Vol. 63, No. 3/4 ISSN 0432-7136

BULLETIN DU GROUPEMENT

d'informations mutuelles

AMPERE

SE CONNAÎTRE, S'ENTENDRE, S'ENTRAIDER

July to December 2014

No. 256/257

Office: ETH Zürich, Laboratory of Physical Chemistry
8093 Zürich, Switzerland, www.ampere.ethz.ch

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Publisher: Gunnar Jeschke, ETH Zurich, Switzerland

Editorial

Dear AMPERE colleagues,

this issue starts a new tradition by articles of the winners of the 2014 AMPERE Prize, Christian Griesinger, and the 2014 Raymond Andrew Prize, Olivier Duss. Starting on pages 12 and 8 you can read their own account on the science that was acknowledged by these awards. There will also be a Raymond Andrew Prize winner in 2015. If you don't want to miss the award ceremony and if you do want to listen to talks about the most exciting research in our field, you must attend EUROMAR 2015 at the beginning of July in beautiful Prague. You can find the most important information on the event on pages 5-7. Don't miss the early-bird registration deadline, which is the same as the final abstract submission date for oral contributions: April 15th 2015.

This issue also contains the final report on NMRCM 2014 in St. Petersburg by V. Chizhik (p.2) and the announcements of further AMPERE events in 2015: the AMPERE NMR School in Zakopane in June (p.4), the 13th International Conference on Magnetic Resonance Microscopy in Munich in August (p.22), and the 9th Alpine Conference on Solid-State NMR in Chamonix in September (p25).

Finally, I want to repeat my invitation to board members or chairs of national or regional European magnetic resonance societies to contact us at ampere@ethz.ch if they are interested in coordination of activities. We shall contact all interested parties right after the next AMPERE Bureau meeting on March 27th 2015 in Zürich.

Gunnar Jeschke
Secretary General of Groupement AMPERE

Report on the International Symposium and Summer School in Saint Petersburg

"Nuclear Magnetic Resonance in Condensed Matter" (NMRCM 2014)

11th Meeting: "Biomolecular NMR and related phenomena".

The Saint-Petersburg University continues a series of annual International Symposiums and Summer Schools in Saint Petersburg "Nuclear Magnetic Resonance in Condensed Matter" (an AMPERE event). The 11th Meeting under the subtitle "Biomolecular NMR and related phenomena" was opened on Monday, July 07, and was closed on Friday, July 11, 2014.
The goal of the meeting was to provide a platform to scientists and students for the use of all aspects of nuclear magnetic resonance methods and techniques as well as computational and theoretical approaches for the solving of various problems in the aria of medicine and biology.
The Meeting was attended by 92 participants from 10 countries (Finland, France, Germany, Japan, Poland, Russia, Sweden, Turkey, Ukraine, USA).
There were 10 lectures, presented by following speakers;

Yu. Bunkov (Grenoble, France)
V.A. Chertkov (Moscow, Russia)
J. Fraissard (Paris, France)
D. Michel (Leipzig, Germany)
V.Yu. Orekhov (Gothenburg, Sweden)
Yu.A. Pirogov (Moscow, Russia)
P.I. Polyakov (Donetsk, Ukraine)
G.V. Mozzhukhin (Istanbul, Turkey)
N.R. Skrynnikov (West Lafayette, USA)
V.I. Volkov (Moscow, Russia)

Moreover, there were 12 contributed talks (20-30') and 35 poster presentations. The organizers published the Book of Abstracts.
Barbara and Uwe Eichhoff (Germany) instituted a prize (300+200 Euro) for the best oral and poster reports of students and postgraduate students. The prizes and proper diploma were awarded by the Organizing Committee to Sergey Sheludiakov (University of Turku, Finland) and Maria Muravyeva (Nizhni Novgorod State University, Russia).

NMRCM 2014 took place at the Petrodvorets Campus of Saint Petersburg State University. Petrodvorets (the satellite-town of Saint Petersburg) is famous for its parks with fountains and palaces. The social program of the conference consisted of a welcome-party and a conference dinner on a boat during "white night".

The information (including abstracts and photographs) on the NMRCM 2011 is available at http://nmr.phys.spbu.ru/.

The NMRCM 2014 was supported by
- Saint-Petersburg University
- Russian Foundation for Basic Research
- Bruker
- "Dynasty" Foundation (Russia)

The information on the next meeting NMRCM will be later (there are certain difficulties for the 2015 year).

Ampere NMR School
14 - 20 June 2015, Zakopane Poland

The AMPERE NMR School, is organized by the Macromolecular Physics Department of Adam Mickiewicz University in Poznań, in collaboration with Centre for European Integration, under the auspices of the Groupement AMPERE.

The School is addressed to young scientists (post graduate students, PhD students and post-doctoral fellows) and is focused on theoretical and experimental aspects of NMR methods, as well as on application of NMR in nanoscience and nanotechnology.

The School covers the following topics:
- solid state and soft matter NMR
- NMR diffusometry and relaxometry
- application of NMR in biology and medicine
- application of NMR material and environmental sciences
- magnetic resonance imaging and spectroscopy
- NMR and quantum information
- theoretical and experimental aspects of dynamic nuclear spin polarization
- advanced NMR techniques

further information can be found under;
http://www.staff.amu.edu.pl/~school/index.html

Euromar 2015

July 5-10, 2015, Prague Congress Centre, Prague, Czech Republic
www.euromar2015.org

Welcome

EUROMAR 2015 will be hosted by Prague. The largest European congress on magnetic resonance will commence in the Prague Congress Center, a place with stunning panorama view of Prague Castle.

EUROMAR 2015 will offer an opportunity to report the latest scientific breakthroughs in magnetic resonance in broad range of scietific fields, ranging from physics and chemistry to biology and medicine. The goal of the conference is to provide a stimulating forum for sharing experience, exchanging ideas, and establishing fruitful collaborations.
On behalf of the organizing committee and the EUROMAR board, I am delighted to cordially invite you to EUROMAR 2015. We will be happy if you will help us to make this congress a memorable scientific and personal event.

Vladimír Sklenář
Chairman
EUROMAR 2015 Conference

Plenary Speakers

Bax, Ad	NIH Bethesda, USA
Edison, Arthur S.	University of Florida, USA
Feigon, Juli	University of California, Los Angeles, USA
Freed, Jack	Cornell University, USA download
Hilty, Christian	Texas A&M University, USA
Kern, Dorothee	Brandeis University, USA
Korvink, Jan	Universität Freiburg, Germany
Ladzihansky, Vladimir	University of Guelph, Canada
Polenova, Tatyana	University of Delaware, USA
Rosay, Melanie	Bruker Biospin, Billerica, USA
Takegoshi, Kiyonori	Kyoto University, Japan
Thiele, Christina	Technische Universität Darmstadt, Germany
Webb, Andrew	Leiden University, The Netherlands

Tutorial Speakers

Blank, Aharon	Technion-Israel Institute of Technology, Israel
Palmer, III, Arthur G.	Columbia University, USA
Parella, Teodor	Universitat Autonoma de Barcelona, Spain

Registration & Abstract Submission

Early-bird registration ends on April 15th, 2015
Abstracts for oral presentations must be submitted by April 15th, 2015
Abstracts for poster presentations must be submitted by May 15th, 2015
Registration will close on June 22nd, 2015

Programme

	Saturday 4 July	Sunday 5 July	Monday 6 July	Tuesday 7 July	Wednesday 8 July	Thursday 9 July
09:00 - 10:20	pNMR management meeting 10:00 - 13:00	pNMR Scientific Meeting 9:30 - 11:30	Plenary session 1	Plenary session 3	Plenary session 5	Plenary session 7
10:20 - 10:50			Coffee Break	Coffee Break	Coffee Break	Coffee Break
10:50 - 12:50		pNMR and Bruker Brunch 11:30 - 12:30	Parallel session 1	Parallel session 3	Parallel session 5	Parallel session 7
12:50 - 14:00		„What's new from Bruker" 12:30 - 14:15	Lunch	Lunch	Lunch	Lunch
14:00 - 16:00	pNMR scientific meeting 14:00 - 18:00	Tutorial Lectures 14:30 - 16:45	Poster Session with Coffee	Poster Session with Coffee	Poster Session with Coffee	Parallel session 8
16:00 - 18:00			Parallel session 2	Parallel session 4	Parallel session 6	Coffee Break Plenary session 8
18:00 - 18:15		Opening and Prize Session 17:00 - 19:00				
18:15 - 18:55			Plenary session 2	Plenary session 4	Plenary session 6	
19:00	pNMR / Bruker sponsored dinner 20:00 - onwards	Welcome Mixer	Bruker Reception	Jeol Reception		Conference Dinner

Raymond Andrew Prize 2014
Oliver Duss

The Scripps Research Institute, La Jolla, California

NMR and EPR as tools for studying the assembly, structure and function of a large protein-RNA complex

An important player in bacterial virulence is the homo-dimeric CsrA-type protein, for which the active protein concentration has to be tightly regulated [1]. At high concentrations, the protein binds to a regulatory structure (ribosome binding site) within a messenger RNA to block its translation into a protein. A fast and efficient way to regulate the available active CsrA protein is its temporal sequestration by a non-coding RNA, which results in translation activation of the corresponding messenger RNA (ncRNA: a RNA which does not code for a protein like a messenger RNA). However, the mechanism of ncRNA-mediated protein sequestration is not understood at the molecular level.

Using a powerful combination of NMR and EPR spectroscopy, we revealed the molecular mechanism of how the ncRNA RsmZ sequesters, stores and eventually releases the CsrA-type protein RsmE.

The ncRNA RsmZ is a 127 nucleotides RNA containing eight potential binding sites for the homo-dimeric RsmE protein (figure 1A). Using isothermal titration calorimetry and electromobility shift assays, we could show that in isolation all the eight sites bind the RsmE protein with low micromolar affinity [2]. However, in context of the full RNA, up to five homo-dimeric RsmE proteins are bound sequentially and cooperatively within a narrow affinity range around hundred nanomolar [3].

Does the binding of the protein to the multiple binding sites on the RNA generate an ensemble of different protein-RNA complexes (RNPs) or is the binding specific and generates a well-defined RNP complex? Simple NMR chemical shift perturbation experiments are in principle well suited to determine where and in which order the RsmE proteins bind the RsmZ RNA. However, the presence of multiple identical homo-dimeric RsmE proteins, which are binding to multiple almost identical RNA sequences, results into severe signal overlap preventing determination of the binding order and topology.

Therefore, we developed a simple approach for multiple segmental isotope labeling of RNA, which permits to isotope label a single protein binding site on the RNA at a time [4, 5]. In this cut & paste approach, a long RNA is in vitro transcribed either unlabeled or with a specific isotope labeling scheme. Then, the labeled and unlabeled RNAs are site-specifically cleaved using splinted RNase H cleavage, generating several

labeled and unlabeled RNA fragments which can subsequently be ligated in any desired combination to obtain full-length segmentally labeled RNAs. Note that RNA fragments can also be combined with short nitroxide spin-labeled RNA fragments for generating long doubly spin-labeled RNAs for long-range distance measurements by the pulse EPR technique DEER (see below) [6]. Performing chemical shift perturbation experiments of differently segmentally isotope labeled RsmZ ncRNAs with the RsmE protein, revealed that, unexpectedly, the five RsmE protein dimers assemble not only sequentially and cooperatively onto the ncRNA RsmZ but also specifically in a well-defined manner (figure 1A).

Interestingly, the RNA is becoming increasingly protected from degradation by RNase E or E. coli whole cell extract upon binding of increasing stoichiometric amounts of RsmE protein [3]. The sequential and well-defined assembly of RsmE and the overlap of RsmE protein binding sites with RNase E cleavage sites explain the increasing nuclease protection conferred by the RsmE protein.

From a biological point of view, the molecular mechanism of assembly and increasing nuclease protection makes much sense. When high concentrations of RsmE protein are present and need to be deactivated, the ncRNA RsmZ is expressed. Because only a single RNA has to be transcribed to sequester (temporarily deactivate) five RsmE proteins, the change in gene expression is fast. Furthermore, the high stability of the protein-RNA complex at high RsmE protein concentrations minimizes RNA degradation allowing the storage of the RsmE protein by the ncRNA RsmZ. Once the relative ratio of RsmE protein to RsmZ RNA decreases, the RsmZ RNA is becoming less stable and can potentially release the protein again.

To understand the molecular basis of the assembly mechanism and the protection of the ncRNA by the RsmE protein from nuclease degradation, structural information is highly desirable. Because such a multi-domain protein-RNA complex is unlikely to crystallize and even if crystallization is possible, crystal packing artifacts could easily distort the structure and topology of the complex, we aimed at determining the structure of a 70 kDa complex between the RsmZ(1-72) RNA and three RsmE protein dimers in solution [3, 6]. The high molecular weight, the large RNA component and the potential dynamic nature of the complex required the use of a modular and combinatorial approach: First, the structures of the isolated domains were solved by NMR spectroscopy [2]. Then, the structure of the entire complex was calculated by including into structure calculation simultaneously the short-range NMR restraints from the isolated domains and the long-range distance constraints between site-specifically introduced spin-labels on the RNA obtained from pulsed EPR spectroscopy. A detailed protocol is presented in figure 1B [6, 7].

Notably, we found that the protein-RNA complex consists of two distinct conformations simultaneously present in solution, which exchange slowly on the NMR timescale (figure 1C). The RNA topologies indicate that the two conformations cannot interconvert unless all the proteins have dissociated from the RNA. This is suggesting that the two protein-RNA complexes form via two parallel assembly pathways.

Why two conformations? At this stage, we can only speculate. It may be possible that two conformations are not required but are a consequence of the RNA being flexible. Optimization of the whole system with a conformationally flexible RNA might have the advantage of efficient sequestration of RsmE from the ribosome binding sites in various topological messenger RNA contexts.

Besides explaining the cooperativity in assembly, the structures also explain how the RsmE protein progressively protects the ncRNA from RNA degradation and finally how the RsmZ/ RsmE RNP eventually could be degraded.

Overall, by combining different biophysical and biochemical methods, we could shed light into how an RNA acts as a protein sponge by efficiently sequestering, then storing and finally releasing the proteins.

[1] T. Romeo, CA. Vakulskas et al., Environmental Microbiology, 2013.
[2] O. Duss, E. Michel et al., Nucleic Acids Research, 2014.
[3] O. Duss, E. Michel, M. Yulikov et al., Nature, 2014.
[4] O. Duss, C. Maris et al., Nucleic Acids Research, 2010.
[5] O. Duss, P. Lukavsky et al., Advances in Experimental Medicine and Biology, 2012.
[6] O. Duss, M. Yulikov et al., Nature Communications, 2014.
[7] O. Duss, M. Yulikov et al., Methods in Enzymology, 2015.

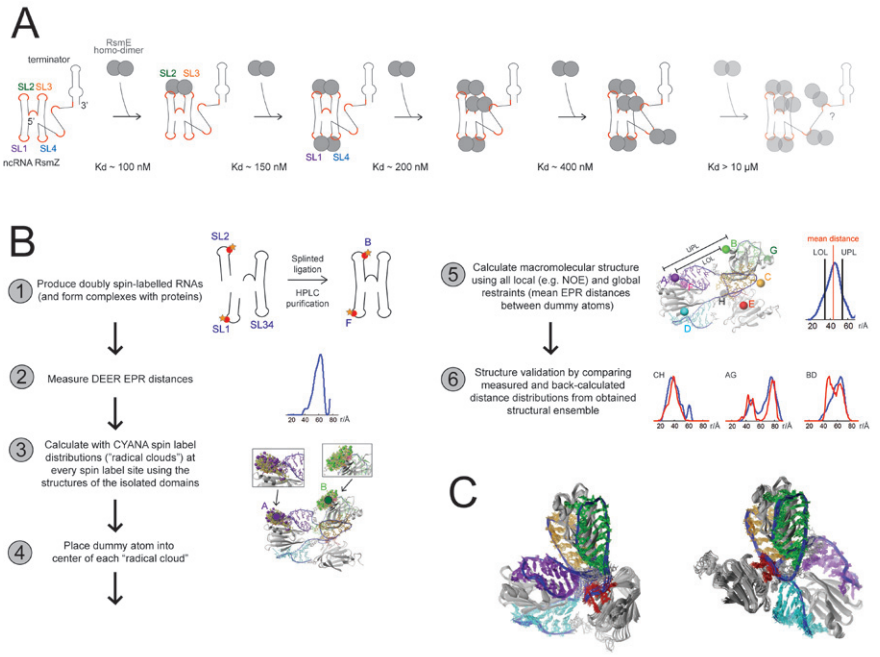

Figure 1: A) Sequential, cooperative and specific assembly of the RsmE/ RsmZ protein-RNA complex. Protein binding sites are shown in red. B) General protocol for structure determination of large RNAs or protein–RNA complexes using EPR-derived distance constraints [6,7]. C) Structural ensembles of both conformations in solution of the 70 kDa protein-RNA complex between the 72 nucleotides non-coding RNA RsmZ (in color) and three homo-dimeric RsmE proteins (grey ribbons) [2,3].

AMPERE Prize 2014
Christian Griesinger

Dept. for NMR-based Structural Biology, Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany

Conformational dynamics control the biological activity of proteins, thereby regulating the essential processes of life. (1,2) Conformational dynamics can be described by ensembles comprising the conformations assumed by a molecule and the measurement of the rates of interconversion between these distinct conformations. These various conformations and the kinetics of interconversion play a major role in molecular interaction, in the thermodynamic stability of functional states; in molecular recognition processes that may change the conformational ensembles; and in allostery and molecular signalling, where correlated motions can transmit information between distant sites in a protein. NMR spectroscopy has the unique possibility of measuring with subatomic resolution conformational ensembles as well as the kinetics of interconversion between its members. My lecture given on the occasion of the Ampere prize in Zürich summarizes the results from my laboratory in this field of research. It addressed three topics:

A) Ensembles of structures and their relation to thermodynamics of binding
B) Rates of interconversion between the ensemble members
C) Correlation of motion between ensemble members that might be the reason for allostery abundantly observed in proteins

A) Structural Ensembles

Residual dipolar couplings (RDCs) have been introduced almost 20 years ago to measure orientations of internuclear vectors in proteins (3,4). Already in the first publication of this then novel parameter Prestegard and colleagues (3) pointed out that RDCs might reveal in addition to the orientation of the internuclear vectors their dynamics. However, the dipolar coupling depends on the two polar angles of the internuclear vector and thus from the measurement of dipolar couplings in a single alignment medium the orientiation cannot be determined unambiguously. Therefore, we (5,6) and Joel Tolman (7) proposed to measure dipolar couplings in several alignment media that would allow to remove the ambiguity of the determination of orientations. Indeed when the measurement is done in at least 5 linearly independent alignment media not only the average orientation of each internuclear vector can be determined, but also the size of the dipolar coupling. This is indicated in Fig.1. More formally, 5 dipolar couplings

determined from linearly independent alignment media can be represented in the matrix equation 1 which can be inverted provided that the alignment matrices are indeed linearly independent.

$$\frac{D_i^{\exp}}{D_{i,zz}} = \sum_{M=-2}^{2} F_{i,M} \left\langle Y_{2,M}\left(\theta,\phi\right)\right\rangle \qquad \text{Eq.1}$$

where $\left\langle Y_{2M}(\theta,\phi)\right\rangle$ are the averaged spherical harmonics for a given NH vector, the angles θ and ϕ are measured in the molecular frame and $D_{i,zz}$ is the dipolar coupling observed for a static vector pointing along the z-ayis of the alignment tensor. The $F_{i,M}$ express the alignment tensor in the molecular frame. Their independence can be evaluated determining the so called condition number which measures the ratio between the largest and the smallest eigenvalue (5,6). The dipolar couplings can be used to determine conformational ensembles of the protein under investigation. Several ensembles have been determined from the NH dipolar couplings using different calculation methods whose characteristics are summarized in Fig.2 (EROS (8), ERNST (9), and EROSII (14)). Principal component analysis of the EROS ensemble reveals that 5 principal components suffice to describe 95% of the motion of the protein (8). The ensemble of structures can then be described as a distribution of points in the multidimensional principal component space. Fig. 3 shows the ensemble described by the coordinates in the two most varying principal components. The ensemble of X-structures that ubiquitin adopts when binding to recognition proteins is depicted as black triangles. The solution ensemble covers all the X-ray structures that ubiquitin adopts in the complexes. Thus all structures that ubiquitin has to adopt in complexes are present in solution already. This is a prerequisite for conformational selection as the binding mechanism of ubiquitin to recognition proteins. Analysis of the conformational ensemble of free ubiquitin suggests two main conformations that are mainly characterized by motion of the loop between the first two β-strands together with the end of the helix, the so called pincer mode (8). Roughly speaking the conformations can be subdivided in those where the pincer is open and those, where the pincer is closed. Since the conformational space of ubiquitin when bound in protein/protein complexes is restricted compared to free ubiquitin it is found to bind either in the open or closed conformation. Proteins binding the open form are therefore called the open binders and those binding the closed from the closed binders. Restricting the ubiquitin ensemble to the closed form should weaken the binding to

open binders and vice versa, restricting the ensemble to the open form should weaken the binding to the closed binders. Introducing mutations distant from the binding interface of ubiquitin indeed leads to a series of mutants which implement such restricted ensembles which either prefer the closed or open conformation. As anticipated the affinity to the open binder Dsk2 is maintained for the open mutants while it is reduced for the closed mutants (Fig. 4) (11). One of the mutants had been described already by David Fushman (12).

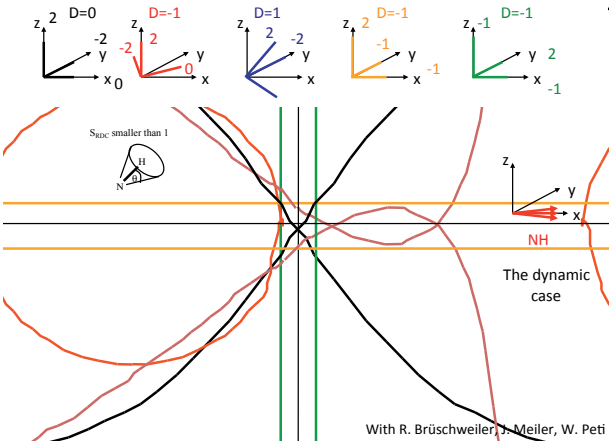

Figure 1
RDCs reveal conformational dynamics: Five linearly independent tensors are assumed for a vector that assumes within the alignment tensor different orientations. Due to the reduction of the dipolar coupling, the iso-dipolar coupling curves drawn on the sphere in the polar representation don't intersect at θ=90, φ = 0 dynamic vector. This fact can be used for the identification of dynamics.

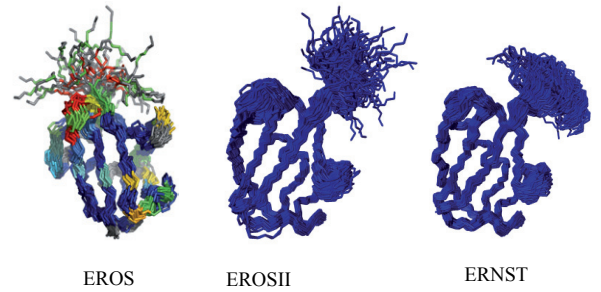

Figure 2
Ensemble nature of ubiquitin in solution: Three ensembles calculated from the same set of dipolar couplings published in reference: EROS (8), EROSII (14) and ERNST (9). Different from many rephe ensemble nature of the protein in solution does not reflect lack of NMR parameters but rather

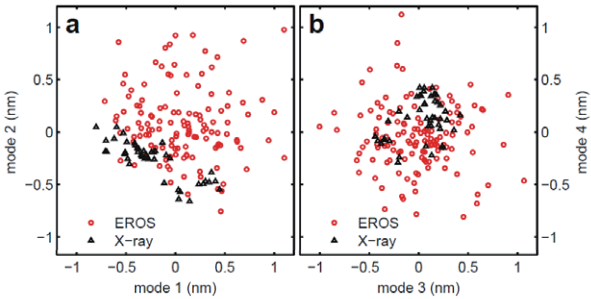

Figure 3
Principal component analysis of EROS (8) ensemble in red dots. The 46 X-ray structures of ubiquitin in complexes with recognition proteins are shown as triangles. Since the bound conformations are represented also by free ubiquitin, conformational selection is a possible mechanism for binding.

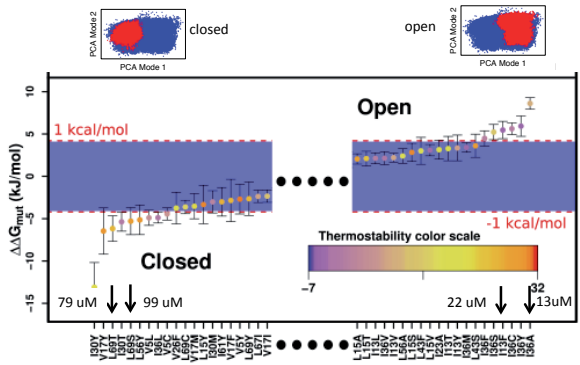

Figure 4
Modulation of binding selectivity by restricting conformational ensembles: Top: predicted conformational ensembles of free ubiquitin (blue) and ubiquitin bound to an open (red) or closed (red) binder. Below: Relative binding affinities of mutants of ubiquitin to ubiquitin binders that recognize either the open or closed form. The experimental binding affinities to the open binder Dsk2 are indicated as numbers and reproduce the predictions. The mutations affect the hydrophobic core but not the binding interface.

B) Kinetics

Dipolar couplings sample orientations of internuclear vectors in membersof structural ensembles that interconvert on any time scale from picoseconds to around 10 ms (Fig. 5). Heteronuclear relaxation rates conventionally measured between nitrogen and hydrogen or carbon and hydrogen sample rates of interconversion between different conformations on a time scale shorter than the correlation time that for the protein ubiquitin is at 4 ns. Slower motion, between around 50 µs and 10 ms can be addressed by relaxation dispersion, that relies on the exchange contribution to the transverse relaxation rate of each nucleus. This exchange contribution is proportional to the square of the difference between the isotropic chemical shifts that each of the conformations have. The rates of interconversion are measured by increasing the radiofrequency field in a $T_{1\rho}$ experiment according to the most simple implementation of the exchange contribution:

$$1/T_{1ex} = R_{1ex} = \frac{\Phi_{ex}}{\tau_{ex}\left(1+\left(\gamma B_1 \tau_{ex}\right)^2\right)} \qquad \Phi_{ex} = p_1 p_2 \left(\omega_1 - \omega_2\right)^2 \qquad \text{Eq.2}$$

Technical limitations of NMR probes set the limit for the fastest rates to be recorded conventionally to around 50 µs leaving a gap of approximately 4 orders of magnitude between 4 ns and 50 µs. The gap in time scales between the correlation time of 4 ns and the fastest accessible in conventional probes of 50 µs can be reduced by increasing the radiofrequency power, which is possible on cryogenic probes (13) due to their high radiofrequency tolerance. This allows to increase the fastest measurable rates to the reciprocal of 4 µs from ^{1}H, 10 µs from ^{13}C, and 25 µs from ^{15}N relaxation dispersion. Together with lowering the sample temperature and thus reducing rates of interconversion between conformation this has made accessible motion in the supra-T_c window that were previously inaccessible. Application of relaxation dispersion methods thus has revealed that a major component of the supra-T_c motion occurs between 1 and 19 µs at 35°C (10). The observed time scale could be linked to the ensemble determined from the residual dipolar couplings described under A) by comparison of the predicted and measured chemical shift variances (Fig. 6).

In a second application of exploiting the increase of the time scales by increase of radiofrequency power side chain motions could be characterized by the comparison of ^{1}H and ^{13}C relaxation dispersion. Observing the terminal methyl groups of the side chains, relaxation dispersion was observed only for the carbons, but not the protons suggesting that the dispersion stems from the rotameric jumps of the side chains. However, the relaxation dispersion Φ_{ex} value observed is too small to allow for the

appreciable populations of at least two rotamers (14). Therefore a more complex rotamer shuffling model had to be introduced (15). This model requires at least two macrostates which feature different populations of the rotameric states of the side chains (Fig. 7). The rotamers still interconvert on a time scale faster than the relaxation dispersion time scale, while the macrostates interconvert with the time scale seen by relaxation dispersion. The relaxation dispersion formula from Eq. 2 has therefore to be modified to that of Eq. 3, in which there are contributions due to change of population of the trans as well as the gauche conformation.

$$1/T_{1ex} = R_{1ex} = \frac{\Phi_{ex}^{shuffling}}{\tau_{ex}\left(1+\left(\gamma B_1 \tau_{ex}\right)^2\right)} \qquad \Phi_{ex}^{shuffling} = \frac{\Delta p_t \Delta p_g \left(\omega_t - \omega_g\right)^2}{4} \qquad \text{Eq.3}$$

C) Correlations

Correlations in conformational interconversions, specifically when proteins fold, have been described by theoreticians, but lack experimental proof (16). Most dynamic NMR parameters are local and focus on a specific bond or atom, such as relaxation parameters. Cross hydrogen bond couplings (17) or chemical shifts (18) also depend on motion and due to their more complex nature are sensitive to larger parts of the surrounding of the involved nucleus and nuclei, respectively. This has been used to measure correlation phenomena in proteins before (15,16). These parameters depend on a correct structural interpretation by quantum chemical methods. Cross correlated relaxation (19) does not require such quantum chemical calculations and the influence of dynamics on this parameter can be interpreted more directly (20) as proposed by Geoffrey Bodenhausen and colleagues on sequential amide NH vectors. Cross validation against these data and additional sequential and intraresidual C_α-H_α/NH cross correlated relaxation revealed that the best fit was obtained using ensembles rather than single structures (Fig. 8) (9). Extensions to evaluate the correlations of motion of further vector pairs are on their way.

D) Conclusion

The work presented summarizes one line of research pursued in my laboratory over at least 15 years starting with Jens Meiler, now at Vanderbildt university, Wolfgang Peti, now at Brown university, and Rafael Brüschweiler, now at Ohio state university. It continued by the meticulous work of Nils-Alexander Lakomek, now a postdoc at ETH Zürich, who won the Andrew prize based on his thesis that included the ensemble description

of ubiquitin. The presented ensembles could be obtained in a very intense collaboration with the theoretical department of my colleague Helmut Grubmüller at the MPI for biophysical chemistry, specifically with Helmut himself, Bert de Groot and Oliver Lange. In addition, in fruitful collaborations with Xavier Salvatella as well as Bryn Fenwick, the ERNST ensemble could be obtained and they determined the amount of correlations from cross correlated relaxation rates measured in my group as well as Geoffrey Bodenhausen's. Finally, David Ban, now a postdoc at St. Jude's hospital, as well as Mike Sabo and Donghan Lee, both now and soon Professors at the university of Louisville, Kentucky, USA, pushed the high power relaxation dispersion experiments that allow these days to measure kinetics down to 4 µs on protons and allowed to derive the population shuffling model for side chains.

Further projects in the group were just mentioned in passing at the end of the prize lecture. They involve the determination of configuration of dynamical small molecules (21), the structure determination of membrane proteins by integration of liquid and solid state NMR as well as X-ray crystallography (22) and finally our most applied project on neurodegeneration as well as neuroprotection (23).

I am grateful to my academic teachers, Prof. Horst Kessler, München, and Prof. Richard R. Ernst, ETH Zürich, and feel deeply honored to have received the Ampere prize on the occasion of the EUROMAR in Zürich, which is not only Richard's university but hosted me to perfection during my doctoral and postdoctoral stay in 1985 and from 1986 till 1989, respectively.

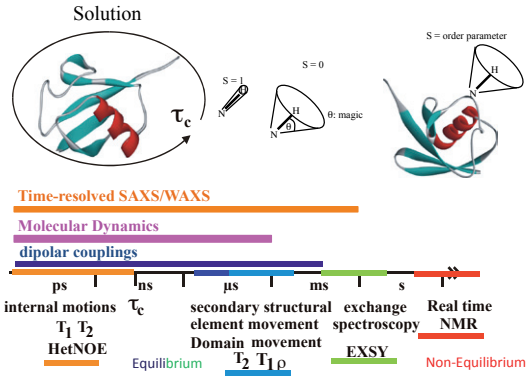

Figure 5
Time scales addressed by different NMR parameters as well as Molecular dynamics (MD) and scattering methods (SAXS/WAXS). Dipolar couplings address the time range up to 10 ms. Relaxation dispersion has been extended from 25 µs down to 4 µs for protons indicated by the darker blue color.

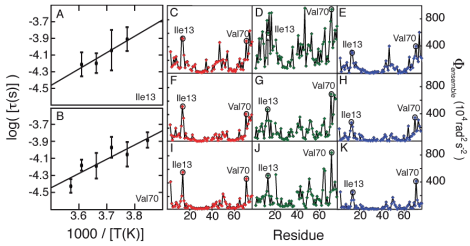

Figure 6 Arrhenius type fit to temperature dependent magnetic relaxation dispersion exchange rates (Table S1) at super-cooled temperatures for Ile13 (A) and Val70 (B). Activation energies of 37 ± 7 kJ/mol and 32 ± 8 kJ/mol were extracted for Ile13 and Val70, respectively. Errors were estimated by Monte Carlo simulation. (C-K): Predicted chemical shift variance (Φensemble) of EROS (C, D, and E), EROSII (F, G, and H) and ERNST (I, J, and K) ensembles. Chemical shifts were extracted from the ensemble members (N=116 for EROS, N = 176 for EROSII, N = 320 for ERNST) with the programs SHIFTX (C, F, and I; red), SHIFTS (D, G, and J; green), and SPARTA (E, H, and K; blue). Residues Ile13 and Val70 showing consistently largest Φensemble through all ensembles and all programs are indicated. It should be noted, that the conformational spread of the ensembles reflects the distribution at 308 K while the experiments were done between 265 and 273 K, which explains the smaller experimental values.

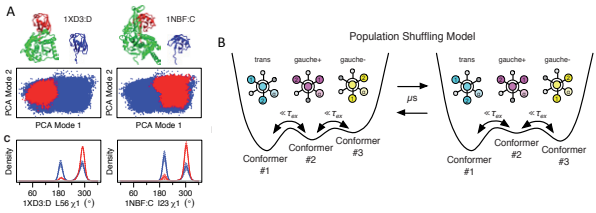

Figure 7
Population shuffling of side chain conformations (15): A) the open and closed conformation of ubiquitin have different side chain populations. B) Relaxation dispersion reveals conformational transitions on a µs time scale between the two macrostates which each feature different populations of the rotamers. In the population shuffling model they interconvert on a ns time scale.

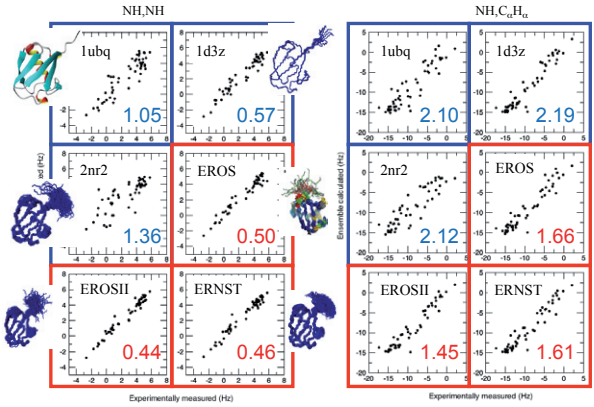

Figure 8
Correlated motion in ubiquitin: Cross validation of various structures and structural ensembles against intraresidual and interresidual cross correlated relaxation rates (9). The deviation of the back-calculated to the experimental values is shown and indicates that the ensembles represent the motion better than single structures.

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The scientific talks will start on Monday morning at 8:30 am.
The last session will finish at 12:30 pm on Thursday 17th September

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- B. H. Meier (Zürich)
- H. Oschkinat (Berlin)
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Scientific Committee
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Other events
2015

19th ISMAR conference	Shanghai (China)	August 16-21 2015