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Stistaite, an extension of the concept of solid solutions

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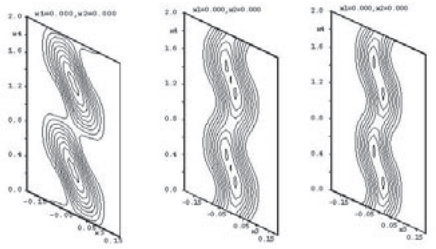
The diffraction patterns from single crystals from Stistaite are dominated by a set of strong reflections that indicate a rhombohedral distortion of a simple cubic lattice. Closer inspection reveals a set of satellites that indicate an incommensurate modulation. The structure of elemental Sb is a simple cubic pattern trigonally distorted by the formation of alternating long and short distances between layers along a cubic <111> direction to yield three long and three short bonds for each Sb atom. The unit cell of elemental Sb is doubled along the trigonal c-axis because of the alternation of interplanar distances. Formally, this may be interpreted as a q-vector of (0 0 1.5) in the rhombohedral unit cell (hexagonal setting). For stistaite, the range of the q-vector is 1.38-1.27 for the composition range 35-55% Sn. The alternating layers of elemental Sb can be interpreted as a saw-tooth like modulation, and for stistaites with a low Sn content, this is largely retained, although the discontinuous portion of the atomic modulation function is smoothened into a sinusoidal region. This corresponds to a part of the structure where Sn and Sb layers alternate with a regular repeat distance.



Modulation functions for Sb (left) stistaite $Sb_{65}Sn_{35}$ (middle) and $Sb_{45}Sn_{55}$ (left)

Keywords: modulated structure, solid solution, stistaite

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Temperature dependence of the modulations in $KNbOB_2O_5$ and $RbNbOB_2O_5$

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$KNbOB_2O_5$ and $RbNbOB_2O_5$ are both members of a family of non-centrosymmetric oxo pyroborates, $AMOB_2O_5$ (A = K, Rb, Cs, Tl; *M* = Nb, Ta) [1-3]. These have attracted considerable interest owing to their potential use as non-linear optical materials. If one considers the structure of $CsNbOB_2O_5$ [4] as the underlying average structure, it is then possible to describe the structures of all other members of that family as modulated variants thereof. The structures of, *e.g*., $KNbOB_2O_5$ [1] and $RbNbOB_2O_5$ [2] have been refined using a super space approach. The structure of $RbNbOB_2O_5$ is incommensurately modulated, despite the apparent value of the modulation wave vector of 2/5 **b*** exactly, while the structure of $KNbOB_2O_5$ refined significantly better as commensurate modulated structure. Using metrics alone it is not straightforward to determine whether a structure is commensurately or incommensurately modulated, however, a variability of the magnitude of the modulation wave vector with composition (*e.g*. for solid solutions) or temperature may be used to resolve the ambiguity. Variable temperature X-ray powder diffraction data were collected for both $KNbOB_2O_5$ and $RbNbOB_2O_5$ at the Australian National Beamline Facility, Photon Factory, Tsukuba, Japan. Diffraction patterns were collected at RT and from 423 K to 1073 K in 25 K steps. The results of the analysis of these data, which supports the previously suggested reason for the modulation, will be presented here.

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Keywords: modulated structure, powder diffraction, temperature dependence

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X-ray structural analysis and biophysical assays in drug discovery

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Examples of the combined use of biophysical assays and X-ray structures of target complexes for drug discovery will be presented.

Keywords: Biacore, drug discovery, ITC

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Studies of protein-protein and protein-RNA complexes by mass spectrometry

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Electrospray ionization mass spectrometry (ESI-MS) enables the determination of not only the molecular sizes of non-covalent macromolecular complexes, but also the binding affinities. This paper shows the advantages of ESI-MS over other techniques in structural studies of biological macromolecular complexes, using as an example our recent analysis of TRAP, trp RNA-binding attenuation protein, and its complexes with RNA and protein ligands. TRAP and its regulator anti-TRAP protein (AT) play the principal roles in controlling tryptophan synthesis in *Bacillus* species. We have characterized both wild-type (wt) and mutant TRAP from *B. stearothermophilus*, and their complexes with RNA or AT by ESI-MS. Wild-type TRAP forms homo-11mer rings. The mutant used carries three copies of the TRAP monomer on a single polypeptide chain, so that it associates to form a 12mer ring with four polypeptides. Mass spectra showed that both the wt TRAP 11mer and the mutant TRAP 12mer can bind a cognate single-stranded RNA. The crystal structure of wt TRAP complexed with AT shows a TRAP 12mer ring surrounded by six AT trimers. However, ESI-MS of wild-type TRAP mixed with AT shows four species with different binding stoichiometries, and the complex observed by crystallography

represents only a minor species in solution; most of the TRAP remains in an 11mer ring form. Mass spectra of mutant TRAP showed only a single species, TRAP 12mer + six copies of AT trimer, which is observed by crystallography. The crystal structure of the TRAP-AT complexes shows that only a 12mer TRAP ring can bind with six copies of AT trimer. These results suggest that crystallization selects only the most symmetrical TRAP-AT complex from the solution, while ESI-MS can take a "snapshot" of all the species in solution.

Keywords: mass spectrometry, transcription regulation, protein interactions

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Characterising protein-ligand binding in support of structure-based drug discovery

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The main stages in drug discovery research are (a). the identification of small molecules that bind to the target (hit id), (b). characterisation of how the hits bind to the target, developing ideas for how the hits may be improved (hits to leads) and (c). the optimisation of the hits to have suitable drug-like properties (lead optimisation). Over the past ten years, the determination of protein structures by X-ray crystallographic methods has had an increasing impact on all three of these stages. Such crystal structures provide exquisite detail on the binding site and mode of interaction. However, this is a necessarily static image of the protein-ligand interaction. It can also take some effort to obtain a suitable protein-ligand complex, so crystallography is not a routine method for compound screening. A series of complementary biophysical methods based on NMR spectroscopy or Surface Plasmon Resonance (SPR) have been developed to support drug discovery These include screening of compounds (including low molecular weight fragments) using SPR or NMR, locating the binding site (and sometimes orientation of binding) of ligands using NMR and characterising the kinetics (on and off rates) of binding using SPR. In this presentation, we will discuss the use of a range of biophysical methods in support of drug discovery research. This will include examples of fragment screening, binding to large protein-protein interaction targets and using on and off rates to rationalise the different in vivo properties of two structurally similar compound series.

Keywords: drug discovery, NMR spectroscopy, surface plasmon resonance

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Mechanism of the cofactor-less urate oxidase: X-ray structures with molecular oxygen or cyanide.

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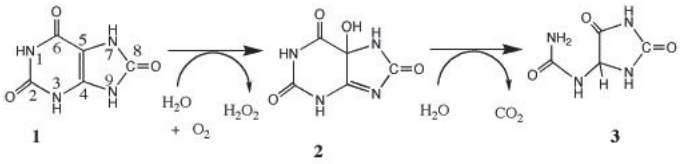
Urate oxidase (UOX) catalytic mechanism is intriguing, since this enzyme catalyzes in presence of molecular oxygen the hydroxylation of ucic acid (1) to 5-hydroxyisourate (5-HIU) (2), with no cofactor or metal ion. 5-HIU is further enzymatically transformed to S-allantoin (3). The X-ray structure of UOX with a competitive inhibitor under pure oxygen pressure has shown that the location of the molecular oxygen advocates for a hydroperoxyisourate intermediate. The X-ray structure of UOX with its natural substrate, uric acid, in presence of cyanide which inhibits the reaction, shows that the substrate, is modified by the enzyme to give the dianion N3- N7-. In this structure, the observed intermediate is likely to be dehydrourate. The different X-ray structures of UOX with a uric acid like inhibitor evidences a water molecule which is likely to be the activated water molecule that abstracts a proton from the urate monoanion. This water molecule will later hydroxylates the dehydrourate intermediate to yield the final product, 5-HIU. A second water molecule hydrogen-bonded to the substrate is also implicated in the reprotonation of the dehydrourate intermediate, leading to 5-HIU.



Keywords: urate oxidase, oxygen pressure, cyanide

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Flavopiridol binding to P-TEFb (CDK9/cyclin T1)

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Flavopiridol is currently in Phase II clinical trials for the treatment of chronic lymphocytic leukemia, the most common of the leukemias. Flavopiridol, derived from a compound originally isolated from the bark of a tree Dysoxylum binectiferum that grows in East Asia, was recognised as having anti-cancer properties as long ago as 1992. It appeared to be a non-specific inhibitor of the cyclin dependent protein kinases (CDKs). More recent work has demonstrated that flavopiridol is a potent inhibitor of P-TEFb, CDK9/cyclin T1, with Ki 3 nM, >10 more potent than its activity against other CDKs. CDK9 regulates transcription through modifications of transcriptional repressors and the C-terminal tail of RNA polymerase. Flavopiridol inhibits transcription therby leading to a decrease in the mRNA levels of many proteins involved in growth and signal transduction that have short lifetimes, including several antiapoptotic proteins. Following improvements in adminstration protocols to overcome scavenging by serum, flavopiridol has given encouraging results in clinical trials. We have recently solved the structure of CDK9/cyclin T1 in complex with flavopiridol. Flavopiridol binds to the ATP site of CDK9 and induces unanticipated structural rearrangements in the glycine rich loop of the protein kinase that bury the inhibitor. These provide a rationale for the strong inhibition of CDK9. Comparison of the mode of action with those for other protein kinase inhibitors will be discussed.

Keywords: flavopiridol, CDK9, protein kinase structure