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Crystal Structure of the HGF β-chain in Complex with the Sema Domain of the Met Receptor
Christian Wiesmann, Jennifer Stamos, *Department of Protein Engineering, Genentech. 1 DNA Way, South San Francisco, USA*. E-mail: chw@gene.com

The Met tyrosine kinase receptor and its ligand, hepatocyte growth factor (HGF), play a key role during development as an important switch that stimulates proliferation, branching and motility. Inappropriate activation of Met signalling promotes invasive growth of many tumor types, which makes Met and its ligand attractive targets for therapeutics. HGF undergoes a maturation cleavage to form a heterodimeric α/β form, which is required for Met activation; however the precise mechanism of Met activation by HGF is still poorly understood. We have solved the crystal structure of the N-terminal 560 residues of the Met receptor in complex with the β-chain of HGF. This fragment of the Met receptor comprises a SEMA domain, a structural motif that is also found in integrins and semaphorins, and a small cysteine rich PSI domain. SEMA domains are 7-bladed β-propellers; the structure shows how HGF-β binds to of the 'bottom' face of this propeller, and identifies residues on Met and HGF that play key roles in this interaction. The structural epitope on HGF-β identified in this crystal structure is in excellent agreement with biochemical and biological studies with HGF and HGF-β mutants.

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Keywords: HGF, met-receptor, sema-domain

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Crystal Structure of Human C-type Lectin-like Oxidized LDL Receptor 1(LOX-1)
Tomoko Ishigaki[a], Izuru Ohki[a], Takuji Oyama [a], Sachiko Machida[b], Shin-ichi Tate[a], Kosuke Morikawa[a], [a]*Biomolecullar Engineering Research Inst., Osaka, Japan.* [b]*National Food Research Inst.* E-mail: ishigaki@beri.or.jp

C-type lectin-like oxidized low-density lipoprotein (LDL) receptor 1, LOX-1, is the major receptor for oxidized LDL (OxLDL) in endothelial cells. LOX-1 plays a critical role in endothelial dysfunction that leads to atherosclerosis. Lox-1 is also expressed in macrophages and smooth muscle cells; these cells progress atherogenesis in sub-endothelial space through interaction with OxLDL in the intima. Thus, LOX-1 is recognized as a therapeutically important target receptor for the pathogenesis of vascular disorder, especially atherosclerosis. To gain the insight into the binding surface structure of LOX-1 to OxLDL, we have determined the crystal structure of the ligand-binding CTLD domain of LOX-1, with a short stalk region connecting the domain to the membrane-spanning region, as a homodimer linked by an inter-chain disulfide bond. . In vivo assays using LOX-1 mutants revealed that the "basic spine", consisting of linearly aligned arginine residues spanning over the dimer surface, is responsible for ligand binding. Single amino acid substitution in the dimer interface caused the severe reduction of LOX-1 binding activity, suggesting that the correct dimmer arrangement is crucial for the binding to OxLDL. Based on the LDL model structure, the possible binding modes of LOX-1 to OxLDL are proposed.
Keywords: atherosclerosis, membrane receptors, three-dimensional protein structure

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The 'Active-like' Structure of the Unphosphorylated Response Regulator StyR
Mario Milani[a], Livia Leoni[b], Giordano Rampioni[b], Elisabetta Zennaro[b], Paolo Ascenzi[b], Martino Bolognesi[c], [a]*'Giannina Gaslini' Institute and INFM, Genova, Italy.* [b]*Department of Biology, University 'Roma Tre', Roma, Italy.* [c]*Department of Biomolecular Sciences and Biotechnology and INFM, University of Milano, Milano, Italy*. E-mail: milani@ge.infm.it.it

The crystal structure of unphosphorylated StyR has been solved at 2.2 Å resolution. StyR belongs to the FixJ family of signal transduction response regulators; it controls transcription of the *styABCD* operon coding for styrene catabolism in *Pseudomonas fluorescens* ST [1]. StyR is composed of an N-terminal regulatory domain (StyR-N), and a C-terminal DNA binding domain (StyR-C). The two domains are separated by an elongated linker α-helix (34 residues), a new feature in response regulator known structures. StyR-C is structured similarly to the DNA binding domain of the response regulator NarL [2]. StyR-N shows structural reorganization of the phosphate receiving region involved in activation/homodimerization: specific residues adopt 'active-like' conformations, and the α4-helix, involved in dimerization of the homologous FixJ response regulator [3], is trimmed to just one helical turn. Overall, structural considerations suggest that phosphorylation may act as an allosteric switch, shifting a pre-existing StyR equilibrium towards the active, dimeric, DNA-binding form.

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Keywords: two-component signal transduction, response regulator, phosphorylation

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Insecticide Selectivity: Structure of a Hemipteran Ecdysone Receptor LBD
Michael C. Lawrence[a], Jennifer A. Carmichael[a], Lloyd D. Graham[b], Patricia A. Pilling[a], V. Chandana Epa[a], George Lovrecz[a], Garry N. Hannan[b], Ronald J. Hill[b], [a]*CSIRO Health Sciences and Nutrition, 343 Royal Parade, Parkville, Victoria 3052, Australia.* [b]*CSIRO Molecular Science, PO Box 184, North Ryde, New South Wales 1670, Australia.* E-mail: mike.lawrence@csiro.au

We report here the X-ray structure of the ecdysone receptor ligand-binding domain (LBD) of the hemipteran *Bemisia tabaci* (silverleaf whitefly) in complex with the ecdysone analogue ponasterone A and compare it with the corresponding known structure from the lepidopteran *Heliothis virescens* ecdysone receptor [1]. Our structure reveals the overall mode of ponasterone A binding is very similar in the two cases, but that the *B. tabaci* ecdysteroid-binding pocket is structured differently to that of *H. virescens* in those parts that are not in contact with ponasterone A. We propose that these differences in the ligand-binding pocket provide a molecular basis for the taxonomic order-selectivity of bisacylhydrazine insecticides [2,3].

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Keywords: insecticides, nuclear receptors, ecdysone

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Structural Basis for Autoinhibition and Activation of eIF2α Protein Kinase GCN2
Anil K. Padyana[a,b], Hongfang Qiu[c], Antonina Roll-Mecak[b,d], Alan G. Hinnebusch[c], Stephen K. Burley[a,b], [a]*Structural GenomiX, Inc., San Diego, CA.* [b]*The Rockefeller University, New York, NY.* [c]*Laboratory of Gene Regulation and Development, NICHD-NIH, Bethesda, MD.* [d]*Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, CA, USA*. E-mail: apadyana@stromix.com

The GCN2 protein kinase (PK) couples the rate of protein synthesis to amino acid stores by phosphorylating eukaryotic

translation initiation factor 2. The autoinhibited form of the GCN2 PK domain is activated in cells starved of amino acids by binding of uncharged tRNA to a histidyl-tRNA synthetase (HisRS)-like domain. Crystal structures of a GCN2 PK dimer have been determined for wild-type and mutant forms in the apo state and bound to ATP or AMPPNP. These structures reveal that autoinhibition results from stabilization of a closed bi-lobate conformation of the apo protein that restricts ATP binding. A hyperactive mutant form of the enzyme (*R794G*) shows a conformational change in the hinge region connecting the N- and C-lobes and significant intra-domain movement that enhances ATP binding and hydrolysis. We propose that interactions between the PK domain and the tRNA-bound form of the HisRS domain remodel the hinge region in a manner similar to the mechanism of enzyme activation by the *R794G* mutation. A hypothetical structural model of a PK_2HisRS_2 tetramer places the kinase hinge near the PK-HisRS interface, poised for allosteric modulation following uncharged tRNA binding.

Keywords: translation regulator, signal transduction, protein kinases

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Domain Closure of the Ligand-binding Core of the AMPA Receptor GluR2: Insights from Agonist and Antagonist Complexes

Christina Kasper, Karla Frydenvang, Bente Vestergaard, Desiree Sprogøe, Helle Hald, Lotte Brehm, Michael Gajhede, Jette Sandholm Kastrup, *Biostructural Research, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Denmark.* E-mail: jsk@dfuni.dk

Ionotropic glutamate receptors (iGluRs) mediate most rapid excitatory synaptic transmission in the mammalian central nervous system and their involvement in neurological diseases has stimulated widespread interest in their structure and function.

We have determined several structures of a ligand-binding core construct of GluR2 (GluR2-S1S2J), which belongs to the AMPA class of iGluRs, in complex with agonists and antagonists. GluR2-S1S2J is composed of two domains (D1 and D2) and the ligands bind within a cleft formed by the domains. AMPA receptor agonists induce distinct conformations of the GluR2-S1S2J by D1-D2 domain closure. In contrast, antagonists stabilize an open conformation of GluR2-S1S2J, resembling the conformation of the *apo* structure.

An excellent correlation exists between domain closure and efficacy of a range of agonists at full-length GluR2, determined by electrophysiology in *Xenopus lævis* oocytes. Together with the various binding modes of agonists and antagonists at GluR2-S1S2J, this will be discussed on the poster.

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Keywords: neurotransmission, receptor-ligand interactions, X-ray crystallography of proteins

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Structural Studies of Kainate Receptor GluR5 Ligand-binding Core Complexes

Jette Sandholm Kastrup[a], Peter Naur[a], Bente Vestergaard[a], Lars K. Skov[a], Jan Egebjerg[b], Michael Gajhede[a], [a]*Biostructural Research, Department of Medicinal Chemistry, Danish University of Pharmaceutical Sciences, Denmark.* [b]*Molecular Neurobiology, Lundbeck A/S, Denmark.* E-mail: jsk@dfuni.dk

Ionotropic glutamate receptors (iGluRs) form a family of ligand-gated ion channels that play a central role in rapid neuronal signaling in the central nervous system. The complex roles of the iGluRs are far from understood in detail but it is generally accepted that these receptors are implicated in a number of psychiatric and neurological disorders such as Alzheimer's, schizophrenia and epilepsy.

A soluble construct of the extracellular ligand-binding core of the GluR5 receptor (GluR5-S1S2), belonging to the kainate class of iGluRs, has been expressed in *Escherichia coli*. The X-ray structure of GluR5-S1S2 in complex with the endogenous neurotransmitter (*S*)-glutamate was recently determined to 1.95 Å resolution. The GluR5-S1S2 structure comprises two domains, trapping (*S*)-glutamate. Interestingly, GluR5-S1S2 forms a dimer with a different arrangement of the two protomers compared to the related GluR2-S1S2J, which belongs to the AMPA class of iGluRs. This structure as well as the current status of the project will be presented on the poster.

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Keywords: neurotransmission, receptor-ligand interactions, X-ray crystallography of proteins

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Unraveling the Binding Mode of the Neutralizing Neuroantibody αD11 to NGF

Sonia Covaceuszach[a], Alberto Cassetta[b], Antonino Cattaneo[a], Doriano Lamba[b], [a]*LaylineGenomics, Trieste.* [b]*Istituto di Cristallografia, CNR Trieste, Italy.* E-mail: covaceu@sissa.it

The monoclonal neuroantibody αD11 is a potent antagonist that prevents the binding of the neurotrophin NGF (nerve growth factor) to its receptors, TrkA and p75, in a variety of systems, most notably in two *in vivo* systems linked to crucial pathological states (*i.e.* Alzheimer disease and AIDS). To elucidate the mechanism of neutralization, structural and functional studies were performed.

The potential therapeutical interest of the antibody was confirmed, demonstrating that it binds both mouse and human NGF with similar affinity. Its epitope was mapped by Sandwich Elisa assay with a pool of mutants. Its Fab fragment was crystallized [1] and the structure was solved at 1.80Å resolution. This structure, that may assist in the humanization without loss in affinity, was docked to NGF on the basis of epitope mapping results.

The present structural investigation along with the crystallographic analysis of the two complexes between NGF and its receptors [2], [3] should provided important insights in the molecular basis of antibody specificity for the NGF antigen and its mode of interaction with the full-length receptor TrkA.

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Keywords: antibody antigen interaction, antibody structure function, neurotrophin

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Towards a Comprehension of the Structure of Mouse proNGF

Francesca Paoletti[1], Sonia Covaceuszach[2], Elisabeth Schwarz[3], Milton Stubbs[3], Rainer Rudolph[3], Antonino Cattaneo[1,2], Doriano Lamba[4], [1]*SISSA-ISAS, Trieste.* [2]*LayLine Genomics, Rome, Italy.* [3]*Martin-Luther Univesity Halle-Wittenberg, Halle (Saale), Germany.* [4]*Istituto di Cristallografia - CNR, Trieste, Italy.* E-mail: paoletti@sissa.it

The neurotrophin NGF (Nerve Growth Factor) is translated as a pro-protein of 27 kDa and cleaved by furin to mature NGF.

NGF is involved in the maintenance and growth of neurons, while the pro-peptide facilitates folding of NGF [1]. ProNGF is the predominant form of NGF in brain [2] and was found to be a high affinity ligand for p75 and to induce p75 dependent apoptosis [3]. The specific receptor for the proNGF is sortilin [4].

We focused on the biophysical biochemical characterization of the mouse proNGF, which was expressed in *E. coli*, refolded and purified. The native structure was proven by fluorescence and circular dichroism. The homogeneity of the protein preparation was tested through dynamic light scattering. We have started a robotic screening for crystallization conditions of the protein and are planning SAXS experiments.

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