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STRUCTURAL GENOMICS AND SIGNALING DOMAINS

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We have begun a small scale structural genomics project aimed at obtaining fold information for the set of sequence families comprising eukaryotic intracellular signaling domains, and exploiting that information to understand biological function and mechanism. The SMART database (**http://smart.embl-heidelberg.de/smart**) is the primary target list for the project. The practical issues of obtaining soluble and crystallizable representatives for each sequence family will be discussed. The question of how much can be learned about signaling mechanisms from elucidating the structure of one representative protein per sequence family will be considered. Examples illustrating issues of principle and practice will be drawn from recent work on VHS and other domains.

Keywords: STRUCTURAL GENOMICS, SIGNAL TRANSDUCTION, PROTEIN TRANSPORT

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DERIVING FUNCTION FROM STRUCTURE - HOW DO WE FARE?

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A Structural Genomics research program at CARB that seeks to infer the function of so-called hypothetical proteins from the structural information has yielded so far some 25 structures. The structures exhibit both previously known and novel folds. I will review the results provide specific examples and assess the power and limits of this approach.

Keywords: STRUCTURAL GENOMICS HYPOTHETICAL PROTEINS

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CRYSTAL STRUCTURE OF SET3, A SUPERANTIGEN-FAMILY PROTEIN FROM A PATHOGENICITY ISLAND IN THE *S. AUREUS* GENOME

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The genomes of *Staphylococcus aureus* and *Streptococcus pyogenes*, two important human pathogens, encode a variety of virulence factors, some of which have been clearly linked to disease. These include toxins such as staphylococcal enterotoxin A (SEA) and toxic shock syndrome toxin (TSST). These form a family of superantigens (SAgs) with a common fold and the ability to severely disrupt the human immune system by binding to both T-cell receptors and MHC class II molecules.

The *Staphylococcus aureus* genome also encodes a cluster of SAg-like genes whose functions are unknown. These genes are clustered on a putative pathogenicity island, implying a role in virulence. We crystallized one of these proteins, SET3, and solved and refined its structure at 1.9 Å resolution (R = 20.5%, R_{free} = 24.0%). SET3 has the characteristic SAg-family fold, consistent with its sequence similarity (26% identity to TSST). Residues implicated in T-cell receptor and MHC binding in the SAgs are changed in SET3, however, and we have shown that SET3 does not have SAg activity. On the other hand, there are strong indications from seroconversion rates and antibody titres that SET3 has a role in pathogenicity. In the crystal, SET3 forms a dimer with a highly positively charged surface. Dimer formation occurs primarily through an extended β-hairpin that differentiates SET3 from other SAg-family proteins. We have shown that SET3 binds to DNA, which stabilizes SET3 dimer formation, but we hypothesize that the 'true' ligands for SET3 are likely to be negatively charged cell surface molecules.

Keywords: VIRULENCE PROTEIN GENOMICS SUPERANTIGEN FAMILY

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THE QUORUM SENSING PROTEIN LuxS: FUNCTIONAL INSIGHTS FROM STRUCTURE

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The high-throughput structure determination platform developed at Structural GenomiX is being utilized to increase the efficiency and effectiveness of the drug-discovery process. Parallelization increases the crystallization success rate and provides structural insights into sequence conservation and variability, of particular interest for antimicrobial design efforts. For example, orthologous proteins are routinely included when crystallizing members of a particular biochemical pathway. As an example of this methodology, we have determined the structure of the bacterial enzyme, LuxS, from quorum sensing pathway 2. LuxS is an attractive target for the development of novel, potentially broad spectrum, antibacterial agents but little was known about the protein beyond its involvement in the production of autoinducer-2 (AI-2), the pathway 2 signaling molecule. We undertook a crystallographic analysis in order to learn more about LuxS function through examination of its conserved structural features. LuxS genes from five bacterial species were selected as initial targets from which three LuxS structures were independently determined. The proteins share a common fold and common homo-dimerization interactions. The putative substrate binding site and zinc-containing catalytic site are also highly conserved. The similarity of the LuxS fold to those of threonyl-tRNA synthetase and mitochondrial processing peptidase suggests possible functional similarities. The structures enabled prediction of specific substrate-binding interactions as well as supported a catalytic mechanism involving zinc-mediated cleavage of the substrate's ribose group.

Keywords: QUORUM SENSING LUXS STRUCTURAL GENOMICS