NMR from Ancient Paper


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XII School on Neutron Scattering (SoNS) "Francesco Paolo Ricci"
Erice 30 April – 9 May 2014

1945 Radiation Laboratory

EDWARD MILLS PURCELL & FELIX BLOCH (NL 1952)

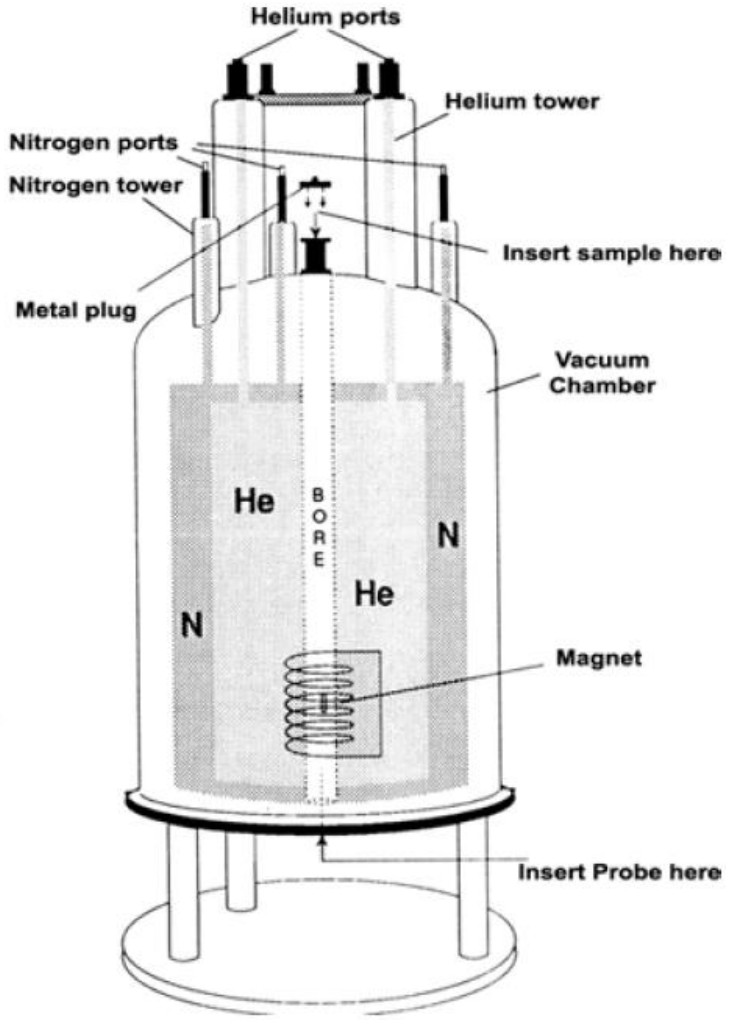
with

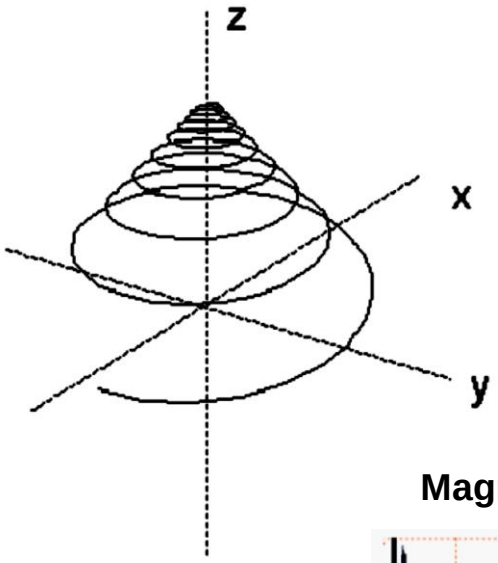
NICOLAAS BLOEMBERGEN (NL 1981)
(First study on water)

The equilibrium recovery of a given nucleus, in a static magnetic field, perturbated by the application of radio-frequency (RF) waves.

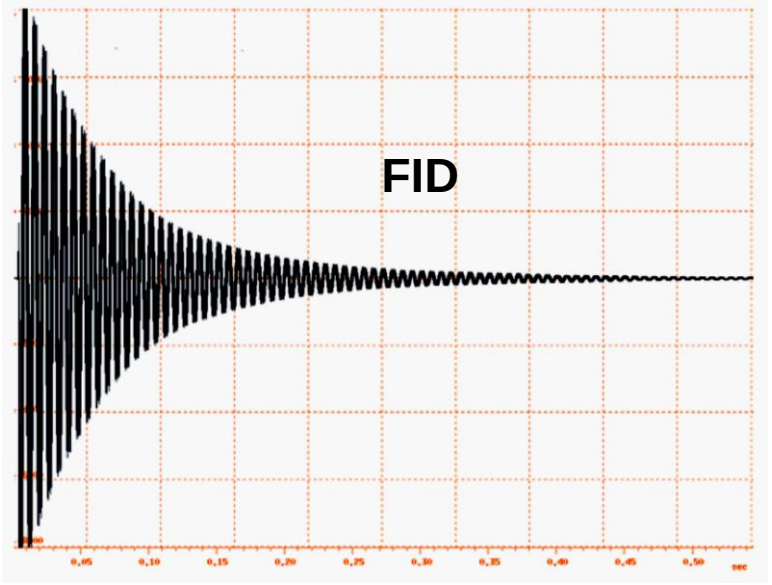
NUCLEAR MAGNETIC RESONANCE

NMR center (CNR-UNIME) for the study of the soft-matter (700 MHz --- 14 Tesla)





Magnetization : return at the equilibrium



NMR PROCESS

The nuclear spins in the Larmor precessional motion (caused by the external field) with a different orientation under the RF effect are collectively oriented along the same direction. Hence, the net effect on the macroscopic magnetization M(ω) will result in a ‘’coherent’’ precessional motion with frequency ω.

SENSITIVITY

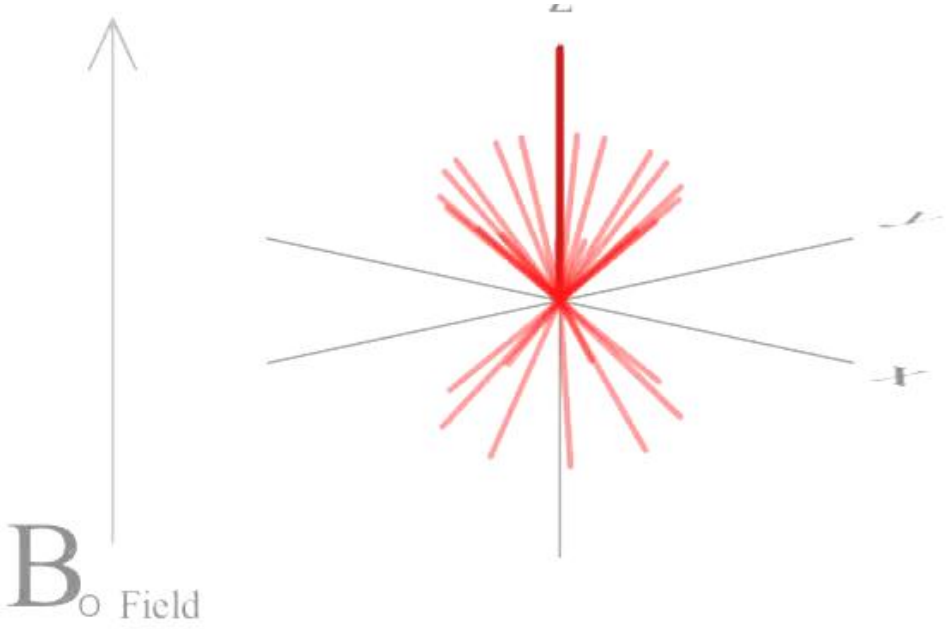
The tecnique sensitivity depends on the magnitude of the external magnetic field being the observable magnetization the difference between the population of the two Zeeman levels (with spin direction parallel and anti-parallel with respect to the direction of the external field). A quantity, this latter, whose ratio depends on the magnetic field intensity B_0 and on the nuclear gyromagnetic ratio (proportional to the nuclear magnetic moment):

(Actual max 10^{-3} ppm -1 GHz)

$$\frac{N_+}{N_-} = e^{-\Delta E/kT} = e^{-\hbar\omega_0/kT} = e^{-\hbar\gamma B_0/kT}$$

All nuclei under the effect of the magnetic field undergo the precessional motion with the Larmor frequency:

$$\omega_0 \equiv \gamma B_0$$



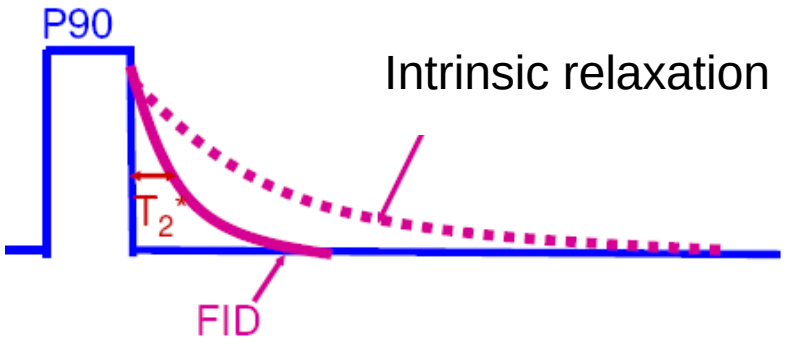
The RF effect results in the coherent dynamics of the net Magnetization M(ω). (The frequency $\nu = \omega - \omega_0$ represents the chemical shift).

The NMR technique can be applied to all nuclei having a non zero magnetic moment; looking to a single or different nuclei simultaneously (1D or ND).

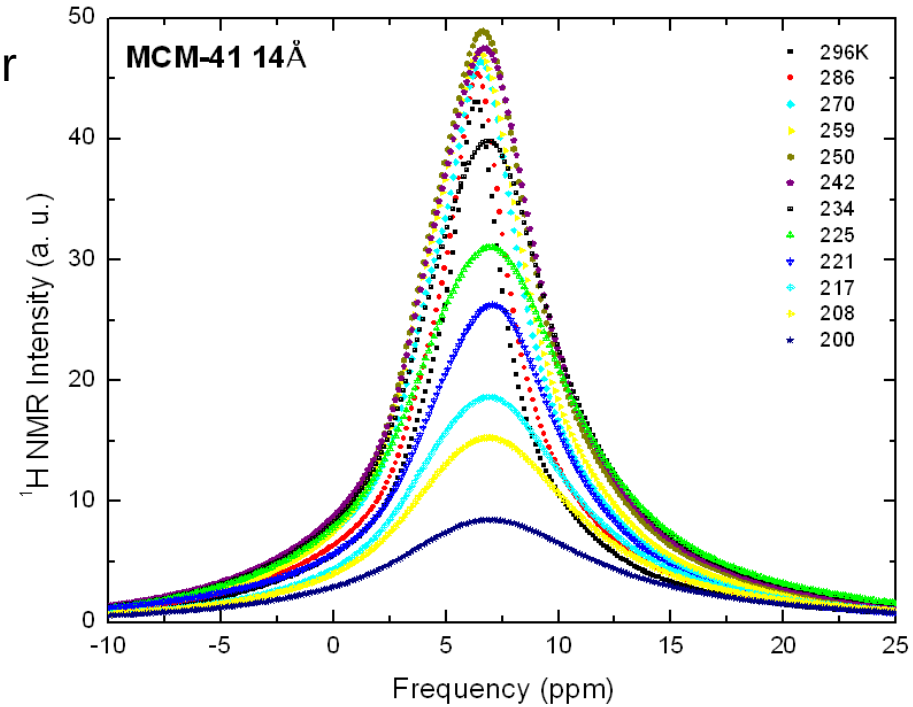
After the applied RF pulses, the Magnetization returns to the equilibrium with characteristic relaxation times. The decay of its transverse component is acquired and Fourier transformed to obtain the spectrum.

OBSERVABLE-1 The Free Induction Decay and Chemical Shift

The frequency shift ($\nu = \omega - \omega_0$) with respect to the Larmor frequency of each nucleus depending on the local field is called "The Chemical Shfit".
Its value and behavior therefore contain information about the local properties of the studied nucleus.



Confined water



With proper pulse sequences it is possible to measure dynamic quantities such as the characteristic relaxation times of the nuclear magnetization and the atomic motion (e.g. mean squared displacement).

OBSERVABLE-2 The Relaxation times, T_2 and T_1

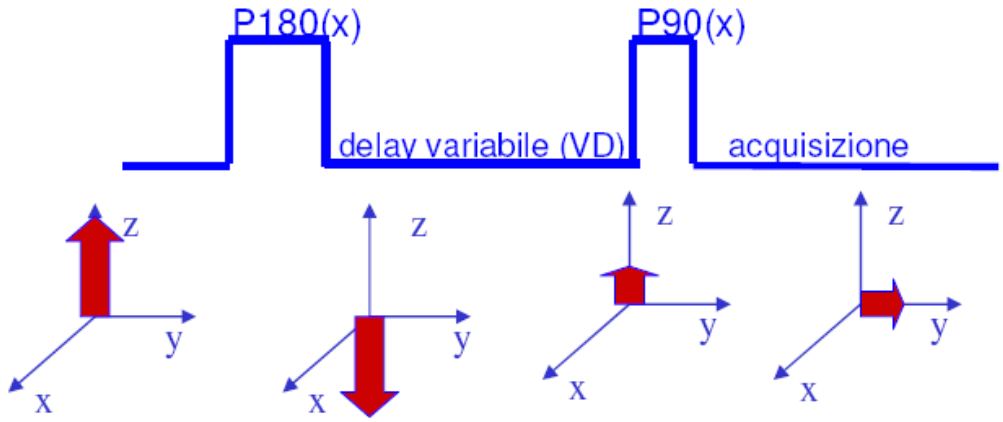
The transversal or spin-spin relaxation time, T_2:
the time required for the M(ω) transverse component (orthogonal to the B_0 direction) to vanish. It measures the dipolar interactions between spins belonging to the same species (i.e. the strength of interplay among the same species). A weaker interaction corresponds to a longer T_2.

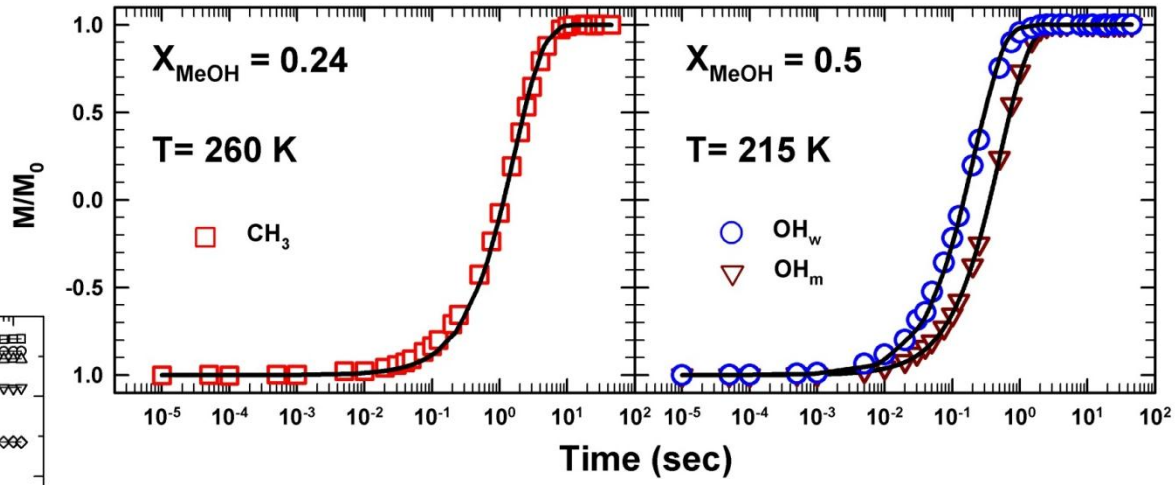
The longitudinal or spin-lattice relaxation time, T_1:
the time required for the M(ω) longitudinal component to recover its equilibrium value after the application of the perturbing pulse sequence. It is a measure of the dipolar interactions of the investigated spins with their surrounding. Its value ranges from 10 to 10^3 of msec for protons in hydrogenated compounds, and usually becomes smaller at lower temperatures.

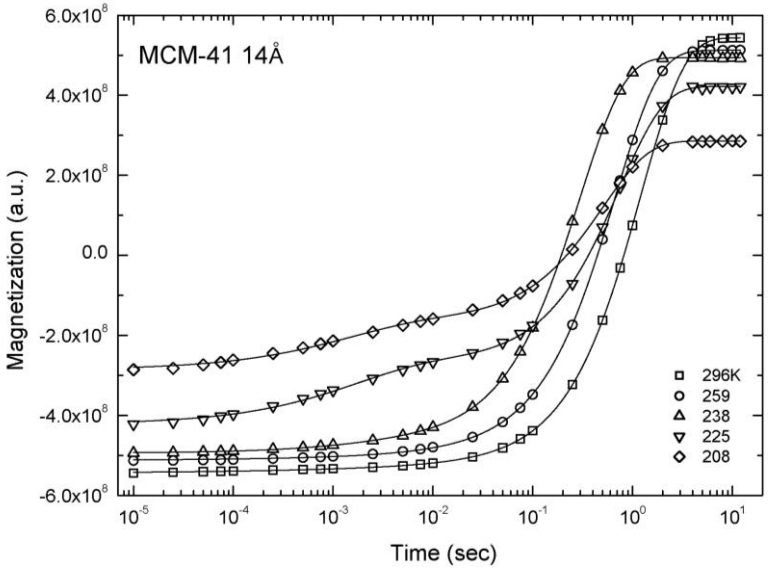
OBSERVABLE-3 The self-diffusion coefficient.

The mean squared displacement (MSD) and the self-diffusion
By means of applied field gradients it is possible to probe the MSD along the field direction, obtaining the coefficient of self-diffusion.

Inversion Recovery & Spin-Lattice (T_1) relaxation

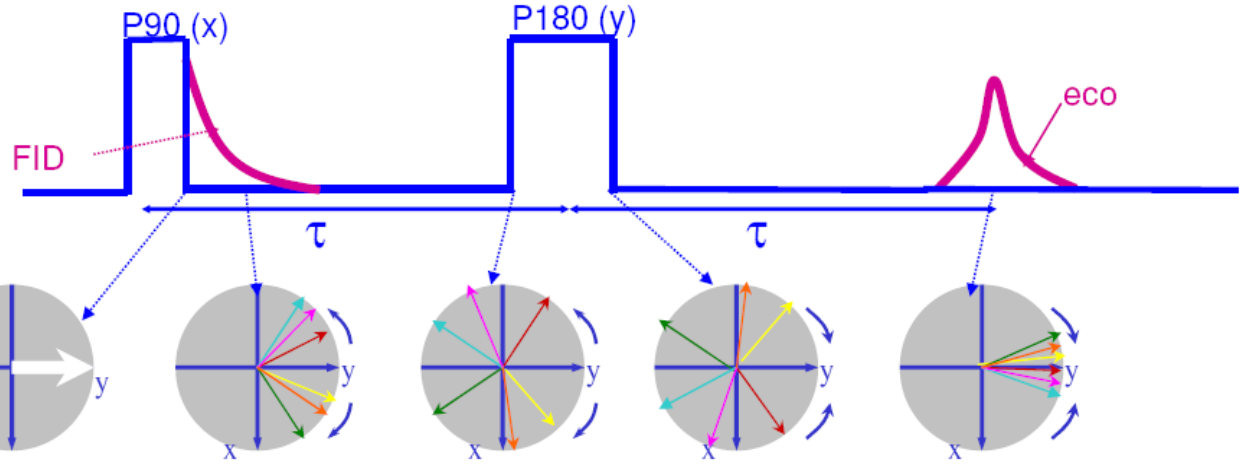




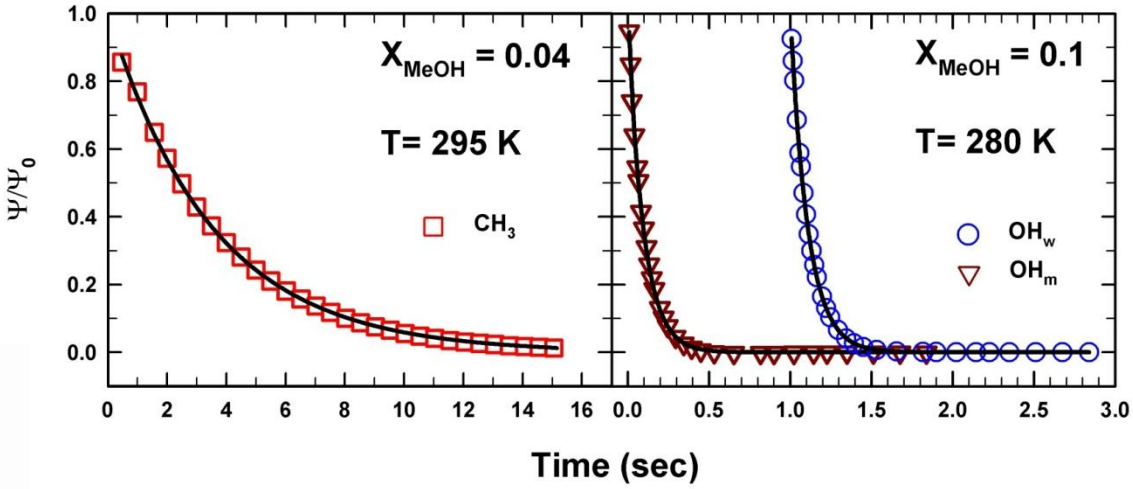


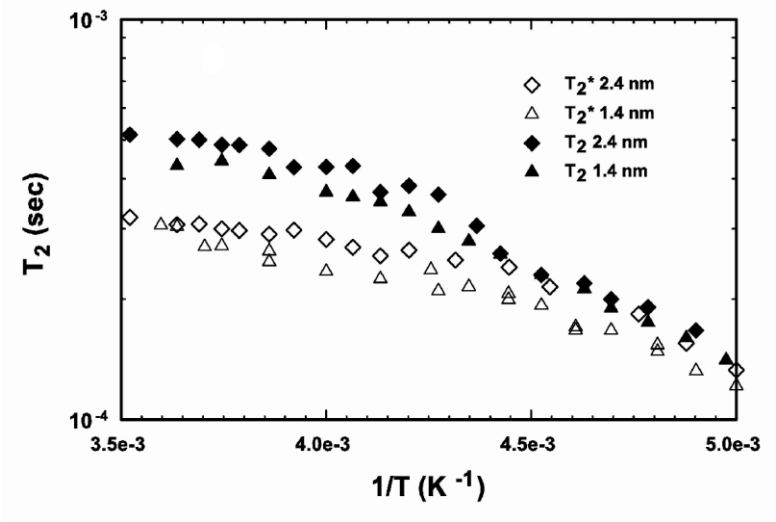
$$M / M_0 = 1 - 2\exp(-t / T_1)$$

Hahn's Echo (T_2):

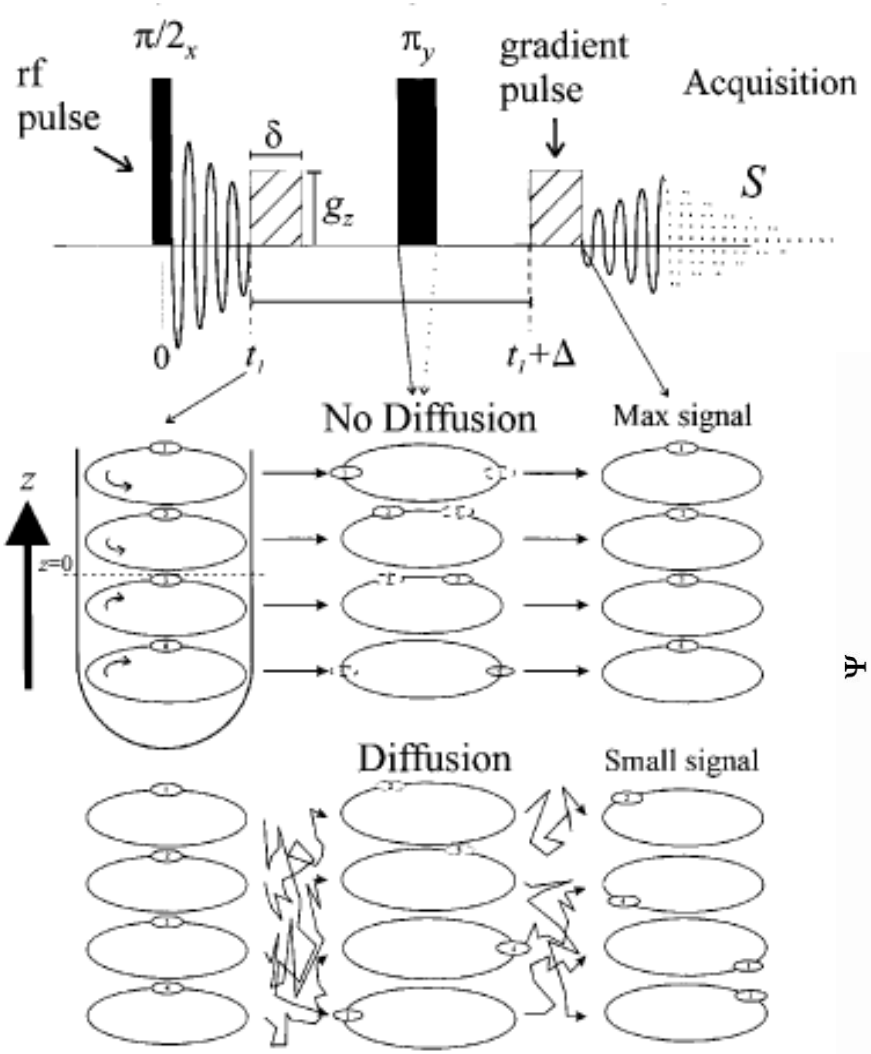


$$\Psi / \Psi_0 = \exp(-t / T_2)$$

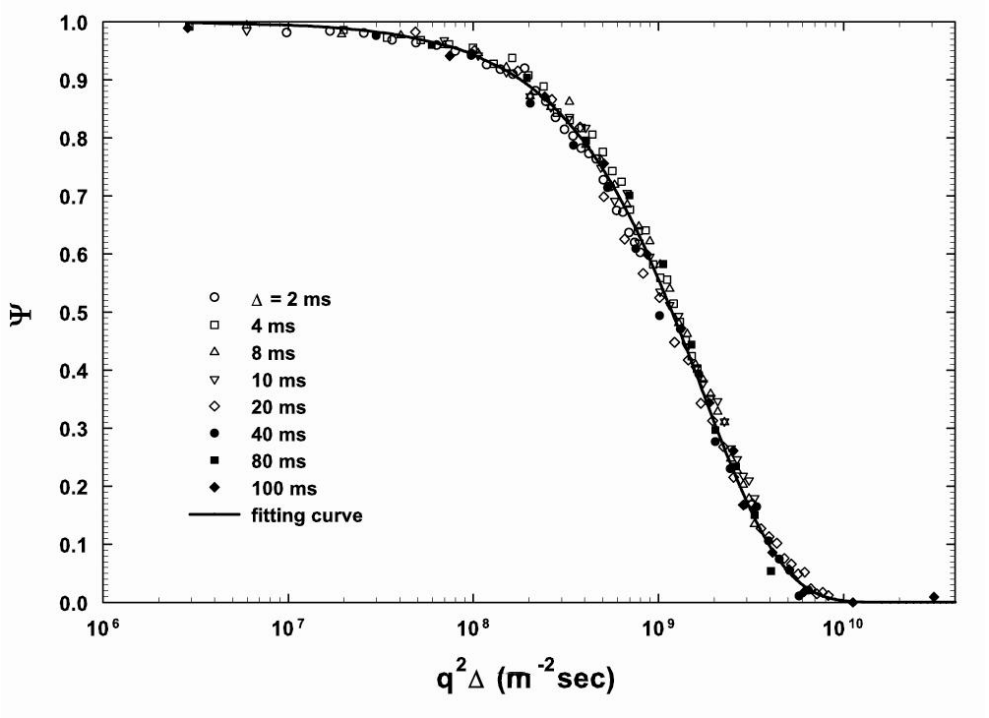




Pulsed Field Gradient and the Self Diffusion



$$\omega_{eff}(\vec{r}) = \omega_0 + \gamma(\vec{g}\cdot\vec{r})$$



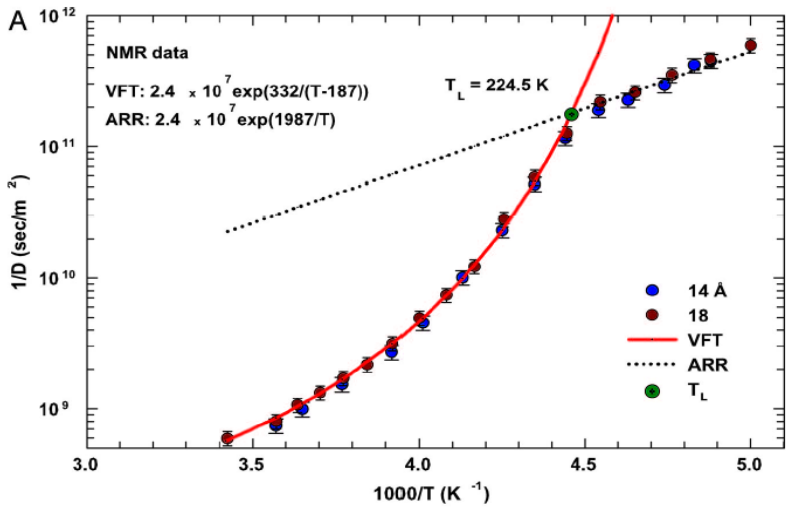
In the case of a gaussian propagator

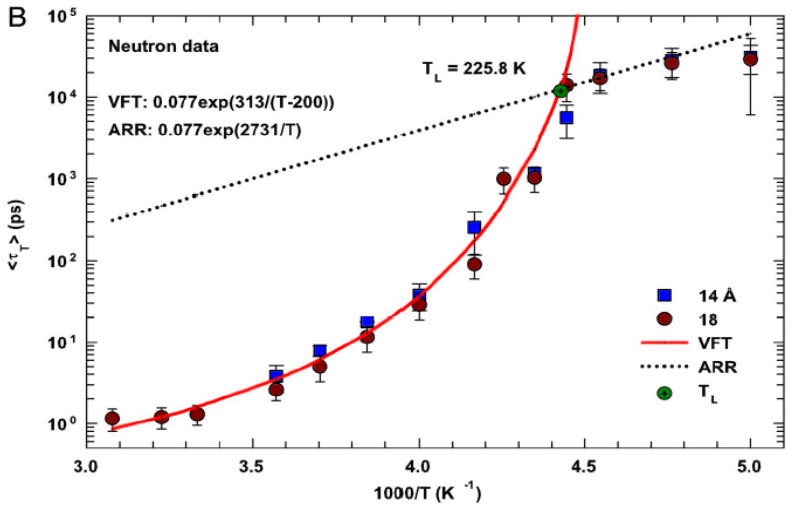
$$\Psi(g\delta,\Delta) = \exp\{-(\gamma\delta g)^2 D\Delta\}$$

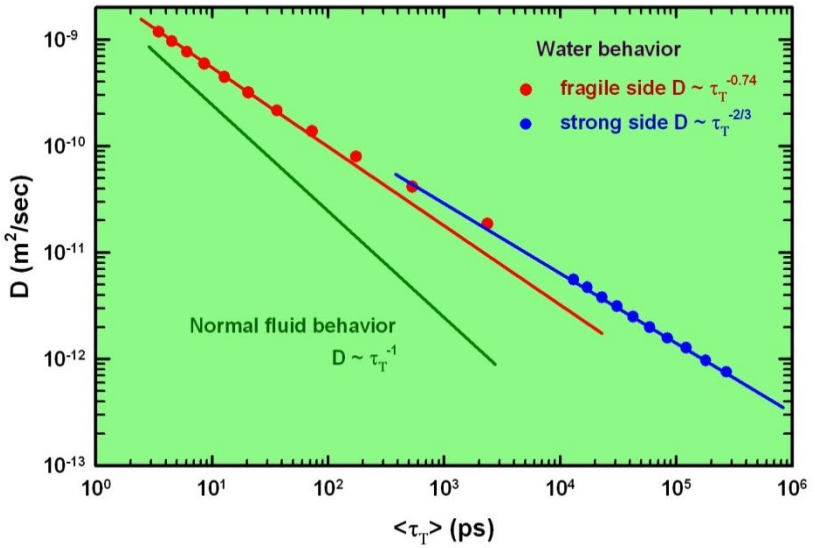
Transport parameters by NMR and Neutrons: The fragile-to-strong (FSC) dynamical crossover

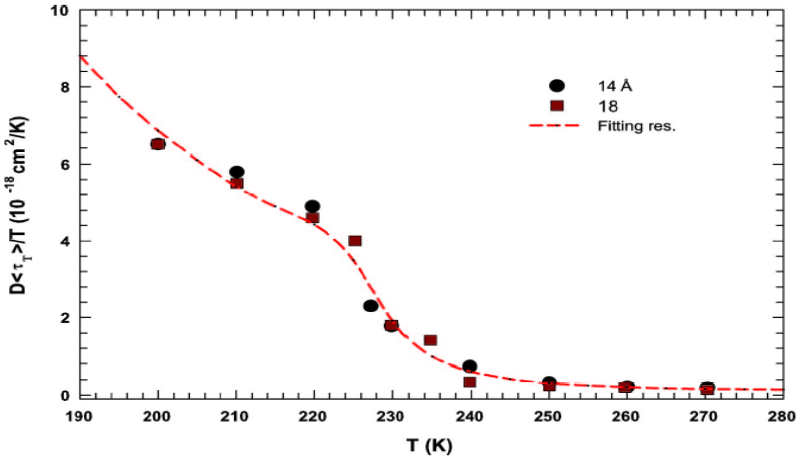
The Stokes-Einstein violation (SEV) just at the FSC crossover temperature T_L.

$$D \cdot \langle \tau_T \rangle / T \sim const$$



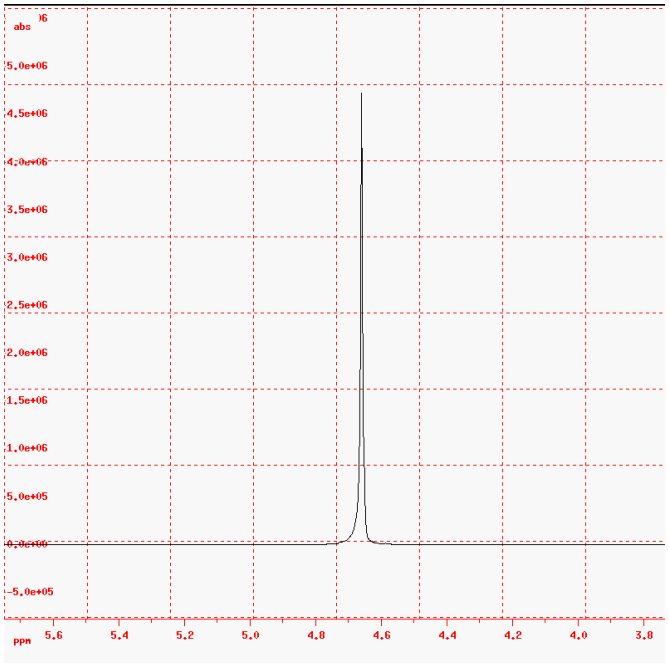






The SEV (a decoupling in the transport parameters) in supercooled liquids is due to dynamical heterogeneities.

A new NMR approach to estimate the configurational specific heat (water case).



The chemical shift δ is an assumed linear response of the electronic structure to the external magnetic field:

$$B_{shifted}(j) = (1 - \delta_j) B_0$$

So, it is connected to the number of possible configuration (j) that a water molecule can assume. This number is inversely proportional to the average number of hydrogen bonds <NHB>, hence the configurational entropy can be written:

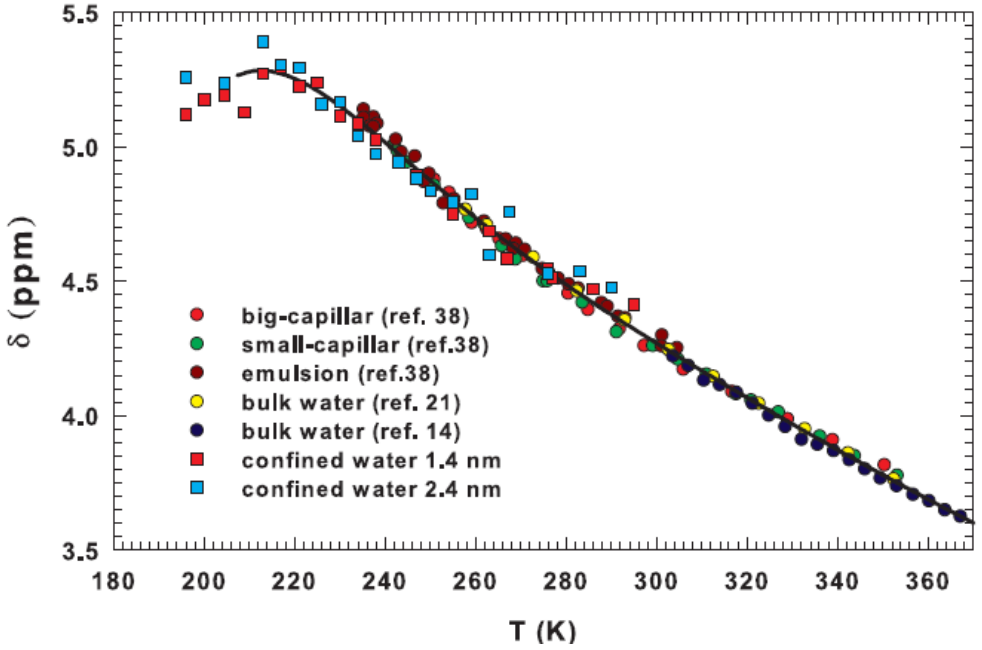
$$S \approx -k_B \ln < NHB >$$

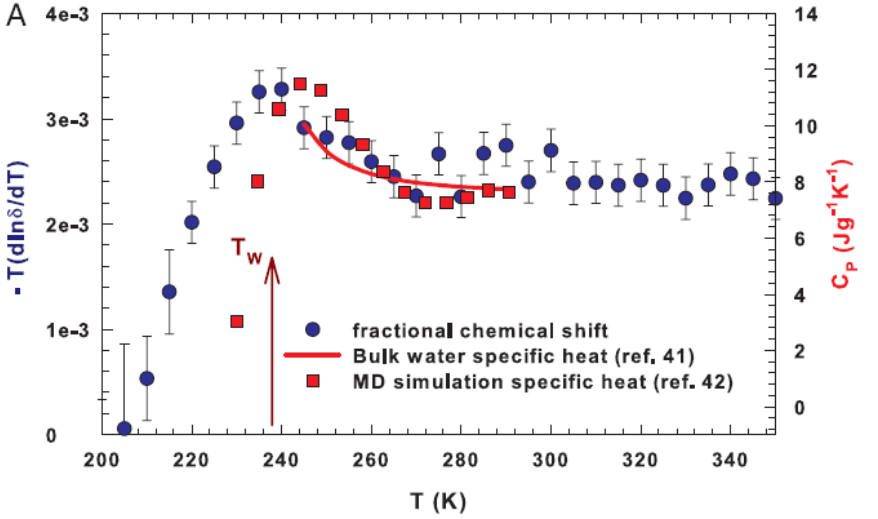
Thus, the derivative of δ with respect to the temperature gives a quantity proportional to the configurational part of the isobaric specific heat

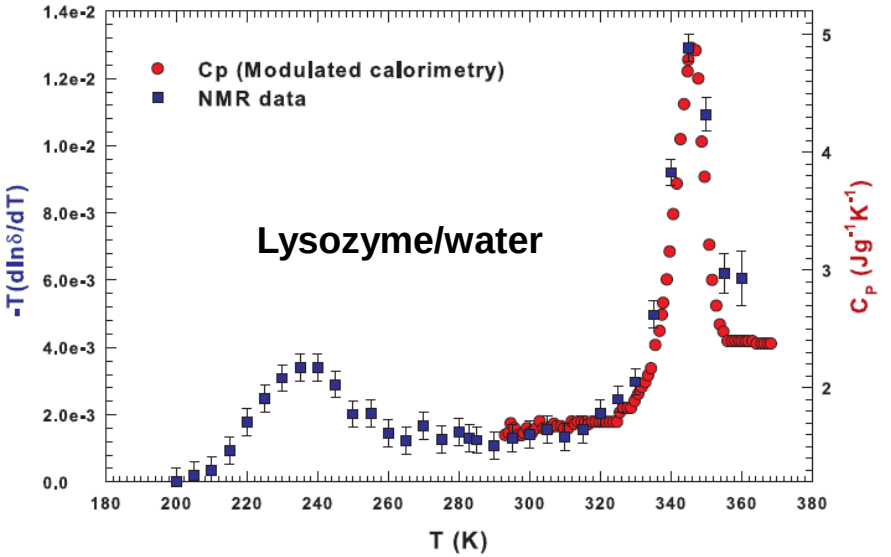
$$-T\left(\frac{\partial \ln \delta(T)}{\partial T}\right)_P \approx -T\left(\frac{\partial \ln < NHB >}{\partial T}\right)_P \approx T\left(\frac{\partial S}{\partial T}\right)_P = C_p(T)$$

Results on supercooled confined water:

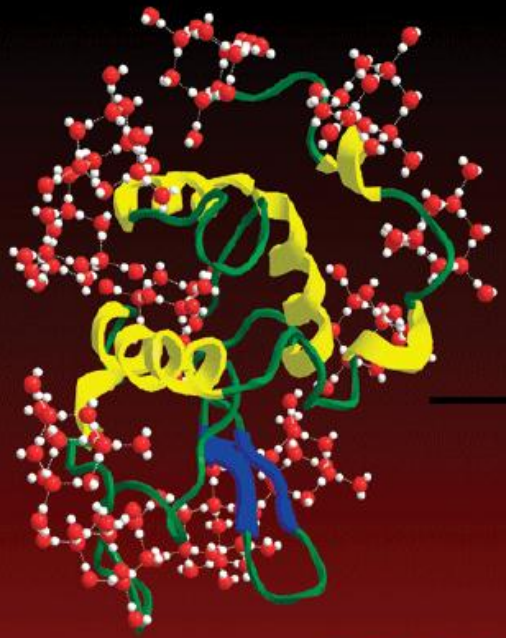
- *NMR Evidence of a Sharp Change in a Measure of Local Order in Deeply Supercooled Confined Water*







F. Mallamace, C. Corsaro, M. Broccio, C. Branca, N. Gonzalez-Segredo, J. Spooren, S.-H. Chen, and H. E. Stanley, PNAS USA, 105, 12725-12729 (2008).



Folded Lysozyme
with water molecules
well organized.

REV.

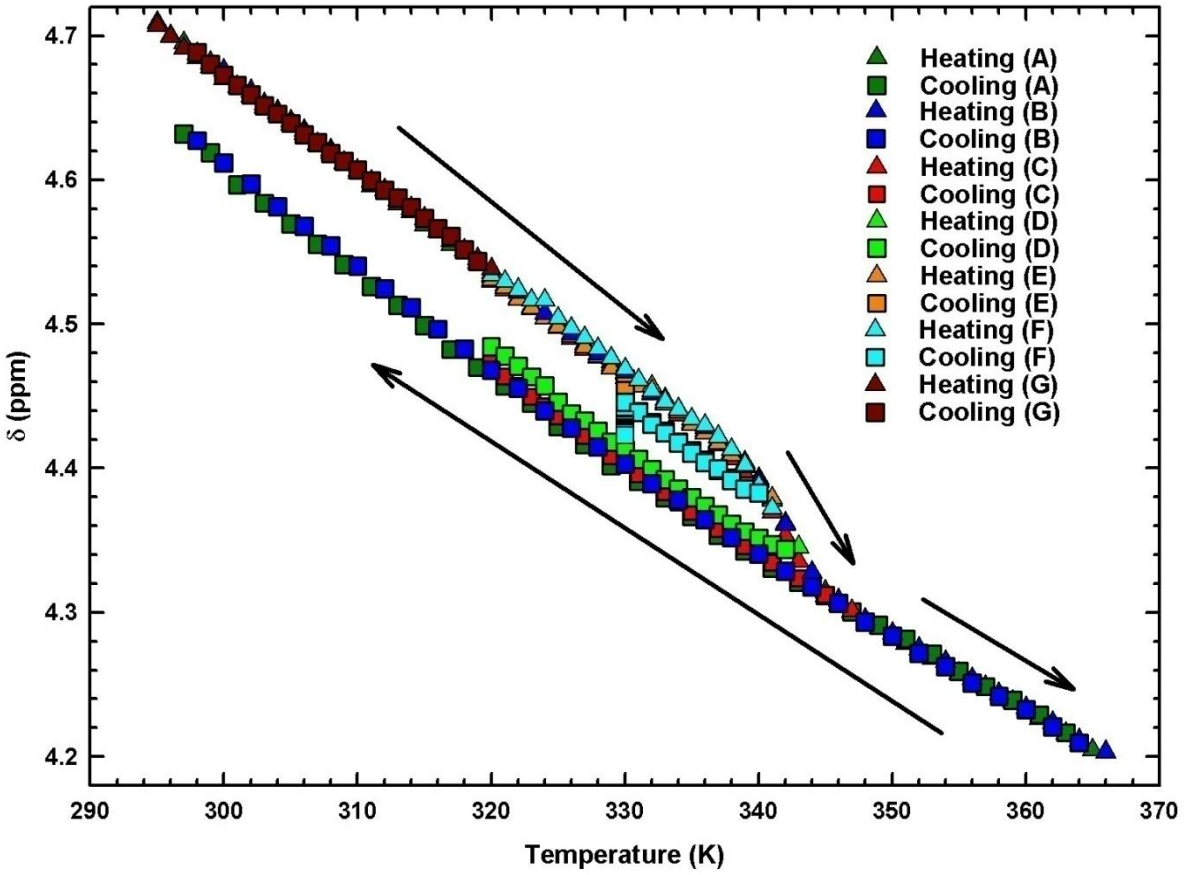
IRREV.

Denatured Lysozyme
with water molecules
not organized.

Results on water-lysozyme system:

The chemical shift of the lysozyme hydration water for different thermal cycles.

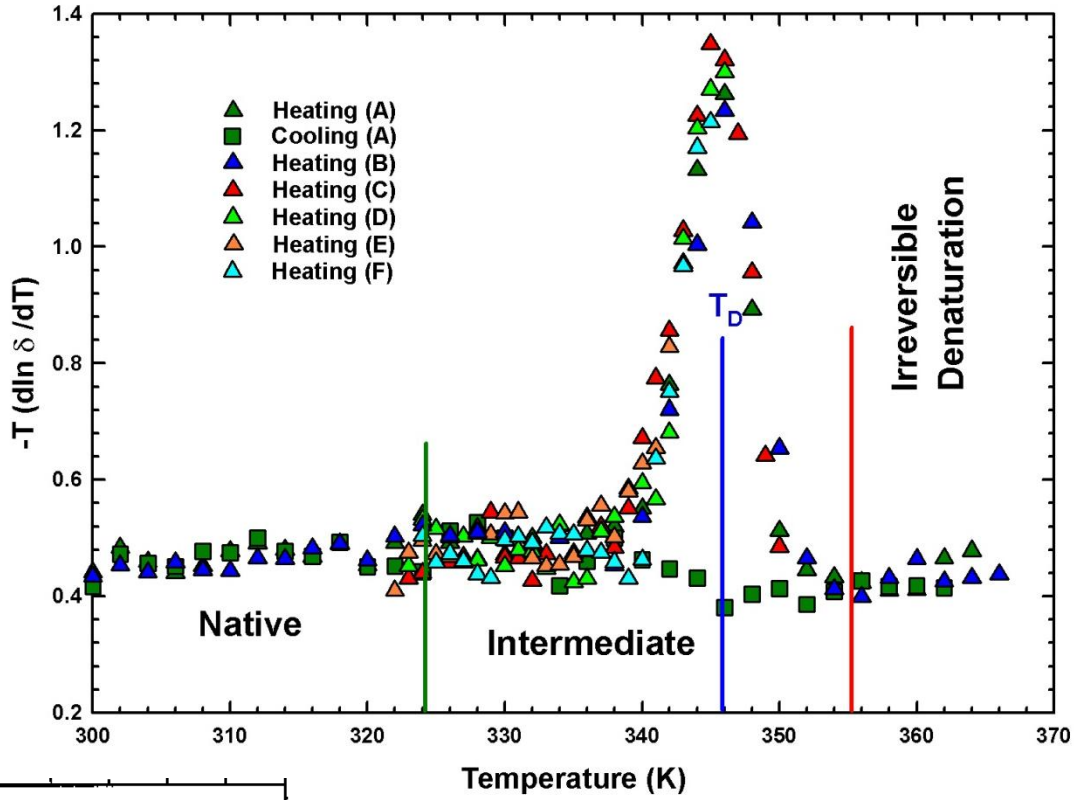
All the heating paths superimpose whereas the cooling paths strongly depend on the thermal history of the system. This influence is much more marked as the final temperature of the heating cycle is close to 346K. Note the sharp change of the slope relative to the heating path between 336K and 346 K. During the cooling phase the data rise almost linearly on decreasing temperature.

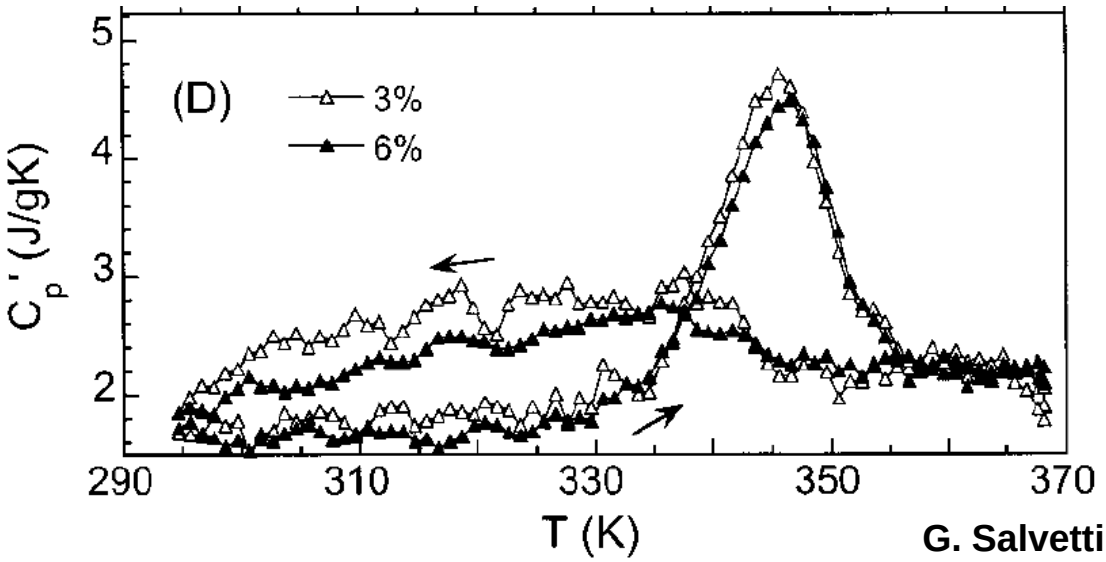


F. Mallamace, C. Corsaro, D. Mallamace, P. Baglioni, H. E. Stanley and S.-H. Chen, JPCB 115, 14280-14294 (2011).

The configurational part of the isobaric specific heat obtained by the chemical shift data as a function of the temperature.

The behavior is in complete agreement with calorimetric measurements on the same system.



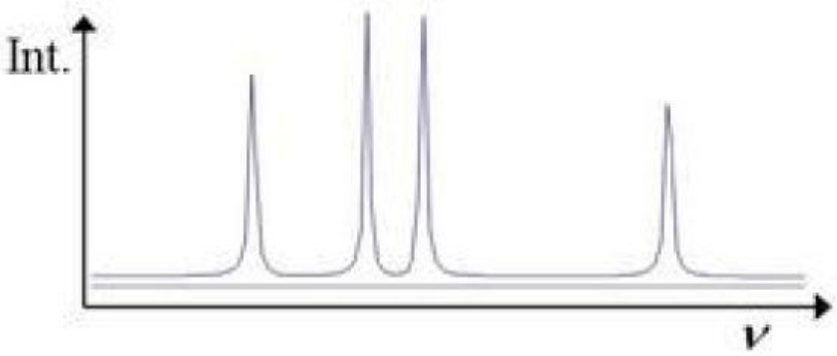


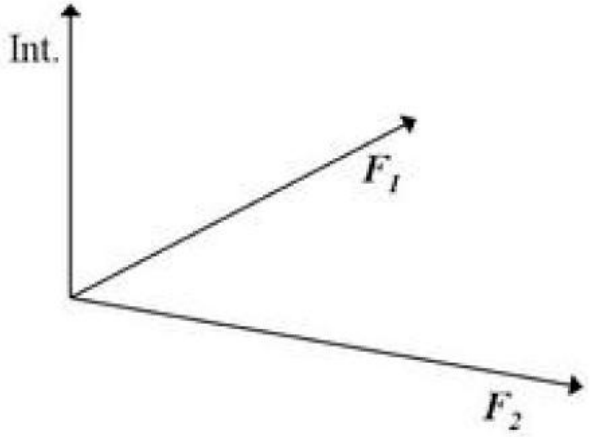
G. Salvetti, E. Tombari, L. Mikheeva, and G. P. Johari, JPCB 106, 6081-6087(2002).

2D NMR spectroscopy

The "normal" NMR spectrum is mono-dimensional or 1D where the plot of an intensity as a function of a frequency, $\nu = \omega - \omega_0$ (or F) of the observed nucleus (e.g. ^{1}H or ^{13}C), is reported.

However, it is possible to perfom NMR spectra where the intensity (or magnetization) is a function of two frequencies (F1 e F2) of the same nucleus or different. This is known as bi-dimensional or 2D NMR and the plot shows an intensity value for each pair of frequency variables. The intensities of the peaks in the spectrum can be represented using a third dimension. More commonly, intensity is indicated using contour lines or different colors.





2D NMR spectra can be divided into two big categories:

1. ***Homonuclear (when the observation nucleus is the same for both frequencies)***
   - **Through bond: COSY (COrrelation SpectroscopY), TOCSY (TOtal Correlation SpectroscopY), 2D-INADEQUATE (Incredible Natural Abundance DoublE QUAntum Transfer Experiment), 2D-ADEQUATE (Adequate DoublE QUAntum Transfer Experiment);**
   - **Through space: NOESY (Nuclear Overhauser Effect SpectroscopY), ROESY (Rotating frame Overhause Effect SpectroscopY)**

2. ***Heteronuclear (when the observation nucleus along the frequencies F2 is different from that on the frequencies F1).***
   - **One-bond correlation: HSQC (Heteronculear Single Quantum Coherence), HMQC (Heteronuclear Multiple-Quantum Coherence)**
   - **Long-range correlation: HMBC (Heteronuclear Multiple Bond Coherence)**

2D NMR spectra provide more information about a molecule than 1D NMR spectra and are especially useful in determining the structure of a molecule, particularly for molecules that are too complicated to work with using 1D NMR.

HR-MAS NMR 2D spectra

COSY (COrrelation SpectroscopY) and TOCSY (TOtal Correlation SpectroscopY) are both 2D homonuclear NMR spectra through bonds, their main difference is in the magnetization coupling (or transfer) between different nuclei.

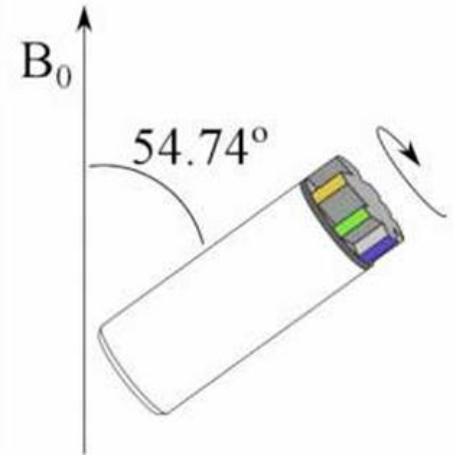
- **In the COSY spectroscopy, M(ω) couples directly only among nearest neighbour nuclei (e.g. in the case of lactic acid : CH_3 and CH).**

- **In the TOCSY case M(ω) instead couples also indirectly between atoms of separate chemical groups providing that they are linked through the observed nucleus or molecular groups containing it. (e.g. the first and final CH_2 of a polymer chain; in our case the sebacic acid chain).**

Their simultaneous analysis in the same system allows the unambiguous identification of a precise simple or complex molecular structures (or metabolite).

High-Resolution Magic Angle Spinning (HR-MAS) NMR

The advantage of this technique is to reduce the line broadening of NMR peaks by tilting samples of a precise angle (about 54.7°) with respect to the direction of the applied magnetic field.
In fact, in this way the Hamiltonian term corresponding to dipolar interactions vanishes and NMR peaks become narrower.
In addition, the rotor is spun at few thousands of Hertz to decrease the line broadening effects due to susceptibility differences within the sample.



SCIENTIFIC REPORTS

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OPEN

SUBJECT AREAS:
CHEMICAL PHYSICS
BIOPOLYMERS
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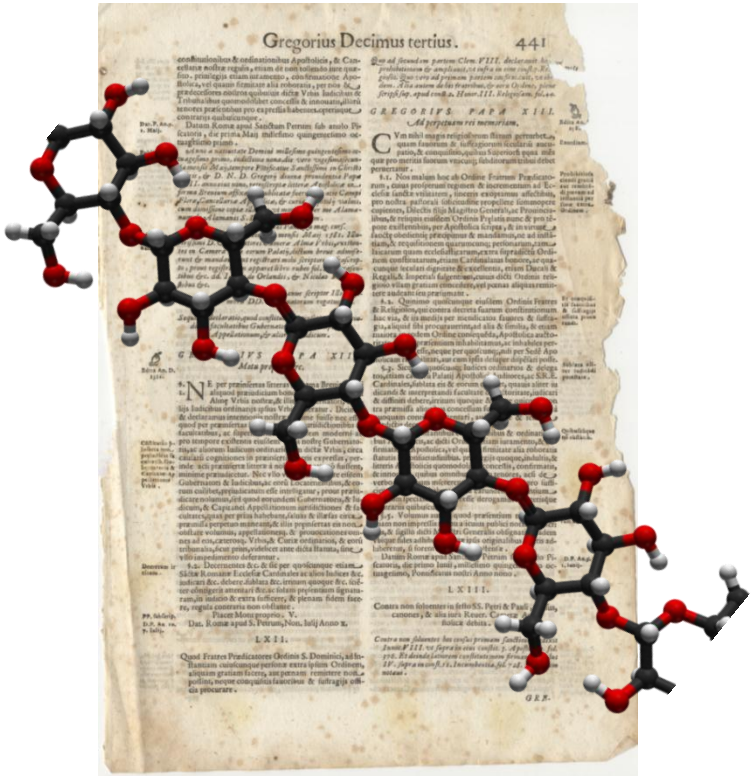
Molecular degradation of ancient documents revealed by ^{1}H HR-MAS NMR spectroscopy

Carmelo Corsaro[1], Domenico Mallamace[2], Joanna Łojewska[3], Francesco Mallamace[1], Luciano Pietronero[4] & Mauro Missori[5]

SEM image of cellulose fibers in ancient paper



Methods

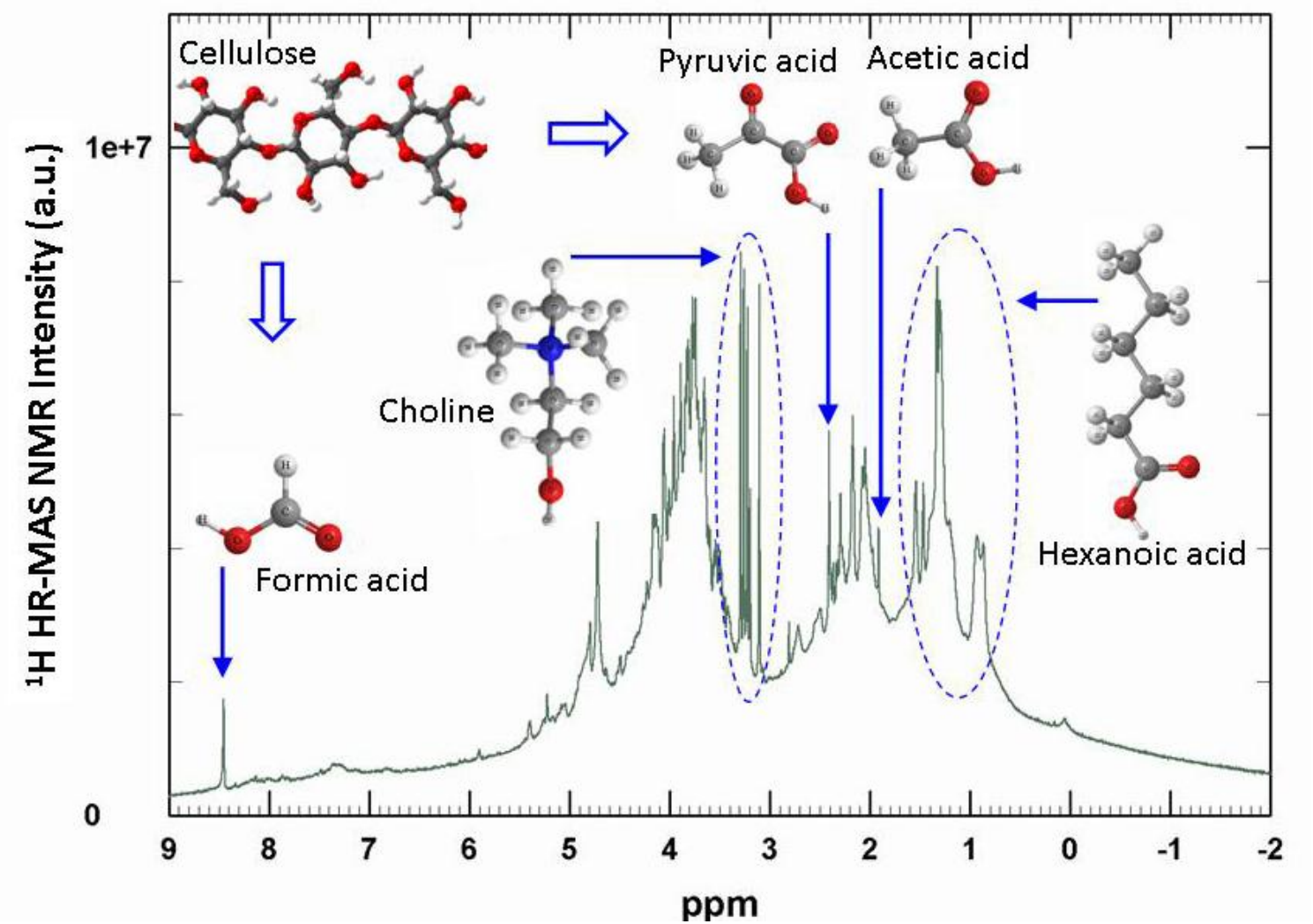
Ancient samples used in this research consist of small pieces of paper, produced in the 15th century in European countries named A1 and B1 (both made in Perpignan, France, in 1413) and A3 (made in Milan, Italy, in 1430), in bad conservation conditions (within a water stain).

Model samples, obtained from Netherlands Organization for Applied Scientific Research (TNO), are made of unbleached cotton linters, containing very low inorganic ingredients (ash content < 0:005% in weight) and no additives or lignin, with high mean degree of polymerization. These samples are named P2, P2C9024 and P2C9048; they were artificially aged in air in a climatic chamber at relative humidity of 59% and temperature of 90°C for 24 and 48 days, respectively. Cellobiose was used as a reference.

^{1}H one and two dimensional NMR experiments on ancient and modern cellulose samples were conducted at ambient pressure by using a Bruker Avance spectrometer operating at 700MHz ^{1}H resonance frequency in the experimental configuration known as Magic Angle Spinning (MAS). Small rectangular pieces, 0.7 x 1.8 cm^2, of about 10mg in weight, of different papers were rolled up, placed in the rotor together with 30μl of D_2O, sealed (with the corresponding insert) and spun at 7000 Hz at the magic angle to increase the spectral resolution.

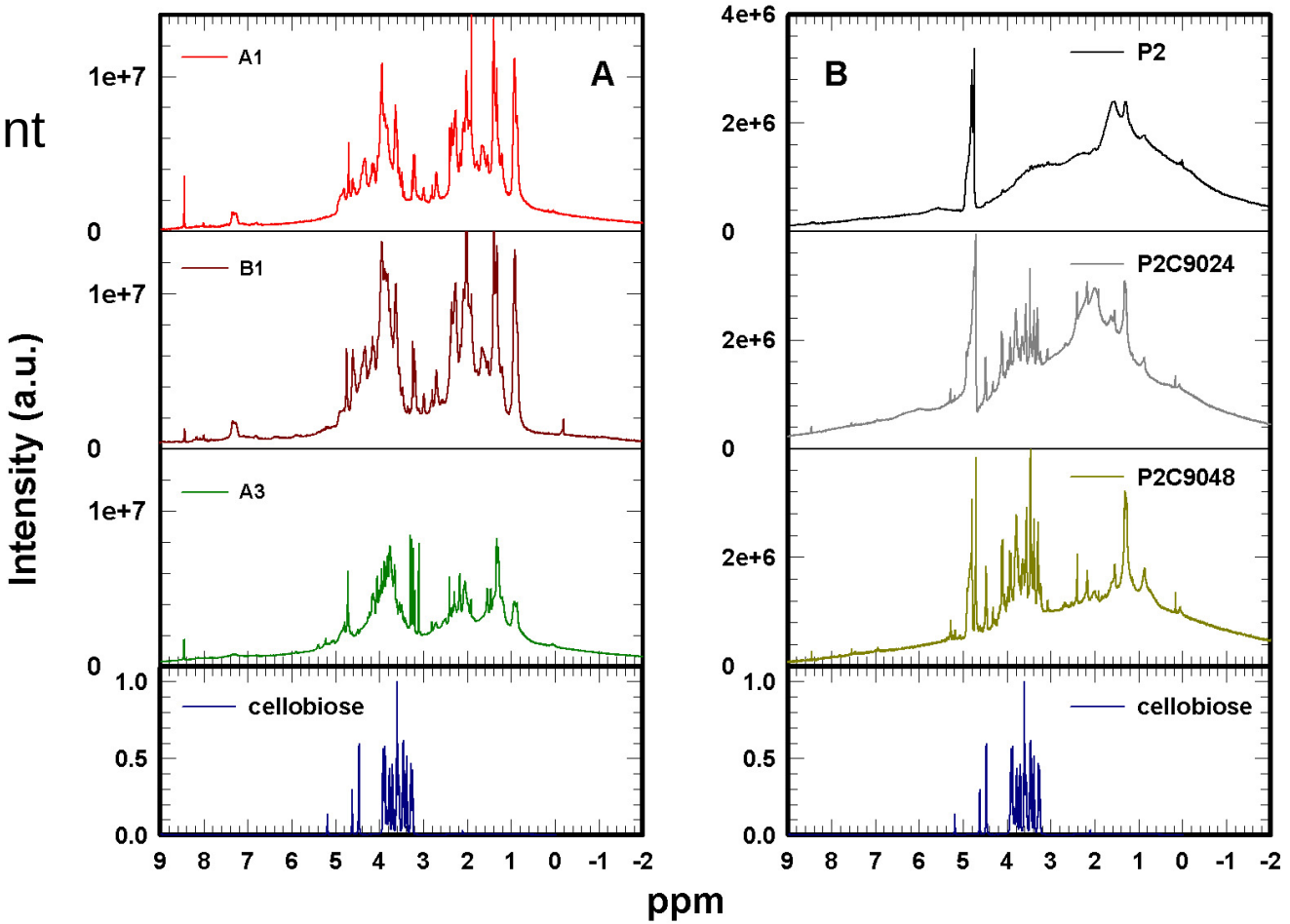
^{1}H HR-MAS NMR 1D spectra of sample A3

(Milan 1430, bad conservation)



^{1}H HR-MAS NMR 1D spectra I (9/-2 ppm)

Ancient



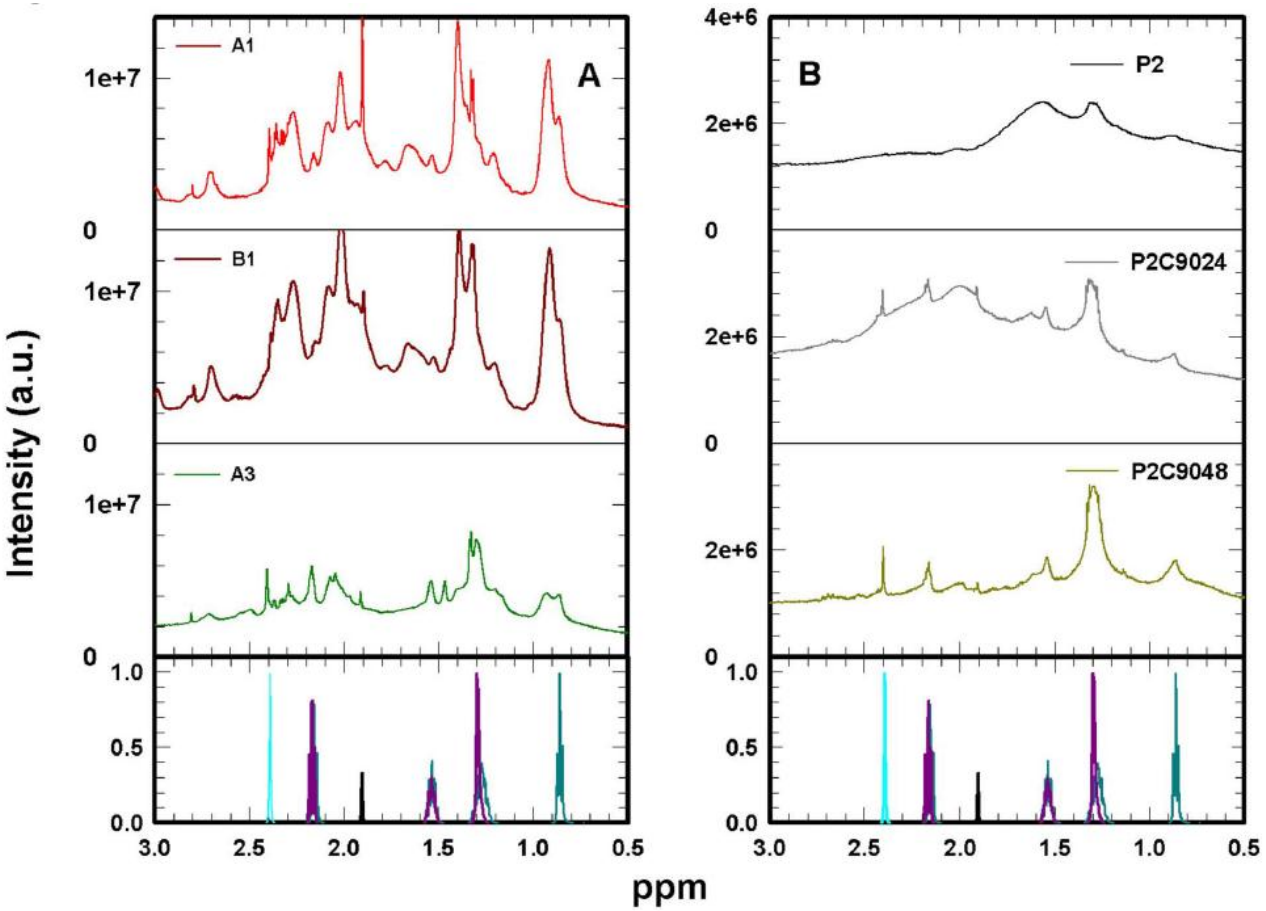
Modern

Degradation processes take place within amorphous regions, which makes ^{1}HR-MAS technique, especially, useful in their study and in recognizing degradation products in ancient samples.

As the paper ages (by applying high temperature and humidity), sharp peak components increase whereas the broad resonances decrease.

^{1}H HR-MAS NMR 1D spectra II (3/0.5 ppm)

Ancient



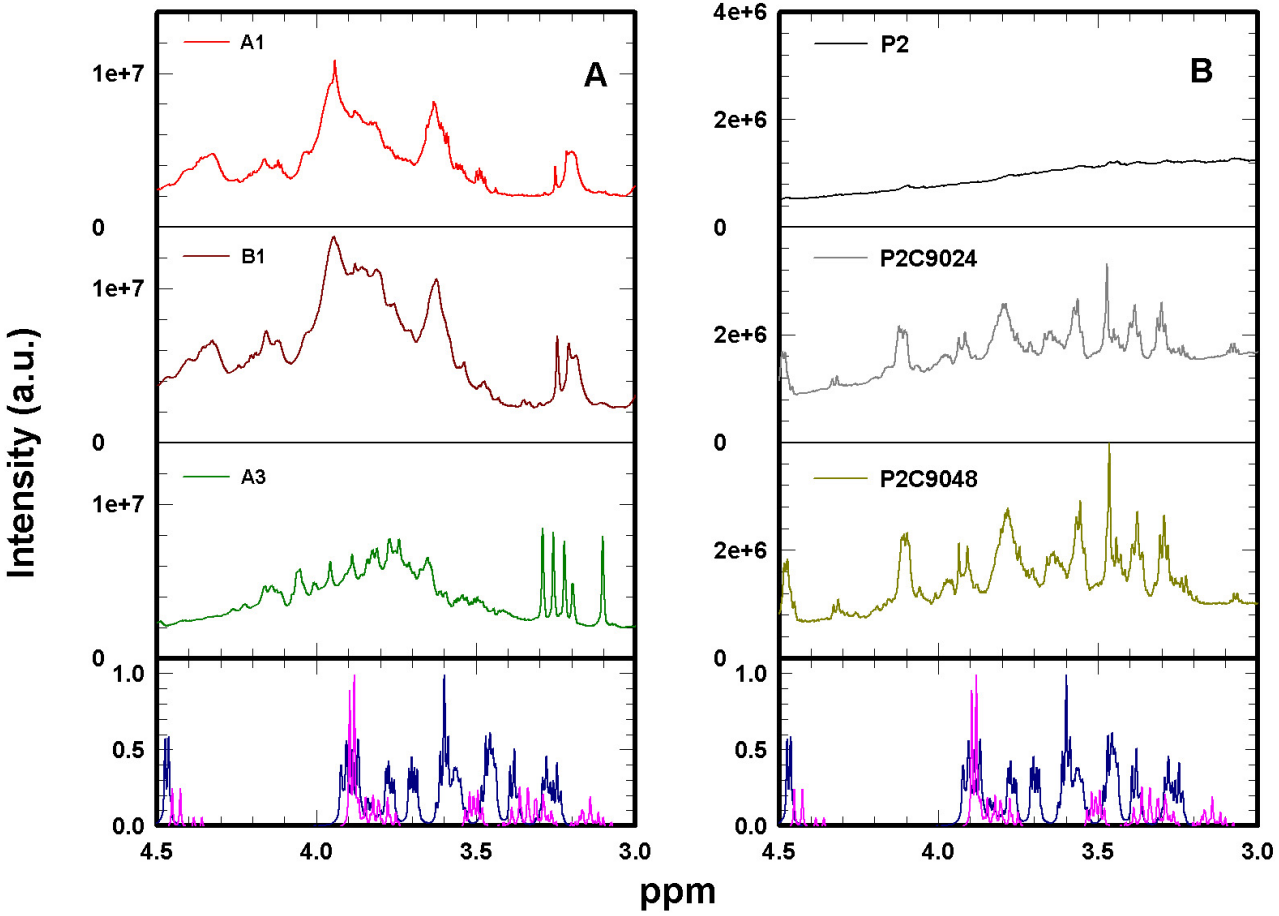
Modern

Region of carboxylic acids: acetic acid (black), hexanoic acid (dark green), pyruvic acid (purple) and sebacic acid (pink).

The presence of carboxylic acids together with the cellobiose-like fragments in modern aged samples, is an indication of coupling between hydrolytic and oxidative degradation routes.

^{1}H HR-MAS NMR 1D spectra III (4.5/3 ppm)

Ancient



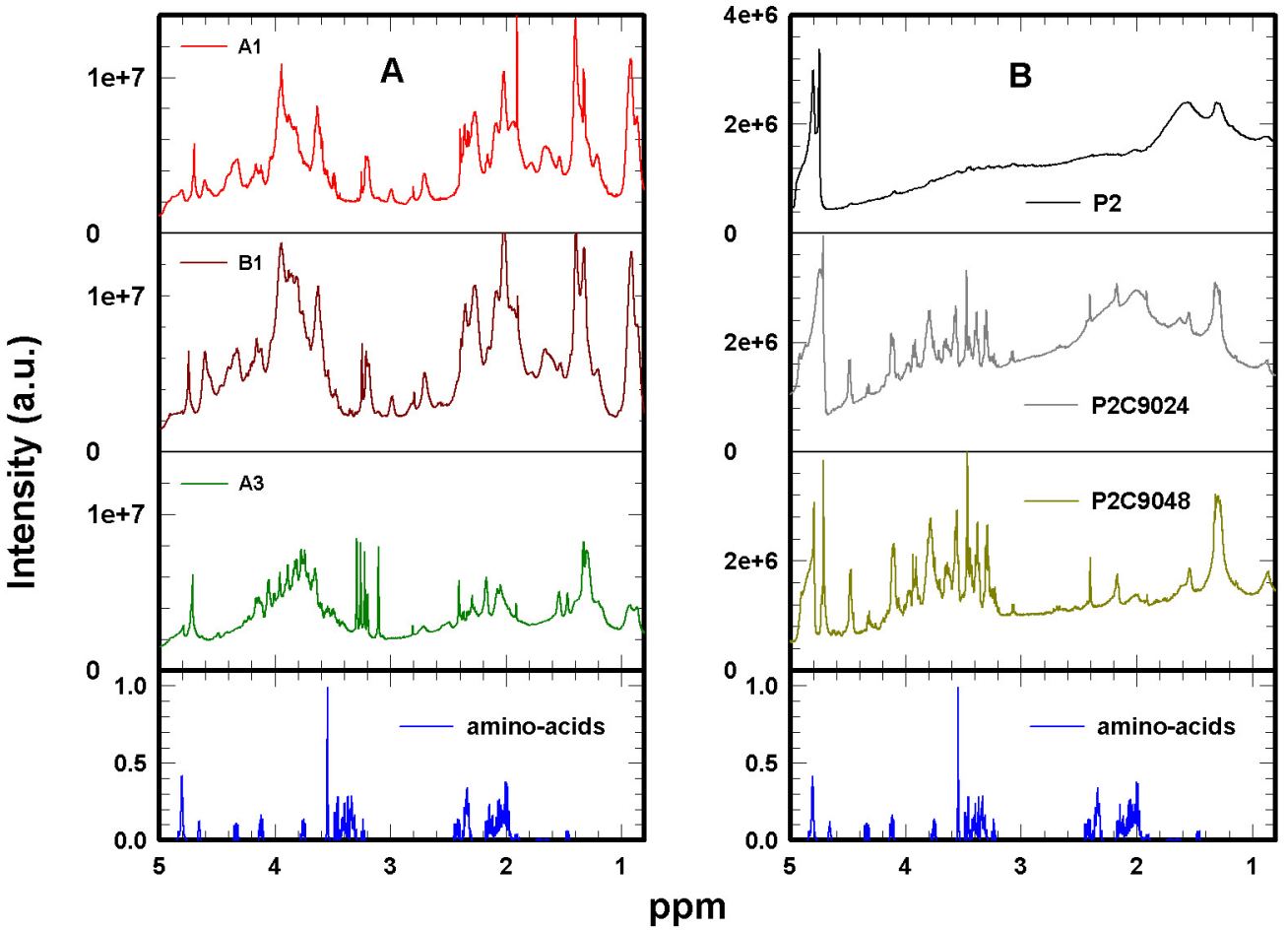
Modern

Region of cellulose (dark blue), and cellobiose (purple).

In ancient samples, the characteristics peaks of cellobiose are less evident. Most intense peaks are likely due to hydroxy-carboxylic acids, such as glucaric and lactic acids. Glucaric acid is derived from the oxidation of D-glupyranose, which can be also oxidized to pyruvic acid and finally transformed in lactic acid.

^{1}H HR-MAS NMR 1D spectra IV (5/1 ppm)

Ancient



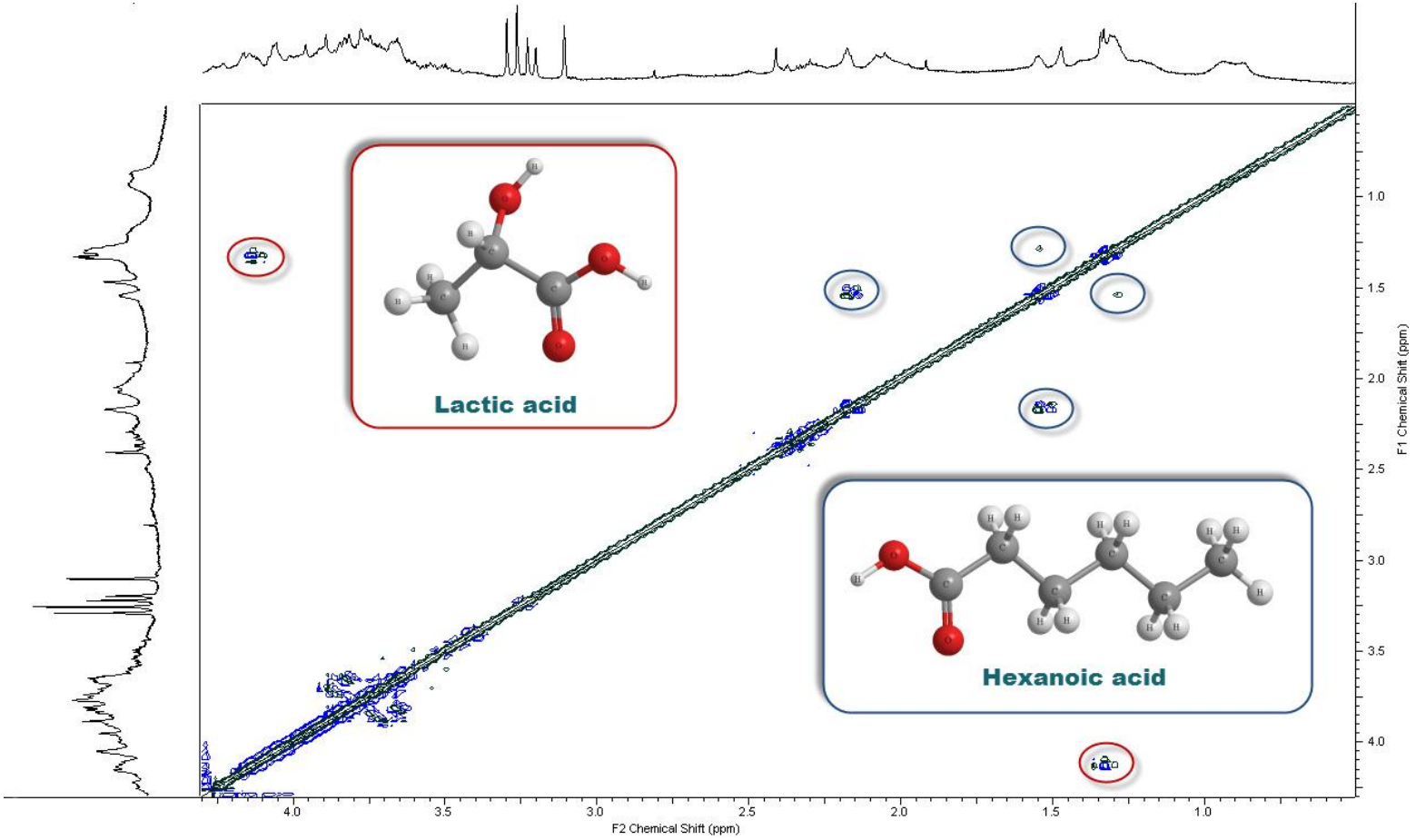
Modern

Region of amino-acids

$$I = 0.329Gly + 0.126Pro + 0.109Ala + 0.095Hyp + 0.074Glu + 0.047Asp + 0.049Arg$$

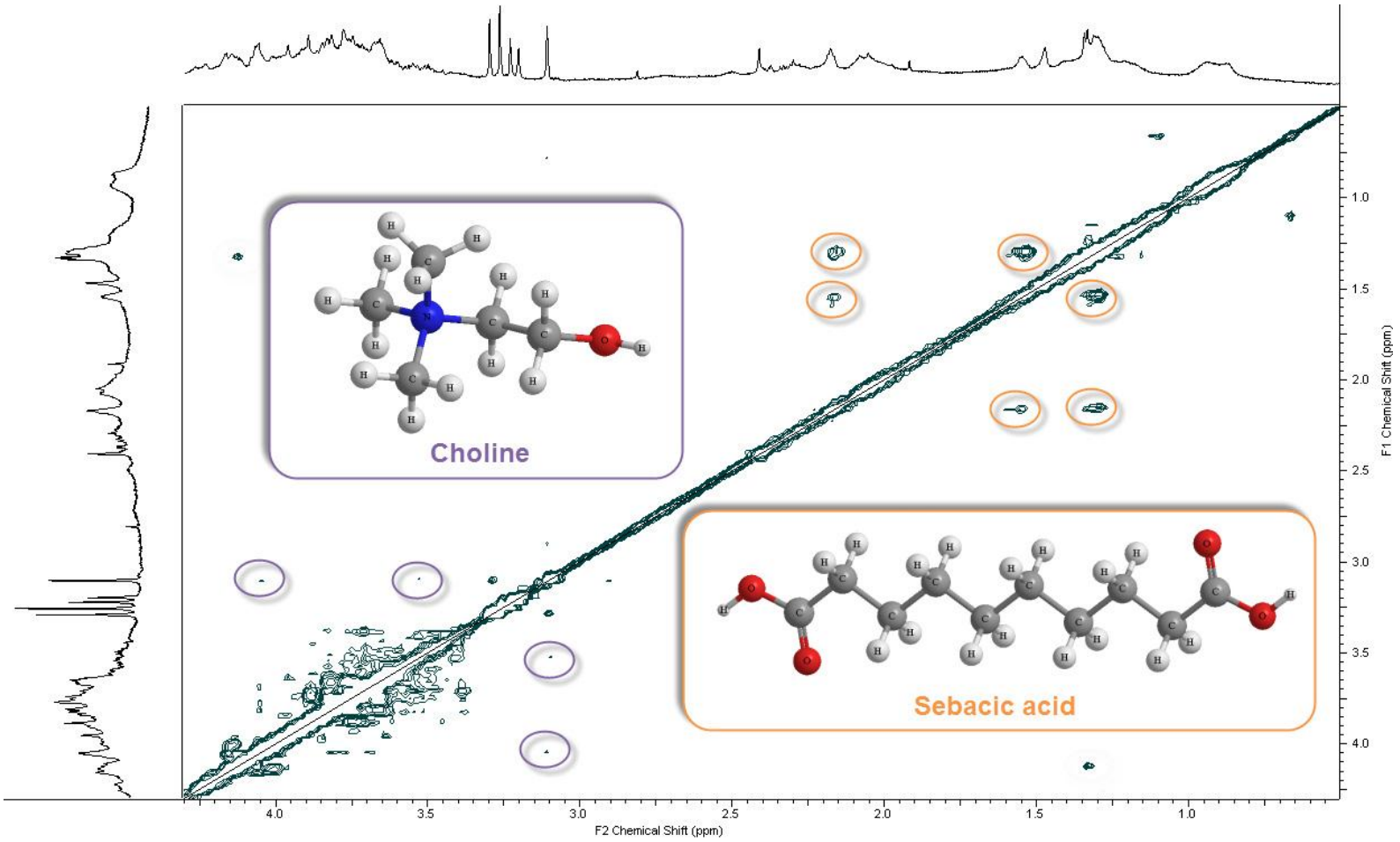
We use the average amino-acid composition for fish and mammal skin, and consider only those present at least at 4.9 wt%. The agreement between spectra of ancient paper and collagen seems better for A1 and B1 samples.

^{1}H HR-MAS NMR 2D spectra: COSY on A3



The cross-peak at (1.*54; 2.16) ppm in sample A3* confirms the presence of hexanoic and adipic acids, whereas that at (1.*3; 4.1) ppm indicates the* presence of lactic acid. In fact, during paper degradation glucose can be broken and oxidized to pyruvic acid, which in turn can be transformed in lactic acid.

^{1}H HR-MAS NMR 2D spectra: TOCSY on A3



Some cross-peaks along the 3.*1 ppm* direction, characteristic of the particular compound found in the A3 sample, are clearly visible.

These should belong to ammonium salts, such as choline, probably due to the use of fish collagen for paper sizing during production.

Conclusions I

- **For the first time, artificially aged paper samples, products of cellulose degradation were individually detected, by this study, in solid paper samples.**

- **Carboxylic acids, together to more complex dicarboxylic and hydroxy-carboxylic acids, were found in all the studied samples.**

- **The results evidence the presence of carboxylic acids in all unaged and artificially aged model samples made of pure cellulose and in naturally aged samples of ancient paper.**

- **When degradation is enhanced in paper by heating at a temperature of 90°*C* in the presence of humidity, a larger set of different acidic products appear in time including low molecular mass aliphatic acids.**

Conclusions II

- Ancient samples show more degradation products of similar structure;
- interestingly, several degradation products can be distinguished in these samples even if they have been kept at room temperature along their life: pyruvic acid, hydroxy-carboxylic acid, such as glucaric and lactic acid, are clearly identified by the NMR spectra;
- this indicates that degradation phenomena in paper materials are driven by complex reactions that take place during centuries even at ambient conditions;
- the knowledge of specific degradation products, when associated with their formation rate, could also give hints concerning condition and also age estimation of ancient materials based on cellulose;
- this knowledge could be used to improve conservation, preservation and restoration of cultural heritage made of cellulose, and suggest for their dating, authentication and provenance.

Thanks for your attention!

NMR spectroscopy is an absorption technique in which the radio-frequency (RF) waves absorbed by the studied nucleus in the presence of an external magnetic field impose proper dynamical changes at nuclear level.