生命医科学特論III

Topics in medical life science III

Omnibus lectures from basic to advanced topics in medical life science

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Topics in medical life science II

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バイオNMR入門

basics of bioNMR

Nuclear Magnetic Resonance （核磁気共鳴法）

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Methods for Protein Structure Determination

method	principle	state of samples	features
Xray crystallography	diffraction	3D crystal	reliability high resolution
NMR	spectroscopy	solution	dynamic and interaction information
Electron microscopy crystallography	diffraction	2D crystal in amorphous ice	suitable for membrane proteins
Electron microscopy Single particle analysis	image analysis + diffraction	solution negative staining or in amorphous ice	no crystals a number of state

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Nobel prizes in the field of NMR

- Rabi, I.I. Physics, 1944: discovery of magnetic resonance
- Bloch, F. & Purcell,E.M., Physics, 1952: chemical shift of ^{1}H resonance
- Ernst, R., Chemistry, 1991: multidimensional FT NMR
- Wuthrich, K., Chemistry, 2002: protein NMR
- Lauterbur, P.C. & Mansfield, P., Medicine, 2003: MRI

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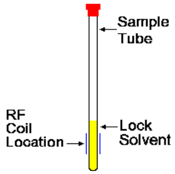
NMR spectrometer

- 超伝導磁石
  superconducting magnet

NMR tube



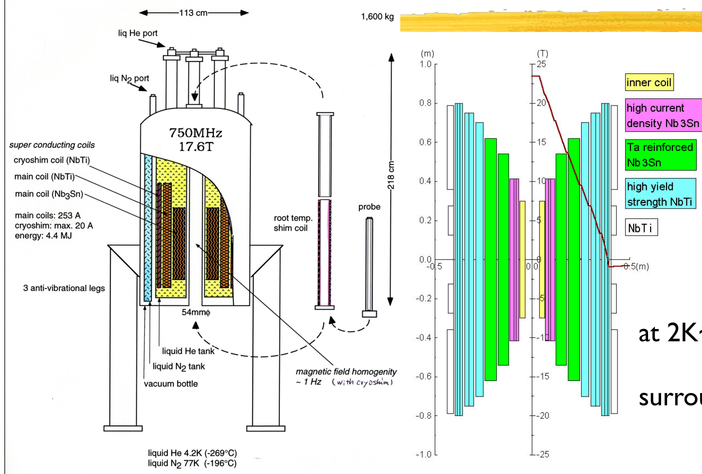
- PC for control

- ＮＭＲ分光器
- spectrometer

A 14 Tesla magnet, but conventionally 600 MHz magnet, because the NMR frequency of [1]H (proton) is 600 MHz in this magnet

超伝導磁石 superconducting magnet

750 MHz Cryostat



超伝導材料

NbTi
Nb_3Sn
NbAl
MgB_2

at 2K~4.2K in liquid He

surrounded by liquid N_2

クエンチ！　Quench!

Sudden loss of superconductivity causes explosive boil-off of liquid helium

superconducting magnet in the world

Bruker (Switzerland) products
1000 MHz = 1GHz

In Japan
950 MHz in Osaka U and Yokohama City U
900 MHz in Nagoya U

superconducting magnet in the world

Bruker Announces Five Ultra-High Field NMR Orders from Europe and Brazil

09/17/2015

BILLERICA, Mass.--(BUSINESS WIRE)-- Bruker (NASDAQ: BRKR) today announced five orders for ultra-high field (UHF) nuclear magnetic resonance (NMR) spectroscopy systems from Europe and Brazil in recent months. These UHF systems have been funded for cutting-edge NMR research in structural biology, intrinsically disordered proteins (IDPs), membrane proteins, macro-molecular complexes and interactions, cell biology, disease research, as well as in advanced materials research.

This Smart News Release features multimedia. View the full release here: http://www.businesswire.com/news/home/20150917005142/en/

Next generation Aeon 1.2 GHz NMR system (Photo: Business Wire).

Bruker defines UHF as NMR systems with ^{1}H proton frequency of 900 MHz or above. Other high-field 700, 800 and 850 MHz orders are not included in the UHF definition. The recent UHF NMR orders include three 900 and 950 MHz systems from Brazil, Switzerland and the UK, with revenue typically within 18 months from order:

- The **Federal University of Rio de Janeiro (UFRJ)** in Brazil is expanding its existing structural biology facility with the addition of a 900 MHz NMR spectrometer. As one of the leading universities in South America, the new 900 MHz system will be available as a regional resource for research in protein structure and dynamics, protein folding and structure of nucleic acids. Professor Fabio C. L. Almeida of UFRJ commented: "Having a 900 MHz will have a strong impact on the development of NMR and structural biology in Brazil and Latin America. It will offer us advantages and capabilities over other techniques in tackling important biological and technological problems."
- The **École Polytechnique Féderale de Lausanne (EPFL)** in Switzerland has ordered a Bruker 900 MHz instrument with the highest field wide-bore (89 mm inner diameter) magnet currently available for solid-state NMR. It will enable EPFL researchers to tackle problems in complex systems such as enzymes, catalytic nanoparticles, active pharmaceutical ingredients and live model organisms.
- The **University of Leeds** in the UK is expanding its Astbury Centre for Structural Molecular Biology with a 950 MHz NMR equipped with a novel ***CryoProbe*** that is now designed for both ^{13}C and ^{15}N direct detection, besides traditional ^{1}H indirect detection. This technology makes the instrument suitable for determining structures, dynamics and interactions of globular proteins, as well as for advanced functional and disease mechanism studies of intrinsically disordered proteins (IDPs). Professor Alex Breeze at the University of Leeds stated: "We are tremendously excited to be installing our new 950 MHz instrument, which will complement our investment in cutting-edge cryo-electron microscopy and other structural techniques. In particular, the combination of 950 MHz field strength and the novel direct-detection and low-volume capabilities of the latest ***CryoProbes*** will allow us to access critical structural and dynamic information on important biological systems and medically relevant targets with the optimum sensitivity and resolution."

Bruker (Switzerland)
1200 MHz = 1.2 GHz

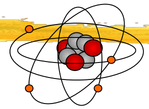
More than 10 orders, but still undelivered

1.3-1.5 GHz magnet is now under development

The higher magnetic field, the better performance?

- magnetic field (B_0) dependency
  - sensitivity ∝ $B_0^{3/2}$
  - resolution ∝ B_0
  - chemical shift anisotropy ∝ B_0
  - magnetic field alignment ∝ B_0^2
  - "NMR time scale" for an exchange process ∝ B_0
- Some measurements are better at low magnetic fields
  - Nucleus with large chemical shift anisotropy
    - ^{13}C carbonyl carbon is best at 600 MHz
    - ^{31}P phosphorous is best at 400 MHz
- Wider spectral width at higher magnetic fields demands higher decoupling power
  - But, fortunately, the adiabatic pulses (Wurst, Chirp etc) eased the restrictions of the high power levels

A nucleus behaves as a small magnet

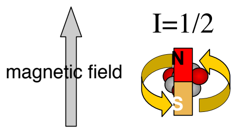


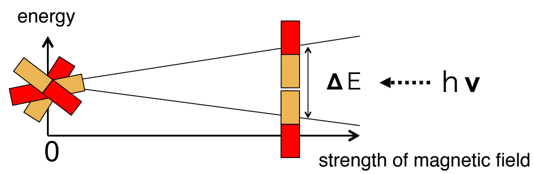
- Some nuclei (not all) have a nuclear spin

hydrogen	spin quantum number I	isotope abundance ratio
^{1}H	½	99.98%
^{2}H	1	0.015%
^{3}H	½	0%

carbon	spin quantum number I	isotope abundance ratio
^{12}C	0	98.9%
^{13}C	½	1.1%
^{14}C	0	0%

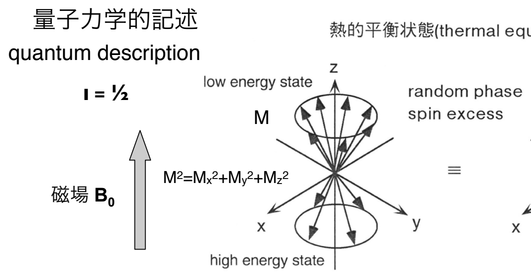
- An NMR active nucleus absorbs or emits electromagnetic wave

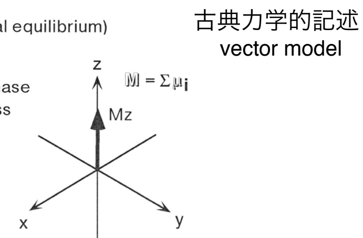




quantum mechanics   classical mechanics

量子力学的記述と古典力学的記述





１個の原子核：微視的な磁気モーメントM
one nucleus is a quantum spin with magnetization

M^2 and M_x, M_y or M_z can determine simultaneously
Any pair of M_x,M_y,M_z cannot determine simultaneously
M_z and M_{x2+y2} can determine simultaneously

アボガドロ数の磁気モーメントの総和：
巨視的な磁化M
Macroscopic magnetization of Avogadro number of nuclei behaves like a classical object
Bloch方程式 dM/dt = M x γB = M x γ(B_0+B_1)

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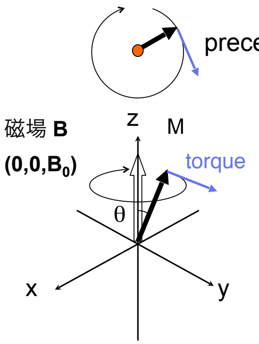
Bloch equation, ブロッホ方程式

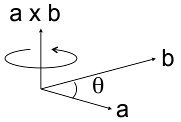


$d M /dt = M \times \gamma B_0$

angular frequency (rad/s)
$\omega_0 = \gamma B_0$

Larmor frequency (Hz)
$\nu_0 = \gamma B_0/2\pi$

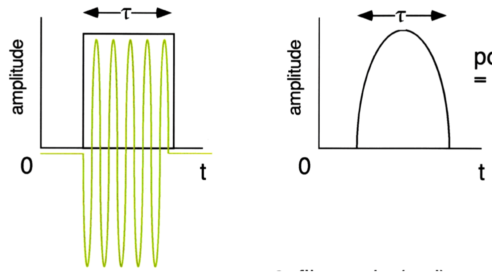
vector product a x b
$| a \times b | = a\ b\ |\sin\theta|$



Pulse, パルス

short-time irradiation of electromagnetic wave

radiofrequency pulse: pulse length (s)
shape
power (magnetic field strength)
$\gamma B_1/ 2\pi$ (Hz)
phase (0-360°)



power factor
= Ashape/Arec

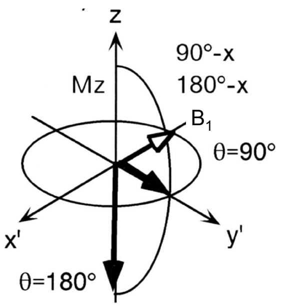
A pulse (B_1) rotates magnetization M along x' or y' axis in real space

$M_x = 0$(constant)
$M_y = M_0 \sin(\gamma B_1 t)$
$M_z = M_0 \cos(\gamma B_1 t)$

precession (nutation) about B_1
→ θ degree pulse

$\theta = \gamma B_1 \tau$

x' and y' axes are in rotating frame



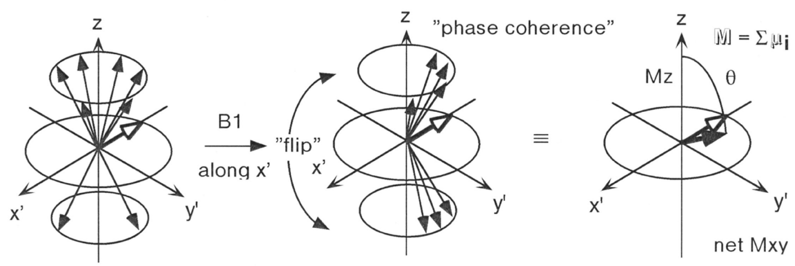
90-deg pulse

180-deg pulse

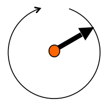
パルスと量子力学的記述

A pulse creates phase coherence in quantum world

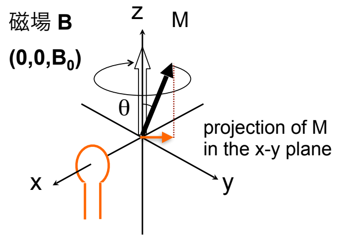
A pulse rotates magnetization M in real world



Magnetization M in the x-y plane creates electromagnetic force in detection coil in an NMR probe



precession、歳差運動

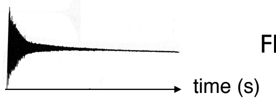


$dM/dt = M \times \gamma B_0$

angular frequency (rad/s)
$\omega_0 = \gamma B_0$

Larmor frequency (Hz)
$\nu_0 = \gamma B_0 / 2\pi$

electromotive force in detection coil



FID (Free induction decay)

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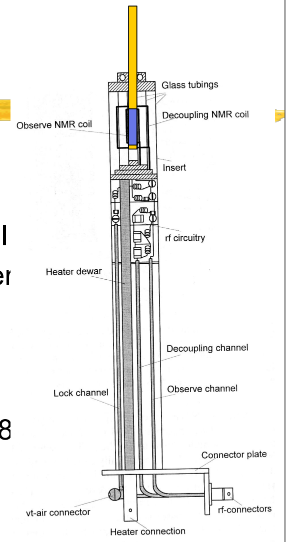
NMR Probe

- various types of probes for various nucleus
- observing nucleus is detected with the inner coil
- decoupling nucleus is irradiated with the outer coil
- various bore size for NMR tubes:
  - 1 mmφ、2.5 mmφ、5 mmφ(common)、8 mmφ



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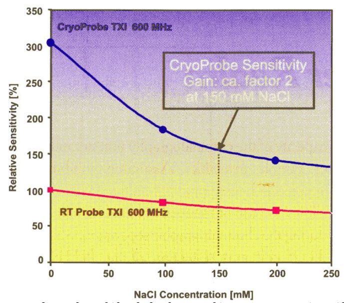
Cryoprobe or Cryogenic probe

- Reduction of thermal noise by cooling the detection coil and preamplifier with cold helium gas (25 K)
→ 4-fold increase in the signal-to-noise ratio



◇good for dilute samples
◇natural abundance measurement
◇single FID → 4D NMR

◆bad with high salt concentrations
◆probe exchange is difficult

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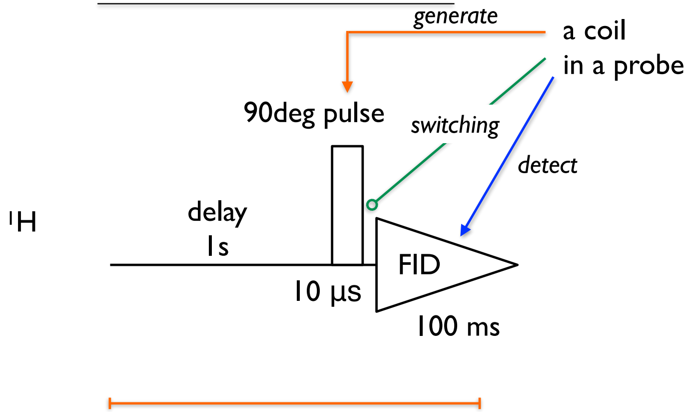
Basic pulse program



repeat *n* times to accumulate FIDs

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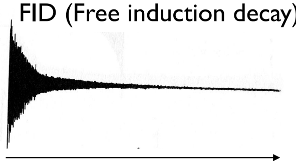
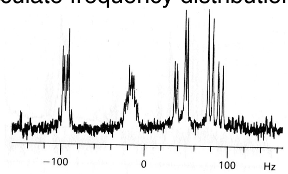
Fourier Transform

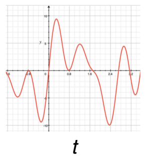
calculate frequency distribution

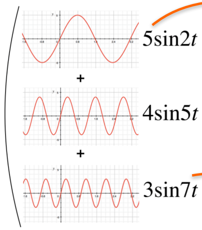
FID (Free induction decay)



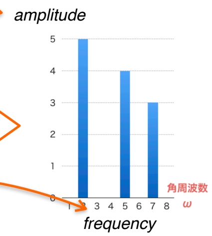
FT







FT



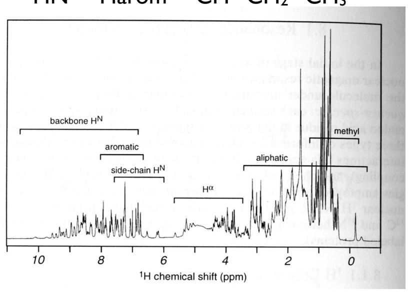
signal intensity as a function of time

signal intensity as a function of frequency

^1H & ^{13}C spectra of a protein

1D ^1H spectrum of a protein

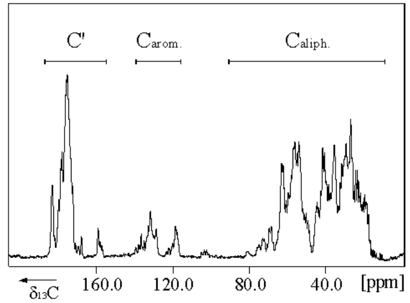
HN Harom CH CH_2 CH_3



ppm

1D ^{13}C spectrum of a protein

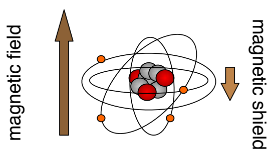
C=O Carom CH CH_2 CH_3

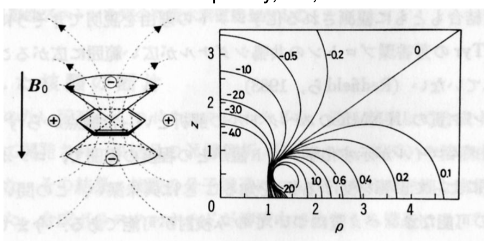


ppm

化学シフト chemical shift

Magnetic shield by electrons → difference in resonance frequency, *i.e.*, chemical shift





the first ^1H NMR of a protein

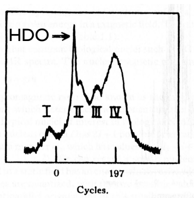
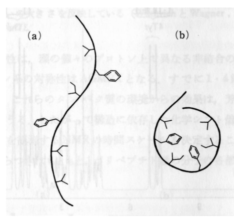
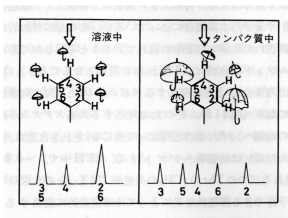


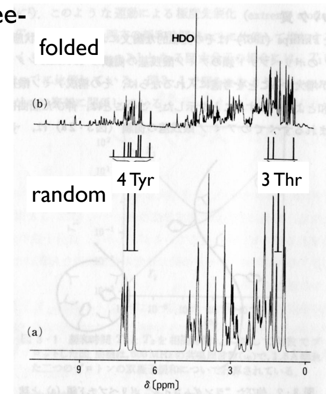
Fig. 1.1 The first ^1H NMR spectrum of an enzyme, ribonuclease, at 40 MHz, obtained in 1957. (Reprinted from Ref. 2 with permission.)

Chemical shift in proteins originates from the three-dimensional fold of proteins

- Charges, double bonds, and aromatic rings in the surrounding environment affect the chemical shift
- Microenvironment depends on the folded three-dimensional structure of proteins



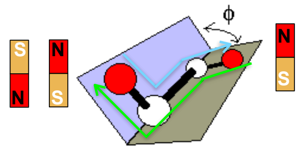


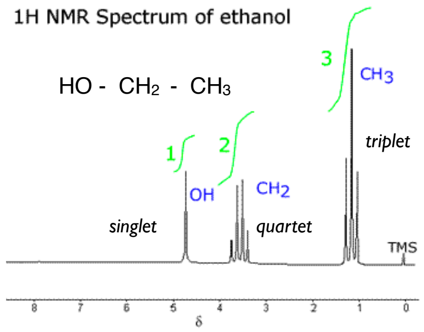


J coupling *or* scalar coupling

Overlap of bonding electrons → J coupling (through bond interaction)

- in Herts (Hz)
- independent from magnetic field





^{1}H in OH is decoupled due to the rapid exchange with the solvent ^{1}H

- J coupling effects are mutual

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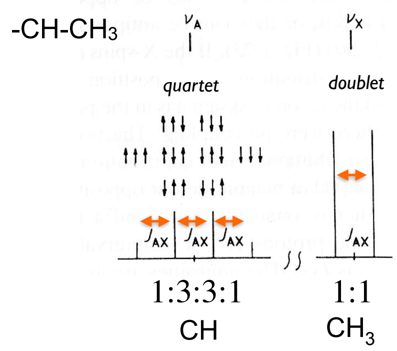
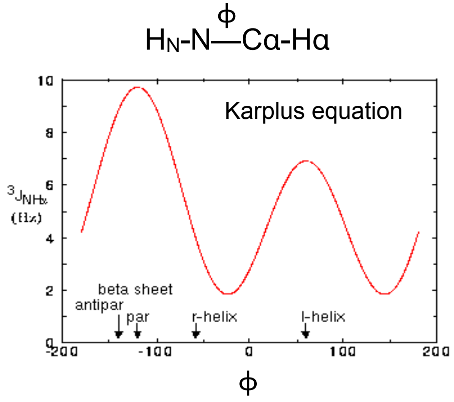
Peak splitting due to J coupling

- number: the n+1 rule
- intensity: binomial distribution （２項分布）
- coupling constant depends on the dihedral angle of the bond





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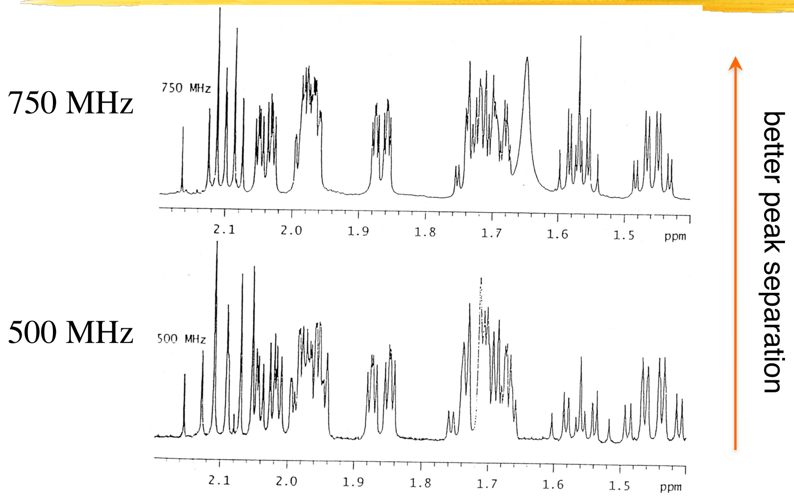
Coupling constant is independent from the magnetic field



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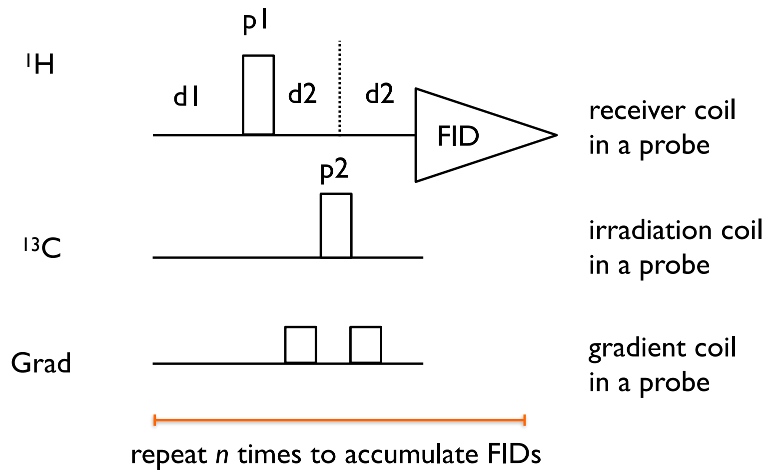
a bit complex pulse program



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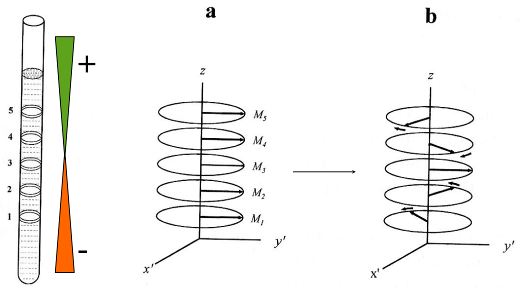
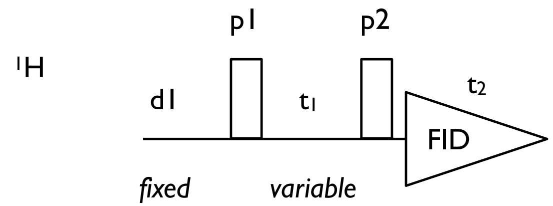
Gradient probe

- generate a gradient of weak magnetic field in a probe
  - magnetic field gradient along x, y, and z directions for a short time (1 ms)



- many important applications: gradient shimming, coherence selection with gradient, removal of unwanted signals, suppression of radiofrequency pulse imperfection

a multi-pulse program for 2 dimensional measurement

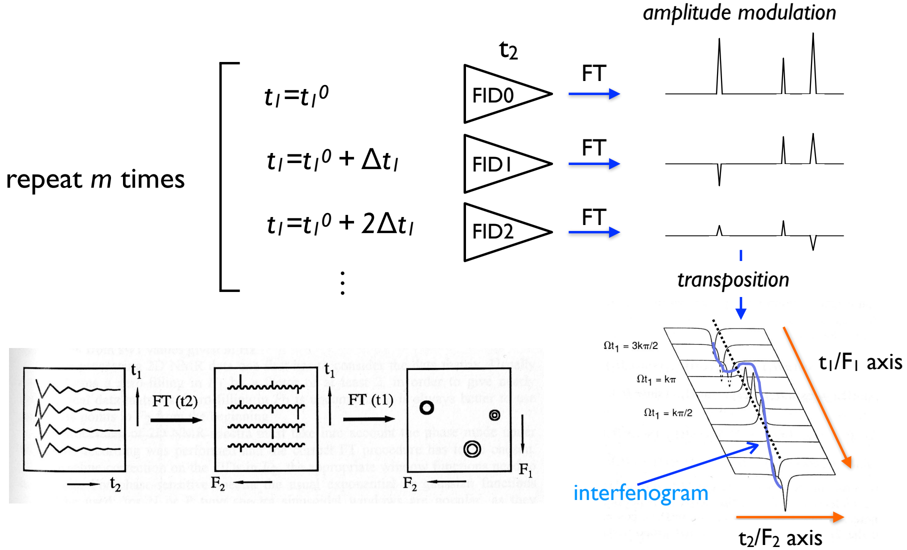


repeat *m* times

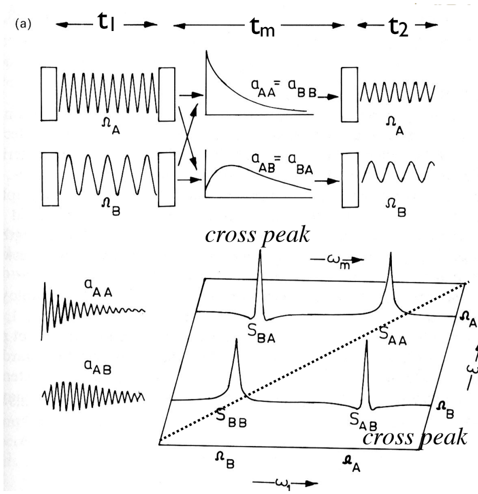
- repeat *n* times — $t_1 = t_1^0$
- repeat *n* times — $t_1 = t_1^0 + \Delta t_1$
- repeat *n* times — $t_1 = t_1^0 + 2\Delta t_1$
- ⋮

Party with Dr. R Ernst (Aug, 1991)

a multi pulse program for 2 dimensional measurement

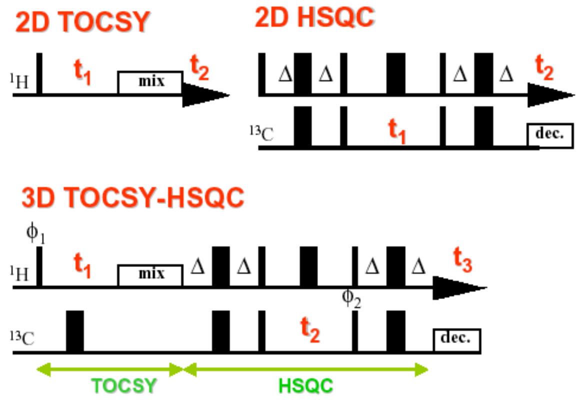


Mixing mechanisms during t_m generate cross peaks

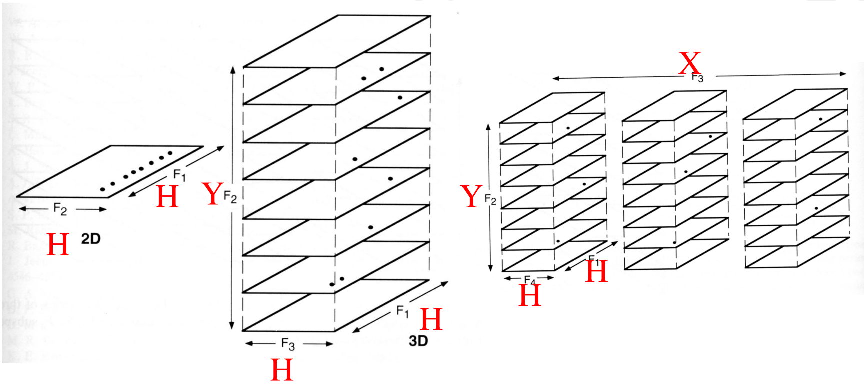


- *J*-coupling
- NOE
- chemical exchange

Pulse programming is easy

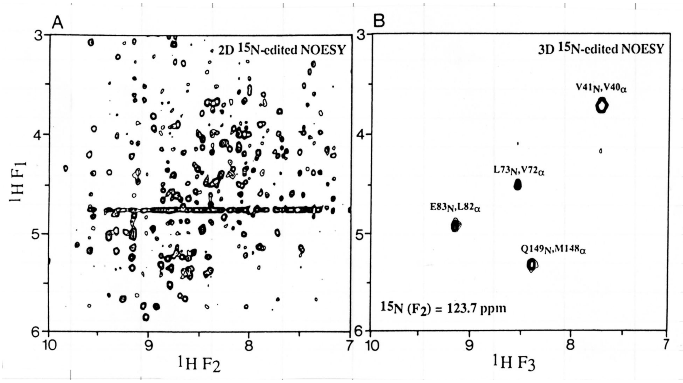


multi-dimensional NMR spectral measurement



X-H ~ H-Y   X-H ~ H-Y   X-H ~ H-Y

Comparison of 2D and a slice of 3D



Protein NMR

- The meaning of Assignment is to assign each NMR peak to a nucleus in the molecule
- sequential assignment （連鎖帰属 ）
  - [^{1}H], [^{1}H,^{15}N]
- triple resonance assignment（トリプル測定帰属）
  - [^{1}H,^{15}N,^{13}C], [^{1}H$_{N}$,^{15}N,^{13}C,^{2}H]

Kurt Wuthrich

Ad Bax

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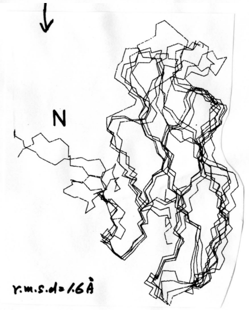
Acknowledged in 1986 as the method of protein structure determination

J. Mol. Biol. (1986) 189, 377–382

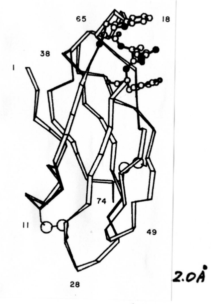
Studies by ^{1}H Nuclear Magnetic Resonance and Distance Geometry of the Solution Conformation of the α-Amylase Inhibitor Tendamistat



Kurt Wuthrich

J. Mol. Biol. (1986) 189, 383–386

Crystal Structure Determination, Refinement and the Molecular Model of the α-Amylase Inhibitor Hoe-467A



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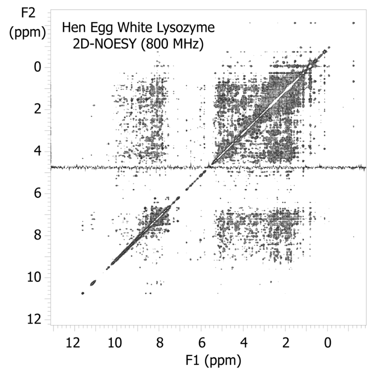
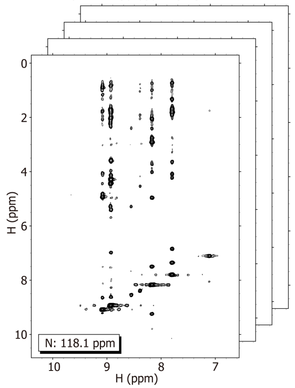
NOESY spectrum

An NOE (nuclear Overhauser effect) peak is observed if the distance of two ^{1}H nuclei is less than 5 Å



2D NOESY



3D ^{15}N-edited NOESY

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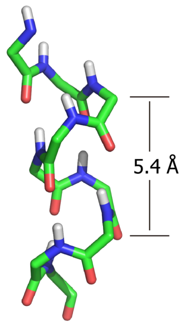
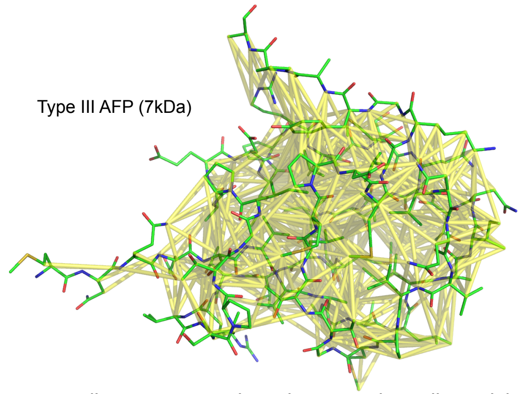
Calculate an ensemble of structures that satisfy the ^{1}H-^{1}H distance information

NOE provides the upper limit of short interproton distances (4 ~ 5 Å)



a pitch of α-Helix is 5.4 Å

Type III AFP (7kDa)



1084 distance constraints shown as the yellow sticks

A large number of NOE peaks is necessary for protein structure determination

40

NMR properties of biological nuclei

γ: gyromagnetic ratio

stable isotopes suitable for protein and nucleic acid NMR (I=1/2)

	1H	2H	3H	12C	13C	14C	14N	15N	16O	17O	18O	31P	32S	33S	34S	35S
spin quantum number I	1/2	1	1/2	0	1/2	0	1	1/2	0	5/2	0	1/2	0	3/2	0	3/2
abundance ratio %	99.99	0.01	RI	98.9	1.1	RI	99.6	0.4	99.76	0.04	RI	100		0.74		RI
$\gamma \times 10^7$	26.7	4.1	28.5	-	6.7	-	1.9	-2.7	-	-3.6	-	10.8	-	2.0	-	
Freq (MHz)	100	15.3	107	-	25.1	-	7.2	10.1	-	13.6	-	40.5	-	7.67	-	

600 MHz in a 600 Mz magnet

60.6 MHz in a 600 Mz magnet

a trick using stable isotopes

protein with natural abundance

stable isotope labeled protein

1H, 12C, 14N → 1H, 13C, 15N

^{13}C glucose
^{15}N ammonium chloride
in a minimal culture medium

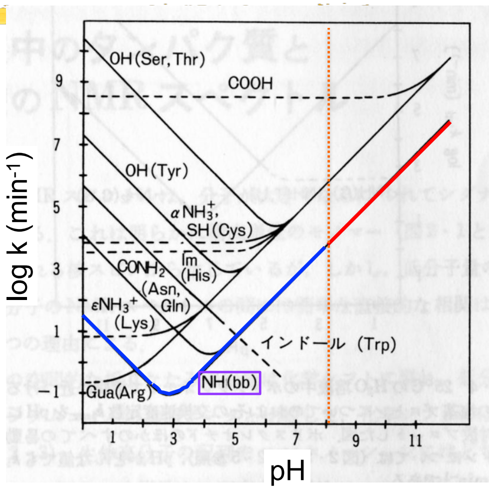
selective observation of one subunit in a complex

1H, 13C, 14N | 1H, 12C, 15N

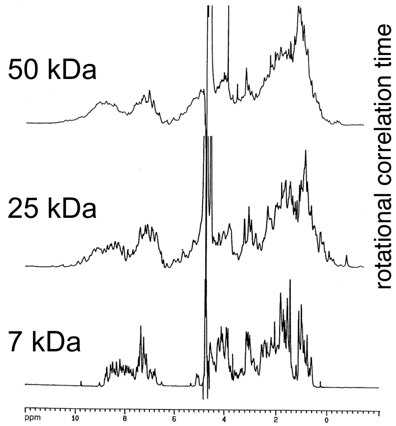
exchangeable protons with bulk water

$k = 10^4$ min^{-1} at pH 8
→
life time of only 6 ms

acidic to neutral pH below 8 is necessary for the measurement of backbone amide protons



Rotational correlation time (∝molecular weight of a protein) determines the line width of NMR spectrum



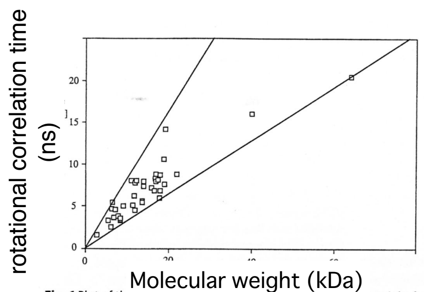
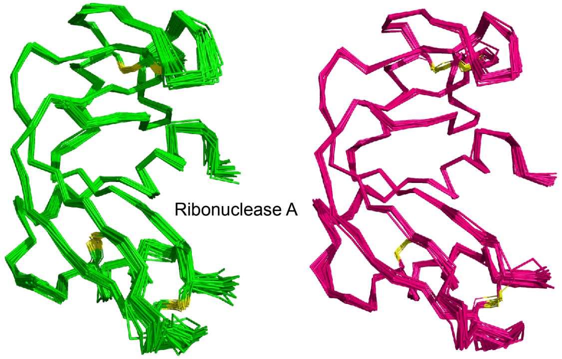


Fig. 1 Plot of the overall correlation time, τ_c, *versus* molecular weight for a set of 39 proteins or protein complexes. The data scaled to 37° were obtained from Table 1 of Dayie *et al.*[36] and supplemented with additional data from three proteins (not scaled), including: flavodoxin from *Anacystis nidulans*[38], the 40,000 M_r yeast eIF4E in a CHAPS micelle (25°)[28], and the 64,000 M_r trp repressor–operator complex (45°)[37]. The two lines indicate the range of correlation times observed in this set. It varies between 0.32 and 0.84 ns/kM_r.

An NMR structure vs crystal structures

a single NMR structure *overlaid X-ray structures*

PDB
2AAS



PDB
1AFK, 1XPS,
8RSA, 1RPH,
1EOS, 1QHC,
1RPF, 1BEL,
1RNW, 1RNX,
1RNQ, 1RNY,
1RNN, 9RSA,
1RNZ, 1RTB,
1RSM, 1RBB,
1RTA, 1DFJ,
1RHA

ensemble of structures in solution snapshots in crystals

a test for checking attendance

•出席を兼ねて，小テストをします．
•馬出キャンパス事前に用紙を配布，終了時に回収
•伊都キャンパスと箱崎キャンパスは，
次のスライドと最後に問題を提示する．
回答は神田までメールで送る
メールアドレス：kohda@bioreg.kyushu-u.ac.jp
今日の１８時まで

•do a test to check attendance
•For attendee in Maidashi campus: pass a test paper at the beginning and collect it at the end of the class.
•For attendee in Ito and Hakozaki campuses: the test will be shown in the next and last slides. Please send your answer to kohda@bioreg.kyushu-u.ac.jp
no later than 18:00 today

Quiz

Choose between two options

- NMR stands for (nuclear mitochondrial response or nuclear magnetic resonance)
- (^{1}H or ^{13}C) frequency is 600 MHz in a 600 MHz superconducting magnet
- The observation of backbone amide protons is possible at pH (3 or 10)

Send your answer to kohda@bioreg.kyushu-u.ac.jp
no later than 18:00, today