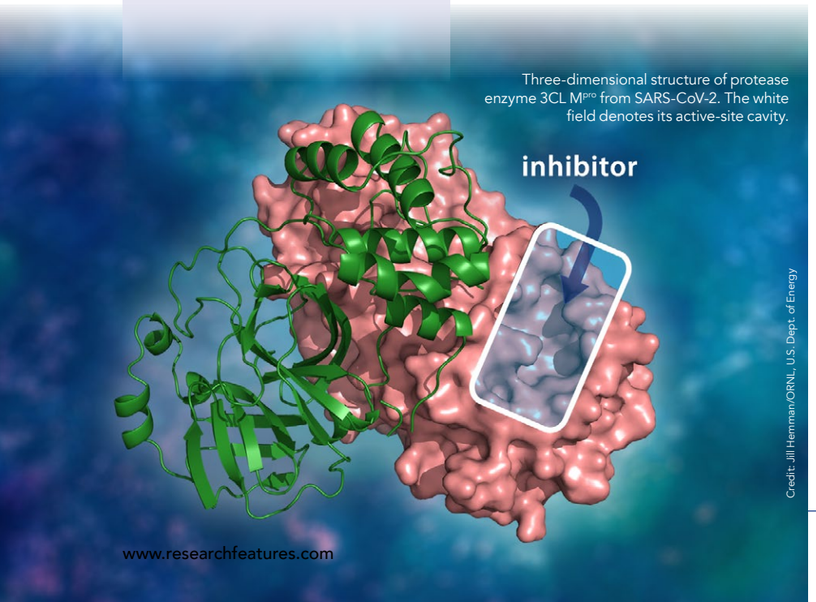
Probing the SARS-CoV-2 main protease active site as a target to inhibit viral replication

SARS-CoV-2 replication involves the synthesis of two large proteins, which are inactive and harmless until the viral main protease enzyme (3CL M^{pro}) uses them as substrates, cleaving them into many smaller, functional products. Dr Andrey Kovalevsky and his team from Oak Ridge National Laboratory propose a design of novel inhibitors and the repurposing of clinical drugs developed to treat other diseases for the treatment of COVID-19. The team uses room temperature crystallography with X-rays and neutrons to guide structure-based and computer-assisted drug design and to assess the ability of drugs to inhibit the SARS-CoV-2 main protease, causing virus replication to stop.

In a relatively short time, COVID-19 has become a pandemic of unprecedented proportions, disrupting travel, economic activity, and social life across every country in the world. The novel human coronavirus SARS-CoV-2 has been identified as the pathogen responsible for COVID-19 and despite intense research, the origins of the virus are still a matter of debate, although a prevailing theory is that it originated in bats. SARS-CoV-2, which has one of the largest viral genomes, with over 30 thousand nucleobases, shares about 80% genomic identity with the earlier SARS-CoV that was responsible for a 2003 outbreak of severe acute respiratory syndrome (SARS), which has been eclipsed by SARS-CoV-2 causing over 2 million deaths in less than a year.

SARS-CoV-2 replication involves the synthesis of two large proteins, known as pp1a and pp1ab, which are inactive and harmless until the viral main protease enzyme (3CL M^{pro}) uses them as substrates, cleaving them into many smaller, active, and functional products. Dr Andrey Kovalevsky and his team from Oak Ridge National Laboratory have joined the pioneering efforts of other scientists around the world in identifying a successful mechanism of action to breach the virus defences. They propose that the main protease from SARS-CoV-2 could prove the ideal target to neutralise the action of the virus by affecting its replication. Along with vaccines and therapeutic antibodies, designing small-molecule therapeutics and repurposing clinical drugs are the two strategies that will create additional intervention options to treat and prevent COVID-19.

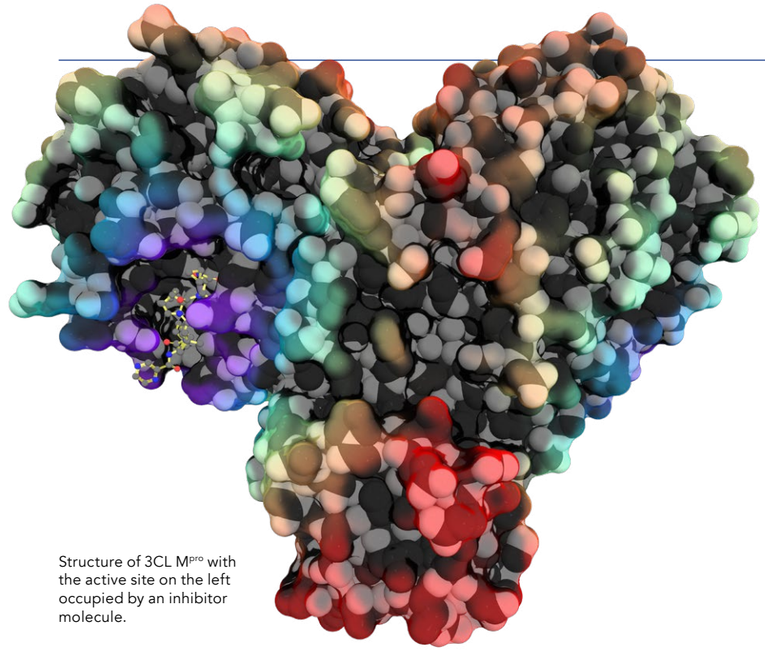


Three-dimensional structure of protease enzyme 3CL M^{pro} from SARS-CoV-2. The white field denotes its active-site cavity.

Credit: Jill Hemman/ORNL, U.S. Dept. of Energy

JAMMING THE VIRAL REPLICATION MECHANISM

Looking for potential drugs among existing clinical enzyme inhibitors is to date considered the most effective route followed by drug design and development in the pursuit of novel treatments. When inhibitor molecules bind to an enzyme's active site with sufficient affinity (or tightness), they will either directly compete with the substrate for access, or they will cause a structural change in the active site, which decreases the affinity of the substrate. As a result of either mechanism, the inhibited enzyme will convert less substrate into product. The overarching goal is to have an inhibitor capable of halting enzyme function to arrest virus replication. Following this principle, the team aims to prove that potent and effective inhibitors of 3CL M^{pro} can directly slow down or stop the degradation of pp1a and pp1ab, which is an essential step for the replication of the virus.



Structure of 3CL M^{pro} with the active site on the left occupied by an inhibitor molecule.

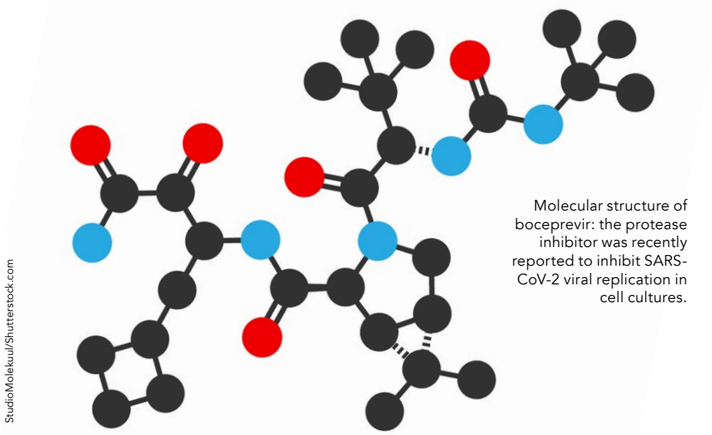
PROBING THE CATALYTIC SITE AT PHYSIOLOGICAL TEMPERATURES

The first step in the rational (structure-based) design of inhibitors is to determine the enzyme's three-dimensional structure and understand its active site architecture at the atomic level. This allows scientists to identify what molecules are more likely to bind to the enzyme and to make predictions about the potency of the interaction. Dr Kovalevsky and his team were the first to determine the room temperature (RT) X-ray crystal structure of ligand-free SARS-CoV-2 main protease containing no small molecules bound. RT crystallography is technically more challenging than its conventional cryogenic counterpart, as it requires the preparation and analysis of larger crystals to counter the radiation damage to the sensitive biological samples that occurs at higher temperatures. However, the technique allowed Dr Kovalevsky and his colleagues to determine the atomic structure of the active site at near-physiological conditions. The RT crystallography studies highlighted the conformational flexibility of the main protease's active-site cavity, which showed significant malleability in accommodating inhibitors not explicitly designed to target this SARS-CoV-2 enzyme, including a variety of bulky chemical groups. Intriguingly, the enzyme achieved the observed plasticity by substantially distorting its shape and size compared with the ligand-free state. Moreover, the extent of the enzyme's shape shifting depended on the bulkiness of the inhibitors.

The team propose that the main protease from SARS-CoV-2 could prove to be the ideal target to neutralise the action of the virus by affecting its replication.

MAPPING THE ENZYME'S ELECTROSTATIC ENVIRONMENT

In another development of their research, Dr Kovalevsky and his collaborators used neutron crystallography to directly observe hydrogen atoms in the protein structure of SARS-CoV-2 main protease. Determining the presence or absence of hydrogen atoms at specific sites on amino acid residues is important to establish what electric charges they possess – negative, neutral, or positive. Importantly, half of all atoms in protein and small molecule drugs are hydrogens. The team's neutron crystallographic study was born out of the data obtained in another study, also published in 2020, that provided valuable information on the oxidation pattern and reactivity of the cysteine amino acids of the protease. An important observation was that the cysteine residue at the catalytic site, where the essential chemistry of cleaving substrate occurs, can be easily oxidised by oxygen always present in water at



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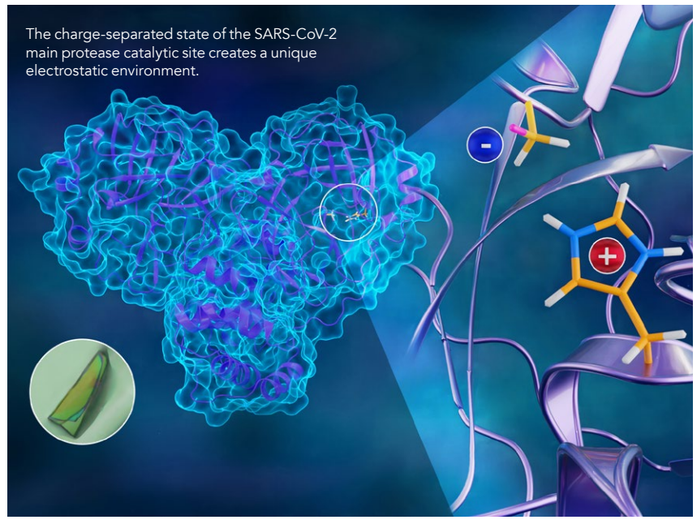
Molecular structure of boceprevir: the protease inhibitor was recently reported to inhibit SARS-CoV-2 viral replication in cell cultures.

room temperature and physiological pH into a rare and highly reactive species, known as peroxysulfenic acid, which provided insight that the catalytic cysteine is very reactive, thus must be negatively charged. Using neutrons, the team validated their hypothesis that the catalytic site adopts a highly polarised and reactive state where the amino acid cysteine is negatively charged and another critical catalytic amino acid, histidine, is positively charged, instead of both being neutral and unreactive, as has always been envisaged. The charge-separated (zwitterionic) state of the SARS-CoV-2 main protease catalytic site creates a unique electrostatic environment that has not been predicted by computer simulations of the enzyme, emphasizing the power of neutron crystallography in creating a complete, accurate atomic map of the protein.

This knowledge provides the scientific community with critical and unique information for structure-assisted and computational drug design that is impossible to obtain with other biophysical techniques at physiologically relevant temperature, allowing precise tailoring of inhibitors to the enzyme's electrostatic environment. Such inhibitors may need to specifically target the negatively charged cysteine and the positively charged histidine in the active site to be effective. Novel inhibitors might also be designed strategically to directly oxidise cysteine residues through a peroxide group, thus inactivating the enzyme.



Credit: Jill Hemman/ORNL, U.S. Dept. of Energy

REPURPOSING OLD DRUGS TO TREAT THE NEW DISEASE

The design of novel antivirals and their development for clinical use, however, may take several years. To make the process quicker and more feasible, Dr Kovalevsky and his team propose using known clinical drugs developed to treat other diseases to assess their ability to bind to the SARS-CoV-2 main protease enzyme.

Dr Kovalevsky and his collaborators analysed how the active-site cavity reacts to the binding of three hepatitis C clinical protease inhibitors – telaprevir, narlaprevir, and boceprevir – and a natural peptide aldehyde, leupeptin. The three hepatitis C repurposed drugs were able to bind to SARS-CoV-2 3CL M^{pro}, and enzyme inhibition kinetics measurements demonstrated that they inhibited the protease when present at micromolar concentrations. Boceprevir and narlaprevir displayed the highest affinities to SARS-CoV-2 3CL M^{pro} and, perhaps, could be looked at as possible treatment options and tested for their ability to inhibit SARS-CoV-2 viral replication.

Dr Kovalevsky and his team were the first to determine the room temperature (RT) X-ray and neutron crystal structures of ligand-free SARS-CoV-2 main protease.

The RT crystallography studies confirmed that the inhibitors have a significant effect on the enzyme's active site cavity geometry and shape. Their affinities for the active site are comparable to those of many other protease inhibitors that had previously been designed to specifically target the SARS-CoV main protease. Interestingly, boceprevir was recently reported to inhibit SARS-CoV-2 viral replication in cell cultures (Fu, L. et al, 2020; Ma, C. et al, 2020).

FUTURE PERSPECTIVES

Dr Kovalevsky and his colleagues use innovative approaches to research the SARS-CoV-2 main protease, an important drug target for the development of inhibitors that can stop virus replication. Their observations provide critical information for structure-assisted and computational drug design, allowing the precise tailoring of molecules to the enzyme's electrostatic environment.

In future developments, the team hopes to design inhibitors that disrupt dimerisation of the enzyme, following the observation that the main protease in SARS-CoV has optimal activity as a dimer and that its enzyme activity is dramatically weakened when dimerisation is halted. Given the structural similarity that exists between SARS-CoV and SARS-CoV-2, it is likely that the dimer interface in the SARS-CoV-2 main protease is also a suitable target for specific dimerisation inhibitors.

The Kovalevsky lab will continue comparing the conformations observed in the ligand-free enzyme at room temperature with the structures adopted by the inhibitor-bound complexes. The flexibility of the enzyme active site observed at room temperature suggests that the ligand-free enzyme conformation at room temperature may provide scientists with the most appropriate structure for investigating drug binding to enable drug design.

Behind the Research

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Research Objectives

Dr Kovalevsky researches SARS-CoV-2 main protease, an established drug target for the design of inhibitors to stop the virus replication.

Detail

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Bio

Dr Andrey Kovalevsky is a structural biologist, biochemist, and drug designer. Dr Kovalevsky received M.Sc with Honours from Kharkov State University, Kharkov, Ukraine, and a Ph.D. from the University at Buffalo, the State University of New York. He worked at Georgia State University and the Los Alamos National Laboratory before joining the Oak Ridge National Laboratory, where he is currently a Senior R&D Scientist. Dr Kovalevsky's current research focuses on studying the structure-dynamics-function relationships in macromolecular drug targets, including the design of protease inhibitors against SARS-CoV-2 and antidotes against nerve agent and pesticide poisoning, and studies of vitamin B_6-dependent enzymes. His approach combines innovative biophysical and computational methodologies, such as X-ray and neutron crystallography at near-physiological conditions, vibrational spectroscopy with neutrons, biomolecular simulations, and structure-based virtual reality (VR)-assisted drug design.

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Collaborators

Dr Daniel Kneller, Dr Leighton Coates, Dr Stephanie Galanie, Ms Gwyndalyn Phillips, Dr Kevin Weiss, Dr Hugh O'Neill

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Personal Response

How are you looking to advance your research?

"The next step in our team's efforts is to determine whether inhibitor binding can alter hydrogen atoms' locations within the SARS-CoV-2 3CL M^{pro}. This is important because if hydrogen atoms relocate, it will change the corresponding amino acids' electric charges, thus completely remodelling the electrostatic environment. Such observations will be another piece of the puzzle in pursuit of small-molecule therapeutics to treat COVID-19. The results our team has obtained so far, and our concurrent work with computational scientists, already give us ideas for the design of novel protease inhibitors, which can be synthesised and studied further."