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Insilico Search for Potential DNA Binding Domain of Human Zinc Finger Protein Sp1

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ABSTRACT

Specificity protein 1 (Sp1) belongs to a family of ubiquitously expressed, C2H2-type zinc finger-containing DNA binding proteins that activate or repress transcription of many genes in response to physiological and pathological stimuli. Specificity protein 1 is considered to be a constitutively expressed transcription factor and has been implicated in the regulation of a wide variety of housekeeping genes, tissue-specific genes, and genes involved in the regulation of growth. In order to determine the binding affinity of Sp1 zinc finger domains, the total energy for each and every possible combination of GC box and Zn finger motifs using Hex server, Model IT software's is calculated. According to the findings of this study, the design of multi-zinc finger proteins with a variety of sequence specificities will be easier to accomplish. Among the three motifs present in Specificity protein 1, motifs 1 and 2 have higher binding affinity than motif 3.

Keywords : GC-box, Zinc finger motifs, SP1, Transcriptional regulation.

I. INTRODUCTION

Transcription factor Sp1 was the first identified member of the Sp/XKLF (Specificity protein/Kruppel-like factor) family. Sp1 protein comprises several domains of which the DNA binding domain is the most conserved among Sp family. The DNA binding domain of Sp1 consists of three contiguous Cys2 His2 Zinc (Zn) fingers and mutational analysis has revealed that Zn fingers 2 and 3 are essential for Sp1 DNA binding activity. Sp1 binds GC-rich elements that are common regulatory elements in promoters of numerous genes (Pore N et al.,2004). Sp1 binds individual Sp1 binding sites as a multimer and is capable of synergic activation on promoters containing multiple binding sites. Sp1 regulates transcription by dynamically recruiting and forming complexes with many factors associated with transcription. Although Sp1 has been described as a transcriptional activator it can also act as a repressor. Activation or repression of transcription by Sp1 depends on the promoter it binds to and on the co-

regulators, it interacts (Chuang JY, Hung JJ 2011). Sp1 had been implicated in the expression of numerous genes involved in many aspects of cellular life such as metabolism, cell growth, differentiation, angiogenesis and apoptosis regulation. This protein is involved in many cellular processes, including cell differentiation, cell growth, apoptosis, immune responses, response to DNA damage, and chromatin remodelling. Post-translational modifications such as phosphorylation, acetylation, glycosylation, and proteolytic processing significantly affect the activity of this protein, which can be an activator or a repressor. The carboxyl terminus of transcription factor Sp1 contains three contiguous Cys2-His2 zinc finger domains with the consensus sequence Cys-X2–4-Cys-X12-His-X3-His.

1) 1.1What is Sp1?

The transcription factor Sp1 (specificity protein 1) belongs to the family of Sp/KLF (Krüppel-like factor) transcription factors. It is the first transcription factor purified and cloned from mammalian cells (Dynan WS, Tjian R,1983). The human Sp1 gene maps to 12q13.1 and encodes a protein of 785 amino acids. The protein contains an N-terminal transactivation domain, which recruits the basal transcriptional machinery complex to the target promoter, and a C-terminal DNA binding domain, which contains three Cys_2His_2-type zinc finger DNA binding motifs required for recognizing GC-rich (GGGGCGGGG) promoter sequences (Wierstra I;2008). Sp1 regulates thousands of genes, such as those encoding vascular endothelial growth factor (VEGF), $p21^{CIP1/WAF1}$, 12(*S*)-lipoxygenase, phosphatase 2A (PP2A), and Sp1 itself. Thus, Sp1 is important for a variety of physiological processes, including angiogenesis, cell cycle progression, inflammation, and senescence (Liu YW; et al., 1997) Dysregulation of Sp1 is observed in many cancers and neurodegenerative disorders. Sp1 gene knockout is embryonic lethal at the 11^{th} day of gestation (Marin M, et al.,1997). It is involved in many processes such as cell growth, apoptosis and immune responses (Yan GZ, Ziff EB 1997). Its role can be switched between that of activator and repressor by processes such as acetylation. The DNA binding domain of Sp1 consists of three contiguous Cys2 His2 Zinc (Zn) fingers and mutational analysis has revealed that Zn fingers 2 and 3 are essential for Sp1 DNA binding activity(Fig 1).

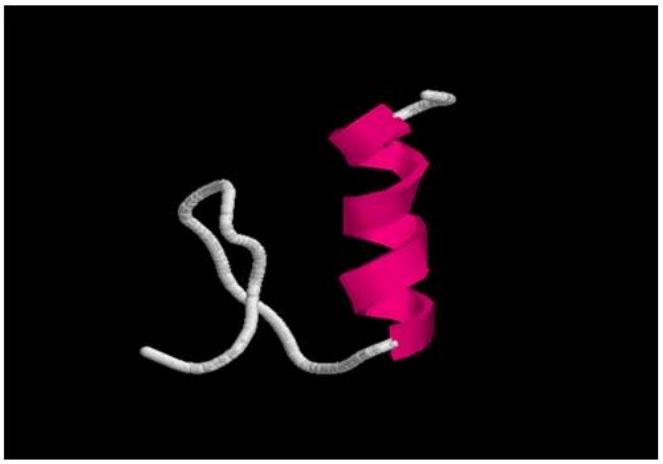

Fig 1: Cartoon structure of SP1

1.2 Zinc Finger Domain:

Zinc-finger domain is a portion of the protein that contains a zinc atom non-covalently bound to certain amino acids. In the case of sp1, the zinc atom is coordinated by 2 Cys and 2 His residues. There are 3 zinc-finger domains in this protein. The general transcription factor Sp1 has a DNA-binding domain that consists of 3 zinc fingers. The C-terminal part of each finger forms alpha-helices that bind DNA; the N-terminal part form beta-sheets. The non-conserved amino acids in the C-terminal side of each finger are responsible for recognizing specific target sites. The C-terminal zinc-finger-3 in transcription factor Sp1 contributes more than the N-terminal zinc-finger-1 in determining Sp1's DNA binding capacity. C2H2 zinc finger motifs are one of the most common DNA-binding motifs found in all eukaryotes. They have a tandemly repeated structure consisting of independent modules with the consensus sequence (Tyr, Phe)-X-Cys-X2,4-Cys-X3,Phe-X5,LeuX2-His-X3–5-His-X2–6(Fig 2).

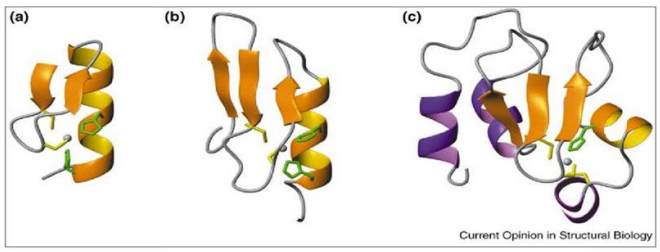

Fig 2:Various Zinc finger motifs representation

A zinc ion tetrahedral binds to invariant cysteines and histidines in each finger domain, forming a compact globular structure. Each finger domain also binds to a three-base pair subsite containing amino acid residues at critical positions in the -helix (Pavletich,N.P. and Pabo,C.O. (1991). The phage display strategy (Reber,E.J. and Pabo,C.O. 1994; Jamieson,A.C et al; 1995) has been used to design zinc fingers with a variety of sequence specificities that are based on the characteristic DNA binding mode. Furthermore, it is anticipated that the multiple connections of the designed zinc fingers will lead to the construction of zinc finger proteins with specificities for any DNA sequence, regardless of its length or base composition, as previously stated(Choo,Y. and Klug,A. (1994)). By examining the selectivity of the first zinc finger for sequences outside the target site, it is possible to gain useful information for the design of such zinc fingers, which can be used to guide the selection of an appropriate second finger (Choo,Y. and Klug,A. (1994).

1.3 GC Box:

GC box is a distinct pattern of nucleotides found in the promoter region of some eukaryotic genes upstream of the TATA box and approximately 110 bases upstream from the transcription initiation site. It has a consensus sequence GGGCGG which is position dependent and orientation independent. The GC elements are bound by transcription factors and have similar functions to enhancers. The SP1 transcription factor contains a zinc finger protein motif, by which it binds directly to DNA and enhances gene transcription. Its zinc fingers are of the Cys_2/His_2 type and bind the consensus sequence 5'-(G/T)GGGCGG(G/A)(G/A)(C/T)-3' (GC box element).

II. Methods

2.1 NCBI : (http://www.ncbi.nlm.nih.gov)

NCBI has had responsibility for making available the GenBank DNA sequence database. NCBI has grown to provide other databases in addition to GenBank. NCBI provides Gene, Online Mendelian Inheritance in Man, the Molecular Modeling Database (3D protein structures), dbSNP (a database of single-nucleotide polymorphisms), the Reference Sequence Collection, a map of the human genome, and a taxonomy browser, and coordinates with the National Cancer Institute to provide the Cancer Genome Anatomy Project. The NCBI assigns a unique identifier (taxonomy ID number) to each species of organism. The amino acid sequence of the sp1 transcription factor is retrieved in the FASTA format from the NCBI server. This server contains the sequences of various proteins and genes, which can be used for comparison and analysis purposes. It also has several tools for the same. In the NCBI homepage, the protein Sp1 is searched against all the databases in NCBI through the 'search' function. From the results, the human sp1 protein is chosen. From the 'download file' option in the sidebar, the amino acid sequence of the protein is retrieved and saved in FASTA format for easy access in further steps. It may also be stored in 'txt' format in case the sequence has to be copied and pasted separately in any other step.

2.2 Scan prosite: http://prosite.expasy.org/scanprosite/

PROSITE is a protein database.[1][2] It consists of entries describing the protein families, domains and functional sites as well as amino acid patterns and profiles in them. PROSITE's uses include identifying possible functions of newly discovered proteins and analysis of known proteins for previously undetermined activity. Properties from well-studied genes can be propagated to biologically related organisms, and for different or poorly known

genes biochemical functions can be predicted from similarities. PROSITE offers tools for protein sequence analysis and motif detection (see sequence motif, PROSITE patterns). It is part of the ExPASy proteomics analysis servers .ScanProsite is an online tool which is used to find specific kind of motifs in an input amino acid sequence by comparing with an in-built library of motif sequences. Here, it is used to find the zinc finger motifs in the sp1 protein's amino acid sequence. After scanning, three such motifs are found in sp1.On entering the ScanProsite tool page, the amino acid sequence is pasted in the search area, and then the 'Start the Scan' button is clicked, which starts the searching process. Even the accession number can be used here instead of pasting the entire sequence. Upon completion, a page displays the different motifs that are present in the input protein sequence.

2.3 DNA binder:

(http://www.imtech.res.in/raghava/dnabinder/submit.html)

DNA binder is a webserver developed for predicting DNA-binding proteins from their amino acid sequence using various compositional features of proteins. The SVM models have been developed on 3 datasets using protein features. SVM(Support Vector Machines) are supervised learning models with associated learning algorithms that analyze data and recognize patterns, used for classification and regression analysis DNA binder allows user to submit more than one sequence for predicting DNA-binding proteins using composition based SVM model (amino acid composition of proteins).In case of PSSM based SVM model (developed using evolutionary information in form of PSSM profile obtained from PSI-BLAST search)server allows to predict one sequence at a time. If user submit more than one sequence, only first sequence will be considered for prediction.

Steps followed in DNA binder:

1)Input sequence: There are two ways of sequence submission. Directly paste into text-box provided or upload the file by using "BROWSE" option. Sequences must be in FASTA format and single-letter code.

2)Prediction approach: There are two approaches of prediction on the basis of input vector used to train SVM are Amino acid composition and PSSM profile

We have used three different datasets and developed SVM models using amino acid and PSSM as input.

- Main dataset**:** This module was developed using DNA-binding and non-binding protein chains. Hence, this module is best suited if domain sequence is submitted for prediction.
- Realistic dataset**:** This module was developed keeping in mind the real-life situation where ratio of DNA-binding and non-binding protein is 1:10. This means it should be ideal choice if high specificity is desired during prediction.
- Alternate dataset**:** If input sequence is full length protein, then this module should be ideal choice because prediction will be done using SVM modules developed using full length protein sequences.

In the main page of this server, the 'Submit Sequence' option is chosen from a sidebar. On this page, a search box is present into which the sequence can be either pasted, or the stored sequence file uploaded. Then, the search parameters, such as the prediction approach to be used, and realistic dataset are fixed and then the prediction is started by clicking on 'Run Prediction'. The prediction process may take some time.The prediction is run for dif combination of zinc finger motifs and scre is obtained.Then the server will provide SVM score ,based on which we find the binding or non binding property and plot the graph.

2.4 Homology modeling:

http://swissmodel.expasy.org/interactive

Homology modeling, also known as comparative modeling of protein, refers to constructing an atomic-resolution model of the "*target*" protein from

its amino acid sequence and an experimental three-dimensional structure of a related homologous protein (the "*template*"). It is a very accurate method used to deduce the three-dimensional structures of proteins. SWISS-MODEL is a tool that utilizes this method. It is used to get PDB files of various motifs and their combinations. SWISS-MODEL is a fully automated protein structure homology-modelling server, accessible via the ExPASy web server, or from the program Deep View (Swiss Pdb-Viewer). The purpose of this server is to make Protein Modelling accessible to all biochemists and molecular biologists worldwide. This server is used to get pdb files of various motifs and their combinations. On this page, the whole sequence can be pasted in the search box or, if the PDB ID is directly entered in the box, the sequence is retrieved by itself and displayed in the box. Then the button 'Search for Templates' is clicked. After searching, a list of templates is displayed, from which the most appropriate template (depending on the job at hand) is chosen and used for building the three-dimensional model for the input amino acid sequence.From the models built we will choose the appropriate model

2.5 Model it server:

http://hydra.icgeb.trieste.it/dna/model_it.html

This server produces a 3D model of DNA molecule given a sequence of maximum 700 nucleotides. We can select either standard DNA conformations (B-DNA, A-DNA) or bent conformations, predicted by a set of structural parameters. The output is a PDB file, that can be visualized by Swiss PDB viewer, rasmol. Models of bent DN (up to 50 base pairs) can be optimized/refined by energy minimization and molecular dynamics. From this server we will get the PDB file of DNA (GC- box). In the search field located on the main page of this server, the sequence of nucleotides (max. 700 nucleotides) is entered. This sequence would have been retrieved from the NCBI website. Then the modelling parameter, such as NMR or X-ray, is chosen depending on the requirement, and then the button 'Send It' is pressed. Upon completion of the modelling process, a PDB file is generated, which can be downloaded and opened in any visualization software such as RasMol.

2.6 Hex server: http://hexserver.loria.fr/

Hex is an interactive **protein docking** and **molecular superposition** program. **It** understands protein and DNA structures in PDB format, and it can also read small-molecule SDF files. Hex calls the two proteins to be docked the "receptor" and "ligand", respectively. These can be uploaded from PDB files on your PC. The Hex Server removes all water molecules and other "hetero" atoms from the input files. During the main docking calculation, Hex rotates each protein about its own coordinate origin, and varies the separation between the two origins. A score is calculated for each orientation, and the highest-scoring orientations are saved and returned to the user. By using this server, we will get docked files for individual motifs. On the main page, we need to upload the PDB file of the receptor and ligand molecule, whose docking we are studying, into their respective fields. Then, the parameters are to be set such as whether we want to use the CPU for processing the docking or the GPU. After the fields are filled, click the 'next' button. Then we will get docking structures of different motifs and DNA.

2.7 Swiss pdb viewer:

http://www.expasy.org/swissmod

Swiss-PdbViewer (aka DeepView) is an application that provides a user-friendly interface allowing to analyze several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface. In tool bar section open pdb file of docked protein obtained from hex server. Now in graphics window we can visualize loaded molecules, which can be rotated, translated and zoomed. In control panel window, selected active layers can be controlled in visualization. It lets you enable the

display of backbones, side chains, labels, molecular surfaces, and ribbons for each set the colors for the different objects on display. After changing colours, force field energy calculation is done which is available under colour section of tool bar window.

III. RESULTS AND DISCUSSION

From NCBI we retrieved the fasta sequence of sp1 protein. The sequence will be as follows:

>gi|38372901|ref|NP_612482.2| transcription factor Sp1 isoform a [Homo sapiens]

MSDQDHSMDEMTAVVKIEKGVGGNNGGNGNGG
GAFSQARSSSTGSSSSTGGGGQESQPSPLALLAATCS
RIESPNENSNNSQGPSQSGGTGELDLTATQLSQGAN
GWQIISSSSGATPTSKEQSGSSTNGSNGSESSKNRTV
SGGQYVVAAAPNLQNQQVLTGLPGVMPNIQYQV
IPQFQTVDGQQLQFAATGAQVQQDGSGQIQIIPG
ANQQIITNRGSGGNIIAAMPNLLQQAVPLQGLANN
VLSGQTQYVTNVPVALNGNITLLPVNSVSAATLTP
SSQAVTISSSGSQESGSQPVTSGTTISSASLVSSQASSS
SFFTNANSYSTTTTTSNMGIMNFTTSGSSGTNSQGQ
TPQRVSGLQGSDALNIQQNQTSGGSLQAGQQKEG
EQNQQTQQQQILIQPQLVQGGQALQALQAAPLSG
QTFTTQAISQETLQNLQLQAVPNSGPIIIRTPTVGP
NGQVSWQTLQLQNLQVQNPQAQTITLAPMQGVS
LGQTSSSNTTLTPIASAASIPAGTVTVNAAQLSSMP
GLQTINLSALGTSGIQVHPIQGLPLAIANAPGDHGA
QLGLHGAGGDGIHDDTAGGEEGENSPDAQPQAG
RRTRREACTCPYCKDSEGRGSGDPGKKKQHICHIQ
GCGKVYGKTSHLRAHLRWHTGERPFMCTWSYCG
KRFTRSDELQRHKRTHTGEKKFACPECPKRFMRSD
HLSKHIKTHQNKKGGPGVALSVGTLPLDSGAGSEG
SGTATPSALITTNMVAMEAICPEGIARLANSGINV
M QVADLQSINISGNGF

From scan prosite we got different zinc finger domains present on Sp1 protein(Fig 3). Totally 3 zinc finger motifs along with their sequence, length and score are predicted. It is shown as follows(Table 1).

Fig 3: Location of 3 zinc finger motifs n Sp1 using prosite scanner

After finding out the zinc finger motifs, whether they are DNA binding OR non-DNA binding are verified using DNA binder tool. From this we will get the SVM score which give the affinity of binding(Fig 4). The SVM score for different combinations of motifs is calculated. Based on the scores obtained the graph using sigma plot is plotted.

Zinc finger motif and their combinations	SVM score for realistic dataset
1	5.354
2	2.253
3	2.889
1&2	3.885
1&3	4.6022
2&3	2.782
1,2&3	3.810

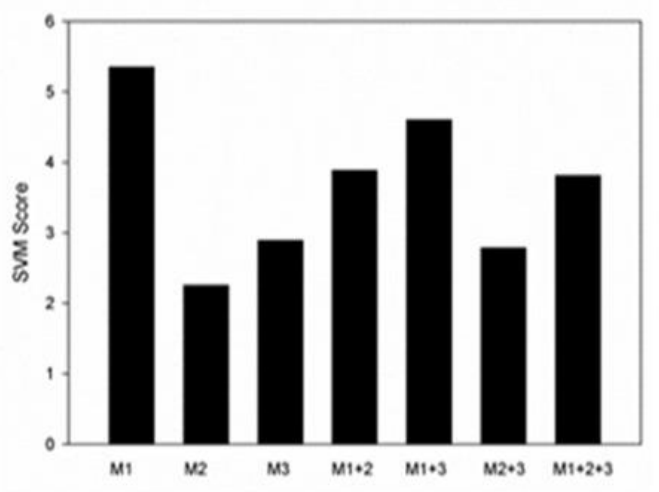

Fig4 : Various zinc finger motifs with their SVM Score with the sigma plot correspondingly.

Now with the help of swiss model we create PDB files for all combinations of motifs. The pdb files are shown in following figures(Fig 5). After creating PDB files for motifs ,we hav to create PDB file for GC –box usind model it server(Fig 6)

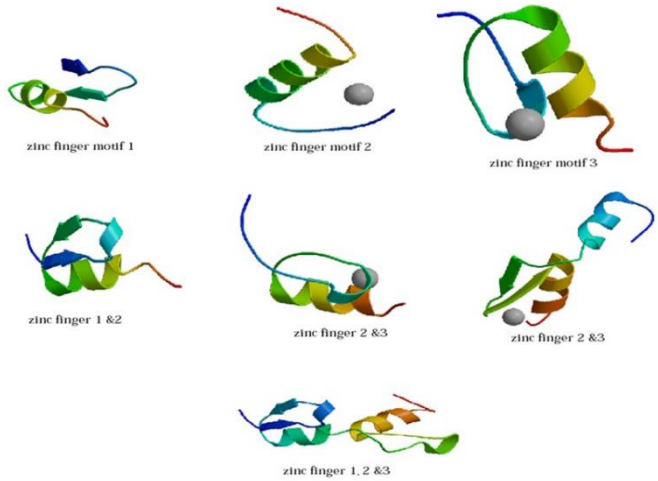

Fig 5:3D structures of receptors obtained from swiss model

After getting the PDB files for motifs and DNA we go for docking by taking ligand as DNA and receptors as different combinations of motifs in Hex online server.Once the docking calculation is complete with default values, you will be forwarded to a results page from which (by default) the best 100 solutions will be made available for download as a compressed PDB file. The first 20 docking orientations are also available as uncompressed individual PDB files. The docking results are as follows(Fig 7).Total energy is calculated for each and every possible combinations of GC box and Zn finger motifs.It has been observed that out of three motifs present in Sp1 motif 1 and 2 shows more binding affinity than motif 3(Table 2).

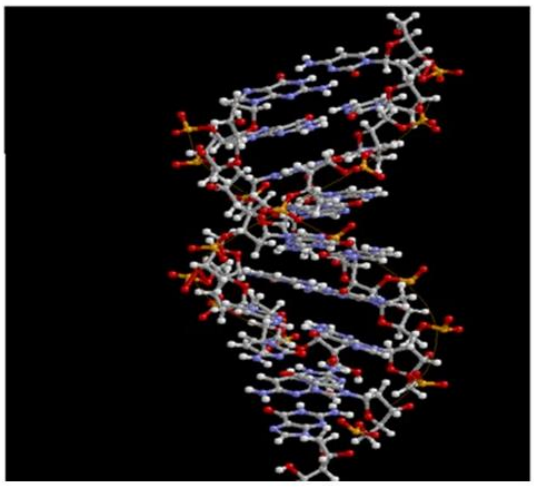

Fig 6:3D structure of GC box obtained from Model-it server

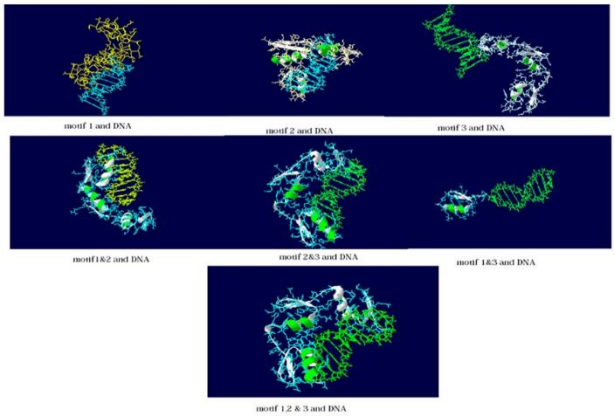

Fig 7 :Docking images of receptor anf ligand reterived from Hex server

Table 1: Totally 3 zinc finger motifs along with their sequence, length and score are predicted

Motif	Motif sequence	Position	Score
Zinc finger motif 1	HICHiqGCGKVYGKTSHLRAHLRWHTGERP	626 - 655	14.087
Zinc finger motif 2	FMCTwsYCGKRFTRSDELQRHKRTHTGEKK	656 - 685	16.082
Zinc finger motif 3	FACPECPKRFMRSDHLSKHIKTHQNKKG	686 - 713	13.131

Table 2: Total energy values of Zinc finger motif binding with GC box.

Zinc finger motifs with DNA (GC box)	Total energy values
M1	-2827.676
M2	-1720.669
M3	-2168.169
M1+M2	-3418.558
M2+M3	-437.843
M1+M3	-1868.679
M1+M2+M3	-2564.256

IV. CONCLUSION

Analysis of binding affinity of Sp1 zinc finger domains has been done and SVM score was calculated. Based on the SVM score homology modelling of Zn finger motifs of Sp1 has been done. Each of the model structure was docked with GC box promoter sequence. Total energy is calculated for each and every possible combinations of GC box and Zn finger motifs.It has been observed that out of three motifs present in Sp1 motif 1 and 2 shows more binding affinity than motif 3. When we calculated the binding affinity motif 1&2 together then we find it shows maximum binding affinity among all possible combinations shown in the result and discussion section. So we have done our work using computational tools to validate our data cloning of different Zn finger protein in bacterial or mammalian system can be done. DNA protein interactions can be studied using various methods like ElectroMobility Assay(EMA), Surface Plasmon Resonance (SPR). Recently, it has been shown by different scientists all over the world that Zn finger proteins and designer Zn finger protein has a role to control the expression of target gene and we have found that there are many reports. which are showing that Zn finger protein is used as recombinant therapeutical molecule. Our Zn finger molecules can also be used as therapeutic molecule if we can manipulate its binding towards DNA or promoter region by using mutational study, we can regulation the gene expression.

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