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Molecular Dynamics Simulations of Protein-Membrane Interactions Focusing on PI3K α and Its Oncogenic Mutants

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William A. Irvine

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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), phosphatidylinositol 4,5 bisphosphate (PIP₂), 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 p110 α -p85 α phosphatidylinositol kinase (PI3K α) complex.

The fourth chapter corresponds to the main focus of this research, the behaviour of wild type PI3K α 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	NVT - Number, Volume, Temperature
APL - Area per Lipid	NPT - Number, Pressure, Temperature
ABD - Adaptor Binding Domain	PI3K - Phosphatidylinositide 3-Kinase
AKT - Protein Kinase B	PIK3CA - PI3K Catalytic Subunit Alpha
BH - Bcl-2 Homology	PC - Phosphatidylcholine
CHOL - Cholesterol	PE - Phosphatidylethanolamine
CYP - Cytochrome P450	PS - Phosphatidylserine
DNA - Deoxyribonucleic Acid	PIP₂ - Phosphatidylinositide 4,5 Bisphosphate
DSSP - Define Secondary Structure of Proteins	PO - Palmitoyl Oleoyl
DP - Dipalmitoyl	PMF - Potential of Mean Force
DO - Dioleoyl	PME - Particle Mesh Ewald
DOP - Deuterium Order Parameter	RMSD - Root-Mean-Square Deviation
E545K - Mutation (E→K) at position 545	RMSF - Root-Mean-Square Fluctuation
GUI - Graphical User Interface	RBD - Ras Binding Domain
GPCR - G-Protein Coupled Receptor	SH2 - Src Homology 2
GTPase - Guanosine Triphosphatase	SO - Stearoyl Oleoyl
HDx - Hydrogen Deuterium Exchange	SA - Stearoyl Arachidonoyl
H1047R - Mutation (H→R) at position 1047	SPC - Simple Point Charge
LINCS - Linear Constraint Solver Algorithm	SASA - Solvent Accessible Surface Area
LJ - Lennard-Jones	SGML - Sphingomyelin
MBD - Membrane Binding Domain	SRS - Substrate Recognition Site
MD - Molecular Dynamics	VDW - Van der Waals
NMR - Nuclear Magnetic Resonance	VPL - Volume per Lipid