that Glu184 is essential for its catalytic activity. We have also determined the residues involved in substrate recognition by single amino-acid substitution experiments based on the structure. These results indicate that a large conformational change of sub-domain is required to exert the muramidase activity. We will discuss a possible PG-hydrolyzing mechanism of FlgJ in flagellar assembly.

1296-Pos

Structure of the Newcastle Disease Virus F Protein in the Post-Fusion Conformation

Xiaolin Wen[1], Kurt Swanson[2,3], Robert A. Lamb[2,3], Theodore S. Jardetzky[1].
[1]Stanford University, Stanford, CA, USA, [2]Northwestern University, Evanston, IL, USA, [3]Howard Hughes Medical Institute, Evanston, IL, USA.

Newcastle disease virus (NDV) is a member of the Paramyxoviridae family. The NDV fusion (F) glycoprotein, which is responsible for merging the viral and cellular bilayers during entry. The X-ray crystal structures have been solved of F proteins in the post-fusion and the pre-fusion conformations, providing atomic level information regarding the conformational transitions accompanying fusion. However, our understanding of the similarities between different F glycoproteins in these two conformational states remains incomplete.

Here, we present the crystal structure of the secreted, uncleaved ectodomain of the NDV F protein. Previous structural analysis of a related NDV F protein was missing key elements of the functional regions of the protein, including two helical segments (HRA and HRB) that assemble into a stable six helix bundle (6HB) in the post-fusion form. We have produced the NDV F protein in pre- and post-fusion conformations, using analogous constructs that produced a pre-fusion PIV5 F structure and a post-fusion HPIV3 F structure. We demonstrate that the two NDV F proteins exhibit the pre-and post-fusion forms through EM analysis and we have solved the crystal structure of the post-fusion form of the NDV F protein. In contrast to the previously determined NDV F structure, our new crystal structure contains the 6HB at the base of the stalk region, consistent with the EM observations and the previously determined HPIV3 F structure. Global superposition of the NDV and HPIV3 structures demonstrates maximum correspondence between distal portions of the structures, with orientation or adjustments in linking domains and the extended HRA stalk. Electrostatic profiles of the NDV, HPIV3, and PIV5 F structures show elements of conserved charge distributions despite significant sequence differences in these glycoproteins, which may be important for their common functionality.

1297-Pos

Structure of DNA Binding Domain of Plant Telomere Binding Proteins Represents Unique Features of Telomere Binding Protein Family

Weontae Lee[1], Sunggeon Ko[1], Hyun-Soo Cho[1], Chaejoon Cheong[2].
[1]Yonsei University, Seoul, Republic of Korea, [2]KBSI, Ochang, Republic of Korea.

Telomeres, the ends of linear eukaryotic chromosomes, are composed of short repeats of G-rich sequences and play essential roles in genome stability with various telomere binding proteins. To characterize the binding mode of plant telomere DNA and telomere binding protein, we determined the structures of DNA binding domain and telomere complex of NgTRF1, atTRF and RTBP1, double strand telomere binding proteins of plants, by multidimensional NMR spectroscopy and X-ray crystallography. We have identified the DNA binding interface of the DNA binding domain of TBPs, which is composed of 4 α-helices by means of chemical shift perturbation analysis. The complex crystal structure of $NgTRF1^{561\ 681}$ and plant telomere DNA $(TTTAGGG)_2$ have shown the molecular details of the interaction between them and we confirmed the interaction biochemically through site-directed mutagenesis. From the comparison with the structure of human telomere binding protein, we tried to show the unique features of plant telomere binding protein in the mode of telomere DNA binding as well as the similarity with the telomere binding proteins in other organisms. To our knowledge, this is the first report of the complex structure of telomere binding protein and telomere DNA in plant.

1298-Pos

Structural and Functional Characterization of an Unusual SH3 Domain from the Fungal Adaptor Protein Bem1

Maryna Gorelik, Ranjith Muhandiram, Alan Davidson.
University of Toronto, Toronto, ON, Canada.

Protein interactions form the basis of most biological processes and in eukaryotes are often mediated by conserved modular domains that recognize linear motifs. Among the most common protein interaction domains is the SH3 domain that generally recognizes PxxP containing peptides. SH3 domains are approximately 60 amino acids long and are composed of five beta strands. We are studying the SH3 domain from the fungal adaptor protein Bem1p that plays an important role during polarized growth and activation of MAPK signaling pathways. This SH3 domain is unusual, even though its sequence conforms to the SH3 domain consensus, it requires an extra 40 amino acids at its C-terminus for folding. Furthermore, in addition to binding PxxP containing peptides, it also binds the Cdc42p GTPase in a PxxP-independent manner. We are using in vitro binding assays and NMR spectroscopy to structurally and functionally characterize this unusual SH3 domain. Contrary to a previous report, we find that the Bem1 SH3 domain can simultaneously bind the Cdc42p GTpase and PxxP-containing peptides and that the binding of one does not affect the affinity for the other. Structural characterization by NMR shows that the extra sequence contains two alpha helices that pack tightly against the SH3 domain and thus form an integral part of the fold. Our findings provide with an example of how a common protein interaction domain can evolve to have additional atypical structural features and associated functions.

1299-Pos

Structural Investigation of a Fibronectin Type III Domain Tandem from the A-band of the Titin

Andras Czajlik, Gary Thompson, Ghulam N. Khan, Arnout Kalverde, Steve W. Homans, John Trinick.
University of Leeds, Leeeds, United Kingdom.

Single molecules of the giant muscle protein titin span half muscle sarcomeres, from the Z-disk to the M-band, and have key roles in sarcomere assembly and elasticity. In the A-band titin is attached to thick filaments and here the sequence shows fibronectin type III and immunoglobulin-like domains. These are mostly arranged in regular patterns of eleven domains called the large super-repeats. The large super-repeat occurs eleven times and this entire region thus forms nearly half of the titin molecule. Through interactions with myosin and C-protein, it is involved in thick filament assembly. We are determining the atomic structure, dynamical properties and the inter-domain arrangement of overlapping double and triple domain fragments of the large super-repeat by NMR spectroscopy. Ultimately, we hope to combine the data to reconstruct the overall conformation of the super-repeat. Here we investigated the A59-A60 domain tandem, which was expressed in bacteria from cDNA. The assignment of the backbone atoms was obtained using triple resonance NMR experiments. An initial structure was determined by backbone chemical shifts and homology modeling using the CS23D and Rosetta software packages. It was refined using RDC data to give realistic models for both domains. As we expected, these are both double-β-sheet sandwich structures characteristic of fibronectin type III domains. We are also carrying out relaxation measurements to probe the dynamics of the domains and their linker region.

1300-Pos

Localization of the Fission Yeast U5.U2/U6 Spliceosome Subunits

Yoshimasa Takizawa, Melanie D. Ohi.
Vanderbilt University Medical School, Nashville, TN, USA.

The spliceosome is a dynamic macromolecular machine that catalyzes the excision of introns from pre-mRNA to generate protein-coding transcripts. The megadalton-sized spliceosome is composed of four small nuclear RNPs (U1, U2, U5, and U4/U6) and numerous pre-mRNA splicing factors. The formation of an active spliceosome is hypothesized to occur in a stepwise manner requiring the assembly and disassembly of large multiprotein/RNA complexes. A promising structural approach to obtain information about spliceosome complexes is single-particle cryo-electron microscopy (cryo-EM), a powerful technique that is ideal for determining the structures of large dynamic complexes at protein concentrations too low for crystallization. Formerly, our group determined structure of the fission yeast U5.U2/U6 spliceosome complex by cryo-EM. This U5.U2/U6 spliceosome complex contains the U2, U5, and U6 snRNAs, pre-mRNA splicing intermediates, U2 and U5 snRNP proteins, the Nineteen Complex (NTC), and second-step splicing factors. However, the location of these subunits in the complex was not determined. Using antibody labeling and single particle EM we are now localizing these individual subunits within the density map of the U5.U2/U6 spliceosome complex. This work now enables us to propose a structural model for U5.U2/U6 organization.

1301-Pos

3D Solution Structure of the C-terminal Chromodomain of the Chloroplast Signal Recognition Particle

Ananthamurthy Koteshwara, Kathir M. Karuppanan, Robyn Goforth, Ralph Henry, Suresh Kumar, K. Thallapuranam.
University of Arkansas, Fayetteville, AR, USA.

Chloroplasts use chloroplast signal recognition particle (cpSRP) pathway to import important cargo like light harvesting chlorophyll protein (LHCP).

cpSRP is unique among SRPs in being devoid of RNA. cpSRP consists of an evolutionarily conserved 54-kDa subunit (cpSRP54) and an unique 43-kDa subunit (cpSRP43). cpSRP43 subunit has four-ankyrin repeat domain at the N terminus and a C-terminal chromo domain (CD). The C-terminal CD of cpSRP43 has been shown to provide interaction sites for the cpSRP54 subunit. In addition, the chromodomain in the cpSRP43 subunit is also believed to be important for the formation of the transit complex with LHCP. In this context, we embarked on the structural characterization of the C-terminal CD using a variety of biophysical techniques including multidimensional NMR spectroscopy. Far UV circular dichroism spectrum of CD shows that the backbone of the protein is predominantly in the helical conformation. 1H-15N HSQC spectrum of CD is well- dispersed suggesting that the protein is structured. Complete resonance assignments (1H, 15N and 13C) in CD have been accomplished using a variety of triple resonance experiments. Chemical shift index plots show that CD is an $\alpha + \beta$ protein. A detailed analysis of the three-dimensional solution structure of CD will be presented. The three-dimensional solution structure of CD provides valuable insights into the molecular mechanism underlying the post-translational transport and integration of LHCP on the thylakoid membrane.

1302-Pos

The PSI SGKB Technology Portal - An Online Database of Structural Genomics Technologies

Lester Carter[1], Maggie Gabanyi[2], Helen M. Berman[2], Paul Adams[1].
[1]LBL, Berkeley, CA, USA, [2]Rutgers, New Brunswick, NJ, USA.

The Protein Structure Initiative (PSI) Structural Genomics KnowledgeBase (SGKB) technology portal is an online database of PSI-derived technologies. Information within the portal will be of use to scientists involved in all branches of molecular biology. Advances are described in all stages of the protein production pipeline, from initial target selection to cloning, expression, structure solution and structure analysis. Information is provided on robotics, high-throughput protocols, and software development.
The url for the website is: http//:technology.lbl.gov/portal/

1303-Pos

Protein Structure Initiative Material Repository (PSI-MR): A Resource of Structural Genomics Plasmids for the Biological Community

Catherine Cormier[1], Jean Chin[2], Joshua LaBaer[1].
[1]Arizona State University, Tempe, AZ, USA, [2]NIH/NIGMS, Bethesda, MD, USA.

The Protein Structure Initiative Material Repository (PSI-MR; http://psimr.asu.edu) provides centralized storage and distribution for the growing collection of more than 80,000 protein expression plasmids created by PSI researchers. These plasmids are an invaluable resource that allows the research community to dissect the biological function of proteins whose structures have been identified by the PSI. The plasmid annotation, which includes the full length sequence, vector information, and associated publications, is stored in a freely available, searchable database called DNASU (http://dnasu.asu.edu). Each PSI plasmid is also linked to a variety of additional resources, including the PSI Structural Genomics Knowledgebase (PSI-SGKB: http://kb.psi-structuralgenomics.org), which facilitates cross-referencing of a particular plasmid to protein annotations and experimental data. Nearly 16,000 PSI plasmid samples are currently available and can be requested directly though the website. The PSI-MR has also developed a novel strategy to avoid the most common concern encountered when distributing plasmids, namely the complexity of material transfer agreement (MTA) processing and the resulting delays this causes. It is in this context that we developed and successfully implemented the Expedited Process MTA, in which we created a network of institutions that agree to the terms of transfer in advance of a material request, thus eliminating the delay researchers would typically encounter while their institution is processing the MTA. Our hope is that by creating a repository of expression-ready plasmids and expediting the process for receiving these plasmids, we will help accelerate the accessibility and pace of scientific discovery.

1304-Pos

How to use the PSI Structural Genomics Knowledgebase to Enable Research

Andrei Kouranov[1], John Westbrook[1], Margaret Gabanyi[1], Yi-Ping Tao[1], Raship Shah[1], Torsten Schwede[2], Konstantin Arnold[2], Florian Kiefer[2], Lorenza Bordoli[2], Paul Adams[3], Lester Carter[3], Wladek Minor[4], Rajesh Nair[5], Joshua LaBaer[6], Helen M. Berman[1].
[1]Rutgers University, Piscataway, NJ, USA, [2]University of Basel, Basel, Switzerland, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [4]University of Virginia, Charlottesville, VA, USA, [5]Columbia University, New York, NY, USA, [6]Arizona State University, Tempe, AZ, USA.

The Protein Structure Initiative Structural Genomics Knowledgebase (PSI SGKB, URL: http://kb.psi-structuralgenomics.org) is a web resource designed to turn the products of the structural genomics and structural biology efforts into knowledge that can be used by the biological community to understand living systems and disease. We will present examples and demonstrate how to use the PSI SGKB to enable biological research. For example, a protein sequence or PDB ID search will provide a list of protein structures from the Protein Data Bank, associated biological descriptions (annotations), homology models, structural genomics protein target information, experimental protocols, and the ability to order available DNA clones. A text search will find technology reports and publications that were created by the PSI's high-throughput research efforts. Web tools that aid in bench top research, such as protein construct design, are also available. Created in collaboration with the Nature Publishing Group, the Structural Genomics Knowledgebase Gateway provides a research library, editorials about new research advances, news, and an events calendar also present a broader view of structural genomics and structural biology. The PSI SGKB is funded by the NIGMS.

1305-Pos

NIGMS PSI:Biology Initiative – High-Throughput-Enabled Structural Biology

Peter C. Preusch, Ravi Basavappa, Jean Chin, Charles Edmonds, Ward Smith, Catherine Lewis.
Nat Inst Gen Med Sciences, Bethesda, MD, USA.

The primary goal of the Protein Structure Initiative:Biology (PSI:Biology) to be funded by the National Institute of General Medical Sciences (NIGMS) is to apply high-throughput structural biology to important biological problems (http://www.nigms.nih.gov/Initiatives/PSI/psi_biology/). This will be accomplished by establishing partnerships between centers for structure determination and biologists with interests in particular proteins or collections of proteins. The PSI:Biology network centers will include: 1) Centers for High-Throughput Structure Determination, 2) Centers for Membrane Protein Structure Determination, 3) the PSI:Materials Repository, and 4) the PSI:Biology Knowledgebase. The partnerships, established through Consortia for High-Throughput-Enabled Structural Biology Partnerships, will define targets for structure determination and provide funds for functional studies in the applicants' laboratories and for a portion of the cost for structure determination in the center. In addition to protein structures and models, the PSI:Biology network will generate and make available reagents and plasmids for expressed proteins to support functional studies in the research community. NIGMS encourages Partnership applications from biologists or groups of biologists with biological questions that will benefit from the determination of relevant protein structures. The PSI:Biology high-throughput approach will enable examination of families of proteins related to the target proteins, an approach that has proven highly successful in generating the first structure of a family member and then allowing many other family members to be modeled. Examples of current partnerships include using structural genomics, modeling and systems biology to generate a three-dimensional reconstruction of the central metabolic network of the bacterium, *Thermotoga maritima*, and the discovery of novel enzymatic mechanisms for the enoylase and amido hydrolase classes of enzymes. Additional opportunities for researchers to join the PSI:Biology network will be provided through ongoing and future program announcements. Researchers may also suggest proteins for structure determination through the PSI Community Nomination site at: http://cnt.psi-structuralgenomics.org/CNT/targetlogin.jsp.

1306-Pos

Technology Development Highlights Generated from the Center for Eukaryotic Structural Genomics

George N. Phillips Jr.,, John Primm, David Aceti, Craig A. Bingman, Ronnie Frederick, Shin-ichi Makino, Francis Peterson, Frank Vojtik, Russel Wrobel, Zsolt Zolnai, Brian Volkman, Brian G. Fox, John L. Markley.
UW Madison, Madison, WI, USA.

The Center for Eukaryotic Structural Genomics (CESG) aims to be the leading center for developing and disseminating tested technologies to efficiently solve structures of eukaryotic proteins. We create, evaluate, and optimize innovative protocols for producing eukaryotic proteins in active forms. We seek to improve the efficiency of all stages from target selection-design to three-dimensional structure determination by X-ray crystallography or NMR spectroscopy, including development of bioinformatic techniques and LIMS tools. Using our protein production platform, we refine methods for improving the yield of structures from high-value targets, in particular proteins from humans and other vertebrates. CESG has a substantial outreach component; more than 400 targets from outside requestors have been accepted for study with a structure success rate of 5%, which compares favorably with the eukaryotic success rates for the