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