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

P.04.12.14

Acta Cryst. (2005). A**61**, C234

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.

[1] Frandsen A., Pickering D.S., Vestergaard B., Kasper C., Nielsen B.B., Greenwood J.R., Campiani G., Fattorusso C., Gajhede M., Schousboe A., Kastrup J.S., *Mol. Pharmacol*., 2005, **67**, 703.

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

P.04.12.15

Acta Cryst. (2005). A**61**, C234

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.

[1] Naur P., Vestergaard B., Skov L.K., Egebjerg J., Gajhede M., Kastrup J.S., *FEBS Lett.*, 2005, **579**, 1154.

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

P.04.12.16

Acta Cryst. (2005). A**61**, C234

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.

[1] Covaceuszach S., Casetta A., Cattaneo A., Lamba D., *Acta Cryst.*, 2004, **D60**, 1323. [2] Wiesmann C., Ultsch M. H., Bass S. H., de Vos A. M., *Nature*, 1999, **401**, 184. [3] He X., Garcia K. C., *Science*, 2004, **304**, 870.

Keywords: antibody antigen interaction, antibody structure function, neurotrophin

P.04.12.17

Acta Cryst. (2005). A**61**, C234-C235

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.

[1] Rattenholl A., Ruoppolo M., Flagiello A., Monti M., Vinci F., Marino G., Lilie H., Schwarz E., Rudolph R., *J. Mol. Biol*., 2001, **305**, 523. [2] Fahnestock

M., Michalski B., Xu B., Coughlin M.D., *Mol. Cell. Neurosc*., 2001, **18**, 210. [3] Lee R., Kermani P., Teng K.K., Hempstead B.L., *Science*, 2001, **294**, 1945. [4] Nykjaer A., Lee R., Teng K.K., Jansen P., Madsen P., Nielsen M.S., Jacobsen C., Kliemannel M., Schwarz E., Willnow T.E., Hempstead B.L., Petersen C.M., *Nature*, 2004, **427**, 843.

Keywords: neurotrophin, protein refolding, biophysical biochemical characterization

P.04.12.18

Acta Cryst. (2005). A**61**, C235

Structural Basis for the Activity and Allosteric Control of Diguanylate Cyclase

Carmen Chan[a], Ralf Paul[b], Dietrich Samoray[a], Nicolas Amiot[c], Bernd Giese[c], Urs Jenal[b], Tilman Schirmer[a], [a]*Structural Biology, Biozentrum, University of Basel.* [b]*Microbiology, Biozentrum, University of Basel.* [c]*Department of Chemistry, University of Basel, Switzerland.* E-mail: carmen.chan@unibas.ch

Recent studies suggest that a novel second messenger, cyclic d-GMP (c-diGMP), is extensively used by bacteria to control multicellular behaviour. This cyclic dinucleotide is synthesised by the diguanylate cyclase (DGC) domain in a reaction that converts two GTP into one c-diGMP and two pyrophosphates. The DGC domain contains a highly conserved GG(D/E)EF sequence motif, and occurs in various combinations with sensory / regulatory domains in bacteria.

We have identified the response regulator, PleD, from *Caulobacter crescentus* as a diguanylate cyclase [1] and have solved its crystal structure in complex with c-diGMP to 2.7 Å [2]. PleD consists of a receiver domain D1 with a phosphorylation site, a receiver-like domain D2, and an effector domain DGC. In the structure, PleD forms a homodimer mediated by D1-D2 interactions. The DGC domain has a similar fold as the catalytic domain of adenylate cyclase but has an active site that reveals different nucleotide binding. The guanine base of c-diGMP is hydrogen bonded to Asn335 and Asp344, while the ribosyl and α-phosphate groups extend over the β2-β3 hairpin that carries the sequence motif. Interestingly, the c-diGMP molecule crosslinks two symmetrically arranged DGC domains from adjacent dimers. We propose that activation of PleD through phosphorylation leads to dimerisation, which allows the two DGC domains of a dimer to align symmetrically for c-diGMP synthesis.

Two intercalated c-diGMP molecules are bound to the domain interface between D2 and DGC. This allosteric binding site explains the observed non-competitive product inhibition. We propose that PleD inhibition is effected by DGC domain immmobilisation to the D1-D2 stem.

[1] Paul R., Weiser S., Amiot N., Chan C., Schirmer T., Giese B., Jenal U., *Genes Dev.*, 2004 **18**, 715. [2] Chan C., Paul R., Samoray D., Amiot N.C., Giese B., Jenal U., Schirmer T., *Proc. Natl. Acad. Sci.*, 2004, **101**, 17084.

Keywords: response regulator, cyclic dinucleotide, allosteric control

P.04.13.1

Acta Cryst. (2005). A**61**, C235

Structural Differences between B and F Subtypes of HIV PR

Mario Sanches[a], Alexander Wlodawer[b], Alla Gustchina[b], Igor Polikarpov[a], [a]*São Carlos Physics Institute, São Paulo University.* [b]*Macromolecular Crystallography Laboratory, NCI-Frederick.* E-mail: mariosan@gmail.com

One of the major problems facing the development of successful therapies against AIDS is the emergence of viral variants that exhibit drug resistance, as well as viral subtypes naturally more liable to development of therapeutic failure. In this work we solved by molecular replacement the crystal structures of four HIV-1 proteases complexed with the inhibitor TL-3: of the subtype B wild type (*Bwt*) at 2.1Å resolution, of the subtype F wild type (*Fwt*) at 2.1Å, and a mutant of each subtype (*Bmut* and *Fmut*) at 1.75Å and 2.80Å, respectively. All crystals were in space group $P6_1$

The mutation V82A in the proteases *Bmut* and *Fmut* causes repacking of the S1' pocket, which rearranges the inhibitor's side chain at the P1' subsite. Our analysis further indicates that some polymorphic substitutions between subtypes B and F could lead to stabilization of naturally flexible regions of subtype F proteases, resulting in an intrinsically less active and drug resistant enzyme. On subtype F proteases the polymorphic substitution M36I leads to the displacement of the loop between residues 35-41, which would cause loss of the flexibility of the flaps and of the loop 76-83 in the active site. Our comparisons further indicate that the polymorphic substitution L89M on non-B subtypes could be equivalent to the L90M resistance mutation on subtype B proteases.

Keywords: HIV retroviral proteases, structural and biological function, biological macromolecules

P.04.13.2

Acta Cryst. (2005). A**61**, C235

X-ray Crystallographic Structure of Virus like Particle from Hyperthermophilic Archaea *Pyrococcus furiosus*

Fusamichi Akita, Chong Khoon Tee, Hideaki Tanaka, Naoyuki Miyazaki, Kazunori Nanba, Yuichiro Nakaishi, Yasuko Ono, Eiki Yamashita, Mamoru Suzuki, Tomitake Tsukihara, Atsushi Nakagawa, *Institute for Protein Research, Osaka University.* E-mail: fusamiti@protein.osaka-u.ac.jp

Hyperthermophilic archaea is grown in an ultimate environment.Interesting protein was discovered from *Pyrococcus furiosus* of them.The proteins were assembled 180 copies subunits into a virus like particle (PfV: *Pyrococcus furiosus* virus), and it has virus like icosahedral symmetry. The atomic structure of PfV was determined at 3.6Å resolution by X-ray crystallography.

Crystallization was performed by hanging-drop vapor diffusion against16-20% MPD. The crystal belongs to the space group of *P*41212 with cell dimensions of *a*=*b*=631.5Å, *c*=351.3Å. Diffraction data was collected at wavelength of 0.9Å crystals at 100K using an imaging plate DIP6040 on beamline 44XU of the SPring-8. A diffraction data set at 3.6Å resolution with 96.8% completeness with an *R*merge of 0.132 was obtained. Phase was determined by the SIRAS (Single Isomorphous Replacement with Anomalous Scattering) method using Tungsten cluster derivative. Phase improvement and extension was performed using the symmetry of a virus and electron density was calculated. The structure model was built based on this electron density map. The CNS program was used refinement against 20-3.6Å intensity data. The crystallographic *R*-factor and free *R*-factor were 0.267, respectively.

PfV subunit was a mixed alpha/beta structure. Three dimensional structure of PfV and bacteriophage HK97 capsid protein were very similar. This suggests that PfV and HK97 have a common ancestor.

Keywords: *Pyrococcus furiosus*, virus like particle, HK97

P.04.13.3

Acta Cryst. (2005). A**61**, C235-C236

Structures of SARS Coronavirus Main Protease Bound by an Aza-peptide Epoxide

Ting-Wai Lee[a], Maia M. Cherney[a], Carly Huitema[b], Jie Liu[b], Lindsay D. Eltis[b], Karen Ellis James[c], James C. Powers[c], Michael N. G. James[a], [a]*CIHR Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.* [b]*Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC V6T 1Z3, Canada.* [c]*School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA*. E-mail: tingwai@ualberta.ca

Soon after the global outbreak of severe acute respiratory syndrome (SARS) in the spring of 2003, a novel coronavirus (CoV) was identified to be the etiological agent of this highly infectious and fatal disease. The main protease (M^{pro}) of this virus is essential for viral replication, and therefore is one of the major targets for the development of anti-SARS agents. We have determined the crystal structures of SARS-CoV M^{pro} unbound in the space group C2, and bound by an aza-peptide epoxide in the space groups C2 and $P2_12_12_1$. These structures show that the peptide binds, like a true substrate, to the substrate-binding and active site of the enzyme, without inducing any significant change in the structure of the enzyme. A covalent bond