

Full Length Research Paper

Epigenetic regulation of PGC1 α in human type 2 diabetes

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Type 2 diabetes mellitus (T2DM) is the most common metabolic disease in the world, reaching epidemic proportions. PPARGC1A mRNA expression is reduced in islets from patients with diabetes 2 and it is influenced by both genetic and epigenetic factor. The epigenetic modification, results as a two fold increase in DNA methylation of the PGC1A promoter of diabetes. This two fold increase in DNA methylation is due to the DNA methyl transferase1 (DNMT1) enzyme. In the present study, methylation activity of DNMT 1 enzyme was inhibited with suitable methylation inhibitors which led to the decrease of the hypermethylation of PGC 1 alpha protein. The interaction study of this modified DNMT1 with PGC 1 alpha leads to the new way of drug discovery in type 2 diabetes.

Key words: Type 2 diabetes mellitus (T2DM), methyl transferase1 (DNMT1), epigenetic modification.

INTRODUCTION

Epigenetics is the study of inherited changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence. Epigenetic modifications, specifically like DNA methylation and histone modification are the key events regulating the process of normal human development (Reik et al., 2001). Type 2 diabetes (T2DM) is characterised by chronic hyperglycaemia as a result of impaired pancreatic beta cell functions and insulin resistance in peripheral tissues that is skeletal muscle, adipose tissue and liver (Yahli et al., 2005). The transcriptional coactivator peroxisome proliferator activated receptor gamma coactivator-1 alpha (protein PGC-1 α ; gene PPARGC1A) is an important factor regulating the expression of genes for oxidative phosphorylation and ATP production in target tissues through coactivation of nuclear receptors (Ling et al., 2008). It was previously shown that the expression of PPARGC1A and a set of genes involved in oxidative phosphorylation are reduced in skeletal muscle from patients with type 2 diabetes. Furthermore, a common

polymorphism, Gly482Ser, in the PPARGC1A gene has been associated with increased risk of type 2 diabetes and an age-related reduction in muscle PPARGC1A expression (Yunhua et al., 2003). Obesity, reduced physical activity and ageing are well known risk factors for type 2 diabetes (T2DM) (Puigserver and Spiegelman, 2003). However, all individuals exposed to an affluent environment do not develop the disease. One likely reason is that genetic variation modifies individual susceptibility to the environment. However, the environment could also modify genetic risk factors by influencing expression of a gene by DNA methylation or histone modifications. Cytosine residues occurring in CG dinucleotides are targets for DNA methylation and gene expression is usually reduced when DNA methylation takes place at a promoter. Whether DNA methylation influences gene expression in target tissues for type 2 diabetes (T2DM) and thereby the pathogenesis of the disease remains to be demonstrated (Puigserver et al., 2003).

Sequencing method is used to determine the pattern of methylation. Treatment of DNA with bisulfate (salt) converts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected. Thus, bisulfite treatment introduces specific changes in the DNA sequence that depend on the methylation status of individual cytosine residues, yielding single-nucleotide resolution information

Abbreviations: PGC1A, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A); DNMT1, DNA methyl transferase 1; T2DM, type 2 diabetes.

about the methylation status of a segment of DNA. Various analyses can be performed on the altered sequence to retrieve this information (Long-Cheng and Rajvir, 2002).

The present study investigated: (1) DNA methylation analysis of PGC1 α ; (2) the modification of the methylation activity of DNMT by methylation inhibitors and (3) the inhibition of the hypermethylation of PGC1 α by modified DNMT1 (protein-protein interaction study).

MATERIALS AND METHODS

Protein Structure

The structure of the enzyme DNMT1 and the PGC 1 α protein was analyzed by SAVS server. The results are shown in Figure 1a and b.

DNA methylation analysis

DNA methylation involves the addition of a methyl group to the 5TH position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring. The process takes place in the so called CpG islands, located in the promoter of the eukaryotic genes. The methylation analysis was done using MethPrimer software. MethPrimer software uses bisulfite sequencing method to determine the pattern of methylation. Treatment of DNA with bisulfate (salt) converts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected. Thus, bisulfite treatment introduces specific changes in the DNA sequence that depend on the methylation status of individual cytosine residues, yielding single-nucleotide resolution information about the methylation status of a segment of DNA. Various analyses can be performed on the altered sequence to retrieve this information (Long-Cheng and Rajvir., 2002); the sequence of the gene was pasted which is methylated, the job was submitted, and the results were gotten as original sequences in the first row and the bisulfate sequences in the next row and the CpG sites are marked as ++ and the unmethylated cytosine residue was converted as uracil, and the methylated cytosine remained as same (<http://www.urogene.org/methprimer/index1.html>).

Modification of methylation activity of DNA methyl transferase1 (DNMT1)

Inhibition of DNMT1 was done, because it is responsible for the hyper methylation of PGC1 α (it transfers the methyl group to the cytosine residue) which causes the type 2 diabetes (T2DM). The inhibitor selection for this DNMT1 was based on the literature search and in that some of the inhibitor compounds are in the phase 1 and phase 2 trial (Goffin and Eisenhauer, 2002). The molecular properties of these inhibitors were analyzed using various softwares. Inhibition was done with the help of docking tools (Schrödinger and autodock). This docking was first done using Autodock and the top six compounds were selected as the input for Schrödinger docking (Akio et al., 2009). Compounds selected for inhibition of DNMT1 were: analogs of azacitidine; hydralazine analogs; decitabine analogs; procainamide analogs and zebularine analogs

Inhibition of DNMT1 using Schrödinger

Glide searches for favorable interactions between one or more

typically small ligand molecules and a typically larger receptor molecule usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule: a protein and a cofactor. GLIDE can be run in rigid or flexible docking modes; the later automatically generates conformation for each input ligand. The combination of positions and orientation of the ligand relative to the receptor, along with its conformation in flexible docking which is referred to as a ligand pose. The ligand poses that GLIDE generates pass through a series of hierarchical filters that evaluate the ligand interaction with the receptor.

Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA nonbonded ligand-receptor interaction energy. Final scoring is then carried out on the energy-minimized poses. Schrödinger's proprietary GLIDE Score multi ligand scoring function is used to score the poses. If Glide Score was selected as the scoring function, a composite Emodel score is then used to rank the poses of each ligand and to select the poses to report to the user. Emodel combines Glide Score non-bonded interaction energy, and for flexible docking, the excess internal energy of the generated energy conformation.

Inhibition of PGC1 Alpha with modified DNMT1

From Protein Data Bank, PDB files were taken. DNMT1 PDB id is 3PT9, length of the protein is 873 residues, resolution is 2.5 Å. PGC 1 α PDB id is 1XB7, length of the protein is 247 and the resolution is 2.5 Å. Based on the resolution and the SAVS server result the PDB files were taken. The Protein - Protein docking was done using ClusPro server.

RESULTS

DNA methylation Analysis

The CpG site was identified from the MethPrimer software. Results were observed. Original sequences in the first row and the bisulfate sequences in the next row and the CpG sites are marked as ++ and the unmethylated cytosine residue was converted as uracil, and the methylated cytosine remained as same; from that we can find the methylated and the unmethylated molecules (Figure 2).

Inhibition using Autodock

The best ligand structures with their binding energy and the interaction of these inhibitors obtained from autodock is shown in Table 1. The best 2 ligand structure and its interaction with the target protein are shown in Figures 3 and 4.

Inhibition using Schrödinger Glide

Glide offers the full range of speed vs. accuracy options, from the high-throughput virtual screening (HTVS) mode for efficiently enriching million compound libraries, to the standard precision (SP) mode for reliably docking tens to hundreds of thousands of ligand with high accuracy, to

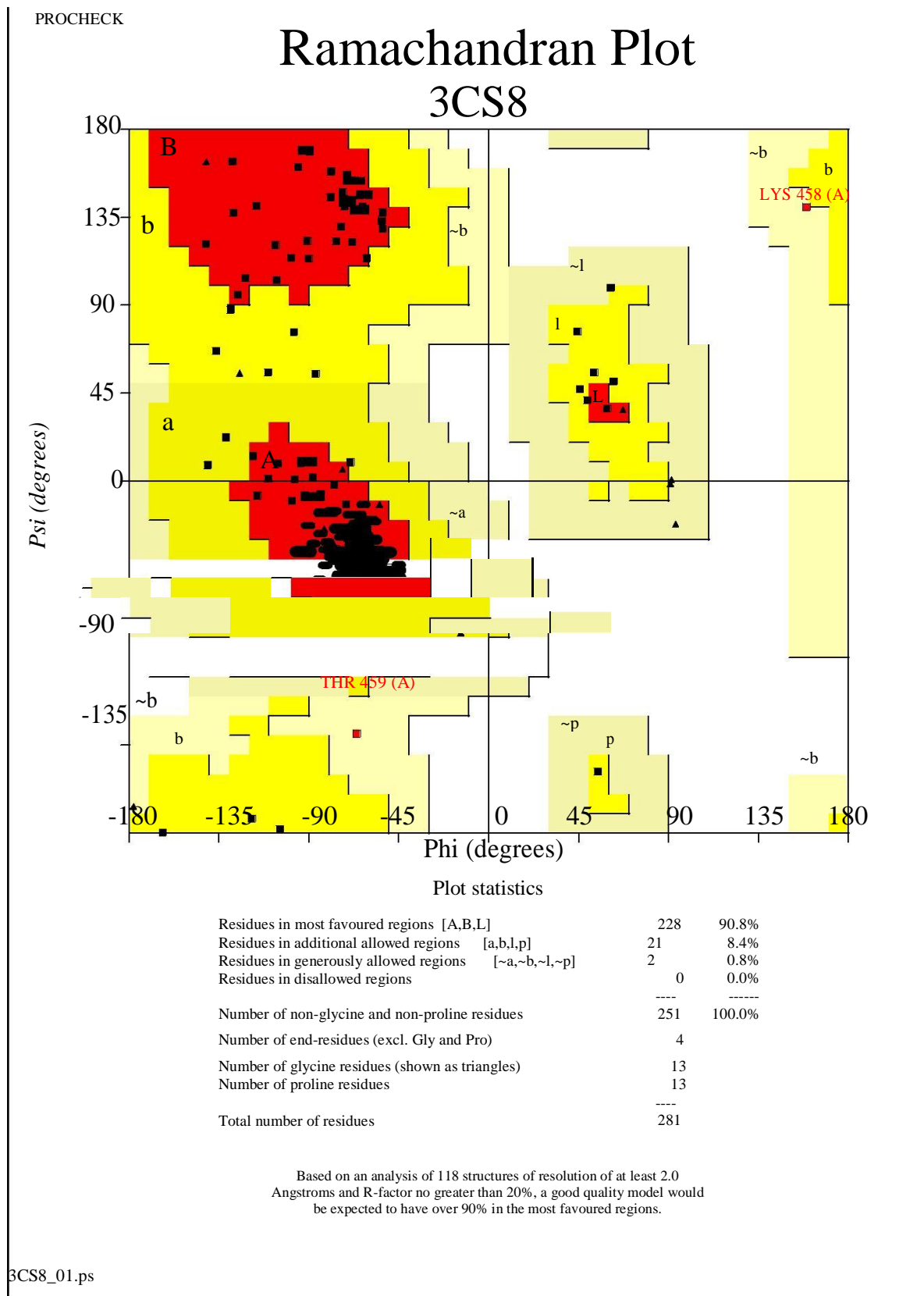


Figure 1 a. PGC1 Alpha. Resolution=2.4; length =275; secondary structure: 62% helical (14 helices; 172 residues); 4% beta sheet (4 strands; 12 residues); active site residues, CYS 285, TYR 473, SER 225, **THR 229**.

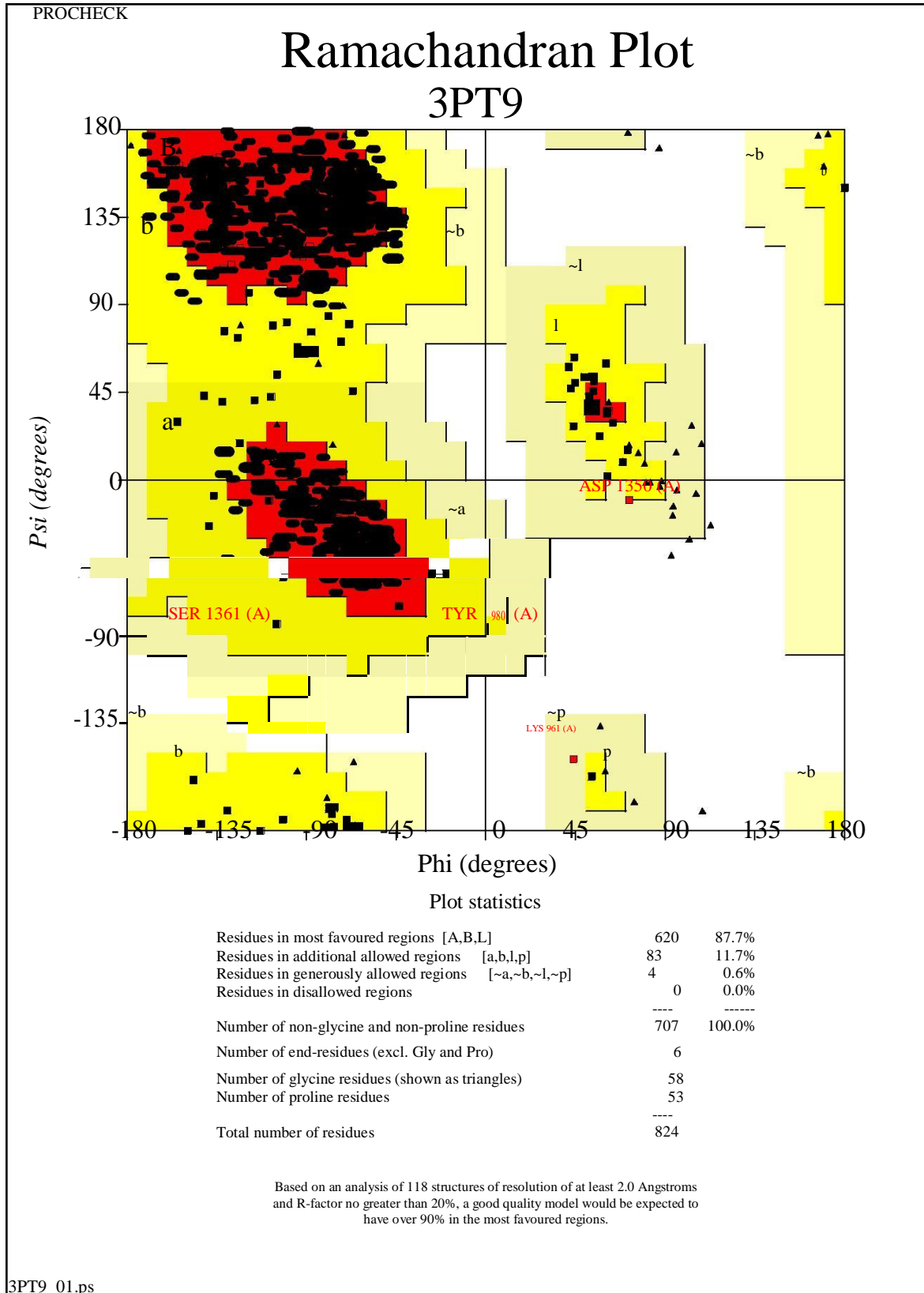
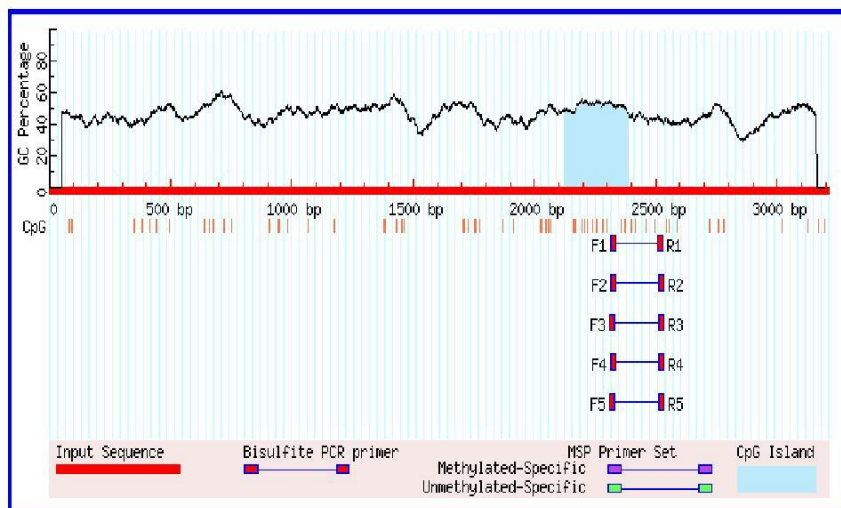


Figure 1b. DNMT1 Resolution =2.5 Å; length = 873 residues; secondary structure 21% helical (30 helices; 188 residues); 25% beta sheet (52 strands; 220 residues); active site residues ARG1187, ARG1181, VAL1189, GLN1212, **SER A1149, GLY A 1150, LEU1189.**

MethPrimer - Design Primers for Methylation PCRs

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MethPrimer result



MethPrimer - Design Primers for Methylation PCRs

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3061 TGTGTGCTTGGTTTAGGGGAAGTATGTGGGTACATGTGAGGACTGGGGGCACCTGACC
:||||:||||:||||:||||:||||:||||:||||:||||:||||:||||:||||:||||:||||:
3061 TGTGTGTTTGGTTTAGGGGAAGTATGTGGGTATATGTGAGGATTTGGGGTATTTGATT

3121 AGAATGCACAAGGGCAAACCAATTTCAAATGGCAGCAGTCCATGAAGACACGCTTAARAC
:||||:++:||||:||||:||||:||||:||||:||||:||||:||||:||||:||||:++:||||:
3121 AGAATGCCTAAGGGTAAATTAATTTAAATGGTAGTACTTTTATGAAGATACGTTTAAAT

3181 CTAGAACITTCARAATGTTTCGTATTCTATTTC
:||||:||||:||||:++:||||:||||:
3181 TTAGAAATTTTAAATGTTTCGTATTTTATTT
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*-----*
* Explanations
*-----*
* Upper row: Original sequence
* Lower row: Bisulfite modified sequence
* (For display, assume all CpG sites are methylated)
* ++ CpG sites
* ::: Non-CpG 'C' converted to 'T'
* >>>>> Left primer
* <<<<<< Right primer
*-----*
```

Figure 2. MethPrimer Output.

the extra precision (XP) mode where further elimination of false positives is accomplished by more extensive sampling and advanced scoring, resulting in even higher enrichment. XP does more extensive sampling than SP. This SP and XP uses rigid docking only; only the ligands structure got moved and docked to protein in different conformations.

The top six ligand molecules obtained from the auto-dock result was given as the input to the glide package and the XP and SP docking was done; the docking score and the glide energy were noted.

Induced fit docking

The active site geometry of a protein complex depends heavily upon conformational changes induced by the bound ligand. The Induced Fit protocol begins by docking the active ligand with Glide. In order to generate a diverse ensemble of ligand poses, the procedure uses reduced van der Waals radii and an increased Coulomb-vdW cut-off, and can temporarily remove highly flexible side chains during the docking step. For each pose, a Prime structure prediction is then used to accommodate

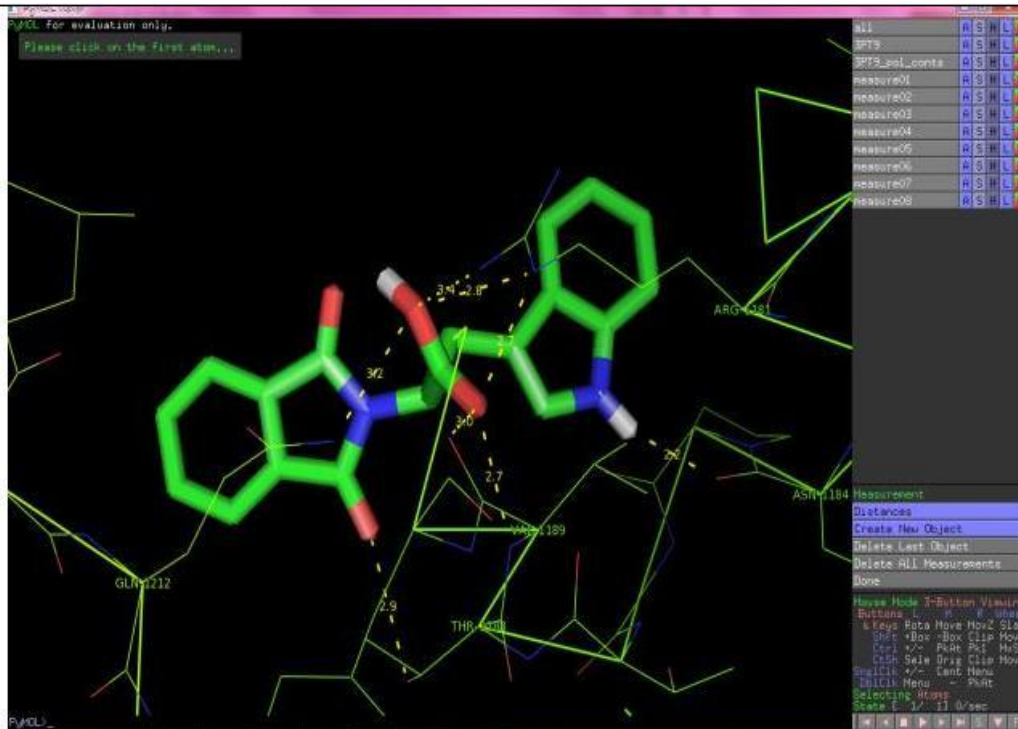


Figure 3. SGS 107 ligand interaction with DNMT1. The best ligand structure which shows the binding energy of **-6.16** with their interaction was viewed using PyMol viewer and the interaction showed are (ARG 1187) O-H...O=2.8, (ARG 1181) N-H...O=2.7, (ARG 1181) N-H...O=3.4, (VAL 1189) N-H...O=3.0, (GLN 1212) N-H...O=3.2, (GLN 1212) N-H...O=3.4, (THR 1188) O-H...O=3.3.

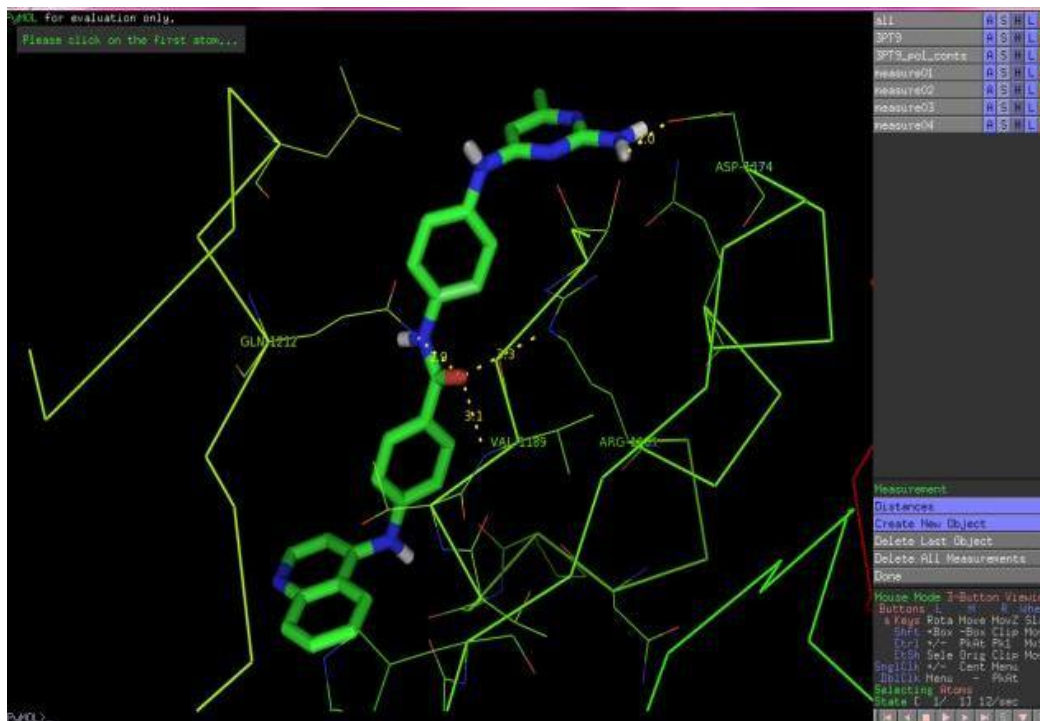


Figure 4. Zebularine ligand interaction with DNMT1. The best ligand structure which shows the binding energy of **-5.73** with their interaction was viewed using PyMol viewer. And the interaction shows are (GLN 1212) N-H...O=2.9 (VAL 1189) N-H...O=3.1, (ARG 1181) N-H...O=3.3.

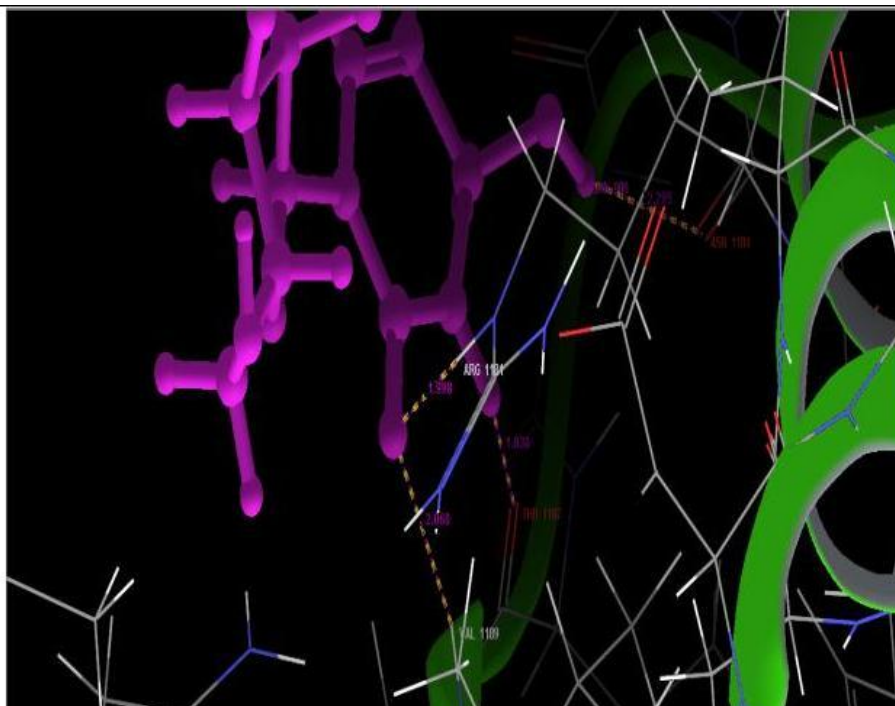


Figure 5. Schrödinger result .The best compound selected from the Schrödinger result was the zebularine molecule and it had the binding energy of -32.8633. The ligand structure shows the interaction of (LEU1189) O-H...O=3.4, (ARG1181) N-H...O=2.9, (GLN1212) N-H...O=3.2, and shows the docking Score of -5.1374.

the ligand by reorienting nearby side chains. These residues and the ligand are then minimized. Finally, each ligand is re-docked into its corresponding low energy protein structures and the resulting complexes are ranked according to GlideScore. Accuracy is ensured by Glide's superior scoring function and Prime's advanced conformational refinement. Simply, we can call it as flexible docking. Both ligand and the protein molecule will move and docked in different conformations. The screened compounds were selected and the induced fit docking was done, and the results are shown below (Figure 5).

Protein-protein docking

Macromolecular docking is the computational modelling of the quaternary structure of complexes formed by two or more interacting biological macromolecules. Protein - protein complexes are the most commonly attempted targets of such modeling. So this protein - protein docking or interaction has the effect to change one protein's function or structure or some of the properties. Based on this technique, the docked DNMT1 protein gets again docked with the PGC 1 alpha protein which may change the hypermethylation process of PGC 1 alpha Protein (Marcotte et al., 1999).

So this protein-protein interaction work was carried out by ClusPro Server.

ClusPro Server

The user can input the PDB codes of the crystal structures of their choice in the receptor and ligand fields, as well as any chain identifiers that they would like to use. Once the PDB files have been uploaded to the server, they are processed into the input files necessary for DOT or ZDOCK, as well as CHARMM minimized for 100 steps with a constrained backbone. The minimized PDBs are then imported to a supercomputer for the running of DOT/ZDOCK, filtering, and clustering. The ClusPro output is shown in the Figure 6.

DISCUSSION

Epigenetics is the study of inherited changes that occur other than DNA sequences level which means the changes does not occur in the sequence. Our lifestyles, food habit, and the environment all these factor make these changes. The epigenetic factors are DNA methylation and histone modification. The major disease caused by this factor is cancer and type 2 diabetes. The DNA hyper methylation of PGC1 alpha protein is responsible for causing the type 2 diabetes (T2DM). This hyper methylation is due to DNMT1 enzymes. Inhibiting the activity of DNMT1 leads to the inhibition of hyper methylation.

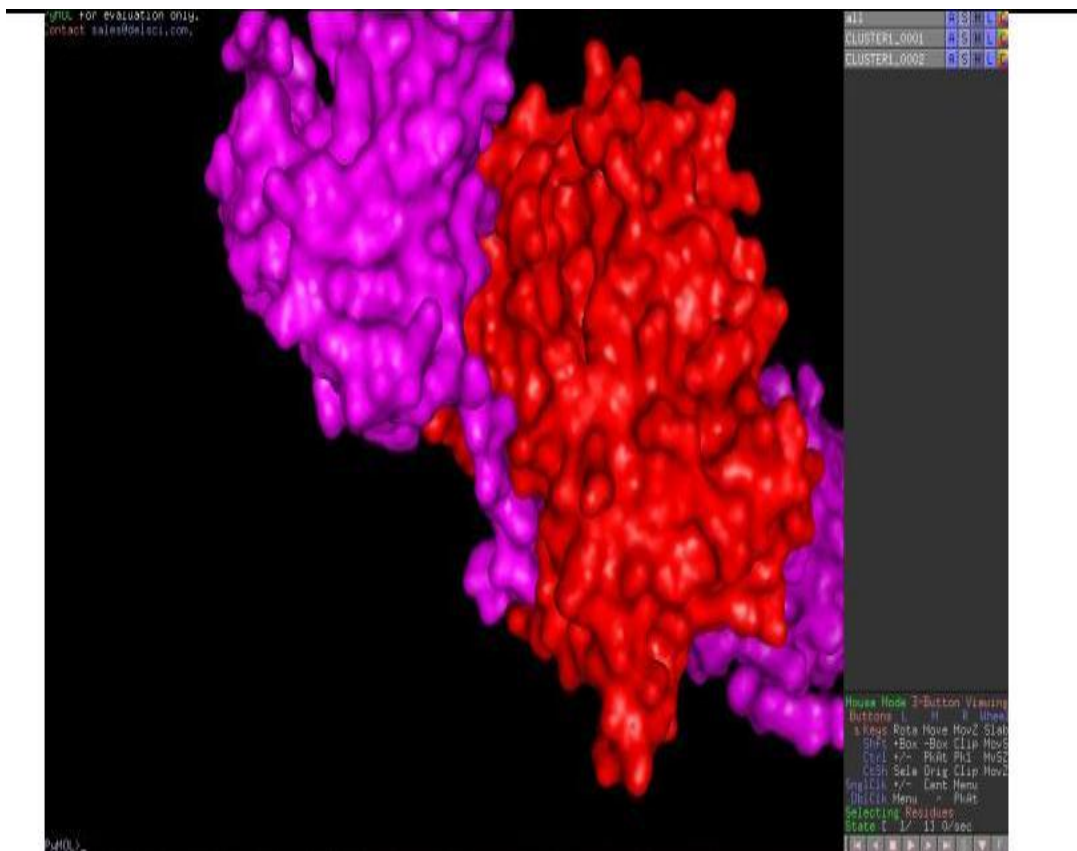


Figure 6. Modified DNMT1 docked with PGC 1 alpha. The two proteins had the interaction with the residues of ARG1187, ARG1181, VAL1189, GLN1212, SER A1149, and SER 225, PHE 226, THR 229, GLY 258, ARG 280. Magenta color = DNMT1; Red = PGC 1 alpha. The ligand molecules are showing the interaction with the active site residues and it has the binding region as in the methyltransferase binding domain.

The inhibition was done by docking methods.

The selection of protein for docking studies is based upon several factors like, it should contain a co-crystal ligand, structure should be determined by X-ray diffraction, and resolution between 2.0-2.5 Angstroms, and out of the 10 entries of DNMT1, 3PT9 was taken for docking analysis (based on the Ramachandran plot statistics) as it showed 620 most favored regions, 83 in additionally allowed region and none of the residue in disallowed regions. The 17 small molecules are obtained by the literature search, and its structure is drawn using chemsketch and its properties are analyzed using Molinspiration tool. Based on the docking results that are based on docking score and interaction, the best inhibitor molecule was found out and it has the binding score more than the native PDB ligand. The ligand molecules show the interaction with the active site residues and it has the binding region as in the methyltransferase binding domain. This domain is responsible for catalyzing the reaction of C-5 cytosine methylation in DNA to produce C5-methylcytosine. So inhibition of this DNMT1 has the ability to decrease the hypermethylation of proteins. The docked DNMT1 protein can inhibit the

methylation property of PGC 1 alpha. Protein - Protein docking was done between docked DNMT1 and PGC 1 alpha protein, which has the interacting region of ARG1187, ARG1181, VAL1189, GLN1212, SER 1149, and SER 225, PHE 226, THR 229, GLY 258, ARG 280; has the same active site residues as inhibited DNMT1. So, we can clearly say that the probability of inhibiting the activity of PGC 1 alpha protein is more in this Protein-protein docking. This approach is used to find the new way of drug discovery for type 2 diabetes.

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