The 7th Alpbach Workshop on: COILED-COIL, FIBROUS & REPEAT PROTEINS

At The Romantikhotel Böglerhof, Alpbach, Austria

Sunday 3rd September – Friday 8th September 2017

7th Alpbach Workshop on: Coiled-coil, fibrous and repeat proteins

SUNDAY SEPTEMBER 3

Arrivals, Reception and Dinner

MONDAY SEPTEMBER 4

09:00 – 10:40 **New developments in coiled coils** (Andrei Lupas)

Andrei Lupas (MPI Tübingen) - Coiled coils - between structure and unstructure
Partho Ghosh (UC San Diego) - Functional essential instability in the M protein coiled coil
Marcus Jahnel (MPI Dresden) - Coiled-coils as molecular motors: multistable polymer engines
Jeni Lauer (MPI Dresden) - Structural Dynamics of the Rab5-Modulated Coiled-Coil Protein EEA1 Revealed by Hydrogen-Deuterium Exchange Mass Spectrometry

10:40 – 11:10 **Tea break**

11:10 – 12:30 **Of α-fibers and β-fibers** (Andrei Lupas)

Birte Hernandez Alvarez (MPI Tübingen) - α/β-coiled coils
Antoine Schramm (Marseille) - Characterization of measles virus phosphoprotein: A coiled-coil domain containing conserved motifs that are crucial for its function
Michelle Peckham (Leeds) - Stable single α helices that do not form coiled coils - what makes them stable?

12:30 - **Lunch**
Afternoon free

17:00 – 17:50 **Flash Presentations for Posters**
17:50 – 18:30 **Posters**

18:30 – 20:00 **Dinner**

20:00 – 21:40 **Coiled coils at the membrane** (Alexander Kros)

Alexander Kros (Leiden) - Studying the interaction of lipopeptides with lipid membranes: the influence of lipopeptide design and its implications for membrane fusion
Rainer Böckmann (FAU Erlangen) - TBA
Niek Crone (Leiden) - The effect of peptide stapling on coiled coil formation and membrane interactions
Ai Niitsu (RIKEN) - Designing autonomous peptide-based membrane pores

TUESDAY SEPTEMBER 5

09:00 – 10:40 **Coiled-coil Design I: structure** (Dek Woolfson)

Dek Woolfson (Bristol) - From rational to computational design of coiled coils and back again
Beate Koksch (Berlin) - TBA
Jana Aupič (Ljubljana) - *De novo* design of a coiled-coil dimer–based switch
Franziska Thomas (Göttigen) - The Heterodimeric Coiled Coil As A Reaction Scaffold

10:40 – 11:10 **Tea break**

11:10 – 12:30 **Coiled-coil Design II: function** (Dek Woolfson)

Aimee Boyle (Leiden) - Coiled-coil peptides with improved metal-binding selectivity
Daniel Mitchell (Sheffield) - Recyclable Bionanostructures from Coiled-coil peptides
Cait Edgell (Bristol) - *De novo* designed coiled coils as programmable protein-protein interaction domains in novel transcriptional regulators

12:30 - **Lunch**
Afternoon free

17:30 – 18:30 **Posters**

18:30 – 20:00 **Dinner**

20:00 – 21:40 **Repeat-protein Design** (Fabio Parmeggiani)

Fabio Parmeggiani (Bristol) - Computational design of custom three-dimensional structures from modular building blocks
Andreas Plückthun (Zürich) - An Alternative Strategy to Generate Binding Proteins
Patrick Ernst (Zürich) - Rigid fusions of DARPins based on shared helix design to facilitate crystallography and enable functional studies
Mohammad ElGamacy (MPI Tübingen) - Computational Design of a Novel Repeat Protein Fold

WEDNESDAY SEPTEMBER 6

09:00 – 10:40 **Designing collagens, polyproline helices and larger assemblies** (Vince Conticello)

Vince Conticello (Emory) - Two-Dimensional Assembly of Collagen-Mimetic Peptides
Jeff Hartgerink (Rice) - Self-assembly and molecular recognition of the collagen triple helix
Emily Baker (Bristol) - Engineering Protein Stability with Atomic Precision in a Monomeric Miniprotein: PPα
Spencer Hughes (Emory) - Self-Assembly of HEAT-Repeat Modules into Nanotubes

10:40 – 11:10 **Tea break**

11:10 – 12:30 **Bundles and barrels** (Vince Conticello)

Stanisław Dunin-Horkawicz (Warsaw) - Fine-tuning of coiled coil design
Nathan W. Schmidt (UCSF)- Designing functional helical bundles by combining existing protein structures
Gustav Oberdorfer (Graz) - Exploring the functionalization of *de novo* helix bundles by deviating from idealism
Guto Rhys (Bristol) - Maintaining and breaking symmetry in homomeric coiled-coil assemblies

12:30 - **Lunch**
Afternoon free

17:00 - 18:30 **Software resources for modelling and analysis** (Andrei Lupas and Dek Woolfson)

Vikram Alva (MPI Tübingen) - The MPI Bioinformatics Toolkit and its application to protein analysis and design
Gevorg Grigoryan (Dartmouth) - Tertiary Alphabet for the Observable Structural Universe
Dmytro Guzenko (Leuven) - CCFold: rapid and accurate prediction of coiled-coil structures
Chris Wood (Bristol) - Parametric Modelling of Repeat Proteins with ISAMBARD and CCBuilder 2.0
Possu Huang, Fabio Parmeggiani, Gustav Oberdorfer (Rosetta community) - *De novo* and data driven design in Rosetta

18:30 – 20:00 **Dinner**

20:00 – 21:40 **Applications of fibrous materials** (Tara Sutherland)

Tara Sutherland (CSIRO) - The use of recombinant honeybee silk for rational design of advanced materials
Benedetto Marelli (MIT) - TBA
Tsunenori Kameda (NARO Tsukuba) - Fibrous silk with coiled coil superstructure produced by the larvae of hornets and its application to useful materials
Christian Borkner (U Bayreuth) - (Ultra-)Thin Spider Silk Coatings - From Structural Assembly to Applications

THURSDAY SEPTEMBER 7

09:00 – 10:40 **Natural coiled coils in signal transduction** (Brian Crane)

Brian Crane (Cornell) - Changes in both the structure and dynamics of highly helical bacterial chemoreceptors modulate activity of their associated histidine kinase CheA
Valentin Gordeliy (Grenoble) - Molecular Mechanisms of Transmembrane Signaling by TCS Sensors
Murray Coles (MPI Tübingen) - One HAMP, two HAMP, three HAMP, more...
Jens Bassler (MPI Tübingen) - The Cyclase Transducer Element (CTE): A coiled-coil hinge that controls adenylate cyclase activity

10:40 – 11:10 **Tea break and Group Photograph**

11:10 – 12:50 **Natural coiled coils in complex biological systems** (Sergei Strelkov)

Sergei Strelkov (Leuven) - Intermediate filament structure: progress and challenges
Robert J Edwards (Duke) - 1600 Å of coiled-coil at 4.3-Å resolution: the skips and bends of a myosin rod atomic model
Kfir Ben-Harush (Ashdod) - The structure and mechanics of nuclear lamins assemblies
Miklos Kellermayer (Budapest) - Structural analysis and nanomanipulation of fibrous proteins with high-resolution aqueous-phase atomic force microscopy

13:00 - **Lunch**
Afternoon Free

17:00 – 19:30 **Protein Design: past, present and future** (Dek Woolfson and Andrei Lupas; Bill DeGrado)

Dek Woolfson (Bristol) - Introduction
Bill DeGrado (UCSF) - De novo design of proteins
Anna Peacock (Birmingham) - Advancing metalloprotein design for new functions and applications
Gevorg Grigoryan (Dartmouth) - Ion transport across the biological membrane by computational protein design
Possu Huang (Stanford) - Building novel molecular platforms from design principles

20:00 – **Romantik Dinner**

FRIDAY SEPTEMBER 12

Departures

The MPI Bioinformatics Toolkit and its application to protein analysis and design.

Vikram Alva[1], Seung-Zin Nam[1], Lukas Zimmermann[1], David Rau[1], Andrew Stephens[1], Jonas Kübler[1], Marko Lozajic[1], Felix Gabler[1], Johannes Söding[2], and Andrei N. Lupas[1]

[1]Dept. of Protein Evolution, Max Planck Institute for Developmental Biology, Tübingen D-72076, Germany

[2]Group for Quantitative and Computational Biology, Max Planck Institute for Biophysical Chemistry, Göttingen D-37077, Germany

The MPI Bioinformatics Toolkit (https://toolkit.tuebingen.mpg.de) is an open and integrative Web service for advanced protein bioinformatic analysis. It includes a wide array of interconnected, state-of-the-art public and in-house tools, whose functionality ranges from the identification of features such as coiled-coil segments (PCOILS, MARCOIL), internal sequence repeats (HHrepID, REPPER), and secondary structure (Quick2D) to the detection of remote homologs of known structure (HHpred) and subsequent generation of structural models (MODELLER). In fact, due to this breadth of its tools, our Toolkit has established itself as an important resource for experimental scientists and as a useful platform for teaching bioinformatic inquiry. Recently, we replaced the first version of the Toolkit, which was released in 2005 and had serviced over 2.5 million external queries, with an entirely new version built using modern Web technologies and with improved features for teaching and collaborative research. In the presentation, we will focus on the usefulness of the Toolkit for the analysis and design of proteins.

De novo design of a coiled-coil dimer–based switch

Jana Aupič[1,2], Fabio Lapenta[1,3], Roman Jerala[1,4]

[1]*National Institute of Chemistry, Department of Synthetic Biology and Immunology, Hajdrihova 19, 1000 Ljubljana, Slovenia*

[2]*Doctoral Study Programme in Chemical Sciences, University of Ljubljana, Ljubljana 1000, Slovenia*

[3]*Interdisciplinary Doctoral Programme in Biomedicine, University of Ljubljana, Ljubljana 1000, Slovenia*

[4]*EN-FIST Centre of excellence, Trg OF 13, 1000 Ljubljana, Slovenia*

Corresponding author: roman.jerala@ki.si

Due to a good understanding of their folding and assembly principles, coiled-coils are a frequent subject of design attempts in the protein design community. Earlier works focused on re-engineering the structure and function of naturally occurring coiled-coils, and building on that knowledge it is now possible to design coiled-coils completely *de novo*. The hallmark of coiled-coils is the heptad repeat sequence HPPHPPP, often denoted as *abcdefg*. Coiled-coil dimer assembly is mediated by hydrophobic interactions between amino acids on *a* and *d* position and salt bridging between residue on *e* and *g* positions[1].

One of the challenges in the protein design is the construction of proteins that undergo a change in conformation in response to environmental cues such as pH, light, redox potential or the presence of specific ligands such as metal ions. A key step towards achieving this goal is the design of a structural element that can accommodate two different conformational states, depending on the external signal. The ability to design the controllable conformational flexibility is especially important for developing designed molecular machines, since the activity of numerous natural proteins was shown to be modulated by conformational changes[2].

We present a rationally designed four heptad peptide that assembles into a parallel dimeric coiled-coil in the presence of Zn(II) ions or upon a pH drop. The secondary structure and thermal stability in different solution conditions were studied using circular dichroism. Oligomerization and dissociation constants were determined using SEC-MALS and ITC, respectively. The reversibility of the designed peptide as well as the effect of other divalent metals on coiled-coil formation was examined.

The designed peptide could be used as a sensing component in biosensors, for reversible biofunctionalization of surfaces or as a versatile tool in synthetic biology. Furthermore, inserting the designed protein into coiled-coil based protein origami cages[3] could allow controllable triggering of the assembly and disassembly.

References:

1. Woolfson, D. N. The Design of Coiled-Coil Structures and Assemblies. Adv Protein Chem 70, 79–112 (2005)
2. Boehr, D. D., Nussinov, R. & Wright, P. E. The role of dynamic conformational ensembles in biomolecular recognition. Nat. Chem. Biol. 5, 789–796 (2009).
3. Gradišar, H. et al. Design of a single-chain polypeptide tetrahedron assembled from coiled-coil segments. Nat. Chem. Biol. 9, 362–6 (2013).

Engineering Protein Stability with Atomic Precision in a Monomeric Miniprotein: PPα

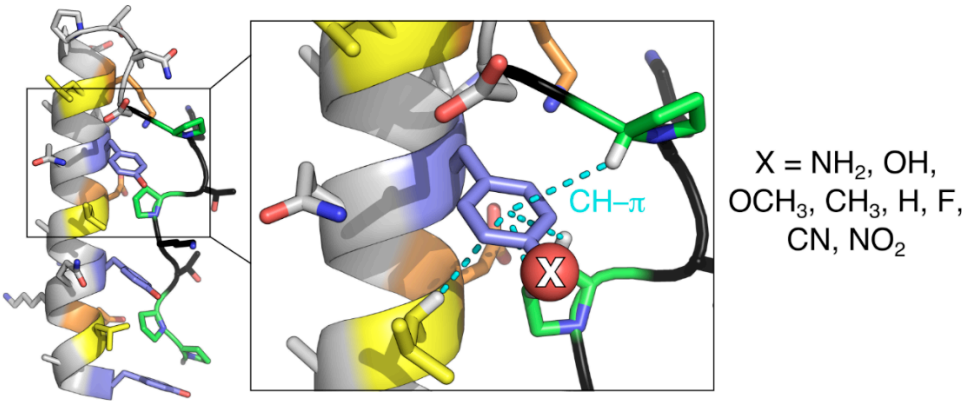
Emily G. Baker,[1]* Christopher Williams,[1,2,$] Kieran L. Hudson,[1,†,$] Gail J. Bartlett,[1] Jack W. Heal,[1] Kathryn L. Porter Goff,[1] Richard B. Sessions,[2,3] Matthew P. Crump[1,2] and Derek N. Woolfson[1-4]*

[1]School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK
[2]BrisSynBio, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol, BS8 1TQ, UK
[3]School of Biochemistry, University of Bristol, Biomedical Sciences Building, University Walk, Bristol, BS8 1TD, UK
[4]Bristol BioDesign Institute, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol BS8 1TQ, UK

[†]Current address: Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, V6T 1Z1, Canada

*Corresponding authors: EGB (emily.baker@bristol.ac.uk) and DNW (D.N.Woolfson@bristol.ac.uk)
[$]Contributed equally to this work.

Abstract:
Miniproteins simplify the protein-folding problem, allowing the dissection of forces that stabilize protein structures. Here we describe PPα-Tyr, a designed peptide comprising an α helix buttressed by a polyproline-II helix.[1] PPα-Tyr is water soluble, monomeric, and unfolds cooperatively with a midpoint unfolding temperature (T_M) of 39 ˚C. NMR structures of PPα-Tyr reveal proline residues docked between tyrosine side chains as designed. The stability of PPα is sensitive to the aromatic residue: replacing tyrosine by phenylalanine, *i.e.* changing three solvent-exposed hydroxyl groups to protons, reduces the T_M to 20 ˚C. We attribute this to the loss of CH–π interactions between the aromatic and proline rings, which we probe by substituting the aromatic residues with non-proteinogenic side chains. Analysis of natural protein structures highlights a preference for proline-tyrosine interactions over other proline-containing pairs, and abundant CH–π interactions in biologically important complexes between proline-rich ligands and SH3 and similar domains.



Tuning non-covalent CH–π interactions in PPα

References:
[1]E. G. Baker *et al*, Engineering Protein Stability with Atomic Precision in a Monomeric Miniprotein. *Nat. Chem. Biol.*, 2017, 13, 764-770.

The Cyclase Transducer Element (CTE): A coiled-coil hinge that controls adenylate cyclase activity

Jens Bassler[1*], Stefanie Beltz[2], Miriam Ziegler[2], Anita Schultz[2], Joachim E. Schultz[2], Andrei Lupas[1*]

[1] Max Planck Institute for Developmental Biology, Dept. Protein Evolution, Spemannstraße 35, 72076 Tübingen, Germany
[2] University of Tübingen, Institute of Pharmaceutical Sciences, Auf der Morgenstelle 8, 72076 Tübingen, Germany
[*] contact: jens.bassler@tuebingen.mpg.de, andrei.lupas@tuebingen.mpg.de

Class III adenylate cyclases (ACs) are signaling proteins that produce the second messenger cyclic AMP (cAMP). Recently, we identified the novel cyclase transducer element (CTE) on the N-terminus of the AC catalytic domain. Biochemical characterization found the element indispensable for the regulation of AC activity in response to upstream receptor activation. Based on the ambigous coiled-coil sequence register of the CTE, we proposed a structural model in which the element acts as a hinge that switches between an experimentally observed bent conformation and a hypothetical coiled-coil conformation, thereby restricting the structural freedom of the catalytic domains to form active dimers (Fig. 1).
In bacterial ACs, a CTE is present whenever the upstream signaling domain is also found in histidine kinases or similar signaling proteins, but absent when not. Thus, our data provides new insights into the evolutionary relationship between ACs and other families of signaling enzymes, as well as a structural rational for the functioning of various laboratory chimeras between them. Notably, the CTE also exists in the adenylate and guanylate cyclases of vertebrates, which are involved in many cellular signaling processes, including the propagation of signals coming from G protein-coupled receptors (GPCRs). This provides indirect evidence for a regulatory function of the membrane anchors of vertebrate ACs and suggests an additional regulatory level to GPCR signaling.

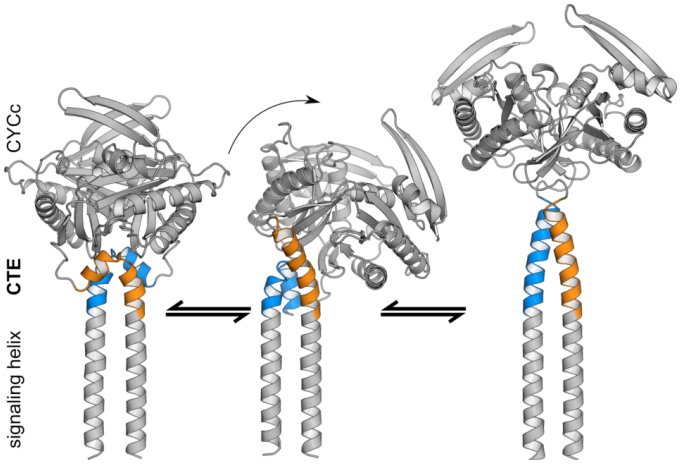


Figure 1. Proposed structural model for the regulation of class III ACs by the CTE (colored). The model progresses through an asymmetric state in which one CTE adopts the straight conformation and the other remains bent.

References:

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3) Bassler, J., Lupas A. N., Schultz, J. E. *in preparation.*

The structure and mechanics of nuclear lamins assemblies

Shani Deri [a],[b], Maayan Khayat[c], Yosef Gruenbaum[e], Ohad Medalia[c,d], Kfir Ben-Harush[a]*

[a] Department of Chemical Engineering, SCE - Shamoon College of Engineering, Jabotinsky 84, 77245, Ashdod, Israel.
[b] Department of Chemical Engineering, Ariel University, 40700, Ariel,, Israel.
[c] Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Ben-Gurion Av. 1, 8410501, Beer-Sheva, Israel.
[d] Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, 8057, Zurich, Switzerland
[e] Department of Genetics, The Hebrew University of Jerusalem, The Edmond J. Safra Campus - Givat Ram, 9190401, Jerusalem, Israel

The nuclear lamina is a meshwork layer apposed to the inner nuclear membrane of all metazoan cell nuclei. It is composed mainly of nuclear lamins that are classified as type V intermediate filament proteins. The organization of nuclear lamins into higher-order assemblies within the cell nucleus play a pivotal role in nuclear structure, mechanics and function. Recently, a seminal study suggested that tissue stiffness and stress increase lamin-A levels within the nucleus providing stabilized nucleus, thus, lamins probably play important role in tissue mechanotransduction. **What are the structures that lamins form in different tissues and how this structural organization is related to the mechanical properties is however still unknown**. Here, the *in-vitro* structure and mechanics of lamin assemblies was studied. Most lamins assemble *in vitro* into paracrystalline structures, though the formation of paracrystals within the nucleus has not been revealed, our investigation has shown that the organization of human type-A lamins into filamentous structure within the nucleus *in-situ* is similar to their organization into paracrystal. To investigate the mechanical properties of lamin paracrystals, we used B-type *C. elegans* nuclear lamins to form macroscopic fibers. Electron cryo-tomography of vitrified sections showed that lamin fibers assembled through the formation of paracrystals network. The relationship between the hierarchical structure and mechanical behvior of lamin fibers was investigated using different lamin constructs that form altered paracrystal structures, including lamins harbor point mutation that cause severe diseases in human. The unique mechanical properties and behavior of lamin fibers revealed here can lead also to new biomaterials in the fields of tissue engineering, medical devices, and possibly a basic understanding of the mechanical properties of intracellular nanomaterials.

Peptide-Lipid Interaction

Kristyna Pluhackova, Sonja Kirsch, Angelica Sandoval-Perez, Rainer Böckmann
Computational Biology, Friedrich-Alexander University of Erlangen-Nürnberg
Erlangen, Germany
E-Mail: rainer.boeckmann@fau.de

The interaction of peptides with lipids is important for a wealth of biological processes, ranging from cell lysis, membrane fusion, transmembrane transport, to signalling and cell-cell communication. Proteins and lipids mutually influence their structure, localization, and mobility and thus function of each other. Using both atomistic and coarse-grained molecular dynamics simulations, we show how a surface-attached coiled-coil [1], or membrane-embedded peptides [2,3] and proteins shape the membrane environment, i.e. induce membrane curvature, and bias the lipid structure and mobility.
The heterogeneity at the membrane/water interface plays a crucial role in the protein-lipid interactions: It yields a directionality, enables a stable anchoring of transmembrane proteins, allows for specific targeting of peripheral proteins to distinct membrane domains, and contributes to protein oligomerization at or in biological membranes. The membrane interface is also the site of action for the first contact of toxins and antimicrobial peptides with the cell. Here we report a novel hydrophobicity scale that allows to quantify the binding strengths of peptides to the membrane-solvent interface. The comparison of peptide toxicity and interfacial membrane binding energy reveals favorable peptide interactions with the headgroups of phosphatidylcholine lipids – the main component of eukaryotic plasma membranes – as the main driving force for the hemolytic activity of peptides.

[1] M. Rabe, C. Aisenbrey, K. Pluhackova, V. de Wert, A. L. Boyle, D. F. Bruggeman, S. A. Kirsch, R.A. Böckmann, A. Kros, J. Raap, B. Bechinger. *A Coiled-Coil Peptide Shaping Lipid Bilayers upon Fusion*. Biophys. J. 11(10):2162-2175 (2016)
[2] A. Sandoval, S. Eichler, S. Madathil, P. J. Reeves, K. Fahmy, and R.A. Böckmann. *The molecular switching mechanism at the conserved D(E)RY motif in class-A GPCRs*. Biophys. J. 111:79-89 (2016)
[3] J. Han, K. Pluhackova, D. Bruns, R.A. Böckmann. *Synaptobrevin transmembrane domain determines the structure and dynamics of the SNARE motif and the linker region*. BBA-Biomembranes 1858:855-865 (2016)

(Ultra-) Thin Spider Silk Coatings - From Structural Assembly to Applications

C. B. Borkner[a], S. Lentz[a], S. Wohlrab[a], E. Möller[a], A. Pellert[a], G. Lang[b] & T. Scheibel[a,c,d,e,f,g]

a – Lehrstuhl Biomaterialien, Fakultät für Ingenieurwissenschaften; b – Professur für Biopolymerverarbeitung, Fakultät für Ingenieurwissenschaften; c – Bayerisches Polymerinstitut (BPI); d – Bayreuther Zentrum für Kolloide und Grenzflächen (BZKG); e – Institut für Bio-Makromoleküle (bio-mac); f – Bayreuther Zentrum für Molekulare Biowissenschaften (BZMB); g – Bayreuther Materialzentrum (BayMAT); Universität Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany

E-Mail: Thomas.Scheibel@bm.uni-bayreuth.de

Materials made of spider silk proteins are known for their high biocompatibility, good biodegradability and lack of immunogenicity and allergenicity [1] Biotechnological production enables high amounts of recombinant spider silk proteins and allows genetic modification of the protein sequence.[2] The negatively charged engineered spider silk protein eADF4(C16) is based on the consensus sequence of *Araneus diadematus* fibroin 4 (ADF4) and contains 16 glutamic acid residues.[2] If glutamic acid is exchanged by lysine (E→K), the positively charged protein eADF4(κ16) is obtained.[3]

Our first objective was to get a deeper insight into self-assembly and folding processes of eADF4(C16) in nano- and microfilms. (Ultra-) thin eADF4(C16) films were produced via spin-coating, and the protein structure as well as surface properties of such films were analyzed depending on film thickness before and after post-treatment with methanol (β-sheet induction). Based thereon, an assembly and folding model depending on the film thickness was proposed.

eADF4(C16) is a promising material for biomedical coating material. The performance of biomaterials largely depends on the materials biocompatibility, which is directly related to unwanted side effects like foreign body response and inflammation, and the potential of interaction of cells with its surface. To avoid these problems, the material's surface properties have to be controlled. The first successful application of eADF4(C16) coatings was reported for silicone breast implants. The risk of periprosthetic fibrous capsule formation was significantly reducedin case of silk coated implants.[4]

Other common polymers for medical applications are polytetrafluoroethylene (PTFE) and polyurethane (PU). In the distinct application of catheters, low or even no cell adhesion is eligible. To influence the properties of existing and commonly used biomaterials and to further increase their biocompatibility, a thin eADF4(κ16)/ eADF4(C16) coating (d = 50 nm) was applied on PU, PTFE and silicone. The positively charged eADF4(κ16) is acting as adhesion promoter between the oxygen plasma treated polymers and negatively charged eADF4(C16) as the outer layer. The coating stability, enzymatic degradation in wound-like and digestive environments as well as cell adhesion behavior were analyzed.[5]

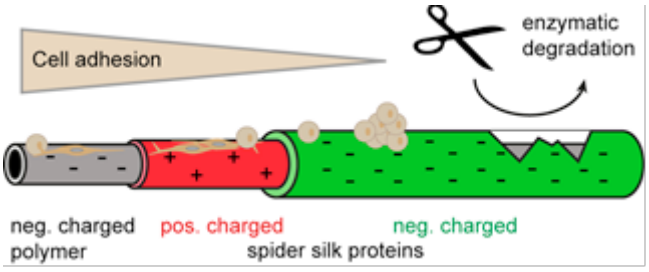

Figure 1: Stable silk protein coating with low cell adhesion properties. Reprinted with permission from Borkner et al. *ACS Biomater. Sci. Eng.* **2017**, *3*, 767-775. Copyright 2017 American Chemical Society.

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Coiled-coil peptides with improved metal-binding selectivity

Aimee L. Boyle, Niek Crone, Guto Rhys, Alexander Kros

Coiled coils capable of coordinating metals have been investigated for more than 25 years, but designs that allow for metal selectivity are rare. Some success has been achieved by exploring the influence of the position of metal-coordinating residues within the heptad repeat,[1] or by favouring a certain metal due to its binding geometry.[2]

Here, we investigate whether metal selectivity can be achieved using a hyperstable coiled-coil scaffold.[3] Such a scaffold should be well-packed and tolerant to mutations, which may allow for improved metal selectivity. This approach allowed us to explore the number of modifications necessary to effect metal-sensitivity in such a scaffold, as well as facilitating investigations into metal-binding affinity and specificity.

A variety of constructs were designed and evaluated using circular dichroism (CD) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and analytical ultracentrifugation (AUC). We found that, with this scaffold, multiple modifications were necessary to effect metal binding and, interestingly, a high degree of selectivity for certain metal ions was observed. The structural basis for this selectivity is currently under investigation and it is anticipated that an understanding of this would allow for specificity to be engineered into such scaffolds, making the peptides attractive for applications such as biosensing.

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One HAMP, two HAMP, three HAMP, more...

Murray Coles, Carolin Ewers and Andrei N. Lupas

It is now over ten years since the first structure of a HAMP domain led us to propose the gearbox model of signal transduction in bacterial transmembrane receptors. Under this model, signals generated in extracellular sensor domains are transmitted to the cytoplasm by rotation of linking helices to interconvert specific coiled-coil packing states. So how has this model stood the test of time? From that single example, the number of HAMP domain structures has grown to dozens. In keeping with the original model, this structural diversity now covers the full range of originally anticipated rotation states. But is there more? We present new structures of the poly-HAMP array from a family of proteins that contain up to 57 contiguous HAMP domains. These form tightly organized, rod-like structures underpinned by an astounding degree of sequence conservation. Further, these structures hint at a new domain of potential signaling states by rotations opposite to those previously observed. Deletion or truncation of poly-HAMP arrays in fungal proteins is associated with morphological defects and resistance to fungicides, suggesting a new role for HAMP domains in direct sensing in the cytoplasm.

Two-Dimensional Assembly of Collagen-Mimetic Peptides

Tao Jiang, Charles Modlin, and **Vincent Conticello**

Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA, USA 30322

Structurally defined materials on the nanometer length-scale have been historically the most challenging to rationally construct and the most difficult to structurally analyze. Sequence-specific biomolecules, i.e., proteins and nucleic acids, have advantages as design elements for construction of these types of nano-scale materials in that correlations can be drawn between sequence and higher order structure, potentially affording ordered assemblies in which functional properties can be controlled through the progression of structural hierarchy encoded at the molecular level. The predictable design of self-assembled structures requires precise structural control of the interfaces between peptide subunits (protomers). However, control of quaternary structure has proven to be challenging to reliably predict as conservative changes in sequence can result in significant changes in higher order, i.e., supramolecular, structure. We have employed simple self-assembling collagen-mimetic peptides as building blocks for the construction of two-dimensional nano-scale assemblies. In contrast to filamentous assemblies (e.g., fibrils, ribbons, and tubes), protein-based two-dimensional assemblies occur relatively infrequently in native biological systems. We have demonstrated that extended and structurally defined two-dimensional assemblies can be constructed through lateral association of chiral rod-like subunits such as the collagen triple helix. The resultant assemblies can exhibit sequence-dependent control of structure, including growth in the lateral and/or axial dimensions. Moreover, the sheet-like assemblies can be integrated with other self-assembled biological structural motifs, such as DNA origami nano-tiles, to afford self-organized hybrid assemblies. Despite the potential for these two-dimensional assemblies as structurally defined nano-scale scaffolds, it remains challenging to reliably predict and control the structure of the assemblies based on sequence-structure correlations at present.

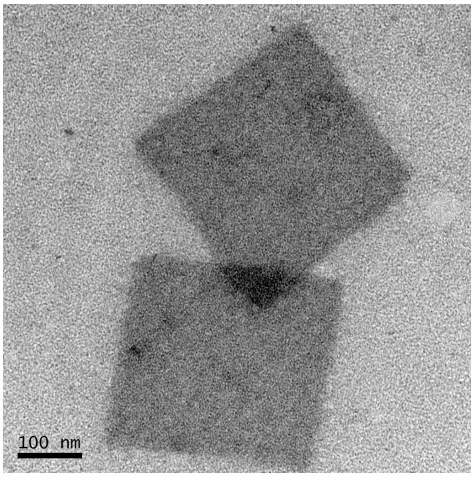

Figure 1. TEM image of two-dimensional nano-sheets derived from self-assembly of charge-complementary collagen peptide **NSIII**.

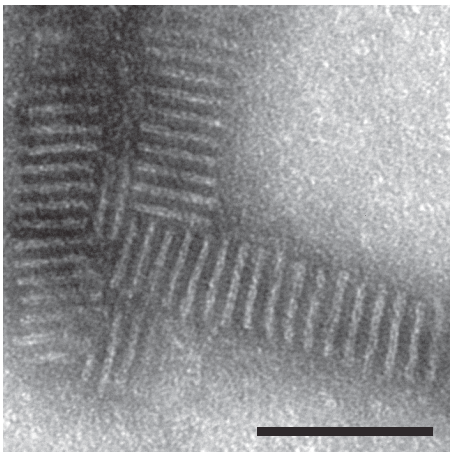

Figure 2. TEM images of nanowires derived from co-assembly of DNA origami nano-tile **TL** and collagen-mimetic peptide **CP^{++}.**

Changes in both the structure and dynamics of highly helical bacterial chemoreceptors modulate activity of their associated histidine kinase CheA

Alise Muok, Nattakan Sukomon, Dipanjan Samanta, Greg E. Merz, Anna R. Greenswag, Peter P. Borbat, Jack H. Freed and **Brian R. Crane**

Department of Chemistry and Chemical Biology, Cornell University, Ithaca NY 14853

Bacterial chemotaxis, the ability of bacteria to adapt their motion to external stimuli, has long stood as a model system for understanding transmembrane signaling, intracellular information transfer, and motility. The sensory apparatus underlying chemotaxis displays remarkably sensitivity, robustness, and dynamic range. Although the molecular components of the chemotaxis system are well characterized in *E. coli*, we still do not fully understand the biophysical mechanisms responsible for function. Investigations of the chemotaxis apparatus are challenging because the sensor comprises an extensive multi-component transmembrane assembly of chemoreceptors, histidine kinases (CheA) and coupling proteins (CheW) that exhibits cooperative structural properties. Several of the components in this assembly, including the chemoreceptors themselves, contain specialized coiled coil regions that play a critical role in propagating conformational signals. We will discuss efforts to understand the structure and dynamics of the chemoreceptor:CheA:CheW complex and how the helical chemoreceptors transmit signals across the membrane to regulate CheA activity. To address these issues, studies have been undertaken on isolated components, reconstituted complexes, and native receptor arrays. Engineered soluble chemoreceptor coiled-coils that mimic natural receptor oligomers have proven particularly useful for studying kinase activation. Evidence will be presented to support the notion that changes to both receptor conformation and dynamics are involved in signal transduction.

The effect of peptide stapling on coiled coil formation and membrane interactions

Niek Crone, Aimee L. Boyle & Alexander Kros

Stapling of peptides has arisen as a useful modification to either restrict their conformation, enhance binding affinity and cellular uptake, or prevent their proteolytic degradation.[1] Two-component stapling methods can be applied to peptides consisting of purely natural amino acids (usually lysine or cysteine residues) to enhance the α-helicity of the peptide and increase its stability to denaturing stimuli.[2] To study the folding dynamics of coiled-coil forming peptides, a series of peptides containing cysteine residues spaced *i* to *i+4* was synthesized, and modified with dibromoxylene to yield structurally isomeric stapled peptides. Circular dichroism (CD) spectrometry showed that these staples increase helicity of the peptides and stability of their coiled-coils, with the strongest effect observed when the staples were positioned close to the C terminus.

In our group, a pair of heterodimeric coiled-coil forming lipopeptides is used to induce membrane fusion between liposomes, and between liposomes and cellular membranes.[3-4] Biophysical studies have shown one of the two peptides to have strong interactions with lipid membranes, and this interaction is assumed to be crucial in the transition from docking of liposomes to fusion of the lipid membranes.[5] In order to induce fusion, it is postulated that these peptides have to switch between coiled-coil and membrane bound conformations; it is likely that the transition between these involves a partially-unfolded peptide. Lipidated versions of these stapled peptides will be tested in this fusion system to determine whether stapling affects fusion.

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W. F. DeGrado
Dept. of Pharmaceutical Chemistry
University of California, San Francisco
555 Mission Bay Blvd. South
CVRI-MC Box 3122
San Francisco, CA 94158-9001, U.S.A.

De novo design of proteins.

De novo protein design, in which one designs proteins beginning from first principles, provides an approach that critically tests our understanding of protein folding and function, while also laying the groundwork for the design of proteins and biomimetic polymers. The de novo design of metalloproteins has proven to be a useful approach for understanding the features in a protein sequence that causes them to fold into their unique three-dimensional structures that bind and tune the properties of their bound cofactors. This talk will focus on the design of proteins that bind porphyrins, dimetal ion cofactors including di-Zn(II), di-Fe(II/III) and di-Mn(II/III) and tetranuclear centers. This talk will focus on principles of protein design and the application to the design of water-soluble proteins that stabilize the formation of organic radicals as well as membrane proteins that function as Zn(II)/proton antiporters.

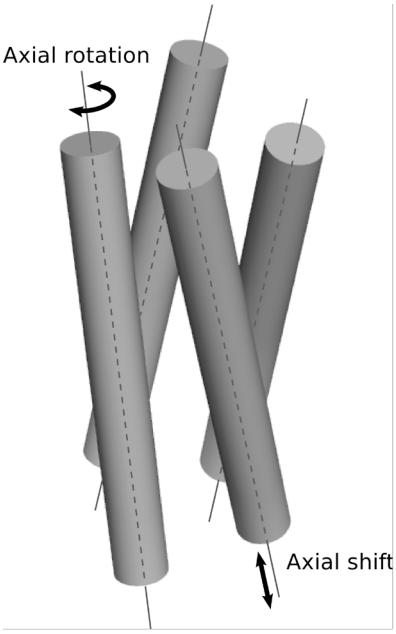
Fine-tuning of coiled coil design

Krzysztof Szczepaniak[1], Jan Ludwiczak[1] and Stanisław Dunin-Horkawicz[1]

[1] Structural Bioinformatics Laboratory, Centre of New Technologies, University of Warsaw, 02-097 Warsaw, Poland

Protein design is a procedure that aims at predicting sequences that will fold to a given backbone structure (target structure). Recent years have shown that computational design techniques can be used to create new coiled coils with well-defined topology. However, experimentally determined structures of designed coiled coils do not always perfectly match the assumed target structures. This raises the question whether these small discrepancies can be avoided or are they simply artefacts of structure determination or measurement procedures.

To address this question, we have investigated the sequence determinants of the hydrophobic core architecture in coiled coils. In a previous study, we have shown that the hydrophobic core in coiled-coil structures can adopt a continuum of configurations, corresponding to the various degrees of rotation of the helices around their axes [1] (see Figure on the right side). In a subsequent work, we identified sequence features determining the helix axial rotation in homo-oligomeric anti-parallel structures and showed that these features can be used to predict hydrophobic core architecture directly from the sequence [2]. Recently, we analyzed another class of structures, namely homo-oligomeric parallel coiled coils, which show little variation in the architecture of the hydrophobic core. To our surprise, we found that also in this class the variation in the axial helix rotation, despite being as small as few degrees, is not an artefact of structure determination or measurement and can be associated with sequence features.



The proposed approach allowing to associate subtle structural changes to sequence features can be used to interpret effects of mutations in a model coiled-coil structure GCN4. We trust that it will be also applicable to tasks such as re-design and *de novo* design of coiled-coil proteins.

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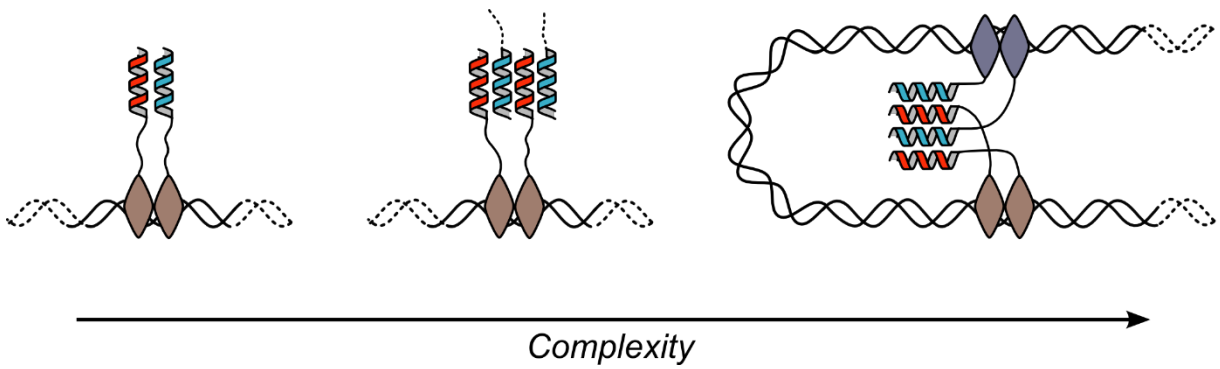
Title: *De novo* designed coiled coils as programmable protein-protein interaction domains in novel transcriptional regulators

Authors: Caitlin Edgell, Abigail Smith, Nigel Savery and Dek Woolfson (University of Bristol)

Abstract: In synthetic biology, genes and their regulatory elements can be combined to make synthetic genetic circuits. When implemented in organisms, these circuits can achieve fine control over the behaviour of that organism. Transcription factors, the proteins that regulate gene expression, are a key component in genetic circuit design. While there are plenty of natural transcription factors, it is also useful to design novel transcription factors with more-desirable and programmable characteristics.

In their simplest form, both activating and repressing transcription factors must interact site-specifically with DNA as well as with other proteins. This is achieved via DNA-binding domains and protein-protein interaction domains, respectively. By combining designed proteins that fulfil these functions, artificial transcription factors can be made. Coiled coils make excellent candidates for the protein-protein interaction domains because their properties can be readily programmed. Furthermore, many natural transcription factors contain coiled coil domains that perform the protein-protein interaction role.

We have implemented *de novo* designed homo- and heteromeric coiled coils of various oligomeric states in transcriptional regulation systems in *E. coli*. These coiled coils robustly fulfil their role as protein-protein interaction domains as well as retaining their designed properties in the complex cellular environment.



Coiled coil-based transcriptional regulators using dimers (left) and tetramers (centre and right) fused to DNA-binding domains.

Robert Edwards, Duke University

1600 Å of coiled-coil at 4.3-Å resolution: the skips and bends of a myosin rod atomic model.

One of the first coiled coil proteins recognized was the muscle protein myosin, which is the motor protein that drives contraction. Myosin is a parallel dimer, and can be divided into two globular "heads" and a long coiled-coil "tail," called the rod domain. The heads contain the motor activity, and can hydrolyze ATP and move actin filaments even when cleaved free of the rod. The coiled-coil rod is very long, ~1600 Å, and contains ~1082 amino acids. The amino acid sequence exhibits the classic coiled-coil heptad repeat, 154 of them, broken only by four skip residues, which are very highly conserved across all species.

To get isolated myosin dimers requires harsh conditions; and in anything near physiological, myosin will polymerize into large filaments, so it's debatable whether myosin really is a dimer, or should more properly be considered a polymer. In the filaments, the rods pack together to form the filament backbone, while the heads project from the surface of the filament. In vivo, the specific geometry of myosin filaments is precisely maintained; however, the exact geometry varies among species. They all share in common a 145-Å axial repeat, at which groups of heads project from the filament backbone. This group of projecting heads is called a "crown" and this is where filaments differ across species, with each species having a specific number of myosin heads projecting from the crown. This gives each type of filament a specific rotational symmetry. Filaments also vary in length across species. In our favorite muscle, the flight muscles of the giant waterbug, the bipolar myosin filaments have 4-fold rotational symmetry, and are about 2.6 μm long, and thus contain ~670 myosin molecules packed into one micro-machine.

We present here a 3D cryo-EM structure of the myosin filament from the giant waterbug, *Lethocerus indicus*. We did this work with two questions in mind, how are the heads docked on the surface of the filament, to be ready to go to work when needed, but resting quietly when not, and how do the rods pack together to form the filament backbone. Resolution within the backbone was especially good and allows us to visualize the entire 1600-Å long rod domain at a resolution of 4-5 Å. We now have an atomic model of the entire myosin molecule, which I'll present and discuss.

Exactly how the rods pack together was previously unknown and the subject of considerable speculation. Our structure clearly shows the rods are packed side-by-side into layers, very similar to an arrangement proposed over 40 years ago, but never before substantiated. Twelve of these layers pack together around a cylindrical core to form the filament backbone. Adjacent layers are staggered by 145 Å, but within each layer the adjacent molecules are axially staggered by 3 x 145 = 435 Å. Thus the structure repeats itself every third crown, and the 12 layers give the filament 4-fold rotational symmetry.

The rod is generally straight, but when considered closely, the actual path of the rod is surprisingly contorted and meanders from side to side circumferentially, and in and out radially, as the rod passes from N-terminus at the filament surface to C-terminus at the filament core. Visually, the filament backbone appears surprisingly open, with subtantial space between rods, but when analyzed, there seems to be an expected amount of contact between rods within each layer. However, there is less contact between layers. Surprisingly, our structure also shows 4-5 non-myosin densities snaking their way among the forest of rods, identity not yet known.

Although the heads are not coiled-coils, they are still an interesting and important part of the structure. As you will see, you can't really discuss one without the other. At this point, our atomic model is a work in progress, which we are still validating and evaluating, so we'll welcome your feedback. But one important conclusion is that myosin is not at all symmetric, despite being a dimer.

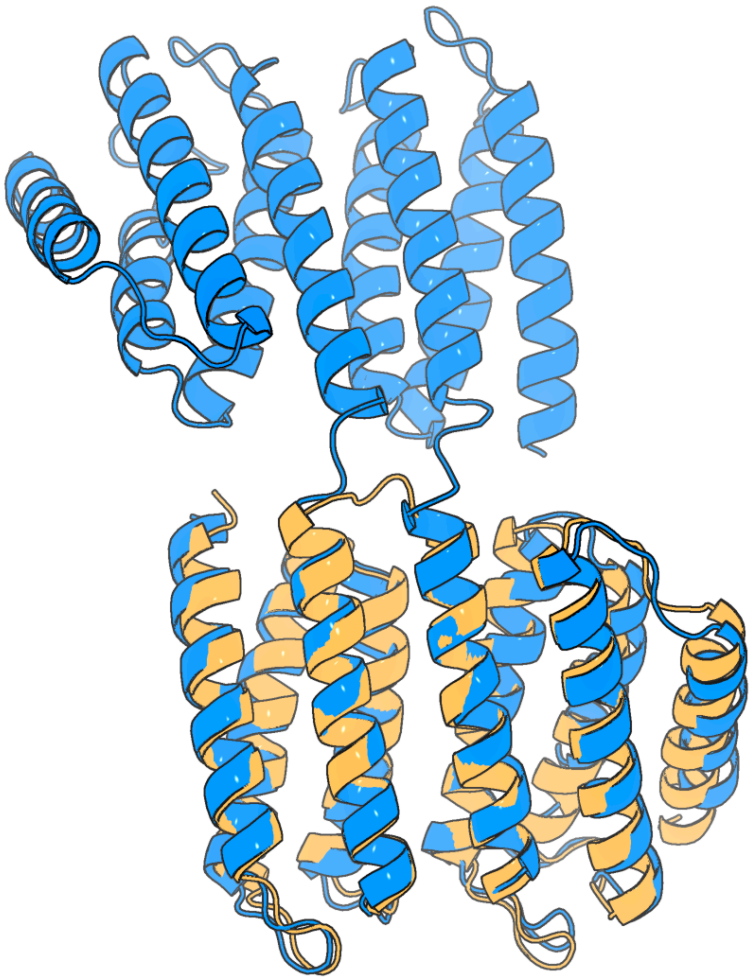
Computational Design of a Novel Repeat Protein Fold

Mohammad ElGamacy, Murray Coles, Hongbo Zhu, Markus Hartmann, Andrei Lupas.

Max Planck Institute for Developmental Biology, Spemannstraße 35, Tübingen 72076, Germany;

mohammad.elgamacy@tuebingen.mpg.de

Assembly of novel proteins from smaller existing pieces can provide an efficient means for protein design and evolution. We present a generalisable approach where robust protein modules can be used as building blocks for the construction of novel topologies. The two-stage-strategy starts by design of the intramolecular interface between arbitrarily posed starting blocks, followed by loop design across the interface. We applied this to construct a repeat protein out of an up-down four-helix-bundle. Topologically, it can be viewed as a back-to-back array of oppositely handed hairpins. Such an architecture offers an open-ended corrugated repeat devoid of net writhe, which has no natural counterpart. The first tested design displayed pH-dependent dimerisation, as it transitions between a domain-swapped dimer at neutral pH and a closed monomer at basic pH. Crystallographic data of the dimeric form showed the interface to agree with the design to 2.1 Å all-atom RMSD, while NMR data collected so far support the preservation of the same interface features in the monomeric form.



Design of a corrugated repeat topology. The designed monomeric form is shown in brown, while the crystal structure of the dimeric form in blue.

Rigid fusions of DARPins based on shared helix design to facilitate crystallography and enable functional studies

Patrick Ernst, Yufan Wu, Alexander Batyuk, Christina Ewald, Peer R. E. Mittl, Annemarie Honegger and Andreas Plückthun

Department of Biochemistry, University of Zurich, Zurich, Switzerland

p.ernst@bioc.uzh.ch

The demand for binding proteins which recognize specific targets and epitopes is increasing in both research and diagnostics. Although they are widely used, classical binding proteins like monoclonal antibodies are facing validation problems and are rather expensive to produce. Novel binding scaffolds have the potential to overcome these problems. In our group we developed Designed Ankyrin Repeat Proteins (DARPins) which are highly stable and can be selected in a high-throughput manner to bind almost any target molecule.

Over the past years we have selected DARPins against more than 400 different target proteins, but the crystallographic validation of these complexes is a major bottleneck. Currently, only 40 DARPin complex structures are deposited in the PDB, and despite their favorable crystallization properties, each complex is still a significant project. While the rigidity of DARPins favors crystal contacts, their small size leaving only a small number of potential crystallization contacts, again limits the success rate.

Here we present a novel design approach which links two DARPins via a rigid helix fusion to increase the size of our target binding DARPins and provide more possibilities for forming crystal contacts. These fusions span a wide range of possible geometries and can be linked in a very modular way. They have been successful in obtaining new crystal structures of complexes which previously failed to crystallize. Some examples will be presented.

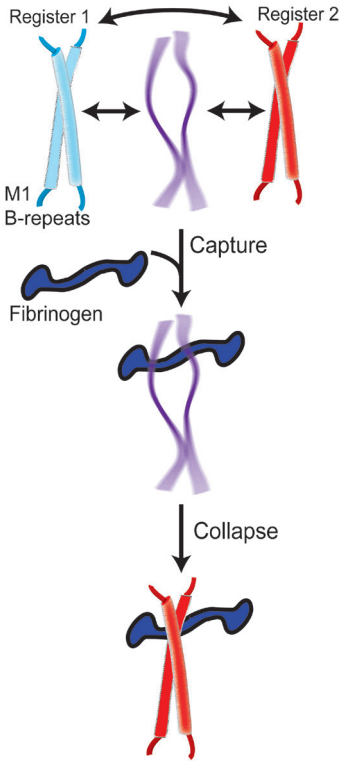
Besides their application as crystallization chaperones, these rigid fusions can also serve as templates for conformational sensors. As an example we present data proving the active-only conformation of a receptor tyrosine kinase linked to about 30 % of breast cancers, thereby shedding light onto mechanistic constraints of receptor activity.

In a last part we expand the concept of rigid helical fusions to designed Armadillo Repeat Proteins (dArmRPs), which are modular peptide binders. By constructing a DARPin-dArmRP-DARPin fusion we were able to obtain the first structure showing full modularity over six consecutive repeats which is not influenced by crystal contacts. Due to its generic design, this construct can now also be used as basis for a crystallization platform to study new pocket designs binding to various new peptides.

Functional essential instability in the M protein coiled coil

Partho Ghosh
University of California, San Diego

The M protein is the antigenically variable, major surface-associated virulence factor of the Gram-positive bacterial pathogen *Streptococcus pyogenes* (or group A *Streptococcus*, GAS). GAS is responsible for mucosal infections, acute invasive diseases, and autoimmune sequelae; the M protein has a significant role in the entire range of these conditions. Throughout nearly its entire ~400 amino acid-length, the M protein is composed of a dimeric, parallel α-helical coiled coil. Puzzlingly, the coiled-coil sequence of the M protein is highly noncanonical and contains destabilizing amino acids, which results in instability at physiological temperature and structural irregularities.



The significance of the noncanonical sequence in the M protein has been revealed by studies of the B-repeats portion of the M1 protein. The B-repeats confer binding to fibrinogen (Fg), an interaction essential to GAS for evasion of phagocytosis. We have shown that M1 B-repeats are conformationally dynamic and interconvert between two alternate coiled-coil registers, which occupy adjacent helical faces. One of the registers (register 1) is incompatible with Fg-binding, while the other (register 2) is compatible. Additionally, the B-repeats have a dissociated and most likely natively unfolded state. The M1 B-repeats were mutated such that they contained amino acids ideal for forming a coiled coil entirely in either register 1 or 2, creating two versions of the M1 protein. Both register 1- and 2-idealized versions of the M1 protein were more stable than wild-type M1 protein. Unsurprisingly, stabilization in the Fg-nonbinding register 1 resulted in a marked loss of Fg-binding. However, surprisingly, stabilization in the Fg-binding register 2 also resulted in a marked loss of Fg-binding. A key observation that explains this counterintuitive result is that Fg-binding was restored to the register 1- and 2-idealized M1 proteins by chaotropic destabilization. This result indicates that instability and dynamics in the B-repeats are essential to Fg-binding. A 'capture-and-collapse' mechanism accounts for this behavior. In this mechanism, a dynamic and intrinsically disordered conformation of the M1 B-repeats is responsible for initially capturing Fg. This dynamic conformation then collapses into a register-2 coiled coil, due to stabilization provided by Fg-binding energy. Thus, the amino acids that destabilize the coiled coil but do not directly contact Fg are as important for Fg-binding as those that directly contact Fg.

We have identified that the M1 B-repeats confer numerous additional functions, including binding LL-37 (an antimicrobial peptide), histones (which have antibacterial activity), and blood group antigens. The multiplicity of functions in the M1 B-repeats would seem to demand a multiplicity of conformations, as created by a noncanonical coiled-coil sequence, rather than the single and regular structure offered by a canonical coiled-coil sequence. The multiplicity of conformations may also serve a masking function in creating a moving target for immune recognition.

Molecular Mechanisms of Transmembrane Signaling by TCS Sensors

Gushchin I.[1,,2,,3], A. Ishchenko[2,4], I. Melnikov[5], V.V. Borshchevskiy[3], V. Polovinkin[1,2,] A. Popov[5]. Engelhard[6], G. Bueldt[3] and V. Gordeliy[1,2,3]

[1]Institut de Biologie Structurale J.-P. Ebel, Université Grenoble Alpes-CEA-CNRS, 38044 Grenoble
[2]Institute of Complex Systems (ICS), ICS-6: Structural Biochemistry, Research Centre Jülich, 52425 Jülich
[3]Moscow Institute of Physics and Technology, 141700 Dolgoprudniy
[4]The Bridge Institute, University of Southern California, Los Angeles
[5]European Synchrotron Radiation Facility, F-38043 Grenoble
[6]Max-Planck Institute of Molecular Physiology, 44227 Dortmund

Abstract: One of the major and essential classes of transmembrane (TM) receptors, present in all domains of life, are sensors of two-component signaling systems (TCS). The structural mechanisms of transmembrane signaling by these sensors are poorly understood.
We will present our recent work on histidine kinases (HKs)[1], parts of TCS. Crystal structures of the periplasmic sensor domain, the TM domain and the cytoplasmic HAMP domain of the *Escherichia coli* nitrate/nitrite sensor HK NarQ in the ligand-bound and mutated ligand-free states will be described. The structures reveal that binding of ligand induces significant rearrangement and piston-like shifts of TM helices. The HAMP domain protomers undergo lever-like motions and convert the piston-like motions into helical rotations. Our findings provide the structural framework for complete understanding of TM TCS signaling and for development of antimicrobial treatments targeting TCS.
In addition we will discuss our X-ray crystallographic work on the complex of sensory rhodopsin II with its cognate transducer and will show in molecular details how the signal propagates within membrane part of the TCS sensor[2,3,4.]

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Tertiary Alphabet for the Observable Structural Universe

Gevorg Grigoryan, Departments of Computer Science, Biological Sciences, and Chemistry, Dartmouth College

Understanding how amino-acid sequence encodes protein structure is a grand challenge of modern biophysics. Chief among the difficulties of describing this sequence-structure mapping is that the space of structural possibilities is immense and complex. We propose that this space should nevertheless be describable as a combination of discrete local structural patterns. We introduce the concept of a TERM (tertiary motif), which encapsulates the full structural environment around a given residue, and show that the protein structural universe is highly degenerate at the level of TERMs. In fact, only 650 TERMs describe over 50% of the structural database at sub-Angstrom resolution. We go on to show that such degeneracy enables the direct quantification of sequence-structure relationships. Local sequence models can be extracted for each TERM contained in a protein structure, based on the many occurrences of the TERM in unrelated proteins, with the overall protein structure described as a combination of these models. We have begun to demonstrate the broad applicability of such a framework: 1) protein design: we have fully redesigned (and validated) protein surfaces using TERM data alone; 2) structure prediction: we found that TERM-based sequence statistics identify accurate models; 3) we have shown that mutational stability changes are predicted quantitatively from TERM data alone. Earlier findings of degeneracies in the protein structure (e.g., for secondary and super-secondary motifs), have greatly advanced computational structural biology. TERM-based mining of structural data is the next logical step that should provide further quantitative insights into sequence-structural relationships.

Ion transport across the biological membrane by computational protein design

Gevorg Grigoryan, Departments of Computer Science, Biological Sciences, and Chemistry, Dartmouth College

The cellular membrane is impermeable to most of the chemicals the cell needs to take in or discard to survive. Therefore, transporters—a class of transmembrane proteins tasked with shuttling cargo chemicals in and out of the cell—are essential to all cellular life. From existing crystal structures, we know transporters to be complex machines, exquisitely tuned for specificity and controllability. But how could membrane-bound life have evolved if it needed such complex machines to exist first? To shed light onto this question, we considered the task of designing a transporter *de novo*. As our guiding principle, we took the "alternating-access model"—a conceptual mechanism stating that transporters work by rocking between two conformations, each exposing the cargo-binding site to either the intra- or the extra-cellular environment. A computational design framework was developed to encode an anti-parallel four-helix bundle that rocked between two alternative states to orchestrate the movement of Zn(II) ions across the membrane. The ensemble nature of both states was accounted for using a free energy-based approach, and sequences were chosen based on predicted formation of the targeted topology in the membrane and bi-stability. A single sequence was prepared experimentally and shown to function as a Zn(II) transporter in lipid vesicles. Further, transport was specific to Zn(II) ions and several control peptides supported the underlying design principles. This included a mutant designed to retain all properties but with reduced rocking, which showed greatly depressed transport ability. These results suggest that early transporters could have evolved in the context of simple topologies, to be later tuned by evolution for improved properties and controllability. Our study also serves as an important advance in computational protein design, showing the feasibility of designing functional membrane proteins and of tuning conformational landscapes for desired function.

CCFold: rapid and accurate prediction of coiled-coil structures

Dmytro Guzenko, Sergei V. Strelkov

Laboratory for Biocrystallography, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Belgium, Dmytro.Guzenko@kuleuven.be

Traditional methods to predict coiled-coil domains and model their structure rely on the presence of a heptad repeat pattern as the main criterion. More recently, additional patterns such as hendecads, quindecads, *etc.* were determined experimentally to be consistent with a continuous coiled coil. Sequences of many naturally occurring coiled coils contain intermixed patterns with sometimes ambiguous assignment of core positions. In such cases, accurate *de novo* structural prediction remains a challenging task. Here we present CCFold, a threading-based algorithm which produces coiled-coil models from protein sequence on input. The algorithm is based on a statistical analysis of experimentally determined structures annotated in the CC+ database and can naturally handle any coiled-coil geometry. We show that CCFold outperforms general-purpose computational folding in terms of accuracy, while being faster by orders of magnitude. This data-driven method makes structural modelling of long coiled-coil domains, such as those found in the intermediate filament dimer and myosin rod, accessible to a wide audience of structural biologists.

Self-assembly and molecular recognition of the collagen triple helix

Jeffrey D. Hartgerink

Rice University Houston, Texas, USA
jdh@rice.edu 1-713-348-4142

Collagen and collagen-like proteins are a ubiquitous family of protein found in viruses, bacteria, fungus and all animals. In humans, collagen accounts for approximately 30% of all protein by mass. Unifying this protein family is its unusual primary amino acid sequence which requires glycine in every third position. Proteins adhering to this primary sequence can self-assemble into the characteristic collagen triple helix. Despite the structure of the triple helix being known for many decades, design rules for the triple helix have developed slowly and have only recently allowed for the assembly of well controlled heterotrimers. Critical for sophisticated helix design are well understood pairwise interactions that allow for stabilization or destabilization to achieve selective self-assembly of triple helices. Our group has used "axial charged pairs" in the collagen triple helix to design selective heterotrimeric helices, non-canonical triple helices with "sticky-ends", and triple helical fibers hundreds of nanometers in length. These designs will continue to grow in their sophistication as additional pairwise interactions are uncovered. A second result of a broader understanding of pairwise interactions in the collagen triple helix is the potential to understand the stability of specific regions of natural collagen helices. Natural collagen has a notably low thermal stability and it is possible to take advantage of this instability to design peptides which will bind specifically to natural collagen. These Collagen Targeting Peptides (CTPs) replace naturally unstable regions of collagen with designed systems which maximize stabilizing interactions. In principle this approach will allow for a variety of applications ranging from selective identification of collagens in tissue to anti-bacterial, anti-fungal and anti-viral therapies. Progress towards these goals will be discussed.

Towards engineering of light-responsive fibers for biomaterial sciences

Julia Hennemann[1], Martin Humenik[2] and Andreas Möglich[1]

[1]Lehrstuhl für sensorische Photorezeptoren, Biochemie II, Universität Bayreuth, Germany
[2] Lehrstuhl Biomaterialien, Fakultät für Ingenieurwissenschaften, Universität Bayreuth, Germany

Light-oxygen-voltage (LOV) proteins serve as blue-light photoreceptors for various physiological adaptations. LOV photosensor domains are small with terminal α helices and a central β-sheet and bind a flavin nucleotide chromophore. Upon illumination by blue light, the flavin cofactor forms a covalent thioether bond with a conserved cysteine residue in the LOV domain, leading to conformational changes within the β-sheet. Those initial light-induced changes are propagated and allosterically coupled to associated effector modules. In particular, signal transduction in several LOV domains goes along with a change in oligomeric state, whereas the association can be either light-promoted or light-repressed. In particular, isolated LOV domains from diatom and algal aureochromes (*Nannochloropsis gaditana* (*Ng*aur) and *Phaeodactylum tricornutum* (*Pt*aur)) undergo light-induced dimerization reactions. This light-promoted reaction has been successfully used in the engineering of allosterically regulated proteins allowing their precise spatiotemporal, non-invasive and reversible control by light. As many biomolecules used in biomaterial sciences associate into higher-order assemblies as part of their function, light is an excellent trigger to control those association reactions. Since the functional properties and assembly of spider silk protein eADF3$(AQ)_{12}$-NR3 depend on dimerization of its N-terminal domain NRN1 initiated by chemical triggers, we propose to control its dimerization upon blue light exposure via an N-terminally located homodimeric LOV domain. If embedded into a fiber, one may modify the number of crosslinks by light and thus alter the mechanical properties such as shear module and stiffness. Moreover, we aim to generate recombinant eADF4(C16)-LOV to establish light-responsive films, capsules, beads, or hydrogels influencing assembly and properties, such as pore sizes and mechanical behavior in a light dependent manner.

α/β-coiled coils

Birte Hernandez Alvarez

Max Planck Institute for Developmental Biology, Tübingen, Germany

Canonical coiled coils are of a highly repetitive and regular structure that is based on the heptad repeat pattern conserved in the amino acid sequence of the single α-helices. Naturally occurring coiled coils often deviate from ideal coils showing unusual periodicities. Known discontinuities that can be accommodated within coiled coil structure include single or successive insertions of one (skip), three (stammer) or four (stutter) additional amino acid residues in the regular heptad pattern. Completing the picture of possible periodicities, we identified and structurally characterized trimeric coiled coil segments showing insertions of six residues (7+6, hexads) and two (7+2, nonads) residues. We find that in either case, the change in periodicity caused by such insertions puts a great strain on the single chains causing a break of coiled coil structure. The α-helices continue as short β-strands which cross each other and form a new triangular shaped secondary structure element, named β-layer. Using GCN4-N16V peptide as a model system, we show that β-layers can be brought about simply by insertion of hexads and nonads in the heptad pattern of a coiled coil. Beside their occurrence as single elements, we find β-layers also in a repetitive arrangement within coiled coil segments of proteins. The crystal structure of such a coiled coil that is mainly composed of alternating α/β elements made from 14 consecutive, almost perfectly conserved nonads shows a new type of protein fiber, referred to as α/β-coiled coil. Searching for a minimalistic α/β-coiled coil built from hexads, we solved the structure of a protein fragment showing three successive β-layers in hexad spacing. Furthermore, we identified conserved sequence motifs for β-layers in different protein families. We find β-layers to be embedded in coiled coils, where they contribute to coiled coil stability, but also to be localized at the N-terminus or C-terminus of coiled coils facilitating the transition from α-helical to β–stranded domains.

Hartmann, M.D., Mendler, C.T., Bassler, J., Karamichali, I., Ridderbusch, O., Lupas A.N., Hernandez Alvarez, B. α/β coiled coils. Elife. **2016**, 15, 5.

Building novel molecular platforms from design principles

Possu Huang
Department of Bioengineering
Stanford University

Research in building *de novo* proteins have elucidated structural principles and engineering insights, leading to efforts in the development of functional molecular platforms for various protein-based biotechnology. I will highlight recent advances in structure-based computational protein design: how we finally succeeded in designing alpha/beta and all-beta barrels, and the principles these model systems have revealed. With our improved ability to custom make protein structures, however, new challenges emerge in functionalizing protein scaffolds for biomedical applications. I will present the idea of designing a general purpose biosensor platform using the methods and novel proteins we have developed in recent years. We hope to engineer new biology-inspired protein platforms that are versatile in their application.

De novo and data driven design in Rosetta

Possu Huang[1], Gustav Oberdorfer[2], Fabio Parmeggiani[3]
[1] Stanford University
[2] University of Graz
[3] University of Bristol

The Rosetta molecular modeling suite[1] is a powerful and versatile software for protein design.
In this presentation we will illustrate some examples of the key protocols for *de novo* design of helical bundles[2] and repeat proteins[3] and how to integrate available information (e.g. coevolution data, structural templates, density maps) in design and structure prediction[4,5].

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Self-Assembly of HEAT-Repeat Modules into Nanotubes

Spencer A. Hughes[1], Edward H. Egelman[2], Joseph S. Wall[3], Xiaobing Zuo[4], Vincent P. Conticello[1]

[1]Department of Chemistry, Emory University, Atlanta, GA 30322, United States.
[2]Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA 22908, United States.
[3]Brookhaven National Laboratory, Upton, NY 11973, United States.
[4]X-ray Science Division, Argonne National Laboratory, Argonne, IL 60439, United States.

Tandem repeat proteins represent potentially useful structural motifs for the construction of synthetic biomimetic assemblies. Using a thermophilic HEAT-repeat protein as a design element, we have produced thermostable and pH-stable nanotubes from self-assembly of a single repeat motif. The constituent peptide, **HEAT-R1**, corresponded to a modified consensus repeat, which was prepared via solid phase peptide synthesis. Self-assembly of HEAT-R1 afforded high aspect-ratio helical filaments of approximately 8.8 nm in outer diameter. Synchrotron SAXS and STEM mass per length measurements were consistent with a hollow cylindrical structure comprising approximately 21 subunits within an axial repeat of 30.8 Å, which corresponded closely to the putative height of a single helix-turn-helix motif associated with the HEAT repeat unit. The hypervariable nature of the inner lumen represents a semi-shielded surface that can be potentially functionalized. Electron cryo-microscopy and subsequent helical reconstruction afforded evidence for a tubular structure in which the asymmetric unit was a dimer of HEAT repeat motifs. In order to promote the formation of more regular structures, concatemers of six and eight HEAT repeat motifs were bacterially expressed, purified, and tested in terms of self-assembly behavior. In the absence of N- and C-terminal capping motifs, the HEAT repeat concatemers self-assembled into closed helical assemblies having roughly the same lateral dimension as those of the single repeat constructs based on negative-stain TEM and SAXS measurements.

Coiled-coils as molecular motors: multistable polymer engines

Marcus Jahnel

Long coiled-coil regions ($L > 30$ nm) are surprisingly often found in proteins that can generate and sense forces. Thus, extended coiled-coil proteins take part in some of the most intricate cellular processes: from vesicle tethering to the transport of cargo, from the maintenance of chromosome structure to the attachment of the kinetochore. Yet how can these slender molecules achieve this in an environment where thermal fluctuations are an essential determinant of any movement?

Recently, we found* that the unusually long membrane tethering protein early endosome antigen 1 (EEA1) — a 220 nm long coiled-coil — is rather rigid and extended when only tethered at its base, yet becomes more flexible upon binding of the small GTPase Rab5 to its free end. Importantly, using a reconstituted tethering system and single-molecule optical tweezer experiments, we demonstrated that this increased flexibility results in an entropic force that can pull two objects together. Moreover, we showed that GTP-hydrolysis acts as a timer for this interaction and that strategic mutations to the coiled-coil can interfere with this effect.

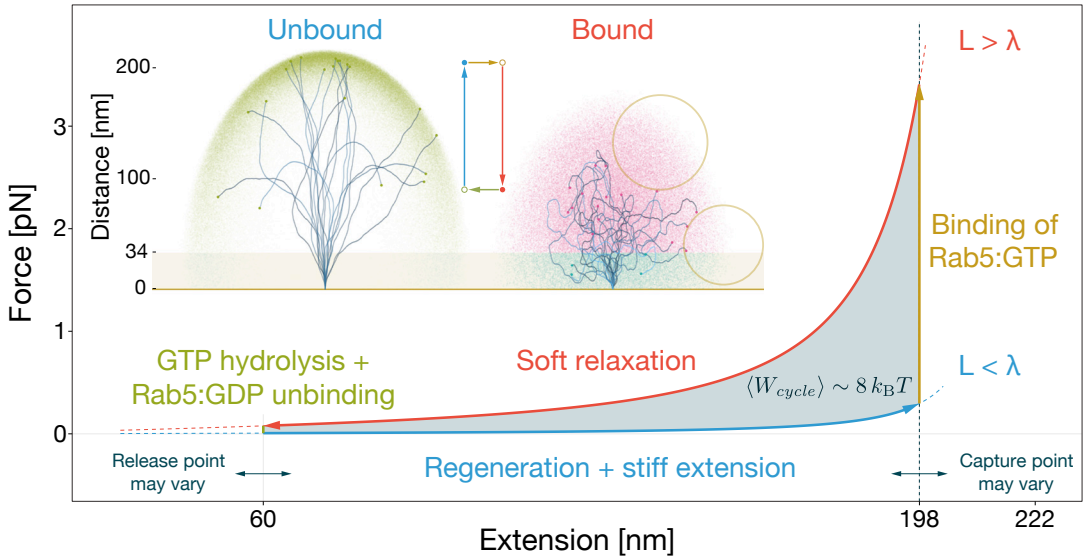


Figure 1: *EEA1 may cycle between more rigid (blue) and more flexible (red) states as a Rab5-dependent molecular motor to capture vesicles from afar and position them closer to the base membrane.*

In modelling our experiments I propose that the coiled-coil protein EEA1 and the signalling molecule Rab5 work together as a two-component molecular motor. In this framework transient NTP-dependent ligand binding events switch a long coiled-coil protein from rigid to flexible (binding) and back (unbinding), while thermal fluctuations drive the slender molecule towards the respective equilibrium conformations (Fig. 1). Modulating how susceptible a biopolymer is to ever present thermal fluctuations could provide an effective mechanism to generate and sense forces on the jiggling molecular level.

* *Murray, D. and Jahnel, M. et al.*, An endosomal tether undergoes an entropic collapse to bring vesicles together. **Nature (2016)**

Fibrous silk with coiled coil superstructure produced by the larvae of hornets and its application to useful materials

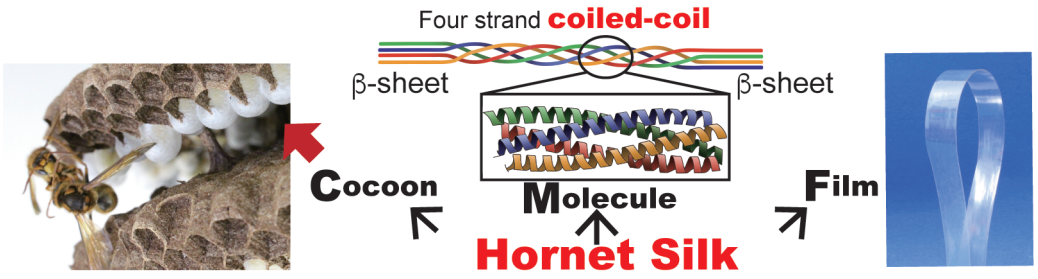
Tsunenori KAMEDA and Taiyo YOSHIOKA

National Agriculture and Food Research Organization (NARO)
Tsukuba, JAPAN

Almost all the silk used in textiles and other industrial materials is obtained from lepidopterans, such as the domestic silkworm (*Bombyx mori*). The silk proteins produced by *Bombyx mori*, are commonly known to have highly repetitive sequences of amino acid units. The dominant crystalline regions in the silk fibers of them consist of the β-sheet structure, so as the silk produced by spiders (*Aranae*). Beside *Bombyx mori*, many holometabolous insects produce a silken cocoon in the late larval stage for protection during pupal metamorphosis. The silk proteins of *Hymenopterans* such as honeybees, bumble bees, bulldog ants, weaver ants, and hornets, are known to show quite different features in their amino acid sequences.[1)] The silk produced by hornet's larvae (**Hornet Silk**) was found to comprise four major proteins and the amino acid sequences were already completely identified by our group.[2)] Each silk protein contains alanine (Ala)-rich domains with a repetition of a heptad sequence motif. Such heptad amino acid sequences tend to form a **coiled coil** structure.[2)3)] Beside X-ray scattering at wide and small angles, we applied cryo-electron diffraction and microscopy to demonstrate the presence of coiled coil structure in silk of the hornet Vespa, and found that α-helical coiled coil and β-sheet complexes are essential structural building elements of hornetsilk proteins.[4)] Our studies on the assembly of the fibrous silk proteins and their internal organization in relation to the primary chain structure suggest a 172 Å pitch supercoil consisting of four intertwined alanine-rich α-helical strands. Supercoils were found to be associated with β-crystals, predominantly localized in the L-serine-rich chain sequences terminating each of the four predominant silk proteins.

We also developed techniques to regenerate the silk in aqueous solution.[5 - 7)] Moreover, they could successfully fabricate transparent and drawn gel films with tensile strengths of up to 170 MPa, and a modulus of 5.5 GPa, higher than those of films prepared from regenerated silkworm silk.[8)] The dominant folding regime was found to be changing from coiled coil (supercoiled) α-helices into β-sheet conformations when mechanically stretched gel film.[8)]

In this study, we examine the detailed structural changes occurring in the coiled coil of stretching hornet silk gel film using wide- and small-angle X-ray scatterings and polarized Fourier transform infrared spectroscopy. On the basis of the time-resolved simultaneous synchrotron X-ray scattering observations monitoring *in situ* the structural changes of regenerated silk material during tensile deformation, it has been successfully shown that the application of tensile force under appropriate conditions induces a transition from the supercoiled α-helices to a *cross*-β-sheet (β) structure keeping unchanged the intertwined character of the four-stranded tertiary superstructure. It has also been clarified that the amorphous protein chains in the regenerated silk material were transformed into the *parallel*-β-sheet (//β) arrangements with varying orientation.



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MempromCC: A new family of membrane-attached coiled coils in prokaryotes and mitochondria

Ioanna Karamichali, Jyoti Adlakha , Marcus D. Hartmann, Birte Hernandez Alvarez, Andrei N. Lupas

Department of Protein Evolution, MaxPlanck Institutefor Developmental Biology, Tübingen D-72076, Germany. Ioanna.karamichali@tuebingen.mpg.de

We have recently discovered a hitherto uncharacterized group of proteins consisting of a helical head, one or multiple β-layer necks, a coiled-coil stalk and a trans-membrane anchor (HNSA architecture). We have named it MempromCC, for **mem**brane-attached **c**oiled-**c**oil proteins of **pro**karyotes and **m**itochondria. While many known protein families have similar architectures, the β-layer neck found between the head and the coiled coil is a unique and persistent characteristic of this group. Bioinformatic analysis of protein sequences with a predicted HNSA architecture showed that, even though the neck appears to be strikingly similar in most sequences and the helical head is conserved in many organisms, MempromCC proteins display great variation in size, sequence and structure. This is particularly conspicuous in the coiled-coil stalks, which show a range of periodicities, lengths, and numbers of β-layers. This variability and lack of convincing sequence similarity across all MempromCC proteins suggest that the group may also include some analogous proteins. However, a core subgroup of proteins from proteobacteria and mitochondria, defined by conserved head sequence motifs, clearly forms a homologous family. Although these proteins have not been functionally characterized and are mostly annotated as hypothetical, two of them, named MCUR1 (or CCDC90A) and Rat1, have been associated with the assembly of membrane complexes in organelles.

Structural analysis and nanomanipulation of fibrous proteins with high-resolution aqueous-phase atomic force microscopy

Miklós Kellermayer
kellermayer.miklos@med.semmelweis-univ.hu
Department of Biophysics and Radiation Biology, Semmelweis University, Budapest
Hungary

Fibrous proteins play a role in a myriad of biological phenomena ranging from the formation of dynamic support templates for cells and subcellular components to cellular motion, muscle contraction and blood coagulation. To gain insight into the structure, dynamics and mechanics of fibrous proteins in a physiologically relevant aqueous environment, we have employed atomic force microscopy (AFM) so that surface-stabilized molecules were gently tapped with short cantilevers excited photothermally at high frequencies and low amplitudes. Furthermore, by nanomanipulation we characterized local interactions and domain stabilities. Several systems were investigated: titin, myosin, fibrin and the tail fibers of the T7 phage.

Titin is a giant protein spanning between the Z- and M-lines of the sarcomere. In the M-line, titin's C-terminus overlaps with that of oppositely oriented titin from the other half of the sarcomere and together with the titin-binding proteins myomesin and M-protein forms an M-line complex with unclear internal structure. We mechanically dissected the M-line complex of single surface-adsorbed titin oligomers with AFM. Loops of filaments with lengths up to 400 nm could be pulled out of the M-line complex. The complex likely has a higher order three dimensional structure involving the packaging of participating filamentous molecules.

In the A-band titin is associated with the myosin thick filament. Titin is thought to serve as a ruler for thick-filament formation via the super-repeat structure of its A-band domains. We tested the titin ruler hypothesis by mixing stoichiometric ratios of titin and myosin under filament-forming ionic conditions and investigating the structure of the formed complexes. Distinct populations of titin oligomers and myosin thick filaments were formed, but complexes of myosin molecules associated in a regular pattern to titin filaments could not be identified. Thus, electrostatically driven self-association is stronger in both myosin and titin than their binding to each other. Conceivably, associated proteins and additional mechanisms are required to modulate and regulate the in situ interactions of titin and myosin.

Fibrin is a meshwork formed by the association of proteolytically activated fibrinogen. The coiled-coil regions of fibrinogen provide elasticity to the meshwork which is thought to influence the stability and protease accessibility of the fibrin clot. Protein density and structural hierarchy severely complicate the exploration of molecular events within the clot. Here we developed a quasi-two-dimensional fibrin meshwork that enables the investigation of fibrin properties via topographical analysis. This stabilized meshwork can be proteolyzed by plasmin, during which the structure and mechanics of individual fibrin fibrils can be followed.

The tail fiber of the T7 phage is a homo-trimer formed of gp17 proteins. In surface-adsorbed T7 particles fixed with glutaraldehyde the tail fibers appeared as a hexagonal array of elbow-like filaments bound to the capsid surface. Intriguingly, the immobilization of the tail fibers coincided with the emergence of free dsDNA, suggesting that the fiber arrangement may be involved in the triggering of viral DNA release.

In summary, high-resolution AFM not only provides valuable insight about the topographical structure of fibrous proteins but enables the dissection of intra- and intermolecular interactions and local structural stability of protein domains.

Studying the interaction of lipopeptides with lipid membranes: the influence of lipospeptide design and its implications for membrane fusion

Alexander Kros*

Dept. Supramolecular Chemistry & Biomaterials, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333CC Leiden, The Netherlands. A.kros@chem.leidenuniv.nl

Abstract:

Membrane fusion, a vital process in all living organisms, is triggered by fusion proteins and their function is key for understanding cellular logistics. An extensively studied class of fusion proteins are SNARE proteins, and as a functional mimic we use lipidated derivatives of the heterodimeric coiled-coil pair E3 (EIAALEK)3 and K3 (KIAALKE)3 to study and control the fusion of liposomes. In this system peptides are tethered to their liposomes via a PEG-spacer and a lipid anchor, and in this contribution the influence of both the PEG-spacer length and the lipid anchor on the fusion process is studied. The effect of these structural modifications on the peptide structure, their interactions with liposomes, and their ability to mediate fusion were studied with lipid- and content-mixing experiments, DLS, and CD spectroscopy, and related to previous research. Our results demonstrate the asymmetric role of the peptides in the fusion process, since the PEG-spacer length affects both roles differently. Lipopeptide E3 is an antenna for lipopeptide K3 to enable docking of the liposomes, and the antenna function is enhanced with longer spacer lengths. Peptide K3 directs the efficiency of the fusion process via peptide-membrane interactions, but the length of the PEG-spacer plays two competing roles during the fusion process. A PEG4/PEG8 spacer length is optimal to destabilize the membrane and forces both membranes in even closer proximity. On the other hand, a PEG12 spacer reach higher fusion efficiencies over time by enhancing the peptide-accessibility. The anchor affects the local membrane characteristics and the peptide structure. The cholesterol anchor enhanced the membrane immersion of peptide K over the DOPE anchor, and thus mediated fusion more efficiently.

Structural Dynamics of the Rab5-Modulated Coiled-Coil Protein EEA1 Revealed by Hydrogen-Deuterium Exchange Mass Spectrometry.

Jeni Lauer[1], Andrei Lupas[2], and Marino Zerial[1]
[1]Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany.
[2]Max-Planck-Institute for Developmental Biology, Spemannstraße 35 – 39, 72076 Tübingen, Germany.

Intracellular trafficking of endosomes requires the cooperation of many soluble and membrane-bound components. The small GTPase Rab5 recruits a set of cytosolic effector molecules on the early endosome necessary for endosome biogenesis. Among them is the early endosome-associated autoantigen 1 (EEA1), whose homodimeric architecture consists of an N-terminal C_2H_2 Zn-finger followed by ~1200 residues of coiled coil, and a C-terminal FYVE domain. EEA1 binding to active Rab5, phosphatidylinositol 3-phosphate (PI3P), and syntaxins 6 and 13, helps regulate endosome tethering and fusion. This study utilized hydrogen deuterium exchange mass spectrometry (HDX-MS) to study the backbone dynamics of EEA1 and how they are regulated by Rab5 binding. Various mutants of EEA1 were generated to modulate coiled-coil stability. This produced valuable insights into how the fine-tuning of the coiled-coil structure controls binding events. We also found allosteric modulation of the coiled-coil structure upon binding Rab5 and noted that this allosteric modulation could be controlled by altering the stability of the coiled-coil.

Increasing sequence diversity in protein design by combining Rosetta with molecular dynamics

Jan Ludwiczak[1,2], Adam Jarmula[2], Stanislaw Dunin-Horkawicz[1]

[1] Structural Bioinformatics Laboratory, Centre of New Technologies, University of Warsaw,

Warsaw, Poland

[2] Laboratory of Bioinformatics, Nencki Institute of Experimental Biology, Warsaw, Poland.

Abstract

Protein design is a procedure for computing natural-like sequences that will fold into a specified structure. It has already been demonstrated that considering the backbone flexibility during the design process positively influences the diversity of the resulting sequences [1]. Rosetta Design, a commonly used software for protein design, allows for the effective exploration of the sequence space, while the molecular dynamics (MD) simulations can thoroughly sample the protein native state conformational space. By combining these two approaches, we developed an iterative design procedure, in which backbone conformational ensembles obtained by clustering of MD trajectories are used as templates for the design. We show that such a combined approach can generate significantly more diverse sequences than currently used procedures. The observed increase in the diversity is achieved without a loss in the quality of sequences, measured as overall resemblance of the designed sequences to natural sequences. In addition, we implemented a MD-based protocol [2] that can be used for assessing the stability of designed models and selecting the best candidates for experimental validation or generating the structural ensembles that can be used as an input for further design simulations. In sum, our results demonstrate that the MD ensemble-based flexible backbone design significantly outperforms the current state-of-the-art methods and thus should be a method of choice for the design of virtually all protein classes, including coiled coils. Finally, to make the procedure accessible for the community we provide a set of easy-to-use scripts for performing the simulations and visualizing the results.

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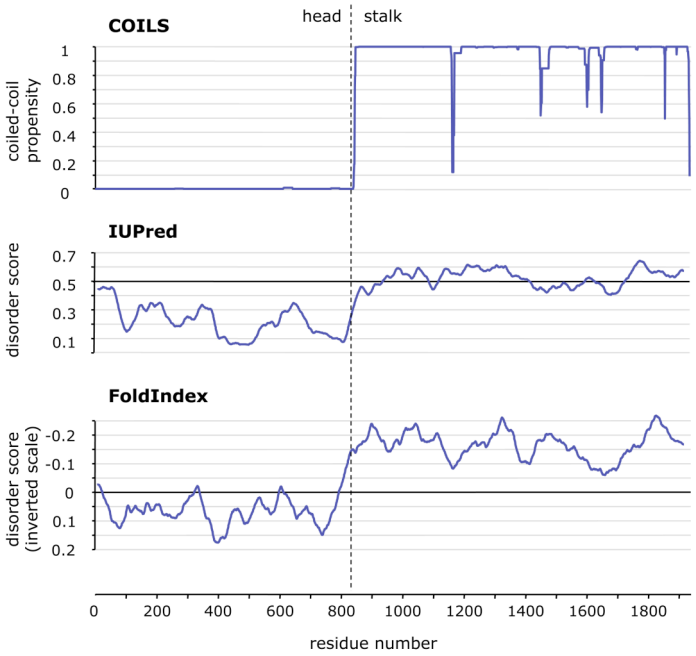
Coiled coils - between structure and unstructure

Jens Bassler, Birte Hernandez Alvarez, Marcus Hartmann, Jörg Martin, Andrei N. Lupas
Department of Protein Evolution, MPI for Developmental Biology, 72076 Tübingen, Germany; andrei.lupas@tuebingen.mpg.de

Due to the regularity of their interactions, coiled coils are frequently very stable proteins and many have been reported to withstand extreme chemical and thermal conditions, even when they comprise chains of little more than 30 residues. Given this thermodynamic stability, it may come as a surprise that coiled coils are close to the unfolded state; in fact, it is not uncommon for them to be mistaken for natively unstructured polypeptides by disorder prediction programs. This failure cuts both ways: highly charged sequences that are largely devoid of hydrophobic residues and lack sequence repeats indicative of coiled-coil structure are often predicted as coiled coils, even though they are most likely unstructured. The longer a coiled coil is, the closer this similarity tends to be.

Using examples from our work on trimeric autotransporters and on histidine zippers, we show that the closeness of the structured and unstructured states can be functionally important. However, we argue that the main reason coiled-coil sequences have evolved to resemble unstructured polypeptides lies in their need to ensure in-register folding of rods that are sometimes many hundreds of residues long. Since packing interactions are structurally the same all along the rod, coiled coils are confronted with many, essentially isoenergetic intermediates that could trap the folding chains out of register if they formed spontaneously. To prevent this, coiled coils have evolved sequences that allow them to be quite stable thermodynamically, once folded, but have kinetic folding barriers that maintain them in an unstructured state until folding has been initiated at a nucleation site and is therefore guaranteed to be in register.



Predictions of coiled-coil propensity and disorder in human myosin heavy chain . The boundary between the globular head domain and the fibrous stalk is marked by a vertical dotted line. The output of FoldIndex is shown on an inverted scale in order to make it directly comparable to the two other programs. The graphs show that the rod is recognized both as a coiled coil (COILS) and as natively unstructured (IUPred and FoldIndex)

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Recyclable Bionanostructures from Coiled-coil peptides

Dr. Daniel E. Mitchell, Dr. Barbara Ciani
University of Sheffield, Western Bank, Sheffield, UK S3 7HF.

Protein self assembly is of interest in a variety of natural systems from the amyloid plaques found in Alzheimer's disease to protein polymers involved in membrane remodelling. For an organism to function correctly, it is vital that these polymers can be recycled into their individual monomer units. In nature, this is achieved by the use of a class of proteins known as AAA^+ ATPases, which generate mechanical force to release subunits from large protein complexes.

Inspired by these natural systems, self assembling peptides have been investigated as a material for a range of different applications including scaffolds for regenerative medicine, drug delivery and even electronics and data storage. In general, the range of functionality and bottom up assembly process makes them highly appealing as a nano-material. This talk will discuss how we can create specific nano-structures from coiled-coil self-assembling peptides, which can be recycled back to their original constituent monomers upon the addition of an ATPase, and the potential applications of this, figure 1.

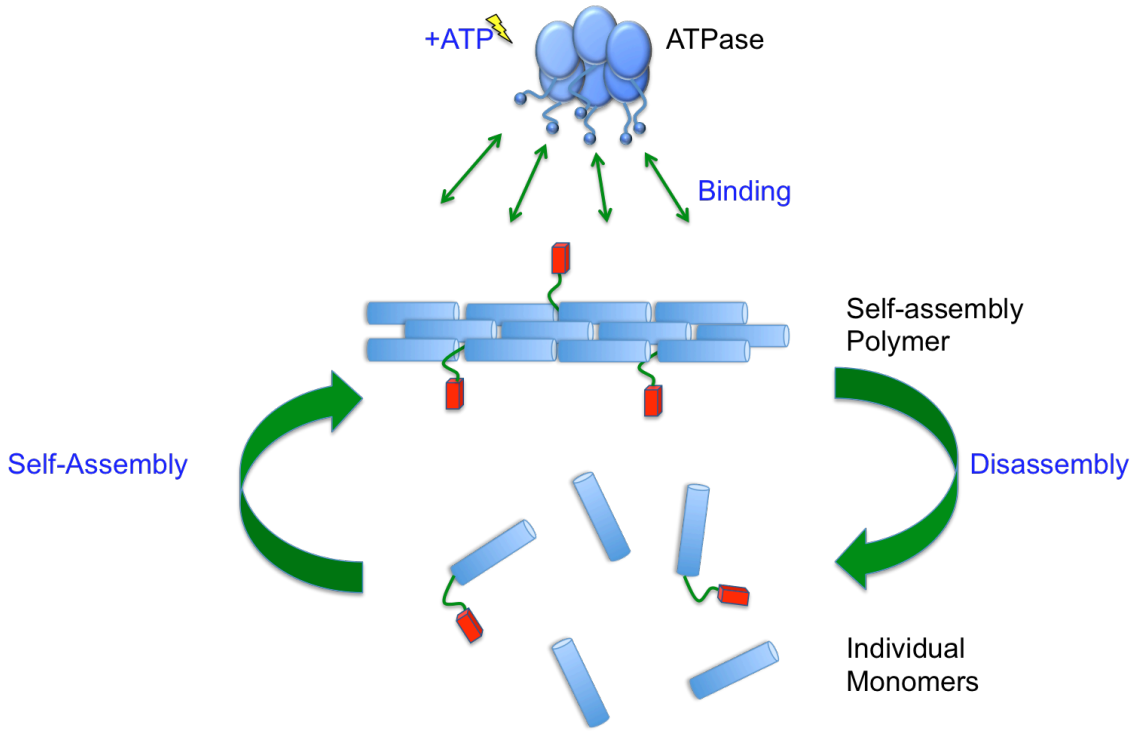

Figure 1. Schematic of our coiled-coil based recyclable system.

Designing autonomous peptide-based membrane pores

Ai Niitsu[1,2], Radhakrishnan K. Mahendran[3], Andrew R. Thomson[2,4], Richard B. Session[5], Yuji Sugita[1], Hagan Bayley[3] and Derek N. Woolfson[2,5]

1. RIKEN
2. School of Chemistry, University of Bristol
3. Department of Chemistry, University of Oxford
4. School of Chemistry, University of Glasgow
5. School of Biochemistry, University of Bristol

Transmembrane protein pores and channels perform crucial functions in transporting ions and bioactive molecules across cell membranes. Successful rational designs of such proteins deliver sequence-structure relationships important to understand protein folding, and add new structural motifs to the protein engineering field[1-2]. However, membrane protein design has been hindered by the poorer understandings of membrane protein structures than those for water-soluble proteins due to the limited high-resolution structural information. To progress work in this field, we design, synthesize, and experimentally and computationally characterize peptide-based, membrane-spanning α-helix barrels. We have recently discovered an α-helical pore-forming peptide (cWza peptide) through redesigning a transmembrane α-helical barrel domain of a natural protein, Wza[3]. The synthesized cWza peptides form a monodisperse barrel comprising eight parallel helices in lipid bilayers (Figure 1). The peptide shows discrete assembly intermediates, and once the pore is formed, it conducts ions and binds small molecules. The cWza peptide pore is the first example of the monodisperse α-helical barrel showing the remarkable stability equivalent to bacterial protein pores. This finding can initiate the engineering of a new class of protein structure and find applications in nanopore technology.

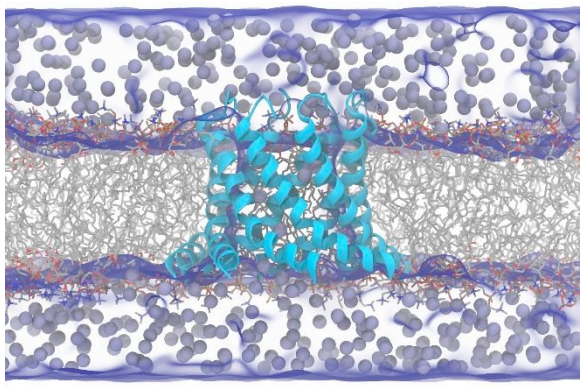

Figure 1. Model structure of the cWza peptide barrel in a molecular dynamics simulation snapshot

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Exploring the functionalization of *de novo* helix bundles by deviating from idealism

Gustav Oberdorfer[1,2], Nathan Rollins[2], David Baker[2]

[1]*Institute of Molecular Biosciences, University of Graz, Humboldtstraße 50/3, 8010, Austria*

[2]*Department of Biochemistry, University of Washington, Seattle, WA, 98195, USA*

Corresponding author: gustav.oberdorfer@uni-graz.at

Computational design of novel protein structures and enzymes is a promising tool to create superior biological materials with tailor-made properties, new pharmaceuticals, complex fine chemicals or renewable fuels. We previously established a procedure for designing proteins with backbones produced by varying the parameters in the Crick coiled-coil generating equations [1]. Combinatorial design calculations using the software suite Rosetta identify low energy sequences for alternative helix supercoil arrangements. After that, loop modeling is applied to connect the designs with lowest energy. The extent to which the designed sequences encode the designed structures is evaluated using large-scale structure prediction, as well as symmetric and asymmetric protein-protein docking calculations. Subsequently, synthetic genes are generated for sequences that converge strongly on the designed structure for experimental characterization. We applied this approach to monomeric and oligomeric helix bundles, some of which infer specificity through explicit design of hydrogen bonds [2,3]. Recently we started to explore the possibility to use the parametric helix-backbone generation to search for arrangements that can be functionalized. In particular, we wanted to design a copper-binding site into *de novo* designed four helix bundles. The metal coordination we used was distorted tetragonal and comprised of three histidine residues and a fourth open site for water coordination. While it was possible to sample a variety of helical backbones that can accommodate this binding site, the side-chain packing arrangements of all these designs showed defects resulting in empty voids in the protein core, owing to the compromise between accommodation of the metal site versus optimal helix-helix packing. Therefore, we expanded our design approach to include side-chain repacking and all-atom minimization steps between each round of sequence design with strong restraints on the binding site. This resulted in irregularities along the helical axis, but aided in side-chain core packing. Six designs that converged on the desired geometry in structure prediction calculations were ordered as synthetic DNA constructs and expressed in *E.coli*. Initial biophysical characterization of these designs suggests that two of them are stable up to 95C and fold into the designed structures. We are currently undertaking further structural investigation of all these designs by X-ray crystallography as well as characterization of the bound metal and its coordination.

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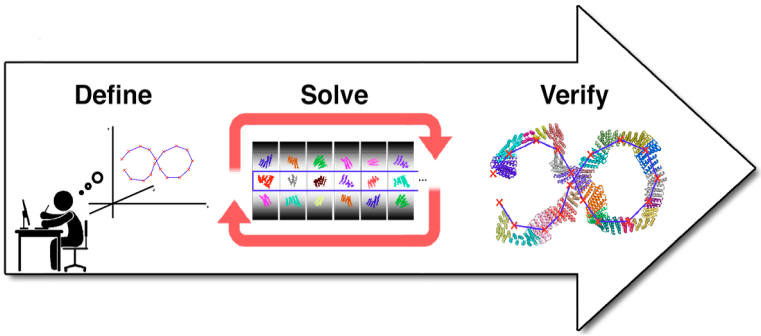
Computational design of custom three-dimensional structures from modular building blocks

Chun-Ting Yeh [1], TJ Brunette [2], David Baker [2], Simon McIntosh-Smith [1], Fabio Parmeggiani [3,4]

[1] Department of Computer Science, University of Bristol, UK
[2] Department of Biochemistry, Institute for Protein Design, University of Washington, Seattle, WA, USA
[3] School of Chemistry, School of Biochemistry, University of Bristol, UK
[4] BrisSynBio, BBSRC/EPSRC Synthetic Biology Research Centre, Bristol, UK



Computational methods have enabled the design of novel protein structures, but they are often still limited to small proteins and symmetric systems[1]. To expand the size of designable proteins while controlling the overall structure, we developed Elfin, a genetic algorithm for the design of novel proteins with custom shapes using structural building blocks derived from experimentally verified repeat proteins[2–4]. By combining building blocks with compatible interfaces, it is possible to rapidly build non-symmetric large structures (> 1000 amino acids) that match three-dimensional geometric descriptions provided by the user. Protein structures with controlled geometry will allow the systematic study of the effect of spatial arrangement of enzymes and signaling molecules, and provide new scaffolds for functional nanomaterials.

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Advancing metalloprotein design for new functions and applications

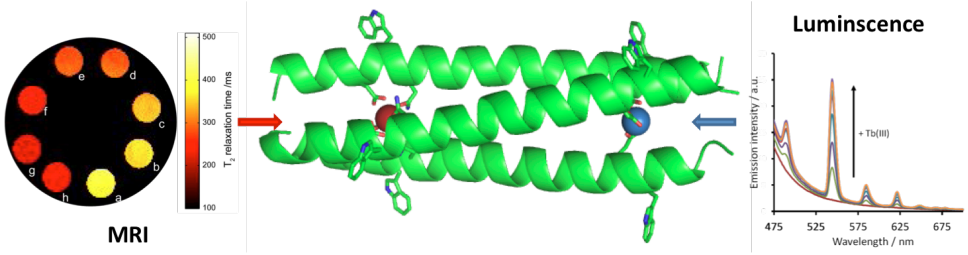
Anna F. A. Peacock

School of Chemistry, University of Birmingham, Edgbaston, B15 2TT, UK.

a.f.a.peacock@bham.ac.uk

De novo designed miniature protein scaffolds, such as the coiled coil, offer exciting opportunities for metal ion coordination [1]. Not surprisingly, due to the protein-like nature of the scaffold, the large majority of *de novo* metallocoiled-coil examples have focussed their efforts on mimicking the active sites of native metalloenzymes. Our approach is to instead use these artificial proteins as novel ligands for the coordination of xenobiotic metals, with no known biological role, with the view to developing functional systems for valuable applications beyond the scope of nature.

We recently reported the design of the first gadolinium coiled coil, which displayed promise as a potential MRI contrast agent [2,3]. We have since interrogated the coordination of various lanthanide ions to our coiled coils and have for the first time shown that we can selectively discriminate between Ln(III) ions based on size. As a result we have now designed coiled coils capable of binding two different Ln(III) ions selectively to two different sites (see Figure), and at a defined distance from one another. The opportunities this affords and the potential applications of this new class of lanthanide coiled coils, will be discussed.



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Stable single α helices that do not form coiled coils - what makes them stable?

*Matthew Batchelor, *Marcin Wolny, *Peter J. Knight, *Emanuele Paci, *Arnout Kalverda, @Dek Woolfson, @Emily Baker, @Gail Bartlett and *Michelle Peckham. *University of Leeds, @University of Bristol

Just over 10 years ago, we discovered that the predicted coiled coil domains of several myosin motor proteins did not form a coiled coil, but instead formed a stable single α-helix or 'SAH' (Knight et al., J. Biol. Chem, 2005). We and others have since found that SAH domains are widespread, and found in many different proteins. These unique domains are highly stable across a range of pH and ionic strength conditions. Their amino acid sequences are rich in charged amino acid residues (Glu (E), Lys (K) and Arg (R)) and we think that their stability arises from a network of stabilising ionic interactions (or salt bridges). We have since discovered that these domains can replace part of the canonical lever in myosin (Baboolal et al., 2009, PNAS), and generate a constant force while elongating (constant force spring behaviour), likely to be important for their cellular function (Wolny et al., J. Biol. Chem. 2015). Through a combination of molecular dynamics simulations, a PDB analysis, and design and characterisation of *de novo* 96-residue polypeptides with 7-residue repeat patterns, we now have a better understanding of how Lys and Arg differentially contribute to the stability of these proteins (Wolny et al., Sci. Rep. 2017). We are currently using NMR to investigate the properties of the SAH domain from myosin 7a in solution, and in particular to try to find evidence for the salt bridges that should be stabilising its structure. Intriguingly, while NMR confirms the helical nature of this peptide, the data so far suggests that these salt bridges are highly dynamic, raising more questions about just how these domains are stabilised.

An Alternative Strategy to Generate Binding Proteins

Andreas Plückthun, Dept. Biochemistry, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland
plueckthun@bioc.uzh.ch www.bioc.uzh.ch/plueckthun

The most frequently used binding proteins used in research are monoclonal antibodies, made by the >40-year-old hybridoma technology, some with questionable performance [1,2]. More recently, recombinant antibodies and non-antibody scaffolds, selected from synthetic libraries, have started to provide access to molecularly defined molecules [3,4]. Nonetheless, all of these approaches require one to treat every target as a completely new project. This is currently unavoidable for folded proteins. However, we hypothesized that for unfolded proteins or unfolded stretches (tags, posttranslationally modified tails, denatured proteins on western blots), termed "peptides" for simplicity, the regularity of the peptide main chain can be exploited. If true, a modular detection system can be devised, which would ultimately allow one to generate a sequence-specific binding protein without experimentation.

The basis of our approach are Armadillo Repeat Proteins [5-15], which bind peptides in a completely extended way, providing a pocket for each side chain, and thus access to a modular approach. Combining evolutionary engineering, NMR, X-ray crystallography and structure-based computation, we have now achieved well crystallizing ArmRPs with bound peptides, picomolar affinities, and a well functioning selection and evolution technology, as well as a portfolio of biochemical and biophysical analysis technologies for the engineered ArmRPs. Progress in the various aspects will be summarized.

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Optimising the design of the PPα Miniprotein

Kate Porter Goff, Dek Woolfson University of Bristol

School of Chemistry, Cantocks Close, University of Bristol, UK, BS8 1TS

Miniproteins have reduced complexity relative to larger proteins. As such, they provide useful scaffolds for probing sequence-to-structure and sequence-to-stability relationships in proteins more generally. Improved understanding of these relationships will facilitate the rational design of new protein folds and assemblies not observed in nature, the so-called 'dark matter' of protein folding space.

Baker *et al* used a fragment based design approach to give PPα, a 34-residue miniprotein comprising an α-helix buttressed by a polyproline II helix.[1] PPα borrows from two natural proteins: a bacterial surface adhesin (AgI/II) (Figure 1, left),[2] and a peptide hormone.[3] Interdigitation of proline residues from the polyproline helix with tyrosine residues presented by the α-helix provides the driving force for assembly and is reminiscent of 'knobs-into-holes' packing.[4] Current work seeks to optimise the design of PPα into completely *de novo* frameworks with alternative connectivities, non-covalent interactions and stabilities. Optimisations in PPα have yielded increased thermal stabilities with midpoints of unfolding transitions (T_m) up to 51 °C vs. 39 °C for the parent. An improved understanding of helix packing between the two types of helix in PPα has been used to create elongated constructs with T_m's ranging 51 to 68 °C.

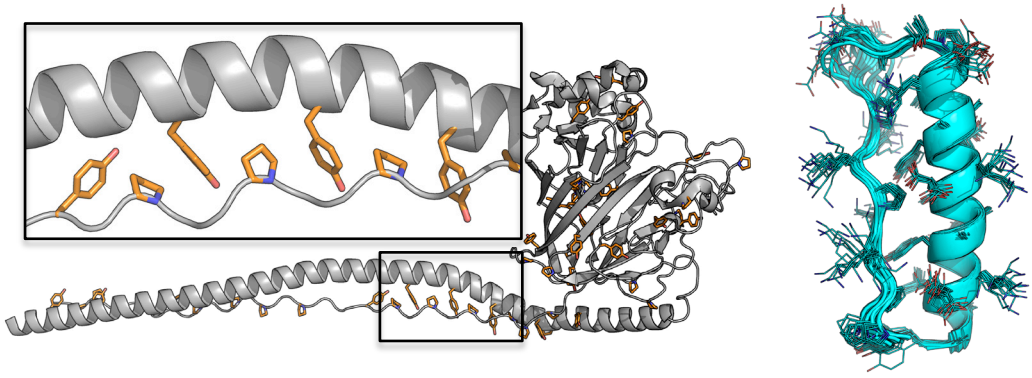

Figure 1. Left: Crystal structure Ag/II (PDB: 3IOX). Right: Overlay NMR ensemble of PPα-Opt.

Future work looks toward using the optimised PPα design to build larger and more complex assemblies with potential applications in biotechnology.

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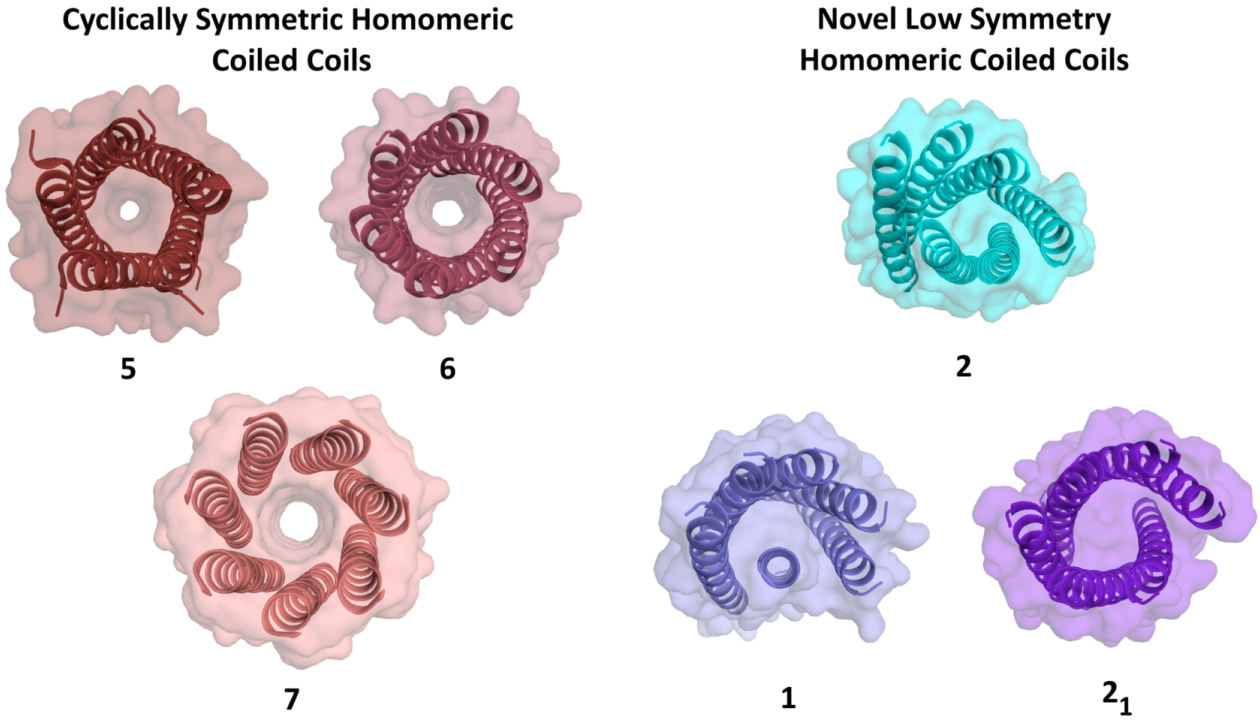
Maintaining and breaking symmetry in homomeric coiled-coil assemblies

Guto G. Rhys,[1,*] Andrew R. Thomson,[1,4] Christopher W. Wood,[1] R. Leo Brady[2] and Derek N. Woolfson[1,2,3*]

[1]School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK
[2]School of Biochemistry, University of Bristol, Medical Sciences Building, University Walk, Bristol BS8 1TD, UK
[3]BrisSynBio, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol BS8 1TQ, UK
[4]School of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK
[*]To correspondence should be addressed: Guto.Rhys@bristol.ac.uk and D.N.Woolfson@bristol.ac.uk

Abstract

Most natural and designed Coiled Coils (CCs) have 2, 3 or 4 helices and simple cyclic (Cn) or dihedral (Dn) symmetry.[1] Increasingly, CCs comprising more than 5 helices are being reported.[2–5] These higher-order CCs are of structural and practical interest as they often have accessible central channels that can be functionalised.[6,7] The cavities are surprising given the drive to maximise buried hydrophobic surfaces during protein folding in water. We have recently discovered that channels can be maintained by the strategic placement of branched aliphatic residues lining the lumen. Otherwise, the structures collapse or adjust to give more-complex multi-helix CCs without simple symmetry. Nonetheless, the structural hallmark of CCs—i.e., knobs-into-holes (KIH) packing of side chains in the core—is maintained leading to classes of CCs not yet observed in nature or accessed by design.



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COILED COIL-MEDIATED SYNTHESIS OF SMALL CYCLIC PEPTIDES IN AN INTRAMOLECULARIZED NCL-LIKE APPROACH

W. Mathis Rink & Franziska Thomas

Institute of Organic and Biomolecular Chemistry, Georg-August-Universität Göttingen
Tammannstraße 2, 37077 Göttingen, Germany
*Corresponding Author: Franziska Thomas, franziska.thomas@chemie.uni-goettingen.de

Abstract

Peptide cyclization is an efficient tool to increase the potency of peptide drugs due to an increase in biostability and conformational constraint. However, the synthesis of small, cyclic peptides is challenging. The *head-to-tail*-cyclization strategy is one of the most commonly applied methods to form a native cyclic peptide structure [1]. To circumvent lavish protection group strategies, the *Native Chemical Ligation* with unprotected peptide thioesters is the method of choice to build up native peptide structures [2]. However, the yields of the lactamizations are poor and the reaction times are often long; except cysteine-rich sequences, in which the thiol groups of the cysteine sidechains accelerate the cyclization reaction due to the formation of peptide thioesters [3]. Our research aims to use a proximity-induced cyclization strategy utilizing the well-understood heterodimeric coiled coil. The generic synthesis protocol includes modification of one coil strand with an N-terminal azide and of the other with an N-terminal thiol. The amino acid sequence to-be-cyclized is C-terminally modified as a thioester and carries an N-terminal cysteine. Furthermore, an alkyne moiety is reversibly attached to a lysine residue close to the N-terminus. First, the N-azide coil is decorated with the peptide thioester of interest using CuAAC ("Click") chemistry [4]. The addition of the thiol-modified coil should then result in transthioesterification followed by coil-coil association to give a hairpin structure as shown in Fig. 1. This results in proximity of the N-terminal cysteine and the peptide thioester furnishing the cyclic peptide by an NCL-like reaction sequence. The N-thiol coil strand is liberated during this process, whereas the cyclic peptide is still attached to the other coil strand. Finally, the carbamate-based linker is cleaved under basic conditions to give the free cyclic peptide.

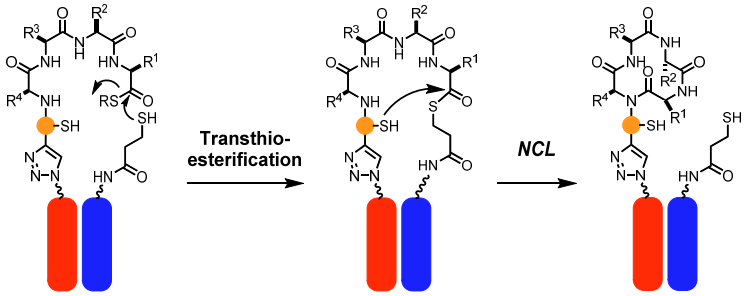

Fig. 1: Concept of the coiled-coil mediated peptide cyclization.

We also present a new NCL-auxiliary, which adds the required sequence independence to this approach.
We aim to establish this method in the synthesis of small cyclic peptides of 3-5 AA and to use the programmability of coiled coils to build-up cyclic peptide libraries.

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Influence of N- and C-terminal domains on the self-assembly of the repetitive core domain of spider dragline silk proteins

Merisa Saric, Joschka Bauer and Thomas Scheibel

Lehrstuhl Biomaterialien, Fakultät für Angewandte Naturwissenschaften, Universität Bayreuth; 95440 Bayreuth, Germany

Spider dragline silk is an intensely studied protein-based material due to its outstanding mechanical properties like high tensile strength, great extensibility and toughness[1]. Spider silk is composed of spidroins (spider fibroin) in a tripartite structure including a highly repetitive core domain and non-repetitive amino- and carboxy-terminal domains[2]. Each terminus plays a significant, independent role in the prevention of protein aggregation within the spiders' gland and controls the self-assembly of spidroins into highly ordered structures in fibrils and fibers[3]. The conversion of the fluid spinning dope into a solid fiber is induced within the spinning duct by ion exchange (sodium chloride against potassium phosphate) and acidification from pH 7.2 to pH 5.7[4]. Hereby, dimerization of N-terminal domains is triggered by protonation of conserved acidic residues resulting in the formation of antiparallel dimers[5]. Together with the parallel orientation of the C-terminal domains, the chains can elongate, and an endless spidroin network resulting in a fiber can be obtained[6, 7].

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Designing functional helical bundles by combining existing protein structures

Nathan W. Schmidt, and William F. DeGrado

Department of Pharmaceutical Chemistry, Cardiovascular Research Institute, University of California, 555 Mission Bay Boulevard South, Box 3122, San Francisco, CA 94158, USA

We describe a procedure for connecting different helical bundle structures into a single helical bundle with minimal disruption to helix geometry using computational protein design. Using this approach we construct single-chain, antiparallel four-helix bundle proteins that contain distinct metal- and porphyrin-binding sites. The designed helical bundles are extremely stable, and both sites bind their ligands with high affinity. The binding sites are close (~10Å separation) to one another in the protein design, and the effects and implications of the arrangement will be discussed. Our approach enables the design of proteins with new functionalities from existing helical bundle structures.

Characterization of measles virus phosphoprotein : A coiled-coil domain containing conserved motifs that are crucial for its function.

Antoine Schramm[1,2], Louis-Marie Bloyet [3], Jennifer Roche[1,2], Carine Lazert [3], Denis Gerlier [3], Sonia Longhi [1,2]

[1] Aix-Marseille Université, Laboratoire Architecture et Fonction des Macromolécules Biologiques, UMR 7257, Marseille, France.
[2] Centre National de la Recherche Scientifique, Laboratoire Architecture et Fonction des Macromolécules Biologiques, UMR 7257, Marseille, France.
[3] Centre International de Recherche en Infectiologie, INSERM U1111, Centre National de la Recherche Scientifique, UMR 5308, Université Lyon 1, Lyon, France.

Abstract :

The Phosphoprotein (P) is a component of the paramyxoviral replicative complex. This protein plays a major role in the viral replication and transcription and connects the viral polymerase (L) to its ribonucleoprotein substrate by protein-protein interaction. P is mainly intrinsically disordered with the exception of the coiled-coil P multimerization domain (PMD) and the X domain (XD). Coiled-coils are repeat-containing proteins that fold into alpha helices that establish tight interactions with each others. Their sequence consists in the repetition of a seven residue motif named heptad. In PMD, a motif of 3 residues occurs between two heptads (stammer). This motif breaks the regular heptad repetition, thus leading to repercussions on the atomic structure of the protein. Indeed, this motif, called “kink”, folds into a 3_{10} helix thereby inducing a distorption into the coiled-coil. Interestingly, this 3_{10} helix is a conserved feature in paramyxoviral PMDs. However, the precise relationships between the structure of the coiled-coil, and especially the presence of the 3_{10} helix, and the function of the protein are still elusive. Many hypotheses can be proposed concerning the kink contribution, it could for instance constitute a motif recognized by the viral polymerase that could favor the formation of the L-P complex, or it could play a structural role by affording local flexibility. In view of unveiling the role and the contribution of the kink to viral transcription and replication, we have undertaken a collaborative project at the interface of functional virology and structural biochemistry.

The design and engineering of transmembrane α-helical barrels for nanopore sensing applications

Alistair Scott, Prof. Dek Woolfson

School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS

Nanopore sensing is an emerging technology that enables the detection and identification of analyte molecules on a single molecule level, based on their ability to block nanoscopic pores. Nanopore sensing of small molecules has been achieved with a number of proteins, but all thus far have been natural β-barrel proteins.

The *de novo* design of pore-forming membrane proteins suitable for nanopore sensing has proved exceedingly challenging.[1] Recent work in our group has demonstrated the first example of stand-alone α-helices (cWza) that assemble autonomously in membranes to form stable, single channels, based on the membrane-spanning domain of the Wza protein.[2] Further, we are also interested in the design of fully *de novo* α-helical membrane proteins, by adaption of computational tools previously used to produce water-soluble barrels.

The design and synthesis of novel, hybrid water-soluble / transmembrane α-helical barrels are underway. It is hoped that such designs could have advantages over earlier efforts, such as enhanced stability and crystallisation properties.

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Coiled-coil-peptide induced cell membrane fusion for drug delivery

Mengjie Shen, Aimee Boyle, René Olsthoorn, Alexander Kros

Coiled-coil motifs are well known for playing fundamental roles in protein structure,[1] but they also play a pivotal role in membrane fusion events.[2] We have developed a model system for membrane fusion, which uses the heterodimeric coiled-coil peptides K [(KIAALKE)n] and E [(EIAALEK)n] (n=3 or 4).[3] These were conjugated to a cholesterol membrane anchoring moiety via a PEG spacer, resulting in constructs named CPK and CPE. Both liposome-liposome and cell-liposome fusion can be achieved with CPK3/E3 and CPK4/E4, respectively.[2,4]

We have shown that fusion is more efficient with longer peptide constructs,[5] therefore we have synthesized 5-heptad variants of the E and K peptides. The variable temperature CD data shows that they have higher melting points and stronger interactions compared to the K3/E3 and K4/E4 variants. We are currently attempting to synthesize the lipopeptides CPK5/CPE5, which may have superior membrane fusion ability, meaning we could use this novel lipopeptide to make drug delivery more efficient, or to facilitate cell-cell fusion.

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Intermediate filament structure: progress and challenges

Sergei Strelkov

Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Belgium

sergei.strelkov@kuleuven.be

The three-dimensional structure of intermediate filaments (IFs) is a fundamental, still largely unresolved problem of structural molecular biology. Functional studies of various IF protein classes are currently florishing, which is not surprising due to their fundamental importance and scores of disease-related mutations detected in IF proteins. Yet the understanding of their 3D structure is clearly lagging behind, despite a long history of research. As I will explain, recently there has been a substantial progress on the atomic structure of the signature central domain of IF proteins which forms a segmented coiled-coil dimer. This progress is due to both experimental crystallographic studies and *in silico* structure prediction. Likewise, new data are becoming available on the atomic structure of the tetramer, the solution species observed for cytoplasmic IF proteins. At the same time, the available evidence on the higher-order IF architecture is still largely coming from the 1990s. I will discuss the recent developments in experiment and modelling that may change this situation in the future. This can not rely on a single method but rather an synergistic use of various approaches. At the same time, both substantial intrinsic disorder in the terminal domains of IF proteins and the polydispersity often observed for assembled filaments add to the complexity of the IF structure challenge.

The use of recombinant honeybee silk for rational design of advanced materials

Tara D Sutherland and Trevor D Rapson

CSIRO, Australia

Precise control of a polymers composition is required for the rational design of advanced materials such as stimuli responsive or multifunctional materials. Recombinant protein systems provide this control, and in contrast to synthetic equivalents are compatible with large scale production. However, despite the promise of proteins for advanced material design, protein-based materials are underrepresented in materials science. Here we describe our efforts to develop recombinant silk proteins that are tolerant of amino acid modifications without compromising the ability to produce the proteins at large scale in recombinant systems or fabricate them into material forms. We describe design of the silk proteins into materials with diverse functional properties ranging from recoverable heme-silk sponges with peroxidase activity, stable nitric oxide-sensing protein films and in electrodes capable of fully reducing oxygen to water.

THE HETERODIMERIC COILED COIL AS A REACTION SCAFFOLD

W. Mathis Rink, Niels B. Paul, Mike C. Groth, and Franziska Thomas

Institute of Organic and Biomolecular Chemistry, Georg-August-Universität Göttingen
Tammannstraße 2, 37077 Göttingen, Germany
*Corresponding Author: Franziska Thomas, franziska.thomas@chemie.uni-goettingen.de

Abstract:

De novo designed biomolecules have a tradition in being applied as models to mimic natural systems or to create new functional assemblies, which work under physiological conditions. Next to the well-understood nucleic acids, some protein folding motifs have been explored extensively with respect to their sequence-to-structure relationships and reliable design rules have been established. Among them the coiled coil is probably best understood, and designs for a variety of coiled-coil assemblies are available.

We aim to use coiled coils as scaffolds to mediate chemical reactions. In this respect, the parallel heterodimeric coiled coil is highly interesting. Due to its heterogeneity it is not simply a dimerization tool but also provides control over the association process. By now, the specificity of the interaction between the two coil strands can be fine-tuned *via* the sequence pattern or chain lengths of the individual coil strands.[1-3] Furthermore, we found that based on the differences in strength of association, coil strands can be readily exchanged to form the more stable coiled-coil assembly. These properties make the heterodimeric coiled coil a perfect building block to develop strategies for proximity-induced reactions and even reaction sequences. Currently, we are focusing on two reaction types: One is peptide cyclization, including head-to-tail and sidechain-to-sidechain cyclization, and consecutive acyl transfer, in other words, a coiled-coil mediated peptide synthesis.

The synthesis of cyclic peptides, especially small cyclic peptides is still challenging and normally requires cumbersome protecting group strategies. This can be facilitated using cyclization chemistry based on chemoselective reactions, such as native chemical ligation.[4,5] However, reaction times are still relatively long and lactamization yields are low. We aim to accelerate the reaction by preformation of the cyclic conformation exploiting heterodimeric coiled-coil association. This approach involves reversible attachment of the to-be-cyclized N-cysteine peptide thioester to one coil strand and subsequent hairpin formation due to association of the other a thiol-functionalized coil strand, and S-to-S acyl transfer. This process induces proximity of the cysteine and the C-terminal thioester moiety, which results in practically immediate peptide cyclization. Attachment of the target peptide is performed either *via* a lysine side chain or *via* an NCL-like peptide backbone auxiliary. We aim to establish this method in the synthesis of small cyclic peptides of 3-5 AA and to use the programmability of coiled coils to build-up cyclic peptide libraries.

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Design and production of natural inspired repetitive recombinant proteins

Winkler A. and Scheibel T.

Lehrstuhl Biomaterialien
Fakultät für Angewandte Naturwissenschaften
Universität Bayreuth
95440 Bayreuth, Germany
E-mail: anika.winkler@bm.uni-bayreuth.de

Fibrous proteins such as collagens or silks have been recently in the focus of research, since the production of novel biocompatible functional materials with varying mechanical properties is important for medical engineering [1, 2].

For determination of molecular mechanisms and structure relevant properties, different proteins were designed on the basis of natural examples. The major ampulate silk of spiders comprises the spidroin MaSp2, which contains various polyalanine and GPGXX motifs in a repetitive core [1]. Based thereon, a [C] module was created containing both motifs and repeated 16-times [3]. Inspired by the lacewing *Mallada signata's* eggstalk protein MalXB2, an [AS] module containing the characteristic KGSA motif was designed and repeated eightfold in a similar manner [4]. The 48 amino acids within the module lead to the same 16-residue periodicity as in the natural protein [4, 5]. Both developed proteins were codon optimized for biotechnological production in *Escherichia coli* [3, 4]. Recently, three eggstalk proteins of *Chrysopa carnea* were identified and produced recombinantly [6]. The byssus of blue mussels mainly consists of collagen-like proteins [7]. So far, the C-terminal flank, dominated by $X(G)_n$ repeats, was recombinantly produced in *E. coli* to investigate its role in the assembly process [8].

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Parametric Modelling of Repeat Proteins with ISAMBARD and CCBuilder 2.0

Chris Wells Wood (University of Bristol) – Alpbach Abstract 2017

A key challenge in protein modelling is to reduce the complexities of 3D-protein folds. One way to achieve this is to describe protein structure using sets of parametric equations, which capture the salient features of these structures using a small number of geometric parameters. At present, this is possible for a subset of proteins, namely repeat proteins. We have developed ISAMBARD (Intelligent System for Analysis, Model Building, And Rational Design), a software package that contains generalised tools for parametric modelling, which can be applied to any parameterisable protein fold. The initial release of ISAMBARD contains parameterisations for α-helical coiled coils, collagen triple helices and α-solenoid proteins. In order to increase accessibility to parametric modelling, we have also developed an easy-to-use web application called CCBuilder 2.0. This provides a simple interface to ISAMBARD, allowing non-experts to model and optimise structures of α-helical coiled coils and collagen triple helices. These models have many applications from aiding molecular replacement for X-ray crystallography, model building and engineering of natural and designed protein assemblies, and through to the creation of completely de novo “dark matter” protein structures. The code for both ISAMBARD and CCBuilder 2.0 is open source and can be downloaded freely (https://github.com/woolfson-group/).

From rational to computational design of coiled coils and back again

Dek Woolfson
Schools of Chemistry and Biochemistry, & Bristol BioDesign Institute
University of Bristol, UK

The vast majority of coiled-coil designs have been based on heuristics or simple rules of thumb learnt from natural proteins or derived empirically through experiment.[1] These rules relate sequence to structure to guide the specification of coiled-coil oligomerization state, strand orientation, partner selection, and, to some extent, stability. This has been extremely informative and productive, and design and engineering is probably more advanced for coiled coils than for any other protein structure.[2] However, to move past the low-hanging fruit of coiled-coil design, and into the *dark matter of protein structures*, we will all have to learn new tricks. To address this we and others—notably the André, Baker, DeGrado, Grigoryan and Harbury groups—have begun to tackle coiled-coil design parametrically using computational methods.[3] For our part, we have developed CCBuilder, which is an easy-to-use web-based GUI;[4] and ISAMBARD, which is a more-versatile Python-based API that is free to download from GitHub and has been written for protein design more generally.[5]

I will describe how a serendipitous discovery of a 6-stranded α–helical barrel,[6] which are rare in nature, led us to develop our computational methods; and how we used these to deliver α–helical barrels predictably.[7] The talk will demonstrate the utility of this approach to make water-soluble protein-like barrels and pores, which we have engineered to form materials, bind small molecules, and catalyse simple reactions.[8,9] Most recently in collaboration with the Bayley lab (Oxford), we have engineered membrane-soluble variants of these α–helical barrels that insert into lipid bilayers and conduct ions in a voltage-dependent manner.[10] I will touch on how the barrels and related structures that we have discovered and built are improving our general understanding of coiled coils.

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Peptide binders based on complementary Armadillo Repeat Protein Fragments

Erich Michel, Department of Chemistry, Zurich, Switzerland; Andreas Plückthun, Department of Biochemistry; Zurich, Oliver Zerbe, Department of Chemistry, Zurich

We have recently discovered that it is possible to reconstitute a designed Armadillo repeat protein (ArmRP) from complementary fragments (Watson et al., Structure 2014). The two fragments, when mixed, will form a complex with nanomolar affinity. The structure of the complex is almost identical to the structure of the entire (single-chain) protein. ArmRP can be split at several places, within repeats as well as between repeats. The affinity of the fragments various between nanomolar and micromolar Kd's, depending on the exact location of the split site.

We have also seen that a repeat protein, that can bind a peptide, can be split into two fragments, and that the reconstituted complex again recognizes the peptide. We then created a small library of C-terminal fragments, that all form complexes with a particular N-terminal fragment. Using NMR in combination with differentially labeled fragments we could demonstrate that upon addition of a peptide, for which only one of the present protein complexes presents a high-affinity binder, the equilibrium is shifted towards the complex that represents the best binder for that peptide.

I present spectroscopic (NMR) and other biophysical data to characterize the underlying proteins and protein complexes. We feel we have discovered a novel interesting property of ArmRP, in that they can be reconstituted from a large number of different complementary fragments, and that interesting biochemical applications may emerge from these systems.

I also demonstrate how segmental labeling of repeat proteins tremendously simplifies their analysis by NMR, and that interesting details of peptide binding to designed ArmRP can be derived from such data.

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Name	Affiliation	E-mail
Adlakha, Jyoti	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	jyoti.adlakha@tuebingen.mpg.de
Alva, Dr. Vikram	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	vikram.alva@tuebingen.mpg.de
Aupič, Jana	National Institute of Chemistry, Synthetic Biology and Immunology, Slovenia	jana.aupic@ki.si
Baker, Dr. Emily Grace	University of Bristol, Chemistry, UK	emily.baker@bristol.ac.uk
Baßler, Jens	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	jens.bassler@tuebingen.mpg.de
Ben-Harush, Dr. Kfir	Shamoon College of Engineering, Department of Chemical Engineering, Israel	kfirb@sce.ac.il
Böckmann, Prof. Rainer	Friedrich Alexander University Erlangen-Nürnberg, Computational Biology, Germany	rainer.boeckmann@fau.de
Borkner, Christian Bruno	University of Bayreuth, Biomaterials, Germany	christian.borkner@bm.uni-bayreuth.de
Boyle, Dr. Aimee	Leiden University, Netherlands	a.l.boyle@chem.leidenuniv.nl
Ciani, Dr. Barbara	University of Sheffield, Chemistry, UK	b.ciani@sheffield.ac.uk
Coles, Dr. Murray	Max Planck Instituite for Developmental Biology, Protien Evolution, Germany	murray.coles@tuebingen.mpg.de
Conticello, Prof. Vincent	Emory University, Chemistry, USA	vcontic@emory.edu
Crane, Prof. Brian R.	Cornell University, Chemistry and Chemical Biology, USA	bc69@cornell.edu
Crone, Niek	Leiden university, Supramolecular & biomaterials chemistry, Netherlands	n.s.a.crone@lic.leidenuniv.nl
Dawson, William Michael	University of Bristol, Chemistry, UK	w.dawson@bristol.ac.uk
DeGrado, William	UCSF, Pharmaceutical Chemistry, USA	william.degrado@ucsf.edu
Dunin-Horkawicz, Stanisław	University of Warsaw, Poland	s.dunin-horkawicz@cent.uw.edu.pl
Edgell, Caitlin Louise	University of Bristol, School of Chemistry, UK	ce1215@bristol.ac.uk
Edwards, Robert Jon	Duke University, Cell Biology, USA	rjpe@cellbio.duke.edu
ElGamacy, Mohammad	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	mohammad.elgamacy@tuebingen.mpg.de
Ernst, Patrick	University of Zurich, Switzerland	p.ernst@bioc.uzh.ch
Ghosh, Partho	University of California, San Diego, Chemistry & Biochemistry, USA	pghosh@ucsd.edu
Gordeliy, Valentin	Institute of Structural Biology, Grenoble, France/FZJ, Juelich, Germany	g.valentin@fz-juelich.de
Grigoryan, Gevorg	Dartmouth College, Department of Computer Science, USA	gevorg.grigoryan@dartmouth.edu
Guzenko, Dmytro	KU Leuven, Pharmaceutical Sciences, Belgium	dmytro.guzenko@kuleuven.be

Hartgerink, Jeffrey D.	Rice University, Chemistry, USA	jdh@rice.edu
Hartmann, Marcus D.	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	marcus.hartmann@tuebingen.mpg.de
Hennemann, Julia	University of Bayreuth, Germany	julia1.hennemann@uni-bayreuth.de
Hernandez, Birte	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	birte.hernandez@tuebingen.mpg.de
Huang, Possu	Stanford University, Bioengineering, USA	possu@stanford.edu
Hughes, Spencer Allen	Emory University, Chemistry, USA	shughe7@emory.edu
Jahnel, Marcus	BIOTEC – TU Dresden & MPI-CBG, Germany	marcus.jahnel@biotec.tu-dresden.de
Kameda, Tsunenori	National Agriculture and Food Research Organization, Japan	kamedat@affrc.go.jp
Karamichali, Ioanna	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	ioanna.karamichali@tuebingen.mpg.de
Kellermayer, Miklós	Semmelweis University, Biophysics and Radiation Biology, Hungary	mszkellermayer@gmail.com
Koksch, Beate	Freie Universität Berlin, Germany	beate.koksch@fu-berlin.de
Kros, Alexander	Leiden university, Supramolecular & biomaterials chemistry, Netherlands	a.kros@chem.leidenuniv.nl
Lauer, Jeni	Max Planck Institute of Molecular Cell Biology and Genetics, Germany	jlauer@mpi-cbg.de
Lehmann, Karin	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	karin.lehmann@tuebingen.mpg.de
Ludwiczak, Jan Marcin	Center of New Technologies, University of Warsaw, Poland	j.ludwiczak@cent.uw.edu.pl
Lupas, Andrei N.	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	andrei.lupas@tuebingen.mpg.de
Marelli, Benedetto	Massachusetts Institute of Technology, Civil and Environmental Engineering, USA	bmarelli@mit.edu
Martinez Goikoetxea, Mikel	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	mmartinez.60@alumni.unav.es
Mitchell, Daniel	University of Sheffield, University of Sheffield, UK	daniel.mitchell@sheffield.ac.uk
Niitsu, Ai	RIKEN, Japan	ai.niitsu@riken.jp
Oberdorfer, Gustav	University of Graz, Institute of Molecular Biosciences, Austria	gustav.oberdorfer@uni-graz.at
Parmeggiani, Fabio	University of Bristol, UK	fabio.parmeggiani@bristol.ac.uk
Peacock, Anna	University of Birmingham, Chemistry, UK	a.f.a.peacock@bham.ac.uk
Peckham, Michelle	University of Leeds, Faculty of Biological Sciences, UK	m.peckham@leeds.ac.uk
Plückthun, Andreas	University of Zurich, Biochemistry, Switzerland	plueckthun@bioc.uzh.ch
Porter Goff, Kate Louise	University of Bristol, School of Chemistry, UK	kp0549@bristol.ac.uk

Rhys, Guto	University of Bristol, School of Chemistry, UK	gr13205@bristol.ac.uk
Rink, Mathis	Georg-August-Universität Göttingen, Organic and Biomolecular Chemistry, Germany	mrink@gwdg.de
Saric, Merisa	University of Bayreuth, Biomaterials, Germany	merisa.saric@bm.uni-bayreuth.de
Schmidt, Nathan Warren	University of California, San Francisco, USA	Nathan.Schmidt@ucsf.edu
Schramm, Antoine	AFMB / CNRS, France	antoine.schramm@afmb.univ-mrs.fr
Scott, Alistair	University of Bristol, School of Chemistry, UK	ali.scott@bristol.ac.uk
Shen, Mengjie	Leiden university, Supramolecular & biomaterials chemistry, Netherlands	m.j.shen@lic.leidenuniv.nl
Soares Pereira, Joana Maria	Max Planck Institute for Developmental Biology, Protein Evolution, Germany	joana.pereira@tuebingen.mpg.de
Strelkov, Sergei	KU Leuven, Pharmaceutical Sciences, Belgium	sergei.strelkov@kuleuven.be
Sutherland, Tara	CSIRO, Australia	Tara.Sutherland@csiro.au
Szczepaniak, Krzysztof Łukasz	Center of New Technologies, University of Warsaw, Poland	k.szczepaniak@cent.uw.edu.pl
Thomas, Franziska	Georg-August-Universität Göttingen, Organic and Biomolecular Chemistry, Germany	fthomas@gwdg.de
Winkler, Anika	University of Bayreuth, Biomaterials, Germany	anika.winkler@bm.uni-bayreuth.de
Wood, Chris Wells	University of Bristol, School of Chemistry, UK	chris.wood@bristol.ac.uk
Woolfson, Dek	University of Bristol, School of Chemistry, UK	d.n.woolfson@bristol.ac.uk
Zerbe, Oliver	University of Zurich, Chemistry, Switzerland	oliver.zerbe@chem.uzh.ch