Crystal Structure of a Conserved Phosphatase Domain of Non-structural Protein-3 (nsp3) from the SARS Coronavirus

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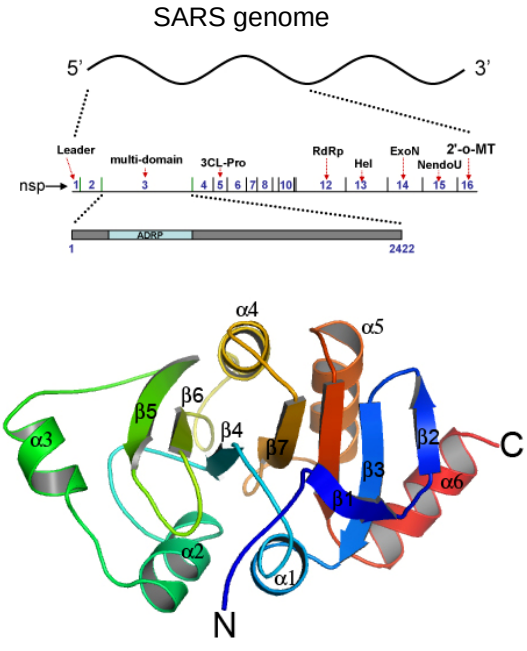
Severe Acute Respiratory Syndrome (SARS) is a debilitating pneumonia-like disease of the upper pulmonary tract in humans whose causative organism is a group II coronavirus (SARS-CoV). An early event in infected cells is the transcription of its genomic RNA into a large (~7000 residues) replicase polyprotein, which is then cleaved into 16 mature proteins known as non-structural proteins or nsps. Several groups have begun to characterize these proteins that form integral components of the SARS replicase/transcriptase complex. The third of these nsps, (nsp3) is a large 213kDa multidomain protein that arises due to cleavage of the polyprotein by the virally encoded papain-like protease or PLpro. It has at least six structural domains apart from a transmembrane domain.

We have embarked on an NIAID funded program that aims to structurally and functionally characterize all the protein domains of the SARS proteome (sars.scripps.edu). The program leverages recent advances made in high-throughput structure determination pipelines like parallel 96-well format cloning, expression and purification of proteins, nanovolume crystallization and remote screening and data collection facilities at synchrotron beamlines. Advances made in robotics and automation at beamlines like the 1-5, 11-1 and 11-3 at the Stanford synchrotron radiation labs (SSRL) and integrated tools like BLU-ICE enable rapid screening of several hundred crystals remotely. A cherished goal of this effort is to use these technologies to rapidly characterize proteomes of emerging disease causing organisms and provide structural templates for the structure-based design of inhibitory molecules.

One of the first structures to result from this effort was the second domain of SARS nsp3. The structure was solved by single-wavelength anomalous dispersion (SAD) method using data collected at the absorption edge of selenium, a commonly employed method in high-throughput structure determination. Native crystals diffracted to 1.4 Å at SSRL beamline 11-1 providing a very high resolution structural model.



The structure revealed a macro-domain-like fold that is commonly seen in a unique class of phosphatases that specifically dephosphorylate to ADP-ribose-1"-Phosphate (Appr). The structure, its organization as a dimer in the asymmetric unit, distribution of electrostatic charge on its surface, the presence of a deep solvent exposed cleft and the conservation of the residues at its putative active site immediately led to experimental validation of this domain as a functional phosphatase specific for Appr substrate and provided important insights into molecular aspects RNA processing and maturation that the virus employs during its replication cycle.

Several other proteins from this virus are in different stages of the structure determination pipeline with the ultimate goal of developing a detailed molecular structure-function-interaction map between the different protein and nucleic-acid components of the SARS proteome and their human cellular counterparts.

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