sample manipulation tasks required in routine crystal screening and data collection. Supplemental ACTOR software for ranking, data collection strategy determination and image processing further increases the value of ACTOR by way of an intelligent framework for automated X-ray diffraction experiments. More recent ACTOR developments incorporate upstream tools used during sample harvesting as well as state of the art sample tracking mechanisms. Additionally, ACTOR software expands downstream to automate structure solution using both 'bind and grind' and SAD methods. Altogether these additions function to enhance and expand the protein crystallographer's toolkit. In this paper we will describe these new hardware and software developments and evaluate their efficacy and reliability for high throughput crystallography.

Keywords: automated data collection, protein crystallography, robotics

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Crystal structure of quinone reductase 2 in complex with the selective inhibitor 5-hydroxyflavone

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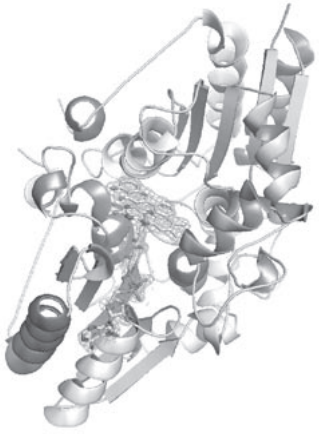
Flavonoids, which are obtained from the diet, are generally considered selective inhibitors for the flavo-enzyme quinone reductase 2 (NQO2). Several studies have reported the therapeutic importance of NQO2 as a target for anti-cancer and anti-malarial agents. Here, the crystal structure of NQO2 in complex with 5-hydroxyflavone is reported. This crystal structure complex resolved at 1.87 Angstrom atomic resolution has provided an explanation of the selective inhibition activity of this particular compound towards NQO2. Binding interactions created by 5-hydroxyflavone with amino acids of NQO2 binding site is solely influenced by the chemical structure of this compound. These interactions enforce the molecule to adopt a certain binding mode which is responsible to exert the compound inhibitory activity towards NQO2. Superimposition studies can be carried out with other structure complexes of NQO2 such as NQO2 − resveratrol crystal structure. This can be exploited for future drug design studies with NQO2.



Keywords: crystal structure, NQO2 inhibitor, 5-hydroxyflavone

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A structural studies of free methionine-(R)-sulfoxide reductase from staphylcoccus aureus

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The reduction of methionine sulfoxide (MetO) is mediated by methionine sulfoxide reductases (Msr). The MsrA(E.C.1.8.4.11), msrB(E.C.1.8.4.12) families can reduce free MetO and MetO within a peptide or protein context. This process is stereospecific with the S- and R-forms of MetO repaired by MsrA and MsrB, respectively. Cell extracts from an MsrA-B- knockout of Escherichia coli have several remaining Msr activities. It has identified an enzyme specific for the free form of Met-(R)-O, fRMsr, through proteomic analysis. The recombinant enzyme exhibits the same substrate specificity and is as active as MsrA family members. The crystal structure of E.coli fRMsr was previously determined. In this study, The X-ray diffraction data of Staphylcoccus aureus fRMsr with DMSO were collected to 1.9Å resolution using synchrotron radiation. The crystals belonged to the hexagonal space group $P6_122$ with cell parameters of $a = b = 89.451$, $c = 88.768$ Å, and $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$. The asymmetric unit contained one molecules of fRMsr, giving a crystal volume per protein mass (Vm) of 0.4 $Å^3$ Da^{-1} and a solvent content of 52.13%. The crystal structure of fRMsr from Staphylcoccus aureus was determined by molecular replacement. The refined model of fRMsr gave *R*factor and *R*free values of 21% and 23%. The structural similarity of the E.coli and Staphylcoccus aureus proteins suggests that most fRMsrs use three cysteine residues for catalysis and formation of a disulfide bond to enclose a small active site cavity.

Keywords: methionine sulfoxide, free methionine-(R)-sulfoxide reductase, asymmetric unit

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New insight into catalytic mechanism of serine proteases from ultra-high resolution X-ray studies

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Serine proteases have been the subject of intensive studies for many years and many aspects of the mechanism of their action have already been revealed. Yet a number of questions still remains, especially concerning hydrogen bonds within the catalytic triad. One of them focuses on the exact nature of the bond between the catalytic histidine and aspartate. Also the role of a conserved hydrogen bond formed by one of the histidine's carbon atoms is unresolved. Ultra-high resolution X-ray structures of alcalase® - a naturally occurring variant of subtilisin Carlsberg - in complexes with chymotrypsin inhibitor 2 (CI-2) wild type and its reactive site mutant M59P, which binds to the enzyme with 10^{-4} lower affinity, have been obtained (at 100K) and analysed. Additionally, an atomic resolution structure of alcalase with native CI-2 at physiologically relevant temperature is discussed. Having structures representing different snapshots of the enzymatic reaction is a standard approach for gaining insight into the catalytic mechanism. When the standard uncertainty of the coordinates is low, as it is at 1Å resolution, even small structural changes can be reliably measured. Such resolution also allows for direct observation of hydrogen atoms in the electron density. A great benefit comes from the possibility to compare their positions

at different temperatures which gives information on the biological relevance of the protein structures solved at cryogenic conditions.

Keywords: serine protease, reaction mechanisms, high-resolution protein structures

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Electrochemically assisted protein crystallization. Applications to protein crystallography

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The electrochemically-assisted crystallization of non-purified (commercial purity) cytochrome c was successfully achieved inside of small cell of a dynamic light scattering (DLS) apparatus. The method combined batch crystallization conditions and an internal electrical field to favor the nucleation stage. This methodology crystallizes commercial cytochrome c without previous isoforms separation, decreasing costs and experimental time to obtain crystals. The effect of the electric field on the aggregation time and on the protein nucleation was observed in real time by means of dynamic light scattering methods. The results showed a marked decrease of the crystallization time (from 45 days to 5 days) highly improving the previous reported method of crystallization. The HPLC signal of re-dissolved crystals of these protein crystals showed that the protein corresponds to the same isoform previously crystallized by micro-seeding methods. The excellent crystal quality of the cytochrome c crystals obtained in the presence of electrical current was confirmed by protein X-ray crystallography reaching 1 angstrom of resolution.

Keywords: cytochrome c, protein electrocrystallization, high-resolution protein crystallography

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Invariom refinement of 5 K 0.66 Å data of the ethanol solvate of gramicidin A

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It is now 20 years since the first structure of Gramicidin D has been reported [1]. Wild type Gramicidin D from bacillus brevis is a mixture of three peptides (Gramicidin A, B and C) each consisting of 15 residues that differ only at position 1 and 11. Gramicidin exhibits antibiotic activity against Gram-positive species by forming ion channels through cell membranes that preferably transport Na^+/K^+. Various solvate structures and ion-complexes of Gramicidin are known to date, as summarized recently [2]. We have re-examined the original ethanol solvate to illustrate the benefits of ultra-high resolution in protein crystallography. To minimize rotational disorder and to maximize resolution we collected data on purified Gramicidin A at a temperature of 5 K at the 3rd generation synchrotron SLS in Switzerland. The resulting Bragg data to 0.66 Angstrom resolution were evaluated with the non-spherical scattering factors of the invariom database [3,4] which is based on the Hansen-Coppens variation of the rigid pseudoatom formalism. Single crystal diffraction data evaluated this way provide a wealth of detailed and accurate information on structure, geometry and electron-density derived properties. For Gramicidin and its biological function an analysis of the electrostatic potential [5] is especially relevant in this respect.

[1] Langs, A.D., Science (1988), 241, 188-191.
[2] Olczak, A., Glowka, M.L., Szczesio, M., Bojarska, J., Duax, W.L., Burkhart, B.M., Wawrzak, Z., Acta Cryst. D63, (2007), 319-327.
[3] Dittrich, B., Koritsanszky, T., Luger, P., Angew. Chem. Int. Ed., (2004), 43, 2718-2721.
[4] Dittrich, B., Huebschle, C.B., Luger, P., Spackman, M.A., Acta Cryst D62, (2006), 1325-1335.
[5] Politzer, P., Murray, J.S. & Preralta-Inga, Z., Int. J. Quant. Chem. (2001), 85, 676-684.

Keywords: charge density, X-ray structure of membrane proteins, synchrotron radiation

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Ultra-high resolution and very cold structure of lysozyme

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Extension of data resolution from high to atomic level reveals a wealth of information about the intricate details of protein structures, important for discussing their chemical and biological behavior or their potential to interact with other molecules. The triclinic form of hen egg-white lysozyme is an example of protein crystals able to diffract beyond the ultra-high resolution limit of 0.8 Å. The ultra-high resolution data measured from these crystals at extremely low temperature of 16 K allowed us to obtain a very accurate model of the molecule. About half of the whole structure displays multiple conformations of the main and side chains. Electron densities for hydrogen atoms and bonding electrons are apparent in many fragments as well as strong indications about protonation states of potentially charged groups. Several discrepancies from the library of geometrical parameters are suggestive for reevaluation of some of such library targets. The structural model will be compared in detail with several available structures of lysozyme obtained with different data resolution limits and temperatures.

Keywords: lysozyme, ultra-high resolution, ultra-low temperature

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Crystal structure of fully oxidized human thioredoxin1 containing disulfide between Cys62 and Cys69

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