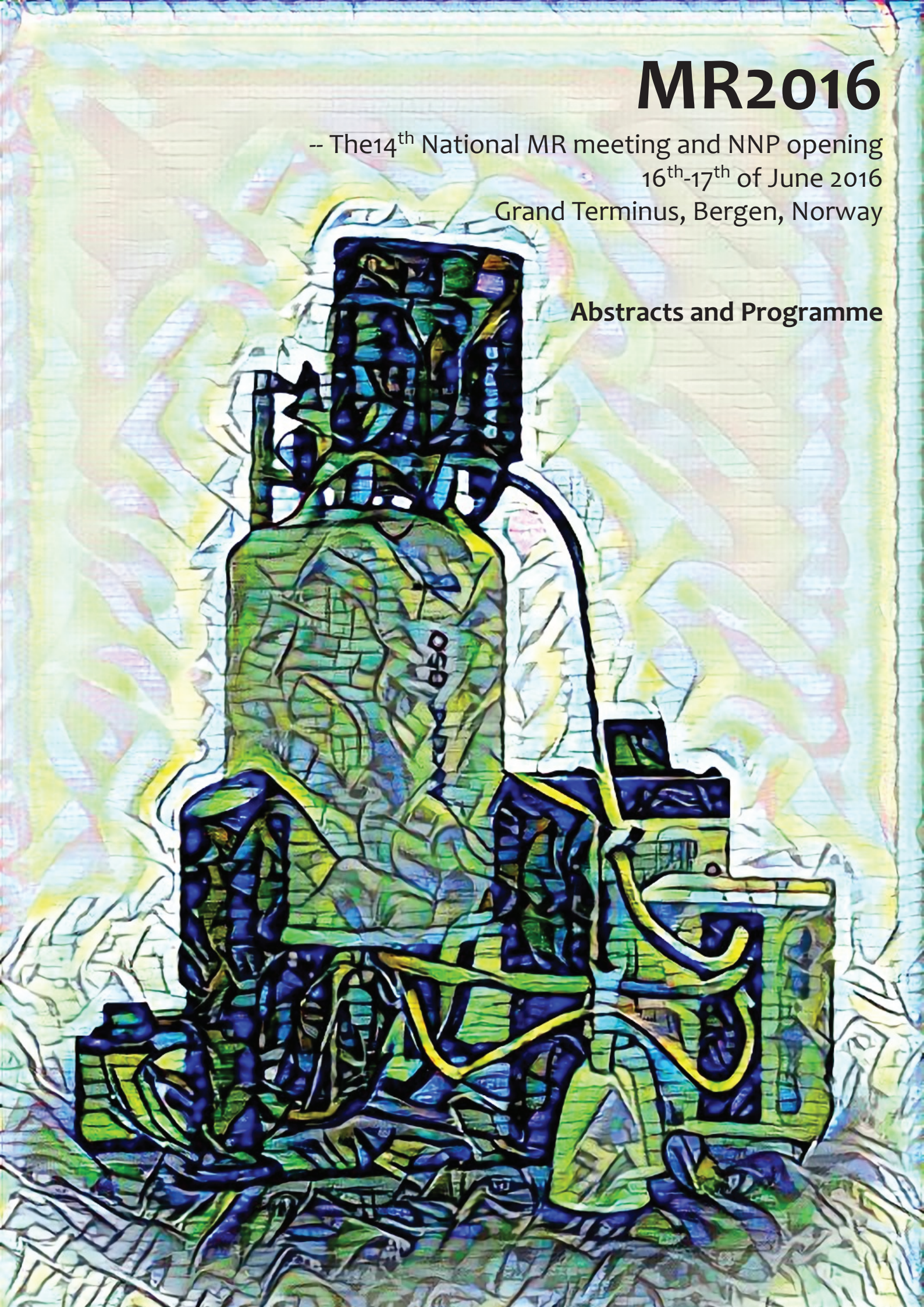
MR2016

-- The14th National MR meeting and NNP opening

16th-17th of June 2016

Grand Terminus, Bergen, Norway

Abstracts and Programme



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Willy Nerdal, Department of Chemistry, University of Bergen
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NNP opening: *JU, O-AB, JCRG, ØH*
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Book of abstracts: *PT, OD, ØH*
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Advisors:
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Samuel Furse, Department of Molecular Biology, University of Bergen

Venue
Hotel Grand Terminus
Terminus Forum and Terminus Hall
Zander Kaaes gate 6
N-5015 Bergen
NORGE

Cover Illustration: *The picture of an 850MHz NMR spectrometer has been subjected to Dreamscope, https://dreamscopeapp.com/*

WELCOME TO MR2016 AND THE OPENING OF NNP

Dear participant of MR2016,

it is a great pleasure to welcome you to Bergen for the biennial Norwegian MR meeting, which will be the 14th of its kind. We hope that you will have both a stimulating and enjoyable stay here with us.

This year will be historic for the Norwegian MR community. We are celebrating the opening of the new Norwegian NMR Platform, NNP. NNP is a national infrastructure platform, hosted by the Department of Chemistry, University of Bergen, in collaboration with UiO, NTNU and UiT. The new platform provides a much needed and long anticipated upgrade in the Norwegian NMR infrastructure and we also hope that it will foster new collaborations and projects. The instrumentation consists of one 850 MHz here in Bergen and two 800 MHz spectrometers at UiO and NTNU, all purchased from Bruker, as well as several instruments at lower field strengths at all sites. The ribbon-cutting marking the opening of the final of the three largest instruments to be inaugurated will be cut on Thursday 16th of June. This ceremony, as well as a celebration, will take place at VilVite, where we are looking forward to hear the lectures of Angela Gronenborn, Finn Aachmann, Bjørn Pedersen and Stefano Ciurli.

In addition to the lectures held at the NNP opening, we have 17 oral presentations and 13 posters which will provide us with insight into magnetic resonance applied to natural and synthetic products, macromolecules, clinical questions, material technology and more. The organization committee is very grateful to everyone who has submitted abstracts, supported the event economically and worked with us to make this happen. We urge you to look at the logos on the back cover of this booklet, so you too know who these generous people are.

Please remember to visit the stands of our business partners in the breaks.
There will be a NSMR General Assembly from 13:00 on Friday. We urge you to participate and vote. Please verify that your membership dues are up to date.

On behalf of the organization committee I wish you a comfortable stay and a stimulating meeting

Øyvind Halskau
MR2016 Committee Leader

GENERAL INFORMATION

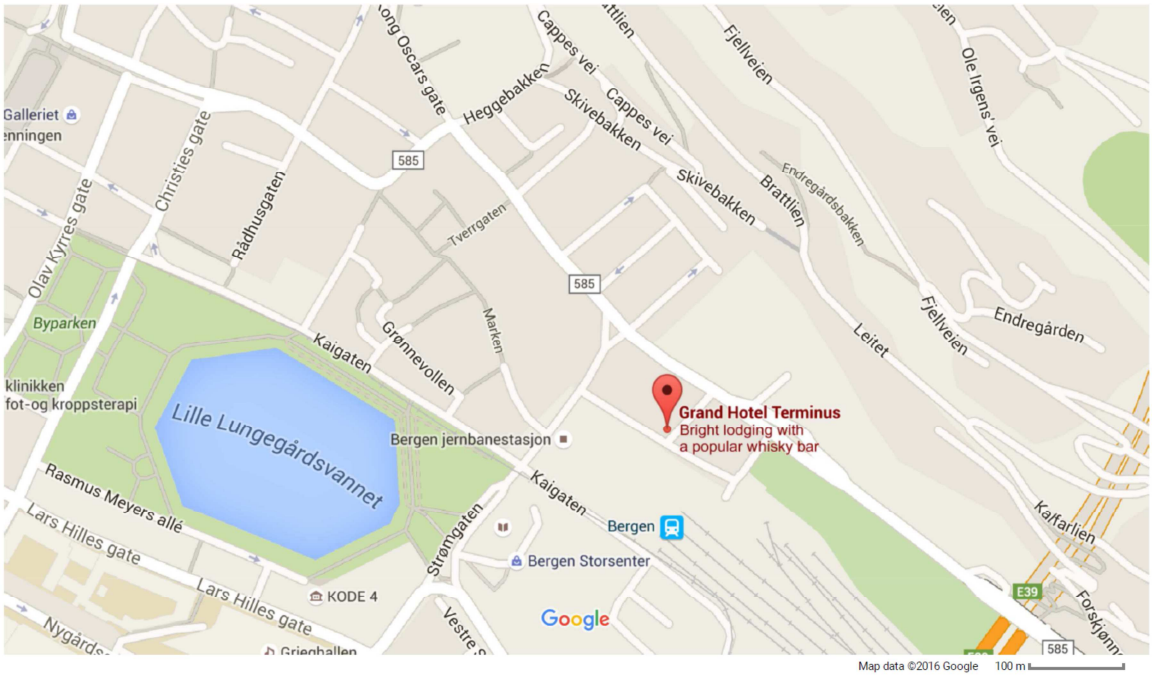
Grand Hotel Terminus has its location near the railway station, airport bus, the bus station, the Bergen Light Rail and the Grieg Hall. It is a mere five minutes' walk from Torgalmenningen Square.

Check in is from 15:00, **check out** should be done by 12:00.

Internet access is free in all areas; please contact hotel staff for access details.

A fitness department/gym, including sauna is available, and it is possible to rent a bike for free – Please make request to hotel staff. Aerobic, Pilates, squash and opportunities for swimming in the new AdO Arena can be accessed in the immediate vicinity of the hotel.

Terminus Whisky Bar offers no less than 1050 selected whiskies, which you can enjoy in an original, classic interior from 1928.



	Start	End	Time	Chair		Presenter
Thursday 16/6 Morning Sessions	08:00	09:00	01:00		Registration and coffee	
	09:00	09:05	00:05		Welcome adress by Øyvind	
	09:05	09:25	00:20	**Øyvind Halskau**	A New Fatty Acid Amide from Streptomyces Species with Insecticidal Activity	Nebosja Simic
	09:25	09:45	00:20		Is ^{31}P NMR the Neglected Nucleus for Lipid Profiling?	Samuel Furse
	09:45	10:05	00:20		Use of nmr spectroscopy in brain metabolism	Frode Rise
	10:05	10:25	00:20		Poster Session	
	10:25	10:45	00:20		Coffee Break and Visit the Sponsors	
	10:45	11:05	00:20		Poster Session	
	11:05	11:25	00:20	**John Georg Seland**	Towards Time-Resolved Quantification of in-vivo GABA Spectroscopy	Gerard Dwyer
	11:25	11:45	00:20		Magnetic Resonanc Imaging for Drug Repurposing in the Treatment of Melanoma Brain Metastasis	Frits Thorsen
	11:45	12:05	00:20		Using Diffusion MRI to Study Demyelination in Cortex and Deep Grey Matter in Animal Model of Multiple Sclerosis	Tina Pavlin
	12:05	13:00	00:55		Lunch	
	13:00	13:30	00:30		**Stroll or Bybane to VilVite**	

Thursday 16/6 NNP opening	Start	End	Time	Chair		Presenter
	13:30	13:35	00:05		Welcome adress by Jarl	
	13:35	13:50	00:15	Jarl Underhaug	Open words by prorektor	Anne Lise Fimreite
	13:50	14:20	00:30		The Start of NMR in Norway	Bjørn Pedersen
	14:20	14:50	00:30		Structures and the Structural Basis of Enzymatic Activity of Several APOBECs	Angela Gronenborn
	14:50	15:10	00:20		Coffee Break	
	15:10	15:40	00:30	Jarl Underhaug	State-of-the-art CryoProbe Technology at High Field Pushes the Limits for Applications From Small Molecules to Bio-NMR	Rainer Kuemmerle
	15:40	16:10	00:30		Urease: a Platform to Integrate Multiple Structural Biology Approaches Towards Drug Design	Stefano Ciurli
	16:10	16:40	00:30		Interaction of a Fungal Lytic Polysaccharide Momooxygenase with Beta-Glucan Substrates and Cellobiose Dehydrogenase	Finn L. Aachmann
	16:40	16:45	00:05		**Stroll to T55A**	
	16:45	18:30	01:45		Ribbon-cutting, cake and sparkling wine	
	18:30	19:00	00:30		**Stroll or Bybane to hotel**	
	19:00	21:00	02:00		Dinner and socializing	

	Start	End	Time	Chair		Presenter
Friday 17/6 Morning Sessions	09:00	09:20	00:20	Aurora Martinez	Interaction Studies of UNG2 with its DNA Replication Fork Partners	Edith Buchinger
	09:20	09:40	00:20		Towards the in vivo Structure of HIV-1 p6	Torgils Fossen
	09:40	10:00	00:20		Pharmacophore Mapping of the ATP-binding Pocket of Protein Kinase DYRK1A with Benzothiazole Fragment Componds by X-Ray and NMR	Johan Isaksson
	10:00	10:20	00:20	Nils Åge Frøystein	The Dynamic Stereochemistry of Opioids	Michal Rachel Suissa
	10:20	10:40	00:20		Coffee break and Visit the Sponsors	
	10:40	11:00	00:20		NMR and Organometallic Chemistry -- Some Examples from Au(III) Chemistry	Marte Sofie M. Holmsen
	11:00	11:20	00:20	Willy Nerdal	NMR projects at MBI -- an overview	Olena Dobrovolska
	11:20	11:40	00:20		Solid-State NMR Studies of Yersinia Adhesin A: From Autotransport Mechansim to Drug Screening	Dirk Linke
	11:40	12:00	00:20		Structure and Dynamics of The Yersinia Adhesin A Membrane-Anchor Domain by NMR	Marcella Orwick Rydmark
	12:00	13:00	01:00		Lunch	
	13:00	14:00	01:00		**Visit the Sponsors and NSMR General Assembly**	

	Start	End	Time	Chair		Presenter
Friday 17/6 Afternoon Sessions	14:00	14:20	00:20	**Tina Pavlin**	Characterization of Catalytic Sites in Microporous Materials Using Solid-State NMR. An ex-situ and in-situ High Temperature Flow Study.	Bjørn Årstad
	14:20	14:40	00:20		Structural Properties of Water Near Solid Surfaces from NMR	Christian Totland
	14:40	15:00	00:20		Characterization of pore Structure Heterogeneity and Liquid Saturation using NMR	John Georg Seland
	15:00	15:20	00:20		Analysis Directly on Biological Tissue with Solid-State NMR	Willy Nerdal
	15:20	15:40	00:20			
	15:40	16:00	00:20			
	16:00	18:00	02:00		Banquet and poster-prize awards	

ORAL PRESENTATIONS

METABOLOMICS

Thursday 9:00-10:05
Chair: Øyvind Halskau

A New Fatty Acid Amide from Streptomyces Species with Insecticidal Activity

Is ^{31}P NMR the neglected nucleus for lipid profiling?

MRI and Metabolomics

CLINICAL AND PRECLINICAL MRI AND MRS

Thursday 11:05 - 12:05
Chair: John Georg Seland

Towards time-resolved quantification of *in-vivo* GABA spectroscopy

Magnetic resonance imaging for drug repurposing in the treatment of melanoma brain metastasis

Using diffusion MRI to study demyelination in cortex and deep gray matter in animal
model of multiple sclerosis

NNP opening

Thursday 13:00 - 16:40
Chair: Jarl Underhaug

The start of NMR in Norway

Structures and the Structural Basis of Enzymatic Activity of Several APOBECs

State-of-the-art CryoProbe Technology at High Field Pushes the Limits for Applications From Small Molecules to Bio-NMR

Urease: a platform to integrate multiple structural biology approaches towards drug design

Interactions of a fungal lytic polysaccharide monooxygenase with β-glucan substrates
and cellobiose dehydrogenase

MACROMOLECULAR NMR session 1

Friday 9:00 - 10:00
Chair: Aurora Martinez

Interaction studies of UNG2 with its DNA replication fork partners

Towards the *in vivo* structure of HIV-1 p6

Pharmacophore mapping of the ATP-binding pocket of protein kinase DYRK1A with benzothiazole fragment compounds by X-ray and NMR

NMR ON NATURAL AND SYNTHETIC COMPOUNDS

Friday 11:00 - 12:00
Chair: Nils Åge Frøystein

The Dynamic Stereochemistry of opioids

NMR and organometallic chemistry – some examples from Au(III) chemistry

MACROMOLECULAR NMR session 2

Friday 11:00 - 12:00
Chair: Willy Nerdal

Solid-state NMR studies of *Yersinia* Adhesin A: from autotransport mechanism to drug screening

Structure and dynamics of the *Yersinia* Adhesin A membrane-anchor domain by nuclear magnetic resonance

MATERIAL SCIENCE AND TECHNOLOGY

Friday 13:30 - 14:50
Chair: Tina Pavlin

Characterization of catalytic sites in microporous materials using solid-state NMR An *ex-situ* and *in-situ* high temperature flow study

Structural properties of water near solid surfaces from NMR

Characterization of pore structure heterogeneity and liquid saturation using NMR

Analysis Directly on Biological Tissue with Solid-State NMR

POSTER SESSION

Posters 1-13
Thursday 10:05 - 11:05

Characterization of catalytic sites in microporous materials using solid-state NMR An *ex-situ* and *in-situ* high temperature flow study

Interactions of a fungal lytic polysaccharide monooxygenase with β-glucan substrates and cellobiose dehydrogenase

Structural effects of copper-binding to a lytic polysaccharide monooxygenase

Towards time-resolved quantification of in-vivo GABA spectroscopy

Experimental comparison of measurement techniques for different molecular weight poly ethylene glycol

Why is HAMLET more toxic to dividing cells?

A continuous- and a discrete spin-spin relaxation rate representation of water in meat – A comparative investigation

Characterization of protein-fatty acid complex

Does the lipid fraction of *Listeria innocua* change as a function of the cell cycle?

Dynamics Study of Human deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase) by Nuclear Magnetic Resonance Spectroscopy

Applications of magnetic resonance imaging within petrophysical rock core analysis

Fatty acids in wild and farmed salmon by use of MRI and solid-state NMR

Hydrolysis of organophosphorus nerve agents by marine DFPases

A New Fatty Acid Amide from Streptomyces Species with Insecticidal Activity

Subramaniam Gopalakrishnan[1], Vijayabharathi Rajendran[1], Sathya Arumugam[1], Hari C. Sharma[1], Srinivas Vadlamudi[1], Ratna Kumari Bhimineni[1], Susana V. Gonzalez[2], Torunn M. Melø[3] and Nebojsa Simic[2]

[1]*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India*
[2]*Department of Chemistry, Norwegian University of Science and Technology (NTNU), Trondheim, Norway*
[3]*Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway*

Helicoverpa armigera is a pest that feeds on more than 300 species of plants, including chickpea, pigeonpea, groundnut, cowpea, cotton, maize and sorghum and a range of vegetable and fruit crops. Thus it inflicts a considerable loss of crops worldwide. The main objective of this study was to isolate and identify the metabolite against it from a previously characterized *Streptomyces* sp. CAI-155.
In a bioactivity guided study, a new compound, N-((S)-1-((4R,5R)-2,2-dimethyl-5-undecyl-1,3-dioxolan-4-yl)-2-hydroxyethyl)stearamide, with potent insecticidal property has been discovered. Its structure and stereochemistry has been elucidated by 1D and 2D NMR experiments and MS spectral data.
The purified metabolite showed 70–78% mortality in 2nd instar *H. armigera* by diet impregnation assay, detached leaf assay and greenhouse assay. The LD50 and LD90 values of the purified metabolite were 627 and 2276 ppm, respectively. The results imply a possibility of using this novel metabolite for pest management in future.
Structure elucidation of the compound was demanding and time consuming task. Challenges during that process will be discussed in the presentation.

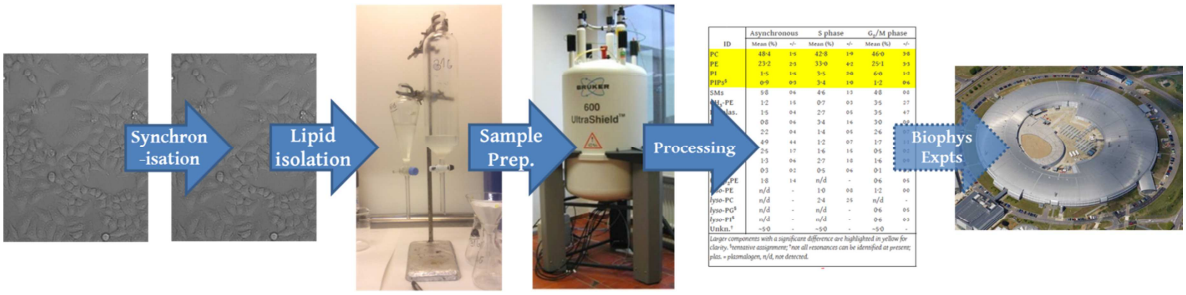
METABOLOMICS-2

Is ^{31}P NMR the neglected nucleus for lipid profiling?

Samuel Furse

Department of Molecular Biology, UiB

Advances in metabolomics over the last two decades have led to an unprecedented insight into the molecular profile of biological systems. Tools for lipidomics in particular have been instructive in profiling both signalling and structural molecular components of cells. However, quantification and structural determination of organic molecules is not always straightforward. ^{31}P NMR offers both structural and quantitative data and is orthogonal to mass spectrometry, and is therefore useful in producing unambiguous lipidomics data. I will discuss the use of ^{31}P NMR for profiling lipid mixtures in the context of my research into the physical role of lipids *in vivo*, with particular relevance to membrane division events in the cell cycle.



METABOLOMICS-3

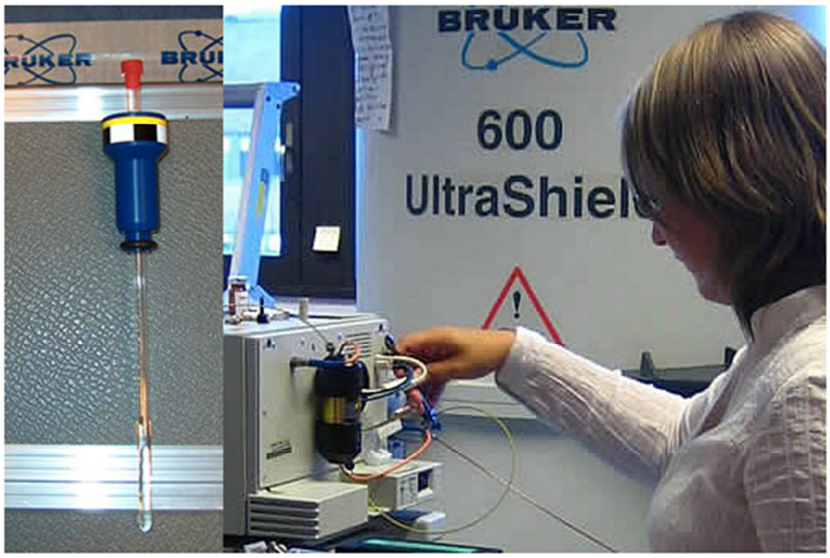
NMR spectroscopy in metabolic studies.

Frode Rise

Department of Chemistry, University of Oslo, PO Box 1033 Blindern, NO-0315 Oslo.

Examples of brain metabolic studies performed with the help of ^{13}C labelling will be presented.

University of Oslo NMR Center - serving the greater Oslo area with NMR science!



Towards time-resolved quantification of *in-vivo* GABA spectroscopy

Gerard Dwyer[1], Alexander Craven[1], Renate Grüner[2,3], Lars Ersland[4], Kenneth Hugdahl[1, 2, 5, 6]

[1]*Department of Biological and Medical Psychology, University of Bergen, Norway,*
[2]*Department of Radiology, Haukeland University Hospital, Bergen, Norway,*
[3]*Department of Physics and Technology, University of Bergen, Norway,*
[4]*Department of Clinical Engineering, Haukeland University Hospital, Bergen, Norway,*
[5]*Division of Psychiatry, Haukeland University Hospital, Bergen, Norway,*
[6]*NORMENT Center of Excellence, University of Oslo, Norway*

The ability to perform quantitative analyses of biochemical compounds *in vivo* with magnetic resonance spectroscopy (MRS) has afforded new insights into biological mechanisms underlying both healthy and diseased states. Of particular interest is the ability to measure GABA, as dysfunction of GABA-ergic transmission has been implicated as a contributing factor in many psychological conditions, including the experience of auditory verbal hallucinations (AVH) in people with schizophrenia. The purpose of this project was to develop a method for performing MRS in a time-resolved or functional manner (i.e. fMRS) in order to measure dynamic changes in GABA in response to tasks or stimuli. To this end, transcranial direct current stimulation (tDCS), a neurostimulation technique that may be used to excite or inhibit neural activity within a given area, was used in a facilitating capacity to increase activity in the posterior superior temporal gyrus (pSTG). Excitatory stimulation has been shown in previous studies to lower local cortical GABA concentration in the area of excitation. To better understand the relation between GABA variations and the experience of auditory hallucinations, of the operational mechanisms of tDCS, and the possibilities for the latter to be incorporated in management of the former, the ability to quantify metabolite variations with improved temporal resolution is crucial.

Typically in MR-Spectroscopy, in order to overcome the inherently low SNR of a single acquisition, a high number of individual spectra are acquired over a long period and averaged together to produce a single time-averaged spectrum. While this approach may be useful for improving SNR, the long scan time it necessitates limits usefulness for time-resolved or functional MRS designs.

Specialized spectroscopy sequences, such as the GABA-specific MEGA-PRESS spectral editing technique exacerbate these problems, as the technique requires the acquisition of two spectra from which a difference spectrum is calculated, effectively doubling scan time and halving temporal resolution. Furthermore, editing techniques are susceptible to issues of co-editing, and variations in editing efficiency due to frequency drift throughout the scan.

Despite the problems fMRS of GABA presents, it has great potential to elucidate biochemical events underlying changes observed both in normal processes and mechanisms of disease.

Here, we present a novel analysis approach for use with GABA-specific MEGA-PRESS spectroscopy data that adaptively combines data from multiple time windows to quantify temporal dynamics. Additionally, some of the challenges posed by acquiring reliable, stable MEGA-PRESS data over an extended sequence will be explored, and finally, an algorithm will be proposed for blind group-wise analysis of such data.

In this experiment, the MEGA-PRESS sequence was used to acquire a series of spectra from the pSTG, an key node in the auditory processing stream which has been implicated in the experience of auditory hallucinations in schizophrenia, amongst other conditions. During this acquisition, transcranial direct current stimulation (tDCS) was used to induce a change in cortical GABA concentration within the area of interest. Previous studies have shown anodal stimulation to decrease local cortical GABA concentration.

With spectra acquired continuously over a ~30 minute period, stimulation was introduced for 10 minutes approximately 10 minutes into the scan thus creating three ~10 minute blocks: Before, during and post stimulation, with each subject undergoing real and "sham" acquisitions in a double-blind design.

Magnetic resonance imaging for drug repurposing in the treatment of melanoma brain metastasis

Frits Thorsen

Kristian Gerhard Jebsen Brain Tumour Research Centre and The Molecular Imaging Centre, Department of Biomedicine, University of Bergen, Bergen, Norway.

Introduction: Around 3.4 million people in Europe will develop cancer every year (1), and up to 40% of these (i.e. 1.5 million people) will develop brain metastases. Untreated, the median survival time is 2-3 months, while aggressive treatment extends survival to 4-12 months. To date, very little has been done to develop drugs specifically targeting brain metastases, and patients with brain metastases have been excluded from clinical trials using drugs to target metastatic disease (2). Thus, there is a need to find new and more effective drugs that can penetrate the blood-brain barrier to treat this large patient group.
Methods: We have developed novel animal models to study brain metastasis, where tumor cells from human melanoma brain metastases or human lung adenocarcinomas are injected into the blood stream of nod/scid mice. We have demonstrated by preclinical, multimodal imaging (MRI, PET/CT, bioluminescence imaging) that the animals develop multiple brain metastases in the brain, similar to what is seen in patients (3-5).
Results and Discussion: From metastatic lesions developing in animal brains, we identified a candidate gene signature list for melanoma brain metastases by deep sequencing. Based on the gene signature list, we found several compounds (already in use on patients for other conditions than cancer), which may improve treatment of brain metastasis.
We showed by multimodal imaging that the cholesterol analogue β-sitosterol effectively inhibited brain metastases and improved survival in our animal models, both on established tumours and in a preventive setting. β-sitosterol broadly suppressed the MAPK pathway via its converging downstream regulators, and effectively reduced mitochondrial respiration through Complex I inhibition. Our results may open new avenues of systemic therapy against metastatic melanoma, as increased mitochondrial respiration is a key mediator of resistance to MAPK-targeted drugs. In conclusion, this broad-spectrum suppression of melanoma brain metastasis strongly encourages further assessment of β-sitosterol as an adjuvant to established MAPK-targeted therapies.

1. Home NCI. SEER Stat Fact Sheets: All Cancer Sites National Cancer Institute Home 2012. Available from: http://seer.cancer.gov/statfacts/html/all.html.
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3. Sundstrøm T, Espedal H, Harter P, Fasmer K, Skaftnesmo KO, Horn S, Hodneland E, Mittelbronn M, Weide B, Beschorner R, Bendner B, Rygh CB, Lund-Johansen M, Bjerkvig R, Thorsen F. Melanoma brain metastasis is independent on LDHA expression. Neuro-Oncol 17:1374-1385, 2015.
4. Sundstrøm T, Daphu I, Wendelbo I, Hodneland E, Lundervold A, Immervoll H, Skaftnesmo KO, Babic M, Jendelova P, Sykova E, Lund-Johansen M, Bjerkvig R, Thorsen F. Automated tracking of nanoparticle-labeled melanoma cells improves the predictive power of a brain metastasis model. Cancer Res 73:2445-2456, 2013.
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Using diffusion MRI to study demyelination in cortex and deep gray matter in animal model of multiple sclerosis

Tina Pavlin

Dep. of Biomedicine, University of Bergen and Dep. of Radiology, Haukeland University Hospital

We have applied a biophysical model of diffusion to study dendrite density and diffusion in cortex and deep gray matter in an animal model of multiple sclerosis. We have performed DTI on mice brains *ex-vivo* at baseline, after 3 and 5 weeks of cuprizone exposure, and 4 weeks after terminating exposure. We observed significant drop in neurite density and increase in intra-axonal diffusion at 3 and 5 weeks of exposure and recovery to baseline values after remyelination. Our study shows the potential of DTI to detect subtle changes in myelin content in gray matter, thereby improving out understanding of the disease.

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Innovation with Integrity

NNP-1

The start of NMR in Norway

Bjørn Pedersen

Skolelaboratoriet i kjemi UiO

The work to get the first NMR-spectrometer to Norway started at Sentralinstitutt for industriell kjemi (SI) by Henry Viervoll and Nico Norman in the early 1950ties. They were both students of Odd Hassel, the first and only Norwegian Nobel laureate in chemistry, and he supported the idea. They succeeded in getting the necessary money from the Norwegian Research Council (NTNF) to buy a dual purpose 60 MHz NMR-spectrometer with a 12”-magnet and a 9.1 GHz EPR-spectrometer with a 6”-magnet from Varian Asc. The spectrometers were installed at SI in the spring of 1960.

But one problem remained: nobody in Norway was trained in the field of NMR and EPR spectroscopy. I was lucky and got the job in the summer of 1959 even though I had never heard of NMR or EPR. I had finished my master’s degree in the autumn a year earlier in inorganic chemistry. I was sent to the Department of Physics at Cornell University and stayed there for ten months working with professor Donald Holcomb and a graduate student Gil Clark. The main work I did was to measure T1 in single crystals of gypsum showing that the relaxation was due to 180 $^{\circ}$flips of the water molecules.

I was back at SI in early September 1960 and found that the spectrometers were quite different from the puls-NMR-spectrometer I had worked with at Cornell. But slowly the technician I was allocated, Merete Lange, and I learned to operate the spectrometers. We also got support from many others working at SI so the working conditions were very good. It was a young staff helping each other. It was also expected that I should get paid projects from Norwegian industry, but most of the projects we worked on were financed from the Norwegian Research Council (NTNF) as I will explain in the talk.

NNP-2

Structures and the Structural Basis of Enzymatic Activity of Several APOBECs

In-Ja L. Byeon, Troy C. Krzysiak, Jinwon Jung, Jinwoo Ahn, Mithun Mitra, Chang-Hyeock Byeon, Kamil Hercik, Jozef Hritz, Judith G. Levin, & Angela M. Gronenborn

University of Pittsburgh, School of Medicine

The APOBEC/AID family of cytosine deaminases plays an important role in the vertebrate innate immune response. These enzymes inhibit the replication of many DNA-based pathogens, such as exogenous viruses and endogenous transposable elements. They function as host restriction factors and display a broad range of activities against endogenous and exogenous retroelements. These proteins contain either one or two domains, comprising a Zn binding motif that is important for functional and structural integrity. Our laboratory has solved NMR solution structures and investigated the structural basis of the enzymatic activity of APOBEC2, APOBEC3A, APOBEC3B and APOBEC 3H. Results of this work will be presented.

Iwatani et al. Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. Nucleic Acids Res., 35, 7096-7108, 2007.
Iwatani et al. HIV-1 Vif-mediated ubiquitination/degradation of APOBEC3G involves four critical lysine residues in its C-terminal domain. Proc. Natl. Acad. Sci. U.S.A. 17, 19539-19544, 2009
Krzysiak et al. APOBEC2 is a monomer in solution: Implications for APOBEC3G models. Biochemistry, 51, 2008-2017, 2012.
Byeon, et al. NMR structure of human restriction factor APOBEC3A reveals substrate binding and enzyme specificity. Nature Comm. 4, 1890, 2013.
Mitra et al. Structural determinants of human APOBEC3A enzymatic and nucleic acid binding properties. Nucleic Acids Res. 42, 1095-1110, 2014
Byeon et al. NMR Structure of the APOBEC3B Catalytic Domain: Structural Basis for Substrate Binding and DNA Deaminase Activity. Biochemistry, in press, 2016

NNP-3

Rainer Kuemmerle, representing Bruker, will present the state-of-the-art within cold probe technology at high fields, and how these advances push the limits for applications on small molecules to bio-NMR.

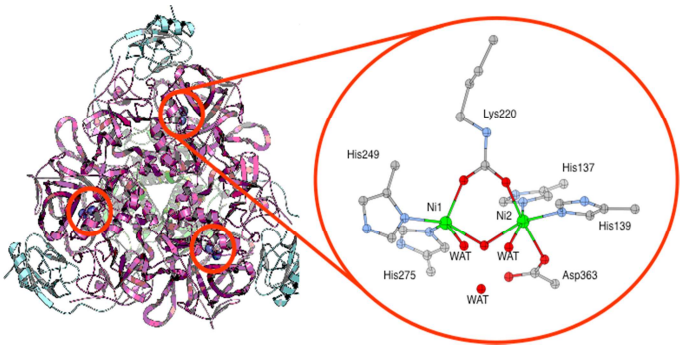
NNP-4

Urease: a platform to integrate multiple structural biology approaches towards drug design

Stefano Ciurli (stefano.ciurli@unibo.it, tel. +39 051 209 6204)

Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna (Italy)

The enzymatic activity of urease, a Ni(II)-dependent enzyme that catalyses the hydrolysis of urea in the last step of organic nitrogen mineralization, has negative consequences for both human health and environment. In particular, urease plays an essential role for the colonization and survival of several deadly ureolytic human pathogens. On the other hand, the large and widespread use of urea as a soil fertilizer for crop production, combined with the high efficiency of soil urease, leads to damage to germinating seedlings and young plants, nitrite toxicity and gaseous loss of urea N as ammonia, with consequent atmospheric pollution, increase of the green house effects, and decreased efficiency of soil fertilization. The development of potent urease inhibitors, necessary to modulate the catalytic activity of this enzyme, requires the knowledge, at the molecular level, of the mechanism of catalysis. In addition, understanding of the homeostatic mechanisms through which the inorganic Ni(II) ion is taken into, or ejected from, the cell to warrant the optimal metal content for the bacterium metabolism, as well as the mechanisms of sensing this ion through the action of metal-dependent transcription factors devoted to the regulation of genes coding for proteins involved in Ni(II) metabolism, would provide additional targets for the development of drugs to fight these ureolytic organisms. This approach is even more important considering the increasing number of human pathogens that are becoming resistant to known antibiotics.



This lecture will describe how an integrated approach using X-ray crystallography, NMR spectroscopy, other biophysical techniques such as calorimetry and light scattering, and computer modelling, can provide information for the design of drugs to modulate the enzymatic activity of urease. The fall out of these discoveries have several implications in the economical, social, environmental and medical aspects of everyday life.

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2. B. Zambelli, F. Musiani, S. Benini, S. Ciurli Acc. Chem. Res. 2011, 44, 520-530.

NNP-5

Interactions of a fungal lytic polysaccharide monooxygenase with β-glucan substrates and cellobiose dehydrogenase

Gaston Courtade[1], Reinhard Wimmer[2], Åsmund K. Røhr[3], Marita Preims[4], Alfons K.G. Felice[4], Maria Dimarogona[5], Gustav Vaaje-Kolstad[3], Morten Sørlie[3], Mats Sandgren[5], Roland Ludwig[5], Vincent G. H. Eijsink[3], Finn L. Aachmann[1]

[1]NOBIPOL, Department of Biotechnology, NTNU Norwegian University of Science and Technology, Trondheim, Norway. [2]Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark. [3]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway. [4]Food Biotechnology Laboratory, Department of Food Science and Technology, Vienna Institute of Biotechnology (VIBT), BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. [5]Department of Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Lytic polysaccharide monooxygenases (LPMOs) are key components of enzymatic biomass (e.g. chitin and cellulose) degradation processes. LPMOs catalyze a reaction cycle that requires copper, molecular oxygen and an electron donor, such as cellobiose dehydrogenase (CDH). Docking models have suggested that residues on the surface of the LPMO, near the copper site, interact with the cytochrome domain of CDH during electron transfer. However, experimental data that could clarify interactions of AA9 LPMOs with their substrates and CDH are lacking.

In this study, we have used NMR and ITC we to study the interactions between a fungal LPMO and three soluble substrates and CDH. In addition, we have analyzed the structure and dynamic features of the LPMO in solution. The results reveal potential adaptations of the LPMO surface to varying substrates and unambiguously show that both the substrate and CDH bind to a patch that is focused around the copper-site. In addition to providing insights into enzyme-substrate interactions in LPMOs, the present observations shed new light on possible mechanisms for electron supply during LPMO action.

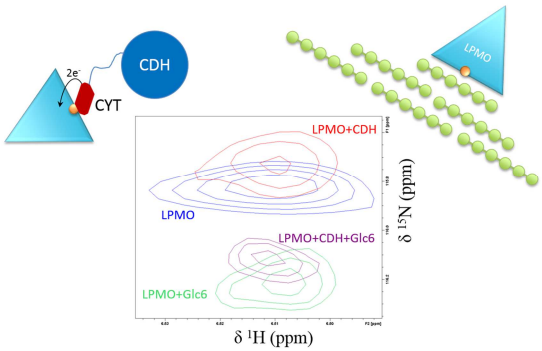

Figure 1: Left: The outer sphere of 12 Å radius represents the region in which ^{1}H signals are expected to be affected by Cu(II) PRE, while the inner sphere of 6 Å radius represents the expected effect on ^{13}C signals. Right: Residues with vanished ^{15}N-HSQC signals upon addition of 50 µM Cu(II) to 1 mM ^{15}N-labeled LPMO are colored blue on the structure.

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Interaction studies of UNG2 with its DNA replication fork partners

Edith Buchinger[1], Bodil Kavli[2], Per Arne Aas[2], Lars Hagen[2], Geir Slupphaug[2], Finn L. Aachmann[1]

[1]Department of Biotechnology, Norwegian University of Science and Technology, 7034 Trondheim, Norway, [2]Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, NTNU, Trondheim, Norway.

In recent years intrinsically disordered proteins (IDPs) have come into the focus of the research community. Prediction shows that up to 33 percent of all functional eukaryotic proteins have an intrinsically disordered segment longer than 25 residues. NMR allows studying these intrinsically disordered proteins or segments at atomic level.

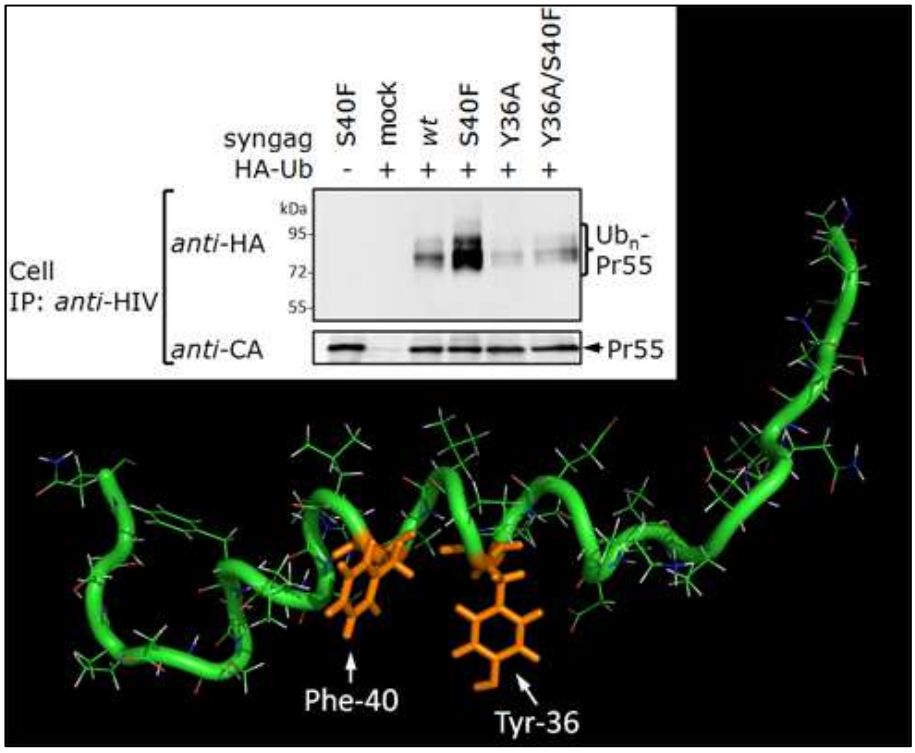
The human uracil DNA glycosylase UNG2 specifically and efficiently removes uracil from DNA in the nucleus. The catalytic C-terminal core domain of UNG2 (85-313 AA) was crystallized both as native protein and in a complex with DNA-uracil. The 85 residue long N-terminal region was predicted to be mainly unstructured which could be confirmed by recent SAXS and NMR analysis. Despite the lack of intrinsic structure, the N-terminal region markedly changes functional properties of UNG2 compared to the catalytic domain alone, including increased preference towards single-strand DNA and localization to replication foci in S-phase. It could be shown that PCNA and RPA associate with the N-terminal region of UNG2 at specific motifs. To what degree these interactions are regulated by post-translational modifications remains, however, elusive.

Both N- and C- terminal UNG2 have now been assigned to allow detailed studies of both domains separately and as well in the full-length protein. Structural rearrangement upon interaction between RPA and N-terminal UNG2 in presence and absence of DNA have been determined at atomic level. Interactions between UNG2 and RPA are measured either on the N-terminal UNG2 or on peptides the aforementioned RPA binding site with domains of RPA known for binding UNG2. Additionally the effects of post-translational modifications of UNG2 on binding to its interaction partners are tested by peptides with these post-translational modifications. Finally, we have studied the effect on the catalytic C-terminal domain of UNG2 when RPA interacts with the unstructured domain using a full-length UNG2.

Towards the *in vivo* structure of HIV-1 p6

Torgils Fossen, Friedrich Hahn, Christian Setz, Melanie Friedrich, Pia Rauch, Sara Marie Solbak, Nils Åge Frøystein, Petra Henklein , Jörg Votteler, and Ulrich Schubert

The HIV-1 p6 Gag protein contains two late assembly (L-) domains that recruit proteins of the endosomal sorting complex required for transport (ESCRT) pathway to mediate membrane fission between the nascent virion and the cell membrane. Here, we show that the mutation S40F, but not the conservative mutation to Asp (S40D) or Asn (S40N), augments membrane association, K48-linked polyubiquitination, entry into the 26S proteasome, and, consequently, enhances MHC-I antigen presentation of Gag derived epitopes. Nuclear magnetic resonance (NMR) structure analyses revealed that the newly introduced Phe-40, together with Tyr-36, causes the formation of a hydrophobic patch at the C-terminal α-helix of p6, providing a molecular rationale for the enhanced membrane association of Gag observed in vitro and in HIV-1 expressing cells. The extended exposure of the S40F mutant to unidentified membrane-resident ubiquitin E3-ligases might trigger the polyubiquitination of Gag. The cumulative data support a previous model of a so far undefined property of p6, which, in addition to MA, acts as membrane targeting domain of Gag.



HIV-1 p6 adopts α-helical structure *in vivo*. Mutation of Ser-40 with Phe creates a hydrophobic domain consisting of and dependent on Y-36 and Phe-40 incorporated in an intact α-helical structure.

Pharmacophore mapping of the ATP-binding pocket of protein kinase DYRK1A with benzothiazole fragment compounds by X-ray and NMR

Johan Isaksson[1], Ulli Rothweiler[1], Wenche Stensen[2], Bjørn Olav Brandsdal[1], Frederick Alan Leeson[1], Richard Alan Engh[1], John S. Mjøen Svendsen[1,2]

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DYRK1A has emerged as a potential target for therapies of Alzheimer's disease using small molecules. Based on the observation of selective DYRK1A inhibition by firefly D-luciferin, we have explored static and dynamic structural properties of fragment sized variants of the benzothiazole scaffold with respect to DYRK1A using X-ray crystallography and NMR techniques. The compounds have excellent ligand efficiencies. The structures show a remarkable diversity of binding modes in dynamic equilibrium. Binding geometries are apparently driven by types of interactions often considered "weak", including "orthogonal multipolar interactions", e.g. F-CO, sulfur-aromat, halogen-aromat. The most stable binding mode or modes in equilibrium are determined by the sum of these interactions, together with the effects of electron withdrawing groups that modulate hydrogen-bonding propensities.
These studies are an excellent example of how NMR data also without isotope labelling are complementary to X-ray structures, and that full structural studies are required to fully interpret the essential physical determinants of binding.

The Dynamic Stereochemistry of opioids

Michal Rachel Suissa*[a], Aud Mjærum Bouzga[b], Per Ola Rønning[a] and Terje Didriksen[b]

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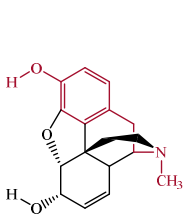
Pain affects us considerably both on the individual and societal levels. The increasing sale of painkillers does not attest to improvement in this field, but rather points to the contrary. A great deal of research has therefore been directed towards conventional and unconventional methods for treating pain. One important group of pharmaceuticals used on a variety of pain types are opioids. Opium products were already in use in Mesopotamia more than 2,000 years ago. A problem with opium products, apart from their good effects, is that they also have a darker side in the form of serious side effects.[i,ii] Therefore, comprehensive investigations searching for selective, non-addictive opioide analgesics have been carried out over the last 50 years. Hundreds of derivatives have been synthesized and tested, and this research has resulted in various derivatives of morphine, agonists and antagonists, as well as synthetic opioids such as Fentanyl, Pethidine and Methadone. Despite this extensive research, we still do not have any derivatives of this family that are free of serious side effects. Finding the bioactive conformation (s) of opioids holds the key to a better understanding of how exactly these molecules bind to the different receptor subtypes of this group. This can in turn be used in design of selective compounds of good analgesic effect but with less serious side effects.

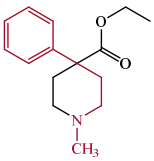
Even though some theoretical work has been conducted in this field[iii,iv], there is still no extensive conformational analysis of this group of molecules.

The conformational freedom of morphine and its charged form morphinum are contained in the conformational preference of the alcohol and amine functions because the carbon back bone is rigid. All together, there are 24 possible conformations, due to inversion of the nitrogen (in the basic forms) and due to rotation around the two hydroxyl groups in both morphine and morphinum.

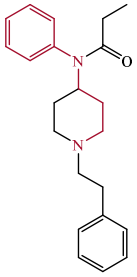
The conformational behaviour of pethidine and fentanyl is far more complex and includes free rotations, nitrogen inversion as well as ring inversion.



Morphine



Pethidine



Fentanyl

NMR and organometallic chemistry – some examples from Au(III) chemistry

Marte Sofie Martinsen Holmsen, Ainara Nova, Yannick Wencke, Eirin Langseth, Sigurd Øien-Ødegaard and Mats Tilset

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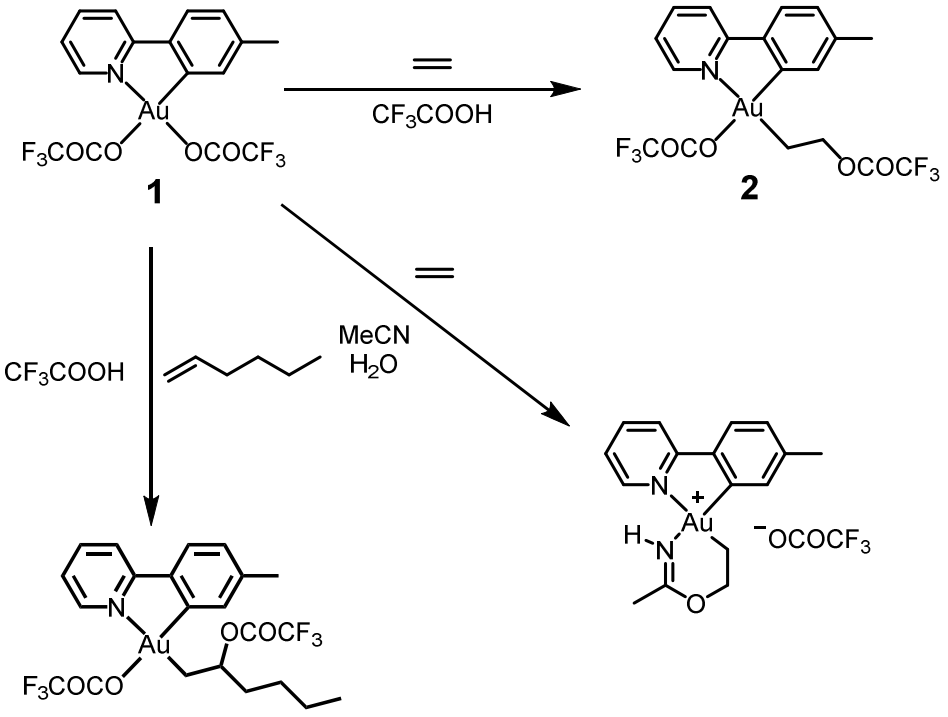
The use of gold complexes in catalysis has become a very popular field of research.[1] Our group recently reported the insertion of ethylene into an Au-O bond.[2] The formal insertion at Au(III) complex 1 takes place into the Au-O bond trans to N of the chelating tolylpyridine ligand furnishing 2. This discovery led to further investigations and the scope of these insertions is now being developed further. The reactivity of Au(III) complex 1 towards a wide variety of alkenes and alkynes has been investigated and recent findings will be presented. Some examples are given in the figure below. NMR is an important tool in organometallic chemistry and this presentation will focus on how NMR was used in order to understand the Au(III) chemistry presented here.



1. A. S. K. Hashmi, F. D. Toste, Modern Gold Catalyzed Synthesis, Wiley-VCH, Weinheim, 2012.
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Selected NMR projects at the Department of Molecular Biology

Olena Dobrovolska

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NMR benefits many ongoing projects at the Department of Molecular Biology. Four projects associated with MBI where NMR plays a role in advancing them will briefly be presented. 1) ErbB3-binding protein EBP1, in vivo showed co-localization with polyphosphoinosities in the nucleolus. In vitro observations using NMR demonstrated the residue-specific binding. 2) Conformational space of misfolding, membrane-disrupting peptides. Misfolding may potentially be triggered by the presence of a lipid membrane. NMR is being used to map the conformational space of misfolding peptides in the presence and absence of a soluble membrane mimic. 3) Epitope- and receptor interface mapping of sTH, a diarrhea-causing heat-resistant peptide from Enterotoxigenic E. Coli. NMR assists in guiding the engineering of a peptide that will not interact with its target receptor in the small intestine, while at the same time being similar enough to be immunogenic. This peptide will be the main component in a prospective vaccine against this type of diarrhea. 4) The bound form of the CW histone recognition module of the ASHH2 methyltransferase. A brief status report on each of project 1-3 will be given, before providing some more details on project 4. CW domains are conserved recognition modules appearing in a range of enzymes associated with epigenetics. Although similar in structure, they are specific to different methylation states of histone tails. A key question is therefore how the different domains confer different binding profiles. ASHH2 is a multidomain enzyme that recognizes and binds to particular methylated lysines on histone tails using its CW domain. In an earlier work (Hoppmann et al., EMBO J 2011) the unbound structure was determined. Further work has led to the hypotheses that the conserved Tryptophans forming part of the K3me1 binding site responds to binding by opening up and changing their angles relative to each other. Moreover, a flexible C-terminal not obviously part of the domain core or binding site, appears to be important for binding and is stabilized upon K3me1 binding. Dynamic data supporting that this is the case is presented, along with a preliminary structure of the bound situation.

Macromolecular NMR-5

Solid-state NMR studies of *Yersinia* Adhesin A: from autotransport mechanism to drug screening

Nandini Chauhan[1], Shakeel Shaheed[2,3], Michael Habeck[3], Barth van Rossum[2], and Dirk Linke[1,3]

[1]Department of Biosciences, UiO, [2]FMP Berlin, Germany, [3]MPI for Developmental Biology, Tübingen, Germany

Adhesion of bacteria to surfaces is essential for many aspects of microbial life: surface recognition and attachment, biofilm formation, and pathogenesis. Adhesion to host tissues is frequently among the first steps in host colonization by pathogens, and adherence is often an essential property for virulence. Adhesins must reach the bacterial cell surface to fulfill their function. Numerous, sometimes highly specific export systems exist for this purpose. In autotransporter proteins of Gram-negative bacteria, export through the outer membrane is mediated by a C-terminal membrane pore domain that auto-exports the N-terminal passenger domain of the same polypeptide chain [1]. Trimeric autotransporter adhesins (TAAs) comprise a widespread family of adhesive molecules in Gram-negative bacteria, many of which are important virulence factors [2]. The are also called Type Vc autotransporters (**Figure 1**). The prototypical trimeric autotransporter is the Yersinia adhesin YadA from Yersinia enterocolitica, which mediates attachment to collagen and other extracellular matrix (ECM) molecules, promotes serum resistance and mediates autoagglutination.

In recent work using mainly solid-state NMR, we showed that the structure of the autotransport domain of YadA contains a conserved region of small residues [3], that we are currently exploiting for mutational studies (unpublished work). We have constructed mutant YadA variants stalled in autotransport that will help us to elucidate the export mechanism through the bacterial outer membrane, and are in the process of solving the structure of this transition-state construct - again using solid-state NMR. Moreover, we are currently working on small-molecule inhibitor screens based on bacterial adhesion in microwell plates, with the aim to find inhibitors that target either the autotransport process (adhesin biogenesis) or the adhesion to ECM molecules (adhesin function).

[1] Linke et al., Trends in Microbiology, 2006
[2] Leo et al., Philosophical Transactions of the Royal Society B, 2012
[3] Shahid et al., Nature Methods, 2012

Structure and dynamics of the *Yersinia* Adhesin A membrane-anchor domain by nuclear magnetic resonance

Marcella Orwick Rydmark

Yersinia Adhesin A (YadA) is a protein found in the membrane of *Yersinia Enterocolitica*, which is involved in a number of food-borne diseases including enterocolitis, acute enteritis, diarrhea, and mesenteric limphadentisis. YadA plays an important role in the ability of Y. enterocolitica to colonize a host, by aiding in the autotransport of a head domain to the cell surface that can stick to host tissues. A recent structure of the YadA anchor domain region was solved with the protein in the crystalline form by ssNMR, where functionally relevant regions were proposed. To understand the dynamics and their functional significance in YadA structure and function, we are currently working towards solving the structure of YadA in both the native lipid environment and in a native-like lipid environment, using both solid state and solution NMR. To date, no known structure of a membrane protein has been solved in the E.coli outer membrane, and very few membrane protein structures have been solved by NMR, particularly in membrane. A primary R&D challenge will be obtaining high quality NMR data in complex environments, and the preliminary amino-acid assignment of the NMR data.

Shahid, S. A et al, Solid-state NMR Study of the YadA Membrane-Anchor Domain in the Bacterial Outer Membrane. Angew. Chem. Int. Ed., 54:12602–12606, 2015.

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[1] Rincon, D. A., Cordeiro, M. N. D. S., Mosquere, R. A. I., Theoretical study of morphine and heroin: Conformational study in gas phase and aqueous solution and electron distribution analysis; J. Quant. Chem. 2010, 110, 2472.
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Characterization of catalytic sites in microporous materials using solid-state NMR. An *ex-situ* and *in-situ* high temperature flow study

Bjørnar Arstad[1], Anna Lind[1], Georgios Kalantzopoulos[2], Fredrik Lundvall[2], David Wragg[2], Helmer Fjellvåg[2]

[1]*SINTEF Materials and Chemistry,* [2]*University of Oslo*

A variant of microporous zeolite materials emerged in the 1980s when Union Carbide (now UOP LLC) discovered a new family of silicoaluminophosphate (SAPO) molecular sieve materials that showed a broad range of structure types and compositions. Among these was SAPO-34, a small pore molecular sieve which has proved to be highly active in the conversion of methanol to olefins a process now being commissioned at several sites in China. However, an omnipresent issue is deactivation of the catalysts by coking and/or structural transformation. Deactivation by coking has been addressed in many studies, but detailed descriptions of the catalyst's structural changes and with the concomitant performance loss are scarce. A well known, but somewhat little described in detail, structural change in SAPOs during use is aggregation of Si atoms to so-called Si-islands with a loss of Brønsted acidity due to the harsh hydrothermal conditions experienced during operation. The present work is a contribution to the understanding of structural changes of acidic sites during and after hydrothermal treatments with focus on changes in Si environments, i.e. the acidic sites in the catalyst.

We have developed catalyst synthesis using 29Si sources in order to greatly improve S:N in our 29Si NMR experiments. The NMR experiments were done using a Bruker Avance III 500 Mhz system with a special in-situ high temperature flow cell, in addition to regular MAS experiments. Steaming of samples were performed to emulate real deactivating processes.

In addition to ex-situ NMR data on systematic hydrothermally treated samples, we have investigated the framework atoms Al, P, and Si and the acidic site (H) from room temperature and up to 300 C at dry or wet flow conditions. These experiments shows initial hydration reactions, structural changes of the acidic sites and different dynamics of protons in the catalyst. Ex-situ 2D ^{1}H-^{29}Si NMR correlation data show that steaming also changes H-Si connectivities. Furthermore the most dynamic protons appear to be those lost first during steaming. Restoration of local hydrogen bonds at ambient conditions appears to take place after heat treatments and there is a coalescence of Si peaks above 100 °C indicating dynamic local structures of the Brønsted acidic sites at elevated temperatures. Complementary in-situ neutron diffraction investigations will be presented if ready.

Structural properties of water near solid surfaces from NMR

Christian Totland

Kjemisk Institutt, UiB

The three-dimensional structure of water is one of the important unsolved puzzles of chemistry. With a lifetime in the range of a few hundred picoseconds, finding suitable experimental techniques to study water structures is a challenging task. One possible exception is when water dynamics are slowed down as a result of surface interactions. We show that under some circumstances, and by creative sample preparation, the orientation dependence of the water H-H dipolar interaction can be used to obtain information on how water molecules orient adjacent a mineral surface. It is further shown that the orientations can be tied to specific low-energy structures which may help explain the origin of the much debated 'hydration forces'.

Characterization of pore structure heterogeneity and liquid saturation using NMR

John Georg Seland

Department of Chemistry, University of Bergen

In a liquid saturated porous sample the spatial inhomogeneous internal magnetic field in general depends on the strength of the static magnetic field, the differences in magnetic susceptibilities, but also on the geometry of the porous network. To thoroughly investigate how the internal field can be used to determine various properties of the porous structure, we present novel multi-dimensional NMR experiments that enable us to measure several dynamic correlations, and where all of the correlations involve the internal magnetic field and its dependence on the geometry of the porous network. We demonstrate how these correlations can be an indicator for pore structure heterogeneity and also how the correlations can be used to determine different saturation states of a porous sample.

Analysis directly on biological tissue with solid-state NMR

Willy Nerdal
Department of Chemistry, University of Bergen

The use of solid-state NMR holds many advantages over the liquid-state approach in that measurement is done directly on biological tissue and all metabolites and lipids can be studied simultaneously without any pretreatment or meticulous extraction procedures. By applying modern instrumentation, sufficient magic angle spinning (MAS) rates and/or other pulse techniques, the resolution is also close to the liquid state approach.

P-1

Characterization of catalytic sites in microporous materials using solid-state NMR. An *ex-situ* and *in-situ* high temperature flow study

Bjørnar Arstad[1], Anna Lind[1], Georgios Kalantzopoulos[2], Fredrik Lundvall[2], David Wragg[2], Helmer Fjellvåg[2]

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P-2

Interactions of a fungal lytic polysaccharide monooxygenase with β-glucan substrates and cellobiose dehydrogenase

Gaston Courtade[1], Reinhard Wimmer[2], Åsmund K. Røhr[3], Marita Preims[4], Alfons K.G. Felice[4], Maria Dimarogona[5], Gustav Vaaje-Kolstad[3], Morten Sørlie[3], Mats Sandgren[5], Roland Ludwig[5], Vincent G. H. Eijsink[3], Finn L. Aachmann[1]

[1]NOBIPOL, Department of Biotechnology, NTNU Norwegian University of Science and Technology, Trondheim, Norway. [2]Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark. [3]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway. [4]Food Biotechnology Laboratory, Department of Food Science and Technology, Vienna Institute of Biotechnology (VIBT), BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. [5]Department of Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Lytic polysaccharide monooxygenases (LPMOs) are key components of enzymatic biomass (e.g. chitin and cellulose) degradation processes. LPMOs catalyze a reaction cycle that requires copper, molecular oxygen and an electron donor, such as cellobiose dehydrogenase (CDH). Docking models have suggested that residues on the surface of the LPMO, near the copper site, interact with the cytochrome domain of CDH during electron transfer. However, experimental data that could clarify interactions of AA9 LPMOs with their substrates and CDH are lacking. In this study, we have used NMR and ITC we to study the interactions between a fungal LPMO and three soluble substrates and CDH. In addition, we have analyzed the structure and dynamic features of the LPMO in solution. The results reveal potential adaptations of the LPMO surface to varying substrates and unambiguously show that both the substrate and CDH bind to a patch that is focused around the copper-site. In addition to providing insights into enzyme-substrate interactions in LPMOs, the present observations shed new light on possible mechanisms for electron supply during LPMO action.

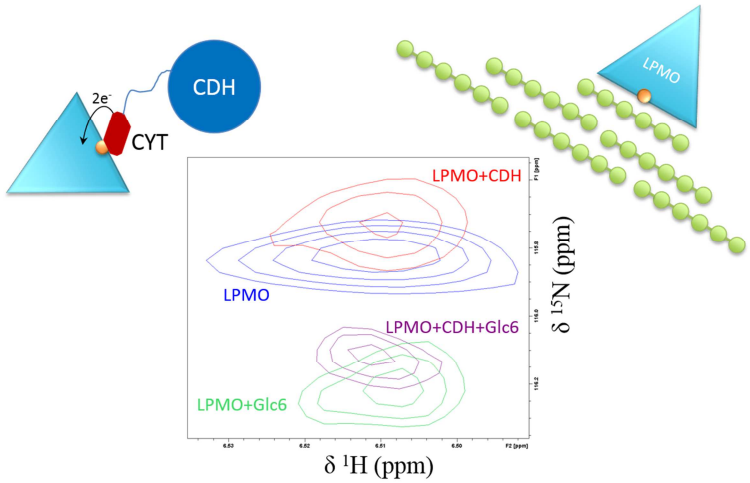

Figure 1: Left: The outer sphere of 12 Å radius represents the region in which ^{1}H signals are expected to be affected by Cu(II) PRE, while the inner sphere of 6 Å radius represents the expected effect on ^{13}C signals. Right: Residues with vanished ^{15}N-HSQC signals upon addition of 50 µM Cu(II) to 1 mM ^{15}N-labeled LPMO are colored blue on the structure.

P-3

Structural effects of copper-binding to a lytic polysaccharide monooxygenase

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Enzymes known as lytic polysaccharide monooxygenases (LPMOs) are abundantly present in biomass-degrading microbes and are currently classified as auxiliary activity auxiliary activity (AA) families 9 (AA9, formerly GH61), 10 (AA10, formerly CBM33), 11 (AA11) and 13 (AA13) (see www.cazy.org). LPMOs have been shown to catalyze the cleavage of glycosidic bonds in polysaccharides through hydroxylation of either carbon within the scissile bond, thus they are of great importance in biorefining. This mode of action requires an external electron donor, molecular oxygen, and the binding of a copper-ion to the LPMO. Whereas x-ray crystallographic studies have been carried out on Cu(I)-LPMOs, solution NMR studies have been solely performed on apo-LPMOs, because of the paramagnetic relaxation enhancement (PRE) brought about by Cu(II). We have used NMR structural and relaxation studies to show that the core structure of an AA10-family apo-LPMO is a compact and rigid β-sandwich with a flat substrate-binding surface that includes the protein N-terminus. Furthermore, 2D NMR titration experiments (^{15}N-HSQC, ^{13}C-aromatic HSQC and ^{13}C-detected COCA) exploiting the PRE indicated that binding of Cu(II) to the LPMO has a marginal structural effect, manifested in minor rearrangements for histidines coordinating the metal ion. Thus showing that apo-LPMOs can be reliably used for NMR structural investigations.

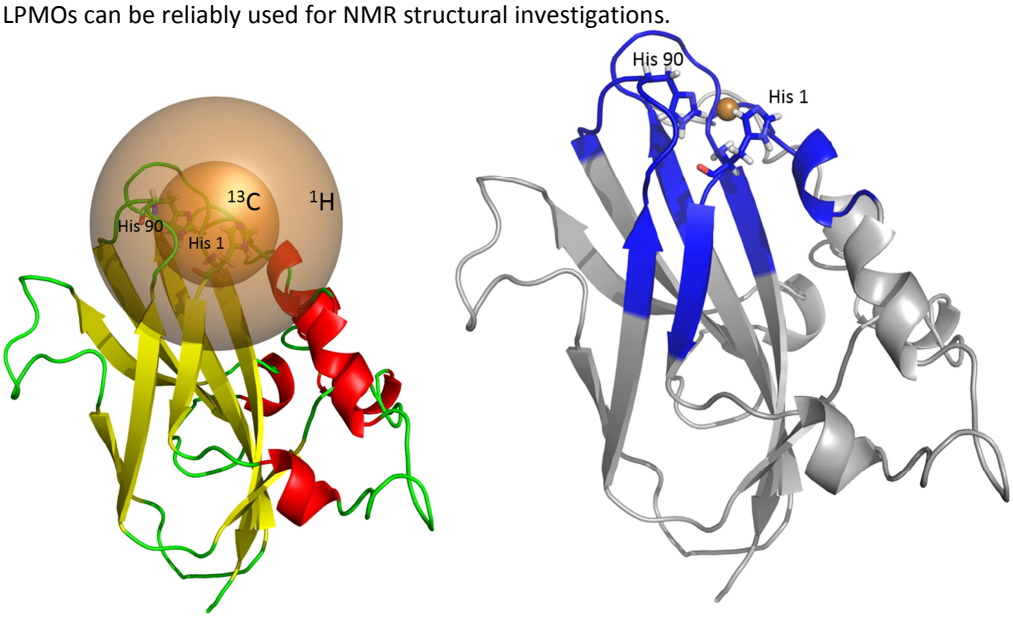

Figure 1: Left: The outer sphere of 12 Å radius represents the region in which ^{1}H signals are expected to be affected by Cu(II) PRE, while the inner sphere of 6 Å radius represents the expected effect on ^{13}C signals. Right: Residues with vanished ^{15}N-HSQC signals upon addition of 50 µM Cu(II) to 1 mM ^{15}N-labeled LPMO are colored blue on the structure.

P-4

Towards time-resolved quantification of *in-vivo* GABA spectroscopy

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The ability to perform quantitative analyses of biochemical compounds in vivo with magnetic resonance spectroscopy (MRS) has afforded new insights into biological mechanisms underlying both healthy and diseased states. Of particular interest is the ability to measure GABA, as dysfunction of GABA-ergic transmission has been implicated as a contributing factor in many psychological conditions, including the experience of auditory verbal hallucinations (AVH) in people with schizophrenia. The purpose of this project is to develop a method for performing MRS in a time-resolved or functional manner (i.e. fMRS) in order to measure dynamic changes in GABA in response to tasks or stimuli. To this end, transcranial direct current stimulation (tDCS), a neurostimulation technique that may be used to excite or inhibit neural activity within a given area, was used in a facilitating capacity to increase activity in the posterior superior temporal gyrus (pSTG). Excitatory stimulation has been shown in previous studies to lower local cortical GABA concentration in the area of excitation. To better understand the relation between GABA variations and the experience of auditory hallucinations, the operational mechanisms of tDCS, and the possibilities for the latter to be incorporated in management of the former, the ability to quantify metabolite variations with improved temporal resolution is crucial. Typically in MR-Spectroscopy, in order to overcome the inherently low SNR of a single acquisition, a high number of individual spectra are acquired over a long period and averaged together to produce a single time-averaged spectrum. While this approach may be useful for improving SNR, the long scan time it necessitates limits usefulness for time-resolved or functional MRS designs. Specialised spectroscopy sequences, such as the GABA-specific MEGA-PRESS spectral editing technique exacerbate these problems, as the technique requires the acquisition of two spectra from which a difference spectrum is calculated, effectively doubling scan time and halving temporal resolution. Furthermore, editing techniques are susceptible to issues of co-editing, and variations in editing efficiency due to frequency drift throughout the scan. Despite the problems fMRS of GABA presents, it has great potential to elucidate biochemical events underlying changes observed both in normal processes and mechanisms of disease. Here, we present a novel analysis approach for use with GABA-specific MEGA-PRESS spectroscopy data that adaptively combines data from multiple time windows to quantify temporal dynamics. Additionally, some of the challenges posed by acquiring reliable, stable MEGA-PRESS data over an extended sequence will be explored, and finally, an algorithm will be proposed for blind group-wise analysis of such data.
In this experiment, the MEGA-PRESS sequence was used to acquire a series of spectra from the pSTG, a key node in the auditory processing stream which has been implicated in the experience of auditory hallucinations in schizophrenia, amongst other conditions. During this acquisition, transcranial direct current stimulation (tDCS) was used to induce a change in cortical GABA concentration within the area of interest. Previous studies have shown anodal stimulation to decrease local cortical GABA concentration.
With spectra acquired continuously over a ~30 minute period, stimulation was introduced for 10 minutes approximately 10 minutes into the scan thus creating three ~10 minute blocks: Before, during and post stimulation, with each subject undergoing real and "sham" acquisitions in a double-blind design. In preliminary analysis, our method shows good potential for capture and quantification of tDCS-induced GABA fluctuations.

P-5

Experimental comparison of measurement techniques for different molecular weight poly ethylene glycol (PEG)

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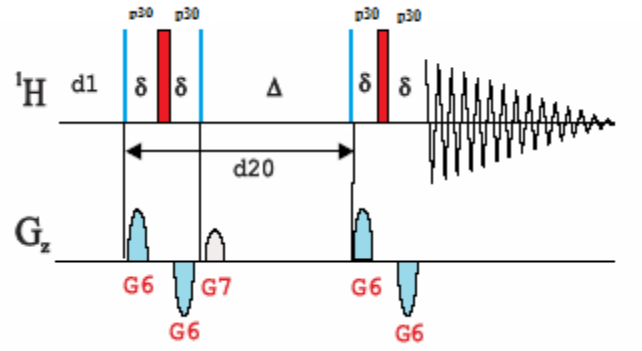
Pulse gradient spin-echo (PGSE) NMR technique (PGSE NMR) is applied to large colloidal and polymer materials. Since first time that diffusion ordered pulse sequence was proposed by Stejskal and Tanner in 1965, the diffusion ordered experiments by NMR have seen great advances. In last few decades many different complicated pulse sequences have been proposed. Among all these sophisticated pulse program the bipolar gradient pulse pair with a spoil gradient pulse (PFG-STEBP) and bipolar longitudinal eddy current delay (PFG-LEDBP) are the most famous one. These pulse sequences with slight changes can be use d as pulse program for 2D diffusion which is called DOSY (Diffusion Ordered SpecroscopY). Dynamic Light Scattering (DLS) techniques are well-known to all polymer scientists. They have been used for determination of hydrodynamic radius (R_h) and the mass average molar mass (M_w). In both DLS and DOSY use the time domain analysis to pursue the Brownian motions of solute molecules in solvent. Then from stock-Einstein the size of molecule can be calculated. We have measured the sizes of PEGs (polyethylene glycol) samples with different molecular weight. Also we have compared the result with theoretical size estimations.



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P-6

Why is HAMLET more toxic to dividing cells?

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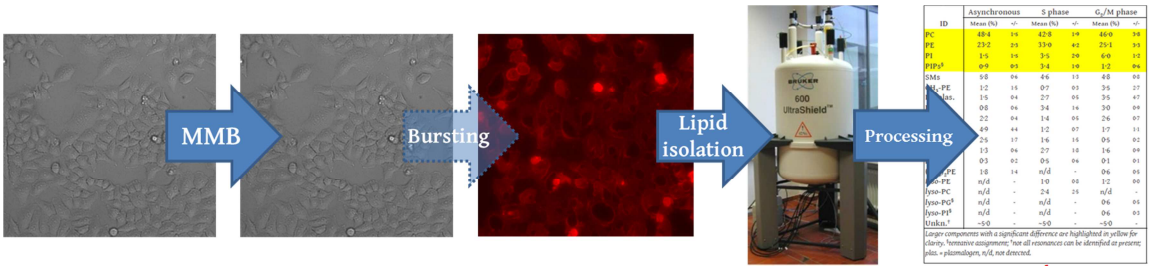
This project is aimed at developing a tool for investigating the interaction of the Human Alpha-lactalbumin Made Lethal to Tumour cells (HAMLET) with membranes. The tool will be used to explore why HAMLET's cytotoxicity to types of rapidly dividing cell appears to be related to its interaction with the plasma membrane (PM)[1-3]. Our hypothesis is that the physical properties of the PM at non-quiescent stages of the cell cycle make it more susceptible to HAMLET. The approach we have adopted is to examine the HAMLET/membrane interactions in PMs of synchronised populations of HeLa S3 cells *ex vivo* through the cell cycle. This requires good cell synchrony, isolation of anchored and purified PMs, quantitative lipidomics, phase behaviour studies and membrane proteomics.
Our novel procedure for synchronising cell cultures (double l-mimosine block, MMB) gives >85% synchrony in the S-phase, ~80% (G2/M) and >75% (G1 phase). Live cultures are treated to preserve the lipid fraction (LF) for isolation, using lipase inhibitors, chaeotropes and freeze-drying. The LF is then isolated from this material using a mixture of organic solvents that is compatible with known lipid chemistry[4]. Lipid profiling uses both ^{31}P NMR and MS to provide high reliability[5] and results from whole-cell studies suggest that during the S-phase the proportion of PE decreases but PI increases. Recent evidence shows that PI induces defects in PC bilayers[6]. We are testing the physical effect of changing the concentration of PI in a PC/PE model system. We are also developing the protocol for isolating adhered PMs with a high purity with respect to other organelles (fluorescence[7]) and assessing changes in the populations of lipid metabolism enzymes (e.g. PI synthase) and the lipids themselves through the cell cycle. Characterising the interaction between HAMLET and the PM as a function of the cell cycle will shed light on the mode of entry of HAMLET into the cell, and indicate whether entry into the cell is a rate-limiting step to toxicity.



ID	Asynchronous		S phase		G_2/M phase	
	Mean (%)	+/-	Mean (%)	+/-	Mean (%)	+/-
PC	48·4	1·9	42·8	1·9	46·0	3·8
PE	23·2	2·5	33·0	4·2	25·1	3·3
PI	1·5	1·5	3·5	2·0	6·0	1·5
PIPs[§]	0·0	0·5	3·4	1·0	1·2	0·4
SMs	5·8	0·4	4·6	1·3	4·8	0·8
Ms-PE	1·2	1·5	0·7	0·3	3·5	2·7
Plas.	1·5	0·4	2·7	0·5	3·5	4·7
	0·8	0·6	3·4	1·4	3·0	0·9
	2·2	0·4	1·4	0·5	2·6	0·7
	0·9	4·4	1·2	0·7	1·7	1·1
	2·5	1·7	1·6	1·5	0·5	0·2
	1·3	0·6	2·7	1·0	1·6	0·6
	0·3	0·2	0·5	0·6	0·1	0·1
-PE	1·0	1·4	n/d	-	0·6	0·5
-PE	n/d	-	1·0	0·6	1·2	0·6
lyso-PC	n/d	-	2·4	2·5	n/d	-
lyso-PG[§]	n/d	-	n/d	-	0·6	0·5
lyso-PI[§]	n/d	-	n/d	-	0·6	0·3
Unkn.[†]	~5·0	-	~5·0	-	~5·0	-

Larger components with a significant difference are highlighted in yellow for clarity. [§]tentative assignment; [†]not all resonances can be identified at present; plas. = plasmalogen, n/d, not detected.

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P-**7**

A continuous- and a discrete spin-spin relaxation rate representation of water in meat – A comparative investigation

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Since meat (muscle) is truly a heterogeneous material composed of a distribution of both pore shapes, pore sizes and NMR surface relaxation sites of various types (and strength) it is not unreasonable to expect the spin-spin relaxation of the "pore" water to be described by a broad range of relaxation times.
Hence, we designed an empirical peak function (characterized by three independent parameters: its width, its mean relaxation rate and its shape factor or skewness) which fitted excellently to any relaxation rate distribution – as derived by an Inverse Laplace Transformation of the observed CPMG response curve. Interestingly, the same CPMG response was found to be excellently represented by a sum of two single exponential decay functions, denoted the "discrete" relaxation rate model.
Interestingly, a detailed analysis suggested that when the fraction (f2) of the slower relaxation rate component (R22) was small (< 5%), the discrete model overestimated f2 by a factor of between 2 and 3 and R22 by nearly 25% as compared to the continuous relaxation rate model. Importantly, these same findings were realized after analyzing corresponding synthetic CPMG response curves, as well, and address the important question, what model is actually representing the reality?

P-8

Characterization of protein-fatty acid complex

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Department of Molecular Biology, University of Bergen

HAMLET is a complex of α-lactalbumin and oleic acid. In 1995 the complex was discovered in a milk-fraction for its selective cytotoxic effect on tumor cells. The cytotoxic effect of the complex is not fully understood, but research has related it to the ability to deposit fatty acids into the cell membrane which becomes perturbed and may collapse from this action. A model proposed by Kaspersen et al. describes the protein-fatty acid complexes as a micelle-like organisation of fatty acid, stabilized by a shell of partially unfolded proteins. This model is based on low-resolution SAXS data. To better understand the mechanism behind the cytotoxicity of HAMLET and to study the molecular properties of the complex at a more detailed level, this study uses an engineered polypeptide in complex with oleic acid as a model. This peptide is derived from parts of α-lactalbumin associated with membrane interaction and fatty acid binding and called A-Cage-C. Results from SEC, DLS, NMR and Trp fluorescence spectroscopy indicates that the complex forms. We will monitor both the chemical environment of the polypeptide backbone (^{13}C, ^{15}N labelling, protein NMR) and that of oleic acid (^{13}C labelling, 1D NMR) to assess how they affect each other. We are now in the process of evaluating the effect of removing excess oleic acid after complex formation by SEC. Preliminary NMR results show that SEC affects both the environment of the fatty acid, and the polypeptide chain, probably due to removal of loosely bound fatty acid. This removal may or may not be desirable from a toxicity point of view, as the fatty acid content of the complex is directly related to its activity. To examine the effect on membranes and cytotoxicity, leakage assay and cell viability assay will be performed.

P-9

Does the lipid fraction of *Listeria innocua* change as a function of the cell cycle?

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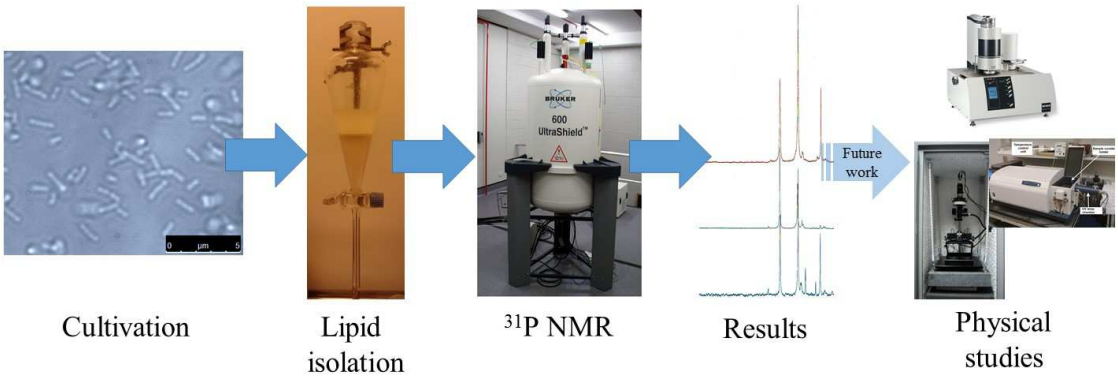
The understanding of the cell cycle of bacteria is important in many fields of research, from biotechnology to medical microbiology. However, there is not yet a clear understanding of the control system for cell division in prokaryotes. Current research does not point to gene expression [1] or to the concentration of structural proteins [2; 3], but structural molecules are essential for both cell division and maturation. Moreover, recent studies have indicated that the composition of the lipid fraction of Escherichia coli [4] change through the cell cycle. As all changes is cell membrane are required to allow cell division, and it is has been shown that lipid profile varies in bacterial populations [5], we developed the hypothesis that that in Gram positive bacteria changes in lipid profile may also be linked to cell elongation and cytokinesis.
Using rifampicin to arrest cultures, an updated procedure for isolating the lipid fraction and quantitative ^{31}P NMR to determine phospholipid composition, we have collected evidence that the phospholipid composition of *L. innocua* NCTC 11288 changes through the cell cycle. The phosphatidylglycerol (PG) fraction remains constant, however the phosphatidylethanolamine and cardiolipin (CL) fractions fall by about 50% during elongation, where lysyl-derivatives of PG and CL increase. These results indicate that lipid synthesis is controlled with respect to the cell cycle. Our further work will explore the physical changes of plasma membrane during these modulations in lipid composition.



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P-10

Dynamics Study of Human deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase) by Nuclear Magnetic Resonance Spectroscopy

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Nuclear-associated deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase) has emerged as a promising drug target in 5-fluorouracil treatment of cancer. Human dUTPase is up-regulated in many human neoplastic cells with bad patient prognosis upon treatment with 5-fluorouracil (5-FU) or its analogues. 1 Elevated expression of dUTPase limits the therapeutic efficacy of the chemotherapy both by decreasing the intracellular dUTP pool and by increasing the levels of dUMP, which in turn is a substrate for Thymidylate synthase (TS), which acts to restore the dTTP:dUTP balance needed for cell proliferation. 2 Suppression of human dUTPase is therefore highly desirable in combination with conventional cancer treatment. 3 A recent report has presented the first in vivo (MX-1 breast cancer xenograft model in mouse) evidence that human dUTPase inhibition enhances the effect of TS inhibitors.4,5In an effort to enable NMR studies of dUTPase function and dynamics, as well as assisting pharmacophore mapping, uniformly triple labeled $^{15}N/^{13}C/^{2}H(50\%)$ human dUTPase has been expressed. The positive effect of partial deuteration, TROSY and stabilizing ligands like 2'-deoxyuridine 5'-α,β-imido-triphosphate (dUPNPP), 5-fluoro-Uracil (5-FU) and in house early hits from our drug development program allowed the observation and assignment of nearly the entire backbone of the enzyme REF. We report, for the first time, a NMR dynamics study of human dUTPase in its apo form and in complex with novel inhibitors with activities in the µM range.

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P-11

APPLICATIONS OF MAGNETIC RESONANCE IMAGING WITHIN PETROPHYSICAL ROCK CORE ANALYSIS

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Magnetic resonance imaging (MRI) is a powerful method in medical imaging and diagnosis. However, it is also successfully applied in material science, and one of the more interesting research areas is within petrophysical rock core analysis. We present data from rock core analysis performed using a 4.7T Biospec MRIscanner. We describe and discuss how MRI can be optimized to characterize different saturation states, study flow processes and evaluate gas hydrate formation in rock cores.

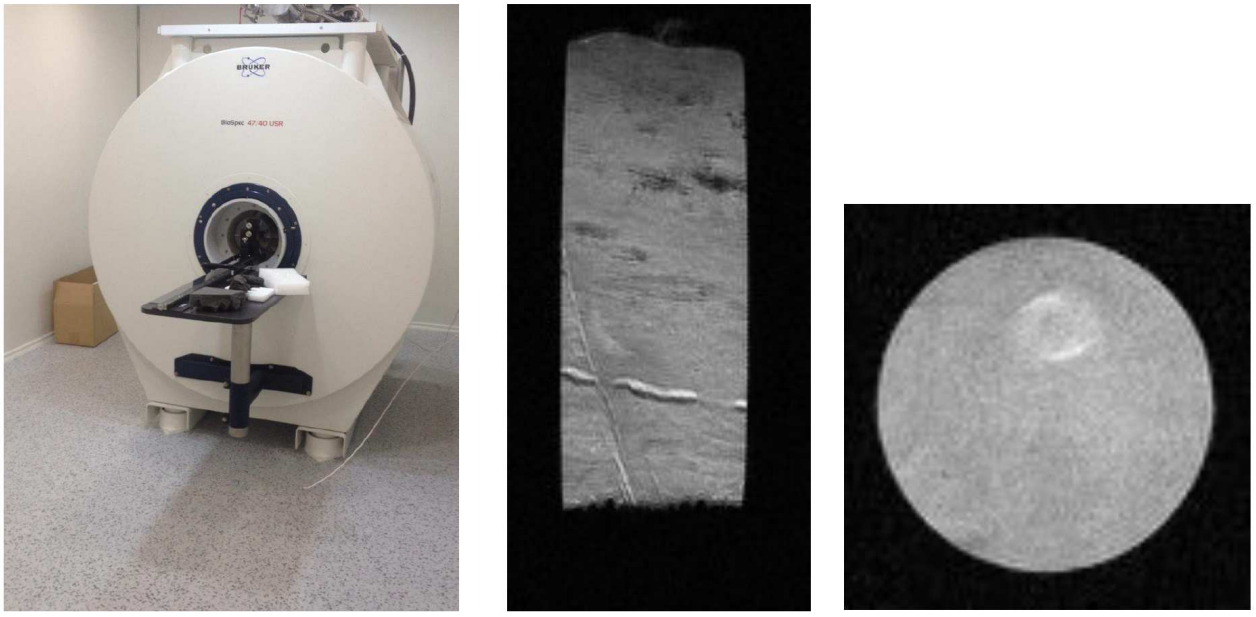

Figure 1: The 4.7T Biospec MRI-scanner from Bruker Biospin located at Statoil ASA, Bergen (left). T2-weighted MR-image of a 100% water-saturated carbonate rock core in the coronal plane (middle) and in the axial plane (right).

P-12

Fatty acids in wild and farmed salmon by use of MRI and solid-state NMR

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Marine omega-3 fatty acids are essential as these fatty acids in very limited quantity can be produced in human cells and need to be supplied through the diet. A good source of omega-3 fatty acids is salmon. Wild salmon eat other fish rich in marine omega-3 fatty acids. The availability of marine omega-3 fatty acids is limited and can`t meet the too needs of a rapidly growing aquaculture industry producing farmed salmon. Therefore, fatty acids from plants have been used. The fatty acids from plants have shorter acyl chains than the marine omega-3 fatty acids as shown in Table 1. We have used solid state NMR and MRI to compare the fatty acids present in wild and in farmed salmon.

Our results show that farmed salmon contains less DHA and EPA, and more ALA and omega-6 than wild salmon. The high content of ALA and omega-6 in the filet is not surprising, given the information in Table 1 and demonstrates that salmon is not capable of converting fatty acids in the feed into marine omega-3 fatty acids in a significant amount. The fatty acid composition of salmon we buy in supermarkets is continuously changing due to developments in fish feeds. The MRI experiments show that in wild salmon, the fat is mainly distributed in the connective tissue, while in farmed salmon, the fat is also present in the meat.

P-13

Hydrolysis of organophosphorus nerve agents by marine DFPases

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The use of enzymes –based decontaminants is of great interest because it has the potential of being both non-toxic and non-corrosive, and simultaneously neutralise organophosphorus nerve agents. With the use of biotechnology new safe enzyme-based decontamination systems can be created. We have used molecular biology and protein engineering tools to enable the re-design of enzymes called diisopropyl fluorophosphatases (DFPases) to degrade various types of organophosphorus nerve agents.
Nerve agents are highly toxic organophosphorus compounds that can be hydrolysed to non-toxic compounds. The hydrolysis rate varies significantly with pH and temperature. Enzymes are able to increase the reaction rate. During the protein engineering phase using a colorimetric test was a conveniently method to screen a large number of enzymes. But for further characterisation of enzyme performance against several nerve agents NMR was the method of choice. Here, we utilised ^{1}H-^{31}P HSQC NMR spectroscopy to examine the activity and stability of the newly engineered DFPases.

The novel enzymes show a broad range of activity against nerve agents, have a wide temperature usability range, work in neutral and basic pH, and can tolerate various organic solvents and soaps.

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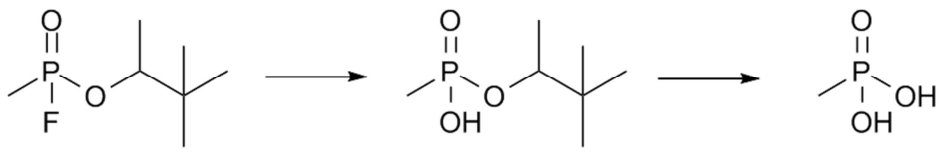

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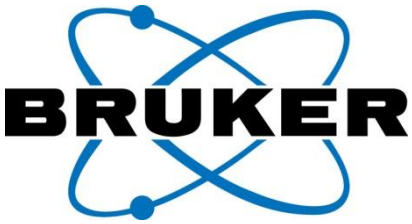

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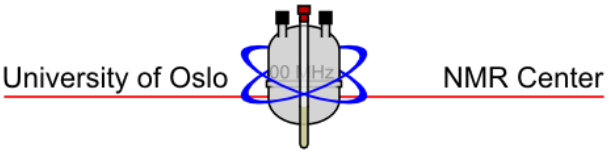

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