Nuclear Magnetic Resonance

– From Basic Physics to Biomedical Applications

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Outline

1. The Dawn of NMR – It is all Physics.
2. Exploiting the power of NMR – A party for all.
   Chemistry, biology, material science, and medicine.
3. Manipulation of nuclear spins – Spin gymnastics.
4. Biomedical applications – Work from our lab.
   - Packaging of SARS CoV nucleocapsid.
   - Mechanism of SUMO mediated signal transduction.
   - Macromolecular dynamics in solid and solution.
5. Look back on a wonderful journey.

1. The Dawn of NMR – A fertile ground for physicists

1924 Pauli proposed the presence of nuclear magnetic moment to explain the presence of hyperfine shift in atomic spectra.

1930 Nuclear magnetic moment was detected using the refined Stern–Gerlach experiment by Estermann.

1939 Rabi et al first detected nuclear magnetic resonance by applying rf energy to a beam of hydrogen molecules.

1946 Purcell et al at Harvard reported nuclear magnetic absorption in parafilm wax.
Bloch et al at Stanford reported nuclear magnetic resonance phenomenom in liquid water.

1940s–60s NMR theories were developed by physicists.

2. Exploiting the power of NMR – A party for all

1949 Chemical shift phenomenon was observed.

1960s

- Ernst and Anderson intrlioduced Fourier Transform technique into NMR that increased NMR sensitivity by orders of magnitude.
- Solid state NMR was revived due to efforts of Waugh at MIT. Application to material and polymer science insoluble proteins etc.
- Biological application became possible due to the introduction of superconducting magnet and high power computers.
- NMR imaging was demonstrated (Lauterbur at Stony Brook).

1970s

- Development of multi-dimensional NMR (Jeneer, Ernest, Bax ..)
- Development of methodologies for determining macromolecular structure (Wüthrich).

1980s and beyond – Exploding applications.

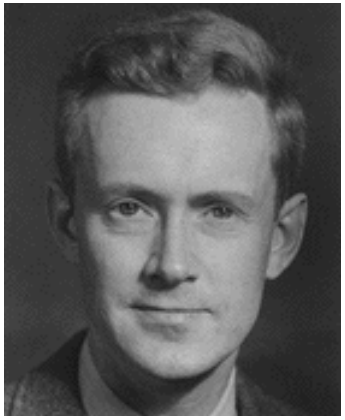
- Methods for characterizing macromolecular structure/dynamics in solution matured.
- Macromolecular structures in solid and gel states become feasible.
- Material science: Zeolites, polymers, fuel cells etc.
  (Clare Grey in Cambridge on Li-Air battery 5x more compact)
- MRI become a powerful clinical imaging modality.
- Functional MRI come to stage.
- Development of several fast NMR methodologies.
- NMR-based Metabolomics.
- ……

Non-trivial applications.

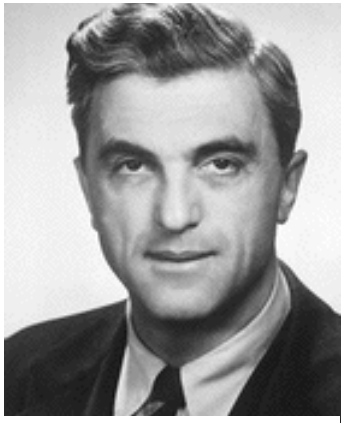
- Each become a sub-discipline by itself.

Nobel Laureates in NMR

Isador I Rabi,
Physics 1944



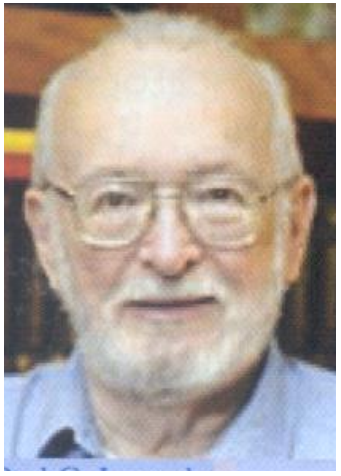
Edward M. Purcell
Physics, 1952



Felix Bloch
Physics, 1952

Richard R. Ernst
Chemistry, 1991

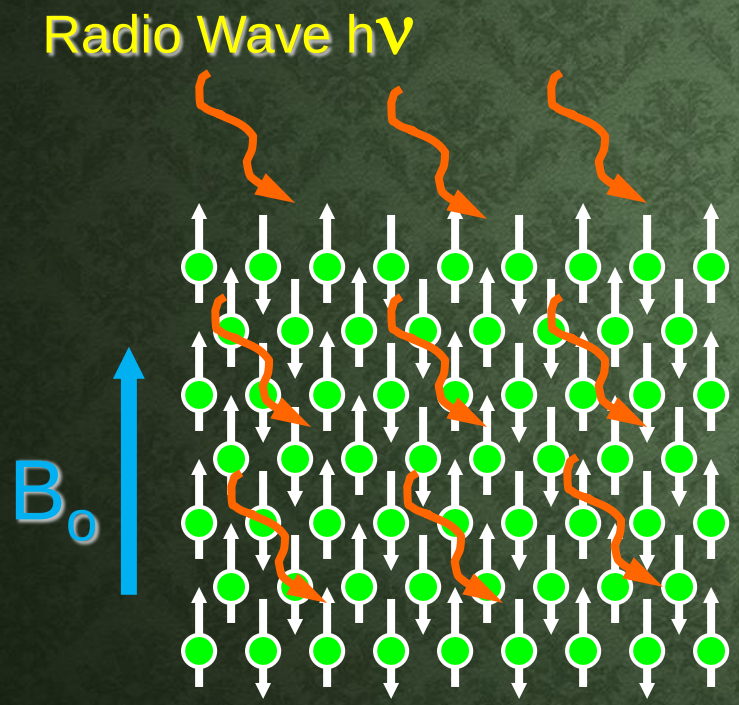
Kurt Wűthrich
Chemistry, 2002

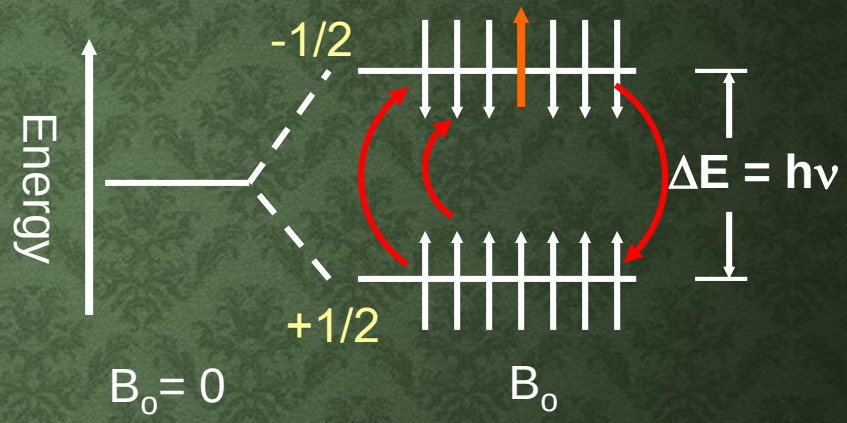


Paul C. Lauterbur
Physiol. Medicine, 2003

Peter Mansfield
Physiol. Medicine, 2003

NMR Spectroscopy





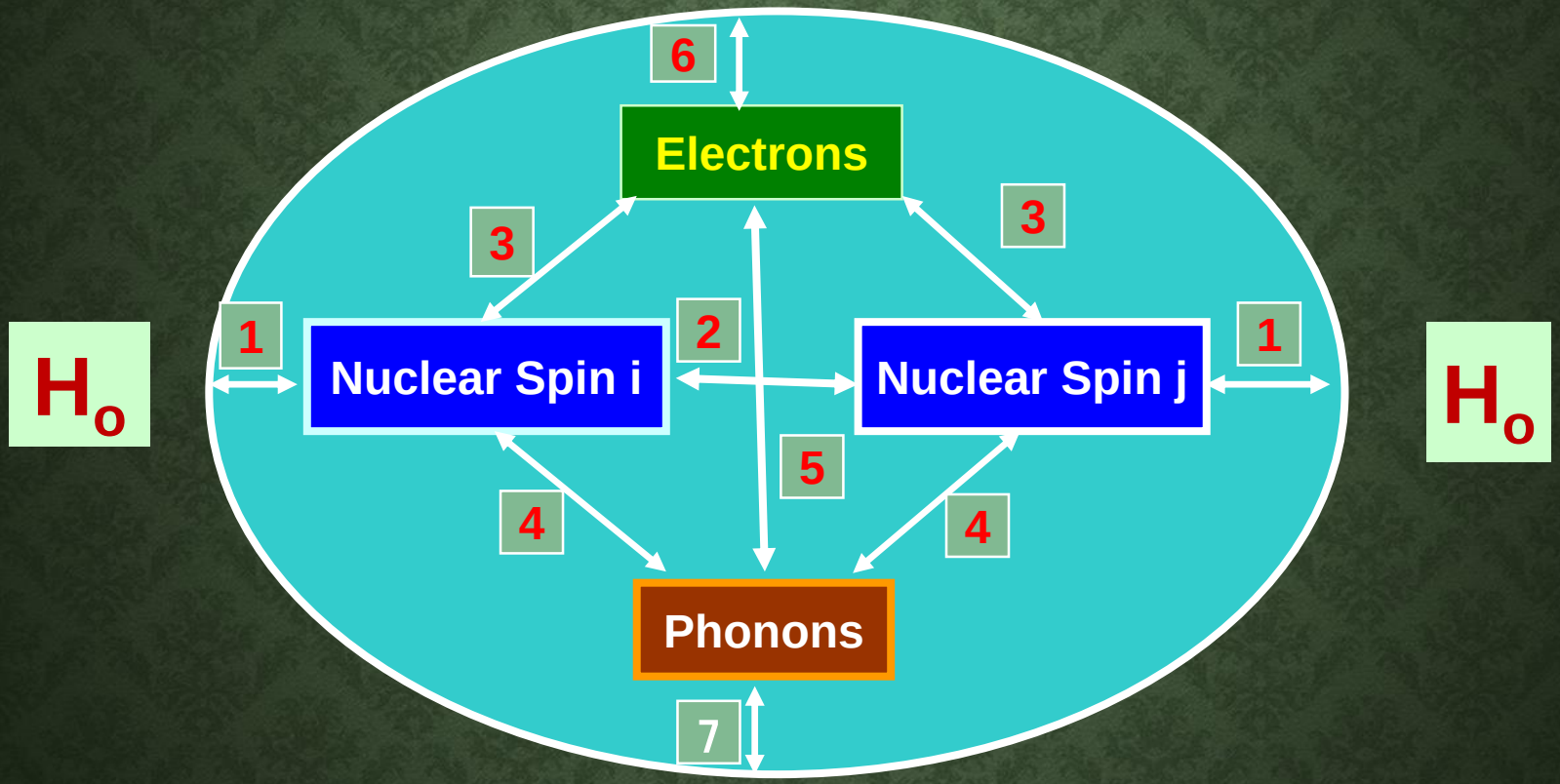
Biologically interested nuclei:
^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P (S=½), ^{2}D (S=1)

Larmor Equation (I = ½):

$$\nu = \gamma B_o / 2\pi$$

ν = Larmor frequency
γ = nuclear gyric ratio
B_o = magnetic field strength

Basic Nuclear Spin Interactions



Dominant Interactions: $H = H_Z + H_{CSA} + H_D + H_Q + H_J + \cdots$

H_z : Zeeman Int.;
H_{CSA} : Chemical Shielding Anisotropic Int.;
H_D= : Dipolar Int.
H_Q : Quadrupolar Int.
H_J : J–Coupling

Basic Nuclear Spin Interactions

Zeeman Interaction (H_z.) (Field depend);

Interaction of nuclear spin with external magnetic field .

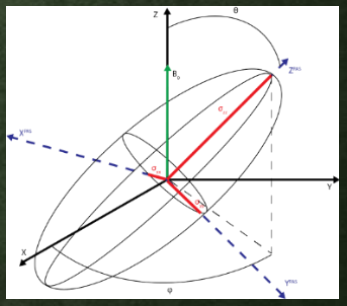
$$H_Q = -\gamma I_Z \cdot Bo$$

Chemical Shielding Anisotropic Interaction (H_{CSA}) (Field dep.);

The nuclear shielding effect of an applied magnetic field, caused by an induced magnetic field resulting from circulation of surrounding electrons

$$H_{CSA} = -\gamma I \cdot \sigma \cdot B_o$$

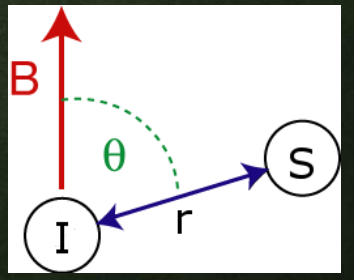
$$\sigma = \begin{pmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{pmatrix}$$



Dipolar Interaction (H_D) (Thru space) (Field indep):

Interaction between adjacent nuclear spins through magnetic dipolar field.

$$H_D = \frac{\hbar\gamma_I\gamma_S}{4\pi^2 r_{IS}^3}[1 - 3\cos^2\theta](3I_zS_z - \vec{I}\cdot\vec{S})$$



Quadrupolar Interaction (H_Q) : (Field indep)

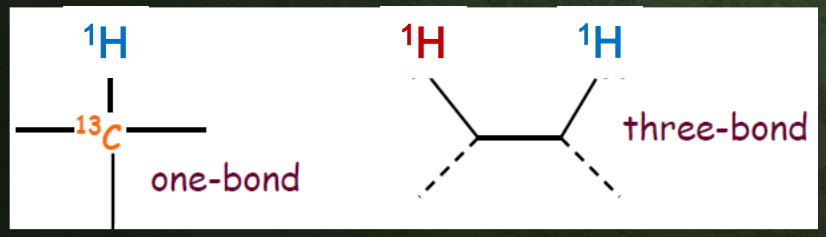
Nuclei with spin > 1/2 have a asymmetric distribution of nucleons (non spherical distribution of positive electric charge)

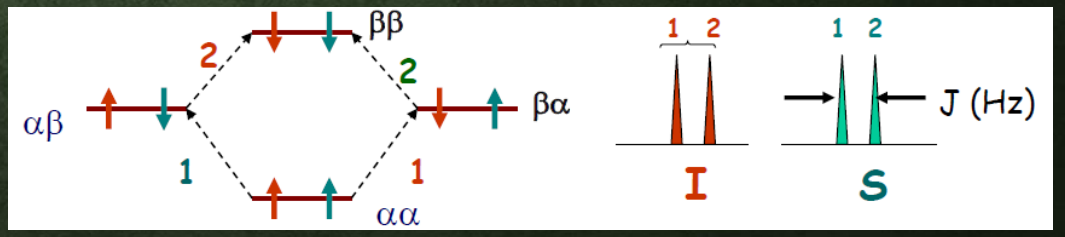
$$H_Q = I \cdot V \cdot I$$

$$V = \begin{bmatrix} V_{xx} & V_{xy} & V_{xz} \\ V_{yx} & V_{yy} & V_{yz} \\ V_{zx} & V_{zy} & V_{zz} \end{bmatrix}$$

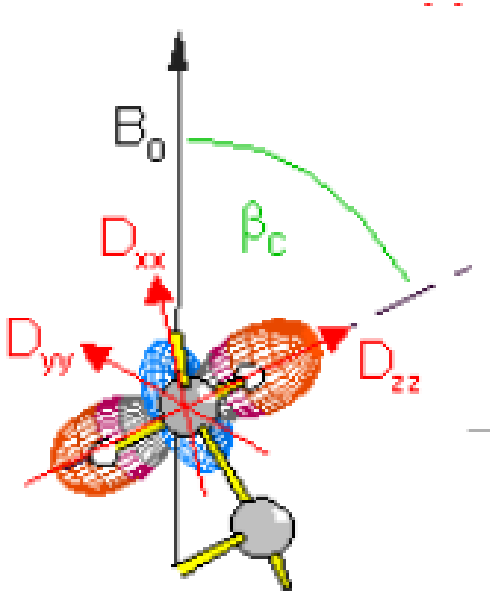
J-Couplings (Thru bond connection) : (Field indep)

Resonance splitting mediated through chemical bonds connecting two spins. It is an indirect interaction between two nuclear spins which arises from hyperfine interactions between the nuclei and local electrons.



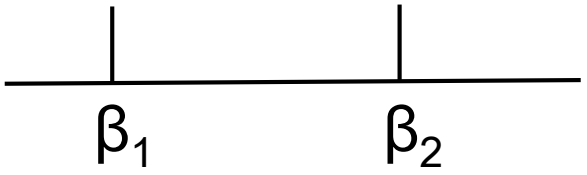


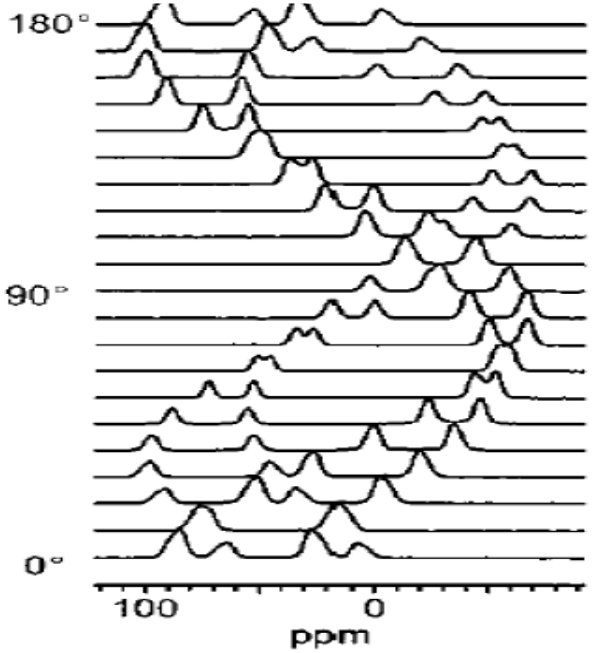
Interaction	Magnitude (Hz) (^{1}H at 2.1T)
Zeeman	10^8
Quadrupole	10^6
Chemical shift	10^3
Dipole	10^3
J-Coupling	10



The resonance frequency of a nuclear spin in single crystal depends on the orientation of the tensorial interaction w.r.t. the magnet field.

Single crystal





NMR spectrum of samples in solid states

Powder patterns

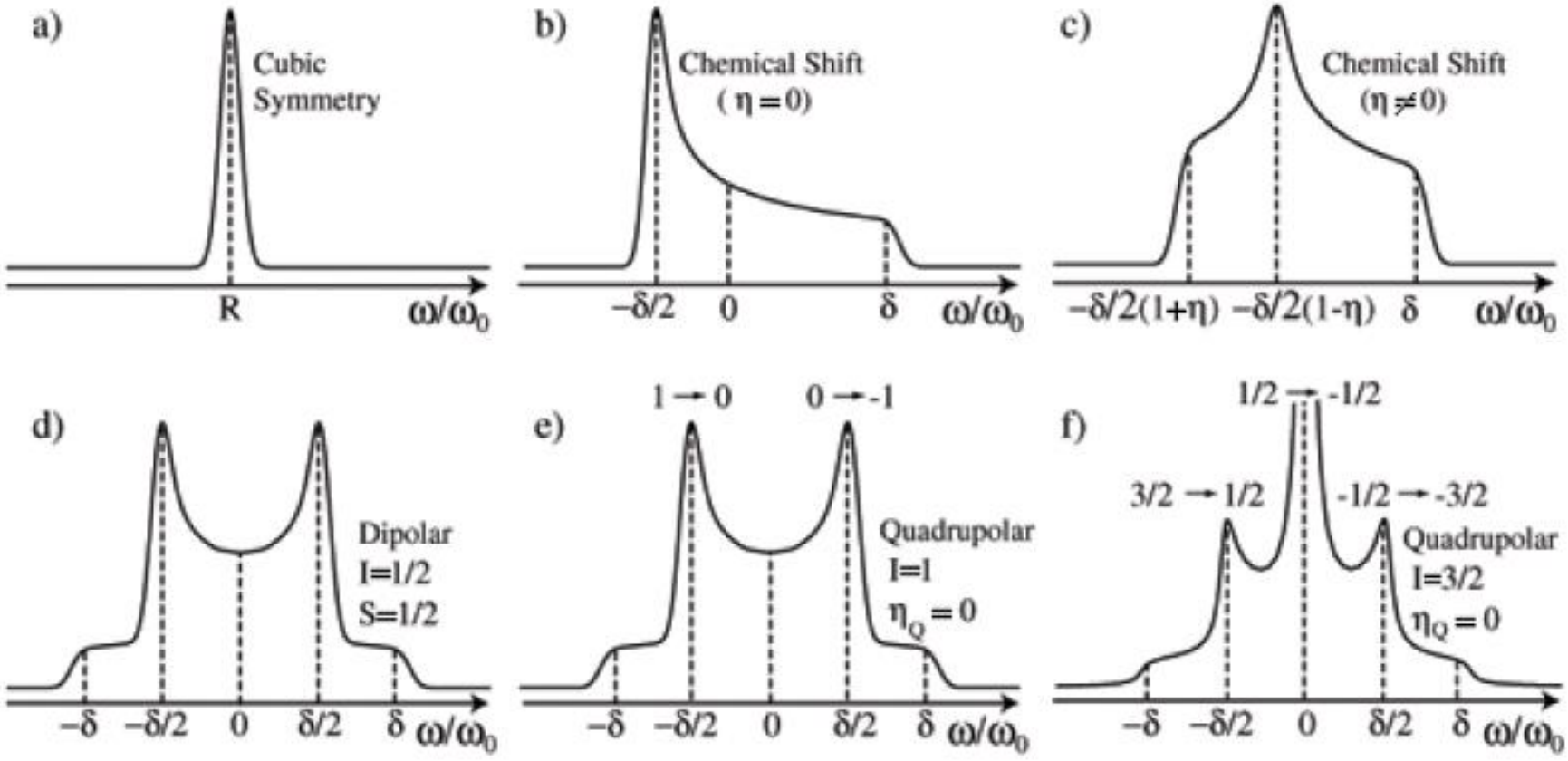


FIG. 1: Calculated NMR spectra of polycrystalline or amorphous samples (powder patterns) corresponding to different NMR interactions: a-c) Chemical Shift under different symmetry conditions; d) Dipolar interaction between two spins 1/2 distant each other by a fixed distance; and e-f) Quadrupolar interaction for spins 1 and 3/2.

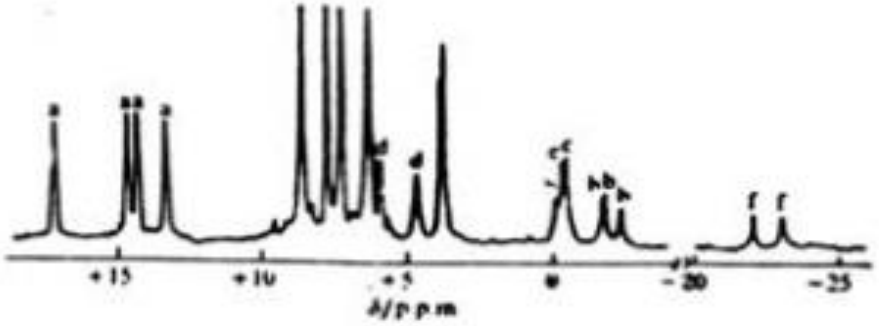
NMR spectra of samples in different states

Small molecules in solution

$\langle H_D \rangle = \langle H_Q \rangle = 0$

$\langle H_{CSA} \rangle = \sigma_{iso}$; $\langle H_J \rangle = J_{iso}$

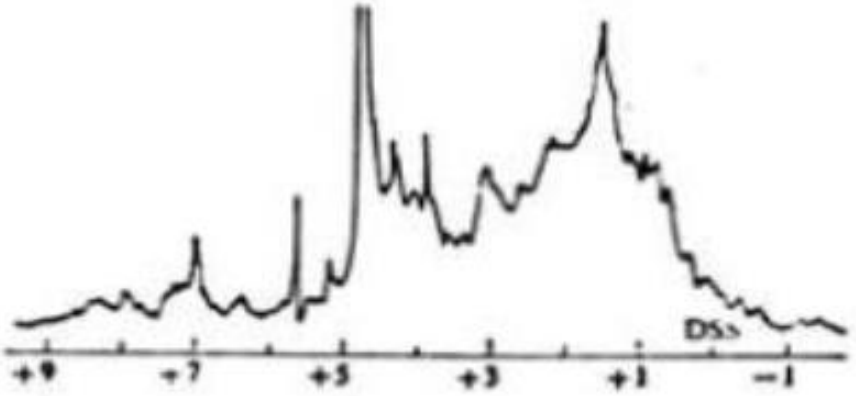
Well–resolved sharp lines



Macromolecules

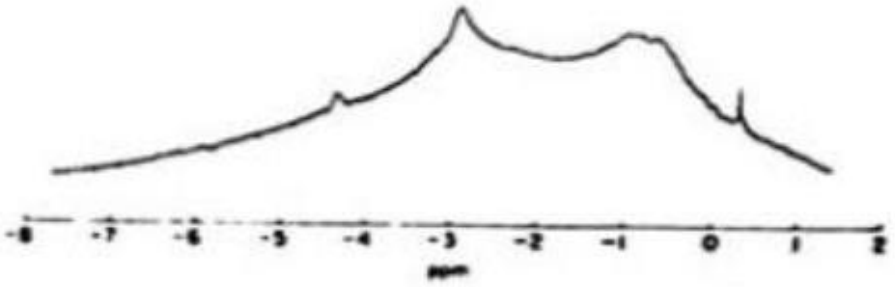
(Slow tumbling)

Broad overlapping



Gel state

(Featureless humps)



1. NMR spectra contains rich information derived from the presence of multiple interactions.
2. Each interaction provide insights into the structure/dynamics of the spin system.
3. It is difficult to quantify the interaction when there are more than one present.

Question:

How to extract the inter-twined interactions ?

➔ Design special pulse sequences to selectively observe/ suppress certain interaction(s)

➔ Spin gymnastics

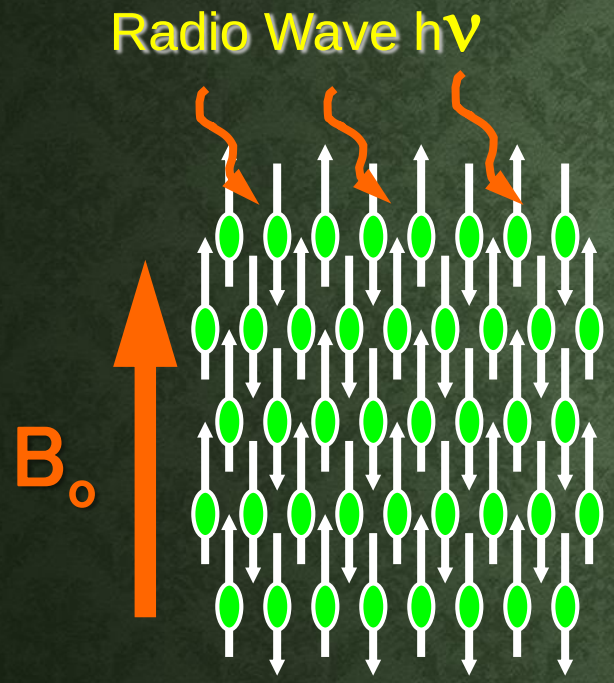
Example: (HSQC)

(2D Heteronuclear Single Quantum Correlation Spectroscopy)

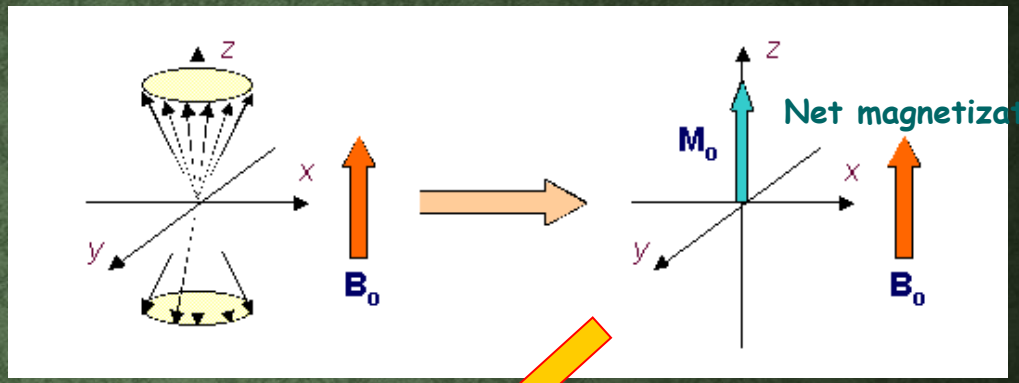
Features:

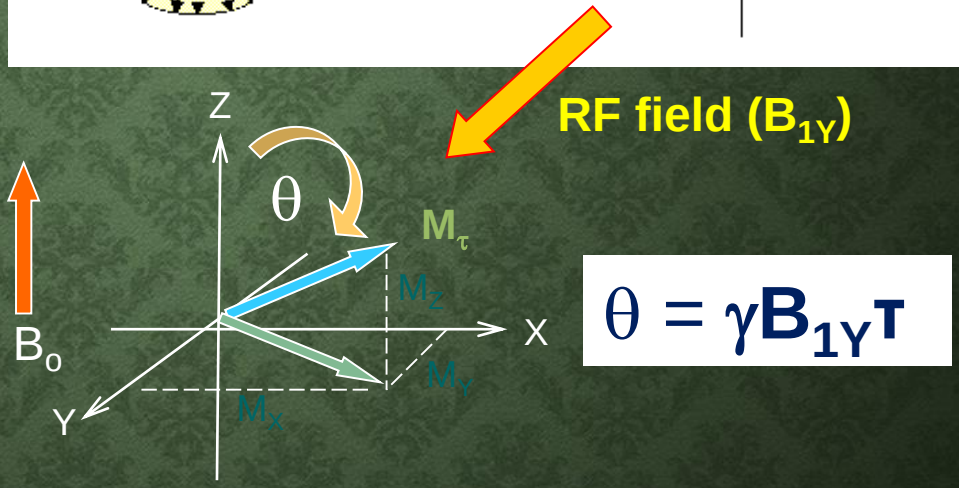
1. Dramatically increased spectral resolution !
2. Dramatically increased sensitivity of insensitive nuclei !
   Enhancement factor $\propto (\gamma_H/\gamma_I)^3$
3. Opened a door for thru-bond sequential resonance assignment (Thru J-coupling).
4. The idea can be extended to higher dimension to include multiple nuclei and field gradients etc

NMR Spectroscopy



Classical view





$$\theta = \gamma B_{1Y} \tau$$

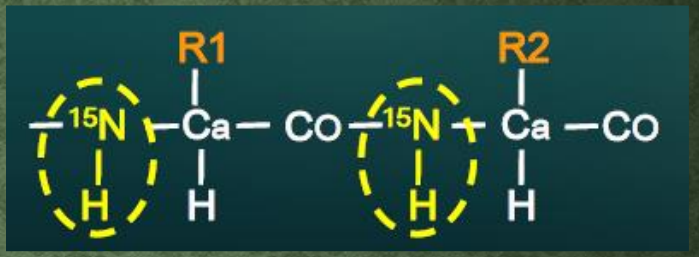
Magnetization will be flipped around Y–axis toward X–Y plane by an angle θ, determined by the RF field strength and the pulse duration.

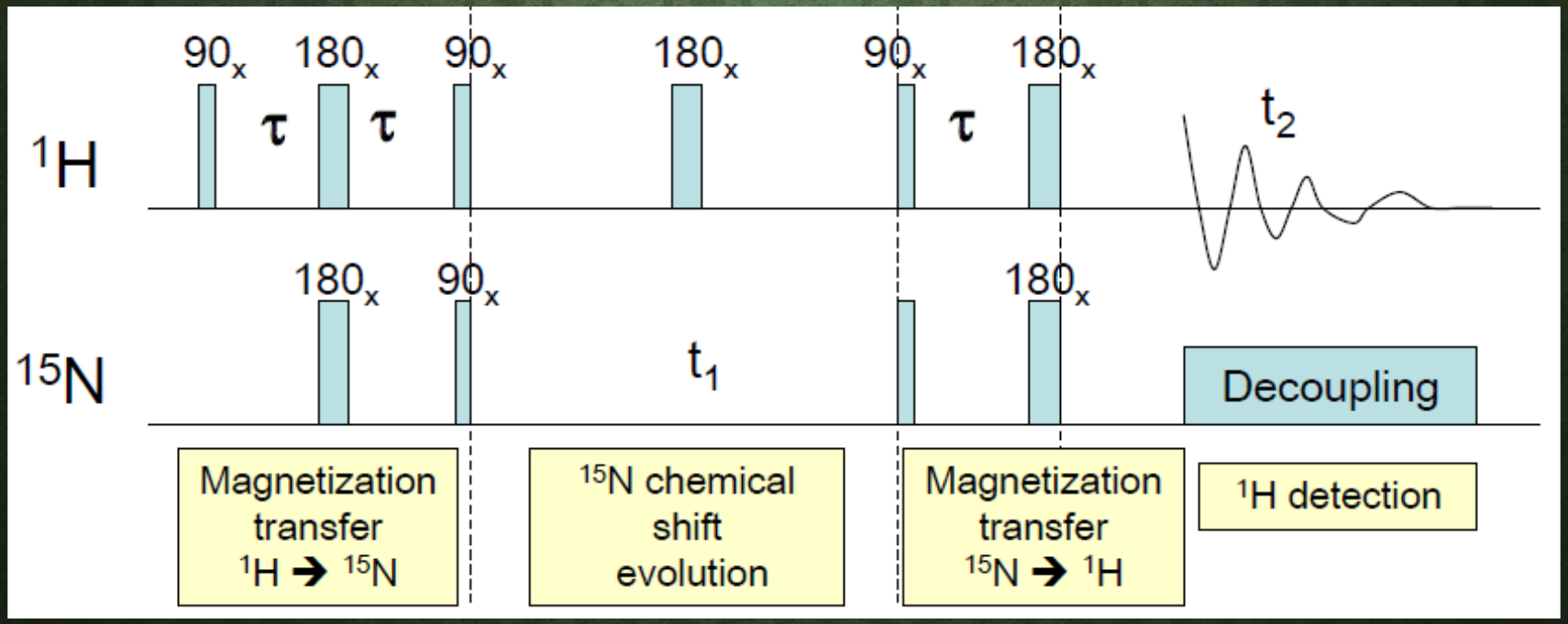
θ = 90° it is call a 90° pulse or π/2 pulse (maximum signal)

θ = 180° it is call a 180° pulse or π pulse (No signal)

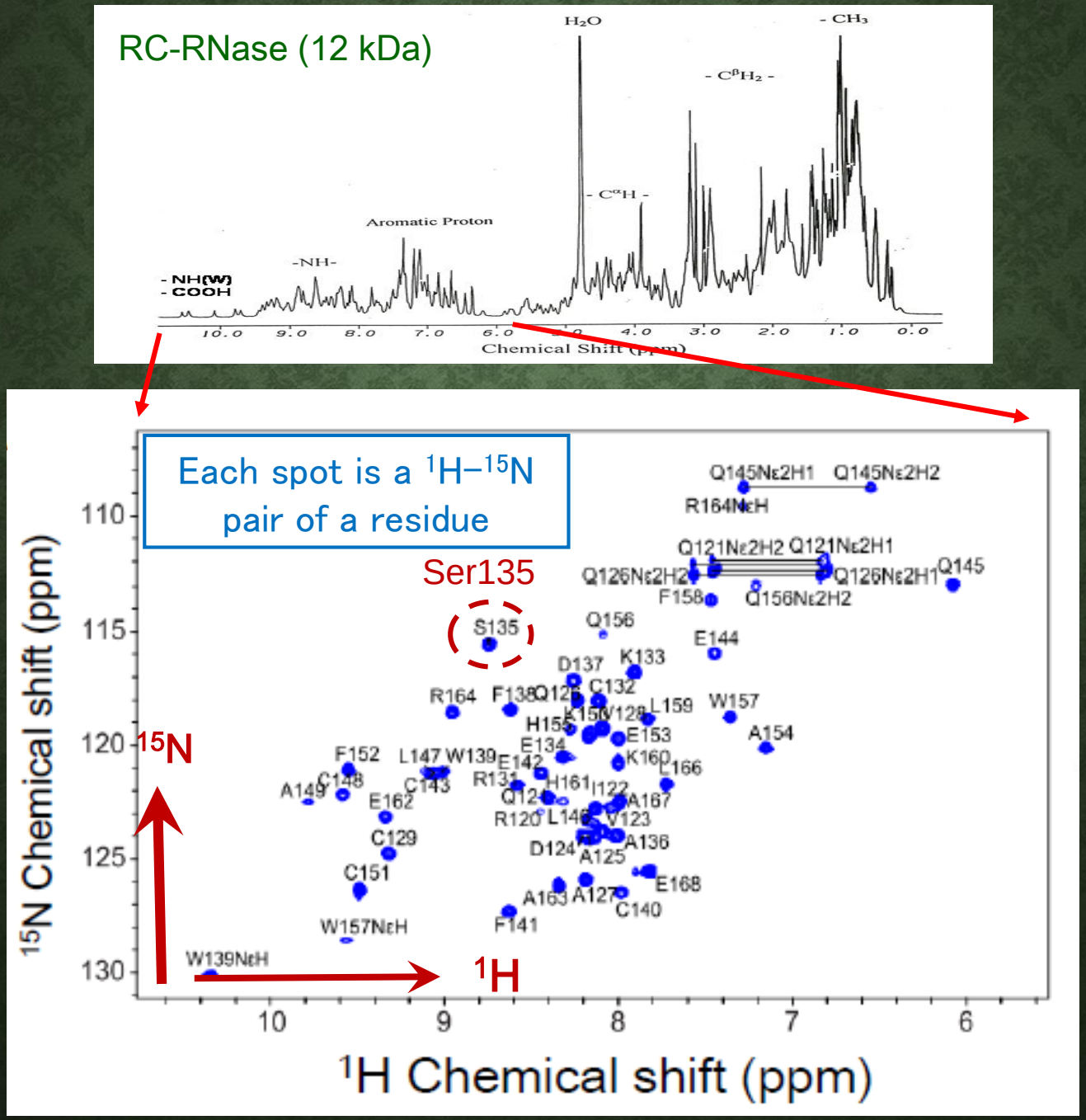
Pulse sequence for ^{15}N–HSQC expt

Protein peptide chain





Efficiency $\propto \sin(2\pi J\tau)$; Maximum transfer when $2\pi J\tau = \pi/2$.


RC-RNase (12 kDa)
H2O
- CH3
- CβH2 -
- CαH -
Aromatic Proton
-NH-
- NH(W)
- COOH
10.0
9.0
8.0
7.0
6.0
4.0
3.0
2.0
1.0
0.0
Chemical Shift (ppm)
Each spot is a 1H–15N pair of a residue
Ser135
15N
1H
15N Chemical shift (ppm)
1H Chemical shift (ppm)
110
115
120
125
130
10
9
8
7
6
Q145Nε2H1
Q145Nε2H2
R164NεH
Q121Nε2H2
Q121Nε2H1
Q126Nε2H2
Q126Nε2H1
Q145
F158
Q156Nε2H2
S135
Q156
E144
D137
K133
C132
R164
F138
Q125
L159
W157
H155
K150
V128
E153
A154
E134
K160
F152
L147
W139
E142
L166
A149
C148
C143
R131
H161
I122
A167
E162
Q124
R120
L146
V123
C129
D124
A136
A125
C151
E168
A163
A127
C140
W157NεH
F141
W139NεH

Biomedical Applications

Molecules ➔ Cell ➔ tissue ➔ Organ ➔ Whole body

1. Chemical Identification:
   A. Identification of metabolites (Metabonomics)
   B. Drug discovery.

2. Macromolecular structure:

3. Macromolecular Dynamics:

4. Magnetic Resonance Imaging (MRI):

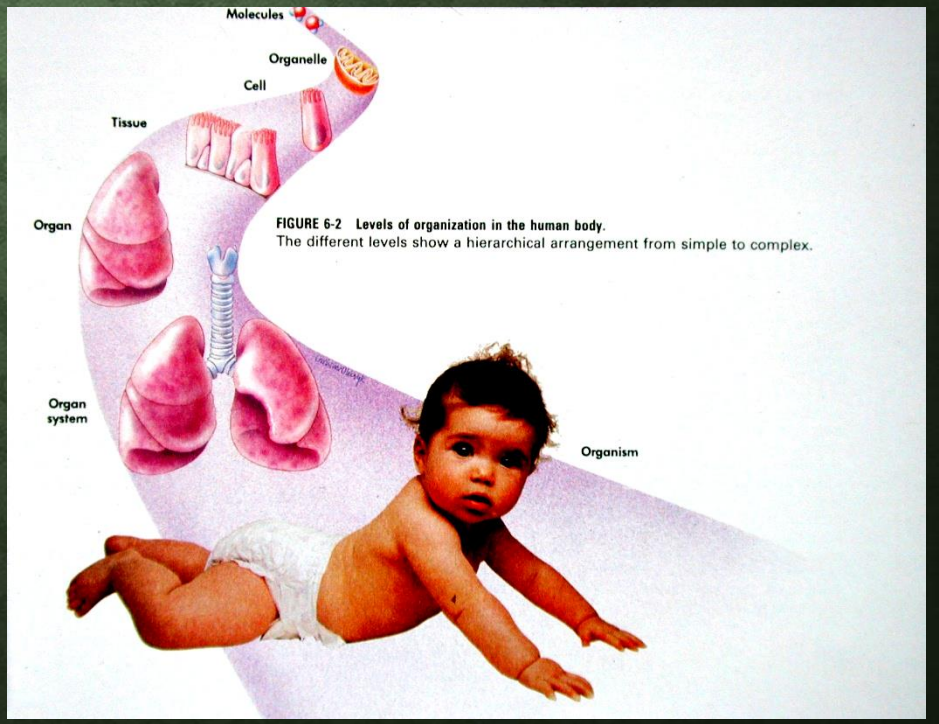
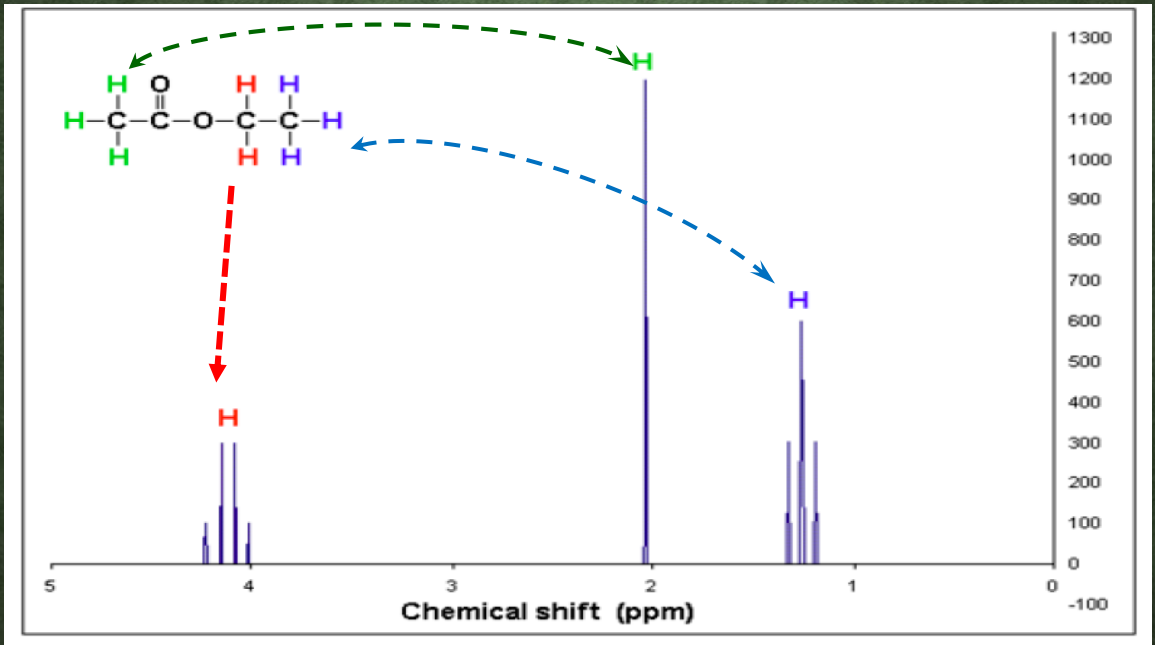


FIGURE 6-2 Levels of organization in the human body.
The different levels show a hierarchical arrangement from simple to complex.

1. Chemical Identification:

Proton spectrum of ethyl acetate



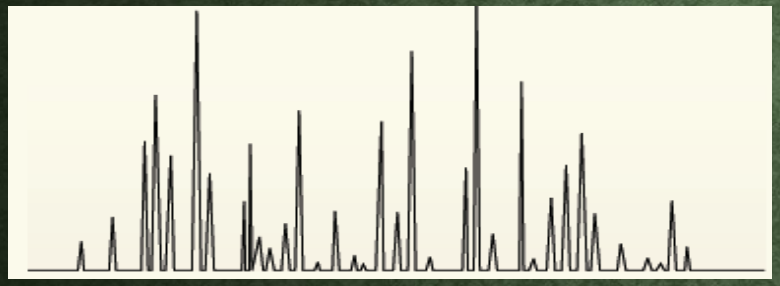
NMR spectrum is the finger print of a chemical

➔ Organic synthesis, natural product identification etc.

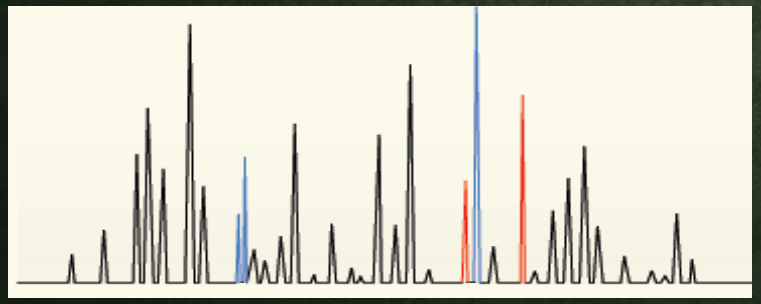
2. Metabonomics – (Nicholson and Lindon, Nature 455, 1054, 2008)

Metabonomics aims to measure the global, dynamic metabolic response of living systems to biological stimuli or genetic manipulation. It seeks an analytical description of complex biological samples and to characterize and quantify all the small molecules in such a sample (Urine, blood, plasma etc).

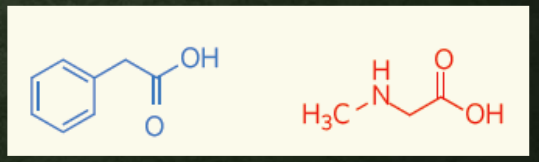
Raw data (Urine, blood etc)



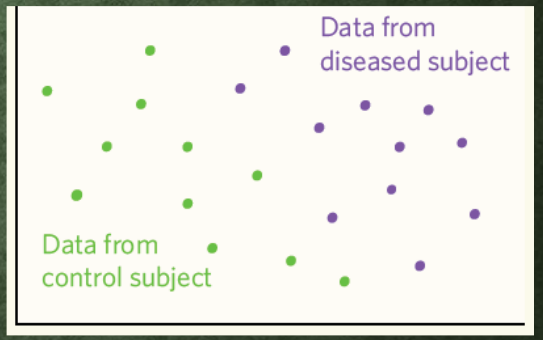
Pattern recognition



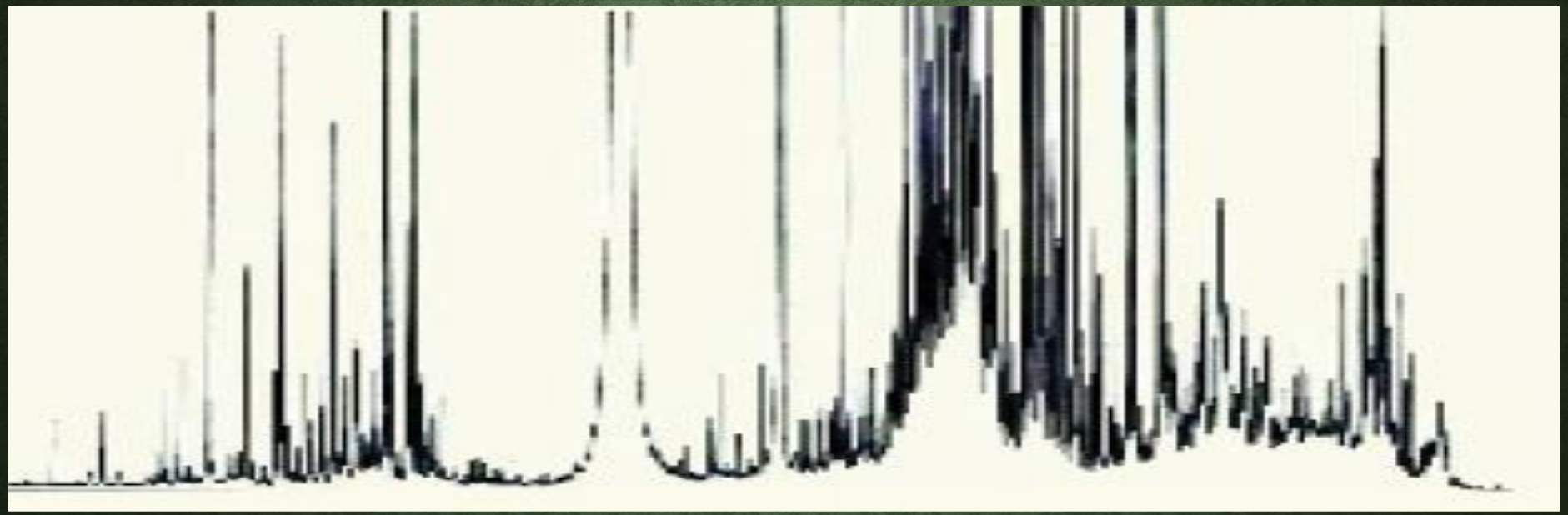
Identify metabolites



Statistical analysis



NMR spectrum of human urine

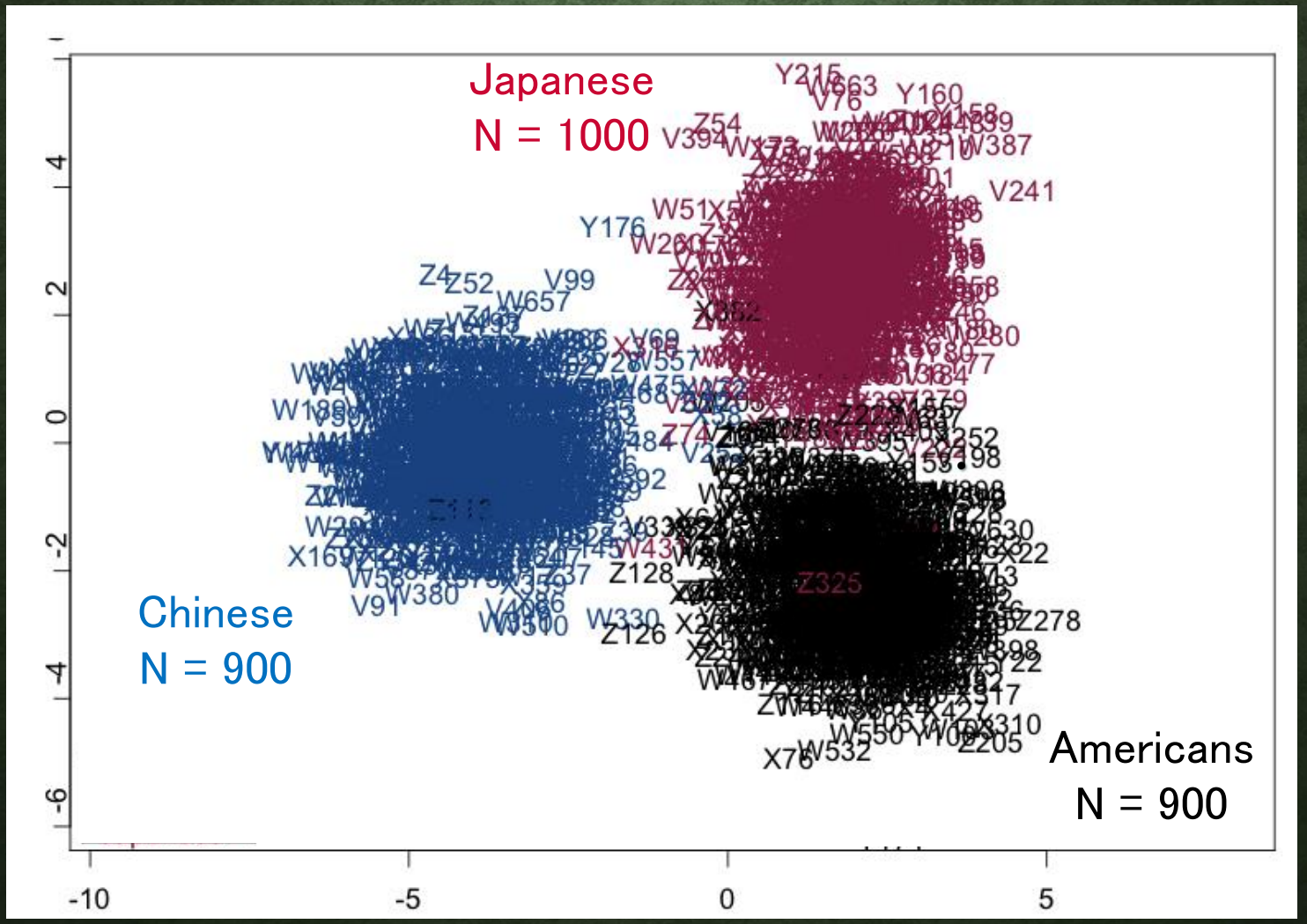


Very complex !

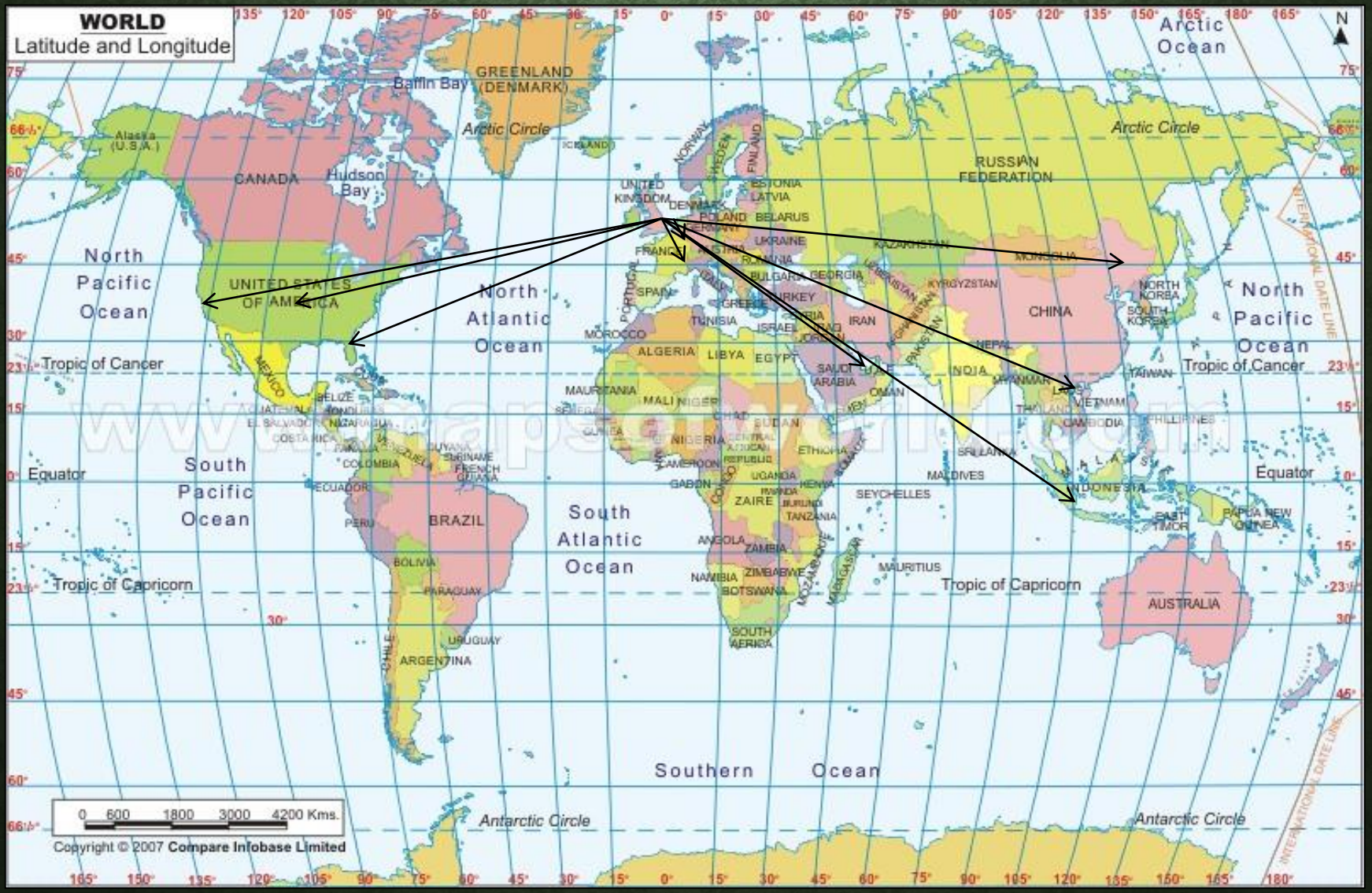
Population studies show:

Metabolic variation is much larger than genetic variation !

(Urinary Metabotypes)



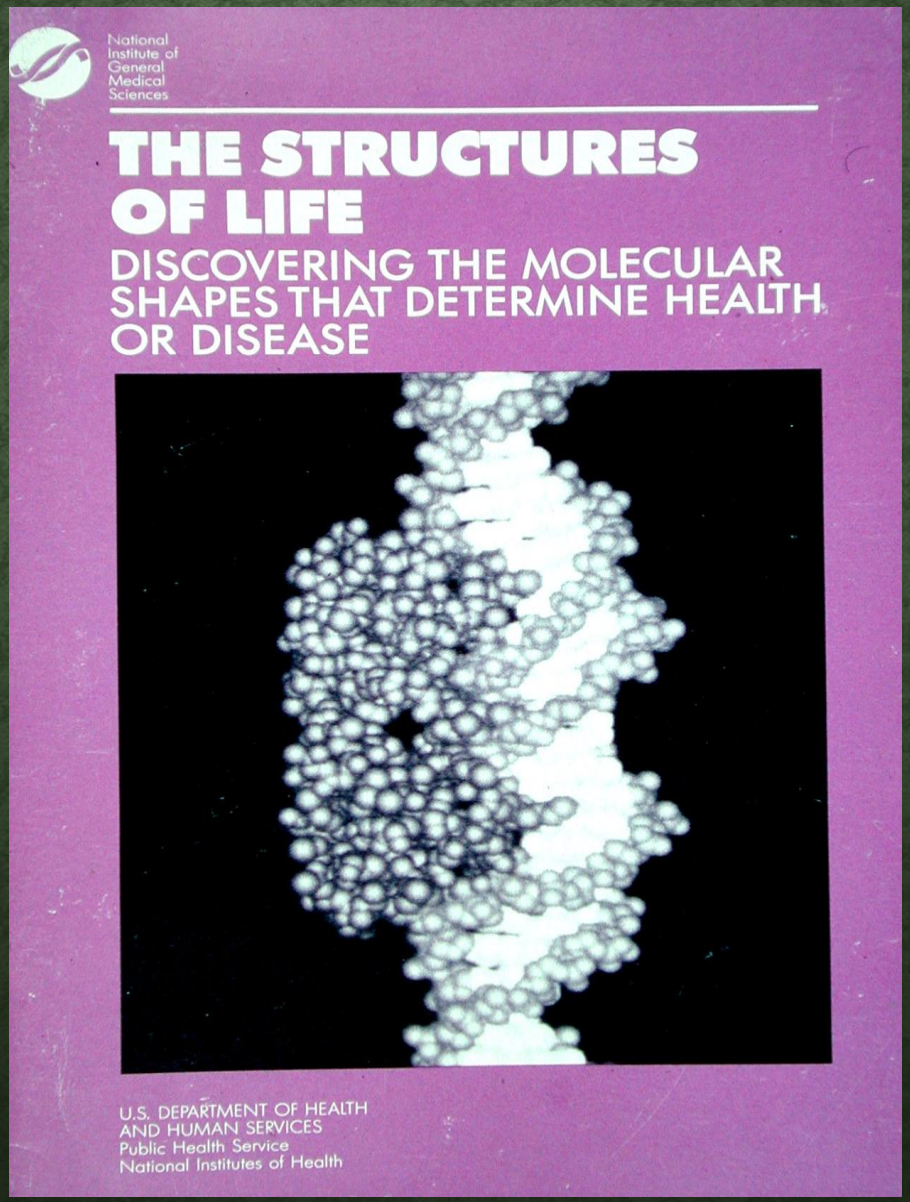
The World Phenome Center network



中研院台灣人體生物資料庫
(Taiwan Biobank)

- Collect and sequencing 300k samples (200K healthy, 100K patients of various diseases).
  (Already Collected over 60k samples now.)
- Perform genome sequence data of all samples for researchers performing other analyses (Data mining).
- Already identified diabetes markers from genome analysis.
- Hope to include NMR- and Mass-based metabonomics data.

2. Macromolecular structure/function



15 February 2001

nature

www.naturejpn.com

the human genome

Nuclear fission
Five-dimensional energy landscapes

Seafloor spreading
The view from under the Arctic ice

Career prospects
Sequence creates new opportunities

naturejobs
genomics special

dup.

16 February 2001

Science

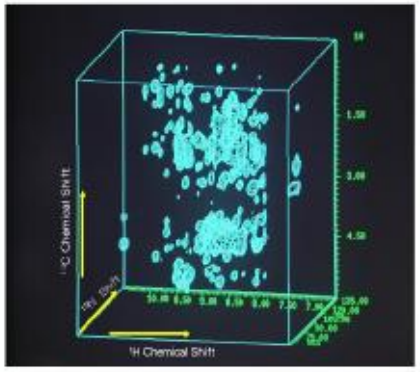
Vol. 291 No. 5507
Pages 1145–1434 $9

THE HUMAN GENOME

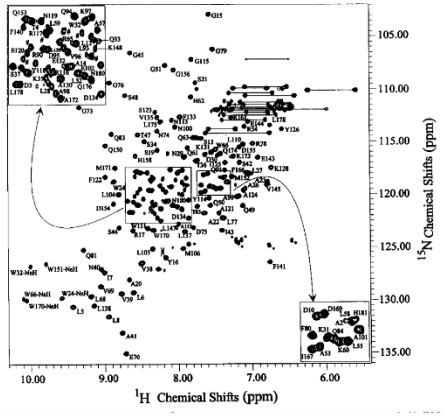
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

Determine Protein Structure by NMR

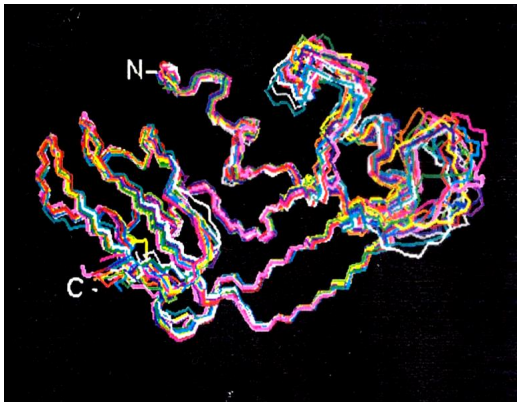
NMR Sample
(1 mM, 0.4 ml)
^{2}H, ^{13}C, ^{15}N-label



Obtain NMR spectra

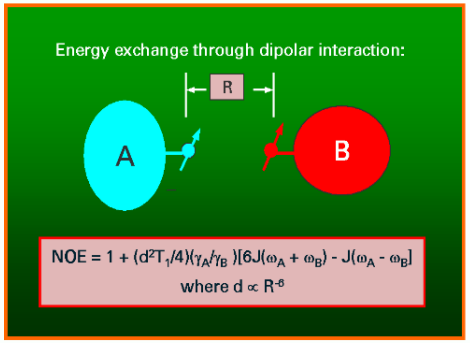


Assign resonances



NMR structures
(Ensemble of 20 structures)

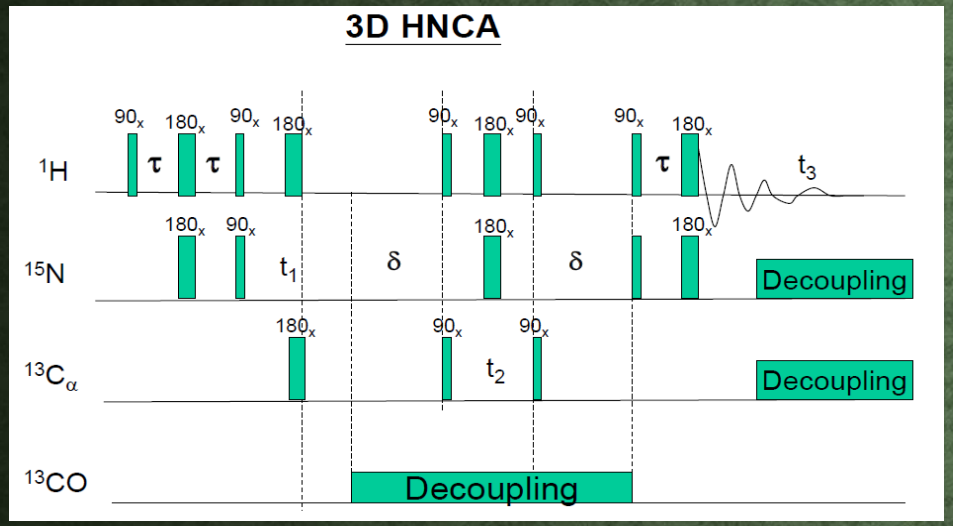
Calculate structures



Obtain restrains
(Distances, angles,
Orientations etc)

Sequential resonance assignments

Heteronuclear multidimensional NMR experiments thru J-coupling

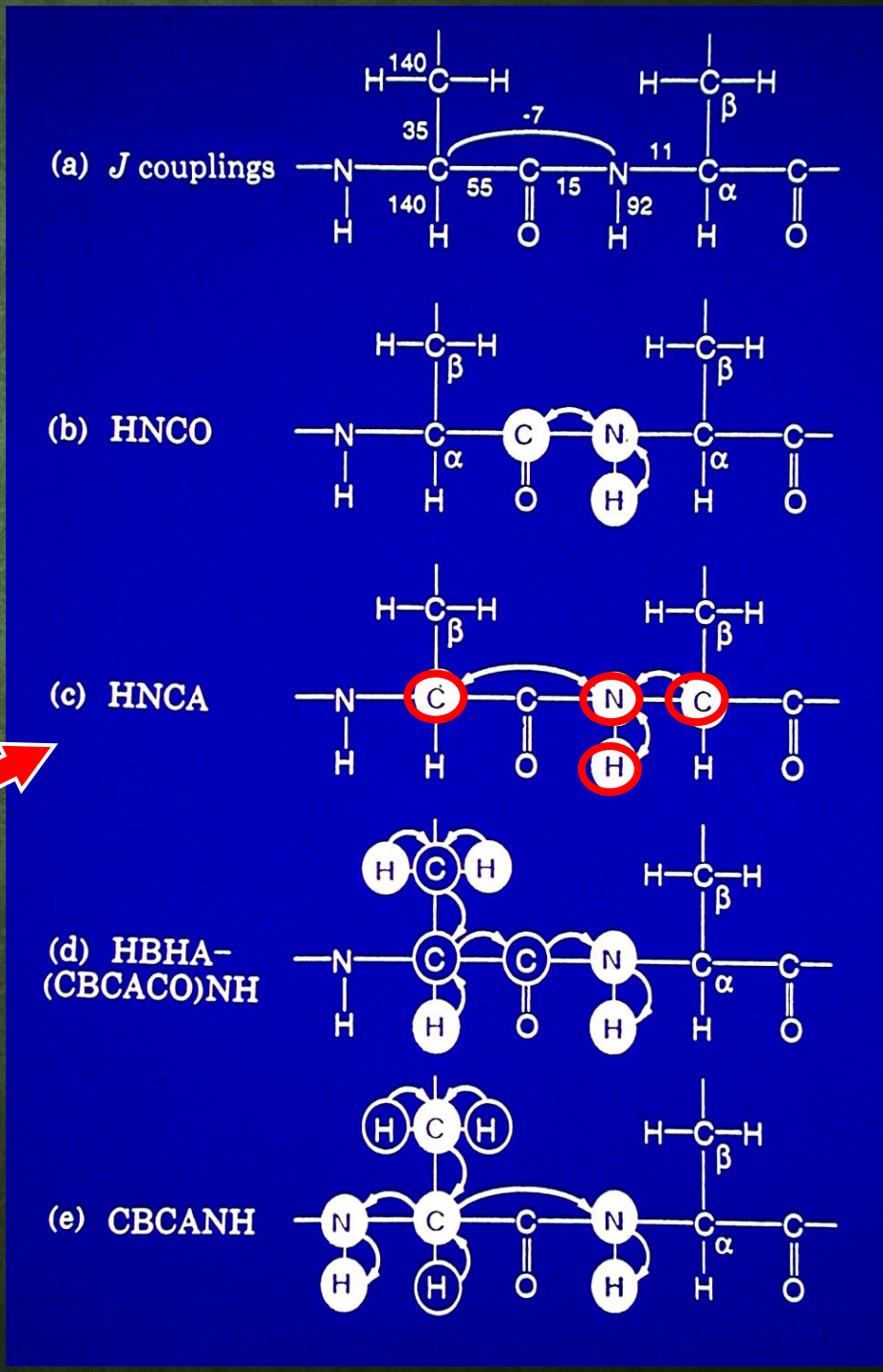


M transfer pathway for HNCA:

^{1}H → ^{15}N → $^{13}C_\alpha$ → ^{15}N → ^{1}H for Detection

→ Detect ^{1}H, ^{13}C, ^{15}N resonances

→ Permit sequential correlation of backbone ^{1}H–^{13}C–^{15}N resonances !!!



Structure Calculation

1. Build a random structure of the given sequence.
2. Energy minimization with least violation by molecular dynamics and simulated annealing to generate many structures.

$$E_{total} = E_{bond} + E_{angle} + E_{improper} + E_{VDW} + E_{cdih} + E_{NOE} + E_{RDC} + \cdots$$

$$E_{bond} = \Sigma k_b (b - b_0)^2; \quad E_{\phi} = \Sigma k_{\phi} (\phi - \phi_0)^2; \quad E_{VDW} = \Sigma k_{ij} [(\sigma_{ij}/r_{ij})^{12} - \sigma ij/r_{ij})^6]$$

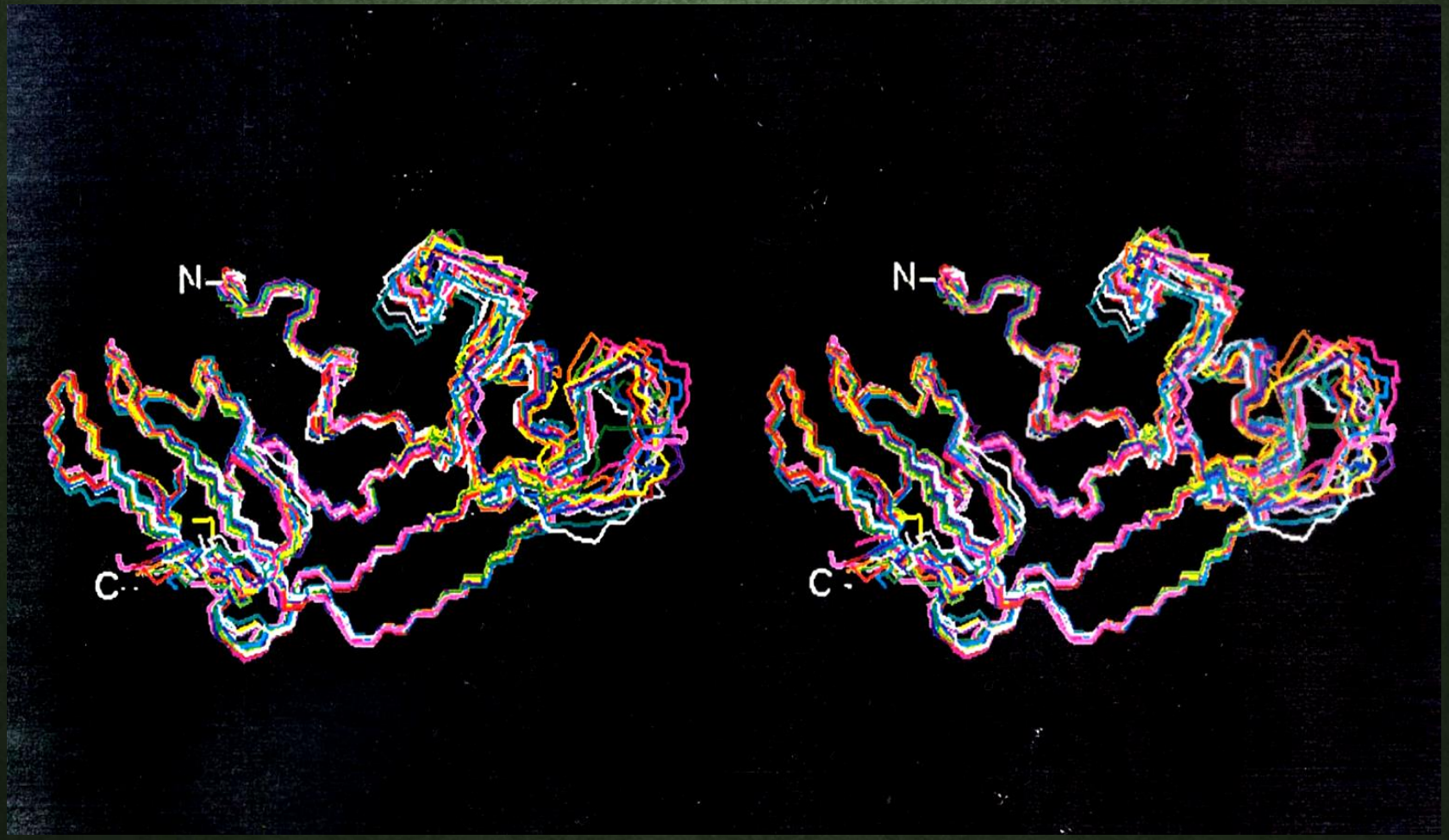
$$E_{improper} = \Sigma k_{impr} (\omega - \omega_0)^2; \quad E_{cdih} = \Sigma k_{cdih} (\Psi - \Psi_0)^2;$$

$$E_{NOE} = \Sigma k_{NOE} (\gamma - \gamma_0)^2; \quad E_{RDC} = \Sigma k_{RDC} (\theta - \theta_0)^2;$$

3. Select 20 structures of least NOE violation (> 0.5 Å).
4. Criteria for good structures:
   a) No NOE violation
   b) RMSD < 0.5 Å
   c) No dihedral angle violation (Ramachandran diagram)

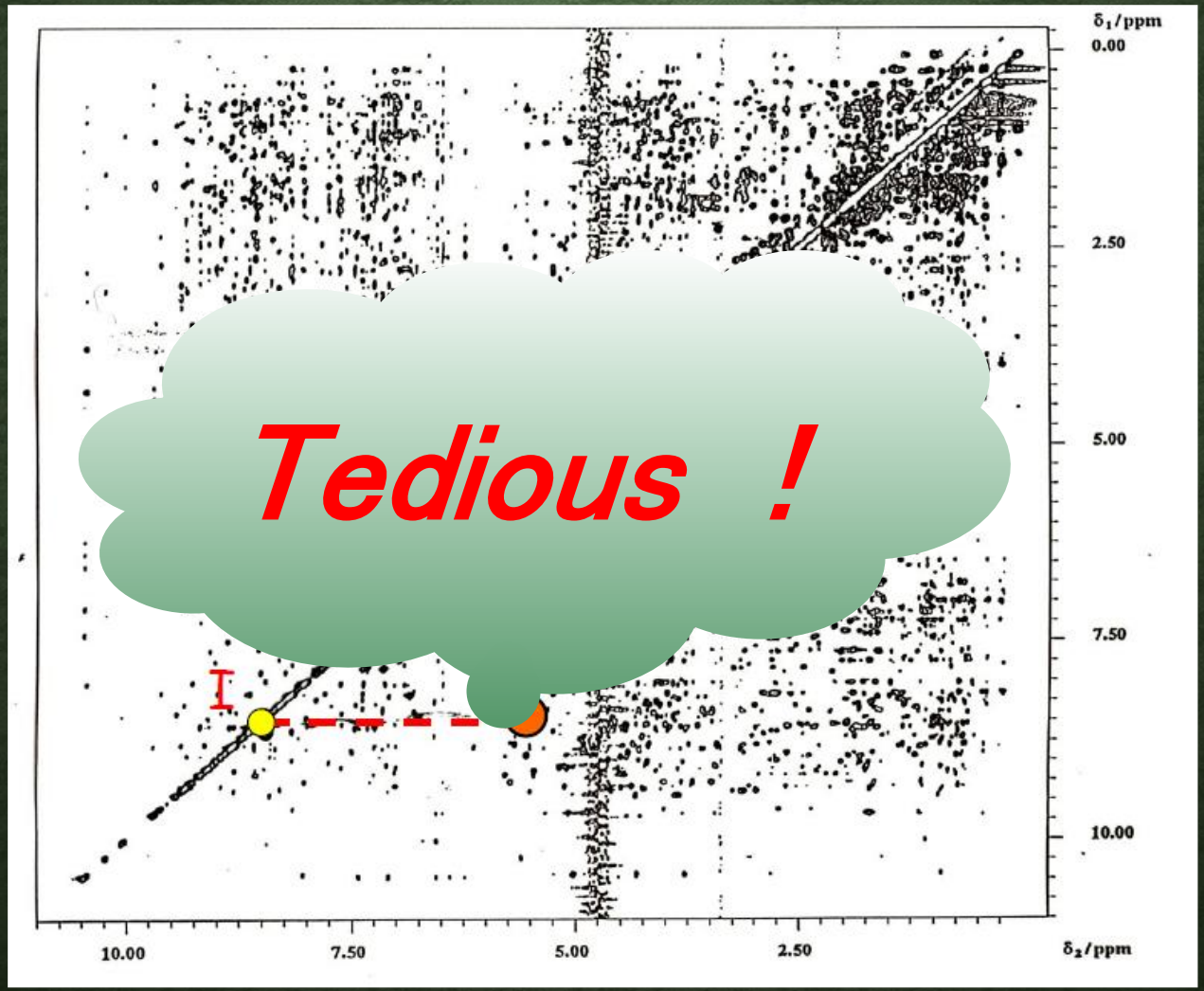
NMR structure of RC-Rnase

Ensemble of a set of lowest energy structures



^{1}H – ^{1}H NOESY spectrum of RC-Rnase

Identify short ^{1}H – ^{1}H distances



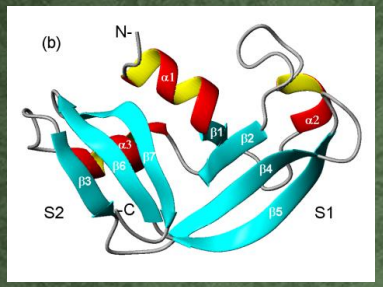
^{1}H chemical shift (ppm)

^{1}H chemical shift (ppm)

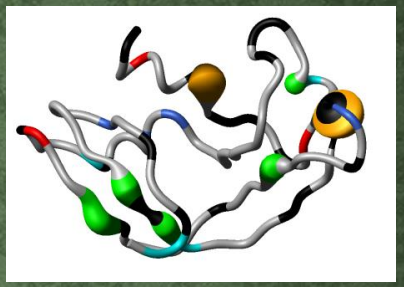
Gallery of structures determined

RC-RNase

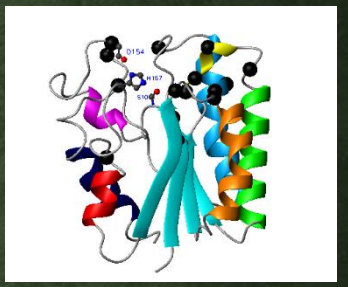
Onconase



Dynamics of onconase

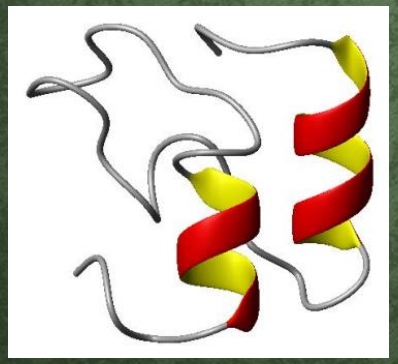


E. Coli Thioesterase

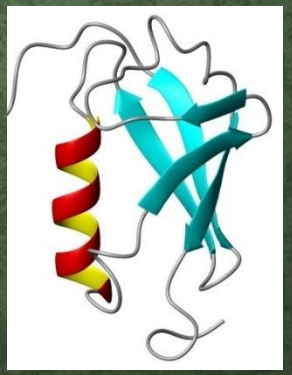


BCKD - LBD

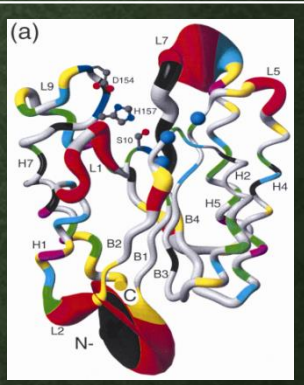
BCKD - SBD



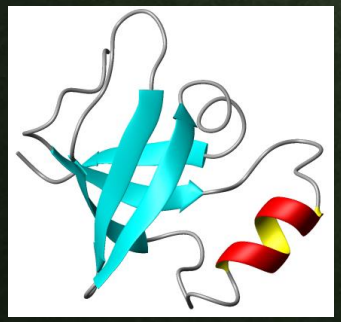
SUMO-3



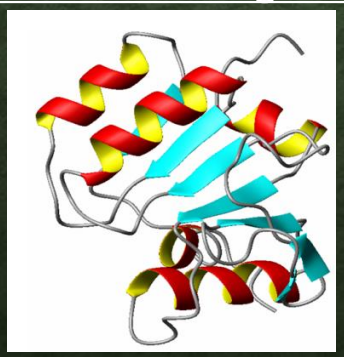
Dynamics-Fast Motion



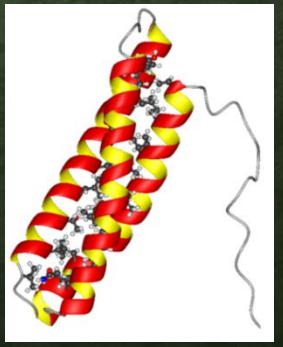
KP Feo A protein



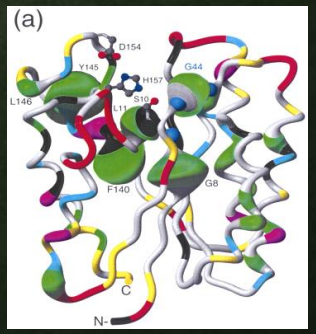
KP CoA Binding Protein



Blo t 5 Allergen

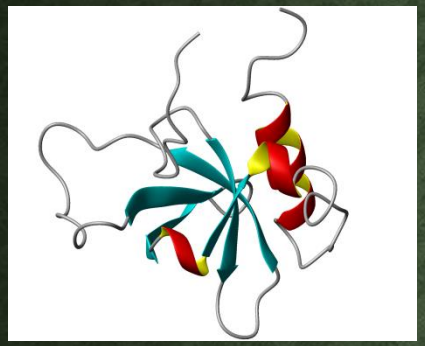


Slow Motion

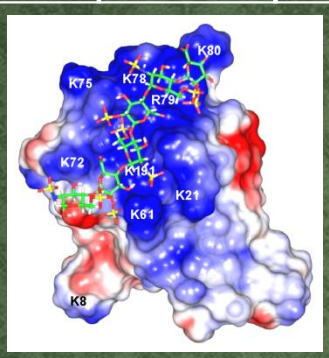


Gallery of structures determined

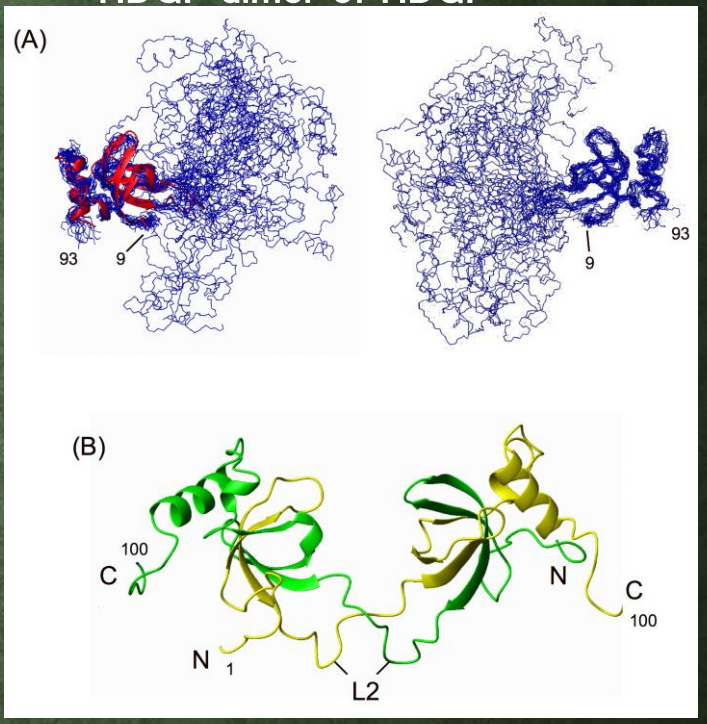
PWWP-domain of HDGF



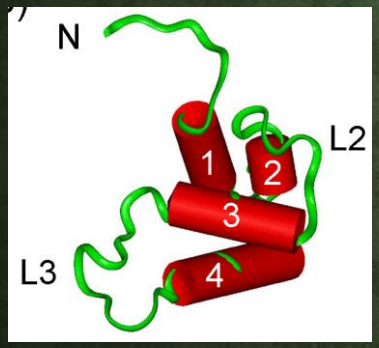
HDGF/heparin complex



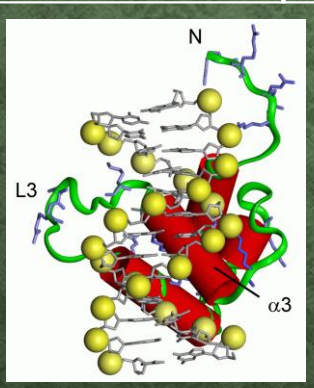
HDGF dimer of HDGF



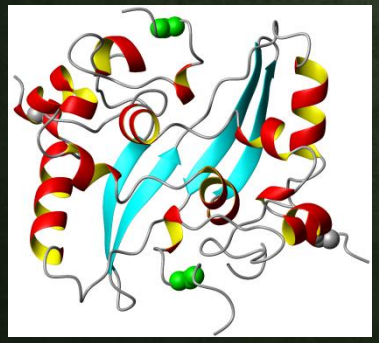
Telomere binding protein



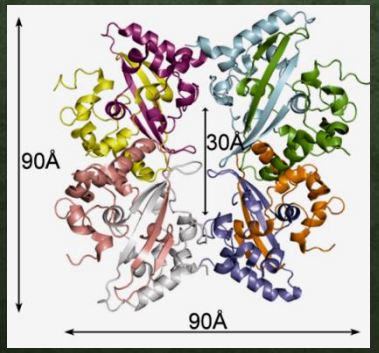
AtTRP/DNA complex



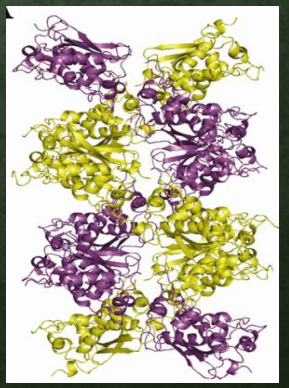
N248-365 of SARS CoV

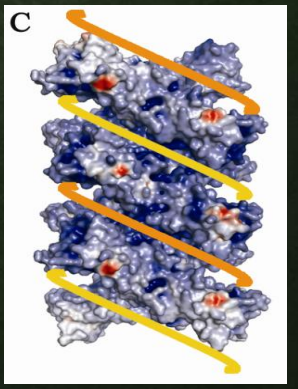


N248-365 of SARS CoV octamer

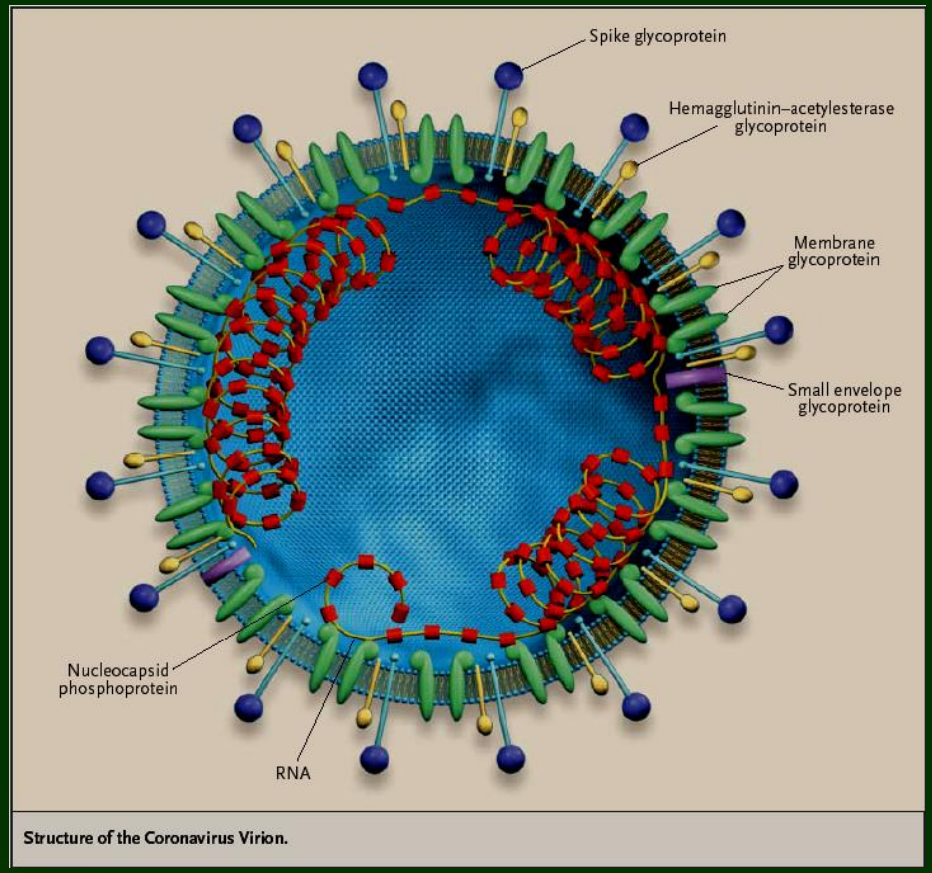


Model of N248-365 of SARS CoV/RNA complex





2.1. Packaging of SARS Coronavirus Ribonucleocapsid

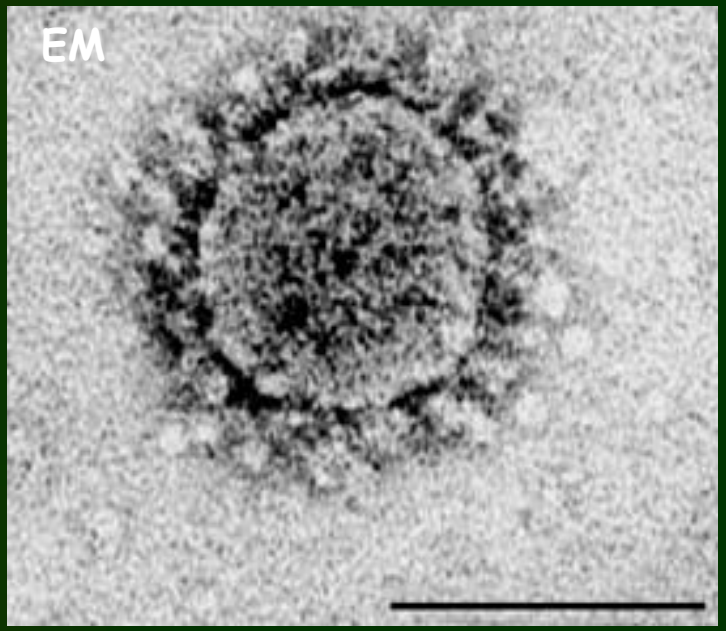


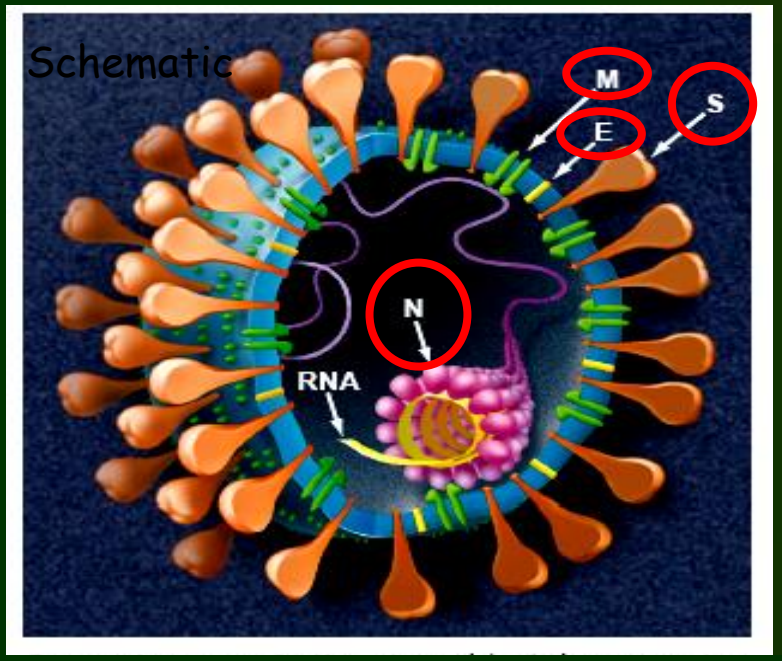
Structure of the Coronavirus Virion.


HI, DOCTOR. I'M GLAD I RAN INTO YOU. I HEARD THAT SHAKING HANDS CAN SPREAD THE SARS VIRUS... IS THAT TRUE?
BUS
STAYSKAL 4/03
TAMPA TRIBUNE

Causative agent – SARS Coronavirus

1. A single stranded plus-sense enveloped RNA virus.
2. Genome of 29,751 nt, containing 14 ORF encoding 28 proteins





Four Structural proteins:

S: Spike protein (1255 a.a.);

M: Membrane protein (221)

E: Envelope protein (76 a.a.)

N: Nucleocapsid protein (422 a.a.)

Nucleocapsid Protein (NP)

- The most abundant viral protein and a major antigenic determinant:
  - ➔ Target for detection and vaccine developments.
- Binds to RNA to form a helical ribonucleoprotein (RNP):
  - ➔ Important in virion assembly, packaging and release.
- Interacts with various host proteins and implicated in functions such as replication and apoptosis etc:
  - Interacts with AP-1 signal transduction pathway ?
  - Interacts with Smad3 and Modulates transforming Growth Factor- Signaling
  - Inhibits Cell Cytokinesis and Proliferation by Interacting with Translation Elongation Factor 1α

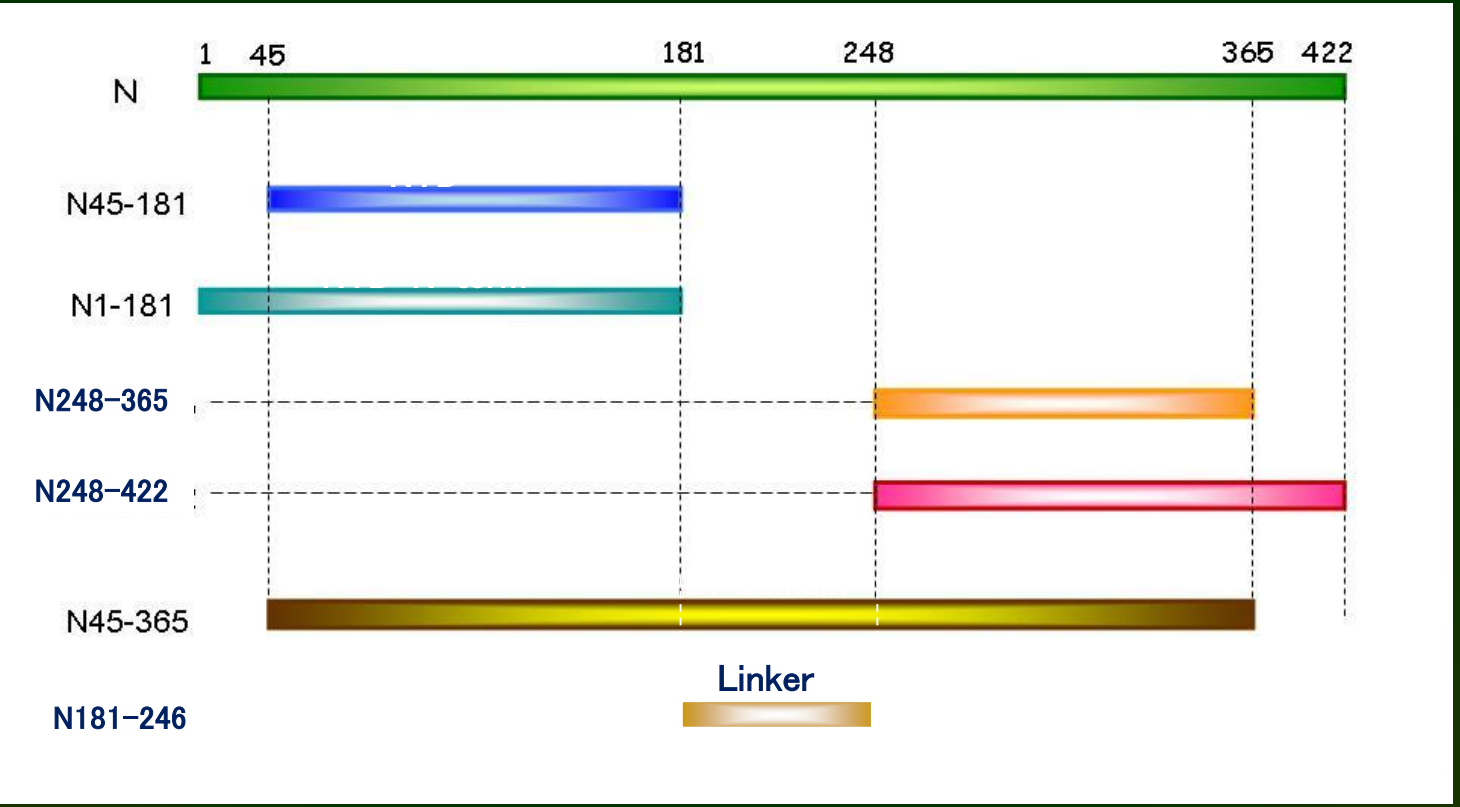
Goal

Unravel the packaging mechanism of helical ribonucleocapsid (RNP) :

1. Dissect N protein domain architecture
2. Probe N protein interaction with RNA.
3. Determine the tertiary structure of N protein.
4. Understand how RNA packs with N protein to form the helical RNP.

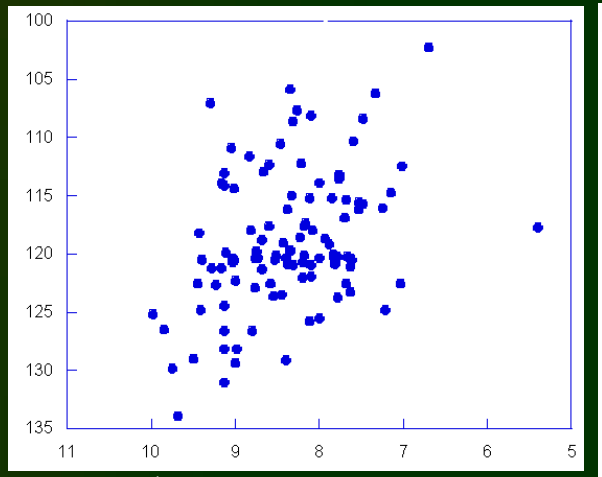
Dissecting Domain architecture of N protein

- The full length protein (422 a.a.) cannot be crystallized and the NMR spectrum is bad
- Divide and conquer – Construct many sub-fragments and characterize their structures.

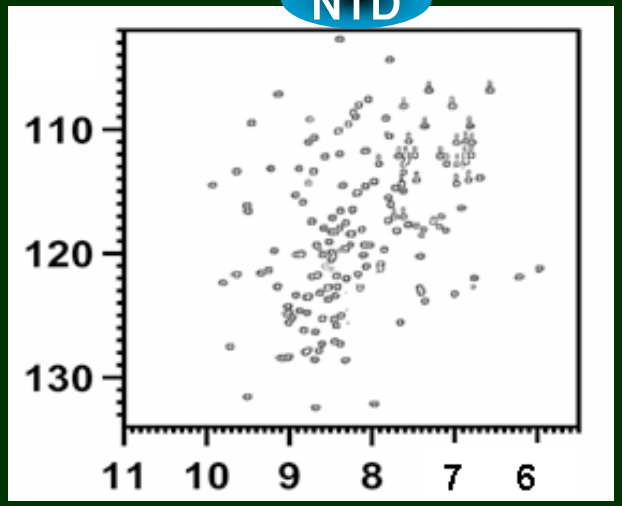


Characterization of protein order by 2D ^{15}N-HSQC

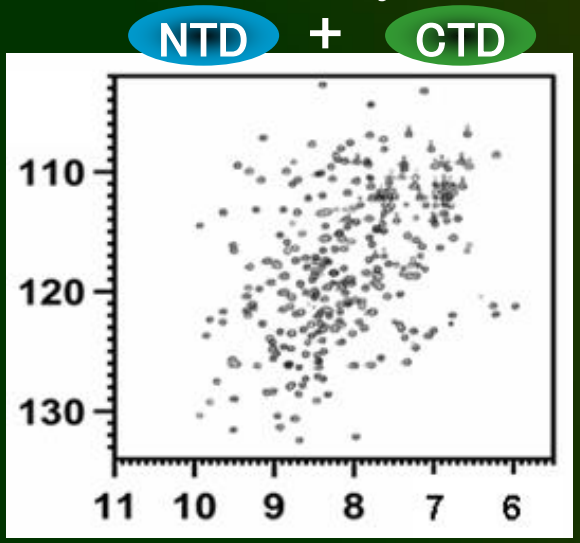
Folded



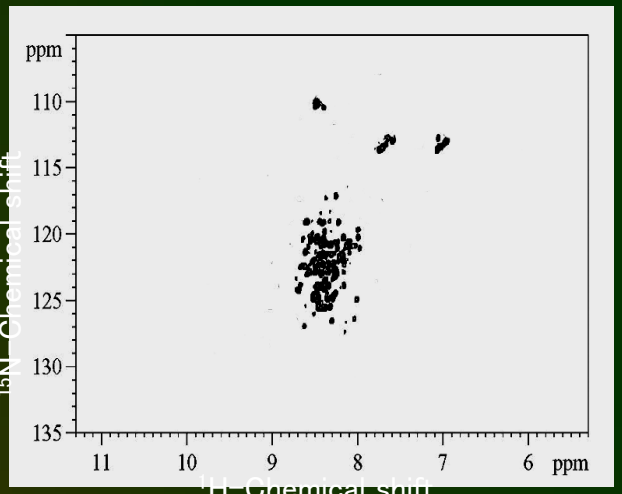
45– 181



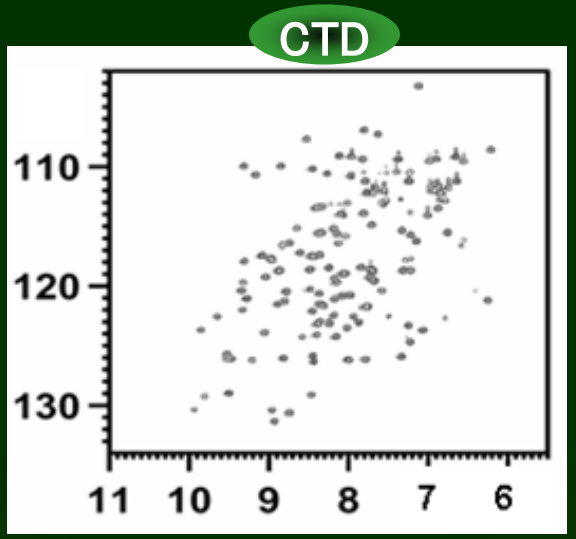
Overlay



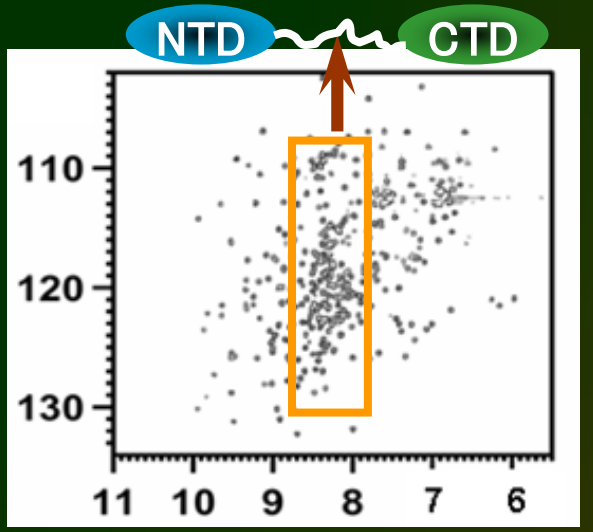
Disordered protein



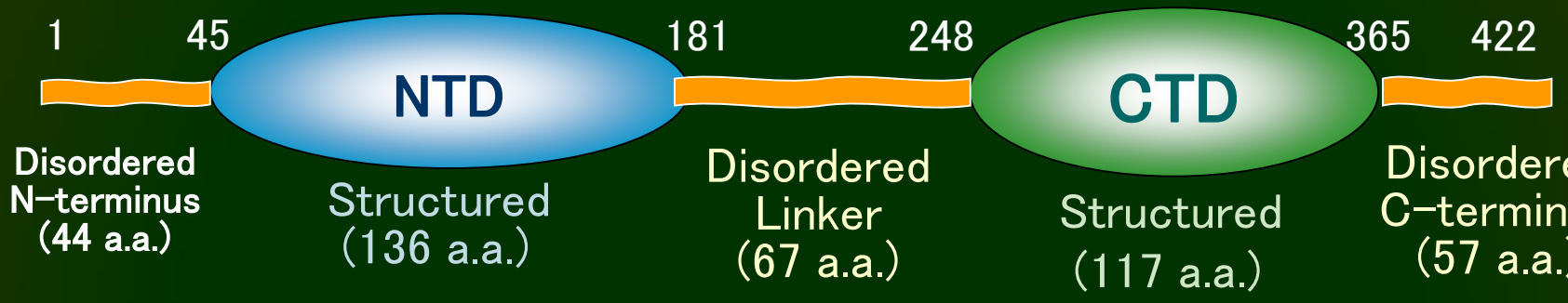
248–365



45–365

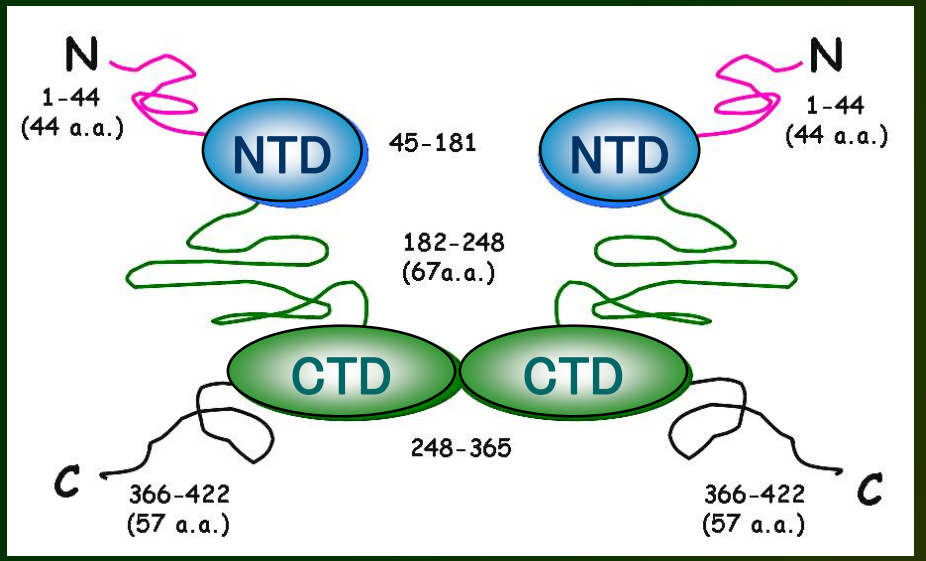


Domain architecture of SARS-CoV NP

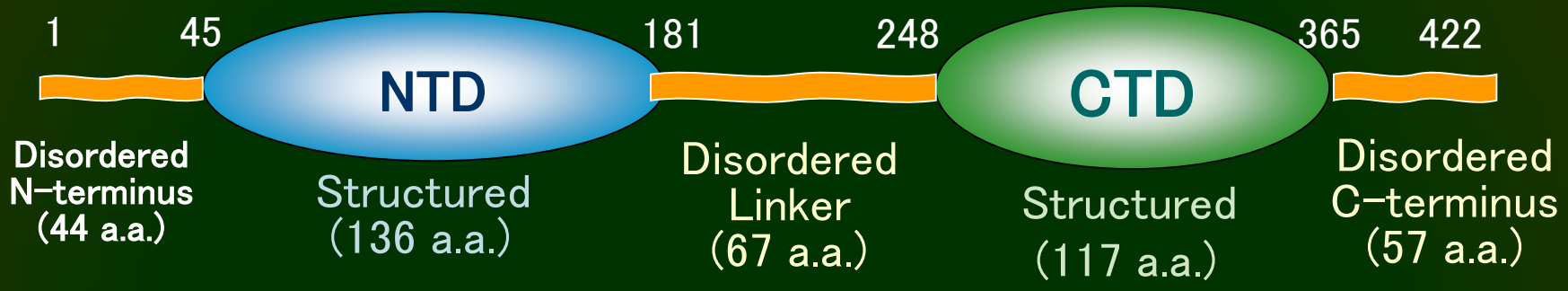


CTD forms a dimer

- Light scattering
- Analytical Ultra- Centrifugation
- Size exclusion chromatography
- Chemical cross linking
- NMR relaxation



Domain architecture of SARS-CoV NP



- ~ 50% of SARS-CoV residues exist in intrinsically disordered state.
- Nucleocapsid proteins belong to a class of proteins with the most disordered residues.

Why ?

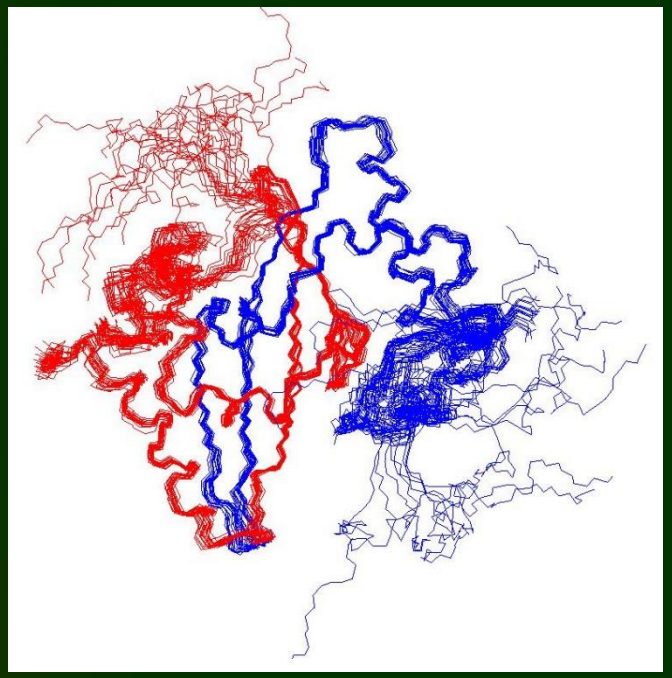
What are the advantages ?

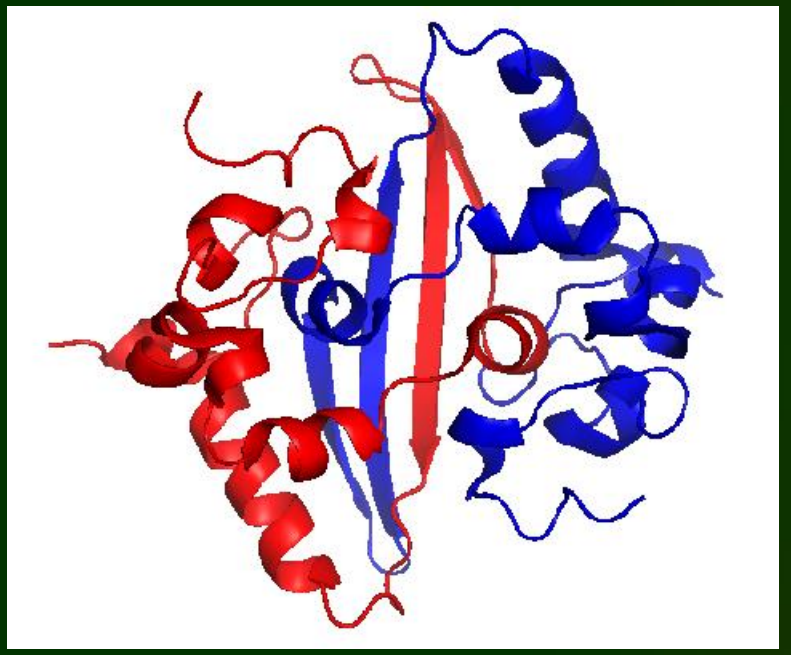
Advantage of intrinsic disorder

1. Increase collision cross section.
2. Adapt to different shapes.
3. Coupled allosteric effect (Multi-valency effect).

NMR Structure Of SARS-CoV NP CTD

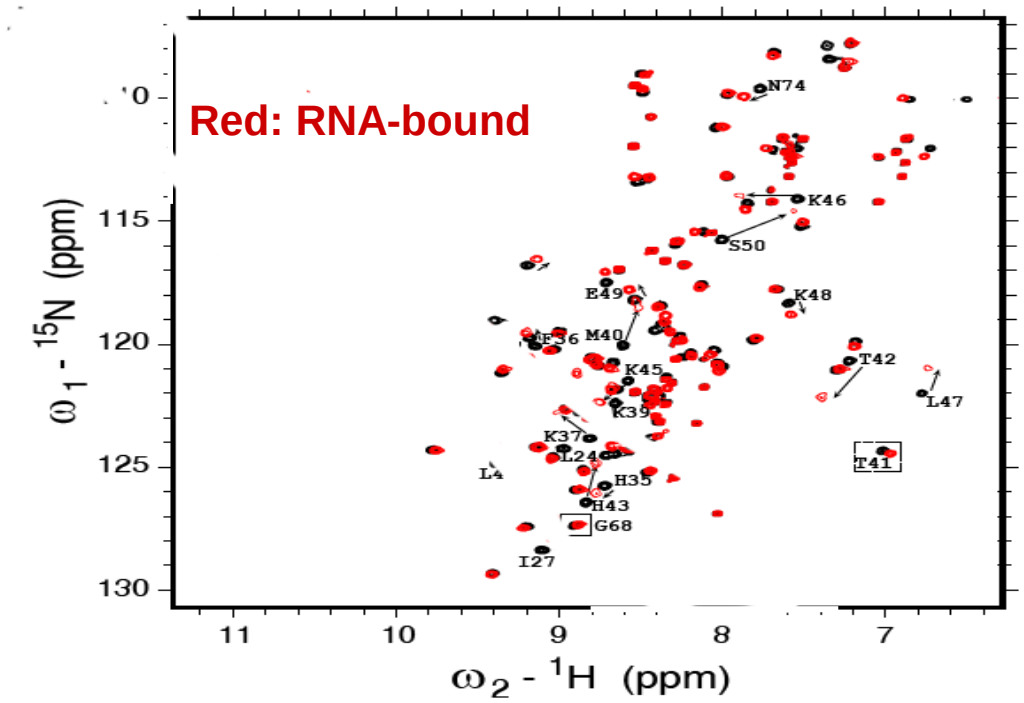
➔ **28 kDa** homo-dimer solved by Stereo-Array Isotope Labeling (SAIL) method (M. Kainosho of Nagoya U)

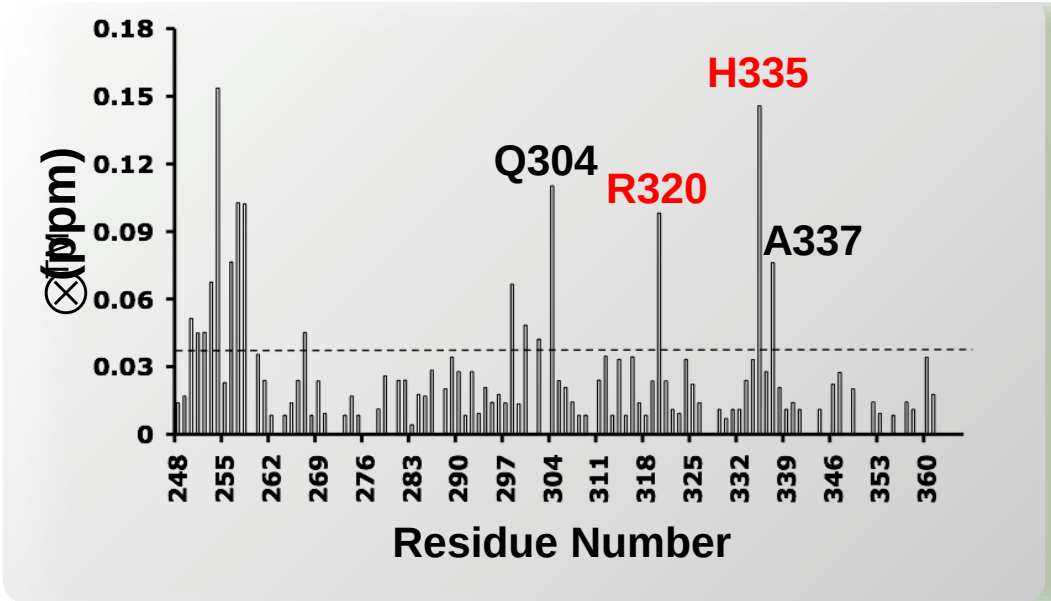


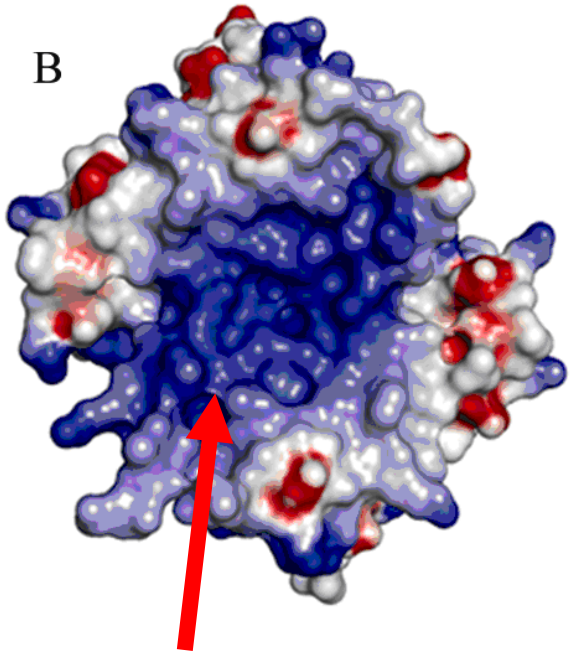


A flatten rectangular domain-swapped dimer

Identification of RNA binding site in CTD



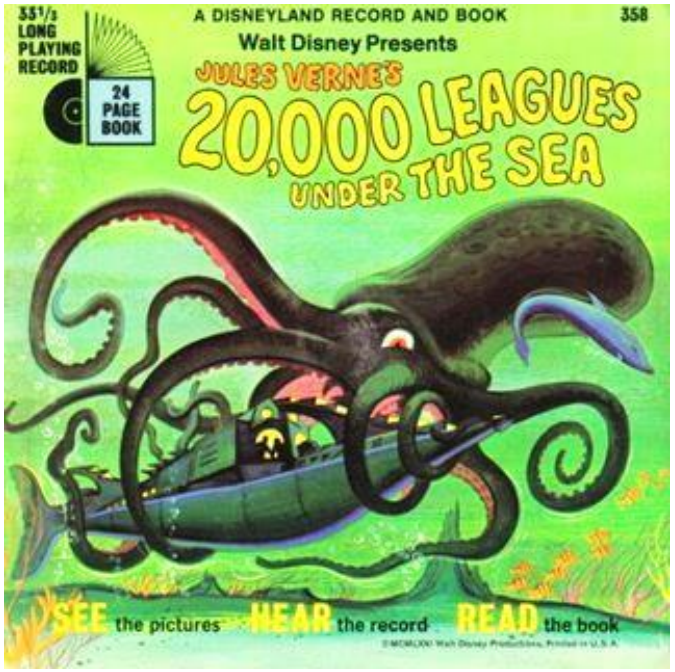




Primary RNA binding site.

N – Nucleic Acid Interaction

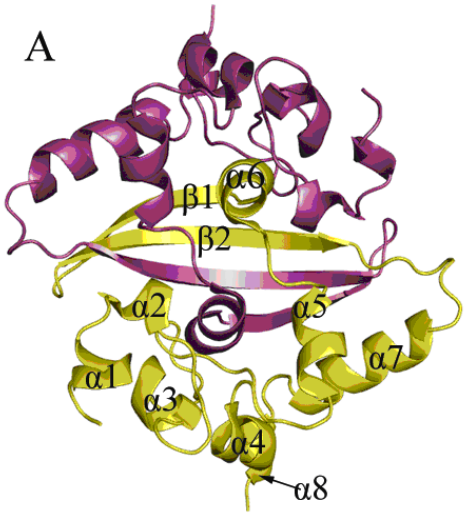
N protein binds to nucleic acid at multiple sites cooperatively, much like an octopus clinching onto it prey.



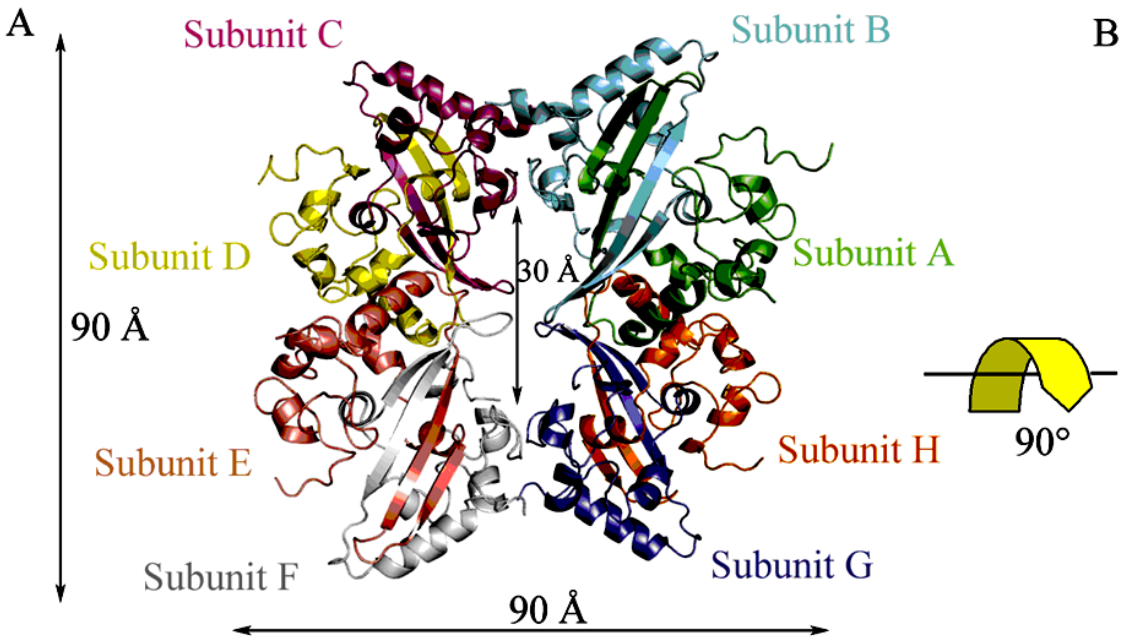
➔ Modular nature and intrinsic disorder are keys to binding cooperativity and RNP packaging

X-ray crystallography

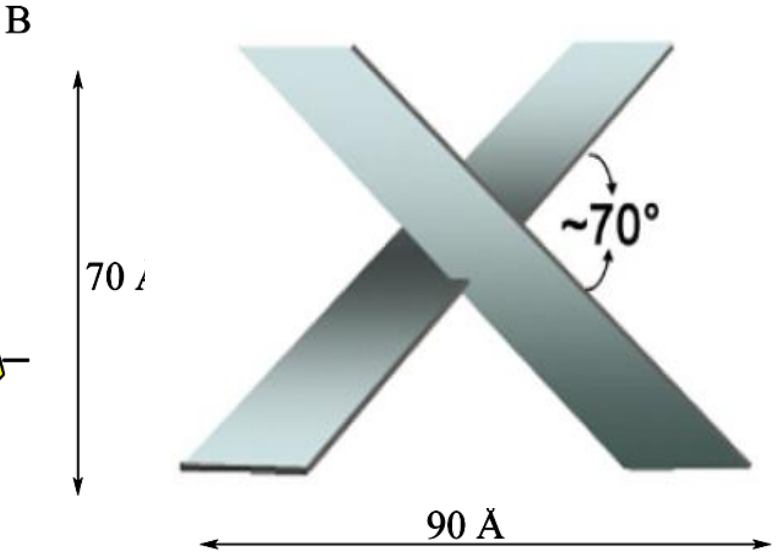
- Structure similar to that determined by NMR.
- CTD packs as an octamer in an unit cell.



Top view

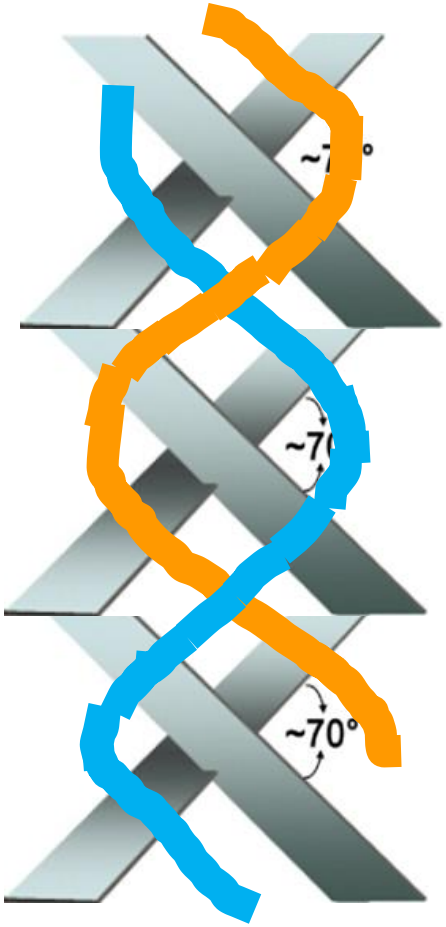


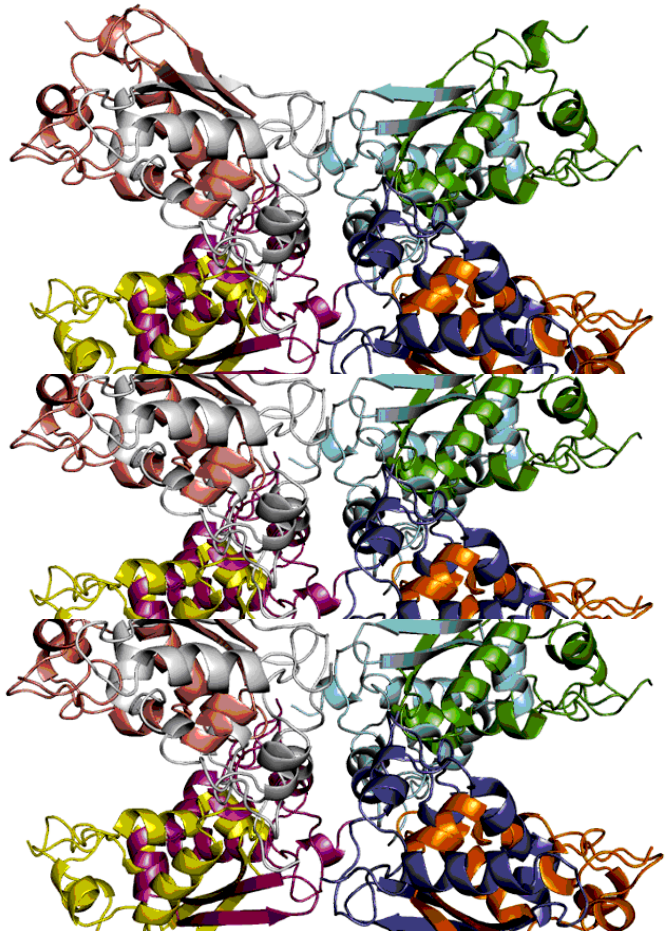
Side view



Crystal packing

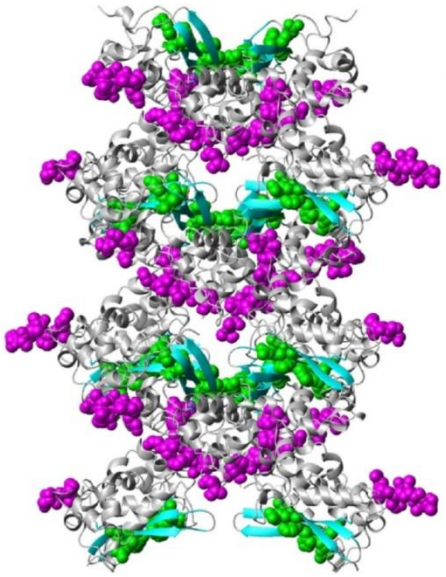
➔ Stacking of 3 octamers forms a complete turn of a left-handed twin helix.



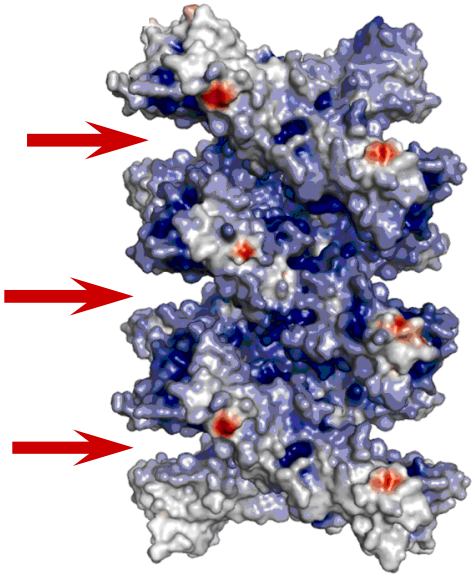


DNA binding sites are located in the positively charged grooves

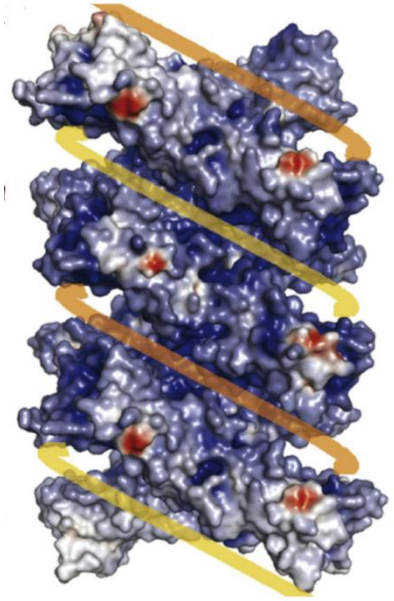
DNA binding site
NMR (magenta)



Surface Charge Potential



RNA binding model



➔ We propose that RNA binds to the Left-handed helix grooves.

Key features of SARS CoV N protein

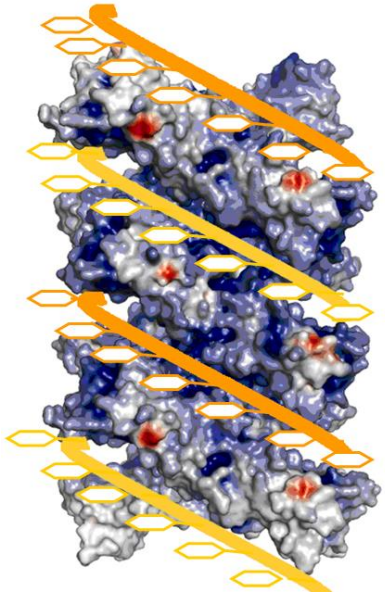
- **A modular protein:** It consist of two structured domains and three disordered segments.
- **It is highly flexible:** ~50% of the residues are intrinsically disordered (ID).
- **A sticky protein:** It binds to RNA at multiple sites cooperatively.
- **The CTD forms a dimer and packs in helical structure in crystal.**

Proposed model of the N/RNA complex

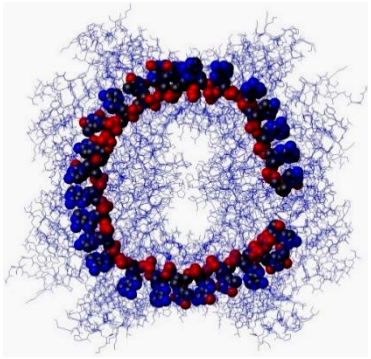
➔ CTD forms the core of the left-handed twin-helix .

➔ Backbone of RNA wraps around CTD core and with bases facing outward.

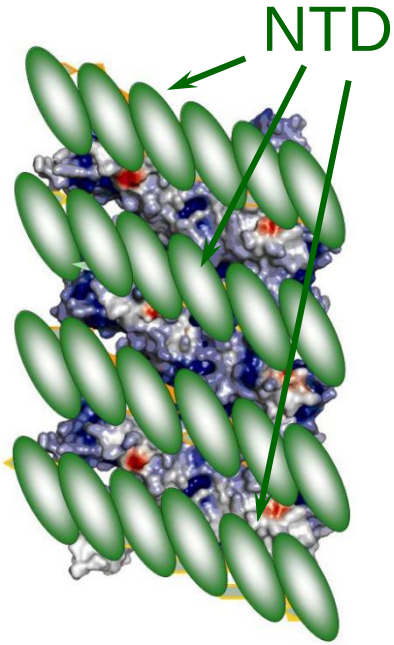
➔ NTD covers the exterior and interacts with the bases.



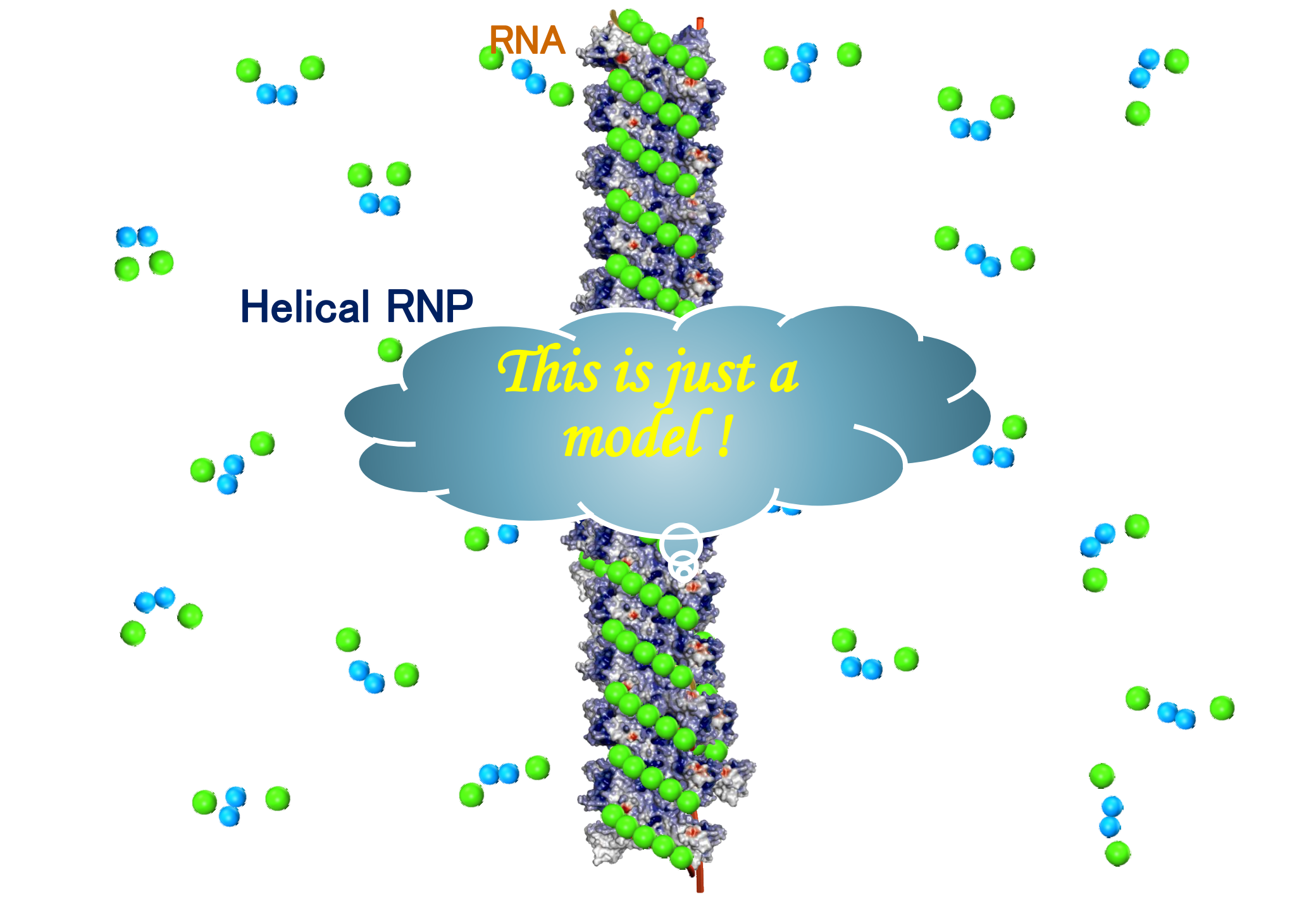
Side view



Top view



N/RNA complex (RNP)


RNA
Helical RNP
This is just a model !

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Dr. Yu-shan Huang (SAXS)

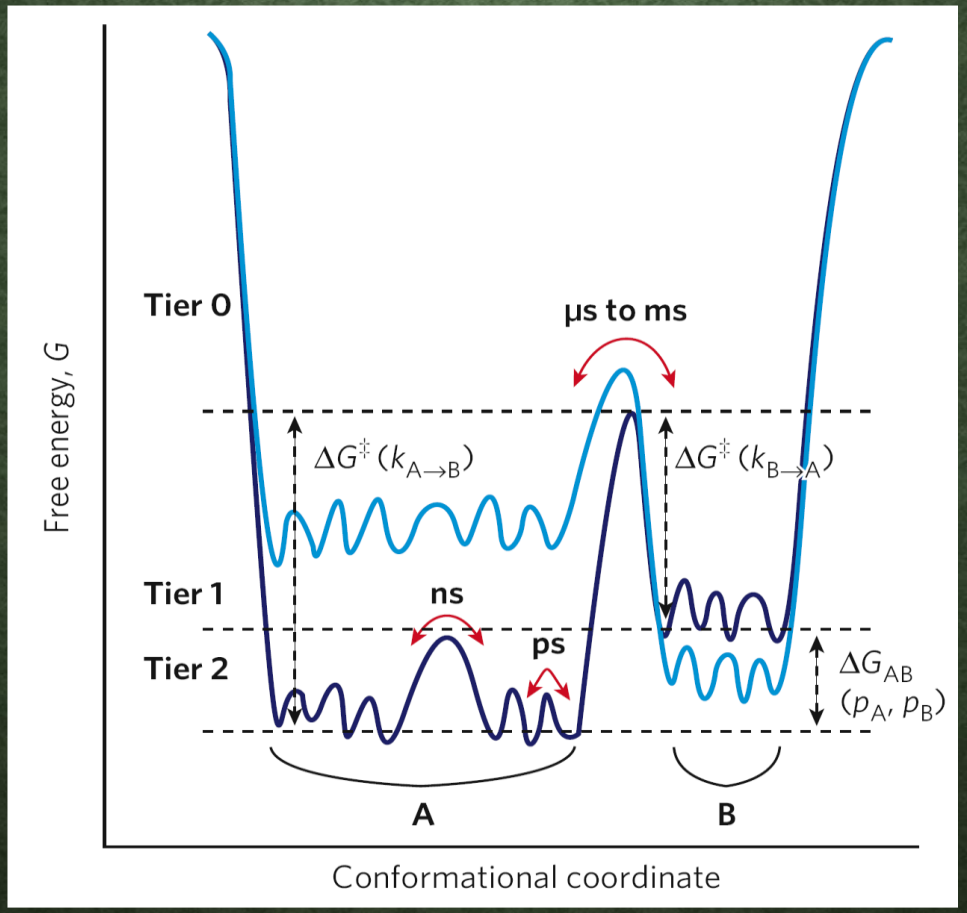
NMR Structure

Prof. Peter Guetert
(RIKEN)

3. Dynamics

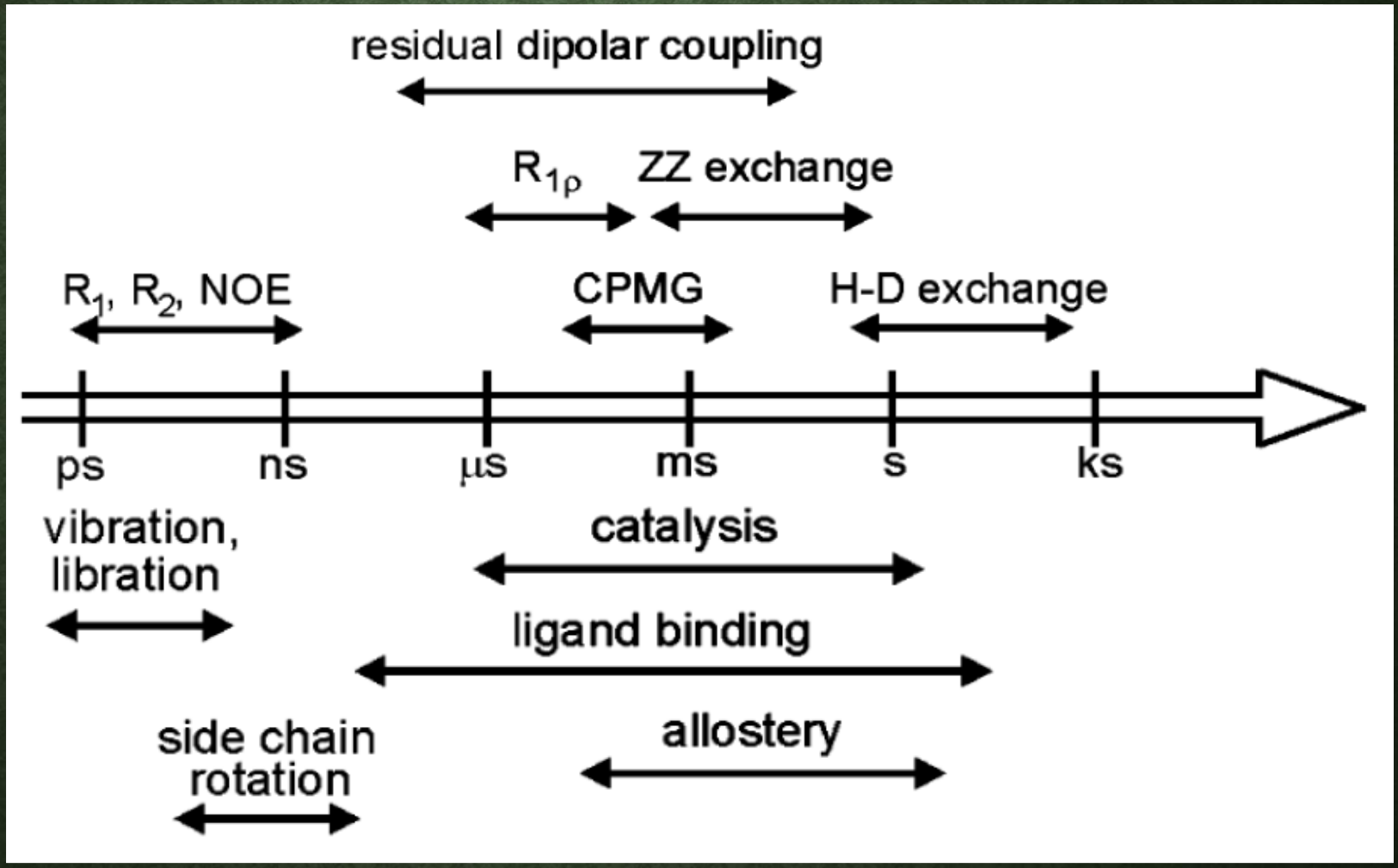
Protein Dynamics

- Energy landscape of protein conformations



Ref. 1. Henzler-Wildman & Kern (2007) Nature 450 :964-72
2. Boehr and Wright (2006) Chem Rev. 106(8):3055-79

Measurement of Macromolecular Dynamics by NMR

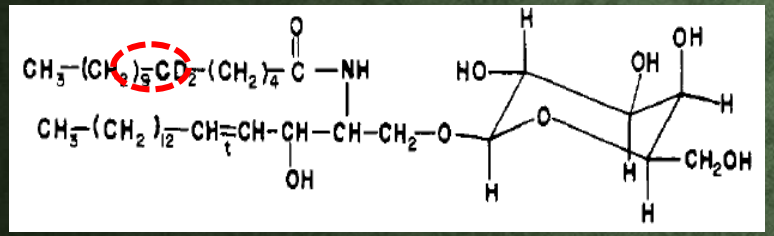


NMR can measure a wide range of dynamic processes

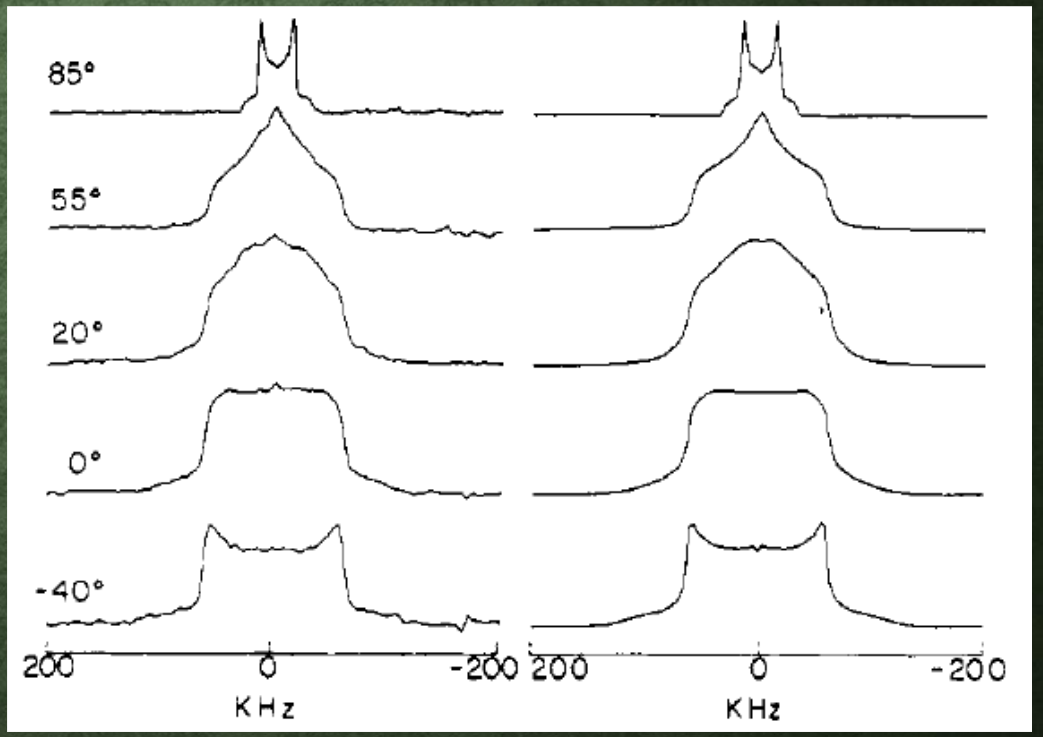
Characterize the restricted rotational isomerization of polymethylene chains by deuterium NMR lineshape simulation

Huang et al J. Am. Chem. Soc. 102, 7377-7379 (1980)

N-palmitoylglactosylceramide



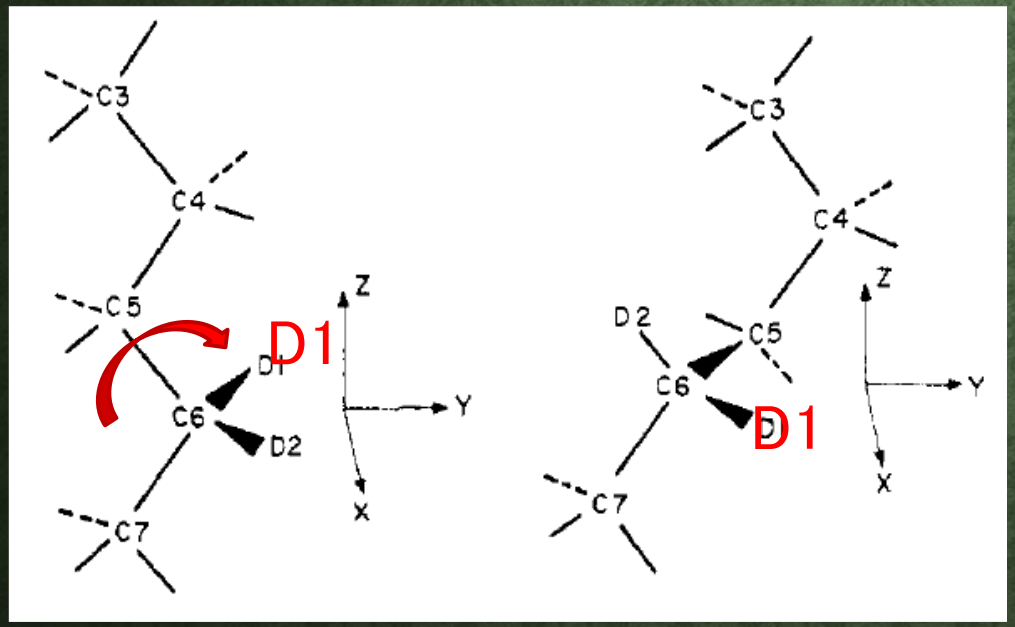
Expt Simulated



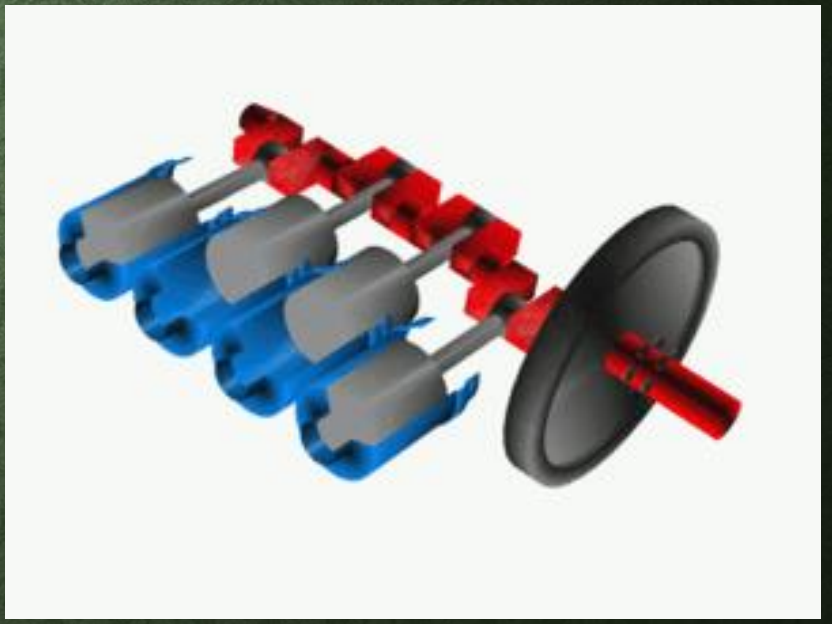
Deuterium quadrupole spectra were simulated with two site flipping model similar to that of the crankshaft motion.

Huang et al J. Am. Chem. Soc. 102, 7377-7379 (1980)

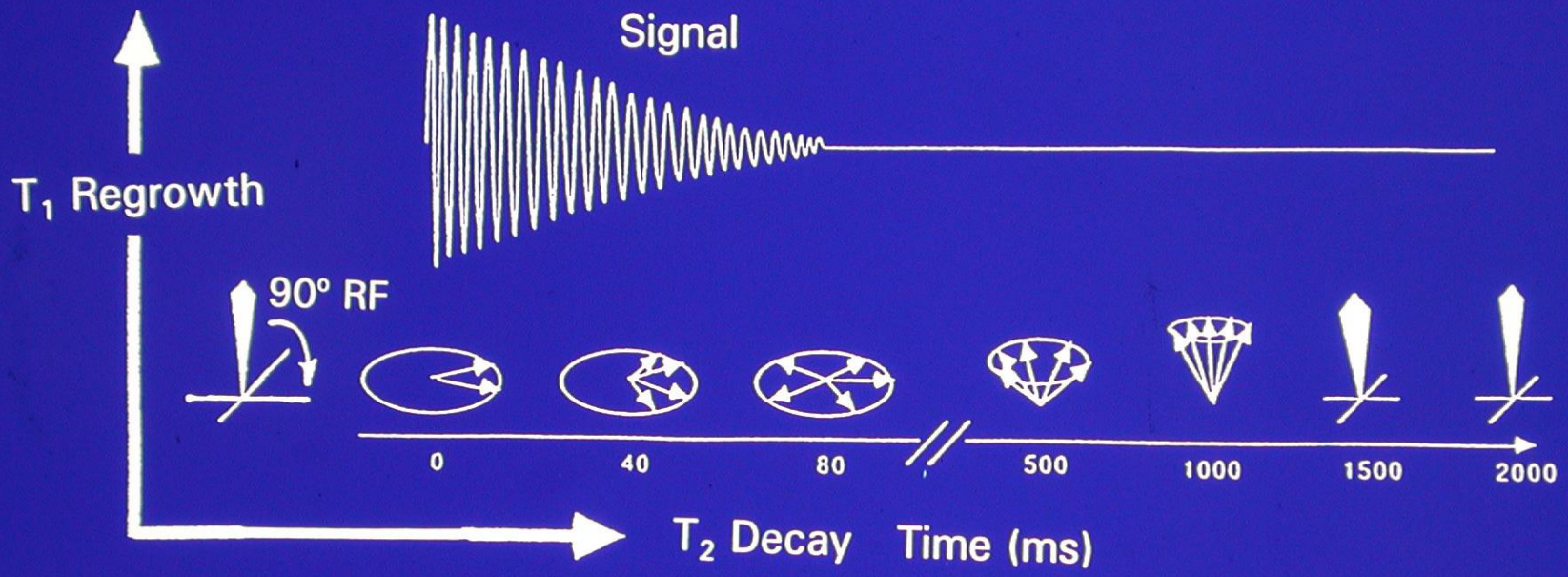
Tetrahedral two site flipping model



Crankshaft motion



Liquid state – NMR Relaxation



Spin-lattice relaxation (T_1) and spin-spin relaxation (T_2) of nuclear spins. Figure shows the evolution of the magnetization after it has been flipped by 90° pulse.

Relaxation Mechanism

Dominated by dipolar and chemical shift anisotropic interactions, and are related to the spectral density functions, $J(\omega)$, by the following equations:

$$R_1 = 1/T_1 = (d^2/4)[J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)] + c^2 J(\omega_N) \quad (1)$$

$$R_2 = 1/T_2 = (d^2/8)[4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)] + (c^2/6)[4J(0) + 3J(\omega_N)] + R_{ex} \quad (2)$$

$$XNOE = 1 + (d^2/4)(\gamma_H/\gamma_N)[6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)]\, T_1 \quad (3)$$

where $d = (\mu_o h \gamma_N \gamma_H / 8\pi^2)(r_{NH}^{-3})$, (Dipolar term)

$c = \omega_N(\sigma_{\parallel} - \sigma_{\perp})/\sqrt{3}$. (Chemical shift term)

μ_o : permeability constant of free space;

h: Planck constant;

γ_i : magnetogyric ratio of spin i;

ω_i: Larmor frequency of spin i;

r_{NH} = 1.02 Å: length of the NH bond vector;

R_{ex}: exchange rate;

$\sigma_{\parallel} - \sigma_{\perp} = -170$ ppm (size of the CSA tensor of the backbone amide nitrogen).

What is J(ω) ? - Modelfree analysis

For a rigid macromolecule undergoing Brownian motion with a rotational correlation time τ_m and local internal motion with rotational correlation time τ_s the spectral density function, J(ω) is given by:

$$J(\omega) = \frac{2}{5}\left[\frac{S^2\tau_m}{1+(\omega\tau_m)^2} + \frac{(1-S_f^2)\tau'_f}{1+(\omega\tau'_f)^2} + \frac{(S_f^2-S^2)\tau'_s}{1+(\omega\tau'_s)^2}\right]$$

S^2: Order parameters (Magnitude of motion)

τ : Correlation times (Speed of motion)

R_{ex} : Chemical exchange rate (Slow motion in ms or μs regime)

→ **Fitting T_1, T_2 and NOE data to determine**

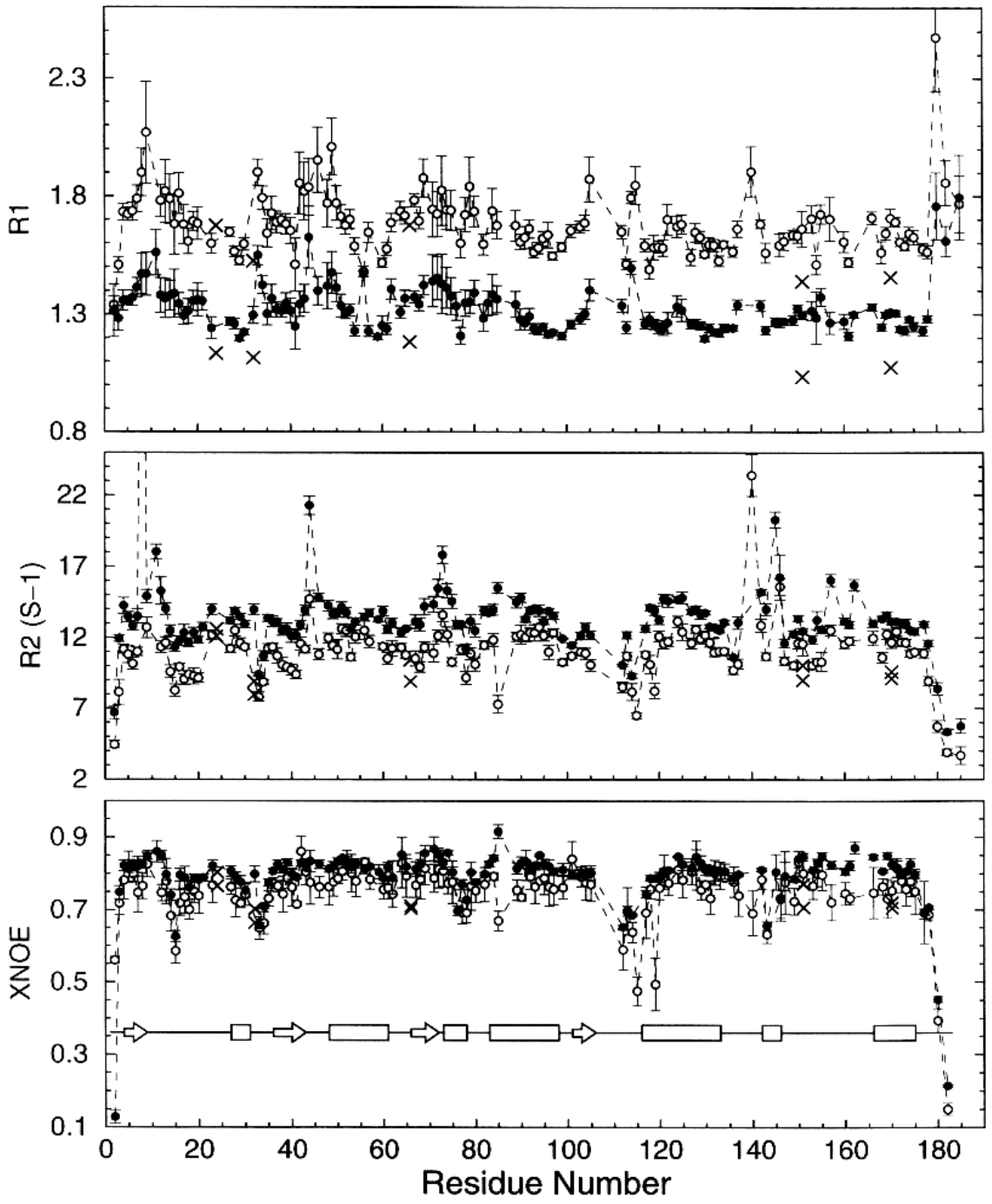
S^2, τ and R_{ex}

Relaxation Data

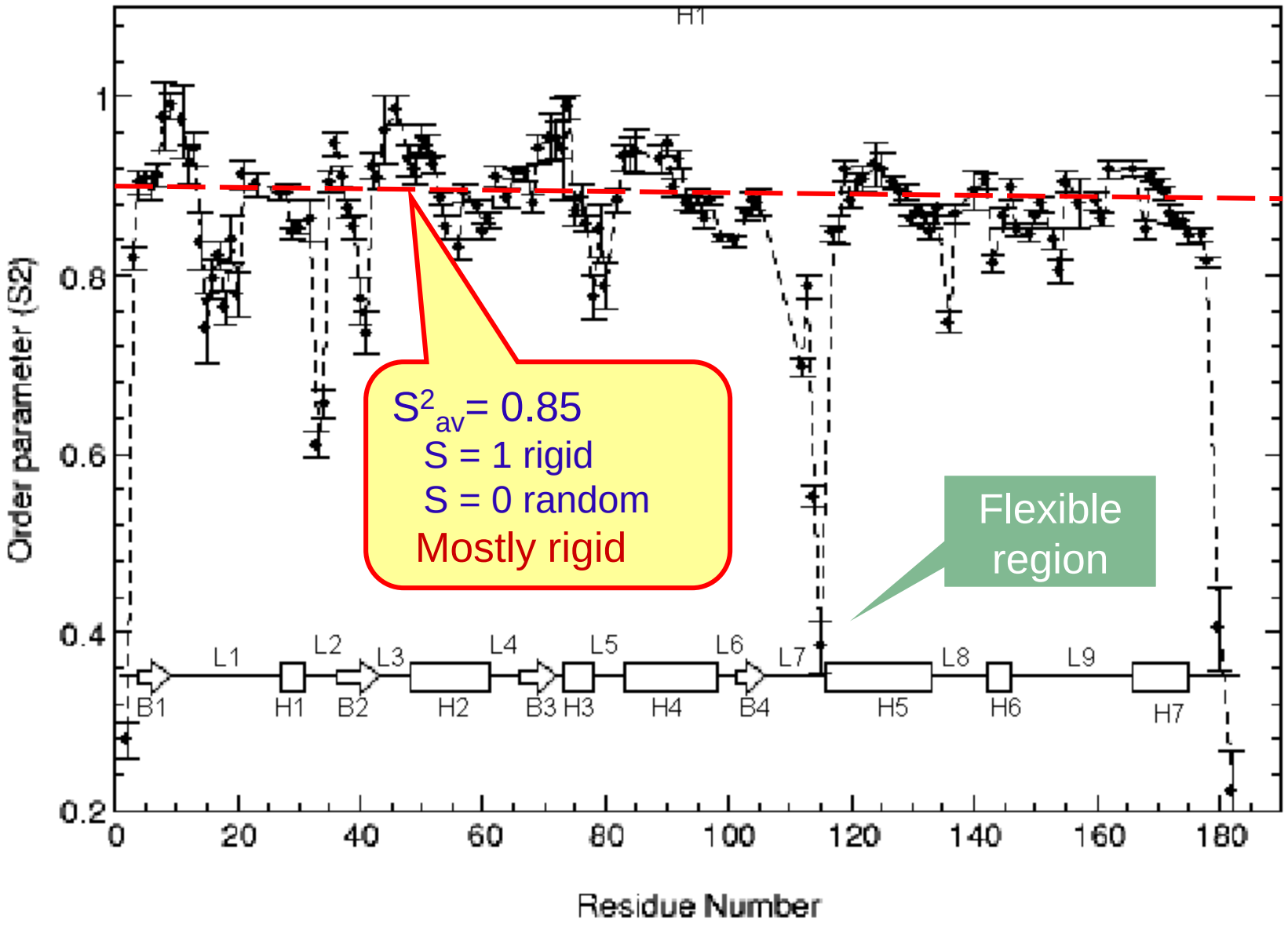
Obtained in two fields:

: 500 MHz

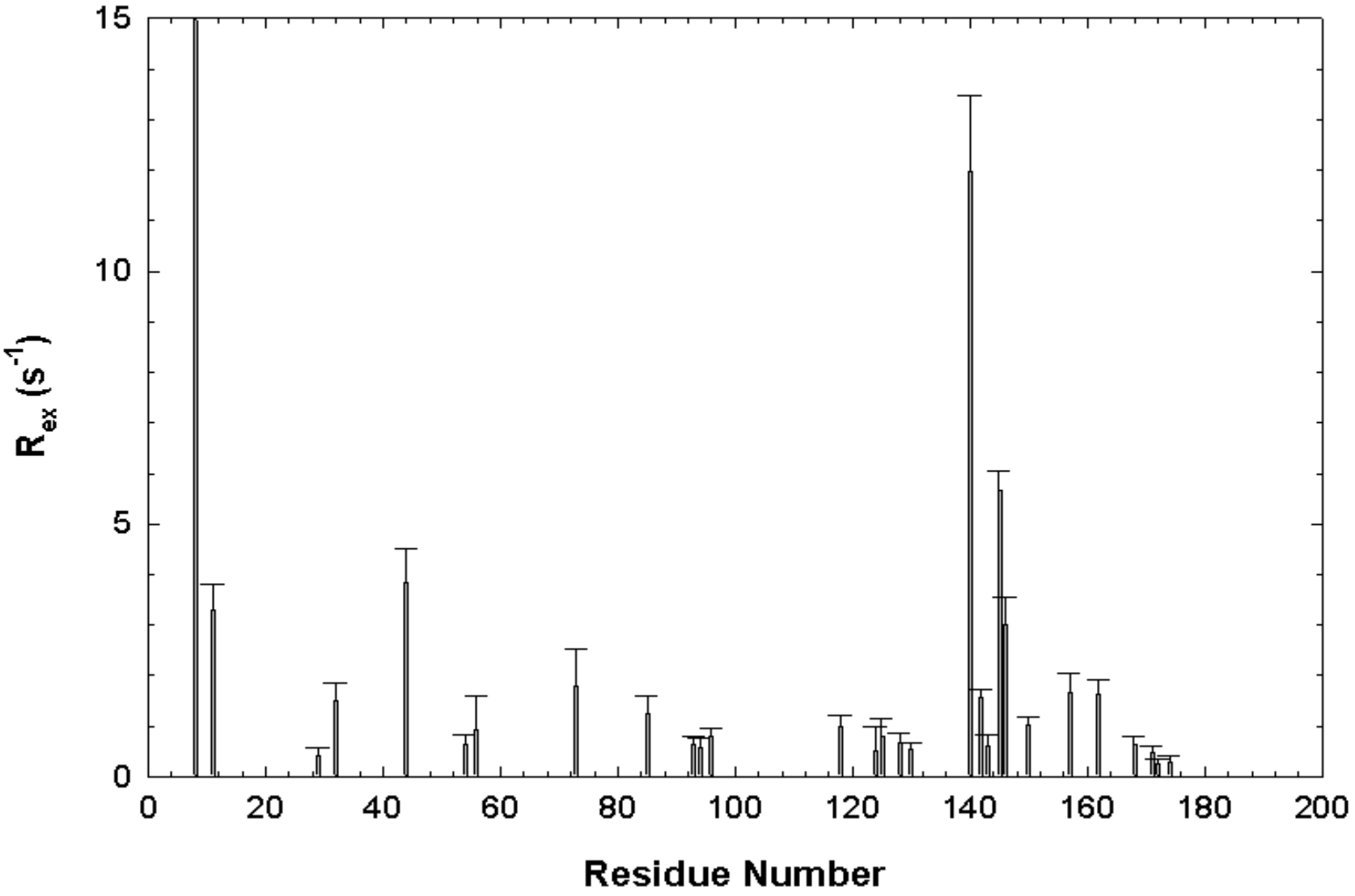
- : 600 MHz



Order parameter

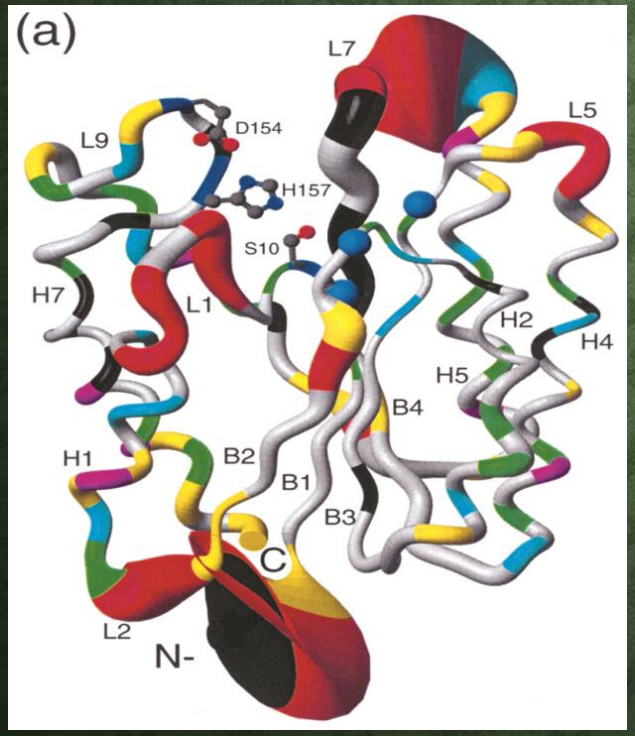


Exchange rate – Residues with low motion



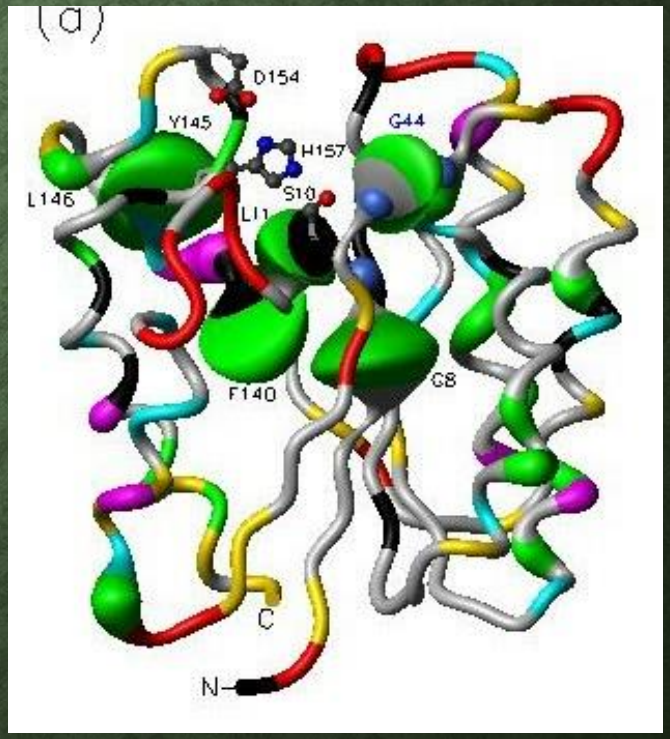
Dynamics of E. coli Thioesterase I

Order parameter



Order parameter

Slow motion



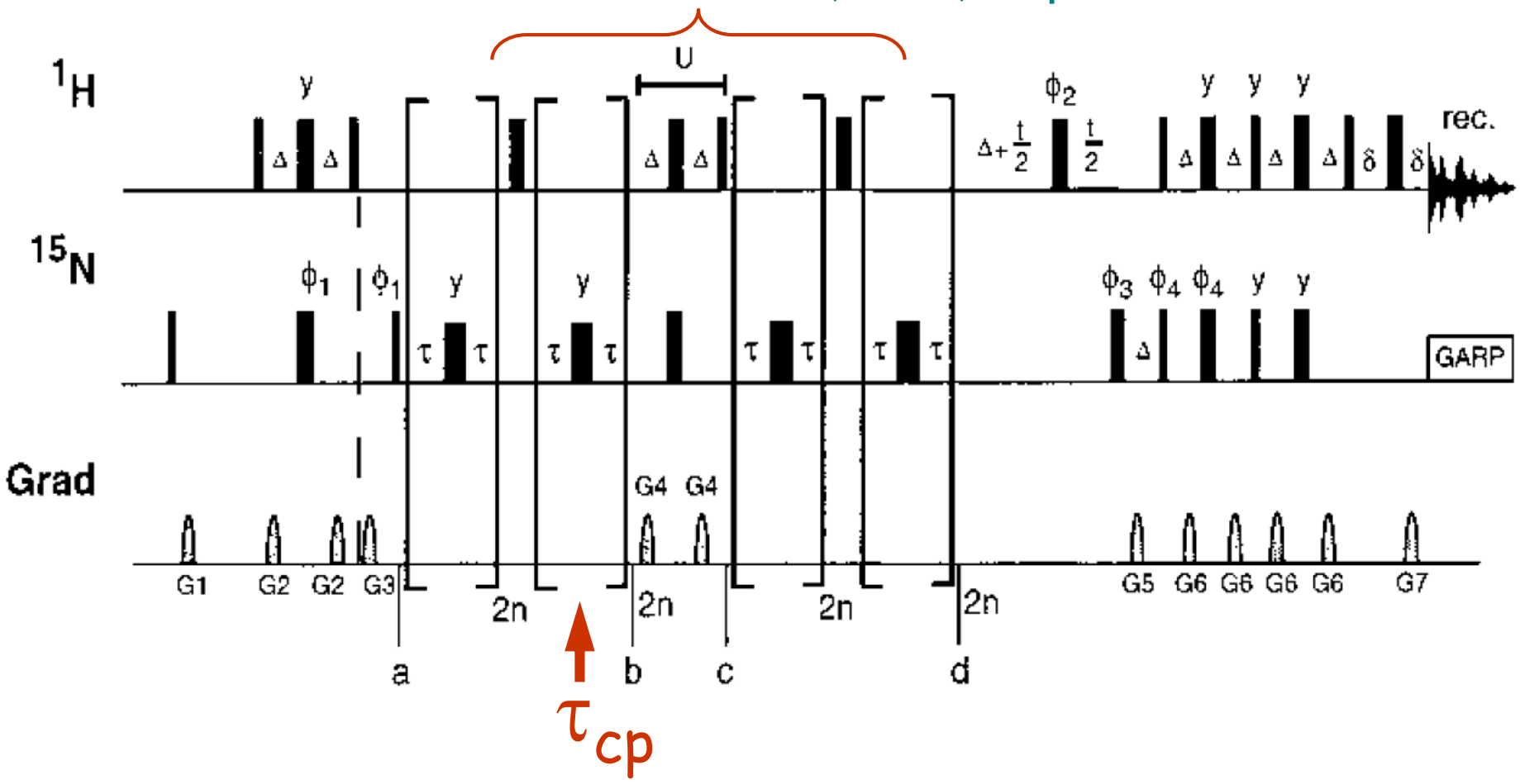
Exchange term

Huang, et al. (2001) J. Mol. Biol. **307**, 1075-1090.

Measuring millisecond time scale motion

Ref. Loria, Rance, and Palmer III (*JACS.* 1999, *121,* 2331–2332)

Carr–Purcell–Meiboom–Gill (CPMG) Sequence

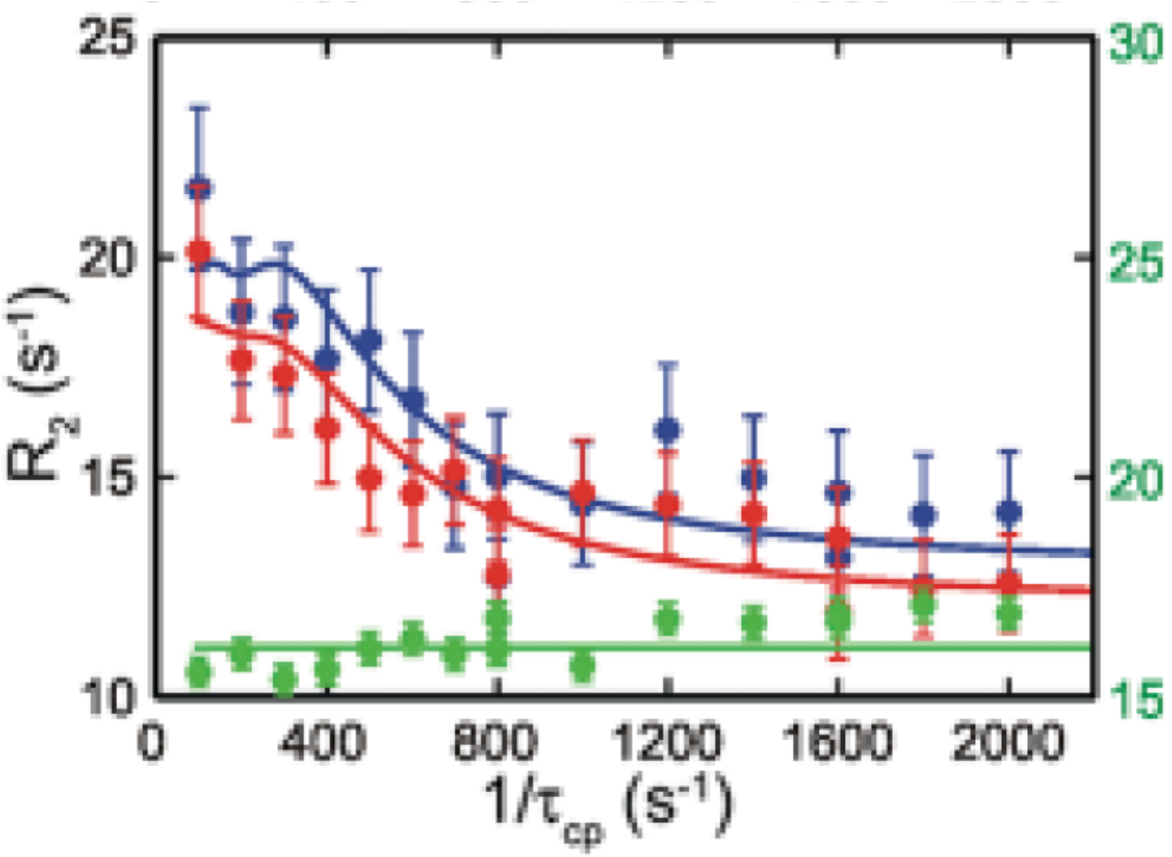


$$R_{2(\tau_{cp})} = \epsilon R_{in} + (1-\epsilon)R_{anti} + R_{ex}$$

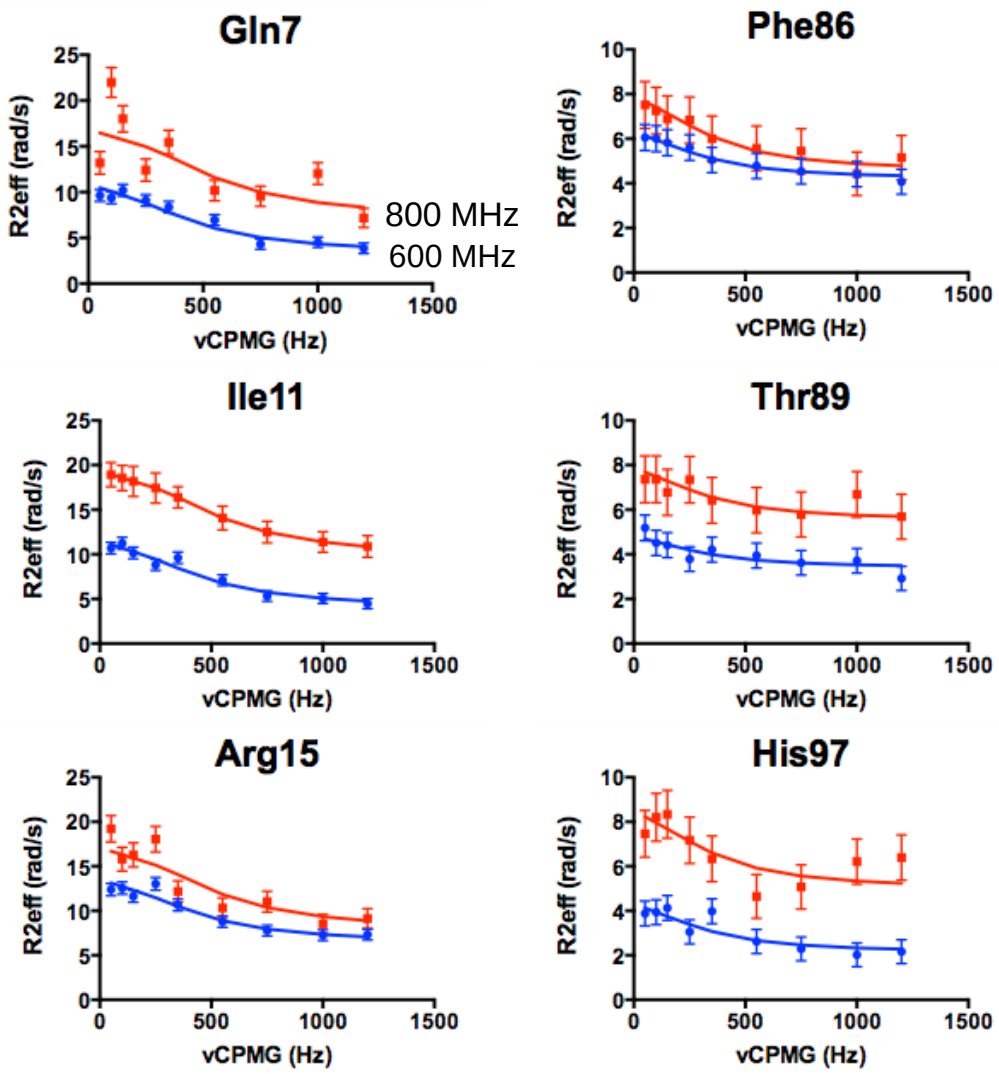
$$R_{ex} = \Phi_{ex}\tau_{ex}[1 - (2\tau_{ex}/\tau_{cp})\tanh(\tau_{cp}/2\tau_{ex})]$$

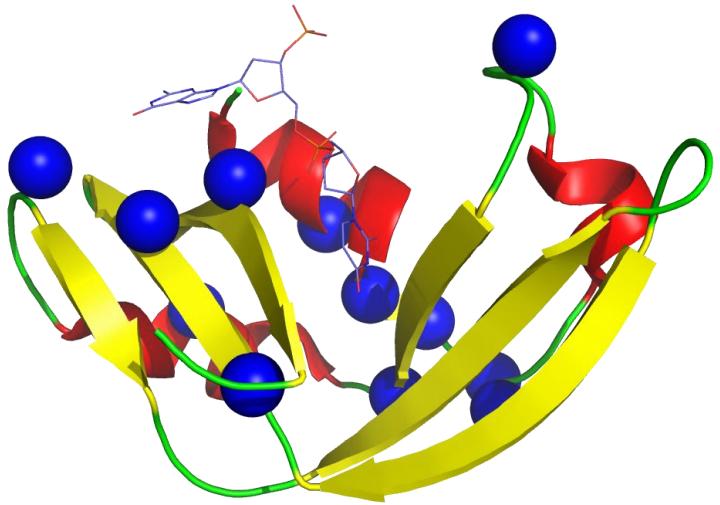
Solve for τ_{ex} for different τ_{cp} (measure 0.5 – 5 ms range)

In which $\Phi_{ex} = (\omega_1 - \omega_2)^2 p_1 p_2$; p_i and ω_i are the populations and Larmor frequencies for the nuclear spin in site i, respectively; and τ_{ex} is the lifetime of the exchanging sites.



Onconase



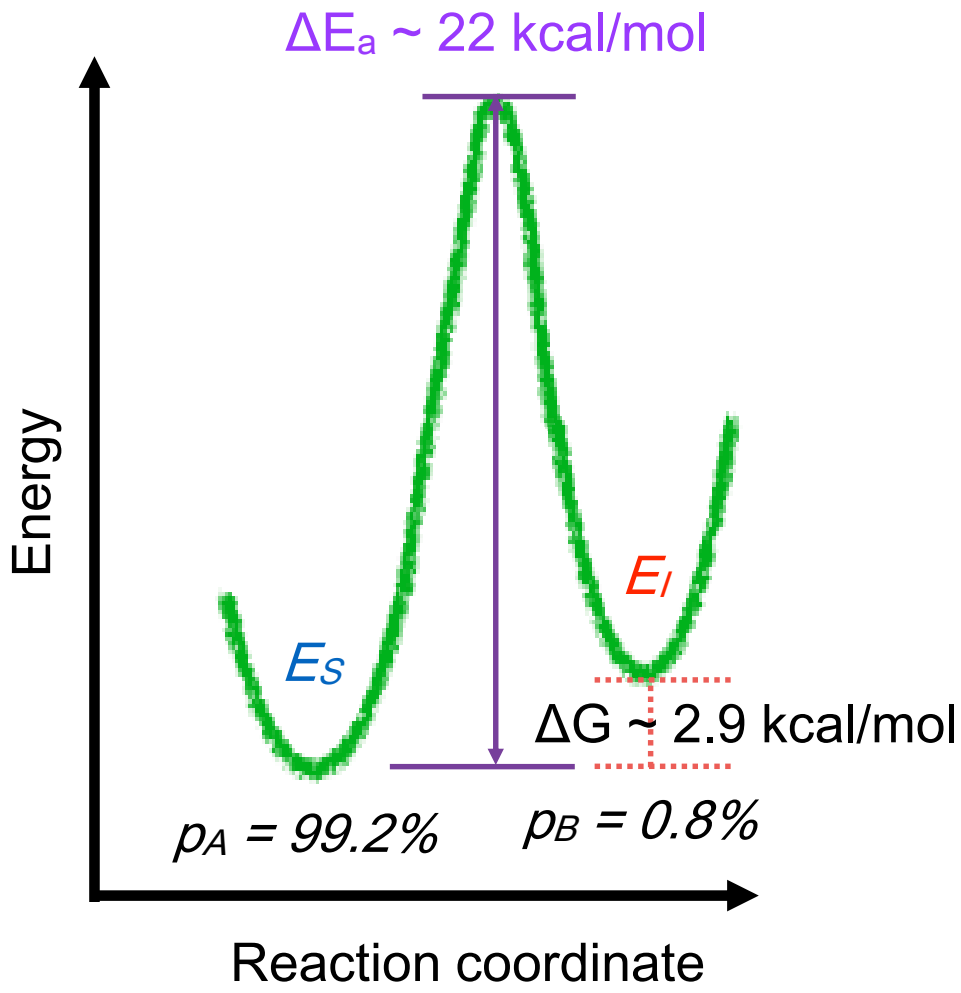


$k_{ex} = 2{,}029 \pm 351\ s^{-1}$

$p_A = 99.2 \pm 0.11\%$

$\Delta G_{eq} \sim 2.9\ kcal/mol$

Catalytic scheme: $E + S \rightleftharpoons ES \rightleftharpoons EI \rightarrow E + P$



Reflection of a Wonderful Journal

1. NMR is a prime example of the importance of basic research. The impact of basic research often takes long time to realize.

2. Science is full of surprises. It is only limited by your imagination.
   Griffin "John Waugh basically invented the field of solid-state NMR when everyone else had left the field because they thought it was never going to work,"

3. Many areas of today's science is inter-disciplinary in nature and a broad knowledge is essential.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