

Functional & Structural Proteomics of SARS: Defining a Rational Response to Emerging Diseases

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Rapid rational therapeutic and prophylactic responses are crucial when faced with new infectious diseases. The emergence of the coronavirus responsible for the Severe Acute Respiratory Syndrome (SARS) tested the utility of post-genomic technologies to characterize and combat this virus. While virus identification and complete genome sequencing took mere weeks, they have been tough acts to follow for drug and vaccine development. We have undertaken a multi-pronged initiative to understand and address precisely this bottleneck using a structural and functional proteomics approach involving bioinformatics, structural biology (X-ray crystallography, NMR, cryo-electron microscopy), genetic approaches (site-directed mutagenesis, antisense functional mapping, microarray-based functional mapping), and macromolecular interaction studies (nanocalorimetry, ligand-fishing techniques, mass spectrometry) to generate a structure-function-interaction map of the entire proteome of the SARS-CoV and its interactions with the host cell. This presents an exciting and comprehensive set of targets for rational, structure-based drug and vaccine design, defining a paradigm adoptable for *any* emerging infectious disease.

We designed multiple constructs of the 28 SARS-CoV ORFs for expression in *E. coli*, baculovirus and mammalian systems. Over 150 constructs have been processed by high-throughout protein expression and purification. Of the 31 expressing constructs, nano-volume crystallization has produced crystal hits for 5. One crystal structure and one NMR structure has been determined. Cryo-electron microscopy has characterized the packing arrangement of the S, M and N proteins in the virion. The challenge is that most SARS proteins are involved in intimate protein-protein, protein-membrane, or protein-RNA interactions which must be understood for a complete description of its biology.

Keywords: structural genomics, viral structure and function, intermolecular interactions