the method to be applied in both crystallography and electron microscopy at resolutions as low as 50 Å.

Additional stereochemical restraints have a modest positive impact in medium resolution crystallographic structures. A hydrogen-bonding restraint that is directionally targeted towards either dipolar or lone-pair interactions is beneficial, in contrast to prior attempts that optimized only dipolar effects. Minimization of the electrostatic potential energy is also beneficial, and more so when calculated by continuum methods rather than by the Coulombic methods previously used. This has been accomplished by combining refinement with numerical solution of the Poisson-Boltzmann equation.

Keywords: refinement, restraint, electrostatic potential

MS90.30.5

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***elNémo*: Using Normal Mode Analysis in Molecular Replacement**

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Normal mode analysis (NMA) is a powerful tool for predicting the possible movements of a given macromolecule. A newly emerging field of NMA in X-ray crystallography is the utilization of normal mode perturbed models as templates for diffraction data phasing through molecular replacement (MR), thus accounting for conformational changes arising for example from ligand binding or different crystallogenic conditions [1]. Given that half of the known protein movements can be modelled by displacing the studied structure using at most two low-frequency normal modes, NMA may have the potential to break tough MR problems in up to 50% of cases. Moreover, even in situations where a MR solution is available, NMA can be used to further improve the starting model prior to refinement, eventually reducing the time spent on manual model construction (i.e. when working with low resolution data sets). Here we present this approach at a number of examples where screening for MR solutions using NMA perturbed templates allowed to obtain a MR solution, whereas MR using the original template failed to yield a model that could ultimately be refined. We outline possible protocols of using NMA in MR and present the web- server *elNémo* [2] for online NMA template generation http://igs-server.cnrs-mrs.fr/elnemo/index.html.

[1] Suhre K., Sanejouand Y.H., *Acta Cryst.*, 2004, D**60**, 796-799. [2] Suhre K., Sanejouand Y.H., *Nucleic Acids Research*, 2004, **32**, W610-W614.

Keywords: crystallography, phasing, normal mode analysis

MS91 ELECTRON CRYSTALLOGRAPHY ON INORGANIC CRYSTALS

Chairpersons: Jacob Jansen, Vera Kletschkovskaia

MS91.30.1

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Present Status of Electron Crystallography on Inorganic Materials

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The most promising alternative to X-rays for structural analysis of extremely small volumes are fast electrons, whose interaction with matter is several orders in magnitude stronger than of X-rays. Thus the two main branches of electron crystallography, electron diffraction structure analysis (EDSA) and (crystallographic) image processing of high-resolution electron microscopy images, are the methods of choice for structural characterisation of small samples and nanocrystalline materials.

Over the last years we have been witness of several new upcoming techniques on instrumentation that have pushed the frontiers of electron crystallography much further. The electron precession beam technique for example, considerably increases the obtainable resolution of any spot electron diffraction pattern and significantly reduces the dynamical contribution to the intensities of zone axis patterns. Thus this method is becoming a very attractive tool for scientists who want to determine crystal structures by EDSA. Another recent breakthrough took place in the field of high-resolution electron microscopy. A new generation of C_s-corrected FEG-TEM's and newly developed software enable to reconstruct the exit wave of crystals with resolution in the sub-Å range from through focus series.

These recent developments are now going to turn electron crystallography – more than 65 years after it's invention by Russian scientists – into a reliable and handy method for structure determination of tiny crystallites and nanosized materials.

Keywords: electron crystallography, inorganic materials, developments in electron crystallography

MS91.30.2

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Structural and Charge-density Studies of Transition-metal Oxides using Convergent-beam Electron Diffraction

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We developed a method to refine crystal structural parameters and charge density using convergent-beam electron diffraction (CBED) [1,2]. The method is based on the non-linear least-squares fitting between full dynamical calculations and experimental intensities of energy-filtered two-dimensional CBED patterns of zeroth-order Laue-zone (ZOLZ) reflections and higher-order Laue-zone (HOLZ) reflections. The HOLZ reflections are essential for the determination of atom positions and Debye-Waller factors and the ZOLZ reflections are utilized for obtaining charge density distributions.

For this purpose, we developed an energy-filter transmission microscope JEM-2010FEF [1], and an analysis program MBFIT [1]. A problem of long computation time needed for dynamical calculations was greatly eased by implementation of parallel computation on a computer cluster [3].

A nanometer scale probe in CBED has great advantages and can be used extensively. Our current targets are perovskite-type transition-metal oxides and related materials with strongly correlated electrons, in which very small domains of twin structures exist and characteristic phase separation occurs easily. In the present talk, analyses of perovskite transition-metal oxides using the CBED method are demonstrated.

[1] Tsuda K., Tanaka M, *Acta Cryst.* 1999, **A55**, 939. [2] Tsuda K. et al., *Acta Cryst.* 2002, **A58**, 514. [3] Ogata Y., et al., *Acta Cryst.* 2004, **A60**, 525.

Keywords: CBED, structure refinement, charge density

MS91.30.3

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Incomensurate Modulated Structure Determination by Combining HREM and ED

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The incommensurate modulated structure can be treated as a multi-dimensional (MD) periodic structure cut with a three-dimensional hyper plane [1]. In the case of one-dimensional modulation the structural modulation reveals directly in the high-resolution electron microscope image projected in the direction perpendicular to the modulation wave vector, but the image resolution is usually insufficient for showing all atoms. In this work it is shown that the electron diffraction data can be utilized to enhance the determined structure resolution by means of the MD direct methods [2].

The electron diffraction pattern consists of main reflections and satellites. The main reflections correspond to the average structure. An arbitrary defocus image is averaged according to the unit cell of basic structure to obtain the average image, and the deconvoluted average image reveals the average structure. Fourier transform of the deconvoluted average image yields phases of low-resolution main reflections for the modulated structure. Phases of high-resolution main reflections for the modulated structure can be derived from the low-resolution phases obtained from the image and the amplitudes from the diffraction pattern. The MD direct-phasing method [2] can be used

for phase extension and refinement of main reflections, and then used for phase extension from main reflections to satellite reflections.

[1] De Wolff P.M., *Acta Cryst. A*, 1974, **A30**, 777. [2] Hao Q., Liu Y.W., Fan H.F., *Acta Cryst*. 1987, **A43**, 820.

Keywords: incommensurate modulated structure, high-resolution electron microscopy, electron diffraction

MS91.30.4

Acta Cryst. (2005). A**61**, C116

Electron Dynamical Diffraction Imaging and Diffuse Scattering by Small Dislocation Loops

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Effects of dynamical scattering of high-energy electrons by elastic fields of interstitial or vacancy loops in a crystalline material provide a convenient means for diffraction contrast imaging. In this presentation we describe new developments in the methodology of simulation of diffraction images and *dynamical* diffuse scattering by small dislocation loops. To simulate diffraction images, a many-beam Howie-Basinki equation approach has been developed where strong dynamical effects as well as the non-parallel propagation of diffracted beams in the crystal are treated using a combination of the adaptive spatial mesh and wave field interpolation techniques. The significance of dynamical diffraction as well as practical applications of the new approach are illustrated by the comparison of simulated and experimentally observed images. The treatment of diffuse scattering includes effects of Kikuchi diffraction on Huang diffuse scattering patterns that we simulate using the atomic displacement fields evaluated using anisotropic elasticity solutions and atomistic modelling.

Keywords: electron microscopy and diffraction, quantitative electron diffraction, dynamical diffraction

MS91.30.5

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Characterization of Nanophases in HRTEM: Fourier Transform and Simulation

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High resolution transmission electron microscopy (HRTEM) was applied to study the microstructure of biomaterials based on calcium phosphates: α-tricalcium phosphate, octacalcium phosphate (OCP) and hydroxyapatite (HAP). Phase analysis at nanolevel was required to evaluate whether the final product included one or several Ca phosphate modifications. Due to high sensitivity of all these compounds to irradiation of the convergent electron beam such local analysis was performed by processing diffractograms (Fourier transform) from HRTEM images with Digital Micrograph software (Gatan). Interpretation of the experimental results was done by the means of simulation of selected area electron diffraction patterns and HRTEM images using JEMS [1], which allows to perform large calculations of dynamical diffraction patterns and HRTEM images for big multiatomic crystallographic unit cells.

HAP nanocrystals (5-20 nm) randomly oriented relatively to each other were identified in plasma sprayed coatings on different substrates. OCP crystals were found to contain HAP inclusions and their sizes were dependent on crystal growth regime. Phase transformation during high temperature synthesis of α-tricalcium phosphate from the β-form has been studied.

[1] JEMS: http://cimewww.epfl.ch/people/Stadelmann/jemsWebSite/jems.html Stadelmann P..

Keywords: electron microscopy and diffraction, simulation, calcium compounds

MS92 EMERGING TECHNOLOGIES FOR STRUCTURAL BIOLOGY

Chairpersons: Sine Larsen, Michael Becker

MS92.30.1

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Diffraction from a Laser-aligned Beam of Hydrated Proteins

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The aim of this work is to solve proteins which cannot be crystallized. An apparatus is under construction at ASU physics (electrons) and at the Advanced Light Source in Berkeley (X-rays) to obtain diffraction patterns from a single-file submicron droplet stream [1]. Each water droplet contains, on average, one protein. The droplets freeze by evaporative cooling to vitreous ice, most of which is allowed to sublimate. The molecules are aligned by a 100 watt CW fiber laser. All three beams , laser, X-rays and droplets, run continuously, and diffraction data is aquired continuously by CCD camera until adequate signal-to-noise is achieved. The laser polarization is then rotated into a new orientation using a quarter-wave plate, allowing tomographic diffraction data collection for three-dimensional reconstruction. The phase problem for the continuous diffraction pattern is solved by novel iterative Gerchberg-Saxton-Fienup methods [2]. Waves scattered by different molecules don't interfere. The requirements of laser power and droplet temperature needed to achieve sub-nanometer resolution and so observe the secondary structure of proteins will be described in detail. Factors which affect the damping of oscillations in the laser beam and momentum transfer by elastic diffraction to a levitated molecule.

[1] Spence J., Doak B., *Phys. Rev Letts*, 2004, **98**, 198102. [2] Spence J. et al, *Acta Cryst A*, 2005, in press. [3] Marchesini S. et al., *Phys Rev.*, 2003, **B68**, 140101(R).

Keywords: proteins, structure, laser alignment

MS92.30.2

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Protein Structures without Crystals

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Many proteins are inherently difficult to crystallize, due to various physical properties, e.g. membrane proteins, due to their large hydrophobic area, or amyloid-forming peptides and proteins due to very strong hydrogen bond networks in combination with hydrophobic interactions. Novel light sources (X-ray free electron lasers) may enable us to obtain structural information from small non-crystalline samples if we are able to gather enough scattering data before radiation damage destroys the sample. On the other end of the spectrum computer simulations of molecular dynamics may be able to predict structures of small proteins based on force fields within the near future. In the current presentation I give an overview of our work in both areas and how they are connected.

Keywords: X-ray, fel, gromacs

MS92.30.3

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Structural Proteomics using NMR in RIKEN Structural Genomics/Proteomics Initiative

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RIKEN Structural Genomics/Proteomics Initiative (RSGI) (http://www.rsgi.riken.jp) was organized by RIKEN Genomics Sciences Center and Harima Institute at SPring-8 in 2001. RSGI has been integrated into the National Project on Protein Structural and Functional Analyses ("NPPSFA" or "Protein 3000"), organized by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), as one center of the program for comprehensive studies. We are now focusing on proteins involved in phenomena of biological and