Targeting Insulin Signalling Pathway and DPP4 for Type-II Diabetes

A Major Project thesis submitted in partial fulfilment of the requirement for the degree of

Master of Technology

In

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Submitted by

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CERTIFICATE

This is to certify that the M. Tech. dissertation entitled **"TARGETING INSULIN SIGNALLING PATHWAY AND DPP4 FOR TYPE-II DIABETES"**, submitted by **Poonam Saini (2k11/BIO/14)** in partial fulfilment of the requirement for the award of the degree of Master of Technology, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by her under my guidance.

The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

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Last but not the least, my heartfelt thanks to the Almighty, my family, colleagues and friends for **their belief and love.**

Poonam Saini

(2k11/BIO/14)

DECLARATION

I hereby declare that the dissertation entitled, "Targeting Insulin Signalling Pathway and DPP4 for Diabetes" submitted in the partial fulfilment of the Master of Technology Degree in Bioinformatics is a record work done by me, during the period January 2013-June 2013 and has not formed the basis for the award of any degree or other similar titles under any University in India or Abroad.

Poonam Saini
(2K11/BIO/14)

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LIST OF ABBREVIATIONS

1. **DPP4** - DIPEPTIDYL PEPTIDASE-4
2. **GSK3B** – GLYCOGEN SYNTHASE KINASE 3 BETA
3. **KCAL/MOL**- KILOCALORIE PER MOLE
4. **MD SIMULATION** – MOLECULAR DYNAMIC SIMULATION
5. **NS** – NANO SECONDS
6. **PTP1B**- PROTEIN-TYROSINE PHOSPHATASE 1B

ABSTRACT

TARGETING INSULIN SIGNALLING PATHWAY FOR TYPE-II DIABETES

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Diabetes has become bitter sweet problem of this world. In one of the shocking disclosure, the 20th annual World Diabetes Congress of the International Diabetic Federation has said that India leads the world in the looming epidemic of diabetes. Out of which Types 2 insulin resistance diabetes account for 90 to 95% of all diabetes .The main force driving this increasing incidence is increase in obesity, the single most important contributor to the pathogenesis of diabetes. Although there are many class of drugs available for the treatment of diabetes like insulin, Sulphonylureas, Metformin,, Acarbose but these therapies have limited efficacy, limited tolerability and significant mechanism-based side effects. Thus, newer approaches are desperately needed. Therefore in our study we have selected Gsk3beta, PTP1b and DPP4 as potential targets for drug designing for diabetes. These proteins are involved in insulin signaling pathway and there over expression results in insulin resistance. Although these targets are already reported in literature as negative regulator of insulin signaling but no drug is available in market till date for the same.In this study, we screened a large dataset of Zinc database against Gsk3b, PTP1Band DPP4 using a high throughput approach. The screening was directed toward catalytic site for Gsk3b and DPP4 while in case of PTP1B allosteric site was considered. We also carried out molecular dynamics simulations of the top-scoring compounds in order to study their molecular interactions with the active site functional residues of the targets and also to assess their dynamic behaviour. We report herein prospective non-covalent type inhibitory molecules which are active against Diabetes. These lead molecules possess improved binding properties and are specific of diabetes.

INTRODUCTION

OBJECTIVE: In this study we have designed specific inhibitors for GSK3b, PTP1B and DPP4.These macromolecules are involved in insulin pathway and are negative regulator of insulin therefore there over expression is related to diabetes.

Diabetes mellitus is a disease in which the body is unable to produce or unable to properly use and store glucose (a form of sugar). Due to which Glucose backs up in the bloodstream resulting in rise of blood glucose level. Type 2 insulin-resistant diabetes mellitus accounts for 90–95% of all diabetes. The main force driving this increasing incidence is a staggering increase in obesity, the most important contributor to the pathogenesis of diabetes. At present, therapy for type 2 diabetes relies mainly on several approaches intended to reduce the hyperglycaemia itself example sulphonylureas (and related insulin secretagogues), drugs of this class increase insulin release from pancreatic islets; metformin, these drugs acts to reduce hepatic glucose production; peroxisome proliferator-activated receptor-g (PPARg) agonists (thiazolidinediones), this class of drug enhance insulin action ; a-glucosidase inhibitors, which interfere with gut glucose absorption and insulin itself, which suppresses glucose production and augments glucose utilization (Moller DE *et.al*.2001). These therapies have limited efficacy, limited tolerability and significant mechanism-based side effects. (Eldar *et.al* 2002) Therefore there is urgent need for finding new molecular targets for diabetes. Within the past few years, our understanding of biochemical pathways related to the development of metabolic syndrome has expanded **(Figure 01).**

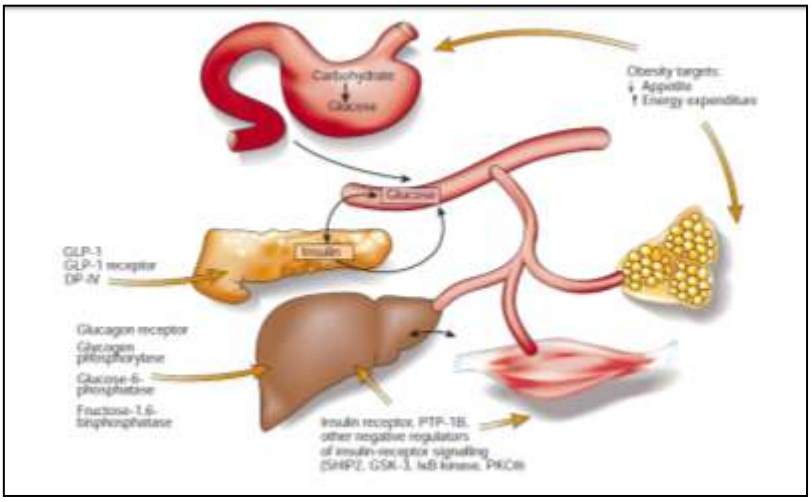


Figure 1: Key Organs and targets involved in diabetes.

(Picture source: Moller DE et.al.2001)

Many of the potential targets are now known within these biochemical pathways. In our study we have targeted the insulin signaling pathway, it is a pathway involve in maintain glucose homeostatic. When high levels of glucose enter the blood stream, insulin is released by beta cells in the pancreas. Insulin then initiates a number of signal pathways in specific muscle and fat cells. These signal pathways are responsible for allowing cells to rapidly increase their ability to uptake glucose from the blood stream. In this work we have targeted GSK3B, PTP1B and DPP4, they are negative regulators of insulin and their over expression is reported to results in insulin resistance (Wiesmann *et.al*2004; Sutton *et.al* 2012). While virtual screening for finding potential inhibitors was done against the catalytic site for DPP4 and GSk3B but for PTP1B allosteric site was selected. These inhibitors were further screened

out on basics of interaction pattern. As the catalytic site of most of kinases is conserved therefore special emphasis was given on specific interactions related to Gsk3b in order to achieve specificity **(Table 1).**

Amino Acid composition in ATP binding site

GSK-3beta	CDK2	FGFR1	VEGFR3
ILE62	.	LEU	LEU
GLY63	.	.	.
ASN64	GLU	GLU	TYR
VAL70	.	.	.
ALA83	.	.	.
LYS85	.	.	.
VAL110	.	ILE	.
LEU132	PHE	VAL	VAL
ASP133	GLU	GLU	GLU
TYR134	PHE	.	PHE
VAL135	LEU	ALA	CYS
THR138	ASP	ASN	ASN
ARG141	LYS	GLU	ASN
GLN185	.	ARG	ARG
LEU188	.	.	.
CYS199	ALA	ALA	.
ASP200	.	.	.

Table 1: Amino acid composition for ATP binding site.

Further all the selected inhibitors were subjected to molecular dynamic simulation in order to study their molecular interactions with the active site functional residues of the targets and also to assess their dynamic behaviour. These selected molecules can further be evaluated using wet lab study in order to calculated there IC-50 values and there potential to act as drug molecules.

REVIEW OF LITERATURE

1. Introduction to Diabetes

In the present scenario one side we see so much of development in infrastructure, education facilities, science and technology's glories stories. On the other hand we are surrounded with many health issues, diseases out of which Diabetes is very common. Diabetes mellitus is a disease in which the body is unable to produce or unable to properly use and store glucose (a form of sugar). Due to which Glucose backs up in the bloodstream resulting in rise of blood glucose level.

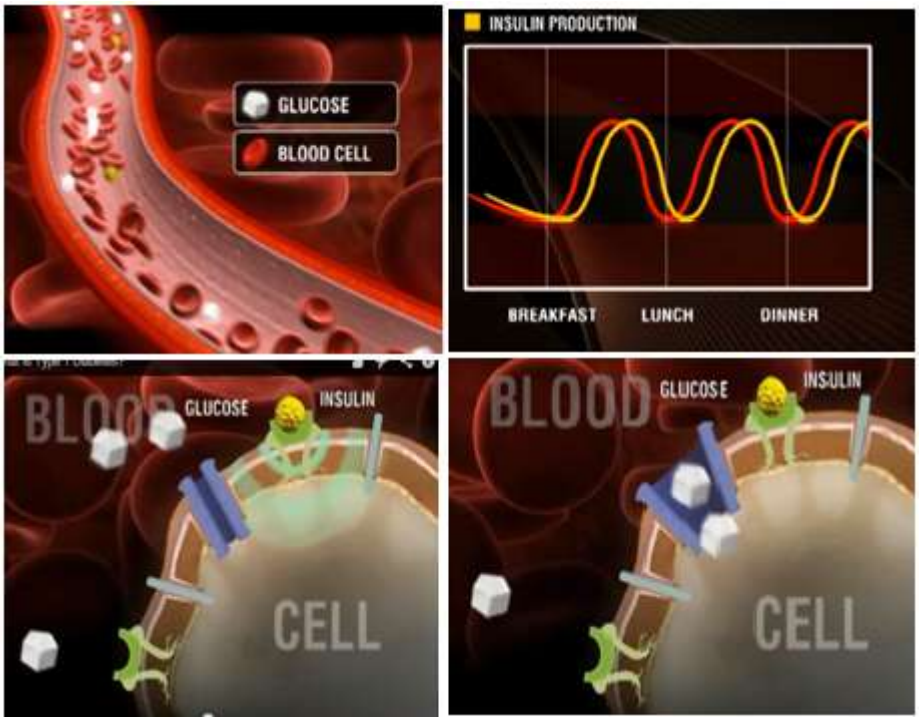


Figure2: Insulin role in Diabetes.

(Picture Credit: http://www.youtube.com/watch?v=_OOWhuC_9Lw#at=50)

In one of the shocking disclosure by The World Health Organization (WHO) in 2012 has said that nearly 200 million people all over the world suffer from diabetes. In India, there are nearly 50 million diabetics, according to the statistics of the International Diabetes Federation. As the incidence of diabetes is on the rise, doctors say, there is a proportionate rise in the complications that are associated with diabetes. WHO also reported that about 80% of the diabetes deaths occur in middle-income countries. To put it more simply, we have crossed the dividing line in which it is a problem associated with individuals, no matter how large this number may be, and is now a very large public health problem, growing astronomically year after year. Evidence shows that in more affluent parts of our country, the prevalence of diabetes is higher in comparison to rural and less affluent areas. The increase in economic status has been linked with the disease. Sedentary lifestyle, intake of polished and processed foods and transoils and stress has been recognized as the causes behind this silent killer. It is actually not a fatal disease and those who suffer from it have nothing to fear so

long as they are able to manage it. But to do so one should have an idea of what it is and how can it be kept under control.

1.1 EPIDEMIOLOGY

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases. Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double (Wild S *et.al* 2004) **(Figure 02).** Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild S *et.al* 2004). The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented (Wild S *et.al* 2004). The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men.

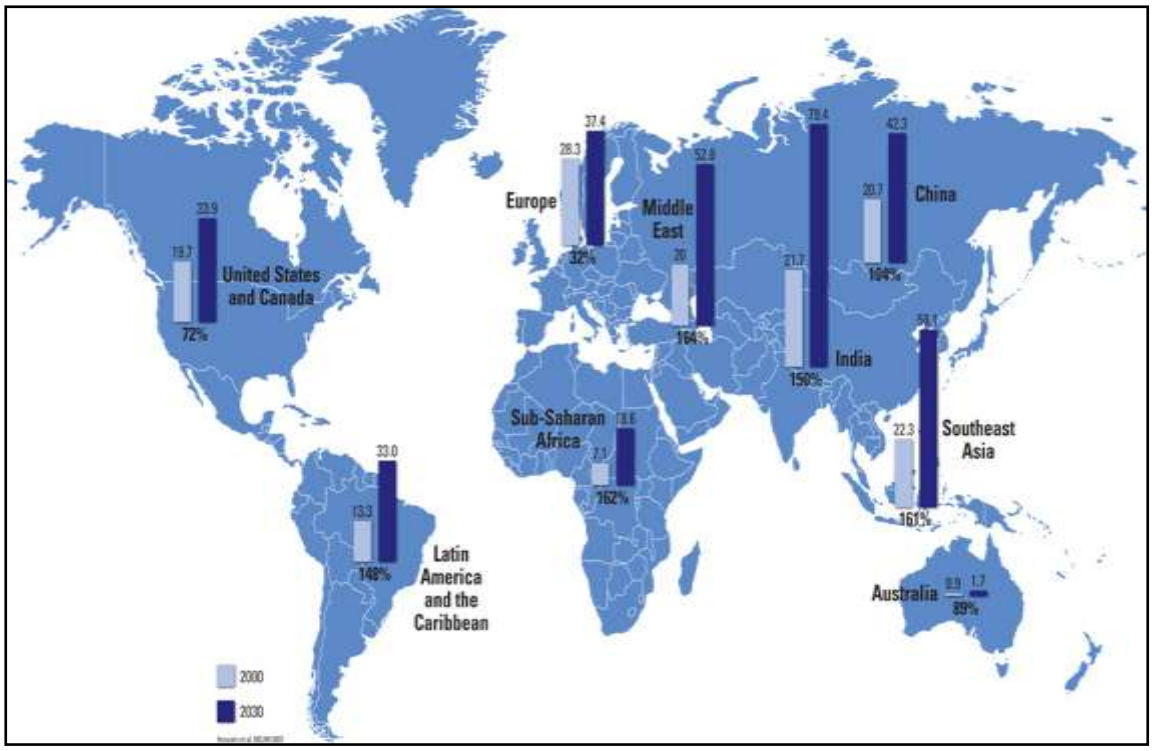


Figure3: Worldwide prevalence of Diabetes

(Picture Source: Hossain et al. 2007)

1.1.1 Diabetes Epidemic in India

- India is facing a twin burden of under-nutrition and over-nutrition: it figures prominently both in the hunger map of the world as well as being the diabetes capital of the world (Mohan V *et.al*, 2007). A country experiencing rapid socioeconomic

progress and urbanization, India carries the highest burden of diabetes with escalating prevalence in both urban and rural populations.(Ramachandran A *etal*, 2009)

- India is facing an epidemic of diabetes, with high prevalence in urban areas. Over the past 30 years, the prevalence of diabetes has increased to 12-18% in urban India and 3-6% in rural India with significant regional variations (Abate N *et al*,2005). These rates in India are 50-80% higher than China (10%).
- Another 14% having prediabetes ─a harbinger of future diabetes─ is as high as diabetes in Indians(Mohan V *et.al*, 2007)
- The difference in prevalence of diabetes across India could be due to dissimilar levels of urbanization and lifestyle factors such as different diets and varying obesity levels (Reddy *et.al* , 2012).
- Significant determinants of diabetes are age, body-mass index (BMI), waist-hip ratio, low physical activity, and family history of diabetes. The driving forces behind the epidemic are urbanization (30%) and economic development with resultant increase in GDP, sedentary lifestyle, western diet, and fast food diet on a background of genetic susceptibility.
- The prevalence of both diabetes and prediabetes increases by age with 60% of Indians having diabetes or prediabetes by age 60 (**Table 2 and Figure 3**).

Diabetes and Prediabetes Burden in India 2011

	Number and Prevalence of Prediabetes%	Number and Prevalence of diabetes%	Total number with diabetes and prediabetes
Tamilnadu	4.8 million (10%)	3.9 million (8%)	8.7 million
Maharashtra	6 million (8%)	9.2 million (13%)	15.2 million
Jharkhand	1 million (5%)	1.5 million (8%)	2.5 million
Chandigarh	0.12 million (14%)	0.13milllion (15%)	0.25 million
Projection for whole of India	62.4 million	77.2 million	139.6 million

Table 2: Diabetes prevalence in India.

(Table information collected from Mohan V et.al, 2007)

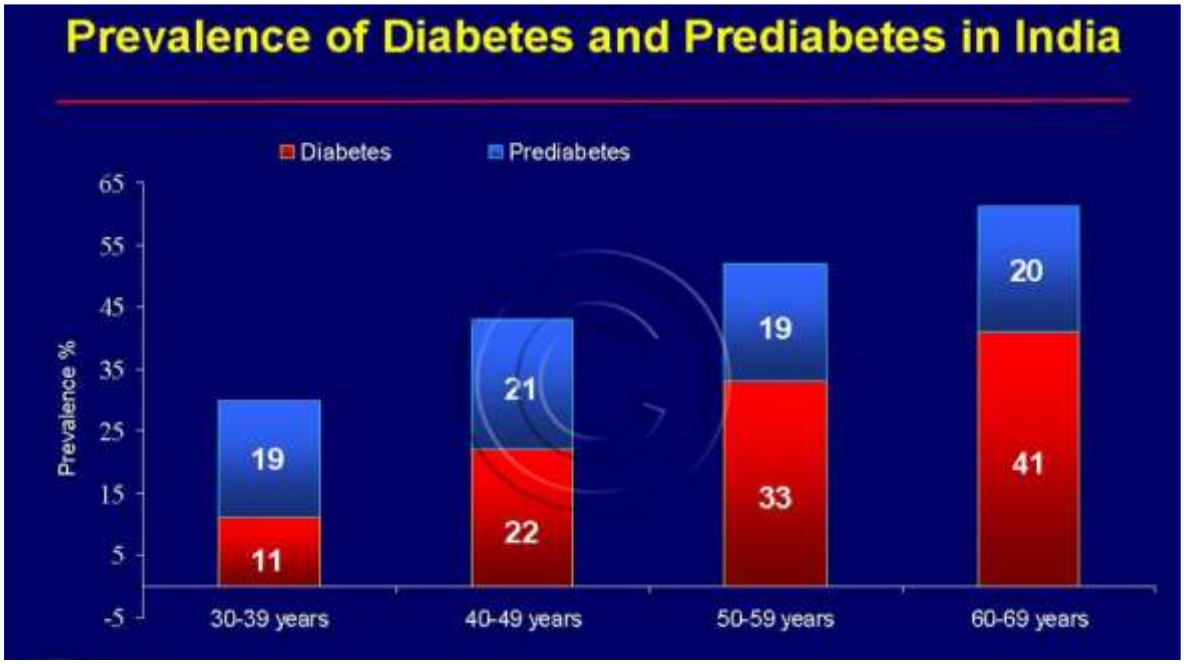


Figure 4: Graph showing Diabetes prevalence with respect to age.
(Picture Source: Decoda study Group 2003)

1.2 Types of Diabetes

To know more about Diabetes let us first begin with its types. Diabetes is basically of two types.

- **Type 1 Diabetes Mellitus**-It is also known as Insulin-dependent diabetes mellitus or Juvenile onset diabetes or simply Diabetes -Type 1.It is an autoimmune disease. An autoimmune disease results when the body's system for fighting infection (the immune system) turns against a part of the body. In this diabetes, the immune system attacks the insulin-producing beta cells in the pancreas and destroys them. The pancreas then produces little or no insulin. Insulin is a hormone released by the pancreas in response to increased levels of blood sugar (glucose) in the blood(Lawrence *et.al* , 2008). Insulin is necessary for glucose to move from the blood to the inside of the cells. Unless glucose gets into cells, the body cannot use it for energy. Excess glucose remains in the blood, and is then removed by the kidneys. There by adding extra work load for kidney. A person who is suffering from type I diabetes must take insulin daily to live. It is also known as Juvenile Diabetes as the onset of it begins in childhood. Children diagnosed with juvenile diabetes are insulin dependent. In some of the Current research juvenile diabetes is considered similar to other disorders such as; rheumatoid arthritis and multiple sclerosis as these are also auto immune diseases.

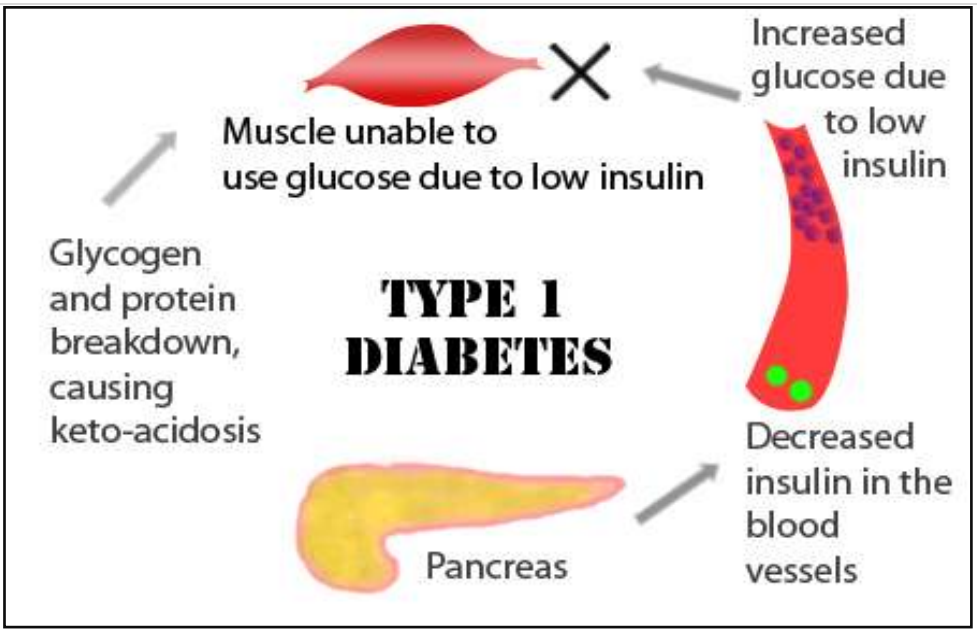


Figure 5: Flow diagram for Type I Diabetes

- **Type II Diabetes Mellitus** –Also known as Noninsulin-dependent diabetes mellitus; or simply Diabetes - Type 2.this one is more common in adults. It is a chronic, life-long disease that results when the body's insulin does not work effectively. A main component of type 2 diabetes is "insulin resistance". This means that the insulin produced by our pancreas cannot connect with fat and muscle cells to let glucose inside and produce energy. This cause hyperglycemia (high blood glucose).Type 2 diabetes usually occurs gradually. Symptoms may include fatigue or nausea, frequent urination, unusual thirst, blurred vision, frequent infections, and slow healing of wounds or sores. Some people have no symptoms. Most people with type 2 diabetes are overweight at the time of diagnosis. However, the disease can also develop in lean people, especially if elderly. Genetics play a large role in type 2 diabetes and family history is a risk factor. However, low activity level, poor diet, and excess body weight (especially around the waist) significantly increase risk for type 2 diabetes. (Lawrence *et.al* , 2008).

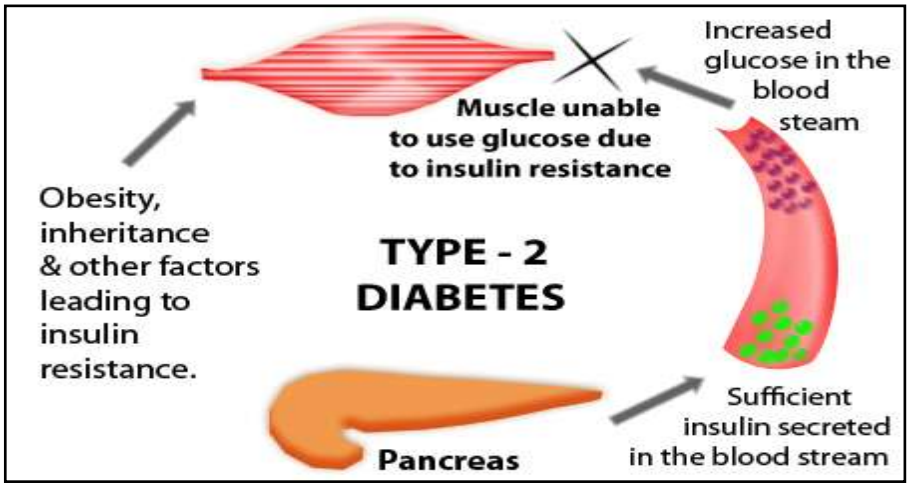


Figure 6: Flow Diagram for Type II Diabetes.

The other less common diabetes are-

Gestational diabetes: Gestational diabetes occurs in pregnant women with no history of diabetes. But it generally disappears after childbirth with 20 to 50 percent chance of developing type 2 diabetes within 5 to 10 years. Like type 2 diabetes, it occurs more often in African Americans, American Indians, Hispanic Americans, and among women with a family history of diabetes. Gestational diabetes mellitus (GDM) is present in around 3-4% of all pregnancies. Gestational diabetes mellitus (GDM) can be associated with significant morbidity and mortality in the foetus and newborn.

- **Diabetes Insipidus**- It is an uncommon condition caused by the inability of the kidneys to conserve water, which leads to frequent urination and pronounced thirst. The amount of water conserved is controlled by antidiuretic hormone (ADH, also called vasopressin).ADH is a hormone produced in a region of the brain called the hypothalamus. It is then stored and released from the pituitary gland, a small gland at the base of the brain. Diabetes insipidus caused by a lack of ADH is called central diabetes insipidus. When diabetes insipidus is caused by failure of the kidneys to respond to ADH, the condition is called Nephrogenic diabetes insipidus. Central diabetes insipidus is caused by damage to the hypothalamus or pituitary gland as a result of surgery, infection, tumor, or head injury. Although rare, central diabetes insipidus is more common than nephrogenic diabetes insipidus. Nephrogenic diabetes insipidus involves a defect in the parts of the kidneys that reabsorb water back into the bloodstream. It occurs less often than central diabetes insipidus. Nephrogenic diabetes insipidus may occur as an inherited disorder in which male children receive the abnormal gene that causes the disease on the X chromosome from their mothers. (Lawrence *et.al* , 2008).

Impaired glucose tolerance (IGT) or Pre-diabetes It is a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of diabetes. However if you have IGT the chances of becoming a diabetic later in life are more, unless the present rise in glucose levels is only because of stress or some other environmental changes.

Secondary diabetes It is a state when diabetes is caused as the result of some other condition or reason, e.g. inflammation of the pancreas, or by the use of certain medication such as diuretics or steroids (a very common cause).

1.3 Symptoms for Diabetes

- **Fatigue**: As the body is unable to use glucose, it begins metabolizing fat. In this process the body has to work harder which results in feeling fatigued and tired. Rapid weight loss: Unable to process the whole of calories in foods, loss of sugar and water in urine contribute to wreight loss.
- **Excessive thirst**: More water consumption is needed to dilute high blood sugar hence a diabetic feel thirsty all the tym.

- **Frequent urination and Excessive hunger:** Body tries to get rid of the extra sugar in the blood by excreting it in the urine. : One of the functions of insulin is to stimulate hunger. Hence higher insulin levels lead to increased hunger.
- **Poor wound healing:** One of the serious implications of diabetes is poor wound healing. High blood sugar levels prevent white blood cells, necessary for blood clotting and fighting against infection from functioning normally,
- **Infections:** Frequent infections result from suppression of the immune system due to presence of excessive glucose in the tissues
- Disturbed mental status: Due to high or low levels of blood sugar the patient feels agitated, irritated and lethargic.
- **Hazy vision**: Hazy vision is not specific for diabetes but is frequently present with high blood sugar levels(Lawrence *et.al* , 2008).

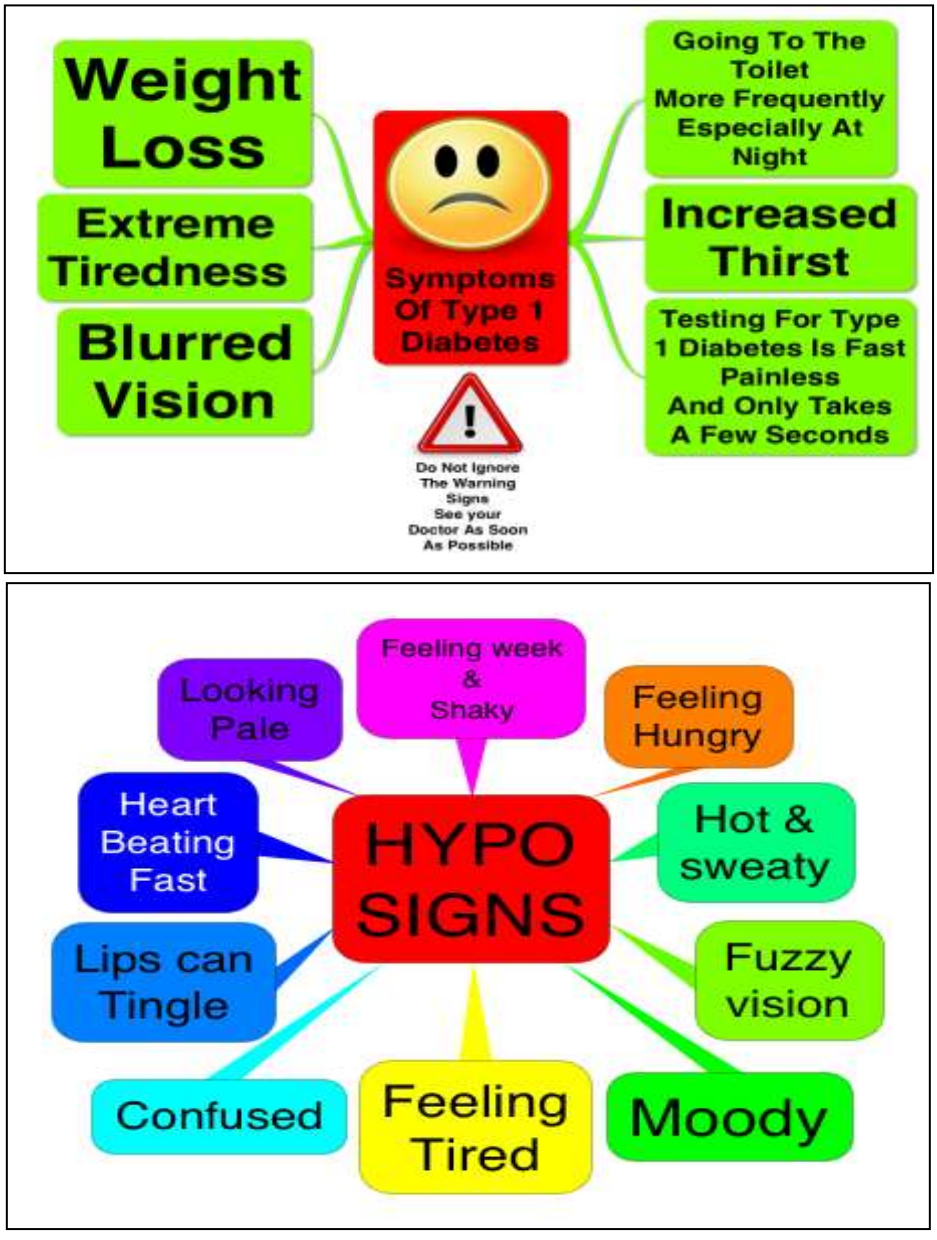


Figure 7: Symptom of Diabetes

(Picture Source: http://www.youtube.com/watch?v=_OOWhuC_9Lw#at=50)

1.4 Impact and causes of diabetes

- Over time, diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage are the result of damage to small vessels, and therefore referred to as micro vascular. Diabetes is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel diseases and this is referred to as macro vascular (Risérus *et.al*, 2009)
- Diabetes is not contagious, which means that we cannot give it to anyone nor can it be caught. "It is believed that the susceptibility to diabetes is passed from generation to generation via genes, but not in any specific pattern. Heredity plays a stronger role in Type 2 diabetes (non-insulin-dependent) than in Type 1 diabetes (insulin-dependent), but the nature of these genetic factors and how they are inherited are not yet understood." (Risérus *et.al*, 2009)

- Type I diabetes is partly inherited, and then triggered by certain infections, with some evidence pointing at Coxsackie B4 virus. A genetic element in individual susceptibility to some of these triggers has been traced to particular HLA genotypes (i.e., the genetic "self" identifiers relied upon by the immune system). However, even in those who have inherited the susceptibility, type 1 DM seems to require an environmental trigger. The onset of type 1 diabetes is unrelated to lifestyle. Type 2 diabetes is due primarily to lifestyle factors and genetics.

1.5 Diabetes Targets and Pathways Involved

Within the past few years, our understanding of biochemical pathways related to diabetes has expanded. Several mechanistic categories for new therapeutic approaches can now be considered(Figure 1).

- First are approaches aimed at reducing excessive glucose production by the liver.
- Second, are the mechanisms to augment glucose-stimulated insulin secretion.
- Third, specific molecular targets in the insulin signalling pathway.
- Fourth, new approaches to obesity and altered lipid metabolism, which offer the prospect of net improvements in insulin action or secretion (Moller DE , *et.al* ,2001)

1.6 Current Therapies Available for Diabetes.

Table 1 Current therapeutic agents for type 2 diabetes

Drug class	Molecular target	Site(s) of action	Adverse events
Insulin	Insulin receptor	Liver, muscle, fat	Hypoglycaemia, weight gain
Sulphonylureas (e.g. glibenclamide) plus nateglinide and repaglinide	SU receptor/ K^+ ATP channel	Pancreatic β-cell	Hypoglycaemia, weight gain
Metformin — biguanides	Unknown	Liver (muscle)	Gastrointestinal disturbances, lactic acidosis
Acarbose	α-glucosidase	Intestine	Gastrointestinal disturbances
Pioglitazone, rosiglitazone (thiazolidinediones)	PPARγ	Fat, muscle, liver	Weight gain, oedema, anaemia

Table 3: Current therapeutic agents for type 2 diabetes.

At present, therapy for type 2 diabetes relies mainly on several approaches intended to reduce the hyperglycaemia itself: sulphonylureas (and related insulin secretagogues), which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production; peroxisome proliferator-activated receptor-g (PPARg) agonists (thiazolidinediones), which enhance insulin action; a-glucosidase inhibitors, which interfere with gut glucose absorption; and insulin itself, which suppresses glucose production and augments glucose utilization (Table 3). These therapies have limited efficacy, limited tolerability and significant mechanism-based side effects. Of particular concern is the tendency for most treatments to enhance weight gain (Zhang, *et.al*, 2000)Several current approaches are also associated with episodes of hypoglycaemia, and few of the available therapies adequately address underlying defects such as obesity and/or insulin resistance. A problem particular to the sulphonylureas is that many patients who respond initially become refractory to treatment over time (‘secondary failures’). Thus, newer approaches are desperately needed. Particular emphasis should be placed on finding and using mechanisms that are dependent on physiological responses (for example, glucose-mediated insulin secretagogues), and that result in weight loss (or lack of weight gain). Also few of the current therapeutic approaches were largely developed in the absence of defined molecular targets or even a solid understanding of disease pathogenesis.

2. GSK 3B Role in Diabetes

Glycogen synthase kinase 3 (GSK-3) is aserine/threonine kinase that was first isolated and purified as an enzyme capable of phosphorylating and inactivating the enzyme glycogen synthase. Beyond its role in glycogen metabolism, GSK-3 beta acts as a downstream regulatory switch that determines the output of numerous signalling pathways initiated by diverse stimuli. One of such pathway where GSK-3 acts a key regulator is type-II diabetes. This serine-threonine kinase may, however, have an important role in the regulation of cell proliferation and apoptosis through its function within the Wnt signalling pathway.

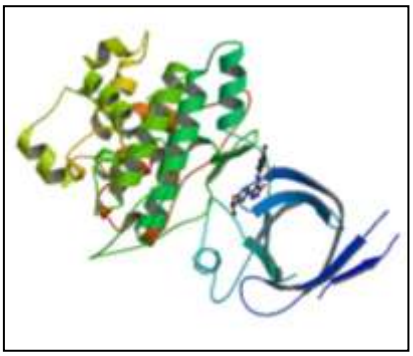


Figure 8: Structure of G3k3b

(Picture Source: RSCB ,PDB ID : 3L1S)

2.1 GSK3B involvement in insulin Pathway

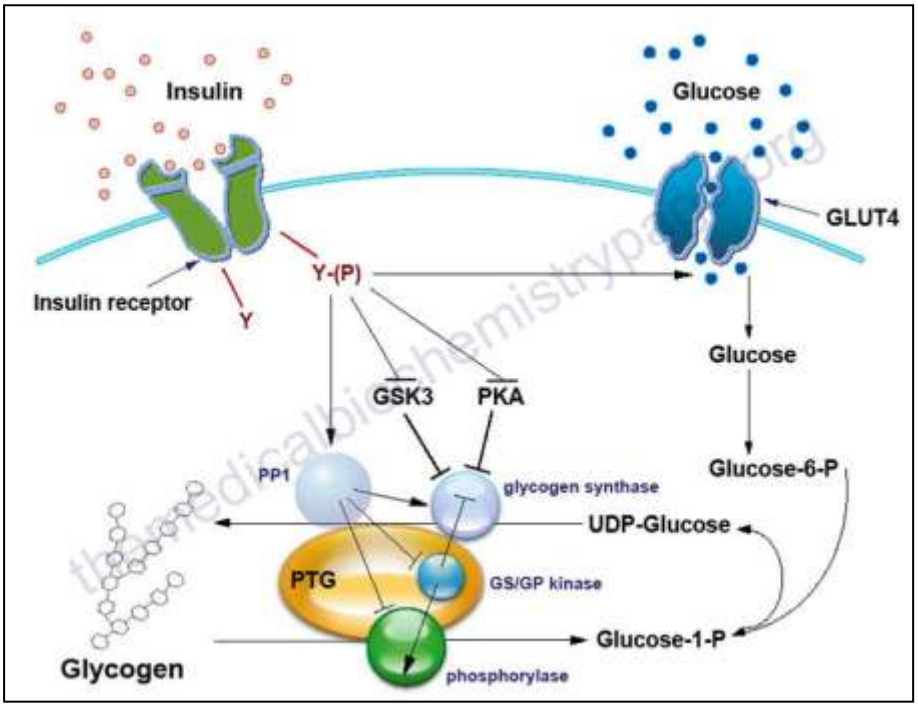


Figure 9: GSk3b role in diabetes.

(Picture Source : http://themedicalbiochemistrypage.org)

- GSK-3 has long been considered as a favorable drug target among the protein kinase family since unlike other protein kinases, which are typically activated by signaling pathways, GSK-3 is normally activated in resting cells, and its activity is attenuated by the activation of certain signaling pathways such as those generated by the binding of insulin to its cell-surface receptor. Activation of the insulin receptor leads to the activation of protein kinase B (PKB, also called Akt), which in turn phosphorylates GSK-3, thereby inactivating it. The inhibition of GSK-3 presumably leads to the activation of glycogen synthesis. The intricate insulin-signaling pathway is further complicated by negative-feedback regulation of insulin signaling by GSK-3 itself, which phosphorylates insulin-receptor substrate-1 on serine residues.
- Therefore, synthetic GSK-3 inhibitors might mimic the action of certain hormones and growth factors, such as insulin, which use the GSK-3 pathway. In certain pathological situations, this scheme might permit the bypassing of a defective receptor, or another faulty component of the signaling machinery, such that the biological signal will take effect even when some upstream players of the signaling cascade are at fault, as in non-insulin-dependent type II diabetes (Chong Gao *et.al* 2011)
- It was found that high activity of GSK-3 impairs insulin action in intact cells, by phosphorylating the insulin receptor substrate-1 (IRS-1) serine residues , and likewise, that increased GSK-3 activity expressed in cells results in suppression of glycogen synthase activity. Further studies conducted in this respect uncovered that GSK-3 activity is significantly increased in epididymal fat tissue of diabetic mice Subsequently, increased GSK-3 activity was detected in skeletal muscle of type II diabetes patients. Additional recent studies further established the role of GSK-3 in glycogen metabolism and insulin signaling (Eldar *et.al*, 2002; Grimes *et.al*, 2001), thereby suggesting that the inhibition of GSK-3 activity may represent a way to increase insulin activity in vivo.

2.2 GSK3B Inhibitors

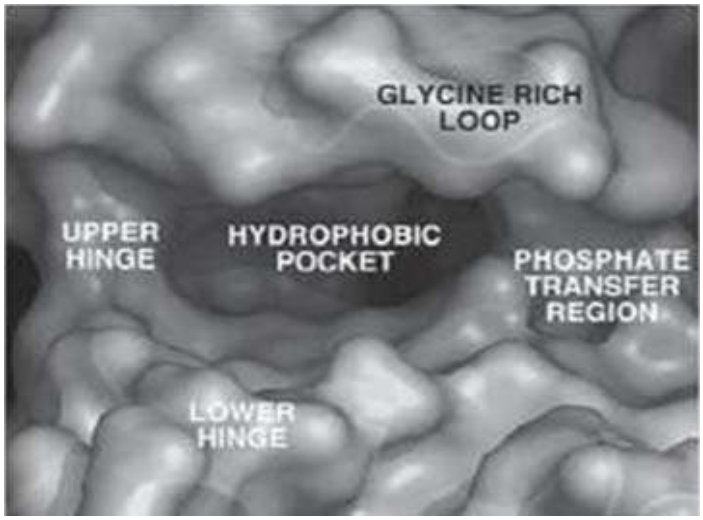


Figure 10: Five distinct pockets in ATP binding site of GSK-3 beta.
(Picture Created using pymol ,PDB id 3L1S)

- The availability of crystal structures of GSK-3b allows structure based lead optimization for the ATP binding site. Although many inhibitors are made of Gsk3b but due to conserved nature of ATP site, specificity is not seen and also no drug is available in market for GSK3b till date. Insulin is a naturally occurring inhibitor of GSK3b as it is involve in negative feed lope.

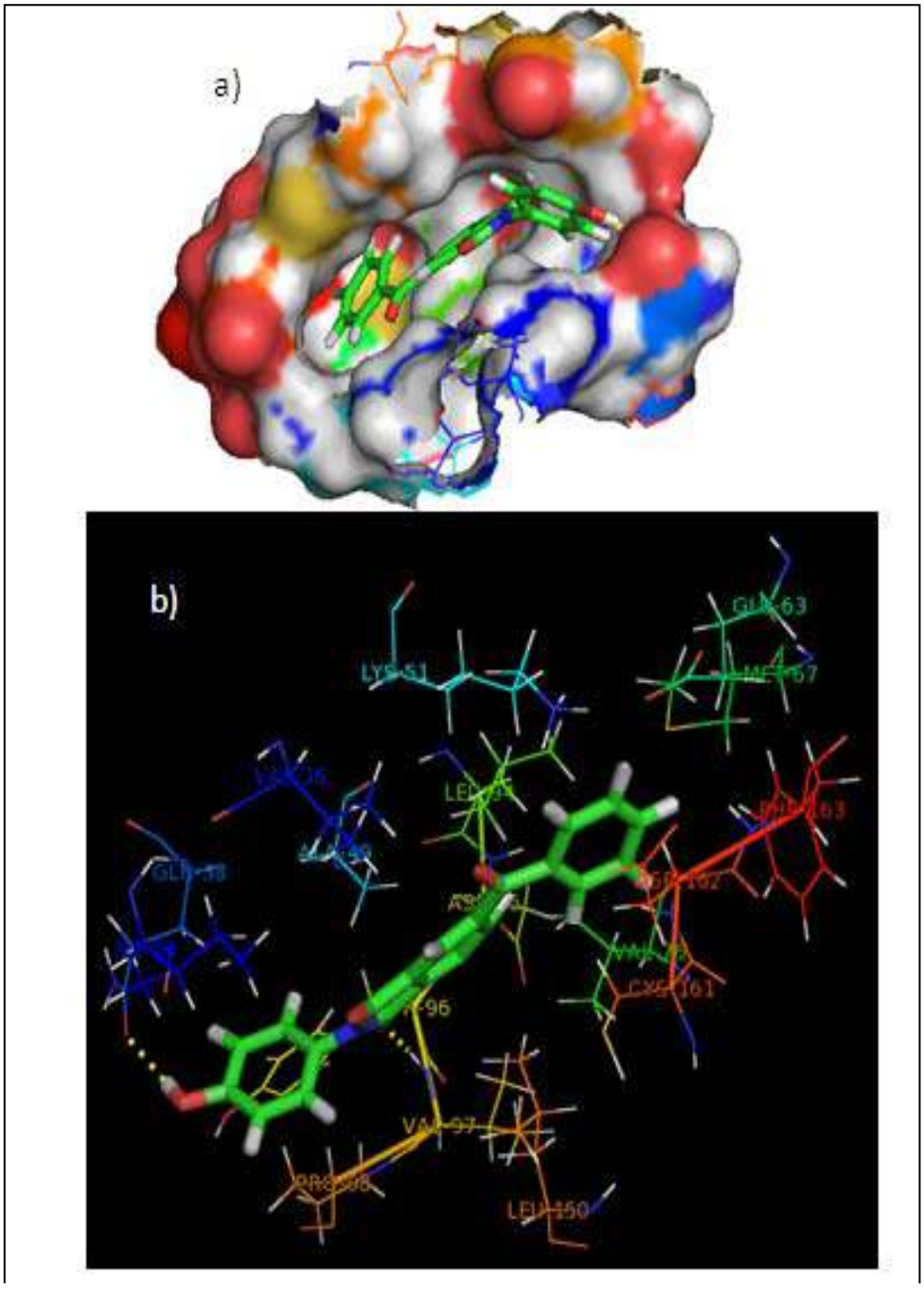


Figure 11: GSK3B with already ready known inhibitor.
(Picture Creted usning pymol. PDB ID 3LIS)

3. DPP4 ROLE IN DIABETES

- **Dipeptidyl peptidase-4** (**DPP4**), also known as **adenosine deaminase complexing protein 2** or **CD26** (cluster of differentiation 26) is a protein that, in humans, is encoded by the *DPP4* gene(Kameoka J, *et.al* ,1993).

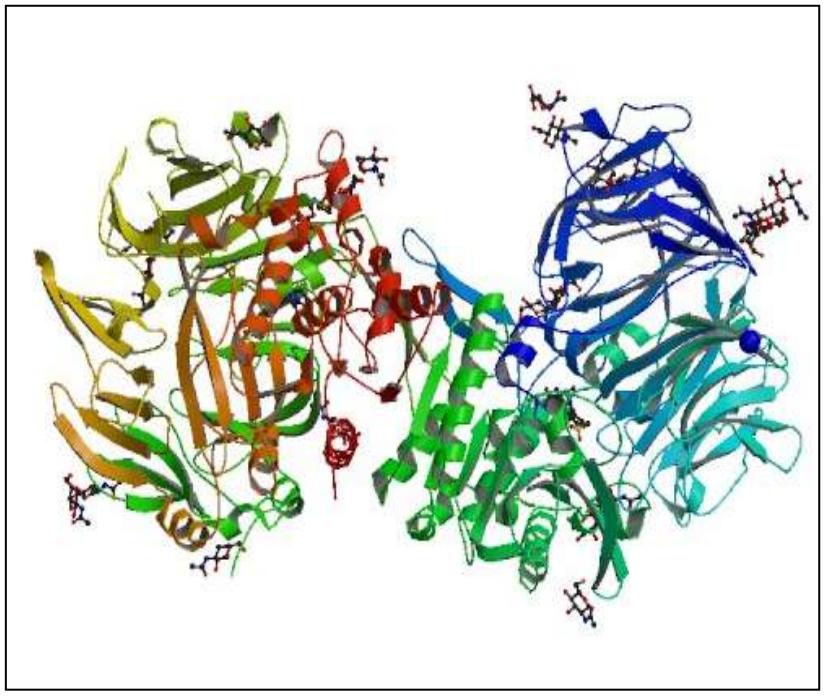


Figure 12: Structure of DPP4

(Picture Source RCSB PDB ID 2HHA)

- The protein encoded by the *DPP4* gene is an antigenic enzyme expressed on the surface of most cell types and is associated with immune regulation, signal transduction and apoptosis. It is an intrinsic membrane glycoprotein and a serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides. It is a rather indiscriminate enzyme for which a diverse range of substrates are known(Chen X ,*et.al* , 2006)The substrates of CD26/DPPIV are proline(or alanine)-containing peptides and include growth factors, chemokines, neuropeptides, and vasoactive peptides.
- DPP4 is involved in diabetes as it down regulates the activity of GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitory peptide) by degrading amino-terminal of these hormones. These hormone act through their respective G-protein-coupled receptors on b-cells to potentiate glucose-stimulated insulin secretion (Drucker, *et.al* 2011).

3.1 DPP4 INHIBITORS

- The introduction of dipeptidyl peptidase 4 (DPP4) inhibitors for the treatment of Type 2 diabetes acknowledges the fundamental importance of incretin hormones in the regulation of glycemia.(Sutton *et.al* ,2012).

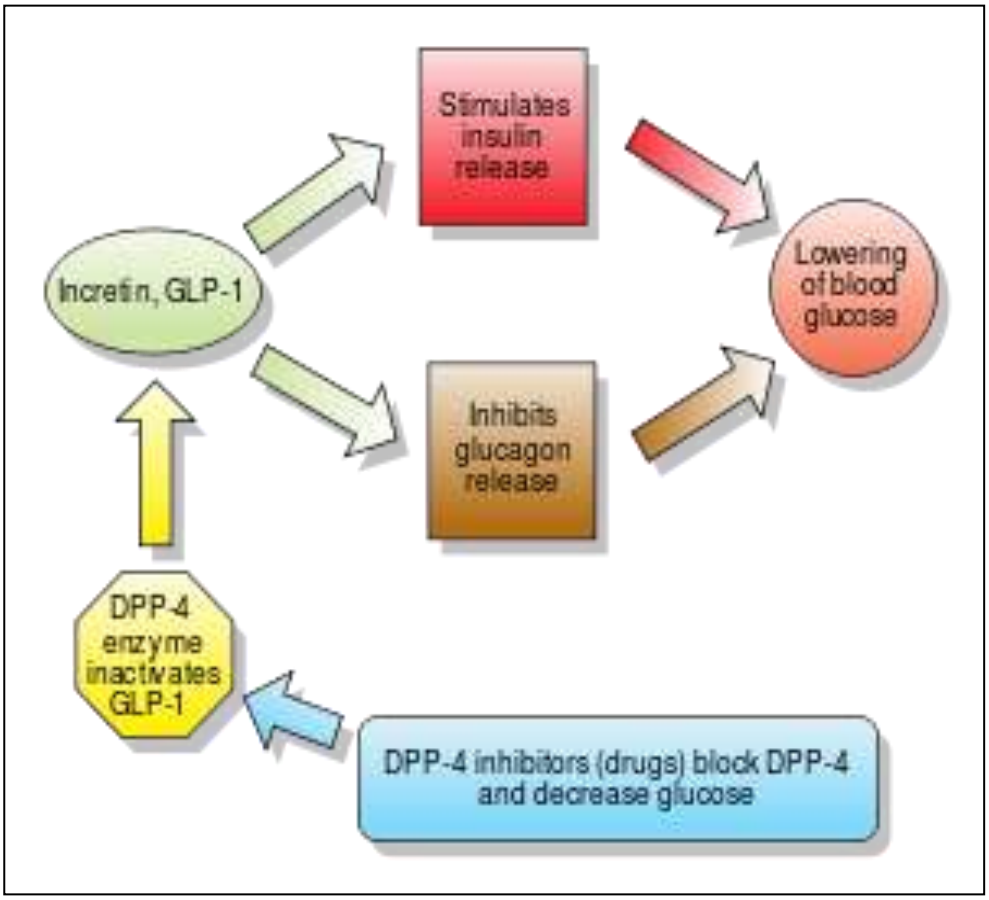


Figure 13: Effect of DPP4 Inhibition

4. PTP1B ROLE IN DIABETES

- PTP1B a key negative regulator of insulin signaling, has emerged as an attractive target for the treatment of type II diabetes.
- PTP1B directly inactivates the insulin receptor (IR) by dephosphorylating tyrosine residues in the regulatory domain and its overexpression inhibits IR signaling.
- The insulin receptor is expressed as a single-chain precursor of 1370 amino acids, containing an α (purple) and a β subunit (pink), which dimerizes in the ER. During its transition through the ER, the receptor is kept in a dephosphorylated state by PTP1B.
- The receptor undergoes further maturation as it transits through the Golgi to the surface of the cell. Insulin stimulation results in internalization of the activated receptor into endosomes which interacts with PTP1B located on the ER. The dephosphorylated/deactivated receptor returns to the plasma membrane or is degraded. (Águeda, *et. al* 2010)
- PTP1B knock out mice are obesity resistant ,type 2 diabetes resistance and found to be in healthy state (fertile , no phenotypic abnormalities).

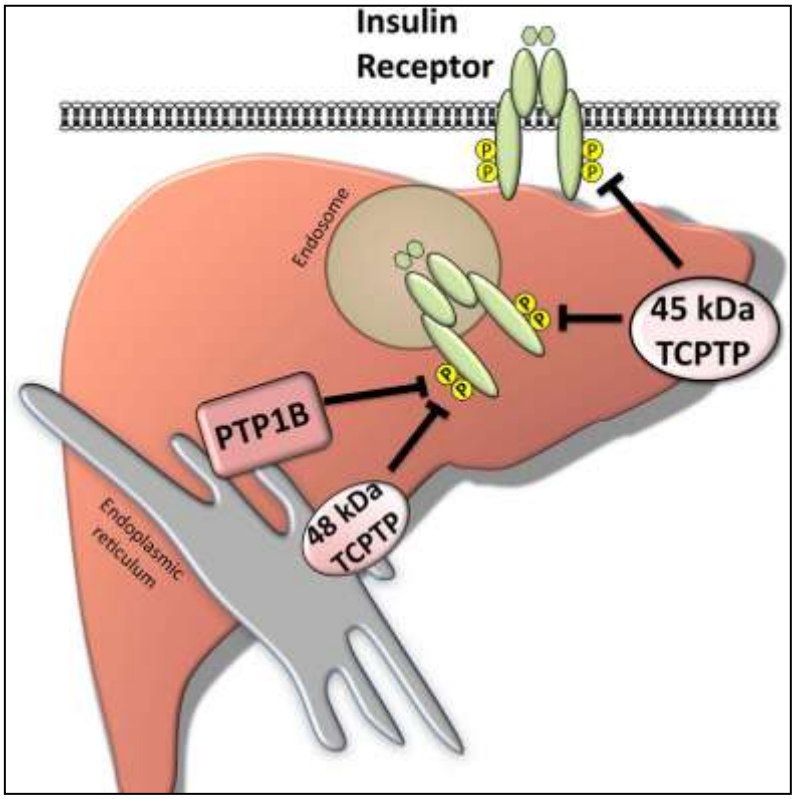


Figure14: PTP1B role in insulin pathway

(Picture source: Protein Phosphatases: From Molecules to Networks volume280)

4.1PTP1B CATALYTIC SITE

- The catalytic site of PTP1B is centered at Cys 215 residue and included WPD loop, which closure is essential for the catalytic mechanism of PTP1B.
- The WPD loop has been shown to play a critical role at two stages of the catalytic cycle.
- First, Asp181 of the WPD loop serves as the proton donor during cleavage of the Tyr(P) P–O bond, and second, Asp181 participates in positioning and activating the water molecule that splits the cysteinyl-phosphate bond in the enzyme-phosphate intermediate.(Bence , *et.al* , 2010).
- At both stages, closure of the WPD loop is essential in bringing Asp181 close to the phosphate group.

4.2 PROBLEM WITH CATALYTIC SITE

- Highly conversed among various phosphatases. It is Relatively shallow site and hence excellent fitting of molecule is not acrhieved. Only highly charged inhibitor show good binding affinity , this result in less permeability and bioavailability.
- Therefore now researcher are targeting allosteric site for inhibition. The allosteric inhibitors block several interactions and side chain movements that are associated with formation of the closed conformation of WPD loop (Hansen , et.al 2005).

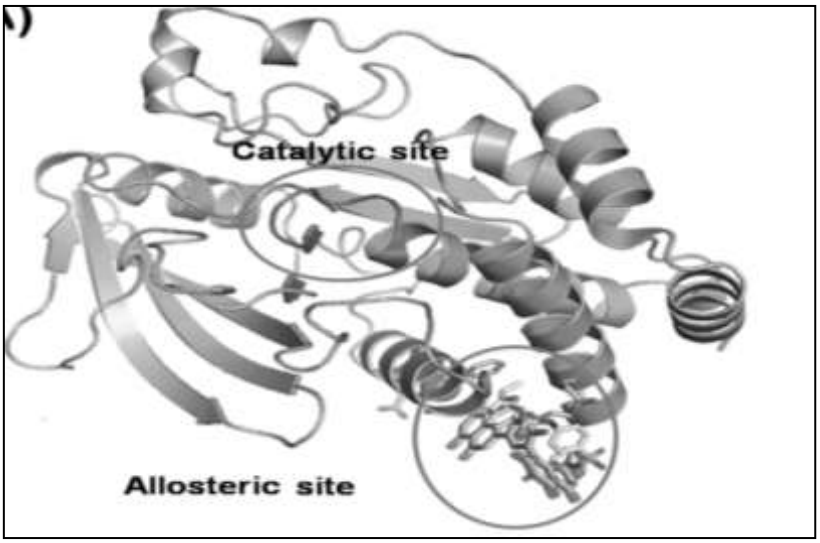


Figure 15: Allosteric and Catalytic Site of PTP1B

4.3Allosteric Site Benefits in PTP1B

- Allosterics site in PTP1B was 1st reported by Hansen group in 2004. This site is 20A away from cys215(a active site residue). It not well conserved. Hence specificity can be easily achieved in this case. No Drug are available in market till date for PTP1B this make this area open for research.
- Few PDBs are available for inhibitor bound PTP1B site namely 1T48, 1T49, 1T4J and none of them show nano-molar activity.

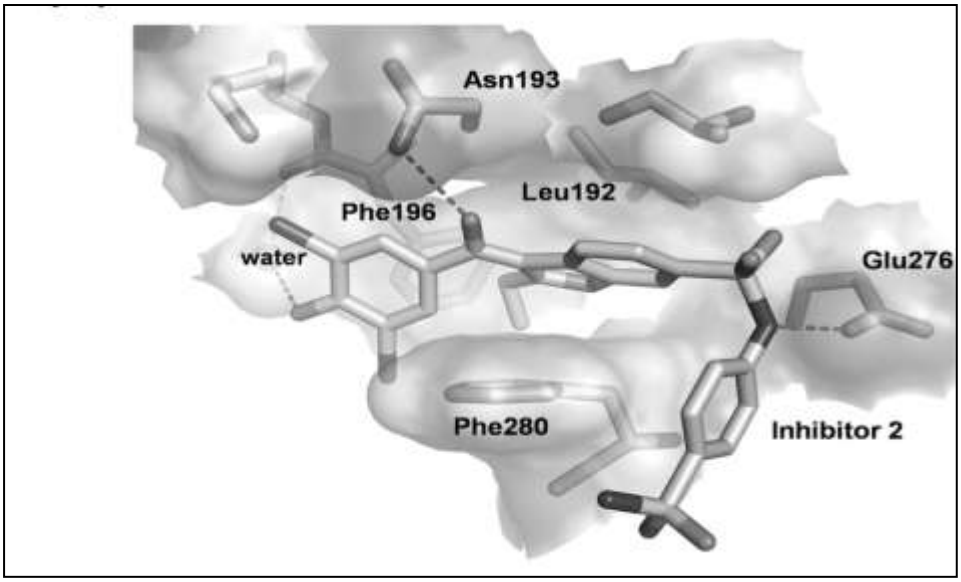


Figure 16: Allosteric site bound to already known inhibitor.
(Picture credit: This picture is created using Pymol and PDB 1T48)

5. INTRODUCTION TO SOFTWARES AND TOOLS USED

5.1 Molecular Docking Softwares

- Molecular docking is a study of how two or more molecular structures, for example drug and enzyme or receptor of protein, fit together. The action of a harmful protein in human body may be prohibited by finding an inhibitor, which binds to that particular protein. Molecular docking softwares are mainly used in drug research industry. The most important application of docking software is virtual screening. In virtual screening the most interesting and promising molecules are selected from an existing database for further research. This places demands on the used computational method; it must be fast and reliable. Another application is the research of molecular complexes.
- These are many softwares available for molecular docking. Some of them are freely available while some of them require purchasable license for their access. These softwares differ in their scoring functions and algorithm they follow like autodock is based on Lamarckian genetic algorithm to create a set of possible conformations.
- The success of a docking program depends on two components: the search algorithm and the scoring function. There are many such algorithm developed and are available for docking but all of them have some limitations and drawbacks. Some software give high score to hydrophobic interaction and some give to hydrogen bonding. Therefore in order to archive good results consensus must be taken.

Brief Description of Docking Softwares used in this project.

5.1.1 AUTODOCK

AutoDock uses Monte Carlo simulated annealing and Lamarckian genetic algorithm to create a set of possible conformations. LGA is used as a global optimizer and energy minimization as a local search method Possible orientations are evaluated with AMBER force field model in conjunction with free energy scoring functions and a large set of protein-ligand complexes with known protein-ligand constants. The newest version 4(vina) should contain side chain exibility. AutoDock has more informative web pages than its competitors and because of its free academic license.

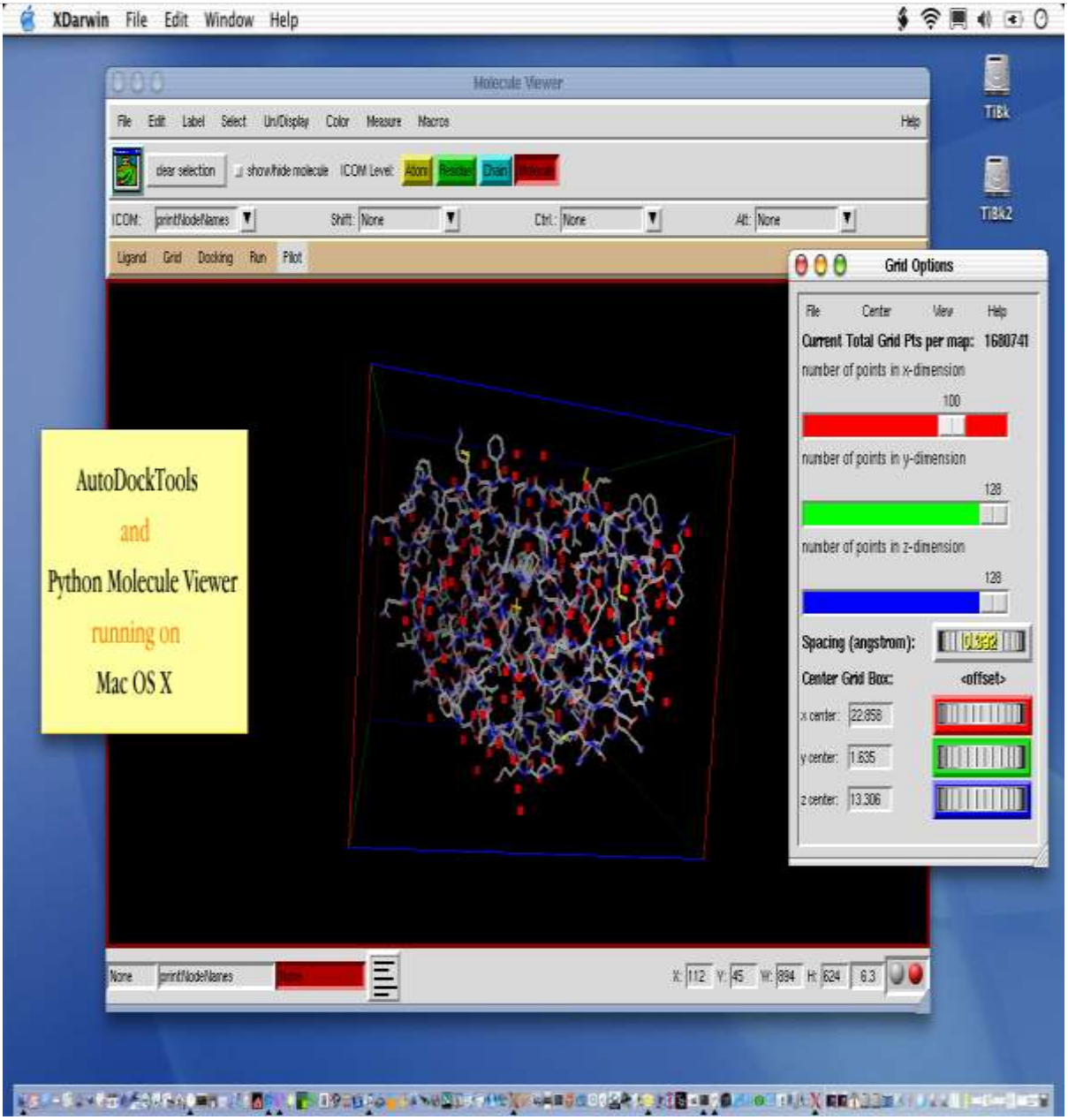


Figure 17: Screenshot of Autodock

5.1.2 GLIDE(SCHRODINGER)

Glide offers the full spectrum of speed and accuracy from high-throughput virtual screening of millions of compounds to extremely accurate binding mode predictions, providing consistently high enrichment at every level. It provides a rational workflow for virtual screening from HTVS to SP to XP, enriching the data at every level such that only an order of magnitude fewer compounds need to be studied at the next higher accuracy level. Glide reliably finds the correct binding modes for a large set of test cases. It outperforms other docking programs in achieving lower RMS deviations from native co-crystallized structures.

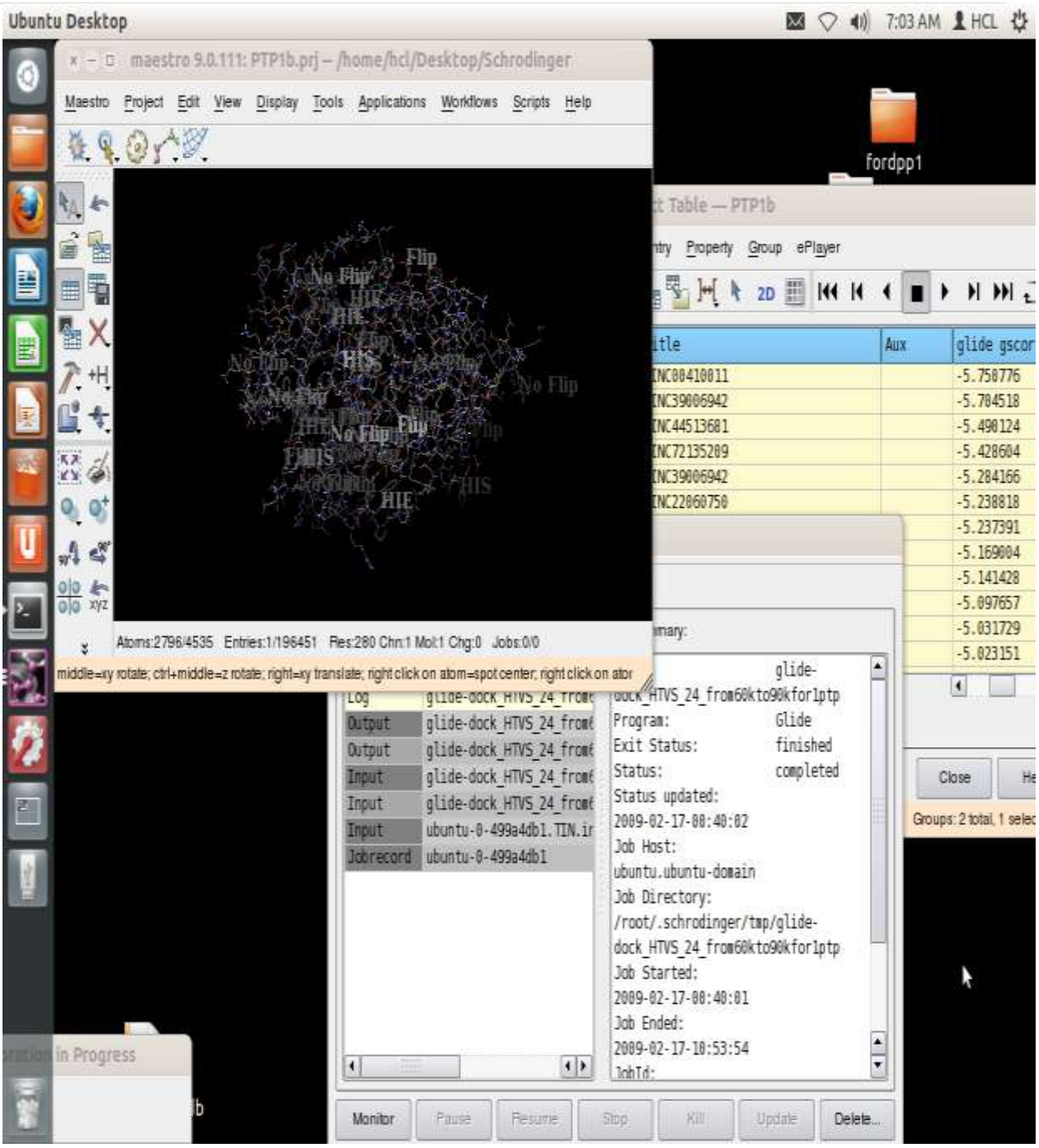


Figure 18: Snapshot of Glide

5.1.3 SANJEENVI

Sanjeevini software has been developed as a computational pathway paving the way expressely towards automating lead design, making any number of known or new candidate molecules out of a small but versatile set of building blocks called templates, screening them for drug likeness, optimizing their geomtery, determing partial atomic charges and assigning other force field parameters, docking the candidates in the active site of a given biological target , estimating the interaction/bidning energy, peforming molecular dynamics simulations

with explicit solvent and salt on the biomolecular target, the candidate and the complex followed by a rigourous analysis of the binding free energy for further optimization. Presetly Sanjeevini is coupled with AMBER and GAMESS for molecular mechanics and quantam mechanics calculations, respectively. There are total of six modules which makes Sanjeevini a complete drug design software. The source codes for all modules are written in FORTRAN, C and C++ computer languages with numerous interfacial UNIX based shell scripts which makes all the modules work like a pipeline such that output of the previous step becomes the input for the next step. The modules under Sanjeevini can also be used independent of the pathway.

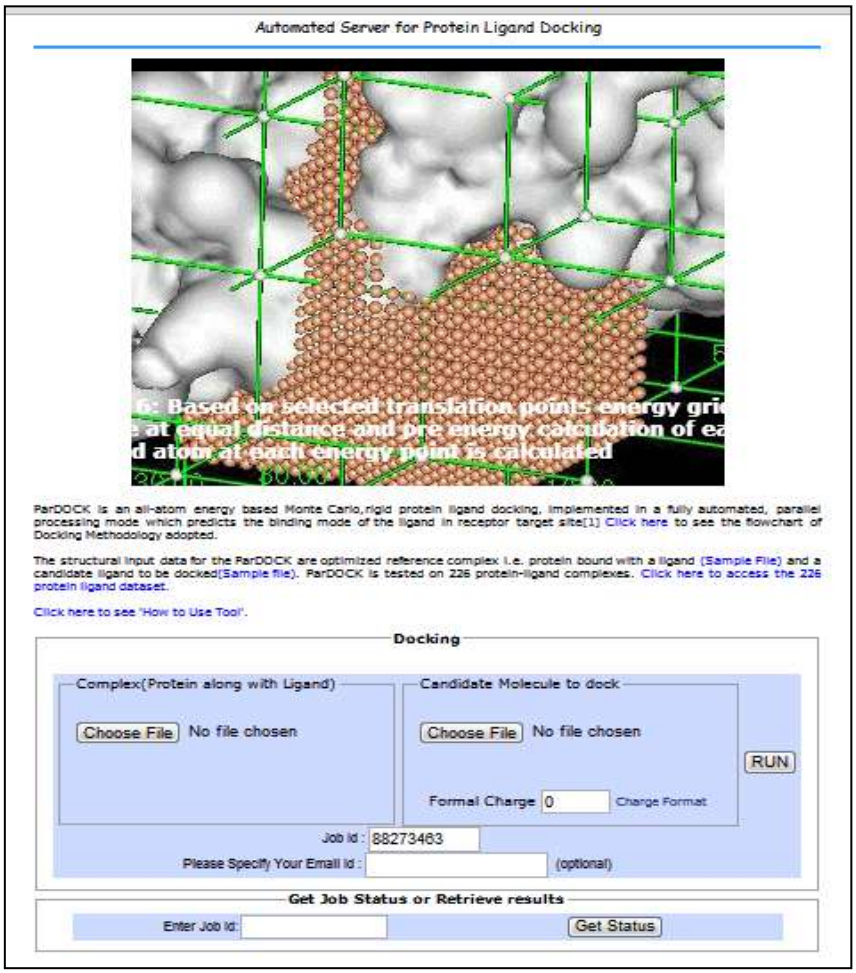


Figure 19: Snapshot of Sanjeevni

5.1.4 SWISSDOCK

SwissDock is a docking web server that addresses the limitations described above. The structure of the target protein, as well as that of the ligand, can be automatically prepared for docking. In addition, the cumbersome syntax of the docking engine is hidden behind a clean web interface providing reasonable alternative sets of parameters as well as sample input files. All calculations are performed on the server side, so that docking runs do not require any computational power from the user. The interpretation of docking results and their integration into existing research pipelines is greatly facilitated by the seamless visualization of docking predictions in the UCSF Chimera molecular viewer, which can be launched directly from the w

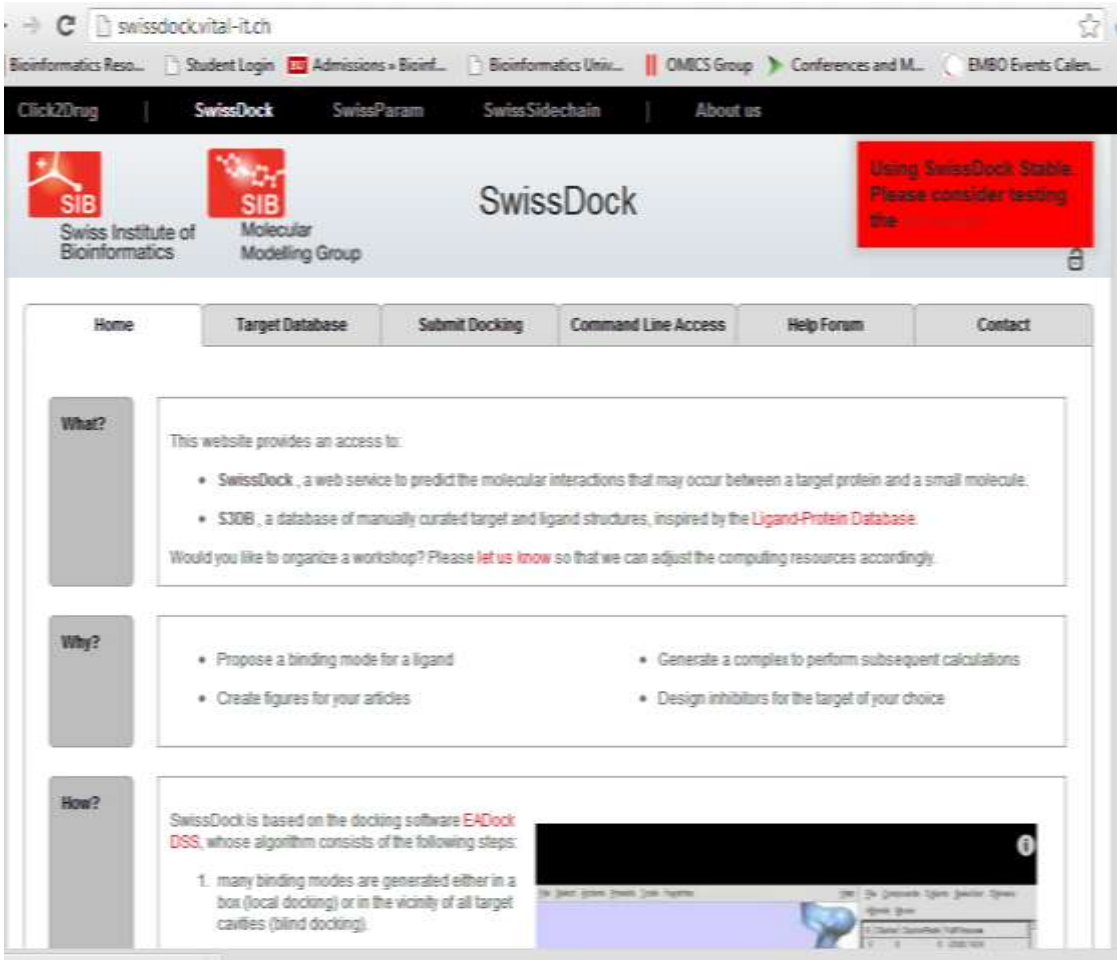


Figure 20: Snapshot of Swiss Dock

5.2 MOLECULAR VISUALIZATION SOFTWARE

5.2.1 Pymol

PyMOLis a free cross-platform molecular graphics system made possible through recent advances in hardware, internet, and software development technology . PyMOL provides most of the capabilities and performance of traditional molecular graphics packages written in C and Fortran . However, its integrated Python interpreter endows it with features and expandability unmatched by any traditional package. PyMOL has been released under a completely unrestrictive open-source software license so that all researchers and software developers can freely adopt PyMOL and then distribute derivative works based on it without cost or limitation**.**

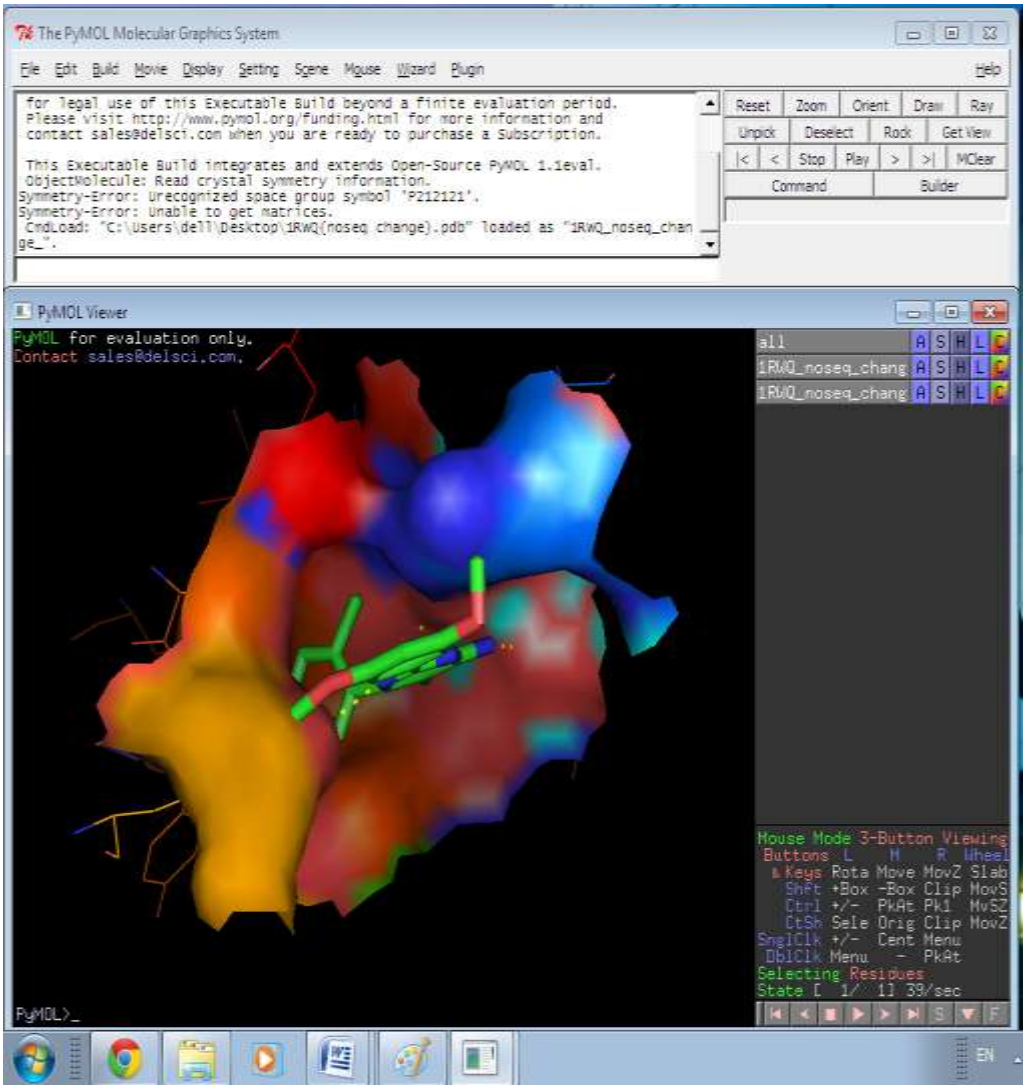


Figure 21: Snapshot of Pymol

5.2.2 ViewerLite

ViewerLite is designed for modeling, visualization, and analysis of biological systems such as proteins, nucleic acids, lipid bilayer assemblies, etc. It may be used to view more general molecules, as VMD can read standard Protein Data Bank (PDB) files and display the contained structure. It provides a wide variety of methods for rendering and coloring a molecule: simple points and lines, CPK spheres and cylinders, licorice bonds, backbone tubes and ribbons, cartoon drawings, and others. It can be used to animate and analyze the trajectory of a molecular dynamics (MD) simulation. In particular, VMD can act as a graphical front end for an external MD program by displaying and animating a molecule undergoing simulation on a remote computer.

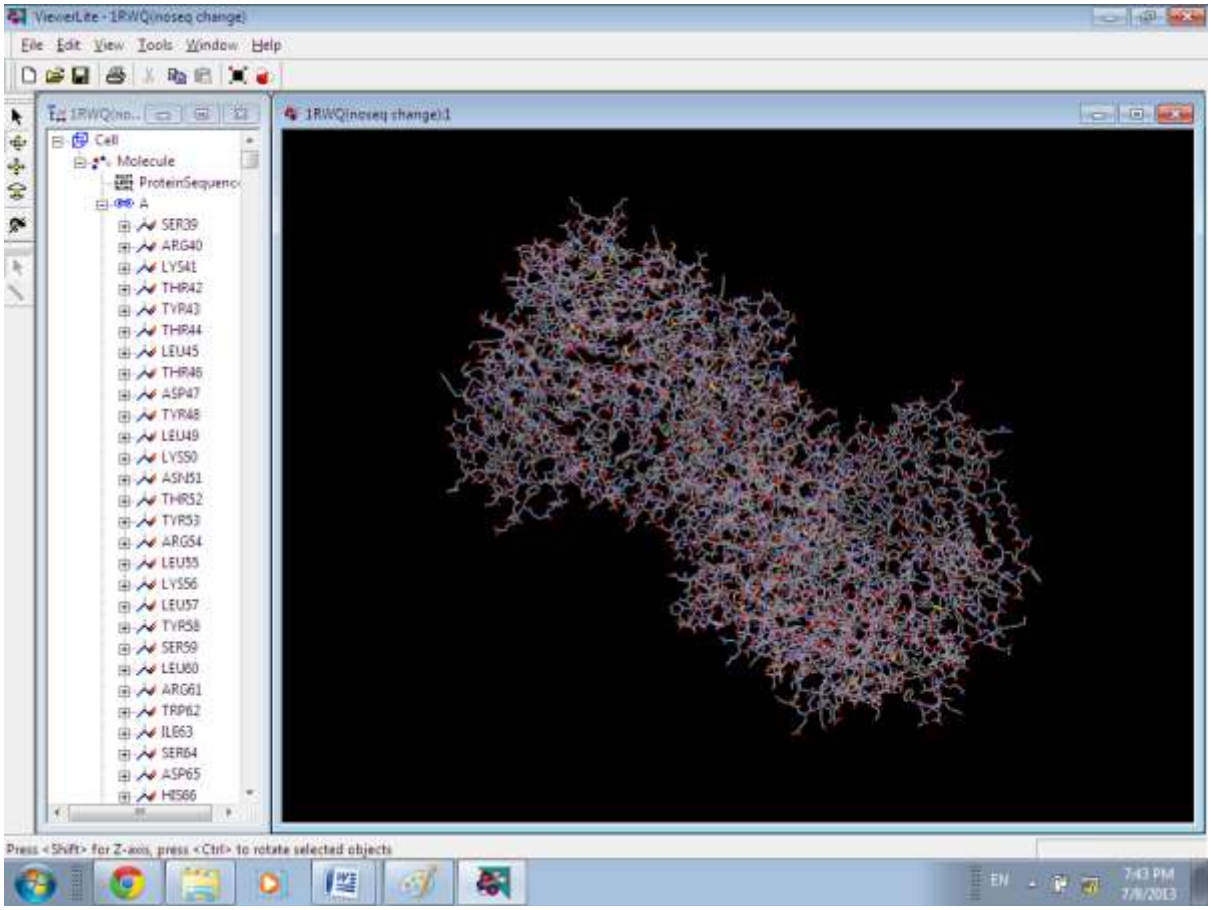
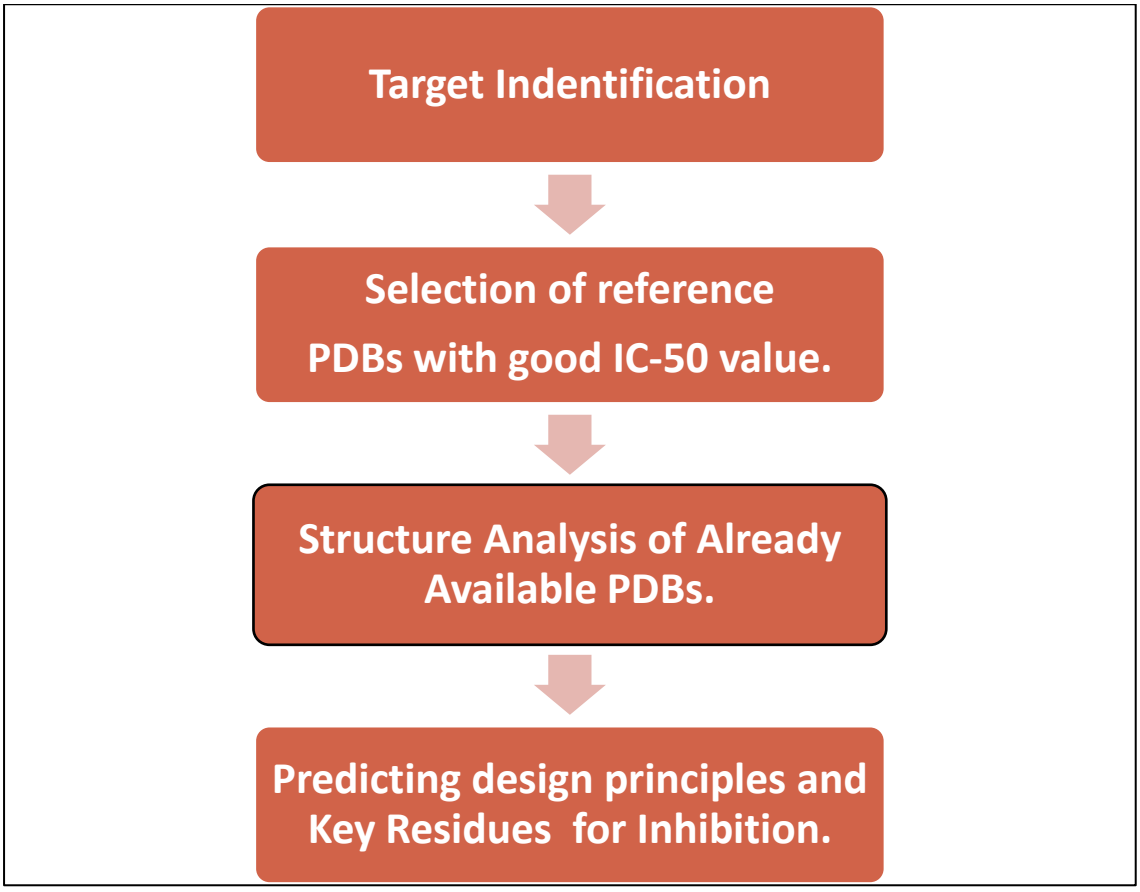


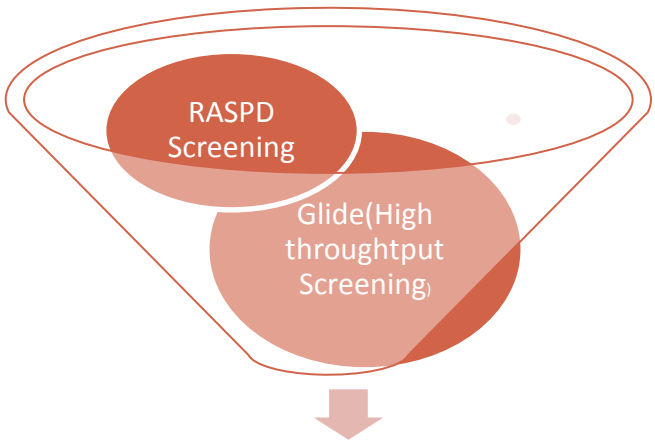
Figure 22: Snapshot of VIewerLite.

METHODOLOGY

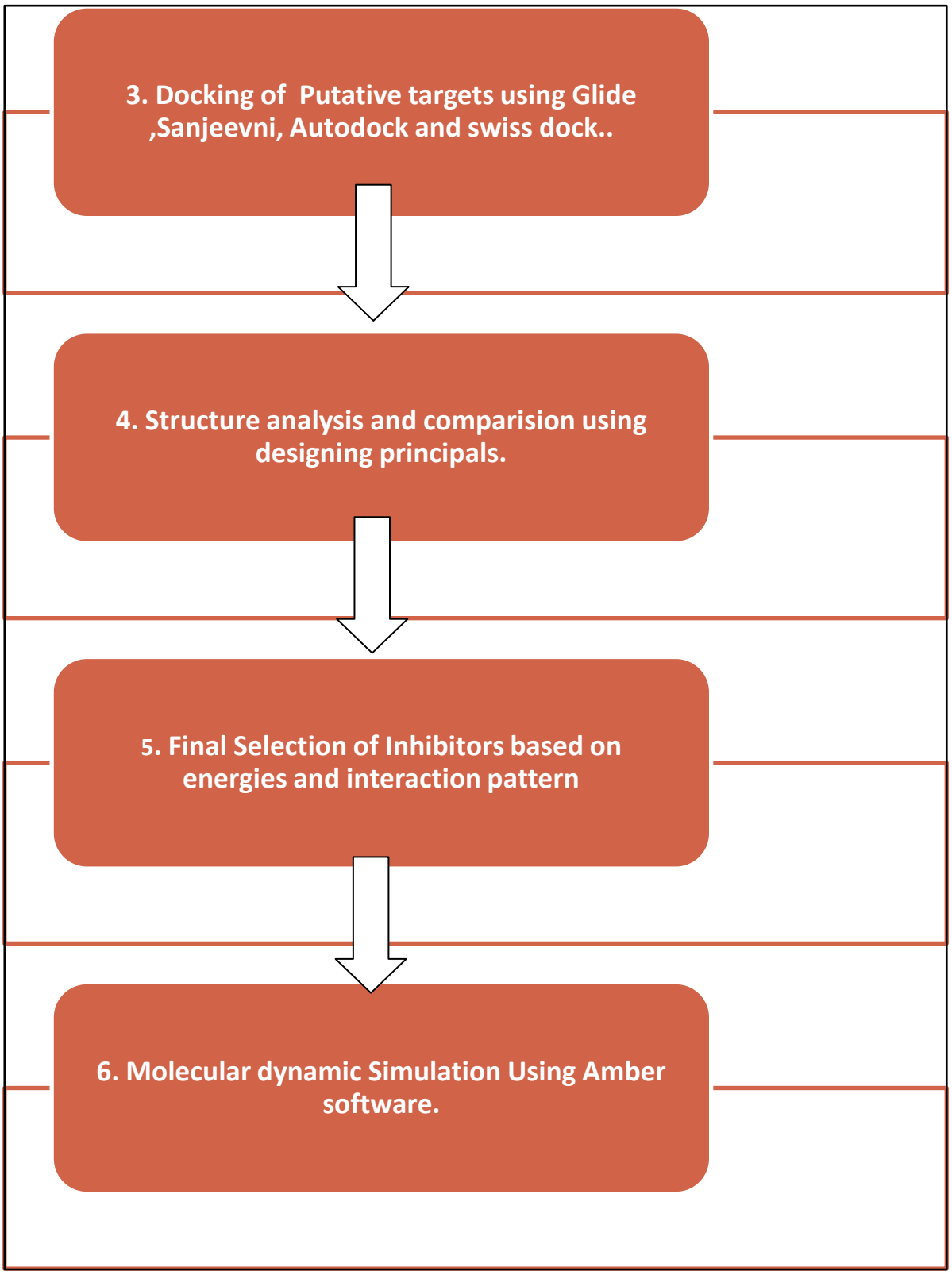
1. Target selection



2. Virtual Screening for Zinc Database ,NCI and Drug Repurposing.



Putative Hit Selected on basics of Energies.


3. Docking of Putative targets using Glide ,Sanjeevni, Autodock and swiss dock..
4. Structure analysis and comparision using designing principals.
5. Final Selection of Inhibitors based on energies and interaction pattern
6. Molecular dynamic Simulation Using Amber software.

RESULTS

1. RESULTS FOR G3K3B

- After Virtual screening and docking finally three molecules were selected as potential inhibitors for G3k3B. In Molecule one and two slight modification was done in order to achieve val135 interaction which is important for inhibition. Safford for selected molecules are shown below.

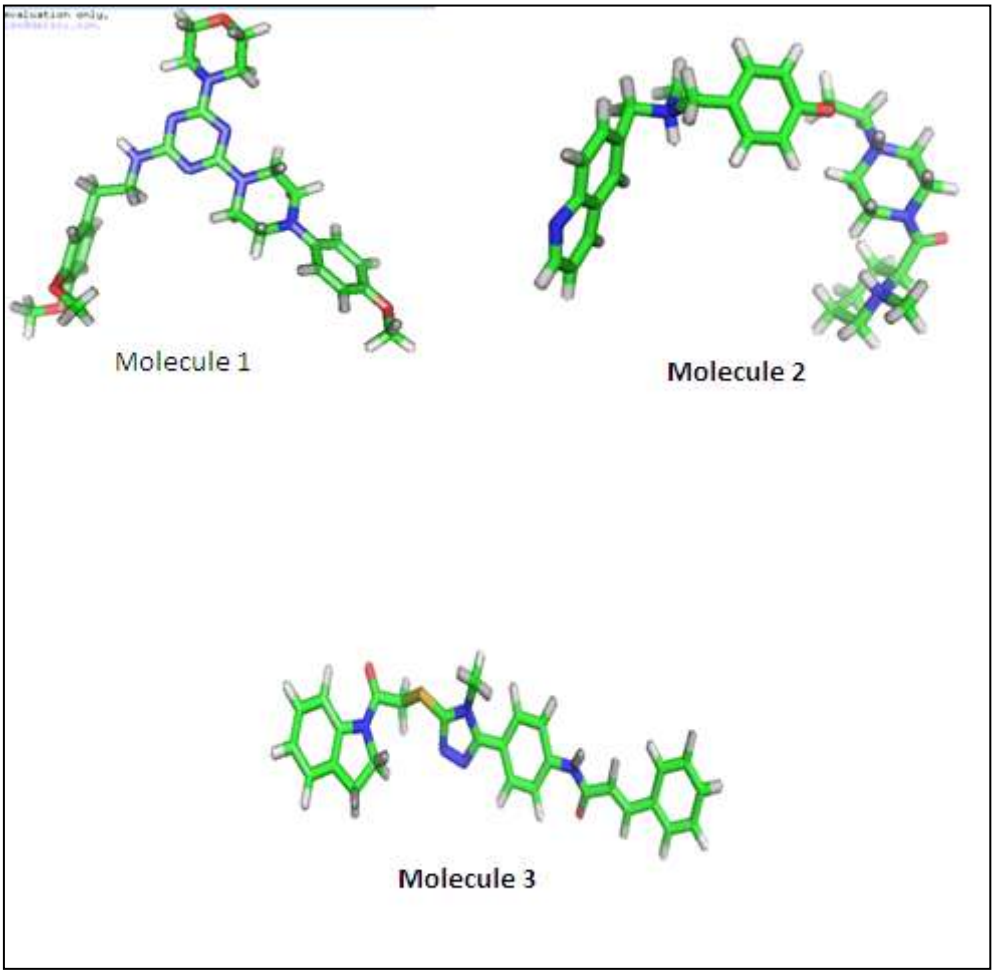


Figure 23: Inhibitors for G3k3B

(*Picture created using Pymol)*

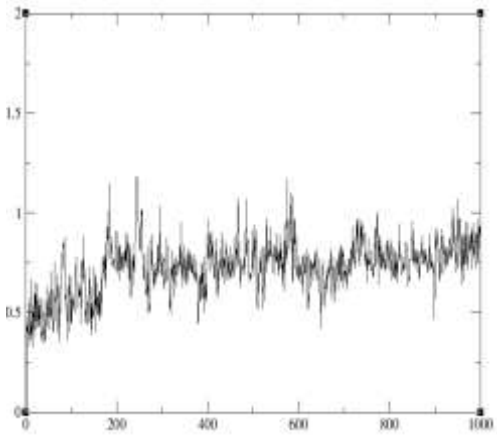
- These molecules not only have good binding energies but also show interactions with key resuidues namely val135, Tyr 136, Asp200, Asp 133.These resuidue are also specific for G3k3b and hence specificity can be archived.

Docking Software	**Sanjeevni** (Energies in kcal/Mol)	**Autodock** (Energies in kcal/Mol)	**Glide** (Energies in kcal/Mol)	**Swiss Dock** (Energies in kcal/Mol)	**Average Energy After MD for 100 snapshots**
Molecule 1	-8.65	-7.48	-7.0	-9.12	-11.19
Molecule 2	-9.11	-7.31	-8.1	-8.88	-10.70
Molecule 3	-7.13	-8.01	-7.33	-8.0	-9.61

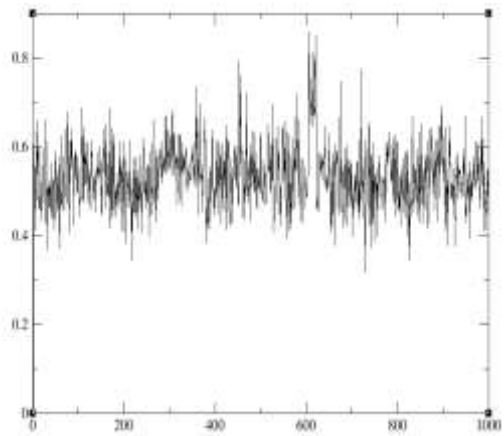
Table 4: Predicted Binding energies for GSk3B

➢ MOLECULAR DYNAMIC SIMULATION RESULT

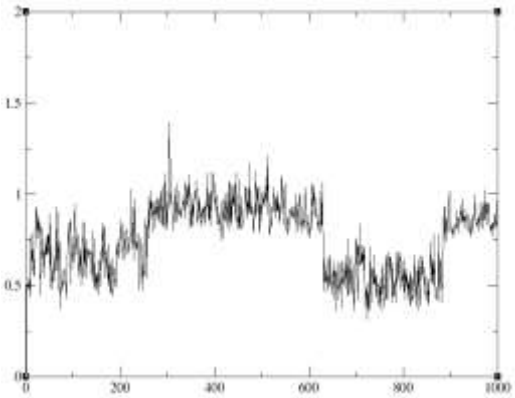
All the molecules were found to be stable and RMSD of less then 2A was Achieved. The average binding free energy calculated during simulationfor 1000 snapshots was above -9kcal for all the molecules.



Molecule1

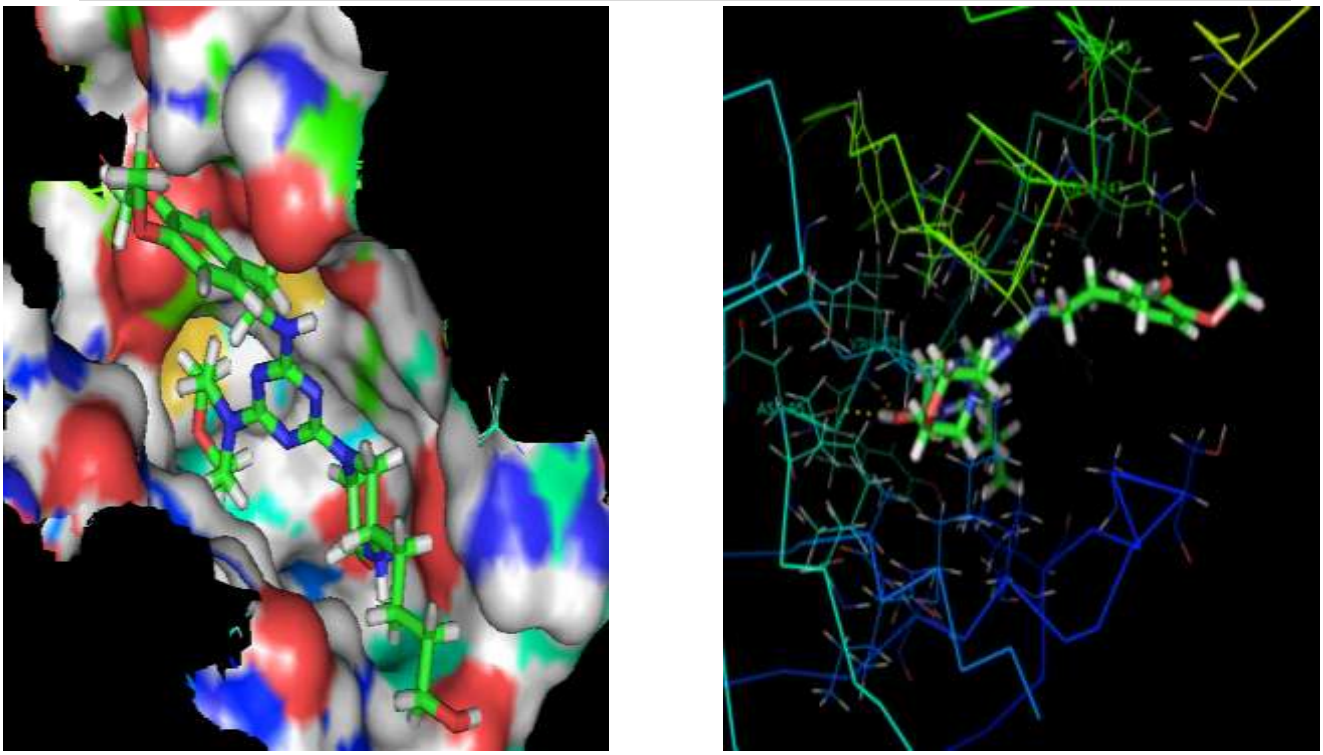


Molecule 2

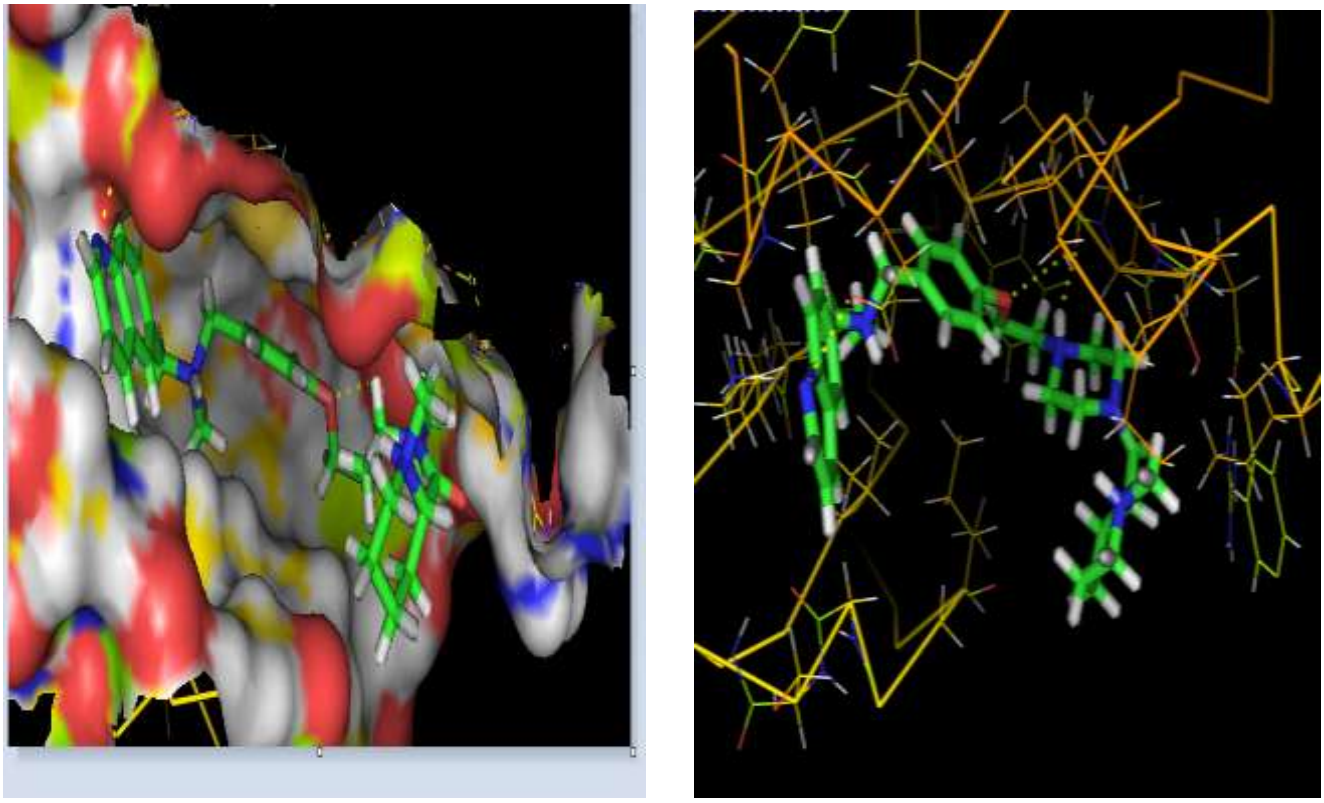


Molecule 3

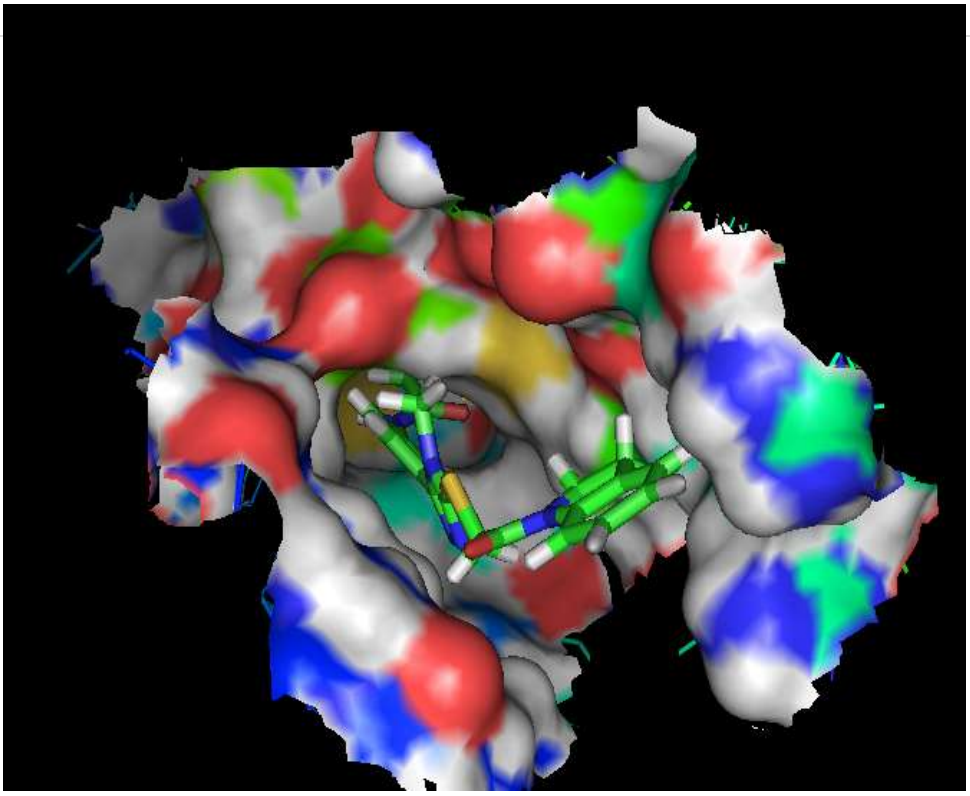
Figure 24: MD simulation graph showing stable tracjectiories for Molecules 1, 2, 3. (Graph represents RMSD vs Time in fsec)

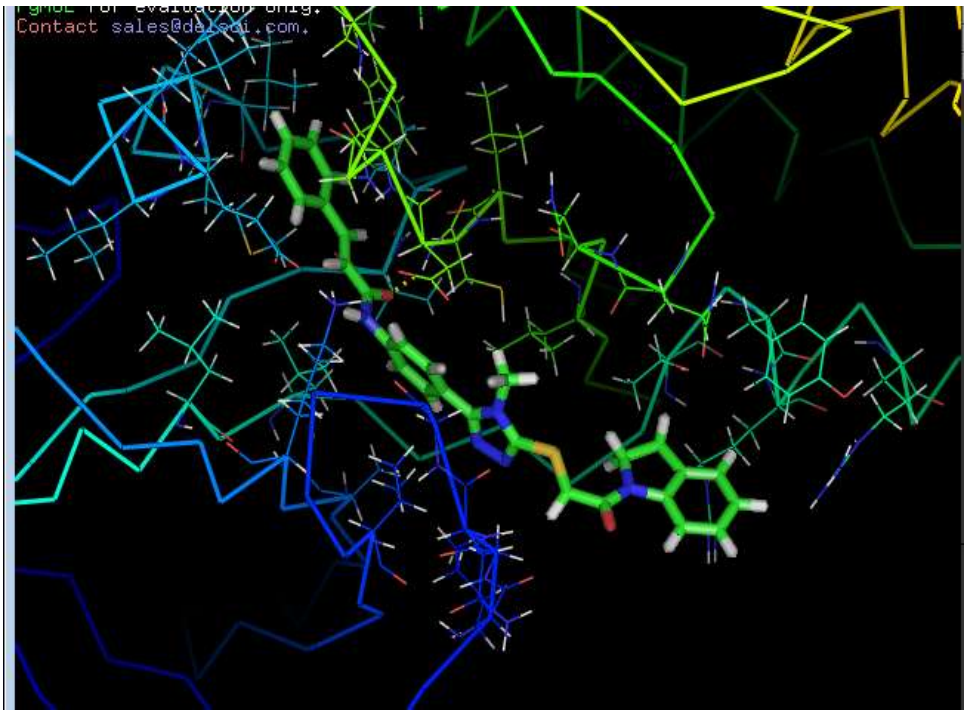


Docked Complex for Molecule 1



Docked Complex for Molecule2





Docked Structure for Molecule 3

Figure 25: Inhibitor bound structures for GSK3B.

2. RESULTS FOR DPP4

- Two highly potent inhibitors were found for DPP4 through virtual screening. These inhibitors give good binding energies and are fitting well in the active site of DPP4.

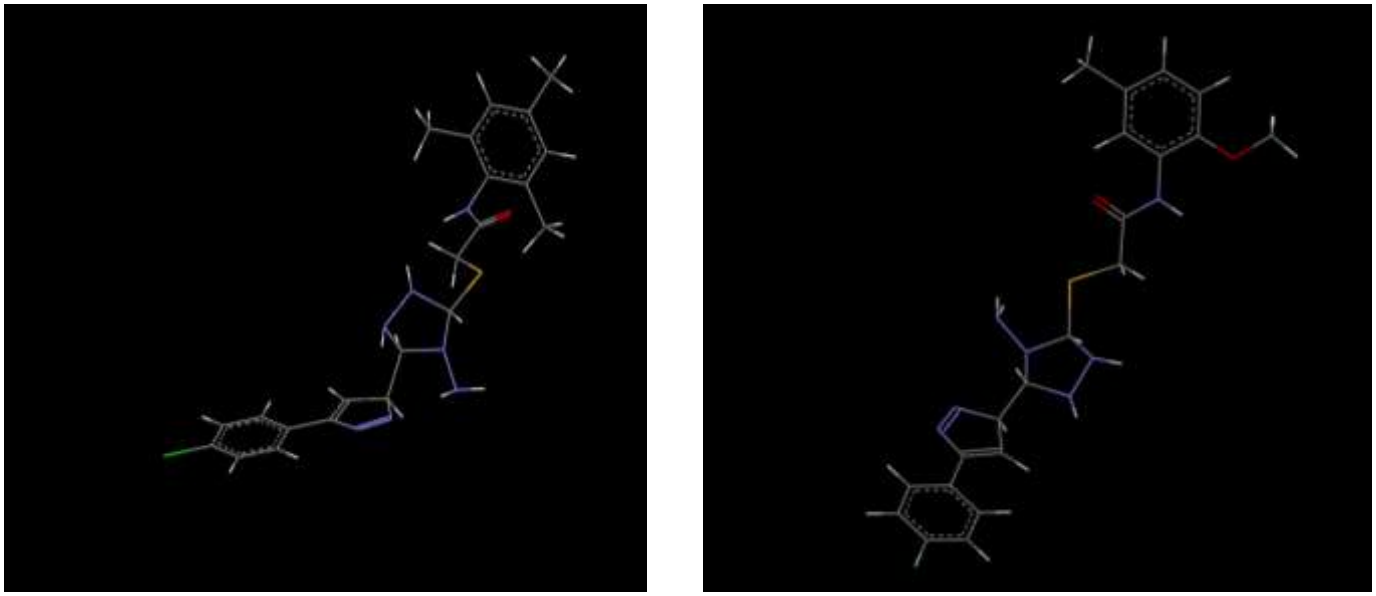


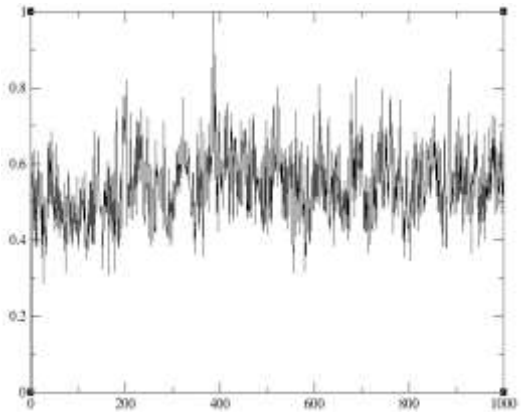
Figure 26: Inhibitor for DPP4

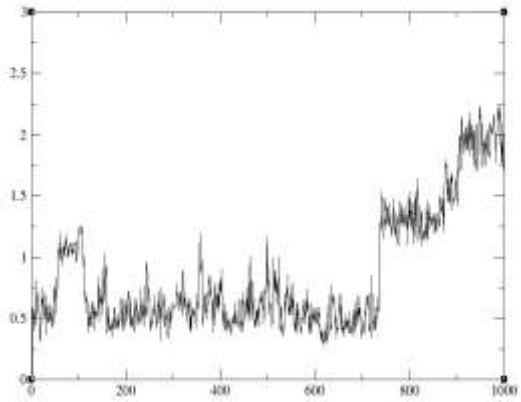
- These inhibitors are forming hydrogen bonding with GLU`205, GLU`206, and Tyr665.

Docking Software	**Sanjeevni** (Energies in kcal/Mol)	**Autodock** (Energies in kcal/Mol)	**Glide** (Energies in kcal/Mol)	**Swiss Dock** (Energies in kcal/Mol)	**Average Energy After MD for 100 snapshots**
Molecule 1	-8.05	-7.88	-8.14	-8.62	-9.99
Molecule 2	-7.17	-8.01	-8.40	-8.03	-9.31

Table 5: Docking Score for DPP4

➢ MOLECULAR DYNAMICS SIMULATION RESULTS

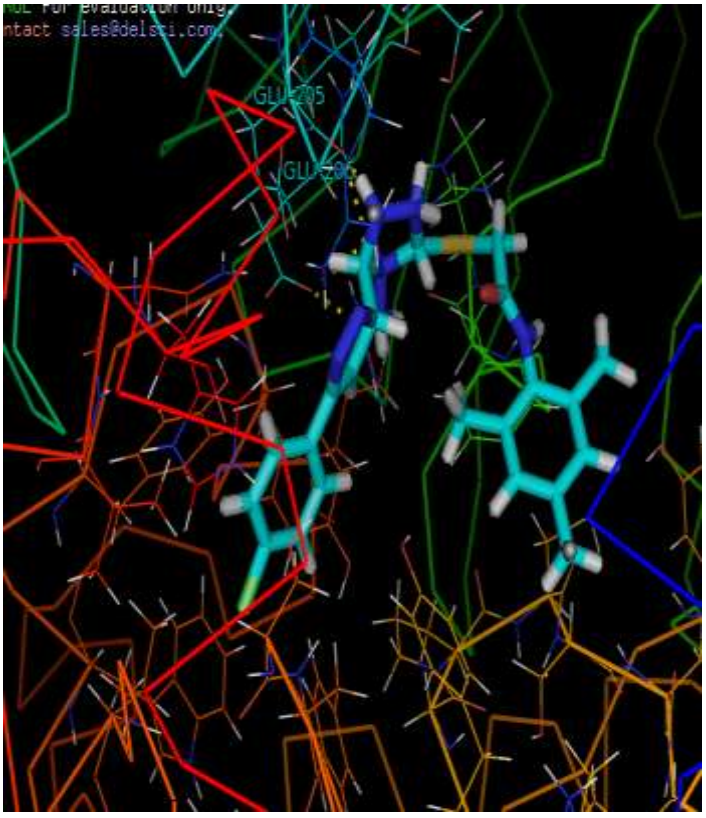


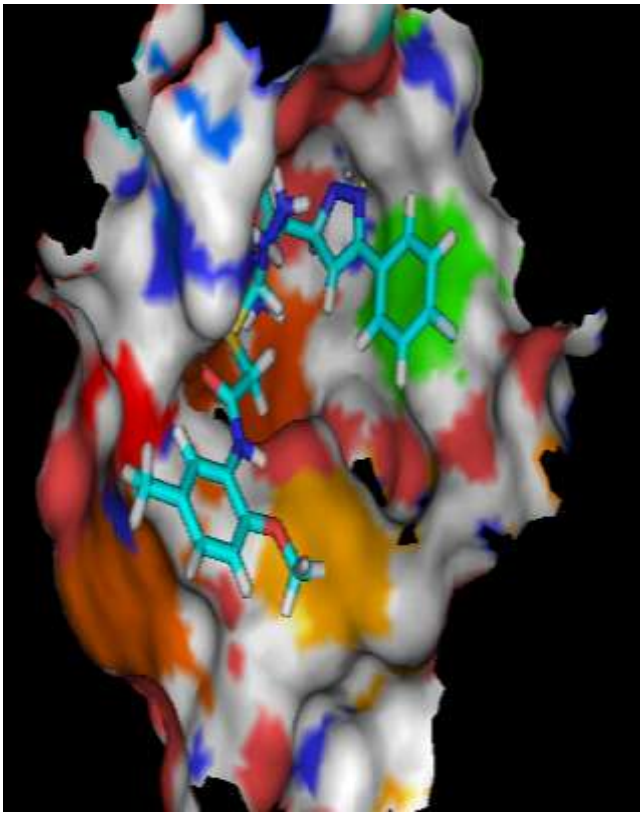


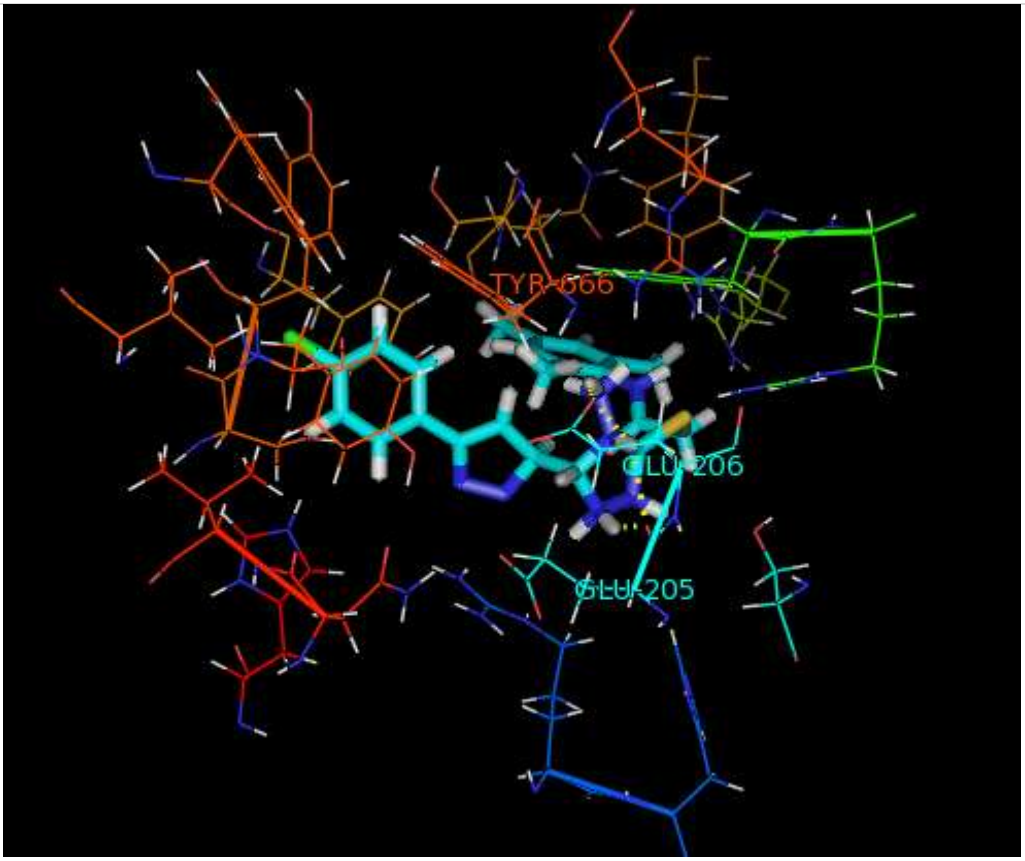
Molecule 1 **Molecule 2**

Figure 28: MD simulation Graph for DPP4
(Graph represents RMSD vs Time in fsec)

➢ DOCKED COMPLEX FOR DPP4







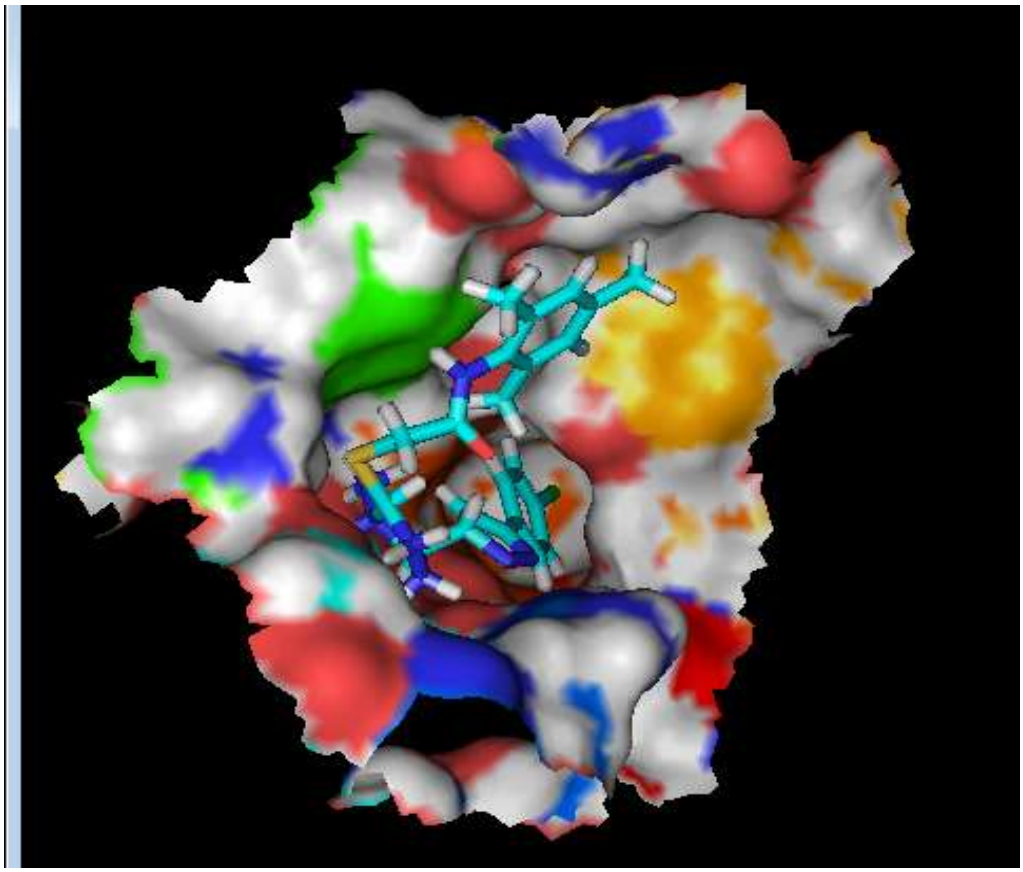


Figure 29: Docked Structures for DPP4

3. RESULTS FOR PTP1B

- **Two Molecules are shortlisted after screening and docking analysis.**

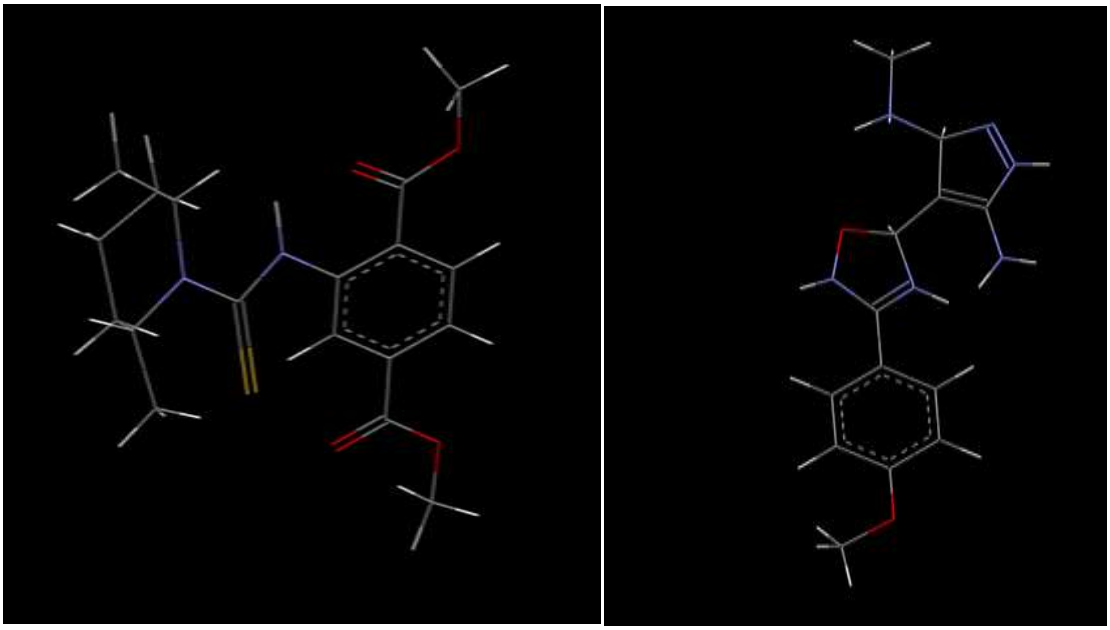
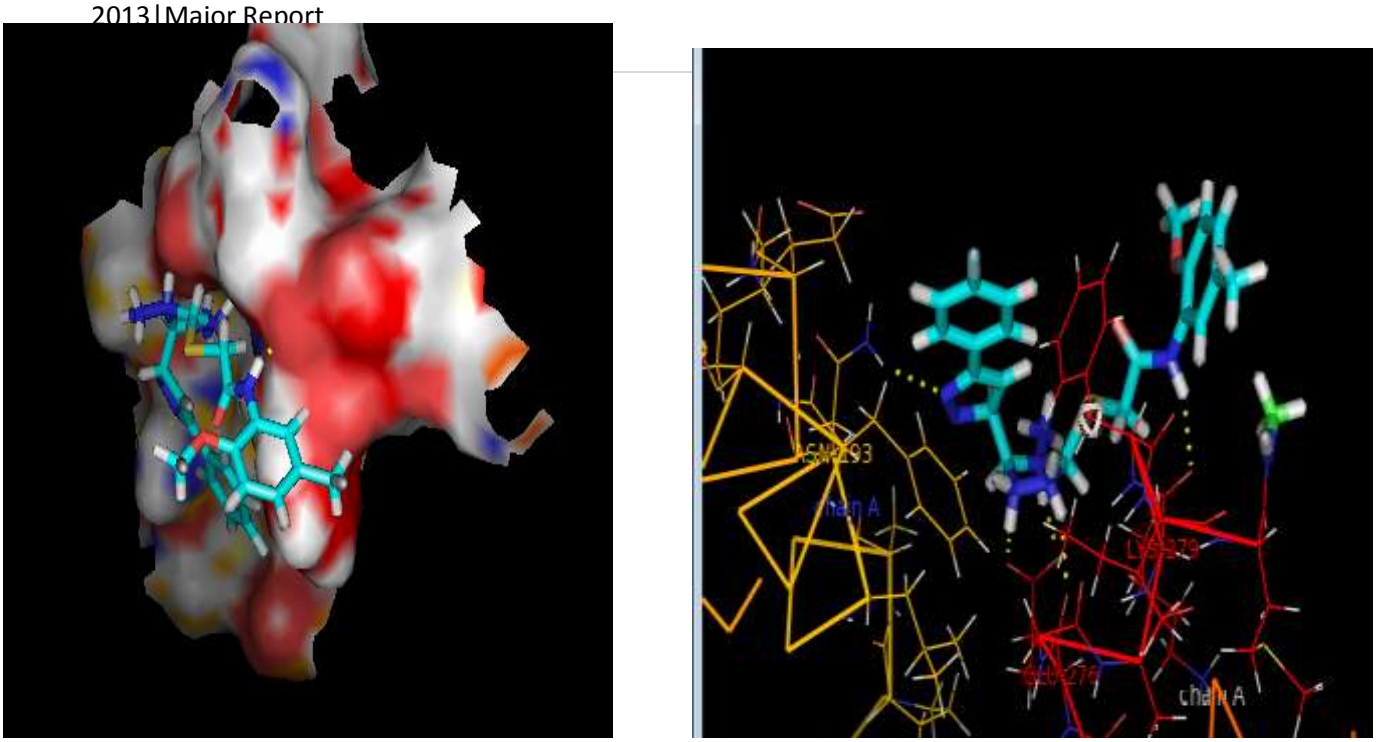


Figure 30: Inhibitor Molecules for PTP1B.

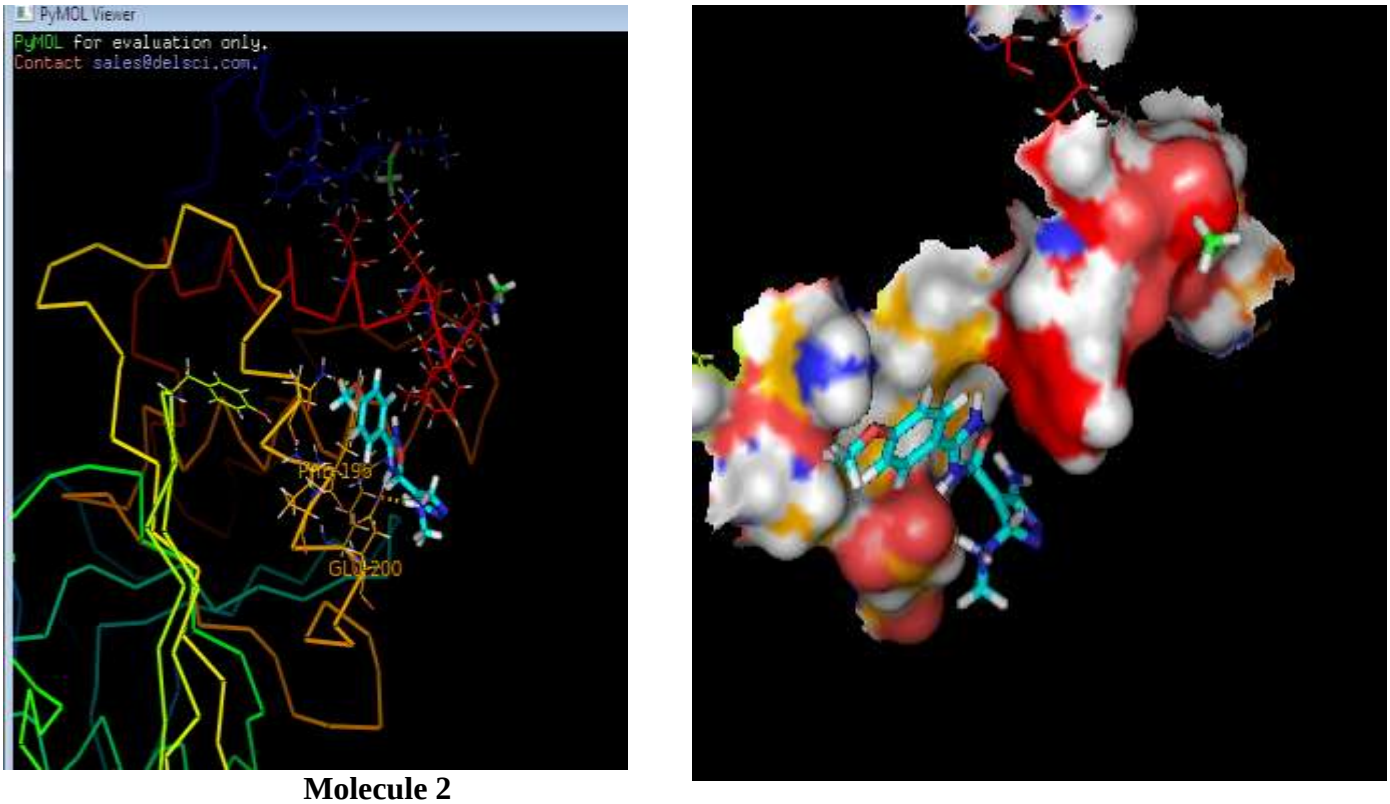
- **These two molecules are giving good binding affinity and are also interacting with key residues for inhibition.**

Docking Software	**Sanjeevni** (Energies in kcal/Mol)	**Autodock** (Energies in kcal/Mol)	**Glide** (Energies in kcal/Mol)	**Swiss Dock** (Energies in kcal/Mol)	**Average Energy After MD for 100 snapshots**
Molecule 1	-7.05	-7.08	-7.14	-7.62	-7.99
Molecule 2	-7.01	-7.0	-7.40	-7.03	-7.31

Table 6: Predicted Binding free energy for PTP1B



Molecule 1



Molecule 2

Figure 31: Docked Structure for PTP1B

➢ MOLECULAR DYNAMICS SIMULATION RESULTS

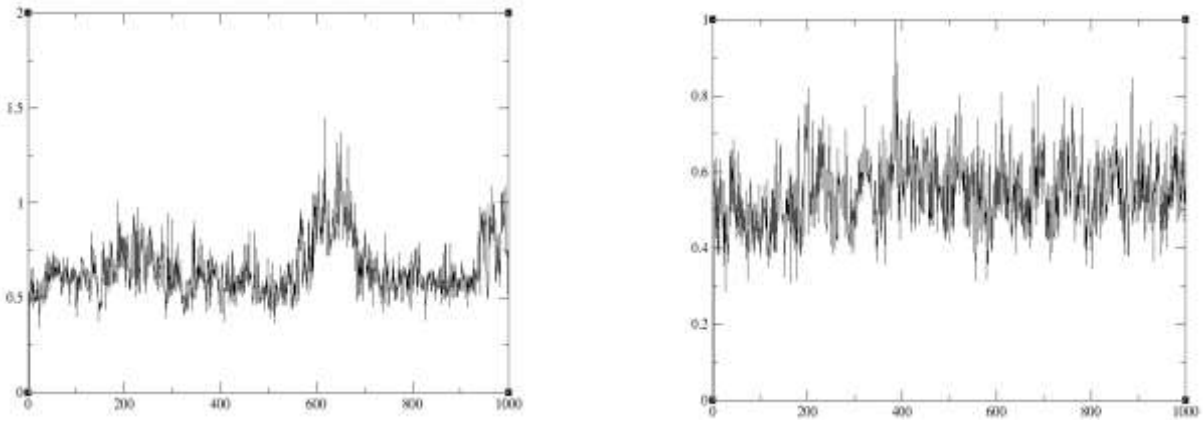


Figure 32: MD simulation Graph for PTP1B.
(Graph represents RMSD vs Time in fsec)

DISCUSSION

1. ATP COMPETITIVE INHIBITORS FOR GSK-3 BETA

Several analyses were done on GSK-3 beta's ATP binding site. It was matched with similar and closely related kinases like CDK, FGFR1 and VEGFR3. A chart showed in Table 1 highlights the amino acid composition of ATP binding site for these four closely related protein kinases. According to this chart, GLY63, VAL70, ALA83, LYS85, LEU188 and ASP200 are the amino acid residues that are conserved in ATP binding site of over a range of protein kinases. Thus, if a molecule is showing interaction with only these above mentioned residues, then specificity issue may come in. Also, ASP133 is replaced by GLU in other protein kinases, which doesn't make much of a difference. Hence, the amino acid residues in the ATP binding pocket of GSK-3 beta that are highly specific to this protein are LEU132, TYR134, VAL135, THR 138, ARG141 and CYS199. **Figure 4** shows a snapshot of a known inhibitor inside the binding pocket of GSK-3beta highlighting the interactions made and the interacting environment.

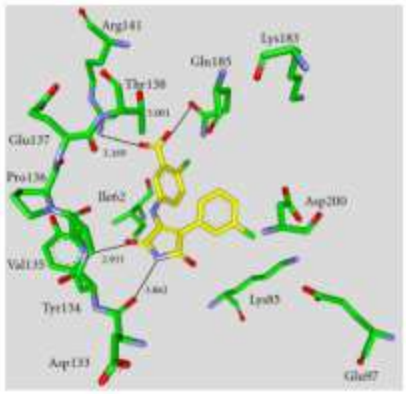


Figure 33: Interaction pattern of already known inhibitor

- After analyzing all the 16 cases, it was observed that amino acid residues LEU132, ASP133, TYR134, VAL135, PRO136, LEU188 and ASP200 were present in all the cases of highly active compounds. This set of residues perfectly matches to the specific amino acid residues mentioned above. Thus, it can be concluded that if a molecule is showing interactions with these residues then it is more probable that it will be having high activity and more specificity as a potent drug candidate. Secondly, it was observed that the residues like ILE62, GLY63, VAL70, ALA83, LYS85, CYS199, THR 138 and ARG141 were present in interacting environment of, if not all then most of the highly active known inhibitor molecules.
- Using this analysis molecules screened by RASPD and GLIDE from ZINC database, NCI database and those obtained after drug repositioning were analyzed for their interaction pattern. In total 12 molecules; 4 screened from ZINC database, 1 from NCI database and rest 7 from Drug repositioning, were analyzed. Their docking score by Sanjeevni and GLIDE was calculated before and after simulations(as shown in results). Also, fit and interaction as well as interaction pattern was reviewed carefully in each case.
- Out of these, three molecules were selected for further optimization process. These molecules were shortlisted based on interaction pattern with the key residues , docking score (obtained from Sanjeevni, Autodock, Glide and swissdock) and molecule fitting in the binding pocket.

PDBID	Interacting Residues	IC 50 (nm)
1Q3D	Val 135, Asp 133	15
1Q3W	Lys 85, Val 135	4
1Q4L	Gln 185, Val 135, Arg 141, Asp 133	76-160
1Q5K	Val 135, Pro 136	41-33000
1Q41	Val 135, Asp 133	22
1UV5	Val 135, Asp133	5
2O5K	Val 135, Asp 133, Arg 220, Arg 141	15
2OW3	Asp 133, Val 135	3
3DU8	Val 135 Lys 85	146
3F7Z	Val 135	65
3F88	Val 135, Asp 200	2.3
3I4b	Val 135, Lys 85, Asp 200	7
3L1S	Val 135, Asp 133, Asp 200	2
3PUP	Val 135, Asp 133, Gln 185	6
3Q3B	Val 135, Asp 133, Lys 85	17.2
3SDO	Val 135, Asp 133	247

Table 7: List of PDBs ID , IC-50 value and Key interacting residues for GSK3B

- After the molecular dynamics run for 40ns stable trajectories were obtained for all the structures and good average binding energies between -8 kcal/mol to -11kcal/mol were obtain for all the structures for the 100 snapshoots as shown in results.

2. DPP4 AND PTP1B INHIBITORS

- The same structure analysis studies as carried out for GSk3B was done for DPP4 and PTP1B.

PDB –ID	IC-50 Values	Interaction given by Ligplot
3Q0T	Not Available	Glu205,Glu206,Tyr662,
4A5S	17 nM	Glu205,Glu206,tyr631,tyr662
2RGU	1 nM	Glu205,tyr631,tyr662
2HHA	122 nM	Gln553,Arg125,Asn710,Tyr662
3O9V	18 nM	Glu205,Glu206,tyr662,tyr547,ser630
3OPM	240 nM	Glu205,Glu206,Tyr662,Trp629
3CCB	30000 nM	Glu205,Glu206,Tyr662
3CCC	8 nM	Glu205,His126,Tyr547,Ser630,Asn689
3HAB	4.2 nM	Glu205,His126,Tyr547,Ser630,Asn690
3O95	5.3 nM	Glu205,His126,Tyr547,Ser630,Asn691
3SWW	4 nM	Glu205,His126,Tyr547,Ser630,Asn692
3SX4	3 nM	Glu205,His126,Tyr547,Ser630,Asn693
2OQV	4 nM	Glu205,His126,Tyr547,Ser630,Asn694
2OAG	3.4 nM	Glu205,His126,Tyr547,Ser630,Asn695
3NOX	2.2 nM	Glu205,His126,Tyr547,Ser630,Asn696
2OQI	3.2 nM	Glu205,His126,Tyr547,Ser630,Asn697
4DSA	Not Available	Glu205,His126,Tyr547,Ser630,Asn698
4DSZ	Not Available	Glu205,His126,Tyr547,Ser630,Asn699
4DTC	Not Available	Glu205,His126,Tyr547,Ser630,Asn700
2OGZ	84 nM	Glu205,His126,Tyr547,Ser630,Asn701
2I78	Not Available	Glu205,His126,Tyr547,Ser630,Asn702
2ONC	13 nM	Glu205,His126,Tyr547,Ser630,Asn703
3G0C	2000 nM	Glu205,His126,Tyr547,Ser630,Asn704
3G0D	5 nM	Glu205,His126,Tyr547,Ser630,Asn705
3G0G	5 nM	Glu205,His126,Tyr547,Ser630,Asn706
1PFQ	Not Available	Glu205,His126,Tyr547,Ser630,Asn707
2AJL	13 nM	Glu205,His126,Tyr547,Ser630,Asn708
2G5P	3.8 nM	Glu205,His126,Tyr547,Ser630,Asn709
2G5T	0.82 nM	Glu205,His126,Tyr547,Ser630,Asn710

Table 8: List of PDBs ID , IC-50 value and Key interacting residues for DPP4

PDB-ID	IC-50 Value	Interaction given by Ligplot
1T48	350000nM	Asn193, Trp290, Leu192, Glu270, Phe 280.
1T49	22000nM	Glu276, Asn193, Phe280,Phe196.
1T4J	8000nM	Glu 276, Asn 193, Leu192

Table 9: List of PDB Ids, IC- 50 values and Interaction pattern of already known inhibitors.

- In case of DPP4 inhibitors Glu205, Glu206, Tyr662, Tyr631, Asn710, Arg125, Tyr752, Try547 were found to be key interacting residues and these residues were also common in all the already known good binders. These residues either forms hydrogen bonding with inhibitor or were contribute to hydrophobic interactions. Using this analysis and docking score finally two molecules were selected from Zinc database. These molecules have good binding energies in range of -8 to -10 kcal /mol and were fitting well in the binding pocket. Further MD simulation stable trajectories, conformed these molecules as good binder for DPP4 active site as shown in results.
- In case of PTP1B inhibitors good interactions were obtained with ASN`193, GLU`276, LYS`279, GLU`200. Although there is no good binder available for PTP1b allosteric site but it is mention in literature, molecule forming hydrogen bonding with Asn193 and occupying site of trp291 block the interactions that are important for closure (active form) of PTP1B. In case of our inhibitors the selected 2 molecules are interacting with Asn193 and are fitting well to occupy site for Trp291 as shown in results. These molecules also show stable trajectories in MD run for 40 ns and hence prove to be good inhibitors for PTP1B allosteric site.

CONCLUSION AND FUTURE PROSPECTIVE.

In this Project we have designed inhibitors for DPP4 Gsk3b and PTP1B. The latter two macromolecules are involved in insulin signalling pathway and are negative regulator of insulin. DPP4 plays important role in diabetes as it regulates activity of GLP-1 (glucagon-like peptide-1) and GIP (gastric inhibitory peptide) by degrading amino-terminal of these hormones. These hormone acts through their respective G-protein-coupled receptors on b-cells to potentiate glucose-stimulated insulin secretion. Therefore blocking activity of G3k3b, PTP1B and DPP4 may results in treatment for diabetes.

- In our study 3 molecules for G3K3B; 2 molecules for DPP4 and PTP1B were designed. These molecules are screened out on basics of whether they are interacting with key residues important for inhibition. The average binding score for these molecules falls in range -7kcal/mol to -9 kcal/mol and stable trajectories are obtained for all the inhibitory molecules.
- Therefore we conclude that molecules reported in this project are good inhibitors for their reported targets and holds potential to act like candidate drugs molecules.

Future Prospective.

- In case of Gsk3b, designing an inhibitory molecule that would block both ATP binding site and Substrate binding site.
- The reported potentials inhibitors for all the targets can be subjected for their activity analysis in cells using in –vitro studies in order to calculate there IC-50 values.
- Designing multi-Target inhibitor which would block the activity of key targets like Gsk3b, PTP1B ,SHIP2 and DPP4 for Diabetes.

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