J Chin Med Assoc

REVIEW ARTICLE

Structure-based approaches against COVID-19

Ta-Chou Huang[a,b], Kung-Hao Liang[a,c,d], Tai-Jay Chang[a,e,f], Kai-Feng Hung[a], Mong-Lien Wang[a,c], Yen-Fu Cheng[a], Yi-Ting Liao[a], De-Ming Yang[a,b,*]

[a]Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [b]Institute of Biophotonics, School of Biomedical Science and Engineering, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [c]Institute of Food Safety and Health Risk Assessment, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [d]Institute of Biomedical Informatics, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [e]Laboratory of Genome Research, Basic Research Division, Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [f]School of Biomedical Science and Engineering, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC

Abstract

The coronavirus disease 2019 (COVID-19) pandemic has had a major impact on human life. This review highlights the versatile roles of both classical and modern structure-based approaches for COVID-19. X-ray crystallography, nuclear magnetic resonance spectroscopy, and cryogenic electron microscopy are the three cornerstones of classical structural biology. These technologies have helped provide fundamental and detailed knowledge regarding severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the related human host proteins as well as enabled the identification of its target sites, facilitating the cessation of its transmission. Further progress into protein structure modeling was made using modern structure-based approaches derived from homology modeling and integrated with artificial intelligence (AI), facilitating advanced computational simulation tools to actively guide the design of new vaccines and the development of anti-SARS-CoV-2 drugs. This review presents the practical contributions and future directions of structure-based approaches for COVID-19.

Keywords: COVID-19; Molecular dynamics simulation; SARS-CoV-2

1. EFFECTIVE STRATEGIES TO CONTROL COVID-19

Many novel technologies were considered for controlling the coronavirus disease 2019 (COVID-19) pandemic. Of them, the top 10 frontier technologies listed by the European Parliamentary Research Service included artificial intelligence (AI), blockchain technology, open-source technology, telehealth, three-dimensional printing, gene-editing tools, nanotechnology, synthetic biology, drones, and robots.[1] These technologies, especially AI, gene editing, and telehealth, can be further integrated into a smart healthcare system.[2] The combination of medical AI with high-throughput viral detection methods, internet information exchanges, and big data analysis can be effective for viral monitoring with acceptable sensitivity and specificity as well as foresighted medical prescriptions.

Advancements in gene editing and viral detection, such as the development of the powerful clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system, have enabled fast and accurate viral detection, thus filling the demand gap due to the limited processing power of the current gold-standard: real-time reverse transcription polymerase chain reaction (RT-PCR). When combined with medical AI, certain clinical diagnostic methods can facilitate precise prognosis prediction and health condition evaluation and, accordingly, prompt the initiation of appropriate treatment. Furthermore, stable and fast 4G Internet connections enable physicians to use telemedicine (ie, remote healthcare or telehealth technologies), thereby accelerating diagnostic evaluation and prescription. A new generation of pharmaceuticals using the Internet of Medical Things can be provided to patients anywhere—at home or in quarantine—without delay. Together, these technologies can decrease both disease severity and mortality rates, thereby ensuring that most patients remain at a mild stage and return quickly to their normal life.

As COVID-19 seems to have turned into a flu-like endemic disease, especially after the Omicron variant, the development of more useful vaccines and therapeutic drugs, especially viral inhibitors, should continue, so that we are prepared for any potential outbreaks in the post-pandemic era.[3] To further our understanding of how severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused COVID-19 and thus prevent or control future outbreaks, we present an overview of the fundamental contributions of methods in structural biology and discuss its combination with modern AI in terms of vaccine design and treatment efficacy.

2. FUNDAMENTAL CONTRIBUTIONS OF STRUCTURAL BIOLOGY ON SARS-COV-2

X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and cryogenic electron microscopy (cryo-EM) are the three major classical structure-based methods. They have been vital since the beginning of the pandemic in helping researchers understand the whole virus and virus–host interactions,[4,5] particularly based on super-high-resolution structures of all viral proteins.[6–8]

** Address correspondence. Dr. De-Ming Yang, Microscopy Service Laboratory, Basic Research Division, Department of Medical Research, Taipei Veterans General Hospital, 201, Section 2, Shi-Pai Road, Taipei 112, Taiwan, ROC. E-mail address: dmyang@vghtpe.gov.tw (D.-M. Yang).*

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2024) 87: 139-141.

Received May 16, 2023; accepted September 28, 2023.

doi: 10.1097/JCMA.0000000000001043

In X-ray crystallography, diffuse X-rays provide detailed atomic information on a crystal structure, thereby helping to identify the ligand-binding site of SARS-CoV-2 nonstructural protein 1,[9] cleavage sites for viral processing and maturation,[10] and the spike receptor-binding domain that binds host cells through the angiotensin-converting enzyme 2 (hACE2) receptor.[11] Various SARS-CoV-2 variants have been reported, and their influence on antibody therapies and existing vaccines over the past 3 years has been determined using X-ray crystallography.[12] An even more detailed structural information can guide the further development of vaccines and drugs against SARS-CoV-2; however, due to the limitation of X-ray diffraction, not every part of the virus can be crystallized in a short time, necessitating the use of NMR spectroscopy and cryo-EM.

X-ray free electron laser (XFEL) is an unconventional and cutting-edge method for studying protein structures. Unlike traditional X-ray crystallography, XFEL can capture high-resolution structural snapshots of biological molecules, including viral proteins, without the need for crystallization. XFEL has been used to study a major SARS-CoV-2 protein, NendoU, which helps the virus to evade the innate immune system by cleaving antisense viral ribonucleic acid (RNA).[13] Researchers have used serial femtosecond crystallography at an XFEL to determine the room-temperature structure of NendoU, thereby highlighting its flexibility and dynamics. They also demonstrated that NendoU maintains its enzyme activity in crystal form, potentially paving the way for the development of more effective antiviral drugs that target allosteric conformational changes and are less prone to resistance.

NMR spectroscopy, which involves the detection of electromagnetic signals with spin frequency from magnetic field changes applied to the atomic nuclei, provides not only structural information but also dynamic features. It was also used to successfully investigate the receptor-binding domain of the SARS-CoV-2 spike protein.[14] NMR spectroscopy studies have challenged the recombinant production of nonstructural proteins of SARS-CoV-2 and called for further development of inhibitors or as a suitable repurposed compound target (valuable resources available at https://covid19-nmr.de/).

Cryo-EM determines the in situ macromolecular structure without the need for crystallization; it was used to successfully reveal the intact spike protein in a lipid bilayer as a product of the fusion reaction, helping better understand the membrane fusion process of SARS-CoV-2.[15] Cryo-electron tomography (cryo-ET) is an advanced imaging technique that allows the visualization of viral molecules. A research team used cryo-ET to study the viral replication compartment of SARS-CoV-2 and elucidate its budding mechanism.[16] They observed RNA filaments within double-membrane vesicles associated with viral replication, proposed a mechanism for membrane bending during virion assembly, and suggested that the virus packages its genome around multiple distinct ribonucleoprotein complexes to accommodate its large genome while maintaining flexibility between them.

3. ADVANCED COMPUTATIONAL APPROACHES TO COVID-19

In general, the potential drug targets of SARS-CoV-2 (and their known structures available in Identifiers in Protein Data Bank, PDB ID) include spike (S) protein (6VXX and 6VSB), nucleocapsid (N) protein (6M3M), envelope (E) protein (7K3G), membrane (M) protein (3I6G), main protease (Mpro, 7C2Q), RNA-dependent RNA polymerase (RdRp, 7D4F), and several nonstructural proteins (NSPS). The inexpensive and convenient way to identify possible molecules that can bind, interact, or even interfere with the sequential functions of these protein targets is through the reprofiling, retasking, repositioning, and rescue of known drugs.

Among these methods, drug repositioning[17] is the most popular and includes three major steps: candidate drug identification, mechanistic evaluation of the drug effect in preclinical models, and evaluation of candidate drugs' efficacy in phase II clinical trials. The first step is crucial with high potential and usually requires various experimental or in silico approaches to be successful. The later theoretical one can be done using molecular simulations without real experiments at an early stage, thus preventing a considerable strain on human resources.

Some novel potential protein targets, such as the granulocyte colony-stimulating factor (CSF3),[18] and known drugs, such as nafcillin, nabumetone, octacosanol, cinametic acid, ascorbyl palmitate, guaifenesin, remdesivir (targeted to RdRp), molnupiravir, and nirmatrelvir,[16] have been recently repurposed and reported as new drugs against SARS-CoV-2. However, the range of drug repositioning is restricted to a limited library of known molecules. Future critical breakthrough points in new drug development may be achieved through modern structural biology–based approaches, especially AI-assisted molecular structural simulation.

The maturity of structural biology along with the progress and integration of medical AI facilitated new strategies against COVID-19, given the major improvements in computational methods such as structural prediction with homology modeling and machine learning and deep learning models, such as MODELLER,[19] RoseTTAFold,[20] and AlphaFold2,[21] as well as the use of molecular dynamics (MD) simulations.

For example, the Amaro Group applied the all-atom MD simulations to elucidate in detail the mechanism underlying the interactions between the SARS-CoV-2 spike protein and the vaccine epitope.[22] They further revealed the functional roles of the glycan coat (N165 and N234, which together regulate the stability of the receptor-binding domain) in inhibiting the binding of spike with hACE2. Their simulation studies predicted the "Closed" status of the spike protein based on a known structure solved by cryo-EM (PBD ID: 6VXX) and the "Open" state based on another structural solved data (also by cryo-EM, PBD ID: 6VSB). These findings provided helpful structural insights into the future development of vaccines and drugs against COVID-19.

Another important SARS-CoV-2 protein is the main protease ($3CL^{pro}$), which is considered a potential drug target because it plays an essential role in the viral life cycle.[23] Structure-based virtual screening highlighted 9 of 1.3 billion compounds from the ZINC15 library, which were further complemented by MD simulations to determine the most potent inhibitor: ZINC000452260308 was thus identified as a lead molecule for new and even next-generation antiviral drugs.

Cao et al[24] used computational protein design to create a small and stable protein that binds tightly to the spike protein, thus preventing its binding to hACE2[24] Furthermore, cryo-EM revealed that the complexes were nearly identical, which could be another starting point for new anti-COVID-19 drugs.

Finally, novel inhibitors can also be designed through docking thousands of anti-human immunodeficiency viruses-1 compounds with protease D and using MD simulations to test the stability of the complexes.[25]

4. FUTURE PERSPECTIVES

All three cornerstones of classical structural biology, namely X-ray crystallography, NMR, and cryo-EM, settled the ways of the anti-COVID-19 war. Advancements in modern structure-based approaches, and their combination with AI technology, have immense value and potential in furthering the design and development of new antivirals or vaccines against SARS-CoV-2.[26–29] The successful experience with structural

biology with COVID-19, including its contributions to nearly all aspects—from the basic protein structure details, mechanism of viral infection and signaling, to the assistance for drug design and development of protein inhibitors—will always remain a major achievement in the history of antiviral development.[30–33]

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science and Technology of Taiwan (MOST 110-2320-B-075-005, MOST 108-2745-8-075-001, MOST 105-2320-B-075-002) and Taipei Veterans General Hospital (V110C-018). This study is based in part on data from the Big Data Center, Taipei Veterans General Hospital (BDC, TPEVGH). The interpretations and conclusions contained herein do not represent the position of Taipei Veterans General Hospital.

REFERENCES

1. Kritikos M. Ten technologies to fight coronavirus. *EPRS: European Parliamentary Research Service* 2020. Available at https://policycommons.net/artifacts/1337141/ten-technologies-to-fight-coronavirus/1944801/. Accessed 18 Oct 2022.
2. Yang DM, Chang TJ, Hung KF, Wang ML, Cheng YF, Chiang SH, et al. Smart healthcare: a prospective future medical approach for COVID-19. *J Chin Med Assoc* 2022;86:138–46.
3. Katzourakis A. COVID-19: endemic doesn't mean harmless. *Nature* 2022;601:485.
4. Bárcena M, Barnes CO, Beck M, Bjorkman PJ, Canard B, Gao GF, et al. Structural biology in the fight against COVID-19. *Nat Struct Mol Biol* 2021;28:2–7.
5. Zhang J, Chen B. Fighting SARS-CoV-2 with structural biology methods. *Nat Methods* 2022;19:381–3.
6. Lynch ML, Snell EH, Bowman SEJ. Structural biology in the time of COVID-19: perspectives on methods and milestones. *IUCrJ* 2021;8:335–41.
7. Yan W, Zheng Y, Zeng X, He B, Cheng W. Structural biology of SARS-CoV-2: open the door for novel therapies. *Signal Transduct Target Ther* 2022;7:26.
8. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020;367:1260–3.
9. Ma S, Damfo S, Lou J, Pinotsis N, Bowler MW, Haider S, et al. Two ligand-binding sites on SARS-CoV-2 non-structural protein 1 revealed by fragment-based X-ray screening. *Int J Mol Sci* 2022;23:12448.
10. Lee J, Kenward C, Worrall LJ, Vuckovic M, Gentile F, Ton AT, et al. X-ray crystallographic characterization of the SARS-CoV-2 main protease polyprotein cleavage sites essential for viral processing and maturation. *Nat Commun* 2022;13:5196.
11. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020;367:1444–8.
12. Wu CR, Yin WC, Jiang Y, Xu HE. Structure genomics of SARS-CoV-2 and its Omicron variant: drug design templates for COVID-19. *Acta Pharmacol Sin* 2022;43:3021–33.
13. Jernigan RJ, Logeswaran D, Doppler D, Nagaratnam N, Sonker M, Yang JH, et al. Room-temperature structural studies of SARS-CoV-2 protein NendoU with an X-ray free-electron laser. *Structure* 2023;31:138–51.e5.
14. Creutznacher R, Maass T, Veselkova B, Ssebyatika G, Krey T, Empting M, et al. NMR experiments provide insights into ligand-binding to the SARS-CoV-2 spike protein receptor-binding domain. *J Am Chem Soc* 2022;144:13060–5.
15. Shi W, Cai Y, Zhu H, Peng H, Voyer J, Rits-Volloch S, et al. Cryo-EM structure of SARS-CoV-2 postfusion spike in membrane. *Nature* 2023;619:403–9.
16. Klein S, Cortese M, Winter SL, Wachsmuth-Melm M, Neufeldt CJ, Cerikan B, et al. SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography. *Nat Commun* 2020;11:5885.
17. Rameshrad M, Ghafoori M, Mohammadpour AH, Nayeri MJD, Hosseinzadeh H. A comprehensive review on drug repositioning against coronavirus disease 2019 (COVID19). *Naunyn Schmiedebergs Arch Pharmacol* 2020;393:1137–52.
18. Fang C, Mei J, Tian H, Liou YL, Rong D, Zhang W, et al. CSF3 is a potential drug target for the treatment of COVID-19. *Front Physiol* 2021;11:605792.
19. Webb B, Sali A. Comparative protein structure modeling using MODELLER. *Curr Protoc Bioinformatics* 2016;54:5.6.1–37.
20. Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, et al. Accurate prediction of protein structures and interactions using a three-track neural network. *Science* 2021;373:871–6.
21. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. *Nature* 2021;596:583–9.
22. Casalino L, Gaieb Z, Goldsmith JA, Hjorth CK, Dommer AC, Harbison AM, et al. Beyond shielding: the roles of glycans in the SARS-CoV-2 spike protein. *ACS Cent Sci* 2020;6:1722–34.
23. Roy R, Sk MF, Jonniya NA, Poddar S, Kar P. Finding potent inhibitors against SARS-CoV-2 main protease through virtual screening, ADMET, and molecular dynamics simulation studies. *J Biomol Struct Dyn* 2022;40:6556–68.
24. Cao L, Goreshnik I, Coventry B, Case JB, Miller L, Kozodoy L, et al. De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* 2020;370:426–31.
25. Arthur DE, Elegbe BO, Aroh AO, Soliman M. Computational drug design of novel COVID-19 inhibitor. *Bull Natl Res Cent* 2022;46:210.
26. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 2020;581:215–20.
27. Ke Z, Oton J, Qu K, Cortese M, Zila V, McKeane L, et al. Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nature* 2020;588:498–502.
28. Peplow M. Cryo-electron microscopy reaches resolution milestone. *ACS Cent Sci* 2020;6:1274–7.
29. Ford CT, Machado DJ, Janies DA. Predictions of the SARS-CoV-2 omicron variant (B 11 529) spike protein receptor-binding domain structure and neutralizing antibody interactions. *Front Virol* 2022;2:830202.
30. Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: structure, biology, and structure-based therapeutics development. *Front Cell Infect Microbiol* 2020;10:587269.
31. Yang H, Rao Z. Structural biology of SARS-CoV-2 and implications for therapeutic development. *Nat Rev Microbiol* 2021;19:685–700.
32. Li G, Hilgenfeld R, Whitley R, De Clercq E. Therapeutic strategies for COVID-19: progress and lessons learned. *Nat Rev Drug Discov* 2023;22:449–75.
33. Gupta Y, Savytskyi OV, Coban M, Venugopal A, Pleqi V, Weber CA, et al. Protein structure-based in-silico approaches to drug discovery: guide to COVID-19 therapeutics. *Mol Aspects Med* 2023;91:101151.