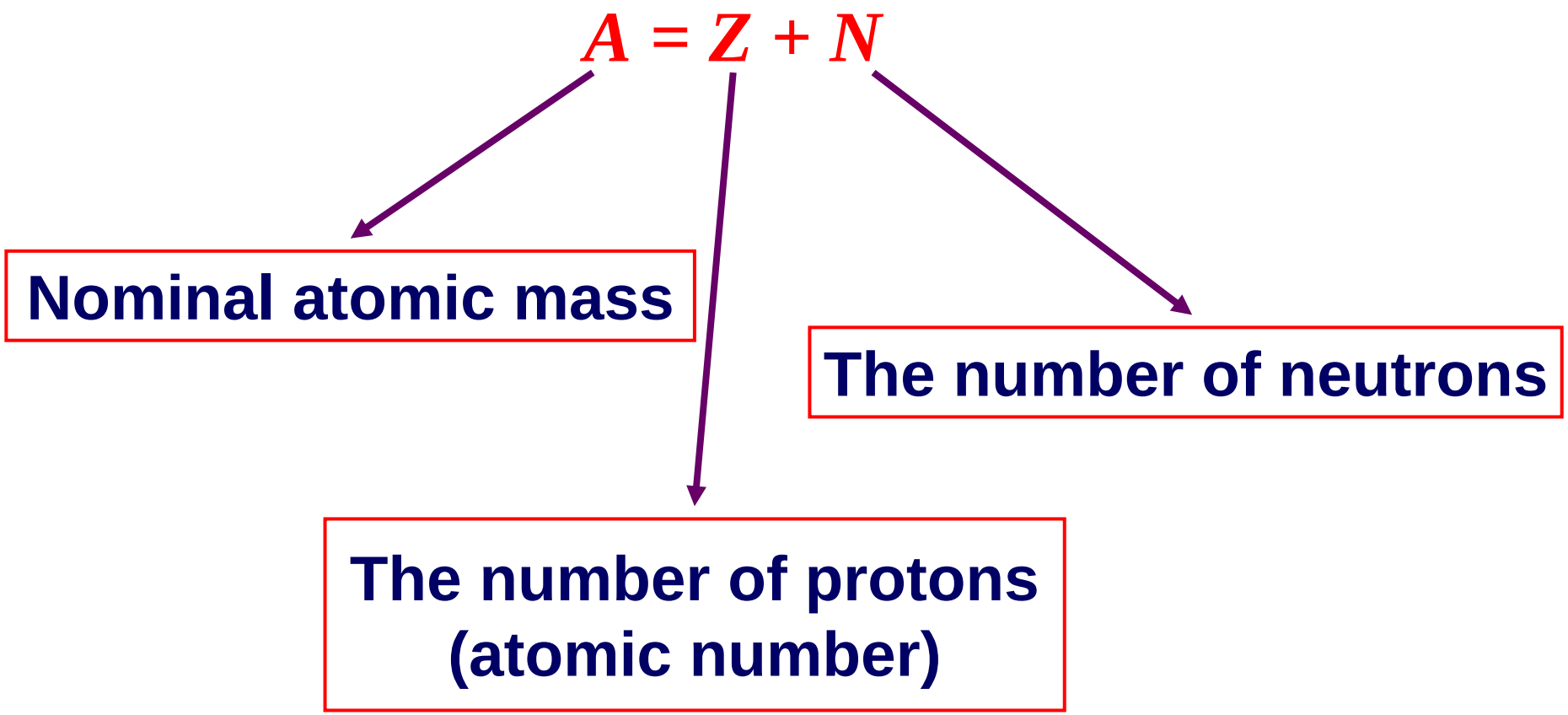
outline

1. NMR principles
2. NMR parameters
3. NMR experiments
4. Determination of NMR solution structure
5. Conclusion

中研院生醫所 N133
Tel.: 02-26523035

- 生物科技可說是近代最重要的一門科學,從遺傳工程的發展，到複製動物的出現，每一次重大的進展都帶給人們無窮的想像；而隨著最近轟動一時的人類基因組計劃(Human Genome Project)的完成，全球的生命科學家又展開了所謂的蛋白質體學(Proteomics)的研究，其目的即是希望能了解人類細胞大約十萬個蛋白質的結構及其在生命體中所扮演的角色及功能，以便設計小分子藥物來調控這些分子的作用.

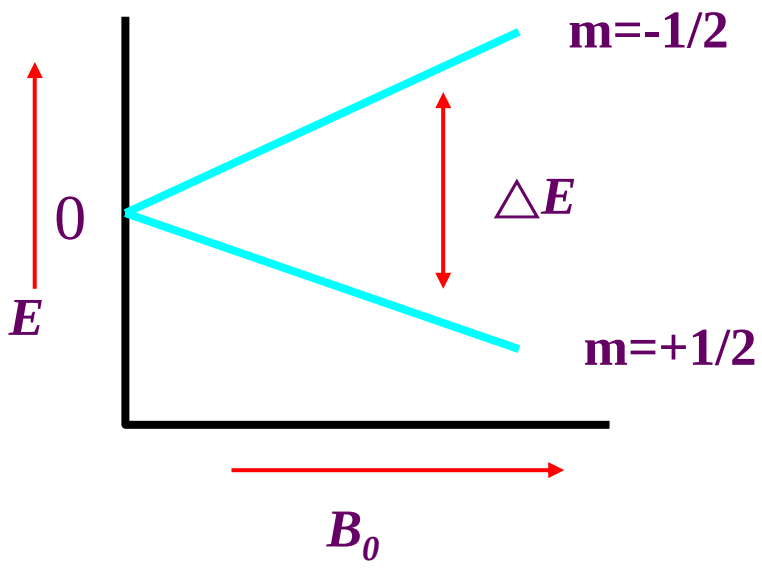
- 前幾年，Dr. K. Wuthrich因其在核磁共振學的努力而獲得了諾貝爾獎的殊榮，也更加確認了核磁共振學在二十一世紀的生命科學領域將扮演一舉足輕重的角色.


$A = Z + N$
Nominal atomic mass
The number of protons (atomic number)
The number of neutrons

- A nucleus with an even mass ***A*** and even charge ***Z***, and therefore also an even ***N***, will have a nuclear spin ***I*** of zero. (^{12}**C**, ^{16}**O** and ^{18}**O**).
- A nucleus with an even mass and odd charge (both ***Z*** and ***N*** odd) will exhibit an integer value of ***I***. [^{2}**H**(***I*=1**), ^{14}N(***I*=1**) and ^{10}**B**(***I*=3**)].
- A nucleus with odd mass (***Z*** odd and N even, or Z even and N odd) will have nuclear spin with an I value that we can express as n/2, where n is an odd integer. [^{1}**H**(***I*=1/2**), ^{13}**C**(***I*=1/2**) and ^{17}**O**(***I*=5/2**)].

Nuclei with $I = 0$ NMR cannot be detected.
Nuclei with $I \neq 0$ NMR can be detected.
Different isotopes of the same element have different nuclear spins, some of which are detectable by NMR, others of which are not.

Number of spin states (2I+1) :



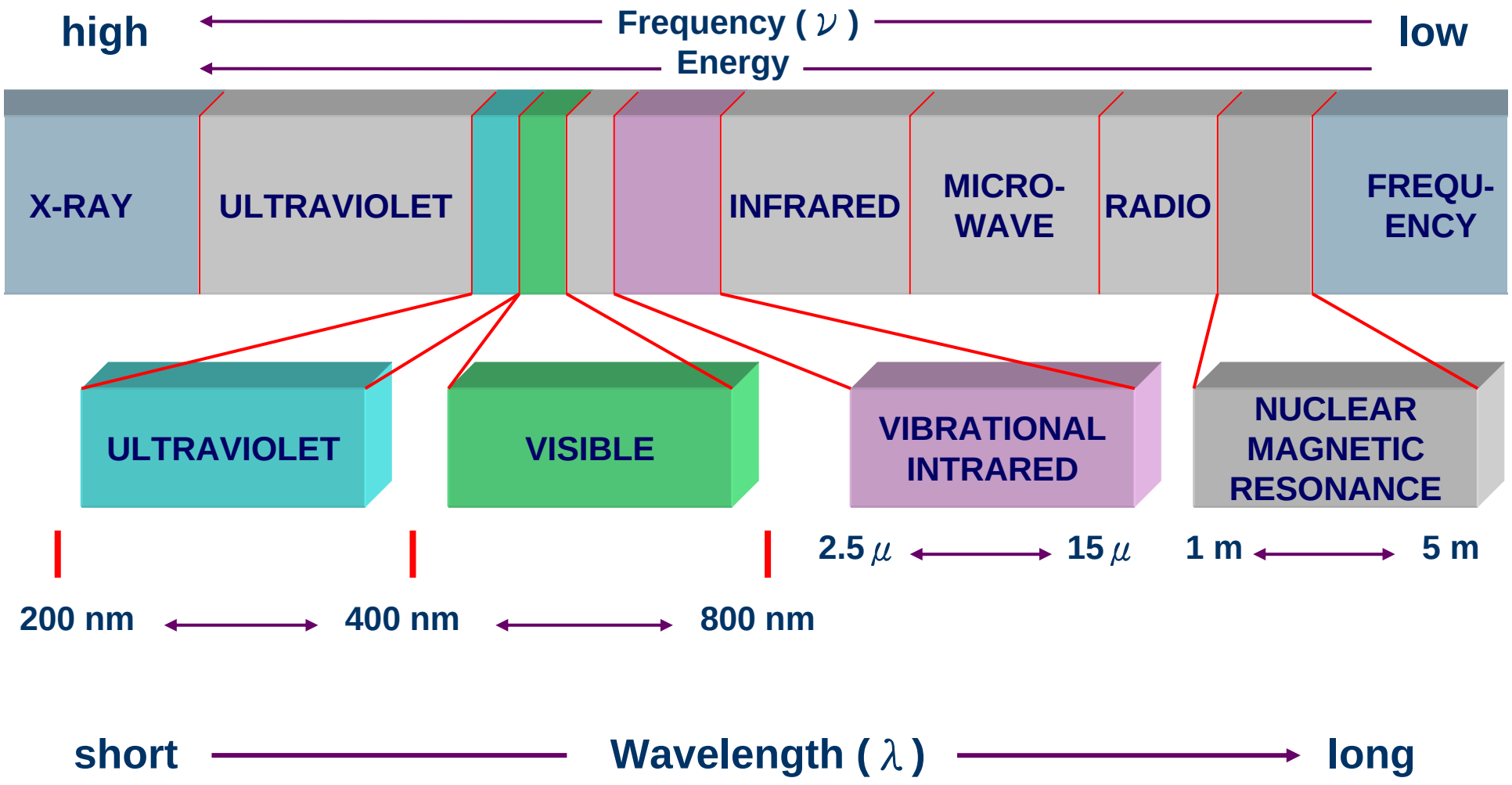
$$E=-mB_0(\gamma h/2\pi)$$

B_0: magnetic field strength
h : Planck's constant
m: spin quantum number
γ : magnetogyric ratio

A nucleus with spin ***I*** can have **2*I*+1** spin states. Each of these states has its own spin quantum number **m**(**m=-*I*, -*I*+1, ・・・, *I*-1, *I***). For nuclei with ***I*=1/2**, only two states are possible: **m=+1/2** and **m=-1/2**.

Properties of nuclei in NMR studies

Isotope	Spin	Frequency (MHz) at 11.74T	Nature abundance (%)	Relative sensitivity
^{1}H	1/2	500.0	99.98	1.0
^{2}H	1	76.7	1.5×10^{-2}	9.65×10^{-3}
^{3}H	1/2	533.3	0	1.21
^{12}C	0	-------	98.89	------
^{13}C	1/2	125.7	1.108	1.59×10^{-2}
^{14}N	1	36.1	99.63	1.01×10^{-3}
^{15}N	1/2	50.7	0.37	1.04×10^{-3}
^{16}O	0	------	~ 100	-------
^{17}O	5/2	67.8	3.7×10^{-2}	2.91×10^{-2}
^{19}F	1/2	470.4	100	0.83
^{31}P	1/2	202.4	100	6.63×10^{-2}

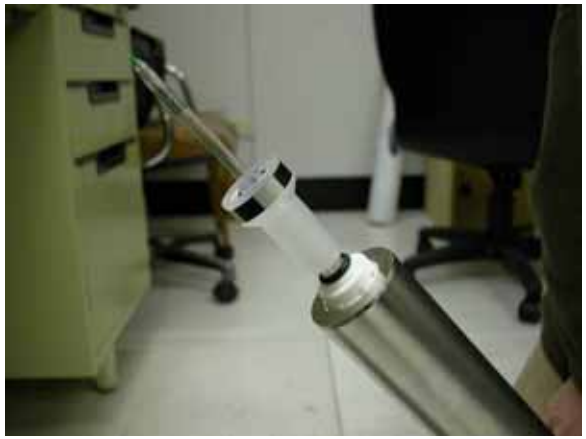


A Portion of the Electromagnetic Spectrum Showing the Relationship of the Vibrational Infrared to Other Types of Radiation.

600 MHz NMR magnet

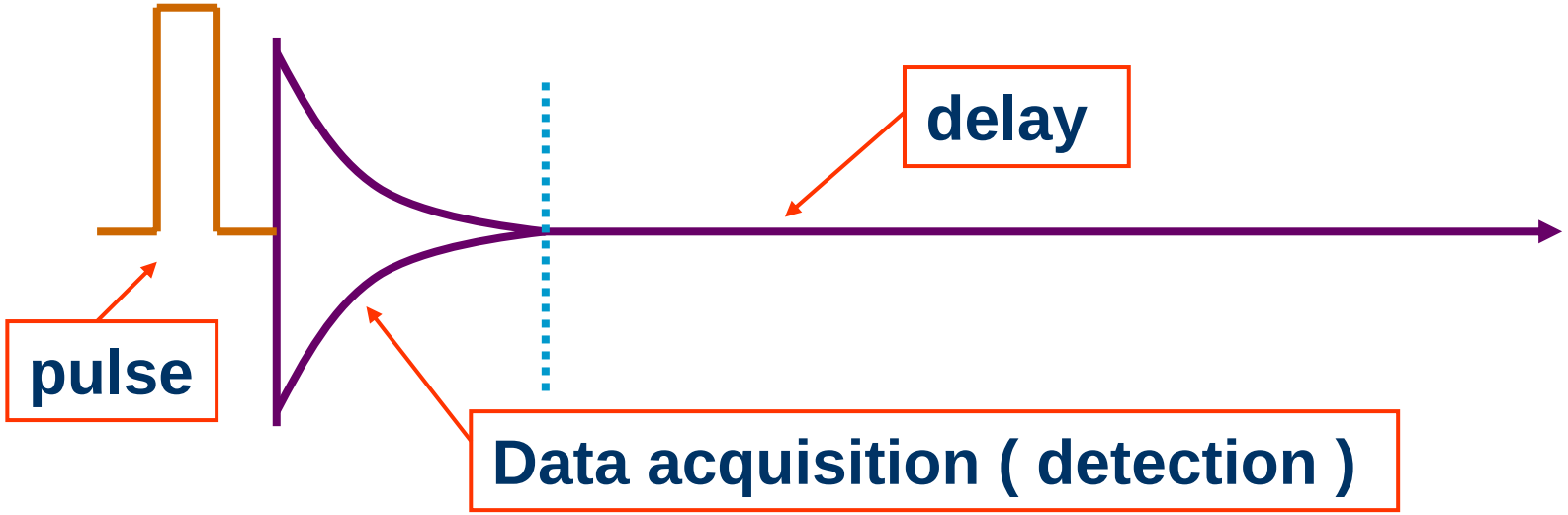
NMR tube and holder

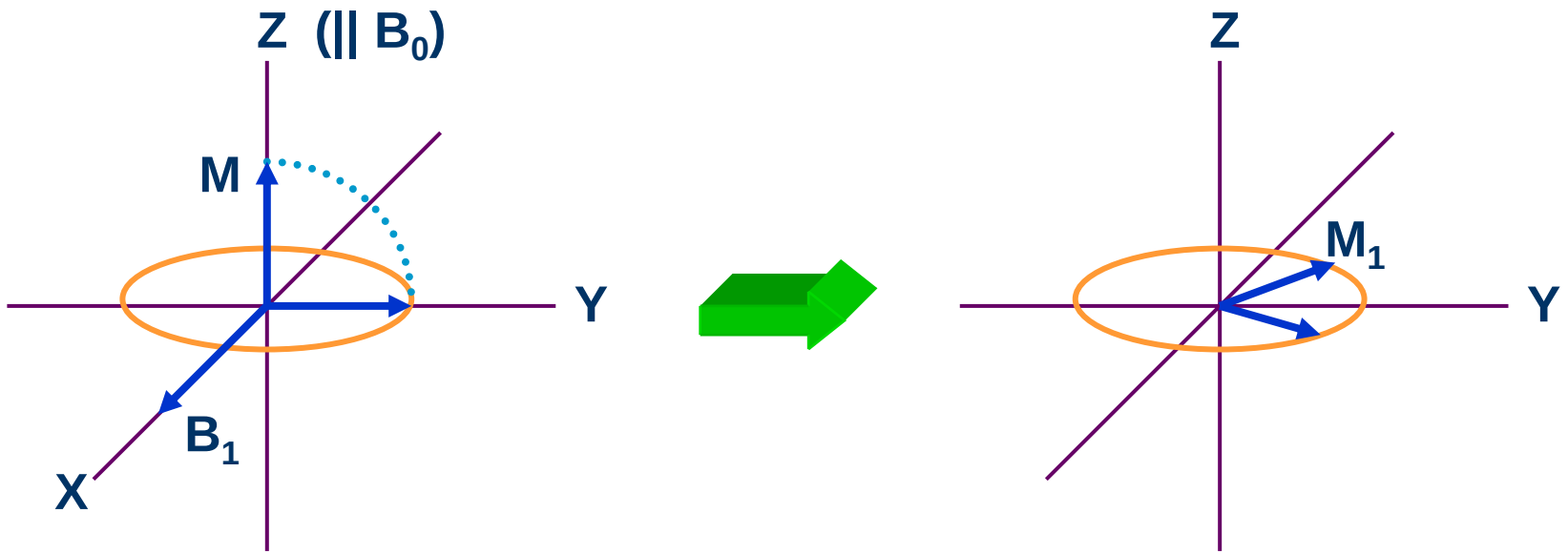
Triple-resonance NMR probe



NMR sample and probe

The normal detection pulse sequence

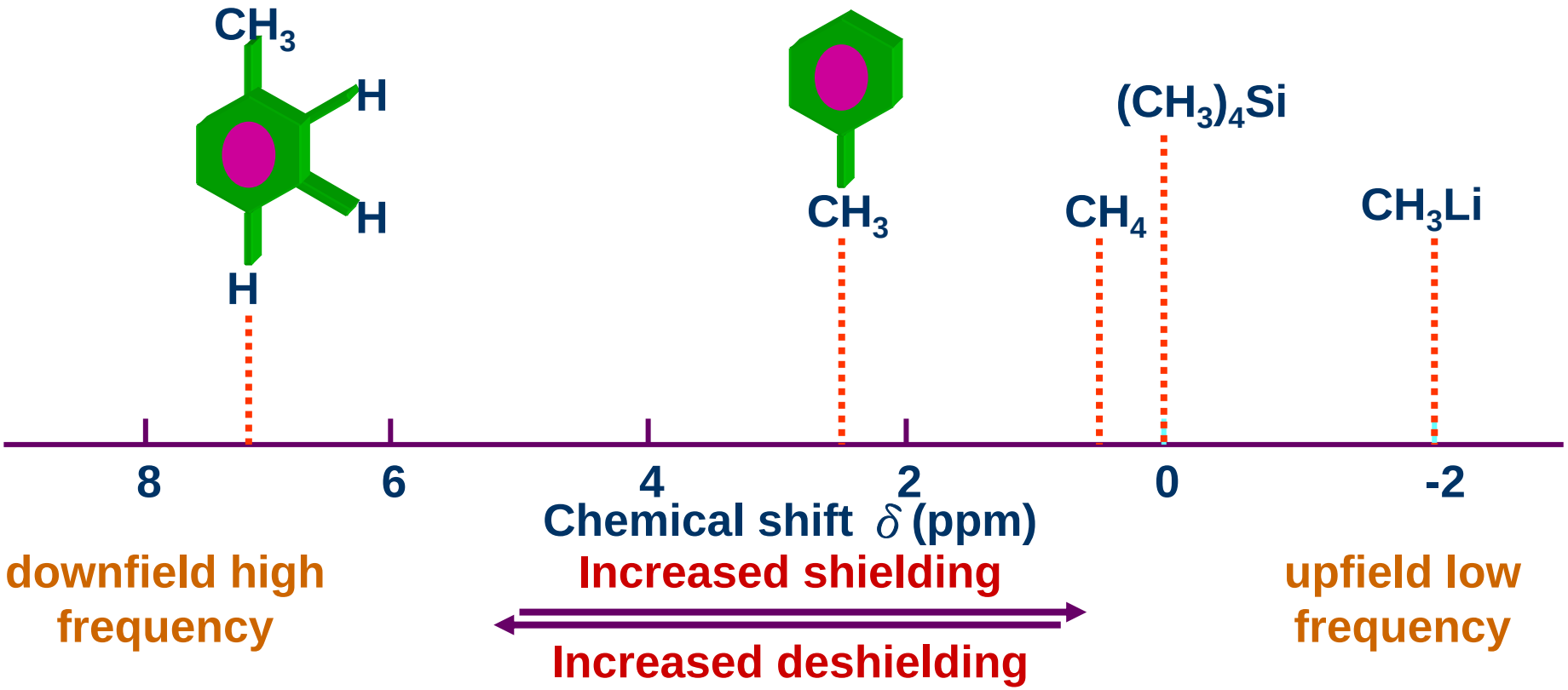




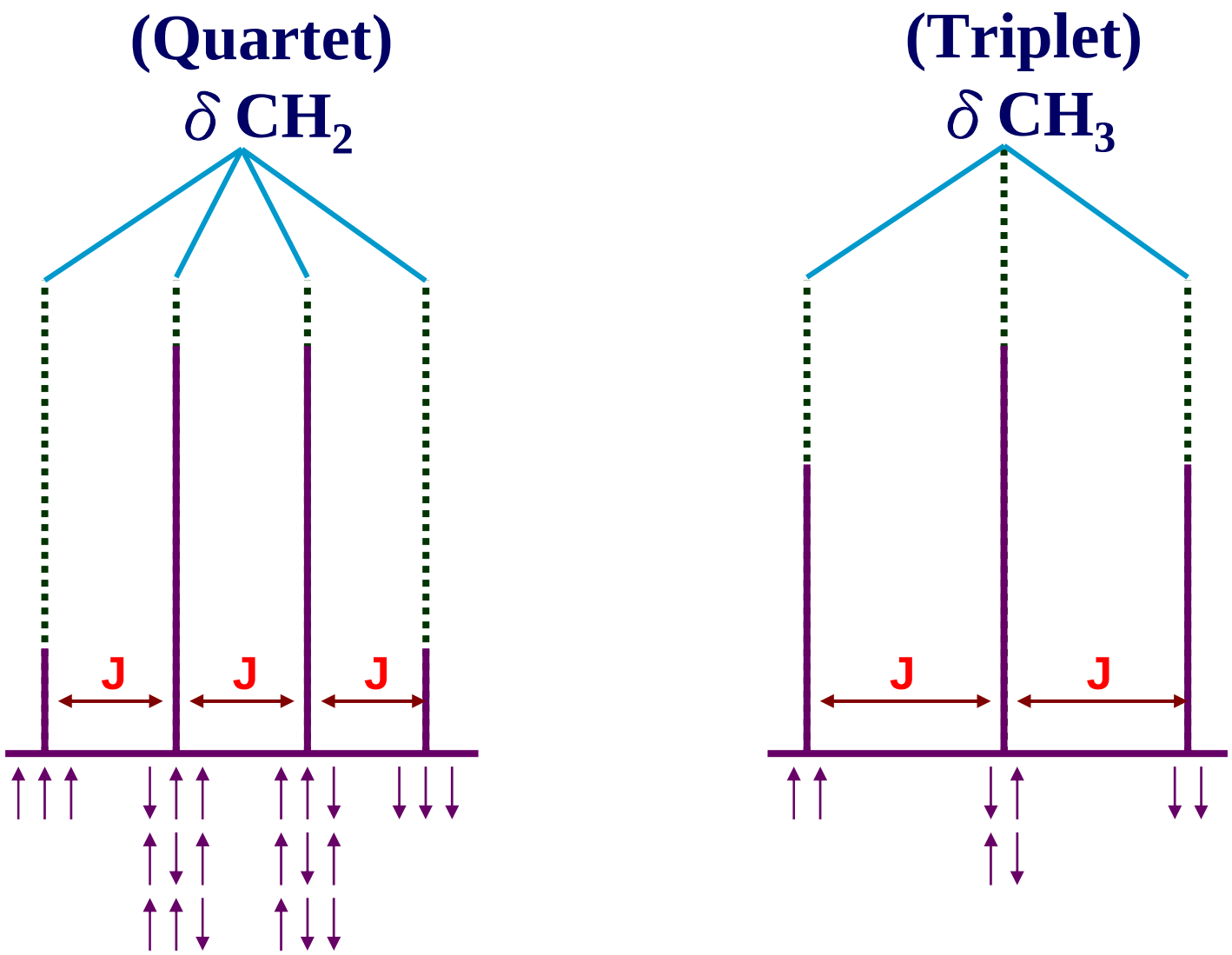
1. Chemical shift (δ) defines the location of a nmr line along the rf axis. It is measured relative to a reference compound. In frequency units the chemical shift is proportional to the applied static magnetic field, and therefore chemical shifts are customarily quoted in parts per million (ppm) units.

$$\Delta\nu = \nu_{signal} - \nu_{reference}$$

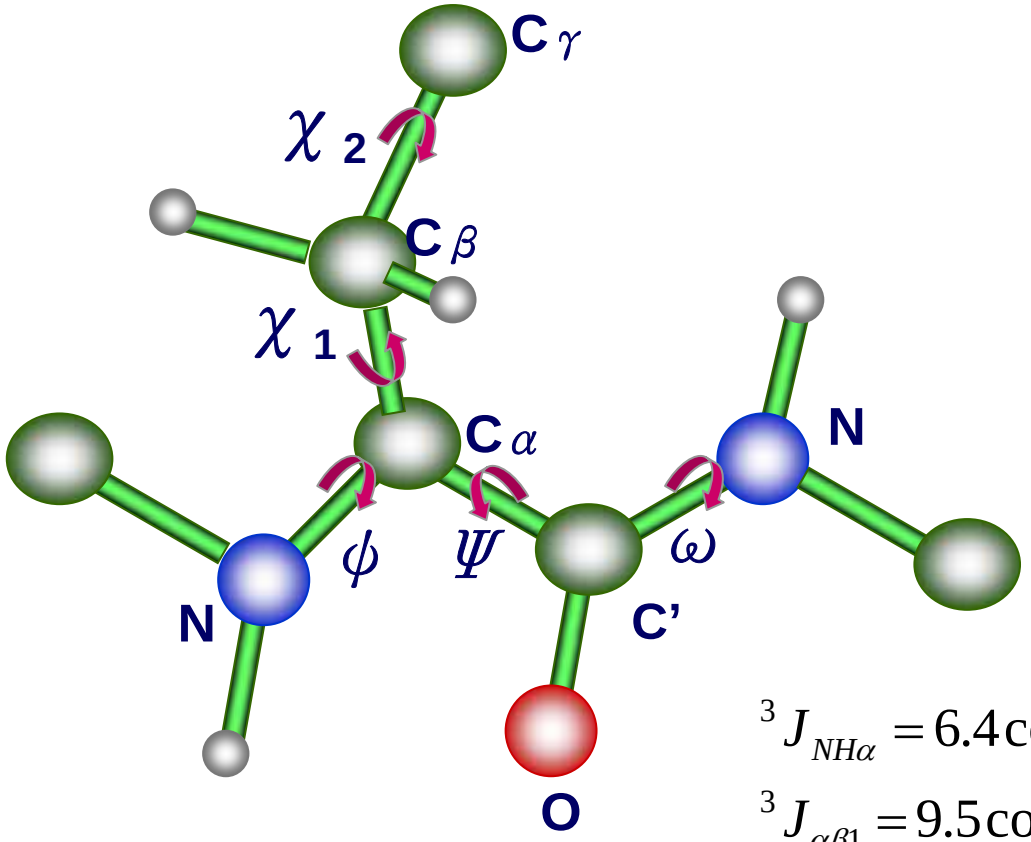
$$\delta(ppm) = (\Delta\nu / \nu_0) \times 10^6$$



2. Spin-spin coupling constant (J) characterize **scalar interactions (through-bond)** between nuclei linked via a small number of covalent bonds in a chemical structure. J is field independent and is customarily quoted in hertz (Hz).



Dihedral Angles: ϕ, Ψ, ω, χ_1, χ_2



$$^3J_{NH\alpha} = 6.4\cos^2(\phi - 60) - 1.4\cos(\phi - 60) + 1.9$$

$$^3J_{\alpha\beta 1} = 9.5\cos^2(\chi_1 - 120) - 1.6\cos(\chi_1 - 120) + 1.8$$

$$^3J_{\alpha\beta 2} = 9.5\cos^2\chi_1 - 1.6\cos\chi_1 + 1.8$$

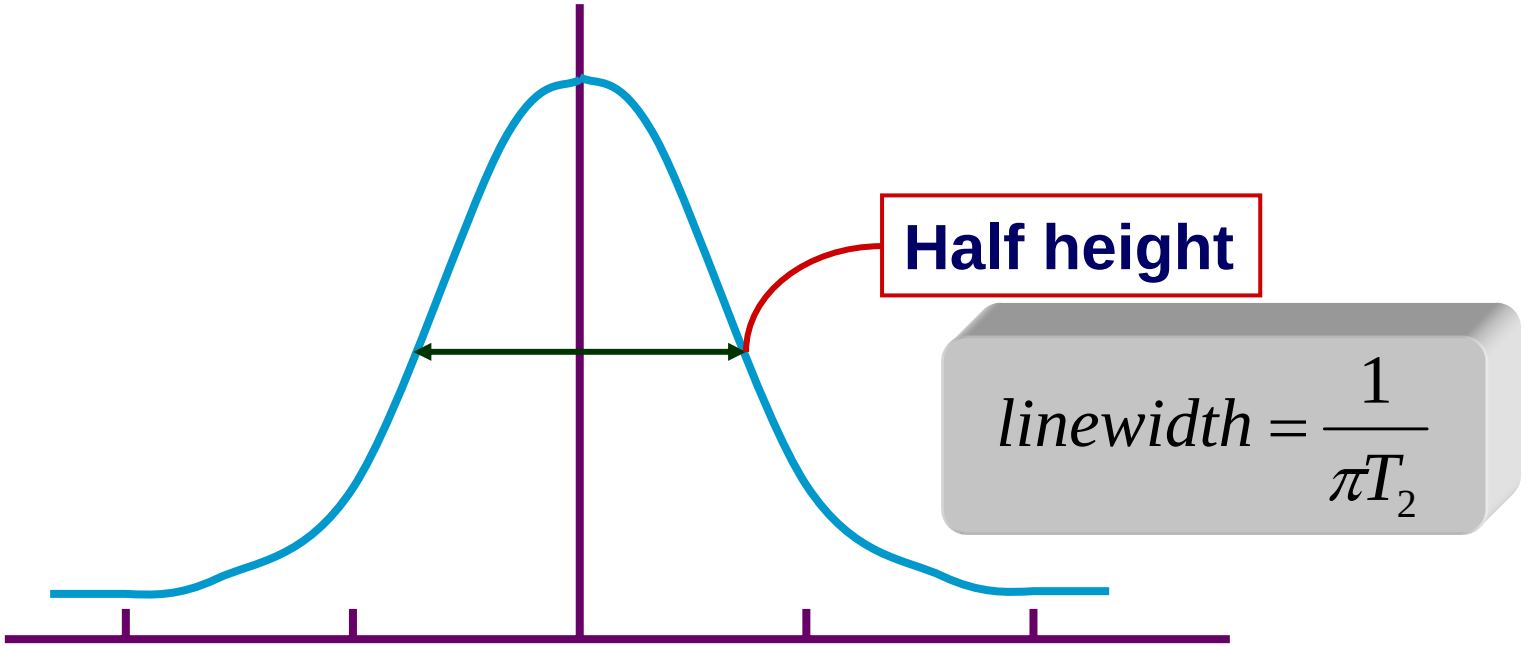
3. Nuclear Overhauser enchancement or Nuclear Overhauser effect (NOE) is the fractional change in intensity of one NMR line when another resonance is irradiated in a double irradiation experiment. Nuclear Overhauser effects are due to **dipolar interactions (through-space) between different nuclei and are correlated with the inverse sixth power of the internuclear disran.**

Nuclear Overhauser enchancement 簡稱**NOE, NOE** 效應是由原子間的偶極-偶極作用**(dipolar-dipolar interaction)** 所造成, 其強度和兩原子的距離的六次方成反比, 一般來說, 當兩個氫原子的距離小於**5Å (10-10 M),** 他們的**NOE** 效應便可在**NOESY**光譜上所觀察到, 而距離越小, 所觀察到的**NOE** 效應便越強, 因此, 以已知距離的一對氫原子的**NOE**為標準(例如苯環上的相鄰氫原子), 我們便可推得所有**NOE**所代表的距離.

$$NOE / NOE_{std} = r_{std}^{6} / r^{6}$$

4. **Longitudinal relaxation time or spin-lattice relaxation time (T_1)** describes the rate at which the magnetization returns to the thermodynamic equilibrium orientation along the static magnetic field after a rf pulse.

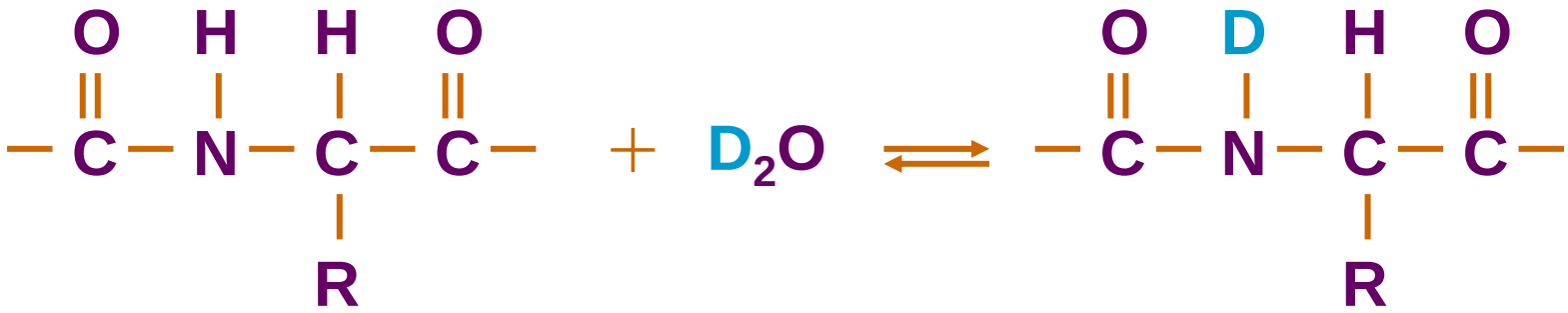
5. **Transverse relaxation time or spin-spin relaxation time (T_2)** describes the decay rate of the effective magnetization observed in the x,y plane after a rf pulse.



6. Labile protons:

$$-NH\ ;\ -OH\ ;\ -SH$$

Most of time, NMR signals of these labile protons can not be seen, due to their fast exchange with H_2O.



However, exchange rate study of amide proton can be used to identify if the amide protons form H-bond or are shielded from the solvent.

Structural information

- **Interproton distances :**

  NOE α R^{-6}

- **Dihedral angles:**

  J-coupling and Karplus equations

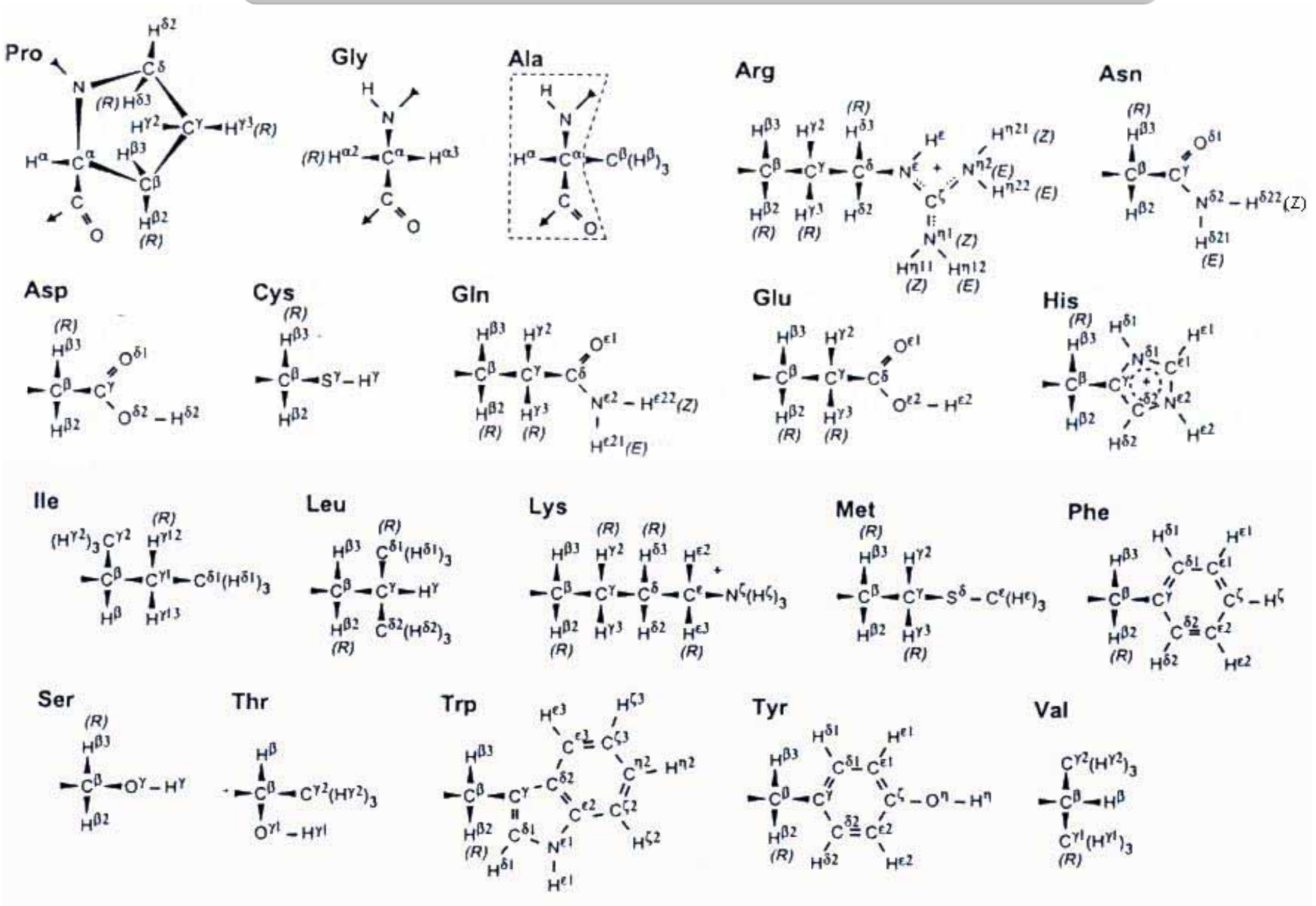
- **Chemical Shift Index (CSI):**

  Chemical shift of $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$

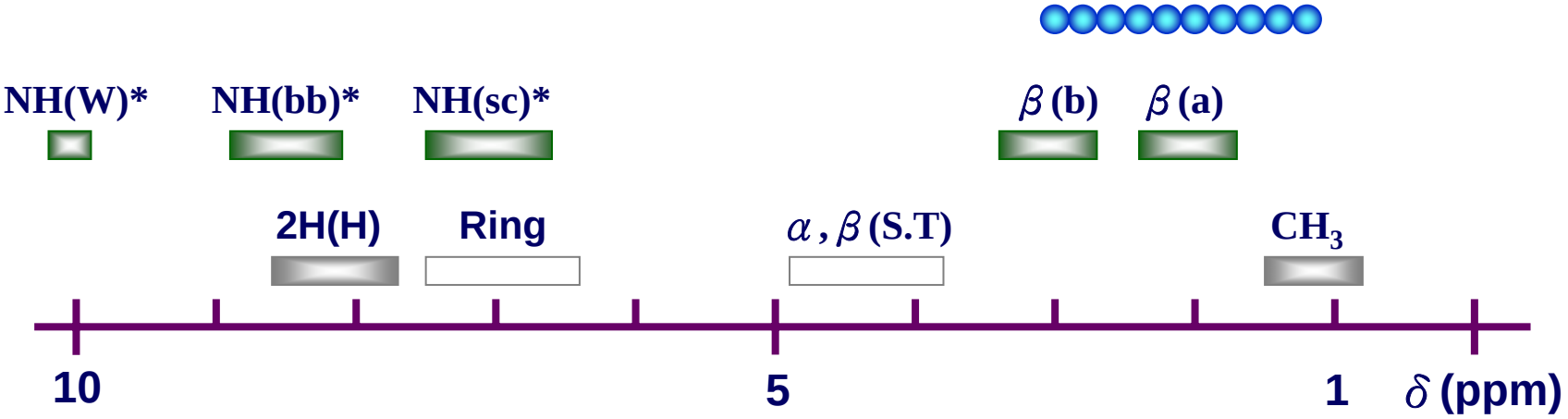
- **Hydrogen bonding:**

  Amide proton exchange rates

Recommended atom identifiers for the twenty common amino acids follow the 1969 IUPAC-IUB guidelines.



Groups of Hydrogen Atoms in the Common Amino Acid Residues with similar Random Coil ^{1}H Chemical Shifts[a].



Code	δ (ppm)	Comments
CH_3	0.9-1.4	
β (a)	1.6-2.3	β H of V, I, L, E, Q, M, P, R, K
β (b)	2.7-3.3	β H of C, D, N, F, Y, H, W
●●●●●	1.2-3.3	Other aliphatic CH
α , β (S.T)	3.9-4.8	All α H, β H of S and T
Ring	6.5-7.7	Aromatic CH of F, Y, W; 4H of H
2H(H)	7.7-8.6	2H of h in the pH range 1-11
NH(sc)*	6.6-7.6	Side chain NH of N, Q, K, R
NH(bb)*	8.1-8.8	Backbone NH
NH(W)*	10.2	Indole NH of W

Parameters for regular polypeptide conformations

	Bond Angle (deg)			Residues per turn	Translation per residue
	ϕ	Ψ	ω		
β	-139	+135	-178	2.0	3.4
β p	-119	+113	180	2.0	3.2
α -helix	-57	-47	180	3.6	1.50
3_{10}-helix	-49	-26	180	3.0	2.00
π -helix	-57	-70	180	4.4	1.15
Polyproline I	-83	+158	0	3.33	1.9
Polyproline II	-78	+149	180	3.0	3.12
Polyglycine II	-80	+150	180	3.0	3.1

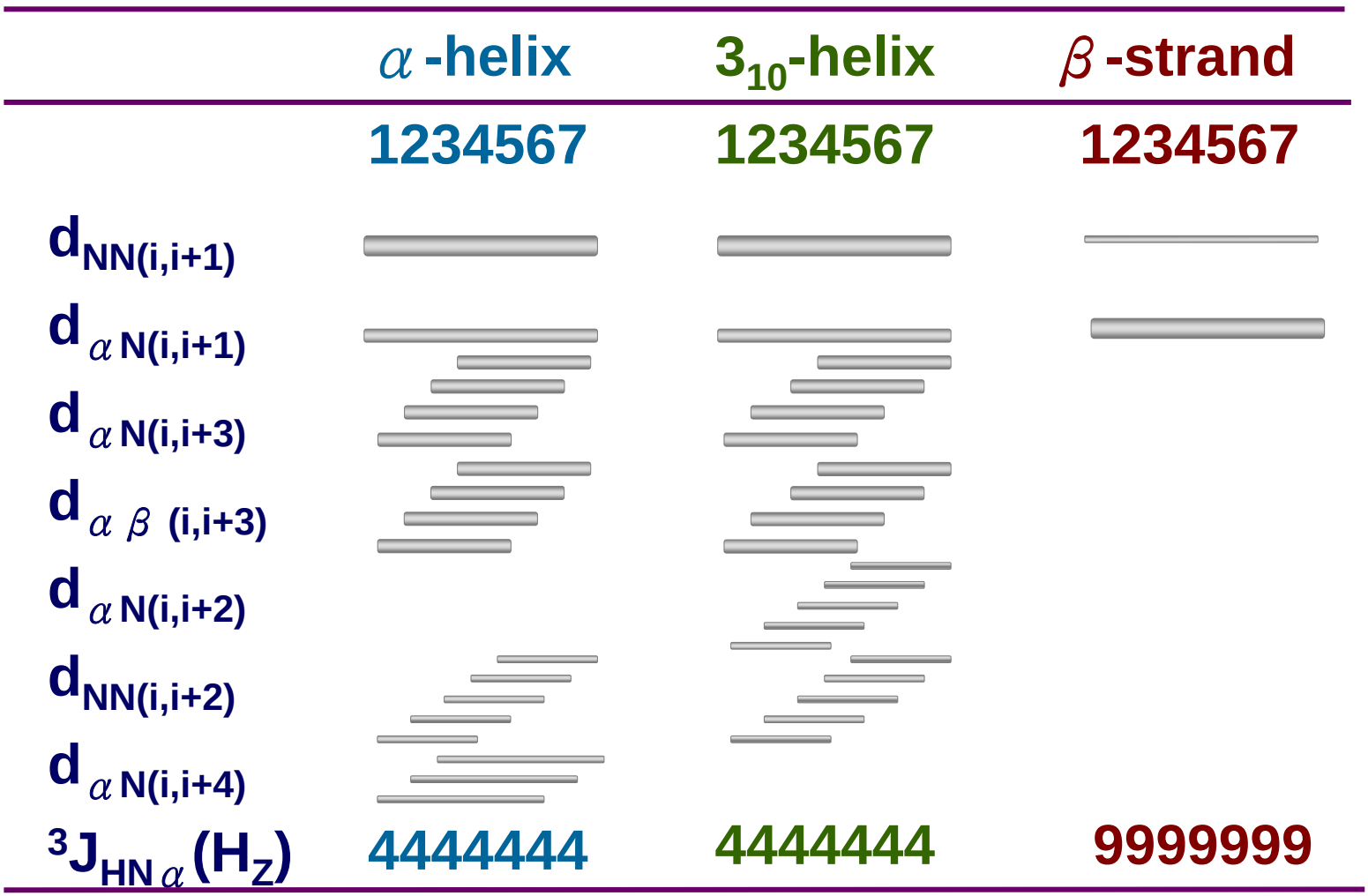
- Adapted from G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.* 23, 283-437(1968); IUPAC-IUB Commission on biochemical Nomemclature, *Biochemistry* 9, 3471-3479 (1970).

Short sequential and medium-range ^1H-^1H distances, vicinal coupling constants, and amide hydrogen exchange rates.

Parameter	α-helix	3_{10}-helix	β	β_P
$d_{\alpha N(i,i)}$	2.6	2.6	2.8	2.8
$d_{\alpha N(i,i+1)}$	3.5	3.4	2.2	2.2
$d_{\alpha N(i,i+2)}$	4.4	3.8		
$d_{\alpha N(i,i+3)}$	3.4	3.3		
$d_{\alpha N(i,i+4)}$	4.2	(>4.5)		
$d_{NN(i,i+1)}$	2.8	2.6	4.3	4.2
$d_{NN(i,i+2)}$	4.2	4.1		
$d_{\beta N(i,i+1)}$	2.5-4.1	2.9-4.4	3.2-4.5	3.7-4.7
$d_{\alpha \beta (i,i+3)}$	2.5-4.1	3.1-5.1		
$d_{\alpha \alpha (i,j)}$			2.3	4.8
$d_{\alpha N (i,j)}$			3.2	3.0
$d_{NN (i,j)}$			3.3	4.0
$^3J_{HN\alpha}(H_Z)$	($\leqq$4)	($\leqq$4)	($\geqq$9)	($\geqq$9)
NH exchange rate	slow	slow	slow	slow

- $d_{\alpha \alpha (i,j)}$, $d_{\alpha N (i,j)}$ and $d_{NN (i,j)}$ refer to interstrand distances.
- The first four residues in the α-helix and the first three residues in the 3_{10}-helix will have fast amide proton exchange rates.
- Every second residue in the flanking strand will have slow amide proton exchange rates

The characteristic patterns of short-range NOEs involving amide, alpha, beta protons observed for ideal α-helices, 3_{10}-helices and β-strand.



- The thickness of the lines is an indication of the intensity of the NOEs
- The values of J coupling are approximate.

Resonance assignment strategies for small proteins

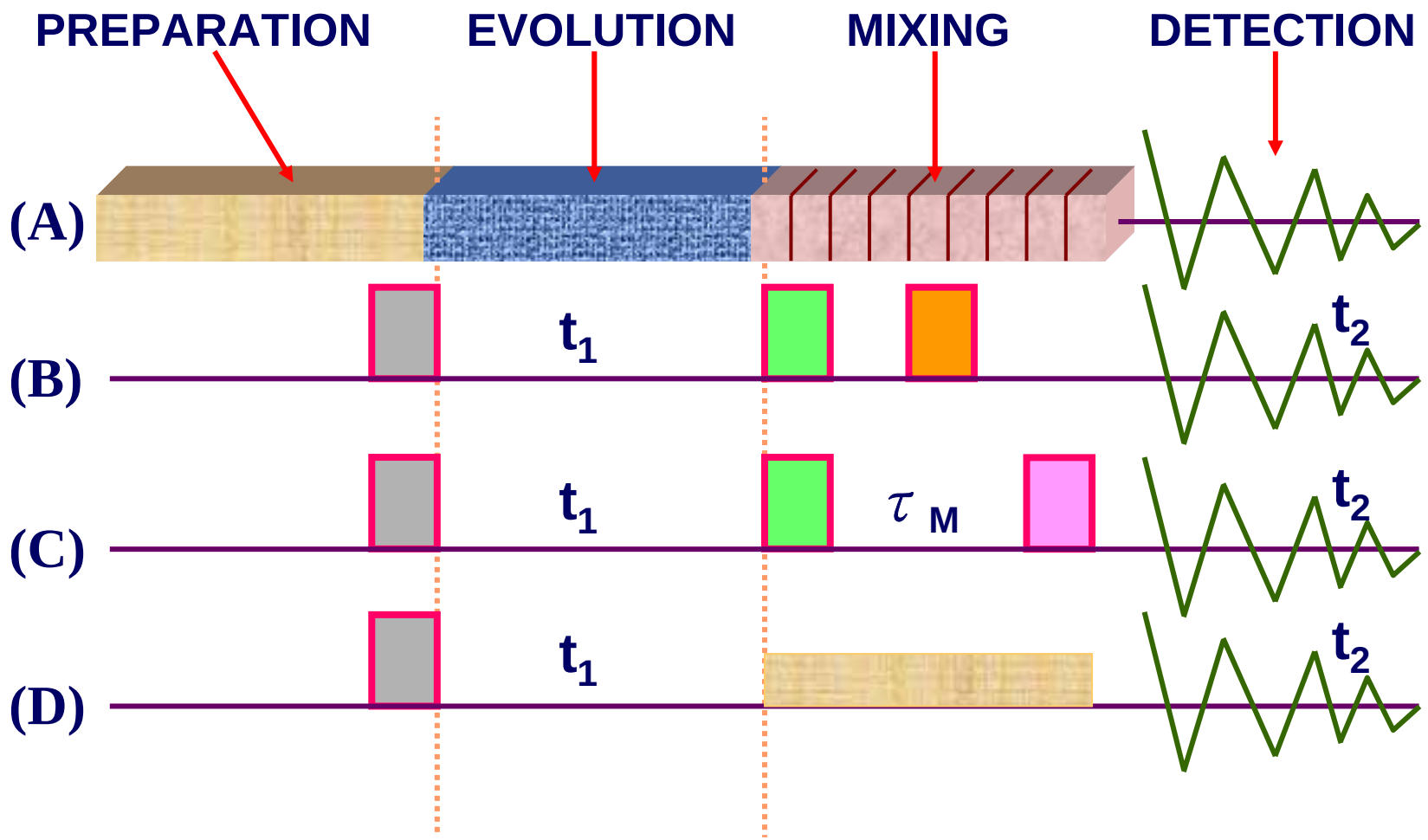
- **Spin system identification :**

  DQF-COSY and TOCSY experiments

- **Sequence-specific assignment:**

  NOESY experiment

For protein < 10 kDa, 2D homonuclear experiments may be sufficient for resolving overlapping NMR resonances.

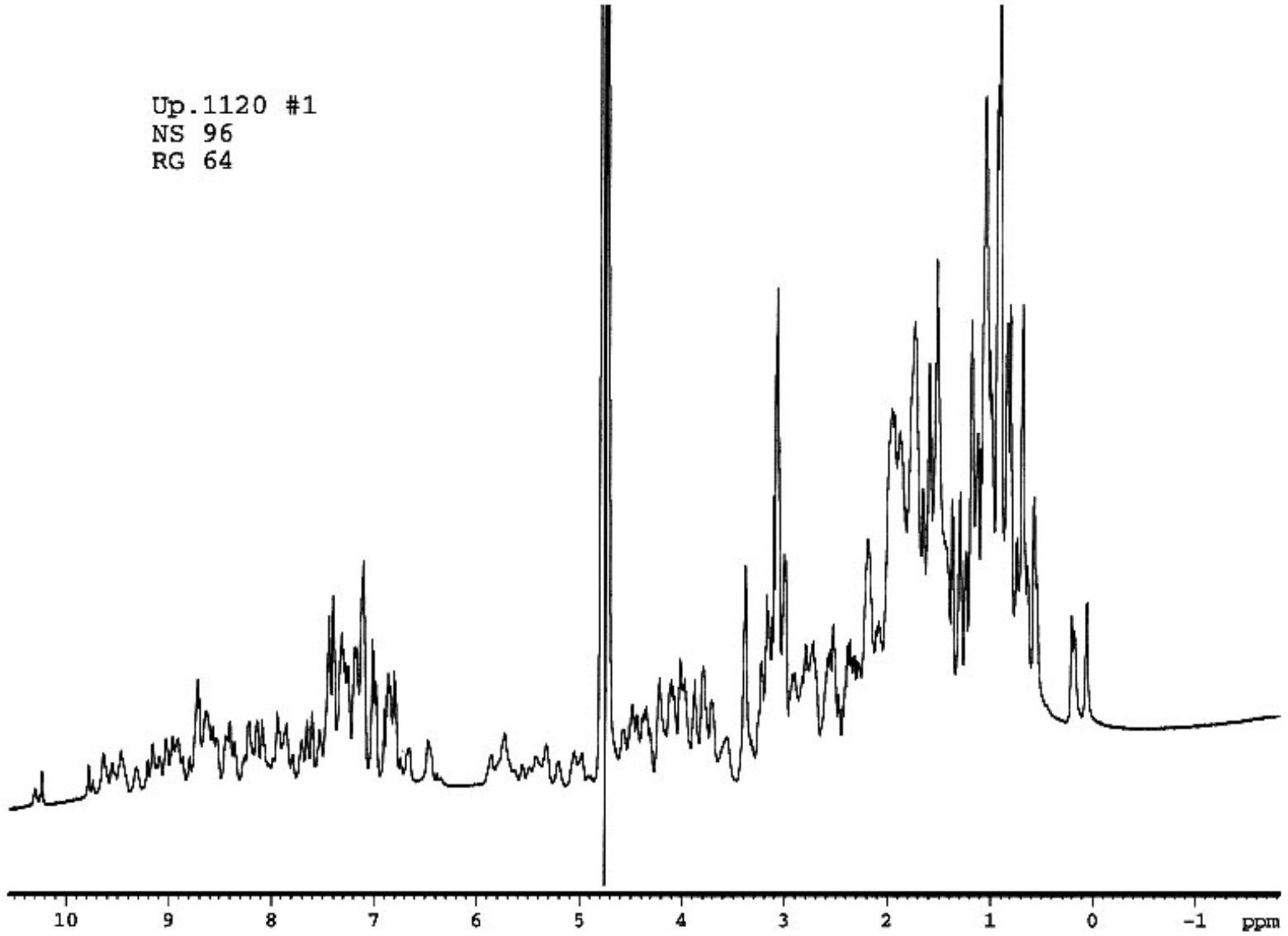


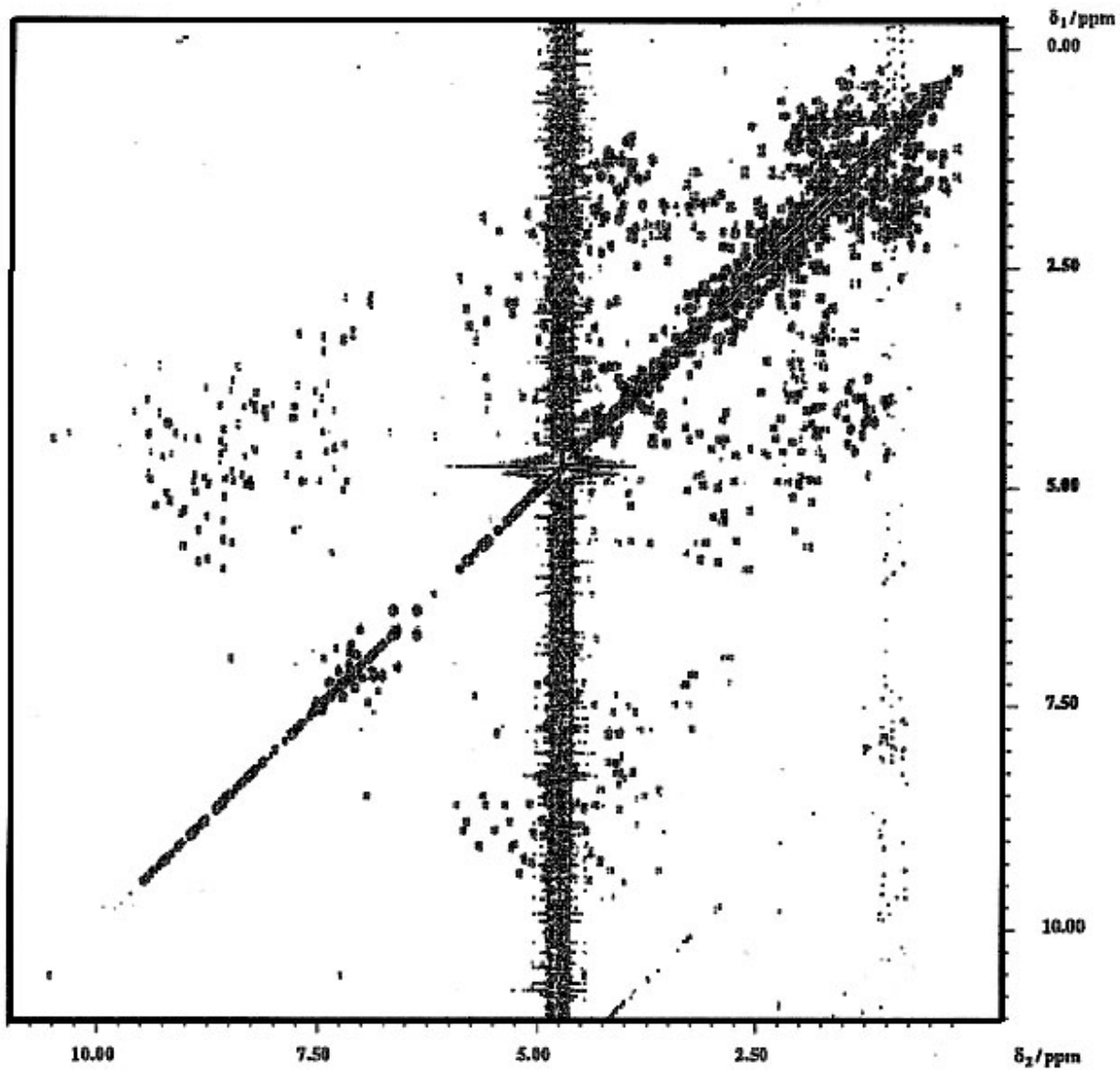
(A) The elements of a generalized two- dimensional NMR experiment

(B) DQFCOSY experiment

(C) NOESY experiment

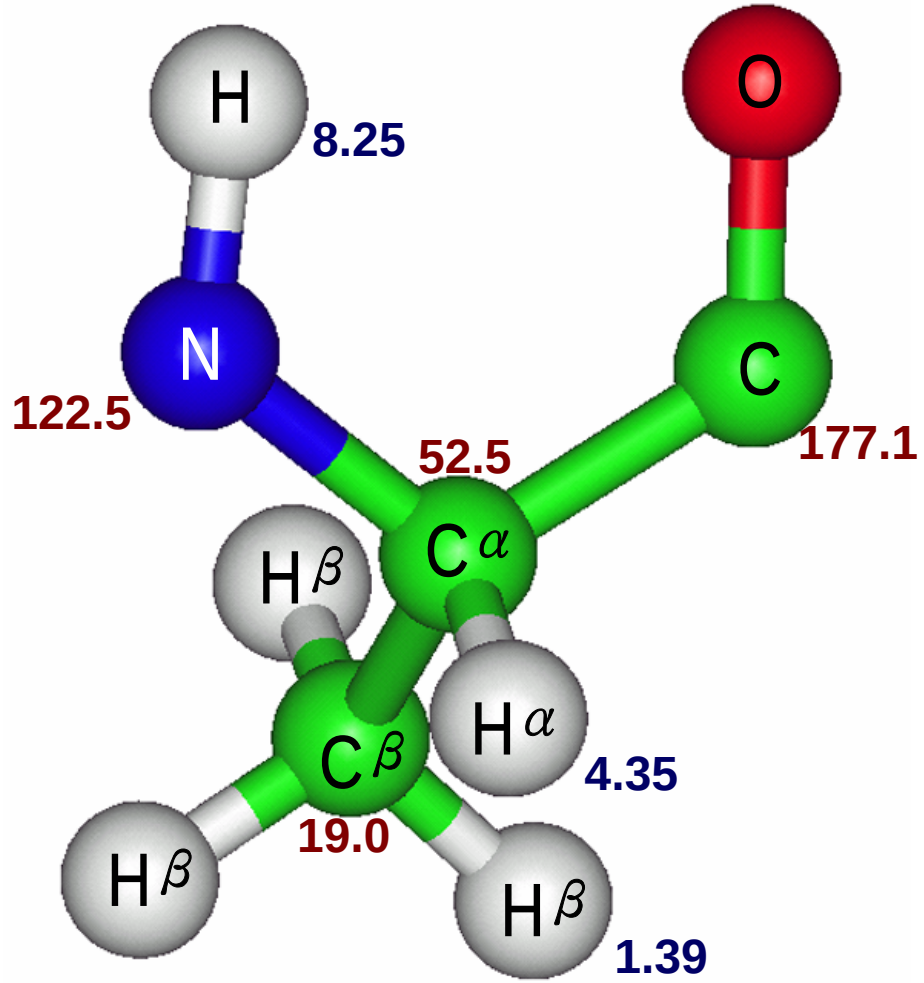
(D) TOCSY experiment

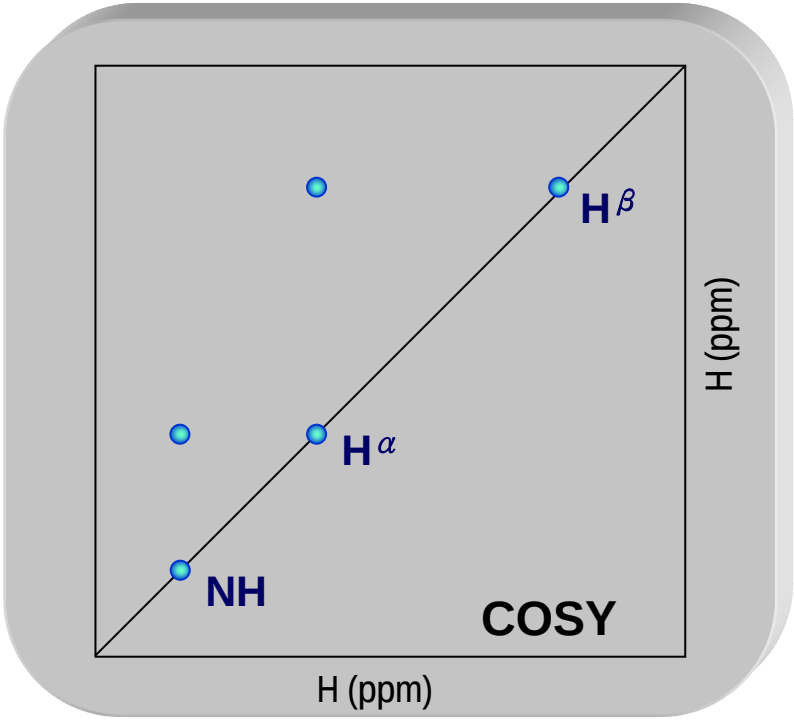

Up.1120 #1
NS 96
RG 64
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

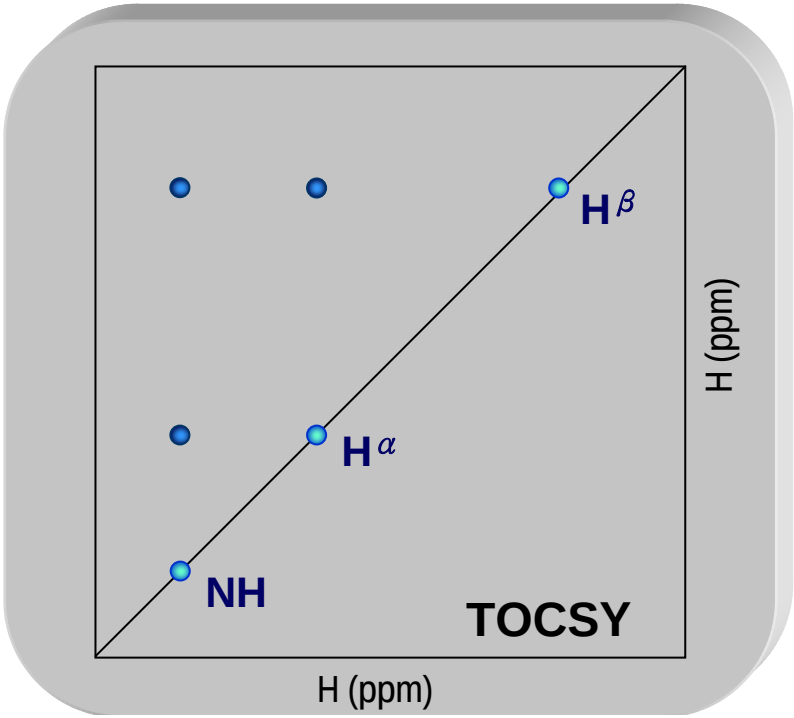


YAS435
exp:13
DQFCOSY

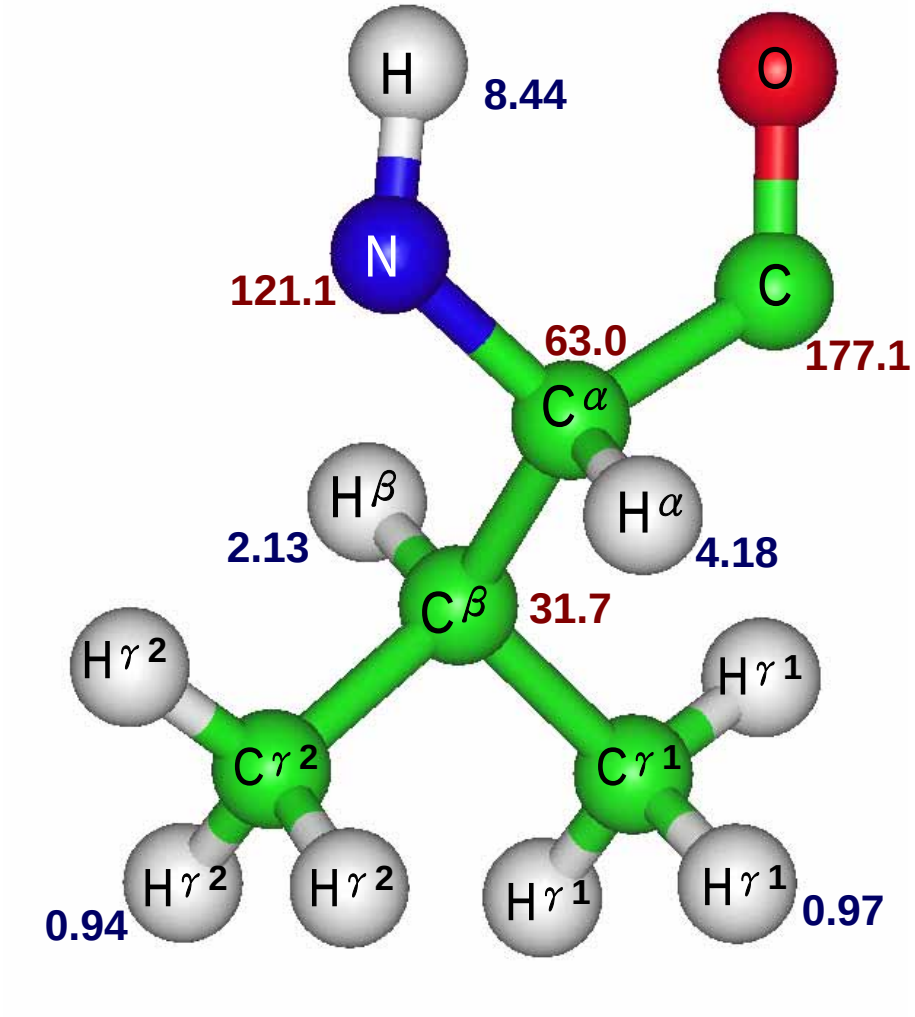
Ala

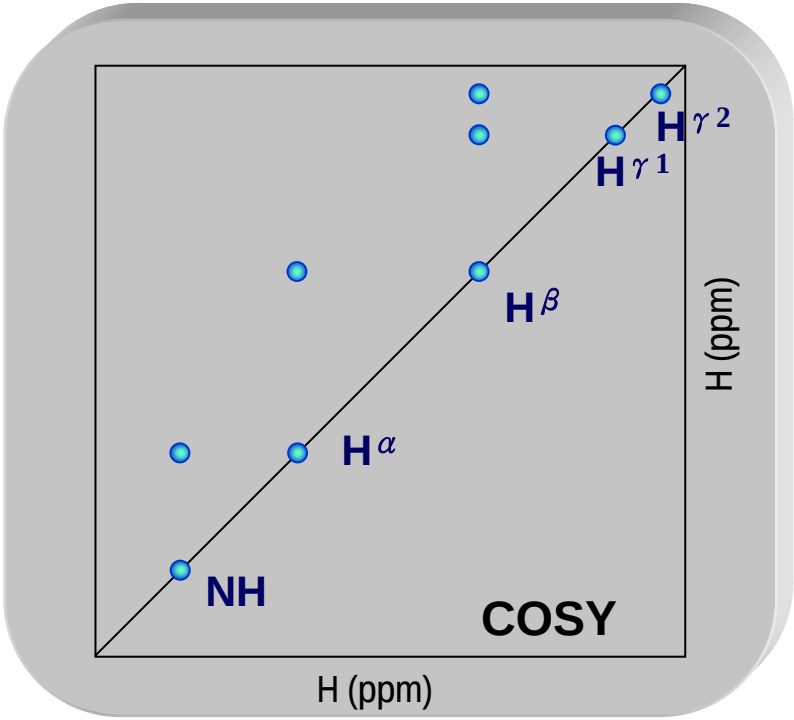

H
8.25
O
N
122.5
C
177.1
52.5
C α
H β
H α
4.35
C β
19.0
H β
H β
1.39

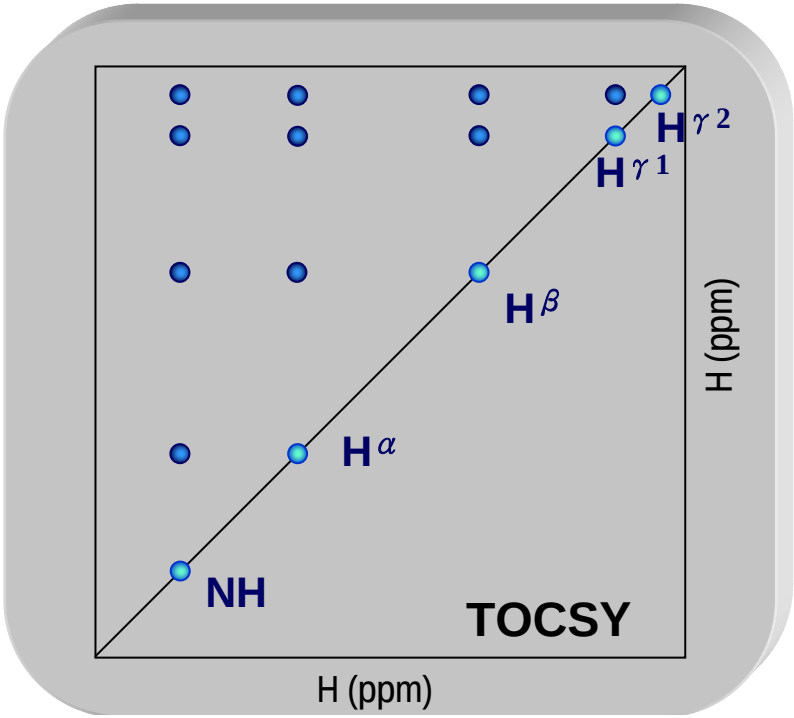

H β
H α
NH
COSY
H (ppm)
H (ppm)


H β
H α
NH
TOCSY
H (ppm)
H (ppm)

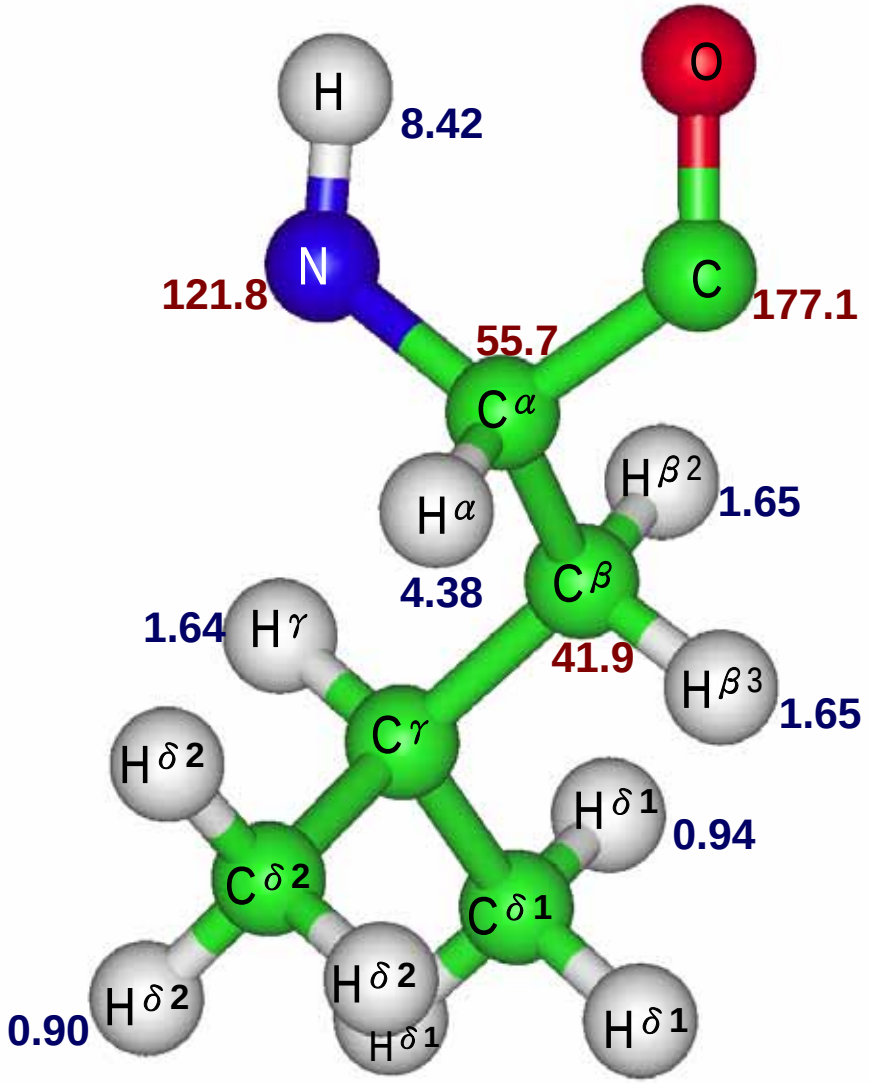
Val

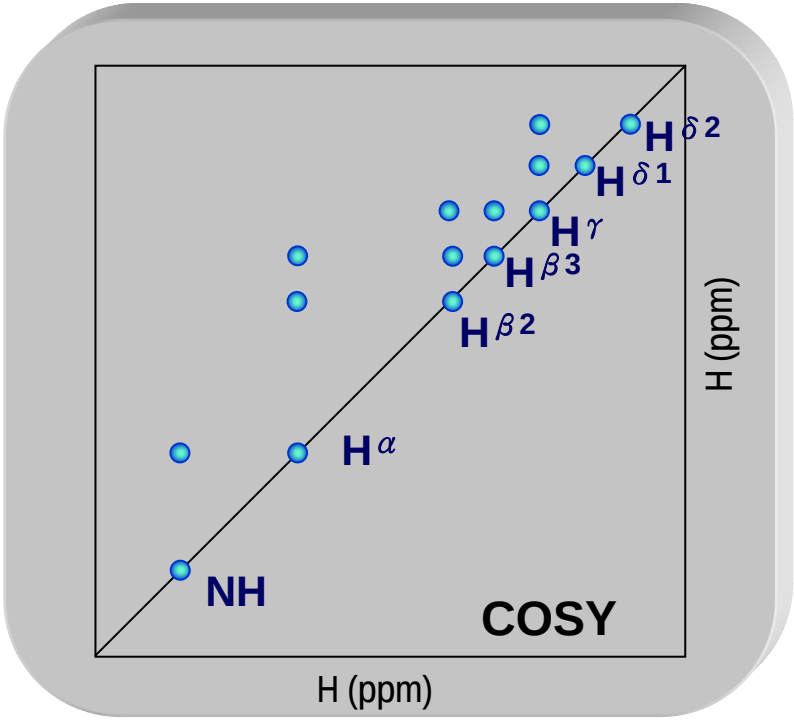

H
8.44
O
N
121.1
C
63.0
177.1
Cα
Hβ
2.13
Hα
4.18
Cβ
31.7
Hγ2
Hγ1
Cγ2
Cγ1
Hγ2
Hγ2
Hγ1
Hγ1
0.94
0.97

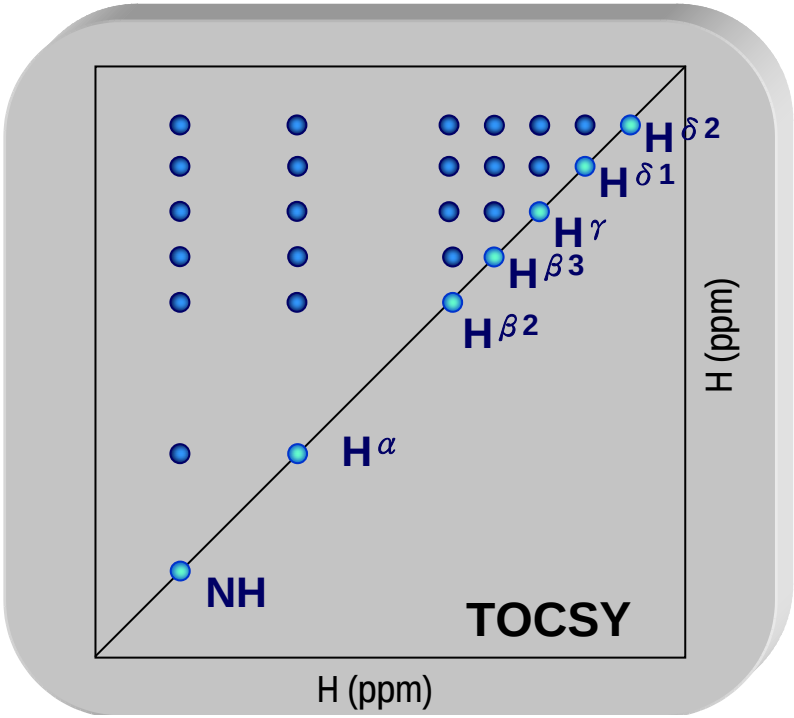

Hγ2
Hγ1
Hβ
Hα
NH
COSY
H (ppm)
H (ppm)


Hγ2
Hγ1
Hβ
Hα
NH
TOCSY
H (ppm)
H (ppm)

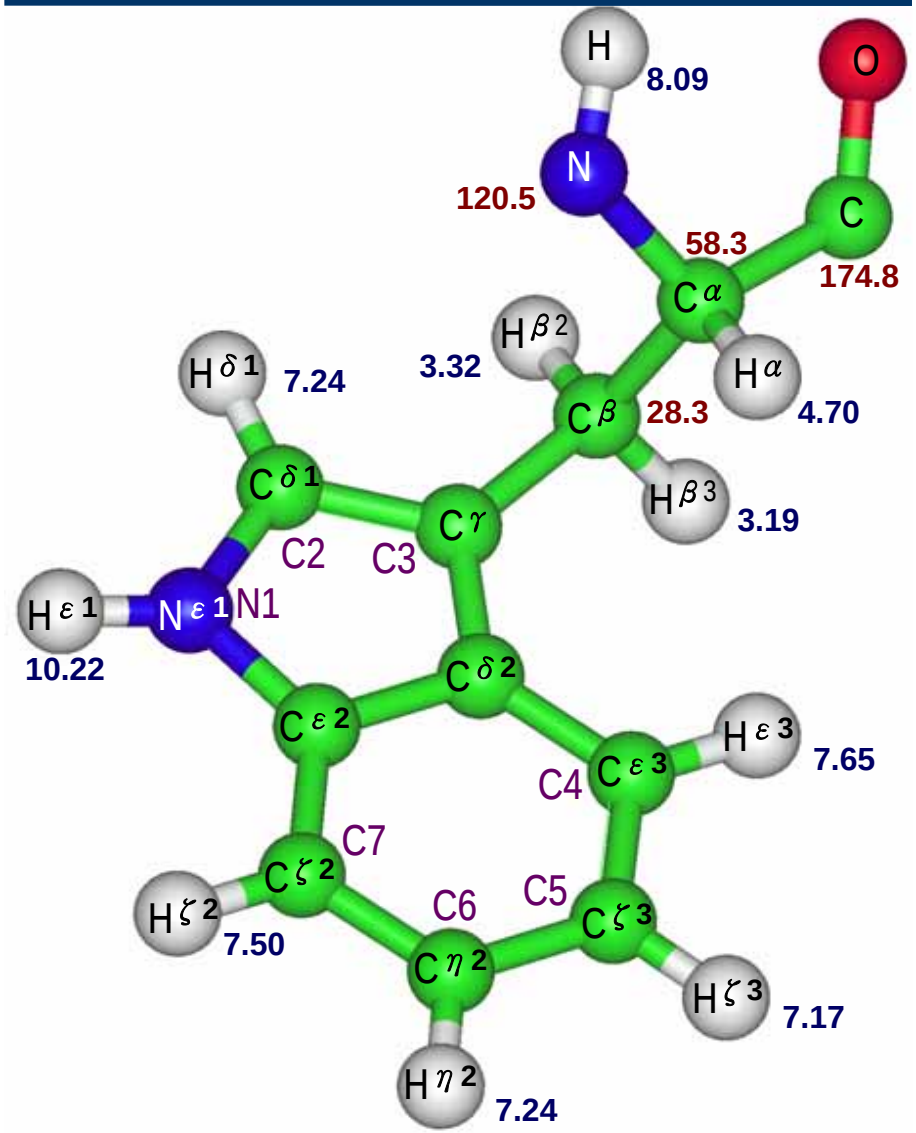
Leu

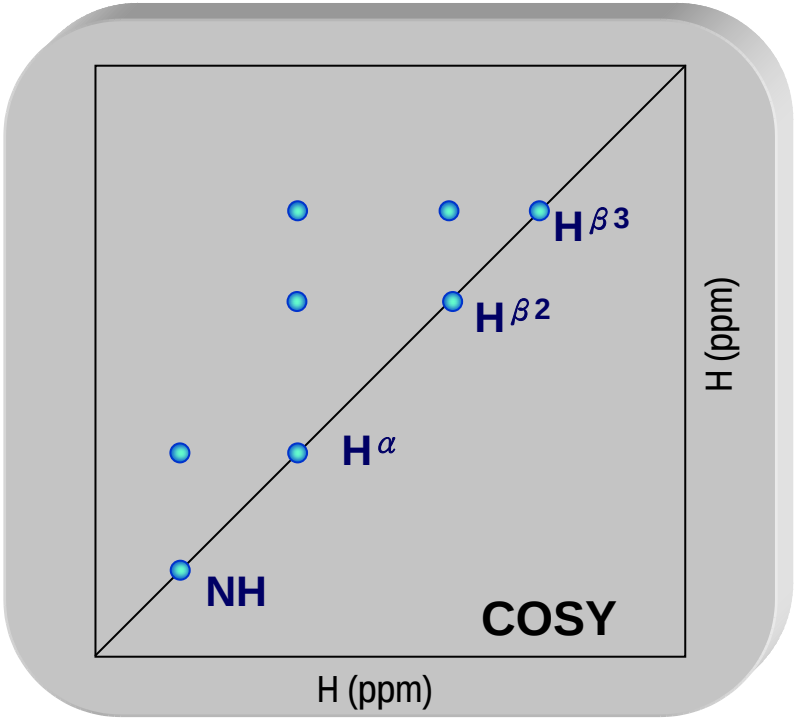

H
8.42
O
N
121.8
C
177.1
55.7
Cα
Hα
4.38
Hβ2
1.65
Cβ
41.9
1.64
Hγ
Hβ3
1.65
Cγ
Hδ2
Hδ1
0.94
Cδ2
Cδ1
Hδ2
0.90
Hδ2
Hδ1
Hδ1

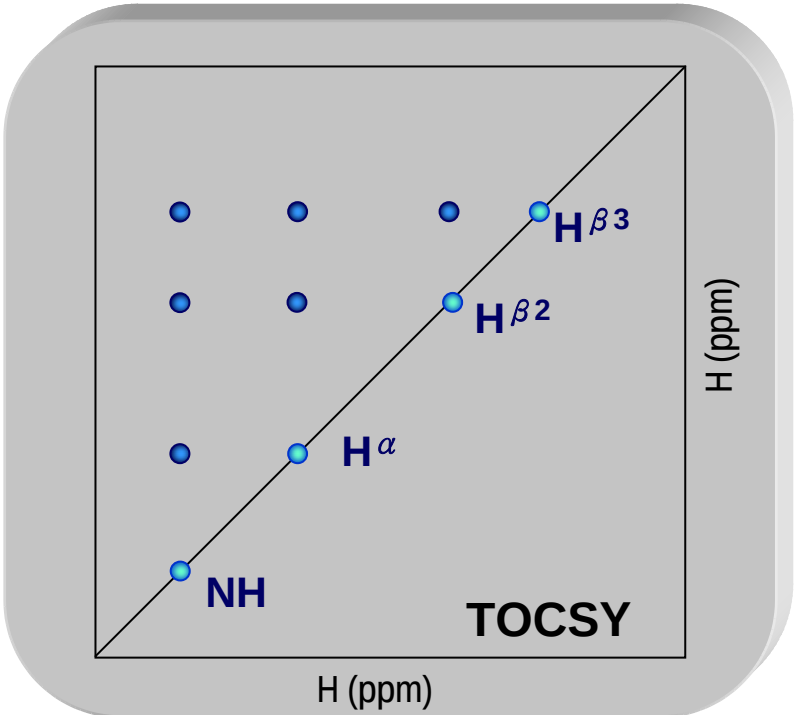

Hδ2
Hδ1
Hγ
Hβ3
Hβ2
Hα
NH
COSY
H (ppm)
H (ppm)


Hδ2
Hδ1
Hγ
Hβ3
Hβ2
Hα
NH
TOCSY
H (ppm)
H (ppm)

Trp

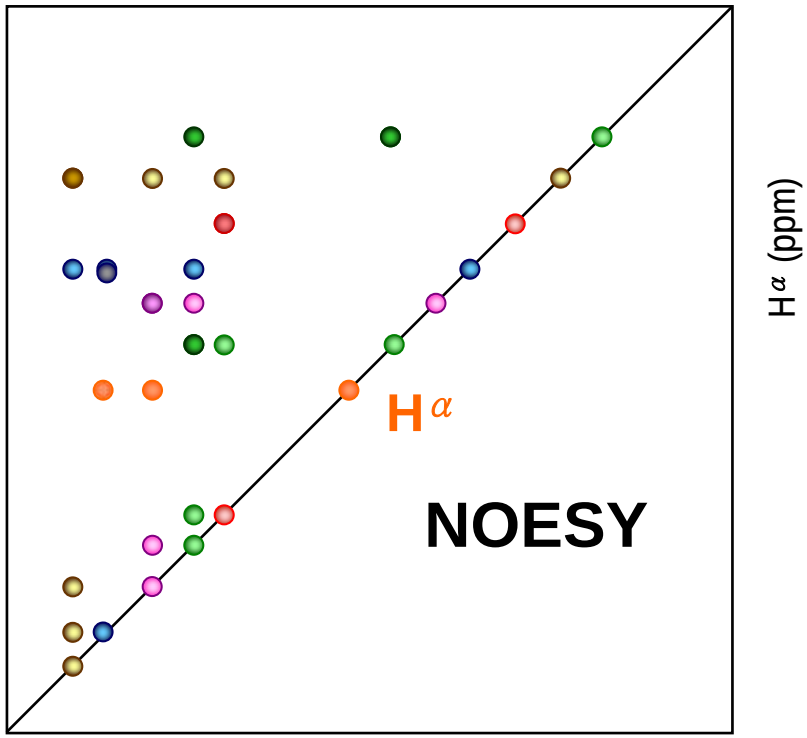

H
8.09
O
N
120.5
C
58.3
174.8
Cα
Hβ2
3.32
Hδ1
7.24
Hα
4.70
Cβ
28.3
Cδ1
Cγ
Hβ3
3.19
C2
C3
Hε1
Nε1
N1
10.22
Cδ2
Cε2
Hε3
7.65
Cε3
C4
C7
Cζ2
Hζ2
7.50
C6
C5
Cζ3
Cη2
Hζ3
7.17
Hη2
7.24

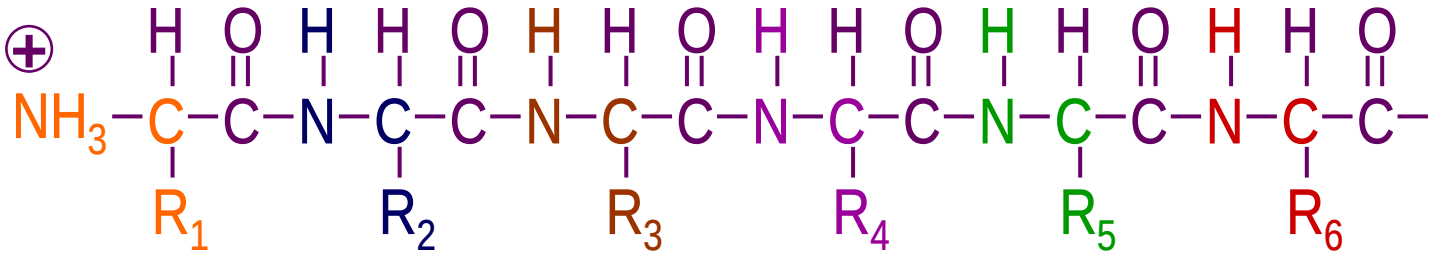

Hβ3
Hβ2
Hα
NH
COSY
H (ppm)
H (ppm)


Hβ3
Hβ2
Hα
NH
TOCSY
H (ppm)
H (ppm)

Fingerprint region

- Phe
- Val
- Gln
- Ala
- Thr
- Ala’





F V Q A T A’

α-helical NOEs $d_{\alpha N}(i,i+3)$, $d_{NN}(i,i+1)$

RC-RNase 2D DQFCOSY at 310K

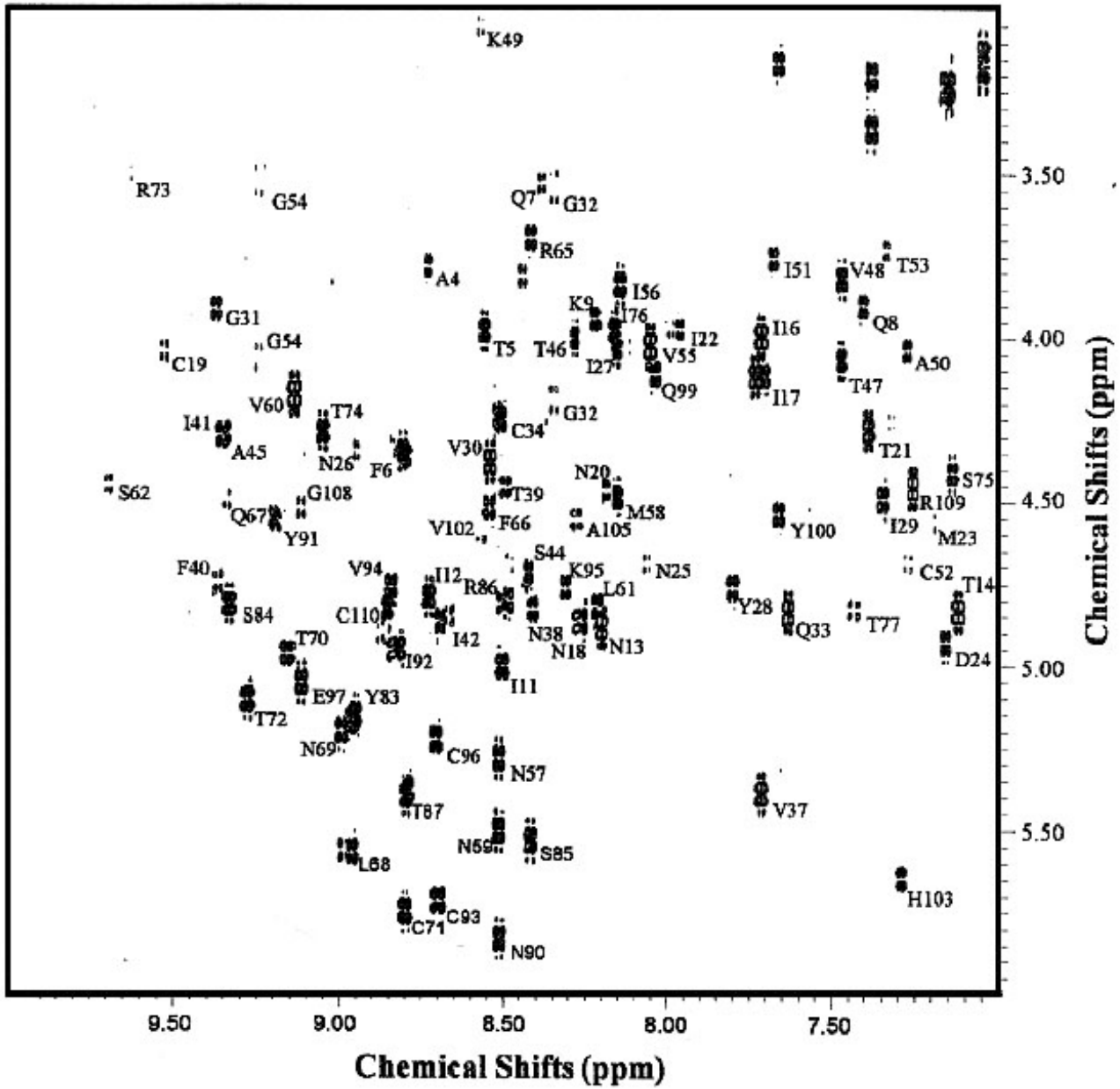
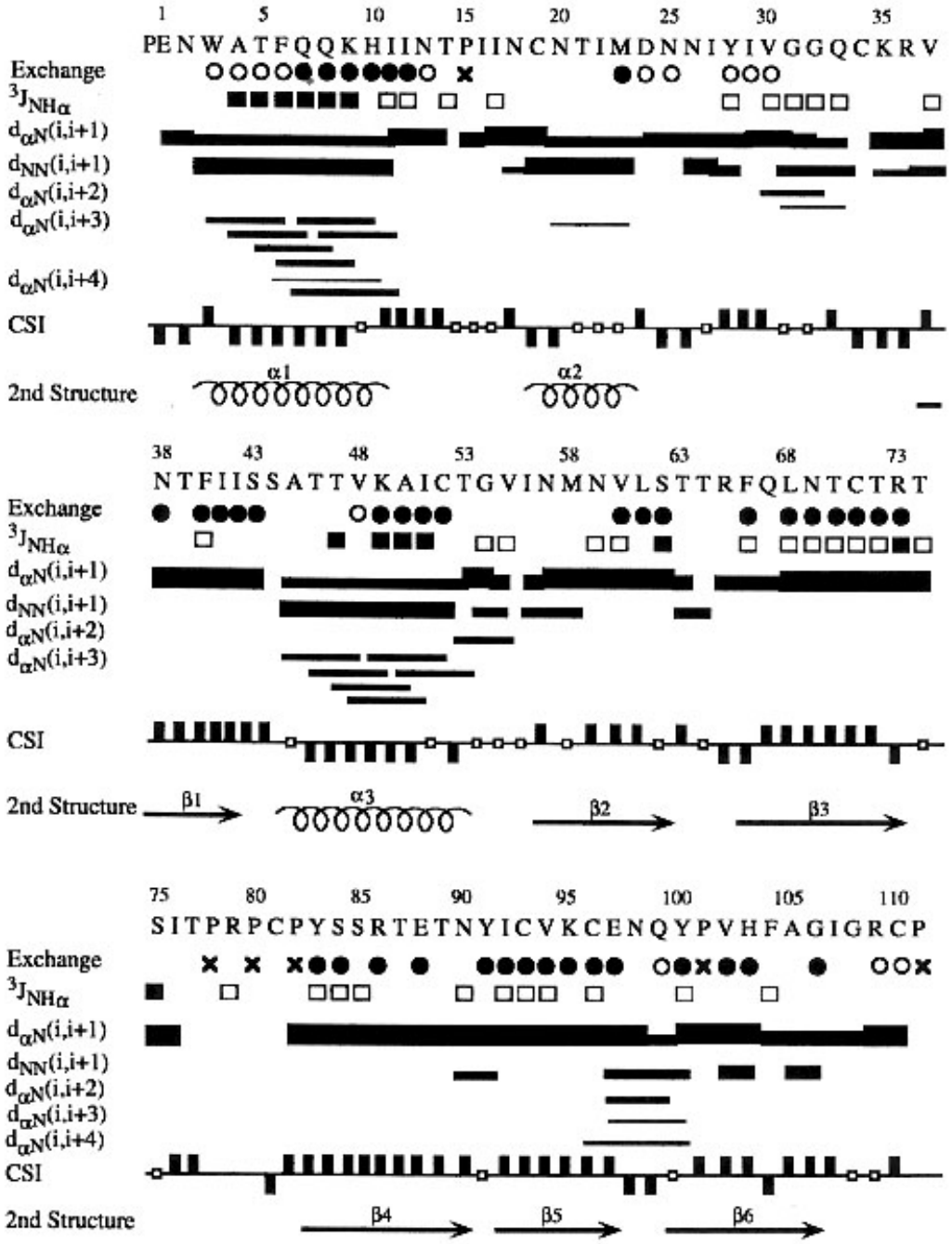


Table 1. ^{1}H chemical shifts for Rc-RNase in 90%/10% H_2O/D_2O at 310 K, pH 3.5, taking TSP resonance (0.00 ppm) as a reference. The chemical shifts of second conformers are parenthesized.

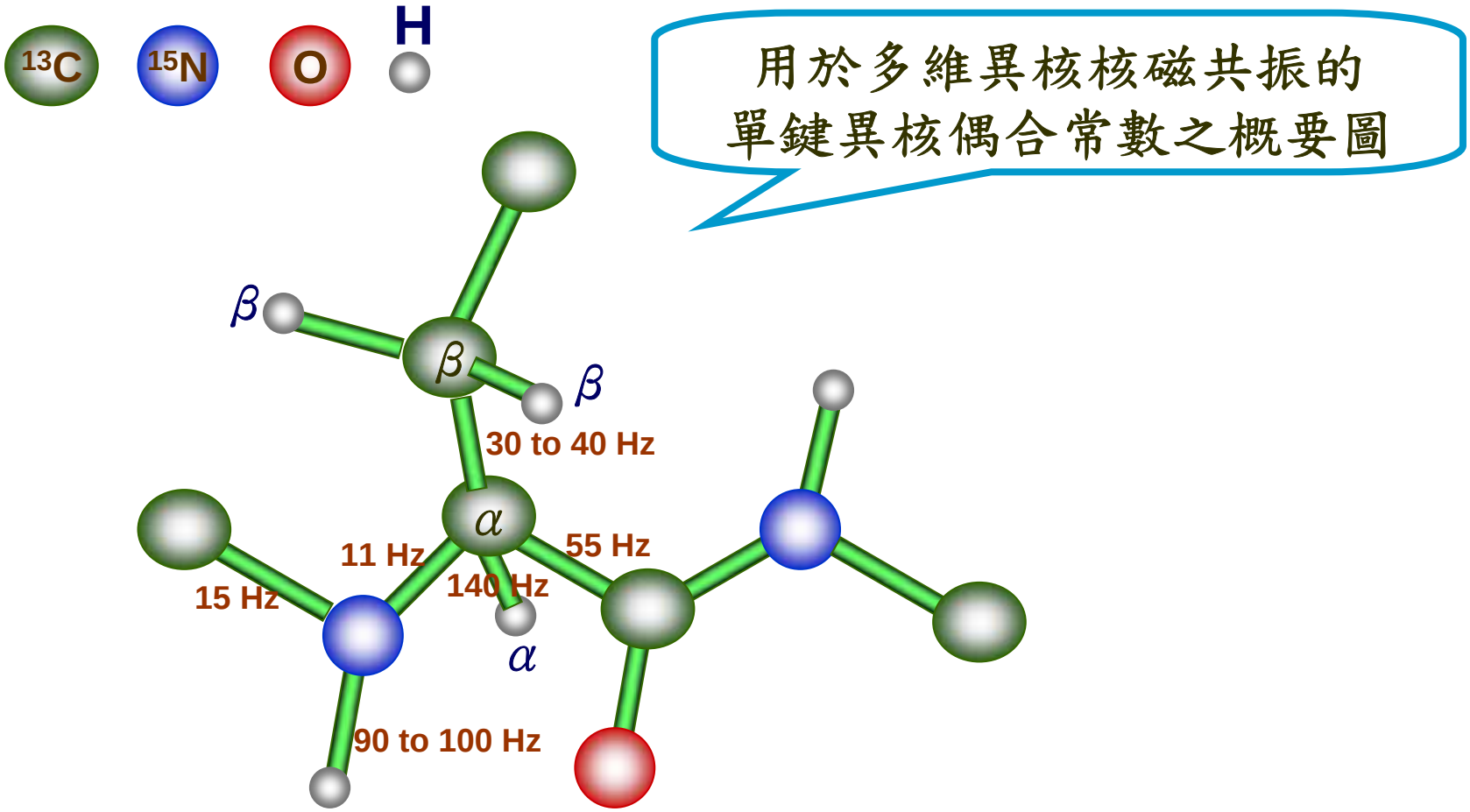
Residues	Chemical Shift (ppm)				
	NH	CαH	CβH	CγH	Others
PE1		3.89			
Asn2	8.04	4.65	3.31, 3.21		$N\delta H_2$ 7.82
Trp3	9.36	5.54	3.17, 3.04		NεH 10.41; 2H 7.14; 4H 8.42; 5H 6.87; 6H 7.37; 7H 7.46
Ala4	8.72	3.80	1.40		
Thr5	8.55	4.00	3.90	1.31	
Phe6	8.83	4.34	3.62, 3.20		$C_{2,6}H$ 7.20; $C_{3,5}H$ 7.01; C_4H 6.83
Gln7	8.39	3.55	1.30, 1.67	1.93, 1.85	
Gln8	7.41	3.91	2.20, 2.03	2.43, 2.43	
Lys9	8.22	3.96	1.44		
His10	7.90	4.65	2.75, 2.19		$C\varepsilon_1H$ 8.52; $C\delta_2H$ 6.59
Ile11	8.50	5.02	2.03, 1.72	1.13	0.95, 0.95
Ile12	8.72	4.81	1.83	1.35, 1.35	0.86
Asn13	8.19	4.90	2.88, 2.88		
Thr14	7.14	4.84	4.17	1.11	
Pro15	4.35	2.23, 2.23	1.96, 1.96		$C\delta H_2$ 3.84, 3.67
Ile16	7.70	4.01	1.76	1.32, 1.03	0.71, 0.62
Ile17	7.72 (7.74)	4.13	1.42		0.71, 0.49
Asn18	8.26	4.88	3.07, 2.62		$N\delta H_2$ 7.54, 7.04
Cys19	9.51	4.06	2.81, 2.43		
Asn20	8.19	4.48	2.98, 2.98		
Thr21	7.39	4.30	4.18	1.23	
Ile22	7.96 (7.99)	3.99	1.53		0.64
Met23	7.19	4.56	1.39, 0.51	2.17, 1.70	
Asp24	7.16	4.94	3.12, 2.63		
Asn25	8.03	4.69	2.85, 2.66		
Asn26	8.94	4.36	2.87, 2.82		$N\delta H_2$ 7.45, 6.81
Ile27	8.15	4.04	1.53		0.55, 0.28
Tyr28	7.80	4.76	3.51, 3.13		$C_{2,6}H$ 6.96; $C_{3,5}H$ 6.54
Ile29	7.35	4.51	2.14		0.69
Val30	8.53	4.40	2.04	1.01, 1.01	
Gly31	9.36	3.93, 3.93			
Gly32	8.34	4.20, 3.56			
Gln33	7.64	4.85	2.09, 1.97	2.35, 2.35	
Cys34	8.43	3.83	2.40, 1.08		
Lys35	8.38	4.25			
Arg36	9.02	3.87	2.08, 1.90	1.79, 1.65	$C\delta H_2$ 3.38, 3.23; NεH 7.37
Val37	7.71 (7.65)	5.41 (5.34)	2.01	0.96, 0.91	
Asn38	8.41	4.84	2.21, 1.92		
Thr39	8.80	4.46			
Phe40	9.38	4.77	3.07, 2.71		$C_{2,6}H$ 7.08; $C_{3,5}H$ 6.93; C_4H 7.03
Ile41	9.36	4.32	1.74	0.78	0.70, 0.58
Ile42	8.68 (8.66)	4.89 (4.87)	1.96	1.27, 1.27	0.74, 0.62
Ser43	8.10	4.65	4.23, 3.26		
Ser44	8.41	4.73	4.27, 4.10		
Ala45	9.34	4.32	1.77		
Thr46	8.28	4.01	4.24	0.98	
Thr47	7.47	4.08	4.41	1.42	
Val48	7.47	3.83	2.40	1.09, 1.06	
Lys49	8.56	3.07	1.80, 1.68	1.03, 0.60	$C\delta H_2$ 1.32, 1.32; $C\varepsilon H_2$ 2.59, 2.28
Ala50	7.28	4.05	1.54		
Ile51	7.68	3.77	1.70	1.00	0.78
Cys52	7.27	4.72	2.85, 2.01		
Thr53	7.34	3.75	4.01	1.20	
Gly54	9.24	4.08, 3.54			
Val55	8.05	4.04	1.87	0.79, 0.79	


1 5 10 15 20 25 30 35
PENWATFQQKHIINTPIINCNTIMDNNIYIVGGQCKRV
Exchange
$^3J_{NH\alpha}$
$d_{\alpha N}(i,i+1)$
$d_{NN}(i,i+1)$
$d_{\alpha N}(i,i+2)$
$d_{\alpha N}(i,i+3)$
$d_{\alpha N}(i,i+4)$
CSI
2nd Structure
α1
α2
38 43 48 53 58 63 68 73
NTFIISSATTVKAICTGVINMNVLSTTRFQLNTCTRT
Exchange
$^3J_{NH\alpha}$
$d_{\alpha N}(i,i+1)$
$d_{NN}(i,i+1)$
$d_{\alpha N}(i,i+2)$
$d_{\alpha N}(i,i+3)$
CSI
2nd Structure
β1
α3
β2
β3
75 80 85 90 95 100 105 110
SITPRPCPYSSRTETNYICVKCENQYPVHFAGIGRCP
Exchange
$^3J_{NH\alpha}$
$d_{\alpha N}(i,i+1)$
$d_{NN}(i,i+1)$
$d_{\alpha N}(i,i+2)$
$d_{\alpha N}(i,i+3)$
$d_{\alpha N}(i,i+4)$
CSI
2nd Structure
β4
β5
β6

Methods for resolving overlapping NMR resonances

- **2D/3D homonuclear NMR experiments such as** 2D-DQFCOSY, 2D-TOCSY, 2D-NOESY, 3D-NOESY-TOCSY**.**

- **2D/3D heteronuclear NMR experiments such as** 2D-^{15}N-HSQC, 3D-^{15}N-NOESY-HSQC **and** triple-resonance experiments (^{1}H, ^{13}C, ^{15}N)**.**

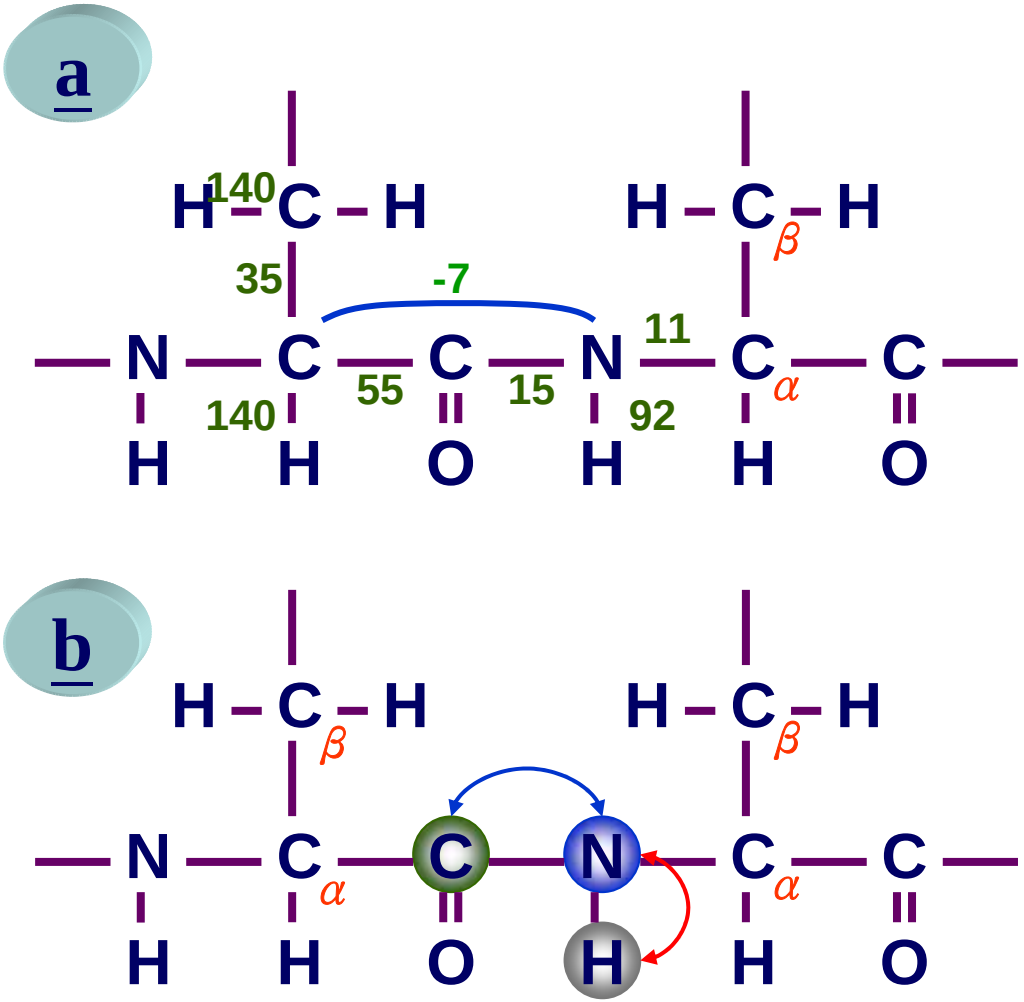

^{13}C
^{15}N
O
H
用於多維異核核磁共振的
單鍵異核偶合常數之概要圖
β
β
β
30 to 40 Hz
α
11 Hz
55 Hz
15 Hz
140 Hz
α
90 to 100 Hz

同位素的標定

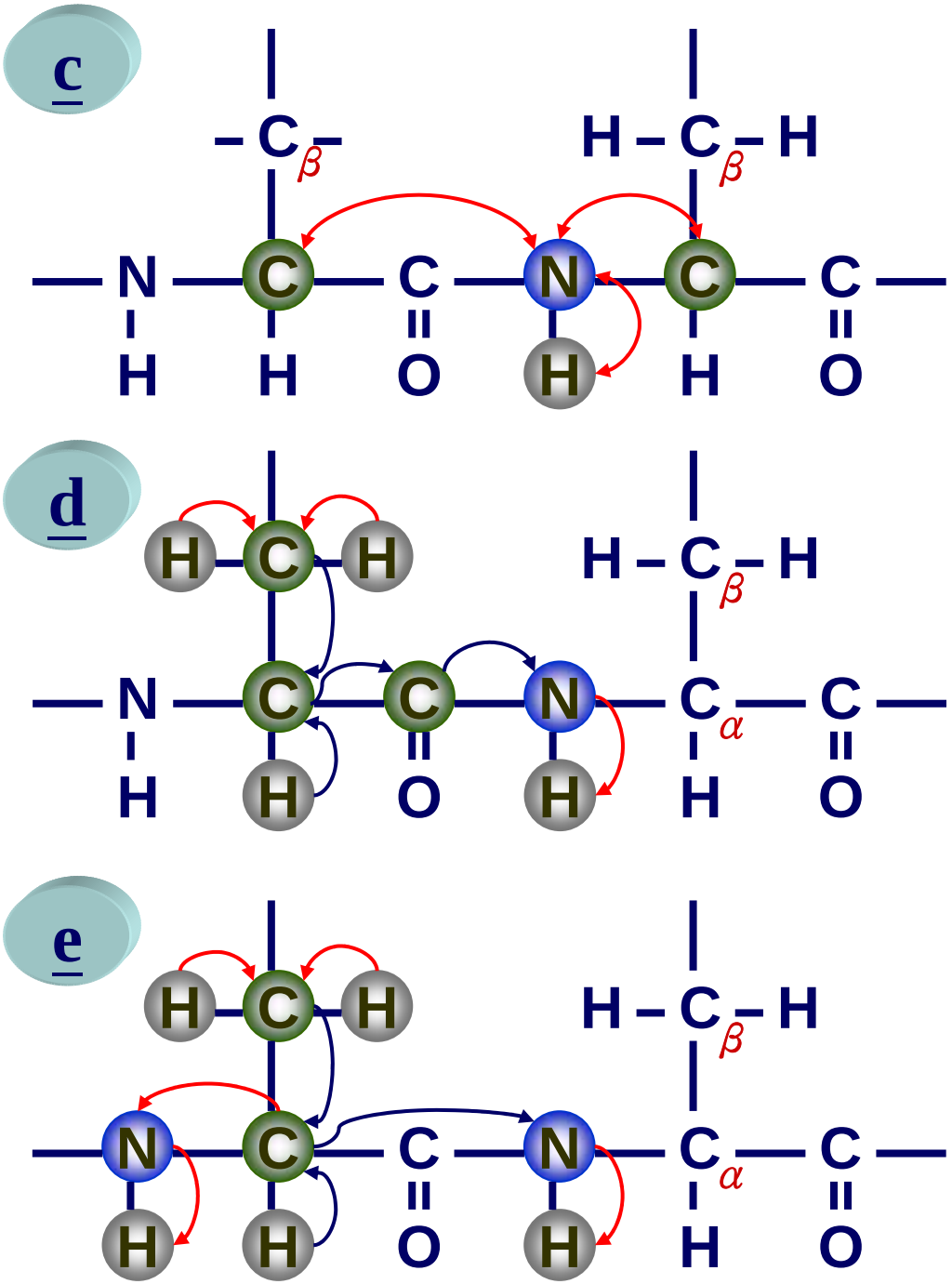
- 因為自然存在的同位素核種：^{15}N 及 ^{13}C 的比例太低, 不足以讓我們進行NMR實驗, 因此人工的同位素標定是必須的.

- 對一般使用大腸桿菌 (E. coli) 表現蛋白質的系統而言, ^{15}N及 ^{13}C 的標定只需要將培養基中的氮和碳的來源, 置換成^{15}N的NH_4Cl (1g/1L) 及 ^{13}C 的 Glucose (2g/1L) 便可達成, 不過^{15}N的NH_4Cl 及 ^{13}C 的 Glucose 造價昂貴, 表現一批 ^{15}N及 ^{13}C 標定的蛋白質所費不貲!! (1 g of $^{15}NH_4Cl$ NT 1200.- ; 1 g of ^{13}C Glucose NT 5400.-)

Act. Chem. **26** 134 (1993) Bax and grzesiek

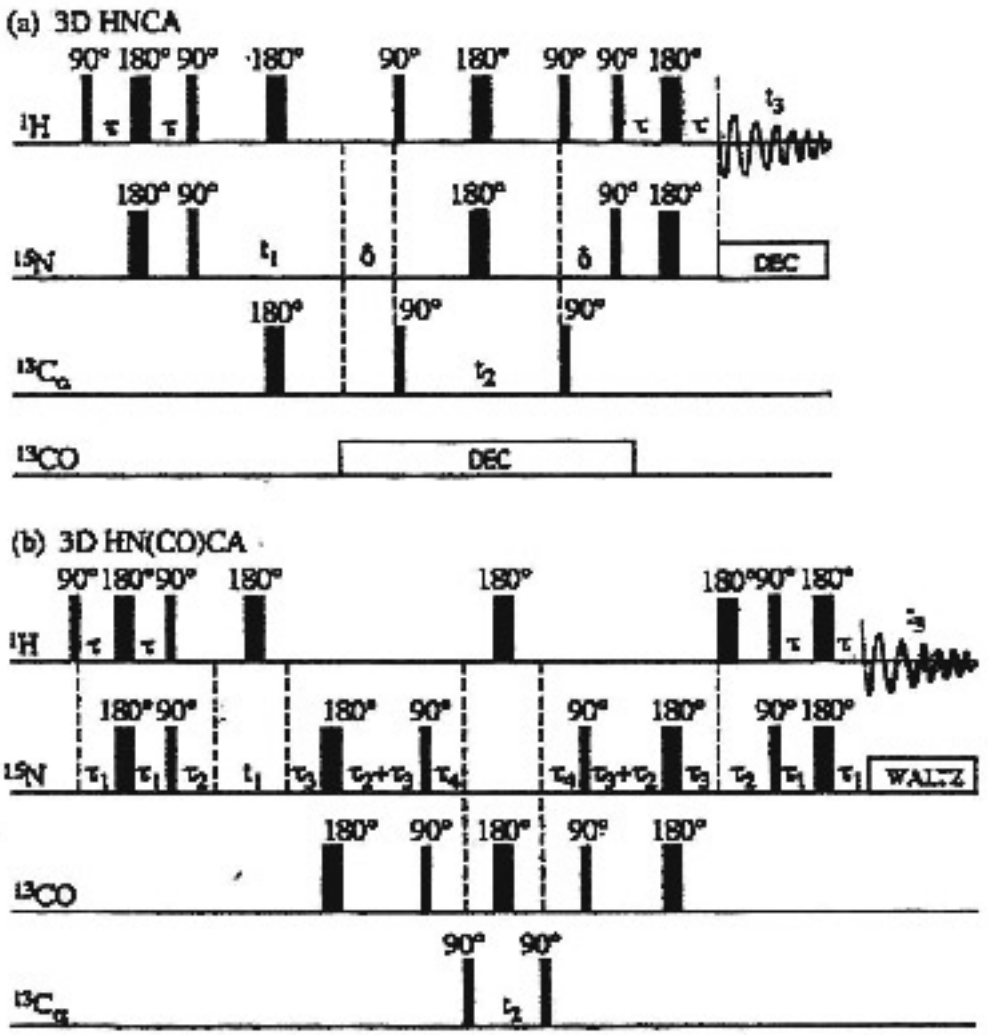


a) A dipeptide segment of a protein backbone with the approximate values for the J couplings which are essential for the assignment procedure in isotopically enriched proteins. (b-e) Schematic diagrams of the nuclei that are correlated in the b) HNCO, …….



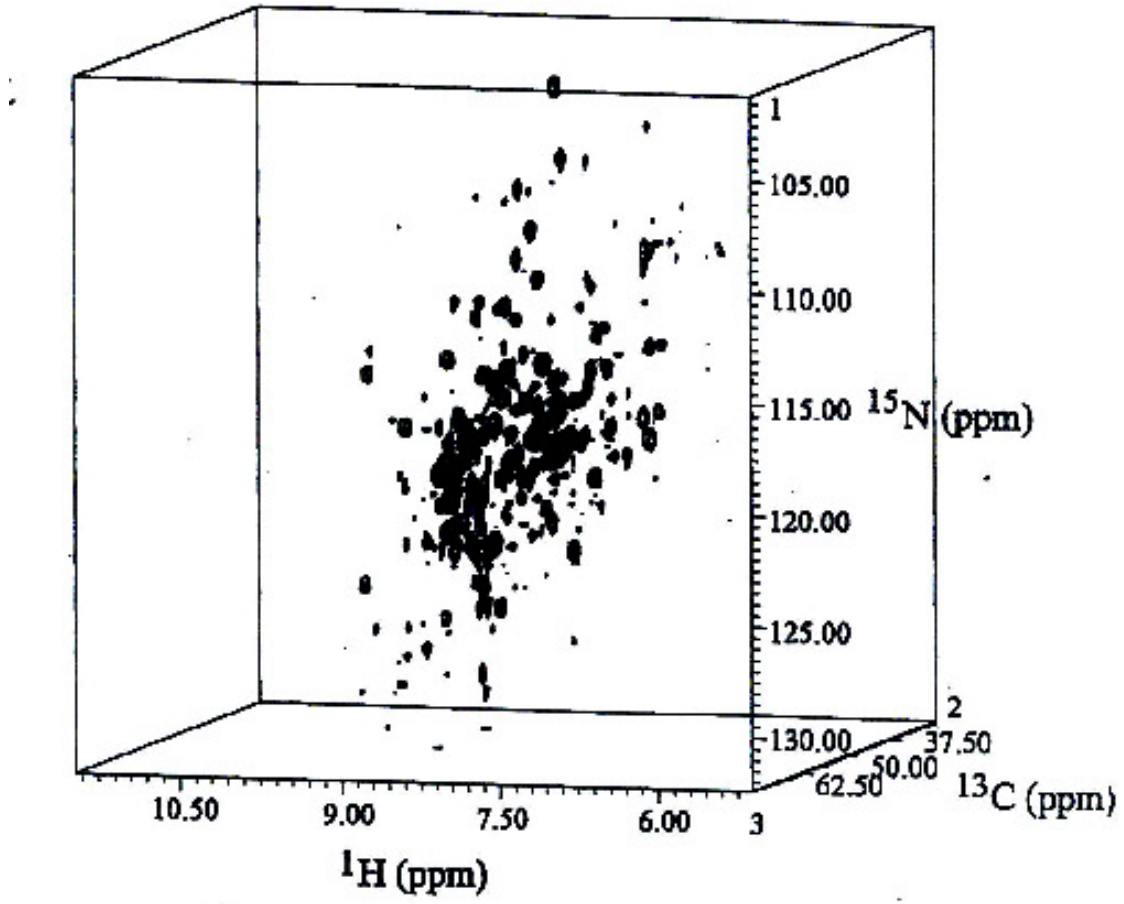
c) HNCA, d) HBHA(CBCACO) NH and e) CBCANH experiments. Nuclei for which the chemical shift is measured in the 3D experiment are marked by solid circles. Nuclei involved in the magnetization transfer pathway, but not observed, are marked by open circles. Magnetization transfer in these experiments is marked by curved solid lines, and the direction of the transfer is marked by arrows.

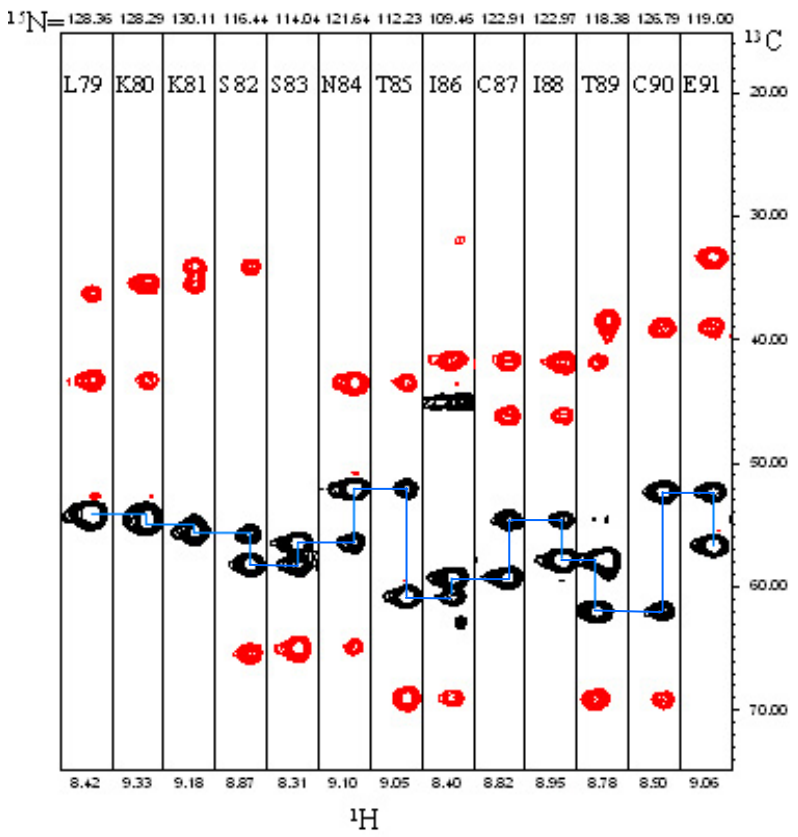
二項常用的三核共振實驗之脈衝圖譜

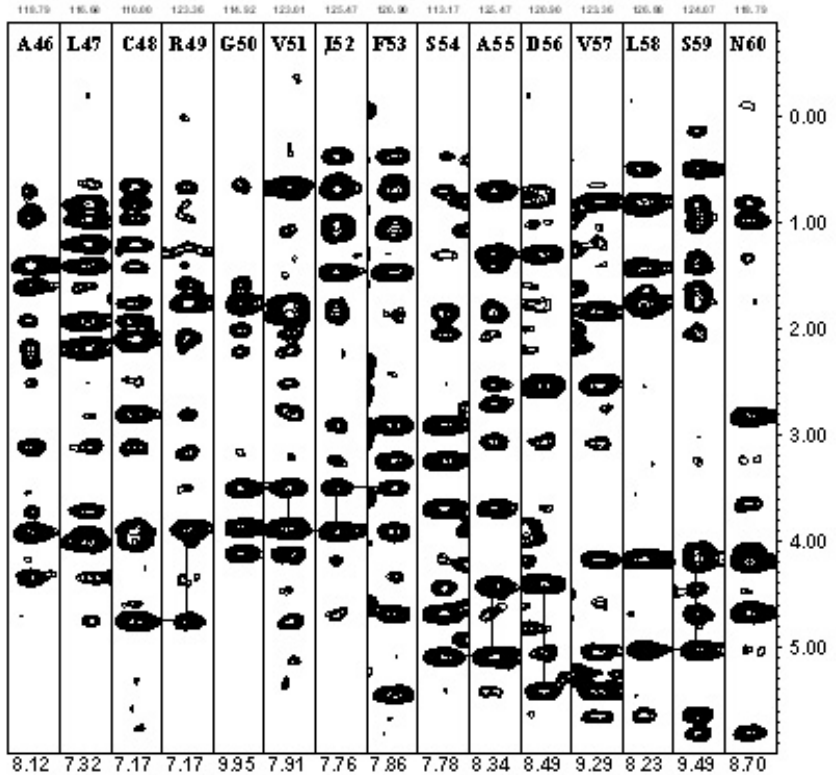


E. Coli Thioesterase I:

3D CBCA(CO)NH




15N
128.36 128.29 130.11 116.44 114.04 121.64 112.23 109.46 122.91 122.97 118.38 126.79 119.00
13C
L79 K80 K81 S82 S83 N84 T85 I86 C87 I88 T89 C90 E91
20.00
30.00
40.00
50.00
60.00
70.00
8.42 9.33 9.18 8.87 8.31 9.10 9.05 8.40 8.82 8.95 8.78 8.50 9.06
1H


A46 L47 C48 R49 G50 V51 I52 F53 S54 A55 D56 V57 L58 S59 N60
0.00
1.00
2.00
3.00
4.00
5.00
8.12 7.32 7.17 7.17 9.95 7.91 7.76 7.86 7.78 8.34 8.49 9.29 8.23 9.49 8.70

Three-dimensional structure determination by simulated annealing using X-PLOR (CNS) program

$$E_{total} = E_{bond} + E_{angle} + E_{improper} + E_{rep} + E_{noe} + E_{tor}$$

Keep the correctness of protein geometry

The energy terms of experimental data

結論

- 核磁共振硬體的進展 ：由於材料科學等方面的進步，目前世售的核磁共振儀已可達到 900 MHz 的等級，使得光譜的解析度更加提昇；而新的超低溫探頭（Cryo-probe）的應用，也大大降低了收集光譜的時間. 目前中研院的核磁共振核心小組（NMR core facility）已有壹台附有超低溫探頭的 800 MHz 核磁共振儀.

- 核磁共振軟體的進展 ：近年來由 Dr. K Wuthrich 所發展的 TROSY 實驗，及由 Dr. A Bax 所發展的 Residual Dipolar Coupling 實驗，使得利用核磁共振決定蛋白質結構的速度加快，蛋白質的大小也更加提昇.

- 綜合以上兩方面的進展，利用核磁共振決定蛋白質結構所需的時間將由以往的1~2年降低到半年到一年，甚至更短；而蛋白質的大小也由以往的 20 kDa 提昇到 30~50 kDa.