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

P.04.10.2

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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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Stevens R.C., Millar D.P., Schultz P.G., Lerner R.A., Janda K.D. R.J., Liles D.C., *Science*, 2000, **290**, 307.
Keywords: antibody structure, photophysics, protein dynamics

P.04.11.3
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Engineering Immune System Glycoproteins to form Uniform Crystalline Lattices
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Interactions of antibodies and Fc receptors (FcR) play a fundamental role in immunity. However, antibodies and FcR are heavily glycosylated, making it difficult to obtain strongly diffracting crystals. Consequently, most structures for these proteins have been determined at low to medium resolutions (<3.0-2.6 Å). Without modifying the carbohydrates, we are developing procedures for generating crystals of immune system glycoproteins that diffract to high resolutions.

A 2.0 Å structure was previously determined for FcγRIIa with a point mutation of Ser to Phe at position 88[1]. The Phe88 side chain is involved in a key lattice contact by completing a hydrophobic pocket that traps a proline "guest ligand" from a symmetry related receptor monomer. As a result the crystals are robust, allowing a variety of receptor glycoforms to be resolved and the structural analysis extended to 1.5 Å. The role of Phe88 in promoting uniform crystalline lattices has been shown by determining the 2.3 Å resolution structure of the "wild-type" (Ser88) FcγRIIa glycoprotein. The Ser88 receptor crystals were fragile with receptors arranged in a different and more loosely packed crystalline lattice compared to Phe88 receptor crystals.

The improved properties of FcγRIIa crystals containing the lattice forming Phe88 mutation may have profound implications for the field of macromolecular crystal engineering.

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Keywords: immune system proteins, cell surface receptors, crystal engineering

P.04.11.4
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Crystal Structure of Leukocyte Ig-like Receptor 9 (LIR9/ILT11 /CD85f)
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Leukocyte Immunoglobulin(Ig)-like receptors (LIRs) are human Ig-like receptors that have activating and inhibitory function in leukocytes. LIRs can be subdivided into three groups: inhibitory, activating and secreted molecules. LIR9 is mainly expressed on monocytes and neutrophils as both membrane bound and secreted forms. The membrane bound LIR9 is an activating receptor with arginine residue in its transmembrane region, and the cross-linking of LIR9 induces activation of monocytes. Whereas LIR1 and LIR2, the inhibitory receptors of LIR family, are known to bind to a broad range of human MHC class I molecules (MHCIs), the binding property of LIR9 is unknown. LIR9 shows less homology with LIR1/2/6 recognizing MHCIs (less than 60% amino acid identity with LIR1/2/6). Here we demonstrated that LIR9 had no or very weak affinities to MHCIs by biosensor analysis. Furthermore, we determined the crystal structure of extracellular domain of LIR9 at 1.9 Å resolution by MAD method. The structure showed large structural differences in the region corresponding to the MHCI binding site of LIR1, resulting in the disability of LIR9 to bind to MHCIs. These results raised the possibility that LIR9 recognizes a non-MHCI ligand.
Keywords: immune regulation, immunoglobulin-like receptor, structural immunology

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The Crystal Structure of Human CD1d with and without α-Galactosylceramide Bound
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The glycolipid α-galactosylceramide binds with high affinity to CD1d and stimulates NKT cells. Here we report the crystal structure of human CD1d in complex with synthetic α-galactosylceramide at 3.2 Å resolution. The structure reveals a tightly fit lipid in the CD1d hydrophobic binding groove, with the sphingosine chain bound in the C' pocket and the longer acyl chain anchored in the A' pocket. Pocket volumes and hydrogen bonds to the glycolipid head group optimize α-galactosylceramide binding to CD1d. The structure of CD1d without lipid is also presented which shows a more open conformation of the binding groove than is seen in lipid-bound CD1d, suggesting a dual conformation of CD1d in which the "open" conformation is more able to load lipids than the lipid-bound "closed" conformation.
Keywords: humanCD1d, empty MHC class I-like protein, α-galactosylceramide

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Structure, Function and Evolution of the Serum Pentraxins
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Wide-ranging studies on the serum pentraxins C-reactive protein and serum amyloid P component are aimed at the investigation of the structural, functional and evolutionary relationships, and of the humoral and cellular recognition properties, of the pentraxins from species ranging from invertebrate to man. Recognition properties of the pentraxins, homologues of which have been found in mammals, fish, amphibians, and invertebrates, include cell wall phospholipids and fungal and bacterial polysaccharides. In the absence of highly developed adaptive immunity, a diverse array of humoral components, such as the pentraxins, provides an essential and effective strategy for recognising and destroying disease-causing pathogens.

The structures of pentraxins from man [1], rat, Mustelis canis and L. polyphemus [2,3,4] reveal variable aggregation of the conserved protomer fold, details of novel binding properties and insights in to the relationships between structural, functional and sequence homology.

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Keywords: pentraxin, innate immunity, evolution

P.04.11.7
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Structural Studies of Human CD81 Extracellular Domain
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CD81 is a four transmembrane protein of 236 amino acids, belonging to the tetraspanin protein family, involved in various