Crystal Structures of 3C and 3C-like Proteases, Thioredoxin Reductase 1, Alpha-fucosidase, Crt M, L-aspartate Beta-decarboxylase, Lipase, 6-phosphogluconate Dehydrogenase, MST3 Kinase, Adenylate Isopentenyltransferase, and Geranyl Pyrophosphate Synthase

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X-ray crystallography was used to investigate the drug targets and proteins involved in critical biological processes. With regards to the drug target, we solved several crystal structures of chymotrypsin-like protease (3C) of human coxsackievirus, 3C-like protease (3CL) human coronaviruses, and 3CL severe acute respiratory syndrome in complex with the inhibitors(1). Here, the presence of specific binding pockets for the residues of peptidomimetic inhibitors explains the binding specificity. These results provide a structural basis for inhibitor optimization and development of potential drugs for antiviral therapies.

Terpyridine-platinum(II) (TP-Pt(II)) complexes are known to possess DNA-intercalating activity and have been regarded as potential antitumor agents. TP-Pt(II) complexes inhibited the human thioredoxin reductase 1 (hTrxR1) activity was inhibited with IC(50) values in the range of 58-78 nM. Additionally, using X-ray crystallography and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, we elucidated that the TP-Pt(II) complexes inhibited hTrxR1 activity by blocking its C-terminal active-site selenocysteine (2). Therefore, TP-Pt(II) complexes possess inhibitory activities against multiple biological targets, and they may be further studied as anticancer agents.

Glycosidases catalyze the hydrolysis of glycosidic bonds and are often therapeutic targets; examples include the influenza virus neuraminidase. and intestinal disaccharidases involved in type II diabetes. Two loops were found to move inward toward the alpha-fucosidase active site to produce a closed conformation of complexes with inhibitors with increasing Ki values from the micro- to nanomolar range (3). Although no further conformational changes in the two loops are observed for inhibitors with sub-nanomolar Ki values, the loops are additionally stabilized by hydrogen bonds and hydrophobic interactions.

The gold color of *Staphylococcus aureus* is derived from the carotenoid staphyloxanthin, a virulence factor for the organism. Here, we report the X-ray crystallographic structure of the most active compound, N-3-(3-phenoxyphenyl)propylphosphonoacetamide (IC_{50} = 8 nM, in cells), bound to CrtM, involved in the first committed step in its biosynthesis, condensation of two farnesyl diphosphate molecules to dehydrosqualene (4).

For the perspective of critical proteins, The type-I PLP enzyme l-aspartate beta-decarboxylase converts aspartate to alanine and CO_2. The crystal structure of a PLP-dependent dodecameric L-aspartate beta-decarboxylase reveals a dodecamer made of six identical dimers arranged in a truncated tetrahedron whose assembly involves tetramer and hexamer as intermediates(5). The structure also suggests that substrate binding triggers conformational changes essential for catalyzing the reaction.

Archaeoglobus fulgidus lipase (AFL) consists of an N-terminal alpha/beta-hydrolase fold domain, a small lid domain, and a C-terminal beta-barrel domain(6). The N-terminal catalytic domain consists of a 6-stranded beta-sheet flanked by seven alpha-helices, four on one side and three on the other side. The C-terminal lipid binding domain consists of a beta-sheet of 14 strands and a substrate covering motif on top of the highly hydrophobic substrate binding site.

6-Phosphogluconate dehydrogenase (6PGDH), the third enzyme of the pentose phosphate pathway, catalyzes the oxidative decarboxylation of 6-phosphogluconate, making ribulose 5-phosphate, along with the reduction of NADP(+) to NADPH and the release of CO_2. Here, we report the first apo-form crystal structure of the pathogenic *Klebsiella pneumoniae* 6PGDH and the structures of the highly homologous *Escherichia coli* K12 6PGDH complexed with substrate, substrate/NADPH and glucose (7).

The MST family is a subclass of mammalian serine/threonine kinases that are related to the yeast sterile-20 protein and are implicated in regulating cell growth and transformation. The MST3 protein contains a 300-residue catalytic domain and a 130-residue regulatory domain, which can be cleaved by caspase and activated by autophosphorylation, promoting apoptosis. Here, five crystal structures of the catalytic domain of MST3 are presented, including a complex with ADP and manganese, a unique cofactor preferred by the enzyme, and a complex with adenine (8).

Cytokinins are important plant hormones, and their biosynthesis most begins with the transfer of isopentenyl group from dimethylallyl diphosphate (DMAPP) to the N6-amino group of adenine by either adenylate isopentenyltransferase (AIPT) or tRNA-IPT. Here, we present the crystal structure of an AIPT-ATP complex from *Humulus lupulus* (HlAIPT), which is similar to the previous structures of Agrobacterium AIPT and yeast tRNA-IPT (9).

Terpenes (isoprenoids), derived from isoprenyl pyrophosphates, are versatile natural compounds that act as metabolism mediators, plant volatiles, and ecological communicators. Divergent evolution of homomeric prenyltransferases (PTSs) has allowed PTSs to optimize their active-site pockets to achieve catalytic fidelity and diversity. Little is known about heteromeric PTSs, particularly the mechanisms regulating formation of specific products. Here, we report the crystal structure of the (LSU.SSU)2-type (LSU/SSU = large/small subunit) heterotetrameric geranyl pyrophosphate synthase (GPPS) from mint (*Mentha piperita*) (10). The LSU and SSU of mint GPPS are responsible for catalysis and regulation, respectively, and this SSU lacks the essential catalytic amino acid residues found in LSU and other PTSs.