Nuclear Magnetic Resonance: Seeing Invisible Things

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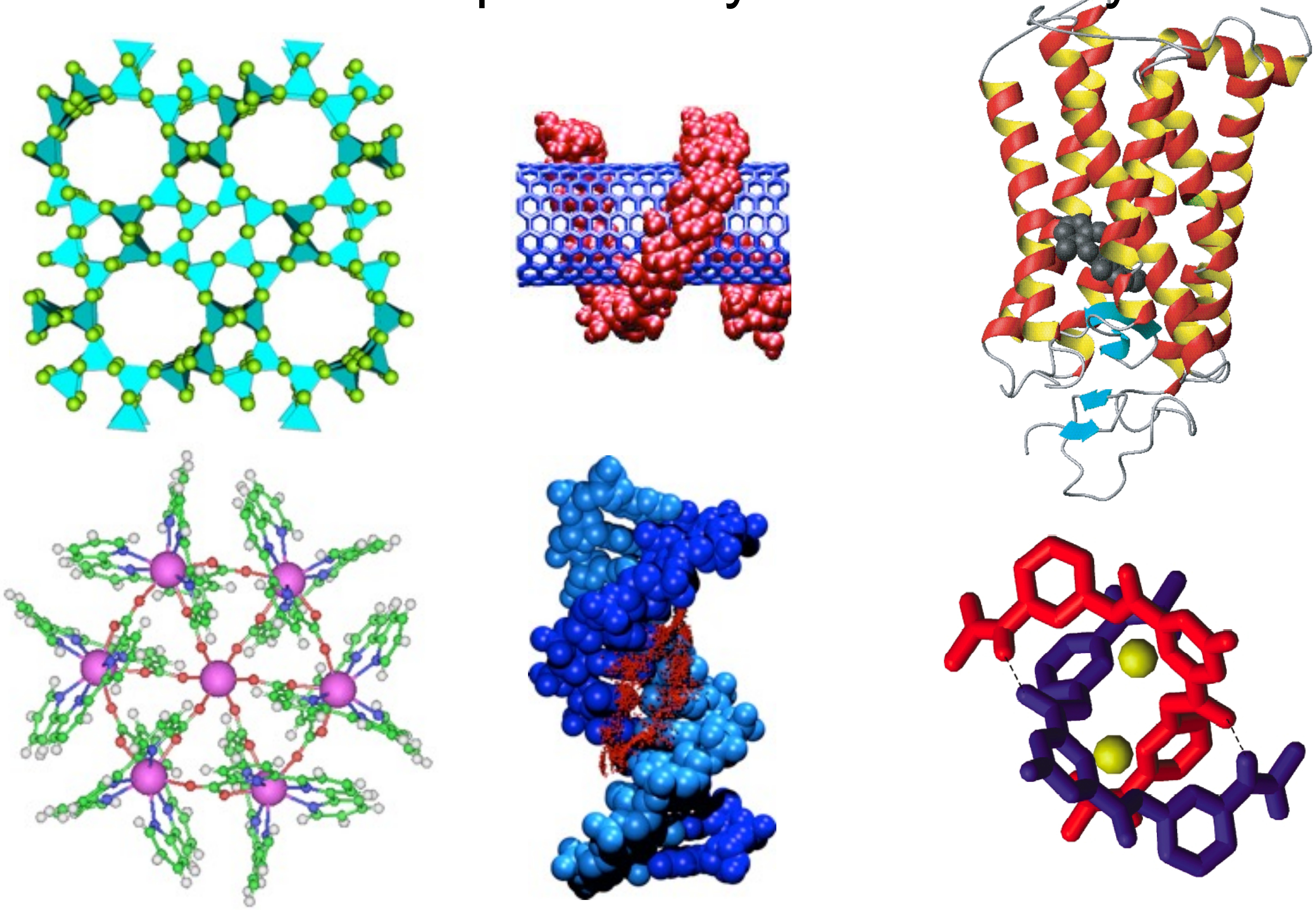
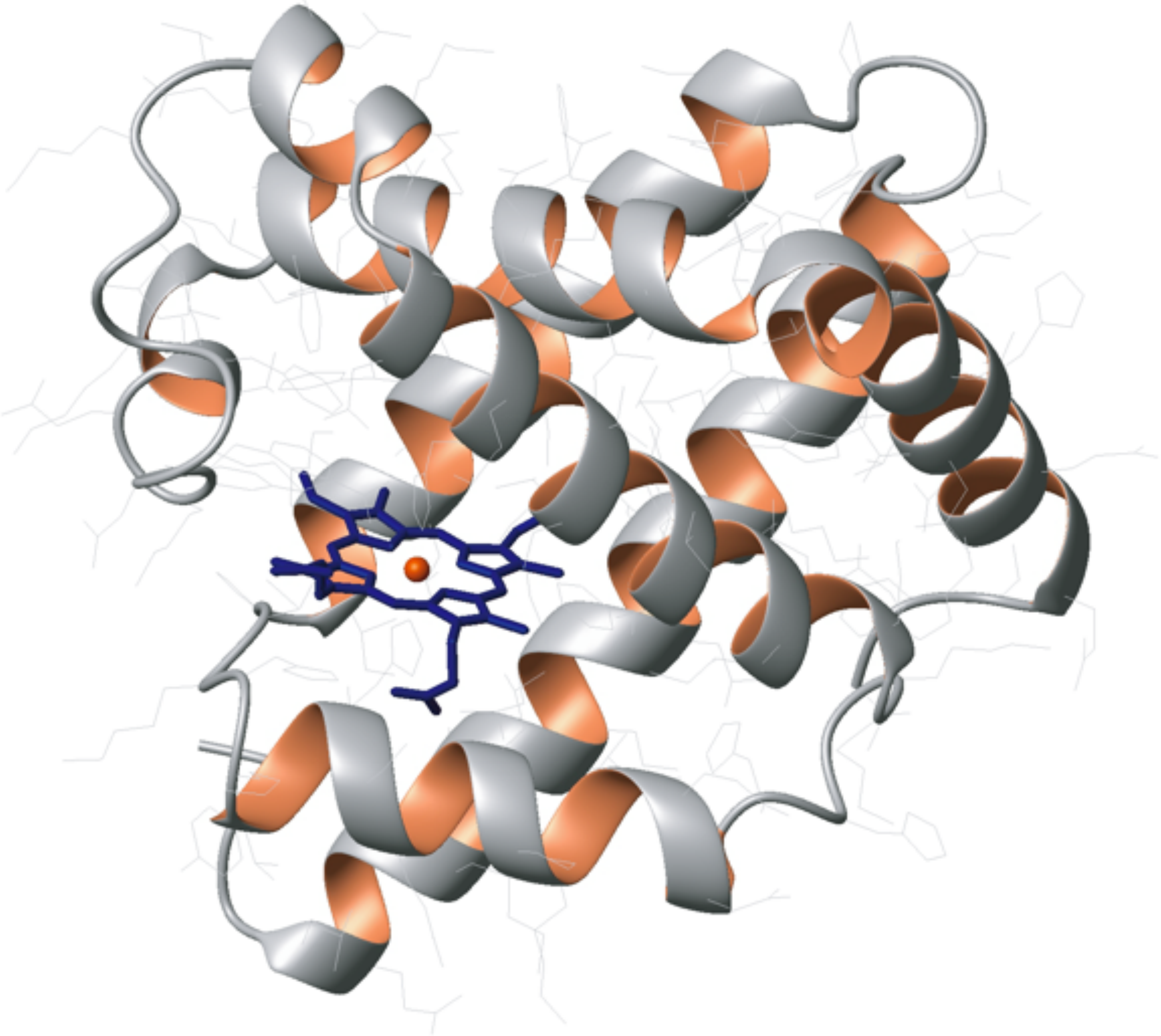
EPFL

Chemicals: Microscopic beauty from curiosity driven research

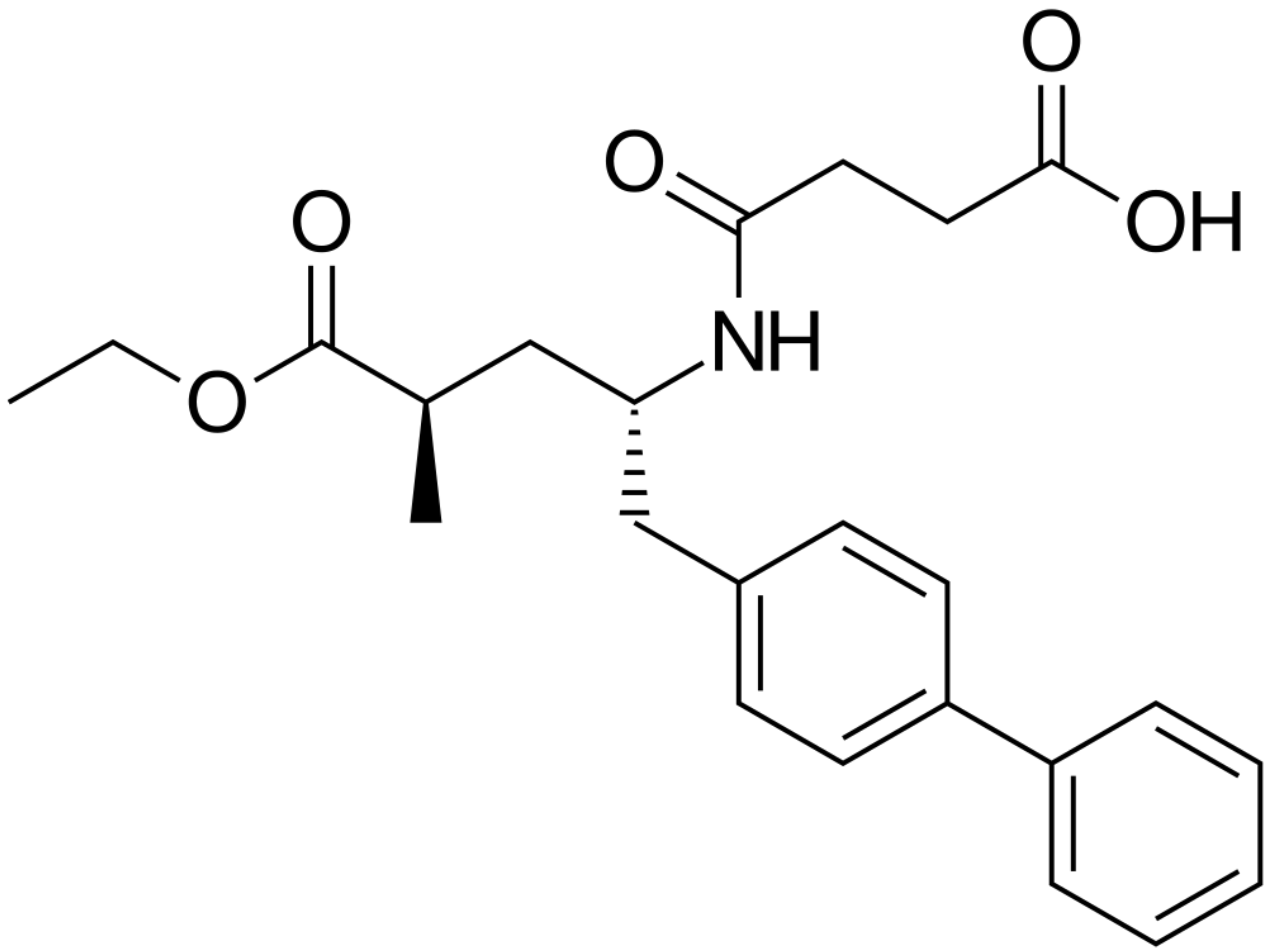


Chemicals: Microscopic beauty from curiosity driven research

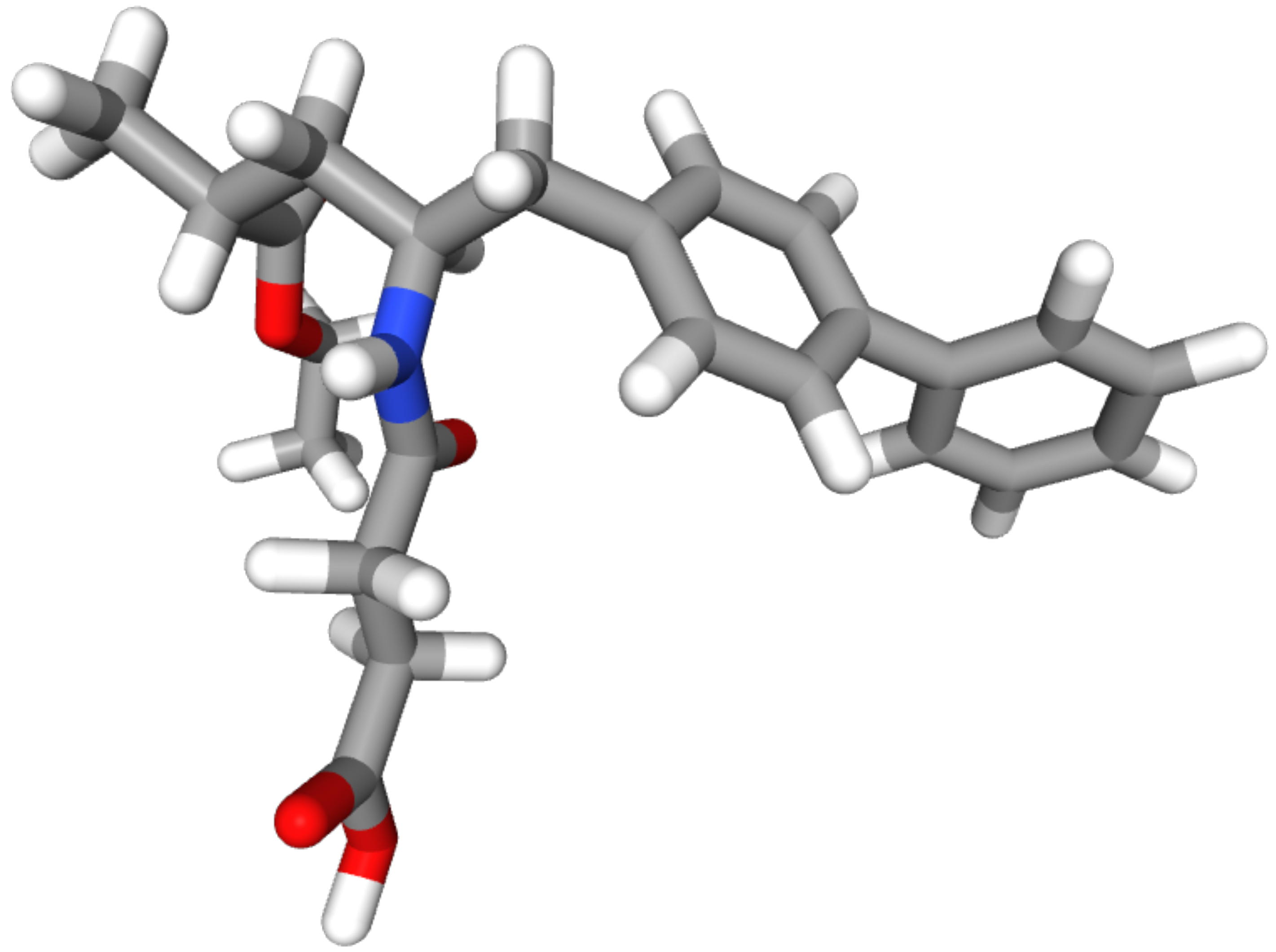


The Inner Life of the Cell

Chemistry Quiz: What's This?



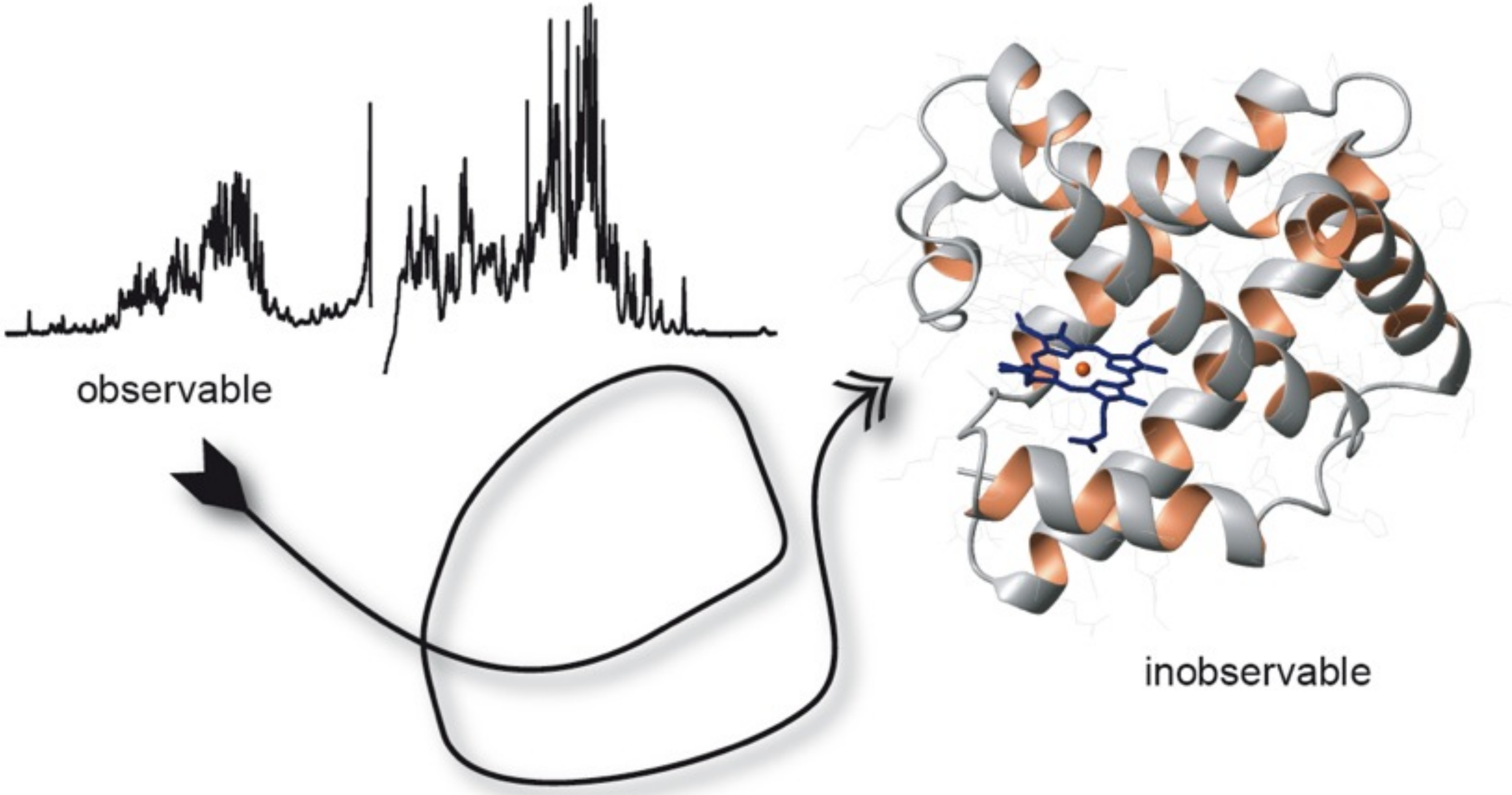
Representation of a Molecule of Sacubitril



← 1.5 nano meters →

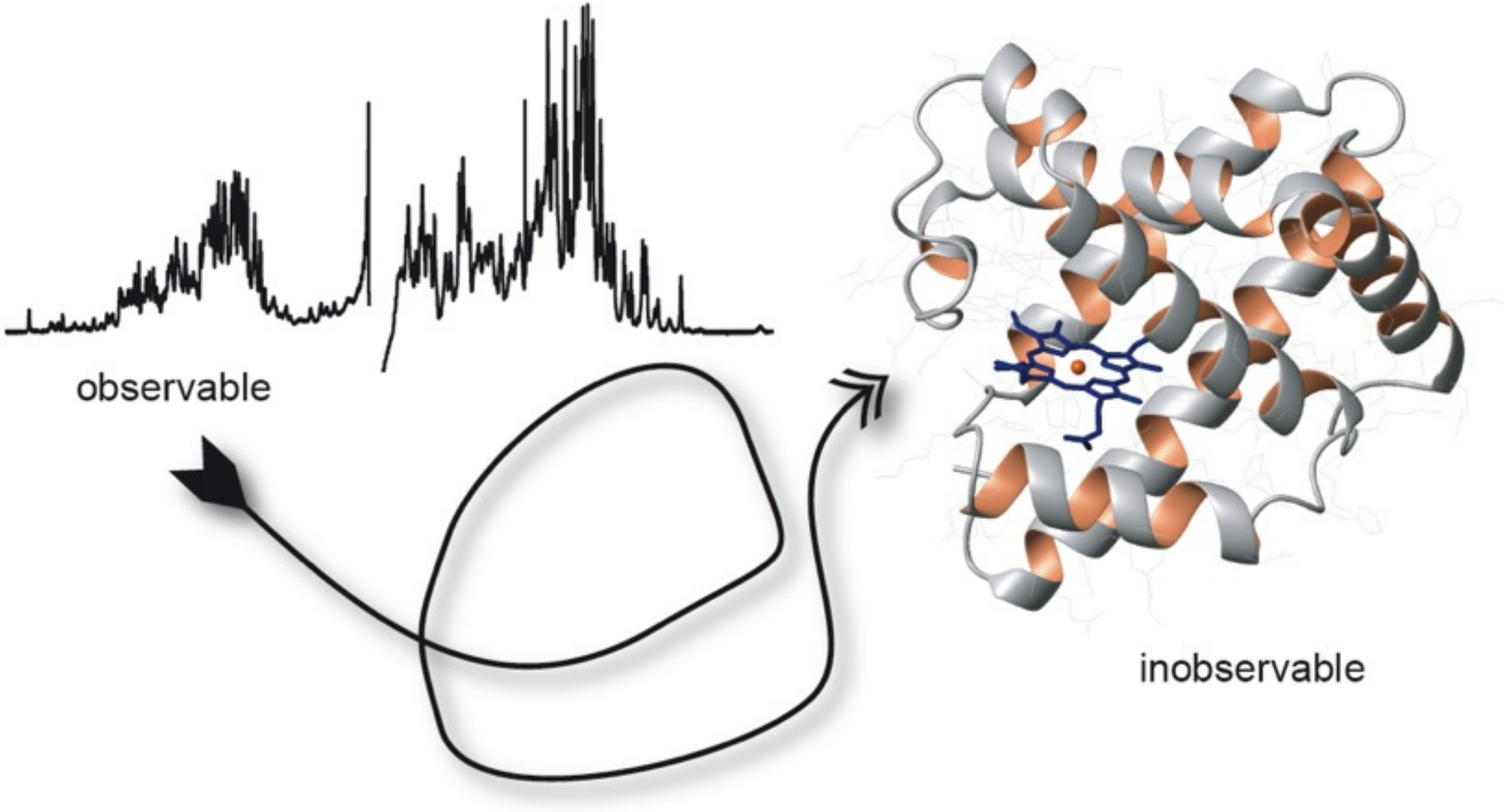
How can we “see” the invisible?

we need spies... **observables** that are more or less direct reporters of molecular structure.



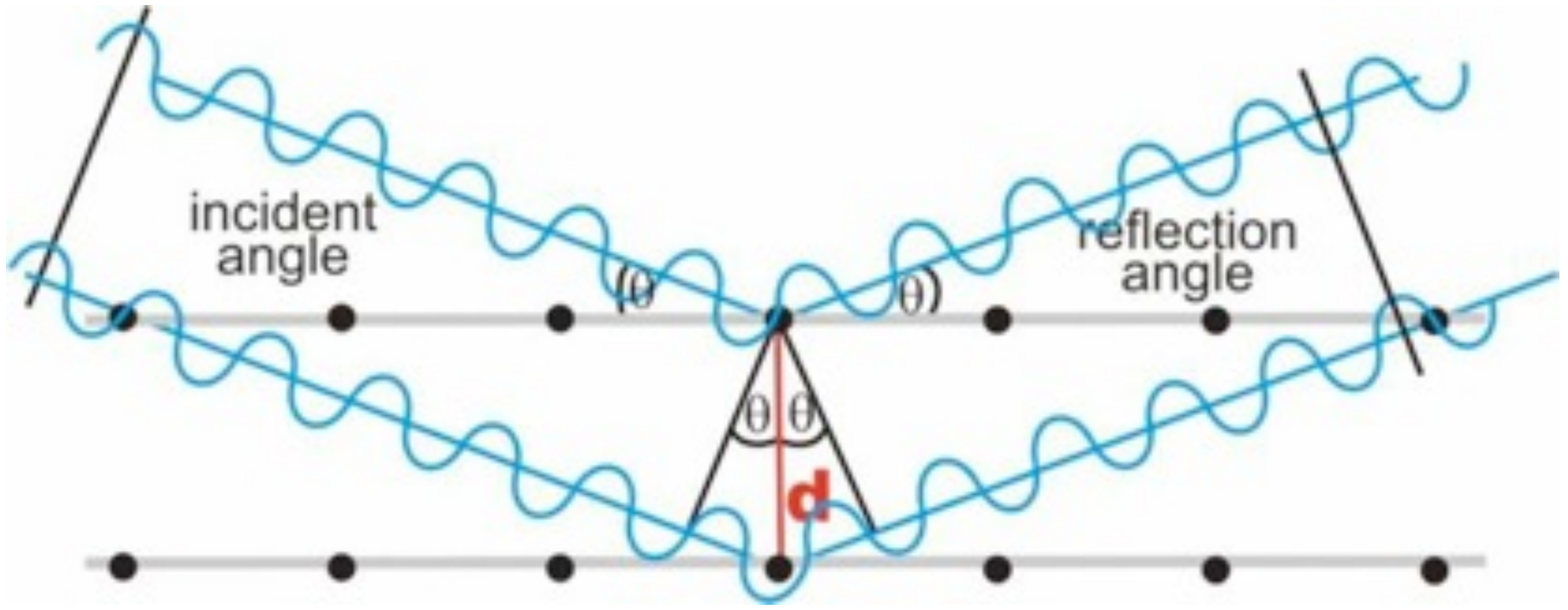
How can we “see” the invisible?

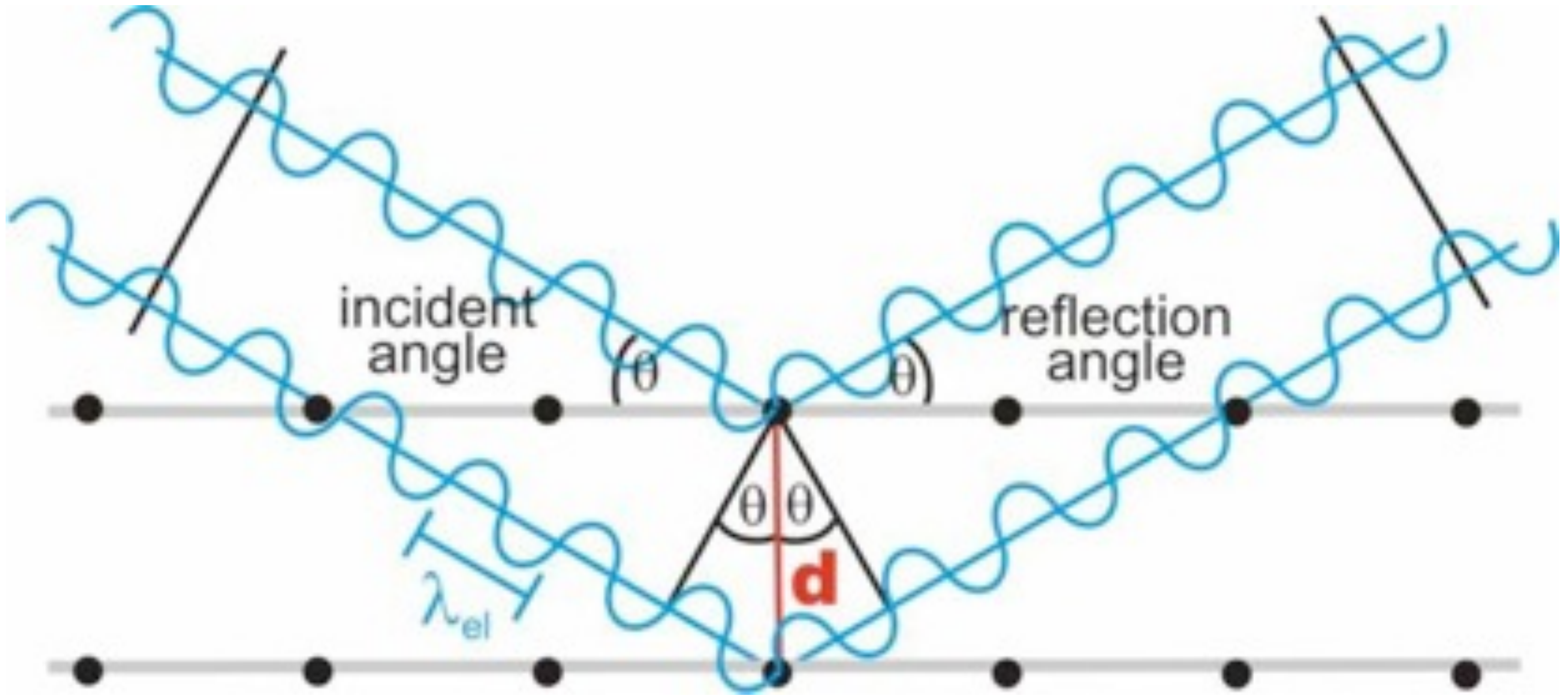
We will **interpret** the **observables** to construct **a model** of the molecular structure.



Observable N° 1:
Bragg Diffraction of X-Rays

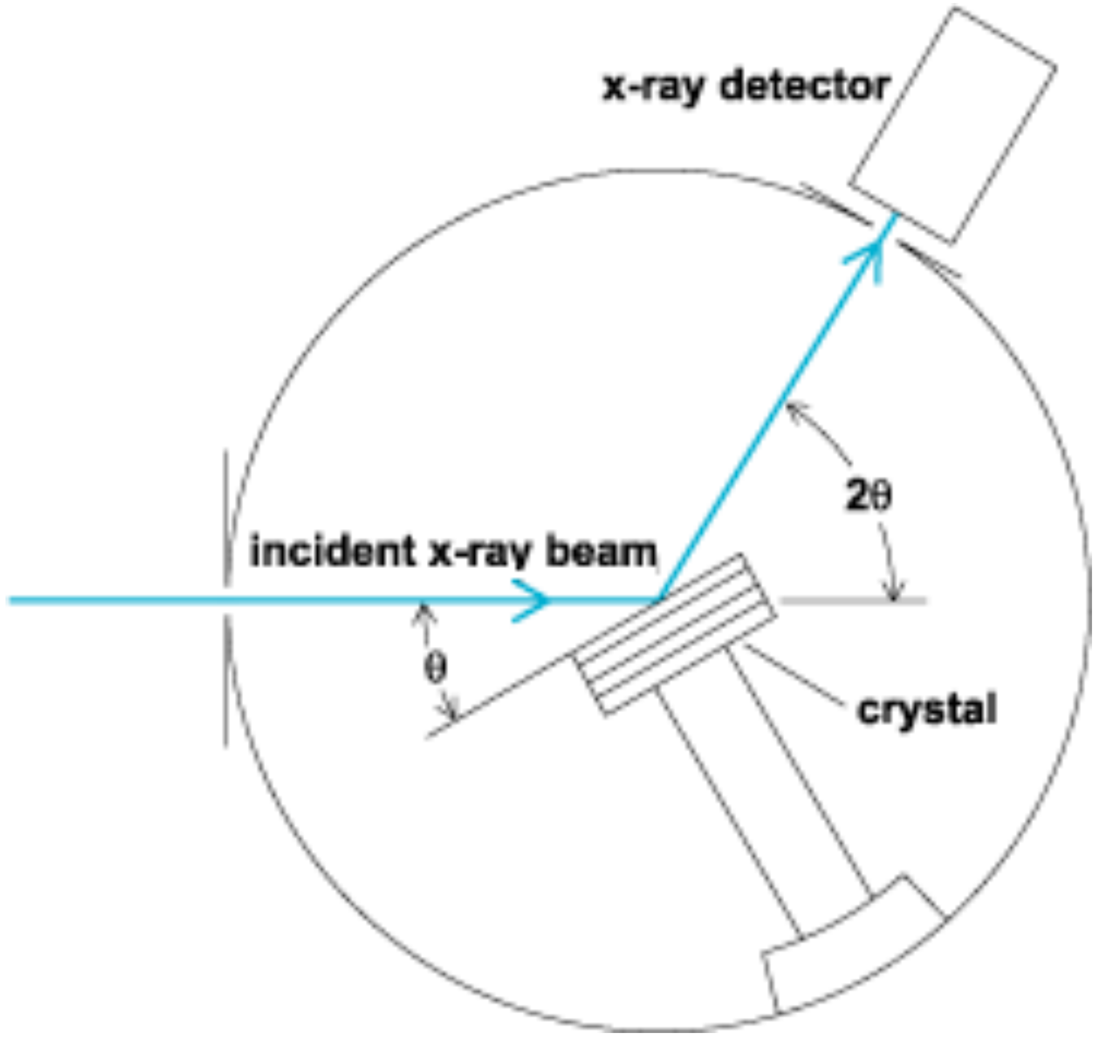
$$n\lambda = 2d\sin\Theta$$





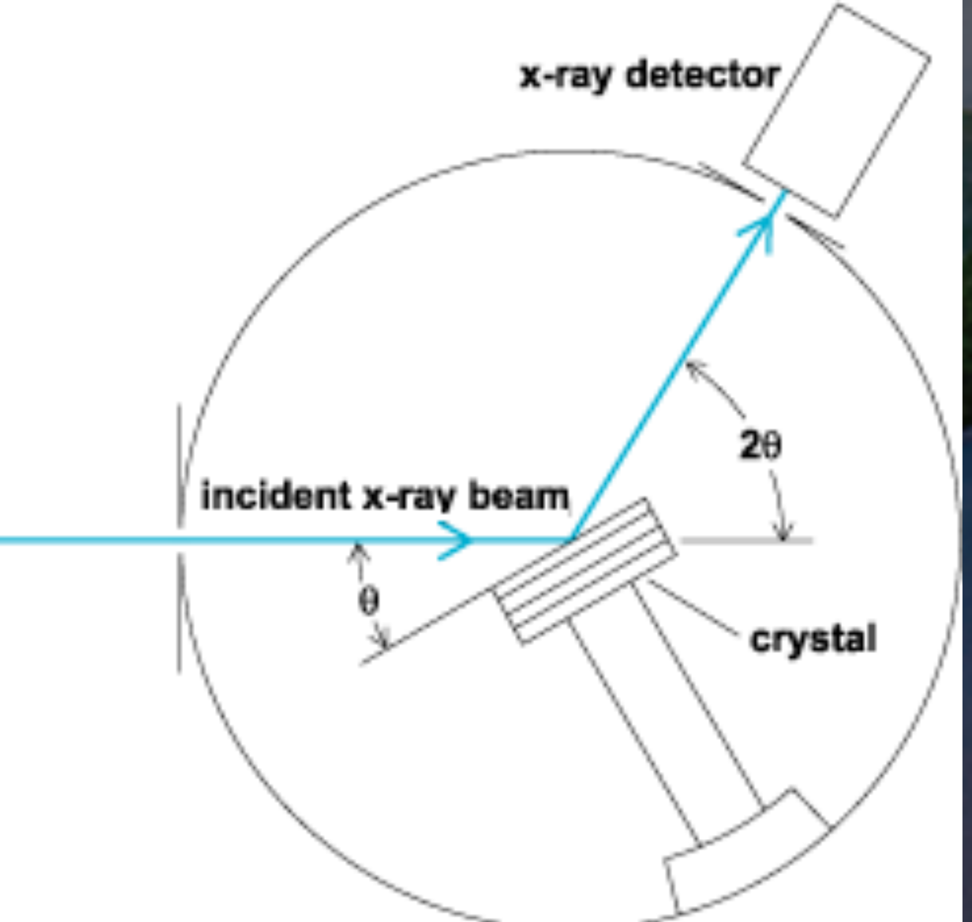
Diffraction of X-Rays: Structure Determination from Single Crystals

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

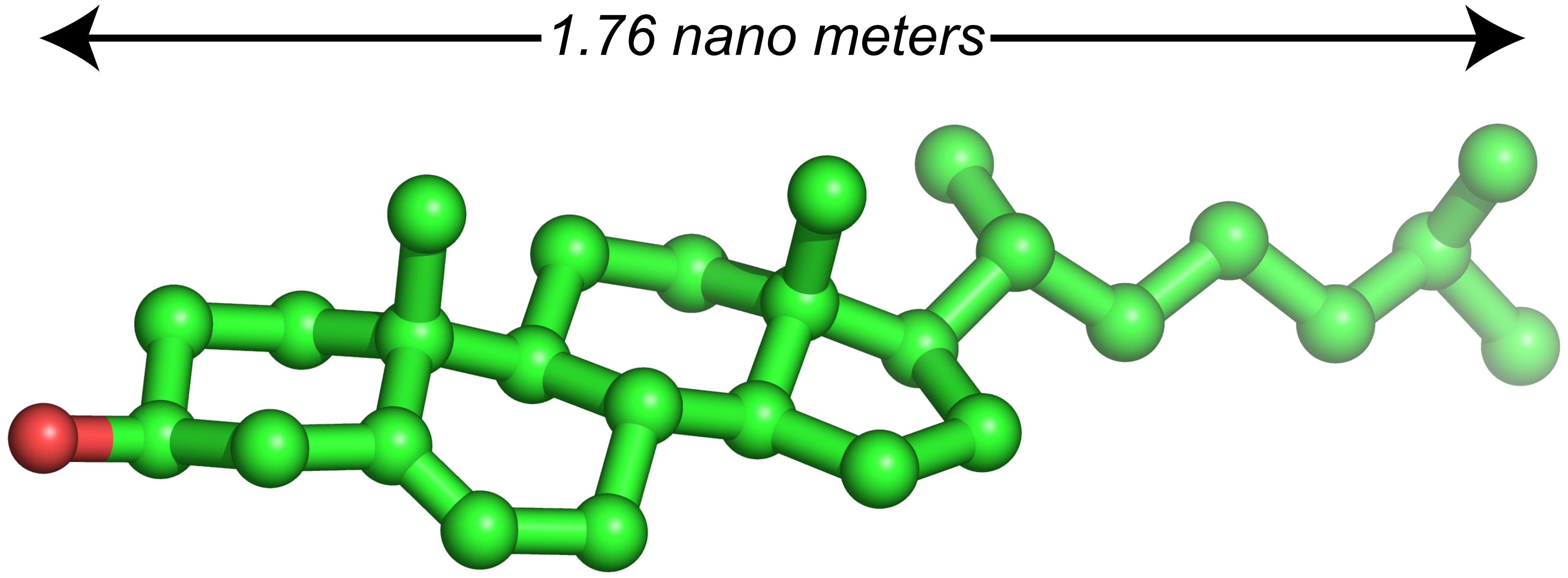


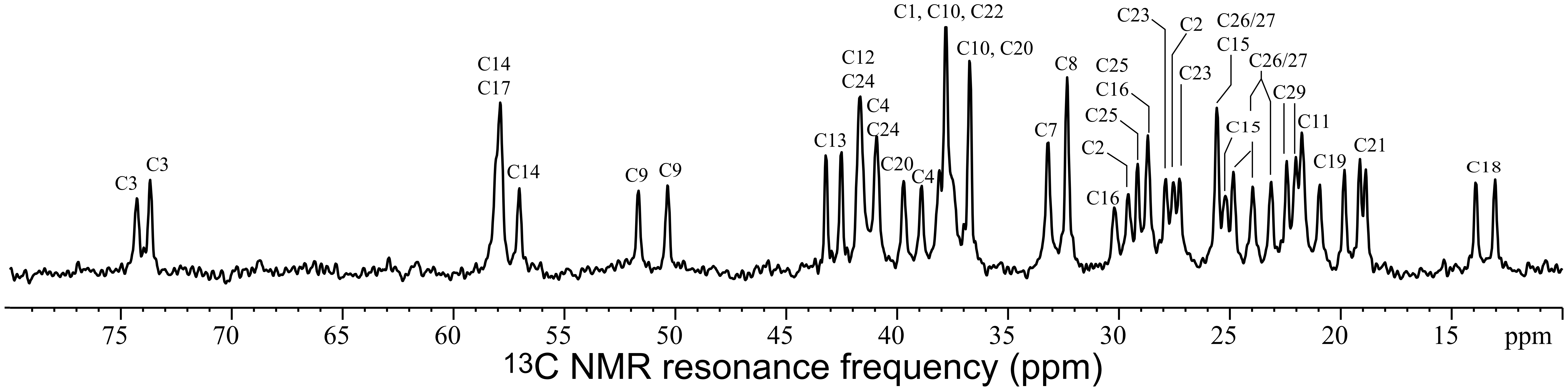
Diffraction will be treated by Prof. Bostedt in the final part of the course

Diffraction of X-Rays: Structure Determination from Single Crystals



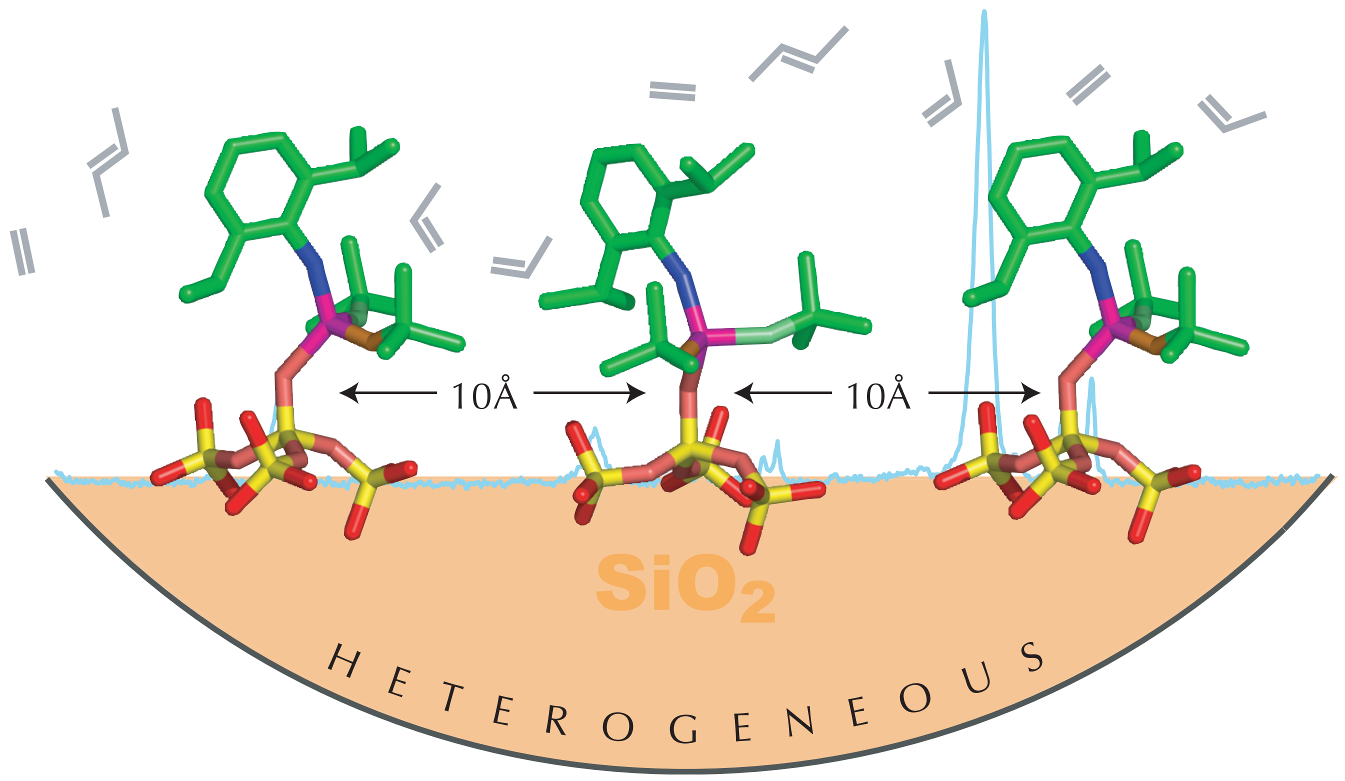
Spectroscopy Provides Eyes for Molecular and Materials Sciences





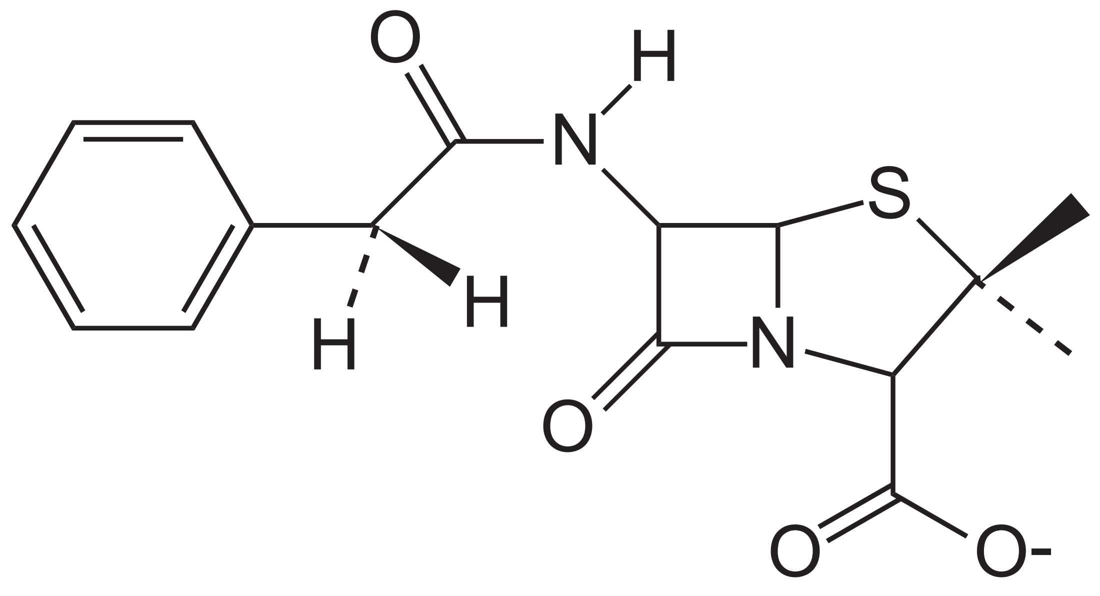
basic chemistry and catalysis

The first spectra from catalysts are recorded in the 1970s, as NMR revolutionizes the way chemists approach multi-step synthesis. In 2006 Schrock wins the Nobel Prize in Chemistry for his development of meta-thesis, which has become central to basic industrial chemistry. In the same year he uses high-field solid-state NMR to validate the mechanism of olefin meta-thesis on a supported catalyst.



from penicillin to taxol: stereochemistry in the drug industry

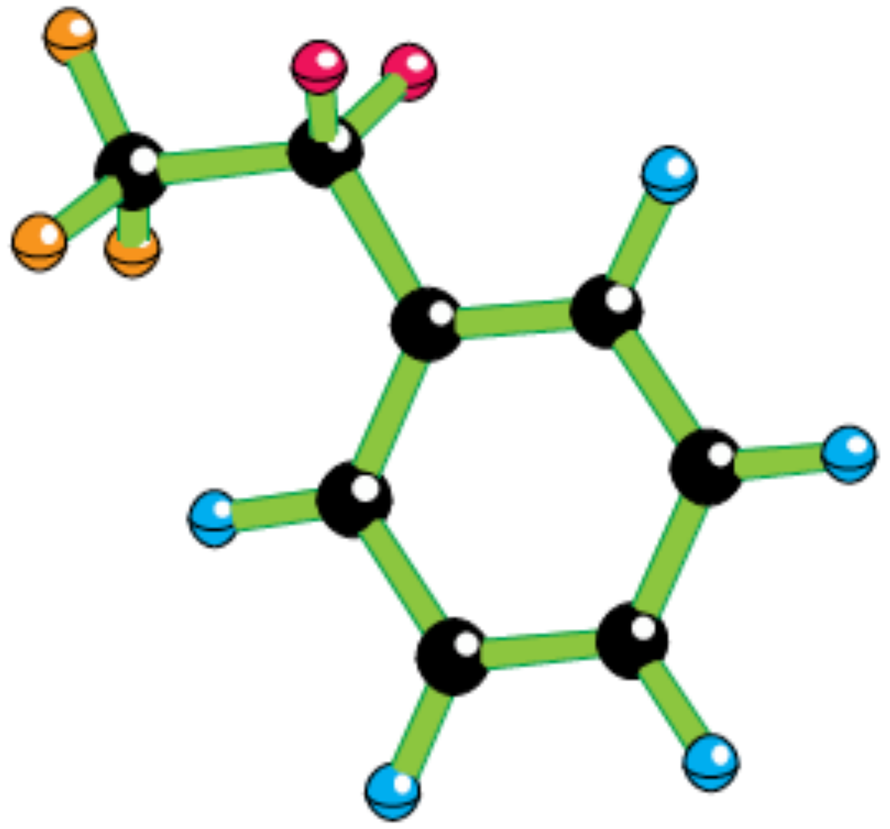
In 1959 Karplus proposes a dependence of H-H coupling constants on dihedral angles. Today this forms the basis for the determination of the stereochemistry of many of the therapeutic drugs on the market, crucial to both their safety and efficiency.

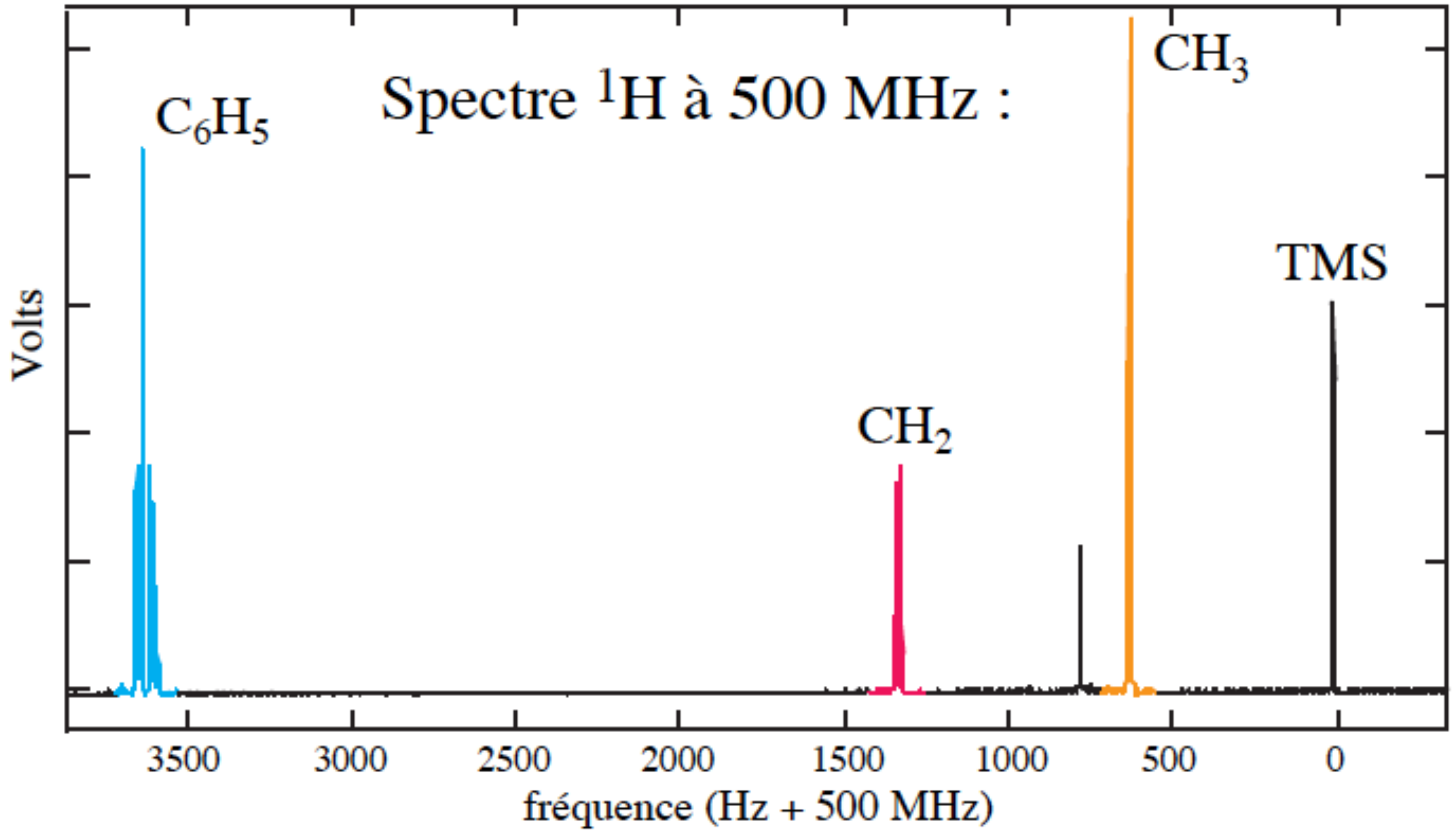


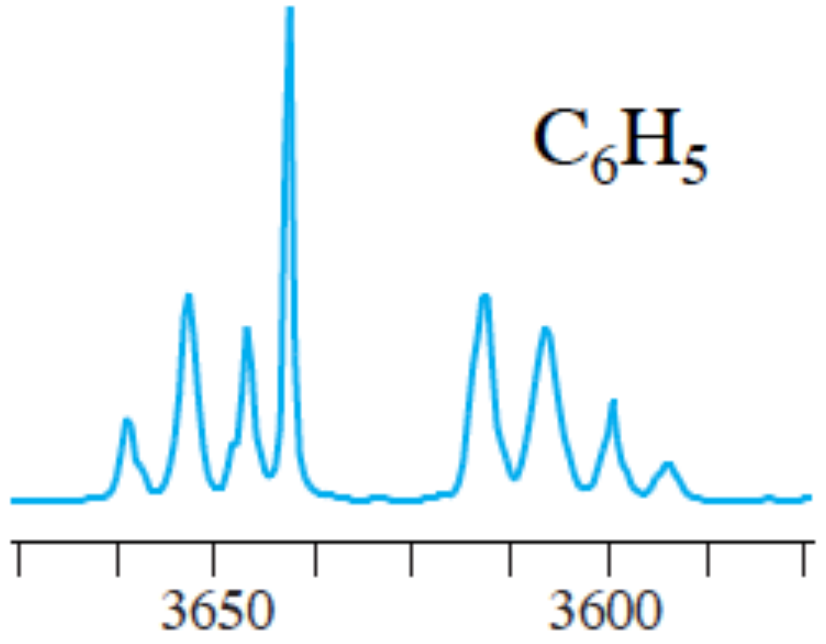
NMR: Is this it?

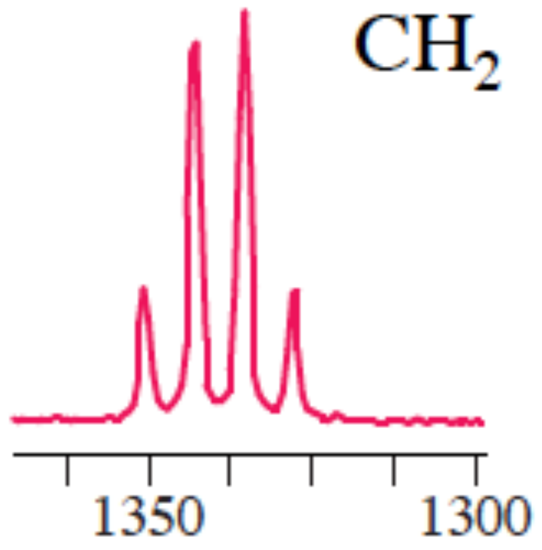
Molécule :

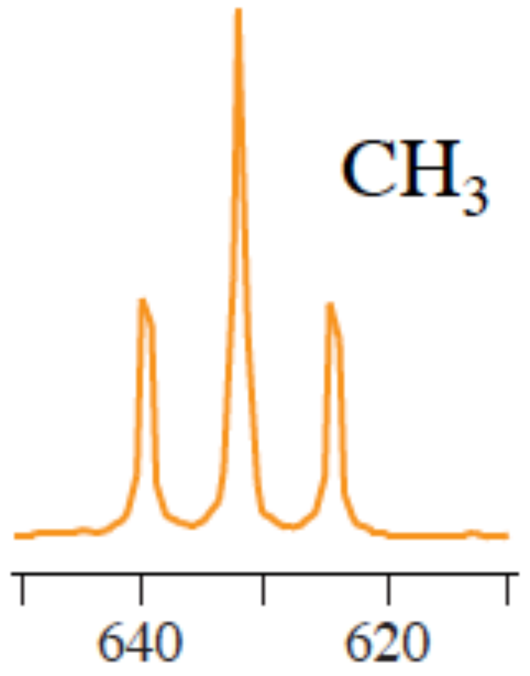
$CH_3 - CH_2 - C_6H_5$



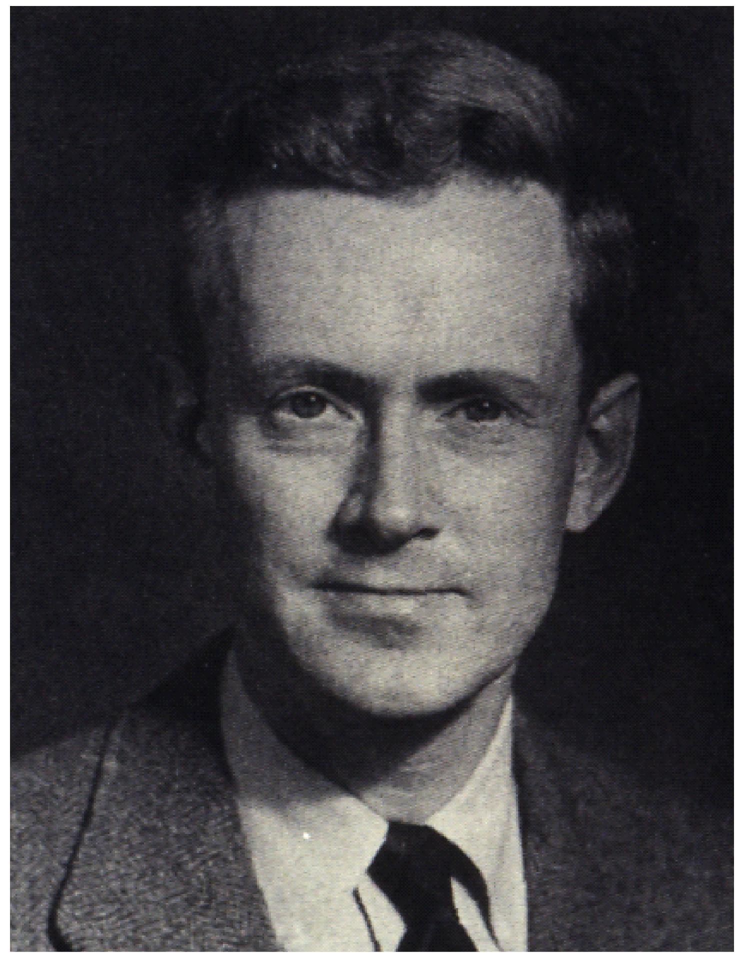




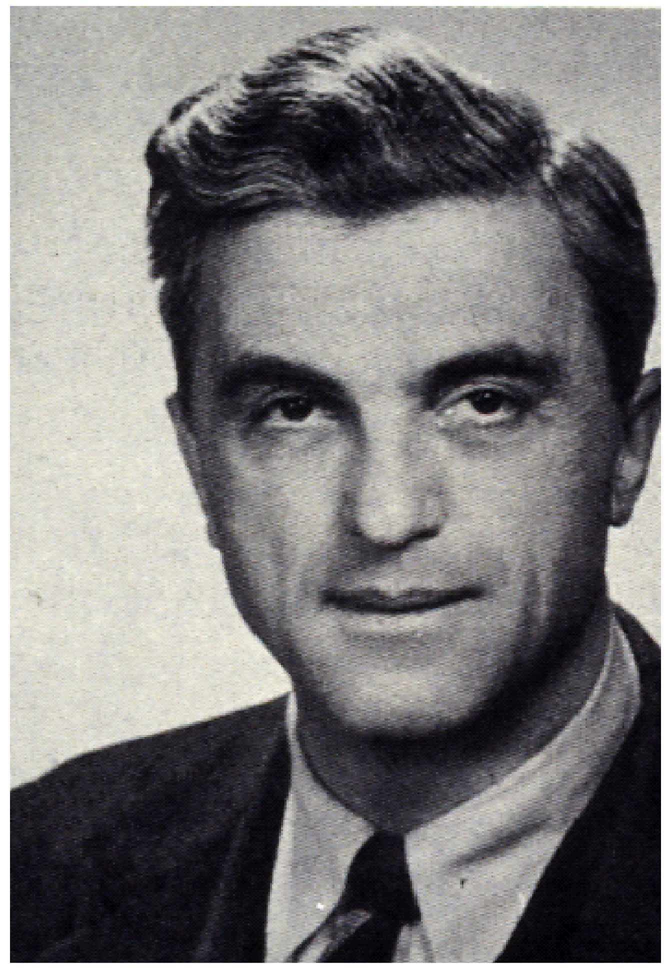




High Field NMR in Condensed Matter

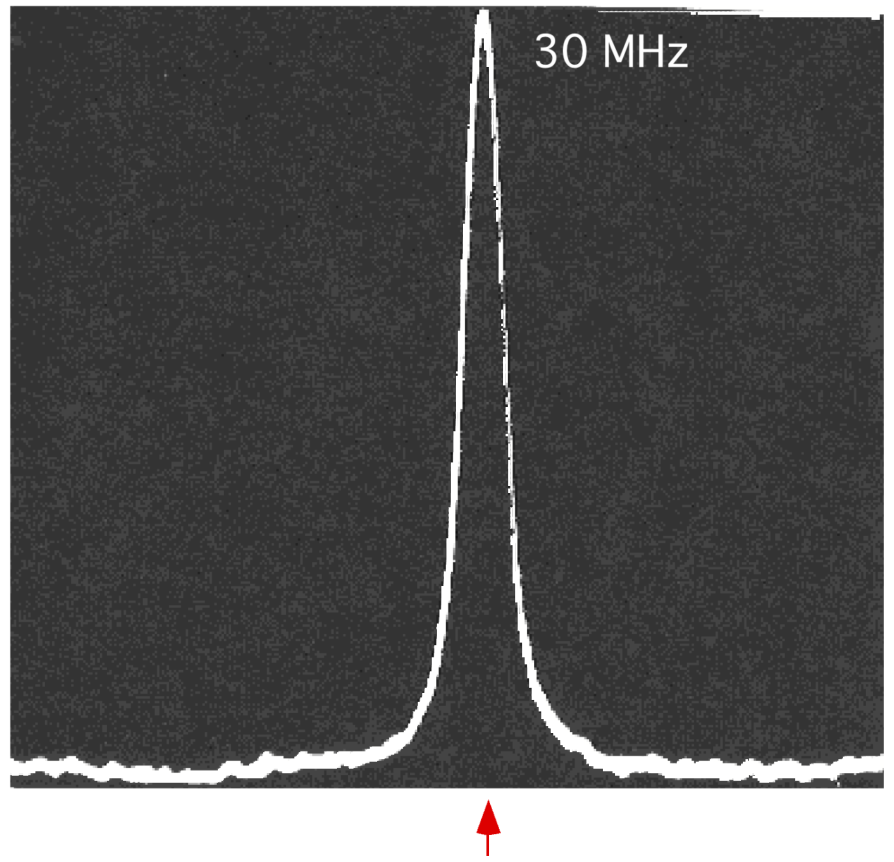


Edward Purcell
at Harvard

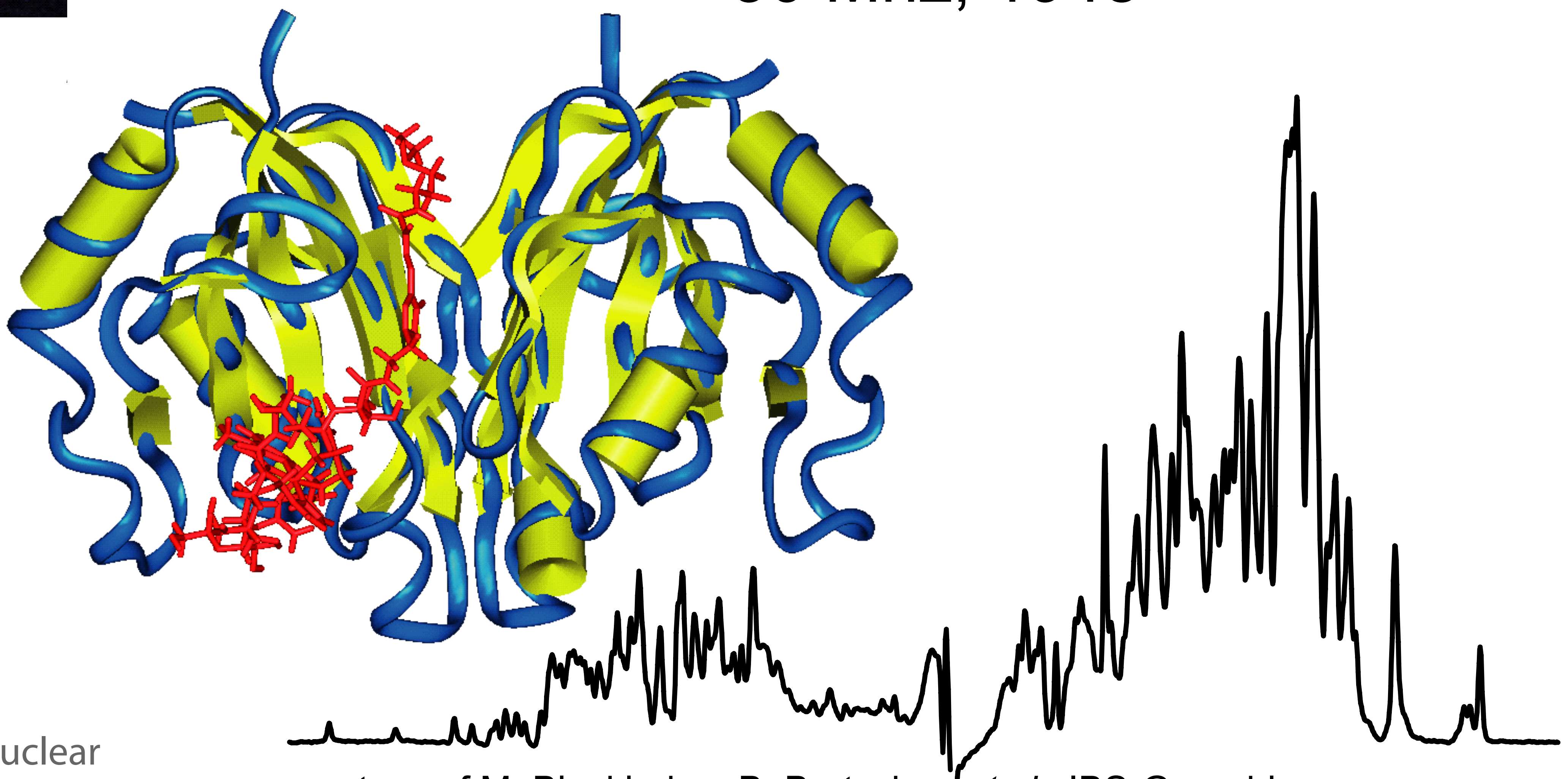


Felix Bloch
at Stanford

$$\omega_0 = -\gamma B_0$$



30 Mhz, 1948

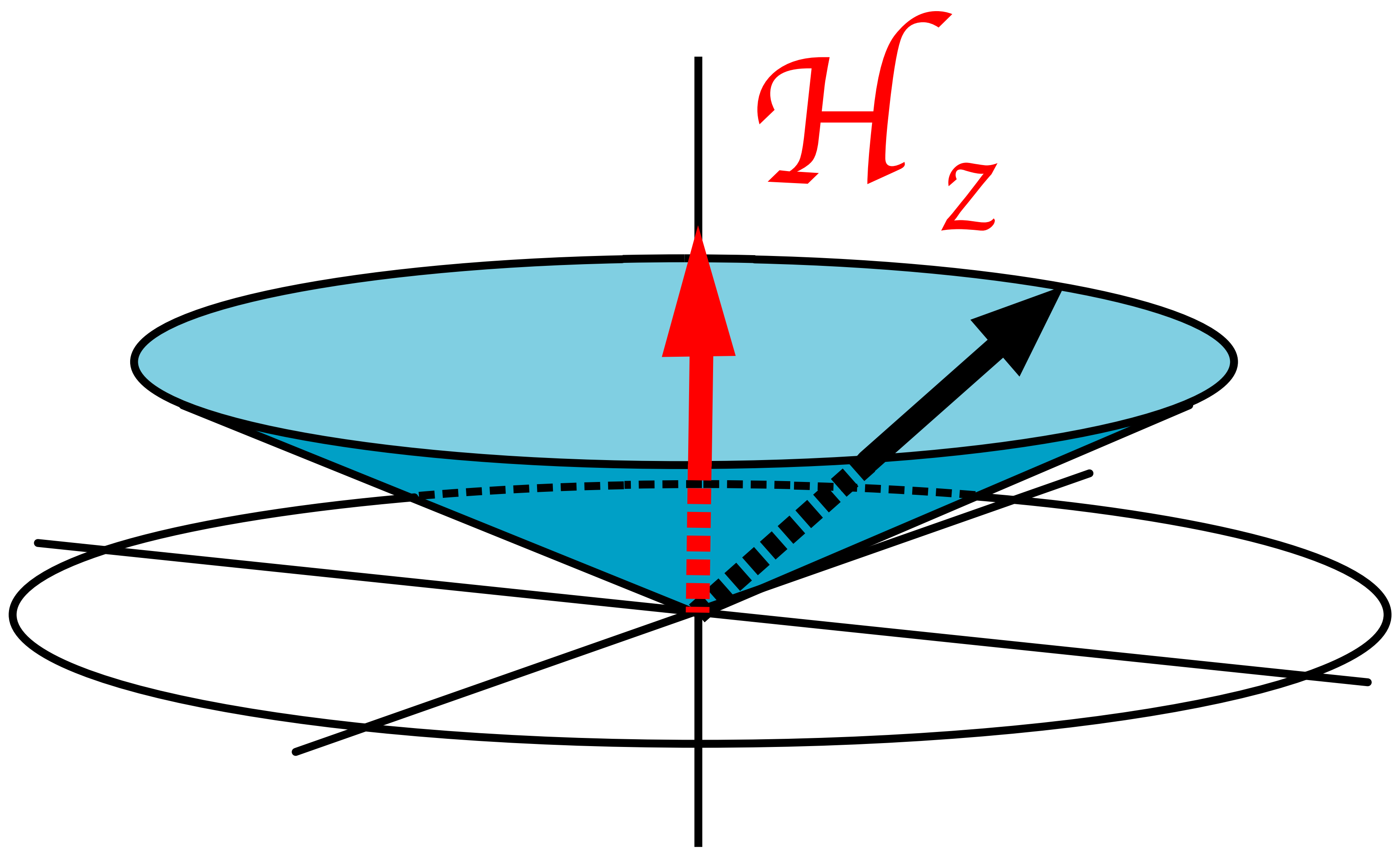


courtesy of M. Blackledge, B. Brutscher, *et al.*, IBS-Grenoble

800 MHz, 2000

The Nobel Prize in Physics 1952
"for their development of new methods for nuclear magnetic precision measurements and discoveries in in connection therewith"

How Can We "See" Nanometer Sized Objects?

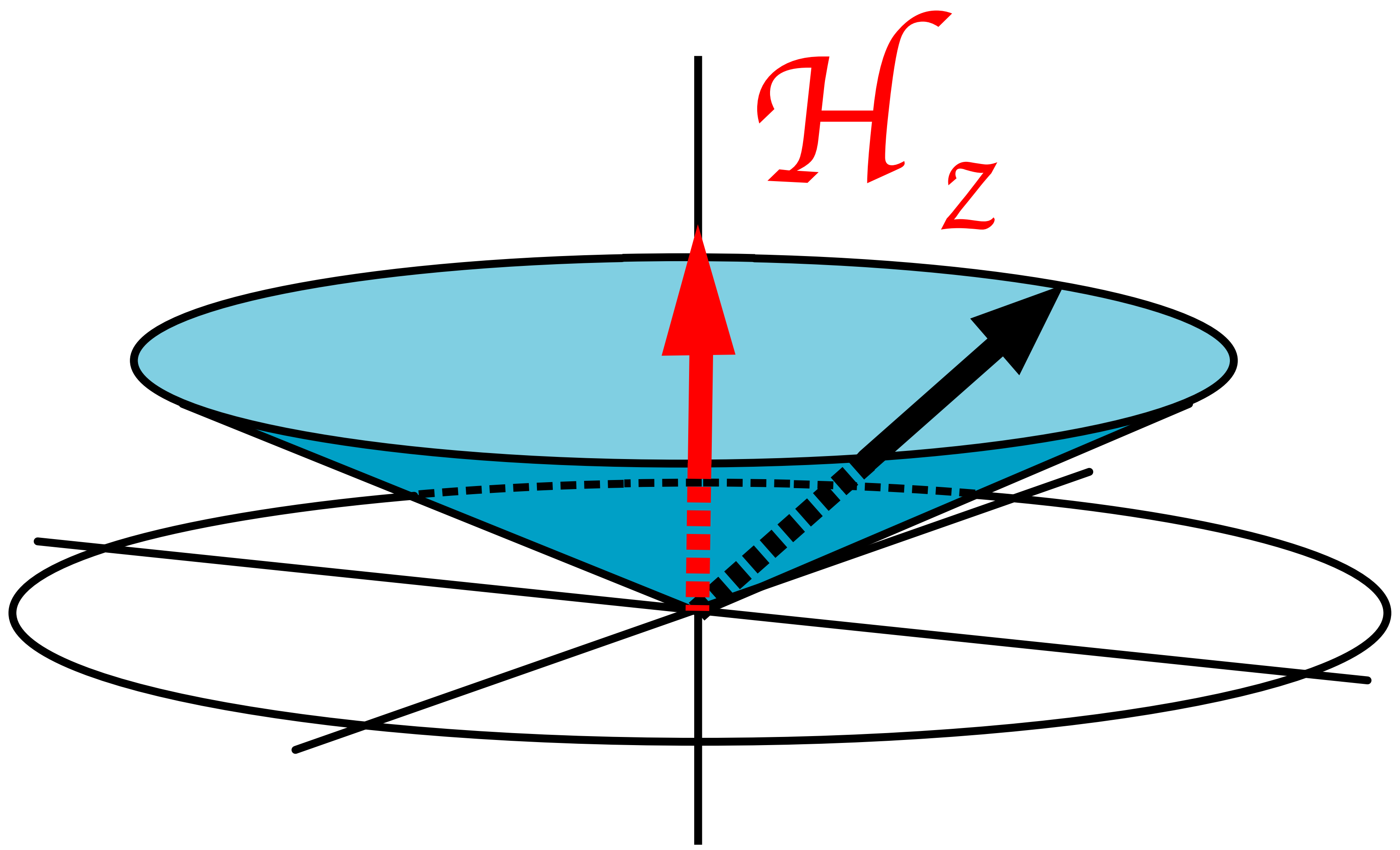


Atoms and molecules have a series of ***quantized properties***.
They exist in well defined quantum states.

The energy differences between states ***depend on the local interactions and the environment***.

Spectroscopy ***detects these energy differences***.

How Can We "See" Nanometer Sized Objects?



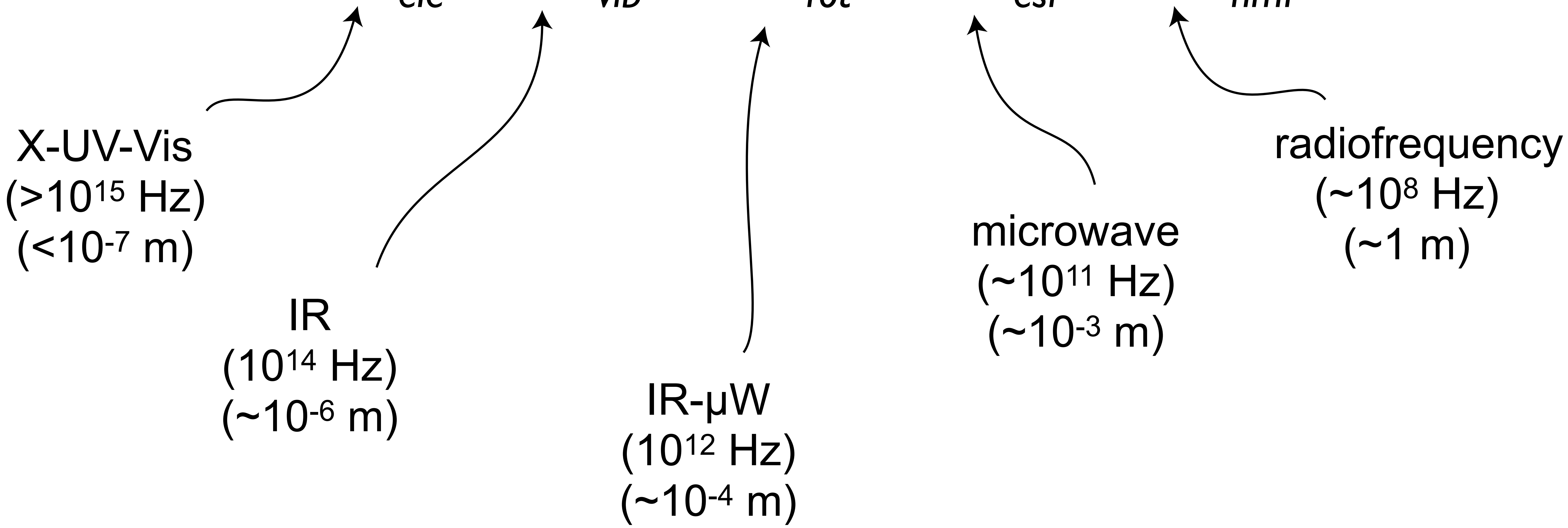
Nuclei have a quantum property called ***spin***.
Spin leads to magnetism.

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.

The System Hamiltonian: The Key

$$\mathcal{H} = \mathcal{H}_{ele} + \mathcal{H}_{vib} + \mathcal{H}_{rot} + \mathcal{H}_{esr} + \mathcal{H}_{nmr}$$



X-UV-Vis
($>10^{15}$ Hz)
($<10^{-7}$ m)

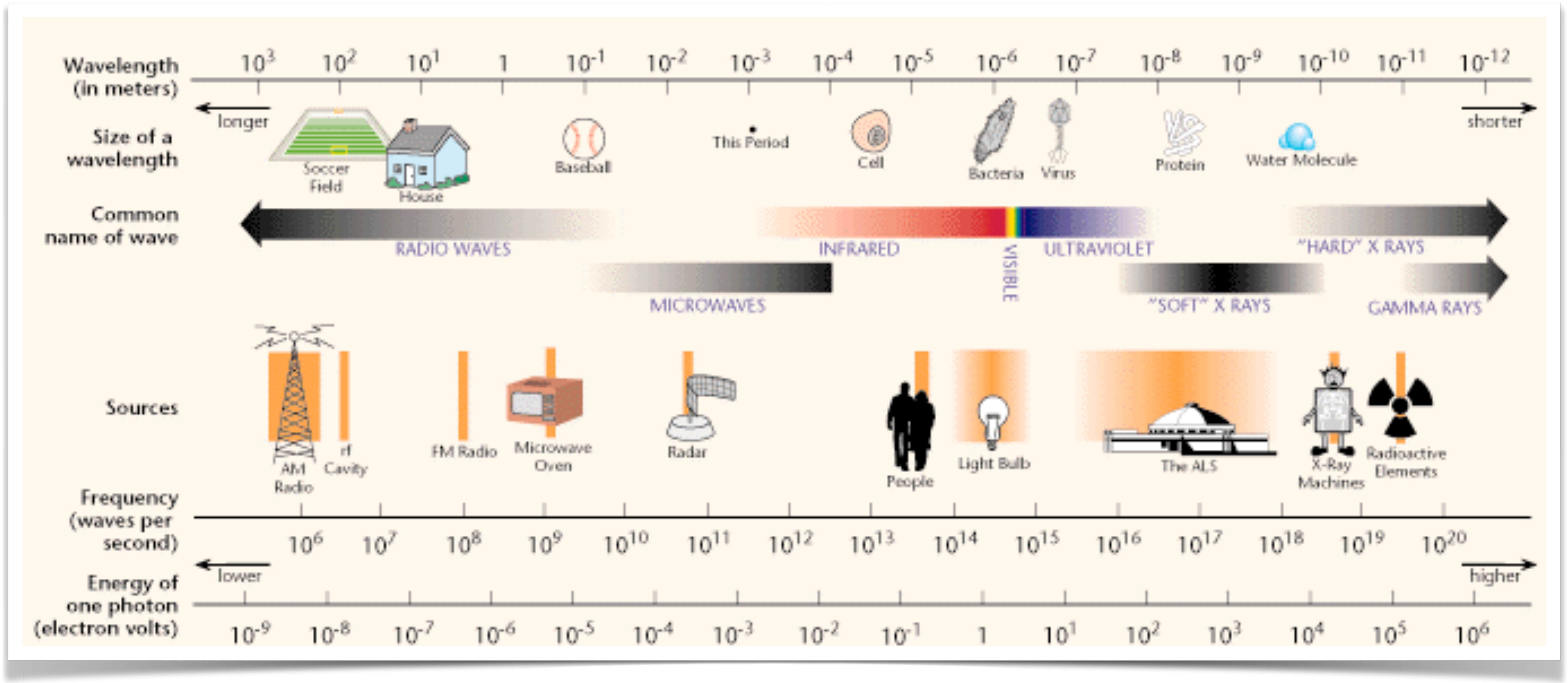
IR
(10^{14} Hz)
($\sim10^{-6}$ m)

IR-µW
(10^{12} Hz)
($\sim10^{-4}$ m)

microwave
($\sim10^{11}$ Hz)
($\sim10^{-3}$ m)

radiofrequency
($\sim10^{8}$ Hz)
(~1 m)

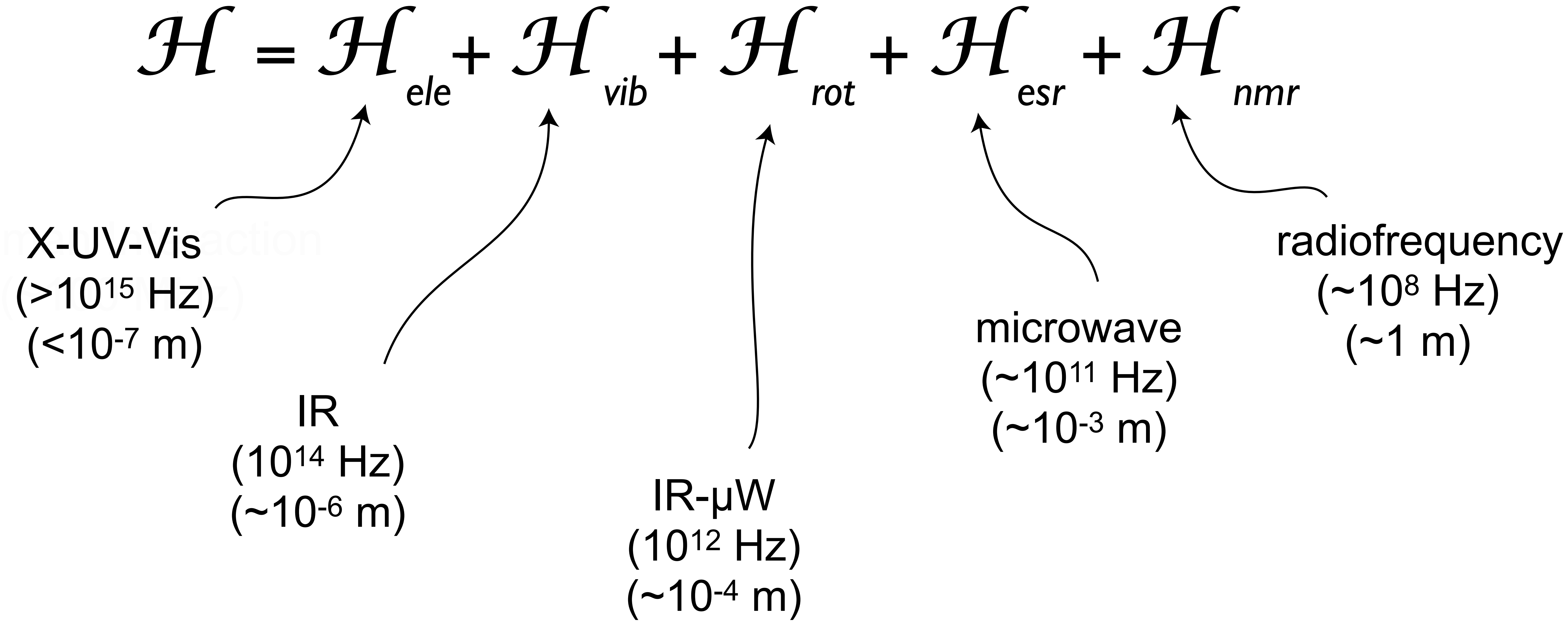
The energy differences between states ***depend on the local interactions and the environment***.



The energy differences between states ***depend on the local interactions and the environment***.

image: http://www.lbl.gov/MicroWorlds/ALSTool/EMSpec/EMSpec2.html

The System Hamiltonian: The Key



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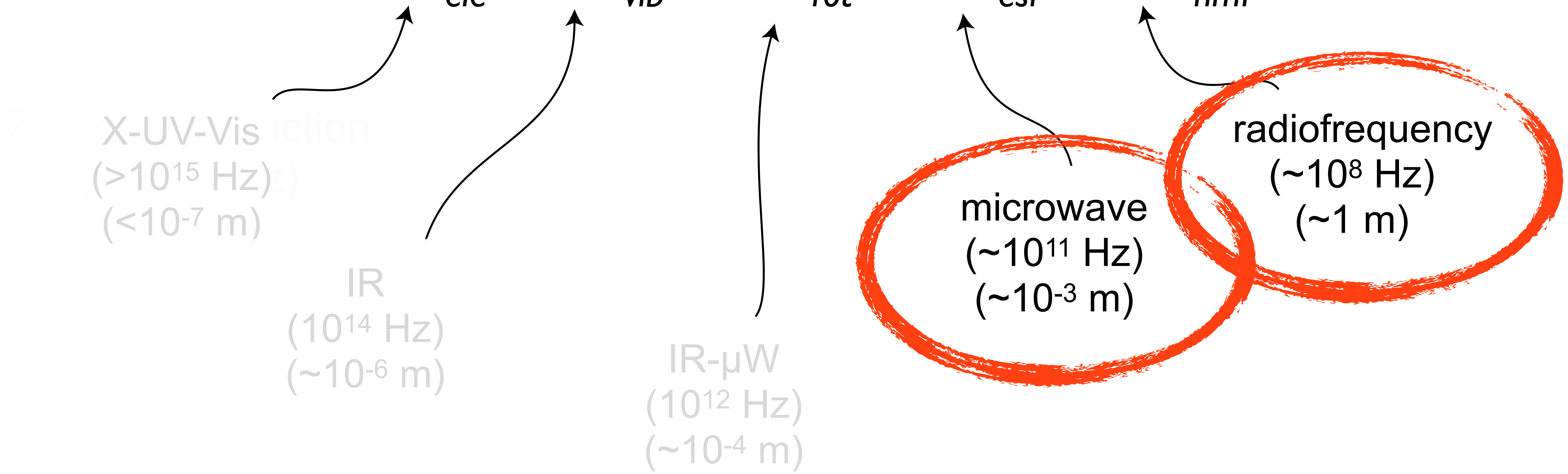


The energy differences between states ***depend on the local interactions and the environment***.

The NMR Hamiltonian: The Key

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

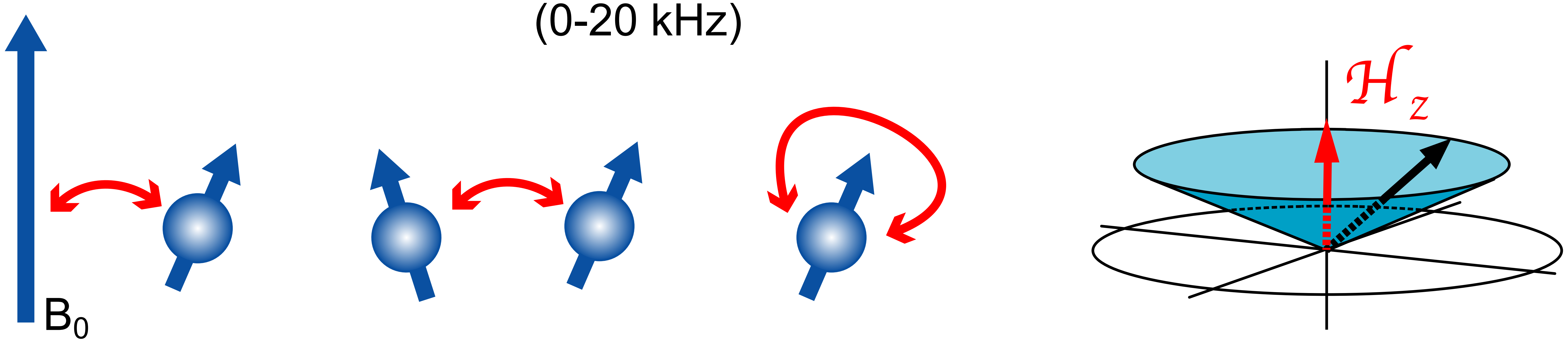
Zeeman Interaction
(~100 MHz)

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:
The dipolar interaction between spins

The NMR Hamiltonian contains several terms, linked to atomic coordinates, electronic structure, or to molecular dynamics.

As an example we remark the magnitude of the dipolar interaction between two nuclear spins j and k is:

$$b_{jk} = -\frac{\mu_0}{4\pi}\frac{\gamma_j \gamma_k \hbar}{r_{jk}^3}$$

If we could measure the dipolar interactions between all pairs of spins, we could deduce the coordinates of the atoms....

More: Malcom H. Levitt, Spin Dynamics, chapter 9.3

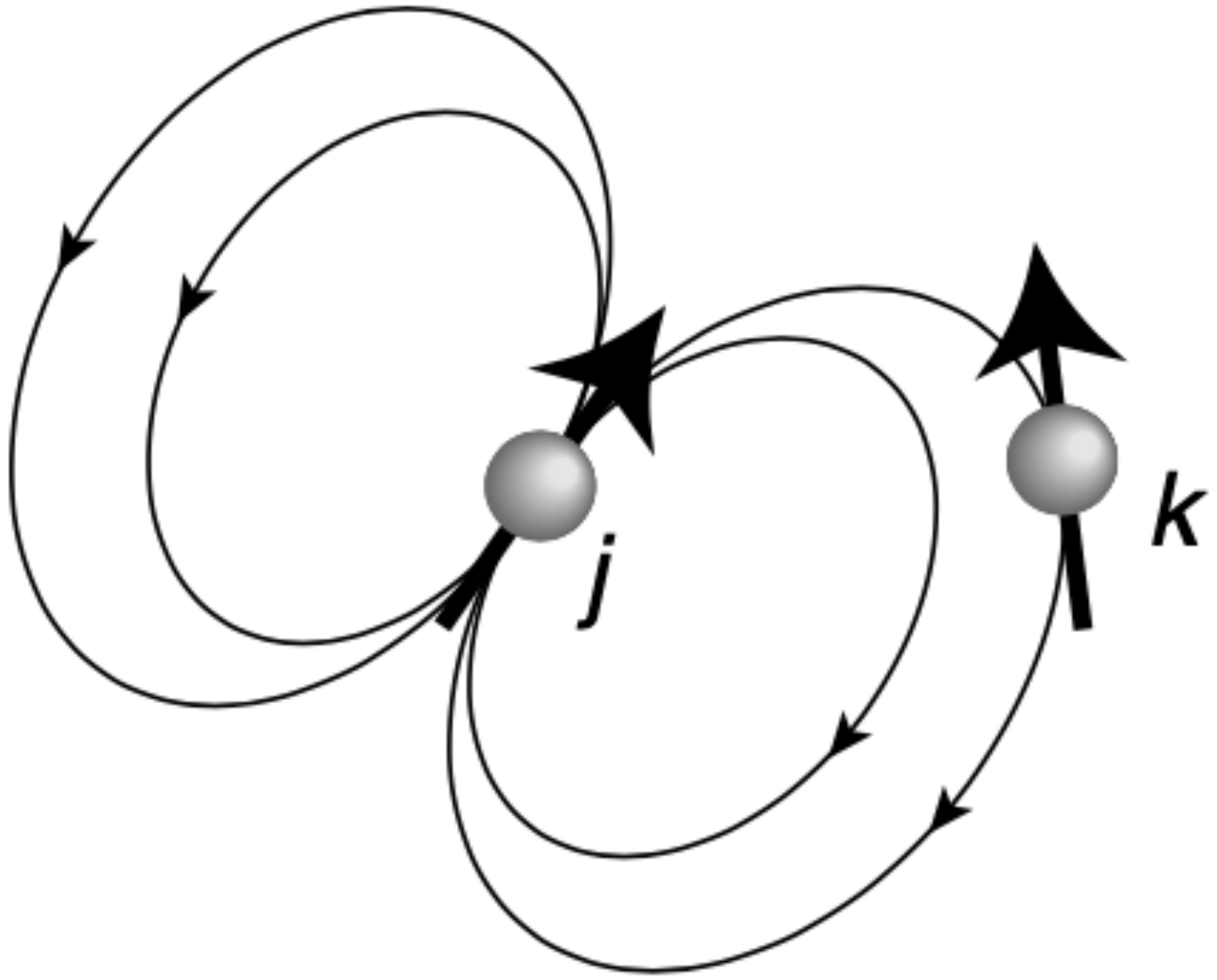
Observable N° 2:
The dipolar interaction between spins

The direct dipole–dipole coupling between spins is conceptually simple. Since each nuclear spin is magnetic, it generates a magnetic field in the surrounding space depending on the direction of the spin magnetic moment. A second nuclear spin then interacts with this magnetic field (and vice-versa). This interaction is called the through-space dipole–dipole coupling, or direct dipole–dipole coupling, because the fields between the nuclear spins propagate through the intervening space, without involving the electron clouds.

The interaction Hamiltonian is:

$$\widehat{\mathcal{H}}_{jk}^{\mathrm{DD,full}} = b_{jk}\left(3(\hat{\mathbf{I}}_j \cdot \mathbf{e}_{jk})(\hat{\mathbf{I}}_k \cdot \mathbf{e}_{jk}) - \hat{\mathbf{I}}_j \cdot \hat{\mathbf{I}}_k\right)$$

where $\hat{\mathbf{I}}_j$ and $\hat{\mathbf{I}}_k$ are the spin angular momentum operators which are proportional to the magnetic moments ($\boldsymbol{\mu}$) of the nuclei, and where $\mathbf{e}_{jk}$ is the unit vector parallel to the line joining the centres of the two nuclei.



More: Malcom H. Levitt, Spin Dynamics, chapter 9.3

Observable N° 3:
The chemical shift

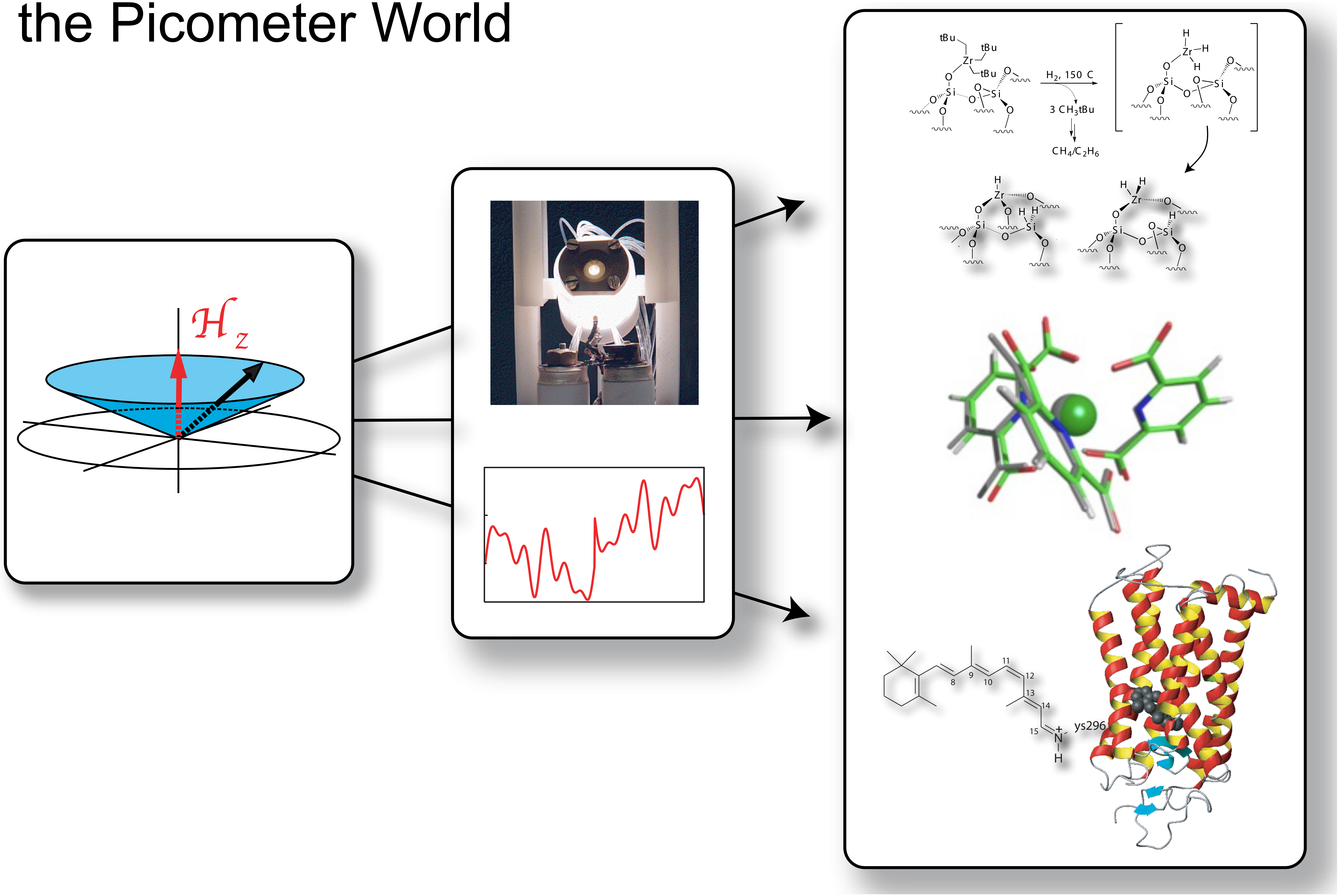
The NMR Hamiltonian contains several terms, linked to atomic coordinates, electronic structure, or to molecular dynamics.

As another example we remark the chemical shift, which is a modification of the resonance frequency with respect to the Larmor frequency, due to the shielding of the nucleus by the electrons.

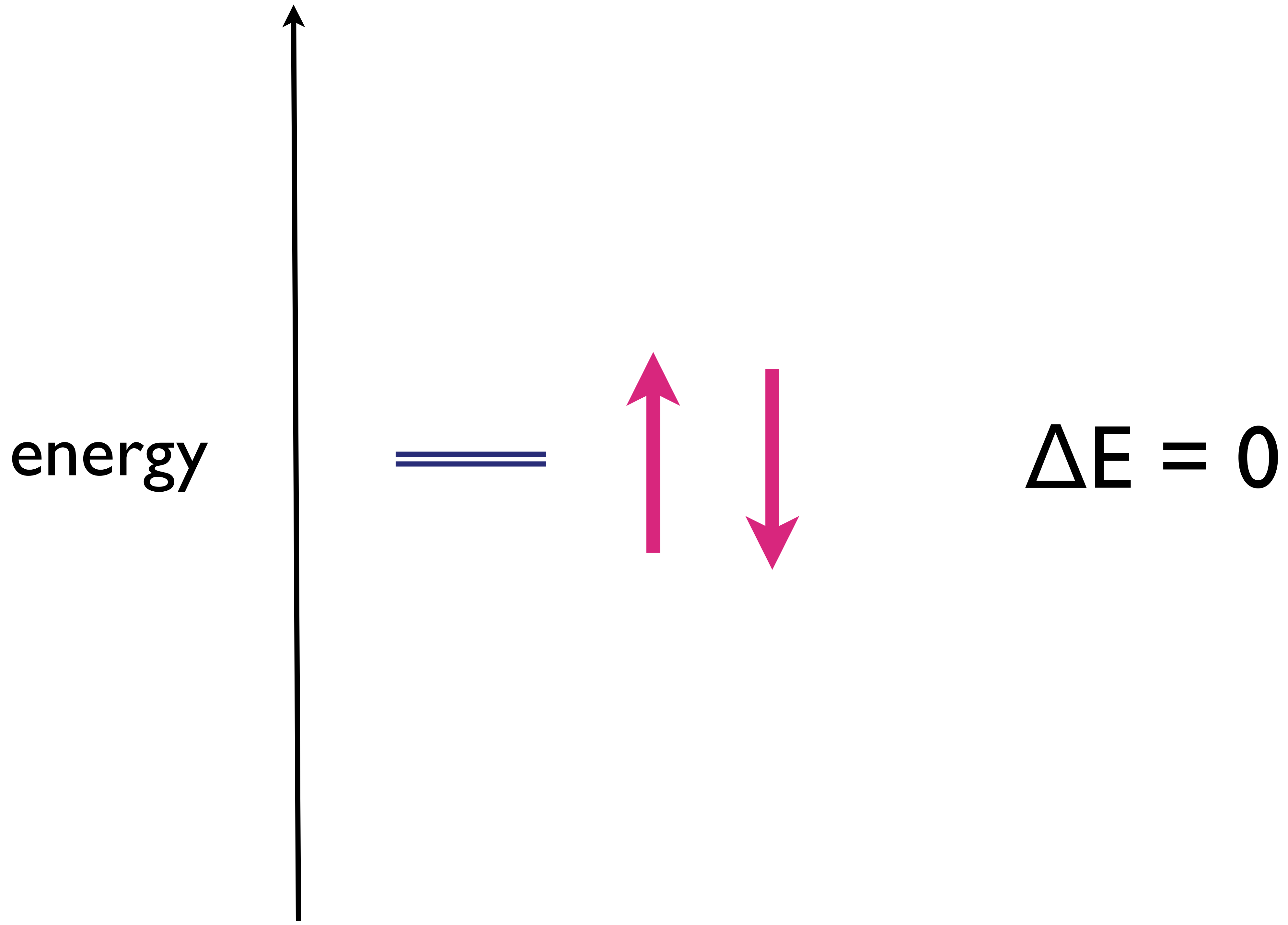
$$\omega = -\gamma(1-\sigma)B_0$$

If we could measure the chemical shifts of all the spins, we could deduce the coordinates of the atoms, **if we knew** the relation between chemical shift and structure....

Spectroscopy: Whispered Messages from the Picometer World

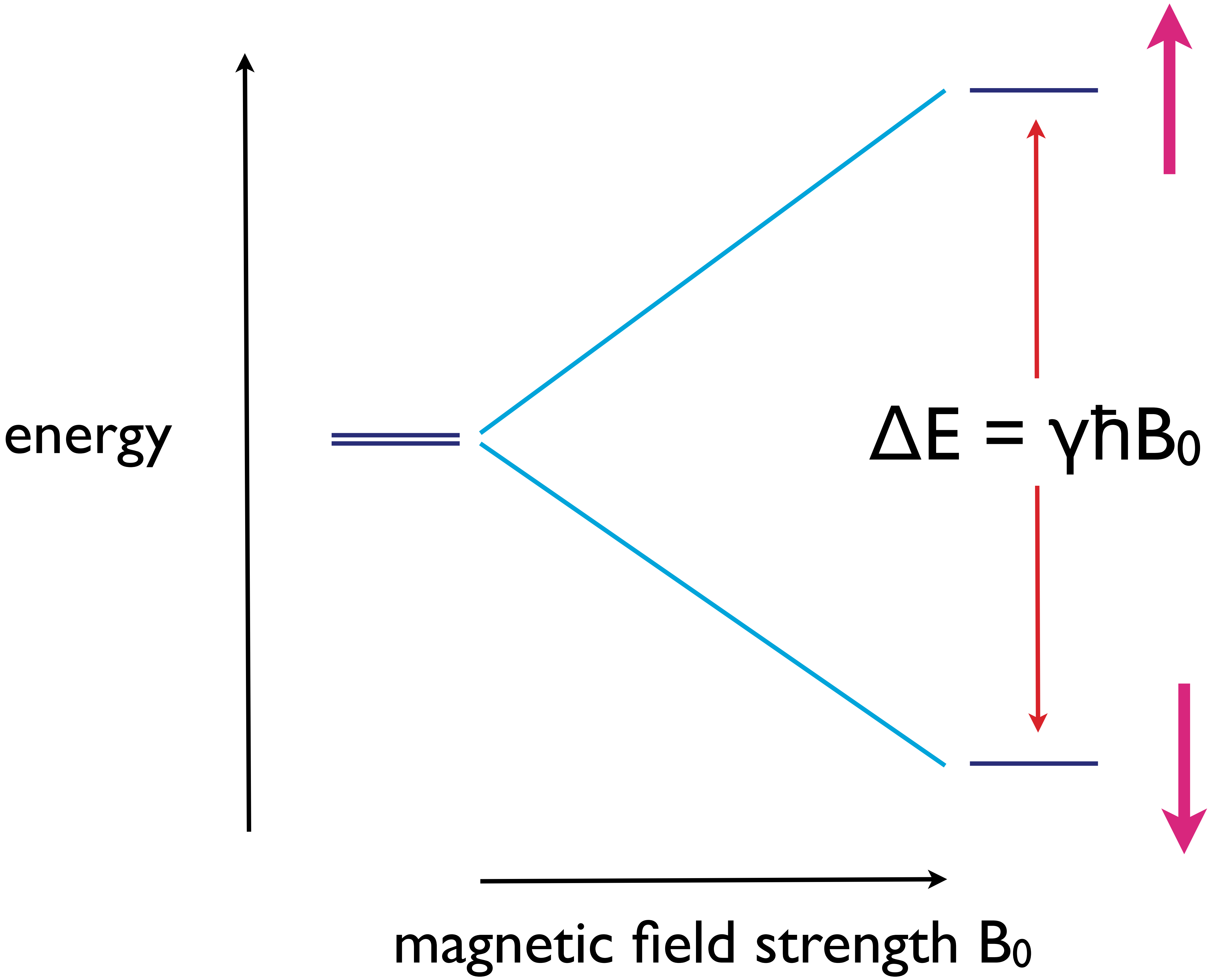


The Zeeman Effect and Radiofrequency Spectroscopy



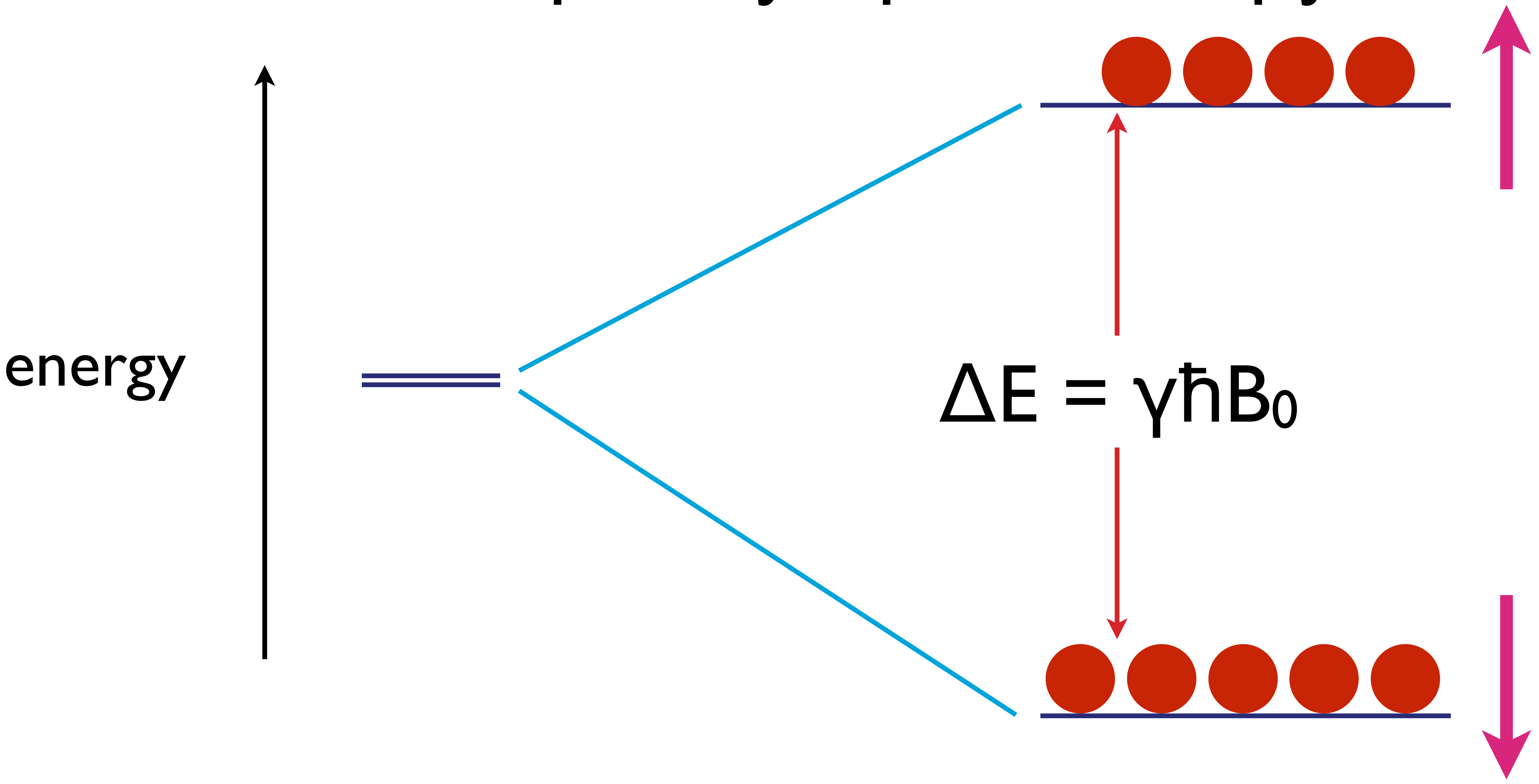
In the absence of an external magnetic field,
the nuclear spin states are degenerate.

The Zeeman Effect and Radiofrequency Spectroscopy



In the presence of an external magnetic field,
the nuclear spin states separated by an energy difference ΔE = γħB0.

The Zeeman Effect and Radiofrequency Spectroscopy

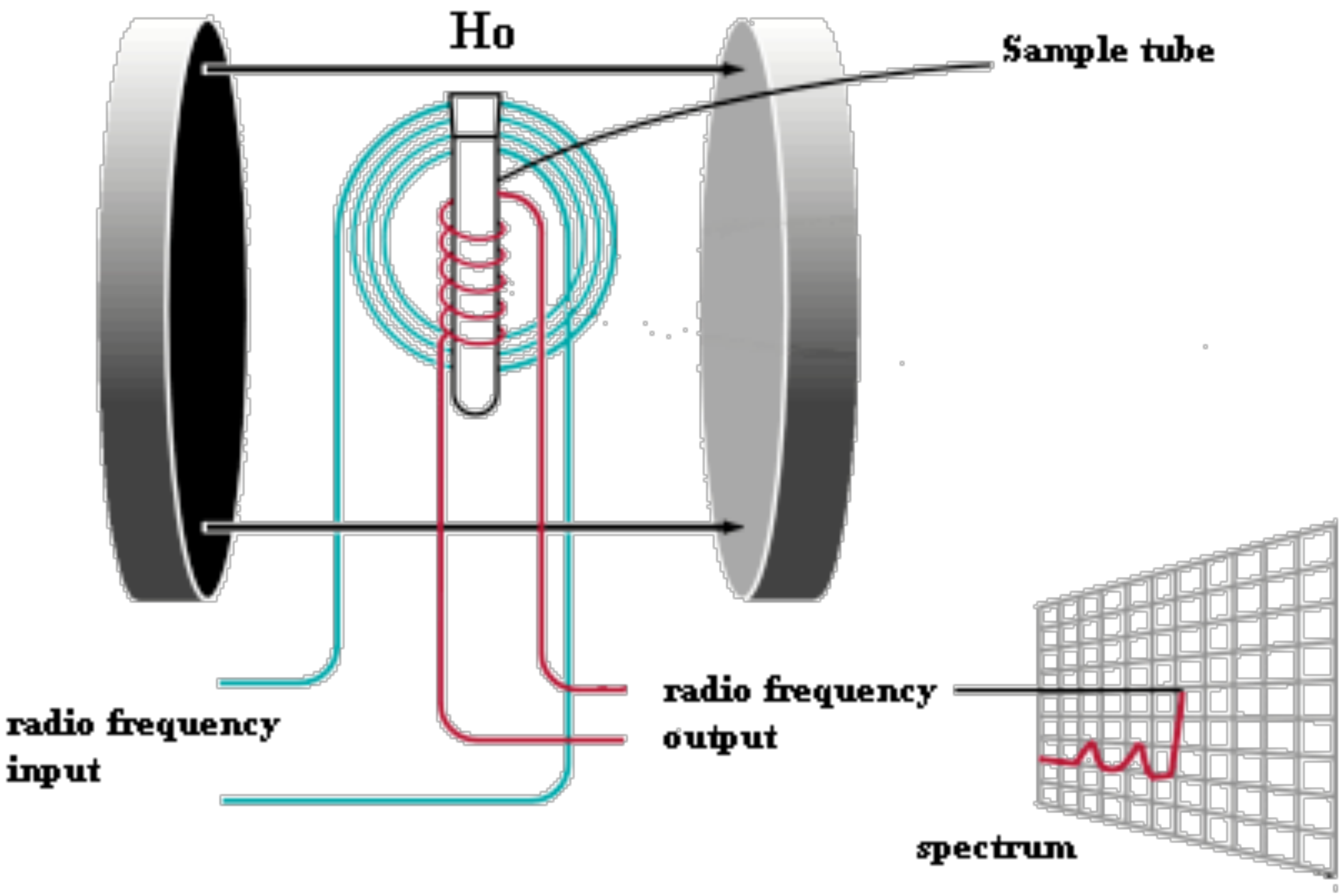


For a spin I = 1/2 two spin states are possible, with a slight preference for the low-energy orientation. **The population of each state is given by the Boltzmann distribution:**

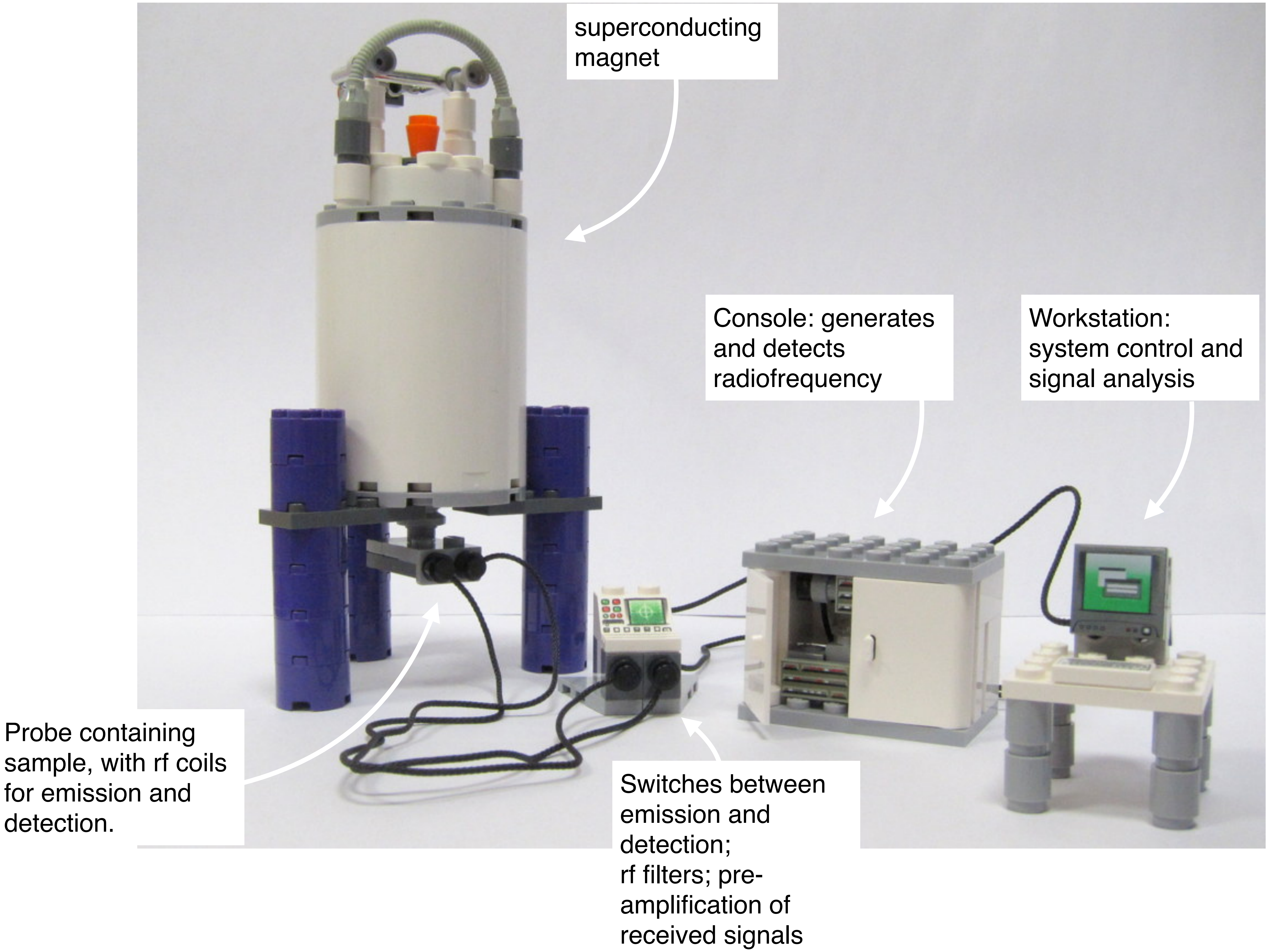
$$N_\alpha/N_\beta = \exp(-\Delta E/k_BT) \sim 1.0001$$

Since NMR frequencies are in the radio frequency range, **the energy difference is very small, and the population difference is also very small**. This is different from most other spectroscopies (e.g. IR, UV-Vis). We note that the stronger the applied magnetic field, the larger the population difference will be, and the more sensitive the NMR experiment.

The NMR Spectrometer



The NMR Spectrometer



superconducting magnet

Console: generates and detects radiofrequency

Workstation: system control and signal analysis

Probe containing sample, with rf coils for emission and detection.

Switches between emission and detection; rf filters; pre-amplification of received signals

https://ideas.lego.com/projects/cc82198a-e937-427f-982a-d8854b126577

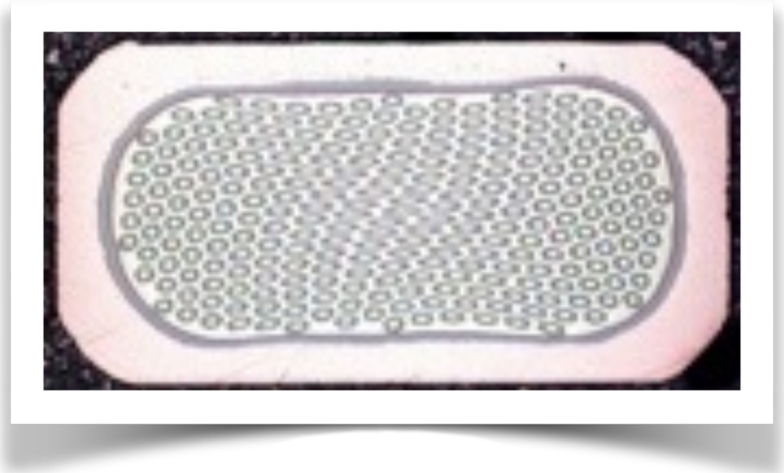
The NMR Magnet

A **magnet** for high-resolution NMR spectroscopy:

1 GHz ^{1}H NMR frequency = **23.5 Tesla.**

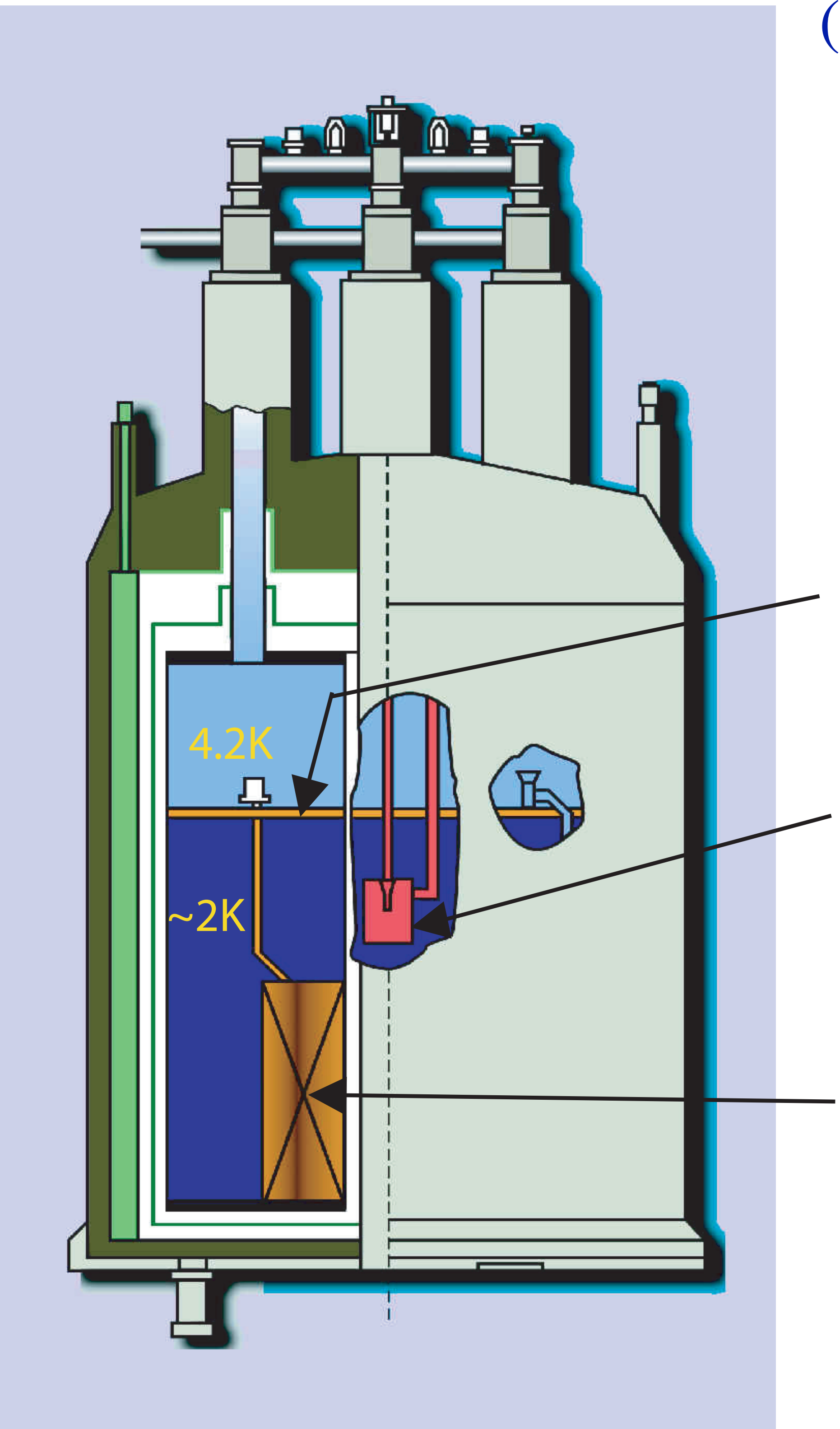
Superconducting coils (no electrical consumption) which allow: to generate a field **stable to within < 1 Hz/hour (10^{-9})**;

and to have a field which is **homogeneous to within < 0.1 Hz (10^{-10}) over a sample volume of ~3 cm^3**



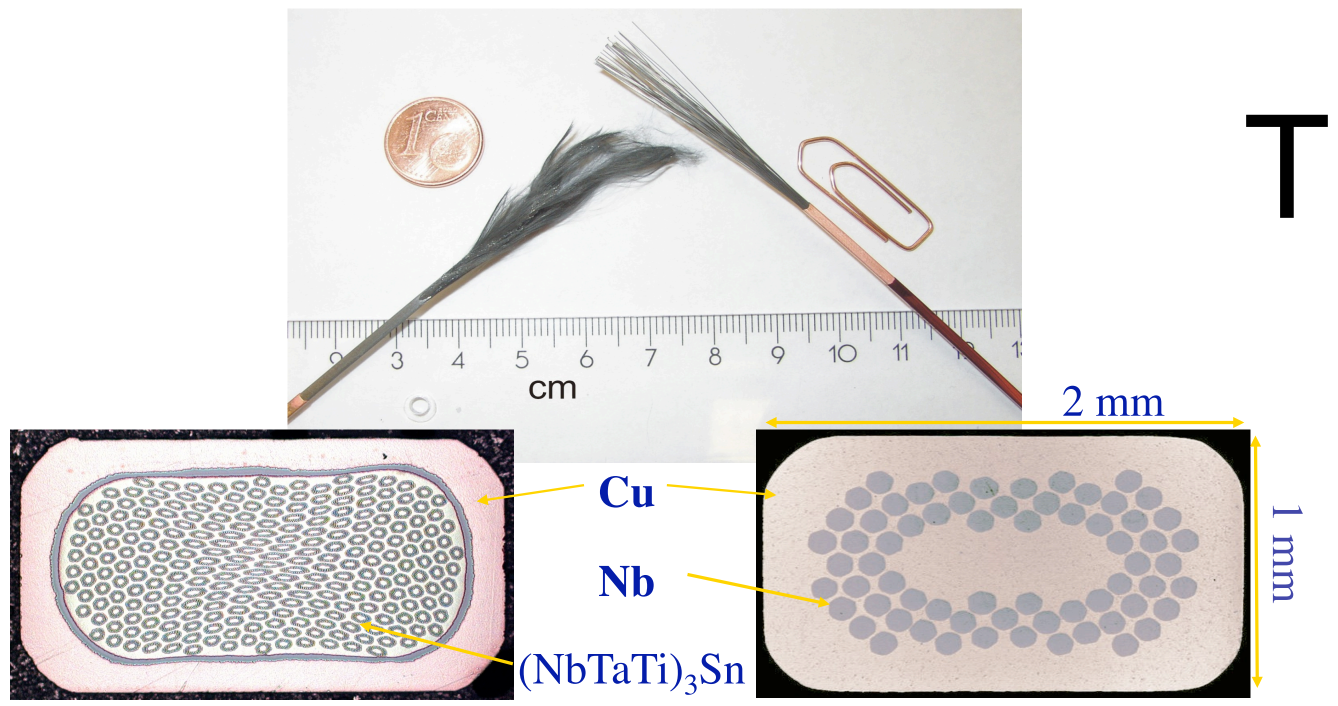
$(NbTaTi)_3Sn$-conductor

~ 50.000 filaments, 5µ



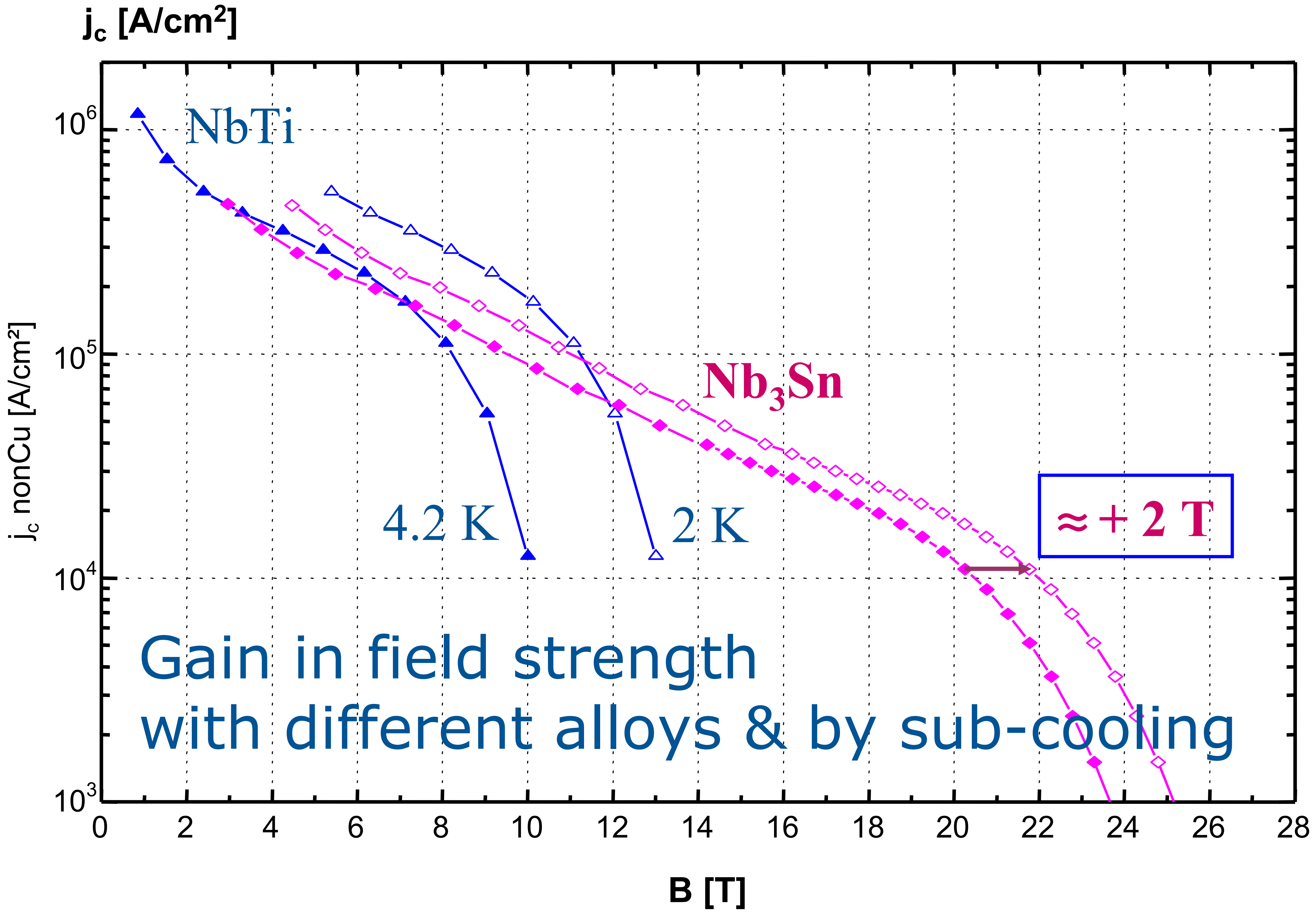
images courtesy of BRUKER BIOSPIN

The NMR Magnet

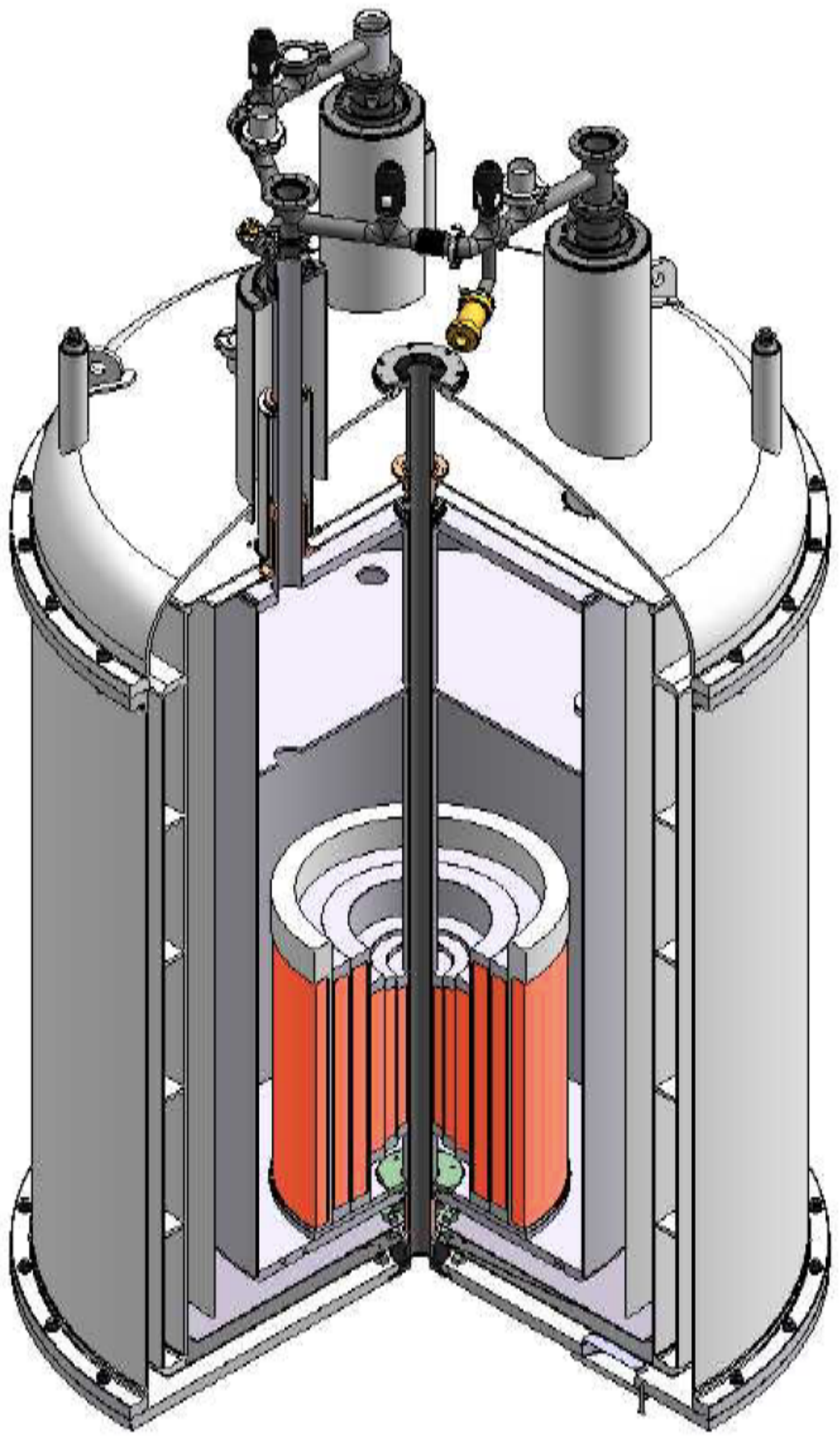


$(NbTaTi)_3Sn$-conductor
~ 50.000 filaments, 5

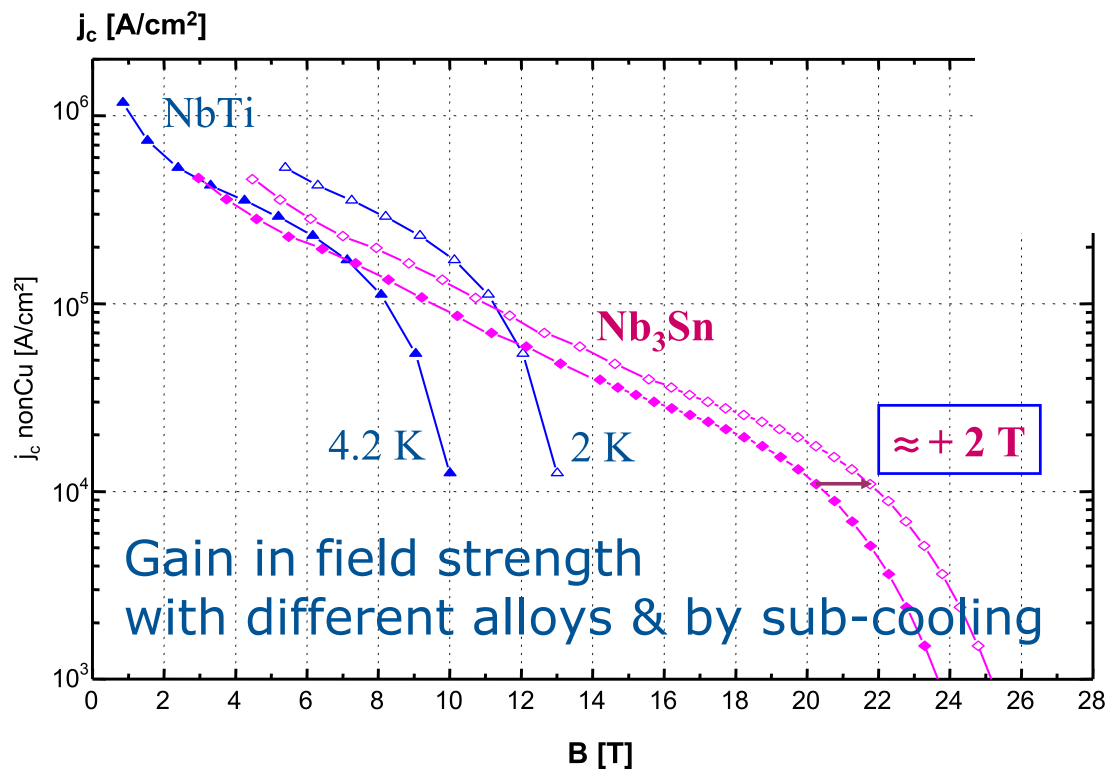
NbTi-conductor
66 filaments, 100



Gain in field strength
with different alloys & by sub-cooling



images courtesy of BRUKER BIOSPIN



Gain in field strength
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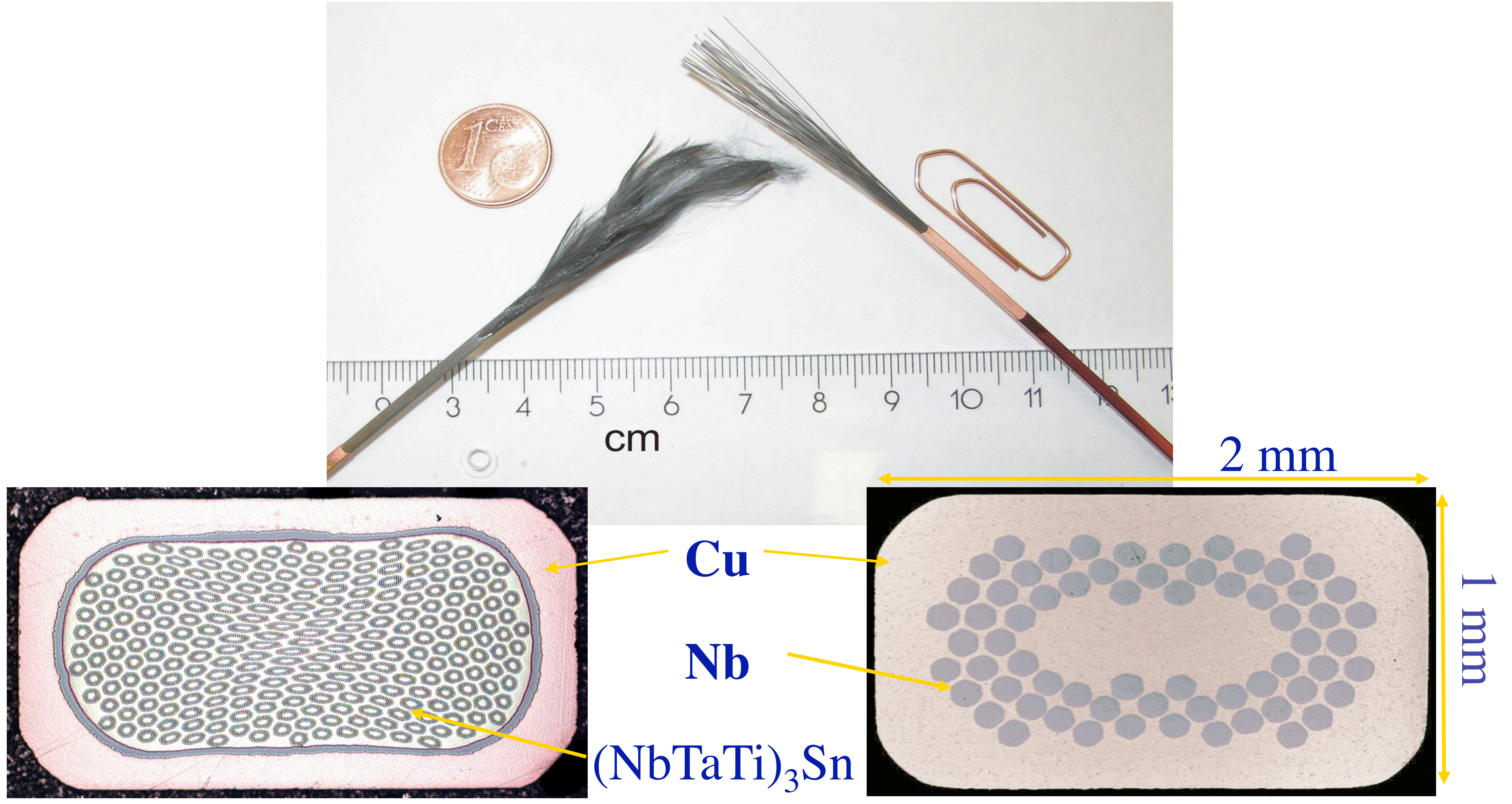
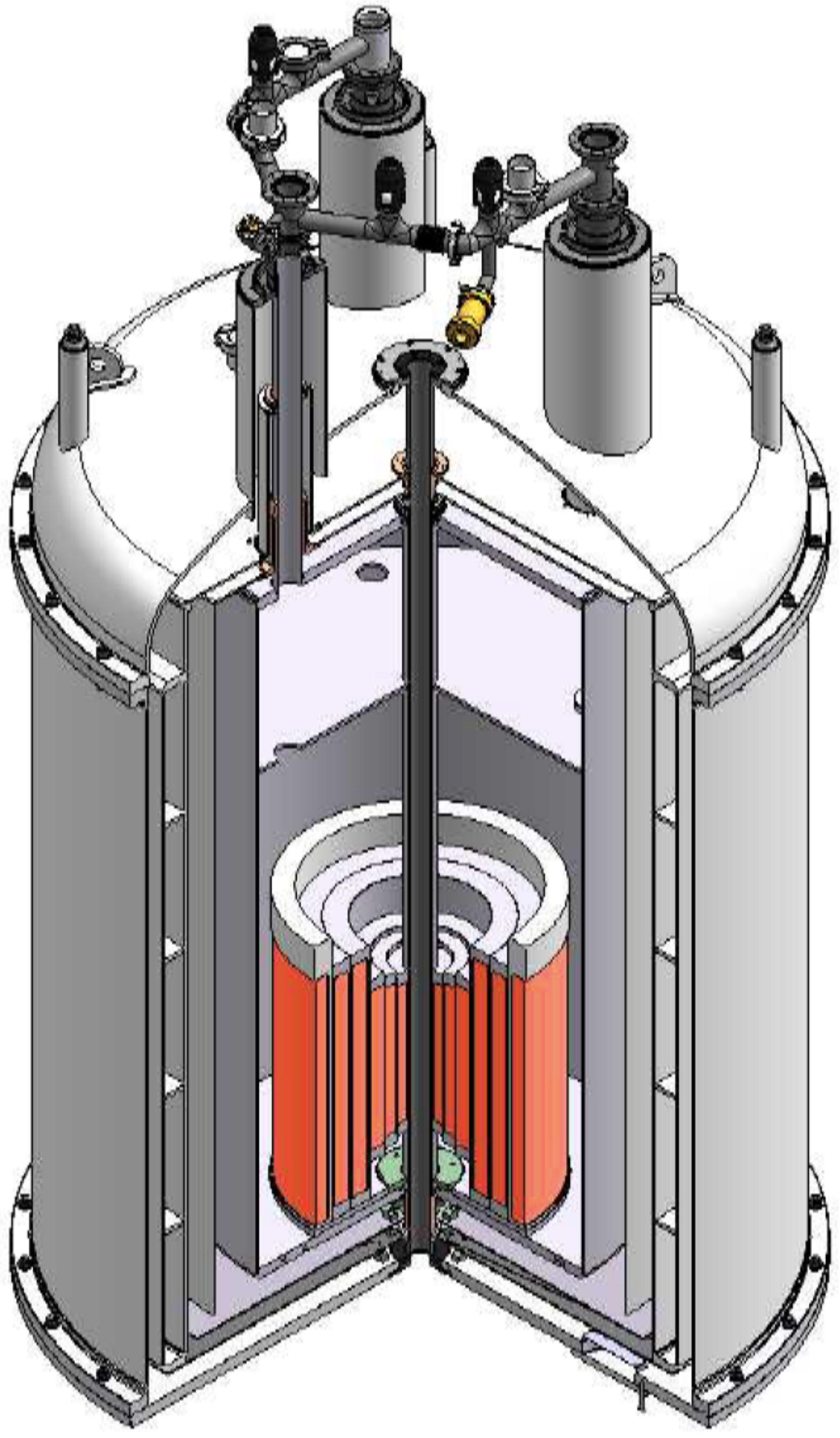
The NMR Magnet



$(NbTaTi)_3Sn$-conductor
~ 50.000 filaments, 5

NbTi-conductor
66 filaments, 100



images courtesy of BRUKER BIOSPIN

The NMR Spectrometer

Liquid N_2

AVANCE
Neo NMR Console

Sample
Temperature
control

WB 21T
Magnet

10.9 T
593 GHz
gyrotron

waveguide

Brennan

The NMR Probe

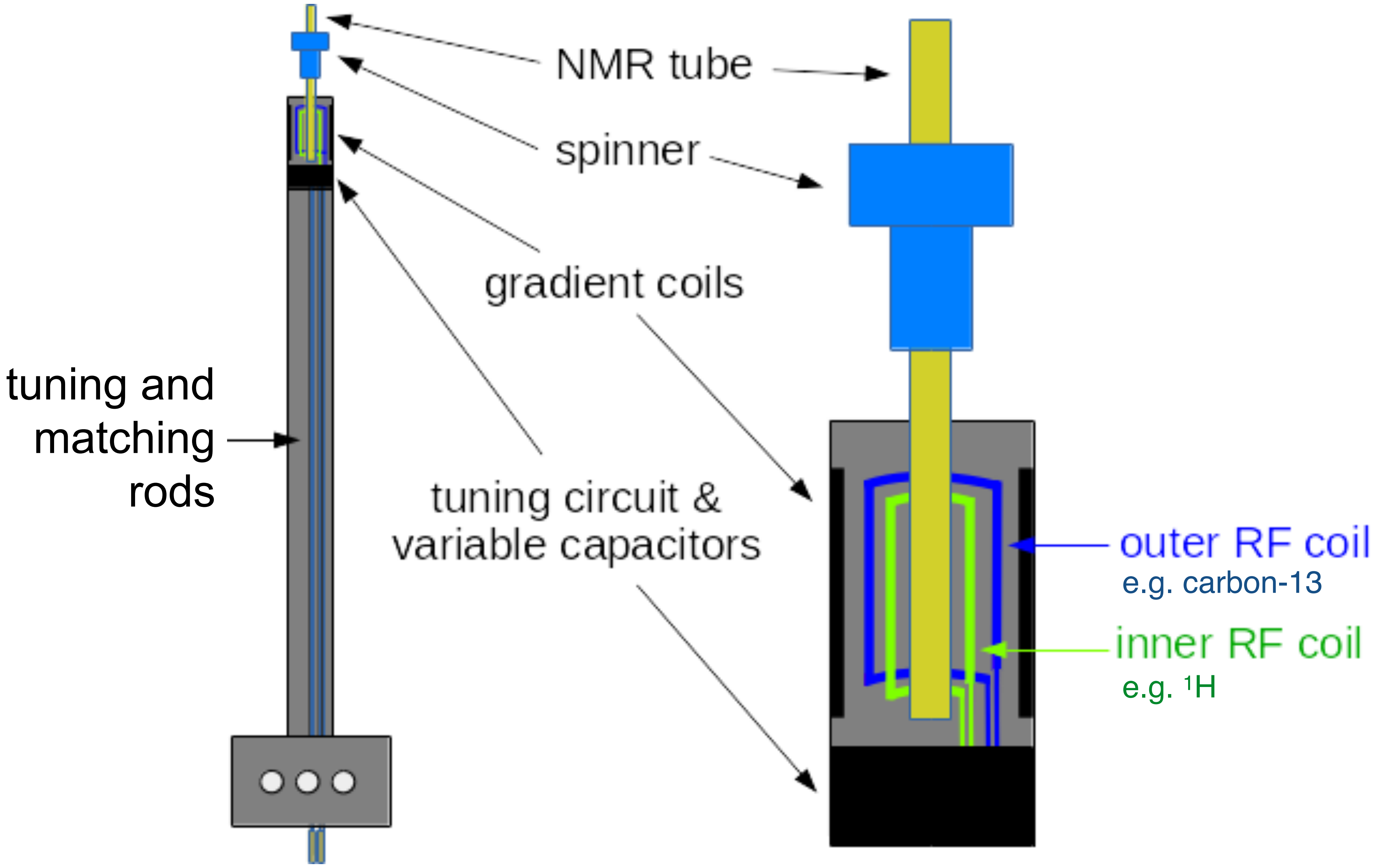
connections for heaters, thermocouples, other sensors, etc,…

RF cables from preamp, one for each nucleus (e.g. ^{1}H, ^{13}C, …)

https://www.uwyo.edu/chemistry/instrumentation/nmr/nmr-probes.html

https://www.ebay.com/itm/BRUKER-500MHz-5mm-NMR-Probe-with-Gradients-Nuclear-Magnetic-Resonance-/232806178548

The NMR Probe



NMR tube

spinner

gradient coils

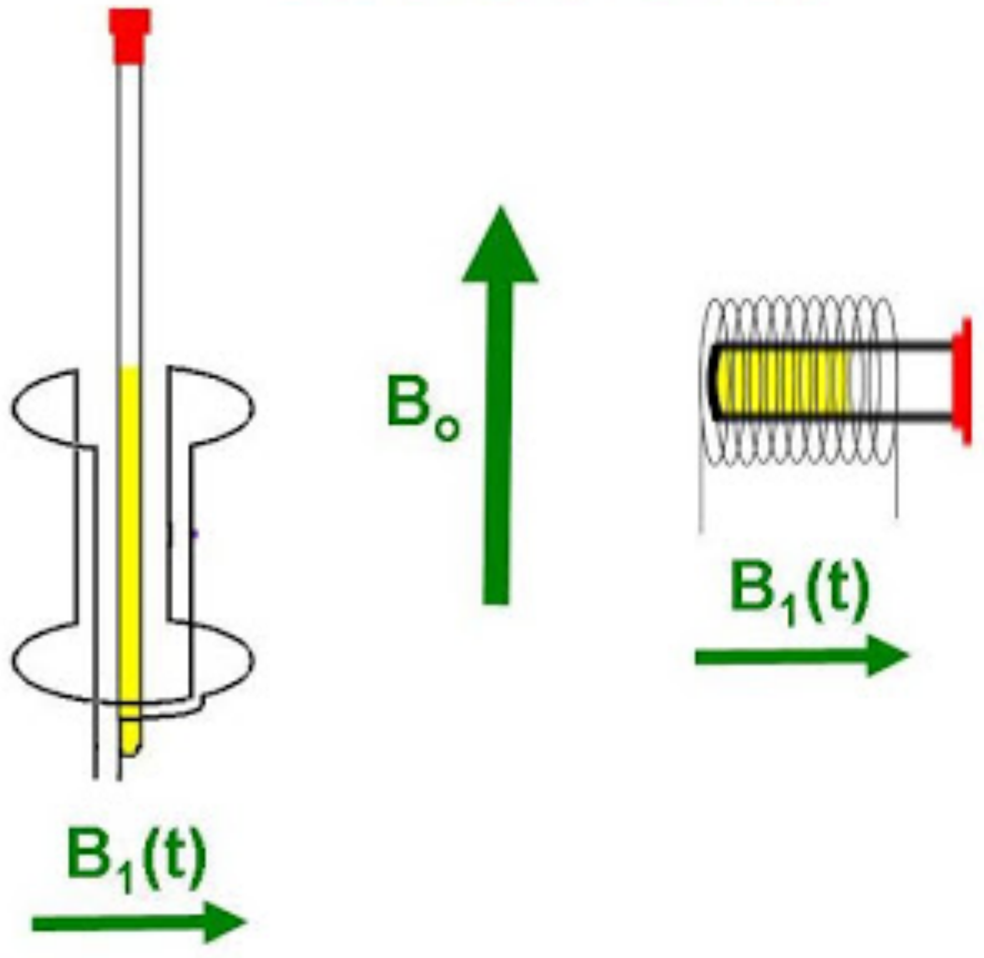
tuning and matching rods

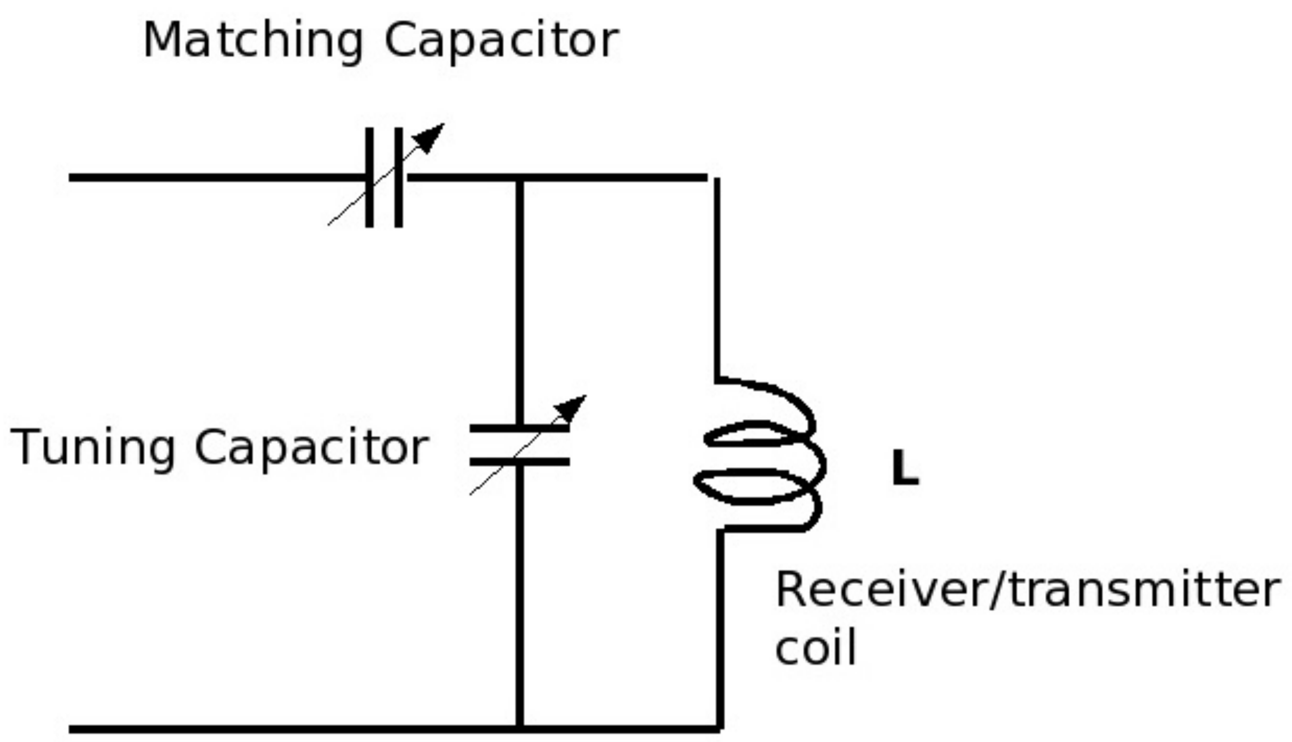
tuning circuit & variable capacitors

outer RF coil
e.g. carbon-13

inner RF coil
e.g. ^{1}H

RF fields in Helmholtz & solenoid coils





http://sopnmr.blogspot.com/2018/11/probes.html
http://u-of-o-nmr-facility.blogspot.com/2008/03/probe-coil-geometry.html
http://www.chem.wilkes.edu/~trujillo/NMR_Course/usask_ca_PROBE_PICTURES/

The NMR Probe

In order for maximum power transfer between two circuit elements, they must be of equal impedance. This is the primary function of the **matching capacitor**. It's task is to match the impedance of the coil/sample system to the external electronics (typically 50 Ohms).

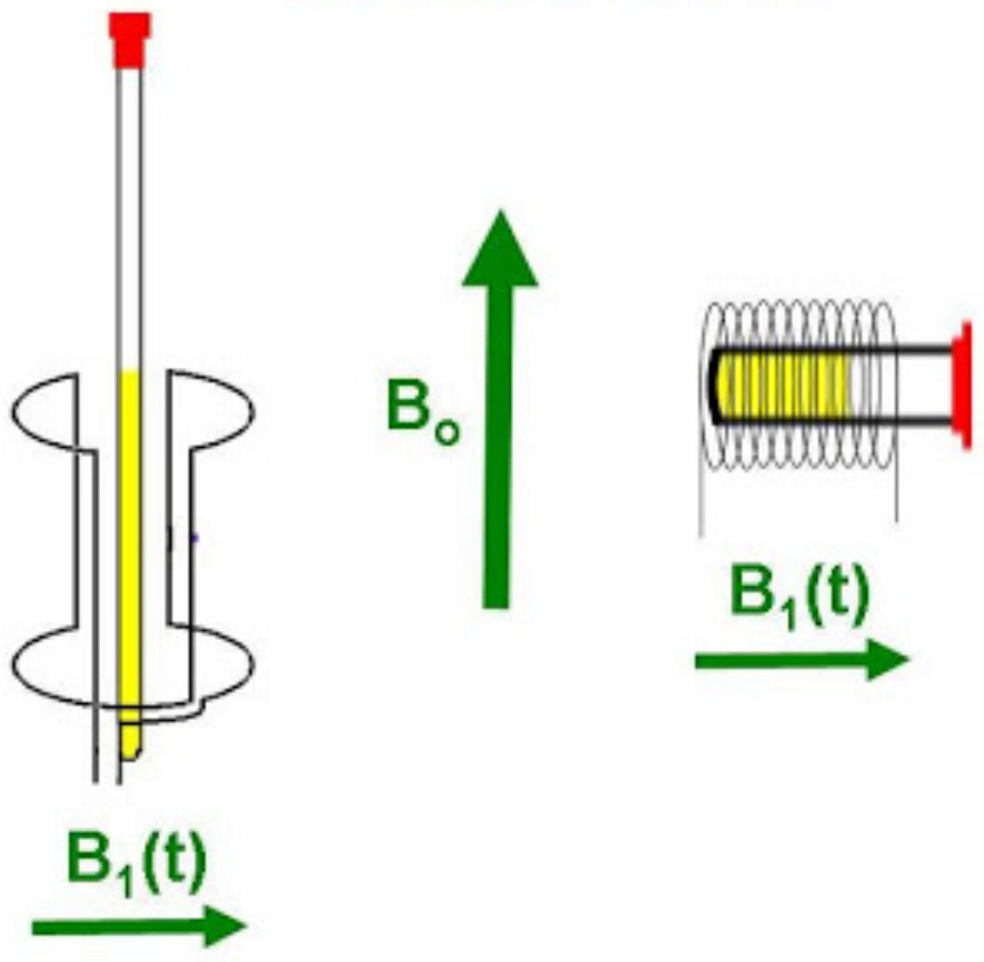
For the parallel circuit of the tuning capacitor and coil (inductance), there is resonant frequency (f) at which electrical energy is most absorbed. This is called a tank circuit; it's resonant frequency is determined by the value of the inductance (L) and the value of the capacitor (C).

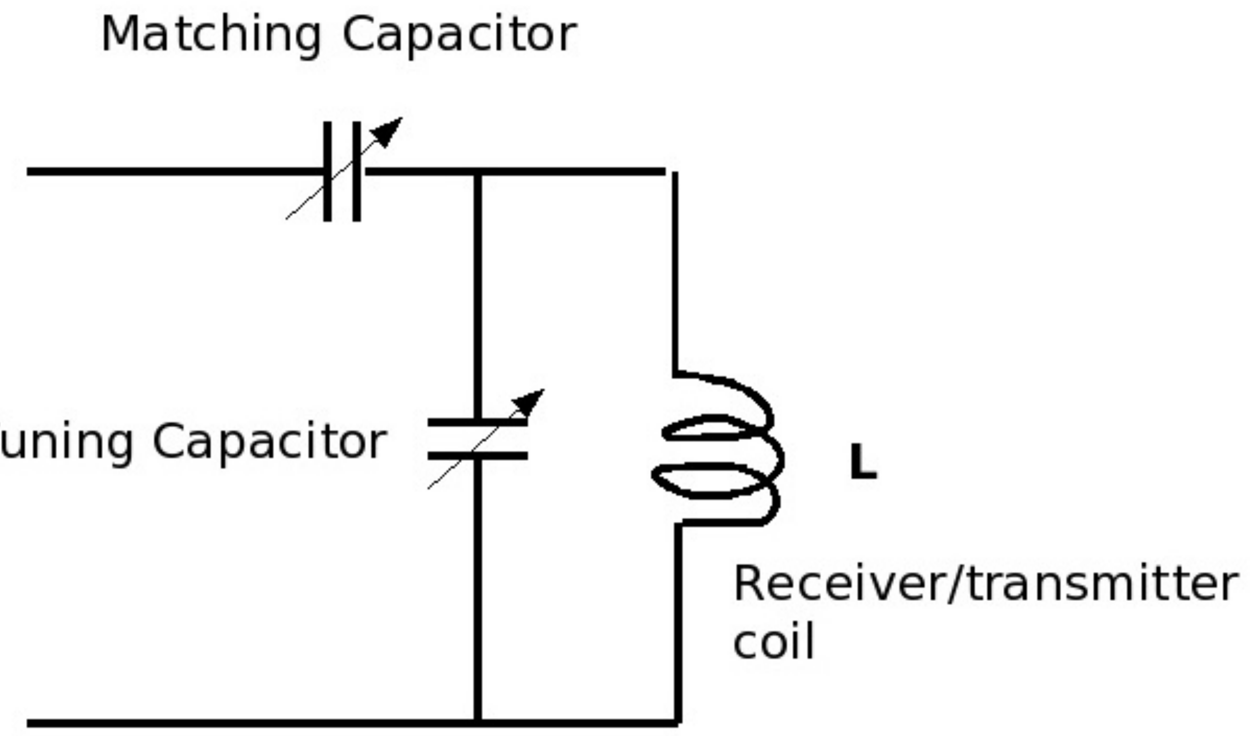
$f = 1/ (2\pi \sqrt{(LC)})$

Usually, it is the capacitor whose value is variable. Thus, **we tune the circuit** to the frequency of interest by adjusting the value of the capacitance so that we can get the strongest signal possible.

Usually the same coil is used for both emission and detection.

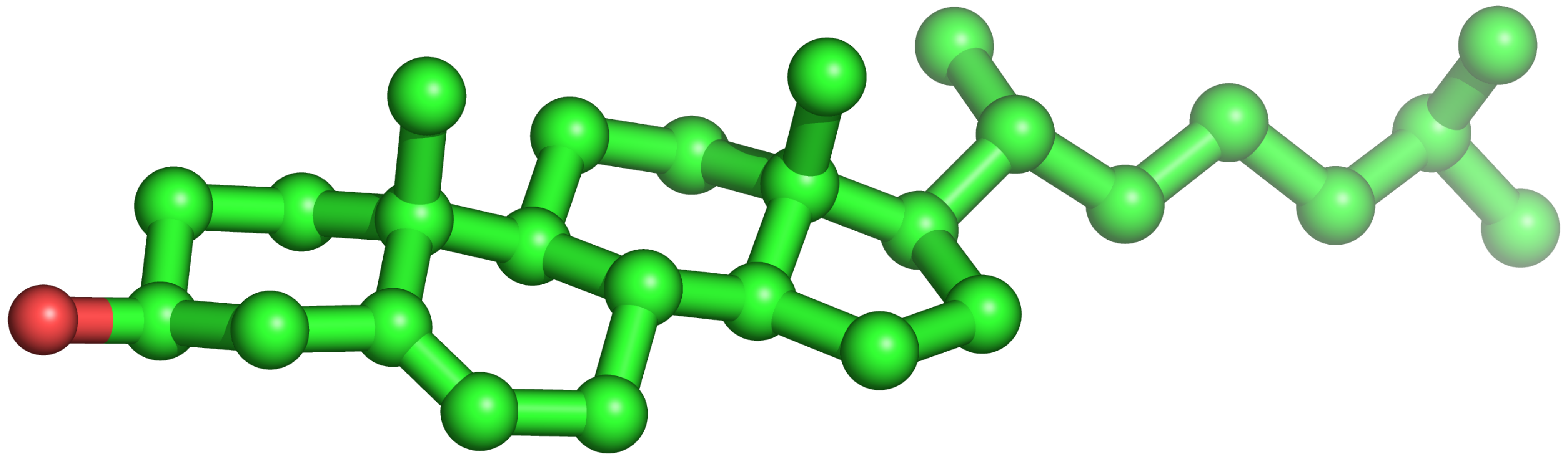
RF fields in Helmholtz & solenoid coils

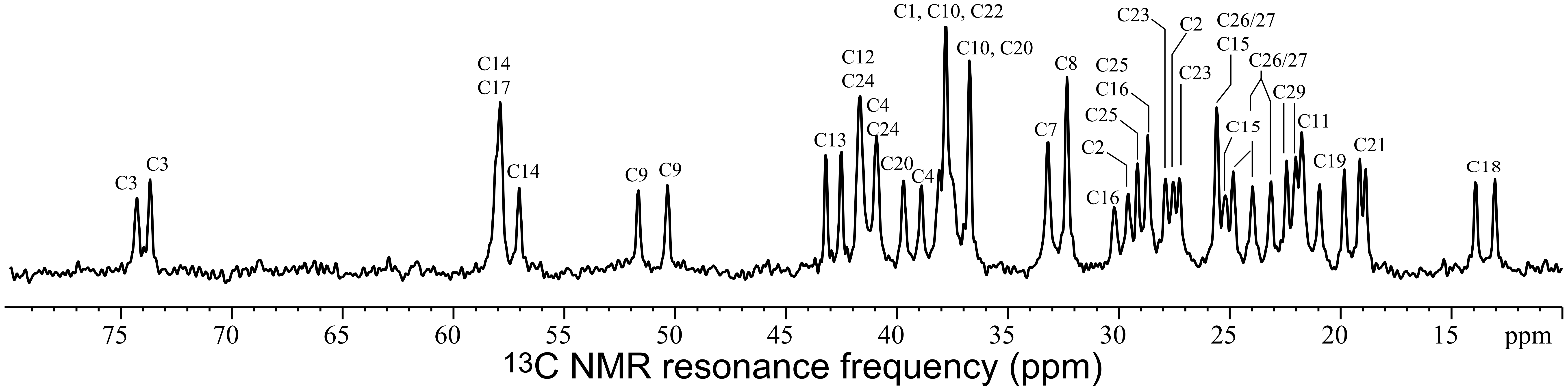




http://sopnmr.blogspot.com/2018/11/probes.html
http://u-of-o-nmr-facility.blogspot.com/2008/03/probe-coil-geometry.html
http://www.chem.wilkes.edu/~trujillo/NMR_Course/usask_ca_PROBE_PICTURES/

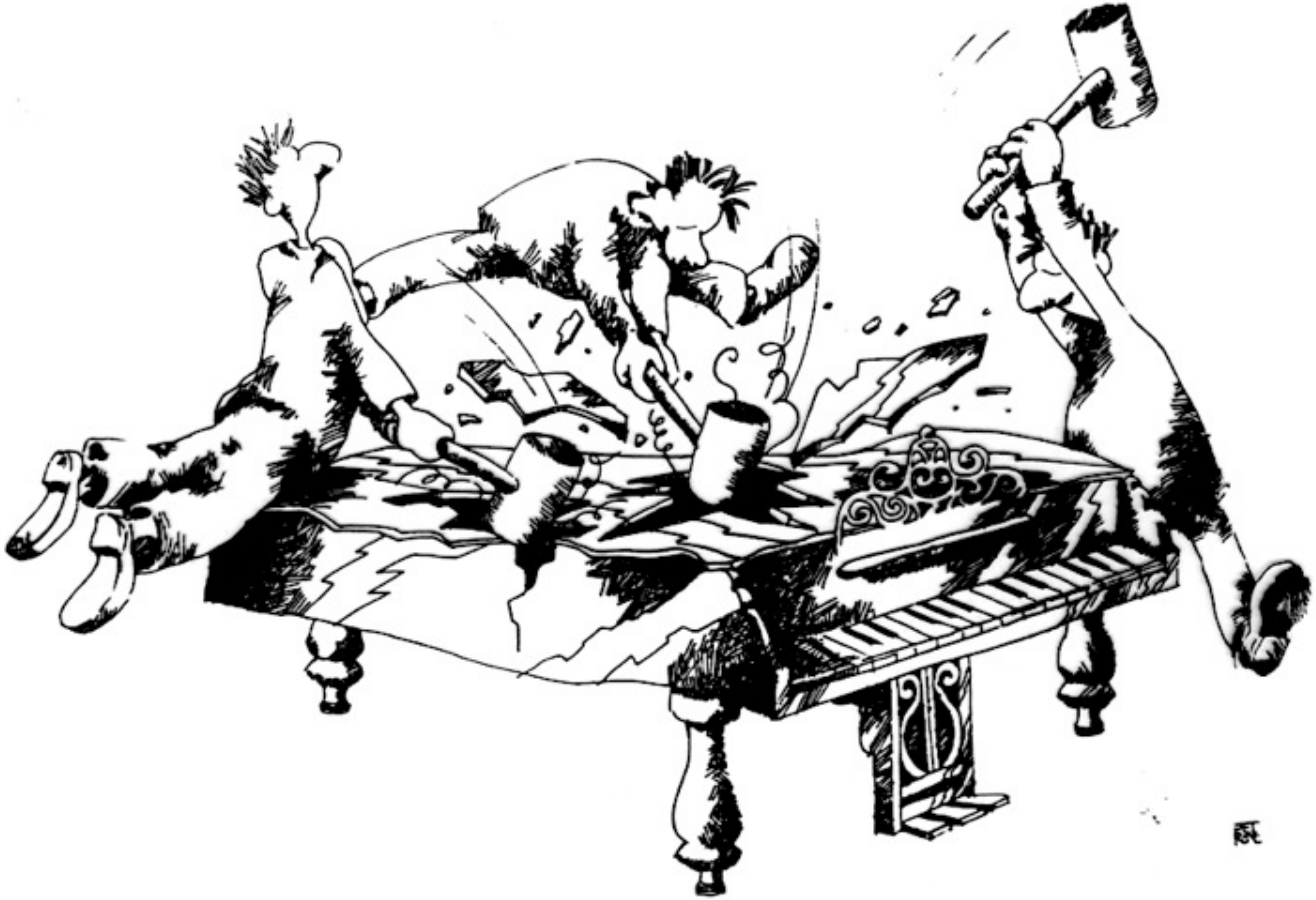
How do we do spectroscopy?





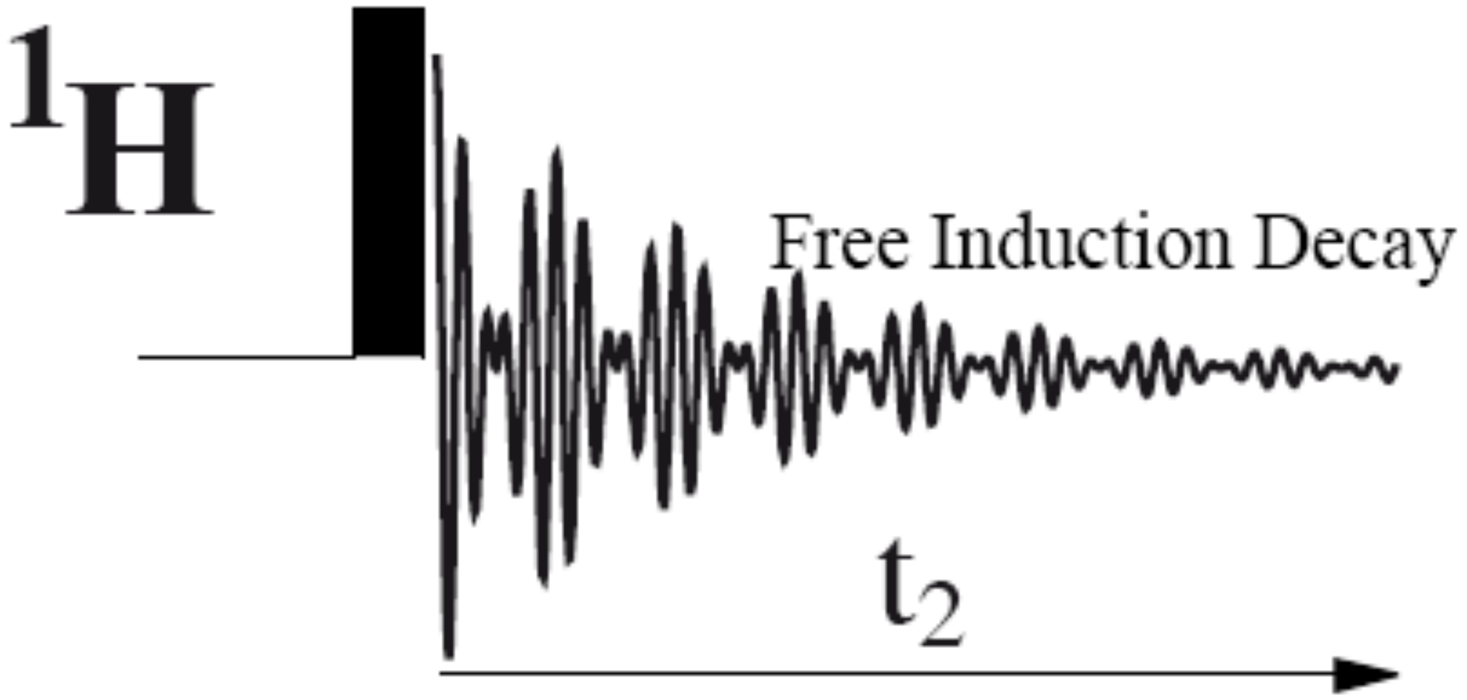
How do we do spectroscopy?

How do we do spectroscopy?



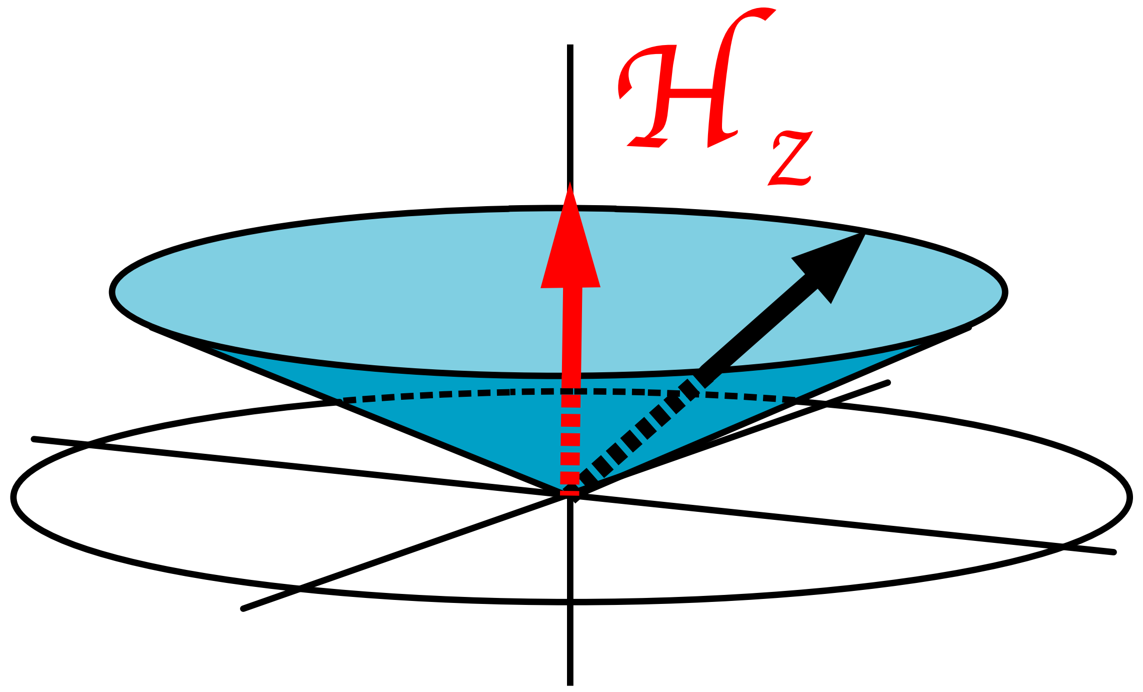
from R. Freeman

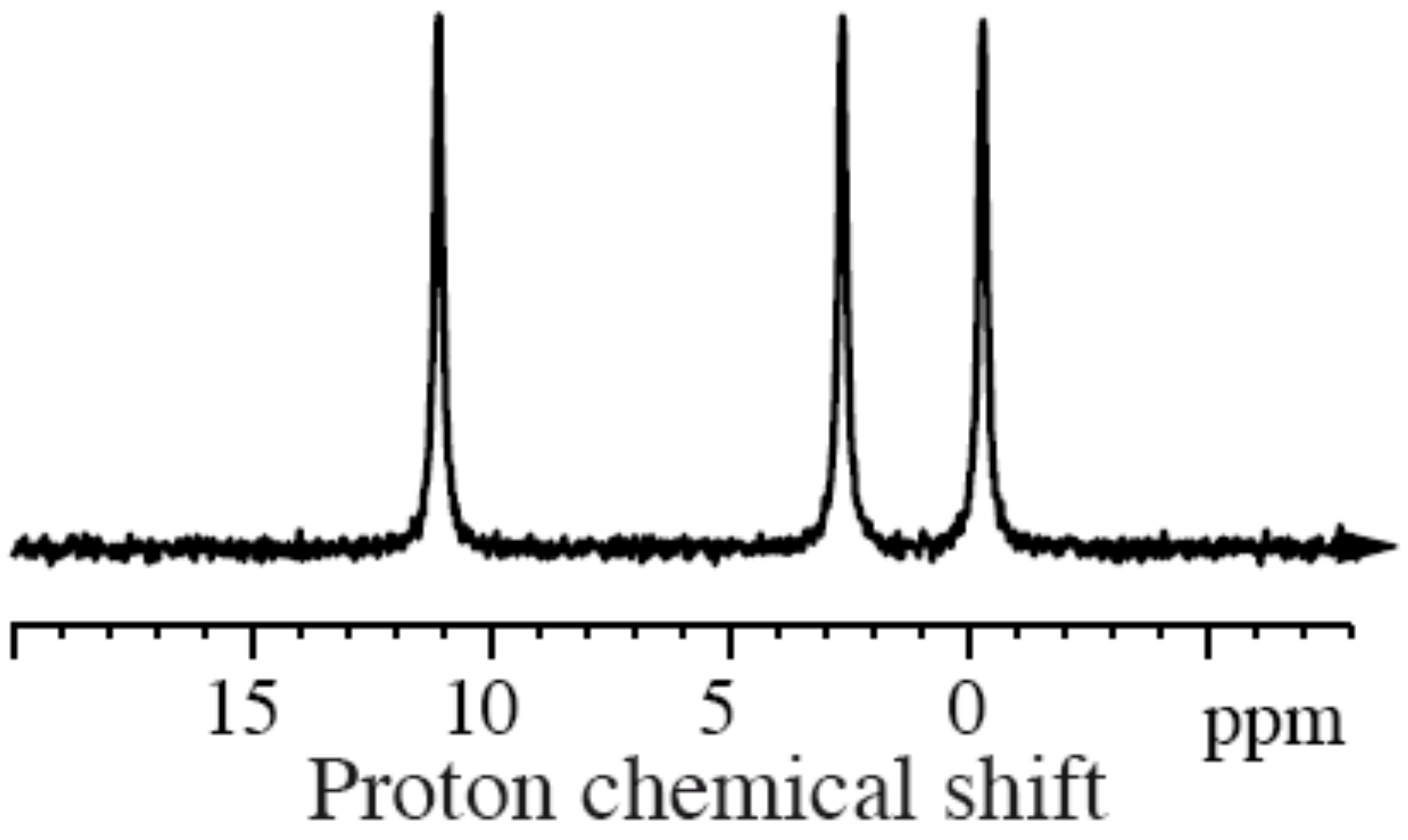
Pulsed Fourier Transform NMR Spectroscopy



Fourier Transformation

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





The NMR Hamiltonian: Spin Cacophony!

$$\mathcal{H}_{nmr} = \mathcal{H}_{z} + \mathcal{H}_{Q} + \mathcal{H}_{D} + \mathcal{H}_{cs} + \mathcal{H}_{J}$$

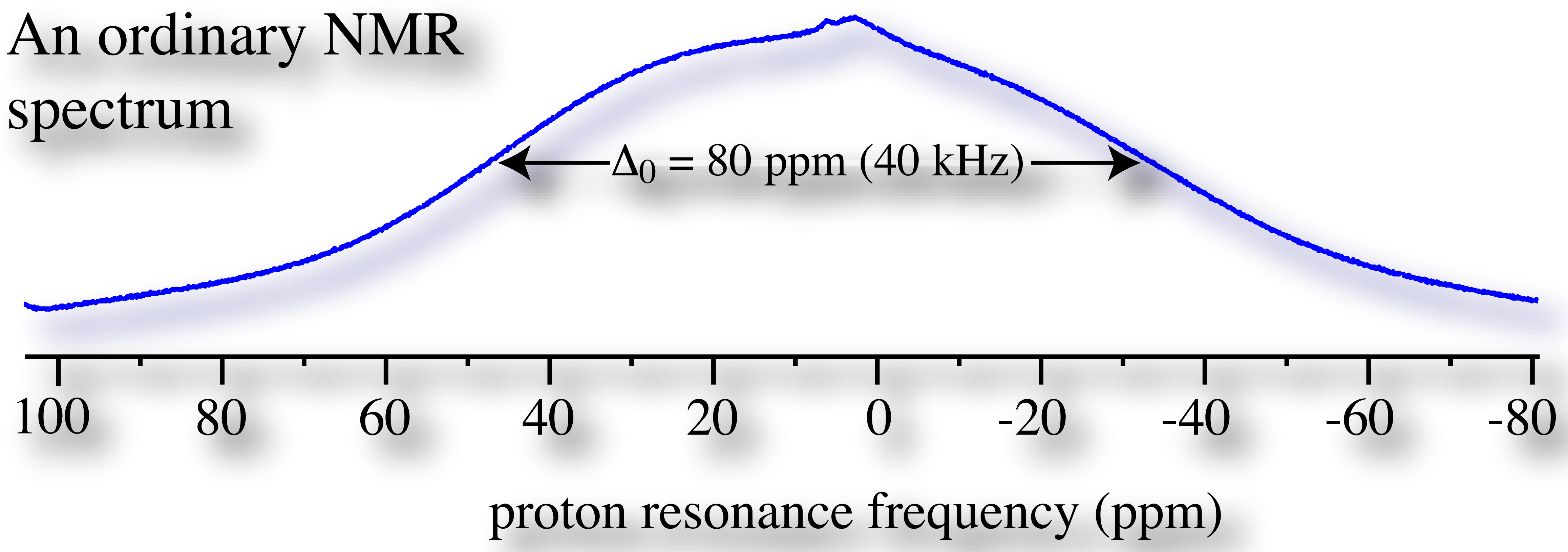
Zeeman Interaction
(~100 MHz)

Quadrupolar Interaction
(0-100 MHz)

Dipolar Interaction
(0-20 kHz)

Chemical Shift
(0-2000 ppm)

Scalar Coupling
(0-200 Hz)


An ordinary NMR spectrum

Dances With Spins

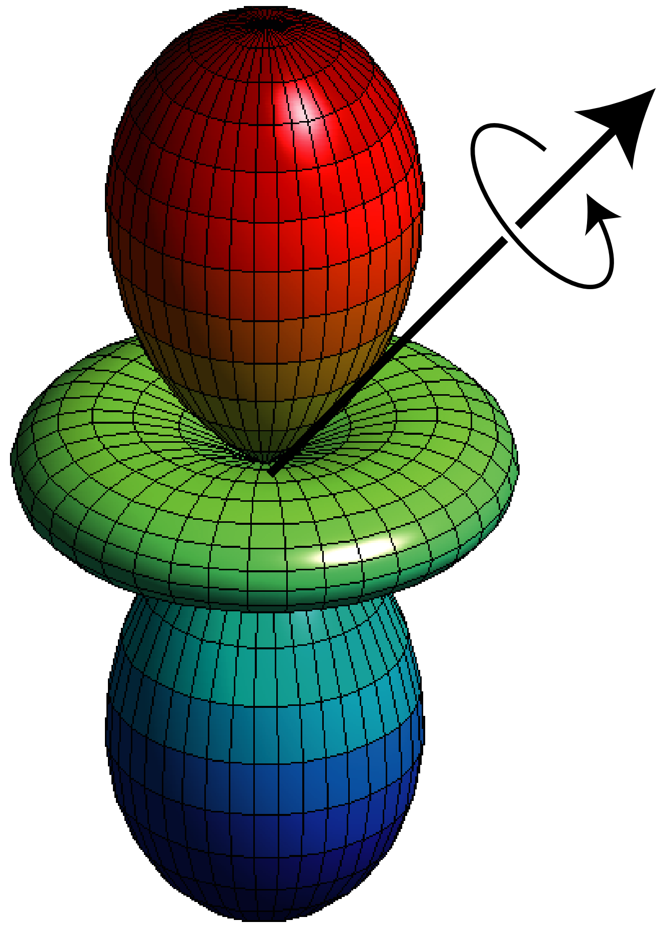
designing effective Hamiltonians

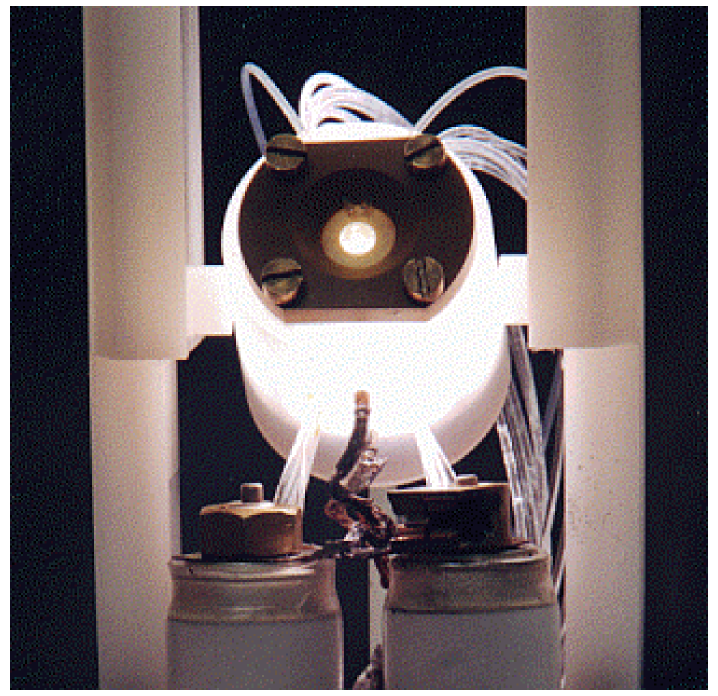
$$\mathcal{H}_{nmr} = \mathcal{H}_z + \mathcal{H}_Q + \mathcal{H}_D + \mathcal{H}_{cs} + \mathcal{H}_J + \mathcal{H}_{ext}$$

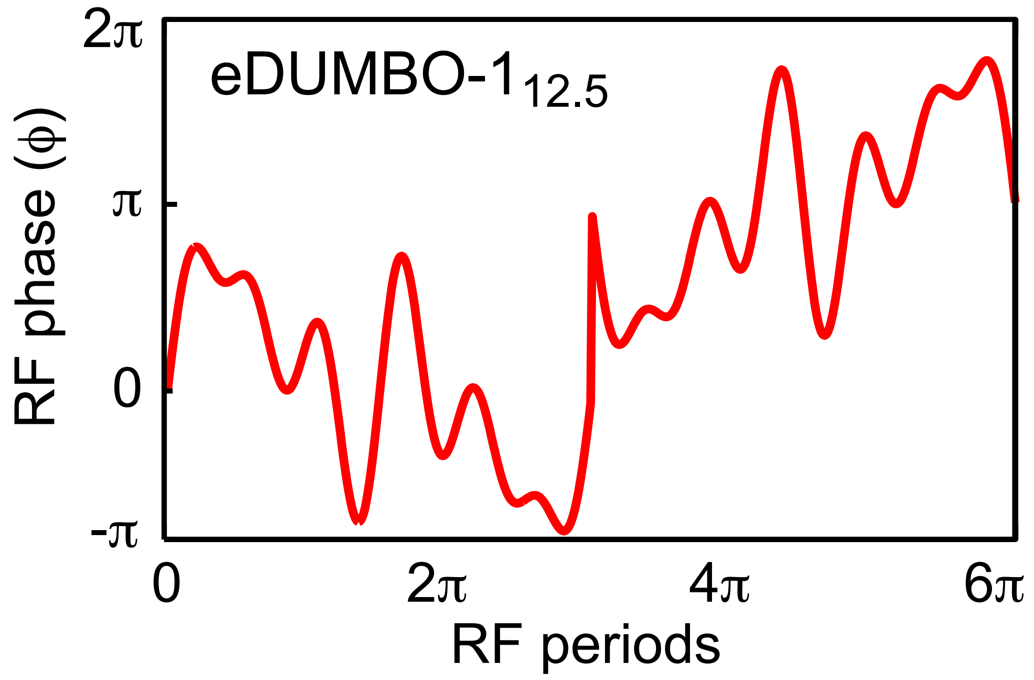
We can ***add rotations*** controlled by the experimentalist. If properly designed these rotations can ***selectively cancel out*** parts of the Hamiltonian.

Rotations in Laboratory Space
(*magic angle spinning*)

Rotations in Spin Space
(*radiofrequency pulses*)





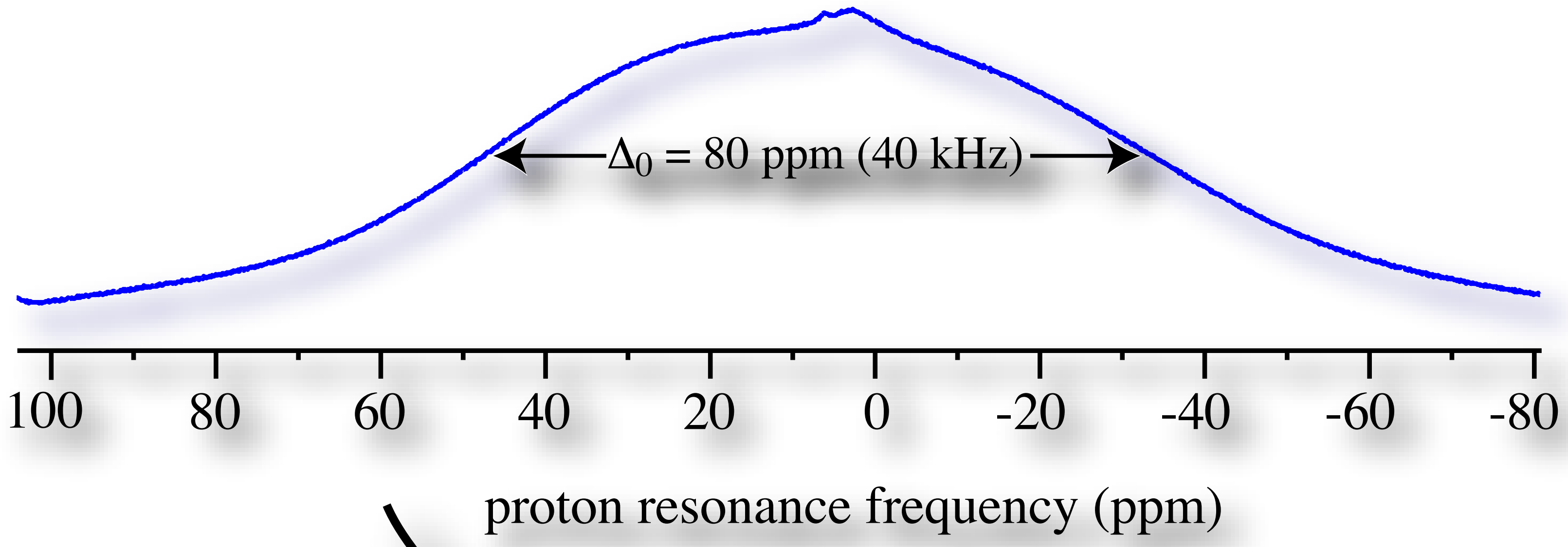


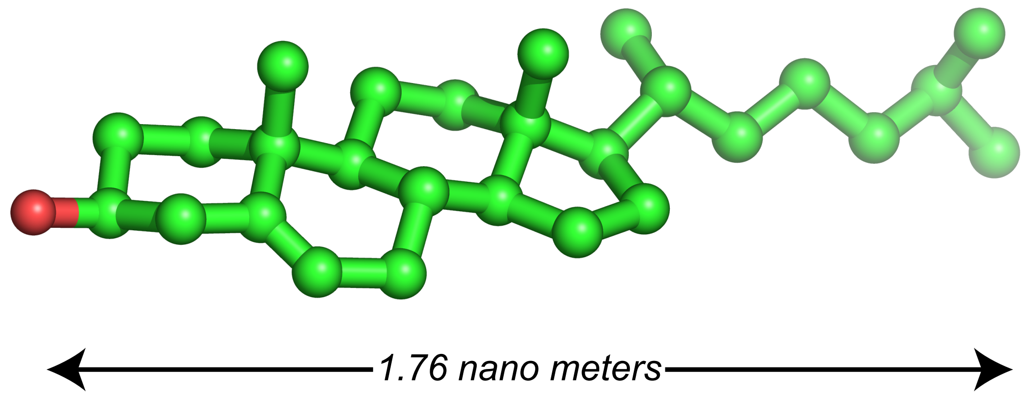
Spatial rotation: 35 000 rev/second
Spin rotation: 150 000 rev/second

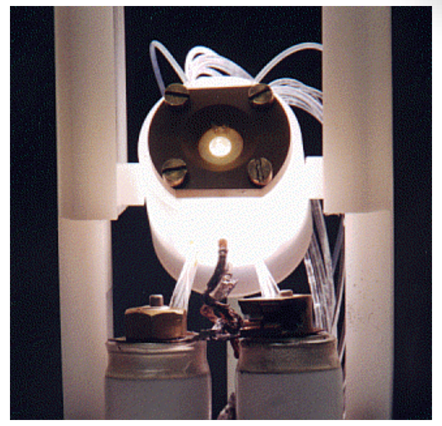
Coherent Averaging: Listening to the Whispered Message

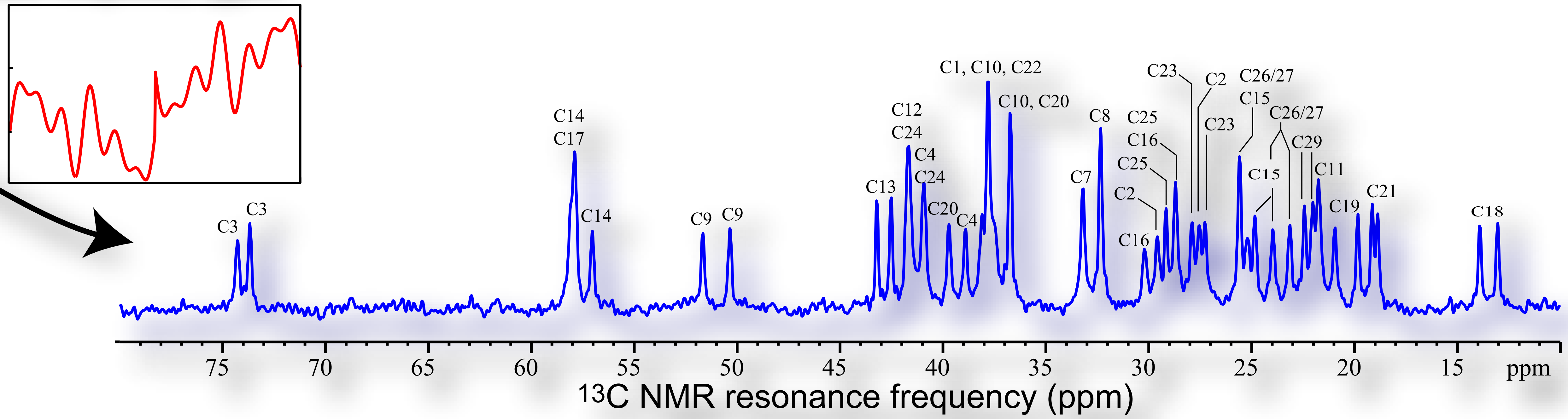
$$\overline{\mathcal{H}}_{nmr} = \mathcal{H}_z + \cancel{\mathcal{H}_Q} + \cancel{\mathcal{H}_D} + \mathcal{H}_{cs} + \cancel{\mathcal{H}_J} + \cancel{\mathcal{H}_{ext}}$$

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





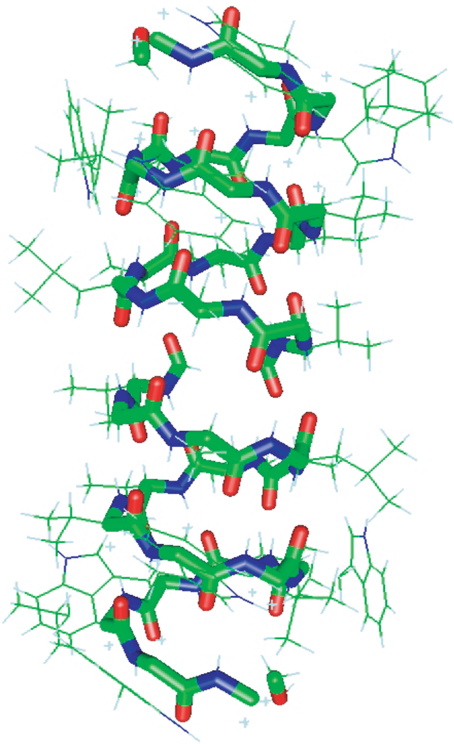




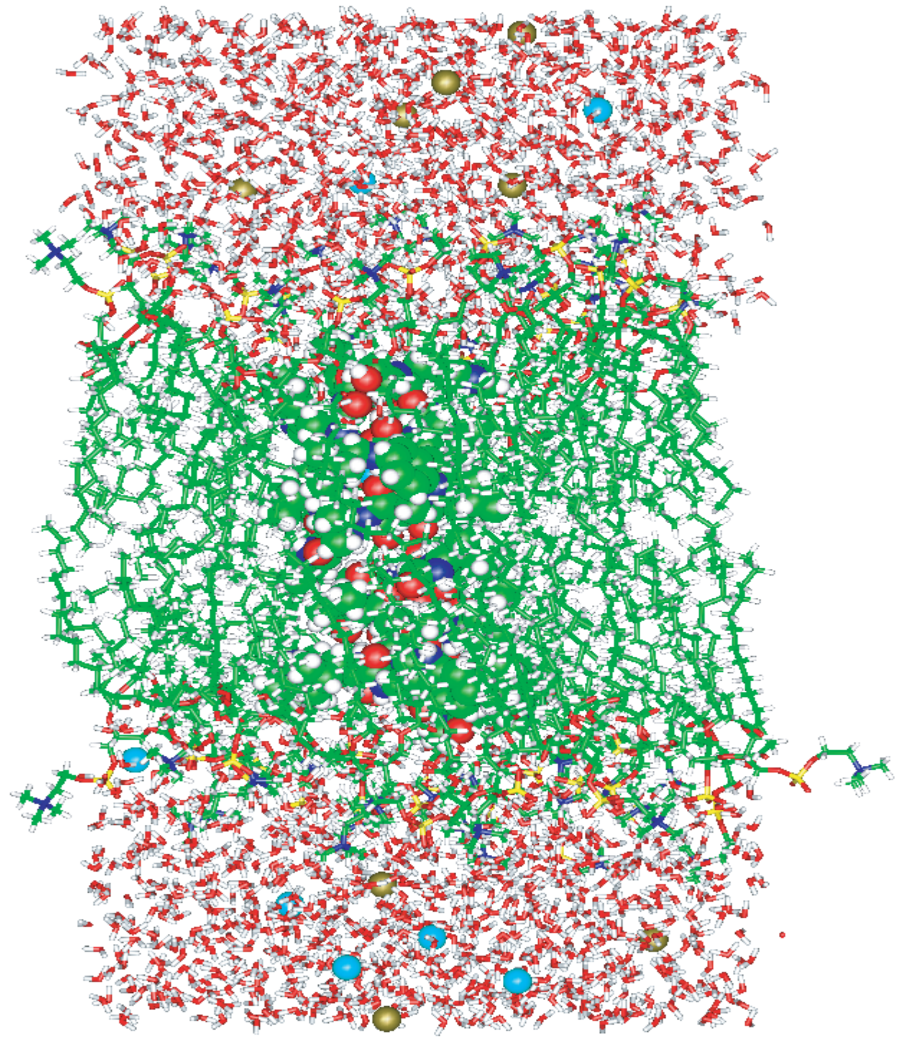
3000 million rotations during one acquisition !!

Multi-dimensional NMR: Pairwise Correlations

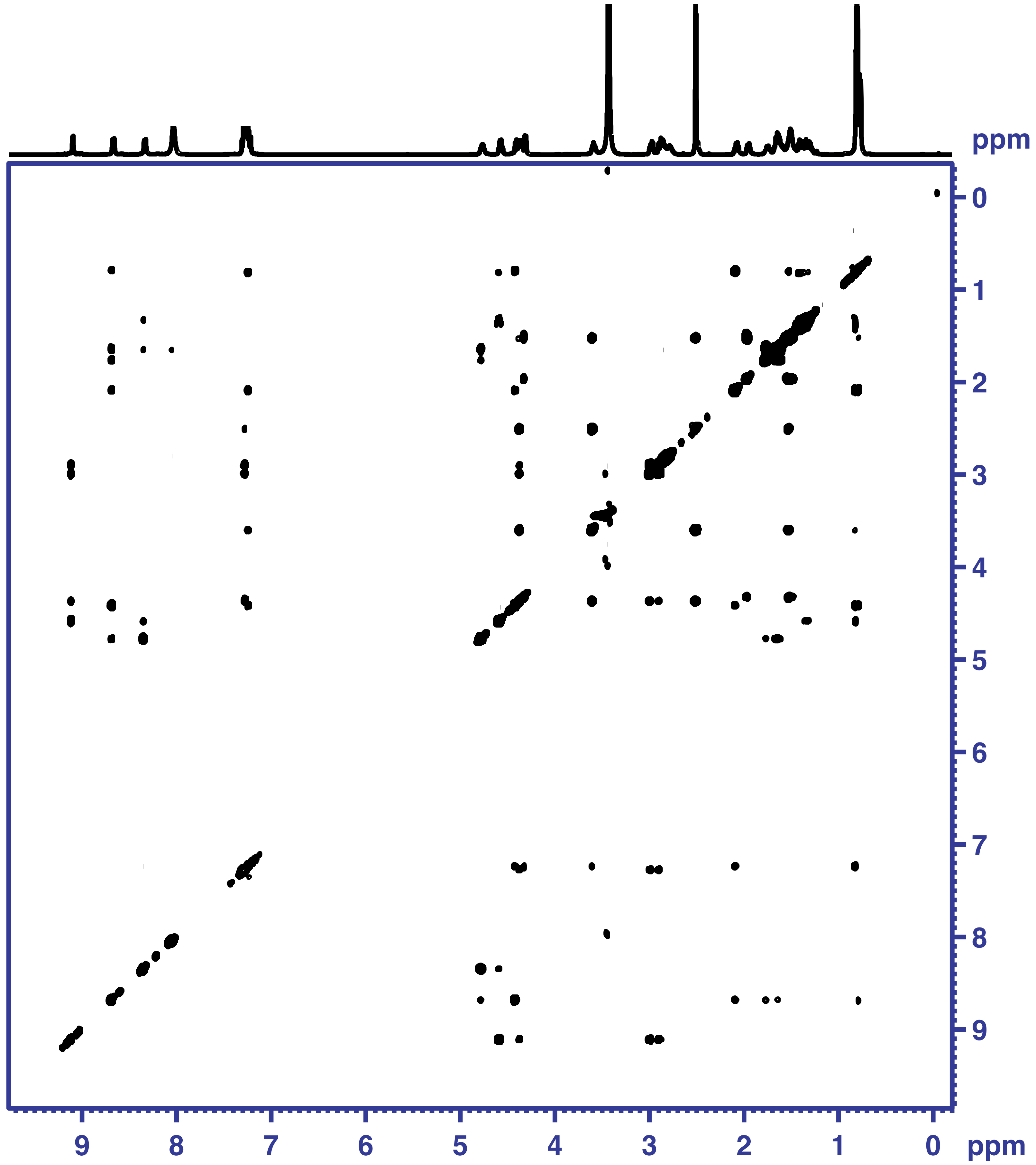
NOESY Spectrum of Gramicidin
The intensity of the cross peaks is related to the inernuclear distance between the two nuclei that are correlated.



Gramicidin A



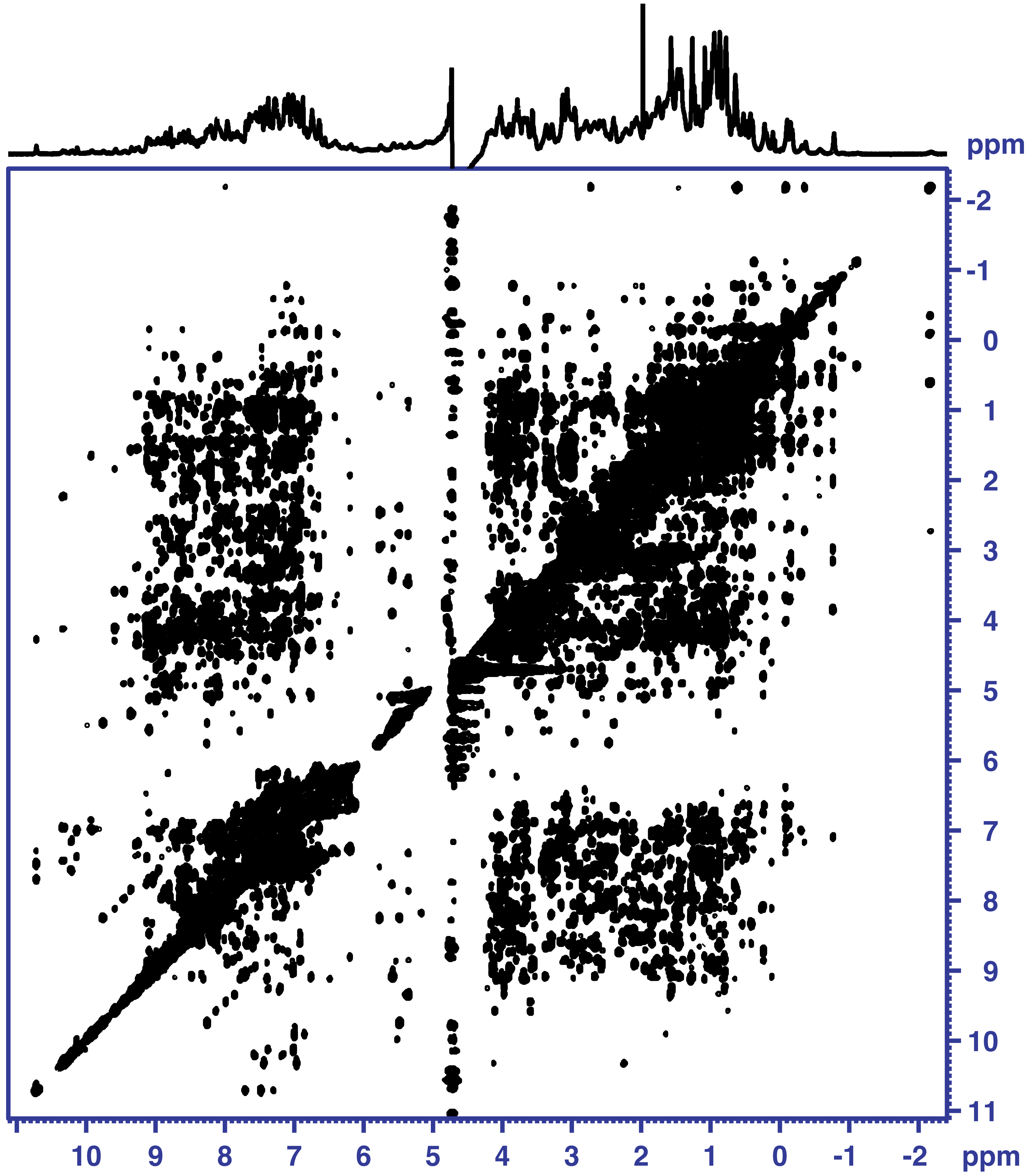
The whole system

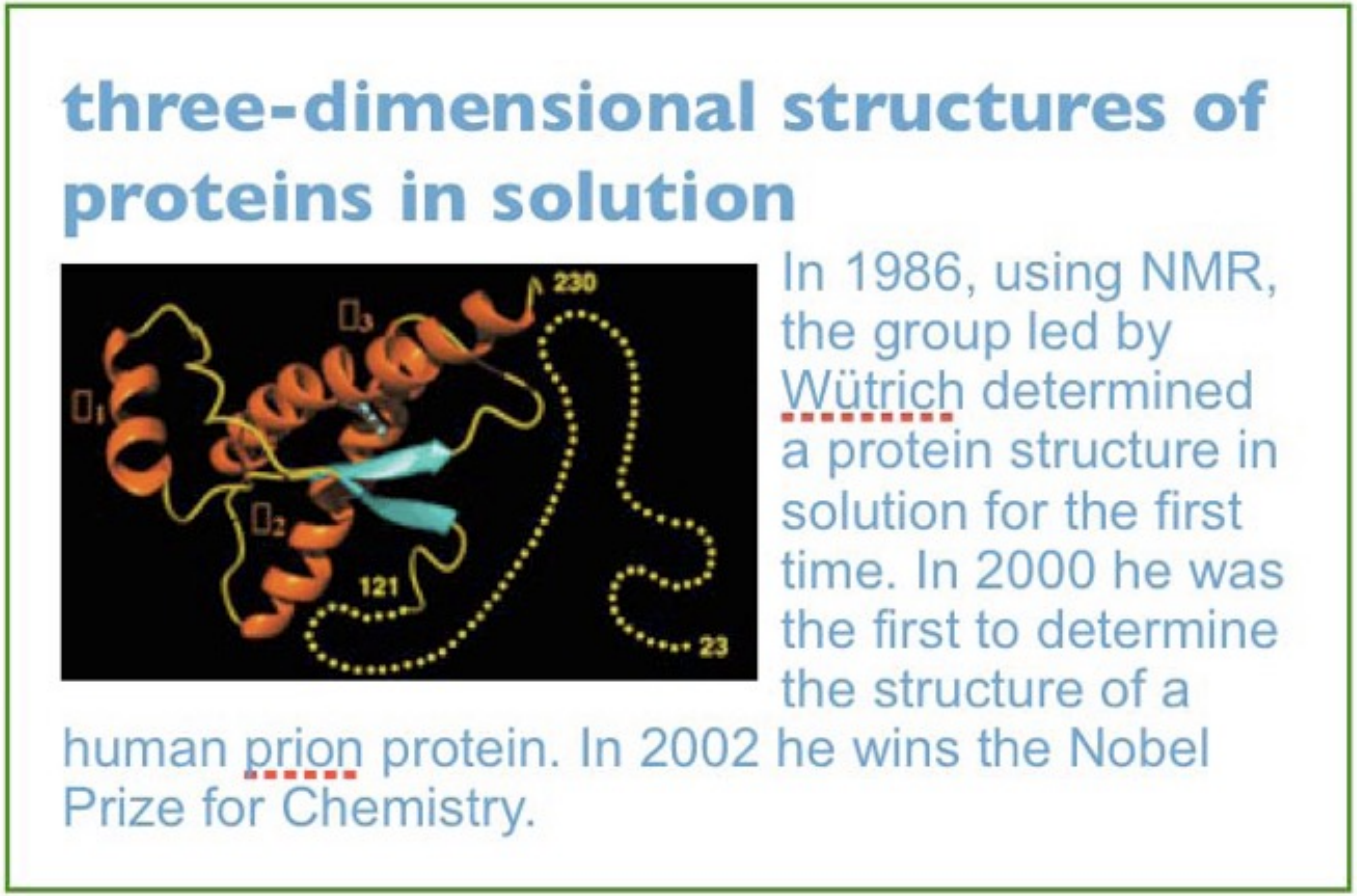


Multi-dimensional NMR: Pairwise Correlations

measuring dipolar couplings

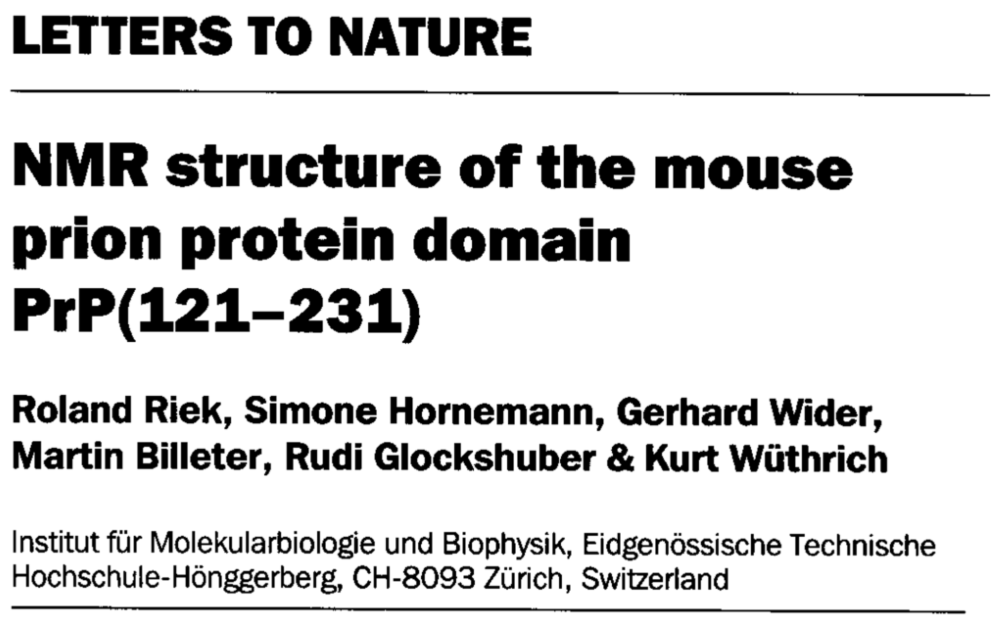
NOESY Spectrum of Lysozyme
The intensity of the cross peaks is related to the inernuclear distance between the two nuclei that are correlated.





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.

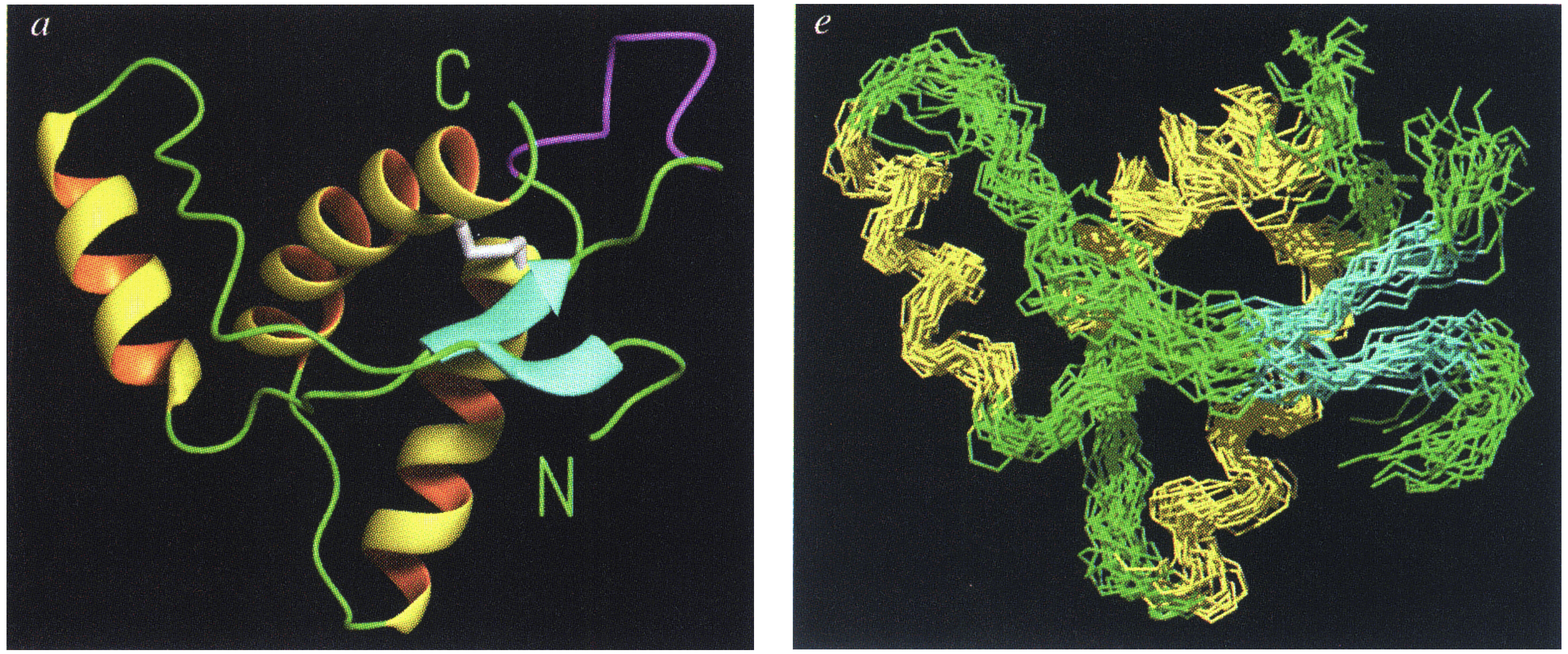


LETTERS TO NATURE

NMR structure of the mouse prion protein domain PrP(121–231)

Roland Riek, Simone Hornemann, Gerhard Wider, Martin Billeter, Rudi Glockshuber & Kurt Wüthrich

Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule-Hönggerberg, CH-8093 Zürich, Switzerland



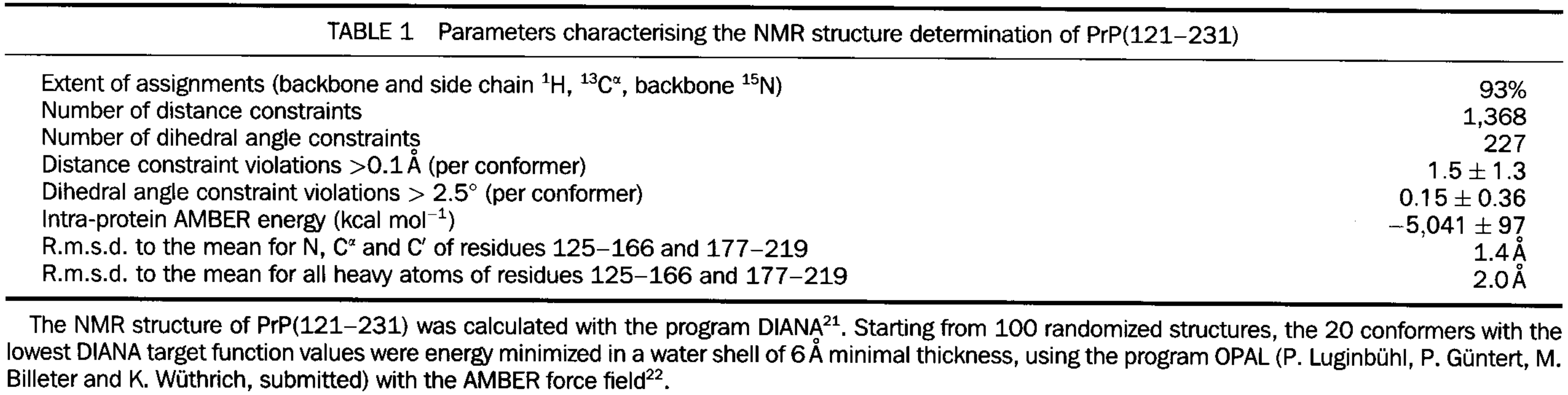
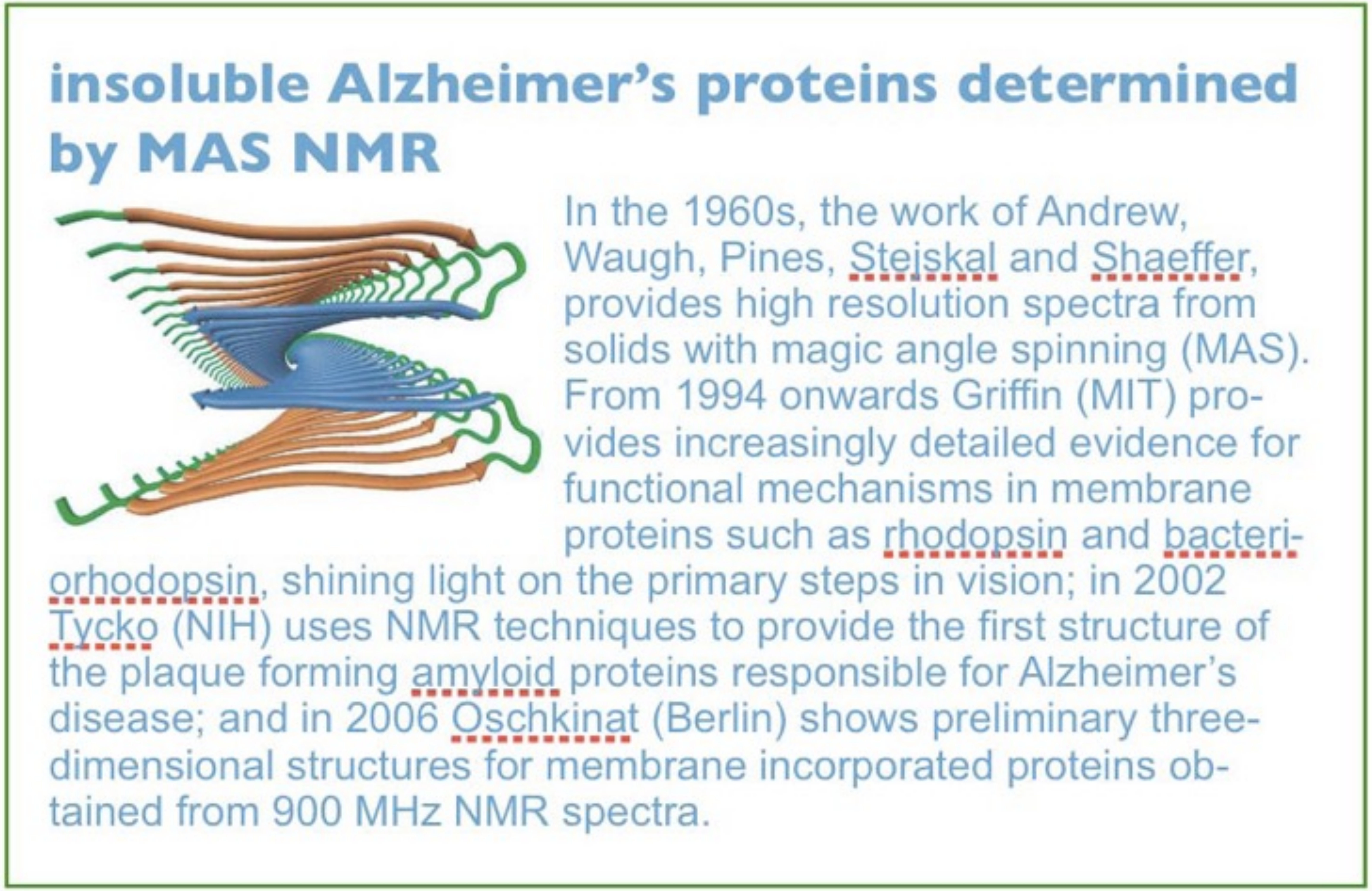


TABLE 1 Parameters characterising the NMR structure determination of PrP(121–231)

Extent of assignments (backbone and side chain ^{1}H, ^{13}C$^\alpha$, backbone ^{15}N)	93%
Number of distance constraints	1,368
Number of dihedral angle constraints	227
Distance constraint violations >0.1 Å (per conformer)	1.5 ± 1.3
Dihedral angle constraint violations > 2.5° (per conformer)	0.15 ± 0.36
Intra-protein AMBER energy (kcal mol^{-1})	−5,041 ± 97
R.m.s.d. to the mean for N, C$^\alpha$ and C′ of residues 125–166 and 177–219	1.4 Å
R.m.s.d. to the mean for all heavy atoms of residues 125–166 and 177–219	2.0 Å

The NMR structure of PrP(121–231) was calculated with the program DIANA[21]. Starting from 100 randomized structures, the 20 conformers with the lowest DIANA target function values were energy minimized in a water shell of 6 Å minimal thickness, using the program OPAL (P. Luginbühl, P. Güntert, M. Billeter and K. Wüthrich, submitted) with the AMBER force field[22].



insoluble Alzheimer’s proteins determined by MAS NMR

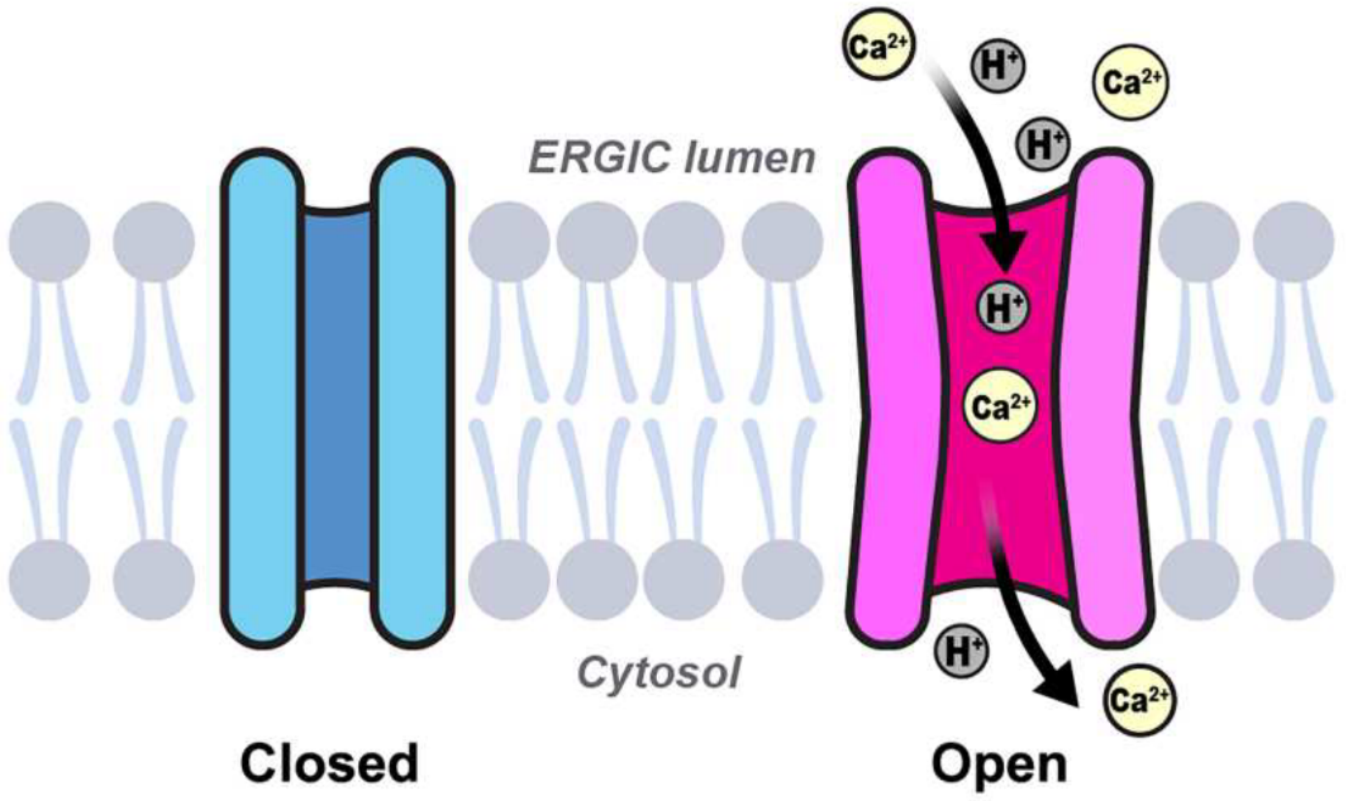
In the 1960s, the work of Andrew, Waugh, Pines, Stejskal and Shaeffer, provides high resolution spectra from solids with magic angle spinning (MAS). From 1994 onwards Griffin (MIT) provides increasingly detailed evidence for functional mechanisms in membrane proteins such as rhodopsin and bacteriorhodopsin,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

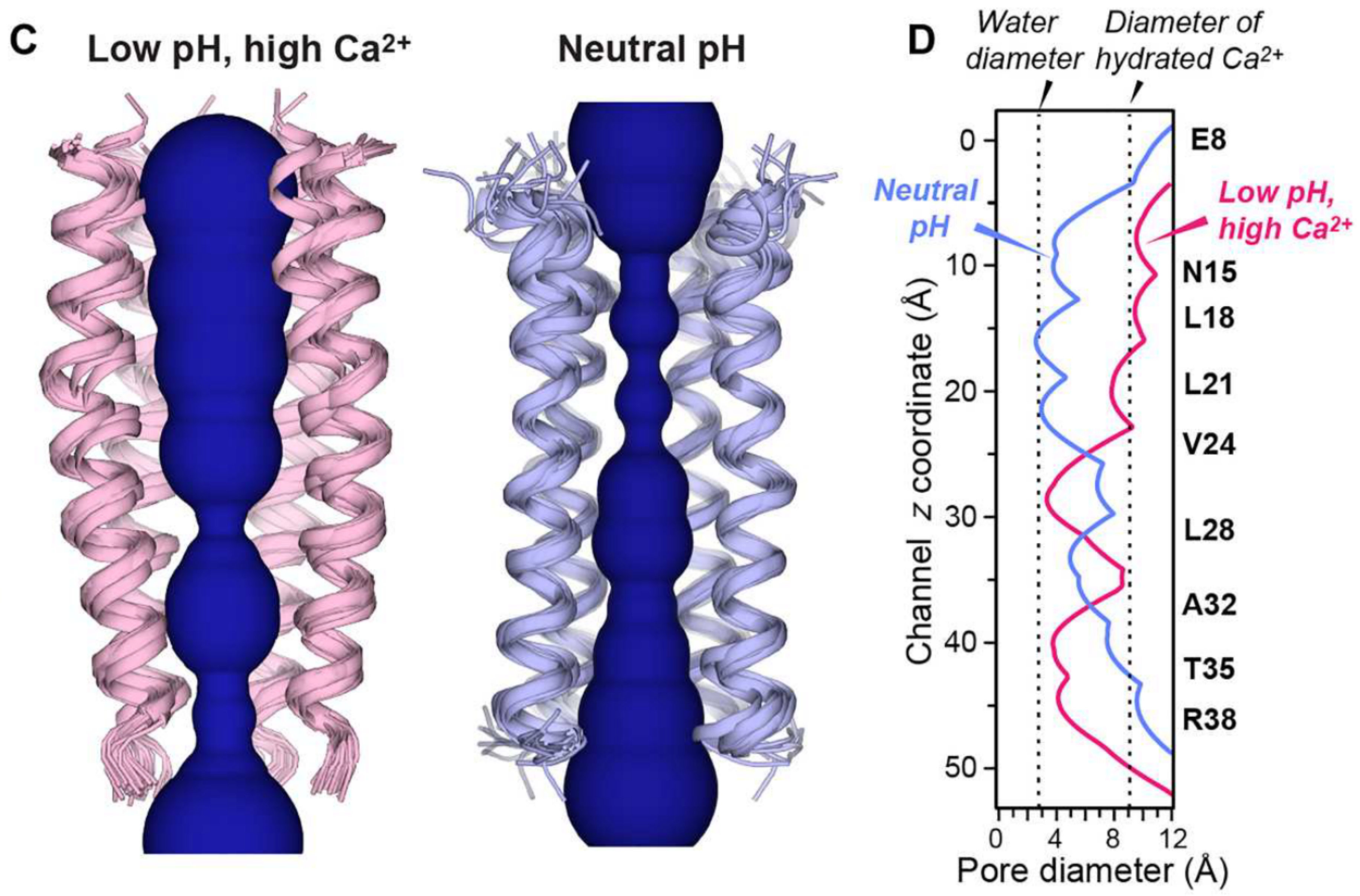
SCIENCE ADVANCES | RESEARCH ARTICLE

CORONAVIRUS

Atomic structure of the open SARS-CoV-2 E viroporin

João Medeiros-Silva, Aurelio J. Dregni, Noah H. Somberg, Pu Duan, Mei Hong*



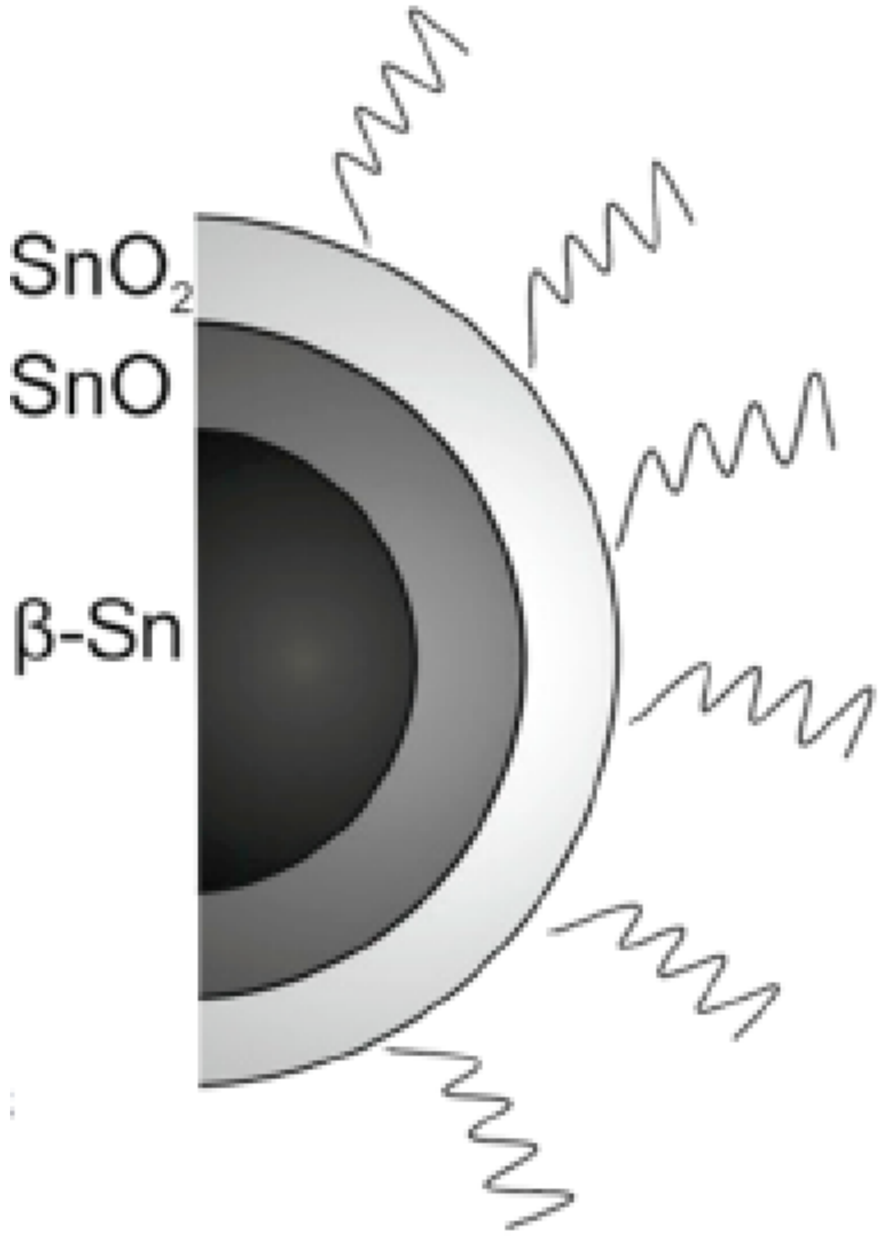


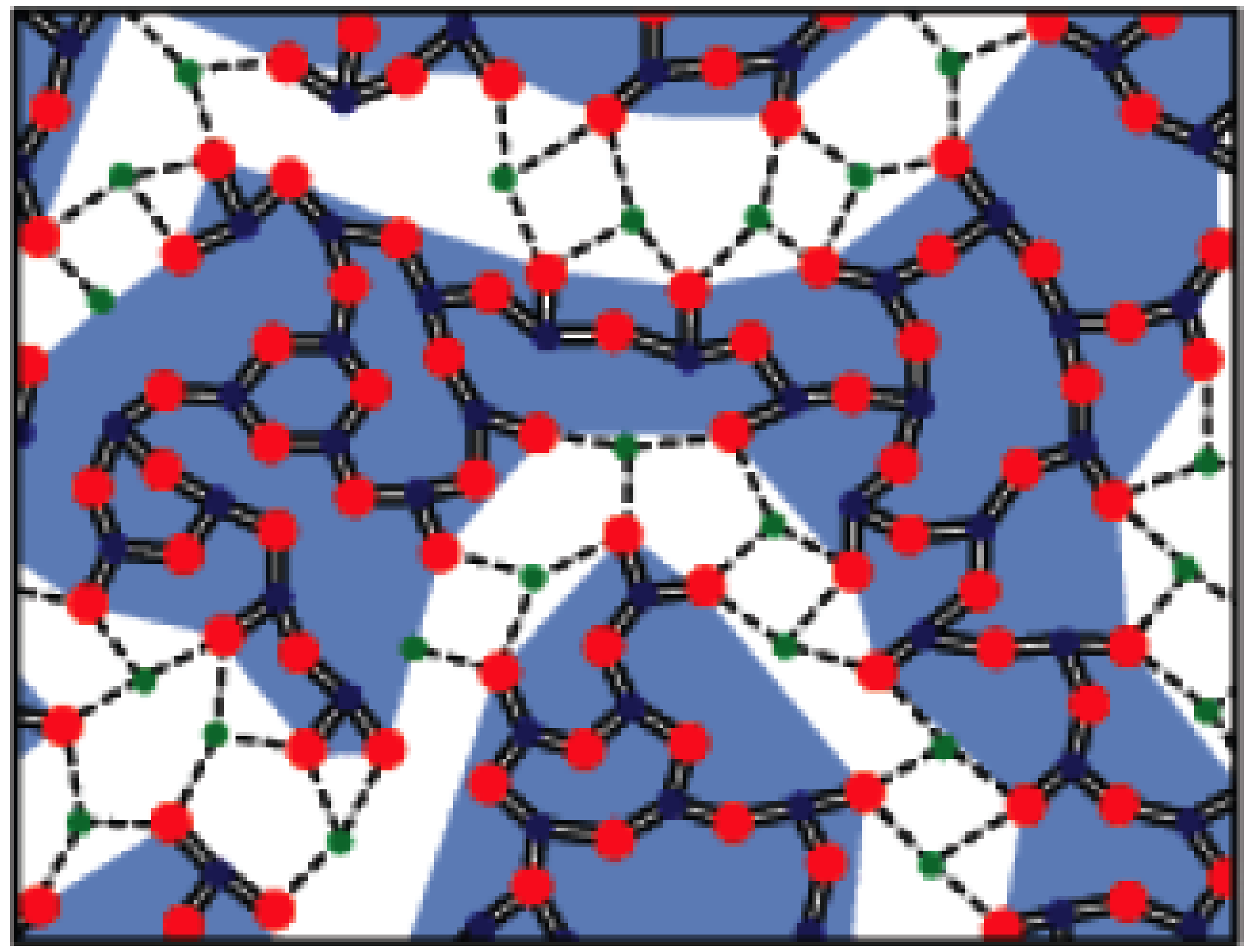
The E channel transports protons and calcium ions, inducing infected cells to launch an inflammatory response that damages tissues and contributes to the symptoms of Covid-19. It is thus an antiviral drug target: if you can stop the channel from sending calcium into the cytoplasm, then you have a way to reduce the cytotoxic effects of the virus.

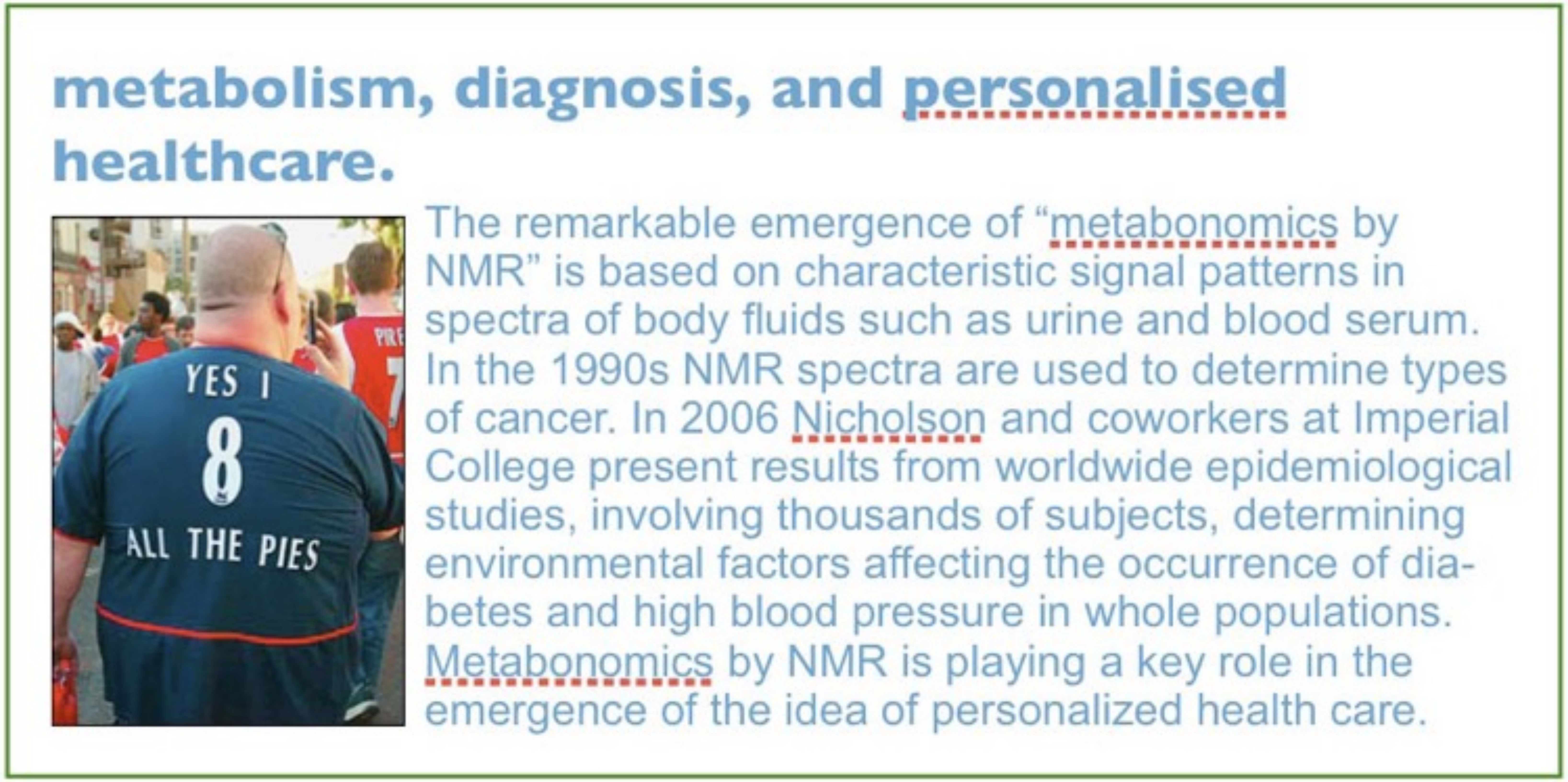
Sci. Adv. **9**, eadi9007 (2023) 13 October 2023

glasses, new materials, and nanosciences

Quadrupolar nuclei have always played a leading role in NMR. Since the 90s oxygen and aluminum NMR studies have continuously contributed to change the understanding we have of the structure and dynamics of glass forming materials and their related molten state. This is now changing the whole way we think about the formation and structure of disordered materials. In 2006 Grey and coworkers use understanding from NMR observations directly to improve the charging rate capacity of lithium nickel magnanese oxide in rechargeable batteries. In 2010 Emsley and coworkers introduce Dynamic Nuclear Polarisation Surface Enhanced NMR Spectroscopy (DNP SENS), and in 2014 they use this new method to solve the structure of core-shell nanoparticles.



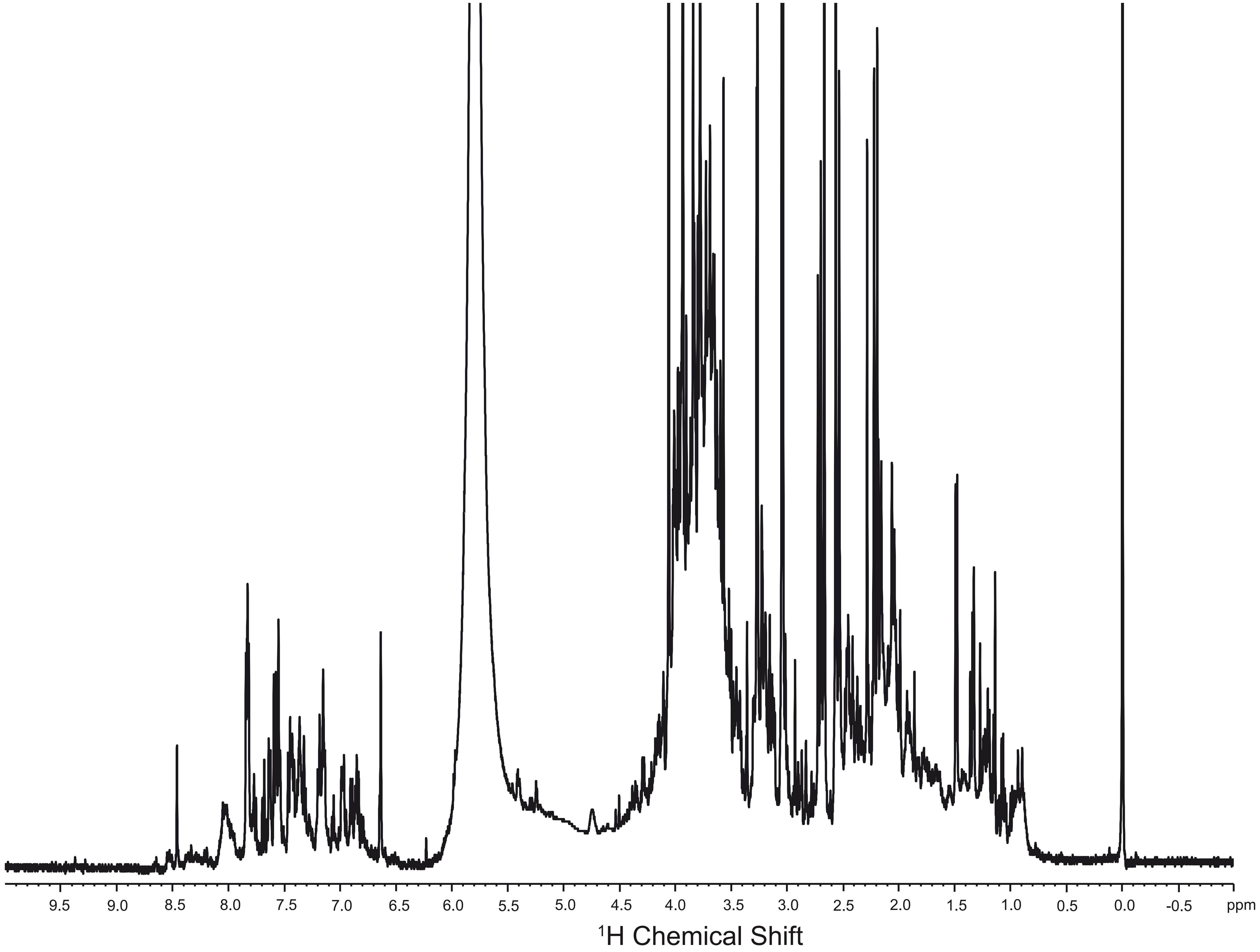




metabolism, diagnosis, and personalised healthcare.

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.

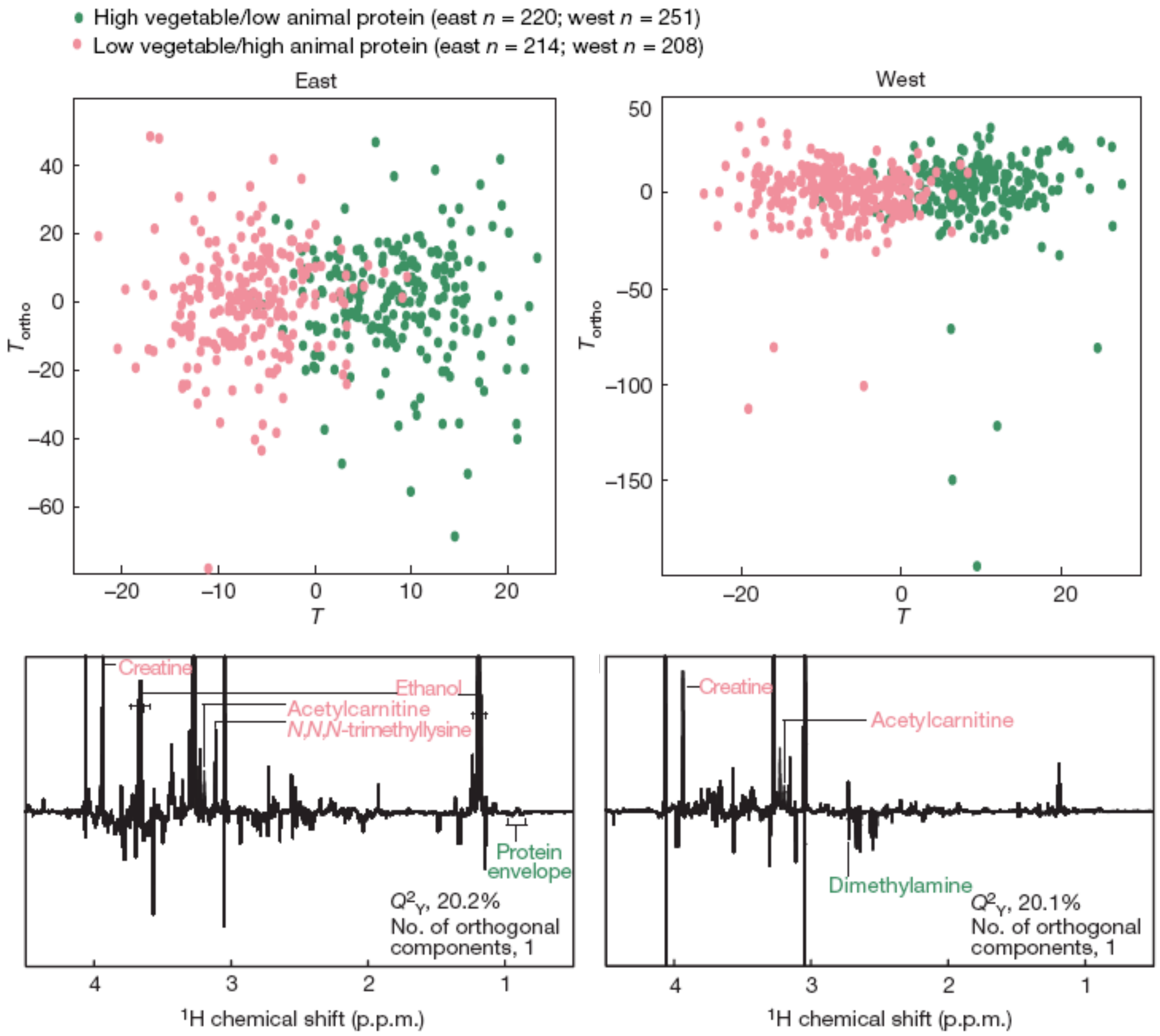
NMR spectrum of human urine



This spectrum provides a “metabolic profile” of the subject.
The study of variations in this profile is called metabolomics/metabonomics

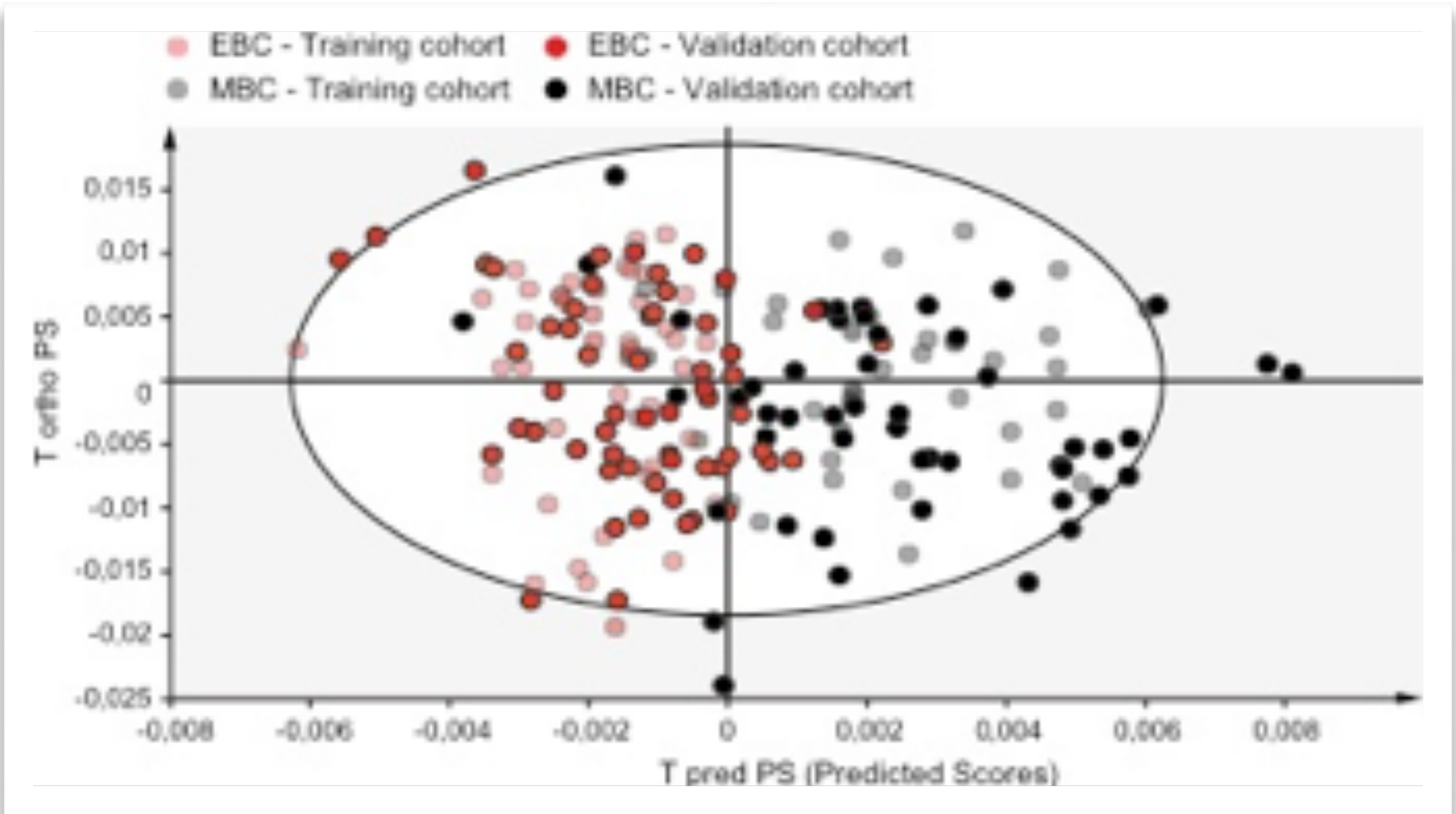
This urine wheel was published in 1506 by Ullrich Pinder, in his book Epiphanie Medicorum.
It describes the possible colours, smells and tastes of urine, and uses them to diagnose disease. (The Royal Library, Copenhagen)

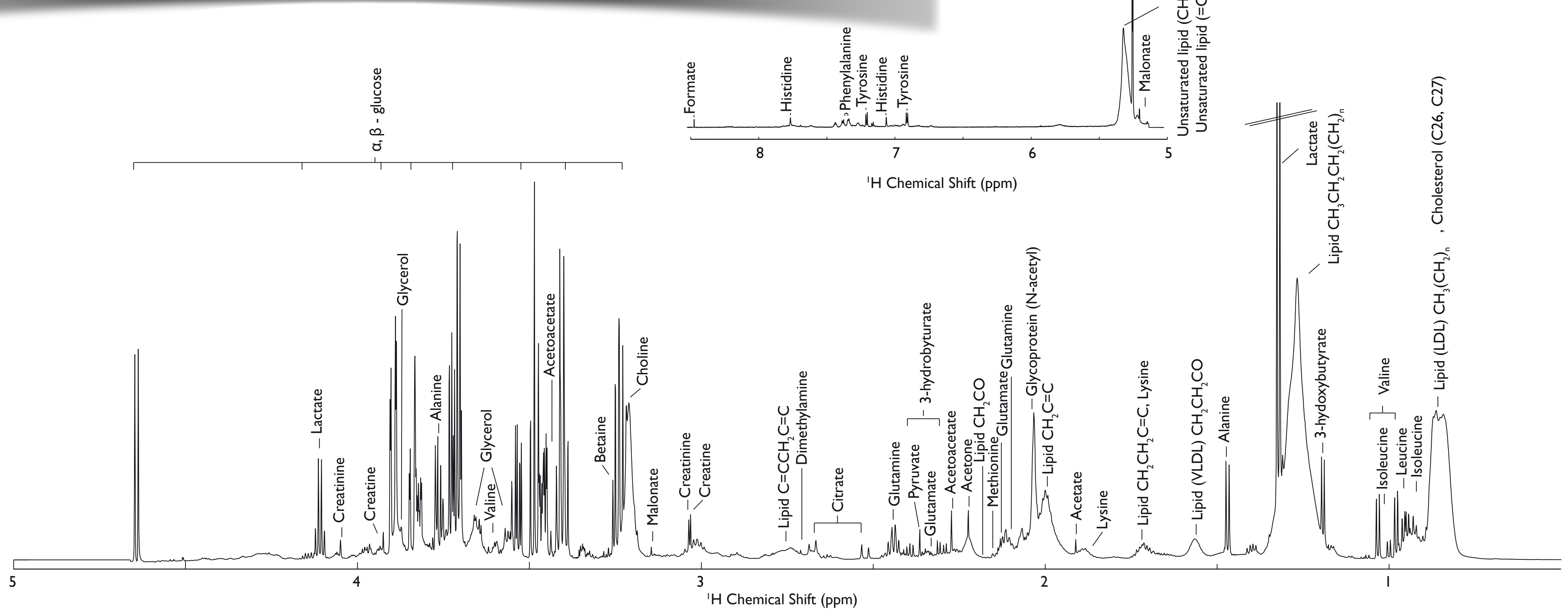
Dietary factors associated to risk of cardiovascular disease



NATURE| Vol 453| 15 May 2008

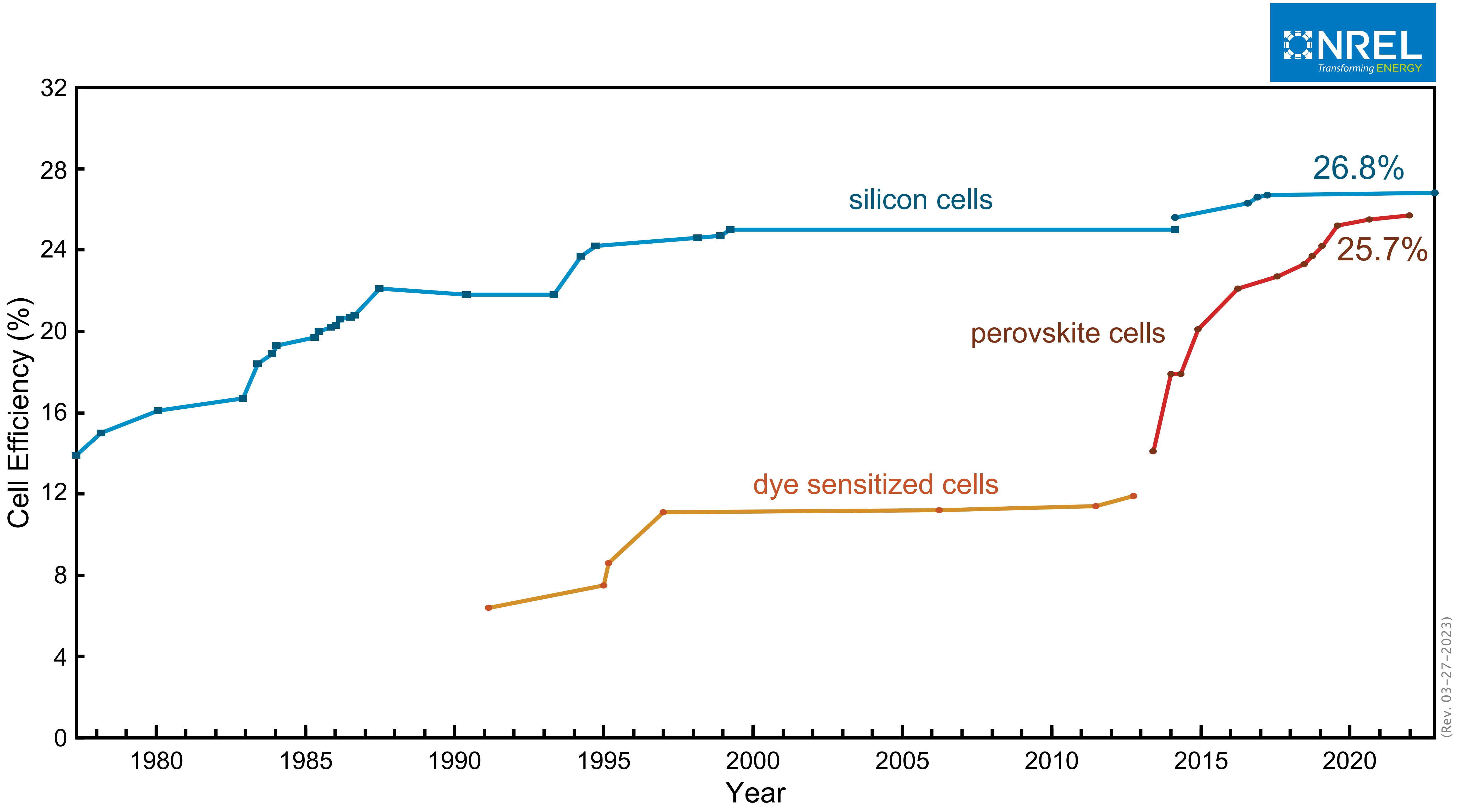
A serum NMR metabolomics fingerprint of advanced metastatic breast cancer



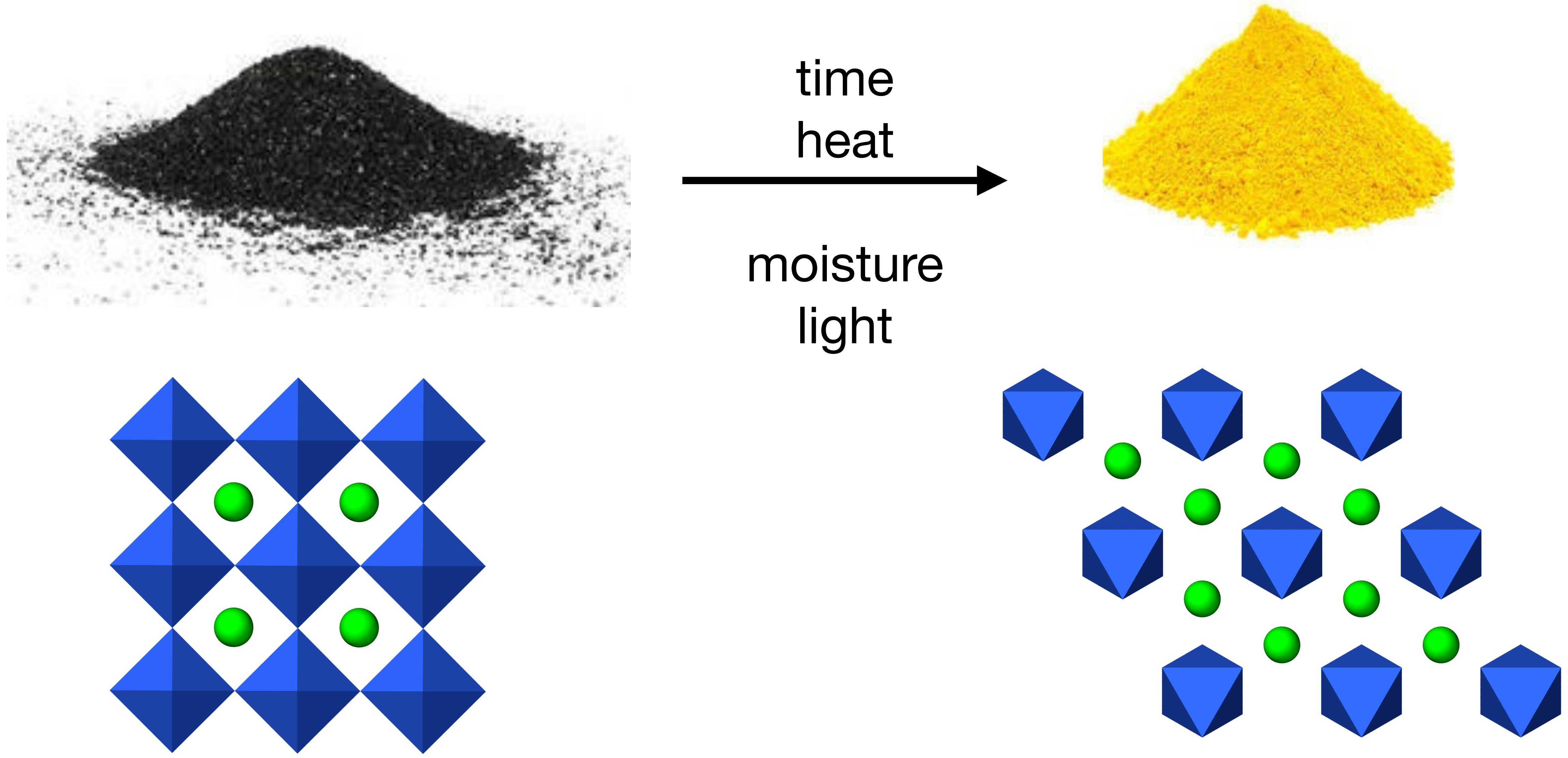


Jobard et al., Cancer Letters, 2014

Perovskite-based cells: the fastest-advancing solar technology to date



Atomic-level mechanism of molecular modulators



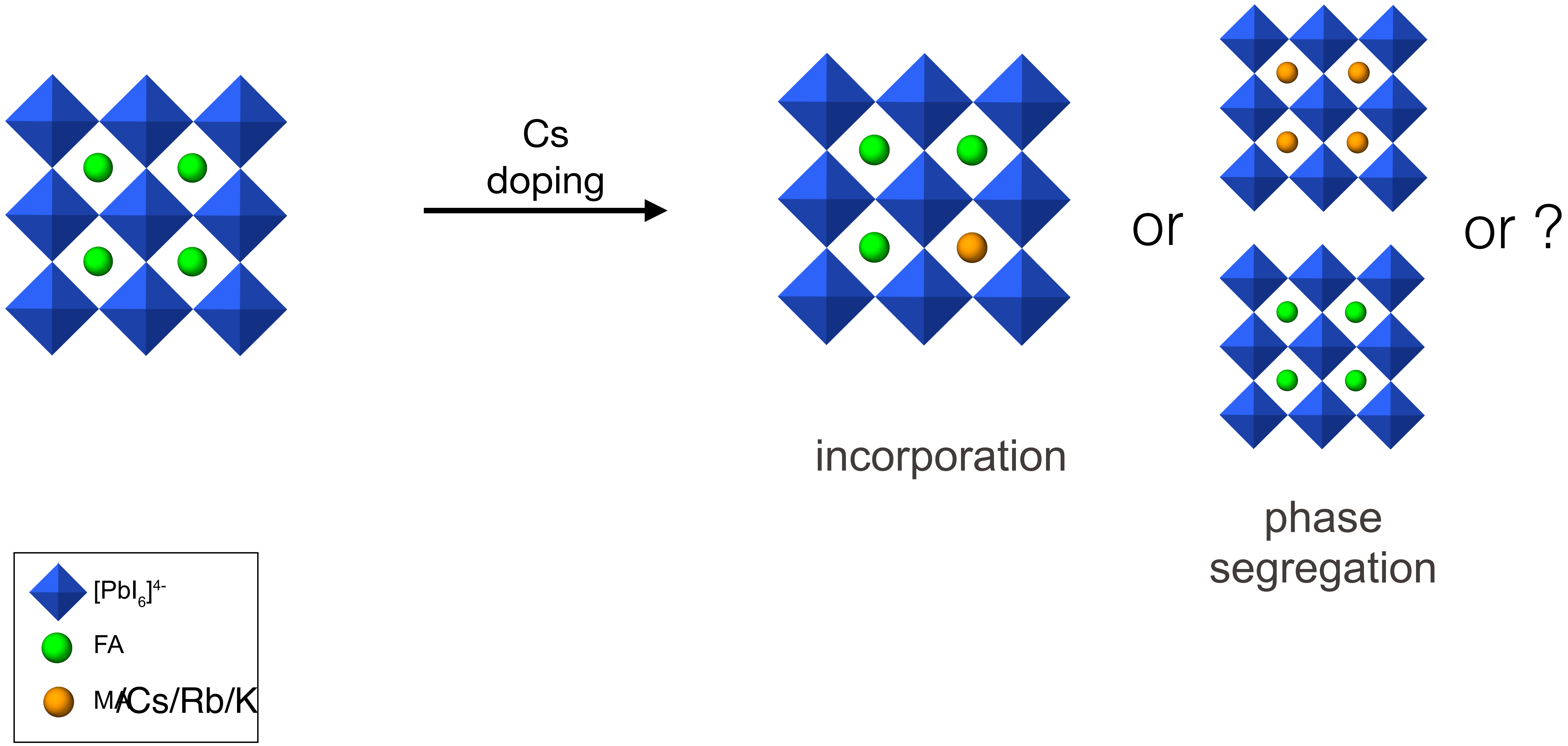
time
heat

moisture
light

α-FAPbI$_3$

δ-FAPbI$_3$

Atomic-level mechanism of molecular modulators



Cs
doping

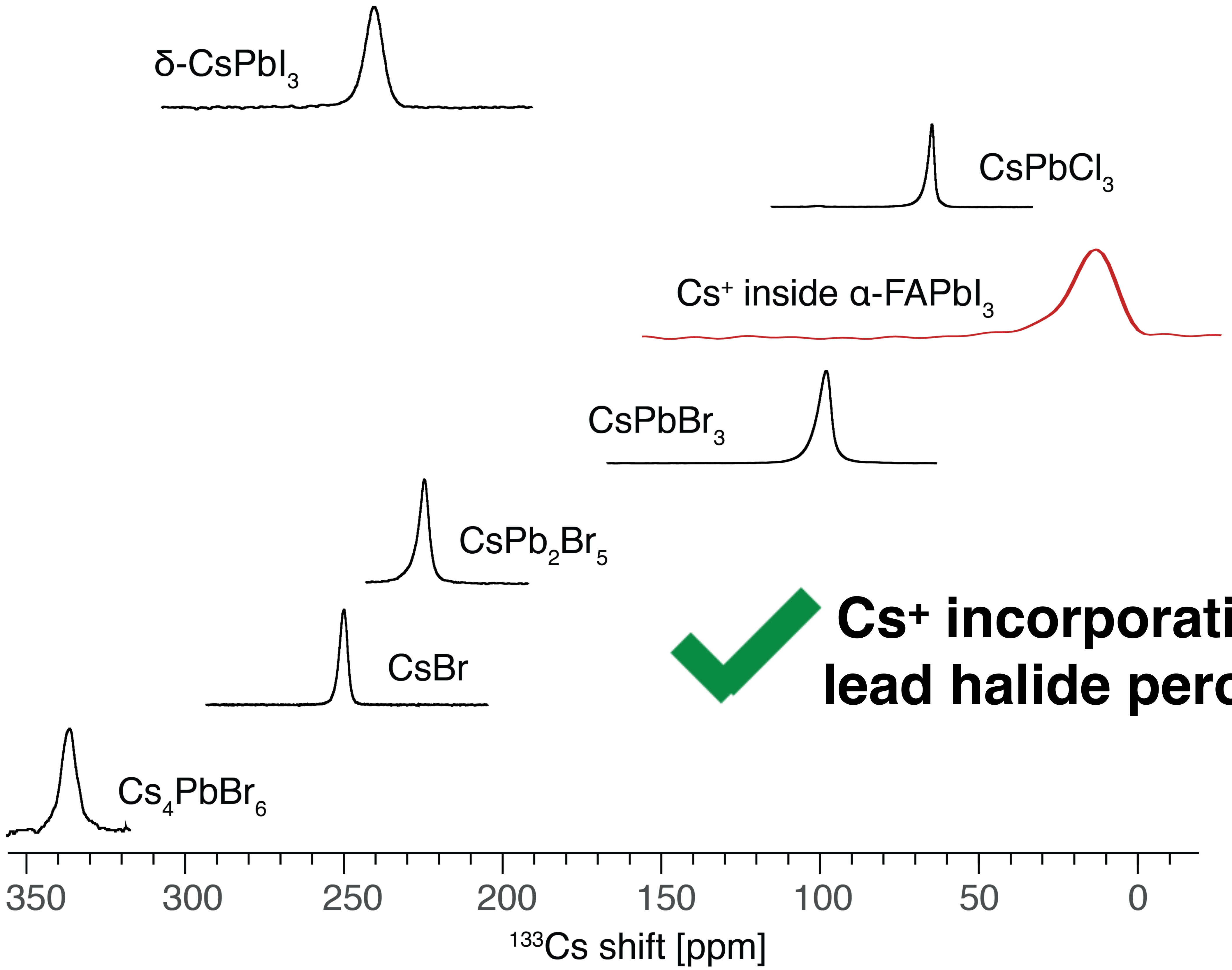
or

or ?

incorporation

phase
segregation

^{133}Cs solid-state MAS NMR of cesium-doped perovskites

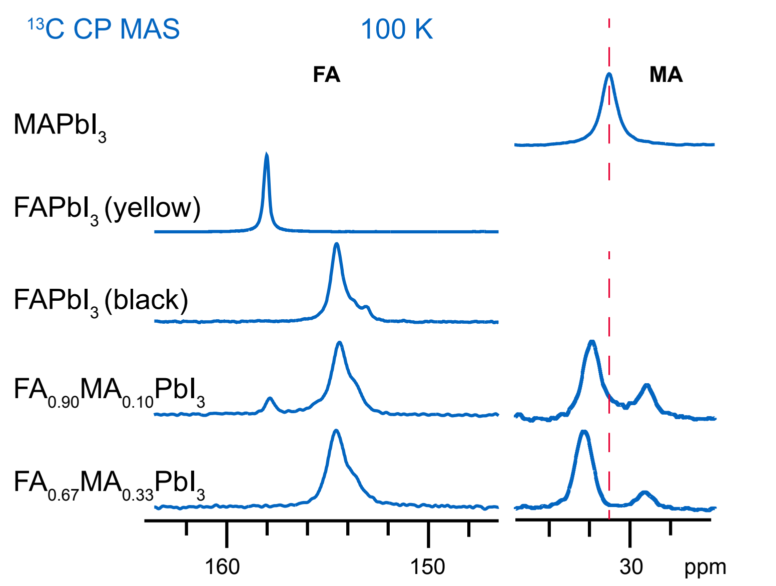


✓ **Cs^+ incorporation into lead halide perovskites**

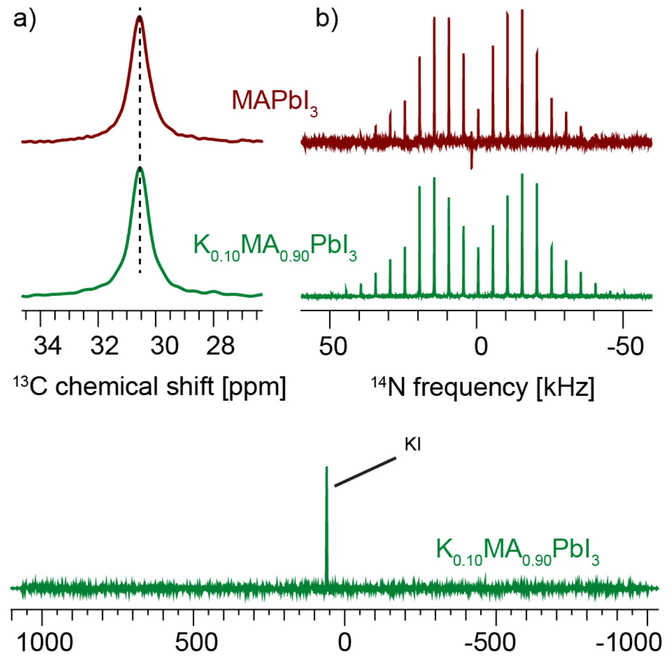
Kubicki, Prochowicz, Hofstetter, Zakeeruddin, Grätzel, Emsley, J. Am. Chem. Soc. **139**, 14173 (2017)

Phase Composition and Cation Doping in Perovskites

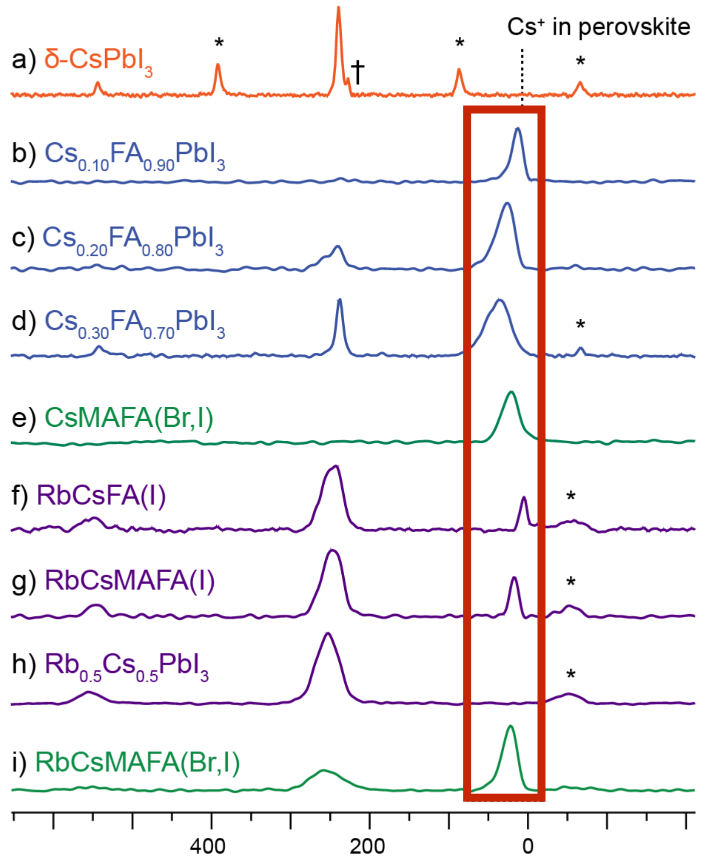
	previous conviction	from NMR
 FA = $(NH_2)_2CH^+$	✓	✓
 MA = $CH_3NH_3^+$	✓	✓
 GUA = $C(NH_2)_3^+$	✗	✓
Cs^+ ●	✓	✓
Rb^+ ●	✓	✗
K^+ ●	✓	✗
Co^{2+} ●	✓	✗



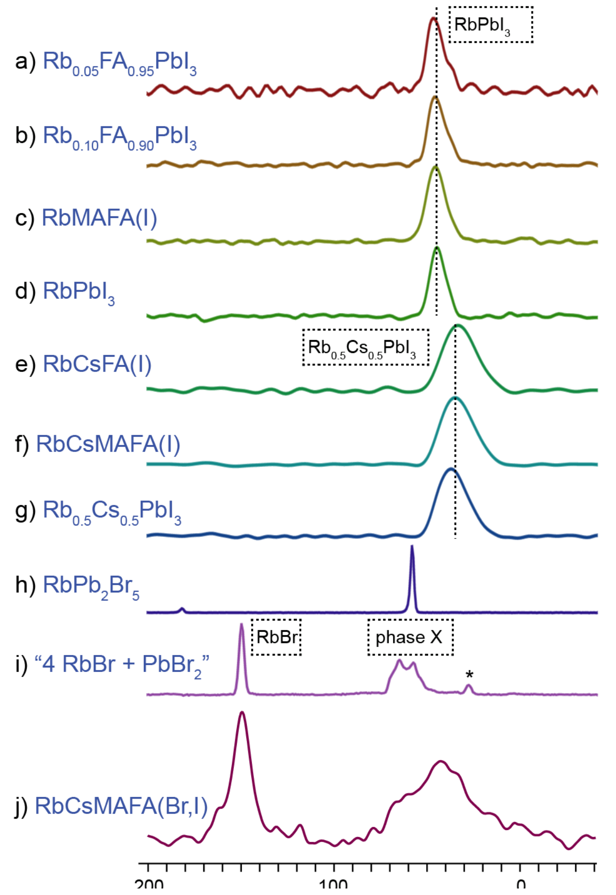
^{13}C chemical shift [ppm]



^{39}K shift [ppm]



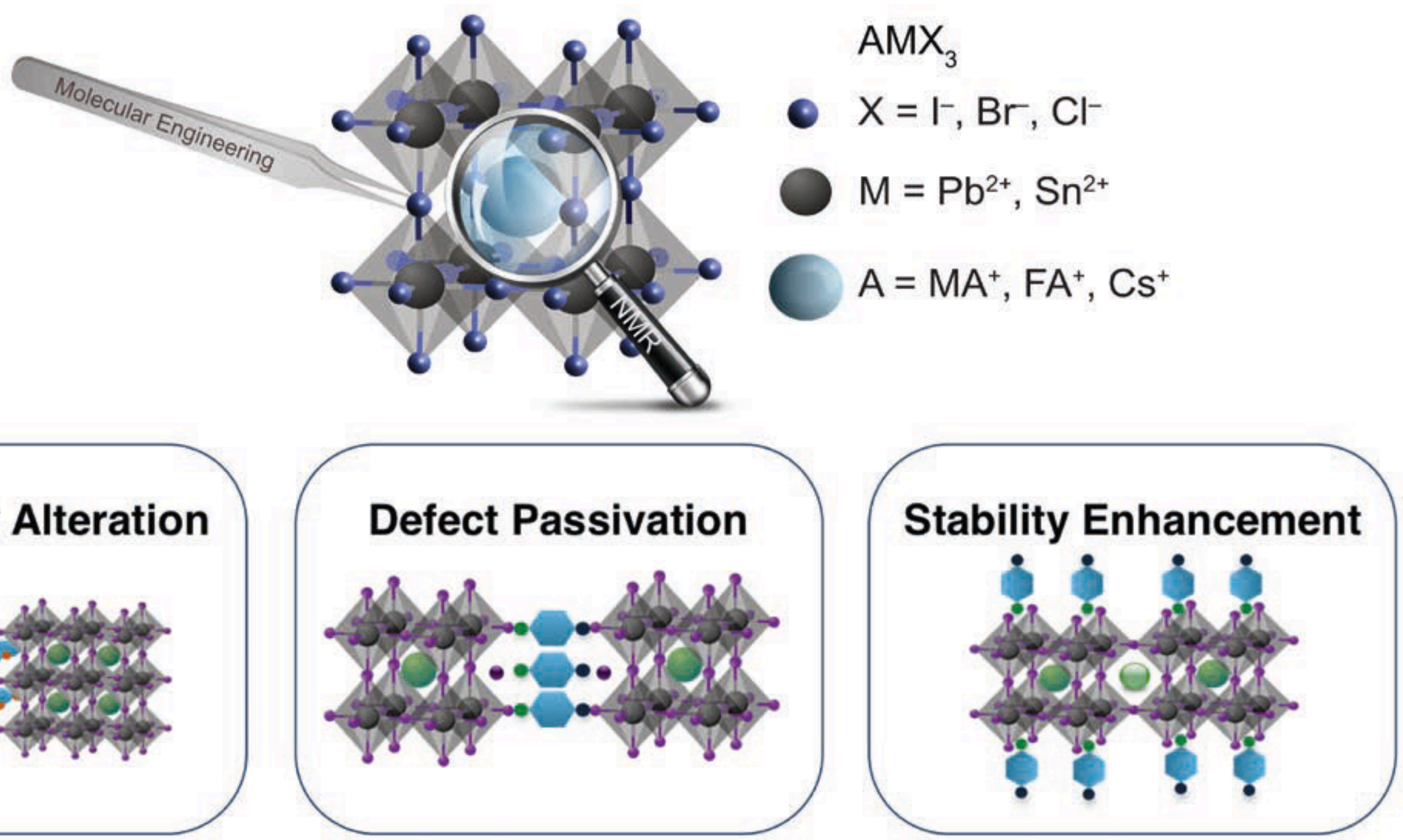
^{133}Cs shift [ppm]



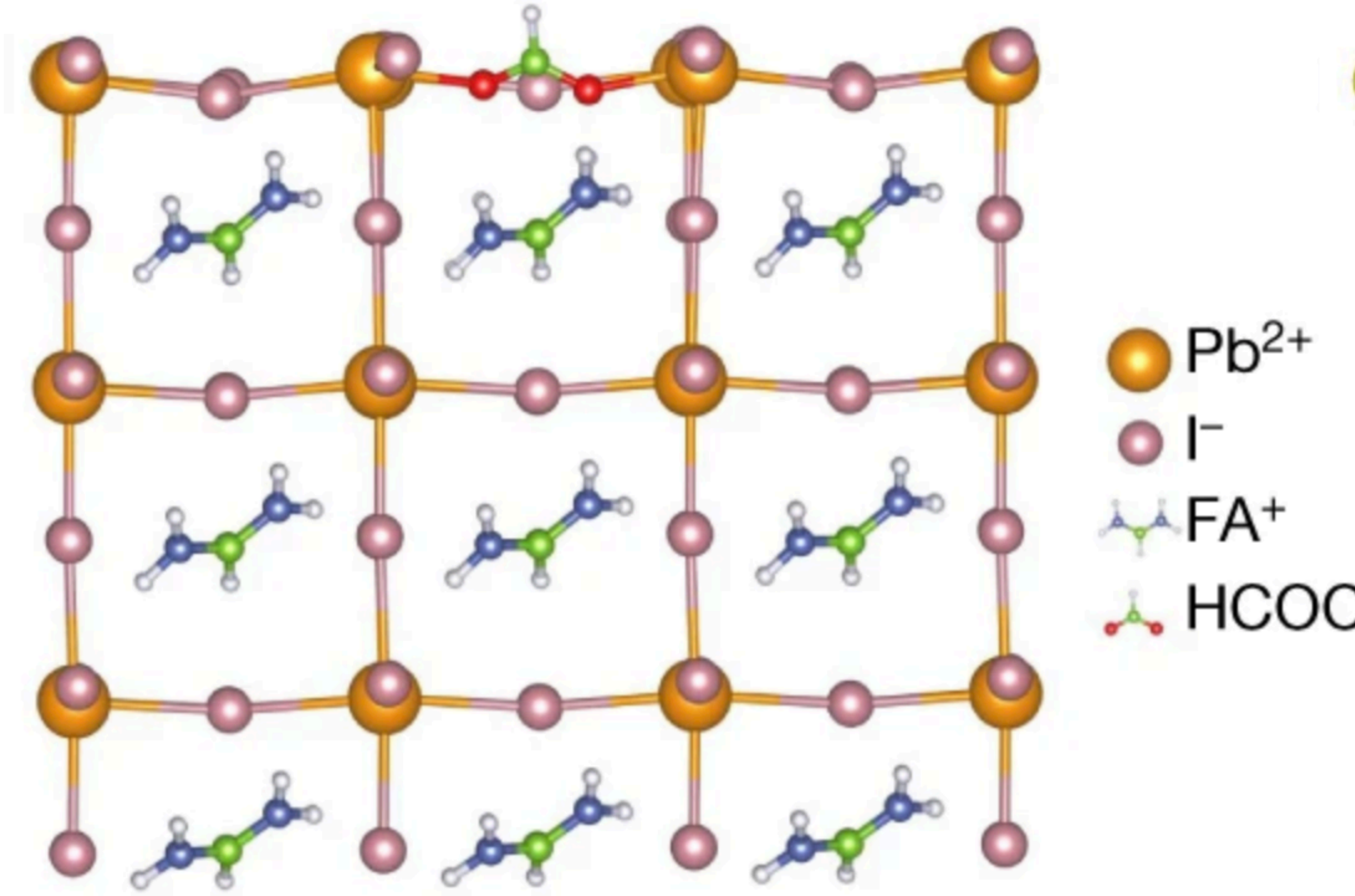
^{87}Rb shift [ppm]

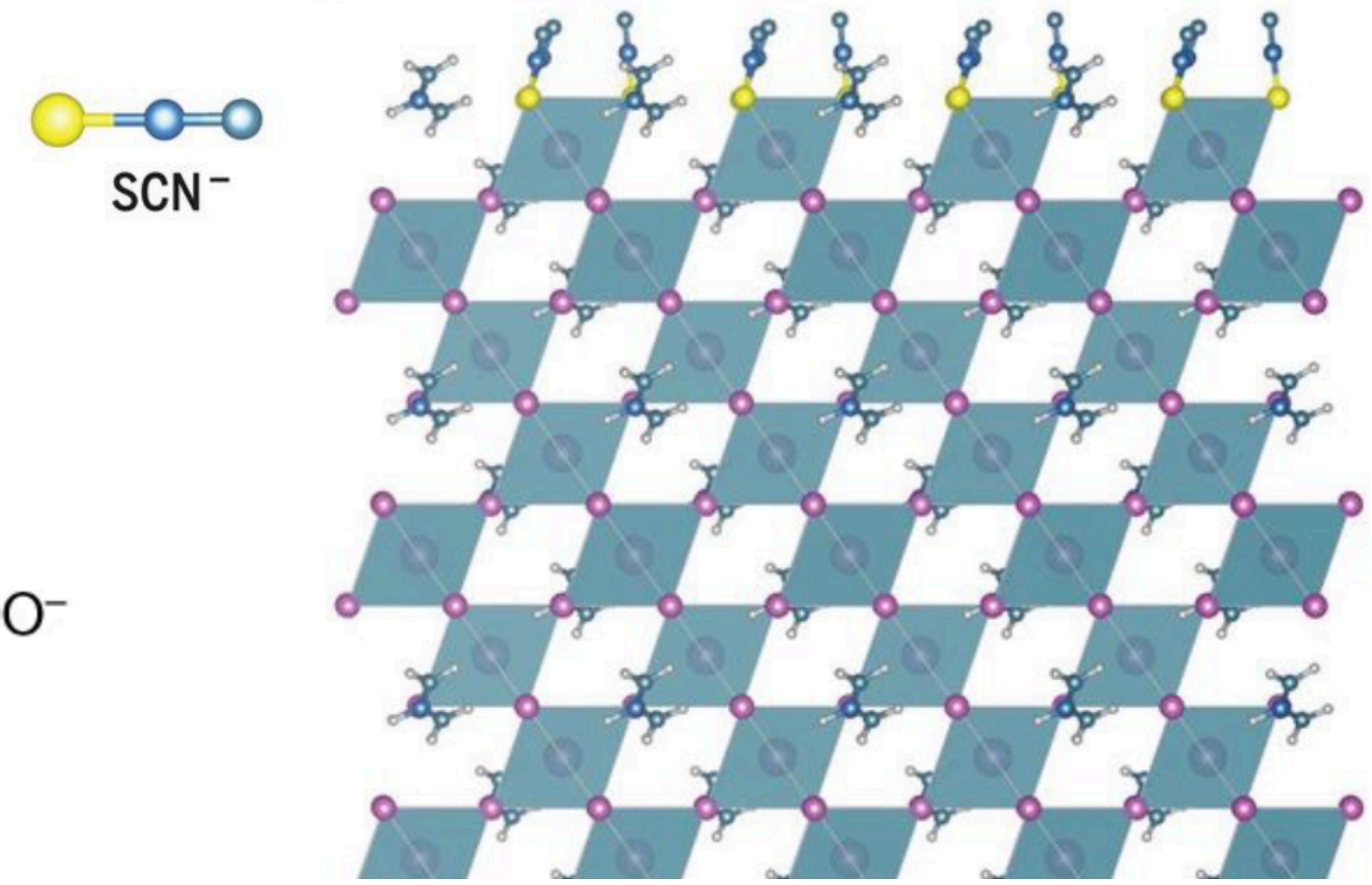
J. Am. Chem. Soc. **139,** 14173 (2017) ; J. Am. Chem. Soc. **140**, 3345 (2018); J. Am. Chem. Soc. **140**, 7232 (2018); Nano Energy **49**, 523 (2018); Nature Comm. **9**, 4482 (2018); Joule **3**, 205 (2019); Nature Comm. **10**, 4686 (2019); ACS Energy Lett **5**, 2964 (2020); Science **370**, 74 (2020) & **others**

NMR determination of the atomic-level mechanism of modulators leads to a paradigm shift in design principles



Atomic-Level Insights from NMR Lead to New Design Strategies that Yield New Record Holding Materials for Efficiency and Stability

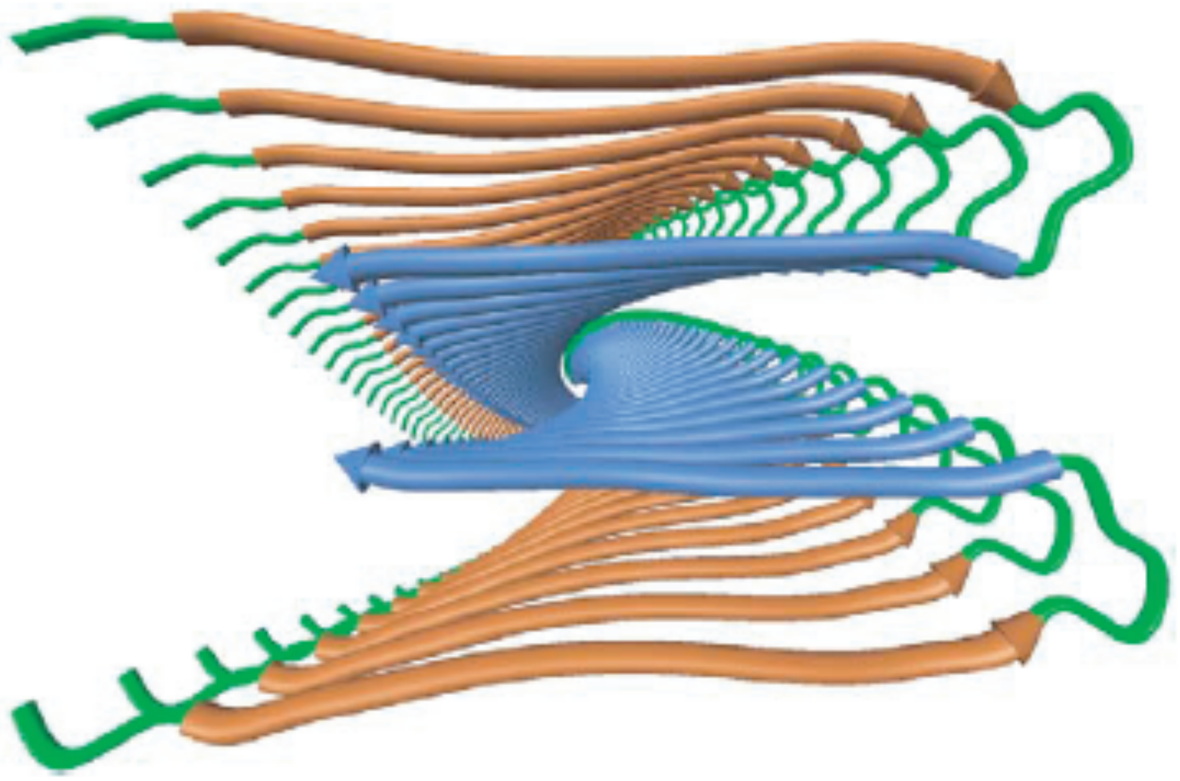


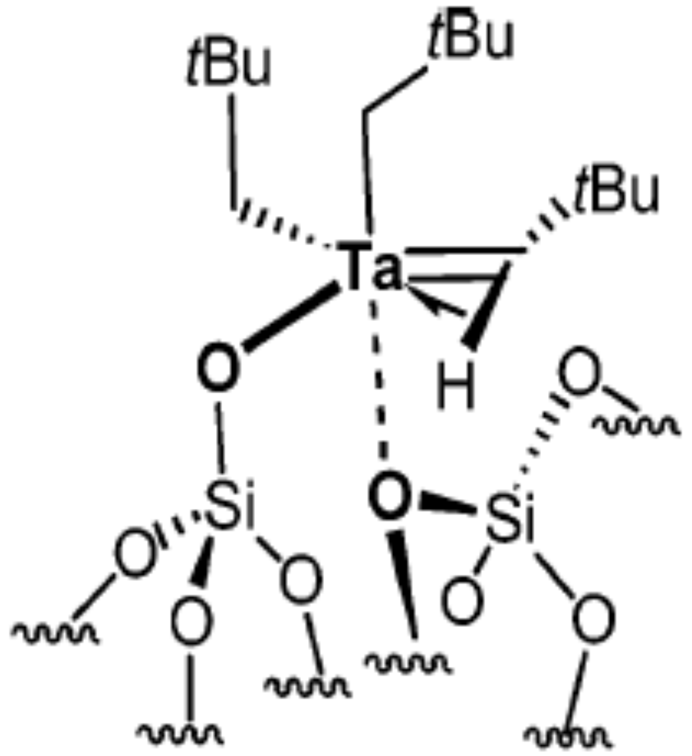


Jeong, *et al.*, “Pseudo-halide anion engineering for α-FAPbI(3) perovskite solar cells,” *Nature*, (2021).

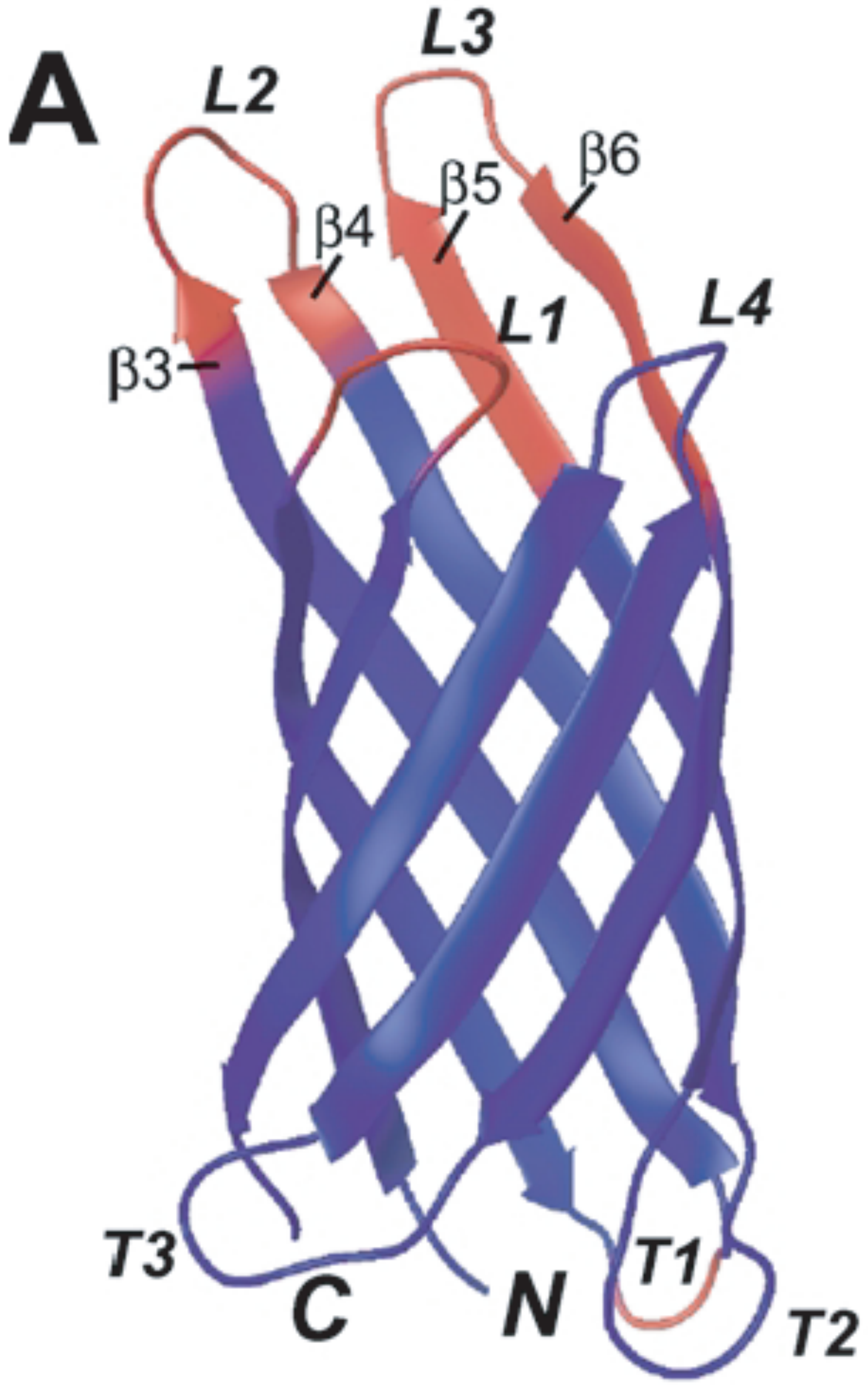
Lu et al., “Vapor-assisted deposition of highly efficient, stable black-phase FAPbI(3) perovskite solar cells,” *Science* **370**, 74 (2020).

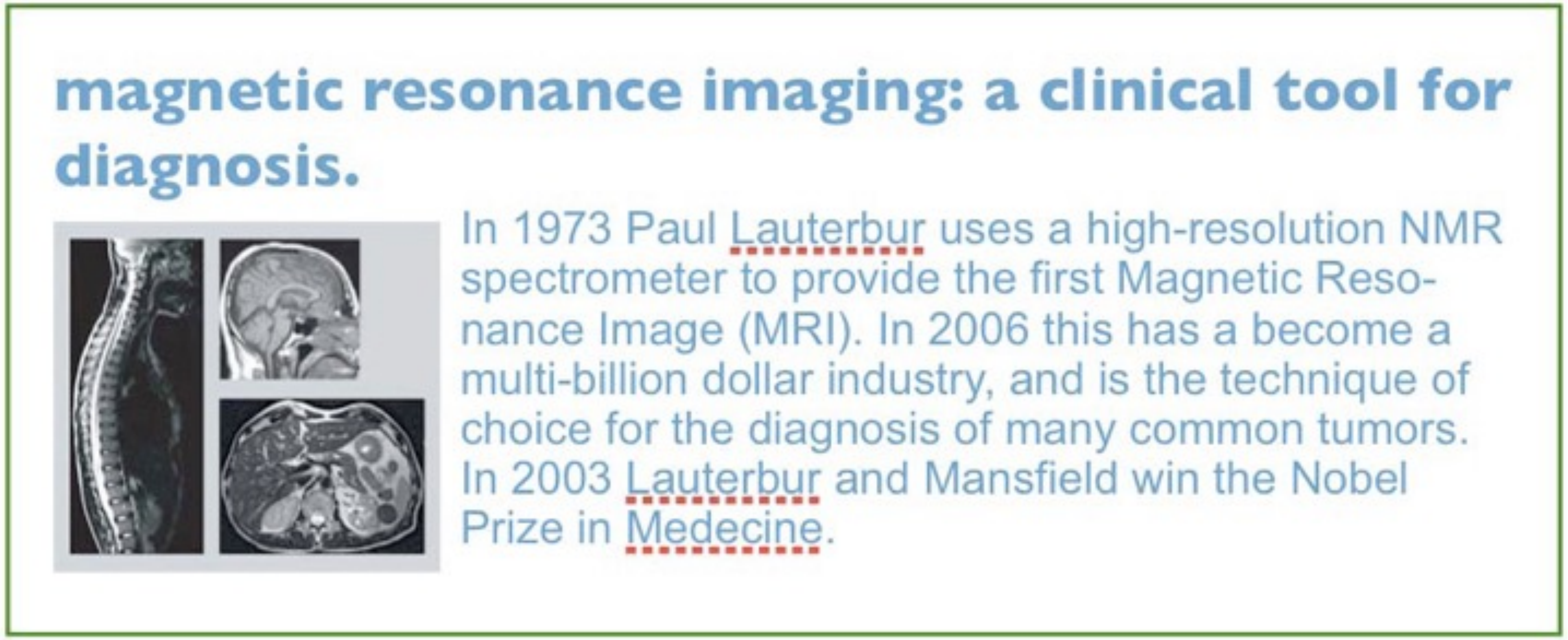
NMR: Is this it?





1a'

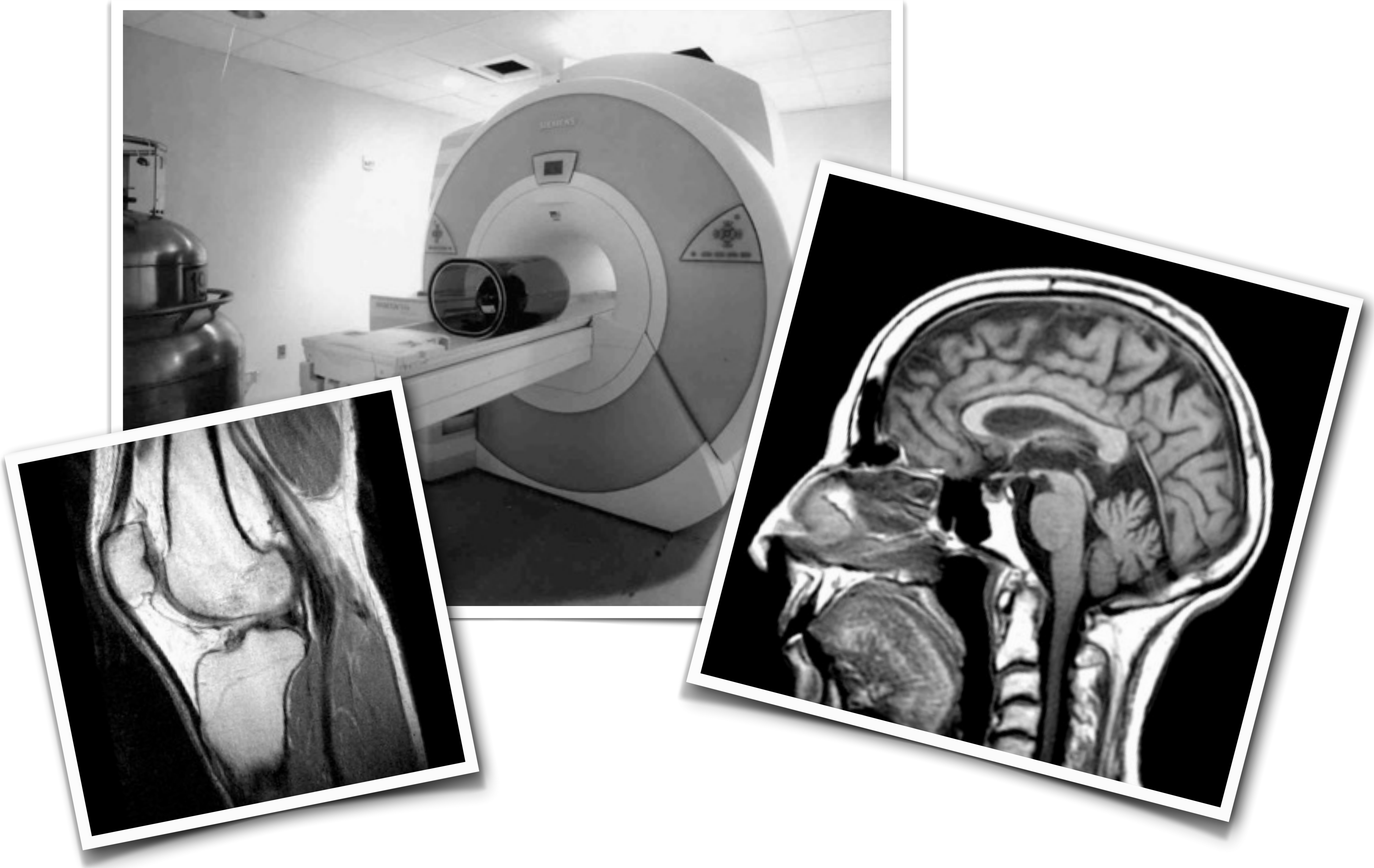




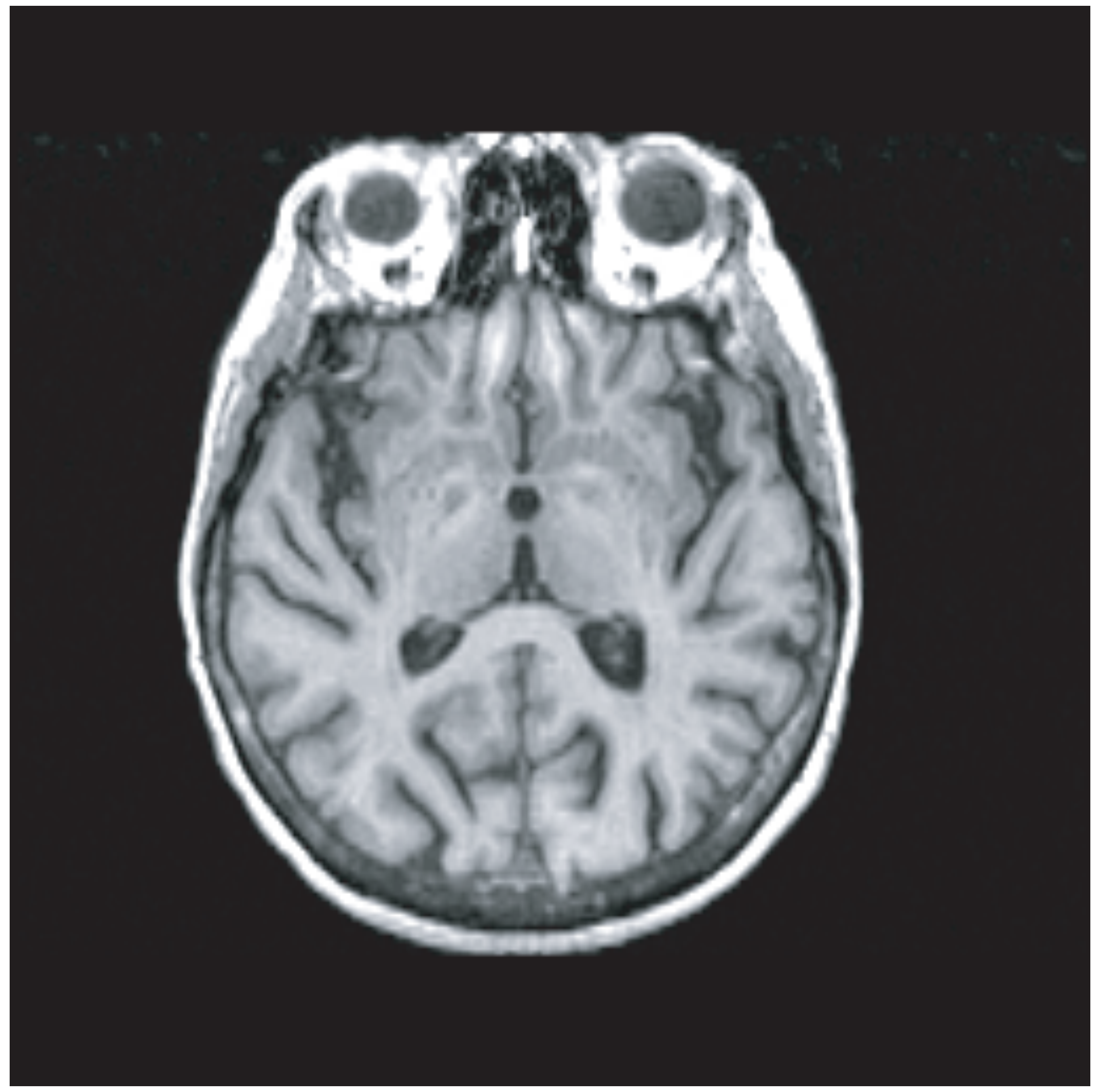
magnetic resonance imaging: a clinical tool for diagnosis.

In 1973 Paul Lauterbur uses a high-resolution NMR spectrometer to provide the first Magnetic Resonance Image (MRI). In 2006 this has a 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.

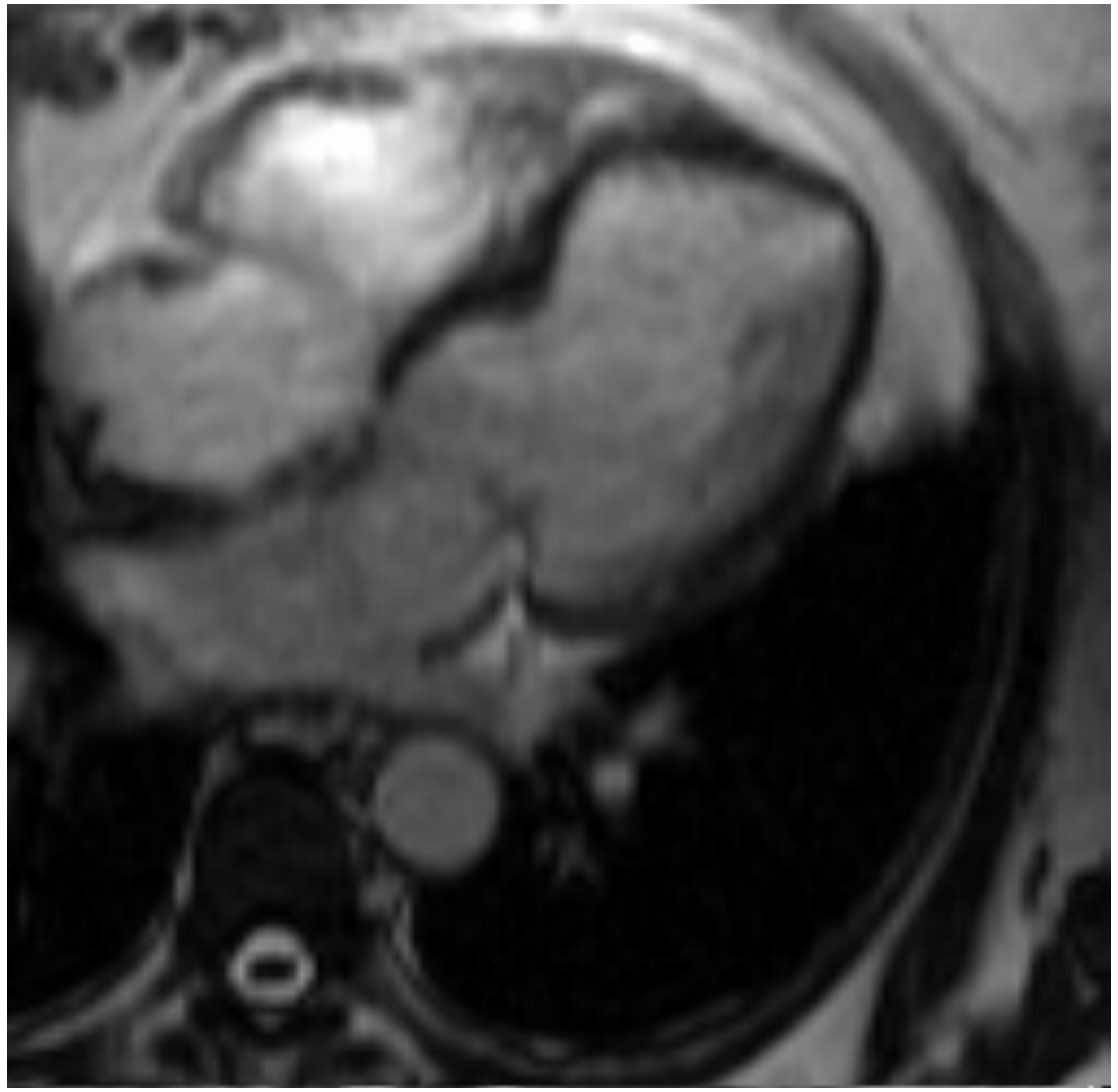
Magnetic Resonance Imaging (MRI)



Images from NMR

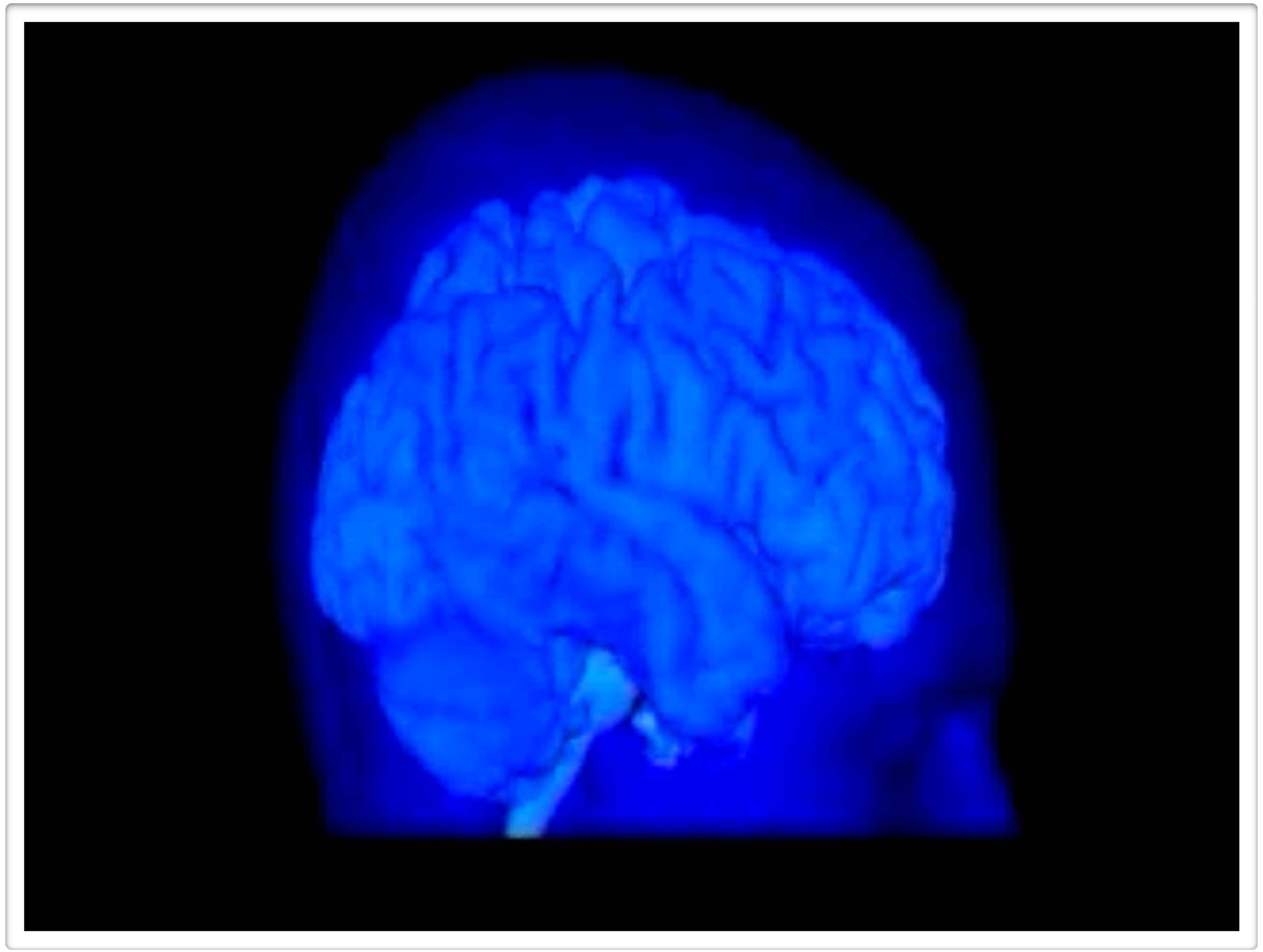


Magnetic Resonance Imaging (MRI)



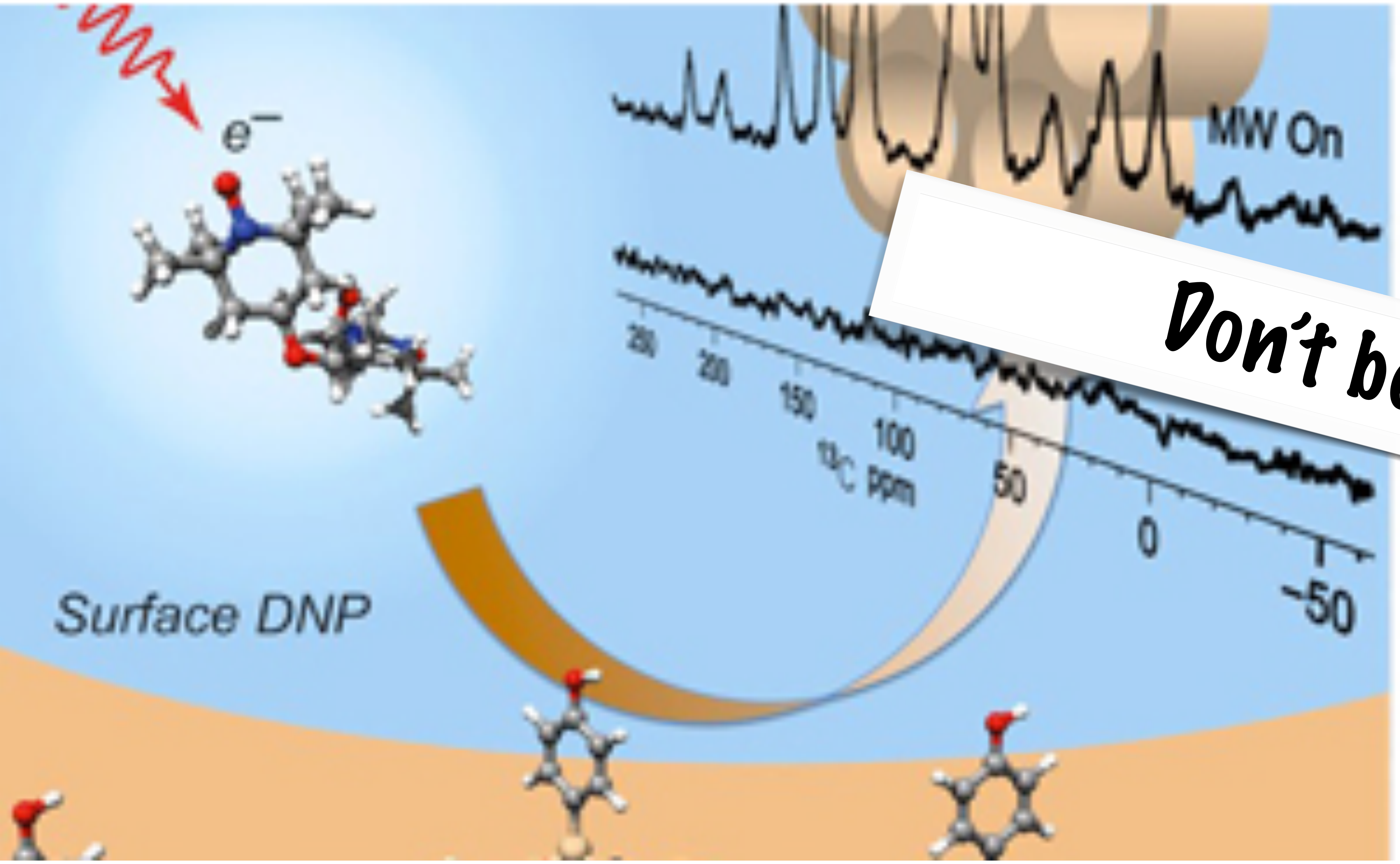
real time imaging

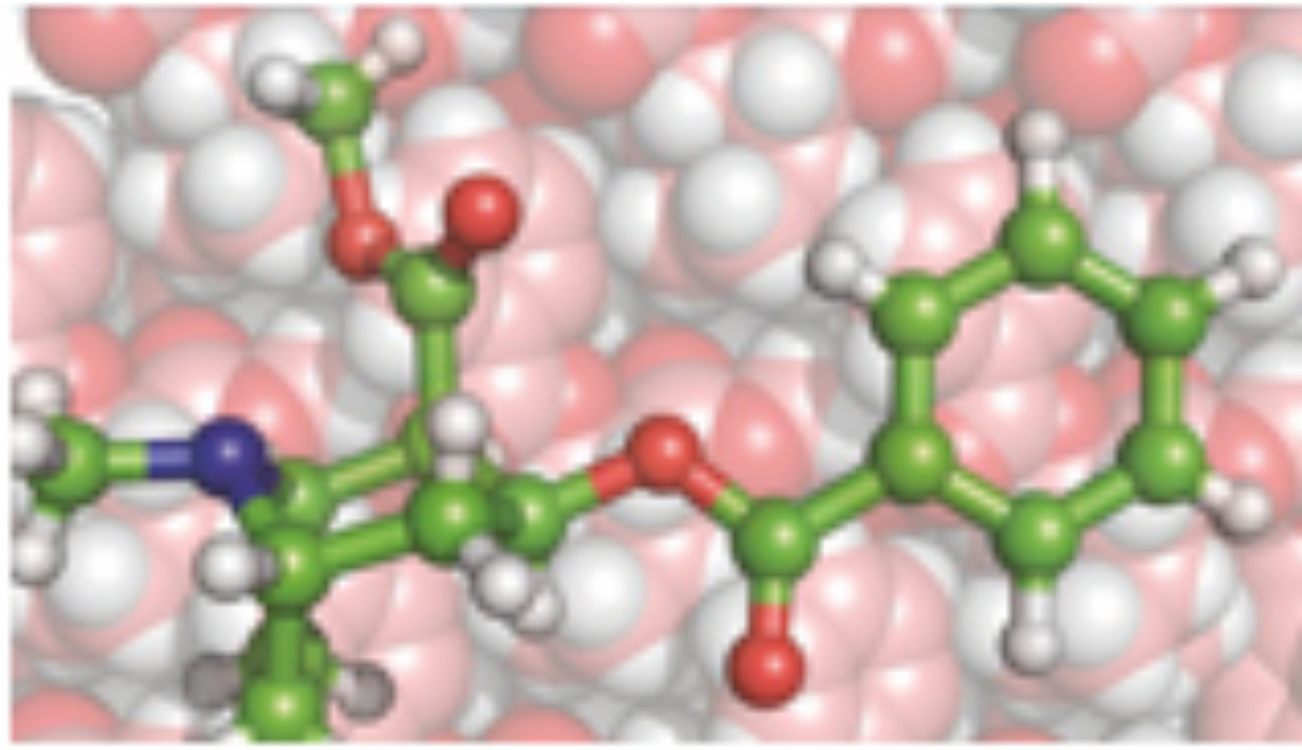
Magnetic Resonance Imaging (MRI)



reading your mind

LRM@EPFL





Don’t be shy! Come see!

LRM

Homework

Before next week's class

read Chapter 2 of
P.J. Hore, "Nuclear Magnetic Resonance," 2nd Edition,
(Oxford University Press, 2015)

A pdf file of the Chapter is available on the course moodle page.

You do not need to read the greyed out parts
(but you can if you want to)

Problem Sets

Problem sets will be completed by working in groups

Each group will work on a different problem set

Each group must submit a joint solution to their problem before the following course.

Solutions will be marked, and will contribute 25% of the final mark for the NMR part of the course.

Corrected solutions to all the problem sets will be posted on Moodle