The majority of structural genomics centers were originally mainly concentrated on the set up of high through put facilities for the structure determination of soluble 'globular' proteins. Some of these centers have been very successful with hundreds of new structures determined. Structural genomics has received strong support in Europe, but the EC projects were organized in a different way and have a somewhat different scope from those in the US, Canada or Japan. European projects concentrated on structure determination of challenging, biologically important protein targets. Membrane proteins and multi-protein complexes are highly underrepresented in structural data banks due to tremendous difficulties in preparing sufficient amounts of material for structural analysis. Our group is participating to two important European projects on the structure determination of protein complexes. The first, 3D repertoire, concerns the structural analysis (X-ray, electron microscopy) of multi-protein complexes from yeast. The second (SpineII) targets human complexes involved in medically important cellular processes. Outline and some results of both projects will be presented.

Protein Conformation

169-Pos Solvent-induced Conformation Changes In Short Alanine-rich Peptides

Kalani J. Seu, Sean M. Decatur

Mount Holyoke College, South Hadley, MA, USA.

Board B1

Alanine-rich peptides, with sequences such as Ac-$(AAAAK)_n$Y-NH_2, are useful tools and models for investigating protein/peptide secondary structure transitions. In water, conformation is length dependent; peptides with n greater than or equal to 4 are largely helical in aqueous solution. Exposure to fluorinated alcohols can both stabilize helix formation and modulate backbone-solvent hydrogen-bonding [1]. Alternatively, there is little known about how these solvents affect the conformation of shorter alanine-rich peptides. As peptide length decreases, residues available for alpha-helix formation also decrease. Short alanine-rich peptides (n = 1) in water are not alpha-helical and instead show indications of having a polyproline II-like structure in their far-ultraviolet circular dichroism (CD) spectra [2]. In the presence of solvents such as 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), the CD spectra lose the signatures of polyproline II. In HFIP/water mixtures, the spectra suggest that the peptides adopt a partially helical conformation, surprising for such a short sequence. In this work we will discuss the results of CD and Fourier-transform-infrared (FTIR) studies of alanine-rich peptides of varying length. The results suggest that the backbone solvent hydrogen-bonding plays an important role in inducing backbone conformation in small peptides.

References

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170-Pos Specificity Of The Helical Conformation Induced By 2, 2, 2, Trifluoroethanol

Nicole Webb, Suresh Kumar, D. Rajalingam, Denise Greathouse

University of Arkansas-Fayetteville, Fayetteville, AR, USA.

Board B2

The specificity of helix-induction in the 15-mers of poly-L-glutamic acid and poly-L-lysine by 2,2,2-trifluoroethanol (TFE) was investigated by circular dichroism (CD), NMR, and FTIR at three pH values: 2, 7, and 13. TFE was observed to promote the induction of helical conformation in poly-L-glutamic acid at pH 2.0, and pH 7.0. Similarly, TFE induces helical conformation in poly-L-lysine at pH 7.0 and pH 13. At pH 7.0, the helical conformation was induced in both poly-L-glutamic acid and poly-L-lysine only at higher concentrations of TFE (> 70 % v/v). At lower concentrations of the fluorinated alcohol, very little or no effect was observed in the backbone conformation of the homopolypeptides. ^{1}H-^{15}N HSQC spectra (obtained at ^{15}N natural abundance, at pH 7.0) of the homopolypeptides showed that profound conformational changes occur in the backbone of the polypeptide chains in higher concentrations of TFE. Analysis of the 2D NMR data in conjunction with those obtained using Far UV CD and FTIR revealed that the helix conformation induced at higher concentrations of TFE is non-specific. The results obtained in this study clearly question the validity of structures of short peptides characterized in high concentrations of organic solvents.

171-Pos Effect of Altered Glycosylation on the Structure of the I-like Domain of β1 Integrin: A Molecular Dynamics Study

Yuemin Liu, Susan L. Bellis, Yuhua Song

The University of Alabama at Birmingham, Birmingham, AL, USA.

Board B3

Glycosylation plays an important role in the regulation of integrin function. Molecular mechanisms underlying the effects of altered glycosylation on β1 integrin structure and function are still largely unknown due to the unavailability of crystal structures of glycosylated integrin. In this study, we used a molecular modeling approach to study the effects of glycosylation on the structure of the I-like domain of the β1 integrin. In particular, we investigated oligosaccharide/peptide interactions using atomically detailed molecular dynamics simulations to observe conformational changes induced by glycosylation. The β1 integrin I-like domain contains three asparagine residues with the appropriate consensus sequence for N-glycosylation. Accordingly, we modeled the I-like domain with all three of these glycans with and without terminal α2–6 sialic acids. Our results demonstrated that the interactions between oligosaccharides and the I-like domain resulted in conformational changes within key functional regions of the I-like domain of β1

integrin including the metal ion-dependent adhesion site, other critical residues for ligand binding, the specificity-determining loop, and the α7 helix. The effect of the tri-glycosylation on the conformation of the I-like domain was the result of oligosaccharides directly interacting with the I-like domain and allosteric effects of the oligosaccharides. In particular, results showed that α2–6 sialylation of the N-linked oligosaccharide side chain played an important role in the intramolecular interaction of the carbohydrate side chain with integrin β1. The results from this study agree with the experimental observations that variant glycosylation regulates integrin function.

Keywords

Integrin β1, I-like domain, glycosylation, sialic acid, molecular dynamics simulation

172-Pos Studying the Peptide Backbone Dynamics of TOAC labeled Phospholamban using EPR Spectroscopic Technique

Harishchandra Ghimire, Gary A. Lorigan

Miami University, Oxford, OH, USA.

Board B4

Phospholamban (PLB) is a 52 amino acid membrane-bound protein which regulates the enzymatic activity of Sarco-endoplasmic reticulum calcium ATPase (SERCA). To study the structure and dynamics of PLB, a spin label 2, 2, 6, 6-tetramethylpiperidine-1 oxyl-4-amino-4 carboxylic acid (TOAC) was attached at different parts of the protein by solid phase peptide synthesis method. Amino acids Ile-45 from the transmembrane domain (amino acids 30–52) and Ser-10 from the cytoplasmic domain (amino acids 1–20) of the full length WT-PLB were replaced with TOAC spin label. Since the TOAC spin label is rigidly coupled to the peptide backbone, it reports more accurately on position, orientation, and dynamics of the peptide backbone. The protein was cleaved, purified by reverse phase HPLC and then inserted in a DMPC/DHPC bicelle to mimic its membrane -associated structure and studied by electron paramagnetic resonance (EPR) spectroscopy. The PLB Ile⋄45 TOAC EPR spectrum at room temperature is very broad due to the slow correlation time of the spin label located in the transmembrane region. In aligned bicelles at higher temperatures, the anisotropic EPR spectra reveal unique lineshapes and hyperfine splittings based upon the topology of the spin-labeled protein with respect to the membrane and the magnetic field. Additionally, a TOAC spin label was placed at position Ser-10 in the cytoplasmic domain for WT-PLB in bicelles and probed with EPR spectroscopy. The spectra from two different domains (cytoplasmic and transmembrane) will be directly compared. Thus, by using aligned EPR spectroscopic techniques we can predict the structural topology of integral membrane proteins.

173-Pos The Activation Pathway Of Src Tyrosine Kinase Studied By The String Method

Wenxun Gan[1], Benoit Roux[2]

[1] *Department of Chemistry, University of Chicago, Chicago, IL, USA*
[2] *Institute for Molecular Pediatric Sciences and Department of Biochemistry & Molecular Biology, University of Chicago, Chicago, IL, USA.*

Board B5

The string method in collective variables is an efficient way to identify transition pathways in complex biological systems. By applying the string method and an ensemble-based sampling technique to the inactive-to-active transition of the human c-Src tyrosine kinase, we find a pathway that represents the most probable transition path of the kinase activation. Starting from the inactive structure, first we generate an initial activation path from a targeted molecular dynamics simulation. The string is represented by connecting a series of discrete images extracted from the initial path. The local drift of each image along the string is estimated from an ensemble of short trajectories and each image is updated correspondingly. After the smoothing and re-parametrization of the string, a set of new images are generated. These steps are repeated until the string is well converged. The free energy along the pathway is calculated to give an estimate of the transition barrier of kinase activation. Analysis of the pathway provides atomistic details of the c-Src kinase activation and may have general implication for the whole tyrosine kinase family.

174-Pos Conformational Changes of the Carboxy-Terminus of CLC-5 in Solution upon Nucleotide Binding

Leigh Wellhauser[1,2], Hsin-Hen Kuo[1], Mohabir Ramjeesingh[2], Charles Deber[1,2], Christine Bear[1,2]

[1] *University of Toronto, Toronto, ON, Canada*
[2] *Hospital for Sick Children, Toronto, ON, Canada.*

Board B6

Mutations within the Cl^-/H^+ transporter ClC-5 lead to Dent's disease, a kidney condition characterized by proteinuria. Several disease-causing mutations in ClC-5 translate into truncations of the carboxy terminus (Ct), highlighting the physiological significance of this region. The ClC-5 Ct forms homodimers and binds adenosine nucleotides as evident by a recent crystal structure [Meyer, S *et al* (2007) *Nat Struct Mol Bio* ***14***:60–7]. As the ClC-5 Ct was not crystallized in the absence of nucleotides it remains unclear whether nucleotide binding leads to conformational changes in the protein. Our previous studies showed that the thermal stability of ClC-5 Ct was enhanced by ATP suggesting that nucleotide binding may alter the oligomeric or conformational state of the protein [Wellhauser, L. *et al* (2006) *Biochem J* ***398***:289–94]. In the present study, sedimen-

tation equilibrium experiments confirmed that the ClC-5 Ct exists in a monomer-dimer equilibrium. Size exclusion chromatography of ClC-5 Ct revealed small shifts of the protein peaks of the absorbance profiles in the presence of nucleotides without a change in the monomer-dimer ratio. Sedimentation velocity revealed a decrease of the frictional coefficient in the absence of nucleotides from 1.55 to 1.46, 1.34, and 1.33 in the presence of ATP, ADP, or AMP respectively, indicating the ClC-5 Ct becomes more compact upon binding nucleotides. Similar concentrations of ATP and AMP competed the photo-affinity labeling with [α^{32}P] 8-N_3-ATP to ClC-5 Ct (IC_{50} 1.70 μM and 2.10 μM respectively), suggesting the change in peptide conformation may not be attributed to differences in nucleotide affinity. Together these results indicate that nucleotide binding leads to a more compact Ct in solution. Future work will focus on uncovering the functional impact of these nucleotide-dependent conformational changes of the Ct on the Cl^-/H^+ transporter activity of full-length ClC-5.

175-Pos Characterization of Protein Molecules by Silicon Nitride Nanopores

Bradley Ledden, Daniel Fologea, David S. McNabb, Jiali Li

University of Arkansas, Fayetteville, AR, USA.

Board B7

We report on the development of silicon nitride nanopore based device for characterization of proteins at the single molecule level. The nanopores are fabricated in a silicon nitride membrane and the diameters of the nanopores are selected to allow one protein molecule to pass. When a voltage bias is applied across the membrane, charged protein molecules can be driven through a nanopore electrophoretically and the translocation of single protein molecules generate characteristic current blockage signals. We demonstrate by measuring the current blockade amplitude, time duration, and the integral of the blockade event, the physical properties of proteins: their size, electrical charge, and conformation can be determined. We report on our studies for proteins including fibrinogen, laminin, collagen, and protein markers at various denaturing conditions.

176-Pos NMR Characterization of a Transmembrane and Cytoplasmic CD4 Fragment and its Interaction with the Soluble Domain of HIV-1 VpU

Marc Wittlich[1], Silke Hoffmann[2], Dieter Willbold[1,2], Bernd W. Koenig[1]

[1] *Heinrich-Heine-Universität, D-40225 Düsseldorf, Germany*
[2] *Forschungszentrum Jülich, D-52425 Jülich, Germany.*

Board B8

Interaction of the cytoplasmic domain of human CD4, which is displayed by CD4+ T-cells, with the cytoplasmic domain of the viral protein VpU plays a critical role in HIV-1 infection. The 70 residue polypeptide CD4tmcyt comprises the transmembrane and cytoplasmic domains of CD4 (residues 372 through 433) preceded by an N-terminal stretch of eight additional amino acids. The 45 residue VpUcyt corresponds to the C-terminal amino acids 39 to 81 of VpU preceded by a GS dipeptide. Both proteins were recombinantly expressed in Escherichia coli in isotope labeled form (^{15}N, ^{13}C) and purified. CD4tmcyt was reconstituted into lipid membranes or membrane mimicking dodecylphosphocholine (DPC) micelles. Close to complete NMR resonance assignment of CD4tmcyt and VpUcyt was achieved in the presence of DPC micelles using three-dimensional NMR spectroscopy. Secondary structure of the two proteins was characterized based on chemical shift analysis, NOE-derived proton-proton distances, and circular dichroism spectropolarimetry. A centrifugation assay was developed for characterization of VpUcyt binding to CD4tmcyt reconstituted in POPC liposomes. VpUcyt contains two α-helices formed by residues 38 to 48 and 64 to 70, respectively, in the presence of DPC micelles. In CD4tmcyt a hydrophobic transmembrane helix extending from M372 to V395 and a short cytoplasmic helix from M407 to R412 are observed. The centrifugation assay shows specific binding of VpUcyt to membrane reconstituted CD4tmcyt indicating the functional relevance of the recombinant polypeptides studied.

177-Pos An Inside View Of Photo Excited PYP Mutants Photoreaction Revealed By A Biosensor In Time Domain

shahbaz J. khan, Javaid Shahbaz Khan[1], Masahide Terazima[3], Mikio Kataoka[2], Miki Harigai[1], Yoshinori Shichida[1], Yasushi Imamoto[1]

Kyoto University, Kyoto, Japan.
[1] *Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan*
[2] *Graduate School of Materials Science, Nara Institute of Science and Technology, Nara 630-0192, Japan*
[3] *Departments of Chemistry, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan*

Board B9

Photoactive yellow protein is one of the most demonstrated and famous photosensor proteins. Various studies have elucidated the protein conformational changes during its photocycle. The formation of long-lived photocycle intermediate pB is accompanied by a global conformational change. We have shown that the diffusion coefficient change measured by the time-resolved transient grating method successfully demonstrated the conformational change which occurs during signaling state formation. However, the exact mechanism and sequence of such changes have not been illustrated so far. The vital role of the amino acid residues involved in the interaction between protein core and N-terminal region in the structure and conformational change of PYP is a focus point of the current research. We studied the conformational changes of mutants for these residues from view point of the diffusion coefficient change at pB formation. The photocycle kinetics of pR_2 to pB' becomes faster and diffusion constants of pB becomes smaller for the site-

An inside view of photo excited PYP mutants photoreaction revealed by a biosensor in time domain

directed mutants as compared with wild-type PYP. However, a disulfide bond introduced between protein core and N-terminal region surprisingly induced the variation in conformation change during signaling state formation and recovery to the ground state. The recovery to the ground state was found to be slower by several folds as compared to wild type PYP. .

178-Pos Electron Microscopic Studies Of The Oligomeric Structure Of Mlc, A Glucose Regulator

Oh Yeun Kwon, Hyesung Jeon

Korea Institute of Science & Technology, Seoul, Republic of Korea.

Board B10

Mlc (makes large colonies) in E. coli is a transcriptional repressor regulating glucose-dependent induction of enzymes and transporters, with the membrane-bound enzyme IIB^{Glc} ($EIIB^{Glc}$). There have been studies showing that Mlc forms tetramers in vitro indicated by size exclusion chromatography, and that the dimeric form of Mlc mutant (R52H) makes a stable structure to bind palindromic DNA. To elucidate the relationship between the oligomeric conformation and function of this protein, the images of purified Mlc in solution were taken using electron microscopy and showed the dimer of dimers which are twisted. The crystal structure of tetrameric conformation could be fit into the image, implicating the importance of biological activity from tetramers. Further structural studies of DNA and/or $EIIB^{Glc}$ complex will lead to a clear explanation of the regulation mechanism.

179-Pos Molecular Dynamics Simulations Of Sarcoplasmic Reticulum Ca2+-ATPase (SERCA) And Spin-labelled Phospholamban

Bengt Svensson, Michel Espinoza-Fonseca, David D. Thomas

Univ. of Minnesota, Minneapolis, MN, USA.

Board B11

Our laboratory is engaged in spectroscopic analysis of structural dynamics of the proteins involved in Ca^{2+} transport and its regulation. In order to interpret and guide these experiments, we have performed molecular dynamics simulations on these proteins in their lipid bilayer environments. The Ca^{2+}-ATPase (SERCA) is an integral membrane protein that uses energy from ATP hydrolysis to pump calcium into the sarcoplasmic reticulum, which relaxes the heart muscle and provides the calcium gradient needed for the next muscle contraction. SERCA is regulated by phospholamban (PLB), an integral membrane protein that inhibits (and aggregates) SERCA unless PLB is phosphorylated. Although crystal structures of the Ca^{2+}-loaded SERCA are available, they represent only a single snapshot of the broad conformational landscape of the protein. To obtain a more complete view, we have performed molecular dynamics simulations of the calcium-bound ($E1.Ca^{2+}$) and calcium-free (E2) of SERCA in an explicit water and lipid environment. The molecular dynamics simulations reveal Ca-dependent domain motions in the protein, particularly the nucleotide-binding and the actuator domains. These motions will be compared with those previously detected by EPR and phosphorescence in this laboratory. We have also performed simulations of CFP-SERCA, a fusion protein containing cyan fluorescent protein linked to the N-terminus of SERCA, in order to interpret fluorescence microscopy data obtained from this construct in living cells. The dynamic interaction of phospholamban with SERCA has been previously studied in this laboratory using EPR spectroscopy and a spin label (TOAC) that reports directly the backbone dynamics of the protein. Therefore, we have performed computational simulations of TOAC-labeled PLB to understand in more detail the backbone dynamics of PLB, as affected by phosphorylation and interaction with SERCA.

This work was supported by NIH (GM27906, AR007612) and the Minnesota Supercomputing Institute.

180-Pos Methods To Determine The Structure Of Intrinsically Disordered Proteins

Joseph E. Curtis, Susan Krueger

National Institutes of Standards and Technology, Gaithersburg, MD, USA.

Board B12

We are developing a suite of computational methods to aide in the design and analysis of small angle scattering data with applications to macromolecular systems. This is achieved by generating an ensemble of macromolecular structures by varying sets of backbone dihedral angles and using mathematical methods to determine structures that have small angle scattering spectra that are consistent with experiment. We have used these tools to predict structures for the HIV-1 Gag protein under high salt conditions. The methods are applicable to the analysis of small-angle scattering spectra (X-ray or neutron) and can be applied to study intrinsically disordered proteins and other biopolymers in solution.

181-Pos Conformational Changes of the ClpA Hexamer

Grzegorz Piszczek[1], Gian Marco De Donatis[2], Michael R. Maurizi[2], Ann Ginsburg[1]

[1] *NHLBI/NIH, Rockville, MD, USA*
[2] *NCI/NIH, Rockville, MD, USA.*

Board B13

The *E. Coli* ClpA chaperone is active as a hexameric ring of subunits that unfolds native proteins in an ATP-dependent process and delivers the unfolded polypeptides to ClpP protease for degradation. Previous work suggests that switching between different conformational states, triggered probably by nucleotide hydrolysis, plays an important role in both unfoldase activity and in substrate translocation. Additionally, calorimetric titrations of ClpA hexamer with

SsrA target peptide performed previously in our laboratory have suggested that peptide binding may also trigger large conformational changes within the ClpA hexamer. To gain insight into the overall conformational changes of the ClpA hexamer upon peptide binding, we have used sedimentation velocity measurements to determine sedimentation coefficient (S) distributions of ClpA in the absence and presence of SsrA. To study conformational changes of ClpA in different nucleotide states single point mutations in ClpA nucleotide binding Walker-B motifs of domains D1 and D2 were introduced. The double D1/D2 ClpA mutant forms stable hexamers in the presence of ATP, as confirmed by analytical ultracentrifugation, but has no detectable ATPase activity. Analytical ultracentrifugation studies indicate that peptide-bound ClpA exhibits a strikingly more narrow distribution of sedimentation coefficients than does ClpA sedimenting in the absence of the peptide. Similar results are obtained for the wild-type ClpA and for a deletion mutant lacking the N-terminal domain, which also binds SsrA peptide. Thus conformational changes in the D1 and D2 domains forming the hexameric ring, rather than different orientations of the N-terminal domains are responsible for observed overall shape changes of the hexamer. Morever, results obtained with the D1/D2 ClpA mutant indicate that in the presence of ATP, the hexamer has a more open conformation, resulting in lower apparent S values than those measured in the presence of the nonhydrolyzable ATP analogue, ATPgammaS.

182-Pos Calcium Produces A Conformational Change In The Complex Of Calmodulin And The Carboxy Region Of The Plasma Membrane Calcium Pump

Sergei Y. Venyaminov[1], Adelaida G. Filoteo[1], John T. Penniston[2], Emanuel E. Strehler[1], Franklyn G. Prendergast[1]

[1] *Mayo Clinic/Foundation, Rochester, MN, USA*
[2] *Massachusetts General Hospital, Boston, MA, USA.*

Board B14

The plasma membrane calcium pump (PMCA), an ion transporter, is critically important in living cells. It helps maintain the calcium concentration of cells at resting levels at which a multitude of signaling pathways occur. The pump is generally at an auto inhibited state which is relieved by its interaction with calmodulin. The 151-residue C-tail of PMCA4b (4btail) was expressed and purified by calmodulin affinity chromatography for structural studies by CD spectroscopy. CD spectra of the 4btail, calmodulin, and their 1:1 complex were measured in the far- and near-UV spectral ranges in the presence and absence of Ca^{2+}. In the presence of Ca^{2+}, calmodulin shows an increase of helical structure ~21% over the apo-state. The 4btail does not change its secondary structure (far-UV CD spectra) or tertiary structure (near-UV CD spectra) in the presence of Ca^{2+}. The spectral properties of 1:1 mixing of the apo calmodulin and apo 4btail are completely additive - this result demonstrates the absence of any change in its secondary or tertiary structure upon the interaction of these two proteins in the apo-state. When mixed in the presence of Ca^{2+}, the calmodulin and 4btail form 1:1 mole/mole complex which shows ~23% increase of helical structure over the arithmetic sum of calcium states for both proteins.

183-Pos Monitoring Of Small Conformational Changes By High-precision Measurements Of Hydrodynamic Radius With 2-focus Fluorescence Correlation Spectroscopy (2ffcs)

Joerg Enderlein[1], Thomas Dertinger[2], Iris von der Hocht[3], Anastasia Loman[3], Ingo Gregor[3], Karl-Wilhelm Koch[4], Konstantin Komolov[4], Felix Koberling[5], Rainer Erdmann[5]

[1] *Eberhard Karls Univ Tuebingen, Tuebingen, Germany*
[2] *University of California Los Angeles UCLA, Los Angeles, CA, USA*
[3] *Forschungszentrum Juelich, Juelich, Germany*
[4] *University of Oldenburg, Oldenburg, Germany*
[5] *PicoQuant GmbH, Berlin, Germany.*

Board B15

We report on our application of a new fluorescence-correlation spectroscopy technique, 2-focus-FCS, for measuring the hydrodynamic radius of molecules with sub-Angstrøm precision. The method is applied of monitoring conformational changes of proteins upon ion binding. In particular, we present measurements on Ca2+-binding of recoverin. Recoverin belongs to the superfamily of EF-hand Ca2+-binding proteins and operates as a Ca2+-sensor in vertebrate photoreceptor cells, where it regulates the activity of rhodopsin kinase GRK1 in a Ca2+-dependent manner. The protein undergoes conformational changes upon Ca2+-changes that are reflected as changes in their hydrodynamic radius. By using 2fFCS we were able to resolve hydrodynamic radius changes of ca. one Angstrøm and used the Ca2+ dependence of this radius for recording binding curves in solution. We compare our results with those obtained by other techniques.

184-Pos Molecular Shape, Oligomerization And Ligand-induced Conformational Change Of Heterotrimeric AMP-activated Protein Kinase

Uwe Riek[1,2], Roland Scholz[2], Peter Konarev[3,4], Arne Rufer[5], Marianne Suter[2], Alexis Nazabal[6], Marco Gregorini, Mohamed Chami[7], Shirley A. Muller[7], Dietbert Neumann[2], Michael Hennig[5], Renato Zenobi[6], Andreas Engel[7], Dimitri Svergun[3,4], Theo Wallimann[2], Uwe Schlattner[1]

[1] *Inserm, U884, LBFA, University Joseph Fourier, Grenoble 1, Grenoble, France*
[2] *Cell Biology, ETH Zurich, Zurich, Switzerland*
[3] *EMBL, DESY, Hamburg, Germany*
[4] *Russian Academy of Sciences, Moscow, Russian Federation*

[5] *F. Hoffmann-La Roche AG, Basel, Switzerland*
[6] *Organic Chemistry, ETH Zurich, Zurich, Switzerland*
[7] *M.E. Müller Institute for Structural Biology, University of Basel, Basel, Switzerland.*

Board B16

Intracellular sensors of cellular energy and nutrient status are emerging as key players in the regulation of cell metabolism in health and disease. Heterotrimeric AMP-activated protein kinase (AMPK) participates in the control of cellular and whole body energy balance by sensing and reacting to an increasing AMP/ATP ratio within a complex upstream and downstream signalling network. Recently, we developed a bacterial coexpression strategy for functionally intact full length mammalian AMPK with a His-tag. Here we report on

(i) the bacterial expression of untagged mammalian AMPK isoform combinations, all containing the γ1 subunit,
(ii) a fully automated 4-dimensional purification protocol, and
(iii) a detailed biophysical characterization of AMPK heterotrimers by small angle X-ray scattering in solution (SAXS), transmission and scanning transmission electron microscopy (TEM, STEM) and mass spectrometry (MS).

AMPK in solution at low concentrations (< ~1 mg/ml) largely consisted of individual heterotrimers in TEM analysis, revealed a precise 1:1:1 stoichiometry of the three subunits in MS, and behaved as ideal solution in SAXS. At higher AMPK concentrations, SAXS revealed a concentration-dependent, reversible dimerization of AMPK heterotrimers, and the formation of higher oligomers. The occurrence of these species was confirmed by STEM mass measurements. In the lower AMPK concentration range, addition of 5′-AMP resulted in a decrease of the radius of gyration by about 5% in SAXS, indicating a global conformational change induced by ligand binding. Both SAXS by single particle reconstruction, and TEM by single particle averaging, showed a similar molecular shape of AMPK heterotrimers revealing elongated, flat particles with protrusions and indentations.

185-Pos Second-harmonic Generation For Studying Structural Motion Of Biological Molecules In Real Time And Space

Joshua Salafsky

Biodesy LLC, Burlingame, CA, USA.

Board B17

Second-harmonic generation (SHG) is a nonlinear optical technique that is well known in physics and chemistry for its ability to detect molecules on surfaces and quantitatively determine their average tilt angle. By labeling biomolecules with an SH-active dye probe, SHG detection is extended to any biological molecule, opening up new avenues for drug discovery and basic research. Labeled biomolecules placed on a surface produce a baseline SHG signal: conformational change, induced by a drug or a ligand, alters the average tilt angle of the probe, and the measured SH intensity. SHG requires only a tiny amount of sample (10^5 – 10^6 biomolecules in a focal spot size of 30 microns) and it is directly sensitive to structure in real time. For basic research, SHG is a viable complement to NMR for detecting a biomolecule's conformational change and for measuring kinetics. In well-defined systems in which the biomolecules are oriented, SHG could be used to measure the angular change of probe in real time. Second-harmonic-active unnatural amino acids (e.g., Aladan) are attractive alternatives to exogenous labels for quantitative measurements and *in vivo* studies. As a tool for drug discovery, SHG offers a screening platform for identifying allosteric or conformation-specific inhibitors, without the need for a crystal structure. Results with a variety of protein targets will be presented, including an integrin, amyloids and kinase. Importantly, SHG is sensitive enough to distinguish between different conformations, even when the protein is labeled non-site-specifically. Because the technique can be scaled up for high-throughput drug screening, it should play an important role in identifying new compounds that induce or stabilize specific target conformations.

186-Pos Small Angle X-ray Scattering of Viral Fusion Proteins

Jessica Lamb[1], Shoshannah Roth[1], Xiangyun Qiu[2], Suzette Pabit[1], Victor Chu[3], Kurt Andresen[4], Gary Whittaker[1], Lois Pollack[1]

[1] *Cornell University, Ithaca, NY, USA*
[2] *NIH, Bethesda, MD, USA*
[3] *Stanford University, Stanford, CA, USA*
[4] *Colgate University, Hamilton, NY, USA.*

Board B18

Many viruses gain entry to target cells by means of fusion proteins. These proteins, which coat the surface of a viral capsid, merge the viral membrane with the target cell membrane, effectively delivering the components to the cell. In the classic example, influenza HA, action of the fusion peptide is triggered by the low pH of the endosome. In an effort to better understand this process we have measured various pH states of viral fusion proteins with SAXS. SAXS can help us distinguish large conformational changes which are part of the fusion process. We present the results here.

187-Pos Cooperative Binding of ATP and RNA Induces a Closed Conformation in DEAD Box RNA Helicases

Bettina Theissen[1], Anne R. Karow[1], Jürgen Köhler[2], Airat Gubaev[1], Dagmar Klostermeier[1]

[1] *University of Basel, Biozentrum, Basel, Switzerland*
[2] *University of Bayreuth, Bayreuth, Germany.*

Board B19

RNA helicases are energy coupling enzymes that convert the energy of ATP hydrolysis into structural changes in their RNA substrates. They mediate a multitude of RNA rearrangements in transcription, splicing, RNA editing, translation, RNA export, ribosome assembly,

and RNA degradation. DEAD box helicases form the largest class of RNA helicases. Structurally, they consist of a conserved helicase core comprising two RecA-like domains connected by a flexible linker. In some cases, N- or C-terminal regions flanking the core confer substrate specificity or mediate interactions with other proteins. Most of the conserved helicase signature motifs line the cleft between the two core domains, and an opening and closing of this cleft during the helicase cycle has been postulated but not shown experimentally.

Single molecule FRET experiments with the *B. subtilis* DEAD box helicase YxiN carrying donor and acceptor fluorophores on different sides of the inter-domain cleft reveal an open helicase conformation in the absence of nucleotides, or in the presence of ATP, or ADP, or RNA. In the presence of ADP and RNA, the open conformation is retained. By contrast, cooperative binding of ATP and RNA leads to a compact helicase structure, proving for the first time that the ATP and ADP bound states of RNA helicases display substantially different structures only when the RNA substrate is bound. These results establish a closure of the inter-domain cleft in the helicase core at the beginning of the unwinding reaction. Furthermore, they open up avenues to follow ATP-induced conformational changes in the catalytic cycle of RNA helicases in real time and to understand the role of these movements in RNA unwinding. Altogether, they suggest a conserved mechanism of energy conversion among DEAD box helicases across kingdoms.

188-Pos The Subtle Nature of Allosteric Activation is Highlighted by Fluorescence Spectra and Anisotropy Data From Variants of Glycogen Phosphorylase b

Andrew Bigley, Gregory D. Reinhart

Texas A&M Univ, College Station, TX, USA.

Board B20

Glycogen phosphorylase contains nine native tryptophan residues per subunit as well as a cofactor, pyridoxal-5-phosphate, at the active site. The fluorescence of these intrinsic fluorophores can be used as a reporter on structural alterations due to allosteric activation by AMP as well as mutations to the primary structure. Intrinsic tryptophan spectra from the truncate Δ2-17 and the site directed mutant K544E (both previously shown to have altered kinetic properties) match the spectra for wild-type glycogen phosphorylase b indicating that the over all structures are similar. The pyridoxal-5-phosphate cofactor remains stabily bound to a lysine side chain via a Shiff's base in the active site. The Shiff's base exists as an equilibrium between two forms. The spectral properties of the cofactor depend on the form of Shiff's base, and the equilibrium between forms is sensitive to the local environment at the active site. In wild-type phosphorylase b, the cofactor spectrum is altered due to the binding of AMP at the allosteric site. The spectra for both Δ2-17 and K544E are distinct from wild-type and do not show sensitivity to AMP despite being activated to similar extents. Steady-state anisotropic data confirms that the changes seen with each mutant are unique from each other and wild-type further confirming that the alterations at the active site are different for each mutant and are independent of activation. These data highlight the subtle nature of allosteric activation and indicate that perturbations due to binding of an allosteric ligand can be specific to only a portion of the active site rather than requiring some global alteration of tertiary structure.

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189-Pos The Role of the Quaternary Shift in the Allosteric Regulation of Phosphofuctokinase from B. Stearothermophilus

Rockann Mosser, Manchi Reddy, James C. Sacchettini, Gregory D. Reinhart

Texas A&M Univ, College Station, TX, USA.

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Phosphofructokinase (PFK) is an important regulatory enzyme in glycolysis that catalyzes the conversion of fructose-6-phosphate (F6P) and MgATP to fructose-1,6-bisphosphate and MgADP. *Bacillus stearothermophilus* PFK (BsPFK) is a homotetramer that is allosterically inhibited by phosphoenolpyruvate (PEP) which binds along one dimer-dimer interface while F6P binds along the other dimer-dimer interface. The only overall quaternary structure deviation between the substrate bound and the inhibitor bound structures of wild-type BsPFK is a quaternary shift, which is defined as a 7° rotation about the substrate binding site interface. Located along the substrate binding interface and involved in numerous inter-subunit hydrogen bonds is the residue D12, which is a completely conserved residue among 149 prokaryote PFKs and is not directly involved in catalysis or the binding of any ligands. When compared to wild-type, D12A BsPFK shows a 100 fold increase in the binding affinity for PEP, a 50 fold decrease in the binding affinity for F6P, and surprisingly the coupling is not greatly affected. Two crystal structures of D12A BsPFK have been solved, with one enzyme structure bound to its inhibitor PEP and the other enzyme structure free of any ligands. These two crystal structures allow for a direct comparison of PEP binding in D12A BsPFK. Both of the structures reveal the enzyme in the inhibitor bound conformation. These structural data along with the kinetics data for D12A BsPFK suggest that the role of the quaternary shift may be involved more in ligand binding than in the allosteric response of the enzyme, contrary to previous proposals. Funding came from NIH grant GM33261 and the Welch Foundation.

Protein Dynamics - I

190-Pos Structural Characterization Of Engineered pH-sensitive Allostery

Martin Sagermann, Richard Chapleau, Elaine DeLorimier, Yun Yong Lei

Univerity of California, Santa Barbara, CA, USA.