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RESEARCH ARTICLE

AN ASSESSMENT OF THE INTERACTION BETWEEN INSECT BRAIN PROTEIN AND NON-STRUCTURAL PROTEIN OF CORONAVIRUS USING IN-SILICO ANALYSES

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Abstract

Corona is an avian and mammalian Ribovirus which generally evade host immune mechanism hampering respiratory tract of the host. Structurally it has an envelope of protein enclosing a single-stranded positively coiled RNA genome. Coronavirus uses around 4-5 different classes of protein for its replication in the host cell. Due to the high mutation rate of the viral genome development of a vaccine against corona has been a difficult task. In the current scenario, the world is facing a problem with CoViD-19 as the biggest pandemic. Several combinations of drugs like Hydroxychloroquine, Plaquine, Chloroquine, etc. targeting viral protein have been utilized for controlling viral infection. The possibility of insect brain proteins was checked against the selected non-structural proteins of CoViD-19 that take an active role in the replication of the virus in the host. Molecular docking methodology plays an important role in predicting interaction between the insect proteins with non-structural protein (nsp) of coronavirus. The results predict good bonding affinity with possible interaction as hydrogen bond and salt bridge between antibacterial protein and nsp of CoViD-19 indicating as afuture alternative medications.

Introduction:-

Coronavirus was first discovered as an infectious bronchitis virus (IBV) found in the respiratory tract of domesticated chicken. Later in the 1940s two other varieties of the corona were discovered namely, mouse hepatitis virus (MHV) and transmissible gastroenteritis virus (TGEV) (McIntosh, 1974). However, several human coronavirus has been discovered since the 1960s the most recent being Middle East respiratory syndrome coronavirus (MERS-CoV) (de Groot et al., 2013) in 2012, and Severe acute respiratory syndrome-related coronavirus (SARS-CoV-2) (Gorbalenya et al., 2020) in 2019. SARS-CoV-2 is composed of four structure protein namely, spike protein, envelope protein, membrane protein, and nucleocapsid proteins. Nucleocapsid covers genomic RNA whereas envelope, spike, and membrane protein together form a viral envelope (Wu wt al., 2020). Apart from these, they have 16 non-structural protein (nsp), each of these plays multiple roles including its replication, host translational inhibitor, host association, etc. The primary functions that direct coronavirus RNA synthesis and processing reside in nsp7 to nsp16, the 3 proteins nsp3, nsp4, nsp6 are predicted to possess a transmembrane domain that is involved in membrane anchoring of replication complex (Snijder et al., 2016;Oostra et al., 2007). A protein nsp4 participates in the assembly of virally-induced cytoplasmic double-membrane vesicles

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necessary for viral replication whereas nsp7 forms a hexadecamer with nsp8 (8 subunits of each) that may participate in viral replication by acting as a primase. Alternatively, it may synthesize substantially longer products than oligonucleotide primers Or may be required to activate the RNA-synthesizing activity of Polymerase (Uniprot, Decemebr 2019). A biomolecule targeting these two proteins may help in inhibiting their replication mechanism which in turn may stop viral infections.

Insect proteins isolated from brain tissue lysate of cockroach have indicated control on the growth of drug-resistant bacteria like MRSA and MRSS. They are also found to show the effect on pathogens like *S.typhi, P.aeruginosa, K.pneumoniae*, etc (Sagar and Jayaprada, 2015). Interaction of this protein with nsp4 and nsp7 can give a ray of hope for controlling replication of SARS-CoV-2 in host cells. Protein docking is the task of calculating the 3D structure of a protein complex from its unbound or model-built subunits. Although proteins are intrinsically flexible, many protein docking algorithms begin by assuming that the proteins are rigid and they use geometric hashing (Bachar et al., 1993) or fast Fourier transform (FFT) correlation techniques (Katchalski-Katzir et al., 1992) to find a relatively small number of putative docking orientations which may be refined and re-scored using more sophisticated techniques. In recent years, several protein docking programs have been made available with web servers like ClusPro (Comeau et al., 2004), GRAMM-X (Tovchigrechko and Vakser, 2006) and ZDOCK (Chen et al., 2003). Docking reports obtained from these servers are always in .pdb format which can beanalyzed in detail using different servers like PDBsum, pyMol (Laskowski et al., 2018), LIGPLOT (McDonals and Thornton, 1994), HBPLUS(Wallace and Laskowski, 1995), etc.

Material and Method:-

Protein modeling:

7 Antibacterial proteins were isolated from the brain tissue lysate of cockroach (Siddharth and Jayaprada, 2015). Of these, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Transferrin were considered for docking study with nsp4 and nsp7. For molecular docking amino acid sequence was procured from UniProt (Uniprot-Consortium, 2011) database (accession no. 343965965 and 372292427) and considered for structural homology modeling in SWISS MODEL online portal (Aartjan et al.,2010). The structure of nsp4 and nsp7 was directly procured from the SWISS MODEL repository database of SARS-CoV-2 (Swiss Model repository, 2019).

Protein-Protein docking:

The protein structures were uploaded in GRAMM-X and allowed for Docking. The results of protein interaction obtained between virus protein nsp7, nsp4 with antibacterial protein transferrin and GAPDH respectively were analyzed using EMBL-EBI's PDBsum sever.

Binding energy prediction:

HAWKDOCK server was used to identify the best binding pose for protein-protein in docking based on binding free energy score. The more the negative value more is the interaction and complex suitable to bind. The .pdb model of GRAMM-X docked protein-protein interaction was uploaded for the calculation of binding energy based on Molecular Mechanics/Generalized Born Surface Area (MM/GBSA).

Results:-

Single structure model of nsp4 and three models of nsp7 were obtained which were labelled as nsp7.1,nsp7.2 and nsp7.3 from SWISS MODEL. (Figure 1)

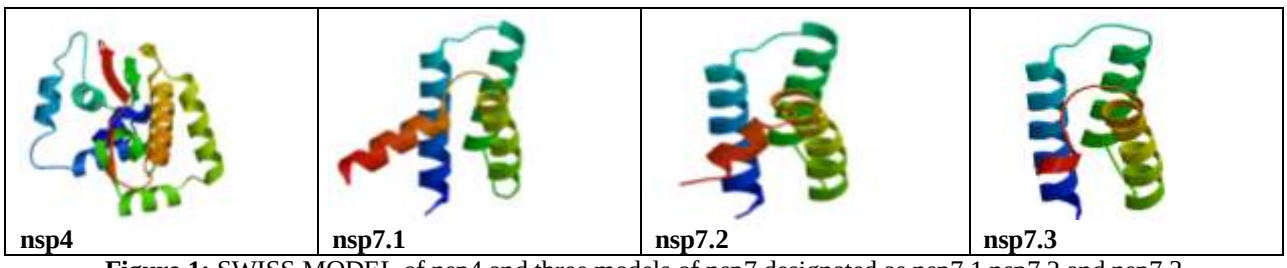


Figure 1: SWISS MODEL of nsp4 and three models of nsp7 designated as nsp7.1,nsp7.2 and nsp7.3

The GRAMM-X docking results represented a detailed interaction reports for GAPDH and Transferrin with nsp4,nsp7.1, nsp7.2 and nsp7.3 as following: (using PDBsum)

Interaction of GAPDH with nsp4:

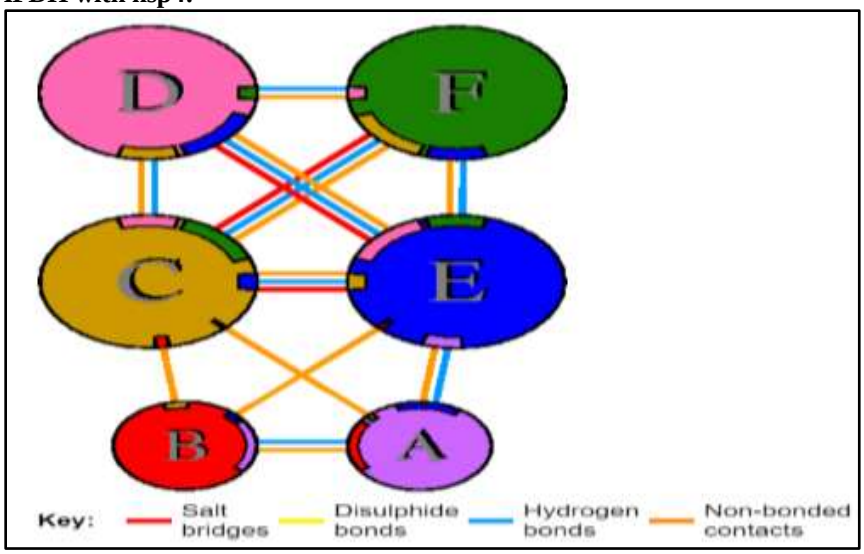


Figure 2:- Picturesque depiction of interacting GAPDH and nsp4.

Above picture represents general interface summary of interaction. Where chain A and chain B are of nsp4 and Chain D, Chain C, Chain E and Chain F are of GAPDH.

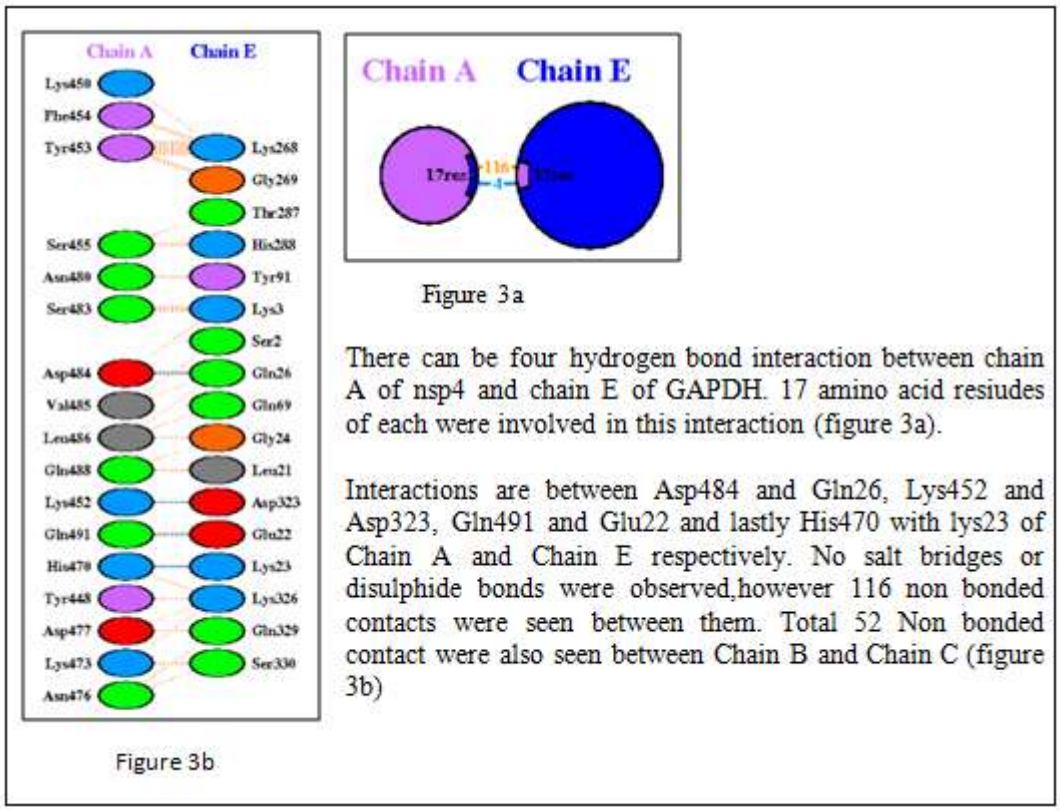


Figure 3b

Figure 3a

There can be four hydrogen bond interaction between chain A of nsp4 and chain E of GAPDH. 17 amino acid resiudes of each were involved in this interaction (figure 3a).

Interactions are between Asp484 and Gln26, Lys452 and Asp323, Gln491 and Glu22 and lastly His470 with lys23 of Chain A and Chain E respectively. No salt bridges or disulphide bonds were observed,however 116 non bonded contacts were seen between them. Total 52 Non bonded contact were also seen between Chain B and Chain C (figure 3b)

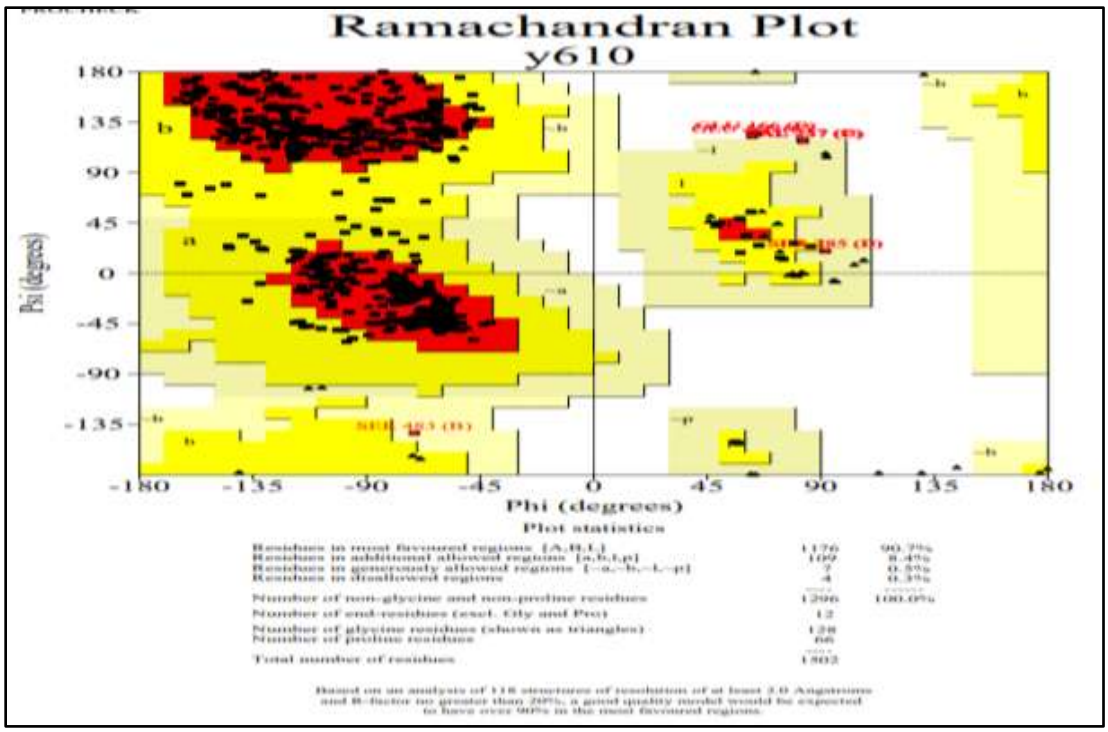


Figure 4:- PROCHECK analyses report of Ramachandran plot for interaction of nsp4 with GADPH. Interaction of GAPDH with nsp7.1:

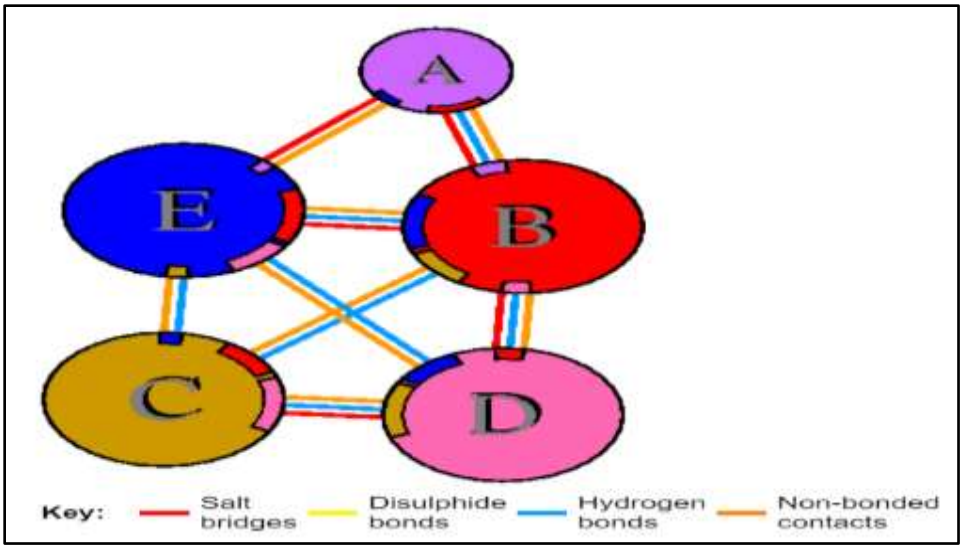


Figure 5:- picturesque depiction of interacting GAPDH and nsp7.1

Above picture represents general interface summary of interaction. Where chain A is nsp7.1 and Chain B, Chain C, Chain D and Chain E are of GAPDH.

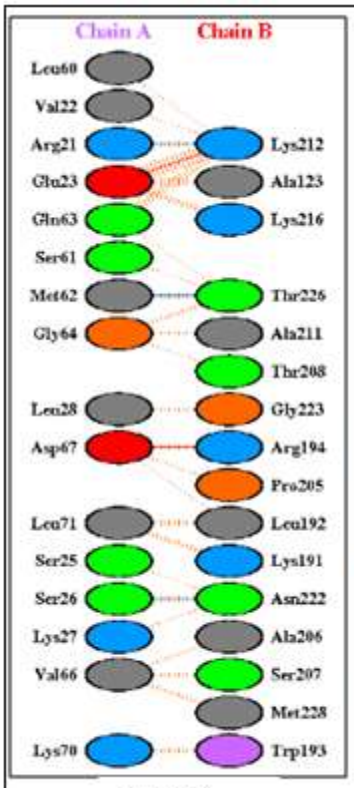


Figure 6.

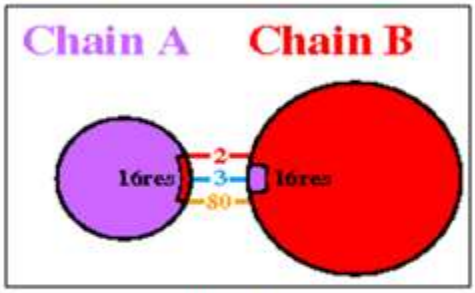
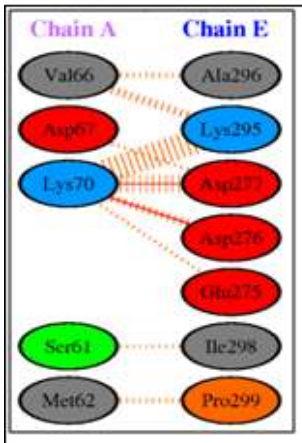


Figure 6a.

There can be three hydrogen bond interaction and two salt bridges are seen between chain A of nsp7.1 and chain B of GAPDH. 16 amino acid resiudes of each were involved in this interaction.(figure 6a)

Hydrogen bond is between Arg21 and Lys212, Met62 and Thr226, Ser26 and Asn222, whereas Salt bridge is between Glu23 and Lys212 and lastly Asp67 and Arg194 of Chain A and Chain B respectively. No disulphide bonds were observed,however 80 non bonded contacts were seen between them. (figure 6b)



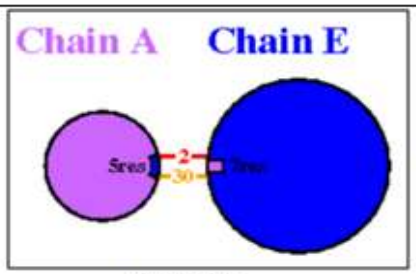


Figure 7a.

There can be two salt bridges are seen between chain A of nsp7.1 and chain E of GAPDH involving 5 and 7 amino acid resiudes respectively(figure 7a)

Figure 7b.

Salt bridge is between Lys70 and Asp277 and Lys70 and Asp276 and Chain A and Chain E respectively. No disulphide bonds were observed,however 30 non bonded contacts were seen between them.(figure 7b)

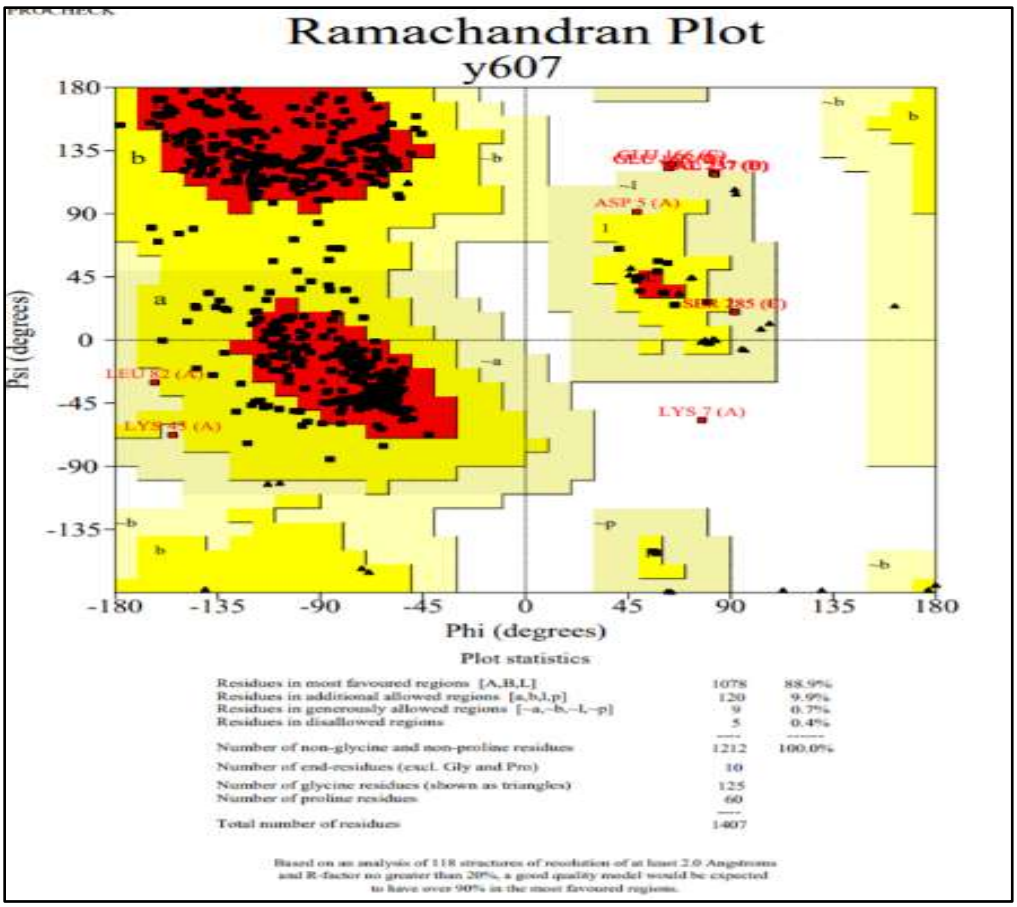


Figure 8:- PROCHECK analyses report of Ramachandran plot for interaction of nsp7.1 with GADPH

Interaction of GAPDH with nsp7.2:

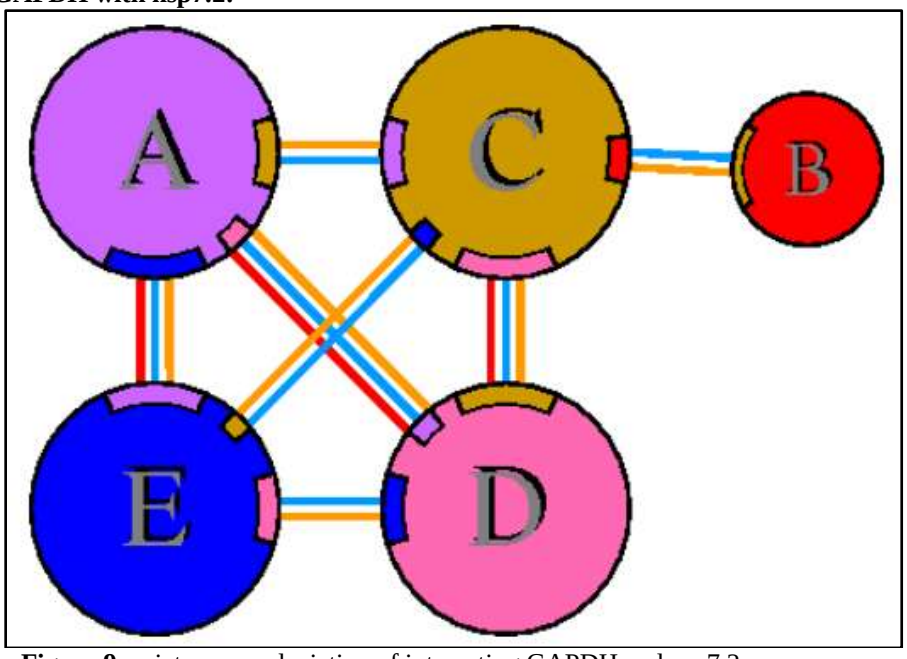
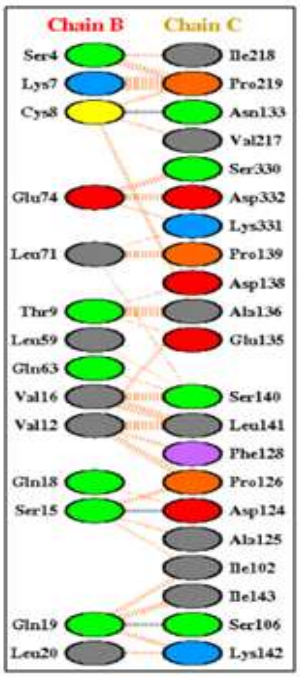


Figure 9:- picturesque depiction of interacting GAPDH and nsp7.2.

Above picture represents general interface summary of interaction. Where chain B is nsp7.2 and Chain B, Chain C, Chain D and Chain E are of GAPDH.



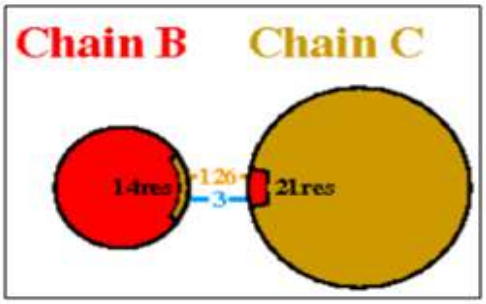


Figure 10a.

There can be 3hydrogen bonds between chain B of nsp7.2 and chain C of GAPDH involving 14 and 21 amino acid residues respectively (figure 10a)

There are 3 hydrogen bonds seen between Cys8 and Asn133,Ser15 and Asp124 and lastly Gln19 and Ser106of Chain B and Chain C respectively. No salt brige or disulphide bonds were observed,however 126 non bonded contacts were seen between them. (figure 10b)

Figure 10b.

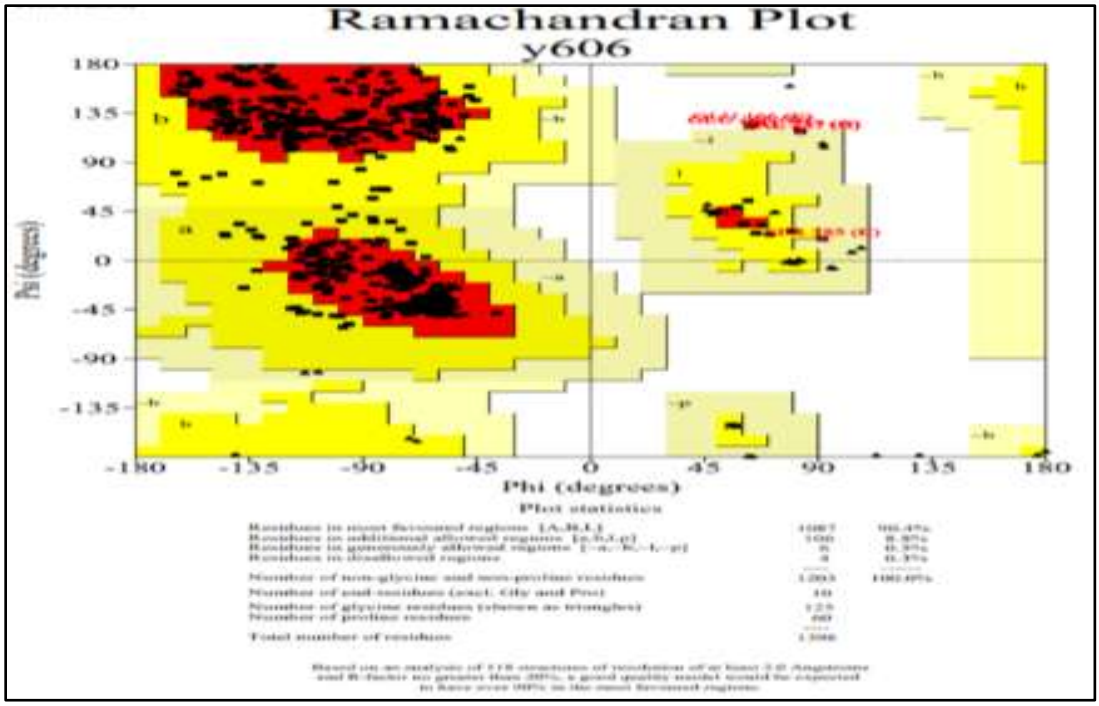


Figure 11:- PROCHECK analyses report of Ramachandran plot for interaction of nsp7.2 with GADPH.

Interaction of GAPDH with nsp7.3:

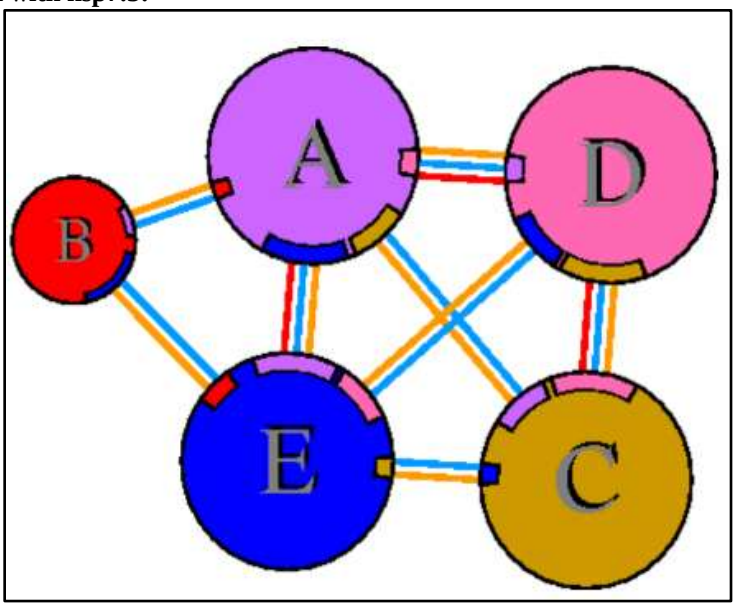


Figure 12: picturesque depiction of interacting GAPDH and nsp7.3.

Above picture represents general interface summary of interaction. Where chain B is nsp7.3 and Chain B, Chain C, Chain D and Chain E are of GAPDH

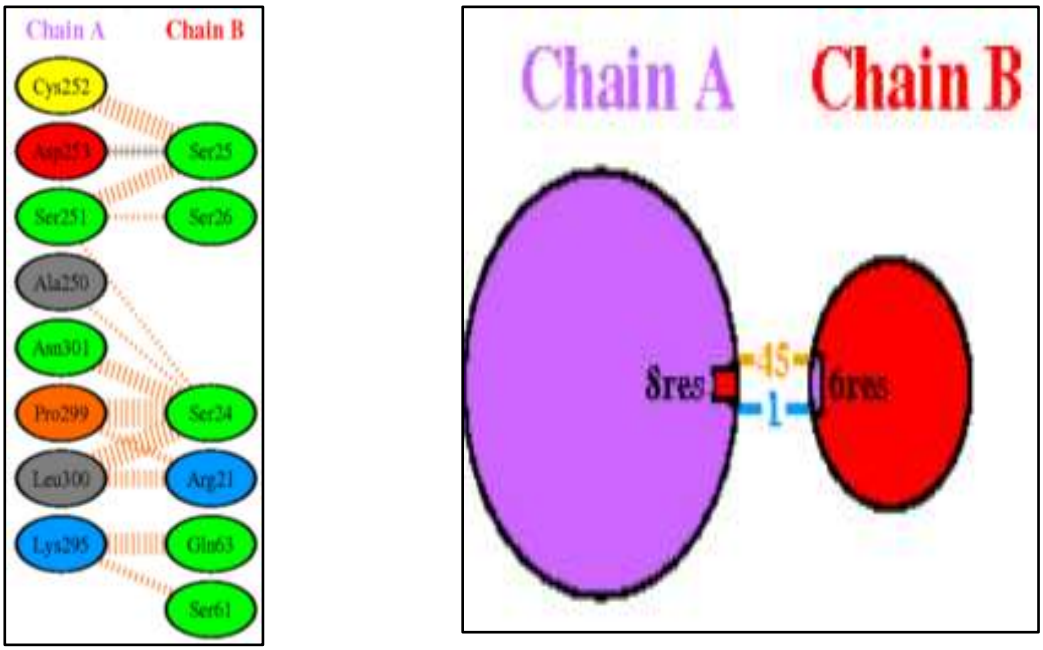


Figure 13a.:- There can be one hydrogen bond are seen between Asp 253 of chain A of GAPDH and Ser25 of chain B of nsp7.3 involving 8 and 6 amino acid resiudes respectively. No salt bridge or disulphide bonds were observed, however 45 non bonded contacts were seen between them (figure 13a and 13b)

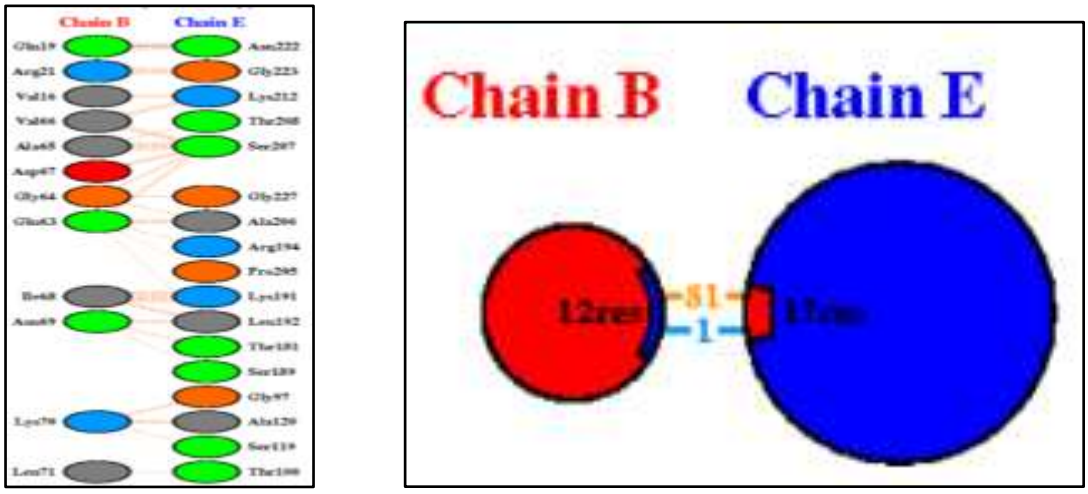


Figure 14a.:- There can be one hydrogen bond are seen between Gln 19 of chain B of nsp7.3 and Asn222 of chain E of GAPDH involving 12 and 17 amino acid resiudes respectively. No salt bridge or disulphide bonds were observed, however 81 non bonded contacts were seen between them (figure 14a and figure 14b)

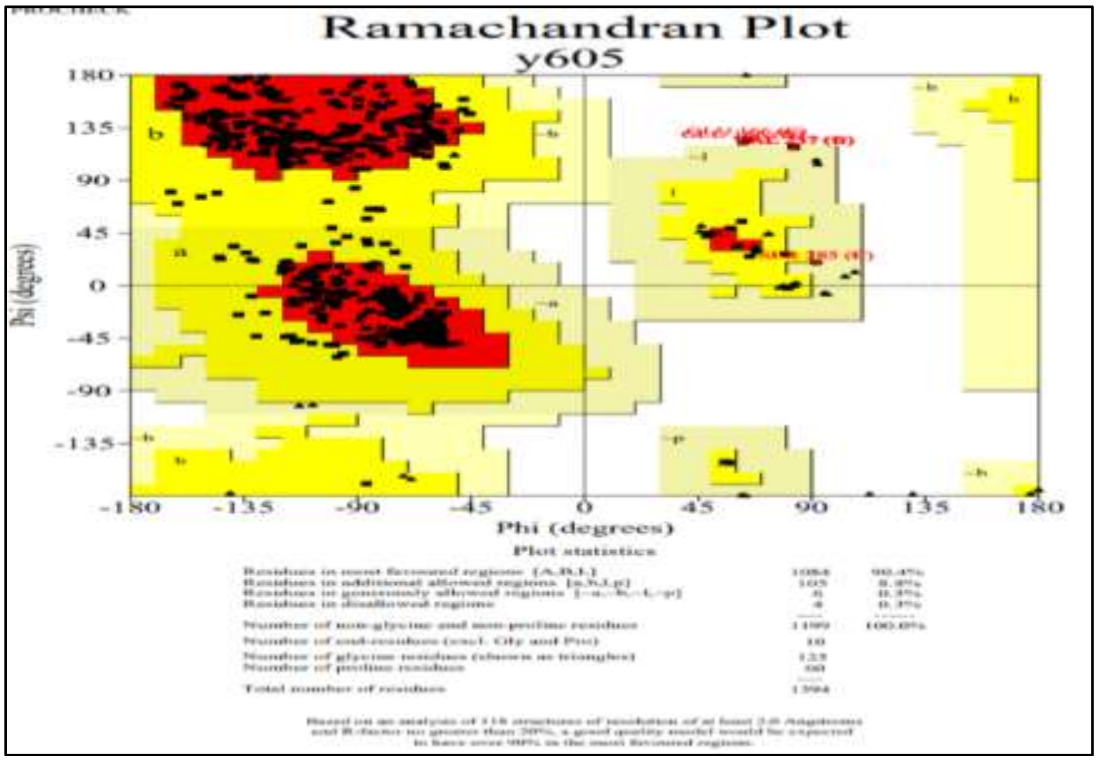


Figure 15:- PROCHECK analyses report of Ramachandran plot for interaction of nsp7.3 with GADPH.

Interaction of Transferrin with nsp4 :

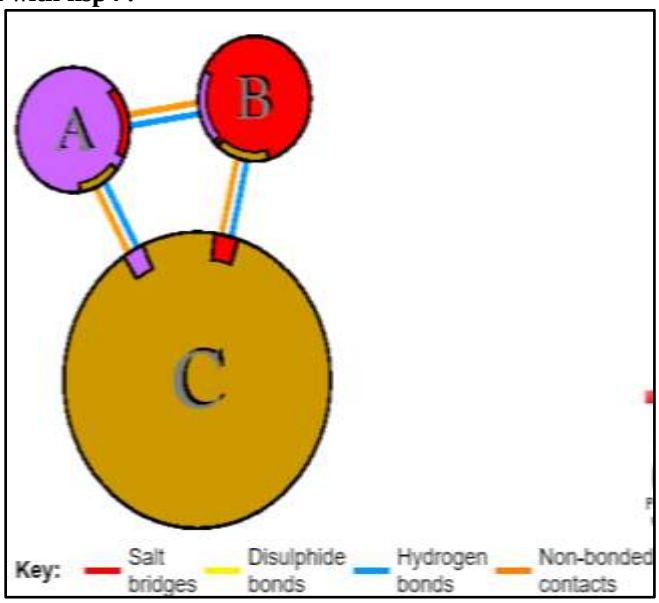


Figure 16:- Picturesque depiction of interacting transferrin and nsp4.

Above picture represents general interface summary of interaction. Where Chain A and Chain B is nsp4 and Chain C is of Transferrin

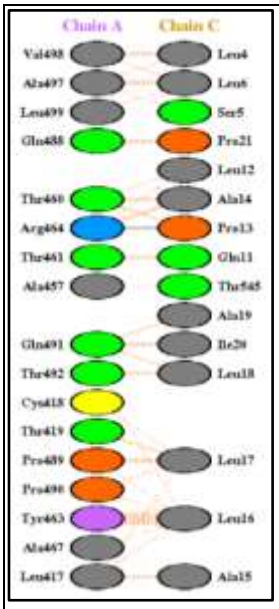


Figure 17b.

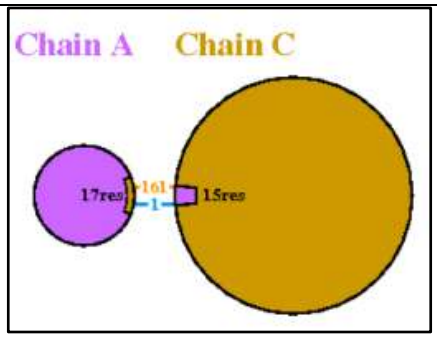


Figure 17a.

There is one hydrogen bond seen between Arg464 of chain A of nsp4 and Pro13 of chain C of Transferrin involving 17 and 15 amino acid resiudes respectively. No salt bridge or disulphide bonds were observed, however 161 non bonded contacts were seen between them
(figure 17a and 17b)

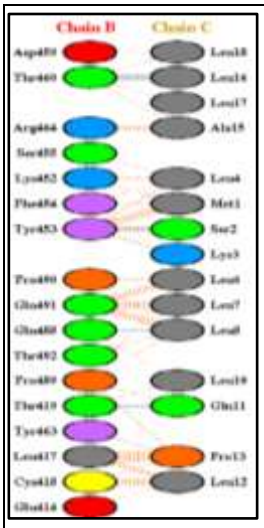


Figure 18b.

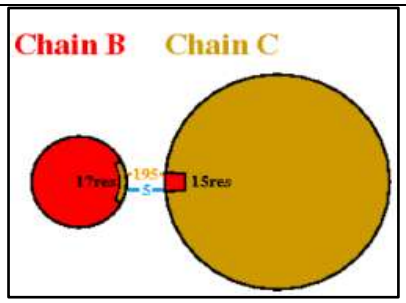


Figure 18a.

There are 5 hydrogen bonds seen, two between Thr460 and Leu16, one between Tyr453 and Ser2, Gln488 and Leu8, Thr419 and Gln11 each of chain B of nsp4 and Chain C of transferrin invovling 17 and 15 amino acid residues respectively. No salt bridge or dishulphide bonds were observed, however 195 non-bonded contacts were seen between them.(figure 18a and 18b)

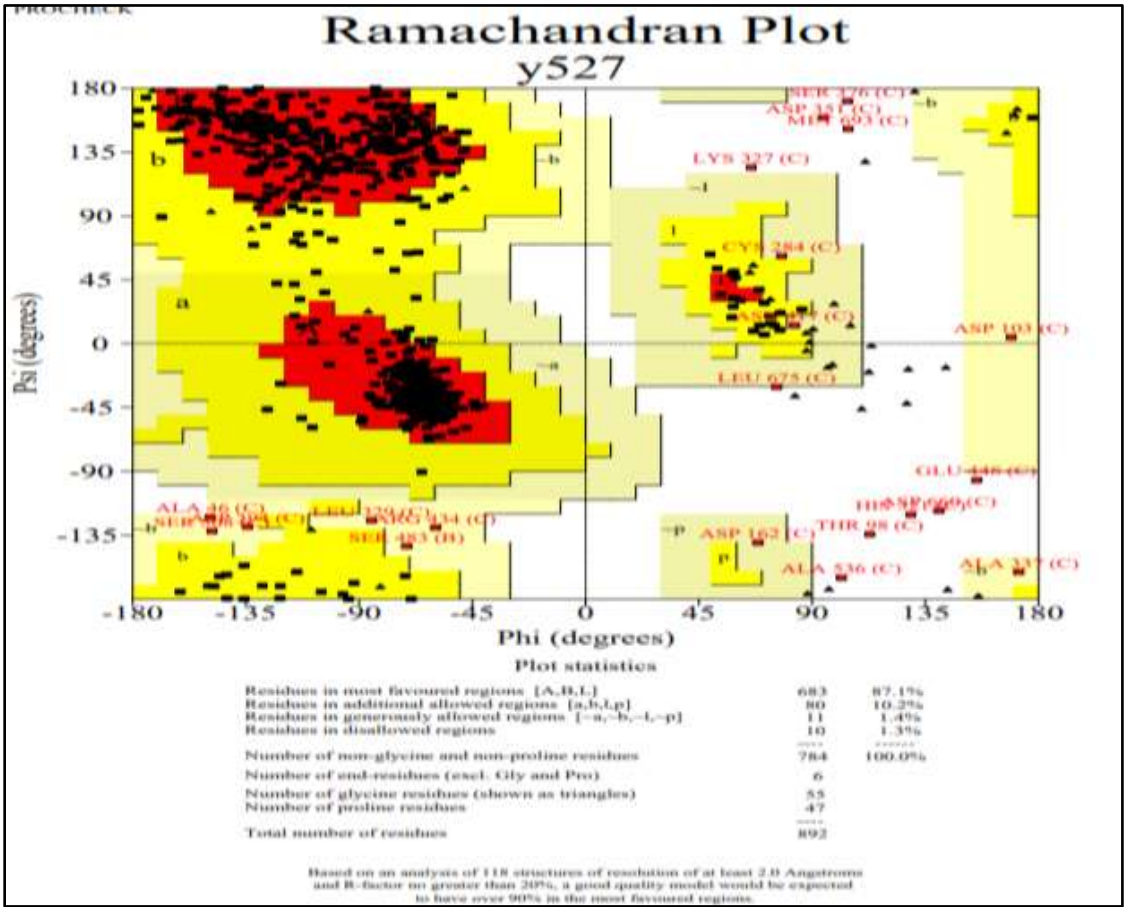


Figure 19:- PROCHECK analyses report of Ramachandran plot for interaction of nsp4 with Transferrin.

Interaction of Transferrin with nsp7.1 :

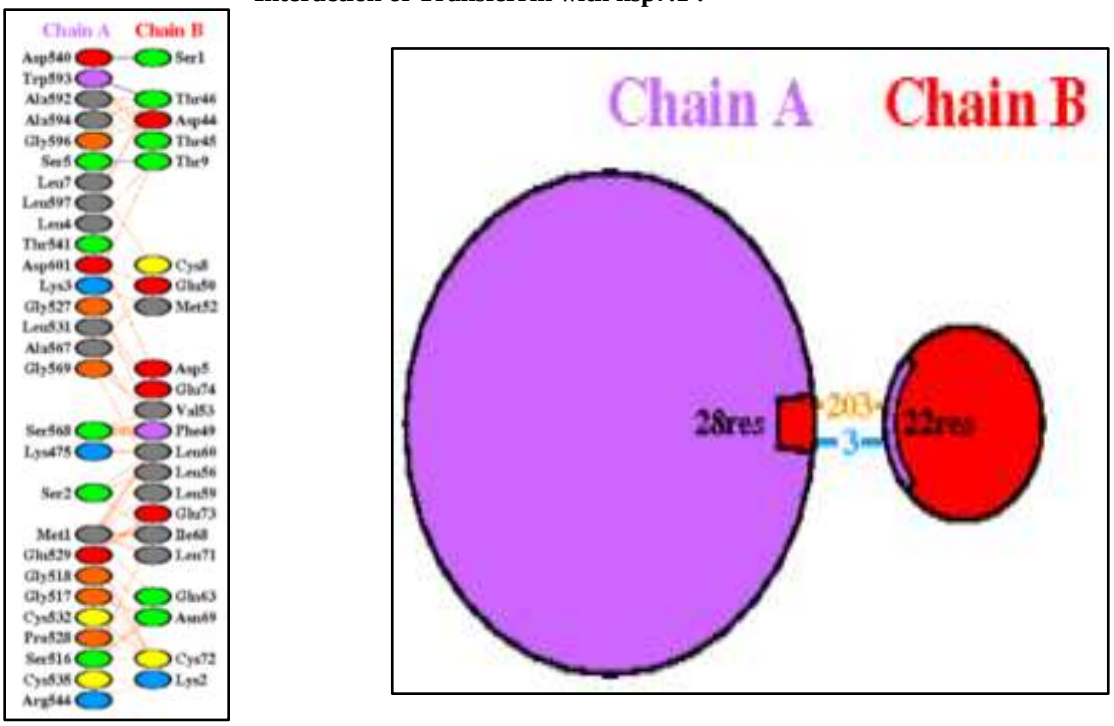


Figure 20a:- Chain A representing Transferrin and Chain B representing nsp7.1 show 3 hydrogen bond between them involving 28 and 30 residues respectively. There are 3 hydrogen bonds are seen, between Asp540 and Ser1, Trp593 and Thr46, Ser5 and Thr9, chain A of Transferrin and Chain B of nsp7.1 involving 28 and 22 amino acid residues respectively. No salt bridge or dishulphide bonds were observed, however 203 non-bonded contacts were seen between them.(figure 20a and 20b)

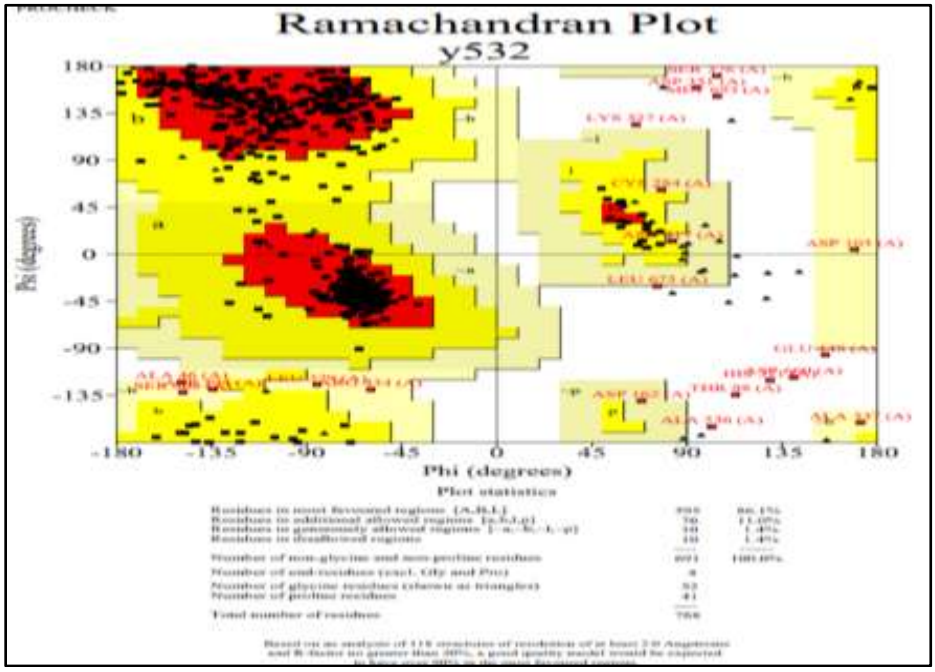


Figure 21:- PROCHECK analyses report of Ramachandran plot for interaction of nsp7.1 with Transferrin Interaction of Transferrin with nsp7.2.

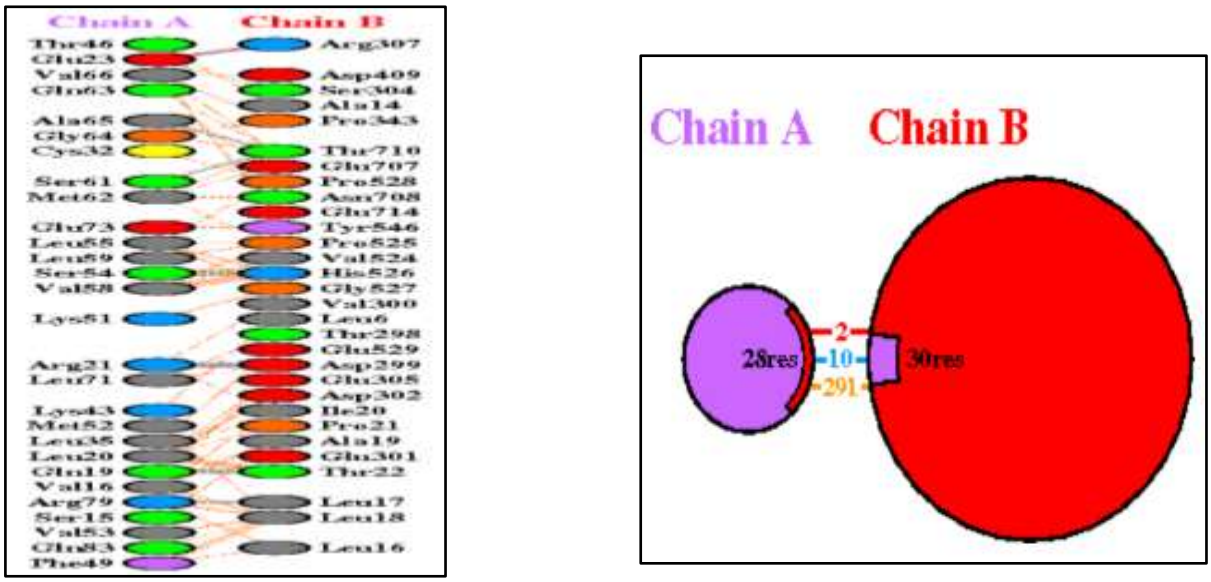


Figure 22a:- Chain A representing nsp7.2 and Chain B representing Transferrin show 2 salt bridge and 10 hydrogen bond between them involving 28 and 30 residues respectively. Salt bridge is seen between Glu63 and Arg307, Arg21 and Asp299 and one hydrogen bond is seen been Glu63 and Arg307, Ala65 and Thr710, Ser61 and Thr710, Arg79 and Leu17 each and two hydrogen bond between Ser54 and His526, Arg21 and Asp299, Gln79 and Thr22 of Chain A and Chain B respectively.

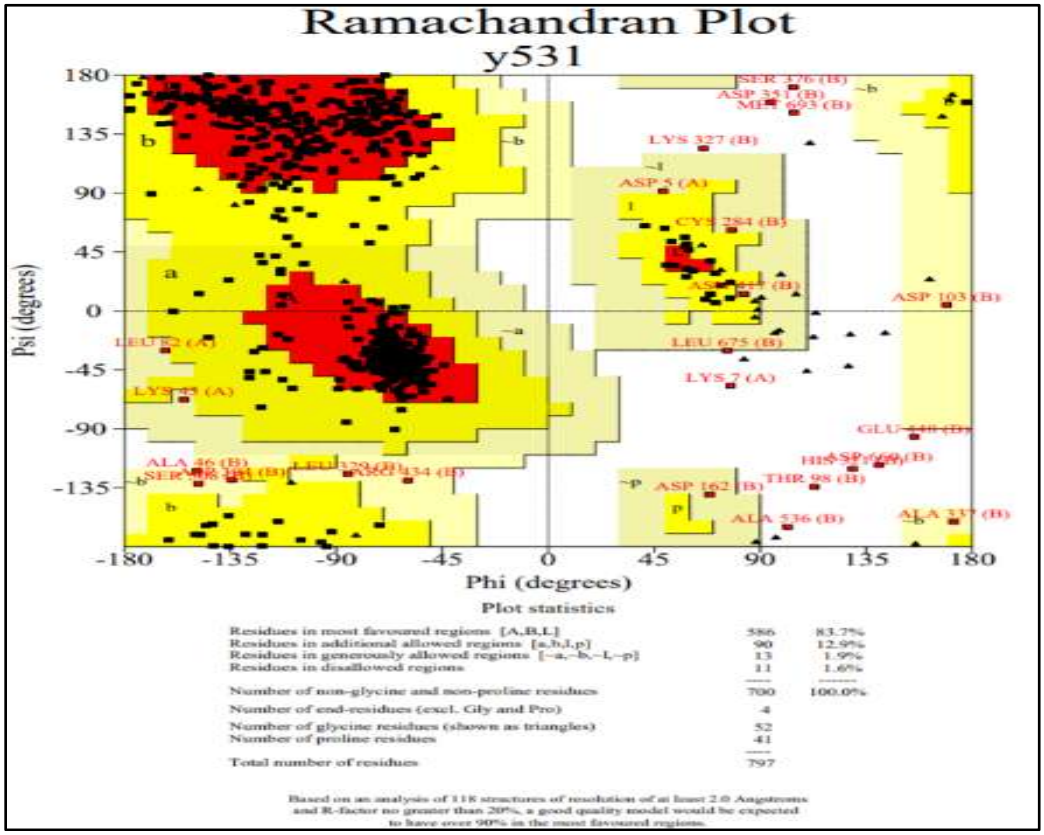


Figure 23:- PROCHECK analyses report of Ramachandran plot for interaction of nsp7.2 with Transferrin

Interaction of Transferrin with nsp7.3 :

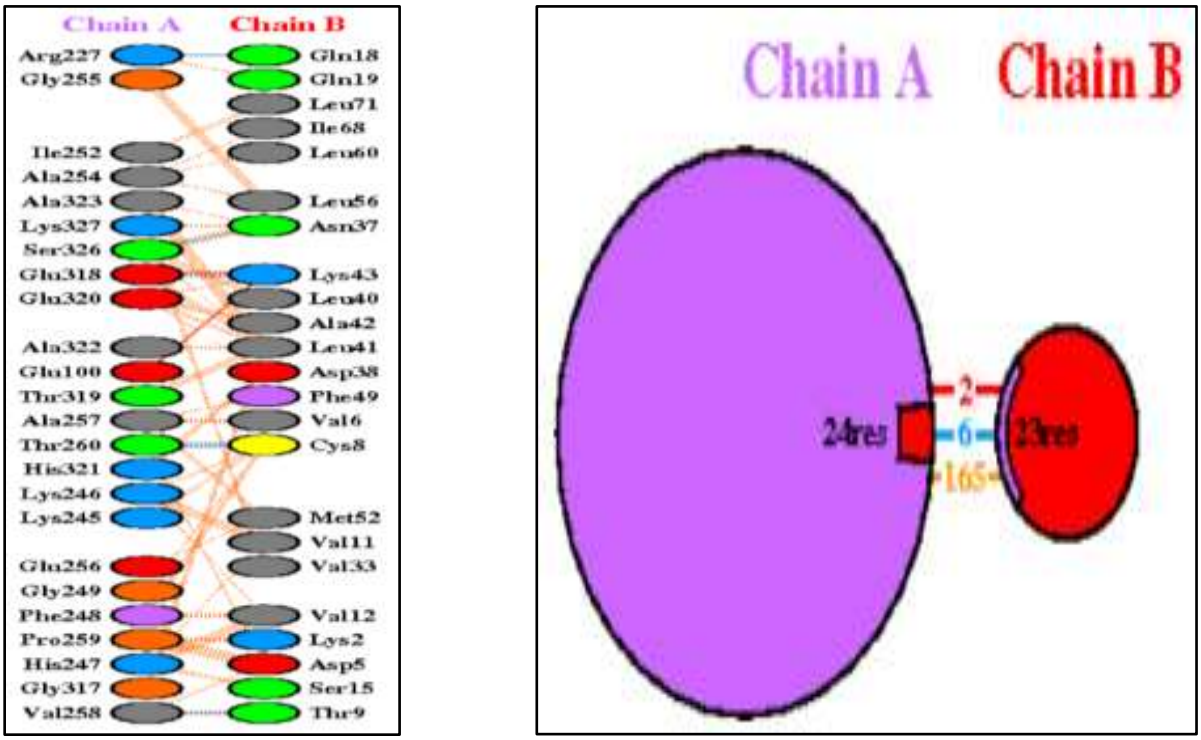


Figure 24a:- Chain A representing transferrin and Chain B representing nsp7.3 show 2 salt bridge and 6 hydrogen bond between them involving 24 and 23 residues respectively. Salt bridge is seen between Glu318 and Lys43, Glu100 and Lys43. One hydrogen bond is seen between Arg227 and Gln18, Glu318 and Lys43, Ser326 and Asn37, Val258 and Thr9 whereas two hydrogen bonds are seen between Thr260 and Cys8 of Chain A and Chain B respectively.

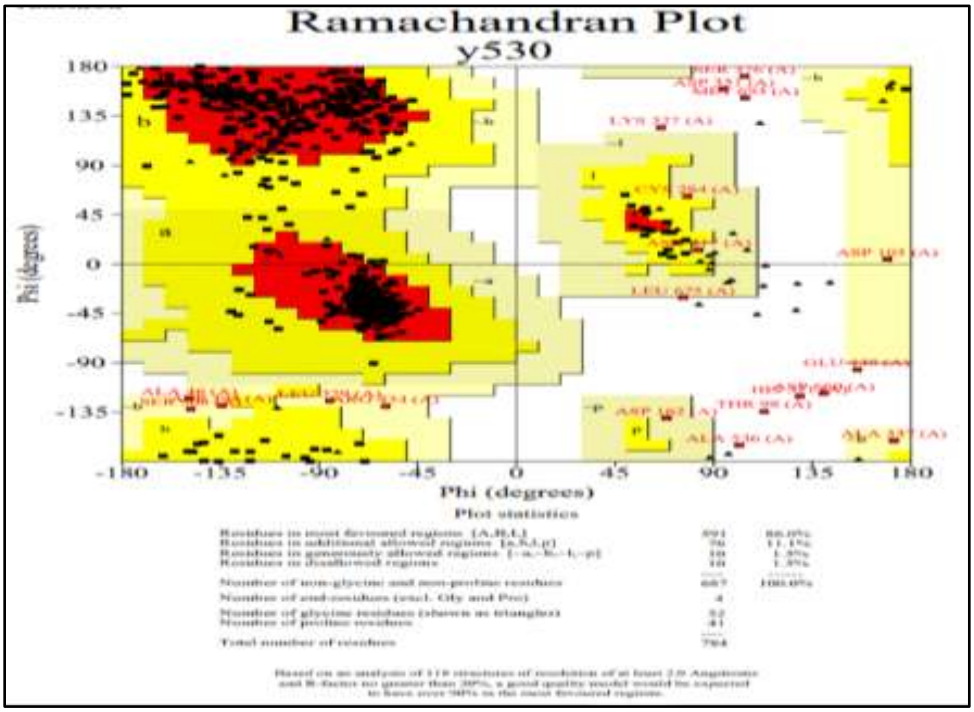


Figure 25:- PROCHECK analyses report of Ramachandran plot for interaction of nsp7.3 with Transferrin.

Table 1:- Binding energy of Transferrin and GAPDH with nsp4 and different models of 7 of SARS-CoV-2/COVID-19.

Protein interaction	Binding energy in kcal/mol
Gapdh-nsp4	-589.99
Gapdh-nsp7.1	-463.73
Gapdh-nsp7.2	-436.58
Gapdh-nsp7.3	-456.69
Transferrin-nsp4	-42.86
Transferrin-nsp7.1	-4678.10
Transferrin-nsp7.2	-4659.62
Transferrin-nsp7.3	-4680.22

The binding energy defines the affinities and stability of the bond between protein and ligand. Complexes providing the lowest binding energy per area square of interaction tends to show maximum binding efficiency. With the above data, we can conclude that transferrin shows its best interaction with the third model of nsp7 and GAPDH shows its best interaction with the first model of nsp7. Interaction of GAPDH and Transferrin with nsp4 is also significant.

Discussion:-

The increasing havoc of human infection by a coronavirus has become a hot subject of research for scientists worldwide. Best control measures that have been concluded by WHO are to take precautions for the spread of pandemic like covering your mouth while coughing and sneezing, sanitization of hand and infected areas, etc. However, this doesn't help in curing the infected patients. Human infected by SAR-CoV-2 needs to be registered with strong antiviral chemicals. Coronavirus attaches host by spike protein which gets cleaved by host protease allowing the entry of virus by endocytosis or by direct fusion of the virus with host membrane. The genomic of the virus thus entered in host cell gets translated and proteases of itself cleaves this protein leading to the development of multiple nsp (Fehr and Perlman, 2015). Zinc inhibits RNA synthesis of coronavirus. A more than 50% reduction of overall RNA-synthesis was observed at a Zn2+ concentration of 50 µM, while less than 5% activity remained at a Zn2+ concentration of 500 µM (Aartjan et al.,2010). 3CLP is an essential viral protein for the viral replication cycle, and as a result, becomes an attractive target for anti-SARS drug development (Yang et al.,2003;Anand et al., 2003; Yang et al., 2005). 3CLP inhibitors are among the first SARS-CoV inhibitors that were discovered by screening compound libraries using a fluorogenic peptide as the substrate and with structure-based design on the basis of the crystal structures of the product-bound form of 3CLP (Ramajayam et al.,2011,Kuo et al., 2009 ,Ramajayam et al., 2010a,Hsu et al., 2004). The compounds identified include zinc or mercury conjugates (Lee et al., 2007;Wu et al.,2004), C2-symmetric diols (Shao et al., 2007), peptidomimetic α,β-unsaturated esters (Shie et al., 2005a), anilides (Shie et al., 2005b), benzotriazole (Wu wt al., 2006), N-phenyl-2-(2-pyrimidinylthio) acetamide (Tsai et al., 2006), biphenyl sulfone (Lu et al., 2006), glutamic acid and glutamine peptides possessing a trifluoromethylketone group (Shao et al., 2008), pyrimidinone (Ramajayam et al., 2010b), and pyrazoleanalogs that can also inhibit 3Cpro of picornaviruses CV-B3 (coxsackievirus), EV-71 (enterovirus) and RV-14 (rhinovirus) (coronavirus and picornavirus dual inhibitors) (Kuo et al., 2006,Ramajayam et al., 2010a). A broad-spectrum drug α-Ketoamides have been used for controlling the replication of coronavirus. This drug inhibits the replication of Corona and Enterovirus (Zang et al., 2020). Antibiotics cannot control virus replication and this is been known since viral discovery. Molecular docking is an in silico method used to identify the interaction of anticipated molecule with the target protein. The salt bridge that is observed between Arg21 and Asp299 of nsp7.2 and transferrin also Asp67 and Arg197 of nsp7.1 and GAPDH may play a very important role in the disruption of viral replication (Shen et al., 2011). HAWKDOCK server uses Molecular -Mechanics/Generalized Born Surface Area (MM/GBSA), which are more theoretically rigorous than scoring functions, have been widely used to predict binding free energies and identify correct binding conformations for protein-protein systems (Goaqi et al., 2019). Molecular docking helps us understand possible interaction between peptide and ligand. However, it doesn't confine similar interaction in vivo.

Conclusion:-

GRAMM-X docking shows promising interaction of antibacterial protein GAPDH with nsp4 and all three models of nsp7 of SAR-CoV-2. Transferrin an iron carrier protein, also showed interactions with nsp4 and all models of nsp7. Docking results showed no disulfide bonds. The obtained salt bridge and hydrogen bond interactions give us scope to consider the antibacterial protein as posible sources of drugs that can controlling replication of coronavirus.

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