Gesellschaft Deutscher Chemiker

Magnetic Resonance Division

35th FGMR Discussion Meeting & Joint Conference of the German, Italian and Slovenian Magnetic Resonance Societies

Advanced Magnetic Resonance – Methods and Applications

Combined with the Workshop

New Frontiers in Sensitivity for EPR Spectroscopy: from Biological Cells to Nano Materials

Frauenchiemsee, September 9 to 14, 2013

HelmholtzZentrum münchen
German Research Center for Environmental Health

GIDRM
Gruppo Italiano Discussione Risonanze Magnetiche

SLONMR

Technische Universität München

35th FGMR Discussion Meeting and Joint Conference of the German, Italian and Slovenian Magnetic Resonance Societies

including tutorials on

Optimum Control and Pulse Sequence Optimization;
CcpNmr; CS- and autoNOE-ROSETTA;
From Spin Hamiltonian to NMR spectra

Frauenchiemsee Island, Germany

September 9th – 12th, 2013

SPP1601 Annual Meeting

New frontiers in sensitivity for EPR spectroscopy: from biological cells to nano materials

Frauenchiemsee Island, Germany

September 12th – 14th, 2013

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Contents

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2. Organization

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http://www.fgmr2013.de

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Till Maurer, San Francisco

3. General Information

Venue / Registration / Conference Office

The meeting will take place in the monastery "Frauenwörth" on the island of Frauenchiemsee.

Locations:

Plenary talks: **Aula**

Parallel talks: **Aula** and **Musiksaal**

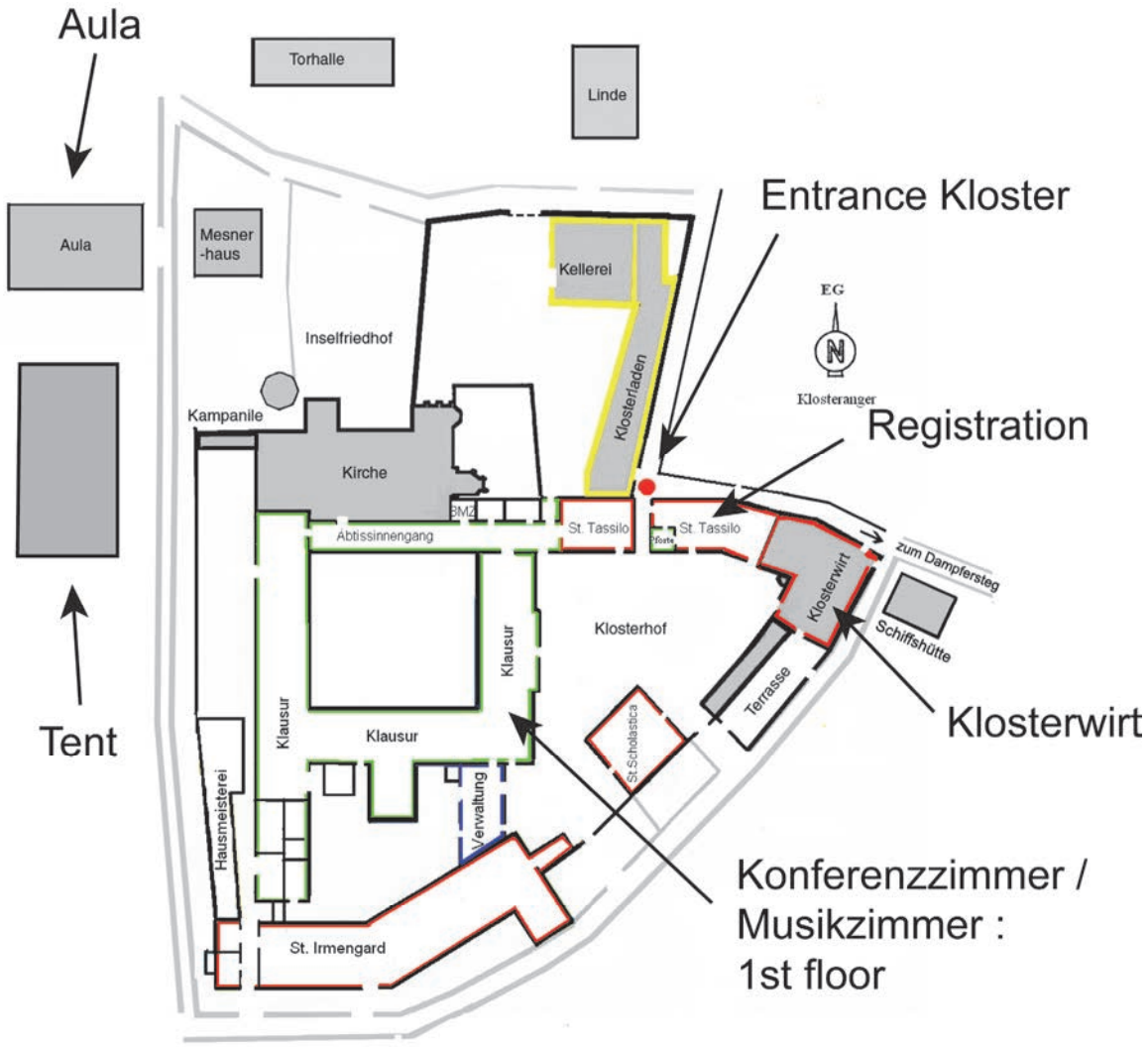
Poster sessions and vendor exhibitions: **Tent**

Conference office:

Mon, Sep 9: **Reception desk of the monastery**

Tue-Thu, Sep 10-12: **Aula**

Conference locations in/around the monastery



Registration / Conference Office:

Monday, Sep 9, 2013 (Reception Monastery)	13:00 – 21:00 h
Tuesday, Sep 10, 2013 (Aula)	8:00 – 19:00 h
Wednesday, Sep 11, 2013 (Aula)	8:00 – 18:00 h
Thursday, Sep 12, 2013 (Aula)	8:00 – 13:00 h

Posters

All posters will be displayed for the whole duration of the conference in the Tent next to the Aula. Posters can be mounted from Monday 13h on and should be removed by Thursday, 13h. Presenting authors are requested to be at their poster during the scheduled poster sessions.

***Poster Session 1**, Tue 19:30-21:00, odd-numbered posters*

***Poster Session 2**, Wed 12:00-13:30, even-numbered posters*

Internet

WLAN is available at the conference site. Details will be provided on site.

Coffee breaks, Lunch, Dinner

Coffee is provided during the coffee breaks in the **Tent**.

Participants who have purchased the meal package or those staying on the island with full board:

- Lunch will be provided in the **Tent**
- Dinner will be served in the restaurant of **Klosterwirt**

Breakfast for all participants with accommodation on the island will be served in the restaurant of **Klosterwirt**.

Participants without meal package: There are a few restaurants, beergardens and food stands on the island.

Conference Dinner (Wed 19:30) is included for all registered particpants and will be served at the restaurant of **Klosterwirt**.

Conference Dinner

Wednesday Sep 11th 2013, 19:30 at **Klosterwirt**

Drinks in the Catacombs

Every evening after the conference program particpants can buy drinks in the Catacombs of the abbey.

Shuttle bus and ferry during the conference

Shuttle bus service

If you have requested the shuttle bus service: the bus will bring you from the following hotels to the ferry port in Gstadt:

- Kloster Seeon: leaving at 7:20h
- Hotel Lambach: leaving at 7:35h
- Hotel Malerwinkel: leaving at 7:40h

Every evening after the conference program, the shuttle service will pick you up from the ferry port in Gstadt, arriving with the special ferry (see schedule below) and drop you off at these hotels.

Ferry

Participants who do not stay on the island will receive ferry tickets to connect from Gstadt to the Island in the morning and evening. **Please return unused tickets to the organizers (conference office)** so that we can have them reimbursed.

After the conference program a **special ferry trip** will be available every evening for conference participants:

Monday & Tuesday: 22:30 h

Wednesday: 23:30 h

Ferry schedule (workdays):

Gstadt > Fraueninsel

7:20 | 8:05 | 8:50 | 9:20 | 9:50 | 10:20 * | 10:50 | 11:20 | 11:50 | 12:20 * | 12:50 | 13:20 | 13:50 | 14:20 * | 14:50 | 15:20 * | 15:50 | 16:20 | 16:50 | 17:20 * | 17:50 | 18:20 * | 18:50 | 19:55 * | 21:00 *

Fraueninsel > Gstadt

6:05 (weekdays only) * | 7:05 * | 7:40 * | 9:00 | 9:30 | 10:00 | 10:10 | 10:30 * | 11:00 | 11:05 | 11:30 * | 11:35 | 12:00 | 12:20 | 12:30 * | 13:00 | 13:05 | 13:30 * | 13:55 | 14:00 | 14:30* | 14:40 | 15:00 | 15:30 * | 15.35 | 16:00 | 16:25 | 16:30 * | 17:00 | 17:05 | 17:30 * | 18:00 | 18:30 | 18:30 * | 19:00 | 20:05 * | 21:15 * |
Special conference ferry: 22:30 (Mon, Tue) / 23:30 (Wed)

*ferry departure/arrival to Nordsteg!!

Note: The ferry trip from Gstadt to Hauptsteg takes ~10 min.

	Monday	Tuesday		Wednesday		Thursday	Friday	Saturday
	9.9.	10.9.		11.9.		12.9.	13.9.	14.9.
08:30		**Plenary I: Frontiers in Magnetic Resonance** *(Chair: Bernd Reif)*		**Plenary III: Small Molecules & Polymers** *(Chair: Christina Thiele)*		**Plenary V: Magnetic Resonance in Structural Biology** *(Chair: Henriette Molinari)*		
		Lucia Banci Florence		**Burkhard Luy** Karlsruhe		**Volker Dötsch** Frankfurt		
		Kevin Brindle Cambridge		**Marco Geppi** Pisa		**Enrica Bordignon** Zürich		
		Hartmut Oschkinat Berlin		**Ruth Gschwind** Regensburg		**Robert Bittl** Berlin		
						Paul Rösch Bayreuth		
10:00		**Coffee**		**Coffee**		**Coffee**		
10:30		**Plenary II: Imaging** *(Chair: Axel Haase)*		**Parallel 5: Nucleic Acids** *(Chair: Luisa Mannina)* [Aula]	**Parallel 6: Biomedicine & Food** *(Chair: Enzo Terreno)* [Musiksaal]	**Plenary VI: Frontiers in Magnetic Resonance II** *(Chair: Steffen Glaser)*		**EPR Satellite Meeting**
	Arrival	**Silvio Aime** Torino		**Teresa Carlomagno** Heidelberg	**Raffaele Lamanna,** Rotondella	**Mario Chiesa** Torino		
		Oliver Speck Magdeburg		**Mirko Cevec** Ljubljana	**Lucia Calucci** Pisa	**Mario Piccioli** Florence		
		Leif Schröder Berlin		**Mario Schubert** Zürich	**Roberto Consonni** Milano	**Marina Bennati** Göttingen		
				Cristina Airoldi Milano	**Wolfram Gronwald** Regensburg	**Jörg Wrachtrup** Stuttgart		
				Elke Duchardt-Ferner Frankfurt	**Parallel 8: BioNMR - Applications** *(Chair: Sonja Dames)* **Silke Wiesner** Tübingen			
12:00		**Sandwich lunch** [Tent]		**12:00-13:30 Postersession II & Sandwich lunch** [Tent]		**Sandwich lunch** [Tent]		
13:30	**14:30-17:00 TUTORIALS** **14:30-15:30** **Steffen Glaser** [Musiksaal] Optimum Control & Pulse Sequence Optimization **Geerten Vuister** [Konferenzzimmer] CCPNMR **16:00-17:00** **Stan Sykora** [Musiksaal] From Spin Hamiltonian to NMR spectra **Oliver Lange** [Konferenzzimmer] CS & autoNOE ROSETTA	**Parallel 1: BioNMR - Methods** *(Chair: Tobias Madl)* [Aula]	**Parallel 2: Polymers & Materials** *(Chair: Jörn Schmedt auf der Günne)* [Musiksaal]	**Parallel 7: Materials & Small Molecules** *(Chair: Marion Menzel)* [Aula]	**Parallel 8: BioNMR - Applications cont'd** *(Chair: Sonja Dames)* [Musiksaal]	**G-NMR** **12:30-15:30** [Aula]	**EPR Satellite Meeting** Thu 12.9 15:35 until Sat 14.9. 12:00	
		Stephan Grzesiek Basel	**Michael Ryan Hansen** Mainz	**Bernhard Blümich** Aachen	**Clemens Glaubitz** Frankfurt			
		Michael Assfalg Verona	**Hellmut Eckert** Münster	**Michele Chierotti** Torino	**Giovanna Musco** Milano			
		Salvatore Bubici Pavia	**Christiane Wolff** Darmstadt	**Cindy Elschner** Dresden	**Nils Lakomek** Göttingen			
		Adele Mucci Modena	**Gregor Mali** Ljubljana	**Alexander Schnegg** Berlin	**Daniel Cicero** Rome			
		Hans-Robert Kalbitzer	**Marco Sette** Rome	**Vito Gallo** Bari	**Maxim Yulikov** Zürich			
		Adam Lange Göttingen	**Kay Saalwächter** Halle	**Noemi Proietti** Rome	**Markus Zweckstetter** Göttingen			
15:30		**Coffee**		**Coffee**				
16:00	**Registration** [Reception Desk Monastery]	**Parallel 3: Dynamics** *(Chair: Horst Kessler)* [Aula]	**Parallel 4: Computation & Structure** *(Chair: Oliver Lange)* [Musiksaal]	**Plenary IV: Pharma & Bio** *(Chair: Stefano Mammi)*				
		Christian Griesinger	**Alessandro Bagno** Padova	**Fabio Arnesano** Bari				
		Mauro Botta Alessandria	**Matthias Köck** Bremerhaven	**Wolfgang Jahnke** Basel				
		Wolfgang Baumann Rostock	**Christiane Ritter**	**Carla Marchioro** Verona				
		Philipp Neudecker Düsseldorf	**Bert de Groot** Göttingen	**Janez Plavec** Ljubljana				
		Jochen Balbach Halle	**Michael Nilges** Paris					
18:00	**Welcome Reception** [Klosterwirt]	**Dinner** [Klosterwirt]		**Anna Laura Segre Fellowship Presentations (GIDRM)**		**EPR Satellite Meeting** Thu 12.9 15:35 until Sat 14.9. 12:00		
19:00	**Opening remarks** [Aula]							
19:15	**Plenary I: Frontiers in Magnetic Resonance** *(Chair: Michael Sattler)* **Gerhard Wagner** Boston							
19:45	**Plenary Awards** (Chair: Wolfgang Jahnke) **FGMR Ernst Awards** **Introduction:** ***Wolfgang Jahnke*** **Mariusz Jaremko, Łukasz Jaremko** (MPI Göttingen) **Yesu Feng** (Duke Univ., Durham, USA) **Franz Schilling** (TUM, Munich) **GIDRM Medal** **Introduction:** ***Henriette Molinari*** **Gennaro Esposito** (Udine)	**19:30-21:00 Poster Session I** [Tent]		**19:30 Conference Dinner** [Klosterwirt]				
		20:30-22:00 FGMR member meeting [Aula]	**20:30-22:00 GIRDM member meeting** [Musiksaal]	**21:30 After Dinner Lecture** [Aula] **Horst Kessler** Garching				
	Drinks in the Catacombs (on your own)	**Drinks in the Catacombs** (on your own)		**Drinks in the Catacombs** (on your own)				

4. Scientific Program

4.1. Tutorials

Optimum Control and Pulse Sequence Optimization

Steffen J. Glaser

Department of Chemistry, TU München, Garching, Germany

The field of Optimal Control Theory offers powerful analytical and numerical tools to explore the ultimate performance limits of pulse sequences. In the last decade, these tools not only provided pulse sequences of unprecedented quality and capabilities, but also new analytical and geometrical insight and a deeper understanding of pulse optimization problems. Efficient numerical algorithms make it possible to develop robust time-optimal or relaxation-optimized pulse sequences, taking into account experimental limitations and imperfections, such as maximum pulse amplitudes, maximum pulse power, pulse inhomogeneity as well as transient effects associated with the switching of pulse amplitudes and phases.
In this tutorial, important concepts of Optimal Control Theory will be introduced and applications to uncoupled and coupled spins will be presented. In this context, the classification of optimization tasks plays an important role. Examples of typical control problems to be discussed are motivated by applications in NMR spectroscopy of small molecules and of large biomolecules, as well as electron spin resonance spectroscopy and magnetic resonance imaging. These include ultra-broadband and band-selective pulses, coherence transfer experiments and novel decoupling sequences.

Straightforward calculation and validation from CcpNmr

Prof. Geerten W. Vuister

Department of Biochemistry, University of Leicester, Henry Wellcome Building, Lancaster Road, Leicester LE1 9HN, United Kingdom

The course will demonstrate the CcpNmr suite as a straightforward way to submit calculation jobs. CCPN provides a single interfaces to submit calculations to many different programs directly from CCPN projects. You can run different calculations from the same data set, without having to learn additional interfaces or prepare separate input for each program, and read the results back in the CCPN project. We will demonstrate two examples:
- Executing the CING structure validation program directly from within CcpNmr Analysis.
- Using the prototype CcpNmr WMS workflow manager to submit multiple parallel structure calculations for ARIA, CYANA, UNIO, and CS-Rosetta from a single input specification.

From Spin Hamiltonian to Spectra - a Tutorial

Stanislav Sykora

Extra Byte, Via Raffaello Sanzio 22C, 20022 Castano Primo, Italy

This tutorial targets primarily new NMR spectroscopists who wish to know more about spin Hamiltonian and spin systems, the origin and rules of strong coupling phenomena, and about how spectra can be simulated and fitted. Though rigorous quantum approach will be used, it will be done in an unobtrusive way, and prior knowledge of the quantum-mechanical apparatus will not be required. On the other hand, the tutorial is not going to "avoid" mathematics; on the contrary, it is intended to provide a mathematical basis sufficient to enable the participants to write their own ad-hoc code for spectra simulation, understanding of basic spectral features, and easy formulas for moderate second order effects. The tutorial syllabus is very rich, maybe excessively so. To alleviate the time-limit problem, an extensive set of materials will appear on the Author's website (ebyte.it/library/SpinTutorialKit.html). The kit will include descriptive materials (slides with accompanying texts and comments, appendices and tables, pre-requisite math, articles), references, a spectra simulation and fitting Utility (Windows, no graphic), a courtesy demo edition of Mnova from Mestrelab, sample spectra, illustrations, free code snippets, etc. The kit pages will start appearing from mid of July and will keep building up until the end of August. Would-be tutorial participants are advised to visit the pages and get acquainted with the "kit". The syllabus includes 20' of free discussions (out of 60') which are considered essential for the success of the tutorial. Should the time limit not allow a complete, live coverage of the whole syllabus, participants will be referred to the online pages for any skipped sections, while preserving the time allotted for discussions.

CS-Rosetta and autoNOE-Rosetta

Oliver Lange

Department Chemie, TU München, Garching, Germany

This tutorial will cover CS-Rosetta structure calculations with and without additional RDC or NOE data based on the python based csrosetta-toolchain downloadable from www.csrosetta.org. In detail we will cover 1) installing of the toolbox including the fragment picker 2) structures from chemical shifts alone 2.1) analysing chemical shift data with TALO+, fragment picking 2.2) setup of basic CS-RASREC/CS-Rosetta calculation 2.3) analysis of CS-Rosetta calculation 3) CS-Rosetta calculations with additional restraints 3.1) adding RDC data 3.2) adding distance restraints (NOE) 4) automatic NOE assignment (autoNOE-Rosetta) 4.1) preparing the input data 4.2) carrying out autoNOE-Rosetta calculations 4.3) post-analysis of autoNOE-Rosetta calculations.

G Schot et al., J. Biomol. NMR; OF Lange et al., Proc. Natl. Acad. Sci. USA, 2012, 109, 10873–10878; OF Lange, D Baker, Proteins, 2012, 80, 884–895; S Raman et al., Science, 2010, 327, 1014–1018; Y Shen et al., Proc. Natl. Acad. Sci. USA, 2008, 105, 4685–4690.

4.2. Ernst Awards 2013

Mariusz Jaremko, Łukasz Jaremko

Department for NMR-based Structural Biology,
Max Planck Institute for Biophysical Chemistry, Göttingen

Cold denaturation of a protein dimer monitored at atomic resolution

Mariusz Jaremko, Łukasz Jaremko, Hai-Young Kim, Min-Kyu Cho, Charles D Schwieters, Karin Giller, Stefan Becker, Markus Zweckstetter,
Nature Chemical Biology 9, 264 (2013)

Yesu Feng

Department of Chemistry, Duke University, Durham

Accessing long-lived nuclear singlet states between chemically equivalent spins without breaking symmetry

Yesu Feng, Ryan M. Davis, Warren S.Warren,
Nature Physics 8, 831 (2012)

Franz Schilling

Department of Chemistry, Technische Universität München, Garching

Tailored real-time scaling of heteronuclear couplings

Franz Schilling, Steffen J. Glaser,
Journal of Magnetic Resonance 223, 207 (2012)

4.3. SPP 1601 Annual Meeting 2013

"New frontiers in sensitivity for ESR spectroscopy: from biological cells to nano materials"

The annual meeting of the DFG priority program (SPP) 1601 „*New frontiers in sensitivity for EPR spectroscopy: from biological cells to nano materials*“ will take place at Frauenchiemsee after to the annual discussion meeting of the FGMR. The SPP program, started in Mai 2012, brings together 25 EPR groups working in different areas of chemistry and physics for an period of three-year, which is intended to be extended to a total six years.

The workshop, organized by Marina Bennati (MPI & University of Göttingen, coordinator of the program), will comprehend research talks from the members of the SPP to develop collaborations and new research within the network. In addition, lectures from internationally renowned scientists and reviewers of the SPP complement the program. This-year special features will be lectures from several guests from US funded by a new initiative between the DFG and NSF for the development of EPR spectroscopy.

Topics extend from methodical developments in pulse excitation schemes with microwaves, innovative detection schemes for EPR or electron spins up to THz spectroscopy. An important aspect is the combination of the methodical developments with the application of EPR to new fields of biological or material science, such as the investigation of *in cell* protein-protein interactions, enzymatic mechanisms, catalytic processes on single-crystalline surfaces or the light-induced degradation of thin-film solar cells.

All presentations are by invitation only. The participation is free of charge for visitors of FGMR 2013.

Invited lectures will be given by:

Gail Fanucci (Univ.Gainsville)
Steve Lyon (Princeton Univ.)
Neil MacKinnon (Univ. Freiburg)
Mark Sherwin (UC Santa Barbara)
Stefan Stoll (Univ. Washington, Seattle)
Christiane Timmel (Univ. Oxford)

4.4. Program Schedule

Monday, September 9, 2013

	Tutorials	
14:30	**Steffen Glaser** (München) Optimum Control & Pulse Sequence Optimization	Musiksaal
14:30	**Geerten Vuister** (Leicester) Calculation and validation from CCPNMR	Konferenz
16:00	**Stan Sykora** (Castano Primo) From Spin Hamiltonian to Spectra	Musiksaal
16:00	**Oliver Lange** (München) CS-Rosetta and autoNOE-Rosetta	Konferenz
16:00	**Registration**	Abbey´s Reception
18:00	Welcome Dinner	Klosterwirt
19:00	**Welcome / Opening remarks**	
	Plenary I: Frontiers in Magnetic Resonance *Michael Sattler*	Aula
19:15	**Gerhard Wagner** (Boston) Gain in Resolution, SNR and Detection Sensitivity by Poisson-Gap Sampling and Reconstruction with Iterative Soft Thresholding for Enabling Studies of Large Proteins	
19:45	**Presentation of the Ernst-Awards** *Wolfgang Jahnke*	Aula
19:45	Introduction and Award Presentation	
20:00	**Mariusz Jaremko, Łukasz Jaremko** (Göttingen) Cold denaturation of a protein dimer monitored at atomic resolution	
20:20	**Yesu Feng** (Durham) Accessing long-lived nuclear singlet states between chemically equivalent spins without breaking symmetry	
20:40	**Franz Schilling** (Garching) Tailored real-time scaling of heteronuclear couplings	
	Presentation of the GIDRM Medal *Henriette Molinari*	Aula
21:00	Introduction	
21:15	**Gennaro Esposito** (Udine) The BLUU-Tramp application: problems and solutions	
	Drinks in the Catacombs (on your own)	Catacombs

Tuesday, September 10, 2013

	Plenary I: Frontiers in Magnetic Resonance *Bernd Reif*	Aula
8:30	**Lucia Banci** (Florence) NMR in Molecular Systems Biology: from structures to function	
9:00	**Kevin M. Brindle** (Cambridge) Imaging cancer metabolism with hyperpolarised ^{13}C labelled cell substrates	
9:30	**Hartmut Oschkinat** (Berlin) About DNP of Proteins and MAS NMR on membrane proteins and with four nuclei	
10.00	*Coffee Break*	
	Plenary II: Imaging Axel Haase	Aula
10:30	**Silvio Aime** (Torino) Exploring new routes to high sensitivity probes for MR-Molecular Imaging	
11:00	**Oliver Speck** (Magdeburg) High Resolution Neuroimaging at 7T	
11:30	**Leif Schröder** (Berlin) Saturation Transfer Applications of Exchangeable, Hyperpolarized Xenon	
12:00	*Lunch*	

	A: BioNMR-Methods *Tobias Madl* — *Aula*	**B: Polymers & Materials** *Jörn Schmedt auf der Günne* — Musiksaal
13:30	**Stephan Grzesiek** (Basel) Protein interactions and function studied by novel NMR methods	**Michael Ryan Hansen** (Mainz) Characterizing Morphology and Molecular Packing in Polymer-Fullerene Blends by Solid-State NMR
13:50	**Michael Assfalg** (Verona) NMR investigation of the equilibrium partitioning of a water-soluble bile salt protein carrier to phospholipid vesicles	**Hellmut Eckert** (Münster) Recoupling homonuclear dipole-dipole interactions in multi-spin systems: applications to inorganic ma-terials
14:10	**Salvatore Bubici** (Pavia) Fast Field Cycling Relaxometry: Moving from Re-search towards Industrial Applications	**Christiane Wolff** (Darmstadt) Studies of a photochemical model system using a new LED based NMR Illumination Device
14:25	**Adele Mucci** (Modena) Evaluation of the effects of cryopreservation on Modena Biobank tissue samples through HR-MAS NMR	**Gregor Mali** (Ljubljana) NMR spectroscopy of molecules embedded within microporous and mesoporous materials
14:45	**Hans Robert Kalbitzer** (Regensburg) Distinct Conformational States of the Alzheimer β-Amyloid Peptide can be Detected by High Pressure NMR Spectroscopy	**Marco Sette** (Rome) Quantitative 2D-NMR analysis of lignins
15:00	**Adam Lange** (Göttingen) Molecular Machines studied by solid-state NMR	**Kay Saalwächter** (Halle) Local deformation of polymer chains as reflected in proton dipole-dipole coupling distributions
15:30	*Coffee Break*	
	A: Dynamics *Horst Kessler* — Aula	**B: Computation & Structure** *Oliver Lange* — Musiksaal
16:00	**Christian Griesinger** (Göttingen) New methods for measuring protein dynamics	**Alessandro Bagno** (Padova) Predicting the NMR spectra of paramagnetic mole-cules by DFT Calculations
16:20	**Mauro Botta** (Alessandria) Coupling fast water exchange to slow molecular tumbling in Gd3+ chelates:1H and 17O NMR relaxo-metric studies	**Matthias Köck** (Bremerhaven) Approaches for the determination of the relative configuration of natural products
16:40	**Wolfgang Baumann** (Rostock) Quantification by selective excitation	16:35 **Christiane Ritter** (Braunschweig) The structural basis for the autocatalytic conversion of the antiviral signaling protein MAVS into its active form
16:55	**Philipp Neudecker** (Düsseldorf) Solution Structure of an Intermediate State in Pro-tein Folding and Aggregation	16:50 **Bert de Groot** (Göttingen) Ensemble refinement and collective dynamics from combined NMR and MD
17:15	**Jochen Balbach** (Halle) Ex vivo NMR during human cell cycle control	17:10 **Michael Nilges** (Paris) Inferential structure determination from hybrid data
18:00	Dinner — Klosterwirt	
19:30	**Poster Session 1 (odd numbers presenting)** — Tent	
20:30	FGMR member meeting — Aula	GIRDM member meeting — Musiksaal
21:00	Drinks in the Catacombs (on your own) — Catacombs	

Wednesday, September 11, 2013

	Plenary III: Small Molecules & Polymers *Christina Thiele* Aula	
08:30	**Burkhard Luy** (Karlsruhe) Small Molecule Structure Determination Using Anisotropic NMR-Parameters	
09:00	**Marco Geppi** (Pisa) Structure, polymorphism and dynamics of small organic molecules by solid state NMR: the surprising case of Ibuprofen	
9:30	**Ruth Gschwind** (Regensburg) Intermediates and Interactions in Organo-, Photo- and Transition Metal Catalysis	
10:00	*Coffee Break*	
	A: Nucleic Acids & Proteins *Luisa Mannina* Aula	**B: Biomedicine & Food** *Enzo Terreno* Musiksaal
10:30	**Teresa Carlomagno** (Heidelberg) The structure of a 400 kDa RNP enzyme from an integrated structural-biology approach	**Raffaele Lamanna** (Rotondella) Proton NMR: a tool for assessing food preservation strategies
10:50	**Mirko Cevec** (Ljubljana) Diversity of RNA molecules	**Lucia Calucci** (Pisa) Boron nitride nanotubes as MRI contrast agents
11:10	**Mario Schubert** (Zürich) RNA resonance assignment strategies based on chemical shift statistics	**Roberto Consonni** (Milano) NMR studies in food authentication
11:25	**Cristina Airoldi** (Milano) NMR study of ligand-receptor interactions in non-homogeneous systems	**Wolfram Gronwald** (Regensburg) State of the art analysis of metabolomic data in the context of human diseases
11:40	**Elke Duchardt-Ferner** (Frankfurt) Functional insights into ligand binding specificity and the binding mechanism of the neomycin sens-ing riboswitch	**Parallel 8: BioNMR - Applications** *Sonja Dames* Musiksaal **Silke Wiesner** (Tübingen) Methionine scanning as a new tool for methyl NMR studies
12:00	**Poster Session 2 (even numbers presenting)** Tent	
	Lunch (sandwiches in the tent)	
	A: Materials & Small Molecules *Marion Menzel* Aula	**B: BioNMR – Applications (cont)** *Sonja Dames* Musiksaal
13:30	**Bernhard Blümich** (Aachen) Compact NMR Spectroscopy	**Clemens Glaubitz** (Frankfurt) Understanding the Functional Mechanism of Membrane Proteins by (DNP-enhanced) Solid-state NMR
13:50	**Michele Chierotti** (Torino) Combined Solid-state NMR and XRPD approach for solving structures of powdered samples: some ex-ample	**Giovanna Musco** (Milano) tr-NOE on human cancer cells, Metadynamics and MD in the Design of isoDGR-Based αVβ3 Antagonists
14:10	**Cindy Elschner** (Dresden) Monitoring of early bone formation with Nuclear Magnetic Resonance Imaging	**Nils Lakomek** (Göttingen) NMR Relaxation Measurements for Membrane Proteins: Internal Dynamics of the Homotrimeric HIV-1 Viral Coat Protein gp41 on Multiple Time Scales
14:25	**Alexander Schnegg** (Berlin) Advanced Electrically Detected Magnetic Reso-nance for Photovoltaic Research	**Daniel Cicero** (Rome) Search and discovery of new low-temperature active enzymes from Antarctic microorganisms
14:45	**Vito Gallo** (Bari) Heteronuclear NMR spectroscopy in the Platinum coordination chemistry	**Maxim Yulikov** (Zürich) Human carbonic anhydrase II as a model receptor-ligand system: localization and orientation of a sul-fonamidic inhibitor by Gd(III)-nitroxide DEER and paramagnetic NMR
15:00	**Noemi Proietti** (Rome) Non invasive applications of portable NMR sensors with micrometric resolution for studying of Cultural Heritage	**Markus Zweckstetter** (Göttingen) Protein folding and unfolding at atomic resolution
15:30	*Coffee Break*	

	Plenary IV: Pharma & Bio *Stefano Mammi*	Aula
16:00	**Fabio Arnesano** (Bari) Monitoring the fate and transport of platinum anti-cancer drugs by NMR spectroscopy	
16:30	**Wolfgang Jahnke** (Basel) Tuning bone affinity into fragment-derived lead compounds: a general strategy for targeting drugs acting on bone	
17:00	**Carla Marchioro** (Verona) Versatility of qNMR in Drug Discovery and Devel-opment	
17:30	**Janez Plavec** (Ljubljana) Four-stranded DNA structures with unique loops and overhangs	
18:00	**Anna Laura Segre Fellowship Presentations (GIDRM)**	Aula
19:30	Conference Dinner	Klosterwirt
21:30	**After Dinner Lecture:** Horst Kessler (Garching)	Aula
	Drinks in the Catacombs (on your own)	Catacombs

Thursday, September 12, 2013

	Plenary V: Magnetic Resonance in Structural Biology *Henriette Molinari*	Aula
8:30	**Volker Dötsch** (Frankfurt) Combination of cell-free synthesis and NMR for the structure determination of membrane proteins	
8:55	**Enrica Bordignon** (Zürich) EPR reveals the conformation of active Bax during apoptotic cell death	
9:20	**Robert Bittl** (Berlin) A structural model for the full-length blue light-sensing protein YtvA from Bacillus subtilis, based on EPR spectroscopy	
9:45	**Paul Rösch** (Bayreuth) Anfinsen and the Art of Bacterial Transcription	
10:10	*Coffee Break*	
	Plenary VI: Frontiers in Magnetic Resonance II *Steffen Glaser*	Aula
10:30	**Mario Chiesa** (Torino) Advanced EPR Methods in Surface Chemistry and Catalysis. The Role of Hyperfine Techniques	
10:55	**Mario Piccioli** (Florence) Paramagnetic NMR for the understanding of the biology of special metal cofactors in proteins	
11:20	**Marina Bennati** (Göttingen) Double resonance techniques in EPR and NMR: from sensitivity enhancement to applications in biological science	
11:45	**Jörg Wrachtrup** (Stuttgart) Single spin nuclear magnetic resonance	
12:10	*Lunch*	
12:30	G-NMR	Aula
15:35	EPR Satellite Meeting (until Saturday, Sep 14, 12:00)	

SPP 1601 Annual meeting

New frontiers in sensitivity for EPR spectroscopy: from biological cells to nano materials

Thursday, September 12, 2013

15:35	**Opening** *M. Bennati*
15:40 – 17:30	***Session* 1: New pulse methods**
	T. Prisner (Frankfurt) New pulsing schemes for EPR and DNP
	S. Glaser (München) Optimal control of spin dynamics in magnetic resonance
	A. Doll (Zürich) New developments in ultrawideband EPR
	R. Rizzato (Göttingen) Cross-polarization edited ENDOR
	P. Höfer (Bruker Biospin, Karlsruhe) Bruker developments towards new tools in EPR spectroscopy
18:00	*Dinner*
19:30 – 21:15	***Session 2: THz-EPR***
	M. Sherwin (Santa Barbara) Free-electron-laser based pulsed EPR
	S. Zvyagin (Dresden) Recent developments of the high-field ESR facility at the Dresden High Magnetic Field Laboratory
	P. Neugebauer (Stuttgart) Improving the sensitivity of THz frequency domain magnetic resonance
	J. Nehrkorn (Berlin) FD-FT THz-EPR on high spin iron states in Myoglobin and Hemoglobin

Friday, September 13, 2013

8:00	*Breakfast*
8:45 – 10:45	***Session 3: Microresonators***
	S. Lyon (Princeton) Enhancing pulsed-EPR sensitivity with superconducting micro-resonators and cryogenic preamps
	D. Suter (Dortmund) Microresonators for EPR
	E. Reijerse (Mülheim) Construction and characterization of microresonators for cw, time resolved and pulse EPR
	A. Pöppl (Leibzig) Sensitivity enhancements in CW-EPR - studies with EPR-silent dielectric resonators and planar microresonators at non-ambient conditions
	N. MacKinnon (Freiburg) Magnetic resonance micro-engineering: enabling new applications
	Coffee break
11:00 – 11:50	***Session 4: Theory***
	S. Stoll (Seattle) Improving EPR simulation methods
	U. Gerstmann (Paderborn) Theoretical EPR fingerprint of coordination defects at silicon related interfaces
12:00	*Lunch*
13:15 - 14:30	Guided tour of the island

14:45 – 15:50	***Session 5: In cell/ in vivo EPR***
	J. Klare (Osnabrück) Approaches toward distance measurements between spin labels in vivo
	M. Drescher (Konstanz) Intracellular EPR spectroscopy
	A. Godt (Bielefeld) Compounds for the evaluation of Gd(3+) complexes as spin labels
	Coffee break
16:05 – 17:50	A. Berndhäuser (Bonn) Synthesis of trityl based spin-labels
	G. Fanucci (Gainesville) Protein conformational sampling from pulsed EPR spectroscopy
	Session 6: New application in materials
	T. Risse (Berlin) Paramagnetic defects on single crystalline surfaces
	E. Erdem (Freiburg) Propeties of defects in ZnO
18:00	*Dinner*
19:30	**General Assembly** *Report from US activities, chairs G. Gerfen, C. Böhme*

Saturday, September 14, 2013

8:00	*Breakfast*
8:45 – 10:25	***Session 7: New detection methods***
	C. Timmel (Oxford) A step-change in sensitivity: how cavity based methods are impacting on condensed phase transient absorption spectroscopy of shortlived radicals
	K. Kartaschew (Bochum) Light induced magnetization detected by magnetic force microscopy
	S. Väth (Würzburg) Charge carrier extraction scheme for electrically detected EPR
	B. Naydenov (Ulm) Demonstrating detection of several nuclear spins by using a single nitrogen-vacancy center in diamond
	Coffee break
10:45 – 11:50	M. Dressel (Stuttgart) Broadband electron spin resonance from 500 MHz to 40 GHz using superconducting co-planar waveguides
	A. Schnegg (Berlin) Spin dependent transport in silicon solar cells monitored by advanced EDMR
	J. Behrends (Berlin) Transient EDMR: recent progress and challenges ahead
	Lunch and Departure

4.5. List of Posters

The posters can be mounted starting Monday, Sep 9 13:00h in the Tent and will be on display for the whole duration of the conference. Please remove the posters by Thursday, Sep 12, 13:00.
Posters should be presented during the Poster Session 1 (odd numbers, Tue 19:30-21:00h) and Poster Session 2 (even numbers, Wed 12:00-13:30h).

Biological Solid State NMR

P1 Interactions of biosilicate and organic molecules in diatom cell walls studied by solid-state NMR spectroscopy
Dorothea Schleuter, Stephan Brückner, Eike Brunner

P2 Sweet secrets – the trehalose coating effect on the internal dynamics of the cold shock protein
Christiane Hackel, Tatyana Zinkevich, Peter Belton, Anja Achilles, Detlef Reichert, Alexey Krushelnitsky

P3 Solution and solid-state NMR characterization of immunoglobulin light-chain amyloid fibrils and their interactions with EGCG
Manuel Hora, Cardine Nokwe, Johannes Buchner, Bernd Reif

P4 Solutions of Supramolecular Assemblies in the Magic-Angle-Spinning Rotor
Andi Mainz, Tomasz L., Religa, Remco Sprangers , Rasmus Linser, Lewis E. Kay, Bernd Reif

P5 Structural investigation of EGCG-induced Alzheimer's disease Aβ oligomers and their interaction with metal ions
Vanessa Morris, Juan Miguel Lopez del Amo, Bernd Reif

P6 Characterization of aggregates formed by the Alzheimer's peptide amyloid-β in the presence of small molecules
Elke Prade, Juan-Miguel Lopez del Amo, Peter Hildebrand, Bernd Reif

P7 Study of the self-aggregation proprieties of the diabetes type II human Islet Amyloid Polypeptide (hIAPP)
Diana C. Rodriguez Camargo, Kostas Tripsianes, Riddihiman Sarkar,Joaquim Mendes, Jasmin Schubert, Burghard Cordes, Tobias Kapp, Antje Wittkopf, Aphrodite Kapurniotu, Bernd Reif

P8 Magic Angle Mis-Adjustment Compensated by Mutual Cancellation of Dipole-Dipole and Chemical Shift Anisotropy
Riddhiman Sarkar, Diana Carolina Rodriguez, Katrin Krzak, Bernd Reif

P9 NMR Spectroscopic Investigations of Supermolecular Proteins and Aggregates in Alzheimer's Disease
Maria Stavropoulou, Andi Mainz, Bernd Reif

Computational Methods

P10 Evolving solid-state MAS NMR experiments using Genetic Algorithms
Matthias Bechmann, John Clark, Angelika Sebald

P11 Towards structure determination of membrane proteins based on sparse NMR data
Tatjana Braun, Oliver F. Lange

P12 Optimal control pulse design for broadband sustainment of singlet states
Matthias D. Budzynski, Franz Schilling, Steffen J. Glaser

P13 Analysis of high pressure induced conformational changes of TmCSP and Byr2-RBD using AUREMOL tools
Nina Danilova, Bärbel Kieninger, Philipp Schneider, Carolina Cano, Konrad Brunner, Martin Rheinard Arnold, Ryo Kitahara, Ralph Elsner, Fritz Huber, Kazuyuki Akasaka, Werner Kremer and Hans Robert Kalbitzer

P14 Solvent PRE-ROSETTA: *de novo* structure prediction using solvent paramagnetic relaxation enhancements
Christoph Hartlmüller, Tobias Madl

P15 Classification Algorithms in Context of NMR based Metabolomics
Jochen Hochrein, Matthias S. Klein, Helena U. Zacharias, Juan Li, Gene Wijffels, Horst Joachim Schirra, Rainer Spang, Peter J. Oefner, Wolfram Gronwald

P16 Modeling of Xe- Bound Human Prion Protein Based on NMR data
D.G. Nair, M. B. Aguiar, W. Kremer, S. Schwarzinger, W. Malloni and H.R. Kalbitzer

P17 ISIC and PERMOL – multifunctional tools for the determination of protein structuresIdea, Implementation in AUREMOL and possible Applications
Bärbel Kieninger, Nina Danilova, Carolina Cano, Konrad Brunner, Philipp Schneider, Silvia de Sanctis, Harald Donaubauer, Tobias Harsch, Hans Robert Kalbitzer

P18 Joint refinement against X-ray and NMR data with REFMAC5
Vito Calderone, Claudio Luchinat, Garib Murshudov, Giacomo Parigi, Enrico Ravera, Mauro Rinaldelli

P19 Rotational tumbling of uncoupled domains in flexible multi-domain proteins and macromolecular complexes
Nasrollah Rezaei-Ghaleh, Frederik Klama, Francesca Munari, Markus Zweckstetter

P20 Effects of NMR Spectral Resolution on Protein Structure Calculation
Suhas Tikole, Victor Jaravine, Vladislav Yu. Orekhov, Peter Güntert

P21 Simulating the time-evolution of sparse spin-1/2 systems
Robert Zeier

P22 Protein structure calculation using CS-Rosetta
Zaiyong Zhang, Oliver F. Lange

Dynamics

P23 Structural analysis of Lysine-20 monomethylation by Set-8 on Histone H4 peptides
Moritz Fölsing, Anna-Winona Struck, Jason Micklefield, Bernd Meyer, Thomas Hackl

P24 Oxygen-17 Dynamic NMR Study of the Pr-DOTA Complex
Luca Fusaro, Michel Luhmer

P25 Dynamic NMR Investigation of N-Me Group Rotation in Dimethylated Arginines
Gerd Gemmecker, Raphael Peltzer, Kostas Tripsianes, Michael Sattler

P26 Ernst angle revisited through the use of optimal control
Marc Lapert, Elie Assemat, Dominique Sugny, Steffen Glaser

P27 NMR study of conformational dynamics of the splicing factor U2AF65
Carolina Sanchez Rico, Lisa R. Warner, Michael Sattler

P28 Balance between electrostatic and hydrophobic interactions in protein complex formation revealed by paramagnetic NMR spectroscopy
Sandra Scanu, Johannes M. Förster, Timmer M., G. Matthias Ullmann and Marcellus Ubbink

P29 NMR Investigation into Structure and Dynamics of Human Blood Group A Glycosyltransferase
Sophie Weißbach, Lena L. Grimm, Thorsten Biet, Monica M. Palcic, Thomas Peters

EPR / Hyperpolarization

P30 PELDOR based Trilateration of Paramagnetic Metal Ions in Biomacromolecules
Dinar Abdullin, Nicole Florin, Gregor Hagelueken, Olav Schiemann

P31 Synthesis of Trityl spin-labels
A. Berndhäuser, H. Arp, O. Schiemann

P32 EPR Experiments on a Genetically Encoded Spin Label
Julia Borbas, Moritz Schmidt, Daniel Summerer, Malte Drescher

P33 Synthesis of EPR pulse sequences on a fast arbitrary waveform generator
Andrin Doll, Gunnar Jeschke

P34 Monitoring Kinetics of Enzymatic ATP Consumption by EPR Spectroscopy
Christian Hintze, Stephan Hacker, Andreas Marx, Malte Drescher

P35 Multifrequency EPR Studies of Photoactive Donor-Acceptor Systems
Hideto Matsuoka, Shinichi Mizutani, Chika Watanabe, Seigo Yamauchi

P36 Application of EPR Methods for a Wide Range of Chemical Questions
Yaser NejatyJahromy, Siavash Saeidpour, Hamed Alaei, Dinar Abdullin, Erik Schubert, Andreas Meyer, Olav Schiemann

P37 Cross-polarization edited ENDOR
Roberto Rizzato, Ilia Kaminker, Shimon Vega, Marina Bennati

P38 α-Synuclein binds alpha-helically to mitochondria
Marta Robotta, Patrick Korf, Hanne R. Gerding, Christiaan Karreman, Stefan Schildknecht, Marcel Leist, Vinod Subramanian, Malte Drescher

P39 EPR spectroscopic studies of hemin peptide binding
E. Schubert, H. Brewitz, T. Kühl, D. Imhof, O. Schiemann

P40 Systematic comparison of sampling strategies for static gradient CW EPR microscopy at low SNR using an iterative SIRT image reconstruction method
Martin Spitzbarth, Malte Drescher

P41 Towards nanomolar NMR detection through SABRE hyperpolarization
Marco Tessari, Nan Eshuis, Niels Hermkens, Bram van Weerdenburg, Martin Feiters, Floris Rutjes, Sybren Wijmenga

P42 Distance and orientation measurements with DEER/PELDOR at 95 and 263 GHz
Igor Tkach, Soraya Pornsuwan, Marina Bennati

Imaging

P43 High Efficiency Compact Helium Liquefaction and Recycling for NMR Systems
Tobias Adler, Shi Li, Jeremy Terry, Cesar Chialvo, Jost Diederichs, Martin Krugler, Stefano Spagna, Richard Reineman, Conrado Rillo

P44 Impact of the organic coating on the relaxometric properties of GdF_3 nanoparticles
Fabio Carniato, Lorenzo Tei, Kalaivani Thangavel, Mauro Botta

P45 Dendrimersomes: a new vesicular nanoplatform for theranostic applications
Miriam Filippi, Marisa Ferraretto, Gilberto Mulas, Jonathan Martinelli, Lorenzo Tei, Mauro Botta, Silvio Aime, Enzo Terreno

P46 In vivo MRI visualization of the intratumor release of Doxorubicin from liposomes by non-focused ultrasound
S.Rizzitelli, P.Giustetto, J.C. Cutrin, V. Menchise, C.Boffa, M.Ruzza, D. Delli Castelli, S.Aime, E.Terreno

Materials

P47 Sol-gel-derived thiol-functionalized oligosilsesquioxanes: influence of synthesis
Evgeny Borovin, Benjamin Papendorf, Emanuela Callone, Sandra Diré

P48 Investigating polymers shape-memory behaviour through solid-state NMR
Silvia Borsacchi, Katia Paderni, Massimo Messori, Maurizio Toselli, Francesco Pilati, Marco Geppi

P49 Solid-State NMR Spectroscopy of Chiral Metal-Organic Frameworks
Herbert C. Hoffmann, Philipp Müller, Mohan Padmanaban, Silvia Paasch, Irena Senkovska, Frank Glorius, Stefan Kaskel, Eike Brunner

P50 Cryogen-Free Superconducting Magnet Design for NMR
D. Pooke, J. de Feijter, M. Mallett, S. Bubici

P51 NMR Relaxation-Viscosity Relationship in PEO Melts
Jacques Leblond, Marc Fleury, Benjamin Nicot, Madeleine Djabourov, Salvatore Bubici

P52 Combination of 13C variable contact time solid state NMR and Principal Components Analysis (PCA) for geographical traceability of wood
Emanuela Callone, Ilaria Santoni, Anna Sandak, Jakub Sandak, Sandra Dirè

P53 Fast Field Cycling Relaxometry: Moving from Research towards Industrial Applications
Salvatore Bubici, Rebecca Steele, Gianni Ferrante

P54 Method for NMR Relaxometry on Hetero-nuclei at Very Low Fields
S. Bubici, R. Steele, G. Ferrante

P55 Coupling Solid-State NMR with GIPAW ab-initio calculations in metal hydrides and borohydrides
Federico Franco, Marcello Baricco, Michele Chierotti, Roberto Gobetto, Carlo Nervi

P56 Phase transitions in polymeric luminescent indicators with a threshold temperature through variable-temperature SSNMR and fluorescence spectra
Francesca Martini, Silvia Borsacchi, Giacomo Ruggeri, Andrea Pucci, Marco Geppi

P57 Interaction of electrolyte molecules with porous carbon materials: Characterization by NMR Spectroscopy
Silvia Paasch, Lars Borchardt, Martin Oschatz, Stefan Kaskel, Eike Brunner

P58 Distorting cages in an ion conductor
V. R. Celinski, D. Jardón Álvarez, S. Ebbinghaus, J. Schmedt auf der Günne

P59 The Grotthuß-Mechanism in Nanoporous Silicates and its Implications to Diffusion Studies in Natural Porous Media
Eva Paciok, Agnes Haber, Maxime Van Landeghem, Bernhard Blümich

P60 Processing additives for BHJ solar cells: a morphological study based on solid-state NMR, AFM and Raman microscopy
S. Spera, M. Salvalaggio, A. Savoini, R. Marinacci, C. Carbonera and A. Cominetti

Nucleic Acids

P61 Structural investigation of inosine-edited RNA duplex and its interaction with RNA-induced silencing complex component p100
Alexander Beribisky, André Dallmann, Kostantinos Tripsianes, Martin Ruebbelke, Lisa Warner, Michael Sattler

P62 Experimental evidence of the existence of the G-triplex and its three-dimensional structure
Linda Cerofolini, Stefano De Tito, Marco Fragai, Andrea Giachetti, Claudio Luchinat, Ettore Novellino, Antonio Randazzo

P63 Structural Investigation of pri-miR-18a by NMR Spectroscopy
Johannes Günther, Joka Pipercevic, Hamed Kooshapur, Andre Dallmann, Michael Sattler

P64 Cytosine residues in a G-rich region lead to formation of noncanonical structures
Vojc Kocman and Janez Plavec

P65 G-quadruplex interior is accessible to water molecules
Peter Podbevsek, Jaka Zavasnik, Janez Plavec

P66 Long-lived intermediates formed by G-rich strands in the absence of cations
Primož Šket, Slavko Čeru, Iztok Prislan, Jurij Lah, Janez Plavec

P67 Cation localization and movement within d(TG8T)4 G-quadruplex in solution
Marko Trajkovski, Janez Plavec

P68 Comparing substrate specificity of 2'deoxyguanosine-binding riboswitch classes by NMR spectroscopy and thermodynamics
A. K. Weickhmann, A. Wacker, E. Duchardt-Ferner, H. Keller, S. Schmidtke, H. Schwalbe, J. Wöhnert

Pharma and Food

P69 ^{1}H-NMR and HR-MAS-^{1}H-NMR as useful tools for the quali/quantitative characterization of cocoa beans and derived products.
Acquotti Domenico, Marseglia Angela, Palla Gerardo, Augusta Caligiani

P70 Authentication of medicines by Nuclear Quadrupolar Resonance (NQR) Analysis. A portable system for non-destructive control of packaged pharmaceutical products
G.Ferrante, R. Cernuschi, R. Gatti, R. Rolfi, K. Althoefer, J. Barras, G. Kyriakidou, J. Smith

P71 Novel platinum compounds with potential for cancer therapy: study of their interaction with model lipid membranes.
Simona Samaritani, Maciej Baginski, Lucia Calucci, Valentina Censi, Jacek Czub, Lukasz Nierzwicki, Milosz Wieczor, Claudia Forte

P72 Distribution of organic additives in micellar solution of surfactants: A PFG NMR diffusion study
Jonas Fuchs, Monika Schönhoff

P73 Solid-state NMR for the characterization of crystal forms of pharmaceutical compounds
K. Gaglioti, M. R. Chierotti, F. Grifasi, R. Gobetto and U. Griesser

P74 Study of chemical interactions between antiretroviral drugs Efavirenz and Tenofovir Disoproxil Fumarate
Elionai Cassiana de Lima Gomes, Maria Irene Yoshida, Wagner da Nova Mussel, Jarbas Magalhães Resende, Silvia Ligório Fialho, Jamile Barbosa, Marco Geppi

P75 Characterization of Protected Designation of Origin Lambrusco Wines of Modena using NMR Spectroscopy and Multivariate Statistical Analysis
Riccardo Graziosi, Giulia Papotti, Davide Bertelli, Maria Plessi

P76 Pharmaceutical co-crystals of niclosamide: a solid-state NMR approach
F.Grifasi, M.R.Chierotti, K.Gaglioti, R.Gobetto, D.Braga, E.Dichiarante, F.Grepioni, L.Maini

P77 Structural and nutritional properties of pasta from *Triticum monococcum* and *Triticum durum* species. A combined 1H NMR, MRI and digestibility investigation
Maristella Gussoni, Fulvia Greco, Gabriella Pasini, Mauro A. Cremonini and Roberto Consonni

P78 The effects of intra- and postoperative ischemia on the metabolic profile of clinical tissue specimens monitored by NMR
Stefano Cacciatore, Xiaoyu Hu, Christian Viertler, Marcel Kap, Gerwin A. Bernhardt, Hans-Jörg Mischinger, Peter Riegman, Kurt Zatloukal, Claudio Luchinat, Paola Turano

P79 NMR Methodology in Food Science
Luisa Mannina, Anatoly P. Sobolev, Noemi Proietti, Donatella Capitani

P80 Conformation of Contryphan-Vn S8W, a newly synthesized cyclic peptide and the role of proline isomerization
Ridvan Nepravishta, Walter Mandaliti, Sonia Melino and, Maurizio Paci

P81 Characterization and classification of propolis extracts by HR-NMR
Giulia Papotti, Davide Bertelli, Riccardo Graziosi, Maria Plessi

P82 Biochemical assessment of RBCs during storage in blood bank conditions
T.A. Pertinhez, P. Berni, E. Casali, L. Lindner, R. Baricchi, A. Spisni

P83 Urinary metabolic profiling of rats treated with curcumin, using ^{1}H NMR and HPLC-MS
Elisabetta Schievano, Matteo Stocchero, Maria Clauser, Emmanuel Ndoum, Stefano Mammi, Stefano Dall'Acqua

P84 NMR characterization of the binding of small ligands and functionalized liposomes to Aβ peptides
Erika Sironi, Cristina Airoldi, Francisco Cardona, Angela Lompo, Barbara La Ferla, Francesco Nicotra

Polymers

P85 Excited Triplet States and Molecular Geometry of Oligo-PPEs Investigated by Pulsed EPR
Patrick Korf, Christian Hintze, Friederike Schütze, Stefan Mecking, Malte Drescher

P86 Complex Coacervation of Polyelectrolytes Studied by Spin-Label EPR Spectroscopy
Uwe Lappan, Brigitte Wiesner, Ulrich Scheler

Small Molecules

P87 NMR Discrimination of Pure Atropoenantiomeric Quinazolinone Derivatives
Silvia Capacchi, V. Mileo, A. Pappani, A. M. Capelli and S. Catinella

P88 Different solid forms of Sodium Ibuprofen: a combined Solid State NMR, X-Ray Crystallography and DSC study
Elisa Carignani, Silvia Borsacchi, Eleonora Macedi, Patrizia Rossi, Paola Paoli, Luca Bernazzani, Marco Geppi

P89 Endometabolomics of B cell differentiation
Zucchelli Chiara, Mari Silvia, Jose Manuel Garcia-Manteiga, Roberto Sitia, Musco Giovanna

P90 ^{13}C NMR of a Single Molecule Magnet: Analysis of Pseudocontact Shifts and Residual Dipolar Couplings
Marko Damjanovic and Markus Enders

P91 NMR Kinetic and Mechanistic Study of Sulfoxide-Mediated α-Arylation of Carbonyl Compounds
Christophe Fares, Xueliang Huang, Mahendra Patil, Walther Thiel, Nuno Maulide

P92 Investigation of the conformational space of a Diarylethene derivative using residual dipolar couplings
Maic Fredersdorf, Robert Göstl, Stefan Hecht, Christina M. Thiele

P93 STD-NMR experiments on tumor cells to investigate RGD ligand-membrane protein interactions
Ileana Guzzetti, Donatella Potenza, Francesca Vasile, Laura Belvisi, Monica Civera, Ilaria Silvestri, Umberto Piarulli, and Cesare Gennari

P94 Pure Shift HSQC Measurements with perfectBIRD Decoupling - a Method to Decouple Diastereotopic Protons
Lukas Kaltschnee, Andreas Kolmer, István Timári, Ralph W. Adams, Mathias Nilsson, Katalin E. Kövér, Gareth A. Morris, Christina M. Thiele

P95 Mechanistic Investigation of the 1,4-Addition Reaction of Organozinc Reagents Catalysed by Chiral Phosphoramidite-Copper Complexes
Carina Koch, Felicitas von Rekowski, Ruth M. Gschwind

P96 Conformational analysis of small organic molecules using NOE and RDC data: A Discussion
Andreas Kolmer, Christina Thiele

P97 The stability of N-Heterotetracenes towards light and oxygen as investigated by NMR spectroscopy
Andreas Kolmer, Nicole Anderl, Matthias Rehahn, Christina Thiele

P98 An NMR Study on Metal-Coordinated White Phosphorus
Piero Mastrorilli, Vito Gallo; Mario Latronico, Maurizio Peruzzini, Vincenzo Mirabello, Dietrich Gudat, Sebastian Heinl, Manfred Scheer

P99 The Spin Density Distribution in a Symmetrically Substituted Bissilylene Radical Cation
Andreas Meyer, Marius Arz, Martin Straßmann, Alexander Filippou, Olav Schiemann

P100 Lyotropic Liquid Crystalline Phases from Supramolecular Polymers as Orienting Media for NMR Spectroscopy
Nils-Christopher Meyer, Martin Leyendecker, Christina Marie Thiele

P101 NMR spectroscopic Characterization of Unsymmetrical Cube-Octameric Silsesquioxanes (COSS)
Volker Schmidts, Sascha Knauer, Sebastian Hörner, Olga Avrutina, Harald Kolmar, Christina M. Thiele

P102 Metabolic changes during cellular senescence investigated by NMR spectroscopy
Claudia Gey, and Karsten Seeger

P103 A Multinuclear NMR characterization of Pt(II)-Pt(IV) intermediate formed during I_2 oxidation of dinuclear Pt(II)-Pt(II)
Stefano Todisco, Andersson Arias, Juan Forniés, Consuelo Fortuño, Antonio Martin, Mario Latronico, Piero Mastrorilli

P104 NMR investigations on OR2, an inhibitor of Aβ aggregation
Daniela Valensin, Chiara Nesti, Riccardo De Ricco, Marek Luczkowski, Henryk Kozlowski

P105 Paramagnetism in free-base metallocenylporphyrins
A.Vecchi, D. O. Cicero, V. Conte, B.Floris, P.Galloni, V. N. Nemykin

P106 Intercomparison of quantitative 1H NMR
Mariangela Vezzoso, Vito Gallo, Pasquale Scapicchio

P107 NMR analysis of human biofluids reveals metabolic changes related to the development of acute kidney injury following cardiac surgery
Helena U. Zacharias, Gunnar Schley, Jochen Hochrein, Matthias S. Klein, Carmen Köberle, Kai-Uwe Eckardt, Carsten Willam, Peter J. Oefner, Wolfram Gronwald

Structural Biology

P108 The role of DJ-1 in apoptosis: an NMR study of its interactions toward the design of inhibitors of the anti-apoptotic protein Bcl-X_L
Carlo Baggio, Mattia Sturlese, Massimo Bellanda, Stefano Mammi

P109 NMR studies of cerato-populin: investigating the differences in fungal PAMP induced resistance.
Baroni F., Pazzagli L., Cappugi G.,Scala A.,Martellini F.,Franzoni L., Pertinhez T.A.,Spisni A.

P110 Structural investigation of the SF1/U2AF65/U2AF35 complex utilizing paramagnetic LBT-gained PCSs
Katja Barthelmes, Yun Zhang, Tobias Madl, Cameron Mackereth, Frank Gabel, Michael Sattler

P111 Progress in the Investigation of the Helical Human Membrane Protein Hv1 by liquid-state NMR.
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P141 NMR analysis of heme-regulatory motifs
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P143 Bile acid binding protein-rhodamine host-guest system: NMR and optical characterization of a fluorescent bio-nanomaterial
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P144 Does the staggered-rotamer model do what we think it does?
Jürgen M. Schmidt and Frank Löhr

P145 Investigations of the cysteine-rich region of type VII collagen with CD- and NMR-spectroscopy
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P146 Metabolic characterization of SH-SY5Y cells and effects of interferon-α exposure
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P147 A NMR-based model of the electron-transfer complex between ferredoxin and [FeFe]-hydrogenase required for hydrogen production
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P148 Conformational states of the ADP ribosylation factor 1 and its importance for the interaction with regulators and effectors
Christian Hundshammer, Tanja Meierhofer, Michael Spoerner

P149 Structural studies of ubiquitylation intermediates
Timo Strohaeker, Fabian Renschler, Karolina Hyz, Silke Wiesner

P150 **Structural studies of monothiol Grx1 from the parasite *Trypanosoma brucei***
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P151 **High pressure NMR spectroscopy and drug development**
P. N. Sunilkumar, I. C. Rosnizeck, C. E. Munte, V. Kropf, M. Spoerner and H. R. Kalbitzer

P152 **Identification of β-microglobulin residues involved in Transglutaminase reaction**
Tomhardt, A., Seeger, K.

P153 **Novel NMR approaches for the characterization of heme centers in proteins**
Mario Piccioli, Paola Turano

P154 **Achieving linear independent alignments through encodable loop lanthanides binding tags**
Dominic Wagner, Katja Barthelmes, Kai Schnoor, Henry Jonker, Harald Schwalbe

P155 **Application of ^{13}C broadband pulses to excitation, refocusing and decoupling for high field**
Lisa Warner, Franz Schilling, Thomas Skinner, Steffen Glaser, Michael Sattler

P156 **Delivery of isotope-labeled proteins into eukaryotic cells for in-cell NMR experiments**
S. Zanzoni, M. Sega, M. D'Onofrio, M. Assfalg, R. Chignola, and H. Molinari

5.1
Abstracts – Talks

Gain in Resolution, SNR and Detection Sensitivity by Poisson-Gap Sampling and Reconstruction with Iterative Soft Thresholding for Enabling Studies of Large Proteins

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Commonly used linear sampling of indirect dimensions in 3D and 4D NMR spectra cannot proceed to maximum evolution times needed to match natural line widths. This can only be achieved with non-uniform sampling (NUS) where we can now routinely record and reconstruct 4D NUS spectra with less than 1% of the indirect points sampling up to 120 ms evolution times, within six days of measuring time. Recording this experiment linearly would take 500 days. Thus, NUS is needed to exploit the power of modern NMR instruments. NUS can also increase the capability of detecting weak signals, such as in 3D or 4D NOESYs. Here we compare time-equivalent US and NUS experiments. If we record only 1% of the indirect points we can collect 100 times more scans per increment in the same amount of time. This increases both, the SNR and the probability of detecting weak peaks. This can be shown in simulations and experimentally To utilize these benefits it is important to apply optimal sampling schedules that focus on time-domain regions where the signal is strongest, to avoid large gaps, and to maximize randomness. We achieve this by selecting sampling gaps randomly according to a Poisson distribution centered at zero. Finally, we need a high-fidelity reconstruction method. After working with and developing new reconstruction methods we have come up with a reliable and very fast approach based on the Iterative Soft Thresholding principle, hmsIST. It doesn't create significant artifacts, yields correct peak heights and is very fast so that high-resolution 4D spectra can be recorded and reconstructed routinely within a day.

Cold-denaturation of a protein dimer monitored at atomic resolution.

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Protein folding and unfolding are crucial for a range of biological phenomena and human diseases. Defining the structural properties of the involved transient species is therefore of prime interest. Using a combination of cold-denaturation with nuclear magnetic resonance spectroscopy we reveal detailed insight into the unfolding of the homodimeric repressor protein CylR2. Seven three-dimensional structures of CylR2 at temperatures from 25 °C to -16 °C reveal a progressive dissociation of the dimeric protein into a native-like monomeric intermediate followed by transition into a highly dynamic, partially folded state. The core of the partially folded state appears critical for biological function and misfolding [1].

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Accessing long-lived nuclear singlet states between chemically equivalent spins without breaking symmetry

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Hyperpolarized magnetic resonance imaging enables real-time monitoring of metabolites at concentration levels not accessible by conventional MRI techniques. However, the short T1 lifetime of hyperpolarized signal largely hurdles its broad application1. Nuclear spin singlet state has been shown to extend the effective T1 by more than an order of magnitude in favourable cases2-4. Previous method to store signal into the singlet state relies on the chemical shift difference between the 2 spins5. Here we show that in more general spin systems such as CC'HnHn', the singlet state between the 13C2 spins can be accessed without chemical shift difference6. Instead, the intrinsic scalar coupling difference, ΔJCH enables interconversion between 13C bulk magnetization and the singlet state population. More importantly, very similar strategy can transfer proton magnetization into and out of the singlet state2. The obvious advantages include a much better SNR of proton detection versus 13C detection; potentially faster and higher hyperpolarization achieved on proton versus 13C; and lastly, the total pulse sequence includes only proton pulse, which can be easily implemented on conventional clinical scanners. Interestingly, this strategy is also immune to perturbation caused by chemical shift difference between the 13C2 spins if there is; therefore, this method to access the singlet state through ΔJCH may largely extend the T1 lifetime of multiple classes of molecules and thus, broaden the application of hyperpolarization to in vivo molecular imaging.

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Tailored real-time scaling of heteronuclear couplings

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Heteronuclear couplings are an extremely valuable source of molecular information, encoded in the multiplet splittings of an NMR spectrum. RF irradiation on one coupled nuclear spin allows modifying the coupling strength, scaling down the multiplet splittings in the spectrum observed at the resonance frequency of the other nuclear spin. Those decoupling pulse sequences are oftentimes used to collapse a multiplet into a singlet and can therefore simplify NMR spectra significantly. Off-resonance continuous wave (CW) decoupling has been studied since the early days of NMR and has been introduced as a means of obtaining correlation information prior to the advent of 2D-NMR [1]. More recently, it has been used extensively for chemical shift monitoring in pseudo-multidimensional NMR, where dimensionality can be increased without the need for the time-consuming process of acquiring an additional dimension [2]. However, in CW off-resonance decoupling, the effective scaling of the scalar coupling constant is a nonlinear function of frequency offset. As a result, the accuracy of the back-calculated chemical shift is strongly dependent on the chosen decoupling frequency.

Scaling of **H**eteronuclear couplings by **O**ptimal **T**racking (SHOT) refers to the numerical optimization of a decoupling pulse based on optimal control algorithms which is then applied synchronously during the data acquisition [3,4]. This provides a pre-defined offset dependence in the effective coupling constant J_{eff} that can be chosen virtually arbitrarily. SHOT pulses can thereby overcome limitations that have been imposed to chemical shift encoding via off-resonance CW decoupling, most importantly solving the problems of non-linear chemical-shift dependence of the residual coupling, and the sensitivity towards B_1 miscalibrations. In an experiment we show that a 1D SHOT $\{^1H\}$-^{13}C experiment yields comparable information to a 2D HSQC and can give full assignment of all coupled spins. It is important to note that the effective *J*-splitting can be scaled to values larger than *J,* which in the directly detected frequency direction to the best of our knowledge is reported for the first time. Both simulations and experiments confirm that actual *J*-couplings which are lower or higher than the coupling constant used for defining the offset-profile J_{opt} are either scaled up or down to the offset dependent value of J_{opt}. This property of SHOT pulses implies that the actual coupling constant *J* does not need to be known for the calculation of chemical shift correlations, allowing a correlation experiment within a single shot. SHOT pulses allow chemical shift correlation experiments within a single scan making them perfectly suited for hyperpolarized fast NMR experiments tailored to the specific needs of the molecule under investigation [5].

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The BLUU-Tramp application: problems and solutions

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Describing the equilibrium unfolding landscape of a protein is of fundamental relevance when non-native states play a crucial role in biological function or disease. The characterization of the thermodynamics or kinetics of the phenomena involving proteins is a direct way of addressing their function. To monitor these phenomena we have devised a new NMR approach that explores the conformational landscape of a protein. By studying the isotope exchange of the backbone amides of a protein dissolved in deuterated water or vice versa, the thermodynamic or kinetic stability of the conformation of each single residue, within the whole protein native state, can be measured. We have proposed, instead, a new experiment, BLUU-Tramp (Biophysics Laboratory University of Udine Temperature ramp), that enables exploring the two-dimensional time-temperature domain in a single experimental session (Rennella et al. 2012), where the isotope exchange data can be collected simultaneously as a function of time and temperature by directly executing a synchronized temperature ramp throughout the time acquisition of the exchange data. Besides testing the method with different proteins under different experimental conditions, the basic version of the experiment is also being improved to overcome problems such as protein precipitation during the temperature ramp, peak tracking efficiency, global, subglobal and local opening assessment. A few examples will be illustrated to account for the state of art of the current development.

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NMR in Molecular Systems Biology: from structures to function

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NMR is an unique tool for describing functional processes at atomic level in a cellular context. Metal transfer occurs through protein-protein interactions[1,2] with the metal transfer determined by affinity gradients[3]. Studying the processes for the biogenesis and transport of iron-sulfur clusters we have also to deal with paramagnetic centers which dramatically affect the NMR spectra, thus requiring tailored experiments. The structural and electronic properties of the metal sites can be complemeted with EPR spectroscopy. The power of NMR, integrated with other spectroscopic techniques, will be presented for a few pathways responsible for copper trafficking in the cell and for the biogenesis of iron-sulfur proteins. New major advancements in in-cell NMR4 and in the characterization of highly paramagnetic systems5 will be discussed within an integrated approach.

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Imaging cancer metabolism with hyperpolarised ^{13}C labelled cell substrates

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We have been developing methods for detecting the early responses of tumours to therapy [1], including MR imaging of tumour cell metabolism *in vivo* using hyperpolarized ^{13}C-labelled cell metabolites [2,3]. Nuclear spin hyperpolarization can increase sensitivity in the MR experiment by >10,000x. This has allowed us to image the location of labelled cell substrates and, more importantly, their metabolic conversion into other metabolites. These substrates include pyruvate, lactate, glutamine, glutamate, fumarate, bicarbonate, ascorbate and glucose.

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About DNP of Proteins and MAS NMR on membrane proteins and with four nuclei

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Solid-state NMR enables the investigation of heterogeneous, complex biological samples at high resolution. In future, typical applications will include studies of molecular machines at work. A major factor enabling such investigations is dynamic nuclear polarisation (DNP), which was introduced to increase signal-to-noise by one or two orders of magnitude. The application of dynamic nuclear polarisation (DNP) requires further optimization of samples, experimental parameters and concepts. Spectra with reasonable line width are obtained on membrane-integrated complexes. Various technical aspects of the application of DNP to protein systems will be discussed. During the DNP process, electron polarization is transferred to the surrounding core nuclei and subsequently to the bulk nuclei. This process depends on several factors such as; relaxation behavior, proton concentration, spin-diffusion and type of the nucleus. In order to improve the quality of DNP spectra, the possibility of DNP at higher temperature is discussed. An overview is given over current experimental problems and possible solutions.
A major application of DNP involves investigations of the nascent chain within and directly after leaving the ribosome. The initial folding events are not yet well understood, and this investigation provides an NMR view onto this important process. Prefolding saves several chaperone steps and protects against enzymatic degradation, so it is important to understand this mechanism. There is currently too little atomic resolution data to confirm the nascent chain's folding state. Labeled nascent chain peptide comprising the signals peptide of DsbA is prepared within unlabeled ribosome, and using DNP NMR data, a picture about the secondary structure of this nascent chain in the tunnel is derived. Populations of coil-like structures are dominantly observed within the tunnel, with very low populated a-helical segments in the DsbA section of the emerging peptide.
Further studies involve an ABC-Transporter that imports positively charged amino acids into bacteria. Studies by solid-state NMR on samples including native bilayers and by solution NMR involving also the substrate binding protein indicate a new transport cycle for the transporter.
In the context of protein studies, experiments involving four nuclei are presented, and approaches to make structure determination more straightforward.

Exploring new routes to high sensitivity probes for MR-Molecular Imaging

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The possibility of carrying out Molecular Imaging protocols by means of MRI is very attractive for the superb anatomical resolution that is attainable by this technique. However, MRI suffers from an intrinsic insensitivity with respect to the competing imaging modalities that has to be overcome by designing suitable amplification procedures based on the development of reporting units endowed with an enhanced sensitivity and on the identification of efficient routes of accumulation of the imaging probes at the sites of interest. [1] Besides paramagnetic agents, much attention is currently devoted to two new classes of frequency-encoding probes, the CEST agents (CEST = Chemical Exchange Saturation Transfer) and the class of hyperpolarized molecules. The use of frequency-encoding agents opens the interesting perspective of detecting more than one agent in the same anatomical region. [2] All together, the recent developments in the design and testing of new probes have significantly improved the potential of MRI in the field of Molecular Imaging applications.

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High Resolution Neuroimaging at 7T

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Human brain imaging research has driven whole body high field MR development. The increased SNR but also the increased susceptibility effects can be exploited for higher resolution in particular for structural and functional imaging. In in vivo imaging very high resolution is meeting a number of challenges. Most prominently, motion degrades the achievable effective resolution in long imaging times. Real time motion detection allows to prospectively correct subject motion and update the imaging volume continuously during the acquisition resulting in previously unmatched high spatial resolution. For functional imaging, the geometric fidelity of the echo planar imaging sequence is further limiting the assignment of activation to anatomy. With fast and accurate mapping of the local imaging point spread function, geometric distortions can be corrected. Together with sub-millimeter single-shot acquisitions the delineation of cortical layers is in reach providing fundamentally new non-invasive information about human brain function.

Saturation Transfer Applications of Exchangeable, Hyperpolarized Xenon

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Xenon-129 is an extremely sensitive NMR probe as illustrated by its large chemical shift range in many different molecular environments. Though a noble gas, it comes with sufficient solubility in aqueous solutions and can reversibly bind to host structures like cryptophanes. These induce a large temporary change in resonance frequency upon reversible binding and thus make these nuclei interesting candidates for saturation transfer experiments. Further combination with hyperpolarization approaches allow for designing functionalized contrast agents or 'biosensors' for molecular imaging. Xenon-based sensors have been developed to detect the presence of a certain analyte or to reveal various biochemical binding events. Beyond the aim to develop targeted agents, studies using unfunctionalized xenon hosts also include various applications. Examples of different NMR saturation transfer signatures of reversibly bound xenon will be presented and their potential biomedical applications will be discussed. This method called Hyper-CEST (chemical exchange saturation transfer with hyperpolarized nuclei) allows high sensitivity detection of Xe biosensors that is not achievable with conventional NMR contrast agents. Recent results including the smashCEST approach with snap-shot MRI will be presented to illustrate the ~19000-fold sensitivity enhancement that comes on top of the hyperpolarization approach. Such studies allow preserving molecular specificity in the imaging signal by encoding the chemical shift dimension in a reasonable amount of time.

Protein interactions and function studied by novel NMR methods

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We extensively use residual dipolar coupling, paramagnetic labeling, scalar couplings and other NMR parameters to characterize folded and unfolded protein states in a quantitative manner. We will give several recent examples. Scalar couplings across hydrogen bonds (H-bonds), which report on the electronic overlap between donor and acceptor orbitals, present a highly sensitive measure of H-bond geometry. Using H-bond scalar couplings, we have recently mapped the pressure and temperature dependent deformation of ubiquitin's H-bond network [1]. We recently also obtained atomic details of the pressure-assisted, cold-denatured state of ubiquitin at 2500 bar and 258 K by high-resolution NMR techniques [2]. This state has structural propensities, which are very similar to ubiquitin's alcohol-denatured (A-) state. At non-denaturing concentrations of methanol, a complete transition from the native to the A-state can be achieved at ambient temperature by varying the pressure from 1 to 2500 bar. This method should allow highly detailed studies of protein folding transitions in a continuous and reversible manner. Abelson kinase is an important drug target in the treatment of chronic myelogenous leukemia. We have characterized the solution conformations of the kinase domain in complex with different inhibitors [3]. The NMR analysis has also helped to locate the interaction side of a new class of allosteric inhibitors that can overcome resistance to ATP-binding-site inhibitors [4]. We will show recent results on these interactions.

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NMR investigation of the equilibrium partitioning of a water-soluble bile salt protein carrier to phospholipid vesicles

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We applied NMR spectroscopy to study the partitioning of a water-soluble bile acid binding protein (BABP) between its free and lipid-vesicle-bound states.[1,2] The data indicated a reversible interaction of BABP with anionic vesicles occurring in a very slow exchange regime on the NMR time scale. The addition of the physiological ligand to the protein-liposome mixture was capable of modulating the interaction, shifting the equilibrium towards the membrane-dissociated ligand-bound protein. As the membrane-bound protein was NMR-invisible, the signals of the free biomolecule were analyzed to obtain quantitative information on binding affinity and steady-state kinetics. The approximate binding epitope was demonstrated from results on BABP samples in which different positively charged lysine residues were mutated to neutral alanines. H/D exchange measurements indicated a higher exposure to solvent for the core amino acid residues in the liposome-bound state. We finally explored exchange saturation transfer experiments to derive information about the NMR-invisible state.

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Fast Field Cycling Relaxometry: Moving from Research towards Industrial Applications

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Fast Field Cycling Relaxometry (FFCR) is a NMR technique used to determine the longitudinal relaxation time (T_1) over a range of B_0-fields spanning about six decades, from about 10^{-6} Tesla up to ~ 1 Tesla, or 3 Tesla, without varying the frequency of the spectrometer [1,2]. The main information that can be extracted from nuclear magnetic resonance dispersion (NMRD) curves,T_1 or $R_1=1/T_1$ versus the Larmor frequency ω (ω=γB_0, γ is the gyromagnetic ratio),is that concerning molecular motions characterized by temperature-activated frequencies and described by means of the spectral density J(ω).

The data obtained may, therefore, be correlated directly to the physical/chemical proprieties of complex materials. The use of radio frequency allows the easy penetration of most materials, thus permitting, the exploration of slow dynamics which are often difficult to study in heterogeneous materials (including liquids, solids and gels) by other spectroscopic methods.

Furthermore, the benefit of exploring the range of low Larmor frequencies is to detect typical relaxation features associated with molecular processes characterized by very long correlation times, such as molecular surface dynamics and collective effects. The FFCR technique shows greatest potential where the characteristics of a sample depend intimately on the molecular dynamics and / or the state of aggregation.

Herein we show developments in the FFCR method and discuss some contributions of NMRDprofiles towards the fundamental understanding of classes of materials and phenomena predicted by theoretical models. We show in practice how relaxation experiments can be applied for qualitative structural diagnostics in solutions, quantitative structural determinations, recognitions of weak intermolecular interactions and studies of molecular mobility. The findings that have been established more recently are noteworthy for their potential industrial use in quality assessment and off-line process monitoring.

FFCR is a technique which has remained as a research tool and practically unexploited in industry, and thus an important challenge is the transfer of this important technique towards more industrial applications.

Evaluation of the effects of cryopreservation on Modena Biobank tissue samples through HR-MAS NMR

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The application of high-resolution magic angle spinning (HR-MAS) NMR spectroscopy to the analysis of intact tissue biopsies and surgery samples dates back to 1997. It represents, at present, an actively investigated field, and it is used to obtain the metabolic fingerprints of tissues. It has been shown to differentiate between normal and neoplastic tissue in the case of breast, brain, kidney, colon cancer and other malignancies in the upper gastrointestinal tract. Nevertheless, it is difficult to run HR-MAS analysis immediately after surgical resection or biopsy, and samples are currently frozen in liquid nitrogen and then stored to -80 °C. Some studies reporting the effect of sample ischaemia, spinning time and measurement temperature on the metabolic profile of tissues can be found.[1] Nevertheless, another important issue to be addressed is the effect of cryopreservation time on the metabolic profile of tissues. Cryopreservation represents a more and more common practice, due to the widespread of tissue BioBanks. To gain a deeper insight into metabolic changes that occur during the storage, samples of Modena BioBank were analyzed through HR-MAS NMR after 1, 6 and 12 months from collection. The results of this study will be discussed.

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Distinct Conformational States of the Alzheimer beta-Amyloid Peptide can be detected by High Pressure NMR Spectroscopy

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Amyloid fibrils in the brain consisting of the beta amyloid peptide are a lead marker of Alzheimer's disease. A deeper understanding of the mechanism of fibril formation may help to design drugs for preventing the deposition of amyloid, therefore the existence of specific conformations of monomeric Abeta is potentially important. Abeta-monomers in aqueous environment are usually assumed to occur in a disordered state. In contrast, by high pressure NMR spectroscopy we detect two main conformational states at atmospheric pressure, a compactly folded state 1 and a partly unfolded state 2 with relative populations of 0.7 and 0.3, respectively [1]. Pure random-coil like structures were not detected. The pressure response indicates an ordered structure between amino acids 16 to 24 and 30 to 37 in state 1. Abeta-fibrils depolymerise at high pressure, the dissociation constant of monomers from the fibrils increases by two orders of magnitude at 200 MPa and 283 K. The partial molar volume of the monomer unit changes by 101 ml/mol with binding. The thermodynamic data suggests that state 1 is responsible for fibril elongation.

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Molecular Machines studied by solid-state NMR

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Gram-negative bacteria use a molecular machinery called the type three secretion system (T3SS) to deliver effector proteins to host cells. Our research group has recently solved an atomic model of the extracellular T3SS needle of Salmonella typhimurium (Loquet et al., Nature, 2012). Concurrently, a high-resolution cryo-electron microscopy density map of the T3SS needle of Shigella flexneri was obtained by Fujii et al. (PNAS, 2012). Modeling of the Shigella needle subunit protein to fit the EM density produced a model incompatible with the atomic model of the Salmonella needle in terms of secondary structure and subunit orientation. We then determined directly the secondary structure of the Shigella needle subunit using solid-state NMR, and its orientation using in vitro and in vivo immunogold labeling in functional needles. We found that Shigella subunits adopt the same secondary structure and orientation as in the atomic model of Salmonella, and we generated a homology model of the Shigella needle consistent with the EM density (Demers et al., PLOS Pathogens, 2013). Here, we will discuss our recent efforts in obtaining higher-resolution structures of Shigella and Salmonella needles. We will also present proton-detected solid-state NMR experiments on perdeuterated T3SS needles. A set of five 3D correlation experiments allowed for the unambiguous assignment of the backbone resonances. Finally, we will show first results of DNP experiments on uniformly ^{13}C-labeled needles.

Characterizing Morphology and Molecular Packing in Polymer-Fullerene Blends by Solid-State NMR

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Polymers with extended pi-conjugation show promising applications in flexible electronic devices due to their semi-conducting properties. In organic solar cells (OSCs), the light-harvesting material consists of a pi-conjugated polymer with a low band gap that is blended with an electron-accepting material, such as fullerenes (PCBM), forming a bulk heterojunction. In this contribution, I will give examples on how solid-state NMR can enable unique information about the nano-scale structuring for such two component systems, e.g., OSCs based on PCPDTBT-PCBM, PBTTT-PCBM, and P3HT-PCBM blends [1-3].

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Recoupling homonuclear dipole-dipole interactions in multi-spin systems: applications to inorganic materials

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A large number of dipolar recoupling techniques have been used for internuclear distance measurements in homo- and heteronuclear two-spin systems. While the extension to multi-spin interactions is straightforward in the case of heteronuclear spin systems, using for example rotational echo double resonance (REDOR) spectroscopy [1], the homonuclear case is complicated by dipolar truncation and chemical shift anisotropy effects. Recently, a new solid state NMR technique has been introduced for coupled homonuclear I = ½ systems that permits a facile treatment of the multi-spin case [2,3]. Recoupling is accomplished in the form of an effective double quantum Hamiltonian created by a symmetry-based POST-*C*7 sequence [4] consisting of two excitation blocks, attenuating the signal (intensity S'). For comparison, a reference signal S_0 with the dipolar recoupling absent is generated by shifting the phase of the second block by 90º relative to the first block. As in rotational echo double resonance (REDOR), the homonuclear dipole-dipole coupling constant can then be extracted from a plot of the normalized difference signal $(S_0-S')/S_0$ versus dipolar evolution time. The method is conceptually related to the established approach of measuring double quantum coherence build-up curves [5], but differs from the latter by directly measuring the "left-over" z-magnetization following the action of double quantum Hamiltonian as a function of mixing time. It has been denoted DQ-DRENAR ("**d**ouble-**q**uantum based **d**ipolar **r**ecoupling **e**ffects **n**uclear **a**lignment **r**eduction"). DRENAR has been successfully validated for various crystalline model compounds representing a wide range of dipolar coupling strengths, spin geometries, and chemical shift anisotropies. Applications to a variety of inorganic materials (glasses, ceramics, hybrid materials) are discussed.

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Studies of a photochemical model system using a new LED based NMR Illumination Device

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NMR spectroscopy is a non-destructive and quantitative method for in situ monitoring of reactions. For the study of photochemical reactions (like the one shown in the picture below) an irradiation source is essential.[1] So far there are several solutions for an illumination device inside the spectrometer, for example by modifying the probe itself or by using an optical fibre guiding the light.[2, 3] While several setups use laser as irradiation source, a new illumination setup was published this year using cheap and exchangeable LEDs and a sandblasted fibre tip.[4] Using this new setup we study the photochemical equilibrium of a modified spiropyrane and merocyanine by irradiation of light with varying wavelengths. The amount of merocyanine in the photo-stationary-state obtained through this illumination method is compared to the amount obtained by illuminating outside of the spectrometer. By changing the wavelength of the irradiating light the equilibrium composition of the sample can be easily manipulated and the kinetics of these composition shifts can be monitored.

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NMR spectroscopy of molecules embedded within microporous and mesoporous materials

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Zeolite-like porous materials, known for several decades, and metal-organic framework materials, a new type of porous materials, are two extremely interesting families of materials that exhibit promising properties for several fields of application including ion-exchange, catalysis, separation, gas and heat storage, sensing and drug delivery. Nuclear magnetic resonance spectroscopy has in many cases played a crucial role in understanding of the formation of this type of materials and in their detailed structural description. In this contribution the potential of solid-state NMR spectroscopy for elucidating the location of the molecules within the pores and for studying the interactions of these molecules with the porous frameworks shall be demonstrated by several examples. The examples will include water and solvent molecules embedded within metal-organic frameworks, and drug molecules incorporated into zeolite-like mesoporous silicates.

Quantitative 2D-NMR analysis of lignins

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One crucial component within the efforts aiming at the valorisation of the so far under-utilized biomass component lignin is the knowledge about its structural features. Only an exact knowledge about the nature of the linkage motifs allows a targeted chemical modification of the intact lignin oligomers. Since these structural features heavily depend on the source of the lignin – in terms of its geographical origin, its biological origin, and the process under which it was obtained –methods leading to the determination of the most important structural aspects are needed.

We have recently published aspects of our on-going work on optimising various NMR-based characterisation methods. We developed an analytical protocol based on ^{1}H-^{13}C HSQC NMR measurements (QQ-HSQC) that allowed the quantisation of the various structural motifs present in a given lignin (Crestini *et al.* 2011, Sette *et al.* 2011). The method proved to be successful when applied to the characterization of milled wood lignins.

More recently, with the aim at developing a routine NMR-based method for identification and quantification of structural features in lignin, we developed simpler approach based on the $HSQC_0$ pulse sequence (Hu *et al.* 2011). $HSQC_0$ of lignins allows the quantitative evaluation of the structural motifs *without* the need of expert knowledge of NMR techniques. In the present communication we present the details of QQ-HSQC *vs* $HSQC_0$ in lignin characterization.

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Local deformation of polymer chains as reflected in proton dipole-dipole coupling distributions

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This contribution first reviews recent advances in the bias-free determination of homonuclear dipole-dipole couplings (DDC) constants and even their distributions in inhomogeneous samples. Long-distance structural constraints or fast anisotropic dynamics lead to small DDC values and thus large potential errors related to data distortion arising from transverse relaxation effects. These can be accounted for by suitable intensity normalization procedures in double-quantum experiments under low-resolution static or high-resolution MAS conditions alike [1-3]. Here, we present a recent application to the study of stretched elastomers. These contain fluctuating polymer chains, within which proton DDCs are not averaged to zero, leaving a finite low value reporting on the state of stretching of a given chain. This opens new perspectives to study local strain distributions in deformed rubbers, and enables a more quantitative understanding of the entropic elastic response of these materials, assessing the validity of models of rubber elasticity. Focusing on the most prominent application of nanoparticle-filled elastomers as found in car tires, we could show that polymer chains are deformed more ("overstrain"), and more inhomogeneously, in such materials [4].

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New methods for measuring protein dynamics

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The possibility to explore dynamics of proteins specifically ubiquitin which promiscuously recognizes many binding partners will be presented based on the accurate measurement of anisotropic parameters such as residual dipolar couplings (1). This approach allows to characterize at unprecendented detail the ground state ensemble of the protein (2). Rates of interconversion between ensemble members have been measured by low temperature relaxation dispersion, dielectric relaxation (3) and temperature jump SAXS and were compared to MD results (4). Further, with high-power relaxation dispersion measurements (5) it is possible to characterize motion kinetically to one digit µs time scales. With these measurements motion on the time scale of 10 µs at 35°C is detected uniformly in the backbone (detected on the amides) and side chains (detected on methyl groups) of ubiquitin. The amplitude of the relaxation dispersion can be reproduced assuming a backbone conformation dependent variance in the population of the side chain rotamers resulting in a rotamer shuffling. The backbone conformational changes occur on the same time scale as the side chain rotamer redistribution, namely, 10 µs time suggesting that the two processes are correlated. Finally, the benefit of using high power for conventional CPMG experiments will be demonstrated with the HEROINE experiment.

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Coupling fast water exchange to slow molecular tumbling in Gd^{3+} chelates: ^{1}H and ^{17}O NMR relaxometric studies

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It is now well established the relaxivity of Gd3+ chelates is relatively well described by Solomon, Bloembergen and Morgan theory. This in turn assists our interpretation of the limits of the relaxivity of the low molecular weight Gd3+ chelates used in clinical practice. These agents tumble rapidly in solution which limits their relaxivity. When this rotational restriction is lifted by making the chelates tumble more slowly the relaxivity continues to be limited by the slower than optimal water exchange kinetics of the chelate. Thus it has been widely understood that in order to maximize relaxivity it is necessary to both slow molecular tumbling and optimize water exchange.[1] To probe the potential gains in relaxivity that can be achieved through the optimization of both these parameters we have prepared and characterized several Gd-based systems with sizes ranging from discrete molecules to inorganic nanoparticles. In particular, the following examples will be illustrated: a) amphiphilic DOTA-like Gd3+ chelates loaded into lipidic nanoparticles; b) selective anchoring of GdDOTA-like complexes on the external surface of organo-modified mesoporous silica nanoparticles;[2] c) GdF3 nanoparticles (< 5 nm) coated with citrate molecules;[3] d) the unexpected case of two isomeric Gd3+ chelates, in which the one with the slowest water exchange affords the highest relaxivity.

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Quantification by selective excitation

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NMR is popular for quantitative determinations despite some difficulties. Mixtures of several components might be difficult to handle. Using non-deuterated solvents, to broaden the practical applicability, creates additional problems related to radiation damping and intercombination sidebands, here it is almost impossible to get reliable quantitative information from standard ^{1}H NMR spectra. One may eliminate such unwanted signals by signal suppression techniques. But, depending on available NMR hardware, the suppression might be incomplete or otherwise manipulate the intensity of the interesting signals due to insufficient selectivity. Our alternative approach: rather than eliminating unwanted signals, we selectively excite the resonance of interest by shaped pulses. This procedure gives good results, even in combination with an external reference for signal intensity calibration [1]. We used it to determine the saturation concentration of hydrogen in eight undeuterated organic solvents [2] and found that the results and their accuracy are comparable to those obtained by other methods. None of the concentrations exceeded 3.3 mmol L^{-1}. This method is helpful in all cases where quantification of low concentrated species is difficult due to a multitude of other components and where it is not feasible to use an internal standard for quantification, e.g. in situ measurements of reacting systems or process analytics. We are currently exploring the scope of application.

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Solution Structure of an Intermediate State in Protein Folding and Aggregation

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Protein folding intermediates have been implicated in amyloid fibril formation involved in neurodegenerative disorders. However, the structural mechanisms by which intermediates initiate fibrillar aggregation have remained largely elusive. We used CPMG relaxation dispersion NMR spectroscopy to determine the atomic-resolution structure of a 2% populated, on-pathway folding intermediate of the A39V/N53P/V55L Fyn SH3 domain. To this end, backbone chemical shifts and RDCs/RCSAs of the "invisible" intermediate reconstructed from CPMG experiments were used as experimental input for chemical shift restrained molecular dynamics simulations via the CamShift approach [1]. The C-terminus remains disordered in this intermediate [2], thereby exposing the aggregation-prone N-terminal strand. Accordingly, mutants lacking the C-terminus and thus mimicking the intermediate fail to safeguard the folding route and spontaneously form beta-sheet-rich fibrillar aggregates with a diameter of several nm and affinity for Congo red. The structure provides a detailed picture of the non-native interactions stabilizing an aggregation-prone intermediate under native conditions and insight into how such an intermediate can derail folding and initiate fibrillation.

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Ex vivo NMR during human cell cycle control

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Cyclins and cyclin-dependent kinases (CDKs) are key regulators of the human cell cycle. CDK4/6 assemble with D-type cyclins for progression from the G1 to S-phase and are negatively controlled by CDK inhibitors of the INK4 family. $P19^{INK4d}$ belongs to this inhibitor family, which gets posttranslationally phosphorylated. In earlier studies [1-3] we could show *in vitro* by glutamate substitutions, mimicking phosphorylation, local unfolding of 2 of the 5 ankyrin repeats of $p19^{INK4d}$. This prevents CDK4/6 inhibition. Now, we succeeded to follow this regulation in much more detail and very close to the *in vivo* situation by simply adding crude cell extracts from various cell lines (HeLa, HEK-293, MDA-MB-231 etc.) to the isotope labeled $p19^{INK4d}$. The NMR read out directly reveals, which serine residues get phosphorylated and consequently which ankyrin repeats locally unfold. By synchronizing the cells and by employing CDK inhibitors, the so far unknown kinases of $p19^{INK4d}$ could be identified. Dephosphorylation by subsequent addition of a phosphatase confirms the integrity of $p19^{INK4d}$ in the cell extracts by fully native NMR spectra. These high resolution data furthermore revealed, which serine has to become phosphorylated to induce ubiquitination and subsequent proteasomal degradation of CDK inhibitor $p19^{INK4d}$ and thus progression of the cell cycle to the next phase. These *ex vivo* NMR methods correlate cell biology methods with structural biology.

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Predicting the NMR spectra of paramagnetic molecules by DFT Calculations

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All basic NMR textbooks warn the reader against attempting to run the spectrum of a paramagnetic substance. Indeed, running the ^{1}H NMR spectrum of a paramagnetic substance using standard acquisition parameters leads to unpredictable, but most often disappointing, results, from awfully broad lines (often at very uncomfortable chemical shifts), to nothing at all. In this communication, we show that DFT calculation of structural and spectroscopic paramaters (orbital shieldings, *g*-factor and hyperfine couplings) leads to the prediction of contact and pseudocontact shifts and line widths which can greatly aid in the experimental setup and assignment of such spectra [1-3]. Examples from organic and inorganic molecules and complexes will be presented.

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Approaches for the determination of the relative configuration of natural products

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The determination of the relative and absolute configuration of natural products is essential to understand their biological activity and to allow their procurement through total synthesis. But so far, there is no general method for the secure assignment of the relative or even the absolute configuration of non-crystallizable natural products. The method of choice for small molecules with several stereogenic centers is the combination of distance geometry (DG) and distance bounds driven dynamics (DDD) calculations using NOE/ROE-derived distance restraints (r). The most important aspect of the fc-rDG/DDD method is the possibility for the configurations to change during the simulation (floating chirality, fc). One of the most investigated complex natural products over the past decade was palau'amine. Since none of the palau'amine congeners have been crystallized, there was a special demand on NMR spectroscopy for this structurally very complex class of natural products. Brown algae of the genus Cystoseira are known to produce various linear meroditerpenes. The isolation of several new members from the brown alga Cystoceira baccata led to a strong indication for a general revision of the bicyclo[4.3.0]nonane system. Since the number of NOEs was not sufficient for an unambiguous assignment of the relative configuration by fc-rDG/DDD, residual dipolar couplings were used to refine the structures. The absolute configuration of the new compounds was assessed by comparison of the circular dichroism spectra to the calculated spectra.

The structural basis for the autocatalytic conversion of the antiviral signaling protein MAVS into its active form

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Upon virus infection, the innate immune response is triggered by RIG-I like helicases, which bind viral RNA. Signals are transduced by the mitochondrial adaptor protein MAVS, which forms high molecular weight, detergent insoluble aggregates upon activation, and in this conformation induces the dimerization of IRF3 and production of type I interferon. Previous studies showed that the N-terminal CARD domain (caspase activation and recruitment domain) of MAVS is responsible for the aggregation. In vitro, MAVS CARD domains assemble into long, fibrillar structures. In cell-based assays, these in vitro formed CARD domain fibrils are sufficient to convert endogenous, unstimulated MAVS into functional aggregates, giving rise to IRF3 dimerization. Here, we have employed magic angle spinning solid-state NMR and other biophysical techniques to elucidate the structural basis of the CARD-domain mediated activation of MAVS in antiviral signaling. We present the solid-state NMR structure of the MAVS CARD domain in its fibrillar form and compare it to the solution NMR structure of the monomeric CARD domain. The structural basis for the initiation of this autocatalytic conversion of MAVS CARD domains by Rig-I will be discussed.

Ensemble refinement and collective dynamics from combined NMR and MD

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Dynamics underlies a multitude of protein functions. The simultaneous determination of structure and dynamics of biomolecules, however, remains a challenge for structural biology. Two examples will be presented that aim to characterize the structural dynamics at the microsecond timescale. For the RS peptide, using a combination of MD and a large body of NMR data, we characterize the ensemble changes upon phosporylation. The peptide undergoes a transition from an unstructured state in the unphospphorylated form to an arch-like structure in the phosphorylated form. For ubiquitin, an extensive set of both backbone and sidechain relaxation dispersion have been recorded, showing a large number of atoms (>30) with microsecond fluctuations. These atoms are distributed throughout the structure and nearly all show the same exchange rate, which suggests that ubiquitin undergoes collective motion involving both the backbone and side chains. A collective mode has been derived from MD simulations that explains the majority of the relaxation dispersion data, thereby providing a mechanistic view of this mode of slow collective dynamics.

Inferential structure determination from hybrid data

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The multiple sources of structural data used in hybrid structure determination of macromolecular complexes poses new challenges: data may be much more sparse than in high resolution methods; data sets from heterogeneous sources may be of highly different and unknown quality and may be mutually inconsistent; data sets from different sources may have very different information content; and data are in general averaged over large ensembles and long measurement times. In addition, structural knowledge of different components of the complex may be available to rather different degrees. Hence, with hybrid methods, the treatment of structure determination as an inference problem is even more important than in high resolution structure determination. For NMR structure determination, we introduced an approach that treats structure determination rigorously as an inference problem, based on Bayesian probability theory, and implemented in the program ISD (Rieping et al., Science 309, 303-305, 2005). We will here discuss how this framework can be adapted for structure determination from hybrid data. Our approach pays particular attention to the appropriate modeling of experimental outliers. We present examples with data from NMR, chemical cross-linking / mass spectrometry and SAXS experiments. This work is funded by the French government ("Investissement d'Avenir" project bip:bip) and the European Union (ERC advanced grant BayCellS).

Small Molecule Structure Determination Using Anisotropic NMR-Parameters

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Anisotropic NMR parameters like residual dipolar couplings (RDCs), residual quadrupolar couplings (RQCs), and residual chemical shift anisotropies (RCSAs) are very powerful structural parameters that allow the conformational, configurational, and constitutional analysis of small molecules [1,2]. If chiral alignment media are used, even the distinction of enantiomers is possible with the approach. Novel technical developments, like the introduction of useful alignment media and imaging of alignment will be presented. A special focus will be on novel HSQC-type experiments like the ASAP- HSQC for very fast detection and the CLIP-RESET for homonuclear decoupled measurement of one-bond couplings.

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Structure, polymorphism and dynamics of small organic molecules by solid state NMR: the surprising case of Ibuprofen

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Ibuprofen (IB) is a drug used in many popular formulations. In spite of having been largely investigated, its structural and dynamic behaviour, as well as its polymorphism, show several still unknown aspects. Since few years, our research group has been applying a variety of solid state NMR techniques to shed light on these properties for both the acidic and Na-salt, either optically-pure and racemic, forms of IB [1-5]. The aspects that will be shown include: (i) the phase properties as a function of temperature studied by ^{13}C CP-MAS; (ii) the very different molecular dynamic behaviour between IB and Na-IB, which has been investigated by ^{13}C spectra and CSA, ^{1}H and ^{13}C relaxation over a very broad temperature range, quantitatively determining motional parameters for every individual fragment; (iii) the effects of vibrations on ^{13}C CSA, on one side estimating vibrational amplitudes, on the other side allowing the understanding of some common differences between DFT-calculated and experimental ^{13}C chemical shift principal values. The groups of M. Levitt (Southampton), P. Paoli (Florence), B. Mennucci and L. Bernazzani (Pisa) are greatly acknowledged.

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Intermediates and Interactions in Organo-, Photo- and Transition Metal Catalysis

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The detection and characterization of intermediates in catalytic reactions is crucial for the understanding of mechanisms and the rational optimization of reaction conditions. However, in many rapidly expanding fields of asymmetric catalysis, mechanistic studies as well as structural investigations on intermediates or intermolecular interactions are scarce. Based on our experiences to detect organocatalytic intermediates in enamine and Brønsted acid catalysis, new results about dienamine intermediates will be presented. There, structural features of several catalytic systems suggest significant contributions of CH–π and π–π interactions. The relevance of these "weak" CH–π and π–π interactions is presented on the example of phosphoramidite palladium complexes. For the first time a general method will be presented for measuring the individual contributions of noncovalent interactions within transition-metal complexes and dissecting them from electronic effects. On Pd complexes it will be shown that modulations in extended CH–π and π–π interaction interfaces provide a ΔΔG value that is significant for stereoselection [1,2]. As third and interwoven topic our recent results in the field of photocatalysis will be presented. There, our LED based NMR illumination device for mechanistic studies on photocatalytic reactions will be introduced, which turned out to be versatile and simple, yet surprisingly powerful[3]. Finally, initial mechanistic studies about photocatalytic reactions e.g. with flavin and Ru(bpy)3Cl2 will be discussed.

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The structure of a 400 kDa RNP enzyme from an integrated structural-biology approach

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The post-transcriptional modifications of ribonucleotides occur during the biosynthesis and processing of the pre-rRNA transcripts. 2'-OH ribose methylation in eukaryotes and archaea is carried out by the box C/D small nucleolar RNA-protein complex (s(no)RNP). The archaeal box C/D sRNP complex is symmetric and constituted by three core proteins (L7ae, Nop5p and Fibrillarin) assembled around the methylation guide sRNA containing two similar conserved motifs: box C/D and box C'/D'. The guide sRNA in the complex base pairs with complementary substrate RNAs and selects the methylation site. Two structural models have been proposed for the box C/D sRNP complex. Following X-ray crystallography, it was suggested that the catalytically active complex is constituted by two copies of each protein and one molecule of the guide RNA [1]. The second model, derived from single-particle negative stain electron microscopy (EM), proposes that four copies of each core protein are assembled around two copies of the guide sRNA [2]. Here we present the structure of the catalytically active box C/D sRNP complex in solution (400 kDa) obtained by the combination of solution state NMR, small angle neutron scattering (SANS), FRET and restraints based molecular modeling. Our structure supports the existence of a pseudo-tetrameric complex and offers insights in the mechanisms of methylation and in the specificity of the enzyme.

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Diversity of RNA molecules

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RNA molecules have different roles and structures. In this presentation NMR studies of several RNAs will be shown, ranging from microRNAs to RNA G-quadruplexes. MicroRNAs (miRNAs) are produced from endogenous hairpin RNA precursors by enzyme Dicer. *let-7* miRNA imperfectly binds to two conserved complementary sites within 3'-untranslated region of *lin-41* mRNA and regulates developmental timing of nematodes from late larval stages to adults. Mutations showed that the base-pairing, asymmetric internal loops and bulges are important for this regulation.
RNA G-quadruplexes are another interesting RNA structures. They are more stable than DNA G-quadruplexes due to the less hydrated structure and the increased intra-molecular interactions afforded by the 2´-OH groups of the riboguanines. The ribose sugar renders RNA G-quadruplexes more heat stable however it also restricts the diversity of structure by disfavouring anti-parallel conformation. The parallel folding topology of RNA G-quadruplexes is preserved irrespective of the exact loop sequence, loop length, molecularity, stabilizing cation, number of quartets, or physical state. The most probable reason for this monomorphic nature is that the riboguanines involved in tetrad formation usually do not adopt *syn* conformation a prerequisite for formation of an anti-parallel quadruplex.

RNA resonance assignment strategies based on chemical shift statistics

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Chemical shifts are important reporters of structural information and their assignment is a prerequisite for any structure determination protocol of biomolecules. Whereas correlations between the chemical shifts and local structure are well established for proteins, such correlations between RNA chemical shifts and structure have not sufficiently been investigated yet. NMR assignment of RNAs is still challenging, mainly due to severe chemical shift overlap. The introduction of $^{13}C/^{15}N$ labelling facilitates slightly assignment strategies but due to the insensitivity of sequential through-bond correlations and severe overlap in the 13C dimension this did not lead to a breakthrough that would be comparable to $^{13}C/^{15}N$ labelling in protein NMR. We present here an assignment strategy for unlabeled double-stranded RNA that makes use of ^{1}H and ^{13}C chemical shift statistics. The approach is illustrated with several RNAs using exclusively 2D NOESY, 2D TOCSY and a natural abundance ^{13}C-HSQC. In contrast to the classical assignment protocol in which ambiguities lead to numerous assignment possibilities that need to be verified, our protocol avoids many ambiguities, is more straightforward and much faster. Our method will be very useful for characterizing siRNAs, for RNA construct optimization of stem-loop RNAs, e.g. for NMR structure determination of protein-RNA complexes or in the context of a modular approach to determine larger RNA structures.

NMR study of ligand-receptor interactions in non-homogeneous systems

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Receptor-ligand interaction studies provide structural information essential for the comprehension of biological phenomena and for the rational design of new molecules able to interact and modulate the activity of bio-macromolecules of pharmacological and biomedical interest, as well as for the development of new diagnostic tools. In some cases the analysis of non-homogenous samples, such as systems containing membrane proteins, nanoparticles, amyloid aggregates, cells, biocompatible materials, is required. These samples might not be soluble enough for solution NMR spectroscopy and, moreover, their intrinsic properties may also hamper the employment of HR-MAS techniques. Thus, we are now developing new NMR tools for the study of receptor-ligand interactions in non-homogenous media. In particular we have created an artificial receptor immobilized on a resin to verify the feasibility to study lectin-carbohydrate interactions in a sample containing an insoluble receptor[1]. At the same time we have developed a new methodology for the characterization of molecular recognition processing working on living cells deriving from solid tissues[2].

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Functional insights into ligand binding specificity and the binding mechanism of the neomycin sensing riboswitch

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Riboswitches are gene regulatory RNA elements located in the 3'-UTR of messenger RNA. In order to ensure efficient metabolic regulation, riboswitches are required to bind to their respective ligands with high affinity and specificity. An extreme example for this is the remarkable ligand specificity of the synthetic neomycin sensing riboswitch which discriminates around 500fold against the replacement of one single ligand amino group by a hydroxyl group. We show here that ligand specificity is encoded on the structural dynamics rather than on the structure level. Employing dedicated NMR techniques we traced in detail the internal structural and dynamic network of the neoswitch to show that the loss of key intermolecular interactions changes not only the local dynamics within the ligand binding pocket but also affects remote parts of the RNA leading to a large scale destabilization of the regulatory inactive complex. Our investigations led to the introduction of experiments for the rapid identification of hydrogen bonds involving the phosphate backbone in RNA, the expansion of a well know RNA folding motif, the U-turn, and to the proposal of a ligand binding mechanism by conformational selection.

Proton NMR: a tool for assessing food preservation strategies

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Food quality and properties are continuously changing from the production to the consumption instant. The speed and the extent of these changes depend on the strategies used to store the foodstuff. Monitoring food quality along the food life is crucial for the choice of the appropriate storage strategies and for the evaluation of the shelf life. Chemical and in particular NMR profiling is a powerful method to evaluate food stability along its life when different preservation strategies are considered. In particular, the correct choice of the appropriate packaging system is crucial for food preservation. There are three main aspects of food-packaging interaction, namely food stability, possible migration of exogenous compounds from package to food and package stability upon food contact, and all of them can be addressed by appropriate NMR techniques. In this work the chemical stability of several foods has been analysed by 1H NMR profiling in different types of packaging (traditional, biodegradable, active). The migration of contaminants and active compounds from package materials is studied by appropriate food simulants before and after food contact. Finally, the stability of the polymers is analyzed by solid state NMR.

Boron nitride nanotubes as MRI contrast agents

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Boron nitride nanotubes (BNNTs) are very promising materials for applications in biomedicine [1]. Aiming at their use in the clinical practice, trackability of BNNTs through standard in vivo imaging methods is highly desirable for both biodistribution studies and diagnostics. We have reported that BNNTs with superparamagnetic properties imparted by Fe nanoparticles as residues of the preparation procedure have the potential to be powerful T_2 contrast-enhancement agents for MRI at high field [2]. However, although the same BNNTs have been shown to be cytocompatible and well tolerated by several cell lines, a high concentration of metal impurities may give rise to toxicity issues. To avoid these problems, BNNTs without metal impurities were recently doped with Gd and tested as contrast agents for MRI at 7 Tesla [3]. Such Gd-doped BNNTs revealed to be good T_2 contrast-enhancement agents as well, prompting us to investigate the mechanism underlying water proton longitudinal and transverse relaxivity in the presence of BNNTs with the aid of both relaxation time analysis at 7 Tesla and Fast Field-Cycling measurements.

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NMR studies in food authentication

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Quality assessment is the most relevant requirement in food development. Particular attention from EU and national government organizations is dedicated to protect and to assure quality and uniqueness of “national products”, endowed with particular organoleptic properties. Different analytical techniques are growing progressively, to protect foods from frauds and not allowed imitations or mislabeling. The use of sophisticated analytical techniques like high resolution NMR should, in principle, assures the preservation of quality parameters. In this work, examples of geographical assessment and quality determination of different foods will be presented, highlighting the strong capability of the NMR based metabolomic approach coupled with chemometrics.

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State of the art analysis of metabolomic data in the context of human diseases

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Extracting relevant biomedical information from large NMR-based metabolomic datasets is of considerable complexity. Therefore, the application of optimal analysis techniques is of prime importance. Here we will summarize recent developments of our group in this regard. Prior to multivariate data analysis, it is important to minimize contributions from unwanted biases and experimental variance. We could show that Quantile and Variance Stabilization Normalization are well suited for this task [1]. One important next step is the classification of samples into known classes of disease. It was demonstrated for example in the context of various kidney diseases that results strongly depend on the choice of the employed algorithm [2,3]. One key component is the precise determination of metabolite concentrations, which in many cases is still hard to accomplish due to severe signal overlap and other interferences. For this, the software algorithm MetaboQuant for automated compound quantification from pre-processed 1D and 2D HSQC NMR spectral data including extensive routines for error detection and validation of results has been developed [4].

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Compact NMR Spectroscopy

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The first chemical-shift resolved NMR spectrum has been measured at Varian with a 0.76 T electro-magnet at 0.1 ppm resolution [1]. Today NMR spectra are measured at high field to benefit from wider a frequency range for the chemical shift and higher sensitivity and low-field NMR spectrometers are hardly in use anymore. In the past years, permanent magnet technology has been explored to build stray-field magnets for relaxation measurements with applications in the oil industry and for materials testing [2]. The lessons learned from shimming such magnets have lead to the development of ultra-compact permanent magnets for high-resolution 1H NMR spectroscopy of solutions [3] and the first commercial desktop NMR spectrometers, which are produced by an increasing number of manufacturers. Despite its low sensitivity, low-field desktop NMR spectroscopy holds great promise for chemical analysis and real-time spectroscopy of small molecules in the chemistry laboratory and under the fume hood [4]. The development of compact NMR and the use of bench-top NMR spectroscopy for reaction monitoring under the fume hood is illustrated.

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Combined Solid-state NMR and XRPD approach for solving structures of powdered samples: some example

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The world of crystal forms (not only polymorphs, hydrates and solvates but also salts and co-crystals...) is rapidly growing due to the important applications it can find in many industrial areas from agricultural and pigments to pharmaceutical.[1] Under the impetus of the green chemistry and of solvent-free processes, often new crystal forms are achieved by means of mechanochemical reactions (simple grinding or wet grinding) which, however, lead to microcrystalline powdered products not suitable for single crystal X-Ray Diffraction. Thus, alternative methods are required for their characterization. Here we present a combined solid-state NMR, XRPD and computational approach for solving structures of microcrystalline samples obtained by grinding or kneading. While XRPD reveals long order data, solid-state NMR, through a NMR crystallography analysis, unravels number of independent molecules and their symmetry, proximities and distances, connections and orientation relations, namely all information on local and intermediate length scales. Selected examples from polymorphs (barbituric acid)[2] to co-crystals (lidocaine with dicarboxylic acid)[3] will be presented where solid-state NMR, through several 1D and 2D (^{1}H-^{13}C FSLG Hetcor and ^{1}H DQ CRAMPS) experiments, has been foundamental for the structure solution.

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Monitoring of early bone formation with Nuclear Magnetic Resonance Imaging

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New bone formation takes place in two main stages, referred to as primary and secondary ossification [1]. Bone healing analysis is typically accomplished with histomorphometrical or X-ray based methods [2–4]. NMR Imaging has the advantage to allow the visualization and differentiation of the newly formed bony tissue at the beginning of the mineralization process, where an appropriate depiction with X-ray methods is not possible yet. The present study was executed with two different craniomaxillofacial defect animal models. One is an evaluation of the mandibular implant healing process; the second experimental study was performed with a cranial defect model. Early bone formation was traced after two, four and eight weeks and one and three weeks, respectively. We used proton density contrast and T1 weighted images for soft tissue differentiation and quantification. The capture of the whole soft tissue was performed by the proton density, whereas with T1-images the amount of fatty tissue, corresponding to the (newly formed) bone marrow, was determined. Hard tissue was identified and quantified indirectly by the quantity of pixels with no NMR signal. For tissue differentiation the effect of the choice of thresholds was discussed.

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Advanced Electrically Detected Magnetic Resonance for Photovoltaic Research

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To bridge the huge gap, between our present day photovoltaic (pv) use and its enormous undeveloped potential; thin film solar cells (TFS) have been developed. Latter devices can be deposited directly on inexpensive substrates like glass and are superior with regard to material consumption, energy payback time and cost effectiveness. However, TFS employ sophisticated design concepts, incorporate complex materials of varying morphology and interlaced interfaces. As a result they exhibit a multitude of different loss mechanisms leading to conversion efficiencies being far below the physical limits. Device limiting recombination and trapping mechanisms in TFS are frequently accompanied by stable or transient paramagnetic states, which renders electron paramagnetic resonance (EPR) and its current detected analogue electrically detected magnetic resonance (EDMR) the methods of choice to study these processes. EPR and EDMR may be employed to characterize transport determining paramagnetic states via their g- and hyperfine-tensors, uncover their role in spin dependent charge transport processes and finally locate them even in multilayer TFS. In order to demonstrate these capabilities we show how pulsed and cw EMDR in combination with novel density functional theory methods may be employed to uncover the role of transport determining dangling bond and band tail states in spin dependent recombination and hopping processes in fully processed TFS devices, incorporating amorphous (a-Si:H) and microcrystalline (µc-Si) silicon layers.

Heteronuclear NMR spectroscopy in the Platinum coordination chemistry

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Heteronuclear NMR spectroscopy is the technique of choice for studying transition metal complexes in solution. Among the measurable parameters, chemical shifts are particularly sensitive to molecular composition, conformation, environment and temperature. Heteronuclei such as ^{19}F, ^{31}P and ^{195}Pt offer a wide variety of applications including structural elucidation, relaxation studies, dynamics, kinetics and mechanistic studies. In the framework of our recent studies on coordination chemistry of Platinum, we have obtained a rich collection of compounds endowed with fluorine and/or phosphorus containing ligands.[1-4] Such species allowed for registration of very informative NMR spectra, especially when experiments such as ^{19}F-^{195}Pt HMQC were recorded. In particular, more data regarding the uncommon +1 and +3 oxidation states of Pt are now available. In this presentation, new achievements on ^{195}Pt NMR will be presented, comprising the relationship between ^{195}Pt chemical shift and the geometry of the metal center.

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Non invasive applications of portable NMR sensors with micrometric resolution for studying of Cultural Heritage

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There is an always growing understanding that the characterization of the state of conservation, the knowledge of the causes of degradation of materials aimed at lengthening the life time of the artefacts, are mandatory in the correct safeguard of Cultural Heritage. Recently NMR techniques have been increasingly applied to investigate, characterize and monitor objects of interest for Cultural Heritage [1-2]. An actual breakthrough has surely been the development of portable NMR instrumentation. These devices can be applied directly on large objects such as paintings, monuments, fully preserving the integrity and the dimension of the object under investigation. The measured NMR parameters are important to establish the state of degradation of objects, to evaluate the performances of consolidation and water repellent treatments on porous materials, to quantitatively map the dampness in wall paintings. A further development of portable NMR devices is the availability of sensors with microscopic spatial resolution for investigating the layer structure of artefacts with micrometric resolution. Specific cases of portable NMR application to the state of degradation of artefacts will be shown to illustrate the potentialities of unilateral NMR devices in the Cultural Heritage field.

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Methionine scanning as a new tool for methyl NMR studies

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Methyl NMR spectroscopy is a powerful tool for studying protein structure, dynamics, and interactions. Yet difficulties with resonance assignment and the low abundance of methyl groups in a protein can preclude detailed NMR studies, particularly the determination of continuous interaction surfaces. Here we present a straightforward strategy called methionine scanning that overcomes the aforementioned problems of methyl NMR spectroscopy. We systematically substitute solvent-exposed residues with reporter methionines in the expected binding site and perform chemical shift perturbation (CSP) experiments using methyl-TROSY spectra. Using these mutants, we can instantaneously assign all newly arising reporter methyl signals, determine interaction surfaces on a per-residue basis, and investigate the importance of each individual mutation for ligand binding. We have applied our methionine scanning methodology to a number of protein complexes including high-molecular weight systems such as the 11S activator-proteasome complex. Our data show that methionine scanning significantly extends the applicability, information content, and spatial resolution of methyl NMR studies.

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Understanding the Functional Mechanism of Membrane Proteins by (DNP-enhanced) Solid-state NMR

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Solid-state NMR offers unique possibilities to gain mechanistic insight into the function of membrane proteins within the lipid bilayer. Here, two different case studies will be presented and discussed. (i) The proteorhodopsin (PR) family consists of hundreds of integral membrane proteins, which occur almost ubiquitously in bacteria near the ocean's surface and show a high level of adaptation to their local environment, especially in terms of light absorption. Proteorhodopsins could fulfil a whole range of potential functions from light-driven proton pumping to signalling and sensing. The proteins have been studied by solid-state NMR supported by dynamic nuclear polarisation. Mutation-induced colour tuning effects will be discussed based on their dynamic, functional and structural basis revealed by spectroscopic means. (ii) In the second part of the talk, time-resolved solid-state NMR data on diacylglycerolkinase from E. coli and the use of lipid cubic phases for kinetic studies will be presented and its implications for the understanding of lipid regulators will be discussed.

tr-NOE on Human Cancer Cells, Metadynamics and MD in the Design of isoDGR-Based αVβ3 Antagonists

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Integrin αvβ3 is highly expressed on tumour cells surface during angiogenesis, thus playing a pivotal role in cancer growth and metastatic spread. RGD and the newly identified isoDGR motifs specifically target tumours via αvβ3. Efficacy of targeting approaches relies on ligand-receptor molecular characterization in the natural membrane environmentl. Here we show that it is possible to apply tr-NOE directly on human cancer cells to prove selective binding of ligands to αvβ3 [1]. We will also present a computational approach based on metadynamics and docking simulations to evaluate in silico the αvβ3 binding properties of isoDGR, DGR and RGD-containing cyclopeptides. The computational predictions have been validated through flow cytometry analysis and tr-NOE spectra on human cancer cells [2]. Finally, coupling molecular dynamics simulation with size-exclusion chromatography, flow cytometry analysis and immunofluorescence microscopy, we demonstrate that isoDGR-based cyclopeptides inhibit receptor allosteric activation. These findings hold major promises for drug design, based on the intrinsic ability of the isoDGR motif to block receptor activation. Conceivably, isoDGR-based drugs might replace the current generation of integrin-binding compounds, representing a promising solution in designing integrin antagonists, devoid of intrinsic paradoxical activation effects. [3]

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NMR Relaxation Measurements for Membrane Proteins: Internal Dynamics of the Homotrimeric HIV-1 Viral Coat Protein gp41 on Multiple Time Scales

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In the current model, HIV virus to host cell fusion is driven by a conformational change in the gp41 viral coat protein from an extended prefusion (or so-called prehairpin) intermediate during virus-to-host-cell docking to a trimer of hairpins that forms an antiparallel six-helical bundle (6HB) arrangement. Structural information on gp41 has so far been limited to individual domains, mainly in the postfusion conformation. Here we have studied the internal dynamics of a construct of gp41,1-194 comprising the fusion peptide, N-terminal and C-terminal heptad repeat (NHR and CHR) and transmembrane region, immersed in DPC micelles. An optimized set of 15N R1, R1rho relaxation and 15N-{1H} NOE measurements, using a TROSY-detection scheme, has been developed which alleviates systematic errors due to water-saturation and cross-correlated relaxation effects, particularly acute in per-deuterated systems [1]. 15N relaxation as well as PRE and SAXS data reveal a high degree of internal dynamics of gp41 on different timescales and are compatible with a prehairpin intermediate that samples a range of relative CHR vs NHR orientations, possibly in exchange with a low population of the late-fusion 6HB [2].

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Search and discovery of new low-temperature active enzymes from Antarctic microorganisms

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Cold-adapted organisms and their enzymes offer a large variety of real and potential applications in different fields of biotechnology. With the aim of discovering new enzymes with activity at low temperatures we undertook the analysis of proteins belonging to *Bizionia argentinensis* (BA), a newly discovered bacterium [1]. A particularly convenient protocol based on NMR experiments [2] was set up in order to identify the best candidates for structural determination. In this way, more than 30 proteins were classified as good candidates for three-dimensional structure determination. The hypothesis is that although the amino acidic sequence is not conserved, structural homology will allow the functional classification of the proteins and the discovery of new enzymes. We have already determined the structure of two proteins with unknown function belonging to BA: BA42 (by NMR) and C24 (by X-ray crystallography). In this talk, the first insights into the possible biological function of the these two newly discovered proteins will be presented.

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Human carbonic anhydrase II as a model receptor-ligand system: localization and orientation of a sulfonamidic inhibitor by Gd(III)-nitroxide DEER and paramagnetic NMR

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The DEER experiment on spectroscopically orthogonal Gd(III)-nitroxide spin pairs allows to selectively detect one type and pump the other type of paramagnetic centers [1]. This is a useful feature in studies of biomolecules with a tendency to agglomerate [2], or when multiple distance measurements are required within the same nano-object [3]. Protein complexes with smaller molecules are one important target for such orthogonal labeling. We labeled a model protein (hCA II) with a non-conventional very rigid Gd(III)-M8-DOTA label [4]. The nitroxide is introduced by a sulfonamide derivative with high affinity to the hCA catalytic center. The positions of Gd(III) and nitroxide paramagnetic centers from paramagnetic NMR experiments are correlated to the Gd(III)-nitroxide distances measured with DEER. The possibility to extract information on the orientation of the inhibitor molecule from the intermediate frequency data (Q band, 35 GHz) is verified experimentally. The work is supported by SNF (Grant No. 200021_121579 and 200021_130263).

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Protein folding and unfolding at atomic resolution

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Partially folded species have a key role in the folding, unfolding and misfolding of proteins. Even for small proteins, protein folding intermediates can slow the rate of folding and lead to misfolding of proteins implicated in neurodegenerative disorders. Owing to their transient nature, however, the structural characterization of protein folding intermediates is a challenge. Importantly, decreasing temperatures can induce unfolding of proteins in a noncooperative fashion - so-called cold-denaturation - and intermediate states can be sufficiently populated to allow their structural characterization. Using a combination of cold denaturation with NMR spectroscopy, we revealed detailed insight into the unfolding of the homodimeric repressor protein CylR2 [1]. Seven three-dimensional structures of CylR2 at temperatures from 25 °C to −16 °C showed a progressive dissociation of the dimeric protein into a native-like monomeric intermediate followed by transition into a highly dynamic, partially folded state. The core of the partially folded state seemed critical for biological function and misfolding [1].

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Monitoring the fate and transport of platinum anticancer drugs by NMR spectroscopy

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Membrane transporters and channels govern the cellular influx/efflux of ions, nutrients, and drugs. The Pt-based anticancer drug cisplatin (cis-$[PtCl_2(NH_3)_2]$) exploits the natural transport routes for essential Cu ions. The plasma membrane Cu permease Ctr1 is involved in cisplatin uptake and sensitivity, whereas the Menkes and Wilson Cu ATPases mediate drug resistance through efflux and/or vesicular sequestration. The Cu chaperone Atox1 was proposed to act as a switch in the Pt relay system between vesicular structures, where Cu ATPases are located, and the nucleus, where cisplatin forms adducts with DNA which are at the basis of antitumor activity [1,2]. NMR spectroscopy represents a valuable tool to study xenobiotic transport and metabolism. In the case of cisplatin, chemical shift sensitivity of ^{195}Pt (natural abundance) and ^{15}N (labeled ammine ligands) can be exploited to identify other metal donor atoms. In turn, selective labeling of protein side chains allow to monitor, in detail, the reaction steps leading to drug transport or inactivation. Finally, thanks to recent development of in-cell NMR spectroscopy, it is possible to probe intracellular drug delivery and interaction with Cu proteins [3,4]. From this study it emerges that cisplatin may interfere with normal Cu trafficking and homeostasis, the success of chemotherapy depending on a balance between beneficial versus undesired side effects.

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Tuning bone affinity into fragment-derived lead compounds: a general strategy for targeting drugs acting on bone

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Nitrogen-containing bisphosphonates (N-BPs) are widely used drugs for treating bone loss associated with diseases such as osteoporosis. Their remarkable safety and efficacy stem in part from their high affinity to bone mineral that enables drug accumulation at the desired site of action. However, FPPS inhibitors have also shown potential in other diseases, but a broader use of the N-BP class of drugs has been limited by its selective uptake in the skeleton. We recently described allosteric, non-bisphosphonate inhibitors of FPPS which lack affinity to bone, and can thus be explored for their anti-tumor and cholesterol-lowering potential [Nat Chem Biol 6, 660 (2010)]. Another novel and promising avenue opened up by the discovery of these allosteric FPPS inhibitors comprises the design of non-bisphosphonate FPPS inhibitors which display affinity to bone, but to a much lesser extent compared to N-BPs. Such compounds could potentially become better drugs for the treatment of bone diseases as they may display an improved oral bioavailability and a more even distribution throughout the skeleton. Here we report the attachment of a monophosphonate functionality to allosteric inhibitors of FPPS. Taking advantage of an NMR-based bone binding assay [ChemMedChem 5, 770 (2010)], we show that the bone affinity of these compounds can be tuned to the desired degree. Targeting a drug to the diseased tissue or organ is an attractive concept for improving efficacy and reducing adverse effects.

Versatility of qNMR in Drug Discovery and Development

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NMR techniques have a huge impact on the Pharmaceutical Industry ranging from classical structural determination of main compounds, by-products and impurities, screening studies with insights on the ligand and on the binding site, metabolism and toxicity studies. The role of quantitative NMR (qNMR) has become essential in several fields, also thanks to the development of methods alternative to the classical internal standard based ones. As an example, the use of qNMR for reaction monitoring and in situ yield determination at early stages of the drug development allows obtaining either relative or absolute concentration values. In particular, the recent increase in the use of qNMR for the direct assessment of chemical purity can potentially reduce the uncertainty when relative response factors for chromatographic methods are not available (e.g. lack of analytical standards), or application is limited (e.g. UV-transparent drugs and/or related impurities). Additional impact derives also from the use of NMR on the early metabolite identification and quantitation, with the objective to understand compound metabolic stability and drug interaction potential directly from in vitro studies. Biosynthesis, LC-MS and NMR in combination allow identification of major metabolites with limited efforts (e.g. no need for labelled compounds), and provide pure metabolites as standards for activity, reactivity or toxicity investigations. Some examples to cover the above aspects will be presented, with focus on application and impact of the resulting data.

Four-stranded DNA structures with unique loops and overhangs

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One of the secondary structures adopted by G-rich nucleic acid sequences are G-quadruplex. G-quadruplexes have been implicated in many vital cell mechanisms which makes them a potential therapeutic target. They typically consist of four G-rich tracts within DNA and RNA sequences. The monomeric form of d[TAG3CG3AG3AG3A2] originating from the first intron of the N-myc gene comprises three G-quartets that are bridged with three single nucleotide propeller-type loops. The dimeric G-quadruplex is characterized by consecutive stacking of six G-quartets. Equilibrium between the two forms is controlled by concentrations of K+ ions and/or oligonucleotide. [1] Oligonucleotide d[G3ATG3ACACAG4ACG3] with four G-tracts folds into a single intramolecular antiparallel (3+1) G-quadruplex with propeller, diagonal and edgewise loops of different lengths.[2] G-quadruplexes require cations for their formation, structural integrity and stabilization. Dynamics of 15NH¬4+ ion movement occurs on a range of timescales and has been shown to correlate with structural features of G-quadruplexes.[3] Structures formed by d(TG3T) and its analogs containing a 5'-5' or 3'-3' inversion of polarity sites revealed that the inter-quartet cavities at the inversion of polarity sites bind ammonium ions less tightly.[4]

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Combination of cell-free synthesis and NMR for the structure determination of membrane proteins

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Cell-free expression systems are ideal for the production of proteins that cannot be overexpressed in E. coli cells. In particular for NMR spectroscopy, cell-free expression provides major advantages since it allows for amino acid type selective labeling with only a minimum of metabolic scrambling. We have developed several labeling protocols that allow us to obtain the backbone assignment of membrane proteins in an efficient way. The TMS-labeling (Transmembrane segment enhanced labeling) is based on the fact that 60% of the amino acids of the transmembrane helices consist of only six different amino acid types. Double labeling membrane proteins with these six amino acids produces large consecutive stretches of labeled amino acids that can be analyzed with standard triple resonance experiments with a significantly reduced peak overlap. In addition, we have developed a combinatorial labeling scheme that allows us to assign specific amino acids. Using these methods we have obtained the backbone assignment for the C-terminal fragment of presenilin, the catalytic component of the gamma-secretase which is responsible for cleaving the amyloid precursor protein and have determined its three-dimensional structure. In addition, we have used these labeling schemes to determine the structure if proteorhodopsin, a retinal binding protein with seven transmembrane helices.

EPR reveals the conformation of active Bax during apoptotic cell death

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Apoptosis is a genetically programmed process which plays a central role in the balance between death and proliferation in tissue homeostasis. The intrinsic pathway which is activated by viral infections, DNA damage, oncogene activation, and it is strictly orchestrated by several members of the Bcl-2 (B-cell lymphoma-2) protein family. Here we focus on pro-apoptotic members (e.g. Bax) which oligomerize at the outer mitochondrial membrane (MOM) and directly participate in the membrane permeation which leads to release of cytochrome c and apoptosis. Some structural insights are available for the conformational changes of Bax upon activation, and the newly discovered structures of truncated dimeric Bax in detergent [1] shed some light on the active form. However there is a pressing need for the determination of 3D structures of the activated oligomeric Bax in membrane environment. Here we show how site-directed spin labeling EPR can follow the conformational changes in Bax [2] triggered by Bid in the presence of liposomes or isolated mitochondria (3). Based on the newly available structural data and on the inter- and intra- molecular distances determined by EPR, a first model of the active Bax molecules in the oligomer is presented [3].

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A structural model for the full-length blue light-sensing protein YtvA from *Bacillus subtilis,* based on EPR spectroscopy

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A model for the full-length structure of the blue light-sensing protein YtvA from *Bacillus subtilis* has been determined by EPR spectroscopy, performed on spin labels selectively inserted at amino acid positions 54, 80, 117 and 179. Our data indicate that YtvA forms a dimer in solution and enable us, based on the known structures of the individual domains and modelling, to propose a three-dimensional model for the full-length protein. Most importantly, this includes the YtvA N-terminus that was so far not identified in any structural model. Our data are in agreement with the crystal structure of an engineered LOV-domain protein, YF1 that shows the N-terminus of the protein to be helical and to fold back in between the β-sheets of the two LOV domains, and argue for an identical arrangement in YtvA. While we could not detect any structural changes upon blue-light activation of the protein, this structural model now forms an ideal basis for identifying residues as targets for further spin labelling studies to detect potential conformational changes upon irradiation of the protein.

Anfinsen and the Art of Bacterial Transcription

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The general rule introduced by Anfinsen that a unique amino acid sequence under specific environmental conditions determines a unique protein fold [1] was and still is a subject of discussions. Whether or not amyloidogenic proteins such as prions or the β-amyloid peptide of Alzheimer's Disease violate Anfinsen's rule is a matter of debate, however, these proteins usually change folds and aggregate, so that a noticeable concentration of misfolded protein is not present in solution.
To really contradict Anfinsen's rule, a protein should be able to exist in two different folds in the same solution, but so far even designing a protein fold that obtains a different structure if 50 % of the amino acids are replaced posed a major challenge [2].
Recently we found the bacterial protein RfaH to clearly violate Anfinsen's rule, as the C-terminal domain (CTD) of this protein can exist in an all-α state as well as in an all-β conformation in the same solution environment [3]. As these two states are entirely different structurally as well as functionally, the term Transformer Protein (TFP) was introduced to describe this unprecedented phenomenon [4].
Nature takes advantage of this conformational transformation in the regulation of bacterial transcription. Each of the two entirely different conformations of the CTD possesses its own distinct function, the α-helical state is autoinhibitory for N-terminal domain (NTD) attachment to RNA polymerase, whereas the β-barrel state is able to recruit ribosomes *via* attachment to the ribosomal protein S10, a structural protein in the small ribosomal subunit. This makes RfaH a regulated functional analog of the transcription:translation coupling factor NusG [5].

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Advanced EPR Methods in Surface Chemistry and Catalysis. The Role of Hyperfine Techniques

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The importance of surface paramagnetic species owes much to that of surface phenomena which are involved in numerous areas of chemistry and material science such as heterogeneous catalysis, photochemistry and, in general terms, nano-sciences and technology. In the present contribution recent results illustrating various approaches and opportunities related to the use of EPR in surface chemistry and heterogeneous catalysis, with emphasis on the application of hyperfine techniques (HYSCORE and ENDOR) will be discussed. In particular examples will be presented concerning the activation of small molecules via surface to molecule electron transfer and the characterization of surface transition metal ions in porous materials.

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Paramagnetic NMR for the understanding of the biology of special metal cofactors in proteins

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Structure/function relationships in metalloproteins are efficiently studied via NMR. However, when the metal cofactor is a paramagnetic species, standard protocols for biomolecular structure determination usually fails. NMR approaches must be tailored according to the electronic properties of each metal cofactor [1]. Iron sulfur cluster and heme proteins are special metal cofactors, used by Nature for as many purposes as possible [2]. We will show some results concerning the mechanisms of heme shuttling between heme carrier protein HasA and membrane receptor HasR [3], and some Iron Sulfur Cluster containing proteins, involved in the assembly of FeS clusters [4].

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Double resonance techniques in EPR and NMR: from sensitivity enhancement to applications in biological science

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Modern EPR spectroscopy relies on a repertoire of many different pulsed and time-domain techniques to manipulate the electron spins and disentangle their interactions. We are employing double resonance techniques such as electron-electron double resonance (DEER/PELDOR) and electron-nuclear double resonance (DNP and ENDOR) to obtain structural information in biomolecules at the atomic and at the nanometer length scale. This talk will overview our recent contributions to enhance sensitivity and resolution of these techniques particularly at high EPR frequencies. For electron-electron double resonance we are exploring the capability of orientation selection in PELDOR/DEER at 94 and 263 GHz for structural determination of RNA and peptides [1]. In a second kind of experiments, we have been investigating polarization transfer mechanisms between electron and nuclei for sensitivity enhancement in DNP and ENDOR. One major effort of this field is directed toward the development of new pulse techniques to improve the performance of current polarization transfer schemes. We are examining coherent electron-nuclear polarization transfer [2], which was recently proposed in the context of dynamic nuclear polarization. Initial results and perspectives are discussed.

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Single spin nuclear magnetic resonance

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Nanoscale detection of EPR and NMR signals is an upcoming technology. By using single spins as novel sensors signals form nanoscale volumes can be acquired. In certain cases spin probes are about to reach sensitivities for as few as 10 nuclear spins. Averaging times and spectra resolution can be improved significantly by using quantum algorithms for readout and signal processing as well as storage. By using nuclear storage spins as well as single shot readout it is expected that NMR signals of single spins can be recorded with high spectral resolution. The talk will describe progress towards that goal.

5.2
Abstracts – Posters

Interactions of biosilicate and organic molecules in diatom cell walls studied by solid-state NMR spectroscopy

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Diatoms form silica cell walls exhibiting extraordinary material properties and delicate patterns on the micro- and nanometer scale[1]. The exact mechanisms of the cell wall formation are an interesting matter of research. Several biomolecules have been found to be included in or attached to the diatom silica. Among them are polyamines, silaffins and silacidins, all of which are able to induce silica precipitation in vitro und thus are probably also responsible for silica condensation in vivo[2-4]. Chitin networks and microrings of silaffin-like proteins, the so-called cingulins have been found in the silica of *Thalassiosira pseudonana*. Both might act as organic templates for the mineral phase[5,6]. Solid-state nuclear magnetic resonance spectroscopy is well-suited to study both the silica and the organic phase. Moreover, solid-state NMR spectroscopy is capable of selectively highlighting the organic-inorganic interface by polarization transfer experiments. Here, we describe ^{13}C-^{29}Si correlation experiments based on double-CP in high magnetic fields. ^{13}C, ^{15}N and ^{29}Si isotope labelling of diatom cell walls increases the NMR sensitivity and allows the detection of selective ^{13}C-^{29}Si polarization transfer and even the measurement of 2D ^{13}C-^{29}Si HETCOR spectra. ^{13}C-^{29}Si REDOR experiments also become feasible.

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Sweet secrets – the trehalose coating effect on the internal dynamics of the cold shock protein

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We present a comprehensive analysis of the effect of trehalose on structure and dynamics of the cold shock protein (Csp) [1]. Two samples - Csp and Csp embedded in a glassy trehalose matrix - were studied at various rehydration levels with four different solid state NMR experiments which are sensitive to different time scales. A combination of T1 relaxation experiments (nanoseconds), T1ρ relaxation experiments (microseconds), DIPSHIFT experiments (microseconds and faster) [2] and CODEX experiments (milliseconds to seconds) [3] provide abundant and rigorous data for a detailed characterization of the effect of trehalose on structural properties and protein dynamics. Derived from our results we can draw the following picture: a) trehalose coating preserves the protein structure to be more native in comparison with the dehydrated lyophilized protein powder even though there are still non-native hydrogen bonds present. b) trehalose coating slows down the motion of the Csp backbone N-H groups in the nanosecond and microsecond range while the motional amplitude remains constant. c) upon adding water to the Csp-trehalose system, water molecules assemble between the protein and the trehalose matrix.

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Solution and solid-state NMR characterization of immunoglobulin light-chain amyloid fibrils and their interactions with EGCG

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Immunoglobulin light-chain amyloidosis (AL amyloidosis) is a rare disease caused by aggregation of free antibody light-chains in the serum. The amyloid aggregates deposit in the circulatory system and inner organs, mainly nerves, kidneys, liver and heart. Accounting for the immense sequence variability of immunoglobulins, only a small fraction of light-chains is amyloidogenic. It is the aim to identify properties, that render a light-chain sequence prone to aggregation. Recent studies have shown positive effects of the green tea polyphenol (-)-epi-gallocatechine gallate (EGCG) on the condition of AL-amyloidosis patients [1]. Since EGCG was also reported to interfere with fibril formation of other peptides, e.g. α-synuclein or amyloid-β, similar effects can be expected for immunoglobulin light-chains [2]. In contrast to the intrinsically unstructured α-synuclein and amyloid-β, light-chain monomers have a native, globular fold. Our solution NMR analysis have shown specific interactions between light-chains and EGCG. We characterize the interactions that result in a destabilization of the fold. In addition, we report first solid-state NMR experiments of variable domain light-chain amyloid fibrils.

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Solutions of Supramolecular Assemblies in the Magic-Angle-Spinning Rotor

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Various processes in the living cell are regulated by very large protein machineries with molecular weights in the megadalton regime. However, structural investigations of these large systems by solution-state NMR are challenging due to their slow rotational diffusion. Here we describe an approach, which opens new perspectives in the investigation of soluble supramolecular modules by magic-angle spinning (MAS) NMR. The absence of rotational diffusion in MAS-induced protein sediments allows typical solid-state NMR techniques without the need of invasive precipitation or crystallization procedures. The combination with protein deuteration, proton-detection and paramagnetic relaxation enhancement enabled us to observe and to assign backbone amide resonances of a 20S proteasome assembly with a molecular weight of 1.1 MDa. Similarly, we used the approach to characterize the polydisperse and highly dynamic small heat-shock protein αB-crystallin (600 kDa) with respect to its copper-dependent chaperone activity and its mechanism of binding destabilized substrate proteins.

Structural investigation of EGCG-induced Alzheimer's disease Aβ oligomers and their interaction with metal ions

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Protein misfolding and aggregate formation are linked to many currently incurable human diseases, including Alzheimer's disease. Fibrils formed by the β-amyloid (Aβ) peptide are the major component of the plaques found in human brains affected by Alzheimer's disease. However there has been a recent shift in the field, recognising that small oligomeric assemblies of the peptide are likely responsible for neurotoxicity. These oligomers have been difficult to study as they are transiently populated and may form heterogenous populations. The green tea catechin compound, epigallocatechin-3-gallate (EGCG), has been shown to interact with Aβ and mitigate its toxicity. This molecule stabilizes oligomers, opening up many possibilities for structural and functional investigation of these key players in the Alzheimer's disease cascade. We are investigating these structures using NMR spectroscopy in concert with other biophysical techniques. The interaction of Aβ with other catechin molecules is also being studied. Metal ions such as copper and zinc have been found in amyloid deposits and have a postulated role in the toxicity of amyloid aggregates, by generation of toxic reactive oxygen species. We are investigating the interactions between metal ions and Aβ in the presence or absence of EGCG using both solution and solid-state NMR. Solid-state NMR spectroscopy is a promising technique for structural investigation of Aβ oligomers, and our overall aim is to determine the structure and interactions of Aβ oligomers in order to better understand their role in Alzheimer's disease.

Characterization of aggregates formed by the Alzheimer's peptide amyloid-β in the presence of small molecules

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Insoluble amyloid species of the Alzheimer's peptide amyloid-β (Aβ) are the main constituents of amyloid plaques, which are the primary cause of neurodegeneration. It is therefore of high interest to study the interaction of Aβ with inhibitory small molecules interfering with the aggregation properties of the peptide. The molecule investigated in this study is the non-steroidal anti-inflammatory drug (NSAID) sulindac sulfide, which has been shown to affect Aβ populations.[1,2] Solution-state NMR experiments demonstrate a reduction of Aβ40 solubility in the presence of the NSAID, and the production of insoluble species. To date, MAS solid-state NMR is the only method that allows analyzing these insoluble protein aggregates on a molecular level. In addition, seeded fibrils show pronounced chemical shift deviations in the presence of sulindac sulfide, which can be specifically assigned to atoms in the Aβ40 sequence. This information, combined with molecular modeling approaches allows the identification of the ligand binding site, and deviation of a docking model. Understanding the detailed mechanism of interaction between Aβ40 and small molecules may assist in designing drugs targeting amyloidogenic proteins.

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Study of the self-aggregation proprieties of the diabetes type II human Islet Amyloid Polypeptide (hIAPP)

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Fatal cell degenerative diseases like type II diabetes are correlated with oligomerization, aggregation, and fibril formation of the hormone, Islet Amyloid Polypeptide (hIAPP) or amylin. It is considered the primary culprit for pancreatic cell loss in type II diabetes patients[1]. Therefore insight into the structure of hIAPP aggregates is of fundamental importance in order to better understand the action of small molecules, which can potentially dissolve protein aggregates and modulate cell toxicity. We established an expression and purification procedure that allows us to obtain a full length uniformly isotopically enriched hIAPP with amidation at the C-terminus and no additional residues at the N-terminus. We apply Solution state NMR to characterize the interaction between the monomeric form of hIAPP and small molecules. Furthermore we carried out MAS solid-state NMR experiments in presence and absence of small molecules which alter fibril morphology.

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Magic Angle Mis-Adjustment Compensated by Mutual Cancellation of Dipole-Dipole and Chemical Shift Anisotropy

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Asymmetric doublets for ^{15}N-^{1}H spin system have been observed for mobile residues in microcrystalline proteins in solid state NMR, which resemble TROSY effects (1-3) in solution NMR. However in the solid state, the mutual cancellation of the dipole-dipole interaction (DD) and the chemical shift anisotropy (CSA) originates from the combination of coherent and incoherent contributions (4). Under fast Magic Angle Spinning (MAS), it is possible to suppress the coherent contribution for heavily deuterated samples. Here we show that these TROSY type experiments are less prone to mis-calibration of magic angle. These experiments are particularly of importance for solid state NMR system, which require flipping of the stator in order to insert the sample and for samples with intrinsically high resolution that are sensitive to magic angle mis-calibration.

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NMR Spectroscopic Investigations of Supermolecular Proteins and Aggregates in Alzheimer's Disease

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The major constituents of the extracellular plaques in Alzheimer's disease (AD) are amyloid-β40 (Aβ40) peptides [1]. Other proteins, including small heat-shock proteins (sHsps), like αB-crystallin (αB), are also found colocalized with Aβ40 peptides in extracellular plaques, functioning as molecular chaperones [2]. In presence of αB, Aβ fibril formation is abolished. Instead oligomeric Aβ aggregates are formed. Oligomeric Aβ species are considered causative for AD pathogenesis. Interestingly, Aβ40 and αB are able to bind Cu(II) [1, 3]. The binding of Cu(II) was shown to modulate the oligomeric architecture and the chaperone activity of αB [2]. In order to understand the mechanism of interaction between Aβ40, αB and Cu(II), we employ a combination of MAS solid-state NMR, solution-state NMR and electron microscopy. Solution – state NMR experiments are performed to characterize the tertiary complex consisting of Aβ, αB and Cu(II). We report first solid – state NMR experiments, which demonstrate that αB induced Aβ aggregates are accessible for NMR structural investigations.

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Evolving solid-state MAS NMR experiments using Genetic Algorithms

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The degrading effects of chemical shielding anisotropy (CSA) on the performance of dipolar recoupling MAS NMR experiments is a well known fact. The interference of CSA with the spin dynamics is particularly troublesome when the aim of an experiment is to determine accurate internuclear distance information as provided by the direct dipolar coupling interaction. Distance information is typically extracted from the polarisation transfer characteristic between coupled spins by taking advantage of the ~r-3 dependence of the direct dipolar coupling. Any influences on the transfer other than by the dipolar coupling will inevitably cause a misinterpretation of the data in terms of internuclear distance. This problem with data interpretation usually occurs in combination with a severe loss of spectral signal intensity in the presence of considerable CSA. Here we demonstrate how nature-inspired genetic algorithms can be used to approach this interference conundrum from a numerical point of view. We have chosen genetic algorithms as a type of heuristic, stochastic search algorithms based on the mechanics of natural genetics. We evolve homonuclear direct dipolar recoupling experiments that, in the end, are widely independent of any CSA influence and provide very high spectral intensity. At the same time it is demonstrated that genetic algorithms offer the means to easily implement optimisation of multiple objectives simultaneously. This especially important since a well performing NMR experiment typically requires multiple objectives to be met (high efficiency, various degrees of broadbandedness, low-power irradiation, small number of pulses, etc.)

Towards structure determination of membrane proteins based on sparse NMR data

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Membrane proteins constitute one of the main protein classes and account for roughly 30 percent of the proteins in the human body. They play crucial roles in several processes ranging from basic small-molecule transport to sophisticated signaling pathways. Due to this wide variety of functions, over 50 percent of all medicinal drugs have membrane proteins as their receptor. However, as their structure is very hard to determine experimentally, membrane proteins comprise only less than two percent of the atomic-resolution structures available today. Structural determination of membrane proteins by NMR remains a challenge and usually leads to sparse distance restraints which are not sufficient for common structure calculation programs. CS-ROSETTA (Chemical-Shift-ROSETTA) is a structure prediction protocol that is able to incorporate various NMR data, e.g. chemical shift data, residual dipolar couplings and NOE distance restraints to guide structure calculations. In this work, we develop and benchmark a structure determination protocol based on the CS-ROSETTA methodology for membrane proteins for which only sparse NMR data is available.

Optimal control pulse design for broadband sustainment of singlet states

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We suggest a novel approach to spin-locking of long-lived spin states that achieves direct design of singlet-sustainment pulses by numerical optimization of rf pulses employing the GRAPE tracking algorithm [1]. As a result, Broadband Optimized Sustainment of Singlet States (BOSS) is demonstrated for exemplary molecules with both strongly and weakly coupled proton spin pairs. Simulations and experiments show an increase in singlet-sustainment bandwidth by a factor of over two when compared to the most effective conventional composite pulse decoupling sequence, namely MLEV-16, at the same average rf power. For the molecule (*E*)-1-bromo-2-iodoethene, with J = 13.4 Hz and $\Delta\nu$ = 29.8 Hz, the singlet is sustained for more than a minute over a bandwidth of ca. 2 kHz, whereas MLEV-16 achieves only ca. 1 kHz, both at $B_{1,RMS}$ = 500 Hz. Tailored low-power BOSS-pulses may be of interest for extending the lifetime of hyperpolarized metabolites in-vivo.

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Analysis of high pressure induced conformational changes of TmCSP and Byr2-RBD using AUREMOL tools

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Given a limited amount of experimental data, obtaining additional information using computational structure prediction tools can drastically speed up the NMR structure determination process. Here we describe a novel application of a number of AUREMOL tools to predict the high pressure structure of the protein TmCSP and to identify different states of the structure of the Ras-binding-domain (RBD) of Byr2. Measurements under high pressure give us the possibility to investigate states of a protein which are only weakly populated under normal conditions.It is possible to differentiate between regions that show a significant change in experimental amide proton and nitrogen chemical shifts and those that show no or only little change in chemical shift corresponding to no structural changes. This was done for both proteins-TmCSP and Byr2-RBD-and the approach and the results are presented here. For TmCSP, a limited set of additional experimental data was available, and a new method was tested to predict the high pressure structure of this protein. For the regions with no conformational changes distances, dihedral angles and H-bond restraints were extracted by the PERMOL tool from the given well-defined structure of 1G6P. In combination with the experimental data a structural bundle was calculated.

Solvent PRE-ROSETTA: *de novo* structure prediction using solvent paramagnetic relaxation enhancements

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De novo protein structure prediction by computational approaches has been shown recently to provide accurate structural models. The often poor convergence of these approaches has been partially circumvented by inclusion of experimental data such as NMR chemical shifts. However, without laborious collection of additional experimental NMR restraints such as NOEs or RDCs these approaches are still limited to small biological systems. To overcome some of the current limitations we included experimental solvent paramagnetic relaxation enhancements (PREs) in the *de novo* protein structure prediction program ROSETTA. As long as chemical shift assignments are available, experimental solvent PRE data can be easily obtained without the need of modifying the sample and provide quantitative information about solvent accessibility of spins In our approach, solvent PREs are used i) directly in the Monte-Carlo based folding algorithm and ii) to re-score the final models. An efficient method for back-calculating solvent PRE data was implemented and incorporated into the ROSETTA framework. We applied our protocol to a benchmark of small- to large-size proteins and show that direct refinement against solvent PRE data improves structural accuracy and precision significantly. We show that solvent PREs provide a new class of restraints in *de novo* structure prediction programs that are easily accessible and applicable to any kind of protein. In particular for challenging systems such as protein complexes and in cases when only sparse data is available, our approach promises significant time savings and significantly improved quality of *de novo* structure predictions based on NMR data.

Classification Algorithms in Context of NMR-based Metabolomics

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The importance of metabolite measurement by means of NMR spectroscopy is constantly growing in biomedical classification. For this, the selection of the best suited classification approach is of prime importance. In total, six binary classification algorithms in combination with different feature selection approaches were systematically assessed by the use of nested cross-validation [1]. The performance of the algorithms was compared on five NMR fingerprinting data sets measured from urine, serum, plasma and milk. A combination of a t-score-based feature filtering with either Support Vector Machines or Random Forests performed well on most of the data sets, while the performance of the other investigated methods varied between data sets. Advantages of Support Vector Machine-based approaches include finding of a global minimum and simple geometric interpretation. Key advantages of the Random Forest methodology are its robustness against overfitting and the suitability for parallel computing.

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Modeling of Xe- Bound Human Prion Protein Based on NMR data

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Transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative disorders that affect humans and other mammals. TSEs are associated with the accumulation of fibrils of misfolded prion protein (PrP)[1]. For an understanding of the conversion of normal protein to infectious isoform the identification of conformational intermediates in the misfolding pathway is crucial. We have already identified two conformational intermediates by high pressure NMR spectroscopy[2,3].We used the noble gas Xe to explore the structure and dynamics of the hydrophobic cavities in PrP [4]. ^{1}H- ^{15}N HSQC experiments were carried out at different Xe partial pressures and the chemical shift perturbation was analyzed as a function of the Xe concentration. It showed Xe binding at three different cavities in the folded core of the protein that are present in the PDB structure (ID: 2KUN). However, only one of the cavities in the structure is large enough to accept a Xe atom. The dissociation constants of Xe determined by NMR vary from cavity to cavity. In the present work, the structure of Xe-bound PrP has been modelled by a combination of docking and molecular dynamics methods using the determined xenon-protein interaction data. The modelled structure is then compared with the known PDBs to find the difference in conformation due to Xe-association and tried to correlate this with the mechanism of prion formation.

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ISIC and PERMOL – multifunctional tools for the determination of protein structures. Idea, implementation in AUREMOL and possible applications

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Knowledge about the three dimensional structure of proteins is the key for understanding the biochemical processes of life. The program PERMOL which is part of the software package AUREMOL was developed to extract information about the conformation of a known protein in the form of restraints – specifically distances between atoms, dihedral angles and H-bonds - and then transfer this information to an unknown protein. These restraints can be used for molecular dynamics as restrictions of the configuration space. We tested the functionality of PERMOL which was originally developed for homology modeling for other interesting calculations: For structure improvement, e.g. if a set of NMR data is too small to confidently define the structural bundle; for combining information from different sources like NMR or XRAY experiments [3] by using the module ISIC; for the analysis of conformational changes of proteins when temperature and/or pressure vary, and as an alternative method for docking peptides to proteins.

AUREMOL[1] is a software tool for protein structure determination from NMR data, which speeds up and automates the whole process
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Joint refinement against X-ray and NMR data with REFMAC5

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Paramagnetic restraints are long range restraints, highly informative on the structure and the mobility of proteins, and can thus be used to monitor whether their structure in solution is consistent with a crystal model and, in this case, to refine it. Crystal and solution structures can in fact be different due to the absence in solution of crystal packing forces or absence in the solid state of conformational heterogeneity due to crystallization of the protein in a single conformation. The NMR restraints can be profitably used directly together with the crystallographic data for assessing the existence of a structural model in agreement with both sets of data. We propose a strategy based on the inclusion of pseudocontact shifts and residual dipolar couplings in one of the most widely used program for protein refinement against X-ray data: REFMAC5. We have shown that single refined structures very similar to the crystal models can be calculated for three regularly folded proteins, in good agreement with the experimental NMR data; on the contrary, for a protein like calmodulin it is not possible to achieve a good joint fit of the NMR data without worsening the agreement of the crystallographic data.

Rotational tumbling of uncoupled domains in flexible multi-domain proteins and macromolecular complexes

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The rotational motion of biomolecules reports on intra- and inter-molecular protein interactions and protein multimerization and is therefore important to understand protein function. Calculation of rotational tumbling times in flexible modular systems, however, is difficult. We have developed a new approach, called HYdrodynamic CoUpling of Domains (HYCUD), for prediction of rotational correlation times. HYCUD is based on the assumption that deceleration of rotational motion in flexible multi-domain proteins strongly depends on hydrodynamic drag forces. We demonstrate that HYCUD accurately predicts rotational correlation times in a wide variety of systems, including monomeric and dimeric heterochromatin protein 1, two-domain variants of the protein GB1, two- and three- copper-binding domain constructs of Wilson disease protein, and a 300 kDa heterochromatin protein 1-nucleosome complex. The HYCUD method provides insights into the motional behavior of multidomain proteins in their free form and within flexible supramolecular complexes.

Effects of NMR Spectral Resolution on Protein Structure Calculation

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Adequate digital resolution and signal sensitivity are two critical factors for protein structure determinations by solution NMR spectroscopy. Achieving maximum digital resolution is usually limited by the practically available measurement time. We developed a method utilizing non-uniform sampling for balancing digital resolution and signal sensitivity, and performed a large-scale analysis of the effect of the digital resolution on the accuracy of the resulting protein structures. Structure calculations were performed as a function of digital resolution for about 400 proteins with molecular sizes ranging between 5 and 33 kDa. The structural accuracy was assessed by atomic coordinate RMSD values from the reference structures of the proteins. We show that high resolution is equally important for proteins of every molecular size. The chemical shift spectral overlap dependent on the corresponding spectral digital resolution can be a predictor of the resulting structural accuracy. Our results show that for every molecular size a minimal digital resolution, corresponding to the natural linewidth, needs to be achieved for obtaining the highest accuracy possible for the given protein size using state-of-the-art automated NOESY assignment and structure calculation methods.

Simulating the time-evolution of sparse spin-1/2 systems

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We simulate the time-evolution of multi-spin systems. The memory requirements arising from an exponentially growing state space are managed by assuming that the density matrix (or the coherence vector) stays sparse during the simulation. We replace the customary matrix exponentiation with optimized computations in structure-constant Lie algebras. This allows us to better account for efficiency and sparsity while increasing the number of spins. We present examples of coherence transfers on several tens of spins.

Protein structure calculation using CS-Rosetta

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During developing the protein structure calculation software CS-Rosetta, we met a particular target—Ar3436A. It doesn't look like difficult but we couldn't get the correct calculation at the beginning. After manually analysis, we find some of the chemical shift assignments of this protein are not correct. Generally, It is estimated that as many as 20% of the proteins have improperly referenced resonances and about 1% of them are misassigned. To avoid the effect of low-quality of chemical shift assignments, on one hand, our group is building an accurate and structure independent method for automated resonance assignment just using easily prepared inputs. On the other hand, CS-Rosetta is proving to be robustness against partially wrong chemical shift assignments.

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Structural analysis of Lysine-20 monomethylation by Set-8 on Histone H4 peptides

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The methyltransferase Set8 catalyzes the monomethylation of hisone 4 K20. Set8 is most abundant in the late G2 and M phase of cell cycle and monomethylation of H4K20 has been shown to have an important role in gene regulation, chromatine structure and cell cycle control. It has been shown by an alanin scan on different peptides that Set8 recognizes a specific motive 17RHRKVL22(R23) which consists of 6 or 7 amino acids. In particular the role of R23 has not been established so far.[1,2] We have investigated the methylation of various parts of the histone 4 chain. Peptides consisting of 6 to 14 aminoacids have been synthesized and their methylation by Set8 using S-Adenosyl-L-Methione as cosubstrate has been monitored using time dependent proton NMR-spectroscopy. The resulting progess curves could be fitted by use of the integrated Michaelis-Menten equation and its solution with the Lambert W-function. Saturation transfer difference (STD) and transfer NOESY experiments were carried out to determine the dissociation constants KD and further investigate the geometry of the substrate in the binding pocket of Set8. The STD experiments confirmed the binding motive that consists of the hexapeptide sequence 17RHRKVL22. However, the kinetic data shows that at least the R23 is necessary for a faster enzymatic methylation by Set8. In order to investigate the role R23 in K20 methylation the binding mode of the peptides and their geometry in the binding pocket are to be determined.

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Oxygen-17 Dynamic NMR Study of the Pr-DOTA Complex

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The complex between ^{17}O-enriched DOTA (tetraazacyclododecanetetraacetic acid) and praseodymium(III) (Pr^{3+}) was studied at different pH levels by variable-temperature ^{17}O NMR. The spectra feature signal broadening that reveals the inter-change of the coordinated and non-coordinated oxygen atoms of the carboxylate groups. The observed linewidth were deconvolved into contributions from quadrupole relaxation, paramagnetic relaxation enhancement effects and chemical exchange. The present ^{17}O dynamic NMR study provide the first quantitative experimental data characterizing the carboxylate internal rotation in a DOTA chelate of a lanthanide metal ion. The activation entropy is negligible and the activation enthalpy is found to range between between 66 and 77 kJ mol^{-1}, depending on the pH and the presence of free Pr^{3+} ions in solution. The fluxional behavior of the carboxylate groups is not expected to significantly affect the residence time of the coordinated water molecule and is probably not related to the interconversion between the diastereomeric forms of the Pr-DOTA complex.

Dynamic NMR Investigation of N-Me Group Rotation in Dimethylated Arginines

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Symmetrically and asymmetrically N-dimethylated arginine (sDMA / aDMA) are known to act as recognition motifs for binding to Tudor protein domains. Published data suggest different binding modes of sDMA in different Tudor domains [1]. We have investigated the rotational barriers of the guanidine C-N partial double bonds by dynamic NMR over a wide temperature range. With methanol or a 50:50 methanol/water mixture as solvent, it was possible to take NMR data as low as 200 K and 230 K, resp., thus freezing out any high-barrier bond rotations. For sDMA and aDMA only a single barrier was observed in the temperature range between 200 and 310 K, with a rotation barrier of ca. 12 kcal/mol for sDMA and ca. 9 kcal/mol for aDMA. Substitution of the HCl salt with the HABS salt (p-hydroxy-azobenzene-p'-sulfonate) could partially mimic the aromatic binding cage known from sDMA binding pockets in Tudor domains. Indeed, with sDMA.HABS in DMSO/water, a slightly higher barrier of ca. 13 kcal/mol was determined. The observed barriers were assigned to rotations about the Nε-Cζ bond; for the Cζ-Nη bond rotations (the one observed in the different Tudor binding modes), no barrier could be observed, i.e., these barriers should be clearly below 8 kcal/mol.

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Ernst angle revisited through the use of optimal control

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The quality of the measured spectrum in Nuclear Magnetic Resonance strongly depend of the Signal to Noise Ratio. Instead of doing a single measure, one way to improve it, is to repeat many times the experiment. The standard method consist in exciting the system with a hard pulse then measure the signal. This hard pulse is characterized by the well known Ernst angle introduce in 1966 [1]. Here we propose to see if improvement of the SNR is possible based on the analysis of Bloch equation. We treat the case of unbounded control, i.e., no limit on the amplitude is considered. In a first step we will describe some special properties of the Bloch equation. More precisely we will present the concept of Magic plane (also called singular set [2]) and the Steady State Ellipsoid. In the second part we will introduce the Steady State Synthesis which associates to each point of the Bloch ball a control which maximize the SNR. To conclude we will answer to following : - What is the best control to maximise the SNR ? - Is the Ernst angle a good solution or an optimal solution?

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NMR study of conformational dynamics of the splicing factor U2AF65

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Protein–RNA interactions play essential roles in gene regulation and RNA metabolism. While high-resolution structures have revealed principles of RNA recognition by individual RNA binding domains (RBDs), the presence of multiple RBDs in many eukaryotic proteins suggests additional modes of RNA recognition by combination and cooperation of these interactions. U2AF65 is a multi-domain splicing factor which has a key role in the assembly of splicing complexes, since a polypyrimidine (Py) tract RNA sequence at the 3′ end of introns is recognized by the tandem RNA recognition motif (RRM) domains (RRM1–RRM2) of U2AF65 [1, 2]. The focus of this research is to study the dynamics and different conformations of one of the most relevant multi-domain proteins involved in pre-mRNA splicing, the auxiliary factor U2AF65, which recognizes the 3′-splice-site-associated polypyrimidine tract RNA. By means of T1 and T2 NMR relaxation experiments we investigate the dynamics of the RRM1-RRM2 domains at ps-ns time scale to unveil the internal motions that are carrying out during the free and bound to RNA states. We are also using PRE measurements to investigate the effects of salt and temperature onto the conformational ensemble of the free and bound RRM1-RRM2.

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Balance between electrostatic and hydrophobic interactions in protein complex formation revealed by paramagnetic NMR spectroscopy

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The formation of a productive protein complex is a stepwise process, in which the free components evolve to the final complex passing through a transient, lowly-populated encounter state. For a long time the first step of association was thought to be exclusively driven by long-range electrostatic interactions. Experimental evidences and theoretical studies questioned this assumption and suggested also a role of hydrophobic interactions in protein association. To study the contribution of the different forces we study the highly dynamic complex formed by plastocyanin and cytochrome f,1 two redox partners in oxygenic photosynthesis, for which both electrostatic and hydrophobic interactions were shown to contribute to the stabilization of the final complex. Through the combination of paramagnetic relaxation enhancement NMR techniques and Computational Methods we were able to visualize the presence of hydrophobic interactions in the encounter state2 and to elucidate the contribution of either electrostatic or hydrophobic forces to the formation of the encounter complex.3

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NMR Investigation into Structure and Dynamics of Human Blood Group A Glycosyltransferase

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Glycosyltransferases (GTs) are an important class of enzymes, which are responsible for the transfer of an activated donor-sugar to an acceptor. The resulting glycoproteins and glycolipids play important roles in various biological processes such as cell-cell recognition. It is known that reactions catalyzed by GTs are accompanied by complex conformational transitions of corresponding substrates and enzymes. But there are no experimental data that resolve the underlying protein dynamics. Human blood group A glycosyltransferase (GTA) serves as a model to analyze the protein dynamics using NMR spectroscopy. GTA is responsible for the last step of the A antigen synthesis. It transfers the activated GalNAc residue from UDP-GalNAc to the H-antigen acceptor. It is known from crystal structures that there are two flexible regions in GTA with three possible conformations: open (no ligand), semi-closed (one ligand) and closed (two ligands). $^{1}H,^{15}N$-HSQC spectra show that various peaks of GTA shift upon titration with the donor substrate UDP and the acceptor H antigen. To further characterize the dynamics a partial backbone assignment of this flexible 70 kDa homo-dimer is needed. The recorded 3D triple resonance spectra are of high quality and the assignment is currently underway in our laboratory. The micro- to millisecond time scale dynamics are studied with relaxation dispersion experiments. GTA has 10 Isoleucines that are evenly spread in the protein and can give insights into the methyl side chain dynamics. First experiments show relaxation dispersion for some of the methyl groups.

PELDOR based Trilateration of Paramagnetic Metal Ions in Biomacromolecules

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Metal ions play an important role in the catalysis and folding of proteins and some ribozymes. Their localization within the three-dimensional fold of a biomacromolecule is therefore an important aim in understanding of structure-function relationships. The present work introduces an Electron Paramagnetic Resonance based trilateration approach for the localization of paramagnetic metal ions. This approach implies the use of spin labels, introduced to the biomacromolecule by site-directed spin labeling [1], as reference points. The distance constraints between the reference points and the metal center are measured by Pulsed Electron-Electron Double Resonance [2] and used latter to solve the trilateration problem via the mtsslTrilaterate program [3]. The EPR-based trilateration is tested on the Cu^{2+} center in azurin. The results of the trilateration are compared with the crystallographic data. It is shown that the precision of the method is 0.16 nm for 6 distance constraints. The influence of the number and precision of the distance constraints and the protein model quality on the results of trilateration is examined.

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Synthesis of Trityl spin-labels

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Triarylmethyl-radicals (Trityl) are highly persistent compounds which are a main interest in current development of spin-labels for EPR-based distance measurements. Compared to nitroxide spin-labels, which are presently the most commonly used spin-label for distance measurements, they show several advantages. Most important among these are their narrow spectral width, their comparatively long relaxation time and their persistence even in reducing environments, which give rise to the hope that they may in future be used for pulsed roomtemperature measurements under truely biological conditions. Here, we describe synthetic approaches to trityl spin-labels carrying a methanethiosulfonate-group as linker for the specific purpose of labeling proteins for EPR distance measurements.

EPR Experiments on a Genetically Encoded Spin Label

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Recent publications demonstrate the ability of EPR to provide structural, dynamical and functional data on biomacromolecules in cells. Of particular interest are measurements of conformational mobility and distance measurements in the nanometer range. The advantages of the method are sensitivity, selectivity, the lack of any limitation imposed by the size of macromolecule, and the possibility to get information on mixtures of conformations via analyzing distance distributions. However, so far, these approaches require microinjection of spin-labelled macromolecules. Moreover, the biomolecules transferred to cells by these means have limited access to natural mechanisms of cellular processing like folding, localization, posttranslational modification and natural decay. In this project, so far, a modified lysine amino acid containing 2, 2, 5, 5, -tetramethyl-pyrrolin-1-oxyl-label was genetically encoded in E.coli, and incorporated into various positions in GFP and TRX mutants. EPR spectroscopy on extracted proteins was used to find out the optimum work-up protocol for obtaining sufficient amount of labeled proteins, in terms of optimal bacterial strain, incubation conditions, expression times and cell lysis and purification temperatures. EPR measurements were also conducted on bacterial cell pellets, where the intracellular spin label is present in free form, as AMP-ester esterified to tRNAPyl and potentially incorporated into amber-terminated, chromosome-encoded host proteins. The results suggest that by careful choice of measurement conditions it is possible to selectively observe the signal of the target protein in cellula.

Synthesis of EPR pulse sequences on a fast arbitrary waveform generator

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On a traditional pulsed EPR spectrometer, pulse shaping is limited to switching on and off a set of microwave channels. Since fast arbitrary waveform generators (AWGs) with timing resolution below 1 ns became available in the past few years, the amplitude and phase of microwave pulses can be controlled precisely. Pulse shaping, as applied in NMR with great success, can therefore be adopted to specific problems in EPR spectroscopy. Due to the fact that an AWG opens up several new ways to design an experiment, the interface between the user and the experiment gains in complexity. Here, we report on our approach of using an AWG to synthesize pulse sequences as a whole. In this concept, the AWG generates all involved excitation pulses as well as synchronization triggers for external hardware. The entire pulse sequence is therefore sliced into a set of digital waveforms, each complemented with playback instructions, such as for instance the number of individual waveform loops. Before starting an experiment, the user provides the pulse sequence as an event-based data structure with all intended timings in nanoseconds. Hierarchical parameter variations on this data structure facilitate complex pulse sequences. Based on this data structure and a set of AWG- and spectrometer-specific constraints, the digital waveforms are calculated and preloaded onto the AWG. Once an experiment is started, control is based on counting acquisition events on the detection device. We discuss some of the limitations of this procedure and demonstrate preliminary experimental results. The experiments were acquired on a basic spectrometer employing a heterodyne transceiver.

Monitoring Kinetics of Enzymatic ATP Consumption by EPR Spectroscopy

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Life uses ATP in cells to supply processes with energy. It also is an important signaling molecule that helps regulating the metabolism of cells and transmits extracellular signals to trigger intracellular processes. Therefore, many enzymes use ATP as an energy or phosphate transferring substrate. Due to this ubiquitous occurrence, the kinetics of enzymatic consumption of ATP is of high interest. Unfortunately, existing methods of investigation are hindered by their need of auxiliary reagents, since these might interfere with the enzymatic process, and thus invalidate the results. In this work a new tool to monitor the kinetics of enzymatic consumption of ATP by applying EPR spectroscopy is presented. For this purpose doubly spin labeled ATP analogues have been synthesized. Their inter-spin distance ranges up to 3.8 nm as determined by DEER. Due to the high flexibility of the linkers this distribution is very broad and has considerable probability below 1.5 nm. Hence, the corresponding cw EPR spectrum exhibits dipolar broadening as long as the ATP remains intact. After enzymatic cleavage of the ATP, both labels are separated spatially and the dipolar broadening vanishes. The spectrum of a sample with only a fraction of the ATP split is a superposition of the spectra of these two extremes. After proper data analysis, the ratio between cleaved and intact ATP can be extracted from the spectrum. By measuring time resolved cw EPR spectra, the kinetics of ATP consumption are studied. Thus, one can investigate the inhibition and stimulation of enzymes when consuming ATP without the need for auxiliary reagents. In contrast to fluorescence spectroscopy, EPR can also be applied in opaque media.

Multifrequency EPR Studies of Photoactive Donor-Acceptor Systems

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Photoactive donor-acceptor systems have been extensively studied for their application to organic photovoltaic cells. In particular, much attention has been focused on conjugated polymer/fullerene blend systems, which provided the main progress in improving photovoltaic cell efficiency. In this work, EPR spectroscopy has been employed to investigate molecular orientations and spin dynamics in thin films. Morphology of the active layer comprising donor and acceptor components is one of the critical factors in the performance optimization of solar cells. This is partially due to a relationship between the polymer morphology and the mobility of charge carriers. We observed angular dependent EPR spectra for the blend films after chemical doping in iodine vapor. The angular variations related to the morphology depended on annealing procedures. We also prepared thin-film photovoltaic cells based on the blend system, and investigated a relationship between the cell performance and the morphology.

Application of EPR Methods for a Wide Range of Chemical Questions

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EPR has demonstrated its potential as a versatile spectroscopic method of choice, owing mainly to its rare combination of sensitivity and selectivity compared to complementary spectroscopic methods. Throughout the years, the introduction of FT methods, multiple resonance approaches, and ever-increasing higher frequencies has made EPR a strong tool for probing into the properties of small and macro- molecules. In AK Schiemann and under the EPR service project SFB 813-Z1, we utilize different EPR techniques to investigate a diverse set of spin centers. Recently tackled questions include the orientation of side chains in thiophene polymers, the distribution of electron spin in a py-Aza radical, the kinetics of decomposition reactions of phosphorus containing complexes, the electronic structure of a NHC substituted bissilylene radical cation, and finally, the determination of spin multiplicity and unpaired electron distribution in the ground state of a series of inorganic molecules containing different transition metals. We herein report on the latest progress of a selection of aforementioned projects.

Cross-polarization edited ENDOR

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Electron nuclear double resonance is a fundamental technique in EPR spectroscopy that directly detects hyperfine transitions of nuclei coupled to a paramagnetic center. Despite its wide use, spin-sensitivity and restricted spectral resolution in powder samples pose limitations of this technique in modern application fields of EPR. In this contribution we examine the performance of an ENDOR pulse sequence that utilizes a preparation scheme different from conventional Davies ENDOR. The scheme is based on electron-nuclear cross polarization (eNCP), which requires concomitant microwave (MW) and radio-frequency (RF) irradiation satisfying specific matching conditions between the MW and RF offsets and the hyperfine coupling. Changes in nuclear polarization generated during eNCP can be detected via a conventional ENDOR read-out sequence consisting of a RF -pulse followed by EPR-spin echo detection. Using 1H BDPA as a standard sample, we first examine the CP matching conditions by monitoring the depolarization of the electron spin magnetization. Subsequently, so-called CP-edited ENDOR spectra for different matching conditions are reported and analyzed based on the provided theoretical description of the time evolution of the spin density matrix during the experiment. The results demonstrate that CP-edited ENDOR provides additional information with respect to the sign of the hyperfine couplings. Furthermore, the sequence is less sensitive to nuclear saturation effects encountered in conventional ENDOR.

α-Synuclein binds alpha-helically to mitochondria

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α-Synuclein, an intrinsically-disordered protein associated with Parkinson's disease, interacts with mitochondria, and can produce mitochondrial dysfunction including impairment of complex I, oxidative stress, mitochondrial lipid abnormalities, and mitochondrial fission. However, the details of physiological and pathophysiological synuclein-mitochondria interactions of these protein remain poorly defined. We probed the interaction of α-synuclein with isolated mitochondria by using site-directed spin labeling in combination with pulsed electron paramagnetic resonance spectroscopy (EPR). The obtained experimental data reveal that α-synuclein bound to mitochondria is in an α-helical arrangement and the N-terminal part of the protein in crucial for binding. The results advance our understanding of α-synuclein's interactions with mitochondrial membranes and thus should help elucidate the role of the protein in the mitochondria dysfunction associated with neurodegenerative diseases.

EPR spectroscopic studies of hemin peptide binding

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Prophyrines are part of essential biological functions like photosynthesis or respiration. Especially, iron porphyrines (Fe^{2+}, Fe^{3+} = heme, hemin) are prosthetic groups in a variety of enzymes like cytochromes, myoglobin and hemoglobin in which the hemin group is permanently bound.[1] However, hemin can also interact transiently with proteins and upon binding regulate them.[2]Via database search possible transient hemin binding motifs were identified and the corresponding peptides were synthesised by a combinatorial library approach. These peptide sequences contained cysteine, histidine and tyrosine which are known to bind directly to the iron ion.[3] But the interaction between the peptide and the hemin is also influenced by the other amino acids in the sequence which do not bind the iron ion directly.[4]In this regard, we investigate a wide range of peptide sequences with EPR spectroscopy in order to identify binding motifs, binding strength, and consequences for the electronic structure of hemin. Based on the *cw* X-band EPR experiments, the peptides were categorised in three groups according to their affinity to hemin and their spin states were assigned. An iron transporter protein called FeoB exihibts one of these sequences and binding of hemin to it could be followed by EPR.

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Systematic comparison of sampling strategies for static gradient CW EPR microscopy at low SNR using an iterative SIRT image reconstruction method

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Performing EPR microscopy experiments at low signal to noise ratio is a trade-off between image quality and measurement time, which has an upper limit depending on the equipment and experimental requirements. There is also a trade-off between the higher resolution obtained by larger magnetic field gradients and better signal to noise ratio at lower magnetic field gradients. In this work, the effects of a wide range of sampling strategies on the reconstructed image quality has been studied using an iterative SIRT reconstruction method and filtered backprojection on artificially degraded experimental data and simulated phantom data, where the number of projection angles, the number of sample points per projection angle and the signal to noise ratio have been varied.

Towards nanomolar NMR detection through SABRE hyperpolarization

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NMR spectroscopy has proven to be an extremely important analytical tool in the field of (bio)chemistry and medicine. However, it suffers from an inherent low sensitivity due to the small population difference between nuclear spin states at thermal equilibrium. Hyperpolarization techniques have been developed to overcome this inherent insensitivity by creating non-Boltzmann spin state distributions. Signal Amplification By Reversible Exchange (SABRE)[1], uses a nuclear spin isomer of molecular hydrogen, para-hydrogen, as source of polarization. Para-hydrogen can interact reversibly with a substrate molecule through a metallo-organic complex, resulting in greatly enhanced signals of unmodified substrate molecules in solution. So far, SABRE has been used at relatively high concentrations, mostly millimolars of substrate. Hereby we present a method to extend the applicability of SABRE to dilute samples, making the detection of analytes in the low micromolar concentration range possible in a single scan experiment. Detecting these concentrations by NMR spectroscopy is remarkable and promising for the application of NMR to trace analysis.

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Distance and orientation measurements with DEER/PELDOR at 95 and 263 GHz

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PELDOR spectroscopy is a well-known technique to measure inter-spin distances in a nanometer range. Besides this, if applied at high fields/frequencies (95 and 263 GHz), the technique exhibits an enhanced orientation selectivity. Thus, it can be used to provide information on the relative orientation of spin-labels in a pair, assuming those are rigidly oriented in a studied bio-macromolecule. Nevertheless, general applicability of the method is hampered by difficulties related to the performance of a two frequency experiment at such frequencies. Furthermore, the inherent symmetry of the involved interactions complicates the analysis of the orientation selective data. We attempt to refine the technique, and in general, to extend the applicability of high field DEER/PELDOR by implementing a dual-mode resonator and by increasing the frequency of the measurements. Our recent results on two representative biological systems, i.e. an RNA and an α-helical peptide[1], permit to explore the feasibility of the approach. We show how the performance of the method can be improved by enhancing resolution toward orientation selectivity and by setting proper constraints for the orientation analysis. Finally, initial results[2] and further prospects of using spin-pairs with different spectral properties for selective distance measurements at high fields are discussed.

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High Efficiency Compact Helium Liquefaction and Recycling for NMR Systems

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For NMR labs, helium consumption is characterized by low daily boil off at a few liters per day, and significant large boil off due to periodic transfers from storage dewars to NMR systems. The traditional, industrial scale helium liquefaction and recycling system, which is designed to produce a minimum of 50 liters per hour, is not a feasible solution for NMR labs. Recent advancements in helium liquefaction technology saw the developments of a new type of compact liquefier, which provides an ideal solution for NMR research labs. The portable ATL 160 helium liquefier utilizes advanced Gifford-McMahon 2-stage cryo-cooler technology to reach a liquefaction rate of 22 Liters/Day with a power consumption of only 7 kW. The challenge in helium recycling for NMR systems, especially for 800 and 900 MHz systems, is the capture of transfer boil off. The transfer to the 4.2 K reservoir, and subsequent pumping on the 2.4 K inner core, result in large volumes of helium gas that need to be recovered and stored. The ATL High Pressure Recovery plant offers an integrated solution to this need. This is shown by a High Pressure Recovery project at the Department of Chemistry at the University of Georgia.

Impact of the organic coating on the relaxometric properties of GdF_3 nanoparticles

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Gd-based MRI probes allow delivering to the site of interest a large number of paramagnetic ions, thus increasing the technique sensitivity.[1] Paramagnetic nanoparticles (NPs) can incorporate different functionalities for imaging or therapeutic delivery. Among the NPs studied, GdF_3 attract interest due to their high relaxivity (r_{1p}). Ultrasmall GdF_3 coated with citrate, EDTA, EDTA-PEG and polyacrylate (PAA) were prepared and their relaxometric behaviour was investigated aiming to unravel the mechanisms underlying the magnetic interaction with water. The ^{1}H (R_1) and ^{17}O (R_2) NMR relaxometric properties of NPs were investigated. ^{1}H nuclear magnetic relaxation dispersion profile of citrate-based GdF_3 NPs present a shape with a peak centred at ca. 100 MHz. This is consistent with a slowly rotating system with a rotational correlation time, τ_R, of a few hundreds of ps.[2] Similar behaviour is found for PAA and EDTA-PEG-based GdF_3. Instead, EDTA-based GdF_3 shows a profile characterized by a decrease of r_{1p} with increasing frequency. ^{17}O NMR R_2 data as a function of temperature for citrate-based GdF_3 suggest the presence of two water molecules in the Gd inner sphere. In addition, the reduced transverse relaxation rates increase with decreasing temperature as also observed for complexes with fast inner-sphere water exchange. This combined analysis indicates a significant contribution from water molecules H-bonded to the coating in addition to highlighting the predominant role of the Gd^{3+} ions on the surface.

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Dendrimersomes: a new vesicular nanoplatform for theranostic applications

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Dendrimersomes are a new class of nanovesicles constituted by amphiphilic Janus dendrimers[1]. Unlike liposomes and polymersomes, the potential of dendrimersomes in biological applications (e.g. in molecular imaging) has not been explored yet. Here, we report the preparation (by using the solvent injection method) and in vitro characterization of dendrimersomes loaded with MRI and optical probes. The probes were encapsulated in the aqueous core or incorporated in the bilayer through the synthesis of a novel dendrimer covalently conjugated to a Gd-complex. The addition of a small amount (5 % in moles) of DSPE-PEG2000-carboxylate into the dendrimeric membrane significantly increased the stability of the vesicles due to electrostatic repulsion. Vesicles size ranged from 150 to 200 nm (PDI < 0.2). Both Gadoteridol and Carboxyfluorescein were encapsulated with good efficiency. Relaxometric studies showed a relatively fast water exchange across the vesicle bilayer, and a high longitudinal relaxivity was measured for the vesicles incorporated with the dendrimer-based Gd-complex. The results obtained indicate that dendrimersomes assembled from Janus dendrimers have potential to represent a new nano-platform for molecular imaging experiments, particularly in the field of theranosis.

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In vivo MRI visualization of the intratumor release of Doxorubicin from liposomes by non-focused ultrasound

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Doxorubicin is one of the most used drugs to treat solid tumors, but shows a very high toxicity. To reduce side effects and improve tumor accumulation, liposomal forms of the drug have been developed.However, the ability of liposomes to deeply diffuse in the tumor is still debated, and several methods to promote the release of the drug at the target site have been proposed. A promising approach is to induce the release of the drug just after the drug administration when most of the drug is still circulating in the tumor vasculature. We have recently demonstrated, in vitro and in vivo, that the local application of pulsed low intensity non-focused ultrasound (pLINFU) can trigger a mechanical non heat-mediated release from liposomes.Then,if a water-soluble paramagnetic Gd-complex is co-encapsulated with the drug,MRI can offer the valuable opportunity to guide the release process and monitoring the therapeutic outcome. Under pLINFU exposure, the in vitro release profile of the 2 agents was very similar. Mice exposed to pLINFU showed a significantT1 contrast enhancement in the tumor. The tumors of the treated mice displayed a much slower growth.Confocal microscopy and histology were carried out on tumors.In addition to optimize the imaging performance (and the therapeutic outcome) of the method, further studies will be carried out to use imaging for the in vivo quantification of the released drug.

Sol-gel-derived thiol-functionalized oligosilsesquioxanes: influence of synthesis

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We present here the sol-gel synthesis and characterization of thiol-functionalized Si-based Nano-Building Blocks (NBBs). The oligosilsesquioxanes were prepared by the controlled hydrolysis-condensation reaction of 3-mercaptopropyltrimethoxysilane (McPTMS), exploiting the in-situ water production (ISWP) [1,2] by esterification reaction of alcohols with chloro-acetic acid (ClAcA). The Si-based NBBs structural evolution has been studied by FT-IR, ^{1}H, ^{13}C and ^{29}Si NMR. The condensation starts with an extensive non-random cyclization and proceeds with the formation of cage-like structures. The condensation degree and the NBB structure are strongly affected by reaction conditions. The formation of cyclic or linear species can be specifically controlled using different condensation promoters, e.g. trifluoroacetic acid (TFA) or dibutyldilauryltin (DBTL), and changing the molar ratio silane/carboxylic acid, which rules out the hydrolysis ratio. For McPTMS/ClAcA<3, the preferential formation of small cyclic species is observed. On the contrary, for McPTMS/ClAcA>3, branched cages and ladder-like structures are detected. The mercaptopropyl chain is preserved in the final NBBs regardless of the reaction conditions. The thiol-functionalized oligosilsesquioxanes can be chemically assembled for the preparation of patterned coatings for microfluidics, and grafted to Au substrates for exploiting the surface Plasmon resonance phenomenon in sensing applications for chemicals and biomolecules.

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Investigating polymers shape-memory behaviour through solid-state NMR

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Shape-memory materials have the property of "memorising" a macroscopic shape and, after being deformated and fixed into a temporary shape, are able to relax back to the memorised stress-free shape following a thermal, electrical, or environmental stimulus. Sheding light onto the properties of these interesting materials at the nano-metric scale and understanding the "molecular" mechanisms of shape-memory behaviour is an important task. Solid-state NMR is one of the few techniques potentially able to achieve this objective [1], but up to now only very few papers have been published. In this work we have deeply investigated novel alkoxysilane-terminated poly(ε-caprolactone) materials, exhibiting shape memory behaviour, whose preparation and shape memory properties have been recently described [2]. Thanks to the application of many different solid-state NMR techniques for the investigation of both spectral and relaxation properties of several nuclei (^{29}Si, ^{13}C, ^{1}H), it has been possible to obtain a deep understanding of the phase and molecular structural, dynamic and orientational ordering properties of these materials, and follow their change during the shape memory behaviour.

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Solid-State NMR Spectroscopy of Chiral Metal-Organic Frameworks

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Metal-organic frameworks (MOFs) represent a new class of porous organic-inorganic solids. Chiral recognition is possible using NMR spectroscopy, for example by using chiral shift agents. For the first time, we demonstrate here the use of a chiral solvating agent (1-phenyl-2,2,2-trifluoroethanol, TFPE) in order to detect chirality in a chirally modified MOF applying solid-state NMR spectroscopy [1]. UMCM-1 is a MOF compound, which contains mesopores as well as two different linkers: BDC and BTB [2]. The BDC linkers were organochemically modified resulting in prochiral iPr-chirBDC and Bn-chirBDC acid [2]. The linkers were utilised for synthesizing iPr-chirUMCM-1 or Bn-chirUMCM-1, respectively [3]. Chiral shift agents rely on the fact that the chiral substrate forms distinguishable complexes with the two enantiomers of the chiral shift agent. These differences can be detected by NMR spectroscopy. To prove the chirality of iPr-chirUMCM-1 and Bn-chirUMCM-1, an in situ solid-state ^{13}C NMR (ssNMR) method was developed. The chirUMCM-1 MOFs were loaded with TFPE, serving as a chiral solvating agent. Loading of both chiral MOFs with the two different enantiomers of TFPE results in significant differences of the resulting ^{13}C CP MAS NMR spectra.

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Cryogen-Free Superconducting Magnet Design for NMR

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For NMR applications, fields above 1.5 Tesla are only practical with superconducting magnets. It has long been thought that the challenges of vibration, stability, and robustness made traditional superconducting magnets impractical for industrial NMR applications. We report the development and successful operation of cryogen-free High Temperature Superconducting (HTS) magnets for NMR operating at 4.7 T (200MHz) and 3 T (125MHz) that address these issues. To overcome the burden of helium and nitrogen refills, cryogen-free magnets are attractive for materials research applications such as beamline magnets and hard disk drive materials research. The availability of high capacity HTS conductors has made development of magnets suitable for NMR application possible. NMR systems using self-shielded HTS-NMR magnets have now accumulated several years of successful operation in five different countries. In particular the 3T system for relaxometry, provides a vital tool in the development of new contrast agents for magnetic resonance imaging. Magnets were manufactured using BSSCO superconductors and exhibit homogeneity of 0.25 ppm before electric shimming for FT-NMR applications and 15 ppm for variable field relaxometry application. High capacity cryocoolers enable rigid coil supports to be used whilst maintaining operating temperatures at 15-30 K, where the wire performance can sustain the desired high fields. HTS magnets do not operate in a persistent-current mode, special high stability power supplies are used to operate the magnet at any desired field strength. These magnets will form the basis of a new class of NMR instrument for industrial environments, enabling innovative solutions that combine variable field relaxometry with FT-NMR. Future developments will include higher fields for both variable field relaxometry and FT-NMR.

NMR Relaxation-Viscosity Relationship in PEO Melts

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We present a combined viscosity-NMR study on a model system, a melt of polyethylene oxide polymers of increasing molecular weights Mw ranging from 0.06 Mc to 12 Mc relative to the critical mass above which entanglements occur. On these polymers, we measured viscosity, NMR self-diffusion, T1ρ, T1 dispersion and T2 at 23.7 MHz. For Mw > Mc, we show that the rheology of these liquids behaves as expected from the dynamics of entangled linear polymers. Besides viscosity, proton longitudinal and transversal relaxation times clearly display the entanglement transition at Mc ~ 4000 g/mol. The expected power laws are verified both for viscosity and diffusivity versus molecular weight. However, temperature and viscosity dependences of the transverse relaxation time T2 are different from simple liquids and we established a new relationship combining T1 and T2 to predict viscosity at a Larmor frequency of 23.7 MHz. The new viscosity relationship can be explained qualitatively from the calculation of the correlation time function together with the reptation theory. For the calculation of the correlation time function, the intra-molecular interaction term is deduced from literature (fast motion of the CH2 ethyl group and slower motion of the skeleton bonds). The inter-molecular interaction term is the most important and gives the dependence with the molecular weight. It has been calculated using the formulation proposed by Kimmich and Fatkullin (1998). With a unique set of parameters, we were able to reproduce NMR dispersion data collected at various frequencies. An important consequence of the theory is that the new viscosity relationship is only valid in a narrow frequency bandwidth around 23 MHz.

Combination of 13C variable contact time solid state NMR and Principal Components Analysis (PCA) for geographical traceability of wood

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The goal of this research was to highlight intra-species differences of wood samples from different European areas by means of spectroscopic and chemometric methods. This approach has been previously used in the agro-food research field (wine, oil, green tea traceability). Wood is a complex mixture of three polymeric components (cellulose, hemicellulose and lignin), whose interconnectivity and homogeneity can be affected by weather, temperature, altitude/latitude, soil as well as silvicultural activities to some extent. 13C VCT MAS NMR analyses were used here for highlighting those possible differences. Spruce samples from Finland, Poland and Italy were selected according to the results of MIR analysis to reduce intrinsic variability. Experimental samples were prepared in a form of rolled thin slices in order to avoid fragmentation due to milling. Rather negligible differences were found directly in 13C CPMAS spectra of wood samples in relation to provenance. Discrimination was however possible by assessing TCH and T1ρ(H) values, and it was clearly evidenced by exploiting chemometric methods, as PCA. Our preliminary conclusions reveal that samples from Finland appear to be significantly different than the ones from other provenances. Samples from Poland and Italy seem to be less homogeneous, but still distinguishable. It was confirmed therefore that wood records geographical and climatic conditions during growth. Finally, 13C VCT CPMAS NMR seems to be applicable to detect such differences and can be a tool for traceability of wood.

Fast Field Cycling Relaxometry: Moving from Research towards Industrial Applications

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Fast Field Cycling Relaxometry (FFCR) is a NMR technique used to determine the longitudinal relaxation time (T1) over a range of B0-fields spanning about six decades, from about 10-6 Tesla up to ~ 1 Tesla, or 3 Tesla, without varying the frequency of the spectrometer [1,2]. The main information that can be extracted from nuclear magnetic resonance dispersion (NMRD) curves, R1=1/T1 versus the Larmor frequency, is that concerning molecular motions characterized by temperature-activated frequencies and described by means of the spectral density.The data obtained may be correlated directly to the physical/chemical proprieties of complex materials. The use of radio frequency allows the easy penetration of most materials, thus permitting, the exploration of slow dynamics which are often difficult to study in heterogeneous materials by other spectroscopic methods. Furthermore, the benefit of exploring the range of low Larmor frequencies is to detect typical relaxation features associated with molecular processes characterized by very long correlation times, such as molecular surface dynamics and collective effects. The FFCR technique shows greatest potential where the characteristics of a sample depend intimately on the molecular dynamics and / or the state of aggregation. Herein we show developments in the FFCR method and discuss some contributions of NMRDprofiles towards the fundamental understanding of classes of materials and phenomena predicted by theoretical models. We show in practice how relaxation experiments can be applied for qualitative structural diagnostics in solutions, quantitative structural determinations, recognitions of weak intermolecular interactions and studies of molecular mobility. The findings that have been established more recently are noteworthy for their potential industrial.

Method for NMR Relaxometry on Heteronuclei at Very Low Fields

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Up to now most applications of NMR relaxometry involved the study of protons due to the low sensitivity of other nuclei and to technical difficulties mainly related to signal to noise ratio problems caused by the low acquisition frequency. The technique of Fast Field Cycling permits the direct observation of hetero-nuclei with low receptivity and detectability, due to the fact that the magnetic field strength can be switched without the need to vary the frequency of the spectrometer. Fast Field Cycling Relaxometry is a NMR technique used to measure the spin relaxation curves over a range of B0-fields, spanning about six decades, from about 10-6 Tesla up to ~ 1 Tesla or 3 Tesla, thus information concerning molecular motions characterized by temperature-activated frequencies and described by means of spectral density can be obtained directly. This multi-nuclear Approach expands the potential of Fast Field Cycling NMR applications and allows exploration of the field dependence of the T1 of important hetero-nuclei within substances, especially at low Larmor frequencies, where other conventional NMR experiments present severe signal-to-noise ratio degradation. The FFCR technique allows investigation of the content and /or the ability to characterize compounds containing important NMR-sensitive nuclei, such as 2H, 7Li, 13C, 19F, 31P, 23Na. The possibility of measuring nuclear spin relaxation, on nuclei other than 1H, over a wide range of frequencies presents a new advance in the possible applications of TD-NMR and the possibility for new channels of research. Herein we show the applicability of FFCR for the acquisition of the longitudinal relaxation rate as a function of the applied magnetic field strength of some important hetero-nuclei.

Coupling Solid-State NMR with GIPAW ab-initio calculations in metal hydrides and borohydrides

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An integrated experimental-theoretical approach for the solid-state NMR (SSNMR) investigation of a series of Group I and II metal hydrides and borohydrides, such as NaH, LiH, $NaBH_4$, MgH_2, CaH_2, $Ca(BH_4)_2$ and $LiBH_4$, is illustrated, mainly focusing on the computation of the ^{1}H, ^{23}Na, ^{11}B, and ^{6}Li SSNMR parameters by means of the GIPAW method in these systems, and aiming to check the reliability of the approach for modelling of NMR spectra. Periodic lattice calculations were performed by means of the plane-wave method, adopting the DFT Generalized Gradient Approximation (GGA) with the PBE functional, as implemented in the Quantum Espresso package (v. 4.3.2). Projector Augmented Wave (PAW), including the Gauge-Including Projected Augmented-Wave (GIPAW) method [1] for SSNMR calculations was used, adopting both Rappe-Rabe-Kaxiras-Joannopoulos (RRKJ) ultrasoft pseudopotentials (PP) and new developed pseudopotentials. This new set of PAW PP was validated by comparison not only with experimental data, but also with computed data achieved employing others sets of PP already available. In particular, the predicted SSNMR ^{1}H, ^{23}Na, and ^{6}Li chemical shift values obtained by using PAW PP are analogous to those derived by employing the standard PP, whereas the former allow a substantial improvement of the ^{11}B chemical shift estimation. Moreover, our computed results revealed to reliably reproduce the ^{11}B MAS experimental spectrum of commercial, $Ca(BH_4)_2$, distinguishing the two phases (α and β) contained in it [2].

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Phase transitions in polymeric luminescent indicators with a threshold temperature through variable-temperature SSNMR and fluorescence spectra

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In this work we investigated the phase transitions of poly(lactic acid) (PLA) in polymeric threshold temperature luminescent indicators containing BBS chromophores [1] through the combination of ^{13}C CP/MAS spectra and the analysis of ^{1}H FID acquired at different temperatures. Indeed, ^{1}H T_2 relaxation time has the property to increase monotonically with the degree of mobility of the system, and the fitting of the ^{1}H FID acquired under on resonance condition, i.e. low magnetic field, allows to identify domains characterized by different ^{1}H T_2, and therefore by different phase and dynamic properties [2]. The phase behaviour of PLA domains under heating was characterized in detail in both the neat polymer and the final indicators, and possible correlations with the luminescent properties of the indicators were identified. Financial support by POLOPTEL project, Fondazione Cassa di Risparmio di Pisa, is acknowledged.

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Interaction of electrolyte molecules with porous carbon materials: Characterization by NMR Spectroscopy

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The present contribution describes the NMR spectroscopic characterization of different porous carbon materials of well-defined pore size loaded with electrolyte molecules. In order to establish a correlation between the NMR parameters and the pore diameter, carbon materials of very well-defined and uniform pore size varying in a relatively wide range from 4.5 nm down to 0.6 nm were synthesized and characterized by ^{129}Xe NMR spectroscopy in order to study the accessibility of the pore system. The samples were then loaded with 1M $TEABF_4$ in acetonitrile or pure acetonitrile and investigated by solid-state ^{1}H, ^{11}B, and ^{13}C NMR spectroscopy. It can be shown, that the chemical shift of NMR active nuclei located at molecules adsorbed in porous carbon materials of comparable degree of sp^2 hybridization is correlated with the pore size. Furthermore, the interpretation of NMR data delivers information about the distribution of electrolyte molecules inside the pores. The influence of different electrolyte concentrations and the time dependence of the adsorption process is investigated by means of various NMR techniques including 2D exchange spectroscopy.

Distorting cages in an ion conductor

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In the past years, the crystal Mayenite ($Ca_{12}Al_{14}O_{33}$) has drawn attention for being a good ion conductor with possible uses as a transparent conductive oxide, catalyst, ion emitter or oxygen sensor [1]. Its structure features in average five vacant cages and a with an O^{2-} ion filled one. Occupation of a cage leads to a distortion of the structure, which brings forth two aluminum sites. We were able to resolve them by ^{27}Al-NMR. It is possible to replace the O^{2-} ion by different anions, such as Cl^-, F^-, OH^-, O_2^- or H^- and even electrons [2]. ^{27}Al-NMR was used to investigate the introduction of different anions into the cages. Furthermore, we developed a mathematical framework for the purpose of line-shape analysis based on the method of moments which is suitable for noisy spectra. Finally, high temperature measurements were performed and an activation energy was obtained.

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The Grotthuß-Mechanism in Nanoporous Silicates and its Implications to Diffusion Studies in Natural Porous Media

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The measurement of T_2-T_2 exchange on low-field NMR platforms is a most straightforward tool to reveal the mechanism of diffusive mass transfer in porous systems [1,2]. Applied to water in Stöber-silicates, two relaxation sites were identified: the silicate surface holding 25% of water, and the pore water holding 75%. Employing our recently developed model to simulate diffusive relaxation exchange [3], we were able to derive the precise kinetic exchange rates at different temperatures. The according exchange activation energy was found to be 7 kJ/mol, which is strong evidence that the Grotthuß mechanism of chemical proton exchange is the *only* mechanism for proton migration in nanoporous silicates, and that translational diffusion of water is largely inhibited [4]. Considering the large silicate surfaces in natural porous media, our results imply that any method that uses proton diffusion as the sole measure for the general mass transfer in such systems, may lead to wrong conclusions.

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Processing additives for BHJ solar cells: a morphological study based on solid-state NMR, AFM and Raman microscopy

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BHJ solar cells comprising conjugated polymers and fullerene derivatives have demonstrated good performances that must be increased in order to be competitive with the traditional silicon solar cells. In order to achieve higher power conversion efficiencies, a possibility is the incorporation of di-substituted alkanes in the casting solution. The mechanism through which they work is still uncertain: they do not react with the components of the active layer, but probably they induce modifications of the morphology, strictly correlated to the solar cell performances. That's why we started a comparative study on the blends formed by PCBTDPP (copolymer carbazole-diketopyrrolopyrrole, a “low bandgap” polymer) and PC70BM. In one blend we added 1,8-diiodooctane (Bl_DIO) as processing additive, while a second one was obtained without it (Bl). We based our study on the comparison of the results obtained with three powerful analytical techniques: solid-state NMR measurements of relaxation times, T1(13C) and T1(1H), AFM and Raman microscopy. By means of 1H NMR relaxation studies an increase of the domain sizes has been observed in Bl_DIO and parallel to this result, the AFM images show that here the surface of the blend is significantly rougher than in Bl. In the Raman spectra of Bl_DIO an intensification of a band at 1620 cm-1 ascribable to PCBTDPP is indicative of a morphological modification induced by the DIO.

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Structural investigation of inosine-edited RNA duplex and its interaction with RNA-induced silencing complex component p100

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The protein p100 (also known as SND1 or Tudor-SN), a component of the RNA-induced silencing complex, is involved in binding and cleaving inosine-edited RNA duplexes thereby excluding them from downstream events in RNA interference. This function is performed by p100's two tandem pairs of staphylococcal nuclease (SN) domains that were shown to be implicated in edited RNA binding and cleavage. However many key aspects of this interaction remain unclear. What is the structural basis for the discrimination between edited and unedited RNA sequences? Does the p100 possess RNA binding capability alone or also have RNAse activity? If so, which structural aspects modulate this activity? To address these questions, we set out to solve the three-dimensional structure of the inosine-edited RNA substrate of p100 by NMR spectroscopy as well as study the structural changes that occur in the p100 protein upon RNA binding. Multidimensional NMR experiments were used to conduct a backbone assignment of the p100 39 kDa construct containing SN domains 3 and 4 up to 85%. There is a strong indication that an interaction between these two domains takes place, which contributes to the overall dual domain fold. To further investigate the p100/edited RNA duplex binding, this interaction will be studied by titrations, measurements of relaxation and residual dipolar couplings, as well as by electrophoretic mobility shift assays.

Experimental evidence of the existence of the G-triplex and its three-dimensional structure

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Nucleic acids can adopt a variety of biologically relevant structures different from the standard Watson-Crick double helix, that can be constituted by one up to four strands. Base stacking and hydrogen-bond interactions, such as the Hoogsteen pairings, are at the basis of the stabilization of these non-canonical tertiary structures. Among these higher-order structures, G-quadruplexes have certainly played a major role in recent years because they are involved in a large number of biological processes, such as the maintenance of genome stability, and have also been exploited as attractive therapeutic targets for the development of anticancer drugs. Recent metadynamics studies on the folding/unfolding process of the thrombin binding aptamer (15-TBA, 5'-GGTTGGTGTGGTTGG-3') suggested the formation of an energetically stable novel structural motif, the G-triplex, obtained from the G-quadruplex by the detachment of the extremity at the 3'-end[1]. A truncated form of 15-TBA, involving the last strand (5'-GGTTGGTGTGG-3') has been synthetized and analysed by NMR. Here we report the first experimental structure at the atomic resolution level of this new G-triplex folding. Experimental distances and dihedral restraints have been measured and implemented in the program X-plor NIH for the structure calculation.

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Structural Investigation of pri-miR-18a by NMR Spectroscopy

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MicroRNAs (miR) are important negative regulators of protein expression. Their impaired function is linked to severe diseases such as cancer. Recent findings show, that pri-miR-18a, a miR precursor, is regulated posttranscriptionally by hnRNP A1 [1], an auxiliary protein factor, which is involved in splicing regulation. Pri-miR-18a is is a RNA stem-loop consisting of 71 nt, and part of a larger cluster on chromosome 13 (oncomir 1). We are studying the structure of the 71nt pri-miR-18a by NMR spectroscopy. We show that the loop region of pri-miR-18a, to which hnRNP A1 is binding, is highly flexible by examination of the truncated samples of the loop region of pri-miR-18a. As it is challenging to analyze large RNA molecules due to spectral overlap in NMR spectra, we employ segmental isotope labeling of the RNA. This protocol [2] includes gene assembly, *in vitro* T7 RNA transcription as well as ribozyme and RNaseH enzyme cleavage followed by religation of the different segments. Segmental ^{13}C-, ^{15}N-double labelled samples reduce spectral overlap and facilitate the NMR data analysis. Progress with this method and NMR analysis of fragments of the full-length and fragments of 18a RNA will be presented.

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Cytosine residues in a G-rich region lead to formation of noncanonical structures

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There is plenty of evidence showing that it is possible for G-rich DNA sequences to form G-quadruplex secondary structures which can be involved in several essential biological processes [1]. The basic building blocks of G-quadruplexes are G-quartets, which are comprised of four Hoogsteen H-bonded guanine residues. Residues involved in a core of a G-quadruplex structure can be connected together by loop regions. Unusual structural motifs and variability in loop regions are especially interesting since they could represent specific binding sites for other biomolecules and small therapeutic agents. If the sequences with the potential to form G-quadruplexes also contain cytosine residues in the same strand aditional structural motifs become possible. The cytosine residues can be part of loop regions which protrude into solution or associate with guanine residues into Watson-Crick GC base pairs. In addition GCGC-quartets exhibiting different H-bonding geometries can be formed. Both, the GC base pairs and GCGC-quartets are highly susceptible to the surrounding conditions such as presence of different cations or water molecules [2, 3]. The folding possibilities can be further expended by the formation of different base pairs which do not belong to the most common Watson-Crick or Hoogsteen types.

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G-quadruplex interior is accessible to water molecules

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G-rich oligonucleotides containing also cytosine residues can form G-quadruplexes where G-quartets are flanked by GC Watson-Crick base pairs. While cations are required for the stabilization of G-quartets, their role in the stabilization of flanking GC base pairs was unclear. Solution state NMR was used to explore the coordination of ammonium cations within a bimolecular $d(G_3CT_4G_3C)_2$ G-quadruplex. The structure of the G-quadruplex exhibits antiparallel strands with edge-type loops. Two G-quartets in the core of the structure are flanked on each side by two Watson-Crick GC base pairs. The topology is equivalent to the solution state structure of the same oligonucleotide in the presence of Na^+ and K^+ ions [1,2]. A single ammonium ion binding site was identified between adjacent G-quartets albeit three sites were expected. The remaining potential cation binding sites between G-quartets and GC base pairs are occupied by water molecules. To the best of out knowledge this is the first observation of long-lived water molecules within a G-quadruplex structure. The flanking GC base pairs adopt a coplanar arrangement and do not require cations to neutralize unfavorable electrostatic interactions amongst proximal carbonyl groups. A relatively fast movement of ammonium ions from the inner binding site to bulk with the rate constants of 21 s^{-1} was attributed to the lack of hydrogen bonds between adjacent GC base pairs and the flexibility of the T_4 loops.

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Long-lived intermediates formed by G-rich strands in the absence of cations

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G-quadruplexes are higher order secondary structures formed by guanine-rich DNA sequences that can be found in biologically significant regions of the genome such as telomeres, immunoglobulin switch regions and promoter regions of eukaryotic cells. They have also been associated with human diseases, as therapeutic targets in drug design and in applications as nanomolecular devices. The presence of cations such as K^+ or Na^+ seems to be essential for the formation of G-quadruplexes, due to their role in reducing repulsions amongst guanine carbonyl oxygen atoms within G-quartets and additionally enhancing base-stacking interactions. With the use of solution-state NMR spectroscopy and other experimental techniques (PAGE, TDS, UV, CD, DSC) we have studied the behavior of guanine-rich sequences and explored their features in an environment almost completely free of G-quadruplex promoting cations. Experimental data has shown the formation of a new structure, which can be considered as an intermediate on the way to folding into G-quadruplexes. It is interesting to note that the guanine bases are not held together by Hoogsteen hydrogen bonds like in G-quartets but rather by alternative base pairing. Our study, where G-rich intermediates were characterized in detail and their kinetic roles determined, provides an important step in elucidating general principles by which G-quadruplexes adopt their native folds.

Cation localization and movement within $d(TG_8T)_4$ G-quadruplex in solution

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Guanine-rich DNA can adopt G-quadruplexes - noncanonical structures comprised of stacked G-quartets, which exhibit potentially applicable characteristics for designing novel DNA-based nanomaterials and nanodevices. We used solution-state NMR to study the role of cation localization and mobility within G-quadruplex structures comprised of a number of G-quartets.[1] In the presence of $^{15}NH_4^+$ ions $d(TG_8T)$ adopted two tetramolecular G-quadruplexes in a 3:1 ratio at room temperature. The essential and distinctive feature of each of the two G-quadruplex species adopted by $d(TG_8T)$ was anti/syn dispositions along the glycosidic torsion angle of G2 residues. Heteronuclear NMR experiments enabled insights into cation localization within $d(TG_8T)_4$ exhibiting eight stacked G-quartets. With the use of 2D ^{15}N–^1H HSQC spectra we identified seven discrete binding sites of $^{15}NH_4^+$ ions within G-quadruplex structures, which demonstrated that all inter-quartet cavities along the central channels were completely occupied by cations. Exchange rates for cation movement, which were determined at room temperature showed that $^{15}NH_4^+$ ions moved an order of magnitude faster from the binding site between two G-quartets both comprised of all guanine residues in an anti glycosidic conformation with respect to the binding site between all-syn and all-anti G-quartets.

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Comparing substrate specificity of 2'deoxyguanosine-binding riboswitch classes by NMR spectroscopy and thermodynamics

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Riboswitches are non-coding RNA sequences that bind small metabolites with high affinity and selectivity and thereby regulate gene expression. Several classes of 2'deoxyguanosine-binding (2'dG) riboswitches have been identified which differ only slightly in their primary sequences but significantly in their ligand affinities and specificities[1]. Only the X-ray structure of the class Ia 2'dG-riboswitch has been solved so far. We investigated the binding specificity of three 2'dG-binding riboswitches (2'dGIa, 2'dGIIa and 2'dGIIb) by NMR and ITC. Almost all imino proton signals could be assigned and the comparison of chemical shifts in 1H-15N-HSQC spectra showed that the tertiary structures of all three riboswitches are overall very similar. Moreover, the preformation induced by magnesium addition of the class II riboswitches was investigated, using the unbound and magnesium-bound spectra. We found that 2'dGIa and 2'dGIIb are similar in preformation, in contrast to 2'dGIIa. The binding affinity to 2'dG at high and low salt showed a pronounced magnesium dependence for all three riboswitches. We also showed that the riboswitches 2'dGIa and 2'dGIIb bind 2'dG with higher affinity than 2'dGIIa. The investigation of the ligand specificity showed, that all riboswitches discriminate against the amino group of the ligand, while they differ with respect to substitutions of the sugar moiety.

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^{1}H-NMR and HR-MAS-^{1}H-NMR as useful tools for the quali/quantitative characterization of cocoa beans and derived products

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We present here the results of four-years studies about the use of high resolution 1H NMR to improve the knowledge about cocoa (Theobroma cacao L.) composition. Cocoa is produced through a multi step process involving cocoa beans fermentation, drying and roasting. A secure method for the quality control of traded cocoa beans would be desirable. Moreover, today's consumers increasingly require high-quality cocoa products, as mono-origin chocolates, but few cocoa producers declare the real geographical origin. Our results showed that 1H NMR combined with chemometric analysis can be useful to solve some important analytical problems related to cocoa beans quality, for example to successfully distinguish non-fermented or partly fermented cocoa beans by well-fermented cocoa beans: this is of outmost importance because the composition of cocoa beans, which is one of the most important factors influencing the taste and flavor of the cocoa products, strongly depends on the fermentation level. The possibility of using 1H NMR as a "fingerprint" in the definition of geographical origin has been also explored, showing that Arriba and Criollo beans, from Ecuador and Grenada, form groups separated from beans coming from African regions. 1H NMR was also useful to follow the modifications occurring during cocoa beans transformation to chocolate. Accurate simultaneous quantification of aminoacids, polyalcohols, organic acids, sugars, methylxantines, catechins, phenols was also achieved. Finally, HR_MAS 1H NMR permitted to obtain spectra comparable to liquid NMR without any sample manipulation, allowing a very rapid control of cocoa beans.

Authentication of medicines by Nuclear Quadrupolar Resonance (NQR) Analysis. A portable system for non-destructive control of packaged pharmaceutical products

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Counterfeit and substandard medicines constitute a serious threat to public health, because they provide little protection from disease and, worse, can expose consumers to major harm. Bad drugs pose potential threats around the world, it is estimated that upwards of 15% of all medicines in the marketplace are counterfeit. The chemical analysis of drugs suspected to be counterfeit is a crucial step in a series of measures that should accompany the overall action against this criminal trade and the analytical techniques that produce a "fingerprint" may be successful. Detection technologies vary in sample throughput, sensitivity, specificity, cost per sample, comprehensiveness of the information produced, and technical expertise required to complete the analysis. An additional constraint could be placed as to where the chosen method should be applicable in the field, or if the sample should be preserved during analysis. Nuclear quadrupole resonance (NQR) is a solid-state radio frequency (RF) spectroscopic technique that can be used to specifically detect the presence of quadrupolar nuclei, such as 14N, an element contained in many narcotics and drugs. It also offers a clear advantage over the other technologies in that RF can penetrate even multiple layers of packaging material, allowing for scans to be carried out without the need to remove pharmaceutical products from their packaging. Herein we show the development of a robust, economical and portable Quadrupole Resonance (QR) device for the non-invasive, non-destructive authentication of packaged pharmaceutical products, particularly bottles and blister packs.

Novel platinum compounds with potential for cancer therapy: study of their interaction with model lipid membranes

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The investigation of the interaction of drugs with lipid membranes is fundamental in understanding the drug transport across biological membranes be it either by passive diffusion or mediated by transport proteins. Platinum complexes, as cisplatin, oxaliplatin and carboplatin, are widely used in chemotherapy being potent antitumor drugs; these drugs, however, have dose-limiting side effects. Great effort is thus dedicated to finding drugs with similar or even higher antitumor efficacy but lower toxicity. With this aim, new platinum complexes (cis-[dichloro(carbonyl)(triphenylphosphino)platinum(II)] and trans-[dichloro{N,N-bis(2-hydroxyethyl)amino}(triphenylphosphino)platinum(II)]) were synthesized and their activity was tested for their antiproliferative activity in vitro against HeLa, A549 and H460 tumor cells. The drug-membrane interaction was investigated by means of NMR spectroscopy using DMPC phospholipid bilayers as model membranes. In particular, ^{31}P, ^{13}C and ^{2}H solid state spectra were recorded at different temperatures from 20°C to 40°C both on static samples and on samples rotating at the magic angle, obtaining information on the phase structure, phase transitions and structural and dynamic changes in the phospholipid upon interaction with the platinum complexes. The results were compared with those obtained by all-atom molecular dynamics (MD) simulations and MD-based free energy computations.

Distribution of organic additives in micellar solution of surfactants: A PFG NMR diffusion study

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Aqueous micellar solutions of either cationic, anionic or nonionic surfactants with dodecyl tails and aromatic additives are investigated by pulsed-field gradient NMR. Employing a fast exchange model, the micelle/water partition coefficient (f) is calculated from diffusion coefficients of both surfactant and additive. Additives with hydrophobicity, as specified by the octanol/water partition coefficients P_{OW}, in a medium range are chosen where a significant dependence of f on log(P_{OW}) has been found. The relevance of specific interactions of substituents of the aromatic additives with the surfactant's head group versus the relevance of the additives overall hydrophobicity is explored. In addition interactions of the different head groups with the aromatic ring system are investigated. While typically the critical micelle concentration (cmc), the water solubility, the packing parameter and other properties depend on the shape and charge of the surfactant, here a novel incorporation coefficient is introduced, which describes the concentration dependence of the micelle/water partition coefficient. This coefficient allows comparisons of values of f for different surfactants with very different cmc, which can be analyzed only at very different concentrations. With the partition coefficient and the incorporation coefficient the distribution equilibrium of aromatic additives in solution of surfactants can be characterized. In general, we observe a clear dependence of partition coefficient and of incorporation coefficient on hydrophobicity of the guest molecule, but we also found unexpected interactions between special aromatic additives and the anionic surfactant caused by structural features.

Solid-state NMR for the characterization of crystal forms of pharmaceutical compounds

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The possibility of obtaining polymorphs, co-crystals or salts provides a tool for tuning physicochemical properties of a solid molecule without changing its peculiar activity. This is important for several application fields such as pharmaceutical, explosives, foods, pigments because the possibility to form salts and/or co-crystals can improved the solubility and the thermal stability. Here we report the solid-state NMR characterization of several crystal forms of some molecules of pharmaceutical interest obtained by means of "solvent free" methods. In particular we focused on the tolfenamic acid (TA) a potent, well-tolerated non-steroidal anti-inflammatory drug with a low water solubility and high permeability in the cell[1]. 1D (^{13}C and ^{15}N CPMAS, ^{13}C and ^{15}N NQS, ^{1}H MAS) and 2D (^{13}C-^{1}H FSLG-HETCOR) solid-state experiments were acquired in order to understand: number of independent molecules in the unit cell, ionic of molecular character of the adduct, hydrogen bond strength [2], formation of a salt rather than co-crystal [3]… IR, Raman, DSC and TGA complete the characterization for evaluating the effects of the microscopic changes on the macroscopic properties.

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Study of chemical interactions between antiretroviral drugs Efavirenz and Tenofovir Disoproxil Fumarate

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Acquired immune deficiency syndrome (AIDS) is a degenerative disease of the immune system caused by the human immunodeficiency virus (HIV). Tenofovir Disoproxil Fumarate and efavirenz are among the most used drugs for the AIDS treatment. Therefore, Solid State Nuclear Magnetic Resonance (ssNMR), Differential Scanning Calorimetry (DSC) and Thermo Optical Analysis (TOA) were used to study possible interactions between these drugs. ^{1}H-decoupled ^{13}C CPMAS experiments have been performed for the two pure components and the mixture in 1:1 w/w ratio at 4 kHz in a 400 MHz Bruker DRX spectrometer. The mixture spectrum showed significant changes in chemical shift values when compared with spectra of pure drugs, especially in the signals relating to deficient electron carbon atoms of both tenofovir disoproxil fumarate and efavirenz. These results suggest a possible incompatibility between the drugs, which was confirmed by DSC and TOA. Those techniques have evidenced significant shifts on the melting points of both drugs in the mixture, if compared with their analysis in pure state.

Characterization of Protected Designation of Origin Lambrusco Wines of Modena using NMR Spectroscopy and Multivariate Statistical Analysis

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Lambrusco are a Protected Designation of Origin (PDO) red wines produced in the province of Modena, one of the most relevant wine-producing region in Italy. For these wines also exists a production code, establishing the ampelographic composition depending on their geographical and historical region of origin. The application of NMR to food as quality control is a subject of great interest and represents an important tool to counter adulteration and sophistication [1]. In the present work we have employed NMR spectroscopy as a molecular fingerprint technique and quality control tool, allowing us to evaluate the authenticity of analyzed wines. Using ^{1}C NMR, ^{13}H−^{13}C HMBC, ^{1}H−^{13}C HSQC analyses as well as literature data, it has been possible to identify several metabolites. We have also applied several pattern recognition procedures, both unsupervised and supervised, to data sets obtained from ^{1}H NMR, to achieve a reliable classification of the different wine samples. PCA was performed to verify the intrinsic variation in the data sets, while PLS-DA was applied to maximize separation between samples. In conclusion, it has been possible to successfully classify the samples depending on their varietal origin using ^{1}H NMR coupled with chemometrics, and to identify the most relevant compounds, responsible for the varietal discrimination.

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Pharmaceutical Co-Crystals of Niclosamide: A Solid-State NMR Approach

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Over the last decade, the design of pharmaceutical co-crystals emerges as a potential method for enhancing the bioavailability of drugs with low aqueous solubility, increasing their Intrinsic Dissolution Rate (IDR), which is one of the main challenges for the pharmaceutical industry[1]. Solid-State NMR (SSNMR) can supply the necessity to analyse noncovalent interactions, especially in powder samples, for which Single Crystal X-Ray Diffraction cannot be employed. In this communication we report the solvent-free synthesis and the powder characterization of different crystal forms of niclosamide (HNic), an API with poor solubility, belonging to Salicilamide class: 4 new salt co-crystals, a classic co-crystal and a salt. The peculiarity of salt co-crystals is the concomitant presence of HNic both as a neutral component and as a salt co-former, which interact via hydrogen bond formation. All samples were investigated by combining experimental solid-state techniques known to provide complementary information on powdered samples: X-Ray Powder Diffraction, SSNMR, IR and Raman. Differential Scanning Calorimetry, Thermo-Gravimetric Analysis, IDR and Powder Dissolution measurements complete the characterization for evaluating the effects of the microscopic changes (weak interactions, etc) on the macroscopic properties (thermal stability and bioavailability). The obtained results indicate that the formation of salt co-crystals provides a reliable route for modifying the HNic intrinsic dissolution rate, in these cases improving the IDR until a factor of 24.

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Structural and nutritional properties of pasta from *Triticum monococcum* and *Triticum durum* species. A combined ^{1}H NMR, MRI and digestibility investigation

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Two different kinds of pasta, differing in botanical species (Triticum turgidum ssp. durum (cv. Saragolla), vs Triticum monococcum ssp. monococcum (cv. Monlis) and processing were studied to investigate a relationship between starch digestibility -enzymatically determined- and microstructure -MRI determined by T_1 and T_2 relaxation and diffusion (D). MRI experiments (4.7 T) were performed on cooked (11-15-20 min) pasta. Combined with ^{1}H NMR analysis (11.7 T) of the chemical compounds released during cooking, MRI data highlighted different microstructure for the two varieties: significantly shorter relaxation and D suggested a more compact structure for Saragolla. Relaxation showed a biexponential decay for T_1, while a single decay for T_2. To shed light on the MRI results, samples cooked in both water and D_2O were compared, confirming the assumed models and showing up a different hydration level. Digestibility results highlighted a lower starch hydrolysis rate in Monlis. All results suggested the protein network to protect starch granules in Saragolla, so influencing the gelatinization process.

The effects of intra- and postoperative ischemia on the metabolic profile of clinical tissue specimens monitored by NMR

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In order to monitor the impact of ischemia on the metabolic profile of human liver tissue during and after surgery, high-resolution magic-angle-spinning (HR-MAS) ^{1}H NMR was used to obtain the metabolic profiles of 162 human liver samples. The NMR profiles changed as a function of intraoperative warm ischemia (WI) and post-resection cold ischemia (CI) time, and provide a fingerprint of the ischemia. In both cases, the observed changes were mainly due to changes in the concentration of sixteen metabolites, although to different extents. NMR profiles proved to have predictive value for distinguishing different ischemia time points with high accuracies. The ischemia NMR profile may represent a useful tool to monitor pre-analytical effects and therefore could contribute to improvements of quality control in biobanks. Furthermore, the study demonstrates the feasibility of controlling pre-analytical processes for metabolomic studies of liver diseases.

NMR Methodology in Food Science

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The increasing ability of high field NMR spectroscopy to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics and food chemistry [1]. 1H high field NMR spectroscopy has shown to be a valuable tool for the qualitative and quantitative analysis of the metabolic profiling of food stuff. The quantitative analysis of the metabolic profiling along with the application of a suitable statistical analysis has allowed food characterization in terms of geographical origin, genetic origin and farming. The potential of NMR spectroscopy to detect food adulterations has been also demonstrated. Here, the NMR methodology used to study foodstuffs is discussed reporting some significant examples [1,2,3].

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Conformation of Contryphan-Vn S8W, a newly synthesized cyclic peptide and the role of proline isomerization

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Contryphan-Vn (NH2-GDCPWKPWC-CONH2) is a conotoxin present in the venom of Conus ventricosus (1). The solution structure of this cyclic peptide characterized by the presence a D-Trp residue in fifth position (2) has been determined. In the new cyclic peptide analogous of the Contryphan-Vn with the S8W substitution the influence of the indole ring of residue 8Trp on the structural stability of the ring structure, and on the isomerization of the prolines and, eventually, on activity of the peptide was to be evaluated. The chemical shifts dependence upon temperature of the amide protons revealed their involvement in intramolecular hydrogen bonds. All the structural constrains obtained by coupling constants and by 2D ROESY experiments led to the determination of the 3D structure by rMD simulation. The substitution S8W leads to some differences with Contryphan Vn : i) the absence of the stable salt bridge between 2Asp and 6Lys ii) the increase of cis isomer of the only 4Pro at higher temperatures. Accordingly with the NMR results, the intrinsic fluorescence of the Contryphan Vn S8W changes upon temperature. The structural differences between Contryphan Vn S8W and Contryphan Vn are due to the conformational constraints induced by cyclization and to the importance of the W8 in the hydrophobic cluster.

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Characterization and classification of propolis extracts by HR-NMR

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Propolis is a resinous substance collected by bees from exudates of different plants, rich in health-relevant phenolic compounds [1]. This study demonstrates that it is possible to use HR-NMR for the simultaneous recognition of 12 typical phenolic compounds (apigenin, chrysin, galangin, kaempferol, quercetin, naringenin, pinocembrin, pinostrobin, caffeic acid, cinnamic acid, p-coumaric acid and ferulic acid) [2] in propolis extracts, using appropriate tools for spectra pre-treatment [3] and analysis, and to verify if the same technique was able to classify propolis according to the harvesting method. A simple 1H-NMR sequence was used for phenolic compounds identification. Sixty-five propolis samples were used to test the proposed identification procedure. Ten out of 12 considered compounds were identified as statistically significant in most of the samples. For the propolis classification according to the harvesting method, the ethanolic extracts were initially analysed for quantification of the main bioactive substances, balsams and waxes. The 1H-NMR and heteronuclear multiple bond correlation spectra were then acquired and analysed by multivariate statistical techniques. The best model was obtained using the 1H-NMR by analysing the spectral region between 4.50 and 13.00 ppm (predictive capacity: 96.7%).

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Biochemical assessment of RBCs during storage in blood bank conditions

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Blood transfusion is a well-established therapy. Nevertheless current protocols are rarely subjected to a detailed assessment of efficacy and safety. The reason is the absence of reliable biochemical quality indicators able to correlate between the effective therapeutic capacity with metabolic risk level. For decades the RBCs quality control standard was the hemolysis during the storage and in vivo recovery and survival [1]. Clinical studies highlighted a correlation between the age of RBC transfused and increase in hospital permanence length, post-operative infections, multiple organ failure and mortality [2]. Aim of this study is the comparison of RBCs metabolic pattern over a 42 days storage period in blood bank conditions, with and without leukodepletion. Metabolites NMR analysis was carried out on RBC supernatant and on RBCs lysate after protein depletion by ultrafiltration. Analysis of RBC metabolites presented some challenges due the presence of high amount of additives in the storage medium. Nonetheless, it turned out to be feasible. The study of the RBCs supernatant allowed to follow the consumption of the preserving additives and the detection and quantification of up to 30 metabolites released by RBCs. NMR spectra of RBCs lysate provided complementary information on some biochemical pathways and set the basis to build a time dependent RBCs metabolic profile. Knowledge of RBC metabolic modifications during storage offers a great potential to the development of new protocols for both conservation and patient oriented therapy.

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Urinary Metabolic Profiling of Rats Treated with Curcumin, using ^{1}H NMR and HPLC-MS

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Curcumin, a hydrophobic polyphenol derived from the rhizome of Curcuma longa, has shown several pharmacologic effects including anti-inflammatory, antimicrobial, antioxidant and anti-cancer activity. Despite being one of the most widely studied plant extracts, its mode of action in vivo remains unclear. The aim of this study is to evaluate the effects of the supplementation of curcuma extract on the metabolic status of healthy rats. A metabolomic strategy based on ^{1}H-NMR and HPLC–MS data in conjunction with statistical analysis was applied to rat urine. Urine samples of twelve rats, randomly divided into a control- and a curcumin-treated group, were collected on day 1, 5, 9, 14, 19 and 25 during the animal experiment. ^{1}H-NMR and HPLC-MS measurements were performed on all urine samples and data were used for statistical analysis. Significant changes in metabolic profiles were observed, caused by both curcuma extract supplementation and aging. Spectral profiles of individual metabolites as a function of diet were obtained by independent deconvolution of NMR and HPLC-MS data, using Parallel Factor Analysis (PARAFAC) and Batch Statistical Process Control (BSPC). Several metabolites were identified that show different profiles between the two groups.The different levels of these metabolites may provide an indication of the biochemical pathways that are sensitive to curcumin supplementation. Our findings indicate a new approach for the evaluation of the bioactivity of plant extracts in a healthy subject model.

NMR characterization of the binding of small ligands and functionalized liposomes to Aβ peptides

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The amyloid peptides Ab1-40 and Ab1-42 represent one of the main molecular targets for the development of potential drugs for the treatment of Alzheimer's Disease(AD).[1]One possible strategy to develop a therapy against AD is to target Ab peptides with small molecules able to bind them and slow down their process of aggregation.We generated a small library of glycofused aromatic tricyclic compounds, tetracycline analogues, and we verified their ability to bind Ab oligomers by STD-NMR and trNOESY experiments. [2]Fluorescent derivatives of the ligands that displayed the best affinity were synthesized, and the ability of these derivatives to bind Ab oligomers and to stain amyloid deposits in brain mouse tissues was demonstrated.[3]Moreover, since Ab peptide ligands should reach their target inside the brain passing through the blood brain barrier, functionalized liposomes were prepared linking one of the best tricyclic ligands to their surface by click chemistry. The ability of these functionalized liposomes to interact with Ab oligomers was then verified by NMR waterlogsy experiments in solution.

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Excited Triplet States and Molecular Geometry of Oligo-PPEs Investigated by Pulsed EPR

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Poly(para-phenyleneethynylene)s (PPE) are versatile polymers that are synthetically easily accessible. They exhibit high fluorescence quantum yields and are very photostable. Their photophysical properties are heavily dependent upon their surrounding. Colloidally stable particles constituted by block copolymers of PPEs are useful for live multicolor cell imaging because of their high fluorescence intensity and tunable absorbance and fluorescence. Despite their chemically and photophysically interesting properties, PPEs are to date mainly characterized as macroscopic bulk materials, only. Owing to their application in organic electronics and cell microscopy their microscopic and electronic properties are of high interest. Herein we report the investigation of the photo-excited triplet state of rod-like Oligo-PPEs via time-resolved EPR with synchronized UV laser flash excitation. The lifetime, relaxation rates and populations of the triplet sublevels are quantitatively analyzed at cryogenic temperatures in a glassy toluene matrix. The photo-physical properties are studied depending on the size of the delocalized π-electron system by varying the number of repeat units in the Oligo-PPEs. In addition, their microscopic material properties are studied in particles with confined size constituted by block coplymers of PEGylated Oligo-PPEs. DLS of these particles suggests that the rod-like Oligo-PPEs have to bend to fit into the particles. This indicates that the usually rigid Oligo-PPEs are not stiff anymore under certain circumstances. The microscopic molecular geometry is investigated by EPR distance measurements in solution, in bulk material as well as in particles to elucidate this contradiction.

Complex Coacervation of Polyelectrolytes Studied by Spin-Label EPR Spectroscopy

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EPR spectroscopy is a powerful technique for the study of structure and dynamics of paramagnetic species. The so-called spin label technique employs stable radicals which are covalently attached to diamagnetic macromolecules. Rotational dynamics of such spin labels on time scales between 10 ps and 1 µs can be characterized by basic and fast CW EPR spectroscopy. The dynamics of the spin labels is influenced by the restricted motion of the sidegroup bearing the label and local polymer backbone motions at the point of the covalent attachment of the label. Polyelectrolytes are a fascinating class of macromolecules that exhibit various interesting phenomena due to the presence of dissociated ionic groups along the macromolecular chain. Recently, we demonstrated that the spin-label technique can be used to study the formation and stability of polyelectrolyte multilayers [1]. Here we present the application of labeled macromolecules to study the complex coacervation of polyelectrolytes. A nitroxide spin label has been covalently attached to P(E-alt-MA). The segmental rotational mobility of the spin-labeled polyanion and the internal rotation of the spin label were determined simulating the line shapes of experimental EPR spectra as a function of temperature. The complex formation of this weak polyanion with the strong polycation PDADMAC was studied as a function of mixing ratio, pH and ionic strength of the solution. If the spin-labeled polyanion is the excess component, the spectrum of a slow-motion component is superimposed by the spectrum of a fast-motion component. In the opposite case, the spectra are dominated by a slow-motion component.

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NMR Discrimination of Pure Atropoenantiomeric Quinazolinone Derivatives

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We studied a few quinazolinone derivatives present as racemates of atropisomers to test the capability of chiral reagents to discriminate isomers in solution. In fact, it is very well known in literature that chiral reagents can enhance shielding/deshielding effects and help to resolve diastereoisomers and/or enantiomers.[1-4] At first, both mono-dimensional and two-dimensional NMR sequencies were used for an in-depth characterization of our compound (1H NMR, 13C NMR, 2D NMR COSY and NOESY, and HSQC and HMBC experiments). Subsequently, chiral reagent (1R)-1-(anthracen-9-yl)-2,2,2-trifluoroethanol (R-Pirkle) or (2R)-2-(6-methoxynaphthalen-2-yl)propanoic acid (R-Naproxene) were added to the atropoenantiomeric solution. The introduction of chiral reagents was carried out to produce diastereoisomeric association complexes in solution, possibly presenting remarkable shift effects unobserved in the starting atropoenantiomeric mixture. Among the chiral reagents investigated, (1R)-1-(anthracen-9-yl)-2,2,2-trifluoroethanol (R-Pirkle) reagent produced interesting results giving rise to characteristic splittings due to the two atropodiastereoisomers in solution, while (2R)-2-(6-methoxynaphthalen-2-yl)propanoic acid (R-Naproxene) proved to be uneffective. The results obtained will be deeply discussed in the poster.

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Different solid forms of Sodium Ibuprofen: a combined Solid State NMR, X-Ray Crystallography and DSC study

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Sodium Ibuprofen is a well known non-steroidal anti-inflammatory drug used in the treatment of pain and fever. Despite its large use, a complete understanding of the behavior of the different solid forms and racemic species of this compound still needs investigations. Herein we present a study exploiting the combination of solid-state NMR, DSC methods and X-ray crystallography (single crystal and microcrystalline powder) on the racemic misture (R/SNaIb) and the enantiomerically pure (SNaIb) Sodium Ibuprofen. Both these samples give a stable di-hydrate crystalline phase at room temperature and atmospheric humidity. First the behavior of the di-hydrate phases has been investigated, with particular interest in their transitions depending on external conditions: temperature and different atmospheres (air or N_2). Afterwards, the analysis focused on the obtained anhydrous forms, with peculiar attention to the chiral properties of the phases. Solid-state NMR ^{13}C CP-MAS spectra of all the investigated forms have been recorded. These measurements allowed a detailed comparison among all the forms to be performed, higlighting differences and similarities between the same molecular moieties in the different solid forms. The combination of the data obtained from the different techniques allowed interesting structural and phase behavior to be identified and discussed.

Endometabolomics of B cell differentiation

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A deep geno-proteomic metamorphosis is activated to promote differentiation of B lymphocytes to plasma cells (high specialized antibody-secreting cells)[1]. We performed a complete metabolic study on B cell differentiation through induction of primary B cells by LPS treatment and analysis by NMR and GS-MS of both endo- and exo-metabolome for 4 days post LPS. We thus analyzed the metabolic changes occurring during cell activation (day 0), proliferation/differentiation (day 1-2), IgM synthesis/secretion (day 3), apoptosis (day 4). Exometabolome data have been already published [2] and herein the endometabolomic results are shown. Multivariate statistical analyses, metabolites assignment and quantification by NMR and GS-MS revealed changes in key metabolomic pathways (glycolysis, RNA-DNA turnover, etc) which clearly distinguish the four phases, highlighting hallmarks of activation/proliferation (lactate, phosphocholine, etc), differentiation and Ig synthesis/secretion (Glu, Cys, GSH, etc). The endometabolome analysis confirmed the exometabolomic results and allowed a complete overview of the metabolomic perturbations. The combined use of NMR and GS-MS allowed the identification of a wide range of metabolite classes and thus of perturbed metabolomic pathways upon differentiation. For small hydrophilic molecules, where both the techniques are responsive, we found a good agreement between the two analytical platforms.

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^{13}C NMR of a Single Molecule Magnet: Analysis of Pseudocontact Shifts and Residual Dipolar Couplings

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Paramagnetic triple decker complexes of lanthanides are promising Single Molecule Magnets (SMMs), with many potential uses.[1] Some of them show preferable relaxation behavior, which enables the recording of well resolved NMR spectra. These axially symmetric complexes are also strongly magnetically anisotropic, and this property can be described with the axial component of the magnetic susceptibility tensor, χ_a. For triple decker complexes with phthalocyanine based ligands, the Fermi contact contribution is small. Hence, together with the axial symmetry, the experimental chemical shifts in ^{1}H and ^{13}C NMR spectra can be modeled easily by considering pseudocontact and orbital shifts alone. This results in the determination of the χ_a value, which is also responsible for molecular alignment and consequently for the observation of residual dipolar couplings (RDCs). A detailed analysis of the experimental ^{1}H-^{13}C and ^{1}H-^{1}H couplings revealed that contributions from RDCs (positive and negative) and from dynamic frequency shifts (negative for all observed couplings) have to be considered. Whilst the pseudocontact shifts depend on the average positions of ^{1}H and ^{13}C nuclei relative to the lanthanide ions, the RDCs are related to the mobility of nuclei they correspond to. This phenomenon allows for the measurement of the internal mobility of the various groups in the SMMs.

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NMR Kinetic and Mechanistic Study of Sulfoxide-Mediated α-Arylation of Carbonyl Compounds

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An NMR kinetic and mechanistic study was undertaken on a sulfoxide-mediated α-arylation of a β-keto ester in order to determine its reaction pathway and to highlight important reaction intermediates. Using high-sensivity measurements, the time evolution of the ^{13}C signal intensities of all starting materials, products and intermediates was evaluated and could be accurately fit to a model reaction involving six different reaction rates. A full characterization of a short-lived intermediate was also achieved at low temperature (253K) and revealed an unexpected polycyclic compound, indicating a bond reorganization from an late-stage, unstable dearomatized intermediate. This NMR investigation, along with DFT calculations, were instrumental in revealing a common reaction pathway between this α-arylation and a seemingly unrelated sulfur(IV)-mediated ylide transfer.[1]

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Investigation of the conformational space of a Diarylethene derivative using residual dipolar couplings

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Diarylethenes (DAEs) are a prominent member of photochromic molecules which can exist in a ring-open and a ring-closed form. The switching between both isomers is induced by UV and VIS light, respectively.[1] It is proposed that the efficiency of the switching depends on the population of the parallel (p) and anti-parallel (a-p) conformation of the ring-open form. Only the a-p conformation leads to the ring-closed form during the photocyclization process. We have chosen one modified DAE derivative, synthesized by the Hecht group, oriented the sample in a weak alignment medium (our new cross-linked PBLG gel)[2] and started investigating the conformational space by residual dipolar couplings (RDCs). In addition to (CLIP)-HSQC[3] for one bond carbon-proton couplings, the heteronuclear long-range HETLOC[4] and the 1,1-ADEQUATE[5] experiment for coupled carbon pairs are used to obtain a total of 8 RDCs.

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STD-NMR experiments on tumor cells to investigate RGD ligand-membrane protein interactions

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NMR experiments (transferred NOE and Saturation Transfer Difference) were used to shed light on the binding epitope of a small library of DKP - RGD peptidomimetics [1] with integrin $alpha_V beta_3$, expressed on the membrane of ECV304 bladder cancer cells. The NMR results were supported by docking calculations of these ligands in the active site of $alpha_V beta_3$ integrin receptor and were compared to the results of competitive $alpha_V beta_3$ receptor binding assays and competitive ECV304 cell adhesion experiments.[2] The different stereochemistry and the different substitution at the nitrogen atoms of the scaffold strongly influences the conformations adopted in the free-state and the binding mode of these ligands in the active site of the integrin receptor.

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Pure Shift HSQC Measurements with perfectBIRD Decoupling - a Method to Decouple Diastereotopic Protons

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The discrimination of compounds which exhibit similar structural features, such as configurational isomers, can easily be prevented by signal overlap. In such cases signal dispersion in indirect spectral dimensions is likely to be inefficient in achieving signal separation as the differences in chemical shifts observed can be quite small, possibly smaller than either the resolution of the indirect dimension or the multiplet width imposed by J-coupling. To diminish these problems pure shift techniques hold great potential, as they can provide both high resolution and J-coupling suppression in the direct dimension. HSQC experiments with homonuclear decoupling in the direct dimension have recently been presented[1]. The BIRD decoupling element[2] employed by these techniques leads to a significant simplification of the spectra obtained, making them well suited for the study of complex problems. However it fails to suppress geminal scalar couplings, resulting in “irreducible” doublet signals for diastereotopic protons. As shown here, full homonuclear decoupling is possible even in the case of diastereotopic protons, if BIRD decoupling is combined with a perfect echo element[3]. The incorporation of the resulting perfectBIRD decoupling element into HSQC experiments suited for precise one-bond RDC-measurements is presented.

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Mechanistic Investigation of the 1,4-Addition Reaction of Organozinc Reagents Catalysed by Chiral Phosphoramidite-Copper Complexes

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Due to high enantioselectivity, nearly quantitative yields and relatively low cost, the 1,4-addition reaction - catalysed by a chiral phosphoramidite-copper complex - is a widely used method for the formation of new C-C-bonds.[1-4] Recently our group was able to elucidate the temperature-dependent complex structures in solution. Using a catalytic system consisting of chiral phosphoramidite ligands and copper(I) salts, we were able to identify complex C2 as the precatalytic complex of this reaction. This new structural motif shows a trigonal/tetrahedral coordination on copper, and thus offers a free coordination side for the transmetalation reagent.[5-7] After this elucidation we focused on the transmetalation step, which is postulated as first step in the proposed mechanism after the addition of an organozinc reagent to the precatalytic system.[7] In our studies we are using variable temperature NMR spectroscopy (170-230 K) and a combination of 1D and 2D spectra. After a screening of different organometallic reagents (MeLi, ZnR2; R=Et, Me, Ph) we are able to present the first direct experimental proof of such a transmetalated species.

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Conformational analysis of small organic molecules using NOE and RDC data: A Discussion

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For the determination of three-dimensional structures of rigid molecules in solution by NMR spectroscopy, one usually uses dihedral angles determined from scalar coupling constants via the Karplus equation, distances from the NOE, and recently the angular information from RDC data. The structure of flexible molecules is much more difficult to determine. In this case, only the average of the NMR parameters is accessible. This complicates the structure determination. If one succeeds though, important information about the structure and dynamics of the molecule under investigation can be obtained in this way. Due to the different information content of NOE and RDC and the different behavior in conformational averaging, both interactions have advantages and disadvantages. Depending on the molecule, a consideration of only one of the two parameters therefore may be sufficient. It was recently proposed that the observation of only one distance determined by NOE in strychnine leads to a distance value which can only be explained by conformational flexibility of strychnine [1]. One of the first examples that show that RDC data can yield information on the flexibility of a molecule is α-methylene-γ-butyrolactone [2]. In both cases, however, only either NOE or RDC data were considered. Therefore, the combined examination of NOE and RDC data shall be shown on selected example molecules, including a discussion of the strengths and weaknesses of both methods.

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The stability of N-Heterotetracenes towards light and oxygen as investigated by NMR spectroscopy

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N-Heterotetracenes are promising compounds for various applications. Especially their stability towards light and/or oxygen in solution is of special interest. There are two essential pathways possible for a decomposition of those compounds: Oxidation or dimerization. For some applications, oxidation must be avoided. Both pathways lead to a variety of decomposition products and may even occur simultaneously. This leads to a lot of new signals in proton NMR spectra. To be certain about the reason for decomposition, the new signals have to be assigned to the possible products of these pathways. This poses a great challenge due to low solubility and little or no visible couplings in NMR spectra. The exclusion of the appearance of oxidation processes therefore can be complicated. We studied the stability and the pathway of decomposition of two N-Heterotetracenes in solution. Using a comparison of experimental and calculated carbon chemical shifts, we were able to determine whether dimerization or oxidation or both takes place. With the help of 2D NMR experiments, we succeeded to assign the important proton signals of the decomposition products. The poster will show the results of this study.

An NMR Study on Metal-Coordinated White Phosphorus

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Variable temperature NMR and $^{31}P\{^{1}H\}$ EXSY experiments in solution and in the solid state were carried out for nine mono-hapto tetraphosphorus transition metal complexes based on ruthenium, osmium, iron, rhenium or manganese [1]. For most of the species, it was ascertained that the metal-coordinated P_4 molecule experiences a dynamic process consisting in a tumbling movement of the P_4 cage while remaining chemically coordinated to the central metal. For two ruthenium complexes, MAS ^{31}P NMR experiments revealed that the dynamic processes observed in solution (*i.e.* rotation and tumbling) take place also in the solid state [2]. Eyring plots of VT NMR data gave the activation parameters for the dynamic processes in solution [1-3].

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The Spin Density Distribution in a Symmetrically Substituted Bissilylene Radical Cation

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The heavier homologues of alkenes have attracted much research interest in the last years. The chemical behaviour of such compounds is completely different from the that of their carbon counterparts. Consequently, this is also true of their electronic structure which can be investigated to rationalize the behaviour of these moieties. The one electron oxidation of heavier alkene homologues leads to the interesting observation that even symmetrically substituted radical cations of this class of compounds assume non-symmetrical structures in single crystals. The reasons for this result have so far not been investigated and furthermore, it remains unclear if the non-symmetric structure is retained in solution as well. In this work, CW and pulsed EPR spectroscopy has been used to address these questions for the case of an NHC substituted bissilylene radical cation.

Lyotropic Liquid Crystalline Phases from Supramolecular Polymers as Orienting Media for NMR Spectroscopy

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The determination of conformations by NMR usually involves distances from the NOE and dihedral angles from ^{3}J couplings. It is, however, often complicated by either absence of NOE data and/or ^{3}J coupling data. The recently reintroduced residual dipolar couplings (RDCs) provide complementary information to these conventional NMR restraints. RDCs belong to the class of anisotropic NMR parameters and the compound in question needs to be oriented with respect to the magnetic field in order to be able to observe them.[1] For organic solvents there are two known concepts strained induced alignments in a gel (SAG) or lyotrop liquid crystalline phases (LLCPs) of rigid polymers like homopolypeptides, polyguanidines or polyacetylenes.[2] While the swelling of SAGs is time consuming the preparation of the polymers for the LLCPs is quite complex. Here we want to present a new orienting medium based on supramolecular polymers.[3] Supramolecular polymers are formed through the self-assembling of the monomers via non-covalent interactions. These polymers have the advantage of a simple and fast preparation. We use benzene-1,3,5-tricarboxyamides (BTAs) with long alkyl chain.[4] BTAs form in organic apolar solvents LLCPs. This phases show some beneficial properties like weak alignment and nearly no polymer residue signal in the spectra.

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NMR-Spectroscopic Characterization of Unsymmetrical Cube-Octameric Silsesquioxanes (COSS)

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Polyhedral oligosilsesquioxanes (POSS) have long been studied as models for silica surfaces and other silica-bound catalytic systems. Due to the synthetic availability and flexibility in the choice of the flanking residues, POSS have found use in a wide range of applications, e.g. nano composite materials or homogenous catalysts.[1] Furthermore, their utility as scaffolds for biological applications has been recently demonstrated.[2,3]Here, novel fluorescent tags are presented, based on cube-octameric silsesquioxanes (COSS) with the general formula $(SiO_{1.5})_8R'_nR_{8-n}$. Due to the unsymmetric substitution pattern, the silicon sites of the core silica polyhedron are chemically inequivalent and the structure of the resulting nanoparticles is readily studied by NMR spectroscopy. The R' side chain carries a fluorescent marker, while the other corner substituents are varied to modulate stability, solubility and chemical behavior. Using ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{29}Si HMBC measurements a full assignment of all resonances, including the non-isochronous Si sites, was conducted, which confirmed the integrity of the cage-like core structure. Moreover, the effects of flanking residues as well as possible hydrolysis and decomposition pathways could be further investigated.[4]

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Metabolic changes during cellular senescence investigated by NMR spectroscopy

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Cellular senescence is an important tumour suppression mechanism and contributes to organismic ageing. Senescent cells are characterized by a stable arrest of proliferation. The sencescent state can be reached by different stresses, e. g. overexpression of oncogenes or loss of telomeres due to replication. Senescent cells are still metabolically active and influence neighbouring cells and tissues. To identify metabolites that are representative for cellular senescence, methanol/chloroform extracts of a human embryonic lung fibroblast cell line (WI-38) have been analysed with NMR spectroscopy. Replicative senescence, DNA damage-induced senescence (etoposide treatment) and oncogene-induced senescence (hyperactive RAF kinase) as triggers for senescence have been investigated. Senescent and control cells can be discriminated according to their metabolite pattern. The ratio of glycerophosphocholine to phosphocholine is increased during senescence and independent of the trigger [1]. The alterations are opposite to the well-known changes in cells undergoing malignant transformation. Since increased glycerophosphocholine levels implicate a key role of phospholipid metabolism in cellular senescence we analysed the lipid phases with 1D and 2D proton NMR spectroscopy. The observed changes in the choline metabolism emphasize the role of senescence in tumour suppression.

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A multinuclear NMR characterization of Pt(II)-Pt(IV) intermediate formed during I_2 oxidation of dinuclear Pt(II)-Pt(II)

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The oxidation of asymmetric dinuclear complexes $[NBu_4]$-$[(R_F)_2Pt(\mu\text{-}PPh_2)_2Pt(N\text{^}L)]$ (N^L: benzoquinolinate **1**, hydroxyquinolinate **2** and picolinate **3** with iodine in dichloromethane was explored. The reaction afforded new dinuclear species of formula $[(R_F)_2Pt(\mu\text{-}I)(\mu\text{-}PPh_2)Pt(P,N\text{-}PPh_2)]$ (**4-6**) where the formation of a new aminophosphane ligand for complex **4** and aminophosphinito ligands for complexes **5-6** occurred. Carrying out the oxidation of **1** in acetone the reaction does not evolve to give the species **4** but affords the product of oxidative addition Pt(II)-Pt(IV) **1***.[1,2] In this contribution the behavior in solution of the Pt(II)-Pt(IV) intermediate **1*** will be described. Complex **1*** was fully characterized in solution by multinuclear NMR spectroscopy. The ^{195}Pt NMR spectrum gives information about the oxidation state of platinum. The ^{1}H ^{31}P HMQC experiment allowed to assign the signals of ortho-protons of phenyl rings and by combining information revealed from ^{1}H NOESY EXSY spectrum an interesting dynamic behavior was ascertained which permitted us to explain the pathway of conversion of intermediate **1*** to give complex **4**.

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NMR investigations on OR2, an inhibitor of Aβ aggregation

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Alzheimer's disease (AD) is the main cause of dementia in the elderly. AD is characterized by the presence of amyloid plaques in the brain tissue, progressive dementia and cortical atrophy. Amyloid plaques are mainly composed by insoluble aggregates of the β-Amyloid peptide (Aβ). The morphology and kinetics of formation of Aß aggregates is affected by many factors, including pH, temperature, ionic strength and concentration of metals [1]. It's well known that the Aß central region, (residues 16-20, KLVFF) plays a key role in Aβ aggregation [2]. In addition modified synthetic peptides based on residues 16-20 of Aß are able to prevent Aß conversion to ß-sheet-rich aggregated structures [3, 4]. In particular it has been shown that the peptide RGKLVFF-NH2 (hereafter called OR2) is able to inhibit both Aß oliogomerization and aggregation [4]. In this study, the behavior of OR2 in solution has been investigated by mainly using NMR and CD spectroscopy with the aim to elucidate the molecular events initiating Aß oligomerization. The influence of membrane mimicking environments has been also evaluated in order to determine any preferred peptide conformation.

Paramagnetism in free-base metallocenylporphyrins

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We have been recently involved in the characterization of both metal free and metallated 5, 10, 15, 20-tetraferrocenylporphyrin (H2TFcP and MTFcP) which showed interesting mixed valence states and reversible electrochemical behavior both in solution and on surface[1-4]. The easy accessibility to the monocationic porphyrin (H2TFcP+) is of particular interest because of the charge transfer among ferrocenyl units which gives rise to an intense inter-valence charge transfer band in the NIR region of the spectrum. This peculiar absorption can be used in the construction of redox-driven optical sensors and switches in the NIR. $(H2TFcP^{+})(PF6^{-})$ was synthesized in good yield and fully characterized through variable temperature ^{1}H NMR experiments as well as diffusion ordered spectroscopy and two-dimensional experiments. Although the presence of a FeIII center is expected to give rise to a strong paramagnetic effect, spectra are slightly perturbed only at very low temperatures suggesting a strong delocalization of the charge all over the molecule.

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Intercomparison of quantitative ^{1}H NMR

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NMR spectroscopy is widely recognized as one of the analytical techniques suitable for reliable quantitative determinations.[1] Despite the advantages offered by NMR, its use for quantification measurements is significantly less widespread than that of other techniques and is limited to academia and specific industrial areas. Recently, the network of the laboratories of the Italian Chambers of Commerce became interested to performance of NMR spectroscopy in purity determination of selected substances. Thus, a national intercomparison was organized involving 33 laboratories from universities, research institutes and companies all following a well defined protocol. Samples contained four common pesticides (Aldicarb, Methamidophos, Pirimicarb, Oxadixyl) dissolved in deuterium oxide and were prepared according to ISO Guide 34 by Lab Instruments S.r.l., a company operating under ISO 9001:2000 and ISO 14001 certifications and ISO 17025 accreditation. In this presentation, design of the NMR experiments, uncertainty measurement evaluation and general statistical data will be presented and discussed. It will be demonstrated that NMR spectroscopy is suitable for validated quantification methods.

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NMR analysis of human biofluids reveals metabolic changes related to the development of acute kidney injury following cardiac surgery

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Patients undergoing cardiac surgery are at risk of developing acute kidney injury (AKI), being associated with high morbidity and mortality. For identifying novel biomarkers capable of predicting the development of AKI after cardiac surgery with cardiopulmonary bypass use, urine[1] and plasma specimens were collected at various time points from 106 patients undergoing cardiac surgery at the University Clinic of Erlangen-Nuremberg and analyzed by means of NMR based metabolomic fingerprinting. 1D NMR urine spectra were statistically analyzed by Support Vector Machine based classification in combination with Quantile Normalization and t-test based feature selection techniques and allowed the reliable prediction of patient outcome with regard to the development of postoperative AKI. Especially, for severe cases of AKI more than 80% prediction accuracy was obtained. Predictions were based on a small subset of biomarkers, including both endo- and exogenous compounds. This study underscores the capability of NMR in combination with bioinformatics for the identification of novel biomarkers in association with disease status and for gaining new insights into pathomechanisms.

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The role of DJ-1 in apoptosis: an NMR study of its interactions toward the design of inhibitors of the anti-apoptotic protein Bcl-X_L

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DJ-1, the product of the PARK7 gene, is a protein associated with Parkinson disease and cancer. It is a multifunctional protein. It was identified as an oncoprotein involved in tumorigenesis because high expression of DJ-1 is a causative risk factor for prostate cancer, ovarian carcinoma, breast cancer, and lung carcinoma. However, the mechanisms underlying such functions still remain barely known. There is some evidence that DJ-1 is a potential new interaction partner of Bcl-X_L, one of several anti-apoptotic proteins in the Bcl-2 family that is implicated in the survival of tumor cells. Proteins in this family are central regulators of programmed cell death, and members that inhibit apoptosis, such as Bcl-X_L and Bcl-2, are overexpressed in many cancers and contribute to tumor initiation, progression, and resistance to therapy. To identify the minimal peptide sequence of DJ-1, able to bind the BH3 cleft of Bcl-X_L and to inhibit its anti-apoptotic function, we cleaved DJ-1 enzymatically and subsequently tested the affinity of the digestion products through a fluorescence polarization assay. To validate the binding of the active peptides resulting from the digestion of DJ-1, the technique of choice is NMR. NMR techniques were also used to perform a screening of a library of fragments with the aim to identify an inhibitor of Bcl-X_L.

NMR studies of cerato-populin: investigating the differences in fungal PAMP induced resistance

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Plant pathogenic fungi secrete several non-catalytic proteins involved in various aspects of the pathogenesis process. Among these, cerato-platanin (CP) was first identified and characterized as a PAMP (pathogen-associated molecular pattern) [1,2]. A sequence homology search revealed a set of fungal Cys-rich secreted proteins that have been grouped in the CP family. They induce synthesis of phytoalexins, overexpression of defence-related genes, H_2O_2 and NO production, markers of defence activation [3]. The core member of this family, CP (from *C. platani*) shows a double psi beta-barrel fold [2]. A CP-orthologous, cerato-populin (Pop1), produced by *C. populicola* with 73% of similarity is under study. Though CP and Pop1 are both host defence inducers, Pop1 shows a slower and weaker defence induction than CP [4]. Our aim is the definition of Pop1 NMR-derived solution structure and the characterization of the backbone dynamics of both CP and Pop1. Preliminary studies showed that Pop1 is thermally stable and monomeric. ^{15}N, ^{13}C and ^{1}H resonances have been assigned and the dynamic features will be presented. By comparing the structure and dynamics of CP and Pop1 we expect to obtain useful hints to understand the molecular aspects of Pop1 defence induction activity.

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Structural investigation of the SF1/U2AF^{65}/U2AF^{35} complex utilizing paramagnetic LBT-gained PCSs

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Alternative splicing of pre-mRNA sequences is a key regulating step in eukaryotic gene expression. The splicing process is assisted by various splicing factors (SFs) and small nuclear RNAs which in complex form the spliceosome. The first step involves the formation of complex E, in which the U2 auxiliary factor (U2AF) and SF1 play an important role for the recognition of the 3' splice site branch point, the polypyrimidine (Py) tract of the pre-mRNA consensus sequence, and organization of the spliceosome assembly. Structural and functional investigations were performed for SF1/U2AF65 and the U2AF65/U2AF35 heterodimer both free and in complex with intron RNA. The aim of our project is now to investigate the structure of the entire complex SF1/U2AF65/U2AF35 including the branch point and Py tract RNA by NMR. A multi-domain conformational selection pathway was revealed for the binding event of the tandem RRM12 domains of U2AF65 to the Py tract RNA. The conformational equilibrium between the open and closed conformation functions as a molecular rheostat that correlates Py tract RNA length and functional strength to U2 snRNP recruitment during spliceosome assembly. However, the relative domain orientations of RRM12 in both states remain unclear. Therefore, we will incorporate chemical and encodable Lanthanide-binding tags (LBTs) into the tandem RRM1,2 domains to introduce paramagnetic lanthanides for measuring paramagnetic effects.

Progress in the Investigation of the Helical Human Membrane Protein Hv1 by liquid-state NMR

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We are very interested in the investigation of a helical human membrane protein, the human voltage gated proton channel (Hv1 [1] or VSDO [2]). Hv1 plays an important role in the human innate immune system. Its predicted structure differs considerably from other cation channels. It is build only of a domain that can sense voltage but lacks the pore domain, which is essential for other cation channels. We want to investigate this protein with liquid-state nuclear magnetic resonance (NMR) spectroscopy. It is a 273 residue protein, which dimerises, and exhibits a partially overlapped [15N, 1H]-TROSY spectrum. Since the transmembrane domain of Hv1 (TM-Hv1) is monomeric and still functional as a proton channel[3], this domain will be investigated first. TM-Hv1 was expressed in functional form in E. coli. Of the backbone resonances 84% are assigned. A large number of medium and long-range constraints have been collected for structure calculations. The goal is to understand the voltage-sensing and the proton permeation pathway of this unique channel.

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Role of the N-Terminal Tail of 1-C-Grx1, an Essential Iron-Sulfur Protein from the Pathogenic Protozoan *Trypanosoma Brucei*

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Glutaredoxins (Grxs) are small thiol proteins, belonging to the thioredoxin-fold superfamily. They can be distinguished into two major groups: dithiol glutaredoxins and monothiol glutaredoxins (1-C-Grxs). African trypanosomes, responsible for the African trypanosomiasis, encode three monothiol glutaredoxins: 1-C-Grx1, 1-C-Grx2 and 1-C-Grx3. Trypanosoma brucei 1-C-Grx1 lacks oxidoreductase activity, but experimental evidence suggests a possible role of 1-C-Grx1 in the biogenesis of iron sulfur clusters, playing an essential role in the iron metabolism of trypanosomes. The protein was initially considered to form non covalent homodimers and it was hypothesized that an N-terminal segment of 35 residues was responsible for the dimerization. The solution structure of a 1-C-Grx1 mutant lacking this sequence has been solved in our laboratory: this domain shows a very similar structure to other glutaredoxins and it has been proven, by 15N relaxation experiments, to be monomeric. Relaxation data were acquired for the non-truncated protein, to obtain information on backbone dynamics and to confirm the initial hypothesis on its dimeric nature. However, this analysis revealed that the protein rotational correlation time is consistent with the protein being monomeric, as confirmed also by MALS experiments. This finding opens the question, discussed here, of the role of the N-terminal tail that we proved to be largely unstructured.

NMR- and CD-based interaction studies suggest a general role for the FATC domain as membrane anchor of phosphatidyl-inositol-3 kinase-related kinases (PIKKs)

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Phosphatidylinositol-3 kinase-related kinases (PIKKs) regulate cellular processes such as DNA repair, RNA surveillance, and cell growth and metabolism. Various mutagenesis studies have shown that the C-terminal FATC domain is important for PIKK regulation. Based on the structural characterization of the redox- and lipid-binding properties of the FATC domain of the ser/thr kinase target of rapamycin (TOR), it contains a redox-sensitive membrane anchor (1,2). Since the FATC domains of all known PIKKs are rather hydrophobic and especially rich in aromatic residues, we analyzed if the ability to interact with lipids and membranes may be a general property. Here, we present NMR- and CD-data for the FATC domains of human SMG1, human ATM, human ATR, human TRRAP, and human DNA-PKcs that indicate that all can interact with different membrane-mimetics and only may have different preferences for membrane properties such as surface charge, curvature, and lipid packing (4). For ATM and DNA-PKcs we used a newly established procedure that relies on the direct use of GB1-tagged proteins (3). For the others we used commercially obtained peptides. Except for the oxidized form of the TOR FATC that forms an α-helix that is followed by a disulfide-bonded loop (1), the FATC domains of the other PIKKs are mostly unstructured in the isolated form and only significantly populate α-helical secondary structure upon interacting with membrane-mimetics (4).

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Cu(I) binding to the N-terminal regions of α- and β-Synuclein

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The synucleins are a family of rapidly growing proteins, that are involved in numerous neurodegenerative pathologies, as well as in various types of cancers.[1-3] While αS is widely studied and their connection to Parkinson's disease is well established, actually was discovered that wild-type βS have an inhibitory effect on αS aggregation,[4] a property that can be exploited in the development of novel therapeutics against various neurological pathologies. Metal ions probably represent the link between the pathological mechanism of protein aggregation and oxidative damage. While αS-Copper(I)/(II) interactions is already known [5,6], βS behavior in presence of these ions is much less investigated. Only the Cu(II) binding difference between αS and βS was recently studied, showing the occurrence of very similar binding domains.[7] The aim of this work is to characterize the conformation propensities of the N-terminal region (1-15) of βS. βS 1-15 behaviors in presence and in absence of metal ions (Cu(I),Ag(I),Hg(II)) and SDS was analyzed and compared to αS 1-15 through NMR investigations.

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Structural analysis of protein-protein interactions in glycosomal biogenesis in Trypanosoma brucei

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The protozoa Trypanosoma brucei (Tb) infects human and causes every year thousands cases of fatal sleeping disease in Africa. Trypanosomatids couple glycolytic and peroxisomal function in the glycosome. They are vesicle-like organelles that enclose enzymes involved in lipid and reactive oxygen species metabolism as well as glycolytic enzymes. The factors that govern the biogenesis and function of glycosomes/peroxisomes are named peroxins (Pex proteins). In human, malfunction of peroxins is associated with numerous severe disorders. Pex5 and Pex14 are two of the conserved peroxins essential for peroxisomal and glycosomal biogenesis. Pex5 recognizes cargo molecules in the cytoplasm and subsequently targets them to the organelle by forming a docking complex with Pex14 at the membrane. Although the Pex5/Pex14 interaction is conserved and functionally important, sequence variations suggest distinct structural features of the Pex5/Pex14 protein-protein interface. The three-dimensional structure of TbPex14 was determined in solution and the binding to human and T. brucei derived Pex5 peptides was characterized using NMR-spectroscopy and fluorescent polarization assays.

Applying a segmental labeling approach to multi-domain Heat Shock Protein 90 to study the interactions with a client protein

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Studying large proteins (>30 kDa) by NMR is challenging. NMR spectra of large proteins suffer from broader line widths due to reduced tumbling times and increased spectral overlap. In order to reduce spectral overlap in we have employed segmental isotope labeling, whereby one domain is isotopically enriched with NMR active nuclei while one or more remaining domains contain natural abundance nuclei. We have optimized protocols using expressed protein ligation with Sortase A. The essential chaperone protein Heat Shock Protein 90 (Hsp90) is a three domain 90 kDa protein. Hsp90 is active as a homodimer in solution. Hsp90 has been extensively studied and structures of some conformational states are known. However, Hsp90 undergoes large conformational changes during its catalytic cycle where molecular details of these motions are poorly understood. Here, we employed segmental labelling on Hsp90 to study by NMR. We analyse NMR data comparing isolated and multi-domain constructs and study the interaction with client proteins of Hsp90 using segmentally isotope-labeled samples. We have observed differences in multi-domain Hsp90 compared to its individual domains in binding of nucleotides and client proteins. Additionally, in our studies we have included a charged linker region which is deleted in previous crystallographic studies. Interestingly we have observed that the linker interacts with specific regions on each domain which is dependent on whether the domains are isolated or linked together.

Interactions of cytoplasmic retinol-binding proteins with phospholipid vesicles: insights into the physiological function

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Vitamin A plays a key role in vision, cell growth and differentiation. In the cell, retinol has several fates: (a) it can be stored as retinyl ester of fatty acids through the action of lecithin-retinol acyl transferase (LRAT) [1]; (b) most non-esterified retinol is bound to cellular carriers (CRBP); (c) it can enter the oxidative pathway for the synthesis of retinoic acid, through the action of retinol dehydrogenases (RDH) [2]. CRBP-I is ubiquitous, whereas the homologous CRBP-II is expressed solely in the enterocytes. Our current understanding of these processes remains largely incomplete, but there is evidence that the membrane-bound LRAT and RDH are inactive towards the protein/ligand complex, suggesting that the membrane microenvironment may trigger retinol transfer from the holo protein to the enzymes. To address this hypothesis we have performed a suite of NMR experiments with CRBP-I and CRBP-II in the presence of model membranes composed of either anionic or zwitterionic phospholipids, at varying protein:lipid molar ratios and ionic strength. The results will be discussed, in comparison with our previous data collected in buffer [3, 4]. All these studies may help to understand certain aspects of the physiological functions of CRBPs.

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How is the ATPase activity of the minichromosome maintenance complex regulated by a small winged helix domain?

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The minichromosome maintenance (MCM) complex plays a central role in the replication of DNA in Eukarya and Archea. It serves as helicase and forms a hexameric ring-shaped complex on the DNA to be replicated. The high sequence homology and the relative simplicity of the archeal MCM compared to the eukaryal MCM renders it a valuable model to study the structure-function relationship of this essential component of the replication machinery. Even though considerable structural information for the archeal MCM was available, such information was missing for their C-terminal domain. We determined the solution structure of the MCM C-terminal domain of S. solfataricus and of M. thermautotrophicus. Both domains adopt a winged-helix (WH) fold with a less ordered N-terminal extension. However, their C-terminal Wing 1 element is shortened and the Wing 2 element appears missing. This sets the archeal WH apart from its MCM6 eukaryotic homologue. In contrast to most other WH domains, the MCM C-terminal WH domain does not bind DNA. However, published and our own biochemical data indicate a regulatory function of the MCM WH towards the MCM ATPase activity, located in the central AAA+ core domain of MCM. The structural basis of this regulatory function is not known. An intramolecular protein-protein interaction between the WH and the AAA+ domains seems likely. We are currently addressing by biochemical and NMR means how the presence of the WH domain controls the ATPase activity of the MCM core domain. The recent results of this structure-function analysis will be presented.

Oxidative protein folding in mitochondria by NMR

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Disulfide bond formation is an essential step in oxidative folding and necessary for proteins to achieve their native conformation. Specifically an oxidative pathway in the mitochondrial intermembrane space is operative which involves the transfer of a disulfide from a specific protein to the substrate. Proteins targeted to the intermembrane space are trapped by a disulfide exchange mechanism that involves an electron cascade from the substrate to MIA40, then on to ALR, and finally to O_2 via cytochrome c. Using NMR, we found that the substrate protein is largely unfolded in the cytoplasm and that MIA40 interact with a specific region of the substrate protein and induces a conformational transition, from an unstructured to an α-helical state, upon the formation of an intermolecular disulphide bond. MIA40 protein is then released in the reduced states and it has to be regenerated to a functional state through transfer of electron to ALR. The mechanistic basis of MIA40-ALR interaction at atomic resolution has been characterized. ALR contains a folded FAD-binding domain at the C-terminus and an unstructured N-terminal domain that is able to perform a dual function in two cellular compartments. A specific region of the N–terminal domain guides the interaction with MIA40 hydrophobic cleft. The hydrophobicity-driven binding of this region ensures precise protein–protein recognition needed for an efficient electron transfer process.

The LOV domain from *Brucella* LOV-HK: The role of the C-terminal helical flanking region in the light-to-signal propagation

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LOV domains are blue-light-activated signaling modules present in a wide range of sensory proteins. Light modulates the virulence of the bacteria *Brucella abortus* through a LOV-histidine kinase (LOV-HK). The *Brucella* LOV domain adopts the α/β PAS domain fold and dimerizes through the hydrophobic β-scaffold, which appears as a key element in the light activation [1]. According to secondary structure predictions, *Brucella* LOV-HK harbors a C-terminal helix (Jα) contiguous to the LOV core that is estimated to be 34 residues long with no sequence similarity to other known LOV proteins. To explore the functional importance of the Jα helix and to gain insight into the *Brucella* LOV-HK light activation mechanism, we performed structural studies with a stable construct consisting of the LOV core and the 37 C-terminal residues (LOVJα). We found that LOVJα, as the LOV core, is dimeric in solution and slowly returns to the dark state after being illuminated. The Jα helix is shorter than predicted, followed by an unstructured region to the C-terminus. The Jα helix is flexible and exposed to solvent. Its conformation does not change upon illumination. These results suggest that the Jα helix in the LOVJα construct is not participating in the signal transduction, though the situation may be different in the full-length protein. The implications of these results in the context of others LOVJα proteins are discussed.

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Structural investigation and immunogenic epitope prediction of *B. pseudomallei* bp-1050 protein

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BPSL1050 is a 14.2 KDa protein produced by *Burkholderia pseudomallei* responsible for melioidosis [1]. Herein we solved the solution structure of bp-1050, consisting of three helices packing on a four-stranded beta sheet composed of three parallel, one anti parallel strands and two flexible loops. Structures extracted from the NMR bundle were used as starting poses to perform three independent molecular dynamics simulations coupled to Matrix of Local Coupling Energy method to predict immunogenic epitopes. Polyclonal antibodies against full length bp-1050 and synthetic immunogenic epitopes have been generated. Epitope mapping methods identified the protein portion involved in immunogenic recognition. Agglutination assays open new perspectives in the application of the derived polyclonal antibodies as potential diagnostic tools for melioidosis.

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Structural characterization of lantibiotic immunity proteins

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Lantibiotics are peptide-derived antibiotics that inhibit the growth of Gram-positive bacteria mainly via interactions with lipid II and lipid II-dependent pore formation in the bacterial membrane. Therefore the Gram-positive producer strains need to protect themselves against their own lantibiotics. Little is known about this immunity mechanism on the molecular level. The expression of the immunitiy proteins SpaI (*B. subtilis*) or NisI (*L. lactis*) alone is sufficient to confer immunity against subtilin or nisin. Interestingly there is no cross-immunity between SpaI and NisI, despite the high sequence and structural similarity of the lantibiotics. In order to elucidate this highly specific immunity mechanism we solved the structure of a 15 kDa biologically active fragment of SpaI by NMR which is the first structure of any LanI protein. NMR investigations of a full length construct of SpaI lacking the diacylglycerol anchor suggest that the 30 N-terminal amino acids are unfolded in the absence of a membrane. However, this N-terminal stretch interacts with liposomes in NMR titration experiments.[1] We are on the way to solve the NMR-structure of NisI. Our results are the first step on the way to understand the immunity mechanism of subtilin and nisin producing strains on a structural level at atomic resolution.

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mtsslSuite: In Silico Spin Labelling, Trilateration and Distance-Constrained Rigid Body Docking in PyMOL

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Nanometer distance measurements based on Electron Paramagnetic Resonance (EPR) methods in combination with site directed spin labelling are powerful tools for the structural analysis of macromolecules. The software package mtsslSuite provides scientists with a set of tools for the translation of experimental distance distributions into structural information. The package is based on the previously published mtsslWizard (G. Hagelüken, R. Ward, J.H. Naimsith, O. Schiemann Appl. Magn. Reson. 2012, 42, 377-391) software for in silico spin labelling. It includes a new version of MtsslWizard that has improved performance and now includes additional spin labels. Moreover, it contains applications for the trilateration of paramagnetic centers in biomolecules and for rigid body docking of subdomains of macromolecular complexes. The mtsslSuite is tested on a number of challenging test cases and its strengths and weaknesses are evaluated.

Abstract redrawn

Molecular mechanisms of FOXO4 transcriptional activation

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The canonical Wnt pathway is a complex protein network which controls cell fate. The protein β-catenin is a key player in Wnt signal transduction. Under oxidative stress, β-catenin activates the class O forkhead box transcription factor 4 (FOXO4), a largely disordered protein, that steers the cell into quiescence[1], thereby counteracting the transcriptional output of Wnt signaling. FOXOs are considered bona fide tumor suppressors. Unsurprisingly, interference with FOXO activation has been linked to colorectal, hepatic and pancreatic cancer. Our research focuses on the molecular mechanisms of FOXO4 transcriptional activation by β-catenin and the regulation thereof by an intricate 'code' of posttranslational modifications (PTMs)[2]. We combine optimized labeling strategies[3], NMR and SAXS to gain detailed perspectives on these molecular mechanisms. We present the structural basis of FOXO4 activation by β-catenin and find that under normal cell homeostasis FOXO4 is auto-inhibited by its disordered C-terminal tail. Under oxidative stress, β-catenin disrupts this auto-inhibited state. Further results for the regulation of transcription activation via the PTM ‘code’ are presented.

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Structural characterization of TRPP1 protein

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The TRPP1 Protein is of particular interest, playing an important role in the formation of the polycystic kidney disease and in the mechanosensory function of the cilium. Despite this, the exact function is unclear. One reason is the unclear definition of various states of the protein. Here the function of one dimerization site is examined. It is known that TRPP1 forms oligomers under various conditions. Recently the necessary conditions for the dimer formation were determined. The NMR structural analysis of the interaction is presented.

Structural characterization of ion channel TRPP1 and its interaction with mDia1

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Mutations in the gens coding for the calcium channel TRPP1 play an important role in the development of the Autosomal dominant polycystic kidney disease (ADPKD) [1]. In tubular kidney cells it is located in the cilia and is involved in the mechanosensory system of the cells. Calcium binding to the C-terminal domain influences the conformational state of the channel and has a regulatory function. [2] The C-terminal domain of TRPP1 forms different oligomers under various conditions. Recently the necessary conditions for the dimer formation were determined. The NMR structural analysis of the dimerization is presented. The dimerization site of TRPP1 interacts with mDia1 and thus links the channel to the cytoskeleton. By chemical shift perturbation studies the interaction surfaces of the two proteins has been mapped and used to calculate a three-dimensional structure of the complex.

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Structural insights into the N-terminal domain of human frataxin provided by the stable intermediate FXN45-210

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Frataxin (FXN) is a mitochondrial Iron-binding protein critical for iron metabolism and antioxidant protection, whose deficiency is associated with Friedreich ataxia. The FXN mRNA is translated to a precursor that is processed to at least two isoforms, FXN42–210 and FXN81–210. Human cells normally contain both isoforms showing different biochemical properties and functional roles [1]. The structure of the C-terminal domain of FXN, solved both by NMR and X-ray crystallography, consists of an elongated domain in which two helices pack against an antiparallel β-sheet. Complications from proteolysis and degradation of the protein's N-terminus hindered the characterization beyond residues 88–210, though studies on FXN61-210 and FXN81-210 indicate that the N-terminus is unfolded and highly dynamic. However, recently FXN45-210 was reported to be stable and nearly complete resonance assignments of these residues were published [2]. With the aim of providing a structural basis for understanding the biological role of the human FXN N-terminus we carried out an NMR characterization of FXN45-210, complemented with molecular dynamics calculations. We discuss the results in the context of the biochemical effects that may contribute to the pathophysiology of Friedreich ataxia.

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Rearrangements in structure of prion protein caused by single point mutation in human genome

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The development of transmissible spongiphorm encephalopathies is associated with the conversion of the cellular prion protein (PrPC) into the misfolded, pathogenic isoform (PrPSc). In human genetic forms of these diseases, mutations in the globular C-terminal domain of PrP(PrPC) are hypothesized to favor spontaneous generation of PrPSc in specific brain regions, leading to neuronal cell degeneration and death. Our recent NMR studies were focused on structural characterization of different truncated recombinant human (Hu) PrPs carrying the pathological Q212P, V210I mutations and protective E219K polymorphism. While Q212P mutation is linked to GSS the V210I mutation is linked to genetic CJD. The naturally occurring E219K polymorphism in the HuPrP is considered to protect against sCJD. We have demonstrated that the determined structures of variants consist of unstructured N-terminal part (residues 90-124) and well-defined C-terminal domain (residues 125-228). Analysis and comparison with the structure of the WT Hu-PrP revealed that although structures share similar global fold, mutations introduces some local structural differences. The NMR structures offer new clues on the earliest events of the pathogenic conversion process and could be used for the development of antiprion drugs.

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Elucidating the structural evolution of a tissue-specific splicing factor

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The Drosophila LS2 protein has arisen from a gene duplication event of dU2AF50 (large subunit of splicing factor U2AF) and is preferentially expressed in the testes of Drosophila.LS2 promots alternative splicing by preventing dU2AF50 from binding to the poly-pyrimidine tracts. Despite sharing high sequence similarity (55% identity), these two proteins exhibit very different RNA binding specificities (recognition of guanosine vs. pyrimidine-rich RNAs respectively) as well as splicing activities (repression vs. activation in alternative splicing respectively). Presence of the enriched LS2 target transcripts in testes suggests its possible role in testes function, gamete production, and cellular regulation. More interestingly, LS2 also employs small subunit of U2AF (dU2AF38) for its function, similar to its progenitor dU2AF50. To understand molecular details of the RNA recognition and mechanisms of multi-domain co-operativity upon binding different RNA targets we are investigating the structure of LS2 free and bound to RNA and will compare this to U2AF. This study will provide insights on how these highly related proteins are evolved with unique properties, and possibly will reveal a path for the evolution of new splicing factors. We have optimized protein expression/purification by testing various LS2 constructs for the structural studies by NMR and currently assigning spectra for the RNA binding domains of LS2.

Design of novel Ras state 1(T) inhibitors

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The guanine nucleotide binding protein Ras is a key component in many signal transduction processes regulating differentiation, apoptosis and proliferation. The protein works as a molecular switch cycling between an inactive GDP bound state (D) and an active GTP bound state (T). The active state interacts with effectors. In approximately 30 % of all human tumors the Ras protein carries a point mutation either at position 12, 13 or 61.[1]^{31}P NMR spectroscopy revealed the existence of two distinct conformations of the active Ras protein.[2,3] State 2(T) represents the effector binding state, whereas state 1(T) has a lower affinity to effectors.[4] With metal-cyclen and metal-BPA complexes we could identify so-called state 1(T) inhibitors which selectively stabilize state 1(T) conformation. So far, the successful inhibition of Ras effector interaction could be shown in vitro.[5,6] In order to increase selectivity and affinity we modified the complexes synthetically and now we can present promising novel inhibitors characterized by NMR spectroscopy. We could show that stabilization of the weak effector binding state 1(T) by small molecules is a novel strategy to inhibit Ras-effector interactions and therefore represents a promising strategy for cancer treatment.

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Structural Characterization of a Sub-millisecond Protein Folding Event: Evidence for Intermediate Formation

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We present experimental data on the denaturant-induced refolding of TC5b, a small polypeptide exhibiting a well-defined globular structure and sub-millisecond folding kinetics; a fact, which has made it an attractive testing ground for biomolecular MD simulations. Using multidimensional NMR spectroscopy together with heteronuclear relaxation experiments performed on the 6 M urea-denatured state of both TC5b and a structurally optimized point mutant, we highlight the importance of side chain interactions for ultrafast and productive refolding. NOE cross-peaks between Trp and some aliphatic amino acid side chains exhibiting both native and, more surprisingly, also non-native character are detected. Also, an enhancement of the nucleation site's hydrophobicity leads to the detection of not only additional non-random interactions but also a concomitant acceleration of the refolding rate constant. To further investigate TC5b's folding behaviour, the data is complemented with het. relaxation rate constants, het. NOE measurements and a reduced spectral density mapping, determined at intermediate concentrations of urea. The results give strong evidence for the formation of a protein folding intermediate, populated to a significant extent during the progression of folding of both constructs. In summary, our observations further emphasize the importance of pre-existing hydrophobic interactions involving sequence-remote side chains as a crucial prerequisite for fast folding and increased thermodynamic stability of the native state. More importantly, the results of urea titration experiments demonstrate that even during a sub-millisecond folding event, intermediate formation can occur without significant deceleration of the overall folding process.

Scanning the conformational space of inactive Ras by means of high pressure NMR

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Conformational dynamics represent an essential prerequisite of adequate protein function. In a molecular switching cycle the guanine nucleotide binding protein Ras alternates between two major conformations, an active GTP-bound and an inactive GDP-bound state. As a consequence of this cycle the protein has to adopt additional conformations including protein-protein interacting states. In solution all of these states coexist concurrently at equilibria with characteristic Gibbs free energies and specific volumes. High pressure NMR spectroscopy is suitable to monitor different protein conformations at atomic resolution by shifting the equilibria towards low populated excited states [1]. In our previous ^{31}P and [^{1}H-^{15}N] HSQC high pressure NMR studies we already identified several intermediate states of active Ras bound to the GTP-analogue GppNHp [2]. Here we present ^{31}P and [^{1}H-^{15}N] HSQC high pressure NMR data on the inactive GDP-bound Ras. We provide evidence for the existence of several inactive Ras conformations expanding our knowledge about its conformational space. Since Ras is a promising target for the treatment of over 30 % of all cancer types [3], the stabilization of its conformational states by means of high pressure may enable their structural resolution and provide putatively drugable target structures.

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Protein-Protein and Protein-Ligand Interactions through NMR

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Binding events of proteins with biological molecules are the key for understanding biological processes. Targeting protein–protein and protein–ligand interactions is of fundamental importance in structure-based drug design. NMR has become a powerful and versatile tool for characterizing at an atomic level the interaction of protein partners, which represent biologically relevant targets for drug discovery. NMR has been also developed into a mature technique for the identification of small molecule ligands for macromolecular targets. The identification of high affinity ligands of target proteins usually requires an initial screening of extended libraries of low affinity compounds followed by their optimization into drugs for therapeutic intervention. We present here an approach that combines biotechnology and advanced NMR tools for studying the interactions of “difficult” proteins involved in neurodegenerative and oncology diseases.

Ca2+-dependent Conformational Changes in a C-terminal Cytosolic Domain of Polycystin-2

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The PKD1 and PKD2 genes are the genes that are mutated in patients suffering from autosomal dominant polycystic kidney disease. The human PKD2 gene codes for a 968-aminoacid long membrane protein called polycystin-2 that represents a cation channel whose activity can be regulated by Ca2+ ions. By CD, fluorescence, and NMR spectroscopy, we have studied a 117-amino acid-long fragment of the cytoplasmic domain of polycystin-2, polycystin-2-(680–796) that was proposed to contain a Ca2+-binding site. NMR structure determination reveals the existence of two Ca2+ binding sites in polycystin-2-(680–796) arranged in a typical and an atypical EF-hand motif. In the presence of Ca2+ the protein forms a dimer. The calcium affinity of the protein was determined by fluorescence and NMR spectroscopy. At 293 K, the KD values for the high and low affinity sites are 55_M and 179_M, respectively.

Conformation and polymerisation of the C-terminal cytosolic domain of TRPP1 are regulated by the Ca^{2+}-concentration

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The *PKD1* and *PKD2* genes are the genes that are mutated in patients suffering from autosomal dominant polycystic kidney disease [1]. The human *PKD2* gene codes for a 968-aminoacid long membrane protein called TRPP1 (former polycystin-2) that represents a cation channel whose activity can be regulated by Ca^{2+} ions [2]. By NMR spectroscopy, we have studied a 117-amino acid-long fragment of the C-terminal cytoplasmic domain of TRPP1 (TRPP1 680–796) that was shown to contain two EF-hands, one canonical (AA 763-774) and one atypical (AA 727-739) [3]. NMR diffusion measurements show that the polymerization of the domain is strongly calcium dependent, in the absence of calcium the domain forms mainly tetramers, at high calcium concentrations dimers can be detetcted. Since the Ca^{2+} affinity of the domain is also strongly pH-dependent, under physiological conditions polymerization state is also regulated by the cytosolic pH.

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Cellular trafficking of metallo-drugs: an integrated structural biology approach

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Cu transporters also mediate cellular uptake and resistance to Pt-drugs [1,2]. We investigated the interaction of cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) with the plasma membrane Cu permease Ctr1 and the cytosolic Cu chaperone Atox1. Ctr1 is a sensitive system to monitor the interaction with metal ions on the surface of living cells, by exploiting the disorder of the N-terminal domain and the selective 13C-methyl labeling of Met side chains. Cisplatin binds to Met-rich motifs at the N-terminal extracellular domain of Ctr1, with Pt coordinated to three Met sulfur atoms and a fourth ligand which can be either a chloride or a hydroxyl oxygen [3]. A structural model of this adduct was obtained by QM/MM based on 195Pt NMR, CD, and EXAFS data [4]. Cisplatin also binds to Cys motifs of Atox1 retaining the ammine ligands essential for antitumor activity. In-cell NMR spectroscopy and ICP-MS were used to probe intracellular drug delivery and the interaction of cisplatin with the CXXC motif of Atox1 in living *E. coli* cells. Atox1 overexpression is shown to have a protective role against cisplatin cytotoxicity by reducing DNA platination and improving cell viability [5].

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Lanthanide chelating carbohydrate conjugates are useful tools to characterize carbohydrate conformation in solution and sensitive sensors to detect carbohydrate-protein interactions

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The use of paramagnetic ions with lanthanide binding tags (LBTs) for obtaining new NMR parameters with structural information has been extended in the last few years for protein structure determination[1], allowing to deduce key conformational and dynamic information in different elegant examples.[2] In order to extend this methodology, we[3] and others[4] have recently reported on the use of a chemically well-defined LBT to perform structural studies of carbohydrates. Here we present the application of a 1-β-aminolactose-LBT to the NMR study of carbohydrates-lectins interactions. Paramagnetic effects have been effectively transferred from the carbohydrate to the protein, providing information about location and topological aspects of the protein bound-state. Low affinity ligands or even transient protein-carbohydrate interactions can be detected.

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Structure determination of non-ribosomally synthesized antibiotic precursors

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Vancomycin and Teicoplanin are glycopeptide antibiotics used for treating gram-positive bacterial infections. These clinically important biomolecules function through interaction with the cell wall precursor peptidoglycan peptidyl units. This interaction blocks cell wall synthesis. The glycopeptide antibiotics are synthesized by multimodular biocatalysts, the nonribosomal peptide synthetases (NRPSs). As a step in understanding the molecular mechanisms and dynamics of the synthesis machinery we determined the structure of the aglycone of vancomycin in its free form and bound to a cell wall fragment. This structure is compared to the corresponding uncyclized heptamer precursor and the aglycone of teicooplanin. Our results indicate that the precursors have a more concave structure than the final antibiotic. Thus, they may adapt more easily to the following steps of the enzymatic synthesis machinery.

Synthesis and Structural Characterization of the Antimicrobial Peptide Miticin C and its Derivatives

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Highly polymorphic transcripts of the antimicrobial peptide Myticin C have been discovered in Mytilus galloprovincialis[1]. It is a 40 residue peptide(4.4 kDa mature peptide) whose conformation, still undetermined, most probably depends on a conserved array of 8 Cys. In this project we have undertaken the synthesis and purification of the peptide in order to study its functional and structural features with CD and NMR. Structural studies of Myticin C and its derivatives might help to understand its mechanism of action as an antimicrobial peptide. Initially, we focused our attention on the structural characterization of a peptide corresponding to the 19-40 portion of Myticin C, in which 3 out of 5 Cys were replaced with Ser to retain a single disulfide bond. The characterization was carried out in saline solutions and in the presence of DPC and SDS micelles as membrane-mimetic environments. In the latter systems, the peptide shows different behavior depending on the surface charge. In the presence of DPC micelles, a well-defined β-sheet structure is formed, as expected on the basis of secondary structure predictions. The NMR study was conducted in these conditions to determine the three-dimensional structure.

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NMR analysis of heme-regulatory motifs

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Heme:protein interactions have been a subject of interest in the past decade because of their vital role in diverse molecular and cellular processes. Here, we present two studies on heme:peptide complex formation: (I) A combinatorial peptide library screening allowed to identify heme-binding motifs of short length (9-mers) with cysteine, histidine and tyrosine as prominent iron-coordinating amino acids. In addition, cysteine-proline (CP) motifs are often observed in heme binding. Therefore, we solved the structure of a CP motif containing 23 amino acid peptide in complex with gallium-protoporphyrin-IX as a heme mimick. This 23-mer is present in the enzyme dipeptidyl-peptidase-8 sequence. The NMR results indicate the binding of the protoporphyrin-IX to the centrally located cysteine while the proline ring acts as a spacer preventing backbone:ppIX van-der-Waals clashes. (II) Voltage-gated potassium channels are responsible for the regulation of several ion conductance pathways. Their inactivation event is one of the most critical event that shapes the presynaptic action potential and regulation of synaptic transmission in the CNS. Heme was shown to play a role in the inactivation and regulation of the potassium channel Kv1.4. The results of NMR studies of a 63 residue heme-binding peptide derived from the Kv1.4 sequence are presented.

Structure and Dynamics of BPSL1445 from *Burkholderia pseudomallei*

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Burkholderia pseudomallei (Bp) is a pathogenic bacterium responsible for melioidosis, a severe endemic disease in Far East [1]. Melioidosis causes septicemia and organ failure, with a high mortality rate. Antibiotic treatments are largely ineffective due to multi-drug resistance. The mechanisms of virulence and host resistance of Bp are still poorly characterized, thus limiting the availability and efficacy of suitable vaccines against melioidosis [2]. Vaccines derived from attenuated Bp mutants induce protective immunity in a murine model of melioidosis, suggesting that some mechanisms of organism protection against the disease exist [2]. The high risks related to these vaccines, though, strongly limit their application. Considerable efforts have been thus devoted to the development of non-living melioidosis vaccines. 49 protein antigens from Bp were recently detected in sera from melioidosis infected patients, opening new perspectives in the generation of effective meliodosis vaccines [3]. One of these candidates is the protein antigen BPSL1445. Here we present the solution structure of BPSL1445 and its dynamical characterization obtained by 15N spin relaxation analysis. The structure will constitute the starting point for future molecular dynamics which will be performed to identify possible epitopes suitable for non-living vaccines production.

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Bile acid binding protein-rhodamine host-guest system: NMR and optical characterization of a fluorescent bio-nanomaterial

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New strategies are requested for the preparation of bio-inspired host-guest complexes to be employed in technologically relevant applications, as sensors and optoelectronic devices. We report here a new approach employing a single monomeric protein as host for the strongly fluorescent rhodamine dye. The selected protein, belonging to the intracellular lipid binding protein family, fully encapsulates one rhodamine molecule inside its cavity forming a host-guest complex stabilized by H and p-hydrogen bonds and favourable hydrophobic contacts, as revealed by the NMR derived structural model. The protein-dye solutions are easily processable and form homogeneous thin films exhibiting excellent photophysical and morphological properties, as derived from photoluminescence and AFM data. The obtained results represent the proof of concept of the viability of this bio host-guest system for the development of bio-inspired optoelectronic devices.

Does the staggered-rotamer model do what we think it does?

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If we agree that it takes four J-coupling measurements in order to determine a torsion angle unambiguously [1], why then do people traditionally attempt to retrieve amino-acid side-chain rotamers on the basis of only two J-coupling constants? Just how trustworthy can the population parameters for those staggered states be? We demonstrate that the popular staggered-rotamer model in its classical form describes rotamer equilibria inadequately. Counterintuitive population parameters are sytematically obtained even if extended or complete sets of up to 6 or 9 coupling constants per residue are available. This is a model-inherent consequence of referring both to only three fixed equi-spaced angles and to only two focal values of Jtrans and Jgauche associated with these [2]. A Karplus equation is notoriously not being applied in the process and, in fact, the bimodal curvature of the coupling-angle dependency is being missed entirely in the classic rotamer analysis. The issue will be highlighted with J-coupling data acquired for side chains in the enzyme RNase T1. We also devise a numerical procedure that, albeit incapable of fixing the problem, does help ameliorate it.

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Investigations of the cysteine-rich region of type VII collagen with CD- and NMR-spectroscopy

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Type VII collagen (Col7) is the major component of the anchoring fibrils in the skin. These Col7 fibrils are essential for skin stability, that is highlighted by mutations leading to a heritable skin blistering disease and a Col7 related autoimmune disease. Col7 has a central collagenous domain that is flanked by two non-collagenous domains with subdomains homolog to protein interaction domains. Despite the biological importance of Col7 there is a basic knowledge gap on structural information. We investigate Col7 for a better understanding of the skin architecture and the connection between different skin layers. A Col7 subdomain with homology with the von-Willebrand-factor, the vWFA2 domain, interacts with the preceding FNIII9 subdomain and type I collagen [1,2]. A cysteine-rich region is found at the junction of the vWFA2 domain and the collagen triple helix. Within this transitional region exists a missense mutation leading to skin blistering. CD spectroscopy proves triplehelix formation of a model peptide of the cysteine-rich region that is supported by temperature dependent NMR spectra. We currently establish a purification protocol for a recombinant expressed peptide. Production of ^{15}N-, ^{13}C-labelled peptide lays the basis for investigating the arrangement of the disulfide bridges. Our results will provide the basis for further structural and functional studies of Col7, that will lead to a better understanding of the pathogenesis of related skin blistering diseases.

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Metabolic characterization of SH-SY5Y cells and effects of interferon-α exposure

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The human neuroblastoma SH-SY5Y cell line is a third successive subclone of the SK-N-SH line, originally established from a bone marrow biopsy of a neuroblastoma patient [1]. These cells possess many characteristics of neurons, and they represent one of the most-used models for studying cellular events and mechanisms involved in neurotoxicity and neurodegeneration even in neuroprotection. Interferons are cytokines endowed with a pleiotropic spectrum of biological properties, including immunomodulation, antiviral and proinflammatory activity. Interferon (IFN)-α is a type I IFN that may have broad-ranging actions in the brain, affecting neuronal differentiation, survival and synaptic plasticity. We previously demonstrated that a 72 hours exposure to IFN-α induces early apoptosis in SH-SY5Y cells. This prompted us to investigate the metabolic profile of the SH-SY5Y cells using HR-MAS NMR Spectroscopy after a 72 hours exposure to IFN-α to explore the metabolic changes that characterize these cells. Moreover, since a metabolic characterization of this extensively used cell clone is still lacking, we analyzed the metabolic profile of the SH-SY5Y in standard growth conditions. Results show some interesting changes in metabolites, such as choline containing compounds, creatine and glutamate. Our goal will be to relate the metabolic changes to IFN-α exposure.

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A NMR-based model of the electron-transfer complex between ferredoxin and [FeFe]-hydrogenase required for hydrogen production

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Hydrogenases are central to the exploitation of photosynthetic organisms for hydrogen production. Because of its simplicity, the [FeFe]-hydrogenase HydA1 from the green algae *Chlamydomonas reinhardtii* is particularly attractive for understanding the catalytic mechanism of [FeFe]-hydrogenases. It is known to interact with the ferredoxin PetF that acts as the electron-donor linking the hydrogenase to the photosystem. The interaction of HydA1 and PetF has already been studied using site-directed mutagenesis. It has been shown that HydA1 competes with the ferredoxin-$NADP^+$-reductase (FNR) for electrons from PetF. This competitive interplay between HydA1 and FNR determines the efficiency of hydrogen production in algae. To allow for the directed modification of the electron transfer to HydA1, the interactions of FNR and HydA1 with PetF have been studied by NMR spectroscopy. Furthermore, the solution NMR structure of PetF has been calculated. This structure is used as a starting structure for docking PetF to HydA1 with Haddock and all available distance restraints. Comparison of the PetF/HydA1- with the PetF/FNR-interface indicates three PetF-residues as only involved in the PetF/FNR-interaction. Mutation of these positions to alanine yields PetF-mutants with a weaker FNR- and an unaltered HydA1-affinity. Such mutants could potentially result in an increased algal hydrogen evolution.

Conformational states of the ADP ribosylation factor 1 and its importance for the interaction with regulators and effectors

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Guanine nucleotide binding proteins (GNB-proteins) play an essential role in cellular signal transduction and transport processes. They act as molecular switches cycling between an inactive GDP-bound form and an active GTP-bound form. For several members of the Ras superfamily of GNB-proteins equilibria of conformations could be shown for both, the active as well as inactive form. We present data on the ADP ribosylation factor 1 (Arf1) a key regulator of vesicular traffick at the Golgi. Arf1 is also involved in signalling cascades necessary for cancer progression and migration [1]. In order to investigate conformational states of Arf1, we modulate the equilibrium using modifications of the bound nucleotide or the amino acid sequence [2,3]. Treatment with GdmCl or the presence of high pressure is used to stabilize single conformations. Using ^{31}P NMR spectroscopy as well as [^{1}H,^{15}N] HSQC experiments in combination with biochemical studies we characterize conformational dynamics as well as their importance for Arf1 interaction with regulators and downstream effectors. These intrinsic conformational equilibria of active GNB-proteins seem to be a fine-tuning mechanism of regulation and by that an interesting target for the modulation of protein activity by small molecules.

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Structural studies of ubiquitylation intermediates

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Ubiquitylation is an essential post-translational modification in eukaryotes. The reaction pathway requires at least three enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-protein ligase (E3). E1 activates the C-terminal glycine residue (G76) of ubiquitin in an ATP-dependent reaction and later forms a thioester linkage through an active-site cysteine. Ubiquitin is then passed on to a conserved active-site cysteine of the E2 through a transesterification reaction. In case of the HECT E3 ubiquitin ligases a second transesterification step occurs and ubiquitin gets transferred from E2 to an active-site cysteine residue of the E3 HECT domain. Eventually HECT E3 ligases attach ubiquitin to a lysine of target proteins. Thereby, an isopeptide bond between G76 and the target lysine residue is formed. Structural studies of ubiquitylation intermediates are hampered by the intrinsic instability of all the thioester compounds. Thioesters are prone to hydrolysation rendering them inaccessible to NMR studies or crystallization assays. In order to characterize the structure of a ubiquitin thioester with a HECT domain of an E3 enzyme, we overcome the instability of the thioester by forming disulfide compounds between a C-terminal mutant of ubiquitin (G76C) and the catalytic cysteine of the HECT domain. The disulfide linkage is stable and mimics the bond distances of thioester bonds. We show here that we can form these Ubiquitin-HECT domain disulfides under non-reducing condition and that we can purify them to homogeneity. Furthermore, we present initial NMR spectra of a Ubiquitin-HECT domain disulfide providing the first structural insight into a HECT reaction intermediate in solution.

Structural studies of monothiol Grx1 from the parasite *Trypanosoma brucei*

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Glutaredoxins (Grxs) are ubiquitous enzymes conserved throughout all the kingdoms of life. Grxs take part in a wide variety of biological processes, the most important being the maintenance of the redox status of cysteine-containing proteins through the reduction of both intra-molecular and mixed disulphides with glutathione of protein cysteinil groups [1].Trypanosomatids are parasitic protozoa implicated in severe disease in the South American and African continents. These parasites exploit a unique thiol-dependent redox system based on bis(glutathionyl)spermidine (trypanothione) rather than glutathione. This peculiarity, together with the pivotal role of monothiol glutaredoxins in iron- and iron-sulfur metabolism, makes this proteins potential candidates as drug targets [2]. Recently, we solve the structure of a deleted form of Tb1-C-Grx1 missing the first 75 amino acid (PDB code:2LTK)[3]. Here, we present a structural characterization of the mature form of Tb1-C-Grx1 including a non-structurated N-terminal tail.

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High pressure NMR spectroscopy and drug development

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Proteins exist in solution as equilibrium of different conformers. The low populated conformers are seldom detected by normal spectroscopic methods. High pressure NMR techniques are very useful for the study of protein conformational equilibrium within the folded state ensemble [1]. Here we describe a generally applicable strategy for the modulation of protein-protein interactions by small compounds, based on the assumption that structural sub-states with different ligand binding affinities are coexisting and are only selected by the binding ligand. The proto-oncogene Ras is the prototype member of the Ras superfamily [2]. In light of the multiple state model, at least three conformational sub-states of the activated GNB protein Ras•Mg2+•GTP must coexist in solution, states that correspond to the complexes with GEFs (1(T)), effectors (2(T)), and GAPs (3(T)). Two of the main conformational states (1(T) and 2(T)) of Ras•Mg2+•GppNHp can be directly observed ^{31}P NMR spectroscopy [3]. We were able to identify four of the expected sub-states from high pressure HSQC NMR spectra. The protein-protein interactions of Ras can be modulated by small compounds that bind to the conformational sub-states. The study focused on the Ras system leads to a novel generally applicable strategy for the development of allosteric inhibitors of protein-protein interactions. The conformational sub-states of Ras (T35S).GppNHp protein are also discussed here.

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Identification of β-microglobulin residues involved in Transglutaminase reaction

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β-microglobulin (βMg) is found on the cell surface of mammalian cells and is the small subunit of the major histocompatibility complex class I molecules as well as of the neonatal Fc receptor (FcRn). The FcRn is a target for treatment of autoimmune diseases since saturation of the receptor with high dosis of IgG leads to a symptom reduction. This is based on prevention of binding of the pathogenic autoantibodies to the receptor and faster degradation of autoantibodies.βMg is a substrate of transglutaminases and can be polymerized by them [1]. The aim of our study is the modulation of the function of the FcRn. Therefore we want to investigate if dimerisation of the FcRn is possible by dimerisation of βMg and how this influences binding properties of the receptor to IgG. Cloning and expression of human βMg in *E. coli.* as well as of the FcRn have been successful. The proteins are localized in inclusion bodies. Therefore already published protocols [2],[3] for purification of the proteins are used. Purification and refolding of βMg is successful as proven by NMR spectroscopy. Using different substrates amino acids were identified that are affected by transglutaminase reaction. After identification of the lysine and glutamine residue that are linked during the transglutaminase reaction, dimerisation of FcRn via βMg will be investigated. With dimerised FcRn binding properties to IgG will be analysed.

[1] Fésüs L. et al., *JCB* 1981 **89**, 706-710 [2] Andersen J.P. et al., *JIM* 2008 **331**, 39-49 [3] Esposito G. et al., *Protein Science* 2000 **9**, 831-845

Novel NMR approaches for the characterization of heme centers in proteins

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The reactivity and functionality of heme proteins are determined by the coordination environment, by the interaction of axial ligands, and by surface properties modulating the interaction partner molecules. We want to review new solution and solid state experiments that have been developed during the last decade. We will show how the combination of novel NMR approaches based on heteronuclear detection and tailored ^{1}H NMR can be used to characterize heme centers also in “non-conventional” coordination environments making reference to a few case example heme proteins. The use of NMR for the characterization of the protein surface properties and implications for protein reactivity will also be discussed.

Achieving linear independent alignments through encodable loop lanthanides binding tags

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In biomolecular NMR-spectroscopy, Residual Dipolar Couplings (RDCs) have become valuable parameters for the study of structure and dynamics of biomolecules in the solution state. Methods based on RDCs from at least three to five linear independent alignments have been developed to characterize motions occurring on the supra-tau_c timescale. Yet, for the majority of biomolecules, it proves to be a great challenge to find a set of sufficiently suited alignment media. Previously, we engineered encodable lanthanide binding tags as a versatile tool for chemical biology, X-ray crystallography and NMR-spectroscopy into loop regions of Interleukin 1b. We here present, that the paramagnetic alignment obtained in differently attached loop LBTs is sufficient to construct five orthogonal linear combinations of RDCs for Interleukin 1b.

Application of ^{13}C broadband pulses to excitation, refocusing and decoupling for high field

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The ^{13}C chemical shifts of carbons found in proteins and nucleic acids span a frequency bandwidth that ranges from 22.5 kHz at 14.1 T (600 MHz ^{1}H) to 45 kHz at 28.2 Tesla(1.2 GHz ^{1}H). At higher magnetic fields, the conventional pulses used for excitation, refocusing and decoupling fail to cover the full bandwidth within the power limits of modern probes, thus decreasing the ability to excite all nuclei and to decouple multiplet splitting of peaks. Adiabatic broadband decoupling pulses provide low-power broadband decoupling, but are plagued with intense sideband artifacts. As the spectrometer fields increase, the need for a robust, low power, broadband ^{13}C decoupling pulse becomes greater. Here, we present low power ^{13}C decoupling pulses designed with optimal control (OC) theory and broadband refocusing pulses that cover greater than 47 kHz. We show the advantages of using low power OC decoupling scheme and broadband refocusing pulses in ^{1}H, ^{13}C HMQC and sup>13C-edited ^{1}H, ^{1}H NOESY spectra. The OC decoupling pulses are compared with GARP and WURST. Further, we propose the use of these broadband pulses in a suite of optimized pulse sequences intended for ^{1}H, ^{13}C HMQC and ^{13}C- edited/filtered ^{1}H, ^{1}H NOESY and ^{13}C, ^{13}C NOESY spectra collected at high fields.

Delivery of Isotope-Labeled Proteins into Eukaryotic Cells for *In-Cell* NMR Experiments

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Protein–protein and protein-ligand interactions play an essential role in many biological processes such as signal transduction, gene transcription and cell regulation. Studying the mechanisms and role of those interactions under physiological or pathological conditions provides guidelines for developing novel therapeutic compound and strategies. In-cell NMR spectroscopy represents a powerful approach to investigate at atomic level the conformation, interactions and dynamics of isotope labeled macromolecules within their natural and highly complex cellular environments. This method has been successfully applied to proteins overexpressed in bacteria cells, but the lack of strong promoters in eukaryotic cells doesn't allow the expression of amounts of isotopically enriched protein detectable by in-cell NMR. Therefore the application of in-cell NMR to eukaryotic cells is still limited by the difficulty to deliver proteins at high concentrations into cells. Although some approaches such as microinjection, cell-penetrating peptides (CPP) and pore-forming bacterial toxin Streptolysin O have been used for introducing 15N labeled proteins into cytosol of living cells, successful delivery largely depends on the physicochemical properties of the protein to be delivered. In this context, the present study is focused to identify and to implement a suitable method in order to deliver different molecules directly into the cytosol for in-cell NMR observation. Preliminary results will be presented and discussed.

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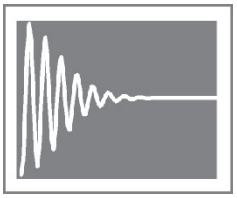

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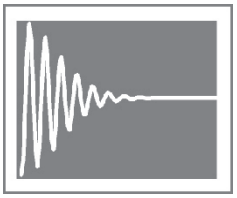

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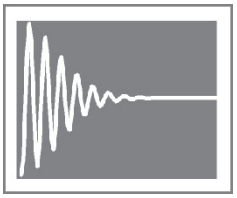

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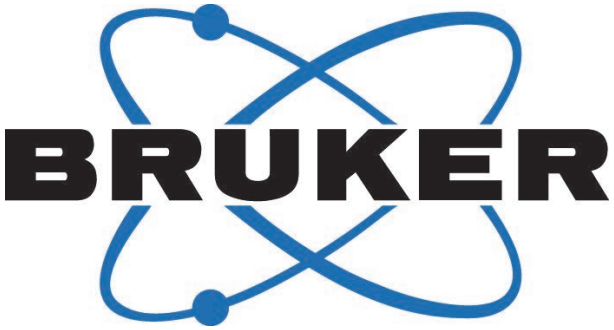
	Monday	Tuesday		Wednesday		Thursday	Friday	Saturday
	9.9.	10.9.		11.9.		12.9.	13.9.	14.9.
08:30		**Plenary I: Frontiers in Magnetic Resonance** *(Chair: Bernd Reif)*		**Plenary III: Small Molecules & Polymers** *(Chair: Christina Thiele)*		**Plenary V: Magnetic Resonance in Structural Biology** *(Chair: Henriette Molinari)*		
		Lucia Banci Florence		**Burkhard Luy** Karlsruhe		**Volker Dötsch** Frankfurt		
		Kevin Brindle Cambridge		**Marco Geppi** Pisa		**Enrica Bordignon** Zürich		
		Hartmut Oschkinat Berlin		**Ruth Gschwind** Regensburg		**Robert Bittl** Berlin		
						Paul Rösch Bayreuth		
10:00		**Coffee**		**Coffee**		**Coffee**		
10:30		**Plenary II: Imaging** *(Chair: Axel Haase)*		**Parallel 5: Nucleic Acids & Proteins** *(Chair: Luisa Mannina)* [Aula]	**Parallel 6: Biomedicine & Food** *(Chair: Enzo Terreno)* [Musiksaal]	**Plenary VI: Frontiers in Magnetic Resonance II** *(Chair: Steffen Glaser)*		**EPR Satellite Meeting**
		Silvio Aime Torino		**Teresa Carlomagno** Heidelberg	**Raffaele Lamanna,** Rotondella	**Mario Chiesa** Torino		
		Oliver Speck Magdeburg		**Mirko Cevec** Ljubljana	**Lucia Calucci** Pisa	**Mario Piccioli** Florence		
		Leif Schröder Berlin		**Mario Schubert** Zürich	**Roberto Consonni** Milano	**Marina Bennati** Göttingen		
				Cristina Airoldi Milano	**Wolfram Gronwald** Regensburg	**Jörg Wrachtrup** Stuttgart		
				Elke Duchardt-Ferner Frankfurt	**Parallel 8: BioNMR - Applications** *(Chair: Sonja Dames)* **Silke Wiesner** Tübingen			
12:00		**Sandwich lunch** [Tent]		**12:00-13:30 Postersession II & Sandwich lunch** [Tent]		**Sandwich lunch** [Tent]		
13:30	**14:30-17:00 TUTORIALS** **14:30-15:30** **Steffen Glaser** [Musiksaal] Optimum Control & Pulse Sequence Optimization **Geerten Vuister** [Konferenzzimmer] CCPNMR **16:00-17:00** **Stan Sykora** [Musiksaal] From Spin Hamiltonian to NMR spectra **Oliver Lange** [Konferenzzimmer] CS & autoNOE ROSETTA	**Parallel 1: BioNMR - Methods** *(Chair: Tobias Madl)* [Aula]	**Parallel 2: Polymers & Materials** *(Chair: Jörn Schmedt auf der Günne)* [Musiksaal]	**Parallel 7: Materials & Small Molecules** *(Chair: Marion Menzel)* [Aula]	**Parallel 8: BioNMR - Applications cont'd** *(Chair: Sonja Dames)* [Musiksaal]	**G-NMR** **12:30-15:30** [Aula]	**EPR Satellite Meeting** Thu 12.9 15:35 until Sat 14.9. 12:00	
		Stephan Grzesiek Basel	**Michael Ryan Hansen** Mainz	**Bernhard Blümich** Aachen	**Clemens Glaubitz** Frankfurt			
		Michael Assfalg Verona	**Hellmut Eckert** Münster	**Michele Chierotti** Torino	**Giovanna Musco** Milano			
		Salvatore Bubici Pavia	**Christiane Wolff** Darmstadt	**Cindy Elschner** Dresden	**Nils Lakomek** Göttingen			
		Adele Mucci Modena	**Gregor Mali** Ljubljana	**Alexander Schnegg** Berlin	**Daniel Cicero** Rome			
		Hans-Robert Kalbitzer	**Marco Sette** Rome	**Vito Gallo** Bari	**Maxim Yulikov** Zürich			
		Adam Lange Göttingen	**Kay Saalwächter** Halle	**Noemi Proietti** Rome	**Markus Zweckstetter** Göttingen			
15:30		**Coffee**		**Coffee**				
16:00	**Registration** [Reception Desk Monastery]	**Parallel 3: Dynamics** *(Chair: Horst Kessler)* [Aula]	**Parallel 4: Computation & Structure** *(Chair: Oliver Lange)* [Musiksaal]	**Plenary IV: Pharma & Bio** *(Chair: Stefano Mammi)*				
		Christian Griesinger	**Alessandro Bagno** Padova	**Fabio Arnesano** Bari				
		Mauro Botta Alessandria	**Matthias Köck** Bremerhaven	**Wolfgang Jahnke** Basel				
		Wolfgang Baumann Rostock	**Christiane Ritter**	**Carla Marchioro** Verona				
		Philipp Neudecker Düsseldorf	**Bert de Groot** Göttingen	**Janez Plavec** Ljubljana				
		Jochen Balbach Halle	**Michael Nilges** Paris					
18:00	**Welcome Reception** [Klosterwirt]	**Dinner** [Klosterwirt]		**Anna Laura Segre Fellowship Presentations (GIDRM)**		**EPR Satellite Meeting** Thu 12.9 15:35 until Sat 14.9. 12:00		
19:00	**Opening remarks** [Aula]							
19:15	**Plenary I: Frontiers in Magnetic Resonance** *(Chair: Michael Sattler)* **Gerhard Wagner** Boston							
19:45	**Plenary Awards** (Chair: Wolfgang Jahnke) **FGMR Ernst Awards** **Introduction:** ***Wolfgang Jahnke*** **Mariusz Jaremko, Łukasz Jaremko** (MPI Göttingen) **Yesu Feng** (Duke Univ., Durham, USA) **Franz Schilling** (TUM, Munich) **GIDRM Medal** **Introduction:** ***Henriette Molinari*** **Gennaro Esposito** (Udine)	**19:30-21:00 Poster Session I** [Tent]		**19:30 Conference Dinner** [Klosterwirt]				
		20:30-22:00 FGMR member meeting [Aula]	**20:30-22:00 GIRDM member meeting** [Musiksaal]	**21:30 After Dinner Lecture** [Aula] **Horst Kessler** Garching				
	Drinks in the Catacombs (on your own)	**Drinks in the Catacombs** (on your own)		**Drinks in the Catacombs** (on your own)				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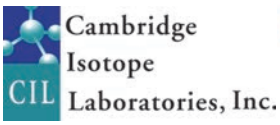
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	Monday	Tuesday		Wednesday		Thursday	Friday	Saturday
	9.9.	10.9.		11.9.		12.9.	13.9.	14.9.
08:30	Arrival	Plenary I: Frontiers in Magnetic Resonance		Plenary III: Small Molecules & Polymers		Plenary V: MR in Structural Biology	EPR Satellite Meeting	EPR Satellite Meeting
10:00		Coffee		Coffee		Coffee		
10:30		Plenary II: Imaging		Parallel 5: Nucleic Acids & Proteins [Aula]	Parallel 6: Biomedicine & Food [Musiksaal] Parallel 8: BioNMR - Applications	Plenary VI: Frontiers in Magnetic Resonance II		
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13:30	14:30-17:00 TUTORIALS [Musiksaal] [Konferenzzimmer]	Parallel 1: BioNMR - Methods [Aula]	Parallel 2: Polymers & Materials [Musiksaal]	Parallel 7: Materials & Small Molecules [Aula]	Parallel 8: BioNMR - Applications cont'd [Musiksaal]	G-NMR 12:30-15:30 [Aula]		
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	Drinks in the Catacombs (on your own)	Drinks in the Catacombs (on your own)		Drinks in the Catacombs (on your own)				