# EXECUTIVE SUMMARY

## of

## UGC sponsored major research project [File No: 42-769/2013(SR)]

### (01.04.2013 - 31.06.2017)

**Title:** ***Preparation of unilamellar vesicles and study their interaction with ions: a bio-mimetic system.***

Principal Investigator: **Dr. Sanat Karmakar**

Department of Physics, Jadavpur University, Kolkata - 700 032

Emails: sanat@phys.jdvu.ac.in / sanat.karmakar@jadavpuruniversity.in

Lipid bilayer is the basic building block of all biological membranes. Phospholipids are the major constituent of the bio-membranes. Therefore, phospholipid bilayer serves as an excellent model system of the membranes. The complexity of bio-membrane has led to the development of a wide variety of simpler model systems. As model systems, unilamellar vesicles and solid supported bilayers are widely used for studying structure and function of membranes at physiological condition. The effect of various types of ions on model membranes has enticed the significant interest due to their immense biological significance. Among all ions alkali metal ions, such as $Na^+$ , $K^+$ are the most abundant cations in eukaryotic cells. Influence of other alkali metal ions, such as $Li^+$, $Rb^+$ and $Cs^+$ is also physiologically relevant and are of biomedical importance. In this project, we have systematically investigated the effect of various alkali metal ions with negatively charged phospholipid membranes.

As a model membranes, we have prepared large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV) and solid supported bilayers from zwitterionic, negatively and positively charged phospholipids. Size distributions of large unilamellar vesicles have been confirmed using dynamic light scattering. Zeta potential and effective charges per vesicle in the presence of various alkali metal ions have been estimated from the measured electrophoretic mobility. We have studied the binding kinetics and estimated the binding constant of ions with the membranes using isothermal titration calorimetry. We have also determined the intrinsic binding constant from the zeta potential using electrostatic double layer theory. The reasonable and consistent value of the intrinsic binding constant of Na+, found at moderate NaCl concentration (10–100 mM), indicates that the Gouy–Chapman theory cannot be applied to very high (> 100 mM) and very low (< 10 mM) electrolyte concentrations. The isothermal titration calorimetry study has revealed that the net binding heat of interaction of the negatively charged vesicles with monovalent alkali metal ions is small and comparable to those obtained from neutral phosphatidylcholine vesicles. The overall endothermic response of binding heat suggests that interaction is primarily entropy driven. The entropy gain might arise due to the release of water molecules from the hydration layer vicinity of the membranes. Therefore, the partition model which does not include the electrostatic contribution suffices to describe the interaction. The binding constant of Na+ (2.4 ± 0.1 $M^{-1}$), obtained from the ITC, is in agreement with that estimated from the zeta potential (~2.0$M^{-1}$) at moderate salt concentrations. Our results suggest that hydration dynamics may play a vital role in the membrane solution interface which strongly affects the ion–membrane interaction.

The effect of counterions on the interaction of the $Na^+$ ion with phospholipid membranes has also been investigated. The intrinsic binding constant of $Na^+$ in the presence of various counterions, such as $Cl^-$ , $Br^-$ and $I^-$ was derived from the zeta potential using Gouy Chapman theory at moderate salt concentrations (10-100 mM).The effect of counterions on the binding affinity of $Na^+$ follows the order $Br^- > I^- > Cl^-$ . Fluorescence spectroscopy using a lipophylic dye nile red was systematically studied in order to gain insights into the locations of ions and the effect of ions of the bilayers properties. The results of the fluorescence lifetime and anisotropy experiments suggest that among all anions, $I^-$ adsorbs and penetrates into the membrane, indicating a significant effect of $I^-$ ion compared to other anions. This result is in agreement with the earlier simulation study on the system.

In summary, the affinity of ion adsorption to the negatively charged membranes follows the sequence $Li^+ > Na^+ > K^+ > Cs^+ > Rb^+$ Absorption of cations to the negatively charged membranes is electrostatic in nature. However, overall enothermic response, as evidenced from isothermal calorimetry study, indicates entropy driven ion-membrane interaction. Hydration dynamics at the solvent-membrane interface might be the primary source of entropy gain. The decrease in fluorescence lifetime and increase in anisotropy of the membrane sensitive probes nile red indeed suggests that the solvent relaxation dynamic plays an important role in the ion-membrane interaction.

## Publications from the project

1. **Pabitra Maity**, Baishakhi Saha , Gopinatha Suresh Kumar , **Sanat Karmakar**, Binding of monovalent alkali metal ions with negatively charged phospholipid membranes, *Biochim. Biophys. Acta: Biomembrane*, 1858 (2016), 706-714.

2. **Pabitra Maity**, Baishakhi Saha , Gopinatha Suresh Kumar , **Sanat Karmakar**, Effect of counterions on the binding affinity of $Na^+$ ion with phospholipid membranes, *RSC Advances,* 6 (2016), 83916.

3. Amrita Basu, **Pabitra Maity**, Prasanta Karmakar, **Sanat Karmakar**, Preparation of Giant Unilamellar vesicles and Solid Support Bilayer from Large Unilamellar Vesicles: A Model System of Biological Membranes, *Journal of Surface Science and Technology*, 32 (2016), 83-90.

4. Achinta Sannigrahi, **Pabitra Maity**, **Sanat Karmakar**, and Krishnananda Chattopadhyay, Interaction of KMP-11 with Phospholipid Membranes and Its Implications in Leishmaniasis: Effects of Single Tryptophan Mutations and Cholesterol, *The Journal of Physical Chemistry B*, 121, (2017), 1824-1834.

5. Animesh Halder, Baishakhi Saha, **Pabitra Maity**, Gopinatha Suresh Kumar, Deepak Kumar Sinha and **Sanat Karmakar,** Lipid chain saturation and the cholesterol in the membrane affect the spectroscopic properties of lipophilic dye nile red, Submitted to *Spectrochim Acta* ***Spectrochimica Acta Part*** *A: Molecular and Biomolecular Spectroscopy*.

6. **Pabitra Maity**, Baishakhi Saha , Gopinatha Suresh Kumar , **Sanat Karmakar**, Interaction of cations and anions with phospholipids membranes: A comparative study: The manuscript under preparation.