INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Membrane Binding Mechanism of Phosphoinositide Interacting Domains and Development of Their Inhibitors

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SHORT ABSTRACT

The content of this thesis entitled "***Membrane Binding Mechanism of Phosphoinositide Interacting Domains and Development of Their Inhibitors***" have been divided into five chapters based on the results of experimental work carried out during the research period. The introductory chapter (**Chapter1**) describes the role of phosphoinositides (PIP) in cell signaling, several PIP binding domains including PX, PH domains and small molecule based non-lipid antagonists those are used as inhibitors in PIP-PH interactions. **Chapter 2** determines the roles of basic and hydrophobic residues of Tks5-Phox homology domain in membrane binding. In **Chapter 3,** we have determined the mechanistic insight into PIP binding properties of Lpd-PH domain. **Chapter 4** demonstrates the inhibition of phosphatidylinositol-3,4,5-trisphosphate binding to AKT pleckstrin homology domain by 4-Amino-1,2,5-oxadiazole derivatives. In **Chapter 5,** we have demonstrated the inhibitory mechanism of triazole-based small molecules on phosphatidylinositol-4,5-bisphosphate binding pleckstrin homology domain. To elucidate the role of basic and hydrophobic residues of Tks5-PX and Lpd-PH domain in membrane binding, we quantitatively determined the binding parameters using a number of biophysical studies including surface plasmon resonance (SPR), fluorescence resonance energy transfer (FRET) and monolayer penetration analyses. Mutational studies revealed that presence of basic residues within the PIP-binding pocket and hydrophobic residues at the putative membrane binding surface of the PX and PH domain are significant for its PIP-dependent membrane binding properties. Under normal growth conditions, cellular localization patterns of the PX and PH domain and its mutants in A549 cells are consistent with their *in vitro* membrane binding properties. We also used a series of small molecule antagonists for PI(3,4,5)P_3/PH-domain and PI(4,5)P_2/PH-domain interaction and determined their inhibitory effect by using competitive-surface plasmon resonance (SPR) analysis. To elucidate their binding selectivity, we also used PI(3,4,5)P_3, PI(3,4)P_2, PI(4,5)P_2 specific PH-domains. For further understanding of their PH-domain inhibition mechanism, we also performed various physicochemical analyses. The results showed that these water-soluble compounds do not significantly interact with the model membranes. The oxadiazole and N′-hydroxy moieties of the compounds are essential for their exothermic interaction with the PH-domains and their bindings do not alter the secondary structure of the PH-domain. Further, this idea can be extended for PIP/PH-domain interactions based drug development.