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Viewpoint

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Structural Genomics Support for Infectious Disease Drug Design

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Structural biology (SB) has greatly affected our understanding of protein form and function, from the elucidation of macromolecular structure to providing atomic-level understanding of complex biochemical, regulatory and immune mechanisms. Recent structural genomics (SG) approaches have accelerated progress, providing tens of thousands of new protein structures and providing insight into their function, even in the absence of significant amino acid sequence conservation. The high-throughput structure determination afforded by SG can also facilitate drug design by guiding refinement of small-molecule inhibitors through active site investigations using ligand and fragment-bound structures, sometimes leveraging protein orthologues to guide drug design.[1] The aim of this viewpoint is to introduce two SG consortia focused on supporting the infectious diseases (ID) community and to encourage medicinal chemists to utilize resources available to support the development of bioactive molecules directed against infectious disease organisms to fill the urgent need to develop drugs for the treatment and eradication of these pathogens.

The Seattle Structural Genomics Center for Infectious Disease (SSGCID) and the Center for Structural Genomics of Infectious Diseases (CSGID) were established in 2007 with National Institute of Allergy and Infectious Diseases (NIAID) funding, with a mission of solving three-dimensional protein structures from category A, B, and C pathogens, as well as emerging and re-emerging infectious disease organisms. While targets were initially selected by the consortia, most are now nominated by the scientific community and include known drug targets, potential drug targets, virulence and resistance factors, pathogenesis-associated targets, infection markers, vaccine candidates, markers of infection, and targets associated with innate immunity. To date, the Centers combined have entered more than 20000 targets into their structure determination pipelines from 55 bacterial, 25 eukaryotic, and 26 viral genera. Target genes are PCR amplified, cloned, and screened for expression, mainly in *Escherichia coli* but also in eukaryotic systems. Soluble proteins are purified in milligram quantities, screened for crystallization, and analyzed by X-ray diffraction using in-house source and off-site synchrotron beamlines. Small proteins that fail to crystallize are queued for structure determination by NMR. To date, the Centers have solved nearly 1500 protein structures. Below, we present three examples of ongoing drug development projects undertaken by the consortia in collaboration with the scientific community.

Macrophage infectivity potentiators (Mips) belong to a class of essential virulence factors called immunophilins that are found in a range of pathogens. The Mip subclass of FK506-binding proteins (FKBPs) are promising drug targets on the basis of their known roles in infection and their susceptibility to known inhibitors. The SSGCID has supported a drug development project in collaboration with the University of Würzburg, the U.K. Defense Science and Technology Laboratory, the University of Exeter, and the University of Western Australia. This collaboration initially characterized the three-dimensional structure of *Burkholderia pseudomallei* FK506-binding protein 12 (FKBP12)[2] and subsequently supported the identification of novel pipecolic acid derivatives, initially described by Juli et al.,[3] that reduce pathogenic cytotoxicity in cells.[4] The project has progressed to hit-to-lead optimization, and results from SSGCID were instrumental in securing funding from the NATO Science for Peace & Security (SPS) Programme to further develop the compounds into formulations ready for testing in preclinical mouse studies.

Inosine 5′-monophosphate dehydrogenase (IMPDH) is found in organisms from all kingdoms of life. It is a branch point between adenine and guanine nucleotide metabolism, a rate-limiting step in GMP biosynthesis, and is an important drug target. In collaboration with investigators at Brandeis University and the University of Houston, the CSGID has determined structures illuminating the structural differences between the human enzyme and those from pathogenic microbes that indicate inhibitors of the enzyme can be developed into new antibiotics.[5−7] Importantly, IMPDH from the protozoan parasite *Cryptosporidium parvum* is more closely related to the bacterial enzymes, and inhibitors have been developed that have high affinity (low nanomolar) for the *Cryptosporidium* enzymes with little activity against the human enzyme.[6,7] As part of this collaboration, the CSGID has determined the structures of 26 IMPDH proteins from different human pathogens in complexes with ligands and inhibitors. These structures provide a guide to rational design of more

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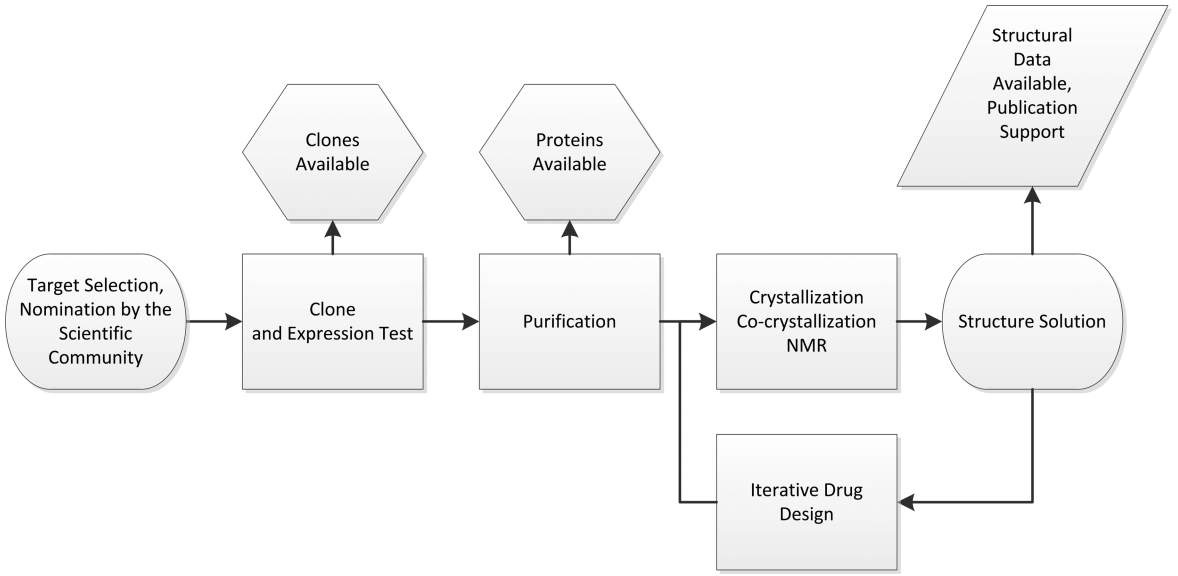

Figure 1. Flowchart of collaborative projects in the SSGCID and CSGID pipelines.

potent and selective drugs against bacterium and parasite IMPDHs.

Both the SSGCID and the CSGID are members of the Structure-guided Drug Discovery Coalition (SDDC), a consortium of SG and screening centers, as well as drug-discovery scientists funded by the Bill & Melinda Gates Foundation with the aim to deliver early leads to malarial, tubercular, and neglected disease drug candidates to preclinical development partners.[8,9] SDDC's approach seeks to combine the strengths of phenotypic and target-based drug discovery. Following this precept, work is undertaken on targets with good genetic validation of essentiality and where there is a whole-cell active small molecule that acts through the target (resistance mapping) and where tractable chemistry is available to mount a hit-to-lead project on the target, supported by structure-guided medicinal chemistry. For the malaria program, two projects are in hit-to-lead development and are being tested for activity and specificity versus a human version of the enzyme. Hits were identified from both fragment screening and phenotypic active libraries, and cocrystal structures were determined to guide chemistry efforts and lead optimization. The tuberculosis program has identified lead series in two projects, which have good pharmacokinetic attributes and are now being tested in in vivo infection models. The neglected diseases program was initiated in November 2014, and target selection is underway.

The CSGID and SSGCID work to actively engage researchers by accepting nominations for structure determination, providing materials and data at no charge and providing publication support (Figure 1). Targets are nominated through submission to the Target Request pages at www.csgid.org and www.ssgcid.org and, once approved by NIAID, are entered into the structure determination pipeline. Applicants are provided access to a password-protected portal for tracking target status. All structures solved are made publicly available, through submission to the Protein Data Bank (PDB). Both Centers work to actively engage as well as collaboratively interpret and publish results from the successful structure determinations together with researchers. To date more than 800 publications cite CSGID or SSGCID structures. Researchers are encouraged to use the Centers' Target pages to search for proteins of interest among the >20000 already in the SSGCID or CSGID structure determination pipelines. Sequence-verified expression clones and/or purified proteins can be provided free of charge to any member of the scientific community for research purposes. These materials can be requested from the Clones/Materials pages of the Centers' Web sites and require only acceptance of terms and conditions before distribution.

In summary, structural biology and structural genomics can aid the medicinal chemist in the evaluation and development of small molecules to combat infectious diseases. The SSGCID and CSGID resources are available to provide support for structure-guided drug design projects.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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