then deliver into the arginine-rich groove. Second, our data show that the region centered around the protrusion is crucial for RNA binding and presumably is the major RNA binding site. The side chains of the arginine residues in this region are pointing towards each other, suggesting that this region may clamp the RNA into the groove. Third, we have found that an arginine rich region at the other end of the groove is also important for RNA-binding. Since 24-27 RNA nucleotides bind to an influenza NP molecule, the RNA is expected to make further contacts with NP in addition to binding along the arginine-rich groove. This work may lead to the design of inhibitors for perturbing the transcription and replication of influenza virus.

Keywords: infectious diseases, nucleoprotein, influenza virus

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Co-crystallization and X-ray studies of HIV-1 Vpr-Importin-alpha and Vpr-inhibitor complexes

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Viral protein R (Vpr) of HIV-1 is a small nuclear protein (14KDa) of 96 aa and has 3 regions consisting of aH1 (residues between 17 and 33), aH2 (38 and 50) and aH3 (56 and 77). Vpr plays various roles in viral infection and cellular functions, and is also known as one of the possible mediators of the nuclear localization of preintegration complex. In a previous study, we showed that Vpr interacts with Importin-a through the aH1 and aH3 regions and that the interaction via aH1 is essential for entry into the nucleus but also for HIV-1 replication of macrophages. Crystal structures of the Vpr in complexes with Importin-a and inhibitors will therefore lead to discovery of novel lead compounds of HIV-1. Vpr (17-74 and 17-81 residues) and Importin-a were expressed as recombinant GST fusion proteins in E.coli. Both proteins were purified by glutathione sepharose 4B column chromatography, and GST was cleaved by Prescission protease. Vpr was further purified by applying Electro-Eluting system with non-reduced condition. After loading to size exclusion column, each protein was buffer exchanged and concentrated to 5mg/mL (Vpr) and 10mg/mL (Importin-a), respectively. Crystallization conditions were determined and optimized in each protein. Co-crystallization and X-ray diffraction trials are under way to determine complex crystal structures of Vpr-Importin-a and Vpr-Inhibitors.
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Keywords: HIV-1, preintegration complex, protein-inhibitor co-crystallization

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New rearrangement in GroEL due to a 22 rotation between the heptameric rings

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Folding, trafficking, maintenance, and degradation of proteins are all processes that depend on the assistance of molecular chaperones. Chaperones are proteins whose function is to assist other proteins in achieving proper folding. Many are heat shock proteins, that is, proteins expressed in response to elevated temperatures or other cellular stresses. Among these, GroEL is a double-heptameric 800 kDa toroid, made of identical subunits that contains two central cavities, one in each ring that can accommodate proteins up to 60 kDa. GroES is a single-ring heptamer that binds to GroEL in the presence of ATP or ADP. In this way, the complex GroES-GroEL forms a hydrophobic cavity where the substrate is folded and is subsequently returned to the medium. Interactions between the two rings in GroEL result in the allosteric regulation of ATP hydrolysis, binding, and release of folding substrates and the cochaperonin GroES. In order to gain information about the signalling pathway associated to cooperativity in this protein and to better understand the role of the interface in the allosteric communication, two different mutants that lack negative cooperativity were studied: GroELE434K and GroELE461K. Crystallographically solved structures of these mutants explain the role of the interface between the rings in the allosteric communication and help to describe the conformational changes that are the cause of the different behaviour of the mutants. On the other hand, regions that stay unaltered during the functional cycle were found. The studies conclude that: i)together with en-bloc domain movements, allosterism is held in GroEL by the combination of rigid and deforming regions within subunits and ii) salt bridge pathways control allosteric communication in GroEL.

Keywords: GroEL, chaperonin, allosterism

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Characterization of the Munc18-Syntaxin protein interaction

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The SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins facilitate vesicle docking and fusion by forming SNARE complexes. These complexes are formed by the interaction of cognate SNAREs found on opposing membranes during fusion. The Sec/Munc (SM) family of proteins are a group of

regulatory proteins essential for this process. SM proteins are thought to regulate fusion by interaction with the SNARE protein Syntaxin (Sx). Different binding modes for the SM-Sx interaction have been observed: binding to the closed conformation (neuronal system [1]) binding to the N-terminus of the Sx protein (yeast Golgi-ER system [2] and GLUT4 system [3],[4]) and to the SNARE ternary complex (yeast exocytotic system [5]). The role of SM proteins in vesicle fusion has been disputed partly due to the different binding modes. Binding to the closed mode inhibits the formation of the ternary complex, negatively regulating vesicle fusion. Whereas binding via the N-terminus of Sx facilitates binding to the ternary complex and positively regulates fusion [3]. Our research focus is on Munc18c and Sx4, the SM and Sx protein respectively, involved in GLUT4 vesicle transport to the plasma membrane in response to insulin signalling. We have used biophysical techniques such as ITC and small angle scattering (SAS) to further characterize the molecular details of the Munc18c-Sx4 interaction. The findings using these techniques, specifically the possible conformational changes of Sx4 on binding to Munc18c will be discussed.

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Keywords: protein-protein interactions, biophysical methods, SAS

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Atomic-level models of the bacterial carboxysome shell

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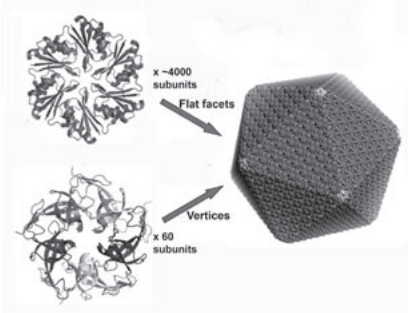
The carboxysome is a bacterial microcompartment, roughly 1000Å in diameter, that sequesters enzymes involved in carbon fixation. The shell is built from several thousand protein subunits and resembles a viral capsid. We have previously solved the crystal structures of hexameric carboxysome shell subunits, which suggest their roles in forming flat facets of the polyhedral shell (Kerfeld, *et al.*, *Science* 2005). There are three structures of homologous hexameric shell proteins solved from one class of carboxysome (beta-type) and one structure solved from another class of carboxysome (alpha-type) (Tsai, *et al.*, *PloS Biol* 2007). The comparison of these hexameric proteins shows interesting characteristics in sheet packing and residue interactions between adjacent hexamers. Furthermore, the recently determined structures of CcmL and CsoS4A subunits from the two classes of carboxysomes possess predominantly β-sheet structures and assemble as pentamers whose size and shape are compatible in forming icosahedral vertices of the protein shell. Combining these pentamers with the hexamers gives two plausible, preliminary atomic models for the carboxysome shell (Tanaka, *et al.*, *Science* 2008).



Keywords: bacterial organelles, carboxysome, CO_2 fixation

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Coordination structure of two Cl^--binding sites in oxygen-evolving photosystem II

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Photosystem II (PSII) performs light-induced electron transfer and water-splitting reactions, leading to the formation of molecular oxygen which is essential for the survival of most of the life on the earth. The components of oxygen-evolving PSII from cyanobacteria include 17 membrane-spanning subunits, 3 hydrophilic, peripheral subunits, and over 70 cofactors such as chlorophylls, carotenoids, Mn, Ca, Fe, and plastoquinones, which give rise to a total molecular mass of 350 kDa for a monomer. The structure of PSII has been reported at 3.8-3.0 Å resolution by X-ray crystallographic analysis. The catalytic center for water splitting of PSII, namely, the oxygen-evolving complex, is located in the membrane surface and composed of 4 Mn atoms and 1 Ca atom coordinated by the protein matrix of PSII. Cl^- is one of the essential cofactors for oxygen evolution of PSII, and is closely associated with the Mn_4Ca-cluster. Its detailed location and function, however, have not been identified. In order to elucidate the coordination structure and function of Cl^- in PSII, we substituted Cl^- with Br^- or I^- in a PSII dimer from *Thermosynechococcus vulcanus*, and analyzed the crystal structure of Br^- or I^--substituted PSII. Our results showed that two Cl^- are bound to each PSII in positions surrounding each side of the Mn_4Ca-cluster at equal distances to the Mn atoms (7.0 Å) as well as to the Ca (10.0 Å). Among these two Cl^-, one is located in the entrance of a proton exit pathway, and another one is located close to the backbone of CP43-Glu354, the side chain of which is coordinated to the Mn_4Ca-cluster. These results well explain the various Cl^--effects on PSII oxygen evolution observed, and provides a basis for fully understanding the mechanism of oxygen evolution.

Keywords: photosynthesis, membrane proteins, anomalous diffraction

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Crystallization and structure determination of the phycobilisome complex

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