a controlled temperature series. By comparing the results from the two crystals, effects of radiation damage could be distinguished from those of temperature, and differences in behavior of the two forms of ferritin were also examined.

As has been previously reported [2, 3], it was found that the increase in unit cell dimensions is linear with dose. Our results show that the irreversible effects of dose could be distinguished from the reversible temperature-induced effects.

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Keywords: ferritin, radiation damage, thermal cycling

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Mitigation of Radiation Damage to Protein Crystals using a Helium Cryostream

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Experiments at APS Beamline 14BM-C (BioCARS) with D-xylose isomerase crystals tested the effect of cryogen temperature in minimizing radiation damage at synchrotron beamlines. Data were collected using cryogenic helium (Pinkerton Device) and nitrogen (Oxford Industries CryoJet). Helium data were collected at 15 K, 50 K and 100 K on similar quality crystals. Nitrogen data were collected at 100 K. Multiple crystals were used at each temperature. Initial data were collected on each crystal followed by a 10 minute exposure. Data collection and exposure cycles were completed for at least 6 cycles. Crystal statistics showed significant reduction in radiation damage at the lowest data collection temperatures (15 K helium stream). In particular, the signal-to-noise estimate, $I/\sigma(I)$, of the highest resolution shell showed progressively less deterioration as temperature of the cold stream decreased. The average decay in $I/\sigma(I)$ for the highest resolution shell was 52 % for 15 K crystals, 63 % for 50 K crystals and 75 % for 100 K crystals. The results indicate that manifestations of radiation damage appear less rapidly at lower temperatures and the effects of radiation damage can be partially mitigated at very low temperatures particularly for high resolution data.

Keywords: radiation damage, synchrotron, temperature

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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 cyroprotectants 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 at 77 K. 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 at the level of a few tenths of an angstrom, which is comparable to the typical structural changes always observed upon flash cooling at ambient pressure. 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

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The Crystal Structures of HcpB and –C: Two Proteins with Sel1-like Repeat Architectures Involved in the Modulation of Innate Immune Response

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Many epsilon-proteobacteria, such as *Helicobacter pylori*, *Campylobacter jejuni* and *Wollinella succinogenes*, settle in the stomachs of higher organisms, requiring proteins to resist the harsh conditions of this ecological niche. The family of Helicobacter cysteine-rich proteins (Hcp) was identified using the method of comparative genome analysis and seems to be specific for this class of microorganisms. So far this family of secreted proteins was lacking a functional and structural description. Using recombinant HcpA, -B, -C and –E we identified high anti-Hcp antibody titers in the sera of *H. pylori* positive patients, confirming that these proteins are expressed *in* vivo [1]. The crystal structures of HcpB and –C were refined at 2 Å resolution and serve as the prototype structures for the large family of Sel1-like repeat proteins [2, 3]. The structure of HcpC reveals a peptide-binding mode that is strikingly similar to TPR proteins suggesting that Hcp proteins might be involved in protein-protein interactions. This hypothesis is corroborated by the secretion of high IFN-gamma and IL12 levels from naïve mouse splenocytes upon addition of recombinant HcpA [4], indicating an implication in the modulation of innate immune response and survival of *H. pylori* in the human stomach.

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Keywords: innate immune response, structural genomics, protein-protein interactions

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Structural Basis for Blue and Purple Fluorescence of Antibody-stilbene Complexes

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A panel of antibodies was generated against *trans*-stilbene[1] in order to explore the influence of protein environment on the excited electronic states of a chromophore. When irradiated by UV-light, stilbene readily undergoes photochemical *trans/cis*-isomerization and exhibits only weak fluorescence. In presence of these antibodies however, the electronically-excited stilbene affords strong fluorescence which is likely the result of precluding isomerization in the antibody pocket due to tight binding of the stilbene. Interestingly, antibody 19G2 exhibits the largest red-shift and a tenfold increase in fluorescence lifetime compared to the other purple-fluorescent antibodies.

Crystal structures of both blue (19G2) and purple (25C10) fluorescent antibodies in complex with stilbene have been determined to elucidate their different fluorescence properties and the mechanism of spectral tuning. In combination with biochemical and spectroscopic techniques, we are probing the unusually strong fluorescence of 19G2 compared to 25C10.

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