Synopsis

The plasma membrane is highly organized and specialized molecular assembly of lipids and proteins and is indispensable for the existence of cells. It imparts identity to a cell and acts as a barrier between cell interior and extracellular space. The membrane therefore plays a crucial role in communication between cells and their environments. The membrane is selectively permeable in nature and acts as a platform for initiation of various physiologically important processes such as trafficking, signaling and host-pathogen interactions. Since a significant portion of integral proteins is embedded in membranes, it is likely that properties of lipids and membranes could influence organization and function of membrane proteins. Lipid-protein interactions in membranes therefore play a significant role in proper functioning of membrane proteins. Cholesterol is an important lipid in this context since it is known to regulate the function of membrane proteins. Cholesterol is often found distributed non-randomly in domains in biological and model membranes. Many of these domains (sometimes termed as 'lipid rafts') are believed to be important for the maintenance of membrane structure and function. The idea of such specialized membrane domains assumes relevance in the cellular context since physiologically important functions have been attributed to these domains.

The G-protein coupled receptor (GPCR) superfamily is the largest and most diverse family of transmembrane proteins involved in signal transduction across membranes. GPCRs are prototypical members of the family of seven transmembrane domain proteins and include >800 members which together constitute ~5% of the human gene. They are involved in the generation of various physiological processes by responding to a diverse array of stimuli such as biogenic amines, peptides, glycoproteins, lipids, nucleotides, and even

photons. GPCRs act as a communication platform by transferring information from the extracellular environment to cell interior through the cell membrane. GPCRs transmit signals across the plasma membrane *via* their interactions with guanine nucleotide binding proteins called G-proteins. Since GPCRs regulate multiple physiological processes, they have emerged as major targets for the development of novel drug candidates in all clinical areas. It is estimated that up to 50% of clinically prescribed drugs act as either agonists or antagonists of GPCRs.

The serotonin$_{1A}$ (5-HT$_{1A}$) receptor is an important seven transmembrane domain receptor and belongs to the largest family of receptors called serotonin receptor family. The serotonin$_{1A}$ receptor is the first serotonin receptors to be cloned as an intronless genomic clone (G-21) of the human genome. The human serotonin$_{1A}$ receptor is composed of 422 amino acids and is characterized by molecular weight of ~46 kDa and an isoelectric point of 8.8. Although no crystal structure of the serotonin$_{1A}$ receptor is available yet, hyropathy plots of amino acid sequences predict that the receptor contains seven hydrophobic stretches that could possibly be membrane spanning α-helices. Serotonergic signaling plays a key role in the generation and modulation of almost every brain processes such as mood, perception, reward, anger, aggression, appetite and memory. Disruption of serotonergic signaling therefore has been implicated in the pathogenesis of various neuronal disorders. The serotonin$_{1A}$ receptor agonists and antagonists have been therefore shown to possess potential therapeutic effects in anxiety-or stress-related disorders. The serotonin$_{1A}$ receptor therefore serves as an important target in the development of therapeutic agents for neuropsychiatric disorders such as anxiety and depression.

Membrane cholesterol has been shown to modulate the function of a number of membrane proteins including physiologically important GPCRs. Two mechanisms have been proposed by which cholesterol can modulate

protein function. The first mechanism involves direct/specific interaction of cholesterol with the membrane protein, which could induce a conformational change in the protein. The recent report showing the presence of cholesterol molecule between helices in the crystal structure of β_2-adrenergic receptor could represent potential evidence for such mechanism. Alternatively, cholesterol can modulate protein function by altering the membrane physical properties in which the protein is embedded. The focus of the work presented in this thesis is to understand the role of lipid-protein interactions in modulating the function of membrane proteins. For this, the function of the serotonin$_{1A}$ receptor has been monitored in membranes with varying cholesterol content under various physicochemical stress conditions. In order to explore the mechanism by which cholesterol can modulate protein function, membrane organization and dynamics have been explored, upon modulating membrane cholesterol and protein content, using fluorescent probes such as pyrene and NR12S. In addition, calcium spikes induced by the serotonin$_{1A}$ receptor have been analyzed to explore the specificity of calcium signaling induced by the serotonin$_{1A}$ receptor. A brief outline of these studies is provided in this synopsis.

Effect of membrane cholesterol on stability of the human serotonin$_{1A}$ receptor

An interesting feature from a number of recently solved crystal structures of GPCRs is the close association of cholesterol in the receptor structure. For example, high resolution crystal structures of GPCRs such as rhodopsin, the β_1-adrenergic receptor, β_2-adrenergic and A_{2A} adenosine receptor all show closely associated cholesterol molecules. In this context, our laboratory recently proposed that cholesterol binding sites in GPCRs could represent nonannular binding sites at inter or intramolecular (interhelical) protein interfaces. Interestingly, cholesterol has been previously reported to

improve stability of the β_2-adrenergic receptor, and appears to be necessary for crystallization of the receptor. The cholesterol analogue, cholesterol hemisuccinate, has been shown to stabilize the β_2-adrenergic receptor receptor against thermal inactivation. Although cholesterol sensitivity of the serotonin$_{1A}$ receptor constituted one of the early reports in the area of GPCR-cholesterol interaction, the effect of membrane cholesterol on the stability of the receptor was not explored. In order to understand the role of membrane cholesterol in the stability of the human serotonin$_{1A}$ receptor, the ligand binding function of the receptor was monitored in membranes of varying cholesterol content under conditions such as high temperature, extreme pH and proteolytic degradation [Chapter 2]. The results showed that membrane cholesterol stabilizes ligand binding of the serotonin$_{1A}$ receptor under various physicochemical stress conditions. These results would be helpful in understanding the function of the serotonin$_{1A}$ receptor under various stress conditions and could provide useful insight into future efforts to crystallize the receptor.

Organization and dynamics of hippocampal membranes utilizing pyrene and NR12S

Organization and dynamics of cellular membranes in the nervous system play a crucial role in the function of neuronal membrane receptors. Earlier work from our laboratory has established native membranes, prepared from the bovine hippocampus, as a convenient natural source for studying the serotonin$_{1A}$ receptor. Interestingly, our laboratory has earlier shown the requirement of membrane cholesterol in modulating ligand binding activity of the serotonin$_{1A}$ receptor. In order to correlate these cholesterol-dependent functional changes with alterations in membrane organization and dynamics, the organization and dynamics of hippocampal membranes and their modulation with cholesterol and protein content was monitored utilizing characteristic fluorescence properties of pyrene [Chapter 3] and NR12S

[Chapter 5; see later]. The results using pyrene showed that the polarity of the hippocampal membrane is increased upon cholesterol depletion, as monitored by change in the ratio of pyrene vibronic band intensities. This was accompanied by an increase in lateral diffusion, measured as an increase in the pyrene excimer/monomer ratio. Since NR12S is a recently developed probe, we characterized fluorescence properties of NR12S in membranes of different phases utilizing sensitive fluorescence techniques [Chapter 4]. We demonstrated that fluorescence emission maximum, anisotropy, and lifetime of NR12S are dependent on phase of the membrane. Interestingly, NR12S exhibited significant REES that appeared to be dependent on phase of the membrane. These results suggested that NR12S is very effective in distinguishing different phases in model membranes with various fluorescence approaches. Further, the role of membrane cholesterol and proteins was monitored in the organization and dynamics of hippocampal membranes using NR12S [Chapter 5]. We showed that fluorescence emission maximum, anisotropy, and lifetime of NR12S are dependent on cholesterol content of the membrane. NR12S exhibited significant REES that appeared to be dependent on membrane cholesterol content. In addition, it was demonstrated that proteins do not contribute much to organization and dynamics of membranes. This was evident from similar values of REES, lifetime and anisotropy in hippocampal membranes and liposomes prepared from these membranes. These results could be relevant in understanding the complex spatiotemporal organization of neuronal membranes, and could have functional implications in neuronal diseases such as SLOS which is characterized by defective cholesterol biosynthesis leading to metabolic cholesterol depletion.

Analysis of serotonin-stimulated calcium signaling in live cells

Ligand binding to the serotonin$_{1A}$ receptor activates two important signaling pathways by activating heterotrimeric G-proteins. Activation of Gα_i

results in the reduction of cAMP whereas activation of βγ initiates calcium signaling. Our laboratory has earlier characterized the serotonin$_{1A}$ receptor mediated cAMP signaling and its modulation by cholesterol content and actin cytoskeleton. In addition, it was demonstrated that the receptor mediated signaling is correlated well with dynamics of the receptor. In order to explore the specificity of calcium signaling induced by the serotonin$_{1A}$ receptor, the calcium spikes induced by the receptor in cells were analyzed [Chapter 6]. A set of parameters for calcium transients were described that could provide novel insight into mechanisms responsible for maintaining the specificity of signals by shaping calcium transients. Results suggested that the maximum fold change in cytosolic calcium concentration and the kinetics of calcium release from intracellular calcium stores into the cytoplasm depend on ligand concentration. In addition, it was demonstrated that the amplitude, duration and area of spike vary with its number (position) in the temporal profile of calcium. Importantly, we observed variation in the amplitude, duration and area of the respective spikes induced by different ligands.

In Chapter 7, we have concluded the results of chapters 2-6 and discussed the future avenues that would be helpful in understanding the function of the serotonin$_{1A}$ receptor.