THE
NMR
NEWSLETTER

No. 499
April 2000

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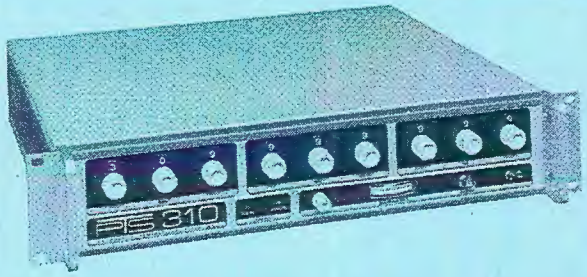

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FORTHCOMING NMR MEETINGS

Gordon Research Conference on Magnetic Resonance, **June 17-22, 2001**, Roger Williams University, Bristol, Rhode Island (note the new, improved location !!!). Contacts: Rob Tycko, Chair, 301-402-8272, tycko@helix.nih.gov, and Kurt Zilm, Vice-Chair, kurt.zilm@yale.edu. Site description and application information available at http://www.grc.uri.edu.

15th European Experimental NMR Conference, Leipzig, Germany, **June, 2000**. For information, see http://eenc.uni-leipzig.de.

XEMAT 2000, a Conference on "Optical Polarization and Xenon NMR of Materials", Sestri Levante, Italy, **June 28-30, 2000**. For information, see http://www.mater.unimib.it/xemat2000/

NMR Course: Part 1 - NMR-based Metabonomics; Part 2 - Hyphenated Spectroscopic Techniques, Imperial College, London, England, **July 10-14, 2000**; Contact: Hersha Mistry,Centre for Continuing Education, Imperial College, 526 Sherfield Building, Exhibition Road. London, SW7 2AZ, UK. Tel: +44 (0)20 7594 6884; Fax: +44 (0)20 7594 6883; Email: h.mistry@ic.ac.uk; Website: http://www.ad.ic.ac.uk/cpd/nmr.htm

Royal Society of Chemistry: 15th International Meeting on NMR Spectroscpy, Durham, England, **week of July 8-13, 2001**; Contact: Mrs. Paula Whelan, The Royal Society of Chemistry, Burlington House, London W1V 0BN, England; +44 0171 440 3316; Email: conferences@rsc.org\

SMASH-2000, Argonne, IL, **July 16-19, 2000**. Contact: G. E. Martin (gary.e.martin@amu.pnu.com). See Newsletter 493, 21.

42nd Rocky Mountain Conference on Analytical Chemistry, Omni Interlocken Resort, Broomfield, CO, **July 31 – August 3, 2000**. NMR Symposium Chair: Lucio Frydman, Univ. of Illinois at Chicago, Dept. of Chemistry (M/C 111) 845 West Taylor St., Room 4500, Chicago, IL 60607-7061; 312-413-1053; Fax: 312-996-0431; lucio@samson.chem.uic.edu

continued on inside back cover

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March 16, 2000
(received 3/24/2000)

Prof. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA
94303

Dear Barry,

^{2}H NMR Detection of Solid [3α-$^2H_1$]cholesterol

Our recent solid state ^{2}H NMR studies of cholesterol molecular organization in membranes prepared from [3α-$^2H_1$]cholesterol and 1,2-diarachidonylphosphatidylcholine (20:4-20:4PC) reveal substantially different behavior of the cholesterol molecule in comparison to saturated phosphatidylcholines. The spectrum shown in figure 1a is the ^{2}H NMR powder pattern characteristic of rapidly reorienting [3α-$^2H_1$]cholesterol with its molecular axis nearly parallel to the bilayer normal. After a large number of acquisitions (100,000) the poor signal to noise indicates very little of the [3α-$^2H_1$]cholesterol is being detected, however. The work of others demonstrated that the presence of solid [3α-$^2H_1$]cholesterol would go unnoticed in experiments designed to detect membrane-intercalated [3α-$^2H_1$]cholesterol[1,2]. The delay time between pulse sequences, τ_r = 15s in fig. 1b allows ample relaxation of the solid [3α-$^2H_1$]cholesterol signal (T_1 = 3s) in addition to that from membrane-intercalated [3α-$^2H_1$]cholesterol. No component for solid [3α-$^2H_1$]cholesterol is discernable from the spectrum in fig. 1a where τ_r = 75ms. On the basis of relative integrated intensities, the estimated membrane intercalation of [3α-$^2H_1$]cholesterol is only 16.7 mol% in the polyunsaturated membrane which is in stark contrast to > 50mol% for saturated and mono-unsaturated membranes[3].

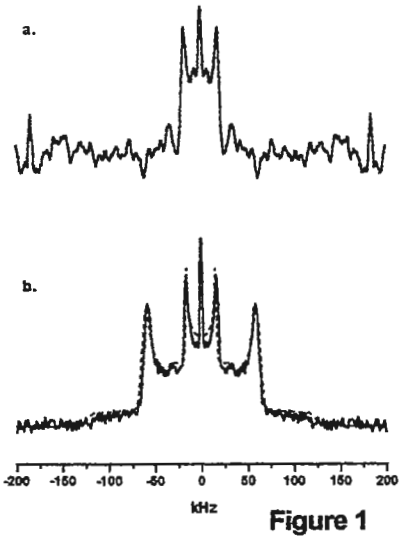

Figure 1

1. Monck,M.A., *et al.* (1993) *Biochemistry* 32:3081-3088

2. Ruocco,M.J. *et al.* (1996) *Biophys.J.* 71: 1776-1788

3. Brzustowicz,M.R. *et al.* (1999) *FEBS Lett.* 451: 197-202

Sincerely,

Michael R. Brzustowicz

Stephen R. Wassall

Please credit this contribution to the account of B.D. Nageswara Rao.

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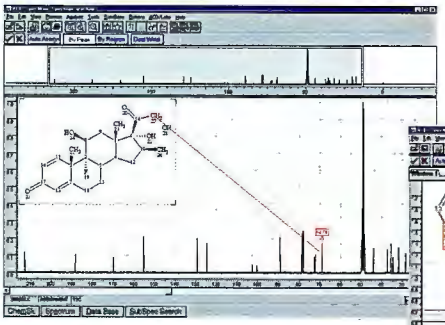

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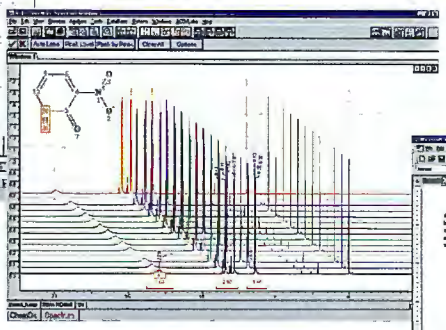

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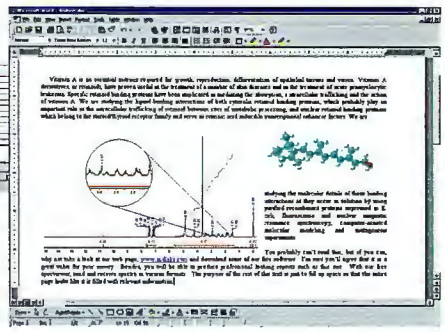

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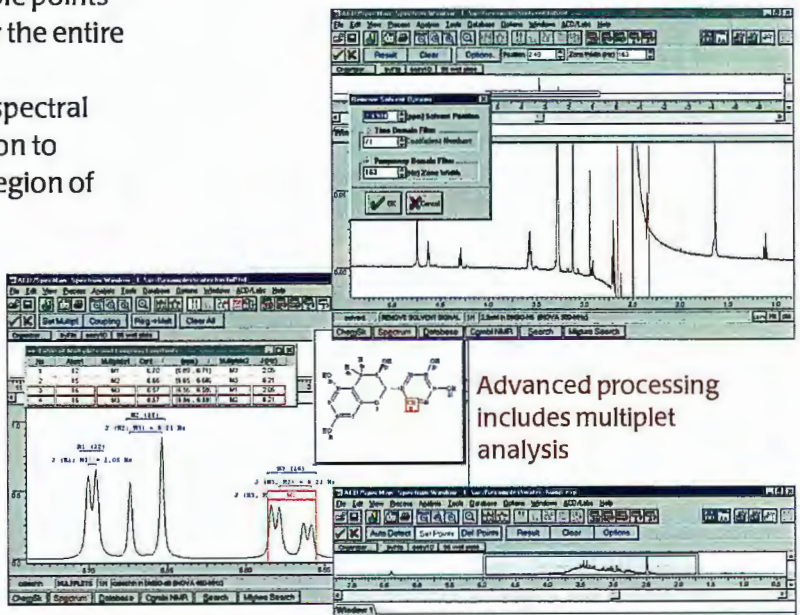

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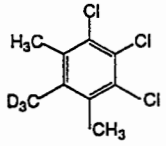
13 March 2000
(received 3/20/2000)

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA
USA 94303

NMR Crystallography – Quantifying Disorder in Molecular Crystals

Dear Barry,

Solid-state NMR spectroscopy has established itself as an important complementary method to that of X-ray diffraction for studying solid structures. Here we wish to address the problem of quantifying orientation disorder in molecular crystals, something that X-ray crystallography cannot always do.



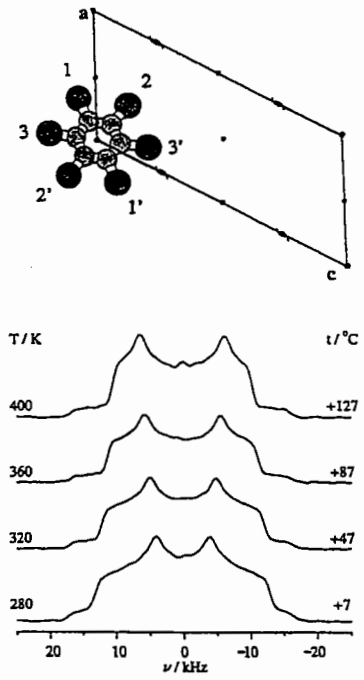


Fig. 1 Top: view of TCTMB unit cell down b axis; bottom: temperature evolution of powder spectra within the monoclinic phase.

As a specific example we consider trichloro-trimethyl-benzene (TCTMB), which at room temperature crystallizes in a monoclinic structure, with two types of symmetry related molecules per unit cell. X-ray measurements [1] suggest complete orientation disorder of this phase, i.e. the methyl and chlorine groups have equal probabilities to occupy the six corners of the benzene core – they are X-ray indistinguishable.

The deuterium NMR spectrum of a powder sample of TCTMB, specifically deuterated in the central methyl groups (TCTMB-d_3), at several temperatures, is shown in Fig. 1. It exhibits a typical line shape of a quadrupolar tensor with a full span of ~38kHz and a finite asymmetry parameter,

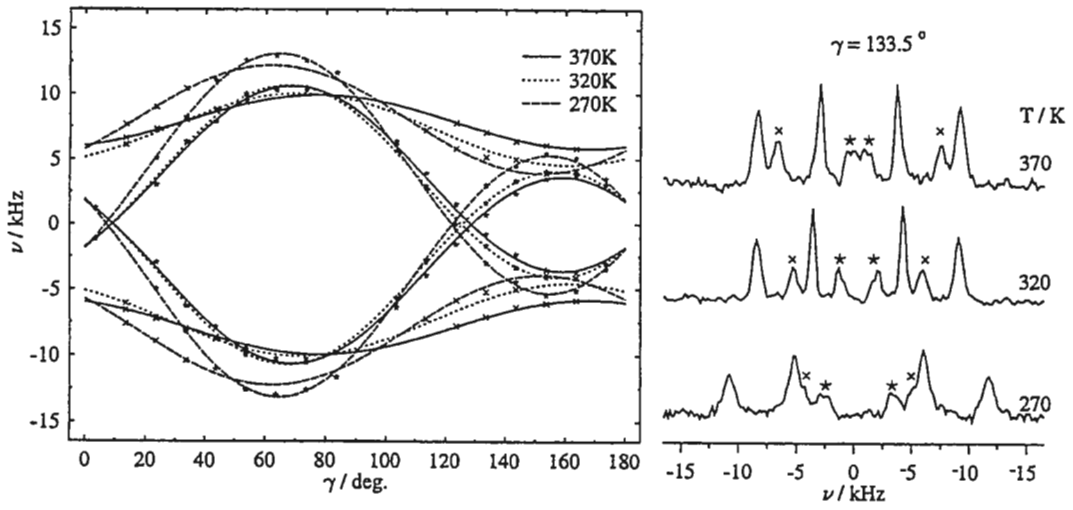


Fig. 2 Right: Spectra of a twin crystal of TCTMB-d3 at the indicated temperatures. Left: Full rotation pattern of the labelled doublets for the three indicated temperatures.

whose value decreases with increasing temperature. This implies that: (i) There is fast spinning of the methyl groups, as well as fast planar reorientation (on the NMR time scale) of the whole molecules. (ii) The dynamic disorder is not complete, i.e. not radially symmetric, as suggested by the X-ray results. Rather, the different orientations, labeled 1, 2 and 3 shown in the projection of the unit cell at the top of Fig. 1, are differently populated and their occupation numbers are temperature dependent. (Note that due to the site inversion symmetry the populations along the direction pairs 1 1', 2 2' and 3 3', are equal). As it turns out it is not possible to derive the population of the three sites from the powder spectrum alone. To do so a full set of single crystal rotation spectra are needed.

In Fig. 2 are shown examples of spectra of a twinned TCTMB-d_3 crystal, at a particular orientation of the magnetic field, but at different temperatures. Two (monoclinically related) strong and two weak doublets are observed due, respectively, to the larger and smaller twins. It may be appreciated that the position of the lines is indeed temperature dependent. The rotation patterns for the labelled pairs at the three indicated temperatures is plotted on the left hand side of the figure. Analysis of these rotation patterns yields the average quadrupole coupling tensor, first in the Standard Orthogonal System (SOS) and then, by a proper transformation, in a Molecular Fixed Frame (MF). The MF was chosen so that the direction labeled 1 corresponds to the x-axis and the normal to the molecular plane to the z-axis.

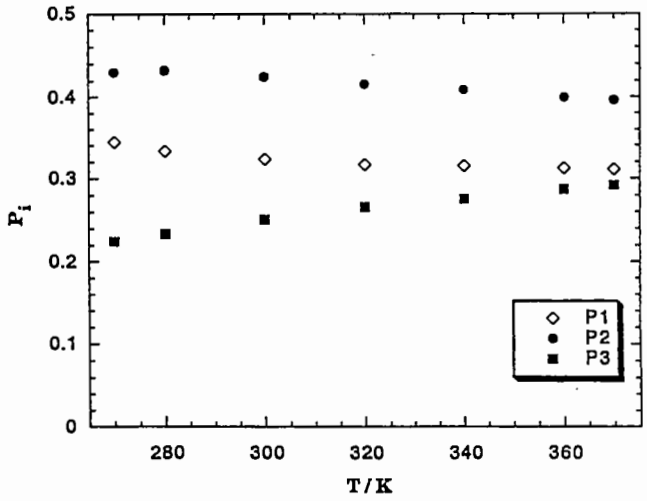


Fig. 3 Site populations p_i in the monoclinic phase of TCTMB as function of the temperature.

In this frame the elements $\langle Q_{xz} \rangle$ and $\langle Q_{yz} \rangle$ of the experimental quadrupole tensor vanish, and the full tensor can be expressed as the following sum of the local tensors:

$$\langle \mathbf{Q} \rangle = p_1\mathbf{Q}_1 + p_2\mathbf{Q}_2 + p_3\mathbf{Q}_3$$

where the p_i's are the fractional populations (of sites i and i') and the $\mathbf{Q}_i$'s are the corresponding quadrupole tensors, all expressed in the same frame (MF). Since the latter are known the p_i's can be calculated. These are plotted, as function of the temperature in Fig. 3.

The results show that the disorder in TCTMB is far from complete and demonstrate the power of single crystal NMR spectroscopy as a complement to the X-ray method.

Sincerely,

Thomas Bräuniger Raphy Poupko Zeev Luz

[1] R. Fourme, M. Renaud, and D. Andre, *Mol. Cryst. Liq. Cryst.* **17**, 209 (1972)

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Dr. BL Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

February 23, 2000
(received 3/2/2000)

Unidentified frightful object (UFO) in mouse microimaging.

Dear Professor Shapiro,

For several months we are dealing with micro imaging on mice. In spite of a relatively low sensitivity and provided no kinetic studies have to be achieved, we found that it was possible to get fair images at 2 Tesla using 50mT/m gradients. In *Figure 1*, one may observe slices obtained with a multislice gradient echo sequence with 1,5 mm thick, 256x128 matrix,
$T_E = 7$ ms, $T_R = 90$ ms; total imaging time : 11s. The given scale is in millimeter.

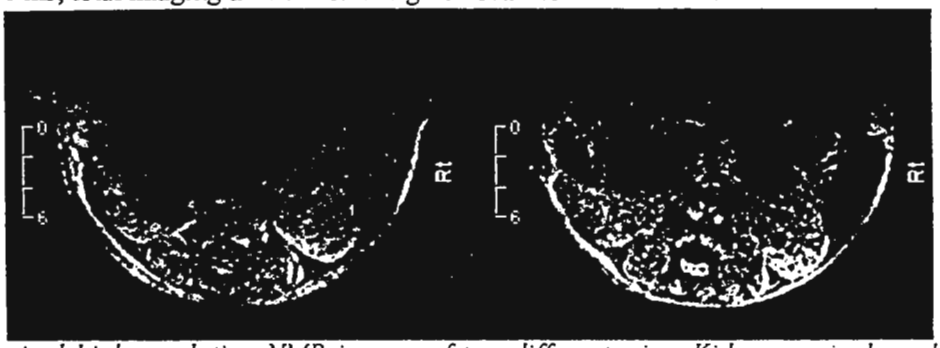

Figure 1 : Abdominal high resolution NMR images of two different mice. Kidneys, spinal cord, dorsal muscles are clearly delineated. UFO appears on the left.

Surprisingly, some mice present a (relatively!) huge black hole inside the abdomen *(Figure 1-left)* – Apparently the cavity should be a dilated stomach. Nevertheless it appears rather strange – and certainly frightful – in this study, this phenomena occurs only on 2 mice in a group of 23. Generally black holes are smaller since they are caused by bowel gases. *(Figure 1 – right)*. We do not know the reasons of this anatomical feature [some of us suggest that it is due to spinachs ingestion, iron rich food which can create artefacts in MRI.]
The experiments reported here were done in accordance with our institution rules for animal models investigations.

Yours sincerely,

André BRIGUET

Linda CHAABANE

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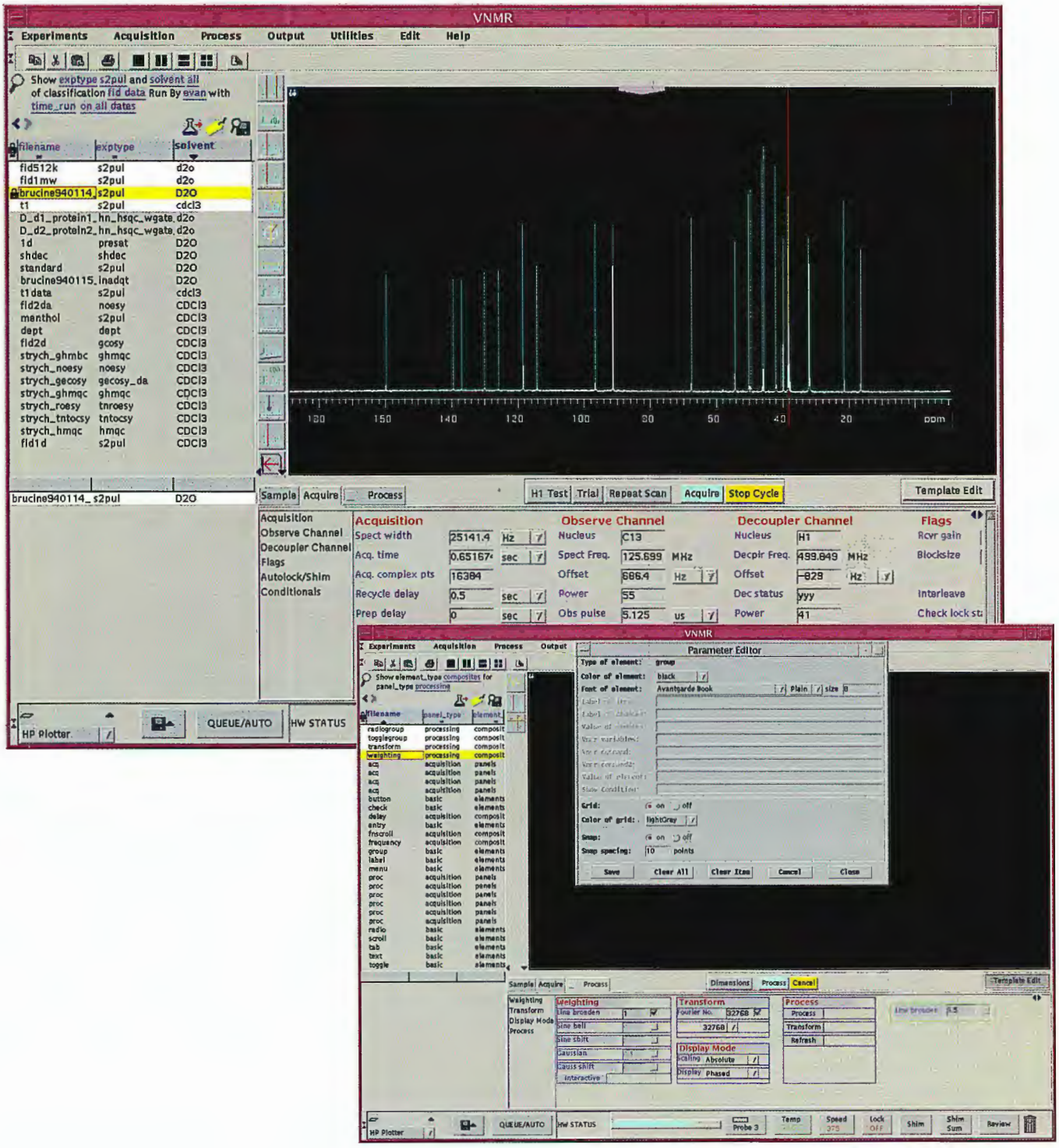

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(received 3/24/2000)

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Observing Dipolar Couplings using Double Quantum Spectra.

Dear Barry,

I've recently stared using dilute liquid crystal solutions to partially orient proteins in the magnetic field. As pointed out in a paper from Professor Art Pardi's group, homonuclear dipolar couplings can be used to transfer magnetization in almost exactly the same way as J-couplings. They suggested using an experiment analogous to TOCSY, but the double-quantum experiment should also be useful for observing homonuclear dipolar couplings. This is because:

i) The mixing time can be tuned to maximise the signals from couplings of a specific value

ii) There are no axial peaks, so dipolar couplings can be observed between protons with very similar chemical shifts.

Figure 1 shows part of a homonuclear double-quantum spectrum of a 90-residue protein. The protein was 0.25mM in a 2.3% CetylPyridinium Chloride / Hexanol / Sodium Bromide lamellar phase giving a D_2O splitting of 10Hz. The mixing time was 30ms and the water signal was suppressed using WATERGATE. The experiment was recorded for 30 hours at 35°C on a Varian Inova 500 Spectrometer.

At the top of the figure are amide-Hα correlations due to J-couplings, but at the bottom of the figure three amide-amide correlations can be observed. These are through-space correlations due to dipolar couplings between the amide protons.

A further advantage of the double quantum experiment is that the mixing time can be made selective. Couplings will not evolve between pairs of protons where only one is inverted by the 180° pulse during the mixing period. If the 180° pulse is made selective for a set of protons, then only couplings within that set will be active. This maximises the amount of signal contributing to the couplings of interest. Figure 2 illustrates this selective approach. The experiment was recorded in exactly the same conditions as figure 1, except that the mixing period included an excitation sculpting element of two 10ms WURST-2 pulses to selectively invert the amide and aromatic protons. In the resulting spectrum, only correlations between amide or aromatic protons are observed. The improved sensitivity of this version allows observation of two couplings which were below the noise in the non-selective experiment.

The sensitivity of the experiment is disappointing, at least to me, but it may be of greater use with sharper signals such as methionine methyls. At higher signal-to-noise ratios it should be possible to calculate values of the couplings by comparing the antiphase lineshape in this experiment with in-phase lineshapes from other experiments such as NOESY (the MOSAIC or 'Titman-Keeler' method).

I'd like to thank Stuart Aitken here at Zeneca for preparing the protein used in this work, and Professor Jens Jørgen Led of Copenhagen University where I started looking at these methods. Please credit this to Zeneca's account.

Yours,

Peter Howe.

Peter Howe.

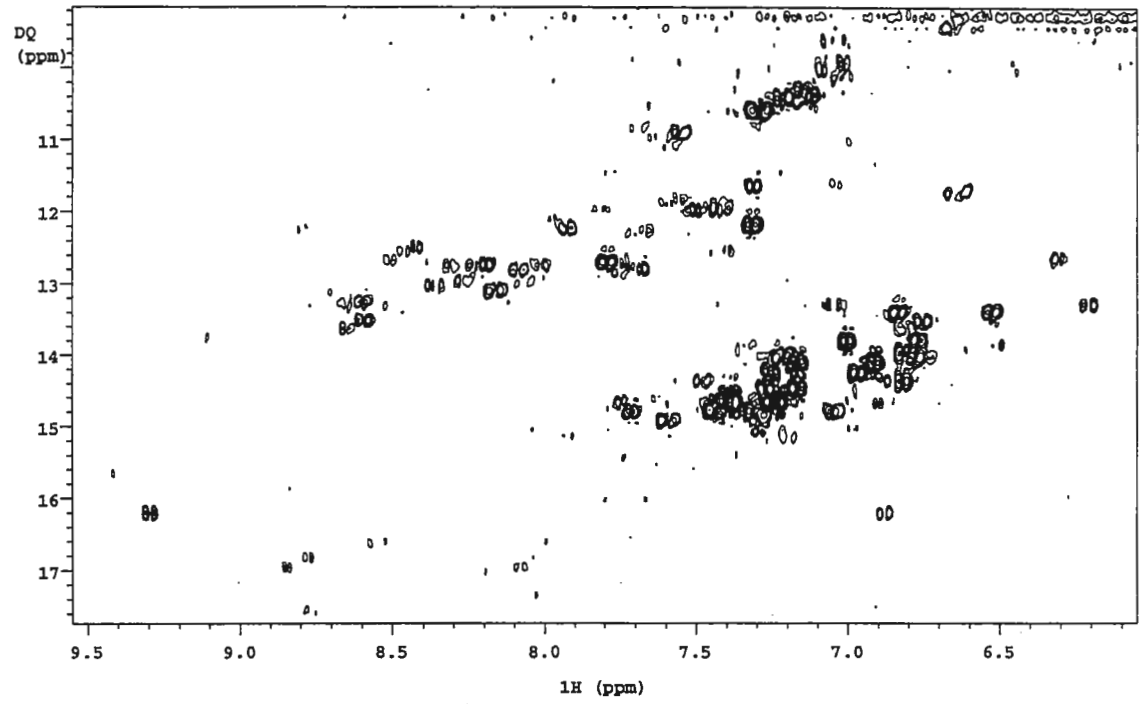

Figure 1 DQ Spectrum.

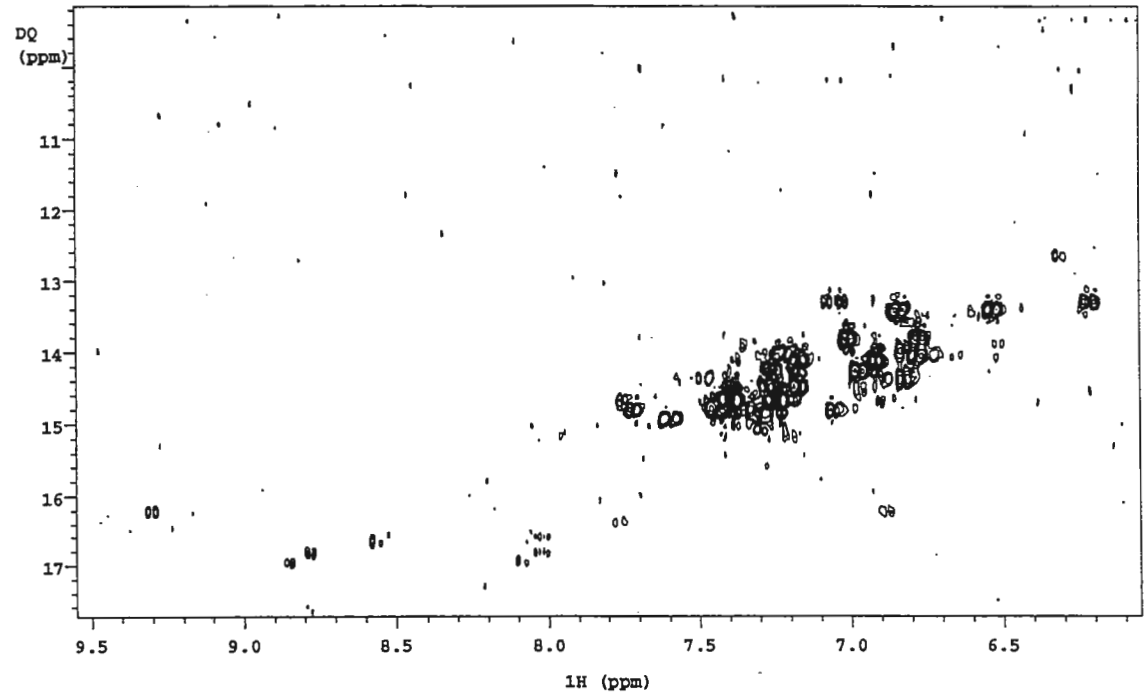

Figure 2 Selective DQ spectrum.

UNIVERSITY OF ALBERTA

Dr. B.L. Shapiro
966 Elsinore Court
Palo Alto, CA 94303
USA

21 March 2000
(received 3/22/2000)

Rapid Analysis of Weak Protein Dimerization from Backbone Amide ^{15}N-T_2's

Dear Barry,

To a first approximation, the transverse relaxation of a backbone amide ^{15}N is given by:

$$\frac{1}{T_2} \approx \frac{D_{NH}}{2}\left[4J(0)\right] \times S^2\tau_c + \frac{4}{15}C\left[J(0)\right] \times S^2\tau_c$$

for an isotropically tumbling protein, where $1/T_2$ is in units of s^{-1}, the correlation time (τ_c) is given in seconds, and S^2 is the Lipari-Szabo order parameter, D_{NH}=1.3 x 10^9 (rad/s)^2, C=0.9 x 10^9 (rad/s)^2 at a ^{1}H frequency of 500 MHz. For well-defined regions in proteins, S^2 ~ 0.85 at about 30 °C, and we get:

$$\frac{1}{T_2} = 1.11\tau_c \qquad [1]$$

where τ_c is given in nanoseconds to give $1/T_2$ in s^{-1}. For a protein that dimerizes weakly, exchange between monomer and dimer is rapid, and observed T_2's will be a weighted average between monomer and dimer. Minimizing the fraction of dimer in solution is extremely desirable from the standpoint of T_2 relaxation. We test for dimerization based on the τ_c sensitivity of T_2. For weak dimerization, the following equilibrium applies:

$$[\mathrm{M}][\mathrm{M}] = [\mathrm{D}] \times K_D \qquad \text{and} \qquad [\mathrm{M}] + 2[\mathrm{D}] = \mathrm{M}_0 \qquad [2]$$

where M is monomer, D is dimer, K_D is the dimer dissociation constant, and M_0 is the total protein concentration. From equations 2 we find for the fraction monomer:

$$f_M = \frac{\left(-K_D - \sqrt{K_D} \times \sqrt{K_D + 8\mathrm{M}_0}\right)}{4\mathrm{M}_0} \qquad [3]$$

For a τ_c that is a weighted average between monomer and dimer, we have:

$$\tau_c = \tau_{c,M}\left(f_M + 2\left(1 - f_M\right)\right) \qquad [4]$$

where $\tau_{c,M}$ is the correlation time of monomer. For K_D ~ 1-2 mM, and M_0 varying in the μM-mM range, the fraction monomer (f_M), and therefore τ_c, is dependent on the total protein concentration. Thus, measurement of T_2 as a function of total protein can allow an estimate of K_D and $\tau_{c,M}$. The average T_2 for a protein can be rapidly estimated from the integrated intensity of the ^1H_N envelope of a one-dimensional {^{1}H-^{15}N}-HSQC correlation with a CPMG pulse train for measurement of ^{15}N T_2. Figure 1A shows ^{15}N-T_2 values for the regulatory domains of skeletal and cardiac Troponin C (s- and cNTnC) that depend on protein concentration, with $1Ca^{2+}$-cNTnC showing longer T_2 values at higher protein concentrations than $2Ca^{2+}$-sNTnC, note that dimerization is more severe for $2Ca^{2+}$-sNTnC than $1Ca^{2+}$-cNTnC.

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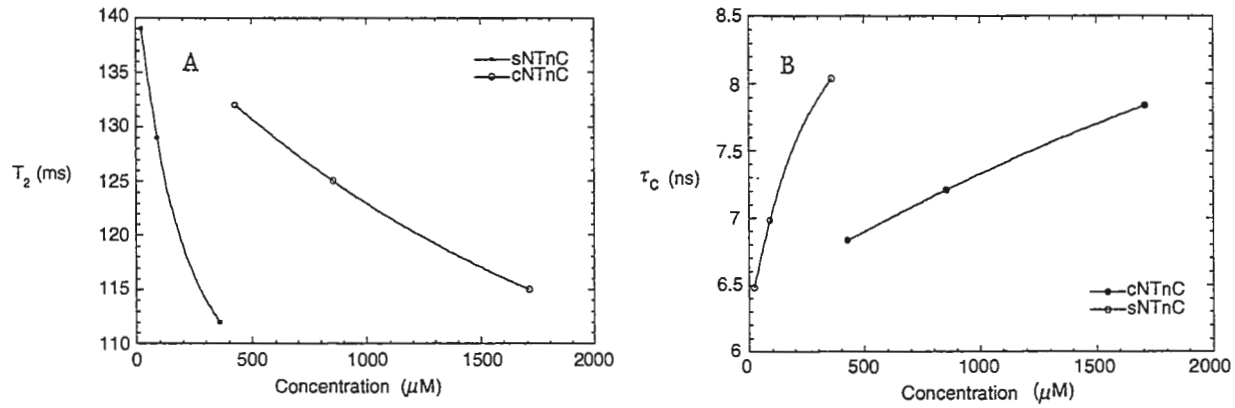

Figure 1. A) Dependence of ^{15}N-T_2 measured from the ^1H_N envelope on protein concentration for $2Ca^{2+}$-sNTnC and $1Ca^{2+}$-cNTnC. The line through the points is a guide for the eye. B) Dependence of τ_c on protein concentration for $2Ca^{2+}$-sNTnC and $1Ca^{2+}$-cNTnC and best fits to a monomer-dimer equilibrium. Rotational correlation times were estimated from the ^{15}N-T_2 values using equation 1. The K_D's are 1.3×10^{-3} and 7.3×10^{-3} for $2Ca^{2+}$-sNTnC and $1Ca^{2+}$-cNTnC, respectively. The values are ~6.3 ns for both s- and cNTnC.

The data in Figure 1B was fit to equations 3 and 4, with K_D and $\tau_{c,M}$ treated as adjustable parameters. The best fits and their associated parameters are shown in Figure 1B. $2Ca^{2+}$-sNTnC has a weaker K_D than $1Ca^{2+}$-cNTnC. K_D and $\tau_{c,M}$, can be used to estimate the fraction of monomer at a given concentration using equation 4. The fitted K_D's for s- and cNTnC are consistent with the hypothesis that the more 'open' structure for $2Ca^{2+}$-sNTnC exposes a larger hydrophobic patch in comparison to $1Ca^{2+}$-cNTnC, and thus dimerizes more readily. The methodology was published as part of the course proceedings for the International School of Structural Biology and Magnetic Resonance[1].

1. Leo Spyracopoulos, Stéphane M. Gagné, and Brian D. Sykes, "Know Your Protein: Effects of Weak Dimerization.", *Proceedings for The International School of Structural Biology and Magnetic Resonance, 4th Course on Dynamics, Structure and Function of Biological Macromolecules.* eds. O. Jardetzky and J.F. Lèfevre, Plenum Press, New York, NY, USA (1999).

Sincerely,

Leo Spyracopoulos

Stéphane M. Gagné

Brian D. Sykes

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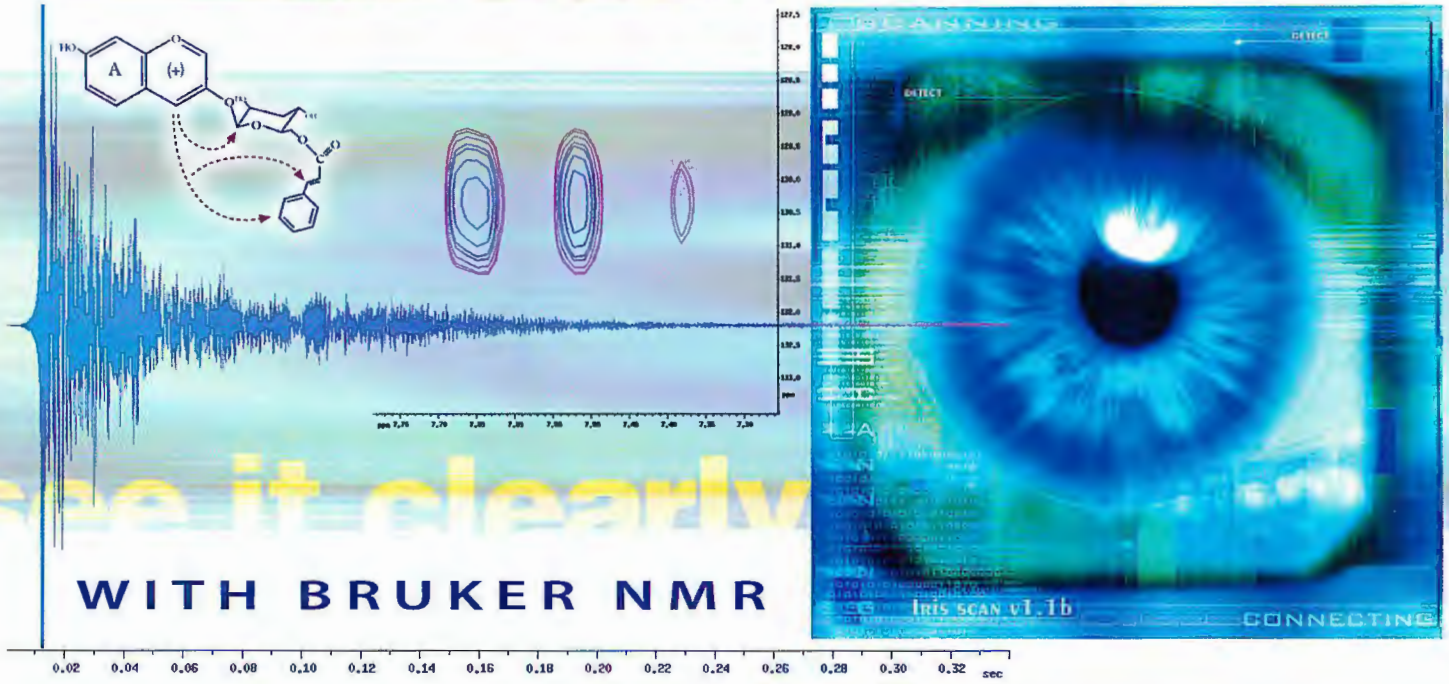

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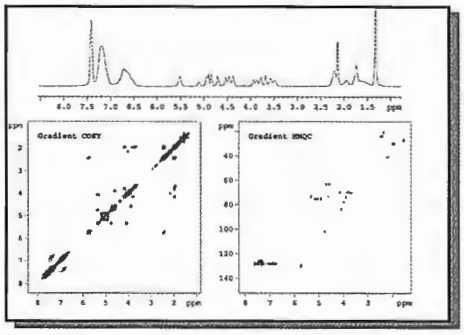

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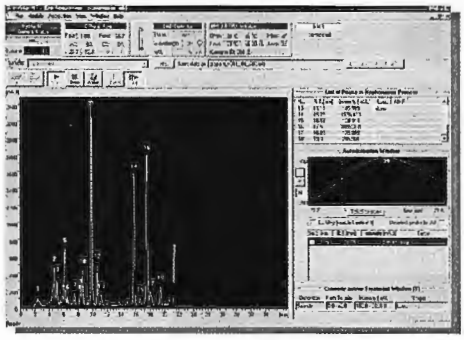

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Dr.B. L. Shapiro
NMR Newsletter

March 3nd 2000
(received 03/03/2000 [sic])

3D structure of microperoxidase –11 by NMR and molecular dynamic studies

Dear Barry,

Recently we had the opportunity to analyse the microperoxidase-11 (MP11), obtained from enzymatic cleavage of cytochrome *c*. As we were not aware of other NMR studies on MP11, after the early papers of Wilson[1] and Williams[2], we decided to examine this structure in details. The renewed interest in microperoxidases, not only as model compounds, but also as oxidation catalysts and potential electronic sensors, prompted us to send our results to Magnetic Resonance in Chemistry.

MP11 is an undecapeptide which retains residues 11-21 (Val-Gln-Lys-Cys-Ala-Gln-Cys-His-Thr-Val-Glu) of the protein and the heme-*c* group attached through thioether bonds at residues 14 and 17. It also retains His18 as a fifth ligand at the iron, while the sixth coordination site can be occupied by exogenous ligands such as water, hydroxide, CN or amines. MP11 exists in the ferric resting state just like the heme enzymes, and is an attractive ferric heme model for the peroxidases, but unfortunately it presents extensive aggregation and complicated high-spin/low-spin equilibria, which can be reduced at low concentration and with the addition of ligands.

We studied MP11 in the paramagnetic Fe(III) low-spin state by NMR and molecular dynamics (MD). Different experimental conditions were examined . In water-methanol and in water-trifluoromethanol mixtures (1mM, pH 7-8, temperature range -5° +5°C) and with various ligands at the sixth coordination site of the iron (NH_3, imidazole, OH, CN), MP11 is predominantly monomeric. This was established by correlation time and dilution experiments . All the protons of the heme and the peptide moiety were assigned by using TOCSY and NOESY experiments, with different spin lock and mixing time values (from 10 to 200 ms) in order to observe the heme or the peptide protons and to evaluate the paramagnetic effect on the peptide frequencies. It was found that the NOE build-up with mixing time is linear up to 50-70 ms for all the residues of the peptide chain with the exception of His18. For this residue, directly bound to the iron, as for the heme methyl and meso protons, the limit is 20-30 ms, then the NOE rapidly decrease.

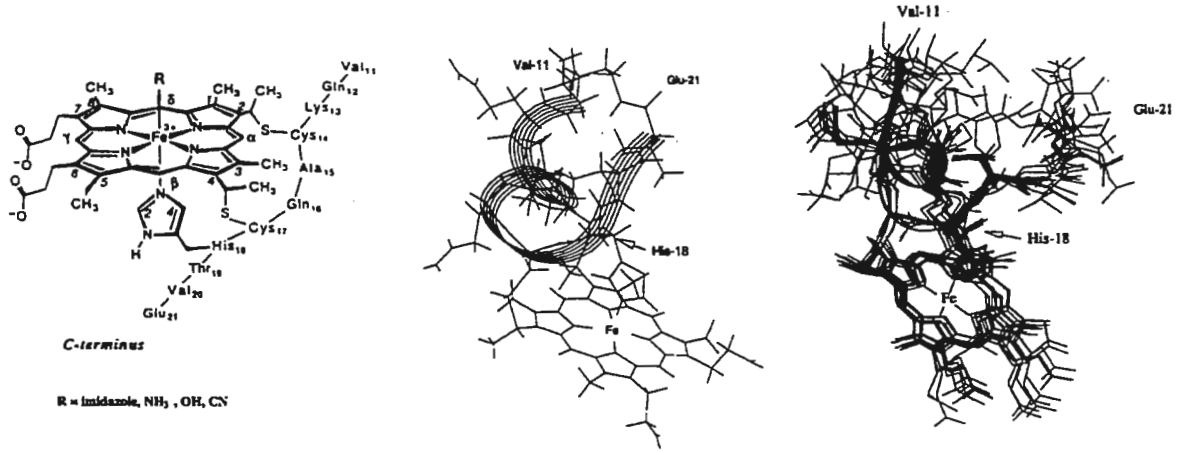
Some stereospecific assignments were performed and the coupling constants between the amide and the α protons were measured. Seventy-one inter-residue and heme-peptide and 40 intra-residue NOE interactions were translated into inter-proton distances by use of Felix software. The reference distance (2.94 Å) for the peptide chain was obtained from the average of the interproton distances αH-NH for those residues where the measured coupling constants allowed the dihedral angles to be define. The distance between one methyl on the porphyrinic ring and the vicinal meso-H (2.8 Å) was taken as reference for the heme moiety and for the heme-peptide interactions. When the distances in the peptide fragment were calculated with the reference adopted for the heme moiety, lower values (but not exceeding 0.6 Å) were obtained; thus we can estimate that the error introduced as a consequence of the paramagnetic effect on the protons close to the iron atom, e.g. His18, should not be larger than this value.

Ten structures, satisfying these distance constraints to within ±0.4 Å, were obtained and showed that the peptide fragment presents a preferred right-handed α-helical structure, with slight deformation at the level of 18 and 19 units. Analysis of the final ten structures using PROCHECK indicated that there are no residues in the disallowed regions of the Ramachandran plot, with ca 60% of residues in the most favoured and ca 40% in the additional allowed regions .

The 3D structure is strongly determined by the His18 axial bond to the iron and by the covalent bond of the two cysteines, but some motions are still allowed. The peptide chain is flexible enough to form the helix and to move over pyrroles I , II and the imidazole ring of His18 can rotate around the N-Fe bond. From the analysis of the MD trajectories we observed that the His ring can oscillate ±30° around the orientation found in cytochrome *c* (i.e. along the α-γ meso axis). The pattern of ^{13}C hyperfine shifts of heme methyls suggests that the orientation of His ring is preserved in MP11 along the α-γ axis. This is supported by MD calculations, which showed that the structures with this orientation are preferred (e.g. ΔE is 104.6 kJ with respect to structures oriented along β-δ axis). The relatively small hyperfine shift found for H-2 proton on the imidazole His ring indicates that the tilt of the major magnetic axis, with respect to the heme normal is very small, suggesting the determinant role of the protein matrix for the tilt of the *z*-axis in heme proteins.

In conclusion, MP11 appears to conserve the most important structural features of the cytochromes active site, i.e. the helix conformation of the inner peptide segment and the orientation of the axial His18 imidazole ring. Since the catalytic activity of the MP11 shows in some cases the same mechanism of peroxydases and cytochrome P-450, should we conclude that the interactions with distal residues have only the role of assistance in modulating the catalytic activity? Another open question is the role of the "tilt" of the major magnetic axis. Extensive efforts have been made to study the mechanism of the catalysis by cytochrome P-450 and by peroxidases in relation to their structures. MP-11 thus appears a good model to study the difference in action and to understand what minimum structure features are important to defining their specific activity.

1. Jehanli AMT, Stotter DA and Wilson MT, Eur. J. Biochem. 1976, **71**, 613.
2. Kimura K, Peterson J, Wilson MT, Cookson DJ, Williams RJP, J. Inorg. Biochem. 1981, **15**,11.



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Department of Chemistry

Professor Gideon Fraenkel

Phone 614-292-4210
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March 8, 2000

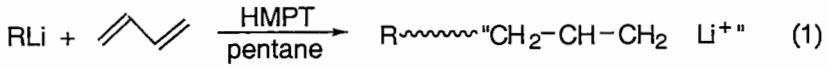
(received 3/15/2000)

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsimore Ct.
Palo Alto, CA 94303-3410

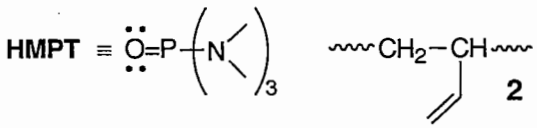
Allylic Lithium Compound
Complexed to HMPT

Dear Barry:

Collaborators at Goodyear Tire and Rubber Co. have observed that butyllithium initiated anionic polymerization of 1,3-butadiene in the presence of nexamethylphosphoramide (**HMPT**), 4X**HMPT**/Li, (1) is an unusually fast reaction. It results in a high degree of vinyl microstruture.



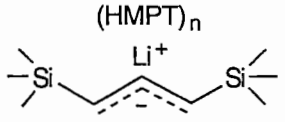
1



2

For those readers not in the business, polymer microstructure describes the distribution of monomers within the polymer. So 80% vinyl microstructure means 80% of the butadiene has polymerized 1, 2, see **2**. The growing "live end" in the anionic polymerization is an allylic lithium compound, see the part in quotation marks in structure **1**. It's nature determines the mechanism of polymerization and ultimately the polymer microstructure and the properties of the resulting rubber.

The Goodyear people wanted to know what kind of an allylic lithium is the live end and is it complexed to HMPT? So we started off studying the possible interaction of 1,3-bis(trimethylsilyl)allyllithium **3**, with **HMPT**. It is already known that **HMPT** complexes



3

with Li^+ at oxygen. Low temperature, 160 K, NMR, ^{13}C, ^{31}P and Li, shows largely one molecular species, one kind each of Li, ^{13}C and ^{31}P with small resonances for more dilute species. Lithium-7 NMR at 160 K is a quintet, 1:4:6:4:1, spacing 7.8 Hz due to $^2J(^7Li, ^{31}P)$ of 7.8 Hz while the ^{31}P NMR is an equal quartet due to the same coupling, see Figs 1 and 2. Thus four **HMPT's** are complexed to each ^7Li. This is the maximum coordination for lithium in such compounds and is tetrahedral. **HMPT** complexed **3** has to be regarded as a separated ion-pair. This structure is unusual for any allylic lithium compound and very likely also applies to the live end in the RLi initiated anionic polymerization of 1,3-butadiene in the presence of **HMPT** with resulting high vinyl microstructure. Perhaps this behavior is characteristic of the chemistry of separated ion pairs in general. We are currently looking at other allylic lithium **HMPT** complexes.

As you may know there is quite an armamentarium of NMR equipment here with a lot of biological (Tsai) and solid state (Grandinetti, Sachleben) work going on. You will be hearing from these people too before long.

Best wishes,

Gideon

Gideon Fraenkel
M. S. Newman Professor of Chemistry

Jose Cabral
Associate Professor
Newark Campus
Ohio State University

1. A. F. Halasa, Wm. Su, Goodyear.

^{31}P NMR

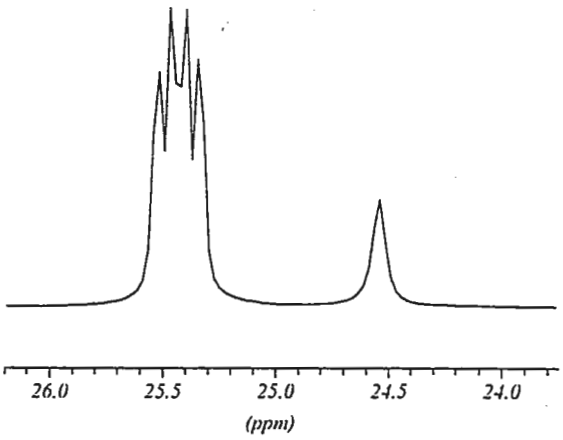


Fig 1

^{7}Li NMR

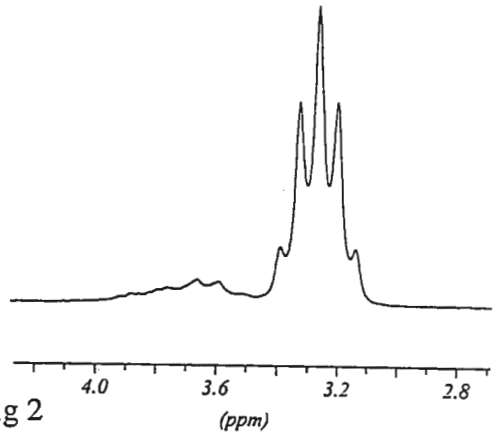


Fig 2

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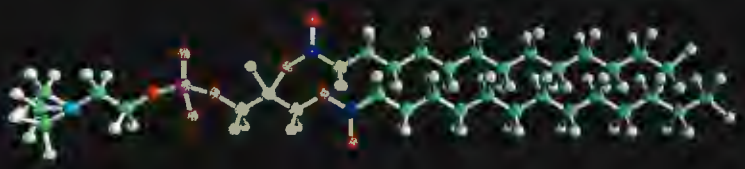

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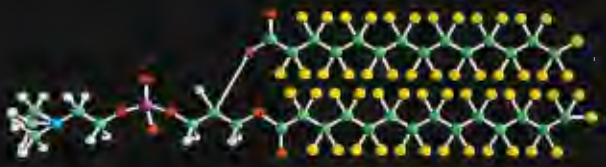

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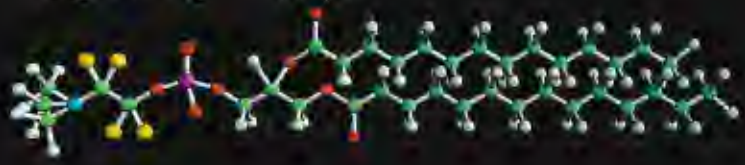

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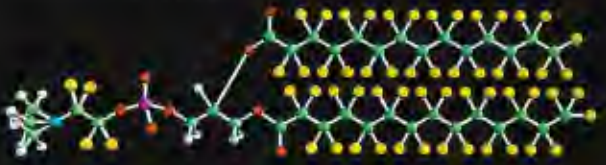
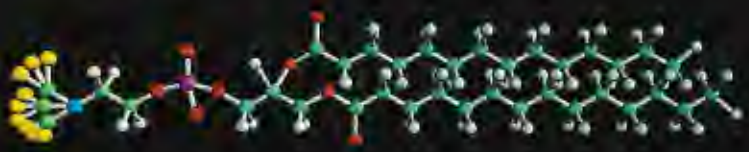


14:0 PC (D58)



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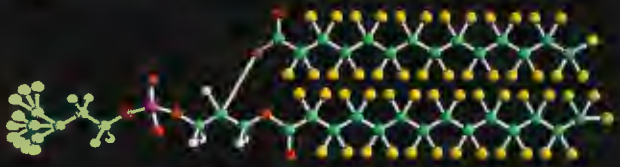

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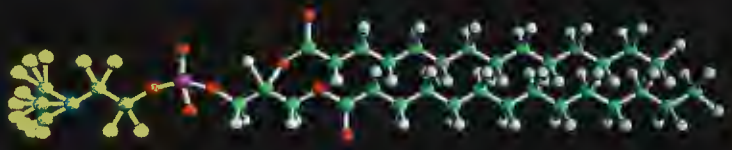

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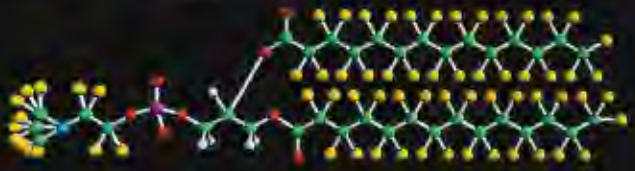

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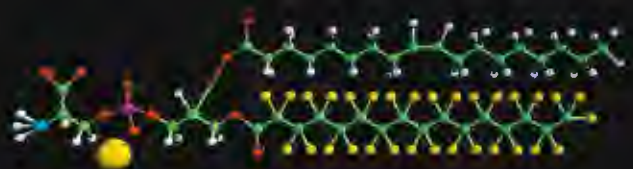
Asymmetric Fatty Acid



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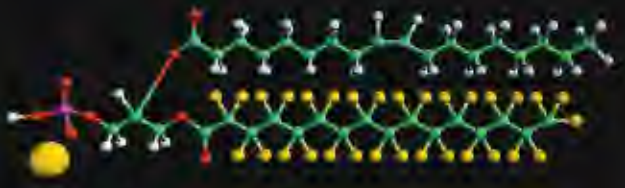

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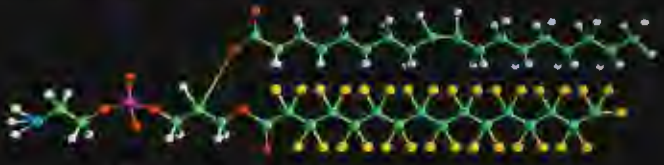

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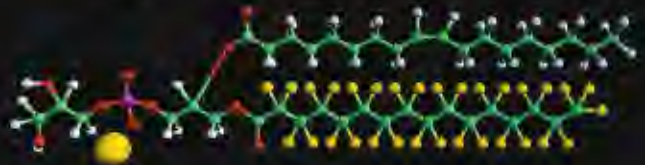

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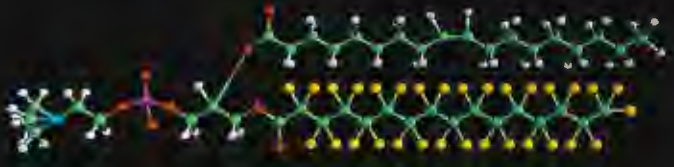

16:0-18:1 PC (D31)

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DAVIS, CALIFORNIA 95616

A Couette Geometry Probe for Space

Feb. 22, 2000
(received 3/14/2000)

Dear Barry,

Emulsions are phase segregated systems with domain sizes which can range from 0.1 micron to 100 microns. They occur in many industrial chemical processes and are common systems in the food we eat. Thus, it is important to understand their physical and rheological properties, as well as the dynamics of their domain sizes. Those dynamics are driven in part by the density difference of the two domains in a gravitational field. This NASA funded project is to study the behavior of emulsions in zero gravity with the eventual goal of putting an experiment on board a space shuttle mission.

As a first step, we have constructed a unique Couette geometry NMR imaging probe. This probe has a number of unique features. The two cylinders are precision ground glass and concentricity is maintained to ± 0.25 mm. The inner cylinder is replaceable and thus wide and narrow gap configurations are possible. The outer cylinder rotates rather than the inner cylinder to prevent formation of instabilities in the flow field at high rotation rates. Drive is provided by a programmable micro-stepper motor capable of 0.007 degree steps. Drive is provided at both ends to prevent a net torque about an axis perpendicular to the cylinder axis. It is built for our 300 MHz horizontal bore imaging system with gradients of 95 G/cm. The housing is machined from ULTEM, which provides a unique combination of machinability, high strength, and low dielectric loss.

We are currently in the testing phase. In particular, we have determined that as the cylinder rotates, the tuning varies ± 25 KHz. Whether this is due to microhetereogeneities in the glass or if the concentricity cited above is insufficient is unclear. In any event, to work around this problem, we are in the process of adding an optical pickup so that data acquisition will be triggered at the same cylinder orientation and thus at the same tuning condition for each revolution. Please credit this contribution to Gerd LaMar's subscription.

Jeffrey H. Walton

Robert L. Powell

Ronald J. Phillips

Stephanie R. Dungan

Nina C. Shapley

Marcos A. d' Avila

The NMR Newsletter - Book Reviews

Book Review Editor: István Pelczer, Dept. of Chemistry, Princeton University, Princeton, NJ 08544

"Introduction to Pulse NMR Spectroscopy"

by

Thomas C. Farrar

Farragut Press, Madison, Wisconsin, 1997*. pp xvii + 210.
ISBN 0-917903-12-9 (softcover) $24.95. 0-917903-13-7 (hardcover) $39.95

*This book is announced as the successor third edition to "Pulse and Fourier Transform NMR Spectroscopy" that was previously published, with Edwin Becker as co-author, by Academic Press.

There are seven chapters: (1) Fundamental Concepts, (2) NMR Pulse Experiments, (3) Instrumentation, (4) Relaxation Mechanisms, (5) Fourier Transform NMR Calculations, (6) Sensitivity Enhancement Techniques, and (7) Two-Dimensional Spectroscopy. There are two appendices: (A) A Brief Review of Vectors, (B) Fundamental Constants, together with tabulations of Symbols and Abbreviations.

The preface is dated April 1997, but even for then the book is not very up-to-date. There is no mention of (for example) gradient pulsing or solid state NMR. Only the basic experiments are featured in the chapter on 2-dimensional spectroscopy.

On the title page the book is called " Pulse Nuclear Magnetic Resonance Spectroscopy. An Introduction to the Theory and *Applications*". The book is weakest in its treatment of *applications*. While there is merit in confining the illustrations to simple molecules such as methyl vinyl ketone, it would have been nice to see examples involving more complex structures. The spectra in the book (at least the softcover edition I examined) are mostly idealized and hand drawn.

There is some evidence (besides the somewhat misleading term in the title) of insufficient editing; thus on the contents page the chapters are listed with Roman numerals (I to VII), while in the text Arabic numerals (1 to 7) are used. It is disappointing to see what was an influential book in the early days of pulse NMR reproduced in this form.

There are strengths in the book in its treatment of the fundamentals of pulse NMR spectroscopy, and the processes involved in NMR experiments. Thus it would be a valuable desk reference for anyone faced with the task of giving a series of introductory lectures. However, I would hesitate to recommend it to a class attending such lectures.

Peter Bladon
Interprobe Chemical Services
Gallowhill House, Larch Avenue
Lenzie, Kirkintilloch
Glasgow G66 4HX
Scotland.

cbas25@strath.ac.uk

UNIVERSITEIT GENT
VAKGROEP ORGANISCHE CHEMIE

Dept Organic Chemistry
Universiteit Gent
Krijgslaan, 281 S4
B-9000 Gent, BELGIUM

Bernard L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto CA
USA 94303

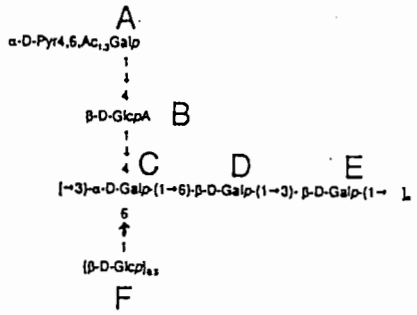
Gent, March 7, 2000
(received 3/25/2000)

NMR EXPERIMENTS FOR THE IDENTIFICATION OF PHYTOPATHOLOGICAL FEATURES IN THE EXO-POLYSACCHARIDE AMYLOVORAN

Dear Barry,

Fire blight is an important quarantine disease on fruit trees and is caused by the bacteria *Erwinia amylovora.* Epidemiological understandig is needed for its control. In the process of infection, the bacterial coat is an essential virulence factor since it participates in the first intimate contact with the host plant system. Structure analysis (1) of the exopolysaccharide amylovoran (EPS) produced by different natural *E. amylovora* isolates revealed the same penta- and hexasaccharide repeating sub-structures when produced by strains infective of *Malaceae* host plants. With fire blight isolates from *Rubus* species, the EPS showed a mixture of hexa- and pentasaccharide substructures. Quantitative data on amylovoran production account for the differences in virulence found for *E. amylovora* strains.

The structure of the pentasaccharides was proposed by Nimtz *et al.* (1) mainly from (i) mass spectral data and (ii) some NMR data, performed on phage-depolymerized amylovoran.The structure of the hexasaccharide was suggested from mass data and limited NMR data.. The penta- and hexasaccharides (the latter now found to be present for 50%) have the same basic pentasaccharide structure Using gHMQC and selective excited TOCSY we could now fully prove that the nature of the supplementary residue F in the hexasaccharide is a β-D-glucopyranosyl group and that its glycosidic linkage is β-1,6 to residue C. The structure of the hexasaccharide is given in folllowing scheme:



The analysis of the spectra is complicated by the fact that the spectra are a mixture of four compounds: residue A is 2,3-di- *O*-acetylated, 2-*O* or 3-*O*-mono-acetylated or not acetylated at all. In solution deacetylation occurs, causing a permanent change of the spectrum.

Sincerely Yours

André De Bruyn Martine Maes, Roger Busson, Piet Herdewijn and Eric Messens

(1) M. Nimtz, et al. Carbohydr. Res. 287 59 - 76 (1996)

NMR COURSES

at Imperial College, London

Contact: Hersha Mistry Tel: 020 7594 6884; Fax: 020 7594 6883
email: h.mistry@ic.ac.uk website: www.ad.ic.ac.uk/cpd/nmr.htm

NMR-based Metabonomics: Drug Toxicity Assessment & Diagnostics

Date: 10 - 11 July 2000
Venue: Imperial College of Science Technology & Medicine,
Exhibition Road, South Kensington, London, SW7 2AZ
Cost: £600
Course Presenters: Professor Jeremy Nicholson
Professor John Lindon
Dr. Elaine Holmes,
together with guest speakers from industry
Course Outline:
1. Application of Metabonomics to Pharmaceutical and Clinical Sciences
2. Introduction to Data Reduction and Basic Chemometric Theory
3. Classification Methodologies
4. Specialist Topics in Advanced NMR Spectroscopy and Pattern Recognition
5. Workshops and Practicals

Who should attend:

The course is designed for members of the pharmaceutical and agrochemical industries who wish to explore the potential of combining 'state of the art' high resolution NMR spectroscopy with appropriate multivariate statistical techniques. The course is strictly limited to 24 participants: early booking is advised.

Hyphenated Spectroscopic Techniques for Bioanalysis

Date: 13 - 14 July 2000
Venue: Imperial College of Science Technology & Medicine,
Exhibition Road, South Kensington, London, SW7 2AZ
Cost: £600
Course Presenters: Professor Jeremy Nicholson
Professor John Lindon
Dr. Elaine Holmes,
together with guest speakers from industry
Course Outline:
1. Overview of Analytical NMR and Hyphenated Techniques
2. Recent Developments in Technology
3. Alternative Hyphenation
4. Workshops and Practicals

Who should attend:

This short course should interest those in Pharmaceutical and Agrochemical Industries responsible for the characterisation of xenobiotic metabolites and impurities/degradation products. NMR experiments covering both in vitro and in vivo systems used in support of discovery and development stages will be considered. Basic practical experience in HPLC, NMR spectroscopy and mass spectrometry would be advantageous, but not essential. Attendees are welcome to provide test problems for the practical sessions (providing advance notice is given to the presenters). Participant numbers are limited to 24.

continued

NMR Spectroscopy of Biofluids & Tissues

Date: 14 - 17 November 2000
Venue: Imperial College of Science Technology & Medicine, Exhibition Road, South Kensington, London, SW7 2AZ
Cost: £900
Course Presenters: Professor Jeremy Nicholson
Professor John Lindon
Dr. Elaine Holmes,
together with guest speakers from industry

Course Outline:
1. Theoretical Aspects of NMR Spectroscopy
2. Application of NMR Spectroscopy to Biofluid Analysis
3. Hyphenated Techniques
4. High Resolution MAS-NMR Spectroscopy of Intact Tissues
5. Data Reduction and Pattern Recognition Analysis
6. Specialist Topics
7. Demonstrations, Practicals and Workshops

Who should attend:

This programme is designed for members of pharmaceutical and agrochemical companies wishing to exploit the modern developments in biological high resolution NMR spectroscopy and related techniques. It is particularly appropriate for applicants working in the areas of drug development and toxicology. Basic practical experience in NMR spectroscopy would be advantageous. The course is strictly limited to 24 participants: early booking is advised.

Position Available

NIH POSTDOCTORAL POSITION AVAILABLE

CARDIOVASCULAR NMR

A postdoctoral position is available immediately for combined work in the Nuclear Magnetic Resonance Unit (Richard Spencer, Chief) and in the Laboratory of Cardiovascular Sciences (Edward Lakatta, Chief) of the National Institute on Aging of the National Institutes of Health in Baltimore, Maryland. Initial work will center on spectroscopic studies of myocardial metabolism related to i) congestive heart failure and ii) β-adrenergic stimulation of the heart. Other research opportunities may be available depending on the interests and background of the successful candidate.

NMR instrumentation consists of a double-resonance Bruker ABX 1.9T/31 cm Biospec with shielded gradients, and a triple-resonance wide-bore Bruker DMX 400 Avance system with microimaging and solids capability.
A background in NMR spectroscopy or imaging of the cardiovascular system is preferred, although applicants with expertise in cardiac or muscle physiology who have the desire to learn NMR techniques are also strongly encouraged to apply. We also invite applications from individuals with experience in other areas of biological NMR.

The appointment will be as an IRTA Postdoctoral Fellow for US citizens, or as a Visiting Fellow for US non-citizens. Accordingly, applicants must have fewer than five years of postdoctoral experience. Interested individuals should send or e-mail their CV and the names, telephone numbers, and e-mail addresses of at least three references to: Dr. Richard Spencer, NMR Unit, NIH/NIA, GRC 4D-08, 5600 Nathan Shock Drive, Baltimore, MD 21224; Tel. 410-558-8226; e-mail: spencer@helix.nih.gov; website: http://www.grc.nia.nih.gov/branches/lci/nmr/nmr.htm

Positions Available

Nova Research, Inc., a high-technology research and development services firm, is currently seeking both Senior and Post-Doctoral level scientists to support the Company's Washington, D.C. based activities. These positions will involve using state-of-the-art instrumentation to conduct both basic and applied research in areas of materials science of importance to the Navy and other government agencies, specifically (i) fundamental solid state NMR investigations of polymer structure and dynamics, particularly polymer miscibility and morphology; (ii) application of advanced solid state NMR techniques, (including hyperpolarized xenon), for the analysis of materials such as polymers, gas hydrates and ceramics, and (iii) magnetic resonance for detection of materials, including detection of explosives by NQR. Positions will be located on-site at the US Naval Research Laboratory. All applicants must possess a PhD in chemistry, physics or closely related discipline, and candidates applying for the Senior Scientist position must have at least 2 years of post-PhD experience in magnetic resonance (NMR, NQR) and in applications to materials chemistry. A strong record of innovation, intellectual independence, scientific leadership, and publications is necessary.

Nova Research offers an exceptional benefits package, and compensation will be commensurate with experience. As positions may require security clearances, applications are limited to US citizens only. Applicants are requested to forward their resumes, salary history, and professional references in confidence to: Nova Research, Inc., Attn: Human Resources, 1900 Elkin Street, Suite 230, Alexandria, VA 22308. EOE.

Position Available

NMR Research Associate

Few health care companies can point to a legacy of achievement more impressive than Pfizer's or look ahead to a future whose potential is as promising. Pfizer is committed to setting the highest standards of quality in products, service and expertise. An opening is available for an NMR Research Associate in Developmental Research at our Pfizer, Inc. Central Research Headquarters in Groton, CT. The Developmental Research NMR group supports all areas of Developmental Research. It is responsible for the characterization of drug candidates, excipients, degradation products and impurities via NMR. The group interacts with other technology and project groups within Developmental Research and throughout Pfizer. The lab is equipped with a 500 MHz liquids NMR spectrometer with gradients, microsample and LC-NMR capabilities. Solids capabilities will be added in the near future. Our group supports an open access lab equipped with 300 MHz and 400 MHz liquids NMR spectrometers with gradients. Numerous additional NMR spectrometers are also available on campus.

As the NMR Research Associate, you will: 1) Elucidate structures and solve scientific questions for small molecules of pharmaceutical interest using multidimensional NMR and LC-NMR; 2) Manage open access NMR spectrometers; 3) Communicate routinely with project chemists throughout Developmental Research; 4) Write technical reports.

Qualifications: The successful candidate will have a B.S./M.S. degree in Chemistry or equivalent and a minimum of two years of NMR experience. Industrial research experience is strongly preferred. Strong communication skills and a demonstrated ability to carry out projects independently are essential. This position provides the opportunity for a motivated individual to contribute in a stimulating professional environment. Pfizer, Inc. offers an excellent salary/benefits package plus an attractive Connecticut shoreline location. Pfizer was recently ranked by FortuneTM as one of the top 100 companies to work for, ranking first in the pharmaceutical industry.

For consideration, send your resume to: Dr. Linda Lohr
Pfizer Central Research
Eastern Point Rd.
Groton, CT 06340-8003.

Address all Newsletter correspondence to:

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303.
650-493-5971* - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

Deadline Dates

No. 500 (May)	28 Apr. 2000
No. 501 (June)	24 May 2000
No. 502 (July)	21 June 2000
No. 503 (Aug.)	25 July 2000
No. 504 (Sept.)	24 Aug. 2000

* Fax: 650-493-1348, at any hour. Do not use fax for technical contributions to the Newsletter, for the received fax quality is very inadequate.

* E-mail: shapiro@nmrnewsletter.com

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Forthcoming NMR Meetings, continued from page 1:

XIX International Conference on Mag. Res. in Biological Systems, Florence, Italy, **August 20-25, 2000**. Contact: Profs. Ivano Bentini or Lucia Banci, Chem. Dept., Univ. of Florence, Via G. Capponi 7, I-50121, Florence, Italy; Phone: +39-055-2757600; Email: icmrbs@lrm.fi.cnr.it; Fax: +39-055-2757555; http://www.lrm.fi.cnr.it//icmrbs.html.

NMR Spectroscopy of Biofluids and Tissues, Imperial College, London, England, **November 13-17, 2000**. Contact: Hersha Mistry, Centre for Continuing Education, Imperial College, 526 Sherfield Building, Exhibition Road. London, SW7 2AZ, UK. Tel: +44 (0)20 7594 6884; Fax: +44 (0)20 7594 6883; Email: h.mistry@ic.ac.uk; http://www.ad.ic.ac.uk/cpd/nmr.htm

Royal Society of Chemistry: 15th International Meeting on NMR Spectroscopy, Durham, England, **week of July 8-13, 2001**; Contact: Mrs. Paula Whelan, The Royal Society of Chemistry, Burlington House, London W1V 0BN, England; +44 0171 440 3316; Email: conferences@rsc.org\

Additional listings of meetings, etc., are invited.

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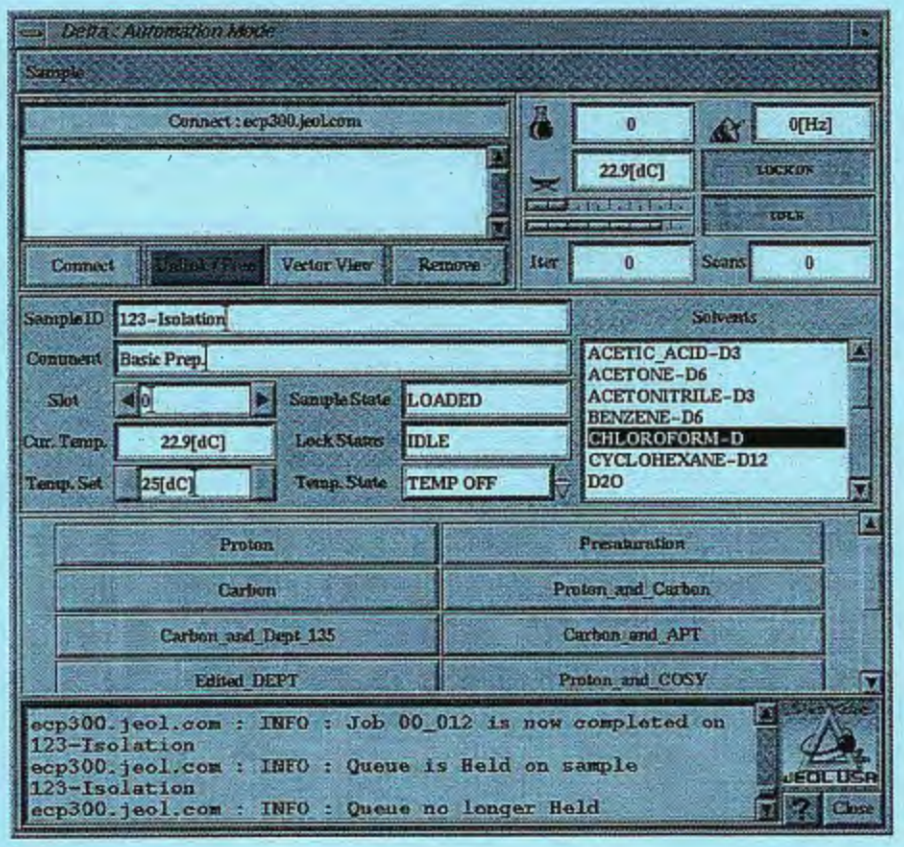

The **Eclipse+** NMR Spectrometer can be operated anywhere there is a computer on the local network. The **Single Window Automation** pictured above can be used with a single mouse click to select the sample from the auto-sample changer, gradient shim on any probe, run the selected experiment, and plot the data on any network postscript printer. Need more data, click another button and the **Eclipse+** is off to do your work - and you have not left your office. Contact us at nmr@jeol.com or visit or web site at www.jeol.com.

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email: nmr@jeol.com www.jeol.com

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