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

Acta Cryst. (2005). A**61**, C47

When Macromolecular Crystallography Meets high Pressure Techniques…

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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

MS33.26.5

Acta Cryst. (2005). A**61**, C47

High Pressure Cooling of Protein Crystals without Cryoprotectants

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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

Acta Cryst. (2005). A**61**, C47

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

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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].

[1] Saunders,V.R., Dovesi R., Roetti C., Orlando R., Zicovich-Wilson C., Harison N.H., Doll K., Civalleri B., Bush I.J., D'Arco Ph., Llunell M., *CRYSTAL2003 user's manual*, University of Torino, Torino, 2003. [2] http://www.hpcx.ac.uk/about/newsletter/HPCxNews02.pdf, pages 10-12. [3] Spackman M. A., Mitchell A. S., *Phys. Chem. Chem. Phys.*, 2001, 3, 1518.

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

MS34.26.2

Acta Cryst. (2005). A**61**, C47-C48

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

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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

proposed as more suitable indices. However, both matrices are generally not directly amenable to experimental determination.

Recently, it has been shown [1-3] how the sign of $\nabla^2\rho$ at **r'** determines whether this point acts as a *source* or as a *sink* for ρ at any other point **r** in a system, with the effectiveness as a source or as a sink being related to $|\nabla^2\rho|$ and to the inverse of the distance between the two points, $\rho(\mathbf{r}) = \int -(1/4\pi)\nabla^2\rho(\mathbf{r'})|\mathbf{r}-\mathbf{r'}|^{-1}\cdot d\mathbf{r'} = \int LS(\mathbf{r}, \mathbf{r'})\cdot d\mathbf{r'}$.

The profile of the *local source function* LS (**r**, $\mathbf{r_{bcp}}$) along the bond path is here used to unravel the different mechanisms by which covalent bonding between light or heavy atoms realizes in crystals.

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Keywords: chemical bonding theory, electron density studies, topological properties of charge distributions

MS34.26.3

Acta Cryst. (2005). A**61**, C48

Thermal Motion Analysis via Modern Probability Methods

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Interesting statistical theory advances with crystallographic application possibilities continue to appear. Examples follow:

The Gram-Charlier anharmonic atom model might be replaced by a saddlepoint expansion [1] or a mixture of linear & angular Gaussians.

The refinement matrix from a structure-factor equation in logarithmic form contains a Fisher information matrix [2] with geometric information about interatomic motion coupling.

A stationary Levy stochastic process [3] along a crystal chemistry interaction network may allow atomic displacement models with Levy-jump intramolecular components and Levy-drift intermolecular rigid-body-motion components.

Spatial point processes [4], e.g. the familiar Gibbs process, allow attraction/avoidance calculation for point particle systems. More general marked point process [4] network node systems with thermal ellipsoid pair Radon-Nikodym derivative[5] couplings seem feasible.

Feasibility studies for a subset of the examples will be discussed.

[a] ORNL operated by UT-Battelle, LLC, under DOE DE-AC05-00OR22725. [1] Jensen J.L.,*Saddlepoint Approximations*, Oxford, 1995. [2] Amari S.I., Nagaoka H., *Methods of Information Geometry*, Oxford, 2000. [3] Sato K.I., *Levy Processes and Infinitely Divisible Distributions*, Cambridge, 1999 [4] Daley D.J., Vere-Jones D., *Introduction to the Theory of Point Processes*, *Volume I*, Springer, 2003. [5] Johnson C.K., "Crystallographic Topology 2: Overview and work in progress", *Trends in Mathematical Physics*, Alexiades V., Siopsis, G. Eds, AMS/IP, 1999.

Keywords: anharmonic refinement models, stochastic processes, fisher information matrix

MS34.26.4

Acta Cryst. (2005). A**61**, C48

Images of Unpaired Electron Density in Molecular Crystals Obtained using Experimentally Constrained Wavefunctions

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Unpaired electrons are responsible for many of the magnetic properties of crystalline systems. Traditionally, unpaired electrons in crystalline systems have been imaged using the technique of polarised neutron diffraction (PND). However, these experiments are difficult, and relatively few data are obtained compared with X-ray diffraction measurements.

In this talk I will present and compare images of unpaired electron density in molecular crystals. These images are obtained from a molecular or cluster Hartree-Fock wavefunctions, which have been constrained to reproduce X-ray diffraction data, to reproduce PND data, and to reproduce both of these data simultaneously.

Two crystalline systems will be considered: a system displaying the photomagnetic LIESST effect, and a simple molecular magnetic system. The difficulties and results will be discussed, including the physical meaning of the orbital energies, and their shapes.

Keywords: quantum crystallography, unpaired electron density, constrained wavefunctions

MS34.26.5

Acta Cryst. (2005). A**61**, C48

Holographic Principles of Molecular Structure and Electron Density Calculations

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Electron densities of molecules obey a holographic principle: in a non-degenerate ground state any small positive volume of the electron density cloud contains the complete information about the entire molecular structure [1]. This holographic theorem provides the constraint on the applications of various electron density fragmentation methods, including fuzzy density fragmentation methods aimed at potential advances in the crystallographic structure refinement process [2], the analysis of quantum chemical functional groups of molecules [3], detailed molecular shape analysis [4], and providing the foundations for linear scaling, ab inito quality macromolecular quantum chemistry computational methods, applied to various proteins [5-7]. Some new advances in these fields will be reviewed.

[1] Mezey P.G., *Mol. Phys*., 1999, **96**, 169. [2] Mezey P.G., *Adv. Molec. Structure Res*.,1998, **4**, 115. [3] Mezey P.G., *Adv. Quant. Chem.*, 1996, **27**, 163. [4] Mezey P.G., *Shape in Chemistry: an Introduction to Molecular Shape and Topology*, VCH, New York, 1993. [5] Mezey P.G., *Int. J. Quant. Chem.*, 1997, **63**, 39. [6] Mezey P.G., *Pharmaceutical News*, 1997, **4**, 29. [7] Exner T.E., Mezey P.G., *J. Comput. Chem.*, 2003, **24**, 1980.

Keywords: holographic theorem, molecular shape, fuzzy density fragments

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MS35 POLYTYPISM AND TWINNING

Chairpersons: Giovanni Ferraris, Elena Belokoneva

MS35.26.1

Acta Cryst. (2005). A**61**, C48

Recent Aspects of the Theory of Oriented Crystal Associations

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Despite its long history, twinning is far from having disclosed all its secrets. Although the definition of twinning is unambiguous, it is still sometimes used in a less appropriate way. The typical example is that of "cell-twinning", a phenomenon by which homogeneous modular structures derive from iso- or heterochemical archetypes with a possible chemical modification at the interface. Modules in cell-twins are related by space-groupoid operations (defined in point space) and the edifice is homogeneous, whereas individuals in twins are related by point group operations (defined in vector space) and the edifice is heterogeneous [1].

The classical reticular theory of twinning had to be extended to include cases not fitting the original classification, leading to a finer subdivision of Friedel's categories [2]. The most recent extension concerns the coexistence of up to three sublattices, which correspond to different types of non-merohedric twinning resulting in an effective twin index (degree of quasi-overlap of lattice nodes) significantly higher than the classical twin index [3].

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Keywords: cell-twinning, geminography, twinning