Neutron crystallography to inform drug design targeting SARS-CoV-2 main protease

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COVID-19, caused by SARS-CoV-2, remains a global health threat after two years of the pandemic even with available vaccines and therapeutic options. The viral main protease (M^{pro}) is indispensable for the virus replication and thus is an important target for small-molecule antivirals. Computer-assisted and structure-based drug design strategies rely on atomic scale understanding of the target biomacromolecule traditionally derived from X-ray crystallographic data collected at cryogenic temperatures. Conventional protein X-ray crystallography is limited by possible cryo-artifacts and its inability to locate the functional hydrogen atoms crucial for understanding chemistry occurring in enzyme active sites. Neutrons are ideal probes to observe the protonation states of ionizable amino acids at near-physiological temperature, directly determining their electric charges – crucial information for drug design. Our room-temperature X-ray crystal structures of M^{pro} brought rapid insights into the reactivity of the catalytic cysteine, malleability of the active site, and binding modes with clinical protease inhibitors.

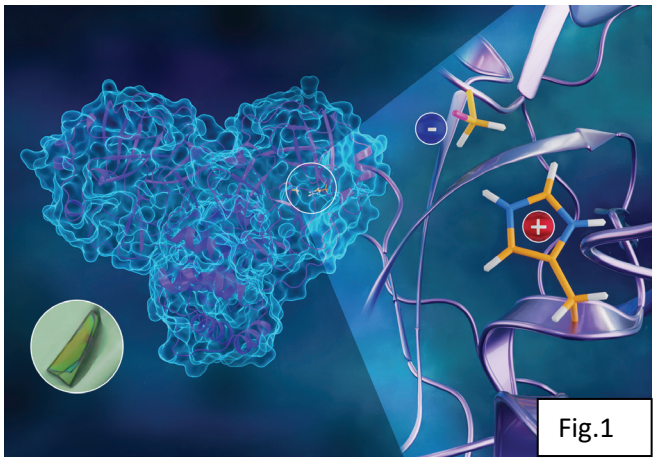

Fig.1

The neutron crystal structures of ligand-free and inhibitor-bound M^{pro} were determined allowing the direct observation of protonation states of all residues in a coronavirus protein for the first time [1,2]. At rest, the catalytic Cys-His dyad exists in the reactive zwitterionic state (Fig. 1), with both Cys145 and His41 charged, instead of the anticipated neutral state. Covalent inhibitor binding results in modulation of the protonation states. This information was used to design nanomolar hybrid reversible covalent inhibitors with robust antiviral properties. High-throughput virtual screening, utilizing ORNL's supercomputing capabilities, in conjunction with *in vitro* assays identified a lead noncovalent compound with sub-micromolar affinity. The neutorn structure of M^{pro} in complex with the noncovalent inhibitor was used in a structure-activity relationship (SAR) study guided by virtual reality structure analysis to novel M^{pro} inhibitors with imporved affinity to the enzyme [3]. Our research is providing real-time data for atomistic design and discovery of M^{pro} inhibitors to combat the COVID-19 pandemic and prepare for future threats from pathogenic coronaviruses.

[1] D.W. Kneller et al. Unusual zwitterionic catalytic site of SARS-CoV-2 main protease revealed by neutron crystallography. *J. Biol. Chem.* **295**, 17365-17373 (2020).

[2] D.W. Kneller et al. Direct observation of protonation state modulation in SARS-CoV-2 main protease upon inhibitor binding with neutron crystallography. *J. Med. Chem.* **64**, 4991-5000 (2021).

[3] D.W. Kneller et al. Structural, electronic and electrostatic determinants for inhibitor binding to subsites S1 and S2 in SARS-CoV-2 main protease. *J. Med. Chem.* **64**, 17366-17383 (2021).

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