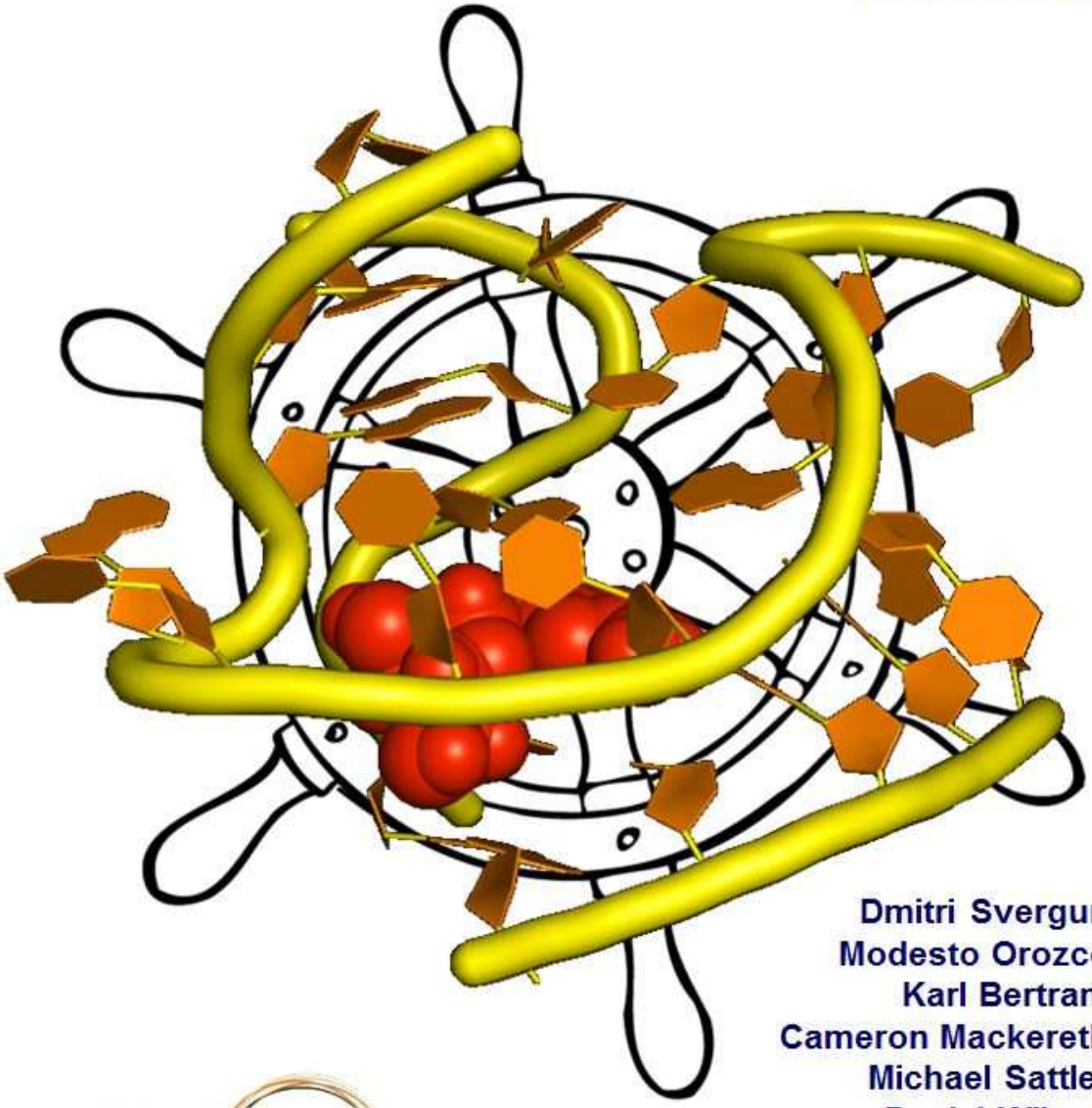
RNA Structural Biology

28.-29.11.
Forschungskolleg Humanwissenschaften
Bad Homburg



Dmitri Svergun
Modesto Orozco
Karl Bertram
Cameron Mackereth
Michael Sattler
Daniel Wilson
Mark Helm
Olav Schiemann
David Lilley

SFB 902

Monday, 28.11.2016		Tuesday, 29.11.2016	
Registration, Welcome Coffee		09:00 – 10:30	PIs meet invited Speakers
		Session 3 – Chair: Hetzert/Schwich	
		11:00 – 11:30 h	Michael Sattler (Student Choice)
		11:30 h	Christina Helmling
		11:45 h	Sven Warhaut
12:00 h: Sandwiches		**12:00 h: Lunch**	
12:45 h	Welcome	**12:45 – 14:00 h: Student Interaction Session, Coffee & Snacks**	
Session 1 - Chair: Hummer			
13:00 h	Dmitri Svergun		
13:30 h	Henrik Gustmann		
13:45 h	Valentin Hodirnau		
14:00 h	Modesto Orozco	*Session 4 – Chair: Prisner*	
		14:00 h	Martin Hengesbach
14:30 h	Lukas Stelzl	14:30 h	Olav Schiemann
14:45 h	Nicole Erlenbach		
15:00- 15:30 h	***Coffee Break***	15:00 h	Claudia Grytz
		15:15 h	Monu Kaushik
Session 2 - Chair: Wöhnert		15:30 h	David MJ Lilley
15:30 h	Karl Bertram		
16:00 h	Nusrat Qureshi	16:00- 16:30 h	***Coffee Break***
16:15 h	Antje Wolter		
16:30 h	Cameron Mackereth	*Session 5 – Chair: Schwich/Hetzert*	
		16:30 h	Daniel Wilson
17:00 h	Samarth Thonta Setty	17:00 h	Johannes Braun
17:15 h	Nicolas Jaé	17:15 h	Elke Duchard-Ferner
17:30 h	Konstantinos Stellos	17:30 h	Mark Helm (Student Choice)
PIs Dinner: La Vecchia Banca		***Dinner for all at the FKH & Goodbye***	

Inhalt

Small-angle X-ray synchrotron scattering to study protein and RNA structure in solution

Dmitri I. Svergun

European Molecular Biology Laboratory, Hamburg Outstation c/o DESY, Notkestr. 85, D22603 Hamburg, Germany

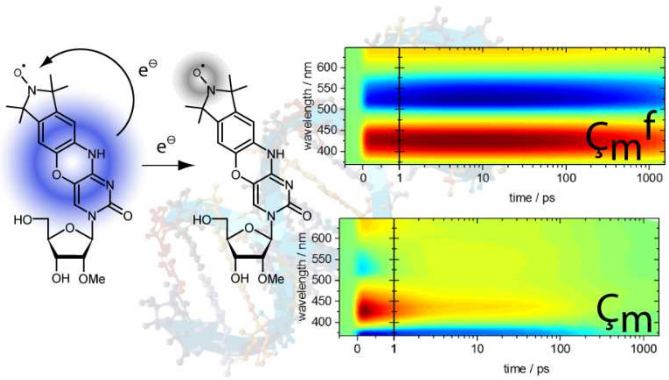
Small-angle X-ray scattering (SAXS) experiences a renaissance in the studies of macromolecular solutions allowing one to analyze the structure of native particles and complexes and to rapidly assess structural changes in response to variations in external conditions. Novel data analysis methods significantly enhanced resolution and reliability of the structural models provided by the technique [1]. Automation of the experiment, data processing and interpretation converted solution SAXS on high brilliance synchrotrons into a streamline tool for large scale structural studies in molecular biology. The technique is also applicable to medical formulations allowing direct use for pharmaceutical industry. SAXS is readily combined the high resolution methods like crystallography and NMR, but also with cryo-EM and complementary biophysical and biochemical techniques. Rapid validation of predicted or experimentally obtained high resolution models in solution, identification of biologically active oligomers and addition of missing fragments to high resolution crystallographic models are possible. For macromolecular complexes, quaternary structure can be effectively analyzed in terms of rigid body movements/rotations of individual subunits, if their structures are available. SAXS can also be applied to characterize the solution states of flexible and intrinsically disordered macromolecules and to quantitatively analyze mixtures of states in combination with on-line size-exclusion chromatography. Recent developments at the EMBL SAXS beamline P12 (Petra-3, Hamburg) will be reviewed and applications of the novel methods to analyze solution structures of proteins and various RNA molecules will be presented.

1. Graewert MA, Svergun DI. (2013) Impact and progress in small and wide angle X-ray scattering (SAXS and WAXS). Curr Opin Struct Biol. 23:748-54.

A multistep quenching mechanism yields a fluorescent RNA-label in disguise

Henrik Gustmann

A steady state and time-resolved UV-vis characterization was performed for the RNA spin-label Çm-spin and its fluorescent precursor Çm^f in aqueous solution. Çm and Çm^f are designed for EPR or fluorescence studies of RNA. Beside steady state absorption, fluorescence and phosphorescence measurements as well as quantum yield determinations the photodynamics of both labels were studied by femtosecond time-resolved absorption spectroscopy and time correlated single photon counting (TCSPC).



It was thus possible to show and quantify the fluorescence quenching caused by the nitroxide group of Çm. The fact that the signal decay of the spin-label is nearly 10 times faster than for the fluorescence label arouses interest in the underlying quenching mechanism. Spectroscopic and theoretical data indicate that the fluorescence quenching is due to a multistep mechanism, including electron transfer, internal conversion as well as intersystem crossing steps. Based on this detailed understanding its photo-physical properties Çm^f can now be deployed as a fluorescent label in time-resolved investigations of RNA strands. We examined a series of labeled RNA single and double strands as test systems. Here it was possible to distinguish between single and double strands and between purine and pyrimidine bases as direct neighbors of the fluorophore by various steady state and time-resolved spectroscopic methods.

References

Gustmann, H., Reuss, A. J.; Lefrancois, D.; Gophane, D. B.; Braun, M.; Dreuw, A.; Sigurdsson, S. T.; Wachtveitl, J.

Structure of RNA polymerase I transcribing rDNA genes

Valentin Hodirnau

RNA polymerase I (Pol I) synthesises ribosomal RNA (rRNA) and regulates cell growth of eukaryotic cells. We study the structure of Pol I in its active, transcribing conformation by two different cryo-electron microscopy methods: cryo-electron tomography by which we visualize whole Miller trees, depicting the nascent transcription products resulting from transcribing Pol I enzymes which are connected to DNA and single-particle analysis that even resolves the active center that binds DNA and RNA. The structures suggest a model for the regulation of transcription elongation where contracted and expanded polymerase conformations are associated with active and inactive Pol I states, respectively.

Atomistic simulations of nucleic acids

Modesto Orozco

Atomistic simulations, and in particular Molecular Dynamics (MD) are becoming instrumental tools to study nucleic acids in a variety of environments. The central core of MD simulations is the force-field, a simple set of equation that links the coordinates of a system with its stability. I will briefly summarize the efforts of our group in refining force-fields, emphasizing the problems intrinsic to the extra layer of complexity produced by the presence of the 2'OH group in RNA.

Kinetics of RNA folding from Molecular Dynamics Simulations

Lukas S. Stelzl

Molecular dynamics (MD) simulations hold the promise to resolve the folding mechanism of RNA in atomistic detail. The folding of RNA is however slow on the MD timescale and powerful enhanced sampling methods have to be employed. Enhanced sampling methods can provide free energy surfaces for biomolecular folding, but these methods typically alter the dynamics, making it difficult or impossible to extract kinetic and mechanistic information directly. Here we show that with replica-exchange molecular dynamics (REMD), one can not only sample equilibrium properties, but also extract kinetic information. For systems that strictly obey first-order kinetics, the procedure to extract rates is rigorous. For actual molecular systems, whose long-time dynamics is captured by kinetic rate models, such as a rapidly folding peptide fragment and a slowly folding RNA, we obtain accurate rate coefficients. Extracting kinetics from REMD realizations of RNA folding will enable new mechanistic investigations, and provide a basis for the validation of simulations by comparison to experimental rates.

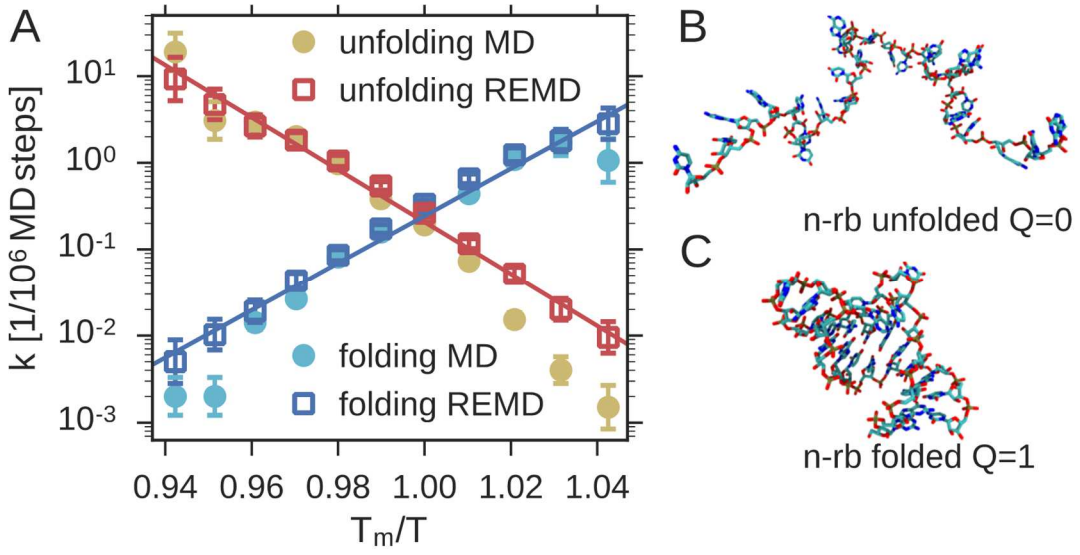


Figure: Rate coefficients for the folding and unfolding of the neomycin riboswitch (n-rb) from MD (closed symbols) and REMD (open symbols) as function of inverse temperature T_m/T, where T_m is the melting temperature (A). Arrhenius fits are shown as lines. (B) Unfolded and (C) folded n-rb. Time and temperature are reported in reduced units for simulations with a coarse-grained model of n-rb.

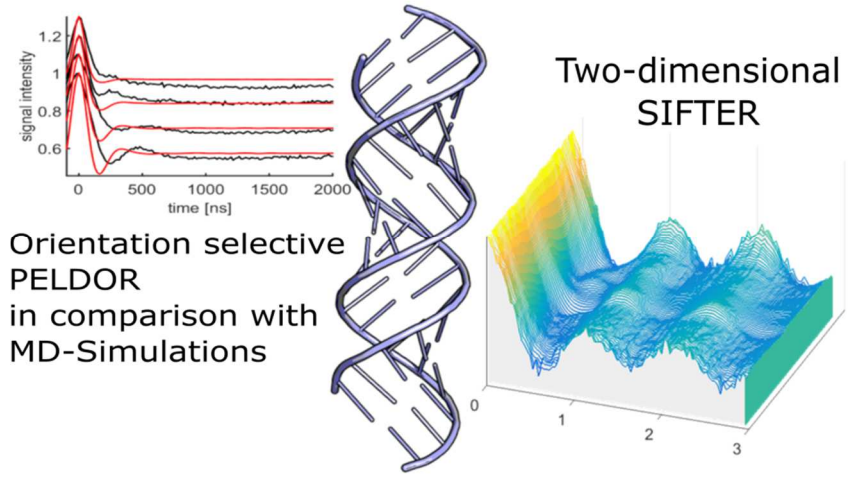
Conformational Dynamics of Nucleic Acids by Orientation Selective Pulsed EPR

Nicole Erlenbach

Orientation selective PELDOR (Pulsed Electron Electron Double Resonance) measurements on nucleic acids with rigid spin-labels provide crucial information about their structure and dynamics.[1] Experiments with the rigid spin label Ç have already yielded a mechanical model for the dynamics of dsDNA.[2]

Experimental PELDOR signals can also be used as sensitive benchmark data for evaluation of MD simulations on nucleic acid molecules. We found that recent force field updates constitute a significant improvement for modeling of dsDNA molecules. MD simulations show that dsDNA undergoes twist-stretch and bending motions in solution, which provides a more detailed picture of the conformational dynamics of dsDNA. For dsRNA, the comparison of first PELDOR experiments with MD simulations show larger deviations between experiments and theory, suggesting that further optimization of the force fields is required for accurate description.

With broadband excitation pulses, 2D-SIFTER (Single Frequency Technique for Refocusing Dipolar Couplings)[3] allow to obtain the orientation selective data set by direct Fourier transform of the solid echo signal. Such experiments offer improved S/N for the determination of the angular information between two rigidly attached nitroxide spin labels. Initial 2D SIFTER measurements and simulations on a model compound and a dsRNA



molecule will be presented.

References

Prisner, T.F.; Marko, A.; Sigurdsson, S.T., *JMR* 252, 187–198 (2015)
Marko, A.; Denysenkov, V.; Margraf, D.; Cekan, P.; Schiemann, O.; Sigurdsson, S. T.; Prisner,T. F., *JACS* 133, 13375 (2011).
Doll, A.; Jeschke, G., P*CCP*, 17,18(2016)
Erlenbach, N.; Stelzl, L.; Bowen, A.; Schöps, P.; Hummer G.; Prisner, T.F.

Structural characterization of the rS1-protein and its mRNA complexes

Nusrat Qureshi

The ribosomal protein S1 (rS1) is a RNA binding multi-domain protein, consisting of six imperfect OB fold repeats, with varying sequence homology [1]. It plays a key role in the process of translation initiation, as it facilitates recognition of most, if not all, mRNAs by ribosomes [2]. Its modular organization allows rS1 to bind diverse mRNAs by transient, non-sequence-specific interactions and, simultaneously, to establish contacts to proteins. Recently, solution NMR structures of individual domains were solved [3, 4]. However, to date, there is still no structure available regarding the RNA-binding region. Our aim is to structurally characterize the RNA binding region of rS1 by solution-state NMR and to study its interaction with the translation initiation region (TIR) of the 5´-UTR of an mRNA, comprising an adenine dependent riboswitch (ASW). Therefore, in accordance to Bisaglia *et al.* [5] we designed five multi-domain constructs, by stepwise truncating the RNA binding region from the N- and C-terminal ends of the protein and analyzed their functionality with electrophoretic mobility shift assays. We found the two-domain construct rS134 (E187-S356) to be the shortest RNA binding construct and are now completing the resonance assignment, in order to solve the structure. In addition, we studied its interaction with the expression platform of the ASW by solution NMR. The timescale for this interaction appears to be within the intermediate to fast exchange time regime. This finding indicates a dynamic interaction between the protein and the RNA, which is characterized by relative high k_{off}-values and is in accordance with previous studies [1]. As a mediator of translation initiation, the rS1-protein has to bind and prepare the mRNA for the interaction with the ribosome. Subsequently it has to dissociate from the mRNA, to enable the formation of the translation initiation complex.

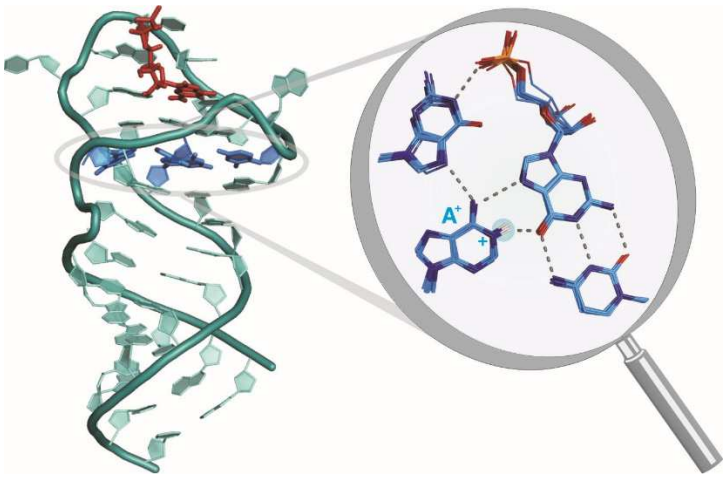
References

[1] Subramanian, A.R. *Prog. Nucleic Acids Res.* (1983) *28*, 101-142.
[2] Hajnsdorf, E.; Boni I. V. *Biochimie* (2012) 94, 1544-1533.
[3] Salah, P.; Bisaglia, M.; Aliprandi, P.; Uzan, M.; Sizun C.; Bontems, F. *Nucleic Acids Res.* (2009) *37*, 5578-5588.
[4] Giraud, P.; Crechet, J. B.; Uzan, M.; Bontems, F.; Sizun C. *Biomol. NMR Assign.* (2014) 9, 107-111.
[5] Bisaglia, M. Laalami, S. Uzan, M. Bontems, F. *J. Biol. Chem.* (2003) 278, 15261-15271.
Qureshi, N.; Fürtig, B.; Jonker, H.; Schwalbe, H.

The Structure of a GTP-bound Aptamer Reveals a Complex RNA-Fold and a Stably Protonated Adenine

Antje Wolter

The GTP class II aptamer is one of 11 structurally and sequentially diverse GTP-binding RNA aptamers. Despite its small size (34 nt) it binds GTP with a K_D in the nanomolar range[1]. We initiated structural studies of the aptamer GTP-complex by solution NMR in order to gain insight into the structural diversity of GTP recognition by different aptamers[2].



We solved the structure of the RNA-GTP complex by solution NMR with a low overall rmsd (0.5 Å). GTP binds to the apical loop of the aptamer and is recognized in a base triplet containing an intermolecular Watson-Crick base-pair and a sugar edge interaction with an adenine. The base of the ligand binding site consists of an intramolecular base triplet with a similar architecture. In addition, the complex structure features a base quadruplet adjacent to the base of the ligand binding site. In this quadruplet, a guanosine imino group forms a hydrogen bond to a phosphate group oxygen. Moreover, an adenosine is stably protonated at its N1 position with an NMR-observable imino proton resonance. The pK_a for the protonated adenine in this base quartet is ~ 9.0 and thus shifted by more than five pH units compared to free adenosine (3.7). Interestingly, the free RNA exists as a partially pre-folded conformational ensemble already containing the protonated adenine and the base quartet at pH 5.3 in the presence of Mg^{2+}. This suggests that the ligand selects the prefolded, protonated state of the aptamer for binding and stabilizes its structure even further.

References

[1] Carothers, J. M.; Oestreich, S. C.; Davis, J. H.; Szostak, J. W. *J. Am. Chem. Soc.*, 126, 5130-5137 (2004)
[2] Wolter, A. C.; Duchardt-Ferner, E.; Nasiri, A. H.; Hantke, K.; Wunderlich, C. H.; Kreutz, C.; Wöhnert, J. *Biomol. NMR Assignments,*10, 1,101-105 (2016)

Molecular details of neuron- and muscle-specific alternative splicing

Cameron Mackereth

The expression of tissue-specific splicing factors contributes to alternative splicing regulation during development. We have investigated the atomic details of pre-mRNA binding by several proteins involved in both muscle and neuron biology in the nematode C. elegans. The solution structure and molecular details of RNA-binding of the muscle-specific SUP-12 protein has led to a series of in vivo splicing assays in live worms using a fluorescent two-colour reporter system built on the isoform regulation of the EGL-15 fibroblast growth factor receptor. In neurons, we have similarly looked at the association of the MEC-8 splicing factor with mec-2 pre-mRNA. The first RNA recognition motif (RRM) domain of MEC-8 is homologous to the RRM domains from Drosophila couch potato and the human protein RBPMS (RNA-binding protein with multiple splicing). Apart from roles in early development, MEC-8 is integral to worm mechanosensory function via the regulation of the mec-2 gene. Recent crystal structures have revealed the key elements in the formation of the RNA-bound MEC-8 protein, and we have determined the critical elements of the RNA sequences bound by MEC-8 in target pre-mRNA. These and other studies continue to build a molecular-based understanding of tissue-specific alternative splicing which we have coupled with in vivo assays and strategic perturbation of protein-RNA interaction

High-throughput screening for splicing regulatory regions

Samarth Thonta Setty

Alternative splicing (AS) is a co- or post-transcriptional process where one gene gives rise to multiple isoforms. This ‘split and combine’ step has increased eukaryotic proteome diversity manifold and has been implicated in several diseases given its pervasive impact. Many proteins recognize distinct splicing regulatory regions across the transcriptome and their combinatorial interplay results in a complex ‘splicing code’. However, the implication of these splicing regulatory regions in shaping the splicing outcome remains poorly understood.

Here, we have established a high-throughput method to screen splicing regulatory regions to comprehensively identify and characterize all such regions that determine a particular splicing decision. As a proof-of-principle, we performed the screen with a minigene harbouring a disease-relevant alternative exon of the RON receptor kinase gene, whose skipping isoform is implicated in cancer progression by promoting epithelial-to-mesenchymal transition. A library of thousands of randomly mutagenized minigene variants was transfected as a pool into human HEK293T cells and the spliced isoforms were subsequently analysed by RNA sequencing. Importantly, a barcode sequence was used to tag the minigene variants and thereby linked mutations to their corresponding spliced products. Using this approach, we identified many previously unknown regulatory regions, in addition to already known regulatory regions. In summary, this novel screening approach introduces a tool to study the relationship of RNA sequence variants and their impact on splicing outcome, offering new insights into the fundamental workings of alternative splicing regulation as well as the relevance of mutations in human disease. This will help in reconstructing the underlying regulatory networks and will provide a blueprint for ultimately cracking the splicing code.

References

Thonta Setty, S.; Braun, S.; Busch, A.; Ebersberger, S.; König, J.; Zarnack, K.

The Antisense Transcript of GATA6 Interacts with the H3K4me3 Deaminase LOXL2 and Epigenetically Regulates Endothelial Gene Expression

Nicolas Jaé

Although the human transcriptome is mainly non-coding, little is known about the function of long non-coding RNAs. Currently, a broad functional spectrum -including miRNA-sponging, splicing modulation, or recruitment of chromatin modifiers- is suggested. Regarding endothelial cells, the response to hypoxia and the regulation of angiogenic activity are key events in the context of several diseases, however, the involvement of lncRNAs is not conclusively defined.

By using RNA-seq, we identified the lncRNA GATA6-AS to be up-regulated by hypoxia in HUVECs. Functional studies revealed that silencing of GATA6-AS strongly impairs sprouting angiogenesis *in vitro* and cellular fractionation showed a predominant nuclear localization. Therefore, we characterized GATA6-AS in more detail: First, by using antisense purification and MS, as well as RIP and pulldowns assays, we identified the chromatin modifier LOXL2 (H3K4me3 deaminase) as associated protein. Second, gene expression profiling identified ~71% of GATA6-AS-regulated genes to be inversely regulated by LOXL2 silencing, including numerous angiogenesis-related genes, e.g. ICAM1. Third, GATA6-AS silencing lead to increased co-IP efficiencies of the ICAM1 promoter in LOXL2 chromatin immuno-precipitations and in parallel reduced global H3K4me3 levels. In summary, these results strongly argue for GATA6-AS acting as negative regulator of nuclear LOXL2.

References

Jaé, N.; Neumann, J.; John, D.; Uchida, S.; Krüger, M.; Dimmeler, S.

Decoding protein-RNA recognition in gene regulation using integrated structural biology

Michael Sattler

Institute of Structural Biology, Helmholtz Zentrum München, Neuherberg, Germany;
Center for Integrated Protein Science Munich and Biomolecular NMR, Technische Universität München, Garching, Germany

RNA plays essential roles in virtually all aspects of gene regulation. These processes involve the recognition of *cis* elements in the RNA, which provide binding sites for RNA binding proteins (RBPs). Most eukaryotic RBPs are multi-domain proteins that comprise multiple structural domains for protein-RNA but also protein-protein interactions. To unravel the molecular basis of these interactions and the underlying RNP (ribonucleoprotein) code we employ integrated structural biology. Solution techniques such as NMR-spectroscopy and SAXS/SANS are combined with X-ray crystallography to study the structural mechanisms and dynamics of the molecular interactions of multi-domain RNA binding proteins. Examples will be presented that highlight the role of conformational dynamics in RNA recognition and protein-protein interactions during eukaryotic pre-mRNA splicing, translational regulation and RNA stability.

NMR structural profiling of transcriptional intermediates reveals riboswitch regulation by metastable RNA conformations

Christina Helmling

The I-A type 2'-deoxyguanosine sensing (2'dG) riboswitch from the bacterium Mesoplasma Florum regulates the expression of ribonucleotide reductase genes.[1–3] At high cellular concentrations of 2'dG, gene expression is repressed as a result of premature transcription termination. In the thermodynamic equilibrium of the full-length mRNA, the 2'dG riboswitch adopts the terminator conformation regardless of whether the ligand is present. This finding suggests kinetic riboswitch control, where both ligand-binding by the aptamer domain and antiterminator formation are co-transcriptional events. In order to mimic the progress of transcription, we determined the secondary structure adopted by 25 transcriptional intermediates and characterized their ligand binding properties by applying a procedure developed to rapidly screen the secondary structure of RNA by NMR.[4] The results show that co-transcriptional folding allows the transcription to progress and facilitate gene expression by extending a thermodynamically favored intermediate structure. This intermediate structure has to be adopted within the transcription of 24 nucleotides. In the presence of ligand, full ligand binding can only be observed directly after the aptamer is transcribed over a sequence of 10 nucleotides. We find that even the full-length riboswitch only reaches 65% binding efficiency compared to the aptamer domain alone. Our data provide an essential understanding for the coupled thermodynamic and kinetic folding pathway by revealing distinct time frames, during which regulatory decisions must be made.

References

[1] Kim, J. N.; Roth, A.; Breaker,R. R., *Proc. Natl. Acad. Sci. U. S. A.* , 104, 16092–16097(2007)
[2] Wacker, A.; Buck, J.; Mathieu, D.; Richter, C.; Wöhnert, J.; Schwalbe, H., *Nucleic Acids Res.* , 39, 6802–12 (2011)
[3] Pikovskaya, O.; Polonskaia, A.; Patel, D. J.; Serganov A., *Nat. Chem. Biol.* , 7, 748–55 (2011)
[4] Helmling, C.; Keyhani, S.; Sochor, F.; Fürtig, B.; Hengesbach, M.; Schwalbe, H., *J. Biomol. NMR* DOI 10.1007/s10858-015-9967-y (2015)

Two perspectives of functional RNA dynamics: NMR and single-molecule FRET spectroscopy of the adenine-sensing riboswitch thermostat

Sven Warhaut

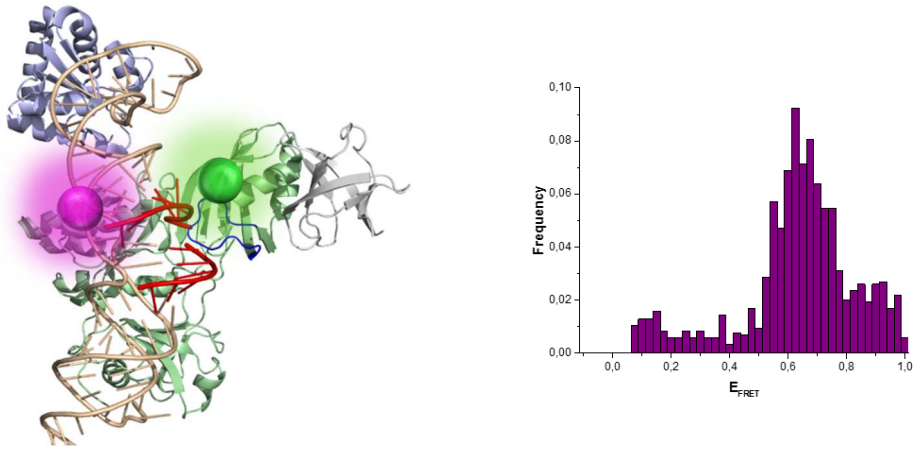
Riboswitches are mRNA elements that regulate the expression of downstream genes by ligand-induced conformational switching between gene-OFF and gene-ON states. The *add* adenine riboswitch (Asw) from the human-pathogenic marine bacterium *Vibrio vulnificus* is a riboswitch thermostat that achieves temperature-compensated regulation of translation with a 3-state mechanism. In this mechanism, a secondary structure pre-equilibrium between the adenine-binding incompetent apoB and the adenine-sensing apoA conformation is coupled to the ligand binding equilibrium between the apoA and the holo conformation. [1] We characterized the ligand-dependent folding of the full-length (112-nucleotide) *add* Asw using NMR and single-molecule FRET (smFRET) spectroscopy. By NMR of the RNA imino protons we observed that the adenine-induced folding of apoA to holo reduces the fractional population of apoB and liberates the ribosome binding site. smFRET demonstrated that long-range tertiary structure interactions of the *add* Asw are confined to the aptamer domain, which exhibits a heterogeneous L2/L3-kissing loop motif between undocked and docked states in the ligand-free and the ligand-saturated form at near-physiological Mg^{2+} concentrations. NMR and smFRET thus provided consistent and complementary views on the adenine-directed multi-state conformational dynamics of this 3-state riboswitch.

References

[1] Reining, A.; Nozinovic, S.; Schlepckow, K.; Buhr, F.; Fürtig, B.; Schwalbe, H., *Nature* (2013), 499(7458), 355-359.
Warhaut, S.; Mertinkus, K.; Höllthaler, P.; Fürtig, B.; Heilemann, M.; Hengesbach, M.; Schwalbe, H.

Stepwise conformational changes during H/ACA RNP assembly and catalysis

Martin Hengesbach



H/ACA RNP complexes catalyze the pseudouridylation of target ribosomal and spliceosomal RNAs in a sequence-specific manner. The pseudouridine synthase Cbf5, three auxiliary proteins (Nop10, L7Ae, Gar1) and a guide RNA constitute the core H/ACA complex, and the target RNA is recruited via base-pairing to the guide RNA. Despite a body of structural data on H/ACA RNP complexes from various organisms, information on structural dynamics throughout the cycle of assembly, substrate recruitment and catalytic turnover are sparse.
We established pipelines to site-specifically label RNA as well as proteins in catalytically active H/ACA complexes for smFRET spectroscopy. By assessing several inter- and intramolecular distances, we show how stepwise assembly of the complex distorts the guide RNA to facilitate substrate binding. We find that upon incorporation into various subcomplexes, RNA conformation differs significantly from the conformation found in the active, fully assembled complex.
Analysis of various structural elements (Gar1, thumb-loop domain) as well as different substrate RNA analogues allows us to identify various states of conformational arrangement. We can correlate these states with predicted intermediates of the respective reaction pathways, and show that distinct conformational rearrangements are correlated with catalysis. Our results explain in detail the functional contribution of individual proteins and their domains for H/ACA-mediated pseudouridylation.
In summary, we unravel the conformational dynamics during stepwise assembly and catalytic turnover in H/ACA complexes with unprecedented detail, improving our understanding of the structural dynamics of site-specific pseudouridylation.

References

Hanspach, G.; Schmidt, A., Hengesbach, M.

EPR-Based Distance Measurements for Structure Determination of RNAs

Olav Schiemann

Institute of Physical and Theoretical Chemistry, University of Bonn, Wegelerstr. 12, 53115 Bonn, Germany,
e-mail: schiemann@pc.uni-bonn.de

RNA full-fills very different roles within cells, it transfers the genetic information from the DNA to the ribosomes, it can act as a catalyst and is involved in gene regulation. In order to understand how RNA is able to achieve this their structures and dynamics need to be known. Thus, methods are required which can yield structural and dynamics information on the required length and time scale. Electron Paramagnetic Resonance (EPR) spectroscopy offers various techniques to unravel local structures on an atomic scale or the arrangement of domains on the nanometer scale. The latter one usually requires the introduction of spin labels commonly nitroxides.

In the talk, a new nitroxide spin label will be presented, which can be attached to RNA via simple click chemistry either with the RNA on the solid support or in solution. It will be shown that this approach enables labeling short RNAs as obtainable from solid RNA synthesizer but also for labeling of large RNA constructs using ligation technologies. Pulsed Electron-Electron Double Resonance (PELDOR or DEER) and cw EPR mesasurments reveal that the label itself is flexible at room temperature but enables orientation selective distance measurements in the frozen state. In the end an approach will be presented by which metal ions like e.g. Mn(II) maybe localized within the folds of RNA using site directed spin labeling and nanometer distance measurements.

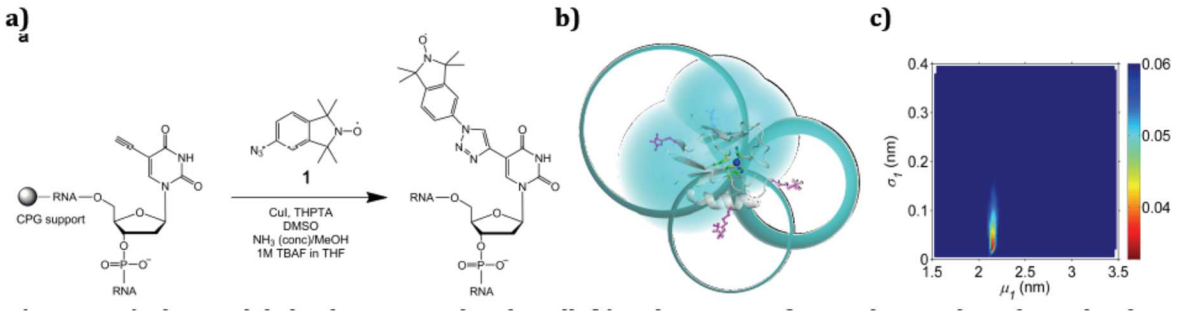
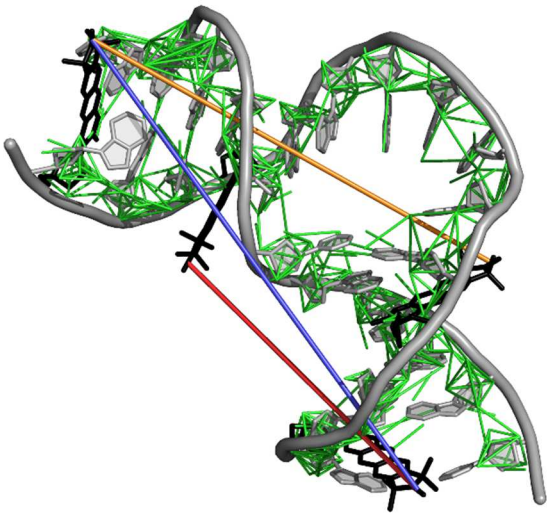


Figure 1: a) The spin label to be presented in the talk. b) Trilateration of a metal ion within a biomolecular structure. c) Error plot of a nanometer distance measurement used for the Trilateration.

Ensemble determination of a bent DNA molecule in atomic resolution: A combined EPR/NMR approach

Claudia Grytz

Distance measurements between a pair of spin labels attached to nucleic acids using Pulsed Electron-Electron Double Resonance (PELDOR also called DEER) spectroscopy is a complementary tool to other structure determination methods in structural biology. If the rigid **Ç** spin label[1], a deoxycytidine analog, is incorporated pairwise into DNA or RNA molecules, not only the distance but also the mutual orientation of the two **Ç** spin labels can be determined by PELDOR. [2,3] Thus, the information about the orientation of secondary structure elements of the nucleic acids can be revealed and used as restraints for structure determination. Since **Ç** is rigidly attached, it can also directly report the conformational heterogeneity[3,4] which is an extremely challenging task to investigate in structural biology. Here, we demonstrate the application of the **Ç** spin label and the obtained PELDOR restraints combined with NMR restraints to determine the global structure and the conformational dynamic of a bent DNA molecule.



References
[1] Grytz, C. M.; Cekan, P.; Smith, A. L.; Barhate, N.; Robinson, B. H.; Sigurdsson, S. T., *Nucleic Acids Res.*, 36, 5946 - 5954 (2008)
[2] Schiemann, O.; Cekan, P.; Margraf, D.; Prisner, T. F.; Sigurdsson, S. T.; *Angew. Chem. Int. Ed.*, 48, 3292-3295 (2009)
[3] Marko, A.; Denysenkov, V.; Margraf, D.; Cekan, P.; Schiemann, O.; Sigurdsson, S. T; Prisner, T. F., *JACS*, 133, 13375-13379 (2011)
[4] Grytz, C. M.; Marko, A.; Cekan, P.; Sigurdsson, S. T.; Prisner T. F., *PCCP*, 18, 2993 – 300
[5] Kazemi, S.; Marko, A.; Cekan, P.; Güntert, P.; Sigurdsson, S. T.; Prisner, T. F.

Dynamic nuclear polarization enhanced solid state NMR using bis-Gd^{3+} polarizing agents

Monu Kaushik

MAS NMR is an important technique in structural biology. Dynamic nuclear polarization (DNP) drastically improves sensitivity of MAS NMR thereby furthering its applicability to new areas of research. A variety of polarizing agents (PAs) have been developed for efficient DNP for high sensitivity gains. This work is based on a relatively new class of PAs: high-spin transition metal ions such as Gd^{3+}, Mn^{2+}. These PAs can substitute intrinsically bound diamagnetic ions in biomolecules with no (or insignificant) effect on their structure, enabling the possibility to obtain site-specific information. Earlier we have demonstrated use of Mn^{2+} as endogenous PA for a hammerhead RNA molecule leading to significant sensitivity enhancement. However, the polarization transfer mechanisms for these PAs are yet to be understood.

Although featuring isotropic Zeeman interaction the high-spin systems of Mn^{2+} and Gd^{3+} experience strong zero-field (electron quadrupolar) interaction; furthermore, Mn^{2+} suffers from hyperfine interaction to the metal nucleus. Both invoke solid effect (SE) at low PA concentration owing to their narrow EPR central transition linewidth. Deviation from pure SE and contribution of cross effect (CE) is observed for high PA concentration (shorter inter-molecular distance). In our attempts to shed light on underlying polarization transfer mechanisms, bis-Gd rigid model complexes are studied. We are able to probe inter-spin distance dependence of DNP field profiles and signal enhancements by varying molecular tether length between the chelator moieties. Eventually, a theoretical model for CE DNP via high-spin PAs is developed. This research extends our understanding of CE DNP via metal ions under MAS and enables us to investigate specifically tailored PAs for efficient CE under conditions where the use of bis-nitroxides is limited (e.g. for in-cell applications).

References

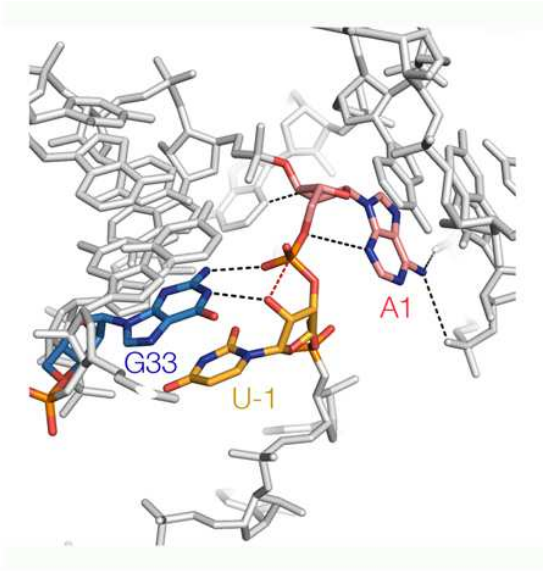
Kaushik, M.; Qi, M.; Godt, A.; Corzilius, B.

Multiple catalytic strategies of a nucleolytic ribozyme

David M. J. Lilley

University of Dundee, Dundee DD1 5EH, UK.

The nucleolytic ribozymes employ multiple processes that contribute to catalysis - (1) facilitation of an in-line trajectory, (2) stabilization of the phosphorane transition state, (3) activation of the O2' nucleophile, and (4) facilitation of the departure of the leaving group. There are multiple ways in which ribozymes achieve this. The twister ribozyme exhibits all four processes to accelerate its cleavage reaction. It uses general acid-base catalysis, mediated by the nucleobases of G33 and A1 as general base (process 4) and acid (process 3) respectively. The latter is highly unusual, as A1 is located immediately 3' to the scissile phosphate, using its highly acidic N3 as the proton donor. A 100-fold stereospecific phosphorothioate effect at the scissile phosphate is consistent with a significant stabilization of the transition state (process 1) by interaction of G33 N2 with the scissile phosphate. A1 is accommodated in a specific binding pocket that raises its pK_a towards neutrality, juxtaposes its N3 with the O5' to be protonated (process 4), and helps create the in-line trajectory required for nucleophilic attack (process 2). Thus the entire structure of the ribozyme has evolved to generate the local environment that promotes catalysis.



T. J. Wilson and D. M. J. Lilley The evolution of chemistry in the ribozymes. *Science* **323**, 1436-1438 (2009).
Y. Liu, T. J. Wilson, S. A. McPhee and D. M. J. Lilley. Crystal structure and mechanistic investigation of the twister ribozyme *Nature Chem. Biol.* **10**, 739-744 (2014).
T. J. Wilson, Y. Liu, C. Domnick, S. Kath-Schorr and D. M. J. Lilley The novel chemical mechanism of the twister ribozyme *J. Amer. Chem. Soc.* **138**, 6151–6162 (2016).

Structural and mechanistic insight into nascent-polypeptide mediated translational stalling

Daniel N. Wilson

Gene Center and Department of Biochemistry, University of Munich, Germany, Munich, Germany;
Center for integrated Protein Science Munich (CiPSM), University of Munich, Munich, Germany

As the nascent polypeptide chain is being synthesized, it passes through a tunnel within the large ribosomal subunit. Rather than a passive conduit for the nascent chain, accumulating evidence indicates that specific nascent polypeptide chains can modulate the rate of translation and, in some specific cases, even induce translation arrest. In some cases, the nascent polypeptide mediated translational arrest depends on the presence of an additional co-factor, such as an amino acid or antibiotic. I will discuss our latest studies using biochemical and structural biological approaches to provide mechanistic insight into how the nascent polypeptide chain, alone or in concert with a small molecule ligand, can interact with components of the ribosomal exit tunnel to induce translation arrest, either by itself adopting non-productive conformations that prevent subsequent peptide-bond formation, and/or evoke allosteric conformational changes that silence the active of the large ribosomal subunit, namely the peptidyl-transferase center.

Mining for new cis regulatory elements in messenger RNAs

Johannes Braun

Many posttranscriptional regulatory pathways involve sequence and/or structural elements within the untranslated regions (UTRs) of the mRNA. To identify novel regulatory elements, we predicted conserved RNA structures in human UTRs using the algorithm *Dynalign*. Putative elements were functionally characterized by reporter gene assays and qRT-PCR. Using this approach, we identified a novel element in the 3´UTR of uncoupling protein 3 (*UCP3*). UCP3 is a mitochondrial membrane protein associated with obesity and diabetes, which functions in the energy metabolism of the cell. The RNA element contains two AU-rich elements, which differ in sequence and structure, but act in concert in regulating mRNA stability. We identified several canonical RBPs as well as moonlighting enzymes binding to the element *in vitro* and currently evaluate their biological function.

Structural Investigation of Tetramethylrhodamine Binding Aptamers

Elke Duchard-Ferner

RNA aptamers, small synthetic RNA molecules designed to bind specific ligands, offer the possibility of investigate general determinants of ligand-RNA interactions. In particular, high-resolution structures of the RNA-ligand complexes serve to understand the atomistic characteristics governing ligand affinity and specificity.
Recently, a series of RNA aptamers binding to the xanthene dye tetramethylrhodamine (TMR) have been reported. [1] These aptamers vary in structural complexity and affinity. We have investigated the binding properties, the fluorescence characteristics and the structure of several of these TMR aptamers. The structure of one aptamer of higher structural complexity, TMR-3, was investigated further by NMR spectroscopy. The high resolution structure of the TMR-3/TMR complex shows that two stems of the RNA form a continuous helix with TMR intercalating between a G-G base pair and a A-G-C base triple. The ligand is specifically recognized by its carboxylate group. Comparison to other aptamer-ligand complexes suggests common xanthene binding features.

[1] Carothers, J. M.; Goler, J. A.; Kapoor, Y.; Lara, L.; Keasling, J. D. (2008) *Nucl. Acids Res.* 2736-2747.
Duchardt-Ferner, E.; Kreutz, C.; Wöhnert, J.

Speaker List

Speaker	Email
Antje Wolter	wolter@bio.uni-frankfurt.de
Cameron Mackereth	cameron.mackereth@u-bordeaux.fr
Christina Helmling	helmling@nmr.uni-frankfurt.de
Claudia Maria Grytz	grytz@prisner.de
Daniel Wilson	wilson@lmb.uni-muenchen.de
David Lilley	d.m.j.lilley@dundee.ac.uk
Dmitry Svergun	svergun@embl-hamburg.de
Elke Duchardt-Ferner	duchardt@bio.uni-frankfurt.de
Henrik Gustmann	gustmann@theochem.uni-frankfurt.de
Johannes Braun	braunj@bio.tu-darmstadt.de
Karl Bertram	kbertra2@mpibpc.mpg.de
Konstantinos Stellos	Konstantinos.Stellos@kgu.de
Lukas Stelzl	lukas.stelzl@biophys.mpg.de
Mark Helm	mhelm@uni-mainz.de
Martin Hengesbach	hengesbach@nmr.uni-frankfurt.de
Michael Sattler	michael.sattler@tum.de
Modesto Orozco	modesto.orozco@irbbarcelona.org
Monu Kaushik	kaushik@em.uni-frankfurt.de
Nicolas Jaé	Jae@med.uni-frankfurt.de
Nicole Erlenbach	erlenbach@prisner.de
Nusrat Qureshi	qureshi@nmr.uni-frankfurt.de
Olav Schiemann	schiemann@pc.uni-bonn.de
Samarth Thonta Setty	samarth.thonta-setty@bmls.de
Sven Warhaut	warhaut@nmr.uni-frankfurt.de
Victor-Valentin Hodirnau	hodirnau@biophysik.org