Contents

Abbreviations .................................................................................................. i
Synopsis ......................................................................................................... ii
List of publications ........................................................................................ vi
Presentations and abstracts ........................................................................ viii

Chapter 1. Introduction
1.1. Biological Membranes ........................................................................... 2
1.2. Organization and Dynamics of Lipids in Biological Membranes .............. 3
1.3. Organization of Membrane Proteins .................................................... 10
1.4. Dynamics of Membrane Proteins ......................................................... 15
1.6. Peptide Models for Exploring Lipid-Protein Interaction .......................... 17
1.5. Basic Tenets of Lipid-protein Interaction .............................................. 19
1.7. Spectroscopic Approaches in Exploring Lipid-protein Interaction 
with a Special Emphasis on Red Edge Excitation Shift .............................. 21

Chapter 2. Differential Effect of Cholesterol and its Biosynthetic 
Precursors on Membrane Dipole Potential
2.1. Introduction ............................................................................................ 34
2.2. Materials and Methods ........................................................................ 38
2.3. Results .................................................................................................... 42
2.4. Discussion .............................................................................................. 53

Chapter 3. Monitoring Membrane Protein Conformational 
Heterogeneity by Fluorescence Lifetime Distribution Analysis 
using the Maximum Entropy Method
3.1. Introduction ............................................................................................ 58
3.2. Materials and Methods ........................................................................ 61
3.3. Results and Discussion ......................................................................... 65

Chapter 4. Membrane Organization and Dynamics of ‘Inner Pair’ 
and ‘Outer Pair’ Tryptophan Residues in Gramicidin Channel
4.1. Introduction ............................................................................................ 74
4.2. Materials and Methods ........................................................................ 75
4.3. Results .................................................................................................... 80
4.4. Discussion .............................................................................................. 91

Chapter 5. Monitoring Orientation and Dynamics of Membrane-
bound Melittin utilizing Dansyl Fluorescence
5.1. Introduction ............................................................................................ 95
5.2. Materials and Methods ........................................................................ 97
5.3. Results ................................................................................................... 102
5.4. Discussion ............................................................................................ 111
## Contents

### Chapter 6. Membrane Interaction of the N-terminal Domain of Chemokine Receptor CXCR1

6.1. Introduction ..........................................................115  
6.2. Materials and Methods ......................................118  
6.3. Results and Discussion ........................................121  

### Chapter 7. Conclusions and Future Perspectives .................................133

### References .................................................................140
Abbreviations

12-AS: 12-(9-anthroyloxy)stearic acid
12-PC: 1-palmitoyl-2-(12-doxyl)stearoyl-sn-glycero-3-phosphocholine
24-DHCR: 3β-hydroxy-sterol-Δ24-reductase
2-AS: 2-(9-anthroyloxy)stearic acid
5-PC: 1-palmitoyl-2-(5-doxyl)stearoyl-sn-glycero-3-phosphocholine
7-DHC: 7-dehydrocholesterol
7-DHCR: 3β-hydroxy-sterol-Δ7-reductase
CD: circular dichroism
CXCR 1: CXC chemokine receptor 1
Dansyl: 1-dimethylamino-5-sulfonylnaphthalene
DAPC: 1,2-diarachidonoyl-sn-glycero-3-phosphocholine
DFT: density functional theory
di-8-ANEPPS: 4-(2-(6-(dioctylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl)-pyridinium inner salt
DLPC: 1,2-dilauroyl-sn-glycero-3-phosphocholine
DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphocholine
Dns-melittin: dansyl-labeled melittin
DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine
DPhPC: 1,2-diphytanoyl-sn-glycero-3-phosphocholine
DTPC: 1,2-ditetradecyl-sn-glycero-3-phosphocholine
GPCR: G-protein coupled receptor
gA: gramicidin A
LED: light emitting diode
LUV: large unilamellar vesicle
POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
PC: phosphatidylcholine
MOPS: 3-(N-morpholino)propanesulfonic acid
MEM: maximum entropy method
NBD: 7-nitrobenz-2-oxa-1,3-diazol-4-yl
REES: Red edge excitation shift
SLOS: Smith-Lemli-Opitz Syndrome
SUV: Small unilamellar vesicle
Tempo-PC: 1,2-dioleoyl-sn-glycero-3-phosphotempocholine
TCSPC: Time correlated single photon counting
TM: Trans-membrane
TRES: time-resolved emission spectra
Synopsis

Biological membranes are two-dimensional, non-covalent, cooperative supra-molecular and anisotropic assemblies of lipids and proteins. They allow cellular compartmentalization, and act as the interface through which cells sense the environment and communicate with each other. Membranes confer an identity to a cell (and its organelles) and represent an ideal milieu for the proper function of membrane proteins and constitute the site of many important cellular functions such as signal transduction and pathogen entry. Roughly half of biological processes occur on, in or around the cell membranes. Biological membranes host membrane proteins which comprises almost 30% of the (human) genome. It is only natural that biological membranes are not just passive bystander in membrane protein function, rather they constitute the site for pharmacological intervention. Apart from membrane proteins which are targeted by 60% of the pharmacological drugs the physicochemical properties of membranes, such as structure, organization and dynamics, have been implicated in various pharmacological effects. This very idea of modulating physiology by altering membrane physicochemical properties has lead to 'membrane-lipid therapy' which constitutes a new approach in molecular medicine.

Lipid crystallography has revealed that roughly half of the thermal thickness of lipid membranes consists of the lipid-water interface, a region of 'tumultuous chemical heterogeneity'. Membrane interface is characterized by motional restriction and intermediate polarity (compared to bulk aqueous medium and monotonous hydrocarbon interior). Diverse range of spectroscopic measurements have established the existence of a motional and polarity gradient along the perpendicular to the plane of the bilayer which modulate the organization and dynamics of membrane proteins. A detailed description of membrane organization, dynamics and lipid-protein interaction using various spectroscopic approaches (with an in-depth discussion on Red Edge Excitation Shift) is provided in Chapter 1.

One of the less explored aspect of membrane electrostatics is membrane dipole potential. Dipole potential is the potential difference within the membrane bilayer, which originates due to the nonrandom arrangement of lipid dipoles and water molecules at the membrane interface. Membrane (lipid) composition has been shown to influence dipole potential. Cholesterol, a representative sterol in higher eukaryotic membranes, is known to increase membrane dipole potential. In Chapter 2, we explored the effect of immediate (7-DHC and desmosterol) and evolutionary (ergosterol) precursors of cholesterol on membrane dipole potential, monitored by the dual wavelength ratiometric approach utilizing the probe di-8-ANEPPS. Our results show that the effect of these
precursors on membrane dipole potential is very different than that observed with cholesterol, although the structural differences among them are subtle. These results assume relevance since accumulation of cholesterol precursors due to defective cholesterol biosynthesis has been reported to result in several inherited metabolic disorders such as the Smith-Lemli-Opitz Syndrome (SLOS). Interestingly, cholesterol (and its precursors) has negligible effect on dipole potential in polyunsaturated membranes. We interpret these results in terms of non-canonical orientation of cholesterol in these membranes. Our results constitute the first report on the effect of biosynthetic and evolutionary precursors of cholesterol on dipole potential, and imply that a subtle change in sterol structure can significantly alter the dipolar field at the membrane interface.

Although membrane proteins constitute a large portion of a typical proteome and represent major drug targets, our understanding of these processes at the molecular level is still limited. This is due to the fact that obtaining high resolution crystal structure of membrane proteins in their native (membrane-bound) conditions continues to be a challenge. Further, dynamic information of membrane proteins over a wide range of spatiotemporal scales are also scarce. In this backdrop, spectroscopic approaches based on suitably chosen model peptide-membrane systems provide complementary information.

Gramicidin, an antimicrobial peptide isolated from the soil bacterium Bacillus brevis, is a prototypical ion channel. The ion channel peptide gramicidin serves as an excellent model for monitoring membrane protein conformation and dynamics due to a number of reasons. It has been shown that the tryptophan residues in gramicidin are crucial for its channel function. Gramicidin exhibits two major conformations in membranes: the channel conformation (functional form) and the non-channel conformation. In the channel confirmation, tryptophan residues are clustered in the membrane interfacial region (a noteworthy feature, shared by many membrane proteins such as the K+ channel), while they are spread across the bilayer normal in the non-channel conformation. Interestingly, the non-channel conformation can be converted to the channel conformation, since the latter is thermodynamically more stable. In Chapter 3, we have analyzed conformational heterogeneity in membrane-bound gramicidin using fluorescence lifetime distribution analysis of tryptophan residues by the maximum entropy method (MEM). MEM represents a model-free and robust approach for analyzing fluorescence lifetime distribution. In this work, we show that fluorescence lifetime distribution analysis using MEM could be a convenient approach to monitor conformational heterogeneity in membrane-bound gramicidin in particular and membrane proteins in general. Lifetime distribution
analysis by MEM therefore provides a novel window to monitor conformational transitions in membrane proteins.

Gramicidin has four functionally important tryptophan residues at 9, 11, 13 and 15 positions. In Chapter 4, we have investigated the effect of substituting two of the four tryptophans by phenylalanine residues on the organization and dynamics of gramicidin in membranes. We have selectively substituted the outer (Trp-13 and 15) and inner (Trp-9 and -11) pairs of tryptophan residues. Our results show that the fluorescence properties of these analogs are different from native gramicidin. The analogue with the outer pair of tryptophans exhibits red shifted emission maxima, while the analog with the inner pair shows blue shifted emission maxima compared to native gramicidin. Interestingly, both of these analogs exhibit red edge excitation shift (REES). Fluorescence lifetimes are higher for both the analogs compared to native gramicidin. This could be due to the release of stacking interactions between tryptophans 15 and 9. Depth analysis by the parallax method shows that the inner pair (Trp-9 and -11) tryptophans localize deep in the hydrocarbon interior while the outer pair tryptophans (Trp-13 and 15) localize in the interfacial region of the membrane. We further show that the outer pair of tryptophan residues exhibits a heterogeneous lifetime distribution and slow anisotropy decay while the inner pair of tryptophan residues exhibit relatively homogeneous lifetime distribution and faster anisotropy decay. Our results assume significance in understanding the role of tryptophans in membrane protein organization and dynamics.

Melittin is a cationic hemolytic peptide isolated from the European honey bee, Apis mellifera. Melittin is a classic example of amphiphilic membrane-interacting peptide. In spite of a rich literature, there is no consensus regarding the orientation of melittin in membranes. In chapter 5, we used a melittin analogue (Dns-melittin) that is covalently labeled at its amino terminal (Gly-1) with the environment-sensitive 1-dimethylamino-5-sulfonylnaphthalene (dansyl) group to obtain information regarding the orientation and dynamics of the amino terminal region of membrane-bound melittin. Our results show that the dansyl group in Dns-melittin exhibits REES in membranes of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), implying its localization in a motionally restricted region of the membrane. This is further supported by wavelength-dependent anisotropy and lifetime changes, and time-resolved emission spectra characterized by dynamic Stokes shift, which indicates relatively slow solvent relaxation in the excited state. Membrane penetration depth analysis using the parallax method shows that the dansyl group is localized at a depth of \(~18\) Å from the center of the bilayer in membrane-bound Dns-melittin. Further analysis of dansyl and tryptophan depths in Dns-melittin shows that the tilt angle between the
helix axis of membrane-bound melittin and the bilayer normal is \(~70^\circ\). Our results therefore suggest that melittin adopts a pseudo-parallel orientation in DOPC membranes at low concentration.

Interestingly several proteins have been identified that interact with the membrane as loose arrays of domains. Such conformations easily escape classical high-resolution structural analysis and the information gleaned from peptides may therefore be instructive for understanding the functioning of such membrane proteins. Further, structural studies have mainly focused on the transmembrane \(\alpha\)-helices, describing their amino acid composition and packing interactions in considerable detail. In contrast, the parts of the proteins located in the membrane-water interface region have received little attention.

The N-terminal domain of chemokine receptors constitutes one of the two critical ligand binding sites, and plays important roles by mediating binding affinity, receptor selectivity, and regulating function. In Chapter 6, we monitored the organization and dynamics of a 34-mer peptide from the N-terminal domain of the CXC chemokine receptor 1 (CXCR1), a G-protein coupled receptor, in membranes by utilizing a combination of fluorescence-based approaches and surface pressure measurements. Our results show that the CXCR1 N-domain 34-mer peptide binds to vesicles of DOPC and upon binding, the tryptophan residues of the peptide experience motional restriction and exhibit red edge excitation shift of 19 nm. Interestingly, a control peptide with identical amino acid composition but, with a scrambled sequence did not show any binding to membranes. These results are further supported by increase in fluorescence anisotropy and mean fluorescence lifetime upon membrane binding. These results demonstrate membrane interaction of the N-terminal domain of CXCR1 and gain relevance in context of the emerging role of cellular membranes in chemokine signaling.

Chapter 7 describes an overall discussion of the work presented in the thesis and some possible future experiments. I have chosen not to include some part my research work on (i) Dipolar relaxation within the protein matrix of the green fluorescent protein, (ii) Orientation and dynamics of a novel fluorescent cholesterol analogue in membranes of varying phase, (iii) Organization and dynamics of tryptophans in the molten globule state of bovine \(\alpha\)-lactalbumin utilizing wavelength-selective fluorescence approach, (iv) Exploring the effects of graded hydration of bovine \(\alpha\)-lactalbumin in reverse micelles and (v) Organization and dynamics in micellar structural transition monitored by pyrene fluorescence; since these topics are only loosely connected with the theme of the thesis.
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Publications


15. Haldar, S., Chaudhuri, A., Gu, H., Koepppe, R.E., Kombrabail, M., Krishnamoorthy, G. and Chattopadhyay, A. “Organization and Dynamics of “Inner Pair” and “Outer Pair” Tryptophan Residues in Gramicidin Channel” (manuscript in preparation)


17. *Haldar, S., Kombrabail, M., Krishnamoorthy, G., and Chattopadhyay, A. “Monitoring Depth-Dependent Heterogeneity in Membranes by Fluorescence Lifetime Distribution Analysis” (manuscript in preparation)


* Not included in the thesis


potential", in National workshop on Fluorescence Correlation Spectroscopy & Biophotonics, NEHU, Shillong, India.
