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 (Mpro) is indispensable for the virus replication and thus is an important target for the design and development of small-molecule antivirals. Computer-assisted and structure-based drug design strategies rely on atomic scale understanding of the target biomacromolecule traditionally derived from 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 protonation states of ionizable amino acids at near-physiological temperature, directly determining their electric charges and hence accurately mapping the active site electrostatics – crucial information for drug design. Our room-temperature X-ray crystal structures of Mpro brought rapid insights into the reactivity of the catalytic cysteine, malleability of the active site, and binding modes of various protease inhibitors. The neutron crystal structures of ligand-free and inhibitor-bound Mpro 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. This information was used to design nanomolar hybrid reversible covalent inhibitors with robust antiviral properties. High-throughput virtual screening, utilizing available supercomputing capabilities, in conjunction with in vitro assays identified a lead noncovalent compound with sub-micromolar affinity. The neutron structure of Mpro in complex with the noncovalent inhibitor was used in a structure-activity relationship (SAR) study guided by virtual reality structure analysis to novel Mpro inhibitors with improved affinity to the enzyme. Our research is providing real-time data for atomistic design and discovery of Mpro inhibitors to combat the COVID-19 pandemic and prepare for future threats from pathogenic coronaviruses.