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