Structure of SARS-CoV-2 papain-like protease PLpro reveals a framework for antiviral inhibitor design

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) papain-like protease (PLpro) is essential for the virus replication and covers multiple functions (1,2). In this context PLpro is a most interesting drug target to identify compounds that inhibit the activity and can be further optimized towards drugs to cure Covid-19 in the future. Beside the cysteine-protease activity, PLpro has the additional and vital function of removing ubiquitin and ISG15 (Interferon-stimulated gene 15) from host-cell proteins to aid coronaviruses in their evasion of the host innate immune responses. Therefore, in terms of drug discovery investigations PLpro is thus an excellent drug target allowing a two-fold strategy, to identify compounds that inhibit viral replication and strengthen the immune response of the host in parallel. To establish a framework allowing an efficient and high throughput screening of compounds to identify inhibitors, we first expressed, purified and crystallized PLpro (Fig. 1); determined and refined the native crystal structure to atomic resolution of 1.42 Å (Fig. 2, pdb code: 7NFV).

Further, we initiated screening via co-crystallization utilizing a library of 2.500 selected natural compounds, obtained from ICCBS Karachi, and identified first potential inhibitors binding to a site that has been previously shown to bind to the ISG15 molecule, refined structures were deposited with pdb codes: 7OFS, 7OFT, 7OFU. Comparing the PLpro-ligand complex structures with the PLpro-ISG15 complex crystal structure (pdb code: 6XAA) clearly shows that several regions of the Ubiquitin fold domain move dynamically, showing functional flexibility to accommodate the ligands (Fig. 3). Corresponding structural data and details, as well as on-going structural efforts to identify new antiviral compounds to combat the coronavirus spread will be presented.

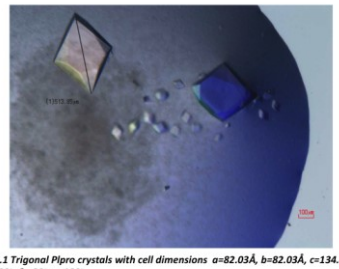

Fig.1 Trigonal Plpro crystals with cell dimensions a=82.03Å, b=82.03Å, c=134.45Å, α=90°, ß= 90°, γ=120°

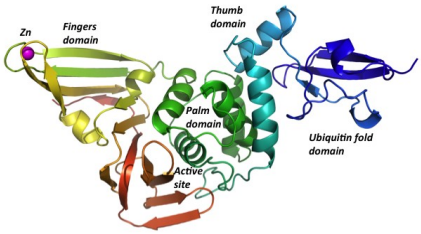

Fig.2 X-ray crystal structure of Plpro illustrating the four domains and the active site. The Zinc ion is represented as a magenta sphere

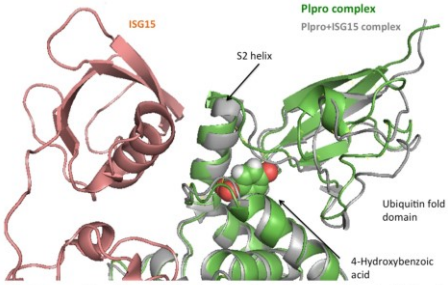

Fig. 3 Superposition of Plpro + 4-hydroxybenzoic acid complex structure (green) with Plpro in complex (grey) with ISG15 molecule (salmon pink). It can be clearly seen that the Ubiquitin fold domain, S2 helix move dynamically to accommodate the ligand

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