Summary of the Dissertation

The first half of this thesis is dedicated to dynamic and structural studies on the interaction between the C-terminus of the N-Ras protein with phospholipid membranes using solid-state NMR. In this work, three semi-synthetic N-Ras proteins were constructed by replacing the native palmitoyl chain with hexadecyl chain to give better stability. To facilitate the NMR study, the C-terminal N-Ras peptide was labeled in an alternating pattern with ^{13}C and ^{15}N to increase signal intensity and to suppress the background signals from the lipid matrix. Furthermore, the hexadecyl chain of the peptide was labeled with ^{2}H to study the dynamic behavior of the lipid chain in the phospholipid membrane. The proteins were synthesized using the chemical biology approach, through ligation of chemically synthesized labeled C-terminal lipidated peptide with the non-labeled N-terminal water-soluble N-Ras protein. The ligation strategy was done through the Michael addition of cysteine at C-terminal of soluble N-Ras protein to MIC group of labeled lipidated peptide. This synthetic method gave moderate yield which were sufficient for the solid state NMR study.

Different MAS NMR experiments such as Bloch-decay ^{13}C experiment, CP ^{13}C experiment, ^{13}C-^{13}C and ^{13}C-^{1}H correlation experiments were performed to establish the $^{13}C\alpha$, $^{13}C\beta$, ^{13}CO and $^{1}H_{\alpha}$ chemical shifts for the C-terminus of the membrane-bound N-Ras protein. The chemical shifts for the C-terminus of the membrane-bound N-Ras proteins determined in the MAS NMR experiments provided the input parameters for the structural calculations. The program package TALOS was used for the empirical prediction of Φ and Ψ backbone structure of the membrane-binding domain of Ras from the isotopic chemical shifts.

A simulated annealing protocol was used for structural calculations of the C-terminus of the membrane-bound lipidated Ras protein based on the torsion angle obtained from TALOS prediction. The computer simulation revealed that the global fold of the C terminus of the N-Ras protein in the membrane resembles a horseshoe. The membrane anchor of Ras has no regular α-helical or β-sheet motifs. The membrane topology of the

protein is determined by hydrophobic interactions between the lipidated cysteine and hydrophobic side chains. The lipid-binding domain is linked to the GTP-binding N-terminal region of the protein by a linker domain, which is putatively flexible. In contrast to H-Ras, there is currently no known structure (or no available structural studies) of the C-terminus of N-Ras. However, considering that there is 92 % sequence homology between H-Ras and N-Ras it can be assumed that these structures are very similar.

Deuterium-labeled hexadecyl lipid chain at cysteine 181 of N-Ras protein was used to obtain the dynamic information of the N-Ras lipid chain's interaction with phospholipid membranes. The dynamic results calculated from ^{2}H NMR indicate that the chain inserts into the hydrophobic membrane interior with almost no energy cost, thereby largely retaining its configurational entropy and leaving the host matrix relatively unchanged. The dynamic properties of this lipid chain completely differ from that of the phospholipid membrane, registering a comparatively low order parameter and high motional amplitude inside the phospholipid membrane.

The backbone and side chain molecular mobility of the seven isotopically labeled C-terminal amino acids of Ras were studied by ^{13}C MAS NMR spectroscopy. The order parameter of backbone and side chain was determined by 2D *dipolar coupling chemical-shift correlation experiment* (DIPSHIFT). The order parameter of farnesylated cysteine 186 is about 60% higher than that of hexadecylated cysteine 181. This observation suggested that the farnesyl modification of cysteine 186 is relatively rigid. In summary, the C-terminus of membrane-associated full length Ras protein shows a versatile dynamics.

The second half of the thesis dealt with the identification of peptide inhibitors of Rab GGTase, which is an enzyme of Rab proteins. The lead compound of the peptide inhibitors is the natural product Pepticinnamin E. The synthesis of peptides library employing hydrazine linker has shown to be an efficient method to generate a large compound library in a short time. By using this traceless linker, around 380 derivatives of Pepticinnamin E were generated. Two oxidation cleavage methods were used to released

the peptides from the solid support, namely, NBS/pyridine, and $Cu(OAc)_2$/pyridine leading to different derivatives at C-terminus of peptides.

Out of these 382 peptide inhibitors, 18 compounds have been found to be active against Rab GGTase, with IC_{50} values between 1 μM to 70 μM. From the structure activity relationship analysis, peptides which contain a long lipid chain at its N-terminus and a moiety with metal chelating ability at C-terminus were found to be the most active substrate to inhibit Rab GGTase activity.

Three compounds, **59**, **80** and **81**, were found to have *in vivo* activity against Rab GGTase. These three peptides were subjected to selectivity screening to determine their potency against FTase, GGTase I and Rab GGTase. These three peptides have shown high inhibitory selectivity of Rab GGTase over FTase and GGTase I. Of which, **81**, which consists of these two features mentioned above, has IC_{50} value of around 1 μM against the enzyme. The kinetic studies of **81** have revealed that the inhibitor is a non-competitive inhibitor of Rab GGTase with respect to the lipid substrate and has a K_d value of around 600 nM.