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STRUCTURAL BIOLOGY

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Special Session on

Structural Biology and Single Molecules

Yuri L Lyubchenko, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Yuri L Lyubchenko

University of Nebraska Medical Center, USA

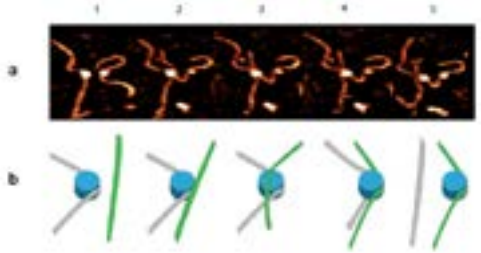
Nanoscale structure and dynamics of centromere nucleosomes

Statement of the Problem: Chromatin integrity is crucial for normal cell development. The cell division process is accompanied by the segregation of replicated chromosome, and chromatin centromeres, specialized segments of chromosomes provide the accuracy of the chromosomal segregation. If the centromere becomes damaged or removed, chromosomes segregate randomly disrupting the cell division process. The centromeres are specifically recognized by kinetochores suggesting that centromeres contain specific structural characteristics. However, these structural details and the mechanism underlying their highly specific recognition remain uncertain.

Methodology & Theoretical Orientation: In this study, we performed direct imaging of CENP-A nucleosome core particles by time-lapse high-speed atomic force microscopy (AFM), enabling us to directly visualize the dynamics of CENP-A nucleosomes. Nucleosomes used for evaluation of DNA wrapping around the histone core were assembled on a DNA substrate containing a centrally positioned 601 motif.

Findings: A broadly dynamic behavior of the DNA flanks was first revealed by analysis of AFM images acquired in ambient conditions. Time-lapse imaging further identified the distinctive pathways unique to CENP-A-nucleosome dynamics that are not shared by H3. The spontaneous unwrapping of DNA flanks can be accompanied by the reversible and dynamic formation of loops with sizes equivalent to a single wrap of DNA. Translocation of CENP-A nucleosomes was observed, with the formation of internal DNA loops along the nucleosome. This process was reversible, settling the core back to its starting position. Additionally, the transfer of the histone core from one DNA substrate to another was visualized, as well as distinctive splitting into sub-nucleosomal particles that was also reversible.

Conclusion & Significance: Altogether, our data suggest that unlike H3, CENP-A is very dynamic, permitting its nucleosome to distort freely and reversibly, which in turn allows a longer-term stability, which may play a critical role in centromere integrity during mitosis and replication.



Biography

Yuri L Lyubchenko is the Professor of Pharmaceutical Sciences at the University of Nebraska Medical Center, Omaha, NE, USA. His research focuses on understanding fundamental mechanisms underlying health and disease, which are key to developing new and more effective diagnostics and medications. This primarily basic research allows him not only to identify new drug targets for small molecule drugs, it also leads to development of the nanotools and methods to discover novel approaches for diagnostic, treatment and disease prevention and to more rapidly determine their efficacy at the molecular level.

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John van Noort, J Proteomics Bioinform 2017, 10:8(Suppl)
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John van Noort

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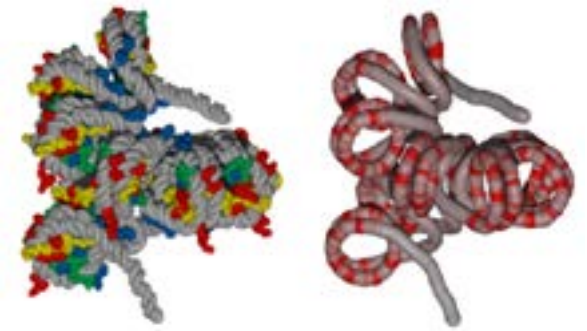
The structure of chromatin; single-molecule experiments on model fibers and real genes

The folding of chromatin defines access to our genes and therefore plays a pivotal role in transcription regulation. However, the structure of chromatin fibers is poorly defined and heavily debated. We used single-molecule techniques to probe and manipulate the dynamics of nucleosomes in individual chromatin fibers. These novel methods were initially applied to synthetic, highly homogeneous nucleosomal arrays and yielded unprecedented insight in the structure and dynamics of chromatin. With single pair Forster Resonance Energy Transfer, we showed that the nucleosome is very dynamic, unwrapping half of its DNA four times per second. Using single molecule force spectroscopy, it was possible to measure the kinetics of this unfolding, both in single nucleosomes and in well-defined arrays of nucleosomes that fold into a 30 nm fiber. Analysis of the unfolding pattern reveals a linker length dependence of the higher order folding. The linker length *in vivo* however varies, and to obtain insight the positioning of nucleosomes we developed a simple statistical physics model that captures sequence dependent positioning effects for both reconstitutions on synthetic DNA and chromatin *in vivo*. We recently developed a method to purify specific chromatin fragments from yeast without crosslinking the fiber while maintaining the complexity that provides functionality to our epi-genome. I will show the first single-molecule force spectroscopy results on intact, native fibers which uniquely probe chromatin structure, composition and variations in it at the single-molecule level.



Biography

Chromatin is the ubiquitous protein-DNA complex that forms the structural basis of DNA condensation in all eukaryotic organisms. Packaging and depackaging of chromatin, called chromatin remodeling, plays a central role in all cellular processes that involve chromosomes such as transcription, replication, recombination and repair. Detailed knowledge of the principles and mechanisms underlying this control of DNA condensation is thus vital for understanding many diseases, including neurological disorders and cancer. The physical mechanisms governing these processes however, are still largely unknown. I am interested in developing and using modern biophysical techniques to unravel the physics behind DNA condensation and its role in transcription regulation.

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Peter Hinterdorfer, J Proteomics Bioinform 2017, 10:8(Suppl)
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Peter Hinterdorfer

Johannes Kepler University Linz, Austria

Deciphering and filming molecular recognition at the nano-scale with AFM

In molecular recognition force microscopy (MRFM), ligands are covalently attached to atomic force microscopy tips for the molecular recognition of their cognitive receptors on probe surfaces. Interaction forces between single receptor-ligand pairs are measured in force-distance cycles. The dynamics of the experiment is varied, which gives insight into the molecular dynamics of the receptor-ligand recognition process and yields information about the binding pocket, binding energy barriers, and kinetic reaction rates. Combination of high-resolution atomic force microscope topography imaging with single molecule force spectroscopy provides a unique possibility for the localization of specific molecular recognition events. The identification and visualization of receptor binding sites on complex heterogeneous bio-surfaces such as cells and membranes are of interest in this context. Considered as the paradigm for molecular recognition are antibodies. They are key molecules for the immune system of vertebrates. The Y-shaped antibody type IgG exhibits C2-symmetry; its Fc stem is connected to two identical Fab arms, binding antigens by acting as molecular callipers. Bivalent binding of the two Fab arms to adjacent antigens can only occur within a distance of roughly 6 to 12 nm. AFM (Atomic force microscopy) cantilevers adorned with an antibody can measure the distances between 5-methylcytidine bases in individual DNA strands with a resolution of 4Å, thereby revealing the DNA methylation pattern, which has an important role in the epigenetic control of gene expression. Moreover, due to their nano-mechanical properties antibodies exhibit "bipedal" walking on antigenic surfaces. The walking speed depends on the lateral spacing and symmetry of the antigens. Importantly, the collision between randomly walking antibodies was seen to reduce their motional freedom. It leads to formation of transient assemblies, which are known to be nucleation sites for docking of the complement system and/or phagocytes as an important initial step in the immune cascade.

Biography

Peter Hinterdorfer performs advanced nanoscopic techniques in nano-bio technology, life science, and medical diagnostics, and has been working on antibody/antigen interactions, transmembrane transporters, virus/membrane interactions, cells of the immune system, nuclear envelope membranes, and bacterial surface layers. He has done pioneering work in single molecule force spectroscopy and has invented a combined topography and recognition imaging technique. Recently he did research with high-speed bio-AFM.

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Notes:

Marco Capitanio, J Proteomics Bioinform 2017, 10:8(Suppl)
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Marco Capitanio

University of Florence, Italy

High-speed optical tweezers for the study of protein-DNA interaction

We developed a constant-force laser trap that allows us to investigate molecular interactions and sub-nanometer conformational changes occurring on a time scale of few tens of microseconds. The method is effective in studying the sequence-dependent affinity of DNA-binding proteins along a single DNA molecule. The improvement in time resolution provides important means of investigation on the long-puzzled mechanism of target search on DNA. In fact, one poorly understood issue in the field of protein-DNA interaction is how proteins weakly interact with non-cognate DNA sequences and how they efficiently find the sequence of interest among an extremely large amount of non-specific sequences. Using our technique, we could discriminate sequence and conformational dependent interactions of a single Lac repressor protein (LacI) on DNA at physiological salt concentrations. The lac operon is a well-known example of gene expression regulation, based on the specific interaction of LacI with its cognate DNA sequence (operator). We observed LacI switching between different interaction modalities on DNA (weak, strong, sliding), depending on the molecule conformation and DNA sequence. We provide a method for measuring 1D-diffusion constants of DNA-binding proteins along DNA with a spatial resolution of about 30 base pairs, observing a broad distribution of 1D-diffusion constants of LacI and sequence-dependent diffusion constants. Our measurements provide a model of target-search and molecular switching mechanism of Lac repressor.

Biography

Marco Capitanio is Senior Researcher at the Department of Physics of the University of Florence, Italy, and Group Leader at the European Laboratory for Non-linear Spectroscopy (LENS). He then obtained his PhD in Physiology. He then joined LENS, a research institute which is part of a European network of laser and spectroscopy facilities. His research interests lie across Physics and Biology. On one hand, his research is focused on the physics of biological systems and on the development of techniques for the study of biology at the molecular scale, with a particular interest on optical methods. On the other hand, he is particularly interested in the molecular mechanisms underlying mechanical regulation of biological systems and the conversion of mechanical signals into changes in gene expression and cell fate.

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Notes:

Gijs Wuite, J Proteomics Bioinform 2017, 10:8(Suppl)
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Gijs Wuite

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Sliding sleeves of XRCC4–XLF bridge DNA and connect fragments of broken DNA

Non-homologous end joining (NHEJ) is the primary pathway for repairing DNA double-strand breaks (DSBs) in mammalian cells. Such breaks are formed, for example, during gene-segment rearrangements in the adaptive immune system or by cancer therapeutic agents. Although the core components of the NHEJ machinery are known, it has remained difficult to assess the specific roles of these components and the dynamics of bringing and holding the fragments of broken DNA together. The structurally similar XRCC4 and XLF proteins are proposed to assemble as highly dynamic filaments at (or near) DSBs. Here we show, using dual and quadruple-trap optical tweezers combined with fluorescence microscopy, how human XRCC4, XLF and XRCC4–XLF complexes interact with DNA in real time. We find that XLF stimulates the binding of XRCC4 to DNA, forming heteromeric complexes that diffuse swiftly along the DNA. Moreover, we find that XRCC4–XLF complexes robustly bridge two independent DNA molecules and that these bridges are able to slide along the DNA. These observations suggest that XRCC4–XLF complexes form mobile sleeve-like structures around DNA that can reconnect the broken ends very rapidly and hold them together. Understanding the dynamics and regulation of this mechanism will lead to clarification of how NHEJ proteins are involved in generating chromosomal translocations.

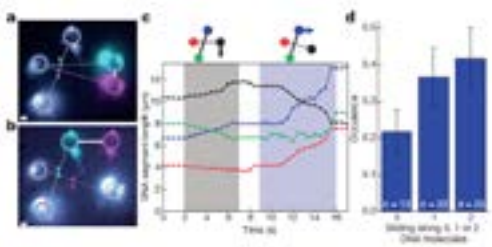

Figure 3 | Mobility of XRCC4–XLF bridges. a, **b**, Overlays of eGFP–XLF logarithmically scaled fluorescence before (cyan) and after (magenta) moving a microsphere (white arrow). DNA molecules were incubated in crossed configuration. Circles denote microsphere positions; dashed lines denote DNA; coloured arrows denote bridge location. Data are representative examples of 13 experiments. **c**, Distance from bridge to bead edges (of complex shown in **a** and **b**) as a function of time. Shaded regions denote bead motion as indicated by the arrows in the cartoons. **d**, Histogram of XRCC4–XLF bridge mobility (both wrapped and crossed configurations). Error bars denote s.e.m.

Biography

Gijs Wuite obtained his PhD in Biophysics in 2000. Since 2001 he leads his own group at the VU University Amsterdam and in 2009 was appointed to full Professor. In his research, he has successfully applied quantitative physical tools to investigate fundamental problems in biology, and to search for the unification of apparently unrelated biological phenomena. Moreover, he has been at the front of recent new and fast developments of biophysical techniques that have enabled visualization, manipulation and control of complex biological reactions. Based on this research work he founded in 2014 a company (LUMICKS) that sell the technology he and his group has developed. His work has appeared in journal such as *Nature, Science, PNAS and Physical Review Letters*. His research has been awarded with the prestigious personal VIDI, VICI and ERC grants. In 2009 Wuite was appointed member of the Young Academy, an independent platform of young top scientists within the Royal Netherlands Academy of Arts and Sciences.

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Dmitrii V Shalashilin, J Proteomics Bioinform 2017, 10:8(Suppl)
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Dmitrii V Shalashilin

University of Leeds, UK

New applications of boxed molecular dynamics: Atomistic simulations of atomic force microscopy experiments and peptide cyclization

New applications of Boxed Dynamics (BXD), an efficient technique to extend the time scale of molecular dynamics and simulate rare events, will be presented. BXD allows analysis of thermodynamics and kinetics in complicated molecular systems. It is a fully atomistic multiscale technique, in which thermodynamics and long-time dynamics are recovered from a set of short-time molecular dynamics simulations. BXD is many orders of magnitude faster than standard MD and can produce well converged results. Previously BXD has been applied to peptide cyclization, solution-phase organic reaction dynamics, and desorption of ions from self-assembled monolayers (SAMs). Here two new applications of BXD will be reported. First atomistic simulations of protein pulling with Atomic Force Microscope AFM) will be presented, where BXD is able to reproduce correctly the Potential of Mean Force (PMF) of a protein pulled in AFM experiments, the experimentally observed force profile and its relationship with the protein structure. Second, an application of BXD to enzymatic peptide cyclization will also be presented, where BXD predicts correctly the cyclizable peptide sequences. All such sequences have a conformation with their C and N termini close to each other. In both applications calculations were done with standard force field without any adjustment of the force field parameters. Thus, BXD proves to be a good predictive tool. It is implemented in CHARMM molecular dynamics code and can be used for many other applications.

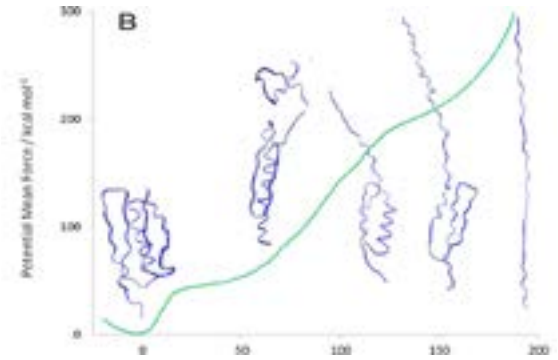

Figure1: Potential of mean force as a function of end-to-end distance calculated with BXD correlates with the structures of the unfolding protein.

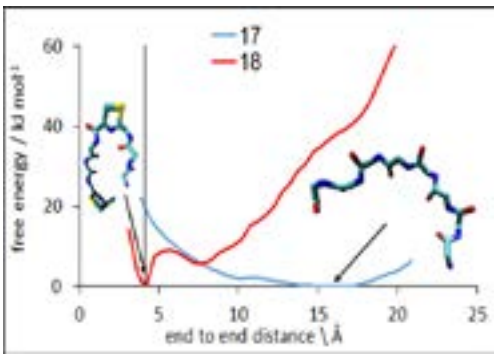

Figure2: PMF as a function of end-to-end distance for two peptides P18 and P17. Only P18, which has a stable conformation with C and N termini close to each other, is cyclizable.

Biography

Dmitrii V Shalashilin is a Professor of Computational Chemistry at the University of Leeds. His research is focused on the development of efficient computational techniques for quantum and classical simulations in chemistry and their applications.

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Scientific Tracks & Abstracts

Day 1

Sessions:

Recent Advances in Structural Biology

Session Chair
Robert Craigie
NIH, USA

Session Co-Chair
Toshiya Senda
High Energy Accelerator Research Organization (KEK), Japan

Session Introduction

Title: **Structural insights from aptamers with base modifications**
Nebojsa Janjic, SomaLogic, Inc., USA

Title: **HIV-1 integrase assembles multiple discrete intasomes that are active for DNA integration *in vitro***
Robert Craigie, National Institutes of Health, USA

Title: **Allosteric control of transcription regulation by nuclear receptors: An integrative structural biology approach**
Dino Moras, IGBMC, France

Title: **Title: Complexes of malaria parasite and human proteins drive formation of cytoadherent assemblies at the surface of infected red blood cells**
John Vakonakis, University of Oxford, United Kingdom

Title: **Microrobotics enables non-contact, fully automated protein crystal harvesting**
David Sargent, ETH Zurich, Switzerland

Title: **The native (Sulfur) SAD method in Photon Factory**
Toshiya Senda, High Energy Accelerator Research Organization (KEK), Japan

Title: **Structural mechanisms of nucleosome recognition as revealed by methyl-TROSY**
Yawen Bai, National Institutes of Health, USA

Title: **New insights into pRN1 priming: Structural changes support specific DNA recognition and catalysis**
Julien Boudet, ETH-Hönggerberg, Switzerland

Title: **A new protocol to investigate conformational population patterns in the enzymatic activity cycle of proteins using molecular dynamics and normal mode analysis**
Luis Paulo. B Scott, Universidade Federal do ABC, Brazil

Title: **Cryo-electron microscopy grid preparation from nanoliter-sized protein samples and single-cell extracts**
Thomas Braun, University of Basel, Switzerland

Nebojsa Janjic, J Proteomics Bioinform 2017, 10:8(Suppl)
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Structural insights from aptamers with base modifications

Nebojsa Janjic
SomaLogic, Inc., USA

Statement of the Problem: The ability to fold into distinct three-dimensional structures is the basis of high affinity and specificity characteristic of aptamer binding to their targets. We have recently introduced base modifications that increase chemical diversity of functional groups front-loaded in randomized nucleic acid libraries from which aptamers are selected. Such modifications have allowed us to identify high-affinity aptamers to many protein targets previously considered "difficult" with conventional nucleic acid libraries. At the same time, our ability to predict the structures of modified aptamers with conventional nucleic acid folding rules was severely compromised, suggesting new rules for folding.

Methodology & Theoretical Orientation: We examined, published structures of sixteen aptamers co-crystallized with their protein targets, including three aptamers with base modifications we reported recently.

Findings: In contrast to small molecules, which are entirely encaged by aptamers, proteins present large surfaces with distinct features that are recognized by complementary surfaces on aptamers. The size of these interaction surfaces is comparable to those observed with antibodies, although for aptamers, the size range is wider on both small and large extremes. The highly flexible phosphodiester backbone allows assembly of known as well as novel nucleic acid motifs into precise three-dimensional structures that orient often discontiguous aptamer regions toward their protein targets in a manner that creates surfaces with exquisite shape complementarity. Base modifications with hydrophobic side chains allow occupancy of distinctly hydrophobic pockets on proteins and create novel structural elements that illustrate the profound role modified nucleotides play in both folding and binding.

Conclusion & Significance: These observations provide compelling structural rationale for the observed high affinity and specificity with which aptamers recognize their protein targets, and show us that the lexicon of structural features accessible to nucleic acid ligands can be vastly expanded with chemical modifications of nucleic acid libraries.

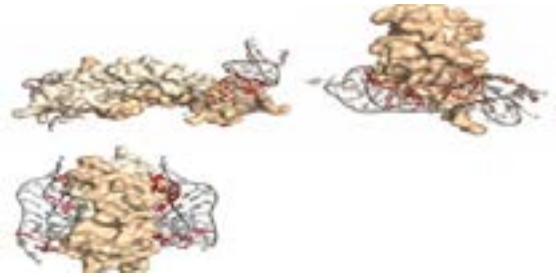

Figure1: *Crystal structures of PDGF-BB (left), IL-6 (middle) and NGF (right) bound to their respective modified aptamers. Modified nucleotides are shown in red.*

Biography

Nebojsa Janjic has been Chief Science Officer at SomaLogic, Inc. since January 2009. Prior to joining SomaLogic, he was a Founder and CSO at Replidyne, Inc., a Biotechnology company focusing on the development of new small-molecule antibacterial agents. Prior to Replidyne, he was Senior Director of drug discovery at NeXstar Pharmaceuticals, where his contributions include the discovery and early development of Macugen, the first aptamer to receive FDA approval and the first VEGF inhibitor developed for the treatment for macular degeneration. As CSO at SomaLogic, he is involved in developing a new generation of modified aptamers and identifying opportunities for their use in science and medicine. He has received his Bachelor's degree in Molecular Biology and PhD in Physical Organic Chemistry from the University of Washington in Seattle and completed his Postdoctoral training at the Scripps Research Institute in La Jolla as a Cancer Research Institute Fellow.

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Robert Craigie, J Proteomics Bioinform 2017, 10:8(Suppl)
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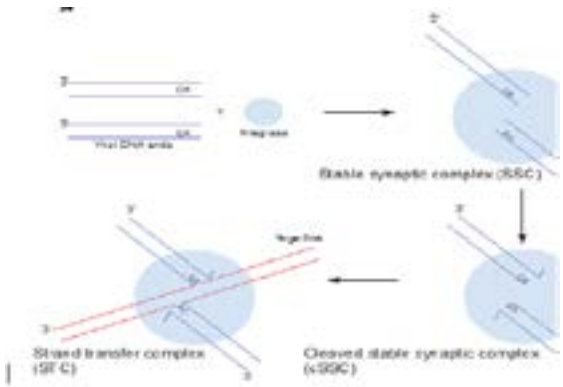
HIV-1 integrase assembles multiple discrete intasomes that are active for DNA integration *in vitro*

Robert Craigie
National Institutes of Health, USA

Statement of the Problem: Integration of retroviral DNA into host DNA is an essential step in the replication of HIV-1 and other retroviruses. Integration is mediated by a nucleoprotein complex (intasome) comprising the virally encoded integrase enzyme and a pair of viral DNA ends. The first intasome on the integration pathway is the stable synaptic complex (SSC) in which a pair of viral DNA ends is bridged by integrase. Within the SSC, integrase then cleaves two nucleotides from the 3' ends of the viral DNA to form the cleaved stable synaptic complex (cSSC). The cSSC captures a target DNA and a pair of transesterification reactions covalently joins viral to target DNA. Currently approved inhibitors of HIV-1 DNA integration target intasomes (specifically the cSSC) rather than free integrase protein, High-resolution structures of intasomes are required to understand their detailed mechanism of action and how HIV-1 can escape by acquiring resistance.

Methodology & Strategy: Although the structures of the individual domains of HIV-1 integrase were determined more than two decades ago, attempts to obtain high-resolution structures of HIV-1 intasomes were unsuccessful. The main obstacles were the propensity of both integrase and intasomes to aggregate and the low efficiency of assembly *in vitro*. We have overcome these problems by developing a hyperactive integrase mutant that assembles intasomes that are amenable to biophysical and structural studies. CryoEM studies of STCs reveal both tetrameric and higher order species that both share a common core architecture with intasomes of related retroviruses. SSCs also assemble both tetrameric and higher order intasomes and both are active for concerted DNA integration *in vitro*.

Conclusions & Significance: The results highlight how a common core intasome architecture can be assembled in different ways. Structures of cSSC intasomes in complex with inhibitors will elucidate their detailed mechanism of action and mechanisms by which HIV-1 can evolve drug resistance.



Biography

Robert Craigie is a Senior Investigator in the National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health, Bethesda, MD, USA. His research has focused on the mechanism of retroviral DNA and the structure and function of proteins and nucleoprotein complexes that mediate it.

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Dino Moras, J Proteomics Bioinform 2017, 10:8(Suppl)
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Allosteric control of transcription regulation by nuclear receptors: An integrative structural biology approach

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Nuclear hormone receptors interact with corepressors, coactivators and other protein cofactors to regulate signal transduction of the basal transcriptional machinery. Most NRs are known to function as dimers and except for the group of oxosteroid receptors (AR, GR, MR, PR) all structural data point to a conserved interface for the ligand binding domains (LBDs) dimers. Allosteric mechanisms control the sequential and ordered binding of nuclear receptors to the various protein effectors and target DNA. The binding of ligands induces structural transitions in the LBDs leading to the release of the corepressors and their replacement by cofactors. The LBD swallows the ligand and shields it from the solvent by closing the pocket with the C-terminal peptide. The agonist/antagonist character of the ligand is then essentially controlled by the position of helix H12 and the stability of the complex. Ligands are modulators of the activation process, their potency being defined by the fraction of time spent in the active conformation. Crystal structures of DNA binding domains (DBDs) bound to different response elements also support the proposal of DNA being an allosteric effector. The architectures of full length receptors bound to DNA fragments and cofactors have been determined by crystallography and in solution using integrative approaches. The later combine structural small angle diffraction methods by X-Rays (SAXS) and neutrons (SANS), optical techniques like FRET with labelled molecules and single particle electron microscopy (cryo-EM). Some common features emerge that rationalize the key role of DNA. The recent advances in cryo-EM allow solution structures determination at near atomic resolution. Conformational equilibrium of NRs in complex with various cofactors are also accessible.

Left: model of NRs heterodimer bound to its DNA target on a nucleosome (from ref 2).
Right: Schematic representation of the LBDs inversion between Inverted and Direct Repeats-bound complexes, illustrating the strong DNA-dependent allostery (from ref 3).

Biography

Dino Moras has completed his Graduation in Chemistry at the University of Strasbourg. While pursuing Post-doc with M G Rossmann, he has contributed to the concept of nucleotide binding domain known as the 'Rossmann fold'. His main scientific contributions are in structural biology, related to the expression of the genetic information: translation of the genetic code by aminoacyl-tRNA synthetases: discovery of the partition of aaRS in two classes and first crystal structure of a class II tRNA-aaRS complex and transcription regulation by Nuclear Receptors: the first crystal structures of the ligand binding domains of two NRs (RXR and RAR) in their apo and liganded form respectively. Presently, his focus is on the molecular mechanisms of regulation using integrative structural biology approaches.

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John Vakonakis, J Proteomics Bioinform 2017, 10:8(Suppl)
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Complex of malaria parasites and human proteins drive formation of cytoadherent assemblies at the surface of infected red blood cells

John Vakonakis
University of Oxford, United Kingdom

Human red blood cells infected by the malaria parasite *Plasmodium falciparum* (iRBC) form dome-shaped ~120 nm-diameter protrusions on their surface, known as 'knobs'. Knobs provide essential presentation platforms for the parasite cytoadherence receptor family P*f*EMP1, which binds ligands on endothelial cells of the blood vessel wall thereby immobilizing iRBC in the microvasculature. The resulting obstruction of blood vessels and disruption of normal circulation causes inflammation and tissue damage that can lead to coma and death. iRBC cytoadherence constitutes the primary mechanism driving morbidity and mortality in *P. falciparum* infections, which account for over 90% of all malaria-related deaths. Despite their importance in malaria pathology the molecular mechanisms underpinning knob formation remain poorly understood. Here, I review recent progress in characterizing knob complexes formed between parasite and parasite-host proteins. Extensive flexibility is common among parasite knob components, which necessitated an integrative approach to resolve these complexes. I will focus on the development of novel *in silico* docking tools suitable for evaluating interactions between folded components and highly charged, very long and flexible protein segments. Our work offers the first glimpse of a molecular model for knobs.

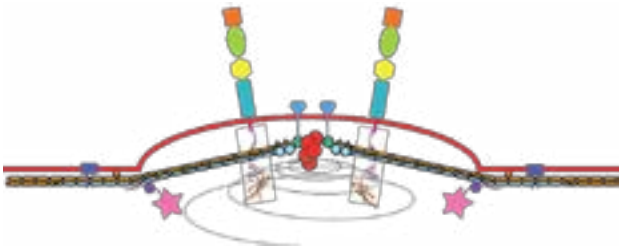

Figure1: *Model of a cytoadherent assembly ('knob') on the surface of P. falciparum-infected red blood cell, depicting recently characterized protein complexes that will be discussed here.*

Biography

John Vakonakis has completed his PhD in Biochemistry at Texas A&M University, where he pioneered the structural analysis of bacterial circadian clock proteins. His Postdoctoral work at the University of Oxford focused on the structural mechanisms underpinning cell adhesion and assembly of the extracellular matrix in animals. He did breakthrough work on the molecular architecture of the centriole organelle during a second Postdoc at the Swiss Light Source, prior to starting his own lab in Oxford Biochemistry. He has been a Marie Currie Fellow, Junior Research Fellow at Trinity College, Oxford, and a Wellcome Trust Research Fellow. He is now Associate Professor in Structural Biology and Biophysics at the University of Oxford, and Fellow in Biochemistry at Lincoln College. Over the last six years his research aims to understand how large molecular machines form in cells, such as the cytoadherence assemblies created upon *P. falciparum*-infection of human erythrocytes.

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David F Sargent, J Proteomics Bioinform 2017, 10:8(Suppl)
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Micro robotics enables non-contact, fully automated protein crystal harvesting

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Statement of the Problem: Most aspects of macromolecular structure determination, from synthesis and purification of materials, through crystallization, data collection and model building, are highly automated. But the recognition, harvesting and cryocooling of crystals reminds of a predominantly manual task. Several concepts, including *in situ* crystallography, are being developed to overcome these difficulties, but frequently impose other restrictions such as data collection strategies. We are developing hardware and software to support crystal harvesting using standard crystallization procedures, thus avoiding such limitations.

Methodology & Theoretical Orientation: We use a magnetically driven mobile, rolling micro robot, the RodBot, to locally move the liquid surrounding crystal. The crystal then passively follows the flow. Crystal position is monitored using low level UV-light. Transport is controlled using flexible algorithms that allow for error-recovery, following stochastic disturbances.

Findings: We demonstrated the effectiveness of the technique using crystals of different geometries and densities in a variety of buffers and cryoprotectants. Even at this developmental stage average harvesting time is reduced compared to manual operations.

Conclusion & Significance: This non-destructive, non-contact method allows crystals to be extracted reliably from the growth droplet in a completely automated process. Harvesting can take place remotely in climate-controlled chambers, ensuring optimal conditions throughout the process with respect to temperature, humidity and composition of the environment. Damage to valuable crystals due to operator jitter or fatigue is eliminated. Incorporation into existing robotics setup for sample handling will also allow increased reproducibility of flash-cooling. Fully automated structure determination pipelines using well-established techniques are now possible and can yield improved data quality at reduced cost.

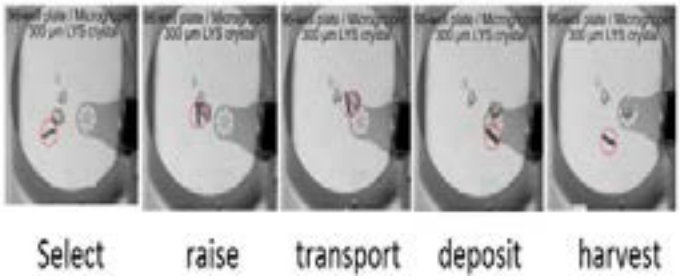

Figure1: *Crystal harvesting using the RodBot micro robot (in red circle). From left: Selecting one of several crystals in a droplet of a 96-well plate; fluid flow from the approaching RodBot raises the crystal off the bottom of the droplet and transports the crystal towards the micro-mount; the crystal is deposited on the micromount, where upon the crystal can be harvested, flash-cooled and stored using a robotic arm.*

Biography

David F Sargent has obtained his PhD in Biophysics from the University of Western Ontario, Canada, followed by Postdoctoral studies at the ETH Zurich and the University of Sydney (Australia). He has extensive experience in macromolecular crystallography at the ETH Zurich, and recently has also been associated with the Multiscale Robotics Laboratory (ETH Zurich) of Bradley J Nelson. He is one of the founders of MagnebotiX, a spinoff of the ETH, which provides tools for magnetic propulsion and guidance at the microscopic scale. The work reported above uses this technology to streamline and accelerate the process of macromolecular crystal structure determination.

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Toshiya Senda, J Proteomics Bioinform 2017, 10:8(Suppl)
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The native (Sulfur) SAD method in photon factory

Toshiya Senda
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Crystallography has been a major method to determine 3D structures of biological macromolecules at atomic resolution. While a new method with cryo-EM is becoming another major technique for the 3D structure analysis, crystallography still has some advantages. Recently, many crystal structures of biological macromolecules are determined by MAD/SAD method with seleno-methionine proteins (SeMet-proteins). Since selenium has an X-ray absorption edge near 1Å, it is convenient to utilize in the MAD/SAD method. While this technique is useful, crystallographers need to prepare SeMet-proteins. If we can develop a method to solve the phase problem without using SeMet-proteins, it would be highly useful for crystallographers. So, we have tried to develop the native SAD (or sulfur SAD) method, in which anomalous signals from sulfur atoms in the native protein are utilized. However, there are some problems in the native SAD method. First, sulfur gives only weak anomalous signals with X-ray typically utilized in protein crystallography (X-ray wavelength of around 1Å). To increase the anomalous signals, we need to use a longer wavelength X-ray than usual. However, since a longer wavelength X-ray shows significant absorption by air, solvent, protein etc., data quality is degraded by the absorption. The native SAD method, therefore, requires a specific system for high quality data collection. To achieve routine utilization of the native SAD method, we have developed a beamline (BL-1A) dedicated for the native SAD method. In BL-1A, we can utilize a long wavelength X-ray (1.9-3.5 Å). Furthermore, the goniometer and X-ray detector are installed inside a He chamber to prevent the absorption problem. Our system enables us to solve crystal structures of proteins by the native SAD method. In the presentation, we will present several examples of crystal structure determination with native SAD. Also, we will mention our unique method for crystal freezing to collect high quality diffraction data required in native SAD experiments.

Figure1: *BL-1A of Photon Factory dedicated to the native SAD phasing*

Biography

Toshiya Senda has completed his PhD from Nagaoka University of Technology (Niigata, Japan) in 1995. He was a Research Associate in Nagaoka University of Technology (1995-2001) and a Senior Researcher in Institute of Advanced Industrial Science and Technology (2001-2012). Now, he is the Director/Professor of Structural Biology Research Center of High Energy Accelerator Research Organization (KEKI) in Japan. He was awarded the CrSJ (Crystallographic society of Japan) award in 2014 (Structural biology studies of CagA from *Helicobacter pylori* and histone chaperon CIA/ASF1).

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Yawen Bai, J Proteomics Bioinform 2017, 10:8(Suppl)
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Structural mechanisms of nucleosome recognition as revealed by methyl-TROSY

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Human genome is packaged into chromatin through association with small positively charged histone proteins. The structural unit of chromatin is the nucleosome, which consists of ~147 bp of DNA and two copies of each of the four core histones (H2A, H2B, H3 and H4). Numerous proteins regulate chromatin structure and function through specific binding to the nucleosome. The structural basis of many of these interactions is unknown. Structural determination of the nucleosome in complex with a protein by X-ray crystallography and single particle cryo-EM has proven to be very challenging in many cases due to difficulties to crystalize them and dissociation of the complex during cryo-processes. On the other hand, the nucleosome is too large (>200 KDa) for structural studies with conventional NMR methods. We have used methyl-TROSY coupled with site-specific mutagenesis and paramagnetic spin labeling to investigate how the nucleosome is recognized by various chromatin factor proteins, including high-mobility group nucleosomal protein, centromere protein C and linker histones. Major results and future perspectives will be presented.

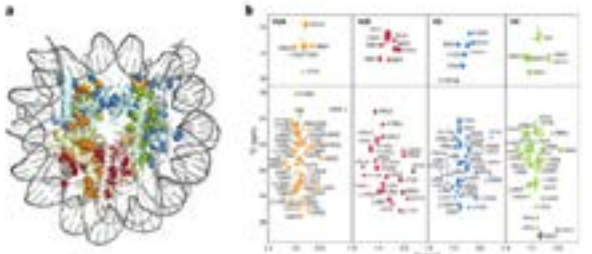

Figure 1. Methyl-TROSY studies of the nucleosome and its interactions with chromatin factors. (a) Crystal structure of the nucleosome: the spheres represent methyl groups in the histones. H2A (orange), H2B (red), H3 (blue) and H4 (green). (b) Methyl-TROSY spectra of the nucleosome and the assignments of its methyl groups.

Biography

Yawen Bai has received his PhD in Biophysics from the University of Pennsylvania Medical School. After Postdoctoral work at the Scripps Research Institute, La Jolla, California, he became an Investigator at the National Cancer Institute of the National Institutes of Health in Bethesda, Maryland since 1997. The research interests of his group include structural studies on protein folding intermediates, histone chaperones, epigenetic specification of centromeres and chromatin folding.

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Julien Boudet et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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New insights into pRN1 priming: Structural changes support specific DNA recognition and catalysis

Julien Boudet[1], **Jean-Christophe Devillier**[2], **Georg Lipps**[2] and **Frédéric H T Allain**[1]
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Primases are single-stranded DNA dependent polymerases that synthesize RNA/DNA primers during replication. A primase, a DNA polymerase and an helicase compose the replication machinery of the archaeal plasmid pRN1. The structure of the archaeal functional primase domain has been solved by X-ray crystallography and it revealed an heteromeric structure with a catalytic prim/pol domain tethered to a novel helix bundle domain. We investigated the NMR structure of the functional pRN1 primase domain in complex with a single-stranded DNA template containing the GTG motif. We showed that the catalytic prim/pol domain of this 38 kDa enzyme is not required for template binding. Intermolecular contacts detected exclusively between the helix bundle domain and the DNA led us to isolate specifically this structurally independent unit. Our results are compatible with a conformational switch between a template-bound open state and a closed active complex. We solved the solution structures of the helix bundle domain in complex with the single-stranded DNA template alone and upon cofactors addition. Affinity measurements validated our structural data demonstrating the importance of residues located in helices 10 and 12 for the interaction with the GTG motif and confirmed the specificity improvement observed upon cofactors binding. In association with functional assays, these novel transient structures bring new perspectives and will help us to characterize the molecular steps required for priming.

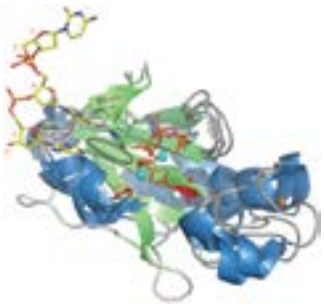

Figure1: *Structural superimposition of catalytic cores from the pRN1 (1RO2), P. horikoshii (1V34), H. sapiens (4LIL) and S. cerevisiae (4LIM) primases as well as the polymerase domain of the M. tuberculosis ligase D (3PKY).*

Biography

Julien Boudet has completed his PhD degree in Structural Biology and Biophysics from the University of Grenoble (Joseph Fourier University) in France under the supervision of Prof. Jean-Pierre Simorre. During his thesis, he has learned nuclear magnetic resonance (NMR) spectroscopy and used this powerful method to investigate proteins and oligonucleotides structures, molecular mechanisms underlying antibiotic resistance and viral proteins interactions. After Graduating, he has joined the group of Prof. Frédéric Allain in ETH Zurich as a Researcher. He has focused his investigations on the DNA replication machinery and, on the primase-mediated catalysis. He set up an innovative computational methods to investigate challenging biological systems and demonstrated the role of cofactors in improving the pRN1 primase specific template recognition.

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Luis Paulo B Scott, J Proteomics Bioinform 2017, 10:8(Suppl)
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A new protocol to investigate conformational population patterns in the enzymatic activity cycle of proteins using molecular dynamics and normal mode analysis

Luis Paulo B Scott
Federal University of ABC, Brazil

Human immunodeficiency virus type-1 protease (HIV-1 PR) is an aspartic protease whose proteolytic activity is essential for cleaving precursor viral polyproteins into individual proteins implied in viral replication. Once HIV enters within a host cell, its RNA is transcribed into DNA through reverse transcriptase, integrated and amplified along with the replication of the host cell's DNA. *Gag* and *gag-pro-pol* genes are transcribed into messenger RNA, translated into *gag* and *gag-pro-pol* precursors proteins in the cytoplasm, and then assembled at the cell surface for budding and formation of the immature viral particles. In this work, we propose a computational protocol to generate and select HIV protease conformations relevant to its function using Normal Mode Analysis (NMA). We have considered structures of the apoenzyme, the protein with its substrate and product and the protein with a drug. This set of structures should reveal large amplitude motions that are critical to the protease activity cycle as: substrate acquisition; substrate cleavage and product release. The apoenzyme presents an increased flap conformational diversity compared to the various complexes, predominantly populated with open flap conformations, that can possibly be related to the substrates acquisition. The enzyme-substrate complexes show more structural diversity than enzyme-product complexes, suggesting a role of these conformational changes in catalytic activity. We present a promising protocol to identify the conformational diversity induced by different types of ligands and that can help the drug design process.

Biography

Luis Paulo B Scott is an Associate Professor in Federal University of UAFBC. He has his expertise in conformational changes and functional movements of macromolecules, specially proteins. Over the last four years, his research group has been financed to investigate molecules related to neurodegeneration and aggregate formation by means of normal mode analysis and molecular dynamics combined. The laboratory coordinated by him has become more and more specialized in the study of macromolecules structural dynamics (functional movements in collaboration with Dr. David Perahia from France.

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Notes:

Thomas Braun et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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Cryo-electron microscopy grid preparation from nanoliter-sized protein samples and single-cell extracts

Thomas Braun, Stefan Arnold, Stefan Albiez, Andrej Bieri, Claudio Schmidli, Anastasia Syntychaki, Luca Rima, Nadia Opara, Shirley Müller, Kenneth N Goldie, Mohamed Chami and **Henning Stahlberg**
University of Basel, Switzerland

Cryo-electron microscopy (cryo-EM) sample preparation techniques ensure that biological specimens can be investigated at physiological conditions in the electron microscope. However, these preparation methods suffer from extensive blotting steps leading to a massive loss of sample and sometimes to partial denaturation of sensitive protein complexes. We have developed a simple method for the almost lossless conditioning and preparation of nanolitre-volumes of biological samples for EM. The method does not involve any blotting steps. A microcapillary is used to aspirate 3 to 20 nanoliters of sample, depending on the experiment. In the figure, the sample is applied (left) and spread (center) on the EM-grid. Real-time monitoring allows the thickness of the water film to be assessed and decreased to the optimum value prior to vitrification (right). We prepared cryo-EM grids of various samples, e.g., bacteriophages and soluble proteins as shown in Figure 1B and C, to demonstrate the usefulness and general applicability of the method. We also showed that high-resolution 3D structures can be calculated from single-particle preparations of a soluble protein. In addition to cryo-EM grid preparation, the versatile method allows nanoliter-sized sample volumes to be conditioned for EM, e.g., negatively stained with heavy metal salts or embedded in trehalose. In addition, we combine the new sample preparation method with a single cell lysis device for adherent eukaryotic cells and image the aspirated cell contents by TEM. To demonstrate the usefulness of this new visual proteomics approach we visualized the changes occurring in single cell proteomes upon heat shocking the cells. Furthermore, we have developed a protein-fishing method based on a magnetic trap and photo-cleavable composite material, to 'fish' untagged proteins from cell lysate by antibodies. This allows target proteins to be isolated from approx. 40,000 cells in 90 min and analyzed by EM.

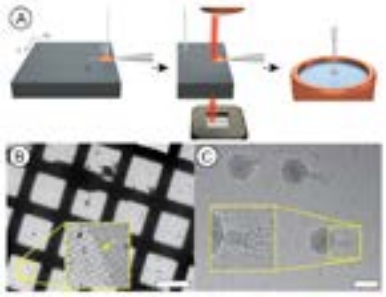

Figure1: *(A) Cryo-EM grid preparation from nanoliter-sized samples (see main text). (B) Example overview, yellow arrows indicate borders of the vitreous ice. Scale bar: 100 µm. (C) Test-sample containing apoferritin particles and bacteriophages at high magnification and defocus (to increase contrast). Inset: twofold enlargement of the indicated region. Scale bar: 80 nm.*

Biography

Thomas Braun has received his PhD in 2002 in Biophysics from the Biozentrum, University of Basel, Switzerland. During his PhD thesis, he has applied high-resolution electron microscopy and digital image processing to study the structure and function of membrane proteins. Subsequently, he has worked on nano-mechanical sensors to characterize the mechanics of membrane proteins at the Institute of Physics, University Basel and the CRANN, Trinity College Dublin, Ireland. He has been working at the Center for Cellular Imaging an Nano Analytics (Biozentrum, University of Basel, Switzerland) since 2009 and is developing new methods for electron microscopy, single cell analysis and nano-mechanical sensors for biological applications.

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Sessions:

Molecular Modelling and Drug Designing

Session Chair
Christina Scharnagl
Technical University of Munich, Germany

Session Co-Chair
Shuanghong Huo
Clark University, USA

Session Introduction

Title: **Structure based drug discovery on membrane protein targets: New developments and advancements**
Michael Hennig, leadXpro AG, Switzerland

Title: **Structure-based drug design of the Eg5 inhibitor NVP-BQS481**
Dirksen E. Bussiere, Novartis Institutes for BioMedical Research, USA

Title: **Does the dynamics of their transmembrane domain qualify bitopic membrane proteins as substrates for intramembrane proteolysis?**
Christina Scharnagl, Technical University of Munich, Germany

Title: **The dynamics of a protein during its insertion into a membrane**
Andreas Kuhn, University of Hohenheim, Germany

Title: **Extract the thermodynamic and kinetic information from protein simulations using dimensionality reduction**
Shuanghong Huo, Clark University, USA

Title: **Dynamics of knotted and entangled neurotoxic polypeptides**
Marek Cieplak, Institute of Physics PAS, Poland

Title: **What docking studies tell us about the role of disordered protein fragments in macromolecular assembly**
Chantal Prévost, CNRS, France

Michael Hennig, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Structure based drug discovery on membrane protein targets: New developments and advancements

Michael Hennig
LeadXpro AG, Switzerland

Today, structure based drug discovery is well implemented in the drug discovery engine of many pharmaceutical companies. Whereas soluble proteins are managed well within the project timelines and portfolio changes in pharmaceutical industry, transmembrane proteins still represent a significant challenge. LeadXpro combines expertise of drug discovery, excellence in high quality solubilized and purified membrane protein science and use of cutting edge biophysical methods like X-ray data collection at synchrotron and FEL sources, single particle cryo-electron microscopy, SPR and others. Strong relationship between leadXpro and Swiss large research facilities like PSI-SLS and SwissFEL as well as C-CINA will enable advances in structure determination of challenging membrane protein drug targets that have not been feasible before. Knowledge of the drug candidate and protein target 3d-structure, together with the full characterization of its interaction by biophysical binding and functional assays will enable to generate novel and better lead molecules for future medicines. Examples of recent developments include the successful fragment screening for the GPCR neutrotensin receptor 1, a fragment screening with 6369 compounds was performed with SPR and 44 hits identified. Finally, 4 selected hits were validated in NMR experiments and computational analysis gave insight into the potential fragment-binding location and interactions to inspire further chemistry efforts. Furthermore, serial crystallography was performed at synchrotron and free electron laser enables structure determination on challenging drug targets. Advantages are (1) analysis at physiologically more relevant room temperature (no freezing of crystals required), (2) low or no radiation damage and (3) the use of very small crystals.

Biography

Michael Hennig is a drug discovery Research Manager with 22 years of experience in pharmaceutical industry. He co-founded and is CEO and Chairman of the board of LeadXpro AG, an emerging biotech company and spin-out of the Paul Scherrer Institute (ETH, Switzerland) that is dedicated to structure based drug discovery of membrane protein targets. Formerly he worked 20 years at Roche research Basel as Global Head and Principle Leader of discovery technologies with responsibility for structure based drug discovery, protein science, assay development and HTS, corporate compound library, stem cell platform. In addition, he is guest Professor at the University of Basel in Structural Biology, gives lecture series in pharmacy, is author of more than 75 paper and lecturer at conferences, inventor of 8 patents in areas of technology, discovery and formulation of drug substances.

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Dirksen E Bussiere, J Proteomics Bioinform 2017, 10:8(Suppl)
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Structure-based drug design of the EG5 inhibitor NVP-BQS481

Dirksen E Bussiere
Global Discovery Chemistry - Novartis Institutes for Biomedical Research, USA

Several biological functions, particularly chromosome segregation, require the generation of motile force. The generation of this force relies heavily on a class of proteins known as motor proteins. Motor proteins such as Kinesin Spindle Protein (KSP), also known as Eg5, utilize the energy derived from ATP hydrolysis to generate motile force. High-throughput screening of Eg5 identified several hits which were non-competitive with ATP with micromolar IC50's capable of inhibiting the motor protein. Using structure-based drug design, these hits were progressed to NVP-BQS481, a clinical candidate with an IC50 of 700 picomolar. The talk will present the design concepts and optimization techniques used to advance the series to the pre-clinical stage.

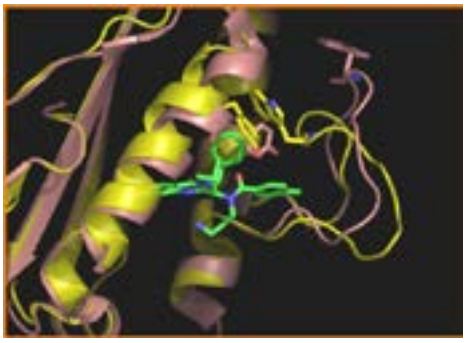

Figure1: *Eg5 compound exosite illustrating conformational changes occurring upon compound binding*

Biography

Dirk Bussiere has his expertise in biochemistry, biophysics, computer sciences and structural biology to the discovery of novel therapeutics for the treatment of disease. He received his BA in biochemistry from Northwestern University, has completed MS in Molecular Biophysics and Biochemistry from Yale University, and PhD in Microbiology, Immunology and Molecular Biophysics from Duke University. He also has an MBA in Entrepreneurship, Management of Technology, and Finance from the Haas School of Business, University of California-Berkeley. He was named a Novartis Leading Scientist in 2007. He is currently the director of the Structural and Biophysical Chemistry group in Global Discovery Chemistry at the Novartis Institutes for Biomedical Research in Emeryville, California.

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Christina Scharnagl, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Does the dynamics of their transmembrane domain qualify bitopic membrane proteins as substrates for intramembrane proteolysis?

Christina Scharnagl
Technical University of Munich, Germany

Integral membrane proteins facilitate communication between the inside of the cell and its exterior. Their transmembrane domains (TMDs) support a diversity of biological functions and exhibit sequence-dependent conformational dynamics on multiple size and time scales. Membrane proteins are notoriously difficult to study by experimental methods. Molecular dynamics (MD) simulations provide a powerful tool of high spatial and temporal resolution that effectively complements experimental methods. Here we focus on the conformational dynamics of the TMD of the amyloid precursor protein (APP). APP is enzymatically hydrolyzed within its TMD by γ-secretase (GSEC), forming toxic Aβ peptides regarded as molecular cause of Alzheimer's disease (AD). Besides APP, GSEC cleaves ~100 single-span membrane proteins within their TMDs, however without obvious consensus sequence. Finding the link between the molecular architecture of the substrate TMDs and cleavage is, therefore, of upmost importance. Because unfolding is obvious to expose the scissile bond, it seems plausible that the TMD itself is optimized for local helix unwinding. However, this view was challenged by our experiments and MD simulations. Our results suggest an alternative model where reaching a cleavage-competent state involves multiple conformational transitions of the substrate/enzyme complex where global conformational plasticity of the substrate TMD is a key determinant. In a first step, we compare the conformational flexibility of a large number of substrate and non-substrate TMDs, as well as TMDs carrying missense mutations related to early onset AD. Knowing the key-dynamical motifs will help to identify new substrates and to elucidate the physiological functions of the protease in the brain and other organs. This work is part of a collaborative research program(https://www.i-proteolysis.de/).

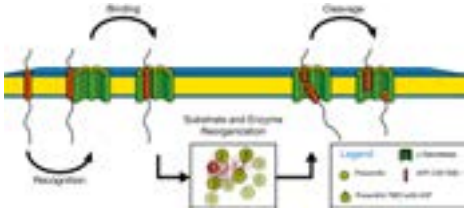

Figure1: *Substrate processing by γ-secretase. The intramembrane protease is a protein complex hydrolyzing substrates within their trans-membrane domains. Transmembrane domain dynamics might be involved in recognition, binding and reorganization steps funnelling the enzyme/substrate complex towards the conformation conducive for cleavage.*

Biography

Christina Scharnagl has her expertise in molecular dynamic simulations of membrane proteins. Her work focuses on biophysical principles of the interdependence of transmembrane helix dynamics, helix-helix binding, and helix-lipid interactions. *In silico* modelling and advanced computational analysis are closely connected to experimental work in research collaborations in order to interpret and guide the experiments and to validate the simulations. The aim of the joint efforts is to understand the impact of these phenomena on multiple biological processes, such as membrane fusion and intramembrane proteolysis.

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Andreas Kuhn et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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The dynamics of a protein during its insertion into a membrane

Andreas Kuhn, Dirk Spann and **Maximilian Haase**
University of Hohenheim, Germany

Most membrane proteins are inserted co-translationally by the Sec-translocase or the YidC/Oxa1/Alb3 insertases. The folding of these proteins occurs within the membrane during the interaction with the insertases. We have purified and reconstituted YidC, the membrane insertase of *Escherichia coli*. The protein spans the membrane 6 times, and the recently solved structure shows a hydrophilic cavity and a greasy slide between the transmembrane segments TM3 and TM5. Hydrophobic residues of TM3 and TM5 interact with the substrate, with a prospective transmembrane segment of an inserting membrane protein as documented by disulfide crossinking experiments. The membrane insertion process can be studied with the reconstituted vesicle system. The purified substrate proteins are solubilized in 10% isopranol or kept unfolded with urea or GuHCl. When the substrate proteins are added to the proteoliposomes by dilution 1:100, they rapidly bind to YidC and become membrane inserted within 2 msec. FRET-based kinetic measurements show that the substrate proteins approach YidC to a close distance during the insertion event. Time-resolved fluorenscence anisotropy shows that the periplasmic domain of YidC moves when a substrate protein was added. This suggests that both the insertase and the substrate protein undergo conformational motions.

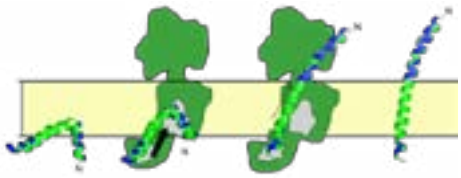

Figure1: *YidC-mediated insertion of the Pf3 coat protein*

Biography

Andreas Kuhn has his expertise in protein folding of membrane proteins. Studies include reconstituted systems with bacterial translocases and insertase, as well *in vivo* studies with *Escherichia coli*. For biophysical experiments, the membrane proteins are purified and their folding is monitored spectroscopically in real time after their addition to liposomes. Andreas Kuhn obtained his PhD from the Universities Basel and Freiburg im Breisgau 1982. After a Postdoc at UCLA with Bill Wickner he continued at the Biozentrum Basel from 1986 to 1989 and accepted a professorship at the University Karlsruhe. Since 1996 he is at the University of Hohenheim in Stuttgart.

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Notes:

Shuanghong Huo et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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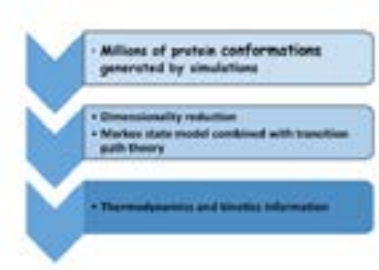
Extract the thermodynamic and kinetic information from protein simulations using dimensionality reduction

Shuanghong Huo and **Gustaf H**
Clark University, USA

In the study of protein thermodynamics and kinetics, it is of paramount importance to characterize protein free energy landscapes. Dimensionality reduction is a valuable tool to complete the task. We have evaluated several methods of dimensionality reduction, including linear and nonlinear methods, such as principal component analysis, Isomap, locally linear embedding, and diffusion maps. A series of criteria was used to assess different aspects of the embedding qualities. Our results have shown that there is no clear winner in all aspects of the evaluation and each dimensionality-reduction method has its limitations in a certain aspect. The linear method, principal component analysis, is not worse than the nonlinear ones in some respects for our peptide system. We have also developed a mathematical formulation to demonstrate that an explicit Euclidean-based representation of protein conformation space and the local distance metric associated to it improve the quality of dimensionality reduction. For a certain sense, clustering protein conformations into macro-clusters to build a Markov state model is also an approach of dimensionality. We have tested inherent structure and geometric structure for state space discretization and demonstrated that the macro-cluster based on inherent structure give a meaningful state space discretization in terms of conformational features and kinetics.



Biography

Shuanghong Huo received her PhD in Computational Chemistry from Boston University. She did her Postdoctoral training at UC-San Francisco. She is a Professor of Chemistry and Biochemistry at Clark University, Worcester, USA. Her research interest is in protein folding, misfolding, and aggregation. Recently, her group is developing dimensionality reduction methods and graph representations of protein free energy landscapes.

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Marek Cieplak, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Dynamics of knotted and entangled neurotoxic polypeptides

Marek Cieplak
Institute of Physics, Poland

We review the physics of processes involving large conformational transformations in knotted proteins in bulk water and then consider folding in ribosomes and unfolding in proteasomes. Formation of a knot is demonstrated to be facilitated by the nascent conditions at the ribosome. Knots in proteins have been proposed to resist proteasomal degradation. Ample evidence associates proteasomal degradation with neurodegeneration. One interesting possibility is that indeed knotted conformers stall this machinery leading to toxicity. However, although the proteasome is known to unfold mechanically its substrates, at present there are no experimental methods to emulate this particular traction geometry. Here, we consider several dynamical models of the proteasome in which the complex is represented by an effective potential with an added pulling force. This force is meant to induce translocation of a protein or a polypeptide into the catalytic chamber. The force is either constant or applied periodically. The translocated proteins are modelled in a coarse-grained fashion. We do comparative analysis of several knotted globular proteins and the transiently knotted polyglutamine tracts of length 60 alone and fused in exon 1 of the huntingtin protein. Huntingtin is associated with Huntington disease, a well-known genetically-determined neurodegenerative disease. We show that the presence of a knot hinders and sometimes even jams translocation. We demonstrate that the probability to do so depends on the protein, the model of the proteasome, the magnitude of the pulling force, and the choice of the pulled terminus. In any case, the net effect would be a hindrance in the proteasomal degradation process in the cell. This would then yield toxicity *via* two different mechanisms: one through toxic monomers compromising degradation and another by the formation of toxic oligomers.

Biography

Marek Cieplak is the Head of Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences in Warsaw, Poland. He completed MS, Department of Physics, University of Warsaw, 1973; PhD, Department of Physics, University of Pittsburgh, 1977; DSc, Department of Physics, University of Warsaw, 1984. His fields of interest are: condensed matter theory (spin waves, spin glasses, porous media, growth processes, atomic friction, river networks, nanofluidics, self-organized nanostructures) and biological physics (large conformational changes of biomolecules within coarse-grained models, especially as induced by stretching, proteins with knots and slipknots, protein folding, dynamics of virus capsids and other multi-proteinic structures such as a cellulosome, interaction of proteins with solids, proteins at air-water interface, modeling of proteasomes, inference of genetic networks from the microarray data). He is the Co-author of textbook "*Theory of Quanta*", Oxford University Press 1992 and has published 250 research papers.

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Chantal Prevost, J Proteomics Bioinform 2017, 10:8(Suppl)
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What docking studies tell us about the role of disordered protein fragments in macromolecular assembly

Chantal Prevost
IBPC - CNRS, France

Statement of the Problem: Many proteins present highly flexible or disordered fragments, either terminal tails or surface loops. Although they often form instable and transient interactions, these fragments play essential roles in regulating macromolecular association or controlling the architecture of supramolecular complexes. The role of their conformational variability in complex formation is poorly understood and requires the development of specific approaches.

Methodology & Theoretical Orientation: We have studied the effect of protein segment conformational variability in protein-protein complex formation as well as peptide docking using theoretical docking approaches. Notably, we have developed a flexible docking method that accounts for the presence of flexible loops, together with analysis protocols that capture the entropic effects associated to structural variability in flexible docking results.

Findings: Whether the flexible segment is a loop or a peptide, we have found that a given mode of association can be stabilized by different conformations of the segment. Alternatively, different loop conformations can stabilize different modes of protein-protein association.

Conclusion & Significance: Tolerance of a binding site to conformational variability, as observed in protein-peptide docking but also in the association of proteins with flexible loops or segments, can play a role in adding a conformational entropy component to the energy of association, thus favoring the initial binding of the flexible fragment to its binding site. For proteins that associate using different binding geometries, either with different partners or along a functional pathway, loop flexibility can also be used to regulate the choice of the binding geometry.

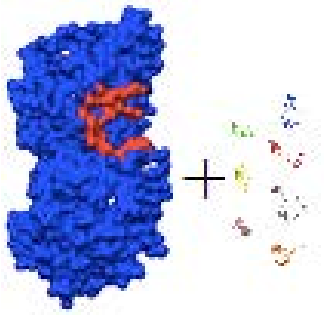

Figure1: *Mapping the interaction energy between the α, β tubulin dimer and the NFL-TBS.40-63 peptide from the docking simulations of seven different conformations of the peptide. The high affinity sites are shown in red, the low affinity sites are in blue. The affinity is defined as the energy-weighted probability of a tubulin surface atom to be involved in a docked interface.*

Biography

Chantal Prevost is a Researcher at the Theoretical Biochemistry Laboratory (LBT) of the French National Research Center (CNRS), in Paris. She has developed a large expertise in studying macromolecular self-assembly *in silico*, either by elaborating new algorithms for flexible proteins docking or by studying fundamental biological processes involving the transition between instable conformational substates. She presently applies this expertise to exploring the architecture or oligomeric assemblies as well as elucidating the mécanismes of homologués recombination, in collaboration with experimental partners.

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Sessions:

3 Dimensional Structure Determination | Computational Approaches | Structural Molecular Biology

Session Chair
Kurt Ballmer-Hofer
Paul Scherrer Institut, Switzerland

Session Co-Chair
Jianyong Li
Virginia Tech, USA

Session Introduction

Title: **Structural analysis of vascular endothelial growth factor receptors reveals drug-targetable allosteric sites regulating angiogenesis**
Kurt Ballmer-Hofer, Paul Scherrer Institut, Switzerland

Title: **A structure complex of a bacterial effort and an interacting protein: Insights into the transfer of virulence effector by pathogenic bacteria**
Jianyong Li, Virginia Tech, USA

Title: **Complex structure of mammalian cytochrome c–cytochrome c oxidase reveals a novel protein-protein interaction mode**
Kyoko Shinzawa-Itoh, Hyogo University, Japan

Title: **Oxidative stress, methionine oxidation, and calmodulin structure and function**
Jeffrey L Urbauer, The University of Georgia, USA

Title: **Molecular mechanism of SHP2 activation by CagA from Helicobacter pylori**
Miki Senda, High Energy Accelerator Research Organization (KEK), Japan

Title: **Integrative approaches to study the structure and motions of DNA sliding clamps**
Alfredo De Biasio, Elettra-Sincrotrone Trieste, Italy

Title: **An atomistic view of microtubule stabilization by GTP**
Liliane Mouawad, Institut Curie, France

Title: **Structure and function of a Chloride Pump Rhodopsin from marine bacteria**
Hyun-Soo Cho, Yonsei University, South Korea

Title: **Construction of structural mimetics of the thyrotropin receptor intracellular domain**
Stanislav Engel, Ben-Gurion University, Israel

Title: **Structural basis of sodium/citrate symporter as a secondary transporter**
Mi Sun Jin, Gwangju Institute of Science and Technology, South Korea

Kurt Ballmer-Hofer et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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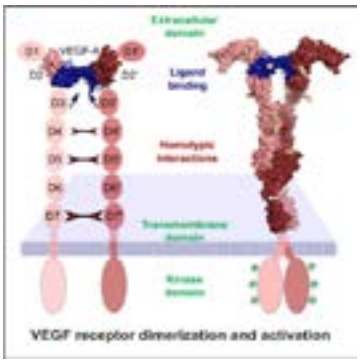
Structural analysis of vascular endothelial growth factor receptors reveals drug-targetable allosteric sites regulating angiogenesis

Kurt Ballmer-Hofer, Sandra Markovic-Mueller, Edward Stuttfeld and **Dragana Avramovic**
Paul Scherrer Institut, Switzerland

Vascular Endothelial Growth Factors (VEGFs) regulate blood and lymph vessel development upon activation of three receptor tyrosine kinases (RTKs), VEGFR-1, -2, and -3. Partial structures of VEGFR/VEGF complexes based on single particle electron microscopy, small angle X-ray scattering, and X-ray crystallography revealed the location of VEGF binding and the spatial arrangement of individual receptor subdomains. Here we describe the structure of the full-length VEGFR-1 extracellular domain (ECD) in complex with VEGF-A at 4 Å resolution. We combined X-ray crystallography, single particle electron microscopy, and molecular modeling for structure determination and validation. The structure reveals the molecular details of ligand-induced receptor dimerization, in particular of homotypic receptor interactions in Ig-domains 4, 5, and 7. Functional analyses of ligand binding and receptor activation confirm the relevance of these homotypic contacts for receptor activation and identify them as allosteric regulatory sites of VEGFR-1. Based on our structural data we also investigated the function of Ig-domains 4, 5 and 7 in VEGFR-2, the primary receptor driving angiogenesis and vasculogenesis in response to VEGF administration. The basic domain structure of VEGFR-2 is very similar to VEGFR-1, the ECD of both receptors consists of 7 Ig-domains, D1-D7. Mutagenesis studies based on the VEGFR-1structure confirmed that Ig-domains 4 and 7 fulfill an essential regulatory function in receptor activation and may thus represent putative targets for pharmacological intervention. We isolated highly specific antibodies and DARPins (Designed Ankyrin Repeat Proteins) specific for domains 4 or 7. A subset of these reagents efficiently blocked receptor activation and inhibited VEGF-dependent signaling *in vitro* in endothelial cell cultures. Most importantly, a domain 4-specific DARPin efficiently blocked vessel development also *in vivo* in a mouse angiogenesis model. In this model endothelial cell spheroids were implanted in matrigel into mice, and cell growth and vessel formation were monitored in the absence and presence of inhibitor. Our study thus revealed a novel approach for therapeutic targeting of aberrant blood vessel development.



Biography

Kurt Ballmer-Hofer focused his research at PSI on the structural and functional analysis of receptor tyrosine kinases, in particular on Vascular Endothelial Growth Factor Receptors, VEGFRs. In collaboration with partner labs his team solved the structures of VEGF ligands, the ligand binding domain of VEGFR-2, and -3, and of the full-length extracellular domain of VEGFR-1 in complex with VEGF. The data of these studies led to the discovery of allosteric receptor regulatory sites in subdomains 4, 5 and 7. Antibodies and DARPins specifically binding to these domains showed strong inhibition of receptor activation and downstream signaling both *in vitro* and *in vivo* in angiogenesis model systems.

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Jianyong Li et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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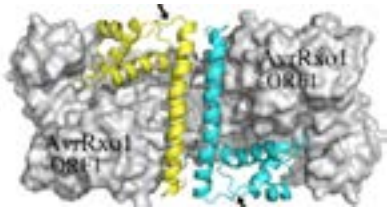
A structure complex of a bacterial effort and an interacting protein: Insights into the transfer of virulence effector by pathogenic bacteria

Jianyong Li, Han Qian and **Binyu Zhao**
Virginia Tech, USA

Bacterial effectors are proteins secreted by pathogenic bacteria into host cells through a type 3 or 4 secretion system. These bacterial effectors may help the pathogens to invade host cells and/or suppress its immune system; thereby promoting their infection, survival and reproduction. Effector proteins are primarily responsible for the pathogenicity of a given bacterial pathogen; therefore, learning the specific mechanism but which their effectors enter into host cells may provide insights for disease prevention. Bacterial Type 3 Secretion System (T3SS) has been extensively studied. It is a needle-like structure made by a number of structural proteins, which is responsible for transfer of protein effector to host cells. The needle tip is ~ 3 nm, which is smaller than the required dimension for most bacterial effectors. Despite some better understanding of the T3SS structure, how their bacterial effectors gain entry to host cells remains speculative. Using a T3SS-dependant effector as a model from *Xanthomonas oryzae* (a bacterium causing serious disease to some essential plants), we determined the structures of an effector protein in complex with a chaperone-like protein. In the genome of *Xanthomonas oryzae*, the coding sequence of effector protein is adjacent to that coding the chaperone-like protein. Our structural analysis indicates that the effector-chaperon complex crystallized as tetramers (1.64 Å resolution). The monomer of the protein effector contains a T4 polynucleotide kinase domain, while the monomer of the chaperon includes a novel kinase binding domain. Our data suggest that the chaperone protein interacts with the protein effector in a manner that helps to stabilize the protein effector and prevents the virulence effect of protein effort from harming the bacteria before being transferred to host cells. Currently, efforts are being made to understand the precise roles the chaperon protein plays during the transfer of protein effector to host cells.



Biography

Jianyong Li is a Biochemistry Professor at Virginia Tech. He has extensive experience in protein-related studies, including protein expression and purification, protein functional determination and protein structure and function relationships. Particularly worth to mention is the functional establishment of some unique yellow genes in insects and structural and function relationship of enzymes involved in kynurenate synthesis in mammals.

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Kyoko Shinzawa-Itoh, J Proteomics Bioinform 2017, 10:8(Suppl)
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Complex structure of mammalian cytochrome c–cytochrome c oxidase reveals a novel protein-protein interaction mode

Kyoko Shinzawa-Itoh
Hyogo University, Japan

Mitochondrial cytochrome c oxidase (CcO) transfers electrons from cytochrome c (Cyt.c) to O2 to generate H2O, a process coupled to proton pumping. To elucidate the mechanism of electron transfer, a crystal structure of the complex of CcO and Cyt.c would be invaluable for mechanistic studies. Two-dimensional (2D) crystals of the mammalian Cyt.c–CcO complex were prepared at higher pH (7.4–9.0) with both proteins in the oxidized state (Osuda et al, 2016), but these 2D crystals could not provide us with a structure of sufficient resolution. We optimized 3D crystallization conditions for ferri-Cyt.c and oxidized CcO at high pH and solved the X-ray structure of the complex at 2.0 Å resolution. The specific interaction between Cyt.c and CcO is stabilized by only six electrostatic interactions between side chains within a small contact surface. From a theoretical calculation based on the complex structure, we identified an electron transfer pathway from the heme c of Cyt.c to CuA of CcO *via* Lys-13 of Cyt.c. Between the two proteins are three water layers, one of which lies between the other two layers without significant direct interaction with either protein. The inter-molecular span between Cyt.c and CcO is longer than those of other complexes by more than 3.0 Å, and the contact surface area of Cyt.c and CcO is smaller than one-third the size of those of other complexes. Cyt.c undergoes large structural fluctuations, using the interacting regions with CcO as a fulcrum. These features of the protein–protein interaction at the docking interface represent the first known example of a new class of inter-protein interaction, which we term "soft and specific". This interaction is predicted to contribute to the rapid association/dissociation of the Cyt.c–CcO complex, which facilitates the sequential supply of four electrons for the O2 reduction reaction.

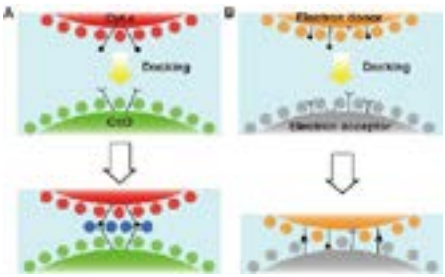

Figure1: *A In the Cyt.c–CcO complex system, water molecules on the surfaces of each protein are preserved to form three layers upon docking, but each protein specifically interacts via the long arms of side chains. B In other ET complex systems, electron donor and acceptor proteins form an ET complex by excluding water molecules on the surface of each protein.*

Biography

Kyoko Shinzawa-Itoh, Associate Professor of University of Hyogo, grew up in Hiroshima Japan. She received her MS degree from Hiroshima University of Graduate School of Integrated Arts and Sciences and her Ph D. in Pharmaceutical Sciences from Hiroshima University of Graduate School of Biomedical & Health Sciences. She worked as assistant professor at the Department of Life Science, Himeji Institute of Technology 1988-2004 and at the Hyogo University of Graduate School of Life Science 2004-2013. She is associate Professor of Picobiology Institute, Graduate School of Life Science University of Hyogo from 2013. She has studied about mitochondrial respiratory complexes.

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Jeffrey L Urbauer, J Proteomics Bioinform 2017, 10:8(Suppl)
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Oxidative stress, methionine oxidation, and calmodulin structure and function

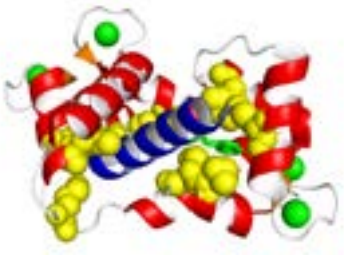
Jeffrey L Urbauer
The University of Georgia, USA

Statement of the Problem: Oxidation of methionine residues in proteins to methionine sulfoxide is a prevalent, reversible post-translational modification. Changes in protein structure and function accompany oxidation due to polarity and steric differences between methionine and the sulfoxide. We are investigating the consequences of methionine oxidation in the regulatory protein calmodulin (CaM), a key calcium signal transducer with nine methionine residues, in hydrophobic pockets of its opposing globular domains, which interact with target proteins. CaM with oxidized methionine residues accumulates under conditions of oxidative stress, and because of its central role in biology, it is important to understand the functional effects of these alterations and their physical origins.

Methodology: Methionine residues in CaM are easily oxidized *in vitro* with hydrogen peroxide. To study the effects of oxidation of specific methionine residues, leucine was substituted for methionine at remaining sites. A combination of functional assays, single molecule studies, and NMR spectroscopy were used to assess functional and structural consequences of methionine oxidation.

Findings: For the best studied case, activation of the plasma membrane Ca-ATPase (PMCA) by CaM, impaired CaM function is due to oxidation of a single C-terminal methionine. Single molecule experiments indicate non-productive binding of oxidized CaM to the PMCA. High resolution NMR studies demonstrate significant structural perturbation in the C-terminal globular domain of oxidized CaM and an inability to anchor the PMCA to this domain.

Conclusion & Significance: The functional effects of methionine oxidation in CaM are highly target dependent, as is the degree to which selective oxidation of particular methionine residues in CaM affects function. The results of CaM activation of the PMCA also indicate that both high-affinity productive and non-productive complexes of oxidized CaM with targets are possible. These facts indicate that a comprehensive understanding of the metabolic consequences of CaM oxidation will be challenging.



Biography

Jeffrey L Urbauer earned Bachelor's and Doctoral degrees in Chemistry from the University of Nebraska-Lincoln. He pursued Postdoctoral studies at the University of Wisconsin-Madison as an NIH Postdoctoral Fellow and at the University of Illinois Urbana-Champaign. He held faculty appointments at the State University of New York at Buffalo, the University of Pennsylvania, and the University of Kansas before joining the faculty in the Department of Chemistry and the Department of Biochemistry and Molecular Biology at the University of Georgia. At the University of Kansas the Mortar Board National College Senior Honor Society awarded him with the Outstanding Educator Award. His research interests include structural biology, protein biophysics and NMR spectroscopy.

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Miki Senda et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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Molecular mechanism of SHP2 activation by CagA from *Helicobacter pylori*

Miki Senda[1], **Takeru Hayashi**[2], **Nobuhiro Suzuki**[1], **Lisa Nagase**[1], **Masanori Hatakeyama**[2] and **Toshiya Senda**[1]
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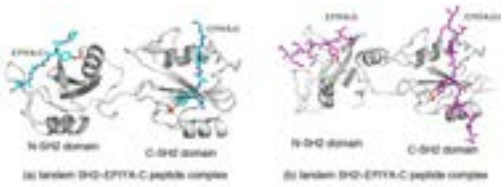
[2]The University of Tokyo, Japan

Helicobacter pylori which is known as a major risk factor of stomach cancer, delivers an effector protein CagA into gastric epithelial cells. CagA then promiscuously interact with host proteins, SHP2 and PAR1b, to deregulate these proteins, potentiating oncogenic signaling. CagA comprises an N-terminal structured region and a C-terminal intrinsically disordered region which interacts with the host proteins. We already determined the crystal structure of the N-terminal region of CagA (1–3). The crystal structure revealed that the basic amino-acid cluster in the N-terminal region is utilized to localized CagA at the inner face of the plasma membrane. After localization, short segments including Glu-Pro-Ile-Tyr-Ala (EPIYA) motif in the C-terminal disordered region are phosphorylated by Src and interact with SHP2 to deregulate its phosphatase activity. In this study, we have analyzed the structure-function relationship of the EPIYA-segments of CagA. Based on the sequence flanking each of the EPIYA motifs, four types of EPIYA segments, A, B, C, and D, have been identified (4). It is already known that combinations of the EPIYA segments are geographically different (Western and East Asian CagA) and affect CagA's oncogenic activity. While Western CagA with EPIYA-A, B, and C segments has weak oncogenic activity, Western CagA with EPIYA-A, B and multiple EPIYA-C segments shows increased oncogenic activity (5). East Asian CagA, which has much higher oncogenic activity than Western ones, typically possesses EPIYA-A, B, and D in the C-terminal region. Our biochemical data revealed that oncogenic activity of CagA is correlated with binding affinity for SH2 domain of SHP2 (SH2_SHP2). We have analyzed the interaction between SH2_SHP2 and the EPIYA-C/D segment using biochemical, crystallographic, and physicochemical methods and revealed two types of activation mechanisms of SHP2. In our presentation, we will report that East Asian and Western CagA utilize two distinct activation mechanisms of SHP2.



Biography

Miki Senda has completed her PhD at 2008 from Nagaoka University of Technology. She is an Assistant Professor of Structural Biology Research Center in High Energy Accelerator Research Organization (KEK). She has several collaborations, in which she has worked as an expert of protein crystallization and crystal quality improvement. She received Oxford Cryosystems Low Temperature Prize at the 63rd Annual Meeting of the American Crystallographic Association (ACA) in 2013.

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Alfredo De Biasio, J Proteomics Bioinform 2017, 10:8(Suppl)
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Integrative approaches to study the structure and motions of DNA sliding clamps

Alfredo De Biasio
Elettra-Sincrotrone Trieste, Italy

Sliding clamps encircle DNA and tether polymerases and other proteins to the genomic template, and are essential factors in DNA replication. Because of the transient interaction that the clamps establish with DNA, the clamp-DNA interface eluded a thorough structural characterization, so that the molecular mechanism for clamp sliding on DNA remained obscure. Here, I will show how the combined use of high-resolution techniques (X-ray crystallography and NMR) and molecular dynamics (MD) simulations allowed to visualize the interactions between the Proliferating Cell Nuclear Antigen (PCNA) – the eukaryotic sliding clamp – and DNA, and to decipher the mechanics of sliding. In addition, recent findings show that the DNA sliding surface of PCNA can be modified to regulate the resistance to DNA damage. From a structural viewpoint, I will reflect on these findings which open a new perspective on PCNA function and offer opportunities to develop tools to manipulate the DNA damage response in cancer treatment.

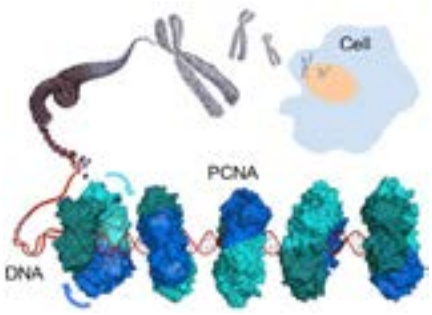

Figure1: *PCNA is a ring-shaped trimeric protein that encircles DNA and binds the polymerases during DNA replication and repair. The integrative use of structural and computational methods allowed to describe the sliding mechanism of PCNA, a spiral motion that keeps the orientation of PCNA relative to DNA invariant.*

Biography

Alfredo De Biasio has work focus on the structure and function of DNA sliding clamps and their complexes operating in DNA replication and repair. He is particularly interested in understanding the mechanisms of sliding of the eukaryotic clamp PCNA, and how these mechanisms are modulated by modifications of the PCNA sliding surface, and the implications in DNA damage avoidance. These problems are tackled by an integrative approach that combines X-ray crystallography, NMR and MD simulations.

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Liliane Mouawad, J Proteomics Bioinform 2017, 10:8(Suppl)
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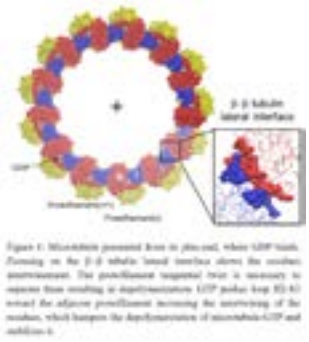
An atomistic view of microtubule stabilization by GTP

Liliane Mouawad
Institut Curie, France

A microtubule is a dynamic system formed of αβ-tubulins. The presence of nonhydrolyzable guanosine-5'-triphosphate (GTP)/guanosine diphosphate (GDP) on the β-tubulins provokes microtubule polymerization/depolymerization. Despite the large number of experimental studies of this dynamical process, its mechanism is still unclear. To provide insights into this mechanism, we studied the first depolymerization steps of GDP/GTP-bound microtubules by normal-mode analysis with the all-atom model. We also constructed a depolymerizing microtubule and compared it to cryo-electron microscopy tomograms (cyro-ET). The results show that during depolymerization, the protofilaments not only curve but twist to weaken their lateral interactions. These interactions are stabilized by GTP, but not evenly. Not all of the interface residues are of equal importance: five of them, belonging to the H2-S3 loop, play a special role; acting as a lock whose key is the γ-phosphate of GTP. Sequence alignments of several tubulins confirm the importance of these residues.



Biography

Liliane Mouawad was always interested in understanding the mechanism of action of proteins or protein assemblies. This understanding may be based on either molecular simulations or on experiments like NMR. But her expertise is primarily in molecular dynamics simulations and more precisely in normal mode analysis (NMA). She has developed several methods going from the calculation of normal modes of very large systems or of images, to the calculation of the pathway between two protein conformations, to the prediction of the compactness of a calcium-binding protein. Recently she was also involved in docking and virtual screening themes, where she has acquired enough expertise to develop a new consensus methodology to overcome some issues observed in these approaches.

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Hyun-Soo Cho, J Proteomics Bioinform 2017, 10:8(Suppl)
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Structure and function of a chloride pump rhodopsin from marine bacteria

Hyun-Soo Cho
Yonsei University, South Korea

Recently, light-driven sodium pump rhodopsin (NaR/KR2/NDQ rhodopsin) and chloride pump rhodopsin (ClR/NTQ rhodopsin) from marine flavobacteria were identified by metagenomics study. One of them, light-driven sodium pump rhodopsin (NaR) structure was determined. The other one we have solved the first crystal structure of a unique class light-driven chloride pump (ClR) from Nonlabens marinus S1-08, at resolutions of 1.57 Å. Like structured Halorhodopsin (HR), ClR can transfer chloride ion from extracellular to cytosol. Although both ClR and HR are same light-driven chloride pump rhodopsin, we found some evidences that ClR and HR are different in structure and mechanism. The structures reveal two chloride-binding sites, one around the protonated Schiff base and the other on a cytoplasmic loop. We identify a "3 omega motif" formed by three non-consecutive aromatic amino acids that is correlated with the B-C loop orientation. Detailed CIR structural analyses with functional studies in E. coli reveal the chloride ion transduction pathway. Our results help understand the molecular mechanism and physiological role of ClR and provide a structural basis for optogenetic applications.

Figure 1: *Chloride ion conductance pathway in ClR*

Biography

Hyun-Soo Cho has research interest in understanding the structural and functional role of various proteins involved in cancer and immune diseases. He is specialized in X-ray crystallography to solve protein structures with other biophysical and biochemical techniques including Cryo_EM recently. His ongoing research projects include various enzymes and receptors especially G-Protein Coupled Receptor (GPCR) related with cancer and immune system.

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Construction of structural mimetics of the thyrotropin receptor intracellular domain

Stanislav Engel
Ben-Gurion University, Israel

Background: Dissecting G protein-coupled receptors (GPCR) signaling in terms of the pathways being activated will boost our understanding of the molecular fundamentals of hormone action. The structural determinants governing the selectivity of GPCR/G protein coupling, however, remain obscure. The selectivity of GPCR/G protein recognition appears to be determined by both specific inter-residue interactions and features related to the overall 3D conformation of the ICD. It appears, therefore, that to elucidate the fundamentals of the selectivity of GPCR/G protein recognition, a comprehensive analysis of the structure-activity relationships of multiple GPCR complexes with different G protein isoforms is required. However, enormous technical difficulties associated with the isolation of functional receptors in quantities required for direct structural studies effectively impede progress in the field.

Methodology: We constructed the functional mimetics of the intracellular domain (ICD) of a model GPCR - thyrotropin receptor (TSHR), based on a unique scaffold, 6-Helix, an artificial protein that was derived from the elements of the trimer-of-hairpins structure of HIV gp41 and represents a bundle of six α-helices.

Findings: The 6-Helix scaffold, which endowed the substituted TSHR ICD elements with spatial constraints analogous to those, found in native receptors, enabled the reconstitution of a microdomain comprising the intracellular loops ICL-2 and ICL-3, which is capable of binding and activating Gα-(s).

Conclusion & Significance: By using a soluble scaffold, which furnishes peptides derived from the GPCR ICD with spatial constraints similar to those, found in native receptors, the reconstitution of a native-like G protein-recognition epitope can be facilitated. The 6-Helix-based mimetics could be used as a platform to study the molecular basis of GPCR/G protein recognition. Such knowledge could lead to the development of novel therapeutic strategies for GPCR-related disorders by targeting the GPCR/G protein interfaces and help counteract cellular dysfunctions *via* focused tuning of GPCR signaling.

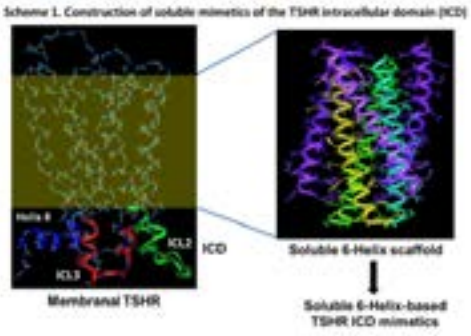

Figure1: *The three conformational states of the KpCitS dimer. (a) Schematic representation of the KpCitS protomer. Two helical hairpins of the transport domain are highlighted in purple. (b) The homodimeric KpCitS structure in different functional states viewed from the membrane plane (top) and from the periplasm (bottom). Citrate is shown as an orange ball-and-stick model. The black oval is a pseudo 2-fold axis, perpendicular to the membrane.*

Biography

Stanislav Engel PhD, is an Assistant Professor in the Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, The National Institute for Biotechnology, Ben-Gurion University in the Negev, Beer-Sheva, Israel. He got his BSc in Biochemistry, MSc and PhD in Biochemistry and Biotechnology Engineering at the Ben-Gurion University in the Negev. Currently, his researches focus on understanding the structural basis of "protein misfolding" diseases, such as ALS, and structure-based drug discovery.

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Mi Sun Jin et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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Structural basis of sodium/citrate symporter as a secondary transporter

Mi Sun Jin[1], **Ji Won Kim**[2], **Subin Kim**[1], **Haerim Lee**[2], **Songwon Kim**[1] and **Jie-Oh Lee**[2]
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The sodium-dependent citrate transporter of *Klebsiella pneumoniae* (*Kp*CitS) belongs to the 2-hydroxycarboxylate transporter (2-HCT) family and allows the cell to use citrate as sole carbon and energy source in anaerobic conditions. We present crystal structures of *Kp*CitS in its citrate-bound outward-facing as well as citrate-free inward-facing state. The structure of the asymmetric *Kp*CitS homodimer containing both outward- and inward-open protomers was also determined. The structures reveal that the *Kp*CitS dimerization domain remains stationary throughout the transport cycle due to an extensive hydrogen bond network as well as hydrophobic interactions. In contrast, its transport domain undergoes a ~35° rigid-body rotation and a ~17 Å translocation perpendicular to the membrane to expose the substrate-binding site alternately to either side of the membrane. Homology models of two other 2-HCT proteins based on the *Kp*CitS structure offer structural insights into their differences in substrate specificity at a molecular level. On the basis of our results and previous biochemical data, we propose that the activity of the 2-HCT family of transporters involves an elevator-like movement in which the transport domain itself traverses the lipid bilayer, carrying the substrate into the cell in a sodium-dependent manner.

Biography

Mi Sun Jin is an Assistant Professor in School of Life Sciences, GIST since 2014. She completed BS in 2002 from Sogang University, MS in 2004 from KAIST, and PhD in 2008 from KAIST (under the supervision of Jie-Oh Lee). She held two Postdoctoral Fellowships; one from 2008-2009 at KAIST (Advisor: Jie-Oh Lee), and other from 2009-2013 from Purdue University (Advisor: Jue Chen). From 2013 to 2014, he worked as Research Specialist in Purdue University.

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Notes:

Sessions:

Frontiers in Structural Biology

Session Chair
Petra Fromme
Arizona State University, USA

Session Co-Chair
Maria Bykhovskaia
Wayne State University, USA

Session Introduction

Title: **Dynamics of biomolecules "In Action" studied with X-ray free electron lasers**
Petra Fromme, Arizona State University, USA

Title: **Protein machinery regulating the synaptic vesicle fusion**
Maria Bykhovskaia, Wayne State University, USA

Title: **Designer Biologics: BMP Chimeras and their clinical potential**
Senyon Teddy Choe, Mogam Institute, Korea and University of California San Diego, USA

Title: **Structural mechanism of partial agonists and antagonists of PPARgamma for use as antidiabetics**
John B Bruning, The University of Adelaide, Australia

Title: **How conformational dynamics descriptors may help in remodeling of allosteric regulation in proteins**
Luba Tchertanov, CMLA ENS, France

Title: **Study on the conformational transition between the alternative and collapsed form of prethrombin-2: Targeted molecular dynamics and free energy sampling**
Sangwook Wu, Pukyong National University, South Korea

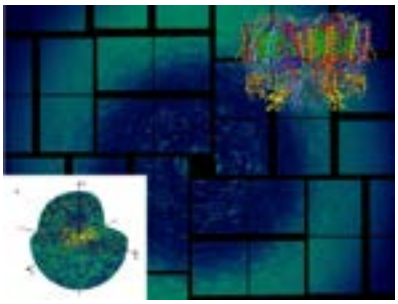
Title: **Structural and molecular dynamics analysis of the super secondary motifs from TIM barrel proteins: Implications for folding and engineering of foldable building blocks for the assembly of TIMs**
Ramakrishna Vadrevu, Birla Institute of Technology and Science, India

Petra Fromme, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Dynamics of biomolecules "In Action" studied with X-ray free electron lasers

Petra Fromme
Arizona State University, USA

Biomolecules are highly dynamic; however, most structures only provide a static picture of the molecule. Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction "snapshots" are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from high energy X-ray free-electron lasers (XFELs), where diffraction is observed before destruction takes effect. The first proof of concept of serial femtosecond crystallography was achieved using Photosystem I, a larger membrane protein complex involved in Photosynthesis as a model system. The structure of non-damaged biomolecules can now be determined, unraveling their function at the atomic scale that include important human membrane-bound receptors. SFX opens a new avenue for determination of protein dynamics, with the goal of molecular movies of biomolecules "in action". First experiments on the proof of principle for time resolved serial femtosecond nanocrystallography have been performed on proteins in Photosynthesis, where first snapshots of steps in water splitting reaction have been observed. A new concept based on continuous X-ray diffraction extends resolution beyond Bragg diffraction and allows for direct phasing of X-ray diffraction data. TR-SFX studies extend to atomic resolution where the first steps in photosensing were recently revealed at a time scale of femtoseconds using the photoactive yellow protein. This pioneering work paves the way for the determination of molecular movies of the dynamics of membrane proteins "at work" in the future. The talk will close with a progress report on the development of compact femto and attosecond X-ray Sources at DESY (AXSIS) and at ASU (CXLS and CXFEL) , which will provide unique new opportunities to study the ultrafast dynamics of reactions in photosynthesis with a combination of X-ray diffraction, X-ray spectroscopy and ultrafast optical spectroscopy.



Biography

Petra Fromme received her masters at the Free University in Berlin in Biochemistry (1985) and then received her doctorate in Chemistry at the Technical University in Berlin (1988) where she then became a professor in 1992. During this time she developed and pursued her fascination with understanding the function of membrane proteins by investigating and determining their atomic structures. In 2002, Dr. Fromme joined Arizona State University as a Professor of Chemistry and Biochemsitry where she has worked with distinguished colleagues from around the world to pioneer a new technique for imaging proteins using extraordinarily powerful x-ray lasers that has the capability to make movies of these fascinating proteins in action.

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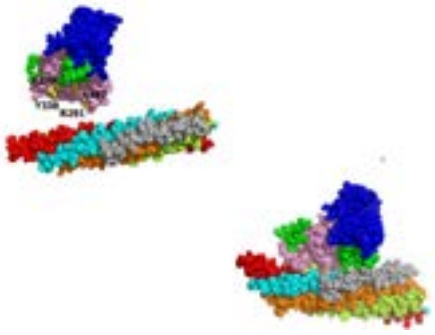
Notes:

Maria Bykhovskaia, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Protein machinery regulating the synaptic vesicle fusion

Maria Bykhovskaia
Wayne State University, USA

Neuronal transmitters are released *via* the fusion of synaptic vesicles with the plasma membrane. Vesicles dock to the membrane *via* a protein complex termed SNARE, which contains membrane attached (t-SNARE) and vesicle attached (v-SNARE) proteins. The fusion occurs in response to a calcium inflow, and the vesicle protein Synaptotagmin (Syt) serves as a calcium sensor. A cytosolic protein Complexin (Cpx) interacts with the SNARE complex, restricting the spontaneous fusion. Although molecular interactions of these proteins have been extensively studied, it is still debated how Syt dynamically interacts with the SNARE protein complex, Cpx, and lipid bilayers to trigger lipid merging. To elucidate these mechanisms, we combined molecular dynamics (MD) simulations with molecular biology and genetic approaches in *Drosophila*. Basing on MD simulations, we created a model of the protein fusion machinery wherein Cpx dynamically interacts with v-SNARE, preventing full SNARE assembly. Our MD simulations also elucidated how Syt interacts with lipid bilayers, causing lipid bulging that may precede the formation of the stalk and the fusion pore opening. Finally, our simulations predicted direct interactions of Syt with the SNARE-bound Cpx. The developed molecular model enabled us to predict new mutations in v-SNARE and Cpx that alter the fusion process. To test these predictions, we generated *Drosophila* lines with single point mutations and investigated how these mutations affect the kinetics of transmitter release. The results of these experiments suggest that our model creates the basis for systematic approach to manipulating the fusion machinery based on theoretical predictions derived from MD simulations.



Biography

Maria Bykhovskaia is an expert in synaptic transmission. Her lab combines molecular modeling and computations with electrophysiology, microscopy, and molecular biology approaches. She holds a Professor's position in the Washington State University. Her PhD training was in protein molecular modeling, and subsequently she used a Postdoc in Computational Neuroscience to initiate a career devoted to the study of presynaptic mechanisms and plasticity. As a PI, she has developed in her lab expertise in electrophysiology, live confocal imaging, and electron microscopy. The lab combines these experimental approaches with mathematical modeling to understand the fundamental mechanisms of release of neuronal transmitters.

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Notes:

Senyon Teddy Choe, J Proteomics Bioinform 2017, 10:8(Suppl)
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Designer Biologics: BMP Chimeras and their clinical potential

Senyon Teddy Choe
[1]Mogam Institute, Korea
[2]University of California San Diego, USA

Discovery of new biologics presents new challenges and opportunities and revenues for biologics are rapidly growing in biopharmaceutical industry. We exploited our detailed structural knowledge of three-dimensional structures of BMPs, their receptors, and their antagonists to engineer synthetic BMP ligands. By use of a novel protein engineering strategy that we termed RASCH (Random Assembly of Segmental Chimera and Heteromers), we have set out to design a synthetic biologic (synbiologics®) for bone and cartilage therapy. In the case of bone fusion, we have used Activin and BMP-2, which are the members of TGF-beta superfamily, to create AB204 (Allendorph et al., 2011). AB204 is a synthetic 50:50 chimera of the two ligands, which indeed shows highly effective bone-forming capability. In the case of cartilage, we have used Activin and BMP-6 to create AB604, again a 50:50 chimera of the two. AB604 shows all the properties of super BMP6, surpassing the functional characteristics of natural BMP6. This approach promises to be a very powerful way to harness different biological functionalities of natural ligands to merge into one synthetic designer molecule such that it goes beyond what Mother Nature could provide a new means to meet various unmet clinical needs.

Biography

Senyon Teddy Choe, is a Professor of Biology and the Founding Director of Drug Discovery Collaboratory at UCSD. He pursued PhD in Biophysics and Medical Physics at Univ. California, Berkeley. He joined the Salk Institute in 1993 as the founding Faculty Member of the Institute's new Structural Biology Laboratory, and remained so through 2015. His research group has focused on understanding how cells talk to each other. An extension of these works explores designing synthetic biologics to modulate stem cells and sick cells directly. His major honors include election in 1999 to the Fellow of American Association for the Advancement of Science. He recently founded joint Center for Biosciences to translate discovery to medical applications to focus on protein engineering and developing new stem cell therapy. He currently leads Mogam Institute for Biomedical Research in Korea aiming for biologics discovery in the areas of infectious diseases and cancer.

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Notes:

John B Bruning et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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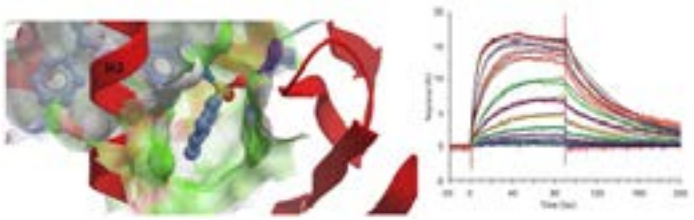
Structural mechanism of partial agonists and antagonists of PPARgamma for use as antidiabetics

John B Bruning[1], **Ted Kamenecka**[2] and **Pat Griffin**[2]
[1]The University of Adelaide, Australia
[2]The Scripps Research Institute, USA

Synthetic full agonists of Peroxisome proliferator-activated receptor gamma (PPARγ) have been prescribed for the treatment of diabetes due to their ability to regulate glucose homeostasis and insulin sensitization. While the use of full agonists of PPARγ has been hampered due to severe side effects, partial agonists and antagonists have shown promise due to their decreased incidence of such side effects in preclinical models. No kinetic information has been forthcoming in regard to the mechanism of full versus partial agonism of PPARγ to date and little structural and dynamic information is available which can shed light on the mechanistic difference between full and partial agonists as well as antagonists. We have used X-ray crystallography, cellular assays, Hydrogen Deuterium Exchange (HDX), and Surface Plasmon Resonance (SPR) to probe the mechanism of several PPARγ partial agonists and antagonists. Our findings demonstrate that not only do partial agonists and antagonists act through distinct transcriptional mechanisms, they also demonstrate differences in structure, dynamics, and kinetics as compared to full agonists.



Biography

John B Bruning completed BSc from Texas A&M University in 1997. He began crystallography in the Laboratory of Yousif Shamoo at Rice University. He worked on the structural mechanism of the human sliding clamp and its interactions with DNA replication proteins. He received PhD in 2005 and completed 2 successful Post-docs. The first was at the Scripps Research Institute from 2005-2007 working on structural studies of nuclear receptors including PPAR, RXR, ER, and TR; second Post-Doc was with Jim Sacchettini in the Houston Medical centre. He was a part of the TB structural genomics consortium. He received his first faculty position at the University of Adelaide in 2012 as a Lecturer. He was tenured in 2015 and promoted to Senior Lecturer in 2016. He was also appointed Adjunct Professor of the Scripps Research Institute in 2016.

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Notes:

Luba Tchertanov et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

How conformational dynamics descriptors may help in remodeling of allosteric regulation in proteins

Luba Tchertanov
CMLA - ENS Cachan, France

Allostery controls nearly all biological processes, and it has been declared by Monod to be "the second secrete of life" after the genome. This universal phenomenon in nature represents a target response on a perturbation (e.g. a ligand binding) leading to a functional change at the target through alteration of the structure or dynamics. Such an event can be described in terms of a large-scale transmission of information between residues. This concept is the cornerstone of our method MONETA that delivers descriptor encoding of the communication network in a protein. Using MONETA, we described the allosteric regulation of several proteins involved in cell signalling. Studying the receptors tyrosine kinases (RTKs), KIT and CSF-1R, and their numerous clinically-relevant mutants, we showed that the allosteric communications between the major regulating fragments in the native proteins were disrupted by the gain-of-function mutations. The diverging impact of equivalent mutations on communication in homologue RTKs permits us to distinguish between the mutation-induced effects that lead to the constitutive activation of KIT and the mutation-induced effects promoted the resistance in CSF-1R. In STAT5s, RTK downstream signalling proteins, we showed the sequence-dependent asymmetry in the STAT5s' communications and their different responses to phosphorylation. Our recent study provided a fascinating illustration of how the binding of agonist ligands controls intrinsic conformational dynamics in human NMDA receptors that stabilize the channel opening. The allosteric binding sites, which were identified by a pocket search at the proteins surface adjacent to the communication pathway, may constitute valid targets for the development of inhibitors able to modulate the function-related communication properties of a protein. Such communication-inspired and communication-targeted modulation may selectively block several activation or post-transduction processes. Our work opens the way to novel and rational strategies for the definition of targets, and the development of efficient target-specific inhibitors.

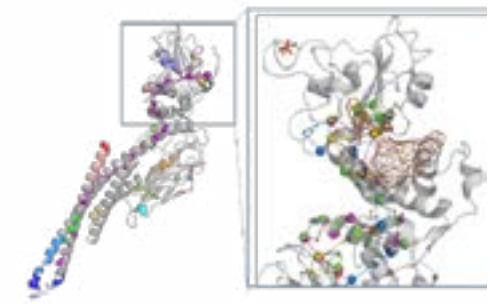

Figure1: *Communication pathway in STAT5 (left) and location of pockets at the protein surface adjacent to the communication pathway (right).*

Biography

Luba Tchertanov is a Research Director at CNRS-France, leader of the Bioinformatics, Molecular Dynamics and Modeling (BiMoDyM) team in Centre Mathématiques et leurs Applications (CMLA-CNRS) at the Ecole Normale Supérieure (ENS) de Cachan. She has multidisciplinary high-level skills, with extensive experience in structural biology, molecular modelling and numerical simulation (more than 100 papers in peer-reviewed journals). She coordinated or contributed as team-leader to different research projects (CEE, ANR, Fondation de France, OSEO, SIDACTION) and industrial contracts (LIPHATECH, the SERVIER Institute, the Pierre FABRE Laboratory, UNILIVER). The research topics are focused on exploration of protein structure–dynamics–function relations. In particular, she is working at the mechanisms of the receptors activation, the mechanisms of resistance to inhibitors, the conformational plasticity and dynamics of inter-molecular interactions and molecular recognition. She is specifically interested in description of allosteric regulation at an atomistic level. Important part of research is dedicated to the development of new methodology and computing tools for description of proteins dynamics.

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Notes:

Sangwook Wu et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Study on the conformational transition between the alternative and collapsed form of prethrombin-2: Targeted molecular dynamics and free energy sampling

Sangwook Wu[1], Hunjoo Myung[2]
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[2]Korea Institute of Science and Technology Information, South Korea

The alternative and collapsed forms of prethrombin-2 are revealed by X-ray crystallogrphy. We analyzed the conformational transition from the alternative to the collapsed form employing targeted molecular dynamics simulation and 2-dimensional free energy landscape using WHAM method. Some hydrophobic residues (W60d, W148, W215, and F227) show a significant difference between the two conformations in the conformational transition process. We show that the four hydrophobic residues undergo concerted movement from dimer to trimer transition *via* tetramer state in the conformational change from the alternative to the collapsed form. Also, we reveal that the concerted movement of the four hydrophobic residues is controlled by movement of specific loop regions behind. In this study, we discuss the difference between the transition path generated by the targeted Mplease let m eD simulation and the transition path with minimum Boltzmann weighting on the two-dimensional free energy surface (FES).

Biography

Sangwook Wu received his B.A. degree in Biochemistry from Yonsei University (Korea) in 1990. After working as a Scientist at Samsung Display Devices (1995-1999), he obtained his Ph.D. in theoretical condensed matter physics from Iowa State University (1999-2005). He joined the computational biophysics lab at UNC-Chapel Hill (Dr. Lee Pedersen) as postdoctoral research associate (2005-2014). From 2014 to the present, he has been a faculty member at Pukyong National University in Korea. His research interests are in the areas of computational dynamics of biological macromolecules.

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Notes:

Ramakrishna Vadrevu et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

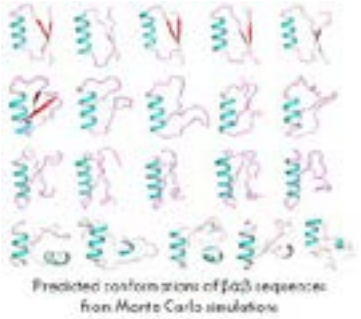
Structural and molecular dynamics analysis of the super secondary motifs from TIM barrel proteins: Implications for folding and engineering of foldable building blocks for the assembly of TIMS

Ramakrishna Vadrevu
Birla Institute of Technology and Science, India

Statement of the Problem: The acquired complex three-dimensional structure of proteins is a culmination of simple structural fragments like α—α, β—β, α—β and β—α units. Thus, tertiary structures can be seen as a combination of basic building block motifs implying that all complex protein structures have evolved from the assembly of small independently folding super secondary structures. The TIM barrel proteins are made up of a regular repeating βαβ motif resulting in the strands and helices in an alternating repetitive pattern. Experimental and theoretical studies have revealed that βαβ unit acts as a minimal unit of stability. The success of designing super secondary motifs that fold in isolation underscores the prospects of designing and or identification of independently folding motifs from the existing protein structures. However, intriguingly, naturally occurring βαβ sequences from proteins that fold independently have not been identified. In our attempts, we addressed the finding of 'needles in hay stick' scenario by an exhaustive sequence and structural space search of the βαβ units from the TIM barrels.

Methodology & Theoretical Orientation: The search approach implemented in this work considered features such as alpha helical propensity, loop length, loop dynamics, residue preferences in loops, long range side chain main chain interactions etc., to shortlist βαβ units with strong propensity to fold in isolation. The prospective βαβ candidates thus shortlisted from the TIM barrels have been further subjected to structure forming tendency employing a combination of Monte Carlo and Molecular dynamics simulations to assess their foldability and stability.

Conclusion & Significance: The prediction of some independently folding βαβ candidates from TIMs are enabling us to experimentally asses their folding and stability. The identification and analysis of independently folding βαβ units that exist naturally will not only provide substantial information on nature's design strategies and evolution of protein conformations but also help to design/engineer novel proteins.



Predicted conformations of βαβ sequences from Monte Carlo simulations

Biography

Ramakrishna Vadrevu received his Master's degree in Physical Chemistry and his PhD degree in Biophysical Chemistry. He spent few years at the Pennsylvania State University and later at University of Massachusetts Medical School as a Postdoctoral Fellow with Prof. C. Robert Mathews. Since 2008 he has been a faculty at the Birla Institute of Technology and Science-Pilani, Hyderabad Campus in the department of Biological Sciences. His research focuses on understanding the role cellular environment on protein stability and folding. His research interests include: protein design and engineering, amyloid material and its applications.

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Notes:

9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

Workshop

Day 2

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Tobias Pfluger, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

Tobias Pfluger

NanoTemper Technologies GmbH, Germany

Solutions for studying protein complexes in structural biology and drug development

Key to understanding the functional role of target proteins is elucidating the structure-function relationship between two proteins or protein complexes. Current analysis is often complex, time-consuming, and lacks data quality. One of the goals of structural biology is to bring together multiple disciplines and methodologies to better characterize proteins and protein complexes. For highly utilized techniques such as cryo-EM, X-ray crystallography and NMR, having the ability to identify and quantify binding affinities as well as analyze conformational and colloidal properties, enables researchers to gain a better understanding of the functional properties of protein targets. During this workshop, you will learn about practical solutions to monitor and optimize protein stability and quality. An overview of the various analytical and biophysical methods commonly used by structural biologist will be discussed. We will share case studies demonstrating the benefits of understanding conformational and colloidal properties of protein complexes and how to use this information for further downstream analysis. Finally, we will share examples of protein-small molecule and protein-protein interactions and novel methodologies that assist researchers in making better decisions.

Biography

Tobias Pfluger studied Chemistry at the Albert Ludwigs University in Freiburg with an emphasis on biochemistry. He received his PhD in Structural Biology investigating the structure and function of membrane proteins involved in cellular signalling cascades. In late 2015, he joined NanoTemper Technologies as an Application Specialist.

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9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

Special Session on

Structural Biology of Biomembranes

John E Baenziger, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

John E Baenziger

University of Ottawa, Canada

Mechanisms underlying lipid-sensing by the nicotinic acetylcholine receptor in both normal and diseased states

The neuromuscular nicotinic acetylcholine receptor (nAChR) is the prototypic member of the pentameric ligand-gated ion channel (pLGIC) superfamily, a superfamily of neurotransmitter receptors that plays a central role in information processing in the brain. It is well documented that nAChR function is exquisitely sensitive to its lipid environment. Lipids influence function by both conformational selection and kinetic mechanisms – they stabilize different proportions of activatable versus non-activatable conformations, and influence the rates of transitions between the different states. In the absence of activing cholesterol and anionic lipids, the nAChR adopts a conformation where agonist binding is uncoupled from channel gating. Lipids likely influence the "coupling" of binding and gating *via* the lipid-exposed transmembrane α-helix, M4. M4 in the neuromuscular nAChR is also the site of both point and truncation mutations that alter expression and/or function leading to congenital myasthenic syndromes. In this seminar, I will focus on the mechanisms by which the peripheral M4 transmembrane α-helix modulates pLGIC function. The M4 C terminus extends beyond the bilayer to interact with key structures that link the agonist binding to the transmembrane gate – referred to here as the coupling interface. We hypothesized that interactions between M4 and the coupling interface are essential to pLGIC function. We show here that such interactions are essential to function in some pLGICs and do participate in lipid-sensing. In the neuromuscular nAChR, however, such interactions between M4 and the coupling interface are less important. Instead, M4 influences function *via* a cluster of polar residues located in the core of the transmembrane domain near the center of the lipid bilayer. Altered M4 structure leads to changes in the energetic coupling between these polar residues, with the changes coupling ultimately propagating to both the gating helix, M2, and the aforementioned coupling interface. Here, we map out the conformational pathway that leads from the lipid-exposed surface of M4 to the channel gate, and thus illustrate how M4 "allosterically" modulates channel function.

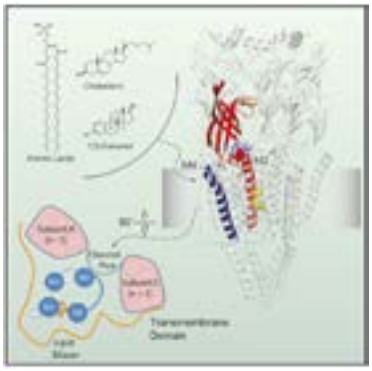

Figure1: *Some allosteric modulators, including lipids, act via the lipid-exposed M4 α-helix of the nAChR. We elucidate the allosteric pathway by which this peripheral structure influences channel gating.*

Biography

John Baenziger is a professor of Biochemistry at the University of Ottawa in Ottawa, Canada. His research is focused on understanding the mechanisms by which lipids influence nicotinic acetylcholine receptor structure and function in both normal and diseased states, with increasing focus on how lipid-nAChR interactions participate in congenital myasthenic syndromes. Dr. Baenziger has served on the editorial board of the Journal of Biological Chemistry. He is the President of the Biophysical Society of Canada and is Treasurer-elect of the International Union of Pure and Applied Biophysics.

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Carsten Mim, J Proteomics Bioinform 2017, 10:8(Suppl)
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9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

Carsten Mim

KTH Royal Institute of Technology, Sweden

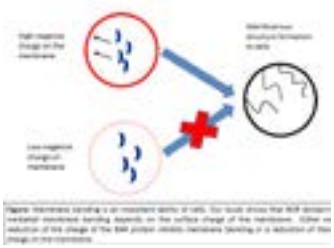
When structure leads to function: Protein complexes at the membrane in endocytosis

Statement of the Problem: The cell bends membranes to generate membrane structures, like the t-tubules in muscles. Bin1/Amphiphysin/Rvs domain proteins are part of the membrane bending machinery and are found in widespread phenomena like endocytosis or cell motility. BAR domain proteins can assemble spontaneously *in vitro* as well as *in vivo*. Which factors regulate the assembly, the membrane tension is a well-studied regulator. In contrast, the role of the membrane composition, as an initiator of membrane bending, is poorly understood.

Methodology & Theoretical Orientation: For this study, we collected electron micrographs to document the membrane bending activity of the BAR protein Bin1. We probed the electrostatic interactions between Bin1 and the membrane by changing the surface charge of the membrane, the ionic strength of the assay and using disease relevant mutants, where the positive charge (K35N) and the negative charge (D151N) are eliminated. The electrostatic interactions between Bin1 and artificial membranes were evaluated by liposome sedimentation. To test how the findings, translate into living cells, we assayed the phenotype of membrane bending-deficient Bin1 mutants in cells that have elevated or reduced levels of negatively charged lipids.

Findings: Our simple, artificial system could reproduce the complex membrane topology present in muscle cells. We focused on the two mutants. We found that in stringent conditions for membrane bending (high ionic strength, low membrane charge) the mutants showed disproportional lower bending activity. These finding were confirmed *in vivo*. We could rescue to mutant phenotype by increasing the membrane surface charge. Conversely, we induced a mutant phenotype in wt Bin1 by lowering the membrane surface charge.

Conclusion & Significance: We established the membrane charge as a novel regulator of membrane tubulation. We speculate that rapid phosphorylation and dephosphorylation of phosphoinositols can act as a switch for induction of membrane bending.



Biography

Carsten Mim has a longstanding interest in membrane and membrane-associated proteins throughout his career. As an experienced Electrophysiologist, he characterized the glutamate transporter EAAT3 and EAAT4. The kinetics of EAAT4 differ from other glutamate transporters, by a voltage sensitive step that slows the turnover rate at hyperpolarized membrane potentials. Further, recorded transient and steady state currents at different temperatures showed that the binding of glutamate is enthalpy-driven unlike the binding of Na+. To visualize membrane: protein complexes, he turned to electron microscopy. His work on the Bin/Amphyphysin/Rvs domain (BAR) protein endophilin in complex with the bilayer resulted in the unexpected discovery that the stability and dynamics of endophilin scaffolds entirely depend on non-specific interactions between amphipathic helices in the bilayer. His findings also provided a first structurally motivated hypothesis how BAR-scaffolds selectively recruit downstream interaction partners through a steric selection mechanism.

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Volodymyr M Korkhov, J Proteomics Bioinform 2017, 10:8(Suppl)
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Volodymyr M Korkhov

Paul Scherrer Institute, Switzerland

Role of the nucleotidyl cyclase helical domain in catalytically active dimer formation

Biography

Volodymyr Korkhov is an Assistant Professor at the Institute of Biochemistry and Paul Scherrer Institute (PSI, Villigen). Prof. Korkhov has been studying various aspects of membrane protein biology throughout his career. As a PhD student at the Institute of Pharmacology, Vienna Medical University, he studied oligomerization of neurotransmitter transporters. He continued research of multidrug and ABC transporters during his postdoctoral training periods at MRC Laboratory of Molecular Biology (Cambridge, UK) and ETH Zurich, respectively. His work on ABC transporter for vitamin B12 from Escherichia coli, BtuCDF, led to a proposal of a complete structure-based mechanism of type II ABC importers. From April 2014, Prof. Korkhov has been leading an independent research group, supported by an SNF Professorship. The overarching topic of research in Prof. Korkhov's group is structure and molecular mechanisms of membrane protein complexes involved in signal transduction.

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Notes:

Qiu-Xing Jiang, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Qiu-Xing Jiang
University of Florida, USA

Structural basis for the lipid-dependent gating of a Kv channel

Human cell membranes are made of both phospholipids and nonphospholipids. The nonphospholipids, such as cholesterol, have no phosphate groups in their headgroup regions and are quite abundant in cell membranes. Mainly due to technical difficulties, quantitative study of possible effects of nonphospholipids on voltage-gated ion channels has been very challenging. Our prior studies have achieved three major developments: 1. a working hypothesis of lipid-dependent gating based on nonphospholipids stabilizing the voltage sensor domain of the KvAP channel in the resting (down) conformation, 2. a novel bead-supported unilamellar membrane system and a new method to stabilize the KvAP channel in the resting state and 3. chemically functionalized carbon films for cryoEM imaging of low abundance complexes by high-affinity selection or of small macromolecular complexes (100-200 kDa) by keeping vitrified ice thinner than usual. The general idea for lipid dependent gating is that the annular lipids around a Kv channel change their arrangements in accompany with the conformational changes of the voltage-sensor domains. Our technical development made it feasible to study the CHOL-dependent gating effects on Kv channels. We studied the CHOL-dependent gating effects on Kv channels in bSUMs. Because almost all known lipid metabolic defects result from dysregulated homeostasis of nonphospholipids, our studies in animal models carrying CHOL metabolic defects will provide the first test of lipid-dependent gating in an *in vivo* physiological setting. Secondly, we apply our ChemiC method to cryoEM study of the 120 kDa KvAP in both an inactivated and a peptide-stabilized down state. The peptides selected from the nonphospholipid-stabilized down state have been showed to recognize the voltage sensors in the right conformation and keep the channels in the right conformation. Our results will reveal the structural basis for the nonphospholipid-induced conformational changes in Kv channels, and unveil connections to the lipid-metabolic defects in humans.

Biography

Qiu-Xing Jiang obtained his PhD in 2002 from the Department of Cellular and Molecular Physiology at Yale University School of Medicine, where he started his work in cryo-electron microscopy in 1999 with Dr. Fred Sigworthis. He is currently heading the Laboratory of Molecular Biophysics and Cell Physiology in Department of Microbiology and Cell Science in the Institute of Food and Agricultural Sciences at University of Florida and he is serving part-time (20%) as the Faculty Director of Electron Microscopy at the Institute of Cross-disciplinary Biotechnology Research at UF. After a short Postdoctoral training at Yale, he finished his Postdoctoral training in structural biology with Dr. Roderick Mackinnon in 2007 before taking an Assistant Professorship position at UT Southwestern Medical Center at Dallas, Texas. He is the recipient of the NIH EUREKA award in 2009, the AHA National Innovative Award in 2012, and the Junior Faculty travel award from GRC Molecular and Cell Biology of Lipids in 2011.

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9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

Scientific Tracks & Abstracts

Day 2

Sessions:

Structural Biology in Complexity Arenas

Session Chair
Charles W. Carter Jr
University of North Carolina at Chapel Hill, USA

Session Co-Chair
Ulf Skoglund
Okinawa Institute of Science and Technology, Japan

Session Introduction

Title: **How does domain motion contribute to transition-state stabilization? Combinatorial thermodynamic cycle analysis of conformational coupling during tryptophan activation**
Charles W. Carter, Jr, University of North Carolina at Chapel Hill, USA

Title: **Structure of Human IgM in complex with the Malaria protein PfEMP1**
Ulf Skoglund, Okinawa Institute of Science and Technology, Japan

Title: **Is nucleoid complexity hence cell diameter limited by the eclipse?**
Arieh Zaritsky, Ben-Gurion University of the Negev, Israel

Title: **Inter-domain communication through intrinsically disordered region (IDR) revealed through the ensemble structure analysis**
Shin-ichi Tate, Hiroshima University, Japan

Title: **Solution NMR relaxation and µs molecular dynamics simulations of dynamic protein-protein and protein-membrane complexes**
Matthias Buck, Case Western Reserve University, USA

Title: **Functional protein conformation networks probed by NMR nanorulers**
Beat Vögeli, University of Colorado at Denver, USA

Title: **On two ways to predict the protein folding process over a chaotic model**
Christophe Guyeux, Université de Bourgogne Franche-Comté, France

Title: **Antibodies as research tools to find new chemical matter**
Marta Westwood, UCB Celltech, UK

Title: **Structural genomics of integral membrane proteins - past successes and future directions**
Brian Kloss, New York Structural Biology Center, USA

Title: **Geometrical principles of homeric β-barrels and α-helices: Applications to modelling amyloid protofilaments**
Steven Hayward, University of East Anglia, UK

Title: **Exploring conformational equilibria of a heterodimeric ABC transporter by electron paramagnetic resonance**
Enrica Bordignon, Ruhr-Universität Bochum, Germany

Title: **Microscopic calculation of conformational thermodynamics in bio-macromolecular complexes**
J. Chakrabarti, S. N. Bose National Centre for Basic Sciences, India

Charles W Carter, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

How does domain motion contribute to transition-state stabilization? Combinatorial thermodynamic cycle analysis of conformational coupling during tryptophan activation

Charles W Carter
University of North Carolina at Chapel Hill, USA

Enzyme mechanisms, especially those that couple NTP hydrolysis to mechanical work and information, use sophisticated dynamic networks to transduce active-site chemistry into domain motions that change binding affinities. We measured and cross-validated the energetics of such networks in B. *stearothermophilus* Tryptophanyl-tRNA synthetase (TrpRS) using both multi-mutant and modular thermodynamic cycles. Coordinated domain motions develop shear in a core packing motif conserved in >125 different protein superfamilies. Multi-dimensional combinatorial mutagenesis showed that four side chains from this "molecular switch" move coordinately with the active-site Mg2+ ion in the transition state for amino acid activation. A modular thermodynamic cycle consisting of full-length TrpRS, Urzyme, and Urzyme plus each of the two domains deleted in the Urzyme gives similar energetics. These complementary experiments establish that catalysis and specificity in full-length TrpRS are both coupled by 5 kcal/mole to: (i) the core packing region where domain movement generates shear, and (ii) the simultaneous motion of the two domains relative to the Urzyme. Theory shows that the minimum action path algorithm estimates thermodynamically meaningful contributions of domain movement to kinetic rates. Correlations between those parameters, the experimental rates, and structural variations induced in the combinatorial mutants confirm that these estimates are realistic. These results validate our previous conclusion that catalysis by Mg2+ ion is coupled to the overall domain motion. Computational free energy surfaces demonstrate that TrpRS catalytic domain motion itself is endergonic but is driven thermodynamically by PPi release. Comparison of the impact of combinatorial mutagenesis on pre-steady state and steady-state rates confirm that dynamic active-site pre-organization endows TrpRS with the elusive conditionality of NTP utilization on domain motion.

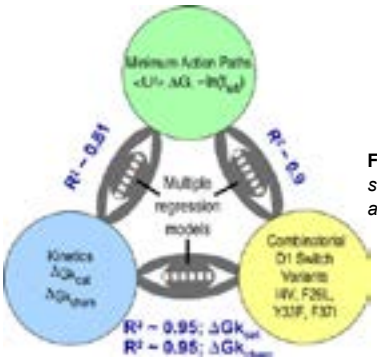

Figure1: *High Correlations between structures (yellow), steady-state kinetics (blue) and computed trajectories (green) for WT and 15 combinatorial variants of typtophanyl-tRNA synthetase.*

Biography

Charles W Carter is an X-ray Crystallographer who studies the origin, evolution, and structural biology of aminoacyl-tRNA synthetases. His research group introduced the use of urzyme-highly conserved structural cores that retain large fractions of the transition-state stabilization free energies of full length enzymes as experimental models of ancestral enzymes.

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Ulf Skoglund, J Proteomics Bioinform 2017, 10:8(Suppl)
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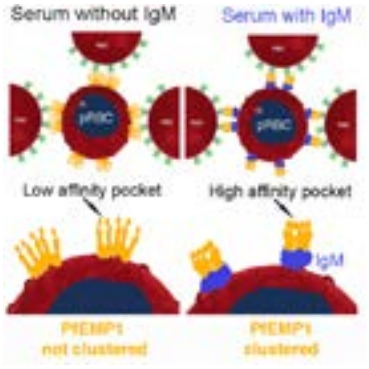
Structure of human IgM in complex with the malaria protein PfEMP1

Ulf Skoglund
Okinawa Institute of Science and Technology, Japan

Children under the age of 5 years have huge malaria burden in endemic area. Increased death in complicated malaria is due to increased sequestration to tissues and agglutination with erythrocytes and cells of our immune system. It is known that parasites that bind to non-immune IgM cause severe malaria due to increased rosetting (agglutination). Using biochemical, parasitology and electron tomography techniques we have identified that PfEMP1, a crescent shaped molecule interacts with human IgM through its bulky C-terminus (membrane proximal) in 1:1 and 2:1 ratio. While the bulky C terminus limits the stoichiometry of this interaction yet clusters parasite molecule PfEMP1 (*P. falciparum* Erythrocyte Membrane Protein-1) to mediate robust host parasite interaction. Structural analysis revealed that PfEMP1 could also preclude the activation of complement mediated lysis of parasite despite IgM deposition on parasitized RBC surface. We also found that IgM although not a rosetting factor enhances this interaction by increasing the strength of this interaction by at least four-fold. In terms of physiological relevance, we need to understand that new born babies have elevated level of IgM and could be more prone to agglutination and hence more deaths due to malaria.



Biography

Ulf Skoglund received his PhD in 1969 at Stockholm University, Sweden. He was a Professor during the years 1996 – 2009 at Karolinska Institute, Stockholm, Sweden. Since 2010, he is a Professor in Structural Cellular Biology at Okinawa Institute of Science and Technology, Okinawa, Japan. He has developed electron-tomographic technologies allowing for images of proteins to be generated so that e.g. X-ray structures can be fitted into the 3D densities. This technique is termed COMET (Constrained Maximum Entropy Tomography). His unit has also developed a large-scale dynamics method that allows for quantitative calculations of molecular movements in solution. Current developments concern the mathematics and improvements of the basic 3D reconstruction principles, as well as work on reconfigurable and high-performance computing. His unit has also been actively pursuing several cell biology projects.

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Notes:

Arieh Zaritsky, J Proteomics Bioinform 2017, 10:8(Suppl)
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Is nucleoid complexity hence cell diameter limited by the eclipse?

Arieh Zaritsky
Ben-Gurion University of the Negev, Israel

Cell width W of *Escherichia coli* is correlated with the mean complexity of its nucleoid, which is expressed as the ratio between the mean times to replicate it and to duplicate the cell aka the number of replication positions n. A set of old, puzzling observations of cell size and dimensions is qualitatively consistent with the view that W is determined by n, and that branching results from breaching a maximum possible value. This maximum nmax is interpreted in terms of a minimal distance possible between successive moving replisomes, so-called eclipse. The data is subject to analytical quantification designed to model the correlations in a way that may (1) shed light on the necessary coupling between the two unique structures in a bacterial cell, nucleoid and sacculus, and (2) lead to decipher the primary signal transduced from DNA to the peptidoglycan biosynthetic pathway. The first approximation is not sufficient to account for the rate at which average cell size rises with time (Po-Yi H and Amir A, personal communication), hence two additional causes are considered to reconcile this discrepancy: loss of division capacity of some DNA-less cells and dependence of the time needed for division on W. A physical signal is invoked, related to transcription/translation of membrane protein genes coupled to membrane-insertion of these proteins termed "transertion", but means to measure the reciprocal stress imposed by transertion strings on both nucleoid and cell envelope are sorely lacking.

Biography

Arieh Zaritsky of Ben-Gurion University's Faculty of Natural Sciences (http://ariehz.weebly.com/) runs a laboratory investigating parallel fields, pure and applied. During his career at BGU (1973-todate), Dr. Zaritsky has instructed over 50 trainees (graduate students and scientists) and was awarded numerous research grants, allowing him to study both fields of expertise. He visited many higher education Institutions around the world and delivered invited lectures related to both research fields at international meetings. After obtaining a distinguished MSc in Genetics at The Hebrew University of Jerusalem (1967), he graduated at Leicester University (1971) and post-doc'ed at The Copenhagen's Institute of Microbiology (1972). Professor Zaritsky is a recognized expert on bacterial cell physiology and bacteriophage multiplication and published over 130 peer-reviewed articles (http://ariehz.weebly.com/articles.html). Dr. Zaritsky Chaired BGU's Life Sciences department (1989-1991) and is an Editorial Board member of Bioengineered and awardee of 1994 Burroughs-Wellcome/ASM Visiting Professorship.

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Shin-ichi Tate, J Proteomics Bioinform 2017, 10:8(Suppl)
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Inter-domain communication through intrinsically disordered region (IDR) revealed through the ensemble structure analysis

Shin-ichi Tate
Hiroshima University, Japan

The functionally relevant inter-domain communication between the domains linked by intrinsically disordered region was explored by NMR in combination with small angle X-ray scattering. Based on the ensemble structure analyses and the numerical simulations to reproduce the chemical shift changes along with the substrate concentration, we have demonstrated how the domains cooperate to enhance the protein function through the substantially dynamic spatial allocation of the domains. Pin1, a proline cis/trans isomerase, comprises two domains linked by 10-residue IDR, one is the substrate biding domain to recognize pSer/pThr-Pro motif and the other is the enzyme domain that rotates the Pro peptide bond in the motif. The enzyme domain shows very limited affinity to the substrate, but its binding ability was enhanced by two orders of magnitude in the presence of the substrate binding domain linked by IDR; in which the inter-domain fly-casting mechanism plays to keep the substrate bound to Pin1 by tossing and receiving the substrate between the domains, once the substrate in bound to either one of the domains. A new functional aspect of IDR will be addressed.

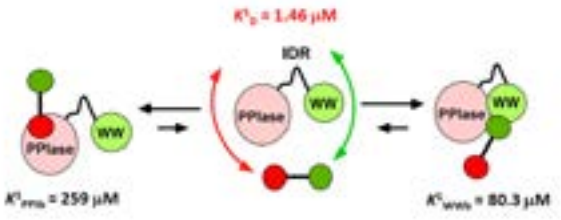

Figure1: *Inter-domain substrate migration mechanism to enhance the binding ability of the enzyme domain by 200-fold. In this process, the IDR roles in prompting the domains to efficiently capture the substrate by allowing them for searching the substrate in a wide space, as analogously described as fly-casting.*

Biography

Shin-ichi Tate has got his PhD degree from the University of Tokyo in 1993. He has the experience as a visiting Researcher at ETH (Prof. K Wühtrich) and NIH (Dr. A Bax). He has been a Professor in the Department of Mathematical and Life Sciences at Hiroshima since 2006. He is now the Director of the Research Center for the Mathematics on Chromatin live Dynamics (RcMcd), and the Dean of the School of Science in Hiroshima University.

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Matthias Buck, J Proteomics Bioinform 2017, 10:8(Suppl)
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Solution NMR relaxation and µs molecular dynamics simulations of dynamic protein-protein and protein-membrane complexes

Matthias Buck
Case Western Reserve University, USA

It is now recognized that protein-protein interactions in solution are often dynamic, especially if the binding affinities are only moderately strong. Dynamics originate in part from the interconversion between structures of the protein complex, e.g. one bound state that is in equilibrium with one or several alternate configurations. We determined the structure of such a complex using NMR restraints and saw the transitions between different configurations in microsecond length all-atom molecular dynamics simulations. Recently, we also studied the dissociation process of mutant complexes which had a weakened primary interaction interface. Those simulations suggested that there is no single dissociation pathway, but that the separation first involves transitions to binding interfaces with fewer/weaker contacts. Comparison is made between experimental NMR relaxation measurements on the ps-ns as well as µs-ms timescale with the microsecond all atom simulations, also in the context of new simulations of the protein association process. The functional significance of the protein complex alternate states and their dynamics are discussed. In a second part of the presentation, we consider a second system involving transient interactions; this time between K-Ras and the lipid bilayer of the plasma membrane. Our recent simulations the full length GTPase at different membranes reveal the underlying rules of the interactions, emphasizing electrostatic contacts but also protein topology. Again, simulations are compared with NMR experiments, carried out at model systems for the membrane.

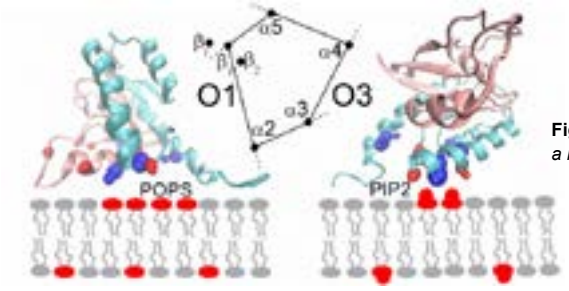

Figure1: *K-Ras4A in two preferred orientations at a membrane containing anionic lipids.*

Biography

Matthias Buck has completed his BA, MA from the University of Cambridge and pursued his DPhil from the University of Oxford. He was a Group Leader since 2002 and Professor since 2014. The Buck laboratory studies two receptor families responsible for cell guidance and positional maintenance (Plexins and Ephrins), both with key involvement in cardiovascular and neuronal development and disease, esp. cancer. They use a wide range of structural biology (solution NMR / x-ray crystallography) and protein biophysical tools (CD, fluorescence spectroscopy, ITC and SPR) in a problem oriented approach. Part of the laboratory also pursues computational modeling and molecular dynamics to provide additional perspective on the problems, provide new insights into the experimental data and to suggest further studies. Small GTPases and their interaction with the plexin receptor cytoplasmic domains has been a major focus of the laboratory and recently they have become very interested in protein-membrane interactions; both the transmembrane regions of the receptors as well as the transient interactions of receptor and GTPase domains with membranes.

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Beat Vögeli, J Proteomics Bioinform 2017, 10:8(Suppl)
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Functional protein conformation networks probed by NMR nanorulers

Beat Vögeli
University of Colorado at Denver, USA

The function of a protein is tightly connected to its conformational network. Often, subtle differences distinguish interchanging states with distinct properties. One major challenge in structural biology is a sufficiently complete description of the structural landscape and the exchange dynamics between structural states at atomic resolution. We have replaced the standard NMR structure determination by an approach that generates multi-state ensembles from a dense network of tight averaged distance restraints derived from exact measurements of nuclear overhauser enhancements. Here, we present the identification of conformational networks harbored by two-human cis/trans isomerases cyclophilin A and Pin1 using the nanorulers provided by eNOEs. We have previously presented an eNOE-based ensemble description of cyclophilin that reveals the presence of a closed and an open state, the latter of which pre-organizes the catalytic site for catalysis. Based on this finding, we demonstrate here a ligand-selective change of the binding affinity to the active site by tuning the dynamics of a highly flexible loop. We show that the binding affinity is increased upon substitution of double glycines to alanines at either of the hinge regions of a loop. The equilibrium distribution is shifted towards more binding-competent conformations. Comparison of the eNOE-based ensembles of the free and ligand-bound WW domain of Pin1 reveals a conformational network that extends into the interface formed with the enzymatically active PPIase domain. This finding may offer an atomic-picture explanation for the previously discovered communication between the two domains.

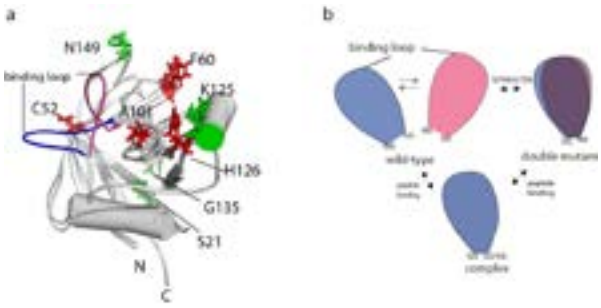

Figure1: *Conformational network of cyclophilin A. a) Structural two-state eNOE ensemble representation of the residues affected by the dynamics of the binding loop shown in the open (blue) and closed state (magenta). b) Mechanistic representation for loop opening and closing for the wildtype, G74A/G75A mutant and the complex.*

Biography

Beat Vögeli has his expertise in nuclear magnetic resonance (NMR) spectroscopy of biomacromolecules. He develops methodology for the elucidation of conformation and communication networks within and between proteins and nucleic acids. He received his PhD degree at the ETH Zürich in the group of Konstantin Pervushin. After a postdoctoral stay at the National Institutes of Health, Bethesda USA, in the group of Ad Bax, he returned to ETH Zürich to become Oberassistant in the group of Roland Riek and Privatdozent. He is currently an Assistant Professor at the University of Colorado at Denver in the Department of Biochemistry and Molecular Genetics.

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Christophe Guyeux, J Proteomics Bioinform 2017, 10:8(Suppl)
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On two ways to predict the protein folding process over a chaotic model

Christophe Guyeux
Université de Bourgogne Franche-Comté, France

In our first theoretical studies about folded self-avoiding walks, we have raised several questions regarding the protein structure prediction problem and the current ways to solve it. In one category of PSP software, the protein is supposed to be synthesized first as a straight line of amino acids, and then this line of amino acids is folded out until reaching a conformation that optimizes a given scoring function. The second category considered that the protein is already in the aqueous solvent, and it does not wait for the end of the synthesis to take its 3D conformation. So they consider SAWs whose number of steps increases until the number of amino acids of the targeted protein end. At each step, the current walk is stretched (one amino acid is added to the protein) in such a way that the pivot k placed in the position that optimizes the scoring function. We have proven that the two sets of possible conformations are different. So these two kinds of PSP software cannot predict the same kind of conformations. We have proven too that the folding process G in the 2D model is chaotic according to Devaney. A consequence of this theorem is that this process is highly sensitive to its initial condition. If the 2D model can accurately describe the natural process, then this theorem implies that even a minute difference on an intermediate conformation of the protein, in forces that act in the folding process, or in the position of an atom, can lead to enormous differences in its final conformation. In particular, it seems very difficult to predict, in this 2D model, the structure of a given protein by using the knowledge of the structure of similar proteins. Let us remark that the whole 3D folding process with real torsion angles is obviously more complex. And finally, that chaos refers to our incapacity to make good prediction, it does not mean that the biological process is a random one.

Figure1: *A conformation that cannot be reached by a folding process on the straight line.*

Biography

Christophe Guyeux has a record of about 120 scholarly publications. Since 2010, he published 43 articles in peer-reviewed international journals (as a co-author, including the top-ranked ones in the areas of Computer Science and interdisciplinary applications, such as AIP Chaos, PLOS ONE, and Clinical Infectious Diseases). He is a co-author of 2 book chapters and 2 scientific monograms. He is also author of 4 software patents, 53 articles that appeared in proceeding of peer-reviewed international conferences. His topics for research encompass Bioinformatics, discrete dynamical systems, and information security. He is currently working as Full Professor at Femto-ST Institute, Université de Bourgogne Franche-Comté, France.

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Notes:

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Antibodies as research tools to find new chemical matter

Marta Westwood
UCB Celltech, UK

Protein-protein interactions (PPIs) are of critical importance in most of biological cellular processes including DNA repair, immune, and allergic responses. Despite their therapeutic relevance, PPIs are intrinsically challenging targets due to complexity of interactions, assay tractability and the lack of well-defined binding pockets at the interacting surfaces. In the quest for small molecule drug candidates targeting PPIs, there have been many different approaches adopted, which include: use of existing lead or drugs, natural products, high-throughput screening and more recently established powerful fragment-based drug discovery. At UCB we have integrated the fragment-based methodology with biological and structural information obtained from antibody-validated protein targets, to develop specific small molecule inhibitors of PPIs. An ensemble of biophysical methods (i.e. SPR, ITC, FRET, MS and ligand-based NMR), corroborated by functional data, were employed to identify and validate fragment hits that constituted the starting point for our PPI inhibitor drug discovery programs. We have also employed antibodies as research tools to hold target proteins in biologically active conformations, aiding the discovery of new small molecules for challenging targets. By holding the target protein in biologically relevant conformations, new sites (in particular allosteric sites), which would otherwise be inaccessible, may become available for binding. The ability to capture the target protein in a specific conformation with high affinity for a significantly long time opens the possibility for a small-fragment molecule screening.

Biography

Marta Westwood has obtained his PhD in Nano-materials from Cranfield University in 2007. In 2011, he has joined Structural Biology group led by Alaistair Lawson at UCB. For the past five years, he has been developing expertise in small molecule drug discovery using a fragment based approach. He has also been involved in the assay development for a novel antibody-enable drug discovery approach. Prior to joining UCB, he was awarded a Post-Doctoral Fellowship at the Institute of Food Research in Norwich to study the mono and multilayer films used to encapsulate active ingredients for a controlled and site-specific delivery within the GIT. During his time at IFR he has worked on numerous projects including a commercial project for Pfizer focused on AFM imaging of proteins and polysaccharides constituting vaccines.

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Brian Kloss, J Proteomics Bioinform 2017, 10:8(Suppl)
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Structural genomics of integral membrane proteins - Past successes and future directions

Brian Kloss
New York Structural Biology, USA

Approximately one-third of all human genes, as well as genes from most other organisms, across all kingdoms of life - encode integral membrane proteins. Nonetheless, the number of integral membrane protein structures solved lags far behind the number of those solved for their soluble counterparts, due primarily to the difficulty of recombinant expression and the instability of membrane proteins once they are detergent-extracted from the lipid bilayer. Over the past 10-20 years, the number of integral membrane protein structures solved, primarily by x-ray crystallography, has increased significantly and structural genomics approaches have played a considerable role in this progress. More recently, advances in cryo-electron microscopy techniques have permitted structures of integral membrane proteins to be determined at resolutions comparable to that of x-ray crystallography, but requiring much smaller quantities of protein. Concurrently, detergents that improve the stability of integral membrane proteins and purification techniques that allow proteins to be extracted and purified in their native lipid environment have also been developed, allowing structural studies of integral membrane proteins to move forward at an exceedingly rapid pace. I will summarize our past integral membrane protein structural biology efforts that employed structural genomics approaches and high-throughput techniques and describe our plans for future structural studies that will continue to make use genomics-based methods, as well as more recently available reagents, techniques and technologies.

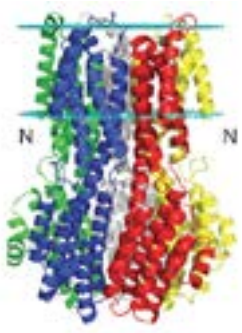

Figure1: *Ribbon diagram showing the structure of the homologue of the human calcium-activated chloride channel bestrophin from Klebsiella pneumoniae.*

Biography

Brian Kloss began his research career as a graduate student in the Laboratory of Carter Bancroft at the Mount Sinai School of Medicine, studying the transcriptional regulation of the pituitary-specific prolactin and growth hormone genes. He went on to do a Postdoc with Michael Young at Rockefeller University, studying the genetic control of circadian rhythms in *Drosophila melanogaster*. Afterwards, he spent almost six years at a biotech startup, helping to develop a cell-based assay for the screening of ligands of GPCRs. For the past ten years, he has been a part of the protein production facility of the Center on Membrane Protein Production and Analysis (COMPPÅ), located at the New York Structural Biology Center. There, he has led a small group focused on the identification, cloning and expression screening of integral membrane proteins of prokaryotic origin, mainly for structural studies.

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Notes:

Steven Hayward, J Proteomics Bioinform 2017, 10:8(Suppl)
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Geometrical principles of homeric β-barrels and β-helices: Applications to modelling amyloid protofilaments

Steven Hayward
University of East Anglia, UK

Examples of homomeric β-helices and β-barrels have recently emerged. Here we have generalized the theory for the shear number in β-barrels to encompass β-helices and homomeric structures. We introduce the concept of the β-strip which comprises neighboring strands, parallel or antiparallel and forms the repeating unit that builds the helix. In this context, the shear number is interpreted as the sum of register shifts between neighboring β-strips. This more general approach has allowed us to derive relationships between the helical width, helical pitch, angle between strand direction and helical axis, mass per length, register shift, and number of strands. The validity and unifying power of the method is demonstrated with known structures including the T4 phage spike, cylindrin, and the HET-s (218-289) prion. The relationships have allowed us to predict register shift and number of strands in transthyretin and Alzheimer β (40) amyloid protofilaments from reported dimensions measured by X-ray fiber diffraction which we have used to construct models that comprise a single strip of in-register β-strands folded into a β-strip helix. The results suggest that both stabilization of an individual β-strip helix as a protofilament subunit and growth of the protofilament by the joining of subunits end-to-end, would involve the association of the same pair of sequence segments at the same register shift.

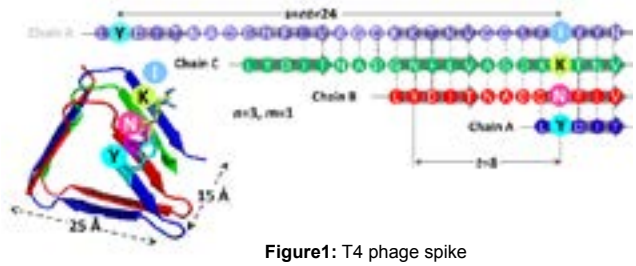

Figure1: T4 phage spike

Biography

Steven Hayward is a Reader in Computational Biology at the University of East Anglia. He uses computational methods, including bioinformatics techniques and simulation, to understand protein structure, dynamics and function. He has focused particularly on protein dynamics and developed the popular DynDom method for the analysis of domain movements in proteins. Recently he has worked on the fundamental structure of amyloid fibrils and works on the development of interactive tools for protein visualization and docking using haptics.

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Notes:

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Exploring conformational equilibria of a heterodimeric ABC transporter by electron paramagnetic resonance

Enrica Bordignon
Ruhr-Universität Bochum, Germany

ABC exporters pump substrates across the membrane by coupling ATP-driven movements of nucleotide binding domains to the transmembrane domains, which switch between inward and outward facing orientations. Understanding their cycle has potential for medical applications because they are involved in multidrug resistance of cancer cells. Site-directed spin labeling electron paramagnetic resonance and the dipolar spectroscopy technique called DEER or PELDOR was used to investigate the conformational transition of the ABC heterodimeric exporter TM287/288 from the hyperthermophile *T. maritima*. The analysis revealed that with nucleotides the transporter exists in an equilibrium between the IF and OF states. ATP binding without hydrolysis was sufficient to partially populate the OF state, and an almost complete conformational shift was observed when nucleotides were trapped in a pre-hydrolytic or post-hydrolytic state. At physiological temperature and without nucleotides, the NBDs disengage asymmetrically while the conformation of the TMDs remains unchanged. Nucleotide binding at the degenerate ATP site prevents complete NBD separation, a molecular feature differentiating heterodimeric ABC exporters from their homodimeric counterparts. Our data suggest hydrolysis-independent partial closure of the NBD dimer, which is further stabilized as the consensus site nucleotide is committed to hydrolysis. A unified mechanism is established, which reconciles the available information for heterodimeric ABC exporters.

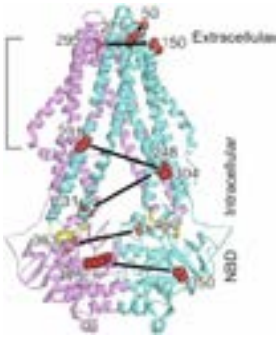

Figure1: *Spin labeling sites in the extracellular, intracellular and NBD regions of TM287/288, represented in the inward-facing apo crystal structure (PDB:4Q4H). TM287 is colored in cyan and TM288 in pink.*

Biography

Enrica Bordignon is an Associate Professor at the Ruhr University of Bochum, where she leads an EPR laboratory dedicated to the study of membrane proteins. She published approximately 50 papers in protein research by EPR methods. Her research interest is understanding the mechanism of action of proteins by EPR.

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Notes:

Jaydeb Chakrabarti et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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Microscopic calculation of conformational thermodynamics in bio-macromolecular complexes

Jaydeb Chakrabarti and **Mahua Ghosh**
S N Bose National Centre for Basic Sciences, India

The microscopic basis of connection between protein conformation and function is a fundamental challenge. Recent experiments show the importance of conformational changes in providing stability to protein complexes. The changes in conformational state have both enthalpy and entropic components. It has been possible to quantify the entropy changes due conformational changes from nuclear magnetic resonance data. Here we show microscopic calculation of both the enthalpy and entropy contribution while protein conformations change from the equilibrium distribution of the dihedral angles of proteins. We have shown that the free-energetically destabilized and entropically disordered residues in each conformation compared to a reference conformation act as binding residue in the given conformation. Here we show that this principle can: 1. ascertain ligand binding of a protein in different conformations, 2. supplement the structure of missing fragments in a protein and 3. serve as a guideline for allosteric changes. All these applications point to tune protein in-silico which would help to design functional materials with protein as building blocks.

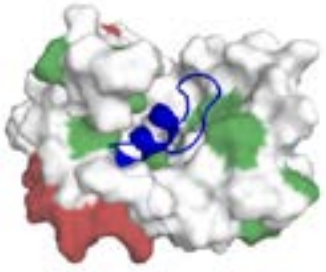

Figure1: *Residue wise data from microscopic calculation of conformational entropy (red: disordered; green: ordered)*

Biography

Jaydeb Chakrabarti is a Theoretical Physicist trained in condensed matter physics. He considers statics and dynamics of soft matter systems including bio-macromolecules. The theoretical methods used in these studies are: (1) Computer simulations based on Molecular Dynamics, Monte Carlo and Brownian Dynamics, (2) Mean field calculations based on classical density functional theories. The goal of his researches is to relate macroscopic properties to microscopic motions.

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Notes:

Sessions:

Signalling Biology

Session Chair
Joachim Krebs
Max Planck Institute for Biophysical Chemistry, Germany

Session Co-Chair
Vesa P. Hytönen
University of Tampere, Finland

Session Introduction

Title: **Calcium, calmodulin and the plasma membrane calcium pump**
Joachim Krebs, Max Planck Institute for Biophysical Chemistry, Germany

Title: **Structural insights into cholesterol regulation of inwardly-rectifying K+ channels**
Irena Levitan, University of Illinois, USA

Title: **Structural aspects of cell signaling**
Carol A. Heckman, Bowling Green State University, USA

Title: **Mechanical stability of Talin Rod controls traction force generation and cell migration**
Vesa P. Hytönen, University of Tampere, Finland

Title: **Photoinduced electron transfer in cytochrome bc1: Kinetics of ubiquinone transfer from the Qo site to the Qi site, and evidence for communication between the monomers in the dimer**
Francis Millett, University of Arkansas, USA

Title: **Mapping energy transfer channels in fucoxanthin–chlorophyll protein complex**
Leonas Valkunas, Vilnius University, Lithuania

Title: **PIP2 modulation of KCNQ1 channels**
Jianmin Cui, Washington University in St. Louis, USA

Title: **LD motif interacting networks in cell-matrix adhesion**
Igor L. Barsukov, University of Liverpool, UK

Title: **Structural conformational changes report biased agonism: The case of Galanin receptors**
Arfaxad Reyes Alcaraz, Korea University, College of Medicine, South Korea

Title: **A biophysical and structural approach to investigate calcium sensor properties of plant calmodulin-like proteins**
Alessandra Astegno, University of Verona, Italy

Title: **Structural insights into the mechanism of how polyphenols suppresses amyloid fibrillation**
He Jianwei, Liaoning University, China

Title: **Type I BIR domain inhibitors in cancer therapy: Designing drugs to modulate the NF-κB pathway**
Federica Cossu, Institute of Biophysics at the National Research Council (IBF-CNR), Italy

Joachim Krebs, J Proteomics Bioinform 2017, 10:8(Suppl)
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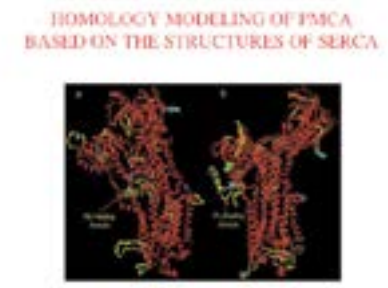
Calcium, calmodulin and the plasma membrane calcium pump

Joachim Krebs
Max Planck Institute for Biophysical Chemistry, Germany

Calcium is the third most abundant metal in nature and a versatile carrier of many signals within and outside the cell. Due to its peculiar coordination chemistry calcium is highly flexible as a ligand which enables it to regulate many important aspects of cellular activity. Calcium can fulfill its many distinct functions onsite and out of the cell due to an integrated network of calcium channels, exchangers and pumps. In this presentation, I will give an overview on our studies of calcium binding proteins, their interaction with protein targets resulting in specific modulations of protein-protein interactions. This will be demonstrated by the interaction of the calcium binding protein calmodulin with one of its targets, the plasma membrane calcium pump, an important regulator of calcium homeostasis of the cell.



Biography

Joachim Krebs has been working in the field of calcium-binding and calcium-transporting proteins for many years. After receiving his PhD, he spent two years as a Post-doctoral fellow in the lab of Prof. RJP Williams at Oxford, UK. In 1977, he has accepted a staff position at the Institute of Biochemistry at the Swiss Federal Institute of Technology (ETH) in Zürich, Switzerland. He was lecturing different courses in Biochemistry and Biophysics and was leading a lab working on the structure-function relationship of calcium-binding and calcium-transporting proteins. After retirement from the ETH he continued his research as a consultant of the Lab of Prof. Christian Griesinger at the MPI in Göttingen, Germany. He has authored, co-authored and edited numerous articles in international journals. Recently he edited a book on "*Calcium: A matter of life or death*" published in 2007. He serves as the Editorial Board Member of *BBA Molecular Cell Research and Archives of Biochemistry and Biophysics.*

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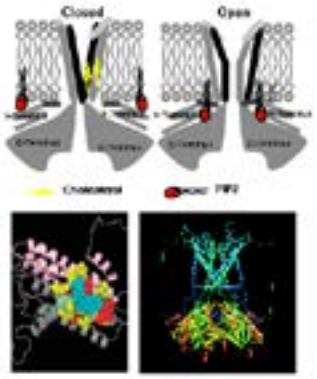
Structural insights into cholesterol regulation of inwardly-rectifying K+ channels

Irena Levitan
University of Illinois, USA

Cholesterol is known to play a significant role in regulating the function of multiple membrane proteins including a growing number of ion channels. Our studies focus on inwardly-rectifying K+ (Kir) channels that are ubiquitously expressed in mammalian cells and are known to play key role in membrane excitability and shear stress sensation. In this study, we have shown that Kir channels are suppressed by loading the cells with cholesterol and enhanced by cholesterol depletion. A series of studies revealed that cholesterol interacts with the channels directly by stabilizing them in a long-lived closed "silent" state and that multiple structural features of the channels are essential for conferring their cholesterol sensitivity. Using a combined computational-experimental approach, we show that cholesterol may bind to two non-annular regions that form hydrophobic pockets between the transmembrane helices of the adjacent subunits of the channel. The location of the binding regions suggests that, cholesterol modulates channel function by affecting the hinging motion at the centre of the pore-lining transmembrane helix that underlies channel gating. In addition, we identified a series of residues in the C and N-terminus of the channel. These are critical for conferring cholesterol sensitivity to the channels, but are not part of the binding sites. These residues form a distinct cytosolic structure, a cholesterol sensitivity belt which surrounds the cytosolic pore of the channel in proximity to the transmembrane (TM) domain, and includes residues whose mutation results in abrogation of the channel's cholesterol sensitivity. Further analysis identified a reversal residue chain comprised of residues that link one of the cholesterol sensitivity belt residues with a distant cytosolic residue that constitute a two-way molecular switch of the channel sensitivity to cholesterol. Further studies are needed to elucidate the connection between cholesterol binding and channel.



Biography

Irena Levitan has completed her PhD and is a Professor of Medicine and Adjunct Professor of Bioengineering at the University of Illinois at Chicago. Her current research focuses on cholesterol regulation of ion channels and cellular biomechanics. Her group has provided the first comprehensive structural insights into cholesterol regulation of K+ channels and the cross-talk between cholesterol and other regulators of these channels. She was named a Guyton Distinguished Lecturer by the Association Chairs of Departments of Physiology for her quantitative and biophysical work on cholesterol modulation of ion channels and how this can affect integrated organ function. She is an author of more than 70 publications and a leading Editor of *Cholesterol Regulation of Ion Channels and Receptors* (Wiley, 2012) and *Vascular Ion Channels in Physiology and Disease* (Springer, 2016).

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Notes:

Carol A Heckman, J Proteomics Bioinform 2017, 10:8(Suppl)
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Structural aspects of cell signaling

Carol A Heckman
Bowling Green State University, USA

Statement of the Problem: Endpoints such as adhesion and motility have been used to infer the function of a protein in cells. These endpoints are unsatisfactory, because a protein can be recruited to different substructures and promote different outcomes in such structures. By defining meaningful endpoints, it is possible to identify a protein's contribution to several different patterns of cell organization and thereby address major problems in biology.

Methodology & Theoretical Orientation: We developed an unbiased method of classifying and quantifying features of fixed, adherent epithelial cells. Primary data, consisting of 102 measures of contour geometry, curvature, relationship to derived model figures, etc., were used to calculate 20 latent factors representing cell features. Factors detect structure by recognizing the relationships between variables. Cells from experiments are classified according to each factor by summing the factor loadings. Filopodia (factor 4) accounted for a larger proportion of cancer-related variance than any other feature. Filopodia are the sensory appendages that are relied on when cells distinguish their more and less adhesive sides. The protrusion defined as factor 7 represented neurites. Even when small, neurites differed from lamellipodia (factor 5). Several factors contribute to ruffling.

Results: Filopodia are down-regulated by three isoforms of protein kinase C (PKC). The effect of PKC ε, a known oncogene, on filopodia is only observed after tumor promoter treatment. The effect is in part due to a PKC ε-mediated increase in ruffling. In cells not treated with tumor promoter, filopodia are down-regulated by isoforms α and ή. PKC α has contrary effects in promoter-treated cells, where it conserves filopodia by suppressing ruffling activity. Activated PKC α may promote filopodia. These activities are consistent with the concept that filopodia are implicated in cell homeostasis. By regulating the prevalence of filopodia, PKC can regulate the way cells react to their surroundings.

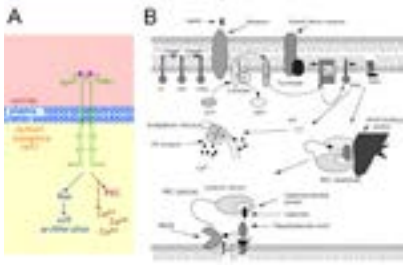

Figure1: *PKC signaling. A) PKC signals downstream of growth factor receptors. B) PKC is also activated by G protein-coupled receptors. When activated, it finds most of its substrates in the vicinity of membranes.*

Biography

Carol A Heckman is an expert on Preneoplasia and image analysis and has developed optical and computational methods suitable for cell feature analysis. Applying these methods, she showed how to deconstruct the cell phenotype and find the features related to oncogenic transformation. About half of the 20 features are useful in distinguishing the phenotypes of normal and cancerous epithelial cells. She has published numerous papers on these topics. The cell features form the basis of an assay to flag chemicals of interest for drug development and identify diseases that can be targeted productively by existing drugs.

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Notes:

Vesa P Hytonen et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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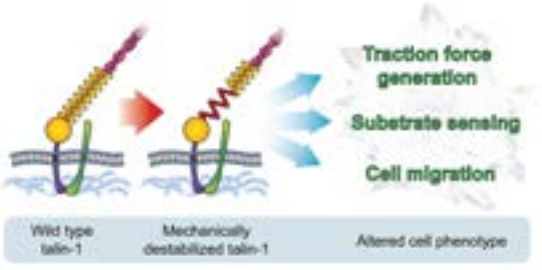
Mechanical stability of talin rod controls traction force generation and cell migration

Vesa P Hytonen[1], Rolle Rahikainen[1], Magdalena von Essen[1], Markus Schaefer[2], Lei Qi[3], Latifeh Azizi[1], Conor Kelly[1], Teemu O Ihalainen[1], Bernhard Wehrle-Haller[4], Martin Bastmeyer[2] and **Cai Huang[3]**
[1]University of Tampere, Finland
[2]Karlsruhe Institute of Technology, Germany
[3]University of Kentucky, USA
[4]University of Geneva, Switzerland

Talin is a central adhesion protein linking β-integrin cytosolic domains to actin fibers. It participates in the transmission of mechanical signals between extracellular matrix and cell cytoskeleton. Talin rod domain consists of a series of mechanically vulnerable α-helical subdomains containing binding sites for other adhesion proteins such as vinculin, actin and RIAM. Force induced unfolding of these rod subdomains has been proposed to act as a cellular mechanosensor, but so far evidence linking their mechanical stability and cellular response has been lacking. We show that stepwise mechanical destabilization of talin rod subdomain increases talin and vinculin accumulation into cell-matrix adhesions and decreases cell migration rate. In addition, mechanical destabilization of talin subdomain was found to decrease cellular traction force generation and to promote the formation of adhesions on fibronectin over vitronectin. Experiments with truncated talin forms confirmed the mechanosensory role of the talin subdomain and excluded the possibility that the observed effects are caused solely by the release of talin autoinhibition. We demonstrate that by modulating the mechanical stability of an individual talin rod subdomain, it is possible to affect traction force generation, ECM sensing and consequently highly coordinated processes such as cell migration. Our results suggest that talin acts as a mechanosensor and is responsible for controlling the cellular processes dependent on mechanical signals and cellular mechanosensing.



Biography

Vesa P Hytonen is a Head of the Protein Dynamics research group in BioMediTech at the University of Tampere, Finland. After graduating as a PhD scholar from the University of Jyväskylä, Finland in 2005, he has conducted Post-doctoral training at ETH Zurich, Switzerland from 2005-2007. He then continued as a Post-Doctoral researcher at the University of Tampere and established independent research group in 2010. He is currently working as Associate Professor at the University of Tampere. His research interests are Mechanobiology, Protein Engineering and Vaccine research and authored more than 100 scientific articles.

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Notes:

Francis Millett, J Proteomics Bioinform 2017, 10:8(Suppl)
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Photoinduced electron transfer in cytochrome bc_1: Kinetics of ubiquinone transfer from the Q_o site to the Q_i site, and evidence for communication between the monomers in the dimer

Francis Millett
University of Arkansas, USA

The electron transfer reactions within wild- type *Rhodobacter sphaeroides* cytochrome bc_1 (cyt bc_1) were studied using a ruthenium dimer to rapidly photo oxidize cyt c_1. It was found that when cyt b_H was initially reduced before the reaction, photooxidation of cyt c_1 led to bifurcated reduction of both the iron-sulfur protein and cyt b_L by QH_2 in the Q_o site, followed by re-oxidation of two equivalents of cyt b_L and cyt b_H. It was proposed that the newly formed ubiquinone diffused through the hydrophobic cavity linking the Q_o site of the reactive monomer A to the Q_i site of the other monomer B, leading to oxidation of cyt b_H in monomer B followed by oxidation of cyt b_L in monomer A by cross-monomer electron transfer. Addition of one equivalent of the Q_i site inhibitor antimycin to the cyt bc_1 dimer had very little effect on any of the electron transfer reactions, while addition of a second equivalent completely inhibited re-oxidation of cyt b_L and cyt b_H. It was also found that addition of one equivalent of the Q_o site inhibitor stigmatellin to the cyt bc_1 dimer completely inhibited all electron transfer reactions in both monomers of the dimer. These experiments are consistent with a half-of-the-sites mechanism in which only one monomer of the dimer is active at a time, implying monomer-monomer interactions. The rapid electron transfer reaction from the ISP to cyt c_1 was found to be greatly decreased by viscosity, indicating a multi-step diffusional mechanism as the iron-sulfur protein rotates from the b state to the c_1 state.

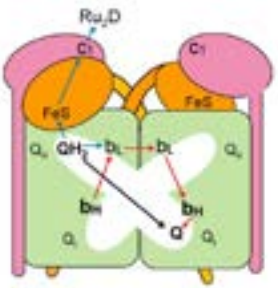

Figure1: *Photoinduced electron transfer in cytochrome bc1: kinetics of ubiquinone transfer from the Qo site to the Qi site, and evidence for communication between the monomers in the dimer*

Biography

Francis Millett received his BS in Chemistry from the University of Wisconsin in 1965, his PhD in Chemical Physics from Columbia University in 1970, and was an NIH Postdoctoral Fellow at California Institute of Technology from 1970-1972. He joined the faculty of the University of Arkansas in 1972, and is now a Distinguished Professor. He developed, together with Bill Durham, the ruthenium photoreduction method which made it possible to measure the kinetics of key steps in electron transfer during mitochondrial oxidative phosphorylation. He has directed collaborative, multidisciplinary research which combines rapid kinetics methods, site-directed mutagenesis, X-ray crystallography, and NMR to investigate protein structure-function relationships.

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Leonas Valkunas, J Proteomics Bioinform 2017, 10:8(Suppl)
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Mapping energy transfer channels in fucoxanthin–chlorophyll protein complex

Leonas Valkunas
Vilnius university, Lithuania

Fucoxanthin–chlorophyll protein (FCP) is the key molecular complex performing the light-harvesting function in diatoms, which, being a major group of algae, are responsible for up to one quarter of the total primary production on Earth. These photosynthetic organisms contain an unusually large amount of the carotenoid fucoxanthin, which absorbs the light in the blue-green spectral region and transfers the captured excitation energy to the FCP bound chlorophylls. Due to the large number of fucoxanthins, the excitation energy transfer cascades in these complexes are particularly tangled. Energy transfer processes and coherent phenomena in the Fucoxanthin–chlorophyll protein complex, which is responsible for the light harvesting function in marine algae diatoms, were investigated at 77 K by using two-dimensional electronic spectroscopy. Experiments performed on femtosecond and picosecond timescales led to separation of spectral dynamics, witnessing evolutions of coherence and population states of the system in the spectral region of Qy transitions of chlorophylls a and c. Analysis of the coherence dynamics allowed us to identify chlorophyll (Chl) a and fucoxanthin intramolecular vibrations dominating over the first few picoseconds. Closer inspection of the spectral region of the Qy transition of Chl c revealed previously not identified, mutually non-interacting chlorophyll c states participating in femtosecond or picosecond energy transfer to the Chl a molecule. Consideration of separated coherent and incoherent dynamics allowed us to hypothesize the vibrations-assisted coherent energy transfer between Chl c and Chl a and the overall spatial arrangement of chlorophyll molecules.

Biography

Leonas Valkunas is the author of more than 350 international publications, head of the department of Theoretical Physics at Vilnius University and Head of the Department of Molecular Compounds Physics at the Center for Physical Sciences and Technology in Vilnius. He is involved in studies of primary processes of photosynthesis such as excitation dynamics and photoinduced charge separation in various photosynthetic systems based on the spectroscopic data and using various theoretical modelling approaches.

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Jianmin Cui, J Proteomics Bioinform 2017, 10:8(Suppl)
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PIP2 modulation of KCNQ1 channels

Jianmin Cui
Washington University in St. Louis, USA

Voltage-gated ion channels generate dynamic ionic currents that are vital to the physiological functions of many tissues. These proteins contain separate voltage-sensing domains, which detect changes in transmembrane voltage, and pore domains, which conduct ions. Coupling of voltage sensing and pore opening is critical to the channel function and has been modeled as a protein–protein interaction between the two domains. However, our data show that coupling in Kv7.1 channels requires the lipid phosphatidylinositol 4,5-bisphosphate (PIP2). We found that voltage-sensing domain activation failed to open the pore in the absence of PIP2. This result is due to loss of coupling because PIP2 was also required for pore opening to affect voltage-sensing domain activation. We identified a critical site for PIP2-dependent coupling at the interface between the voltage-sensing domain and the pore domain. This site is a conserved lipid-binding site among different K+ channels, suggesting that lipids play a significant role in coupling in many ion channels. To further investigate the mechanism of PIP2 mediated VSD-pore coupling, we identified a compound that mimics PIP2 structure and function as a molecular probe. This compound was identified using an in-silico screening approach based on molecular docking of a library of compounds to the PIP2 binding site in a homology model of the Kv7.1 channel. Our results show that this compound can substitute PIP2 in activating the Kv7.1 channel.

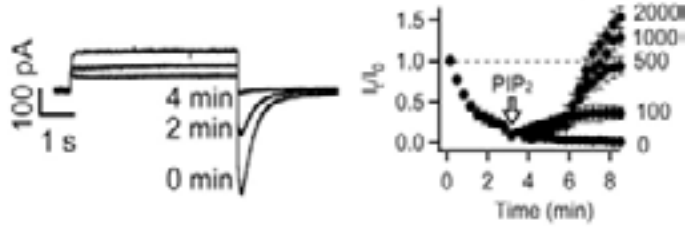

Figure1. *Kv7.1 activation depends on PIP2. Left. Kv7.1 currents in an inside-out patch decreases with time due to PIP2 diffusion out of the membrane patch. Right. Cytosolic PIP2 application (μM) activates Kv7.1.*

Biography

Jianmin Cui is a Professor on the Spencer T. Olin Endowment at Washington University in St. Louis, in the Department of Biomedical Engineering. He received PhD in Physiology and Biophysics from State University of New York at Stony Brook and a Post-Doctoral training at Stanford University. He was an assistant professor of Biomedical Engineering at Case Western Reserve University before moving to St. Louis. His research interests include BK-type calcium-activated potassium channels and IK_s channels.

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Igor L Barsukov, J Proteomics Bioinform 2017, 10:8(Suppl)
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LD motif interacting networks in cell-matrix adhesion

Igor L Barsukov
University of Liverpool, UK

Statement of the Problem: Cell-matrix adhesion requires assembly of large multi-protein complexes linked to the cyto-domains on the integrin adhesion receptors. These complexes dynamically change in response to the adhesion forces and environmental signals. Mechano-sensitive adaptor protein talin couples the force and adhesion signaling by acting as a hub for numerous, often competitive, interactions. The molecular mechanism that controls the co-ordination of the interactions in time and space is currently not understood. The aim of this study is to define the interactions between talin and Rho-GAP Deleted in Liver Cancer 1 (DLC1) that regulates adhesion forces.

Methodology: Structure of the talin/DLC1 complex was solved by X-ray crystallography and the interactions between the proteins analyzed by NMR spectroscopy. Fluorescent imaging was used to define the interactions within the adhesion complexes and cancer cell lines were employed to characterize the effect of the interaction on the biological activity.

Findings: We defined the atomic details of the talin/DLC1 interactions and used this information to identify signaling protein paxillin as a talin ligand. Based on the structural information we designed a range of talin mutants that modulate the interactions and demonstrated that the mutations reduce DLC1 signaling in adhesion.

Conclusion & Significance: Talin recognizes LD motifs in DLC1 and paxillin through a set of well-defined charge interactions. These interactions are like other LD motif interactions previously identified in signaling pathways. These interactions are also like the interactions between talin, RIAM and vinculin that were previously not assigned to the LD motif family. Together, the relatively weak LD motif interactions within the adhesion complex create a protein network that could dynamically respond to the adhesion signals.

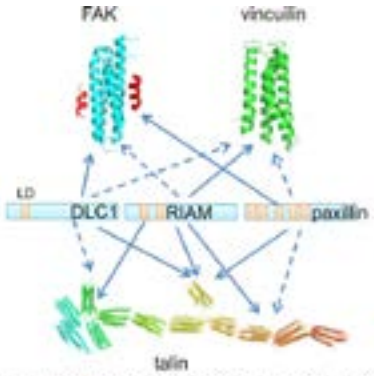

Figure 1. LD motif interactions inter-connecting adhesion proteins

Biography

Igor L Barsukov has expertise in Structural Biology, primarily using NMR spectroscopy and X-ray crystallography. The focus of his research has been on the structure and function of integrin-mediated cell-matrix adhesions, where he directed full structural analysis of the key adhesion proteins talin, leading to the currently widely used model of stretch-dependent talin activation. He is currently extending the model of talin functionality to include competitive, talin based, interaction networks. Recently he identified interactions between neuronal scaffold proteins Shank3 and Ras-family GTPase that regulate integrin activity and have implications for control of synaptic plasticity.

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Arfaxad Reyes Alcaraz, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Structural conformational changes report biased agonism: The case of galanin receptors

Arfaxad Reyes Alcaraz
Korea University, South Korea

Statement of the Problem: G protein coupled receptors (GPCRs), also known as seven-transmembrane receptors are the largest family of cell-surface receptors that communicate extracellular stimuli to the cell interior. To date it has been widely accepted synthetic ligands targeting the same receptor can stabilize multiple active structural conformations having therefore differential signaling that eventually results in different physiological responses a phenomenon better known as biased agonism. However biased agonism might not only be restricted to synthetic ligands but also to endogenous ligands targeting the same receptor which may explain such a ligand redundancy, suggesting the existence of endogenous biased agonism as a physiological mechanism.

Methodology & Theoretical Orientation: The aim of this study was to establish a relationship between conformational changes in galanin receptors and their signaling properties in living cells. For that purpose, we developed a structural complementation assay based on NanoBit technology and a series of conformational fluorescein arsenical hairpin (FIAsH) bioluminescence resonance energy transfer (BRET) biosensors to monitor structural changes of β-arrestin 2 induced by binding with each galanin receptor.

Findings: Here we showed that galanin receptors impose different conformational signatures in β-arrestin, moreover structurally different ligands activating the same receptor imposed different conformations in β-arrestin 2 producing biased signaling.

Conclusion & Significance: Our data provide definite evidence that a receptor activated by structurally different ligands can adopt multiple active conformations. Moreover, this finding also demonstrates that functionally specific structural galanin receptor conformations can indeed be translated to downstream effectors producing a different physiological response.

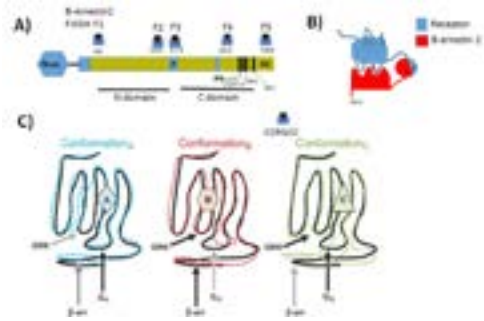

Figure: A) Five NLuc-ß-arrestin 2-FIASH BRET structural biosensors (F1-F5) were constructed by inserting the amino acid motif CCPGCC after amino acid residues 40, 140, 171 263 and 410 of ß-arrestin2. The location of each FIASH motif is shown in relation to the globular N and C domains of ß-arrestin2. B) Diagram of the structural complementation assay used to measure in real time binding between ß-arrestin2 and Galanin receptors. C) Schematic diagram depicting levels of conformational aberration produced in different areas of the receptor upon stabilization of receptor conformations by different agonists. Arrows depict various regions of interaction of the receptor with cytosolic proteins such as different G-proteins and ß-arrestin. It might be surmised that dissimilar conformations affect these various regions to varying degrees causing respective differences in effect for diverse coupling mechanisms.

Biography

Arfaxad Reyes Alcaraz has his expertise in structure and stability of G-protein coupled receptors and passion for improving and creating new drug discovery platforms that greatly contribute in the development of more selective drugs with minor side effects. His studies about biased agonism in galanin receptors helped to understand the relationship between conformational structure of the receptor and its corresponding physiological effect induced by a specific ligand. Recently, he and his co-workers were able to develop a highly selective agonist for galanin receptor 2 with anxiolytic effect *in vivo* which was the base to discover how different ligand structures induce different conformations on the structure of galanin receptors. His works greatly contribute to understand the relationship between structure and function of galanin receptors.

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Alessandra Astegno, J Proteomics Bioinform 2017, 10:8(Suppl)
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A biophysical and structural approach to investigate calcium sensor properties of plant calmodulin-like proteins

Alessandra Astegno
University of Verona, Italy

Calcium is an essential second messenger in plants that regulates various signaling pathways through stimulus-specific Ca^{2+} signatures, which are decoded and converted into a wide variety of biochemical changes by Ca^{2+} sensors. Besides evolutionarily conserved calmodulin (CaM), plants exclusively possess a group of calmodulin-like proteins (CMLs), which play central roles in the coordination of plant responses to different external stimuli. Nevertheless, only few of these proteins have been thoroughly characterized and demonstrated to function as Ca^{2+} sensors. Our research is focused on the investigation of the metal-binding, physicochemical and structural properties of various plant CMLs using complementary biophysical and structural approaches to correlate their properties with the biological activity. We have recently characterized CML36 from *Arabidopsis thaliana*, demonstrating that *in vitro* the protein shows feature consistent with Ca^{2+} sensor function. ITC analysis revealed that CML36 possesses two high affinity Ca^{2+}/Mg^{2+} mixed sites and two low affinity Ca^{2+}-specific sites. Binding of Ca^{2+} to CML36 increases its α-helical content and triggers a conformational change that exposes hydrophobic surfaces necessary for target recognition. Ca^{2+} and Mg^{2+} ions also stabilized the tertiary structure of CML36. Cations binding to the Ca^{2+}/Mg^{2+} mixed sites appear to guide a large structural transition from a loosely packed molten globule apo-state to a well-defined, stable holo-structure. Through *in vitro* binding experiments, we showed that CML36 directly interacts with the N-terminal domain of *Arabidopsis* Ca^{2+}-ATPase isoform 8 (ACA8), a type IIB Ca^{2+} pump localized at the plasma membrane (PM). Moreover, we demonstrated that this interaction promotes ACA8 Ca^{2+}-dependent hydrolytic activity *in vitro*.

Biography

Alessandra Astegno is interested in various aspects of Protein Chemistry, including folding, Evolution and structure-function relationship of proteins and Macromolecular assemblies. She obtained a PhD in Applied Biotechnologies from University of Verona in 2010. She is currently working as an Assistant Professor in Biochemistry at the Department of Biotechnology of the University of Verona. She has a solid background in recombinant protein expression and purification, functional and structural characterization of metallo-proteins as well as PLP-dependent enzymes. Recently, her work focused on the study of calcium signaling in higher plants through biophysical, biochemical and structural characterization of calcium sensor proteins, such as calmodulin and calmodulin like proteins of *Arabidopsis thaliana*.

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He Jianwei, J Proteomics Bioinform 2017, 10:8(Suppl)
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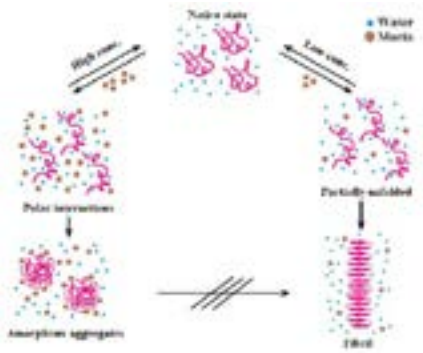
Structural insights into the mechanism of how polyphenols suppress amyloid fibrillation

He Jianwei
Liaoning University, China

Polyphenols, especially natural flavanols, have received considerable public attention in China due to the positive association between food and traditional herbal consumption and beneficial health effects. Flavonoids has been demonstrated to be active inhibitors of fibrillation by amyloidogenic protein. We recently reported the inhibitory activity of Myricetin against HEWL fibril formation, in which Myricetin exhibited a stronger inhibition than the well-characterized polyphenol Quercetin. In contrast to our previous studies using other polyphenols, we find the generation of irregular structural aggregates formed by the binding of Morin to HEWL, which support a novel and distinctive model for how this small molecule inhibits amyloid formation. Moreover, we also demonstrated that EGCG was a potent inhibitor of amyloidogenic cystatin amyloid fibril formation *in vitro*. Through combining experimental and computational data, we could propose a mechanism by which EGCG inhibited the fibrillation of cystatin: EGCG appears to be a generic inhibitor of amyloid-fibril formation, although the mechanism by which it achieves such inhibition may be specific to the target fibril-forming polypeptide. In conclusion, our findings implicate the importance of diet and drink habits as playing a key role in guarding against amyloid fibril formation and promoting healthy aging.



Biography

He Jianwei is a Professor at School of Life Science, Liaoning University, China, and has received MS degree in Biochemistry in 2002, from Yamaguchi University, Japan. He completed his PhD in Bio resource in 2005 at Tottori University, Japan. His research interests include: 1) Using molecular dynamics and biochemical methods to study protein oligomerization progress and the importance of dimers and tetramers in the aetiology of amyloidotic diseases. 2) Mining, screening or designing of novel inhibitors of natural resources against protein misfolding and amyloid aggregation.

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Type I BIR domain inhibitors in cancer therapy: Designing drugs to modulate the NF-dB pathway

Federica Cossu
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Inhibitors of apoptosis proteins (IAPs) constitute a family of conserved proteins whose over-expression enhances cell survival and resistance to anticancer agents. IAPs are E3 ligases, ubiquitylating substrates for the regulation of NF-κB. Furthermore, they sequester caspases to prevent apoptosis. IAPs interactions occur through type I and type II BIR (Baculovirus IAP repeat) domains. Smac-mimetics (SM) mimicking the active N-terminal peptide of Smac-DIABLO, the natural antagonist of IAPs, have been shown to sensitize cancer cells to apoptosis. SM interact with type II BIR domains of IAPs, thus relieving caspases from X-linked IAP (XIAP) inhibitory activity and leading to cellular IAPs (cIAPs) auto-ubiquitylation and proteasomal degradation within minutes from exposure. Although SM are currently promising candidates for cancer therapy, some cancer cell lines present SM-resistance due to renewed cIAP2 activity and re-activation of NF-κB. IAPs-mediated regulation of NF-κB signaling is based on the formation of different protein-protein complexes, regulating ubiquitin-dependent signal transduction cascades. The type I BIR domain from different IAPs has been recognized as a pivotal platform for the assembly of such complexes. We analysed the surface of type I BIR domains (X- and cIAP-BIR1) to identify the hot-spots for the relevant protein-protein interactions. Virtual docking using libraries of compounds returned hits (NF023 and analogues) and can impair BIR1-based complexes with predicted low micromolar affinities that were experimentally confirmed. For this purpose, *in vitro* assays include fluorescence-based and biophysical techniques (thermofluor, microscale thermophoresis, SEC, DLS, SLS). Crystallography on the protein-ligand complexes is the core of the structure-driven approach used for the iterative optimization of specific and selective drug candidates. Treatment of cancer cell cultures with the selected compounds will verify their effects on the modulation of IAPs-dependent signaling cascades. This represents a novel strategy to promote apoptosis in cancer and will unravel new insights on the regulation of NF-κB pathway.

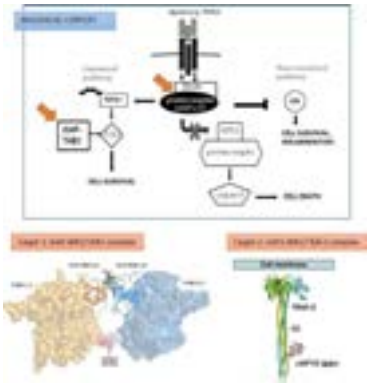

Figure1: *Complexes targeted for innovative cancer therapy: Simplified NF-kB pathway in the upper box: The arrows indicate the protein complexes targeted by our drug design study. Target 1: Crystal structure of XIAP-BIR1 (colored cartoons) in the presence of NF023 (in sticks) superimposed with the crystal structure of BIR1 in complex with TAB1 (colored surfaces, PDB id: 2POP): The compound potentially impairs X-BIR1 dimerization, destabilizing X-BIR1/TAB1 interaction. Target 2: cIAP2-BIR1 is recruited to TRAF2, which is anchored to the TNF receptors. NF023 is shown to impair cIAP2-BIR1/TRAF2 complex in silico, with low micromolar affinities*

Biography

Federica Cossu has always been interested in the field of cancer research, being fascinated by structural studies of crucial macromolecules and protein complexes involved in the cellular processes of cell death/survival. She gathered experience in cloning, expression, purification and crystallization of recombinant proteins, mainly belonging to the field of cancer. From the last few years, she has focused on the structure-based design of small molecules to be developed, as drug candidates directed to pre-clinical studies. The success of this activity is proved by one patent, one award for her PhD thesis and several publications in the field. She progressively improved her knowledge on biophysical techniques for the study of proteins and on the in-silico analysis of protein structures. She has collected experience in European laboratories, including several short visits/experiments at the ESRF synchrotron in Grenoble and at Soleil in Paris. She has been working in lab for ten years, covering various positions within the research group, from Master's degree student to Post-Doctorate. She has been the supervisor of students, also giving lessons on the structural approaches applied to cancer therapy.

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Day 3

Sessions:

Structural Biology in Cancer Research | Current Trends

Session Chair
Guo-Ping Zhou
Gordon Life Science Institute, USA

Session Co-Chair
Tzu-Ching Meng
Academia Sinica, Taiwan

Session Introduction

Title: **The NMR studies of the interaction between polysialic acid (polySA) and the PSTD peptide in ST8Sia IV polysialytranseferase**
Guo-Ping Zhou, Gordon Life Science Institute, USA

Title: **Understanding differential selectivity of arrestins toward the phosphorylation state of G-protein-coupled receptors**
Ozge Sensoy, Istanbul Medipol University, Turkey

Title: **Structural basis for the cooperative allosteric activation of the free fatty acid receptor GPR40**
Stephen M. Soisson, Merck Research Laboratories, USA

Title: **Conformational dynamics revealed by ensemble cryo-EM**
Andrei Korostelev, UMass Medical School, USA

Title: **Structural basis for PTPN3-p38gamma complex involved in colon cancer progression**
Tzu-Ching Meng, Academia Sinica, Taiwan

Title: **Holliday junction resolvase GEN1 functions as a versatile DNA sensor and processor**
Christian Biertümpfel, Max Planck Institute of Biochemistry, Germany

Title: **Monitoring protein structural changes on a proteome-wide scale**
Paola Picotti, ETH Zurich, Switzerland

Title: **Slow domain reconfiguration causes power law kinetics in a two-state enzyme**
Hagen Hofmann, Weizmann Institute of Science, Israel

Title: **Structural and dynamic studies of DENV and ZIKV proteases and its insight into inhibitor design**
CongBao Kang, Agency for Science, Technology and Research (A*STAR), Singapore

Title: **Assembly mechanism of foreign dsDNA-sensing inflammasomes**
Jungsan Sohn, Johns Hopkins University, USA

Title: **Functional divergence in protein families: A co-variation analysis**
Marie Chabbert, University of Angers, France

Title: **RNA-binding domain disorder modulates the RNA destabilizing activity in the TTP family of proteins**
Francesca Massi, University of Massachusetts Medical School, USA

Title: **Role of conformational equilibrium in molecular recognition and capsid assembly: the case of flavivirus capsid proteins**
Fabio C. L. Almeida, Federal University of Rio de Janeiro, Brazil

Title: **Integrative approaches for variant interpretation in coding regions**
Sushant Kumar, Yale University, USA

Title: **Generation of long acting therapies using glycosylated linkers**
Abdulrahman Alshehri, University of Sheffield, UK

Title: **Hydrogen bond interaction with trypanosomal adenosine kinase; ornithine decarboxylase and triose phosphate isomerase could not be involved in the antitrypanosomal activity of stigmasterol: An *in silico* study**
Aminu Mohammed, Ahmadu Bello University, Nigeria

Guo-Ping Zhou, J Proteomics Bioinform 2017, 10:8(Suppl)
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The NMR studies of the interaction between polysialic acid (polySA) and the PSTD peptide in ST8Sia IV polysialytranseferase

Guo-Ping Zhou
Gordon Life Science Institute, USA

The α 2,8-sialyltransferase (ST8Sia) family consists of 6 sia-lytranseferases, which are related to forms of polysialic acid chains (PSA) on neural cell adhesion molecule (NCAM) and NCAM polysialylation, and have important effects on formation of sialic acid storage diseases, neural system diseases and invasive cancers. It has been known that synthesis of PSA chains is catalyzed by two polysialyltransferases, ST8Sia II (STX) and ST8Sia IV (PST). In addition, a polybasic motif of 32 amino acids in both ST8Sia II and ST8Sia IV has been designated as "polysialytransferase domain" (PSTD), which is essential for NCAM polysialylation. In this study, we have determined the 3D structure of the PSTD peptide containing 22 amino acids (22AA) in ST8Sia IV using NMR spectroscopy. This NMR-based model displays that the PSTD domain consists of an α-helical segment, two unstructured domains in both N- and C-terminus, and two three-residue-loops near the C-terminus of the peptide. Our overlaid 2D 1H-15N-HSQC spectra of the 22AA-PSTD peptide show that the amide proton chemical shifts of some amino acids such as I260, I261, H262, R265, L269 and K272 have been changed after polySA was mixed with the PSTD peptide. In addition, the peak intensity of A263, V264, R265, Y267, L269 and K272 were also decreased after adding polySA. However, there is no any change in both chemical shift and the amide proton peak intensity for all other residues located on outside of the helix. Above NMR results indicate a weak interaction exists between the helix of the PSTD and the PolySA, which may play a vital role in modulating biosynthesis of polySA chain and NCAM polysialylation.

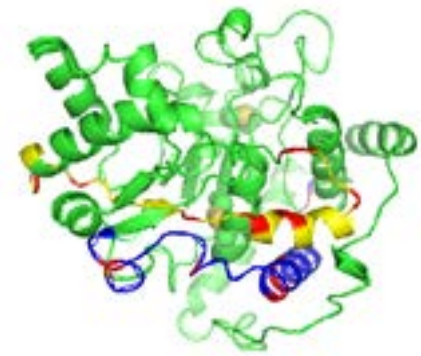

Figure1: *The 3D models of ST8Sia IV. The PSTD domain is shown by yellow. All basic residues in PSTD and PBR motifs are shown by red.*

Biography

Guo-Ping Zhou is currently a Distinguished Professor of Gordon Life Science Institute, USA. He is also an Adjunct Professor of several academics in the United States and China. He received his PhD in Biophysics from University of California at Davis, and completed his Postdoctoral training at Stanford University and Harvard University, respectively. He has determined the 3D NMR structures of some important proteins, protein-DNA complexes, and super lipids. He has successfully introduced the elegant wenxiang diagrams to elucidate the biological mechanisms of protein-protein/ligands interactions observed by NMR. Meanwhile, he has also published many papers in bioinformatics, and edited some special issues on structural biology for several influential scientific journals.

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Understanding differential selectivity of arrestins toward the phosphorylation state of G-protein-coupled receptors

Ozge Sensoy
Istanbul Medipol University, Turkey

Arrestins (Arrs) are a family of four proteins (Arr1- 4) which mediate G-protein-coupled receptor (GPCR) desensitization and internalization by coupling to active and phosphorylated receptor. Recently, they have also been shown to mediate GPCR-independent signaling pathways. The specific functions of Arrs (desensitization vs. G-protein-independent signaling) can be regulated by differential phosphorylation of the receptor, which is known as the *phosphorylation barcode*. The molecular mechanism responsible for formation of a high-affinity complex between an Arr subtype and a GPCR having a certain phosphorylation pattern remains elusive but is crucial for directing the subtype towards a specific functional role, and hence paves the way for development of safer therapeutics with fewer side-effects. As a first step in that direction, we have started with elucidating the activation mechanism of Arr subtypes by carrying out comparative molecular dynamics (MD) studies of the two members of the family, namely Arr1 and Arr3, which exhibit the largest differences in terms of phosphorylation selectivity. In addition, we also modeled and simulated Arr1-R175E mutant, which is known to be constitutively active, and compared it to Arr1 and Arr3 to detect activation-related rearrangements. We found novel structural elements that had not been considered before as determinants for activation and can be targeted with drugs for functional modulation. The emerging model also proposes that activation of Arr1-R175E is connected to perturbation of the well-known region, namely, the polar-core, whereas no changes were observed in that region in Arr3 despite the presence of other activation-related changes. With that, we could propose a structural model to explain the molecular mechanism responsible for markedly reduced selectivity of Arr3 towards phosphorylated GPCRs. Finally, knowledge achieved in this study can also be utilized to modulate Arr binding to GPCRs under disease conditions such as otozomal dominant disorders and congestive heart failure.

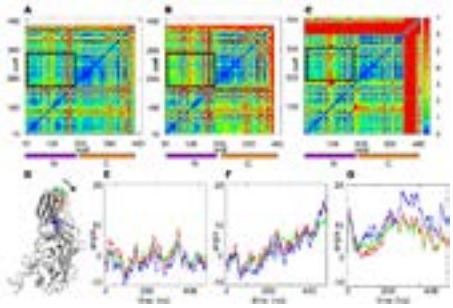

Figure1: *Top, Distance fluctuation maps calculated for Cα-Cα distances along the MD trajectories for (A) Arr1, (B) Arr1-R175E (B) and (C) Arr3. N and C-domains are identified by stripes. The dashed rectangles highlight the distance fluctuations in the N-C interface region. Bottom, Panel D: View of Arr3, with rotation axis perpendicular to the Picture plane and passing through Cα of I321, depicted in black (I306 in Arr1); rotation on this axis in the direction of the arrow corresponds to an increase in the rotation angle. Cα atoms used in quantifying the rotation along the MD trajectory are represented in blue, red, and green. Panels (E-G): Time dependent rotation of the selected residues in Arr1-WT (E), in Arr1R175E (F) and Arr3(G).*

Biography

Ozge Sensoy being a Computational Biophysicist, her research has focused on understanding molecular mechanisms of biologically important problems and providing mechanistic insight at the molecular level. In particular, she has been working with GPCRs and their interacting partners which are responsible for cellular signaling. She works in close collaboration with medicinal chemists to direct them for effective molecular designs. In addition, she is also responsible for testing the efficacy of these molecules *in silico* before transferring them to either *in vitro* or *in vivo* studies. Recently, she has been awarded an international COST (European Cooperation in Science and Technology) grant which is based on developing heterobivalent molecules capable of binding more than one target for treatment of symptoms of Parkinson's disease.

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Notes:

Stephen M Soisson, J Proteomics Bioinform 2017, 10:8(Suppl)
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Structural basis for the cooperative allosteric activation of the free fatty acid receptor GPR40

Stephen M Soisson
Merck Research Laboratories, USA

Clinical studies indicate that partial agonists of the G-protein-coupled, free fatty acid receptor GPR40 enhance glucose-dependent insulin secretion and represent a potential mechanism for the treatment of type 2 diabetes mellitus. Recently identified, full allosteric agonists (AgoPAMs) of GPR40 bind to a site distinct from partial agonists and can provide additional efficacy. Our recent studies have led to a 3.2-Å crystal structure of human GPR40 (hGPR40) in complex with both the partial agonist MK-8666 and an AgoPAM. Surprisingly, the structure reveals a novel lipid-facing AgoPAM-binding pocket outside the transmembrane helical bundle. Comparison with an additional 2.2-Å structure of the hGPR40–MK-8666 binary complex reveals an induced-fit conformational coupling between the partial agonist and AgoPAM binding sites, involving rearrangements of the transmembrane helices 4 and 5 (TM4 and TM5). These structural rearrangements, along with AgoPAM binding, appear to trigger the transition of intracellular loop 2 (ICL2) into a short helix. These conformational changes likely prime GPR40 to a more active-like state and explain the binding cooperativity between these ligands.

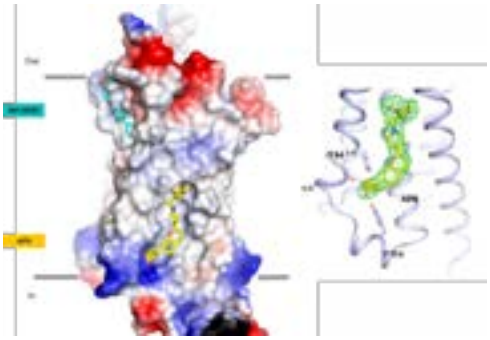

Figure1: *Structural basis for the cooperative allosteric activation of the free fatty acid receptor GPR40*

Biography

Stephen M Soisson, PhD is a Director of Biochemical Engineering and Structure at Merck Research Laboratories in West Point, Pennsylvania (USA). With 25+ years of structural biology experience, he has focused research on elucidating the structural aspects of biological regulatory mechanisms, and applying these insights in the area of structure-based drug design. He has served on the scientific advisory boards of the Structural Genomics Consortium, and the GPCR Consortium.

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Conformational dynamics revealed by ensemble cryo-EM

Andrei Korostelev
UMass Medical School, USA

Virus propagation depends on efficient synthesis of viral proteins by the host translational machinery. Internal ribosome entry sites (IRESs) of viral mRNAs mediate cap-independent initiation. Intergenic-region (IGR) IRESs of *Dicistroviridae* family, which includes the Taura syndrome virus (TSV) and Cricket paralysis virus (CrPV), use the most streamlined mechanism of initiation, independent of initiation factors and initiator tRNA. A tRNA-mRNA like pseudoknot of IGR IRESs binds the ribosomal A (aminoacyl-tRNA) site of the 80S ribosome. The pseudoknot translocates to the P site to allow binding of the first tRNA and initiate translation. Using electron cryo-microscopy of a single specimen, we resolved five ribosome structures formed with the Taura syndrome virus IRES and translocase EEF2 GTP bound with sordarin. The structures suggest a trajectory of IRES translocation, required for translation initiation, and provide an unprecedented view of eEF2 dynamics. The IRES rearranges from extended to bent to extended conformations. This inchworm-like movement is coupled with ribosomal inter-subunit rotation and 40S head swivel. eEF2, attached to the 60S subunit, slides along the rotating 40S subunit to enter the A site. Its diphthamide-bearing tip at domain IV separates the tRNA-mRNA-like pseudoknot I (PKI) of the IRES from the decoding center. This unlocks 40S domains, facilitating head swivel and biasing IRES translocation *via* hitherto-elusive intermediates with PKI captured between the A and P sites.

Biography

Andrei Korostelev is passionate about mechanisms of translation regulation. He received PhD in Michael S Chapman laboratory at Florida State University in 2003 and performed Postdoctoral studies with Harry F Noller in 2004-2010. The Korostelev laboratory at the RNA Therapeutics Institute uses recent advances in biochemical and structural methods to elucidate detailed mechanisms that govern translation and regulation of translation under stress conditions or during disease. Recent work revealed high-resolution "frames" of the motions that the translational machinery undergoes during bacterial stress responses (including the stringent response) and viral infection, as summarized on the laboratory web site: http://labs.umassmed.edu/korostelevlab/research.htm

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Structural basis for PTPN3 - p38gamma complex involved in colon cancer progression

Tzu Ching Meng
Academia Sinica, Taiwan

The Ras signaling cascade acts as a key driver in human colon cancer progression. Among the modules in this pathway, p38gamma (MAPK12) and its specific protein tyrosine phosphatase PTPN3 (PTPH1) are critical regulators responsible for Ras oncogenic activity. However, the molecular basis for their interaction is completely unknown. Here we report the unique architecture of the PTPN3-p38gamma complex by employing an advanced hybrid method integrating X-ray crystallography, small-angle X-ray scattering (SAXS) and chemical cross-linking/mass spectrometry (CX-MS). Our crystal structure of PTPN3 in complex with the p38gamma phosphopeptide presented a unique feature of the E-loop that defines the substrate specificity of PTPN3 towards fully activated p38gamma. The low-resolution structure demonstrated the formation of an active-state or a resting-state complex of PTPN3-p38gamma. We showed a regulatory function of PTPN3's PDZ domain, which stabilizes the active-state complex through interaction with the PDZ-binding motif of p38gamma. Using SAXS and CX-MS approaches, we found that binding of the PDZ domain to the PDZ-binding motif lifts the atypical auto-inhibitory constraint of PTPN3, enabling efficient tyrosine dephosphorylation of p38gamma to occur. Our findings emphasize the potential of structural approach for PTPN3-p38gamma complex that may deliver new therapeutic strategies against Ras-mediated oncogenesis in colon cancer.

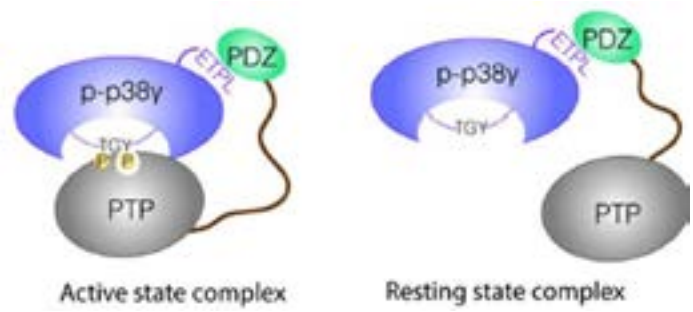

Figure1: *Our structural data suggest the presence of the active state complex (left) and the resting state complex (right) between PTPN3 and p38gamma. The interaction of PDZ domain in PTPN3 and the ETPL motif in p38gamma drives the complex formation. We show that the resting state complex may promote oncogenic signaling involved in colon cancer progression.*

Biography

Tzu Ching Meng has completed his PhD from University of Nebraska Medical Center in 1999 and Post-doctoral studies from Cold Spring Harbor Laboratory in 2003. Since then, he has been working at Academia Sinica, the premier government-funded institution in Taiwan. He is currently a Research Fellow with Professorship jointly appointed by National Taiwan University. He has published more than 40 papers in reputed journals and has been serving as an Advisory Board Member of competitive journals.

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Holliday junction resolvase GEN1 functions as a versatile DNA sensor and processor

Christian Biertumpfel
Max Planck Institute of Biochemistry, Germany

Several DNA repair and maintenance pathways depend on the correct and efficient processing of DNA intermediates by structure-specific nucleases. Human Holliday junction resolvase GEN1 seems to be an enzyme of last resort for recognizing and cleaving a specific range of DNA structures. The crystal structure of human GEN1 in complex with Holliday junction DNA pinpointed to a crucial role of the chromodomain for efficient DNA recognition and cleavage. We further characterized different DNA-binding modes of GEN1 using biochemical methods in combination with structure-guided mutagenesis. The analysis highlights the importance of the arch region to distinguish between different DNA substrates. In addition, we identified a cluster of positive amino acids shadowing the chromodomain to assist the enzyme for robust DNA recognition. Moreover, we directly show that GEN1 operates as a monomer with 5' flap DNA and as a dimer in complex with DNA four-way junctions, which is a unique feature in the Rad2/XPG nuclease family. This linked cleavage mechanism ensures that DNA junctions are resolved in a strictly symmetric manner without altering DNA information. GEN1's DNA recognition features make it a versatile tool for DNA processing and for maintaining genome integrity.

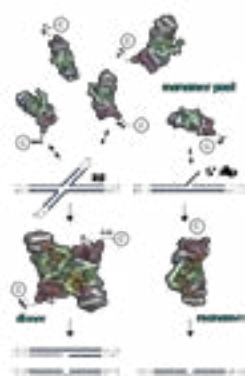

Figure1: *Holliday junction resolvase GEN1 is a monomer in solution and thus, cleavage competent for 5' flap substrates. However, it can only cleave DNA four-way junctions by forming an active nuclease dimer.*

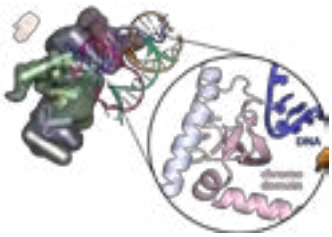

Figure2: *Structure of human Holliday junction resolvase GEN1 in complex with a DNA four-way junction. The nuclease domain is extended by a chromodomain for efficient DNA recognition and cleavage.*

Biography

Christian Biertumpfel obtained his PhD degree from the European Molecular Biology Laboratory (EMBL) and the Ruprecht Karls University of Heidelberg, Germany. His PhD research focused on the crystallization and characterization of Holliday junction resolvases. During his Postdoctoral time at the National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, USA, he could solve a first crystal structure of a Holliday junction resolvase from T4 phages in complex with a DNA four-way junction. Furthermore, together with Wei Yang he determined the structure and mechanism of human DNA polymerase η functioning as a molecular splint. After a short period at the Vaccine Research Center, NIAID, NIH, he moved to the Max Planck Institute of Biochemistry, Martinsried, Germany as a Max Planck Research Group Leader. Recently, the Biertumpfel Lab obtained structural information on the human Holliday junction resolvase GEN1 and they found for the first time a chromodomain extending a nuclease domain.

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Notes:

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Monitoring protein structural changes on a proteome-wide scale

Paola Picotti
ETH Zurich, Switzerland

Protein structural changes induced by external perturbations or internal cues can profoundly influence protein activity and thus modulate cellular physiology. Mass spectrometry (MS)-based proteomic techniques are routinely used to measure changes in protein abundance, post-translational modification and protein interactors, but much less is known about protein structural changes, owing to the lack of suitable approaches to study global changes in protein folds in cells. In my talk, I will present a novel structural proteomics technology developed by our group that enables the analysis of protein structural changes on a proteome-wide scale and directly in complex biological extracts. The approach relies on the coupling of limited proteolysis (LiP) tools and an advanced MS workflow. LiP-MS can detect subtle alterations in secondary structure content, larger scale movements such as domain motions, and more pronounced transitions such as the switch between folded and unfolded states or multimerization events. The method can also be used to pinpoint protein regions undergoing a structural transition with peptide-level resolution. I will describe selected applications of the approach, including 1. The identification of proteins that undergo structural rearrangements in cells due to a nutrient shift; 2. The analysis of *in vivo* protein aggregation; 3. The cell-wide analysis of protein thermal unfolding; and 4. The identification of protein-small molecule interactions (e.g. drug-target deconvolution). I will discuss the power and limitations of the method and possible new directions in structural biology enabled by this emerging approach to protein structure analysis.

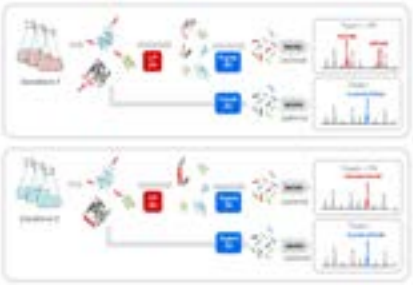

Figure1: *LiP-MS approach. A native proteome extract is incubated with a broad-specificity protease for a short time. Red arrows indicate LiP sites. Protease activity is quenched by shifting the proteome to denaturing conditions and complete trypsin digestion is then performed. A fraction of the same sample is only subjected to the trypsin step. The samples are analyzed by MS and levels of the resulting fully tryptic (FT) and half-tryptic (HT) peptides are compared. A FT peptide containing a LiP cleavage site will be detected in the trypsin control and replaced by two HT halves in the sample subjected to LiP. N and D indicate native and denaturing conditions, respectively.*

Biography

Paola Picotti completed her PhD at the University of Padua (Italy) and then joined the group of Ruedi Aebersold at ETH Zurich (Switzerland), where she developed novel targeted proteomic techniques. In 2011, she was appointed Assistant Professor at ETH Zurich. Her group develops structural and chemoproteomics methods and uses them to study the consequences of intracellular protein aggregation. Paola Picotti's research was awarded an ERC Starting grant, a Professorship grant from the Swiss National Science Foundation, the Latsis Prize, the Robert J Cotter Award, the SGMS Award and the EMBO Young Investigator Award. Main contributions of her group are the development of a structural method to analyze protein conformational changes on a system-wide level, the discovery of novel allosteric interactions, the analysis of the determinants of proteome thermostability and the identification of a novel neuronal clearance mechanism for a protein involved in Parkinson's disease.

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Notes:

Hagen Hofmann et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Slow domain reconfiguration causes power law kinetics in a two-state enzyme

Hagen Hofmann, Iris Grossman Haham, Gabriel Rosenblum and **Trishool Namani**
Weizmann Institute of Science, Israel

Conformational transitions in proteins are typically captured well by rate equations that predict exponential kinetics for two-state reactions. Here, we describe a remarkable exception. The electron-transfer enzyme quiescin sulfhydryl oxidase (QSOX), a natural fusion of two functionally distinct domains, switches between open and closed domain arrangements with apparent power law kinetics. Using single-molecule Foerster resonance energy transfer (FRET) experiments on timescales from nanoseconds to milliseconds, we showed that the unusual open-close kinetics results from slow domain rearrangements in a heterogeneous ensemble of open conformers. While substrate accelerates the kinetics, thus suggesting a substrate-induced switch to an alternative free energy landscape of the enzyme, the power-law behavior is also preserved upon electron load. Our results show that conformational multiplicity with slow sampling dominates the motions of QSOX, thus providing an explanation for catalytic memory effects in other enzymes.

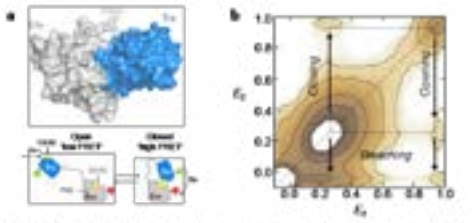

Figure 1: Structure of TbQSOX and domain motions measured with single-molecule FRET. (a) Structure of the enzyme TbQSOX in the closed conformation with Trx domain (blue) and Erv-domain (gray) docked to each other and schematic representation of the open and closed state that correspond to populations with low and high FRET, respectively. (b) Two-dimensional occurrence map for TbQSOX. Off-diagonal cross-peaks arise from opening/closing dynamics of the domains of the enzyme.

Biography

Hagen Hofmann received his PhD from the Martin Luther University Halle-Wittenberg (Germany) in 2008. In the period 2008 - 2014, he was a Postdoctoral Fellow at the University of Zurich in the group of Benjamin Schuler and since 2014 he is heading the “Molecular Systems Biophysics” group at the Weizmann Institute of Science (Israel). He and his group use a broad set of single-molecule fluorescence tools to understand the dynamics of proteins and protein networks on timescales from nanoseconds to hours. In addition, live-cell imaging, *in vivo* single-molecule FRET, and single particle tracking is used to monitor proteins in live cells. His interest ranges from the physics of disordered proteins over coupled binding and folding reactions up to stochastic genetic circuits and regulatory protein networks.

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Notes:

CongBao Kang, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Structural and dynamic studies of DENV and ZIKV proteases and its insight into inhibitor design

CongBao Kang
Agency for Science, Technology and Research, Singapore

Dengue virus (DENV and Zika virus (ZIKV) belong to *Flaviviridae* family which contains important human pathogens. DENV affects people living in tropical and subtropical regions. DENV infection can cause serious diseases such as dengue fever. ZIKV has drawn worldwide attention because of the outbreak in 2015. Viral genome of a *flavivirus* encodes a polyprotein that can be processed into structural and non-structural (NS) proteins by both host and viral proteases. Viral protease is a two-component serine protease formed by a cofactor region (~40 aa) from NS2B and a protease region (~170 aa) from NS3. The NS2B-NS3 protease of DENV or ZIKV is a validated target because of their function in maturation of viral proteins. Structural studies have been conducted for both DENV and ZIKV proteases. For DENV, previous studies have demonstrated that the free protease adopts an open conformation in which the C-terminal part of the NS2B cofactor region stays away from the active site. In the presence of an inhibitor, DENV protease forms a closed conformation in which the C-terminal region of NS2B forms part of the active site and interacts with the inhibitor. Our NMR study reveals that an unlinked DENV protease adopts the closed conformation in solution. Based on the knowledge on DENV protease, several constructs were made for ZIKV protease. Structural studies demonstrated that ZIKV protease adopts the closed conformation in the absence and presence of an inhibitor or substrate. The linker or enzymatic cleavage site present between NS2B and NS3 may affect inhibitor to interact with the active site. Our accumulated studies have shown that the unlinked protease construct can be used for studying protease-inhibitor interactions. We have demonstrated that the unlinked ZIKV protease interacts with different types of inhibitors. Our studies will be helpful for structure-based inhibitor design against both ZIKV and DENV proteases.

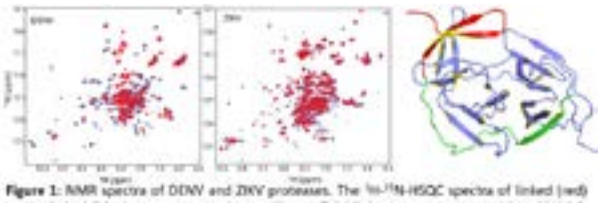

Figure 1: NMR spectra of DENV and ZIKV proteases. The ^{1H}-^{15N}-HSQC spectra of linked (red) and unlinked (blue) proteases are shown. The artificial linker contains nine residues (G$_4$S)$_2$. The structure of unlinked ZIKV protease is shown. The NS2B cofactor region and NS3 (blue) are shown in different colors. The C-terminal region of NS2B which can be affected by the artificial linker is highlighted in red.

Biography

CongBao Kang received his PhD from School of Biological Sciences at Nanyang Technological University (NTU). He was a Research Fellow at Centre for Structural Biology, Vanderbilt University, where he was working on structural determination of disease-related membrane proteins. He is currently the group leader of high End NMR group at ETC. His group is working on protein structure, dynamics and its interaction with potential drug candidates using solution NMR spectroscopy. The goal of his group is to provide structural information of a target protein to the medicine chemists to understand structure-activity relationship of potent compounds. His group is involving in hits identification, hits to lead, and lead optimization steps of the drug discovery process. His is currently working on target-based drug discoveries. The targets include methyltransferases, kinases, ion channels, membrane-bound receptors, protein-protein interactions, and viral proteins.

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Notes:

Jungsan Sohn, J Proteomics Bioinform 2017, 10:8(Suppl)
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Assembly mechanism of foreign dsDNA-sensing inflammasomes

Jungsan Sohn
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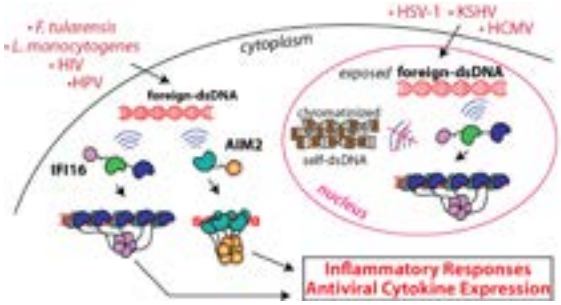
Absent-in-melanoma-2-like receptors (ALRs) detect foreign double-stranded (ds)DNA from invading pathogens and assemble into filamentous signaling platforms termed inflammasome. The ALR filaments play crucial roles in launching antiviral and inflammatory responses against many pathogens (e.g. HIV and HSV); however, persistent ALR complexes are also linked to autoimmune disorders (e.g. Sjogren's syndrome and lupus). Here, by combining solution assays, electron microscopy, and single-molecule methods, we investigate the filament assembly mechanisms of two prototypical ALRs, namely Interferon Gamma Inducible Protein 16 (IFI16) and (absent in melanoma 2) AIM2. (1) IFI16 detects foreign dsDNA both in the host nucleus and cytoplasm. We found that IFI16 uses dsDNA as a one-dimensional diffusion-scaffold to assemble into filaments. The dsDNA-binding HIN200 domains of IFI16 are responsible for tracking dsDNA, while its pyrin domain (PYD) is necessary for filament assembly. Importantly, nucleosomes represent barriers that prevent IFI16 from targeting host dsDNA by directly interfering with its assembly. This unique scanning-assisted assembly mechanism would allow IFI16 to distinguish self- from non-self-dsDNA in the nucleus. (2) AIM2 detects cytoplasmic dsDNA and assembles into an inflammasome. We found that the PYD of AIM2 (AIM2 PYD) drives both filament formation and dsDNA binding. As with IFI16, the size of exposed dsDNA acts a key regulator for the polymerization of AIM2. The helical symmetry of the upstream $AIM2^{PYD}$ filament is consistent with the filament assembled by the PYD of the downstream ASC adaptor, indicating that AIM2 acts as a structural template for polymerizing ASC. Together, our studies provide a unifying paradigm for how ALRs carry out foreign dsDNA-sensing pathways, where generating a structural template by coupling ligand-binding and oligomerization plays a key signal transduction mechanism.



Biography

Jungsan Sohn has his research focused on understanding the molecular mechanism by which human immune system engages invading pathogens. He received BS from University of Michigan, and PhD from Duke University. Upon completing his Post-doctoral training at MIT with Dr. Bob Sauer, he joined the faculty at Johns Hopkins in 2011.

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Notes:

Marie Chabbert, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Functional divergence in protein families: A co-variation analysis

Marie Chabbert
University of Angers, France

Statement of the Problem: Co-variations between positions in a multiple sequence alignment may reflect structural, functional, and/or phylogenetic constraints. Numerous co-variation methods have been developed and may yield a wide variety of results. However, few studies have been undertaken to determine co-variations methods adequate to gain information on functional divergence within a protein family.

Methodology & Theoretical Orientation: We explore co- variation methods for their capability to mine co-varying positions related to the functional divergence in a protein family. To reach this objective, we compare several methods on a model system that consists of three nested sets of about 300, 100, and 20 paralogous sequences of a protein family, the class A of G protein-coupled receptors. The co- variation methods analyzed are based on chi2 scores, mutual information, substitution matrices, or perturbation methods. We analyze the dependence of the co-variation scores on residue conservation, measured by sequence entropy, and the networking structure of the top pairs.

Findings: Out of the four methods that privilege top pairs with intermediate entropy, two favor individual pairs, whereas the other two methods, OMES (Observed minus Expected Squared) and ELSC (Explicit Likelihood of Subset Covariation), favor a network structure with a central residue involved in several high scoring pairs. This network structure is observed for the three sequence sets, making a hierarchical analysis possible. In each case, the central residue corresponds to a residue known to be crucial for the evolution of the protein family and the sub-family specificity. Positions co-varying with this central residue form a few clusters in the receptor 3D structure.

Conclusion & Significance: The central residues obtained with the OMES or ELSC methods can be viewed as evolutionary hubs, in relation with an epistasis-based mechanism of functional divergence within a protein family.

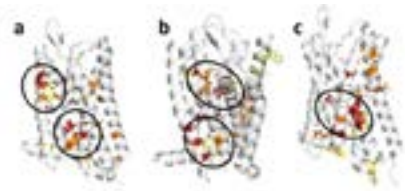

Figure1: *Visualization of co-varying residues in set 1 (a), 2 (b) and 3 (c) on the 3D structure of typical G protein coupled receptors. The color code, from yellow to red, is indicative of the co-variation score with the central residue of the network.*

Biography

Marie Chabbert is a Scientist from the French CNRS (Centre National de la Recherche Scientifique). She has her expertise in molecular modeling and bioinformatics approaches to the structure-function relationship of proteins. She has special interest in deciphering the mechanisms that drove protein evolution and in using evolutionary data to gain structural and functional information on protein families. She is presently working on the G protein-coupled receptors, especially chemotaxis and vasoactive peptide receptors.

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Notes:

Francesca Massi, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

RNA-binding domain disorder modulates the RNA destabilizing activity in the TTP family of proteins

Francesca Massi
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Despite the importance of RNA-binding proteins to gene regulation, our understanding of how their structure and dynamics contribute to their biological activity is limited. In this study, we focus on two related RNA-binding proteins—TTP and TIS11d—that regulate the stability of mRNA transcripts encoding key cancer-related proteins, such as tumor necrosis factor-α and vascular endothelial growth factor. These two proteins display differential folding propensity in the absence of RNA, despite sharing a high sequence identity. We identified three residues located at the C-terminal end of an α-helix that determine the folding propensity of the RNA-binding domain in the apo state. We also showed that stabilization of the structure of the RNA-binding domain is associated with differences in RNA-binding activity *in vitro* and increased RNA-destabilizing activity in the cell. Phylogenetic analysis indicates that this family of proteins has only recently evolved to be able to modulate its biological activity through its dynamic structure. To investigate how three residues determine the folding and stability of the TZF domain we used molecular dynamics and NMR spectroscopy. We observed that a π-π stacking between the side chains of a conserved phenylalanine and the zinc coordinating histidine is essential to maintain the correct tetrahedral geometry between the three cysteines, the histidine and the zinc ion. A hydrogen bond in the C-terminal zinc finger of TIS11d is important to keep the phenylalanine in proximity of the imidazole ring of the zinc coordinating histidine in a conformation that allows for stacking of the side chains. Lack of this hydrogen bond in TTP is responsible for the reduced zinc affinity of the C-terminal zinc finger. Sequence alignment shows that this phenylalanine residue is highly conserved. These results suggest that most CCCH-type zinc finger proteins employ π-π interactions to stabilize the structure of the TZF domain.

Figure1: *The stacking of the aromatic rings of the conserved Phe and of the zinc coordinating His stabilizes conformation of the His in a rotameric state compatible for zinc-binding.*

Biography

Francesca Massi is an Associate Professor in the Department of Biochemistry and Molecular Pharmacology at the University of Massachusetts Medical School. She received her Ph.D. in Chemistry with John E. Straub from Boston University and was a postdoctoral fellow with Arthur G. Palmer at Columbia University. Her research interests include protein function and dynamics studied with NMR spin relaxation experiments and computer simulations.

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Notes:

Fabio C L Almeida, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Role of conformational equilibrium in molecular recognition and capsid assembly: The case of flavivirus capsid proteins

Fabio C L Almeida
Federal University of Rio de Janeiro, Brazil

Proteins are dynamic entities able to move in a wide range of timescales that goes from picoseconds to seconds. Motions that occur in microseconds to seconds define biologically relevant events that are frequently involved in binding, allostery and catalysis. In our laboratory, we used relaxation parameter, relaxation dispersion experiments and molecular dynamic simulation to correlate conformational equilibrium with molecular recognition and catalysis. Dengue and Zika are major arthropod-borne human viral disease, for which no specific treatment is available. The flavivirus capsid protein is the trigger of virus assembly. Capsid proteins are located at the cytoplasm bound to lipid droplets (LD). Binding to LDs are essential for virus assembly. We previously showed that the positively charged N-terminal region of Dengue virus capsid protein prompts the interaction with negatively charged LDs, after which a conformational rearrangement enables the access of the central hydrophobic patch to the LD surface. We also showed the participation of the intrinsically disordered region in binding and possible regulation of capsid assembly. We probed the structure and dynamics of Dengue virus and Zika virus capsid proteins (DENVC and ZkC) by nuclear magnetic resonance. They bind lipid droplets (LD) in the cytoplasm, which mediates virus assembly in an unknown way. We showed that the dynamics of the capsid protein is intrinsically involved in the mechanism of LD and RNA binding and virus assembly. We also measured binding to nucleic acids and probed the assembly using small angle x-ray scattering and negative staining electron microscopy. The understanding and the participation of the intrinsically disordered N-terminal region and its dynamics helped us propose a mechanism for Dengue and Zika virus assembly and to develop a peptide with the potential to block virus assembly.

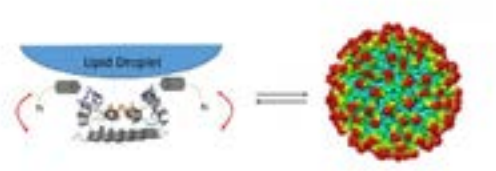

Figure1: *The role of conformational equilibrium in lipid droplets recognition and capsid assembly.*

Biography

Fabio C L Almeida has his expertise in protein structure and dynamics by nuclear magnetic resonance (NMR). He solved the structure of several proteins by NMR. He has important contribution in the structure and dynamics of plant defensins. He and his group showed that despite the conserved folding, defensins display a wide variation in dynamics, which enabled the mapping of binding regions and description of the mechanism of membrane recognition. The group also showed that dynamics are also the key for understanding the mechanism of membrane recognition of antimicrobial peptides. Pre-existent order in flexible peptides permits discrimination between the regions of specific and non-specific binding. His group has also described the structure and dynamics of the water cavity of thioredoxin, which is an essential structural element for catalysis. He is the Director of the National Center of NMR (CNRMN) and President of the Brazilian NMR Association (AUREMN).

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Notes:

Sushant Kumar, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Integrative approaches for variant interpretation in coding regions

Sushant Kumar
Yale University, USA

Statement of the Problem: The exponential rise in next-generation sequencing data is presenting considerable challenges in terms of variant interpretation. Though deep sequencing is unearthing large numbers of rare single nucleotide variants (SNVs), the rarity of these variants makes it difficult to evaluate their potential deleteriousness with conventional phenotype-genotype associations. Furthermore, many disease-associated SNVs act through mechanisms that remain poorly understood. 3D protein structures may provide valuable substrates for addressing these challenges. We present two general frameworks for doing so. In our first approach, we use localized frustration, which quantifies unfavorable residue interactions, as a metric to investigate the local effects of SNVs. In contrast to this metric, previous efforts have quantified the global impacts of SNVs on protein stability, despite the fact that local effects may impact functionality without disrupting global stability (e.g. in relation to catalysis or allostery). In our second approach, we employ models of conformational change to identify key allosteric residues by predicting essential surface pockets and information-flow bottlenecks (a new software tool that enables this analysis is also described). Importantly, although these two frameworks are fundamentally structural in nature, they are computationally efficient, thereby making analyses on large datasets accessible. We detail how these database-scale analyses shed light on signatures of conservation, as well as known disease-associated variants, including those involved in cancer.

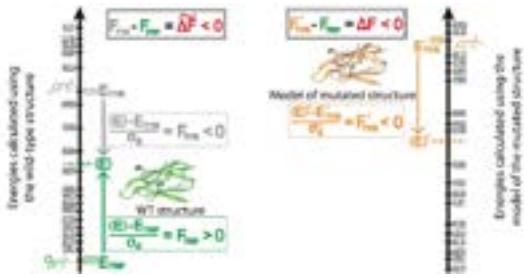

Figure1: *The effect of introducing a typical deleterious SNV (ΔF < 0). Each of the two vertical lines represents an energy-level diagram. Each level on this energy scale corresponds to the total energetic value of the protein if the residue position) were to be occupied by distinct amino acids. The ΔF associated with an SNV is negative if the SNV introduces a destabilizing effect.*

Biography

Sushant Kumar is a Postdoctoral Associate in the Molecular Biophysics and Biochemistry department at the Yale university. He has extensive experience in biological data mining, proteins simulations and cancer genomics. He is particularly interested in integrating genomic variation data and protein structural data to develop novel methods assessing disease variant impact. In past, he has applied coarse-grained models to decipher the role of various physical factors influencing the coupled folding and binding mechanism observed among disordered proteins.

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Abdulrahman Alshehri et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Generation of long acting therapies using glycosylated linkers

Abdulrahman Alshehri, R.J.Ross and **I.R.Wilkinson**
University of Sheffield, UK

Rationale: The current therapeutic drugs such as, growth hormone (GH), granulocyte colony-stimulating factor (GCSF) and leptin require once-daily injections, which are inconvenient and expensive. Therefore, a number of approaches to reducing therapeutic regimens clearance have been tried mainly through conjugation with another moiety. One such technology already being employed is PEGylation; however this has been shown to be non-biodegradable and toxic. A previous study by Asterion has shown that the use of glycosylated-linkers between two GH ligands to create protein-tandems resulted in their glycosylation and an increased molecular weight (MW) whilst maintaining biological activity. The use of this technology using GCSF as an example will be presented, but can be easily applied to other molecules such as leptin.

Hypothesis: The incorporation of variable glycosylated linkers between two GCSF ligands will create a construct with high molecular weight and protected from proteolysis resulting in reduced clearance with out blocking bioactivity.

Methodology: GCSF tandems with linkers containing between 2-8 NAT glycosylation motifs and their respective controls (Q replaces N in the sequence motif NAT so there is no glycosylation) were cloned, and sequenced. Following expression in Chinese hamster ovary (CHO) cells, expressed protein was analysed by SDS-PAGE to confirm molecular weights. *In vitro* bioactivity was tested using an AML-193 proliferation assay. Immobilised Metal Affinity Chromatography (IMAC) was used to purify the protein. Pharmacokinetic and pharmacodynamics properties of the purified GCSF tandem proteins were measured in normal Sprague Dawley rats with full ethical approval.

Results: Purified glycosylated tandems show increased molecular weight above that of controls when analysed by SDS-PAGE. All GCSF tandems show increased bioactivity in comparison to native GCSF. Following intravenous administration to rats, GCSF2NAT, GCSF4NAT, GCSF8NAT containing 2, 4 & 8 glycosylation sites respectively and GCSF8QAT (non-glycosylated GCSF tandem control) showed approximately 3-fold longer circulating half-life compared to that reported for the native GCSF (1.79 hours). Both GCSF2NAT and GCSF4NAT show a significant increase in the percentage of neutrophils over controls at 12 hours post injection. This effect however is short lived as the counts at 24+ hours are not significantly different to controls. GCSF8NAT shows an increase in the percentage of neutrophils that is only significant at 48 hours.

Conclusion: Results show that the use of glycosylated linkers to generate GCSF tandems results in molecules with increased molecular weight, improved *in vitro* bioactivity, longer circulating half-lives and enhanced neutrophilic population when compared to both native GCSF and the non-glycosylated tandem protein.

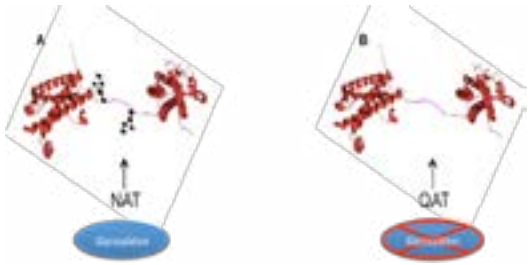

Figure1: *An example of 2NAT glycosylation motifs and its control 2QAT within a flexible linker (Gly4Ser)n between two GCSF ligands. (A) The glycosylation motif 2NAT inserted to the linker (glycosylated linker). (B) Non-glycosylation motif 2QAT control.*

Biography

Abdulrahman Alshehri working at security Forces Hospital, Riyadh, Saudi Arabia for 12 years. I did my Phd at sheffield University, UK. I have been working in creating long acting therapies using different stratigies such as, glycosylated linkers. Using multiple techniquies like PCR, gene cloning, cell culture, pharmacodynamic and pharmacokinetic to generate these therapies.

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Aminu Mohammed et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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Hydrogen bond interaction with trypanosomal adenosine kinase; ornithine decarboxylase and triose phosphate isomerase could not be involved in the antitrypanosomal activity of stigmasterol: An *in silico* study

Aminu Mohammed and **Mohammed Auwal Ibrahim**
Ahmadu Bello University, Nigeria

Stigmasterol has previously been reported to possess antitrypanosomal activity using *in vitro* and *in vivo* models. However, the mechanism of antitrypanosomal activity is yet to be elucidated. In the present study, molecular docking was used to decipher the mode of interaction and binding affinity of stigmasterol to three known antitrypanosomal drug targets viz; adenosine kinase, ornithine decarboxylase and triose phosphate isomerase. Stigmasterol was found to bind to the selected trypanosomal enzymes with minimum binding energy of -4.2, -6.5 and -6.6 kcal/mol for adenosine kinase, ornithine decarboxylase and triose phosphate isomerase respectively. However, hydrogen bond was not involved in the interaction of stigmasterol with all the three enzymes but hydrophobic interaction seemed to play a vital role in the binding phenomenon which was predicted to be non-competitive like type of inhibition. It was concluded that binding to the three selected enzymes, especially triose phosphate isomerase, might be involved in the antitrypanosomal activity of stigmasterol but not mediated *via* a hydrogen bond interaction.

Biography

Aminu Mohammed, an academic staff from Ahmadu Bello University, Zaria-Nigeria obtained his PhD Biochemistry from the famous University of KwaZulu-Natal, South Africa in Biomedical Research Lab. His research interest focus on screening and isolation of potent ingredients/nutraceuticals with antidiabetic or antitrypanosomal potentials from vast wealth of plants located in African region using modern spectroscopic techniques. In addition, we are interested in elucidating the possible mode of actions of extracts, compounds or nutraceuticals derived from the plants using various *in vitro* and *in vivo* models. Presently, we focus on the *in silico* computer simulation and improving bioavailability of spice-derived nutraceuticals as possible antidiabetic or antitrypanosomal agents.

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9th International Conference on

STRUCTURAL BIOLOGY

September 18-20, 2017 Zurich, Switzerland

Young Researchers Forum

Jessica L Thomaston, J Proteomics Bioinform 2017, 10:8(Suppl)
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XFEL structures of the M2 proton channel of influenza A reveal pH-dependent water networks under room temperature conditions

Jessica L Thomaston,
University of California, San Francisco, USA

The M2 proton channel of influenza A is a drug target that is essential for replication of the flu virus. It is also a model system for the study of selective, unidirectional proton transport across a membrane. Ordered water molecules arranged in wires inside the channel pore have been proposed to play a role in the conduction of protons to the four gating His37 residues and the stabilization of multiple positive charges within the channel. Previous crystallographic structures determined using a synchrotron radiation source were biased by cryogenic data collection conditions, and room-temperature data collection was limited by radiation damage. These problems have been avoided through room temperature diffraction at an X-ray free electron laser. Data were collected at an XFEL source to a resolution of 1.4 Å at three different pH conditions: pH 5.5, pH 6.5, and pH 8.0. Here, we examine the ordering of water in the M2 pore within crystals containing only the C_{open} conformation, which is an intermediate that accumulates at high protonation of the His37 tetrad. This allows us to access multiple protonation states of His37 in the C_{open} conformation and probe changes in solvent ordering prior to and following the release of a proton into the viral interior. At pH 5.5, a continuous hydrogen bonded network of water molecules spans the vertical length of the channel, consistent with a Grotthuss mechanism model for proton transport to the His37 tetrad. This ordered solvent at pH 5.5 could act to stabilize the positive charges that build up on the gating His37 tetrad during the proton conduction cycle. The number of ordered pore waters decreases at higher pH, where the C_{open} state is less stable. These studies provide a graphical view of the response of water to a change in charge within a restricted channel environment.

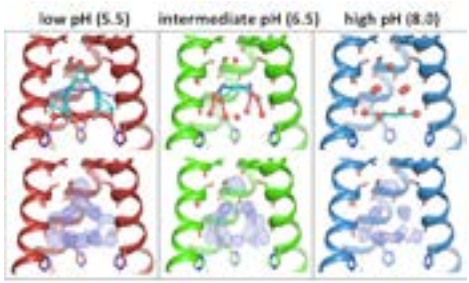

Figure1: *Room temperature XFEL structures of the M2 proton channel transmembrane domain at pH 5.5, 6.5, and 8.0. Solvent ordering is at a maximum at pH 5.5, with fewer ordered waters at pH 6.5 and pH 8.0. A continuous hydrogen bonding network is observed in the low pH condition and could be consistent with a Grotthuss transport mechanism for proton transport when the channel is at maximally conducting pH conditions.*

Biography

Jessica L Thomaston is a PhD candidate in the lab of Professor William DeGrado at the University of California, San Francisco. She studies the structure of the influenza M2 proton using lipidic cubic phase crystallization techniques and x-ray diffraction at synchrotron and XFEL sources. The M2 protein is among the smallest proton channels found in nature and is also a drug target against the flu. Her work focuses on the proton conduction mechanism of the M2 channel, particularly the involvement of water in proton transport and the structural characterization of how drugs and novel inhibitors bind to the channel and block proton conduction.

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Maharani Pertiwi Koentjoro et al., J Proteomics Bioinform 2017, 10:8(Suppl)
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Crystal structure of DNA-binding domain-CbnR with its promoter reveals the basis of the LysR-type transcriptional regulator recognition

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Cupriavidus necator NH9, which can utilize chlorocatechol as a sole carbon and energy source, degrades chlorocatechol with enzymes of the ortho-cleavage pathway. These enzymes are coded in the cbnABCD operon, of which expression is specifically regulated by a LysR-type transcriptional regulator CbnR. CbnR forms a tetramer and can be regarded as a dimer of dimers. The tetrameric CbnR has four DNA- binding domains and these DNA-binding domains recognize approximately 60 bp DNA sequence. The binding sequence is composed of two binding sites, recognition binding site and activation binding site. Each binding site seems to be recognized by two DNA-binding domains in the tetramer. While the crystal structure of the tetrameric CbnR has already been determined, the molecular mechanism of DNA recognition by CbnR remains elusive. We therefore initiated the crystal structure analysis of DNA-binding domain of CbnR in complex with RBS. The crystal structure would give an insight into the molecular mechanism of the CbnR-DNA interaction, which is the first step to understand the gene activation mechanism by LTTR. Here we report the crystal structure of CbnR(DBD) (residues 1 - 87) in complex with RBS, a 25-bp DNA fragment. The crystal structure was determined by the MR-native SAD method at 2.55 Å resolution with Rwork/Rfree of 0.221/0.264. The crystal structure shows that dimeric CbnR(DBD) interacts with RBS. The dimeric CbnR(DBD) adopts essentially the same conformation as that in the tetramic CbnR with the root mean squares deviation of 1.1 Å (174 Cα atoms). The 3α helix and the winged region of the winged-helix turn helix motif in CbnR(DBD) directly interact with the major and minor grooves of promoter sequence, respectively, and the interactions seem to bend DNA by approximately 30°. To further analyse the molecular mechanism of their interaction, biochemical analysis is in progress.

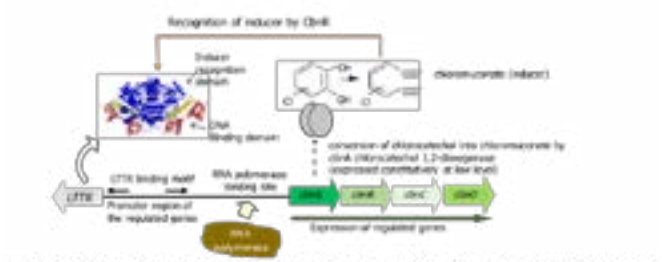

Fig. Binding of tetramer CbnR (CbnR, PDB: 1IXC) crystal structure (2.2 Å) to its promoter region lead to the transcriptional activation of the regulated genes.

Biography

Maharani Pertiwi Koentjoro is a 3rd year PhD student and the Monbukagakusho fellow in the United Graduate School of Agricultural Science, Gifu University, Japan. She has completed her BA from Sepuluh Nopember Institute of Technology, Indonesia, and a Master in Gadjah Mada University, Indonesia. Her research interests include molecular biological and biochemical investigation on bacteria. Currently she is working on structural studies of complex molecular machines that initiates LysR-Type Transcription Regulator in bacteria.

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Delivery methods for free electron lasers: Direct protein crystallization on solid supports economizes sample consumption in serial femtosecond crystallography

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Classical crystallography methods based on synchrotrons usually require crystals of relatively large dimensions, i.e. above 5 micrometres. The recent availability of X-ray free electron laser sources providing femtosecond X-ray pulses of ultrahigh brightness facilitate the investigation of nanocrystals. However, in this case data collection must be performed in the mode of the serial crystallography in so-called diffraction-before-destruction regime because the probed area of the sample is destroyed after the interaction with ultra-intense radiation. As thousands of crystals must be provided sequentially to the XFEL beam, selection of an efficient sample delivery system is crucial to minimize protein consumption during data collection. Delivery methods applied so far include steady streaming liquid jets of the crystal suspension. The application of more viscous media like lipidic cubic phase, agarose or hyaluronic acid matrices has also been demonstrated. However, all these methods use significant amounts of the precious protein, which cannot be recovered even if not directly probed. Recent developments of drop of demand methods or fixed targets allow overcoming this problem. But still, handling of the fragile crystals should be gentle or at best avoided. Microfabricated silicon chips with ultrathin Si3N4 membranes provide the possibility to regularly position crystals on precisely defined spots by direct crystallization using classical vapor diffusion method. The sample consumption is minimal since crystal growth takes place in nanolitre volume cavities. No additional sample transfer is needed, because X-rays are probing the crystals at the spot where they grew on the X-ray-transparent ultrathin amorphous silicon nitride membranes. Assembly with a second chip to form a hermetically sealed sandwich protects specimens from dehydration and facilitates *in situ* diffraction data collection at room temperature, as demonstrated in a synchrotron experiment providing high-resolution patterns.

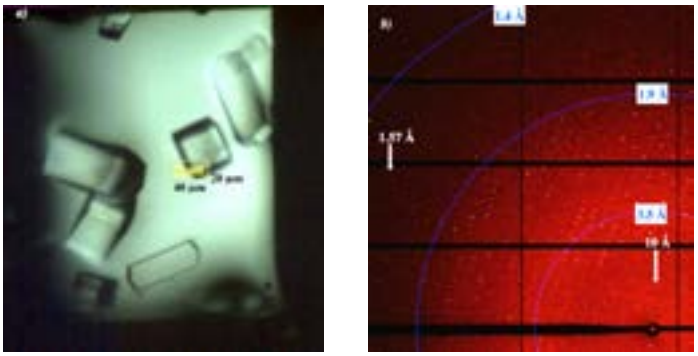

Figure1: *a) Lysozyme crystals enclosed in-between silicon nitride membranes and mounted on a synchrotron beamline goniometer. b) High resolution diffraction pattern obtained by in situ exposure with synchrotron radiation from the crystal as indicated in (a).*

Biography

Nadia L Opara joined the CINA group at the Biozentrum University of Basel and the LMN at PSI in Switzerland in 2014 in the frame of SNI PhD school program, to work on a project aiming at improving sample preparation methods for XFEL-based protein nano-crystallography. Beforehand she completed her Bachelor studies in chemistry and Master program in Molecular Biotechnology at the Lodz University of Technology in Poland.

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The challenge of improve disease treatment with protein engineering. The contributions of X-ray crystallography

Stephanie Bath de Morais and **Tatiana A C B Souza**
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Acute Lymphoid Leukemia is the most common neoplasia in childhood. The multi-therapeutic treatment resulted in remarkable advances in treatment of children, with 90.4% survival rate. L-asparaginase has been a central component of ALL therapy for over 40 years and acts by depleting plasma asparagine. In contrast to the normal cells, tumor cells lack the ability to synthesize asparagine and thus depend on external uptake of this amino acid for growth. Nowadays, three asparaginases are used in therapy: native L-asparaginase II from *Escherichia coli*, a pegylated form of this enzyme and L-asparaginase isolated from *Erwinia chrysanthemi*. Among the commercially available L-asparaginases, the *E. coli* enzyme presents the highest catalytic activity but also the highest toxicity, due to its further ability to hydrolyze glutamine, generating glutamate. Moreover, the immune response in patients under therapy with bacterial asparaginases can result in enzyme neutralization and the need to proceed the treatment with one of the alternative L-asparaginases. Based on the analysis of the available crystal structures we have designed, produced and crystallized E. coli asparaginase with modifications. Crystals diffracted up to 1.65 Å resolution at the Soleil Synchrotron. We combine structural analysis with kinetic and cellular approaches to identify the determinants of *E. coli* asparaginase toxicity. In addition, we have been working on the production of modified human asparaginases for structural characterization, kinetic and anti-leukemic activity assays. The introduction of human asparaginase in ALL treatment would avoid the problems caused by the bacterial enzymes, however a major difficulty in the therapeutic use of human enzyme comes from the fact that human asparaginases need to undergo activation through an auto-cleavage step, which was shown to be a low efficiency process *in vitro*, reducing the enzyme activity. These structural analyses gather insights about how engineering asparaginases can improve ALL treatment.

Figure1: *Schematic methodology of this project execution*

Biography

Stephanie Bath de Morais performs her PhD under Tatiana Souza supervision and coordinate projects involving advances in leukemia treatment advances. She is part of the team since 2013 and has expertise in molecular, structural and cancer biology.

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