

Table of Contents

Abstract	(i)
Declaration	(vi)
Certificate of supervisor	(vii)
Certificate of oral defense	(viii)
Acknowledgements	(ix)
Table of Contents	(xi)
List of Figures	(xx)
List of Tables	(xxxiv)
Abbreviations	(xxxvi)
List of Publications	(xxxviii)
Conference Proceedings	(xxxix)
CHAPTER 1: Motivation & outline of the Thesis	1- 4
1.1 Motivation of the present work	2
1.2 Outline of the thesis	3
CHAPTER 2: Introduction & Review of Literature	5-29
2.1 Breast cancer	6
2.1.1 Classification of breast cancer	6
2.1.2 Therapies	10
2.1.2.1 Endocrine therapies	10
2.1.2.2 Drugs targeting Receptor Tyrosine Kinases (RTKs)	10
2.1.2.3 Chemotherapy and radiotherapy	11
2.1.3 Endocrine therapy resistance	11
2.2 Protein kinases	12
2.2.1 General architecture of protein kinase	13
2.2.1.1 N-terminal lobe	13
2.2.1.2 C-terminal lobe	14
2.2.2 Active and inactive structures of protein kinases	16
2.3 Lemur Tyrosine kinase-3 (LMTK3)	16
2.3.1 The LMTK family	16
2.3.2 LMTK3 and its structure	20
2.3.3 LMTK3 and its association with different types of cancers	21

Table of Contents

2.3.4	Role of LMTK3 in breast cancer	22
2.3.4.1	Implication of LMTK3 in ER α positive breast cancer	22
2.3.4.2	LMTK3 a regulator of ER α	23
2.3.5	Implication of LMTK3 in triple negative breast cancer promotes tumor invasion and metastasis through GRB2 mediated induction of integrin β_1	25
2.3.6	Role of phosphorylation in LMTK3 activation by CDK5 and its contribution in breast cancer progression	27
2.3.7	LMTK3 inhibitors	29
2.4	Main objectives of the thesis	29
CHAPTER 3:	COMPUTATIONAL METHODS	30-70
3.1	Molecular dynamics (MD) simulations	31
3.1.1	Historical Background	32
3.1.2	Theory of molecular dynamics simulation	33
3.1.3	Force field	34
3.1.4	Periodic boundary conditions	36
3.1.5	Long range interactions Ewald sum	37
3.1.6	SHAKE algorithm	38
3.1.7	Temperature and pressure computation and control	39
3.1.8	Water molecule models	40
3.2	Simulation Methodology in AMBER	41
3.2.1	Simulation environment	42
3.2.2	Energy minimization	43
3.2.3	Heating the system	45
3.2.4	Equilibration	46
3.2.5	Production phase	46
3.2.6	Analysis	46
3.3	3D Structure visualization tools	48
3.3.1	Visual molecular dynamics (VMD)	48
3.3.2	UCSF Chimera	48
3.4	Potential of mean force	48

Table of Contents

3.4.1	Umbrella sampling	49
3.4.2	Running the umbrella sampling calculations	50
3.4.3	The Weighted Histogram Analysis Method (WHAM) for free-energy calculations	51
3.5	Binding free energy calculation using Molecular Mechanics energies combined with the Poisson-Boltzmann or Generalized Born and Surface Area continuum solvation method (MM-PBSA and MM-GBSA)	51
3.5.1	Free energy calculation using MM-PBSA/GBSA.pl script	52
3.5.2	Free energy decomposition using MM/PBSA.py script	53
3.6	Molecular docking	55
3.6.1	Docking methodologies	56
3.6.1.1	Rigid ligand and rigid receptor docking	56
3.6.1.2	Flexible ligand and rigid receptor docking	56
3.6.1.3	Steps performed in AutoDock	56
3.7	In silico prediction of protein-protein interaction	58
3.7.1	PatchDock web server	59
3.7.1.1	Patchdock web server: Input, output and user interface	60
3.8	ClusPro web server	62
3.9	PDBsum web server	63
3.9.1	Wiring diagram	64
3.9.2	Topology diagram	65
3.9.3	Protein-protein interfaces	65
3.10	Hot spot residue prediction	67
3.11	Virtual Screening	68
3.11.1	Ligand-based virtual screening	69
3.11.2	Structure-based virtual screening	69
3.11.2.1	Structure based virtual screening using Dock Blaster server	69
3.12	Energy optimized (E-)pharmacophore model generation	69

Table of Contents

CHAPTER 4:	Salient structural features of Human Lemur Tyrosine Kinase-3 (LMTK3) domain from Molecular dynamics simulation study	71-97
4.1	Abstract	72
4.2	Introduction	72
4.3	Materials and Methods	74
4.3.1	Structural modelling and validation of LMTK3 domain	74
4.3.2	Sequence alignment of LMTK3 domain with other kinases	75
4.3.3	Molecular Dynamics (MD) simulation of LMTK3 domain	75
4.3.4	Analysis	75
4.3.5	LMTK3-ATP binding mechanism	77
4.4	Results and Discussions	77
4.4.1	Structural validation of LMTK3 domain	77
4.4.2	Identification of conserved regions in LMTK3 domain using multiple sequence alignment	81
4.4.3	Structural characteristics features of LMTK3 domain	84
4.4.3.1	RMSD analysis	84
4.4.3.2	Radius of gyration	84
4.4.3.3	B-factor analysis and RMSF	85
4.4.3.4	SASA analysis	85
4.4.3.5	Secondary structure analysis	86
4.4.3.6	Hydrophobic contact analysis and Hydrophobicity	89
4.4.3.7	Electrostatic potential	91
4.4.3.8	Hydrogen bond analysis	92
4.4.3.9	Binding cavity	94
4.4.4	ATP binding pocket of LMTK3 domain	96
4.5	Conclusions	97
CHAPTER 5:	Unveiling the transient Protein-Protein interactions that modulate the activity of Estrogen Receptor(ER)-α by Human Lemur Tyrosine Kinase-3 (LMTK3) domain: An <i>in silico</i> study	98-114
5.1	Abstract	99

Table of Contents

5.2	Introduction	99
5.3	Materials and Methods	102
5.3.1	Structural modelling and validation of ER α and LMTK3 domain	102
5.3.2	Protein – Protein Docking	103
5.3.2.1	Rigid docking	103
5.3.2.2	Refinement of Complex Structure	103
5.3.4	Prediction of Interface residues between ER α and LMTK3 domain	103
5.3.5	ER α -MAPK interaction	103
5.3.6	Molecular Dynamics simulation of ER α -LMTK3 complex	104
5.4	Results and Discussions	104
5.4.1	Validation of ER α and LMTK3 structures	104
5.4.2	Protein-Protein interaction study	105
5.4.3	Interaction profile between ER α and MAPK	109
5.4.4	MD simulation study on the ER α -LMTK3 complex	111
5.5	Conclusions	113
CHAPTER 6:	Unveiling the Protein-Protein interactions between GRB2 and LMTK3 that induce integrin β1 during breast cancer progression and metastasis: an <i>in silico</i> study	115-134
6.1	Abstract	116
6.2	Introduction	116
6.3	Materials and Methods	119
6.3.1	Modelling and validation of protein structures	119
6.3.2	Protein-Protein Docking of GRB2 and LMTK3 domain	120
6.3.3	Molecular dynamics simulations of GRB2-LMTK3 complex	120
6.3.4	Hot spot residue identification at GRB2-LMTK3 interface	121
6.3.5	Binding free energy calculation	121
6.4	Results and Discussions	122
6.4.1	Protein-protein interaction at GRB2-LMTK3 interface	122

Table of Contents

6.4.2	Molecular dynamics simulation analysis	127
6.4.3	Hot Spot residues at GRB2-LMTK3 interface	131
6.4.4	Binding free energy using MM-GBSA method	133
6.5	Conclusions	134
CHAPTER 7a:	Unveiling the Transient Protein-Protein Interactions that Regulate the Activity of Human Lemur Tyrosine Kinase-3 (LMTK3) Domain by Cyclin Dependent Kinase 5 (CDK5) in Breast Cancer: An <i>in silico</i> Study	135-150
7a.1	Abstract	136
7a.2	Introduction	136
7a.3	Materials and Methods	138
7a.3.1	Prediction of activation segment and probable phosphorylation sites in LMTK3 domain	138
7a.3.2	Protein – protein docking	138
7a.3.2.1	Rigid docking	138
7a.3.2.2	Refinement of complex structure	139
7a.3.3	Prediction of interface residues between CDK5 and LMTK3 domain	139
7a.3.4	Molecular dynamics simulation of CDK5-LMTK3 complex	139
7a.4	Results and Discussions	141
7a.4.1	Activation segment and phosphorylation sites determined in LMTK3 domain	141
7a.4.2	Protein-protein interaction study	142
7a.4.3	MD Simulation study on the CDK5-LMTK3 complex	147
7a.5	Conclusions	150
CHAPTER 7b:	Effect of activation loop phosphorylation on Human Lemur Tyrosine Kinase-3 (LMTK3) activity: A Molecular Dynamics Simulation Study	151-163
7b.1	Abstract	152
7b.2	Introduction	152

Table of Contents

7b.3	Materials and Methods	154
7b.3.1	Prediction of activation segment and phosphorylation site in LMTK3 domain : A comparative study with other kinases (PKA and ERK2)	154
7b.3.2	<i>In silico</i> phosphorylation and mutation of LMTK3 domain at activation segment	154
7b.3.3	Molecular Dynamics (MD) simulation of phosphorylated, unphosphorylated and mutated LMTK3 domain	155
7b.3.4	Trajectory analysis	155
7b.4	Results and Discussions	156
7b.4.1	Activation segment and phosphorylation site prediction	156
7b.4.2	Molecular Dynamics (MD) simulation analysis	156
7b.4.2.1	Stability and Flexibility analysis from RMSD and RMSF	156
7b.4.2.2	Radius of gyration	160
7b.4.2.3	Energetics	161
7b.5	Conclusions	162
CHAPTER 8:	Structure based virtual screening of high-affinity ATP competitive inhibitors against Human Lemur Tyrosine Kinase-3 (LMTK3) domain- a novel therapeutic target for breast cancer	164-186
8.1	Abstract	165
8.2	Introduction	165
8.3	Materials and Methods	167
8.3.1	Molecular docking and virtual screening	167
8.3.1.1	LMTK3-ATP docking	167
8.3.1.2	Virtual screening and molecular docking of lead compounds for LMTK3 domain	167
8.3.2	Molecular Dynamics (MD) simulation of LMTK3-ATP/lead complexes	170
8.3.3	Free Energy calculation of LMTK3-ATP/lead complexes	171
8.3.3.1	MM-PBSA and MM-GBSA method	171

Table of Contents

	8.3.3.2 Umbrella sampling simulations	171
8.4	Results and Discussions	172
	8.4.1 ATP binding pocket of LMTK3 domain	172
	8.4.2 Potential inhibitors for LMTK3	174
	8.4.3 Bioavailability of LMTK3-inhibitors	179
	8.4.4 MD simulation and free energy calculations	180
	8.4.4.1 MM-PBSA/GBSA free energy calculation	183
	8.4.4.2 Umbrella Sampling	184
8.5	Conclusions	186
CHAPTER 9:	Identification of potential inhibitors against Human Lemur Tyrosine Kinase-3 (LMTK3) domain: An E- pharmacophore approach	187-212
9.1	Abstract	188
9.2	Introduction	188
9.3	Materials and Methods	189
	9.3.1 Structural Modeling and validation	189
	9.3.2 Molecular dynamics simulation of Apo- LMTK3 domain	190
	9.3.3 Steps for E-Pharmacophore model generation	191
	9.3.3.1 Protein preparation	191
	9.3.3.2 Receptor Grid Generation	191
	9.3.3.3 Small molecular library preparation	191
	9.3.3.4 High throughput Virtual Screening (HTVS)	192
	9.3.3.5 Energy Optimized (E)-Pharmacophore model generation	192
	9.3.4 Molecular dynamics (MD) simulation of the LMTK3-hit complexes	192
	9.3.5 Binding free energy calculation and per residue energy decomposition	193
	9.3.6 Potential of mean force (PMF) calculation using Umbrella Sampling simulations method	194
9.4	Results and Discussions	194

Table of Contents

9.4.1	Structural validation of LMTK3 domain	194
9.4.2	Database screening and the molecular interaction studies	197
9.4.3	Binding mode analysis of three best compounds	199
9.4.4	MD trajectory analyses of LMTK3-hit complexes	202
9.4.5	MM-PBSA free energy calculation and per residue energy decomposition	205
9.4.6	PMF calculations	211
9.5	Conclusions	212
CHAPTER 10:	Summary and Future prospects	214-215
10.1	Overview of results	214
10.2	Future prospects	216
	Bibliography	
	Publications (first page)	