

Direct visualization of SARS-CoV-2 main protease electrostatics using neutron crystallography

D Kneller¹, S Galanie², G Phillips³, L Coates⁴, A Kovalevsky⁵

¹Oak Ridge National Lab ²Oak Ridge National Lab, ³Oak Ridge National Lab, ⁴Oak Ridge National Laboratory, ⁵Oak Ridge National Lab

knellerdw@ornl.gov

The SARS-CoV-2 virus that causes COVID-19 induced a worldwide economic and public health calamity in 2020. SARS-CoV-2 possesses an essential cysteine protease (Mpro) which serves as the 'heart' of viral replication and thus is a major target for small-molecule inhibitors. Structural biology strategies historically achieved atomic scale understanding of enzymes from cryogenically preserved samples using X-ray diffraction. Conventional protein X-ray crystallography studies are nevertheless hindered by the possibility of cryo-artifacts and the inability to determine protonation states, whereas neutrons provide an ideal probe to directly visualize protonation states of ionizable residues at near-physiological temperatures. A series of rapid room-temperature X-ray and neutron diffraction studies of Mpro are presented here encompassing our response to the pandemic, providing essential details about Mpro structure, function and, inhibition by reversible covalent and non-covalent inhibitors.

Pre-requisite room-temperature X-ray structures of Mpro were solved while growing the large-volume protein crystals amenable to neutron diffraction experiments. Rapid insights in the early months of the pandemic were provided by describing the inherent structural plasticity of the active site cavity [1]. We discovered that the catalytic Cys145 can be trapped in the rare peroxydisulfenic acid oxidation state at physiological pH while surface cysteines remain reduced indicative of the cysteine's high reactivity [2]. Structural comparisons between clinical HCV protease inhibitors indicated how significant malleability of active site residues operate during induced fit with different inhibitor moieties [3].

The neutron crystal structure of ligand-free Mpro was determined, providing direct observation of protonation states in any cysteine protease for the first time and painting the picture of the active site electrostatics. The non-canonical catalytic dyad of Cys145-His41 exists in the reactive zwitterionic state at rest, with charged thiolate and doubly protonated imidazole side chains [4]. We determined a follow-up neutron crystal structure of deuterated Mpro in complex with a covalent inhibitor to witness the net positive charge of the active site is maintained through rearrangements of protonation states and remodeling of the active site electrostatics [5]. Neutron crystallography of Mpro showcases the importance of accurate experimental models for mechanistic, in silico, and drug design research to better understand pathogens at the atomic level. All structures and results were immediately shared with the scientific community allowing for real-time contributions to fight COVID-19.

[1] Kneller, D. W.; Phillips, G.; O'Neill, H. M.; Jedrzejczak, R.; Stols, L.; Langan, P.; Joachimiak, A.; Coates, L.; Kovalevsky, A. Structural Plasticity of SARS-CoV-2 3CL Mpro Active Site Cavity Revealed by Room Temperature X-Ray Crystallography. *Nat. Commun.* 2020, 11 (1), 7–12. <https://doi.org/10.1038/s41467-020-16954-7>.

[2] Kneller, D. W.; Phillips, G.; O'Neill, H. M.; Tan, K.; Joachimiak, A.; Coates, L.; Kovalevsky, A. Room-Temperature X-Ray Crystallography Reveals the Oxidation and Reactivity of Cysteine Residues in SARS-CoV-2 3CL Mpro : Insights into Enzyme Mechanism and Drug Design . *IUCrJ* 2020, 7 (6). <https://doi.org/10.1107/s2052252520012634>.

[3] Kneller, D. W.; Galanie, S.; Phillips, G.; O'Neill, H. M.; Coates, L.; Kovalevsky, A. Malleability of the SARS-CoV-2 3CL Mpro Active Site Cavity Facilitates Binding of Clinical Antivirals. *Structure* 2020. <https://doi.org/10.1016/j.str.2020.10.007>.

[4] Kneller, D. W.; Phillips, G.; Weiss, K. L.; Pant, S.; Zhang, Q.; O'Neill, H. M.; Coates, L.; Kovalevsky, A. Unusual Zwitterionic Catalytic Site of SARS-CoV-2 Main Protease Revealed by Neutron Crystallography. *J. Biol. Chem.* 2020, jbc.AC120.016154. <https://doi.org/10.1074/JBC.AC120.016154>.

[5] Kneller, D. W.; Phillips, G.; Weiss, K. L.; Zhang, Q.; Coates, L.; Kovalevsky, A. Direct Observation of Protonation State Modulation in SARS-CoV-2 Main Protease upon Inhibitor Binding with Neutron Crystallography. *J. Med. Chem.* 2021. <https://doi.org/10.1021/acs.jmedchem.1c00058>.

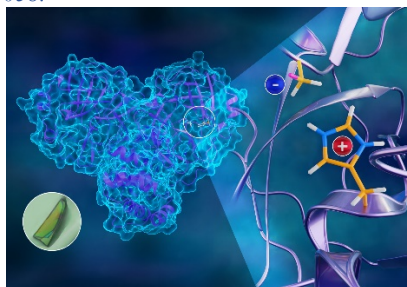


Figure 1