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

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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

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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

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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

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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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