# Molecular Dynamics Simulations of Protein-Membrane Interactions Focusing on PI3Kα and Its Oncogenic Mutants

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# Abstract

The interactions between proteins and membranes are key to many aspects of biological function. Molecular dynamics simulations can provide insight into both atomic-level structural details and energetics of protein-membrane interactions. This thesis describes the development of a physiologically accurate brain lipid bilayer, and its use in molecular dynamics simulations to characterise how proteins that are important drug targets interact with the cell membrane. A method for rapidly identifying the orientation of a protein that interacts most favourably with a membrane was also developed and tested.

The first chapter provides an introduction to molecular dynamics and its role in the context of this research.

The second chapter details the development of a cellular membrane with a physiologically representative brain lipid composition. This was done through the testing of simple systems prior to the construction of two more complex lipid bilayers comprising phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositide 4,5 bisphosphate ($PIP_2$), sphingomyelin, and cholesterol.

The third chapter implements the brain lipid bilayer in the development of a rotational interaction energy screening method designed to predict the most favourable orientation of a protein with respect to the cellular membrane. The functionality of the method was validated through application to two membrane proteins commonly implicated in cancer: the phosphatase and tensin homolog (PTEN), and the p110α-p85α phosphatidyl-inositol kinase (PI3Kα) complex.

The fourth chapter corresponds to the main focus of this research, the behaviour of wild type PI3Kα and two of its oncogenic mutants (E545K and H1047R) with regards to membrane and substrate interaction. It was primarily found that H1047R's increased membrane affinity allowed it to sample a catalytically competent orientation independently of *Ras*, unlike the wild type. Furthermore, it was also found that the position of the C terminal tail with regards to the substrate binding pocket was crucial in the achievement of a catalytically competent position against the cellular membrane.

The fifth and final chapter describes a cytochrome P450 system embedded in a cellular membrane. It was primarily found that the properties of its ingress and egress tunnels depended on the presence or absence of a substrate in the active site.

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# Dictionary of Common Abbreviations

**ATP** - Adenosine Triphosphate
**APL** - Area per Lipid
**ABD** - Adaptor Binding Domain
**AKT** - Protein Kinase B
**BH** - Bcl-2 Homology
**CHOL** - Cholesterol
**CYP** - Cytochrome P450
**DNA** - Deoxyribonucleic Acid
**DSSP** - Define Secondary Structure of Proteins
**DP** - Dipalmitoyl
**DO** - Dioleoyl
**DOP** - Deuterium Order Parameter
**E545K** - Mutation (E→K) at position 545
**GUI** - Graphical User Interface
**GPCR** - G-Protein Coupled Receptor
**GTPase** - Guanosine Triphosphatase
**HDx** - Hydrogen Deuterium Exchange
**H1047R** - Mutation (H→R) at position 1047
**LINCS** - Linear Constraint Solver Algorithm
**LJ** - Lennard-Jones
**MBD** - Membrane Binding Domain
**MD** - Molecular Dynamics
**NMR** - Nuclear Magnetic Resonance
**NVT** - Number, Volume, Temperature
**NPT** - Number, Pressure, Temperature
**PI3K** - Phosphatidylinositide 3-Kinase
**PIK3CA** - PI3K Catalytic Subunit Alpha
**PC** - Phosphatidylcholine
**PE** - Phosphatidylethanolamine
**PS** - Phosphatidylserine
**$PIP_2$** - Phosphatidylinositde 4,5 Bisphosphate
**PO** - Palmitoyl Oleoyl
**PMF** - Potential of Mean Force
**PME** - Particle Mesh Ewald
**RMSD** - Root-Mean-Square Deviation
**RMSF** - Root-Mean-Square Fluctuation
**RBD** - Ras Binding Domain
**SH2** - Src Homology 2
**SO** - Stearoyl Oleoyl
**SA** - Stearoyl Arachidonoyl
**SPC** - Simple Point Charge
**SASA** - Solvent Accessible Surface Area
**SGML** - Sphingomyelin
**SRS** - Substrate Recognition Site
**VDW** - Van der Waals
**VPL** - Volume per Lipid