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Progress in many biologically important projects is hindered by the inability to grow large homogeneous crystals of macromolecules and their complexes. Useful data can be collected from 10-mm or smaller crystals when background is reduced by matching the beam size to the size of the sample crystal. An inexpensive apparatus was implemented for delivering high-flux-density, user-selectable, 5-mm and 10-mm beams to the sample. The device is based on overfilling an aperture placed 30 mm upstream of the sample. The aperture is held within a set of nested tubes that act as downstream and upstream scatter guards. The mini-beam apparatus was integrated in the user program on the 23-ID dual canted undulator beamlines of GM/CA CAT at the APS, and its benefits for both large and small crystals were demonstrated (Sanishvili et al., 2008). The advantages of small beams for small crystals are undisputed; quantitative results will be presented. Small beams also offer many advantages for large, inhomogeneous samples. For example, small beams may reduce refined crystal mosaicities; they can dramatically improve diffraction quality when large beams lead to smeared and/or irregularly shaped spots. Operational simplicity and interchangeability with larger beams allows small beams to be used as probes to identify optimal regions of a crystal for data collection with a larger beam.

Sanishvili, R., Nagarajan, V., Yoder, D., Becker, M., Xu, S., Corcoran, S., Akey, D. L., Smith, J. L., and Fischetti, R. F. (2008). Acta Crystallogr D64, 425-35.

Keywords: micro-crystals, micro-beam, radiation damage

MS.15.2

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Recent developments and success on ID23-2, at the ESRF

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The ESRF ID23-2 beam line is a microfocus beam line fully dedicated for studying macromolecular microcrystals. It is a fixed wavelength beam line using a single bounce Si[111] monochromator; the beam is focused down to 7.5 µm horizontally by 5 µm vertically (FWHM) by Pt-coated silicon mirrors in a Kirkpatrick-Baez (KB) geometry. The experimental setup is composed of a MD2m diffractometer (from MAATEL, under an EMBL patent), a SC3 sample changer [1] and a MarMOSAIC 225 CCD detector. The main challenge for ID23-2 was to provide to the MX user community a beam line with a beam size smaller than 10 µm in diameter while keeping the same "easy-to-use" environment and reliability as the other ESRF MX beam lines (ID14, ID23-1, ID29, BM14) [2]. The beam line has been open to the user community since mid-November 2005. The first year of user operation has demonstrated that the design was basically sound and that the beamline can be used by inexperienced users. Instrumentation developments, data collection strategies and some user results will be presented and discussed.

[1] Cipriani et al. Acta Cryst. (2006) D62, 1251-1259.
[2]http://www.esrf.fr/UsersAndScience/Experiments/MX/About_our_beamlines

Keywords: microcrystals, synchrotron X-ray instrumentation, synchrotron structural biology research

MS.15.3

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Microbeam studies of insect virus polyhedra, infectious protein crystals containing virus particles

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Cypovirus and baculovirus are two unrelated types of insect virus that both produce very unusual infective particles - stable micron sized protein crystals called polyhedra that can remain infective in soil for years. Polyhedra form inside infected larval cells and consist of a body centred cubic lattice of 28kD viral polyhedrin protein molecules. How the growing crystals selectively incorporate virus particles from the complex intracellular 'soup' is intriguing. Cypovirus polyhedra form in the cytoplasm and baculovirus polyhedra form in the nucleus. The amino acid sequences of the corresponding polyhedrin molecules have no evident homology. Despite these differences the unit cells of the two polyhedra have nearly identical 103Å cell dimensions. Polyhedra are unusually stable and easily obtained protein crystals and may in future provide an interesting platform for protein engineering. We are interested in these possibilities and in understanding the structural biology of these unique viral structures. Using samples provided mainly by Hajime Mori at the Kyoto Institute of Technology, we have been collecting micro-beam X-ray diffraction data from viral polyhedra since 2004 in collaboration with Clemens Schulze-Briese at the Swiss Light Source. The ~2Å resolution atomic structure of cypovirus was obtained in 2006 using MIR methods. The talk will summarise the project.

Keywords: cypovirus, polyhedrin, micro-crystallography

MS.15.4

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A new beamline to achieve protein micro-crystallography at SPring-8

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BL32XU, a new undulator beamline at SPring-8 for protein micro-crystallography, is being under construction and will be operated for the National Project from 2010 in Japan. Recently, users' demands for high-quality data collection from protein micro-crystals are increasing as target proteins get large and difficult to be crystallized. Achieving the objective requires very high signal-to-noise ration of diffraction spots from a sample crystal. Spatially brilliant and small beam, of the order of a few or several micro-meters, is proved to achieve the objective at several synchrotron radiation facilities. We designed a micro-focus beamline, BL32XU at SPring-8, which provides dense and micro-sized X-rays. A hybrid in-vacuum undulator developed at SPring-8 will be equipped. X-rays

from the device is monochromatized with liquid-N_2 cooled double crystal monochromator, and will be focused by using K-B mirrors fabricated with Elastic Emission Machinery. Ray trace calculation with the designed configurations shows achievable beam size at sample position corresponds to 1 x 2 μm^2 with 10^{10} phtons/sec. Beam size is designed to be changeable from about 1~25 μm^2 according to designed experiments. The new beamline will largely benefits users by cutting off their wasting time to optimize crystallization conditions especially for smaller and lower quality crystals. The beamline will provide high quality diffraction datasets from micro crystals. Besides, users will be able to probe single-crystal volumes from a heterogeneous protein crystal using the micro-beam. Designed optics and instrumentations to be equipped such as an automated sample changer, advanced software to avoid serious radiation damages and so on will be also presented.

Keywords: protein crystallography, synchrotron X-ray diffraction, synchrotrons

MS.15.5

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Microcrystallography at Diamond: Facilities for crystal optimization and structure determination

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The I24 microfocus macromolecular crystallography beamline at the Diamond Light Source comes into operation in 2008. The beamline is tuneable from 6.5 - 25 keV and offers versatility in beam size and shape at both sample position and detector position by utlizing a two-stage demagnification incorporating a movable final Kirkpatrick-Baez mirror pair. The beamline incorporates a CATS sample mounting robot that will also enable diffraction screening of crystallization conditions in 96 well plates. This facility will provide invaluable feedback for the crystallization efforts in the Wellcome Trust funded Membrane Protein Laboratory at Diamond. Significant design and build effort has been put into versatility, stability and the generation of a low background sample environment for the measurement of diffraction data. The design concepts of I24 will be described and preliminary results from beamline commissioning will be presented.

Keywords: synchrotron X-ray instrumentation, microcrystals, biological macromolecular crystallography

MS.16.1

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Fragment-based drug discovery: From crystal to clinic

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Fragment-based screening is a powerful method for the identification of attractive chemistry start points against specific drug targets. These initial low molecular weight fragment hits typically have uM to mM potency but are shown to be highly ligand efficient. Astex Therapeutics uses its proprietary platform, PyramidTM, utilising high-throughput X-ray crystallography and other biophysical techniques, to identify high quality fragment hits against a broad range of therapeutic targets. The availability of structural information from the screening phase provides a detailed map of the active and secondary fragment binding sites allowing the chemist to design molecules that maximise interaction with the protein target. Rapid progress can be achieving reducing the time taken to identify a clinical candidate by many years. To highlight the success of our approach, AT7519 (a CDK inhibitor) and AT9283 (Aurora inhibitor), which are both currently in Phase I clinical trials will be discussed together with another non-kinase oncology target, HSP90.

Keywords: structure-based drug design, anticancer drugs, X-ray crystallography

MS.16.2

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Role of structures in designing anti-AIDS drugs targeting reverse transcriptase

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HIV-1 reverse transcriptase (RT) is the target for almost half of the approved anti-AIDS drugs. The non-nucleoside (NNRTI) drugs bind RT at an allosteric pocket whereas the nucleoside (NRTI) drugs compete with nucleotides and act as DNA chain terminators. Both NRTIs and NNRTIs are challenged by emergence of drug resistance mutations in RT. Understanding the roles of mutations is important in designing effective drugs. A systematic structure based design of diarylpyrimidine (DAPY) NNRTIs, including the recently approved drug TMC125 (Intelence/etravirine), has revealed that adding strategic flexibility to a drug molecule can help overcome the effects of resistance mutations by reorienting (wiggling) and repositioning (jiggling) in the binding pockets. Our recent high resolution (1.8 Å) crystal structures of wild-type and mutant RT/TMC278 (riplivirine) complexes demonstrate how the DAPY NNRTI TMC278 wiggles and jiggles to fit into the pockets of wild-type, and L100I+K103N and K103N+Y181C mutant RTs. Mechanisms of NRTI resistance are highly complex and structurally distributed over broad areas of RT. The NRTI resistance mutations that discriminate a nucleoside analog from its corresponding nucleotide can occur at steps involving binding to RT, catalytic reaction of polymerization, translocation of nucleic acid after incorporation, and/or through excision. Our current structures of NRTI resistant mutant RT/DNA/dNTP (and analog) complexes help in understanding complementary clinical and biochemical data, and the combined insights will help in developing more effective drug combinations in the clinic and also in designing new and improved NRTIs.

Keywords: HIV, drug resistance, structural flexibility

MS.16.3

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Monoamine oxidases and LSD1: Similar chemistry for neurotransmitter and chromatin modification

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