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Brief Report

Exploring Lectin-Glycan Interactions: A Novel Glycan based Drug Discovery Approach for SARS-CoV-2 and its Challenges

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Abstract: The Severe acute respiratory syndrome-Corona virus-2 (SARS-CoV-2) which is responsible for recurring pandemics takes advantage of host-cell processes including the glycosylation pathway. The heavily glycosylated spike protein assists viruses in attachment and penetration. The N-glycans of N-terminal domain N165 and N234 play a significant role in conformational dynamics of the receptor-binding domain (RBD) with angiotensin-converting enzyme 2 (ACE2). In addition, the deletion of N-glycan sites N331 and N334 have been associated with reduced infectivity. This signifies the importance of targeting the N-glycans making them unavailable for interacting with the ACE2 receptor, ultimately leading to reduced infectivity. These glycans can be specifically targeted and can be used for designing SARS specific drugs or neutralizing molecules. In the current study, lectins Griffithsin, Cyanovirin-N, Cyanovirin homolog, and BanLec H84T were used for targeting N-glycans of the spike protein. Molecular docking programs AutoDock Vina and HADDOCK were used to study lectin-glycan interactions. The interactions look convincible in accordance with the lowest interaction energies best-fit approach but the characteristic feature (scoring functions) of the docking programs are questionable concerning Lectin-Glycan interactions exist in nature. There is a high need of the hour for the development of specific algorithms for docking glycans with a preferential selection of terminal residues.

Keywords: angiotensin-converting enzyme 2; BanLec H84T; Cyanovirin; glycosylation; Griffithsin; algorithms

1. Introduction

The Severe acute respiratory syndrome-Corona virus-2 (SARS-CoV-2) is a single-stranded RNA virus with a large trimeric spike (S) protein extending from the surface of the virion (Walls et al., 2020; Wrapp et al., 2019; J. Zhang et al., 2021). The spike protein assists the virus in attachment and penetration by binding to angiotensin-converting enzyme 2 (ACE2) of lungs, arteries, heart, kidney, and intestines and transmembrane serine protease 2 (TMPRSS2) of pneumocytes (Cui et al., 2022; Yin et al., 2022). These viruses take advantage of host-cell processes including the glycosylation pathways (Duan et al., 2020; Tian et al., 2021; Watanabe et al., 2020) to embellish their protein surface with glycans. Of all the four envelope proteins, spike protein is extensively glycosylated, which is responsible for viral attachment, fusion, and entry (Benton et al., 2020). Recent glycan profile characterization studies have identified 22 N-glycosylation and 2 O-glycosylation sites (Shajahan et al., 2020; M. Wang et al., 2021; Watanabe et al., 2020; Y. Zhang et al., 2021). The glycans are host-specific (Y. Zhang et al., 2021) and composed of high mannose, hybrid and complex type structures. The N-glycans N165 and N234 play an indispensable role in modulating the conformational dynamics of the receptor-binding domain (RBD) thereby influencing ACE2 recognition (Casalino et al., 2020) whereas reduced infectivity is associated with deletion of N331 and N343 glycosylation sites (Li et al., 2020). Similarly, the N-glycans of the S2 subunit may play a significant role in S1/S2 cleavage and membrane fusion priming (Lavie et al., 2022; Peacock et al., 2021). The overall analysis of spike

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proteins reveals the presence of Ser494 in the receptor-binding loop (RBL) of SARS-CoV-2 alone. Which could be a potential O-glycan site in making and could play a prominent role in the recognition of ACE2 and RBD binding dynamics. The molecular dynamic (MD) studies by Rahnama et al., (2021) predicts that O-glycan at Ser494 increases the RBD-ACE2 binding affinity. The N-/O-glycans serve as novel antiviral therapeutic drug targets as they exhibit a pivotal role in viral attachment and penetration (Zhao et al., 2021). The current study focuses on proteins like lectins, which have a high binding affinity to glycans serving as potent antivirals.

Lectins are a group of non-immunogenic proteins, which are ubiquitously available in nature, having a high affinity toward glycans and carbohydrates without modifying them (Casalino et al., 2020; Rüdiger & Gabius, 2001). They play a significant role in numerous biological activities such as antiviral (Gondim et al., 2019; Mitchell et al., 2017), antibacterial (El-Araby et al., 2020; Marques et al., 2018), antifungal (da Silva et al., 2018), insecticidal (Macedo et al., 2015), anticancerous (Hung & Trinh, 2021), mitogenic and apoptosis-inducing activities (K. Wang et al., 2019) due to their high propensity towards glycans. The mechanism of interactions and diversity of consequences concerning lectin-glycan interactions are not clear. The said interactions are being exploited in designing antivirals for enveloped viruses such as Porcine delta coronavirus (Tang et al., 2022), influenza (Covés-Datson et al., 2020), Ebola Virus (Covés-Datson et al., 2019), Human Immunodeficiency Virus (HIV) (Hopper et al., 2017; Zhou et al., 2018), Hepatitis C Virus (HCV) (Takebe et al., 2013). In the current study, lectins Griffithsin, Cyanovirin, and BanLec were investigated against SARS CoV-2.

The hololectins with *jacalin-like* domains Griffithsin (GRFT; 2GUC; Griffithsia species) and BanLec (4PIU; *Musa acuminate)* along with Cyanovirin-N (CV-N; 3GXZ; *Nostoc ellipsosporum*) and its homolog Cyanovirin-N (Cyt-CVNH; 5K79; *Cyanobacterium Cyanothece*) were investigated for their antiviral potency against SARS-CoV-2. These lectins with two/three carbohydrate-binding domains have high specificity toward the mannose residues and usually exist in dimeric form. GRFT has 121 amino acids with three carbohydrate-binding domains having Tyr (28, 69, and 110) and Asp (30, 71, and 112) each playing a significant role in the interaction of GRFT with mannose residues of glycans (Lee, 2019). The due presence of three carbohydrate-binding domains and being accompanied by the 'GGSGG' domain ensures high binding specificity towards mannose residues and makes GRFT a potent broad-spectrum antiviral against the enveloped viruses like HIV, HCV, and CoV (Lee, 2019). The *In-vitro* inhibitory effect of GRFT individually against SARS CoV-2 infection and in combination with inhibitor EK1 has been reported (Cai et al., 2020), which deliberates the necessity of composite drug combinations in nullifying the development of resistance against the drugs. On contrary, CV-N has 101 amino acids with two carbohydrate-binding domains having a tetrad of asparagine and glutamate residues (Asn42, Asn53, Glu41, and Glu56) in them (Bewley & Otero-Quintero, 2001). The disulfide bridges present between Cys8-Cys23 and Cys58-Cys73 play a major role in maintaining the binding site intact. Matei et al., (2016) and their coworkers have identified a new homolog of Cyanovirin-N (Cyt-CVNH; *Cyanobacterium Cyanothece*) from cyanobacterium Cyanothece which has 4-fold higher potency than CV-N against HIV-1. Moreover, the wild-type BanLec with 149 amino acids and two carbohydrate-binding sites is a potent T-cell mitogen (Singh et al., 2014) besides inhibiting viral entry, transcription, and translation. Lectins identified so far just inhibit the viral entry but BanLec inhibits transcription and translation too. With due unique features, it has become the lectin of importance. Swanson et al., (2010)and their co-workers engineered BanLec and successfully created a mutant BanLec F84T and BanLec H84T uncoupling the mitogenicity besides preserving the lectin or antiviral activity. BanLec F84T is less mitogenic whereas BanLec H84T is non-mitogenic. *In vitro* and *In vivo* studies determining the antiviral activity of BanLec against HIV, Influenza and Ebola have been reported. Astonishingly, BanLec not only inhibits viral entry but also replication and transcription of enveloped viruses (Covés-Datson et al., 2019, 2020; Swanson et al., 2010) These studies confirm that BanLec serves as a promising broad-spectrum antiviral, anti-replication, and anti-transcriptional therapeutic drug.

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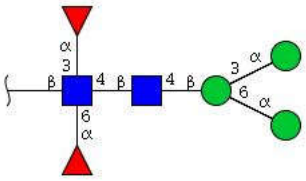
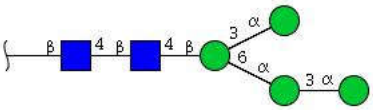
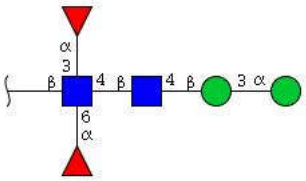
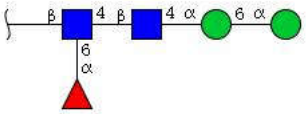
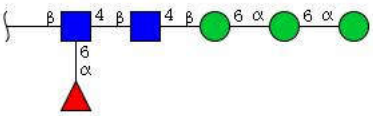
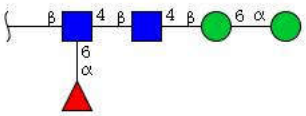
In the current study, we have employed molecular docking approaches for studying their antiviral potentials against SARS-CoV-2.

2. Results and Discussion

The affinity values of glycans docked against the lectins GRFT, CV-N, Cyt-CVNH, and BanLec H84T were obtained using the multiple ligand docking suite of EasyDockVina (doi.org/10.5281/zenodo.3732170) (Supplementary Table I). The glycans were ranked according to their binding affinities against each lectin, from which the top five were considered for manual docking using AutoDock Vina. The symbolic nomenclature of glycans (SNFG), calculation of mass to charge (m/z) ratio, and LINCUS linear description of selected glycans are tabulated (Table I).

Table I. Glycans selected for manual docking using AutoDock Vina (This includes the Glycan ID, SNFG, m/z ratio and LINCUS linear description).

Glycan ID	SNFG	m/z	LINCUS
7		1519.7615	[][b-D-GlcpNAc]{[(3+1)][a-L-Fucp]{}[(4+1)][b-D-GlcpNAc]{[(4+1)][b-D-Manp]{[(3+1)][a-D-Manp]{}[(6+1)][a-D-Manp]{}}}[(6+1)][a-L-Fucp]{}}
12		1375.6828	[][b-D-GlcpNAc]{[(4+1)][b-D-GlcpNAc]{[(4+1)][b-D-Manp]{[(3+1)][a-D-Manp]{}[(6+1)][a-D-Manp]{[(3+1)][a-D-Manp]{}}}}}
14		1315.6617	[][b-D-GlcpNAc]{[(3+1)][a-L-Fucp]{}[(4+1)][b-D-GlcpNAc]{[(4+1)][b-D-Manp]{[(3+1)][a-D-Manp]{}}}[(6+1)][a-L-Fucp]{}}
17		1141.5725	[][b-D-GlcpNAc]{[(4+1)][b-D-GlcpNAc]{[(4+1)][a-D-Manp]{[(6+1)][a-D-Manp]{}}}[(6+1)][a-L-Fucp]{}}
18		1345.6723	[][b-D-GlcpNAc]{[(4+1)][b-D-GlcpNAc]{[(4+1)][b-D-Manp]{[(6+1)][a-D-Manp]{[(6+1)][a-D-Manp]{}}}}[(6+1)][a-L-Fucp]{}}
19		1141.5725	[][b-D-GlcpNAc]{[(4+1)][b-D-GlcpNAc]{[(4+1)][b-D-Manp]{[(6+1)][a-D-Manp]{}}}[(6+1)][a-L-Fucp]{}}

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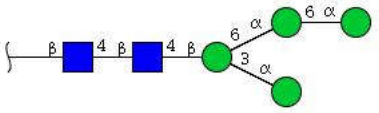
28		1375.6828	[][b-D-GlcpNAc]{[(4+1)][b-D-GlcpNAc]{[(4+1)][b-D-Manp]{[(3+1)][a-D-Manp]{}[(6+1)][a-D-Manp]{[(6+1)][a-D-Manp]{}}}}}

The interacting residues, affinity, and the root mean square deviation (RMSD) values of the selected glycans against lectins are listed in Table II. On visual observation, the glycan molecule was properly fitting into the surface and cavity of the lectins (Fig. I) with well-established interactions. The *in-silico* results look convincing but cannot be verified experimentally as two major factors need to be considered. In *in-silico*, the orientation of the glycan binding to the lectin is not comparable to that available in nature. To be clear, The core of the glycan is readily attached to the protein - making only the terminal residues available to interact with the lectins. The statement is further supported by the papers published till-to-date (Bewley & Otero-Quintero, 2001; Lee, 2019; Matei et al., 2016), which describe the specificity of lectins against the definite *terminal* residues but not the whole glycans. Furthermore, lectins have well characterized carbohydrate-binding regions (CBRs) but in the case of *in silico,* the whole glycan is fitting perfectly on the lectin surface with non-specific interactions. Lokhande et al., (2020) have also evidenced similar results with the molecular docking program FlexX and simulation studies using the Desmond module. Unfortunately, such results can lead to erroneous downstream experiments, which will be catastrophic to the drug discovery community. The best among all the docking programs for studying molecular interactions in humans is HADDOCK (Dominguez et al., 2003; Pagadala et al., 2017), which has also produced similar results. The other flexible docking program AutoDock Vina with Monte Carlo methods, knowledge-based scoring function, and local optimization Broyden-Fletcher-Goldfarb-Shanno (BFGS) method has reproduced the same erroneous results (Trott & Olson, 2010). Regrettably, the above said molecular dynamic (MD) studies have also suggested a similar kind of interaction, which cannot be considered anymore as the molecular docking and simulation programs run on a similar algorithm of finding the best fit with the lowest interaction energies or low-scoring conformations. The scoring functions of docking majorly fall into three categories: (a) force field-based, (b) empirical-based and (c) knowledge-based. The force field-based programs refer to the orientation of the molecules in six dimensional rotational or translational space based on their potential energies (Fischer et al., 2010; Kang et al., 2014). Empirical-based programs predict the best pose with lowest binding affinity (Guedes et al., 2018). Knowledge-based programs construct frequency distributions of the interactions and perform statistical analysis of the complexes (Meng et al., 2011). None of these scoring functions can be employed for studying the interactions of glycans with lectins and/or any other biomolecules. In Pagadala et al., (2017) the accuracy and/or efficiency of docking programs are systematically reviewed.

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Table II. The interacting residues, affinity and RMSD of the glycans against lectins.

Lectin	Glycan ID	Interacting residues	Affinity (kcal/mol)	RMSD	
				L.B.	U.B.
GRFT (PDB ID: 2GUC)	7	Arg81, Ser96, Asn97, Asn60, Ser59, Tyr57, Thr62, Ser73.	-8.6	1.217	2.200
	12	Thr94, Asn97, Ser59, Thr76, Asn60, Ser96, Glu75, Ser73, Arg81.	-8.4	1.463	2.150
	14	Thr62, Ser73, Asn97, Asn77, Tyr57, Ser59, Asn60, Ser96.	-8	1.420	2.049
	17	Ser73, Asn60, Thr62, Asn97, Thr94, Ser96.	-8.4	5.355	11.229
	18	Thr62, Arg81, Asn60, Ser96, Asn97, Asn71, Arg64, Glu75.	-8.6	4.226	13.833
	19	Met78, Arg81, Ser73, Thr62, Thr94, Asn60, Asn97.	-9	1.282	2.209
	28	Ser73, Thr94, Thr62, Asn60, Ser96, Asn97, Ser59, Arg81.	-8.7	3.380	8.819
CVN (PDB ID: 3GXZ)	7	Thr83, Cys58, Cys73, Ser52, Gln50.	-7.7	3.649	11.229
	12	Ser33, Gln50, Ser52, Asp35, Ser38, Leu98.	-7.7	1.757	4.088
	14	Gln50, Cys73, Gln78, Asn53, Thr57, Arg76	-7.5	7.698	14.194
	17	Thr83, Ser38, Asn37, Asp35.	-7	2.380	11.398
	18	Leu98, Thr97, Lys99, Ser33, Asp35, Ser52, Ser38.	-7.9	5.582	15.878
	19	Thr57, Asn53, Cys58, Thr83, Ser52, Pro51, Gln50.	-7.1	9.105	15.487
	28	Leu87, Ile91, His90, Ser33, Lys99, Asp35.	-7.5	3.161	10.365
Cyt-CVNH (PDB ID: 5K79)	7	Thr104, Glu32, Asn31, Tyr107, Thr17, Asn36, Ser34, Ser19	-7.8	0.923	1.561
	12	Ser19, Asp14, Glu32, Thr17, Ser16, Asn36, Ser34	-6.8	3.191	10.390
	14	Ser16, Asn36, Ser34, Thr33, Asn31, Thr104, Glu32, Leu105, Thr17.	-7.7	2.908	12.153
	17	Thr17, Ser34, Asn36, Ser19, Leu105, Glu32, Ser16.	-7.5	2.073	2.676
	18	Lys24, Ser34, Glu32, Ser19, Thr17, Asn36, Thr104, Asn31.	-7.3	4.916	8.247
	19	Leu105, Ser34, Ser19, Asn36, Thr17, Ser16, Thr104, Asn31, Glu32.	-7.6	3.546	6.910
	28	Asp14, Ser19, Asn36, Ser16, Thr12, Glu32, Ser34, Thr17.	-7.1	23.697	27.606
BanLec H84T (PDB ID: 4PIT)	7	Ala17, Asp19, His54, Ser58, Gly56, Gly59, Lys130.	-7.2	2.300	12.497
	12	Ser16, Tyr55, Tyr46, Asp19, His54, Thr52, Arg53, Gly56, Gly128.	-7.1	4.279	11.008
	14	Gly56, Ser58, His54, Thr52, Asp19, Met20, Tyr46, Arg53, Tyr55.	-7.1	5.284	8.281

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17	Phe131, Gly59, Ser58, Lys130, Asp133, Gly128, Ser16.	-6.9	28.881	32.402
18	Met20, Gly56, Gly128, His54, Tyr46, Arg53, Tyr55.	-7.3	27.333	31.705
19	Thr52, Arg53, Ser58, Gly56, Gly128, Gly129, His54, Asp41, Thr50.	-6.6	27.019	29.059
28	Tyr55, Arg53, Gly59, Gly129, Ser58, Gly56, His54.	-7.4	27.058	29.696

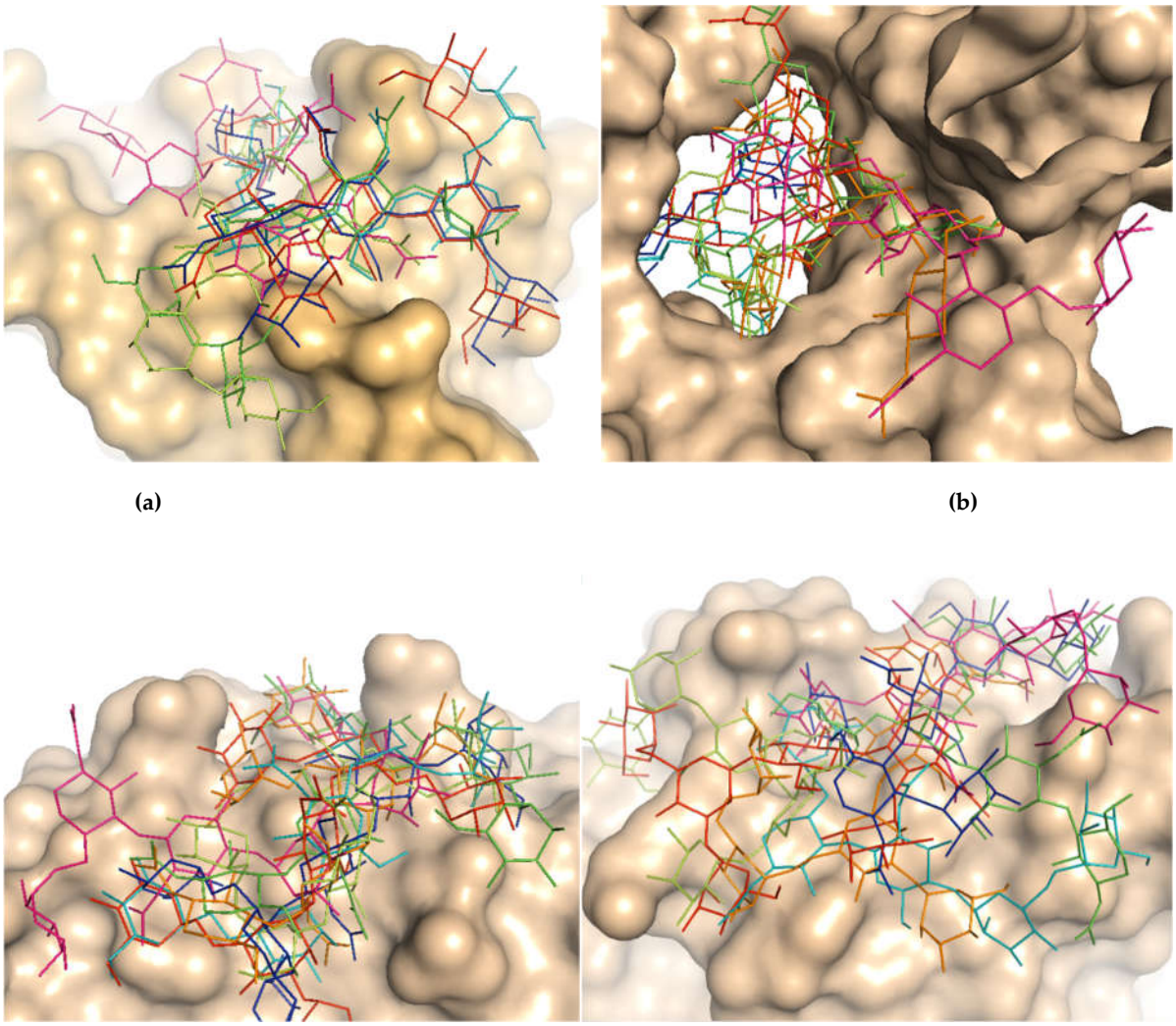

Figure I. The results obtained from docking studies shows the interaction of lectin (a) Griffithsin, (b) Cyanovirin-N, (c) Cyanovirin Homolog, and (d) BanLec H84T with selected glycan 7 (red), 12 (green), 14 (blue), 17 (limon), 18 (hot pink), 19 (teal) and 28 (orange). The ligands can be observed showing non-specific interactions and fitting onto the surface.

Possibilities include the docking of terminal monosaccharides as individual molecules with lectins followed by superposing the bound monosaccharide with the whole polysaccharide or glycoprotein of our interest. The prepared molecule has to be energy minimized before using for further downstream processing. The other way is to nullify the hydroxyl groups of all the saccharides, which are to be excluded from the interaction, and proceed for docking. Chemists, Biologists, and Computer scientists have to come

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forward with sophisticated tools like pdb4amber or antechamber for the generation of output files of the required format and/or specific algorithms for docking glycans with a preferential selection of a particular monosaccharide. On the other hand, the CBR data of lectin specificity from 3D structure analysis, Isothermal titration studies even be further confirmed by either experimentally or MD studies. As observed in the case of Snake gourd seed lectin (SGSL) where although the lectin chain of SGSL has two binding sites in the 1α and 2γ subdomains but only one can retain (2γ) the Me-α-Gal (Chandran et al., 2018). The results from the docking softwares should not be blindly trusted but should be corroborated with the similar structures available in the pdb database.

3. Conclusion

The arena of bioinformatics is taking huge strides but only a minuscule advancement is observed in the area of glyco-informatics. The robust methods of docking in glycobiology are still in infancy. The docking tools available for studying lectin-glycan interactions have their own limitations. The published data using docking and MD tools evidences the low interactions energies based best-fit approach may lead to insignificant docking in the case of glycans or polysaccharides. Hence it's highly suggestible to corroborate the same with crystallized PDB complexes before proceeding with downstream experiments.

4. Materials and Methods:

4.1. Glycan Library Preparation and Selection

The Glycan library was populated from the available PDB files and high-resolution LC-MS/MS studies. The multiple ligand docking program EasyDockVina (doi.org/10.5281/zenodo.3732170) was used to find the affinity values for each glycan against lectins. Glycans with the highest affinity against at least two lectins were selected for a manual docking. Glycan Builder (Damerell et al., 2012) was used to build the Symbolic Nomenclature of Glycans (SNFG), calculation of mass to charge (m/z) ratio, and generation of LINCUS linear description. MGL Tools 1.5.6 was used generation of pdbqt files of glycans required for docking.

4.2. Lectins Selection and Preparation

Lectins with proven antiviral capabilities such as Griffithsin (GRFT; 2GUC) from red alga Griffithsia species, Cyanovirin-N (CV-N; 3GXZ) from cyanobacterium *Nostoc ellipsosporum*, Cyanovirin-N homolog (Cyt-CVNH; 5K79) from cyanobacterium Cyanothece, and BanLec H84T (4PIT) mutant of BanLec (*Musa acuminata*) created by Swanson and his Co-workers were selected for studying the lectin-glycan interactions. BIOVIA Discovery Studio was used for the removal of water, existing ligands, and protein chains wherever necessary. MGL Tools 1.5.6 was used for the addition of polar hydrogens, gastegier charges, kollman charges, and generation of pdbqt files of lectins required for docking.

4.3. Molecular Docking

MGL Tools 1.5.6 was used for the generation of the Grid parameter file. Further, molecular docking of glycans against lectins was carried out using HADDOCK (Dominguez et al., 2003) and AutoDock Vina (Trott & Olson, 2009). The output files were visualized and analyzed using BIOVIA Discovery Studio.

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Abbreviations

RBD, receptor binding domain; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane serine protease 2; HIV, Human Immunodeficiency Virus; HCV, Hepatitis C Virus; CV-N, Cyanovirin; GRFT, Griffithsin; Cyt-CVNH, a new homolog of Cyanovirin-N from cyanobacterium Cyanothece; SNFG, symbolic nomenclature of glycans; RMSD, root mean square deviation; CBRs, carbohydrate binding regions; MD, molecular dynamic; SGSL, Snake gourd seed lectin (SGSL)

References

1. Benton, D. J., Wrobel, A. G., Xu, P., Roustan, C., Martin, S. R., Rosenthal, P. B., Skehel, J. J., & Gamblin, S. J. (2020). Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion. *Nature* |, *588*, 327. https://doi.org/10.1038/s41586-020-2772-0
2. Bewley, C. A., & Otero-Quintero, S. (2001). The potent anti-HIV protein cyanovirin-N contains two novel carbohydrate binding sites that selectively bind to Man8 D1D3 and Man9 with nanomolar affinity: Implications for binding to the HIV envelope protein gp120. *Journal of the American Chemical Society*, *123*(17), 3892–3902. https://doi.org/10.1021/JA004040E/SUPPL_FILE/JA004040E_S.PDF
3. Cai, Y., Xu, W., Gu, C., Cai, X., Qu, D., Lu, L., Xie, Y., & Jiang, S. (2020). Griffithsin with A Broad-Spectrum Antiviral Activity by Binding Glycans in Viral Glycoprotein Exhibits Strong Synergistic Effect in Combination with A Pan-Coronavirus Fusion Inhibitor Targeting SARS-CoV-2 Spike S2 Subunit. *Virologica Sinica*, *35*(6), 857–860. https://doi.org/10.1007/S12250-020-00305-3/FIGURES/1
4. Casalino, L., Gaieb, Z., Goldsmith, J. A., Hjorth, C. K., Dommer, A. C., Harbison, A. M., Fogarty, C. A., Barros, E. P., Taylor, B. C., Mclellan, J. S., Fadda, E., & Amaro, R. E. (2020). Beyond shielding: The roles of glycans in the SARS-CoV-2 spike protein. *ACS Central Science*, *6*(10), 1722–1734. https://doi.org/10.1021/ACSCENTSCI.0C01056/SUPPL_FILE/OC0C01056_SI_006.ZIP
5. Chandran, T., Sivaji, N., Surolia, A., & Vijayan, M. (2018). Ligand binding and retention in snake gourd seed lectin (SGSL). A crystallographic, thermodynamic and molecular dynamics study. *Glycobiology*, *28*(12), 968–977. https://doi.org/10.1093/GLYCOB/CWY072
6. Covés-Datson, E. M., Dyall, J., DeWald, L. E., King, S. R., Dube, D., Legendre, M., Nelson, E., Drews, K. C., Gross, R., Gerhardt, D. M., Torzewski, L., Postnikova, E., Liang, J. Y., Ban, B., Shetty, J., Hensley, L. E., Jahrling, P. B., Olinger, G. G., White, J. M., & Markovitz, D. M. (2019). Inhibition of Ebola Virus by a Molecularly Engineered Banana Lectin. *PLOS Neglected Tropical Diseases*, *13*(7), e0007595. https://doi.org/10.1371/JOURNAL.PNTD.0007595
7. Covés-Datson, E. M., King, S. R., Legendre, M., Gupta, A., Chan, S. M., Gitlin, E., Kulkarni, V. v., García, J. P., Smee, D. F., Lipka, E., Evans, S. E., Tarbet, E. B., Ono, A., & Markovitz, D. M. (2020). A molecularly engineered antiviral banana lectin inhibits fusion and is efficacious against influenza virus infection in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(4), 2122–2132. https://doi.org/10.1073/PNAS.1915152117/SUPPL_FILE/PNAS.1915152117.SAPP.PDF
8. Cui, Z., Liu, P., Wang, N., Cao, L., Cao, Y., Wang, X., Wang, L., Fan, K., Zhu, Q., Wang, K., Chen, R., Feng, R., Jia, Z., Yang, M., Xu, G., Zhu, B., Fu, W., Chu, T., Feng, L., … Xie, X. S. (2022). Structural and functional characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. *Cell*, *185*, 860–871. https://doi.org/10.1016/j.cell.2022.01.019
9. da Silva, P. M., de Moura, M. C., Gomes, F. S., da Silva Trentin, D., Silva de Oliveira, A. P., de Mello, G. S. V., da Rocha Pitta, M. G., de Melo Rego, M. J. B., Coelho, L. C. B. B., Macedo, A. J., de Figueiredo, R. C. B. Q., Paiva, P. M. G., & Napoleão, T. H. (2018). PgTeL, the lectin found in Punica granatum juice, is an antifungal agent against Candida albicans and Candida krusei. *International Journal of Biological Macromolecules*, *108*, 391–400. https://doi.org/10.1016/J.IJBIOMAC.2017.12.039
10. Dominguez, C., Boelens, R., & Bonvin, A. M. J. J. (2003). HADDOCK: A protein-protein docking approach based on biochemical or biophysical information. *Journal of the American Chemical Society*, *125*(7), 1731–1737. https://doi.org/10.1021/ja026939x
11. Duan, L., Zheng, Q., Zhang, H., Niu, Y., Lou, Y., & Wang, H. (2020). The SARS-CoV-2 Spike Glycoprotein Biosynthesis, Structure, Function, and Antigenicity: Implications for the Design of Spike-Based Vaccine Immunogens. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/FIMMU.2020.576622
12. El-Araby, M. M., El-Shatoury, E. H., Soliman, M. M., & Shaaban, H. F. (2020). Characterization and antimicrobial activity of lectins purified from three Egyptian leguminous seeds. *AMB Express*, *10*(1), 1–14. https://doi.org/10.1186/S13568-020-01024-4/FIGURES/5
13. Fischer, N. M., Schneider, W., & Kohlbacher, O. (2010). Rescoring of docking poses using force field-based methods. *Journal of Cheminformatics*, *2*(Suppl 1), P52. https://doi.org/10.1186/1758-2946-2-S1-P52
14. Gondim, A. C. S., Roberta Da Silva, S., Mathys, L., Noppen, S., Liekens, S., Holanda Sampaio, A., Nagano, C. S., Renata Costa Rocha, C., Nascimento, K. S., Cavada, B. S., Sadler, P. J., & Balzarini, J. (2019). Potent antiviral activity of carbohydrate-specific algal and leguminous lectins from the Brazilian biodiversity. *Medchemcomm*, *10*(3), 390–398. https://doi.org/10.1039/C8MD00508G
15. Guedes, I. A., Pereira, F. S. S., & Dardenne, L. E. (2018). Empirical Scoring Functions for Structure-Based Virtual Screening: Applications, Critical Aspects, and Challenges. *Frontiers in Pharmacology*, *9*, 1089. https://doi.org/10.3389/FPHAR.2018.01089
16. Hopper, J. T. S., Ambrose, S., Grant, O. C., Krumm, S. A., Allison, T. M., Degiacomi, M. T., Tully, M. D., Pritchard, L. K., Ozorowski, G., Ward, A. B., Crispin, M., Doores, K. J., Woods, R. J., Benesch, J. L. P., Robinson, C. v., & Struwe, W. B. (2017). The Tetrameric Plant Lectin BanLec Neutralizes HIV through Bidentate Binding to Specific Viral Glycans. *Structure*, *25*(5), 773-782.e5. https://doi.org/10.1016/J.STR.2017.03.015/ATTACHMENT/61E89277-C512-4B09-921E-8386BB310DC5/MMC2.ZIP

17. Hung, L. D., & Trinh, P. T. H. (2021). Structure and anticancer activity of a new lectin from the cultivated red alga, Kappaphycus striatus. *Journal of Natural Medicines*, *75*(1), 223–231. https://doi.org/10.1007/S11418-020-01455-0/FIGURES/5
18. Kang, L., Liu, Z., & Guo, Q. (2014). A force fields-based multi-scale docking method in drug molecular design. *Proceedings 2014 IEEE International Conference on Security, Pattern Analysis, and Cybernetics, SPAC 2014*, 139–142. https://doi.org/10.1109/SPAC.2014.6982674
19. Lavie, M., Dubuisson, J., & Belouzard, S. (2022). SARS-CoV-2 Spike Furin Cleavage Site and S2′ Basic Residues Modulate the Entry Process in a Host Cell-Dependent Manner. *Journal of Virology*. https://doi.org/10.1128/JVI.00474-22
20. Lee, C. (2019). Griffithsin, a Highly Potent Broad-Spectrum Antiviral Lectin from Red Algae: From Discovery to Clinical Application. *Marine Drugs*, *17*(10). https://doi.org/10.3390/MD17100567
21. Li, Q., Wu, J., Nie, J., Zhang, L., Hao, H., Liu, S., Zhao, C., Zhang, Q., Liu, H., Nie, L., Qin, H., Wang, M., Lu, Q., Li, X., Sun, Q., Liu, J., Zhang, L., Li, X., Huang, W., & Wang, Y. (2020). The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell*, *182*(5), 1284-1294.e9. https://doi.org/10.1016/J.CELL.2020.07.012
22. Lokhande, K. B., Apte, G. R., Shrivastava, A., Singh, A., Pal, J. K., Venkateswara Swamy, K., & Gupta, R. K. (2020). Sensing the interactions between carbohydrate-binding agents and N-linked glycans of SARS-CoV-2 spike glycoprotein using molecular docking and simulation studies. *Journal of Biomolecular Structure and Dynamics*. https://doi.org/10.1080/07391102.2020.1851303/SUPPL_FILE/TBSD_A_1851303_SM4721.PDF
23. Macedo, M. L. R., Oliveira, C. F. R., & Oliveira, C. T. (2015). Insecticidal Activity of Plant Lectins and Potential Application in Crop Protection. *Molecules 2015, Vol. 20, Pages 2014-2033*, *20*(2), 2014–2033. https://doi.org/10.3390/MOLECULES20022014
24. Marques, D. N., Almeida, A. S. de, Sousa, A. R. de O., Pereira, R., Andrade, A. L., Chaves, R. P., Carneiro, R. F., Vasconcelos, M. A. de, Nascimento-Neto, L. G. do, Pinheiro, U., Videira, P. A., Teixeira, E. H., Nagano, C. S., & Sampaio, A. H. (2018). Antibacterial activity of a new lectin isolated from the marine sponge Chondrilla caribensis. *International Journal of Biological Macromolecules*, *109*, 1292–1301. https://doi.org/10.1016/J.IJBIOMAC.2017.11.140
25. Matei, E., Basu, R., Furey, W., Shi, J., Calnan, C., Aiken, C., & Gronenborn, A. M. (2016). Structure and Glycan Binding of a New Cyanovirin-N Homolog. *The Journal of Biological Chemistry*, *291*(36), 18967–18976. https://doi.org/10.1074/JBC.M116.740415
26. Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. (2011). Molecular Docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, *7*(2), 146. https://doi.org/10.2174/157340911795677602
27. Mitchell, C. A., Ramessar, K., & O'Keefe, B. R. (2017). Antiviral lectins: Selective inhibitors of viral entry. *Antiviral Research*, *142*, 37. https://doi.org/10.1016/J.ANTIVIRAL.2017.03.007
28. Pagadala, N. S., Syed, K., & Tuszynski, J. (n.d.). *Software for molecular docking: a review*. https://doi.org/10.1007/s12551-016-0247-1
29. Peacock, T. P., Goldhill, D. H., Zhou, J., Baillon, L., Frise, R., Swann, O. C., Kugathasan, R., Penn, R., Brown, J. C., Sanchez-David, R. Y., Braga, L., Williamson, M. K., Hassard, J. A., Staller, E., Hanley, B., Osborn, M., Giacca, M., Davidson, A. D., Matthews, D. A., & Barclay, W. S. (2021). The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. *Nature Microbiology*, *6*(7), 899–909. https://doi.org/10.1038/S41564-021-00908-W
30. Rahnama, S., Azimzadeh Irani, M., Amininasab, M., & Ejtehadi, M. R. (2021). S494 O-glycosylation site on the SARS-CoV-2 RBD affects the virus affinity to ACE2 and its infectivity; a molecular dynamics study. *Scientific Reports*, *11*(1). https://doi.org/10.1038/S41598-021-94602-W
31. Rüdiger, H., & Gabius, H. J. (2001). Plant lectins: Occurrence, biochemistry, functions and applications. *Glycoconjugate Journal 2001 18:8*, *18*(8), 589–613. https://doi.org/10.1023/A:1020687518999
32. Shajahan, A., Supekar, N. T., Gleinich, A. S., & Azadi, P. (2020). Deducing the N- and O-glycosylation profile of the spike protein of novel coronavirus SARS-CoV-2. *Glycobiology*, *30*(12), 981–988. https://doi.org/10.1093/GLYCOB/CWAA042
33. Singh, S. S., Devi, S. K., & Ng, T. B. (2014). Banana Lectin: A Brief Review. *Molecules 2014, Vol. 19, Pages 18817-18827*, *19*(11), 18817–18827. https://doi.org/10.3390/MOLECULES191118817
34. Swanson, M. D., Winter, H. C., Goldstein, I. J., & Markovitz, D. M. (2010). A Lectin Isolated from Bananas Is a Potent Inhibitor of HIV Replication. *Journal of Biological Chemistry*, *285*(12), 8646–8655. https://doi.org/10.1074/JBC.M109.034926
35. Takebe, Y., Saucedo, C. J., Lund, G., Uenishi, R., Hase, S., Tsuchiura, T., Kneteman, N., Ramessar, K., Tyrrell, D. L. J., Shirakura, M., Wakita, T., McMahon, J. B., & O'Keefe, B. R. (2013). Antiviral Lectins from Red and Blue-Green Algae Show Potent In Vitro and In Vivo Activity against Hepatitis C Virus. *PLOS ONE*, *8*(5), e64449. https://doi.org/10.1371/JOURNAL.PONE.0064449
36. Tang, R., Guo, L., Fan, Q., Zhang, L., Wang, Y., Zhang, X., Shi, D., Wu, Y., Shi, H., Liu, J., Chen, J., & Feng, L. (2022). Porcine deltacoronavirus infection is inhibited by Griffithsin in cell culture. *Veterinary Microbiology*, *264*, 109299. https://doi.org/10.1016/J.VETMIC.2021.109299
37. Tian, W., Li, D., Zhang, N., Bai, G., Yuan, K., Xiao, H., Gao, F., Chen, Y., Wong, C. C. L., & Gao, G. F. (2021). O-glycosylation pattern of the SARS-CoV-2 spike protein reveals an "O-Follow-N" rule. *Cell Research 2021 31:10*, *31*(10), 1123–1125. https://doi.org/10.1038/s41422-021-00545-2
38. Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/JCC.21334
39. Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Erratum: Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (Cell (2020) 181(2) (281–292.e6), (S0092867420302622), (10.1016/j.cell.2020.02.058)). In *Cell* (Vol. 183, Issue 6, p. 1735). Cell Press. https://doi.org/10.1016/j.cell.2020.11.032
40. Wang, K., Liu, C., Hou, Y., Zhou, H., Wang, X., Mai, K., & He, G. (2019). Differential apoptotic and mitogenic effects of lectins in Zebrafish. *Frontiers in Endocrinology*, *10*(JUN), 356. https://doi.org/10.3389/FENDO.2019.00356/BIBTEX

41. Wang, M., Shajahan, A., Pepi, L. E., Azadi, P., & Zaia, J. (2021). Glycoproteomics Sample Processing, LC-MS, and Data Analysis Using GlycReSoft. *Current Protocols*, *1*(3), e84. https://doi.org/10.1002/CPZ1.84
42. Watanabe, Y., Allen, J. D., Wrapp, D., McLellan, J. S., & Crispin, M. (2020). Site-specific glycan analysis of the SARS-CoV-2 spike. *Science*, *369*(6501), 330–333. https://doi.org/10.1126/SCIENCE.ABB9983/SUPPL_FILE/ABB9983_WATANABE_SM.PDF
43. Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., Graham, B. S., & Mclellan, J. S. (2019). *Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation*. https://www.gisaid.
44. Yin, W., Xu, Y., Xu, P., Cao, X., Wu, C., Gu, C., He, X., Wang, X., Huang, S., Yuan, Q., Wu, K., Hu, W., Huang, Z., Liu, J., Wang, Z., Jia, F., Xia, K., Liu, P., Wang, X., … Xu, H. E. (2022). Structures of the Omicron spike trimer with ACE2 and an anti-Omicron antibody. *Science*, *375*(6584), 1048–1053. https://doi.org/10.1126/SCIENCE.ABN8863
45. Zhang, J., Xiao, T., Cai, Y., & Chen, B. (2021). Structure of SARS-CoV-2 spike protein. *Current Opinion in Virology*, *50*, 173–182. https://doi.org/10.1016/J.COVIRO.2021.08.010
46. Zhang, Y., Zhao, W., Mao, Y., Chen, Y., Wang, S., Zhong, Y., Su, T., Gong, M., Du, D., Lu, X., Cheng, J., & Yang, H. (2021). Site-specific N-glycosylation Characterization of Recombinant SARS-CoV-2 Spike Proteins. *Molecular & Cellular Proteomics : MCP*, *20*. https://doi.org/10.1074/MCP.RA120.002295
47. Zhao, X., Chen, H., & Wang, H. (2021). Glycans of SARS-CoV-2 Spike Protein in Virus Infection and Antibody Production. *Frontiers in Molecular Biosciences*, *8*. https://doi.org/10.3389/FMOLB.2021.629873
48. Zhou, R., Wang, X., Liu, H., Guo, L., Su, Q., Wang, H., Vasiliadis, T., Ho, W., & Li, J. (2018). GalNAc-Specific Soybean Lectin Inhibits HIV Infection of Macrophages through Induction of Antiviral Factors. *Journal of Virology*, *92*(6), 1720–1737. https://doi.org/10.1128/JVI.01720-17/ASSET/EE814304-64B6-4964-AB89-0EE9060F2C6E/ASSETS/GRAPHIC/ZJV0061833610007.JPEG

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Supplementary Table I Binding affinity values of glycans against lectins (GRFT, CV-N, Cyt-CVNH and BanLec H84T) generated using EasyDockVina (Top 5 values in each row are highlighted in grey).

Glycan ID	Affinity(kcal/mol)			
	GRFT	CVN	Cyt-CVNH	BanLecH84T
1	-7.6	-6.6	-6.4	-6.3
2	-6.3	-4.9	-6.5	-5.6
3	-6.4	-6.3	-6.8	-5.3
4	-6.5	-4.9	-5.7	-5.7
5	-6.4	-5.1	-5.8	-6.2
6	-6.5	-5.3	-5.2	-5.7
7	-8.6	-6.9	-8.4	-7.2
8	-8.6	-6.9	-6.9	-6.8
9	-7	-6.1	-7	-6.8
10	-6.8	-5.8	-6.1	-6.2
11	-8.2	-7	-6.6	-6.8
12	-9	-7.5	-7.4	-7.3
13	-8.3	-6.9	6.9	-6.4
14	-8.8	-8	-7.7	-6.8
15	-8.2	-6.7	-6.6	-6.9
16	-7.6	-6.1	-6	-5.8
17	-8.7	-7.1	-7.7	-7.3
18	-9.2	-7.4	-7.8	-7.5
19	-9.3	-7	-7.3	-7.2
20	-8.2	-6.1	-6.2	-7.2
21	-8.1	-6.2	-6.6	-6.3
22	-7	-5.3	-5.9	-6
23	-6.2	-4.9	-5.8	-5.2
24	-7.7	-6.8	-7.3	-7.1
25	-7.1	-6.1	-6.4	-5.8
26	-5.1	-4.1	-4.9	-4.8
27	-6.7	-5.4	-6.4	-6.2
28	-8.4	-7.6	-8	-7