

FA5-MS38-T01

Monitoring the preparation of protein derivative crystals via Raman-microscopy. Alessandro Vergara.
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Raman microscopy is having increasing applications into molecular biology. Primary structure features can be analyzed, such as Se-Met labeled protein crystals to be used for MAD crystallography [1] or to check heavy metal incorporation in isomorphous derivative crystals. Secondary structure modification can be clearly identified according to relationships between amide band frequencies and Ramachandran angles [2]. Ligand binding [3] and protein dynamics/reactivity can also be efficiently followed. The combined Raman and X-ray crystallography approach reinforces the interpretation of biophysical data. In particular, reactivity and dynamics of metal proteins have been investigated. This approach is particularly informative and well developed for hemoproteins [4, 5].

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Keywords: metal protein, ligand soaking, secondary structure

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Practical variations on the Microseed Matrix Screening (MMS) method in protein crystallisation. Patrick Shaw Stewart, Peter F.M. Baldock, Richard A. Briggs, Stefan A. Kolek. *Opticryst*, see <http://www.opticryst.org>.
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Traditionally, microseeding has been used as an optimization step, i.e. seed crystals are dispensed or transferred into variations of the original crystallization hit solution [e.g. 1, 2]. A novel, systematic approach, referred to as the Microseed Matrix Screening (MMS) method, was introduced by Ireton and Stoddard [3]. This method was automated and further improved by D'Arcy et al.[4], who first used seeding with commercial random screening kits. Experience has shown that MMS used in this way not only produces more hits, it also generates better-diffracting crystals because crystals are more likely to grow in the metastable zone [5]. Systematic users of the method report that it gives a useful improvement in about 75% of cases [6].

Douglas Instruments - as a member of the Opticryst consortium - has investigated several variations in the method. In real-life projects, new crystallization conditions are often sought in an effort to reduce twinning, improve diffraction resolution, find crystals with new space groups etc. Moreover, it is desirable to reduce the number of salt crystals that typically arise from combining the hit solution with all of the

various solutions in a screen. We therefore investigated suspending the seed crystals in alternative solutions (i.e. replacing of the original hit solution, which D'Arcy et al. used). Solutions investigated included protein solution ("preseeding the protein stock"), ammonium sulphate, PEG, NaCl and ethanol. The original hit solution was found to be slightly more effective, but several other solutions also worked well. Note that these alternative solutions reduce the likelihood of crystallization by an additive effect, so they can be used when the original conditions give poor diffraction (especially PEG or NaCl, which are less likely to give salt crystals). Throughout the project, the statistical significance of experiments carried out was increased by focusing on "pregnant" conditions, which were defined as conditions that reliably gave crystals when seeds were present, but which otherwise did not support crystallization.

The stability of seeds in various solutions at room temperature was also investigated. When using the original hit solution (see above), up to 75% of seeds were lost after 3 hours, and 99% after 24 hours.

Obviously the MMS method has the disadvantage that it cannot be used until at least one hit has been obtained. We therefore investigated nucleation with micro-porous glass [7] and zeolites [8]. These materials were less effective than microseeding, but may nevertheless be useful - because they can of course be used in the absence of previous hits.

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Keywords: Crystallization of proteins

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Single Crystal X-ray Diffraction in Laser-Heated Diamond Anvil Cells. L. Dubrovinsky^a, N. Dubrovinskaia^{b,c}, K. Glazyrin^a, M. Merlini^d, M. Hanfland^e, T. Pippenger^b. ^a*Bayerisches Geoinstitut, Universität Bayreuth, Germany.* ^b*Mineralphysik, Institut für Geowissenschaften, Universität Heidelberg, Germany.* ^c*Lehrstuhl für Kristallographie, Physikalisches Institut, Universität Bayreuth, Germany.* ^d*ESRF, Grenoble, France.* ^e*Dipartimento di Scienze della Terra, Università degli Studi di Milano Via Botticelli, Italy.*
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Diamond anvil cell technique is the most successful method of pressure generation capable for working in the multimegabar pressure range. However, there are still a number of problems related to high-temperature experiments in DACs. Laser heating techniques cover a wide P-T field: P>200 GPa, T=1300-5000 K. A sample preparation for laser-heating experiments is relatively easy and there is practically no risk for the diamonds due to heating. However, so far all existing DAC laser-heating systems are stationary, they are linked either to certain equipment (an optical or Raman spectrometer, for example) or a beam-line, and do not allow rotating a cell

during heating with respect to the incident X-ray beam. Studies of crystal structures require mobility of the laser-heating system. We have developed a portable laser heating system for DACs suitable for the single crystal diffraction experiments. In order to test application of the portable laser heating system for single crystal diffraction studies at ID09a we investigated high-P,T behavior of tungsten single crystals up to 40 GPa and 2500 K and silicate perovskite (Mg,Fe)(Si,Al)O₃ at pressures over 75 GPa and 2700 K. The body text font is Times New Roman (size 9) with single spacing and full justification. The total allowed area is 8 x 24 cm.

Keywords: single crystals, high-pressure crystallography, high-temperature X-ray diffraction

FA5-MS38-T04

Progress in Using Short Wavelength Radiation for Chemical Crystallography. Jürgen Graf^a, Bernd Hasse^a, Francesca Fabbiani^b, Thomas Schulz^c, Dietmar Stalke^c, Holger Ott^d, Carsten Michaelsen^a. ^a*Incoatec GmbH, Geesthacht, Germany.* ^b*GWZ, University of Göttingen, Germany.* ^c*IAC, University of Göttingen, Germany.* ^d*Bruker AXS GmbH, Germany.*
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Combining synthetic multilayer mirrors with microfocus X-ray sources has become a standard with in-house X-ray sources for single crystal diffraction as well as a number of applications in powder diffraction. The maximum angle of incidence at which a multilayer mirror reflects is significantly smaller for higher energy radiation, such as Mo-K_α or Ag-K_α radiation than it is for Cu-K_α radiation. This is why synthetic multilayer mirrors traditionally have been used for Cu-K_α radiation or softer wavelengths. Modern deposition technology, however, allows for the reproducible production of high quality multilayer mirrors with smaller *d*-spacing. In consequence, these mirrors reflect higher energy radiation at larger angles of incidence. Combined with the latest generation of microfocus sealed tubes this provides new high-performance low-power X-ray sources for shorter wavelengths.

We will present selected results on the use of these low-power consumption, high-performance sources for Mo-K_α and Ag-K_α radiation in structural chemistry and high-pressure crystallography.

Keywords: instrumentation, high-pressure X-ray diffraction, chemical crystallography

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X-ray Diffraction from Crystals in Crystallization Drops. Tadeusz Skarzynski. *Oxford Diffraction, Yarnton, Oxfordshire, United Kingdom.*
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Characterization of protein crystals with X-rays, in-situ, without needing to extract them from the crystallization plate allows establishing a “base line” for crystal quality, and evaluating resolution limits before crystals are subjected to any manipulation. The in-situ testing also allows quickly distinguishing between salt and protein crystals, test

harvesting, soaking and cryoprotectant conditions and selecting the best crystals for data collection.

We will show how the in-situ testing, both at synchrotron beam lines and using the Oxford Diffraction PX Scanner system for home labs can be used as a powerful tool providing valuable feedback at all stages of macromolecule crystallization. Several examples of significant variation of diffraction properties of crystals grown from the same conditions will be illustrated and discussed highlighting the importance of critical assessment of crystal quality at room temperature. We will show results from crystals grown under oil and in capillary-based devices. Initial trials of using the in-situ diffraction to detect ligand and heavy-atom binding will be presented as well.

Keywords: crystallization process, X-ray methods, in-situ diffraction