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The Structural Domains of the Early B-cell Factors (EBF). Marina I Siponen[a], Magdalena Wisniewska[a], Lari Lehtiö[b], Ida Johansson[a], Helena Berglund[a]. [a]*Structural Genomics Consortium, Department of Molecular Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.* [b]*Division of Pharmacy, Department of Biochemistry and Pharmacy, Åbo Akademi University, Turku, Finland.*
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The human Early B-cell fctor (EBF) family of transcription factors plays a variety of developmental roles [1]. Roles for this four-member family of proteins (EBF1-4) include B-cell development in lymphopoiesis, neuronal development, ***osteogenesis*****,** and adipogenesis. Furthermore, recent studies have started identifying the absence of expressed EBFs in different cancer forms such as leukemias (EBF1) and glioblastomas (EBF3), suggesting a new tumor suppressor role for EBF family members [2]. The structured part of the EBFs is composed of a DNA binding domain (DBD), a TIG (transcription factor immunoglobulin) domain, and an atypical helix-loop-helix (HLH) region. An additional unstructured trans-activation domain is also present in the C-terminus. The EBF proteins show very high homology within the family over the whole structured part, consequently they can bind to specific DNA response elements as homo or hetero-dimers. Although the different domains have been, to a certain extent, biochemically characterized, no structural information was yet available for the EBF proteins. Structural data is of particular importance for this family since they share very low sequence similarity with other transcription factors. Using a domain approach, we have independently solved both the DBD and TIG+HLH domains of human EBF1 and EBF3, respectively. Despite low sequence similarity, the DBD structure reveals a striking resemblance to the DNA binding domains of the Rel homology superfamily of transcription factors. Interesting features of this domain include an atypical zinc binding site, termed Zn-knuckle, a region previously coined as important for specific DNA binding and transcriptional activation [3]. The TIG+HLH domain reveals an interesting dimerisation motif likely involved in protein-protein interactions, with either family members or regulatory partners.

[1] Liberg D, Sigvardsson M, Akerblad P. (2002) *Molecular and Cellular Biology* 22: 8089-8397, [2] Liao D. (2009) *Mol Cancer Res.* 7(12):1893-901, [3] Fields S, Ternyak K, Gao H, Ostraat R, Akerlund J, Hagman J. (2008) *Molecular Immunology* 45**:** 3786-3796

Keywords: Transcription regulation, Biochemistry of DNA proteins, X-ray protein crystallography

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Structural insight into *Neisseria meningitidis* PorB during pathogenesis. Mikio Tanabe[a], Crina Nimigean[b], Tina Iverson[c]. [a]*ZIKHALOmem, Martin-Luther-Universität Halle-Wittenberg, Institut für Biochemie und Biotechnologie, Halle(Saale), Germany.* [b]*Departments of Anesthesiology, Weill Cornell Medical College, New york, NY, USA.* [c]*Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA.*
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PorB is the second most prevalent outer membrane protein in *Neisseria meningitidis*. PorB is required for bacterial survival and neisserial pathogenesis, but is also able to elicit a Toll-like receptor mediated host immune response. During infection, PorB can integrate into host cell mitochondria and likely binds mitochondrial ATP, which is thought to be important for cellular apoptosis. We have determined the x-ray crystal structure of PorB at 2.3 Å resolution. Structural analysis and co-crystallization with substrate molecule suggest three distinct putative solute translocation pathways (non-slective cation, non-selective anion and sugar specific) through the channel pore. Co-crystallization with the ATP analog AMP-PNP suggests that binding of nucleotides regulates these translocation pathways both by partial occlusion of the pore and by restricting the motion of a putative voltage gating loop. PorB, located on the surface of *N. meningitidis*, can be recognized by receptors of the host innate immune system during infection. Features of PorB suggest that Toll-like receptor mediated recognition of outer membrane proteins may be initiated by a non-specific electrostatic attraction.

Keywords: neisseria pathogenesis, outer membrane protein, solute transport

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Structure of the essential enzyme ThiM from the bacterium Staphylococcus *aureus.* Julia Drebes[a], Markus Perbandt[a,c], Carsten Wrenger[b], Christian Betzel[a]. [a]*University of Hamburg, Department of Chemistry, Laboratory for Structural Biology of Infection and Inflammation, c/o DESY, Germany.* [b]*Bernhard Nocht Institute for Tropical Medicine, Department of Biochemistry, Germany.* [c]*University Medical Center Hamburg-Eppendorf, Department of Medical Microbiology, Virology and Hygiene, Germany.*
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Staphylococcus aureus is a commensally existing bacterium that colonizes 20% of healthy adults permanently and up to 50% transiently. Its pathogenicity plays an important role in nosocomial infections affecting immuno-suppressed patients. Symptoms caused by *S. aureus* range from superficial skin lesions up to life threatening conditions like pneumonia or endocarditis [1]. In 2005, *S. aureus* remerged as a major human pathogen due to methicillin resistant *S. aureus* (MRSA) strains and caused more than 18,000 deaths in the U.S.A. Staphylococcal pneumonia contributed to more than 75% of these deaths [2]. Therefore the developement of new and effective drugs against S. aureus is urgently required. Attractive drug targets are preferably metabolitic pathways, which are absent in the host organism.
In terms of strcuture base drug design investigations we have characterized and analysed the structure of ThiM (5-(hydroxyethyl)-4-methylthiazole kinase) from Staphylococcus *aureus*, which is an essential enzyme of the vitamin B1 metabolism. Vitamin B1, in its active form thiamine pyrophosphate (TPP), is a cofactor for several other key enzymes of the carbohydrate and amino acid metabolism [3]. Humans have to acquire Vitamin B1 by dietary uptake, because the metabolic pathway is absent. We intent to analyse the structures of all enzymes involved in the vitamin B1 cascade to allow a most systematic development of potential drugs against S. aureus, Here we present structural insights of ThiM at 2.1 Å resolution.

[1] Lowy F.D., *N Engl J Med.*, 339, 520–532, 1998. [2] Klevens R.M., et al.,. *JAMA*, 298, 1763–1771, 2007. [3] Pohl M., Sprenger G.A., Müller M. *Curr Opin Biotechnol*., 4, 335-42, 2004.

Keywords: MRSA, drug design, Vitamin B1 metabolism

FA1-MS12-P04

Structural Characterization of the Aromatic Monooxygenases PhzO and TcpA. Emerich-Mihai Gazdag[a], Dmitri V. Mavrodi[b], Luying Xun[c], Linda S. Thomashow[b], Wulf Blankenfeldt[a]. [a]*Max Planck Institute of Molecular Physiology Dortmund, Germany.* [b]*Department of Plant Pathology, Washington State University, Pullman, Washington 99164.* [c]*School of Molecular Biosciences, Washington State University, Pullman, Washington 99164-4234.*
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A large number of oxygenases has been isolated and studied over the past decades. They are able to catalyze a wide variety of oxidative reactions such as regio- and stereoselective hydroxylation. Oxidation reactions are difficult to be achieved using chemical approaches, which makes monooxygenases interesting catalysts for biotechnological applications.
Our current work focuses on the structural and biochemical characterization of the two two-component flavine-diffusible monooxygenases PhzO and TcpA. These enzymes belong to an understudied class of monooxygenases that utilize FAD as a cosubstrate rather than as a cofactor.
PhzO from *Pseudomonas aureofaciens* is a monooxygenase that hydroxylates phenazines [1], a class of redox-active bacterial secondary metabolites that act as virulence factors in infections of humans. At the same time, the phenazines also have beneficial effects in the biological control of plant disease because root-colonizing phenazine producers such as *P. aureofaciens* can protect the plant against other microbial pathogens. PhzO is responsible for the generation of a large spectrum of hydroxylated phenazine derivatives that *P. aureofaciens* produces, and insight into its structure/activity relationships may provide opportunities to enhance the plant-protecting properties of this strain.
TcpA from *Ralstonia eutropha*, on the other hand, initiates the sequential dechlorination of polychlorophenols through an oxidative process [2]. A better understanding of the dechlorination procedure could lead to better applications of these polychlorophenol-degrading microorganisms in the bioremediation of polychlorophenols, a pesticide derivative that is one of the most persistent environmental pollutants.
We present here the crystal structures of PhzO and TcpA in the ligand-free form. Comparison the related 4-hydroxyphenylacetate 3-monooxygenase HpaB [3] allows for the modeling of enzyme-substrate complexes and the identification of specificity determinants. Biochemical assays for the investigation of substrate turnover have been developed and demonstrate that at least for the phenazine-modifying PhzO reduction of the substrate precedes hydroxylation.

[1] Shannon M. Delaney, Dmitri V. Mavrodi, Robert F. Bonsall, Linda S. Thomashow, *Journal of Bacteriology*, Jan. 2001, p. 318–327. [2] Luying Xun, Chris M. Webster, *The Journal of Biological Chemistry,* Feb. 2004, pp. 6696–6700. [3] Kim SH, Hisano T, Takeda K, Iwasaki W, Ebihara A, Miki K, *The Journal of Biological Chemistry,* Nov. 2007, pp. 33107-17.

Keywords: phenazine derivatives, polychlorophenols, two-component flavine-diffusible monooxygenases

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Structural and functional studies of phenazine biosynthesis protein PhzE, a 2-amino-2-desoxyisochorismate synthase. QiAng Li[a], Dmitri V. Mavrodi[b], Linda S. Thomashow[b,c], Manfred Roessle[d], Wulf Blankenfeldt[a]. [a]*Max Planck Institute of Molecular Physiology, Department of Physical Biochemistry, Otto-Hahn-Straße 11, 44227 Dortmund, Germany.* [b]*Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430 (U.S.A).* [c]*USDA, Agricultural Research Service, Root Disease and Biological Control Unit, Pullman, WA 99164-6430 (U.S.A).* [d]*European Molecular Biology Laboratory-Hamburg Outstation, c/o Deutsches Elektronen Synchrotron, 22603 Hamburg, Germany.*
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Phenazines are nitrogen-containing heterocyclic pigments produced by a number of bacterial genera, including fluorescent *Pseudomonas*, *Burkholderia*, *Brevibacterium* and *Streptomyces*. Historically, it was believed that phenazines are solely used as redox-active antibiotics in microbial competitiveness. Recently, however, it has been recognized that these compounds have diverse physiological functions because they also act as signalling molecules and also as respiratory pigments under anoxic conditions as met e.g. in the deeper layers of biofilm. This indicates that phenazine biosynthesis may be an attractive target for pharmaceutical intervention [1].Phenazine biosynthesis requires five genes encoding the proteins PhzB, PhzD, PhzE, PhzF and PhzG, which catalyze the reactions responsible for the synthesis of phenazine-1-carboxylic acid from chorismic acid. PhzE catalyzes the first reaction in this pathway, producing 2-amino-2-desoxyisochorimate (ADIC). The enzyme is highly similar to bacterial anthranilate synthases, which are known to be feedback-inhibited by tryptophan. We have therefore studied PhzE by structural and biochemical methods to assess if it acts as a point of allosteric control of the phenazine biosynthesis pathway.We present here the crystal structure of PhzE of *Burkholderia lata* 383 in a ligand-free open and ligand-bound closed conformation at 2.9 and 2.1 Å resolution, respectively. PhzE arranges as an unusual intertwined dimer, which was also confirmed by small angle x-ray scattering. The dimer possesses relatively weak interactions along the dyad axis but makes more intimate contact between the glutamine amidotransferase and ADIC synthase domains of the two opposite chains, leading to the formation of an ammonia transport channel with approx. 25 Å in length. Large structural rearrangements accompany the binding of chorismic acid, which was found converted to benzoate and pyruvic acid in the ADIC synthase active center of the closed form. Unlike anthranilate synthase, PhzE is not allosterically inhibited, which can be attributed to a tryptophan residue of the protein blocking the respective potential regulatory site. Additional electron density in the active center of the GATase1 domain was identified as zinc and it could be demonstrated that Zn^{2+} and Ni^{2+} indeed reduce the activity of PhzE.

[1] Mentel, M., Ahuja, E.G., Mavrodi, D. V., Breinbauer, R., Thomashow, L.S. & Blankenfeldt, W. (2009) *ChemBioChem* 10, 2295-2304.