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

total PSI effort. These eukaryotic targets frequently present unique challenges. All CESG protein production protocols and Technology Dissemination Reports are accessible through the PSI Knowledgebase: http://kb-psi-structuralgenomics.org/KB/ and CESG's website. Selected technology developments are presented here. These include advances in expression vector design, enhanced methodology for cell based and wheat germ cell-free expression systems, new software to improve the quality and reduce time for structure determination by X-ray crystallography and NMR, and optimized techniques for the production of TEV protease for use in our protein production platform. We actively share our advances with the biotechnology, pharmaceutical, and academic communities through collaborations, oral presentations, peer-reviewed articles, submissions to public databases and material distribution channels, including PepcDB, PDB, BMRB, PSI Materials Repository, and technology transfer workshops.

Supported by NIH/NIGMS Protein Structure Initiative grants GM074901 and GM064598. We acknowledge ALL members of the CESG team for their dedicated work.

1307-Pos

Effective Protein Crystallization Screening with Synthetic Zeolite Molecular Sieves as Hetero-Epitaxic Nucleant

Michihiro Sugahara, Yuko Kageyama-Morikawa, Naoki Kunishima.
RIKEN, Sayo-cho, Japan.

Protein crystallization is still a major bottleneck in structural biology. As the current methodology of protein crystallization is a type of screening, it is usually difficult to crystallize important target proteins. The hetero-epitaxial growth from the surface of mineral crystal as a nucleant had been thought to be effective to enhance the chance of protein crystallization. However, generally applicable hetero-epitaxial nucleants for protein crystallization have never been found. Recently, we have reported a protein crystallization method using synthetic zeolite molecular sieves as a hetero-epitaxic nucleant. This method is based on the packing space expansion of protein crystals by a directed nucleation on the material surface, thereby providing new crystal forms with a substantial improvement of diffraction quality in some cases. In this work, a sparse matrix crystallization screening experiment of xylanase from *Trichoderma longibrachiatum* was performed with and without molecular sieves, using a commercially available sparse matrix screening kit. A result of crystallization screening of xylanase showed that molecular sieves promotes the crystallization of xylanase, suggesting that the hetero-epitaxic nucleate approach allows us to improve the effectiveness of the sparse matrix screening of protein crystallization. Interestingly, molecular sieves 5A and 13X provided a new crystal form under the crystallization condition containing zinc, in which a zinc-mediated protein sheet looked favorable for the hetero-epitaxic 3D crystal growth.

1308-Pos

Structural Biology Reveals A New Protein Family from *S.Cerevisiae* with A Novel Fold and Implicated in the Metabolism Control And Drug Resistance

Tatiana Domitrovic[1], Guennadi Kozlov[2], João G. Freire[1], Claudio A. Masuda[3], Marcius S. Almeida[3], Monica Montero-Lomeli[3], Edna Matta-Camacho[2], Kalle Gehring[2], Eleonora Kurtenbach[1].
[1]Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brazil, [2]Department of Biochemistry, McGill University, Montréal, QC, Canada, [3]Instituto de Bioquimica, UFRJ, Rio de Janeiro, Brazil.

We have undertaken a small-scale structural genome project focusing on *S. cerevisiae* ORFs without characterized functional motifs or known primary sequence homologs. The cloning, expression and purification screening of 9 targets sequences, led to the determination of the crystal structure of Yer067w by Multiple Anomalous Diffraction at 1.7 Å resolution. This 20 KDa protein present an alpha-beta fold where the 7-stranded beta-sheet is backed by 4 alpha-helices on one side. Interestingly, a structure-based search using the servers SSM or DALI retrieved only proteins with insignificant superposition scores, indicating that Yer067w represents a novel fold superfamily. The phylogenetic analysis of Yer067w primary sequence homologs showed that this protein belongs to a well-conserved family exclusive to Ascomycetes. To further understand Yer067w role we have searched for functional hints using yeast strains deleted for this gene and its paralog *YIL057C*. Microarray analysis of Δyer067w revealed important modifications in expression of genes related to oxidative phosphorylation, amino acids and lipid metabolism. In a screening for phenotypes, we verified that all mutants presented growth deficiencies in non fermentative carbon sources and Western bolt analysis showed that the presence of both proteins are tightly linked to growth on respiratory substrates or low nutrient conditions, suggesting that both proteins are important to the metabolism in glucose free media. Furthermore, Yer067w mutants revealed an antifungal drug resistance phenotype, presenting an increment of 2 times in the MIC for Nystatin and anphotericin B. This work highlights the importance of functional characterization of unknown ORFs for the comprehension of yeast cells metabolism and for uncover new regulatory elements. Support: FAPERJ, CNPq, CIHR

Protein Aggregates I

1309-Pos

Thermodynamic Instability of A Self-Assembled 16-Residue Alanine-Based Oligopeptide in Aqueous Media: Hydrogel, Fibril, and Beaded Filament Formation

Thomas J. Measey[1], Konstantin Kornev[2], Reinhard Schweitzer-Stenner[1].
[1]Drexel University, Philadelphia, PA, USA, [2]Clemson University, Clemson, SC, USA.

Oligoalanines with greater than ca. 14 residues, which have been doped with charged residues (e.g. lysine), usually adopt α-helical conformations in aqueous media.[1] In contrast, Ac-$(AAKA)_4$-NH_2 aggregates instantaneously when dissolved in aqueous media at concentrations ranging from 70 μM to 7 mM. Evidence from UV circular dichroism (UV-CD) and FTIR spectroscopies suggest a mostly β-sheet like conformation. Kinetics studies of the initially formed conformations suggest a transition from a mostly β-like to a mostly PPII-like conformation, in contrast to typical fibril formation/growth studies. AFM images of freshly prepared samples indicate a porous, tissue-like architecture. Addition of salts (e.g. NaCl) stabilizes this architecture, resulting in the formation of a macroscopic hydrogel. β-sheet and hydrogel formation by such an amino acid sequence is quite unusual, as it does not obey typical rules required for peptide hydrogel formation. In particular, there are no alternating complimentary charges, nor alternating hydrophilic and hydrophobic residues. Without the added stabilization provided by the addition of salts, however, the peptide transitions from a mostly β-sheet-like structure to a PPII-like conformation, possibly via triple helix formation, as evidenced by both electronic and vibrational CD. AFM images suggest the formation of a heterogeneous mixture of assemblies, including bead-like filaments as well as networks of well-defined, intertwined fibrils. Potential applications for peptide hydrogels include drug delivery devices and tissue engineering scaffolds. In this regard, release studies of model peptides which have been trapped within the peptide hydrogel will be presented, as well as rheological characterization of the hydrogel as a function of salt and peptide concentration.

[1] Scholtz, J. M.; Marqusee, S.; Baldwin, R. L.; York, E. J.; Stewart, J. M.; Santoro, M.; Bolen. D. W. *Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2854-2860.

1310-Pos

The Self-Aggregation of A Polyalanine Octamer Promoted by its C-terminal Tyrosine and Probed by A Strongly Enhanced VCD Signal

Thomas J. Measey[1], Kathryn B. Smith[2], Sean M. Decatur[3], Liming Zhao[1], Guoliang Yang[1], Reinhard Schweitzer-Stenner[1].
[1]Drexel University, Philadelphia, PA, USA, [2]Mount Holyoke College, South Hadley, MA, USA, [3]Oberlin College, Oberlin, OH, USA.

The 8-residue alanine oligopeptide, Ac-A_4KA_2Y-NH_2(AKY8), was found to form amyloid-like fibrils upon incubation at room temperature in acidified aqueous solution, at peptide concentrations > 10 mM. The fibril solution exhibits an enhanced VCD couplet in the amide I' band region, which is nearly 2 orders of magnitude larger than typical polypeptide/protein signals in this region. Such intensity enhancements have recently been observed for insulin and lysozyme fibrils.[1] We performed simulations of the VCD and IR amide I' band profile using a simplified excitonic coupling model. Preliminary results suggest that inter-sheet coupling is responsible for the VCD intensity enhancement. The UV-CD spectrum of the fibril solution shows circular dichroism in the region associated with the tyrosine side chain absorption. A similar peptide, Ac-A_4KA_2-NH_2 (AK7), lacking in a terminal tyrosine residue, does not aggregate. These results suggest a pivotal role for the C-terminal tyrosine residue in stabilizing the aggregation state of this peptide. It is speculated that interactions between the lysine and tyrosine side chains of consecutive strands in an antiparallel arrangement, e.g. via cation-π interactions, are responsible for the stabilization of the resulting fibrils. These results offer considerations and insight for the de novo design of self-assembling oligopeptides for biomedical and biotechnological applications, and highlight the usefulness of VCD as a tool to probe amyloid fibril formation.

[1] Ma, S.; Cao, X.; Mak, M.; Sadik, A.; Walkner, C.; Freedman, T.B.; Lednev, I.K.; Dukor, R.K.; Nafie, L.A. *J. Am. Chem. Soc.***2007**, 129, 12364-12365.