The 6th Alpbach Workshop on: COILED-COILS, COLLAGEN & FIBROUS PROTEINS

At The Romantikhotel Böglerhof, Alpbach, Austria

Sunday 8th September – Friday 13th September 2013

6th Alpbach Workshop on: COILED-COILS, COLLAGEN & FIBROUS PROTEINS

PROGRAMME

Sunday 08 September	*Arrivals, Reception and Dinner*

Monday 09 September		
	New Developments in Coiled Coils *Session Chair: Andrei Lupas*	
09.00 – 09.20	Andrei Lupas MPI, Tübingen	*The Histidine Zipper*
09.20 – 09.55	Andrew Carter, MRC-LMB, Cambridge	*Sliding in the dynein stalk coiled coil*
09.55 – 10.15	Dek Woolfson, University of Bristol, UK	*CC+, The Periodic Table and a Basis Set of Coiled Coils*
10.15 – 10.35	Katja Arndt, University of Potsdam, Germany	*Interfering Peptides Targeting Protein-protein Interactions*
10.35 – 11.00	Tea & Coffee Break	
	New Developments in Coiled Coils *Session Chair: Andrei Lupas*	
11.00 – 11.20	Kerstin Reiss, Stehle Lab, University of Tübingen	*Structural Characterization of Reovirus attachment protein σ1*
11.20 – 11.55	Marcus Hartmann, MPI, Tübingen	*α/β Coiled Coils*
11.55 – 12.30	Andrey Kajava, CRBM, Montpellier, France	*Breaking the Amyloidogenicity Code: Bioinformatics Approach to Predict Predisposition to Amyloidosis*
12.30	Lunch Afternoon Free	
17.00 – 18.00	Posters	
	Coiled coils at the membrane *Session Chair: Andrei Lupas*	
18.00 – 18.35	Ben Nichols, MRC-LMB, Cambridge	*Coiled coil proteins in the caveolar coat complex*

18.35 – 19.10	Murray Coles, MPI Tübingen	*Coiled coils as key elements in bacterial transmembrane receptors*
Monday 09 September (continued)		
19.10 – 19.30	Andreas Möglich, Humboldt Universität, Berlin, Germany	*Structure and Function of a Light-Regulated Histidine Kinase*
19.30 – 21.00	Dinner	
	Coiled coils at the membrane *Session Chair: Andrei Lupas*	
21.00 – 21.20	John Walshaw, Institute of Food Research, Norwich, UK	*Identical helix twins do different things- only one on the straight and narrow: A mechanism for oligomer state-shifting in biological switches with multiple heptad registers*
21.20 – 21.40	Ai Yoshinaka, University of Bristol, UK	*Design and engineering of transmembrane α-barrel forming peptides*
21.40 – 22.00	Sergei Strelkov, KU Leuven	*To be confirmed*

Tuesday 10 September		
	Coiled-Coil Design *Session Chair: Dek Woolfson*	
09.00 – 09.20	Dek Woolfson, University of Bristol, UK	*Self-assembled Peptide Cages*
09.20 – 09.55	Roman Jerala, NIC, Slovenia	*Concatenated single-chain coiled-coil segments for the self-assembly of polypeptide polyhedra*
09.55 – 10.15	Michelle Peckham, University of Leeds, UK	*Coiled coils and SAH domains – how to spot the difference*
10.15 – 10.35	Selected short talk	*To be confirmed*
10.35 – 11.00	Tea & Coffee Break	
	Coiled-Coil Design Session Chair: Dek Woolfson	
11.00 – 11.35	Vince Conticello, Emory University, USA	*Nanotubes from self-assembly of coiled coil peptides*
11.35 – 11.55	Antony Burton, University of Bristol, UK	*Towards catalysis in CC-Hex*
11.55 – 12.30	Alexander Kros, Leiden University, NL	*Optimization of coiled coil driven membrane fusion*
12.30 – 12.50	Tim Kükenshöner, University of Potsdam, Germany	*Selection, Design and Analysis of Chimeric D- and L-alpha-Helix Assemblies*
12.50	Lunch Afternoon Free	
17.00 - 1800	Posters	
	Collagens and Collagen Design *Session Chair: Jordi Bella*	
18.00 – 18.35	Jordi Bella, University of Manchester, UK	*Introductory Talk*
18.35 – 19.10	Jeff Hargerink, Rice University, USA	*Axial Charge Pair Interactions Control Collagen Triple Helix Composition, Register and Higher Order Assembly*

19.10 – 19.30	Selected short talk	*To be confirmed*
Tuesday 10 September (continued)		
19.30 – 21.00	Dinner	
	Collagens and Collagen Design *Session Chair: Jordi Bella*	
21.00 – 21.35	John Ramshaw, CSIRO, Australia	*Recombinant Bacterial Collagens as New Materials for Biomedical Applications*
21.35 – 22.00	2x selected short talks	*To be confirmed*

Wednesday 11 September		
	Engineering with fibrous proteins *Session Chair: David Kaplan*	
09.00 – 09.35	David Kaplan, TUFTS, USA	*Designing Proteins for Structure – Functioning and Modeling Considerations*
09.35 – 10.10	Tara Sutherland, CSIRO, Australia	*Insect silks based on coiled coil structural proteins*
10.10 – 10.40	Natasha Burgess & Franziska Thomas, University of Bristol, UK	*Tubes of CC-Hex*
10.40 – 11.00	Tea & Coffee Break	
	Engineering with fibrous proteins *Session Chair: David Kaplan*	
11.00 – 11.35	Anna Rising, Karolinska Institute, Sweden	*pH dependent dimerization of a spider silk protein domain: molecular mechanisms and implications for regulation of protein solubility and assembly*
11.35 – 11.55	Martina Elsner, Universität Bayreuth, Germany	*Spider Silk Particles for Biomedical Applications*
11.55 – 12.30	2 short talks (15+5) or 1 long (25+10) plus 1 short talk (15+5)	*To be confirmed*
12.30	Lunch Afternoon Free	
17.00 – 18.00	Posters	
	Engineering with fibrous proteins *Session Chair: John Ramshaw*	
18.00 – 18.20	Martin Humenik, Universität Bayreuth, Germany	*Silk protein – oligonucleotide conjugates as new nanomaterials*
18.20 – 19.30	1 long talk plus 2 short talks OR 2 long talks plus 1 short talk	*To be confirmed*
19.30 – 21.00	Dinner	
	Engineering with fibrous proteins *Session Chair: John Ramshaw*	
21.00 – 22.00	*3 selected short talks (15+5) or 1 long*	*To be confirmed*

	and 1 short talk	

Thursday 12 September		
09.00 – 12.30	***Natural Coiled Coils*** *Session Chair: John Squire*	
09.00 – 09.10	John Squire, University of Bristol, UK	*Overview*
09.10 – 09.35	Ed Morris, Institute of Cancer Research, London	*Structure of the Human Cardiac Muscle Myosin Filament*
09.35 – 10.00	Massimo Buvoli, University of Colorado, USA	*Myosin Rod Sequence and filament assembly*
10.00 – 10.25	Danielle Paul, Institute of Cancer Research, London	*How the structure of the thin filament changes on Ca^{2+} activation*
10.25 – 10.40	Selected short talk	*To be confirmed*
10.40 – 11.00	Tea & Coffee Break	
11.00 – 11.30	Elisabeth LeRumeur, University of Rennes, France	*Overview: Spectrin-like repeats and Dystrophin*
11.30 – 11.55	Kristina Djinovic Carugo, University of Vienna	*Crystal structure of human muscle α-actinin: insights into regulation of ligand binding and Z-disk assembly*
11.55 – 12.20	Pradeep Luther, Imperial College, London	*Alpha-actinin/actin interaction in Z-bands*
12.20 – 12.30	Selected short talk	*To be confirmed*
12.30	Lunch Afternoon Free	
17.00 – 19.30	***Intermediate Filaments*** *Session Chairs: Andrei Lupas, Dek Woolfson & David Parry*	

17.00 – 17.25	Andrei Lupas	*Introduction*
Thursday 12 September (continued)		
17.25 – 18.25	David Parry, Massey, New Zealand	*Plenary Lecture: Fifty Years of Fibrous Protein Research: A Personal Retrospective*
		Keratin Intermediate Filaments: Differences in the sequences of the Type I and Type II chains explain the origin of the stability of an enzyme-resistant four- chain fragment
18.25 – 18.55	Sergei Strelkov, KU Leuven	*Intermediate filaments as we know them*
18.55 – 19.25	Lars Norlen, Karolinska Institute	*To be confirmed*
20.00	Romantik Dinner	

Friday 13 September		
	Extracellular Matrix *Session Chair: Jo Adams*	
09.00 – 09.35	Jo Adams, University of Bristol, UK	*Thrombospondins and their roles in extracellular matrix*
09.35 – 10.10	Thordur Oskarsson, Heidelberg Institute for Stem Cell Technology & Experimental Medicine	*Extracellular matrix proteins of stem cell niches promote breast cancer metastasis*
10.10 – 10.30	Stefanie Wohlrab, Universität Bayreuth, Germany	*RGD-modified recombinant spider silk proteins for improved cell adhesion and proliferation*
10.30 – 11.00	Tea & Coffee Break	
	Extracellular Matrix *Session Chair: Jo Adams*	
11.00 – 11.35	David Hulmes, IBCP, Lyon, France	*Key roles for coiled coils in procollagen trimerization and collagen fibril assembly*
11.35 – 12.30	Selected short talks	*To be confirmed*
12.30	Lunch & Departures	

Abstracts of Talks and Posters

Josephine C. Adams, University of Bristol

Session: Extracellular Matrix

Talk Title: **Thrombospondins and their roles in extracellular matrix**

Abstract

Thrombospondins and tenascins are families of extracellular matrix glycoproteins that originated at different points in animal evolution, yet both families are characterised by oligomerisation through short- parallel coiled-coil domains and some analogous functions as modulators of cell-extracellular matrix interactions. This talk will introduce thrombospondins and tenascins in the context of recent investigations of the evolution of extracellular matrix in animals. Outcomes of evaluation of LOGICOIL, a new multi-state predictor for coiled-coil domains, with regard to prediction and analysis of the higher-order coiled-coil domains of thrombospondins and tenascins will be presented.

Katja M. Arndt, Molecular Biotechnology, University of Potsdam

Session: New developments in coiled coils

Talk Title: **Interfering Peptides Targeting Protein-protein Interactions**

Abstract

Protein-protein interaction surfaces are attractive yet challenging targets for disease intervention. Coiled-coil motif containing proteins constitute especially attractive targets as they are involved in different diseases and their interaction patters in general are well understood. However, interaction patterns found in natural coiled coils differ significantly from the simplified schemes used in rational design approaches. For this reason, we combined rational design with in-vivo and in-vitro selection systems to generate interfering peptides (iPEP) specifically targeting disease-relevant coiled coil proteins. Different selection strategies as well as the generation of iPEPs against the oncoproteins Jun/Fos, leukemia-associated AF10 as well as melanoma-associated MITF will be discussed.

Poster title: **Improving Coiled Coil Stability while Maintaining Specificity using a Bacterial Hitchhiker Selection System**

Tim Kükenshöner [a) b)], Daniel Wohlwend [c)], Janina Speck [b)], Jean-Christoph Niemöller [b)], Oliver Einsle [c)], Kristian M. Müller [a)] and Katja M. Arndt [a)]

a) Molecular Biotechnology, University of Potsdam, 14476 Potsdam, Germany; Email: katja.arndt@uni-potsdam.de
b) Institute of Biology III, Albert-Ludwigs University of Freiburg, 79104 Freiburg, Germany
c) Institute of Organic Chemistry and Biochemistry, Albert-Ludwigs University of Freiburg, 79104 Freiburg, Germany

Abstract:

Design and selection of peptides targeting cellular proteins is challenging and often yields candidates with undesired properties. Therefore, we devised a new selection system based on the twin-arginine translocase (TAT) pathway of *E. coli,* named hitchhiker translocation (HiT) selection. A pool of coiled coil encoding sequences was designed and selected for interference with the coiled coil domain (CC) of a melanoma-associated basic-Helix-Loop-Helix-Leucine-Zipper (bHLHLZ) protein, the Microphthalmia Associated Transcription Factor (MITF). One predominant sequence (iM10) was enriched which showed remarkable protease resistance, high solubility and thermal stability while maintaining specificity and affinity (Kd ~ 40 nM) towards the target peptide. Additionally, a mutation screen indicated that helices of increased homodimer stability and improved expression rates were preferred in the selection process rather than peptides of solely enhanced target affinity. The solved crystal structure of the iM10/MITF-CC heterodimer (2.0 Å) provided important structural insights and validated our design predictions. Importantly, iM10 did not only bind the MITF coiled coil but also to the markedly more stable bHLHLZ domain of MITF. Characterizing the selected variants of the semi-rational library demonstrated the potential of the innovative bacterial selection approach.

Joschka Bauer, Universität Bayreuth

Poster title: **The role of terminal domains during storage and assembly of spider silk proteins**

Abstract

Spider dragline silk is a protein-based material with extraordinary mechanical properties that is used as stabilizing frame and radii in orb webs. Its unique combination of strength and elasticity exceeds that of many synthetic fibers- like Kevlar™ or steel. Dragline fibers are mainly composed of two protein classes, named major ampullate spidroin 1 and 2 (MaSp). The proteins are stored at very high concentrations of up to 50 % (w/v) inside the spinning gland. The spidroin solution assembles into a solid fiber within milliseconds during the spinning process. This sophisticated transformation is accompanied by environmental changes like ion exchange and acidification as well as the application of shear forces. MaSp are composed of a large repetitive core domain, flanked by smaller non-repetitive terminal domains. The repetitive core, which determines the mechanical properties of the final fibers, consists of up to 100 repeating units, each 40-60 residues long. The highly conserved-non-repetitive termini are folded globular domains comprising approximately 80-160 residues. Here, we present the structural dynamics of both terminal domains in response to changing conditions during the spinning process and the influence of the domains on storage and assembly of MaSp.

Jordi Bella, University of Manchester

Session: Collagens and Collagen Design

Talk Title: Introductory Talk

Abstract

To be provided at the meeting

Christian Bruno Borkner, S. Wohlrab, G. Lang & T. Scheibel, Lehrstuhl Biomaterialien, Universität Bayreuth

Poster title: **Coating of polymers with a recombinant spider silk protein for medical applications**

Abstract

Important polymers for medical applications, such as catheters, are polytetrafluoroethylene (PTFE), polyurethane (PU) and silicone. Due to biofilm formation, cell adhesion and cell growth on the surfaces, there are various problems like incrustations, inflammation and thrombosis.[1] To avoid these problems it is important to change the surface properties, for example by protein coatings. Spider silk, here used as a coating material, shows no toxicity, low/no immune-reactivity, slow biodegradability and weak cell adhesion.[2] We developed a method to deposit stable and water-insoluble eADF4(C16) coatings out of aqueous solution on synthetic polymers, leading to decreasing cell adhesion on the surface. Therefore, spider silk shows a high potential to optimize material properties and functionality.

[1] H. Seiter, K.-P. Schmitz, D. Behrend, Der Urologe [A] 2000 39: 463-468

[2] A. Leal-Egana, T. Scheibel, Biotechnol Appl Bioc 2010 55: 155-167

Natasha Burgess, University of Bristol

Poster Title: **A New Scaffold for de novo Designed Coiled-Coil Nanotubes**

Abstract

The bottom-up design of new 3D protein-based materials remains an intriguing challenge at the interface of synthetic biology and materials science. Our lab has concentrated on elucidating the sequence-to-structure relationship behind self-assembling α-helical coiled-coil peptides; on using this knowledge to assemble a 'toolkit' for designing peptide sequences for specific applications; and, in turn, using this toolkit to address the problem of engineering 3D assemblies.[1-2] In the creation of the toolkit a new a parallel hexameric coiled-coil structure, CC-Hex, was discovered.[3] An exciting feature of CC-Hex is that it has a ~5 Å channel running through it, which has been shown to be capable of supporting point mutations to polar residues. One of our aims now is to develop CC-Hex as a coiled-coil building block to create novel biomaterials. Inspired by previous work from our lab on Self-Assembling Fibres (SAFs), the goal of this research is to create fibrous assemblies of higher complexity, organisation and function than available by SAFs.[4-5] The work that will be presented establishes that CC-Hex can be used to create stable fibrous systems with the added benefit of large and modifiable internal surface areas suitable for materials applications.

References

1)Fletcher J. M. et al. Self-Assembling Cages from Coiled-Coil Peptide Modules. Science 2013 340-595-599

2)Boyle A.L. et al. Squaring the circle in peptide assembly: from fibers to discrete nanostructures by de novo design. J Am Chem Soc 2012. 134(37): 15457-67

3)Zaccai N. R et al. A de novo peptide hexamer with a mutable channel Nat. Chem. Biol. 2011 7: 935-941.

4)D Papapostolou et al. Engineering nanoscale order into a designed protein fiber- Proc. Natl. Acad. Sci. U. S. A. (2007)104: 10853-10858

5)Mahmoud Z. N. et al The non-covalent decoration of self-assembling protein fibers. Biomaterials 2010 31: 7468-7474

Antony Burton, University of Bristol

Poster title: **Accessibility, Reactivity and Selectivity of Side Chains Within a Channel of de novo Peptide Assembly**

Abstract

Recently, we reported the first example of a parallel, hexameric coiled coil CC-Hex.[1] X-ray crystallography revealed a central, hydrophobic channel of 6 Å in diameter, which presents the possibility for using the assembly as a scaffold for enzyme design. Importantly, polar and charged residues can be inserted into the central pore, with the construct maintaining its oligomeric state. Here we show that installed Cys residues can undergo simple organic transformations with small-non-polar alkylating agents with the construct remaining folded as a hexamer as the reaction progresses. The alkylated products of these transformations have been thoroughly characterized by solution phase biophysics, and a 1.9 Å X-ray crystal structure displays the conserved hexameric suprastructure. This investigation shows the construct is able to accept small molecule electrophiles into the channel of a de novo designed protein; a first step towards de novo designed enzymes.

1. Zaccai N. R. et al. Nat. Chem. Biol. 2011 7 935.
2. Burton, A.J. et al. J Am Chem Soc (In Press) (2013)

Massimo Buvoli, MCD Biology and BioFrontiers Institute

Session: Natural coiled coils

Talk title **Myosin Rod sequence and filament assembly**

Abstract

The coiled coil rod of striated muscle myosins divides into 38 repeats of 28 amino acids each that share both a common evolutionary origin and a particular charge profile. Myosin assembly appears to be guided by electrostatic interactions occurring between adjacent rods as well as C-terminal short molecular determinants. To better define the contribution of these elements and their potential interplay, we have carried out repeat swappings and substitutions with a 28 amino acids consensus sequence derived from a myosin rod alignment. The competence of these recombinant myosins in participating into cardiomyocyte thick filament formation will be discussed.

Andrew Carter, MRC LMB-Cambridge

Session: New developments in coiled coils

Talk Title: **Sliding in the dynein stalk coiled coil**

Abstract

Cytoplasmic dynein is a motor protein that uses the energy of ATP hydrolysis to move along microtubules. The motor domain is a ring of AAA+ domains that binds to the microtubule by a 15nm long antiparallel coiled-coil stalk. I will discuss the structural evidence for how ATP binding to the AAA+ domains is communicated to the microtubule binding domain. Our current model is that communication involves a half heptad sliding motion of one helix relative to the other.

Murray Coles, MPI for Developmental Biology, Tübingen

Session: Coiled coils at the membrane

Talk Title: **Coiled coils as key elements in bacterial transmembrane receptors**

Abstract

In 2006 we published the first structure of a HAMP domain, a ubiquitous linking domain in bacterial transmembrane receptors. This showed a dimeric, four-helix bundle with unusual coiled-coil packing, related to canonical packing by axial rotation of all four helices. This led us to propose the gearbox model of HAMP-mediated signalling, whereby the alternate packing forms represent signalling states. In recent years, we have validated this model using structural, biochemical and bioinformatics methods. In particular, we have fused HAMP with a downstrem histidine kinase effector module, leading to a detailed model of kinase regulation, the first time that models of signal transduction and effector regulation have been unified in these receptors. Here we summarise this work, which together with a growing body of literature data, defines an obligate role for dimeric coiled coils in signal generation, transduction and interpretation.

Vincent P. Conticello, Emory University

Session: Coiled-coil and collagen design

Talk Title: **Peptide Nanotubes and Nanosheets derived from Coiled-coil Assemblies**

Abstract

Recent advances in the structural analysis of biological assemblies have provided significant insight into the mechanisms that underlie the function of these complex macromolecular machines. The information obtained from these studies promises an unprecedented opportunity for the creation of novel, functionally programmable materials derived from self-assembly of biological structural motifs. Historically, structurally defined materials on the nanometer length-scale have been the most challenging to rationally construct and the most difficult to structurally analyze. Sequence-specific biomolecules, i.e. proteins and nucleic acids, have an advantage as design elements on the nano-scale in that correlations can be drawn between sequence and higher order structure, potentially affording synthetic materials in which functional properties can be controlled through the progression of structural hierarchy encoded at the molecular level. Chemosynthetic and biosynthetic approaches have been employed to create artificial peptides and proteins of uniform and controllable sequence that self-assemble into a wide variety of structurally defined supramolecular materials, including nanotubes and nano-sheets. A variety of analytical methods including circular dichroism, linear dichroism, transmission electron microscopy, atomic force microscopy, X-ray fiber diffraction and small-angle X-ray scattering have been applied for structural characterization of these materials. The structures and potential of these materials will be discussed in relation to the properties of the peptide structural motifs.

Kristina Djinovic-Carugo, Department of Structural and Computational, Biology Max F. Perutz Laboratories, University of Vienna

Session: Natural coiled coils

Poster title: **Crystal structure of human muscle α-actinin: insights into regulation of ligand binding and Z-disk assembly**

Abstract

α-Actinin is the major component of the Z-disk, where it cross-links actin filaments from adjacent sarcomeres. It is an antiparallel dimer of 200 kDa, containing in each subunit an N-terminal actin binding domain (ABD), a central rod domain assembled from spectrin-like repeats that mediate the antiparallel assembly, and a C-terminal calmodulin-like (CaM-like) domain with 4 EF-hand motifs. Additionally to actin filaments, α-actinin binds multiple other cytoskeletal and signalling proteins. In striated muscle, the tightly defined numbers of α-actinin crosslinks between the antiparallel actin filaments at the Z-disk are organised by specific binding sites on the giant molecular blueprint of the sarcomere, titin. These titin Z-repeats contain a short, hydrophobic, α-actinin-binding motif. To achieve ordered cytoskeletal assemblies, the binding properties of α-actinin must be tightly spatiotemporally regulated, in muscle α-actinin its actin and titin binding properties are regulated by phosphoinositide. Biochemical analyses led to propose previously that the α-actinin - titin interaction is regulated by an intramolecular mechanism, where the short sequence between the ABD and the rod interacts with the CaM-like domain in a pseudoligand complex, acting effectively as an intramolecular autoinhibitor. Here, we present the first complete crystallographic structure of sarcomeric human α-actinin, complemented by small angle X-ray scattering data, electron-electron paramagnetic resonance, and in vivo studies of structure-informed mutants, which give insight into its molecular assembly and Z-disk architecture as well as into the mechanism of α-actinin function and regulation.

Melanie H. Dietrich,[1] Kerstin Reiss,[1] Sarah Katen,[2,3] J. Denise Wetzel,[2,3] Terence S. Dermody,[2,3,4] and Thilo Stehle[1,2]

[1]Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen, Germany
[2]Department of Pediatrics, [3]Elizabeth B. Lamb Center for Pediatric Research, and [4]Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

Poster title: **Receptor binding properties of reovirus attachment protein σ1**

Abstract

Mammalian orthoreoviruses (reoviruses) are non-enveloped, icosahedral viruses with a segmented dsRNA genome. They are mostly non-pathogenic in humans, but they cause a number of disease syndromes in newborn mice. Therefore, they have been used extensively as model systems to study mechanisms of virus-host interactions and pathogenesis.

The outer-capsid protein σ1 mediates attachment to the host-cell surface and is the major target of the neutralizing immune response. The homotrimeric σ1 protein is a filamentous molecule of about 400 Å in length and comprises three distinct domains: the N-terminal tail, the body, and the head. The tail, which is anchored into the vertices of the virion icosahedron, incorporates a heptad-repeat pattern characteristic of α-helical coiled-coil formation. The body consists (mostly) of triple β-spiral repeats, a fold thus far observed only in a small number of viral fiber proteins, including the adenovirus fiber, the avian reovirus sigma C protein, and the bacteriophage PRD1. The globular head domain folds into an 8-stranded β-barrel. Three reovirus serotypes are known, which differ mainly in the sequence of the σ1-encoding gene segment and are represented by prototype strains type 1 Lang (T1L), type 2 Jones (T2J), and type 3 Dearing (T3D).

Reovirus attachment is thought to proceed via a two-step adhesion strengthening mechanism that is initiated by low-affinity binding to a carbohydrate receptor and followed by high-affinity binding to junctional adhesion molecule A (JAM-A). While all reovirus serotypes bind JAM-A using a conserved region in the head domain, glycan engagement occurs using different parts of T1L σ1 and T3D σ1. T1L σ1 binds the GM2 glycan in a shallow groove in the head domain, while T3D σ1 binds α2,3-, α2,6-, and α2,8-linked sialylated oligosaccharides between two β-spiral repeats near the mid-point of the fiber. The recognition of different carbohydrates by different parts of the σ1 molecule may account for serotype-specific differences in spread and tropism. It is unknown whether the large distance between the binding sites for JAM-A and glycan on T3D σ1 is necessary for proper binding of both receptor molecules or whether the protein undergoes a conformational change after binding to its carbohydrate receptor.

The epitopes of σ1-specific antibodies that neutralize reovirus infection and inhibit hemagglutination have been mapped by viral escape mutants and are located in the head domain. Recently, we have determined the crystal structure of a complex between T3D σ1 and the Fab portion of the serotype-specific antibody 9BG5. The target epitope spans two σ1 subunits but does not overlap with the

receptor-binding sites on T3D σ1. This finding suggests that the antibody inhibits reovirus infection by preventing rearrangement of σ1, a process that might be important for reovirus cell entry.

Stanislaw Dunin-Horkawicz, International Institute of Molecular and Cell Biology, Warsaw

Poster title: **From structure to sequence and back again**

Abstract

Coiled coils are protein structural domains comprising α- helices arranged in a regular fashion. The consequence of the regular nature of coiled coils is that their structures can be described fully by parametric equations. Parametrization allows for the quantification of the properties of coiled- coil structures solved experimentally and to the generation of models for coiled coils with predefined properties. The coiled-coil parameters can be divided into two categories: the first concerns whole bundle (e.g. radius, helix axial shift, helix axial rotation), the second characterizes individual helices (e.g. helix radius, residues per turn). The presented study aims at determining the relationship between sequence and structure in four- helical antiparallel coiled coils. To this end, we calculated ~1.000 backbone structural models that sample all theoretically possible helix axial rotation and helix axial shift states. Subsequently , for each model, an amino acid sequence was designed using a two-step procedure in which fixed- backbone design and full-atom relaxation is repeated alternatively. The obtained full-atom structures and the corresponding calculated folding energy scores were used to construct a "designability" landscape, which reveals combinations of helical axial rotation and helical axial shift states that are energetically favorable. Importantly, all the experimentally determined coiled- coil structures occupy these low-energy areas, implying that the proposed computational model is biologically significant. The statistical analysis of the designed amino acid sequences allowed defining features associated with individual regions of the "designability" land scape. These sequence features were generalized into a set of basic rules that govern folding of coiled coils of this type. Moreover, the sequences of naturally occurring coiled coils were found to obey these rules, suggesting the possibility of designing novel coiled-coil structures that will adopt yet unobserved conformations, and predicting the coiled coil conformation directly from the sequence. This, in turn, may have a substantial impact on engineering systems that relay on four-helical coiled-coil structures, e.g. bacterial signal transduction systems.

Martina B. Elsner,[1] Claudia Blüm,[1] Nicolas Helfricht,[2] Andreas Lammel,[1] Georg Papastavrou[2] and Thomas Scheibel[1]

[1]Lehrstuhl Biomaterialien- Fakultät für Angewandte Naturwissenschaften- Universität Bayreuth- 95440 Bayreuth, [2]Lehrstuhl Physikalische Chemie II- Fakultät für Biologie- Chemie und Geowissenschaften- Universität Bayreuth- 95440 Bayreuth

Session: Engineering with fibrous proteins

Talk Title: **Spider Silk Particles for Biomedical Applications**

Abstract

The recombinant spider silk protein eADF4(C16), based on the European garden spider (Araneus diadematus) silk protein ADF4, can be assembled into fibers, non-woven mats, films, hydrogels, capsules and particles. The eADF4(C16) particles show a high potential for drug delivery applications. Particles are produced in an all aqueous process, and their size can be adjusted according to the intended application. For pharmaceutical formulations the stability of the colloidal suspensions, over a large range of electrolyte concentrations, is of importance as well as the particle's surface properties like surface charge and roughness. Measurement of the electrophoretic mobility and direct force measurements demonstrated that the colloidal properties of the spider silk particles are directly related to the amino-acid sequence of the underlying protein. The particles show a high encapsulation potential for low-molecular weight molecules and proteins. For a better control of these properties the effect of crosslinking was studied. It could be shown that processing constitutions have an impact on structural integrity of the particles and related drug loading.

Carolin Ewers, Max Planck Institute for Developmental Biology- Tübingen

Poster title: **Structural Studies of HAMP Domains**

Abstract

To be provided at the meeting

Jeffrey D. Hartgerink, Rice University

Session: Coiled-coil and collagen design

Talk Title: **Axial Charge Pair Interactions Control Collagen Triple Helix Composition, Register and Higher Order Assembly**

Abstract

To be provided at the meeting

Marcus D. Hartmann, MPI for Developmental Biology, Tübingen

Session: New developments in coiled coils

Talk Title: **α/β Coiled Coils**

Abstract

Naturally occurring coiled coils often harbor discontinuities that retain the α-helical structure, but perturb the periodicity of the helical bundle. Nevertheless, the range of periodicities that coiled coils can assume is limited by the degree of supercoiling that their constituent α-helices can tolerate. Here, we describe a novel supersecondary structure element of trimeric coiled coils, which shows us the limits of possible periodicities and also shows how these limits can be circumvented. We name this element the ß-layer, as it breaks the α-helical coiled-coil structure locally by forming short beta strands perpendicularly to the coiled-coil axis. The trimeric arrangement resembles a triangular beta sheet. We studied ß-layers systematically in the GCN4 background and show that they represent the last missing element in understanding all possible periodicities that can be brought about by short insertions into the heptad repeat. Many naturally occurring coiled coils contain ß-layers in a regular repeat structure, forming a new type of fiber: the a/ß coiled coil.

David J.S. Hulmes, IBCP, Lyon, France

Session: Extracellular matrix

Talk Title: **Key roles for coiled coils in procollagen trimerization and collagen fibril assembly**

Abstract

We have recently determined the first 3D structure of a procollagen C-propeptide trimer, from procollagen III (Bourhis et al, 2012, Nature Struct Mol Biol). The structure includes a coiled coil that triggers trimerization during intracellular molecular assembly. In addition, outside the cell, the C-propeptide trimer controls proteolytic processing of procollagen as the rate limiting step in the assembly of collagen molecules into fibrils. This occurs through interactions involving the coiled-coil with procollagen C-proteinase enhancer (Bourhis et al, 2013, Proc Nat Acad Sci). Such interactions are similar to those occurring in diverse biological processes such as complement activation, transport and signalling.

Martin Humenik, Thomas Scheibel Biomaterials, Universität Bayreuth

Session: Engineering with fibrous proteins

Talk Title: **Silk protein – oligonucleotide conjugates as new nanomaterials**

Abstract

Recombinant spider silk protein eADF4(C16), a recombinant variant of Araneus diadematus dragline silk ADF4, undergoes structural changes upon salting out resulting in self-assembly of beta-sheet rich fibrils or particles. Silk fibrils are promising mechanically strong structures for "bottom-up" fabrication of bio-nanomaterials. DNA specific immobilization of different protein-oligonucleotide conjugates is an established approach for bioanalytics and for functionalization of nano-structures based on DNA tiles and origami. Inspired by the advantages of both silk and DNA materials, we synthesized, characterized and self-assembled novel oligonucleotide-spider silk conjugates. The recombinant spider silk protein eADF4(C16) and short oligodesoxynuclotides (ODNs) were site-specifically modified to create azide-alkyne coupling pairs. The protein moieties of prepared ODN-eADF4(C16) conjugates were arranged in parallel, antiparallel and a branched fashion depending on complementarities of hybridized ODNs. Interestingly, simple conjugates and their hybridized constructs retained the ability to assemble into beta-sheet rich fibrils very similar in appearance to those of unmodified protein eADF4(C16). Moreover, fibrils prepared of ODN-eADF4(C16) conjugates enabled specific attachment of neutravidin-modified gold nanoparticles directed by complementary biotin-ODN. The silk-oligonucleotide conjugates allow assembly of hierarchical fibril arrays for distinct applications.

Roman Jerala[1,2], Helena Gradišar[1,2], Sabina Božič[1] and Tibor Doles[1,2]

Session: Coiled-coil and collagen design

[1]Department of Biotechnology, National Institute of Chemistry, Ljubljana, Slovenia; [2]Excellent NMR-Future Innovation for Sustainable Technologies Centre of Excellence, Ljubljana, Slovenia;

Talk Title: **Concatenated single-chain coiled-coil segments for the self-assembly of polypeptide polyhedra**

Abstract

Design of natural protein folds is still beyond our current ability. We can apply an engineering modular approach using well understood polypeptide modules to design self-assembling polyhedra, where the formed coiled-coil dimers form their edges. Orthogonal designed coiled-coil dimers can be concatenate in a defined order which contains the instruction for building a selected topological fold. Graph theory can provide the analysis of different paths leading to the desired structure. The order of orthogonal interacting segments encodes the topology of the assembly, similar to the order of amino acids defining the protein folds. Similar to protein structures only a small fraction of sequences of segments encodes the structures that can self-assemble into the shape that satisfies all the coiled-coil pairing interactions. We will demonstrate the assembly of a polypeptide tetrahedron, prepared in recombinant form in bacteria and verified by several complementary methods. Finally the development of tools and paths for the design of other polyhedra will be presented.

Andrey Kajava, CRBM, Montpellier, France

Session: New developments in coiled coils

Talk Title: **Breaking the Amyloidogenicity Code: Bioinformatics Approach to Predict Predisposition to Amyloidosis**

Abstract

Devastating neurodegenerative diseases such as Alzheimer's disease, Parkingson's Disease, and Huntington's disease are linked to the formation of protein aggregates called amyloid fibrils. There currently exists no cure, and no means of early diagnosis for these diseases. Numerous studies have shown that the ability to form amyloid fibrils is an inherent property of the polypeptide chain. This has lead to the development of a number of computational approaches to predict amyloidogenicity by amino acid sequences. Although these methods perform well against short peptides (~6 residues) they generate an unsatisfactory high number of false positives when tested against longer sequences of the disease-related peptides and proteins [1]. Recently new experimental techniques have shed light on the structure of amyloids showing that the core element of a majority of disease-related amyloid fibrils is a ß-strand-loop-ß-strand motif called ß-arch [2- 3- 4- 5]. Using this information we have developed a new approach to predict amyloidosis from protein sequence [6]. Test of this program has shown that it yields the best known prediction of the disease-related sequences. As whole genome sequencing becomes cheaper, our method provides opportunity to create individual risk profiles for the neurodegenerative, age-related and other diseases ushering in an era of personalized medicine.

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David Kaplan, Tufts University

Session: Engineering with fibrous proteins

Talk Title: **Designing Proteins for Structure, Function and Modeling Considerations**

Abstract

To be provided at the meeting

Miklós Kellermayer, Semmelweis University

Session: Natural coiled coils

Poster title: **High-resolution structure and actin-binding of synthetic myosin thick filaments**

Abstract

The most important tissue of macroscopic biological motion is the striated muscle, in which actin filaments interact with myosin II motor proteins organized into bipolar thick filaments. Although it has been shown that thick filaments polymerize in an ionic-strength-dependent manner, their exact structure and the underlying molecular mechanism are unknown. According to most muscle contraction models, these polymers stochastically interact with actin filaments. However, the details of this interaction are not fully understood.

We examined the structure of synthetic thick filaments (TFs) and their interaction with actin filaments by using atomic force microscopy (AFM), which is capable of high-resolution imaging under aqueous conditions. Thick filaments were synthesized from rabbit back muscle myosin with dialysis at different ionic strengths. The TFs, together with rhodamine-phalloidin labelled F-actin, were adsorbed to mica surface in buffer, their topography scanned with AFM and the fluorescent signal examined with total internal reflection fluorescence (TIRF) microscopy.

While on mica, thick filaments displayed an opened-up arrangement, enabling us to observe the details of the internal structure, such as subfilaments of the shaft, which consisted of helically entwined tail domains. The structural compactness of the TFs depended on ionic strength, as the average width was 103±23- 179±34- 167±38- 162±43- 159±41 nm at 0- 30- 60- 90 and 120 mM KCl- respectively. In the axis and the middle of the filaments at 0 mM KCl a solid core was observed, which contained 2-3 subfilaments. At higher ionic strength we could resolve to 3-10 thinner subfilaments branching out into individual myosin tails by the ends of the filament. Actin filaments did not bind directly to the surface, but the presence of myosin fixed their position and in both TIRF and AFM images they were observable. Their interaction with individual and filamentous myosin molecules could be examined. Although many structural details for example the mechanism of bipolarity need to be clarified the arrangement of myosin molecules in thick filaments is such that the motor domains at their surface can optimally attach to actin filaments.

Balázs Kiss[1], Pasquale Bianco[2] and Miklós S.Z. Kellermayer[1],

[1] MTA-SE Molecular Biophysics Research Group, Department of Biophysics and Radiation Biology, Semmelweis University, Tuzoltó u. 37-47- 1094 Budapest- Hungary. [2]Laboratorio di Fisiologia, DBAG, Dipartimento di Fisica, Polo Scientifico, I-50019 Sesto Fiorentino (FI) Italy

Poster title: **Nanomechanics of desmin filaments explored with force-clamp optical tweezers**

Abstract

Desmin forms the intermediate filament system of muscle cells where it plays important role in maintaining mechanical integrity and elasticity. Although the importance of intermediate-filament elasticity in cellular mechanics is being increasingly recognized- the molecular basis of desmin's elasticity is not fully understood. In the present work we explored the mechanical properties of desmin filaments by using optical tweezers. Desmin- purified from chicken gizzard, was polymerized by the addition of $MgCl_2$. For mechanical manipulation, desmin filaments were captured between two anti-desmin-coated latex beads, one of which was positioned in a force-measuring optical trap and the other one held and manipulated with a moveable micropipette. Alternatively, desmin filaments were previously covalently linked to a carboxy functionalized latex bead and in situ photocrosslinked with an amino functionalized bead. By moving the micropipette away from the trap applying constant pulling rate, typically several micrometer section of the captured desmin filament was stretched. In the force versus extension curves transitions appeared even at low forces, but for forces higher than 50 pN inherent flexibility of polypeptide chains dominates the force response. Non-linear portions of the elastic curves were fitted with the wormlike-chain model, yielding an average 1 nm apparent persistence length. It is conceivable that the non-linear force curves reflect the behavior of unfolded- parallel attached desmin monomers. Contour length and persistence length distributions are not altered significantly in case of covalently attached desmin. Applying constant force upon stretch desmin filaments displayed length fluctuations in a force-dependent manner resulting in an average contour length increment of 14.5 nm. This value is below the contour length of a desmin dimer (i.e. 45 nm); thus it might correspond to the axial lengthening of a coiled-coil domain or might arise due to sliding between staggered dimers. Chemical crosslinking of subunits of the trapped filament with glutaraldehyde shifted the contour length increment-distribution towards a greater average value indicating that sliding between staggered desmin dimers is the most likely scenario during stretch.

Acknowledgement: This study was supported by TAMOP-4.2.1.B-09/1/KMR-2010-0001.

Vid Kocar, National Institute of Chemistry, Slovenia

Poster title: **Directed micro-tissue assembly via genetically introduced cell-surface heterodimerization domains**

Abstract

Modular tissue engineering is an emerging area of research in regenerative medicine. Its focus lies on recreating microstructural forms and features of natural tissues. The basic principle of modular tissue engineering is the preparation of tissue units with well-established three-dimensional architecture and their successive micromanipulation or self-assembly into higher-order artificial tissue structures. Prevalent approaches to the bottom-up formation of modular tissues rely on ad hoc cell surface engineering by means of chemical conjugation to cell surface moieties. However, such methods are intrinsically definitive and do not support additional engineering of tissue remodelling in response to environmental cues. We believe that introducing protein heterodimerization domains via underlying genetic circuits into modular tissue units is the logical advancement of current chemical approaches. To direct the assembly of cell-cell contacts we expressed chimeric coiled-coil containing transmembrane proteins in mammalian cells. Two approaches were used to investigate the ability of coiled-coils to direct formation of intercellular contacts. The first approach was a derivative of layer-by-layer tissue construction where consecutive binding and washing steps were implemented in order to obtain flat stacking of several cell sheets. The second approach aimed at producing spherical aggregates in a mixed suspension of cells expressing complementary coiled-coil segments. Despite current challenges, we believe such a synthetic biology approach to modular tissue engineering might provide a genetically encoded toolbox for future fine tuning of tissue microarchitecture, chemically or light-induced tissue modulation, contact depending cell differentiation and many others.

Alexander Kros, Leiden Institute of Chemistry, Leiden University

Session: Coiled-Coil Design

Talk Title: **Optimization of coiled coil driven membrane fusion**

Abstract

Membrane fusion plays a central role in biological processes such as neurotransmission and exocytosis. This process is defined by the merging of opposing membranes into one, which results in content transfer. Proteins located in opposite membranes form a coiled coil motif, thereby forcing the membranes into close proximity. Subsequently, membrane fusion occurs. In living systems this process is vital as it aids, for example, the transport of proteins between intracellular compartments and the controlled release of neurotransmitters.

In the past years we developed a model system for membrane fusion composed of a pair of complementary coiled coil forming lipidated peptides. Embedded in liposomal membranes these peptides are able to induce rapid, controlled and targeted membrane fusion. Initially we used the heterodimeric peptide pair E/K as designed by Litowski and Hodges. In this presentation, recent developments in optimization of the membrane fusion process by modification of the coiled-coil peptide pair are discussed.

Tim Kükenshöner [a) c)], Urs B. Hagemann [c)], Daniel Wohlwend [b)], Oliver Einsle [b)], Kristian M. Müller [a)] and Katja M. Arndt [a)]

a) Molecular Biotechnology, University of Potsdam, 14476 Potsdam, Germany; Email: katja.arndt@uni-potsdam.de
b) Institute of Organic Chemistry and Biochemistry, Albert-Ludwigs University of Freiburg, 79104 Freiburg, Germany
c) Institute of Biology III, Albert-Ludwigs University of Freiburg, 79104 Freiburg, Germany

Session: Coiled-Coil Design

Talk Title: **Selection, Design and Analysis of Chimeric D- and L-alpha-Helix Assemblies**

Abstract

D-peptides are highly interesting because of their proteolytic stability, however, interactions to natural proteins is poorly understood. Exploiting a previously designed, coiled coil based D/L heterotetramer (L-Base/D-Acid); we randomized the L-helix (L-Base) at residues presumably involved in complex formation. The resulting library, which contained $4.3x10^7$ variants, aimed to stabilize the assembly while using a less repetitive sequence. From phage display selection, one promising peptide (helixA) was enriched exhibiting alterations at 3 out of 8 randomized core (a/d) and 7 out of 8 randomized edge (e/g) positions compared to the original helix. Comparative analysis of both, HelixA and L-Base with D-Acid revealed in either case predominance for heterotetramer formation. As judged by isothermal titration calorimetry, the helices however displayed different patterns of interaction with trimeric intermediate states highly depending on concentration ratios of the peptides. In addition to a significant affinity gain, the helixA/D-Acid thermograms revealed one subspecies less compared to the L-Base/D-Acid assembly, suggesting an improvement of structural integrity forced by the selection process. To gain further insight into the interaction, we mutated single core amino acids as well as entire heptad positions using amino acids Leu, Val, Ile, Ala and Asn. Whereas some mutations completely disrupt complex formation, one Asn insertion promotes an altered folding process of only one transition state indicating heterotrimers. Conclusively, these results have implications for D/L peptide design as they dissect interaction modes of chimeric assemblies and reveal potentials and limitations of structural oligomerization specificity.

Tim Kükenshöner [a) c)], Urs B. Hagemann [c)], Daniel Wohlwend [b)], Oliver Einsle [b)], Kristian M. Müller [a)] and Katja M. Arndt [a)]

a) Molecular Biotechnology, University of Potsdam, 14476 Potsdam, Germany; Email: katja.arndt@uni-potsdam.de
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Elisabeth Le Rumeur[1], Baptiste Legrand[1], Emmanuel Giudice[1], Anne-Elisabeth Molza[1], Aurélie Nicolas[1], Angélique Chéron[1], Christophe Tascon[1], Céline Raguénès-Nicol[1], Jean-François Hubert[1], Mirjam Czjzek[2], Olivier Delalande[1]

[1]Université de Rennes 1, Institut de Génétique et Développement de Rennes (IGDR), Rennes Cedex, France. [2]Station Biologique de Roscoff, UMR 7139 CNRS and Université Pierre et Marie Curie (UPMC), Roscoff, France

Session: Natural coiled coils

Talk Title: **Overview: Spectrin-like repeats and Dystrophin**

Abstract

Duchenne muscular dystrophy (DMD) is caused by the genetic deficit of dystrophin, a large cytoskeleton protein of skeletal muscle included in the DGC sarcolemmal complex. Dystrophin complete deficit in DMD leads to cell degeneration induced by frequent sarcolemma ruptures. Dystrophin is a huge protein of 427 kDa where 85% of the protein is a large central domain constituted by 24 repeats sharing high structural homology with spectrin-like repeats and interacting with partners such as n-nitrous oxide synthase, F-actin, microtubules and lipids. However, in absence of any high resolution structural data of the repeats, the molecular basis of the dystrophin function and interactions is not deciphered and numerous questions still remained.

In this context, we proposed (1) a computational study of all the dystrophin repeats of the central rod domain based on the homology modeling with spectrin repeats and the analysis of their surface properties (Legrand et al., Plos One, 2011, 6:e23819). Four representative tandem repeats were then further studied by molecular dynamics simulation. The repeats appeared as coiled-coils and their surface properties are highly diverse and specific in terms of electrostatics and hydrophobicity making each of them unique. Molecular dynamics simulations revealed specific flexibility or bending properties of several inter-repeat linkers which may constitute small novel “junctions” between sub-domains.

Secondly, (2) we obtained structural data for various in vitro expressed multi-repeat fragments of dystrophin by using Small angle X-ray scattering (SAXS). SAXS is useful for obtaining protein structure of molecules suspended in a buffer solution, which complements the “rigid” structures determined by X-ray crystallography. Due to its low resolution (roughly 15 Å), the methodological way to correlate a SAXS shape to a three dimensional structure when analyzing the data is problematic. To this end, we have been developing a methodological approach using molecular dynamics simulations under SAXS constraints and are now able to solve high-resolution structures of long dystrophin fragments with great confidence. Based on a multi-resolution and interactive docking program (BioSpring), the reconstruction method allowed us to solve for the first time the structure of multi-repeat fragments of the central domain of dystrophin consistent with SAXS data (Figure 1 as an example below) and we proposed all atom models of the whole dystrophin rod domain.

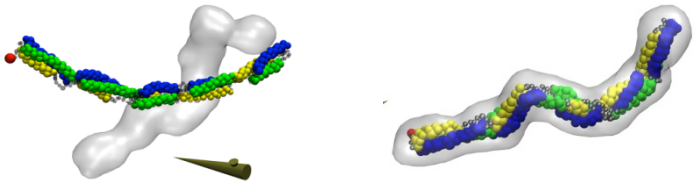

Fig.1. Interactive low-resolution fit of dystrophin model into a SAXS shape (left) - Final R11-15 model structure obtained from interactive docking and before high-resolution MD relaxation (right)

The data showed specific topology of the various fragments with kinks and twists, offering a new view of the dystrophin central domain.

Our models also constitute a rationale molecular plat-form for initiating docking studies with atomic structures of the known partners such as nNOS, F-actin as well as with lipids and to guide site-directed mutagenesis to more precisely and experimentally define the surface involved in the interactions.

Andrei Lupas[1], Birte Hernandez Alvarez[1], Alga Zuccaro[2], Jörg Martin[1], Marcus Hartmann[1]

[1] Max Planck Institute for Developmental Biology, Tübingen, Germany

[2] Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Session: New developments in coiled coils

Talk Title: **The Histidine Zipper**

Abstract

The structure originally proposed for the leucine zipper was explicitly not that of a coiled coil, but of an antiparallel dimer, in which the leucines formed a ridge of residues at the interface, with side-chains interdigitated like the teeth of a zipper [1]. Subsequently, the leucine zipper turned out to be a "leucine ladder", i.e. a canonical, parallel coiled coil with the leucines in position *d* of the heptad repeat, whose sidechains associated laterally into rungs, separated along the core by the residues in position *a*. We found that by replacing the leucines in position *d* with histidine and the residues in position *a* with alanine in the prototypical leucine zipper from the yeast transcription factor GCN4, we could convert it to a true zipper, with an antiparallel orientation of the helices and interdigitation of the histidine side chains. This mode of interaction was first described by the Richardson lab in 1995 as the "ferritin-like Alacoil" [2]. We characterized this interface motif in three natural protein families, where it occurs between consecutive helices in a single polypeptide chain, mediating the formation of helical hairpins rather than dimerization. In two of the families, one of the helices is bifaceted, with the second, offset heptad repeat either in positions *b--e* or *g--c* (type II in the nomenclature of Woolfson et al. [3]), mediating hairpin dimerization.

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Pradeep Luther, Imperial College London

Session: Natural Coiled Coils

Talk Title: **α-actinin/actin interaction in Z-bands**

Abstract

To be provided at the meeting

Lorenz Meinel, University of Wuerzburg

Session: Engineering with fibrous proteins

Talk Title: **Design space for silk-fibroin as a non-covalent drug delivery biopolymer**

Abstract

Silk fibroin (SF) isolated from *Bombyx mori* cocoons has been successfully deployed as a drug delivery system, particularly for sensitive biologicals. SF is a protein consisting of hydrophobic blocks, intermitted and flanked by at physiologic pH negatively charged hydrophilic blocks. The biopolymer is forming inducible β-sheets and can be processed in an aqueous environment. We used protamine as a positively charged model drug at physiologic pH, to study the interaction with SF in solution using isothermal titration calorimetry, turbidimetry, static light scattering, and nanoparticle tracking analysis. These studies mechanistically detail the understanding of SF interaction with biologicals and as a basis for the rational design of SF based controlled drug delivery systems.

Andreas Möglich, Humboldt-Universität zu Berlin

Session: Coiled coils at the membrane

Talk Title: **Structure and Function of a Light-Regulated Histidine Kinase**

Abstract

Excitability, i.e. the ability to perceive and respond to signals, is a basic hallmark of life. Our work aims at quantitatively understanding (analyzing) and at controlling (synthesizing) the underlying signal-transduction systems, specifically those engaged in the detection of light. At the molecular level, light perception is mediated by sensory photoreceptor proteins which comprise photosensor modules that absorb light and effector modules that exert a certain downstream biological activity. Based on structural and sequence data, we have constructed the blue-light-regulated histidine kinase YF1 by recombining a light-oxygen-voltage (LOV) photosensor with an effector module. On the basis of YF1- gene expression in prokaryotes can be up, or down-regulated as a function of blue light, thus affording spatiotemporally precise, reversible and non-invasive control. A crystal structure of YF1 we recently determined at 2.3 Å resolution reveals that the dimeric LOV photosensor module is linked to the catalytic effector modules via coiled-coil connectors. Functional assays pinpoint this region of the structure as crucial for activity and regulation by light; even single amino-acid mutations suffice to completely invert the signal response of gene expression. Structural motifs identified in YF1 widely recur in signal receptors, and the underlying signaling principles and mechanisms may be widely shared between soluble and transmembrane- prokaryotic and eukaryotic signal receptors of diverse biological activity

Edward P. Morris[1], Robert W. Kensler[2], Steve B. Marston[3], John M. Squire[4] and Hind A. AL-Khayat[3].

1. Division of Structural Biology, The Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW3 6JB, U.K 2. Department of Anatomy and Neurobiology, University of Puerto Rico Medical School, San Juan, Puerto Rico 00936-5067, U.S.A. 3. National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London W12 0NN, U.K. 4. School of Physiology & Pharmacology, University of Bristol, Bristol BS8 1TD, U.K.

Session: Natural Coiled Coils

Talk Title: **Structure of the Human Cardiac Muscle Myosin Filament**

Abstract

Human cardiac muscle myosin filaments are assemblies of myosin molecules and accessory proteins. Myosin molecules consist of two head domains and a rod-like coiled-coil domain. Each head domain possesses the ATPase activity necessary for contraction. Cardiac myosin filaments are bipolar with the myosin tails packing together and the heads adopting a 3-stranded quasi-helical arrangement. A detailed knowledge of the structure of human cardiac myosin filaments in the normal relaxed state is likely to be important in understanding how mutations give rise to various cardiomyopathies. To address this issue we have successfully developed a method to isolate myosin filaments from human cardiac muscle that preserves the highly ordered pseudo-helical structure of the relaxed filaments, thus making them amenable to analysis by electron microscopy and single particle image methods. From such samples we have produced a 3D reconstruction of the C-zone of the myosin filament. The 3D reconstruction reveals a 429 Å repeating unit containing three distinct sets of densities on the outer surface of the filament which correspond to the three crowns of myosin heads. Each crown is characterized by features with a close resemblance to the myosin head pairs identified in smooth muscle myosin [1] and in tarantula myosin filaments [2]. Accordingly, atomic models of myosin head pairs derived from the tarantula structure were fitted into each crown using the docking program Uro [3]. The close agreement between the models and the fitted density supports the proposal that the myosin heads of relaxed vertebrate cardiac myosin filaments adopt this configuration [4]. The location and orientation of the docked head pairs allows the pseudo-helical myosin head arrangement within the C-zone of the myosin filament to be defined as well as allowing the identification of the intermolecular interactions between the head pairs on different crowns which stabilize the relaxed conformation of human cardiac myosin filaments [5]. Furthermore, additional density on the surface of the myosin backbone can be assigned to the 39 Å-spaced domains of titin and cMyBP-C.

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Martin Neuenfeldt, University of Bayreuth

Session: Engineering with fibrous proteins

Talk Title: **Artificial Silk Made of Lacewing Egg Stalk Protein**

Abstract

Some lacewing species (*Chrysopidae*) produce egg-stalks comprising beta-sheet-rich proteins composed mainly of serine, glycine and alanine residues. In contrast to parallel beta-sheets in spider silk and *Bombyx mori* silk, the beta-strands in egg stalks are aligned perpendicular to the fiber axis (cross-beta structure). Due to this molecular characteristic egg-stalks possess a high bending stiffness and, at higher humidity, a high extensibility.

The structure of the egg stalk was the first known functional cross-beta structure. X-ray studies also showed the transformation of cross-beta into parallel-beta structure via longitudinal stretching of the stalk fibre.

In the lacewing species *Mallada signata*, the sequences of two egg stalk proteins were identified so far. Both proteins contain repetitive core domains showing a periodicity of 16 amino acid residues. In our group, we recombinantly produced an artificial egg stalk protein comprising a multimerized consensus motif based on these repetitive domains. Currently, artificial egg stalks can only be processed out of organic solvents (e.g. hexafluoroacetone) due to the low solubility in aqueous solutions. Therefore, it is necessary to optimize the protein sequence in order to increase the solubility of the protein.

Ben Nichols, MRC-LMB, Cambridge

Session: Coiled coils at the membrane

Talk Title: **Coiled coil proteins in the caveolar coat complex**

Abstract

Caveolae are an abundant feature of the plasma membrane of many mammalian cell types, and have key roles in mechano-transduction metabolic regulation and vascular permeability. Caveolin and cavin proteins, as well as EHD2 and pacsin 2, are all present in caveolae. How these proteins assemble to form a protein interaction network for caveolar morphogenesis is not known. Using in vivo crosslinking- velocity gradient centrifugation, immuno-isolation and tandem mass spectrometry, we determine that cavins and caveolins assemble into a homogenous 80S complex,which we term the caveolar coat complex. There are no further abundant components within this complex, and the complex excludes EHD2 and pacsin 2. Cavin 1 forms trimers and interacts with caveolin 1 with a molar ratio of about 1:4. Cavins 2 and 3 compete for binding sites within the overall coat complex, and form distinct sub-complexes with cavin 1. The core interactions between caveolin 1 and cavin 1 are independent of cavin 2, cavin 3 and EHD2 expression, and the cavins themselves can still interact in the absence of caveolin 1. Using immuno-electron microscopy as well as a recently developed protein tag for electron microscopy (MiniSOG), we demonstrate that caveolar coat complexes form a distinct coat all around the caveolar bulb. In contrast and consistent with our biochemical data, EHD2 defines a different domain at the caveolar neck. 3D electron tomograms of the caveolar coat-labeled using cavin-MiniSOG, show that that the caveolar coat is composed of repeating units of a unitary caveolar coat complex.

Lars Norlen, Karolinska Institute

Session: Intermediate Filaments

Talk title: **To be provided at the meeting**

Abstract

To be provided at the meeting

Thordur Oskarsson, Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH)

Session: Extracellular matrix

Talk Title: **Extracellular matrix proteins of stem cell niches promote breast cancer metastasis**

Abstract

Metastasis is the spread of cancer cells from their site of origin leading to outgrowth in distant organs and is the principal cause for cancer related deaths. To progress into overt metastasis, disseminated cancer cells must resist a non-permissive environment and maintain viability and growth at distant sites. Increasing evidence suggests that cancer cells adapt by engaging and manipulating the microenvironment, generating a metastatic niche that promotes cancer cell fitness. We show that tenascin C (TNC), an extracellular matrix (ECM) protein expressed in normal stem cell niches, is an important component of the metastatic niche and advances metastatic progression in breast cancer. TNC is initially expressed by the cancer cells within micrometastasis but following metastatic outgrowth and stromal activation, myofibroblasts emerge as a significant source of TNC. The cancer cell derived TNC is required to initiate metastatic colonization and this suggests that cancer cells expressing their own niche components gain a significant advantage. Interestingly, TNC expression in clinical breast cancer samples associates with the expression of periostin (POSTN), another glycoprotein of the ECM. POSTN was recently identified as a component of the metastatic niche and the coexpression with TNC raises the possibility of an interlinked role in the niche to promote metastasis. The ECM components of the metastatic niche support important functions in cancer progression and effectively help cancer cells to colonize distant organs. Further dissection of the metastatic niche and the ECM mediated signaling within the niche, could provide new avenues to therapeutically inhibit metastasis progression and prevent cancer relapse.

David Parry, Massey University

Session: Intermediate filaments

Talk title: **Fifty Years of Fibrous Protein Research: A Personal Retrospective**

Abstract

As a result of X-ray fiber diffraction studies on fibrous proteins and crystallographic data on fragments derived from them, new experimental techniques across the biophysical and biochemical spectra, sophisticated computer modeling and refinement procedures, widespread use of bioinformatics and improved specimen preparative procedures the structures of many fibrous proteins have now been determined to at least low resolution. In so doing these structures have yielded insight into the relationship that exists between sequence and conformation and this, in turn, has led to improved methodologies for predicting structure from sequence data alone. In this personal retrospective a selection of progress made during the past 50 years is discussed in terms of events to which the author has made some contribution.

2nd Talk title: **Keratin Intermediate Filaments: Differences in the sequences of the Type I and Type II chains explain the origin of the stability of an enzyme-resistant four- chain fragment**

Abstract

Previous studies have shown that a strong interaction exists between oppositely directed 1B molecular segments in the intermediate filaments of trichocyte keratins. A similar interaction has been identified as having a significant role in the formation of unit-length filaments, a precursor to intermediate filament formation. The present study is concerned with the spatial relationship of these interacting segments and its dependence on differences in the amino acid sequences of the two-chain regions that constitute the 1B molecular segment. It is shown that along a particular line of contact both chain segments possess an elevated concentration of residues with a high propensity for homodimer formation. The transition from the reduced to the oxidized state involves a simple axial displacement of one molecular segment relative to the other, with no attendant rotation of either segment. This changes the inter-relationship of the two 1B molecular segments from a loosely packed form to a more compact one. After the slippage eight of the cysteine residues in the dimer are precisely aligned to link up and form the disulfide linkages as observed. The two remaining cysteine residues are located on the outside of the dimer and are presumably involved in inter-dimer bonding. The existence of a unique line of contact requires that two chains in the molecule have different amino acid compositions with the clustering of homodimer-favoring residues phased by half the pitch length of the coiled coil.

Danielle M Paul[1], John M Squire[2], Edward P. Morris[1]

[1.] Institute of Cancer Research, London. [2] University of Bristol

Session: Natural coiled coils

Talk title: **How the structure of the thin filament changes on Ca^{2+} activation**

Abstract

Detailed three-dimensional structures of the muscle thin filament in the active and relaxed states are required in order to understand its regulation. To this end we have applied a reference free single particle analysis approach to electron microscope images of negatively stained reconstituted thin filaments from skeletal actin and cardiac tropomyosin and troponin. Two sets of filaments were prepared with and without Ca^{2+}. For image analysis the filaments were segmented into ~800Å long particles centred on the troponin complex. Density attributable to troponin and tropomyosin is readily identifiable in the two-dimensional class averages and the three-dimensional reconstructions. The data have previously been analysed using a model-based single particle method (Pirani *et al.,* 2005, 2006). Our non-model based approach and novel strand averaging procedure (Paul et al 2010, 2004) has enabled us to see the rearrangement of the troponin complex on the thin filament and identify the dynamic regions. Furthermore we have seen a movement of tropomyosin that has not previously been reported. Tropomyosin occupies two different positions on actin in the Ca^{2+}, treated filament and a single position in the Ca^{2+} free state. This has led us to propose a modified steric three-state model of thin filament regulation.

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Michelle Peckham, University of Leeds

Session: Coiled-coil Design

Talk Title: **Stable single alpha helical domains and their relationship to coiled coils**

Abstract

Alpha helices are normally stabilised by interactions with neighbouring secondary structure elements (i.e. coiled-coils). However, we recently showed that many proteins contain stable single α-helical (SAH) domains, usually inserted between two different functional domains. Coiled coil prediction programmes such as PEP-COIL or COILS often erroneously predict these domains to be coiled coil. However, these peptides are rich in charged amino acid residues, do not contain a hydrophobic seam and have a relatively high thermal stability (without neighbouring interactions). To date, the best-studied SAH domains are those from myosins, where they contribute to the mechanical lever of these motor proteins. We have used AFM to investigate the unfolding properties of naturally occurring SAH domains found in myosins. Each SAH domain has been cloned between two I27 domains (where I27 is the 27th immunoglobulin-family domain of titin) in an expression construct that contains five I27 domains, or expressed as an isolated peptide. Both isolated peptides, and SAH domains sandwiched between I27 domains are highly helical, by Circular Dichroism. AFM demonstrates that these SAH domains unfold at low forces (less than ~50pN) and do not have a characteristic unfolding profile. We have also investigated the propensity of 7- 11 and 15 heptad peptides from different regions of the coiled-coil tail of beta-cardiac myosin to form coiled coil. Surprisingly we find that 15 heptad peptides from some regions of the tail do not form coiled coil at all. However, 7 heptad peptides from other regions, which are like SAH domains, relatively rich in charged residues, but also contain a hydrophobic seam, do form coiled coil and may have properties similar to that described for a 'trigger sequence'. Thus sequences rich in charged residues, but with a hydrophobic seam can form stable coiled coils, but those that lack a hydrophobic seam form stable single alpha helices.

Martin Rabe, University of Leiden

Talk Title: Versatility of a Lipid Membrane Tethered Coiled-Coil Motif

Abstract

Recently, coiled coil forming lipopeptides were used to trigger the fusion of Vesicles. The mechanistic details of this process are not well understood as only vesicle docking is not sufficient for merging two lipid bilayers. When tethered to lipid membranes the close proximity to neighbouring peptides and to the polar lipid interface can alter the state of the peptide. In the talk these different states will be discussed on base of experimental results from CD-, IR- spectroscopy and lipid monolayer techniques. Furthermore a possible influence of these states on the fusion mechanism will be discussed.

Title of poster: **Determination of Thermodynamic Data and Molecularity from Melting Curves of Peptide Complexes**

Abstract

Circular dichroism melting curves of coiled coil complexes are often used to determine their thermodynamic binding parameters. In the past, application of this method required the knowledge of the stochiometric factors of the complex and was limited to low molecularities. Here we show the route for the extension of this method to higher molecular orders of any dimension by means of compuational methods, that can be introduced in global curve fitting routines. This enables us to determine the binding energies of tetramers and higher oligomers. Furthermore the combination with Job's method makes it possible to estimate the molecularities of the complexes by direct comparison of different binding models

John Ramshaw, CSIRO, Australia

Session: Collagens and Collagen Design

Talk Title: **Recombinant Bacterial Collagens as New Materials for Biomedical Applications**

Abstract

Yong Y. Peng, Violet Stoichevska, Linda Howell, Geoff J. Dumsday, Jerome A. Werkmeister and John A.M. Ramshaw

CSIRO Materials Science and Engineering, Clayton 3169, Australia.

Collagen has proven safe and effective in numerous medical products, and is now used in tissue engineering as a scaffold. Collagen is usually extracted from animals, frequently bovines, but there are concerns about transmissible diseases. This introduces an opportunity to develop recombinant collagens. However, recombinant mammalian collagens require the functional co-expression of prolyl 4-hydroxylase to achieve collagen stability, which leads to additional complexity.

Collagens all have a characteristic triple-helical structure with a $(Gly\text{-}X\text{-}Y)_n$ repeating sequence. Recently, a few collagen-like sequences have been characterised from bacteria. These collagens lack hydroxyproline, yet form stable triple-helices at 35-38 °C, and can be readily expressed in *E. coli*.

Initial constructs were based on the collagen-like protein Scl2 from *S. pyogenes*. Constructs included the registration domain (V), the collagen domain (CL), but without the C-terminal non-collagen tail. This sequence, of ~237 amino acids in a $(Gly\text{-}Xaa\text{-}Yaa)_n$ repeating sequence, shows limited, if any, biological interaction sites and is consequently a 'blank slate' into which selected functional domains can be inserted. We have previously shown that the monomer of this collagen is non-immunogenic and non-cytotoxic. We have introduced a triple-helical heparin and integrin binding motifs into the Scl2 sequence as examples of new functionalities. In addition, multimeric constructs of the single Scl2 collagen domain have been constructed. The work has now been extended to bacterial collagens from other species.

Trials have shown that these constructs can be readily produced in 2L fermentation using defined media. Under selected conditions, yields for V-CL up to 19 g/L were obtained, with an average of 17 g/L. For multiple constructs, the best yields were obtained with V-CL and yields declined with increasing construct size.

As we can produce bacterial collagen in good yield in fermentation trials, it is important that we have a matching simple, cost effective purification approach that can be used for large scale preparations. Consequently, we have examined an approach that avoids use of columns and affinity tags, which are often expensive to use and are not always easily scaled up. The purification strategy used some of the distinct properties of collagen and collagen-like proteins.

We have examined this approach with a range of bacterial collagen-like materials. The initial step of the purification, after cell rupture, was to acidify the homogenate, typically to between pH 2 and pH 3, selected so that the recombinant collagen remained soluble and in its native state. A substantial proportion of the host *E. coli* proteins are insoluble under these acidic conditions. Cell debris and insoluble proteins were removed by centrifugation and/or filtration. The remaining host proteins were removed by proteolytic digestion, for example using pepsin or papain. Papain allows a fully

animal free system if required. Triple-helical proteins are typically very resistant to proteolysis and are not digested by these present conditions. The non-triple helical V-domains of the constructs, however, are generally removed during proteolysis, removing potentially immunogenic domains from the product. The resulting preparation can be concentrated at neutral pH, with the product normally showing as a single band in SDS-PAGE. However, a further final polishing step may still be necessary, depending on the intended application of the material.

Title of poster / potential 2nd talk: **A new class of animal collagen masquerading as an insect silk**

Abstract

Tara D. Sutherland[1], Yong Y. Peng[2], Holly Trueman[1], Sarah Weisman[1], Shoko Okada[1], Andrew A. Walker[1,3], Alagacone Sriskantha[1], Jacinta F. White[2], Mickey G. Huson[4], Jerome A. Werkmeister[2], Veronica Glattauer[2], Violet Stoichevska[2], Stephen T. Mudie[5], Victoria S. Haritos[1] and John A.M. Ramshaw[2]

[1] CSIRO Ecosystem Sciences, Clunies Ross Street, Acton, ACT 2601,

[2] CSIRO Materials Science and Engineering, Bayview Avenue, Clayton, VIC 3169,

[3] Research School of Biology, Australian National University, Canberra, ACT, 0200,

[4] CSIRO Materials Science and Engineering, Waurn Ponds, Vic 3216,

[5]Australian Synchrotron, Blackburn Road, Clayton, VIC 3168, Australia

Collagen is ubiquitous in the animal kingdom, where it comprises some 28 types that form the extracellular matrix within organisms. More than 50 years ago, Rudall proposed an extracorporeal collagen in the cocoon of certain hymenopteran insects. He showed that the X-ray fibre diffraction pattern of cocoon silk from the gooseberry sawfly had a collagen-like structure. However, amino acid analysis showed that it did not contain any hydroxyproline, which was a characteristic feature of animal collagens. In the present study we have examined the cocoon silk from a related sawfly, the willow sawfly, *N. oligospilus*. X-ray diffraction of the willow sawfly fibres also showed the reflection at 0.286 nm that is characteristic of mammalian collagen axial spacing. Mechanical testing of dry cocoon silk fibres showed that they were stronger than dry collagen fibres from mammalian tissues. Protein and mRNA were isolated from excised silk glands including the secretory cells. The protein extract showed 3 predominant protein bands. The cDNA prepared from the silk gland extract led to the identification of 3 different collagen chains. Each chain had a signal peptide, plus both N- and C-terminal non-collagenous terminal domains. Whereas the collagen domains were of similar length for each sequence, the terminal domains were quite variable in length. The cocoon proteins did not contain any hydroxyproline. MS of tryptic peptides of cocoon proteins were shown to be identical to those in the sequence data, including the non-collagenous domains, showing that these are present in the mature protein. Each protein was also produced as a recombinant product. These individual proteins folded as triple-helices as the collagen domains were resistant to pepsin and gave CD spectra with 220nm maxima of the triple-helix. Although there are 3 distinct chains it is not yet known if these form a heterotrimer or 3 separate homotrimer molecules.

Kerstin Reiss,[1] Sarah Katen,[2,3] Melanie Dietrich,[1] Terence S. Dermody,[2,3,4] Thilo Stehle[1,4]

[1] Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen, Germany
[2] Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America
[3] Elizabeth B. Lamb Center for Pediatric Research, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America
[4] Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America

Session: New developments in Coiled Coils

Talk Title: **Structural characterization of reovirus attachment protein σ1**

Abstract

Mammalian orthoreoviruses (reoviruses) are nonenveloped, double-stranded RNA viruses with an icosahedral capsid. Reoviruses are used as models to study viral replication and pathogenesis. In addition, they are currently being evaluated in clinical trials as oncolytic agents as they preferentially infect and kill tumor cells.

Reovirus outer-capsid protein σ1 is a trimeric fiber molecule that protrudes from the virion surface. σ1 is composed of three distinct domains: the N-terminal tail, the body, and the C-terminal head. The head and body domains specifically engage receptor molecules on the host cell surface to enable initial attachment of the virus to target cells. The interaction between σ1 and its receptors has been partially structurally characterized.

In contrast, no structural information is available about the N-terminal tail domain of σ1. A large portion of the σ1 tail carries a heptad repeat pattern characteristic of the formation of α-helical coiled coils. To obtain structural information about this domain, we determined crystal structures of two large segments of the σ1 tail. The crystal structures confirm that these regions of the σ1 tail form trimeric α-helical coiled coils, in which the σ1 sequences follow the predicted heptad repeat pattern. The trimeric coiled coil is stabilized by a network of interhelical salt bridges and hydrogen bonds.

The crystal structures of the σ1 tail segments can be used to rationalize engineering of chimeric reoviruses that incorporate helical elements from other proteins. For example, such chimeric viruses could be potentially used as vaccines to induce antibodies directed against viral epitopes that form α-helices. Such epitopes in HIV and influenza virus elicit broadly neutralizing antibodies. Therefore, the reovirus σ1 α-helical coiled-coil tail domain may serve as a new platform for development of antiviral vaccines.

Anna Rising[1,2], Nina Kronqvist[1], Marlene Andersson[2], Kerstin Nordling[1], Michael Landreh[3], Kristaps Jaudzems[4], Stefan D. Knight[5], Jan Johansson[1,2]

[1]KI Alzheimer Disease Research Centre, NVS Department, Karolinska Institutet, Novum, 5th floor, 141 86 Stockholm, Sweden. [2]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, The Biomedical Centre, 751 23 Uppsala, Sweden. [3]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden. [4]Latvian Institute of Organic Synthesis, Department of Physical Organic Chemistry, Aizkraukles 21, Riga, LV-1006, Latvia. [5]Department of Molecular Biology, Swedish University of Agricultural Sciences, 751 24 Uppsala, Sweden.

Session: Engineering with fibrous proteins

Talk Title: **pH dependent dimerization of a spider silk protein domain: molecular mechanisms and implications for regulation of protein solubility and assembly**

Abstract

Spider dragline silk formation relies on the conversion of spider silk proteins (spidroins) in helical and/or random coil structures to an insoluble state in which the proteins adopt mainly ß-sheet conformation. Despite their strong tendency to polymerize, spidroins are stored at huge (>300 mg/ml) concentrations in the major ampullate glands [1]. The formation of the silk fiber in the spinning duct involves a lowering of pH, shear forces, dehydration of the dope and changes in ion composition [2]. The spidroins are produced by two types of epithelial cells in the gland [3], and contain three structural units; a non-repetitive N-terminal domain (NT) of about 130 amino acid residues, a central highly repetitive part of several thousands residues composed of poly-alanine/glycine-rich co-blocks, and a C-terminal non-repetitive domain (~110 residues). NT is the most conserved part of spidroins irrespective of spider species and silk type [4], and functions as a pH regulated relay conferring solubility to spidroins at pH 7 and fiber formation at pH 6 [5]. At pH 7 (as in the gland) NT is a monomeric five-helix bundle, while lowering the pH to 6 (as in the duct) causes distinct conformational changes and dimerization [6-9]. In the monomer, a tryptophan is buried between helix 1 and helix 3, while it is largely surface exposed in the dimer. The burial of the Trp side-chain causes a dislocation of the five-helix bundle, yielding a subunit conformation that is incompatible with the dimer structure [6]. The distinct structures of monomeric and dimeric NT indicate that both these states, and the pH driven conversion between them, are relevant for the apparent dual functions of NT - to confer solubility and to promote fiber formation. We are now aiming to design mutants that can trap NT in its monomeric and dimeric state, respectively, and we also aim to harness NT´s high solubility by using it as a tag in the production of recombinant fusion proteins.

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John Squire, Muscle Contraction Group, School of Physiology and Pharmacology, University of Bristol

Session: Natural coiled coils

Talk Title: **Coiled-Coils in Fibrous Proteins - an Overview**

Early work on fibrous proteins sorted them into α- and β-forms and collagen structures. The α-forms comprised the so-called ***k-m-e-f*** group of proteins (keratin, myosin, epidermin, fibrin). They were characterised in X-ray diffraction patterns by strong 0.15 nm and 0.51 nm meridional reflections, an equatorial peak close to 0.98 nm and near-equatorial layer-lines (see Parry *et al*., 2008). When the α-helix was first described by Pauling *et al.* (1951) and its diffraction pattern was considered, the 0.15 nm meridional peak was expected and observed (Perutz, 1951), but, instead of the observed 0.51 nm meridional, a near-meridional reflection at 0.54 nm was expected and the near-equatorial peaks were not explained. As is well known, Crick solved this by suggesting that two or more α-helical chains twisting round each other to form a coiled-coil would explain all the observations (Crick, 1952; 1953a,b). In addition, the amino acids on the line of contact between the chains would be expected to be apolar, thus cementing the chains together through hydrophobic forces. The classic two-chain coiled-coil would then have a repeating pattern of 7 apolar amino acids (the heptad) along this line of contact, as was subsequently confirmed in the case of tropomyosin when its sequence was first determined. At that time coiled-coils were only known in the fibrous proteins, but since then they have been found to be ubiquitous protein folds providing protein zippers, tubes, sheets, spirals, funnels and rings (Lupas and Gruber, 2005). However, today's session is mainly about the natural fibrous proteins. The morning session covers the structures of the coiled-coils found mainly in muscle (myosin, tropomyosin, troponin, α-actinin, dystrophin), whereas this afternoon covers the structures of keratin and other intermediate filaments.

Myosin filaments Myosin filaments occur in all cells, but they are most abundant in muscle where they interact with actin filaments to produce muscular force and movement. Myosin filaments are composed mainly of myosin molecules which have a long two-chain coiled coil rod on one end of which there are two gobular domains (myosin heads) which carry the enzymatic and actin-binding properties of myosin. The myosin rods pack together to form the filament backbones with the heads on the surface. When I came into the muscle field in about 1969 there was structural evidence about myosin filaments in frog muscle (Huxley and Brown, 1967) and insect flight muscle (Reedy, 1968). In both case the evidence was interpreted in terms of a 2-stranded helical arrangement of myosin heads on the filament surfaces. Squire (1971) showed that this was probably not the case and that the helical symmetry of different filaments could well be different. In the end it was found that vertebrate myosin filaments are 3-stranded and insect flight muscle filaments are 4-stranded. Myosin filaments in scallop muscle were found to be 7-stranded, but all these structures are closely related; myosin rods like to pack in a certain way (Squire, 2009). Since then the emphasis has been on trying to define myosin filament structure in detail either by modelling low-angle X-ray diffraction data from well-ordered muscles (AL-Khayat *et al*., 2006) or by using electron microscopy and image processing (Woodhead *et al*., 2005). Early image processing assumed that the filaments were helical, but vertebrate muscle myosin filaments are not in fact perfectly helical and modern methods apply single particle analysis without assuming helical symmetry (AL-Khayat *et al*., 2013). Edward Morris will present the latest results on human myosin filament structure determination by single particle analysis and Massimo Buvoli will discuss the myosin rod sequence and its implications for filament formation.

Actin filaments Actin monomers are globular proteins, but they aggregate to form long helical filaments, which appear like two strings of beads twisting round each other (Hanson & Lowy, 1963). End to end polymers of the 2-chain α-helical coiled coil tropomyosin molecule form two long strings

along the two strands of actin monomers. Tropomyosin is about 40 nm long and associated with each tropomyosin is the regulatory complex troponin. Parry and Squire (1973) and others (Huxley, 1972; Haselgrove, 1972) showed that thin filament regulation occurs because the tropomyosin strands in relaxed muscle block the site on actin where myosin heads want to bind. When vertebrate muscle is activated, Ca^{2+} ions are released into the muscle interior where they bind to troponin and cause the tropomyosin to move away from the myosin binding sites. Quite how this works is not yet known, but Danielle Paul will show the latest results of applying electron microscopy and single particle analysis (Paul *et al*., 2010) to vertebrate muscle actin filaments so that the tropomyosin and troponin can be visualised.

Three-strand spectrin-like coiled-coils Myosin and tropomyosin are both 2-chain coiled coils with the two chains pointing in the same direction. However, other muscle proteins, in the so-called spectrin family, are single chain molecules where the chain folds back on itself twice to form three-strand coiled-coil subunits (Yan *et al*., 1993). Apart from spectrin, such proteins are α-actinin which is found interacting with actin filaments in the muscle Z-line (and elsewhere) and dystrophin which is associated with the muscle membrane and which is modified or missing in patients with some of the muscular dystrophies. Elisabeth LeRumeur will provide an overview of the spectrin superfamily and will present her latest finding on dystrophin. Kristina Djinovic Carugo will present the latest crystal structure of α-actinin and Pradeep Luther will show how α-actinin assembles with actin filaments to form the muscle Z-lines and Z-crystals as in nemaline myopathy.

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Sergei Strelkov, KU Leuven

Session: Intermediate filaments

Talk Title: **Intermediate filaments as we know them**

Abstract

Intermediate filaments are a vital part of the eukaryotic cytoskeleton. Their assembly is driven by the self-association of elementary coiled-coil dimers. Over the decades, a lot of evidence has been collected towards the assembly process, while persistent X-ray crystallographic studies yielded a rather complete atomic description of the dimer. Yet our understanding of the IF, a semi-ordered and delicately controlled structure, remains far from being complete.

Tara Sutherland, CSIRO

Session: Engineering with fibrous proteins

Talk Title: **Insect silks: a new twist on coiled coils**

Abstract

The use of the coiled coil motif as the basis of structural materials is an engineering solution that has convergently evolved in at least five insect lineages,the stinging hymenopterans, sawflies, fleas, lacewings, and praying mantises and retained throughout large radiations of these insect families. We will review the current state of knowledge regarding the molecular structure of these materials, collected from techniques including X-ray scattering, nuclear magnetic resonance and Raman spectroscopy. Recently we have determined the amino acid sequences for multiple classes of these coiled coil silk proteins, enabling secondary and tertiary protein structural predictions to be compared to data from direct biophysical measurements. Finally, we review progress in the production of biomimetic coiled coil materials made using proteins of this type.

Franziska Thomas, Aimée L Boyle[a], Antony J. Burton, Ai Yoshinaka and Derek N Woolfson,[a b]

[a]School of Chemistry, University of Bristol, Bristol,BS8 1TS, UK; [b]School of Biochemistry, University of Bristol, BS8 1TD, UK

Session: New developments in coiled coils

Talk Title: **A Set of de Novo Designed Parallel Heterodimeric Coiled Coils with Quantified Dissociation Constants in the Micromolar to Sub-nanomolar Regime**

Abstract

Helical coiled-coil motifs are amongst the best understood peptide/protein folds. The basic coiled-coil motif is straightforward: sequence patterns of hydrophobic (h) and polar (p) residues – hpphppp, often denoted as abcdefg, direct the folding of amphipathic helices, which associate through their hydrophobic (a/d) faces to form helical bundles.[1]These principles facilitate the development of sequence-to-structure relationships for coiled coils- which can be used as rules to guide the successful de novo design of new peptides and materials. Herein, we present a new set of de novo parallel heterodimeric coiled coils. The parallel orientation is directed by the combination of isoleucine and leucine residues at a and d positions, respectively. But in contrast to previous designs,[2] we have also included two asparagine residues at the central a-position of each coiled-coil helix. As found in previous studies,[3] this additional design feature leads to higher dimer specificity and allows also the defined pairing of helices with different chain length. Heterospecificity is guided by complementary charge-charge interactions between e and g. Our study also includes the dependence of the coiled-coil stability on the length of the peptide chains.[3] To study the influence of the asparagines in the oligomeric assembly of coiled coils, 1D and 2D NMR experiments were undertaken to get information about the asparagine dynamics and the coiled-coil structure. The availability of such a set of peptides adds to our toolkit of peptide building blocks for potential applications in protein engineering- biomaterials design and synthetic biology.[4]

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Andrew Thomson, University of Bristol

Session: New developments in coiled coils

Talk Title: **Higher-Order Coiled-Coil Assemblies**

Abstract

To be provided at the meeting

Holly E. Trueman & Tara D. Sutherland, CSIRO Ecosystem Sciences/Future Manufacturing Flagship

Session: Natural coiled coils

Talk Title: **Lessons from nature: How evolutionary history can aid rational material design**

Abstract

The aculeate insects (ants, bees and wasps) produce a coiled-coil silk that they use to build domiciles, protect larvae and support wax hives. Many of these insects have had their genomes sequenced giving us a unique data set showing the evolutionary history of these silk proteins over the last 150 million years. We use this data to aid in rational design of new information encoding materials.

John Walshaw, Institute of Food Research, Norwich, UK

Session: Coiled coils at the membrane

Talk Title: **Identical helix twins do different things- only one on the straight and narrow: A mechanism for oligomer state-shifting in biological switches with multiple heptad registers**

Abstract

Several molecular switches have been proposed to be based on coiled-coil helices which can adopt different heptad registers in different states. We recently solved the structure of part of Drosophila BicD domain CC3- a homodimeric parallel coiled coil whose N-terminal half exhibits marked asymmetry and two different heptad registers simultaneously- offset by 4 amino acids- on identical polypeptide chains- which thus engage in heterotypic parallel packing. In contrast to other known axial staggers in parallel homodimeric coiled coils such as tropomyosin, the CC3 N-terminal segment is thus staggered by one whole knobs-into-hole packing layer; its regular, essentially in-plane layers are formed by different residues of the two chains of identical sequence. Implicated in this asymmetry is an unusual array of aromatic side chains, one of which is mutated to an aliphatic side chain a long-observed classical point mutation with a dramatic morphological phenotype. The function of BicD (which has highly conserved mammalian homologues) is to mediate transport of its bound cargos (e.g. mRNA-carriers) along microtubules, by means of features in its N-terminal half which bind to the dynein motor/dynactin complex. The dynein/dynactin-binding is thought to be autoinhibited by binding to CC3 of other coiled-coil motifs: CC1 and/or CC2 in the N-terminal region of BicD. We have also shown that cargo-binding is necessary for relief of this autoinhibition. We hypothesize that the observed multiple offset registers represent versatile "half-seams" which as well as the asymmetric dimeric-state, can take part in a proposed higher-order- likely tetrameric state involving CC1/CC2, the basis of the autoinhibition (interfaces between trimeric or tetrameric coiled-coil helices have earlier been described as two half-seams, with each interfacing with one of two neighbouring helices). Our model suggests an explanation of the aromatic-aliphatic point mutation which would favour the uninhibited dimeric state; thus resulting in abnormal levels of motor-mediated mRNA transport with consequences for morphogen distribution. The simultaneous presence of multiple registers in one sequence also has implications for coiled-coil prediction/design.

Alexandra Wasmuth[1]- Aimee L. Boyle[1,2] , Antony J. Burton[1] , Derek N. Woolfson[1,3]

[1]School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK. [2]Faculty of Science, Leiden Institute of Chemistry, Soft Matter Chemistry, Gorlaeus Laboratories, Einsteinweg 55- 2333 CC Leiden, The Netherlands. [3]School of Biochemistry, University of Bristol, University Walk, Bristol BS8 1TD, UK.

Session: New developments in coiled coils

Talk Title**: Design and characterisation of a single-chain multi-coiled-coil protein-like assembly**

Abstract

The design of truly de novo proteins with hitherto unseen structures is extremely challenging. Natural coiled-coil proteins display a characteristic sequence pattern known as the heptad repeat- in which hydrophobic (h) and polar (p) residues are spaced thus hpphppp; these are usually denoted abcdefg. Association to defined and well-characterized states, such as parallel dimers, is directed by hydrophobic and electrostatic interactions involving residues at positions a and d of the heptad repeat- and e and g- respectively.[1] The details of these patterns, or rules for coiled-coil assembly, can be deduced by inspection of natural coiled coils, or empirically through protein engineering, and then used in the de novo design of synthetic peptide systems. In turn, these de novo coiled-coil peptides can be used to generate discrete self-assembling nanostructures. Until recently however, [2] most of these designs focused on unlinked non-covalent assemblies. Here we describe the design and characterisation of a single-chain protein-like assembly comprising multiple coiled-coil modules. This builds on two of our previous studies. First, it uses designs for three orthogonal heterodimers.[3] Second, we link these peptides together using (GN)4 linkers arrived at empirically.[4] The 6-helix construct has been made via the recombinant expression of a synthetic gene in E. coli, and characterised using a combination of biophysical and structural techniques. Studies like this and those of Jerala and co-workers are opening new routes to the design of protein-like assemblies of increased complexity, and with potential applications in synthetic biology.

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S. Wohlrab[1], F. Bauer[1], S. Müller[1], A. Schmidt[1], S. Neubauer[2], H. Kessler[2],A. Leal-Egaña[3] and T. Scheibel[1]

[1]Lehrstuhl Biomaterialien, Fakultät für Angewandte Naturwissenschaften, Universität Bayreuth-95440 Bayreuth, Germany. [2] Institute for Advanced Study, Technische Universität München, 85747, Garching, Germany. [3]Laboratory of the Physics of the Cytoskeleton and Morphogenesis, Batiment, 40-20- 17 Rue Martyrs, 38054 Grenoble, France

Session: Extracellular Matrix

Talk Title: **RGD-modified recombinant spider silk proteins for improved cell adhesion and proliferation**

Abstract

Due to their biocompatibility and biodegradability as well as their remarkable mechanical properties, spider silk have been in focus of biomaterial research for several years. Using biotechnological methods, recombinant spider silk proteins and modified versions thereof have been produced. The ability to produce various two, or three- dimensional morphologies such as microspheres, foams, hydrogels and films, along with processing in aqueous solutions under ambient conditions, enables many promising biomedical applications like implant coatings or wound dressings. In this study, we focused on films made of eADF4(C16) (an analogue of A. diadematus dragline silk ADF4). Since cell attachment is weak on flat eADF4(C16) films, the integrin recognition sequence RGD was introduced either chemically or genetically. These modified silk films resulted in a significantly improved fibroblast adhesion and faster cell proliferation. Moreover, cell growth and orientation can be controlled by producing patterned films out of two different silk proteins.

Christopher W Wood, University of Bristol

Poster title: **Exploring Coiled Coil Folding Space**

Abstract

The recent de novo design of a hexamer expanded the range of classical coiled coil oligomer states, but can the range be further expanded to include higher order assemblies? In an attempt to generate these structures, we describe new computational tools being developed to aid the design process.

Dek Woolfson, University of Bristol

Session: New developments in coiled coils

Talk Title: **CC+, the Periodic Table, and a Basis Set of Coiled Coils**

Abstract

Coiled coils are defined at the sequence level by the heptad and related repeats, and structurally by knobs-into-holes interdigitation of side chains between neighbouring helices. Previously, we developed the SOCKET algorithm to identify the latter in protein X-ray crystal and NMR structure coordinate sets.[1] More recently, we have applied SOCKET to the entire Protein Data Bank and identified >1700 coiled-coil-containing protein structures. These have been used to create a relational database of coiled-coil-positive structures, CC+.[2] CC+ is available on-line.[3] It has a user-friendly GUI with pull-down menus and buttons to facilitate a wide range of searches for all types of coiled-coil structural and sequence motifs. We have also organised the coiled-coil structures into a Periodic Tables of Coiled Coils, based on oligomer state and topology.[4] This highlights the range and variety of coiled-coil structures found (so far) in nature, and also indicates where protein designers might work to generate new examples in this structural class. With the latter in mind, using sequence-to-structure relationships garnered from CC+ and from the literature, we have begun to generate a Basis Set, or Toolkit, of *de novo* design coiled-coil structures. Thus far, this comprises parallel homomeric dimers, trimers and tetramers (CC-Di, CC-Tri and CC-Tet) and a number of heterodimers (CC-Di-AB).[5,6] We have used the toolkit to expand the Basis Set, notably to include a hitherto unseen parallel coiled-coil hexamer, CC-Hex.[7]

Session: Coiled-coil Design

Talk Title: **Self-assembled Peptide Cages**

Abstract

An ability to mimic the boundaries of biological compartments would improve our understanding of self-assembly, and provide routes to new materials for the delivery of drugs and biologicals, and the development of protocells. This talk will describe how we have combined short *de novo* designed peptides to form unilamellar spheres approximately 100 nanometers in diameter.[8] The design comprises two, non-covalent helical bundles from our toolkit of coiled-coil modules, namely a heterodimer (CC-Di-AB) and the homotrimer (CC-Tri).[5,6] These are joined back-to-back via disulfide bonds to render two complementary hubs (hub-A and hub-B), which when mixed form hexagonal networks that close to form cages. We characterised the hubs and the complete assemblies using a combination of CD spectroscopy, analytical ultracentrifugation and dynamic light scattering in solution, and visualised them with a variety of electron and atomic force microscopy methods. Finally, we have been able to model the assemblies at various levels to help understand their folding and closure. This design strategy offers control over the chemistry, self-assembly, reversibility and size of the particles.

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Ai Yoshinaka[1], Mahendran K. Radhakrishnan[2], Hagan Bayley[2], and Derek N. Woolfson[1,3]

[1]School of Chemistry, University of Bristol. [2]Department of Chemistry, University of Oxford. [3]School of Biochemistry, University of Bristol

Session: Coiled coils at the membrane

Talk Title: **Design and engineering of transmembrane α-*barrel* forming peptides**

Abstract

Membrane proteins account for approximately 1/3 of all protein-encoding regions across all genomes.(1) They play a wide variety of roles in biological functions. Notably, channel proteins, which conduct ions and small molecules across membranes perform crucial functions in importing nutrients into cells, exporting waste, transporting bioactive molecules and cell apoptosis. Given their various roles, the successful rational design and engineering of channel proteins could have significant impact in bionanotechnology and synthetic biology. The barrel-like structures of channel proteins are generally based on either β–hairpins or α-helices. Compared with β-barrel proteins, however, transmembrane α-barrels have largely untapped potential in engineering new pores and channels; they remain relatively unexplored as high-resolution structural information is limited.(2-3) To engineer such "α-barrels" we have adopted two approaches. First, truncated natural membrane-spanning α-barrel proteins have been synthesized and characterized by structural biophysics and single-channel-recording methods. Second, a de novo designed water-soluble coiled-coil hexamer (CC-Hex) where 6 self-assembling α-helices form a 0.5 nm channel (4) is being redesigned to form a transmembrane CC-Hex. These approaches will enable us to understand the folding and channel functions of α-barrels, and to obtain novel and mutable transmembrane channels.

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Delegate List

Name	Affiliation	Contact E-mail
Josephine C. Adams	University of Bristol	jo.adams@bristol.ac.uk
Katja Arndt	University of Potsdam- Germany	katja.arndt@uni-potsdam.de
Joschka Bauer	Universität Bayreuth	joschka.bauer@bm.uni-bayreuth.de
Jordi Bella	University of Manchester	Jordi.bella@manchester.ac.uk
Pauline Bennett	King's College London	pauline.bennett@kcl.ac.uk
Aimee Boyle	Leiden University	a.l.boyle@chem.leidenuniv.nl
Sabina Božic	NIC- Slovenia	sabina.bozic@ki.si
Christian Bruno Borkner	Lehrstuhl Biomaterialen- Universität Bayreuth	christian.borkner@bm.uni-bayreuth.de
Natasha Burgess	University of Bristol	N.burgess@bristol.ac.uk
Antony Burton	University of Bristol	antony.burton@bristol.ac.uk
Massimo Buvoli	MCD Biology and BioFrontiers Institute	buvoli@colorado.edu
Andrew Carter	MRC LMB- Cambridge	cartera@mrc-lmb.cam.ac.uk
Murray Coles	Murray Coles	murray.coles@tuebingen.mpg.de
Vincent P. Conticello	Emory University	vcontic@emory.edu
Melanie Dietrich	University of Tuebingen	melanie.dietrich@ifib.uni-tuebingen.de
Kristina Djinovic-Carugo	Department of Structural and Computational- Biology Max F. Perutz Laboratories- University of Vienna	kristina.djinovic@univie.ac.at
Tibor Doles	NIC- Slovenia	tibor.doles@ki.si
Stanislaw Dunin-Horkawicz	International Institute of Molecular and Cell Biology in Warsaw	sdh@genesilico.pl
Martina Elsner	Lehrstuhl Biomaterialien- Universität Bayreuth	martina.elsner@bm.uni-bayreuth.de
Carolin Ewers	Max Planck Institute for Developmental Biology- Tübingen	carolin.ewers@tuebingen.mpg.de
Tímea Feller	Tímea Feller	timea.feller@gmail.com
Helena Gradišar	National Institute of Chemistry- Slovenia	helena.gradisar@ki.si
Jeffrey D. Hartgerink	Rice University	jdh@rice.edu
Marcus D. Hartmann	Max Planck Institute for Developmental Biology	marcus.hartmann@tuebingen.mpg.de
Kieran Hudson	University of Bristol	kieran.hudson@bristol.ac.uk
David J.S. Hulmes	IBCP- Lyon- France	d.hulmes@ibcp.fr
Martin Humenik	Biomaterials- University Bayreuth	martin.humenik@bm.uni-bayreuth.de
Roman Jerala	NIC- Slovenia	roman.jerala@ki.si
Andrey Kajava	CRBM- Montpellier- FRANCE	andrey.kajava@crbm.cnrs.fr
David Kaplan	Tufts University	david.kaplan@tufts.edu
Miklós Kellermayer	Semmelweis University	kellermayer.miklos@med.semmelweis-univ.hu
Balázs Kiss	Semmelweis University- MTA-SE Molecular Biophysics Research Group	balazs.kiss@eok.sote.hu
Vid Kocar	National Institute of Chemistry Slovenia	vid.kocar@ki.si

Alexander Kros	Leiden Institute of Chemistry- Leiden University	a.kros@chem.leidenuniv.nl
Tim Kükenshöner	University of Potsdam - Germany	kuekenshoener@hotmail.de
Elisabeth Le Rumeur	Université de Rennes	elisabeth.lerumeur@univ-rennes1.fr
Andrei Lupas	MPI, Tuebingen	andrei.lupas@tuebingen.mpg.de
Pradeep Luther	Imperial College London	p.luther@imperial.ac.uk
Lorenz Meinel	University of Wuerzburg	l.meinel@pharmazie.uni-wuerzburg.de
Andreas Möglich	Humboldt-Universität zu Berlin	andreas.moeglich@hu-berlin.de
Edward Morris	Institute of Cancer Research- London	edward.morris@icr.ac.uk
Martin Neuenfeldt	University of Bayreuth	martin.neuenfeldt@bm.uni-bayreuth.de
Ben Nichols	MRC-LMB	ben@mrc-lmb.cam.ac.uk
Lars Norlen	Karolinska Institute	lars.norlen@ki.se
Thordur Oskarsson	Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH)	thordur.oskarsson@hi-stem.de
David Parry	Massey University	d.parry@massey.ac.nz
Danielle Paul	Institute of Cancer Research	danielle.paul@icr.ac.uk
Michelle Peckham	University of Leeds	m.peckham@leeds.ac.uk
Martin Rabe	University of Leiden	m.rabe@chem.leidenuniv.nl
John A M Ramshaw	CSIRO- Australia	john.ramshaw@csiro.au
Heinz Redl	Ludwig Boltzmann Institute for Experimental and Clinical Traumatology	office@trauma.lbg.ac.at
Kerstin Reiss	University of Tübingen	kerstin.reiss@uni-tuebingen.de
Anna Rising	Karolinska Institutet	anna.rising@ki.se
John M Squire	University of Bristol	j.squire@imperial.ac.uk
Sergei V. Strelkov	KU Leuven	sergei.strelkov@pharm.kuleuven.be
Tara Sutherland	CSIRO	Tara.Sutherland@csiro.au
Lydia Tabernero	University of Manchester	lydia.tabernero@manchester.ac.uk
Andreas Teuschl	University of Applied Sciences Technikum Wien	teuschl@technikum-wien.at
Franziska Thomas	University of Bristol	chfbm@bristol.ac.uk
Andrew Thomson	The University of Bristol	chzart@bristol.ac.uk
Holly Trueman	CSIRO	holly.trueman@csiro.au
John Walshaw	Institute of Food Research- Norwich-UK	john.walshaw@ifr.ac.uk
Alexandra Wasmuth	University of Bristol	alexandra.wasmuth@bristol.ac.uk
Stefanie Wohlrab	Lehrstuhl Biomaterialien- Universität Bayreuth	stefanie.wohlrab@bm.uni-bayreuth.de
Christopher W Wood	University of Bristol	chris.wood@bristol.ac.uk
Derek Woolfson	University of Bristol	d.n.woolfson@bristol.ac.uk
Ai Yoshinaka	University of Bristol	Ai.Yoshinaka@bristol.ac.uk