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.

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Keywords: HIV retroviral proteases, drug design, structure

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

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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

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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

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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.

[1] Olland, et al., *J. Biol. Chem*., 2002, **277**, 3698-3707. [2] Zhang, et al., *Structure*, 2002, **10**, 69-79.

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

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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

structure of human testis ACE in complex with a novel C-domain specific inhibitor, to 3.0 Å, which reveals detailed information on the interactions of this inhibitor with the active site.

In addition, we have carried out a normal mode analysis that reveals the intrinsic flexibility of tACE about its active site cleft. The intrinsic flexibility suggested by this study indicates a mechanism whereby subaccess could be achieved.

The information obtained in this study will be used in the design of new specific inhibitors of the C-domain of somatic ACE.

[1] Natesh R., Schwager S., Sturrock E., Acharya K., *Nature*, 2003, **421**, 551.

Keywords: glycoproteins, metalloprotein structures, drug targets

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Structural Basis for Potent Inhibition of COX by Resveratrol-A Natural Product in Wine

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Non-steroidal antiinflammatory drugs block the cyclooxygenase activity of prostaglandin-H synthase, also known as cyclooxygenase (COX), the enzyme that mediates biosynthesis of eicosanoids from arachidonic acid. Two enzyme isoforms have been identified: COX-1 which is constitutively expressed, and COX-2, which is inducible. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a phytoalexin found predominantly in grapes and it has both antiinflammatory and cancer chemopreventive activity. One of the mechanisms of action of resveratrol is believed to be mediated through potent inhibition of COX-1 and COX-2 activity. We have determined the x-ray structure of COX-1 co-crystallized with resveratrol to 2.9 Å resolution using synchrotron radiation (BioCARS beamline 14-BM-C) to determine the binding mode of resveratrol in the active site. Using the crystal structures of COX-1/resveratrol and COX-2/flurbiprofen complexes, we performed computational docking studies of resveratrol and its two (3- and 4'-) sulfate metabolites using Dock 4.0.1. Our results indicate that the computed free energy values of binding for each of the docked resveratrol analogs are commensurate with their experimentally determined inhibition constants (K_i). However, the computational modeling results were unable to predict the selectivity in binding of resveratrol and its metabolites to the two enzymatic isoforms most probably due to the slight differences in binding affinities of these molecules for COX-1 or COX-2. This research is funded by grants from the National Cancer Institute (NIH: R03 CA92744-02 and 5 P01 CA48112-10).

Keywords: resveratrol, cyclooxygenase, docking

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Structural Studies of Human α−thrombin

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Thrombin is a member of the serine proteinase family. The structure consists of two chains. Thrombin plays an important role in the coagulation of blood; contact with fibrinogen results in the formation of fibrin that polymerize into a blood clot.

X-ray data have been measured at ESRF ID 14.4, SRS MPW10 and at APS SBC. The four X-ray diffraction data sets achieved thus far are between 1.26 and 1.4 Å based on $\langle F/\sigma(F)\rangle > 2$ and completeness > 50%, two bound with hirugen only, and two with an inhibitor as well. We wish to identify hydrogen atoms at the active site and other key water hydrogens that are involved in the cleavage of fibrinogen.

In addition we wish to use neutron crystallography because the scattering factor for neutrons of deuterium equals that of C, N and O. We have so far grown a large (0.7x0.7x~0.3mm) thrombin crystal. Tests of the diffraction on the ILL LAue DIffractometer are imminent.

Overall, we plan to understand better how inhibitors bind to thrombin, so as to design enhanced drugs.

Keywords: thrombin, hydrogens, neutron diffraction

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Active Structure of FR901451, a Potent Macrocyclic Elastase Inhibitor

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Bacteria produce a lot of bioactive and structurally unimaginable compounds. Among them, FR901451 from *Flexibaccter sp.* No.758 is known to have large tri-macrocyclic structure and to inhibit porcine pancreatic elastase, which in turn resembles the attractive drug target leukocyte elastase [1]. The crystal structure of FR901451 as bound to pancreatic elastase was solved at 2.5 Å resolution. The inhibitor occupies the most prominent subsites S1' to S3 of the elastase and prevents a hydrolytic attack by covering the active center with its rigid ring structure. The observed binding structure may help to design potent elastase inhibitors.

[1] Fujita T., Hatanaka H., Hayashi K., Shigematsu N., Takase S., Okamoto M., OkuharaM., *J. Antibiotics*, 1993, **47**, 1359.

Keywords: elastase inhibitor, macrocyclic compound, protein-inhibitor complex

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Structure of Pteridine reductase (PTR1) from *Trypanosoma brucei*

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Anti-folate resistance in the trypanosomatid parasites is due in part to pterin reductase (PTR1) which is capable of reducing folate. This allows uptake of folate even when the primary enzyme, dihydrofolate reductase, is inhibited, and makes PTR1 an important drug target. The crystal structure of PTR1 from *Trypanosoma brucei* complexed with the cofactor NADPH and the inhibitor methotrexate has been determined to 2.2 Å. The protein structure is closely related to the previously determined *L. major* structure [1], with the cofactor and inhibitor bound in a similar fashion. The methotrexate molecule is significantly better defined in the *T. brucei* structure but there is no indication of increased MTX – protein interaction. A non-conservative Leu-Cys substition close to the active side is observed.

[1] Gourley D.G., et al., *Nature Str. Biol*., 2000, **8**, 521-525.

Keywords: enzyme inhibitor design, biological macromolecules, folate dependent enzymes

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Crystallography as a Tool to Identify the Best Inhibitor in a Complex Mixture

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In this study we crystallized the HIV-1 aspartic protease using an equimolar mixture of four stereoisomeric inhibitors. Fourier maps obtained by high resolution diffraction data (up to 1.3 Å) from