Cryo-EM Trends for Drug Discovery

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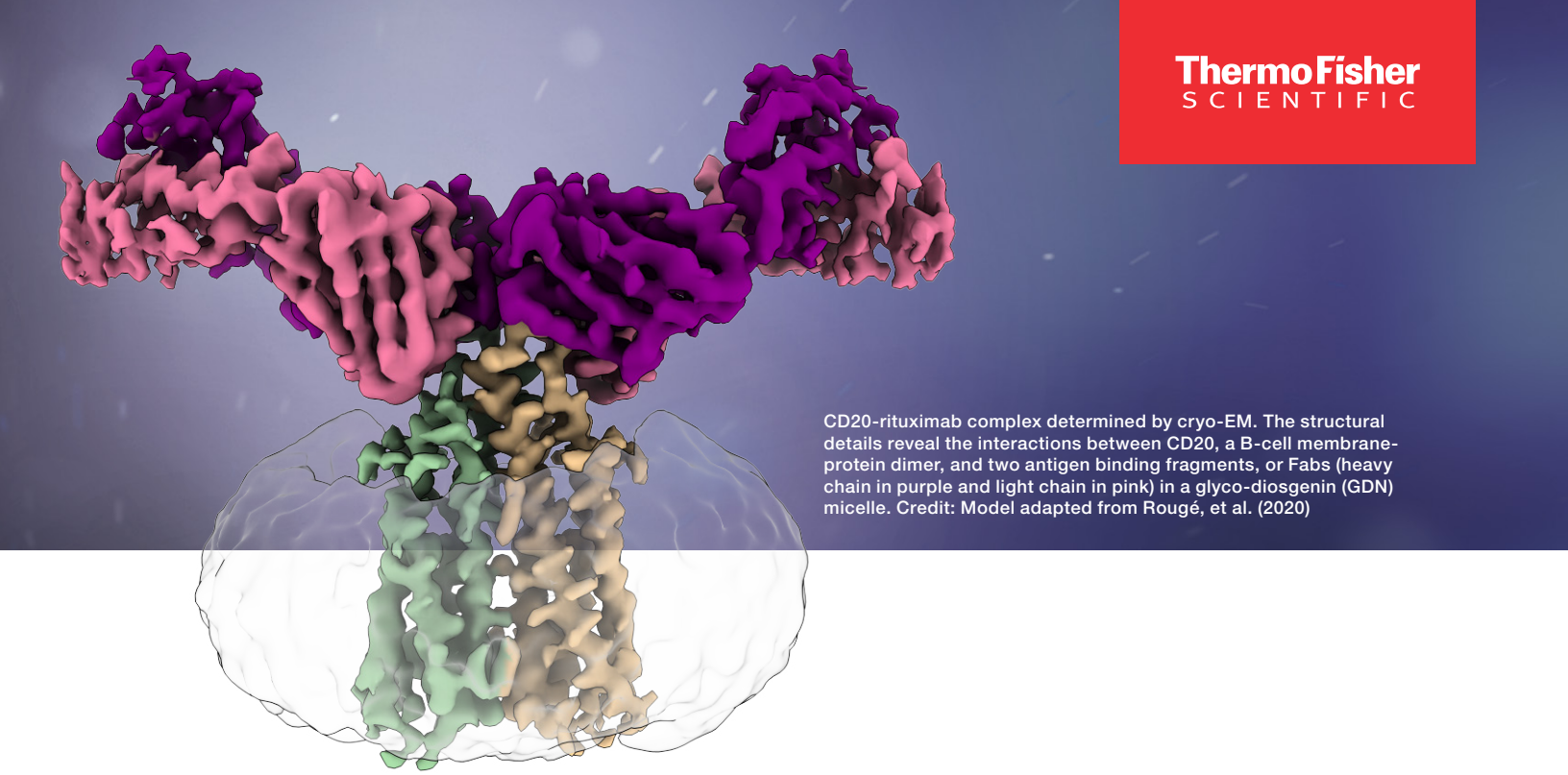

CD20-rituximab complex determined by cryo-EM. The structural details reveal the interactions between CD20, a B-cell membrane-protein dimer, and two antigen binding fragments, or Fabs (heavy chain in purple and light chain in pink) in a glyco-diosgenin (GDN) micelle. Credit: Model adapted from Rougé, et al. (2020)

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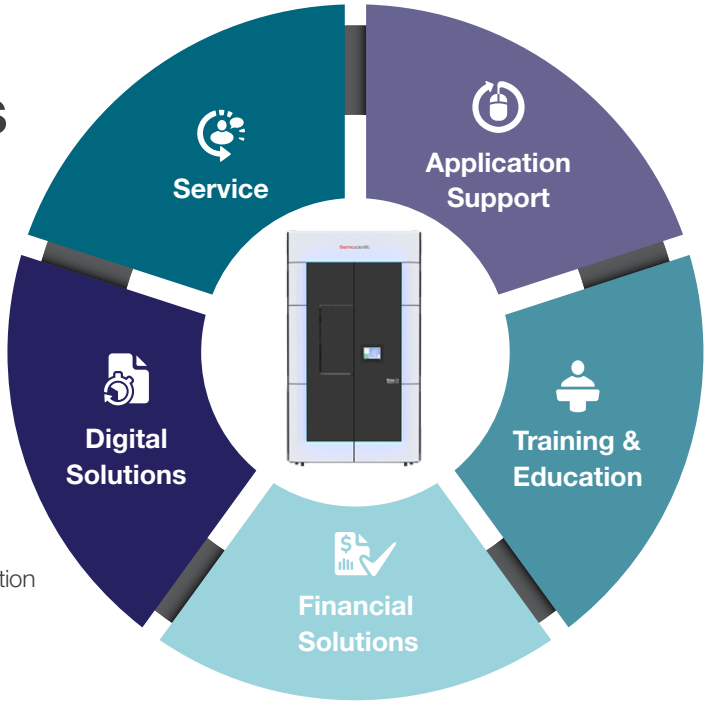

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Cryo-EM Trends for Drug Discovery

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COVER IMAGE: Nakane, T., Kotecha, A., Sente, A. et al. Single-particle cryo-EM at atomic resolution. Nature 587, 152–156 (2020)

Introduction

Cryo-electron microscopy (cryo-EM) has advanced rapidly in the past few years, positioning itself as an invaluable tool for the pharmaceutical industry. Nobel Laureate Richard Henderson predicted that by 2024, more protein structures will be determined by cryo-EM than X-ray crystallography.

Numerous advancements have made cryo-EM better suited for pharmaceutical research. The timeliness and throughput of the workflow has increased dramatically, allowing membrane protein targets to be supported by structural biology, similar to how kinases are supported by crystallography. The publication of GPCR, ion channel, and Covid-19 spike protein structures by multiple competing groups demonstrates the technique's increased ease of use. Cryo-EM is no longer an exhausting Nobel-prize-worthy effort—numerous groups have now integrated this technique into their work.

The Resolution Revolution, and the subsequent Throughput Revolution, made cryo-EM fit into the drug discovery process as smoothly as X-ray crystallography. Will we go beyond that in the next few years? Unhindered by crystal contacts, a cryo-EM sample contains multiple conformations that sample the flexibility of proteins. It's exciting to look forward to drugs that lock one such conformational state intentionally with an allosteric compound or antibody.

For example, deconvoluting the signature assembly of neurodegenerative Tau filaments may inspire and validate desperately needed models for geriatric diseases, finally giving that field the push it needs.

In the past the use of structure biology has been used as a way to strengthen patents but recent publications, showing distinct modes of action for different antibody drugs against the same target, indicate that cryo-EM will likely be used for the antibody discovery and development process much earlier.

This e-Book shows just some of the things that can be done with cryo-EM. We hope you will see how much the technique has matured, and how the microscopes themselves have become more intelligent, enabling users to focus on the science, instead of on the machine.

Hans Raaijmakers, PhD
Staff Scientist, Thermo Fisher Scientific

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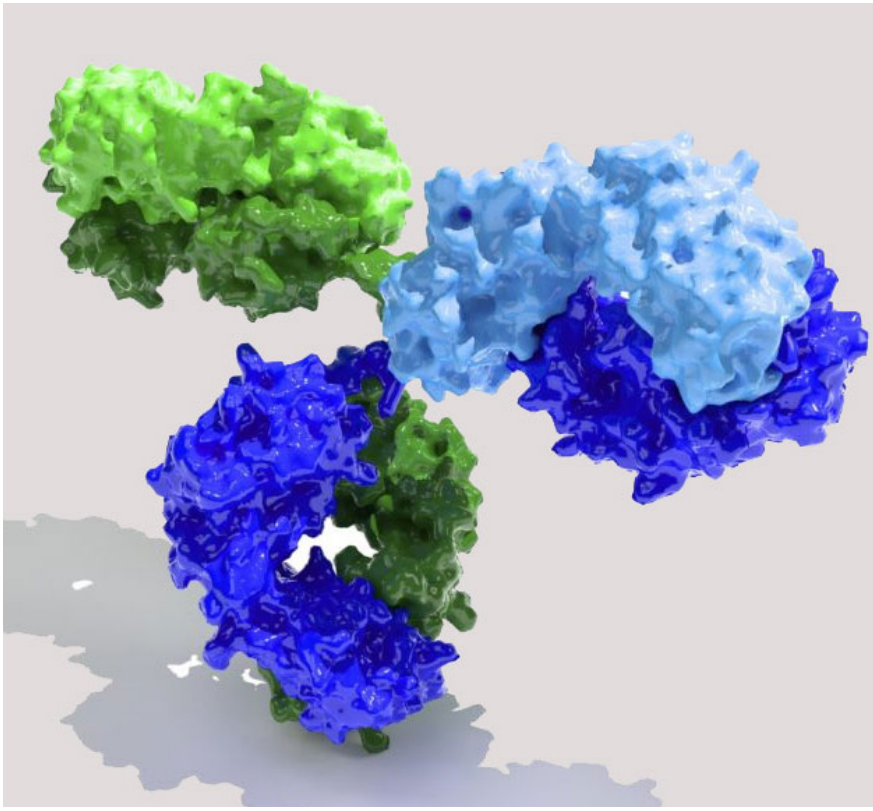
GEN Genetic Engineering & Biotechnology News

Zika and Dengue Neutralizing Human Antibody Distorts Dengue but Not Zika



Source: Indigo Molecular Images, Ltd/Getty Images

Combining cryogenic electron microscopy (cryo-EM) and hydrogen/deuterium exchange mass spectrometry (HDXMS), the researchers visualize the two viruses and explore their movement in presence of the antibody.

Scientists have identified a new way in which antibodies disable viruses. In addition to latching on to antigenic proteins on the surface of a virus and blocking the virus from infecting host cells, a new study shows antibodies can also burrow in and distort the viral surface, effectively preventing the virus from docking on and entering host cells.

An international team of researchers led by scientists at Penn State report this new finding in an article in the journal Cell, titled "Human antibody C10 neutralizes by diminishing zika 2 but enhancing dengue dynamics." The authors show the same antibody can neutralize zika and dengue in two different ways.

"This study reveals a new mode of virus neutralization by antibodies. Antibodies have been traditionally assumed to neutralize their targets by a sole mechanism of blocking the surface, so that the virus cannot access its target receptor site. We have demonstrated that antibodies show additional mechanisms of virus neutralization. They distort their virus targets by burrowing into the target surface," says Ganesh Anand, PhD, associate professor of chemistry at Penn State and co-corresponding author of the paper.

"Furthermore," says Anand, "different concentrations of antibodies elicit different conformational changes in the entire virus particle. This study emphasizes how a

virus represents a moving target for antibodies, which in turn alters the modes of antibody engagement. This also reveals that viruses offer a nonuniform epitope landscape for antibodies to bind and neutralize."

Anand and his colleagues investigate the interactions between human monoclonal antibody (HMAb) C10 and the two disease-causing viruses that the antibody strongly neutralizes.

Combining cryogenic electron microscopy (cryo-EM) and hydrogen/deuterium exchange mass spectrometry (HDXMS), the researchers visualize the two viruses and explore their movement in presence of the antibody.

"The combination of static imaging with dynamic visualization by mass spectrometry is novel and provided orthogonal complementary insights into virus behavior in solution..."

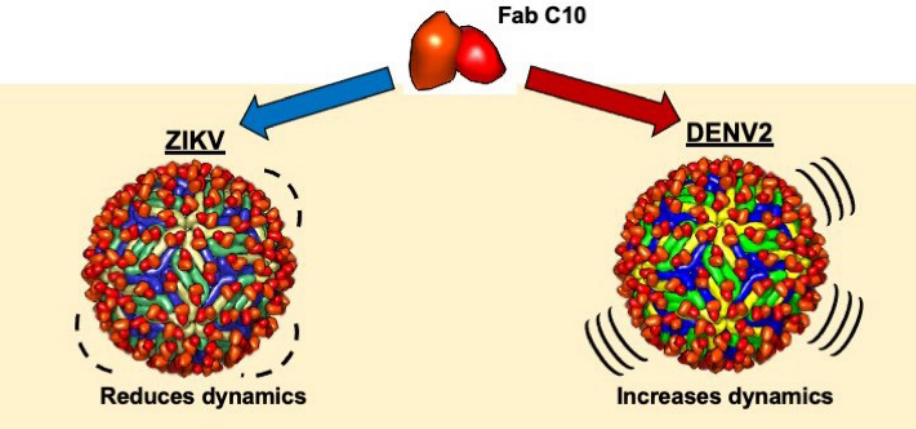
"Cryo-EM involves flash-freezing a solution containing molecules of interest and targeting them with electrons to generate numerous images of individual molecules in different orientations," says Anand. "These images are then integrated into one snapshot of what the molecule looks like. The technique provides more accurate pictures of molecules than other forms of microscopy." The team collected high resolution, static cryo-EM snapshots of the viruses in increasing concentrations of the HMAb C10 antibody.

"The notion that viruses are highly mobile targets, that certain antibodies enhance the virus' mobility and achieve neutralization through distortion came from amide HDXMS. HDXMS is a method that can be leveraged to measure the movements of virus surface proteins," says Anand. HDXMS involves submerging molecules of interest — in this case zika and dengue virus with HMAb C10 antibodies—in "heavy water" that has had its hydrogen atoms replaced with deuterium, hydrogen's heavier isotope.

"When you submerge a virus in heavy water, the hydrogen atoms on the surface of the virus exchange with deuterium," says Anand. "You can then use mass spectrometry to measure the heaviness of the virus as a function of this deuterium exchange. By doing this, we observed that dengue virus, but not zika virus, became heavier with deuterium as more antibodies were added to the solution. This suggests that for dengue virus, the antibodies are distorting the virus and allowing more deuterium to get in. It's as if the virus is getting squished and more surface area becomes exposed where hydrogen can be exchanged for deuterium."

Although HMAb C10 effectively neutralizes both zika and dengue viruses, this HDXMS data shows while the antibody squishes the dengue virus, it does not result in increased incorporation of heavy water in the zika virus, indicating the antibody does not squish the zika surface.

"The combination of static imaging with dynamic visualization by mass spectrometry is novel and provided orthogonal complementary insights into



The same antibody can neutralize zika and dengue viruses in two different ways — one where it binds to the virus and deactivates it (left), which is the traditional way we think about antibody activity, and the other where it burrows in and distorts the virus (right).

[Source: Ganesh Anand, Penn State]

virus behavior in solution," says Anand. "It's like those cartoon flipbooks, where each page has a slightly different image, and when you flip through the book, you see a short movie. Imagine a flipbook with drawings of a racehorse. **Cryo-EM shows you what the racehorse looks like and HDXMS shows you how fast the racehorse is moving. You need both techniques to be able to describe a racehorse in motion.** This complementary set of tools enabled us to understand how one type of antibody differentially affects two types of viruses."

The researchers note, the more antibodies they add, the more distorted the dengue virus particles become, suggesting that the relationship between the quantities of interacting molecules determines the extent of neutralization. At saturating conditions, in which antibodies are added at high enough concentrations to fill all available binding locations on the dengue viruses, the researchers show, 60% of the virus' surface is distorted. This distortion is enough to protect host cells from infection.

"If you have enough antibodies, they will distort the virus particle enough so that it's preemptively destabilized before it even reaches its target cells," Anand says.

When the scientists expose BHK-21 cells—a cell line derived from the kidneys of baby hamsters–to antibody-bound dengue viruses, they see 50–70% fewer cells are infected.

Anand explains, while "antibodies can work by jamming the exits so the passenger cannot get out of the car, we have found a new mechanism in dengue virus where antibodies basically total the car so it cannot even travel to a cell."

Unlike SARS-CoV-2, which has spike proteins protruding in all directions, Anand explains, the surfaces of both zika and dengue are smoother with easily accessible "peaks" known as five-fold vertices and progressively inaccessible "valleys" known three-fold and two-fold vertices.

"Antibodies do not like two-fold vertices because they are very mobile and difficult to bind to," says Anand. "We found that once the five- and three-fold vertices have been fully bound with antibodies, if we add more antibodies to the solution, the virus starts to shudder. There's this competition taking place between antibodies trying to get in and the virus trying to shake them off. As a result, these antibodies end up

burrowing into the virus rather than binding onto the 2-fold vertices, and we think it's this digging into the virus particle that causes the virus to shake and distort and ultimately become non-functional."

Zika is a more stable and less dynamic virus than dengue, which has a lot of moving parts, explains Anand. "Dengue and Zika look similar but each one has a different give. Dengue may have evolved as a more mobile virus as a way of avoiding being caught by antibodies. Its moving parts confuse and throw off the immune system. Unfortunately for dengue, antibodies have evolved a way around this by burrowing into the virus and distorting it."

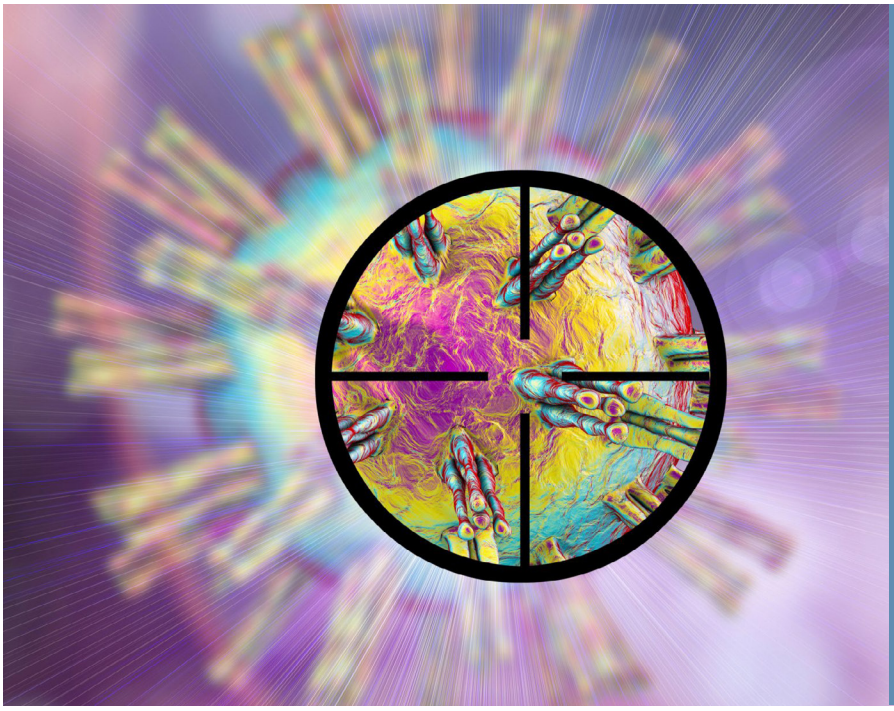
The distortion strategy of virus neutralization is not unique to antibody engagement with dengue and zika viruses. "Dengue is just a model virus that we used in our experiments, but we think this preemptive destabilization strategy may be broadly applicable to any virus," says Anand. "It may be that the antibodies first attempt to neutralize viruses through the barrier method and if they are unsuccessful, they resort to the distortion method."

These new findings could be useful in designing therapeutic antibodies, Anand says. "HMAb C10 antibodies are specific to dengue and zika viruses and happen to be capable of neutralizing zika and dengue viruses in two different ways, but you could potentially design therapeutics with the same capabilities for treating other diseases, such as COVID-19. By creating a therapeutic with antibodies that can both block and distort viruses, we can possibly achieve greater neutralization."

He adds, "You don't want to wait for a virus to reach its target tissue, so if you can introduce such a therapeutic cocktail as a nasal spray where the virus first enters the body, you can prevent it from entering the system. By doing this, you may even be able to use less antibody since our research shows that it takes less antibody to neutralize a virus through the distortion method. You can get better bang for the buck."

The study uncovers a new strategy that antibodies use to disable viruses. Future studies by the group will probe deeper into the terms of this novel engagement. "We are trying to define the first principles of antibody engagement on virus surfaces. We are particularly interested in identifying the rules for destabilizing or distorting antibody design. This novel mechanism would add to the antiviral arsenal. We wish to develop targeted virus-distorting antibodies. We are also interested in correlating virus mutational hotspots with mobile loci on the surface," says Anand.

Coronavirus SARS-CoV-2 Spike Structure: <4 Weeks from Sequence to Published Manuscript



Coronavirus SARS-CoV-2 Spike Structure Mapped at Atomic Scale by Cryo-EM

Source: Kateryna Kon/Science Photo Library/Getty Images

Researchers have determined the first 3D atomic-scale map of the spike glycoprotein of the novel coronavirus (SARS-CoV-2). Mapped by Cryo-EM, this finding from teams at the University of Texas (UT) at Austin and the National Institutes of Health is an essential step to allow researchers to develop vaccines and antiviral drugs to combat the virus.

The work is published in the Science article, "Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation."

The authors determined a 3.5 Å-resolution cryo-EM structure of the S trimer. The predominant state of the trimer, the authors write, "has one of the three receptor-binding domains (RBDs) rotated up in a receptor-accessible conformation." They also show "biophysical and structural evidence that the 2019-nCoV S binds [angiotensin-converting enzyme 2] ACE2 with higher affinity than SARS-CoV S."

Jason McLellan, PhD, associate professor at UT Austin, and his colleagues have spent many years studying other coronaviruses, including SARS-CoV and MERS-CoV. They had already developed methods for locking coronavirus spike proteins into a shape that made them easier to analyze and could effectively turn them into candidates for vaccines. This experience gave them an advantage over other research teams studying the novel virus.

"As soon as we knew this was a coronavirus, we felt we had to jump at it," McLellan said, "because we could be one of the first ones to get this structure. We knew

exactly what mutations to put into this, because we've already shown these mutations work for a bunch of other coronaviruses."

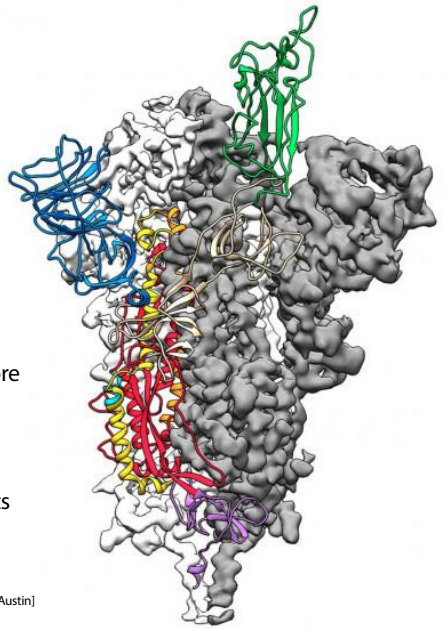
SARS-CoV-2 Spike Protein

The bulk of the research was carried out by the study's co-first authors, PhD student Daniel Wrapp and research associate Nianshuang Wang, both at UT Austin.

Just two weeks after receiving the genome sequence of the virus from Chinese researchers, the team had designed and produced samples of their stabilized spike protein. It took about 12 more days to reconstruct the 3D atomic-scale map of the spike protein and submit a manuscript to Science, which expedited its peer review process.

The molecule the team produced, and for which they obtained a structure, represents only the extracellular portion of the spike protein, but it is enough to elicit an immune response in people, and thus serve as a vaccine.

Additionally, the team tested several published SARS-CoV RBD-specific monoclonal antibodies and found that they do not have appreciable binding to the SARS-CoV-2 S protein, suggesting antibody cross-reactivity may be limited between the two RBDs.



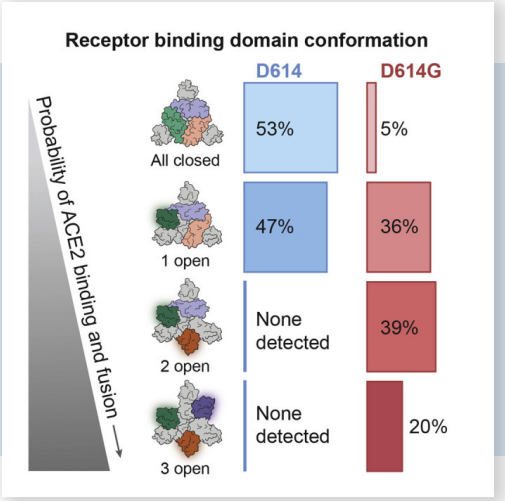
This is a 3D atomic-scale map of the SARS-CoV-2 spike protein. The protein takes on two different conformations—one before it infects a host cell, and another during infection. This structure represents the protein before it infects a cell, called the prefusion conformation.

[Jason McLellan/Univ. of Texas at Austin]

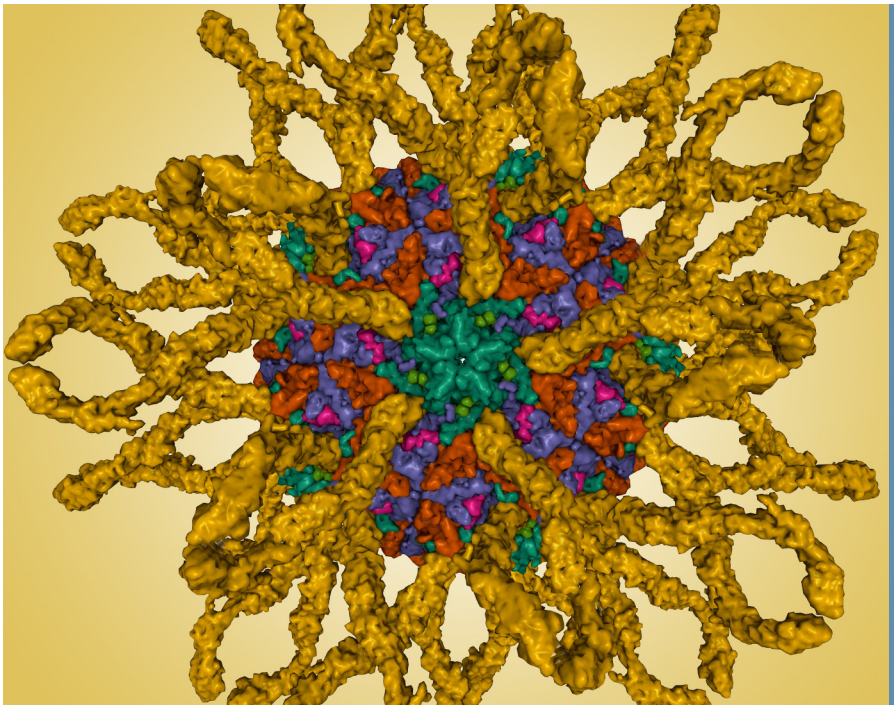
Next, McLellan's team plans to use their molecule to pursue another line of attack against the virus that causes COVID-19, using the molecule as a "probe" to isolate naturally produced antibodies from patients who have been infected with the novel coronavirus and successfully recovered. In large enough quantities, these antibodies could help treat a coronavirus infection soon after exposure. For example, the antibodies could protect soldiers or health care workers sent into an area with high infection rates on too short notice for the immunity from a vaccine to take effect.

Cryo-EM can recognize multiple conformations in one sample. Here, prevalence of conformations of the SARS-CoV-2 spike (S) protein and its variant D614G are compared to support a proposition about infectivity. Read all about it in Cell

Read the Article



Cryo-EM Drives Structure-Based Drug Design



A conversation with Christopher Phillips, PhD, Senior Director, AstraZeneca, and Alexey Rak, PhD, Head, Biostructure and Biophysics, Sanofi, to learn more about this rapidly evolving technology.

Source: vdvornyk/Getty Images

Cryo-EM images frozen-hydrated samples at cryogenic temperatures. This innovative imaging technology reveals the atomic structures of biomolecules, such as large proteins and dynamic protein complexes, providing another avenue to evaluate protein structures and potential drug targets. X-ray crystallography achieves similar resolutions but requires samples to be in crystal form, which may not be possible for certain samples.

GEN spoke to Christopher Phillips, PhD, Senior Director, AstraZeneca, and Alexey Rak, PhD, Head, Biostructure and Biophysics, Sanofi, to learn more about this exciting and rapidly evolving technology and its impact on drug discovery research.

GEN: *What drove your decision to adopt cryo-EM?*

Rak: As head of biostructure and biophysics I pay attention to novel technologies. Cryo-EM helps meet all the imaginable timelines from the receipt of the sample to delivery of the structural information. These timelines are much shorter than for x-ray crystallography. Cryo-EM also requires a smaller amount of material and accepts a larger variation of specimen types. Based on the results we had generated while we were outsourcing use of an instrument, we decided to internalize the technology as it was more cost and time effective. It was a no brainer except for identifying the budget.

Phillips: Structural biology has had a huge impact in the pharmaceutical industry. Structure-based drug design principally has been accomplished using crys-

tallography. But crystallography has some restrictions in determining structures of membrane proteins and large multi-protein complexes that are the targets of many drugs. Cryo-EM expands the target space for structure-based drug design. It allows us to support more drug discovery projects than we can if we only utilize crystallography.

GEN: *Describe the cryo-EM implementation process. Were there major considerations or adoption hurdles? And how did you overcome them?*

Phillips: In Cambridge UK we had a great model for implementing cryo-EM. Formed in April 2016, the Cambridge Pharmaceutical Cryo-EM consortium is a partnership among the Medical Research Council Laboratory of Molecular Biology, University of Cambridge's Nanoscience Centre, and Materials Science Department, Thermo Fisher Scientific and five pharmaceutical companies: Astex Pharmaceuticals, AstraZeneca, GSK, Sosei Heptares Therapeutics, and UCB. The consortium enabled use of the technology and allowed us to have a shared facility to evaluate the use of cryo-EM in drug discovery. Now the pharma partners are making internal investments in cryo-EM as well.

There are a number of constraints to successfully install a cryo-EM. We are in the process of implementing the technology in-house in 2022.

Rak: Cryo-EM is very sensitive to vibration, the magnetic field, the rate of the air replacement and laminar flow and many other factors. You need to be well prepared because the performance of the instrument will reflect the quality of the installation. We worked with the vendor to assess our proposed location that turned out to require some heavy modifications.

Christopher Phillips, PhD, Senior Director, AstraZeneca

To ensure good performance of the instrument we did not wait for delivery. In agreement with the vendor we performed a CWAT (Customer Witness Acceptance Test). We traveled to the production site in the Czech Republic to test the instrument before it was disassembled for shipment to France. This allowed us to inspect the instrument's performance in the ideal environment and then compare that to the performance in the environment we had created.

The first instrument was installed in December 2020 and met all performance criteria. Heavy usage began in January 2021. While we were waiting for delivery, we also had to acquire talent with the required expertise and were able to bring on board personnel with a cumulative 25 years of experience. This was important because cryo-EM is a novel technology, so resources are limited.

The instrument is performing well and justifies the time and investment we spent on the location adaptation. This instrument that was supposed to collect and provide data at 3Å is routinely providing data up to 2.5Å resolution. **This has exceeded our expectations and led to the decision to scale up and internalize two more instruments.**

GEN: *Provide use examples of the impact of cryo-EM on your drug discovery research.*

Rak: During the first SARS epidemic in 2003 cryo-EM technology was not yet available. The SARS gene sequence was available in 2003 and the spike protein structure delivered in 2013, 10 years later. In 2020 after the first SARS-CoV-2 strain was sequenced in just 2-3

weeks the first structure of the spike protein was delivered by a number of teams. Compare the timelines of 10 years to a few weeks.

In drug discovery the advantage of providing information sooner is great for the patients and also introduces a competitive advantage for the companies. Internally, it takes us approximately 2 weeks to determine a structure with cryo-EM. Some structures can be delivered within one week from sample preparation, data acquisition, and data processing through structure elucidation. And there are some records when the structure was delivered within a day. We also have an example where in only ½ day we delivered the structure to answer a pharmacologically-relevant question.

X-ray crystallography performs well and is not yet to be replaced by cryo-EM. More likely they will work in concert for a longer while. Once an x-ray crystallography platform is established and can iteratively provide target-drug bound structures the new structures can be provided within 1-2 weeks. For new targets and those difficult to tackle by crystallization (membrane proteins) cryo-EM can deliver structural information faster. We had an example with an oncology target that delivered a cryo-EM structure within 3 days while it took 2 years to provide it by x-ray crystallography.

Phillips: We are actively doing programs with ion channels, GPCRs, and large multi-protein complexes where cryo-EM provides iterative structures to chemistry teams for structure-based drug design. These are key drug targets where the use of x-ray crystallography would not have been possible.

GEN: *How has the technology advanced and how will its continual evolution provide even more biological insight?*

Rak: We are not in the position to squander money and only invest in robust technologies that can deliver usable pharmacological information. Microscopy is not a new technology. The first electron microscope was developed in the 1930s, but the resolution was not good enough for biological processes. The resolution revolution in cryo-EM made this method applicable in drug discovery in the 21st century.

Alexey Rak, PhD, Head, Biostructure and Biophysics, Sanofi

The technology is evolving rapidly. Even since we acquired our instrument the technology has advanced, and we plan to upgrade our existing instrument. A one-year-old microscope now has the opportunity to perform 1.5 times better, meaning you get the results 1.5 times faster, due to the development of new energy filters and detectors. I would not be surprised to see another major revolution.

The turnover in data collection and processing can still be improved. An analogy is film-based photography. Only after you invested the time to develop the film do you see the results. With the change to digital photography you immediately see the picture. Cryo-EM is at the level of well-developed and advanced film photography. My vision is that we get to the point where on the fly after taking the image you can understand if the sample is good or needs optimization, so you do not waste time collecting data that is not meaningful to structure determination.

With one microscope we generate approximately 1TB of data daily. That comes to half a PB annually. With three instruments we expect to accumulate a volume of 4PB

annually. All our data are now on the cloud. Using cloud computing we have the storage and also the protocols that allow us to install the dedicated software. Since our data and analyses tools are consolidated in one place all of our international users can access it along with the software to process it straightaway.

Data processing consumes a lot of computational power and takes quite long. In collaboration with companies and academia we are developing AI and machine-learning-based algorithms so that you can see the final information sooner and to realize the dream of having cryo-EM perform in a digital way– taking the image, looking at it and seeing your results straightaway.

Phillips: Cryo-EM is a rapidly changing field. A big advancement on the technology side has been the new cameras (detectors) that have come into play, which are sensitive and much faster and speed up the data collection significantly. Although camera advances have been the major change recently, software is improving all the time and there are a number of particularly good EM software packages available now that allow structures to be solved in the time frame that is required for projects. We cannot spend a month determining the structure we need to be able to do it in a few days. Online processing in real time as the data are collected has facilitated the process.

But more improvements are needed, such as in sample preparation techniques – the method of making cryo-EM grids with freezing technologies. These techniques still need to become more robust and reproducible.

GEN: What advice would you give to someone considering implementing cryo-EM?

Phillips: It is a complex process, so you have to have a lot of ducks lined up. To start with, the proteins you are going to be studying by cryo-EM are difficult proteins to make and purify in sufficient quality to study the structure. You have to get your sample preparation and protein chemistry for these difficult targets aligned.

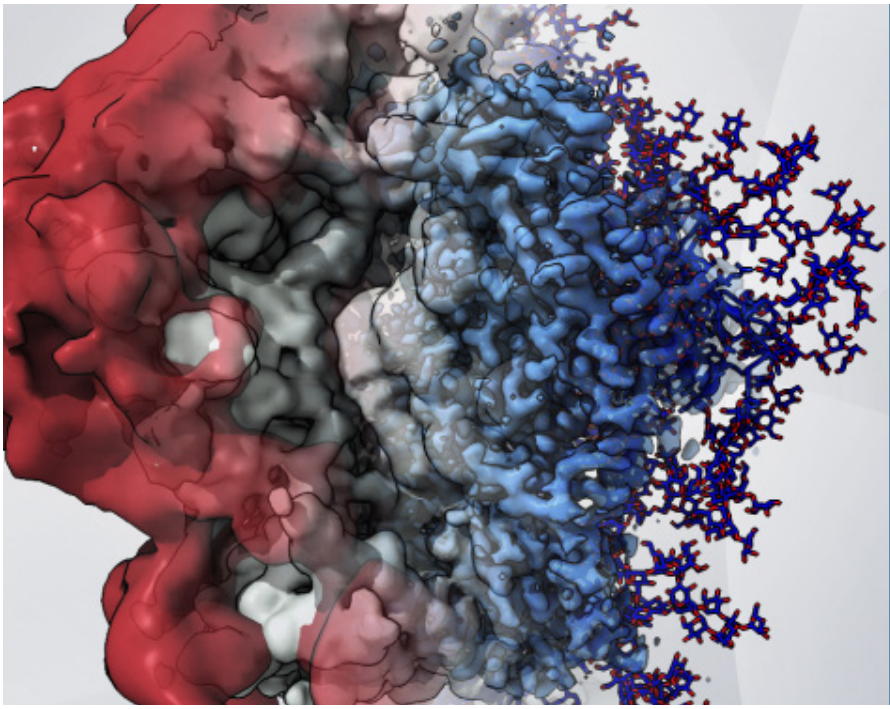
Next, make sure you have a robust microscopy set up. This is a new skill set for many pharma so you need to get the right personnel in place. Finally, there is a large IT component. You have to make sure you are working well with your IT partners to establish the workflows that are required.

Cryo-EM generates a huge amount of data, so you need to have systems in place to manage and handle the data, and process it rapidly to keep up with project demands. You need to have a strong IT infrastructure behind the instrumentation.

Rak: Be bold. Even if you think one instrument is enough you may still want to organize your installation site in a way to accommodate a decision to acquire another instrument. Think twice about saving money on the installation infrastructure. A good installation will lead to good performance and the quality of the results. Also do not ignore the experience of people. We visited a number of places where cryo-EM was installed and tried to avoid the problems they were dealing with so our time could be spent on more creative things than trouble shooting. Lastly, enjoy it.

As you think about this technology more convincing data are being produced all the time. The more people that use cryo-EM the bigger the community, the better the integration and our capability to use this technology in more effective ways to better patients' lives.

HIV Glycans: Shield Shifters and Spike Stabilizers



By mapping HIV's glycan shield, researchers hope to contribute to a better understanding of why antibodies react to some spots on the virus but not others.

Source: Zachary Berndsen, PhD, Ward Lab at Scripps Research

In the fight against infectious diseases, the most daunting foes include viruses that use glycans to fend off antibodies. These glycans form on the outermost spike proteins of HIV and many other viruses, including influenza, Ebola, Lassa, and coronaviruses. Because the glycans are highly mobile, they can seem a blur to antibodies—as well as to scientists hoping to visualize them using conventional atomic-scale imaging technology, that is, conventional cryo-electron microscopy (cryo-EM).

Cryo-EM has been combined with other analytical techniques to visualize HIV's glycan shield. This work, recently accomplished by scientists at Scripps Research and Los Alamos National Laboratory, captured details that were never seen before, including vulnerabilities that could be exploited by new vaccines. Curiously, the new work suggests that HIV glycans play defense and offense simultaneously. According to the scientists, the glycans not only deflect antibodies, they also reinforce the structure of HIV's spike protein, keeping it poised for infection.

Details appeared October 22 in the Proceedings of the National Academy of Sciences, in an article titled, "Visualization of the HIV-1 Env glycan shield across scales." The article describes how the scientists mapped the dense array of N-linked glycans on Env, the HIV-1 envelope glycoprotein.

"Here, we present an integrated approach of single-particle cryo-EM, computational modeling, and site-specific mass spectrometry (MS) to probe glycan

shield structure and behavior at multiple levels," the article's authors wrote. "We found that dynamics lead to an extensive network of interglycan interactions that drive the formation of higher-order structure within the glycan shield."

By mapping HIV's glycan shield, the researchers hope to contribute to a better understanding of why antibodies react to some spots on the virus but not others. The researchers also hope that their findings may guide the design of new vaccines.

The new combined approach revealed the glycans' structure and dynamic nature in extreme detail and helped the team better understand how these complex dynamics affect the features observed in the cryo-EM maps.

"We now have a way to capture the full structures of these constantly fluctuating glycan shields, which to a great extent determine where antibodies can and can't bind to a virus such as HIV," said the study's lead author Zachary Berndsen, PhD, a postdoctoral research associate in the structural biology lab of Scripps Research professor Andrew Ward, PhD, one of the study's corresponding authors.

The Scripps Research team collaborated with the lab of Gnana Gnanakaran, PhD, staff scientist at Los Alamos National Laboratory and the study's other corresponding author. Los Alamos is equipped with high-performance computing resources that enabled fresh approaches for modeling the glycans.

The researchers combined cryo-EM with sophisticated computer modeling and a molecule-identifying technique called site-specific mass spectrometry. Cryo-EM relies on averaging tens or hundreds of thousands of individual snapshots to create a clear image, thus highly flexible molecules like glycans will appear only as a blur, if they show up at all.

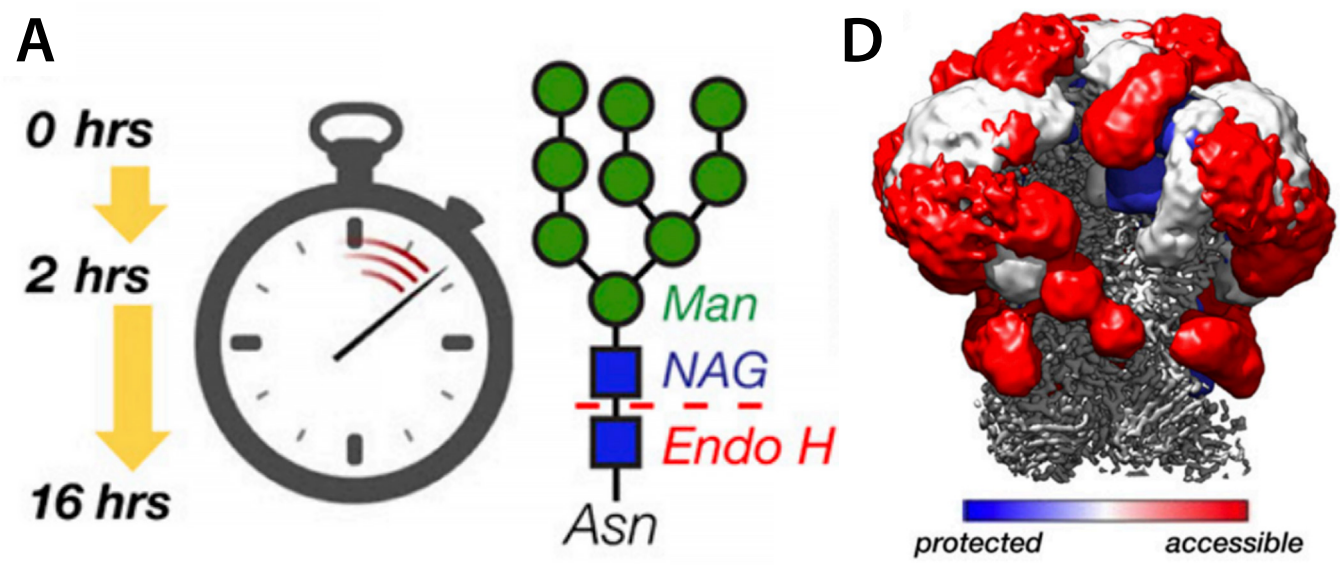
But by integrating cryo-EM with the other technologies, the researchers were able to recover this lost glycan signal and use it to map sites of vulnerability on the surface of Env.

"This is the first time that cryo-EM has been used along with computational modeling to describe the viral shield structure in atomic detail," noted Srirupa Chakraborty, PhD, co-lead author and postdoctoral researcher in the Gnanakaran lab at Los Alamos National Laboratory.

The new combined approach revealed the glycans' structure and dynamic nature in extreme detail and helped the team better understand how these complex dynamics affect the features observed in the cryo-EM maps. From this wealth of information, the team observed that individual glycans do not just wiggle around randomly on the spike protein's surface, as once was thought, but instead clump together in tufts and thickets.

"There are chunks of glycans that seem to move and interact together," Berndsen explained. "In between these glycan microdomains is where antibodies apparently have the opportunity to bind."

Experimental HIV vaccines rely on modified, lab-made Env proteins to elicit antibody responses. In principle,



The most challenging aspect for glycan analysis is to determine occupancy as % present at a certain site. The occupancy determined by mass spec, correlates well with the intensity observed in Cryo-EM maps. Difference maps are used to show which glycans are cleaved first by Endo H, which are cleaved slowly, and which are resistant to 16 hours of Endo H cleavage.

these vaccines' effectiveness depends in part on the positioning and extent of the shielding glycans on these lab-made viral proteins. Therefore, Berndsen and colleagues applied their method to map the glycans on a modified HIV Env protein, BG505 SOSIP.664, which is used in an HIV vaccine currently being evaluated in clinical trials.

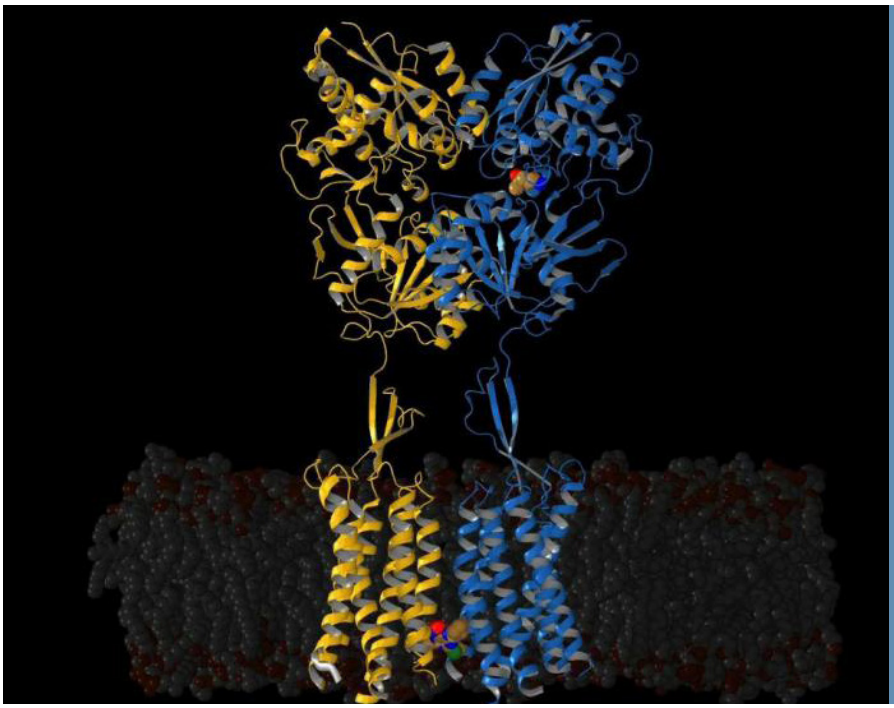
"We found spots on the surface of this protein that normally would be covered with glycans but weren't—and that may explain why antibody responses to that site have been noted in vaccination trials," Berndsen said.

That finding, and others in the study, showed that Env's glycan shield can vary depending on what type of cell is being used to produce it. In HIV's infections of humans, the virus uses human immune cells as factories to replicate its proteins. But viral proteins used to make vaccines normally are produced in other types of mammalian cells.

In another surprise discovery, the team observed that when they used enzymes to slowly remove glycans from HIV Env, the entire protein began to fall apart. Berndsen and colleagues suspect that Env's glycan shield, which has been considered merely a defense against antibodies, may also have a role in managing Env's shape and stability, keeping it poised for infection.

The team expects that their new glycan-mapping methods will be particularly useful in the design and development of vaccines—and not only for HIV. Many of the techniques can be applied directly to other glycan-shielded viruses such as influenza viruses and coronaviruses, and can be extended to certain cancers in which glycans play a key role, the researchers added.

Scientists Develop Map That Reveals the Structure and Movements of GABA Receptor



The scientists hoped to map the structure of $GABA_B$ in both inactive and active states. To their surprise, they found the existence and rough maps of two intermediate states.

$GABA_B$ components GB1 (blue) and GB2 (yellow). Credit: Cornelius Gati/SLAC National Accelerator Laboratory

Gamma aminobutyric acid (GABA) is a naturally occurring amino acid that works as a neurotransmitter in your brain. GABA is considered an inhibitory neurotransmitter because it inhibits certain brain signals and decreases activity in your central nervous system. When released, it binds to neurons at one of two receptors, $GABA_A$ and $GABA_B$, and slows their firing rates. GABA is one place for researchers to start looking to understand neuropsychological ailments. Now scientists from the Department of Energy's SLAC National Accelerator Laboratory, have developed a detailed map of one of GABA's receptors and have discovered not only the structure, but new details of how it moves from its inactive state to active state.

Their study, "Structural basis of the activation of a metabotropic GABA receptor," is published in Nature.

The scientists studied $GABA_B$, using cryo-electron microscopy to take detailed pictures of the molecule. Cryo-electron microscopy uses beams of electrons rather than light to form images of a sample, and then freezing the sample to preserve it under the harsh conditions in an electron microscope.

"Here we present four cryo-electron microscopy structures of the human full-length GB1–GB2 heterodimer: one structure of its inactive apo state, two intermediate agonist-bound forms, and an active form in which the heterodimer is bound to an agonist and a positive allosteric modulator. The structures reveal substantial differences, which shed light on the complex motions that underlie the unique activation mechanism of $GABA_B$," wrote the scientists.

The scientists hoped to map the structure of $GABA_B$ in both inactive and active states. To their surprise, they found the existence and rough maps of two intermediate states. "We didn't even know these states existed," stated Cornelius Gati, PhD, an author of the study and structural biologist at the Department of Energy's SLAC National Accelerator Laboratory.

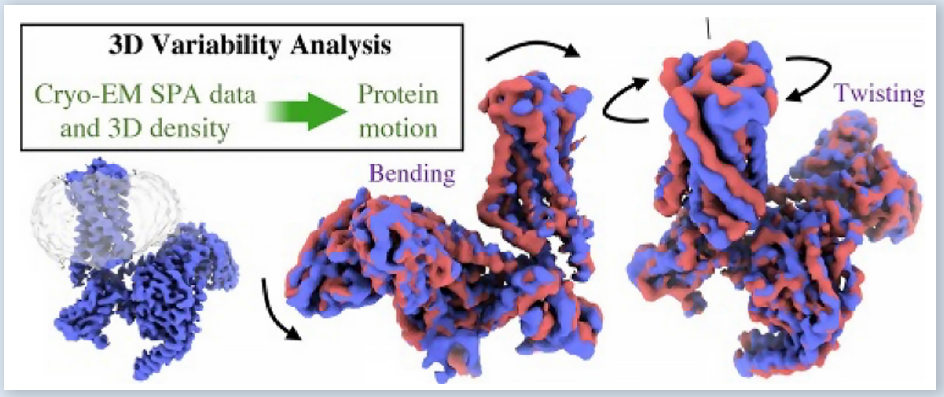
To observe the active state, the team added two molecules with $GABA_B$ and took additional cryo-EM images, which stabilized the $GABA_B$ receptor in its active state. "Our results show that agonist binding leads to the closure of the Venus flytrap domain of GB1, triggering a series of transitions, first rearranging and bringing the two transmembrane domains into close contact along transmembrane helix 6 and ultimately inducing conformational rearrangements in the GB2 transmembrane domain via a lever-like mechanism to initiate downstream signaling. This active state is stabilized by a positive allosteric modulator binding at the transmembrane dimerization interface," the researchers noted.

Being able to see each of those steps along with new details, such as the site where the PAM binds to $GABA_B$, could help researchers design better drugs to treat neuropsychological disease, explained Vadim Cherezov, PhD, a structural biologist at the University of Southern California and co-author of the study.

Their findings about the structure and its transitions between states could help scientists better understand GABA receptors and may eventually lead to better treatments for psychosis and other conditions.

The activation transition for the $GABA_B$ receptor. $GABA_B$ comprises two distinct parts, GB1 and GB2. In the first step toward activation, researchers added an agonist, a GABA-like molecule that brings the pieces of GB1 and GB2 that sit outside the cell together. In the second step, the team added a molecule called a positive allosteric modulator, or PAM, which together with the agonist stabilized $GABA_B$ in its active form.

Credit: Cornelius Gati/SLAC National Accelerator Laboratory.



In Cryo-EM, protein conformations are not restricted by crystal contacts, and all conformations present in the sample can be seen. While modeling this flexibility allows for higher resolution structures, it also gives a unique way to observe the dynamics of the protein.

Read the Article

Visualizing How Drugs Affect Our Neurons

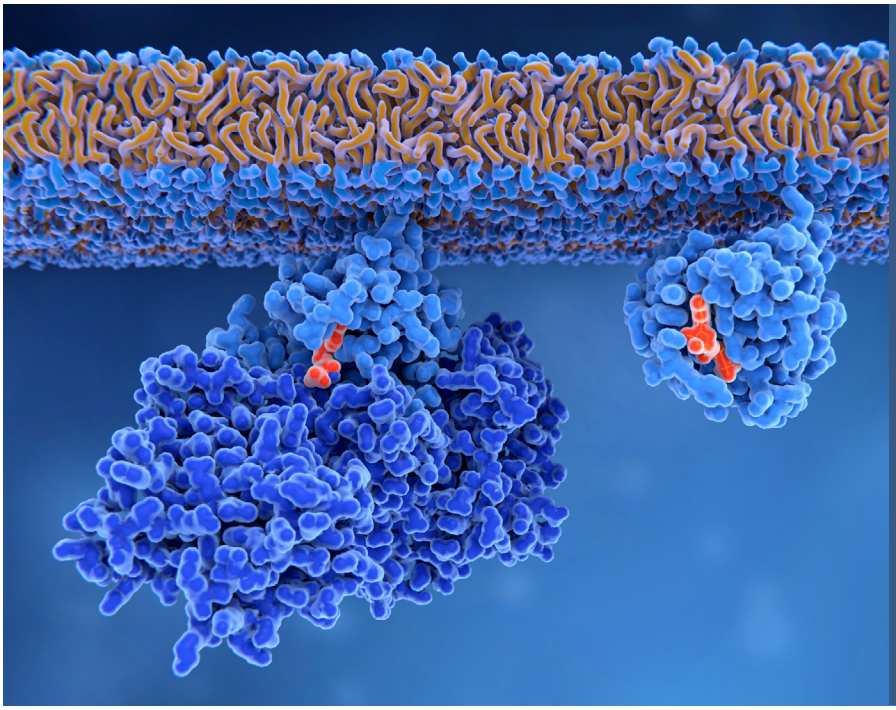


Image Source: selvanegra/Getty Images

Researchers at the MRC Laboratory of Molecular Biology at Cambridge University wanted to fully understand behavior of the $GABA_A$ receptor, particularly its interaction with benzodiazepines such as Valium.

By Hans Raaijmakers and Alex Ilitchev

Our neurons are covered with receptors that bind signaling molecules, also known as neurotransmitters. The activation of these receptors is what drives all neuronal activity, making them highly relevant targets for therapeutics and drugs. A well-known neurotransmitter is gamma-aminobutyric acid, or GABA, which binds to GABA type-A ($GABA_A$) receptors. $GABA_A$ activation has a sedative effect, and these receptors are often activated by anesthetics; disfunction of $GABA_A$ has also been associated with a range of disorders including epilepsy, insomnia, and anxiety.[1] Understanding the behavior of drugs that target $GABA_A$ is not only critical for the development of new pharmaceuticals but also for a clearer understanding of how currently available therapeutics (e.g., drugs such as benzodiazepines and barbiturates) function.

GABA receptors have been challenging targets for traditional structural analysis techniques, such as X-ray crystallography and NMR spectroscopy, as they are not only membrane proteins but also have the inherent structural flexibility of receptors, with different bound/unbound/activated/etc. states. This makes them challenging to crystallize and resistant to bulk analysis. Ultimately, only a few $GABA_A$ complex structures are known, despite their clinical significance, and even fewer at sufficient resolution to inform structure-based drug design.

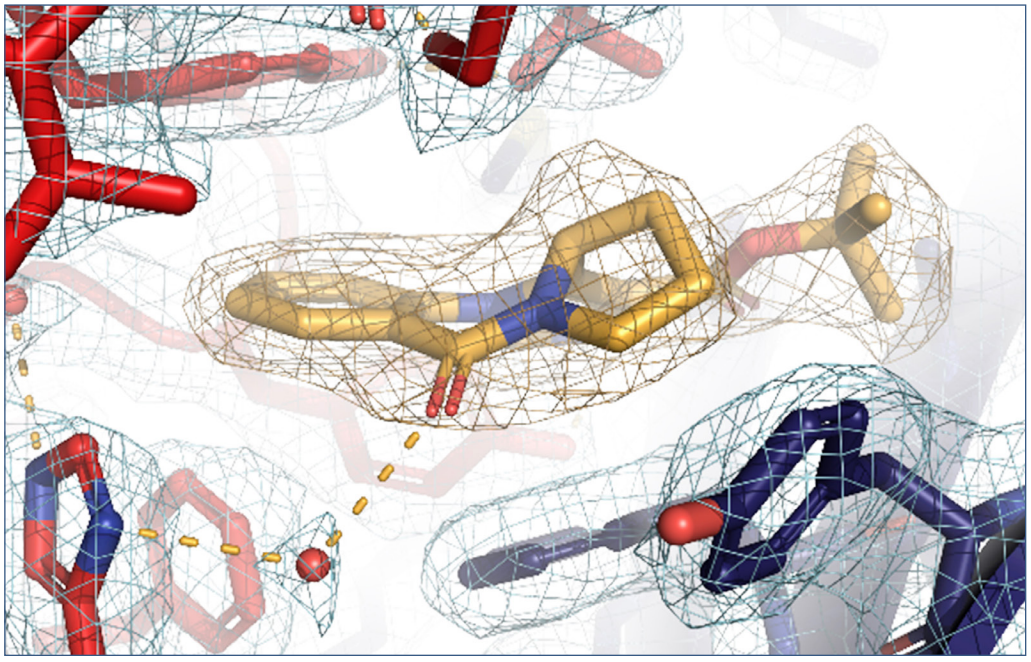
Cryo-electron microscopy of membrane proteins

Cryo-electron microscopy (cryo-EM) describes a group of techniques performed with transmission electron microscopes (TEMs) on cryogenically fixed (vitrified) samples. The key benefit of cryo-EM is that flash-freezing preserves the near-native conformations of the specimens, avoiding extensive sample preparation or crystallization. Cryo-EM single particle analysis, in particular, is well suited for the characterization of receptors and membrane proteins. Individual proteins or protein complexes are embedded in a thin layer of vitreous ice, with individual snapshots taken of each; these images are then recombined into a 3D reconstruction of the sample. Data can even be grouped into structural families (open/unopen, bound/unbound), meaning that conformational flexibility does not prevent analysis. Instead, different conformations can be extracted from the same experiment, providing clear insight into the transitions that the receptor might experience in living systems.

Single particle analysis of ligand-bound $GABA_A$

In the case of $GABA_A$, a collaboration, led by researchers at the MRC Laboratory of Molecular Biology (Cambridge University), wanted to fully understand the mechanical behavior of the receptor, particularly its interaction with benzodiazepines such as Valium (diazepam).[2] While highly efficient sedatives, benzodiazepines can have substantial side effects and carry the risk of addiction. By understanding the specific ways in which these drugs target $GABA_A$, Masiulis et al. hope to inform the development of novel therapeutics that are more effective and carry fewer drawbacks.

Using single particle analysis, they were able to determine several ligand-bound $GABA_A$ structures,



$GABA_A$ receptor in complex with a benzodiazepine-type tranquilizer, visualized with the Thermo Scientific™ Krios™ Cryo-TEM at 2.6 Å resolution.

including the receptor bound to GABA and to two benzodiazepines: Valium and alprazolam. Structures were determined to ~3.5 Å resolution, which is sufficient for the accurate determination of molecular mechanisms; at these scales, ligand-protein interactions can be visualized clearly. They found that both drugs bound to the primary binding pocket, although diazepam exhibited an additional lower-affinity binding site in a different region (the transmembrane domain). Overall, they were able to observe how interactions between the extracellular and transmembrane domains modified the behavior of $GABA_A$ and that signaling occurs in an asymmetric fashion.

Driving structure-based drug design with cryo-EM

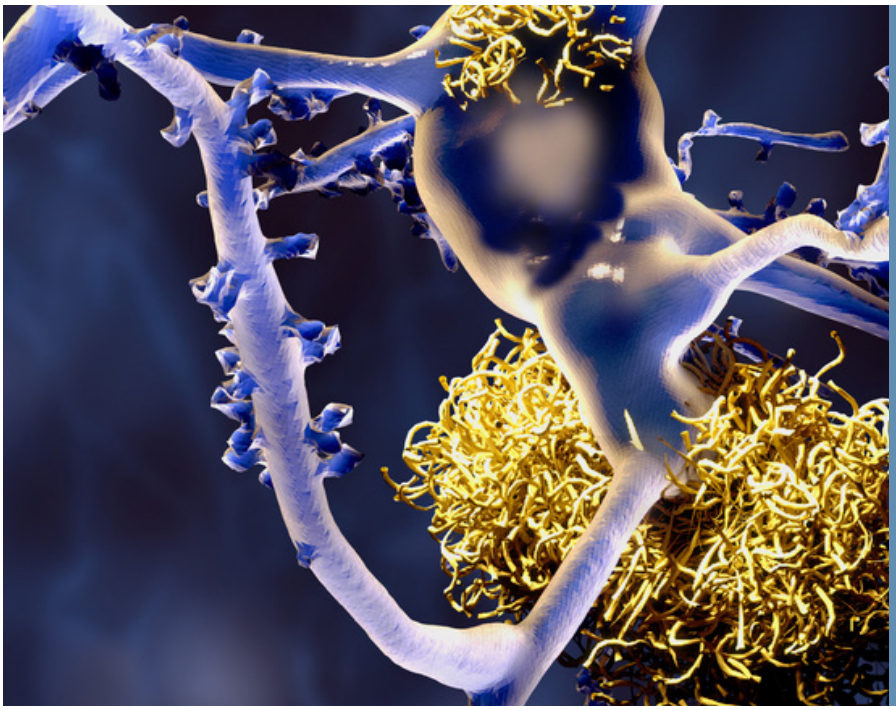
It is important to reiterate that these observations would not be possible without cryo-EM. Single particle analysis is uniquely capable of generating the representative structures of $GABA_A$ that are necessary for binding site identification. Thanks to cryo-EM, biopharmaceutical researchers finally understand the molecular behavior of benzodiazepine and can use this information in the rational design and development of better, more effective therapeutics. The direct observation of molecular interactions opens massive avenues in structure-based drug design, promising to revolutionize how pharmaceuticals are developed.

References

[1] Möhler, H. $GABA_A$ receptors in central nervous system disease: anxiety, epilepsy, and insomnia. J. Recept. Signal Transduct. 26, 731–740 (2006).

[2] Masiulis, S. $GABA_A$ receptor signaling mechanisms revealed by structural pharmacology. Nature 565, 454–459 (2019).

New Clues Uncovered about Toxic Protein Structures in Neurodegenerative Diseases



Source: selvanegra / Getty Images

These abnormal structures, known as amyloid fibrils, are a signature of brain pathology in amyotrophic lateral sclerosis (ALS) and have also been found in several other disorders, including Alzheimer's disease.

Transactive response DNA-binding protein-43 (TDP-43) is a soluble protein that interacts with nucleic acids. However, in several neurodegenerative disorders, this protein forms large, harmful rope-like clumps. These abnormal structures, known as amyloid fibrils, are a signature of brain pathology in amyotrophic lateral sclerosis (ALS). Similar inclusions have also been found in several other disorders, including Alzheimer's disease, cerebral age-related TDP-43 with sclerosis, dementia with Lewy bodies, hippocampal sclerosis, Huntington's disease, and chronic traumatic encephalopathy.

By using cryo-electron microscopy, scientists at the Case Western Reserve University School of Medicine were able to determine the structures of TDP-43. This structural insight provides clues as to how these toxic proteins clump and spread between nerve cells in the brain. Their findings may pave the way for developing new therapeutics to treat diseases such as ALS and frontotemporal dementia (FTD).

Their findings are published in the journal Nature Communications, in a paper titled, "Cryo-EM structure of amyloid fibrils formed by the entire low complexity domain of TDP-43."

"Amyotrophic lateral sclerosis and several other neurodegenerative diseases are associated with brain deposits of amyloid-like aggregates formed by the C-terminal fragments of TDP-43 that contain the low complexity domain of the protein," wrote

the researchers. "Here, we report the cryo-EM structure of amyloid formed from the entire TDP-43 low complexity domain in vitro at pH 4. This structure reveals single protofilament fibrils containing a large (139-residue), tightly packed core."

"These devastating brain disorders that affect tens of thousands of Americans are on the rise worldwide, and there are no effective treatments to stop their progression," stated Witold Surewicz, a professor in the department of physiology and biophysics at the School of Medicine and the study's senior author.

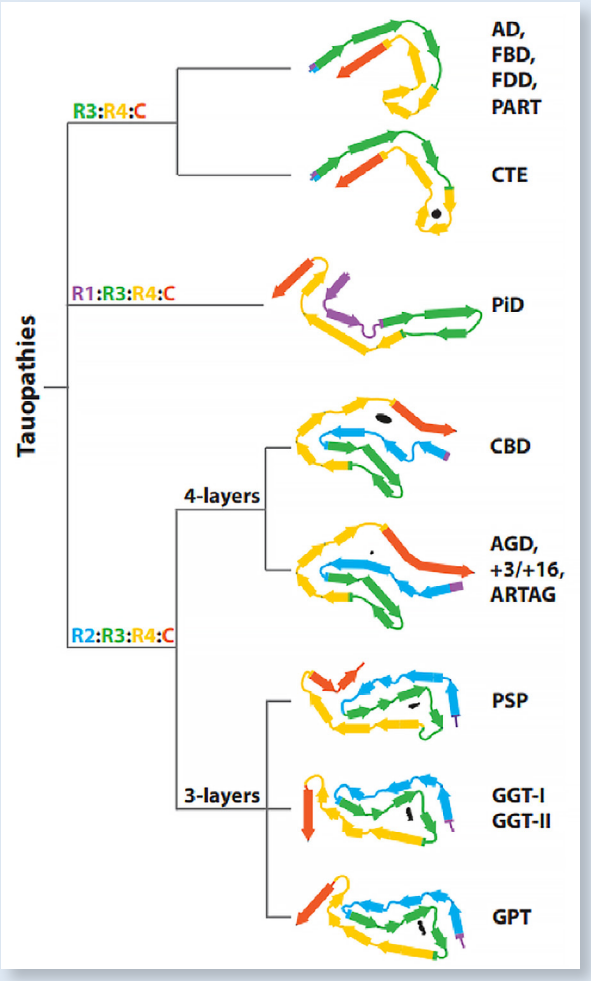
The researchers analyzed thousands of images of fibrils formed in the test tube by the key fragment of TDP-43. They determined the complex architecture of these elongated structures at a resolution close to individual atoms. This structural insight revealed the nature of the template on which more copies of TDP-43 can lock.

The researchers also observed how the fibril structure could be controlled by amino acid mutations in TDP-43 linked to hereditary forms of ALS and FTD, as well as by aging-dependent modifications of the protein.

"The present structure for non-phosphorylated TDP-43 LCD fibrils provides a necessary foundation for future high-resolution structural studies with fibrils containing protein variants with different phosphorylation patterns," concluded the researchers.

Qiuye Li, a graduate student and lead author commented: **"This is really an exciting development because it reveals a mechanism for the growth of these toxic aggregates. This, in turn, provides important clues as to how these aggregates may spread between the cells in affected brains."**

Structure-based Classification of Tauopathies



Structures of tau filaments [. . .] suggest a hierarchical classification of tauopathies on the basis of their filament folds, which complements clinical diagnosis and neuropathology and also allows the identification of new entities. Learn more in Nature...

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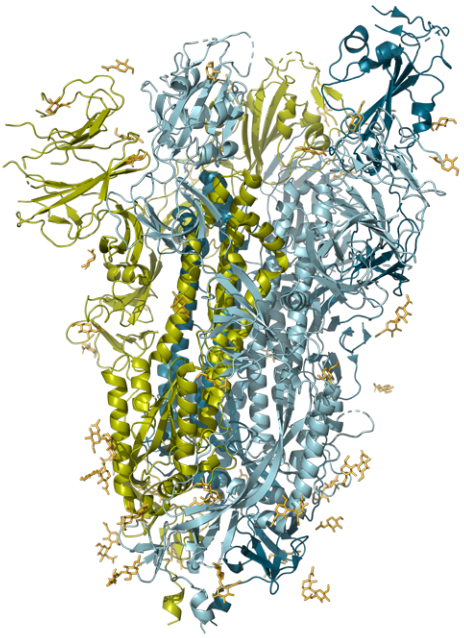

Cryo-EM structure of SARS-CoV-2 (COVID-19) spike glycoprotein in the prefusion conformation, with the three subunits of the trimer in teal, green, and light blue, and glycosylation in yellow. Image created with PDB data (6VSB).

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