Symmetry and Spin: Improving Molecular Imaging with Molecular Hyperpolarization

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1895: X-rays first image opaque media

Hand mit Ringen: print of Wilhelm Röntgen's first "medical" x-ray, of his wife's hand, taken 22 Dec 1895
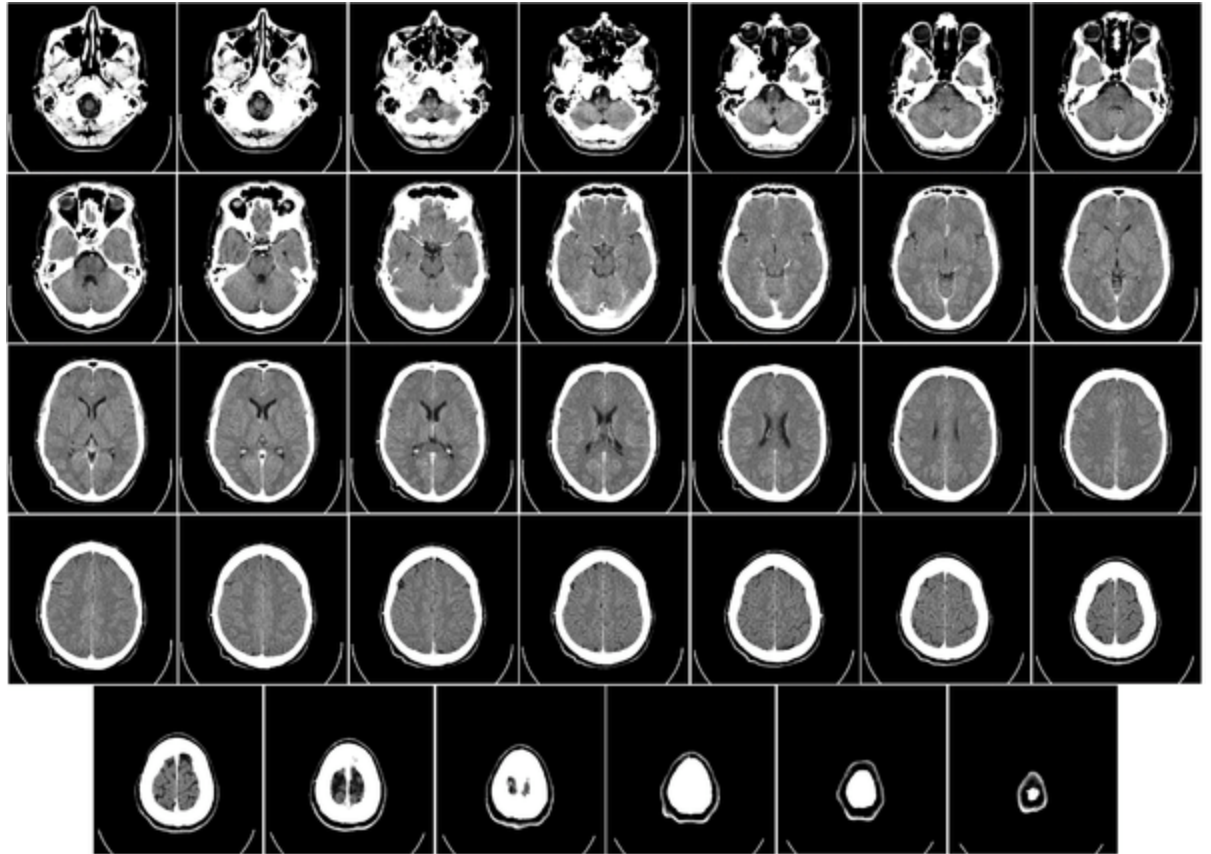

An X-ray picture (radiograph) taken by Röntgen of Albert von Kölliker's hand at a public lecture 23 Jan 1896

Source: Wikipedia

(X-ray) Computed Tomography gives beautiful images of structure, but…

X-ray contrast comes from larger scattering cross section of heavier nuclei.

Contrast agents: Ba, I, Gd, … But does this correlate with disease?

Source: Wikipedia

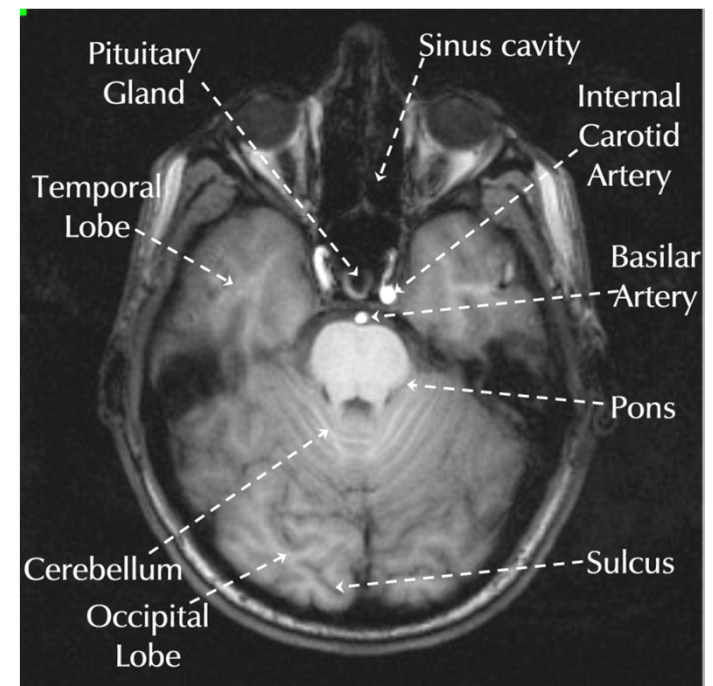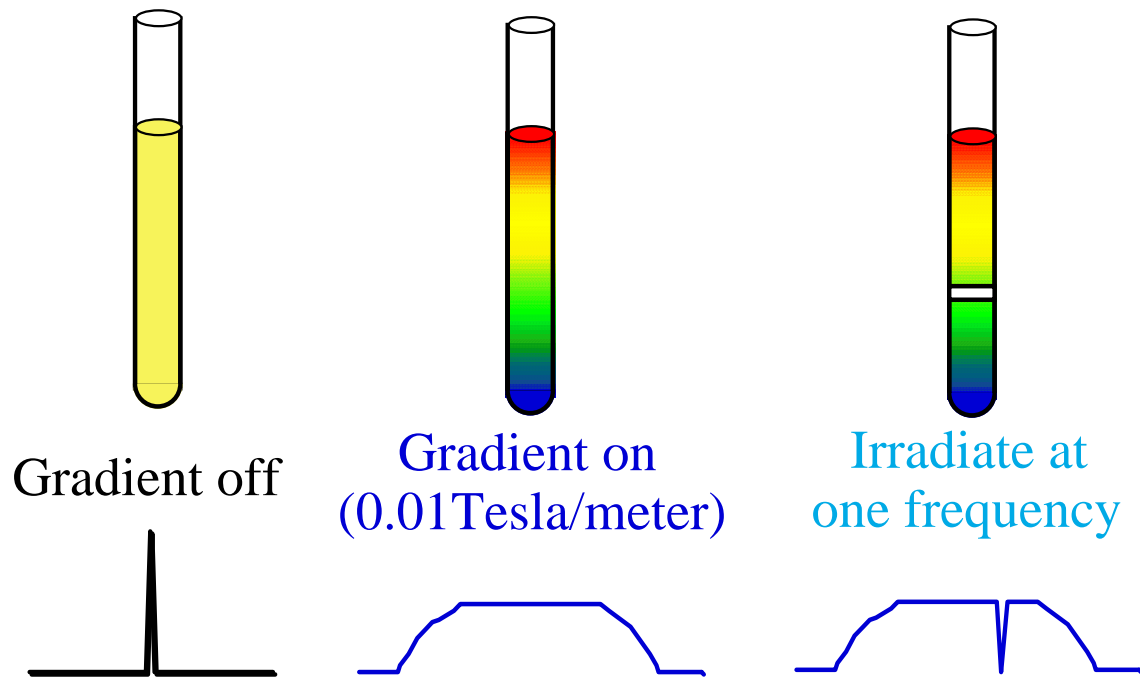# Nuclear magnetic resonance (NMR) is very useful for studying molecules

Basic physics (1920s): atoms have "magnetic dipoles", meaning they act like bar magnets (or spinning charged balls). Commonly called "spin angular momentum"

angular momentum $\vec{J}$

spinning charged sphere

N

S
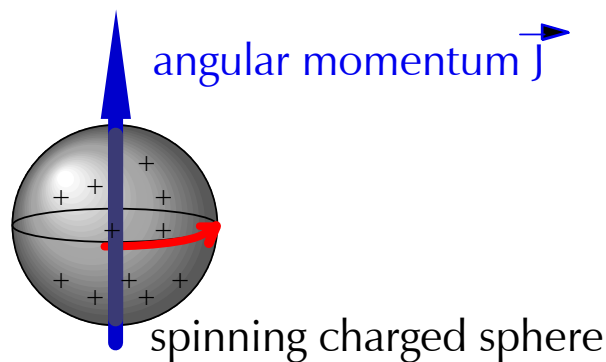
Basic physics (1940s): hydrogen atoms in a magnetic field have two stable states–"spin up" (pointed along the field, like a compass needle) or "spin down"

1960-present: chemistry, materials science, clinical diagnosis, oil well logging, watching thought . . .

Magnetic resonance imaging uses gradients to give high-resolution maps

Gradient off

Gradient on
(0.01Tesla/meter)

Irradiate at
one frequency

Pituitary Gland

Sinus cavity

Internal Carotid Artery

Temporal Lobe

Basilar Artery

Pons

Cerebellum

Sulcus

Occipital Lobe

Magnetic Resonance Imaging-the fundamental challenges

- Weak signal: $\approx 10^{-5}$ fractional magnetization

  Detecting metabolites hard (particularly unlabeled)

  Imaging anything but water difficult

- Water images structure more than function

  Density, relaxation rates, diffusion: physics of 1954

- Tissue-the ultimate complex material

  Susceptibility variations degrade spectral resolution

Breast image (axial)   (sagittal)   Prostate
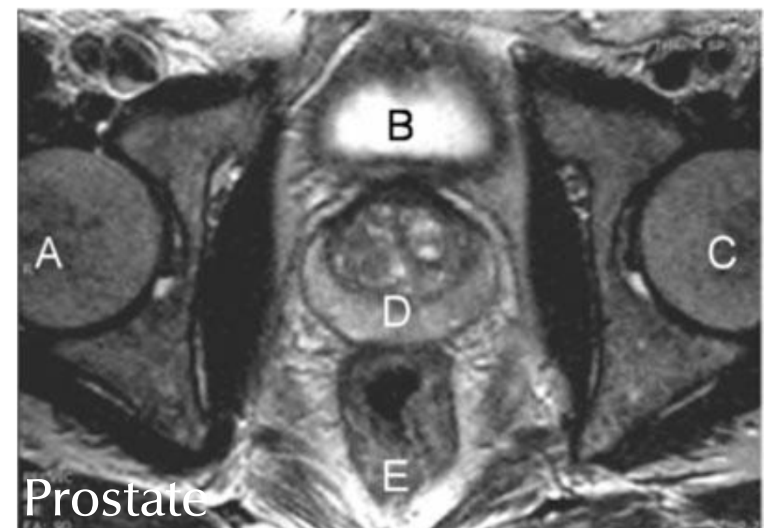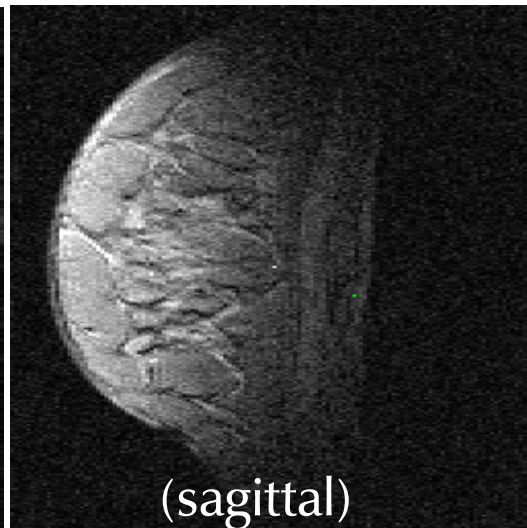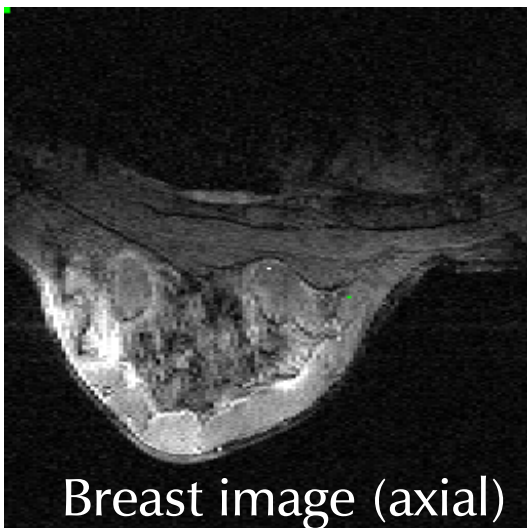
Reuters headline, 7/19/07: "Tiny brain no obstacle to French civil servant"
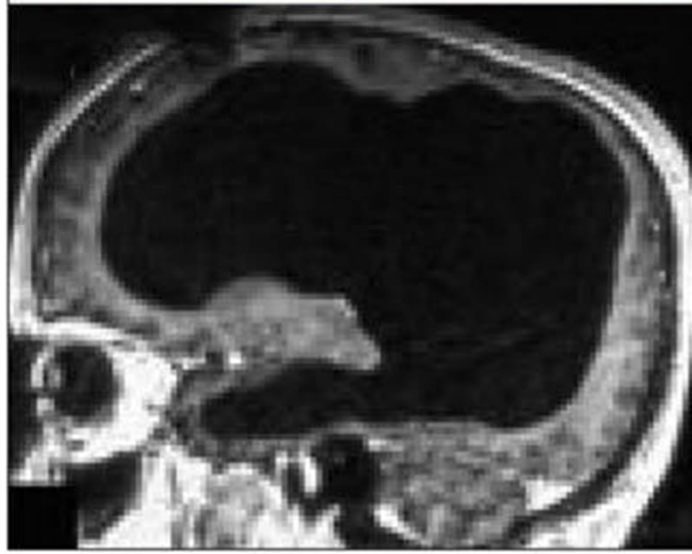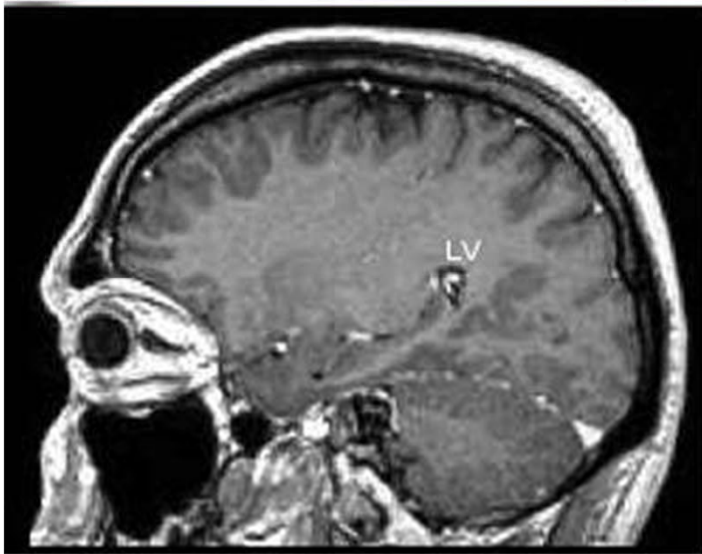
Left: Normal T1-weighted MR image (LV= lateral ventricle) Right: massive ventricular enlargement, in a patient with normal social function. From *L. Feuillet, H. Dufour, J. Pelletier, Lancet 370, 262 (2007).*

"A man with an unusually tiny brain managed to live an entirely normal life despite his condition, caused by a fluid buildup in his skull, French researchers reported … "He was a married father of two children, and worked as a civil servant," Dr. Lionel Feuillet wrote in a letter to the Lancet medical journal.

The man had a shunt inserted into his head to drain hydrocephalus -- water on the brain -- as an infant…the man had an IQ of 75, below average but not considered mentally retarded or disabled, either."
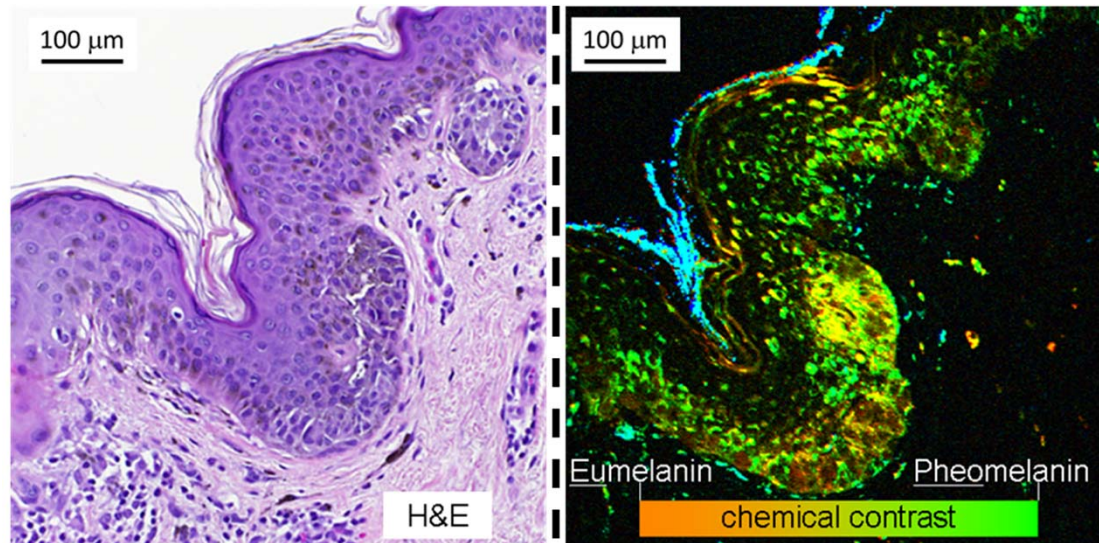
What is "Molecular Imaging?"

- Uses *molecular specific signatures* to image function instead of structure

    Positron emission tomography (e.g. ^{18}F, $t_{1/2}$=2 h); SPECT (γ emitters, e.g. ^{99m}Tc, $t_{1/2}$=6 h)

    Magnetic Resonance Imaging

    Optical Imaging (both surface and deep tissue)

- Molecular Medicine: the real opportunity

    Cost to bring a drug to market >$1B-almost all in clinical trials

- Society for Molecular Imaging meeting

    1st: Boston, 2002 (500 attendees)

- World Molecular Imaging Congress

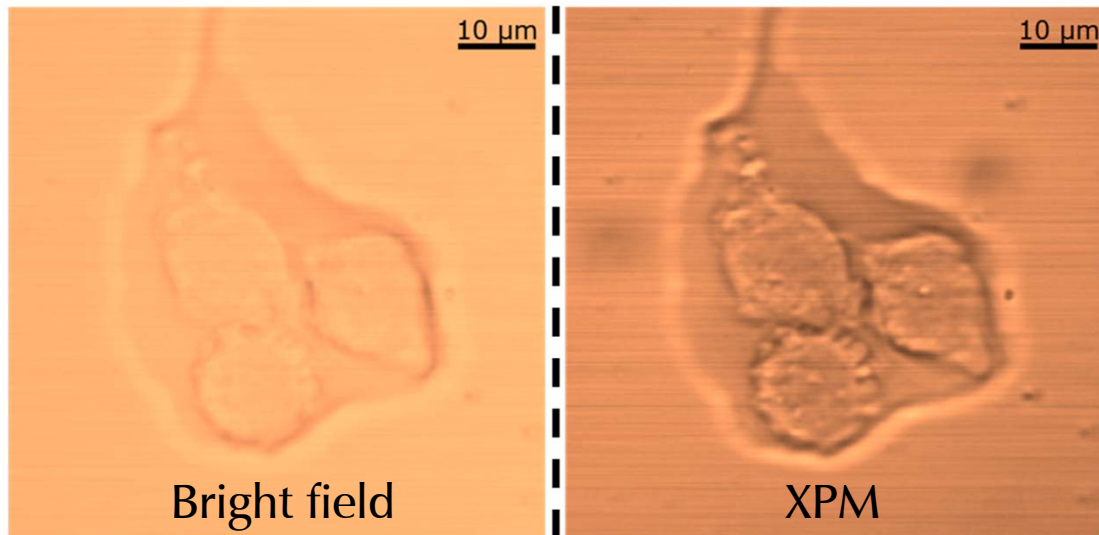   *13th: Seoul, 2014 (3000 attendees)*

Three approaches to practical molecular information in optical and MR imaging

1. Study what is already removed:

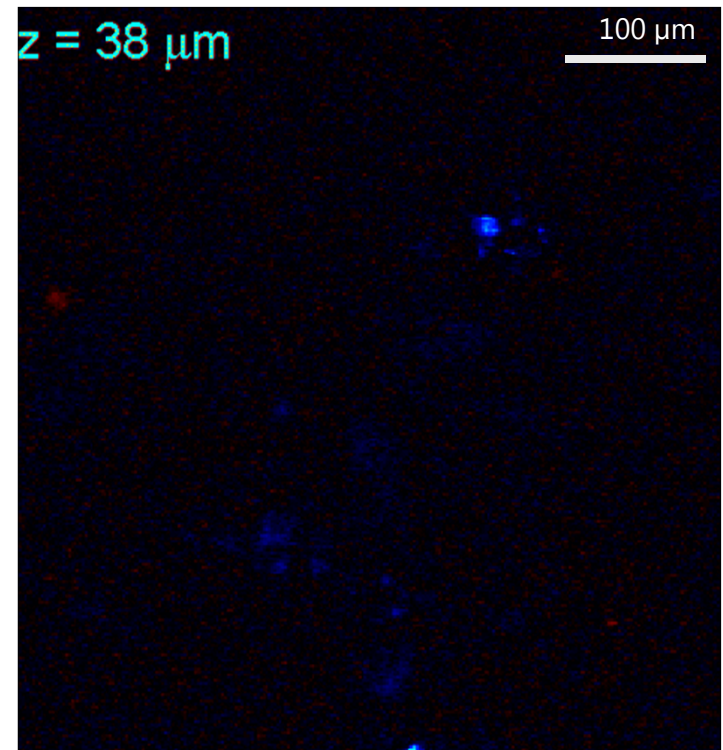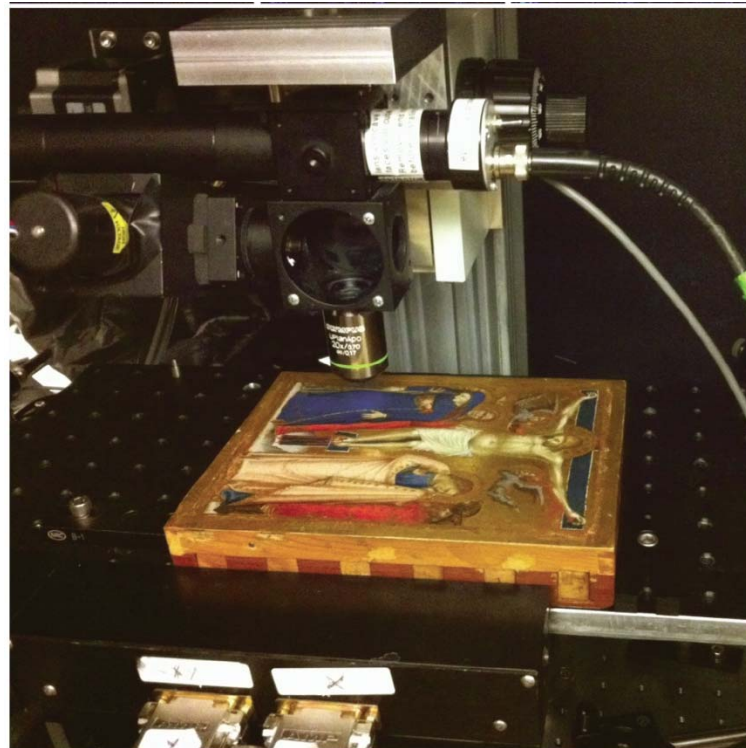Thin sample imaging: new molecular contrast in melanoma histology

Cross-phase modulation imaging through fs pulse shaping: nonlinear phase contrast

Three approaches to practical molecular information in optical and MR imaging

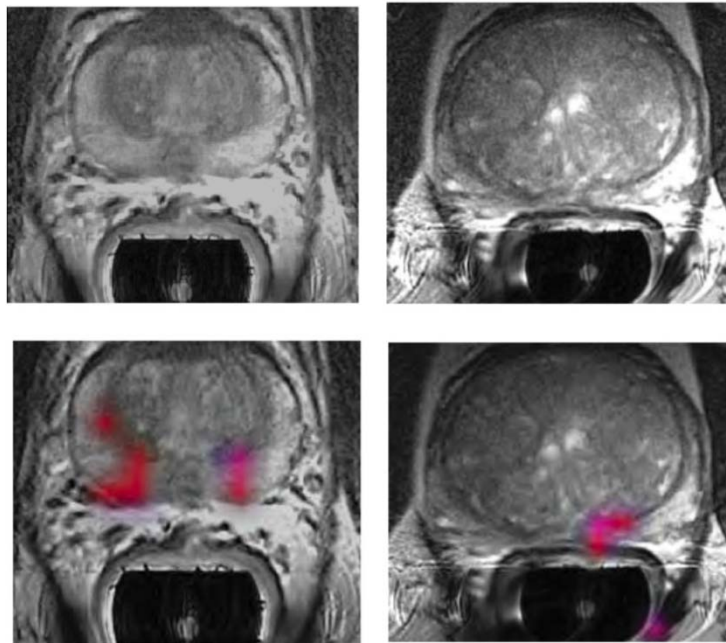– 2. Make better use of *intrinsic contrast*

Nonlinear optics: fs pulse shaping/ pulse train modulation
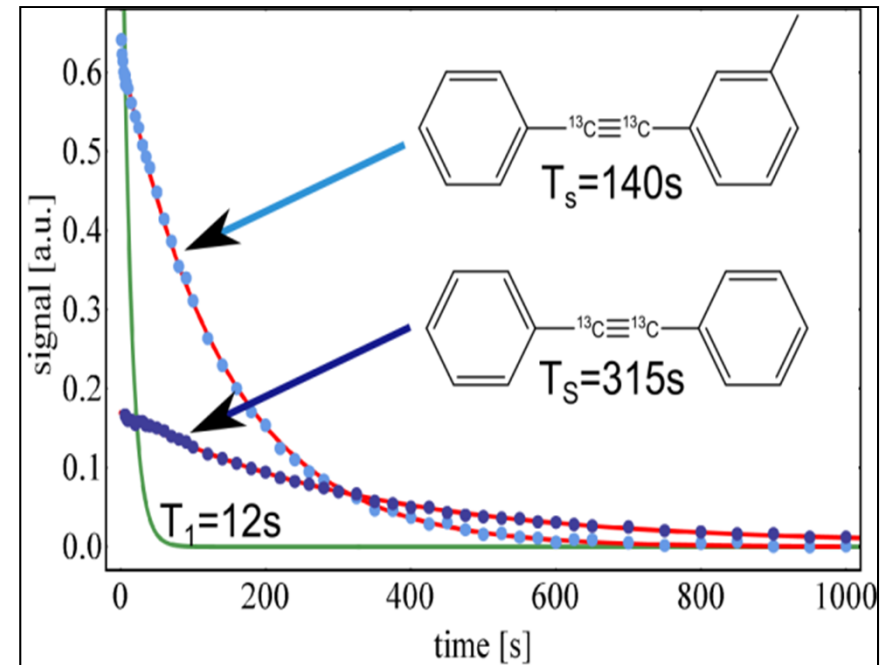
Three approaches to practical molecular information in optical and MR imaging

– 3. Add back what was already there

Hyperpolarized MR imaging with long-lived states

Gray: human prostate MR images Color: high lactate/ pyruvate ratio after injection of HP ^{13}C pyruvate (UCSF /GE).

Our work: families of MR agents or tags with many-minute lifetimes and biocompatibility

NMR and MRI-the fundamental challenges

– Weak signal: ≈10^{-5} fractional magnetization

Detecting metabolites hard (particularly unlabeled)

Imaging anything but water difficult

– Water images structure more than function

Density, T_1, T_2, T_2^*, diffusion: physics of 1954

– Tissue-the ultimate complex material

Susceptibility variations degrade spectral resolution

– Want to do better? Use different spin physics

Hyperpolarization

– Dynamic Nuclear Polarization

-Transfer polarization from electrons to protons at low temperature

-Very general method

Pharmaceutically relevant molecules

Acetaminophen

Conventional NMR

Sensitivity enhancement ≈ 120
S/N enhancement ≈ 7500
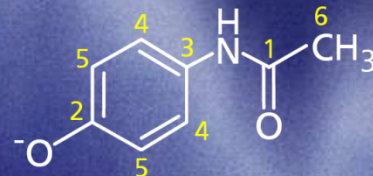
DNP-NMR

Conventional NMR
- 53mM solution
- 3600 scans at 56°C (5 hours)

DNP-NMR
- hyperpolarized at 1.4K for 6 hours
- dissolved in 4ml water ⇒ 1.3mM

Technology ● Solutions ● Knowledge

Hyperpolarization is one of the most exciting new frontiers in MRI

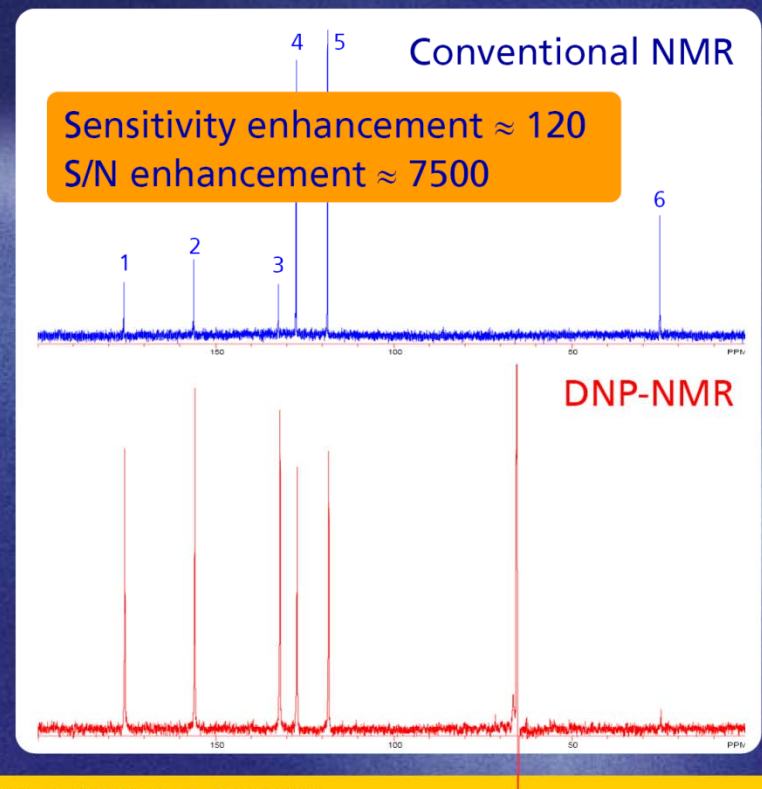**Analysis of Cancer Metabolism by Imaging Hyperpolarized Nuclei: Prospects for Translation to Clinical Research**

John Kurhanewicz[*,1], Daniel B. Vigneron[*,1], Kevin Brindle[†], Eduard Y. Chekmenev[‡], Arnaud Comment[§], Charles H. Cunningham[¶], Ralph J. DeBerardinis[#], Gary G. Green[**], Martin O. Leach[††], Sunder S. Rajan[‡‡], Rahim R. Rizi[§§], Brian D. Ross[¶¶], Warren S. Warren[##] and Craig R. Malloy[***,1]
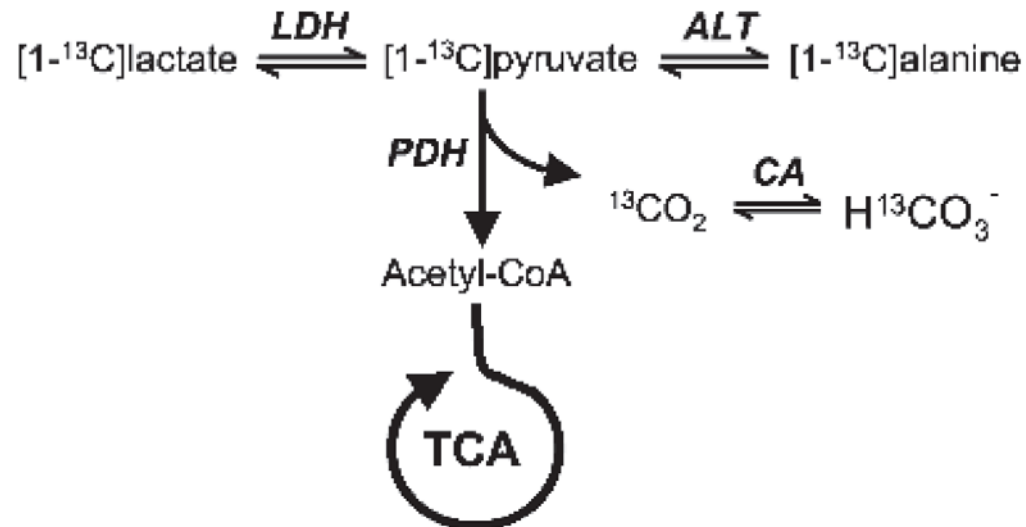
Phase I/II clinical trial going on now with GE/UCSF

New GE clinical polarizer: 10x output, new dose every hour
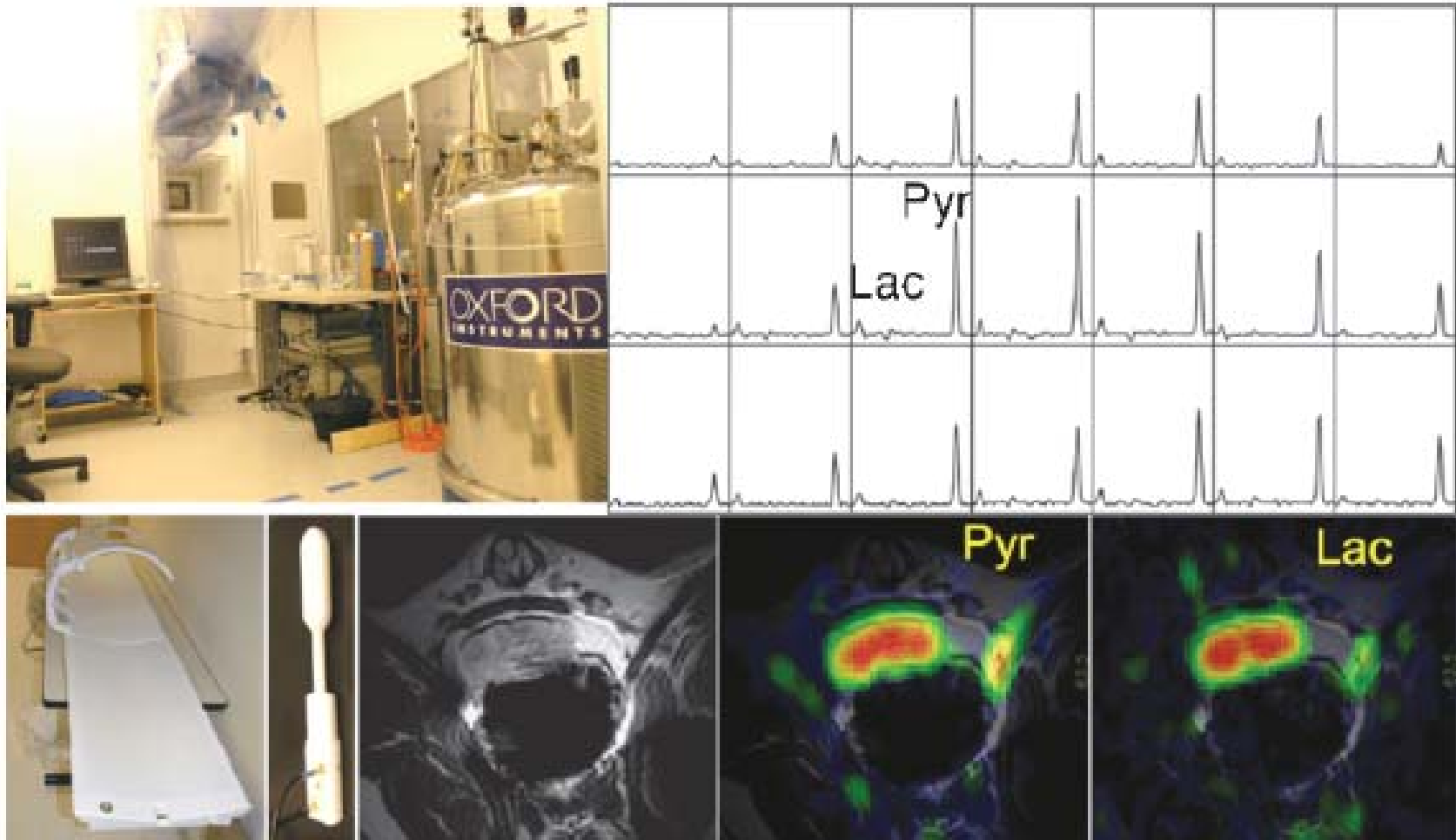
Clinicial applications of hyperpolarization: the potential and challenges

- Delivery in vivo: excellent progress

  GMP system demonstrated

  Several clinically relevant molecules detected in vivo

- Creating hyperpolarization: expensive

  Many different molecules hyperpolarized

  DNP complex and slow (cooling to 1K needed; hour to polarize; >$20M for a clinical system)

  (Talk this afternoon, S12 (16:50) solves this problem)

- General application: limited by spin physics

  Carbon signal lifetime ca. 1 min in favorable cases

  Creates great demands on delivery systems
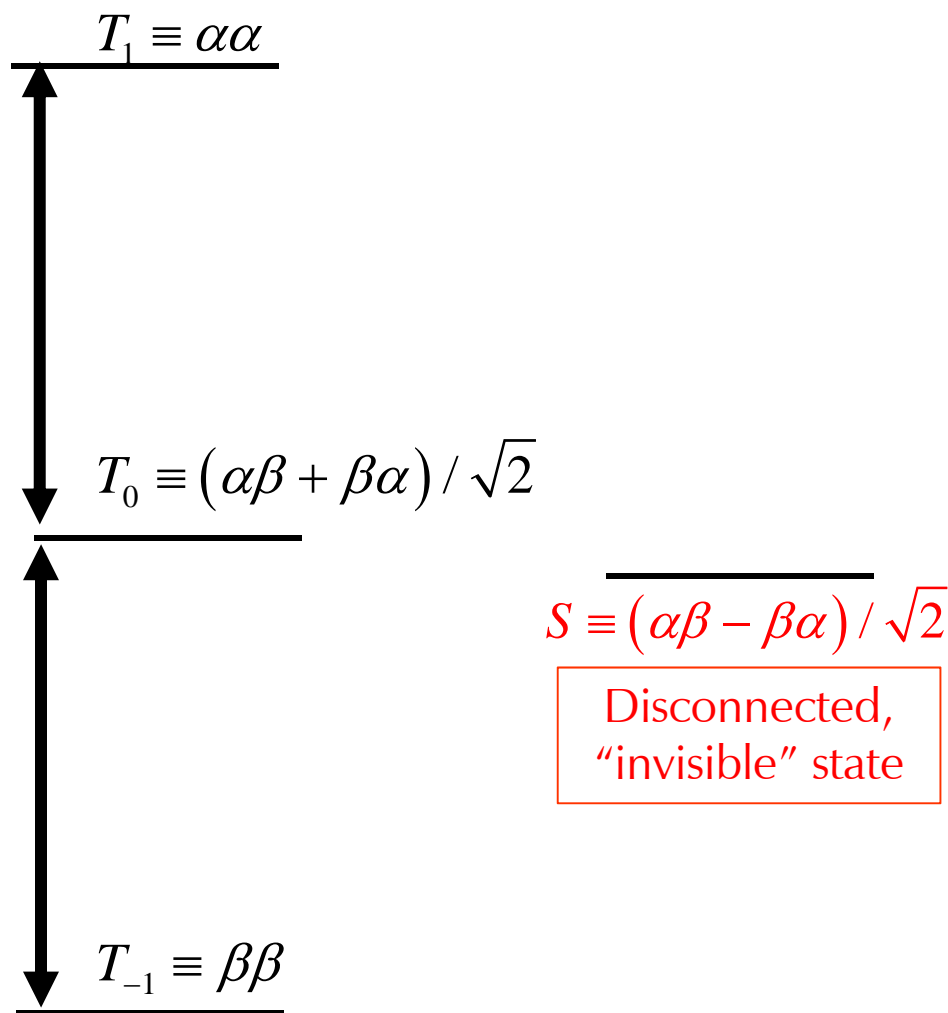
  Limits accessible biochemical pathways

This talk: how do you preserve large population?

Pulse sequences in NMR are very powerful, but...

- Removing inhomogeneous broadening (T_2^*)

  Spin echo (1950, Hahn); multiple echoes (1955-1956, Carr, Purcell, Meiboom, Gill)

- Altering effective Hamiltonian ("static" T_2")

  Dipolar refocusing (1967, Waugh), time reversal (1971, Pines), two-quantum Hamiltonian (1979, W^2)

- *Dozens* of QC/QIP papers (slowly varying T_2)

  Example: dynamic decoupling (Uhrig 2007) removes effect of low-frequency fluctuations in MRI (2009, W^2)

- None of this work preserves populations (T_1)

Two magnetically equivalent spins give a disconnected, long-lived singlet eigenstate

$T_1 \equiv \alpha\alpha$

$T_0 \equiv (\alpha\beta + \beta\alpha)/\sqrt{2}$

$S \equiv (\alpha\beta - \beta\alpha)/\sqrt{2}$

Disconnected, "invisible" state

$T_{-1} \equiv \beta\beta$

Identical spins ($CH_2=CCl_2$)

$\alpha\alpha$

$\alpha\beta$

$\beta\alpha$

$\beta\beta$
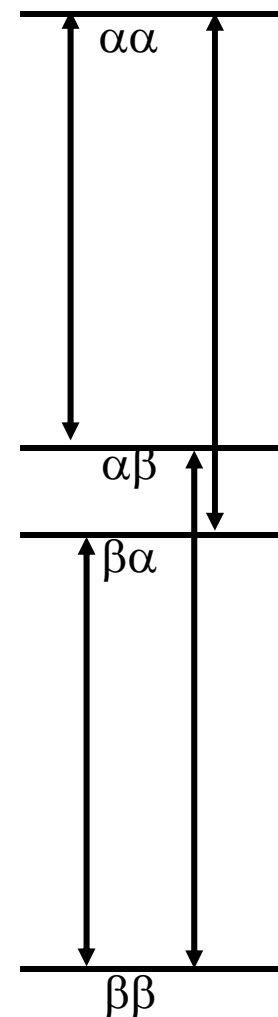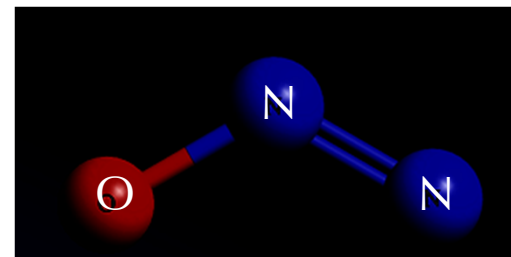
Two different spins ($CHCl=CHBr$)

Hyperpolarization and singlet states offer a potential route to long-lived tracers

Singlet state between *inequivalent spins* offered the first approach to long-lived signal (Levitt et. al. *PRL* **2004,** *92,* 153003; *JACS* **2004,** *126* , 6228).

Two challenges:

*1. Inequivalent spins require spin locking or zero field*

> *Sequence in original spin locking paper: 300 MHz head coil, power dissipation (SAR) is 6000 times legal limit.*

2. Very sensitive to paramagnetic relaxation

Singlet between *chemically equivalent* spins avoids decoupling; self-sustained at any field strength (Warren et. al. *Science* **2009**)
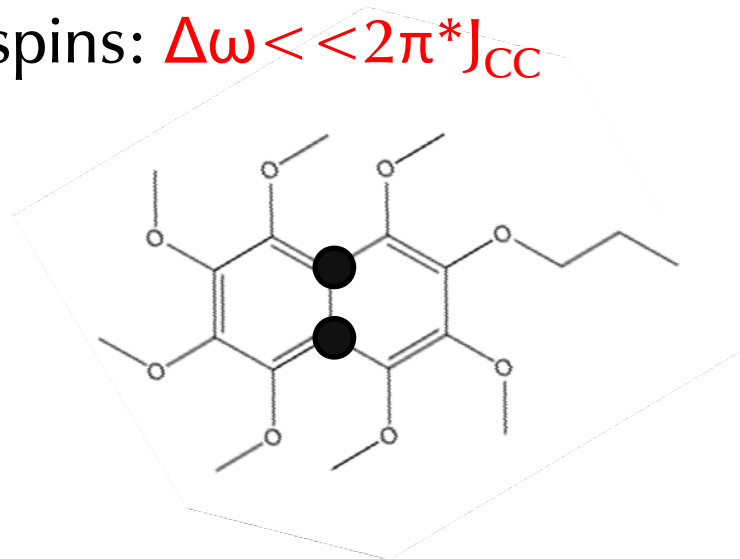
Challenge: required chemistry or field cycling to unlock
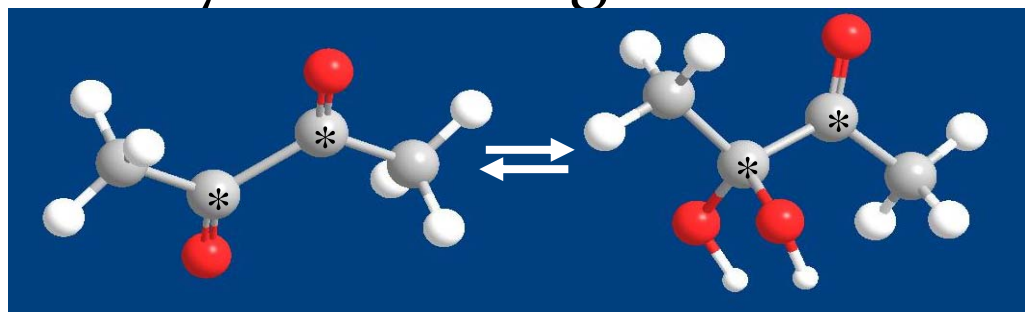
The field has converged: symmetry (or near-symmetry), low-γ, low field best

– Levitt: single pair of near equivalent spins: $\Delta\omega <<< 2\pi * J_{CC}$

Magnetization to Singlet to Magnetization (MSM)
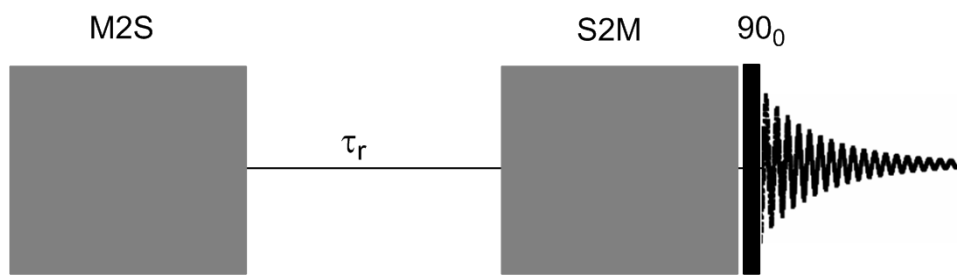
Pileio, Levitt et al, *PNAS* **2011,** *107* (40), 17135-17139

– Warren: *multiple pairs* of *chemically equivalent* spins: $\Delta\omega = 0$ while $\Delta J_{CH} \neq 0$.

$J_{CH} = 6.5 Hz$

$J'_{CH} = 52 Hz$

$J_{cc} = 152 Hz$

Feng, Davis, Warren, *Nature Physics* **2012,** *8*, 831-837

Can we access the singlet state, without breaking chemical equivalence?
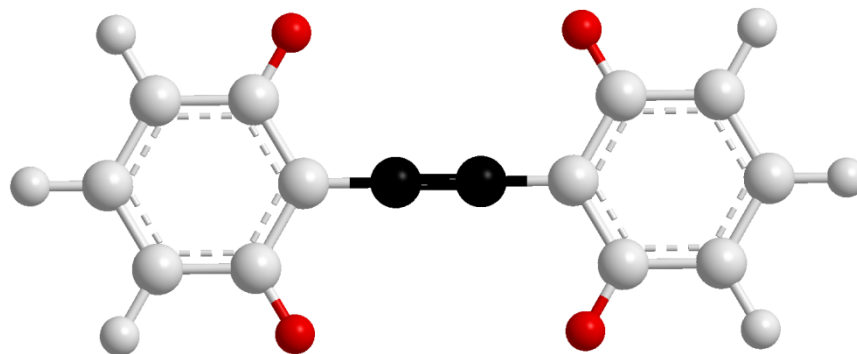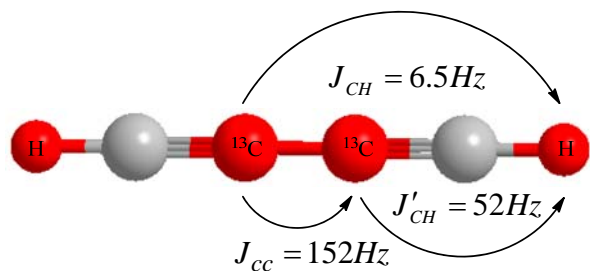
– Chemical equivalence gives a lot of advantages

Active decoupling not needed-reduced power dissipation (clinically acceptable)

Relaxation mechanisms have to break symmetry. If environment is the same, they are reduced

– Doesn't this violate the laws of physics?

No, if the spins are chemically equivalent, but coupled differently to others

There is nothing new about A_2B_2 spin systems-but they are interesting

THE ANALYSIS OF NUCLEAR MAGNETIC RESONANCE SPECTRA

II. TWO PAIRS OF TWO EQUIVALENT NUCLEI[1]

J. A. POPLE,[2] W. G. SCHNEIDER, AND H. J. BERNSTEIN

ABSTRACT

This paper deals with the analysis of the nuclear magnetic resonance spectrum of two pairs of two equivalent nuclei (of spin $\frac{1}{2}$) whose relative chemical shift is of the same order as the spin-coupling constants of the system (A_2B_2 in the notation of Part I). The complete matrix is set up and correlated with the results of McConnell, McLean, and Reilly (4) for the corresponding theory with large chemical shifts.

The proton resonance spectrum of naphthalene is reported and is analyzed as an A_2B_2 system on the hypothesis that spin couplings between protons on different rings are negligible. A complete analysis of the spectrum of o-dichlorobenzene, which represents a further example of an A_2B_2 system, is also given. The spectrum of 1-chloro-2-bromoethane at room temperature is also analyzed as an A_2B_2 system.

Example: diacetylene. Today we call this AA′BB′ or AA′XX′ to accommodate multiple couplings
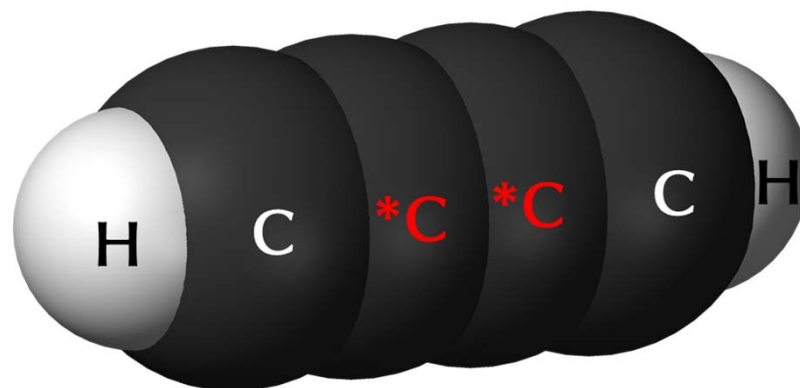
What are the basis states for AA′BB′?
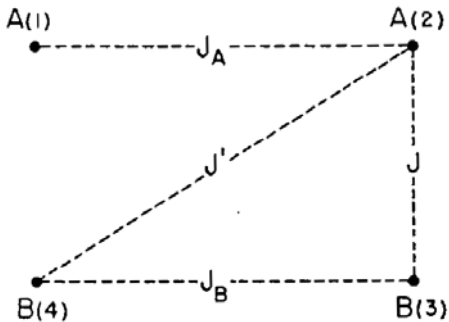
$$K = J_A + J_B, \qquad L = J - J',$$
$$M = J_A - J_B, \qquad N = J + J'.$$

| Function*	A	B	Diagonal matrix elements†	Off-diagonal matrix elements
s_2	$\alpha\alpha$	$\alpha\alpha$	$\eta H_0(2-\sigma_A-\sigma_B)+\frac{1}{2}N$	
$1s_1$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	$\alpha\alpha$	$\eta H_0(1-\sigma_B)$	$(1s_1|\,\mathscr{H}\,|2s_1) = \frac{1}{2}N$
$2s_1$	$\alpha\alpha$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	$\eta H_0(1-\sigma_A)$	
$1s_0$	$\beta\beta$	$\alpha\alpha$	$\eta H_0(\sigma_A-\sigma_B)-\frac{1}{2}N$	$(1s_0|\,\mathscr{H}\,|2s_0) = 0, \quad (2s_0|\,\mathscr{H}\,|3s_0) = \frac{1}{2}L$
$2s_0$	$\alpha\alpha$	$\beta\beta$	$\eta H_0(-\sigma_A+\sigma_B)-\frac{1}{2}N$	$(1s_0|\,\mathscr{H}\,|3s_0) = \frac{1}{2}L, \quad (2s_0|\,\mathscr{H}\,|4s_0) = \frac{1}{2}N$
$3s_0$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$-K$	$(1s_0|\,\mathscr{H}\,|4s_0) = \frac{1}{2}N, \quad (3s_0|\,\mathscr{H}\,|4s_0) = -\frac{1}{2}L$
$4s_0$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	0	
$1s_{-1}$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	$\beta\beta$	$\eta H_0(-1+\sigma_B)$	$(1s_{-1}|\,\mathscr{H}\,|2s_{-1}) = \frac{1}{2}N$
$2s_{-1}$	$\beta\beta$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	$\eta H_0(-1+\sigma_A)$	
s_{-2}	$\beta\beta$	$\beta\beta$	$\eta H_0(-2+\sigma_A+\sigma_B)+\frac{1}{2}N$	
$1a_1$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$\alpha\alpha$	$\eta H_0(1-\sigma_B)-\frac{1}{2}(K+M)$	$(1a_1|\,\mathscr{H}\,|2a_1) = -\frac{1}{2}L$
$2a_1$	$\alpha\alpha$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$\eta H_0(1-\sigma_A)-\frac{1}{2}(K-M)$	
$1a_0$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$-\frac{1}{2}(K-M)$	$(1a_0|\,\mathscr{H}\,|2a_0) = -\frac{1}{2}L$
$2a_0$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$2^{-\frac{1}{2}}(\alpha\beta+\beta\alpha)$	$-\frac{1}{2}(K+M)$	
$1a_{-1}$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$\beta\beta$	$\eta H_0(-1+\sigma_B)-\frac{1}{2}(K+M)$	$(1a_{-1}|\,\mathscr{H}\,|2a_{-1}) = -\frac{1}{2}L$
$2a_{-1}$	$\beta\beta$	$2^{-\frac{1}{2}}(\alpha\beta-\beta\alpha)$	$\eta H_0(-1+\sigma_A)-\frac{1}{2}(K-M)$	

Pairs of equivalent spins and couplings create a versatile class of long-lived reagents

- Two carbons and two hydrogens have highly protected singlet-singlet state $(\alpha\beta-\beta\alpha)_C(\alpha\beta-\beta\alpha)_H$
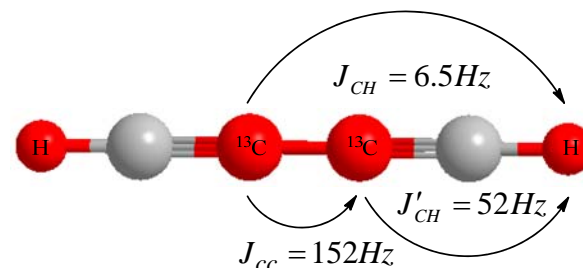
- Triplet-triplet state $(\alpha\beta+\beta\alpha)_C(\alpha\beta+\beta\alpha)_H$, accessible from thermal magnetization or hyperpolarization, has the same symmetry.

- If $J_{CC}+J_{HH}>>J_{CH}-J'_{CH}$ SS and TT stable:

$$H/2\pi\hbar = \begin{array}{c} TT \\ SS \end{array}\left(\begin{array}{cc} \frac{1}{2}(J_{CC}+J_{HH}) & -\frac{1}{2}(J_{CH}-J'_{CH}) \\ -\frac{1}{2}(J_{CH}-J'_{CH}) & -\frac{1}{2}(J_{CC}+J_{HH}) \end{array}\right)$$

$$\begin{array}{cc} TT & SS \end{array}$$

- To invert spins with a weak pulse, *go on resonance*. Here that means give many 180s to only C or only H at the right rate:

$$\tau = 1/(2\sqrt{(J_{CC}+J_{HH})^2+(J_{CH}-J'_{CH})^2})$$
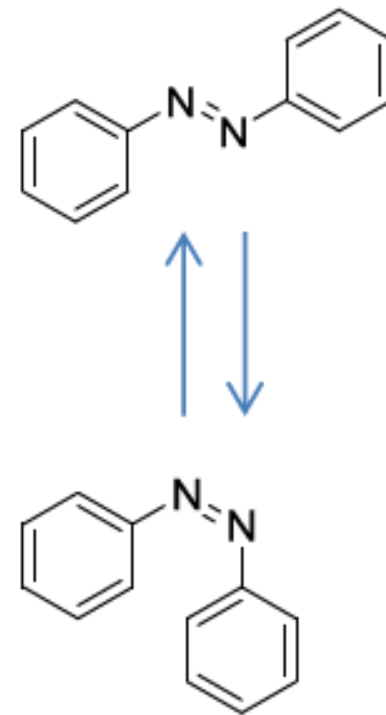
$$n = \pi/(2\arctan((J_{CH}-J'_{CH})/(J_{CC}+J_{HH})))$$

Inversion symmetry seems to be valuable experimentally

– Azobenzene: trans→cis with light

$T_1(nitrogen)=8$ sec (8.4 T)

Trans T_s: 60 ± 10 s; cis T_s: $<T_1$
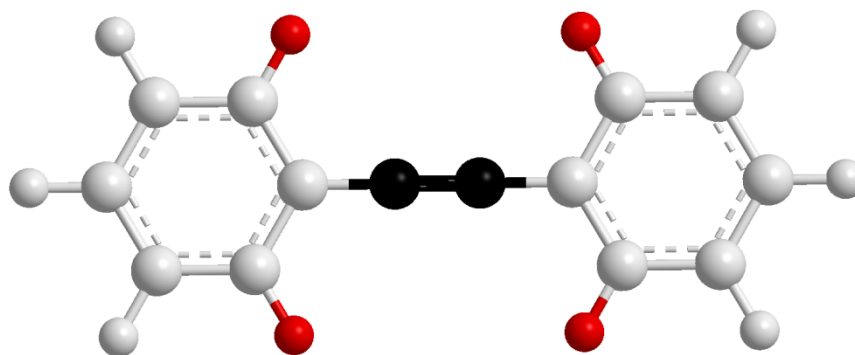
– Diphenylacetylene (8.4T):
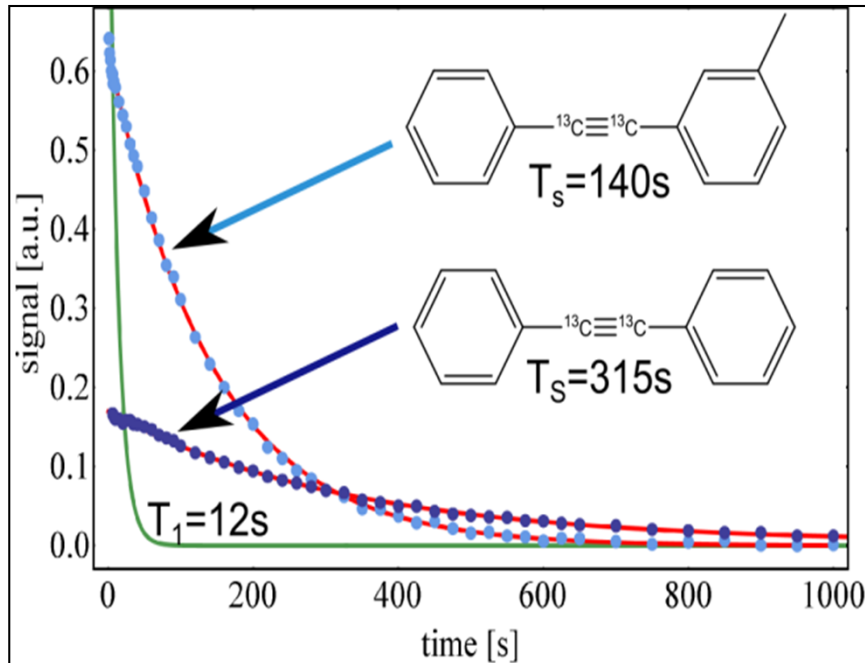
$T_s>300$ s; insensitive to oxygen

Long-lived "markers" as biochemical probes

Singlet polarization lifetime measurements at 8T of $^{13}C_2$-MDPA (light-blue) and $^{13}C_2$-DPA (dark-blue). Longitudinal magnetization relaxes with $T_1 = 12$ s for both compounds, as indicated by the green line.

Current Work

– Attaching diphenyl-$^{13}C_2$-acetylene e.g. onto suberoylanilide hydroximic acid (SAHA), a histone deacetylase inhibitor and anti-cancer drug.

– Classes of drugs that inherently contain diphenyl-$^{13}C_2$-acetylene segments. For example, antibiotic LpxC inhibitors based on a diphenyl-diacetylene (1,4-diphenyl-1,3-butadiyne)-threonylhydroxamate scaffold.
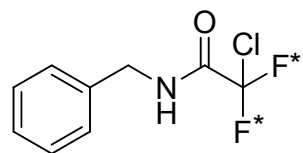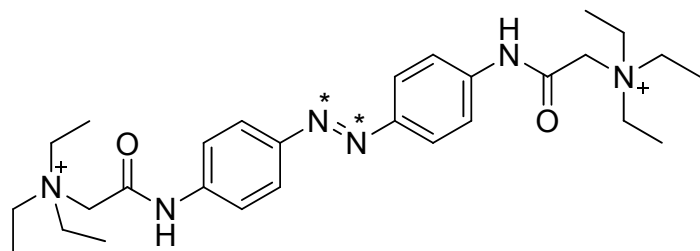
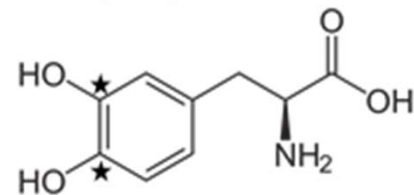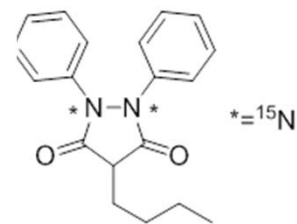# Generality: biologically relevant molecules and molecular tags
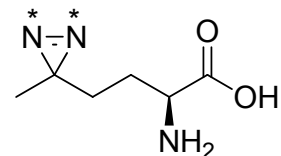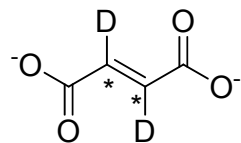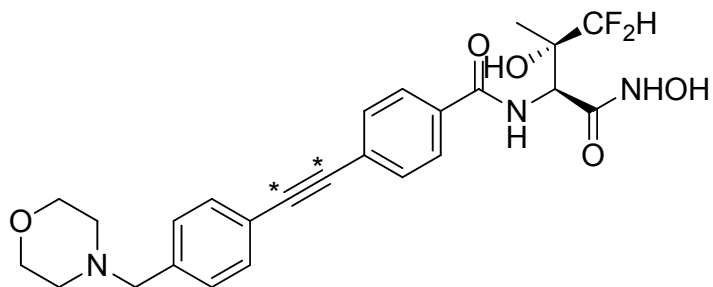
C. J. Lee *et al.*, Chem Biol **18** (2011).
A. Mourot *et al.*, Nature Methods **9** (2012).
E. Christiansen *et al.*, J Med Chemistry **56** (2013).

– Phenbutazone (animal NSAID)
– L-Dopa
– Napthalenes, naphthoquinones, pyridazines
– Maybe separated ^{14}N (pyrazines, adenine?)

AA′X$_2$X$_2$′: many different two-level systems

Γ	$\sum m_H$	2-level systems	
A_g	+1	$\frac{1}{\sqrt{2}}\left(T_{+1}T_0 + T_0T_{+1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{+1}T_0 - T_0T_{+1}\right)S$
	0	$\frac{1}{\sqrt{2}}\left(T_{-1}T_{+1} + T_{+1}T_{-1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{-1}T_{+1} - T_{+1}T_{-1}\right)S$
	-1	$\frac{1}{\sqrt{2}}\left(T_{-1}T_0 + T_0T_{-1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{-1}T_0 - T_0T_{-1}\right)S$
B_{1u}	+1	$\frac{1}{\sqrt{2}}\left(T_{+1}T_0 - T_0T_{+1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{+1}T_0 + T_0T_{+1}\right)S$
	0	$\frac{1}{\sqrt{2}}\left(T_{-1}T_{+1} - T_{+1}T_{-1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{-1}T_{+1} + T_{+1}T_{-1}\right)S$
	-1	$\frac{1}{\sqrt{2}}\left(T_{-1}T_0 - T_0T_{-1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{-1}T_0 + T_0T_{-1}\right)S$
B_{2u}	+1	$\frac{1}{\sqrt{2}}\left(T_{+1}S + ST_{+1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{+1}S - ST_{+1}\right)S$
	-1	$\frac{1}{\sqrt{2}}\left(T_{-1}S + ST_{-1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{-1}S - ST_{-1}\right)S$
B_{3g}	+1	$\frac{1}{\sqrt{2}}\left(T_{+1}S - ST_{+1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{+1}S + ST_{+1}\right)S$
	-1	$\frac{1}{\sqrt{2}}\left(T_{-1}S - ST_{-1}\right)T_0$	$\frac{1}{\sqrt{2}}\left(T_{-1}S + ST_{-1}\right)S$

Eight have the same resonance condition; the two "obvious" states (in red) are different.
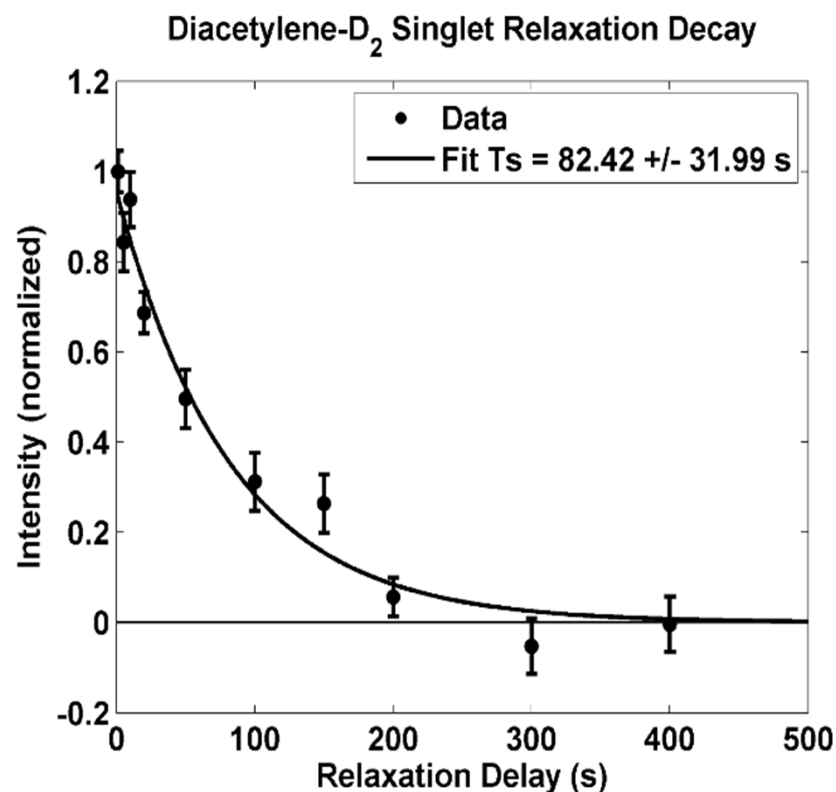
– AA'QQ'' where X is spin 1 (D, ^{14}N)

J_n = 54 Hz J_f = -6.3 Hz

^{1}H ^{13}C ^{13}C ^{1}H

J_{CC} = 154 Hz

$T_S = 49 \pm 23s$

J_n = 8.2 Hz J_f = -0.9 Hz

^{2}H ^{13}C ^{13}C ^{2}H

J_{CC} = 154 Hz

$T_S = 83 \pm 30$ s
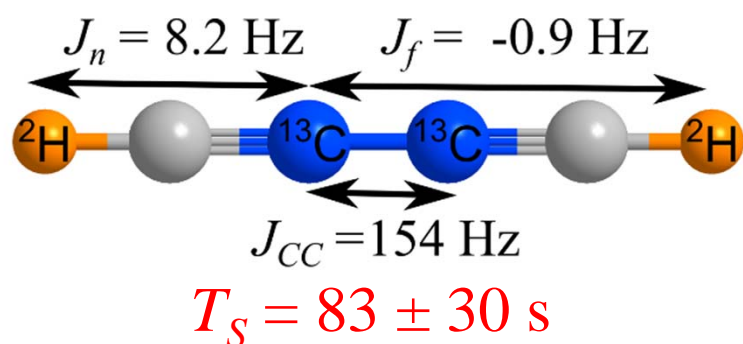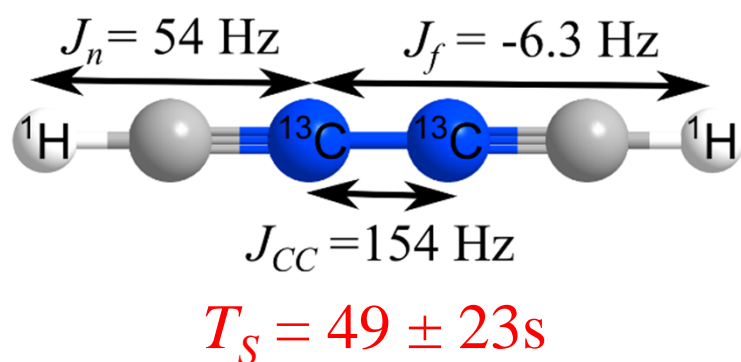
^{13}C $T_1 = 16.7 \pm 0.9$ s, ^{2}H $T_1 = 0.5 \pm 0.2$ s

Deuterated DPA: 500 s at 9.4T (protonated 310 s). All are much longer at 1T.

Diacetylene-D$_2$ Singlet Relaxation Decay

- Data
- Fit Ts = 82.42 +/- 31.99 s

Intensity (normalized) vs Relaxation Delay (s)

Opinion: HP MRI will have broadest impact at low fields with multimodality imaging

– Conventional NMR: $SNR \propto \gamma_{prep} \gamma_{det}^{7/4} B^{7/4}$

  Boltzmann equilibrium $\propto \gamma_{prep} B$; Magnetization $\propto \gamma_{det}$; Induced voltage $\propto \omega = \gamma_{det} B$; coil noise $\propto \omega^{1/4}$

– Conventional MRI: $SNR \propto \gamma_{prep} \gamma_{det} B^1$

  Coil noise $\propto \omega$, dominated by body

– Hyperpolarized MRI: $SNR \propto \gamma_{det}$

  Boltzmann factor eliminated!

– Relaxation times usually much longer at low field; power dissipation lower ($SAR \propto B^2$)

  Compromise (0.5-1.5T) to get a good anatomic and HP image, or marry with CT for even lower field

Relevant papers and Extraordinary People

- *Long lived states:* Science <u>323</u>, 5922 (2009); Nature Physics <u>8</u>, 831(2012); JACS <u>135</u>, 9632 (2013); J Chem Phys. 140(1), 014201 (2014); J Magn Reson <u>239</u>, 81(2014); JACS 2014 (doi: 10.1021/ja505792j)

- *Dynamic Decoupling:* J Chem Phys 131, 204510(2009); MRM <u>69</u>, 1044 (2013)

- *Art/cancer imaging:* PNAS <u>111</u>, 1708 (2014); Sci Transl Med <u>3</u>, 71ra15 (2011)

Other links: www.cmbi.duke.edu