mammalian counterparts, suggesting additional roles other than metal binding and detoxification. Homology modeling of a Cd-binding durum wheat MT (dMT) indicates mammalian-like folds for the metal-binding domains, and *ab initio* calculations yield a DNA-binding like structural motif for the hinge region. Here, we study the structural features of full-length dMT in metal-free (apo) and metal-bound (holo) states using various biophysical and biochemical techniques. Cd-bound dMT is expressed in *Escherichia coli* as a GST fusion protein and cleaved from this tag for further analyses. Small angle X-ray solution scattering measurements and gel-filtration chromatography revealed holo-dMT as a dimer whereas apo-dMT is monomeric as shown by native state mass spectrometry experiments. Far-UV circular dichroism (CD) measurements revealed apo- and holo-dMT as a random coiled protein. Near-UV CD measurements showed two maxima at 246.6 and 266 nm for holo-dMT. The amount of bound Cd was determined as 5±1 M equivalent by inductively coupled plasma optical emission spectroscopy. When Cd metals were stripped, the two CD maxima disappeared and this form of dMT showed CD spectrum similar to that of the acid unfolded dMT, suggestive of disordered structure for the apo form. Interestingly, Kratky plot of the SAXS data lacks the characteristic maximum observed for globular proteins, indicating that Cd-bound dMT is in a molten globule like conformation. Taken together, our results put forward the possibility of dMT as a candidate for an intrinsically unstructured protein.

2749-Pos Thermal Stability Of The TATA Box Binding Protein

César Millán-Pacheco, Víctor M. Capistrán, Nina Pastor

Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico.

Board B52

Protein function implies a compromise between stability and flexibility. Proteins from organisms adapted to high temperatures appear to compensate larger thermal fluctuations with increased stability. We probe the structural, energetic and fluctuation amplitude responses of TBP with 5 ns molecular dynamics simulations at temperatures ranging from 0 to 100°C, carried out in the NPT ensemble at 1 atm. We simulated TBPs from *Pyrococcus*, *Sulfolobus*, *Saccharomyces* and *Arabidopsis*, with unfolding Tms of 101, 85, 60 and 60°C, respectively; *Pyrococcus* TBP has a disulfide bond, and its effect was studied simulating a reduced version of the protein (unfolding Tm of 97°C). The fluctuation amplitudes and free energy of the proteins (estimated within the MM-PBSA formalism) increase with temperature, but not at the same rate for all the systems. Resiliency measures[1] suggest that TBP has used entropic, rather than enthalpic stabilization as a strategy for thermal adaptation; accordingly, the number of intramolecular H-bonds does not change with temperature. The disulfide bond contributes enthalpically to the stability of *Pyrococcus* TBP. The rate of increase of fluctuation amplitudes with temperature at each residue revealed heating-resistant zones, and others that are highly responsive. The first lie at the interface between the β-sheet and helices 2 and 2′, as suggested by [2]; the extent of these zones decreases with the Tm. The latter map to the N- and C-termini, the stirrups and the loop connecting the N- and C-subdomains. The disulfide bond increases the stability of the connections between helix 1 and the β-sheet, and also the loop between strands 3 and 4.

Funding: Macroproyecto UNAM: Tecnologías para la Universidad de la Información y la Computación

References

[1]. Tehei, M. and Zaccai, G. *Biochim. Biophys. Acta* (2005) ***1724***:404
[2]. Koike H, et al. *Structure* (2004) ***12***:157

2750-Pos Biological Thermodynamics: The Thermal Set Point and the Origins of Life

Paul W. Chun

University of Florida Collge of Medicine, Gainesville, FL, USA.

Board B53

Application of the Planck-Benzinger thermal work function to biological systems has demonstrated a basic pattern for life processes, in that there is a lower cutoff point, $<T_h>$, where entropy is favorable but enthalpy is unfavorable, i.e. $\Delta H^o(T_h)(+) = T\Delta S^o(T_h)(+)$, and upper cutoff, $<T_m>$, above which enthalpy is favorable but entropy unfavorable, i.e. $\Delta H^o(T_m)(-) = T\Delta S^o(T_m)(-)$. Only between these two limits, where $\Delta G^o(T) = 0$, is the net chemical driving force favorable for interacting biological processes. In the case of water vapor condensation the compensatory temperatures, $<T_h>$ and $<T_m>$, are 30 K and 380 K. Each living system is made up, in some part, of water. Hence we suggest that the single point at which the system is its most stable, defined as the thermal set point, $<T_S>$, must fall between the limits of 30 K and 380 K. We find that each biological system will exhibit a negative minimum of Gibbs free energy change at this well-defined temperature, $<T_S>$. Each system will have its own unique value of $<T_S>$, where the bound unavailable energy $T\Delta S^o = 0$. At this point, $\Delta H^o(T_S)(-) = \Delta G^o(T_S)(-)_{minimum}$, the maximum work can be accomplished. For water vapor condensation, thermal set point falls at 260 K and $<T_{Cp}>$ at $\Delta Cp^o(T) = 0$ is 130 K. In examining interacting protein systems, it would appear that the heat capacity change of reaction of water within the system determines the behavior of the other thermodynamic functions. It is apparent from the application the Chun approach to studies of numerous biological interactions that the origins of life in any system are inevitably linked to a single, unique thermal set point.

Protein Structure

2751-Pos Structure and Interactions of Mini-B, a Fragment of Surfactant Protein B

Muzaddid Sarker[1], Alan J. Waring[2], Kevin MW Keough[1], Valerie Booth[1]

[1] *Memorial University of Newfoundland, St. John's, NL, Canada,*
[2] *UCLA School of Medicine, Los Angeles, CA, USA.*

Board B54

Lung surfactant, a mixture of lipids and proteins, is essential to life as it reduces the surface tension at the alveolar air-water interface and thus enables normal breathing. Surfactant Protein B (SP-B) plays crucial roles in lung surfactant function and is absolutely necessary for the survival. SP-B is thought to function by facilitating large-scale rearrangement of lipids and stabilizing the structures at various stages of breathing cycle. However, neither the structural basis for this ability nor the physiological ramifications of lipid rearrangements are yet understood, in part because a high-resolution structure of SP-B has not been determined. Mini-B is a peptide fragment of SP-B that has been shown to retain similar activity to the full-length protein in certain *in vitro* and *in vivo* studies. We have used solution NMR to determine the structure of Mini-B initially in organic solvent hexafluoroisopropanol (HFIP) and finally in detergent micelles composed of sodium dodecyl sulfate (SDS) mimicking the physiological environment. In both conditions, Mini-B forms two α-helices connected by an unstructured loop. In the native-like SDS environment, Mini-B possesses a strikingly amphipathic surface. These studies help to define the structural features that underlie SP-B's function and provide a platform to probe its interactions with other components of the lung surfactant system.

2752-Pos Structural Basis for Autoimmunity: Crystal Structure of Human TL1A

Chenyang Zhan[1], Qingrong Yan[1], Yury Patskovsky[1], Wuxian Shi[2], Udupi A. Ramagopal[1], Rafael Toro[1], Huiyong Cheng[1], Michael D. Brenowitz[1], Stanley G. Nathenson[1], Steven C. Almo[1]

[1] *Albert Einstein College of Medcine, Bronx, NY, USA,*
[2] *Case Western Reserve University, Cleveland, OH, USA.*

Board B55

The Tumor Necrosis Factor ligand (TNF) superfamily includes important cytokines that coordinate the development, homeostasis and course of adaptive immune responses. TL1A is a newly described member of the TNF superfamily that is directly implicated in the pathogenesis of several autoimmune diseases, including inflammatory bowel disease, atherosclerosis and rheumatoid arthritis. Here we report the crystal structure of the human TL1A extracellular domain solved to 2.5 Å resolution. TL1A adopts a typical jelly-roll fold, which confirms its classification as a conventional TNF superfamily member. This structural information provides insights into the binding specificity between TL1A and its reported receptors, DcR3 and DR3. In addition, we have investigated disulfide bond formations in recombinant TL1A and have discovered functional TL1A mutants with increased stability.

2753-Pos Potential problems in studying protein-protein interactions using recombinant proteins and spin labeling EPR methods

Chloe Antoniou, Yuanli Song, L. W.-M. Fung

Department of Chemistry, University of Illinois at Chicago, 845 West Taylor St., MC 111, Chicago, IL, 60607, USA.

Board B56

Spin labeling EPR methods have been an important tool in studying protein-protein interactions. Spin labels are often attached to a cysteine residue side chain via a disulfide bond and EPR spectra of the label samples are obtained in the absence and in the presence of the binding partner(s). A potential problem may arise is if the unlabeled binding protein contains glutathione (GSH) molecules. When the protein is obtained from GST-fusion proteins, GSH is often used in the purification process. Some GSH molecules were found to be difficult to remove. We showed that the GSH molecules exchange with the spin labels bound to the protein, rendering artificial, multi-component spectra, making the analysis of protein-protein interactions difficult.

2754-Pos Changing of Function and Structure of Milk Carrier Protein β-lactoglobulin-A upon Interaction with New Anti-cancer Compounds Pd(II) Complexes

Adeleh Divsalar, Ali Akbar Saboury

Instute of Biochemistry and Biophysics, Tehran, Iran (Islamic Republic of).

Board B57

β-lactoglobulin (BLG), the major protein in the whey of ruminant milk is a globular protein with a monomer molecular weight of a bout 18,300. The physiological function of BLG is tentatively considered to be the binding and transportation of small hydrophobic ligands such as retinal and fatty acids. Since Palladium complexes have been reported to show fewer side effects relative to other heavy metal anticancer compounds, in this study a new class of anticancer Pd (II) complexes including 2,2′-bipyridin n-butyl dithiocarbamato Pd(II) nitrate (Com-**1**), 2,2′-bipyridin n-hexyl dithiocarbamato Pd(II) nitrate (Com-**2**), 2,2′-bipyridin octylglycinato Pd(II) nitrate (Com-**3**) and 2,2′-bipyridinglycinato Pd(II) nitrate (Com-**4**) was designed. The aim of this study is to investigate the effects of these ligands on the structure and function of BLG -A using fluorescence and CD techniques. Results of fluorescence studies revealed that the complexes had no dithiocarbamate moiety (Com **3** and **4**) could quench the intrinsic fluorescence emission of the proteins at lower concentrations than those had such moiety (Com **1** and **2**). The far UV-CD studies revealed that the secondary structure of BLG-A did not show any noticeable alteration upon interaction with different of Pd(II)-complexes. The potency of the retinol binding of BLG-A (function studies of the protein) in the

presence of Pd (II) complexes was studied. Results show that in the presence of Pd (II) complexes, the potency of the retinol binding of BLG-A significantly reduced. Our results suggested that Pd (II) complexes addition of dithiocarbamate moiety to structure of Pd(II) complexes probably has fewer effects on the carrier protein structure, while function of the protein significantly changed upon interaction by both of the groups of these ligands.

2755-Pos Characterization of Conformational Changes of Ca^{2+} Independent Bound Annexins Induced by Membrane Curvature

Torsten Fischer[1], Balachandra G. Hegde[1], Harry T. Haigler[2], Ralf Langen[1]

[1] *Department of Biochemistry and Molecular Biology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA,*
[2] *Department of Physiology and Biophysics, University of California-Irvine, Irvine, CA, USA.*

Board B58

Annexins are well-known Ca^{2+}-dependent membrane binding proteins but increasing evidence shows that they are also capable of Ca^{2+}-independent membrane interaction. We had previously shown that pH-dependent membrane interaction of annexin B12 causes major conformational changes. Depending on pH and lipid composition, the resulting structure can either be transmembrane or peripherally-bound. In comparison, Ca^{2+}-dependent binding shows very little conformational change and the structure is almost identical to that of annexin B12 in solution. Recent studies have shown that numerous proteins will change their conformation in response to changes in the headgroup packing density of membrane bilayers. We found that high membrane curvature or membranes with a high degree of lipids with negative intrinsic curvature induce membrane interaction of annexin B12 at neutral pH. Furthermore, EPR analysis shows that the curvature-dependent membrane bound form shares similarities with the pH-dependent transmembrane as well as the recently described peripherally-bound forms of annexin B12. This new interaction of annexin B12 at neutral pH is likely stabilized by the reduced packing density in the headgroup region that results from the membrane or intrinsic curvature. The detailed structure of the curvature-dependent membrane-bound is currently being investigated using novel pulsed EPR approaches. Analogous experiments with A2 and A5 showed that both were able to undergo curvature-dependent membrane interactions. These results suggest that at least some members of the annexin family could act as sensors of reduced membrane curvature or reduced headgroup packing density *in vivo.*

2756-Pos Structure of Membrane-Bound α-Synuclein Combining Modeling with Continuous Wave and Pulsed EPR

Christine C. Jao, Balachandra G. Hegde, Jeannie Chen, Ian Haworth, Ralf Langen

University of Southern California, Los Angeles, CA, USA.

Board B59

α-Synuclein is a presynaptic protein involved in several neurodegenerative diseases. It has been implicated in the modulation of the presynaptic vesicle pool size, the modulation of neurotransmitter release, and synaptic vesicle recycling. α-Synuclein is a disordered protein in solution, but transforms into a helical protein in the presence of membranes. The detailed structure of this physiologically important membrane-bound form remains unknown. In order to investigate this structure, we employed site-directed spin labeling combined with continuous wave (CW) and pulsed EPR spectroscopy. O_2 and NiEDDA accessibilities of 67 singly labeled, membrane-bound α-synuclein derivatives reveal local secondary structure and membrane topology for residues 25 through 90 (including previously published data for residues 59–90). We find that this region forms a single, interfacial helical structure with an unusual periodicity in which 11 amino acids take up exactly 3 turns. In order to get more detailed structural information and to test whether the extended helical structure includes additional N-terminal residues, we measured intramolecular distances using 4-pulse DEER (Double Electron Electron Resonance) experiments. These data together with computer modeling allowed us to arrive at a detailed structural model for membrane-bound α-synuclein. Importantly, this structure is significantly different from that of SDS-bound α-synuclein which, according to high resolution NMR, contains two bent α-helices. One of the reasons for these different structures could be the significantly smaller size of the SDS micelles, which might prevent the formation of a single elongated helix. The continuous helical structure described here could also be applicable to other 11-amino acid-repeat-containing proteins such as apolipoproteins, which wrap around lipid particles of defined size.

2757-Pos Towards An Automatic Classification Of Protein Structural Domains Based On Structural Similarity

Vichetra Sam[1], Chin-Hsien Tai[1], Jean Garnier[2], Jean-Francois Gibrat[2], Byungkook Lee[1], Peter J. Munson[1]

[1] *NIH, Bethesda, MD, USA,*
[2] *INRA - Unite MIG, Jouy en Josas Cedex, France.*

Board B60

Understanding the protein structure universe and establishing evolutionary relationships among them are aided by classification. Well-known classification databases, such as SCOP and CATH, are manually curated at some stage and are different from automatic classifications such as FSSP. One reason for this difference could be the pair-wise similarity scores used in automatic classification. Another possible reason is the clustering procedure used. Here, we explore the degree to which these two factors might affect the final classification.

We use DALI, SHEBA and VAST pairwise structural similarity measures on the ASTRAL40 SCOP C class domains, to investigate a variety of hierarchical clustering procedures. All procedures first construct a dendrogram which progressively joins domains into clusters. The dendrogram is then cut in a variety of ways to produce a partition, which is compared to the SCOP Fold classification.

Wardâ™s method for building dendrograms led to partitions closest to the SCOP fold partition. Two dendrogram-cutting strategies, which explicitly optimize similarity to SCOP, gave an average of 72% true positives at the 1% false positive rate (FPR). Cutting the largest size cluster at each tree-cutting step produced one of the best agreements obtained without using a prior knowledge of SCOP and gave an average of 61% true positives at the 1% FPR. Cutting the longest branch at each step produced one of the worst agreements.

We also developed a method to detect differences that are irreducible by any dendrogram-cutting strategies and found that they were substantial. These observations, as well as visual examination of individual cases, lead us to conclude that the major difference between automatic and manual protein classification arises from the pairwise scores themselves, rather than by an effect of the clustering procedure.

2758-Pos Structural and Functional Analysis of Human Potassium Channel Tetramerization Domain Containing Protein 5 (KCTD5)

Irina Dementieva[1], Valentina Tereshko[1], Zoe McCrossan[1], Dan Araki[1], Nikolaus Grigorieff[2], Elena Solomaha[1], Steve Goldstein[1]

[1] *University of Chicago, Chicago, IL, USA,*
[2] *Brandeis University, Waltham, MA, USA.*

Board B61

The human genome contains a 21 gene family encoding proteins that have an N-terminal BTB/POZ domain and a variable C-terminal domain. The N-terminal domain bears significant sequence homology to the cytoplasmic tetramerization domain (T1) of voltage-gated potassium channels and the gene family is named 'hKCTD' to reflect this fact. Strong preferential expression of hKCTD transcripts in a variety of fetal tissues suggests they may play a role during development. The structure and function of KCTDs has been unclear. Here, we determine the crystal structure of full-length hKCTD5 and its N-terminal core domain by multi-wavelength anomalous diffraction at 3 Å and 1.9 Å resolution, respectively. The overall KCTD5 structure surprisingly reveals a pentameric assembly of subunits with a funnel-shaped, metal transporter-like architecture as seen in CorA with well-defined N- and C-terminal domains and a complex-spanning central cavity. The high resolution structure of the N-terminal domain reveals the basis for pentameric assembly of KCTD subunits showing how it differs from T1 tetramers. As expected, we find human Golgi re-assembling stacking protein 55 (hGRASP55) to co-immunoprecipitate with hKCTD5 on co-transfection in HEK cells as previously reported by Gandhi et al. (Nat Genet, 2006). To further investigate this interaction, we applied surface plasmon resonance (Biacore) to map domains mediating protein-protein interaction with full-length and truncated versions of hKCTD5 and hGRASP55. Kinetic analyses show the proteins to associate with high affinity (KD =0.8±0.1 × 10–6, M-1) and that interaction requires the hKCTD5 C-terminal domain. To-date, we find no evidence that KCTDs assemble with or influence the function of voltage-gated potassium channels.

2759-Pos Expression, Purification and Structural Characterization of CfrA, A Putative Iron Transporter from Campylobacter jejuni

Casey L. Carswell, John E. Baenziger

University of Ottawa, Ottawa, ON, Canada.

Board B62

Campylobacter ferric receptor (CfrA) is a putative siderophore-mediated iron transporter localized to the outer membrane of the enteric food-borne pathogen *C. jejuni.* To gain further insight into CfrA structure and function, we cloned the *cfra* gene from chromosomal DNA and examined the expression of 6-, 8-, and 10-His-tagged constructs under different temperatures and concentrations of the inducer IPTG. Several detergents, including dodecylmaltoside, were effective at solubilizing CfrA from the pelleted membrane fraction, although a substantial portion of the expressed protein was resistant to solubilization and likely targeted to inclusion bodies. FTIR spectra recorded from purified and membrane-reconstituted CfrA are highly characteristic of a beta-sheet protein and are similar to FTIR spectra that have been recorded from other siderophore-mediated iron transporters. Spectral analysis suggests a secondary structure of ~ 50% beta-sheet and ~ 10% alpha-helix. CfrA undergoes relatively extensive peptide hydrogen-deuterium exchange upon exposure to ^2H_2O, suggesting a structure that has substantial exposure to aqueous solvent. The membrane incorporated CfrA is resistant to thermal denaturation at temperatures up to 95°C. Denaturation was achieved by boiling the membrane-reconstituted CfrA for 1 hour. The secondary structure, relatively high aqueous solvent exposure, and high thermal stability are all consistent with a transmembrane beta-barrel structure containing a plug domain. Furthermore, we constructed a homology model of CfrA based on sequence similarity to identify subtle differences in defined regions of the protein. Our data provides the first structural evidence that CfrA is a beta-barrel protein consistent with the structure of siderophore-mediated iron transporter.

2760-Pos Solution Structure and DNA-binding Surface of a Crenarchaea-conserved Chromatin Protein

Yingang Feng[1], Li Guo[2], Li Huang[2], Jinfeng Wang[1]

[1] *Institute of Biophysics, Chinese Academy of Sciences, Beijing, China,*
[2] *Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.*

Board B63

Archaea contain a variety of chromatin proteins consistent with the evolution of different genome packaging mechanisms. Among the two main kingdoms in Archaea, Euryarchaeota synthesize histone homologues, whereas Crenarchaeota have not been shown to possess a chromatin protein conserved at the kingdom level. Recently, we identified a novel chromatin protein Cren7 from *Sulfolobus*. The protein family of Cren7 is conserved in crenarchaea, and has no significant sequence homology with known proteins. We deter-

mined the solution structure and DNA-binding surface of *Sulfolobus solfataricus* Cren7 (SsoCren7) by solution NMR spectroscopy. The structure of SsoCren7 is a SH3-like fold and employs a three-strand beta sheet in DNA binding. Cren7 displays a higher affinity for double-stranded DNA than for single-stranded DNA in the NMR titration experiments. The structure and DNA-binding surface of Cren7 resemble those of Sul7d, a chromatin protein which only exist in *Sulfolobus*, while the two proteins share a very low sequence identity. The structural similarity between Cren7 and Sul7d suggest a conserved kink-and-unwinding mechanism for DNA compacting in crenarchaeal chromatin. Compared to Sul7d, Cren7 have additional flexible loops and lack a C-terminal helix, and has an additional flexible DNA-binding loop and a larger solvent-exposed hydrophobic patch in the DNA-binding surface. These differences give a structural insight into the differences in biochemical properties and DNA-compacting mechanisms.

2761-Pos Fluorescence Studies of Human Erythrocyte Spectrin at the Tetramerization Site

Yuanli Song[1], Nina Pipalia[2], L. W.-M. Fung[1,2]

[1] *Department of Chemistry, University of Illinois at Chicago, 845 West Taylor Street, Chicago, IL 60607, USA;*
[2] *Department of Chemistry, Loyola University of Chicago, Chicago, IL 60626, USA*

Board B64

Fluorescent spectroscopic methods were applied to site-specifically-labeled erythoid spectrin to study conformational changes of the functional partial domain (Helix C') and the junction region of a recombinant alpha spectrin protein upon binding to a beta spectrin partner. This recombinant alpha spectrin protein consists of the sequence of the first 368 amino acid residues of alpha spectrin. Residues 21 to 52, spanning the partial domain and the junction region, were replaced by cysteine residues individually and then labeled with monobromobimane (mBBr). For each mBBr labeled protein, the polarity index and potassium iodide accessibility were determined from fluorescent signals in samples with and without the beta spectrin partner. These parameters were found to exhibit a periodic pattern as a function of residue position. Our results showed that the periodicity remained around 3.6 before and after binding with the beta partner for segments in the partial domain. However, the segment consisting of residues 46–52, the junction region, exhibited a periodicity of about 3.6 only after binding to the beta partner. Our findings demonstrated that the junction region underwent a conformational change from an unstructured conformation to a helical conformation. This conformational change in alpha spectrin upon binding beta spectrin may be related to the association affinity of alpha-beta spectrin to form functional tetramers.

2762-Pos Protein Structure and Dynamics in the Switch I region of Cdc42Hs

Paul D. Adams[1], Adrienne Loh[2], Robert McFeeters[3], Robert Oswald[4]

[1] *University of Arkansas, Fayetteville, AR, USA,*
[2] *University of Wisconsin-Lacrosse, Lacrosse, WI, USA,*
[3] *National Cancer Institute, Frederick, MD, USA,*
[4] *Cornell University, Ithaca, NY, USA.*

Board B65

Effectors and regulators of Cdc42Hs bind to the protein in regions comprising two flexible loops: Switch I (residues 28–40) and Switch II 89(residues 57–74). The structure and dynamics for a construct of Cdc42Hs containing a single-point mutation in the Switch I region, Cdc42Hs(T35A), has been performed. T35 exhibits two predominant conformations depending on: 1) The activation state of Cdc42Hs, and 2) The particular effector or regulator that is bound to the protein. These conformational changes contribute to the structural disorder and increased flexibility of the Switch I region in the wild type protein. The appearance of several residues of Switch I in the 1H, 15N-HSQC spectrum of Cdc42Hs(T35A) not apparent in the spectrum of wild type suggests that, although the Switch I region remains a little more disordered than the remainder of the protein, this region experiences much less conformational freedom in T35A than in wild type. The dynamics studies of Cdc42Hs(T35A)-GDP show that, although Switch I continues to show ps-ns motions, the chemical exchange observed in wild type on the slower μs-ms timescale is lost in Cdc42Hs(T35A). These results suggest that lost flexibility in Switch I results in possible lost orientations in regions of the protein that may be required for optimal binding of effectors important in signal transduction.

2763-Pos Structure of Myosin Ic IQ Motifs in Complex with Calmodulin

Karl R. Schmitz, Kathryn M. Ferguson

University of Pennsylvania, Philadelphia, PA, USA.

Board B66

Myosin Ic is a single-headed unconventional myosin with the ability to interact with membranes and influence membrane dynamics. The C-terminal region of this motor protein functions as a rigid lever arm during the ATPase cycle, translating conformational changes within the motor domain to mechanical force on associated membranes or proteins. Within the lever region are three consecutive IQ motifs, to which calmodulin subunits bind. The interaction between IQ motifs and calmodulin is known to provide an avenue for calcium-dependent regulation of activity, and is thought to do so by affecting the conformation or rigidity of the lever arm. We have determined the crystal structure of the complex formed between apo-calmodulin and the IQ region of myosin Ic. Calmodulin is found to be in a partially open conformation, stabilized primarily through interactions to its N-terminal lobe. The first IQ domain of myosin Ic is unoccupied in the structure, and the entire IQ helix adopts a slight

curvature. This structural data, in conjunction with solution biophysical studies of the complex, yield functional and regulatory insights into the C-terminal lever arm of this protein.

2764-Pos Structural Analysis of β-Turn Tripeptides PGA and PGL by Vibrational Spectroscopy

Laquisha E. Hamilton, Reinhard Schweitzer-Stenner

Drexel University, Philadelphia, PA, USA.

Board B67

It has been postulated that β-turns play an important role in initiating protein folding and in directing the subsequence pathway of folding. Identifying folding initiation sites by studying smaller peptide fragments of proteins for evidence of folded structures has extensive utility. We have measured and analyzed the amide I' band profiles in the polarized Raman, IR, electronic circular dichroisum (ECD), and vibrational circular dichroism (VCD) spectra of Ac-Pro-Gly-X-OH, where X = Ala and Leu in an attempt to obtain the respective spectral characteristics of specific turn structures. PGA and PGL are known to have high β-turn stability of the type I and type II respectively. The peptides were dissolved in D_20 and were prepared at acidic pD values. The ECD results show that PGA and PGL exhibit characteristics of type I and type II turns respectively. The experimental Raman results show that there are three distinct bands with different polarization ratios which indicate excitonic coupling. The experimental results were then compared to theoretical calculations using an excitonic coupling model. Simulation results and calculations on the spectra based on a DFT-calculation performed at a LSDA 6-31G** level of theory indicate that these peptides adopt indeed a type I and type II β-turn conformation.

2765-Pos Nogo66: Receptor Interactions and Lipid-Associated Structure

Jessica R. Schulz, Rosemarie Vithayathil, Melanie J. Cocco

University of California, Irvine, Irvine, CA, USA.

Board B68

Originally characterized for its inhibitory effects in the central nervous system, the protein Nogo (neurite outgrowth inhibitor) has since been implicated in the progression of several other diseases including Alzheimer's and multiple sclerosis. With 1162 amino acids in the human form, Nogo-A is a large membrane protein with several regions shown to cause axon collapse in the CNS. One of these regions, termed Nogo66, is the focus of our studies. Nogo66 is expressed extracellularly in oligodendrocytes and flanked by two putative transmembrane domains. Our study of Nogo66 includes structural characterization using nuclear magnetic resonance (NMR) and receptor-binding assays using phage display and surface plasmon resonance (SPR). Upon characterization of Nogo66 in aqueous solution we found the protein to be highly dynamic, displaying NMR spectra characteristic of both helical and beta sheet secondary structure. In contrast, we find dramatic changes in structure in the context of lipids and detergents that mimic the native environment of Nogo66. Concurrently, using site-directed mutagenesis coupled with phage display we identified key residues of Nogo66 involved in ligand-receptor interaction. We present here our results characterizing the functional mutations of Nogo66 and the structural impact of a lipid environment.

This research was funded by the Roman Reed Research Fund of the Reeve-Irvine Foundation.

2766-Pos Structure-based Insights Into Protein Recognition By B30.2/spry Domain

Bonsu Ku

Postech, Pohang, Republic of Korea

Board B69

As a protein-interacting module, the B30.2/SPRY domain is present in numerous proteins involved in a wide range of biological functions. However, it is not clear whether this domain may recognize a consensus sequence or not. Herein, we report the crystal structure of the B30.2/SPRY domain of GUSTAVUS, a SPRY domain-containing SOCS box (SSB) protein, complexed with a 20 amino acid peptide of the RNA helicase VASA. It is revealed that the peptide-binding site is conformationally rigid and has a pocket, which plays a critical role in the high-affinity interaction between GUSTAVUS and VASA. According to the sequence alignment, the peptide-interacting residues are highly conserved in human SSB family proteins, and this observation led to identify a short sequence motif in prostate apoptosis response-4 (Par-4) recognized by SSB-1. Similar studies can be applied to provide a rational ground to search for multiple target proteins containing a common sequence motif recognized by B30.2/SPRY domain-containing proteins.

2767-Pos Water-mediated Interactions Between Dna And The Phob Dna-binding/transactivation Domain

Tsutomu Yamane, Hideyasu Okamura, Mitsunori Ikeguchi, Yoshifumi Nishimura, Akinori Kidera

Yokohama City University, Yokohama, Japan.

Board B70

The solution structure of the transcription factor PhoB DNA-binding/transactivation domain complexed with DNA was determined by NMR spectroscopy. These solution structures determined conventional structural calculation proceture were refined by using simulated annealing in a periodic boundary box of explicit water with the particle mesh Ewald method. The refined structures provided better convergence and better local geometry compared with the structures determined in a vacuum. The hydrogen bond interactions between the PhoB domain and DNA in the aqueous environment were fully formed. The complex structure was found to be very similar to the crystal structure, particularly at the PhoB-

DNA interface, much more so than expected from the vacuum structure. These results indicate the importance of the proper treatment of electrostatic and hydration influences in describing protein-DNA interactions. The hydration structures observed for the refined structures contained most of the crystal waters as a subset. We observed that various water-mediated PhoB-DNA interactions contributed to the molecular recognition between PhoB and DNA.

2768-Pos Expression Of, And Preliminary Biophysical Characterization Of A Minimal Binding Domain Peptide Of Tsc2 That Binds To The Small Gtpase Rheb

Huimin Liu, Paul Adams

University of Arkansas, Fayetteville, AR, USA.

Board B71

Tuberous sclerosis complex TSC1/TSC2 participates in the control of cell growth. Mutations in either of the gene would result in an autosomal dominant disease that develops benign tumors in a variety of tissue that affects around 1 in 6000 persons. Studies indicated that TSC1/TSC2 suppresses cell growth by inhibiting the mTOR/S6K/4EBP1 signaling pathway through the stimulation of the GTP hydrolysis activity of the Ras GTPase. TSC2 has been shown to be the active component, the C-terminal region of which contains a conserved GTPase activiating protein (GAP) binding domain. It was recently revealed that Rheb, a member of the Ras superfamily GTPases is a direct target of TSC2, and the Rheb-TSC2 complex mediates the mTOR pathway.

We present preliminary studies the on the determination a minimal binding domain peptide of TSC2 for Rheb. A series of peptides truncated from the C-terminal region of TSC2 encompassing the GTPase-binding domain were expressed in bacterial cell line. A peptide of 57 amino acids (TSC2-57) was obtained as the minimal sequence so far has demonstrated similar binding affinity to Rheb *in vitro* to the C-terminal region of TSC2 expressed in mammalian cell line indicated by pull down assay (Castro, 2003). Further studies were carried out to optimize expression, purification and solublization of the peptide. Fluorescence spectroscopy was used to measure the binding affinity of TSC2-57 for Rheb, and to determine thermodynamic parameters. Preliminary NMR studies indicate the peptide is structured upon binding to Rheb. These studies provide the first biophysical account of the binding interaction with TSC2 with Rheb. These results represent a first step towards the structural characterization that is expected to provide molecular details of the Rheb-TSC2 complex's role in biological functionality.

2769-Pos Cw-EPR Based Structural and Functional Characterization of a Molecular Switch

Sascha C.T. Nicklisch

University of Cologne, Cologne, Germany.

Board B72

Molecular switches are known to regulate protein activities, but their detailed understanding is yet scarce. We address the intermolecular dynamics of a secondary transporter (BetP) during its activation process via site-directed spin labeling electron paramagnetic resonance spectroscopy (SDSL-EPR). For this purpose we first created single cysteine mutants at strategic positions within the BetP protein. Subsequently, respective positions were labeled with a nitroxide spinlabel (MTS) and the mobility and accessibility profiles at X-Band (9,7GHz) as well as Q-Band (34GHz) microwave frequencies were analyzed.

Secondary transport systems are major players of the osmotic stress response bacteria have to cope with in their native surrounding. The transporter BetP of the Gram-positive soil bacterium Corynebacterium glutamicum is one of the fastest uptake systems known and predominantly counteracts such stress situations with the uptake of compatible solutes. The carrier possesses distinct cytoplasmic terminal domains that are capable of sensing (C-terminus) the osmotic stress signal as well as modulating (N-terminus) the transport activity. Previous investigations revealed that changes of the internal K+-concentration as a measure for hyperosmotic stress modulate BetP activity. Beside K+, the lipid composition of the membrane has been proven to influence the sensitivity of BetP for osmotic stress.

Our results show that the C-terminal domain of BetP functions as a molecular switch that activates the transporter upon signal perception with respect to hyperosmotic stress. Thereby we elucidated a physiological response under in vivo conditions on the molecular level.

2770-Pos Unfolding for Binding: Interactions of Talin with Vinculin and Actin

Gordon C. K. Roberts[1], Alexandre R. Gingras[1], Benjamin Goult[1], Igor Barsukov[2], David R. Critchley[1]

[1] *University of Leicester, Leicester, United Kingdom,*
[2] *University of Liverpool, Liverpool, United Kingdom.*

Board B73

There are now many examples of 'intrinsically disordered' proteins whose function involves interaction with other proteins or with nucleic acids associated with a transition to a folded, or at least partially folded, state. Much more unusual are proteins that exist in a stable folded conformation but which must *unfold* in order to interact with a partner protein.

We have now described this behaviour in detail in domains from the cytoskeletal protein talin, which is involved in coupling integrins with the actin cytoskeleton in cellular junctions with the extracellular matrix. The globular head of talin contains a FERM domain which binds the β-integrin subunit cytoplasmic domain, and this head is linked to an elongated flexible rod that contains an actin-binding site and also 11 binding sites for vinculin, a protein which may be recruited to stabilize the initial weak integrin/talin/F-actin complexes by cross-linking talin to F-actin.

We have determined solution and crystal structures of eight domains from the talin rod, showing that the rod consists of a series of helical bundles. It is notable that the hydrophobic residues defining vinculin binding sites (VBSs) are buried in the core of these bundles, as they are in the structures of the VBS-vinculin complexes. The formation of the vinculin-talin complex must thus involve structural changes in talin in order for the VBS to become accessible to bind vinculin. We have shown by NMR and EPR spectroscopy that these changes amount to unfolding of the helical bundles. We have also shown that in the 5-helix bundle which contains the actin-binding site the presence of helix 1 negatively regulates actin binding through structural changes in the domain.

2771-Pos X-ray Crystal Structure Of Wild-type LacY

Lan Guan[1], Osman Mirza[2], Gillian Verner[1], So Iwata[3], H. Ronald Kaback[1]

[1] *Department of Physiology, University of California Los Angeles, Los Angeles, CA, USA,*
[2] *Faculty of Pharmaceutical Sciences, University of Copenhagen, Copenhagen, Denmark,*
[3] *Division of Molecular Biosciences, Imperial College London, London, United Kingdom.*

Board B74

Here we describe an X-ray structure of wild-type LacY obtained from crystals formed by manipulating phospholipid content during crystallization. LacY is organized into two 6-helix bundles with 2-fold pseudo-symmetry separated by a large interior hydrophilic cavity containing the side chains important for sugar and H+ binding. Although sugar is not observed, a single substrate-binding site is freely accessible from the cytoplasmic side, but inaccessible from the periplasmic side, which is sealed by tight packing between helices V/VIII, I/VII and II/XI. The wild-type structure exhibits the same global fold as observed in X-ray structures of the conformationally restricted C514G mutant, which binds sugar but cannot catalyze translocation across the membrane. Consistent with biochemical and biophysical studies, the inward-facing conformation of wild-type LacY represents the conformation in the membrane. Thus, the limiting step for transport may be the conformational change leading to an outward-facing conformation that allows access to the binding site from the outside. There is no apparent difference between the wild type and mutant structures despite the heterogeneous population distribution of conformers in the absence or presence of ligand. It seems apparent that a single conformational state, which may represent the lowest free-energy state of LacY, is selected by crystal lattice formation.

2772-Pos Structural And Functional Study Of Ccpn, A Transcriptional Regulator Of The Metabolic Carbon Flux In Gram Positive Bacteria

Denis Chaix[1], Karine De-Guillen[1], Dominique Le Coq[2], Stefan Arold[1], Nathalie Declerck[1]

[1] *CBS, Montpellier, France,*
[2] *Laboratoire de Microbiologie et Génétique Moléculaire, Thiverval-Grignon, France.*

Board B75

CcpN is a newly identified transcriptional repressor involved in the regulation of the metabolic carbon flux in Bacillus subtillis. Under glycolytic conditions of cell growth, CcpN prevents the expression of the gapB and pckA genes, encoding key enzymes of neoglucogenesis. This repressor is composed of an N-terminal Helix-Turn-Helix motif forming the DNA binding domain, followed by two CBS domains forming the regulatory domain. The effector metabolite(s) that modulate(s) CcpN repressor activity is (are) yet unknown, but a regulatory protein partner (YqfL) has been identified. We have undertaken the structural and functional analysis of CcpN in order to determine the molecular basis of its control mechanism. We have solved the crystal structures of the regulatory domain, alone and in complex with different ligands that could potentially modulate the repressor activity. The binding of these molecules in solution was confirmed by NMR foot-printing and Saturation Transfert Difference (STD). No important changes in the tertiary or quaternary structure of the protein could be detected upon ligand binding, as deduced from light or X-ray scattering, limited proteolysis or cross-linking experiments. However, mutations in the ligand binding site lead to a loss of repressor activity in vivo, without altering the protein structural integrity. The results of our current investigations on the effect of amino acid-substitutions and ligand binding on CcpN function will be presented.

2773-Pos Why is the bacterial photosynthesis of *Roseobacter denitrificans* obligat aerob - a structural analysis of the RC-LH1 Core Complex

Steffani Schaefer[1], Richard K. Hite[2], Tom Walz[2], Andreas Labahn[1]

[1] *University of Freiburg, Freiburg, Germany,*
[2] *Harvard Medical School, Boston, MA, USA.*

Board B76

Roseobacter denitrificans belongs to the group of obligate aerobic photosynthetic bacteria. The typical bacterial photosynthesis is an anaerobic process, down-regulated in the presence of oxygen. Although *Roseobacter denitrificans* also produces bacteriochlorophyll a, it is incapable of synthesizing the protein components of the photosynthetic apparatus and performing photosynthetic energy transduction without oxygen. A key step in photosynthesis is the transfer of the light excitation energy from absorbed photons via the light harvesting complex 1 (LH1) to the bacterial reaction center (RC).

With the RC-LH1 complex from *Roseobacter denitrificans,* we have isolated for the first time the core complex from an obligate aerobic photosynthetic bacterium. The core complexes were solubilized with decyl-β-D-maltoside and purified by anion exchange chromatography and sucrose gradient centrifugation steps. Single particle electron microscopy was used to perform a first structural

characterization of the core complex. 30,000 particles were selected from micrographs of negatively stained samples, aligned to each other and classified to produce class averages. The class averages showed different views of the core complex, which could thus be combined to calculate a three-dimensional reconstruction. The density map at 30 Å resolution shows that the LH1 complex surrounding the RC forms a closed circle. Although the resolution of the reconstruction is not yet sufficient to resolve possible gaps in the LH1 ring, a tight ring of LH1 around the RC may prevent the exchange of dihydroquinone and thus light-induced cyclic electron transfer.

2774-Pos High-Resolution Structure of Pentameric Phospholamban by Solution and Solid-State NMR Hybrid Approach

Raffaello Verardi, Nathaniel Traaseth, Lei Shi, Gianluigi Veglia

University of Minnesota, Minneapolis, MN, USA.

Board B77

Phospholamban (PLN) is a 52 residue membrane protein that regulates Ca^{2+} cycle in muscle cells through interaction with the sarco(endo)plasmic reticulum calcium ATPase (SERCA). PLN has two phosphorylation sites at serine-16 ad threonine-17. In the unphosphorylated state, PLN binds to SERCA and lowers its apparent affinity for Ca^{2+}. When PLN is phosphorylated at Ser-16 or Thr-17 by protein kinase A or Calcium/Calmodulin dependent kinase II, respectively, the inhibition of SERCA is relieved. PLN is postulated to exist in an equilibrium between monomeric and pentameric forms. The former is believed to be the active species, whereas the latter has been considered as an inactive storage form. Recent studies have suggested that pentameric PLN can also bind to SERCA and exert an inhibitory effect. High-resolution structure of pentameric PLB by solution NMR was proposed in 2005 but it was in disagreement with our solution and solid-state NMR data on both monomeric and pentameric PLN. Here we report the high-resolution structure of pentameric PLN obtained by a combination of solution and solid-state NMR techniques. Our approach is general and integrates distance constraints derived from different sources (NOE constraints, solid-state NMR topological data, paramagnetic relaxation enhancement experiments, and residual dipolar coupling measurements) to solve the structures and topologies of membrane proteins.

This work was supported by National Institutes of Health Grants GM64742 and HL80081 and American Heart Association Grant 0160465Z (to G.V.) and American Heart Association Grant 0515491Z (to N.J.T.)

2775-Pos Vibrational Structural Markers for Time-resolved Structural Detections of Proteins

Aihua Xie, Beining Nie, Anupama Thubagere

Oklahoma State University, Stillwater, OK, USA.

Board B78

Experimental techniques for time-resolved structural detections of proteins during functions are crucial for structure-function studies of proteins. Few techniques are available for this endeavor. Time-resolved vibrational spectroscopic techniques (including infrared and Raman) contain rich information on protein structural dynamics. The bottleneck is how to extract structural information from vibrational data. Here we report the developments of vibrational structural markers of neutral phenolic groups in tyrosine and other organic compounds. The side chain of tyrosine has been found in the active sites of variety of proteins. Their interactions with other structural elements in proteins are often mediated via hydrogen bonding interactions. Using density functional theory based computational studies together with available experimental data in literature, we have identified three vibrational structural markers that are sensitive to hydrogen bonding interactions. Combined use of two vibrational structural markers, a form of 2D vibrational spectroscopy, allows time-resolved experimental detection of the number and the type of hydrogen bonding interactions of the phenolic groups of tyrosine residues in proteins.

2776-Pos Low Resolution Model of Solubilized Apolipoprotein B-100

Ines Waldner-Scott[1], Alexander Johs[2], Peter Laggner[1], Ruth Prassl[1]

[1] *Institute of Biophysics and Nanosystems Research, Graz, Austria,*
[2] *Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN, USA.*

Board B79

Apolipoprotein B-100 (apoB-100), the single protein moiety of human low density lipoprotein (LDL), is a large amphipathic glycoprotein which plays a triggering role in the progression of atherosclerosis.

Recently, we were able to restore a 3-D low-resolution model of apoB-100 delipidated by the non-ionic detergent Nonidet P-40 (NP-40), using small-angle neutron scattering data in combination with advanced shape reconstruction algorithms [1]. SANS data, collected at beamlines D11 and D22 using the high-flux reactor at the ILL, Grenoble, were processed by the contrast matching technique. Thereby, three-dimensional features of the protein, including its structural flexibility in solution, were obtained without contribution of the detergent. The protein adopts an elongated curved shape of about 55 nm length and is comprised of distinct subdomains

connected by flexible regions. In addition, this low resolution model was used as a template in order to reconstruct a hypothetical spatial arrangement of apoB-100 on LDL, including information derived from a secondary structure prediction.

A relatively new approach for solubilization of membrane-associated proteins is the use of short, lipid-like amphiphilic peptides instead of classical detergents [2]. Accordingly, we have studied apoB-100 stabilized by these components, proving that the secondary structure is preserved to the same extent than native apoB-100 on LDL or solubilized in NP-40. Thus, these novel molecules constitute a very promising new class of surfactants for elucidating the structure of lipid-free apoB-100.

References

[1]. Johs A. et al., (2006) J. Biol. Chem. *281*, 28, 19732–19739.
[2]. Zhao X. et al., (2006) Proc. Natl. Acad. Sci. USA *21*, 103(47), 17707–17712.

2777-Pos MitoNEET Is A Uniquely Folded 2Fe-2S Outer Mitochondrial Membrane Protein Stabilized By Pioglitazone

Mark L. Paddock[1], Herbert L. Axelrod[2], Aina E. Cohen[2], Sandra E. Wiley[3], Melinda Roy[4], Edward C. Abresch[1], Dominique Capraro[4], Anne N. Murphy[3], Rachel Nechushtai[5], Jack E. Dixon[3], Patricia A. Jennings[4]

[1] *Dept. Physics, UCSD, La Jolla, CA, USA,*
[2] *SSRL, Menlo Park, CA, USA,*
[3] *Dept. Pharmacology, UCSD, La Jolla, CA, USA,*
[4] *Dept. Chemistry & Biochemistry, UCSD, La Jolla, CA, USA,*
[5] *Hebrew Univ, Jerusalem, Israel.*

Board B80

Iron sulfur proteins are key players in processes that sustain life on earth - respiration, photosynthesis and nitrogen fixation. MitoNEET, an outer mitochondrial membrane protein (1), has a novel structure containing 2Fe-2S centers (2,3). MitoNEET was originally identified by labeling of mitochondrial membranes with a photoaffinity derivative of pioglitazone, a drug used in type II diabetes treatments (4). Two protomers intertwine to form a unique dimeric structure that constitutes a new fold among not only the ~650 reported Fe-S protein structures but also all known protein folds (3). The protomers form a two-domain structure: a beta cap domain and a cluster binding domain that cradles the two redox active labile 2Fe-2S clusters. Binding of pioglitazone stabilizes the 2Fe-2S clusters of the protein (3). MitoNEET's biophysical properties suggest that it could function in FeS cluster transport and/or redox reactions.

Supported by grants from NIH and DOE.

Dimeric structure of mitoNEET in ribbon representation. The 2Fe-2S clusters are shown as spheres.

References

(1). Wiley *et al* (2007) *PNAS* ***104***, 5318–5323.
(2). Wiley *et al* (2007) *JBC* ***282***, 23745–23749.
(3). Paddock *et al.* (2007) *PNAS* ***104***, 14342–14347.
(4). Colca et al. (2004) *Am J Physiol Endocrinol Metab* ***286***, E252–260.

2778-Pos Structure and Plasticity of Endophilin and Sorting Nexin 9

Qi Wang

Cornell University, Ithaca, NY, USA.

Board B81

Many adaptor proteins containing Bin/Amphiphysin/Rvs (BAR) domains, such as Endophilin and Sorting Nexin 9/SH3PX1 (Snx9), link membrane curvature to various steps in endocytosis, for example assembly of the vesicle budding machinery, scission, and signaling. Snx9 belongs to a large family of phox homology (PX) domain-containing proteins implicated in membrane trafficking and protein sorting. Here we present the crystal structure of the PX-BAR module of Snx9 required for membrane binding and targeting. In the crystal, the PX domains form lobes on either side of the BAR domain dimer yielding an overall compact conformation. The

phosphatidylinositol-phosphate (PIP) binding site on the PX domain extends the moderately concave membrane interacting surface of the BAR domains. Interestingly, Small Angle X-ray Scattering profile reveals that Snx9 adopts a more curved conformation in solution with the PX domains being flexibly connected, in contrast to the solution structure of full-length Endophilin in which the SH3 domains appear to be rigidly located at the distal tips of the BAR domains.

2779-Pos Interfacial Water Controls Protein Conformation

András Dér

Biol. Res. Ctr. Hung. Acad. Sci., Szeged, Hungary.

Board B82

A phenomenological theory of salt-induced Hofmeister phenomena is presented, based on a relation between protein solubility in salt solutions and protein-water interfacial tension. As a generalization of previous treatments, it implies that both kosmotropic salting-out and chaotropic salting-in are manifested via salt-induced changes of the hydrophobic/hydrophilic properties of protein-water interfaces. The theory is applied to describe the salt-dependent free energy profiles of proteins as a function of their water-exposed surface area. On this basis, three classes of protein conformation have been distinguished, and their existence experimentally demonstrated using the examples of bacteriorhodopsin and myoglobin. The results support the ability of the new formalism to account for the diverse manifestations of salt effects on protein conformation, dynamics and stability. It is also shown that the relation between interfacial tension and protein structural stability is straightforwardly linked to protein conformational fluctuations, providing a keystone for the microscopic interpretation of Hofmeister effects. Implications of the results concerning the use of Hofmeister effects in the experimental study of protein function are discussed.

2780-Pos Novel ligand-induced survivin dimer conformation via Replica Exchange Molecular Dynamics (REMD) and receptor-based reverse Virtual Screening (VS)

In-Hee Park[1], Chenlong Li[2]

[1] *Chemical Physics Program, The Ohio Sate University, Columbus, OH, USA,*

[2] *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio Sate University, Columbus, OH, USA.*

Board B83

Survivin is an anti-cancer drug target due to its over-expression in cancer cells. It has two important binding hot spots - the BIR domain (Smac/Diablo binding site) and its dimerization interface. The BIR-domain, representative in all Inhibitor of Apoptosis Proteins (IAPs), conserves the zinc binding structure, suppresses caspases, and ultimately prevents apoptosis. Survivin is unique among IAPs in its formation of a symmetric, bowtie-shaped homodimer structure, held by hydrophobic interactions. Most inhibitor design has been focused on the BIR/Smac binding site. In this study, we probe conformational "plasticity" in the dimerization interface aiming for design and discovery of dimerization inhibitors.

The Abbott laboratory has identified a small molecule binding site near the interface of the survivin dimer through NMR experiments [1]. A benchmarking of the Abbott8 compound binding modes aided in the refinement of the receptor conformation by exploring the survivin conformational flexibility. First, flexible dockings of Abbott8 to the X-ray structure of survivin with key flexible residues (PHE93, PHE101) were carried out, followed by a 'reverse VS' as to dock the Abbott8 ligand to the dimer snapshots from the NTP-MD. The REMD method was utilized to enhance the sampling of the dimer conformations.

A final 'reverse VS' with Abbott8 compound against a large set of survivin conformations sampled via REMD allowed for a reproduction of the NMR experimental binding mode. Compared to the X-ray structure, the BIR-domain is virtually conserved; a prolific change in a survivin alpha-helix allows a new flexible loop conformation better suited for ligand binding.

References

[1]. Wendt, M. D. *et al. Bioorganic & Med. Chem. Lett.* **2007**, *17*(11), 3122–3129

2781-Pos Allosteric Effectors Of HbA Influence Subunit Interactions And Global Dynamics

Gusztav Schay[1], Laszlo Smeller[1], Takashi Yonetani[2], Judit Fidy[3]

[1] *Semmelweis University, Budapest, Hungary,*

[2] *University of Pennsylvania, Philadelphia, PA, USA,*

[3] *Semmelweis University Budapest, Budapest, Hungary.*

Board B84

Luminescence and FTIR spectroscopy studies were performed to unravel the way how the local effect of binding allosteric effectors around the central cavity in the HbA tetramer becomes a signal for oxygen binding affinity in the subunit heme pockets. Both the oxy- and deoxy-states of HbA were studied and the effect of binding Cl, DPG, BZF and IHP was investigated. A method has been elaborated by using high hydrostatic pressure to dissociate the HbA tetramers into dimers without affecting the native structure of the subunits, and to determine the Kd of the tetramer->dimer transition based on Trp fluorescence measurements. The data showed that the Kd is affected both in the oxy- and in the deoxy-states of HbA by the binding of allosteric effectors and the extent of the effect followed the order in their efficiency of influencing oxygen affinity. Temperature dependent phosphorescence lifetime studies (from 10 K to 240 K) based on the signal of Zn-protoporphyrin in substituted HbA have been performed to monitor the effect of binding allosteric effectors on the conformational dynamics of the subunits. The temperature of the

onset of phosphorescence quenching was used to characterize the large scale conformational dynamics of the protein. These motions in the ms range were found sensitive for the binding of allosteric effectors. The results showed that both the fine structure of subunit interface and the large scale motions of conformational dynamics may play role in forming a signal for oxygen binding from the local structural effect of binding allosteric effectors.

Acknowledgements: Support is acknowledged from NIH-FIRCA grant no. TW005924 (T.Y.,J.F.), and Hung. Min. Health ETT no. 512/2006 (J.F.)

2782-Pos Conformational Constrains in the Amelogenin Self-assembly: The Role of PPII Structure in the Thermotropic Liquid Crystal-like Assembly of Amelogenin

Rajamani Lakshminarayanan, Janet Moradian-Oldak

University of Southern California, Los Angeles, CA, USA.

Board B85

Amelogenin is the major extracellular enamel matrix protein whose primary structure is conserved in diverse vertebrates. An important hallmark of amelogenin is its ability to form supramolecular structure, known as nano-sphere, which in turn assembles to form linear arrays. Such assembly of amelogenin is believed to provide an ideal scaffold for the oriented growth of the enamel mineral. In this study we compared the primary structures of various amelogenins and examine their position in the charge vs. hydrophobicity plot. The results showed that all the amelogenins lie at the boundary between completely folded and completely unfolded proteins. Circular Dichroism (CD) spectroscopy of the recombinant full length porcine amelogenin (rP172) was performed at various concentrations and at various temperatures. The secondary structure of rP172 changed significantly with increasing protein concentration. At low concentrations, variable temperature CD experiments indicated the existence of an equilibrium between unordered and PPII structures. We estimated that rP172 contains about 21.5% PPII content at 10 °C and 12.9% at 45 °C. At higher concentrations, β-sheet structure was the dominant conformation. VT-CD experiments at higher concentrations indicated two transitions suggesting significant structural changes upon amelogenin assembly. Isothermal titration calorimetry dilution experiment has been performed at 10, 20, 30, and 37 °C. At low temperatures (10 and 20 °C), the assembled rP172 exhibited a three-step behavior suggesting the formation of spherical assemblies which further associate to form rod-like structures. However, at 30 and 37 °C the assembly exhibited a two-step transition indicating formation of spherical structures only. Since the only difference in the secondary structure of rP172 at various temperatures is the %PPII content, we attribute such structural changes owing to the high PPII content at low temperatures.

2783-Pos Deciphering the Assembly Mechanism of Vimentin Intermediate Filaments by Near-UV Circular Dichroism

Sofia Georgakopoulou[1], Tatjana Wedig[2], Nadine Sachs[2], Harald Herrmann[2], Ueli Aebi[1]

[1] *University of Basel, Basel, Switzerland,*
[2] *German Cancer Research Center, Heidelberg, Germany.*

Board B86

We present a new spectroscopic approach for identifying distinct "signatures" of human vimentin's various stages of association during its assembly into intermediate filaments (IFs). Near-UV circular dichroism (CD) signals recorded during vimentin assembly, probe the distinct near-field interactions of its aromatic amino-acids occurring within the different oligomers and polymers.

We used wild-type vimentin A11 tetramers as starting material and recorded its near-UV CD "signature". Assembly of these tetramers into IFs was induced and the spectral changes were recorded during several hours. A strong band at 290 nm appeared upon initiation of assembly, concomitant with the immediate loss of a band at 278 nm. The latter denotes the longitudinal annealing of the filament units. The appearance of the 290-nm band is caused by ionization of a tyrosine's phenol ring demonstrating the formation of distinct tyrosine-binding polar protein pockets within the IF polymer. This is compatible with a radial compaction of the tetrameric building blocks. After storage at 4°C the 290-nm signal disappeared without recovery of the 278-nm band, indicating breakage of the filament structure into shorter units and loss of some IF-specific tetramer conformations.

These results were complemented by measurements on mutant vimentin variants: a variant that forms short filaments at 21°C which, however, elongate into full-length IFs at 37°C; a tryptophan-free variant; a variant forming only unit-length filaments; a mutant whose assembly is arrested at the dimer level.

Alternatively to x-ray crystallography or electron microscopy, near-UV CD spectroscopy can be used to monitor "structural signatures" of proteins during assembly. Our spectroscopic approach allows continuous monitoring of its overall conformational state in a non-destructive way, under physiological conditions. We can identify protein interactions and states within a protein polymer, which would be difficult to depict by current imaging technologies.

2784-Pos Dissecting The Assembly Mechanism Of Vimentin Intermediate Filaments Using 'Dead-end' Mutant Variants

Norbert Mücke[1], Tatjana Wedig[1], Shirley A. Müller[2], Peter Burkhard[3], Ueli Aebi[2], Harald Herrmann[1]

[1] *German Cancer Research Center (DKFZ), Heidelberg, Germany,*
[2] *Biozentrum, University of Basel, Basel, Switzerland,*
[3] *University of Connecticut, Storrs, CT, USA.*

Board B87

The in vitro assembly of intermediate filament (IF) proteins such as vimentin and desmin into long, 10-nm wide filaments proceeds by three distinct steps: (i) lateral association of tetrameric complexes consisting of two anti-parallel coiled-coil dimers into unit-length filaments (ULFs); (ii) longitudinal annealing of ULFs into short, open filaments; and (iii) filament elongation from short IFs by end-to-end annealing followed by radial compaction to yield mature, highly flexible IFs. We recently investigated a large number of desmin mutations that cause severe muscular dystrophies in man. Interestingly, several of the mutant variants assemble relatively normal until they 'hit a block' indicating that particular amino acid residues become critical for assembly to proceed. In order to dissect the IF assembly mechanism in a complementary, rational way, we designed point-mutated vimentin variants based on theoretical considerations and analyzed those that became 'arrested' upon ULF formation. One of them exhibits a leucine instead of an aromatic amino acid in the d position of the heptad repeat pattern thereby interfering with coiled-coil formation. The ULF-like structures formed by this mutant variant sediment with a sharp peak at 25 S in the analytical ultracentrifuge. Furthermore, mass measurements by scanning transmission electron microscopy (STEM) revealed the mass of the assembled ULF-like particles to increase with the NaCl concentration from 2 MDa (at 50 mM) to 5 MDa (at 160 mM). Hence, this mutant variant allows for fine-tuning of the assembly state of these 'dead-end' structures, thereby offering a more mechanistic insight into vimentin IF assembly by systematic modulation of the assembly conditions. In addition, we are currently performing structural investigations by cryo-electron tomography in order to reveal the relevant dimer-dimer interactions at molecular detail.

2785-Pos Three-Dimensional Reconstruction of von Willebrand Factor Tubules

Ying Wang[1], Ren-Huai Huang[2], Robyn Roth[2], Xiong Yu[1], Angie R. Purvis[2], John E. Heuser[2], Edward H. Egelman[1], Evan J. Sadler[2]

[1] *UVA, Charlottesville, VA, USA,*
[2] *Washington University School of Medicine, St. Louis, MO, USA.*

Board B88

Endothelial cells assemble von Willebrand factor (VWF) multimers into ordered tubules within storage organelles called Weibel-Palade bodies, and tubular packing is necessary for the secretion of VWF filaments that recruit platelets to sites of vascular injury. We have recreated VWF tubules in vitro, starting with domains D1D2 and D'D3. We used the iterative helical real space reconstruction (IHRSR) method to reconstruct them from electron microscopic (EM) images of negatively stained samples. The IHRSR approach is especially powerful when applied to helical filaments that are flexible and heterogeneous in axial rise, which are features of VWF tubules. We extracted 26,131 overlapping tubule segments (each 290 Å in length) and generated different reconstructions after sorting by pitch. The most populated reconstruction consists of a right-handed single-start helix with an axial rise of 25.4 Å and twist of 85.5 degrees per subunit, corresponding to ~4.2 subunits per turn of a 110 Å helix. Other dimensions of the reconstruction include an outside diameter of ~25 nm, inside diameter of ~12 nm, and wall thickness of ~6.5 nm. Reconstructions with different pitches have a similar surface appearance, diameter, and twist (0.2% difference) but a different axial rise. Using the 0.5 Fourier Shell Correlation criterion, the resolution of each reconstruction is ~22 Å. The main limitation on resolution appears to be the continuous variability of pitch. The helical packing of VWF D1D2 and D'D3 proteins provides a framework to investigate the biogenesis of Weibel-Palade bodies and the assembly of homologous multimeric proteins.

2786-Pos Computer Simulation of Protein Translocation Inside the Central Channel of Bacterial Flagellum

Zhongzhou Chen, Anton Arkhipov, Peter L. Fredollino, Klaus Schulten

UIUC, Urbana, IL, USA

Board B89

The bacterial flagellum is made of multiple repeats of a single protein, flagellin. The flagellum is elongated by translocating new flagellin monomers through a central channel within the flagellum to the tip of the filament, where the monomer self-assembles onto the existing structure. The translocation process, driven by a molecular pump inserting flagellin into the flagellar central channel, is essentially unknown. Here we report a computational study that is based on extremely extensive (~2 million atoms) molecular dynamics simulations and mathematical modeling.

The protein translocation process has been studied by computer simulation of a short piece of unfolded flagellin inside a segment (44 flagellin repeats) of flagellar filament. We observed specific interactions between charged residues on the translocating monomer and the C-terminal region of the channel flagellin, which might enhance translocation by preventing salt-bridge interactions within the translocating monomer itself. Sequence alignment of the flagellin reveals a highly conserved SLLX region at the C-terminus, which is much more flexible than the rest of the channel surface residues, and a structurally equivalent co-mutation among different species that preserves the observed protein-channel interactions. We also demonstrate how other features of the flagellin molecule, such as its folding pattern, are designed to facilitate translocation.

The in vivo growth rate of Salmonella flagellum decays exponentially with the increase in length of the flagellum, and when a flagellum is broken, it re-grows with the same length-dependent rate as before. In order to explain these experimental observations, a simple physical model, based on the simulation results, is proposed for the overall protein translocation process.

2787-Pos Self-Assembly, Patterning, and Membrane Protein Insertion in Synthetic Lipoproteins

Daniel Bricarello

UC Davis, Davis, CA, USA.

Board B90

Vascular lipoproteins are spherical or discoidal native nanoparticles consisting essentially of phospholipid and cholesterol encapsulated by a shell of proteins (e.g., apolipoproteins). Depending on the lipid to protein (L/P) ratio and protein type, they range in size from a few nanometers (e.g., high-density lipoproteins or HDL) to several hundred nanometers (e.g., chylomicron). Preliminary exploration of these structures indicates an affinity for spontaneous hierarchical assembly on multiple levels. Solutions of wild-type apolipoprotein and unilamellar vesicles kept near their phase transition temperature form, unprompted, artificial HDL complexes. These native nanoparticles spontaneously self-assemble at solid hydrophilic surfaces forming essentially two-dimensional organized arrays. Amphiphilic surfaces or direct exposure to short-wavelength UV light can be used to further pattern these higher-order assemblies. Current work in our lab is aimed at controlled vectorial incorporation of membrane proteins within single lipid-discs. This ability represents an attractive strategy to sequester single functional proteins in lipidic microenvironments for in vitro preservation of their function.

2788-Pos Gold Fluorescent Nanocluster Synthesis With A Versatile Protein Scaffold

Jennifer L. Belyea[1], Chang Zhong[2], Yuping Bao[2], Jennifer S. Martinez[2], R B. Dyer[1], Dung M. Vu[1]

[1] *Los Alamos National Laboratory, C-PCS, Los Alamos, NM, USA,*
[2] *Los Alamos National Laboratory, MPA-CINT, Los Alamos, NM, USA.*

Board B91

Gold metal nanoclusters (< 1 nm) exhibit strong size-dependent fluorescence emission. They have a potential use as fluorescent reporters in the development of diagnostics and the *in vivo* imaging of cellular processes. In this study, we present work on the synthesis of gold fluorescent nanoclusters using a robust and versatile protein scaffold, the maltose binding protein (MBP). MBP contains an intrinsic molecular recognition site and binding function coupled to a large scale structural response (hinge motion) that can be exploited for nanocluster formation. Modifications of the binding pocket have included substitutions to residues rich in carboxylates, amines, and hydroxyls side chains that are amenable to gold binding. In addition, mutations were also made to enlarge the binding pocket and provide structural constraints on the hinge motion. These modifications have resulted in the formation of blue emissive gold nanoclusters in MBP. Screening of MBP mutants have lead to the identification of specific residues that are responsible for the formation of these gold nanoclusters. The formation of gold nanoclusters is confirmed by fluorescence and MALDI-MS. Binding of gold atoms in the binding pocket is confirmed by Trp fluorescence quenching. Optimization of nanocluster assembly conditions through changes in pH, temperature, reductants, ion strength have resulted in increased fluorescence emission and greater stability.

2789-Pos Nanoscale Structural Analysis of Membrane Protein Arrays using Polarised Neutron Reflection

Anton P. Le Brun[1], Stephen A. Holt[2], Deepan S.H Shah[3], Charles F. Majkrzak[4], Jeremy H. Lakey[1]

[1] *University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom,*
[2] *STFC Rutherford Appleton Laboratory, Didcot, United Kingdom,*
[3] *Orla Protein Technologies Ltd., Newcastle upon Tyne, United Kingdom,*
[4] *NIST Centre for Neutron Research, Gaithersburg, MD, USA.*

Board B92

Proteins can be immobilised on surfaces to make arrays with potential uses in tissue engineering, proteaomics and point of use diagnostic devices. Outer membrane proteins (OMP) from *Escherichia coli* have a beta-barrel structure, making ideal protein engineering scaffolds for building arrays. The proteins can be immobilised onto flat gold surfaces by introducing a cysteine residue into their periplasmic turns. The thiol group of the cysteine will form a strong gold-thiolate bond immobilising the OMP to the surface in a specific and correct orientation. Here we use the transmembrane section of the monomeric protein OmpA (TmOmpA). The Z domain of *Staphylococcus aureus* protein A has been engineered into the N - terminal of a circularly permuted TmOmpA to create OmpAZ. The Z domain can bind immunoglobulin G at its constant region leaving the variable regions free to bind antigen. The structure of this model protein array was probed using polarised neutron reflection (PNR). Polarised neutrons reflect differently from a magnetic metal layer deposited under the gold surface according to their two spin states (spin up and spin down). This provides a method of providing additional scattering length density contrast to very complex layer systems without needing to make any changes to the biological layer. The collection of two complimentary but independent data sets allows for more accurate modelling and the resulting high resolution data will be presented.

2790-Pos Oriented, Chemically Functionalized Amyloid Network With Controllable Mesh Size For Nanotechnology Applications

Árpád Karsai[1], Ünige Murvai[1], Katalin Soós[2], Botond Penke[2], Miklós SZ Kellermayer[1]

[1] *University of Pécs, Pécs, Hungary,*
[2] *University of Szeged, Szeged, Hungary.*

Board B93

Amyloid fibrils are present in the extracellular space of various tissues in neurodegenerative and protein misfolding diseases. The

fibrils may be used in nanotechnology applications, because of their self-assembly properties and stability, provided that they can be chemically addressed and if their growth and orientation can be controlled. Here we show that amyloid β25–35 (Aβ25–35), a toxic fragment of Alzheimer's beta peptide, forms trigonally oriented fibrils on mica. Oriented binding depends on an apparently cooperative interaction of a positively-charged moiety on the Aβ25–35 peptide with the K^+-binding pocket of the mica lattice. By varying K^+ concentration the growth rate and the mesh size of the oriented amyloid fibril network may be tuned. To add chemical reactivity, we used a mutant peptide, Aβ25–35_N27C, the cysteine residue of which may be used for subsequent chemical modifications. We find that Aβ25–35_N27C forms epitaxially growing fibrils on mica which evolve into a trigonally oriented branched network. The binding is apparently more sensitive to cation concentration than that of the wild-type peptide. By nanomanipulating Aβ25–35_N27C fibrils with a gold-coated AFM tip we show that the sulfhydryl of Cys27 is reactive and accessible from the solution. The oriented network of Aβ25–35_N27C fibrils can therefore be specifically labeled and may be used for constructing nanobiotechnological devices.

2791-Pos Surfactant behavior of hydrophobin proteins

Markus B. Linder, Katri Kurppa, Arja Paananen, Geza Szilvay

VTT Technical research centre of Finland, Espoo, Finland.

Board B94

Hydrophobins are surface active proteins produced by filmantous fungi. In nature they help fungi to adapt to their environment by lowering surface tension, acting as protective layers and functioning as adhesives. We have studied hydrophobins belonging to class II. We have shown that they have extraordinary properties as surfactants which have led to industrial applications in for example foam and emulsion stabilization, protein immobilization and protein purification. The films that hydrophobins form at the air water interface were studied by AFM and found to be highly structured consisting of a hexagonal pattern with a repeating unit of 6 mn. Size exclusion chromatography and FRET showed that there is a dynamic formation of oligomers in solution that is analogous to some extent to micelle formation in traditional surfactants. However, important differences were noted between the solution behavior of hydrophobins and traditional surfactants.

2792-Pos Respiratory Chain Supercomplexes in Yarrowia lipolytica Mitochondria

Sergio Guerrero Castillo, Diego González-Halphen, Salvador Uribe-Carvajal

Universidad Nacional Autónoma de México, México, Mexico.

Board B95

In Yarrowia lipolytica mitochondria, the electron flux from NADH to O2 is branched. The alternative components are an external type II NADH dehydrogenase (NDE), and an alternative oxidase (AOX) located on the matrix side. These are single-subunit oxido-reductases not implicated in proton translocation1. Thus, if electrons pass by way of those two enzymes, the oxidation of external NADH would not be an energy-conserving process. This waste of energy may be functional when the amount of energy has to be down regulated, but in the exponential growth phase it is not convenient. To prevent the electron flux between alternative components, either AOX interacts with complex I2,3, or NDE associates with the cytochrome pathway (complexes III and IV). In isolated mitochondria from the wild type and αnubm mutant of Y lipolytica. αnubm has an inactive complex I and NDE is re-localized to the matrix side4 (NDEi). We measured O2 consumption with different respiratory substrates and inhibitors detecting a substrate-dependent inhibition pattern suggestive of NDE association with the cytochrome pathway. From these data, we propose that in wild type but not in αnubm mitochondria, electrons coming from NDE are directed to the cytochrome pathway. AOX appears not to be associated with other complexes. Furthermore, we identified the interaction between NDE with the complexes III-IV in the wild type, by BN, CN-PAGE, in-gel activity assays and mass spectrometry. We also observed supercomplexes5 and a complex V dimer.

References

1. Joseph-Horne et al., (2001). Biochim Biophys Acta. *1504*:179–95;
2. Veiga et al., (2003). FEMS Yeast Res. *3*: 141–148;
3. Krause et al., (2004). J Biol Chem. *279*, 26453–26461;
4. Kerscher y cols. (2001). J Cell Sci. *114*: 3915–21;
5. Schagger H. (2001). Methods Cell Biol. *65*: 231–44

2793-Pos Reduced Tight Assembly of Trpase Tetramer Enhanced PLP Release Leading to Dissociation and Cold Inactivation

Orna I. Almog[1], Anna Kogan[1], Garik G. Gdalevsky[1], Robert S. Phillips[2], Yehuda Goldgur[1], Rivka Cohen-Luria[1], Abraham H. Parola[1]

[1] *Ben Gurion University of the Negev, Beer Sheva, Israel,*
[2] *University of Georgia, Athens, GA, USA.*

Board B96

Tryptophanase (Trpase) from *Escherichia coli* is a pyridoxal phosphate (PLP)- dependent homotetrametic enzyme with a molecular weight of 210 kD. We studied the reversible cold inactivation and cold dissociation of *E. coli* Trpase and its Y74F, C298S and W330F mutants. The incubation of *E. coli* Trpases at 2°C for 20 hr resulted in a significant loss of activity and enhanced the dissociation into dimers. However, incubation for several hrs at 25°C of these Trpases restored their initial activity and the re-association of the dimers into the tetrameric active form. In addition, we found that at 25°C, all apo forms of Trpase markedly (about 70%) dissociated into dimers and further upon cooling to 2°C (about 90%). This is in contrast to the

holo, PLP-bound form. We also determined the crystal structures of the three apo mutants at 1.9Å resolution and compared them to the known structure of holo, wt *P. vulgaris* Trpase in order to unravel the mechanism of reversible cold dissociation of Trpase. Crystal structure analysis revealed that the mutants in their apo form are found in an "open" conformation compared to the "closed" conformation of *P. vulgaris* in its holo form. We show here for the first time that the cold inactivation and dissociation of *E. coli* Trpases is primarily affected by PLP release. The main cause for the enhanced loss of activity of the three mutants is due to the change in the size of the amino acid side chain. This prevents the tight assembly of the active tetramer, making it more susceptible to the cold driven changes in hydrophobic interaction which facilitates the PLP release.

2794-Pos Abnormal Formation And Remodeling Of Fibers Containing Type I Collagen Homotrimers

Sejin Han[1,2], Elena Makareeva[2], N. V. Kuznetsova[2], A. DeRidder[2], M. B. Sutter[2], D.J. McBride[3], J. M. Pace[4], U. Schwarze[4], P. H. Byers[4], R. Visse[5], NagaseH. [5], W. Losert[1], Sergey Leikin[2]

[1] *UMD, College park, MD, USA,*
[2] *SPB, NICHD, Bethesda, MD, USA,*
[3] *School of Medicine, University of Maryland, Baltimore, MD, USA,*
[4] *Department of Pathology, University of Washington, Seattle, WA, USA,*
[5] *Kennedy Institute of Rheumatology Division, Imperial College, London, United Kingdom.*

Board B97

The normal form of type I collagen is an $\alpha1(I)_2\alpha2(I)$ heterotrimer. In fetal tissues, tumors, and several heritable disorders, $\alpha1(I)_3$ homotrimers have been found as well, but their role remains unclear. Here we report abnormal formation and remodeling of fibers containing the homotrimers. Turbidity measurements show five fibrillogenesis steps in hetero- and homotrimer mixtures: (i) early growth, (ii) low plateau, (iii) lag phase, (iv) delayed growth, and (v) high plateau. Only (i) and (ii) occur in homotrimers and only (iii)–(v) in heterotrimers. Confocal microscopy reveals that the homotrimers form spikes emanating from a common center while heterotrimers form an entangled, thread-like fibril network. In mixtures, initial homotrimer spike formation appears to be followed by heterotrimer fibrillogenesis at spike surfaces, resulting in intermediate fibril morphology. Hetero- and homotrimers copolymerize into fibrils, but they segregate at a sub-fibril level, e.g., into separate microfibrils. With increasing homotrimer fraction in the mixture, the solubility of the heterotrimers in equilibrium with fibrils remains constant whereas the solubility of the homotrimers increases, consistent with the segregation and presence of pure heterotrimer microfibrils. The segregation may affect tissue remodeling by matrix-metalloproteinases (MMP-1 and MMP-13). Indeed, we find that the homotrimers are cleaved ~10 times slower than the heterotrimers. The corresponding Km are similar. The lower cleavage rate of homotrimers is associated with a reduced Vmax, indicating a preferential MMP interaction with the $\alpha2(I)$ chain at a rate-limiting step. The uneven cleavage rates may cause accumulation of aberrant homotrimer microfibrils upon remodeling of segregated fibrils by MMP. This may explain, e.g., how a small fraction of the homotrimers can cause a predisposition to osteoporosis, which was recently linked to the $\alpha1(I)$ chain overproduction in individuals with an SP1 polymorphism in the $\alpha1(I)$ promoter region.

Protein Aggregates

2795-Pos Structure of α-synuclein Oligomers by Site-directed Spin Labeling

Yujin E. Kim, Ralf Langen

University of S. California, Los angeles, CA, USA.

Board B98

The misfolding and aggregation of α-synuclein is central to the pathology of Parkinson's disease (PD) and other neurodegenerative disorders. Growing evidence indicates that the primary pathogenic species are the nonfibrillar oligomer rather than mature fibrils. Numerous studies on the aggregation pathway of α-synuclein have described different oligomeric structures, which include spheres, rings, chains and amorphous structures in addition to the fibril. Although various biophysical and biochemical techniques have been used to examine the structure of these oligomeric states of α-synuclein, a direct comparison of the structural conformation between these oligomer types and the fibril has not been shown. In this study, we used electron paramagnetic resonance (EPR) spectroscopy combined with site-directed spin labeling to obtain residue-specific structural information for oligomeric α-synuclein. Using EPR spectroscopy, two different types of oligomeric structures were identified. In addition, we observed that the structures of both types of oligomers do not match the fibril structure, indicating that these oligomers are conformationally unrelated to the fibril. Based on these results, we are testing the hypothesis that these oligomers are not structural precursors to the fibril, but are instead "off-pathway" to fibril formation, providing further support for the suggestion that fibrils may be protective and that the oligomers are the toxic species. This has important implications for the understanding of PD pathology and for the development of disease modifying therapeutics for the treatment of PD and other α-synuclein related diseases.

2796-Pos Elucidating the Mechanism of Amyloid Formation by Horse Apomyoglobin

Angela Pozo Ramajo, Sean Decatur

Mount Holyoke College, South Hadley, MA, USA.

Board B99

Myoglobin is a 153 residue long globular protein responsible for transporting oxygen in muscle tissue. In its native form, myoglobin is composed of eight well characterized α-helices, which collapse into a spherical tertiary structure stabilized by hydrophobic interactions. However, in spite of the high helical content of native