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

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.

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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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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

P4

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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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