This research is to study the lateral distribution of 1-palmitoyl-2-(10-pyreny)decanoyl-sn-glycerol-3-phosphatidylcholine (Pyr-PC) in dimyristoylphosphatidylcholine (DMPC) bilayer membranes. Fluorescence and statistical mechanics are employed. A series of dips were found in the plot of E/M (the ratio of excimer fluorescence to monomer fluorescence) vs. the mole fraction of Pyr-PC. The results can be interpreted in terms of the extended hexagonal super-lattice model. Based on this model, lipids in the Pyr-PC/DMPC binary mixtures are regularly distributed in the membrane. Pressure data further suggest that the bilayer membrane has more free volume at non-critical concentrations and more free volume when the regular distribution (as compared to the irregular region) reaches a local minimum. The computer simulation work further suggests that the area of regular arrangements have local maxima at the critical mole fractions if the repulsive interaction between Pyr-PCs is strong enough.
A. Statement of the problem studied

Lipid lateral distribution in membranes is an old, but not well understood, problem. In theory, lipids can be randomly distributed, domain segregated or regularly distributed in a given two-component membrane. However, convincing experimental evidence for lipid regular distribution is lacking, and little is known about the physical principle governing regular distribution. In this study, lipid lateral distribution in two-component model membranes composed of 1-palmitoyl-2-(10-pyreny)decanoyl)-sn-glycerol-3-phosphatidylcholine (Pyr-PC) and dimyristoylphosphatidylcholine (DMPC) is examined by fluorescence spectroscopy and statistical mechanics. Pressure, temperature, and lipid composition are the experimental variables. In this project, Dr. Chong's (PI) laboratory at Meharry Medical College conducts the experimental work whereas Dr. Sugar (Co-PI) at Mount Sinai Medical Center is responsible for the theoretical calculations.

B. Summary of the most important results

The most significant result obtained in the PI's laboratory is the discovery of E/M dips (Tang & Chong, 1992 Biophys. J.63, 903-910). This result provides the most compelling evidence that Pyr-PC can be regularly distributed in DMPC multilamellar vesicles. We have observed a series of dips, in addition to kinks, in the plot of E/M (the ratio of excimer fluorescence to monomer fluorescence) vs. the mole fraction of Pyr-PC. We have proposed an extended hexagonal super-lattice model to explain the observed dips/kinks. The model states that if the pyrene-containing acyl chains are regularly distributed as a hexagonal super-lattice in the DMPC matrix at a specific concentration Y, then the acyl chains of DMPC can form a regularly distributed hexagonal super-lattice in the membrane at a critical concentration.
Using this model, we found that there is an excellent agreement between the calculated and the observed dips/kinks positions.

Our recent pressure data revealed differences in lipid packing between Pyr-PC/DMPC bilayers at critical mole fractions and those at non-critical mole fractions (Tang & Chong, unpublished results). At pressures below 0.6 kbar and at 30 °C, Pyr-PC/DMPC mixtures are in the liquid-crystalline state. In this pressure range, E/M is found to be almost invariant with pressure for 50 mol% Pyr-PC in DMPC, but decreasing steadily with pressure at neighboring non-critical concentrations. Similar results were observed near 33.3 mol% Pyr-PC in DMPC. Pressure data suggest that Pyr-PC/DMPC mixtures at non-critical mole fractions have more free volume or a higher compressibility than mixtures at critical mole fractions, because pressure produces only volume changes under isothermal conditions and because the decrease in E/M for pyrene derivatives in membranes has been previously reported to result from decreased free volume.

The revealed physical nature of the E/M dips not only gives us a better understanding of lipid lateral organization in membranes but also leads to new theoretical considerations and experimental design for exploring the relationship between lipid regular distribution and membrane function. For example, we have recently found that the activity of phospholipase A2 can be modulated by lipid compositions in the membrane, becoming higher when the area of regular distributions reaches a local maximum (Wei, Wu & Chong, unpublished results). This represents the first evidence that lipid regular distribution has a regulatory role in membrane functions.

Dr. Sugar (Co-PI), on the other hand, made an important contribution in the theoretical aspect. He developed a computer program which simulates, by means of Monte Carlo methods, the lateral distribution of pyrene-labeled molecules in lipid membranes. By assuming long-range repulsive interaction between the labeled chains we were able to get simulated E/M vs. X curves (i.e., excimer to monomer ratio vs. molar ratio of the labeled molecules). In agreement with the experimental data there are kinks or dips in the calculated E/M curves at certain critical concentrations of the labeled molecules. The pattern analysis of the computer generated distributions of the labeled molecules shows that there are areas of the membrane where the labeled molecules are arranged regularly. The computer analysis further shows that the area of regular arrangements have local maxima at the critical concentrations if the repulsion is strong enough. In the case of weak repulsion the area of regular arrangements is a monotone increasing function of the concentration of the labeled chains. In conclusion, when E/M dip is measured at a critical concentration then a cluster of regularly arranged labeled chains is always percolated throughout the membrane; on the other hand, when E/M kink is measured at a critical concentration the cluster of regularly arranged chains is rarely percolated.

During this funding period, Dr. Sugar published a paper entitled "Computer Simulation of 2D-NMR (NOESY) Spectra and Polypeptide Structure Determination". In this work the
support of Army Research Office has been acknowledged because a basic idea of the paper on the
general polypeptidic dynamics came from our earlier work which related directly to the Army
grant. It is very interesting that a mathematical trick which resulted in general explicit
Chem. 95, 7508-7515) leads to the general explicit relationship between jump models of
intramolecular dynamics and spectral densities of 2D-NMR spectra. This result will be useful
for analyzing 2D-NMR spectra of flexible macromolecules.

C. List of all publications and technical reports

Fourier Transforms in the Analysis of Fluorescence Data. 2. Fluorescence of Pyrene-labeled
95, 7516-7523.

Fluorescence Data. 3. Fluorescence of Pyrene-Labeled Phosphatidylcholine in Lipid Bilayer

Formation of Interdigitated Liposomes. A Study with Prodan Fluorescence. Biochemistry,
30, 9485-9491.

Hexagonal Super-Lattices in Pyrene-PC/DMPC Binary Mixtures at Specific Concentrations.
Biophys. J. 63, 903-910.

Sugar, I. P. and Xu, Y. (1992) Computer Simulation of 2D-NMR (NOESY) Spectra and


interdigitation on proton permeability in DPPC vesicles. Biophys. J. in press.

D. List of all participating scientific personnel showing any advanced degrees earned by them
while employed on the project

Mr. Junwen Zeng, a graduate student research assistant supported by this Army grant, received
his Ph.D. degree in Biochemistry from Meharry Medical College in May 1993.