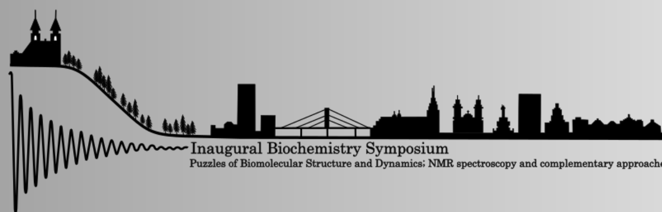

JKU
Institute of Biochemistry

Inaugural Biochemistry Symposium

Puzzles of (Bio)molecular Structure and Dynamics:

NMR spectroscopy and complementary approaches

Location: Uni-Center Loft at Johannes Kepler University Linz, Austria.

Date: 23--24 May 2023

https://bit.ly/3IoQ92O

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1. The symposium is organized by

Institute of Biochemistry

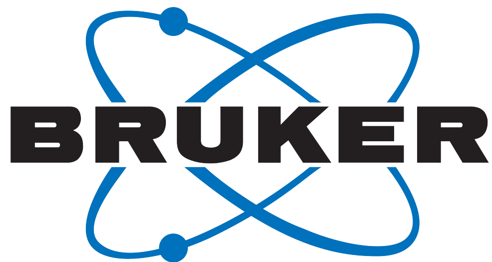
Johannes Kepler University Linz

Altenberger Straße 69

4040 Linz

https://www.jku.at/biochem

2. The symposium is sponsored by



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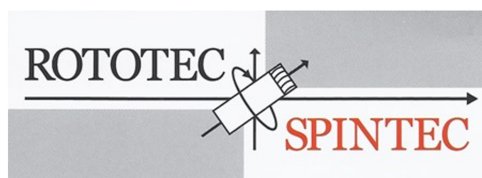

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3. Directions and Traveling

The symposium is held at the Loft of Uni-Center at JKU Linz. For further information go to:

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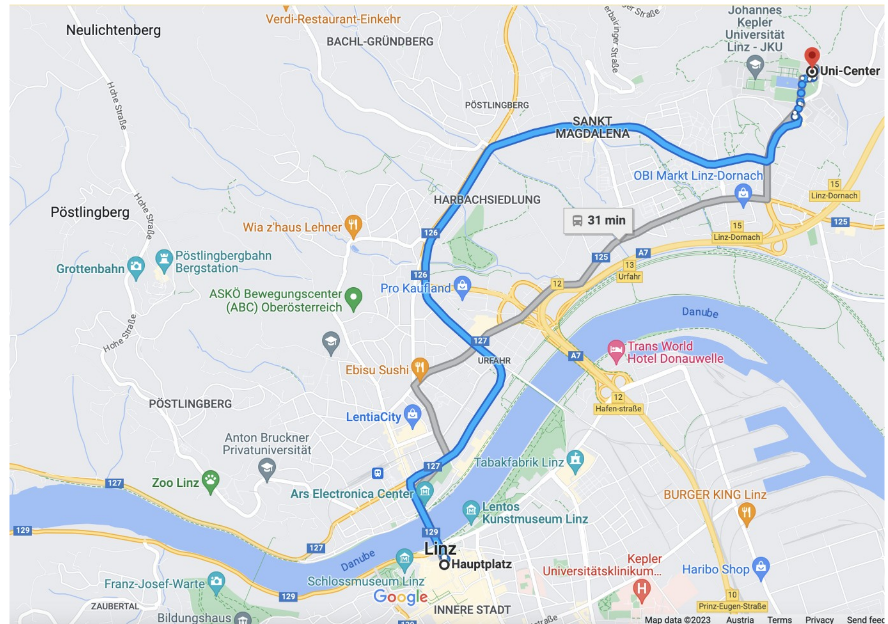
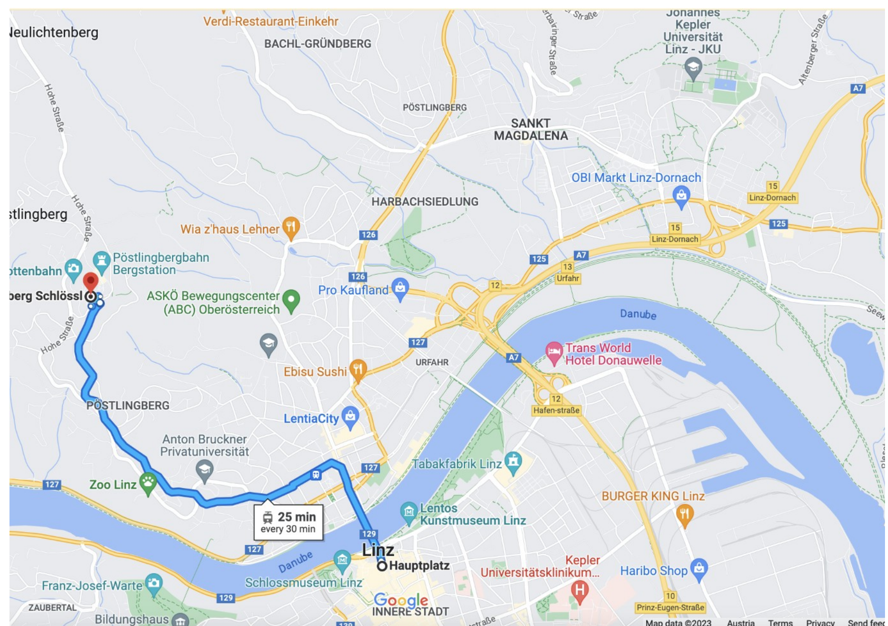


The conference dinner is run at Pöstlingberg Schlößl: https://poestlingberg.at
There is a special tram service to this place departing 23.May at 18:45 from Hauptplatz Linz and returns at 22:45.



4. Program

Location: LOFT, Uni-Center,

Tuesday 23 May 2023

10:00 registration opens + coffee
10:25 opening

Session 1: NMR, metabolites & cells (chair: Adriana Rathner)

10:30 **Marco Tessari**, Radboud Universiteit, Nijmegen (NL)
NMR Discrimination of enantiomers at sub-micromolar concentration via parahydrogen-Induced hyperpolarization
11:10 **Simone Fjordside**, Aarhus University/Novo Nordisk A/S (DK)
Metabolic Profiling of a Hepatic ER Stress Model
11:30 **Claudio Luchinat**, CERM/CIRMMP, Università di Firenze (IT)
Metabolomics by NMR: achievements and perspectives

12:10 lunch & poster session

Session 2: protein structure & dynamics with NMR (chair: Mario Schubert)

13:30 **Claire Raingeval**, Aarhus University (DK)
Accurate extraction of site-specific methyl deuterium quadrupolar patterns: from simulation to experiment
13:50 **Paul Schanda**, Institute of Science and Technology, Klosterneuburg (AT)
NMR in solution and solids: playing with spins and isotopes to resolve biological mechanisms
14:30 **Klaartje Houben**, DSM Science & Innovation, Delft (NL)
Characterization of food proteins and enzymes

15:10 tea break - group photo

Session 3: protein structure & dynamics with X-rays (chair: Robert Konrat)

15:40 **Martin Weik**, IBS, Grenoble (FR)
Making molecular movies of proteins at work using bright X-ray sources
16:20 **Jean van den Elsen**, University of Bath (UK)
Compounds from the cowshed: designing novel complement inhibitors

17:00 free time / check-in at hotel

18:45 Pöstlingbergbahn Special service from Hauptplatz to Pöstlingberg
19:00 Dinner at Pöstlingberg Schlössl
22:45 Pöstlingbergbahn Special service from Pöstlingberg to Hauptplatz
(earlier service at own cost or walking down anytime possible)

Wednesday 24 May 2023

Session 4: dynamics & recognition (chair: Remco Sprangers)

9:00 **Mikael Akke**, Lund Universitet (SE)
Protein–ligand binding studied by NMR

9:40 **Lars Schäfer**, Ruhr University, Bochum (DE)
NMR experiments and MD simulations: A Love Relation?

10:20 coffee break

Session 5: structure & design (chair: Rob Kaptein)

10:50 **Ingemar André**, Lund Universitet (SE)
Design and prediction of the structure of protein containers"

11:30 **Marco Sette**, Università di Roma "Tor Vergata", Rome (IT)
NMR for therapeutical peptides

12:10 lunch & poster session

Session 6: Proteins, RNA, and electrostatics (chair: Norbert Müller)

13:10 **Rolf Boelens**, Universiteit Utrecht (NL)
Structure and dynamics in gene regulation

13:50 **Anthony Mittermaier**, McGill University (CA)
Dynamics of Guanine Quadruplexes

14:30 **Lewis E. Kay**, University of Toronto (CA)
Using magnetic fields to probe electric fields of biomolecules

15:10 closing remarks

15:20 symposium ends + short tours at the Institute of Biochemistry
TNF tower, 5th floor (refreshments & snacks provided)

5. Posters & Poster Abstracts

Poster #	Presenting Author	*Title*
#1	Abi Saad, Marie Jose	*Unlocking the Potential of Allosteric Inhibitors for Future Pandemics: Targeting the Main protease of SARS-CoV-2*
#2	Baumgartner, Manuel	*Investigating the Activation of a Calcium Sensor Protein Dimer Connected at the N-Term*
#3	Buhl, Julie	*The power of co-solute PRE in NMR*
#4	Cabrera, Andrea	*Intermolecular dynamics and allostery of human BRD4-BD1 and -BD2*
#5	Fjordside, Simone	*A Real-Time Approach to Study Cellular Metabolism in Liver Cells*
#6	Gemmecker, Gerd	*Investigating ligand-induced conformational changes in the tRNA-guanine transglycosylase dimer by ^{19}F NMR*
#7	Giri, Malyasree	*Probing electrostatics from salt-dependent hydrogen exchange rates in the unfolded protein state*
#8	Gubensäk, Nina	*Vibrio cholerae's ToxRS Bile Sensing System*
#9	Hejduk, Libor	*Do structural differences of Decorin binding proteins from European Borrelia genospecies influence glycosaminoglycanes binding?*
#10	Holzinger, Julian	*RDCs and the Order of the PROTAC*
#11	Kadeřávek, Pavel	*Domain 1.1 of σA factor of RNA polymerase from Bacillus subtilis beyond its major state conformation*
#12	Krempl, Christina	*Combining NMR and smFRET experiments to study the conformations of the Dcp1:Dcp2 mRNA decapping complex*
#13	Napoli, Federico	*Long-range coupling in a dodecameric enzyme complex unraveled by solid-state NMR*
#14	Pantoja, Christian Felipe	*NMR spectroscopy to study the thermodynamics of liquid-liquid phase separation of human Tau protein*
#15	Raingeval, Claire	*Accurate extraction of site-specific methyl deuterium quadrupolar patterns: from simulation to experimentation*
#16	Rathner, Adriana	*Exploring Interactions of Adhesins from Pathogenic Borrelia*
#17	Rohden, Darja	*Synthesis and Incorporation of Arginine with Specific Isotope Patterns to Study Protein Dynamics by NMR*
#18	Schmalix, Alexander	*Regulation of Nedd4 family E3 ubiquitin ligases through auto-inhibition*
#19	Schneider, Jakob	*Substrate binding of the 65 kDa cytosolic domain of Tom70 studied by NMR spectroscopy*
#20	Tužinčin, D.	*Domain 1.1 of σ A factor of RNA polymerase from Bacillus subtilis beyond its major state conformation*
#21	Wilson, Russell J.	*2D Mechanoresponsive Surfaces for Measuring Cellular Traction Forces*

#1

Unlocking the Potential of Allosteric Inhibitors for Future Pandemics: Targeting the Main protease of SARS-CoV-2.

Marie José Abi Saad[1-2], Nicolas Coudevylle[1], Julien Orts[1]*

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[2]Vienna Doctoral School of Pharmaceutical, Nutritional and Sport Sciences, University of Vienna, Vienna, Austria.

The threat of future pandemics remains a significant global health challenge, as seen in the ongoing COVID-19 pandemic caused by SARS-CoV-2. Recent studies have linked COVID-19 to neurodegenerative diseases [1], highlighting the need for effective therapeutics. While vaccines have been developed, they alone are not sufficient to combat the pandemic. Therefore, identifying drugs that can inhibit SARS-CoV-2 replication, in addition to vaccines, is a promising approach.

This study focuses on the main protease (Mpro) of SARS-CoV-2, which plays a critical role in the virulence of the virus. Currently, only two drugs (remdesivir and paxlovid) have been approved by the FDA for the treatment of COVID-19. However, the protease can mutate, leading to increased resistance to the active ingredients of drugs used for treatment [2]. Allosteric sites are usually less prone to mutations, making them attractive targets for drug discovery. It has emerged as a potential solution to minimise side effects associated with drug interactions within the Mpro binding pocket.

Within the international Covid19-NMR consortium, we established a protocol for Mpro production [3] and performed NMR screening on a library of fragments [4], natural products [5], and FDA-approved drugs. Many FDA-approved drugs appeared to interact within the binding pocket of Mpro, but showed severe aftermaths such as heart failure and cardiotoxicity. Therefore, the search for allosteric inhibitors might be the solution to minimize side effects. So far, six repurposed FDA drugs have shown allosteric inhibitor activity against Mpro [6].

To identify allosteric binding sites and provide the medicinal chemistry community with clear structure-activity relationship information for the rapid improvement of Mpro-targeted drugs, we aim to utilise advanced Nuclear Magnetic Resonance (NMR) and X-ray crystallography techniques. Our research hopes to contribute to the development of effective drugs not only for the current COVID-19 pandemic but also for future ones.

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2. Service R., (2022) Bad news for Paxlovid? Coronavirus can find multiple ways to evade COVID-19 drug., Science, 337.
3. Altincekic N., Orts J., Abi Saad MJ., et al., (2021) Large-scale recombinant production of SARS-CoV-2 proteome for high-throughput and structural biology applications., Frontiers in Molecular Biosciences, 8, 89.
4. Berg H., Abi Saad MJ., Orts J., et al., (3023) Comprehensive Fragment Screening of the SARS-CoV-2 Proteome Explores Novel Chemical Space for Drug Development., Angewandte Chemie, 134 (2022).
5. Wasilewicz A., Kirchweger B., Bojkova D., Abi Saad M.J., et al., Identification of natural products inhibiting SARS-CoV-2 by targeting the viral proteases: a combined in silico and in vitro approach., J. Nat. Prod.
6. Günther S., et al., (2021) X-ray screening identifies active site and allosteric inhibitors of SARS-CoV-2 main protease., Science, 372, 642-646.

*Ass. Prof. Dr. Julien Orts: julien.orts@univie.ac.at

#2

Investigating the Activation of a Calcium Sensor Protein Dimer Connected at the N-Term

Manuel Baumgartner*[a], Agrim Gupta[a], Adriana Rathner[c], Petr Rathner[a], Katharina Röser[a], Marc Fahrner[b], Matthias Bechmann[a], Solomia Pylypchuk[c], Christoph Romanin[b], and Norbert Müller[a,c,d]

[a]*Institute of Organic Chemistry, Johannes Kepler University Linz, Austria*
[b]*Institute of Biophysics, Johannes Kepler University Linz, Austria*
[c]*Institute of Biochemistry, Johannes Kepler University Linz, Austria*
[d]*Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*

The calcium sensor protein, stromal interaction molecule 1 (STIM1), is involved in the activation of the calcium release activated calcium (CRAC) channel [1]. In the activated state, STIM1 spans from the lumen of the endoplasmatic reticulum (ER) to the plasma membrane-bound CRAC channel. The depletion of calcium in the ER, activates STIM1 and leads to its homo-oligomerization and spatial elongation. Three cytosolic coiled-coil fragments of STIM1 (CC1, CC2 and CC3) modulate various intra- and intermolecular interactions involved in the transition between the resting (closed) and active (extended) conformation [2].

As a monomer, the isolated CC1 domain of STIM1 forms a three-helix bundle, which is stabilized by interhelical coiled-coil interactions in solution [3]. We have previously detected interactions between the different coiled-coil domains using chemical shift perturbation monitoring. We constructed artificial dimers by connecting two CC1 fragments at the N-terminal side. These dimers are used to study the role of homo-dimerisation in the activation of STIM1 by comparing their properties to the monomeric protein using NMR spectroscopy.

References

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* Corresponding author: manuel.baumgartner@jku.at

#3

The power of co-solute PRE in NMR

Julie Maibøll Buhl[*a], Benjamin Andreas Wahlqvist[c], Jakub Obuch[b], Petr Hermann[b], Frans A. A. Mulder[a]

[a]*Institute of Biochemistry, Johannes Kepler University Linz, Austria*
[b]*Department of Inorganic Chemistry, Charles University in Prague, CZ*
[c]*Interdisciplinary Nanoscience Center - INANO-Kemi, iNANO-huset Aarhus C, Denmark*

The employment of paramagnetic substances in NMR has been prevalent for many years due to the well-known effects on relaxation, also referred to as paramagnetic relaxation enhancement (PRE). Here, we have explored three applications for co-solute PRE, which span from observations seen in small molecules to a newly proposed method for determining the electrostatic potential in macromolecules. All applications utilize gadolinium-based agent, chosen for the large magnetic moment and stability of the chelates.

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#4

Intermolecular dynamics and allostery of human BRD4-BD1 and -BD2

A. Cabrera[1], N. Coudevylle[1], J. Orts[1*]

[1]*Department of Pharmaceutical Sciences, University of Vienna, Josef-Holaubek-Platz 2, A-1090 Vienna, Austria*

A deep understanding of the structure-activity relationship between proteins and their ligands is crucial for the development of drugs. A thorough description of the intermolecular interactions requires the characterization of geometry (structure) and dynamics of the molecular complex at atomic resolution, including the internal dynamics of both partners and the intermolecular dynamics taking place at the complex interface. However, the quantitative involvement of dynamics has been rarely studied due to the lack of adequate experimental methods at such resolution.

This project aims to establish a quantitative structure-dynamics-activity relationship for protein-ligand interactions. Proteins BD1 and BD2, which are part of the human bromodomain-containing protein 4, will be probed along with several small binders. First, a structural ensemble of proteins in their free and complex forms will be obtained by NMR. NOE build-up curves will be acquired and converted into distances, from which a multiple-state ensemble of structures will be derived. NOE violations will be analyzed to obtain a map at atomic resolution of allosteric communications through motions in the apo- and holo-proteins, which will allow to follow the effect of ligand binding on the protein dynamics. Afterwards, NOESY experiments at several t_{mix} and temperatures will be acquired to derive population distributions from which thermodynamic parameters ΔH and ΔS will be calculated. This will enable for "calorimetry-like" measurements to be obtained, hence determining at atomic resolution the enthalpy and entropy of the protein-ligand interaction.

ITC experiments have been done to derive the global affinity, ΔH, and ΔS of the binding of BD1 and BD2 with ligand ABBV-774. The backbone assignment of both proteins has been completed, as well as relaxation experiments to derive their τ_c. The next milestone is to acquire and interpret the NOE build-up curves to derive the network of distances covering the whole protein (dynamics-activity relationship).

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#5

A Real-Time Approach to Study Cellular Metabolism in Liver Cells

Simone Fjordside*[a]

[a]*Interdisciplinary Nanoscience Center - INANO-Kemi, iNANO-huset Aarhus C, Denmark*

Metabolomic studies deal with metabolites within a biological system. This is a powerful tool, which can be used for phenotypic fingerprinting and quantitative analysis. However, metabolomic *in vitro* studies are performed at static conditions and at exact time points following cell lysis. In-Cell NMR makes it possible to do metabolomic studies directly on living cells in an untargeted and label-free environment.

Currently, the **purpose is to establish a stable mammalian cellular setup in which liver cells are encapsulated in a hydrogel inside the In-Cell NMR bioreactor** and are continuously supplied with fresh medium. The In-Cell NMR bioreactor can be placed inside the NMR spectrometer and NMR spectra are monitored continuously for the entire experimental time. Cell viability is determined in order to compare viability at the beginning and end of the experimental setup. By establishing an In-Cell NMR Bioreactor it will be possible to do real-time metabolomic studies *in vitro*.

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#6

Investigating ligand-induced conformational changes in the tRNA-guanine transglycosylase dimer by ^{19}F NMR

G. Gemmecker[1*], A. Nguyen[2], M. Sattler[1], G. Klebe[2], F. Diederich[3]

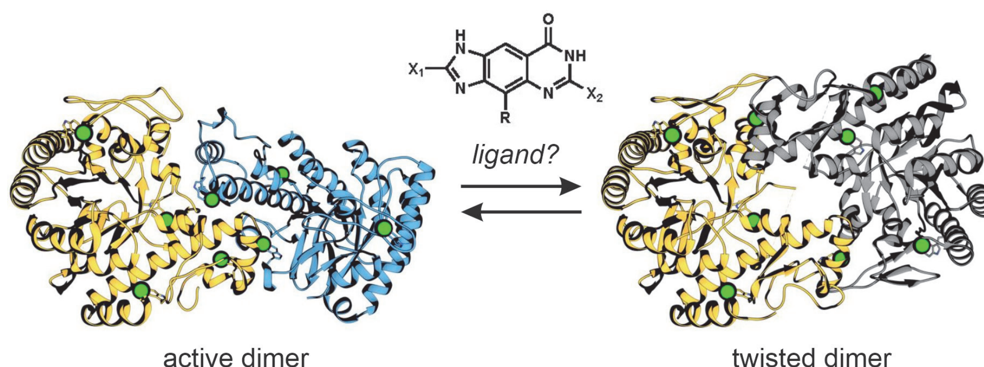
[1] TU Munich, Garching, Germany & HelmholtzZentrum München, Neuherberg, Germany,
[2] Philipps University, Marburg, Germany, [3] ETH Zürich, Zurich, Switzerland

Crystallographic studies have recently shown a novel twisted and functionally inactive form of the homodimeric tRNA-guanine transglycosylase (TGT), a putative target against Shigellosis.

In those X-ray studies, some active-site ligands are exhibiting a 130° twist between the two monomers, leading to a functional loss of TGT. Additonal NMR investigations in solution could verify and quantify ligand-induced shifts of the equilibrium between native and twisted dimer states.

Due to the size of the TGT dimer (86 kDa) we have used ^{19}F-NMR spectroscopy, incorporating 5-fluorotryptophans (5FW) at the four tryptophan positions in wild type TGT. The inhibitor-induced conformation of 5FW-TGT in solution was assessed from ^{19}F-NMR chemical shift perturbations relative to free 5FW-TGT. In 1D ^{19}F spectra, different perturbation patterns could be observed that can be correlated with either a normal or twist-inducing ligand binding.

It could be shown that ligands known to induce the twisted dimer in crystals will lead to a characteristic ^{19}F shift pattern, with a pronounced signal shift for 5F-W178 and 5F-W326. However, other ligands not known to induce the twisted state in crystals were found to show a ^{19}F pattern that implies the existence of a dynamic equilibrium between normal and twisted state. Comparison of various benzoguanine ligand structures hints to a role of the C4 substituent in inducing the twisted state, by de-stabilizing helix αA and the adjacent loop-helix motif near the ligand-bindig site of TGT. The ^{19}F shift pattern of the normal functional 5FW-TGT dimer could only be seen with ligands without any C4 substituent – or with a substituent actually stabilizing helix αA with H-bonds. These findings on the various degrees of twist-induction from different ligands suggest a novel concept for the design of new drug candidates.



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#7

Probing electrostatics from salt-dependent hydrogen exchange rates in the unfolded protein state

Malyasree Giri[1*], Julie Maibøll Buhl[2] and Frans A.A. Mulder[1,2]

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Hydrogen exchange (HX) plays an important role in protein stability and folding. It is important to account for the protein's electrostatics in the interpretation of HX rates as electrostatic effects influence the HX rates which in turn affects the evaluation of protection factors (PFs). The precise measurement of PFs and their meaningful interpretation needs a quantitative study of the influence of electrostatics on HX in the unfolded state. Our objective is to compute electrostatics from salt-dependent hydrogen exchange rates in the unfolded protein state using Phase-Modulated CLEAN Chemical EXchange Spectroscopy (CLEANEX-PM) [1,2]. The CLEANEX-PM indicates ^{1}H signals that transfer the magnetization saturation from the water to ^{1}H amide resonance during the mixing period of the pulse sequence in NMR experiments. We used human α-synuclein (αSN) as a proxy for the unfolded state. It is an intrinsically disordered protein (IDP) of 140 amino acids which contains a small net positively charged N-terminal domain (residues 1–61), a central region (residues 62–95; also known as the nonamyloid-b component region) that is devoid of charged amino acids with two exceptions, and a highly acidic C-terminal tail (residues 96–140). Backbone resonances of the alpha-synuclein at pH 5.8 and 25 °C were assigned using BMRB entry 16543 and ^{15}N-TOCSY-HSQC and ^{15}N-NOESY-HSQC spectra. CLEANEX-PM experiments were performed at 25 °C with different exchange duration times at various pH and salt concentrations. At each pH and salt concentration, a series of 2D (CLEANEX-PM)-FHSQC spectra was recorded with different mixing times to measure the peak volumes V and an FHSQC spectrum was recorded to measure the reference peak volumes V_0. We obtained intensity build-up curves (V/V_0) for a few residues at different pH and salt concentrations. Accurate chemical exchange rates can be obtained from initial slope analysis. The experimentally determined HX protection factors can be interpreted correctly by considering these effects [3]. HX rates determined at various salt concentrations can help to build a comprehensive model which can account for electrostatic effects and can be utilized as quantitative proxies for determining the electrostatic potential around IDPs.

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#8

Vibrio cholerae's ToxRS Bile Sensing System

N. Gubensäk[1]*, T. Sagmeister[1], C. Buhlheller[1], B. D. Geronimo[2], G. E. Wagner[3,4], L. Petrowitsch[1], M. Gräwert[5], M. Rotzinger[3], T. M. I. Berger[1], J. Schäfer[6], I. Usón[7,8], J. Reidl[1,9,10], P. A. Sánchez-Murcia[2], K. Zangger[3,9,10] and T. Pavkov-Keller[1,9,10]*

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[5] EMBL Hamburg; Hamburg, Germany.
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[10] BioTechMed-Graz; Graz, Austria..

Cholera represents a diarrheal disease caused by the Gram-negative bacterium *Vibrio cholerae*. Its environmental persistence causing recurring sudden outbreaks is enabled by *V. cholerae*'s rapid adaption to changing environments involving sensory proteins like ToxR and ToxS. Located at the inner membrane, ToxR and ToxS react to environmental stimuli like bile acid thereby inducing survival strategies e.g. bile resistance and virulence regulation [1]. Currently, transcription factor ToxR is described as main environmental sensor for bile acid [2]. whose activity is enhanced by binding to ToxS [3,4].

In-depth analysis of the ToxRS complex enabled unraveling of an intricate mechanisms governing ToxRS's environmental sensing capabilities. Thereby providing a promising tool for disruption of this vital interaction, ultimately inhibiting *Vibrio's* survival and virulence. Our findings hold far-reaching implications for all *Vibrio* strains that rely on the ToxRS system as a shared sensory cornerstone for adapting to their surroundings.

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#9

Do structural differences of Decorin binding proteins from European *Borrelia* genospecies influence glycosaminoglycanes binding?

Libor Hejduk[1,2], Martin Strnad[1,2], Manuel Baumgartner[3], Filip Dyčka[1], Libor Grubhoffer[1,2], Ján Štěrba[1], Ryan O. M. Rego[1,2,] Norbert Müller[1], Adriana Rathner[4*]

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Adhesion of spirochetes from *Borrelia burgdorferi* sensu lato complex is the crucial step in the initial phase of Lyme disease infection. Decorin-binding proteins (Dbp) are glycosaminoglycan (GAG) binding adhesins exposed on the surface of *borrelia* spirochetes. Dbps are expressed in two homologous forms A and B, both were characterized as main factors of *borrelia* virulence [1]. Based on the previous described differences in binding mechanisms of Dbp-GAG interaction [2], we focused on the relations between structural differences and GAG binding. We aim to describe the structural differences in detail among Dbps from European *Borrelia* species and their particular interactions with different GAGs using solution nuclear magnetic resonance (NMR) spectroscopy at atomic resolution. Almost complete backbone and sidechain assignments of DbpA from *B. Afzelii* and *B. Bavariensis* have been achieved [3] and assignment for other Dbp protein homologues is in progress. Secondary structure propensity based on chemical shifts and backbone dynamics (T_1 and T_2 relaxations, heteronuclear ^{1}H-^{15}N NOE) for both variants were compared with available NMR structures of North American *Borrelia* species. For more accurate assignment of methyl groups we also expressed selectively unlabelled proteins (KIT/ KILT amino acids combinations). We performed initial protein-GAG interaction studies of variants of DbpA/DbpB with different GAGs by NMR titrations and by hydrogen-deuterium exchange mass spectrometry (HDX-MS). Additionally, Kd values of binding were measured by microscale thermophoresis (MST). NMR-based structural and interaction analyses combined with HDX experiments indicate species-specific differences in GAG binding and set the starting point for extensive detailed research of the influence of small structural and dynamic differences and their impact on GAG binding.

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#10

RDCs and the Order of the PROTAC – Determining Conformational Ensembles in Solution

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PROTACs are heterobifunctional molecules which aim to degrade a protein of interest (POI) by hijacking the ubiquitin-proteasome system. They are designed to simultaneously bind to both an E3 ligase and a disease-causing protein in order to form a ternary complex. Ubiquitination of the POI by the E3 ligase, thereby marking it for proteasomal degradation, is induced by their proximity in the PROTAC mediated ternary complex. The linker, which connects the two rather rigid inhibitors, can be alkyl and ether based. It is rather flexible and can exhibit different levels of complexity, from a short three carbon linker to elongated and branched systems. Moreover, the linker characteristics turned out to be important for the desired potency and suitable pharmacokinetic properties. Therefore, linker design is crucial for the efficacy of a PROTAC.

The conformational behaviour of the PROTAC in solution is dependent on the linker composition and furthermore, it is important for the understanding of its free energy of binding to its targets. The use of residual anisotropic magnetic interactions such as RDCs and RCSAs helps us in the structural elucidation of the PROTAC molecule in solution and complements standard methods, such as NOESY NMR. Here, we study a SMARCA VHL PROTAC with a rather short and stiff linker. In a combined approach including the experimental RDC and RCSA values, MD simulations and a maximum entropy approach with theoretical RDC values from the MD trajectory as a prior we could determine the conformational ensemble of the PROTAC molecule in solution.

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#11

Domain 1.1 of σA factor of RNA polymerase from Bacillus subtilis beyond its major state conformation

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#12

Combining NMR and smFRET experiments to study the conformations of the Dcp1:Dcp2 mRNA decapping complex

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Controlled degradation of mRNA is vital for the regulation of gene expression. The removal of the 5' cap structure from the mRNA body is a central step in this process and it is catalyzed by the Dcp1:Dcp2 decapping complex. During the catalytic cycle, Dcp1:Dcp2 undergoes large structural rearrangements. We previously characterized distinct compositions of the complex using NMR relaxation dispersion experiments and found that the apo complex exchanges between the catalytically inactive open and closed conformations, whereas the Edc1 and substrate bound complex adopts a catalytically active conformation [1]. Here, we complement these NMR data with smFRET experiments that only require short data acquisition times and low protein concentrations, which allowed us the examination of protein complexes that are not suited for NMR experiments.

Interestingly, for some of the smFRET fluorophore labeling positions that we used, we found that the conformations that were derived from NMR and smFRET experiments were incompatible. To unravel the cause of these inconsistencies, we recorded NMR experiments on fluorescently labeled decapping complexes. These data reveal that the fluorophore labeling can shift the conformational equilibrium of the complex significantly, likely through interactions between the large fluorescent labels and the protein surface. Based on that, we conclude that a cross-validation of smFRET data is important when studying dynamic complexes.

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#13

Long-range coupling in a dodecameric enzyme complex unraveled by solid-state NMR

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TET2 aminopeptidases is a half-megadalton, homo-dodecameric assembly arranged in a tetrahedral shape. Each of the four facets of the tetrahedron presents a 17Å pore through which unfolded peptides, single α-helices of β-hairpins can access and get processed. The highly-compartmentalized internal organization of TETs is structurally different from any other class of protease complexes, with three circularly-arranged active sites in the catalytic chamber. Gauto et al. [1] recently showed how the functional mechanism of TET aminopeptidases remains elusive, with currently unavailable knowledge about substrate recognition, fixation and product release. Preliminary results seem to suggest the functional coupling of the putative entry pore and the active site, which are 20Å distant. We employ site-directed mutagenesis and solid-state NMR, in order to investigate the existence of long-range allosteric coupling. This includes experiments to probe motion spanning different timescales, such as EXchange SpectroscopY (EXSY), NEar-Rotary-resonance Relaxation Dispersion (NERRD), Rotational-Echo DOuble Resonance (REDOR) and R1 relaxation [2]. Lastly, we use linear combinations of the determined rate constants to characterize protein dynamics, following the recently proposed “detectors” approach [3].

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#14

NMR spectroscopy to study the thermodynamics of liquid-liquid phase separation of human Tau protein

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Liquid-liquid phase separation (LLPS) has emerged as a key mechanism for cell organization and the main promotor of the so-called membrane-less organelles. Intermolecular long-range interactions and entropy-driving processes are important factors behind LLPS. These forces represent the fine balance between enthalpy and entropy contributions. NMR spectroscopy is able to study transitory and long-range interactions along with solvent properties. Using the information provided by paramagnetic relaxation enhancement NMR (PRE-NMR), we demonstrate the essential role played by long-range interactions in the LLPS properties of human Tau protein. Following, we evaluate the implication of entropy-driving processes by monitoring the phase separation mechanism under stepwise temperature increases. Applying an integrative spatially resolved NMR strategy, we monitor the LLPS kinetics and the dehydration mechanism carried out by water. Finally, the obtained equilibrium concentrations were used to report the phase diagram of human Tau protein. The unusual appearance of the phase diagram reveals the complex competition between enthalpy and entropy behind LLPS of biomolecules. Altogether, the presented data reveals the critical factors that control the phase separation of human Tau protein and the valuable use of NMR to study this phenomenon.

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#15

Accurate extraction of site-specific methyl deuterium quadrupolar patterns: from simulation to experimentation

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#16

Exploring Interactions of Adhesins from Pathogenic Borrelia

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Borrelia spirochetes causing Lyme disease, use adhesin proteins to infect host cells via interactions with extracellular matrix components such as decorin (decorin binding proteins, DbpA/DbpB) or fibronectin (RevA). Dbp proteins bind glycosaminoglycans (GAG) and variations in these interactions across genospecies have been linked to tissue tropism. Here we present how small differences in conformations of homologous adhesins from European *Borrelia* affect their affinity towards GAGs.

DbpA, DbpB and RevA proteins from 3 European Borrelia species were prepared as recombinant proteins with uniform and selective labelling schemes. NMR-based secondary structure predictions and backbone dynamics characterization have been carried out for representative proteins. Binding affinities to GAGs were probed for Dbp proteins via NMR titration and HDX-MS. Dbp proteins were able to bind all ligands, but affinities and interacting residues were specific and depend *Borrelia* species. Moreover, the affinities of European Dbps differ significantly when compared with the previously determined values for North American Dbps despite the general structural similarity [3,5]. Our results support the suggested hypothesis of the very selective Dbp-GAGs interplay underlying the different tissue tropisms of *Borrelia*.

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#17

Synthesis and Incorporation of Arginine with Specific Isotope Patterns to Study Protein Dynamics by NMR

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Allostery is a phenomenon observed in numerous proteins, whose primary functions are altered by a chemical modulator bound to an interaction surface, distant to the actual active site [1]. A defined change in the protein's structure is transferred via a communication network of correlated dynamic motions to the active site, resulting in activation or inhibition of the protein's function [2]. Despite the fundamental role of allostery for proper cell function, mechanisms on an atomic level, as well as evolutionary origins are still poorly understood. The main models of allostery assume that this phenomenon is linked to protein dynamics and the interconversion of differently populated conformational states [2]. One valuable tool to study these dynamic processes and to quantitively characterize low-populated energetically high conformational states is protein NMR spectroscopy [3]. The very sensitive change of NMR observables to local structural dynamic processes allows the observation of protein motions at an atomic resolution [3,4]. The project *AlloSpace*, a French-Austrian collaboration funded by the *FWF-ANR*, focuses on allosteric processes in L-lactate dehydrogenases (LDH) and L-malate dehydrogenases 3 (MalDH3). The size of the target proteins necessitates the application of aligned isotope patterns to investigate dynamic properties and protonation states of key residues. In this sense, isolated ^{13}C-^{1}H spin systems in an otherwise highly deuterated environment represent optimal sensors to probe protein dynamics, leading to well-resolved, intense resonances in 2D ^{13}C-^{1}H spectra [5]. The orientation of the conserved residues R109 and R171 are crucial for the enzyme's activity and regulation. Therefore, an 11-step-synthetic route to yield selectively labeled arginine was developed. It was possible to synthesize the target compound starting from aspartate using commercially available and reasonable priced isotopically labeled compounds ($K^{13}CN$, MeOD). By application in *E. coli*-based overexpression systems the compound will help to identify dynamic properties, as well as pK_a values. The resulting protein samples will be investigated by solution-state or magic-angle-spinning NMR to quantify population states of different protein conformations.

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#18

Regulation of Nedd4 family E3 ubiquitin ligases through auto-inhibition

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The post-translational modification of proteins with ubiquitin is involved in nearly all cellular functions in eukaryotes and provides a system for fast and specific regulation by altering activity, localization, interaction surfaces and stability of targeted proteins. Ubiquitination, the attachment of ubiquitin to target proteins, is mediated by the sequential action of three cooperating enzymes, called E1, E2 and E3. In this enzyme cascade, E3 ligases determine the substrate selectivity and are known as key specificity factors in ubiquitin signaling, determining the fate of their substrates. Consequently, E3 ligases themselves are precisely regulated to prevent random ubiquitination reactions. HECT-type E3 ligases of the Nedd4 family, such as Smurf2, Nedd4, Itch and WWP1 are auto-inhibited by intramolecular interaction of their N-terminal C2 and / or WW domains with the catalytic HECT domain. In Smurf2, for example, the C2 domain inhibits HECT domain activity by blocking thioester formation and non-covalent ubiquitin binding, whereas the WW1 domain further enhances the auto-inhibitory interaction [1]. Interestingly, despite the high degree of sequence conservation within the Nedd4 family, the exact mechanisms of auto-inhibition differ between the individual family members. The manipulation of these auto-inhibition mechanisms is an interesting target point for the treatment of diseases associated to ubiquitin signaling. Here, by utilizing methyl TROSY NMR spectroscopy, NMR titration experiments and biochemical assays, we provide new insights on how the auto-inhibition of Nedd4 family ligases is established.

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#19

Substrate binding of the 65 kDa cytosolic domain of Tom70 studied by NMR spectroscopy

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Although mitochondria possess their own genome, the majority of mitochondrial proteins are encoded in the nucleus and have to be sorted to the functional environment by chaperones, co-chaperones and receptors. As mitochondria are essential organelles that are involved in fundamental processes (e.g. oxidative phosphorylation and apoptosis), the targeting of mitochondrial precursor proteins (also referred to as precursor proteins or precursors) is tightly regulated in eukaryotic cells. Still, despite the essential role of this process, the molecular details are elusive.

The main entry gate of precursor proteins at the mitochondrial outer membrane (OM) is the `translocase of the outer membrane´ (TOM) complex and consists of a central receptor protein Tom22 and the pore protein Tom40. The TOM component Tom70 has a complex function, acting as an interaction interface for cytosolic chaperones and precursor proteins[1]. Tom70 is involved in the sorting of these precursor proteins in and through the OM to the intermembrane space (IMS), mitochondrial inner membrane (IM) and mitochondrial matrix. However, until now it is not clear, how the precursor sorting is facilitated by Tom70. Using site-specific ^{1}H-^{13}C-methyl-group labeling of Tom70, we study the dynamic sorting mechanism of Tom70 from *Saccharomyces cerevisiae* and the role of cytosolic chaperones.

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#20

Domain 1.1 of σ^A factor of RNA polymerase from *Bacillus subtilis* beyond its major state conformation

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σ factors of RNA polymerase (RNAP) in bacteria are its key components which recognize promotor sequences and initiate transcription [1]. Domain 1.1 of σ^A factors occupies the primary channel of RNAP and affects the promoter selection process [2,3]. Here we present a study of domain 1.1 of σ^A from the model organism *Bacillus subtilis* and we describe its dynamics at μs—ms timescale using nuclear magnetic resonance together with its thermal unfolding characterized by optical and calorimetric methods. The results show that this domain possesses a natural ability to form a significantly less compact and more flexible state compared to the previously determined structure [4] at biologically relevant temperatures as documented by the secondary structure propensity (SSP) prediction based on NMR data (Figure 1). Interestingly, even the hydrophobic core of the protein is affected by the transition between the states of the protein. The study is complemented with functional characterization of domain 1.1 in transcription in dependence on temperature. It clearly shows that domain 1.1 responds to increases in temperature, supporting the hypothesis where the conformational plasticity of domain 1.1 affects its ability to enter or exit the primary channel of RNAP increasing the transcriptional output at elevated temperatures.

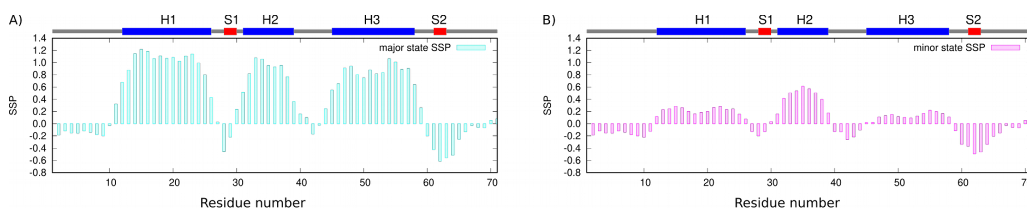

Figure 1: SSP prediction calculated for the previosuly determined structure [4] (A), and the minor state (B). Secondary structures of major state are shown above the graph (alpha helices and beta sheets are shown in blue and red, respectively).

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#21

2D Mechanoresponsive Surfaces for Measuring Cellular Traction Forces

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Cells sense mechanical forces in their local microenvironment through specific attachments to the extracellular matrix (ECM). Despite the essentiality of mechanical forces for basic cellular function, very little is known about the forces that act at the cell-ECM interface and how these forces feed into a variety of intracellular mechanosensing cascades. To improve our understanding of biomechanical processes at the cell-ECM interface, we introduce a library of coiled coil-based molecular force sensors Using atomic force microscope-based (AFM) single-molecule force spectroscopy (SMFS), we have calibrated the rupture force-loading rate dependence of a series of heterodimeric coiled coils with different loading geometries and coiled coil lengths. The mechanically calibrated coiled coils with rupture forces between 20-50 pN have been utilized for the fabrication of mechanoresponsive surfaces that allow for determining the forces that are transmitted across cell integrin-ligand attachments. As a proof of concept, fibroblast cells were allowed to adhere to surfaces containing RGDS-functionalized coiled coil sensors or covalently coupled RGDS (control). We show that all tested force sensors allow initial attachment within 30 min after cell seeding. At time points between 60-120 min, distinct cell spreading behavior is observed for coiled coil sensors with different thermodynamic and mechanical stabilities. These results aid the future design of smart mechanoresponsive 3D materials for investigating cellular mechanosensing processes at the single-molecule level.

6. List of Participants

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