BCMB / CHEM 8190 Biomolecular NMR
GRADUATE COURSE OFFERING IN NUCLEAR MAGNETIC RESONANCE

"Biomolecular Nuclear Magnetic Resonance" is a course intended for all graduate students with an interest in applications of nuclear magnetic resonance (NMR) to problems in structural and functional biology. It will begin with a treatment of the fundamentals that underlie magnetic resonance phenomena and develop this into a basis for experimental design, interpretation of data, and critical reading of the literature.

http://tesla.ccrc.uga.edu/courses/bionmr/

Syllabus

I. Introduction

M 1/9	A. Magnetic properties of nuclei and electrons - precession	5-38 L*
W 1/11	B. RF pulses and spin relaxation - Bloch equations	39-50 L*, 653 L*

II. Instrumentation

M 1/16	MLK Jr. Holiday (no class)	
W 1/18	A. Instrumental considerations - a look at probes	65-76L*
M 1/23	B. Fourier transform methods and data Processing	85-102 L*, 78-101 K*

- Friday Labs are scheduled separately (C122 Davison Life Sciences) – intro to computer systems 1/13, 1/20
- Texts:
  - -"Spin Dynamics - Basics of Nuclear Magnetic Resonance" (2nd edition), M. H. Levitt (L)*
  - -"Protein NMR Spectroscopy, Principles & Practice" (2nd edition), J. Cavanagh, W. J. Fairbrother, A. G. Palmer III, N. J. Skelton. (C)*
  - -"Understanding NMR Spectroscopy" (2nd edition), J. Keeler (K)*

Biomolecular NMR 2014

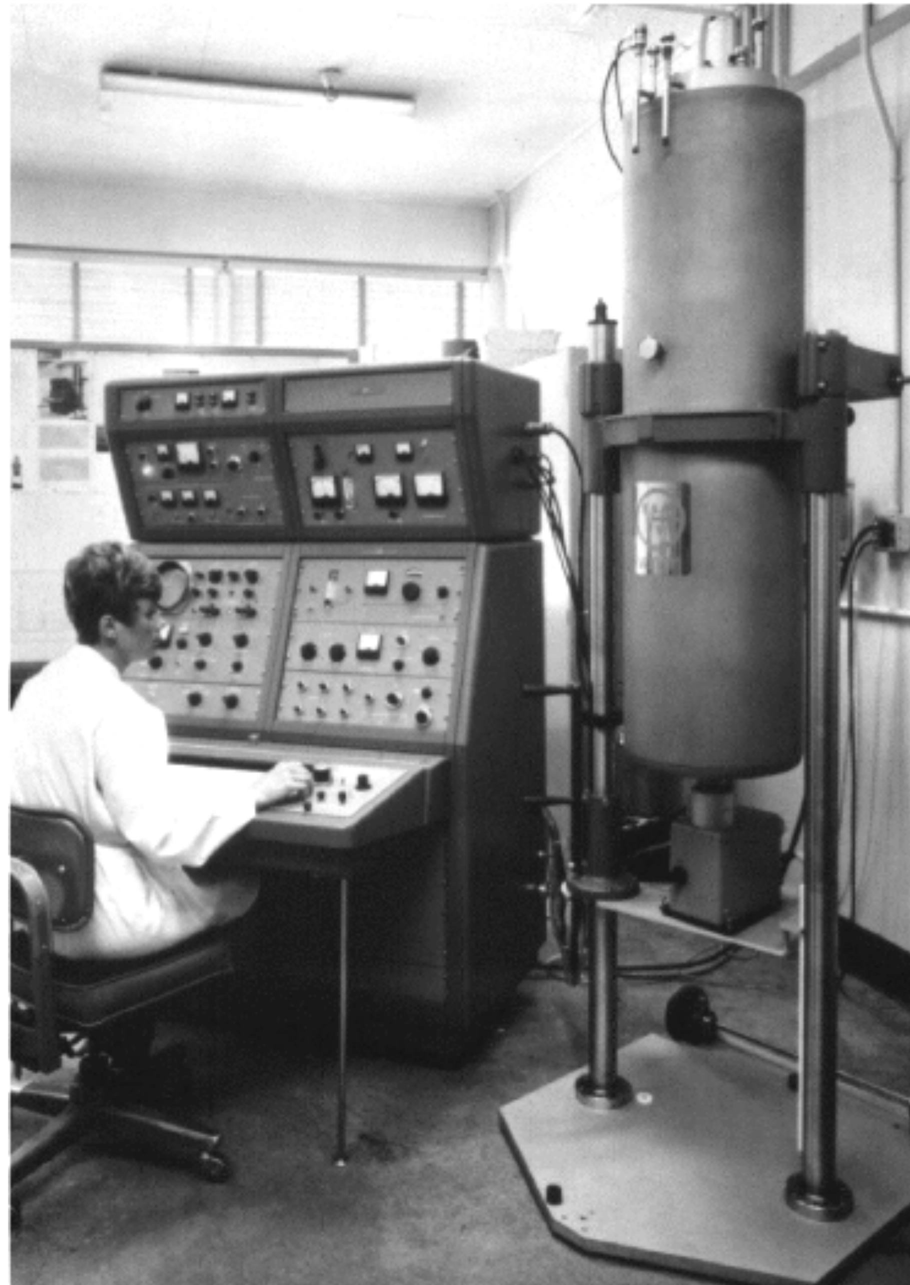
- Biomolecular NMR - short history ~ 1985 first protein structure
- Compared to X-ray ~ 1953 first protein structure
- Today ~ 11 % of structures in the PDB (10,287) come via NMR – higher for nucleic acids
- Unique structural applications – weak associations, partially structured, membrane associations, in-cell observation
- Diverse applications: drug screening, metabolic monitoring, in vivo imaging
- NMR is still an evolving science

NMR Recognition

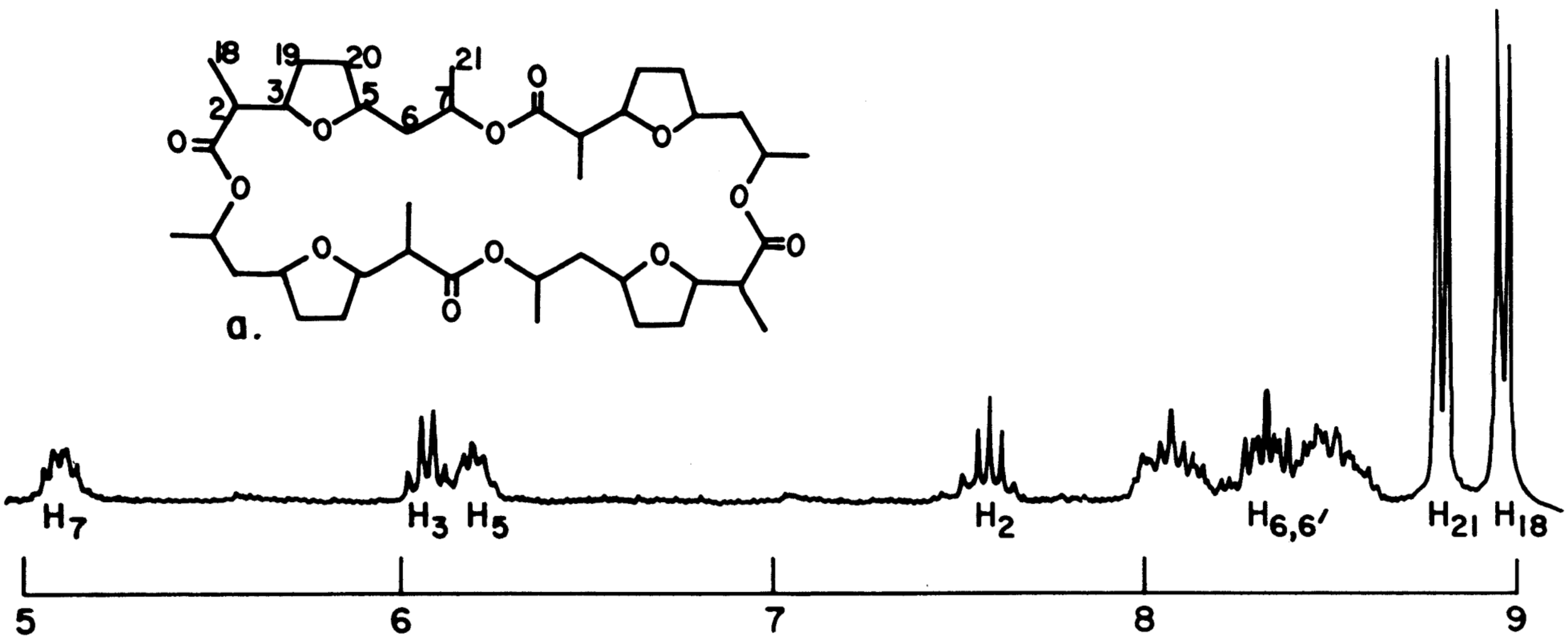
- 1944 – Isidor Isaac Rabi - Nobel Prize in Physics
  - -"for his resonance method for recording the magnetic properties of atomic nuclei"
- 1952 – Felix Bloch and Edward Mills Purcell – Nobel Prize in Physics
  - -"for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith"
- 1991 – Richard Ernst – Nobel Prize in Chemistry
  - -"for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy"
- 2002 – Kurt Wuthrich – Nobel Prize in Chemistry
  - -"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution"
- 2003 – Paul Lauterbur and Sir Peter Mansfield – Nobel Prize in Physiology and Medicine
  - -"for their discoveries concerning magnetic resonance imaging"

Varian HR 220

~1965
Superconducting Magnets Boosted Field Strength Required a Lot of Care And Feeding



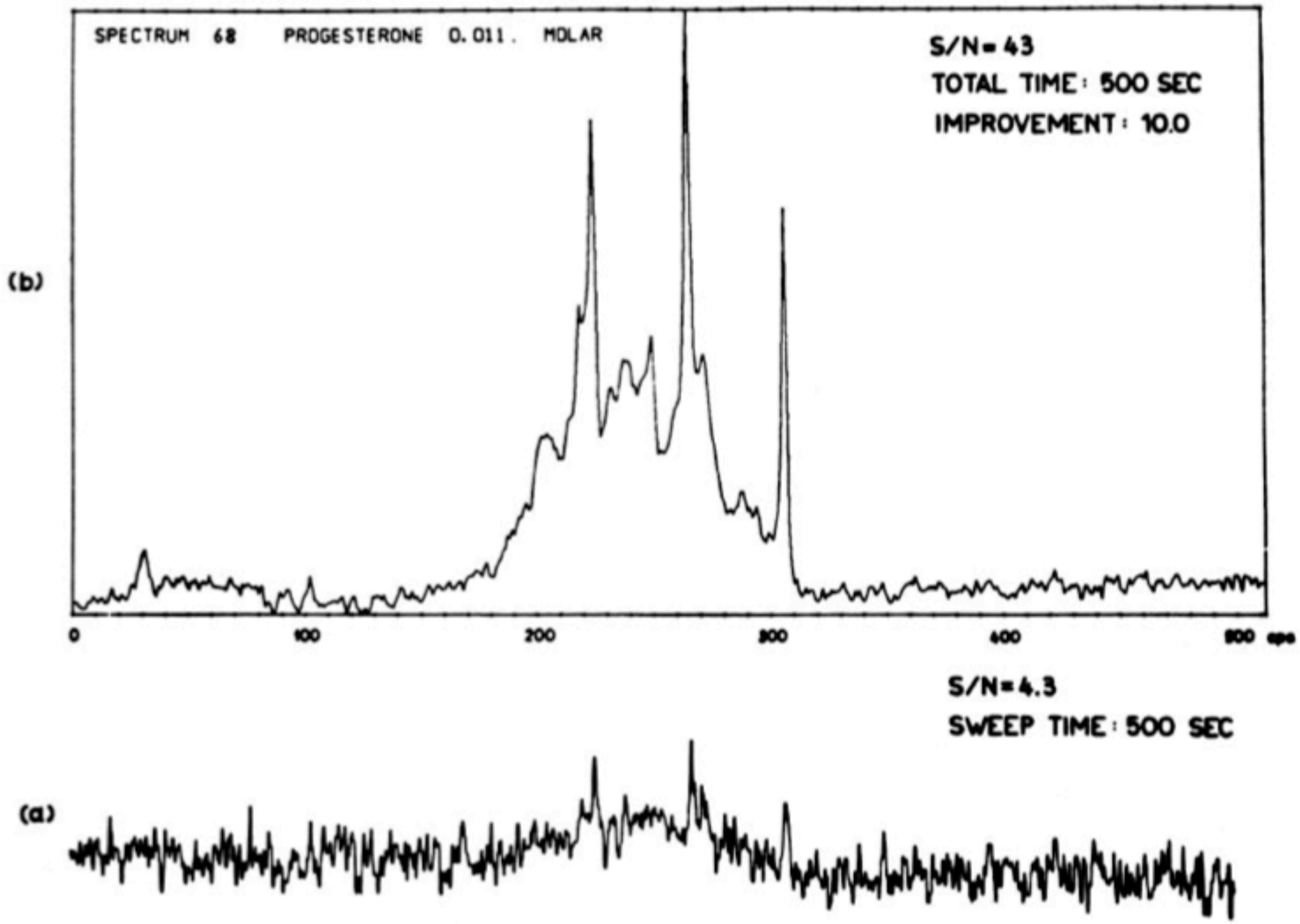
High Field (220 MHz), but Still 1D CW NMR



~1970 Richard Ernst Introduced Multidimensional Pulse FT Methods

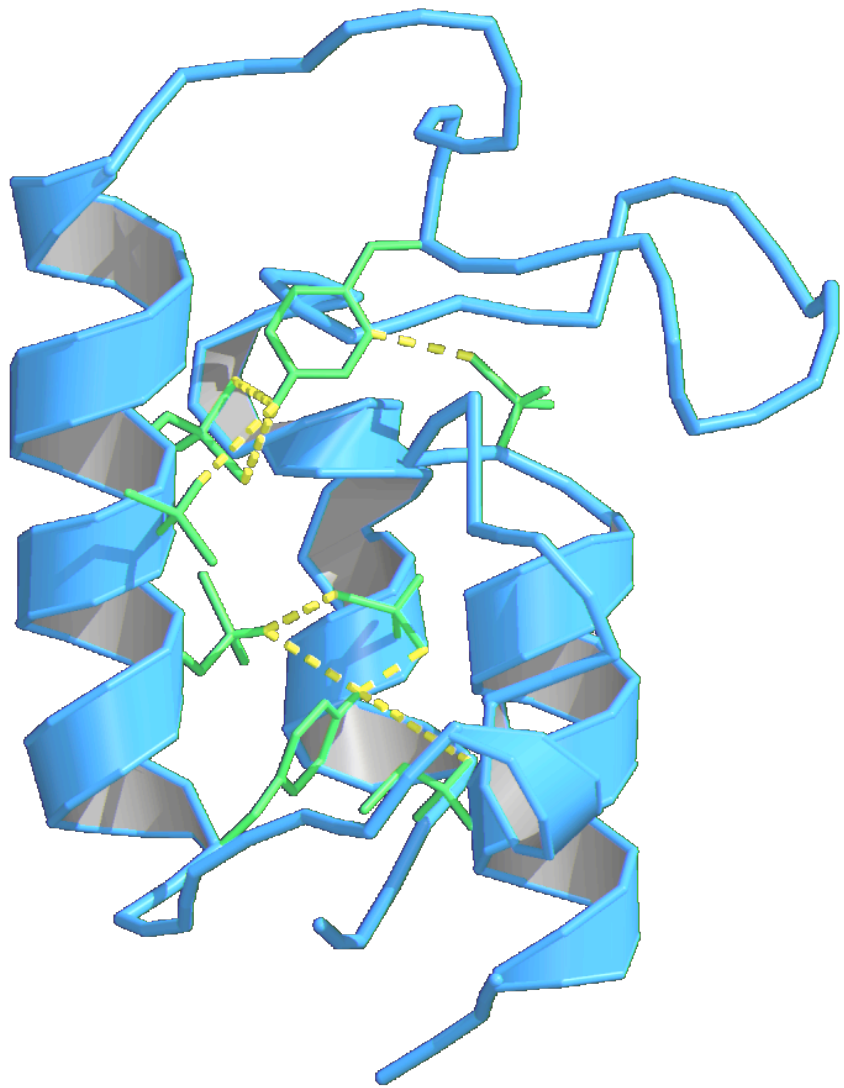
PULSED FOURIER TRANSFORM NMR

Richard Ernst & Wes Anderson, Rev. Sci. Instr. 37, 93 (1966)

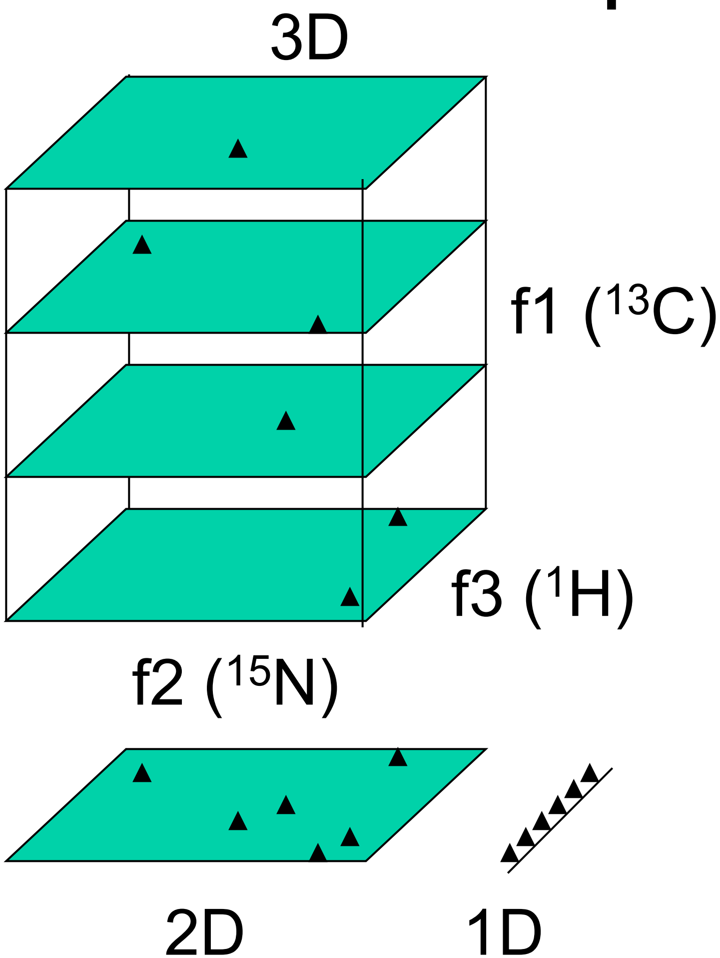


Fourier transform (top) and conventional spectra of 0.011 M progesterone showing sensitivity enhancement by a factor ten

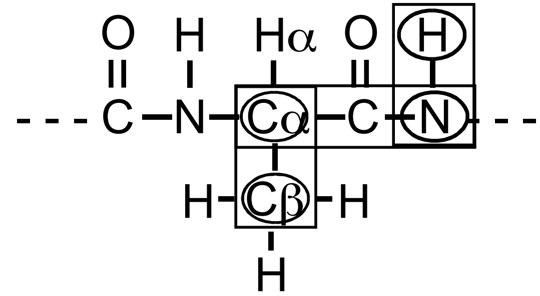
~1982 Kurt Wüthrich: 2D ^{1}H-^{1}H NMR: ~10 kDa Protein assign resonances, collect NOE's, calculate structure



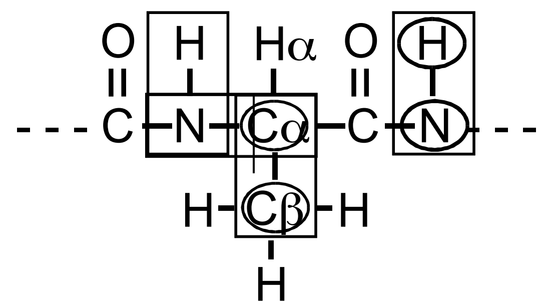
Extension to 3D: Through-bond Correlations in Peptides
Isotope Labeling is Key



HN(CO)CA



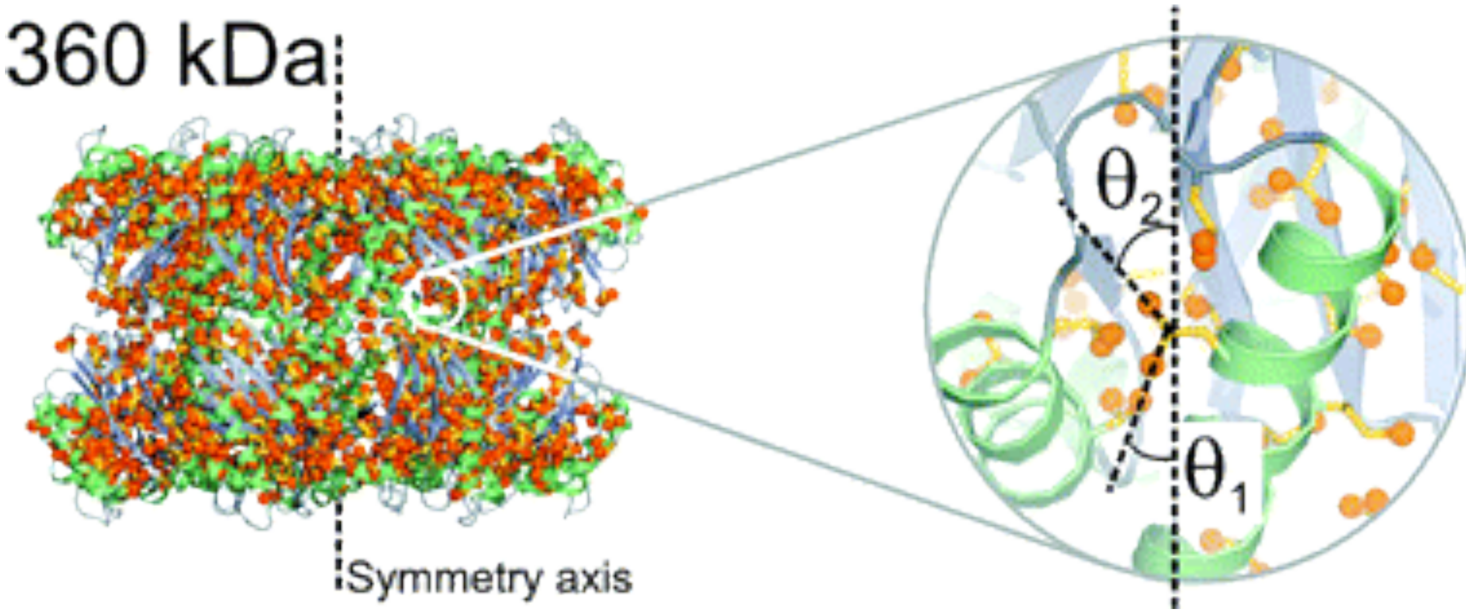
(HB)CBCA(CO)NH



HNCACB

Ikura, M.; Kay, L. E.; Bax, A., (1990) *Biochemistry* 29:4659-4667

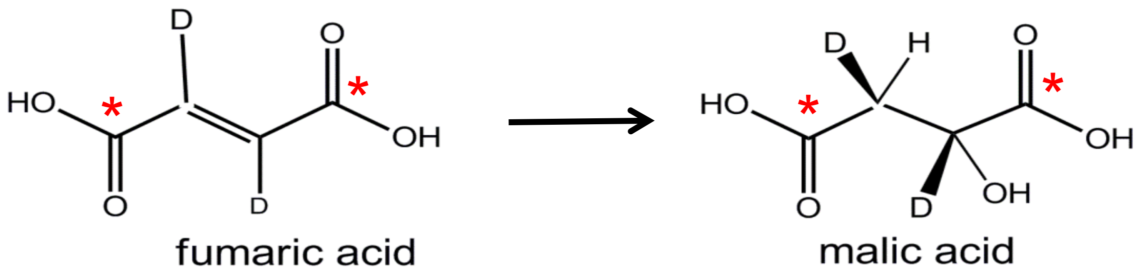
Today Very Large Systems Can Be Studied: Proteasome subunit – active site dynamics

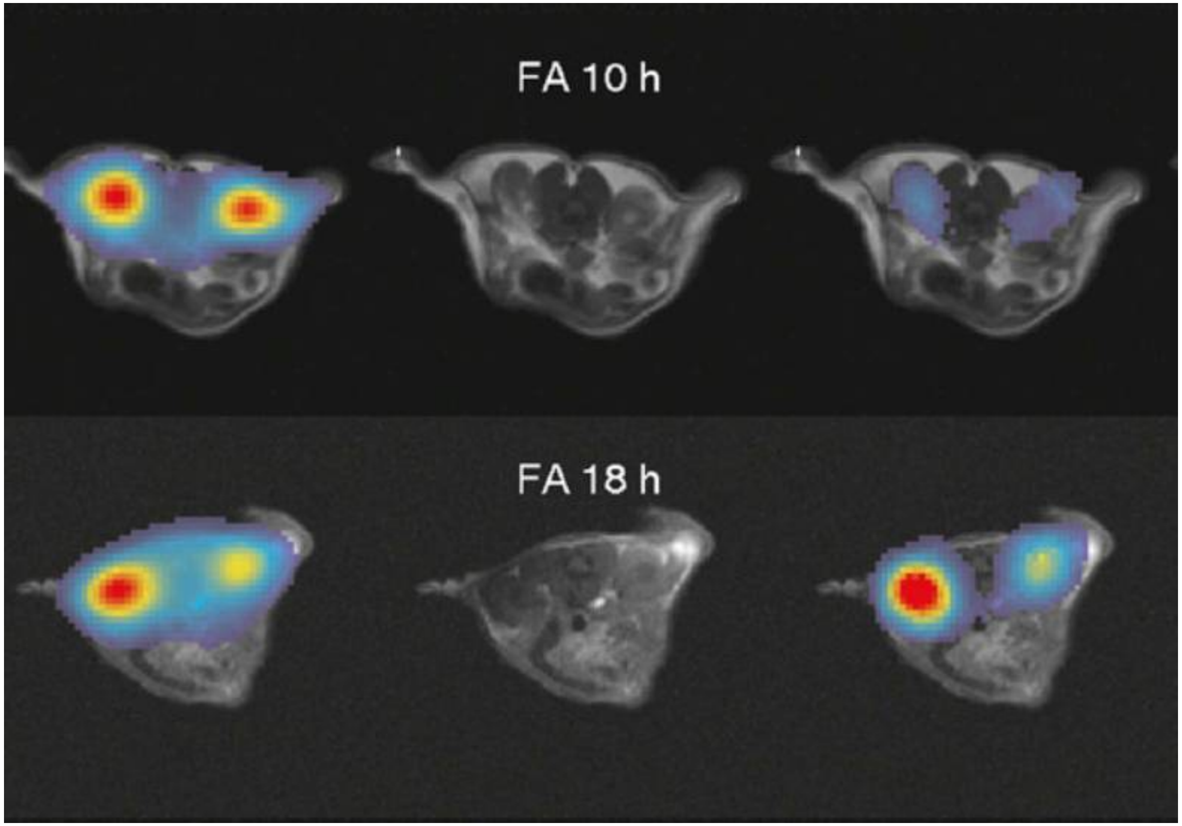


Sprangers, R and Kay, LE, 2007. Probing supramolecular structure from measurement of methyl H-1-C-13 residual dipolar couplings. *Journal of the American Chemical Society* **129:** 12668-+.

Ruschak AM and Kay LE, 2010, Methyl groups as probes of supra-molecular structure, dynamics and function, J Biomol NMR **46:**75-87

NMR Spectroscopy + MRI Monitors Metabolism *in vivo*





Example: Fumaric acid to malic acid conversion indicates onset of acute tubular necrosis of the mouse kidney. Images are 10 and 18 hrs after folic acid induced nephropathy. Left and right images based on signals of carboxyl resonances of fumaric and malic acid respectively.

Kevin M. Brindle, et al. PNAS, 109, 1374-1379, 2012

NMR is widely applicable to structure and function of biomolecules

- Montelione, G. T. & Szyperski, T. (2010). Advances in protein NMR provided by the NIGMS Protein Structure Initiative: Impact on drug discovery. *Current Opinion in Drug Discovery & Development* **13**, 335-349.
- Tzeng, S. R. & Kalodimos, C. G. (2011). Protein dynamics and allostery: an NMR view. *Current Opinion in Structural Biology* **21**, 62-67.
- Felli, I. C. & Pierattelli, R. (2012). Recent progress in NMR spectroscopy: Toward the study of intrinsically disordered proteins of increasing size and complexity. Iubmb Life 64, 473-481.
- Hurd, R. E., Yen, Y. F., Chen, A. & Ardenkjaer-Larsen, J. H. (2012). Hyperpolarized 13C metabolic imaging using dissolution dynamic nuclear polarization. Journal of Magnetic Resonance Imaging 36, 1314-1328.
- Robinette, S. L., Bruschweiler, R., Schroeder, F. C. & Edison, A. S. (2012). NMR in Metabolomics and Natural Products Research: Two Sides of the Same Coin. Accounts of Chemical Research 45, 288-297.
- Gopinath, T., Mote, K. R. & Veglia, G. (2013). Sensitivity and resolution enhancement of oriented solid-state NMR: Application to membrane proteins. Progress in Nuclear Magnetic Resonance Spectroscopy 75, 50-68.
- Goldbourt, A. (2013). Biomolecular magic-angle spinning solid-state NMR: recent methods and applications. Current Opinion in Biotechnology 24, 705-715.
- Manley, G. & Loria, J. P. (2012). NMR insights into protein allostery. Archives of Biochemistry and Biophysics 519, 223-231.
- Qureshi, T. & Goto, N. K. (2012). Contemporary Methods in Structure Determination of Membrane Proteins by Solution NMR. In Nmr of Proteins and Small Biomolecules (Zhu, G., ed.), Vol. 326, pp. 123-185.
- Bardaro, M. F. & Varani, G. (2012). Examining the relationship between RNA function and motion using nuclear magnetic resonance. Wiley Interdisciplinary Reviews-Rna 3, 122-132.

NMR Active Isotopes Exist for Nearly Every Element

http://bouman.chem.georgetown.edu/NMRpt/NMRPerTab.html

Select an element by clicking on it:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
H																H	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	**	Rf	Ha	Sg	Ns	Hs	Mt									

*	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
**	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Review of Spin Properties

- NMR active nuclei possess an intrinsic angular momentum, $\vec{\boldsymbol{I}}$, known as the spin angular momentum. The magnitude is

$$\vec{\boldsymbol{I}} = \hbar\, [\ I\ (I+1)\ ]^{1/2}$$

- Here I is the nuclear spin quantum number (integral or half-integral). If $I = 0$, no spin angular momentum (not NMR active)

- Associated with $\boldsymbol{I}$ is a magnetic moment, $\boldsymbol{\mu}$

$$\vec{\boldsymbol{\mu}} = \gamma\, \vec{\boldsymbol{I}} = \gamma\, \hbar\, [\ I\ (I+1)\ ]^{1/2}$$

- The proportionality constant is the gyromagnetic ratio, γ
- In NMR, larger $\vec{\boldsymbol{\mu}}$ for given $\vec{\boldsymbol{I}}$ (large γ), means more sensitive nucleus

Review of Spin Properties

- In a magnetic field, otherwise degenerate (energetically equivalent) states split into nondegenerate states (known as Zeeman splitting)
- The states are quantized, with the number of states established by the spin quantum number, *I*

$$\# \text{ levels} = 2I + 1$$

- Each of the 2*I*+1 states/levels is associated with a magnetic quantum number, *m*

$$m = -I, -I+1, \ldots, I-1, \mathrm{I}$$

- The component of ***I*** along the *z* axis, I_z, is defined as follows

$$I_z = m\hbar$$

- Thus

$$\mu_z = \gamma I_z = m\gamma\hbar$$

Review of Spin Properties

- The energies of the states resulting from the interaction of the magnetic moment with a magnetic field, $\vec{\boldsymbol{B}}$ are given by

$$E = -\vec{\mu} \bullet \vec{\boldsymbol{B}}$$

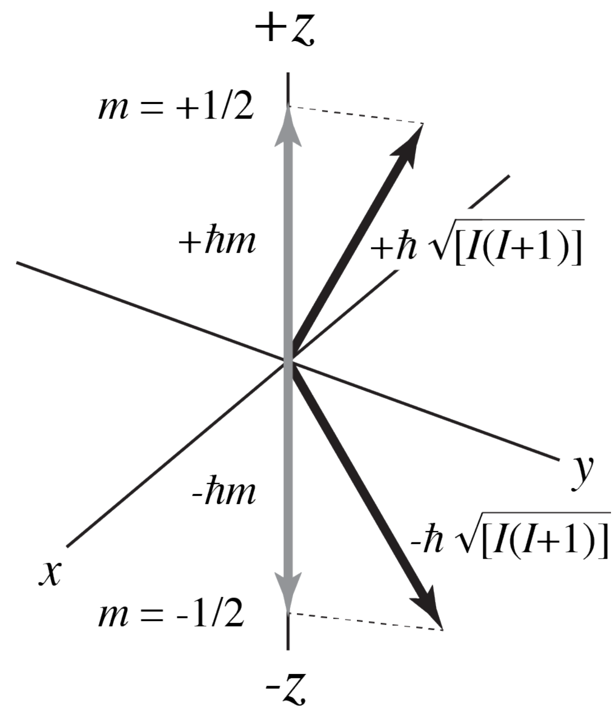
- The energies of the states depend on the orientations of the moments in the magnetic field, hence are proportional to the *scalar* projection of $\vec{\mu}$ on $\vec{\boldsymbol{B}}$ (the dot product), $\boldsymbol{\mu}_z$

$$E = -\,\boldsymbol{\mu}_z B_0 = -\,m\gamma\hbar B_0$$

- Here B_0 is the magnetic field strength

- The $2I+1$ energy levels are equally spaced. The energy difference between any two adjacent levels is

$$\Delta E = \gamma\hbar B_0$$



classical view of directional quantization for spin ½ nuclei

Review of Spin Properties

- The torque exerted by B_0 on the magnetic moments/dipoles promotes precession about the *z*-axis at a frequency given by

$$\upsilon_L = \gamma B_0 / (2\pi) \quad (\textit{Larmor frequency}\text{, in Hz})$$

$$\omega_0 = \gamma B_0 \ (\text{radians/sec})$$

- The energy difference between energy (spin) states can then be written as

$$\Delta E = h\upsilon_L$$

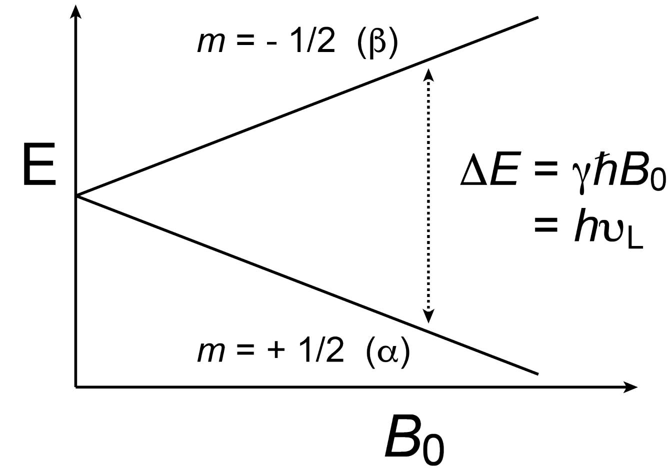
- Transitions between energy (spin) states can be effected by an electromagnetic field with an energy equal to ΔE. This occurs when the frequency of that field, υ_1, is equal to the Larmor frequency (*resonance* condition).

$$\upsilon_1 = \upsilon_L$$

Review of Spin Properties

- For spin 1/2 (I = 1/2), there are $2I$+1 = 2 energy levels, with values of *of m* equal to +1/2 and -1/2, called α and β, with energies

  $E_\alpha = -\frac{1}{2}\gamma\hbar B_0$ $\quad$ $E_\beta = +\frac{1}{2}\gamma\hbar B_0$



- From Boltzman statistics, the population ratio of these states can be estimated

$$\frac{N_\beta}{N_\alpha} = \exp\left(\frac{-\Delta E}{k_B T}\right) \approx 1 - \left(\frac{\Delta E}{k_B T}\right) \approx 1 - \left(\frac{\gamma\hbar B_0}{k_B T}\right)$$

  - example: ^{1}H, 300 °K, 5.875 Tesla (250 MHz)

$$\frac{N_\beta}{N_\alpha} = 1 - \frac{26.7519\times10^7\times1.0546\times10^{-27}\times5.875}{1.3805\times10^{-16}\times300} = 0.99996$$

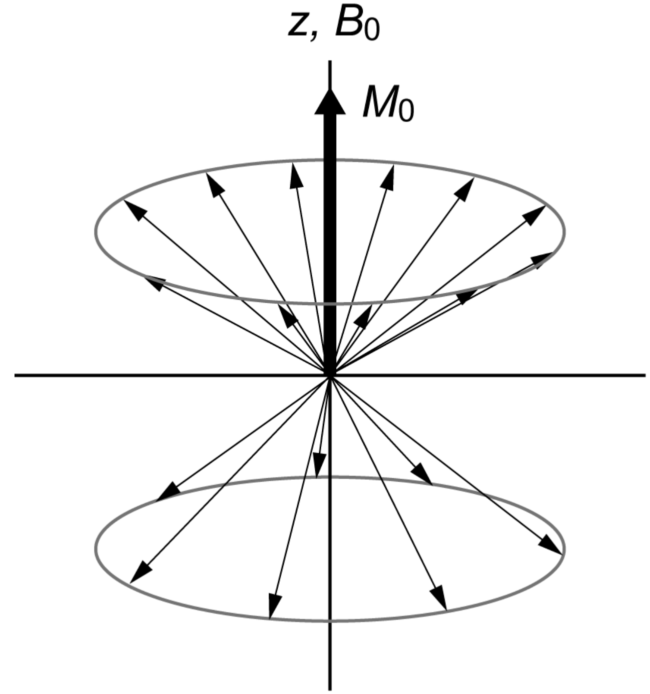
- ΔE is small, so the populations of α and β are nearly equal, and the macroscopic magnetization is small: *NMR is insensitive*

Review of Spin Properties

- The sum of the z-components of the nuclear dipoles in an ensemble gives the macroscopic (bulk) magnetization, M_0

$$M_0 = \gamma\hbar \sum_{m=-I}^{I} mN_m \quad (\text{recall } \mu_z = m\gamma\hbar)$$

$$M_0 \approx \frac{N\gamma^2\hbar^2 B_0}{k_B T(2I+1)} \sum_{m=-I}^{I} m^2 \approx \frac{N\gamma^2\hbar^2 B_0 I(I+1)}{3k_B T}$$

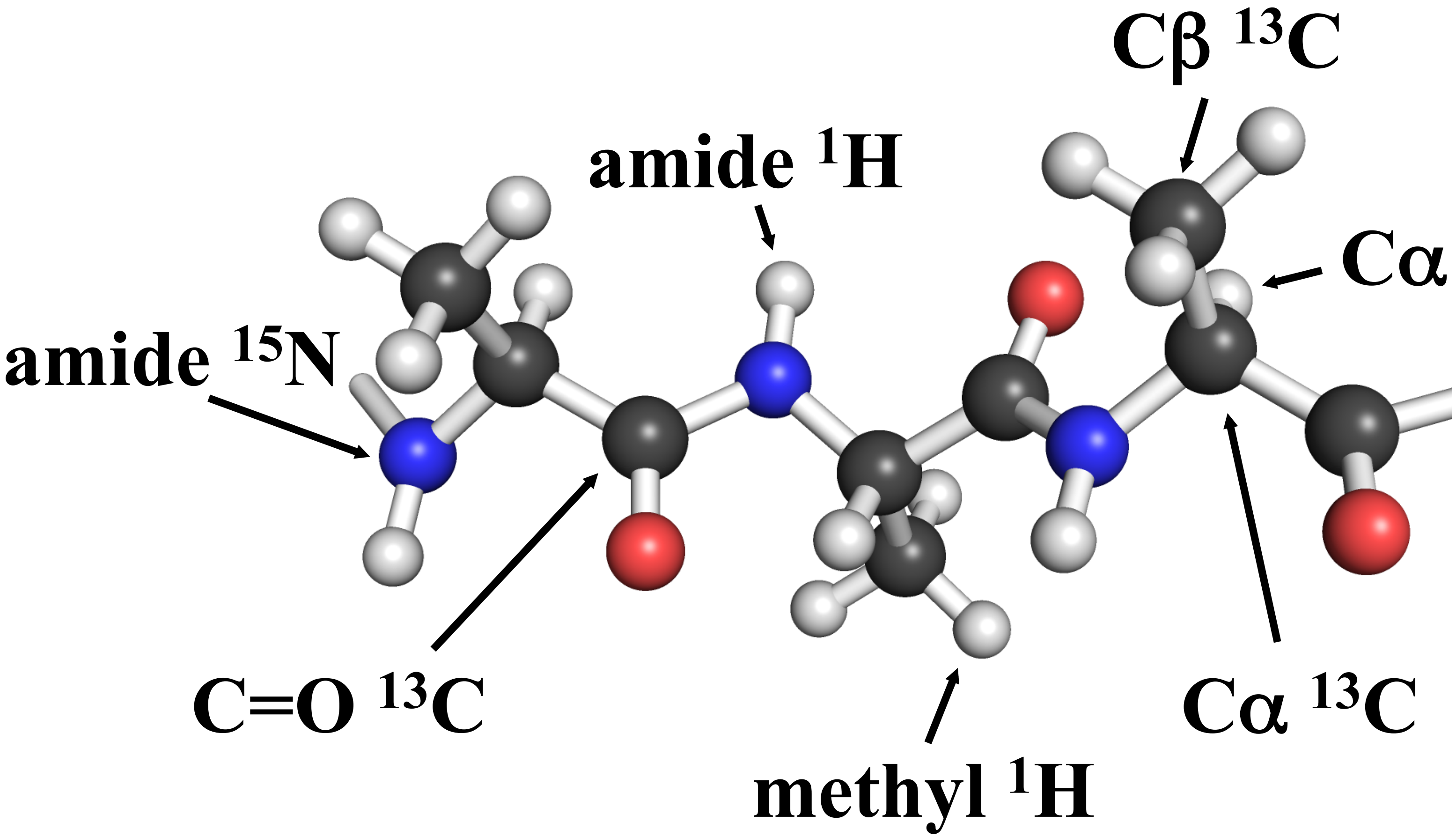


- Note: dependence on γ^2, linear dependence on B_0, dependence on isotopic abundance (N)

Spin ½ Nuclei are Most Useful in Biomolecular NMR

	^{1}H	^{13}C	^{15}N	^{19}F	^{31}P
Spin	1/2	1/2	1/2	1/2	1/2
Natural abundance	99.985%	1.108%	0.37%	100%	100%
Magnetogyric ratio ($\gamma/10^{7}$, rad T^{-1} s^{-1})	26.7519	6.7283	-2.7126	25.1815	10.8394
Relative sensitivity	1.00	1.59×10^{-2}	1.04×10^{-3}	0.83	6.63×10^{-2}
Relative receptivity	1.00	1.76×10^{-4}	3.85×10^{-6}	0.83	6.63×10^{-2}
Magnetic moment (μ/μ_N)	4.8372	1.2166	-0.4903	4.5532	1.9601
Quadrupole moment	0	0	0	0	0
Resonance frequency (MHz)	100	25.144	10.133	94.077	40.481

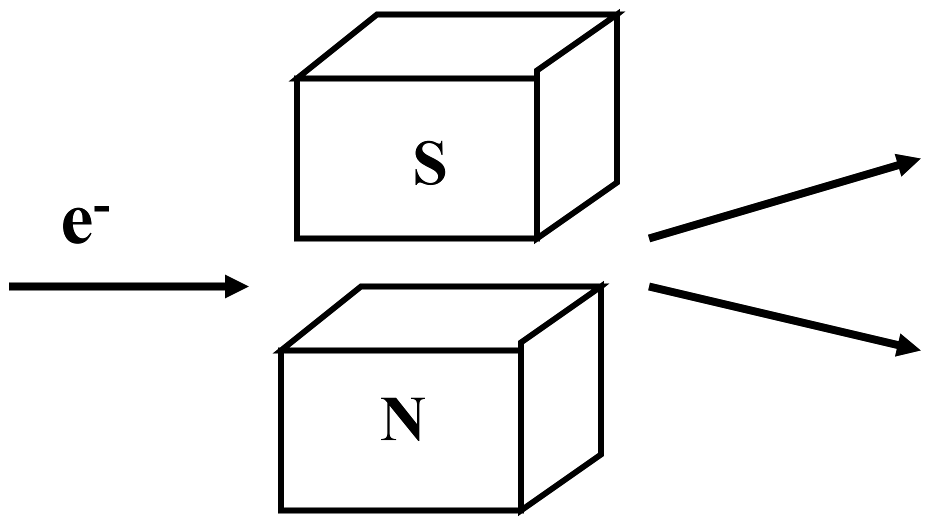
Polypeptides are Rich in NMR Active Nuclei



Nuclear Properties

- Not all nuclei have magnetic moments, Why?
- Not all nuclei are equally abundant, Why?
- Spins vary, Why?
- Magnetogyric ratios vary, Why?

Fundamental Particle Properties



Beam in field gradient

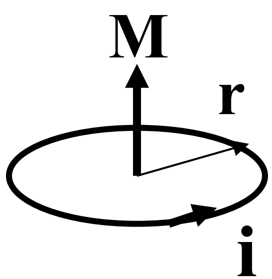
Stern Gerlach experiment:

- **demonstrated particles (electrons) possess an intrinsic angular momentum, and it is quantized**

- **Na atom - 1 unpaired electron**
  Two spots implies quantized moments: +/- 1/2
  protons and neutrons are also spin 1/2 particles

Understanding Magnetic Moments

- Current Loop Model: classical analogy to connect "spin" to magnetic moment
- Can get reasonable estimate of γ for electron



Area, S

$$\vec{M} = i\,\vec{S}$$

i in Coulomb s^{-1}
S in m^2
M in JT^{-1} (Tesla)

Estimates: $i = -ev/(2\pi r)$, $S = \pi r^2$, M (or μ) $= -erv/2$

$$\vec{\mu} = -e(\vec{r} \times \vec{v})/2, \quad \vec{L} = m_e\, \vec{r} \times \vec{v}, \quad \vec{\mu} = -e/(2m_e)\, \vec{L} = \gamma\, \vec{L} = \gamma h/(2\pi) l$$

$\gamma = -g\,(e/(2m_e))$, g = Lande g factor

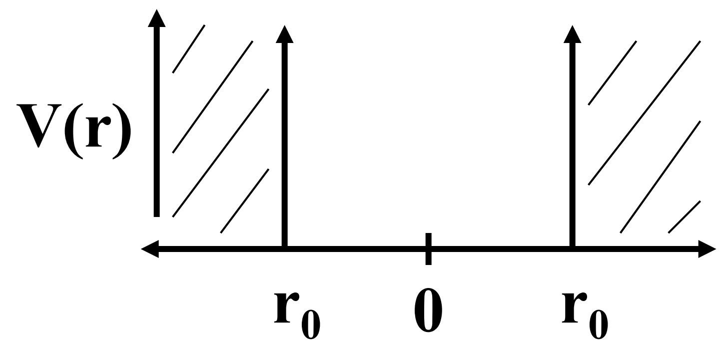
Values of Particle Magnetogyric Ratios

Electron: $g \approx 2$, $\gamma_e = -17.7 \times 10^{10}\ T^{-1}s^{-1}$

Proton: expect $1/m_p$ dependence, 1/2000 and positive
$2.7 \times 10^{8}\ T^{-1}s^{-1}$

Neutron: similar mass to proton
$-1.8 \times 10^{8}\ T^{-1}s^{-1}$

Heavier Nuclei: the Shell Model



Analogous to shell model for atomic electrons

Some rules:

a) spherical particle in a box potential

$\psi = R_{nl}(r)\, Y_l^m(\theta,\phi)$, $E(n,l)$

ladder of energy levels like H atom, but all ls allowed

l=0, 1, 2, 3 for "s", "p", "d", and "f" like atomic case

b) strong coupling of spin and orbit angular momentum

quantized total: $j = l \pm 1/2$ for spin 1/2 particle

larger j, lower energy (usually)

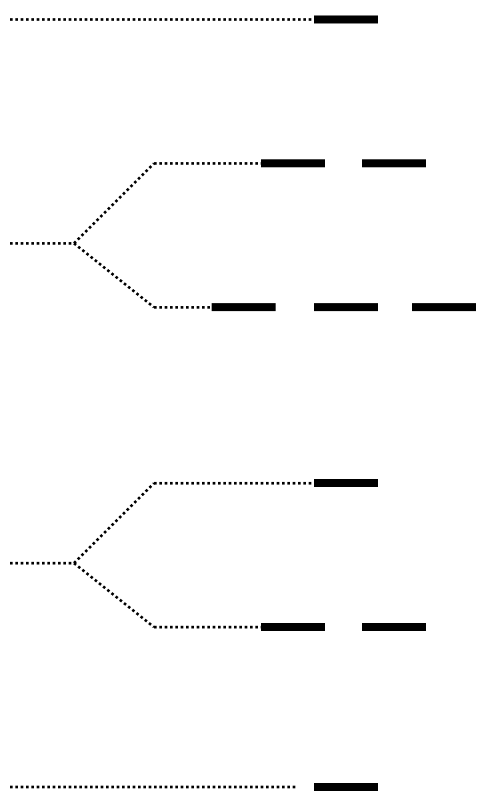
Shell Model Rules Continued

c) Treat protons and neutrons separately and fill from bottom up assuming 2j + 1 degeneracy

d) Assume particle pair strongly within levels: only unpaired spins count - total spin angular momentum given by j of level for unpaired spin

e) sign of moment depends on sign of moment for fundamental particle (+½ for proton, -½ for neutron) but changes sign when moment subtracts instead of adds to l in giving j

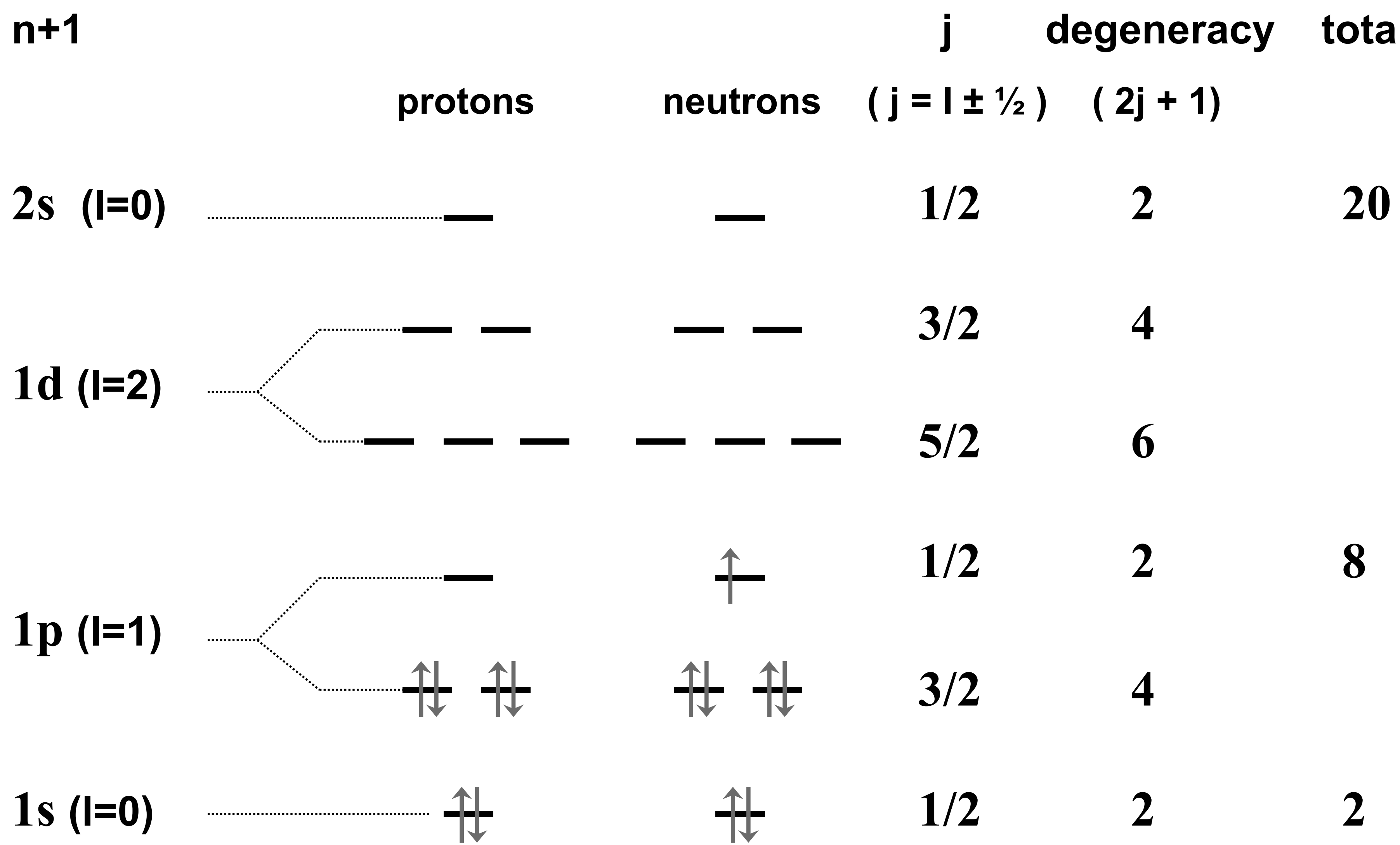
Energy Level Diagram



n+1	j (j = l ± ½)	degeneracy (2j + 1)	total
2s (l=0)	1/2	2	20
1d (l=2)	3/2	4	
	5/2	6	
1p (l=1)	1/2	2	8
	3/2	4	
1s (l=0)	1/2	2	2

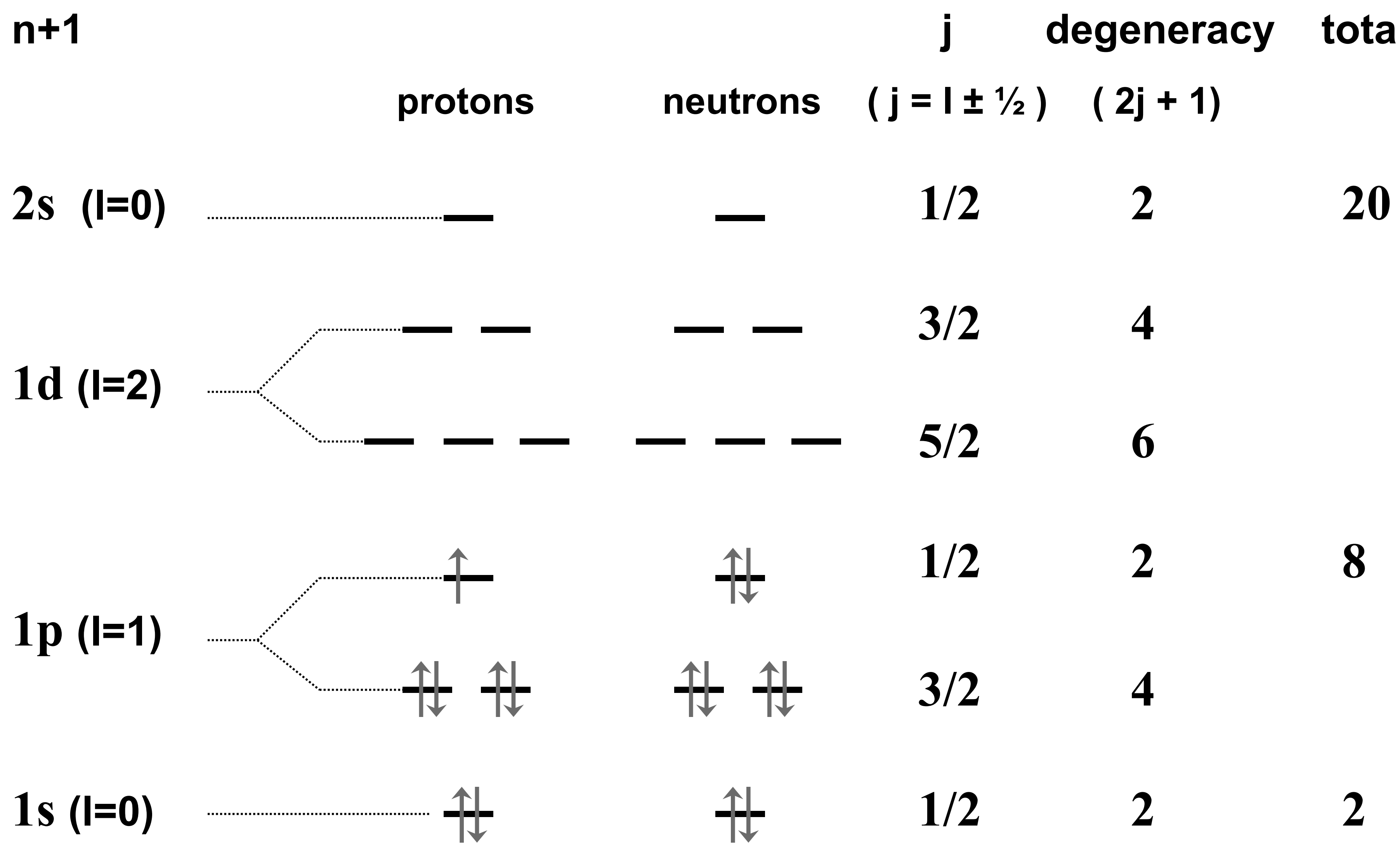
Example: $^{13}_{6}C$ (6 protons, 7 neutrons)

- unpaired neutron (-1/2) in $1p_{1/2}$ (j = 1-1/2 = 1/2), so spin=1/2, positive γ

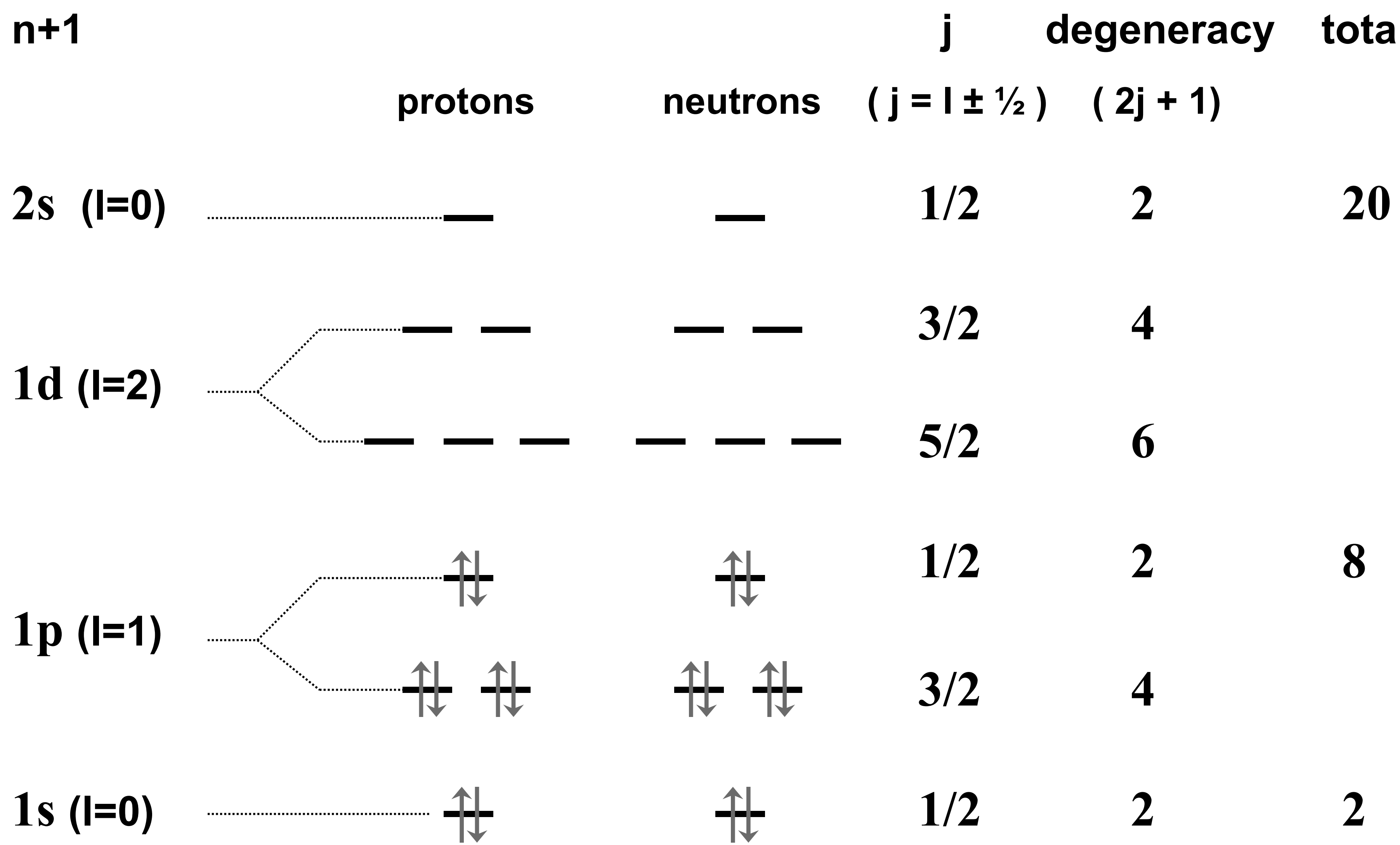


Example: $^{15}_{7}N$ (7 protons, 8 neutrons)

- unpaired proton (+1/2) in $1p_{1/2}$ (j = 1-1/2 = 1/2), so spin=1/2, negative γ



Example: $^{16}_{8}O$ (8 protons, 8 neutrons, two magic numbers), spin = 0
- highly stable (99.76% of all oxygen on Earth)



Particle Physics / Spin

Proton Spin Mystery Gains a New Clue:

https://www.scientificamerican.com/article/proton-spin-mystery-gains-a-new-clue1/