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The giant muscle protein titin extends over one half of the muscle sarcomere. In its largest isoform titin comprises more than 38,000 residues and about 300 domains. Its structural complexity does not allow the application of classical structural biology methods to determine its overall architecture. Therefore, we have decided to chop the protein into smaller fragments and determine the high resolution structures of representative parts. Within this endeavor, we have also become interested to consider known ligands involved in the titin interactome for structural/functional analysis. Over the last decade, we determined structures of the N-terminal assembly complex (Zou et al., 2006), from the I-band (Mayans et al., 2001; Vega et al., unpublished) and from the A-band including the kinase domain and down-stream signaling complexes (Mayans et al., 1998; Muller et al., 2006; Muller et al., 2007; Muller et al., unpublished; Chen et al., unpublished). The available data allow modeling a large part of the titin proteome and to interpret available low resolution data of the entire titin filament. Combined with complementary functional data, our findings reveal key structural/functional relationships of titin and its interactions partners. Structural biology results from a related sarcomeric filament protein, myomesin (also known as mini-titin) will be presented in a separate contribution. Some of the data have been published recently (Pinotsis et al., 2008).

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Keywords: muscle proteins, protein/protein interactions, kinase structure

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Ion channel structures by single particle analysis using EM: Sodium and TRP channels, IP3 receptor

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Six-transmembrane (6-TM) type channels are plasmamembrane integral components of cellular signaling pathways conserved in almost all species including animals, plants, and some kinds of prokaryotes. These channels selectively permeate cations in response to various signals. In excitable and non-excitable mammalian cells, 6-TM cation channels play fundamental roles, including the generation of action potential and its transmission, the regulation of intracellular ion concentrations, and the activation of signaling cascades by humoral or mechanical pathways. We have recently determined the structure of four different 6-TM type cation channels: the voltage-sensitive sodium channel[1], the IP3 receptor[2], the TRPC3[3] and TRPM2[4] channels, using single particle analysis from cryo-EM images. The basic structure of the molecules was shown to be similar: a bell-like shape composed of a relatively small extracellular (or luminal) domain, a protein-dense transmembrane domain, and an expanded cytoplasmic domain. However in detail, the cytoplasmic architectures are quite different from each other and are diversely evolved to their specific physiological functions.

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Keywords: electron microscopy analysis, ion channel structures, three-dimensional, image reconstruction

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The assembly process of the double-layered capsids of phytoreoviruses

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Viruses in the family *Reoviridae* have an inner core with a large interior cavity that contains the 10 to 12 segmented double-stranded RNA as a genome, and a transcriptional complex that includes proteins with RNA polymerase, helicase, guanylyltransferase and transmethylase activities and an RNA-binding protein. The core particle is surrounded by one or two layers of outer capsid proteins. During X-ray crystallographic and Cryo-electorn microscopic studies of the structural organization of *Rice dwarf virus*, a member of the genus *Phytoreovirus* in the family *Reoviridae*, we have identified possible structural mechanisms that allow creation of a large cavity inside a double-layered spherical particle that consists of heterologous proteins with different lattices. The viral particle seems to be created in a genetically economical manner, with the sealing of joints between inner-layer proteins by a second layer of proteins, suggesting the organization of a rigid protein layer that separate from and, probably, protects the interior of the virus from the cytoplasmic environment within infected cells. Procedure of the virus assembly was analyzed combined with molecular cytopathological data in virus infected cells.

Keywords: virus assembly, viral structure and function, virus host interactions

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A new virus structure: The nucleosome-like organization of the filamentous archaeal virus AFV1

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Double-stranded DNA viruses infect Archae living in hot springs at temperature above 80 ℃ . They are radically different in their properties from viruses that infect Bacteria and Eukarya. Not only are the shapes of these viruses different to all other viruses found on Earth, but ca 90% of their putative genes do not have any homologs in other viruses or cellular life forms (1). Their ecological, morphological and genomic originality raises intriguing questions about their biology and their origins. Combination of electron microscopy and X-ray crystallography has revealed a nucleosome-like organization for the lipothrixvirus Acidianus Filamentous Virus 1 (AFV1) that has not been yet observed for any linear viruses. The three-dimensional reconstruction of AFV1 core has revealed a left-handed helical structure similar to those in the eukaryal nucleosome. In this architecture, one of the two major structural proteins, the basic ORF132 might form a histone-like central proteic core with the DNA super helix wrapped around. The second structural protein, ORF140, is located peripherically. We propose that its helical and elongated N-terminus, charged positively, is in contact with DNA, probably binding major grooves. The globular C-terminus domain contains an amphilic helix and harbours buried octyl-glucoside molecules. It might, together with associated lipids, form the outer coat of the virus. Structural characterizations of these fascinating DNA archaeal viruses, already described with the virus STIV (2), contribute to a recent upsurge of interest in the evolution of virus in general.

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Keywords: virus structure, X-ray macromolecular crystallography, electron microscopy

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Structural studies of Holliday junction resolvases from bacteriophages, archaea and yeast

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Holliday junctions (HJ) are central intermediates in repair and reorganization of DNA by homologous recombination. HJ-resolving enzymes (also known as HJ resolvases) mediate the resolution of the 4-way junction by introducing symmetrical nicks in opposing strands. Members of this ubiquitous family of structure-specific endonucleases function as dimers and require divalent cations for cleavage. We have previously reported the crystal structure of a HJ complex of Phage T4 endonuclease VII (EndoVII), an enzyme, which is involved in mismatch repair and the resolution of branch points prior to packaging of DNA into the phage head. The conformation of the EndoVII-bound HJ represents a hybrid between the standard stacked-X and square-planar conformations, demonstrating how the junction is recognized and distorted by an induced-fit mechanism. We have also solved the crystal structures of cruciform cutting enzyme 1 (Cce1) from Candida glabrata and of the HJ cutting enzyme (Hjc) from the hyperthermophile archaeon Archaeoglobus fulgidus at 3 and 1.7Å resolution, respectively. They represent two structurally distinct resolvase families with the same biological function, but exhibiting clearly different substrate specificities. Recently we have determined the structure of a HJ complex of A.fulgidus Hjc at 3.2Å resolution. Surprisingly, in this complex two Hjc dimers are bound to the junction, which exhibits an essentially undisturbed stacked-X conformation. Common features as well as striking differences in the mode of junction binding and recognition among the structurally characterized members of the resolvase family will be discussed.

Biertuempfel, C., Yang, W. & Suck, D. Nature, 2007, 449, 616-620.

Keywords: Holliday junction resolvases, endonuclease VII, Hjc, Cce1, crystal structure

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Charge density based ligand design

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Recently we synthesised and experimentally determined the charge density in molecular species to gain insight in their reactivity and coordination behaviour. Not to judge on the structure-reactivity relationship by mere bond lengths comparison we rather relay on the topological analysis on the basis of high resolution data and a multipole refinement. Various topics are addressed in the talk:
S=N bonds in polyimidosulfit ylides are rather easy to cleave because they are electrostatically reinforced S+—N— single bonds rather than hetero-olefin bonds and the formal S=C double bond is a polar S+—C— single bond with no ylenic contribution;
The same is valid for P=N bonds. Reducing of iminophosphoranes to phosphanes is feasible under the right conditions, even though they are known to be thermodynamic sinks;
The metallaphosphane [Me2Al(mu-Py)2P] contains a divalent P(III) atom with two lone pairs at the central phosphorus atom. Following this finding the P-atom in the protonated phosphanide PPy2(H) can be employed in mu-bridging mimicking a 4-electron donor;
Multipole refinement of an alpha-lithiated benzyl silane provides insight in the electronic situation and thus the observed stereochemical course of transformations. Surprisingly the negative charge generated at the carbanion hardly couples into the phenyl ring;
The Laplacian distribution around the boron atom in [{$Cp(CO)_2Mn$}$_2$(mu-BtBu)] with its three VSCCs clearly shows the difference between the borylene ligand and the carbonyl ligand. The complex has to be classified as a dimetalloborane with no Mn-Mn bond rather than a borylene complex.

Keywords: ligand design, charge density studies, structure and function

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Non-linear optical properties & structure determination by combining X-ray data and QM wavefunctions

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