bind to a site on the surface of the thumb domain. A cyclohexyl and phenyl ring substituents, bridged by an indole moiety, fill two closely spaced pockets whereas a carboxylate substituent forms a salt bridge with an exposed arginine side chain. In the apoenzyme, the inhibitor binding site is occupied by a small alpha-helix at the tip of the N-terminal loop that connects fingers and thumb domains. Thus, these molecules inhibit the enzyme by preventing formation of intramolecular contacts between these two domains and consequently precluding their coordinated movements during RNA synthesis. Our structures identify a novel mechanism by which a new class of allosteric inhibitors inhibit the HCV polymerase and open the way to the development of novel antiviral agents against this clinically relevant human pathogen. Furthermore, the structures reveal a mechanism of inhibition, with the inhibitor displacing part of the fingertip loop anchoring fingers to the thumb, which may be relevant also for the inhibition of other viral RNA dependent RNA-polymerases.

**Keywords: HCV, NS5B, polymerase**

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**Structural Parameters Influencing the Affinities and Effectiveness of Ribosomal Antibiotics**

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Residing in the large ribosomal subunit and stretching from the site of peptide bond formation, to the other end of the particle, the protein exit tunnel provides the path of the emerging nascent proteins. Being of utmost importance for life, the ribosomal tunnel is targeted by a large number of antibiotics, belonging to the macrolide and ketolide families, which bind to a specific pocket made exclusively of RNA and act by blocking the tunnel, thus hampering nascent protein progression.

High-resolution crystal structures of several antibiotics, belonging to the various branches of these families as well as of compounds possessing characteristic properties of both the macrolides and ketolides, allowed parameterization of the specific contributions of the different nucleotides comprising the macrolide binding pocket. Analysis of these structures shed light on basic issues of antibiotics selectivity and provided the structural basis for the mechanisms of antibiotics resistance.

Comparative analysis of antibiotics binding modes to the eubacterial pathogen model, Deinococcus radiodurans, and to the archaea Haloarcula marismortui, which shares properties with eukaryotes and prokaryotes, showed that despite the overall conservation of the ribosome, phylogenetic and conformational variations in antibiotics binding pocket allow their selectivity, thus facilitating their therapeutical usage.

**Keywords: ribosomal tunnel, antibiotics, protein synthesis**

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**Multiple Inhibitor Co-crystal Structures of the Human Topoisomerase I Covalent DNA Complex bound to a Series of Structurally Diverse Anti-cancer Compounds**

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Topoisomerases are ubiquitous enzymes that relieve the torsional stress of DNA generated by nuclear processes such as replication and transcription. All topoisomerases act through a conserved tyrosine residue to cleave the DNA phosphodiester backbone and form a covalent phosphotyrosine intermediate. After cleavage, the broken DNA strand can rotate around the unbroken strand to either wind or un-wind DNA. The phosphodiester backbone is restored in a reversal of the transesterification reaction.

The transient top1-DNA covalent complex is a validated target for the development of anti-cancer compounds. Several structurally diverse families of chemical compounds have been discovered which specifically bind to and trap the transient top1-DNA covalent complex, which eventually results in cell death.

We report the X-ray crystal structures of the human top1-DNA complex bound with representative members of several families of anti-cancer compounds including: camptothecins, homo-camptothecins, indenoisoquinolines, indolocarbazoles and minor groove binding top1 poisons. Two distinct binding sites are identified, one for intercalating compounds such as camptothecin, and another for minor groove binding ligands. The planar nature of the intercalating compounds allows them to stack between DNA base pairs at the site of single-strand cleavage. These new X-ray structures will aid the rational design of completely novel structural classes of anticancer drugs.

**Keywords: topoisomerase I, camptothecin, DNA complex**

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**Fragment-based Screening by X-ray Crystallography: An Alternative to High-throughput Screening**

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Screening of libraries of small molecules (or drug fragments) by X-ray crystallography offers an alternative approach to discovering novel active site binders for enzymes, which may be used as a starting point in a drug discovery programme. This method can identify unique fragments with a potency in the millimolar range, and which are not found by most enzyme assay screening methods. Many of these compounds show efficient binding for their size. The use of crystallography as a screening tool gives access to precise structural data on identification of fragment binding, and this information can be used as a starting point for rational optimization of the fragment into a potent inhibitor. This may then be used as a potential lead compound for drug discovery. This method is illustrated with examples from two kinase projects [1].

[1] Hartshorn M.J., Murray C.W., Cleasby A., Frederickson M., Tickle I.J., JhotiH., *J. Med. Chem*., 2005, **48(2)**, 403-413.

**Keywords: protein crystallography application, drug discovery and design, kinase**

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**Inspecting the Pharmacophore of Protein Kinase CK2 with Tetrabromobenzimidazoles**

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CK2 is a highly pleiotropic protein kinase whose high constitutive activity is suspected to cooperate to neoplasia. Here the crystal structures of the complexes between CK2 and three new selective tetrabromobenzimidazole derivatives inhibiting CK2 with $K_i$ values between 40 and 400 nM are presented. The ligands bind to the CK2 active site in a different way with respect to the parent compound tetrabromobenzotriazole. They enter more deeply into the cavity establishing halogen bonds with the backbone of Asp114 and Val116 in the hinge region. A detailed analysis of the interactions highlights a major role of the hydrophobic effect in the binding of this class of inhibitors. In contrast polar interactions are responsible for the different orientation of the molecules in the active site which ultimately influences the extent of the accessible surface area buried to the solvent.

**Keywords: protein kinases, CK2, inhibitors**

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*Acta Cryst*. (2005). A**61**, C247
**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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*Acta Cryst*. (2005). A**61**, C247
**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**

**P.04.15.28**
*Acta Cryst*. (2005). A**61**, C247
**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