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Editors

# Biomembrane Frontiers

Nanostructures, Models, and the Design of Life

Volume 2

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ISBN 978-1-60761-313-8 e-ISBN 978-1-60761-314-5  
DOI 10.1007/978-1-60761-314-5  
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2009927494

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## PREFACE

The book, *Biomembrane Frontiers: Nanostructures, Models, and the Design of Life*, a volume in the *Handbook of Modern Biophysics* series, is based on a workshop held on the 20th and 21st of March 2008 at the University of California Davis. Unlike other meeting monographs, the book presents the exciting frontiers of biomembrane research for both expert and student colleagues interested in research at the interface of biology and physics.

The idea of the workshop originated from discussions about how to create an effective outreach for the NSF-NIRT joint project “Aerogel and Nanoporous Materials for Biomolecular Applications” between the Longo, Faller, and Risbud groups at UC Davis and the groups of Curt Frank at Stanford and Joe Satcher at Lawrence Livermore National Laboratory. In the project we interacted with researchers from diverse backgrounds and hoped to create an opportunity to foster a multi- and interdisciplinary exchange of ideas. Thus, the workshop idea was conceived.

The workshop brought together experts working on many different aspects of biological membranes: from theory and simulation, to supported model bilayers, and to clinical applications. Several material scientists working on the interactions of biological membranes with biological or nonbiological materials also participated. Such a diverse set of experts in one meeting is unusual, as the different communities of theorists and experimentalists working on model membranes and real biological systems are typically quite distinct and do not often interact. Very few, if any, conferences take up the challenge of embracing a broad range of research interests. The chapters of the volume reflect the dynamic synergism of the diverse research interests in biomembrane research and present invaluable, leading ideas to a broad community of researchers and students.

At the workshop, the lively discussion made clear that everybody learned from this unique interaction with colleagues from several disciplines. It was obvious that many aspects of membranes cut across a variety of disciplines and that only research using a combination of ideas and techniques can facilitate real progress.

Several of the speakers invited their graduate students to the workshop. A large number of local graduate students and faculty also participated. Indeed, the graduate students benefited from examining the common theme of membranes from many different perspectives. These students also presented impressive work during poster sessions. The workshop had more than 70 participants.

The book is arranged topically. It leads from theory to applications. After an introduction by Harden McConnell on the history of lipid complexes over the last century, several chapters on theoretical and computational descriptions of membranes follow. Even within this subgroup there is already great diversity. Studying membranes at many different time and length scales is important and requires a wide variety of theoretical approaches to address them. The next block of chapters deals with techniques and applications in model membranes of increasing complexity. In this area there is always the compromise between the simplicity necessary to understand the system in as much detail as possible and the complexity to mimic real biological membranes as realistically as possible. The final chapters address questions of biological and clinical importance involving real membranes.

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The workshop and ultimately this book would not have been possible without the dedicated support of a number of people: these are, of course, first and foremost, the speakers and graduate student poster presenters at the workshop and the authors of the chapters. They volunteered their time and effort to make this workshop a success. Specifically, we would like to thank our graduate students Allison Dickey, Emel Goksu, Clark Henderson, Matthew Hoopes, Monica Lozano, Barbie Nellis, Mike Skaug, Juan Vanegas, and Chenyue Xing for their help in organizing and running the workshop. In particular, we would like to acknowledge the organizational talent of Jenny McDonald. Finally, we would like to thank the NSF-NIRT program and the Graduate Group in Biophysics at UC Davis for their financial support, and Springer Science+Business Media for the opportunity to publish this book.

Roland Faller, Thomas Jue, Marjorie Longo, and Subhash Risbud

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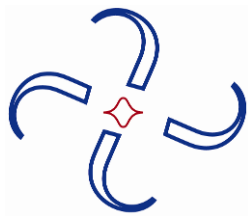
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# PERSPECTIVES: COMPLEXES IN LIQUIDS, 1900–2008

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## INTRODUCTION

The interplay of chemical and phase equilibria is one of the classical problems in physical chemistry, having literature citations as far back as the early 1900s. Phase diagrams of particular interest have been those for ternary liquid mixtures (and alloys) that form closed loops. These loops correspond to composition regions in which two liquids are formed. These diagrams were sometimes interpreted in terms of the reversible formation of compounds, or complexes between components of the mixtures. This subject is now of renewed interest in connection with the discovery of liquid–liquid immiscibility in monolayer and bilayer membranes composed of phospholipids and cholesterol. Specific ternary mixtures of phospholipids and cholesterol in bilayers have closed-loop phase diagrams, and have also been interpreted in terms of the formation of complexes. Immiscibility is found in model mixtures of defined composition, and also in lipids extracted from cell membranes and in lipid blebs from animal cells. The observed consolute critical temperatures of some of these mixtures are not far from temperatures characteristic of animal cells, raising the possibility of biophysical significance.

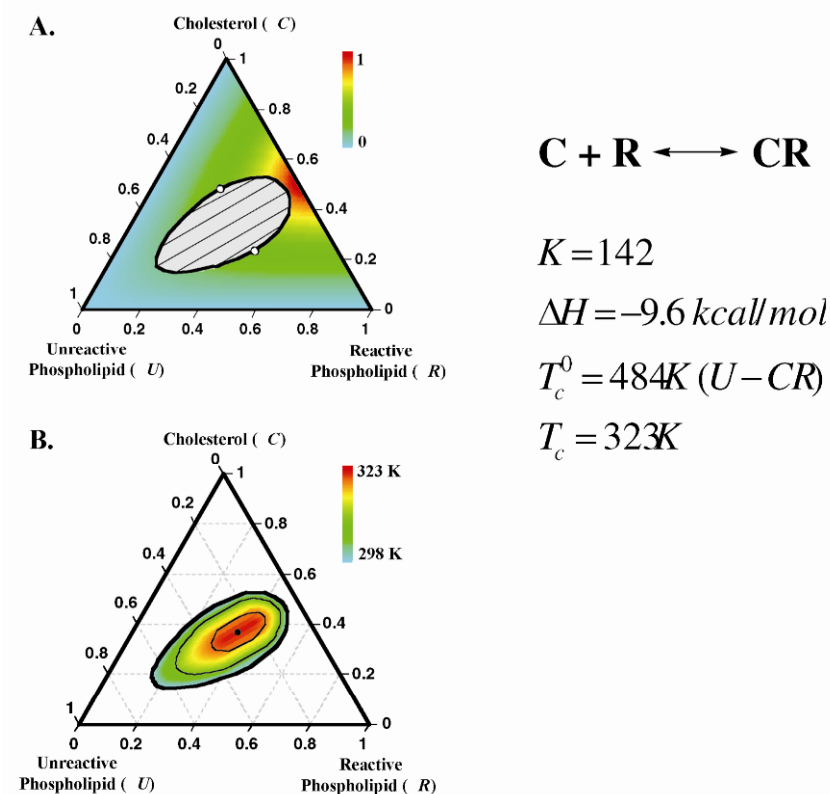
The purpose of this discussion will be to describe simple models of intermolecular interactions that have been used to model liquid–liquid immiscibility in monolayers and bilayers.

## COMPLEXES AND THE ORIGIN OF LIQUID–LIQUID IMMISCIBILITY

Our interest in complex formation between cholesterol and phospholipids originated with attempts to model phase diagrams observed for monolayer mixtures at the air–water interface

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[1,2]. In our calculations we followed the earlier general theoretical studies of Corrales and Wheeler [3]. Much earlier investigators were also interested in nonideal concentrated liquid mixtures [4]. Of particular interest were closed-loop phase diagrams that describe liquid–liquid immiscibility in ternary mixtures [5]. One example is the phase diagram for a ternary mixture of phenol, acetone, and water. At temperatures between 65 and 92°C any pair of these substances is completely miscible, but mixtures containing all three components simultaneously give rise to two liquid phases. An explanation, doubtless oversimplified, is that (a) acetone and phenol form a complex, and (b) this complex is immiscible with water.



**Figure 1.** Calculated phase diagram for a ternary lipid mixture in which two components — *C* (cholesterol) and *R* (saturated phospholipid) — react reversibly to form a 1:1 molecular complex, and in which this complex undergoes repulsive interactions with the third component, *U* (dioleoylphosphatidylcholine). Please visit <http://www.springer.com/series/7845> to view a high-resolution full-color version of this illustration.

A number of ternary lipid mixtures in bilayers also show closed-loop phase diagrams, as shown in the work of Veatch and Keller [6]. The ternary lipids usually consist of cholesterol, a saturated phospholipid, and an unsaturated phospholipid. We proposed that this immiscibility is related to the formation of “condensed complexes” between cholesterol and the saturated phospholipid [7], by analogy with the model used earlier for monolayers [1,2]. Figure 1 gives the result of a theoretical calculation of the phase diagram of a ternary lipid mixture: cholesterol

(C), dipalmitoylphosphatidylcholine (DPPC, or “reactive” phospholipid (R)), and dioleoylphosphatidylcholine (DOPC, or “unreactive” phospholipid (U)) [8]. In constructing this diagram it was assumed that cholesterol and DPPC form a complex, and that this complex tends to be immiscible with DOPC. The diagram in Figure 1 seeks to model the experimental results of Veatch and Keller for the phase diagrams of ternary lipid mixtures containing cholesterol and phospholipids [6]. Of course, over the years there have been many earlier qualitative proposals for the formation of cholesterol–phospholipid complexes, but there have been very few attempts at quantitative studies. (See [2] for extensive references to these earlier proposals.)

One can summarize some of the theoretical methods that can be used to calculate the thermodynamic phase diagrams yielding liquid–liquid immiscibility with the following formulas.

Two components, mean field repulsion:

$$F = -TS + |a|X_A X_B. \quad (1)$$

Three components, mean field attraction:

$$F = -TS - |a|X_A X_B, \quad (2)$$

Two, three, or more components, mean field plus complexes:

$$F = -TS - kTX_{\text{cpx}} \ln K + |a|X_{\text{cpx}} X_{\text{DOPC}}. \quad (3)$$

Ising lattice model:

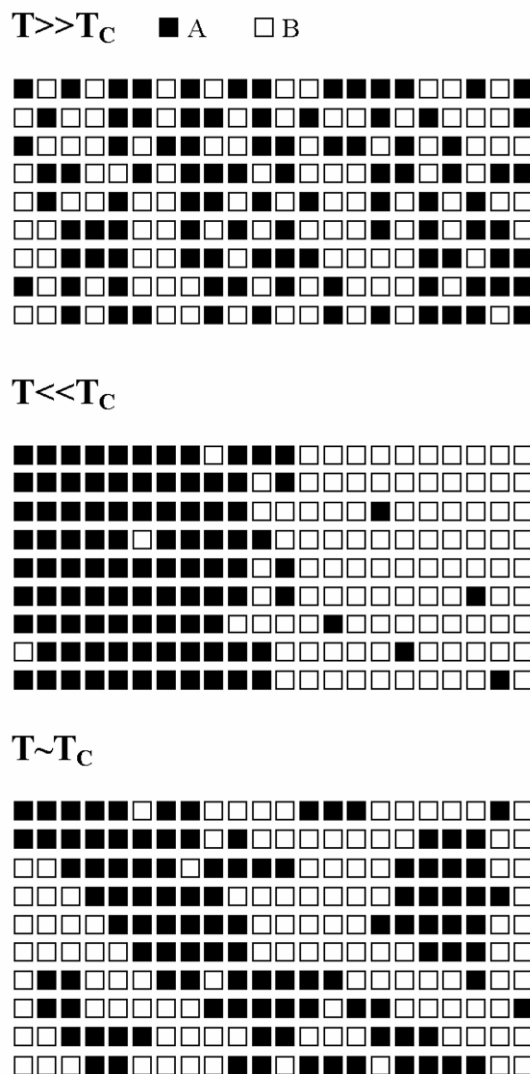
$$E = -\sum_{i<j} J_{ij} (s_i s_j - 1). \quad (4)$$

In Eqs. (1)–(3),  $-TS$  gives the contribution of the entropy of mixing to the free energy, and  $|a|$  is a parameter that represents a mean-field repulsion (Eqs. (1) and (3)) or attraction (Eq. (2)) between molecules. Equation (3) was used in constructing the phase diagram in Figure 1; here  $K$  is the equilibrium constant describing the formation of a complex between cholesterol and DPPC, and the parameter  $|a|$  describes the putative repulsion between the complex and DOPC. The quantities  $X$  represent the mole fractions of components.

Equation (1) is the most elementary equation that can be used to model liquid–liquid immiscibility, in this case between two components A and B. In this model there is a competition between the entropy that favors random mixing and the energy term proportional to  $|a|$  that favors a separation of the A and B molecules. The mean field term  $|a|X_A X_B$  has the form of a long-range interaction between A and B molecules. There are usually no long-range interactions in bilayers. The Ising model, described in Eq. (4), can also be discussed in terms of A and B molecules, and does not involve a mean-field free energy.

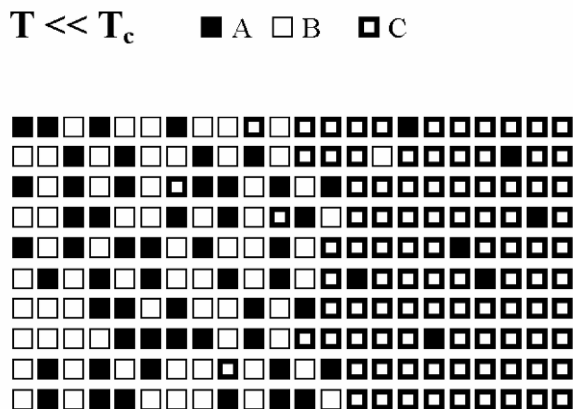
In the Ising model, spins with possible values of  $S_i = \pm 1$  are placed on a square lattice, where the nearest neighbor interactions are repulsive when neighboring spins have opposite values,  $S_i = -S_j$ . Otherwise, there is no interaction. One can also describe this Ising problem in terms of the interactions between molecules A and B. That is, equal numbers of molecules A and B are placed on a square lattice. Molecules A and B repel one another. This gives rise to the temperature-dependent “liquid–liquid” phase separation sketched in Figure 2, for which the critical transition temperature is  $T_c$ .





**Figure 2.** Schematic representation of the Ising problem in terms of molecules A and B where A and B repel one another. At high temperatures the distribution of molecules is dominated by the entropy of mixing, whereas at lower temperatures the phase separation into a liquid rich in A and a liquid rich in B is dominated by the repulsive AB interaction. At the critical transition temperature,  $T_c$ , there is long-range composition correlation in the sense that molecules of one type are most likely to be surrounded by molecules of the same type, that is, A (B) molecules are most likely to be surrounded by A (B) molecules.

It has recently been shown that ternary lipids mixtures containing cholesterol, as well as animal cell lipids, have properties (critical exponents) that conform to this 2D Ising model [9,10]. A lattice model can also be used in describing complex formation, as illustrated in Figure 3. Here molecules A and B react reversibly to form a complex C, and this complex undergoes repulsions with both A and B. Some models, termed “decorated lattice models,” can describe complex formation and can be mapped mathematically on the Ising model [3].



**Figure 3.** Phase separation can also be described by means of a lattice model, including complex formation. Here phase separation is brought about by the reaction of A and B to form a complex C, where C undergoes a repulsive interaction with A and B.

Clearly, a number of thermodynamic models can be used to describe liquid–liquid immiscibility in membranes containing phospholipids and cholesterol. Some of these models explicitly postulate the formation of molecular complexes. In these concentrated, condensed liquids molecular pairs may preferentially associate with one another due to repulsive interactions among other molecules. (Note the preferred association of the A molecules with one another in Fig. 2 due to the repulsion between the A and B molecules.) In general we use the term “complex” formation between molecules X and Y to describe molecular liquids in which the probability of finding neighboring XY molecules exceeds that expected for random mixing, irrespective of whether or not isolated pairs of X and Y “attract” one another.

We find the idea of complexes to be chemically intuitive, and to facilitate the calculations of the shapes of phase diagrams, chemical activities, heat capacities, and NMR spectra. The NMR spectra of bilayers containing cholesterol and phospholipids may reflect the kinetics of formation and dissociation of complexes (for leading references, see [11]).

## OUTLOOK

Our objective in using complexes to interpret the physical chemical properties of phospholipid-cholesterol mixtures is twofold. First, as briefly summarized above, this is a convenient intuitive first-approximation. Second, the idea of complex formation is likely to be appropriate to other weak but relatively specific reversible lipid–protein and protein–protein interactions in cell membranes. References [1,2,6–11] provide many references to earlier as well as more recent work in this field.

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