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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

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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

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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