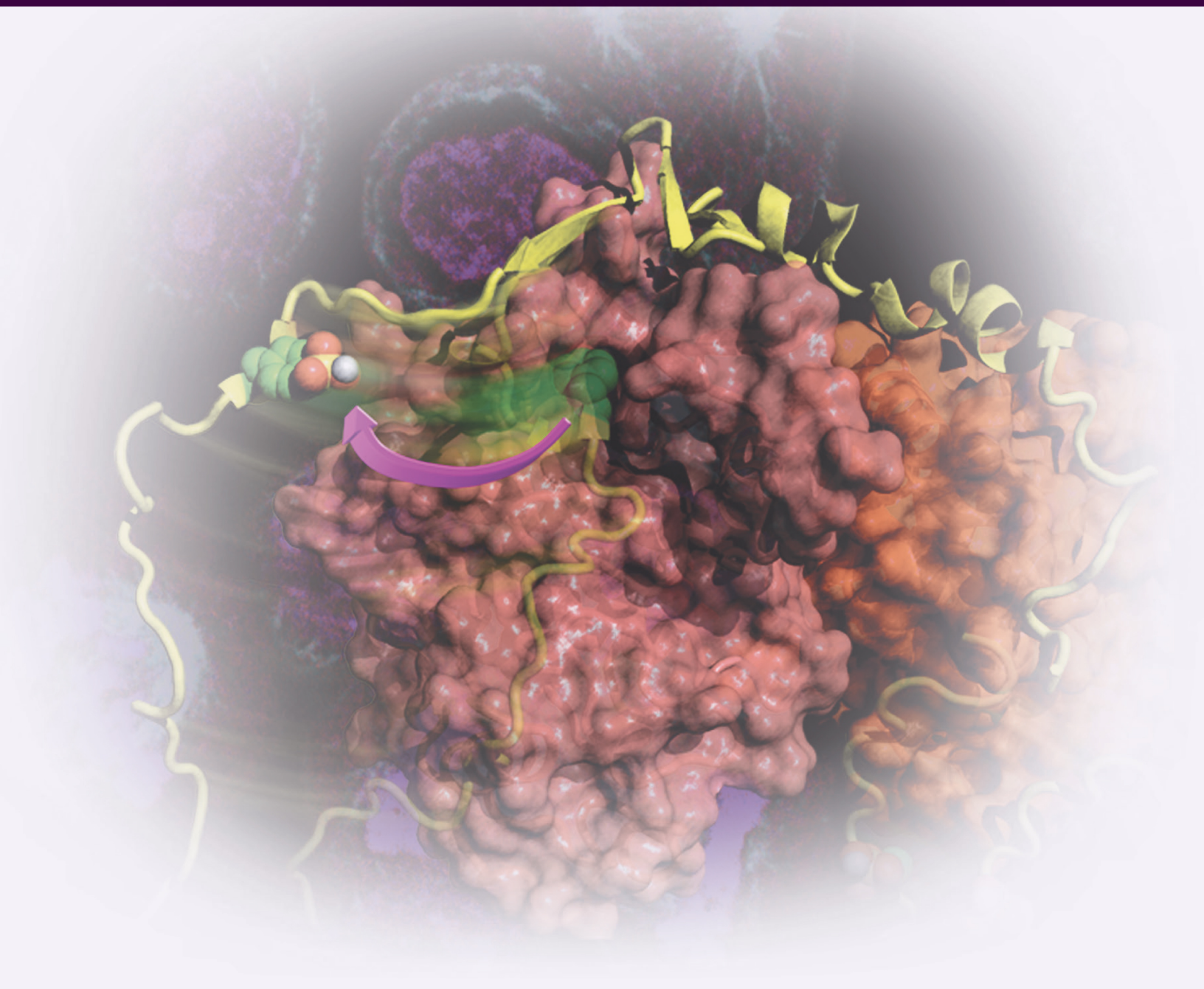
Disordered Motifs and Domains in Cell Control

OCTOBER 11–15, 2014 | DUBLIN, IRELAND



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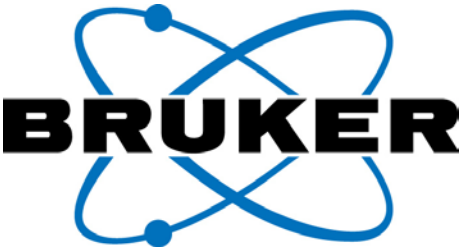

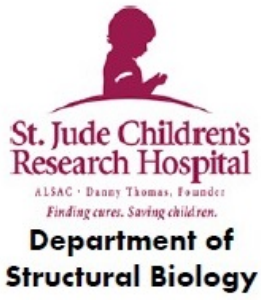

October 2014

Dear Colleagues,

On behalf of the Biophysical Society, we would like to welcome you to the *Disordered Motifs and Domains in Cell Control* meeting. Interest in intrinsically disordered proteins (IDPs) has exploded in recent years. It is now widely recognized that ~50% of human proteins are IDPs, or contain disordered regions, and that disorder is often essential for function. Furthermore, disorder is prevalent in the proteomes of most higher organisms. However, despite this broad awareness, in most cases we lack knowledge of the molecular functions associated with protein disorder. Consequently, numerous unresolved questions remain relating to the contribution of IDPs to biological processes in living systems. An emerging theme is that many disordered protein regions contain short linear motifs, or somewhat longer disordered domains, that mediate biomolecular interactions and thus drive biological function. These disordered motifs and domains are the subject of this thematic meeting.

While we recognize the importance of disordered motifs and domains in the function of proteins, and there are more and more examples where the molecular details of their biological functions are understood, in general we currently can only speculate about their roles in the vast swaths of disorder within proteomes. At this meeting, structural biologists, biophysicists, cell biologists, systems biologists, computational biologists and bioinformaticians will assemble to reveal how disordered motifs and domains drive biological function. Key questions to be addressed include: What are the physical features of disordered motifs and how do these mediate their functional interactions? And how are these interactions regulated? Given our current knowledge of disordered motifs and domains, how can we identify others within uncharacterized regions of proteomes? And can their functions be predicted? How diverse are the molecular mechanisms associated with disordered motifs and domains? What are the links between the dynamics and conformational heterogeneity of disordered protein regions and function? What types of structures do disordered motifs and domains form? How diverse are the length scales of these structures? What are the selective pressures that have given rise to disordered protein regions through evolution? And how are the functions of disordered motifs and domains altered in disease? By bringing together scientists with widely ranging expertise and perspectives, we seek to collectively address these questions and transform our understanding of the roles of protein disorder in biology.

This meeting offers a diverse program covering all aspects of the field with almost 40 lectures and over 50 posters. We hope to expand everyone's view of disordered motifs and domains within proteins and achieve synergy to drive the field forward in future years.

Most of all, we wish everyone a great meeting!

Sincerely yours,

Anna Akhmanova, Norman Davey, Ashok Deniz, Richard Kriwacki, and Sonia Longhi
The Organizing Committee

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Registration

The registration and information desk is located in Gandon Central in the Davenport Hotel. Registration hours are as follows:

Saturday, October 11	4:00 PM – 5:30 PM
Sunday, October 12	8:00 AM – 5:00 PM
Monday, October 13	8:00 AM – 5:00 PM
Tuesday, October 14	8:00 AM – 5:00 PM
Wednesday, October 15	8:00 AM – 12:00 PM

Instructions for Presentations

Presentation Facilities

A data projector will be made available in the Auditorium. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

Poster Sessions

1) All poster sessions will be held in Gandon North. Posters in each poster session will be on display from 8:00 AM – 10:00 PM on the day of the assigned poster session.

   Poster Session I
   All posters scheduled for Poster Session I should be set up in the morning of October 12 and MUST be removed by 10:00 PM the same day.

   Poster Session II
   All posters scheduled for Poster Session II should be set up in the morning of October 13 and MUST be removed by 10:00 PM the same day.

2) During the poster presentation sessions, presenters are requested to remain in front of their posters to meet with attendees.

3) A display board measuring 3 feet wide by 6 feet high will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the program book.

4) All posters left uncollected at the end of the meeting will be discarded.

Coffee Break
Coffee breaks will be held in Gandon South where tea and coffee will be provided free of charge to all participants.

Internet
Hi-speed WIFI access is available in the meeting rooms of the Davenport Hotel and the sleeping rooms of the Davenport Hotel, Alexander Hotel, and Mont Clare Hotel.

Smoking
Smoking is not permitted inside the buildings of the Davenport Hotel, Alexander Hotel, or Mont Clare Hotels.

Meals
The welcome reception, coffee breaks, poster receptions, and lunches (October 11-14) are included in the registration fee. Lunches and poster receptions will be held in Gandon North. A full Irish breakfast is included in the room rate at the following locations for guests staying at Society contracted hotels:
Davenport Hotel: Lanyon's Restaurant
Alexander Hotel: Caravaggio's Restaurant
Mont Clare Hotel: Gold Smith's Restaurant

Social Events
Welcome Reception with light hors d'oeuvres will be held in Ascot Suite at the Alexander Hotel on Saturday, October 11 from 6:00 – 7:30 PM. An optional walking tour of Dublin's City Center will take place Tuesday, October 14 and an optional tour of the Guinness Storehouse will take place Wednesday, October 15 at 2:00 PM. Sign up for the Guinness tour at the registration desk, individual payments will be processed when you arrive at the Guinness Storehouse.

Name Badges
Name badges are required to enter all scientific sessions and poster sessions. Please wear your badge throughout the conference.

Contact
If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from October 11 – October 15 during registration hours. You may also contact Amy Robinson at ARobinson@biophysics.org or call the Alexander Hotel at 353 1 607 3900 and ask for Amy's room.

Disordered Motifs and Domains in Cell Control
Davenport Hotel
Dublin, Ireland
October 11-15, 2014

PROGRAM

All scientific sessions and poster presentations will be held in Gandon North and South unless otherwise noted.

Saturday, October 11, 2014

4:00 – 6:00 PM	Registration/Information	**Gandon Central**
6:00 – 7:30 PM	Opening Reception	**Ascot Suite, Alexander Hotel**

Sunday, October 12, 2014

8:00 AM – 5:00 PM	Registration/Information	**Gandon Central**
	Session I: Regulation of Motif Interactions through Post-Translational Modification Co-Chairs: Richard Kriwacki, St. Jude Children's Research Hospital, USA & M. Madan Babu, University of Cambridge, United Kingdom	
9:00 – 9:10 AM	Welcome/Opening Remarks	
9:10 – 9:40 AM	Peter Wright, The Scripps Research Institute, USA *Intrinsic Disorder, Posttranslational Modification, and Signaling Complexity*	
9:40 – 9:55 AM	Diana Mitrea, St. Jude Children's Research Hospital, USA* *Acidic Patches in Nucleophosmin Recruit*	
9:55 – 10:25 AM	Stephan Feller, Institute of Molecular Medicine, Germany *Signal Computation in Large Multi-protein Complexes Assembled on Mostly Disordered Platform Proteins*	
10:25 – 10:55 AM	Coffee Break	**Gandon South**
10:55 – 11:25 AM	Richard Kriwacki, St. Jude Children's Research Hospital, USA *Structural Biology of Disordered Motifs in Regulation of Apoptosis and Cell Division*	

**Short talks selected from among submitted abstracts*

11:25 – 11:40 AM	Birthe Kragelund, University of Copenhagen, Denmark* *Intrinsic Disorder in Membrane Proteins*
11:40 AM – 12:10 PM	Mart Loog, University of Tartu, Estonia *Multisite Phosphorylation Networks in Cdk1-dependent Cell Cycle Regulation*
12:10 – 2:00 PM	Lunch **Gandon North**
	Session II: Methods in Molecular and System Level Studies in Motif Biology Co-Chairs: Susan Taylor, University of California, San Diego, USA & Rohit Pappu, Washington University of St. Louis, USA
2:00 – 2:30 PM	Jin Wang, State University of New York, Stony Brook, USA Changchun Institute of Applied Chemistry, China *Specificity and Affinity Quantification of Flexible Recognition from Underlying Energy Landscape Topography*
2:30 – 3:00 PM	Jörg Gsponer, University of British Columbia, Canada *Fast Computational Identification of MoRFs in Protein Sequences*
3:00 – 3:15 PM	Perdita Barran, The University of Manchester, United Kingdom* *The Use of Mass Spectrometry to Determine the Disordered Content of Proteins*
3:15 – 3:45 PM	Zhirong Liu, Peking University, China *Interaction Specificity of Intrinsically Disordered Proteins*
3:45 – 4:15 PM	Coffee Break **Gandon South**
4:15 – 4:45 PM	Martin Blackledge, Institut de Biologie Structurale, France *Relating Conformational Flexibility to Cellular Function in Intrinsically Disordered Viral and Signalling Proteins*
4:45 – 5:00 PM	Iris Antes, Technical University of Munich, Germany* *Computational Prediction of Protein-Peptide Binding*
5:00 – 5:30 PM	Norman Davey, University of California, San Francisco, USA *The Discovery and Characterization of a Novel Class of APC/C Activator Binding Motif Required for Ordered Cyclin Destruction and Spindle Assembly Checkpoint Integrity*
5:30 – 6:00 PM	Keith Dunker, Indiana University, USA *Close Encounters of the Third Kind: Disordered Binding Domains*

**Short talks selected from among submitted abstracts*

6:00 – 8:00 PM Dinner on own

8:00 – 10:00 PM Poster Session I

Monday, October 13, 2014

8:00 AM – 5:00 PM Registration/Information **Gandon Central**

Session III: System-wide Identification of Functional Motifs; How Much Biology Are We Missing?
Co-Chairs: Jörg Gsponer, University of British Columbia, United Kingdom & Toby Gibson, European Molecular Biology Laboratory, Germany

9:00 – 9:30 AM M. Madan Babu, University of Cambridge, United Kingdom
Use of Host-like Peptide Motifs in Viral Proteins Is a Prevalent Strategy in Host-Virus Interactions

9:30 – 9:45 AM Ylva Ivarsson, Uppsala University, Sweden*
Interaction Profiling Using Phage Peptidomes

9:45 – 10:15 AM Alan Moses, University of Toronto, Canada
Tinkering with Signaling: Evolution of Short Linear Motifs in Disordered Regions

10:15 – 10:45 AM Coffee Break **Gandon South**

10:45 – 11:15 AM Anna Akhmanova, Utrecht University, The Netherlands
Control of Protein Localization to Microtubule Tips by Disordered Motifs

11:15 – 11:45 AM Denis Shields, University College Dublin, Ireland
Enigmas of Protein Disorder and Motif Evolution in Viruses and Across Diverse Species

11:45 AM – 12:00 PM Pia Harryson, Stockholm University, Sweden*
Plant Stress: Membrane Binding of the Disordered lant Dehydrins

12:00 – 12:30 PM Philip Kim, University of Toronto, Canada
Novel Drug Leads: Highly Parallel Screening of Disordered Peptide Motifs for Phenotypic Effects in Cells

12:30 – 2:00 PM Lunch **Gandon North**

**Short talks selected from among submitted abstracts*

Session IV: Molecular Hand-Offs During a Protein's Lifetime: From Ribosome to Proteasome
Co-Chairs: Anna Akhmanova, Utrecht University, The Netherlands & Sonia Longhi, CNRS at University of Axi-Marseille, France

2:00 – 2:30 PM	Brenda Schulman, St. Jude Children's Research Hospital, USA *Dynamic Mechanisms Underlying Ubiquitin Ligation*
2:30 – 3:00 PM	Edward Lemke, European Molecular Biology Laboratory, Germany *Decoding Protein Plasticity from Single Molecules to Large Complexes*
3:00 – 3:15 PM	Marie-France Carlier, CNRS, France* *Control of Actin Filament Assembly by Multifunctional WASP-Homology 2 (WH2) Domains*
3:15 – 3:45 PM	Shu-ou Shan, California Institute of Technology, USA *Decision Making and Molecular Interplay during Protein Biogenesis*
3:45 – 4:15 PM	Coffee Break **Gandon South**
4:15 – 4:45 PM	Elisar Barbar, Oregon State University, USA *Protein Disorder and Polybivalency in Allosteric Regulation of Large Molecular Machines*
4:45 – 5:00 PM	Kuan Wang, Academia Sinica, Taiwan* *Intrinsically Disordered Titin PEVK Motifs: The Interplay of Force, Form, and Function of an Ion-exchange Driven Elastomer*
5:00 – 5:30 PM	Kylie Walters, National Cancer Institute, USA *Riding with the Ubiquitin Ticket*
5:30 – 5:45 PM	Gary Daughdrill, University of South Florida, USA* *Disorder and Residual Helicity Alter p53-Mdm2 Binding Affinity and Signaling in Cells*
5:45 – 6:15 PM	Harvey McMahon, MRC Laboratory of Molecular Biology, United Kingdom *Maturation of Clathrin-coated Vesicles Requires Dynamic Instability and Processivity*
6:15 – 8:00 PM	Dinner on own
8:00 – 10:00 PM	Poster Session II

**Short talks selected from among submitted abstracts*

Tuesday, October 14, 2014

9:00 AM – 5:00 PM	Registration/Information	**Gandon Central**
	Session V: Dynamics within Protein Complexes and Roles in Regulation Co-Chairs: Brenda Schulman, St. Jude Children's Research Hospital, USA & Keith Dunker, Indiana University, USA	
9:00 – 9:30 AM	Toby Gibson, European Molecular Biology Laboratory, Germany *Regulation by In-complex Molecular Switching*	
9:30 – 9:45 AM	Abel Garcia-Pino, Vrije Universiteit Brussels, Belgium* *Entropic Exclusion Determines Allostery in a Major Family of Intrinsically Disordered Bacterial Transcription Factors*	
9:45 – 10:15 AM	Jane Dyson, The Scripps Research Institute, USA *Role of Functional Disorder in Large Protein Complexes*	
10:15 – 10:45 AM	Coffee Break	**Gandon South**
10:45 – 11:15 AM	Liesbeth Veenhoff, University of Groningen, The Netherlands *Disordered Linker Regions for Sorting of Transmembrane Proteins*	
11:15 – 11:30 AM	Vincent Hilser, Johns Hopkins University, USA* *Parallel Tuning of Activation and Repression in Intrinsic Disorder-Mediated Allostery*	
11:30 AM – 12:00 PM	Susan Taylor, University of California, San Diego, USA *PKA: Dynamic Assembly of Macromolecular Signaling Complexes*	
12:00 – 3:00 PM	Lunch and Free Time	
	Session VI: Motifs, Multi-Valency and Membrane-less Organelles in Cells Co-Chairs: Peter Tompa, Vlaams Institute of Biotechnology, Belgium & Peter Wright, The Scripps Research Institute, USA	
3:00 – 3:30 PM	Dirk Görlich, Max Planck Institute for Biophysical Chemistry, Germany *Nup98 FG-Domains from Diverse Species Spontaneously Phase-Separate into Hydrogels with Exquisite NPC-like Permeability*	
3:30 – 3:45 PM	Stephanie Weber, Princeton University, USA* *Inverse Size Scaling of the Nucleolus by a Concentration-dependent Phase Transition*	
3:45 – 4:15 PM	Tanja Mittag, St. Jude Children's Research Hospital, USA *The Role of Multivalent Interactions of Tumor Suppressor SPOP with Gli3 in Regulating Ubiquitination*	

**Short talks selected from among submitted abstracts*

4:15 – 4:30 PM	Chi Pak, University of Texas Southwestern, USA* *Discerning Sequence-encoded Mechanisms of De Novo Nuclear*	
4:30 – 5:00 PM	Coffee Break	**Gandon South**
5:00 – 5:15 PM	Patrick Farber, Hospital for Sick Children, Canada* *Phase Separation of a Disordered Protein in the Formation of Membrane-less Organelles*	
5:15 – 5:45 PM	Roy Parker, University of Colorado, Boulder, USA *Assembly and Functions of mRNP Granules in Eukaryotic Cells*	

Wednesday, October 15, 2014

Session VII: Motifs that Drive Disease: A Biological Double-edged Sword
Co-Chairs: Tanja Mittag, St. Jude Children's Research Hospital, USA & Ashok Deniz, The Scripps Research Institute, USA

9:00 – 9:30 AM	Gilles Trave, University of Strasbourg, France *The Oncoproteins of Human Papillomaviruses: Instances of Viral Strategies for Hijacking of Host Motifs*	
9:30 – 9:45 AM	Brian Bothner, Montana State University, USA* *The C-Terminal Domain of Hepatitis B Virus Capsid Protein has Mastered being a Jack-of-all-trades*	
9:45 – 10:15 AM	Ashok Deniz, The Scripps Research Institute, USA *Single-Molecule Biophysics of Proteins Disordered and Misfolding*	
10:15 – 10:45 AM	Coffee Break	**Gandon South**
10:45 – 11:15 AM	Rohit Pappu, Washington University of St. Louis, USA *Modulation of Huntingtin Exon 1 Interactions through Synergy between Polyglutamine Tracts and Flanking Sequence Motifs*	
11:15 – 11:45 AM	Sonia Longhi, CNRS at University of Axi-Marseille, France *How Order and Disorder within Paramyxoviral Nucleoproteins and Phosphoproteins Orchestrate the Molecular Ballet of Transcription and Replication*	

Session VIII: Motif Biology: State of Understanding and Future Directions
Chair: Norman Davey, University of California, San Francisco, USA

**Short talks selected from among submitted abstracts*

11:45 AM – 12:15 PM	Peter Tompa, Vlaams Institute for Biotechnology, Belgium *Towards Describing IDP Function by Dynamic Structural Ensembles*
12:15 – 12:45 PM	Closing Remarks/Farewell
2:00 PM	Optional Guinness Storehouse Tour

SPEAKER ABSTRACTS

Intrinsic Disorder, Posttranslational Modification, and Signaling Complexity

Peter E. Wright.
The Scripps Research Institute, La Jolla, CA, USA.

Intrinsically disordered proteins (IDPs) mediate critical regulatory functions in the cell, including regulation of transcription, translation, the cell cycle, and numerous signal transduction events. The lack of stable globular structure confers numerous functional advantages, including, paradoxically, both binding promiscuity and high specificity in target interactions. IDPs play a central role in dynamic regulatory networks, where their propensity for posttranslational modifications, their rapid binding and dissociation kinetics, and their ability to interact with multiple target proteins makes them well adapted for precise transduction of cellular signals. The role of IDPs in dynamic cellular signaling will be illustrated by reference to pathways regulated by the general transcriptional coactivators CBP and p300, the tumor suppressor p53, and oncoproteins from adenovirus and human papillomavirus. CBP and p300 are central nodes in eukaryotic transcriptional regulatory networks and transcription factors must compete for binding to the limiting concentrations of CBP/p300 present in the cell. Many intrinsically disordered proteins contain multipartite interaction motifs that perform an essential function in the integration of complex signaling networks. The role of multipartite binding motifs and posttranslational modifications in regulation of signaling pathways will be discussed.

Acidic Patches in Nucleophosmin Recruit Nucleolar Proteins via Arginine-Rich Linear Motifs

Diana Mitrea[1], Richard W. Kriwacki[1,2].
[1]St. Jude Children's Research Hospital, Memphis, TN, USA, [2]University of Tennessee Health Science Center, Memphis, TN, USA.

The multifunctional protein Nucleophosmin (NPM1) localizes primarily to the nucleolus, where it is involved in ribosome biogenesis, tumor suppression and nucleolar stress response. NPM1 functions as a nucleolar chaperone for several proteins and is part of the ribonucleoprotein complexes. Utilizing a combination of bioinformatics, biophysical and structural approaches, we identified short linear motifs enriched in arginine in a large number of the known NPM1 nucleolar interactors (Mitrea et. al PNAS (2014), 111:4466) and show that these short linear motifs interact with highly conserved acidic patches on NPM1. Interestingly, tumor suppressor protein ARF which suppresses NPM1-dependent ribosome biogenesis competes for the same binding regions on NPM1 utilized for interactions with ribosomal proteins. Rigorous understanding of the molecular mechanism utilized by NPM1 to switch between nucleolar partners will provide critical insight into the regulation of nucleolar functions and structure.

Signal Computation in Large Multi-protein Complexes Assembled on Mostly Disordered Platform Proteins

Stephan Feller.
Imm, Zamed; Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany.

Receptor tyrosine kinases transmit signals through multi-protein complexes that contain large multi-site docking (LMD) proteins. The LMD proteins function as essential assembly platforms for sophisticated computational units in the cytoplasm of metazoan cells. Intensely studied examples of LMD proteins with critical roles in major cancers are the Gab family proteins Gab1 and Gab2. They display well-folded N-terminal pleckstrin homology (PH) domains, followed by long tail regions, which are supposedly entirely unstructured. How Gab proteins facilitate the computation of cross-talking pathways has remained a mystery. How is it possible that largely disordered proteins organize efficient, highly sophisticated signal computation units? The answer seems to be, that they may be not as 'chaotic' as previously thought. We have recently made two observations that challenge the idea of entirely intrinsically disordered Gab protein tails. Firstly, we have shown by biophysical methods that segments of the Gab tail regions can form helices (PPII, 3-10; Harkiolaki et al. 2009, Structure). Secondly, we have evidence from peptide array overlay blots and mutational studies that the long tail of the Gab1 protein can interact with the Gab1 PH domain at several sites (N-terminal folding nucleation [NFN] hypothesis; Simister et al. 2011, PLoS Biol). This should generate loop regions where functionally defined subcomplexes can assemble. When these subcomplexes then interact, cross-talk between multiple pathways can occur (Lewitzky et al. 2012, FEBS Lett). Interestingly, the helical tail segments serve as critically important docking sites for adaptors coupling the intracellular 'nanocomputers' to oncogenic receptors. They may thus be "Achilles' heels" that could be targeted in human cancers. Uncoupling oncogenic receptors from their intracellular signalosomes should simultaneously affect several pathways, creating in essence a multi-potent inhibitor. Initial attempts to synthesise such new inhibitory compounds are under way.

Structural Biology of Disordered Motifs in Regulation of Apoptosis and Cell Division

Ariele V. Follis[1], Jerry Chipuk[2], Fabien Llambi[3], James Asciolla[2],Yongqi Huang[1], Mi-Kyung Yoon[1], Steve Otieno[1], Moreno Lelli[4], Mi-Kyung Yun, Max Tsytlonok[5], Hugo Sanabria[6], Yuefeng Wang[1], Brett Waddell[1], Cheon-Gil Park[1], Siva Vaithiyalingam[1], Diana M. Mitrea[1], Stephen W. White[1], Peter Tompa[5,7], Claus Seidel[6], Doug Green[2], **Richard W. Kriwacki**[1,8]

Departments of [1]Structural Biology, and [3]Immunology, St. Jude Children's Research Hospital, USA; [2]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [4]Centre de RMN à Très Hauts Champs, France; [5]VIB Structural Biology Research Center (SBRC), Vrije Universiteit Brussel, Brussels 1050, Belgium; [6]Institut für Physikalische Chemie, Lehrstuhl für Molekulare Physikalische Chemie, Heinrich-Heine-Universität, Düsseldorf, Germany; [7]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest 1519, Hungary; [8]Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Sciences Center, Memphis, TN, USA.

Conserved linear motifs frequently occur within disordered regions of proteins and, through interactions with other biomolecules (e.g., proteins, nucleic acids, lipids, metabolites, etc.), contribute to function in myriad biological processes. Our studies have shown that dynamics within motifs, both in their free states and when bound to their functional targets, are critical for the transmission of signals within regulatory pathways. In particular, posttranslational modifications serve to switch motif function by altering their interactions. We will describe the roles of dynamic, disordered motifs in regulation of apoptosis and cell division. Studies of a disordered motif in p53 have revealed the roles of posttranslational modifications in mediating interactions with and activation of the apoptotic effector, BAX. Studies of the cell cycle regulators, p21 and p27, have shown i) that motif dynamics within functional complexes is critical for signal transmission, and ii) that subtle differences in motif topology can lead to dramatic differences in regulatory behavior. Our studies illustrate the structural, dynamic, and functional complexities of motifs within disordered regions of proteins and, in general, advance our understanding of disorder-function relationships for proteins.

Intrinsic Disorder in Membrane Proteins

Birthe B. Kragelund, Gitte W. Haxholm, Louise F. Nikolajsen, Ruth Hendus-Altenburger. University of Copenhagen, Copenhagen N, Denmark.

Intrinsically disordered regions (IDRs) in membrane proteins play central roles in cellular signaling processes and like their structured protein counterparts, they engage in interaction networks of regulatory nature. Intracellular domains of many membrane proteins contain large IDRs of importance for function and with numerous predicted as well as confirmed phosphorylation sites. Due to their lack of globular structure insight into their structure-function relations have been crucially lacking. Using NMR spectroscopy, biophysics and cell-biology we have deciphered regulatory roles of intrinsic disorder in cytokine receptors and in ion transporters with direct links to phosphorylations. The interplay of intrinsic disorder and phosphorylation in these proteins highlights specific space and temporal effects in scaffolding including interplay with some of the major signaling pathways such as JAK2/STAT and MAPK-signaling.

Multisite Phosphorylation Networks in Cdk1-dependent Cell Cycle Regulation

Rainis Venta[1], Andreas Doncic[2], Ervin Valk[1], Mardo Kõivomägi[1], Jan Skotheim[2], **Mart Loog**[1]. [1]Institute of Technology, University of Tartu, Tartu, Estonia, [2]Stanford University, Stanford, CA, USA.

Multisite phosphorylation of proteins is a powerful signal processing mechanism whose diverse possibilities are not well understood. In this process, multiple phosphates are added in either a random or defined order to kinase substrates. When a crucial set of key sites becomes sufficiently phosphorylated, a downstream signaling switch is triggered. Multisite phosphorylation plays a pivotal role in processing CDK (cyclin-dependent kinase) signals to ensure temporal regulation of cell cycle events. The key factor that controls this process is the phospho-adaptor Cks1. It binds to phosphorylated threonines and enhances CDK-dependent phosphorylation of neighboring sites. This event occurs several times to process the phosphorylation of the entire network of sites. As the phosphorylation sites are located in disordered segments of the targets, or in disordered proteins, the cyclin-CDK-Cks1 complex acts as a catalytic scaffold whose rate of multisite phosphorylation is determined by how well the fixed spatial orientation of the docking pockets on the scaffold fits with the linear pattern of phosphorylation sites and docking sites in the substrates. We demonstrate this phenomenon on Sic1, a G1/S inhibitor of Cdk1 in budding yeast. The phosphorylation events in Sic1 lead to the generation of phosphodegron motifs that are recognized by the ubiquitination machinery and thereby mark Sic1 for destruction. We show that the network of sites in Sic1 is processively phosphorylated by S-phase cyclin-Cdk1-Cks1. The processivity is modulated by phosphorylation/dephosphorylation of a priming site and a diversional site by two kinases and a phosphatase of stress pathways. Both the priming site and the diversional site compete for binding to Cks1. This mechanism demonstrates how external signals can be integrated into the Cdk1 control system via multi-branched signal-processing modules based on multisite phosphorylation networks. Such transistor-like modules are possibly ubiquitous and could regulate many cellular events.

Specificity and Affinity Quantification of Flexible Recognition from Underlying Energy Landscape Topography

Jin Wang.
Stony Brook University, Stony Brook, USA.

The flexibility in biomolecular recognition is essential and critical for many cellar activities. Flexible recognition often leads to moderate affinity but high specificity, in contradiction with the conventional wisdom that high affinity and high specificity are coupled. Furthermore, quantitative understanding of the role of flexibility played in biomolecular recognition quantitatively is still challenging. Here, we meet the challenge by quantifying the intrinsic biomolecular recognition energy landscapes with and without flexibility through the underlying density of states. We quantified the thermodynamic intrinsic specificity by the topography of the intrinsic binding energy landscape and the kinetic specificity by association rates. We found that the thermodynamic and kinetic specificity are strongly correlated. Furthermore, we found that the flexibility decreases the binding affinity on one hand but, increases the binding specificity on the other hand, and the decreasing or increasing proportion of affinity and specificity are strongly correlated with the degree of flexibility. This shows more (less) flexibility leads to weaker (stronger) coupling between affinity and specificity. Our study provides a theoretical foundation and quantitative explanation of the previous qualitative studies on the relationships among flexibility, affinity and specificity. In addition, we found that the folding energy landscapes are more funneled with binding, indicating that binding helps folding of the investigated dimers. Finally, we demonstrated that the whole binding-folding energy landscapes can be integrated by the rigid binding and isolated folding energy landscapes in weak binding flexibility. Our results provide a novel way to quantify the flexibility and specificity in biomolecular recognition.

Fast Computational Identification of MoRFs in Protein Sequences

Jörg Gsponer.
University of British Columbia, Canada

Intrinsically disordered regions of proteins play an essential role in the regulation of various biological processes. Key to their regulatory function is often the binding to globular proteins domains via molecular recognition features, MoRFs, in a process known as disorder-to-order transition. Predicting the location of MoRFs in protein sequences is an important computational challenge. We introduce $MoRF_{CHiBi}$, a new machine learning approach for a fast and accurate prediction of MoRFs in protein sequences.

The Use of Mass Spectrometry to Determine the Disordered Content of Proteins

Rebecca Beveridge[1], Kamila Parcholarz[2], Jason Kalapothakis[2], Cait MacPhee[2], **Perdita Barran**[1].
[1]The University of Manchester, Manchester, United Kingdom, [2]The University of Edinburgh, Edinburgh, United Kingdom.

In the last decade mass spectrometry (MS) coupled with electrospray ionisation (ESI) has been extensively applied to the study of intact proteins and their complexes. Solvent conditions, for example pH, buffer strength and concentration, affect the observed desolvated species; the ease of altering such extrinsic factors render ESI-MS an appropriate method by which to consider the range of conformational states that proteins may occupy including natively folded, disordered and amyloid. Rotationally averaged collision cross sections of the ionised forms of proteins, provided by the combination of mass spectrometry and ion mobility (IM-MS), are also instructive in exploring conformational landscapes in the absence of solvent. We have selected 19 different proteins, both monomeric and multimeric, ranging in mass from 2846 Da (melittin) to 150 kDa (Immunoglobulin G) and we consider how they present to the mass spectrometer under different solvent conditions. Mass spectrometery distinguishes which of these proteins are structured from those that contain regions of disorder by considering two experimental parameters; Dz (the range of charge states occupied by the protein) and DCCS (the range of collision cross sections that the protein is observed in). We provide a simple model which allows the theoretical prediction of the smallest and largest possible collision cross sections based on the volume of the amino acids in the sequences, and we compare these calculated parameters with the experimental values. the intensities of ions in the mass spectra is used to provide occupancy of conformational states allowing us to qualitatively predict the potential energy landscape of each protein. This empirical approach to assess order or disorder has more accuracy than theoretical methods based on the amino acid sequences for the chosen systems, and could provide an initial route to characterisation.

Interaction Specificity of Intrinsically Disordered Proteins

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Interaction specificity of proteins is critical to their cellular function. Due to their chain flexibility, whether intrinsically disordered proteins (IDPs) possess high specificity is in debate. We conducted some investigations on this question. Firstly, by combining an analysis on mutant data in the literature and a simulation with a coarse-grained model, we found that that the enthalpy–entropy compensation for disordered protein complexes was more complete than that for ordered protein complexes. Interactions of IDPs are more malleable than those of ordered proteins due to their structural flexibility in the complex. Secondly, we performed extensive all-atom simulations on the segment 370-409 of the oncoprotein c-Myc and its binding to an inhibitor. Upon binding of the ligand, c-Myc remained disordered. The ligand was found to bind to c-Myc at different sites along the chain and may be described as “ligand clouds around protein clouds”, which is different from the more rigid cases that usually result in a dominant bound structure. Finally, the drug design concerning IDPs is briefly discussed.

Relating Conformational Flexibility to Cellular Function in Intrinsically Disordered Viral and Signalling Proteins

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Proteins containing long (>50aa) intrinsically disordered regions (IDRs) comprise 50% of eukaryotic proteomes, and represent extreme examples where protein flexibility plays a determining role in function. The development of a meaningful molecular descriptions of IDRs remains a key challenge for contemporary structural biology. We have developed approaches to map the conformational energy landscape explored by IDRs using experimental NMR (1), providing calibrated procedures that make extensive use of cross-validation to test the predictive capacity of the resulting ensemble descriptions (2,3).
We now use these tools to investigate the role of disorder in functional protein complexes involving IDRs. The study of protein-protein interactions involving IDRs poses a number of intriguing questions regarding recognition at the molecular level. Although few systems have been experimentally characterised, the structural plasticity of IDRs is thought to provide functional modes that are inaccessible to folded proteins. The replication machinery of paramyxoviruses represents a paradigm of IDP-mediated interactions, with the highly (>70%) disordered tetrameric Phosphoprotein initiating transcription and replication via its interaction with the disordered domain of the Nucleoprotein (4-6). Similarly the JNK signalling pathway exhibits extensive disorder, with specificity apparently controlled by disordered MAP kinase domains containing annotated linear motifs. In both cases post-translational modification plays a role in the functional interaction. In combination with the approaches outlined above, spin relaxation and chemical exchange measurements are used to characterize structure, dynamics and kinetics of these highly dynamic complexes in solution and in their native physiological environments.

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Computational Prediction of Protein-Peptide Binding

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Protein-peptide interactions are crucial for many important biological processes, especially in the context of signal transduction and protein-protein assembly. In addition, peptides also serve as natural inhibitors for proteins and therefore are often used as lead structures in pharmaceutical research. Prominent examples for peptide-based drugs are the inhibitors of viral proteases [1]. There exist very few computational approaches, which allow a structure-based prediction of protein-peptide binding, especially for larger peptides with more than 5 amino acids and surface-exposed binding sites. We have developed a two-stage method for this purpose:
First, we predict the peptide's binding site on the protein's surface, which is important for many biologically relevant protein-peptide interactions for which the structure of the bound complex is not known. Second, we perform a throughout sampling of the peptide in the predicted binding site to identify the bound protein-peptide complex conformation using two methods: IRECS [2] and DynaDock [3], both allowing for an efficient description of the protein's flexibility during protein-peptide assembly and thus fully flexible docking.
The procedure was evaluated on a set of 20 different protein-peptide complexes and allows the successful prediction of bound protein-peptide complex structures (RMSD/exp. structure < 2.5 Â) for peptides with up to 16 amino acids starting from the unbound protein structure if available. The methodology was meanwhile successfully applied to predict Hsp70, TRAF2/6, and MHC peptide binding [4].

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The Discovery and Characterisation of a Novel Class of Anaphase Promoting Complex (APC/C) Activator-binding Motif Required for Ordered Substrate Destruction and Spindle Assembly Checkpoint (SAC) Integrity

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The APC/C ubiquitin ligase regulates mitosis by degrading specific proteins at specific times. The CDC20-like family members, CDC20 and CDH1, act as substrate recruitment subunits for the APC/C. How CDC20 and CDH1 temporally order the degradation of their substrates is a key problem in the control of cell division. Here, we have used a bioinformatics approach to identify a novel class of SLiM that mediates binding between a number of mitotic regulators and members of the CDC20-like family. We have named the motif the ABBA motif, as it is present in the A-type Cyclins, the SAC components BUBR1 and BUB1, and the CDH1 inhibitor ACM1. We demonstrate that Cyclin A, BUB1 and BUBR1 bind competitively to the same site on the APC/C co-activator CDC20. Furthermore, we show that the ABBA motif is required for Cyclin A destruction when the SAC is active. These observation provide a mechanism by which Cyclin A is degraded when the APC is inhibited by the SAC through competition with the central SAC component BUBR1. We also show that the functional homologue of Cyclin A in yeast, CLB5, contains an ABBA motif and, similarly to Cyclin A, the CLB5 ABBA motif is required for the correct ordering of protein destruction during mitotic exit in yeast. Finally, we show that the ABBA motifs in BUBR1 and BUB1 are required to recruit CDC20 to unattached kinetochores and for the SAC to work at full strength. Thus, we have identified a motif conserved through evolution that connects the recognition of APC/C substrates with the SAC, and is integral to the correct timing of protein destruction in mitosis.

Close Encounters of the Third Kind: Disordered Binding Domains

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While developing intrinsically disordered protein (IDP) predictors (1), we noticed that our training set proteins contained several examples for which false positive predictions of structure exhibited strong overlap with binding sites for protein partners (2). We called such sites molecular recognition features (MoRFs) (3) and we developed a collection of MoRF predictors (4-6). In the selected examples, MoRFs were rather short, typically less than 15 residues in length. In parallel studies we identified longer partner-binding disordered regions that we called disordered binding domains, several of which matched hidden Markov models called Pfams (7). Thus, we studied Pfams that are predicted to be disordered (8). We will present our in-progress work on predicted-to-be disordered Pfams that, contrary to the IDP predictions, are found to be structured in the Protein Data Bank.

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Use of Host-like Peptide Motifs in Viral Proteins Is a Prevalent Strategy in Host-Virus Interactions

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Viruses interact extensively with host proteins, but the mechanisms controlling these interactions are not well understood. We present a comprehensive analysis of eukaryotic linear motifs (ELMs) in 2,208 viral genomes and reveal that viruses exploit molecular mimicry of host-like ELMs to possibly assist in host-virus interactions. Using a statistical genomics approach, we identify a large number of potentially functional ELMs and observe that the occurrence of ELMs is often evolutionarily conserved but not uniform across virus families. Some viral proteins contain multiple types of ELMs, in striking similarity to complex regulatory modules in host proteins, suggesting that ELMs may act combinatorially to assist viral replication. Furthermore, a simple evolutionary model suggests that the inherent structural simplicity of ELMs often enables them to tolerate mutations and evolve quickly. Our findings suggest that ELMs may allow fast rewiring of host-virus interactions, which likely assists rapid viral evolution and adaptation to diverse environments.

Interaction Profiling Using Phage Peptidomes

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Phage display is a powerful technique for specificity profiling of peptide-binding domains. Using highly diverse combinatorial peptide phage libraries, the method is suited for the identification of high affinity ligands with inhibitor potential. A complementary but considerably less explored approach is the proteomic peptide phage display where expression products from exquisitely designed oligonucleotide libraries are displayed on phage particles. Proteomic phage display can be used to uncover protein-protein interactions of potential relevance for cell function. The method is particularly suited for the discovery of interactions between peptide binding domains and their target motifs
We recently generated phage libraries displaying all human C-terminal sequences using custom oligonucleotide microarrays and used them to interrogate interactions of human protein-95/disks large/zonula occludens-1 (PDZ) domains. We successfully identified novel PDZ domain interactions of potential relevance to cellular signaling pathways and validated a subset of interactions with a high success rate. I will present our recent results on how the combination of combinatorial and proteomic peptide phage display can be used to elucidate preferences of peptide binding domains and to identify targets of biological relevance.

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Tinkering with Signaling: Evolution of Short Linear Motifs in Disordered Regions

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Short linear motifs are a major class of functional elements commonly found in intrinsically disordered protein regions and are known to be important for transient signaling interactions. Evolutionary changes in these motifs could underlie differences in signaling and protein regulation between related species. We've been studying the patterns of evolution of short linear motifs in disordered regions, and I will describe two of our ongoing projects. First, we systematically identified short linear motifs that were present in single copy ancestral proteins, but were lost in one of the two paralogs after gene duplications. We find that short linear motifs are lost more often in paralogs than in single copy genes, suggesting that divergence of short linear motifs in disordered regions is a general mechanism for changes in function after gene duplications. Second, we serendipitously discovered a lineage-specific docking site in a highly conserved protein kinase. Taking advantage of this system, we have examined how new docking motifs appear in existing substrate proteins, and how new substrates are co-opted in this signaling network. This gives us a simple model for how signaling complexity increases during evolution.

Control of Protein Localization to Microtubule Tips by Disordered Motifs

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Growing microtubule ends are associated with a large variety of proteins, known as +TIPs (plus end tracking proteins), which connect microtubules to different cellular structures and regulate microtubule dynamics. The core components of the +TIP network are the members of the End Binding (EB) family, which can autonomously recognize growing microtubule ends and recruit to them numerous, structurally diverse factors. A large group of +TIPs bind to EB proteins through natively unstructured basic and serine-rich polypeptide regions containing a short core motif SxIP (serine-any amino acid-isoleucine-proline). We have performed a proteome-wide search for mammalian SxIP-containing +TIPs by combining biochemical and bioinformatics approaches and identified a set of EB partners that have the capacity to accumulate at the growing microtubule ends, including protein kinases, a small GTPase, membrane- and actin-associated proteins. Proteome-wide analysis of EB partners showed that the SxIP motif is the major microtubule tip localization signal. The knowledge of the properties of this motif made it possible to address the function of microtubule plus end association in different proteins. Our study demonstrates that disordered motifs are key players in the highly complex +TIP interactome. The approaches used in our study can be applied to many other classes of protein motifs to discover their proteome-wide occurrence and their contribution to the global protein interaction networks.

Enigmas of Protein Disorder and Motif Evolution in Viruses and Across Diverse Species

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Current dogma suggests that disorder is rampant in mammalian biology. However, we have little understanding of what factors determine its distribution. The degree of viral protein disorder across different viruses is not well understood. We surveyed predicted disorder across 2,278 available viral genomes in 41 families, and correlated the extent of disorder with genome size and other factors. Protein disorder varies strikingly between viral families (from 2.9% to 23.1% of residues), and also within families. However, this substantial variation did not follow the established trend among their hosts, with increasing disorder seen across eubacterial, archaebacterial, protists, and multicellular eukaryotes. For example, among large mammalian viruses, poxviruses and herpesviruses showed markedly differing disorder (5.6% and 17.9%, respectively). Viral families with smaller genome sizes have more disorder within each of five main viral types (ssDNA, dsDNA, ssRNA+, dsRNA, retroviruses), except for negative single-stranded RNA viruses, where disorder increased with genome size. However, surveying over all viruses, which compares tiny and enormous viruses over a much bigger range of genome sizes, there is no strong association of genome size with protein disorder. We conclude that there is extensive variation in the disorder content of viral proteomes. While a proportion of this may relate to base composition, to extent of gene overlap, and to genome size within viral types, there remain important additional family and virus-specific effects. Differing disorder strategies are likely to impact on how different viruses modulate host factors, and on how rapidly viruses can evolve novel instances of SLiMs subverting host functions, such as innate and acquired immunity.

We also present findings on the dynamics of evolution of SLiMs and their cognate binding domains across many biological kingdoms, to understand better the origin of mechanisms of motif recognition.

Plant Stress: Membrane Binding of the Disordered Plant Dehydrins

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Sudden temperature changes and vanishing supply of water present major challenges to plant survival, growth and productivity. This project investigates the biological and molecular function of the stressed induced dehydrin proteins to broaden our understanding on how plants adapt to drought, temperature changes or salty soils. Although highly expressed during stress is the molecular function of dehydrins yet unclear. Dehydrins are intrinsically disordered proteins expressed under water-related stress in plants. As a clue to their function, some dehydrins are found to interact in an orderly manner with negatively-charged lipids, supporting the idea of a key role in safeguarding membrane integrity. We have earlier reported that this lipid interaction is modulated electrostatically by global and local charge. Of particular interest is the pronounced effect of local charge that shed light on how dehydrin function is regulated in vivo: membrane binding and vesicle assembly is controlled by protonation of histidine switches flanking the conserved K-segments. However, the lipid binding capacity varies among the 4 dehydrins in this study, from some showing low/no binding examples of strong specific binding. These data indicate a functional diversity among the plant dehdyrins.

Novel Drug Leads: Highly Parallel Screening of Disordered Peptide Motifs for Phenotypic Effects in Cells

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Intrinsically disordered (ID) peptides/proteins have crucial roles in the control of cell growth and proliferation and numerous disordered proteins are associated with human diseases such as cancer and immunodeficiency disease. In particular, disordered regions harbour numerous short peptide regions (known as linear motifs) that often function as binding sites. Such motifs mediate a large fraction of protein-protein interactions, especially in signalling, and should thus be good targets for therapeutic intervention. Although numerous studies have attempted to develop potential therapeutic peptides for decades, canonical small-scale screens make it difficult to generate effective and selective anti-tumor peptides. Here, we develop a high-throughput human peptide library screen for peptides inhibiting tumor cell growth. About 400 unique peptide sequences were isolated that exhibited anti-proliferative effects. We observed that these peptides can directly inhibit cancer cell growth without affecting normal cells (targeted therapy) and we identified the protein-protein interaction targets of our top peptides. Using a number of orthogonal experimental techniques, we confirmed that the disruption of these interactions is their likely mode of action, thus identifying novel putative (and known) anti-apoptotic interactions as well as their inhibitors. The identified interactions are ideal new targets for therapeutic intervention while our peptides serve as potential lead compounds. We thus demonstrate that our high-throughput human peptide screen can be a valuable tool to develop novel anti-cancer drugs. Furthermore, our technology can be a powerful means to elucidate the roles of the multitude of disordered peptide motifs.

Dynamic Mechanisms Underlying Ubiquitin Ligation

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Post-translational modification by ubiquitin-like proteins (UBLs) is a predominant eukaryotic regulatory mechanism. The vast reach of this form of regulation extends to virtually all eukaryotic processes that involve proteins. UBL modifications play critical roles in controlling the cell cycle, transcription, DNA repair, stress responses, signaling, immunity, plant growth, embryogenesis, circadian rhythms, and a plethora of other pathways. UBLs dynamically modulate target protein properties including enzymatic activity, conformation, half-life, subcellular localization, and intermolecular interactions. Moreover, the enzymatic process of UBL ligation to proteins is itself dynamic, with the UBL moving between E1/E2/E3 enzyme active sites and ultimately to a target. With roughly 300 members, the largest E3 family consists of Cullin-RING ligases (CRLs), which regulate a staggering number of biochemical pathways and biological processes. CRL activity is under fascinating conformational control, with different orientations of the catalytic RING domain mediating different activities. In my presentation, I will discuss recent results from the lab addressing how the dynamic conformations underlie regulation of and by this large family of dynamic ubiquitin E3 ligases.

Decoding Protein Plasticity from Single Molecules to Large Complexes

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Intrinsically disordered and phenylalanine-glycine rich nucleoporins (FG-Nups) form a selective permeability barrier inside the nuclear pore complex (NPC): Large molecules can only cross the central channel of the NPC when piggybacked by nuclear transport receptors (NTRs) that specifically interact with FG-Nups. These FG-Nups, however, display complex and non-random amino acid architecture and possess repeatedly occurring FG-motifs flanked by distinct amino acid stretches. How such heterogeneous sequence composition relates to function and how homotypic interactions between such disordered stretches, and transient heterotypic interactions with folded transport receptors could give rise to a transport mechanism is still unclear. To address this challenge we developed a combined fluorescence correlation and time resolved polarization spectroscopy approach to study the binding properties of the IDP nucleoporin153 (Nup153) to NTRs. The detection of segmental backbone mobility of Nup153 within the unperturbed complex provided a readout of local, region specific, binding properties that are usually masked in measurements of the whole IDP. Binding affinity of functionally and structurally diverse NTRs to distinct regions of Nup153 differed by orders of magnitudes - a result with implications for the diversity of transport routes in nucleocytoplasmic transport. Furthermore, synergistic molecular dynamics simulations permitted visualization of previously unknown steps and binding modes during Nup•NTR interactions at atomic resolution. These results have molecular implications for the diversity of transport routes within nucleocytoplasmic transport and on how nuclear transport can pursue specifically and very fast inside the nuclear pore complex.

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Control of Actin Filament Assembly by Multifunctional WASP- Homology 2 (WH2) Domains

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WH2 domains are widespread, intrinsically disordered, short (30 amino-acids) protein motifs that fold upon binding to actin. They are present, in single or repeated motifs, in about 100 proteins that play a role in cell motility and morphogenetic processes by regulating actin assembly. They all bind actin similarly, in particular inserting an amphipathic α-helix in a hydrophobic pocket at the shear zone between actin subdomains 1 and 3 at the barbed face of the actin monomer. Thus, they display a large variety of functions, from sequestration of monomeric actin or profilin-mimicking activity to regulation of filament barbed end dynamics and filament severing. We have analyzed the structural basis for the multifunctionality of several WH2 domain proteins like N-WASP, Spire, Cordon-bleu, VASP and the Vibrio cholerae effector VopF. We show that 1) discrete sequence variations underlie differences in function ; 2) binding of WH2 to barbed end terminal subunits allows barbed end capping by Spire, an essential feature of its synergy with formin 2 in meiosis ; 3) a combination of profilin-like activity, filament severing and ADP-G-actin sequestering generates enhanced filament dynamics and oscillatory polymerization of actin ; 4) dimerization of WH2 domains allows filament barbed end tracking and potential processive assembly in VASP and VopF.

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Decision Making and Molecular Interplay during Protein Biogenesis

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Accumulating data show that the ribosome exit site is a crowded environment where a variety of protein biogenesis factors interact with a nascent protein. Accurate decision-making must be made at the ribosome exit site to ensure that the nascent protein engage the correct factors, and thus enter the proper biogenesis pathway. The molecular mechanisms by which these decisions are made have remained elusive. To address this question, we investigated the molecular interplay between the signal recognition particle (SRP), a conserved protein-targeting machine that mediates the localization of proteins to the cellular membrane, and trigger factor (TF), a major co-translational chaperone in bacteria. The results reveal multiple mechanisms by which TF influences cargo selection by the SRP, and provide a conceptual framework to understand molecular interplay in the crowded environment at ribosome exit site.

Protein Disorder and Polybivalency in Allosteric Regulation of Large Molecular Machines

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Cytoplasmic dynein is an essential microtubule-based motor that controls diverse cellular processes ranging from mitotic spindle assembly to axonal transport. An intriguing feature of this multi-subunit complex is the regulation of its activity and various functions depends on multiple protein complexes that bind at the intrinsically disordered N-terminus of the intermediate chain subunit, located quite distant from the motor end of the complex. The N-terminal domain of the intermediate chain IC is also the site of binding to LC8, a dimeric protein known to promote structural organization of its disordered partners by enhancing either the partner self-association or binding affinity to other dimeric proteins. This work combines NMR and isothermal titration calorimetry to investigate the residue level allosteric communication between yeast orthologs of LC8 (Dyn2) and the p150Glued subunit (Nip100) of the dynein regulator, dynactin. The N-terminal domain of yeast IC (Pac11) is primarily a disordered monomer except for a single alpha helix (SAH) that projects as an elongated structure and forms the recognition site for Nip100. The SAH domain is followed by two recognition sites for Dyn2 separated by a 20-residue linker that includes a nascent helix. Dyn2 binding induces structural changes in Pac11 localized to the linker helix which interestingly is the same helix that is significantly affected by Nip100 binding. We propose that multiple Dyn2 sites shift the population states of disordered Pac11 to an aligned active conformation but rather than enhancing binding of Pac11 to Nip100, they facilitate allosteric regulation by aligning the linker helix that is part of the Nip100 binding dynamic network. The conservation of protein disorder among IC orthologs underscores the importance of structural transitions triggered by bivalent light chains for propagation of long range conformational changes.

Intrinsically Disordered Titin PEVK Motifs: The Interplay of Force, Form, and Function of an Ion-exchange Driven Elastomer

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We describe the interplay between elasticity and ensemble structures of intrinsically disordered proteins, using the PEVK segment of the giant (~3-4 MDa) elastic protein titin as a model. Titin PEVK is an highly extended scaffold protein segment encoded by splicing of more than 100 exons and is thought to integrate mechanical stress and SH3-mediated signaling pathways (J. Biol. Chem,281, 27539-556, 2006).

Solution and gel phase NMR, single molecule force spectroscopy and steered molecular dynamics simulations were used to investigate the ensemble structures and ion-pair interactions of an engineered 15-mer polyprotein, consisting of 15 identical titin PEVK modules, with affinity tags at both ends to facilitate nanomechanical measurements by single molecule atomic force microscopy.

Using the NMR data for the polypeptide backbone and a subset of possible long range interactions, we were able to simulate an ensemble of representative structures and to simulate the mechanical stretching of a trimer and compare it to the stretching of the single polyprotein by atomic force microscopy. The stretching simulations showed that the attractive ionic interactions are in constant flux, leading to an elastic behavior similar to entropic polymers. Some of the simulations showed the exact force signature as those seen in experimental force spectra, and the force-bearing events arise from either transient hydrophobic residues, depending upon the trajectory of the stretching polymer.

The current work tightly integrates both experiments and simulations to provide a more complete understanding of how intrinsically disordered ampholytes behaves as an elastic element by an intrachain ion-exchange mechanism. Our insights of nanomechanical properties of this intrinsically disordered protein are useful in the understanding of how force modulates function and in the design of elastic functional elastic biomaterials.

Riding with a Ubiquitin Ticket

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The compact 76 amino acid protein ubiquitin is used to signal for a broad spectrum of cellular events. This modification is diversified by expansion into a ubiquitin polymer, formed through eight possible linkages. Ubiquitin receptors contribute to determining the outcome of ubiquitination by their specificity for distinct ubiquitin polymers. This talk will present new interactions involving ubiquitin receptors and ubiquitin polymers, as well as functional implications. By using NMR spectroscopy, we have found protein dynamics and disorder to play distinct and defining functional roles.

Disorder and Residual Helicity Alter p53-Mdm2 Binding Affinity and Signaling in Cells

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The p53 transactivation domain (p53TAD) is an intrinsically disordered protein (IDP) domain that undergoes coupled folding and binding when it interacts with partner proteins like the E3 ubiquitin ligase, Mdm2, and the 70 kDa subunit of replication protein A, RPA70. The secondary structure and dynamics of six closely related mammalian orthologues of p53TAD were investigated using nuclear magnetic resonance (NMR) spectroscopy. Clustering analysis showed that the divergence in transient helical secondary structure of the p53TAD orthologues is more extensive than the amino acid sequence divergence. In contrast, strong correlations were observed between the backbone dynamics of the orthologues and the sequence identity matrix, suggesting that the dynamic behavior of IDPs is under positive evolutionary selection. Mutating conserved prolines that flank the Mdm2 binding site to Alanines doubled the level of transient helical secondary structure in this region. This doubling of transient helical secondary structure increased the *in vitro* binding affinity between p53TAD and Mdm2. The *in vivo* binding affinity between full-length p53 and Mdm2 was also increased in the proline mutants. This increase in binding affinity disrupted the expression of p53 target genes and inhibited the ability of cells to arrest in G1 following radiation induced DNA damage. Taken together our results demonstrate that the transient helical secondary structure of p53TAD has been finely tuned by evolution and disrupting this structure has deleterious effects on target gene expression and cell fate decisions.
This research was supported by the Deutsche Forschungsgemeinschaft (Emmy Noether grant PS1794/1-1 to PS), the Association pour la Recherche contre le Cancer (postdoctoral fellowship to FXT), the European Union FP7 (Marie Curie CIG to AL), the American Cancer Society (RSG-07-289-01-GMC to GWD) and the National Science Foundation (MCB-0939014 to GWD).

Maturation of Clathrin-coated Vesicles Requires Dynamic Instability and Processivity

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Clathrin-mediated endocytosis (CME) is a dynamic process that is important for synaptic vesicle retrieval, receptor uptake and virus internalisation. More than thirty interacting proteins have been identified in this pathway, their interactions studied *in vitro* and the temporal recruitment patterns mapped *in vivo*. How does a complicated network of protein interactions give rise to the emergent property of clathrin-coated vesicle budding? We find the network is wired for processivity via overlapping short motifs in intrinsically disordered protein domains binding to competing adaptors (FCHo2 and AP2). This inherent competition gives rise to dynamic instability, constant co-existence of assembly and disassembly, and the arrangement of competition between modules determines directionality. I will discuss the two major functional consequences of disrupting the CME network. While the network is robust and withstands minor perturbations, there is a correlation between the strength of CME inhibition and the way the network has reacted. We will explore the implications of this analysis.

Regulation by In-Complex Molecular Switching

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Proteomics has shown us that regulatory proteins spend most, often all, of their time in large macromolecular complexes. This suggests that to understand cell regulation, we need to understand the processes that occur within these complexes. The knowledge now being won about the role of natively disordered polypeptide and short linear motifs, suggests that these protein modules are assembled into molecular switch devices within these complexes. We have reviewed and classified these switches by mechanism. We collate motif switches in the switches.ELM database. In the talk, I will discuss the nature of cell regulation by molecular switching and how we might move forward the computational representation of regulatory pathways and complexes. In particular, it needs to be understood that protein complexes are units of biochemical function as, at a different scale, are individual peptide modules (domains, motifs): Regulatory proteins themselves are not, however, meaningful units of biochemical function but are vehicles for bringing concatenated assemblies of functional peptide modules into the regulatory complexes.

Entropic Exclusion Determines Allostery in a Major Family of Intrinsically Disordered Bacterial Transcription Factors

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Phd is the paradigm of a recently discovered transcription regulation mechanism known as conditional cooperativity. Under normal conditions transcription of classic type II toxin-antitoxin operons occurs through a complex mechanism that allows for the toxin to act as a co-repressor at low toxin:antitoxin ratios and become an activator at high toxin:antitoxin ratios. To address how Phd recognizes its binding sites, we determined the crystal structures of phage P1 Phd in complex with its operator box. The DNA-binding domain of the Phd dimer interacts with DNA in a novel fashion where α-helix α1 “reads” the target sequence and the backbone of α-helices α1 and α2 interact with the phosphate backbone. Moreover the wing regions defined by loop b2b3 of each monomer bind to the minor groove of the DNA tethering the DNA to the protein. These wing contacts communicate the N-terminal region of the protein to the intrinsically disordered C-terminus and may explain the allosteric cross-talk between toxin binding and DNA regulation. Our data reveal the intrinsically disordered domain of Phd acts as a master regulator by subjecting the system to either negative or positive cooperativity, depending on the occurring interaction. In absence of Doc, the intrinsically disordered part of the repressor bound to the DNA acts as a "veil" covering the second site and precluding the binding of a second Phd molecule, resulting in strong negative cooperativity and weak repression. When Doc is present it acts as an anchor point for the second Phd that folds upon binding leading to positive cooperativity and strong repression. Such cooperativity switch enables the condition-specific tuning of transcription that regulates the operon.

Role of Functional Disorder in Large Protein Complexes

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The eukaryotic proteome contains proteins that populate a wide variety of structural forms. Fully folded proteins are the most familiar from structural studies, but disordered and partly folded forms are also functional. Some proteins and protein domains are completely disordered, and this disorder is required for their correct function. Cellular signaling is one area where a number of functional disordered and partly ordered proteins play a part. We have been exploring the role of disorder in the interactions of the transcription factor NFκB with its inhibitor protein IκBα by measuring NMR spectra of component domains of these proteins. Disorder and partial order in IκBα contribute to the affinity and specificity of its interaction with NFκB, particularly in its competition for DNA-bound NFκB. Disorder and partial order are also observed in NFκB, adding to the complexity of its interactions with both IκBα and DNA. These studies illustrate the role of flexibility in the interactions of polypeptide chains as they interact to perform their functions.

Disordered Linker Regions for Sorting of Transmembrane Proteins

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Traffic of membrane proteins to its proper membrane compartment depends on sorting signals encoded on the membrane proteins. We studied sorting of membrane proteins to the inner membrane of the nuclear envelope in *Saccharomyces cerevisiae* and found that the transport of the membrane proteins Heh1 (Src1) and Heh2 depends on a sorting signal that is composed of a nuclear localization signal (NLS) and a long intrinsically disordered (ID) linker (Meinema et al., Science 2011). We proposed a transport mechanism in which the ID linker dodges into the NPC scaffold to enable interactions inside the NPC at a distance from the membrane. We followed up on this work and present structural, biochemical and in vivo microscopy data showing the membrane proteins are embedded in the membrane during transport, the NLS of Heh2 has unique properties and mutational analysis of the ID linker to probe role of flexibility and charge. We noted that ID linkers are also present in some membrane proteins that reside in membrane junctions or contact sites between the endoplasmic reticulum (ER) and the plasma membrane (PM). We show that the localization at the cell periphery of two *S. cerevisiae* proteins, Ist2 and Ssy1, depends on the presence of a plasma membrane binding domain, an ID linker region of sufficient length and a transmembrane domain that most likely resides in the endoplasmic reticulum. We conclude the ID regions play a relevant role in bridging adjacent heterologous membranes.

In both targeting routes, to the inner membrane of the nuclear envelope and to the PM-ER junctions, the role of the ID linker may be to present the sorting signal away from the crowded membrane region and to resolve the restriction to 2D movements of membrane embedded proteins.

Parallel Tuning of Activation and Repression in Intrinsic Disorder-Mediated Allostery

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Intrinsically disordered proteins (IDPs) present a functional paradox because they lack stable tertiary structure, but nonetheless play a central role in signaling. Like their structured protein counterparts, IDPs can transmit the effects of binding an effector ligand at one site to another functional site, a process known as allostery. Because allostery in structured proteins has historically been interpreted in terms of propagated structural changes that are induced by effector binding, it is not clear how IDPs, lacking such welldefined structures, can allosterically affect function. Here we show mechanistically, using human glucocorticoid receptor (GR) as a model, how IDPs transmit signals allosterically through a probabilistic process that originates from the simultaneous tuning of both activating and repressing ensembles of the protein. Moreover, GR modulates this signaling by producing translational isoforms with variable disordered regions. These results provide a functional explanation for the prevalence of splice sites and post-translational modufication sites within ID segments.

PKA Signaling: Linkers, Loops, and Intrinsically Disorder Regions Control Allostery

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The PKA catalytic subunit was the first protein kinase to be crystallized and gave us our first glimpse of the fold that describes all protein kinases, which represent one of the largest gene families. The two lobes that comprise the core are flanked by N-terminal and C-terminal tails which wrap around both lobes. By capturing all the stages of catalysis in crystal structures we appreciate the dynamic nature of these tails, how they wrap around the N-lobe and how they undergo order-disorder transitions as part of the catalytic cycle. Ordering the activation loop by the addition of a critical phosphate is another fundamental order/disorder transition that is a conserved feature of most protein kinases. These dynamic features allow the kinases to function as highly regulated molecular switches. In the case of PKA, the assembled C-subunit is packaged with regulatory subunit dimers that contain the cAMP binding domains. The cyclic nucleotide binding domains are highly dynamic allosteric signaling modules that have been conserved at all stages of biology, and once again the fundamental features of this domain have been elucidated by crystal structures of the isolated subunits. However, it is not until one sees full-length R_2C_2 tetramers of the different PKA isoforms that one appreciates the importance of allostery for PKA signaling. The order/disorder transitions of the linker regions of the R-subunit are an essential feature of this regulation that is so critical for every mammalian cell.
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Nup98 FG-Domains from Diverse Species Spontaneously Phase-separate into Hydrogels with Exquisite NPC-like Permeability

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The permeability barrier of nuclear pore complexes (NPCs) conducts massive transport mediated by shuttling nuclear transport receptors (NTRs) and, at the same time, suppresses an intermixture of nuclear and cytoplasmic contents. In Xenopus, it relies foremost on the intrinsically disordered FG-repeat domain of Nup98. We now analyzed Nup98 FG-domains from evolutionary distant eukaryotes representing mammals, lancelets, insects, nematodes, fungi, plants, amoebas, ciliates, and excavates. We observed that dilute aqueous solutions of these FG-domains spontaneously phase-separate into characteristic "FG-particles" with hydrogel properties. Phase separation required neither sophisticated experimental procedures nor auxiliary eukaryotic factors, but occurred already during recombinant FG-domain expression in bacteria. The Nup98 FG-phases displayed essentially the same permselectivity as authentic NPCs: They posed effective barriers towards inert macromolecules and yet allowed far larger NTR·cargo complexes to enter rapidly. FG-particles even reproduced the known phenomenon that large cargo-domains inhibit NPC-passage of NTR·cargo complexes and that cargo-shielding and an increased NTR: cargo surface-ratio can override this inhibition. Their exquisite sorting selectivity and intrinsic assembly propensity suggest that Nup98 FG-phases form also in authentic NPCs and indeed account for the permeability properties of the pore.

Inverse Size Scaling of the Nucleolus by a Concentration-dependent Phase Transition

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Cells must coordinate the size of their structures across a range of length scales as they grow and divide. Indeed, many organelles, such as the nucleus, mitochondria, mitotic spindle and centrosome, exhibit size scaling, a phenomenon in which organelle size depends linearly on cell size. However, the mechanisms of organelle size scaling remain unclear. Here, we show that the cell size-dependent assembly of the nucleolus, a membrane-less organelle important for cell size homeostasis, arises from an intracellular phase transition. We find that nucleolar size directly scales with cell size in early *C. elegans* embryos. Surprisingly, however, when embryo size is altered, we observe *inverse* scaling: nucleolar size increases in small cells and decreases in large cells. We demonstrate that this seemingly contradictory result arises from maternal loading of a fixed number of nucleolar components, which condense into nucleoli only above a threshold concentration. Such concentration-dependent phase transitions provide a mechanistic link between organelle size and cell size and may represent a general principle underlying the functional organization of the cell.

The Role of Multivalent Interactions of Tumor Suppressor SPOP with Gli3 in Regulating Ubiquitination

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Multivalent protein interactions can give rise to avidity effects, ultrasensitivity, phase separation and spatial organization. The tumor suppressor SPOP, the substrate adaptor of a ubiquitin ligase, self-associates into large oligomers. We predict that its predominantly intrinsically disordered substrate Gli3 contains many weak SPOP binding motifs. Multivalency of SPOP and Gli3 for each other, i.e. the ability of SPOP oligomers to bind many SPOP binding motifs in Gli3 and of Gli3 molecules to interact with many SPOP monomeric units, suggests a highly concentration-dependent interaction.

We used a peptide microarray, NMR spectroscopy, fluorescence methods and analytical ultracentrifugation (AUC) to identify SPOP binding motifs in Gli3. Importantly, even very weak motifs contributed substantially to ubiquitination demonstrating their functional relevance. Using AUC, size exclusion chromatography, and static light scattering, we demonstrated that two distinct dimerization domains in SPOP mediate the formation of concentration-dependent higher-order SPOP homo-oligomers. Our thermodynamic characterization supports an isodesmic self-association model, indicating that the size of the oligomers is limited only by protein availability. Importantly, our full description of SPOP's valency as a function of concentration now permits the quantitative characterization of its interactions with multivalent binding partners.

Does the multivalent Gli3/SPOP interaction give rise to avidity effects in small complexes or to large cross-linked complexes? We show that Gli3 and SPOP co-localize into nuclear "bodies" in cells suggesting that these "bodies" may be mediated by (1) specific oligomerization through defined interfaces in SPOP; and (2) the weak multivalent interactions between Gli3 and SPOP. It has been suggested that concentration-dependent assembly/disassembly of large, multivalent complexes provides a mechanism for fine-tuning signaling cascades. Multivalent interactions of SPOP with its substrates may be an avenue to regulate levels of proteins critical in development and proliferation in a concentration-dependent manner.

Discerning Sequence-encoded Mechanisms of *de novo* Nuclear Puncta Formation by the Disordered Nephrin Intracellular Domain

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Phase separation leads to the formation of diverse nuclear and cytoplasmic puncta. The connection between sequence-encoded information and phase separation is poorly understood. We show that the disordered nephrin intracellular domain (NICD) , forms liquid-like nuclear puncta (nephrin puncta). We have used emerging rules for sequence-disorder relationships to quantify the impact of sequence patterns on forming nephrin puncta. These puncta require a combination of multivalent acidic motifs and hydrophobic motifs. The patterning and charge density of acidic motifs regulate phase separation leading to distinct classes of puncta. Hydrophobic motifs are also required for puncta formation. Deletion of these motifs is disruptive to puncta formation whereas the puncta are robust to sequence shuffling within the motifs. Although key proteins found in (nuclear) paraspeckle bodies colocalize to nephrin puncta, our evidence suggests that nephrin puncta are novel structures formed *de novo*. Our cellular measurements and computer simulations suggest that nephrin puncta, which form through liquid-liquid demixing, are likely to be driven by counterion-mediated diminution of long-range electrostatic repulsions between acidic motifs and non-specific short-range attractions mediated by hydrophobic motifs. Our studies suggest that non-specific multivalent interactions may be generally used, notably by disordered proteins, to promote the formation of known or novel phase separated cellular bodies. Further, our work suggests rules that connect sequence patterns within disordered proteins to their ability to phase separate in cells.

This work was supported by grants from the NIH and Welch Foundation, and by the Howard Hughes Medical Institute.

Phase Separation of a Disordered Protein in the Formation of Membrane-less Organelles

Patrick Farber[1], Timothy J. Nott[3], Ashok Sekhar[2], Evangelia Petsalakis[3], Eden Fussner-Dupas[3], David Bazett-Jones[1], Andrew Baldwin[4], Lewis Kay[2,1], Julie Forman-Kay[1].
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Intrinsically disordered proteins and regions (IDPs/IDRs), which do not have stable secondary and tertiary structure, are capable of adopting different structural states. Many IDPs/IDRs populate conformationally heterogeneous monomeric states or engage in discrete interactions with other proteins, leading to folding upon binding or retaining significant disorder in the bound state. Others are involved in large-scale association having different degrees of order, from more defined fibers, to variably networked gels and to disordered liquid demixed states or droplets. These latter have been suggested to provide the physical basis for cellular membrane-less organelles such as the nucleolus and RNA granules.

We have studied the N-terminal disordered region of Ddx4, an RNA DEAD-box helicase that is essential for formation of a class of membrane-less organelles termed nuage or germ granules functioning in spermatogenesis. When expressed in HeLa cells, the protein forms spherical, micron-sized, liquid-like cellular organelles. *In vitro*, it phase separates to form droplets with similar morphological and dynamic properties to the organelles observed in cells. Phase separation is sensitive to salt, pointing to the importance of electrostatic interactions. The sequence features of the disordered N-terminus of Ddx4 underlying phase separation include clustering of charged residues into blocks of net positive and negative charge, with over-representation of FG/GF pairs and RG/GR pairs within the positive blocks. Perturbations of these properties disrupt phase separation, pointing to multi-valent cation-pi interactions playing an important role. Solution NMR data are consistent with a disordered liquid-like state. The transient sampling of multi-valent interactions in self-association of Ddx4 extends previous observations of dynamic multi-valent interactions in discrete complexes of IDPs/IDRs. The insights obtained from these biophysical studies of Ddx4 will be valuable for developing a general understanding of the biogenesis and disassembly of membrane-less cellular organelles.

Assembly and Functions of mRNP Granules in Eukaryotic Cells

Roy Parker[1,2], Ross Buchan[3], Saumya Jain[3], David Protter[2], Briana Van Treeck[2], Robert Walters[2], Carolyn Decker[1,2], Jennifer Garcia[2], Sarah Mitchell[2], Siddharth Shukla[2].
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Stress granules and P-bodies are conserved cytoplasmic aggregates of non-translating mRNPs implicated in the regulation of mRNA translation and decay, and are related to RNP granules in embryos, neurons and in some neurodegenerative diseases. In a genetic screen using bakers yeast, we identified 125 genes that affect the dynamics of P-bodies and/or stress granules including multiple components of the vacuolar ATPase, THO/TREX and prefoldin complexes. Analyses of additional mutants, including Cdc48 alleles, provide evidence that stress granules can be targeted to the vacuole by autophagy. An area of current research is understanding the mechanisms that drive stress granule and P-body assembly and clearance and how that affects mRNA function.

The Oncoproteins of Human Papillomaviruses: Instances of Viral Strategies for Hijacking of Host Motifs

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Papillomaviruses are small oncogenic DNA viruses infecting the epithelia of mammals, birds and reptiles. "High-risk" papillomaviruses (hrm-HPVs) are the main cause for cervical cancer and are also often involved in head and neck cancers and in some cutaneous tumours. The oncogenic properties of papillomaviruses are mainly due to two "oncoproteins", E6 and E7, which promote proliferation and immortalisation and perturbate the adhesion properties of the infected cells. E6 and E7 proteins bind to large numbers of target proteins controlling cell proliferation (Rb, cyclins...), cell adhesion (PDZ domain proteins), protein degradation (E6AP, Cullin) and cell death (p53, BAK, Bax...). E6 and E7 proteins possess 150 and 100 residues, respectively. Interestingly, E6 and E7 mainly act by hijacking Small Linear Motifs (SLiMs) which normally mediate protein-protein interaction networks within the host. Both E6 and E7 extensively employ motif mimicry strategies, as observed for many other viruses (see review by Davey et al., Trends Biochem Sci. 2011). In particular, E7 contains a LxCxE motif which binds to diverse members of the Rb family, while E6 contains a C-terminal motif recognizing PDZ domains. E6 also employs a less usual strategy, which consists in capturing within its target host proteins acidic helical motifs containing the consensus LxxLL, which include some "LD motifs" found in various proteins involved in cell adhesion and polarity control. The LxxLL and PDZ hijacking properties of E6 and their biological implications will be discussed in detail in the light of recent structural and functional data obtained in our laboratory.

The C-Terminal Domain of Hepatitis B Virus Capsid Protein has Mastered being a Jack-of-all-trades

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Hepatitis B Virus (HBV) is a serious human pathogen: 350 million people suffer from chronic HBV infection and 600,000 die from it annually. Assembly of a HBV virion begins with formation of an RNA-filled T4 icosahedral capsid. Using moves akin to a contortionist, the circular dsDNA genome of the mature virus is reverse transcribed within the confines of the capsid. Extensive structural, biophysical, and cellular characterization have revealed that the capsid protein takes an active part in assembly, recruitment of the viral polymerase, the process of reverse-transcription, and intracellular trafficking. Functional analysis of HBV core particles has associated these biological roles directly with the C-terminus of the capsid protein. In an interesting twist, one set of functions require the C-terminus to be on the exterior of the capsid, while other functions place this domain on the interior. The 34 amino acid C-terminal domain is rich in arginine residues, is subject to phosphorylation, and contains a nuclear localization signal. Our work is being conducted using a series of mutant forms of capsid protein and a variety of biophysical techniques including hydrogen-deuterium exchange mass spectrometry. We have shown that the C-terminal domain reversibly unfolds leading to transient externalization. Phosphorylation of this linear motif can initiate reorganization of packaged nucleic acid and alters protein dynamics of the particle. The nucleoprotein core of HBV capsids is a complex machine controlled by a multifunctional linear domain that interacts with nucleic acids, kinases, and transport proteins all in days work.

Single-Molecule Biophysics of Proteins Disordered and Misfolding

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Intrinsic Protein Disorder is increasingly recognized as a functionally important and prevalent feature in biology. The special biophysics and chemistry of disordered protein motifs are believed to play a key role in protein biology, function and misfunction in the cell. However, the structural complexity inherent in these systems often limits the biophysical information available from many conventional ensemble methods. We adapt and devise novel and state-of-the-art single-molecule fluorescence methods with unique capabilities to uncover hidden structural and kinetic information about such complex systems, by minimizing the averaging intrinsic to ensemble techniques. We will discuss examples of our single-molecule studies which uncover new details of the complex landscapes for disordered protein association and folding, features that relate to their function and misfunction. Overall, we will highlight some of the unique capabilities of single-molecule methods to map dynamic structural complexity in such systems, thus providing insight critical to a fundamental understanding of biology.

Modulation of Huntingtin Exon 1 Interactions through Synergy between Polyglutamine Tracts and Flanking Sequence Motifs

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Polyglutamine expansions in the huntingtin protein cause Huntington's disease. N- and C-terminal segments that include a 17-residue amphipathic stretch N17 and a 38-residue proline-stretch C38 flank the polyglutamine tract in huntingtin. Mutant transcripts of the huntingtin gene are aberrantly spliced and yield toxic N-terminal fragments that span the exon 1 encoded region of huntingtin. Our in vitro biophysical and computational studies demonstrate that N17 and C38 act as gatekeeping linear sequence motifs. They modulate the driving forces for and mechanisms of polyglutamine-mediated aggregation of exon 1 spanning fragments of huntingtin. Additionally, C38 interacts directly with profilin and its affinity for profilin is enhanced by polyglutamine-mediated aggregation. Our results come from a combination of in vitro, in silico, and in cell investigations. They highlight the importance of polyglutamine aggregation-mediated enhancement in C38-proflin interactions for wild type polyglutamine lengths. These results also suggest a model for the interplay between protein-aggregation that has functional relevance for wild type polyglutamine lengths versus pathological aggregation for expanded polyglutamine tracts.

How Order and Disorder within Paramyxoviral Nucleoproteins and Phosphoproteins Orchestrate the Molecular Ballet of Transcription and Replication

Sonia Longhi.
AFMB, UMR 7257, CNRS and Aix-Marseille University, France

The nucleoproteins and phosphoproteins of measles, Nipah and Hendra viruses provide an excellent model system to study the functional impact of disordered motifs. The non-segmented, single-stranded RNA genome of these paramyxoviruses is encapsidated by the nucleoprotein (N) within a helical nucleocapsid. Transcription and replication are carried out onto this ribonucleoproteic complex by the viral RNA dependent RNA polymerase that consists of a complex between the large protein (L) and the phosphoprotein (P). The P protein serves as an essential polymerase co-factor as it allows recruitment of L onto the nucleocapsid template. Tethering of L relies on the interaction between the C-terminal X domain (XD) of the P protein and the C-terminal, intrinsically disordered domain (N_{TAIL}) of N. This latter is disordered not only in isolation but also in the context of the nucleocapsid, being partly exposed at the surface of this latter. Within N_{TAIL}, a short motif, serving as molecular recognition element, has been identified and the mechanisms of its interaction with XD thoroughly investigated. In particular, we have shown that binding to XD triggers α-helical folding of this motif, while the majority of N_{TAIL} remains "fuzzy". Random mutagenesis studies showed that this motif is poorly evolvable, implying that its sequence has already been naturally optimized for interaction with XD. Compared to N, the P protein has a higher modular organization, consisting of alternating disordered and structured regions. Among these latter is a coiled-coil region responsible for P multimerization. Using X-ray crystallography and SAXS we recently showed that these coiled-coil regions exhibit considerable differences in the quaternary structure and in the extent of disorder. The functional implications of these findings will be discussed.

Towards Describing IDP Function by Dynamic Structural Ensembles

Peter Tompa.
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Intrinsically disordered proteins and complex multidomain proteins are characterized by dynamic ensembles of conformations that cannot be unequivocally described by traditional static terms of structural biology. These states of proteins are critical in understanding their function at the atomic level, which will eventually lead to extending the structure-function paradigm to establish “unstructural biology” as a new field (1). The functional importance of structural dynamics and complexity necessitates new standards and protocols for the description of structural ensembles, also termed “supertertiary” structure in the case of very large proteins composed of a combination of folded and disordered elements (2). Here we will 1) outline the development of a new database (pE-DB) that is designed to hold structural ensembles of proteins (3), 2) show through a few examples (PSD95, CBP) current experimental efforts to describe structural complexity at the supertertiary structural level, and 3) describe a novel bioinformatics tool, DynaMine, developed for predicting backbone dynamics from amino acid sequence.

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2) Tompa, P. (2012) On the supertertiary structure of proteins. Nature Chem. Biol. 18, 597-600
3) Varadi, M. et al. (2014) pE-DB, a database of protein structural ensembles. Nucl. Acids. Res. epub
4) Cilia E. et al. (2013) From protein sequence to dynamics and disorder with DynaMine. Nature Commun. 4: 2741.

POSTER ABSTRACTS

POSTER SESSION I
Sunday, October 12
8:00 PM – 10:00 PM
Gandon Suite North

All posters being presented in Poster Session I should be set up the morning of October 12 and MUST be removed by 10:00 PM.

Abdel Aouacheria	1-POS	Board 1
Andrea Soranno	2-POS	Board 2
Ashley Phillips	3-POS	Board 3
Boris Shneyer	4-POS	Board 4
Ciara Kyne	5-POS	Board 5
Claudiu Gradinaru	6-POS	Board 6
Dana Reichmann	7-POS	Board 7
David Langelaan	8-POS	Board 8
Diego Tesauro	9-POS	Board 9
Eleanor Dickinson	10-POS	Board 10
Elliot Drew	11-POS	Board 11
Martha Cyert	12-POS	Board 12
Gitte Haxholm	13-POS	Board 13
Hadar Amartely	14-POS	Board 14
Harald Herrmann	15-POS	Board 15
Irina Suarez	16-POS	Board 16
Jeffrey Mugridge	17-POS	Board 17
Jihun Lee	18-POS	Board 18
Katharina Mandel	19-POS	Board 19
Konstantin Turoverov	20-POS	Board 20
Leah Randles	21-POS	Board 21
Louise Nikolajsen	22-POS	Board 22
Luca Ferrari	23-POS	Board 23
Lucía Chemes	24-POS	Board 24

1-POS Board 1

Evidence for a Broader Definition of BH3 Motifs Previously Known to Exist Only in Disordered and Globular Apoptosis Regulators

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BCL-2 proteins control cell death by apoptosis through a complex network of protein-protein interactions. This protein group is formed by a family of homologs related to BCL-2 (which contain BH1, BH2 and BH3 motifs), and by BH3-only proteins, which bind tightly to the BCL-2-like family members via their BH3 motif. Pro-apoptotic and anti-apoptotic BCL-2 homologs promote or prevent mitochondrial disruption, whereas classical BH3-only proteins (seven in humans, excluding Bid) act upstream of mitochondria to connect various stress stimuli to the BCL-2-regulated apoptotic pathway. BH3 motifs have three notable features, (1) a short length of about 20 amino acids, (2) a loose consensus sequence, and (3) they are present both in proteins adopting (or predicted to adopt) a defined 3D fold, such as the globular protein BCL-2, and in intrinsically disordered proteins (IDPs) such as the seven classical BH3-only proteins. In the unstructured BH3-only proteins, the BH3 motif is located in the context of disordered regions and adopts an alpha-helical structure upon binding to the BCL-2 globular domain, whereas in BCL-2 and its homologs, the BH3 motif is contained within an amphipathic alpha-helix that contributes to the overall domain architecture. Furthermore, the BH3 motif is not only involved in protein-protein interactions of intrinsically disordered BH3-only proteins with BCL-2-like globular domains but also in protein-protein interactions between globular domains (of the BCL-2-type). BCL-2 homologous proteins thus represent binding sites (receptors) for BH3 motifs, whether embodied in another BCL-2 homolog or within an IDP. Based on these features, we provide computational analyses of publicly available protein sequences and experimental evidence that the BH3 motif is a functional entity of broader scope not specific to BCL-2 proteins or cell death regulators.

2-POS Board 2

Single-molecule Spectroscopy Reveals Polymer Effects of Disordered Proteins in Crowded Environments

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Intrinsically disordered proteins (IDPs) are involved in a wide range of regulatory processes in the cell. Owing to their flexibility, their conformations are expected to be particularly sensitive to the crowded cellular environment. Here we use single-molecule Förster resonance energy transfer to quantify the effect of crowding as mimicked by commonly used biocompatible polymers. We observe a compaction of IDPs not only with increasing concentration, but also with increasing size of the crowding agents, at variance with the predictions from scaled-particle theory, the prevalent paradigm in the field. However, the observed behavior can be explained quantitatively if the polymeric nature of both the IDPs and the crowding molecules is taken into account explicitly. Our results suggest that excluded volume interactions between overlapping biopolymers and the resulting criticality of the system can be essential contributions to the physics governing the crowded cellular milieu.

3-POS Board 3

α-Synuclein D^2: The Disorderly Disordered Parkinson's Disease Amyloid

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Parkinson's disease is the 2nd most common neurodegenerative disorder worldwide, affecting approximately 2% of the world population over 65. Current UK spending is ~£3.3billion and with those aged over 65 predicted to double by 2050, a treatment is quickly becoming of paramount importance.

α-Synuclein is an amyloidogenic intrinsically disordered protein implicated in Parkinson's disease aetiology, whose potential functions range from enzyme regulation to synaptic vesicle release. α-Synuclein co-exists in conformations ranging from disordered monomers to β-sheet rich fibrils. Our aim is to unravel the structures of the potentially druggable early aggregation species using multiple gas phase techniques, to inform a drug discovery process whose disordered subject limits the use of traditional techniques.

Ion Mobility Mass Spectrometry (IMMS) is a hybrid mass spectrometry based gas phase electrophoretic technique which generates a rotationally averaged Collision Cross Section (CCS) for each conformer. Native Mass Spectrometry (MS) and IMMS have the ability to study the structure and conformational dynamics of complex protein samples.

Under gentle Nano Electrospray Ionisation conditions (nESI) α-Synuclein presents ions, over a wide charge state range in positive and negative mode, characteristic of its intrinsically disordered nature. The CCS of α-Synuclein monomers range from ~1000$Å^2$ to ~3000$Å^2$, again demonstrating its conformational flexibility. nESI also reveals small aggregate populations up to pentamer.

MS and IMMS highlight the conformational plasticity of α-Synuclein, including its susceptibility to solution condition modification, genre-defining levels of day-to-day variation and the lack of a solution phase conformational response following in vitro aggregation. Crosslinking-IMMS has also been used to directly sample the abundance solution phase conformers. Our results are compared to established biophysical techniques.

These results highlight the advantages of mass spectrometry-based approaches in determining transient structural forms and their application to studying the aggregation of amyloidogenic proteins.

4-POS Board 4

Unconventional Myosin 19 is a Novel Mitochondrial Membrane-anchored Molecular Motor and a Regulator of the Mitochondrial Network

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Mitochondria undergoing continuous cycles of fission and fusion create a very dynamic network, which is essential for its proper functions. Mitochondria long-range motility relies on microtubule motors such as kinesin and dynein, however actin filaments and myosins have also been shown to interact and support mitochondria localization and dynamics. Recently, the actin-dependent motor Myosin 19 was found to localize to the mitochondria. However, the interaction of endogenous Myosin 19 with the mitochondria, the molecular details of this interaction, and its physiological role remain unknown. We initiated our investigation with bio-informatics analysis to have revealed a putative transmembrane (TM) region in the tail domain of Myosin 19, which is flanked with positive residues and predicted to form an amphiphilic α-helix. We demonstrate using subcellular fractionation, phase separation and carbonate extraction that endogenous Myosin 19 is anchored to the OMM revealing its amphiphilic nature. Furthermore, we show using proteinase K and carboxypeptidase Y digestion of purified mitochondria that Myosin 19 has an Ncyt-Ccyt topology. Remarkably, the predicted TM region is essential and sufficient to target Myosin 19 to the mitochondria. We show that Myosin 19 overexpression disrupts the mitochondrial network and causes it to be restricted to the perinuclear region. Interestingly, starvation causes Myosin 19 to re-localize to the tips of actin protrusions in the cell periphery. To this end, we present strong evidence that Myosin 19 is a monotopic membrane protein localized to mitochondria and may provide a linkage between the mitochondria and the actin cytoskeleton. The altered localization may reveal an additional role for Myosin 19 which is linked to mitochondria function and regulation. These results support the contribution of the actin cytoskeleton and myosin to mitochondrial dynamics.

5-POS Board 5

Residue-level Insights into α-synuclein Aggregation

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The structural properties of intrinsically disordered proteins (IDP's) give them unique binding abilities.[1] For example, IDP's have increased conformational freedom and large "capture radii", allowing them to rapidly encounter partner proteins.[2] IDP flexibility can also facilitate induced folding upon binding.[1,2] Such disparate binding abilities allow IDP's to interact with a number of partners, making them important regulators of macromolecular assemblies in cells.[1,3] Erroneous IDP interactions therefore have drastic consequences *in vivo*. For example, IDP-mediated aggregates (amyloids) are pathological hallmarks of many neurodegenerative diseases.[3] Although the molecular-level mechanisms of amyloid formation remain poorly understood, evidence suggests that the process of IDP aggregation in vitro is similar to that observed in biology.[3] Thus, studies of IDP aggregation under simplified conditions will improve our understanding of amyloid formation in cells. Similarly, studies of IDP interactions with ligands that promote or inhibit aggregation can reveal sites of importance in the aggregation process.[4]

α-synuclein (α-syn) is an IDP that forms the major component of cytoplasmic amyloids associated with Parkinson's disease, multiple system atrophy and dementia.[3] Here, we describe the NMR characterization of α-syn's interaction with a known inhibitor of α-syn aggregation. The interaction between α-syn and a macrocyclic molecule will also be discussed. The implications for α-syn self-association will be addressed.

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6-POS Board 6

The Effect of Intrachain Electrostatic Repulsion on the Conformational Disorder and Dynamics of the Sic1 Protein: A Single-molecule Study

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In yeast, the cyclin-dependent kinase inhibitor Sic1 is a disordered protein that, upon multi-site phosphorylation, forms a dynamic complex with the Cdc4 subunit of an SCF ubiquitin ligase. To understand the multi-site phosphorylation dependence of the Sic1-Cdc4 interaction, which ultimately leads to a sharp cell cycle transition, the conformational properties of the disordered Sic1 N-terminal targeting region were studied using single-molecule fluorescence spectroscopy. Multiple conformational populations with different sensitivities to charge screening were identified by performing smFRET and FCS experiments in non-denaturing salts and ionic denaturants. Both the end-to-end distance and the hydrodynamic radius decrease monotonically with increasing the salt concentration, and a rollover of the chain dimensions in high denaturant conditions is observed. The data was fitted to the polyelectrolyte binding-screening model, yielding parameters such as the excluded volume of the uncharged chain and the binding constant to denaturant. An overall scaling factor of ~1.2 was needed for fitting the data, which could imply that Sic1 cannot be approximated by a random Gaussian chain. Fluorescence correlation spectroscopy reveals Sic1 structure fluctuations occurring on both fast (10-100 ns) and slow (~10ms) time scales, with the fast phase absent in low salt solutions. Our data provide direct evidence that long-range intrachain electrostatic repulsions are a significant factor for the conformational landscape of Sic1, and support the role of electrostatics in determining the overall shape and hydrodynamic properties of intrinsically-disordered proteins.

7-POS Board 7

How Do Intrinsically Disordered Chaperones Work?

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The ability of cells to sustain and recover after stress conditions depends on a well-developed network of protein chaperones. Recently, a new class of intrinsically disordered (ID) chaperones, including the redox-regulated chaperone Hsp33, was discovered. This unique class of ATP-independent chaperones serves as the first line of defense in problematic stress conditions that cause both broad protein unfolding and inactivation of essential housekeeping chaperones. One common feature of this class of chaperones is their ability to rapidly convert large parts of their structure into unfolded protein segments in response to stress conditions. This state of native disorder seems to be crucial for their role in preventing protein aggregation by binding partially unfolded clients and releasing them once stress conditions are abolished.

This mode of action raises fundamental questions regarding the role of intrinsic disorder in chaperones function, regulation and specificity. To address these questions we used a highly conserved redox-regulated chaperone, Hsp33, as a model protein. The activation of Hsp33 is triggered by oxidation, which leads to unfolding of ~40% of its structure. To understand a mechanism of substrate recognition and role of structural plasticity, we, at first, extended characterization of the Hsp33 chaperones to eukaryotes. In this study, we characterized a novel eukaryotic homologue of Hsp33 in Trypanosoma Brucei. Silencing of this chaperone leads to increase in sensitivity to heat and oxidative conditions in the Trypanosoma parasites. In addition, to define a role of sequence specificity in chaperone activity, we designed a chimera Hsp33 chaperone by replacing a large region of its binding site by a non-related sequence originated from a non-Hsp33 protein. Remarkably, the chimera protein exhibited a significant chaperone activity. This finding challenges the current consensus that function relates directly to protein sequence, but rather to its structural elements.

8-POS Board 8

Investigating the Structural Basis for Recruitment of CBP/p300 by E2A

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E2A is a transcription factor that is essential for proper regulation of B-lymphocyte development and disruption of E2A through a t(1;19) chromosomal translocation is associated with acute lymphoblastic leukemia. The product of the t(1;19) translocation is the oncoprotein E2A-PBX1 which contains the unstructured activation domains (AD) of E2A and most of the PBX1 protein, including the DNA binding domain. Previous work from our group has determined that leukemogenesis primarily requires AD1 of E2A and the KIX domain from CBP/p300. However, the activation domains of E2A are able to recruit CBP/p300 in a cooperative manner, suggesting that other modules of CBP/p300 may be involved in forming the E2A:CBP complex. The objective of this study was to isolate other domains of CBP/300 which recognize E2A. Using complementary biophysical techniques such as peptide microarrays, isothermal titration calorimetry, pull-down experiments and nuclear magnetic resonance spectroscopy we have determined that AD1 of E2A adopts a helical structure when it binds to the Taz2 domain of CPB/p300. These results illustrate how the unstructured activation domains of E2A are able to recognize multiple domains of CBP/p300, allowing for cooperative recruitment of CBP/p300 by E2A.

9-POS Board 9

CD and NMR Conformational Preferences of Intrinsically Disordered Amphiphilic Peptides: A New Class of Potential Targets in Drug Discovery

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Owing to the large panel of biological functions of peptides and their high specificity and potency, the development of peptide-based therapeutic and diagnostic tools has received increased interest. Peptide amphiphiles (PAs) are an emerging class of molecules in which a bioactive peptide is covalently conjugate to a hydrophobic moiety1. Due to the coexistence in the molecule of a hydrophilic peptide sequence and a hydrophobic group, PAs are able to self-assemble spontaneously into a variety of nanostructures, such as monolayers, bilayers, and vesicles. In this work we have synthesized predicted by MEDOR2 disordered peptide sequences3 functionalized by alkyl chains, and connected by ethoxylic-based linker. The structural properties in solution of these new PAs were investigated using CD, NMR and DLS. The presence of the alkyl chains induces not only the self-assembly of these new PAs into supramolecular aggregates but also a gain of structure within the disordered peptide. The design of supramolecular systems, generated by joining a disordered peptide and a lipophilic moiety, could drive the disordered peptide to fold into a stable structure. This structural modification could be a promising route to develop a new class of bio-molecules for processes in which a specific conformational rearrangement is required.

10-POS Board 10

The Use of Ion Mobility Mass Spectrometry to Probe Modulation of Function of p53 by a Small Molecule Inhibitor

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The transcription factor, p53 is heavily implicated in tumour suppression pathways, blocking tumour development by triggering cellular senescence or apoptosis. This partially disordered protein is implicated in over 50% of tumours, its function often rendered inert by overexpression of the ubiquitin E3 ligase MDM2, also intrinsically disordered. p53 binds to MDM2 directly via their respective N-terminal domains. Release of p53 from MDM2 would reactivate the tumour suppressor, providing an attractive cancer therapy drug target. The drug candidate RITA has been shown to restore wild-type p53 function in tumour cells by preventing the p53/MDM2 interaction. In contrast to the previously reported inhibitor Nutlin which binds MDM2, RITA binds to the p53 N-terminus. It is hypothesised that RITA binds outside of the p53/MDM2 binding cleft, allosterically exerting its effect via a conformational change in p53.
This study aims to probe the mechanism of RITA inhibition of p53 using a multi-technique approach. We employ the gas phase techniques Mass Spectrometry (MS) and Ion Mobility-Mass Spectrometry (IM-MS) to study the conformational space occupied by both wild-type and mutant N-terminal p53 during RITA inhibition. Hydrogen deuterium exchange coupled to MS (HDX-MS) is used to study the interaction in the solution phase. IM-MS measurements reveal that RITA binds to p53 N-terminus transiently and with low affinity. Despite this it reconfigures the wild-type protein to a unique compact structure. We hypothesise that this conformational change in p53 prevents MDM2 from binding. HDX-MS data reveals the highly flexible nature of p53 N-terminus and highlights the unique capability of IM-MS to capture transient structural intermediates in dynamic protein systems.

11-POS Board 11

Protein Design for Decreased Disorder

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Disorder-to-order transitions are the basis for the promiscuity and diversity of many interactions seen in intrinsically disordered proteins (IDPs), leading to the ubiquity of intrinsic disorder in signalling and regulatory proteins. However, the complexity of IDP dynamics present a unique challenge to the structural characterisation of these proteins. Using the Small Hydrophilic Endoplasmic Reticulum associated Protein (SHERP) from the parasite L. major, protein design principles have been applied to explore the protein's disorder-to-order transitions both computationally and in vitro. A number of sequences were identified which significantly decrease protein disorder and a strong relationship between decreasing disorder and increasing energetic stability is seen in SHERP mutants. This has allowed for the identification of key residues involved in structural transitions and the design of mutant proteins which preserve key features of the wild-type ordered structure.

12-POS Board 12

The Calcineurin Signaling Network Evolves Via Conserved Kinase–Phosphatase Modules That Transcend Substrate Identity

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To define the first functional network for calcineurin, the conserved Ca^{2+}/calmodulin-regulated phosphatase, we systematically identified its substrates in *S. cerevisiae* using phosphoproteomics and bioinformatics, followed by co-purification and dephosphorylation assays. This study establishes new calcineurin functions and reveals mechanisms that shape calcineurin network evolution. Analyses of closely related yeasts show that many proteins were recently recruited to the network by acquiring a calcineurin-recognition motif. Calcineurin substrates in yeast and mammals are distinct due to network rewiring but surprisingly are phosphorylated by similar kinases. We postulate that co-recognition of conserved substrate features, including phosphorylation and docking motifs, preserves calcineurin-kinase opposition during evolution. One example we document is a composite docking site that confers substrate recognition by both calcineurin and MAPK. We propose that conserved kinase-phosphatase pairs define the architecture of signaling networks and allow other connections between kinases and phosphatases to develop and establish common regulatory motifs in signaling networks.

13-POS Board 13

Intrinsically Disordered Cytoplasmic Domains of Two Cytokine Receptors Mediate Novel Interactions with Membranes

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Class 1 cytokine receptors regulate essential biological processes such as metabolism, reproduction and growth, through complex intracellular signaling networks[1,2]. The structural platform for understanding their functions is currently incomplete as structure-function studies of the intracellular domains (ICDs) are critically lacking. We have used nuclear magnetic resonance spectroscopy in combination with other biophysical techniques to present the first comprehensive structural characterization of any cytokine receptor ICD. We show that the ICDs of the human prolactin and growth hormone receptors are intrinsically disordered throughout their entire lengths. We further show that they interact specifically with hallmark lipids of the inner plasma membrane leaflet through conserved hydrophobic and basic motifs resembling immuno T-cell receptor activation motifs (ITAMs). Substituting either the basic or hydrophobic residues resulted in reduced binding to membranes. Based on these results, we propose a model where cytokine receptor ICDs associate with the membrane to confine intracellular signaling at the membrane interface, thereby increasing the efficiency of signaling. Our findings will impact future structure-function studies of cytokine receptors and provide a missing link to address differential signaling implicating the membrane as an active component.

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14-POS Board 14

The STIL Protein Contains Intrinsically Disordered Regions that Mediate its Protein-protein Interactions

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STIL is a centrosomal protein that participates in centrosomal biogenesis and controls normal cell division. Its expression is crucial for the development of tissues, especially embryonic. Overexpression of STIL is correlated with formation of numerous types of cancer. Despite its importance, the mechanism of action of STIL is not clear and no structural or quantitative information is available about the protein. Here we performed structural and biophysical characterization of the central domain of STIL. We divided the protein to several fragments and developed, for the first time, protocols for expression and purification of the recombinant central domains of STIL: STIL4 200-450, STIL5 450-700 and STIL6 500-650. SEC, CD and computational disorder predictions revealed that the central domain of STIL is mostly disordered. This region mediates the interactions of STIL with the CHFR protein: Peptide array screening and fluorescence anisotropy revealed CHFR-derived peptides that bound STIL IDR with nanomolar affinity. The intrinsic disorder may provide STIL the flexibility required for its function. The structural properties of STIL combined with the large number of interactions with its different partners make STIL likely to be a scaffold protein.

15-POS Board 15

The Non-structured Amino-terminal Domain of Intermediate Filaments Powers Assembly

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Intermediate filament (IF) proteins are principal structures of the metazoan cytoskeleton. The IF multi-gene family represents fibrous proteins consisting of a central alpha-helical "rod" domain flanked by non-alpha-helical amino- (“head”) and carboxy-terminal (“tail) domains. IF-proteins form robust but highly flexible fibers and networks, both in the cytoplasm and the nucleus, that are not soluble under physiological conditions. Their role for the mechanical properties of cells is being elucidated by recent work quite convincingly. However, the assembly mechanism is still not completely understood, in particular the role of the non-structured “head” is by no means clear. At the molecular level, IF-proteins form coiled coils that laterally associate in an anti-parallel fashion to yield tetrameric complexes, which are stable under low ionic strength conditions. Hence, the assembly module for IFs is non-polar. The principal reaction for the elongation of IF proteins is a “head-to-tail” association of the “rods” with a 2 to 4 nm overlap. The “head” domain is essential for assembly, both laterally and longitudinally, as headless IF-proteins form only dimers under tetramer conditions, and tetramers under filament forming conditions. In order to investigate the contribution of individual parts of the “head” domain in these two assembly reactions, we have subjected the fibroblast-specific IF-protein vimentin to systematic truncation by recombinant techniqiues within the “head” domain. The assembly properties were followed by analytical ultracentrifugation, electron and atomic force microscopy in vitro and by transfection of the corresponding cDNA clones into vimentin-free fibroblasts generated from vimentin-(-/-) embryos. We demonstrate that the first 20 amino acids of the 85 amino acid-long "head" are dispensable for both tetramer and IF formation. Further truncation abolishes filament-forming capacity completely. Truncation of more than 40 amino acids impairs tetramer formation indicating the “head” is needed for orderly coiled-coil alignment.

16-POS Board 16

Intrinsically Disordered dsRNA Binding Domain of *Arabidopsis thaliana* DCL1 Folds in the Presence of Substrate RNA

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DCL1 is the ribonuclease that carries out miRNA biogenesis in plants. The enzyme has two tandem double stranded RNA binding domains (dsRBDs) in its C-terminus, which are essential for the enzyme function *in vivo*.

By means of fluorescence anisotropy assays, we show that the first of these domains (DCL1-A) binds precursor RNA fragments when isolated, and cooperates with the second domain in the recognition of substrate RNA.

Remarkably, despite showing RNA binding activity, DCL1-A is intrinsically disordered. We produced four different constructs of the protein, spanning the isolated domain and including surrounding regions. The domain is unstructured in every case. We explored different solution conditions to test what could lead the domain to acquire an ordered structure, and found that it folds when bound to its substrate dsRNA. By acquiring a set of standard NMR spectra, we assigned ca. 90% of the backbone resonances corresponding to the free unfolded and bound folded protein. Analysis of NMR data of the free protein shows it transiently explores secondary structure elements on the C-term end that could be essential for its capability of binding to the substrate. We have calculated the structure of the folded protein in complex with dsRNA employing CS-Rosetta. The structure corresponds to a canonical dsRBD, bearing some differences. One of the three RNA binding regions is missing, but affinity for the substrate is not affected. Finally in the presence of excess dsRNA we observe an intermediate unfolded bound species. ZZ exchange experiments show that this unfolded form is in slow exchange with the folded form. Based on these results, we propose a binding mechanism and discuss functional implications.

17-POS Board 17

Short Linear Motifs and Activation of Dcp2-mediated mRNA Decapping

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Cellular regulation of messenger RNA (mRNA) is crucial for proper gene expression. 5'-to-3' mRNA decay is one of the major pathways used by eukaryotes to regulate mRNA levels and carry out mRNA quality control. A critical step in the 5'-to-3' decay pathway is the removal of the protective methyl-guanosine cap found on the 5'-end of all eukaryotic mRNA, which commits the transcript to rapid degradation. Cleavage of the cap structure is catalyzed by the conserved decapping enzyme Dcp2, in combination with protein coactivators that modulate decapping activity. Many of these coactivators employ unstructured, short linear motifs to bind and activate the decapping enzyme. Dcp2 is a dynamic enzyme and one of the ways disordered motifs in coactivators may accelerate catalysis is by shifting Dcp2 conformational equilibria to promote the active conformation of the decapping complex. Here we present in vitro biophysical (NMR) and biochemical (enzymology) data characterizing Dcp2 function in the presence of disordered coactivator motifs. We propose a conserved model of Dcp2 activation in which short linear coactivator motifs contact Dcp2 near the active site and promote the active conformation of the decapping enzyme.

18-POS Board 18

Multivalent Interaction of Higher-order Oligomers of SPOP with the Transcriptional Activator Gli3

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Multivalent interactions, in which each binding partner has multiple binding sites or binding motifs for the other protein, can lead to the assembly of large higher-order complexes allowing for subcellular organization. Speckle-type POZ protein (SPOP), recently identified as a novel tumor suppressor, localizes to nuclear puncta and is a substrate adaptor of a cullin3-RING ubiquitin ligase (CRL). It recruits substrates to the CRL and promotes their ubiquitination and downstream degradation. SPOP self-associates into large higher-order homo-oligomers through its two independent oligomerization domains, rendering it multivalent for substrates. Some SPOP substrates, such as Gli3, reportedly harbor many SPOP binding motifs. Here, we identified SPOP binding motifs in Gli3 and determined that SPOP self-associates indefinitely through an isodesmic self-association mechanism. Using a variety of biophysical methods including dynamic light scattering (DLS), composition gradient multi-angle light scattering (CG-MALS), biolayer interferometry (BLI) and cellular assays we studied the multivalent interaction between SPOP and Gli3. We investigated self-association and substrate binding of SPOP mutants found in cancers comparing physiological and pathological SPOP properties. We determined factors promoting SPOP localization in nuclear punctate structures. The formation of higher-order complexes may result in subcellular organization but may also lead to activity enhancement through high local concentrations of components, and ultrasensitive regulation of signaling pathways.

19-POS Board 19

Re-Assembling Gab Family Protein-based Signalling Complexes

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Gab family proteins are large multi-site docking proteins serving as dynamic assembly platforms for signal processing protein complexes. Gab proteins contain a well-folded N-terminal pleckstrin homology (PH) domain, followed by a long tail region predicted to be largely disordered. However, the molecular details of how Gab facilitates the computation of specific biological responses remain largely unstudied. Upon receptor activation multiple tyrosine residues within the tail of Gab become phosphorylated and serve as binding sites for signalling proteins including SHP2, Crk, PLCγ and PI3 kinase. Two highly-conserved RxxK epitopes with helical secondary structure serve as critically important binding sites for the adaptor protein Grb2 [1], connecting the Gab proteins to a plethora of functionally distinct receptors.

Our study aims to investigate the structure and dynamics of Gab protein-based signalling complexes by attempting a step-wise assembly of recombinantly expressed key proteins. Initially, we are focusing on the caspase-generated Gab1 fragment p35Gab1 [2], which contains binding sites for Grb2, c-Met, and PI3 kinase. We co-purified p35Gab1 and Grb2 and studied the properties of the p35Gab1–Grb2 complex by biophysical techniques. In preliminary atomic force microscopy (AFM) and electron microscopy (EM) experiments, we detected clear signals for discrete, defined asymmetrical particles, which have dimensions consistent with that of Grb2 bound to additional protein material, and thus are likely to represent the p35Gab1–Grb2 complex. These initial findings suggest to us that it may be feasible to build even larger complexes containing Gab proteins bound to several interaction partners and to study their structural, biophysical and biochemical properties. Further structural analyses of step-wise assembled signalling protein complexes at higher resolution are planned to provide a first glimpse into the virtually unknown molecular architecture of these signalling network hub protein assemblies.

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20-POS Board 20

G-Actin is Quasi-Stationary State Stabilised by Ca Ion, While Polypeptide Chain of Actin Determines Its Inactivated State

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Actin is a ubiquitous and multifunctional protein. It is one of the main components of the system of muscle contraction, it forms the cytoskeleton, it is found in the cell nucleus in which, except for the motility and scaffold functions, actin acts as a regulator protein that participates in the processes of transcription and chromatin remodeling. From one hand G-actin is surely globular protein, it is even called so, yet by multifunctionality and ubiquity it resembles intrinsically disordered proteins. So what is it? Careful consideration of the unfolding-folding behavior of G-actin was a crucial step for answering this question. EDTA-induced calcium removal from actin and action of various denaturing agents (pH, temperature, and chemical denaturants), all result in the formation of the so-called inactivated actin (I-actin), which is a complex compact oligomer containing 14-16 subunits. G-actin denaturation in vitro is a completely irreversible process. Therefore, I-actin represents the thermodynamically stable state of a polypeptide chain, information about which is encoded in the amino acid sequence of this protein. Although it is believed that I-actin is functionally inactive, one cannot exclude a possibility that the functional importance of this form of actin is not established as of yet. G-actin, represents a thermodynamically instable, quasi-stationary state, which is formed in vivo as a result of complex posttranslational energy-intensive folding events controlled and driven by cellular folding machinery.
This work was supported by Program MCB RAS, grant RSF 14-24-00131

21-POS Board 21

E2 Ubiquitin-Conjugating Enzyme Interacts with Ubiquitin Receptor Rpn13

Leah Randles, Kylie J. Walters.
National Cancer Institute, Frederick, MD, USA.

Regulated protein degradation by proteasome is essential and contributes to a large spectrum of cellular events, including cell cycle progression, DNA repair, and signal transduction. Substrate ubiquitination by a 3-step E1-E2-E3 enzymatic cascade can signal for proteolysis by proteasome, which houses two ubiquitin receptors in its 19S regulatory particle, Rpn13 and S5a. Prior to proteolysis, substrates are deubiquitinated and Rpn13 recognizes ubiquitin with an N-terminal domain and deubiquitinating enzyme Uch37 with a C-terminal domain. We have found Rpn13 N-terminal domain to bind a disordered portion of an E2 ubiquitin-conjugating enzyme that functions in cell cycle progression. This poster will present functional and physical interrogations into this Rpn13/E2 interaction.

22-POS Board 22

The Intracellular Domain of the Human Growth Hormone Receptor is Intrinsically Disordered and Interacts with Lipids Characteristic of the Inner Plasma Membrane Leaflet

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The growth hormone receptor (GHR) is a single-pass transmembrane protein belonging to the type I cytokine receptor family. The GHR lacks intrinsic kinase activity and therefore depends on the activity of constitutively associated Janus Kinase 2 (JAK2) for signaling. GHRs exist as pre-formed inactive dimers in the membrane and binding of growth hormone (GH) leads to formation of active ternary 2:1 receptor/hormone complexes. Binding of GH has been shown to induce conformational changes in the extracellular and transmembrane domains leading to separation of the lower transmembrane domains and thereby separation of associated JAK2s. This separation results in release of intermolecular inhibitory interactions between the associated JAK2s, leading to apposition of kinase domains and signal transduction through the JAK-STAT pathway. Whereas numerous structures are available of complexes of the extracellular domain, no structural characterization is available for the intracellular domain (ICD) of the GHR. Using nuclear magnetic resonance (NMR) spectroscopy we show that the ICD of the human growth hormone receptor is intrinsically disordered over its entire length without long-range contacts. We have identified several transiently populated α-helices distributed along the chain, which are separated by extended structures. Using small unilamellar vesicles (SUVs) and NMR spectroscopy we have shown that the membrane proximal part of the GHR-ICD specifically interacts with lipids characteristic of the inner leaflet of the plasma membrane, but not with outer leaflet lipids. The lipid interaction domain (LID) spans 87 residues with three transient helices and has an architecture that is analogous to a similar domain identified in the human prolactin receptor. We propose a role for the LID:membrane interactions in signaling efficiency of the receptor.

23-POS Board 23

Chaperoning of Aggregation Prone Stretches

Luca Ferrari[1], Magdalena Wawrzyniuk[1], G. Elif Karagöz[1,2], Madelon M. Maurice[3], Stefan G. Rüdiger[1].
[1]Utrecht University, Utrecht, Netherlands, [2]UCSF, San Francisco, CA, USA, [3]UMC Utrecht, Utrecht, Netherlands.

Protein aggregation is a common landmark of many human diseases, yet its molecular basis remains poorly understood. The aggregation-prone stretches involved in this process are usually present buried within folded domain or otherwise buffered by the cellular environment. Mutations can either expose buried stretches or introduce new aggregation-prone sites, as observed for cancers involving tumor suppressors in signaling pathways.

We recently characterized the interaction between the molecular chaperone Hsp90 and the aggregation prone stretches of Tau, an intrinsically disordered protein involved in Alzheimer's disease (Karagöz et al., Cell 2014). The described interaction together with the importance of Hsp90 in late folding events suggest a pivotal role of this chaperone in the aggregation prone stretches dynamics.

The aim of our study is to understand how these dynamics occurs in folded proteins, folding intermediates and intrinsically disordered substrates.

To address this question we applied fluorescence techniques. We selectively labelled the Hsp90 binding region of Tau to unravel how this chaperone alters aggregation kinetics. The role of co-chaperones and ATP cycle were taken into account.

We turned to intrinsically disordered, aggregation-prone tumor suppressors, to test whether the paradigms gained from our study also apply to other molecular processes. We analyzed analysed Hsp90 interactions with members of the Wnt signalling cascade that are destabilized by cancer mutants. Indeed we found that cancer mutations in the destruction complex of the Wnt cascade cause protein aggregation and as a result tumour growth. The aggregation phenotype correlates with changes in the interactome, suggesting a causal link between aggregation and cancer phenotype.

Our study provides insights into molecular basis of protein aggregation and its deleterious effects on cellular activities. Our results shed also light on the counter intuitive concept of the interaction between intrinsically disordered proteins and the folding machinery of the cell.

24-POS Board 24

Binding Properties of Linear Motif-mediated Viral Retinoblastoma-target Protein Interactions

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[1]Leloir Institute-IIBBA CONICET Av. Patricias Argentinas

Many proteins from pathogenic viruses have intrinsically disordered (IDP) domains harboring short linear motifs that target cellular functions. The LxCxE and E2F motifs mediate high-affinity binding to the retinoblastoma (Rb) tumor suppressor, promoting cell cycle progress and efficient replication of the viral genome. However, persistent expression of viral proteins can lead to cell transformation and cancer. Interference with host functions is key to understanding viral pathogenesis. However, current insight into structure-function relationships of viral proteins is still scarce. A review of experimentally reported instances revealed LxCxE and E2F motifs presence in viral and cellular partners. However, viral instances are located exclusively within IDP regions and enriched in sequence features enhancing binding affinity, suggesting they may have evolved to allow for effective competition with cellular interactions. Cellular instances are located in exposed loops or helices, suggesting a stronger entropic cost for binding. Solution binding studies of the LxCxE and E2F motifs from four cellular and viral Rb targets showed that the E2F2 transcription factor motif bound Rb with high affinity (K_D = 12 nM). Opposed to the two-state behavior of the HPV E7 LxCxE site, binding involves slow conformational rearrangements of the E2F2 motif. The individual LxCxE and E2F motifs from the adenovirus E1A protein presented K_D values in the 200 nM range, suggesting that the full-length protein has sub-nanomolar affinity and that both E1A and HPV E7 compete effectively for binding with the low affinity (K_D = 10 μM) histone deacetylase protein. These results suggest that intrinsic disorder may favor the evolution of both conformational and sequence features within viral linear motifs, endowing viral proteins with high affinity interactions that interfere effectively with host functions, and stress the need for a combined structure-function and evolutionary analysis of pathogenic virus-host interactions.

POSTER SESSION II
Monday, October 13
8:00 PM – 10:00 PM
Gandon Suite North

All posters being presented in Poster Session II should be set up the morning of October 13 and MUST be removed by 10:00 PM.

Magdalena Wawrzyniuk	25-POS	Board 1
Maksym Tsytlonok	26-POS	Board 2
Manuel Luitz	27-POS	Board 3
Matthew Gage	28-POS	Board 4
Michael Fealey	29-POS	Board 5
Mohammad Mofrad	30-POS	Board 6
Monica Zoppè	31-POS	Board 7
Navaneethakrishnan Krishnamoorthy	32-POS	Board 8
Noa Lahav	33-POS	Board 9
Pawel Antonik	34-POS	Board 10
Rainer Bomblies	35-POS	Board 11
Rebecca Beveridge	36-POS	Board 12
Riccardo Marabini	37-POS	Board 13
Sarah Rauscher	38-POS	Board 14
Sarah Shammas	39-POS	Board 15
Sebastian Broendum	40-POS	Board 16
Steven De Gieter	41-POS	Board 17
Sylvia Röstin	42-POS	Board 18
Takashi Nagata	43-POS	Board 19
Tim Verschueren	44-POS	Board 20
Timothy Cross	45-POS	Board 21
Veranika Zobnina	46-POS	Board 22
Veronika Csizmok	47-POS	Board 23
Alexandre Chenal	48-POS	Board 24

25-POS Board 1

Disordered Proteins in the Eyes of a Molecular Chaperone

Magdalena Wawrzyniuk[1], Luca Ferrari[1], Elif Karagöz[1,3], Madelon M. Maurice[2], Stefan G. Rüdiger[1].
[1]Utrecht University, Utrecht, Netherlands, [2]UMC, Utrecht, Netherlands, [3]UCSF, San Francisco, CA, USA.

The Hsp90 family constitutes the most abundant cytoplasmic molecular chaperone system, which assists late stages of protein folding. Recently, we obtained a structural model of Hsp90 in complex with Tau, an intrinsically disordered protein [1]. This complex reveals how a disordered protein looks like in the eyes of a chaperone. Based on this paradigmatic interaction, we set out to extract general themes of Hsp90 substrate recognition, which aims to provide a general mechanistic view on why and when a molecular chaperone car recognize intrinsically disordered proteins. We developed an algorithm to identify stretches of similar properties in other disordered proteins. Based on this, here we present a bioinformatic tool for screening for potential Hsp90 binding sites among intrinsically disordered proteins. We further tested the predictions experimentally for a subset of substrates. As first target, we focused on the instrinsically disordered scaffold proteins of the destruction complex of the Wnt signaling cascade.

Reference
[1] Karagöz GE, Duarte AM, Akoury E, Ippel H, Biernat J, Morán Luengo T, Radli M, Didenko T, Nordhues BA, Veprintsev DB, Dickey CA, Mandelkow E, Zweckstetter M, Boelens R, Madl T, Rüdiger SGD. Hsp90-Tau complex reveals molecular basis for specificity in chaperone action (2014) Cell 156, 963-74.

26-POS Board 2

Single-molecule Fluorescence Reveals How Dynamics within Cdk2/CyclinA-bound p27 Mediate Signal Transmission

Maksym Tsytlonok[1], Hugo Sanabria[3], Yuefeng Wang[2], Cheon-Gil Park[2], Suren Felekyan[3], Katherina Hemmen[3], Peter Tompa[1], Claus Seidel[3], Richard Kriwacki[2].
[1]VIB Brussels, Brussels, Belgium, [2]St. Jude Children's Research Hospital, Memphis, TN, USA, [3]Dusseldorf University, Dusseldorf, Germany.

In the classical structure-function paradigm the function of a protein is associated with its three-dimensional structure. However, recent studies using single-molecule FRET, NMR and other techniques have questioned the idea that proteins are static molecules. This phenomenon is exacerbated especially in the proteins that are fully or partially unstructured, termed intrinsically disordered proteins (IDPs). The intrinsic flexibility of IDPs affords functional advantages in molecular recognition. One of the most prominent examples is the disordered polypeptide p27: It is able to regulate eukaryotic cell division by interacting with a number of cyclin-dependent kinase (Cdk)/cyclin complexes, as well as with other nuclear and cytoplasmic targets. Phosphorylation of p27 by oncogenic kinases contributes to tumorigenesis in several human cancers. The crystal structure of p27 bound to Cdk/Cyclin complex and ensemble measurements provide molecular details of the specificity and the sequential induced-folding mechanism of this interaction. However, the mechanistic details of the dynamic processes associated with the phosphorylation of p27 required for the degradation of p27 are not completely understood. By applying single-molecule fluorescence spectroscopy in combination with biochemical data, we will provide a basis for understanding how dynamics within Cdk2/CyclinA-bound p27 mediate signal transmission. Our results show that p27 exhibits dynamics in its bound conformation and upon phosphorylation several domains are sequentially released. These observations explain the mechanism of how intra- and inter-molecular phosphorylation of partially activated Cdk2/CyclinA are regulated during the cell-cycle.

27-POS Board 3

From Chaos to Order: The Association Pathway of RNAse-S

Manuel Luitz, Rainer Bomblies, Martin Zacharias.
Technical University of Munich, Garching, Germany.

Intrinsically disordered proteins can undergo transitions to ordered folded states upon association with a receptor protein. The transition pathway from unbound to bound complex is often difficult to determine experimentally. In case of the RNAse-S system a small S-peptide, disordered in the unbound state, forms an alpha-helical structure upon binding to the S-protein partner. The resulting complex forms an active RNAse-S enzyme. Molecular dynamics (MD) and advanced sampling approaches were used to investigate the coupled folding and binding of the S-peptide at atomic resolution. In agreement with xperiment significant conformational fluctuations of the isolated S-Peptide compatible with a disordered state were found. In order to identify residues which contribute most to the complex affinity and to find possible key contacts which are formed first on the route to the folded complex we performed in silico alanine scanning on the all residues of S-Peptide. Phe8 was identified as an anchor residue which contributes most to the binding free energy along with Met13 and His12 contributing slightly less to affinity. A pulling imulation on the centers of masses of S-peptide and S-Protein revealed an unfolding pathway with an initial opening of the S-Peptide helix followed by a subsequent dissociation of the key contacts. Based on these findings we could trigger complex formation in several extended MD simulations by closing the key contact of Phe8 with S-Protein and a disordered initial S-peptide. The subsequent

S-peptide folding process resulted in helix folding emerging from a specific hydrogen bonding network to stabilize the final helical peptide structure. Comparison with coil-helix transitions of the S-peptide in solution allowed the characterization of important interactions with the S-protein that stabilize and promote helix folding.

28-POS Board 4

Characterizing the Function of the Disordered Region in Soluble Guanylyl Cyclase

Candice Benally, Parul Singh, **Matthew Gage**.
Northern Arizona University, Flagstaff, USA.

Signaling by nitric oxide (NO) is a key component in cardiovascular heath as well as links to a growing number of other diseases such as cancer and diabetes. The primary receptor for NO is soluble Guanylyl Cyclase (sGC), a heterodimeric protein that is upregulated by binding of NO to the heme group in the β-subunit. Activation of sGC results in the conversion of GTP to cGMP and decreased sCG activity is linked to atherosclerosis, aging, loss of memory, acute ischemia, while increased sGC catalytic activity is associated with endothelial cell proliferation, vasodilation, cell motility and survival. The activation, catalytic activity and structure of sGC have been widely studied but the mechanism behind sGC regulation remains poorly understood. Both subunits of sGC contain four distinct functional domains: an H-NOX, a Per Arnt Sim (PAS), a coiled-coiled and a C-terminal catalytic domain. Interestingly, N-terminus of the α-subunit also contains a 70 amino acid intrinsically disordered region (sGC-IDR) with a previously undetermined function. Using a yeast two-hybrid screen, our lab has identified putative interactions between sGC-IDR and both an E3 ubiquitin ligase and the muscle protein titin. These interactions suggest roles for the sGC-IDR in both localization and degradation of sGC. Experiments are ongoing to characterize the role the sGC-IDR plays in both potential functions.

29-POS Board 5

Disorder Allosterically Propagates and Modulates Calcium Ligation of Synaptotagmin I

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Synaptotagmin I (Syt I) is a vesicle localized integral membrane protein responsible for sensing the calcium influx that triggers fast synchronous release of neurotransmitter. Syt I consists of a transmembrane helix, a cytosolic 60 residue tether region, and two C2 domains that bind calcium and acidic phospholipids. How Syt I senses and subsequently allosterically conveys its calcium ligation state to other fusion machinery proteins and membrane lipids to synchronize exocytosis is incompletely understood. We believe that allosteric propagation of calcium ligation is mediated in part by intrinsic disorder. The tether region between the transmembrane helix and first C2 domain (C2A) consists of 83% disorder-promoting residues suggesting it represents an intrinsically disordered region with potential for modulating activity of the adjacent C2 domains. We probed this tether region for intrinsic disorder and found that, in aqueous solution, it is unstructured with the dramatic allosteric effect of switching C2A's calcium binding mode from one devoid of cooperativity to one with cooperativity. With further analysis we discovered that in the presence of lipid vesicles whose composition mimics a synaptic vesicle, the extent of allosteric coupling between the tether (which becomes weakly folded) and C2A regions is enhanced. The energetic sign of this allosteric coupling was positive, indicating the tether and C2A are more stable together than apart at the membrane. This is a key feature of the allosteric coupling as it provides a means of transducing the calcium ligation signal along the synaptic vesicle surface through tether region ordering. Together, these finding indicate that Syt I harbors an intrinsically disordered region that not only has potential to modulate calcium sensing for triggered release, but also provides a means to allosterically propagate calcium ligation to other lipid and protein modulators of membrane fusion.

30-POS Board 6

Disordered FG-repeat Domains in the Nuclear Pore Complex

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Motivation: Disordered FG-repeat domains coat the inner layer of the nuclear pore complex (NPC) and are believed to play a central role in the selectivity barrier function of the NPC, exclusively controlling the vital traffic of macromolecules into and out of the nucleus [1]. The confinement of the FG-repeat domains to the central channel, the delicate and compact nature of the NPC, and fast and high throughput of bidirectional traffic in it have challenged the investigation of these disordered domains. Computational and theoretical modeling approaches offer a useful alternative towards understanding the disordered FG-repeat domains in the NPC [2,3].

Objective: We aim to explore the conformational behavior of the disordered FG-repeat domains in the NPC central channel.

Methods: 3D coarse-grained models of the yeast central channel are develped with all 11 known FG nucleoporins (Nups). The exact sequence of the FG Nups disordered domains are extracted and their length, hydrophobicity, charge, and the native grafting density are incorporated in the model.

Results: Our results show that the FG-motifs of these disordered domains are mainly concentrated towards the central part of the channel, while charged residues are predominantly localized near the central channel wall. Depending on the nuclear pore diameter, FG-repeats can either make a channel-filling hydrogel or a thick lubricating layer, consistent with two differing models proposed in the literature.

References:
[1] Jamali et al. (2011) Nuclear Pore Complex: Biochemistry and Biophysics of Nucleocytoplasmic Transport in Health and Disease. International Review of Cell and Molecular Biology. 287: 233-286.

[2] Moussavi-Baygi et al. (2011) Biophysical Coarse-Grained Modeling Provides Insights into Transport through the Nuclear Pore Complex. Biophysical Journal. 100(6):1410-1419.

[3] Azimi et al. (2013) Higher Nucleoporin-Importinβ Affinity at the Nuclear Basket Increases Nucleocytoplasmic Import. PLoS One. 8(11):e81741.

31-POS Board 7

What's Disorder? Proposal for a New Name to Define 'Disordered' Motifs

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Scientific Visualizatio Unit - IFC, Pisa, Italy.

The definition of protein motifs which are not amenable for crystallization as 'disordered' is based on the historical origin of their identification. Fortunately, in the last several years, much information has been acquired on these motifs, making it unnecessary to keep the original definition.

I argue that there are several reasons why such definition is improper, and suggest that the community engage in the search for a new name. For one, the fact that the (part of) protein cannot be forced in to crystal has no biological relevance: in nature proteins do not generally form crystals. Also, biology is quite 'disordered', at all levels: rules exists, that are naturally originating form physics laws, but then high level combinations of different rules can produce extremely variable and elastic new rules, so that very rarely two events develop in the same 'ordered' fashion. Third, the idea of disorder contains a negative meaning (lack of order) which implies a judgement, that I consider unwarranted in a scientific setting. Finally 'disorder' is a very general name, often used as synonym for disease, that makes it very hard to perform literature searches, and often confuses people, especially those not involved in the field.

I therefore suggest that a new name is created, with the consensus of the community, based on the important features that have been attributed to these motifs in the years of research dedicated to them. The new name can be based on one of several concepts, can be a good sounding acronym, or can be a totally invented word.

Meeting participants can enjoy a creative exercise while discussing the opportunity of changing the disorder word.

32-POS Board 8

Bedside to Bench: Molecular Modeling of Double Mutation of cMyBP-C Resulting in Severe Phenotype

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The gene MYBPC3 encodes cardiac myosin binding protein-C (cMyBP-C), a multi-domain (C0-C10) protein. Mutations in the gene are one of the major factors for causing inherited hypertrophic cardiomyopathy (HCM). Here, we describe molecular modelling of a double mutation in cMyBP-C in a patient with severe phenotype of HCM and the mutations are in the complex C1(E258K)-motif-C2(E441K). The 3D structure for the motif was modelled. The complex C1-motif-C2 was constructed and both the double and single mutations were introduced in the complex. Molecular dynamics simulations were performed for 10ns for the complexes. The results showed that the E258K and E441K in isolation can predominantly affect the native domain as well as the nearby motif via conformational changes, when they exist together it resulted in an additive effect. These changes involved potentially important regions in the motif such as phosphorylation sites. The mutations also modified the inter-domain interface of C1-motif-C2 and altered the surface electrostatic properties. This study suggests that the double mutation in the regulatory N-terminal of cMyBP-C could interfere with the binding to neighbouring domains and with other sarcomeric proteins such as actin and myosin, thus explaining a putative mechanism for severe phenotype in our patient.

33-POS Board 9

The Regulation of Non Muscle Myosin II by the Disordered Non-helical Tailpiece

Noa Lahav[1], Shoshana Ravid[2], Assaf Friedler[1].
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Non-muscle myosin II (NMII) mediates cellular processes that require contractility. It is composed of a globular motor domain in its N-terminal region and a coiled-coil rod domain in the C-terminal region and undergoes dynamic filament assembly-disassembly cycles to facilitate its activities. NMII isoform C (NMII-C) has a disordered 55-residue C-terminal tailpiece (residues 1946-2000), which regulates the filament assembly of NMII-C. The N-terminal region of the tailpiece (residues 1946-1967) has a net positive charge of +7 while its C-terminal region (residues 1968-2000) has a net negative charge of -10. We previously demonstrated that a peptide corresponding to the positive part of the non-helical tailpiece promotes filament assembly of NMII-C. Phosphorylation of two specific threonine residues (positions 1957 and 1960) in the positively charged region of the tailpiece introduces negative charges with consequent shift from attraction between NMII-C rods that leads to filament assembly, to repulsion leading to inhibition of filament assembly. We synthesized phosphorylated peptides derived from the positively charged region of the tailpiece, in which the relevant threonines were replaced by phosphothreonines. We found that a single phosphorylation on either of the two threonines resulted in inhibition of NMII-$C_{1296\text{-}1854}$ filament assembly. The positively charged $tailpiece_{1946\text{-}1967}$ and its derivatives modulate the filament assemble of NMII-C and thus may serve as lead compounds against diseases resulting from mutations that disrupt the NMII filament assembly such as cancer.

Refernces:
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(2) Ronen, D., Rosenberg, M. M., Shalev, D. E., Rosenberg, M., Rotem, S., Friedler, A., & Ravid, S. (2010),
J Biol Chem, 285(10), 7079–7086.
(3) Rosenberg, M. M., Ronen, D., Lahav, N., Nazirov, E., Ravid, S., & Friedler, A. (2013),
J Biol Chem, 288(14), 9779–89.

34-POS Board 10

NMR Study of the 30 kDa Fucose-binding Lectin from *Ralstonia solanacearum*

Pawel Antonik[1,2], Alex Volkov[3], Michaela Wimmerova[4], Ursula Broder[1], Nico Van Nuland[3], Peter Crowley[1].
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RSL (30 kDa trimer) is a fucose-binding lectin,[1] which is found in *Ralstonia solanacearum*, a worldwide distributed plant pathogen, responsible for wilting in a wide range of crops.[2]
NMR experiments were carried out to assign the $^{1}H/^{15}N$ HSQC spectrum of RSL, a useful "fingerprint" for future studies. RSL dynamics are being studied in addition to numerous biological processes pertaining to folding and assembly, ligand binding and molecular recognition.[3] Wt-RSL presents an extraordinarily strong thermostability from 86°C in the free form to 96°C in the bound form.[4] Both NMR dynamics and SDS-PAGE experiments show how the stability and flexibility of RSL change in the presence of different monosaccharides.
The results will aid the identification of inhibitors of RSL that function by blocking the sugar-binding site. Such inhibitors could be further developed to prevent pathogen binding in crops.

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35-POS Board 11

The Mechanism of pKID-KIX Complex Formation Studied by Molecular Dynamics Simulations

Rainer Bomblies, Manuel Luitz, Martin Zacharias.
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The intrinsically disordered kinase inducible domain (KID) of the transcription cofactor CREB forms a kinked two-alpha-helices motif upon serine phosphorylation and subsequent binding to the transcription factor CBP. The effect of phosphorylation and the contribution of individual amino acid residues to the coupled folding and binding process are still not fully understood. An exhaustive sampling of the accessible conformational space of the unbound disordered state is difficult by unrestraint continuous molecular dynamics (cMD) simulations since substates are stable for hundreds of nanoseconds. We thus determined the conformational preferences of the unbound phosphorylated and non-phosphorylated KID with a novel method of sampling where the root mean square deviation (RMSD) of a set of chosen distances (dRMSD) is used as the reaction coordinate. Hamilton-Replica Exchange MD umbrella sampling (US) with this reaction coordinate allows sampling of a significantly larger fraction of the conformational space than cMD. Our dRMSD-US simulations show that KID phosphorylation promotes a more helical structure, mainly around the phosphorylation site, in turn facilitating pKID binding to KIX. To identify key binding residues in pKID we performed computational alanine-scanning with free energy calculations in explicit water. The major contributions arise from the hydrophobic residues of both helices, most importantly Leu141 in helix B and Leu128 in helix A each contributing more than 10 kJ/mol to the binding affinity. These residues might form initial key contacts prior to the structural transition into the bound state.

36-POS Board 12

A Mass Spectrometry-Based Framework to Identify (Non)-Structural Order in p27

Rebecca Beveridge[1], Yongqi Huang[2], Rohit V. Pappu[3], Richard Kriwacki[2], Perdita E. Barran[1].
[1]University of Manchester, Manchester, United Kingdom, [2]St. Jude Children's Research Hospital, Memphis, TN, USA, [3]Washington University, St. Loius, MO, USA.

In the last ten years mass spectrometry (MS) coupled with electrospray ionisation (ESI) has been extensively applied to identify proteins and elucidate stoichiometry of protein complexes, often without the need for labels. Because desolvated species are affected by solvent conditions such as pH, buffer strength and concentration, ESI-MS is an appropriate method by which to consider the range of conformational states that proteins may occupy including natively folded, disordered, denatured and amyloid.

Rotationally averaged collision cross sections of the ionised forms of proteins, provided by the combination of mass spectrometry and ion mobility (IM-MS), are also instructive in exploring conformational landscapes in the absence of solvent. We compare these experimental parameters to a simple model which allows the prediction of the theoretical smallest and largest possible collision cross sections based on the volume of the amino acids in the sequences. Consideration of the occupancy of conformational states (based on the intensities of ions in the mass spectra) allows us to qualitatively predict the potential energy landscape of each protein. This empirical approach to assess order or disorder has more accuracy than theoretical methods based on the amino acid sequences for the chosen systems, and could provide an initial route to characterisation.

Here we apply IM-MS analysis to the intrinsically disordered protein p27. We compare the range of rotationally averaged collision cross sections of the wildtype protein with various permutants, to investigate the effects of different distributions of charge. We also monitor conformations of the different permutants when they are sprayed from buffers of different ionic strengths and relate this back to the charge distribution.

37-POS Board 13

Intrinsically Disordered Ribonucleotide Reductase Inhibitors in *S.cerevisiae*

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Ribonucleotide reductase (RNR) catalyzes the rate-limiting step in the deoxyribonucleotide biosynthesis pathway and it is an extremely important enzyme during DNA replication and damage. It is widely accepted that its dysregulation leads to unbalanced dNTPs levels in the cell and hence onset of mutations that ultimately can lead to various cancers. Recently, and in two different yeast strains (*S. cerevisiae* and *S.pombe)* it has been demonstrated that a family of intrinsically disordered proteins (IDPs) exist that can inhibit RNR activity. Although RNR inhibition is believed to be common to these proteins their sequences diverge significantly and additional functions are suggested to be present and shared in subsets of these proteins. In this study we have used *S.cerevisiae* as a model organism and NMR spectroscopy as the main technique to delineate common structural and dynamical features of three RNR inhibitors, Sml1, Dif1 and Hug1. The aim is to identify common interaction motifs and key residues important for maintaining the disordered state. Through the description of the unfolded state, the disordered state and from functional characterization, we aim to address the protein non-folding problem.

38-POS Board 14

Structural Ensembles of Intrinsically Disordered Proteins Depend Strongly on Force Field

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Intrinsically disordered proteins (IDPs) fulfill many biological roles and are important drug targets. However, they are poorly understood relative to the wealth of structural information available for globular proteins. Their structural characterization presents a formidable challenge to both theory and experiment: the structure of an IDP must be described as a structural ensemble of many interconverting conformations.

Here, we use molecular dynamics simulations to obtain structural ensembles of two IDPs: (1) a 50-residue peptide derived from Nsp1p, which is an FG-nucleoporin responsible for the selectivity of the nuclear pore complex and (2) a 20-residue RS-repeat peptide derived from serine/arginine-rich-splicing-factor 1, which is crucial in RNA splicing. Because force fields for polypeptides have been developed primarily to study folded proteins, it is not clear how accurately they can model disordered states. We therefore performed simulations using four force fields: amber99sb*-ildn, amber ff03w, CHARMM22*, and CHARMM36. We performed replica exchange (RE) simulations for a total of 150 microseconds per force field. To minimize the computational cost of these simulations, we developed an algorithm that yields a temperature ladder for which the mean first passage time between the lowest and highest temperature is minimal.

The structural ensembles we obtain for both the FG and RS peptides differ markedly between force fields with respect to hydrogen bonding, radius of gyration, and secondary structure, and are sufficiently converged to make such a comparison. Importantly, secondary structure content differs more on average between force fields than between the two peptide sequences. Thus, disordered peptides appear to be particularly sensitive to force field selection, much more so than globular proteins. A comparison to NMR data on the RS peptide is ongoing, and will shed light on which of these force fields offers the most accurate description.

39-POS Board 15

Coupled Folding Upon Binding of Transcription Factors and Allostery within the KIX System

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IDPs are overrepresented in processes such as signalling and transcription, where proteins often interact with a range of partners. One much-studied key hub protein is the coactivator CBP/p300, whose folded KIX domain binds a number of different intrinsically disordered transcription factors at two separate sites on its surface. The interaction of KIX with several of its ligands has been well studied by equilibrium methods, and structural information is available for many of the complexes. We have performed comparative stopped-flow experiments to determine the association and dissociation rates for six different disordered ligands, shedding light on the mechanism of these coupled folding and binding reactions. These are shown to be the fastest protein-protein interactions yet reported (without long-range electrostatic attraction). We further describe the general mechanistic basis for the positive allostery between the two binding sites of KIX.

40-POS Board 16

Phosphorylation Downstream of the PIP-Box Motif in Spd1 Regulates Association with PCNA

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Ribonucleotide Reductase (RNR) is ubiquitously involved in controlling the rate of mutation in cells and understanding its regulation is very important to improve our understanding of cancer and how it develops. In S. pombe, one way by which RNR is regulated is by a small intrinsically disordered inhibitory protein called Spd1. During DNA replication, Spd1 is degraded to allow synthesis of dNTPs. A key step in Spd1 degradation is the interaction with Proliferating Cell Nuclear Antigen (PCNA). Spd1 contains a short linear motif known as the PCNA interacting protein-box (PIP-box). For many PCNA-binding proteins, this interaction is mediated via their PIP-Box motif and mutation studies have confirmed that the PIP-Box in Spd1 is important for its interaction with PCNA. To resolve how this motif in Spd1 mediates the interaction with PCNA, we have structurally characterized Spd1 and the interaction between Spd1 and PCNA using Nuclear Magnetic Resonance spectroscopy and hydrogen-to-deuterium exchange mass spectrometry. We show that the Spd1 PIP-Box forms a transient α-helix in the unbound state and phosphorylation of Spd1 downstream of the PIP-Box destabilizes this α-helix. Phosphorylation also abolishes the interaction between Spd1 and PCNA and therefore we propose a model where phosphorylation of Spd1 regulates the interaction with PCNA. Furthermore, we discuss the role of the altered α-helix propensity in this process. Regulation of Spd1 degradation allows the cell to control RNR activity and thereby maintain a balanced level of dNTPs to minimize the rate of mutation in the cell.

41-POS Board 17

Molecular Basis behind the Regulation of Activity of the Novel Kinase Doc by the Antitoxin Phd

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Fic proteins (Filamentation induced by cAMP) are ubiquitous in all domains of life and play a critical role in a myriad processes, such as bacterial pathogenesis. These proteins are defined by a conserved FIC domain and catalyze AMPylation (transfer of AMP) of target proteins. Doc is a Fic-like protein that belongs to the phd/doc toxin-antitoxin module and was shown to inhibit bacterial translation via phosphorylation of Elongation Factor Tu. Here we show how the intrinsically disordered C-terminal domain of the antitoxin Phd regulates the activity of Doc. Doc adopts a rather dynamic ensemble of conformations in its free state, which collapses into a more condensed and rigid structure upon binding of Phd. The intrinsically disordered Doc binding region of Phd (residues 52-73) harbors two distinct segments with distinct functionalities. The hydrophilic segment (Phd65-73) sterically competes with ATP binding and acts as a direct inhibitor. On its own, its affinity is weak, binding occurs with high on and off rates and does not lead to los of dynamics in Doc. On the other hand, the hydrophobic segment encompassing residues 52-64 (Phd52-64) does not overlap with the active site of Doc but binding is tight and induces the same structural changes as observed for the complete binding region of Phd. The collaborative action of both segments results in an intrinsically disordered locking mechanism suggested to prevent binding of the catalytic NTP. Our findings represent a novel structural paradigm for the plasticity of the catalytic mechanisms used by the active centers of enzymes and their cognate neutralization mechanisms.

Methods: Isothermal titration Calorimetry, Surface Plasmon Resonance, Small Angle X-ray scattering, X-ray crystallography.

42-POS Board 18

Stress Induced Proteins in Plants: Studies of the Intrinsically Disordered Dehydrins

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Plants can activate an array of responses when they have to survive different environmental stresses such as low temperature or drought. One such response is the induction of stress proteins, for example the late embryonic abundant (LEA) proteins. Although the precise functions of these proteins are unknown, the general hypothesis is that they stabilise membranes, acts as metal sponges, stabilise other proteins or other cellular structures by preventing them from denature due to stress. Group 2 of the LEA proteins, the dehydrins, constitute a class of intrinsically disordered proteins that are expressed under condition of stress in plants. Characteristic to the dehydrins are some highly conserved stretches of 7-17 residues that are repetitively scattered in their sequence, the K-, S-, Y- and lysine rich segments. We have shown that the dehydrin Lti30 (K6 dehydrin) interacts and even aggregates negatively charged phospholipid vesicles and that it is detectable with light microscopic pictures and absorbance measurements. This interaction is depending on the pairs of histidins that are flanking the K-segments of Lti30. Rab18 (Y2SK2 dehydrin) shows a weak interaction, while Cor47 (SK2 dehydrin) and Lti29 (SK2 dehydrin) showed no binding at all.

In this study we investigates how calcium and zinc can modify the binding of the dehydrins to phospholipid vesicles. Calcium enhanced the binding to phospholipid vesicles for the dehydrins that already showed binding capacity (Lti30 and Rab18), while it was having no effect for Cor47 and Lti29. Zinc was affecting all four dehydrins and promoted binding and aggregation of phospholipid vesicles by Cor47 and Lti29. Zinc was even increasing the aggregation of negatively charged vesicles when Lti30 and Rab18 were added.

43-POS Board 19

NMR Study of the Interaction between MDM2 and a Peptide Selected by mRNA Display

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OBJECTIVE: MDM2 is an oncoprotein who binds to the intrinsically disordered region (IDR) of tumor suppressor p53 and inhibits its functions. p53 prevents tumorigenesis through cell cycle arrest or apoptosis of cells in response to cellular stress such as DNA damage. Thus, inactivation of p53 by MDM2 leads to the development of several human cancers. Peptides that mimic the MDM2-binding region of p53-IDR reportedly restore the anti-cancer activity of the p53 pathway. We aimed to obtain the peptide that has a higher potency and affinity to MDM2 than the known ones and elucidate the mechanism of the strong binding.

RESULTS: We screened for the MDM2-binding peptide from large random peptide libraries in two stages by means of mRNA display. The obtained 12-mer peptide, which we named MIP, exhibited higher affinity to MDM2 than the reported ones as confirmed by SPR. Experiments using living cells showed that MIP has superior binding activity and potency to prevent tumor cell growth. We have then determined the structure of MIP and MDM2 in the bound form by solution NMR method.

CONCLUSIONS: Comparison between the structures of MIP:MDM2 complex and previously reported p53 peptide:MDM2, DI:MDM2, and PMI:MDM2 complexes showed that the stronger binding and higher anti-MDM2 activity observed for MIP can be explained as due to its enlarged binding surface over previously reported peptides. Additionally, MIP was found to have tendency to form some secondary structure in the hydrophobic solution environments. These findings provide fruitful hints for drug discovery against diseases caused by abrogation of the function of intrinsically disordered proteins.

44-POS Board 20

Mass Spectrometry Combined with Chemical Probing of Flexible Proteins

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Mass spectrometry can be used in combination with chemical probing techniques to characterize the flexible regions of proteins. Often used methods for protein characterization, such as x-ray crystallography and electron microscopy, are not always suited for the study of flexible (disordered) or highly dynamic proteins. We propose a combination of covalent solution labelling which targets the exposed, solvent accessible surface of proteins and complexes, with subsequent analysis by mass spectrometry (digestion followed by LC-MS/MS).

The use of different reagents, time-titration and varying reaction conditions results in differences in probing patterns. The comparison of the extent, rate and location of modifications between the denatured and native state of a protein can tell us something about which regions are structured, as well as about the solvent accessibility of the surface area and by extension the flexibility of a protein. Selective modification of amino acids, e.g. histidines with diethylpyrocarbonate or lysines with dimethylamine borane, as well as type 0 crosslinkers induces only a limited number of modifications which ensures the structure integrity and avoids complex data. We also explore chemical as well as fast laser-induced (FPOP = Fast Photochemical Oxidation of Proteins) hydroxylation of the accessible protein surface.

Optimization of these reactions as well as the combination with other MS-based techniques, such as ion mobility and top-down fragmentation, allow us to gain more insight into the structure and interactions of proteins. This makes mass spectrometry in combination with chemical probing particularly suited for the study of flexible proteins. We will highlight these different approaches using a panel of proteins with known degrees of structural flexibility and disordered regions (IDR).

45-POS Board 21

Disordered Domains in *Mycobacterium tuberculosis* Membrane Proteins Associated with Cell Division

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ChiZ of Mycobacterium tuberculosis (Mtb Rv2719c) is a small (164 amino acid residue) membrane protein having a single transmembrane helix, an N-terminus of 65 residues and a C-terminus of 78 residues. The TMHMM v2.0 prediction software on the Tuberculist website predicts that the N-terminus is in the periplasm and PONDR-FIT suggests that the N-terminus with 14 proline and 13 arginine residues is disordered. It has been functionally denoted as a peptidoglycan hydrolase and potentially this disorder is necessary for binding to the heterogeneous peptidoglycan layer of the cell wall.

The cell wall is particularly complex in Mtb and during cell division it is critical for the bacillus to maintain the integrity of the cell wall. In part it is this cell wall that is responsible for the latent state of the bacillus that necessitates drug treatment over at least a 6 month period. Today there are rapidly increasing extreme drug resistant strains that are resistant to all of the front line drugs. As a result of the unique cell wall, many proteins involved in cell division are associated with hydrolyzing and reforming the cell wall including the peptidoglycan layer. Many of these proteins including FtsW, PonA1, PonA2, PbpB, RipA and ChiZ appear to have intrinsically disordered domains. Many other proteins involved in cell division including FtsK, Wag31, PknA, PknB, FtsQ, CwsA, Rv2164c are also predicted to have intrinsically disordered domains. Solution NMR spectra and spectral assignments of the N-terminal ChiZ domain in a disordered state have been obtained. Preliminary data in the presence of peptidoglycan will be shown. Solid-state NMR spectroscopy of lipid bilayer preparations and solution NMR spectroscopy in detergent micelles of CwsA will be shown leading to an initial structure of this Mtb cell division protein.

46-POS Board 22

Structural Interactions between Integrin αIIbβ3 and Calcium- and Integrin-binding Protein 1

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Integrins are heterodimeric transmembrane receptors that coordinate cell adhesion, migration, and extracellular matrix assembly. Platelet-specific integrin αIIbβ3 plays a critical role in hemostasis and thrombosis, and regulation of its activation state represents a promising novel target for anti-platelet therapy. Two highly conserved motifs in the intracellular cytoplasmic tails of integrin αIIbβ3 (KVGFFKR in αIIb and LLITIHD in β3) maintain the receptor in a default inactive state through weak interactions. Platelet activation initiates a cascade of intracellular events that lead to spatial separation of the cytoplasmic tails and activation of integrin αIIbβ3. The KVGFFKR motif of the αIIb cytoplasmic tail is also a target for multiple intracellular proteins which regulate platelet responses. It has been suggested that CIB-1 (calcium- and integrin-binding protein 1) activates integrin αIIbβ3 and maintains the activated state of the receptor by covering the KVGFFKR motif of αIIb and preventing it's association with β3. Apo-CIB-1 is a molten globule, but upon binding of divalent ions (Ca^{2+} or Mg^{2+}) it adopts stable conformation and increases it's affinity to the αIIb cytoplasmic tail. C-terminal extension of the Ca^{2+}-CIB-1 structure undergoes large conformational changes upon binding to αIIb and regulates CIB-1 specificity by partially covering the binding pocket in an unbound ligand-free state. Extensive experimental studies on molecular interactions of CIB-1 with αIIb have been reported, but the structure of the CIB-1/αIIb complex remains unclear. Molecular docking, virtual screening and molecular dynamics simulations were used to elucidate and analyze the structure of CIB-1/αIIb complex and to discover molecules interacting with the binding region of CIB-1.

47-POS Board 23

The Human hCdc4/Fbw7 has a Multisite Phosphorylation-dependent Dynamic Interaction with its Binding Partner c-Jun Facilitating Tight Cell-cycle Control

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Cancer-associated proteins, including the oncoprotein c-Jun, are highly enriched in disordered protein regions that mediate complex regulation of cell cycle and other signalling pathways. The complexity of regulation is often facilitated by dynamic interactions of disordered proteins, not leading to a static ordering upon binding. The transcription factor c-Jun is targeted for degradation by the human orthologue of Cdc4 (hCdc4). We hypothesize that the hCdc4 has a multisite phosphorylation-dependent dynamic interaction with its binding target c-Jun as it contains many possible weak CPD (Cdc4 phosphodegron) sites. We assigned the backbone resonances in non-phosphorylated and phosphorylated states of two overlapping c-Jun constructs containing 4 (Jun123) and 6 (Jun276) CPD sites and performed structural and binding studies. Our data show that both Jun123 and Jun276 are disordered, and interestingly Jun276 has 10 fold greater binding affinity to the isolated WD40 domain of Cdc4 than Jun123. The NMR analysis shows broadening on many phosphorylated residues in both Jun123 and Jun276, without significant ordering of Jun upon binding. Together, our data demonstrate that a multisite, dynamic interaction occurs between the Cdc4 and c-Jun. To determine how multiple CPD sites affect Jun stability in cells, we transfected HeLA cell with WT and different CPD site mutants Jun and followed the degradation of Jun. The preliminary data show that degradation of individual N-terminal mutants is similar to WT, degradation of the protein mutated on all four N-terminal sites is slower, and degradation of C-terminal mutants and the all sites mutant is even slower, consistent with our hypothesis of the importance of multiple CPD sites. These results provide a detailed picture of interactions of the cancer-associated hCdc4 and facilitate understanding of the oncogenic process mediated by Jun.

48-POS Board 24

Molecular Crowding Stabilizes both the Intrinsically Disordered Calcium-free State and the Folded Calcium-bound State of an RTX Protein: Implication for PROTEIN Secretion

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Ligand-induced disorder-to-order transition plays a key role in the biological functions of many proteins that contain intrinsically disordered domains. Here, we present data on an RTX (« Repeat in ToXin ») protein, RC_L, an IDP that folds upon calcium binding. RTX motifs are calcium-binding nonapeptide sequences that are found in more than 250 virulence factors secreted by Gram-negative pathogenic bacteria. Using a combination of biophysical approaches, we showed that RC_L exhibits the hallmarks of intrinsically disordered proteins in the absence of calcium. Calcium binding triggers a strong reduction of the mean net charge, dehydration and compaction, folding and stabilization of secondary and tertiary structures of RC_L. Moreover, RC_L is an attractive model to investigate the effect of molecular crowding because it offers the opportunity to characterize the crowding effects on the same protein under two drastically distinct folding states. Macromolecular crowding affects most chemical equilibria in living cells by sterically restricting the available space. We showed that the crowding agent Ficoll70 did not affect the structural content of the apo-state and holo-state of RC_L but increased the protein affinity for calcium. Besides, Ficoll70 strongly stabilizes both states of RC_L, increasing their half-melting temperature (ΔTm), without affecting enthalpy changes. The power law dependence of the [illegible] allowed the estimation of the Flory exponent of the thermally unfolded states. Altogether, our data suggest that, in the apo-state as found in the crowded bacterial cytosol, RTX proteins adopt extended unfolded conformations that may facilitate protein export by the secretion machinery. Subsequently, calcium gradient across bacterial cell wall and crowding also enhances the calcium-dependent folding and stability of RTX proteins once secreted in the extracellular milieu.

Articles on this topic:
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Circular Dichroism, Synchrotron Radiation CD, Fourier Transform Infrared Spectroscopy, Nuclear Magnetic Resonance, Fluorescence, Size Exclusion Chromatography followed by UV, RI, Static Light Scattering and Intrinsic Viscosity, Analytical Ultra Centrifugation, Quasi-Elastic Light Scattering, Electrophoretic Mobility, Small-Angle X-ray Scattering.