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Keywords: in-situ powder diffraction, phase transitions, phase diagram

MS32.26.4
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Structure Solution of Thermal Decomposition Compounds using Laboratory X-rays
Thierry Bataille, *Laboratoire de Chimie du Solide et Inorganique Moléculaire (UMR 6511 CNRS), Institut de Chimie, Université de Rennes 1, Rennes, France.* E-mail: thierry.bataille@univ-rennes1.fr

Powder X-ray diffraction at non ambient conditions is developing tremendously, thanks to the rapid progresses in diffraction techniques, methods and software. The present study focuses on the structure solution of inorganic powdered compounds resulting from thermal transformations, using the Bragg-Brentano optics with a conventional X-ray source. Some features related to *in situ* powder data collection are discussed. They include the sample surface displacement, which generates errors on peak positions for pattern indexing, the thermal stability of the products upon heating and the problem of line overlap. Indeed, the latter may arise from diffraction line broadening generated by the crystallite fragmentation during the thermal transformation. This is a major limiting factor for solving the crystal structure, since it strongly affects the structure solution with the direct methods and global optimisation approaches. The influence of the microstructure on the structure solution of the decomposition compound γ-$Zn_2P_2O_7$ is illustrated by a study from simulated patterns.

Representative examples of *ab initio* structure determination of thermal decomposition products will be described, such as those obtained by dehydration reactions of open-framework oxalate and phenylphosphonate materials, and by degradation of nitrate and squarate compounds.
Keywords: structure determination, thermal decomposition, powder diffraction

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Structure Solution of Single-Element Molecules from Pair Distribution Function
Pavol Juhas[a], David M. Cherba[b], Phillip M. Duxbury[a], William Punch[b], Simon J.L. Billinge[a], *[a]Department of Physics and Astronomy, [b]Department of Computer Science and Engineering, Michigan State University, East Lansing, MI, USA.* E-mail: juhas@pa.msu.edu

Recent developments of synchrotron x-ray and neutron instruments and acquisition techniques allowed fast and precise measurements of experimental Pair Distribution Functions (PDFs) from molecules, crystals and disordered materials. However, it is usually complicated to extract the structure information from PDF data, and the data processing typically involves a tedious testing of a series of structure models. Therefore it is desirable to find a better way how to analyze PDF data. For single-atom molecules the PDF curves can be converted to a table of inter-atomic distances, which transforms the PDF curve-fitting to a molecular conformation problem. We have developed several algorithms on reconstruction of single atom molecules and tested them with artificial and experimental distance data.
Keywords: ab-initio structure determination, pair distribution function, molecular structure

MS33 SOFT CONDENSED ORGANIC-BIOLOGICAL MATERIALS UNDER PRESSURE

Chairpersons: Roger Fourme, Wilson Poon

MS33.26.1
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Exploring the Configurational Landscape of Biomolecular Systems under Extreme Conditions
Roland Winter, *University of Dortmund, Physical Chemistry I - Biophysical Chemistry, Otto-Hahn-Straße 6, D-44227 Dortmund, Germany.* E-mail: winter@pci.chemie.uni-dortmund.de

Lipid bilayers, which provide valuable model systems for biomembranes, display a variety of polymorphic phases, depending on their molecular structure and environmental conditions, such as pH, ionic strength, temperature and pressure. By using spectroscopic and diffraction techniques, the temperature and pressure dependent structure and phase behaviour of simple lipid bilayers as well as binary and ternary (raft) lipid mixtures have been studied. Neutron small-angle scattering, two-photon excited fluorescence microscopy, and FT-IR spectroscopy were used to study also the lateral organization of phase-separated lipid membranes and the influence of peptide incorporation. Moreover, applying the pressure-jump relaxation technique in combination with time-resolved spectroscopic and diffraction techniques, the kinetics of various lipid phase transformations was investigated. The technique was also be applied to study other biomolecular structural transformations, such as protein folding. We present data on the pressure-induced un/refolding of various proteins. A thermodynamic approach is used for determining the stability of proteins as a function of both temperature and pressure and express it as a three-dimensional free energy surface. Morover, the effect of various chaotropic and kosmotropic cosolvents on the temperature- and pressure-dependent structure and stability of proteins is discussed. Finally, recent advances in using pressure for studying misfolding, aggregation and fibril formation (amyloidogenesis) of proteins (e.g., insulin, PrP) will be discussed.
Keywords: high pressure, membranes, proteins

MS33.26.2
Acta Cryst. (2005). A**61**, C46
Probing two Heads Configuration of Heavy Meromyosin by High-pressure SAXS Technique
Tetsuro Fujisawa[a], Shigeo Kuwamoto[a], Yuichiro Maéda[a], Yoh Okamoto[b], *[a]Laboratory for Structural Biochemistry, RIKEN Harima Institute/SPring-8, Harima, Japan. [b]Muroran Institute of Technology.* E-mail: fujisawa@spring8.or.jp

We are studying multiple conformations of myosin, by employing the high-pressure X-ray scattering (HP-SAXS). High hydrostatic pressures would shift the equilibrium between conformations. The heavy meromyosin (HMM), a chymotryptic product of myosin, is known to have two heads with one long tail, and HP-SAXS is highly sensitive to the two head orientations. We have carefully optimized the solvent condition for minimal deterioration of HMM both due to aging and radiation damage. The experiments were done at BL45XU-SAXS (SPring-8, Harima) using a compact high-pressure cell [1].

Under 0.1-200 MPa, no structural change was observed that points to that the asymmetric configuration of two heads was rather stable [2]. Above 250 MPa, HP-SAXS pattern of HMM irreversibly changed. At room temperature the change is kinetically controlled, while at -12 °C under 200 MPa HMM structure was equilibrated. The pressure treated samples were all reversible in terms of actin binding and intrinsic Trp fluorescence. We will report on the solution structure of HMM based on HP-SAXS under the low temperature and high- pressure condition.

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Keywords: high-pressure research, SAXS and SANS synchrotron, proteins muscle

MS33.26.3
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SAXS Investigations of Conformation and Stability of Eye Lens Proteins under Pressure
Stéphanie Finet[a], Fériel Skouri-Panet [b], Annette Tardieu [c], *[a]ID2-ESRF, Grenoble, France. [b]IMPMC, Paris, France. [c]PBSF-P6-IM, Paris, France.* E-mail: finet@esrf.fr

We have combined small angle X-ray scattering (SAXS) and a high-pressure cell to study the effect of pressure, temperature and pH,

on the conformation and the stability of γ- and α-crystallins. α-, β- and γ-crystallins are the main components of mammalian eye lenses and their structural and associative properties are responsible for lens transparency. γ are monomers (21 kDa, up to 80% sequence identity), whereas α are large hetero-oligomers of about 800kDa. The C-terminal domain of α belongs to the ubiquitous superfamily of sHSPs (small heat shock proteins): upon stress, they are able to incorporate the non-native proteins to prevent their aggregation.

High-pressure experiments performed with α-crystallins have shown a partially reversible change in size from 2 to 3kb at room temperature, and this effect was enhanced by the combination of temperature and pressure. In the case of γ-crystallins, pressure and temperature needed to be combined with pH, and the results depend upon the different γ itself. Crystallins are known to be exceptionally stable *in vivo* since they are synthesised to last for life. They therefore represent an extreme case of stability versus unfolding and these results have shown that these proteins (mainly beta strands) are also stable upon pressure.

Keywords: high-pressure SAXS, crystallins, conformation changes

MS33.26.4

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When Macromolecular Crystallography Meets high Pressure Techniques…

Eric Girard[a], Richard Kahn[b], Mohamed Mezouar[c], Anne-Claire Dhaussy[d], Tianwei Lin[e], John E. Johnson[e], Roger Fourme[a], [a]*Synchrotron SOLEIL, St Aubin, France.* [b]*Institut de Biologie Sructurale, Grenoble, France.* [c]*ESRF, Grenoble, France.* [d]*CRISMAT, Caen, France.* [d]*SCRIPPS, La Jolla, USA.* E-mail: eric.girard@synchrotron-soleil.fr

Until recently, only two crystal structures of small proteins at high pressure below 200 MPa generated in a Be cell were published [1,2]. The lack of structural data at high pressure was due mainly to the cumulated complexities of high-pressure containment and crystallography. A technical breakthrough was achieved with a set-up at the ESRF ID30/ID27 beamline combining a diamond anvil cell, ultra-short wavelength (0.33 Å) X-rays from undulators and a large imaging plate [3]. The accessible pressure range was increased by nearly one order of magnitude. The quality of diffraction data collected under high pressure achieved usual standards.

We will present the technical advances as well as scientific results that we have obtained. In particular, scientific results will focus on the first crystal structure of a complex macromolecular assembly under high pressure, the Cowpea Mosaic Virus capsid at 330 MPa [4], demonstrating that high pressure macromolecular crystallography can now be considered as a mature and general technique.

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Keywords: high-pressure, X-ray crystallography, macromolecules

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High Pressure Cooling of Protein Crystals without Cryoprotectants

Chae Un Kim, Sol M. Gruner, *162 Clark Hall, Physics Department, Cornell University, Ithaca, NY, USA.* E-mail: ck243@cornell.edu

The flash cooling of protein crystals is the best known method to effectively mitigate radiation damage in macromolecular crystallography. To prevent physical damage to crystals upon cooling, suitable cryoprotectants must usually be found, a process that is time-consuming and, in certain cases unsuccessful. Recently we have developed a novel method to cryocool protein crystals without the need for penetrative cryoprotectants. In the new method, each protein crystal is pressurized up to 200 MPa (2000 atm) in He gas at 10 ˚C. The crystal is then cyrocooled under pressure and the pressure was released while the crystal is kept cooled. Results are presented for two proteins that have been flash-cooled at ambient pressure and pressure-cooled, in all case without penetrating cryoprotectants. For glucose isomerase, the flash-cooled crystal diffracted to only 5.0 Å and mosaicity could not be estimated but the pressure-cooled one diffracted to 1.05 Å with 0.39˚ mosaicity. For thaumatin, the flash-cooled crystal diffracted to only 1.8 Å with 1.29˚ mosaicity but the pressure-cooled one diffracted to 1.15 Å with 0.11˚ mosaicity. The protein structures show that the structural perturbation by pressure is very small. A mechanism on the pressure cooling is proposed involving the dynamics of water at high pressure and high density amorphous (HDA) ice.

Keywords: high pressure cooling, cryocrystallography, crystallography of biological macromolecules

MS34 Advances in Computational Methods for Electron Density Studies

Chairpersons: Louis Farrugia, Tibor Koritsanszky

MS34.26.1

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Ab initio Quantum-mechanical Calculation of Electron Charge-density in Crystals

Roberto Orlando[a], Bartolomeo Civalleri[b], Roberto Dovesi[b], Piero Ugliengo[b], [a]*Dept. Science and Advanced Technologies, University of Eastern Piedmont, Alessandria, Italy.* [b]*Dept. Chemistry IFM, University of Turin, Italy.* E-mail: roberto.orlando@unipmn.it

The *ab initio* quantum-mechanical CRYSTAL code [1] is one of the tools available for the calculation of the electronic structure and properties of crystals. It is based on a description of the wavefunction in terms of linear combinations of atomic orbitals (LCAO), which permits an easy interpretation of the electronic structure and a direct comparison with molecular fragments.

A large variety of properties of matter in the condensed phase can be calculated with the present release of the code, CRYSTAL03, even for systems of considerable size: a calculation of the electronic structure of the crambin protein ($P2_1$, 92 amino acid residues per cell) has been attained recently [2].

Molecular crystals are an important area of application of CRYSTAL. The use of a basis set of atomic orbitals is convenient for the calculation of the lattice energy and the characterization of hydrogen bonds, where the modifications in the electron charge density of the molecules due to the formation of the crystal can be investigated, along with their effect on the structure factors [3].

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Keywords: charge density, ab initio calculations, molecular crystals.

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Beyond $\nabla^2\rho_b$: Chemical Bond Analysis using the Local Form of the Source Function

Carlo Gatti, Luca Bertini, Fausto Cargnoni,*CNR-ISTM, Milan, Italy.* E-mail: c.gatti@istm.cnr.it

The sign of the Laplacian of the density at the bond critical point, $\nabla^2\rho_b$, has been largely used for discriminating the closed-shell-like ($\nabla^2\rho_b>0$) from the shared-shell-like ($\nabla^2\rho_b<0$) interactions. This dichotomous bond classification has the merit of being simple, but it has also proved to be often inadequate. This is the case of bonding between heavy atoms missing the outermost regions of charge depletion and concentration in their atomic Laplacian distributions and/or the case of interactions having very low $|\nabla^2\rho_b|$, a fact which makes the sign of $\nabla^2\rho_b$ quite indeterminate and the use of $\nabla^2\rho_b$, as the only classification index, deceiving. Other quantities, based on the first and/or the second order density matrices, have in these cases been