STRUCTURE VALIDATION AND MUTATION PREDICTION IN LEPTIN RECEPTOR (OB-RB)

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Abstract:

Leptin mediates various cellular processes through the receptor (OB-Rb) located at the cell membrane. Leptin receptor (LR) belongs to the Class 1 cytokine receptor family with four cysteine receptor homology domains (CRP). Development of the three-dimensional structure of LR will make the easier understanding of the atomic interaction of LR with leptin. Hence, in the present study, we aimed to model a three-dimensional structure of OB-Rb's cytokine receptor homologous domain (CRH2) and construct various mutated models to identify potential therapeutic molecules. We analyzed the structure of the CRH2 domain of the LR using SWISS-PORT Database and validated the designs. No significant changes were observed in the mutated forms except a slight structural alternation in the replacement model of cysteine with alanine. The detailed analysis of the molecular mode of Leptin/CRH2 interactions and identifying several residues help to choose selective LR agonistic and antagonist in the therapeutic point of view.

Keywords: Leptin, Leptin receptor, CRH2 domain validation

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1. Introduction:

Leptin, the adipocyte peptide hormone, is a crucial player in energy homeostasis and body weight control, and its structure resembles the 4-α-helical bundle cytokines [1]. The adipocytes secrete leptin in mammals, and its concentration in the blood positively correlates with the mass of the white adipose tissue [2]. In recent years, it has become clear that leptin is a pleiotropic molecule and has direct and diverse effects on different tissues [3]. It also influences many functions such as angiogenesis, hematopoiesis, immune, and inflammatory responses [4, 5, 6, 7]. Leptin shows close structural similarity with IL-6, granulocyte colony-stimulating factor (G-CSF), and other long-chain cytokines such as growth hormones [8].

Leptin binds to a membrane protein, called leptin receptor (LR), encoded by the DB gene. It is a class I cytokine receptor family composed of four cytokine receptor homologous domains (CRH), an Ig-like domain, a transmembrane segment, and a C-terminal cytoplasmic domain in the long isoform [9]. The report indicates that the leptin receptor shows the highest sequence similarity with the receptors of the IL-6 family and with the G-CSFR [8]. The extracellular part of the leptin receptor in humans contains seven structural domains [10]. Domains 1 consists of residues from 62– 178, domain two from 235–328, and a fibronectin type III fold, and together it forms a cytokine receptor homology module (CRH), named CRH1. On the other hand, the third domain consists of 329–427 amino acids and has an immunoglobulin (Ig)-like fold. The fourth and the fifth domains comprised residues from 428–535 and 536–635, respectively, also have a fibronectin type III fold and a second CRH, named CRH2. Domains 6 and 7 adopt a fibronectin type III fold. The presence of an Ig-like domain between the two cytokine receptor modules is again similar to the G-CSF and IL-6 family receptors [8]. Various approaches have explored the identification of CRH2 as the main high-affinity binding site for leptin with the receptor [11]. The Ig-like domain is strictly required for the JAK2 phosphorylation and concomitant STAT3-dependent signaling [9, 12].

The information on the three-dimensional structure of OB - Rb would be an essential parameter in understanding the atomic interactions of leptin with its receptor and the mechanism of the receptor activation. Hence, in this study, we constructed a three-dimensional model of the CRH2 domain of OB - Rb based on its sequence similarity to the four-helix bundle cytokine family. The model of OB - Rb presented here provides a basis for understanding the molecular recognition interactions of leptin by its receptor, particularly by the CRH2 domain, which might involve to a maximum during the leptin receptor activation. The approach also opens up an avenue for designing molecules on therapeutic purpose towards the interruption of leptin binding to its receptor and further activation.

2. Materials and Methods

Structural analysis of the CRH2 domain of the leptin receptor

Sequence retrieval and homology modeling

Sequence retrieval carries out from the SWISS-PROT Database for the target sequence corresponding to the leptin receptor. The selected query sequence consists of a long isoform of the leptin receptor protein of humans, which carries 1165 amino acids (Accession No. P48357.2). Homology modeling was performed using the SWISS-MODEL platform by submitting the target sequence to the automated mode in the SWISS-MODEL workspace. Four types of models with similar homology modeled structures have been obtained. The maximum identity is found in the 2Q7nA LIF (Leukemia Inhibitory Complex) model that consists of 234-630 residues of leptin receptor and is homologous to a fragment from LIF. This particular region corresponding to residues 234-630 of OB - Rb consists of the CRH2 domain and evaluates the ligand-binding affinity with the receptor.

Validation of the structure and the pairwise alignment

The structure of the fragment mentioned above has been validated by using WWW.NIH MBI Laboratory for Structural Genomics and Proteomics software. The homology modeled structure is submitted to find out the secondary structure, Ramachandran plot, and atomic contacts. Next, the homology modeled sequence and the template sequences are submitted to find the conserved region within the target. They are observed by comparing the obtained pairwise results from the CLUSTAL-W program. The highly conserved region has a cysteine at 352 positions in OB- Rb and plays a critical role in stabilizing the structure. The residue is mutated at alanine, glycine, and serine positions, and then the structure is validated to evaluate the impact.

Mutation prediction and structure validation of mutated residues

Prediction of mutation

The mutation prediction is performed by submitting the homology modeled PDB file to the

WHAT IFWeb Interface using the mutation prediction option. The residue is mutated by specifying the amino acid number and mentioning the amino acid name. It provides the mutated structure that can be viewed through RasMol (version 2.7) and can identify the altered regions. The conserved cysteine has mutated to alanine, glycine, and serine, and the mutated structures are viewed and considered for further analysis.

Structure validation of four mutated residues

The mutated structure with cysteine is replaced by alanine, glycine, and serine and validated using the PROCHECK. The secondary structure, Ramachandran plot, and the atomic contacts of the validated structures are analyzed. The methodology was carried as described below. The target protein sequence (Leptin receptor-long isoform) has been retrieved from the SWISS-PROT database and submitted for homology modeling. The modeled structures are validated for their stability, accessibility, atomic contacts, and secondary structure prediction. The pairwise alignment is made by considering the homology modeled sequence with the template sequence. Conserved amino acid, the cysteine, is identified from the pairwise alignment and is replaced by various amino acids such as alanine, glycine, and serine. The mutated structures are validated for their stability, accessibility, atomic contacts, and secondary structure prediction.

3. Results

Structural analysis of leptin receptor (OB-Rb) and its binding to the ligand

The model is constructed by the automated modeling programmer SWISS-MODEL server (http://swissmodel.expasy.org). To obtain a structural framework for the automated modeling, we selected the member of the four-helix bundle cytokine receptor complex, especially the Leukemia Inhibitory Factor (LIF) receptor (domain 1- 5) (2Q7nA), and the structured data obtained in the PDB form. Figure 1.A shows the Rasmol view of the model I, in which three motifs are numbered as 1, 2, and 3 as observed. Detailed analysis of the OB- R has indicated that the CRH2 domain has four cysteine residues. In addition, the cysteine at 352 is highly conserved in all known species and suggests significant recognition for the ligand.

Further, the above model is validated with the PROCHECK program to validate Ramachandran plot and atomic contacts and identify the secondary structure and disulfide bonds. The Ramachandran plot is drawn for the model I (Figure 1.B) shows the maximum number of residues in the allowed region and a very few residues in the disallowed region. Among the total number of 397 residues, 23.3% show an additional permitted region. The favored part is threefold higher (69.3%) than the compared, whereas the allowed region has only two percent of the disallowed region. Figure 1.C1, C2, C3, and C4 represents the residual properties of model I. The model shows the maximum serine residues (44) and 33 numbers for leucine and valine. It also shows five numbers of methionine residues and is the least among identified residues. Among the total identified serine residues, 11 are located in the unfavor regions. Even though both the leucine and valine residues show a maximum at the unflavored region, the leucine exhibits eight residues compared to valine having only 4. There are 30 numbers of proline identified with eight at the unfavor region and match with the leucine in the same region.

The secondary structure of model I shows a maximum of β–strand (34.76%) followed by α-helices (9.07%). The accessible region in the model is found to be less when compared to the buried region. However, the data has to be further refined and reviewed in detail to understand better the function of the presumed ligand-binding domain of the leptin receptor. In all the models, we could find unbound cysteine residues. We observed three cysteines in the CRH2, of which the cysteine located at 352 is identified as the most conserved one. In order to study the importance of the conserved cysteine, the amino acid is replaced by alanine, serine, and glycine. The three-dimensional view of the cysteine replaced models are represented as models 1.A, 1.B, and 1.C, respectively. In addition, the data obtained for all these structures are examined with PROCHECK.

Replacement of cysteine with alanine (Model 1.A)

Model 1.A is obtained by mutating the cysteine into alanine. Figure 2.A represents the Rasmol view of the altered model. The secondary structure of this model shows a maximum of β-strand (32.25%) followed by α-helices (10.58%). The maximum number of turns is obtained (4.28%) in model 1. A when compared to the other mutated models. This model is more similar to the constructed model 1 in the case of accessible and buried regions. The Ramachandran plot (Figure 2.B) did not show any significant difference between model 1 and model 1.A. Similarly, no difference is observed in the properties of all the residues except an increase and decrease in the number of alanine and cysteine residues (Figure 2.C.1, C.2, C.3, and C.4).

Replacement of cysteine with serine (Model 1.B)

Model 1.B is obtained by mutating the cysteine into serine. Figure 3.A represents the Rasmol view of the altered model. Interestingly, it is observed with

a similar increase in β- strand (34.76 %) and a decrease in α-helices (9.07%).

Replacement of cysteine with glycine (Model 1.C)

Model 1.C is obtained by mutating the cysteine into glycine. Figure 4.A represents the Rasmol view of the altered model. In the analysis of amino acid residues seen in the Ramachandran plot (Figure 4.B) after replacing the cysteine residue with glycine, an increase in one unfavoured region in glycine is observed compared to Model 1. In addition, this model shows a secondary structure of 34.51% of β-strand, 11.34% of α-helices, and 3.7 % β–turns (Figure 4.C.1, C.2, C.3, and C.4).

Altogether, the analysis emphasizes no significant changes in the allowed region in the Ramachandran map in all these mutated structures. However, the three-dimensional structure is slightly altered in those models in which the cysteine is replaced with alanine and serine. Nevertheless, no change is observed when the cysteine is replaced with glycine. The ligand-binding region (red) is closer with region 2 in the structure mutated with serine, resulting in steric interaction due to the bulky side chain of alanine/serine.

4. Discussion

The structure of leptin and its receptors are known to conserve among diverse organisms, including humans, mice, horses, dogs, fishes, etc. [3]. However, the functional significance of the conservation among the varying organisms is not clear. Furthermore, leptin receptor has a complex situation in various isoforms such as short, soluble, and long forms and occurs in different tissues [4]. Therefore, understanding the structure and function of the long-form leptin receptor could be crucial in understanding the signaling pathways induced by the adipocyte hormone leptin.

A model has been constructed to understand the leptin binding mode with its receptor using the SWISS-MODEL program with the aid of the structural framework of the LIF receptor domain. The data shows that the CRH2 contains four cysteine residues. The one located at 352 positions is highly conserved and recognized as the vital region for ligand binding and follows the earlier report [3]. Thus, we could also observe four cysteines in the CRH2 binding domain, and the cysteine located at the 352nd position is noted as conserved. To find out the relevance of the conserved cysteine, we mutated the structure by replacing the cysteine with various amino acids such as alanine, serine, and glycine. The Ramachandran maps of mutated forms indicate that a slight change is observed in the 3D structure of the model when the cysteine is replaced by alanine and serine. On the other hand, no difference is observed when the structure is mutated with glycine. Apart from this observation, we are not able to find any significant changes in the mutated structures. Leptin exerts pleiotropic effects while leading various physiological and pathological processes [5, 6, 7]. The diversity of leptin action is believed to be the differential mode of the binding character of leptin with its various forms of receptors located in the plasma membrane [13, 14, 15]. The JAK2 and STAT, especially the STAT3 and STAT5 proteins, are essential for leptin receptor signaling and are highly conserved across vertebrates [16]. Six isoforms of leptin receptors have been generated in mammals by alternate splicing of transcripts from a single leptin receptor gene [17].

5. Conclusion:

In summary, we modeled a structure to detail leptin's molecular, atomic interaction with its long-form receptor and identified several residues that critically contribute to this interaction.

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Conflicts of interest: None of the authors had any conflict of interest that could affect the performance of the work or the interpretation of the data.

6. References

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Figure legends

Figure 1- The Rasmol view, Ramachandran Plot, and Residual properties of the constructed model. The CRH2 domain shows four cysteine residues with highly conserved residue at the 352nd

position. It also shows the maximum number of strands (34.76%).

Figure 2 – The Rasmol view, Ramachandran Plot, and Residual properties of the mutated model 1.A. Replacement of cysteine from the 352nd position by alanine. No significant change is observed except a variation in the number of alanine and cysteine residue compared to the proposed CRH2 domain.

Figure 3 - The Rasmol view, Ramachandran Plot, and Residual properties of the mutated model 1.B. Replacement of cysteine from the 352nd position by serine. No significant changes are observed except an increase in the strands and a decrease in theα helices.

Figure 4 - The Rasmol view, Ramachandran Plot, and Residual properties of the mutated model 1.B. Replacement of cysteine from the 352nd position by glycine. An increase in one unfavoured region is observed.

Figures

Figure 1

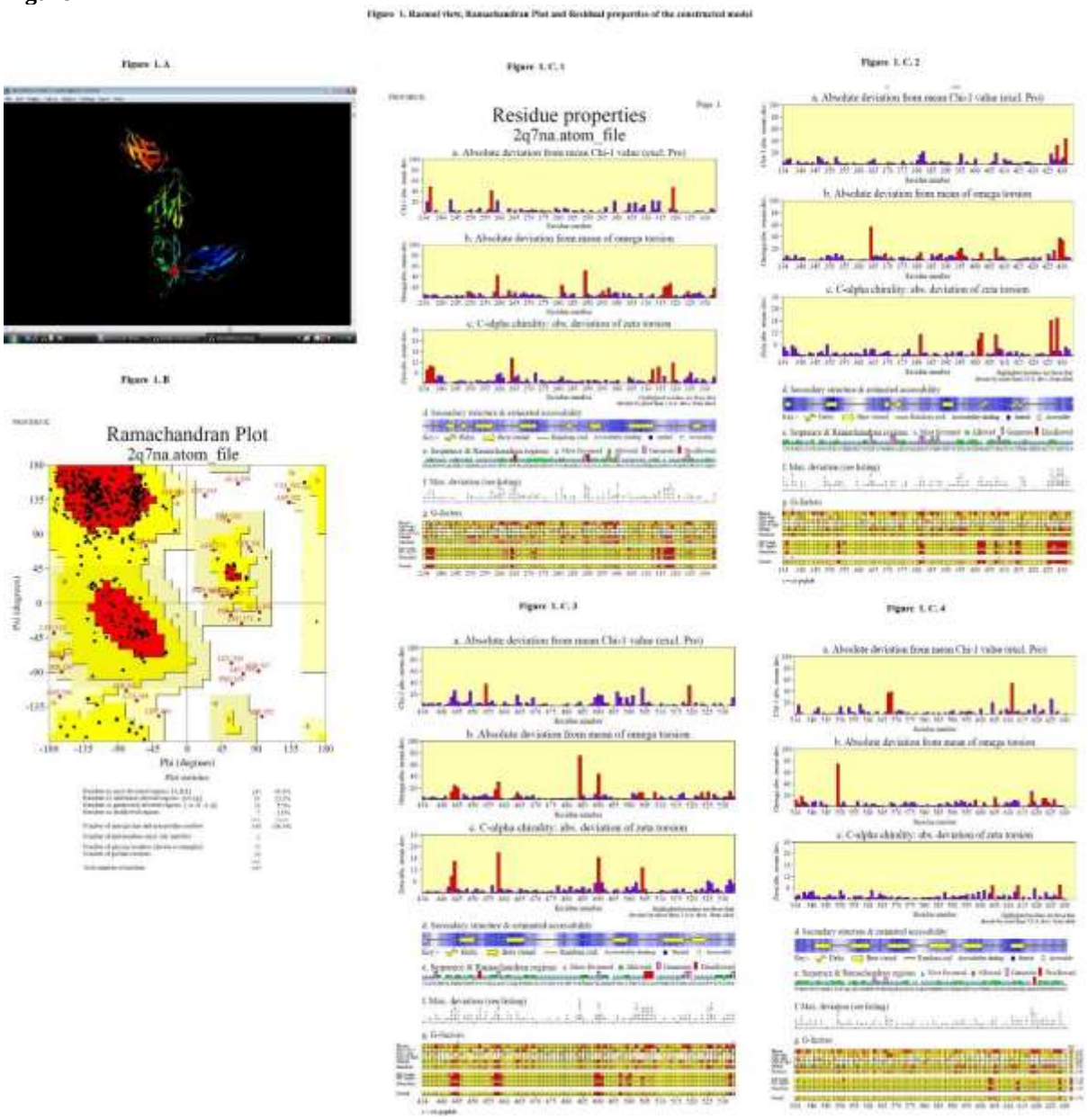

Figure 2

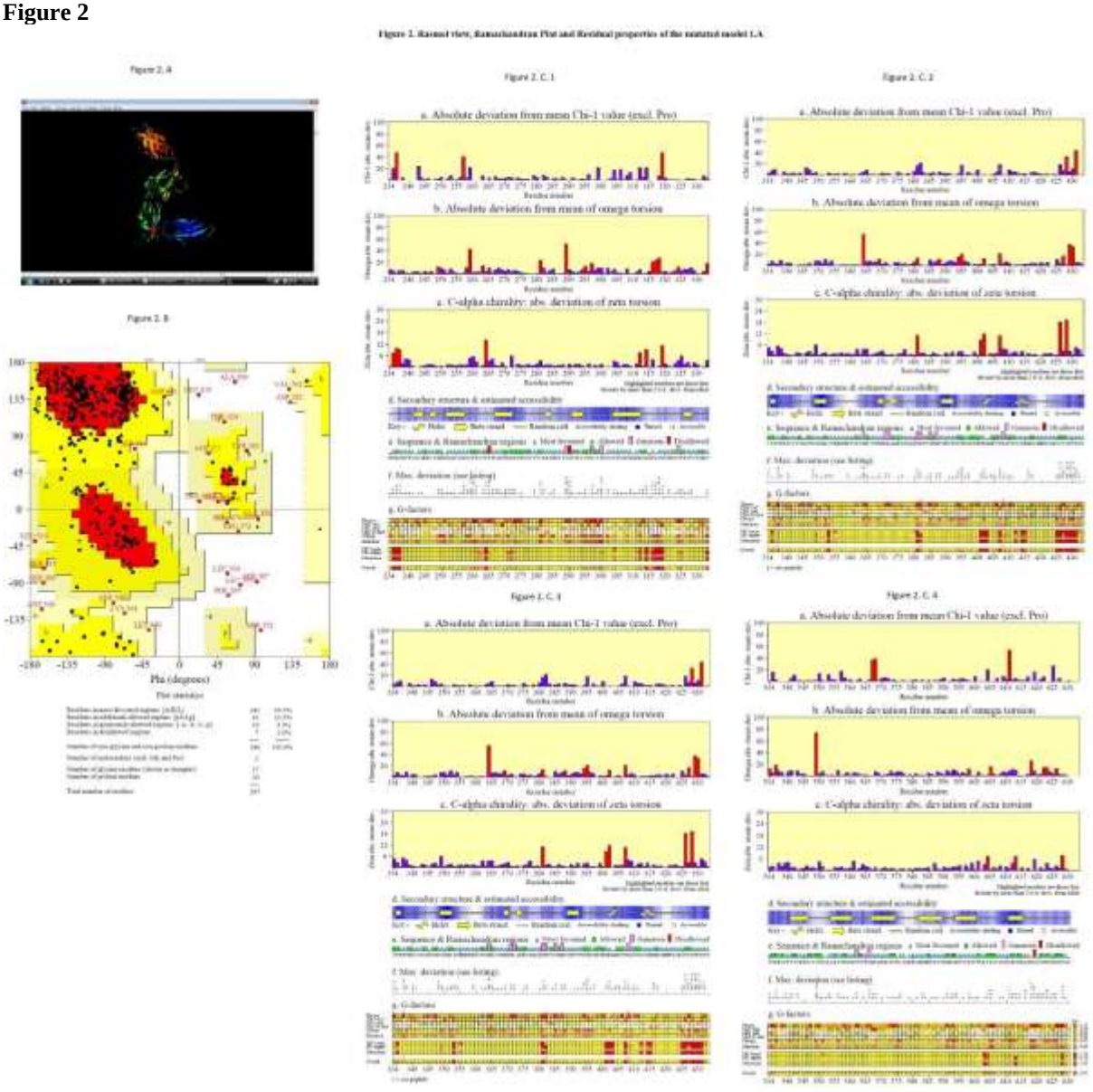
Figure 2. Ramachandran Plot and Residual properties of the mutated model 1.A
Figure 2. A
Figure 2. B
Phi (degrees)
Figure 2. C. 1
a. Absolute deviation from mean Chi-1 value (excl. Pro)
b. Absolute deviation from mean of omega torsion
c. C-alpha chirality: abs. deviation of zeta torsion
Figure 2. C. 2
a. Absolute deviation from mean Chi-1 value (excl. Pro)
b. Absolute deviation from mean of omega torsion
c. C-alpha chirality: abs. deviation of zeta torsion
Figure 2. C. 3
a. Absolute deviation from mean Chi-1 value (excl. Pro)
b. Absolute deviation from mean of omega torsion
c. C-alpha chirality: abs. deviation of zeta torsion
Figure 2. C. 4
a. Absolute deviation from mean Chi-1 value (excl. Pro)
b. Absolute deviation from mean of omega torsion
c. C-alpha chirality: abs. deviation of zeta torsion

Figure 3

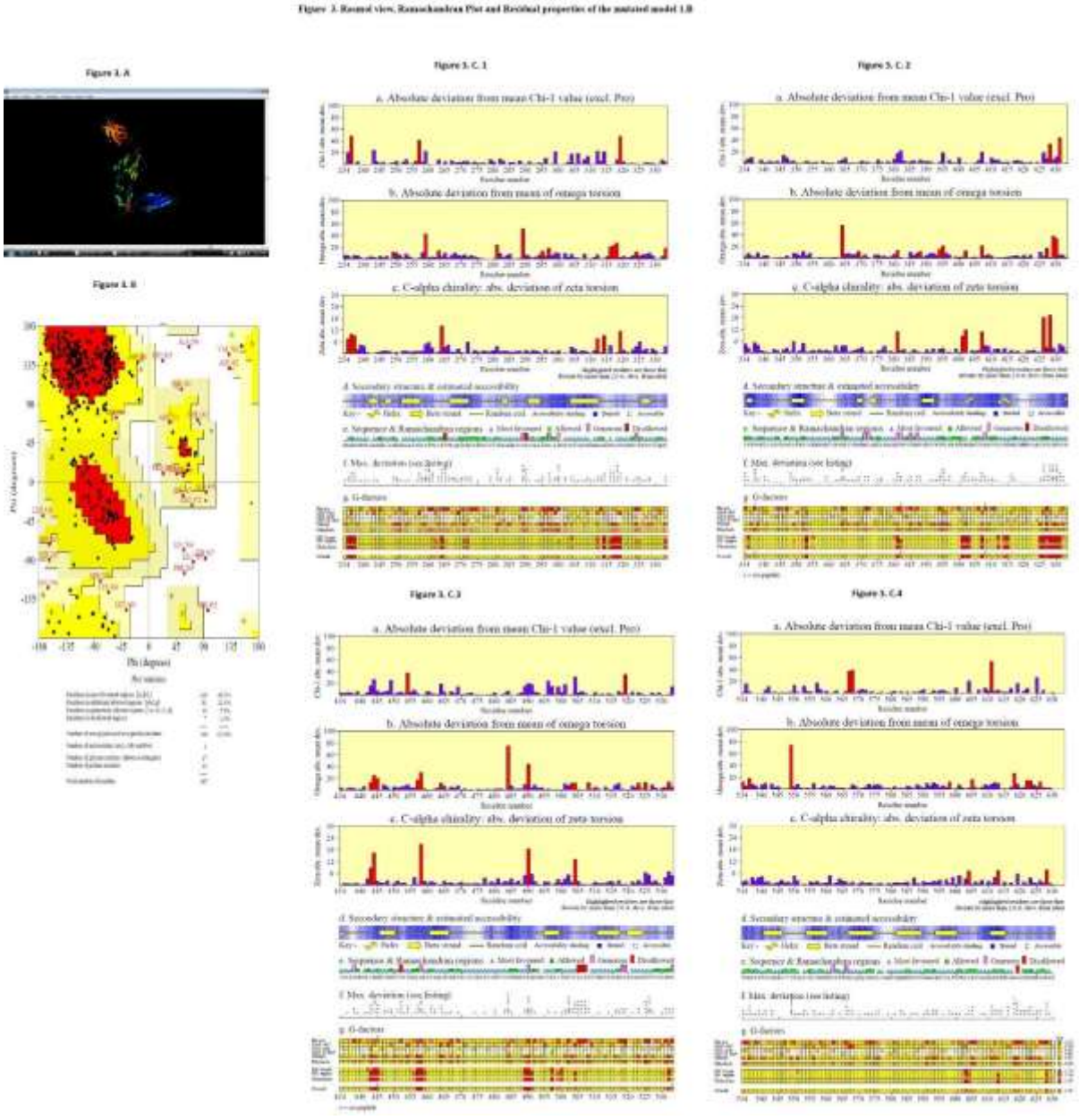

Figure 4