16 of their cysteines. Although no clear evidence exists for a physiological function of mammalian CRISP found mainly in the epididymis and salivary glands, snake venom CRISP are known to inhibit smooth muscle contraction and cyclic nucleotide-gated (CNG) ion channels. The structure of CRISP-*a* from *Naja atra* is determined at 1.58-Å resolution using the sulfur-SAD method and consists of unique disulfide patterns and two distinct structural domains: a protease sandwich fold (N-terminal) and an ion channel-blocking BgK toxin fold (C-terminal). With one positively charged cluster found at water accessible helix regions next to the Ser-His-Glu triad of the protease domain, heparin binding plays a role in regulating CRISP-*a* activity. As important residues identified to block CNG and K^+ channels of other toxin homologues are located at one face of the ion channel-blocking domain, the structure provides a basis for rational design of a peptide blocker of the CNG channel. The ion channel-blocking domain and heparin-binding site of CRISP-*a* are suggested to play a chaperone role in leading it to the site of protease action for remodeling of the extracellular matrix in mammalian cells.

Keywords: sulfur-SAD phasing, toxin CRISP structure, heparin

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Structure of Parasporin-1, a Novel Bacterial Cytotoxin against Human Cancer Cells

Toshihiko Akiba[a], Tokio Ichimatsu[b], Hideki Katayama[b], Tetsuyuki Akao[b], Osamu Nakamura[b], Eiichi Mizuki[b], Michio Ohba[c], Kazuaki Harata[a], *[a]BIRC, AIST, Tsukuba. [b]BFRI, FITC, Kurume. [c]Grad. Sch. Agric., Kyushu Univ., Fukuoka, Japan.* E-mail: k-harata@aist.go.jp

The crystal structure of parasporin-1 from *Bacillus thuringiensis* strain A1190 has been determined at 1.76 Å resolution. Parasporin-1 belongs to the Cry protein family, which includes insecticidal pore-forming toxins successfully utilized in agriculture; however, the protein is not insecticidal but specifically toxic to particular types of cultured human carcinoma cells. This strict selectivity suggests its potential use as an anti-cancer drug.

Parasporin-1 has a three-domain architecture common to available structures of other insecticidal Cry proteins; the main chain of each domain is superimposed reasonably well with their counterparts in spite of low sequence homology. Significant deviations are found in a few limited regions. Of particular interest is the N-terminal extension upstream of domain 1, which clamps the domain to domain 2 and which presumably disable the transformation of the domain necessary for pore formation. Among the available Cry protein structures, only the inactive Cry2Aa protoxin has an analogous structure. These observations along with biochemical results [1] suggest that parasporin-1 may act as a simple ligand to activate an unidentified signaling pathway leading to malfunction of membrane channels rather than as a pore-forming toxin.

[1] Katayama H., et al., *J. Biochem.*, 2005, **137**, 17.

Keywords: pore-forming toxins, anticancer biochemistry, receptor recognition

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Structure of Diol Dehydratase Reactivating Factor – A Novel Molecular Chaperone

Naoki Shibata[a], Koichi Mori[b], Naoki Hieda[b], Mamoru Yamanishi[b], Yoshiki Higuchi[a], Tetsuo Toraya[b], *[a]Graduate School of Science, University of Hyogo, Japan. [b]Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Japan.* E-mail: shibach@sci.u-hyogo.ac.jp

Diol dehydratase and glycerol dehydratase are adenosylcobalamin-dependent enzymes that catalyze the conversion of 1,2-propanediol, 1,2-ethanediol and glycerol to the corresponding aldehydes. Glycerol, a physiological substrate for the enzyme, inactivates the enzyme in an irreversible manner. Diol dehydratase reactivating factor is a molecular chaperone, reactivating the inactivated diol- and glycerol dehydratases in the presence of AdoCbl, ATP and Mg^{2+}. Here we report the crystal structures of ADP-bound and nucleotide-free forms of diol dehydratase reactivating factor.

Initial electron density map of the selenomethionine-substituted ADP-bound form was obtained from the MAD diffraction data collected at the BL38B1 beam line, SPring-8, Japan. Diffraction data sets for native ADP-bound and nucleotide-free crystals were collected at the BL41XU beam line, SPring-8, Japan.

Structure of nucleotide-free diol dehydratase reactivating factor is similar to that of nucleotide-free glycerol dehydratase reactivating factor reported by Liao *et al*. [1]. The ADP-bound form of diol dehydratase reactivating factor shows rearrangement of domains with respect to its nucleotide-free form.

[1] Liao, et al., *Structure*, 2003, **11**, 109.

Keywords: diol dehydratase reactivating factor, molecular chaperone, crystal structure

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Structural and Functional Analysis of PDI–related Proteins

Madhumati Sevvana[a], Qingjun Ma[a], Kathrin Barnewitz[b], Chaoshe Guo[b], Hans-Dieter Söling[b], David M. Ferrari[b], George M. Sheldrick[a], *[a]Lehrstuhl für Strukturchemie, University of Göttingen. Department of Neurobiology, [b]MPI, Göttingen, Germany.* E-mail: msevvana@shelx.uni-ac.gwdg.de

Protein Disulfide Isomerase[PDI]–related proteins are residents of the endoplasmic reticulum and are involved in several functions, some of which include redox and chaperone activities. Their function involves several non-covalent weak interactions with specific epitopes on substrate proteins. The molecular basis of these interactions has not been understood until recently [2].

We recently elucidated the first crystal structure of such a eukaryotic PDI–related chaperone, Wind from Drosophila [1]. It has been identified that Wind binds Pipe (a 2-*O*-sulfotransferase) *in vitro*. A putative peptide binding site has been mapped on the b'-domain for substrate binding with the requirement of the integrity of a surface on the d'-domain. Crystal structures of several Wind–mutants and their complexes with the peptides mimicking the Pipe binding site were elucidated giving some clues about the binding mechanism. Further, the structure of a mammalian orthologue of Wind, Erp28 has been solved, suggesting a functional role for the structural conservation between the proteins.

[1] Ma Q., Guo C., Barnewitz K., Sheldrick G. M., Söling H. D., Uson I., Ferrari D. M., *JBC*, 2003, **278**, 44600. [2] Barnewitz K., Guo C., Sevvana M., Ma Q., Sheldrick G. M., Söling H. D., Ferrari D. M., *JBC*, 2004, **279**, 39829.

Keywords: chaperone, protein disulfide isomerase, Wind

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TPR Repeat Domain of O-linked GlcNAc Transferase: Similarities to Importin Alpha

Martin Jinek[a], Jan Rehwinkel[a], Brooke Lazarus[b], Elisa Izaurralde[a], John A. Hanover[b], Elena Conti[a]. *[a]European Molecular Biology Laboratory, Heidelberg, Germany. [b]National Institute of Diabetes and Digestive and Kidney Disease, NIH, Bethesda, USA.* E-mail: jinek@embl.de

Addition of N-acetylglucosamine (GlcNAc) is a ubiquitous form of intracellular glycosylation, catalyzed by the conserved O-linked GlcNAc transferase (OGT). OGT contains an N-terminal domain of tetratricopeptide (TPR) repeats that mediates the recognition of a broad range of target proteins. Nuclear pore complex components are major OGT targets, as OGT depletion by RNAi results in the loss of GlcNAc modification at the nuclear envelope. To gain insight into the mechanism of target recognition, we solved the crystal structure of the homodimeric TPR domain of human OGT, containing 11.5 TPR repeats[1]. The repeats form an elongated superhelix. The concave surface of the superhelix is lined by absolutely conserved asparagine residues, in a manner reminiscent of the peptide-binding site of importin α. Based on this structural similarity, we propose that OGT employs an analogous molecular mechanism to recognize its targets.

[1] Jinek M., Rehwinkel J., Lazarus B.D., Izaurralde E., Hanover J.A., Conti E., *Nat. Struct. Mol. Biol.*, 2004, **11**, 1001.
Keywords: glycosylation, TPR repeat, protein-protein interactions

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Structural Studies of Quinolinate Synthase
Erika Soriano, Ethan Settembre, Tadhg P. Begley, Steven E. Ealick, *Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA*. E-mail: evs4@cornell.edu

Nicotinamide adenine dinucleotide (NAD) is an essential cofactor in several metabolic pathways and has recently been shown to play a role in several signaling pathways [1]. Consequently, there is great interest in the biosynthesis of NAD. Quinolinate is the universal precursor in the de novo biosynthesis of NAD and can be synthesized from either tryptophan in the case of eukaryotes or aspartate in most prokaryotes [2].

The aspartate pathway begins with L-aspartate oxidase which converts aspartate to iminoaspartate. Quinolinate synthase (QS) catalyzes the condensation of iminoaspartate and dihydroxyacetone phosphate to form quinolinic acid [3]. This enzyme has been difficult to characterize due to either instability or inactivity when it is overexpressed and purified.

QS is the last enzyme in this pathway to be structurally characterized. We have determined the crystal structure of QS at 2.8 Å resolution. The crystal structure and sequence alignments provide insights into the details of the active site and the enzyme's evolution.

[1] Berger F., Ramírez-Hernández M.H., Ziegler M., *Trends Biochem. Sci*., 2004, **29**, 111. [2] Magni G., Amici A., Emanuelli M., Raffaelli N., Ruggieri S., *Adv Enzymol. Relat. Areas Mol. Biol.*, 1999, **73**, 135. [3] Nasu S., Gholson R.K., *Biochem. Biophys. Res. Commun.*, 1981, **101**, 533.
Keywords: quinolinate synthase, NAD biosynthesis, quinolinate

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A Second FMN-binding Site in Yeast CPR Suggests a Novel Mechanism of Electron Transfer by Diflavin Reductases
Larissa M. Podust, Michael R. Waterman, *Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, USA.* E-mail: larissa.m.podust@vanderbilt.edu

NADPH-cytochrome P450 reductase (CPR) transfers two reducing equivalents from NADPH via FAD and FMN to the large super family of microsomal cytochrome P450 monooxygenases (CYPs) in one-electron transfer steps. Mechanism of electron transfer by diflavin reductases remains elusive and controversial. We determined the crystal structure of CPR from *Saccharomyces cerevisiae*, which is functionally active toward its physiological substrate cytochrome P450 and discovered a second FMN-binding site at the interface of the connecting and FMN-binding domains. We propose that during catalytic turnover a single FMN molecule shuttles twice between two protein sites that accommodate two different semiquinone forms, neutral (blue) and anionic (red). Oscillating between two sites FMN presumably swings along the interface between the reductase domains circumscribing about half a circle of the 10 Å radius around invariant D187 as the center of rotation, so that the FMN N5-reference atom relocates approximately 20 Å, while the ribityl moiety remains within interaction distances from the carboxyl of D187 and T71. Yeast CPR loses the ability to support the catalytic function of CYP51 upon substitution of D187 or T71 with alanine. We believe that the proposed mechanism will move forward our understanding of electron transfer by diflavin reductases (including nitric oxide synthase (NOS)) since these electron transporters are highly homologous genetically, structurally, and functionally to CPR.
Keywords: diflavin reductase, electron transfer, FMN-binding

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Identification and Purification of a Soluble Region of BubR1
Dohyun Han[1], Jongchul Yoon[1,2], Yup Kang[3,2], Kyunggon Kim[1], Jungeun Park[1], Youngsoo Kim[1,4], *Division of [1]Molecular Genomic Medicine and [4]Cancer Research Institute, Seoul National University. [3]Insitute for Medical Sciences, Ajou University School of Medicine.* E-mail: hdh03@snu.ac.kr

The mitotic checkpoint complex (MCC) ensures the fidelity of chromosomal segregation, by delaying the onset of anaphase until all sister chromatids have been properly attached to the mitotic spindle. In essence, this MCC-induced delay is achieved via the inhibition of the anaphase-promoting complex (APC). Among the MCC components, BubR1 plays two major roles in the functions of the mitotic checkpoint. First, BubR1 is able to inhibit APC activity, either by itself or as a component of the MCC. Second, BubR1 activates mitotic checkpoint signaling cascades.

To determine the structure of BubR1, we obtained a soluble BubR1 constructs using a three-step expression strategy. First, we obtained two constructs from BLAST sequence homology searches, both of which were expressed abundantly in the inclusion bodies. Second, we adjusted the lengths of the two constructs by secondary structure prediction, thereby generating partially soluble constructs. Third, we optimized the solubility of the two constructs by modification at the C-terminus. Finally, we obtained a highly soluble BubR1 protein via the *E. coli* expression system.

This report may provide insight into the design of highly soluble constructs of insoluble multi-domain proteins.
Keywords: protein secondary structure analysis, cell-cycle proteins, solubility

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Structural Basis for the Cell-specific Activity of NGFI-B/Nurr1 Ligand-binding Domains
Ralf Flaig[a], Holger Greschik[b], Carole Peluso-Iltis[b], Dino Moras[b], *[a]Diamond Light Source, UK. [b]IGBMC, Strasbourg, France.* E-mail: ralf.flaig@diamond.ac.uk

NGFI-B is a ligand-independent orphan nuclear receptor of the NR4A subfamily that displays important functional differences with its homolog Nurr1. In particular, the NGFI-B ligand-binding domain (LBD) exhibits only modest activity in cell lines in which the Nurr1 LBD strongly activates transcription. To gain insight into the structural basis for the distinct activation potentials, we determined the crystal structure of the NGFI-B LBD at 2.4 Å resolution. Superimposition with the Nurr1 LBD revealed a significant shift of the position of helix 12, potentially caused by conservative amino acid exchanges in helix 3 or helix 12. Replacement of the helix 11-12 region of Nurr1 by that of NGFI-B dramatically reduces the transcriptional activity of the Nurr1 LBD. Mutation of M414 in helix 3 to leucine, or of L591 in helix 12 to isoleucine (the corresponding residues found in NGFI-B) significantly affects Nurr1 transactivation. Swapping the helix 11-12 region of Nurr1 into NGFI-B results in a modest increase of activity. These observations reveal a high sensitivity of LBD activity to changes that influence helix 12 positioning. Mutation of hydrophobic surface residues in the helix 11-12 region (outside the canonical co-activator surface constituted by helices 3, 4 and 12) severely affects Nurr1 transactivation. Together, our data suggest that a novel co-regulator surface that includes helix 11 and a specifically positioned helix 12 determine the cell type-dependent activities of the NGFI-B and the Nurr1 LBD.
Keywords: ligand-binding domain, transcription, NGFI-B

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Structure of a Cell Polarity Regulator, an aPKC and Par6 PB1 Domain Complex
Yoshinori Hirano[a], Sosuke Yoshinaga[a], Ryu Takeya[b], Nobuo N. Suzuki[a], Masataka Horiuchi[a], Motoyuki Kohjima[b], Hideki Sumimoto[b], Fuyuhiko Inagaki[a], *[a]Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Hokkaido University, N-12, W-6, Kita-ku, Sapporo, Japan. [b]Medical Institute of Bioregulation, Kyushu Universuty, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Japan.* E-mail: y-h@pharm.hokudai.ac.jp