Review article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Clin Exp Vaccine Res 2024;13:202-217
https://doi.org/10.7774/cevr.2024.13.3.202
pISSN 2287-3651 • eISSN 2287-366X

Insights into structural vaccinology harnessed for universal coronavirus vaccine development

Chin Peng Lim[1,2], Chiuan Herng Leow[2], Hui Ting Lim[2], Boon Hui Kok[2], Candy Chuah[3], Jonas Ivan Nobre Oliveira[4], Malcolm Jones[5], Chiuan Yee Leow[1]

[1]School of Pharmaceutical Sciences and [2]Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Gelugor; [3]Faculty of Medicine, Asian Institute of Medical Science and Technology University, Bedong, Malaysia; [4]Department of Biophysics and Pharmacology, Bioscience Center, Federal University of Rio Grande do Norte, Natal, Brazil; [5]School of Veterinary Science, The University of Queensland, Gatton, Australia

Received: April 7, 2024
Revised: May 10, 2024
Accepted: May 15, 2024

Corresponding author: Chiuan Yee Leow, PhD
School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia
Tel: +60-182567188, Fax: +60-46570017
E-mail: yee.leow@usm.my

No potential conflict of interest relevant to this article was reported.

The authors acknowledge the funding support provided for this work by the Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme with Project Code: FRGS/1/2022/SKK0/USM/02/5 and FRGS/1/2021/SKK06/USM/02/12.

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 perfusion 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]

 https://doi.org/10.7774/cevr.2024.13.3.202

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]
Espript	http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.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://mobyle.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://pacomplex.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.

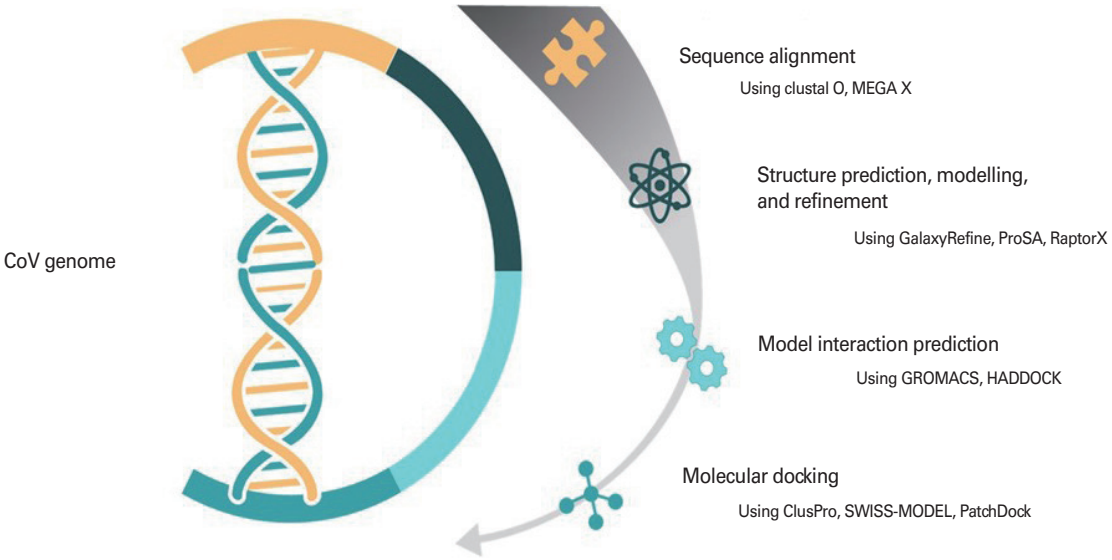

Fig. 1. Schematic workflow of universal coronavirus vaccine development based on structural vaccinology approach.

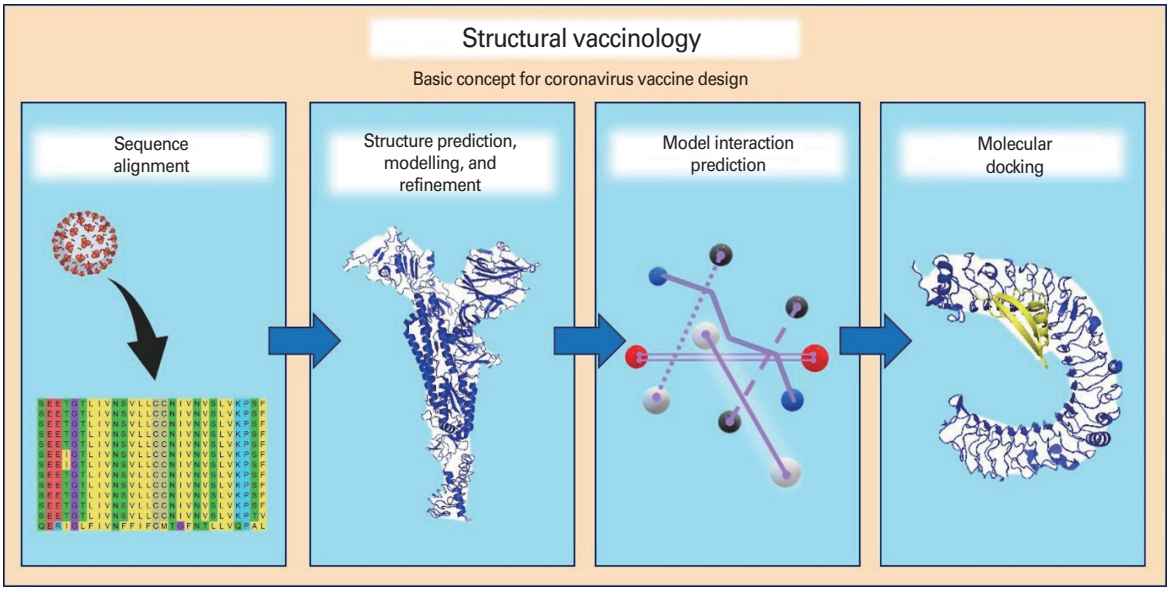

Fig. 2. Structural vaccinology: basic concept for coronavirus vaccine design.

 https://doi.org/10.7774/cevr.2024.13.3.202

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.

ORCID

Chin Peng Lim *https://orcid.org/0000-0002-5789-7109*
Chiuan Herng Leow *https://orcid.org/0000-0003-0849-428X*
Hui Ting Lim *https://orcid.org/0000-0002-0937-467X*
Boon Hui Kok *https://orcid.org/0000-0002-6075-8265*
Candy Chuah *https://orcid.org/0000-0001-6113-5070*
Jonas Ivan Nobre Oliveira *https://orcid.org/0000-0003-1646-921X*
Malcolm Jones *https://orcid.org/0000-0002-0035-6141*
Chiuan Yee Leow *https://orcid.org/0000-0002-6919-5921*

References

1. Lim CP, Kok BH, Lim HT, et al. Recent trends in next generation immunoinformatics harnessed for universal coronavirus vaccine design. Pathog Glob Health 2023;117:134-51.
2. Nuccitelli A, Cozzi R, Gourlay LJ, et al. Structure-based approach to rationally design a chimeric protein for an effective vaccine against Group B Streptococcus infections. Proc Natl Acad Sci U S A 2011;108:10278-83.
3. Schneewind O, Missiakas D. Structural vaccinology to thwart antigenic variation in microbial pathogens. Proc Natl Acad Sci U S A 2011;108:10029-30.
4. Dormitzer PR, Grandi G, Rappuoli R. Structural vaccinology starts to deliver. Nat Rev Microbiol 2012;10:807-13.
5. Swanson KA, Settembre EC, Shaw CA, et al. Structural basis for immunization with postfusion respiratory syncytial virus fusion F glycoprotein (RSV F) to elicit high neutralizing antibody titers. Proc Natl Acad Sci USA 2011;108:9619-24.
6. Beernink PT, Shaughnessy J, Braga EM, et al. A meningococcal factor H binding protein mutant that eliminates factor H binding enhances protective antibody responses to vaccination. J Immunol 2011;186:3606-14.
7. Pajon R, Beernink PT, Granoff DM. Design of meningococcal factor H binding protein mutant vaccines that do not bind human complement factor H. Infect Immun 2012;80:2667-77.
8. Beernink PT, Granoff DM. Bactericidal antibody responses induced by meningococcal recombinant chimeric factor H-binding protein vaccines. Infect Immun 2008;76:2568-75.
9. da Silva MK, Fulco UL, Junior ED, Oliveira JI. Moving targets: COVID-19 vaccine efficacy against Omicron subvariants. Mol Ther 2022;30:2644-5.
10. Marty AM, Jones MK. The novel Coronavirus (SARS-CoV-2) is a one health issue. One Health 2020;9:100123.
11. Rappuoli R, De Gregorio E, Del Giudice G, et al. Vaccinology in the post-COVID-19 era. Proc Natl Acad Sci USA 2021;118:e2020368118.
12. Dormitzer PR, Ulmer JB, Rappuoli R. Structure-based antigen design: a strategy for next generation vaccines. Trends Biotechnol 2008;26:659-67.
13. Rappuoli R, Bottomley MJ, D'Oro U, Finco O, De Gregorio E. Reverse vaccinology 2.0: human immunology instructs vaccine antigen design. J Exp Med 2016;213:469-81.
14. Pallesen J, Wang N, Corbett KS, et al. Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. Proc Natl Acad Sci U S A 2017;114:E7348-57.
15. Juraszek J, Rutten L, Blokland S, et al. Stabilizing the closed SARS-CoV-2 spike trimer. Nat Commun 2021;12:244.
16. Bangaru S, Ozorowski G, Turner HL, et al. Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate. Science 2020;370:1089-94.
17. Hsieh CL, Goldsmith JA, Schaub JM, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science 2020;369:1501-5.
18. He L, Lin X, Wang Y, et al. Single-component, self-assembling, protein nanoparticles presenting the receptor binding domain and stabilized spike as SARS-CoV-2 vaccine candidates. Sci Adv 2021;7:eabf1591.
19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10.
20. Pearson WR, Lipman DJ. Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 1988;85:2444-8.
21. Brusic V, Petrovsky N. Immunoinformatics and its relevance to understanding human immune disease. Expert Rev Clin Immunol 2005;1:145-57.
22. Wang L, Jiang T. On the complexity of multiple sequence alignment. J Comput Biol 1994;1:337-48.
23. Edgar RC, Batzoglou S. Multiple sequence alignment.

Curr Opin Struct Biol 2006;16:368-73.
24. Stanfield RL, Wilson IA. Protein-peptide interactions. Curr Opin Struct Biol 1995;5:103-13.
25. Lohning AE, Levonis SM, Williams-Noonan B, Schweiker SS. A practical guide to molecular docking and homology modelling for medicinal chemists. Curr Top Med Chem 2017;17:2023-40.
26. Xiang Z. Advances in homology protein structure modeling. Curr Protein Pept Sci 2006;7:217-27.
27. Chothia C, Lesk AM. The relation between the divergence of sequence and structure in proteins. EMBO J 1986;5:823-6.
28. Anasir MI, Poh CL. Structural vaccinology for viral vaccine design. Front Microbiol 2019;10:738.
29. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov 2007;6:404-14.
30. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 2011;7:146-57.
31. Waqas M, Haider A, Sufyan M, Siraj S, Sehgal SA. Determine the potential epitope based peptide vaccine against novel SARS-CoV-2 targeting structural proteins using immunoinformatics approaches. Front Mol Biosci 2020;7:227.
32. Chaudhuri A. Comparative analysis of non structural protein 1 of SARS-CoV2 with SARS-CoV1 and MERS-CoV: an in silico study. J Mol Struct 2021;1243:130854.
33. Ghorbani A, Zare F, Sazegari S, Afsharifar A, Eskandari MH, Pormohammad A. Development of a novel platform of virus-like particle (VLP)-based vaccine against COVID-19 by exposing epitopes: an immunoinformatics approach. New Microbes New Infect 2020;38:100786.
34. Akhand MR, Azim KF, Hoque SF, et al. Genome based evolutionary lineage of SARS-CoV-2 towards the development of novel chimeric vaccine. Infect Genet Evol 2020;85:104517.
35. Madeira F, Park YM, Lee J, et al. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Res 2019;47(W1):W636-41.
36. Rahman MS, Hoque MN, Islam MR, et al. Epitope-based chimeric peptide vaccine design against S, M and E proteins of SARS-CoV-2, the etiologic agent of COVID-19 pandemic: an in silico approach. PeerJ 2020;8:e9572.
37. Banerjee A, Santra D, Maiti S. Energetics and IC50 based epitope screening in SARS CoV-2 (COVID 19) spike protein by immunoinformatic analysis implicating for a suitable vaccine development. J Transl Med 2020;18:281.
38. Rakib A, Sami SA, Islam MA, et al. Epitope-based immunoinformatics approach on nucleocapsid protein of severe acute respiratory syndrome-coronavirus-2. Molecules 2020;25:5088.
39. Srivastava S, Kamthania M, Singh S, Saxena AK, Sharma N. Structural basis of development of multi-epitope vaccine against Middle East respiratory syndrome using in silico approach. Infect Drug Resist 2018;11:2377-91.
40. Ayyagari VS, T C V, K AP, Srirama K. Design of a multi-epitope-based vaccine targeting M-protein of SARS-CoV2: an immunoinformatics approach. J Biomol Struct Dyn 2022;40:2963-77.
41. Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. Bioinformatics 2007;23:2947-8.
42. Bhatnager R, Bhasin M, Arora J, Dang AS. Epitope based peptide vaccine against SARS-COV2: an immune-informatics approach. J Biomol Struct Dyn 2021;39:5690-705.
43. Jakhar R, Kaushik S, Gakhar SK. 3CL hydrolase-based multiepitope peptide vaccine against SARS-CoV-2 using immunoinformatics. J Med Virol 2020;92:2114-23.
44. Papadopoulos JS, Agarwala R. COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics 2007;23:1073-9.
45. Awadelkareem EA, Mohammed NO, Gaafar BB, Ali SA. Epitope-based peptide vaccine design against spike protein (S) of novel coronavirus (2019-nCoV): an immunoinformatics approach. Res Sq [Preprint] 2020 Jun 9. https://doi.org/10.21203/rs.3.rs-30076/v1
46. Panda PK, Arul MN, Patel P, et al. Structure-based drug designing and immunoinformatics approach for SARS-CoV-2. Sci Adv 2020;6:eabb8097.
47. Kuraku S, Zmasek CM, Nishimura O, Katoh K. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res 2013;41(Web Server issue):W22-8.
48. Cuspoca AF, Diaz LL, Acosta AF, et al. A multi-epitope vaccine against SARS-CoV-2 directed towards the Latin American population: an immunoinformatics approach. Res Sq [Preprint] 2020 Sep 2. https://doi.org/10.21203/rs.3.rs-70414/v1
49. Bailey TL, Gribskov M. Combining evidence using p-values: application to sequence homology searches. Bioin-

formatics 1998;14:48-54.
50. Rehman HM, Mirza MU, Ahmad MA, et al. A putative prophylactic solution for COVID-19: development of novel multiepitope vaccine candidate against SARS-COV-2 by comprehensive immunoinformatic and molecular modelling approach. Biology (Basel) 2020;9:296.
51. Sievers F, Wilm A, Dineen D, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 2011;7:539.
52. Sievers F, Higgins DG. Clustal Omega, accurate alignment of very large numbers of sequences. Methods Mol Biol 2014;1079:105-16.
53 Rice P, Longden I, Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. Trends Genet 2000; 16:276-7.
54. Heo L, Park H, Seok C. GalaxyRefine: protein structure refinement driven by side-chain repacking. Nucleic Acids Res 2013;41(Web Server issue):W384-8.
55. Jain N, Shankar U, Majee P, Kumar A. Scrutinizing the SARS-CoV-2 protein information for designing an effective vaccine encompassing both the T-cell and B-cell epitopes. Infect Genet Evol 2021;87:104648.
56. Khairkhah N, Aghasadeghi MR, Namvar A, Bolhassani A. Design of novel multiepitope constructs-based peptide vaccine against the structural S, N and M proteins of human COVID-19 using immunoinformatics analysis. PLoS One 2020;15:e0240577.
57. Yazdani Z, Rafiei A, Yazdani M, Valadan R. Design an efficient multi-epitope peptide vaccine candidate against SARS-CoV-2: an in silico analysis. Infect Drug Resist 2020; 13:3007-22.
58. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res 2007;35(Web Server issue):W407-10.
59. Bhattacharya M, Sharma AR, Patra P, et al. Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-COV-2): immunoinformatics approach. J Med Virol 2020;92:618-31.
60. Sanami S, Zandi M, Pourhossein B, et al. Design of a multi-epitope vaccine against SARS-CoV-2 using immunoinformatics approach. Int J Biol Macromol 2020;164: 871-83.
61. Almofti YA, Abd-elrahman KA, Gassmallah SA, Salih MA. Multi epitopes vaccine prediction against severe acute respiratory syndrome (SARS) coronavirus using immunoinformatics approaches. Am J Microbiol Res 2018;6:94-114.
62. Shi J, Zhang J, Li S, et al. Epitope-based vaccine target screening against highly pathogenic MERS-CoV: an in silico approach applied to emerging infectious diseases. PLoS One 2015;10:e0144475.
63. Abdelmageed MI, Abdelmoneim AH, Mustafa MI, et al. Design of a multiepitope-based peptide vaccine against the E protein of human COVID-19: an immunoinformatics approach. Biomed Res Int 2020;2020:2683286.
64. Mahapatra SR, Sahoo S, Dehury B, et al. Designing an efficient multi-epitope vaccine displaying interactions with diverse HLA molecules for an efficient humoral and cellular immune response to prevent COVID-19 infection. Expert Rev Vaccines 2020;19:871-85.
65. Joshi A, Joshi BC, Mannan MA, Kaushik V. Epitope based vaccine prediction for SARS-COV-2 by deploying immuno-informatics approach. Inform Med Unlocked 2020; 19:100338.
66. Cheng J, Randall AZ, Sweredoski MJ, Baldi P. SCRATCH: a protein structure and structural feature prediction server. Nucleic Acids Res 2005;33(Web Server issue):W72-6.
67. Naz A, Shahid F, Butt TT, Awan FM, Ali A, Malik A. Designing multi-epitope vaccines to combat emerging coronavirus disease 2019 (COVID-19) by employing immuno-informatics approach. Front Immunol 2020;11: 1663.
68. Bhattacharya D, Nowotny J, Cao R, Cheng J. 3Drefine: an interactive web server for efficient protein structure refinement. Nucleic Acids Res 2016;44(W1):W406-9.
69. Sarkar B, Ullah MA, Johora FT, Taniya MA, Araf Y. Immunoinformatics-guided designing of epitope-based subunit vaccines against the SARS coronavirus-2 (SARS-CoV-2). Immunobiology 2020;225:151955.
70. Devi A, Chaitanya NS. In silico designing of multi-epitope vaccine construct against human coronavirus infections. J Biomol Struct Dyn 2021;39:6903-17.
71. Moura RR, Agrelli A, Santos-Silva CA, et al. Immunoinformatic approach to assess SARS-CoV-2 protein S epitopes recognised by the most frequent MHC-I alleles in the Brazilian population. J Clin Pathol 2021;74:528-32.
72. Kuriata A, Gierut AM, Oleniecki T, et al. CABS-flex 2.0: a web server for fast simulations of flexibility of protein structures. Nucleic Acids Res 2018;46(W1):W338-43.
73. Rahman N, Ali F, Basharat Z, et al. Vaccine design from the ensemble of surface glycoprotein epitopes of SARS-

CoV-2: an immunoinformatics approach. Vaccines (Basel) 2020;8:423.
74. Tahir Ul Qamar M, Rehman A, Tusleem K, et al. Designing of a next generation multiepitope based vaccine (MEV) against SARS-COV-2: Immunoinformatics and in silico approaches. PLoS One 2020;15:e0244176.
75. Kolinski A. Protein modeling and structure prediction with a reduced representation. Acta Biochim Pol 2004; 51:349-71.
76. Blaszczyk M, Jamroz M, Kmiecik S, Kolinski A. CABSfold: server for the de novo and consensus-based prediction of protein structure. Nucleic Acids Res 2013;41(Web Server issue):W406-11.
77. Tahir Ul Qamar M, Shahid F, Aslam S, et al. Reverse vaccinology assisted designing of multiepitope-based subunit vaccine against SARS-CoV-2. Infect Dis Poverty 2020;9:132.
78. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res 2018;46(W1):W363-7.
79. Kumar TA. CFSSP: Chou and Fasman secondary structure prediction server. Wide Spectr 2013;1:15-9.
80. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera: a visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605-12.
81. Goddard TD, Huang CC, Meng EC, et al. UCSF ChimeraX: meeting modern challenges in visualization and analysis. Protein Sci 2018;27:14-25.
82. Pettersen EF, Goddard TD, Huang CC, et al. UCSF ChimeraX: structure visualization for researchers, educators, and developers. Protein Sci 2021;30:70-82.
83. Meng EC, Goddard TD, Pettersen EF, et al. UCSF ChimeraX: tools for structure building and analysis. Protein Sci 2023;32:e4792.
84. Tahir Ul Qamar M, Saleem S, Ashfaq UA, Bari A, Anwar F, Alqahtani S. Epitope-based peptide vaccine design and target site depiction against Middle East respiratory syndrome coronavirus: an immune-informatics study. J Transl Med 2019;17:362.
85. Mukherjee S, Tworowski D, Detroja R, Mukherjee SB, Frenkel-Morgenstern M. Immunoinformatics and structural analysis for identification of immunodominant epitopes in SARS-CoV-2 as potential vaccine targets. Vaccines (Basel) 2020;8:290.
86. Abd Albagi SO, Al-Nour MY, Elhag M, et al. A multiple peptides vaccine against COVID-19 designed from the nucleocapsid phosphoprotein (N) and spike glycoprotein (S) via the immunoinformatics approach. Inform Med Unlocked 2020;21:100476.
87. Chen Z, Ruan P, Wang L, Nie X, Ma X, Tan Y. T and B cell epitope analysis of SARS-CoV-2 S protein based on immunoinformatics and experimental research. J Cell Mol Med 2021;25:1274-89.
88. Enayatkhani M, Hasaniazad M, Faezi S, et al. Reverse vaccinology approach to design a novel multi-epitope vaccine candidate against COVID-19: an in silico study. J Biomol Struct Dyn 2021;39:2857-72.
89. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 2001;305:567-80.
90. Hallgren J, Tsirigos KD, Pedersen MD, et al. DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks. BioRxiv [Preprint] 2022 Apr 8. https://doi.org/10.1101/2022.04.08.487609
91. Jyotisha, Singh S, Qureshi IA. Multi-epitope vaccine against SARS-CoV-2 applying immunoinformatics and molecular dynamics simulation approaches. J Biomol Struct Dyn 2022;40:2917-33.
92. Bency J, Helen M. Novel epitope based peptides for vaccine against SARS-CoV-2 virus: immunoinformatics with docking approach. Int J Res Med Sci 2020;8:2385-94.
93. Sarkar B, Ullah MA, Araf Y, Rahman MS. Engineering a novel subunit vaccine against SARS-CoV-2 by exploring immunoinformatics approach. Inform Med Unlocked 2020;21:100478.
94. Ward JJ, McGuffin LJ, Bryson K, Buxton BF, Jones DT. The DISOPRED server for the prediction of protein disorder. Bioinformatics 2004;20:2138-9.
95. Corral-Lugo A, Lopez-Siles M, Lopez D, McConnell MJ, Martin-Galiano AJ. Identification and analysis of unstructured, linear B-cell epitopes in SARS-CoV-2 virion proteins for vaccine development. Vaccines (Basel) 2020; 8:397.
96. Craig DB, Dombkowski AA. Disulfide by Design 2.0: a web-based tool for disulfide engineering in proteins. BMC Bioinformatics 2013;14:346.
97. Chauhan V, Rungta T, Rawat M, Goyal K, Gupta Y, Singh MP. Excavating SARS-coronavirus 2 genome for epitope-based subunit vaccine synthesis using immunoinformatics approach. J Cell Physiol 2021;236:1131-47.
98. Robert X, Gouet P. Deciphering key features in protein

structures with the new ENDscript server. Nucleic Acids Res 2014;42(Web Server issue):W320-4.
99. Zhang J, Liang Y, Zhang Y. Atomic-level protein structure refinement using fragment-guided molecular dynamics conformation sampling. Structure 2011;19:1784-95.
100. Ko J, Park H, Heo L, Seok C. GalaxyWEB server for protein structure prediction and refinement. Nucleic Acids Res 2012;40(Web Server issue):W294-7.
101. Kumar J, Qureshi R, Sagurthi SR, Qureshi IA. Designing of nucleocapsid protein based novel multi-epitope vaccine against SARS-COV-2 using immunoinformatics approach. Int J Pept Res Ther 2021;27:941-56.
102. Samad A, Ahammad F, Nain Z, et al. Designing a multi-epitope vaccine against SARS-CoV-2: an immunoinformatics approach. J Biomol Struct Dyn 2022;40:14-30.
103. Herrera LR. Immunoinformatics approach in designing a novel vaccine using epitopes from all the structural proteins of SARS-CoV-2. Biomed Pharmacol J 2020;13:1845-62.
104. Heo L, Lee H, Seok C. GalaxyRefineComplex: refinement of protein-protein complex model structures driven by interface repacking. Sci Rep 2016;6:32153.
105. Ko J, Park H, Seok C. GalaxyTBM: template-based modeling by building a reliable core and refining unreliable local regions. BMC Bioinformatics 2012;13:198.
106. Linding R, Russell RB, Neduva V, Gibson TJ. GlobPlot: exploring protein sequences for globularity and disorder. Nucleic Acids Res 2003;31:3701-8.
107. Sen TZ, Jernigan RL, Garnier J, Kloczkowski A. GOR V server for protein secondary structure prediction. Bioinformatics 2005;21:2787-8.
108. Kumar N, Sood D, Chandra R. Design and optimization of a subunit vaccine targeting COVID-19 molecular shreds using an immunoinformatics framework. RSC Adv 2020;10:35856-72.
109. Scott WR, Hunenberger PH, Tironi IG, et al. The GROMOS biomolecular simulation program package. J Phys Chem A 1999;103:3596-607.
110. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics 2008;9:40.
111. Safavi A, Kefayat A, Mahdevar E, Abiri A, Ghahremani F. Exploring the out of sight antigens of SARS-CoV-2 to design a candidate multi-epitope vaccine by utilizing immunoinformatics approaches. Vaccine 2020;38:7612-28.
112. Khan S, Shaker B, Ahmad S, et al. Towards a novel peptide vaccine for Middle East respiratory syndrome coronavirus and its possible use against pandemic COVID-19. J Mol Liq 2021;324:114706.
113. Kumar A, Kumar P, Saumya KU, Kapuganti SK, Bhardwaj T, Giri R. Exploring the SARS-CoV-2 structural proteins for multi-epitope vaccine development: an in-silico approach. Expert Rev Vaccines 2020;19:887-98.
114. Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng 1995;8:127-34.
115. Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011;51:2778-86.
116. Wang D, Mai J, Zhou W, et al. Immunoinformatic analysis of T- and B-cell epitopes for SARS-CoV-2 vaccine design. Vaccines (Basel) 2020;8:355.
117. Wu S, Zhang Y. LOMETS: a local meta-threading-server for protein structure prediction. Nucleic Acids Res 2007;35:3375-82.
118. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018;35:1547-9.
119. Xu D, Zhang Y. Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. Biophys J 2011;101:2525-34.
120. Gupta R, Jung E, Brunak S. NetNGlyc 1.0 server: prediction of N-glycosylation sites in human proteins. Kongens Lyngby: DTU Bioinformatics; 2004.
121. Steentoft C, Vakhrushev SY, Joshi HJ, et al. Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology. EMBO J 2013;32:1478-88.
122. Blom N, Sicheritz-Ponten T, Gupta R, Gammeltoft S, Brunak S. Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. Proteomics 2004;4:1633-49.
123. Blom N, Gammeltoft S, Brunak S. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. J Mol Biol 1999;294:1351-62.
124. Petersen B, Lundegaard C, Petersen TN. NetTurnP: neural network prediction of beta-turns by use of evolutionary information and predicted protein sequence features. PLoS One 2010;5:e15079.
125. Laskowski RA, Hutchinson EG, Michie AD, Wallace AC, Jones ML, Thornton JM. PDBsum: a web-based database of summaries and analyses of all PDB structures. Trends Biochem Sci 1997;22:488-90.
126. Lizbeth RG, Jazmín GM, Jose CB, Marlet MA. Immunoin-

formatics study to search epitopes of spike glycoprotein from SARS-CoV-2 as potential vaccine. J Biomol Struct Dyn 2021;39:4878-92.
127. Thevenet P, Shen Y, Maupetit J, Guyon F, Derreumaux P, Tuffery P. PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. Nucleic Acids Res 2012;40(Web Server issue): W288-93.
128. Baruah V, Bose S. Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV. J Med Virol 2020;92:495-500.
129. Singh S, Singh H, Tuknait A, et al. PEPstrMOD: structure prediction of peptides containing natural, non-natural and modified residues. Biol Direct 2015;10:73.
130. Kaur H, Garg A, Raghava GP. PEPstr: a de novo method for tertiary structure prediction of small bioactive peptides. Protein Pept Lett 2007;14:626-31.
131. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc 2015;10:845-58.
132. Laskowski RA, Watson JD, Thornton JM. ProFunc: a server for predicting protein function from 3D structure. Nucleic Acids Res 2005;33(Web Server issue):W89-93.
133. Wallner B, Elofsson A. Can correct protein models be identified? Protein Sci 2003;12:1073-86.
134. Jones DT. Protein secondary structure prediction based on position-specific scoring matrices. J Mol Biol 1999; 292:195-202.
135. McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. Bioinformatics 2000;16:404-5.
136. Benkert P, Tosatto SC, Schomburg D. QMEAN: a comprehensive scoring function for model quality assessment. Proteins 2008;71:261-77.
137. Wang S, Li W, Liu S, Xu J. RaptorX-Property: a web server for protein structure property prediction. Nucleic Acids Res 2016 8;44(W1):W430-5.
138. Kim DE, Chivian D, Baker D. Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 2004;32(Web Server issue):W526-31.
139. Pontius J, Richelle J, Wodak SJ. Deviations from standard atomic volumes as a quality measure for protein crystal structures. J Mol Biol 1996;264:121-36.
140. Almagro Armenteros JJ, Tsirigos KD, Sonderby CK, et al. SignalP 5.0 improves signal peptide predictions using deep neural networks. Nat Biotechnol 2019;37:420-3.
141. Levin JM, Robson B, Garnier J. An algorithm for secondary structure determination in proteins based on sequence similarity. FEBS Lett 1986;205:303-8.
142. Geourjon C, Deleage G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Comput Appl Biosci 1995;11:681-4.
143. Yang Y, Faraggi E, Zhao H, Zhou Y. Improving protein fold recognition and template-based modeling by employing probabilistic-based matching between predicted one-dimensional structural properties of query and corresponding native properties of templates. Bioinformatics 2011;27:2076-82.
144. Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res 2003;31:3381-5.
145. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 1997;18:2714-23.
146. Radivojac P, Vacic V, Haynes C, et al. Identification, analysis, and prediction of protein ubiquitination sites. Proteins 2010;78:365-80.
147. Krieger E, Joo K, Lee J, et al. Improving physical realism, stereochemistry, and side-chain accuracy in homology modeling: four approaches that performed well in CASP8. Proteins 2009;77(Suppl 9):114-22.
148. Peng J, Xu J. RaptorX: exploiting structure information for protein alignment by statistical inference. Proteins 2011;79(Suppl 10):161-71.
149. Kallberg M, Margaryan G, Wang S, Ma J, Xu J. RaptorX server: a resource for template-based protein structure modeling. Methods Mol Biol 2014;1137:17-27.
150. Yang J, Roy A, Zhang Y. Protein-ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. Bioinformatics 2013;29:2588-95.
151. Abraham MJ, Murtola T, Schulz R, et al. GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 2015;1:19-25.
152. van Zundert GC, Rodrigues JP, Trellet M, et al. The HADDOCK2.2 web server: user-friendly integrative modeling of biomolecular complexes. J Mol Biol 2016;428:720-5.
153. OriginLab Corporation. Northampton (MA): OriginLab Corporation; 2016.

154. Liu IH, Lo YS, Yang JM. PAComplex: a web server to infer peptide antigen families and binding models from TCR-pMHC complexes. Nucleic Acids Res 2011;39(Web Server issue):W254-60.
155. Tina KG, Bhadra R, Srinivasan N. PIC: protein interactions calculator. Nucleic Acids Res 2007;35(Web Server issue):W473-6.
156. Kozakov D, Hall DR, Xia B, et al. The ClusPro web server for protein-protein docking. Nat Protoc 2017;12:255-78.
157. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res 2005;33(Web Server issue):W363-7.
158. Case DA, Cheatham TE 3rd, Darden T, et al. The Amber biomolecular simulation programs. J Comput Chem 2005;26:1668-88.
159. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins 2006;65:712-25.
160. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 2009;30:2785-91.
161. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31:455-61.
162. Kurcinski M, Jamroz M, Blaszczyk M, Kolinski A, Kmiecik S. CABS-dock web server for the flexible docking of peptides to proteins without prior knowledge of the binding site. Nucleic Acids Res 2015;43(W1):W419-24.
163. Blaszczyk M, Kurcinski M, Kouza M, et al. Modeling of protein-peptide interactions using the CABS-dock web server for binding site search and flexible docking. Methods 2016;93:72-83.
164. Kozakov D, Beglov D, Bohnuud T, et al. How good is automated protein docking? Proteins 2013;81:2159-66.
165. Cheeseright T, Mackey M, Rose S, Vinter A. Molecular field extrema as descriptors of biological activity: definition and validation. J Chem Inf Model 2006;46:665-76.
166. Bauer MR, Mackey MD. Electrostatic complementarity as a fast and effective tool to optimize binding and selectivity of protein-ligand complexes. J Med Chem 2019;62:3036-50.
167. Kuhn M, Firth-Clark S, Tosco P, Mey AS, Mackey M, Michel J. Assessment of binding affinity via alchemical free-energy calculations. J Chem Inf Model 2020;60:3120-30.
168. Devaurs D, Antunes DA, Hall-Swan S, et al. Using parallelized incremental meta-docking can solve the conformational sampling issue when docking large ligands to proteins. BMC Mol Cell Biol 2019;20:42.
169. Mashiach E, Schneidman-Duhovny D, Andrusier N, Nussinov R, Wolfson HJ. FireDock: a web server for fast interaction refinement in molecular docking. Nucleic Acids Res 2008;36(Web Server issue):W229-32.
170. Lee H, Heo L, Lee MS, Seok C. GalaxyPepDock: a protein-peptide docking tool based on interaction similarity and energy optimization. Nucleic Acids Res 2015;43(W1):W431-5.
171. Friesner RA, Banks JL, Murphy RB, et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 2004;47:1739-49.
172. Halgren TA, Murphy RB, Friesner RA, et al. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem 2004;47:1750-9.
173. Tovchigrechko A, Vakser IA. GRAMM-X public web server for protein-protein docking. Nucleic Acids Res 2006;34(Web Server issue):W310-4.
174. Tovchigrechko A, Vakser IA. Development and testing of an automated approach to protein docking. Proteins 2005;60:296-301.
175. Singh A, Copeland MM, Kundrotas PJ, Vakser IA. GRAMM web server for protein docking. Methods Mol Biol 2024;2714:101-12.
176. Weng G, Wang E, Wang Z, et al. HawkDock: a web server to predict and analyze the protein-protein complex based on computational docking and MM/GBSA. Nucleic Acids Res 2019;47(W1):W322-30.
177. Yan Y, Zhang D, Zhou P, Li B, Huang SY. HDOCK: a web server for protein: protein and protein-DNA/RNA docking based on a hybrid strategy. Nucleic Acids Res 2017;45(W1):W365-73.
178. Macindoe G, Mavridis L, Venkatraman V, Devignes MD, Ritchie DW. HexServer: an FFT-based protein docking server powered by graphics processors. Nucleic Acids Res 2010;38(Web Server issue):W445-9.
179. Zhou P, Jin B, Li H, Huang SY. HPEPDOCK: a web server for blind peptide-protein docking based on a hierarchical algorithm. Nucleic Acids Res 2018;46(W1):W443-50.
180. Xu X, Yan C, Zou X. MDockPeP: an ab-initio protein-

peptide docking server. J Comput Chem 2018;39:2409-13.
181. Hospital A, Andrio P, Fenollosa C, Cicin-Sain D, Orozco M, Gelpi JL. MDWeb and MDMoby: an integrated web-based platform for molecular dynamics simulations. Bioinformatics 2012;28:1278-9.
182. Eswar N, Webb B, Marti-Renom MA, et al. Comparative protein structure modeling using Modeller. Curr Protoc Bioinformatics 2006;Chapter 5:Unit-5.6.
183. Phillips JC, Hardy DJ, Maia JD, et al. Scalable molecular dynamics on CPU and GPU architectures with NAMD. J Chem Phys 2020;153:044130.
184. Duhovny D, Nussinov R, Wolfson HJ. Efficient unbound docking of rigid molecules. In: Guigo R, Gusfield D. editors. Algorithms in bioinformatics. Berlin: Springer Berlin Heidelberg; 2002. p. 185-200.
185. Campos DM, Silva MK, Barbosa ED, Leow CY, Fulco UL, Oliveira JI. Exploiting reverse vaccinology approach for the design of a multiepitope subunit vaccine against the major SARS-CoV-2 variants. Comput Biol Chem 2022;101:107754.
186. Kozakov D, Brenke R, Comeau SR, Vajda S. PIPER: an FFT-based protein docking program with pairwise potentials. Proteins 2006;65:392-406.
187. Xue LC, Rodrigues JP, Kastritis PL, Bonvin AM, Vangone A. PRODIGY: a web server for predicting the binding affinity of protein-protein complexes. Bioinformatics 2016;32:3676-8.
188. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph 1996;14:33-8.
189. Pierce BG, Wiehe K, Hwang H, Kim BH, Vreven T, Weng Z. ZDOCK server: interactive docking prediction of protein-protein complexes and symmetric multimers. Bioinformatics 2014;30:1771-3.
190. Camacho CJ, Gatchell DW, Kimura SR, Vajda S. Scoring docked conformations generated by rigid-body protein-protein docking. Proteins 2000;40:525-37.
191. Comeau SR, Gatchell DW, Vajda S, Camacho CJ. ClusPro: an automated docking and discrimination method for the prediction of protein complexes. Bioinformatics 2004;20:45-50.
192. Connolly ML. Solvent-accessible surfaces of proteins and nucleic acids. Science 1983;221:709-13.
193. Campos DM, Fulco UL, de Oliveira CB, Oliveira JI. SARS-CoV-2 virus infection: targets and antiviral pharmacological strategies. J Evid Based Med 2020;13:255-60.
194. Campos DM, Oliveira CB, Andrade JM, Oliveira JI. Fighting COVID-19. Braz J Biol 2020;80:698-701.
195. Centers for Disease Control and Prevention. COVID Data Tracker [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2023 [cited 2023 May 17]. Available from: https://covid.cdc.gov/covid-data-tracker/#variant-proportions
196. Yee PT, Laa Poh C. Impact of genetic changes, pathogenicity and antigenicity on Enterovirus- A71 vaccine development. Virology 2017;506:121-9.
197. Peeters M, Toure-Kane C, Nkengasong JN. Genetic diversity of HIV in Africa: impact on diagnosis, treatment, vaccine development and trials. AIDS 2003;17:2547-60.
198. Koff WC, Berkley SF. A universal coronavirus vaccine. Science 2021;371:759.
199. Wall EC, Wu M, Harvey R, et al. Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination. Lancet 2021;397:2331-3.
200. Ahmed SF, Quadeer AA, McKay MR. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. Viruses 2020;12:254.
201. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 2020;11:1620.
202. Lee CH, Koohy H. In silico identification of vaccine targets for 2019-nCoV. F1000Res 2020;9:145.
203. de Oliveira Campos DM, da Silva MK, Silva de Oliveira CB, Fulco UL, Nobre Oliveira JI. Effectiveness of COVID-19 vaccines against Omicron variant. Immunotherapy 2022;14:903-4.
204. Stanekova Z, Vareckova E. Conserved epitopes of influenza A virus inducing protective immunity and their prospects for universal vaccine development. Virol J 2010;7:351.
205. Giurgea LT, Han A, Memoli MJ. Universal coronavirus vaccines: the time to start is now. NPJ Vaccines 2020;5:43.