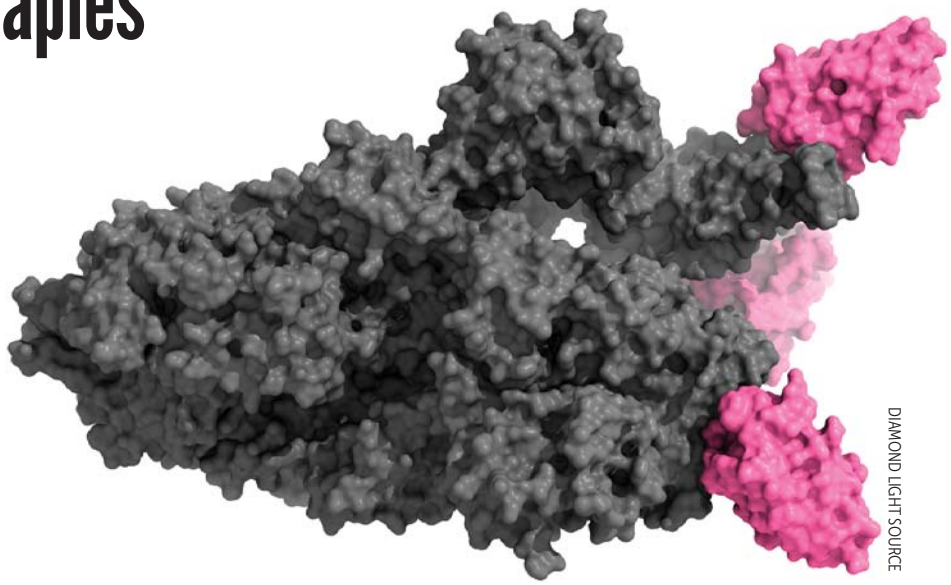
Cats and llamas could offer a path to coronavirus therapies

Computation and experimentation also yield possible therapeutic compounds for COVID-19.



DIAMOND LIGHT SOURCE

THE SPIKE PROTEIN OF THE SARS-COV-2 VIRUS (gray) is shown with three small antibodies (pink) attached to its receptor binding domains. The spike attaches at the left to the viral membrane (not shown).

As the world anxiously awaits development of one or more vaccines to tame the SARS-CoV-2 virus, other research continues at a feverish pace to find effective treatments for the disease it causes, COVID-19. That work, in which physicists and chemists are deeply involved, has made significant strides in the past several months and has turned up a few surprises.

Researchers at the University of Alberta reported at the August virtual meeting of the American Crystallographic Association that a dipeptide-based protease inhibitor used to treat a fatal coronavirus infection in cats also blocks replication of the SARS-CoV-2 virus in samples of monkey lung tissue. Joanne Lemieux, a biochemist at the university, says the antiviral, known as GC373, works by blocking the function of the main protease (M^{pro}), an enzyme that cleaves the polyproteins translated from viral RNA into individual proteins once it enters human cells.

Lemieux says GC373 has been shown to have no toxic effects in cats. Anivive, a California company that develops pet medicines, has applied for US Food and Drug Administration approval to begin trials in humans. Lemieux's group crystallized the M^{pro} in combination with the drug and produced three-dimensional images of how the drug binds strongly to the active pocket on the enzyme. Although GC373 should be effective in its current form, the group is planning further crystallography experiments at the Stanford Synchrotron Radiation Lightsource (SSRL) and the Canadian Light Source to see if a reformulation could optimize it for human use, she says.

At the end of April, SSRL opened a new beamline that has one of the smallest beam sizes and highest brightnesses of any in the world devoted to structural molecular biology and x-ray macromolecular crystallography. "We'll be able to use smaller crystals, collect higher-quality data, get a better signal-to-noise ratio, and collect more data sets per hour" than ever before, says Ian Wilson of Scripps Research, which provided some of the funding for the beamline. Stanford University, several private foundations, and the National Institutes of Health also contributed support.

The search for potential COVID-19 treatments couples experimental and computational efforts. In one example, collaborators at Brookhaven National Laboratory and Stony Brook University developed a complex computational model detailing the sequence of how the viral spike protein attaches to the human host cell. Kerstin Kleese van Dam, director of Brookhaven's Computational Science Initiative, says that when the spike is in its usual down, or inward, position, it is protected from the immune system. The spike becomes more vulnerable when it shifts to the up, or outward, position required to enter the host cell.

The team identified a pocket on the spike that changes shape during the transition from down to up, and compound screening simulations uncovered several molecules that might attach to the pocket and block cell invasion. "It's a bit like wedging a door open," van Dam says. "You might be able to wedge the spike into an upward position, so the immune system can attack it." Chemists are now working to synthesize the predicted molecules. *In vitro* experiments hopefully will confirm the compounds' activity. If the results are positive, light sources or other instruments will probe how the molecules bind to the viral protein.

Jim Brase leads Lawrence Livermore National Laboratory's computational effort on the coronavirus. He says the lab's considerable unclassified computing assets have been running physics-based models to probe the molecular dynamics between proteins and compounds in a search for drug candidates and antibodies that will counter the coronavirus. Anti-

bodies are proteins that bind to and neutralize alien proteins. "You have two very complex molecules and you are trying to see how they come together and what the energy of their binding strength is," Brase says. "These are typically million-atom calculations, and you have to let [the atoms] jiggle around and settle into their lowest energy states." The process must be repeated hundreds of times to obtain the average binding energy. "Then you move to the next potential configuration. If you have a thousand of those, you are doing a lot of calculations."

A commercial vendor manufactures the computer-designed antibodies for Livermore and Sandia National Laboratories, where researchers assay them to determine if they bind to their SARS-CoV-2 targets. Artificial intelligence (AI) is used in winnowing the field of potential new molecules from the nearly infinite number of possible combinations of amino acids.

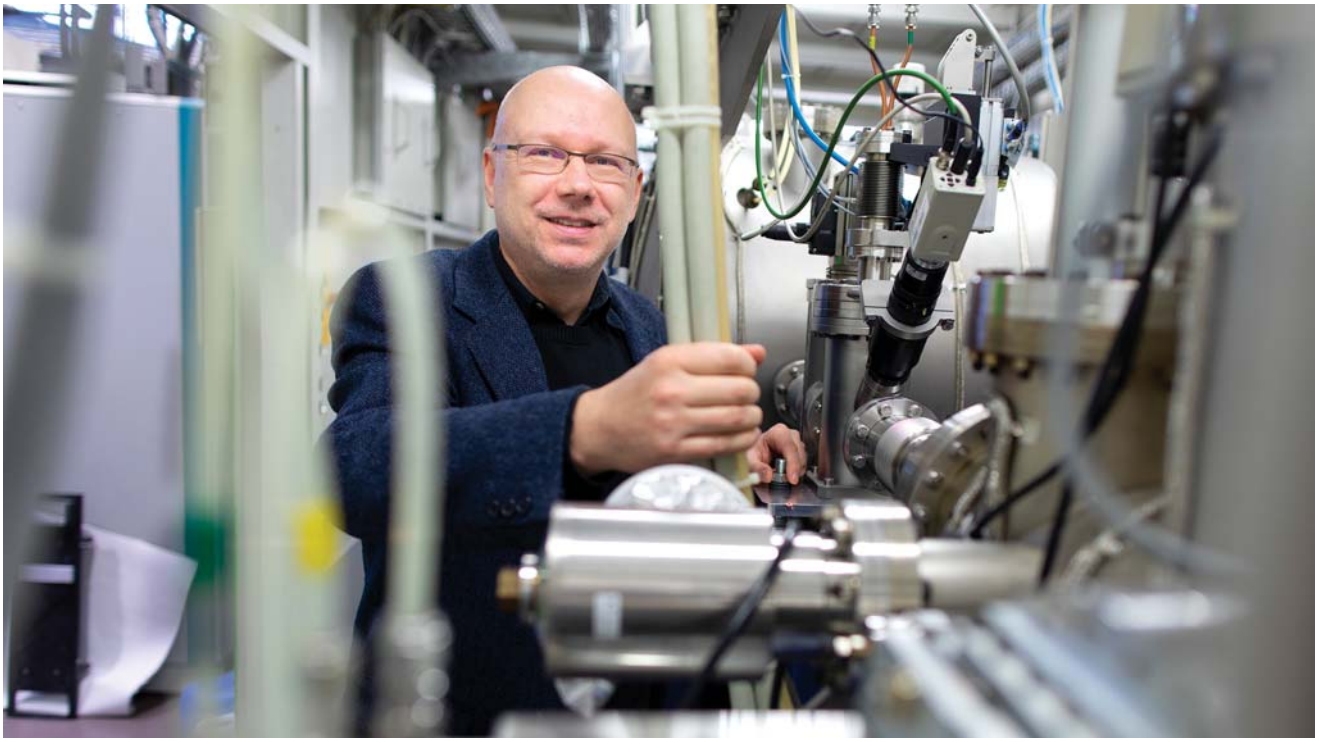
Models developed by the Accelerating Therapeutics for Opportunities in Medicine consortium, comprising Livermore, GlaxoSmithKline, and other university and government entities (see PHYSICS TODAY, January 2018, page 27), will be used to predict the safety and pharmacokinetics of candidate compounds to become drugs, Brase says.

At Los Alamos National Laboratory, structural biologist Julian Chen is modeling the interactions between the spike protein and the angiotensin-converting enzyme 2 (ACE2) on the human cell surface that is the path to infection. "We know that viruses make use of the path of least resistance to get into the cell," he says. "SARS-CoV-2 certainly did not evolve specifically to bind to a human ACE2 receptor, so it's a good enough interaction, but likely not an optimal one." It should be possible to design molecules that can outcompete the virus in binding with ACE2, thereby preventing infection, he says.

The COVID-19 High Performance Computing Consortium, launched in March, brings together 7 Department of Energy national labs with 8 NSF-funded supercomputing centers, NASA, 11 high-tech industry giants, more than a dozen US universities, and institutions from Japan, South Korea, Sweden, Switzerland, and the UK. Brase says a panel reviews and ranks requests for computational time from investigators around the world. A committee then matches accepted proposals with the consortium members' available computing resources. Many proposals require more resources than any one member can spare. In those cases, multiple members will contribute time, he says. About 80 projects are underway.

The European Commission is funding an 18-institution coronavirus-fighting consortium known as Exscalate4CoV that includes supercomputing centers at Cineca, Italy's supercomputing consortium; the Barcelona Supercomputing Center; and Germany's Jülich Research Center. The Swiss Institute of Bioinformatics contributes 3D models of the viral proteins, and the Fraunhofer Institute for Molecular Biology and Applied Ecology provides a drug-repurposing library of 6000 compounds and biochemical assays. Screenings of crystallized viral proteins in combination with newly discovered molecules are performed at Italy's

HZB



MANFRED WEISS, director of the macromolecular research group at the BESSY II light source, stands beside a crystallography beamline.

Elettra Synchrotron Trieste, KU Leuven in Belgium, and Poland's International Institute of Molecular and Cell Biology.

FELs enter the picture

Since chloroquine was first proposed as a potential treatment for COVID-19, multiple clinical studies have shown the drug and its derivates to be ineffective and to have harmful side effects. But Claudio Masciovecchio, head of scientific programs at the FERMI free-electron laser (FEL) at Elettra, says he's not ready to write off chloroquine just yet. One recent study showed it to interfere with the spike–ACE2 interaction. He says the FERMI FEL's ability to probe the low-frequency vibrations that are responsible for the biochemical activity of a molecule or drug may settle the issue. Researchers from the University of Bologna, ETH Lausanne, and the University of California, Irvine, are collaborating with FERMI scientists to gain further insight to the interaction. Complementary experiments are to take place this month on Elettra's synchrotron.

SLAC, home to SSRL, is reopening its x-ray FEL (XFEL), the Linac Coherent Light Source (LCLS), this month after an extended shutdown for an upgrade. The LCLS produces x rays in femtosecond pulses at a brightness up to 10 billion times that of a synchrotron. The intensity allows studies of protein crystals that are too small for synchrotrons. The extreme shortness of XFEL pulses allows diffraction patterns to be recorded before radiation damage occurs. Additionally, XFELs can probe crystals at temperatures more relevant to physiological conditions, whereas crystallography at synchrotrons is typically performed at liquid-nitrogen temperatures.

But FELs haven't been able to match the productivity of synchrotrons, where automated sample handling allows high-throughput screening of potential drug compounds that are crystallized together with viral proteins. SLAC scientist Alex Batyuk says the LCLS is now being outfitted to speed experiments. "We know beam time at XFELs is very limited, and we'd like to make it more available for such critical and medically relevant targets, especially at this time," he says.

Progress at light sources

A number of promising compounds for drug treatments have been identified at the UK's Diamond Light Source, which, in addition to screening compound libraries and making the results publicly available, since March has been screening libraries of existing drugs and compounds approved for use in humans for activity against several SARS-CoV-2 proteins. (See PHYSICS TODAY, May 2020, page 22.) Martin Walsh, Diamond deputy director of life sciences, says the results won't be released until the molecules can be confirmed to be safe. "Fingers crossed, we hope to have something in the public domain in the autumn," he says.

In one collaboration with Diamond and Oxford University, the startup Exscientia conducted an initial AI-enabled computational screening of the 15 000 compounds in the ReFRAME drug-repurposing library, which is funded by the Bill and Melinda Gates Foundation. The light source was used to discern the structures of the selected molecules combined with viral proteins.

Diamond also joined forces with the COVID Moonshot, a crowdsourced drug development effort spearheaded by PostEra, a San Francisco–based medicinal chemistry design startup. In that effort, Diamond assessed the activity of more than 900 compounds that were synthesized by Moonshot partners from a list of 10 000 candidates proposed by hundreds of researchers. Diamond obtained structures of 129 of those compounds combined with proteins.

An initial screening of 1200 selected commercially available compounds at the BESSY II light source in Berlin has found "a couple of relatively weakly bound hits," says Manfred Weiss, director of the lab's macromolecular research group. Although those small compounds "are useless for any form of therapy," he says, "they serve as the basis to find larger compounds that bind more strongly and exert some biological effect." Of the 25 to 30 larger follow-up compounds procured, 5 or 6 bind more strongly, he says. Collaborators at the University of Lübeck will next assay them for biological effect.

Llamas' contribution

Finding or manufacturing antibodies may present a more expedient path to a COVID-19 treatment. Antibodies don't enter cells, so they have fewer safety issues, and potential side effects are of less concern than those of drugs. Compared with a drug–protein interaction, an antibody has the entire surface area of the virus to which it could potentially bind, notes Sean McSweeney, director of Brookhaven's Center for Biomolecular Structure.

At Brookhaven's National Synchrotron Light Source II, researchers are performing small-angle x-ray scattering on antibodies in solution. Though the technique produces lower-resolution images than crystallography does, changes to the buffers and other solution components can alter the scattering pattern and yield information about the interactions. "If you have a potential antibody and a target, you can look at them separately through scattering, and together in solution you will see an envelope and a shape that is different," says McSweeney.

Diamond is part of a UK collaboration that last month reported a potential COVID-19 therapy in so-called nanobodies derived from llamas. The surprising contribution of the South American camelid arises from their antibodies' small size: about one-quarter that of humans. That feature increases opportunities for the antibodies to fit into the binding pockets of antigens, in comparison to more complex human antibodies. Nanobodies are more stable, so they could potentially be stored for longer periods after production. And they can be delivered directly to the lungs by an inhaler.

Research dating to the 2002 outbreak of the severe acute respiratory syndrome (SARS) coronavirus found that llamas developed antibodies when exposed to that virus's spike protein. In a 13 July paper in *Nature Structural and Molecular Biology*, scientists at the Rosalind Franklin Institute, Oxford University, Diamond, and Public Health England reported producing a nanobody that bound tightly to the spike protein of SARS-CoV-2 in the laboratory.

Experiments involving camelid antibodies and the spike protein also are underway at the Australian Synchrotron, where the first structure of the SARS-CoV-2 nonstructural protein-9 was produced. Dene Littler and colleagues at Monash University have identified a potential binding site on the NSP9 protein that is thought to be involved in RNA binding.

PAC-MAN

A different approach to COVID-19 therapeutics is being developed at Stanford and Lawrence Berkeley National Laboratory (LBNL). Using the gene-editing tool CRISPR, Stanford bioengineering professor Stanley Qi last year developed a prophylactic antiviral for influenza he called PAC-MAN. In March, Qi began working with Michael Connolly, a principal scientific engineering associate at LBNL's Molecular Foundry, to develop a system for delivering PAC-MAN to the cells of COVID-19 patients.

PAC-MAN is composed of a virus-killing enzyme and a strand of guide RNA, which commands the enzyme to destroy specific nucleotide sequences in viral genomes. Connolly has been developing synthetic molecules known as lipitoids that can deliver PAC-MAN to patient cells by encapsulating the enzyme inside tiny nanoparticles the size of a virus.

In April, Qi and Stanford colleagues demonstrated a type of lipitoid that self-assembled with DNA and RNA into PAC-MAN carriers in a sample of human epithelial lung cells. According to Qi, the system reduced the amount of synthetic SARS-CoV-2 in solution by more than 90%. The Stanford team plans to test in an animal model the PAC-MAN/lipitoid system against a live SARS-CoV-2 virus. The interdisciplinary team will be joined by collaborators at New York University and Karolinska Institute in Stockholm.

Connolly says other efforts at the Molecular Foundry include finding new sensors and viral binding materials that could aid in diagnostics and new materials for personal protection equipment.

For obvious reasons, most coronavirus research has been with recombinant viral proteins instead of the complete virus. While some researchers, including Lemieux and her colleagues at the University of Alberta, were able to test their compounds against the live virus, others, including Masciovecchio, say an inability to work with SARS-CoV-2 has hindered their research. "We are working on protocols which have to be accepted by the health authorities to bring the real virus into Elettra," he says. "It would be really helpful to measure real viruses. It's safe so far as the sample container is secure."

Regardless of their success in fighting the current outbreak, researchers say their work will be highly useful in other viral outbreaks that are sure to come. The main protease of SARS-CoV-2, for example, differs little from that of earlier coronaviruses. "If we find a drug that deactivates the protease of CoV-2, it more than likely deactivates the original SARS as well," says Diamond's Walsh. "And if another similar coronavirus SARS virus emerged, we could quickly have a therapeutic against that."

David Kramer