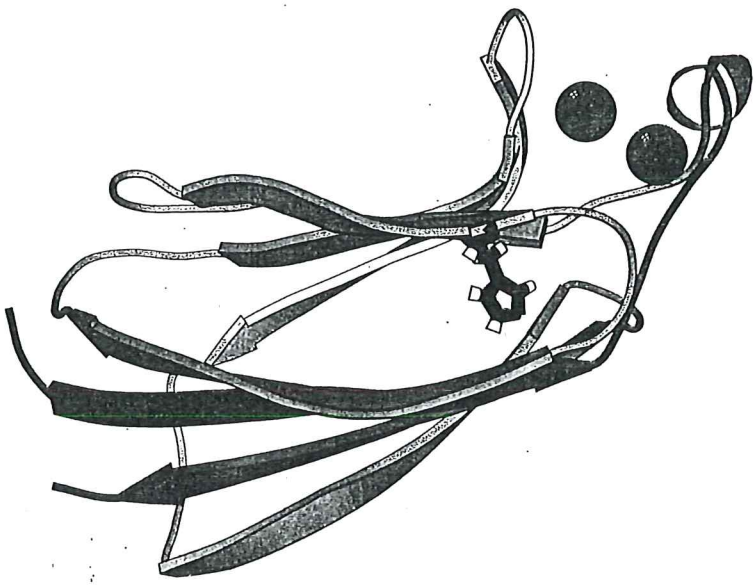
Det ~~tredje~~ syvende

Nasjonale NMR-møtet



Det kalsiumbindende domenet i cPLA2

Radisson SAS Resort, Beitostølen 9 - 10 januar 2002

Det tredje

Nasjonale NMR-møtet

Radisson SAS Resort,
Beitostølen 9 - 10 januar 2002

Institutt for kjemi,
NTNU,
7. januar 2002

Velkommen

Sist det nasjonale NMR-møtet ble arrangert på Geilo, 5.-6. januar 2000, bestemte Samarbeidsutvalget for NMR at Trondheim skulle stå ansvarlig for møtet i 2002. I skrivende stund er det uklart om vi har et Samarbeidsutvalg for norsk NMR eller ikke. Norges forskningsråd (NFR) gir ikke lenger støtte til opprettholdelse av utvalget, og søknader om utstyr prioriteres av den enkelte FOU-virksomhet. Dette gjør det ikke lett å fremme forslag om utstyr og prosjekter som har felles interesse for flere norske NMR brukere. En av hensiktene med dette møtet kan da være at vi får muligheter til å orientere hverandre om hva som skjer på NMR-fronten innen de forskjellige institusjonene.

Vi kan glede oss over noen lyspunkter i norsk NMR. Universitetet i Tromsø har kontrahert et 600 MHz instrument for høyoppløsningsstudier, og ved NTNU og Universitetet i Oslo investeres det i høyoppløsning NMR-utrustning til ulike formål. Medisinsk seksjon ved MR-senteret, Trondheim, går til innkjøp av et helkropps 3 Tesla system for MRI/MRS formål. En annen gledelig utvikling er et større fokus på bruk av lavfelt NMR ved FoH-enhetene i Norge, og vi har til og med fått det første "gründerfirma" innen dette segmentet av NMR, lokalisert til Harstad.

Internasjonalt går utviklingen stadig videre. Som eksempler kan det nevnes at det første kommersielle 21 Tesla (900 MHz) instrumentet for høyoppløsningsstudier er installert ved "The Scripps Research Institute", San Diego, USA. Ultra høyfelts-utstyr sammenkoplet med nye NMR-metoder som TROSY (Transverse Relaxation Optimized Spectroscopy) gjør at stadig større makromolekyler kan studeres. Samtidig ser en at 750- 800 MHz instrumenter blir tatt i bruk for studier av proteiner i fast fase. Er dette noe for norske forskningsmiljøer? Er dette kompetanse som det vil være nyttig å bygge opp sett i relasjon til prosjektet Funksjonell genomforskning i Norge- FUGE?
Også innen MRI/MRS går utviklingen for helkroppsinstrumenter i retning av stadige kraftigere magneter. Fra Universitetet i Minnesota er det i det siste publisert en rekke anatomiske bilder og spektra av mennsker tatt opp på 7 Tesla.
Investeringene for slike systemer er formidable og kravet til kompetansen for å drive slikt utstyr er høy. Skal norske forskere få tilgang til slikt utstyr i Norge, kreves det et stort nasjonalt løft.

Trondheim 7. januar 2002.

Henrik W. Anthonsen John Georg Seland Jostein Krane

Onsdag 9 januar

1300	-	1400	Lunch/innkvartering
1415	-	1420	Velkommen ved Prof. Jostein Krane, NTNU,
			Ordstyrer: Einar Sletten
1420	-	1455	Present status of flow-NMR. Jens Christian Madsen, BRUKER BioSpin Skandinavia AB
1455	-	1515	Karotenoider Mono- og di-karbokation av β,β-karoten Karoten fosfolipider, Jostein Krane , Inst. Kjemi, NTNU
1515	-	1535	Utplassering av en VNMRF, Henrik W. Anthonsen, Inst. Kjemi, NTNU
1535	-	1555	$^{1}H^{15}N$ NMR Studies on the kinetics of the reaction between antitumor platinum complex trans-EE and nucleotides. Yangzhong Liu, Univ. Bergen
1555	-	1630	Kaffepause med poster presentasjoner
			Ordstyrer: Bjørn Pedersen
1630	-	1715	Structural studies of the membrane-bound conformations of α- lactalbumin using hydrogen exchange monitored by band-selective, homonuclear decoupeled TOCSY. Øyvind Halskau, Univ. Bergen
1715	-	1745	Structure elucidation of Kalata B1. Lars Skjeldal
1745	-	1805	^{1}H chemical shift effects on oligonucleotides upon platinum binding. J. Vinje, Department of Chemistry, University of Bergen
1805	-	1825	NMR-studies of a polyelectrolyte-polyelectrolyte complex in a medical formulation. Are Kristiansen, Pronova Biomedical as
1825	-	1930	Poster presentasjoner med forfriskninger
2000	-		Middag

Torsdag 10 januar

Ordstyrer: Eddy Walter Hansen

0900	-	0945	New Aspects of Contrast Agent Development Pål Rongved Amersham Health
0945	-	1005	Multivariat analyse - Anvendelse og nytteverdi på biologiske NMR-data Tone Frost Baathen, SINTEF Unimed MR
1005	-	1050	MR i mus og rotte. Christian Brekken, Post doc., SINTEF Unimed MR, Trondheim.
1050	-	1120	Kaffepause / poster

Ordstyrer: Dagfinn Aksnes

1120	-	1140	Proton dekoplet ^{19}F NMR spektroskopi av medikamenter. Oddbjørn Sæther, Medisin, NTNU
1140	-	1200	Bruk av NMR spektroskopi til analyse av molekylære forandringer ved ulike sykdommer og skader i øyet, Øystein Risa, Medisin, NTNU
1200	-	1220	Kombinerte diffusjons- og relaksasjonsmålinger for karakterisering av heterogene systemer John Georg Seland, NTNU, Institutt for Kjemi
1220	-	1300	Diskusjon
1300	-	1400	Lunch

Postere henges opp så snart deltagerene kommer og tas ned ved lunsj torsdag.

FOREDRAG

Present status of flow-NMR.

Jens Christian Madsen, BRUKER BioSpin Skandinavia AB

Utplassering av en virtuell kjernemagnetisk resonans fasilitet, VNMRF

Frank Antonsen og Håkon Rueslåtten, Statoil, Trondheim
Henrik W. Anthonsen, Geir H. Sørland, John G. Seland og Jostein Krane,
Institutt for Kjemi, NTNU

Ved Statoils forskningssenter på Rotvoll, Trondheim er det plassert en lavfelts NMR magnet. Denne magneten brukes ikke bare av forskere i Trondheim, men også av personer som ikke er bosatt i Trondheim. For å redusere reisevirksomheten blir det satt opp en *virtuell NMR fasilitet*, VNMRF.

Dette er et system som gjør det mulig å bruke NMR instrumenter over lengre avstander, samt en rekke andre funksjoner som letter samarbeid mellom grupper som er geografisk spredt.

Vårt system baserer seg på et tilsvarende system satt opp av "Environmental Molecular Sciences Laboratory" (EMSL) i USA.

Karotenoider

Mono- og di-karbokation av β,β-karoten
Karoten fosfolipider

Jostein Krane, Bjart Frode Lutnæs, Bente Janette Foss, Liv Bruås, Vassilia Partali, og Synnøve Liaaen Jensen

Norges teknisk-naturvitenskapelige universitet
Institutt for kjemi

Magnetisk resonans (EPR og NMR) har vist seg å være velegnet for strukturoppklaring av de ustabile intermediatene som dannes når β,β-karoten behandles med Lewis-syrer i organiske løsningsmidler.

Eksempler på bruk av NMR for strukturoppklaring av tre isomere av karoten fosfolipid vil bli demonstert der acyl- og fosforyl-migrering representerer en kompliserende faktor for tolkning av spekterene.

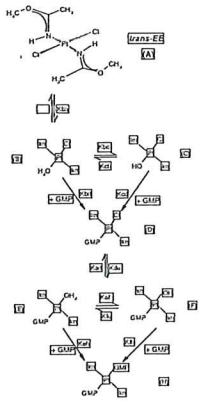
^{1}H ^{15}N NMR Studies on kinetics of the reaction between antitumor platinum complex *trans-EE* and nucleotides

Yangzhong Liu[1], Giovanni Natile[2], and Einar Sletten[1]

[1]Department of Chemistry, University of Bergen, Allegt.41, N-5007 Bergen, Norway.
[2]Department of Pharmaceutical Chemistry, University of Bari, I-70125, Bari Italy.

The recently developed antitumor platinum drug, *trans-EE* has been intensively studied for its significant activities and different binding mode from conventional *cis*-form platinum complexes to the targets DNA. Structural studies of *trans-EE* DNA adducts have been carried out in our group recent years. The kinetics of reaction between *trans-EE* and GMP was studied in this work.

The reactions were monitored on 2D ^{1}H ^{15}N NMQC NMR spectra by using ^{15}N enriched *trans-EE* sample. The following scheme shows the reaction pathway and various products. Each adduct in the scheme displayed a distinct signal on HMQC NMR spectra. Three major products, D, E and H in the scheme were separated by HPLC and studied on NMR previously in reference (Metal-Based Drugs, 2000. 7, 169) in which they were labeled as M1, M2 and M3. Other adducts could not be distinguished on HPLC. The data collected from the integration were processed on SCIENTISTS program and the rate constants were obtained.



Scheme: Reaction pathway. Iminoether ligand was abbreviated as Im.

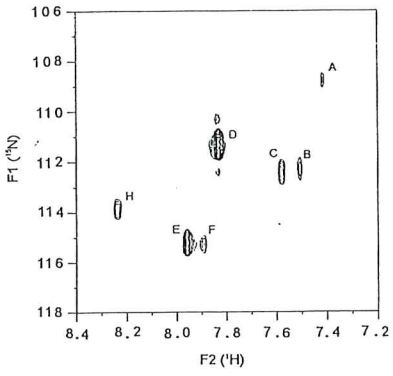


Figure. 2D ^{1}H ^{15}N NMQC NMR spectra from the reaction between 2.2 mM GMP and 0.9 mM ^{15}N *trans-EE* for 7hours at 298 K

Structural studies of the membrane-bound conformations of α-lactalbumin using hydrogen exchange monitored by band-selective, homonuclear decoupeled TOCSY.

[a]Halskau, Ø., [b]Agasøster, A. V., [c]Martínez, A, [d]Frøystein, N. Å.

[a]*University of Bergen, Årstadveien 19, 5009, Bergen, Norway*
[b]*University of Oslo, Blindern, Oslo, Norway*
[c] *University of Bergen, Årstadveien 19, 5009, Bergen, Norway*
[d] *University of Bergen, Allegt. 41, N-5007, Bergen, Norway*

Elucidation of protein structure at atomic resolution is a requirement for the detailed understanding of biochemical processes. Our research is motivated by the need to understand the interaction between water-soluble proteins and cellular membranes. This important class of biochemical activity is involved in cellular communication[1], drug-bacteria interaction[2], the induction of apoptosis[3,4] and the folding of integral membrane proteins[5]. The progress in this area is hampered by experimental difficulties; the slow tumbling of phospholipid vesicle-protein complexes causes loss of all the spectral detail needed for structural elucidation.
We have developed an experimental approach that avoids this problem. The well-characterised milk protein bovine α-lactalbumin (BLA) interacts reversibly with vesicles composed of 1,2- dioleoylphosphatidylglycerol and egg-yolk lechitin (1:1) in a pH controlled reaction. The protein is allowed to exchange in D_2O for incremented amounts of time while bound to vesicles. The labile backbone amide protons will exchange at different rates, depending on the local structural stability and amount of protection from the solvent[6]. These rates can thus be used to gain structural information about the membrane-protein complex. The exchange-rates for this membrane-bound sample are compared to rates derived from a similarily treated set of samples of the native protein.
The amount of resonances in the spectrum of even a small protein, requires the adoption of 2D or multidimensional NMR techniques. The experimental times of these techniques are, however, long compared to the exchange rates of most labile backbone amide protonos. To solve this problem, we used BAnd-Selective Homonuclear Decoupled TOtal Correlation SpectroscopY, which allowed us to select a suitable spectral window containing most of the crosspeaks from the labile backbone amide protons[7]. This reduced acquisition time from about 20 hours to 70 minutes. Additionally, water suppression using the DPFGSE on the Watergate-5 suppression scheme was included[8,9].
We were able to determine the properties and structure of BLA when bound to the vesicles and propose a detailed mechanism for the steps necessary for effecient membrane association. Further research into the role of phospholipid composition on the complexed structure of BLA and the protein's ability to interact indicates that it is possible to stabilise more than one conformer of BLA in a membrane environment. This is supported by the work of Bañuelos and Muga[10], and has relevance for cancer research[3].

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Prof. Lars Skjeldal
IKB, NLH
1430 ÅS

Structure elucidation of Kalata B1.

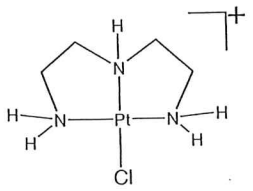
Oldenlandia affinis (DC) is a tropical, perenial plant, which grows across the African continent near equator. It has been used in African folk medicine for long time. In Zaire and in the Central African Republique it has been used to accelerate the uterine contractions during labour, and amongst the Zulus in South-Africa against asthma and heart disease. In Zaire the mothers drink a decoction from the overground parts of the plant during the delivery. They call it "Kalata-kalata", the same name as they use for the plant.
Dr. Lorents Gran brought the plant to Norway and isolated a polypeptide with 29 amino acids in 1972. This polypeptide was named Kalata B1.
Prof. Knut Sletten did the sequence work in 1974, and found it to be a cyclic polypeptide with three disulfide linkages. Many efforts have been done in order to elucidate the disulfide pattern, but in 2001 we suceeded to find it by high field NMR.
The three dimensional structure was solved by use of a 750 MHz instrument, and the structure is deposited in the pdb bank as 1JJZ.

^{1}H chemical shift effects on oligonucleotides upon platinum binding.

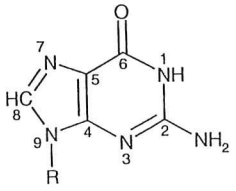
J. Vinje[a], *E. Sletten*[a], *T. Brown*[c], *J. A. Parkinson*[b], *P. J. Sadler*[b].

[a]*Department of Chemistry, University of Bergen, Norway*
[b]*Department of Chemistry, University of Edinburgh, UK*
[c]*Biological and Medical Sciences Building, University of Southampton, UK*

In the analysis of platinum complexes binding to DNA chemical shifts of the protons on the DNA bases are often used to find the platination site, and also in some cases to predict the conformation of the platinated site [1]. We have studied three different DNA oligomers reacting with ^{15}N labeled $[PtCl(dien)]^+$ in H_2O. The base sequence of the oligomers and the numbering of the bases are $[d(T_1A_2T_3G_4G_5T_6A_7C_8C_9A_{10}T_{11}A_{12})]_2$ (I), $[d(T_1A_2T_3G_4G_5A_6T_7C_8C_9A_{10}T_{11}A_{12})]_2$ (II) and $[d(TATGGCCATA)]_2$ (III). The kinetics of the reactions were studied by 2D [^{1}H, ^{15}N] HMQC experiments and showed a specific platination of G4N7 in (I), in (II) both G4N7 and G5N7 were platinated at approximately the same reaction rate and in (III) only G4N7 was platinated. After the reaction sample (I) and (II) were stored for some month, during this time rearrangement reactions occurred, and these products were separated by HPLC and analyzed by 1D ^{1}H, 2D ^{1}H NOESY and 2D [^{1}H, ^{15}N] HMQC experiments. There were two platinated products in both the samples. In the first product the G4H8 signal was shifted downfield by 0.3 ppm and in the second G4H8 was shifted downfield by 0.6 ppm. In both the products the G5H8 signal was not shifted. A much used proof of platination at guanine N7 is a downfield shift of about 0.5 ppm of the guanine H8 on the platinated base [2]. These results therefor indicate that G4N7 is platinated in both products. But after closer inspections of the 2D ^{1}H NOESY maps they showed that in the first product there was crosspeaks between G5H8 and one of the amine groups of the $[PtCl(dien)]^+$ and no crosspeaks between G4H8 and $[PtCl(dien)]^+$. In the second product the situation was opposite, crosspeaks between G4H8 and one of the amine groups of the $[PtCl(dien)]^+$ and no crosspeaks between G5H8 and $[PtCl(dien)]^+$. This shows that G5N7 is platinated in product number one and G4N7 in number two. The exceptionally about this result is that there is a platination of a guanine that don't show any shift of it's base proton.



[PtCl(dien)]$^+$



Guanine

References.
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"NMR-studies of a polyelectrolyte-polyelectrolyte complex in a medical formulation"

Are Kristiansen,
Pronova Biomedical as, Gaustadalleen 21, N-0349 OSLO, akristiansen@pronova.no

A medical formulation, containing the polysaccharides chitosan and heparin, has been studied using basic NMR techniques to evaluate its structure and dynamic behavior. The formulation has the appearance of a paste or a gel, and was studied using both H_2O and D_2O as solvent. The complex displayed ^1H NMR signals that were not observed from the native polymers. Some interesting isotope effects were observed, but these have not been fully understood. Clearly, only a fraction of the protons in the periogel was observed using solution state NMR methodology, *i.e.* the fraction having the most mobility, while protons in the true immobilized gel material were not observed. However, the observable fraction displayed significant signs of interaction, hence it was possible to draw some qualitative conclusions on the nature of the complex. The observable fraction of the gel material has not been estimated or quantified, thereby precluding any quantitative conclusions. Magic angle spinning was employed to reduce magnetic susceptibility induced linebroadening and weak dipolar interactions, however, no significant differences from solution state NMR spectra were observed. The data strongly suggest that the complex is formed by stable electrostatic polyelectrolyte-polyelectrolyte interactions.

"New Aspects of Contrast Agent Development"

Pål Rongved, Amersham Health

ABSTRACT

Contrast agents (CA) play an important role in medical imaging in X-ray, Magnetic Resonance Imaging (MRI) and ultrasound. Even if medical imaging using magnetic resonance principles based on biological hydrogen may give good anatomical and morphological information, it is a consensus in the MR community that CA may gain diagnostic information that is not achievable without them. Enhanced contrast may be used to visualise diseased areas, improving a better choice of therapy. In addition, the pharmacokinetics and dynamics of biodistribution of the agents may gain information about functionality of organs and cells.

The contrast agents approved for human use today are low-molecular, water-soluble extracellular fluid (ECF) agents. There is trend in development of agents with a pharmacokinetic profile that is more favourable for optimal delineation of many types of pathology with alterations in tissue and organ blood volume, e.g. ischemia, infarction and tumour vascularization. Other trends in CA development are targeting based on molecular mechanisms, MR based on other nuclei than ^{1}H and ways of increasing the signal intensity to improve sensitivity.

In addition to a reasonable preparation process and userfriendliness, an agent useful for these indications should (1) have specificity for the target yielding sufficient contrast duration for the imaging procedure, they should (2) have no adverse reactions and (3) have a safe excretion within a reasonable time. These subjects will be more thoroughly discussed in the lecture.

Multivariat analyse -
Anvendelse og nytteverdi på biologiske NMR–data

Tone Frost Bathen, SINTEF Unimed MR, N-7465 Trondheim

Multivariat analyse av biologiske NMR data ble først brukt av Jeremy Nicholson og John Lindon på slutten av 80-tallet. Anvendelsen har økt de siste årene, både innenfor MRS og MRI, men har ofte en ulik rolle for de to typene data. Multivariat analyse av MRI data har gjerne som formål å utvikle teknikker som kan være et hjelpemiddel i tolkning av bilder, for eksempel i gjenkjennelse og beskrivelse av strukturer. Når det gjelder spektroskopi legges det ofte mer vekt på å skille mellom spekter fra ulike klasser av prøver, samt datareduksjon for å gjøre viktig informasjon mer synlig og tolkningen enklere.

Dette innlegget vil ta for seg multivariate analyser av MRS data generelt, gi en kort oversikt av metoder og vise noen eksempler hvor multivariat analyse er anvendt.

MR i mus og rotte

Christian Brekken, Post doc., SINTEF Unimed MR, Trondheim.

Små dyremodeller, spesielt genetisk modifiserte mus, tas i stadig større grad i bruk som verktøy i kreftforskning (prevensjon, diagnostikk, terapi, etc.). Potensialet som ligger i bruken av slike modeller er fortsatt stort. MR avbildning (MRI) og i økende grad spektroskopi (MRS) er teknikker som gir god mulighet for ikke-invasive anatomiske, funksjonelle, biokjemiske, genetiske og/eller farmakologiske studier in vivo. Her presenteres noen aktuelle eksempler på slike studier fra utenlandske forskningsmiljøer og fra MR-senteret i Trondheim.

Proton dekoplet ^{19}F NMR spektroskopi av medikamenter

Oddbjørn Sæther[1, 2], Jostein Krane[1], Øystein Risa[1], Olav Haraldseth[2], Anna Midelfart[1, 2]
[1]Fakultet for naturvitenskap og teknologi, [2]Det medisinske fakultet, NTNU.

Deteksjonsgrensen for fluorforbindelser i en Bruker Avance DBX 100 BioSpec (B_0 = 2.35 T) ble etablert, i den hensikt å undersøke muligheten for *in situ* monitorering av fluorinerte medikamenter i øyet ved hjelp av ^{19}F NMR spektroskopi. En 25 mm overflatespole ble brukt for ^{19}F, og "time-share" proton dekopling ble implementert i en eksperimentell modell basert på et fantomøye. Med vårt oppsett kunne man med en opptakstid på 30 min detektere 0.1-0.2 µmol medikament.

Bruk av NMR spektroskopi til analyse av molekylære forandringer ved ulike sykdommer og skader i øyet

Øystein Risa[1], Oddbjørn Sæther[1, 2], Jostein Krane[1], Anna Midelfart[1, 2]

[1]Fakultet for naturvitenskap og teknologi, [2]Det medisinske fakultet, NTNU.

Høyoppløselig NMR spektroskopi har et stadig større nedslagsfelt innenfor analyse av biologiske væsker og vev. I dette arbeidet brukes først og fremst ^{1}H NMR spektroskopi til undersøkelser av forandringer i den metabolske profilen i ulike deler av øyet ved sykdommer (grå stær), skader (etseskader) og medikamentell behandling (steroider). Særlig vekt er lagt på grå stær som er en av de vanligste øyesykdommene. Årsaken til grå stær er uklar, men blant risikofaktorer finner vi; UV-stråling, røyking, alkohol, diett, alder og noen medisinske preparater. Det er av stor interesse å finne en eller flere biologiske fellesnevnere for sykdomsprofilene slik at bedre forebyggende arbeid kan iverksettes. NMR spektroskopi kan hjelpe oss til å danne et mer helhetlig bilde av hva som skjer med metabolitter i øyet når sykdommen oppstår eller skaden inntreffer. Optimering av gamle og nye NMR metoder er nødvendig for å kunne trekke mest mulig informasjon ut av prøvene. Dette i tillegg til identifisering av de ulike molekyler fra NMR spektra og tolkning av samlede resultat har gitt oss nyttig informasjon rundt biologiske responser i øyet.

KOMBINERTE DIFFUSJONS– OG RELAKSASJONSMÅLINGER FOR KARAKTERISERING AV HETEROGENE SYSTEMER

John Georg Seland, Geir Humborstad Sørland, Henrik Anthonsen og Jostein Krane

Institutt for kjemi, Norges teknisk–naturvitenskaplige Universitet, NTNU
7491 Trondheim

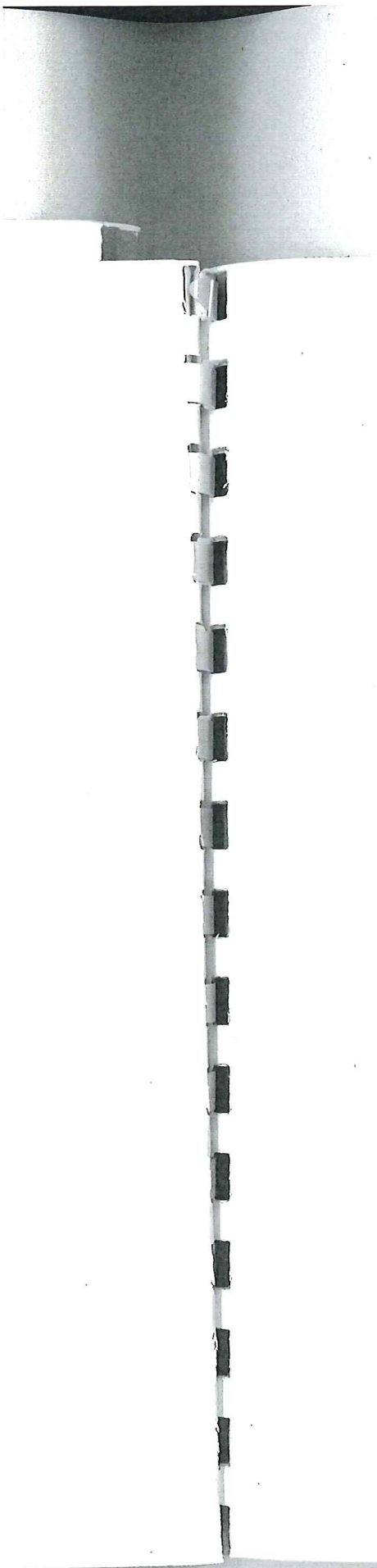
I heterogene systemer kan interne magnetiske felt gradienter føre til forbredning av linjebredden, og dermed gjøre det vanskelig å spektralt oppløse de ulike komponentene. Ved analyse av relaksasjonskurver fra heterogene systemer, kan derfor informasjon både angående mobilitet til de ulike komponentene, og hva slags omgivelser komponentene befinner seg i, være vanskelig tilgjengelig.

Når såkalte Pulsed Field Gradient (PFG) eksperimenter kombineres med relaksasjonseksperimenter i systemer med komponenter av ulik mobilitet, er det mulig å trekke ut mer informasjon sammenlignet med hva som er tilgjengelig i et ordinært relaksasjonseksperiment.

Puls sekvenser som gjør det mulig å separat måle transvers relaksasjon for hver av komponentene i heterogene multikomponent systemer blir presentert.

Eksperimenter som er utført i modellsystemer av olje og vann i tettpakkede glasskuler viser at det er mulig å separere signalene fra olje og vann på grunnlag av deres ulikhet i mobilitet. T_2–attenuasjonene for olje og vann kan dermed bestemmes hver for seg. Dette gjør det blant annet mulig å lettere bestemme fuktegenskapene til et slikt system.

Metodene kan anvendes på alle typer systemer som har komponenter som varierer tilstrekkelig i mobilitet, slik som olje og vann i porøs stein, og fett og vann i biologiske systemer.



POSTER

Structural basis for protein-ligand recognition. ^{1}H NMR study on the binding of the cofactor tetrahydrobiopterin with aromatic amino acid hydroxylases and with nitric oxide synthase .

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(6R)-L-*erythro*-5,6,7,8-tetrahydrobiopterin (BH_4) is the cofactor for the enzymatic hydroxylation of the aromatic amino acids: phenylalanine, tyrosine, and tryptophan, catalyzed by the nonheme iron-dependent aromatic amino acid hydroxylases, using dioxygen as additional substrate. This superfamily includes phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), which catalyzes the catabolism of L-phenylalanine, the synthesis of catecholamines and the synthesis of serotonin, respectively. BH_4 also plays the cofactor role in nitric oxide synthases (NOSs). Nitric oxide synthases are cytochrome P_{450}-like heme enzymes that utilize BH_4, FMN, and FAD as cofactors to catalyze the NADPH-dependent oxidation of L-arginine to citrulline and nitric oxide. In the present study, a more detailed structural information for complexes formed with each of these enzymes and BH_4, has been attempted to be solved by ^{1}H NMR spectroscopy and molecular modeling techniques. Binding of the pterin cofactor was monitored by observation of magnetization transfer between protons of the protein and the ligand due to magnetic dipole-dipole interactions (transfer nuclear Overhauser effect, TRNOE). Distances of BH_4 protons to the iron in the hydroxylases were estimated by the paramagnetic probe-T_1 method. Intramolecular proton-proton NOEs between protons on the ligand in free and bound states have been measured to provide distance constraints, and these have been used in distance geometry calculations and docking of the cofactor into its binding site in the enzymes. Similar to BH_4 in solution at neutral pH, the two hydroxyl groups of the side chain at C6 seem to adopt a *cis* conformation when bound to the three hydroxylases, but re *trans* when bound to NOS. Moreover, evidence has been obtained for the observed differences in conformation when BH_4 is free in aqueous solution and when bound to the enzymes. These differences in conformation seem to be related to the differences in primary sequences at the pterin binding sites. Thus, the specific conformations may reveal the differential regulation elicited by the cofactor.

Acknowledgments. The work was supported by the Research Council of Norway and L. Meltzer høyskolefond.

Measuring fat and water in minced beef meat

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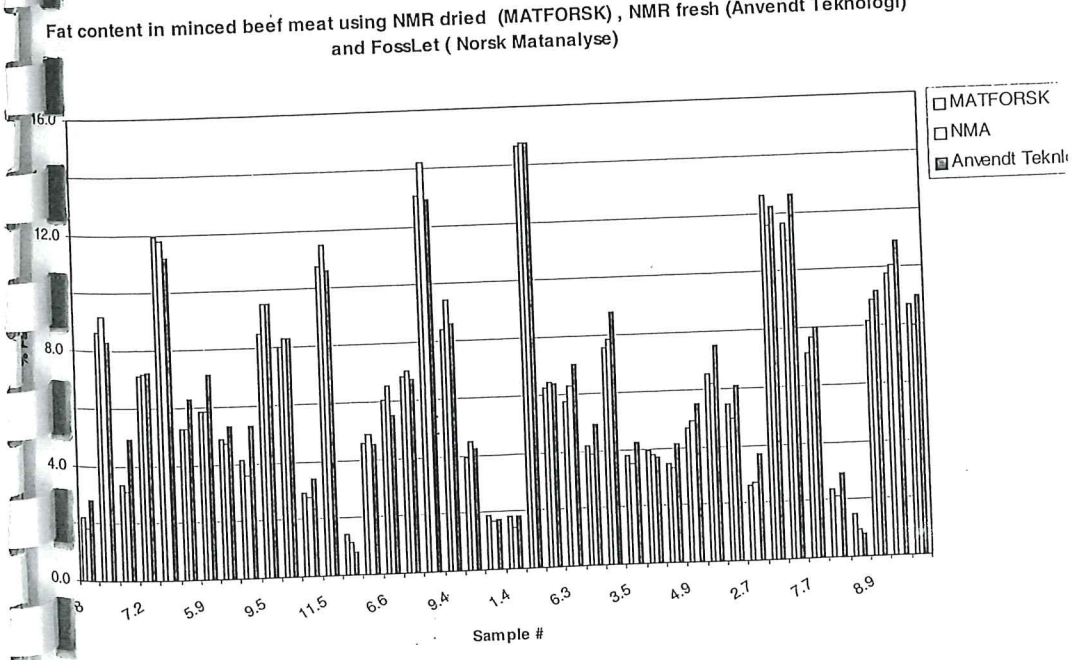
The use of low field NMR is a fast and accurate alternative to the use of solvent extraction and drying, to determine the content of fat and water in a biological system.

On 3 sets, each containing 42 samples of minced beef meat where the fat content varies from less than 1 % and up to 14 %, the fat content has been measured either by NMR on fresh tissue, NMR on dried tissue, or using solvent extraction (Foss-let). Comparison of the 3 methods for determination of the fat content shows satisfactorily agreement between the different methods.



Anthocyanins from tart cherries

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Department of Chemistry, University of Bergen, Allégt. 41, 5007 Bergen

Four anthocyanins were isolated from acidified, methanolic extract of tart cherries (*Prunus cerasus*) by partition against ethyl acetate followed by column chromatography using Amberlite XAD-7 and Sephadex LH-20 material. Two of the structures were elucidated by one- and two-dimensional NMR spectroscopy to be the 3-*O*-(2"-*O*-β-glucopyranosyl-6"-*O*-α-rhamnopyranosyl-β-glucopyranoside) and 3-*O*-(2"-*O*-β-glucopyranosyl-β-glucopyranoside) of cyanidin. The structure of the other two anthocyanins were determined by on-line UV-Vis spectroscopy and co-chromatography with authentic anthocyanins to be the 3-*O*-glucoside and 3-*O*-(6"-*O*-rhamnosylglucoside) of cyanidin.

Anthocyanins from blue flowers of *Salvia farinacea*

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Anthocyanins have been isolated from the acidified methanolic extract of blue flowers of *Salvia farinacea* by successive use of different chromatographic methods. The complete structure of the two major anthocyanins were identified by a combination of 1D and 2D NMR techniques and electrospray MS to be the 3-*O*-[6-*O*-(*p*-coumaroyl)-β-glucopyranoside]-5-*O*-β-glucopyranosides of malvidin and delphinidin. Among the minor pigments, three anthocyanins including the novel pigment, malvidin 3-*O*-[6-*O*-(caffeoyl)-glucoside]-5-*O*-β-glucoside, were tentatively identified.

Diffusion studies of materials confined in porous crystallites

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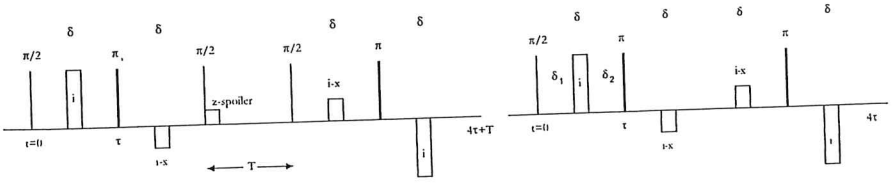
The study of mass transport and diffusion in confined geometry is particularly important when developing materials for practical applications such as catalysts, selective adsorbents or membranes. The possibility of interpreting and correlating molecular mass transport phenomena in solids depends critically on knowledge of the surface structure and regularity. NMR in particular, has become an increasingly important method for investigating molecular motion and transport in porous solids.

When measuring diffusion in heterogeneous media it has become evident that a bipolar form of the pulsed field gradient sequence is a powerful method for reducing the cross term between the applied and internal magnetic field gradient and in reducing the eddy current dead time [1]. The measured diffusivities might be underestimated without the use of a bipolar sequence, and errors due to eddy current field are likely to occur.

If we are studying systems where porous grains or crystallites are of the same order of magnitude as the distance traveled by the molecules during the pulse sequence, it is important to keep the diffusion time short. The distance traveled by the molecules during the pulse sequence will be reduced with shorter diffusion times. Depending on the crystallite size it will then be possible to extract true intracrystallite diffusion coefficients instead of a diffusion coefficient that is significantly affected by restrictions at the crystallite surfaces.

In this work we have used two different pulse sequences:



a 13-interval and a 11-interval sequence [2], respectively. Two different porous systems have been investigated; ethane confined in H-ZSM-5 crystallites (mean crystallite diameter larger than 20 μm) and ethane or cyclohexane confined in MCM-41 crystallites (mean crystallite diameter larger than 14 μm). The true intra-crystalline diffusivity was obtained by using the short diffusion time model [3] and extrapolating to zero observation time. A high diffusion rate was observable over a wide temperature region.

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Three-dimensional NMR Studies of ^{13}C / ^{15}N Labelled Human Ubiquitin

Halvard Hårklau, Henrik W. Anthonsen og Jostein Krane
Institutt for Kjemi, NTNU

We have employed several three-dimensional NMR experiments for the study of a sample of ^{13}C / ^{15}N double labelled human ubiquitin.

Among the different experiments we have applied double resonance ^{1}H / ^{15}N experiments like 3D-NOESY-HSQC and 3D-TOCSY-HSQC. Triple resonance ^{1}H / ^{13}C / ^{15}N experiments as HNCA, HN(CO)CA and HNCO have also been used.

UVB radiation effects on the metabolites, diffusion of water and the T_1 and T_2 relaxation times in rat lens

Ø. Risa, H.W. Anthonsen, J.G. Seland, J. Krane, A. Midelfart
Medisin, NTNU

UV-irradiation may damage the lens by several mechanisms such as protein cross-linking, DNA damage, membrane damage which in the end may lead to cataract. In attempt to better understand the mechanisms of UV-cataract formation, different NMR spectroscopy methods has been used to study the metabolic profile and physical properties in the lens. By using HRMAS ^{1}H NMR spectroscopy on intact rat lenses we observed significant changes in concentration for several metabolites after developing UV-induced cataract. Additional physical information was provided by measuring the water diffusion coefficient and the T_1 and T_2 relaxation times which all are parameters that can be indicators of molecular organisation in the lens. These results may give valuable knowledge to the correlation between the metabolic changes and the physical alterations caused by UV-irradiation in the lens.

VNMRF

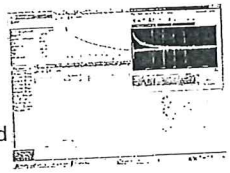
Utvikling av en virtuell kjernemagnetisk resonans fasilitet

Henrik W. Anthonsen, Geir H. Sørland,
John G. Seland og Jostein Krane
Institutt for Kjemi, NTNU

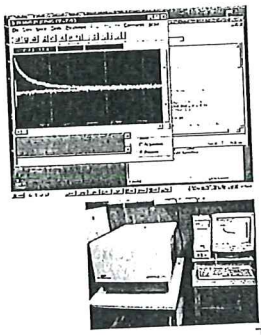
STATOIL
Frank Antonsen og Håkon Rueslåtten
Statoil, Trondheim

VNMRF - et system for fjernstyrt samarbeid

-Et sentralt NMR instrument - flere eksterne brukere
-Dele skjermbilde / NMR instrument
-Audiovisuell kontakt
-Dele notater i en elektronisk notatbok



Harstad



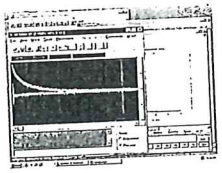
Trondheim

Hvorfor en VNMRF?

-Dyre instrumenter
-Folk lever på ulike steder
-Økt samarbeid med andre grupper
-Kontrollere status på et eksperiment

Teknisk implementering

-Ikke avhengig av operativsystem
-Kan brukes over et vanlig modem
-Vi kan tilpasse systemet til ulike dataformater



Stavanger

Vårt system er basert på et konsept utviklet av "Environmental Molecular Sciences Laboratory" (EMSL) i USA. Dette opplegget er godt utprøvd i en rekke ulike prosjekter.

Characterization of Mesoporous Materials by ^{1}H NMR

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The last decade, several workers have investigated the freezing/melting point depression of water confined in porous media by monitoring the ^{1}H NMR intensity of the non-frozen liquid as a function of temperature.[1-3] Three investigations, employing cyclohexane and benzene as probe molecules, have also been reported.[2-4]

In this work, we have used acetonitrile to investigate the pore size distribution of six different mesoporous silica materials with nominal diameters ranging from 40 to 300 Å.[5] Acetonitrile melts at 227 K and undergoes a solid-solid phase transition at 217 K. However, no structural information on acetonitrile appears to be available.

The liquid and solid components of the adsorbate were distinguished, on the basis of the spin-spin relaxation time T_2, by employing a simple Carr-Purcell spin-echo sequence. NMR gives a more detailed picture of the pore size distribution than N_2 sorption measurements revealing three well-defined peaks. However, the peak at the lower pore size, which reflects the surface layer, can be removed by increasing the pulse spacing in the spin-echo experiment.

The geometrical restrictions and surfaces have a large influence on both the phase behaviour and the dynamics of the confined molecules. We have, therefore, investigated the NMR line-shapes and dynamics of acetonitrile confined within 60 and 200 Å pores and discussed the results with reference to the bulk material. The confined samples clearly reveal two components giving rise to a narrow line superimposed on a broad complex line. The two components are attributed to the liquid-like surface layer, and the solid at the interior of the pore. The relaxation times and the diffusivity of the confined samples are significantly reduced in the liquid state owing to more restricted molecular motions. However, whereas surface interactions appear to play an important role in slowing down the translational and tumbling motions, the rotation of the methyl group seems to be little affected.

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Mg(II)-mediated Binding of Ciprofloxacin to a DNA-oligomer: NMR and Molecular Docking Results

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Ciprofloxacin is a typical second-generation fluoroquinolone, a dominant class of antibacterial agents. The fluoroquinolones act by inhibiting the activity of DNA Gyrase, an enzyme essential to all bacteria. The mechanism is still not fully understood, but it is generally accepted that fluoroquinolone, DNA and DNA Gyrase form a ternary complex[1]. There have also been reports that Mg(II) might play a regulatory role for this complex formation[2]. Therefore the interaction between ciprofloxacin and a DNA decamer both in the presence and absence of Mg(II) was studied by high-field NMR (600 MHz) spectroscopy.

In the absence of Mg(II), the drug bound mainly in the major groove. Intercalation, as proposed by Palú[3] et. al., was excluded as there were no detectable distortion of the nucleic acid bases. 2D NOESY spectra showed strong crosspeaks between protons on the piperazine ring of ciprofloxacin and the anomeric proton on the sugar moiety of the nucleotides, indicate a shift to minor groove binding upon addition of 2 mM Mg(II). Iterated Docking results show indeed that the minor groove has the lowest-energy conformation.

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DNA binding of NUCKS.

Lars Skjeldal, Henrik Anthonsen, , Tormod Skauge, and Anne Carine Østvold.

Use of NMR in detection of ^{13}CO turnover in CO-Dehydrogenase from Rhodospirillum rubrum

Lars Skjeldal, Jongyun Heo, Christopher R. Staples and P.W. Ludden

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