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Special Issue: Pushing boundaries of cryo-EM

Opinion

Contributions of single-particle cryoelectron microscopy toward fighting COVID-19

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Single-particle cryoelectron microscopy (cryo-EM), whose full capabilities have been realized only within the past decade, has had a pivotal role in the fight against COVID-19. This is due to the technique's intrinsic power to depict both structural and dynamic features of molecules; in this case, of the spike protein of SARS-CoV-2. By now, numerous cryo-EM studies have furthered our understanding of spike protein–angiotensin-converting enzyme 2 (ACE2) receptor interactions, which has informed the design of effective vaccines, and have enabled the characterization of neutralizing antibody binding sites, which will lead to the design of novel therapeutics as the virus evolves.

Highlights

The fight against the COVID-19 pandemic relies to a great extent on cutting-edge research in structural biology.

Cryo-EM has made major contributions in elucidating structures of virus and host proteins.

Cryo-EM has played a crucial role in the development of vaccines and antibodies.

Structural biology in combating diseases

We are now living through a time that will be remembered by historians and by generational family memory for its vast impact on our economy, on the way we live, and on our culture. Much of this is still merely visible in outline and difficult to extrapolate. Because of the worldwide spread of COVID-19 caused by SARS-CoV-2, the impact is felt by all of humankind. We have learned from history books that, over the past millennium at least, epidemics such as the plague have inevitably brought profound – if not revolutionary – changes in the order of the societies inflicted with them. The last pandemic on a similar scale, the Spanish flu in 1918, was overshadowed by the devastations wrought by the First World War that ended in the same year, and its societal impact has therefore been difficult to tell apart from that of the war.

Our chances of success in fighting this pandemic differ greatly from those in previous times because we are much better prepared. The reason for this is the unprecedented knowledge revolution that has taken place in the middle of the 20th century and onwards: we uncovered the unity of life and much of the molecular basis of life processes in all organisms. Particularly, we know how cells receive their instructions for making proteins and sustaining their metabolism from a genetic blueprint in the form of DNA. We know that the current pandemic is caused by a virus that, as many other viruses, exploits and subverts the molecular apparatus of the host to its own gain following a universal playbook we are able to recognize. That body of knowledge, accumulated by research in molecular genetics, molecular biology, and structural biology, has transformed and empowered medicine profoundly. What it means is that, unlike the people who lived through the 1918 pandemic, we are no longer powerless in the global fight against a viral disease.

Paramount in this revolution of medicine have been research endeavors we refer to under the rubric of structural biology; endeavors whose aim is to elucidate the structural basis of life processes on the atomic scale. On that sub-light microscopic level of structure, molecular interactions can be understood and even simulated in detail using the laws of Newtonian mechanics.

For decades, starting with the groundbreaking work of Max Perutz on the structure of myoglobin and hemoglobin [1], X-ray crystallography has been the dominant method for molecular structure

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research. To date, it has given us more than 140 000 atomic structures of biological molecules, all readily accessible in the public database, the Protein Data Bank (PDB)[i,ii]. Application of this method of structural research is, however, restricted to molecules that can be induced to form highly ordered crystals. This limitation has resulted in the exclusion of many other molecules important for medicine, particularly membrane-bound channels and receptors. Another limitation lies in the fact that X-ray crystallography seldom captures molecules in their (multiple) native states. Such limitations do not exist in the recent technique of single-particle cryo-EM, which was recently highlighted by the award of the 2017 Chemistry Nobel Prize[iii] [2].

Although the methods for cryopreparation of sample and computational methods for data analysis and reconstruction go back all the way to the 1980s, atomic resolution could not be achieved for asymmetric structures until the development of novel single-electron-detecting cameras [3–5] and their commercial introduction in 2012. It is fortuitous [6], in hindsight, that the technique was ready in time to help in the combat against several viruses implicated in recent deadly epidemics – Ebola (2014–2016) [7–9], Zika (2015–2016) [10,11], dengue (2019–2020) [12–16], MERS-CoV (2012–2015) [17–20], and now SARS-CoV-2.

In the following, we wish to highlight the crucial role this technique has played in combating SARS-CoV-2, by helping elucidate the structures of both the virus and of the host molecules they interact with as the virus seeks entry into the cell to engineer its takeover. Specifically, both the rapid developments of mRNA-based vaccines and effective antibody therapies have drawn from knowledge gained by single-particle cryo-EM. It will become readily apparent that the most important contributions of the technique have been twofold: first, the capture of the spike protein in its different conformational states and the inference these structures allow to draw on the dynamics of the molecule as it interacts with the host, and second, the characterization of complexes formed by the spike protein with receptors and antibodies.

Virus–host interaction

The key to both the development of vaccines and neutralizing antibodies has been in understanding the way the virus gains entry into the host cell during infection. For this to happen, the viral spike glycoprotein must first recognize and latch on to ACE2; a receptor on the cell surface [21,22]. Knowledge of this interaction has been gained by single-particle cryo-EM studies capturing the structures of the isolated spike protein in its pre- and postfusion configurations, and structure determination of the complex formed by spike protein with ACE2 [18,19,23–25].

It is remarkable that the structure and dramatic conformational changes of the spike protein preceding the fusion event were already understood before the onset of the COVID-19 pandemic caused by SARS-CoV-2 [18,19]. This is due to the close similarity of SARS-CoV-2, in structure and mode of infection, to SARS-CoV-1 and MERS-CoV, as revealed by the studies of the Ward, McLellan, and Veesler groups [18,19,22,23]. Briefly, these studies of coronaviruses have shown that the spike protein is a trimer comprising structurally identical monomers, each composed of two subunits, S1 and S2. The spike protein is a metastable fusion machine. On the surface of the virus, spike exists predominantly in the prefusion form, ready to fuse with host when they bind ACE2. However, due to its metastability, some spikes will dissociate the S1 subunit and trigger the conformational changes in S2 associated with fusion. Critically, it is the prefusion conformation that must be recognized by the immune system to fight infection. In the prefusion conformation, the subunit S1 conformation alternates between two conformations in which its receptor-binding domain (RBD) is either in an ‘up’ (receptor-accessible) or ‘down’ (receptor-inaccessible) position (Figure 1). Binding to ACE2 in the accessible position leads to the fusion event.

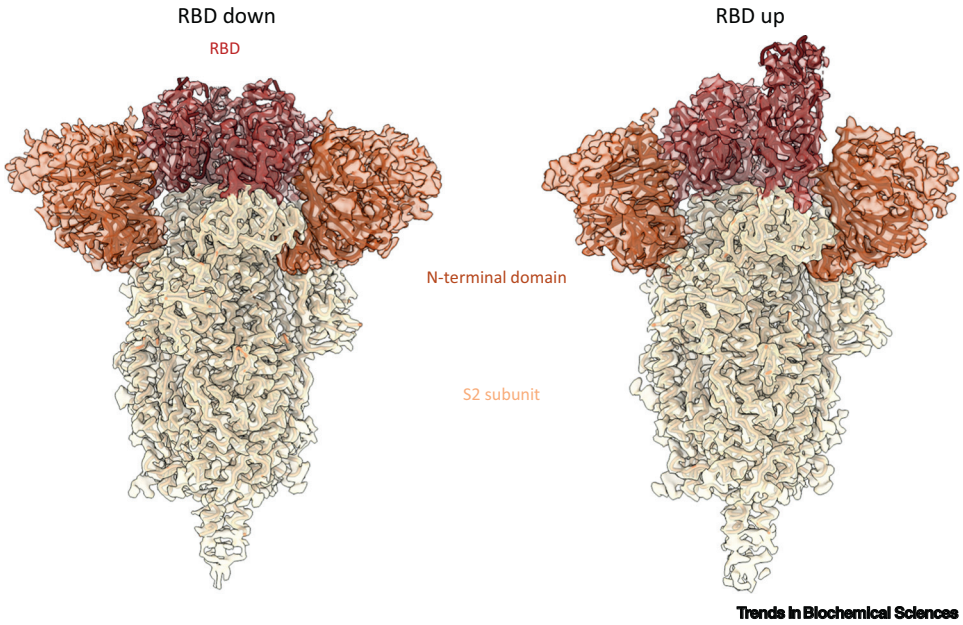

Figure 1. Cryoelectron microscopic reconstruction of SARS-CoV-2 spike protein in two conformations. RBD: receptor binding domain. PDB IDs 6VXX (RBD down) and 6VYB (RBD up) [22].

To gain a detailed understanding of the dynamic interaction between spike protein and receptor, molecular dynamics simulations taking the cryo-EM structure of the spike protein as their starting point have been recently performed [26]. Sztain and coworkers achieved times in the 100-μs range for an all-atom simulation by using the weighted ensemble path-sampling strategy. Their simulations indicated that a glycan gate controls the move from the down to the up position of the RBD. These results received experimental confirmation from a different type of analysis of McLellan's cryo-EM dataset, reported in the same paper: ManifoldEM, which uses a geometric machine-learning method, manifold embedding, in the analysis of cryo-EM data from a large ensemble of molecules in thermal equilibrium. It yields a tabulation of occupancies across conformational states and, eventually, a map of the molecule's energy landscape [27].

Development of vaccines

The development of the first two successful vaccines against COVID-19, by Pfizer and Moderna, have relied on a strategy that had been devised in the fight against the similar coronaviruses, SARS-CoV-1 and MERS-CoV, several years ago [19,28]. As noted previously, the similarity between the three viruses extends from the structural similarity to the similarity of the way the viruses infect the host. In particular, this strategy of vaccine development makes use of mRNA that encodes the spike protein. When mRNA coding for spike protein is injected, the body's ribosomes make many copies of this protein, which in turn elicit the body's immune response, making the body equipped to fight off the real virus whenever it tries to enter later on [29].

It is fascinating to see how the detailed knowledge of the spike protein's interactions with ACE2 gained in cryo-EM studies of SARS-CoV-1 and MERS-CoV just a few years before the onset of COVID-19 paved the way to the development of highly effective mRNA-based vaccines against SARS-CoV-2. Using cryo-EM, Andrew Ward's group at The Scripps Research Institute, in collaboration with Jason McLellan's group then at Dartmouth, first working with the common cold HKU1

coronavirus [28], and later with MERS-CoV [17], discovered that a pair of point mutations to proline will prevent formation of a helix associated with the post-fusion conformation [17]. Such S2P spikes – spikes with the double proline mutation – are almost exclusively in the prefusion conformation in which the virus elicits the immune response, paving the way to a successful vaccination strategy. Now fast forward to the beginning of January 2020, when the first cases of COVID-19 were reported in the USA. Within 2 weeks of receiving the genetic sequence of the newly found virus from China, McLellan – now at the University of Texas at Austin and collaborating with Barney Graham at National Institutes of Health (NIH) – was able to visualize the spike protein by cryo-EM and show that the same trick for stabilizing the active form works for COVID-19 that had been found effective for SARS-CoV-1 and MERS-CoV [23].

Characterization of antibodies

Before emergency approval for the first vaccines was given by the US Food and Drug Administration (FDA) [30,31], much of the clinical focus was on development of effective treatments for COVID-19. While hydroxychloroquine and remdesivir dominated the headlines [32–34], physicians were, and still are, using monoclonal antibodies to treat high-risk cases of COVID-19 [35–37]. These treatments involve injecting patients with antibodies identified from convalescent COVID-19 patients that specifically target the spike protein; work made possible by the discovery of stabilizing mutations discussed previously [38–45]. Overall, these studies have shown that effective neutralizers target either the RBD or the N-terminal domain (NTD) – domains located near the top of the spike (Figure 2).

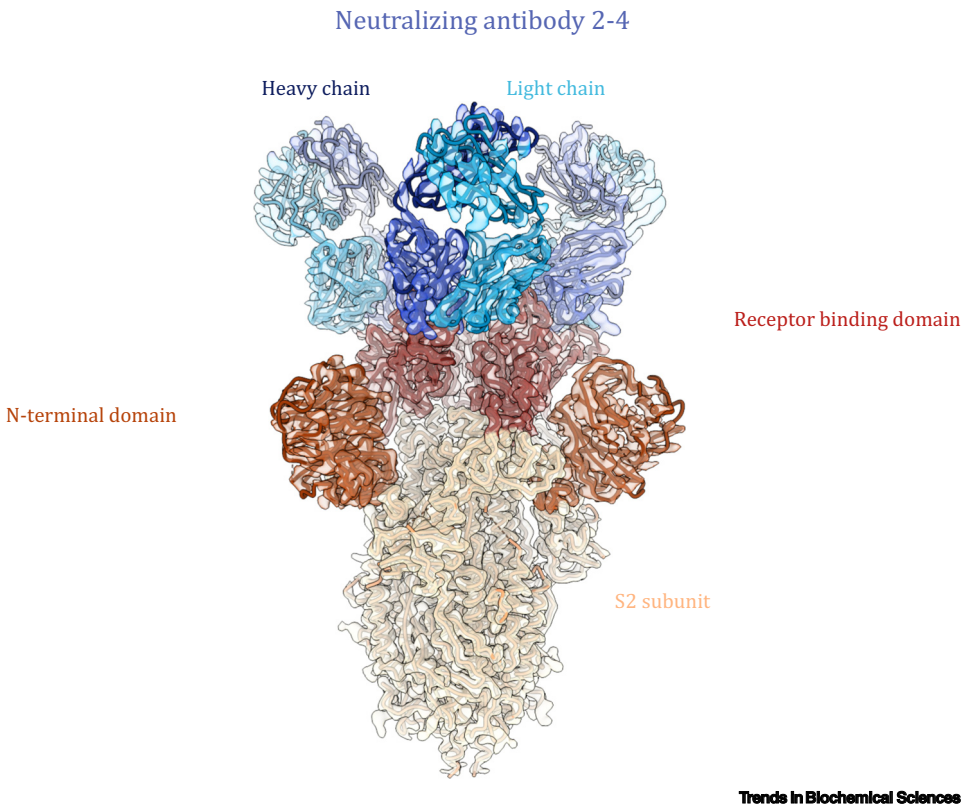

Figure 2. Cryoelectron microscopic structure of a neutralizing antibody bound to the SARS-CoV-2 spike protein. PDB ID 6XEY [41].

The most effective RBD-directed antibodies neutralize SARS-CoV-2 by blocking RBD interactions with ACE2 [38,41,42,44–46] Several genetic classes of antibodies are known to arise in common among humans in response to SARS-CoV-2 infection [46–49]; members of which have been produced as therapeutics. Some RBD-directed antibodies bind only with RBD in the down position, thus locking the spike in a conformation that cannot bind ACE2 [46,49]. Others bind to the side of RBD and can bind with RBD in the up position [41,49–51]. In both cases, neutralization is thought to arise through interference with ACE2 interaction.

The other region vulnerable to neutralization by antibodies is the NTD. Neutralizing antibodies targeting the NTD are just as potent as those targeting the RBD [41], but they neutralize through an as-yet unknown mechanism. There are several possibilities for this action: (i) it blocks the prefusion to postfusion conformational transition, as was seen with a MERS-CoV antibody [52]; (ii) it blocks interaction with one of the other proposed molecules that the virus may use to infect cells [53–55]; (iii) it may interfere with some later step in the infection process, after the virus has entered the cell; or (iv) the binding of the antibody to NTD affects the mobility of the RBD allosterically, that is, through conformational changes that propagate over a long distance [56]. Cryo-EM has revealed that these antibodies target a single supersite [57,58] in a highly prevalent response. The presence of a single vulnerable site may be due to the large number of sugars shielding much of the NTD, or it may be due to the need for antibodies to approach the viral spike from a particular angle or to induce or prevent a specific conformational change [57]. Most recent data appear to imply the first of these modes of action, namely inhibition of the fusion process after binding to ACE2 [59].

Understanding the variety of ways antibodies can bind to and neutralize the virus is critical in designing antibody therapies against emerging variants of concern (VOCs). Unfortunately, many mutations in these VOCs are within the epitopes of the reproducible antibody classes, consistent with the idea that human immune pressure is the driving force in the generation of mutations [60]. One approach to mitigating this problem was to develop therapies that are cocktails of multiple antibodies; an approach taken by Regeneron [36]. If the mutation giving rise to the variant reduces the efficacy of one antibody, another antibody in the cocktail may still work.

Concluding remarks

The study of proliferating variants has driven much of the recent cryo-EM work on SARS-CoV-2 neutralizing antibodies. This work has elucidated the atomic positions of the mutations [61,62]; helped explain what is driving these mutations [51]; and most importantly, solved structures of antibodies that are capable of neutralizing the variants, thus making them ideal candidates for improved therapeutics [51,63].

Overall, the advent of cryo-EM has revolutionized our ability to quickly visualize and understand viral proteins, their conformational states, and interactions with receptors and antibodies. The speed at which such information became available for SARS-CoV-2 during the COVID-19 pandemic was breathtaking. For HIV, by comparison, the first structures of pieces of the viral spike appeared about a decade after the onset of the AIDS pandemic [64], and those of the complete viral spike appeared a decade later [65–67]. The advent of cryo-EM has defined a 'new normal' in which such structural information can be acquired in weeks or months, rather than decades or years. Cryo-EM has thus become a critical pillar in the protection against pandemic threats (see Outstanding questions).

Outstanding questions

Integration of techniques, such as genetic sequencing, cryo-EM, and molecular dynamics described in this article, has enabled rapid understanding of virus–host interactions. How will the increasing toolbox of genomics and structural tools (e.g., by addition of cryo-electron tomography) shed further light on these interactions and viral lifecycles? What new therapeutics can be derived from these observations?

As technologies for cryo-EM continue to develop (i.e., more reproducible sample preparation, enhanced detectors, and increased accessibility to equipment and expertise), how quickly can the scientific communities contribute to combatting future pandemics? Further, with these advances, how can the global scientific community contribute to ongoing battles with known infectious diseases?

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Declaration of interests

The authors have no interests to declare.

Resources

[i]www.rcsb.org/

[ii]www.rcsb.org/stats

[ii]www.nobelprize.org/prizes/chemistry/2017/press-release/

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