Danish NMR-meeting

January 19–20

2015

The annual Danish NMR-meeting is this year arranged in Lund by the Natural Product Research group at the Department of Drug Design and Pharmacology. http://danish-nmr-meeting.dk

Programme & Abstracts

36th Danish NMR meeting January 19-20, 2015

Programme

Monday 19/1-2015

11:00 – 12:00 Arrival and registration

12:00 – 13:00 Lunch

13:00 – 13:05 Opening remarks

Session 1: Metabolomics and SAR, session chair: Anders Malmendal

13:05 – 13:50 **Pasi Soininen** (University of Eastern Finland): Six years of high-throughput serum NMR metabolomics – towards large-scale epidemiology and genetics.

13:50 – 14:10 **Reinhard Wimmer** (Aalborg University): Structure-aided Rational Design of Antimicrobial Peptides.

14:10 – 14:30 **Santosh Lamichhane** (Aarhus University): NMR-based metabolomics of human feces.

Poster session 1: Odd numbered posters presented

14:30 – 15:00 Coffee served in the exhibition/poster area

14:30 – 15:00 Poster presentations.

Session 2: Metabolism and Small molecules, session chair: Reinhard Wimmer

15:00 – 15:45 **Ursula Sonnewald** (Norwegian Institute of Science and Technology): How can ^{13}C nuclear magnetic resonance spectroscopy be used to study cerebral metabolism?

15:45 – 16:05 **Poul Erik Hansen** (Roskilde University): NMR and Structure-function relationships.

16:05 – 16:25 **Andreas Bergner** (LOT-QuantumDesign GmbH): Affordable Benchtop NMR Spectroscopy for the Laboratory.

Poster session 2: Even numbered posters presented

16:25 – 17:05 Coffee served in the exhibition/poster area

16:35 – 17:05 Poster presentations.

Session 3: Small molecules and applications, session chair: Poul Erik Hansen

17:05 – 17:25 **Kenneth Kongstad** (University of Copenhagen): Recent Advances in LC-NMR Techniques – Fighting fungi and Type 2 Diabetes.

17:25 – 17:45 **Henrik Pedersen** (Lundbeck): NMR on mass limited samples.

17:45 – 18:05 **Andrew Benie** (Novo Nordisk): The formulation challenge – automation unlocked.

18:05 – 18:25 **Jens Christian Madsen** (Bruker): New developments in Bruker Biospin's product line.

Dinner

19:00 – ? Classy conference dinner and poster prize awards.

Tuesday, 20/1-2015

09:00 – 09:05 Messages from the organizers

Session 4: Solid-state NMR, chair: Thomas Vosegaard

09:05 – 09:50 **Daniel Topgaard** (Lund University): Diffusion MRI methods inspired by solid-state NMR

09:50 – 10:10 **Nicholas Balsgart** (Aarhus University): A new method for high-throughput detection and quantification of phospholipids in complex mixtures.

10:10 – 10:30 **Morten Kjærulff Sørensen** (Aarhus University): Development and exploitation of cost-efficient multinuclear NMR – Characterization of fuel oil onboard ships, monitoring of NPK in animal slurry, and high-resolution MAS solid-state NMR at low-field instrumentation.

10:30 – 10:50 **Anders Bodholt Nielsen** (Aarhus University): Broadband DREAM and RESPIRATION-CP.

10:50 – 11:10 Coffee

Session 5a: Proteins, chair: Birthe Kragelund

11:10 – 11:30 **Frans Mulder** (Aarhus University): Measurement and Modeling of Electrostatic Interactions in Disordered Proteins.

11:30 – 11:50 **Christoph Weise** (Umeå University): Coupled folding and membrane-binding of the disordered linker of Yersinia YscU.

12:00 – 13:00 Lunch

Session 5b: Proteins, chair: Kaare Teilum

13:00 – 13:20 **Yuichi Yoshimura** (Aarhus University): Easy and unambiguous sequential assignments of intrinsically disordered proteins by correlating the backbone ^{15}N or $^{13}C'$ chemical shifts of multiple contiguous residues in highly resolved 3D spectra.

13:20 – 13:40 **Katrine Bugge** (University of Copenhagen): Digging into the fat layer to search for the structural and mechanistic secrets of cytokine receptor transmembrane domains.

13:40 – 14:00 **Jakob T Nielsen** (Aarhus University): Solution structure of the apo- and metal-bound form of the heavy metal binding domain of a PIB-type copper-ATPase.

Closing remarks

14:00 – Closing remarks, departure.

Posters

Poster 1.

Small molecule compounds effect on α-synuclein.

Camilla Bertel Andersen, Nikolai Lorenzen, Cagla Sahin, Martin Kurnik, Frans A A Mulder, Daniel E Otzen.

Poster 2.

ssNMR studies of the local environments in layered double hydroxides.

Line B. Petersen, Nicholai D. Jensen, Andrew S. Lipton, Vadim Zorin, Ulla Gro Nielsen.

Poster 3.

NMR-based metabolomics for identification of α-amylase inhibitors in rowan berries (*Sorbus* spp.).

Sofie L. Broholm, Simone M. Gramsbergen, Nils T. Nyberg, Anna K. Jäger, and Dan Stærk.

Poster 4.

Characterization of phosphate sequestration by a lanthanum modified clay: a combined solid-state NMR, EXAFS and PXRD study.

Line Dithmer, Andrew S. Lipton, Kasper Reitzel, Terence Warner, Daniel Lundberg and Ulla Gro Nielsen.

Poster 5.

Solid State MAS ^{13}C NMR Chemical Shifts In Biomimetic Cu(I/II) Compounds.

Vibe Jakobsen, Jonas Sundberg, Vickie McKee, Ulla Gro Nielsen, and Christine J. McKenzie.

Poster 6.

NMR chemical shifts of common laboratory trace impurities originating during protein production and purification.

Lars Alf Jensen, Frans Mulder.

Poster 7.

Ligand binding to ^{15}N-labeled HSA constructs expressed in *Pichia*.

Camilla Kejlberg, Frans Mulder, Jan Kristian Jensen, Zebin Hong.

Poster 8.

High-resolution α-glucosidase and radical scavenging profiling combined with HPLC-HRMS-SPE-NMR for identification of bioactive constituents in crude extract of *Pueraria lobata*.

Bingrui Liu, Kenneth T. Kongstad, Nils T. Nyberg, Sun Qinglei, Anna K. Jäger, and Dan Staerk.

Poster 9.

High-resolution snake venom inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of antivenomous constituents in *Clausena excavata*.

Yueqiu Liu, Nils Nyberg, Dan Stærk, and Anna K. Jäger.

Poster 10.

Identification of Fungal Plasma Membrane H^+-ATPase Inhibitors in *Lecaniodiscus cupanioides* by HPLC-HRMS-SPE-NMR.

Ida K. Straadt, Kenneth T. Kongstad and Dan Stærk.

Poster 11.

Membrane-binding of the intrinsically-disordered linker between transmembrane and cytosolic domains of the Yersinia secretion-switch protein YscU.

Christoph Weise.

Poster 12.

Temperature dependence of domain motion and internal mobility of T4 lysozyme.

Mengjun Xue, Michael Wulff Risør, Yuichi Yoshimura, Frans Mulder.

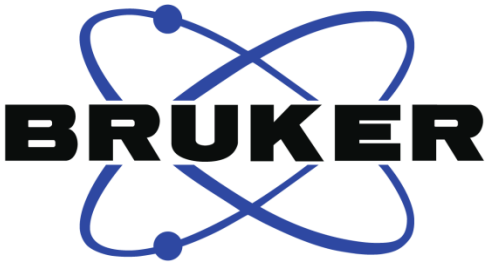
Poster 13.

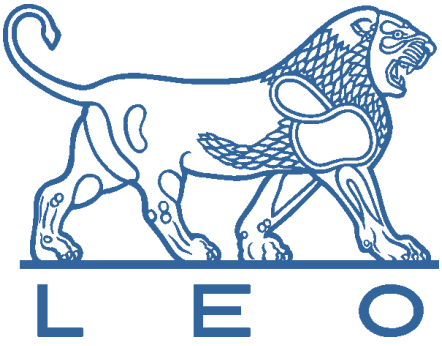
Improving Heteronuclear Dipolar Decoupling Sequences using π pulses.

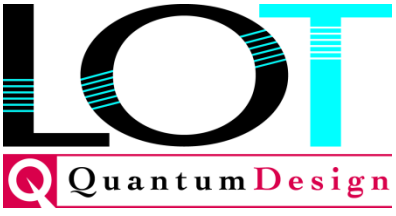
Asif Equbal, Niels Chr. Nielsen

Sponsors

We are very thankful for the sponsorship from the following companies. The sponsorships have enabled free participation for MSc students and invitation of speakers.







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Abstracts

Six years of high-throughput serum NMR metabolomics – towards large-scale epidemiology and genetics.

Pasi Soininen, University of Eastern Finland

Metabolic profiling (metabolomics) is increasingly used to provide insights into the molecular underpinnings of common diseases such as diabetes and cardiovascular disease, and it holds also potential to improve current methods for risk assessment and prognostics. Yet metabolomics will be truly useful in epidemiology and genetics only if quantitative data on specific, identified metabolites are available [1].

Towards these goals, we have set up an automated high-throughput platform for human serum NMR metabolomics [2] that has been used to analyse over 200,000 samples during the past 6 years. The methodology features absolute quantification of specific molecular identities including lipoprotein subclass profiling, lipid constituents such as polyunsaturated fatty acids, as well as quantification of various small molecules including amino acids and glycolysis precursors. These molecular data relate to multiple biological pathways and metabolic functions in health and disease, including the metabolic signatures of insulin resistance, obesity, and other common risk factors [3,4].

This novel line of molecular epidemiology allows a more detailed molecular understanding of biochemical pathways and disease pathologies. Our results also demonstrate improved risk prediction for cardiovascular disease and all-cause mortality [5,6]. The technological characteristics of the metabolomics platform will be presented in relation to applications in epidemiological studies on cardiometabolic diseases.

[1] Soininen et al. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Cardiovascular Epidemiology and Genetics. Circ. Cardiov. Genet. 2014;accepted.

[2] Soininen et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst. 2009;134:1781-5.

[3] Kujala et al. Long-term leisure-time physical activity and serum metabolome. Circulation. 2013;127:340-8.

[4] Würtz et al. Metabolic signatures of insulin resistance in 7,098 young adults. Diabetes. 2012;61:1372-80.

[5] Würtz et al. High throughput quantification of circulating metabolites improves prediction of subclinical atherosclerosis. Eur. Heart J. 2012;33:2307

[6] Fischer et al. Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons. PLoS Med. 2014;11:e1001606.

Structure-aided Rational Design of Antimicrobial Peptides.

Reinhard Wimmer, Aalborg University

NMR-based metabolomics of human feces.

Santosh Lamichhane, Aarhus University

Metabolomic analyses of fecal material are gaining increasing attention because the gut microbial ecology and activity have impact on the human phenotype and regulate host metabolism.

Sample preparation is a crucial step. In this work an optimized method for extraction and analysis of fresh feces by proton NMR spectroscopy is presented. In addition, the potential of NMR-based metabolomics is illustrated on a dietary intervention study (n = 12) to explore the impact of polydextrose (PDX), an insoluble fiber, on the human fecal metabolome.

This presentation will demonstrate that proton NMR spectroscopy is a useful technique for metabolite profiling of feces and for testing compliance to dietary fiber intake in intervention trials. In addition, it will be demonstrated how correlation analyses based on NMR spectra can provide novel information about associations between fecal metabolites and activity of specific gut bacteria.

How can ^{13}C nuclear magnetic resonance spectroscopy be used to study cerebral metabolism?

Ursula Sonnewald, Norwegian Institute of Science and Technology

^{13}C Nuclear magnetic resonance spectroscopy (NMRS) is a useful tool for the study of brain metabolism in particular for astrocyte-neuronal interactions. It allows the simultaneous and separate detection of signals from different compounds as well as different atoms in a given molecule, so that metabolism via specific path-ways can be determined. As an example, ^{13}C from [1-^{13}C]glucose is incorporated via the pyruvate dehydrogenase reaction into the C2 of acetyl-CoA, then into the C4 of α-ketoglutarate and finally into C4 of glutamate and glutamine and the C2 of GABA, which can be measured by ^{13}C NMRS. In addition to determining the amount of label in a given position in a molecule such as glutamate, the amount of molecules with label in different positions (isotope isomers or isotopomers) can be obtained. Furthermore, it can be determined if a ^{13}C atom has a ^{13}C labelled neighbour due to the magnetic interaction between adjacent ^{13}C nuclei. Using ^{13}C NMRS after injection of [1-^{13}C]glucose it has been calculated that acetyl CoA derived from glucose is predominantly metabolized in the neuronal tricarboxylic acid cycle, however, acetate is selectively metabolized by astrocytes. Thus, by simultaneous injection of [1-^{13}C]glucose and [1,2-^{13}C]acetate followed by ^{13}C NMRS analysis of brain extracts information about neuronal and astrocytic metabolism can be obtained in the same animal. Using this method, in combination with animal models of neurological or psychiatric diseases details about glial-neuronal metabolic disturbances can be found.

NMR and Structure-function relationships.

Poul Erik Hansen, Roskilde University

Structures of tautomeric compounds such as usnic acid and tetracycline will be discussed based on isotope effects on chemical shifts and DFT calculations.

Affordable Benchtop NMR Spectroscopy for the Laboratory.

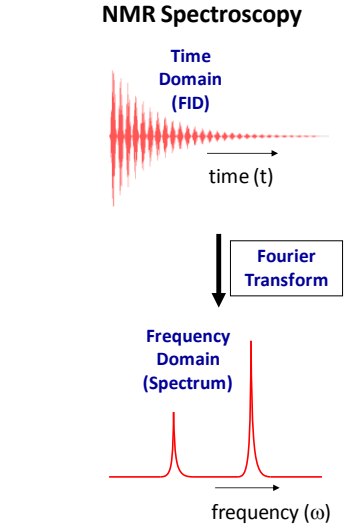
Andreas Bergner, LOT-QuantumDesign GmbH

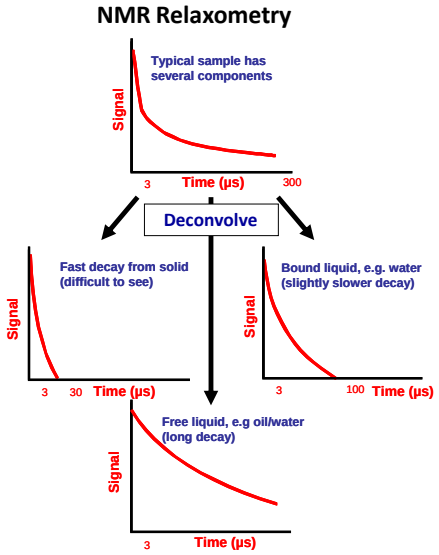
Nuclear Magnetic Resonance (NMR) Spectroscopy, along with Mass Spectrometry, is an important technique for structural characterization thus has become a standard tool for organic chemistry. Unfortunately time on an NMR instrument is expensive, even for routine analysis, because of the associated running costs. The super-conducting magnet requires regular cryogen fills of liquid helium and nitrogen, the former becoming an increasingly scarce and therefore expensive commodity. In addition, it normally requires an expert to run and maintain the instrument.

In contrast, benchtop NMR instruments based on permanent magnet technology have low running costs as they are cryogen-free; these instruments are commonly used for measurement of oil, moisture, solid fat and fluorine content for process and quality control. Permanent magnets constructed from rare earth metals have been developed recently which are smaller, lighter and have much better performance than traditional AlNiCo magnets.

We will present in our talk the latest development in benchtop NMR systems that do not require liquid Helium or Nitrogen. We will show two different NMR systems for *a*) NMR relaxometry and *b*) high resolution NMR spectroscopy.

We will also have a running NMR spectroscopy system at our booth and we are more than happy to run customer samples during the conference.





Recent Advances in LC-NMR Techniques – Fighting fungi and Type 2 Diabetes.

Kenneth Kongstad, University of Copenhagen

The hyphenation of HPLC and NMR is a milestone in complex mixture analysis approaches and in particular the natural product research area has benefitted immensely. While the HPLC-NMR technique had great potential it was limited to major components due to sensitivity issues. The introduction of a solid phase extraction module to the HPLC-NMR setup revolutionized the technique and allowed for structural elucidation of even minor metabolites directly from crude extracts [1, 2].

This talk will present the most recent development in the field of LC-NMR techniques: The high-resolution bioassay/HPLC-HRMS-SPE-NMR platform, which allows for rapid identification of biologically active constituents directly from complex mixtures [3]. This novel approach to natural product based drug discovery will be exemplified through our functional food program focusing on type 2 diabetes [4] as well as ongoing efforts to discover antifungal metabolites targeting the fungal H^+ pump [5].

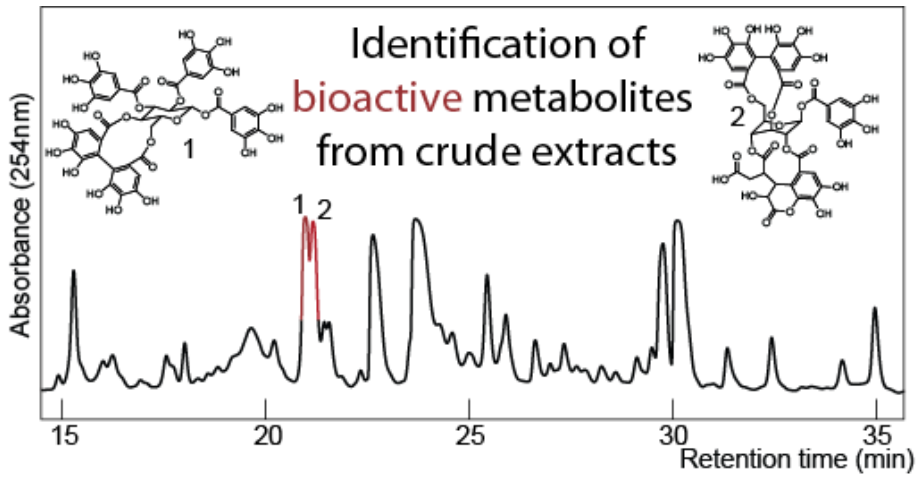
[1] Sprogøe, K.; Stærk, D.; Jäger, A.K.; Adsersen, A.; Hansen, S.H.; Witt, M.; Landbo, A.-K. R.; Meyer, A.S.; Jaroszewski, J.W. *J. Nat. Prod.* **2007**, *70*, 1472-1477

[2] Johansen, K. T.; Wubshet, S. G.; Nyberg, N. T.; Jaroszewski, J. W. *J. Nat. Prod.* **2011**, *74*, 2454-2461

[3] Wiese, S.; Wubshet, S.G.; Nielsen, J.; Staerk, D. *Food Chem.* **2013**, *141*, 4010-4018

[4] Schmidt, J.S.; Lauridsen, M.B.; Dragsted, L.O.; Nielsen, J.; Staerk, D. *Food Chem.* **2012**, *135*, 1692-1699

[5] Kongstad, K. T.; Wubshet, S.G.; Johannesen, A.; Kjellerup, L.; Winther, A.-M.L.; Jäger, A.K.; Staerk, D. *Food Chem.* **2014**, *62*, 5595-5602



NMR on mass limited samples.

Henrik Pedersen, Lundbeck

In pharma the NMR lab often has to deal with mass limited samples, e.g. isolated drug metabolites and isolated impurities from production or development. Together with high sensitivity NMR equipment, optimal NMR sample format and preparation is important to get sufficient 1D and 2D NMR spectra for structure elucidation. In this talk practical examples of this will be presented.

The formulation challenge – automation unlocked

Andrew Benie, Novo Nordisk

NMR is an extremely versatile tool in the development of pharmaceuticals as it provides detailed information about composition without the need for separations or extraction.

Consequently NMR should be a perfect tool for examining the stability of early stage formulations, however to date it is underutilised in this regard. If we wish to study stability directly in the NMR tube then we need to make use of the same grade of materials that would be used in the final product. This precludes the use of deuterated compounds as they have different purity profiles; furthermore the presence of D_2O alters the kinetics of degradation pathways.

Consequently if we want to use NMR then we either need to use an external locking or measure without lock. The latter is by far the cheapest both in terms of material and labour. Here I present a surprisingly simple strategy for obtaining high quality & reproducible NMR spectra in full automation unlocked.

New developments in Bruker Biospin's product line.

Jens Christian Madsen, Bruker

A brief overview of the recent developments concerning magnets, probes, console, and software will be given.

Diffusion MRI methods inspired by solid-state NMR.

Daniel Topgaard, Lund University

A wide range of porous materials, from lyotropic liquid crystals to brain tissue, contain anisotropic pores with varying sizes, eccentricities, and degrees of alignment on mesoscopic length scales. A complete characterization of the material requires estimation of all these parameters, but unfortunately their effects on the detected MRI signal are hopelessly entangled when using standard diffusion MRI methods.

This presentation will give an overview of our recent work in adapting solid-state NMR methods to a diffusion MRI context and resolve the effects of pore size, anisotropy, and orientation.

Our approach builds on the formal analogy between the chemical shift and diffusion anisotropy tensors, which means that classical solid-state NMR techniques, such as magic-angle spinning, have diffusion MRI equivalents [1]. In its simplest form, magic-angle spinning of the diffusion-weighting vector allows for estimation of the distribution of isotropic diffusivities free from the confounding influence of anisotropy. Joint analysis of the directional and isotropic data yields quantitative estimates of both the microscopic anisotropy and the orientational order of the domains [2]. With numeric optimization of the pulse sequence [3], the methods can be implemented on clinical MR scanners, for instance giving information about the shapes of randomly oriented cells in brain tumors [4].

[1] S. Eriksson, et al., J. Magn. Reson., 226, 13-18, (2013).

[2] S. Lasič, et al., Front. Physics, 2, 11, (2014).

[3] D. Topgaard, Microporous Mesoporous Mater., 178, 60-63, (2013).

[4] F. Szczepankiewicz, et al., Neuroimage, 104, 241-252 (2015).

A new method for high-throughput detection and quantification of phospholipids in complex mixtures.

Nicholas Balsgart, Aarhus University

Development and exploitation of cost-efficient multinuclear NMR – Characterization of fuel oil onboard ships, monitoring of NPK in animal slurry, and high-resolution MAS solid-state NMR at low-field instrumentation.

Morten Kjærulff Sørensen, Aarhus University

I will present the main results of my recently PhD thesis concerning our development and application of multinuclear cost-efficient NMR.

This includes four subsections:

i) Magic-angle spinning solid-state NMR on low-field instrumenttion (0.45 T). Here, our ^{31}P MAS NMR study of K_2PO_3F is shown as an example of the performance taking advantage of the different B_0 field dependencies of the spin interactions.

ii) The development of a ^{27}Al NMR sensor for onboard ship detection of aluminosilicate zeolites (so-called catfines) in marine heavy fuel oil. With this 1.5 T NMR sensor a high precision is demonstrated for ppm-level detection of catfines in good agreement with reference measurements from commercial laboratories.

iii) Natural-abundance ^{17}O NMR in a 1.5 T permanent magnet.

iv) The application of mobile ^{14}N, ^{31}P, and ^{39}K NMR for monitoring of N, P, and K in animal slurry for agricultural fertilization.

All four applications of multinuclear cost-efficient NMR is described, and excellent performance of the instruments is demonstrated in good agreement with the relevant simulations or reference measurements.

Further reading for *i)-iii)*:

[1] M.K. Sørensen, O. Bakharev, O. Jensen, H.J. Jakobsen, J. Skibsted, N.C. Nielsen, "Magic-angle spinning solid-state multinuclear NMR on low-field instrumentation", Journal of Magnetic Resonance 238 (2014) 20-25.

[2] M.K. Sørensen, M.S. Vinding, O.N. Bakharev, T. Nesgaard, O. Jensen, N.C. Nielsen, "NMR Sensor for Onboard Ship Detection of Catalytic Fines in Marine Fuel Oils", Analytical Chemistry 86 (2014) 7205-7208.

[3] Sørensen et al. Magn. Reson. Chem. In press: Natural abundant ^{17}O NMR in a 1.5 T Halbach magnet.

Broadband DREAM and RESPIRATION-CP.

Anders Bodholt Nielsen, Aarhus University

The presentation will contain a novel theoretical description by employing Floquet Theory which can give insight into important aspects of hetero- (RESPIRATION-CP) and homonuclear (Broadband DREAM) dipolar recoupling sequences for MAS solid-state experiments. Non-ideal pulse effects will be analysed which cannot directly be done by Average Hamiltonian Theory (AHT).

Measurement and Modeling of Electrostatic Interactions in Disordered Proteins.

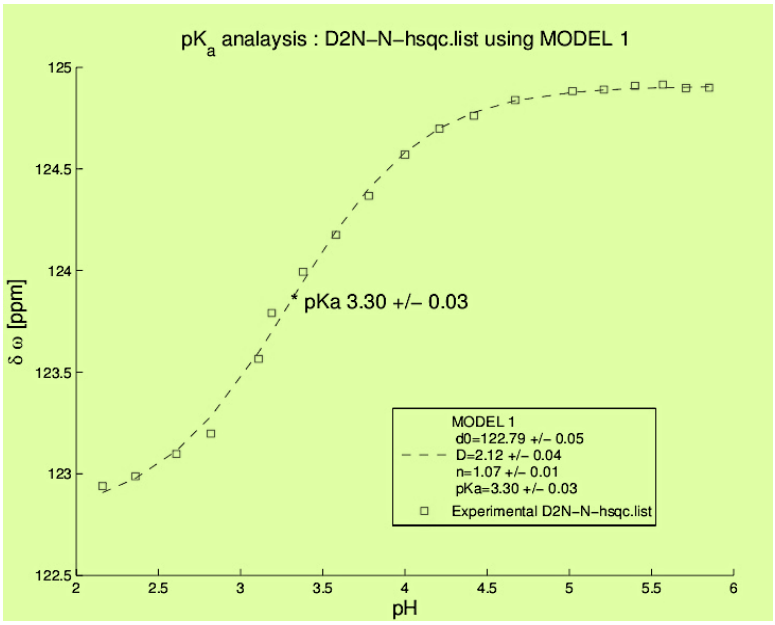
Frans Mulder, Aarhus University

We have used the IDP alpha-synuclein as a test case for the residue-specific measurement of side chain protonation states as a function of pH. High-resolution titration curves were obtained, which allow for the determination of binding affinities (pKa constants), as well as the distinctive observation of stretching and asymmetry due to semi-local electrostatic interactions.

pKa shifts up to 0.6 units from model values were observed, in agreement with previous studies [1,2]. A Monte Carlo model that predicts pKa constants based on Gaussian chain statistics and comprehensive Coulomb interactions is able to recount the course features in the experimental data, but fails to describe it in closer detail. The latter observation suggests that pH dependent NMR data offer the potential to post-select and refine IDP structural (ensemble) models.

[1] Y.J. Tan, M. Oliveberg, B. Davis, A.R. Fersht J. Mol. Biol. 254 980–992 (1995)

[2] R.L. Croke, S.M. Patil, J. Quevreaux, D.A. Kendall, and A.T. Alexandrescu Prot. Sci. 20 256–269 (2010)



Coupled folding and membrane-binding of the disordered linker of Yersinia YscU.

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Pathogenic bacteria of the genus *Yersinia* use the type three secretion system (T3SS) to defeat cells of the host immune system [1,2]. The T3SS includes a needle-like apparatus responsible for protein translocation, secreted effector proteins that disrupt targeted host cells, and auxiliary proteins including chaperones and regulators assisting during needle assembly and subsequent effector secretion. YscU is an auxiliary protein associated with the bacterial inner membrane and implicated in the switch from secretion of needle proteins to later components. YscU consists of a transmembrane domain and a cytoplasmic domain ($YscU_C$) containing a folded core and a linker to the transmembrane domain.

All X-ray crystal structures of $YscU_C$ to date indicate that the linker is highly unstructured (see e.g. [3]), but we reasoned that secondary structure might be stabilized through association with the negatively charged bacterial inner membrane. We therefore evaluated the effect of lipid vesicles on the secondary structure by CD spectroscopy, and employed solution NMR experiments in the presence of SDS micelles to describe the protein conformation, its stability, and its exposure to solvent within micelles [4].

The role of positive linker residues on secretion and the acquisition of structure were then probed by alanine mutagenesis combined with secretion and CD assays. The tantalizing results of combined NMR, CD and molecular biology provide valuable guidance for further investigations into the structure and role of YscU within the T3SS complex.

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Easy and unambiguous sequential assignments of intrinsically disordered proteins by correlating the backbone ^{15}N or $^{13}C'$ chemical shifts of multiple contiguous residues in highly resolved 3D spectra.

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Sequential resonance assignment strategies are typically based on matching one or two chemical shifts of adjacent residues. However, resonance overlap often leads to ambiguity in resonance assignments in particular for intrinsically disordered proteins (IDPs).

We investigated the potential of establishing connectivity through the three-bond couplings between sequentially adjoining backbone carbonyl carbon nuclei ($^3J_{C'C'}$), combined with semi-constant time chemical shift evolution, for resonance assignments of IDPs. Extended sequential connectivity strongly lifts chemical shift degeneracy of the backbone nuclei in disordered proteins. We show here that 3D (H)N(COCO)NH and (HN)CO(CO)NH experiments with relaxation-optimized multiple pulse mixing correlate up to seven adjacent backbone $^{15}N^H$ or $^{13}C'$ nuclei, respectively, and connections across proline residues are also obtained straight-forwardly. Multiple, recurrent long-range correlations with ultra-high resolution allow $^1H^N$, $^{15}N^H$, and $^{13}C'$ resonance assignments to be completed from a single pair of 3D experiments.

Digging into the fat layer to search for the structural and mechanistic secrets of cytokine receptor transmembrane domains.

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The prolactin receptor (PRLR), growth hormone receptor (GHR) and erythopoietin receptor (EPOR) are members of the cytokine receptor family. Currently, the cross-membrane signal transduction through these receptors is an enigma; no structures of any class I cytokine receptor transmembrane domain (TMD) has been solved till now, nor has a general mechanism been established to account for the propagation of binding signal.

With NMR spectroscopy as the main tool we are investigating the structural characteristics of PRLR-TMD, GHR-TMD and EPOR-TMD with the goal of elucidating their role in cross-membrane signal transduction. We have acquired residue-specific structural information on the three TMDs and calculated a structure of PRLR-TMD. Our data suggests similar structural behaviors of the three related TMDs, such as a weak, but highly specific, propensity to homodimerize.

Solution structure of the apo- and metal-bound form of the heavy metal binding domain of a PIB-type copper-ATPase.

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ATPases are essential for transporting heavy metals. Previously, the structure of a Cu^+-ATPase was determined by X-ray crystallography [1], but noticeably the important structure of the N-terminal domain binding the heavy metal (HMBD) could not be resolved. Here we present the solution structure of the HMBD in the metal-free and -bound form using NMR spectroscopy.

The chemical shifts were analyzed to discern structural features, provide dynamical characterization and follow the binding of Ag^+.

The HMBD structure is composed of two flexible ends and a compact structured core built of an anti-parallel beta-sheet with three strands connected by two turns. The metal ion is bound sandwiched in between the two turns coordinated to three sulfur atoms in two Met and a Cys residue. The HMBD structure rearranges slightly in the turns to accommodate the metal ion.

[1] P. Gourdon et al, "Crystal structure of a copper-transporting PIB-type ATPase", Nature 475, 59-64 (2011)