Yusung-gu, Daejeon, Daejeon, 305-806, Korea (S), [2]Department of Chemistry, KAIST, Daejeon, Korea 305-701, E-mail：jwhwang@kribb.re.kr

Human thioredoxin1 (hTRX1) is a small 12-kD oxidoreductase enzyme consisting of 105 amino acids and containing a dithiol/disulfide active site with multiple cellular functions. This enzyme has activity as a cellular reductase by a dithiol-disulfide exchange reaction using two cysteine residues (Cys32 and Cys35) in the conserved active site sequence. Apart from the two cysteines, there are three additional conserved cysteines, Cys62, Cys69, and Cys73 in the mammalian TRX, which have not been known to their biological functions. Although it has been identified that the Cys73 residue is involved in dimerization of hTRX via an intermolecular disulfide bond formation between Cys73 of each monomer in the oxidized state, biological function of the Cys62 and Cys69 residues in the non-active remain to be fully elucidated. In the previous paper, researchers proposed that the formation of a disulfide bond between Cys62 and Cys69 could give a way to transiently inhibit hTRX activity for redox signaling or oxidative stress. Furthermore, they proposed a model structure of the non-active site disulfide in the hTRX. Here, we present the high-resolution crystal structure of fully oxidized hTRX1, which shows an intramolecular disulfide bond between Cys62 and Cys69. The disulfide bond formation disengages a helix proximal to the active site and results in a conformational change of the hTRX enzyme, providing a structural basis for understanding the regulation mechanism of redox signaling or oxidative stress.

Keywords: human thioredoxin1, intramolecular disulfide, redox signaling

## P04.23.439

*Acta Cryst*. (2008). A**64**, C367

### Joint neutron and X-ray diffraction studies at 293 K of antifreeze protein

Andre Mitschler[1], Mathew Blakeley[2], Michael Haertlein[2], Christophe Mueller-Dickmann[3], Alexandre Popov[3], Eduardo Howard[4], Isabelle Haertlein[2], Alberto Podjarny[1]
[1]IGBMC, 1 rue Laurent Fries, Illkirch, CUS Strasbourg, 67404, France, [2]ILL, 6 rue Jules Horowitz, Grenoble, France, [3]ESRF, 6 rue Jules Horowitz, Grenoble, France, [4]IFLYSIB, 59 N 789, La Plata, Argentina, E-mail：mitschler@gmail.com

Type III Antifreeze Proteins (AFPs) are small globular monomeric proteins (66 aa, M.W.=7kDa), which are highly homologous. Their shared antifreeze property is linked to a network of hydrogen bonding between a specific lattice plane on ice and several conserved, polar and solvent accessible amino acids located along a flat Ice-Binding Surface (IBS). We shall present our developments: 1) - Neutron Laue data collection on the new LADI III (ILL) on an ab-initio fully deuterated tiny crystal (volume = 0.12 $mm^3$, resolution = 2 Å), including production of fully deuterated protein, crystallization by macro-seeding in $D_2O$. The ratio resolution/volume is similar to the Human Aldose Reductase [1]. 2) - X-ray diffraction at Synchrotron ESRF beamline ID29 on a fully deuterated crystal of the same crystallization batch at a resolution of 1.05 Å, necessary to carry out a joint Neutron - X-ray refinement like for Human Aldose Reductase h AR [2]. 3) Specific H labelling on Leucine and Isoleucine of the fully deuterated protein, in order to create a contrast useful for specific phasing methods for neutron diffraction data. ( Human Frontier Science Program).

Ref. 1. Hazemann, Blakeley et al., Acta Cryst, D61,1413,2005.
Ref. 2. Blakeley, Ruiz et al , PNAS, 105, 1844,2008.

Keywords: neutron diffraction, antifreeze protein, perdeuteraton

## P04.23.440

*Acta Cryst*. (2008). A**64**, C367

### X-ray induced perturbation in an ultra-high resolution protein structure

Kazuki Takeda, Kouji Kusumoto, Yu Hirano, Kunio Miki
Kyoto University, Graduate School of Science, Department of Chemistry, Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto, 606-8502, Japan, E-mail：ktakeda@kuchem.kyoto-u.ac.jp

Positions of hydrogen atoms and orientations of water molecules are important to functions of proteins. However, such information from protein crystals is easily disturbed by radiation damage. The damage can not be prevented completely even in the data collection at cryogenic temperatures. Therefore, influence of X-rays should be estimated exactly in order to bring out meaningful information from crystallographic results. Diffraction data from a single crystal of the high-potential iron-sulfur protein (HiPIP) from *Thermochromatium tepidum* were collected at SPring-8, and were merged into three data sets as exposure to X-rays. The maximum absorption doses were estimated to be $4.5 \times 10^5$, $9.0 \times 10^5$ and $1.4 \times 10^6$ Gy for the three data sets. Structures analyzed at 0.70 Å show detailed views of X-ray induced perturbation such as positional changes of hydrogen atoms of a water molecule. We will discuss about initial steps of radiation damage from the ultra-high resolution analysis.

Keywords: electron transfer, high-resolution protein structures, radiation damage

## P04.23.441

*Acta Cryst*. (2008). A**64**, C367-368

### High resolution crystals of human hematopoietic & lipocalin-type prostaglandin D synthases in space

Hiroaki Tanaka[1], Koji Inaka[2], Toshiharu Tsurumura[3], Kosuke Aritake[3], Masaru Sato[4], Sachiko Takahashi[1], Mari Yamanaka[1], Naoki Furubayashi[2], Erika Hirota[1], Satoshi Sano[4], Tomoyuki Kobayashi[4], Tetsuo Tanaka[4], Yoshihiro Urade[3]
[1]Confocal Science Inc., Wakamatsu Building 7F, 3-3-6 Nihonbashi Hon-cho, Chuo-ku, Tokyo, 103-0023, Japan, [2]Maruwa Foods and Biosciences, 170 Tsutsui-cho, Yamatokoriyama, Nara, 639-1123, Japan, [3]Osaka Bioscience Institute, 6-2-4, Furuedai, Suita-shi, Osaka, 565-0874, Japan, [4]Japan Aerospace Exploration Agency, 2-1-1 Sengen, Tsukuba, Ibaraki, 305-8505 Japan, E-mail：tanakah@confsci.co.jp

Hematopoietic and lipocalin-type prostaglandin (PG) D synthases (H-PGDS and L-PGDS) are responsible for production of PGD2, which acts as an allergic inflammatory mediator[1] and an endogenous sleep-promoting substance[2], respectively. The specific inhibitors of each enzyme are important for suppression of various diseases. To obtain high-quality crystals for structural analysis, we crystallized both H-PGDS and L-PGDS by using a counter-diffusion method under a microgravity environment on the International Space Station (ISS). The three-dimensional structure of human H-PGDS has already been determined in a complex with an H-PGDS inhibitor HQL-79 (PDB ID: 2CVD)[3]. Since novel H-PGDS inhibitors with affinities 100-fold higher than HQL-79 have recently been developed, we obtained high quality crystals of human H-PGDS in complexes with those novel inhibitors by using PEG 6000 as a precipitant in microgravity. The crystals showed diffraction up to 1.2

Å resolution with R-merge 5.7, mosaicity 0.295 and completeness 86.9%[4]. On the other hand, L-PGDS crystals were previously obtained with citrate as a precipitant and diffracted to 2.0 Å (PDB ID: 2CZT). In microgravity, we obtained L-PGDS crystal which diffracted up to 1.0 Å resolution with R-merge 0.064, mosaicity 0.16 and completeness 98.8%.

References
[1] Urade, Y. et al. (1989). J. Immunol. 143, 2982-2989.
[2] Qu, W.-M. et al. (2006). Proc. Natl. Acad. Sci. USA. 103(47), 17949 − 17954.
[3] Aritake, K. et al. (2006). J. Biol. Chem. 281, 15277-15286.
[4] Takahashi, S. et al.(2008) Acta Cryst. F, submitted.

Keywords: atomic resolution crystallography, inhibitor and drug design, microgravity crystallization

## P04.23.442

*Acta Cryst*. (2008). A**64**, C368

### Protein helix-dipole calculations based on experimental electron densities

Dorothee C. Liebschner, Benoit Guillot, Christian Jelsch, Claude Lecomte
Faculte des Sciences et Techniques, LCM3B, BP 239, Boulevard des Aiguillettes, Vandoeuvre-les-Nancy, Lorraine, 54506, France, E-mail：dorothee.liebschner@lcm3b.uhp-nancy.fr

Since the 1970's, it has been assumed that the alpha helix represents a macro dipole. This is due to the dipole moment of the individual peptide units, which are aligned parallel to the helix-axis and thus form a macro-dipole. The helix-dipole is guessed to have effects upon the functioning and structure of proteins [1]. However, the exact dipole strength is still unclear. Estimations include only the atomic charges of the main chain and not of the side chains. Dielectric measurements of the dipole strength using helical oligopeptides in solution have been made [2], but lack of precision as the percentage of the peptides with helical conformation is unknown. Here, we propose an estimation of the alpha-helix-dipole referring to experimental electron density distributions. The electron density of isolated amino acid compounds has been refined with the program MoPro (Molecular Properties, [3]) using the Hansen and Coppens multipole formalism [4] to create an experimental electron density distribution database (ELMAM, [5]). The atomic charges described in the ELMAM database are then transferred to the protein. The dipole moment of the alpha-helix is calculated either by using the transferred charges or, if the protein diffracts at subatomic resolution below 0.8 Å, by using charges that are refined against X-ray diffraction data.

[1] Hol W., Adv. Biophys. (1985) 19, 133-165
[2] Schwarz G., Savko P., Biophys. J., 39 (1982), 211
[3] Jelsch C., Guillot B., Lagoutte A., Lecomte C., J. Appl. Cryst. (2005) 38, 38-54
[4] Hansen N., Coppens P., Acta Cryst. (1978) A34, 909-921
[5] B. Zarychta, V. Pichon-Pesme et al., Acta Cryst. (2007). A63, 108-125

Keywords: helix-dipole, subatomic resolution, electron deformation density

## P04.23.443

*Acta Cryst*. (2008). A**64**, C368

### Charge density analysis of human aldose reductase active site

Benoit Guillot[1], Christian Jelsch[1], Alberto Podjarny[2], Claude Lecomte[1]
[1]LCM3B, Faculté des Sciences et Techniques, Vandoeuvre-lès-Nancy, France, 54506, France, [2]IGBMC, Structural Biology and Genomics Department, Strasbourg, France, E-mail：benoit.guillot@lcm3b.uhp-nancy.fr

Human aldose reductase (hAR) is a NADPH dependent enzyme involved in diabetes complications. Crystals of hAR in complex with NADPH and the Idd594 inhibitor diffract up to 0.66Å resolution [1]. Such subatomic resolution X-ray data allows, after a restrained IAM refinement, the observation of significant residual deformation electron density on covalent bonds. This residual electron density peaks are the deviation from the spherical approximation of the atomic electron clouds, due to chemical bonding. The Hansen & Coppens [2] multipolar model, implemented in the MoPro [3] software, allows taking into account the deformation electron density. Hence a constrained multipolar refinement of hAR has been performed, leading to decreased atomic thermal motion amplitudes and better stereochemistry [4]. Here we present the continuation of this work: a precise, unconstrained, charge density analysis focusing on hAR active site. Deformation electron densities of the NADPH cofactor, the Idd594 inhibitor and the catalytic amino acids have been modelled using the multipolar formalism. Then a topological analysis of hAR active site electron density has been performed in order to derive informations related to the inhibitor binding and the catalytic mechanism.

1 Howard E. et. al., Prot. Struct. Funct. & Gen. 2004. 55, 792-804.
2 Hansen, N.K., Coppens, P., Acta. Cryst. 1978. A34, 909-921.
3 Guillot, B., Lecomte, C., Podjarny, A., Jelsch, C. Acta. Cryst. D, in press.
4 Jelsch, C., Guillot, B., Lagoutte A., Lecomte C., J. Appl. Cryst. 2005. 38, 38-54.

Keywords: charge density, high-resolution crystallography, protein ligands

## P04.23.444

*Acta Cryst*. (2008). A**64**, C368−369

### Ultra-high resolution structure of endopolygalacturonase determined by X-ray and neutron diffraction

Tetsuya Shimizu[1,2], Toru Nakatsu[1,2], Nobutaka Shimizu[2,3], Mamoru Sato[4], Kazuo Kurihara[5], Kazuo Miyairi[6], Toshikatsu Okuno[6], Nobuo Niimura[7], Masaki Yamamoto[2,3], Hiroaki Kato[1,2]
[1]RIKEN SPring-8 Center, Division of Synchrotron Radiation Instrumentation, 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, [2]RIKEN, SPring-8 center, 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, [3]SPring-8/JASRI, 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo, 679-5148, Japan, [4]Graduate School of Integrated Science, Yokohama City University, 1-7-29 Suehirocho, Tsurumi-ku, Yokohama 230-0045, Japan, [5]JAEA, Tokai, Ibaraki 319-1195, Japan, [6]Faculty of Agriculture and Life Sciences, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan, [7]Graduate School of Science and Engineering, Ibaraki University, 2-1-1 Bunkyo, Mito, Ibaraki 310-8512, Japan, E-mail：shimizu@spring8.or.jp

Endopolygalacturonases (endoPGs) are inverting glycosidases that