



Insights into structural vaccinology harnessed for universal coronavirus vaccine development

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Structural vaccinology is pivotal in expediting vaccine design through high-throughput screening of immunogenic antigens. Leveraging the structural and functional characteristics of antigens and immune cell receptors, this approach employs protein structural comparison to identify conserved patterns in key pathogenic components. Molecular modeling techniques, including homology modeling and molecular docking, analyze specific three-dimensional (3D) structures and protein interactions and offer valuable insights into the 3D interactions and binding affinity between vaccine candidates and target proteins. In this review, we delve into the utilization of various immunoinformatics and molecular modeling tools to streamline the development of broad-protective vaccines against coronavirus disease 2019 variants. Structural vaccinology significantly enhances our understanding of molecular interactions between hosts and pathogens. By accelerating the pace of developing effective and targeted vaccines, particularly against the rapidly mutating severe acute respiratory syndrome coronavirus 2 and other prevalent infectious diseases, this approach stands at the forefront of advancing immunization strategies. The combination of computational techniques and structural insights not only facilitates the identification of potential vaccine candidates but also contributes to the rational design of vaccines, fostering a more efficient and targeted approach to combatting infectious diseases.

Keywords: Immunoinformatics, Human coronaviruses, Vaccinology, Molecular structure, Universal coronavirus vaccine

Introduction

Pathogenic microorganisms constantly adapt to evade host immune responses, posing a challenge to the development of effective vaccines [1]. Structural vaccinology, an interdisciplinary field encompassing structural biology, immunology, and bioinformatics, has emerged as a promising approach for comprehending immune responses and designing vaccines [2-4]. This field originated from the groundbreaking work of scientists in the late 20th century who recognized the significance of elucidating the three-dimensional (3D) structures of immune-related proteins. By leveraging structural information, scientists aim to engineer more stable, homogeneous, and efficiently produced vaccine antigens. Significant progress has been made in this regard, including the development of the respiratory syncytial virus F subunit antigen [5], and the design of a group B *Streptococcus pilus*-based fusion protein [2] and an improved

Neisseria meningitidis serogroup B single-domain fHbp antigen, both capable of eliciting immunity against a broader range of antigenic variants compared to their wild-type counterparts [6-8]. These advancements pave the way for a deeper understanding of the structural basis of immunogenicity and immunodominance, ultimately enhancing vaccine efficacy in the long run.

With the advancement in next-generation sequencing technology, there have been rapid technological advances in the field of molecular sciences that have led to a substantial increase in the number of nucleotide and protein sequences derived from genome sequencing projects. These sequences are now carefully curated in extensive databases so that scientists around the world with internet access and appropriate software can easily access and use this wealth of data. The availability of advanced informatics tools has greatly improved the efficiency and time required for species and variant identification, particularly in the context of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. In this context, structural vaccinology has emerged as an indispensable approach that uses sequence alignment techniques to reveal similarities between both homogeneous and heterogeneous proteins. In this way, it facilitates the discovery and analysis of critical protein features and contributes to the development of effective vaccines [9]. When scientists at Fudan University and their collaborators made public the genome sequence of SARS-CoV-2 responsible for the coronavirus disease 2019 (COVID-19) pandemic in January 2020, laboratories worldwide were prepared for the challenge [10,11].

The concept of structural vaccinology is about optimizing the antigens selected for vaccine development [12]. Massive

amounts of high-throughput structural information generated by structure determination technologies such as nuclear magnetic resonance spectroscopy, X-ray crystallography, and cryogenic electron microscopy are readily available in online databases, especially protein structures [4]. In view of this, the designed vaccine antigens or candidates can be improved in terms of immunogenicity, stability, homogeneity, and production efficiency by taking into account the findings of structural biology and immunology [4,12,13]. Table 1 illustrates some applications of the structural vaccinology approach in the study of human coronaviruses [14-18].

Structural modeling is usually preceded by standard alignment methods such as BLAST [19] or FASTA method [20]. In principle, protein sequence analysis offers several aspects, including identification of conserved patterns of functional domains in related proteins, prediction of secondary structures and 3D structure, and prediction of protein functions [21]. Subsequently, this leads to understanding and determining the evolutionary background of the species under study, based on the analyzed sequences [22]. In multiple sequence alignment, the sequences of selected proteins are arranged in a rectangular array to determine whether specific regions are homologous, superimposable, or perform common functions [23]. The resulting sequence similarities are important for generating high-quality models with considerable accuracy when it comes to molecular modeling, especially homology modeling.

Molecular modeling provides a detailed understanding of the specific 3D structures and interactions between macromolecules such as proteins [24]. In homology modeling, a 3D structure is constructed from a protein sequence of interest

Table 1. Applications of structural vaccinology approach in human coronavirus research

Virus	Target protein	Outcome	Reference
MERS-CoV	S protein ectodomain	S ectodomain in complex with G4 showed optimal prefusion conformation and enhanced expression while generating high NAb titers.	[14]
SARS-CoV-2	S protein	Four substitutions with low surface exposure (D614N, A892P, A942P, and V987P) were introduced, resulting in a 6.4-fold higher yield, no heterologous trimerization domain, improved stability and properly folded conformation.	[15]
SARS-CoV-2	Full length S protein	SARS-CoV-2-3Q-2P-FL immunogen contained some modifications, including RRAR to QQAQ at S1/S2 polybasic cleavage site, as well as two proline substitutions at residues K986 and V987. As a result, this immunogen was stable, homogeneous, and predominantly in prefusion conformation.	[16]
SARS-CoV-2	S protein	HexaPro acquired higher expression yield than S-2P by a factor of 9.8, heat stress resistance and ability to preserve the prefusion conformation. It also displayed notable responses to human convalescent sera and receptor-binding domain-specific monoclonal antibody CR3022.	[17]
SARS-CoV-2	S protein	HR2-deleted glycine-capped spike (S2GΔHR2) induced higher NAb titers than S2P by 2-fold and exhibited augmented thermostability.	[18]

MERS, Middle East respiratory syndrome; S, spike; Nab, neutralizing antibody; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HR2, heptad repeat 2.

with reference to the existing crystal structure of a homologous protein as a template. Molecular docking, including analysis of receptor-ligand interactions, can then be performed using the homology model [25]. A sequence identity of at least 35% is considered a rule of thumb for reliable homology modeling [26,27]. The quality of the template structure is generally determined by the prediction accuracy. A resolution of more than 3.5 Å indicates that the template structure is less “accurate” and more perturbations have formed in the crystal structure during the prediction process [25]. To identify the position of conserved domains within a protein, 3D modeling is essential. The conserved domains of antigens are selectively exposed as epitopes via the structural vaccinology approach [28]. Unlike T cell epitopes, which lack a defined 3D structure, it is extremely important for epitopes recognized by neutralizing antibodies [29]. Most neutralizing epitopes derive from residues consisting of noncontiguous primary structure, resulting in 3D electrostatic landscapes that can be targeted in vaccination strategies. By using high-quality 3D structures, antigens can be engineered to outperform native molecules in terms of production efficiency and storage, thereby reducing distribution costs [12].

In molecular docking, the interaction between a small molecule and a protein is modeled at the atomic level, which in turn provides insight into how small molecules approach and bind to target proteins in a 3D manner and the biochemical processes that take place during the interaction. Docking basically comprises two elements: the search of conformational space for ligand binding, including the respective orientation and the position and scoring assessment of the binding affinity [25]. There is a compromise between docking accuracy and time to find the lowest energy conformation, which is assumed to suggest a biologically relevant orientation. Under this assumption, more search time will raise the possibility of finding the lowest energy conformation. However, it comes with two drawbacks: entropies are overlooked, and it is not always applicable for transition state models [25].

Prior knowledge of the location of the binding site indeed enhances docking efficiency and accuracy. Otherwise, the sites can be reviewed by comparing of the target protein with similar proteins in terms of function or bound ligands [30]. In this paper, we will review and explain *in silico* tools for sequence alignment, structure prediction, modeling, and refinement, as well as model interaction prediction and molecular docking, for the development of coronavirus peptide vaccines. We used keywords such as “immunoinformatics,” “coronavirus,” and “vaccine” and all existing human coronaviruses were included in the scope.

Sequence Alignment

As informatics has advanced, sequence alignment tools have evolved and improved greatly [31-50] (Table 2). Clustal Omega (Clustal O) was developed for rapid and accurate multiple sequence alignments of a large number of protein, DNA, and RNA sequences [51]. Like the older versions, Clustal X and Clustal W, Clustal O performs the basic progressive alignment in which sequences are arranged in growing subalignments. The order of the alignments is then determined using guide trees. A guide-tree is a clustering of sequences created according to the pairwise distances between sequences, with closely related sequences aligned, followed by the less related sequences [52]. It outperforms most widely used rapid methods in terms of alignment accuracy [51]. Waqas et al. [31] analyzed the genomic sequences of all structural proteins with the help of Clustal O to identify the conserved regions. Sequences of proteins of interest were studied for the conservation among all pathogenic human coronaviruses in akin manner, for example, nsp1 [32].

The European Molecular Biology Open Software Suite, or EMBOSS, is a free, open-source analysis software that is particularly useful in the field of molecular biology. This software automatically handles data in many formats. It also allows transparent retrieval of sequence data from the Internet. EM-

Table 2. List of servers and software for sequence alignment and comparison

Tool	Web address	Reference	Articles reviewed
Clustal omega (Clustal O)	https://www.ebi.ac.uk/Tools/msa/clustalo/	[35]	[31,32,36-40]
Clustal X/W	http://www.clustal.org/clustal2/	[41]	[33,37,42,43]
Constraint-based multiple alignment tool (COBALT)	https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi	[44]	[45]
EMBOSS Water pairwise sequence aligner	https://www.ebi.ac.uk/Tools/psa/emboss_water/	[35]	[33,46]
MAFFT	https://mafft.cbrc.jp/alignment/server/	[47]	[34,48]
MAST	http://meme-suite.org/tools/mast	[49]	[37,50]

BOSS has integrated a variety of programs and tools for purposes such as sequence alignment, database searching with sequence patterns, protein motif identification, and domain analysis [53]. Alignment of nucleotide sequences of spike (S) protein as well as antigenicity determination were performed using EMBOSS for accessible SARS-CoV-2 isolates [33].

Structure Prediction, Modeling, and Refinement

In addition to commonly used tools such as GalaxyRefine, Protein Structure Analysis (ProSA), and RaptorX, other tools were also used for peptide vaccine development [31,32,34,36-40,42,43,46,51-147] (Table 3). GalaxyRefine is a web server that deals with model structure refinement to improve the global and local structure quality of the model. The models generated by structure prediction servers, e.g., I-TASSER and ROSETTA, are uploaded to the server in Protein Data Bank (PDB) format for refinement. Essentially, the server reconstructs the side-chain conformations and continuously relaxes the structure using molecular dynamics simulations after side-chain repacking perturbations. It then generates four additional models that most closely resemble the initial model in terms of structural elements and loops [54]. In general, notable improvement in the quality of predicted structures generated by different tools, such as I-TASSER and Phyre2, based on structural proteins or even whole viral proteome, was reported after the refinement by GalaxyRefine [55-57].

ProSA is generally used to verify 3D models of protein structures. The range of applications includes the detection of errors in experimentally determined structures and theoretical models, as well as the determination of the stability of engineered proteins. The overall quality score for an input structure is displayed in a graph that includes the scores of all experimentally determined protein chains currently available in the PDB, and then displays the relationship between the input structure and experimental protein structures. Problematic parts of a model are identified by calculating local quality scores and displayed in a 3D structure with color codes. The goal of developing ProSA is to promote the use of the analysis tool in the early stages of structure determination and refinement to validate structures prior to submission to the PDB [58]. Based on the studies by Bhattacharya et al. [59] and Sanami et al. [60], S protein-based vaccine constructs displayed great quality, determined by their respective Z-scores.

In the RaptorX server, a new threading scoring function is applied through statistical learning to measure the compatibility between a target sequence and a template structure. To increase alignment accuracy, RaptorX considers the correlation between protein features and structural information for proteins with sparse sequence profiles [148]. In addition, the server evaluates the quality of information content in the sequence profiles based on the number of non-redundant homologs available for the target sequence and a template structure to further optimize the appropriate modeling strategy for the target. In short, the secondary and tertiary structure generated by RaptorX can be used for further analyses such as epitope prediction, protein docking and protein-protein interaction studies [149]. The prediction of 3D structures was conducted using RaptorX, either to locate epitopes of interest or binding residues in structural point of view [61,62] or to prepare for the docking against immune receptors like human leukocyte antigen (HLA) alleles [63].

Model Interaction Prediction and Molecular Docking

Numerous tools are available for interaction model prediction (Table 4) [32,36,39,42,46,60,64,67,70,71,73,74,87,88,91,93,101,150-155] and molecular docking (Table 5) [32,34,36-40,42,43,46,55-57,59,60,63-65,69-71,73,77,85,86,88,91-93,97,101-103,108,111-113,116,126,128,156-189]. ClusPro is a server that uses automated rigid-body docking and discrimination algorithms to filter and group docked conformations by cluster properties. Filtering means selecting the centers of the high-occupancy clusters of low-energy structures by analyzing the empirical free energy [190]. In general, the server performs three steps: (1) collection of billions of conformations as samples for rigid-body docking, (2) determination of the largest cluster representing the most likely models of the complex based on root mean square deviation (RMSD)-based clustering of 1,000 lowest-energy structures, and (3) refinement of the selected structures by energy minimization. The rigid-body docking step uses PIPER16, a docking program based on the Fast Fourier Transform correlation approach that does not require information about the structure of the complex [156]. ClusPro is capable of predicting near-native complexes for a variety of proteins, including enzyme-inhibitor, antibody-antigen, and signal transduction complexes [191]. The docked complex of vaccine construct and Toll-like receptor (TLR)-3 generated via ClusPro displayed

Table 3. List of servers and software for structure prediction, modeling, and refinement

Tool	Web address	Reference	Articles reviewed
3Dpro	http://scratch.proteomics.ics.uci.edu/	[66]	[60,67]
3Drefne	http://sysbio.rnet.missouri.edu/3Drefine/	[68]	[55,60,69-71]
CABSflex	http://biocomp.chem.uw.edu.pl/CABSflex2/submit	[72]	[73,74]
CABSfold	http://biocomp.chem.uw.edu.pl/CABSfold/	[75,76]	[77]
CASTp	http://sts.bioe.uic.edu/castp/index.html	[78]	[70]
CFSSP	https://www.biogem.org/tool/chou-fasman/	[79]	[36]
ChimeraX	https://www.cgl.ucsf.edu/chimerax/	[80-83]	[31,32,38,42,43,46,63,77,84-88]
DeepTMHMM	https://dtu.biolib.com/DeepTMHMM	[89,90]	[34,84,87,91-93]
DISOPRED	http://bioinf.cs.ucl.ac.uk/disopred/	[94]	[95]
Disulfide by Design	http://cptweb.cpt.wayne.edu/DbD2/index.php	[96]	[34,69,70,93,97]
Esript	http://esript.ibcp.fr/ESript/cgi-bin/ESript.cgi	[98]	[34]
FG-MD	https://zhanglab.ccmb.med.umich.edu/FG-MD/	[99]	[34]
GalaxyLoop	http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=LOOP	[100]	[91,101]
GalaxyRefine	http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE	[54]	[36,39,40,43,55-57,67,74,77,88,91,93,101-103]
GalaxyRefineComplex	https://seoklab.github.io/GalaxyRefineComplex/	[104]	[103]
GalaxyTBM	http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=TBM	[105]	[88,103]
GlobPlot	http://globplot.embl.de/	[106]	[103]
GOR4	https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_gor4.html	[107]	[34,60,88,93,103,108]
GROMOS96	http://www.gromos.net/	[109]	[36,46]
I-TASSER	https://zhanglab.ccmb.med.umich.edu/I-TASSER/	[110]	[32,34,40,56,70,101,102,111-113]
LigPlot+	https://www.ebi.ac.uk/thornton-srv/software/LigPlus/	[114,115]	[31,40,116]
LOMETS	https://zhanglab.ccmb.med.umich.edu/LOMETS/	[117]	[32,40]
MEGA X	https://www.megasoftware.net/	[118]	[38,61,63,84,87,92,95]
ModRefiner	https://zhanglab.ccmb.med.umich.edu/ModRefiner/	[119]	[34,36,39,77]
NetNGlyc	https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0	[120]	[111]
NetOGlyc	https://services.healthtech.dtu.dk/service.php?NetOGlyc-4.0	[121]	[97,111]
NetPhos	https://services.healthtech.dtu.dk/service.php?NetPhos-3.1	[122,123]	[111]
NetTurnP	https://services.healthtech.dtu.dk/service.php?NetTurnP-1.0	[124]	[69]
PDBsum	http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html	[125]	[67,74,77,126]
PEP-FOLD	https://mobylipe.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3	[127]	[31,38,46,65,69,74,84,87,92,102,116,126,128]
PEPstrMOD	http://osddlinux.osdd.net/raghava/pepstrmod/nat_ss.php	[129,130]	[39]
Phyre2	http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index	[131]	[36,37,57,86,97,111]
ProFunc	https://www.ebi.ac.uk/thornton-srv/databases/profunc/index.html	[132]	[37]
ProQ	https://proq.bioinfo.se/cgi-bin/ProQ/ProQ.cgi	[133]	[32]
ProSA	https://prosa.services.came.sbg.ac.at/prosa.php	[58]	[32,36,38,40,42,59,60,70,74,77,88,91,93,97,101,102]
PSIPRED	http://bioinf.cs.ucl.ac.uk/psipred/	[134,135]	[40,42,64,69,74,77,87,91-93,95,101,102,108,113]
PyMOL	https://pymol.org/2/	-	[31,32,34,36,37,40,42,57,59,74,77,84,87,91,92,101-103,116]
Qualitative model energy aNalysis (QMEAN)	https://swissmodel.expasy.org/qmean/	[136]	[39]
RaptorX	http://raptorx6.uchicago.edu/	[137]	[36,39,61-63,69,70,77,91,93,97,101,111,112]
Robetta	https://robetta.bakerlab.org/	[138]	[38,64,111]
SAVES	https://servicesn.mbi.ucla.edu/SAVES/	[139]	[32,36,38,40,42,56,57,59,64,69-71,74,91,93,101,103,111,113]

(Continued on next page)

Table 3. Continued

Tool	Web address	Reference	Articles reviewed
SignalP	https://services.healthtech.dtu.dk/services/SignalP-5.0/	[140]	[95]
SIMPA96	https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_simpa96.html	[141]	[93]
SOPMA	https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html	[142]	[38,62,74,93,97,102]
SPARKS-X	https://sparks-lab.org/server/sparks-x/	[143]	[59]
SWISS-MODEL	https://swissmodel.expasy.org/	[144]	[36,37,39,43,67,71,73,74,86,97,126]
Swiss-PdbViewer	https://spdbv.vital-it.ch/	[145]	[38,43,63,70]
UbPred	http://www.ubpred.org/	[146]	[111]
YASARA	http://www.yasara.org/minimizationserver.htm	[147]	[67,102]

Table 4. List of servers and software for model interaction prediction

Tool	Web address	Reference	Articles reviewed
COACH	https://zhanglab.ccmb.med.umich.edu/COACH/	[150]	[32]
GROMACS	http://www.gromacs.org/	[151]	[32,36,39,46,60,64,70,71,74,87,88,91,93,101,108,111]
Haddock (guru interface)	https://alcazar.science.uu.nl/services/HADDOCK2.2/haddockserver-guru.html	[152]	[32,36,67,73,74]
Originlab	https://www.originlab.com/	[153]	[87]
PACOMPLEX	http://pacocomplex.life.nctu.edu.tw/	[154]	[42,111]
PIC (Protein interactions calculator)	http://pic.mbu.iisc.ernet.in/	[155]	[101]

Table 5. List of servers and software for molecular docking

Tool	Web address	Reference	Articles reviewed
AMBER20	https://ambermd.org/	[158,159]	[102,112]
Autodock	https://autodock.scripps.edu/	[160]	[39,63,85]
AutoDock Vina	http://vina.scripps.edu/	[161]	[38,39,46,85,102]
CABSdock	http://biocomp.chem.uw.edu.pl/CABSdock	[162,163]	[71]
ClusPro	https://cluspro.bu.edu/home.php	[156,164]	[42,46,55-57,60,64,69,70,91,93,97,101-103,108,111,112,126]
Cresset Flare	https://www.cresset-group.com/software/flare/	[165-167]	[86]
DINC	http://dinc.kavrakilab.org/	[168]	[65]
FireDock	http://bioinfo3d.cs.tau.ac.il/FireDock/	[169]	[40,69,73,88,93,112,116]
GalaxyPepDock	http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=PEPDOCK	[170]	[36,56,103]
Glide	https://www.schrodinger.com/glide	[171,172]	[111]
GRAMM	https://gramm.compbio.ku.edu/gramm	[173-175]	[77]
HawkDock	http://cadd.zju.edu.cn/hawkdock/	[176]	[37,69,93]
HDOCK	http://hdock.phys.hust.edu.cn/	[177]	[70]
HEX	http://hexserver.loria.fr/	[178]	[43,108]
HPEPDOCK	http://huanglab.phys.hust.edu.cn/hpepdock/	[179]	[86,92]
MDockPeP	http://zougrouptoolkit.missouri.edu/mdockpep/	[180]	[86]
MDWeb	https://mmb.irbbarcelona.org/MDWeb/	[181]	[65]
Modeller	https://salilab.org/modeller/	[182]	[57,88,126]
NAMD	https://www.ks.uiuc.edu/Research/namd/	[183]	[55,126,128]
PatchDock	https://bioinfo3d.cs.tau.ac.il/PatchDock/	[157,184]	[34,39,40,59,65,69,73,88,93,108,112,116,185]
PIPER	https://www.schrodinger.com/products/piper	[186]	[113]
PRODIGY	https://bianca.science.uu.nl/prodigy/	[187]	[32,36,69,103]
VMD	https://www.ks.uiuc.edu/Research/vmd/	[188]	[32,88,126]
ZDOCK	http://zdock.umassmed.edu/	[189]	[37,128]

high binding affinity, indicated by the presence of multiple hydrogen bonds between interacting residues [64].

PatchDock is a geometry-based molecular docking algorithm. The main task is to find docking transformations that have good molecular shape complementarity with large interface regions and fewer steric clashes. The Connolly dot surface representation of the molecules is divided into concave, convex, and flat patches [192], and complementary fields are paired to generate transformation candidates. The quality of the transformations is then determined by geometric fit and atomic desolvation energy. Redundant solutions

are eliminated by RMSD clustering [157]. For instance, one of the designed vaccine constructs exhibited lower binding energy with receptors including angiotensin converting enzyme 2 and TLR-8 as well as HLA alleles, implying its relevance from the immunological perspective [34]. Also, short epitope (ITLCFTLKR) was tested for docking against HLA class I alleles (HLA-A*11:01, HLA-A*68:01), in which significant binding was present in the docked complexes as a result [65]. The general workflow of coronavirus genomic analysis for vaccine development via structural vaccinology approach is shown in Figs. 1 and 2.

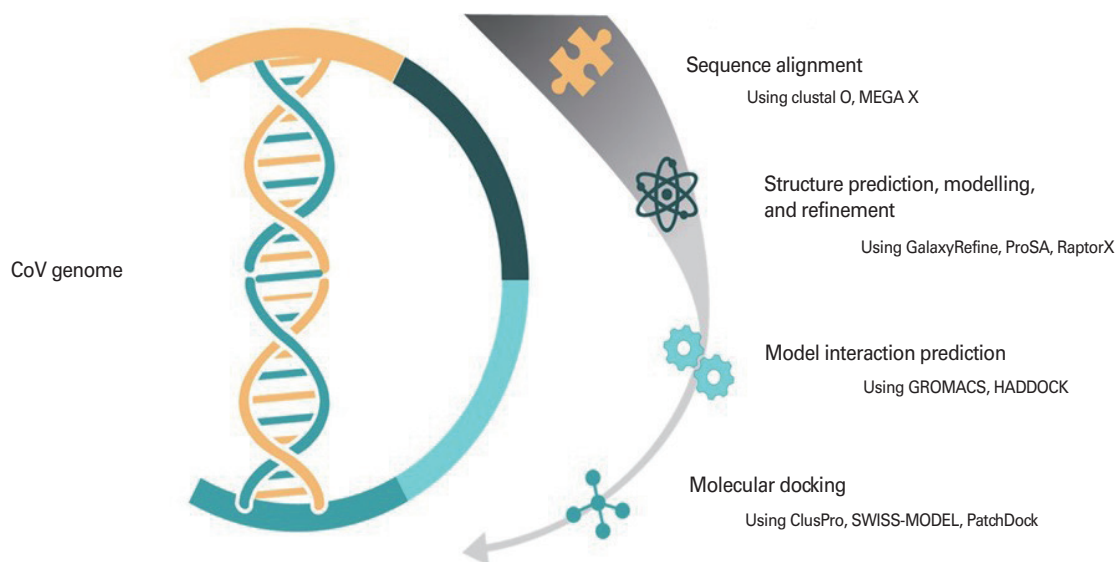


Fig. 1. Schematic workflow of universal coronavirus vaccine development based on structural vaccinology approach.

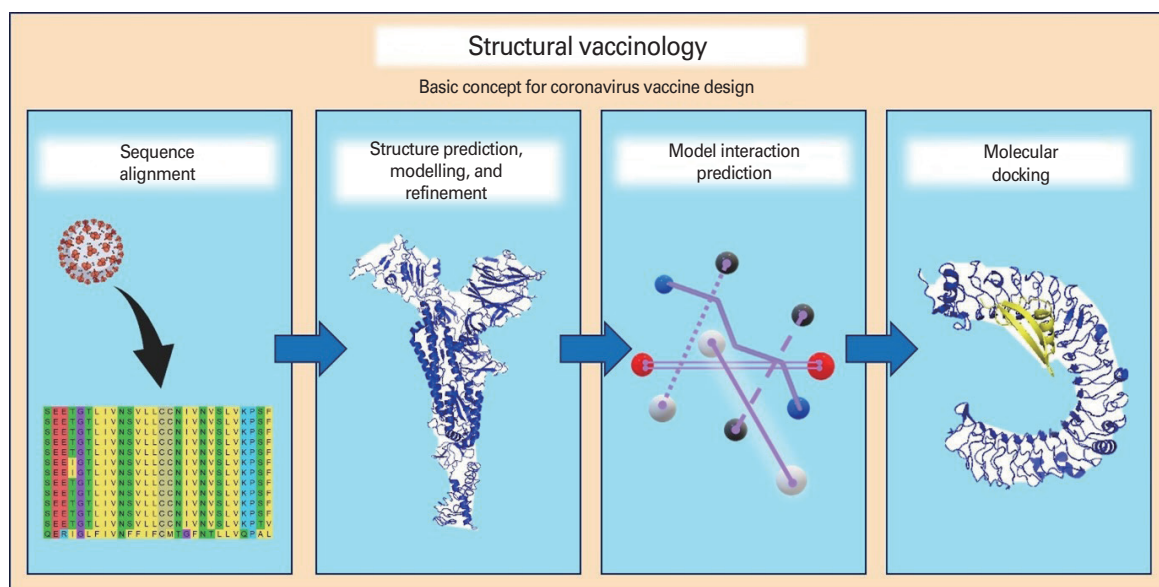


Fig. 2. Structural vaccinology: basic concept for coronavirus vaccine design.

Future Prospects of Coronavirus Vaccine Development

Given the rapid emergence of new variants of SARS-CoV-2, there is an urgent need to accelerate scientific research to develop effective treatment and therapy without delay [193,194]. Since May 17, 2023, the original Omicron variant has been replaced by circulating subvariants, including XBB.1.5, XBB.1.16, and XBB.1.9.1 [195]. Therefore, the process of screening and analyzing coronaviruses for pathogenicity and evolution needs to be accelerated. Genomic changes essentially result in sequence and structural changes in translated proteins. Substitutions or deletions affecting critical amino acids lead to changes in protein conformation and thus to different antigenic properties. These properties are then manifested in different immunogenic responses in patients [196,197]. In addition, the high mutation rate of coronaviruses has significant implications that render existing COVID-19 vaccines less effective or even ineffective. Indeed, a significant decline in neutralizing antibody titers against the Beta and Delta variants was observed shortly after the appearance of Omicron [198,199]. This calls for rational design of a universal vaccine that targets conserved regions and provides long-lasting and broad protection against multiple coronavirus strains. Some studies have suggested that structural proteins are ideal candidates for universal vaccines because potential T- and B-cell epitopes are mostly found in these proteins [200-202]. Moreover, the proteins are exposed to the host environment during infection. Therefore, stimulation of protective immunity largely depends on these proteins. In response to frequent viral mutations, information on the potential sites of nucleotide alterations and conserved regions should be updated and shared to eliminate mutation-prone regions in the proteins of coronavirus vaccine candidates [203].

The relatively rapid development and availability of COVID-19 vaccines compared with earlier vaccines is a clear indication of the significant advances in contemporary biomedical and engineering technology. In this context, immunoinformatics and structural modeling using supercomputing and machine learning play a critical role in the rapid identification of common antigenic targets in coronaviruses. Databases that contain continuously updated genetic sequences of coronaviruses from human and animal isolates are advantageous for mapping the evolution of the virus. As it is currently the greatest public health threat, it is undoubtedly critical to develop universal vaccines that are highly effective and

cross-protective against SARS-CoV-2 variants. Conserved T- and B-cell epitopes serve as a key element in the development of a universal vaccine [204]. Moreover, it is shocking to learn that this pandemic is estimated to cost between \$8 and \$16 trillion worldwide, and this figure is approximately 500 times higher than that required to prevent the next pandemic [198]. Global efforts and collaboration are indeed a must to make this large project scientifically feasible. In fact, the scope of a universal vaccine should include all known human coronaviruses, including potential ones that could cross the “species barrier” and threaten human health in the future [205].

Conclusion

Structural vaccinology harnesses its strength in exploring and predicting functional domains and features within the 3D structure of proteins, offering a potent avenue for generating novel antigens that elicit optimal and broad immune responses. By identifying common features between different types of proteins, conserved patterns can be revealed, leading to the creation of accurate and reliable molecular models in molecular modeling studies. This in turn enables comprehensive analysis of specific 3D structures and protein interactions. A prominent technique within molecular modeling is homology modeling, which enables the construction of accurate 3D structures based on the known crystal structure of a related protein. Homology modeling allows researchers to effectively predict the structure and behavior of proteins of interest. Subsequently, molecular docking techniques can be applied using the homology model. Molecular docking allows detailed study of atomic-level interactions between small molecules and proteins, providing valuable insights into how these molecules approach and contact target proteins in a 3D manner, thereby influencing biochemical processes. Molecular docking involves two critical aspects: exploration of conformational space and orientation for ligand binding, and assessment of binding affinity through positional alignment and scoring evaluation. This comprehensive approach helps to understand the complex interplay between molecules and proteins, paving the way for the development of new therapeutics and vaccines. By harnessing the power of structural vaccinology and molecular modeling techniques, significant advances have been made in the field of COVID-19 vaccine research. These advances have not only accelerated the identification of potential vaccine candidates, but also improved our understanding of the intricate mechanisms underlying

viral protein interactions. Further integration of structural vaccinology and molecular modeling holds great promise in addressing new viral threats and developing effective interventions to combat infectious diseases.

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