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Structure determination of NEMO(NF-; κ B essential modulator) UBAN domain

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NF-κB(nuclearfactor-κB)is a family of trascription factors which play essential roles in regulation of the gene expression in innate or adaptive immune responses. NF-κB proteins are kept inactive in the cytoplasm by binding of inhibitory molecules, IκBs, in the resting cells. Inducing stimuli trigger phosphorylation of the IκBs by a kinase complex (IKK) which leads to their ubiquitylation and degradation. NEMO (also called IKKγ) as a regulatory component of the IKK complex is required for the NF-κB activation in most circumstances. The activation mechanism by NEMO remains unclear, although, its ubiquitylation and oligomerization are proposed to be involved in this process. The minimum ubiquitin binding domain is highly conserved among NEMO and ABIN proteins and is named as UBAN (Ubiquitin Binding domain in ABIN proteins and NEMO). This domain corresponds to the coil-Zipper (CoZi) region in NEMO which is known as the minimum oligomerization domain, as well. Here we report the X-ray crystallographic structure of the COZi domain of NEMO at 2.8 Å resolution. The structure reveals a parallel, highly interacting coiled-coil, homo-dimer all through the CoZi domain.

Keywords: protein X-ray crystallography, NF-κB, NFκB essential modulator

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A structural basis for MHC class I associated susceptibility to multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune disease affecting the central nervous system with characteristic symptoms including motor dysfunction, ataxia and visual and sensory impairment. The events leading to disease initiation remain incompletely understood but it is thought that disease develops in genetically susceptible individuals and requires additional environmental triggers [1]. Linkage studies consistently reveal the MHC region to harbour susceptibility loci and the DR2 haplotype (HLA-DR2a, HLA-DR2b and HLA-DQ6) remains the strongest genetic risk identified to date. However linkage studies stratified for DR2 indicate additional susceptibility loci within the MHC region. In particular, the MHC class I allele HLA-A*0301 has been found to double the risk of developing MS, independently of DR2, whilst individuals positive for both the HLA-A*0301 and DR2 alleles exhibit an enhanced disease risk exceeding the sum of the individual contributions. Here we report the crystal structure of HLA-A*0301 in complex with a candidate autoantigen from proteolipid protein (PLP 45-53). Comparison of this structure to that of the MHC class I molecule HLA-A*0201, which exerts a dominant protective effect from disease and approximately halves the relative risk of disease development [2, 3], offers insight into the structural basis of MHC class I associated susceptibility to multiple sclerosis.
[1] Sospedra et al., *Annu Rev Immunol*, 23: 683 − 747 (2005)
[2] Fogdell-Hahn et al., *Tissue Antigens*, 55: 140-148 (2000)
[3] Harbo et al., *Tissue Antigens*, 63: 237 − 47 (2004)

Keywords: multiple sclerosis, autoimmunity, MHC class I

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Crystal structure of the human IL-15/IL-15R α complex

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Interleukin (IL)-15 contributes to $CD8^+$ T-cell memory acquisition and natural killer cell generation, whereas IL-2 has a pivotal role in the expansion and functions of regulatory and activated effector T cells. The IL-15 and IL-2 receptors belong to the common γ (γ c) cytokine receptor family and share signal-transducing β and γc subunits. Within the γc-chain family, the IL-15 and IL-2 receptors are unique insofar as specificity is provided by a third, higher affinity "private" receptor α-subunit. Whereas the private IL-2Rα subunit is co-expressed with β and γc on T- and B-cells ostensibly to allow cell-autonomous signaling, IL-15Rα is expressed *in trans* on antigen-presenting cells. The 1.85 Å crystal structure of the human IL-15/IL-15Rα complex accounts for the specificity of cytokine recognition, highlighting the high degree of electrostatic and geometric complementarity between the acidic receptor-binding surface of IL-15 and the basic ligand-binding region of IL-15Rα, and the essential role of binding-site waters in forming this very high affinity complex (Kd = 38 pM) [1]. In spite of very low IL-15/IL-2 sequence homology and the distinct architectures of the cytokine-binding "sushi" domains of each receptor, the receptor binding foot-prints of each cytokine, and therefore the topologies of both the IL-15/IL-15R α and IL-2/IL-2Rα complexes, are remarkably similar. Overall, there appear to be no structural obstacles to the transpresentation of either cytokine. Our findings suggest that antigen-experienced IL-2Rα^+ T cells could, in principle, enhance IL-2Rα^- T cells responses *via* the direct presentation of IL-2.
[1] Chirifu, M., et al. (2007) *Nature Immunology* 8, 1001-1007

Keywords: structural determination of cytokine complexes, molecular recognition, protein-protein interactions

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Hematopoietic protein tyrosine phosphatase (HePTP): Molecular determinants of substrate specificity

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Cells of the immune system have inherently high levels of tyrosine phosphorylation. In fact, they express more genes encoding protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) than nearly any other cell type. One PTP expressed exclusively in cells of the immune system is hematopoietic protein tyrosine phosphatase (HePTP). HePTP functions to negatively regulate the activation of T cells, and it does so by dephosphorylating and inactivating its only known substrates, the mitogen-activated protein kinases (MAPKs) Erk1/2 and p38. The importance of this regulation is highlighted by the fact that dysregulation of HePTP is associated with several diseases of the immune system, including acute myelogenous leukemia and non-Hodgkins lymphoma. In order to understand the molecular basis of the HePTP:MAPK interaction, we have generated a series of HePTP substrate-trapping mutants (STMs) in order to selectively populate an HePTP:MAPK peptide dephosphorylation complex. STMs have low catalytic activities yet retain high affinities for their substrates. We first determined the biochemical and biophysical characteristics of these HePTP STMs to identify those STMs most suitable for structural studies. We then crystallized and determined the structures of several HePTP STMs in both their apo forms and in complex with two distinct substrate peptide mimetics, e.g. peptides that correspond to the singly- and dually-phosphorylated activation loop of the MAPK Erk2. Finally, we describe how this biochemical, biophysical and structural data has allowed us to identify, for the first time, the structural features of HePTP that confer such a high degree of specificity for its MAPK substrates.

Keywords: hematopoietic protein tyrosine phosphatase (HePTP), extracellular signal-regulated kinase (Erk), T cell activation

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Structure of *Escherichia coli* tyrosine kinase Etk reveals novel activation mechanism

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While protein tyrosine kinases (PTKs) have been extensively characterized in eukaryotes, far less is known about their emerging counterparts in prokaryotes. The inner-membrane Wzc/Etk protein belongs to the bacterial PTK family, which plays a critical role in regulating the polymerization and transportation of virulence-determining capsular polysaccharide (CPS). The kinase utilizes a unique two-step activation mechanism centering on the intraphosphorylation of a tyrosine residue, although the specific detail remains unknown. Herein we report the first crystal structure of a bacterial PTK, the C-terminal kinase domain of *E. coli* tyrosine kinase (Etk) at 2.5Å resolution. The folding of the Etk kinase domain in bacteria differs markedly from that in eukaryotic PTKs. Based on the structure and supporting mass spectrometric evidence of the PTK observed, a unique activation mechanism is consequently proposed that involves the regulation of the phosphorylation of a single tyrosine residue at position 574 and its specific interaction with a previously unidentified key arginine residue at position 614 (R614) to unblock the active site.

Keywords: protein tyrosine kinases, pathogenic bacterial mechanism, polysaccharides

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Crystallographic analysis of response regulator protein from *Desulfovibrio vulgaris* Hildenborough

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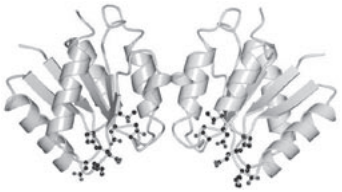
Sulfate-reducing bacteria *Desulfovibrio vulgaris* can generally use hydrogen and organic acids as electron donors for sulfate reduction. Sulfate reduction and hydrogen oxidation are spatially separated in the cytoplasm and periplasm, respectively. It has been proposed that electron transport linking periplasmic hydrogen oxidation to cytoplasmic sulfate reduction is mediated through the high-molecular-mass cytochrome redox protein complex (the Hmc complex). Two genes (rrf1 and rrf2) encoding response regulator proteins with a putative function in the regulation of gene expression are present immediately downstream from the structural genes of the hmc operon. The deletion of the rrf1, 2 genes gives rise to increased hmc operon expression. In order to understand the regulation mechanism of Hmc complex expression, we tried to determine the crystal structure of the response regulator proteins (Rrf1 and Rrf2). We cloned the rrf1and rrf2 genes from *Desulfovibrio vulgaris* Hildenborough and successfully overexpressed, purified and crystallized Rrf1 protein. Here we report the crystal structure of Rrf1 at 2.1 Å resolution.



Keywords: signal transduction, transcription regulation, X-ray crystallography of biological macromolecules

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Crystal structure of human ERK1 kinase mono-phosphorylated at Tyr204

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ERK is a member of MAP kinase family that regulates cell growth and differentiation in response to extracellular stimulation. ERK consists of two major isoforms, ERK1 and ERK2, which have a high degree of amino acid sequence homology and are different from each other in the intravital behavior. In order to investigate *in vivo* function of ERK1, we determined the crystal structure of human ERK1 complexed with 5-iodotubercidin, a potent inhibitor. Purified ERK1 was identified as auto-phosphorylated protein at Tyr204 by Western blot experiments. Crystals of the complex were obtained using a reservoir solution of 30% PEG4000, 0.2 M lithium sulfate,