AMPERE Biological Solid-State NMR School

Online Phase: January 13, 2021 – May 26, 2021

Combined Attendance/Online Week:
Berlin, 14.06. -18.06.2021
MDC.C, AXON 1, Robert-Rössle-Str. 10, 13125 Berlin

Dear biological solid-state NMR enthusiast, we will have our biannual Mallorca Solid-State NMR School again in 2021, and invite all of you to participate. This time, it is a bipartite course, with an online school of basics from January to May 2021, and a week of attendance in June 2021. Registration for combined virtual and real event 40 €, including AMPERE membership. Depending on the pandemic situation, the workshop in Berlin will be held under safe, distance-enforcing conditions and participant attendance in Berlin may be limited to 40 persons. The lectures will also be transmitted. The week in Berlin is geared towards the exchange of experience and nitty-gritty experimental details as well as for networking among PhD students and postdocs. Students are strongly encouraged to present a poster, but it is of course not required for participation since the course is also designed for newcomers in biological solid-state NMR.

The course program in the online school (virtual) will start at the 13.01.2021 and will consist of introductory lectures twice a month on Wednesdays from 15:00 to 16:00. There will be a 60 minutes presentation and 15 minutes for the discussion/question session, as well as exercises supplied. Afterwards virtual poster presentations of two participants will take place, 10 mins presentation, 10 mins discussion. The discussion will be in free format as in front of a poster at a conference, however, people need to register for discussion according to their scientific preferences to simplify the procedure.
In the following week, the exercises on the topic of the lecture will be discussed in about an hour, again on Wednesdays, 15:00, and solutions presented. Between lecture and exercise discussions a webpage will be open for questions to the exercises, and lecturers and teaching assistants will answer and discuss in chat mode.
All attendees agree to join the conference dinner on Wednesday, June 16, either in person or via the web. Tables will be arranged either outside in Berlin or online.

Registration:
Please send an email to: **steuer@fmp-berlin.de**. This should include the full name, affiliation (full address) and and in case you would like to present a poster, send the title. You will receive a request for payment after confirmation of registration. Places are limited.

Program

Online Phase:

13.01 **Quantum Mechanics *Beat Meier***

Topics: Repetition of basic quantum mechanics. Emphasis on matrix forms and the description of spin variables. Density operator description and Liouville-von Neumann equation. Rotating-frame transformation.

20.01 ***Exercises*** Quantum Mechanics

27.01 **Anisotropic Interactions and MAS *Beat Meier***

Topics: Basic NMR Hamiltonians. The calculation of spectra. Introduction to anisotropic NMR interactions. Spectra of single crystals and powder samples. Description by cartesian vs. spherical tensors.

03.02 ***Exercises*** Anisotropic Interactions

10.02 **Time-Dependent Hamiltonians *Matthias Ernst***

Topics: General structure of Hamiltonians in spherical-tensor notation, Origin of time-dependent Hamiltonians, sample rotation (MAS), interaction-frame transformations (rotating frame).

17.02 ***Exercises*** Time-Dependent Hamiltonians

Topics: Euler rotations and transformations from the PAS to the LAB coordinate system. Interaction-frame transformations.

24.02 **Solving Time-Dependent Hamiltonians *Matthias Ernst***

Topics: Average Hamiltonian Theory for discrete time periods and for continuously changing Hamiltonians, Floquet theory for single and multiple time dependencies. Some simple examples.

03.03 ***Exercises*** Solving Time-Dependent Hamiltonians

Topics: Calculate AHT for the examples calculated in the previous exercise. Calculate the Fourier components of the Hamiltonian needed in Floquet theory.

10.03 **Linewidths Basics/Relaxation *Beat Meier***

Topics: Possible sources for line broadening. Linewidth and resolution. ^{13}C detection vs. proton detection. Second moment considerations. Line intensity and relaxation during polarization transfer periods.

17.03 ***Exercises*** Linewidths basics

30./31.03 **Intro and Exercises in Product Operator Calculations for Biochemists *Hartmut Oschkinat***

Topics: What are product operators, how are they derived, how are pulses, chemical shifts, and couplings treated? Calculation of simple pulse sequence elements. Procedures for phase cycling, selection of coherence transfer pathways. Exercises cover calculations of the effects of simple pulse sequence elements, the COSY, DQF-COSY and INEPT experiments, comparison to recorded spectra.

07.04 **SIMPSON *Thomas Vosegaard***

Topics: Basics of numerical simulations. Considerations on choosing the right space - Hilbert space or Liouville space. Powder averaging and efficient propagation will be presented. Existing simulation software will be reviewed. Examples on more customized numerical simulations are given.

14.04 ***Exercises*** SIMPSON

21.04 **Introduction into EPR *Enrica Bordignon***

Topics: Site-directed spin labeling methods, types of labels (nitroxide, Gadolinium, trityl, copper), how to extract dynamics, water accessibility and interspin distances via EPR.

28.04 ***Exercises*** EPR

05.05 **Dynamic Nuclear Polarisation *Bob Griffin***

Topics: Basic theory of dynamic nuclear polarization, pulsed and continues microwave irradiation, polarization transfer mechanisms, first applications.

12.05 ***Exercises*** Dynamic Nuclear Polarisation

19.05 ***Huub de Groot*** The Challenges of Modern Structural Biology.

26.05 **Installation of CCPN *Vicky Higman and Anja Böckmann***

Combined Attendance/Online Week:

The lecture slots are 1h30 slots, some include exercises and sometimes lectures are 2x45 mins, with a separate exercise section.

	Sunday 13.06.21	**Monday 14.06.21**	**Tuesday 15.06.21**	**Wednesday 16.06.21**	**Thursday 17.06.21**	**Friday 18.06.21**
9:00-10:30		Decouplin/ Recoupling Theory *Ernst*	Carbon Detection/ Assign-ments/CCPN *Böckmann/Meier*	The Spectrome-ter/Probes *Engelke*	DNP *Griffin*	Structure Calcu-lation Basics *Bardiaux/Higman*
10:30-11:00		Coffee	Coffee	Coffee	Coffee	Coffee
11:00-12:30		Decoupling/ Recoupling *Vosegaard*	Proton Detection & Fast MAS *Meier/Böckmann*	Sample Prep Bacteria/ Cell-free *Böckmann/Osch-kinat*	Integrative Ensemble Calculations *Bonomi*	Structure *Oschkinat/Meier*
12:30-14:00		Lunch	Lunch	Lunch	Lunch	Lunch
14:00-15:30		Exercises *Ernst/Vosegaard*	Assignment Proce-dures/CCPN *Böckmann/ /Higman*	Relaxation/ Dynamics *Reif*	Students Poster Talks (online)	Low Populated States *Jensen* EPR in Structural Biology *Bordignon*
15:30-16:00		Coffee	Coffee	Coffee		In cell MAS NMR *Baldus*
16:00-17:30		Pulse Sequences *Polenova*	Exercises *Polenova/Meier*	Integrative Struc-tural Biology *A. Lange*	Students Pos-ter Talks (online)	
17:30-18:00	Wel-come Mixer (out-doors)	Questions	Questions (30 mins) Students Poster Talks	Conference Din-ner (outdoors)	Questions (online)	

<u>Monday, 14.06.2021</u>

Decoupling/Recoupling Theory *Matthias Ernst*

Topics: Short repetition of time-dependent Hamiltonians and how to solve them. Application of AHT and Floquet theory to understand decoupling and recoupling processes. Simple example cw irradiation on one spin - resonance conditions, residual coupling terms, spin diffusion. Residual coupling terms and resonance conditions in different decoupling sequences. Principles of recoupling sequences: discrete pulses, cw irradiation, complex pulse sequences.

Decoupling/Recoupling *Thomas Vosegaard*

Topics: More practical information to decoupling and recoupling sequences. Examples of commonly used pulse sequences and what they do.

Decoupling/Recoupling Exercises *Matthias Ernst and Thomas Vosegaard*

Topics: Simpson simulation to illustrate decoupling/recoupling. Simple decoupling in a I2S three-spin system under MAS. Analysis of importance of the various cross terms (cw, TPPM, XiX). Simple recoupling using cw irradiation. Simulation of more complex recoupling sequences (C/R)?

Pulse Sequences *Tatyana Polenova*

Topics:

<u>Tuesday, 15.06.2021</u>

Carbon Detection/Assignments *Anja Böckmann and Beat Meier*

Topics:

Spin systems, amino acids, BMRB, typical chemical shifts for secondary structure elements, ^{13}C detection: experiments for the identification of spin systems (DARR, NCA, NCACB, CCC, transfers used), spin systems in spectra, how to find them in DARR and homonuclear 3D spectra, NCA and NCACB, sequential walk and experiments allowing it (NCACO, NCOCA, CANCO), CCPN examples for a sequential walk, assignment completeness and quality check, back prediction of NCA and DARR 20 & 100 ms, ^{1}H detection: experiments detected on protons with examples in CCPN, different possible transfers, efficiencies, assignment strategy, opportunities for assignments provided by amino-acid specific labeling.

Proton Detection & Fast MAS/Assignment Procedures/CCPN *Beat Meier, Anja Böckmann, Vicky Higman*

Topics: Proton spectra at fast MAS: where does the linewidth come from? description of the coherent effect with AHT, incoherent effects under fast MAS, what limits the spinning frequencies, proton detection: resolution as a function of the spinning frequency, proton detection: sensitivity as a function of the spinning frequency, what is the optimum spinning frequency? The influence of the magnetic field strength, hNH experiment: deuterated samples or full protonation? hCH experiments: CH, CH2 and CH3 groups, CP at fast MAS, spin diffusion at fast MAS, proton detection for relaxation measurements, examples for a number of proteins.

Assignment Procedures/CCPN Practicals *Anja Böckmann, Beat Meier, Vicky Higman*

Topics: Hands-on assignments with CCPN – Carbon/proton detected.

<u>Wednesday, 16.06.2021</u>

The Spectrometer/Probes *Frank Engelke*

Topics:

Sample Prep Bacteria/ Cell-free *Hartmut Oschkinat and Anja Böckmann*

Topics: Expression protocols for *E.coli*, protein deuteration, how to achieve selective labelling: feeding amino acid precursors, glycerol & glucose isotopomers, amino acids. Pitfalls: transamination, amino acid catabolism, Krebs-cycle, proton exchange characteristics. Cell-free protein synthesis for NMR samples, expression tests, purification, labeling, assemblies, lipid reconstitution, sample to NMR (how?).

Relaxation/Dynamics *Bernd Reif*

Topics:

1) Protons in solid-state NMR

1H MAS frequency dependent and B0 dependent intensities, amide protons in perdeuterated samples, methyl labeling, sample heating due to MAS and decoupling; solvent suppression schemes; scalar coupling based triple resonance experiments; assignment strategies; detection of hydroxyl, imidazole, amino group protons; proton distance restraints; multidimensional deuterium NMR spectroscopy; off-magic angle spinning; application of proton detection to amyloids and membrane proteins.

2) Dynamics

Observables for the quantification of dynamics; derivation relaxation superoperator; derivation expression for dipolar T1 relaxation; model-free Lipari-Szabo analysis; experiments to quantify deuterium quadrupolar tensors; 15N-T1, order parameters, cross-correlated relaxation, R1rho, heteronuclear NOEs; influence of spin diffusion on relaxation parameters; TROSY in the solid-state.

Integrative Structural Biology *Adam Lange*

Topics:

<u>Thursday, 17.06.2021</u>

DNP *Bob Griffin*

Topics:

Integrative Ensemble Calculations *Max Bonomi*

Topics:

<u>Friday, 18.06.2021</u>

Structure Calculation Basics *Benjamin Bardiaux and Vicky Higman*

Topics: Restraints (distances, dihedral angles), manual/automated calculations, simulated annealing, structure and restraints validation, CCPN-ARIA, symmetry (oligomers and fibrils).

Structure *Hartmut Oschkinat and Beat Meier*

Topics:

Low Populated Protein States *Malene Ringkjobing-Jensen*

Topics: Results on IDP interactions, stuff looking at low populated ring flipping intermediates combining NMR and X-ray crystallography.

EPR in Structural Biology: from in vitro to in cell EPR *Enrica Bordignon*

Topics: How to detect conformational changes of membrane proteins via EPR and unveil the structural effects induced by different environments: comparison of EPR

data in detergent, liposomes, nanodiscs and physiological membranes in cells. An example will be shown on the potential of combining EPR with cryo-EM and single molecule fluorescence spectroscopy to monitor the kinetics of the conformational transition in a Mega-Dalton protein.

In cell MAS NMR ***Marc Baldus***

Topics: