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 Mpro) uses them as substrates, cleaving them into many smaller, active, and 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 Mpro) 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 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.

**REPLICATION MECHANISM**

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 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.

**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...
Behind the Research

Hepatitis C repurposed drugs were able to inhibit main protease of SARS-CoV-2, as detailed in a recent study published in Nature Communications (Fu, L., Ye, F., Feng, Y. et al. 2020). Boceprevir and GC76 are known inhibitors of SARS-CoV-2 main protease, and the researchers have discovered that these drugs can also inhibit SARS-CoV-2 main protease with high affinity.

This research, led by Dr. Andrey Kovalevsky, is part of the broader effort to repurpose existing drugs for the treatment of COVID-19. The team’s work has been supported by the Department of Energy, Oak Ridge National Laboratory, and the National Institute of Allergy and Infectious Diseases.

In their study, the researchers used room-temperature X-ray crystallography to reveal the oxidation and reactivity of the catalytic cysteine residues in SARS-CoV-2 main protease. This information has provided insights into the mechanism of action of the drug and has helped to identify new targets for drug development.

The researchers also suggest that this approach could be applied to other viral proteases, such as those of HIV and SARS-CoV-1, which could lead to the development of new drugs for the treatment of those diseases.

Dr. Kovalevsky and his team are working on using these findings to develop more effective treatments for COVID-19 and other viral infections. They are also exploring the potential of using these drugs in combination with other antiviral agents to achieve synergistic effects.

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References

Fu, L., Ye, F., Feng, Y. et al. (2020). Boceprevir and GC76 efficaciously inhibit SARS-CoV-2 by targeting its main protease. Nature communications, 11, 4417. Available at: https://doi.org/10.1038/s41467-020-16954-7


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 Mpro. This is important because if hydrogen atoms release, it will change the corresponding amino acids’ electric charges, thus completely remodeling the electrostatic environment. Such observations will be another piece of the puzzle in pursuit of small-molecule therapeutics to treat COVID-19. The results of our team have been obtained so far, and our concurrent work with computational scientists, already gives us ideas for the design of novel protease inhibitors, which can be synthesized and studied further.