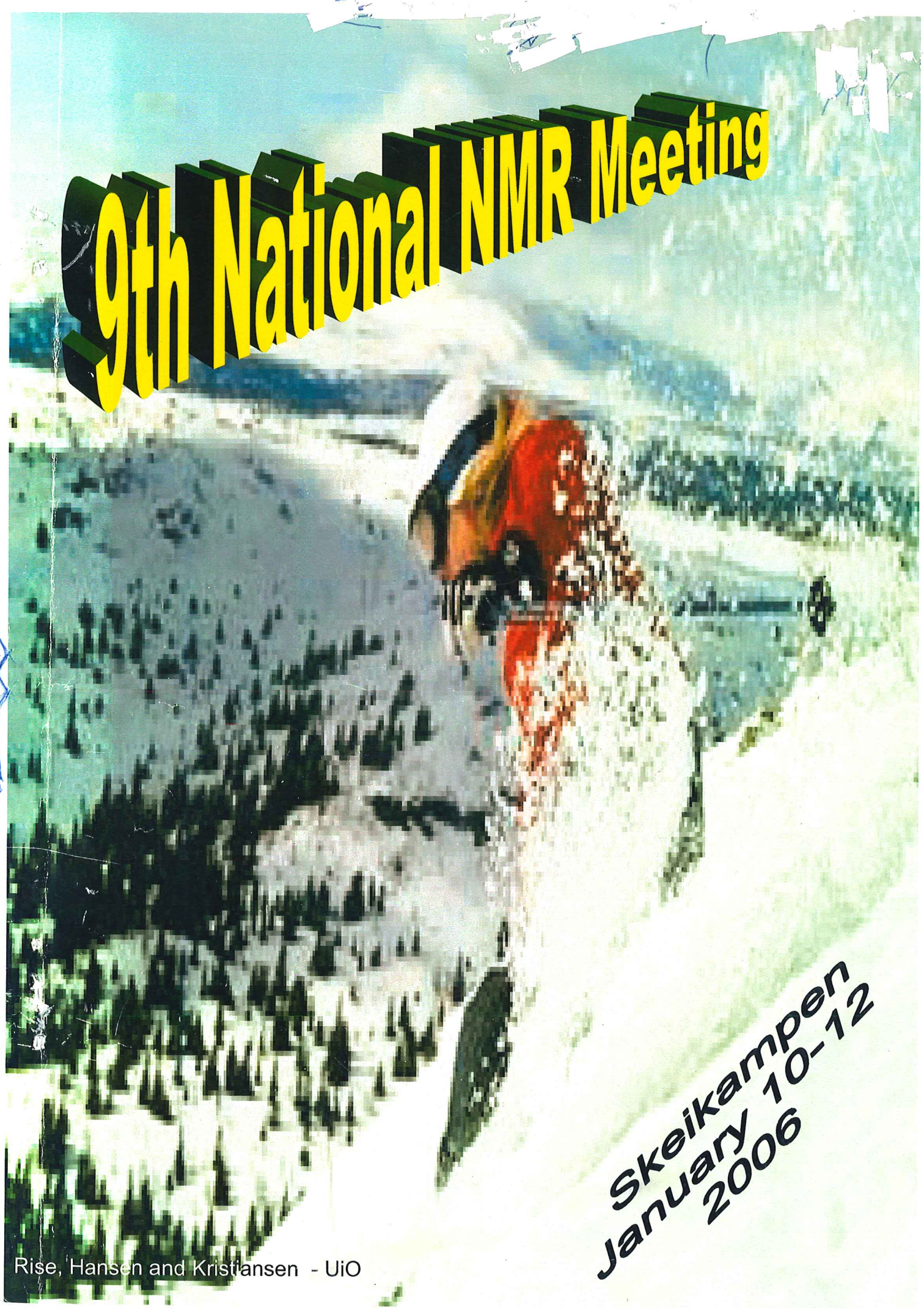
# 9th National NMR Meeting



Skeikampen
January 10-12
2006

Rise, Hansen and Kristiansen - UiO

## Welcome to the 9th National NMR meeting

One important feature regarding scientific meetings is to nurture contact with colleagues and friends and to establish scientific networks for future collaboration. In particular, research scholars and master students are encouraged to attain the meeting in order to exchange ideas and to share their scientific challenges with other researchers in the (national) NMR community by poster- or lecture contributions, or simply by taking part in private communications. It is well known that scientific knowledge, insight and creation of ideas most frequently are brought forth by participating in such combined social and scientific arrangements. For many of us such a meeting represents an notable and essential motivation factor in our daily, scientific struggles.

The sequence of scientific presentations in this meeting was not structured or methodically ordered. Actually, they were chosen (on purpose) randomly with the objective that anyone should find something of interest within each of the scientific sessions.

Noting that nearly 50% (19 persons) of you contribute with an oral presentation, the organizing committee wants to thank each one of you for the great interest and enthusiasm you demonstrate to make this meeting possible.

Frode Rise  Eddy W. Hansen  Per Eugen Kristiansen

On behalf of the organizing committee at the Chemistry Department of UiO.

## Previous National NMR Conferences

| TIME | PLACE | ORGANIZERS |
|---|---|---|
| 14 - 17 mars 1983 | Oppdal | J. Krane, AVH, UNIT |
| 10 - 12 mai 1989 | Geiranger | J. Krane, AVH, UNIT<br>(Norsk – Svensk NMR-diskusjonsgruppe) |
| 7- 8 oktober 1993 | Ustaoset | T. Skjetne, MR-senteret, Sintef Unimed |
| 16 - 17 oktober 1995 | Trondheim | T. Skjetne, MR-senteret, Sintef Unimed |
| 7- 8 januar 1998 | Fefor Hotell, Vinstra | D. W. Aksnes og Einar Sletten, UiB |
| 5 - 6 januar 2000 | Highland Hotell Geilo | B. Pedersen og F.Rise, UiO |
| 9- 10 januar 2002 | Radisson Hotell, Beitostølen | H. W. Anthonsen, J. G. Seland og J. Krane, NTNU |
| 12 - 14 januar 2004 | Quality Hotel Hafjell | D. W. Aksnes og W. Nerdal, UiB |

# Scientific Program
## National NMR Meeting; January 10 – 12, 2006

### Tuesday

| | | |
|---|---|---|
| 09.00 – 14.00 | **Registration** | |
| 14.00 – 15.00 | **Lunch** | |
| 16.00 – 16.05 | **Welcome** | |
| | **Lectures** | **Chairman E. Hansen** |
| 16.05 – 16.45 | **Einar Sletten**; *DNA oligonucleotides form three-way junctions containing a helical dinuclear iron(II) supramolecular cylinder* | |
| 16.50 – 17.10 | **J. G. Seland**;*Studying water exchange in biological tissue using two dimensional relaxation and diffusion measurements* | |
| 17.15 – 17.35 | **T. Pavlin;** *Noninvasive measurements of gas exchange in a 3D fluidized bed* | |
| 18.00 – 19.30 | Poster-presentation | |
| 20.00 - | Dinner | |

### Wednesday

| | | |
|---|---|---|
| - 13.00 | **Skiing?!** | |
| 13.00 – 14.00 | **Lunch** | |
| | **Lectures** | **Chairman D. Aksnes** |
| 14.00 – 14.40 | **Alexander Dikiy;** *Biomolecular NMR for investigation of protein structure and function: some examples.* | |
| 14.50 – 15.30 | **Torgils Fossen**; *Solution Structure of the Human Immunodeficiency Virus Type 1p6 Protein* | |
| 15.35 – 15.55 | **Oliver Geier;** *Acquisition weighted $^{31}P$ SLOOP chemical shift imaging in the human heart* | |
| 15.55 – 16.30 | **Coffee/poster** | |
| | **Lectures** | **Chairman E. Sletten** |
| 16.30 – 16.50 | **H.S. Haugen;***The three-dimensional NMR structure of the pediocin-like bacteriocin (antimicrobial peptide) curvacin* | |
| 16.55 – 17.25 | **Chen Song;** *Lipid microdomains in bilayer of dipalmitoylphosphatidyl-choline (DPPC) and 1-stearoyl-2-docosahexaenoylphosphatidylserine (SDPS) and their Perturbation by Chlorpromazine : A $^{13}C$ and $^{31}P$ Solid-State NMR Study* | |
| 17.30 – 18.00 | **T.C.B. Andersen**; *Itk SH3 domain binds the T cell specific adapter protein (TSAd) in a non conventional way* | |
| 18.05 – 18.25 | **Lars Skjeldal;** *NMR structure of an oxidized 2Fe-2S ferredoxin* | |
| 18.30 – 19.30 | Election of new members to the "National NMR Liaison committee" | |
| 20.00 - | **Dinner, (B. Pedersen og D. Aksnes;** *Hvordan kom NMR til Norge?)* | |

### Thursday

| | | |
|---|---|---|
| | **Lectures** | **Chairman A. Dikiy** |
| 09.30 – 10.00 | **H. Holmsen;** *Use of NMR and other physicochemical methods to study interactions between psychotropic drugs and glycerophospholipids* | |
| 10.05 – 10.25 | **Morten Bruvold**;*Changes in $T_1$ and $T_2$ Components in Global Ischemic Rat Heart Tissue* | |
| 10.30 – 10.50 | **K. Larsen**; Clarification of the Stereochemistry of DTX-1 | |
| 10.55 - 11.15 | **B. F. Lutnæs**; *Archaeal C80 isoprenoid tetraacids responsible for naphtenate deposition in crude oil processing* | |
| 11.20 - 11.40 | **K.K. Dao**; *Effects of sodium perchlorate on the oligomeric state of the regulatory subunit of PKA studied by NMR* | |
| 11.45 – 12.05 | **Ø. Halskau;** *Triple resonance spectroscopy of 56 kDa dimeric scaffolding protein 14-3-3* | |
| 12.10 - 12.50 | **Torgils Fossen**; *Characterization of novel flavonoid classes by NMR spectroscopy* | |
| 13.00 – 14.00 | **Lunch** | |

## List of participants

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# ABSTRACTS
# (LECTURES AND POSTERS)

# Dynamic NMR Studies of cyclohexane and cyclopentane confined in mesoporous MCM-41

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## 1. Introduction

The spin-lattice ($T_1$) and spin-spin ($T_2$) relaxation times and the diffusivity are very sensitive to changes in the molecular mobility and environment. Thus, NMR is a well-established method for studying the dynamics of adsorbed species [1]. If crystallites of sufficient size can be obtained (≥10 μm), intracrystallite NMR parameters might be observed without complications from boundary or inter-particle effects.

In the present work, the $T_1$ and $T_2$ relaxation times and diffusivities of cyclohexane and cyclopentane confined in a MCM-41 solid are studied.

## 2. Experimental

The crystalline MCM-41 solid with pore diameter 3.2 nm was prepared under acidic conditions. The NMR measurements were carried out on a Bruker Avance DMX 400 spectrometer using the inversion-recovery and CPMG sequences and a modified stimulated echo sequence [2]. To eliminate the signal attenuation caused by spin diffusion in internal field gradients, the spacing between the 180° pulses of the CPMG sequence was set to 0.6 ms. The length of the gradient pulses (δ) and the spacing between the RF pulses (τ) were always fixed to 0.5 and 1.1 ms, respectively. The value of the z-storage time (Δ) was 4 and 8 ms for cyclopentane and cyclohexane, respectively. The natural logarithm of the echo attenuation $\ln(I/I_0)$ was plotted vs. the square of the field gradient strength $g^2$, and $D$ was extracted from the initial part of the curve.

## 3. Results and discussion

The rotational and translational dynamics of cyclohexane and cyclopentane confined in the MCM-41 solid were studied as a function of temperature by measuring the $T_1$ and $T_2$ relaxation times and the diffusivities [3]. Filling degrees of 60%, 80% and 104% were used when investigating the confined samples. The $T_1$ behaviour is remarkably similar for the two adsorbates although we compare compounds exhibiting different bulk phases at corresponding temperatures. $T_1$ and $T_2$ increase clearly with increasing pore filling, presumable as a result of a reduced contribution from surface relaxation.

The diffusivity of confined cyclopentane decreases markedly with increasing pore filling in contrast to the situation for cyclohexane. In the former case, however, the diffusion experiment detects the diffusivity of all the liquid in the pores, while in the latter case only the diffusivity of the surface layer is monitored below the depressed melting point. For confined cyclopentane, the activation energy is 14-15 kJ $mol^{-1}$, as compared to 9.9 kJ $mol^{-1}$ for the bulk liquid.

A high diffusion rate is observed over a wide temperature range in the surface layer of confined cyclohexane, even below the plastic-brittle solid transition point. Indeed, the diffusivity is about three orders of magnitude larger than in the plastic bulk phase. The high mobility of the surface layer is also reflected in the relatively low activation energy (22-23 kJ $mol^{-1}$), in comparison with the plastic bulk phase (44 kJ $mol^{-1}$).

## 4. References

[1] P.J. Barrie, Annual Rep. NMR Spectr. 41 (2000) 256-316.

[2] G.H. Sørland, B. Hafskjold, O. Herstad, J. Magn. Reson. 124 (1997) 172.

[3] D.W. Aksnes, K. Førland, M. Stöcker, Micropor. Mesopor. Mater. 77 (2005) 79.

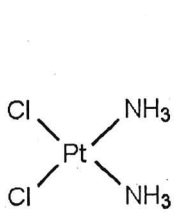
# Reaksjonsmekanisme studiar av *trans*-Pt(II) iminoeterar

**Alvheim C., Vinje J. and Sletten E.**

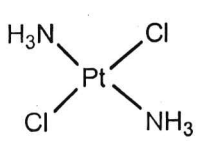
*Kjemisk institutt, Universitetet i Bergen.*

Eit av dei mest brukte medikament for behandling av kreft ved hjelp av kjemoterapi er cisplatin - eit heller enkelt metallkompleks av Pt(II), sjå Figur 1. Det er kjent at cisplatin bind seg til DNA molekylet og hindrar celledeling, men ein kjenner ikkje til detaljar i reaksjonsmønsteret [1]. Det er også uklart kvifor nokre platinakompleks er effektive mot kreft medan andre ikkje. To avgjerande faktorar i denne samanhengen er: (i) strukturen til platina-DNA kompleksa som blir danna, og (ii) kor raskt platina komplekset reagerer med DNA og andre biomolekyl. *Trans* isomeren av cisplatin (kalla transplatin) har ikkje antitumor aktivitet. Ein trur dette kjem av at strukturen til transplatin-DNA komplekset og reaksjonskinetikken til transplatin ikkje er optimal i forhold til å indusera celledaud i kreftceller. Kven av dei to faktorane som er mest avgjerande for at transplatin ikkje effektivt kan hemma celledeling er enno ikkje avklart.

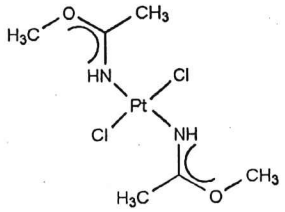
I seinare tid er det syntetisert analogar av transplatin som har vist lovande antitumor aktivitet. Ein type av slike aktive transplatin analogar er Pt(II) kompleks det ei eller begge amingruppene på transplatin er byta ut med iminoeter. Tidlegare NMR studiar av ein Pt(II) iminoeter som innehaldt to iminoeter ligandar (*trans-EE*) viste svært låg reaktivitet mot dobbelstreng DNA, medan reaksjonshastigheita mot enkelstreng DNA var liknande den funne for cisplatin [2]. To nye Pt(II) iminoeter kompleks som berre inneheld ei iminoetergruppe blir for tida studert (*trans-E* og *trans-Z*). Det er forventa at desse reagerer raskare enn *trans-EE* med dobbeltstreng DNA. Dette då *trans-E* og *trans-Z* ikkje har meir enn ei sterisk krevjande iminoetergruppe, og dermed lettare kan nå det indre av DNA duplexen der nukelobasane er plassert (dette er bindingssete for Pt(II) kompleks).



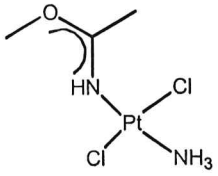

Cisplatin



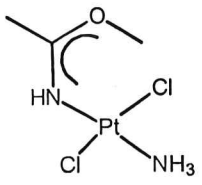

Transplatin




*Trans-EE*




*Trans-E*




*Trans-Z*

**Referansar:**

(1) Wang, D.; Lippard, S. J. *Nature Rev. Drug Disc.* **2005**, *4*, 307.
(2) Vinje, J.; Intini, F. P.; Natile, G.; Sletten, E. *Chem. -Eur. J.* **2004**, *10*, 3569.

# Itk SH3 domain binds the T cell specific adapter protein (TSAd) in a non conventional way.

**Andersen TCB[1], Spurkland A.[1], Andreotti A[2], Karlsson BG[3]**

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The T cell specific adapter protein (TSAd) is expressed in activated T cells. The function of this novel adapter molecule is still not well defined. However, existing data points to a role for TSAd in modulating T cell receptor induced signaling events. TSAd has been cloned as a binding partner for several intracellular kinases, including the Interleukin-2 tyrosine kinase Itk. Itk participates in early signal transduction in T cells, and has recently been shown to play a role in migration and actin polymerization in T cells. We wanted to determine the binding requirements for TSAd to Itk, and whether TSAd modulates Itk function. By pulldown assays we have found that TSAd binds to Itk only via the Itk SH3 domain, and that this binding requires the TSAd amino acids 257-274, including one classic PXXP motif. 15N labelled samples of the murine and human Itk SH3 domains were titrated with the synthetic peptide (IPVPRHRPAPRPKPSNP) or the two halves (IPVPRHRP and PRPKPSNP, respectively). The binding was followed in 2D (1H, 15N) HSQC NMR experiments by observing the chemical shift changes of the NH groups of the SH3 domain.
Interestingly these experiments showed that it is the IPVPRHRP half of the peptide that interacts with Itk SH3 and not the PRPKPSNP which includes the PXXP motif. Mapping of the interaction information onto the known structure of murine Itk SH3 shows that the site of TSAd peptide binding on Itk SH3 is as expected localized around the conserved tryptophan W208, however the actual structure remains to be calculated. Analyses to determine the functional significance of interaction of TSAd with Itk are ongoing.
*This work was supported by EMBIO, Univerity of Oslo.*

# Sorption energetics and diffusion of methyl salicylate in chloro-butyl rubber.

**Bakken E,[a] Hansen EW[b] and Gran HC[a]**

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The present study is part of a larger project that focuses on physical protection against chemical warfare agents (cwa) and decontamination of personnel and equipment exposed to cwa. A respirator is in this context essential in order to protect the lungs and the respiratory tract from vapour hazard, and it is desirable that a respirator can be cleaned after contact with vapour or liquid agents. The respirators used by the Norwegian Army are made of chloro-butyl rubber (chloro-isobutylene-isoprene rubber, CIIR), and the present focus is transport of cwa in CIIR.

To avoid experiments with toxic agents methyl salicylate (MS) was used as a simulant for sulphur mustard gas (HD). The solubility of MS in CIIR was measured using a simple glass set-up, and the Henry's-law constant at 333 K was obtained by extrapolating the results to infinite dilution. Absorption isotherms at 303, 318 and 333 K were calculated from the Henry's-law constant and the Flory-Huggins regular solution model.[1,2] The (partial molar) enthalpy of absorption of MS(g) and the (partial molar) enthalpy of mixing of MS(l) and CIIR have been derived. Diffusion coefficients of MS in CIIR were measured by both NMR and thermogravimetry, and the concentration of MS as a function of time and distance into CIIR were subsequently calculated from Fick's second law. Finally, the present concentration profiles were integrated to obtain the total mass of MS in bulk CIIR.

1 P. J. Flory, *Principles of Polymer Chemistry*, chap. 12, Cornell University Press, Ithaca, New York, 1953
2 D. R. Paul og Y. P. Yampolskii, *Polymeric Gas Separation Membranes,* chap. 2, CRC Press, Boca Raton, Florida USA, 1994

# Changes in $T_1$ and $T_2$ Components in Global Ischemic Rat Heart Tissue

**Bruvold M, Seland JG, Sissel S, Bruruk H and Jynge P.**

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Water in biological tissue is affected by different processes during ischemia (1,2). Cell swelling and contracture alter distribution of myocardial water between the intracellular and extracellular compartments. This study aims to follow the progress of water redistribution by relaxographic measurements (3) of multiple $T_1$ and $T_2$ components in excised rat heart tissue.

Ventricular myocardium was excised and relaxography (Maran Ultra, Resonance Instruments Ltd, 23MHz, 37°C) was undertaken using Saturation Recovery ($T_1$) every 2.5 min or Carr-Purcell-Meiboom-Gill ($T_2$) every minute during the first 60 min after excision. The data were analysed using an Inverse Laplace Transformation revealing the distribution of relaxation components present in the myocardium as relaxograms (3).

The use of $T_2$-relaxography is promising in describing the status of ischemic progress and other processes altering the water distribution in biological tissue. In addition, the $T_1$-value of tissue, influenced by the presence of intracellular manganese ions, could be used in combination with $T_2$-relaxography to obtain information about equilibrium water exchange between intracellular and extracellular compartments during ischemic progress.

**References:**

[1] Jennings RB, Reimer KA and Steenbergen C. Myocardial Ischemia and Reperfusion: Role of Calcium.
In: Control and Manipulation of Calcium Movement. Raven press (1985)
[2] Hearse DJ, Garlick PB, Humphrey SM. *Am J Cardiol*, **39**(7), 986-993, (1977)
[3] C. Labadie *et al. J Magn Reson Series B*, **105**, 99, (1994).

# Effects of sodium perchlorate on the oligomeric state of the regulatory subunit of PKA studied by NMR

**Dao KK[1], Frøystein NÅ[2], Keen J[1], Døskeland SO[1] & Martinez A[1]**

*[1]Department of Biomedicine and [2]Department of Chemistry, University of Bergen, Norway.*

The crystal structures of the dimeric form of the regulatory subunit of the cAMP-dependent protein kinase A type I (RI) in complex with ligands (Rp-cAMPS and Sp-cAMPS) are available (PDB 1NE4 and 1NE6). It was shown that the N-terminal segment (residues 109–117) can make the RI subunit forming a weak homodimer even in the absence of its dimerization domain. Our studies aim to study the RI in its unliganded and liganded states, in order to understand the relationship and conformationals changes related to dimerization and ligand binding. Using NMR spectroscopy we attempt to identify the subunit interface in solution. The pH 7.4 NOESY spectrum shows the degeneracy of the proton signals due to the equilibrium between monomer and dimer, thus preventing structure determination of the unliganded monomeric state. By using guanidine hydrochloride and sodium perchlorate in titration experiments the dissociation and conformational transition is induced and can be monitored by 1D $^{1}$H NMR and 2D $^{1}$H-$^{1}$H NOESY experiments. Dissociation of the dimer and appearance of the monomeric form was followed by analytic size exclusion chromatography and by the corresponding chemical shift change and narrowing of the most-upfield signals on the 1D $^{1}$H NMR spectrum of wild-type RI. Also, some signals disappear upon formation of monomers. Thus, guanidine hydrochloride and sodium perchlorate seem to stabilize the folded monomeric form of protein and can be effective to aid in the assignment of the NMR spectra of this important protein.

# Biomolecular NMR for investigation of protein structure and function: some examples

**Dikiy Alexander**

*Department of Biotechnology ,NTNU, 7491 Trondheim*

During the lecture, it will be shown how high resolution NMR spectroscopy can be applied for structural and functional studies of metallo-proteins. The first example concerns NMR studies of strongly paramagnetic oxidized plastocyanin, copper-containing blue-copper protein. Application of advanced NMR techniques resulted in determination of the first solution structure of a Cu(II)-containing protein. The approach proposed in our investigation allowed us to monitor and to assign paramagnetic NMR signals as broad as 800 KHz! NMR investigations of recombinant human purple acid phosphatase will be used to demonstrate how NMR spectroscopy can be applied for functional characterization of proteins.

# Cisplatin Interaction with Lipid Bilayers
# A Solid-State NMR Study

**Dyrøy K and Nerdal W**

*Department of Chemistry, University in Bergen*

Cisplatin is used in cancer chemotherapy. Cisplatin has to pass through the cellmembrane on its way to DNA, where it binds and destroys the cancer tumour. Unfortunately, many patients develop resistance to cisplatin chemotherapy. The cellmembrane is basically a double layer of phospholipids and can be vital in the development of cisplatin resistance. In this study we employ phospholipid membranes of DSPC (1,2-Distearoyl-sn-glysero-3-phosphocholine) with and without cisplatin. We monitor the $^{13}C$, $^{1}H$ and $^{31}P$ nuclei by solid-state NMR on a 500 MHz spectrometer.

# Characterization of novel flavonoid classes by NMR spectroscopy

**Fossen T, Jordheim M, Bjorøy Ø, Holmberg MH, Rayyan S and Andersen ØM**

*Department of Chemistry, University of Bergen, Allegt. 41, N-5007 Bergen, Norway*

Flavonoids comprise the largest group of naturally occurring polyphenolic compounds. More than 8000 flavonoids, including 550 anthocyanins, have hitherto been identified. Correlated to the developments in NMR instrumentation including higher fields, cryogenic probes, improvements of NMR techniques, as well as improved methods for isolation of plant pigments, new anthocyanins with increasing structural complexity have in recent years been characterized. Using mild treatment during extraction and isolation, followed by extensive use of multidimensional NMR and MS we have identified several new anthocyanin classes.

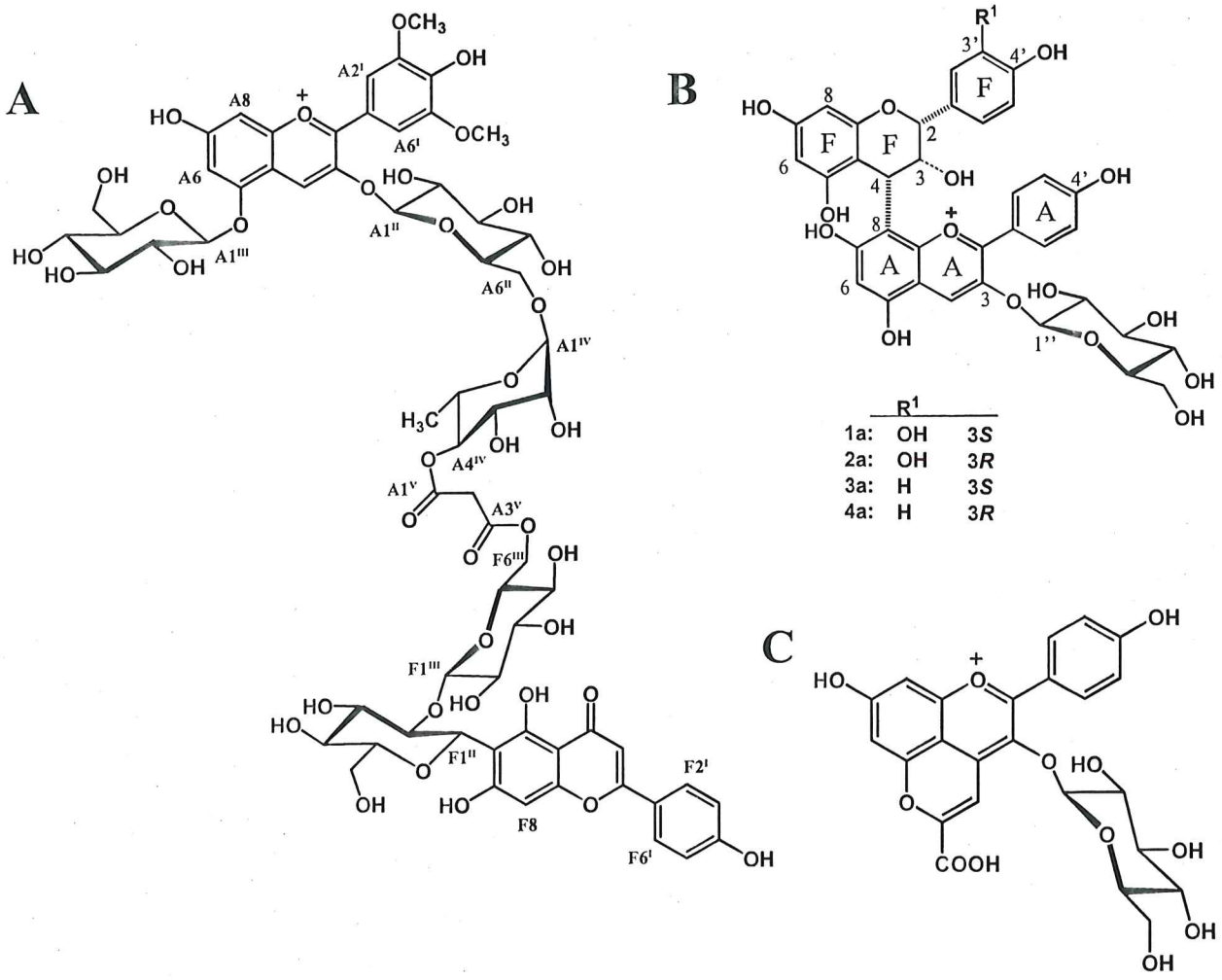


*Figure 1. Structures of (malvidin 3-O-(6II-O-α-rhamnopyranosylAIV-β-glucopyranosideAII)-5-O-β-glucopyranosideAIII) (apigenin 6-C-(2II-O-β-glucopyranosylFIII-β-glucopyranosideFII)) malonateAV (AIV-4→AV-1,FIII-6→AV-3) (A) isolated from leaves of Oxalis triangularis, flavan-3-ol(4α→8)pelargonidin 3-O-β-glucopyranosides (B) and 5-carboxypyranopelargonidin 3-O-β-glucopyranoside (C) isolated from strawberries.*

# Solution Structure of the Human Immunodeficiency Virus Type 1p6 Protein

**Fossen T[‡#], Wray V[‡], Bruns K[‡], Rachmat J[‡], Henklein P[$], Tessmer U*, Maczurek A[§], Klinger P[§], and Schubert U[§]**

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The HIV-1 p6 protein represents a docking site for several cellular and viral binding factors and fulfils major roles in the formation of infectious viruses. To date, however, the structure of this 52 amino acid protein, by far the smallest lentiviral protein known, either in its mature form as free p6, or as the C-terminal part of the Pr55 Gag polyprotein, has not been unravelled. We have explored the high resolution structure and folding of p6 by CD and NMR spectroscopy. Under membranous solution conditions p6 can adopt a helix-flexible-helix structure: a short helix-1 (14-18) is connected to a pronounced helix-2 (33-44) by a flexible hinge region. Thus, p6 can be subdivided into two distinct structural and functional domains: Helix-2 perfectly defines the region that binds to the virus budding factor AIP-1/ALIX indicating that this structure is required for interaction with the endosomal sorting complex required for transport (ESCRT). The PTAP motif at the N-terminus, comprising the primary late (L) assembly domain which is crucial for interaction with another cellular budding factor, Tsg101, does not exhibit secondary structure. However, the adjacent helix-1 may play an indirect role in the specific complex formation between p6 and the binding grove in Tsg101. Moreover, binding studies by NMR demonstrate that helix-2, which also comprises the LXXLF motif required for incorporation of the HIV-1 accessory protein Vpr into budding virions, specifically interacts with the Vpr binding region, indicating that under the specific solution conditions used for structure analysis p6 adopted a functional conformation.

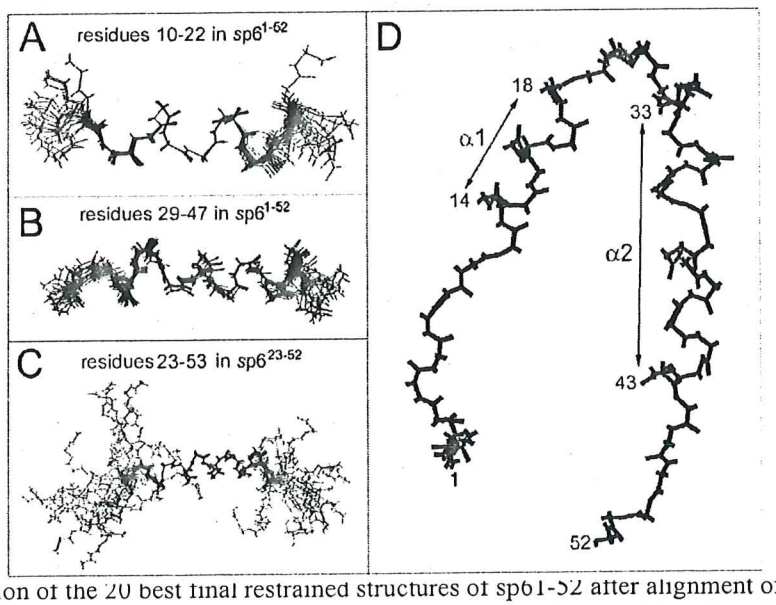


Figure 1. (A) Supposition of the 20 best final restrained structures of sp61-52 after alignment of the backbone atoms of residues Ser-14 to Gly-18. Shown are structures comprising residues Pro-10 to Thr-22. (B) Supposition of the 20 best final structures of sp61-52 after alignment of the backbone atoms of residues Lys-33 to Ser-43. Shown are structures comprising residues Glu-29 to Ser-47. (C) Supposition of the 20 best final structures of sp623-52 after alignment of the backbone atoms of residues Lys-33 to Leu-44. Structures comprising all residues are shown. (D) Central structure of sp61-52 as represented by the structure that shows the lowest average rmsd value to all other final structures.

# Acquisition weighted $^{31}$P SLOOP chemical shift imaging in the human heart

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Phosphorous magnetic resonance spectroscopy has revealed to be a potential method for the investigation of the high-energy phosphate metabolism and cardiac diseases, such as dilated cardiomyopathy and coronary disease. In addition it can be applied as a tool to distinguish between viable and non-viable myocardial tissue. In this context chemical shift imaging is the most widely-used method. In the majority of all cases only relative changes in phosphocreatine (PCr), adenosine triphosphate (ATP) and inorganic phosphate were determined.
Due to the fact that $^{31}$P metabolites can only be found in low concentrations and the $^{31}$P nucleus provide a low sensitivity large voxel size comparable to the size of the anatomical structures has to be chosen.
Because of this and the inconvenient shape of the spatial response function, the signal of a voxel placed in the human myocardium is usually contaminated by signal from surrounding tissue. To improve the shape of the SRF a k-space filter can be applied. But in turn this affects the spatial resolution and decreases the sensitivity. A better approach is to apply the k-space weighting during the acquisition, respectively use a density weighted sampling scheme [1, 2]. This has shown to improve quality both of chemical shift imaging and common magnetic resonance imaging in comparison to techniques with a non-weighted k-space sampling. Nevertheless using this method the localization is still bound to the shape of the voxels and how they are arranged in relation to the anatomical structure.
In contrast to that the SLOOP ("spectral localization with optimal pointspread function") method allows us to obtain spectra which match the anatomic structure of the examined organs. Furthermore the SLOOP algorithm allows us to determine absolute concentratins of 31P high energy metabolites.
The purpose of this study was to apply the SLOOP (spectral localization with optimal point spread function) [3] evaluation to $^{31}$P acquisition weighted chemical shift imaging of the human myocardium and compare the findings with results obtained using SLOOP and standard CSI.
Our findings reveal that that the SLOOP evaluation can be applied to acquisition weighted chemical imaging. The results obtained for acquisition weighted chemical shift imaging and for standard chemical imaging are in good agreement. Furthermore show the results achieved by acquisition weighted chemical imaging for all metabolites a smaller standard deviation than those achieved by standard chemical shift imaging.

**References:**

1. Pohmann R, von Kienlin M. Accurate Phosphporous Metabolite Images of the Human Heart by 3D Acquisition-Weighted CSI. Magn Reson Med 2001;45:817-826.
2. Köstler H, Beer M, Landschütz W, Buchner S, Sandstede J, Pabst T, Kenn W, Neubauer S, von Kienlin M, Hahn D. Fortschr Röntgenstr 2001;173:1093-1098.
3. Meininger M, Landschuetz W, Beer M, Seyfarth T, Horn M, Pabst T, Haase A, Hahn D, Neubauer S, von Kienlin M [1999] Magn. Reson. Med.41:657–663.

# Triple resonance spectroscopy of 56 kDa dimeric scaffolding protein 14-3-3

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14-3-3 is an important scaffolding protein that has more than a hundred reaction partners [1]; among these are the aromatic amino-acid hydroxylases which catalyse rate limiting steps in the synthesis of important neurotransmitters [2]. Tryptophan hydroxylases catalyses the hydroxylation of tryptophan, which is required for the production of serotonin and melatonin [3]. Tyrosine hydroxylase catalyses a step in the production of catecolamine neurotransmitters, such as dopamine, noradrenaline and adrenaline [2]. These two hydroxylases has been reported to bind to membranes [4]. We want to study the structural determinants for the interaction of 14-3-3 with its partners, including tryptophan and tyrosine hydroxylases. Moreover, we also aim to elucidate how 14-3-3 affects these hydroxylases ability to bind to membranes. To do this we have embarked on a detailed structural and dynamic analysis of 14-3-3 using state of the art NMR. Since 14-3-3 probably is in equilibrium as a dimer of 56 kDa in the cell, and definitely so at concentrations required for NMR, we have triple-labelled the protein with $^{2}H$, $^{13}C$ and $^{15}N$. We have accumulated 3D NMR data, reducing linewidths due to large protein size using transverse relaxation optimized spectroscopy versions of triple-resonance techniques HNCA and HNCOCA [5,6]. On the basis of these data, we here present a partial assignment of the protein.

[1] Fu, H, Subramanian, RR & Masters, SC (2000) *Annu Rev Pharmacol Toxicol* 40:617-47.
[2] Fitzpatrick, PF (2003) *Biochemistry* 42:14083-91.
[3] Martínez, A, Knappskog, PM & Haavik, J (2001) *Curr. Med. Chem.* 8:1077-1091.
[4] Thorolfsson, M, Doskeland, AP, Muga, A & Martinez, A (2002) *FEBS Lett* 519:221-6.
[5] Byeon, IJ, Li, H, Song, H, Gronenborn, AM & Tsai, MD (2005) *Nat Struct Mol Biol.*
[6] Salzmann, M, Pervushin, K, Wider, G, Senn, H & Wütrich, K (1998) *Proc. Natl. Acad. Sci. USA* 95:13585-13590.

# Combined NMR Relaxation and Cryogenic NMR to Probe Pore-Architecture (Water Confined in Porous Polymer Particles)

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## 1. Introduction

The object of the present work is to probe the pore morphology or pore-structure of porous polymer particles by combining NMR cryoporometry and NMR relaxometry. The polymer particles were prepared by Dynal Biotech and pre-saturated with water before packed into 10 mm NMR tubes, and sealed. All NMR measurements were performed on a 23.5 MHz MARAN Ultra NMR instrument.

## 3. Results and discussion

The $T_2$-distribution of pore-confined water (Figure 1, Left) reveals essentially one or two distinct and separate peaks

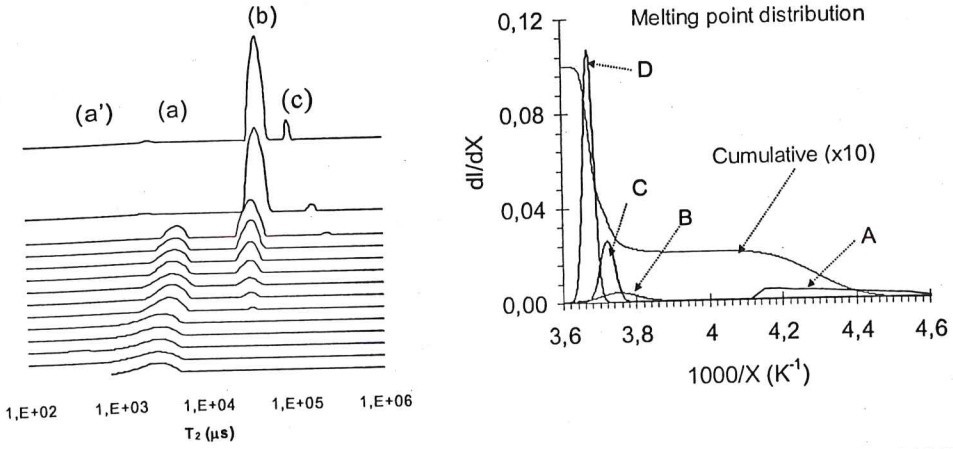
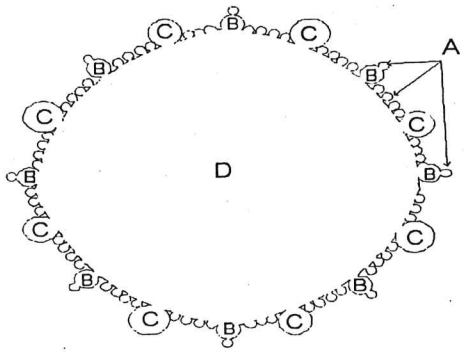



**Figure 1**. Relaxation time distribution of pore confined water as a function of temperature; 238 K, 242 K, 247 K, 252 K, 257 K, 265 K, 266 K, ….., 271 K (from bottom to top; left). The observed intensity versus temperature curve (IT-curve) and the derived melting point distribution curves A, B, C and D are shown on the right.

By carefully analyzing the experimental data in Figure 1 the following pore structure is derived [5].




## References

[1]. E. W. Hansen et al, J. Phys. Chem., 2005, in press.

# The three-dimensional NMR structure of the pediocin-like bacteriocin (antimicrobial peptide) curvacin A

**Haugen HS, Fimland G, Nissen-Meyer J & Kristiansen PE**

*Department of Molecular Biosciences, University of Oslo*

The 3D structure of the membrane-permeabilizing 41-mer pediocin-like bacteriocin *(antimicrobial peptide) curvacin A produced by lactic acid bacteria has been studied by NMR spectroscopy. In DPC micelles, the cationic and hydrophilic N-terminal half of the peptide forms an S-shaped β-sheet-like domain stabilized by a disulfide bridge and a few hydrogen bonds. This domain is followed by two α-helices: a hydrophilic 6-mer helix between residues 19 and 24 and an amphiphilic/hydrophilic 11-mer helix between residues 29 and 39. There are two hinges in the peptide, one at residues 16-18 between the N-terminal S-shaped β-sheet-like structure and the central 6-mer helix and one at residue 26-28 between the central helix and the 11-mer C-terminal helix. The C-terminal helix is the only amphiphilic/hydrophilic part of the peptide and is thus presumably the part that penetrates into the hydrophobic phase of target-cell membranes. The hinge between the two helices may introduce the flexibility that allows the helix to dip into membranes. The helix-hinge-helix structure in the C-terminal half of curvacin A clearly distinguishes this peptide from the other pediocin-like peptides whose structures have been analyzed. This suggests that curvacin A along with the structural homologues enterocin P and carnobacteriocin BM1 belong to a subgroup of the pediocin-like family of bacteriocins (antimicrobial peptides).

# Use of NMR and other physicochemical methods to study interactions between psychotropic drugs and glycerophospholipids

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*Departments of *Biomedicine and **Chemistry, University of Bergen*

We showed previously that chlorpromazine (CPZ) interferes strongly with the poly-phosphatide (PPI) metabolism in human platelets. Since these cells do not have $D_2$ recep-tors, which CPZ is supposed to interact with, we supposed that the drug interacted with membrane phospholipids since CPZ is a cationic, amphiphilic molecule. Studies with monolayers (Langmuir), fluorescence, calorimetry, surface plasmon resonance as well as solid state and solution ($^{13}C$ and $^{31}P$) NMR clearly showed that CPZ interacts with acidic, but not neutral glycerophospholipids and that the interaction increased with the degree of unsaturation of the acyl chains.

Solid state (MAS) $^{13}C$-NMR showed that addition of 10 mol-% CPZ to liposomes containing dipalmitoyl phosphatidylcholine (DPPC)/ pig brain phosphatidylserine (PBPS) [54/36 mol-%] at 37 °C caused a 5-15 ppm downfield shift of 30 % of the acyl chain resonances, while at 25 °C an interaction with the serine head groups was noted. Further studies revealed that CPZ preferentially binds to the phosphate group of PBPS and less binding to the serine carboxyl group. $^{31}P$-NMR studies indicated an effect of the anisotropic motion of the serine group that was intermolecular rather than intramolecular and that CPZ made a complex with PS. Reverse phase HPLC analysis of the PBPS showed that it contained the 18:0/18:1 (SOPS, 49%), 18:0/22:6 (SDPS, 28%) and some minor molecular species. NMR studies showed that there was very little interaction between CPZ and SOPS as compared to PBPS in identical mixtures with DPPS, strongly indicating that it was the SDPS species that interacted strongly with CPZ; this was later confirmed using authentic SDPS. $T_1$-relaxation measure-ments made it clear that the saturated acyl chain carbons and the choline head groups were not affected by CPZ. Increased signal intensity and $T_1$ values showed that CPZ caused reduced mobility of $C_4$ and $C_5$ of the 22:6 acyls, indicating direct interaction of CPZ $_4$-$C_5$ double bond. Finally, certain resonances in the DPPC/SDPS liposomes disappeared by CPZ indicating SDPS microdomains in these liposomes that disappears by CPZ addition.

We have extended some of our platelet PPI metabolism and physicochemical studies (not yet NMR) to include other psychotropic drugs than CPZ: trifluoperazine, prochlorperazine, haloperidol, pimozide, clozapine, olanzapine and quietiapine. All did interfere with PPI metabolism and altered the structure of negatively charged glycerophospholipids, although to different degrees, with quietiapine and olanzapine showing the least interaction. We suggest that alteration of glycerophospholipid structure by which these amphiphilic drugs alter the function membrane-embedded proteins such as receptors and enzymes.

# Interactions between the anti-cancer drug cisplatin and a total lipid extract from pig brain investigated by solid-state NMR methods

**Jensen M and Nerdal W**

*Department of Chemistry, University of Bergen*

Cis-diamminedichloroplatinum (II) (cisplatin) is a widely used chemotherapeutic agent with significant activity against cancers of the testis, ovary, head, neck and lung. Unfortunately, the drug has severe side-effects like nausea, ear damage and vomiting. Many patients may develop resistance as well, rendering the drug useless. The mechanisms of interaction between DNA and cisplatin leading to apoptosis and eventually cell-death are well known. The mechanisms leading to side-effects and drug-resistance are less known. This study aims to reveal knowledge about the mechanisms leading to side effects or drug resistance.

As a model membrane, a total lipid extract from pig brain (LEB) obtained from AvantiLipids is used. The lipid extract is reacted with cisplatin and solid-state NMR spectra from both pure LEB and a LEB/cisplatin mixture containing 30 weight % cisplatin are investigated.
NMR experiments include the following methods:
Static $^{31}P$ to obtain the Chemical Shift Anisotropy and Magic Angle Spinning experiments on $^{31}P$, $^{13}C$ and $^{1}H$. $T_1$ relaxation measurements of $^{31}P$ and $^{13}C$. 2D NMR experiments like COSY may also be included.

# Structure and Mode of Action of the Membrane-Permeabilizing Antimicrobial Peptide Pheromone Plantaricin A

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*Department of Molecular Biosciences, University of Oslo , Norway.*
*Running title: Plantaricin A Structure and Mode of Action*

The three-dimensional structure in dodecylphosphocholine micelles of the 26-mer membrane-permeabilizing bacteriocin-like pheromone plantaricin A (PlnA) has been determined by use of nuclear magnetic resonance spectroscopy. The peptide was unstructured in water, but became partly structured upon exposure to micelles. An amphiphilic α-helix stretching from residue 12 to 21 (possibly also including residues 22 and 23) was then formed in the C-terminal part of the peptide, while the N-terminal part remained largely unstructured. PlnA exerted its membrane-permeabilizing antimicrobial activity through a non-chiral interaction with the target cell membrane, since the D-enantiomeric form had the same activity as the natural L-form. This non-chiral interaction involved the amphiphilic α-helical region in the C-terminal half of PlnA, since a 17-mer fragment that contains the amphiphilic α-helical part of the peptide had antimicrobial potency that was similar to that of the L- and D-enantiomeric forms of PlnA. Also the pheromone activity of PlnA depended on this non-chiral interaction, since both the L- and D-enantiomeric forms of the 17-mer fragment inhibited the pheromone activity. The pheromone activity involved, however, also a chiral interaction between the N-terminal part of PlnA and its receptor, since high concentrations of the L-form (but not the D-form) of a 5-mer fragment derived from the N-terminal part of PlnA had pheromone activity. The results thus reveal a novel mechanism whereby peptide pheromones such as PlnA may function. An initial non-chiral interaction with membrane lipids induces α-helical structuring in a segment of the peptide pheromone. The peptide becomes thereby sufficiently structured and properly positioned in the membrane interface, thus enabling it to engage in a chiral interaction with its receptor in or near the membrane water interface. This membrane-interacting mode of action explains why some peptide pheromones/hormones such as PlnA sometimes display antimicrobial activity in addition to their pheromone activity.

# Clarification of the C-35 Stereochemistry of DTX-1

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†) *National Veterinary Institute, Pb 8156 Dep., N-0033 Oslo, Norway*
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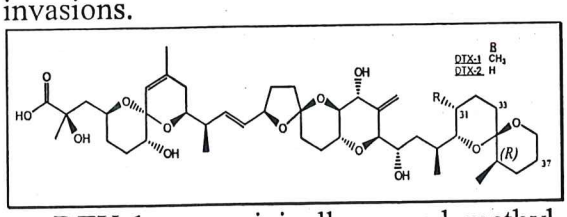
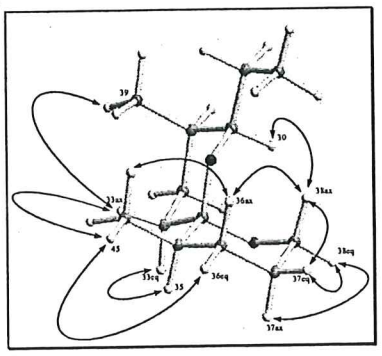
Mussels and seafood are enjoyed around the world as food and delicacies. Okadaic acid and dinophysistoxins (DTXs), a group of polyethers that sometimes contaminate filter-feeding shellfish, may cause symptoms classified as diarrhetic shellfish poisoning [1]. The alga *Dinophysis acuta* is one pro-genitor of okadaic acid, DTX-1 and 2, and is found in coastal waters of Norway [2]. Algal blooms are known to cause problems for the marine farming industry, and a broader knowledge of the toxins is needed in order to understand and ameliorate these algal invasions.




DTX-1 was originally named methyl-35(*S*)-okadaic acid [3] and several papers support this structure [4]. We now have evidence that the methyl group is equatorial, and that the stereochemistry at C-35 is *R*. The elucidation for DTX-1 was performed using 1 mg of pure toxin sample on a Bruker AV 600 MHz NMR-spectrometer at UiO. DTX-2 appears to have the same stereochemistry as DTX-1 at C-35 [4].

*NOESY* spectra of DTX-1 in $CD_3OD$ and $CDCl_3$ were acquired consecutively, and enough supportive evidence collected to define the C-35 stereochemistry. The use of a cryo probe together with the 600 MHz spectrometer provided sufficient signal separation and sensitivity to make this structural assignment. Analysis of $^{13}C$, *DEPT135*, gs-*HMBC* and gs-*HSQC* spectral data also indicated that some of the published chemical shifts for DTX-1 may be incorrect.



*Molecular model of DTX-1 showing terminal rings with NOE correlations.*

1. Yasumoto, T.M., M.; Oshima, Y.; Sano, M.; Matsumoto, G. K.; Clardy, J., *Diarrhetic shellfish toxins.* Tetrahedron, 1985. 41(6): p. 1019-1025.
2. Miles, C.O., *A novel Pectenotoxin, PTX-12, in Dinophysis Spp. and Shellfish from Norway.* Chem. Res. Toxicol., 2004. 17(11): p. 1423-1433.
3. Michio Murata, M.S., Hiroshi Sugitani, Yasukatsn Oshima, and Takeshi Yasumoto, *Isolation and Structural Elucidation of the Causative Toxin of the Diarrhetic Shellfish Poisoning.* Bulletin of the Japanese Society of Scientific Fisheries, 1982. 48(4): p. 549-552.
4. Tingmo Hu, J.D., David Jackson, Julie Marr, Eugene Nixon, Stephen pleasance, Michael A. Quilliam, John A. Walterdand Jeffrey L.C. Wright, *Isolation of a New Diarrhetic Shellfish Poison from Irish Mussels.* Journal of Chemical Society, Chem. Comm., 1992(1): p. 39-41.

# Archaeal $C_{80}$ isoprenoid tetraacids responsible for naphthenate deposition in crude oil processing

**Lutnæs BF,[a] Brandal Ø[b], Sjöblom J[b] and Krane J[b]**

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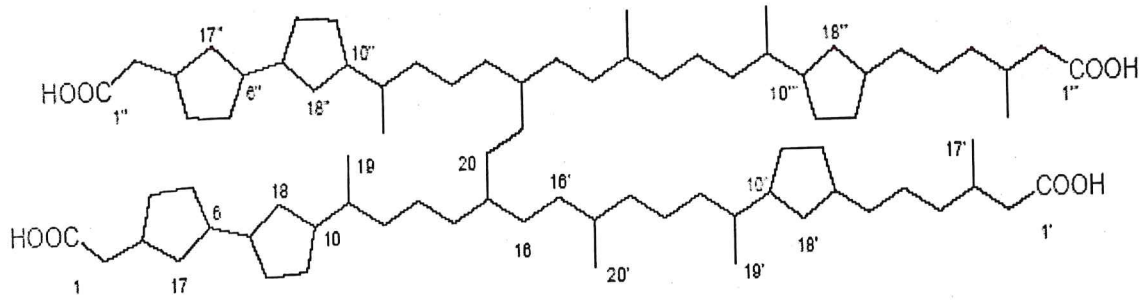
The formation of solid deposits in oil-water separators during crude oil processing is a serious problem in many oilfields, causing production irregularities accompanied by expensive production shutdowns.
Salts of naphthenic acids, a group of carbocyclic carboxylic acids, are the main contributor to the deposits, in addition to inorganics. It was recently demonstrated that naphthenic tetracarboxylic acids, which constitute only a few ppm of the crude oil, are responsible for the formation of naphthenate deposits[1,2]..Dispite a high research effort in the oil industry, the structures of these naphthenic tetraacids have escaped detection.
Here we report the naphthenic tetraacids to be a novel family of $C_{80}$ isoprenoid tetrameric carboxylic acids, as elucidated by NMR and mass spectroscopy. The tetraacids have a 20-bis-16,16'-biphytane carbon skeleton, which points to an archaeal origin. The tetraacids possess unique surfactant properties,[3] and may be produced by oil degrading archaea for use as biosurfactants, or may originate from molecular fossils.




1. T. D. Baugh, N. O. Wolf, H. Mediaas, J. E. Vindstad and K. Grande, *Preprint - ACS, Division of Petroleum Chemistry*, 2004, **49**, 274.
2. T. D. Baugh, K. Grande, H. Mediaas, J .E. Vindstad and N. O. Wolf, *Proceedings - SPE 7th Int. Symp. on Oilfield Scale*, Aberdeen, SPE/IADC 93011, 2005.
3. Ø. Brandal, A.-M. Hanneseth, P. V. Hemmingsen, J. Sjöblom, J. Kim, R. R. Rodgers and A.G. Marshall, *J. Dispersion Sci. Technol.* in press.

# Phytochemical studies on the aerial parts of *Blepharis aspera* (Acathanceae)

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**Abstract**

During the ongoing study on the chemistry of plants growing on heavy metal mineralised soils and heavy metal accumulating plants in Botswana, 4 secondary metabolites were isolated from the aerial parts of Blepharis *aspera* (Acantanceae) and characterised by spectroscopic means (1D and 2D NMR, and mass spectrometry). The four isolated compounds are 3, 4-dihydroxy benzoic acid (**1**), 4-hydroxy-3-methoxybenzoic acid (**2**), 4′, 5, 6, 7-tetrahydroxy flavone (**3**) and *n*-docosyl-*trans*-ferulate (**4**). Compounds **1**, **2** and **3** were isolated from the ethyl acetate extract and compound **4** was isolated from the dichloromethane extract.

*Keywords: Blepharis aspera*, Acathanceae, 3, 4-dihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4′, 5, 6, 7-tetrahydroxy flavone, *n*-docosyl-*trans*-ferulate

# Noninvasive measurements of gas exchange in a 3D fluidized bed

**Pavlin T[a*], Wang R[a,b], Mair R[a,b], McGorty R[a], Rosen MS[a], Cory DG[b], Walsworth RL[a]**

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[b]*Department of Nuclear Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02138, USA*

Granular media are collections of small solid particles that are ubiquitous in many industries as well as in nature. These particles are solids, but under certain conditions a collection of them will tend to behave like a liquid. Some examples of granular materials include food products such as rice, corn and salt, building materials like sand, gravel and soil; and chemicals such as aluminum powder, plastic spheres and pharmaceuticals. Diagnosing the dynamics of these materials is relevant to a wide range of scientific and engineering problems.

One of the most commonly encountered dynamic granular processes is gas fluidization. Gas fluidization is a process in which solid particles experience fluid-like suspensions in a stream of upward flowing gas [1, 2]. Most commercial gas-fluidized beds operate in the bubbling fluidization regime, in which bubbles emerge at the bottom of the bed and expand while rising up along the bed. Bubbles help to agitate the bed to achieve better mixing, and to enhance chemical reaction rates in processes where the particles act as catalysts or reaction constituents.

The efficiency of fluidized bed processes depends greatly on the rate at which the fluidizing gas comes into contact and reacts with the solid phase. Generally, this rate will depend on the rate of gas exchange between the bubble and the emulsion phase (interstitial space between the closely-packed solid particles), and the gas exchange between the emulsion and the adsorbed phase (micro-porous surface of the solid particles).

Despite the wide applications of gas-fluidization, the understanding of the dynamics of solid particles and fluidizing gas is far from complete. Such systems are difficult to model numerically due to the large number of degrees of freedom and inelastic collisions among the particles [3]. In addition, a typical three-dimensional bed is opaque, resulting in difficulties using light scattering or sound waves to probe bed behavior below the surface [4]. We present a novel method that uses existing NMR technology and laser-polarization of noble gases (xenon) to non-invasively measure the gas-exchange rates between bubble, emulsion and adsorbed phases in a three-dimensional gas-fluidized bed of alumina particles. An NMR model is derived from the Bloch equations that yields both bubble-emulsion and emulsion-adsorbed phase gas exchange rates from the variation of NMR signals in exchange experiments. A fluidized bed apparatus incorporating an RF coil has been designed and constructed for operation in a standard vertical bore superconducting magnet, and provides a convenient avenue to test and verify the model. Susceptibility differences between the bubble and the emulsion phase enabled us to use T2-contrastbased MR sequence to measure bubble-emulsion exchange rate, while emulsion-adsorption exchange rate was measured utilizing chemical shift of adsorbed xenon molecules. The experimental fluidized bed system and NMR measurements, when interpreted with the NMR model, yield exchange rate values that conform to intuition and prior invasive measurement on similar experimental systems.

[1] D. Kunii. *Fluidization Engineering.* Butterworth-Heinemann, 1991.
[2] J.F. Davidson and D. Harrison, *Fluidized Particles,* Cambridge University Press, 1963.
[3] R. Jackson. *The Dynamics of Fluidized Particles.* Cambridge University Press, 2000.
[4] J. Z. Xue, et al., *Diffusion, Dispersion, and Settling of Hard Spheres.* Phys. Rev. Lett., 69 (11), 1992.
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# Hvordan kom NMR til Norge?

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- Når og hvor kom det første NMR-spektrometeret til Norge.
- Hvem tok initiativet og hvorfor?
- Hvordan kunne man ta opp et NMR-spektrum uten datamaskin?
- Hva brukte man NMR til i begynnelsen?

Vi har fulgt utviklingen av NMR i Norge fra 1960 og forteller her historien frem til ca 1980 slik vi har opplevd den.

# NMR-analysis of the 3-D structure of the two-peptide bacteriocin lactococcin G

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Lactococcin G is a bacteriocin that consists of two peptides, the α peptide with 39 residues and the β peptide with 35 residues. Both peptides are required in about equal amounts in order for the bacteriocin to permeabilize target-cell membranes and thereby exert its antimicrobial activity. As determined by CD-spectroscopy, the two peptides interact with each other and become structured – adopting a partial amphiphilic α-helical conformation – when exposed to membrane-like entities [1]. In this study, NMR-spectroscopy has been used to analyse the 3-D structure of the lactococcin G peptides separately upon exposure to dodecylphosphocoline micelles. To obtain $^{15}N$ labelled peptides for NMR-analysis, the peptides were expressed as fusion proteins using an *E. coli* expression system specially designed for expression of $^{15}N$- and $^{13}C$-labelled peptides [2]. The peptides were fused to the C-terminal end of the immunoglobulin-binding domain of the streptococcal protein G (GB1) via a linker that contains a methionine residue, thereby enabling specific chemical cleavage of the fusion protein with CNBr. The peptides and linkers were inserted as gene fusions to the GEV2-vector which is a vector developed for expression of GB1 based on pET system[3]. *E. coli* cells containing this modified GEV2-vector were grown on a defined medium (M9) containing $^{15}NH_4Cl$ as the sole source of nitrogen. The peptides were sequentially assigned by using $^{15}N$ resolved three-dimensional NOESY- and TOCSY- HSQC. Additional information was provided by two-dimensional NOESY and TOCSY spectra. The calculation of the structures were based on the H-H distance restrains from the $^{15}N$-NOESY-HSQC and the NOESY spectra combined with angular restraints calculated on the basis of the chemical shifts.

[1] Hauge HH, *et al.* (1998) Eur J Biochem., 25:565-72.
[2] Koenig BW, Rogowski M & Louis JM (2003) J. Biomol. NMR 26:193-202.
[3] Huth JR, et al. (1997) Protein Sci, 6: 2359-2364.

# Studying water exchange in biological tissue using two dimensional relaxation and diffusion measurements

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Biological tissue can be regarded as a porous system, where water is confined in different compartments, and with equilibrium water exchange between these compartments. In a single pixel of a $^1H_2O$ MRI image the observed signal is a sum of signals from water molecules in different magnetic environments. Effects from tissue inter-compartmental equilibrium water exchange can therefore be quite significant in the quantitative analysis of various in vivo MRI data.

Unfortunately, water signals from different tissue compartments as detected by NMR are isochronous, and cannot be separated by differences in chemical shift. However, one way of investigating equilibrium water exchange effects is to add a contrast agent that changes the relaxation time of one tissue component compared to the others, a method known as Relaxographic Imaging (1). The total relaxation behavior will depend on the change in relaxation rate for the particular compartment, and to which extent the water in other compartments can access the contrast agent through water exchange. In addition, using two dimensional correlation measurements between diffusion and relaxation it is possible to separate the relaxographic signals due to their difference in diffusivities, a method that has been successfully applied in petrophysical systems (2-5).

In this study we have investigated water compartments and equilibrium water exchange in excised rat myocardium enriched with different amounts of intracellular manganese. To identify the individual water compartments we used diffusion-$T_2$ and $T_1$-$T_2$ correlation measurements, which were analyzed using Inverse Laplace Transformations in two dimensions (6). The results show that using this method it is possible to identify intracellular and extracellular water compartments on a very detailed level.

1. C. Labadie, *et al.*, *J. Magn. Reson. B* **105**, 99, (1994).
2. M. D. Hurlimann and L. Venkataramanatan, *J. Magn. Reson.* **157**, 31, (2002).
3. J. G. Seland, *et al.*, *Appl. Magn. Reson.* **24**, 42, (2003).
4. G. H. Sørland, *et al.*, *Appl. Magn. Reson.* **26**, 417, (2004).
5. J. G. Seland, *et al.*, Phys. Rev. E **70**, 051305, (2004).
6. Venkataramanan L, *et al.*, *IEEE Trans. Signal Process.* **50**, 1017, (2002).

# Controlling LC-SPE-NMR Systems

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There are several stages of the LC-SPE-NMR process that should be monitored closely to ensure a successful experiment, for instance analyte break-through and compound transfer from the LC-SPE to the NMR probe. Analyte breakthrough on SPE cartridges prevents analyte enrichment which is required for performing NMR analysis on compounds present at low or moderate concentrations. In this study, analyte breakthrough monitoring was performed with a UV detector and later a mass spectrometer placed after the SPE unit. Breakthrough occurrences were often observed after only 5 or less trappings on SPE cartridges with our original system. However, when lowering the flow rate over the SPE system and using SPE cartridges packed with alternative stationary phases, the number of trappings possible increased 5-fold.
To increase control over the SPE-NMR transfer, an gradient pump-UV system was used to elute compounds trapped on an SPE to an NMR probe. The analyte band would be placed in the active volume of the probe by a stop-flow mechanism.
The modified LC-SPE system was coupled on line with a micro-coil NMR probe and off-line with an NMR cryo-probe, and was used for analysis of degradation products of the insecticide monuron, present in the low ppm range.

# NMR of a cyclic macropeptide

**Skjeldal L**

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3D structure of a macrocylic peptide was determined by use of 1D an 2D proton NMR experiments. 1H-15N HSQC was performed, without isotope incorporation, on a Bruker AvanceII 600 MHz instrument, equipped with a TXI probe.

# Structure elucidation of a paramagnetic protein.

**Skjeldal L**

*IKBM, Norwegian University of Life Sciences, UMB*

By use of paramagnetic NMR, a histidine ligating to the iron-sulfur cluster in T4MoC ferrdoxin was shown to be protonated in the oxidized state. Hyperfine shifted signals were detected at both redox states.

# NMR structure of an oxididized 2Fe-2S ferredoxin

**Skjeldal L, et al.**

*IKBM, Norwegian University of Life Sciences, UMB*

The Rieske ferredoxin component, T4MoC, from *Pseudomonas mendocina* KR1 is a 112-amino acid iron-sulfur protein. The 2Fe-2S cluster is ligated to two cysteines and two histidines, and the cluster is paramagnetic in both redox states at room temperature. The NMR structure was determined from 2D and 3D $^{1}H$, $^{13}C$, and $^{15N}$ NMR data. The structural model was refined through simulated annealing by molecular dynamics in torsion angle space with input from 1650 experimental restraints, including 1264 inter-proton distance restraints obtained from NOE's, and 113 dihedral angle ($\varphi$,$\psi$) restraints. The T4MoC structure consists of 10 β-strands arranged in three anti-parallell β-sheet topology with the iron-sulfur cluster exposed to water. The structure is deposited in the Protein Data Bank as 1SJG.

# DNA oligonucleotides form three-way junctions containing a helical dinuclear iron(II) supramolecular cylinder.

**Sletten E and Cerasino L**

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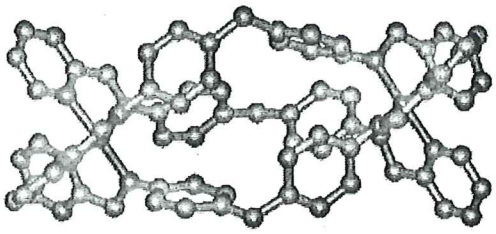
A long-term goal is to design drugs that can bind selectively to chosen sequences of DNA. Within biological systems the interactions between specific enzymes and DNA regulate the production of proteins. Sequence-specific code recognition is achieved through surface motifs which interact with either the minor or, more commonly, the major groove of DNA in a non-covalent fashion. The ability to artificially stimulate or prevent the processing of the genetic code is an important goal and offers new opportunities for disease prevention or control. Synthetic agents that target the major groove of DNA with recognition through non-covalent surface motifs therefore have the potential to be a powerful new tool.

A tetracationic supramolecular cylinder, $[Fe_2L_3]^{4+}$ ($L=C_{25}H_{20}N_4$), with a triple-helical architecture has been found to bind strongly to DNA [1]. NMR investigations have been carried out to clarify the mode of binding of the cylinder to several DNA oligonucleotides. Quite unexpectedly, the cylinder is shown to promote the formation of a three-way junction (TWJ) involving three strands of deoxyribopolynucleotides. $^1H$ NMR resonances for the cylinder show that the two-fold symmetry is lifted while the three-fold symmetry around the cylinder axis is retained. The NOE contacts between the helical cylinder and DNA unambiguously show that one end of the cylinder is fitted into the centre of the three-way junction experiencing a three-fold symmetric hydrophobic environment. The 1: 3 cylinder/DNA stoichiometry estimated from 1D NMR supports a molecular model of a three-way junction composed of three strands.




1. Meistermann, I., Moreno, V., Prieto, M.J., Molderheim, E., Sletten, E., Khalid, S., Rodger, P.M., Peberdy, J.C., Isaac, C.J., Rodger, A., Hannon, M.J. *Proc. Natl. Acad. Scien.* (2002) **99**, 5069-5074.
2. Moldrheim, E., Hannon, M.J., Meistermann, I., Rodger, A., Sletten, E. *J. Biol. Inorg. Chem.* (2002) **7**, 770-780

Acknowledgment: EU –Research Training Network, EU-COST D20, Norwegian Research Council

# Lipid microdomains in bilayer of dipalmitoylphosphatidylcholine (DPPC) and 1-stearoyl-2-docosahexaenoylphosphatidylserine (SDPS) and their Perturbation by Chlorpromazine : A $^{13}C$ and $^{31}P$ Solid-State NMR Study.

**Song C and Nerdal W**

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The polyunsaturated fatty acid docosahexaenoic acid (DHA, c22:6, n3) is found at a level of about 50% in the phospholipids of neuronal tissue membranes and appears to be crucial to human health. Dipalmitoyl phosphatidylcholine (DPPC, 16:0/16:0 PC), 1-palmitoyl-2-oleoylphosphatidylserine (POPS) and the DHA containing 1-stearaoyl-2-docosahexenoyl phosphatidylserine (SDPS) were used to make DPPC (60 %)/POPS (29 %)/SDPS (11 %) bilayers with and without 10 mol % chlorpromazine (CPZ), a cationic, amphiphilic phenothiazine derivative. The $T_1$ relaxation measurements make it clear that the saturated acyl chains carbons (palmitic, stearic and most of the oleic chain) and the choline headgroup are not affected by CPZ addition. The observed increased signal intensity and longitudinal relaxation times ($T_1$-values) of DHA indicate the reduced mobility of $C_4$ and $C_5$ caused by CPZ binding. Resonances which are only present in $^{13}C$ NMR spectrum of the DPPC (60 %)/SDPS (40 %) sample and disappear in presence of 10 % CPZ are likely to be due to the special interface environment, e. g. the hydrophobic mismatch, at the interface of DPPC and SDPS micro domains in the DPPC/SDPS bilayer. In itself, the appearance of resonances at novel chemical shift values is a clear demonstration of a unique chemical environment and existence of lipid micro domains in the DPPC (60 %)/SDPS (40 %) bilayer. The findings of the study presented here suggest CPZ bound to the phosphate of SDPS will slow down and partially inhibit such a DHA acyl chain movement in the DPPC/SDPS bilayer and perturb the micro domains. This would affect the area occupied by an SDPS molecule and probably the thickness of the bilayer where SDPS molecules reside as well. It is quite likely that such CPZ caused changes can affect the function of proteins embedded in the bilayer.

# Analysis of rock core plugs at different states by NMR

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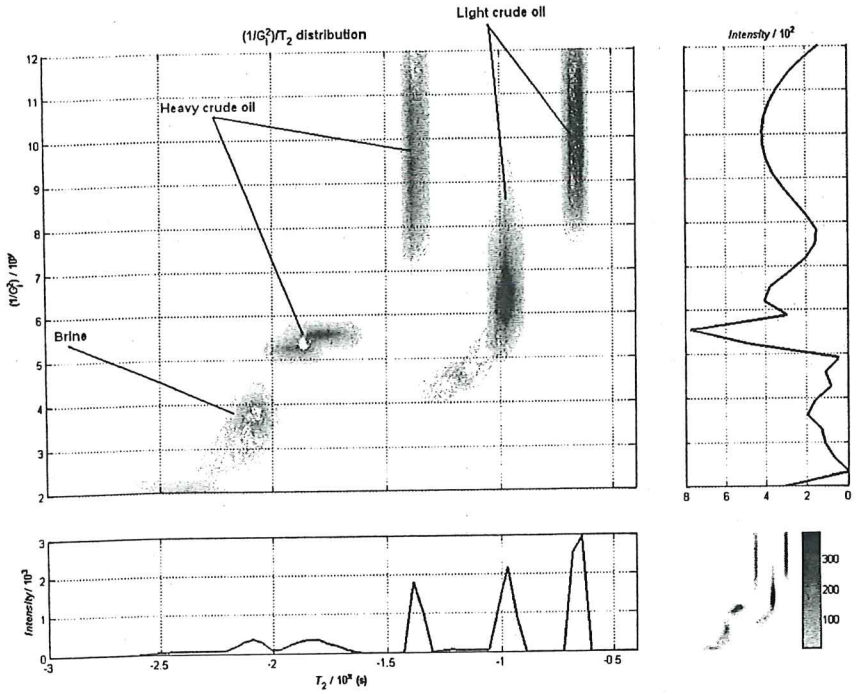
The use of proton Nuclear Magnetic Resonance (NMR) yields a non-invasive technique for the study of rock core plugs either at fresh state or saturated with different loadings of crude oil and/or brine. Over the paste decades, the technique has been successfully applied on rock core plugs for porosity measurements and pore size distributions (in units of $T_2$-values). Several other methods have been tried with more limited success, as the determination of tortuosity. This method often fails because the life time of the NMR signal is to short for probing the heterogeneity of the sample.

Here we present a set of methods that extract more information from the rock core plugs when compared to standard applications. We also show that the use of higher applied magnetic field, i.e. better signal to noise, improves the quality of the NMR data such that new information is resolved.

*Key words: Low Field NMR, Pulsed Field Gradients, Relaxation Times, Pore Size Distributions, Internal Gradients, Rock Core Analysis, 2 Dimensional Inverse Laplace*



# A novel *Fusarium* metabolite with biological activity

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2-amino-14,16-dimethyl-octadecan-3-ol, a new sphingosine analogue toxin in the fungal genus *Fusarium* [1] was purified from the methanolic extract from the rice culture of a *Fusarium avenaceum* strain, which had been isolated from Norwegian grain. The metabolite was discovered by bio-assay guided fractionation of culture extracts and was cytotoxic against the porcine kidney epithelial cell line PK-15. Other *Fusarium* species that were found to produce the compound in rice culture include *F. tricinctum*, *F. langsethiae* and *F. poae*.
The results from the structure elucidation using 1D and 2D NMR spectroscopy and mass spectroscopy will be presented.
2-amino-14,16-dimethyl-octadecan-3-ol has previously been isolated as a metabolite of a *Paecilomyces* species and NMR spectral data for this compound have been reported as part of a production procedure Patent Application [2]. However, it cannot be ascertained if the total stereochemistry of the metabolite is the same when it is isolated from the *Paecilomyces* species and from *Fusarium avenaceum.*

[1] Uhlig, S., Petersen, D., Flåøyen, A.,Wilkins, A. *Toxicon* 46 (2005) 513-522
[2] Rokuro, M., Satoshi, O., Kazuro, S., *PCT Int. Appl.* (2003). Novel substance FKI-0550 and process for producing the same, WO2001P1057820011204[WO03048373]

# The Inverse Laplace algorithm of Bernhard Kaufmann applied to NMR Relaxation Curves

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Bernhard Kaufmann [1] has developed an algorithm for fitting a sum of exponentials to a curve, often referred to as the Inverse Laplace transform. The idea of the algorithm is to express the sum of exponentials as a linear combination of powers of the independent variable and successive integrals of the sum. The algorithm is compact, fast and non-iterative, and is therefore a good choice when facing the numerically difficult Inverse Laplace problem.

An implementation is hereby presented in which cross validation is used to find the optimal number of components present in a given curve. The method has been tested on real NMR data sets, and shows good performance.

References
1. Bernhard Kaufmann: Fitting a Sum of Exponentials to Numerical Data
eprint arXiv:physics/0305019
05/2003, *http://arxiv.org/abs/physics/0305019*

# Bernhard Kaufmanns algoritme for invers Laplacetransform andvendt på relaksasjonskurver fra NMR

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Bernhard Kaufmann [1] har utviklet en algoritme for å tilpasse en sum av eksponensialledd til en kurve, ofte omtalt som invers Laplacetransform. Kjernen i algoritmen er å uttrykke summen av eksponensialledd som en lineærkombinasjon av potenser av den uavhengige variabelen, og suksessive integraler av summen. Algoritmen er kompakt, rask og ikke-iterativ. Den er derfor et godt valg når den numerisk vriene inverse Laplacetransformen skal takles.

Her presenteres en implementasjon som bruker kryssvalidering for å finne det optimale antallet komponenter som er tilstede i en gitt kurve. Metoden er testet på målte NMR-data, og viser seg å fungere godt.

Litteratur:
1. Bernhard Kaufmann: Fitting a Sum of Exponentials to Numerical Data
eprint arXiv:physics/0305019
05/2003, *http://arxiv.org/abs/physics/0305019*