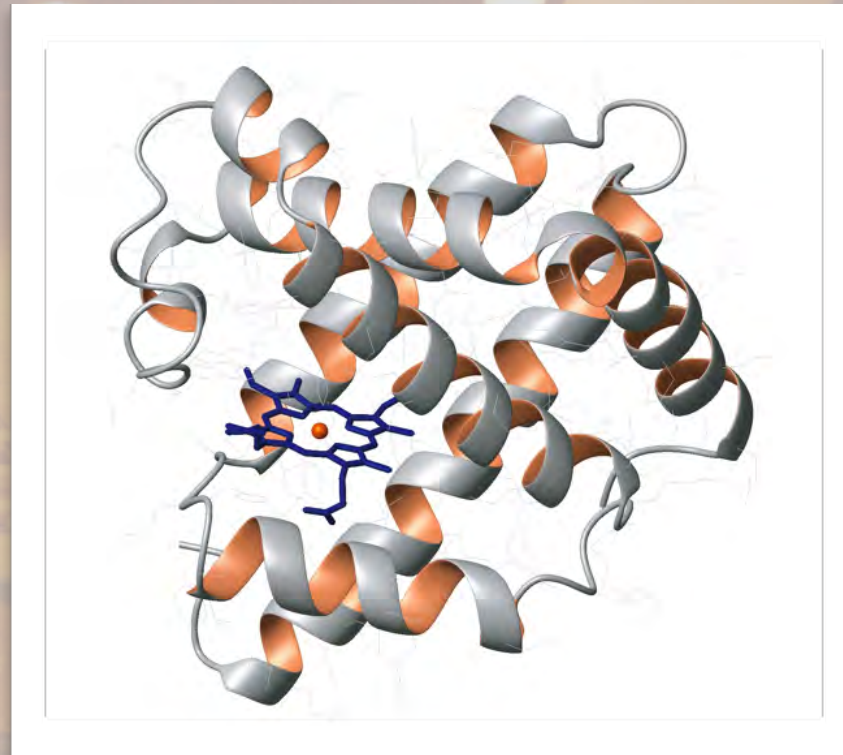
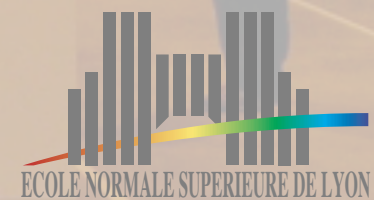


Résonance Magnétique Nucléaire: Seeing Invisible Things



Lyndon Emsley,
Université de Lyon, Centre de RMN à Très Hauts Champs



imagine

... si l'on pouvait transformer le CO₂ dans l'atmosphère en pétrole, combattant ainsi le changement climatique et fournissant des ressources pour les générations futures ...

(le développement durable)

Centre de RMN à Très Hauts Champs à Lyon

imagine

... si l'on pouvait réparer les dommages infligés
par la maladie d'Alzheimer, rendant les
souvenirs d'enfance perdus ...

(la biologie structurale)

Centre de RMN à Très Hauts Champs à Lyon

imagine

... si l'on pouvait diagnostiquer et traiter des
maladies graves avant qu'elles ne se
déclarent ...

(sciences biomédicales)

Centre de RMN à Très Hauts Champs à Lyon

imagine

... que vous êtes un rêveur ...

(john lennon)

Centre de RMN à Très Hauts Champs à Lyon

aujourd'hui

... la recherche peut transformer ces
rêves en réalité ...

$\omega_1, \omega_2 = \iint s(t_1, t_2) \exp\{-i(\omega_1 t_1 + \omega_2 t_2)\} dt_1 dt_2$
 $I(\omega_1, \omega_2) = \iint s(t_1, t_2) \exp\{-i(\omega_1 t_1 + \omega_2 t_2)\} dt_1 dt_2$
 $\mathcal{H}_D = \frac{1}{2} \frac{\mu}{\gamma} \frac{\hbar^2 \gamma_1 \gamma_2}{3} (1 - 3 \cos^2 \theta) (3 I_{1z} I_{2z} - \vec{I}_1 \cdot \vec{I}_2)$
 $\mathcal{H}_D = \frac{1}{2} \frac{\mu}{\gamma} \frac{\hbar^2 \gamma_1 \gamma_2}{3} (1 - 3 \cos^2 \theta) (3 I_{1z} I_{2z} - \vec{I}_1 \cdot \vec{I}_2)$
 $\mathcal{H}_D = \frac{1}{2} \frac{\mu}{\gamma} \frac{\hbar^2 \gamma_1 \gamma_2}{3} (1 - 3 \cos^2 \theta) (3 I_{1z} I_{2z} - \vec{I}_1 \cdot \vec{I}_2)$
 $\mathcal{H}_D = \frac{1}{2} \frac{\mu}{\gamma} \frac{\hbar^2 \gamma_1 \gamma_2}{3} (1 - 3 \cos^2 \theta) (3 I_{1z} I_{2z} - \vec{I}_1 \cdot \vec{I}_2)$

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la rmn

... ne peut résoudre aucun de ces défis seule ...

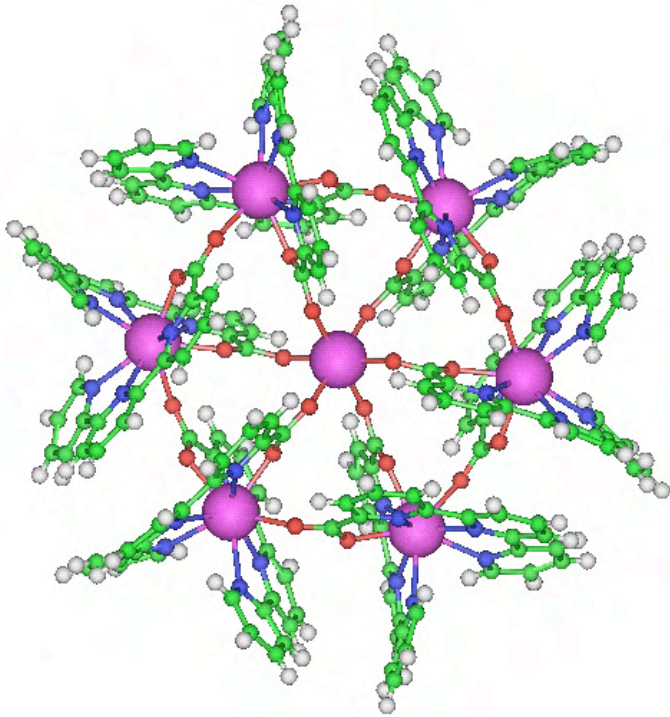
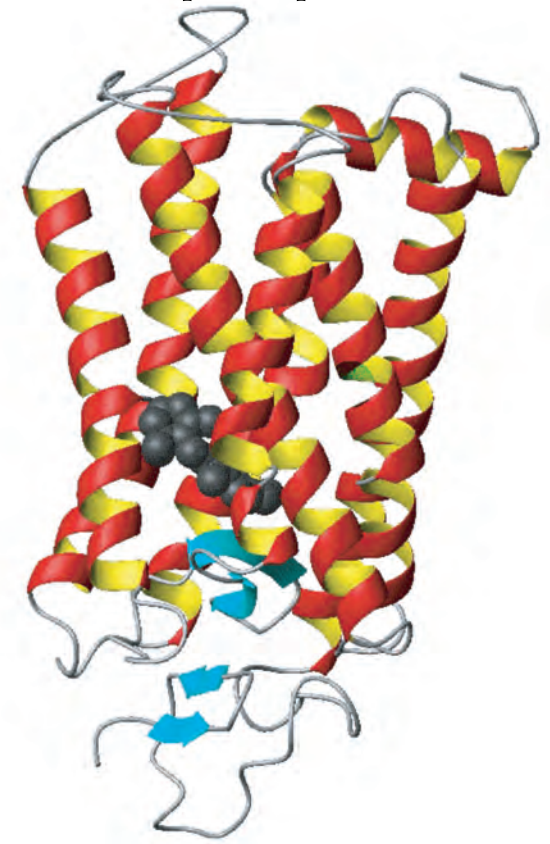
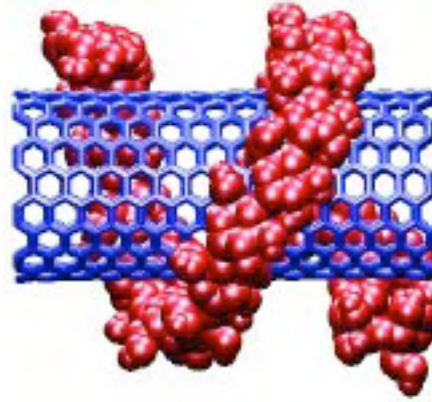
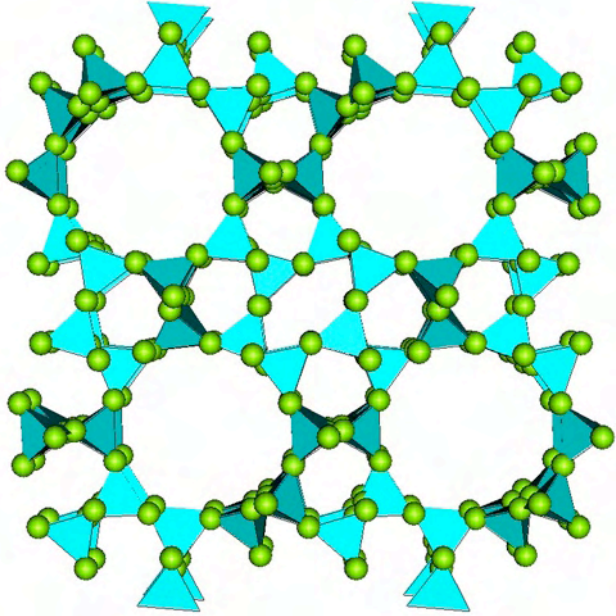
Centre de RMN à Très Hauts Champs à Lyon

la rmn

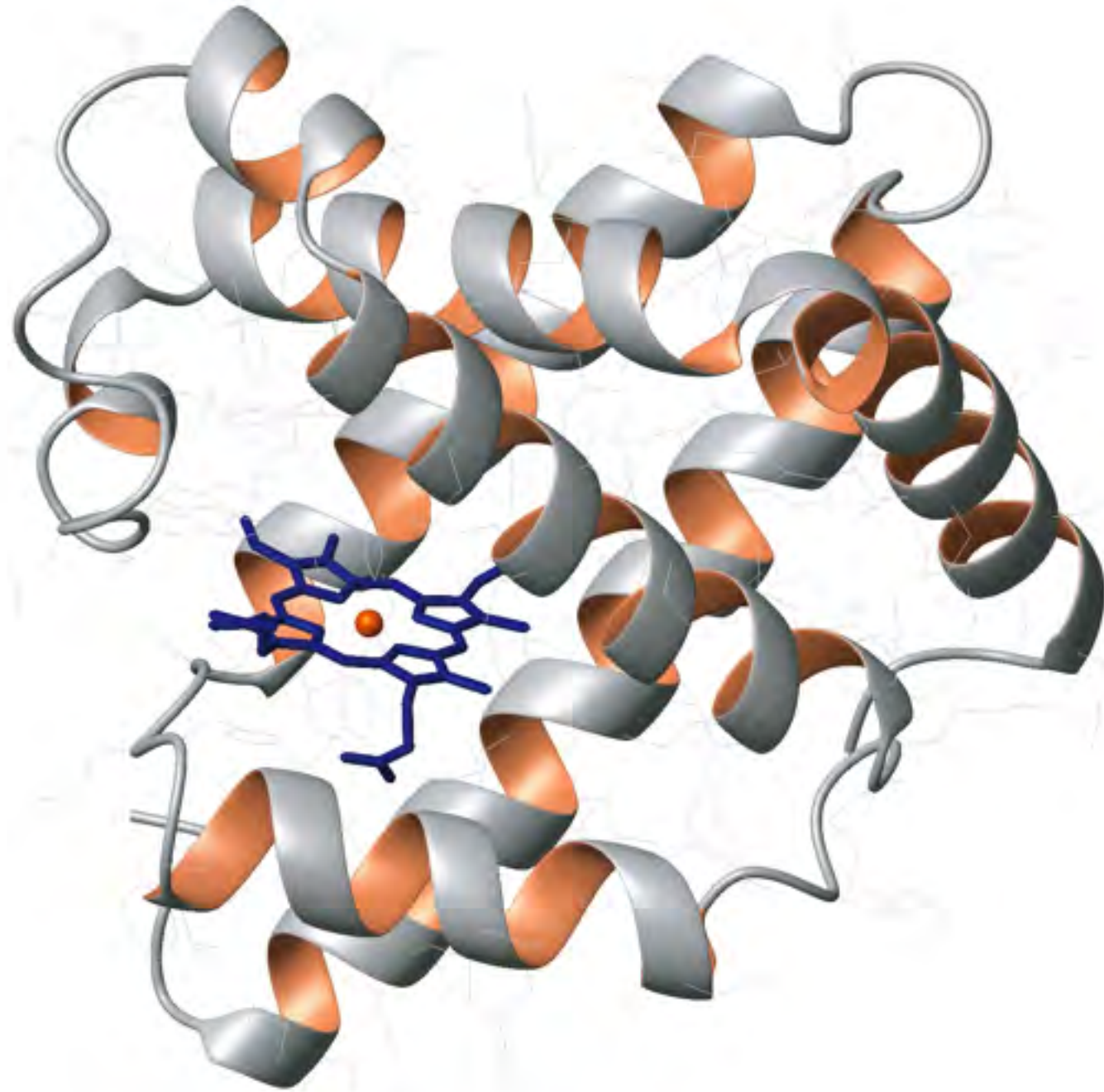
... mais aucun de ces défis sera vaincu
sans la rmn ...

Centre de RMN à Très Hauts Champs à Lyon

Produits Chimiques: La beauté microscopique de la recherche par curiosité



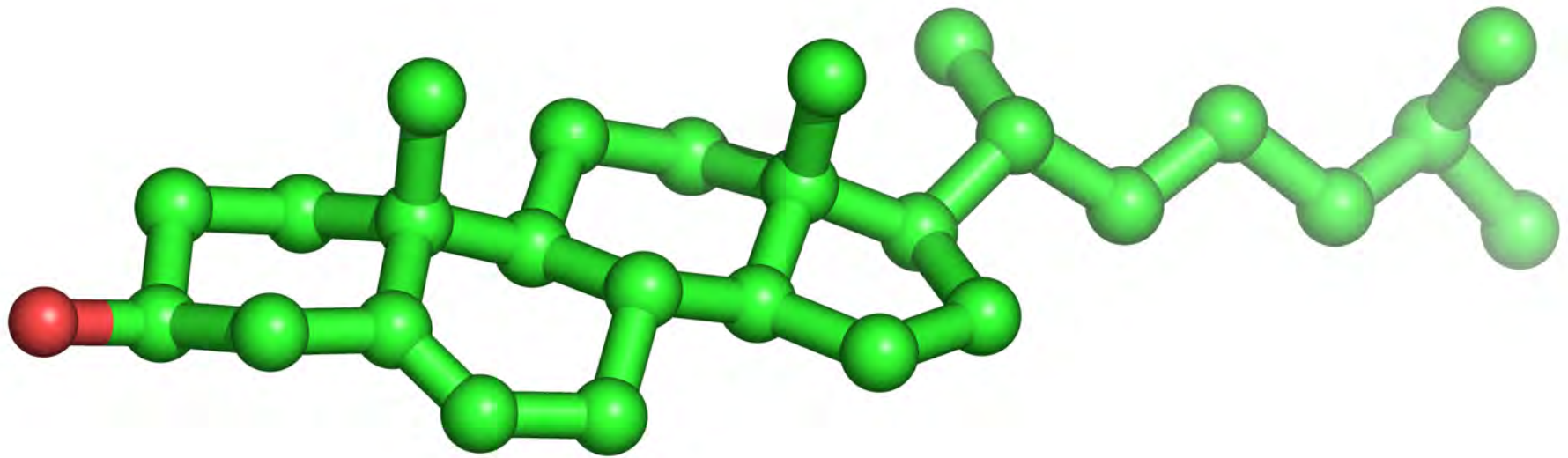
Produits Chimiques: La beauté microscopique de la recherche par curiosité



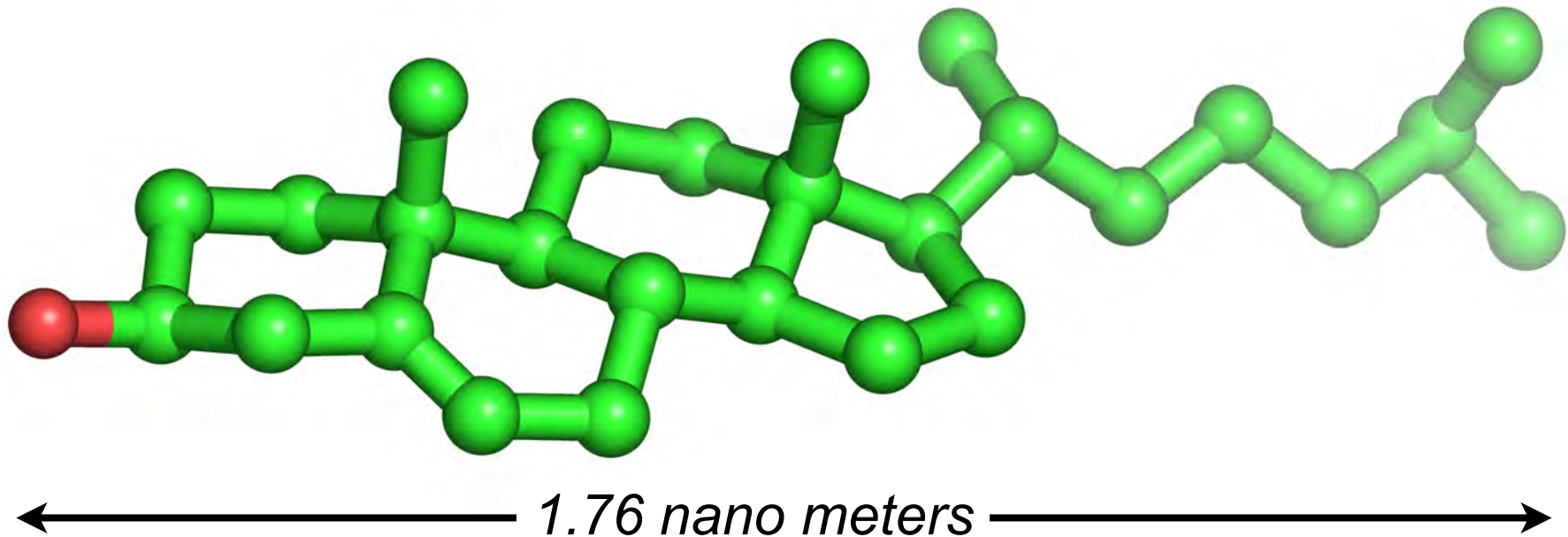


Ribosome

Représentation d'une Molécule de Cholestérol



Représentation d'une Molécule de Cholestérol



Représentation d'une Molécule de Cholestérol (Taille Réelle)

(rappel: 1 nano meter = 0.000000001 meters)

Représentation d'une Molécule de Cholestérol (Taille Réelle)

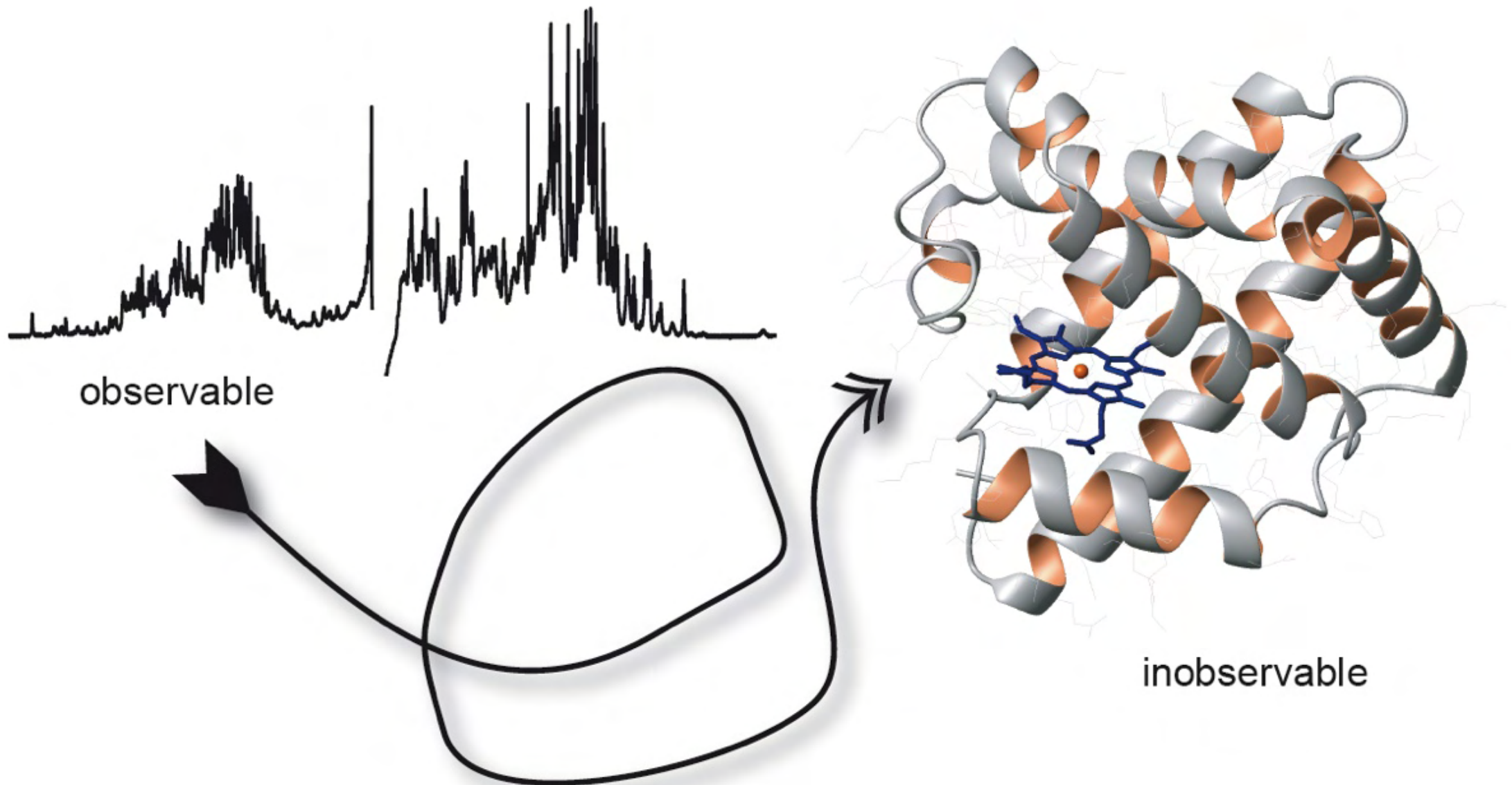
look very,
very closely??



(reminder: 1 nano meter = 0.000000001 meters)

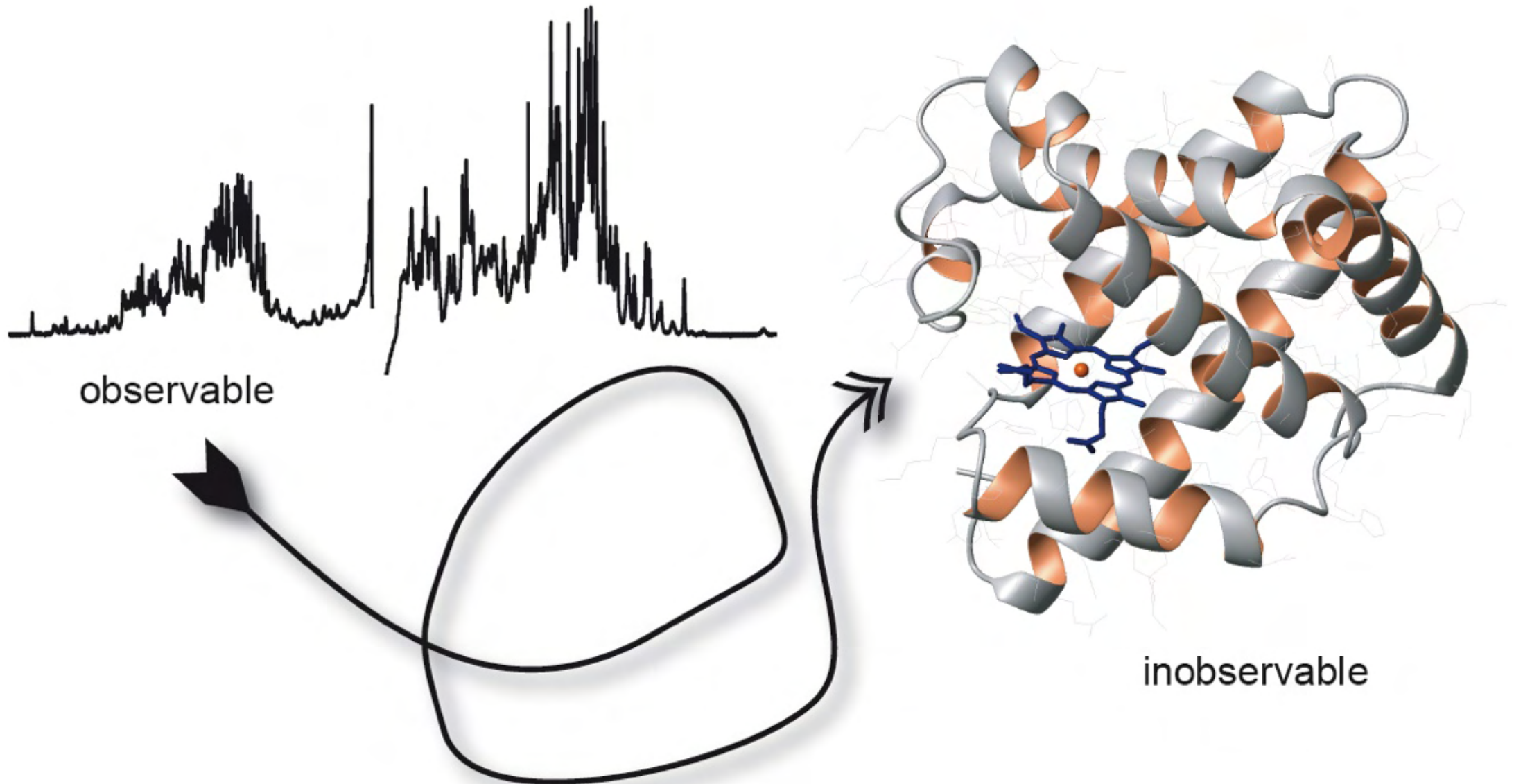
Comment Voir l'Invisible?

*il nous faut des espions... des **observables** qui sont des rapporteurs plus ou moins directs de la structure moléculaire.*



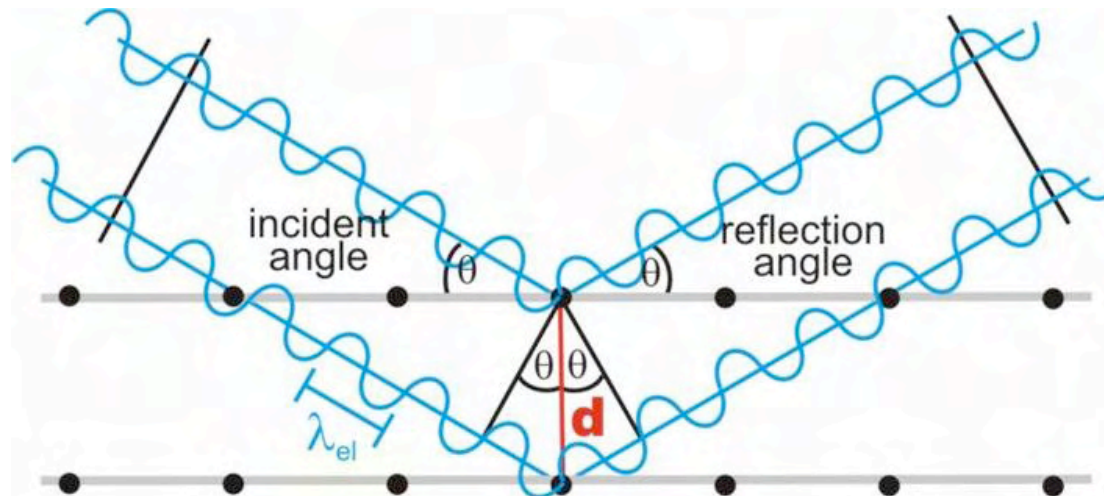
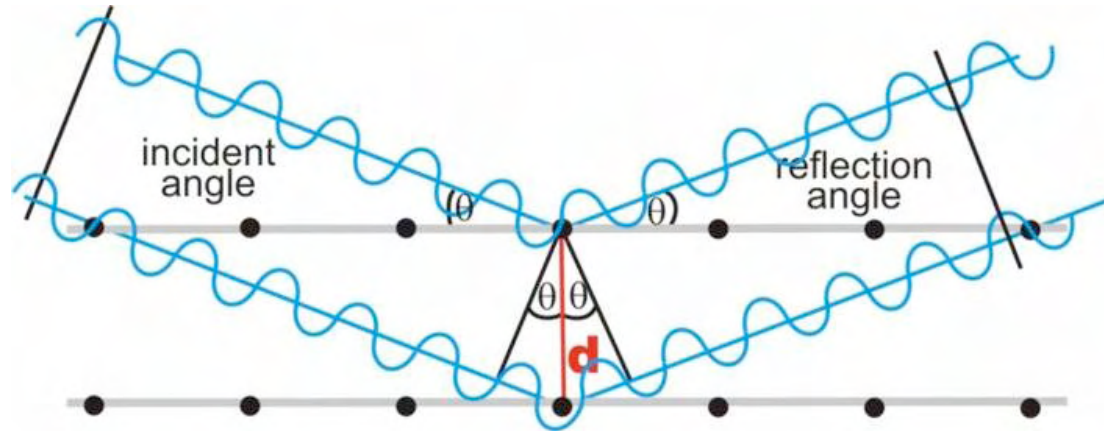
Comment Voir l'Invisible?

Nous allons *interpréter* les *observables* pour construire *un modèle* de la structure moléculaire.



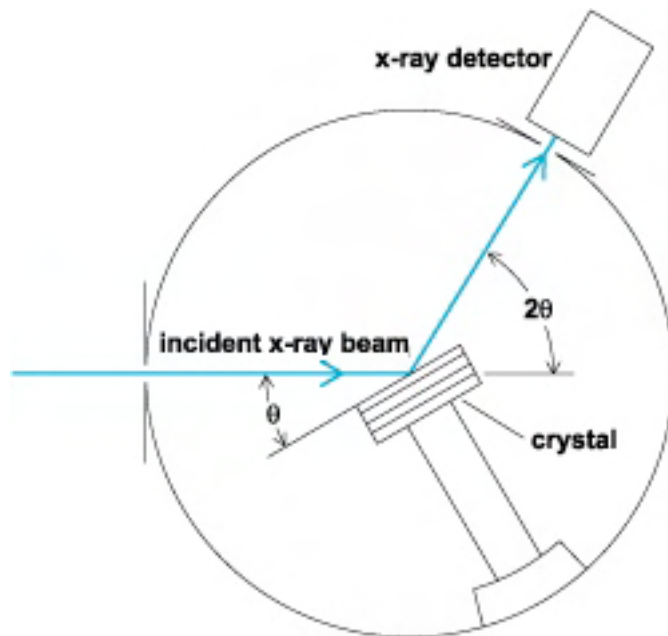
Observable N° 1: Diffraction Bragg de Rayons-X

$$n\lambda = 2d\sin\theta$$



Diffractomètre de Rayons-X: Détermination de Structure de Cristaux Uniques

$$n\lambda = 2d\sin\theta$$

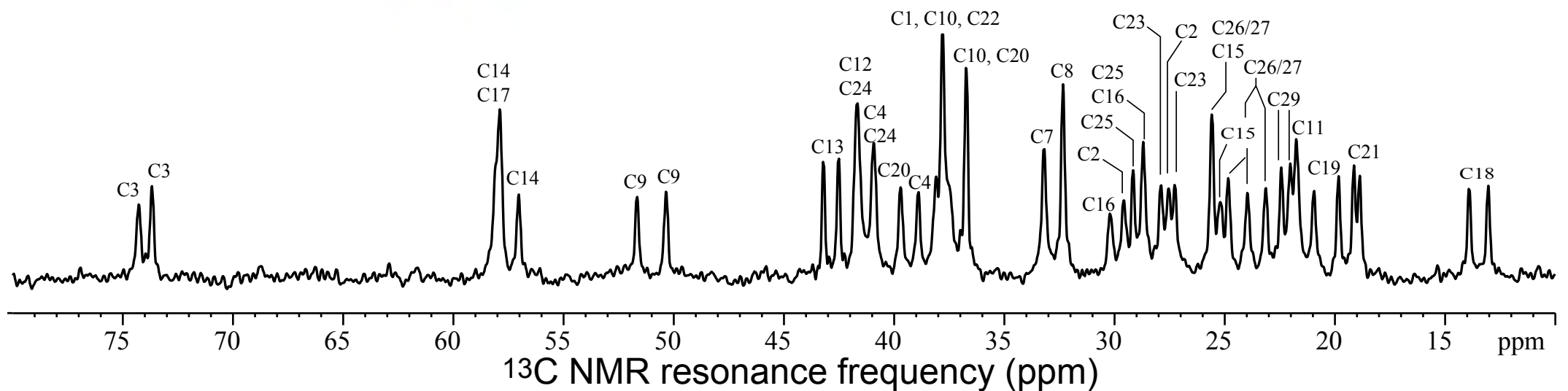
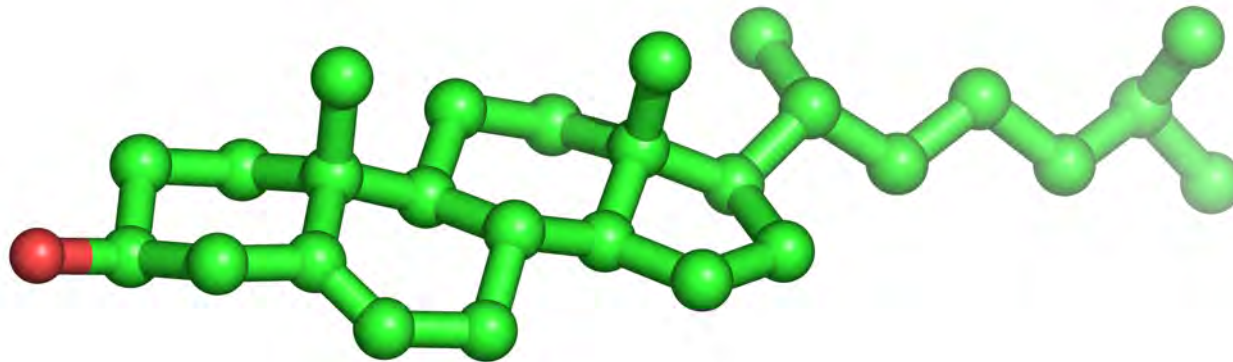


Diffractomètre de Rayons-X: Détermination de Structure de Cristaux Uniques

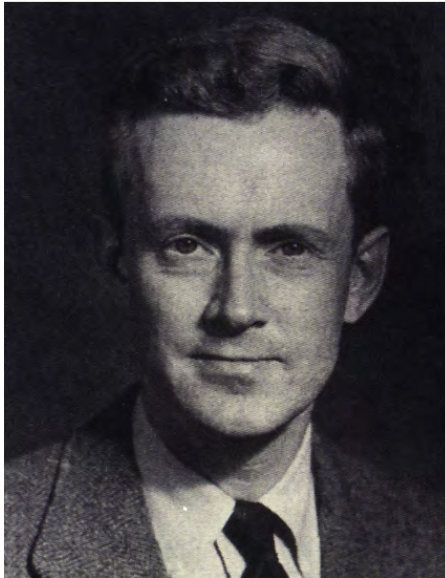


Une Alternative: La spectroscopie fournit des yeux aux sciences des matériaux et des molécules

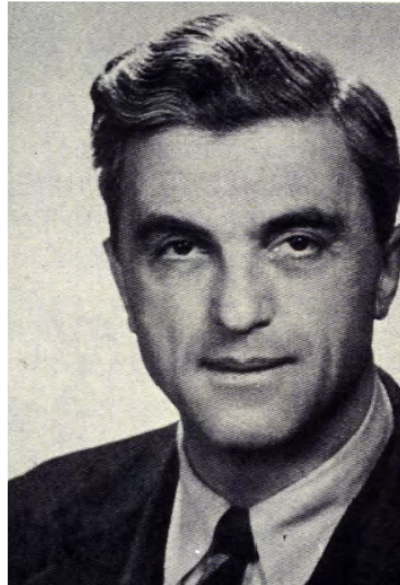
← 1.76 nano meters →



La RMN haut champ de la matière condensée

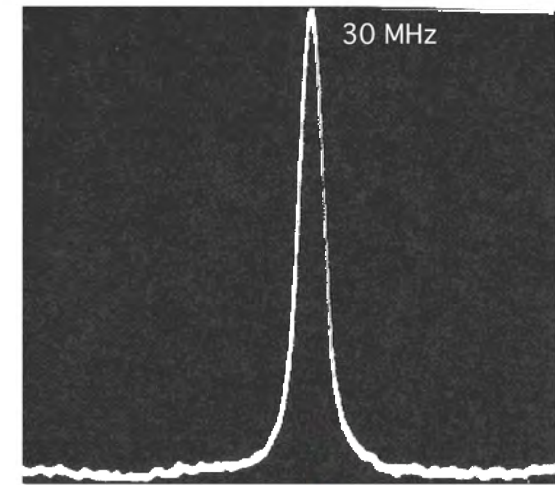


Edward Purcell
at Harvard

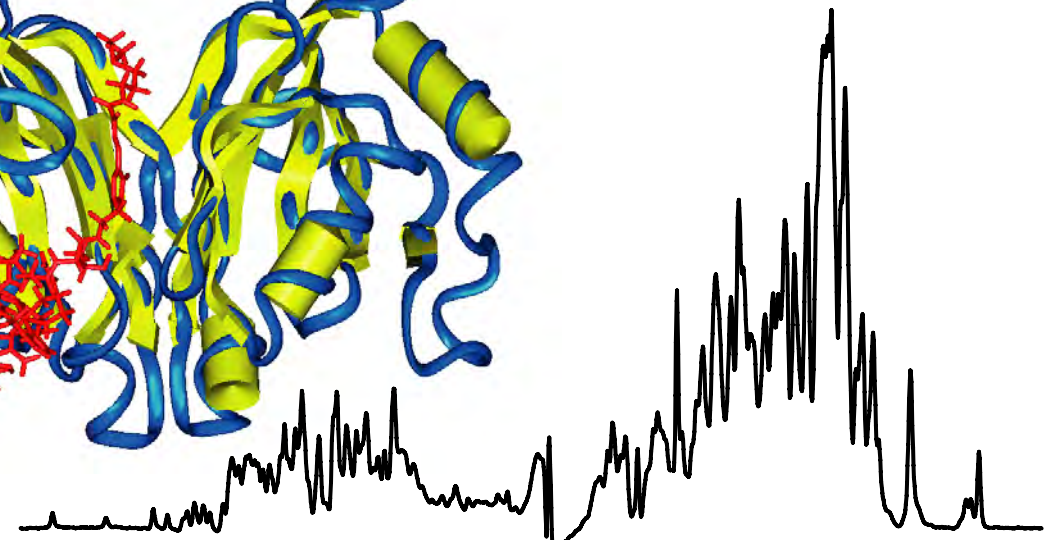
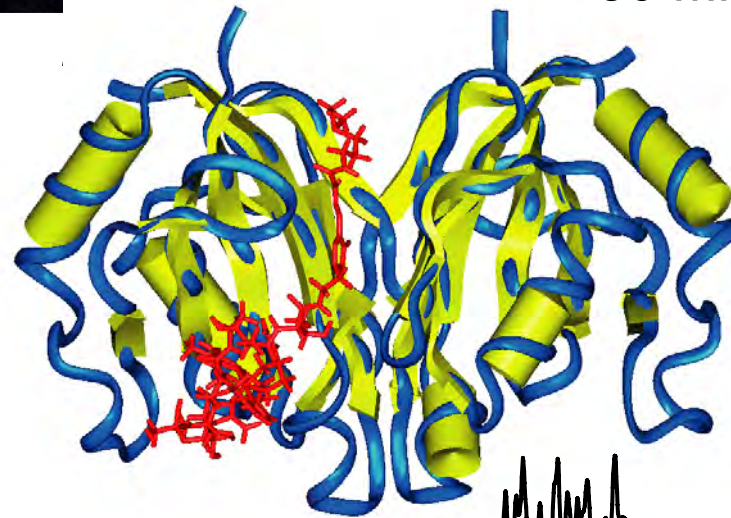


Felix Bloch
at Stanford

$$\omega_0 = -\gamma B_0$$



30 Mhz, 1948



courtesy of M. Blackledge, B. Brutscher, *et al.*, IBS-Grenoble

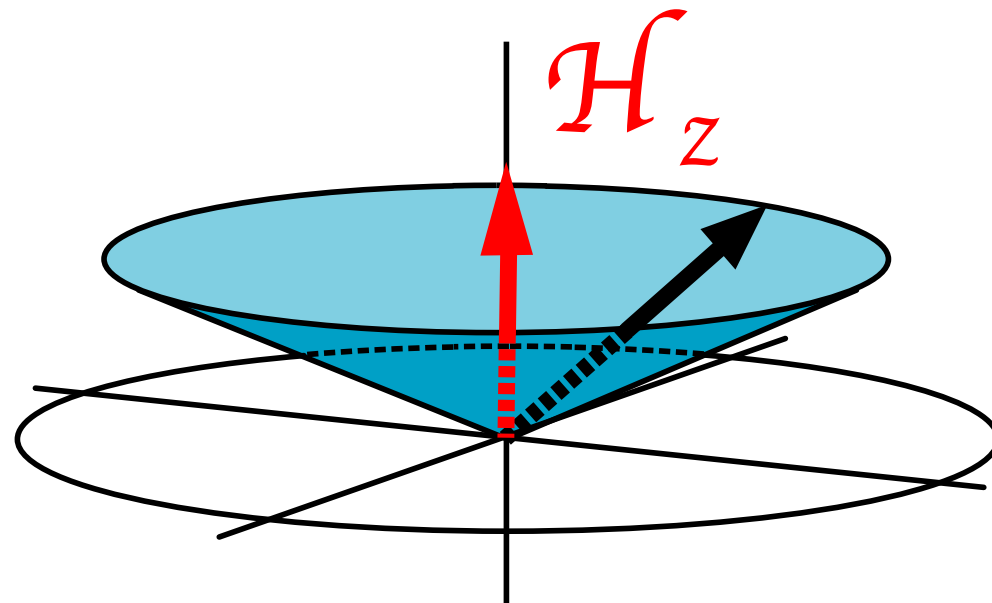
800 MHz, 2000



The Nobel Prize in Physics 1952

"for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith"

Comment est ce qu'on peut "voir" des objets de taille nanométrique?



Nuclei have a quantum property called ***spin***.
Spin leads to magnetism.

The spin ***precesses*** around the total magnetic field with ***frequencies that depend on the local interactions and environment.***

Nuclear Magnetic Resonance Spectroscopy detects that precession.

L'hamiltonien RMN: La clé

$$\mathcal{H} = \mathcal{H}_z + \mathcal{H}_Q + \mathcal{H}_D + \mathcal{H}_{cs} + \mathcal{H}_J$$

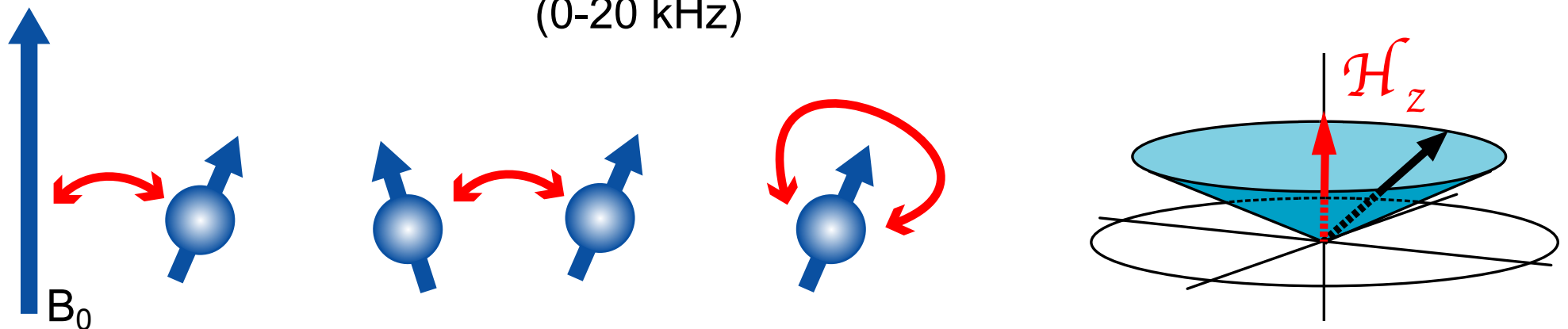
Zeeman Interaction
(~100 MHz)

Quadrupolar Interaction
(0-100 MHz)

Dipolar Interaction
(0-20 kHz)

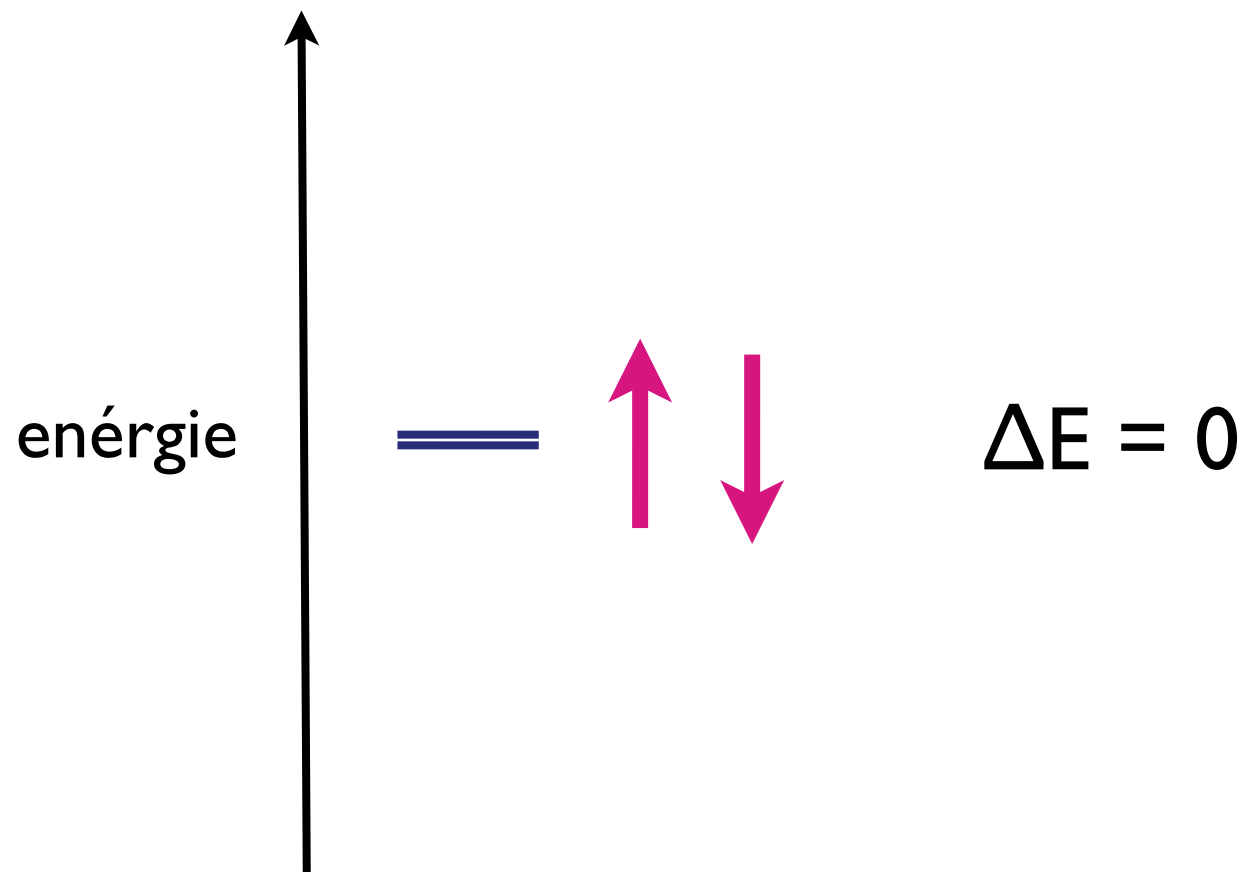
Chemical Shift
(0-2000 ppm)

Scalar Coupling
(0-200 Hz)

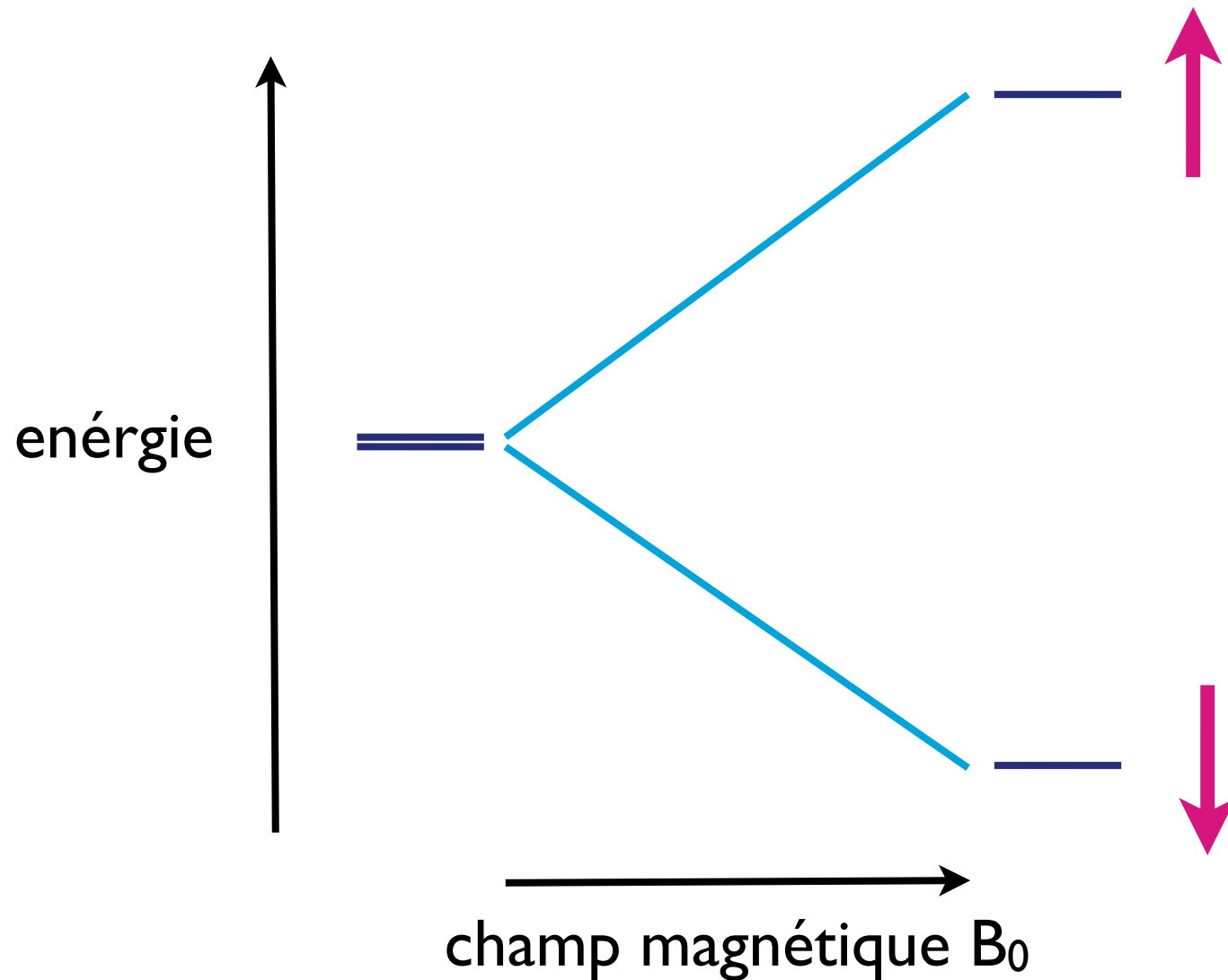


The spin **precesses** around the total magnetic field with **frequencies that depend on the local interactions and environment.**

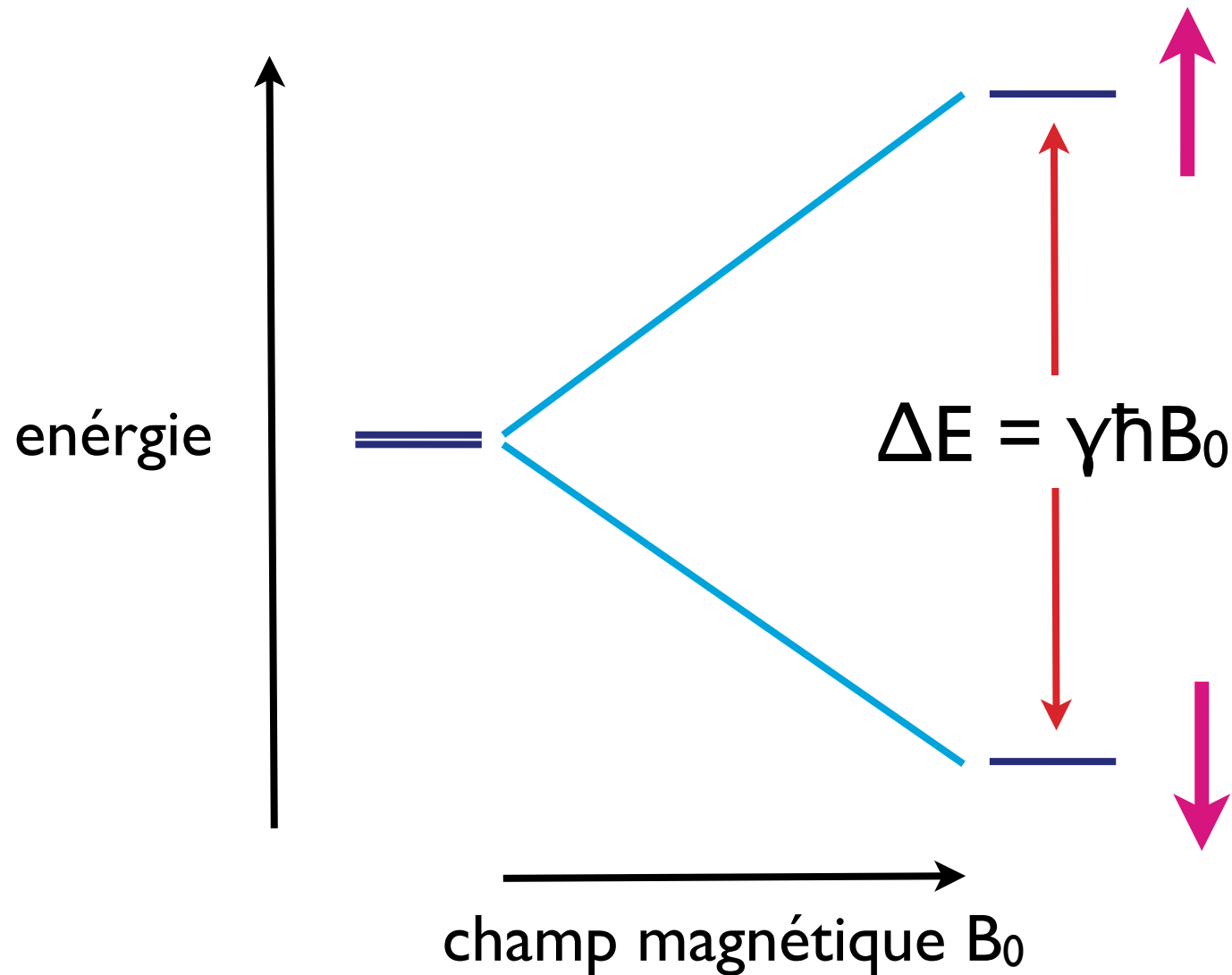
L'Effet Zeeman et Spectroscopie de Radiofréquence



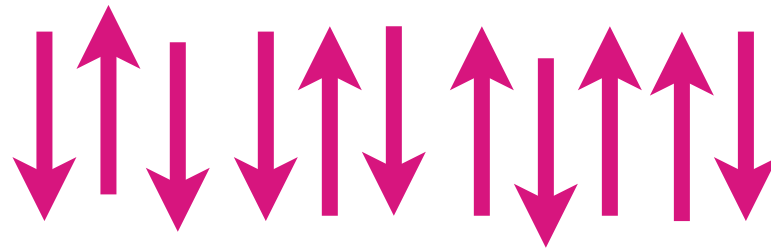
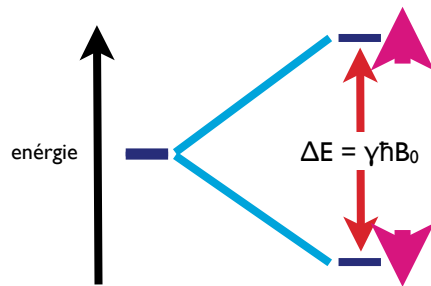
L'Effet Zeeman et Spectroscopie de Radiofréquence



L'Effet Zeeman et Spectroscopie de Radiofréquence



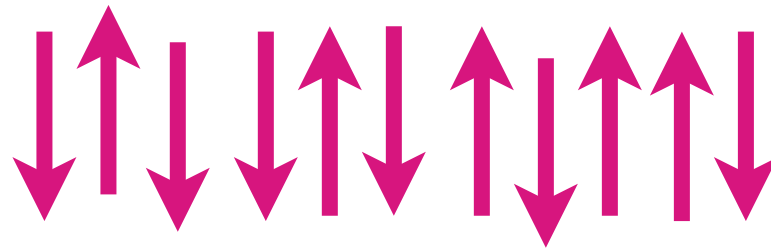
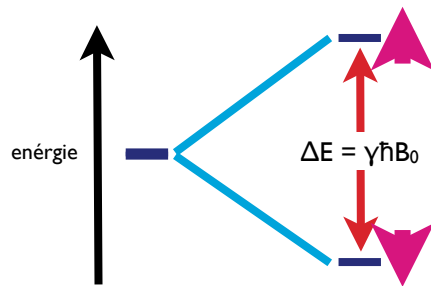
L'Effet Zeeman et Spectroscopie de Radiofréquence



Deux orientations sont possibles, avec un léger difference en faveur de l'orientation de l'orientation anti-parallèle

$$N_a/N_p = \exp(-\Delta E/k_B T) \sim 1.0001$$

L'Effet Zeeman et Spectroscopie de Radiofréquence



Plus le champ magnétique sera intense, plus le moment macroscopique sera élevé, et plus on pourra étudier des systèmes petits et complexes

$$N_a/N_p = \exp(-\Delta E/k_B T) \sim 1.0001$$

L'hamiltonien RMN: La clé

$$\mathcal{H} = \mathcal{H}_z + \mathcal{H}_Q + \mathcal{H}_D + \mathcal{H}_{cs} + \mathcal{H}_J$$

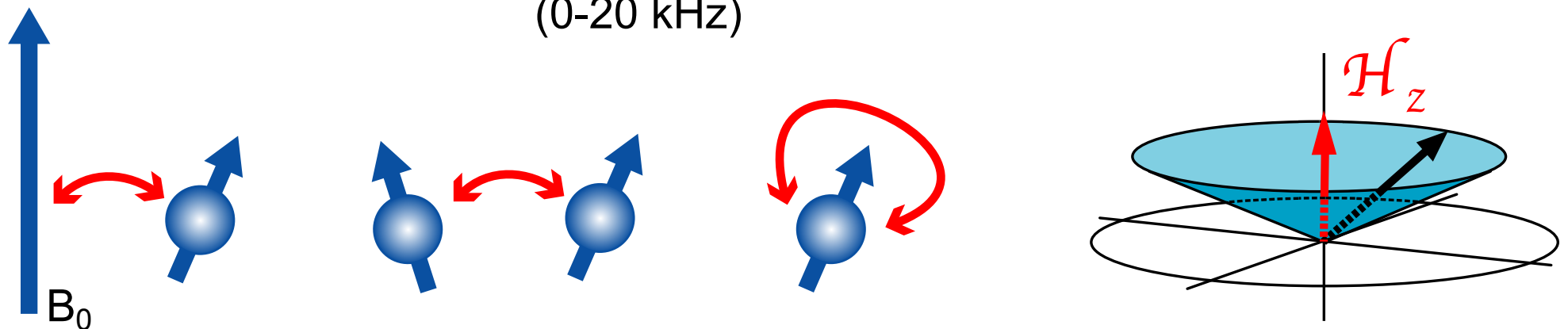
Zeeman Interaction
(~100 MHz)

Quadrupolar Interaction
(0-100 MHz)

Dipolar Interaction
(0-20 kHz)

Chemical Shift
(0-2000 ppm)

Scalar Coupling
(0-200 Hz)



The spin **precesses** around the total magnetic field with **frequencies that depend on the local interactions and environment.**

Observable N° 2: L'interaction dipolaire entre spins

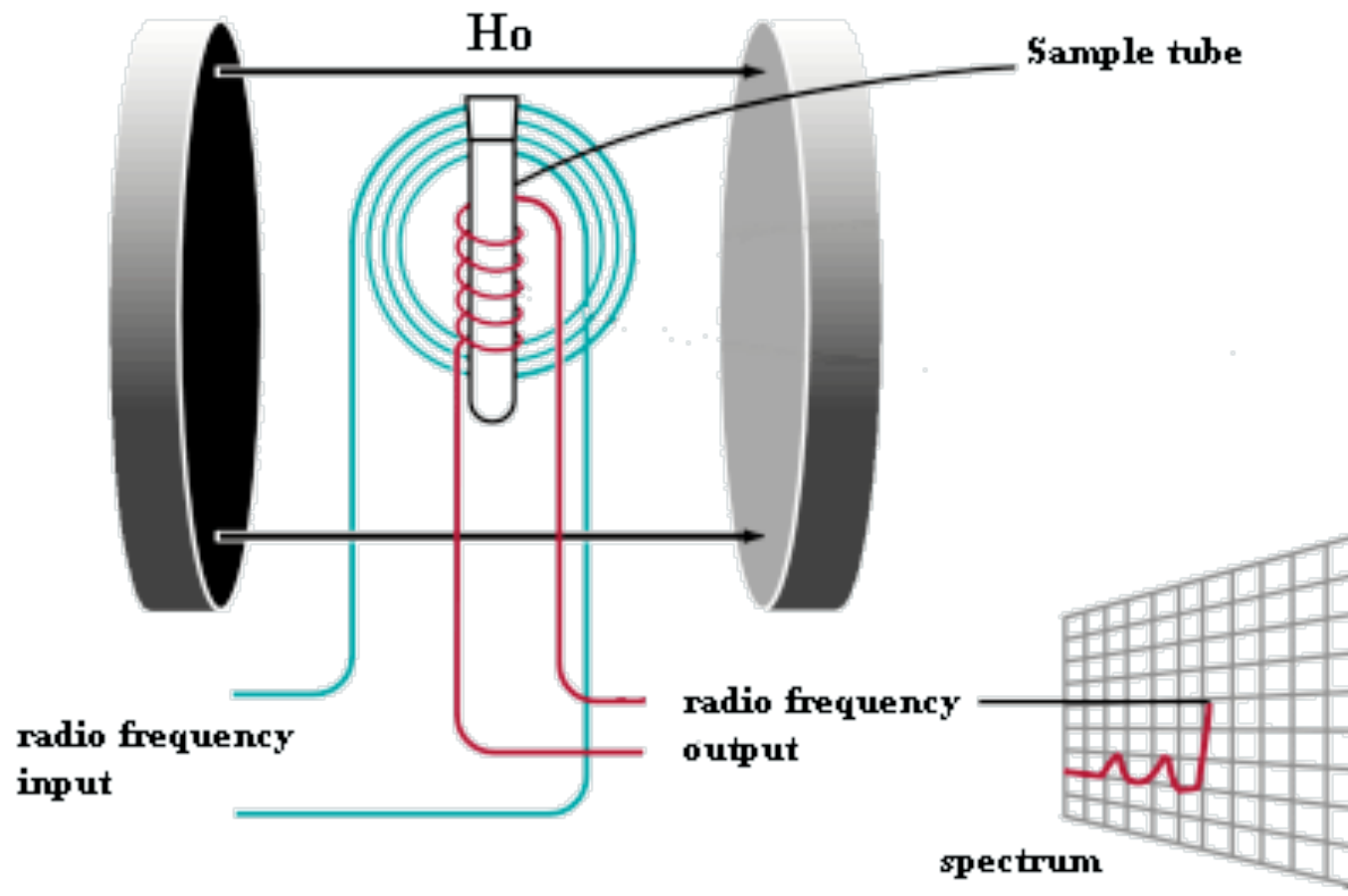
L'hamiltonien RMN contient plusieurs termes qui sont reliés aux coordonnées des atomes, à la structure électronique, ou à la dynamique de la molécule.

En particulier on remarque l'interaction dipolaire:

$$b_{CH} = -\frac{\mu_0 \hbar^2 \gamma_C \gamma_H}{4\pi r_{CH}^3}$$

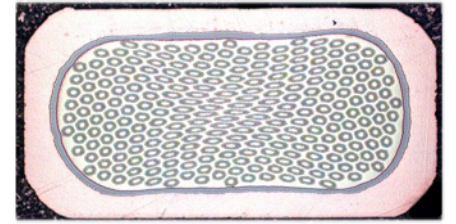
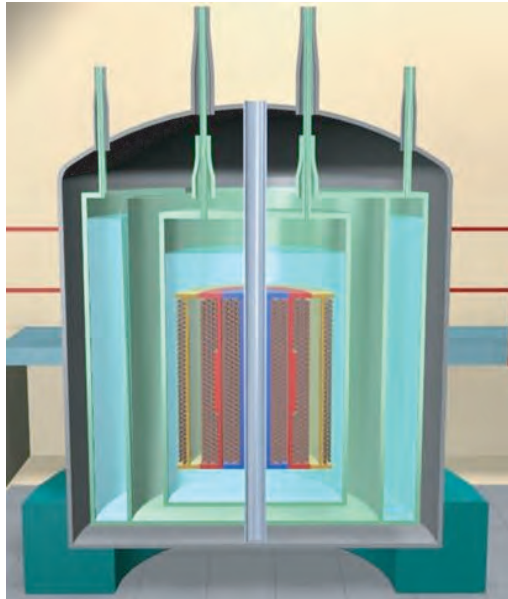
Si nous pouvions mesurer les interactions dipolaires entre toutes les pairs de spins, on pourrait en déduire les coordonnées des atomes....

Le spectromètre RMN

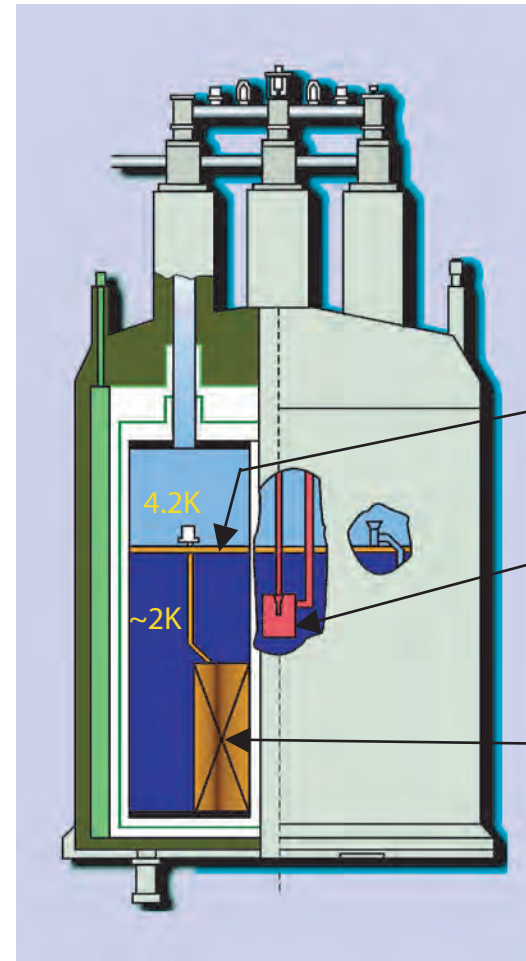


L'aimant RMN

un aimant RMN haut résolution:
1 GHz = 23,5 Tesla
superconductrice
(0 consommation d'électricité)
stabilité du champ < 1 Hz/heure (10^{-9})
homogénéité du champ à 10^{-10} sur un
volume de 3cm^3



$(\text{NbTaTi})_3\text{Sn}$ -conductor
~ 50.000 filaments, 5μ



Thermal Barrier

Joule-Thompson
Cooling Unit

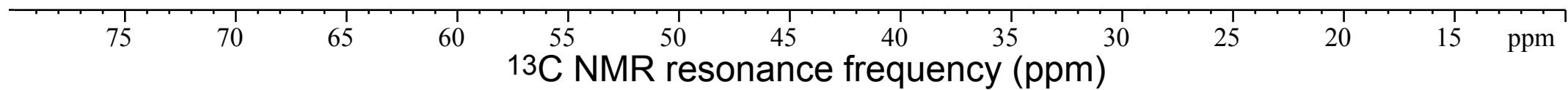
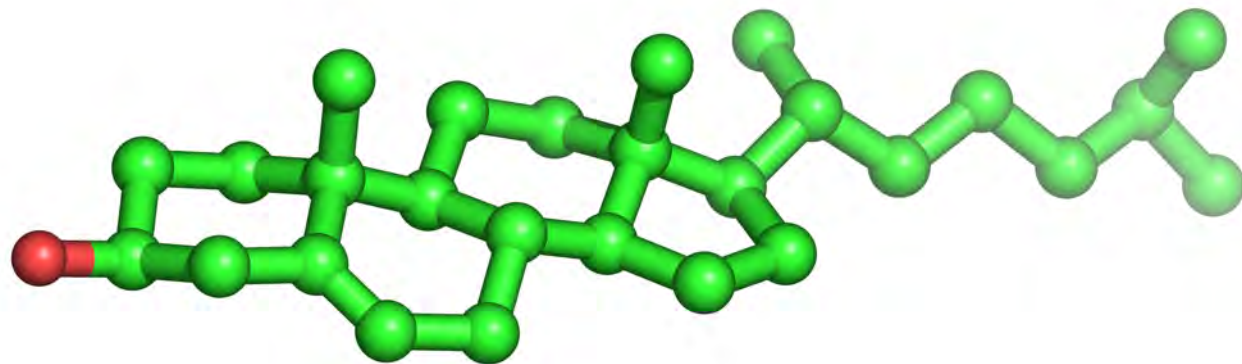
Superconducting
Magnet Coil

images courtesy of  **BRUKER
BIOSPIN**

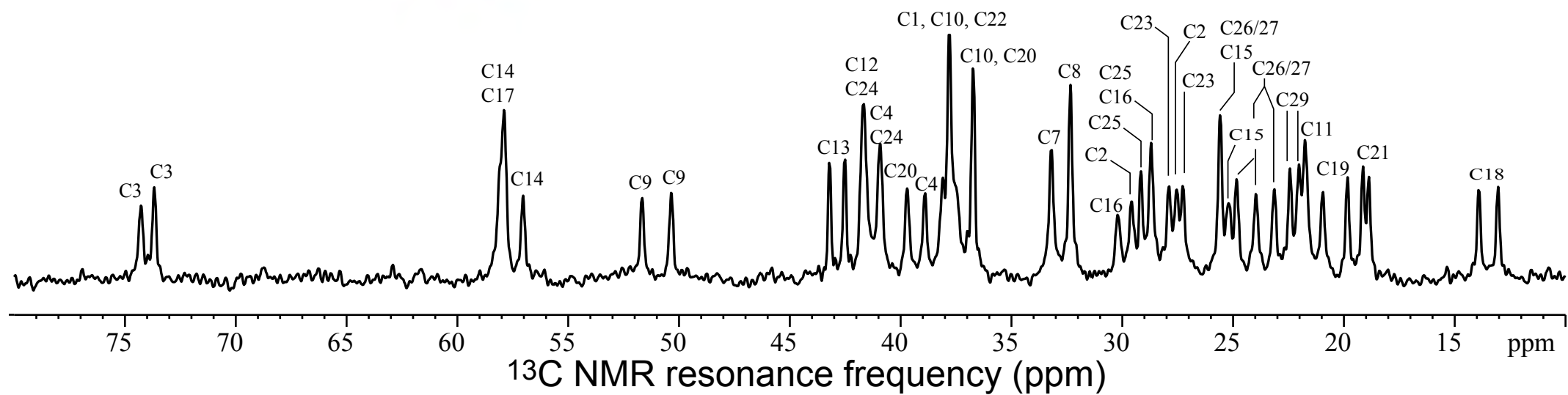
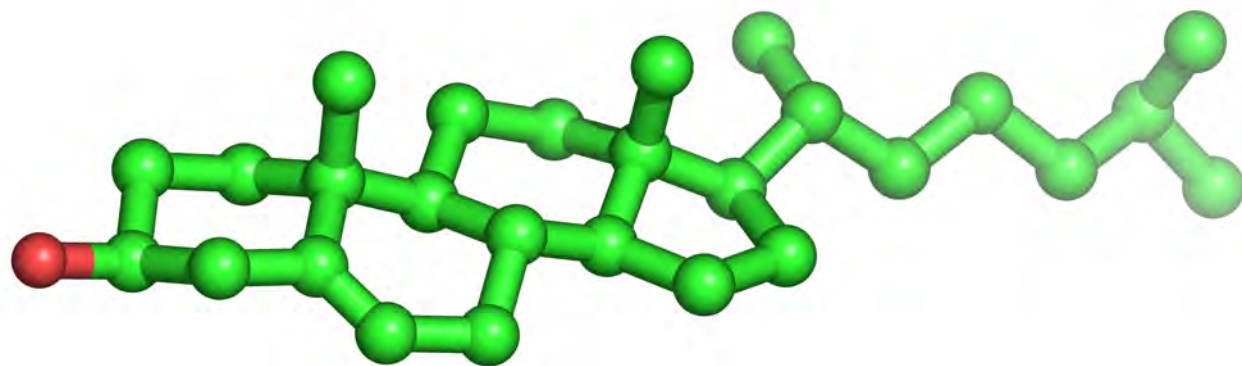
Le spectromètre RMN



Comment Faire de la Spectroscopie?



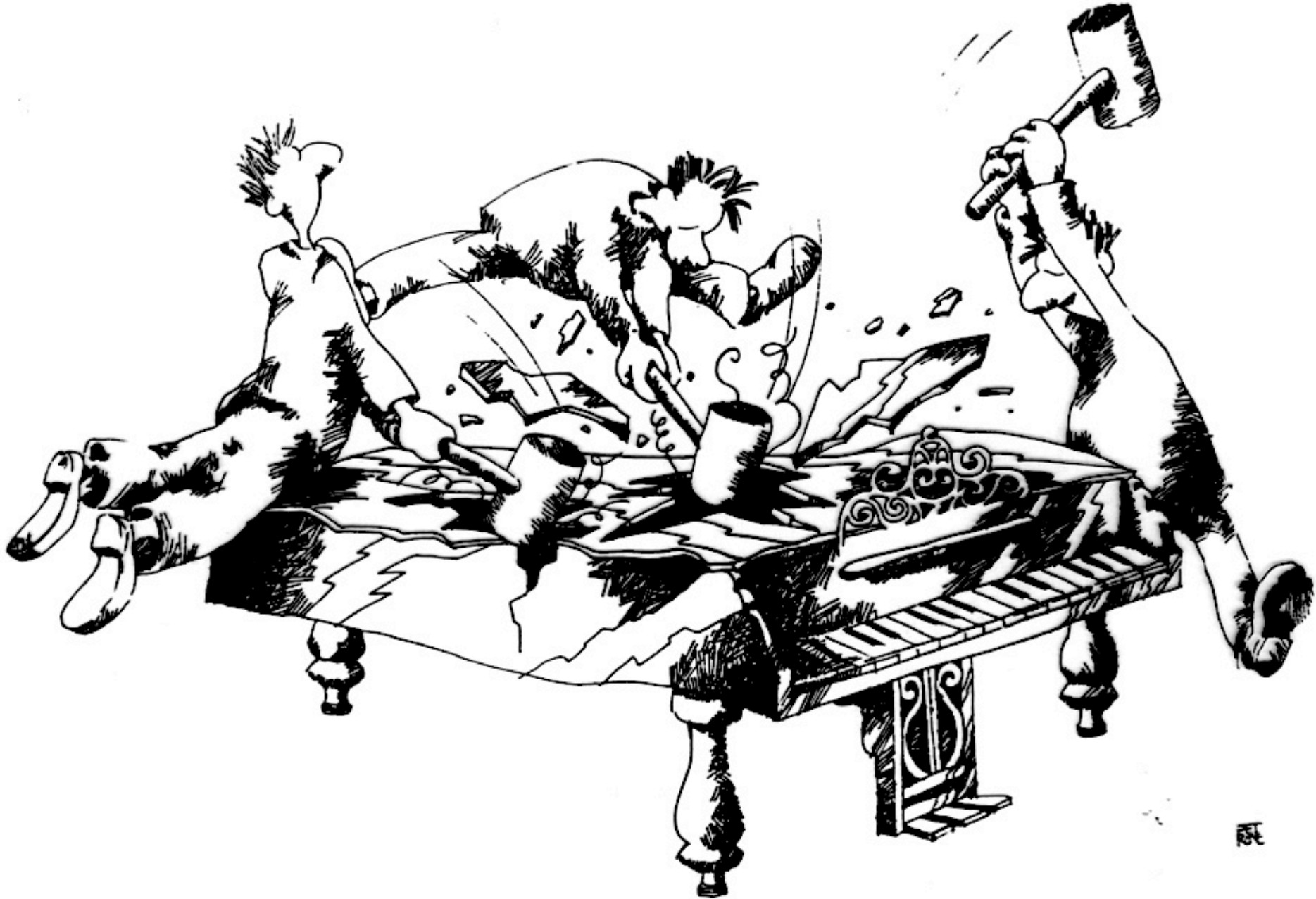
Comment Faire de la Spectroscopie?



Comment Faire de la Spectroscopie?

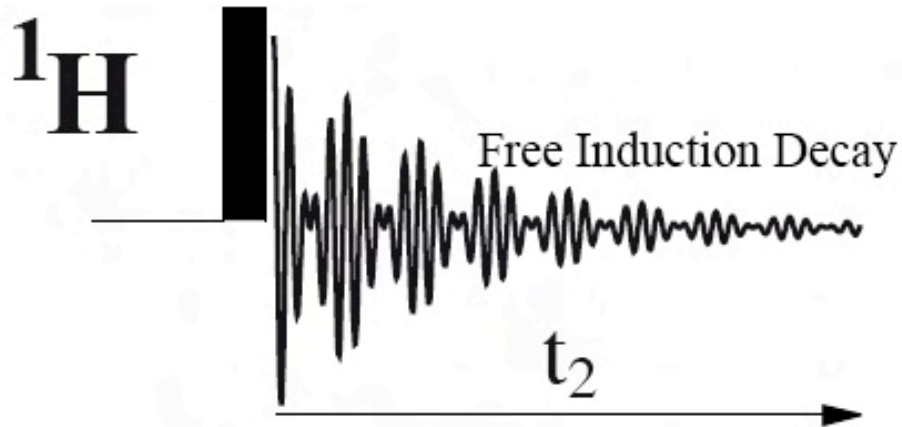


Comment Faire de la Spectroscopie?



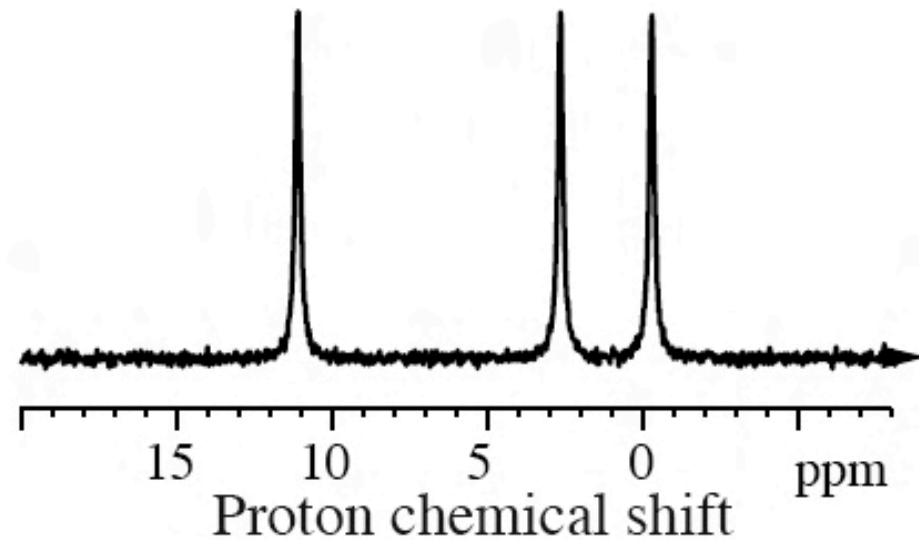
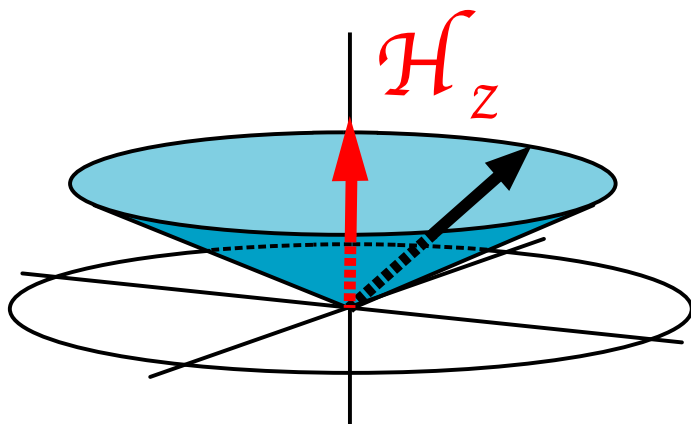
from R. Freeman

La RMN impulsionnelle par transformé de Fourier



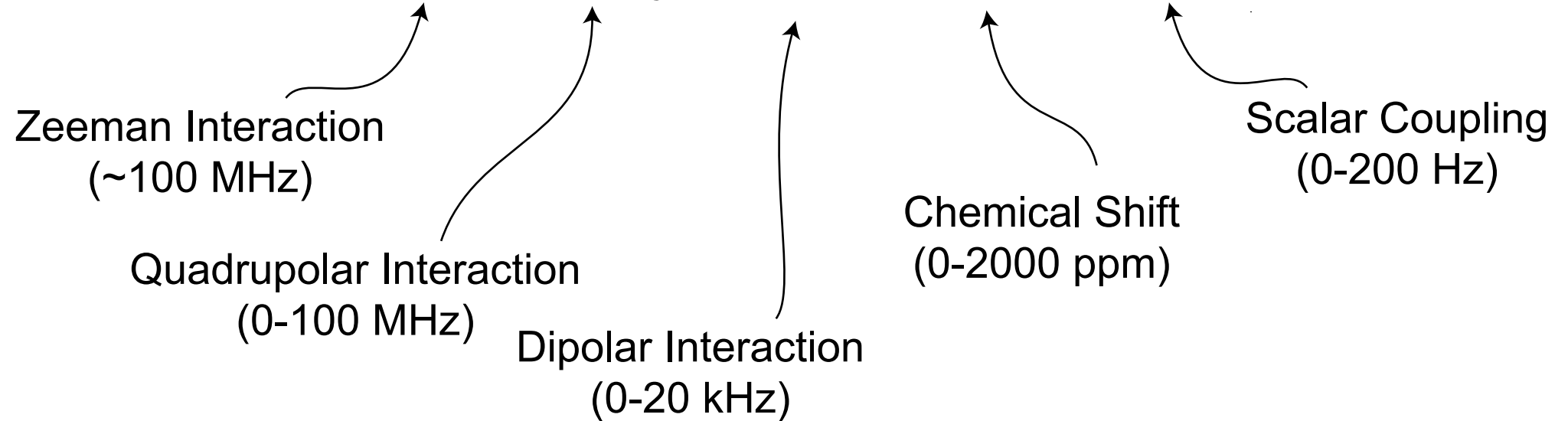
Fourier Transformation

$$I(\omega) = \int S(t) \exp\{-i\omega t\} dt$$

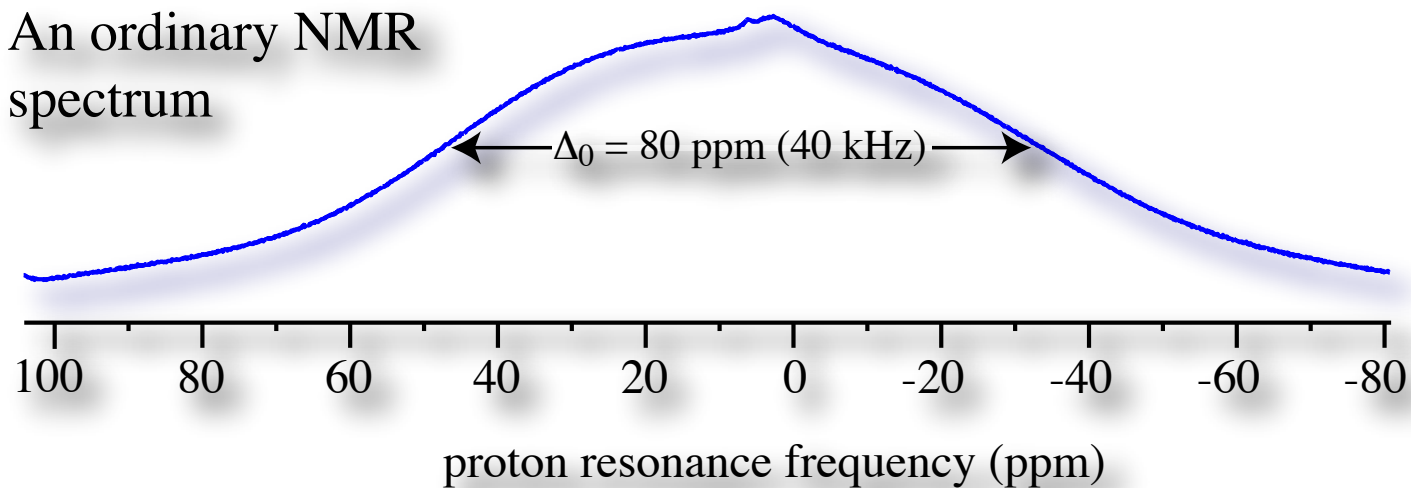


L'hamiltonien RMN: La cacophonie des spins

$$\mathcal{H} = \mathcal{H}_z + \mathcal{H}_Q + \mathcal{H}_D + \mathcal{H}_{cs} + \mathcal{H}_J$$



An ordinary NMR spectrum



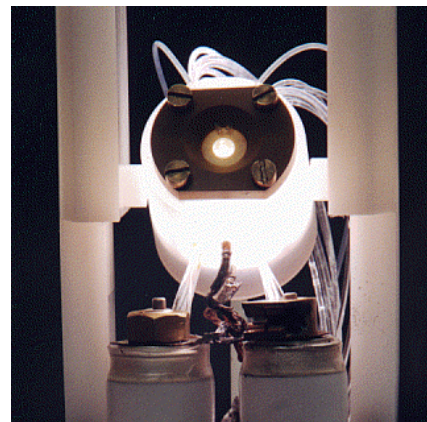
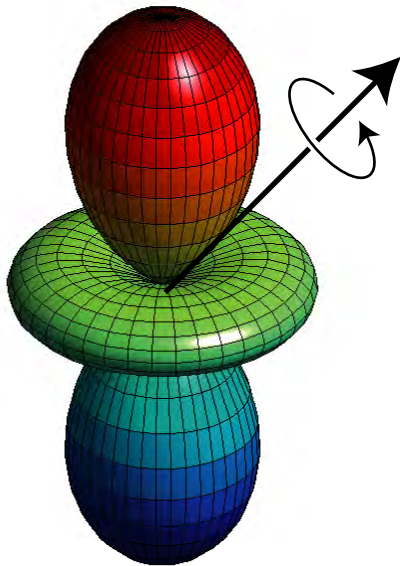
Danse avec des Spins

designing effective Hamiltonians

$$\mathcal{H} = \mathcal{H}_z + \mathcal{H}_Q + \mathcal{H}_D + \mathcal{H}_{CS} + \mathcal{H}_J + \mathcal{H}_{ext}$$

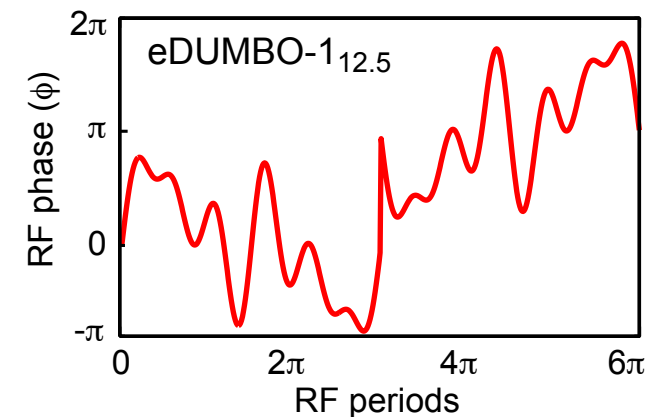
We can **add rotations** controlled by the experimentalist. If properly designed these rotations can **selectively cancel out** parts of the Hamiltonian.

Rotations in Laboratory Space
(*magic angle spinning*)



Spatial rotation: 35 000 rev/second
Spin rotation: 150 000 rev/second

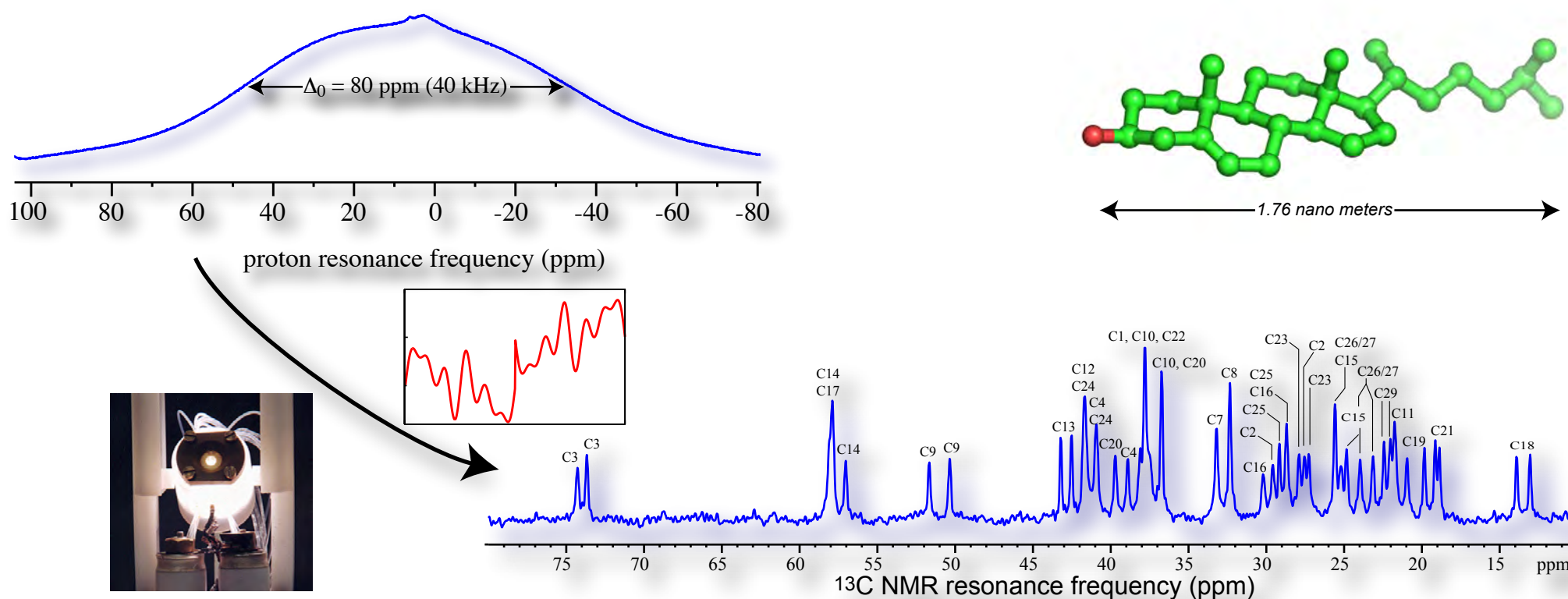
Rotations in Spin Space
(*radiofrequency pulses*)



Moyenne cohérente : l'écoute du message chuchoté

$$\overline{\mathcal{H}} = \mathcal{H}_z + \cancel{\mathcal{H}_Q} + \cancel{\mathcal{H}_D} + \mathcal{H}_{cs} + \cancel{\mathcal{H}_J} + \cancel{\mathcal{H}_{ext}}$$

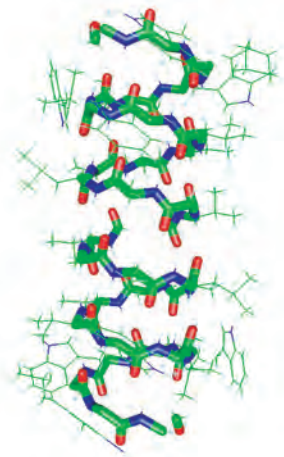
We can *add rotations* controlled by the experimentalist. If properly designed these rotations can *selectively cancel out* parts of the Hamiltonian.



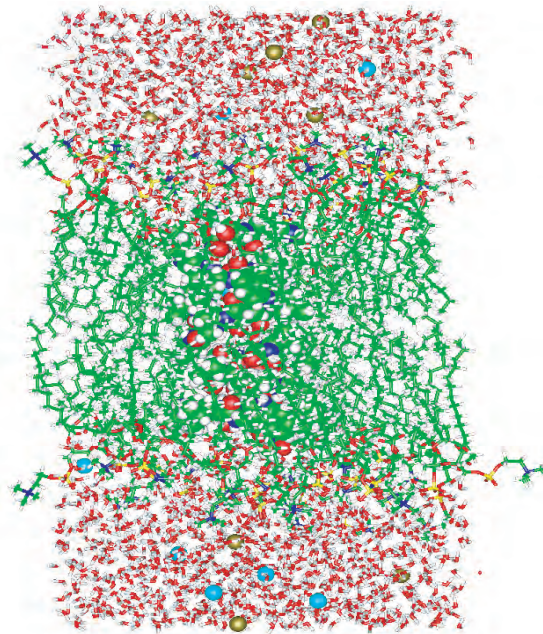
3000 millions de rotations pendant un expérience!!

RMN multi-dimensionnelle: résolution de corrélations par paire.

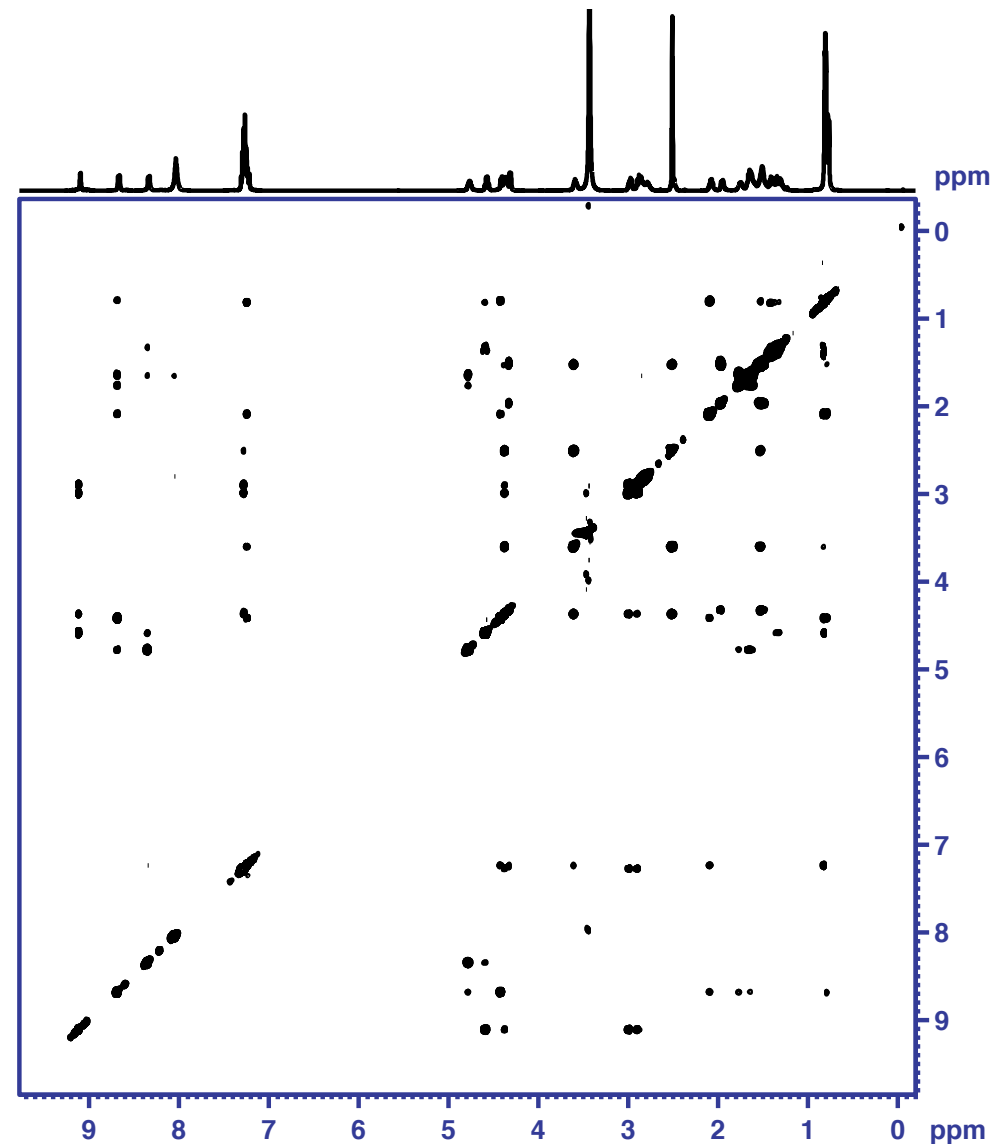
NOESY Spectrum of Gramicidin
The intensity of the cross peaks is related to the internuclear distance between the two nuclei that are correlated.



Gramicidin A



The whole system



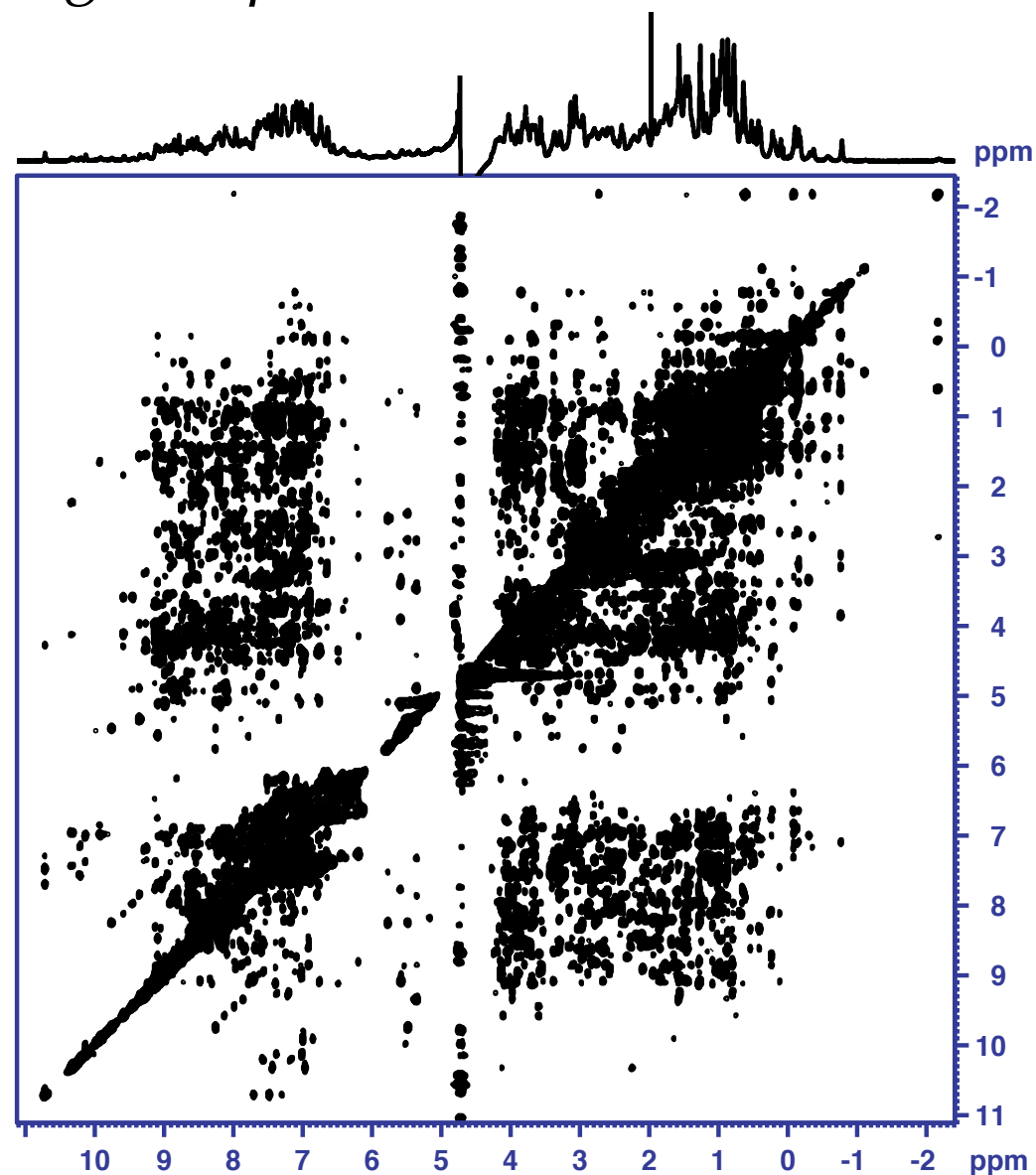
(more of this and related topics in RMN Structurale)

RMN multi-dimensionnelle: résolution de corrélations par paire.

mesure de couplages dipolaires

NOESY Spectrum of Lysozyme

The intensity of the cross peaks is related to the internuclear distance between the two nuclei that are correlated.



(more of this and related topics in RMN Structurale)

RMN multi-dimensionnelle: détermination structurale?

un protocole de détermination structurale par RMN

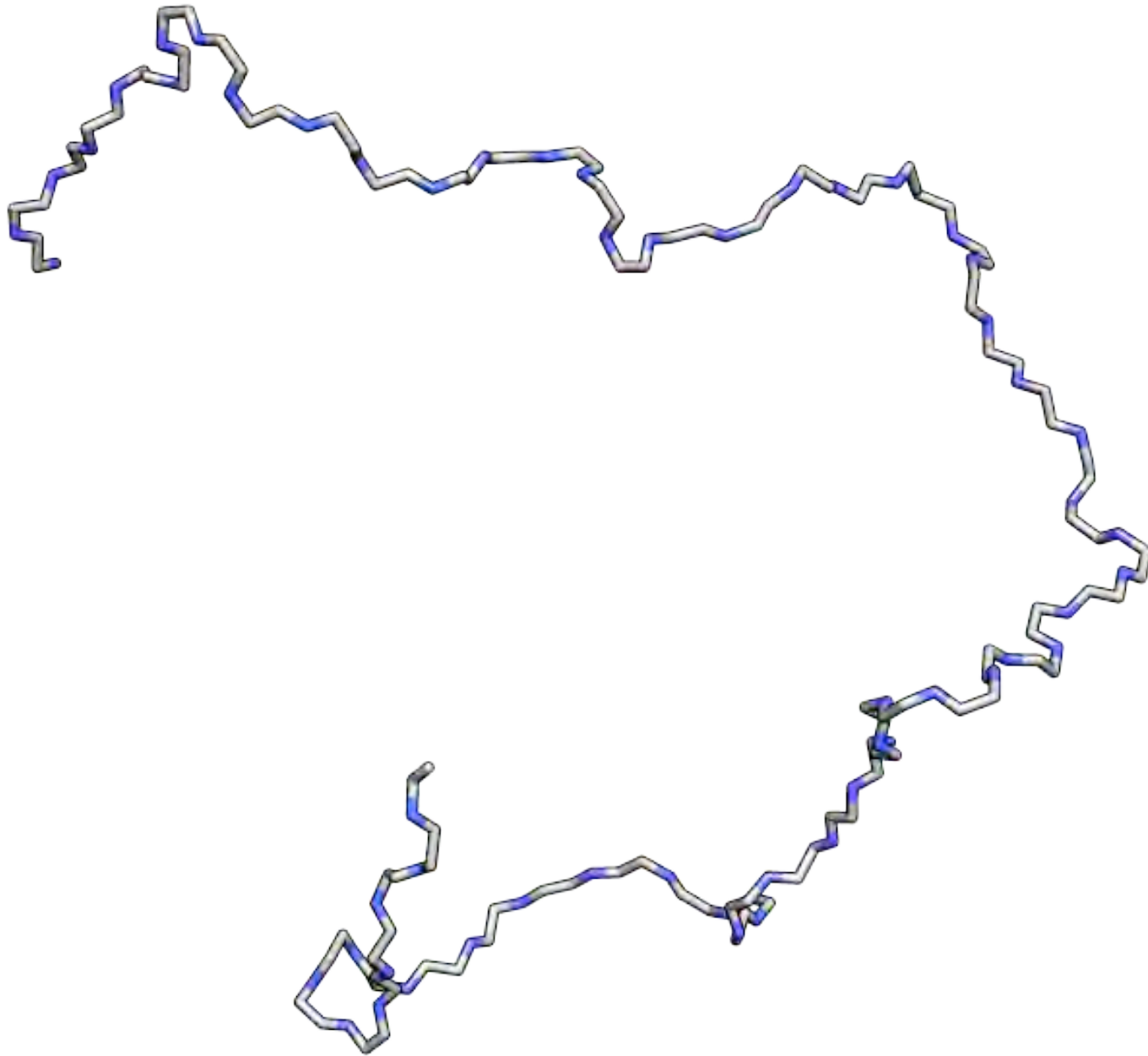
- 1. préparation d'un échantillon*
- 2. attribution des résonances à des noyaux*
- 3. mesure de couplages dipolaires*
- 4. modélisation de la structure?*

RMN multi-dimensionnelle: détermination structurale?

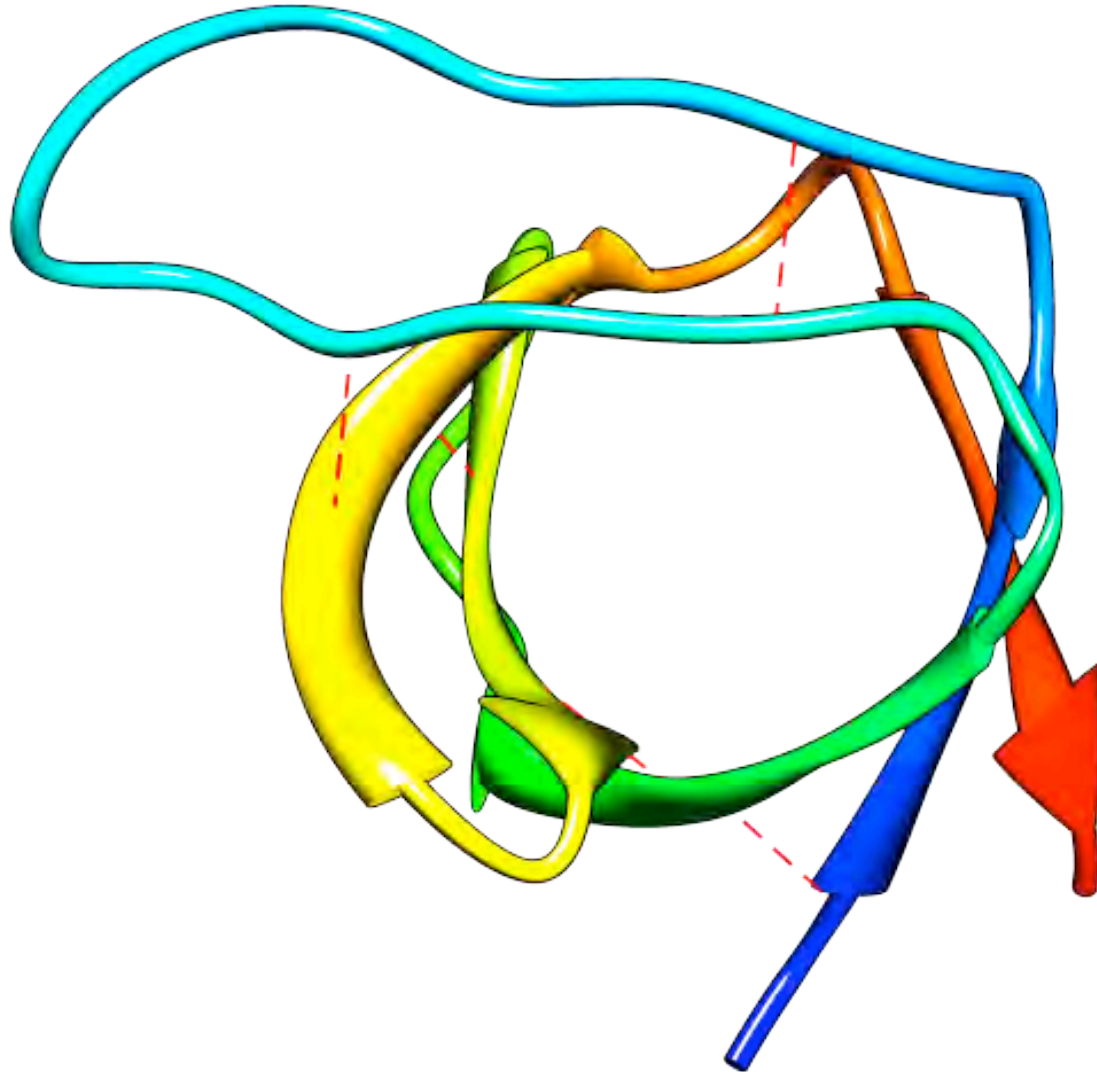
*une fois que nous avons **identifié une observable**,
et une fois que nous **savons la mesurer**,
comment va t-on **construire le modèle de la structure**?*

La modélisation moléculaire sous contrainte expérimentale.

De la contrainte expérimentale au modèle structural



De la contrainte expérimentale au modèle structural



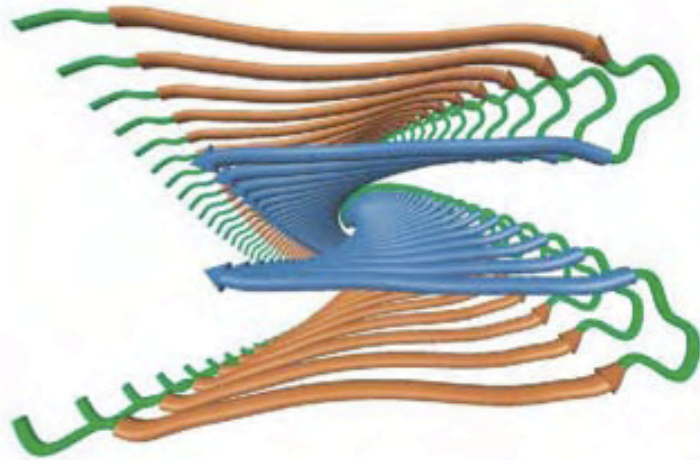
three-dimensional structures of proteins in solution



In 1986, using NMR, the group led by Wütrich determined a protein structure in solution for the first time. In 2000 he was the first to determine the structure of a

human prion protein. In 2002 he wins the Nobel Prize for Chemistry.

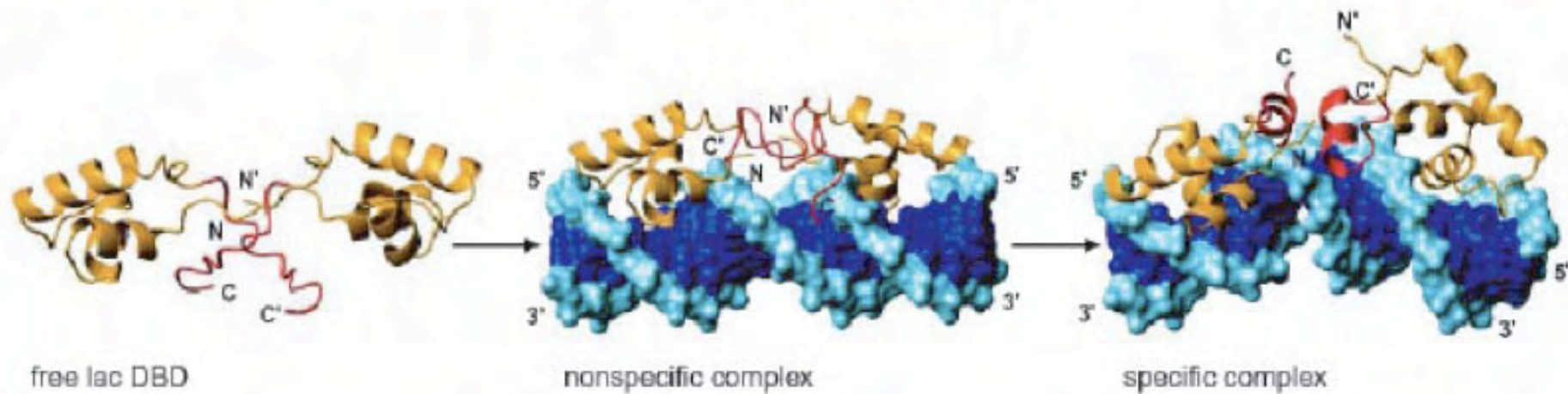
insoluble Alzheimer's proteins determined by MAS NMR



In the 1960s, the work of Andrew, Waugh, Pines, Stejskal and Shaeffer, provides high resolution spectra from solids with magic angle spinning (MAS). From 1994 onwards Griffin (MIT) provides increasingly detailed evidence for functional mechanisms in membrane proteins such as rhodopsin and bacteri-

orhodopsin, shining light on the primary steps in vision; in 2002 Tycko (NIH) uses NMR techniques to provide the first structure of the plaque forming amyloid proteins responsible for Alzheimer's disease; and in 2006 Oschkinat (Berlin) shows preliminary three-dimensional structures for membrane incorporated proteins obtained from 900 MHz NMR spectra.

solving the DNA recognition puzzle



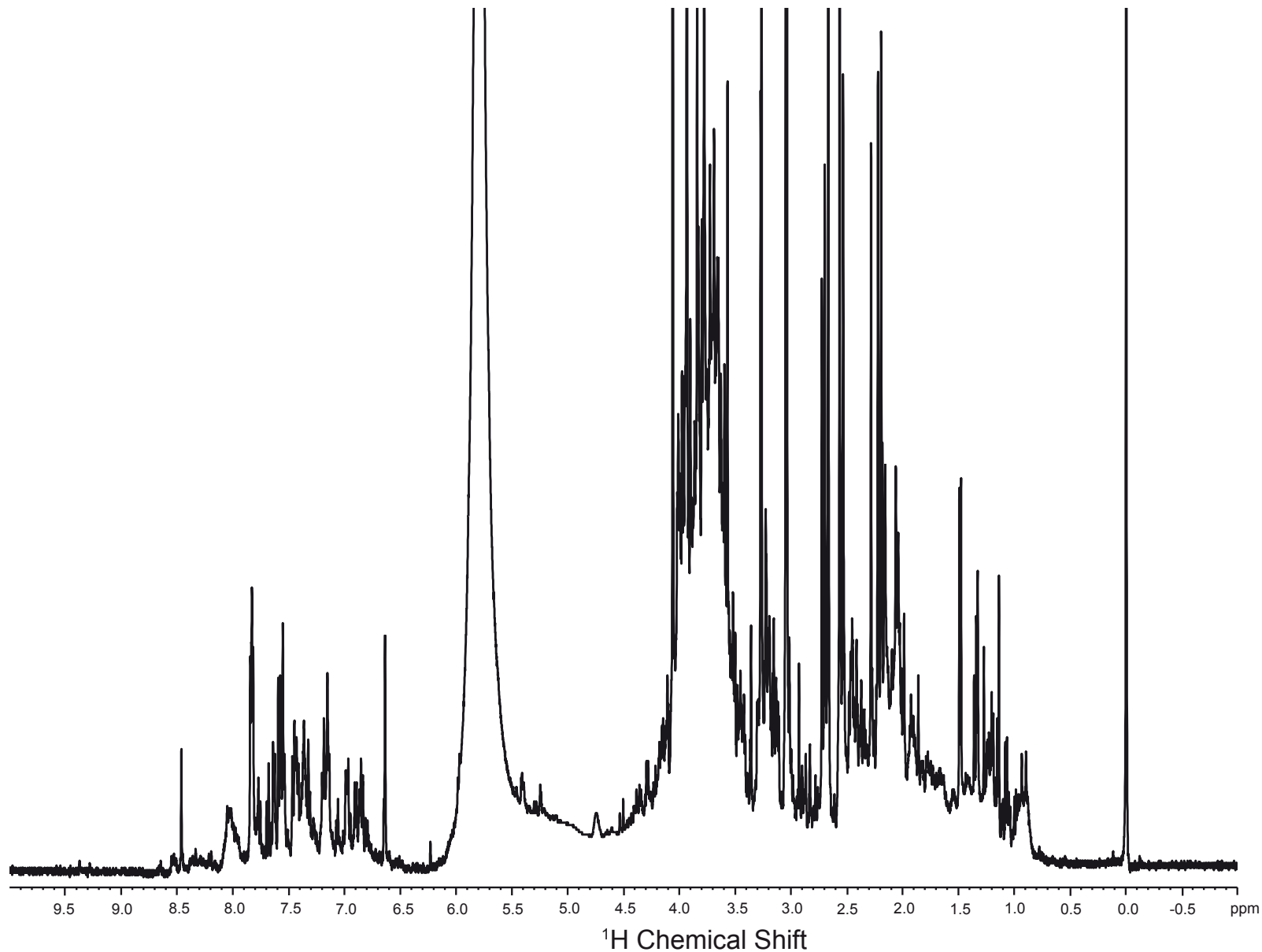
NMR spectra are first obtained from DNA and RNA oligomers in the early 1970s. In 2004, Kaptein uses 900 MHz NMR of protein-DNA complexes to determine the kinetics and structural changes that allow proteins to find their recognition sites in extended DNA sequences.

metabolism, diagnosis, and personalised healthcare.

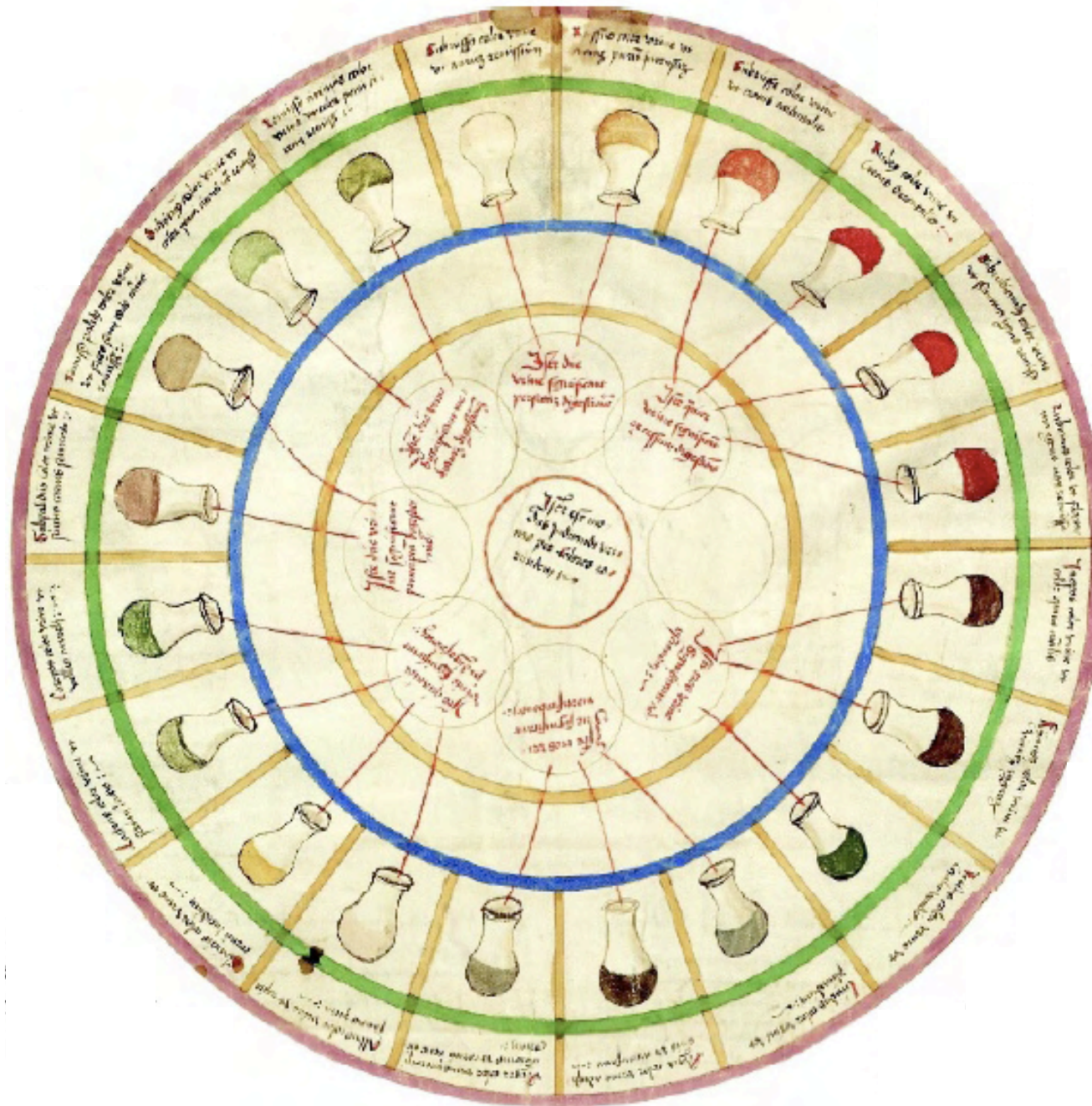


The remarkable emergence of “metabonomics by NMR” is based on characteristic signal patterns in spectra of body fluids such as urine and blood serum. In the 1990s NMR spectra are used to determine types of cancer. In 2006 Nicholson and coworkers at Imperial College present results from worldwide epidemiological studies, involving thousands of subjects, determining environmental factors affecting the occurrence of diabetes and high blood pressure in whole populations. Metabonomics by NMR is playing a key role in the emergence of the idea of personalized health care.

Spectre RMN d'urine humain



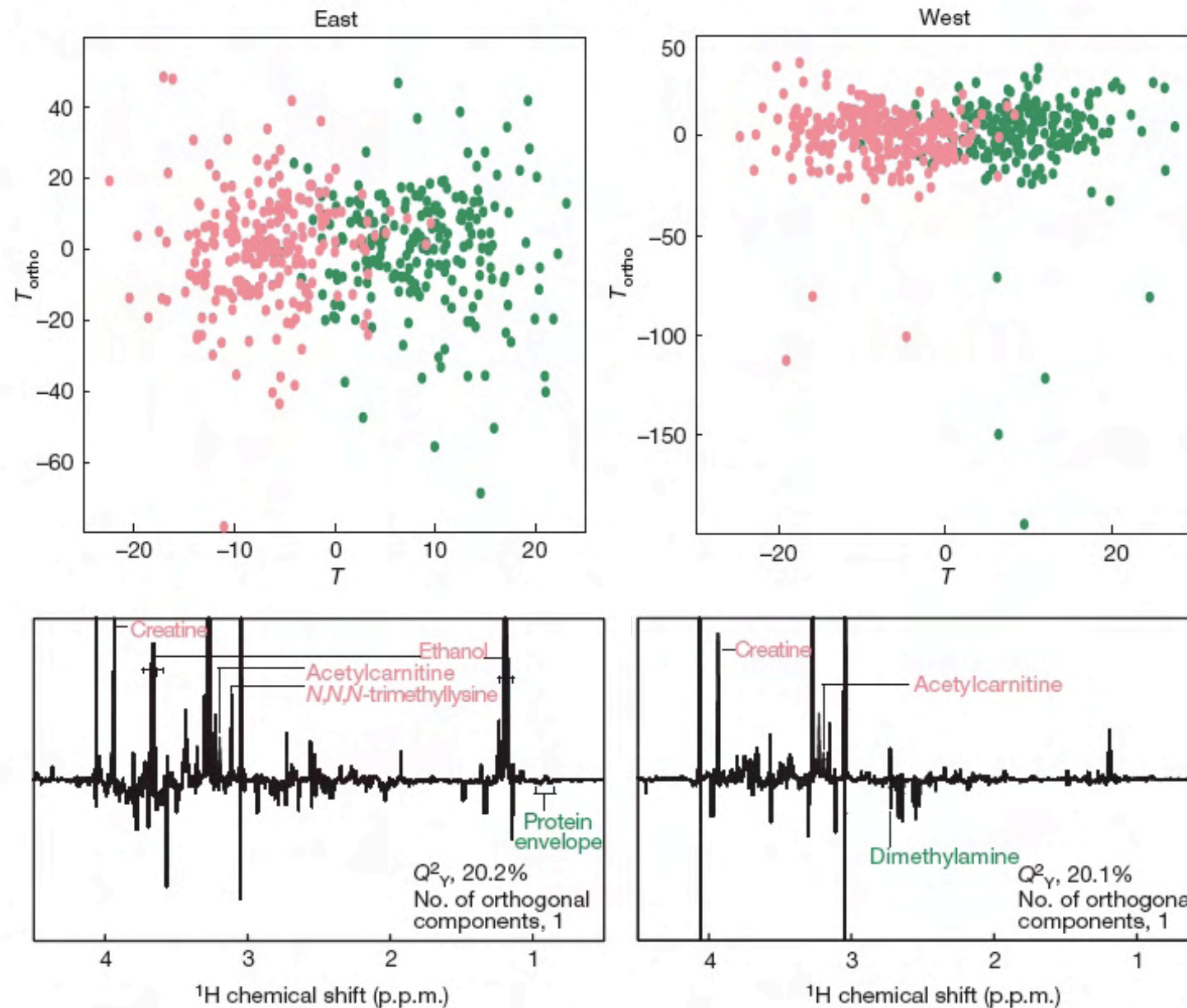
*Ce spectre fourni un "profil métabolique" du sujet.
L'étude de variations dans ces profils s'appel la métabonomique.*



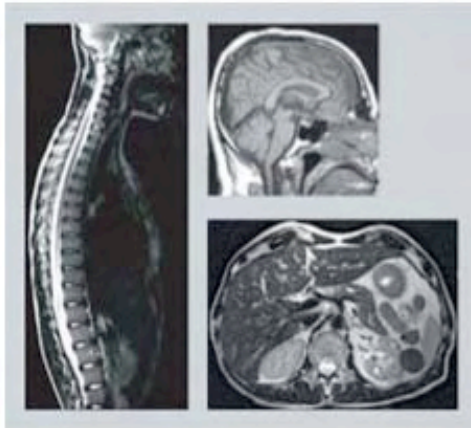
This urine wheel was published in 1506 by Ullrich Pinder, in his book Epiphanie Medicorum. It describes the possible colours, smells and tastes of urine, and uses them to diagnose disease. (The Royal Library, Copenhagen)

Dietary factors associated to risk of cardiovascular disease

- High vegetable/low animal protein (east $n = 220$; west $n = 251$)
- Low vegetable/high animal protein (east $n = 214$; west $n = 208$)

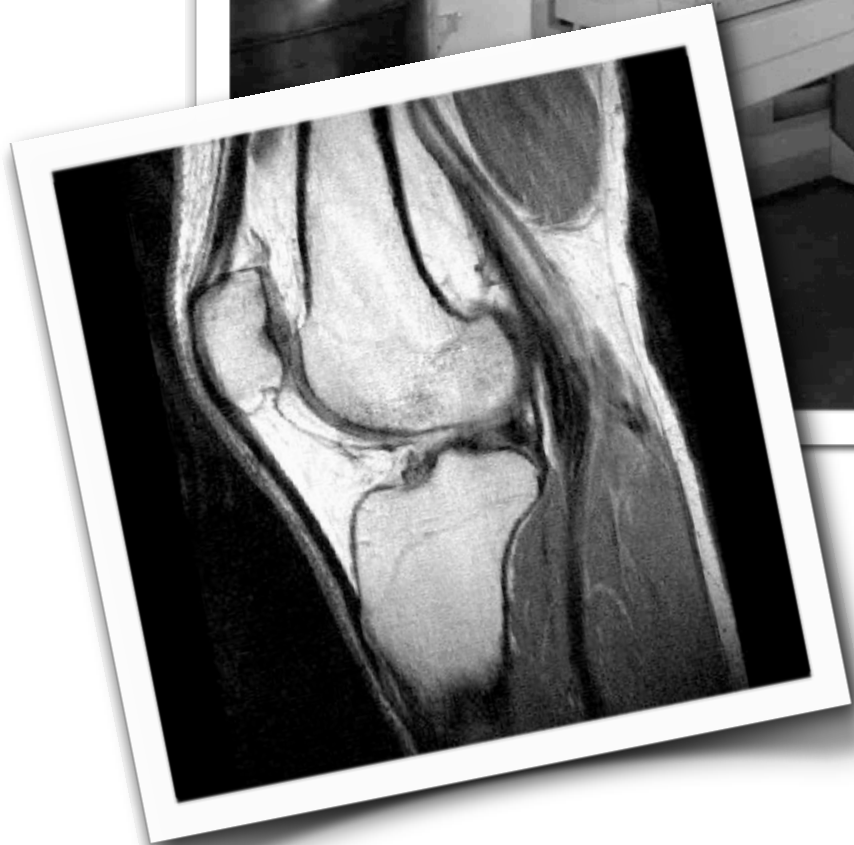


magnetic resonance imaging: a clinical tool for diagnosis.

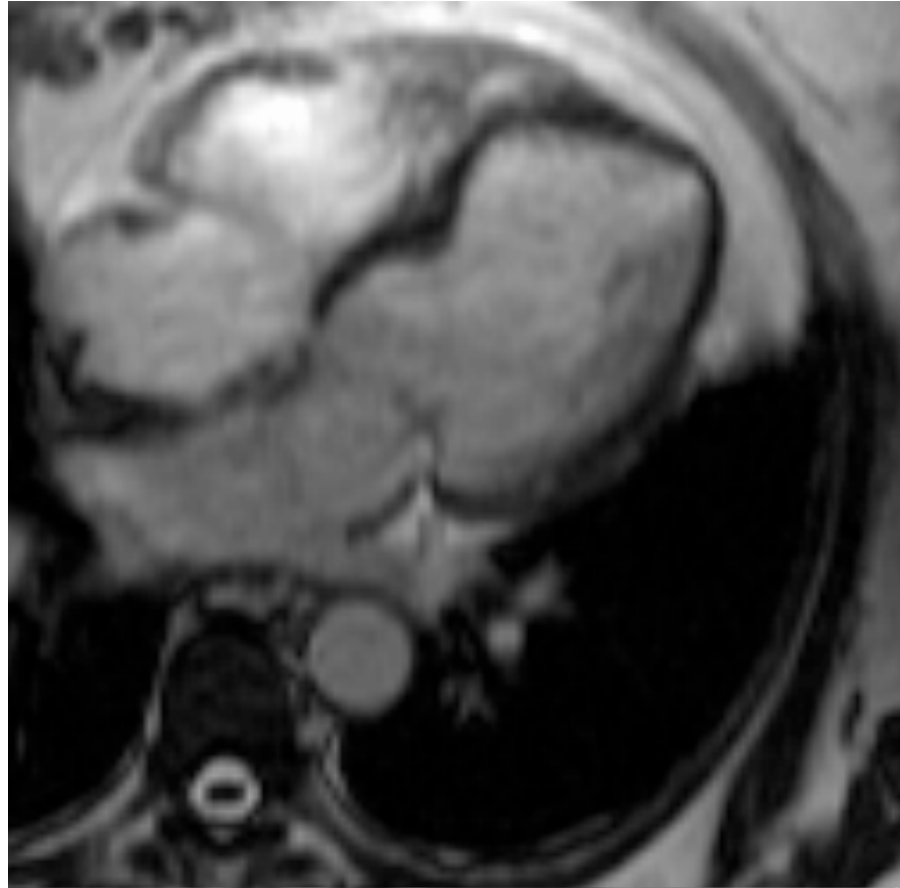


In 1973 Paul Lauterbur uses a high-resolution NMR spectrometer to provide the first Magnetic Resonance Image (MRI). In 2006 this has become a multi-billion dollar industry, and is the technique of choice for the diagnosis of many common tumors. In 2003 Lauterbur and Mansfield win the Nobel Prize in Medecine.

Imagerie par Résonance Magnétique (IRM)

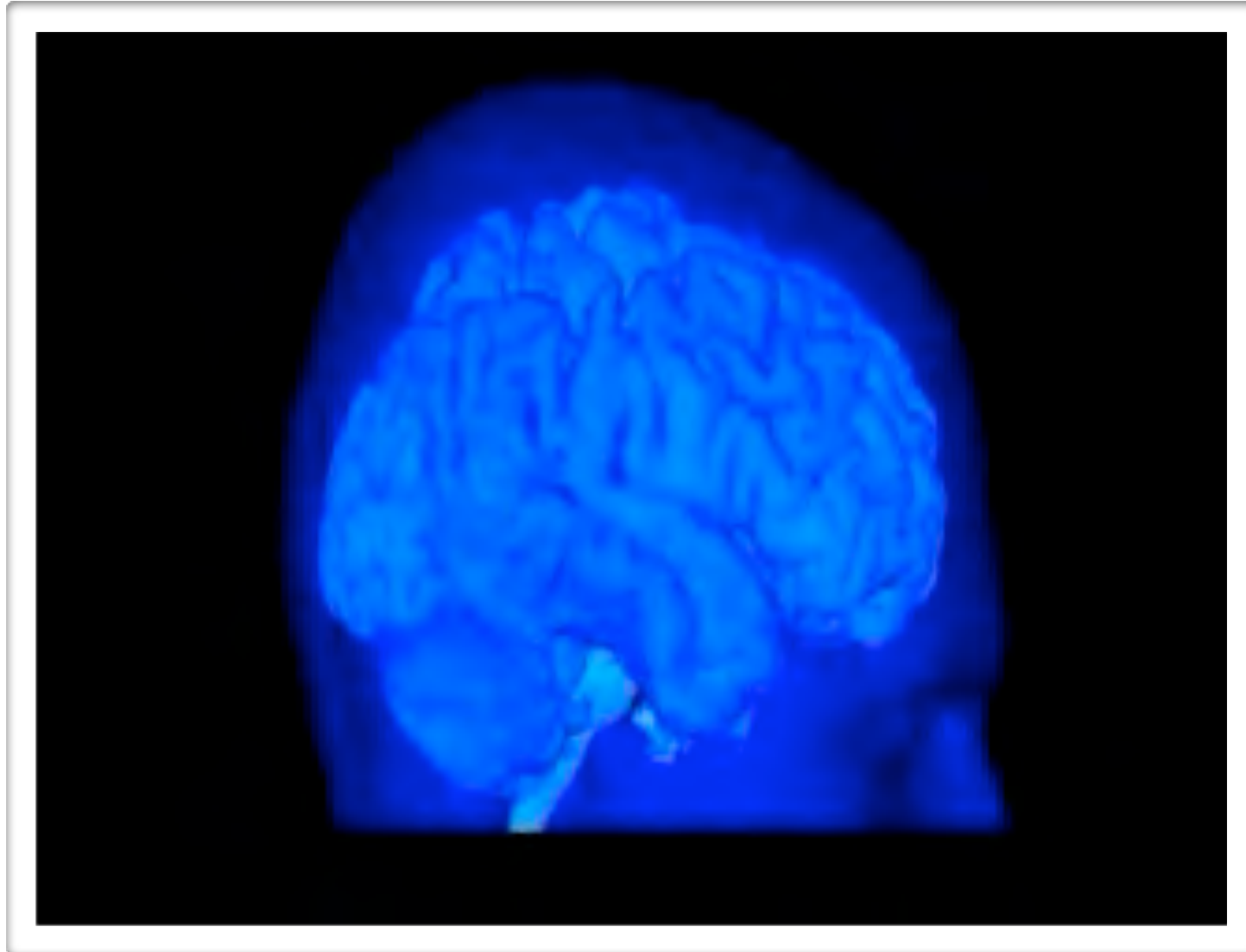


Imagerie par Résonance Magnétique (IRM)



regard comment ça marche!

Imagerie par Résonance Magnétique (IRM)



lisant dans vos pensées

Centre de RMN à Très Hauts Champs à Lyon



Large Scale Facility, User Access: www.ralf-nmr.fr



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