"DEVELOPMENT OF NOVEL VACCINE DEVELOPMENT STRATEGY BASED ON MULTIPLE RECEPTORS INTERACTION WITH CoV2 SPIKE PROTEIN"

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT
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Submitted by
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June - 2022

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CERTIFICATE

I hereby certify that the project dissertation titled **'Predicting potential vaccine development from binding of multiple binding receptor with spike protein'** which is submitted by **Aakriti Kumari**, **2K20/IBT/01**, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

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CANDIDATE'S DECLARATION

I, Aakriti Kumari, 2K20/IBT/01, student of M.Tech (Industrial Biotechnology), hereby declare that the project dissertation titled **'Predicting potential vaccine development from the binding of multiple receptors with spike protein'** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree, diploma associateship, fellowship or other similar title or recognition.

Place: New Delhi
Date: 26 May 2022

Aakriti Kumari
2K20/IBT/01

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Aakriti Kumari

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ABSTRACT

A severe acute respiratory syndrome known as covid19 was a threat to mankind globally in the year 2020 and 2021. To date, 48 crores confirmed cases have been reported according to WHO. The spike protein of coronavirus is essential for host cell recognition and host infection. It engages ACE2 for entry into the host cell. However, there is growing evidence that the spike protein and other structural protein binds with multiple receptors for viral infection. The key to tackling this pandemic is to understand the receptor recognition by virus and its variability in host cell infection and associated side effects. Our studies predicted interaction between S protein and multiple human receptors, Toll-like receptors (TLR), Mannose receptor, Heat shock protein HSPA5 or glucose-regulated protein (GRP78), and Ezrin, using molecular docking and structural bioinformatics. Carbohydrate moieties present outside the surface of spike protein which is known to be the driving force for internalization, inflammation, and infection of the virus. Our Docking result shows that HSPA5 has the most proficient binding with spike protein after ACE2 where threonine residue of the spike protein of A chain docked with Arginine of the receptor. Understanding the binding sites for receptors with spike protein, nucleocapsid protein, membranous protein, and envelope protein may help to fight the disease and find a better vaccine coverage area. Hence, our research article provides better vaccine coverage by finding the amino acid binding site using in-Silico methods.

KEYWORDS: SARS CoV2, protein-protein docking, spike protein, ACE2, TLR, mannose, Ezrin, HSPA5, binding energy, docking score

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CHAPTER 1 – INTRODUCTION

SARS CoV2 rapidly appeared in late December 2019 in China, Wuhan city, and was declared a pandemic by (the World health organization) WHO in march 2020[1][2]. It has caused more infections, death, and economic disruption than in SARS CoV 2002-2003. The origin of the virus is unclear; however, many scientists believe that it has originated from bats and is transmitted from bats to humans via seafood [3]. Four strain of beta coronaviruses is alpha, beta, gamma, and delta. Human coronavirus has seven different strains HKU1, OC43,229E, and NL63 which cause the common cold, and SARS CoV2 – SARS CoV which is responsible for Covid19[4]. SARS CoV2 has a positive single RNA strand as its genetic material and require mammals and human as host to replicate [5][6]. S1 and S2 are monomers of S protein which is a homotrimer and have three peculiar domains(i) Intracellular domain (ii) transmembrane and (iii) Extracellular [12]. S1 subunit forms a receptor-binding motif comprising of the receptor-binding domain and N-terminal domain and a study shows this motif is attached to ACE2 for entry and infection in humans [7]. S2 subunit has a helix domain, an attached domain and a dimer domain that facilitates the connection between the host cell membrane and virus [8] [9]. Transmembrane serine protease 2 cleaves the furin cleavage site located between S1 and S2 for a briefing of spike protein, important for infection [10] [11] As shown in literature, another proteolytic cleavage is present in the S2 subunit which is utilized by fusion peptide, for host cell fusion and penetration[1][6][7].

Some population of patients with comorbidities like diabetes, blood pressure, and heart disease is at greater risk of getting infected by covid19[12] [13]. For the pathogenesis of these comorbidities, our body has renin-angiotensin. The predominant metabolite of the renin-angiotensin system is angiotensin2 (AngII) which is an exclusive vasoconstrictor for blood pressure, diabetes, and heart disease [14] [15] Angiotensin-converting enzyme(ACE2) counterbalanced the production of AngII. ACE2 is utilized by SARS CoV2 for entry and infection of alveolar epithelial cells [16]. A study based on qualitative theory states that patients with comorbidities take AngII receptor blockers and ACE inhibitors could increase the binding sites of SARS CoV2 and lead to severity of coid19 [17] [18]. However, studies reported that S protein may injure multiple receptors for its binding with the host the t cell and cause infection in intra and extrapulmonary immune and non-immune cells deficiency [19] [20] [21]

In Silico studies shows the binding within S protein and Toll-receptors (TLRs; TLR1, TLR2, and TLR8), Ezrin, Heat shock protein A5(HSPA5), Mannose receptor. Moreover, Receptors like Ezrin and DPP4 are yet to be confirmed whether they bind to SARS CoV2 protein or not. Thus these receptors and proteins facilitate receptor-dependent blocking of the S-protein and activate viral infection. So, we performed docking between receptors and protein to predict amino acid binding residue and compared it with ACE2 as a reference. our research article provides potential therapeutic targets for covid19, which are present at the binding sites of multiple receptors.

1.1 Receptors involved in SARS CoV2

Angiotensin-converting enzyme 2(ACE2)

Angiotensin is a Decapeptide ion converted by an angiotensin-converting enzyme into octapeptide AngII, which activates the AngII type I receptors to subdue contractility. Literature shows that this conversion leads to increased fibrosis, inflammation, thrombosis, and pulmonary damage. Heptapeptide angiotensin (Ang1-7) is produced by conversion of ACE2 to AngII because it results in the production of Mas receptor and protects from lung injury [22][23]. So it can be said that an imbalance of ACE2 and Ang (1-7) decreases AngII and leads to acute lung injury [22]. This is reported in major heart patients and causes severe symptoms. Majorly all research supports the fact that spike protein uses ACE2 as a host for viral replication [10] [11]. S1 subunit of S protein contains receptor binding motif, the catalytic ectodomain N –terminal present extracellularly also called as the peptidyl domain of ACE2 which leads to the formation of SARS CoV2/ACE2 complex [10]. SARS CoV2 complex for endosome by undergoing endocytosis due to activation of serine protease 2 by SARS CoV2 for S briefing [11]. The viral strand of RNA enters the cytoplasm for transcription and translation in the encapsulated form of the endosome [11]. At the same time if the patient gets infected with SARS CoV2 the level of AngII increases in plasma concentration. Interleukin 6 and 1 in human lung cells and tumor necrosis factors are released from activated B-cells of infected patients which stimulate kappa light chain enhancer [24][25]. Combining these studies of ACE2 suggests the inactivation of Ang (1-7)/MasR pathway due to activation of AngII which trigger pro-inflammatory cytokines, which leads to hyperactivation of ACE2 in patients infected with SARS CoV2.

Heat shock protein A5(HSPA5) or glucose-regulated protein 78(GRP78)

It is a chaperone protein present in the ER and controls the unfolded protein response for maintenance and protein surveillance, this cell undergoes stress by the accumulation of incorrectly folded protein. Literature showed that HSPA5 is present in the lumen of ER, activation of TF 6, inositol enzyme and enhancing protein folding, initiate cell death [26,27]. Unfolding of protein results in GRP78 which translocate into the plasma membrane. After translocation GRP78 can recognize RNA virus via the substrate-binding domain [28]. In response to pulmonary trauma and injury, damaged epithelial cells release GRP78 in infected Covid patients. Inflammation in Covid patients can be increased because of GRP78 acts as a damp for (TLR2 AND 3) [29][30]. So various literature on GRP78 shows that it can act as a viral entry into the host and in Silico studies prove the binding affinity of GRP78 with S protein.

Ezrin

This is encoded by the EZR gene which belongs to Ezrin-radixin the eosin family [31]. Due to the Deletion of genes in Ezrin, and B-cells, there is an increase in inflammation and key anti-inflammatory markers [32]. The role of Ezrin during viral infection can be studied in (HIV-1) [33]. By the inhibition of unnecessary membrane, fusion GRP78 facilitates the viral entry into the host. Spike protein uses the FERM domain to interact with GRP78[34]. Ezrin is an agonist

that could be the systematic approach to inhibiting SARS CoV2 viral entry. Our Silico study shows the binding of affinity of Ezrin with spike protein but there needs to be more research on Ezrin for SARS CoV2 infection.

Mannose receptors

It is CD206 glycoprotein which is expressed on dendritic cells and macrophages [35]. MR extracellular domain contains 8CRD which particularly binds to mannosylated residues [36] [37]. But the interaction of mannose and spike protein study is not yet clear, this may be due to the presence of mannose present in S protein [38]. Bioinformatics studies show that the N-terminal domain S1 subunit where mannose glycosylated positions showed their presence on the S protein which interacts with the mannose receptor [39]. Literature shows that highly mannosylated regions of the S protein showed strong binding affinity by the CRD of MR in kidney cells [38]. This should be noted that the severity of Covid 19 increases due to the highly mannosylated region of S protein which binds to the CDR region of MR.

Toll-like receptors (TLRs)

TLR is an integral protein part of innate immunity which hosts foreign pathogens [41] [42]. TLR is also called the first line of defense against invading pathogens [40]. TLRs contain 3 structural compounds (i) an Intracellular N-terminal domain(ii) a transmembrane domain (iii) an extracellular C domain rich in leucine repeats which bring variety to TLRs [43]. To eliminate and neutralize invading pathogens, TLR responds to an inflammatory response by PAMP (pathogen-associated molecular patterns [41] [42]. Injured, stressed, and necrotic cells independent of infection release DAMP (damaged associated molecular patterns), where TLR responds to these DAMP [44] [45]. TLR 3,4 and TLR (1,2,4-10) are adapters inducing (TRIF) pathways and factor-88 dependent pathways respectively are compulsory among TLR pathways, independent of the origin of the activating ligand[46][47]. TLR is expressed in the human respiratory system displaying heterogeneity in different cell inhabitants [40]. The presence of TLR on the cell surface is shown in molecular docking studies and has demonstrated a strong binding affinity of S protein with TLR 2, 4, and 6 [48]. Vaccination reporting particularly TLR agonist and antagonist may have a systematic approach for the moderate immune response that prevent viral disease and enhance viral immunity for removal in Covid-19 patient. Further, we have done the docking of specific TLR with spike protein to study binding energy.

TLR2/6

They are heterodimers that recognize specific glycoproteins and contribute to inflammatory response during viral infection. Thus this specifies a limited role in antiviral immunity [49][50]. Serum collected from Covid patients has shown increased levels of TLR 2/6 found in the periphery of mononuclear cells. Thus literature shows that a direct association between DAMP and TLR and are not able to mediate inflammatory response [46][47]. CD36 on the virus mediates its entry upon recognition by TLR 2/6[51]. It can be postulated that the gateway for S protein is provided by the trafficking of TLR2/6 present on the cell surface. Recent studies state that MMG11 and CuCpt which are TLR2 and TLR6 inhibitors respectively can be potential covid19 therapeutics [52] [54]. Combining the in-vivo and in-vitro studies, it

can be postulated that CuCpt22 can reduce the organic phenomenon of IL-6 in lung epithelium cells.

TLR 3

It is responsible for recognizing dsRNA and mediates viral immunity by interacting with viral PAMP. Activation of NF-K beta and interferon regulatory factor 3 or 7 are done exclusively by TLR-3[54]. This activation results in the release of IL-6,8 and TNF-alpha [55]. Recent studies from alveolar epithelial cells from an infected person of Covid state that 48hour post-treatment with synthetic poly (I: C) can reduce the viral burden. Due to this treatment, TLR3 rapid antiviral response and is prime accused in the immune system [56].

TLR 4

It recognizes lipopolysaccharide from bacterial gram-negative immunity [57]. For unregulated chronic inflammatory response and autoimmune disease, TLR4 responds to DAMP. The level of TLR4 was found to be high from the samples of infected Covid patient. This study is supported by one of the reports of Covid patients which shows the high performance of cytokines and chemokines which are released by TLR4 activation pulmonary pathogenesis [58]. Furthermore, due to the recognition of pathogens by TLR4, it can be the new initial position of used by SARS CoV2 independent of ACE2 expression. S1 subunit of spike protein has an affinity for TLR4.

TLR 5

Flagellin used for motility in gram-negative bacteria is found to interact with TLR5 which results in subsequent NF-KB has driven sensitivity, through conscription of MYD88and causing severity in Covid patients that can be used for treatment for Covid patients [59]. Our research showed successful docking of TLR5 with the S1 subunit of S protein.

TLR 7/8

They are located on intracellular organelles same as TLR3, which recognize single-stranded RNA and elicit pro-inflammatory responses [60]. TLR 7 and 8 share the same homology and are reported as 2 different receptors. ssRNA of HIV-1, MERS, and influenza virus has been reported to activate TLR5 for viral entry. The pro-inflammatory response releases chemokine, cytokine, INF1, and INF2 upon recruitment of TLR5[62]. It is yet unclear, whether TLR7/8 interacts directly with spike protein but it still can be used for therapeutic targets as its ability to sense ssRNA. Bioinformatics analysis revealed a genomic analysis of ssRNA of SARS CoV2 shows large fragments that could be identified by TLR7/8[61]. Signal transduction by lung epithelial cells by TLR7/MyD88 pathway is necessary for IFN production [63].

CHAPTER 2- REVIEW OF LITERATURE

2.1 Severe acute respiratory syndrome: A brief overview

The first CoV was found in embryos of chicken in 1937, known as infectious bronchitis virus (IBV). Numerous CoV was found in a number of animals and birds which classified them into four divisions of the mammalian linked alpha, beta CoV, and the bird associated Gamma, delta CoV. Their presences in different animals show that they are zoonotic in origin and are infected from animals to humans.

Taxonomical classification

Family: Coronaviridae

Subfamily: Orthocoronaviridae

Order: Nidovirales

Subordination: Cornidovirineae

Scientists have extracted a complete whole-genome sequence from five patients when Wuhan city was infected by the pneumonia epidemic. The final output shows 79.5% whole genetic sequence identity with the coronavirus strain in bats having SARS strain named Bat CoV RaTG13. So it can be predicted that coronavirus was originally coined from bats and gradually transmitted to different humans and animal hosts. They were first identified as beta coronavirus SARS CoV2 and discovered to be closely associated with SARS CoV2 which was responsible to cause outbreaks in 2002 and 2004. There are 4 types of coronaviruses, two alpha coronaviruses (NL63 and 229E) and two beta coronaviruses KU1 and OC43), they circulate in humans and cause the common cold. SARS CoV2 emerged in the Wuhan city of China in December 2019, it was named as 2019 novel coronavirus (2019-nCoV) and when more genetic information was made available it was named SARS CoV2 and soon became a global threat in the early 2020s, as a result, WHO declared it a pandemic in April 2020.

2.2 Structure of SARS CoV2

Among RNA viruses, SARS CoV2 has the largest genome that is about (27-32 Kb). It has enveloped genome which contains 3 structural proteins Spike protein(S), Membranous protein(M), an Envelope protein(E), nucleocapsid protein present outside the genome, and contains 16 non-structural proteins (nsp1-16). RNA processing and replication mediated by Nsp1. The signaling pathway in host cells is controlled by Nsp2. The translated protein is separated by Nsp3. Transmembrane domain2 is present in Nsp4 and modifies ER. Replication of polyprotein is done by Nsp5. The transmembrane domain is Nsp6. Nsp7-8 form template primer RNA. Nsp9 form ssbinding protein. Nsp10 is for cap methylation of viral RNA.Nsp12 contains RNA-dependent RNA polymerase. The zinc-binding domain is present in Nsp13. Nsp14 has proof edited exonuclease activity. Nsp15 contains Mn+ endoribonuclease activity. Nsp16 has O' ribose methyltransferase. Protein trafficking, replication, transcription, and translation are major functions of NSPs to inhibit host defenses Infection is suppressed by mRNA splicing and interrupted protein synthesis by binding of NSP16 to U1, U2 domain ds SnRNAs.

Spike glycoprotein

Spike glycoprotein is the key point for entry into host cells. A homotrimer structure is formed by spike glycoprotein outside the viral surface. It is an attractive therapeutic target due to its critical entry point for coronavirus. S1 and S2 subunits are the two functional parts of the S protein. N-terminal domain(NTD) and receptor-binding domain(RBD) are present in the S1 subunit. Binding of receptor on host cell is performed by S1 subunit. Fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT) are present in S2 subunit. Spike glycoprotein is activated by the fusion of membranes of viruses and host cells; host proteases cleave the spike glycoprotein. N-linked glycan is critical for proper folding, neutralizing antibodies, and decorating the spike protein trimers extensively.

The receptor-binding domain

RBD is present in the spike protein of SARS CoV2 that recognizes the receptor ACE2 and in some cases multiple receptors also. RBD is the chief reason for antiviral compounds and antibodies. Two structural domains are present in the RBD: the core and the external subdomains. A highly conserved domain is a core subdomain. RBD comprises five beta-strands which are joined by Disulphide bonds and arranged in an antiparallel manner. The external subdomain is stabilized by a disulfide bond, Binding site of ACE2 is present in the peptidyl site of the N-terminal domain which is formed by two subunits of RBM and ACE2. RBM provides the space for the binding of receptors inside its concave part. The binding of host cell receptor and spike protein leads to conformational changes in RBD, like the formation of hinge which leads to two states, “down” conformation and “up” conformation. SARS-CoV-2 could not recognize the ACE2 on the host cells in the down conformation.

2.3 Multiple binding Receptor for SARS CoV2

Studies suggest that ACE2 shows strong binding with the RBD domain of SARS CoV2. Then the S protein is dominated by serine protease TMPRSS2, which releases the S protein subunit S2 to fuse the viral and cellular membrane. Then the viral gene enters the cell and reproduces more viruses. Our studies show that besides ACE2 there are multiple receptors present outside the host cell which have shown strong binding with the RBD domain of SARS CoV2 such as TLR, mannose receptor, GRP78, and Ezrin. The binding site of this receptor with Spike glycoprotein predicts that these areas can be used for vaccine development in the future for Covid patients.

2.4 Coronavirus in humans

In the late 1960s, first human coronavirus case was registered and were named as HCoV-229E and HCoV-OC43. HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1 are the four types of endemic that circulate worldwide in the human population. Patients who complained about the respiratory problem in these endemic are found to show mild symptoms of the common cold in the upper and lower respiratory tract. Asymptomatic infections have also been described. Acute respiratory failure can also occur in individuals with existing pulmonary diseases and immunosuppressed patients. The situation completely changed with the

appearance of the SARS-CoV. Studies suggest that SARS-related CoV are found in bats and civet cats, hence it can be predicted that firstly it is transmitted from cats to humans, followed by human-to-human transmission. Since 2004 no human infections are reported with the original SARS virus, another CoV dangerous for humans emerged in 2012.

2.5 Novel vaccine strategy for SARS CoV2

Spike glycoprotein is a vigorous choice for vaccine design. There are five kinds of vaccine strategies mRNA vaccine, replication-defective viral vector vaccine, inactivated pathogen vaccine, protein subunit vaccine, and virus like a particle. Other than these vaccine strategies the binding site of various receptors with spike protein, envelope protein, nucleocapsid protein, and membranous protein can also be used for vaccine design. Pattern recognition receptors(PRRs) is used to detect the host innate immune system in vaccine candidate and recognize the pathogen-associated molecular patterns(PAMP). Dendritic cells, monocytes, and neutrophils patrol throughout the body and attract vaccine antigens from pathogen-associated patterns. Host cells detect the pathogen and become activated through pattern recognition receptors (TLR plays an important role).

CHAPTER 3- METHODOLOGY

2.1 Sequences and data retrieval

FASTA form sequence of spike protein with protein ID YP_009724390.1 from NCBI accession number NC_045512 was downloaded [64]. The sequence was pasted on the SWISS model and spike protein with 100% sequence identity and PDB ID 6XR8 was downloaded. Structure of receptors ACE2(PDB ID 1R42), Mannose (PDB ID 6INN), Ezrin (PDB ID 1N12), HSPA5(PDB ID 3LDL), TLR2(PDB ID 2Z80), TLR3(PDB ID 2AOZ), TLR4 (PDB ID 2Z63), TLR5(PDB ID 4NX9), TLR6 (PDB ID 4OM7), TLR7 (PDB ID 6LVX), TLR8 (PDB ID 7R52) from RCSB PDB bank was downloaded.

2.2 Databases

2.2.1 Swiss model

It provided the 3-D structure of structural spike protein in PDB format based on a high similarity percentage sequence matched to the reference sequence [65]

2.2.2 HEX 8.0

This is used for protein-protein docking of spike protein with human receptors for analyzing E-total and H-bonds involved in the binding between ligand (spike protein) and receptor [66]

2.2.3 HDOCK

It is a powerful pipeline for protein-protein docking which is template-based modeling. It is different from other docking platforms because it supports amino acid sequence as input and also adjusts ligand binding. The template sequences of receptor and ligand were submitted for 3analyzing binding energy of amino acid residue, docking score, and RMSD (in Armstrong). HDOCK provides the top 20 docked models of provided template, out of which model with the lowest RMSD value was chosen because studies state that the lowest RMSD value provides a perfect docking structure [67]

2.2.4 Pymol

The result of HDOCK was analyzed with Pymol for the binding surface between receptor and ligand [68]

CHAPTER 4- RESULT AND DISCUSSION

Attachment of Spike glycoprotein of SARS CoV2 with ACE2, Mannose, HSPA5, Ezrin, TLR 2,3,4,5,6,7,8 are shown in figure 1,2,3,4,5a,5b,5c,5d,5e,5f,5g respectively. Docking is performed by HDOCK and visualized by Pymol software. Alpha helix is shown by helix while arrows show beta-sheets. Hydrogen bonding is shown by yellow dots.

Figure1: Human angiotensin converting enzyme 2 act as receptor(green) binds with spike glycoprotein of SARS CoV2(blue). Yellow dots show the docking interface region

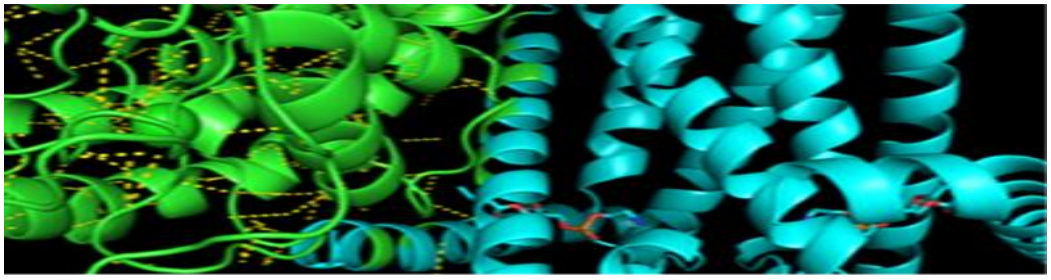


Figure2: Ezrin receptor(blue) binds with spike glycoprotein(purple). Yellow dots show the docking interface region.

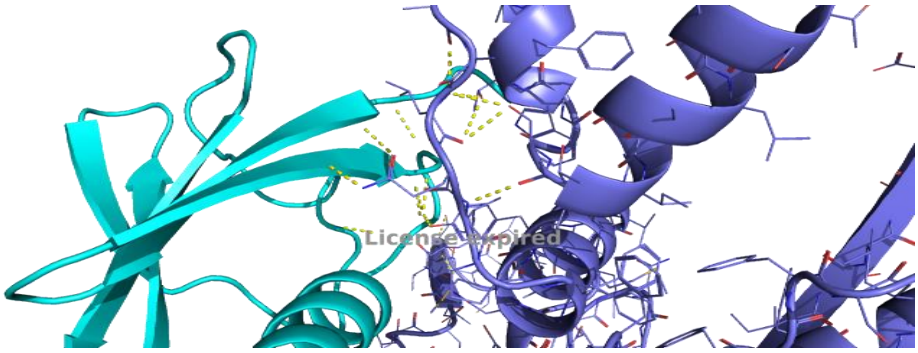


Figure3: HSPA5 or GRP78 act as receptor (yellow) binds spike glycoprotein(green). purple dots show the docking interface region.

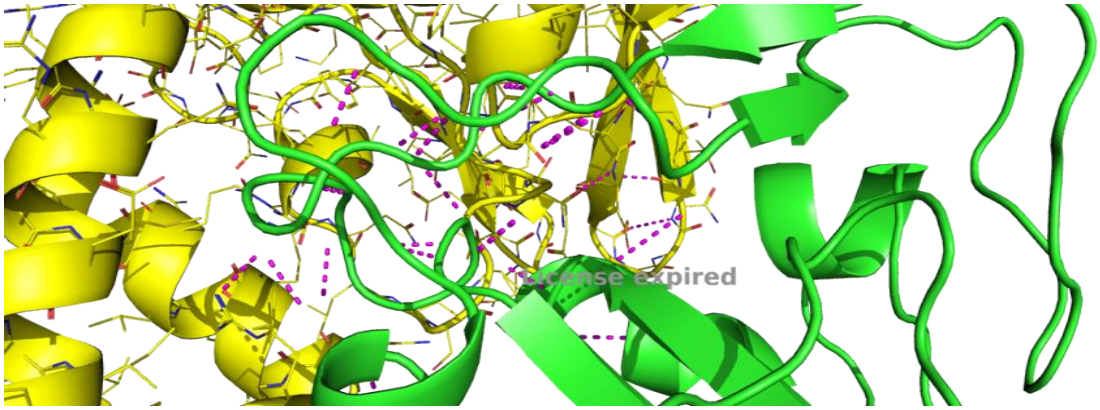


Figure4: Mannose receptor (green) binds with spike glycoprotein(blue). Yellow dots show the docking interface region.

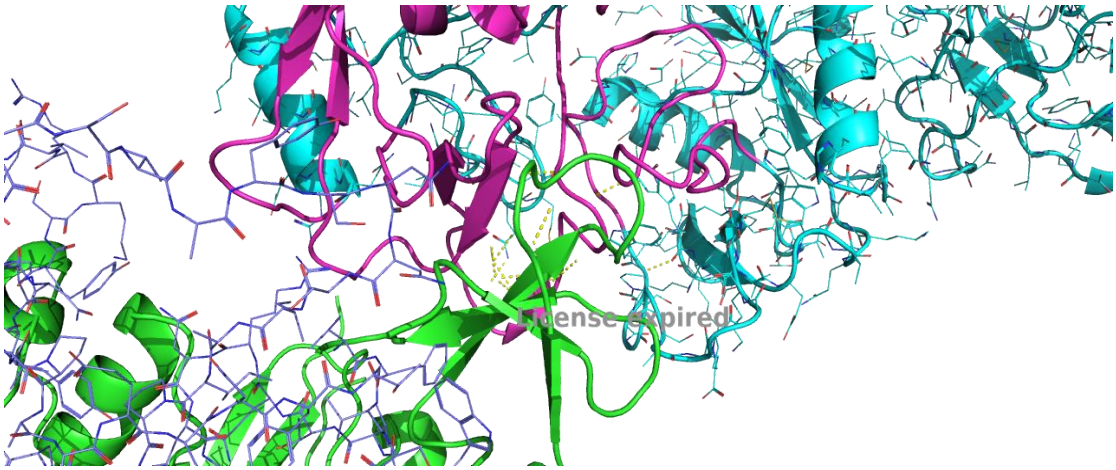


Figure5: TLR2 as receptor binds(purple) with spike glycoprotein(green). Yellow dots show the docking interface region.

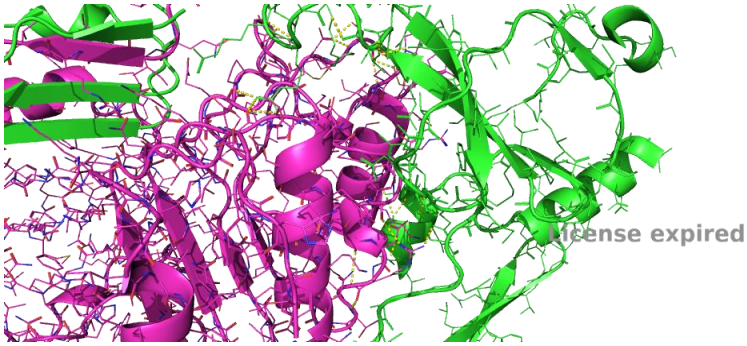


Figure5a: TLR3 as receptor binds (purple) with spike glycoprotein (green). Yellow dots show the docking interface region

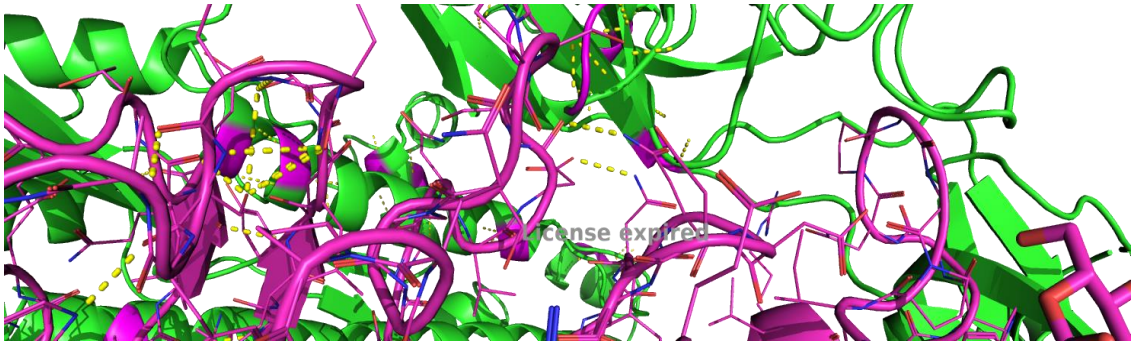


Figure5b: TLR4 act as receptor (purple) binds with spike protein(blue). Yellow dots represent docking interface region

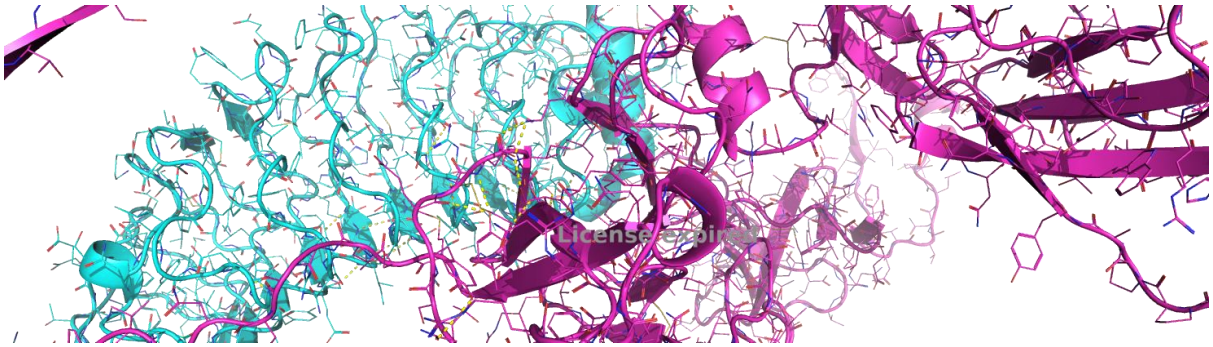


Figure5c: TLR5 act as receptor (blue) with spike protein (green). Yellow dots represent interface region.

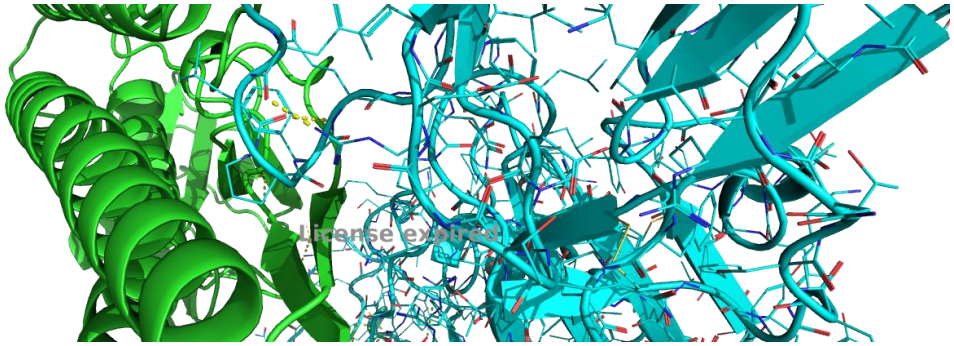


Figure5d: TLR6 act as receptor(blue) with spike protein (green). Yellow dots represent interface region.

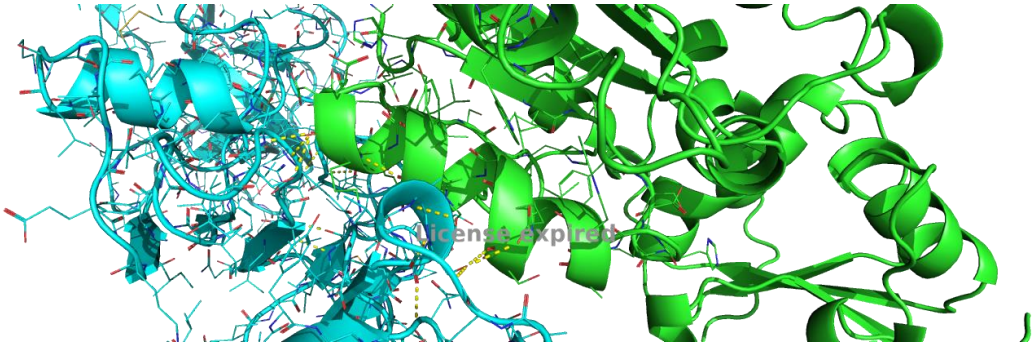


Figure5e: TLR7 act as a receptor (purple) binds with spike protein (green). Yellow dots represent docking interface region.

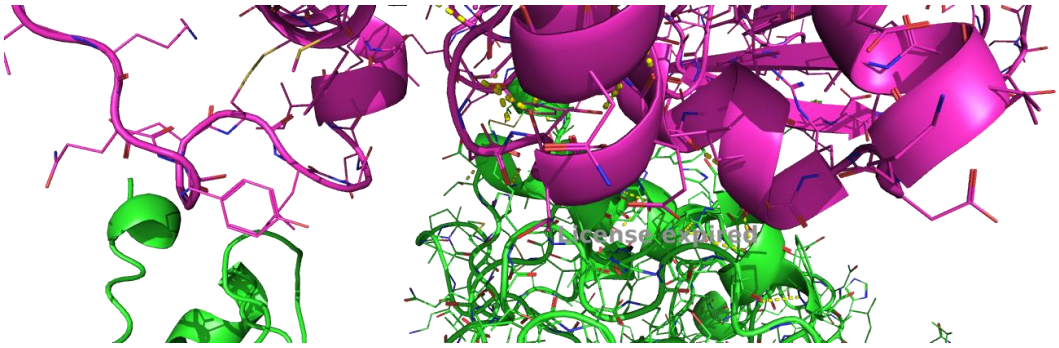
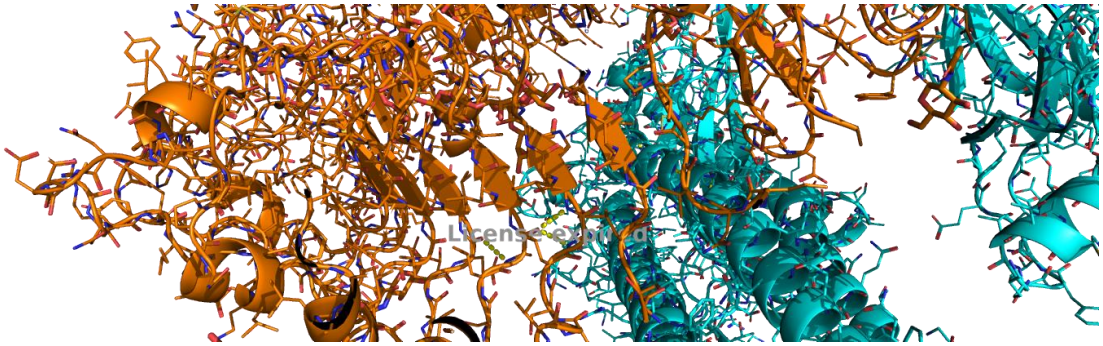


Figure5f: TLR8 act as receptor(orange) binds with spike protein(blue). Yellow dots represent docking interface region.



Several hydrogen bonds involved in the binding of these receptors and spike glycoprotein are shown in table1 and 1a, which shows that HSPA5 or GRP78 has a maximum number of hydrogen bonds 199 as compared to all other receptors. Table2 summarizes the binding energy, docking score, and amino acid involved in binding of spike protein and receptors, where it has been found that HSPA5 has the highest docking score of -213.37 among arginine and threonine in receptor and ligand respectively. Further, Ezrin, TLR6/7/8 showed no hydrogen bonding and TLR3 shows the lowest docking score between receptor and ligand.

Tables1: HEX8.0 protein-protein docking result: First-row show receptors present in human and the column shows spike glycoprotein in SARS CoV2

Spike protein/ Receptors	ACE2		HSPA5		Ezrin		Mannose	
S – protein	E-total	H-bonds	E-total	H-bonds	E-total	H-bonds	E-total	H-bonds
	-452.3	137	-241.4	199	-70.98	0	-323.8	70

Table1a: First column shows protein- protein docking of TLR receptor with Spike protein

Receptors/Spike protein	E-total	H-bonds
TLR2	-594.24	143
TLR3	-746.51	27
TLR4	-616.15	57
TLR5	-860.30	57
TLR6	-445.53	0
TLR7	-123.53	0
TLR8	-62.65	0

Table 2: Hdock result: Docking of human receptors with Spike glycoprotein of SARS CoV2

Receptor	Amino acid position		Amino acid residue		Chain		Binding energy	Docking score	RMSD
	R	L	R	L	R	L			
TLR2	319	647	ARG	TYR	B	A	4.998	-288.54	314.8
TLR3	372	1093	SER	GLY	A	A	4.951	-351.99	362.38
TLR4	497	670	ASN	ILE	A	A	4.992	-295.67	322.95
TLR5	147	577	GLN	ARG	A	A	4.958	-239.96	271.20
TLR6	725	25	HIS	PRO	B	A	4.991	-258.85	317.13
TLR7	65	124	THR	THR	B	A	4.999	-305.46	295.81
TLR8	278	1130	ILE	ILE	B	A	4.970	-276.62	303.75
MANNOSE	287	1127	ASN	ASP	C	A	4.919	-253.94	566.95
HSPA5	261	500	ARG	THR	A	A	4.985	-213.17	308.76
ACE2	453	107	TYR	VAL	A	A	4.819	-270.00	200.60
EZRIN	23	903	ASN	ALA	B	A	4.998	-216.22	214.80

This study compares the binding efficiency of different receptors with spike glycoprotein of SARS CoV2 which indicates that when the genetic material of the virus binds with HSPA5 or GRP78 leads to more complications in Covid patients. After going through several research articles it was deduced that GRP78 acts as a receptor for Mucorales (fungal hyphae). CoTH3 and CoTH2 are spore coat proteins for fungal hyphae that act as ligands for cell surface GRP78. It is found that diabetic patient has increased level of spore coat protein and as a result mucormycosis. Hence, Anti-GRP78 and Anti-CoTH3 can be used as drugs to treat mucormycosis [69]. Mannose-binding lectin increases the level of respiratory tract infection in children, greater mortality impaired lung infection leads to cystic fibrosis, hospitalization rate also increases with respiratory tract infection related to chronic pulmonary disease and high risk of pneumococcal disease [70]. Ezrin peptides can be used as immune modulators to restore effective immunity in covid-19 patients. These peptides can enhance the adaptive immune response to resolve the infection and prevent re-infection. It can save critically ill Covid patients [71]. ACE2 controls many pathophysiological effects in humans from fluctuating blood pressure, cardiovascular function, cell death, and proliferation to host cell responses. It can be said that ACE2 is a better vaccine coverage to treat Covid patients with antibodies against ACE2 which are designed based on epitopes of ACE2 with viral docking sites. Therefore, ACE2 pulmonary enzymatic activity should be given special attention before the antibody is tested preclinically and re-expressed due to activation of TLR, as a result, this adaptor molecule induces the production of type1 IFNs and inflammatory cytokines [72]. Several TLR is involved in sensing PAMP from SARS CoV2, which contribute to the antiviral response against SARS CoV2 infection. Asthma in Covid patients occurs due to dysfunction in TLR. Covid 2nd wave increases heart patients, Statins are used in cardiovascular disease. Studies showed that the efficacy of statins is affected by the occurrence of the TLR4. Acute lung injury and a higher probability of organ failure and death occur due to TLR1. Increase in susceptibility to pneumonia due to TLR5 and increase risk of asthma due to TLR6/7/8[73]. All this research showed that receptors can be visualized as a better coverage area for vaccine manufacture.

CHAPTER 6 – CONCLUSION

Spike protein is the major part of virus internalization in the host cells. ACE2 was known to be the only receptor used by SARS CoV2 spike protein for entry of host cells. Our study supports this hypothesis that spike glycoprotein can utilize mannose, HSPA5, Ezrin, and TLR as a receptor for entry of host cells other than ACE2. HSPA5 is an enzyme for protein unfolding which results in translocation of GRP78 and binds to RNA in the substrate-binding domain. Our docking results show strong binding efficiency with spike protein as compared to other receptors with mucormycosis (black fungus) in Covid patients. Ezrin is another receptor used by spike protein with its FERM domain and its agonist results in protection from reinfection of Covid. Heptapeptide is an angiotensin enzyme is produced by conversion of ACE2 to AngII from lung injury and results in heart complications in Covid patients. The mannosylated region of spike protein binds to the CDR region of the Mannose receptor and increases the Covid severity. Spike protein has been found to bound with TLR2/3/4/5/6/7/8 especially with TLR5, this results into NK-Fb driven inflammation signalling of MYD88 and severity in Covid patients. Our study suggests that binding sites of spike protein, nucleocapsid protein, membranous protein and envelope protein with these multiple receptors may show the better vaccine coverage area for SARS CoV2. We look forward to these receptor binding sites a novel therapeutic agent for the treatment of Covid-19.

CHAPTER 7- REFERENCES

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