MS1-05 **High Pressure Freezing of Macromolecular Crystals.** Anja Burkhardt,[a] Martin Warmer,[b] Armin Wagner,[c] Saravanan Panneerselvam,[a] Athina Zouni,[d] Carina Glöckner,[d] Jingshan Ren,[e] Elisabeth E. Fry,[e] David I. Stuart,[ce] Rudolph Reimer,[b] Heinrich Hohenberg,[b] Alke Meents,[a] *[a]DESY, Germany, [b]Heinrich-Pette-Institute, Leibnitz Institute for Experimental Virology, Germany, [c]Diamond Light Source, United Kingdom, [d]Max-Volmer-Laboratory for Biophysical Chemistry and Biochemistry, Technical University Berlin , Germany, [e]Division of Structural Biology, University of Oxford, United Kingdom*
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X-ray induced radiation damage to biological samples can be reduced by more than 100 times by cooling the samples to cryogenic temperatures [1], [2]. Macromolecular crystals can contain up to 90% of solvent (mainly water). Cryocooling of such crystals requires cryoprotectants such as glycerol or ethylene glycol in order to suppress hexagonal ice formation and to convert the water to amorphous ice (vitrification). The search for optimal cryoconditions can be very time and crystal consuming, especially for large unit cell systems such as viruses. Such crystals usually have weak crystal contacts and tend to crack due to osmotic shock when soaked in a solution of a different ionic strength [3]. In addition, the crystal quality is often degraded upon conventional flash-cooling even if adequate cryoprotectants have been found. Thus, there is a big demand for alternative cryocooling techniques in macromolecular crystallography (MX). A promising approach which allows sample vitrification without cryoprotectants is high pressure freezing (HPF). In MX, this technique has been applied to different proteins of medium molecular weight and unit cell size [4], [5], but successful HPF on large unit cell systems with weak crystal contacts has not been reported to date. HPF is also well established in the field of cryo-electron microscopy (cryo-EM) for cryofixation of cells or tissue [6]. In our work, a standard HPF protocol from cryo-EM was optimized for macromolecular crystals [7]. The crystals are cryocooled at 210 MPa and 77 K using a Baltec HPM 010 instrument. This HPF device provides high cooling rates which allow vitrification of the solvent inside the crystals and the surrounding growth solution. The crystals can therefore be directly cryocooled in their mother liquor without the need for cryoprotection. Our HPF protocol was applied to several macromolecular crystals of different crystal symmetry, unit cell size and solvent content. Crystals of hen egg-white lysozyme, cubic porcine insulin, the membrane protein photosystem II (PSII) and Bovine Enterovirus 2 (BEV2) were high pressure frozen giving crystals of very good diffraction quality. The non-cryoprotected PSII crystal diffracted down to 4.5 Ĺ and showed a mosaicity of 0.22° after HPF. At 100 K a full data set was collected from a single position of a BEV2 crystal, a system which could not be cryoprotected using conventional soaking protocols. The HPF BEV2 crystal diffracted to a resolution of 2.5 Ĺ and possessed mosaic spreads of 0.25°. In summary, our HPF protocol is ideally suited for cryocooling large unit cell systems with weak crystal contacts which are difficult or even impossible to cryoprotect.

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MS2-01 **Visualising rapid structural changes in photosynthetic reaction centres with XFEL radiation.** David Arnlund[a], Linda Johansson[a], Gergely Katona[a], Erik Malmerberg[a], J. Davidsson[b], Anton Barty[c], Ilme Schilchting[d], Sebastian Boutet[e], Petra Fromme[f], John Spence[e], Henry Chapman[b], Richard Neutze[a].
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Time-resolved structural studies of protein reaction dynamics aim to elucidate the conformational changes occurring in proteins and thereby elucidate the chemical details of their reaction mechanism. I will introduce this sub-field of Structural Biology by briefly presenting structural results from time-resolved Laue diffraction studies of a photosynthetic reaction centre performed using synchrotron radiation [1]. I will then touch upon the implications of new approaches to time-resolved structural biology which can emerge from the revolutionary new approach of ultrafast serial femtosecond crystallography (SFX) at X-ray free electron lasers [2,3]. I will outline the potential benefits of single-shot time-resolved SFX studies from micro-crystals at an X-ray free electron laser. Finally, I will discuss the potential of ultrafast time-resolved Wide Angle X-ray Scattering (WAXS) at an XFEL. The example of the bacterial photosynthetic reaction centre will be used throughout to illustrate these emerging approaches to time-resolved structural biology.

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