Neutron crystallography for drug design targeting SARS-CoV-2 viral proteins

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COVID-19, caused by SARS-CoV-2, is a global health and economic catastrophe. The viral main protease (M^{pro}) is indispensable for SARS-CoV-2 replication and thus is an important target for small-molecule antivirals. Computer-assisted and structure-guided 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 an ideal probe to observe the protonation states of ionizable amino acids at near-physiological temperature, directly determining their electric charges – crucial information for drug design. Our X-ray crystal structures of M^{pro} collected at near-physiological temperatures brought rapid insights into the reactivity of the catalytic cysteine, malleability of the active site, and binding modes with clinical protease inhibitors. 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. At rest, the catalytic Cys-His dyad exists in the reactive zwitterionic state, with both Cys145 and His41 charged, instead of the anticipated neutral state. Covalent inhibitor binding results in modulation of the protonation states, retaining the overall electric charge of the M^{pro} active site cavity. In addition, high-throughput virtual screening in conjunction with *in vitro* assays identified a lead non-covalent compound with micromolar affinity, which is being used to design novel M^{pro} inhibitors. 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.

Keywords: SARS-CoV-2, main protease, neutron crystallography, protonation states, drug design.