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Structure of Guinea Pig 11β Steroid Dehydrogenase 1 with Glycyrrhetinic Acid
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11β steroid dehydrogenase 1(11βHSD1) catalyzes the conversion of glucocorticoid cortisone to cortisol, amplifying the local concentration of cortisol in select tissues. Increasing evidence in the literature implicates 11βHSD1 in the metabolic syndrome consisting of diabeties, visceral obesity, and hyperlipidemia[1]. In addition, inhibition of 11βHSD1 ameliorates hyperglycemia and increases insulin sensitivity in diabetic animal models[2]. 11βHSD1 is thus a target for drug intervention in diabetes. We present the structure of Guinea Pig 11βHSD1 with Glycyrrhetinic Acid, a natural product inhibitor. We also discuss the mechanism of 11βHSD1 in relation to other steroid dehydrogenases and the implications of the structure for structure based drug design.

[1]Masuzaki H., Paterson J., Shinyama H., Morton N., Mullins J., Seckl J., Flier J., *Science*, 2001, **294**, 2166. [2] Alberts P., Nilsson C., Selen G., Engblom L.O., Edling N.H., Norling S., Klingstrom G., Larsson C., Forsgren M., Ashkzari M., Nilsson C.E., Fiedler M., Bergqvist E., Ohman B., Bjorkstrand E., Abrahmsen L.B., *Endocrinology*, 2003, **144**, 4755.
Keywords: diabetes, structure-based drug design, dehydrogenase steroid nucleotide

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Farnesyl Pyrophosphate Synthase: Clinical Target for Bone Diseases
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Osteoporosis affects one in three women and one in five men over the age of 50. Bisphosphonate therapy, which inhibits bone resorption, reduces the risk of fracture by 50% within one year.

Nitrogen-containing bisphosphonates are known inhibitors of farnesyl pyrophosphate synthase (FPPS) and are currently used to treat osteoporosis, Paget's disease of the bone, and malignant bone tumors. FPPS resides at a branchpoint of the isoprenoid pathway due to the fact that the farnesyl pyrophosphate product can undergo either chain-elongation or cyclization, or may be utilized for protein prenylation. Since the post-translational addition of a farnesyl moiety is essential to activate many intracellular signaling proteins, inhibition of FPPS leads to apoptosis. Why some nitrogen-containing bisphosphonates are more potent inhibitors, and hence more effective drugs, is poorly understood.

The structure of human pyrophosphate synthase in complex with magnesium and the bisphosphonate risedronate shows the binding mode for this important class of inhibitors. Risedronate occupies the chain-elongation site but not the isopentenyl pyrophosphate site. Two aspartate clusters chelate the magnesiums that mediate ligand binding and are involved in catalysis. Although predictions suggested two inhibitors binding to each protein chain, isothermal titration calorimetry and the crystal structure clearly indicate a one-to-one stoichiometry.

Since this is the first example of a mammalian FPPS, it will provide the basis for more accurate structure-assisted drug design.
Keywords: transferases, protein-drug interaction, drug design

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Structure of *S*-Adenosyl-L-Homocysteine Hydrolase from *Plasmodium falciparum*
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The human malaria parasite *Plasmodium falciparum* is responsible for the death of more than a million people each year. The emergence of strains of malaria1 parasite resistant to conventional drug therapy has stimulated searches for antimalarials with novel modes of action. *S*-Adenosy1-L-homocysteine hydrolase (SAHH) is a regulator of biological methylations. Inhibitors of SAHH affect the methylation status of nucleic acids, proteins, and small molecules. *Plasmodium falciparum* SAHH (PfSAHH) inhibitors are expected to provide a new type of chemotherapeutic agent against malaria. Despite the pressing need to develop selective PfSAHH inhibitors as therapeutic drugs, only the mammalian SAHH structures are currently available. Here, we report the crystal structure of PfSAHH complexed with the reaction product adenosine [1].

[1] Tanaka N., et al., *J. Mol. Biol.*, 2004, **343**, 1007-1017.
Keywords: crystal structure, malaria, drug design

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Crystal Structure of Oxido Squalene Cyclase
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Oxido Squalene Cyclase (OSC) catalyses the synthesis of the steroid scaffold which is a key step in the synthesis of cholesterol. In a single highly selective reaction OSC forms lanosterol from the linear substrate oxido squalene.

The 2.1Å structure of this human monotopic integral membrane protein shows how OSC is inserted to the membrane. The hydrophobic substrate can reach the active site that is deeply buried in the center of the enzyme through a channel that opens into the membrane. The structure gives also new insights into the way OSC catalyzes the interesting cyclization reaction. Analysis of the mode of inhibitor binding to the active site cavity will help in the design of new OSC inhibitors as anticholesteremic drugs.

Also the high level expression, purification and crystallization of this human membrane protein will be described. Analytical ultra centrifugation was used to characterize the aggregation state of OSC and was helpful in predicting crystallizability.
Keywords: cholesterol, cyclase, membrane protein

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Crystal Structure of the N-terminal Ankyrin Repeat Domain of Human RNase L
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Ribonuclease L (RNase L) is implicated in both the molecular mechanisms of interferon action and the fundamental control of RNA stability in mammalian cells. RNase L is catalytically active only after binding an unusual activator molecule containing a 5'-phosphorylated 2',5'-linked oligoadenylate, (pp)p(A2'p5')2A (2-5A), in the N-terminal half. RNase L consists of three domains, namely the N-terminal ankyrin repeat domain, the protein kinase homology domain, and the C-terminal ribonuclease domain. The N-terminal ankyrin repeat domain is responsible for 2-5A binding, and the C-terminal domain is responsible for catalytic activity.

We have determined the crystal structure of the N-terminal ankyrin repeat domain (ANK) of human RNase L complexed with the activator 2-5A at 1.8 Å resolution [1]. The ANK folds into eight ankyrin repeat elements and forms an extended curved structure with a

concave surface. The 2-5A molecule is accommodated in the concavity and interacts with ankyrin repeats 2 to 4. Two structurally equivalent 2-5A binding motifs are found at repeats 2 and 4. The structural basis for 2-5A recognition by ANK is essential for designing stable 2-5As with a high likelihood of activating RNase L.

[1] Tanaka N., Nakanishi M., Kusakabe Y., Goto Y., Kitade Y., Nakamura K.T., *EMBO J.*, 2004, **23**, 3929.

Keywords: 2-5A system, ankyrin repeat, interferon

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Crystal Structures of Autocrine Motility Factor Complexed with Inhibitors

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Autocrine motility factor (AMF), a tumor-secreted cytokine, stimulates cell migration *in vitro* and metastasis *in vivo*. AMF is identical to the extracellular cytokines neuroleukin and maturation factor and, interestingly, to the intracellular enzyme phosphoglucose isomerase. Cytokine activity of AMF is inhibited by carbohydrate compounds, as they compete for AMF binding with the carbohydrate moiety of the AMF receptor, which is a glycosylated seven-transmembrane helix protein. Crystal structure analyses and site-directed mutagenesis studies of human AMF revealed that the regions important for the enzymatic function of AMF/PGI overlap those for the cytokine function of AMF [1].

Here we have determined the crystal structures of the various length of inhibitor-bound AMF at high resolution and assayed the inhibitory activities of the various inhibitors. These data provide an insight into the lead compound design of more effective AMF inhibitors.

[1] Tanaka N., Haga A., Uemura H., Akiyama H., Funasaka T., Nagase H., Raz A., Nakamura K.T., *J. Mol. Biol.*, 2002, **318**, 985.

Keywords: cancer, metastasis, tumor

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LTB_4 12-hydroxydehydrogenase/15-oxo-PG 13-reductase and Indomethacin Complex

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The bi-functional leukotriene B_4 12-hydroxydehydrogenase/15-oxo-prostaglandin 13-reductase (LTB_4 12-HD/PGR) is essential for eicosanoid inactivation. It catalyzes the first irreversible reactions in each eicosanoid inactivation and the activity may be regulated by a protein with SH3 domain. LTB_4 is oxidized to 12-oxo-LTB_4, and 15-oxo-PGE_2 are reduced to 13,14-dihydro 15-oxo-PGE_2. Some none steroidal anti-inflammatory drugs inhibit LTB_4 12-HD/PGR activity. Here we report the structure of LTB_4 12-HD/PGR with $NADP^+$ and indomethacin. The indomethacin binds to the 15-oxo-PGE_2 binding site, indicating that the indomethacin competitively inhibits LTB_4 12-HD/PGR. The chloro-benzene moiety of indomethacin enters into the hydrophobic pore that is the recognition site of ω-chain of 15-oxo-PGE_2, and the carboxyl group of indomethacin interacts with Arg56 and Tyr262. The architecture is identical to those of indomethacin and cyclooxygenase complexes. The result may be useful for further development of cyclooxygenase inhibitor.

Keywords: anti-inflammatory compounds, dehydrogenases, protein-inhibitor binding

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Automated Structure Refinement for High-throughput Ligand Detection with BUSTER-TNT

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The use of crystallography for the discovery of lead compounds often involves a large number of experiments with different soaking or co-crystallization trials. The subsequent refinement and analysis of the resulting datasets can be time-consuming and tedious. Since the crystallographic parameters (resolution, space group, cell parameters) are quite often similar, this task is ideally suited for automation.

We present a method (**autoBUSTER**) that automates the refinement, solvent model update, ligand detection and analysis. Centered around the BUSTER-TNT program [1,2], it requires a minimal amount of user input. Although it can be used at any resolution and for any kind of macromolecular structure, it is tuned to the refinement of protein structures at better than 2.8 Å resolution.

The knowledge of any (possibly) bound ligand can be given (a) explicitly by supplying a PDB file of dummy atoms that describes the assumed binding site, or (b) by letting the system automatically analyze the residual density of difference Fourier maps. A unique feature of BUSTER-TNT is used, where the various masks describing the known fragment, the bulk solvent and the missing part can be given independently from each other. The results show that this can greatly enhance the capability of uniquely defining any bound ligand.

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Keywords: refinement, ligands, automation

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Crystallographic Studies of Novel Inhibitors of β-Lactamases

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Bacterial expression of β-lactamases is the most widespread resistance mechanism to β-lactam antibiotics. There is a pressing need for novel, non-β-lactam inhibitors of these enzymes [1]. Our efforts to overcome bacterial resistance mechanisms have been directed towards novel, non β-lactam inhibitors of AmpC β-lactamase, a class C enzyme responsible of resistance to antibiotics treatment in gram-negative bacteria.

Through a structure-based approach, we discover novel inhibitors for this enzyme, with covalent mechanism of action such as boronic acid derivatives and with no-covalent, competitive mechanism of action, such as thiophene-2-carboxylic acid derivative [2].

In one case we were able to extend the inhibitory activity towards class A β-lactamases, obtaining a broad spectrum, highly potent inhibitor.

Some inhibitors were active in cell culture, reversing resistance to the third generation cephalosporin ceftazidime in bacterial pathogens expressing AmpC and did not up-regulate β-lactamase expression in cell culture.

The structure-based design, synthesis, biological evaluation and the crystallographic studies of such novel inhibitors will be described.

[1] Cosgrove S., Carmeli Y., *Clin. Infect. Dis.*, 2003, **36**, 1433-1437. [2] Tondi D., Morandi F., Bonnet R., Costi M. P., Shoichet B. K., *J. Am. Chem. Soc.*, 2005, **127(13)**, 4632-4639.

Keywords: enzyme inhibition, drug resistance, X-ray complexes