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In Silico Molecular Docking Study for Inhibitor Designing Against SARS-CoV-2 Spike Protein

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Abstract

Background: The severe acute respiratory syndrome caused by the infection of novel coronavirus (SARS-CoV-2) and responsible for present global pandemic. Till now, there is no approved treatment available for dealing with SARS-CoV-2 infection. The virus infection is caused by the binding of spike (S) glycoprotein on the cell receptor ACE2.

The objectives of this study were to characterize spike (S) glycoprotein [6XRA] and cell receptor ACE2 [1R42] for structure analysis, validation and design a peptide inhibitor that could potently inhibit SARS-CoV-2 fusion and entry by using a pseudo typed-virus system.

Material and Methods: The pdb file of spike protein [6XRA] and ACE2 [1R42] were retrieved from PDB. The pdb files were used for designing of ribbon structure model using 3D structure system. Visual investigation of the protein crystal structure was performed on the UCLA-DOE system and validation of model by using PROCHECK server. The quality of model was estimated by using ProSA and QMEAN server. HDOCK server was used for protein–protein docking.

Results: The structure and function of spike protein [6XRA] and ACE2 [1R42] predicted by *in silico* modeling studies. SARS-CoV-2 spike protein [6XRA] model corresponding to probability conformation with 89.8% residue of core section, and ACE2 [1R42] model with 88.9% residue of core section in φ-ψ plot that specifies accuracy of prediction model. The ProSA Z-score score -6.25 and -13.13 for 6XRA and 1R42 respectively; indicates the good quality of the model. Molecular dynamic simulation and docking studies explained that, the inhibitor interacts effectively at the ACE2 binding site of S protein. Hence, proposed HR2-based peptide (4MOD) inhibitor could potently inhibit SARS-CoV-2 fusion and entry by using a pseudo typed-virus system.

Conclusion: The crystal model useful to characterize the structure and functional aspect of both spike protein [6XRA] and human cell receptor ACE2 [1R42]. The proposed HR2-based peptide (4MOD) inhibitor could potently inhibit SARS-CoV-2 fusion and entry by using a pseudo typed-virus system. These results lay the groundwork for future inhibitory peptide drug design

Keywords: SARS-CoV-2, COVID-19, φ-ψ plot, ACE2, 6XRA, Docking

Introduction

The novel coronavirus reports from Wuhan, China (December 2019) reveal that, SARS-CoV-2 infection spread globally and causes severe acute respiratory syndrome[1]. The *Coronaviridae* family viruses recognized as the causative agent of respiratory disease and nominated as the SARS-associated coronavirus[2]. The SARS-CoV-2 infection cause

COVID-19 (Coronavirus Disease 2019)[3]. The COVID-19 reported from 221 countries around the world and territories. Till 19 May, according to WHO, confirmed cases were 16,49,12,329 and more than 34,19,039 deaths reported worldwide. In India the active cases were 2,52,28,996 and 2,78,719 death were reported according to COVID-19 Dashboard of India till dated 19 May 2021.

Globally, a total of 1,407,945, 776 vaccine doses have been administered and estimated mortality risk is ~2% and ~5.6% in diabetes [4].

The genome sequencing result reveal that SARS-CoV has polyadenylated RNA of 29.7 kb[4]. During clinical trial, after treatment with lopinavir/ ritonavir, little or no coronavirus titters were detected in infected patient[5]. The clinical trial reports suggested about the apparent efficacy of Chloroquine phosphate against COVID-19 associated pneumonia[6].

The surface spike (S) glycoprotein of coronaviruses mediates the recognition of human cell ACE2 receptor and fusion, thereby play essential role in initiating infection[1,33]. Fever, dry cough, myalgia, headache, and diarrhoea are the most consistent clinical symptoms of COVID-19 with multisystemic involvement[7,8]. The sequence analysis, structure modeling and biological data management is possible using *in-silico* methods [9,10].

In the present study, spike protein [6XRA] and cell receptor ACE2 [1R42] were characterized for structure analysis, validation and designed a HR2-based peptide (4MOD) inhibitor that could potently inhibit SARS-CoV-2 fusion and entry by using a pseudo typed-virus system.

Materials and Methods

Homology Modeling: The pdb file of spike protein [6XRA] and ACE2 [1R42] were retrieved from PDB. Both the pdb files were used for designing of ribbon structure model using 3D structure server (https://swissmodel.expasy.org/assess).

Model Reputation: UCLA-DOE used for quality examination of protein model[11]. The structural model validates by using PROCHECK[12,13] server and the results indicated that the model was reliable[14]. Overall G-factor, residue positions in φ-ψ plot regions analysis was used for the selection of suitable model [15,16]. The protein stability was analysed by using QMEAN (version 3.1.0) [17, 18] and ProSA [19] Z-score.

Molecular Docking: Interaction of ligand molecule on target protein at binding / active site studied by using molecular docking tools[20]. The ligand / protein-protein docking was done by using HDOCK server. HDOCK working based on algorithm of template-free and template-based docking, and its provides service of homology modeling, macro-molecular or protein–protein docking[21].

Results

Model Building: The target and template were aligned with the help of homology modeling[22]. 3D ribbon model of spike protein [6XRA] and human cell receptor ACE2 [1R42] generated using 3D structure server (https://swissmodel.expasy.org/assess) [Figure 1].

Model Reputation: SARS-CoV-2 spike protein [6XRA] model corresponding to probability conformation with 89.8% residue of core section, 9.9 % of allowed section and 0.3 % residue of outer section in φ-ψ plot. The human cell receptor ACE2 [1R42] model corresponding to probability conformation with 88.9% residue of core section, 9% of allowed section and 1.9 % residue of outer section in φ-ψ plot [23] (Figure 2a, b). The findings show that protein models were reliable [24, 25].

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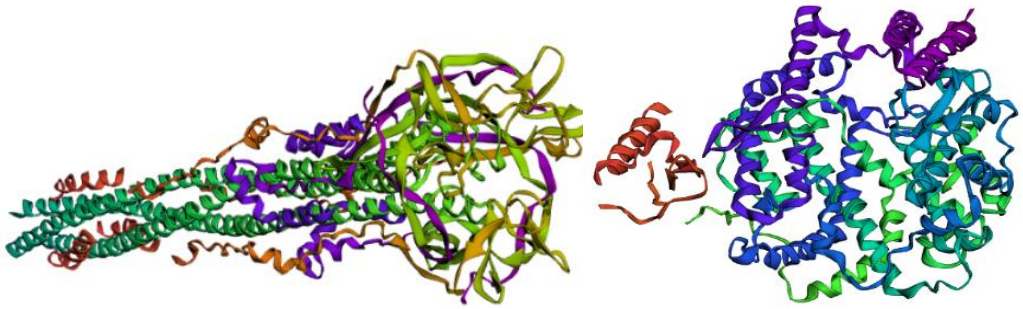
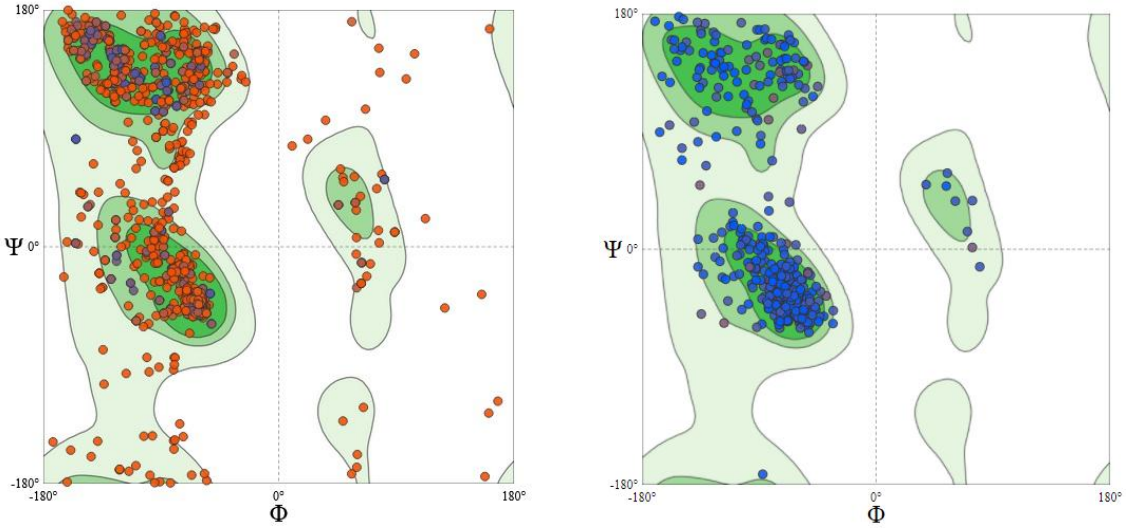
Fig 1. 3D ribbon structure model of (a) SARS-CoV-2 spike protein [6XRA] and (b) human cell receptor ACE2 [1R42] generated using 3D assessment server



(a) (b)

Fig 2. (a) φ-ψ plot of human cell receptor SARS-CoV-2 spike protein [6XRA]. Total number of residues were 843 (89.8%) in favoured [A, B, L], 93 (9.9%) in allowed [a,b,l,p] and, 3 (0.3%) in generously allowed regions. (b) φ-ψ plot of human cell receptor ACE2 [1R42]. Total number of residues were 521 (88.9%) in favoured, 53 (9%) in allowed and, 11 (1.9 %) in generously allowed regions.



(a) **(b)**

Validation of Model: The potential errors in the 3D model of spike protein [6XRA] human cell receptor ACE2 [1R42] were identified using ProSA. The archived ProSA Z-score score -6.25 and -13.13 for 6XRA and 1R42 respectively; specifies following: energy deviation and inclusive model quality (Figure 3,4). Z-score values specifies less erroneous structures model [19, 26]. QMEAN Z-score indicates protein model reliability (Figure 3,4) [9, 27].

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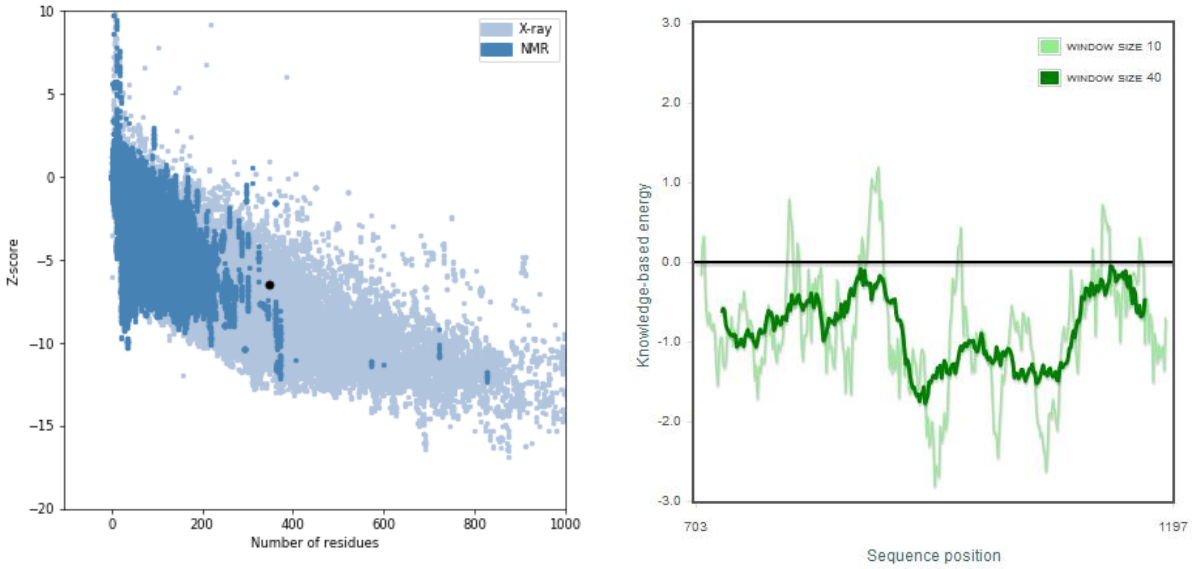
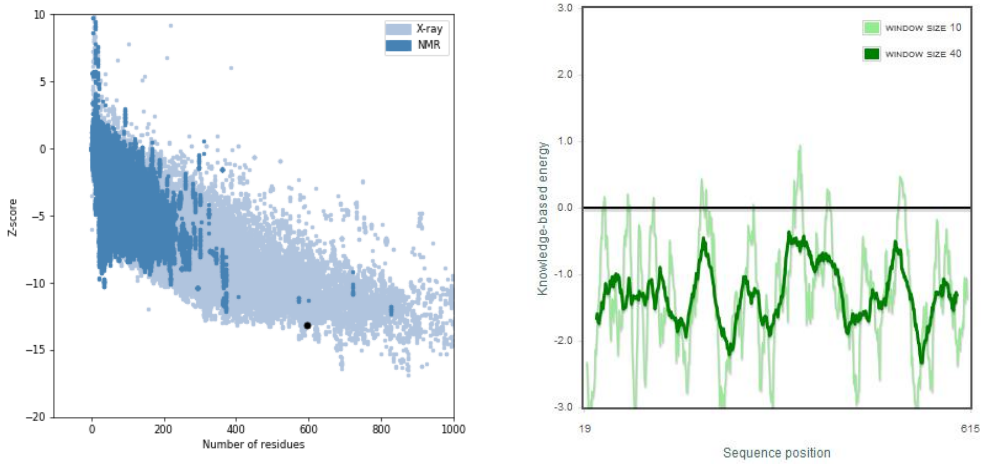
Fig 3. ProSA service examination of SARS-CoV-2 spike protein [6XRA] inclusive model quality (a) and limited model quality (b)



(a) **(b)**

Fig 4. ProSA service examination of human cell receptor ACE2 [1R42] inclusive model quality (a) and limited model quality (b)



(a) **(b)**

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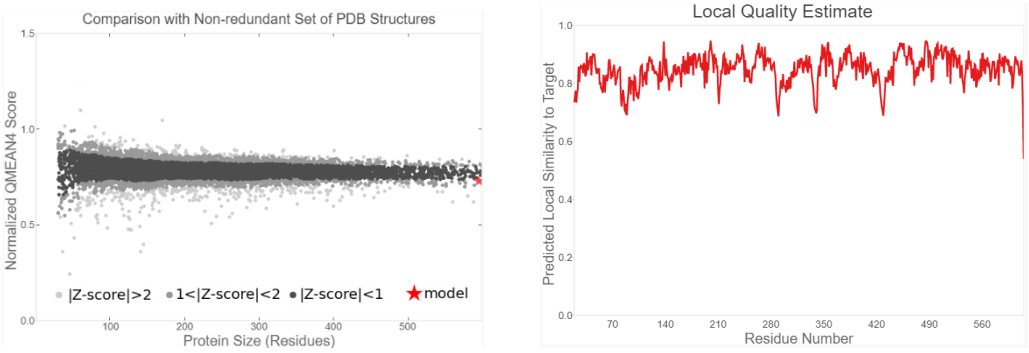
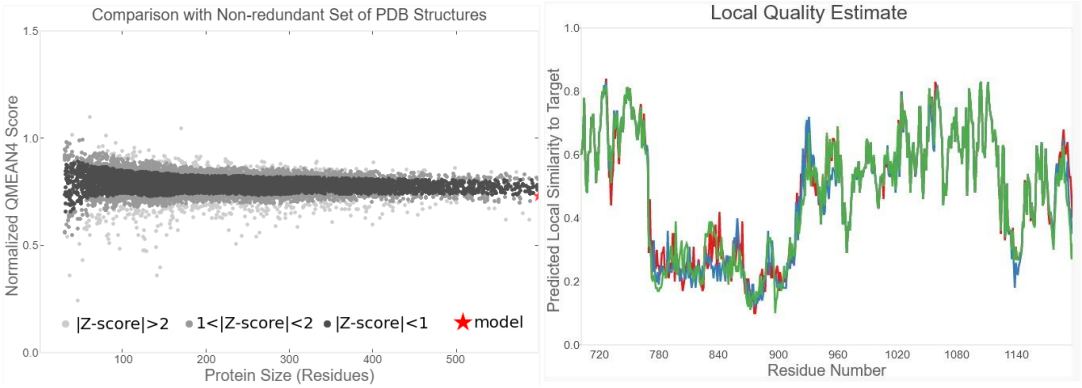
Fig 5. The QMEAN scores of human cell receptor ACE2 [1R42]. Plot showing Z-score (a). Local quality model for estimation of local summarily to target (b).



(a) **(b)**

Fig 6. QMEAN scores for biological unit reference set of SARS-CoV-2 spike protein [6XRA]. Plot showing Z-score (a). Local quality model for estimation of local summarily to target (b).



(a) **(b)**

The QMEAN Z-score -0.93 and -0.90 observed for spike protein [6XRA] and human cell receptor ACE2 [1R42] respectively. The Z score is near to 0 and depicts acceptable values [28]. The density plot for QMEAN score was used to assess the validity of the model, which was shown to be predictable between 0 and 1 (Figure 5, 6). The QMEAN scores for a biological unit position set that was used to evaluate oligomeric proteins are shown in figures 5 and 6. Multiple sets of Z-values for different parameters occurred when comparing the QMEAN values to the non-redundant protein library. The diversion of total energy of spike protein [6XRA] and human cell receptor ACE2 [1R42] were measured by using Z-score [29].

Variance of total structural energy also evaluates by Z-score with respect to random conformation's energy dispersion. The local distance difference test (lDDT) was carried out to assess the local precision and stereochemical plausibility of the models. Local Distance Difference Test (lDDT) score lengths of human cell receptor ACE2 [1R42] was 0.07297 and 0.19982 for spike protein [6XRA], specifies very reliable structure. The lDDT assesses the validation of stereochemical acceptability and differences in distance between atoms in the model [9, 30].

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Fig 7. HDOCK protein–protein docking model. Predicted protein receptor 1R42 is signified by ribbon structure surface and highlighted in rainbow. The predicted and native ligand 4MOD structure is highlighted in green

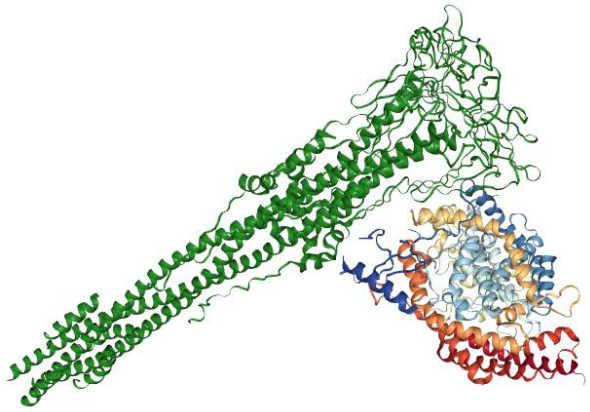

Table 1. Complex template statistics

Molecule	PDB ID	Chain ID	Align length	Coverage	Seq_ID (%)
Receptor	1R42	A	597	0.905	100.0
Ligand	4MOD	A	64	0.058	56.2

Table 2. Summary of the Top 10 Models. Row 1 (ranks of the models), Row 2 (energy scores of docking), Row 3 (ligand RMSDs from the input or modeled structures)

Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-288.28	-279.62	-276.09	-273.90	-273.09	-261.40	-256.33	-255.53	-255.10	-253.87
Ligand RMSD (Å)	231.41	230.89	254.61	231.98	255.19	321.28	334.43	238.52	336.30	312.00

Discussion

The docking model was built by using corresponding complex template information, with the template having Seq_ID of > 30% and sequence coverage of > 0.7 for the ligand and receptor is considered reliable [21]. For both receptor (1R42) and ligand (4MOD) coverage and Seq_ID value are more than standard values, therefore thought its reliable. By and large, models tend to be dependable with test structure for grouping character of >50% [31] (Figure 7; Table 1).

The top 10 docking models summary shown in table 2. The outline ordinarily contains three columns: the positions (ranks), docking vitality (energy) scores, and ligand RMSDs from the input or modeled structures. The template-free docking brought about within the beat 10 calculations, where the model 2 incorporates precision with an RMSD of 230.89 Å from the crystal structure (Figure 7; Table 1).

With this concept, identified an HR2-based peptide (4MOD) that could potently inhibit SARS-CoV-2 fusion and entry by using a pseudo typed-virus system. These results lay the groundwork for future inhibitory peptide drug design [32].

Till presently, there's no affirmed treatments are accessible for treatment of coronavirus disease. The association of spike (S) glycoprotein to ACE2 cell receptor leads infection [33]. In the present study, the spike protein [6XRA] and cell receptor ACE2 [1R42] characterized for structure analysis and validation

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and designed a HR2-based peptide (4MOD) inhibitor could potently inhibit virus fusion and entry [32].

The spike protein [6XRA] model corresponding to probability conformation with 89.8% residue of core section, and ACE2 [1R42] model with 88.9% residue of core section in φ-ψ plot that specifies accuracy of prediction model [9, 23]. The ProSA Z-score score -6.25 and -13.13 for 6XRA and 1R42 respectively; indicates the good quality of the model[19]. Molecular dynamic simulation and docking studies explained that the inhibitor ties effectively at ACE2 binding location of S protein. Hence, proposed HR2-based peptide (4MOD) inhibitor could potently inhibit SARS-CoV-2 fusion and entry by using a pseudo typed-virus system. These results lay the groundwork for future inhibitory peptide drug design.

Conclusion

The functional characteristics of SARS-CoV-2 spike protein [6XRA] and human cell receptor ACE2 [1R42] could be predicted by the generated modes for structure analysis and validation and designed a HR2-based peptide (4MOD) inhibitor could potently inhibit SARS-CoV-2 fusion and entry by using a pseudo typed-virus system using computational modeling approach. PROCHECK, ProSA, and QMEAN strategies show reliability of model. HDOCK server used for template-free and based protein–protein docking. These findings lay the groundwork for future inhibitory peptide drug design.

Ethical Issues

Research project approved by the ethics committee of Pacific Institute of Medical Sciences, Sai Tirupati University, Udaipur- 313003, Rajasthan, INDIA as part of Ph.D research work.

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