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A drug repurposing approach targeting SARS-CoV-2 PLpro in the fight against Covid-19

Papain-like protease (PLpro) coded by SARS-CoV-2 genome is a key enzyme in viral replication. This enzyme is a subdomain of the viral non-structural protein 3 (nsp3) [1]. PLpro is a cysteine protease which recognizes and cleaves the LXGG consensus sequence of both viral and host proteins showing deubiquitinating and deISGylating activity on key proteins in cytokines response pathways, which relates this enzyme to a major inflammatory response and immune system evasion [2,3]. PLpro has been drawing interest as a pharmacological target to fight the pandemic emergency [4].

The aim of the project is to identify novel inhibitors of PLpro providing biochemical and structural information to support rational drug discovery and repurposing campaigns. Thanks to a collaboration started within the Exscalate4Cov consortium (www.exscalate4cov.eu), an HT-in vitro repurposing screening was conducted by ITMP in Hamburg. Activity assays were performed on recombinant proteins expressed in *E. coli* and successfully purified at the Elettra Protein Facility. Different WT and mutated constructs were produced of the PLpro catalytic domain and of a longer sequence (PLpro_L) containing the nucleic acids binding domain (NAB) at the C-terminal. The PLpro_L construct was probed in the repurposing screening while the catalytic domain was used in the reconfirmation assay. A list of active compounds was selected and further characterized in our lab by TSA and by setting up numerous crystallization trials. So far, crystals obtained resulted to be of the apo form, indicating that the protein is difficult to crystallize in complexed with a ligand. The PLpro_L low resolution structure has been recently determined by SAXS, considering both wild type and two frequent mutants of the SARS-CoV2 Delta variant [5]. The overall shape is much elongated and interestingly the mutations induce higher flexibility of the molecule in solution. Crystallization of PLpro_L in apo and holo forms are still on development with the aim to characterize the binding, together with biophysical binding properties in a drug development optics.

Primary author: MORASSO, Stefano (Elettra Sincrotrone Trieste)

Co-authors: Dr STORICI, Paola (Elettra Sincrotrone Trieste); COVACEUSZACH, SONIA (CNR - Istituto di Cristallografia); Mrs KUZIKOV, Maria (ITPM Hamburg); Dr GRIBBON, Philip (ITPM Frankfurt); Dr ZALIANI, Andrea (ITPM Hamburg); Dr AMENITSCH, Heinz (University of Graz); Ms GLABBAI, Barbara (Elettra Sincrotrone Trieste)

Presenter: MORASSO, Stefano (Elettra Sincrotrone Trieste)

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