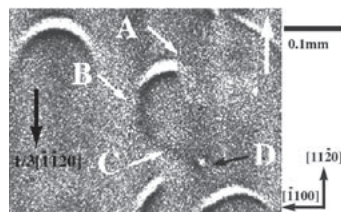


Phy 27 (1988) 169.

Keywords: activity, MBE, gallium nitrides

**MS.49.6***Acta Cryst.* (2008). A64, C89**Contrast of dislocations in 4H-SiC by SR topography in grazing-incidence geometry**Hirofumi Matsuhata<sup>1</sup>, Hiroataka Yamaguchi<sup>1</sup>, Toshiyuki Ono<sup>2</sup>, Bin Chen<sup>3</sup>, Takashi Sekiguchi<sup>3</sup><sup>1</sup>National Institute of Advanced Science and Technology, Laboratory of Energy Semiconductor Electronics, 1-1-1, Umezono, Tsukuba, Ibaraki, 305-0032, Japan, <sup>2</sup>Central Research Laboratory, Hitachi Ltd, 1-280 Higashi-Koigakubo, Kokubunji Tokyo 185-8601, Japan, <sup>3</sup>National Institute for Materials Science, Namiki, Tsukuba 305-0044, Japan, E-mail: h.matsuhata@aist.go.jp

Dislocations near surface of 4H-SiC were observed using synchrotron radiation topography in the Bragg case with grazing-incidence geometry. Figure is an image of basal-plane dislocation half-loop at  $g=11\text{-}28$ ,  $\lambda=0.15\text{nm}$  on Si-face. The (0001) plane is tilted towards the  $[-1\text{-}120]$  direction by 8 degrees from the surface. In this condition, lattice defects within 10  $\mu\text{m}$  depth are observed. Along this dislocation line, bright contrast at A, dark and bright asymmetric line at B, and dark contrast at C are observed. Absence of contrast can be seen at B at  $g=1\text{-}108$ , and so that B is a screw dislocation part. We have observed migrations of dark dislocations in specimens after forward-bias degradation effect, in which Si-core dislocations are known to move. Thus we concluded that C is Si-core, A is C-core edge dislocation, and the Burgers vector is  $1/3[-1\text{-}120]$ . The observed dark and bright contrast is discussed to be similar effect described by Ando and Kato (1970). By applying this rule we could identify uniquely 6 different Burgers vectors for all basal-plane dislocations and threading edge dislocations at only one diffraction condition. Ando and Kato: *J. Appl. Cryst.* 3 (1970) 74.



Keywords: wide-bandgap semiconductors, dislocations, topography X-ray

**MS.50.1***Acta Cryst.* (2008). A64, C89**Decoding homophilic recognition specificity of Dscam, a neuronal receptor with thousands isoforms**

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The Dscam gene gives rise to thousands of diverse cell surface receptors thought to provide homophilic and heterophilic recognition specificity for neural development and immune responses. Mutually exclusive splicing allows for the generation of sequence variability in three immunoglobulin (Ig) ecto-domains (D2, D3, D7). X-ray structures of the N-terminal four Ig domains (D1-D4) of four distinct Dscam isoforms have been determined. The structures

reveal a horseshoe configuration, with variable residues of D2 and D3 constituting two independent surface-epitopes on either side of the receptor. All these four isoforms engage in homo-dimerization coupling variable domains D2 with D2 and D3 with D3 using the same epitope. The recognition specificity has been analyzed to decode how sequence and local conformation of these two variable domains contribute to homophilic interaction. The structure of the third Ig-like domain D7 has also been determined in the form of D7-D8 fragment for several isoforms. A general view of how these variable Ig domains embedded in thousands receptor isoforms offer homophilic recognition for neuronal wiring has been provided.

Keywords: dscam receptor, decoding recognition specificity, thousand isoforms

**MS.50.2***Acta Cryst.* (2008). A64, C89**Crystal structure of the [2Fe-2S] transcriptional activator SoxR bound to DNA**Kunio Miki<sup>1,2</sup>, Satoshi Watanabe<sup>1</sup>, Akiko Kita<sup>1</sup>, Kazuo Kobayashi<sup>3</sup><sup>1</sup>Kyoto University, Department of Chemistry, Graduate School of Science, Sakyo-ku, Kyoto, Kyoto, 606-8502, Japan, <sup>2</sup>RIKEN Spring-8 center at Harima Institute, Koto 1-1-1, Sayo, Hyogo 679-5148, Japan, <sup>3</sup>Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan, E-mail: miki@kuchem.kyoto-u.ac.jp

SoxR functions as a sensor of oxidative stress such as superoxide and nitric oxide. It exists as a dimer with each subunit containing a [2Fe-2S] cluster. Reversible oxidation of the [2Fe-2S] cluster activates SoxR to enhance the production of various antioxidant proteins through the *soxRS* regulon. SoxR belongs to the MerR family of transcriptional activators, target promoters of which have an unusual 19 or 20 bp spacer between the -35 and -10 operator elements. In the active state, SoxR and other MerR family proteins activate transcription from unique promoters by distorting the DNA conformation. In order to elucidate structural features of the iron-sulfur cluster of SoxR and the transcriptional activation mechanism, we have determined the crystal structures of SoxR and its complex with DNA in the oxidized (active) state [1]. The overall structure of SoxR consists of a DNA binding domain, a dimerization helix and an Fe-S cluster binding domain. The dimerization helix forms an antiparallel coiled-coil, stabilizing the SoxR dimer. The structures reveal that the [2Fe-2S] cluster of SoxR is unusually solvent-exposed and surrounded by an asymmetric environment, suggesting that the asymmetrically charged environment is a key factor of redox-dependent conformational changes of SoxR and the target promoter. The DNA structure is shown to be sharply bent at the middle and unwound by 3-bp, compared to a B-form DNA. Based on comparison of the target promoter sequences of the MerR family, the present structures shows an activated promoter conformation with a 20-bp spacer in the MerR family.

[1] Watanabe S, Kita A, Kobayashi K, Miki K., *Proc Natl Acad Sci USA*, 2008, 105, 4121.

Keywords: SoxR protein, MerR family, transcription factors

**MS.50.3***Acta Cryst.* (2008). A64, C89-90**Hybrid LRR technique and crystal structures of the toll-like receptor complexes**

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Toll-like Receptors (TLRs) are central to vertebrate innate immune responses. To facilitate soluble expression and crystallization of human TLRs with bound ligands, we have developed a novel technique that we term the Hybrid LRR Technique. The hagfish VLR proteins were chosen as the fusion partners and connected to human TLRs at the conserved LxxLxLxxN regions. The hybrid LRR technique neither interrupts function of TLR nor causes substantial structural changes. TLR4 and MD-2 form a heterodimer that recognizes LPS from Gram negative bacteria. TLR2 in association with TLR1 or TLR6 responses to microbial lipoproteins and lipopeptides. The crystal structures reveal that TLR1, 2 and 4 are atypical members of the LRR family and are composed of N-terminal, central and C-terminal domains. The beta sheet of the central domain shows unusually small radii and large twist angles. MD-2 binds to the concave surface of the N-terminal and central domains of TLR4. The interaction with Eritoran, a candidate anti-sepsis drug, is mediated by a hydrophobic internal pocket in MD-2. Binding of the tri-acylated lipopeptide, Pam3CSK4, induced the formation of an m shaped heterodimer of the TLR1 and TLR2 ectodomains whereas binding of the di-acylated lipopeptide, Pam2CSK4 did not. The three lipid chains of Pam3CSK4 mediate the heterodimerization of the receptor; the two ester-bound lipid chains are inserted into a pocket in TLR2, while the amide-bound lipid chain is inserted into a hydrophobic channel in TLR1. An extensive hydrogen bonding network, as well as hydrophobic interactions, between TLR1 and TLR2 further stabilize the heterodimer. We propose that formation of the TLR dimer brings the intracellular TIR domains close to each other to promote dimerization and initiate signaling.

Keywords: protein structure, TLR, LRR

### MS.50.4

*Acta Cryst.* (2008). A64, C90

#### Crystal structure of the sodium pump at 3.5 Å

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The Na<sup>+</sup>,K<sup>+</sup>-ATPase, the sodium-potassium pump, was first described in 1957 by Jens C. Skou (1) - a discovery for which he was awarded the Nobel prize in Chemistry in 1997. The Na<sup>+</sup>,K<sup>+</sup>-ATPase belongs to the P-type ATPase family, and via formation and break-down of phosphoenzyme intermediates it derives the energy from ATP hydrolysis to pump Na<sup>+</sup> out of the cell and K<sup>+</sup> into the cell, thereby energizing the plasma membrane with steep electrochemical gradients for these key cations. The Na<sup>+</sup>,K<sup>+</sup>-ATPase is a heterotrimeric complex composed of an  $\alpha$ ,  $\beta$  and  $\gamma$  chain that all contain transmembrane segments. A complete native dataset was obtained at 3.5 Å resolution on the X06SA beam line at the Swiss Light Source (SLS). The brilliant light source present at SLS was necessary to obtain useful data from these very weakly diffracting crystals. A low resolution molecular replacement solution allowed us to identify two heavy-atom derivatives by difference-Fourier analysis forming the basis for MIRAS phasing at 7 Å resolution. The crystal form has 75%

solvent and contains two-fold NCS. Careful density modification with NCS and inter-crystal averaging was applied and extended the MIRAS phases to 3.5 Å resolution thus allowing for model building and refinement of the structure. This the first structure of the Na<sup>+</sup>,K<sup>+</sup>-ATPase in the K<sup>+</sup>/Rb-bound form contains a nearly complete model of the  $\alpha$ -subunit and shows the location of the transmembrane helices of the beta and gamma subunits associated with alpha. Two strong peaks in the anomalous difference Fourier map pinpoint the position of two occluded Rb<sup>+</sup> sites (K<sup>+</sup>)(2).

References:

- (1) J. C. Skou (1957) *Biochim Biophys Acta.* 2,394-401
- (2) Morth et al. (2007). Crystal structure of the sodium-potassium pump. *Nature.* 450,1043-9

Keywords: sodium pump, ATPase, membrane protein

### MS.50.5

*Acta Cryst.* (2008). A64, C90

#### Structural studies of pre-mRNA 3'-end processing

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Most eukaryotic messenger RNA precursors (pre-mRNAs) must undergo extensive maturational processing, including 3'-end cleavage and polyadenylation. Despite the characterization of a large number of proteins that are required for the cleavage reaction, the identity of the endoribonuclease is not known. In addition, very little structural information is available for the proteins involved in 3'-end processing, which include the cleavage and polyadenylation specificity factor (CPSF), cleavage stimulation factor (CstF), cleavage factors I and II (CFI and CFII), and poly(A) polymerase. This 3'-end processing machinery also plays an important role in transcriptional termination by RNA polymerase II. We have recently determined the crystal structures of the 73 kD and 100 kD subunits of CPSF (1), as well as the 77 kD subunit of CstF (2). Our studies provide direct structural and biochemical evidence that CPSF-73 is the endoribonuclease for the cleavage reaction and reveal a dimeric association of the CstF-77 subunit that may be crucial for pre-mRNA 3'-end processing. Our latest results on other factors in this machinery will also be presented. Crystallization of CPSF-100 and CstF-77 required *in situ* proteolysis (3,4), and this attractive technique of crystallization will be described as well. Supported in part by a grant from the NIH.

References:

1. C.R. Mandel, S. Kaneko, H. Zhang, D. Gebauer, V. Vethantham, J.L. Manley & L. Tong. (2006). *Nature*, 444, 953-956.
2. Y. Bai, T.C. Auperin, C.-Y. Chou, G.-G. Chang, J.L. Manley & L. Tong. (2007). *Mol. Cell*, 25, 863-875.
3. C.R. Mandel, D. Gebauer, H. Zhang & L. Tong. (2006). *Acta Cryst.* F62, 1041-1045.
4. Y. Bai, T.C. Auperin & L. Tong. (2007). *Acta Cryst.* F63, 135-138.

Keywords: protein complex, ribonucleases, protein crystallization and *in situ* proteolysis