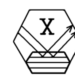

Session VI - Saturday, September 26

L33

STRUCTURAL STUDIES OF AvaII RESTRICTION ENDONUCLEASE - RNA/DNA HYBRID COMPLEX

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Restriction endonucleases naturally target DNA duplexes. However, a systematic screen has shown that a minority of enzymes can also cleave RNA-DNA hybrids[1]. The mechanistic basis of this promiscuity with respect to the sugar backbone of the substrate is not understood. We have crystallized AvaII, one of the enzymes that can cleave RNA/DNA hybrids, and present an apo-structure (with the RNA/DNA duplex bound in a non-productive conformation) together with models for the productive complexes with DNA/DNA and DNA/RNA duplexes, which shed light on the RNA/DNA hybrid cleaving activity of the enzyme.

1. I. A. Murray, S. K. Stickel, R.J. Roberts, *Nucleic Acid Res.*, **38(22)**, (2010), 8257-8268.

This work was supported by the National Science Centre (NCN, UMO-2011/02/A/NZ1/00052, N N301 028934 and N N301 425038).

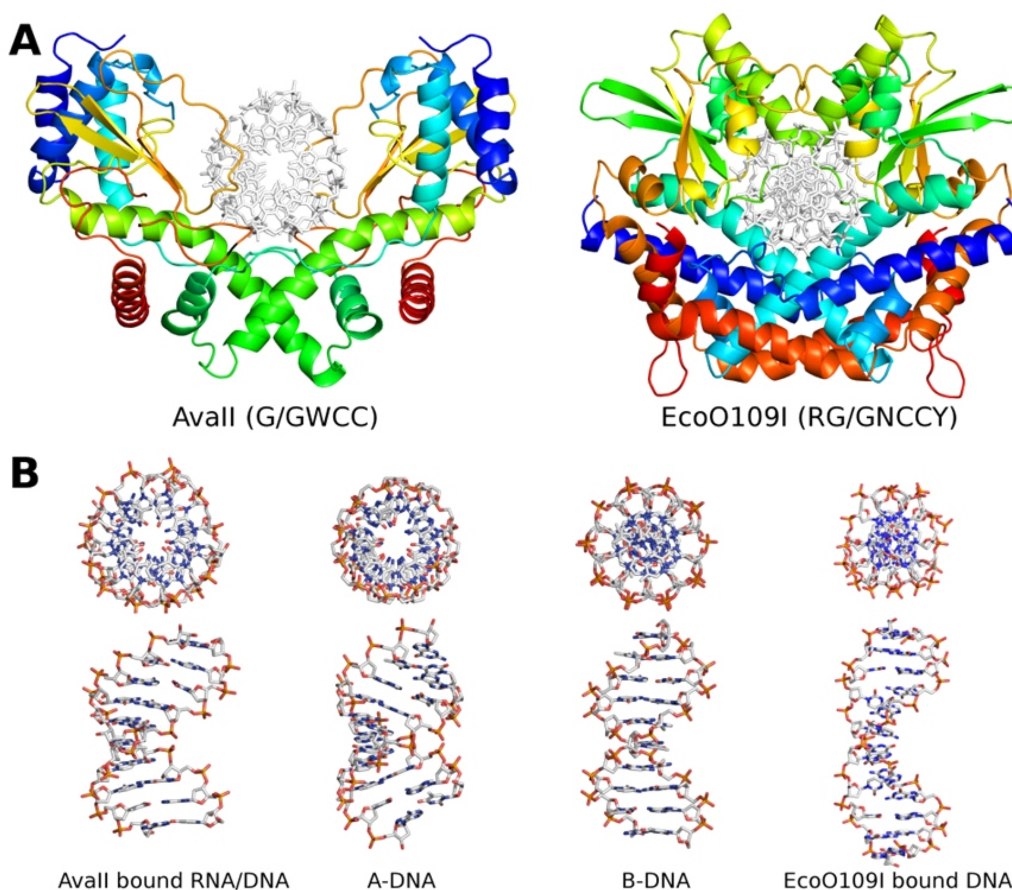
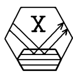


Figure 1. **A)** Overall structures of AvaII and EcoO109I nucleic acid complexes. The proteins are coloured from N- (blue) to C-termini (red). **B)** The conformation of RNA-DNA duplex in the AvaII complex in comparison with A-DNA, B-DNA and DNA duplex in the EcoO109I complex.



L34

STRUCTURAL CHARACTERIZATION OF THE SARCOMERIC Z-DISK FATZ: -ACTININ-2 COMPLEX

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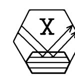
The sarcomeric Z-disc defines the lateral borders of the sarcomere and has been seen primarily as an important structure for mechanical stability. The core of a Z-disc consists of actin filaments coming from adjacent sarcomeres which are crosslinked by -actinin molecules. Apart from its major components, -actinin and F-actin, mature Z-disks comprise a number of other proteins. They are regarded as one of the most complex macromolecular structures in biology, acting as a platform for a dynamic web of interactions essential for muscle contraction and its homeostasis.

FATZ-1 (filamin-C -actinin telethonin Z-disk binding protein) is described as a node of protein-protein interactions, including associations with -actinin-2, filamin-C, myotilin, telethonin, calcineurin, ZASP/Cypher, and aciculin [1]. FATZ: -actinin-2 complex seems to appear in the early stage of the myofibrillogenesis [2], suggesting that FATZ acts as a protein adaptor that recruits to organize other proteins at the Z-disk. FATZ is predicted to be an intrinsically disordered protein, a property that allows it forming many different protein-protein complexes. Our aim here is to elucidate the structural basis of its complex formation with -actinin-2.

A combination of X-ray crystallography, SAXS, biochemical, and molecular biological techniques was used to understand the molecular mechanism of how FATZ-1 binds to -actinin-2. ITC experiments together with limited proteolysis and mass spectrometry techniques identified FATZ-1 binding sites on -actinin-2, providing new insights into the *modus operandi* of this protein complex in the Z-disk. Structure-based mutants were designed that specifically abrogate this complex formation.

Moreover, our preliminary X-ray diffraction data on FATZ-1: -actinin-2 complex at 3.7 Å resolution show the conformational state of -actinin-2 bound to FATZ, which suggests orientation diversity of the former [3]. Work on crystal structure refinement, crystallization conditions, and FATZ-1 constructs for complex formation is ongoing.

1. G. Faulkner, A. Pallavicini, A. Comelli, M. Salamon, G. Bortoletto, C. Ievolella, S. Trevisan, S. Kojic, F. Dalla vecchia, P. Laveder, G. Valle, G. Lanfranchi *J. Biol. Chem.,* **275**, (2000), 41234-42.
2. W. Jushuo, S. Nathan, M. Balraj, Z. Qiang, C. Ju, M. S. Jean, W. S. Joseph *Cell Motil. Cytoskeleton* 61 (2005), 34-48.
3. E. de A. Ribeiro, N. Pinotsis, A. Ghislen, A. Salmazo, P. V. Konarev, J. Kostan, B. Sjöblom, C. Schreiner, A. A Polyansky, E. A. Gkougkoulia, M. R. Holt, F. L. Aachmann, B. Zagrovi, E. Bordignon, K. F. Pirker, D. I. Svergun, M. Gautel, K. Djinovic-Carugo *Cell* **159** (2014), 1447-60.



L35

STRUCTURAL BASIS OF NON-MUSCLE -ACTININ REGULATION BY CALCIUM

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-Actinin functions as scaffolding protein crosslinking actin filaments into actin bundles. This is achieved via a particular antiparallel dimeric topology in which each monomer comprises an N-terminal actin binding domain (ABD), a connecting segment (NECK), a central rod domain built by spectrin-like repeats, and a C-terminal calmodulin-like domain with four EF hand motifs (EF12-EF34). While muscle -actinin isoforms (2 and 3) are regulated by phosphoinositides, the non-muscle isoforms (1 and 4) are controlled by calcium binding to EF hands [1]. Accordingly, calcium concentration $>10^{-7}$ M reduces or even abolishes their actin bundling capacity. However, the molecular mechanism of this regulation remains unknown. We hypothesise that calcium binding to EF12 induces a conformational change that has an impact on the position of ABD actin binding. To address this question we focused on -actinin isoform 2 from parasite *Entamoeba histolytica* (ehACTN2), which shows high sequence identity with human non-muscle homologs [2]. It also contains a shorter rod domain (two spectrin-like repeats vs. four in human proteins), thus representing an ancestral form of -actinin.

Low-speed actin co-sedimentation assays using ehACTN2 revealed a significant decrease of actin bundling activity when the experiment was performed in the presence of calcium. We further used the recently published crystal structure of hACTN2 to design mutations on ehACTN2 disrupting contacts between NECK and EF34, which abolished completely its bundling capacity. The same result was obtained for a protein deletion variant lacking EF34. Circular dichroism showed identical secondary structure conformation for both "NECK" mutant and EF34 deletion variant when compared to that of wild type protein. In addition, these experiments evinced a substantial increase in stability of the wild type ehACTN2 upon calcium binding. The stabilizing effect of calcium was further corroborated by limited proteolysis experiments in which a calcium-insensitive mutant was cleaved significantly faster than wild type protein.

Structural analysis in solution by SAXS revealed essentially the same conformation for wild type ehACTN2 in the absence and presence of calcium as well as for calcium-insensitive mutant. However, both the "NECK" mutant and the EF34 deletion variant showed a less extended, more globular conformation, suggesting variable orientations of ABD. Finally, the crystal structures of calcium-bound wild type ehACTN2 and of calcium-insensitive mutant were determined, showing an overall architecture similar to that of hACTN2. Most interestingly, unlike in hACTN2, EF12 (which coordinates calcium) is placed between the two spectrin-like repeats, while the EF34 wraps around NECK. In addition, the calcium-insensitive mutant evinces flexibility for ABD and certain regions of EF12-EF34. To sum up, we hypothesize that calcium binding introduces a conformational change that is transmitted from EF12 to EF34, which subsequently affects the flexibility of NECK and reduces orientation sampling for ABD. These results highlight the importance of the proper positioning and flexibility of ABD for actin bundling.

1. KS Foley and PW Young, *Biochem. J.* **459** (2014), 1-13.
2. A Virel, B Addario, and L Backman, *Mol. Biochem. Parasitol.* **154** (2007), 82-9.

L36

STRUCTURAL STUDIES ON THE SYNAPTIC ACTIVE ZONE SCAFFOLD

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Synaptic vesicles (SV) fuse at specialized membrane compartments called active zones (AZs). The intracellular face of a presynaptic AZ membrane is decorated by an electron-dense scaffold, termed cytomatrix at the AZ (CAZ; Figure 1) [1]. In recent years, an evolutionarily conserved set of large, multi-domain proteins operating as major scaffold building blocks at the AZs was identified: Syd-2/Liprin-á, RIM, RIM-binding-protein (RBP) and ELKS family proteins, such as the protein Bruchpilot (BRP) in *Drosophila melanogaster* [2].

Neither the structural rules, by which these AZ scaffolds assemble, nor the roles of these scaffolding proteins for the SV exo-/endocytic cycle are presently well understood. To address these questions, we started to identify folded regions and interacting domains in AZ scaffold components and to elucidate the structures of individual units and sub-complexes by X-ray crystallography.

1. Haucke, V., Neher, E., and Sigrist, S.J. (2011) Protein scaffolds in the coupling of synaptic exocytosis and endocytosis. *Nat Rev Neurosci* **12**, 127-138.
2. Südhof, T.C. (2012) The presynaptic active zone. *Neuron* **75**, 11-25.

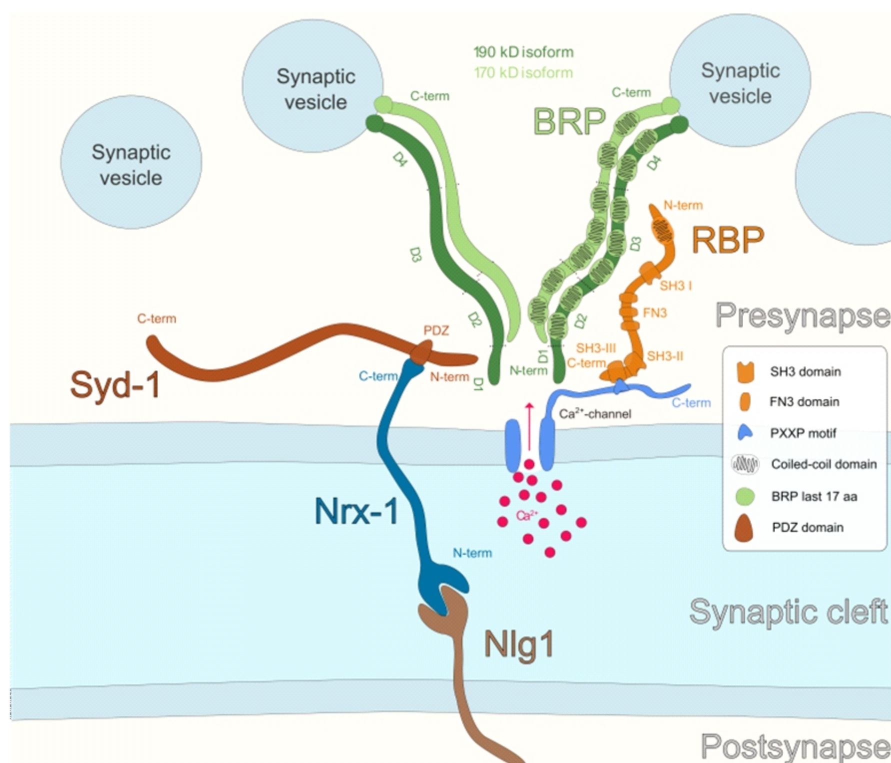
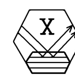


Figure 1. Scheme of a *Drosophila* AZ, showing some of the major scaffolding proteins in the CAZ.



L37

STRUCTURAL STUDIES ON THE SELECTIVITY OF SNF BINDING TO U1 AND U2 snRNAs IN *DROSOPHILA MELANOGASTER*

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The spliceosome is a large and highly dynamic RNA-protein molecular machine that is assembled from five complex RNA-protein subunits (the snRNPs U1, U2, U4, U5 and U5) and many non-snRNP proteins. The U2 snRNP contains the U2B" and U2A' proteins in vertebrates, or SNF and U2A' in most other metazoans. U2B" and SNF bind directly to stem loop IV (SLIV) of U2 snRNA, using their N-terminal RNA recognition motifs (RRM). SNF is also a protein component of the U1 snRNP, where it binds to SLII of U1 snRNA. In vertebrate U1 snRNP, a different protein, U1A, binds SLII. Only in the U2 snRNP do SNF and U2B" use their RRMs to bind to both SLIV and to the protein U2A'. Using proteins and RNAs from *Drosophila melanogaster*, we determined crystal structures of (i) SNF alone, (ii) the binary SNF-SLII^{U1} complex, (iii) the binary SNF-U2A' complex and (iv) the ternary SNF-U2A'-SLIV^{U2} complex to address two specific questions concerning U1 and U2 snRNP assembly. First, in organisms that use SNF, how is U2A' excluded from the U1 snRNP? Second, in organisms that use U2B", why is the U2B"/U2A' complex found only in the U2 snRNP?

1. Price, S. R., Evans, P. R. & Nagai, K. Crystal structure of the spliceosomal U2B"-U2A' protein complex bound to a fragment of U2 small nuclear RNA. *Nature* **394**, 645-650, doi:10.1038/29234 (1998).
2. Oubridge, C., Ito, N., Evans, P. R., Teo, C. H. & Nagai, K. Crystal structure at 1.92 A resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin. *Nature* **372**, 432-438, doi:10.1038/372432a0 (1994).

L38

GRISELIMYCINS, NOVEL ANTI TUBERCULOSIS AGENTS: STRUCTURAL INSIGHTS INTO THE MODE OF ACTION, RESISTANCE AND BIOSYNTHESIS

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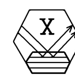
Tuberculosis remains a major global health problem that caused an estimated 1.5 million deaths in 2013 [1], whereby a growing percentage is multidrug resistant tuberculosis. Novel anti TB agents with distinct mechanism of action are urgently needed. Griselimycins (GMs) from *Streptomyces caelicus* were found to exhibit bactericidal effects almost exclusively on mycobacteria and were thus developed as promising anti-TB agents showing exceptional potential in *in vivo* mice models [2].

Aiming to identify the mode of action of these novel antibacterial lead substances we analyzed the biosynthetic gene cluster of GMs and found that the GM producing strain *S. caelicus* harbors an additional variant of the *dnaN* gene (encoding the sliding clamp of DNA polymerase) in the GM biosynthetic gene cluster. Upon heterologous overexpression, this variant confers GM resistance to GM sensitive *Streptomyces*. While the common DnaN of the producer strain seems to be inhibited by GMs, this is not the case for the additional variant, which thus represents a mechanism of GM self-resistance and hence lead to the identification of bacterial DnaN as a novel target for a natural product. As it represents an important building block and the only non-proteinogenic amino acid incorporated in GMs, we also studied the biosynthesis of (2S, 4R)-4-methylproline by *S. caelicus.* Here, we focused our research on the hydroxylation of the starter substrate L-leucine as this step determines the stereochemistry of the 4-methylproline incorporated in GMs.

Mycobacterial DnaN and both sliding clamp variants of the GM producer strain were heterologously expressed, purified and crystallized in the presence of GMs. In addition, binding of GMs to the proteins was characterized by SPR. The crystal structures clearly show the binding of GMs and give insight into their mode of action [2]. In the case of the *S. caelicus* proteins, the structures point also to a potential mechanism of GM self-resistance. Furthermore, we could solve the crystal structure of the leucine hydroxylase MPS1 in complex with its substrates. Due to substrate turnover in co-crystallization setups, we were also able to solve the structure of MPS1 in complex with its reaction products, and could thus explain its stereospecificity.

1. World Health Organization, *Global Tuberculosis Report 2014*, (2014).
2. A. Kling, P. Lukat, D. V. Almeida, A. Bauer, E. Fontaine, S. Sordello, N. Zaburannyi, J. Herrmann, S. C. Wenzel, C. König, N. C. Ammerman, M. B. Barrio, K. Borchers, F. Bordon-Pallier, M. Brönstrup, G. Courtemanche, M. Gerlitz, M. Geslin, P. Hammann, D. W. Heinz, H. Hoffmann, S. Klieber, M. Kohlmann, M. Kurz, C. Lair, H. Matter, E. Nuermberger, S. Tyagi, L. Fraisse, J. H. Grosset, S. Lagrange, R. Müller, *Science*, **348**, (2015), 1106.



L39

CRYSTAL STRUCTURE OF A COMMON GPCR-BINDING INTERFACE FOR G PROTEIN AND ARRESTIN

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G-protein-coupled receptors (GPCRs) transmit extracellular signals to activate intracellular heterotrimeric G proteins (G   ) and arrestins [1]. For G protein signalling, the G   C-terminus (G CT) binds to a cytoplasmic crevice of the receptor that opens upon activation [1, 2, 3] A consensus motif is shared among G CT from the Gi/Gt family and the 'finger loop' region (ArrFL1–4) of all four arrestins [5]. Here we present a 2.75 Å crystal structure of ArrFL-1, a peptide analogue of the finger loop of rod photoreceptor arrestin, in complex with the prototypical GPCR rhodopsin [5]. Functional binding of ArrFL to the receptor was confirmed by UV/Vis spectroscopy, competitive binding assays and Fourier transform infrared spectroscopy. For both G CT and ArrFL, binding to the receptor crevice induces a similar reverse turn structure, although significant structural differences are seen at the rim of the binding crevice. Our results reflect both the common receptor-binding interface and the divergent biological functions of G proteins and arrestins [5].

1. Hofmann KP, Scheerer P, Hildebrand PW, Choe HW, Park JH, Heck M, Ernst OP. A G protein-coupled receptor at work: the rhodopsin model. *TIBS* 2009; **34**, 540-.
2. Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, Hofmann KP, Ernst OP. Crystal structure of opsin in its G-protein-interacting conformation. *Nature* 2008; **455**, 497-.
3. Choe HW, Kim YJ, Park JH, Morizumi T, Pai EF, Krauss N, Hofmann KP, Scheerer P, Ernst OP. Crystal structure of metarhodopsin II. *Nature* 2011; **471**, 651-.
4. Kim YJ, Hofmann KP, Ernst OP, Scheerer P, Choe HW, Sommer ME. Crystal structure of pre-activated arrestin p44. *Nature* 2013; **497**, 142-.
5. Szczepek M, Beyričre F, Hofmann KP, Elgeti M, Kazmin R, Rose A, Bartl FJ, von Stetten D, Heck M, Sommer ME, Hildebrand PW, Scheerer P. Crystal structure of a common GPCR-binding interface for G protein and arrestin. *Nat Commun*. 2014; **5**:4801-.

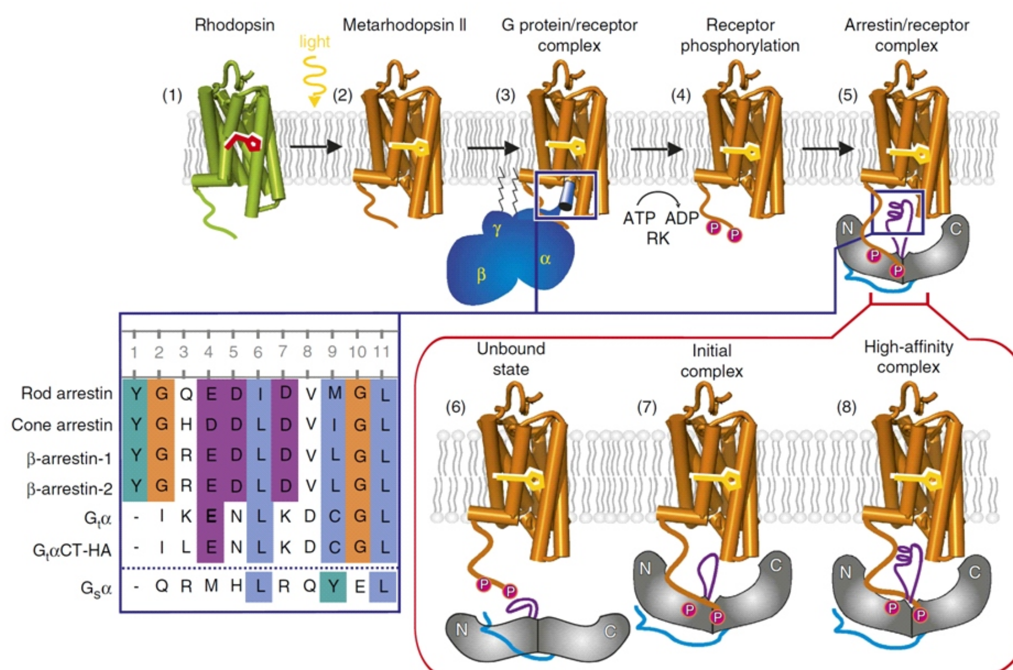

Figure 1. Overview of rhodopsin signal transduction and deactivation. (1) Dark-state bovine rhodopsin with inverse agonist 11-*cis*-retinal. (2) Light-activation and formation of metarhodopsin II (Meta II) with agonist all-*trans*-retinal [3]. (3) Coupling to the heterotrimeric G protein [2, 3]. (4) Phosphorylation of the activated receptor by its specific kinase. (5) Rod photoreceptor arrestin binding to the phosphorylated receptor. Inset (red box): (6) Basal arrestin approaches phosphorylated Meta II. (7) Interaction with receptor-attached phosphates activates arrestin [4]. (8) Tight binding of the receptor by arrestin (induces further structural changes in arrestin finger loop) [5]. Inset (indigo box): Sequence alignment of proposed common sequence motif: rod photoreceptor arrestin (Arr1, residues 68–77), cone photoreceptor arrestin (Arr4, residues 63–72), -arrestin-1 (Arr2, residues 64–73), -arrestin-2 (Arr3, residues 65–74), wild-type Gt CT (residues 340–349), high-affinity variant Gt CT-HA (residues 340–349), Gs (residues 385–394).