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In Silico Structure Analysis of Type 2 Diabetes Associated Toll Like Receptor-4 (TLR4)

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Abstratct – TLRs are evolutionary conserved Pattern-Recognition Receptors (PRRs) that play a key role in the activation of innate immune response by recognizing highly conserved pathogen-associated molecular patterns (PAMPs), such as the Lipopolysaccharide (LPS) component of gram-negative bacteria. The role of TLR4 genes are associated with type 2 diabetes mellitus in several populations. In this paper modeller was used for homology modelling of human TLR4 (O00206.2). The TLR4 Human Md-2-E.Coli (3FXI) and crystal structure of Mouse TLR4/md-2 (3VQ1) were selected as a template for homology modelling. Ramachandram plot of TLR4 (O00206.2) has 81.2% residue in the most favored region, while template TLR4 (3FXI) has 69.1% and template TLR4 (3VQI) has 77.8% in the most favored region. 3-D structure validation of TLR4 was done by RAMPAGE and SAVES tools. 3D structure of Human TLR4 suggested the active site remains conserved among family members and the major interaction are similar to those observed for the homologous templates.

Keywords: In Silico, Type 2 Diabetes, TLR4, Homology Modelling.

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM), previously known as non-insulin-dependent diabetes (NIDDM), is a complex heterogeneous group of metabolic conditions characterized by increased levels of blood glucose due to impairment in insulin action and/or insulin secretion (Das & Elbein, 2006). Toll-like receptor 4 is a protein (M. wt. 95kDa) that in humans is encoded by the TLR4 gene located on the chromosome no. 9. Polymorphisms in genes that encode proteins related to the innate immune system, such as the toll-like receptors (TLRs), could influence the immune response as well as the development of T2DM (Bagarolli, et. al., 2010).

TLRs are evolutionary conserved Pattern-Recognition Receptors (PRRs) that play a key role in the activation of innate immune response by recognizing highly conserved pathogen-associated molecular patterns (PAMPs), such as the Lipopolysaccharide (LPS) component of gram-negative bacteria (Meylan, et. al., 2006, Arancibia, et. al., 2007). Human orthologs of TLR4 are known to comprise at least 10 members (Zarember & Godowski, 2002). Each TLR family member recognizes a specific pathogen component and upon activation, triggers a signaling cascade leading to the production of inflammatory cytokines, releasing of antimicrobial peptides and activation of the adaptive immune response (Akira, et. al., 2001, Takeda & Akira, 2005).

TLR4 recognizes LPS as a ligand (Takeda & Akira, 2005) and is expressed in macrophages, airway epithelia, adipose tissue, skeletal muscle, pancreas and vascular endothelial and smooth muscle cells (Shi, et. al., 2006). It also interacts with endogenous ligands such as free-fatty acids, heat shock proteins 60 and 70, fibrinogen and fibronectin, which are elevated in T2DM patients (Shi, et. al., 2006, Sasu, et. al., 2001, Smiley, et. al., 2001). Homology modelling refers to the modelling of protein 3D structure using a known experimentally determined structure of homologous protein as a template. Homology modelling provides structural information, that is important to understand of protein function, dynamics, interactions with ligands (Barry, et. al., 1994). In the present study, we used protein homology modelling

(Lima, et. al., 2012) to determine the structure of human TLR4.

MATERIAL METHODS:

The protein sequence of human TLR4 (O00206.2) was retrieved from GeneBank-NCBI (www.ncbi.nlm.nih.gov) in the FASTA format. The sequence Alignment was performed via BlastP against Protein Data Base (PDB) to search the best template for TLR4 protein modelling. Homology modelling of human TLR4 protein was performed by MODELLER using crystal structure of homologous template human TLR4-human MD-2-E.coli LPS Ra complex (3FXI) and crystal structure of homologous template mouse TLR4/md-2/Lipid IVa complex (3VQ1). The PBD file of human TLR4 (O00206.2) was generated by using PyMOL.

Molecular Modelling of TLR4

The SAVES v5.0 server provides a visual analysis of the quality of a putative crystal structure of protein. Verify 3D expects this crystal structure to be submitted in PDB format. RAMPAGE program was used for visualizing and assessing the Ramachandran plot of human TLR4 (O00206.2) and its homologous template. The model was selected on the basis of various factors such as overall G-factor, number of residues in core that fall in generously allowed and disallowed region in Ramachandran plot (figure 1,2,3). The model was further analyzed by QMEAN (Benkert, et. al., 2009, Novotny, et. al., 1998) and ProSA (Wiederstein & Sippl, 2007). ProSA was used for the display of Z-score and energy plots.

RESULTS AND DISCUSSION

Building of protein Model: To build this model, BLAST was done with the maximum E-value allowed for template being 5e-^{62}. Sequence alignment of human TLR4 (O00206.2) protein by using the BLAST revealed sequence homology with TLR4 Human Md-2-E.Coli (3FXI) 100% identity and crystal structure of Mouse TLR4/md-2 (3VQ1) 62% identity, which was selected as template for the model building of TLR4 (O00206.2) protein. Total 604 residues (72% of query sequence) have been modelled with 100% and 62% confidence by the highest scoring template.

Model Reputation: The human TLR4 (O00206.2) model showed good stereo chemical property in terms of overall G-factor value of -0.23. G-factor value indicating that geometry of the model corresponds to the probability conformation with 81.2% residue in the most favoured region of the Ramachandran plot showing high accuracy of model predicted (Prajapat, et. al., 2014). The number of residues in allowed region is 18.1%, generally allowed region 0.5% and disallowed region 0.2%.(figure 1a,1b). A similar approach was also used for homologous templates TLR4 Human Md-2-E.Coli (3FXI) and its Ramachandran plot has 69.1% residue in the most favoured region, 28.9% residue in the allowed region, 2.1% residue in the generally allowed region and none of the residue in the disallowed region. (figure 2a,2b). The homologous templates of Mouse TLR4/md-2 (3VQ1) has Ramachandran plot in which 77.8% residue in the most favoured region, 20.9% residue in the allowed region, 0.7% residue in the generally allowed region and 0.5% residue in the disallowed region (figure 3a,3b).

The Ramachandran plot has 90% residue in most favoured region has a good quality (Morris, et. al., 1992) therefore the target model of TLR4 has consider in good quality model due to 81.2% residue in the most favoured region.

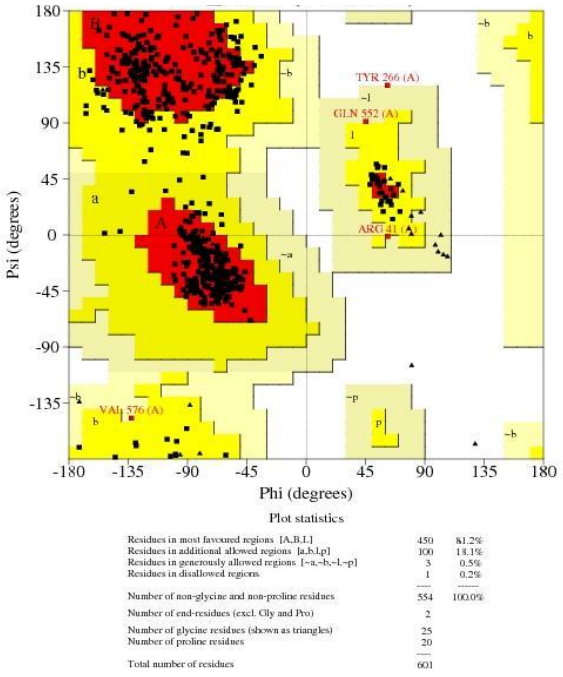


Figure 1a: Ramachandran plot of 3D model of human TLR4 (O00206.2)

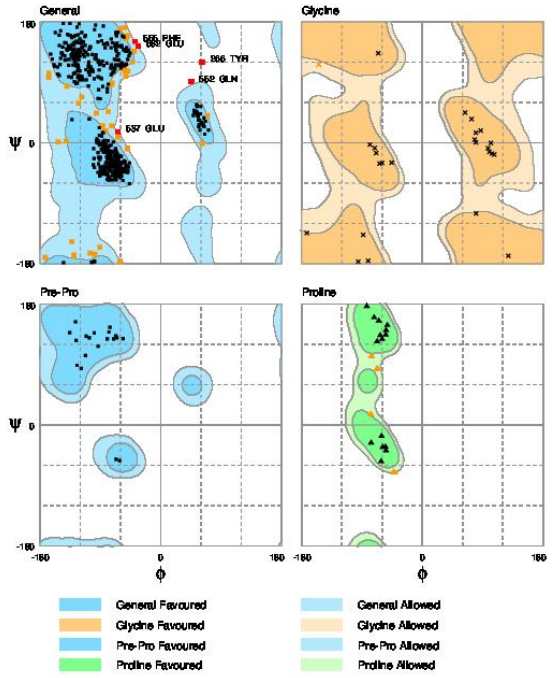


Figure 1b: Non-proline residues and non-glycine residues regions

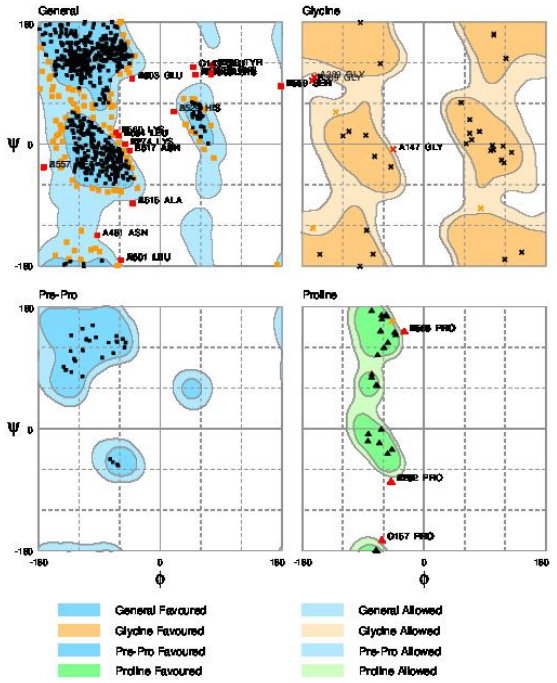


Figure 2b: Non-proline residue and non-glycine residue regions

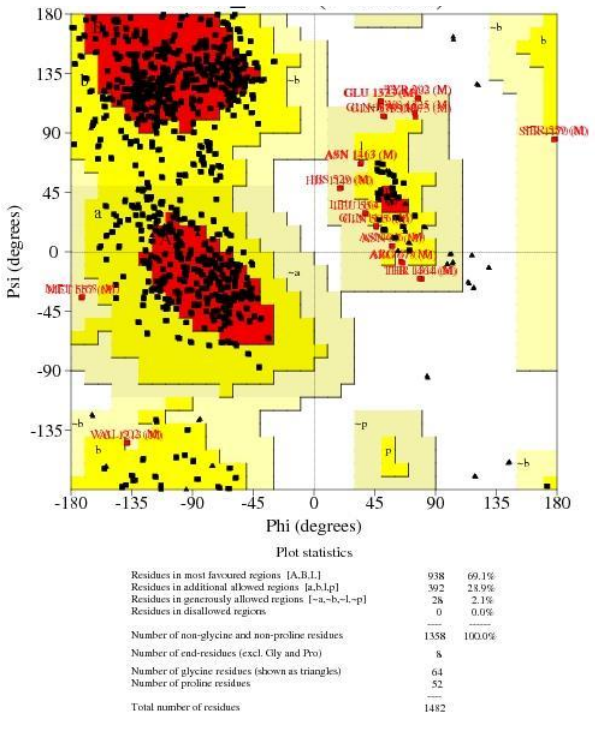


Figure 2a: Ramachandran plot of 3D model of template human TLR4- human MD-2-E.coli LPS Ra complex (3FXI)

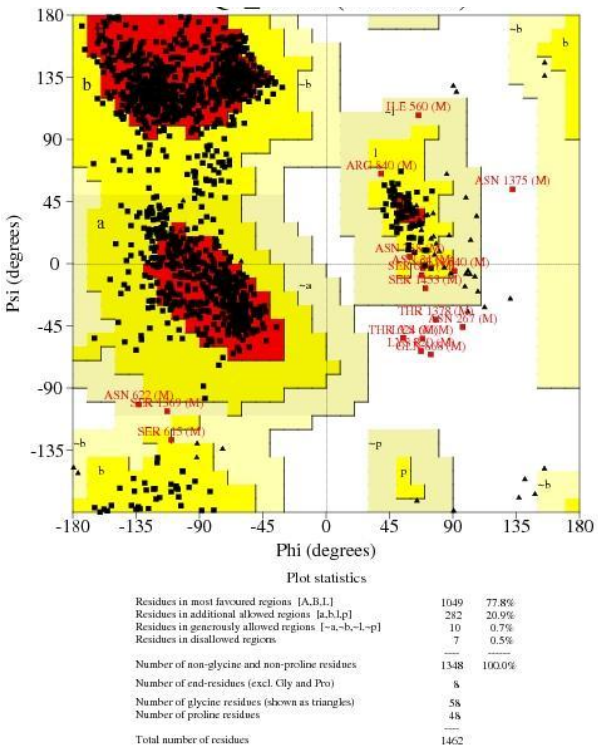


Figure 3a: Ramachandran plot of 3D model of template mouse TLR4/MD- 2/lipid IVa complex (3VQ1)

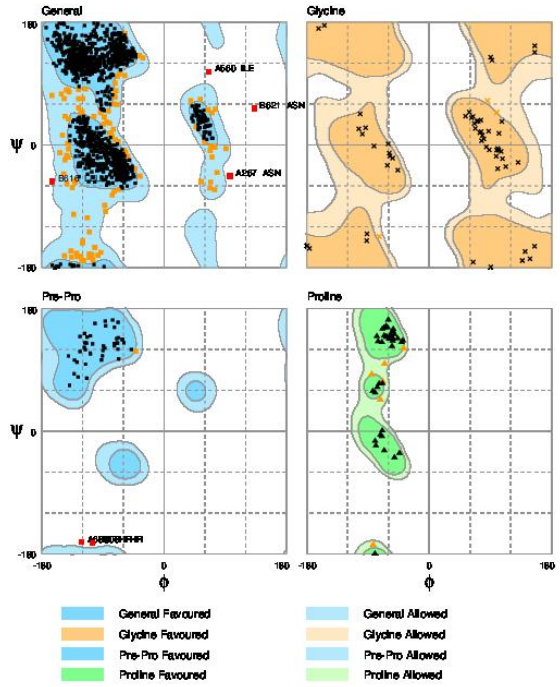


Figure 3b: Non-proline residues and non-glycine residue region

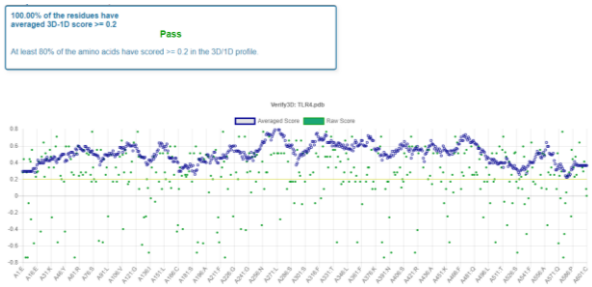


Figure 4: Verified 3D graph of human TLR4 (O00206.2)

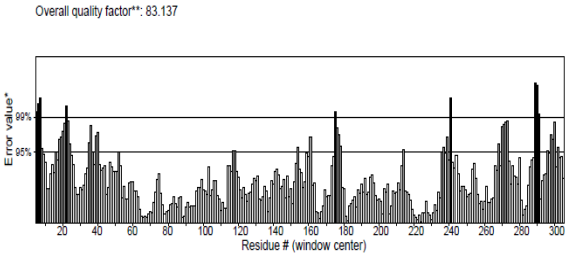
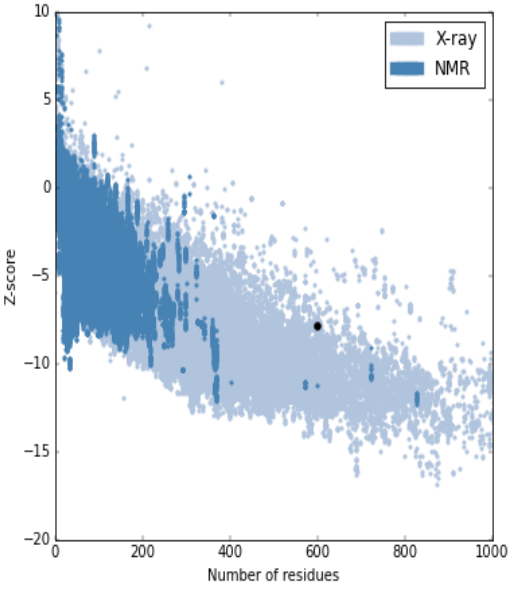


Figure 5: Errat graph of human TLR4 (O00206.2)

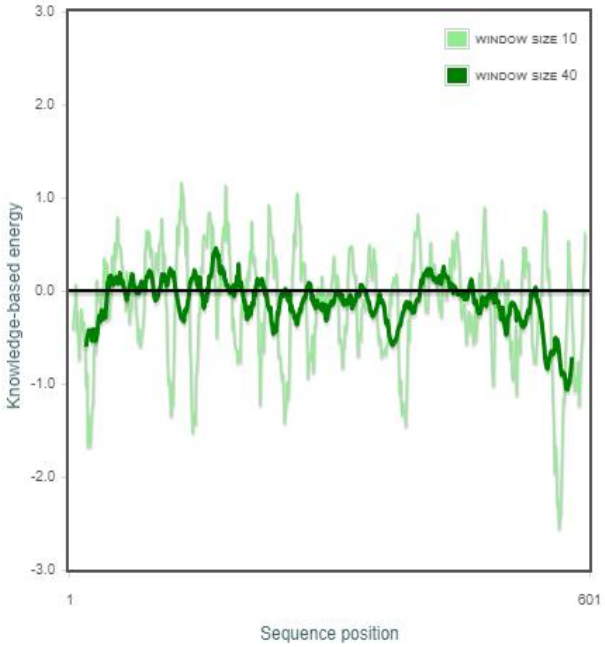
The verified 3D high score 0.81 for human TLR4 (O00206.2) indicate that environmental profile of the model is good (figure 4). In the verified 3D plot, 100% of the residue have an averaged 3D-1D score >=0.2. The profile score above zero in the verified 3D graph is corresponds to an acceptable environment of the model (Bowie, et. al., 1991, Luthy, et. al., 1992)

The Errat is a program used in protein structure determined by crystallography. The two line are draw on the Errat is indicate the confidence with which it is possible to rejection region that exceed the error vaule . Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolutions (2.5 to 3A) the average overall quality factor is around 91%. The overall quality factor for human TLR4 (O00206.2) was 83.137 (figure 5).

Model Validation: ProSA tool was used for the potential errors detection of protein structure based on energy plot and display in three- dimensional manner. ProSA model of human TLR4 (O00206.2), human TLR4- human MD-2-E.coli LPS Ra complex (3FXI) and mouse TLR4/MD- 2/lipid IVa complex (3VQ1) protein for potential errors (figure 6,7and 8). The ProSA web z-scores of protein chains in PDB determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue) with respect to their length. The plot shows only chains with less than 1,000 residues and a z-score of 10.The z-scores of human TLR4 (O00206.2), human TLR4-human MD-2-E.coli LPS Ra complex (3FXI) and mouse TLR4/MD- 2/lipid IVa complex (3VQ1) are highlighted as large dots. The human TLR4 (O00206.2) ProSA Z-score was -7.82 for (Figure 6),for template human TLR4- human MD-2-E.coli LPS Ra complex (3FXI) ProSA Z-score was -7.81 (figure 7) and for template mouse TLR4/MD- 2/lipid IVa complex (3VQ1) ProSA Z-score -7.99 that indicates the overall model quality of target and template (Figure 8) measures the deviation of the total energy of the structure with respect to an energy distribution derived from random conformations (Teilum, et. al., 2005).

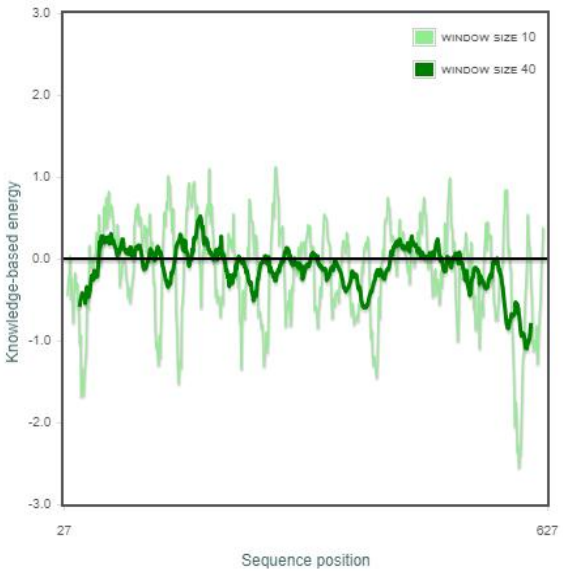


(a)



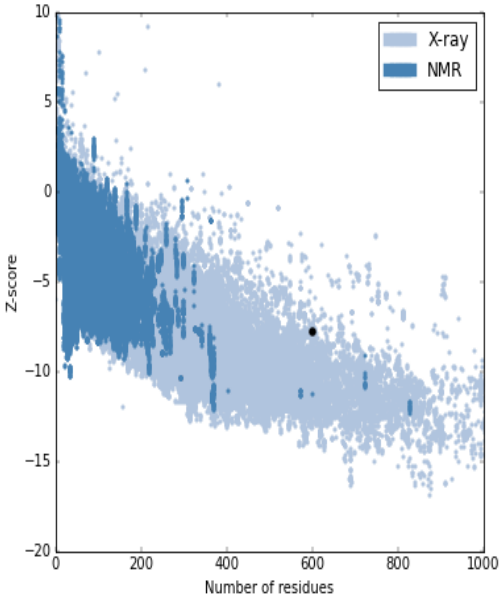
(b)

Figure 6: ProSA web service analysis of human TLR4 (O00206.2). (a) Overall model quality and (b) local model quality.

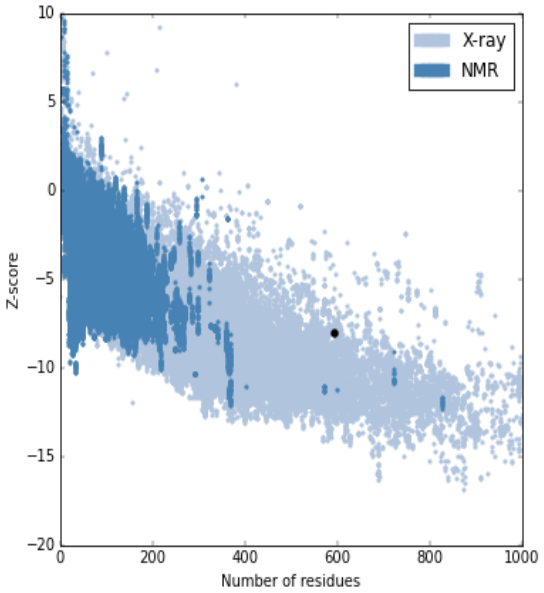


(b)

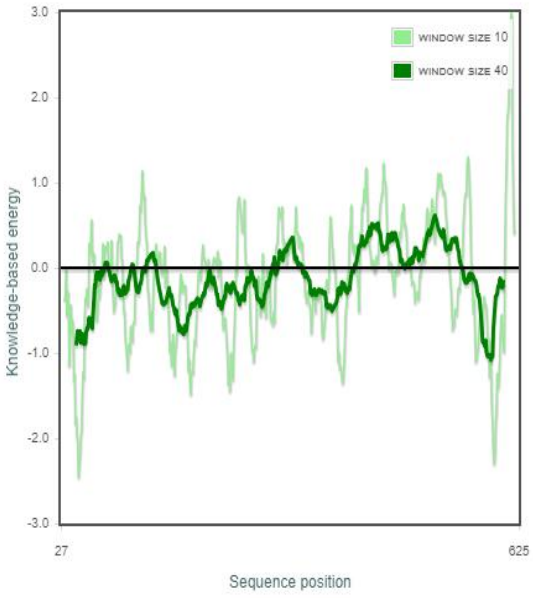
Figure 7: ProSA web service analysis of template human TLR4- human MD-2-E.coli LPS Ra complex (3FXI). (a) overall model quality and (b) local model quality.



(a)



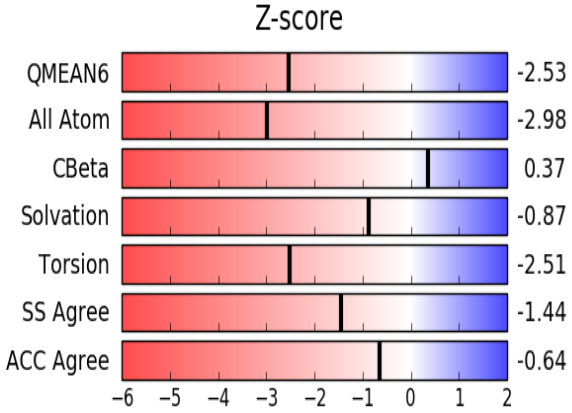
(a)



(b)

Figure 8: ProSA web service analysis of template mouse TLR4/MD- 2/lipid IVa complex (3VQ1). (a) overall model quality and (b) local model quality.

The QMEAN Z- score of human TLR4 (O00206.2) -2.53 (Figure 9), template human TLR4- human MD-2-E.coli LPS Ra complex (3FXI) QMEAN Z-score is -3.06 (Figure 10) and for template mouse TLR4/MD-2/lipid IVa complex (3VQ1) QMEAN Z-score is -1.10 (figure 11). which very closed to the value of 0 and this shows the fine quality for the models [15; 21]. A comparison between the normalized QMEAN score (0.40) and protein size in non-redundant set of PDB structures in the plot revealed different set of Z-values for different parameters such as C-beta interactions (-0.37), interactions between all atoms (-2.98), solvation (-0.87), torsion (-2.51), SSE agreement (-1.44) and ACC agreement (-0.64) (Figure 9; Table 1).



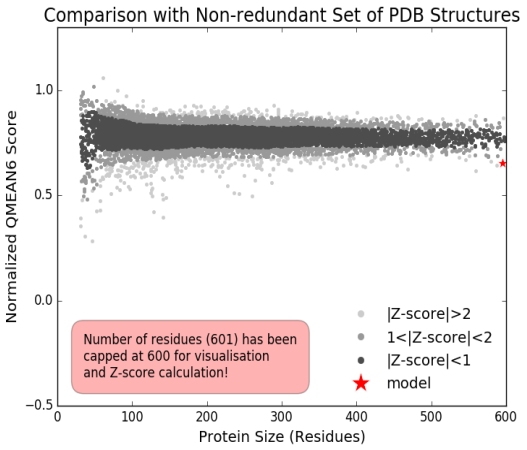
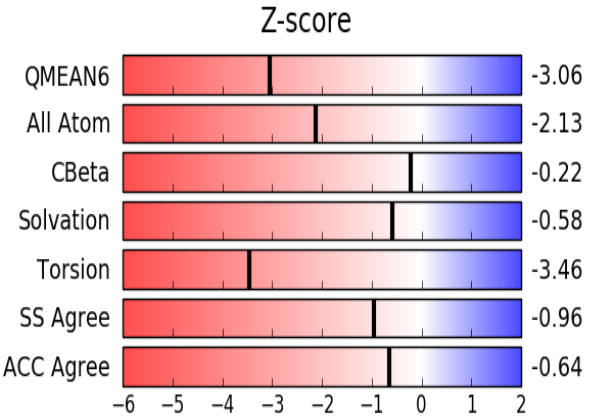


Figure 9: Plot showing the Z-score of human TLR4 (O00206.2)



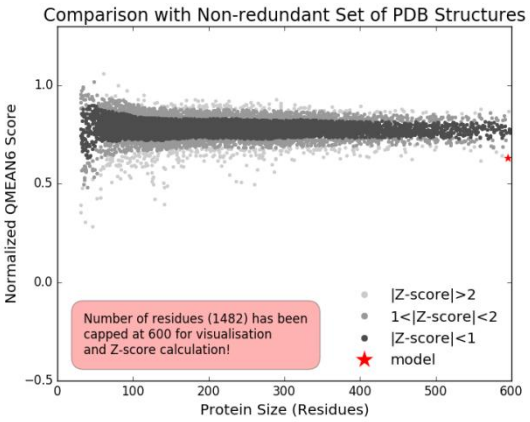


Figure 10: Plot showing the Z-score of template human TLR4- human MD-2-E.coli LPS Ra complex (3FXI)

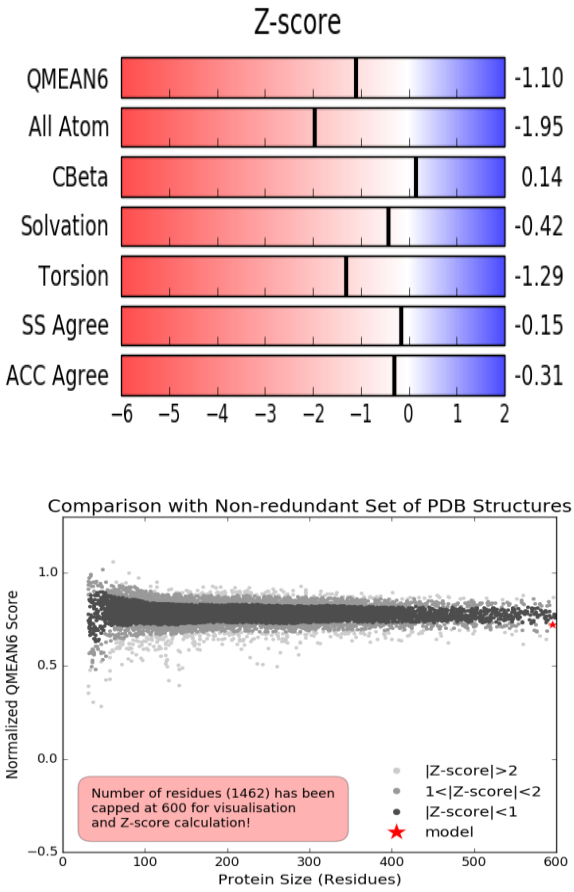


Figure 11: Plot showing the Z-score of template mouse TLR4/MD- 2/lipid IVa complex (3VQ1)

Table 1. Table showing the QMEAN value as well as Z-score

Accession No / PDB access code	protein	Z- Scores						
		QMEAN	All atom	CBeta	Salvation	Torsion	SSE agree	ACC agree
O00206.2	TLR4	-2.53	-2.98	0.37	-0.87	-2.51	-1.44	-0.64
3FXI	TLR4-human MD-2-E.coli LPS	-3.06	-2.13	-0.22	-0.58	-3.46	-0.96	-0.64
3VQ1	mouse TLR4/MD-2/lipid IVa complex	-1.10	-1.95	0.14	-0.42	-1.29	-0.15	-0.31

CONCLUSION

The human TLR4(O00206.2)protein model was obtained through homology modelling and the main interaction are similar to those observed for homologous template human TLR4- human MD-2-E.coli LPS Ra complex (3FXI) and mouse TLR4/MD-2/lipid IVa complex (3VQ1). The human TLR4 (O00206.2) model showed overall G-factor value of -0.23 with 81.2% residue in the most favoured region Ramachandran plot and its homologous templates human TLR4- human MD-2-E.coli LPS Ra complex (3FXI) 69.1% residue in the most favoured region and mouse TLR4/MD- 2/lipid IVa complex (3VQ1) have 77.8% residue in the most favoured region that indicates high accuracy of model predicted. The human TLR4 (O00206.2) ProSA Z-score was -7.82, homologous templates 3FXI WAS -7.81 and 3VQ1 was -7.99. That indicates overall model quality and measures the deviation of total structural energy with respect to an energy distribution derived from random conformations. We hope that, these results will be useful for the designing of inhibitors for TLR4 (O00206.2) and understanding of the mechanism of inhibition at the molecular level.

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