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Dual Polarization Interferometry in Macromolecular Crystallisation Diagnostics. Emmanuel Saridakis[a], Attia Boudjemline[b], Marcus J. Swann[b], Irene M. Mavridis[a], Naomi E. Chayen[c], [a]*Institute of Physical Chemistry, N.C.S.R. "DEMOKRITOS", Athens 15310, Greece,* [b]*Farfield Group Ltd, Crewe CW1 6GU, U.K.* [c]*Biomolecular Medicine, Dept. of Surgery and Cancer, Faculty of Medicine, Imperial College London, SW7 2AZ, U.K.*
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Dual Polarization Interferometry (DPI) is a waveguide based interferometer technique, which is well established for the characterisation of surface layers. This method allows the correlation of changes in refractive index to the thickness and density of surface layers immediately above a waveguide surface. It has therefore been applied in a wide range of areas from polymer multilayer deposition to measuring protein orientation, interactions and conformational changes. DPI was found to also be an effective diagnostic tool for macromolecular crystallisation, which may allow the early and efficient detection of conditions that are most likely to lead to crystals, more economically than by conventional screening. It may in addition provide information useful to the study of nucleation as well as to crystal optimisation. It is shown that DPI can be used to discriminate between the formation of crystalline and amorphous material in real time, in a dialysis setup. This setup allows the screening of a continuous or step-wise concentration gradient of a precipitating agent in one trial. Real-time DPI monitoring of such a trial provides therefore information as to whether this particular precipitant or precipitant/buffer combination is worth pursuing and optimising for crystal production. We have examined in detail the differences between DPI measurements from crystallising versus non-crystallising (clear and amorphously precipitating) protein solutions and have found what seems to be an unequivocal 'signature' of crystallisation events versus amorphous precipitation or clear solution.

Keywords: protein crystallisation, crystallogenesis, Dual Polarisation Interferometry

FA5-MS38-P08

Optimization of the packing in the haloalkane dehalogenase crystal by seeding. Yukari Sato[a], Ryo Natsume[a], Masataka Tsuda[b], Yuji Nagata[b] and Toshiya Senda[c], [a]*Japan Biological Informatics Consortium,* [b]*Graduate School of Life Sicences, Tohoku University, Japan,* [c]*Advanced Industrial Science and Technology, Japan*
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The preparation of crystals that diffract to atomic resolution is a bottleneck in protein structure determination. Seeding is known to be a useful method for protein crystallization and for the optimization of crystal growth, since this method does not require the spontaneous formation of a crystal nucleus. Heterogeneous seeding is used when the protein cannot crystallize. It is also known that the quality of crystals can be improved by repeating the seeding cycle (serial seeding). However, the mechanism by which the heterogeneous and serial microseedings improve crystallization (or crystal quality) remains elusive. To reveal the mechanism underlying crystallization with the heterogeneous and serial microseeding methods, we analyzed the crystal structures of two haloalkane dehalogenases: 1) His-DbjA, which crystallized spontaneously, and 2) DbjA, which crystallized in the combination of heterogeneous and serial microseedings.
Haloalkane dehalogenases (EC 3.8.1.5) are key enzymes for the microbial degradation of halogenated aliphatic pollutants. The enzyme DbjA from *Bradyrhizobium japonicum* USDA110 has novel substrate specificity for β-methylated haloalkanes [1]. Our initial attempt to crystallize DbjA gave no crystals, while a DbjA variant with a histidine tag at the C-terminus (His-DbjA) successfully crystallized [2]. The use of His-DbjA as a seed in heterogeneous microseeding yielded DbjA crystals. The obtained crystal, however, diffracted to approximately 12 Å. To improve the crystal quality, microseeding was subsequently repeated four times using crystal seeds that were prepared from a crystal diffracting to the highest resolution among the crystals obtained in each microseeding cycle. This procedure finally yielded a crystal diffracting to 1.85 Å resolution. Although His-DbjA and DbjA were crystallized under the same conditions except microseeding for DbjA, their space groups were different. The asymmetric unit of His-DbjA contains two dimers ($P2_12_12$), while that of DbjA contains one ($C2$). Crystal packing analysis with computer graphics showed that the crystal lattice of the $C2$ crystal can be assembled vertically on both the (100) and (010) planes of $P2_12_12$ without steric hindrances, suggesting that the crystal growth of DbjA can be initiated from the cell planes of His-DbjA. We postulate that the improvement of crystal quality by serial microseeding occurs when a crystal seed with (mostly) uniform $C2$ lattices was selected for crystal growth from numerous crystal seeds comprised of different types of lattices ($C2$ and $P2_12_12$). This study showed that heterogeneous seeding coordinates molecular packing by providing cell planes of the initial seed for the protein of interest, and that repeating the microseeding can increase the chance to produce crystals of proper molecular packing. The combination of these methods will open the way to obtain crystals that diffract at high resolution.

[1] Sato, Y. et al. *Appl. Envrion. Microbiol*, 2005, 71, 4372. [2] Sato, Y. et al. *Acta. Cryst.* F, 2007, 63, 512.

Keywords: Seeding, packing, Protein crystal growth

FA5-MS38-P09

Oxygen vacancy contribution on the polarization dependent DAFS of Rutile TiO_2. M. Zschornak[ab], C. Richter[a], D. Novikov[c], H. Stöcker[ad], T. Leisegang[a], S. Gemming[b], D.C. Meyer[d], [a]*Structural Physics, TU Dresden, Germany,* [b]*Materials Research, FZ Dresden-Rossendorf, Germany,* [c]*DESY/HASYLAB Hamburg, Germany,* [d]*Exp. Physics, TU Bergakademie Freiberg, Germany*
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Energy and polarization dependent *Diffraction Anomalous Fine Structure* (DAFS) also known as *Anisotropic Anomalous Scattering* (AAS) can be employed in addition to *X-ray Absorption Fine Structure* (XAFS) in order to study electronic transitions from core states to unoccupied states [1]. Here, we present results from resonant X-ray diffraction experiments on

TiO_2 (rutile modification), space group (136) $P4_2/mnm$, carried out in the vicinity of the Ti-*K* absorption edge. The influence of oxygen vacancies on the anomalous scattering signal (see e.g. [2]) has been studied on a series of 10 x 10 x 1 mm^3 single crystal wafers of different vacancy concentrations obtained by annealing at a temperature of 800°C in a vacuum of about 10^{-6} mbar for different durations. Considered reflections include the 'forbidden' 001 and allowed 111 reflection. Simulations by means of *ab-initio* modeling of the near-edge transitions and an interpretation of the far-edge resonances based on vacancy-induced static Ti displacements from high- to low-symmetry positions will be presented.

[1] V.E. Dmitrienko, E.N. Ovchinnikova: *Acta Cryst. A56* (2000) 340.
[2] V.E. Dmitrienko, K. Ishida, A. Kirfel, E.N. Ovchinnikova: *Acta Cryst. A61* (2005) 481.

Keywords: DAFS, anomalous scattering methods, resonant scattering

FA5-MS38-P10

Automated Seeding for the Optimization of Crystal Quality. Naomi Chayen[a], Lesley Haire[b] and Sahir Khurshid[a], [a]*Biomolecular Medicine, Imperial College London, UK,* [b]*National Institute for Medical Research, Mill Hill, UK*
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With the advent of structural genomics a variety of crystallization techniques have been automated and applied to high throughput, yet seeding which is the most common and successful optimization method is still being performed predominantly manually. The aim of this study was to devise simple automated seeding techniques that could be applied in a routine manner using existing robots and without the requirement of specialised tools. Two alternative protocols for automated seeding experiments are described. One involves the delivery of micro-crystals from stock to target wells using the robot dispensing tip as a seeding tool. The second harnesses an animal whisker as the seeding tool. Larger, better ordered crystals were obtained using both techniques.

Keywords: protein crystallization, automation, seeding

FA5-MS38-P11

Upside Down Protein Crystallization. Naomi Chayen[a] and Sahir Khurshid[a], [a]*Biomolecular Medicine, Imperial College London, UK*
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A technique leading to the formation of the highest ever diffracting crystals of 'Human Myosin Binding Protein C' (MyBPC) is described. This method was initially designed to facilitate the use of the microbatch method in microgravity. The crystallisation vessels currently employed for microgravity crystallisation are non-optimal with regards to cost, sample volume, size and ease of use. The utilisation of microbatch experiments is a favourable alternative in each respect: To date, the use of microbatch has not been pursued due to concerns of oil leakage. To address this issue, a novel approach is described where the microbatch plates are inverted throughout the duration of the experiment. The findings intimate the application of the microbatch method to space flight and the potential to significantly increase the output of microgravity crystallisation research. Furthermore, crystallisation in the inverted position was found to be enhanced with crystals of the target MyBPC diffracting to the best ever obtained resolution of 1.2Å. It is proposed that this can be attributed to the negation of drop contact with the crystallisation vessel enabled by this method in a manner similar to containerless crystallisation.

Keywords: protein crystallization, optimization, microbatch

FA5-MS38-P12

Electronic Carbon-Nanotube-Based Materials for Protein Crystallization. Naomi E. Chayen[a] Lata Govada[a], Piyapong Asanithi[b], Emmanuel Saridakis[c], Izabela Jurewicz[b], Eric W. Brunner[b], Rajesh Ponnusamy[d], Jamie A. S. Cleaver[c], Alan B. Dalton[c], and Richard P. Sear[c] , [a]*Biomolecular Medicine, Department of Surgery and Cancer Oncology, Faculty of Medicine,Imperial College London, London SW7 2AZ, United Kingdom,* [b]*Department of Physics and Chemical and Process Engineering, Faculty of Engineering and Physical Sciences, University of Surrey, Guildford, Surrey GU2 7XH, United Kingdom,*[c]*Institute of Physical Chemistry, NCSR "DEMOKRITOS", Ag. Paraskevi 15310, Athens, Greece,* [d]*Institute of Biochemistry, University of Lubeck, Ratzeburger Allee 160, 23538 Lubeck, Germany*
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The use of porous materials as nucleants for protein crystallization have been well documented over the past decade. Harnessing this porosity alongside the surface chemistry has the potential to yield nucleants of greater efficacy. To this end, we report on the first use of carbon-nanotube-based films. The nanotubes induced crystal nucleation in the metastable zone of the phase diagram for a range of proteins including the targets 'Human Cardiac Myosin Binding Protein-C' (MyBPC) and 'Nonstructural Protein 9 of the Transmissible Gastroenteritis Virus'. Furthermore, crystals of 'MyBPC' diffracted to a resolution of 1.6 Å improving on the previous limit of 3.0 Å. Thus, nanotube-based films are very promising candidates for future crystallisation trials of intractable proteins.

Keywords: protein crystallization-1, nucleants-2, carbon nanotubes-3

FA5-MS38-P13

Attenuated Total Reflection-FT-IR Spectroscopic Imaging of Protein Crystallization. Naomi E. Chayen[a], Lata Govada[a], K. L. Andrew Chan[b], Roslyn M. Bill[c], Sergei G. Kazarian[b], [a]*Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, SW7 2AZ, United Kingdom,* [b]*Department of Chemical Engineering,*