factor (EGF)-like module, whereas β-NX has a single LG domain. Recently several groups have reported crystal structure of β-NX and NL complex revealing a unique binding with 2:2 stoichiometry. However these crystal structures do not provide significant insights into the synaptic signal transduction triggered by β-NX/NL interaction. We determined the crystal structure of β-NX/NL complex at 3.5Å resolution in different crystal form, possibly mimicry their molecular clustering of synaptic cleft. We also report the crystal structure of a single repeat segment of α- NX at 2.3Å resolution (i.e. LG-EGF-LG segment). Comparison of these structures stolongly suggested that α- and β-NX have the different clustering mechanism mediated by the interaction with their ligands NL.

Keywords: structures of biomolecules, cell adhesion, complexes

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Preliminary X-ray analysis of MEK1/ERK2 complex

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The Ras/Raf/MEK (mitogen-activated protein kinase) /ERK (extracellular-signal- regulated kinase) pathway plays a key role in governing cell proliferation, differentiation and survival. The pathway represents an attractive drug target in proliferative diseases. The aim of the present study is to clarify the mode of the interaction between MEK and ERK by analyzing the crystal structure of MEK1/ERK2 complex. A detailed study of the three-dimensional structure of the complex should provide information to design novel and target-specific drugs against proliferative diseases. MEK1 and ERK2 were expressed in *E. coli* with GST tags at their N-termini. The lysate including GST-tagged MEK1 was centrifuged to produce a crude extract, which was then loaded onto a GST-affinity column. By on-column cleavage using precision protease, the desired protein was obtained. Subsequent purification by anion exchange chromatography on a MONO Q column yielded two peaks which were assigned as homologous MEK1. ERK2 was purified by a similar procedure and also split into two peaks on a MONO Q column. For each of four complexes obtained by combining two MEK1 and two ERK2, conditions of crystallization were searched using the commercially available sparse-matrix screening kits. Optimization of crystallization conditions for X-ray crystallography is currently progress.

Keywords: MEK, ERK, crystallization

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Structural biology of a nuclear import of proteins by transportin 1

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The transport of macromolecules between the nucleus and the cytoplasm through nuclear pore complexes (NPCs) is mediated via several transport pathways by transport receptors that are most commonly members of the importin-β family. Transport receptors form complexes with their transport substrates (cargoes) through cognate nuclear localization signals (NLSs) for import substrates or nuclear export signals (NESs) for export substrates, and target substrates to NPC components termed nucleoporins. Transport directionality and interactions between the transport receptor and substrate are regulated by RanGTP and, in the nuclear import system, binding of RanGTP to the receptor in the nucleus is associated with substrate dissociation. Of the several transport pathways, the best characterized is an import pathway mediated by importin-β (karyopherin-β1). Transportin 1 (Trn1) (karyopherin-β2) is a transport receptor that belongs to the importin-β family and has 24% sequence similarity to importin-β. Here we describe four crystal structures of human Trn1 in a substrate-free form as well as in the complex with three NLSs (hnRNP D, JKTBP and TAP, respectively). Our data have revealed that (i) Trn1 has two sites for binding NLSs, one with high affinity (Site A) and one with low affinity (Site B), and NLS interaction at Site B controls overall binding affinity for Trn1, (ii) Trn1 recognizes the NLSs at Site A followed by conformational change at Site B to interact with the NLSs, and finally, (iii) a long flexible loop, characteristic of Trn1, interacts with Site B, thereby displacing transport substrate in the nucleus. These studies provide deep understanding of substrate recognition and dissociation by Trn1 in import pathways.

Keywords: importin, nuclear transport, nuclear pore complex

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Structures of starch binding domain of R. oryzae glucoamylase reveal an amylosic binding model

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Glucoamylase hydrolyzes starch and polysaccharides to β -D-glucose. Rhizopus oryzae glucoamylase (RoGA) consists of two functional domains, an N-terminal starch binding domain (SBD) and a C-terminal catalytic domain, which are connected by an O-glycosylated linker. The SBD of RoGA belongs to the carbohydrate binding modules (CBMs) family 21 (RoGACBM21). The crystal structures of SBD and the complexes with a cyclic carbohydrate, β-cyclodextrin and a linear carbohydrate, maltoheptaose were determined at 1.25, 1.8, and 2.3 Å resolution, respectively. The overall structures of SBD belong to a β-sandwich fold with an immunoglobulin-like architecture. Two carbohydrate binding sites, sites I and II, were determined on the surface of SBD, where site I is a flat and broad hydrophobic binding region created by the aromatic residues, Trp47, Tyr83, and Tyr94; site II is a protruded and narrow binding environment formed by Tyr32 and Phe58. Besides the hydrophobic interaction, two unique polyN loops comprising consecutive asparagines also participate in the sugar binding. To elucidate the mechanism of polysaccharide binding, a number of mutants were constructed and characterized by the quantitative binding isotherm and Scatchard analysis. In addition to sites I and II, a continuous binding surface through Tyr67 and Tyr93 might be essential for long-chain polysaccharide binding. An amylosic binding model for RoGA was proposed.