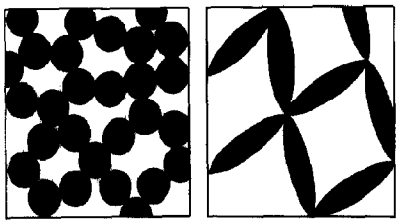

Fig. 3. Demonstration of the effect of domain asymmetry upon attaining the percolation threshold.

them. The result is a reduction in the translational diffusion coefficient, D_t, over distances that are very much larger than the characteristic size of the phase B domains. Theoretically, the reduction in D_t is a function of the mass fraction of the obstacle phase (phase B) and the mobility of its domains relative to the particle mobility [8–12], as well as of the shape of the obstacle domains [13,14]. What is true for phase B domain obstacles applies equally well to other obstacles such as protein molecules or molecular aggregates that are impenetrable to the test particle and dispersed in the major phase.

In the case of mobile obstacle domains, if the obstacle mobility is larger than the test-particle mobility, or when the obstacles are immobile, long-range D_t for the particle drops linearly with the mass fraction of the obstacle phase. In the first case $D_t = 0$ only when all of the system is in phase B and in the latter case $D_t = 0$ when the percolation threshold is attained. If obstacle mobility and particle mobility are comparable, reduction of D_t is a non-linear monotonic function of the phase B mass fraction and $D_t = 0$ only when the whole system is in phase B.

The percolation threshold is also a monotonic non-linear function of obstacle asymmetry. Asymmetric domains (obstacles) attain the percolation threshold at a lower mass fraction than symmetric domains. This is seen in Fig. 3.

The only demonstration of the phenomenon of percolation in the plane of membranes has been in lipid bilayer model systems composed of binary lipid mixtures in which there is a co-existence of solid and fluid phases in the same bilayer [15–20] and in one bilayer system composed of a ternary mixture of two phospahtidylcholines and cholesterol [21]. The fluorescence recovery after photobleaching (FRAP) technique (for a review of the method see ref. [22]) was used in these studies to measure the long-range lateral diffusivity of fluorescent lipid analogues which had been previously shown to be soluble in only fluid lipid phases. Probe diffusion was examined over several μm distances as a function of temperature and chemical composition of the bilayers. The method permits the detection of percolating and non-percolating states from an evaluation of re-

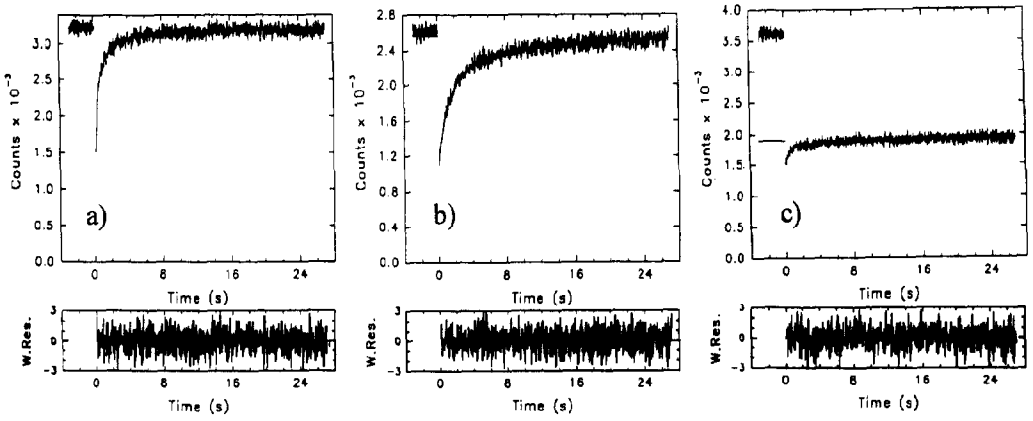

Fig. 4. Application of the FRAP technique to lipid bilayers with phase separations. The figure shows some typical experimental curves for diffusional recovery of fluorescence in (a) a bilayer with a single fluid phase, (b) a bilayer in which the solvent fluid phase is continuous but in which there are probe-impenetrable domains of a second (dispersed) phase, and (c) a bilayer in which the solvent fluid phase is disconnected. Reproduced with permission from ref. [20].

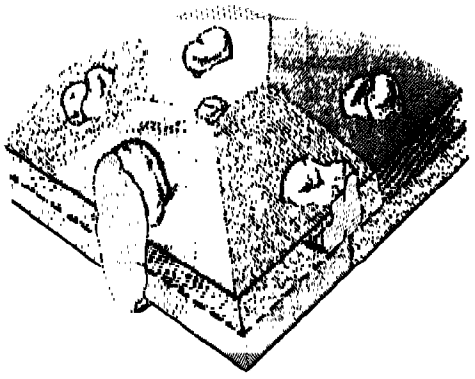

Fig. 5. A proposed modification of the fluid mosaic model in which the protein mosaic is superimposed upon a mosaic of co-existent lipid phase domains.

covery of fluorescence intensities at "infinite" time after photobleaching [15] or characteristic recovery times [20] for fluorescence recovery. Fig. 4 shows some typical experimental curves.

On the basis of these experiments it was possible to define percolation threshold lines in the temperature-composition phase diagrams for the bilayer systems examined in regions of these diagrams where gel and fluid phases co-exist. These results are included in Fig. 1. Application of the "lever rule" allows a calculation of the mass fraction of each phase at the percolation threshold. It is clear that this fraction is variable from system to system and even within the same system and that the shape of gel phase domains can in some cases be extremely asymmetric (axial ratios of as low as 0.03 if the domains are considered to be elliptical). One conclusion that is evident from these results is that it is possible in certain cases to have a major phase (upto 75% of the mass of the system) divided into non-connected domains by a minor phase which, due to the strong asymmetry of its domains, forms a quasi-two dimensional reticulum in the membrane plane.

In general, phase co-existence in membranes may lead to a mosaic not just of components as originally proposed by Singer and Nicolson [1] but also to a mosaic of phase domains (see Fig. 5). Such a situation leads to an effective spatial separation of membrane components due to preferential solubilities of these in one or the other phases. This type of mosaic has important consequences for bimolecular reactions that occur in membranes as well as for several other characteristics of these systems (for a brief review see ref. [23]) which will not be discussed here.

5. Phase separations and bimolecular reactions in membranes

The consequences of percolation and diffusion in membranes with phase separations has been

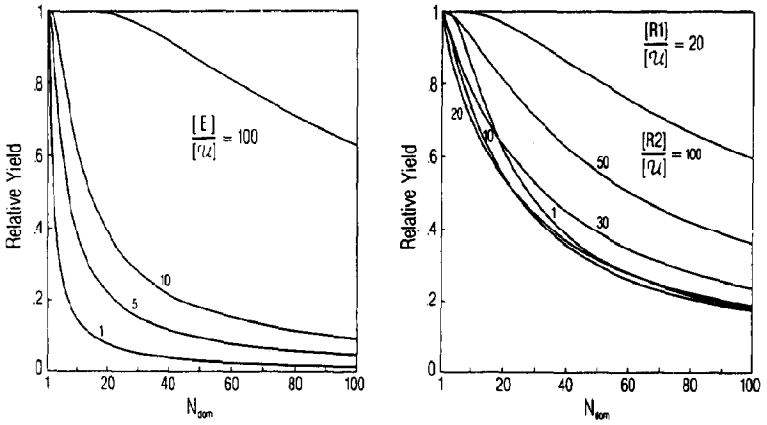

Fig. 6. Theoretical simulations of bimolecular reaction yields in membranes with phase separations. Two types of reactions have been considered: (left) a simple enzymatic reaction in which E + R → E + P (where E is the enzyme, R the reactant and P the product); (right), a classical bimolecular reaction of the type R1 + R2 → P (where R1 and R2 are reactants and P is the product). Reproduced with permission from ref. [24].

examined from a point of view of reaction yields for several types of reactions that are of biological interest and are known to occur in membranes [24]. In the case of reactants that are dissolved in non-connected domains, separated by other domains or structures that are impenetrable, the reaction yield is simply a function of the probability of encountering the reacting species in the same domain. A relative reaction yield can be defined as the ratio of the reaction yield in a phase separated system in which the solvent phase is dispersed as non-connected domains to the yield in a system in which the solvent phase is continuous. The results of theoretical simulations for two common types of membrane-associated reactions are shown in Fig. 6. It is evident that for cases where the number of reacting molecules is limited (which is often the case in biological membranes) or the number of dispersed solvent-phase domains is large, the effects can be quite dramatic.

A membrane that exists close to its percolation threshold may respond rapidly to very small perturbations of its physical and chemical milieu by crossing the threshold in one direction or another thereby making biochemical reactions that occur in it possible or impossible. Crossing the percolation threshold can thus be viewed as a switching mechanism in biochemical processes that occur at the level of the biological membrane with its consequences for cell physiology.

Acknowledgements

This work was supported in part by the Junta Nacional de Investigação Científica e Tecnologica (JNICT) through the STRIDE program (project No. STRDB/CEN/513/92) and by the Commission of the European Communities through the Human Capital and Mobility programme (contact No. EBBCHRXCT920018). I am grateful to various colleagues at the Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany (upto 1991) and at the Universidade do Algarve, Faro, Portugal, for their collaboration over the past six years during which this work was realised. Special thanks are due to Thomas E. Thompson, Dieter Hallmann, Eurico Melo and Paulo Almeida.

References

[1] S.J. Singer and G.L. Nicolson, Science 175 (1972) 720.
[2] M.K. Jain, in: Membrane fluidity in biology, ed. R.C. Aloia (Academic Press, New York, 1983) p. 1.
[3] J.F. Tocanne, L. Dupou-Cezanne, A. Lopez and J.F. Tournier, FEBS Letters 257 (1989) 10.
[4] M. Glaser, Curr. Opin. Struct. Biol. 3 (1993) 475.
[5] P.F. Devaux, Curr. Opin. Struct. Biol. 3 (1993) 489.
[6] J.A.F. op den Kamp, Ann. Rev. Biochem. 48 (1979) 47.
[7] J.W. Gibbs, Trans. Connecticut Acad. 3 (1877) 108, 343.
[8] M.J. Saxton, Biophys. J. 39 (1982) 165.
[9] M.J. Saxton, Biophys. J. 52 (1987) 989.
[10] M.J. Saxton, Biophys. J. 56 (1989) 615.
[11] A.K. Harrison and R. Zwanzig, Phys. Rev. A 32 (1985) 1072.
[12] H. Van Beijeren and R. Kutner, Phys. Rev. Letters 55 (1985) 238.
[13] W. Xia and M.F. Thorpe, Phys. Rev. A 38 (1988) 2650.
[14] E.J. Garboczi, M.F. Thorpe, M.S. de Vries and A.R. Day, Phys. Rev. A 43 (1991) 6473.
[15] W.L.C. Vaz, E.C.C. Melo and T.E. Thompson, Biophys. J. 56 (1989) 869.
[16] W.L.C. Vaz, E.C.C. Melo and T.E. Thompson, Biophys. J. 58 (1990) 273.
[17] T. Bultmann, W.L.C. Vaz, E.C.C. Melo, R.B. Sisk and T.E. Thompson, Biochemistry 30 (1991) 5573.
[18] W.L.C. Vaz, Comments Mol. Cell. Biophys. 8 (1992) 17.
[19] P.F. Almeida, W.L.C. Vaz and T.E. Thompson, Biochemistry 31 (1992) 6739.
[20] P.F. Almeida, W.L.C. Vaz and T.E. Thompson, Biochemistry 31 (1992) 7198.
[21] P.F. Almeida, W.L.C. Vaz and T.E. Thompson, Biophys. J. 64 (1993) 399.
[22] T.M. Jovin and W.L.C. Vaz, in: Methods in enzymology, Vol. 172, eds. S. Fleischer and B. Fleischer (Academic Press, New York, 1989) p. 471.
[23] W.L.C. Vaz and P.F. Almeida, Curr. Opin. Struct. Biol. 3 (1993) 482.
[24] E.C.C. Melo, I.M.G. Lourtie, M.B. Sankaram, T.E. Thompson and W.L.C. Vaz, Biophys. J. 63 (1992) 1506.