as an attractive target for inhibitor drug design to increase the efficacy of treatment [2].

Drug resistance remains a limiting factor in cancer chemotherapy and thus understanding the mechanisms of this effect represents an essential step in improving cancer treatment. There are many reports correlating over-expression of GST and reduced sensitivity to chemotherapy in lung, liver, breast, ovarian, and other forms of cancer[1]. GSTs are hypothesized to catalyse conjugation of GSH to anticancer drugs forming inactive conjugates, therefore, decreasing efficacy in treatment. The precise mechanisms responsible for the development of resistance to these commonly used anti-cancer agents is currently unknown. Gaining insight, through structural studies by X-ray crystallography, of this enzyme complexed to these compounds, will aid in the design of effective, and specific, inhibitors.

One of the major aims of this work is to determine the 3D structures of these complexes and subsequently pursue structure-based drug design of human GST pi class enzyme (hGSTP1-1) with the hope of discovering potent specific inhibitors. I have collected over 25 data sets of GST complexed to a range of compounds, several of which have been solved and the structures completed. The structure of the hGSTP1-1 in complex with these compounds will identify critical residues which will aid drug design of novel, therapeutic, GST inhibitors.

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Keywords: anticancer drugs, inhibitor design, protein crystallography drug design

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X-ray Crystallograpy of the Antiepileptic Drug Zonisamide with CA II

Angela Casini[a], Giuseppina De Simone[b], Carlo Pedone[b], Jochen Antel[c], Andrea Scozzafava[a], Claudiu T. Supuran[a], [a]*Dipartimento di Chimica, Università degli Studi di Firenze, Florence, Italy.* [b]*Dipartimento di Chimica Biologica-Sezione Biostrutture and Istituto di Biostrutture e Bioimmagini-CNR, University of Naples "Federico II", Naples, Italy.* [c]*Solvay Pharmaceuticals, Hannover, Germany.* E-mail: angela.casini@tiscali.it

Zonisamide, **ZNS**, is a widely used antiepileptic drug [1] whose mechanism of action is still not fully clarified. Recently a patent[2]claimed that ZNS is also effective for reducing weight in obese subjects and for treating eating disorders.

In a previous study we showed by means of solution and X-ray studies, that another sulfonamidic antiepileptic drug, Topiramate, is a strong inhibitor of physiologically relevant human carbonic anhydrase (hCA) [3].Thus we decided to investigate the interaction of ZNS with the CA isozymes involved in lipogenesis and other metabolic processes, through the crystallographic analysis. Here we report the X-ray crystal structure of the complex ZNS-hCA II at a resolution of 1.70 Å, showing that the ZNS participates in the classical inhibitory interactions with the Zn(II) ion and with specific residues in the active site of the hCA II.

Thus the activity of this drug in different metabolic pathways must be reconsidered also according to its possibility of interaction with different CAs.

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Keywords: drug design, sulfonamides, carbonic anhydrase

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Structures of 5-methylthioribose Kinase: Catalytic Mechanism and Drug Design

Shao-Yang Ku[a,b], Patrick Yip[b], Kenneth A. Cornell[c], Michael K. Riscoe[d], P. Lynne Howell[a,b], [a]*Department of Biochemistry, University of Toronto.* [b]*Hospital for Sick Children, Toronto, Canada.* [c]*Department of Chemistry, Boise State University, Boise, USA.* [d]*Department of Chemistry, Portland State University, Portland, USA.* E-mail: syku@sickkids.ca

The essential amino acid methionine plays critical roles in a variety of cellular functions but is energetically costly to synthesize. As a consequence, pathways to salvage methionine have evolved in almost all organisms. 5-methylthioribose (MTR) kinase is a key enzyme in this pathway in microorganisms and certain plants, and the absence of a mammalian homolog suggests that the enzyme is a good target for the design of novel antibiotics against MTR kinase containing pathogens and selective herbicides. Recombinant *B. subtilis* MTR kinase has been expressed, purified and crystallized with the detergent CHAPS, and structures of the apo enzyme, ADP, ATP and ATP-MTR complexes have been determined. The first structure was determined by MAD technique using holmium in complex with ADP as the phasing derivative. The structure of MTR kinase has a eukaryotic protein kinase fold, and is similar to 3',5'-aminoglycoside phosphotransferase and choline kinase. Structures of MTR kinase with and without its substrate reveal local conformational flexibility and illuminate a detailed catalytic mechanism of the enzyme. These structures also provide a blueprint for future structure or mechanism based drug design.

Keywords: protein crystallography drug design, methylthioribose kinase enzymatic mechanism, methionine recycing pathways

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Dobexilate as a Lead Compound in Angiogenesis Inhibition Search

Israel Sánchez, Rosa Maria Lozano, Antonio Romero, Guillermo Gimenez, *Department of structure and protein function, CIB-CSIC, Madrid, Spain.* E-mail: israelsf@cib.csic.es

Fibroblast growth factors (FGFs) are powerful angiogenic polypeptides, whose mitogenic activity requires the presence of heparin-like compounds. Inhibition of angiogenesis-promoting factors such as fibroblast growth factor is considered to be a potential procedure for inhibiting solid tumor growth. Although several peptide-based inhibitors are currently under study, the development of antiangiogenic compounds of small molecular size is a pharmacological goal of considerable interest. We have study the effect of dobexilate *in vitro* and *in vivo* in order to find a minimum compound capable of inhibiting angiogenesis and tumor growth. Cell cultures as well as animal model experiments have shown clearly an angiogenesis suppressing effect event at low concentration as 50 μM. To provide structural information of this process we have solve the three-dimensional structure of a dobexilate-FGF complex. The structure gave us a clear image of the antiangiogesis mechanism of the dobexilate molecule which consists in the steric hindrance of interaction between the FGF molecule and the low affinity membrane receptor of this molecule in the plasma membrane, hampering in this way the beginning of the signalling cascade. Further studies of different groups in the minimal dobexilate structure could give us a more powerful and less toxic antiangiogenic compound using the disruption of the interaction of FGFs with heparin and heparan sulphates as its principal mechanism.

Keywords: FGFs, antiangiogenesis, dobexilate

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HIV Protease Inhibition Seen by X-ray Diffraction and Molecular Dynamics

Jindřich Hašek, Karel Zimmermann, Tereza Skálová, Jan Dohnálek, Jarmila Dušková, Hana Petroková, Eva Vondráčková, *Institute of Macromolecular Chemistry Academy of Sciences, CZ16206 Praha 6, Czech Republic.* E-mail: hasek@imc.cas.cz

Binding of inhibitor into the complex with HIV protease is accompanied by a large movement of protease flaps. X-ray crystallography shows the stable inhibited protease complexes [1], or unliganded proteases with "semi-opened" flaps and larger atomic displacement parameters. This work inspects conformational mobility

of the HIV protease flaps and the process of inhibitor unbinding using molecular dynamics (Amber, Oral) [2] under influence of external forces (with variable magnitudes and directions) making the system pass through different conformations in a reasonable computer time. Energies of individual components of the system were monitored to judge on feasibility of the states acquired. For unliganded protease, the flaps can accommodate a large range of opened conformations allowing direct entry of inhibitor into the binding cleft. Therefore, the protease without inhibitor is in a relaxed state with its flaps in large spectrum of conformations of similar energy. On the complexation the protease flaps close over the inhibitor as necessary and thus can accommodate inhibitors of different sizes. Forced unbinding of inhibitor simulated with HIV protease in a "water box" shows that the flaps stick to the inhibitor and follow it up to a large distance from the protein. The energy profiles show that the process of unbinding has many steps and must be slow relatively to the natural movement of the flaps to keep their deformation energies low. The project was supported by GA AV CR KJB4050312 and MSMT 1K05008.

[1] Petroková H., et al., *Eur.J.Biochem*, 2004, **271**, 4451. [2] Zimmermann K., *J. Comput. Chem*., 1991, **12**, 310-319.

Keywords: HIV retroviral proteases, drug design, structure

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Series of HIV-1 Protease Nanomolar Inhibitors; Binding to WT and Mutant Protease

Tereza Skalova, Jan Dohnalek, Jarmila Duskova, Hana Petrokova, Eva Vondrackova, Jindrich Hasek, *Institute of Macromolecular Chemistry, Czech Academy of Sciences, Prague, Czech Republic.* E-mail: skalova@imc.cas.cz

HIV protease cleaves polyprotein of immature human immunodeficiency virus and contributes thus to formation of active mature virus. Inhibition of HIV protease is one of the ways which are used to break life cycle of HIV and several inhibitors of HIV protease are already used as drugs against AIDS in clinical practice.

A series of chemically similar pseudo-tetrapeptide inhibitors of HIV-1 protease (K_i in the range from 0.1 to 1000 nM, [1]) was selected for structural analysis. The inhibitors have different peptide bond isosteres and they differ in amino acid residue in P2' binding position. Binding to wild type protease and to mutants A71V, V82T, I84V or L63P, A71V, V82T, I84V was compared.

It was found that, in binding pockets S1' – S3', binding stays similar in the series of nine structures and low B factors were refined. On the contrary, flexibility and variability exists in the P1 binding position and in the peptide bond isostere region.

Acknowledgement: The research was supported by the Grant Agency of the Academy of Sciences of the Czech Republic, project KJB4050312.

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Keywords: HIV retroviral proteases, HIV drug design, macromolecular crystal structure

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Crystallization and X-ray Analysis of the Catalytic Domain of Human PDE 3B

Sangita B. Patel[1], Jeffrey P. Varnerin[2], Michael R. Tota[2], Scott D. Edmondson[1], Emma R. Parmee[1], Christine Chung[1], Suresh B. Singh[1], Anthony Mastracchio[1], Joseph W. Becker[1], Giovanna Scapin[1], [1]*Department of Medicinal Chemistry, Merck & Co., Rahway, NJ 07065.* [2]*Department of Obesity and Metabolic Disorders, Merck & Co., Rahway, NJ 07065.* E-mail: sangita_patel@merck.com

The catalytic domain of human phosphodiesterase 3B has been cloned, expressed in *Escherichia coli*, and purified in the presence of the PDE3 inhibitors IBMX (3-isobutylmethylxanthine) or MERCK1 by affinity chromatography. Initial screening of crystallization conditions for these complexes in the hanging-drop vapor-diffusion mode resulted in three different crystal forms, all characterized by quite large unit cell parameters, elevated solvent content and poor diffraction quality. Subsequent optimization of these conditions led to crystals that diffract to 2.4 Å and belong to space group C2, with unit cell parameters a=146.7, b=121.5, c=126.3 Å, β=100.6°. Rotation function analysis indicates that the asymmetric unit contains four copies of the monomeric enzyme, corresponding to a solvent content of 64% [1]. The structures of the catalytic domain of human PDE3B in complex with IBMX and MERCK1 have been solved to 2.4 Å using these optimized crystals. These structures explain the dual cAMP/cGMP binding capabilities of PDE3, provide the molecular basis for inhibitor specificity, and can supply a valid platform for the design of improved compounds [2].

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Keywords: phosphodiesterase 3B, protein crystallography, drug design

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Crystal Structure of Nicotinic Acid Mononucleotide Adenylyltransferase from *Pseudomonas aeruginosa* in its Apo and Substrate-complexed Forms

Hye-Jin Yoon, Se Won Suh, *Department of Chemistry, Seoul National University, Korea*. E-mail: yoonhj@snu.ac.kr

The enzyme nicotinic acid mononucleotide adenylyltransferase is essential for the synthesis of nicotinamide adenine dinucleotide and is a potential target for antibiotics. It catalyzes the transfer of an adenyl group from ATP to nicotinic acid mononucleotide to form nicotinic acid adenine dinucleotide. In order to provide missing structural information on the substrate complexes of NaMN AT and to assist structure-based design of specific inhibitors for antibacterial discovery, we have determined the crystal structures of *Pseudomonas aeruginosa* in three states, i.e., the NaMN-bound form at 1.7 Å resolution and ATP-bound form at 2.0 Å as well as its apo-form at 2.0 Å. They represent crucial structural information necessary for better understanding of the substrate recognition and the catalytic mechanism. Structural comparisons of the substrate-complexes with the apo enzyme indicate that there is little conformational change upon binding each of the substrates. Our structures indicate that a conformational change is necessary to bring the two substrates closer together for initiating the catalysis. We suggest that such a conformational change likely occurs only after both substrates are simultaneously bound in the active site.

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Keywords: nicotinic acid mononucleotide adenylyltransferase (NaMN AT), nicotinamide adenine dinucleotide (NaMN), ATP

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Structure of a Glycosylation Mutant of Testis ACE bound to a novel Inhibitor

Jean Watermeyer[a], E.D. Sturrock[a], B.T. Sewell[b], [a]*Division of Medical Biochemistry, UCT, South Africa.* [b]*Electron Microscope Unit, UCT, South Africa.* E-mail: jean@science.uct.ac.za

Human angiotensin-converting enzyme (ACE) is vital to the regulation of blood pressure. ACE inhibitors are commonly used for the treatment of cardiac disease. Structural information about ACE has only been elucidated recently, with the solution of a crystal structure of human testis ACE (tACE)[1].

We have determined the structure of a glycosylation-deficient mutant of tACE, to 2.9 Å. The structure reveals a predominance of α-helices with the active site located deep in the cavity that separates the two sub-domains. This is in agreement with the structure of a native form of tACE that was published recently. We have also solved a