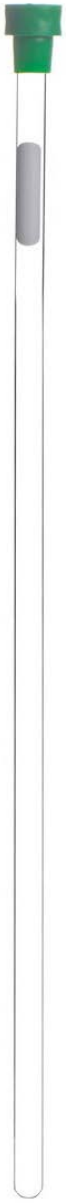

VARIAN

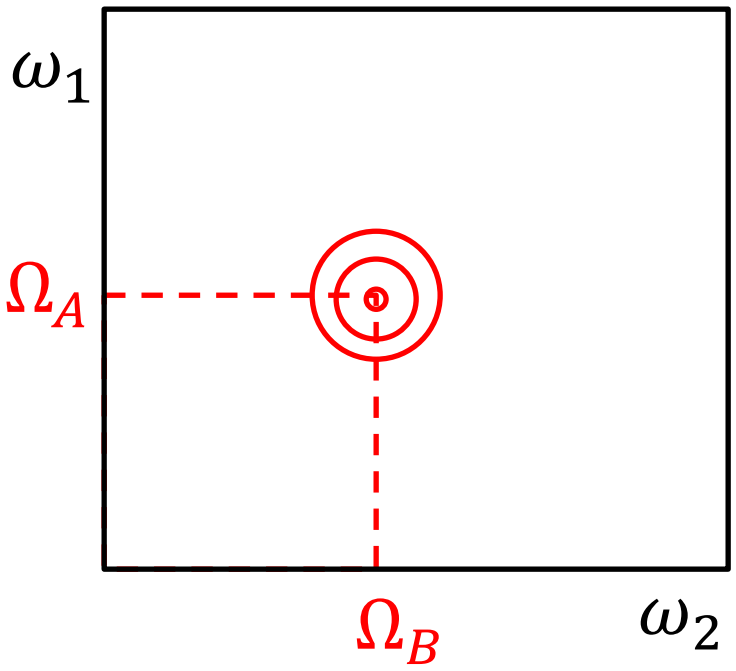


- Typical NMR tube diameter: 5 mm
- Sample volume: > 600 µL
- Smaller volume (200-300 µL) in Shigemi tubes
- Concentration for structure determination: several hundred µM
- Lower concentration for ligand binding
- Signal to noise ratio (S/N) $\alpha \sqrt{n}$
- Cryoprobe: electronics cooled to ~ 20K, higher S/N (up to 4 fold), lower concentrations possible
- Higher S/N (& better resolution) at higher magnetic field
- Any buffer but preferably salt < 200 mM, pH < 7.5
- For homonuclear experiments, phosphate buffer or deuterated buffer
- 5-10% deuterated solvent (^2H_2O) for lock

2-dimensional (2D) NMR spectroscopy

Correlation between nuclear dipoles

- Through-bond interactions (J-coupling) < 4 bonds apart
- Through-space interactions (nuclear Overhauser effect, NOE) < 5 Å apart



Common 2D homonuclear (^{1}H) NMR experiments

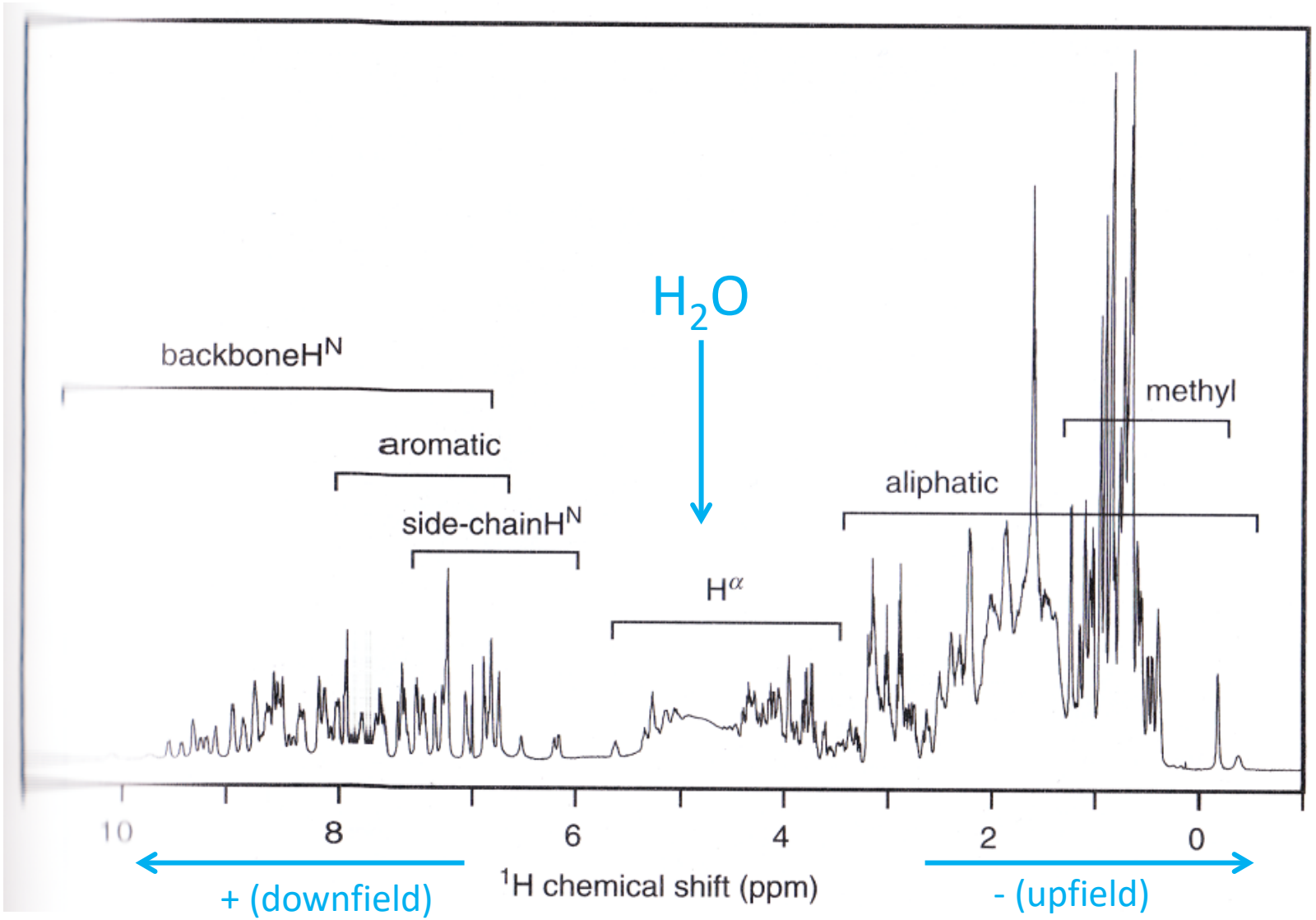
- Useful for proteins up to ~ 100 residues
- Also useful for small(er) molecules

COSY (COrrelation SpectroscopY): crosspeaks between J-coupled spins (2 or 3 bonds apart)

TOCSY (Total Correlation SpectroscopY): crosspeaks within J-coupled networks (spin systems)

NOESY (NOE SpectroscopY): crosspeaks between spins close in space (< 5Å apart)

1D NMR spectrum



Cavanagh, J., Fairbrother, W.J., Palmer, A.G.III, Rance, M., Skelton, N.J. Protein NMR Spectroscopy: Principles and Practice, 2nd edition, 2007, Academic Press

TABLE 1
RANDOM COIL ^{1}H CHEMICAL SHIFTS FOR THE 20 COMMON AMINO ACIDS WHEN FOLLOWED BY ALANINE

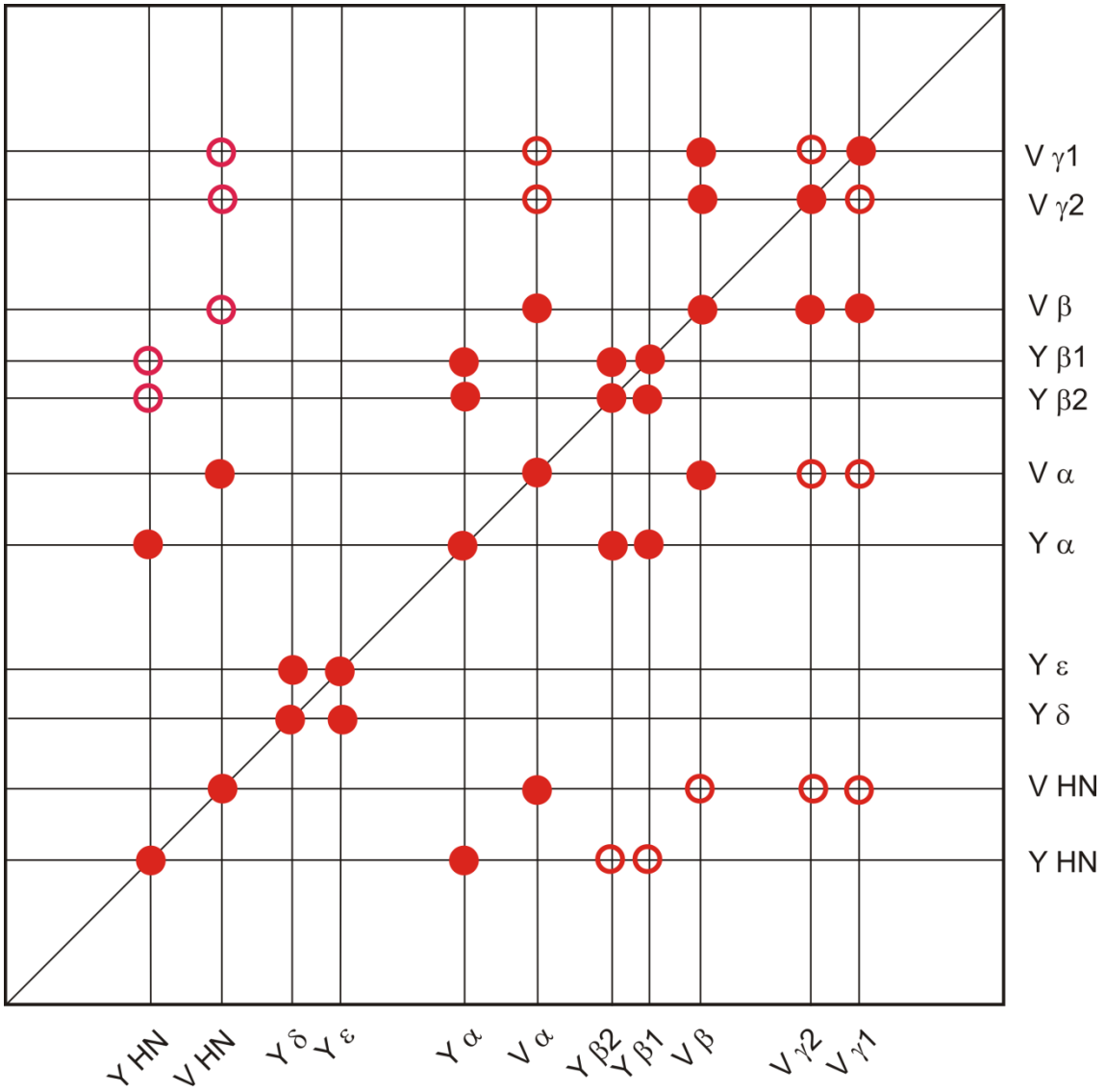
Residue	NH	H^{α}	H^{β}	Others
Ala	8.24	4.32	1.39	
Cys (reduced)	8.32	4.55	2.93, 2.93	
Cys (oxidized)	8.43	4.71	3.25, 2.99	
Asp	8.34	4.64	2.72, 2.65	
Glu	8.42	4.35	2.06, 1.96	γCH_2 2.31, 2.31
Phe	8.30	4.62	3.14, 3.04	2,6H 7.28; 3,5H 7.38; 4H 7.32
Gly	8.33	3.96		
His	8.42	4.73	3.29, 3.16	2H 8.58; 4H 7.29
Ile	8.00	4.17	1.87	γCH_2 1.45, 1.16; γCH_3 0.91; δCH_3 0.86
Lys	8.29	4.32	1.84, 1.75	γCH_2 1.44, 1.44; δCH_2 1.68, 1.68; εCH_2 2.99, 2.99; εNH_3^+ 7.81
Leu	8.16	4.34	1.62, 1.62	γCH 1.59; δCH_3 0.92, 0.87
Met	8.28	4.48	2.11, 2.01	γCH_2 2.60, 2.54; εCH_3 2.10
Asn	8.40	4.74	2.83, 2.75	γNH_2 7.59, 6.91
Pro	–	4.42	2.29, 1.94	γCH_2 2.02, 2.02; δCH_2 3.63, 3.63
Gln	8.32	4.34	2.12, 1.99	γCH_2 2.36, 2.36; δNH_2 7.52, 6.85
Arg	8.23	4.34	1.86, 1.76	γCH_2 1.63, 1.63; δCH_2 3.20, 3.20; εNH 8.07
Ser	8.31	4.47	3.89, 3.87	
Thr	8.15	4.35	4.24	γCH_3 1.21
Val	8.03	4.12	2.08	γCH_3 0.94, 0.93
Trp[a]	8.25	4.66	3.29, 3.27	2H 7.27; 4H 7.65; 5H 7.18; 6H 7.25; 7H 7.50
Tyr	8.12	4.55	3.03, 2.98	2,6H 7.14; 3,5H 6.84

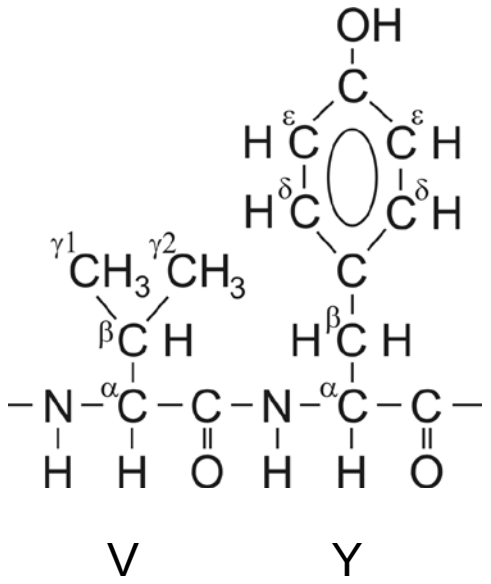
Chemical shifts are referenced to internal DSS at 25 °C, pH ~5.0.
[a] Measured using a peptide with free N- and C-termini.

Wishart DS, Bigam CG, Holm A, Hodges RS, Sykes BD. 1H, 13C and 15N random coil NMR chemical shifts of the common amino acids. I. Investigations of nearest-neighbor effects. J Biomol NMR. 1995 Jan;5(1):67-81.

COSY (COrrelation SpectroscopY): crosspeaks between J-coupled spins ●

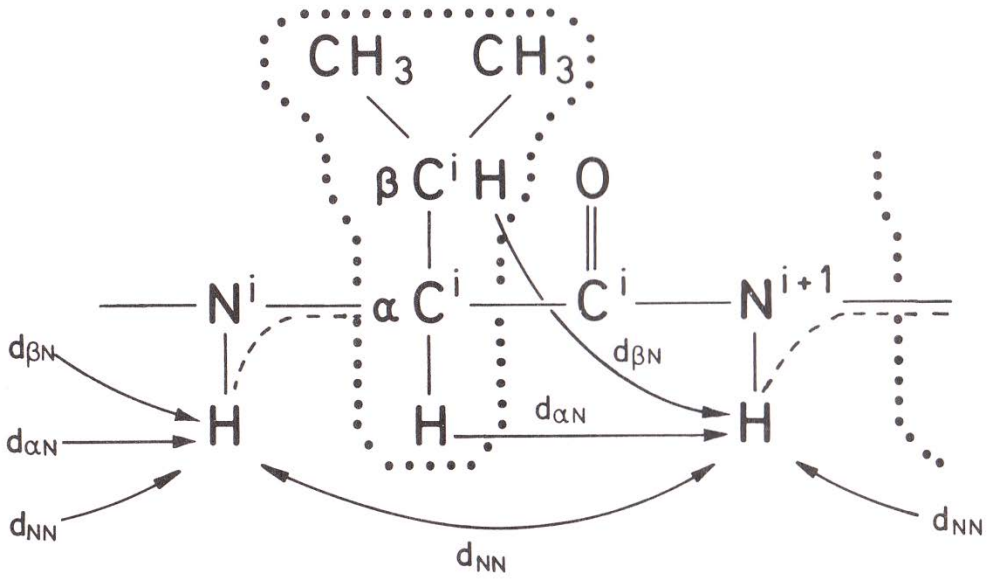
TOCSY (Total Correlation SpectroscopY): crosspeaks within J-coupled networks ● + ○





NOESY (NOE SpectroscopY): crosspeaks between spins close in space (< 5Å apart)

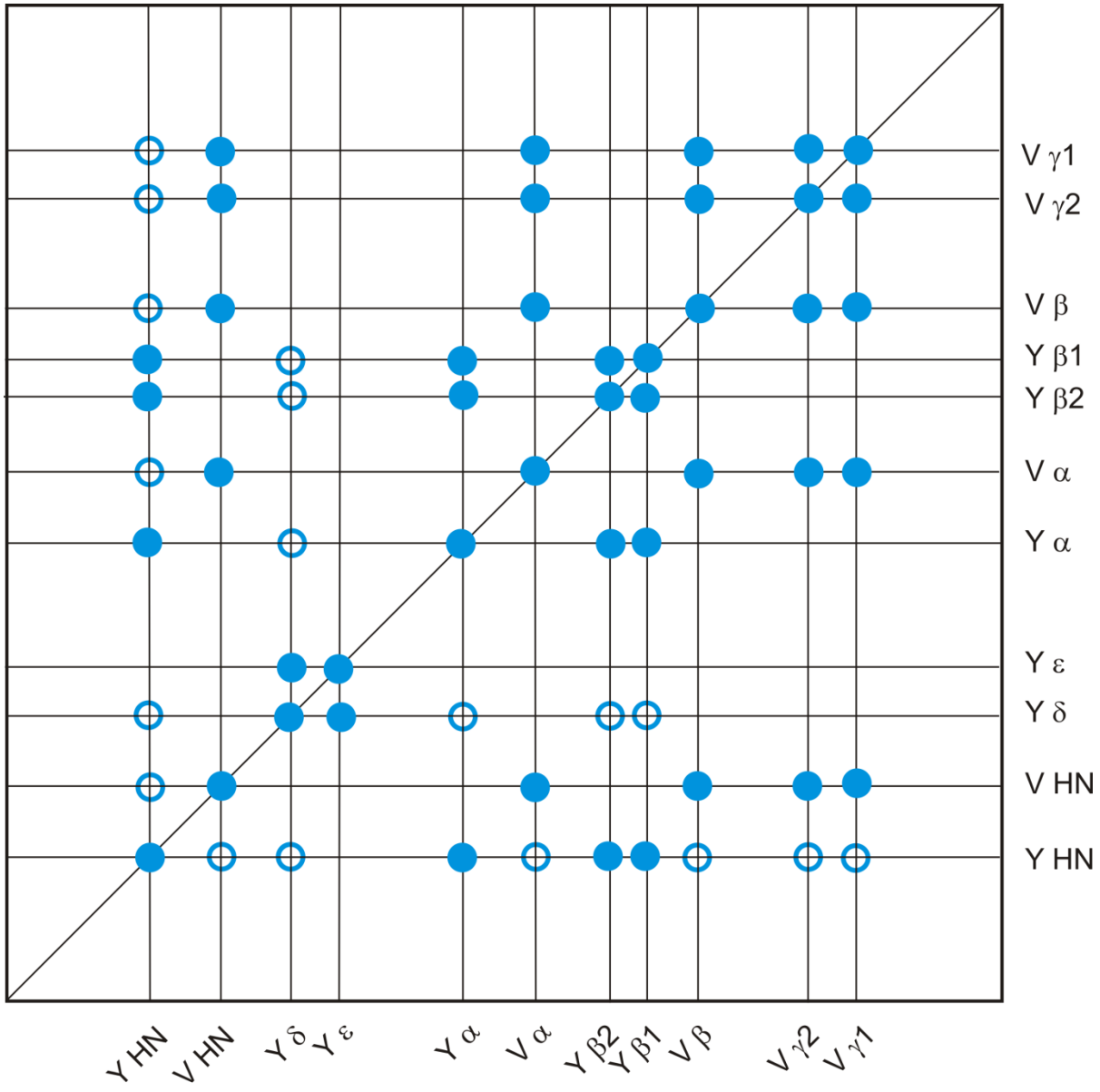
- sequential assignment: connect sequential spin systems (find neighbors)
- structural information

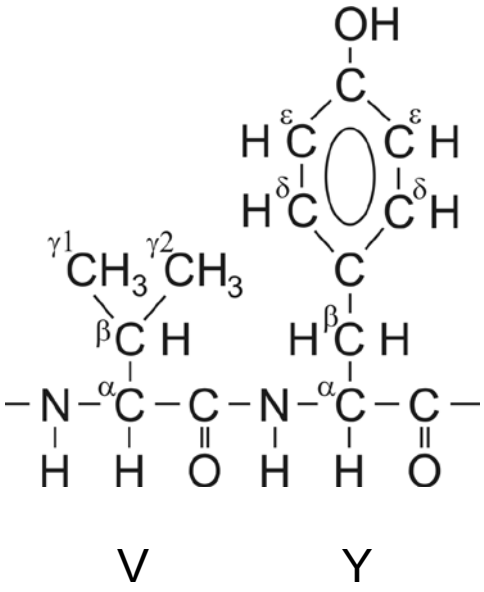


Wüthrich, K. NMR of Proteins and Nucleic Acids, 1986, John Wiley & Sons

NOESY ● + ○

COSY+TOCSY ●



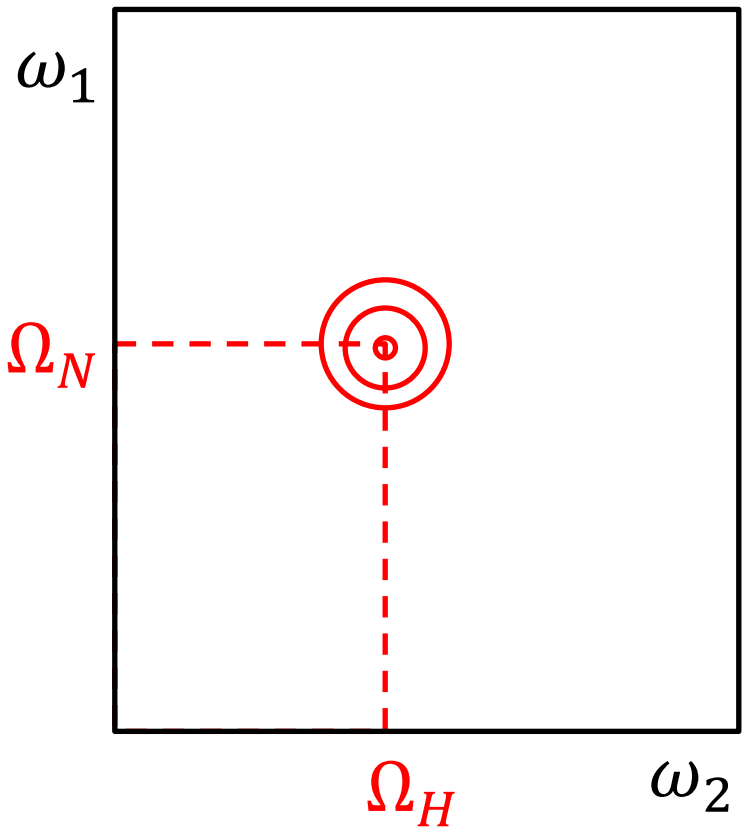


Common 2D heteronuclear NMR experiments

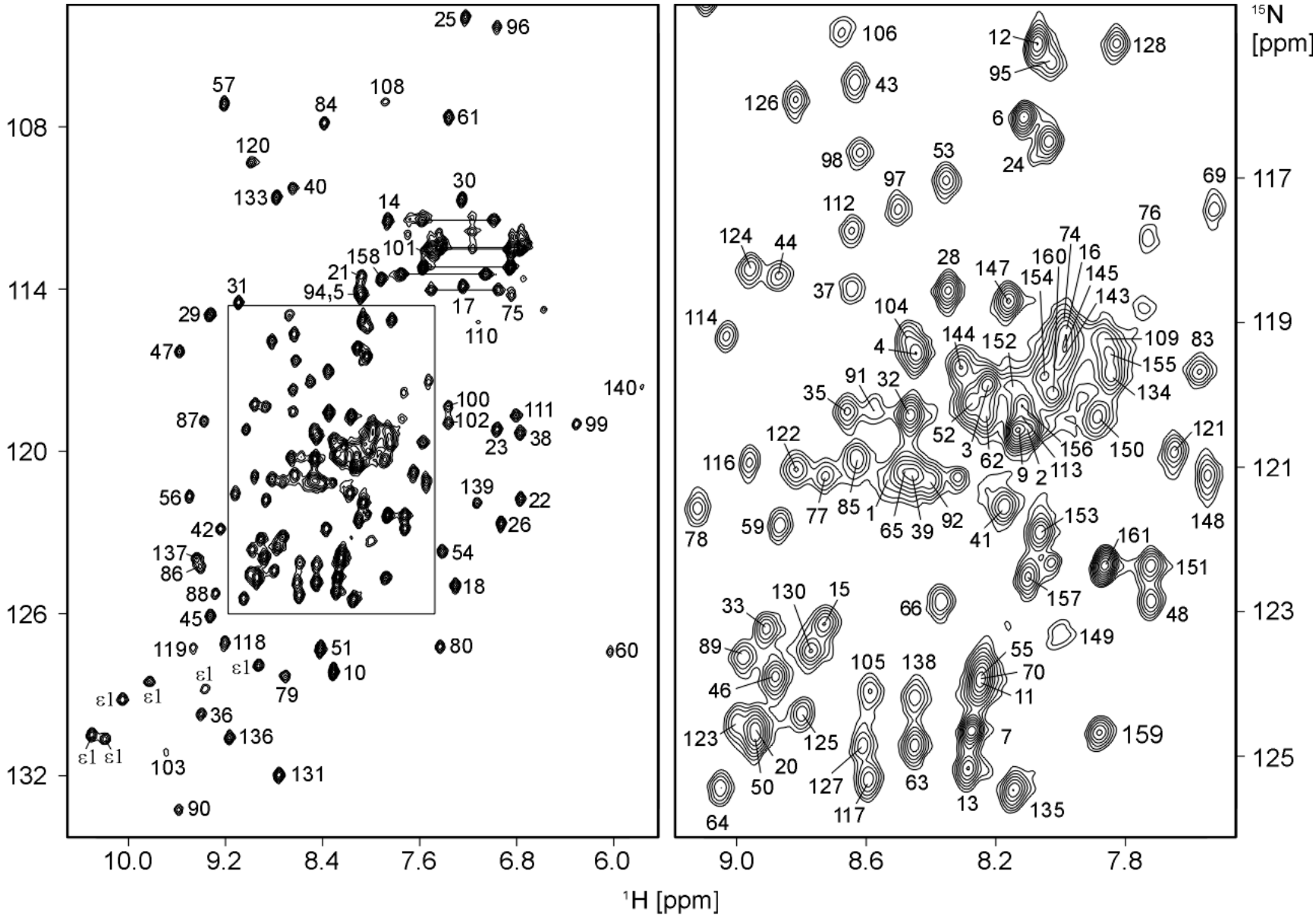
- Useful for both small and large proteins
- Typically performed on proteins labeled with ^{15}N and/or ^{13}C
- To incorporate ^{15}N or ^{13}C into proteins, the proteins are expressed in *E. coli* or yeast in a growth medium that only contains ^{15}N or ^{13}C

^{1}H-^{15}N HSQC (Heteronuclear Single Quantum Correlation / Coherence): crosspeaks between ^{1}H and ^{15}N atoms that are directly bonded

^{1}H-^{13}C HSQC: crosspeaks between ^{1}H and ^{13}C atoms that are directly bonded

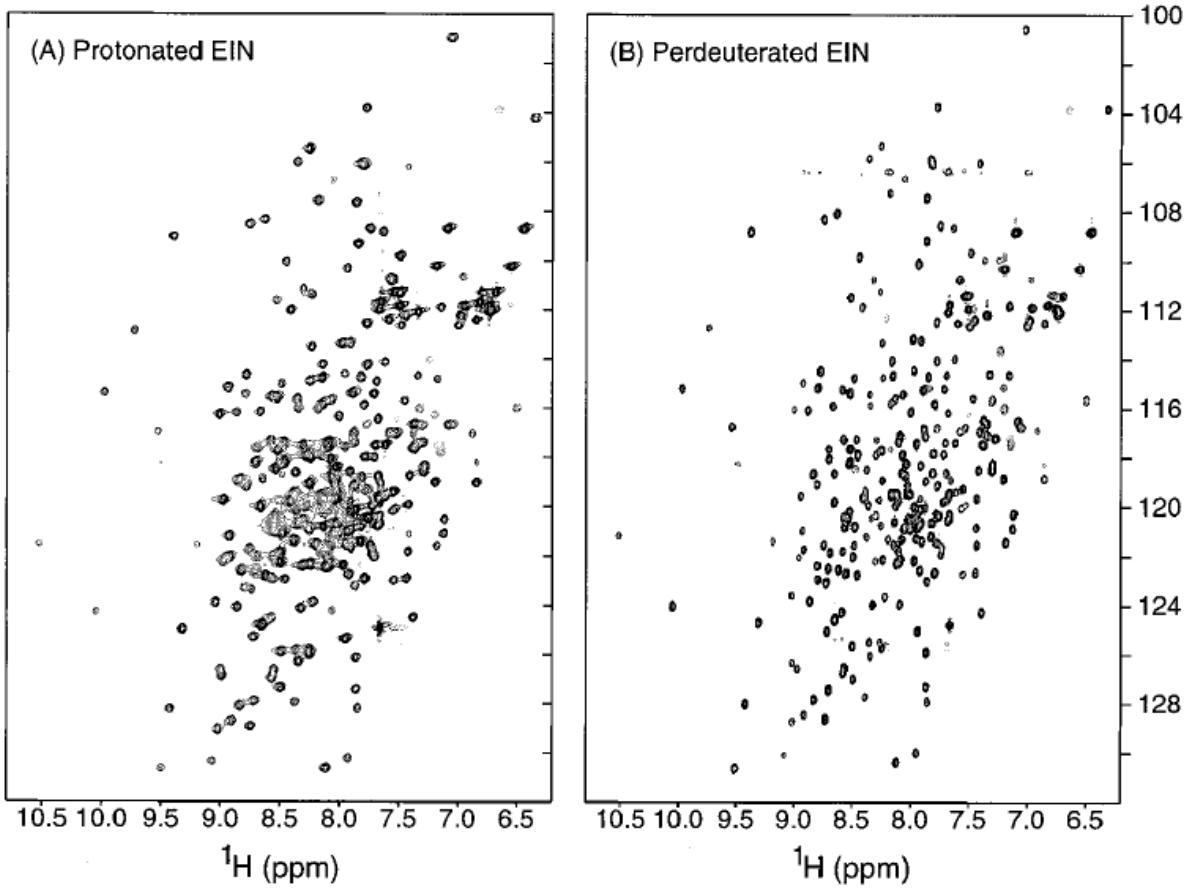


^{1}H-^{15}N HSQC spectrum of a protein with ~ 160 residues



Large proteins (or other molecules or molecular assemblies)

- ^{15}N and ^{13}C labeling combined with partial or complete ^{2}H labeling (deuteration) to reduce relaxation

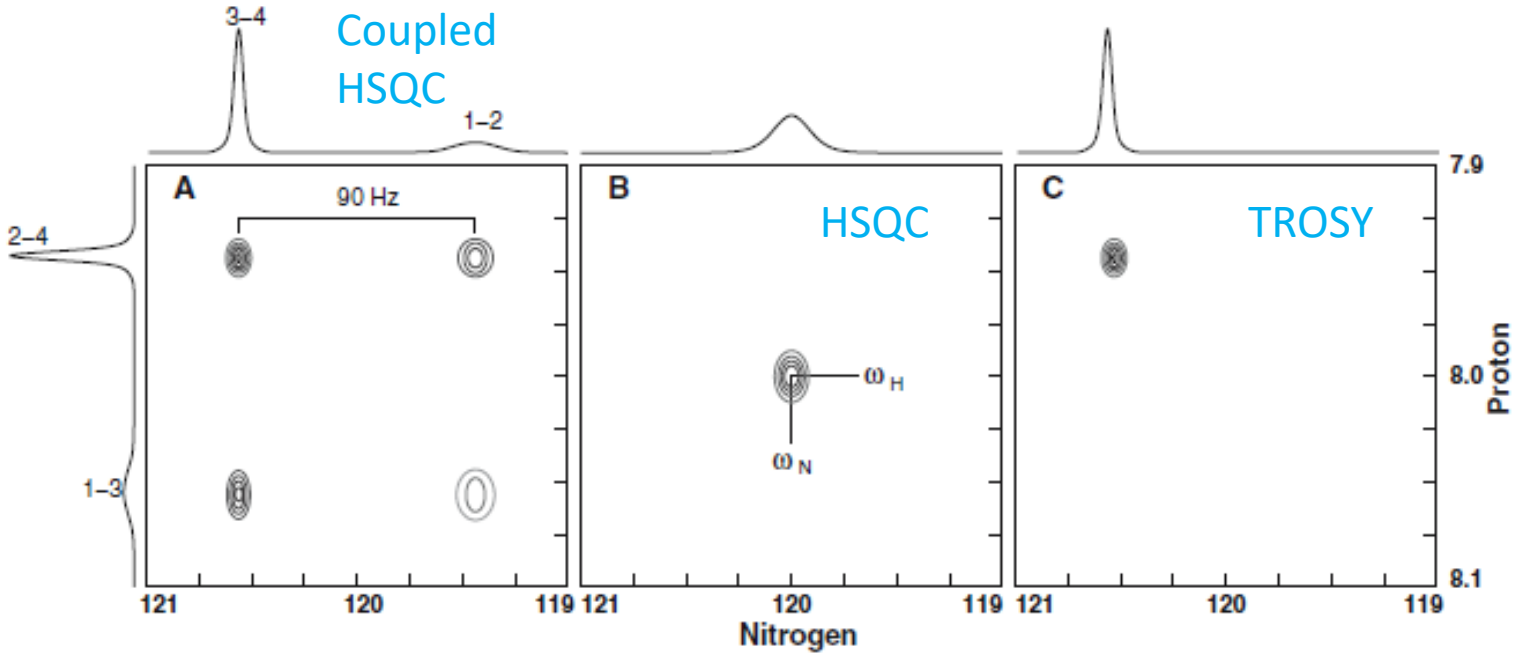


Garrett DS, Seok YJ, Liao DI, Peterkofsky A, Gronenborn AM, Clore GM. Solution structure of the 30 kDa N-terminal domain of enzyme I of the Escherichia coli phosphoenolpyruvate:sugar phosphotransferase system by multidimensional NMR. Biochemistry. 1997 Mar 4;36(9):2517-30.

Large proteins (or other molecules or molecular assemblies)

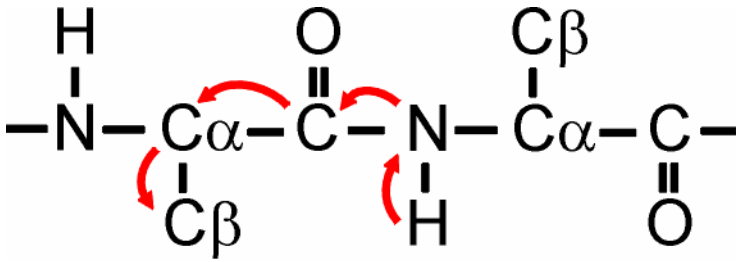
TROSY (Transverse Relaxation-Optimized SpectroscopY):

- similar to HSQC
- sharper peaks for large molecules
- at high magnetic fields

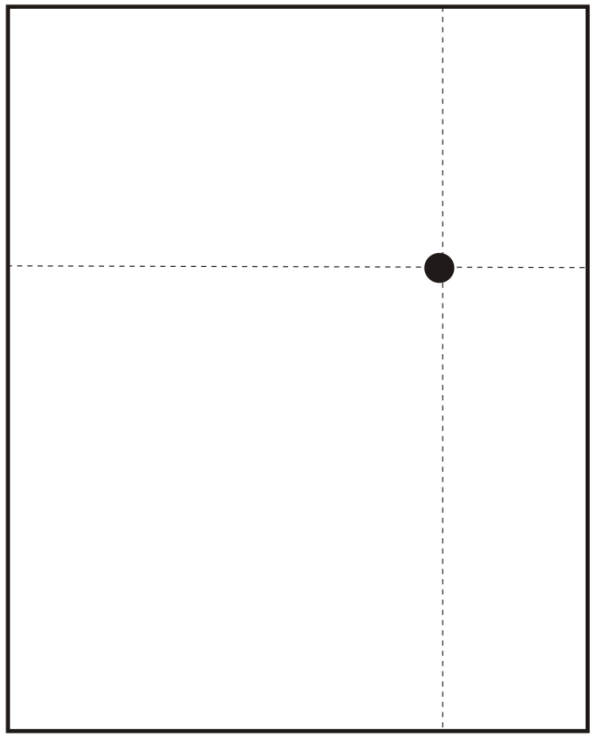


Rule, G.S. and Hitchens, T.K. Fundamentals of Protein NMR Spectroscopy, 2006, Springer

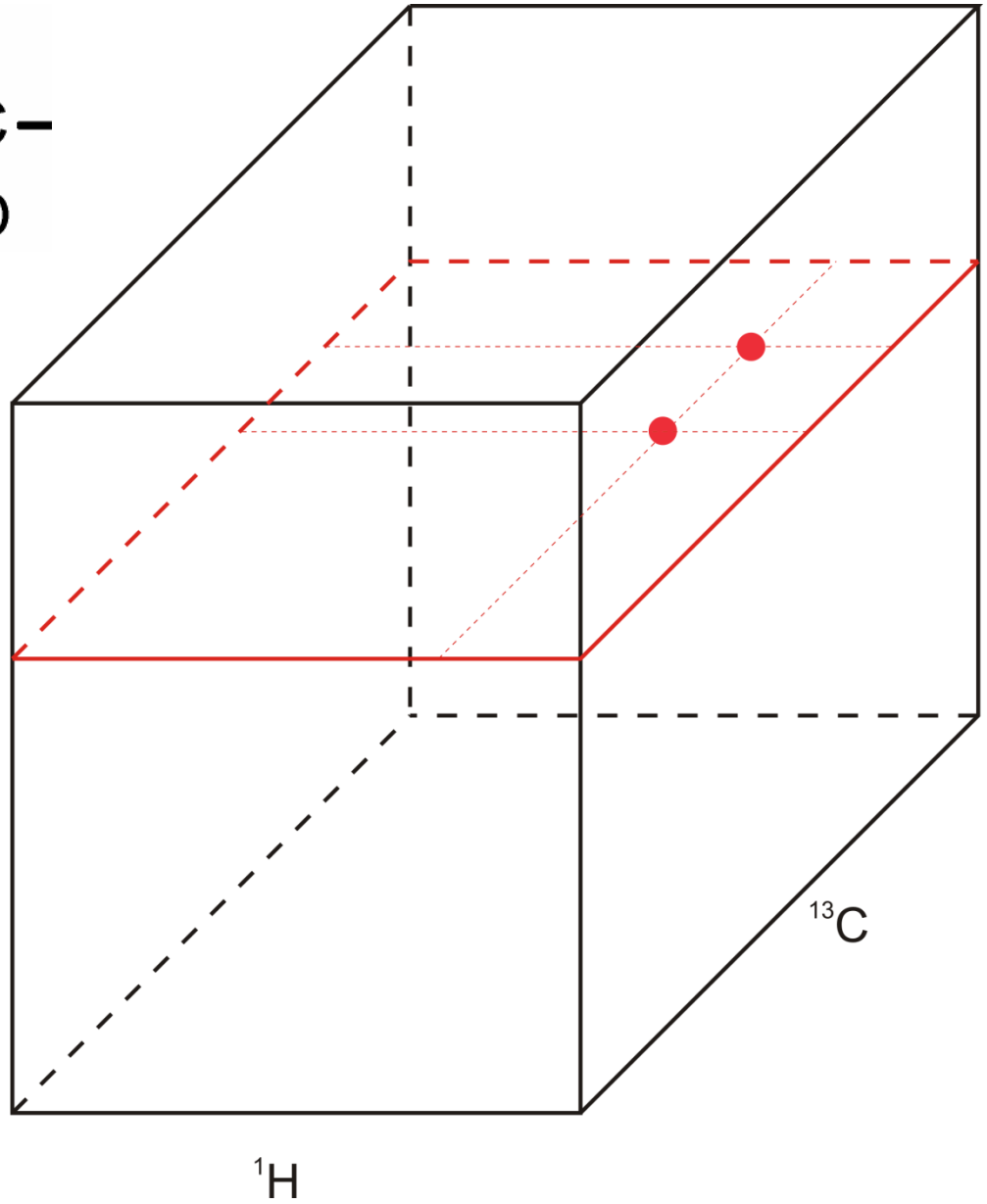
3D NMR spectroscopy



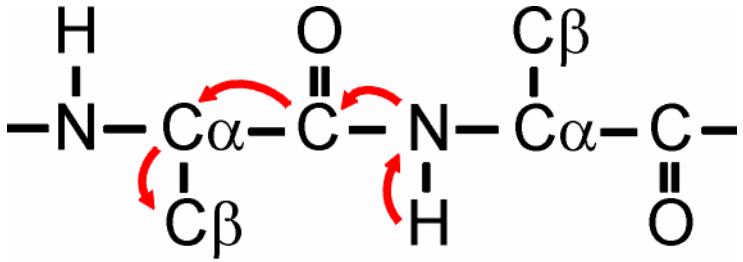
2D ^{1}H-^{15}N HSQC

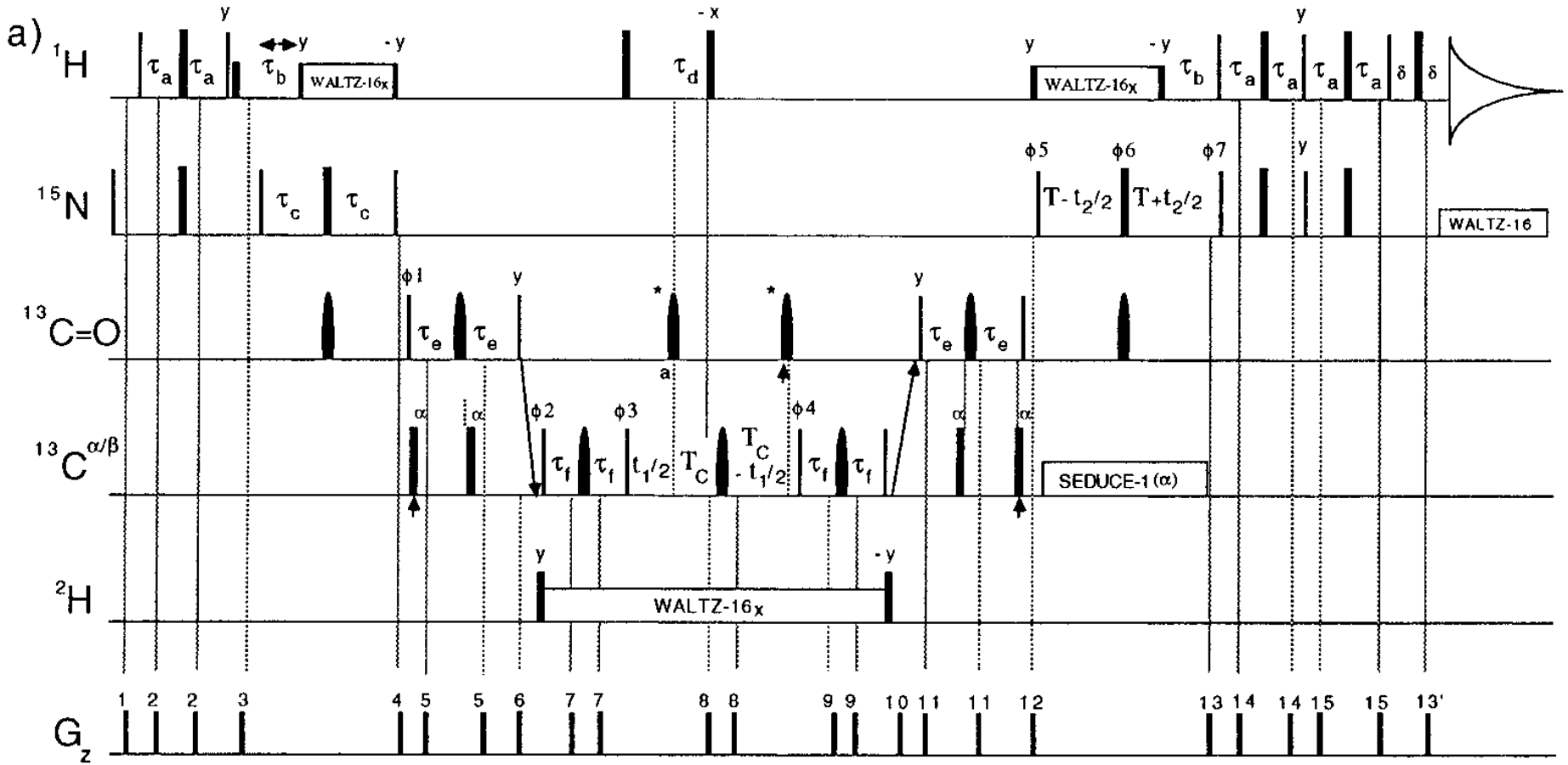


CBCACONH

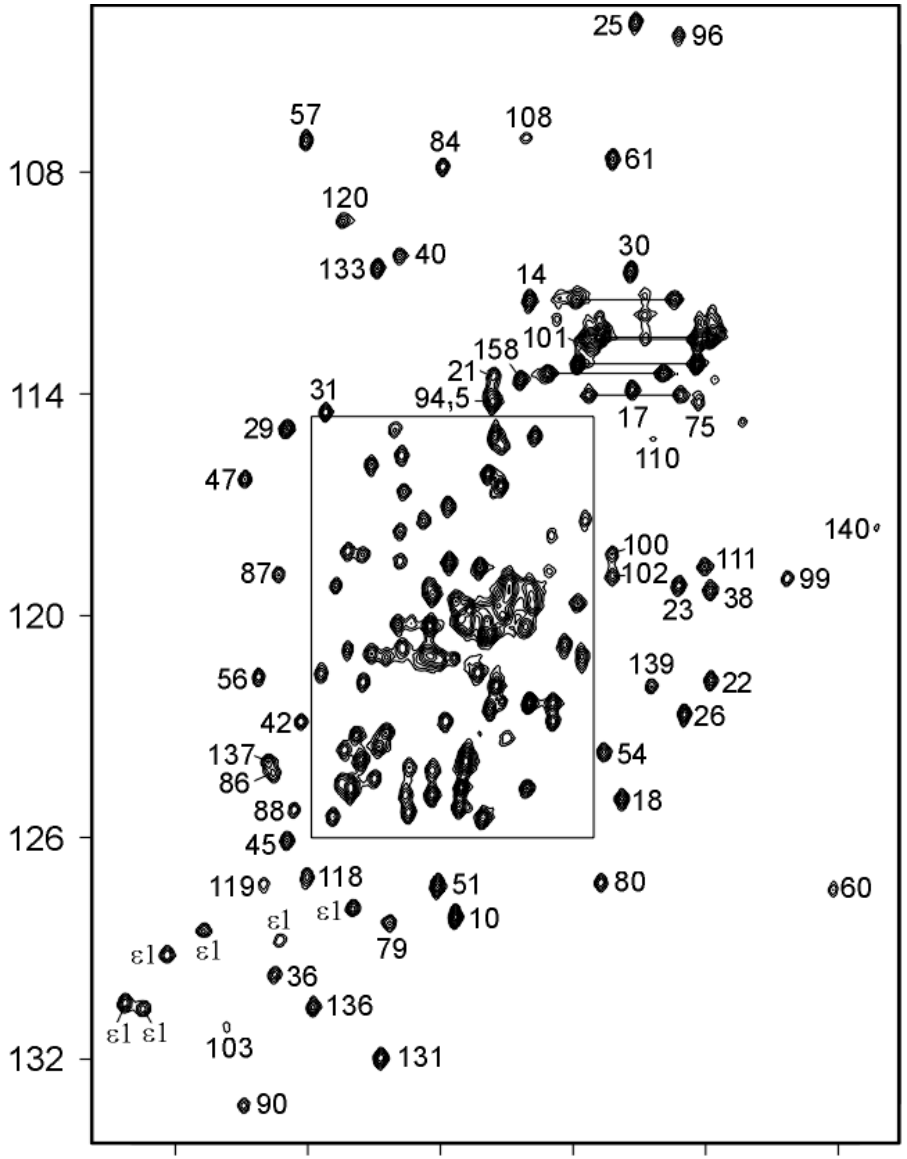


HNCOCACB

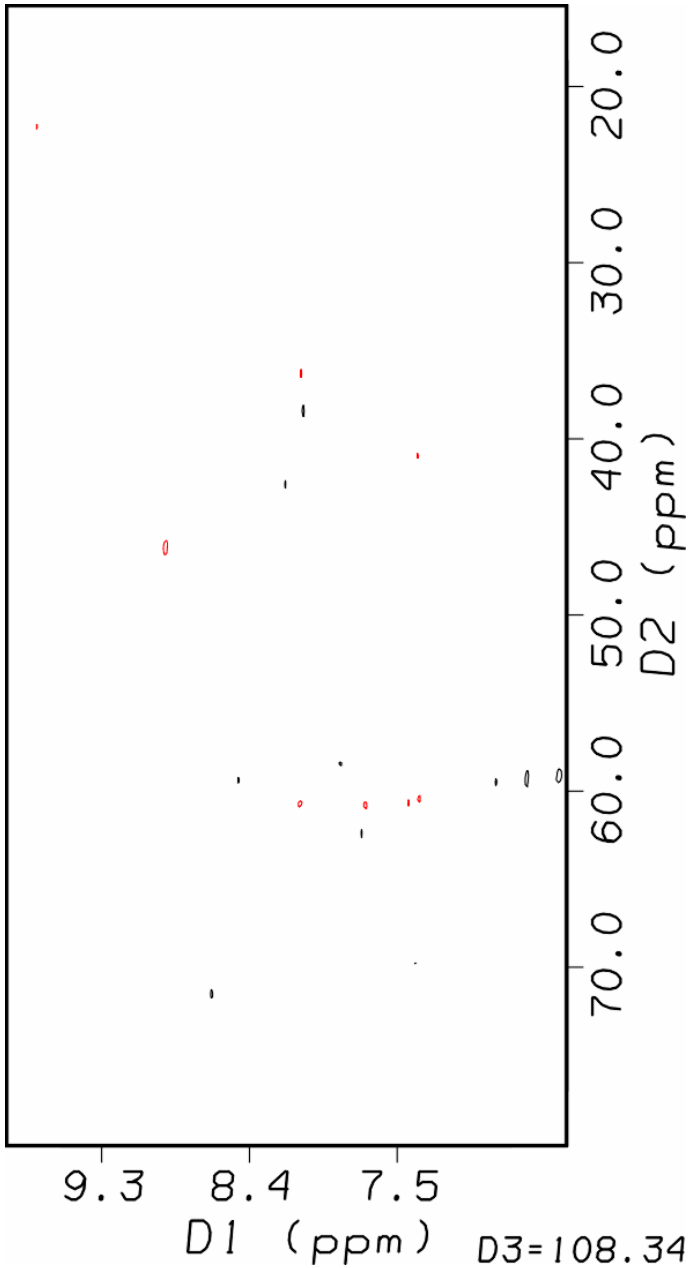

H
O
Cβ
N
Cα
C
N
Cα
C
Cβ
H
O


a)
^{1}H
^{15}N
^{13}C=O
^{13}C$^{\alpha/\beta}$
^{2}H
G$_z$
WALTZ-16x
WALTZ-16
SEDUCE-1 (α)
τa τb τc τd τe τf
T- t2/2
T+t2/2
t1/2
Tc
Tc - t1/2
φ1 φ2 φ3 φ4 φ5 φ6 φ7
δ

2D ^{1}H-^{15}N HSQC



3D HNCOCACB



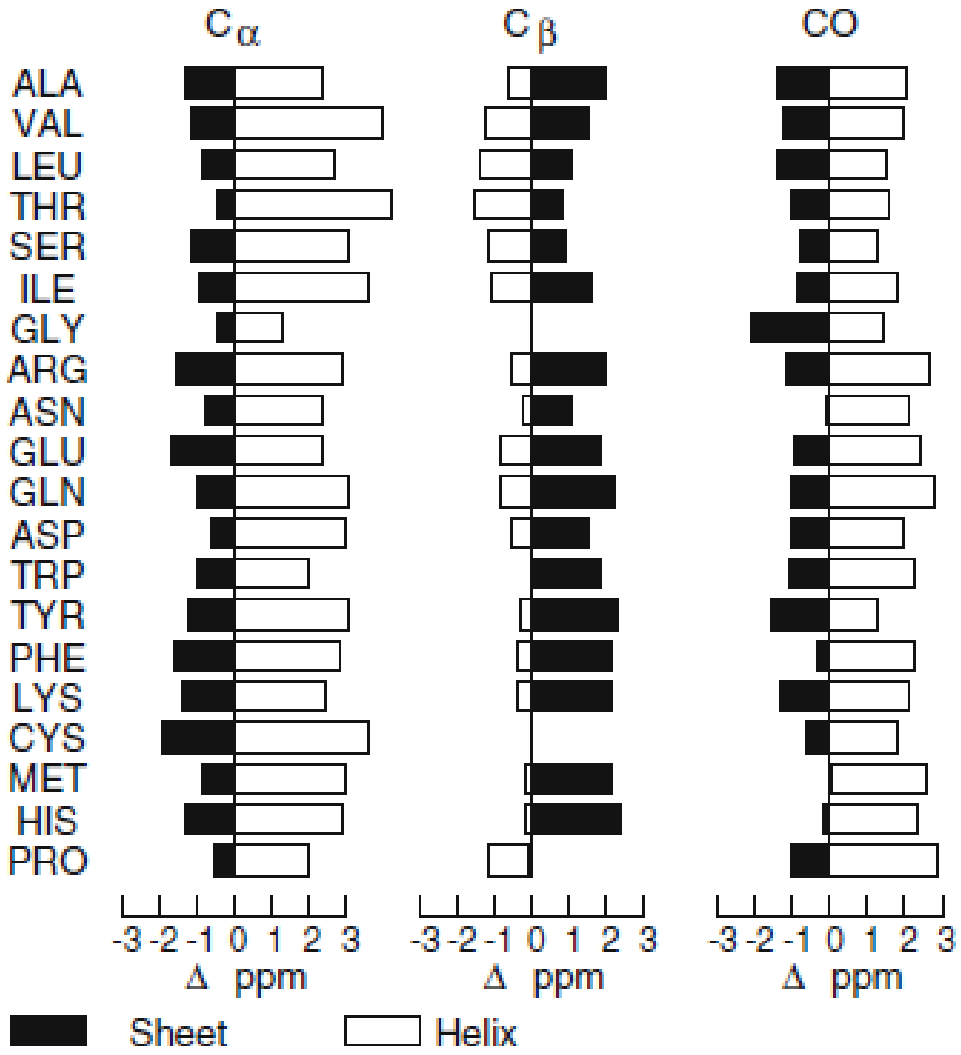
RANDOM COIL ^{13}C CHEMICAL SHIFTS FOR THE 20 COMMON AMINO ACIDS WHEN FOLLOWED BY ALANINE

Residue	C=O	C^{α}	C^{β}	Others
Ala	177.8	52.5	19.1	
Cys (reduced)	174.6	58.2	28.0	
Cys (oxidized)	174.6	55.4	41.1	
Asp	176.3	54.2	41.1	γCO 180.0
Glu	176.6	56.6	29.9	γCH_2 35.6; δCO 183.4
Phe	175.8	57.7	39.6	1C 138.9; 2,6CH 131.9; 3,5CH 131.5; 4CH 129.9
Gly	174.9	45.1		
His	174.1	55.0	29.0	2CH 136.2; 4CH 120.1; 5C 131.1
Ile	176.4	61.1	38.8	γCH_2 27.2; γCH_3 17.4; δCH_3 12.9
Lys	176.6	56.2	33.1	γCH_2 24.7; δCH_2 29.0; εCH_2 41.9
Leu	177.6	55.1	42.4	γCH 26.9; δCH_3 24.9, 23.3
Met	176.3	55.4	32.9	γCH_2 32.0; εCH_3 16.9
Asn	175.2	53.1	38.9	γCO 177.2
Pro	177.3	63.3	32.1	γCH_2 27.2; δCH_2 49.8
Gln	176.0	55.7	29.4	γCH_2 33.7; δCO 180.5
Arg	176.3	56.0	30.9	γCH_2 27.1; δCH_2 43.3; εC 159.5
Ser	174.6	58.3	63.8	
Thr	174.7	61.8	69.8	γCH_3 21.5
Val	176.3	62.2	32.9	γCH_3 21.1, 20.3
Trp[a]	176.1	57.5	29.6	2CH 127.4; 3C 111.2; 4CH 122.2; 5CH 124.8; 6CH 121.0; 7CH 114.7; 8C 138.7; 9C 129.5
Tyr	175.9	57.9	38.8	1C 130.6; 2,6CH 133.3; 3,5CH 118.2; 4C 157.3

Wishart DS, Bigam CG, Holm A, Hodges RS, Sykes BD. 1H, 13C and 15N random coil NMR chemical shifts of the common amino acids. I. Investigations of nearest-neighbor effects. J Biomol NMR. 1995 Jan;5(1):67-81.

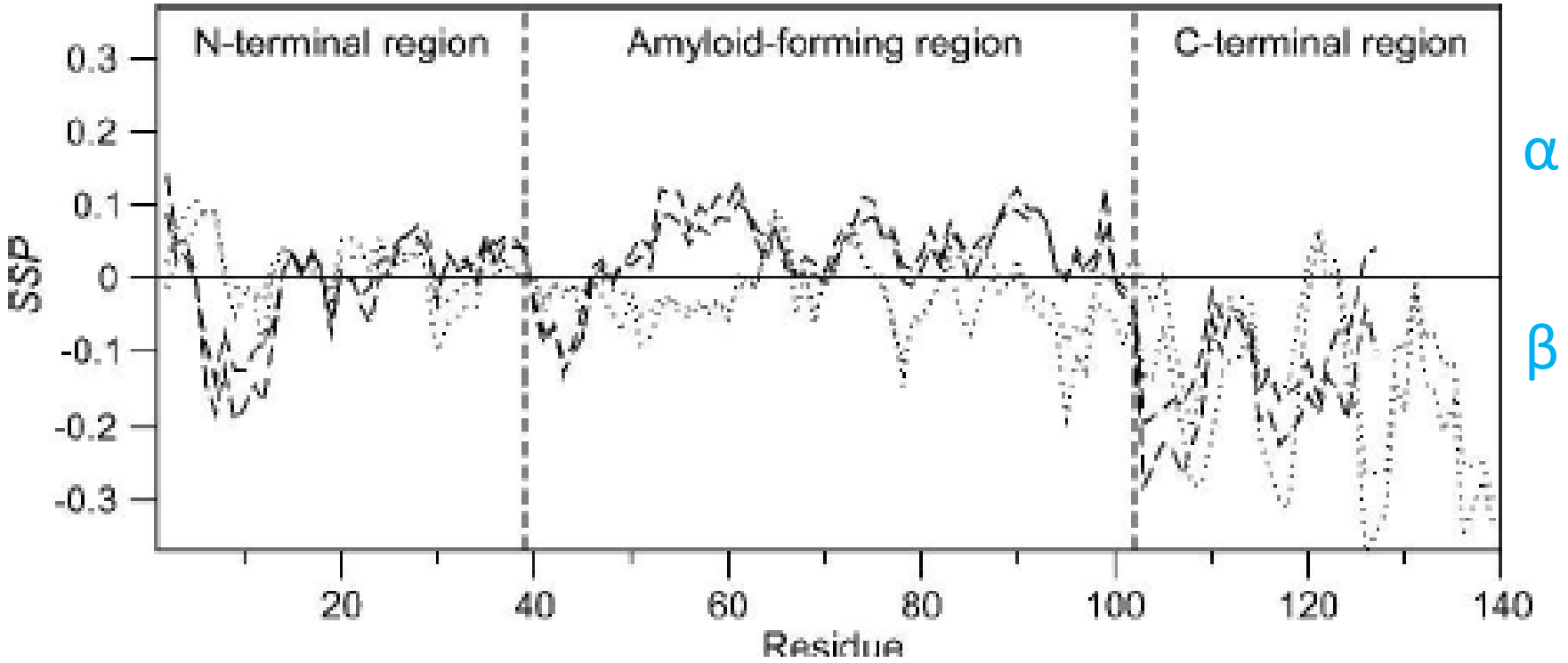
^{13}C chemical shifts are sensitive to secondary structure

→ Prediction of Φ and Ψ dihedral angles



Rule, G.S. and Hitchens, T.K. Fundamentals of Protein NMR Spectroscopy, 2006, Springer

^{13}C chemical shifts can be used to estimate secondary structure propensity in intrinsically disordered proteins

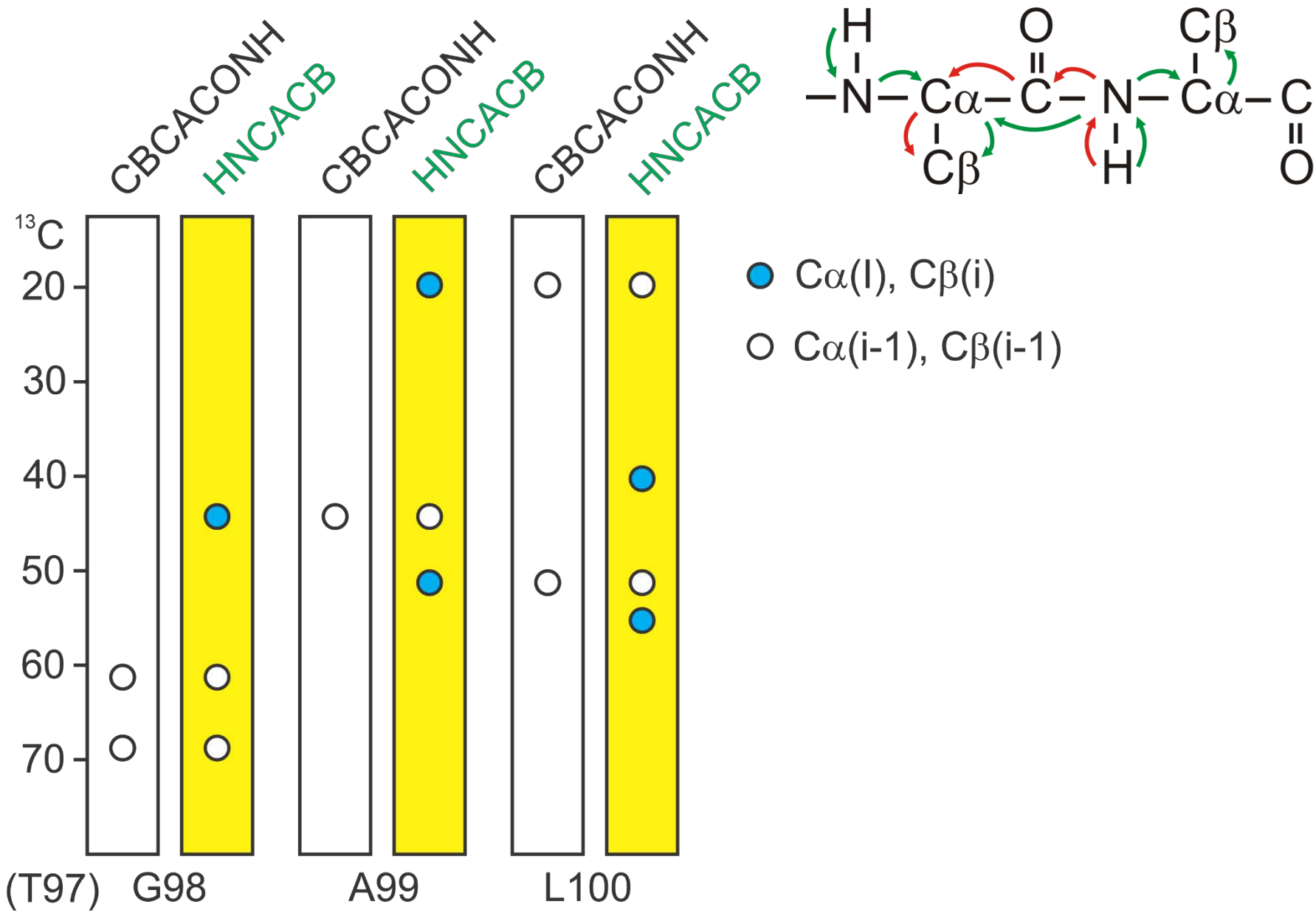


Marsh JA, Singh VK, Jia Z, Forman-Kay JD. Sensitivity of secondary structure propensities to sequence differences between alpha- and gamma-synuclein: implications for fibrillation. *Protein Sci*. 2006;15(12):2795-2804.

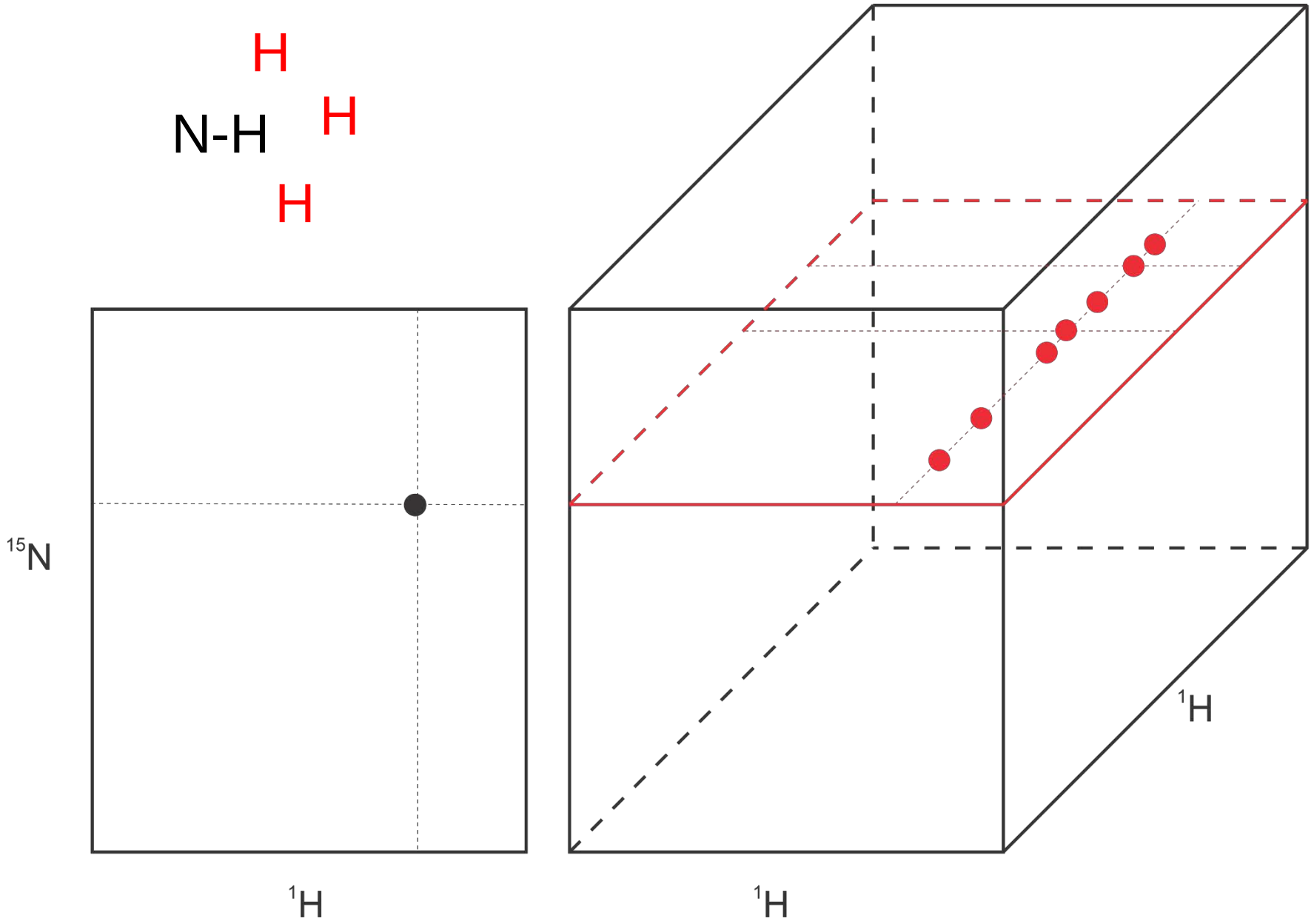
Examples of heteronuclear 3D experiments

HNCO	HN(i), N(i), C'(i-1)
HN(CA)CO	HN(i), N(i), C'(i)+C'(i-1)
HNCA	HN(i), N(i), Cα(i)+Cα(i-1)
HN(CO)CA	HN(i), N(i), Cα(i-1)
HNCACB	HN(i), N(i), Cα(i)+Cβ(i)+Cα(i-1)+Cβ(i-1)
CBCA(CO)NH	HN(i), N(i), Cα(i-1)+Cβ(i-1)
C(CO)NH	HN(i), N(i), all C(i-1)
H(CCO)NH	HN(i), N(i), all HC(i-1)
CCH-TOCSY	HC(i), C(i), all C(i)
HCCH-TOCSY	HC(i), C(i), all HC(i)
15N-edited TOCSY	HN(i), N(i), all H(i)
15N-edited NOESY	HN(i), N(i), H(<5Å)
13C-edited NOESY	HC(i), C(i), H(<5Å)

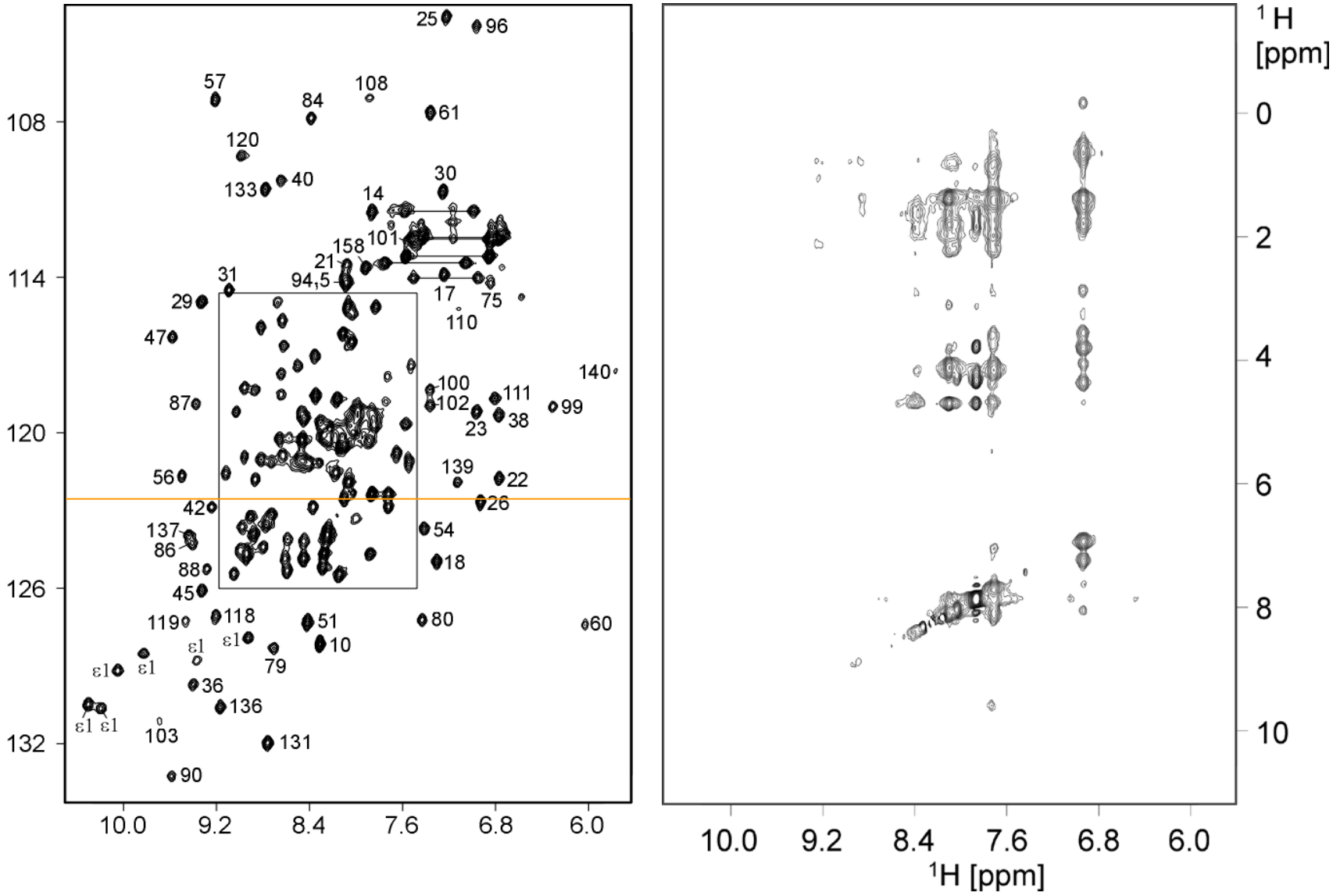
Sequential assignment using 3D heteronuclear experiments



3D ^{15}N-edited NOESY: Distance information

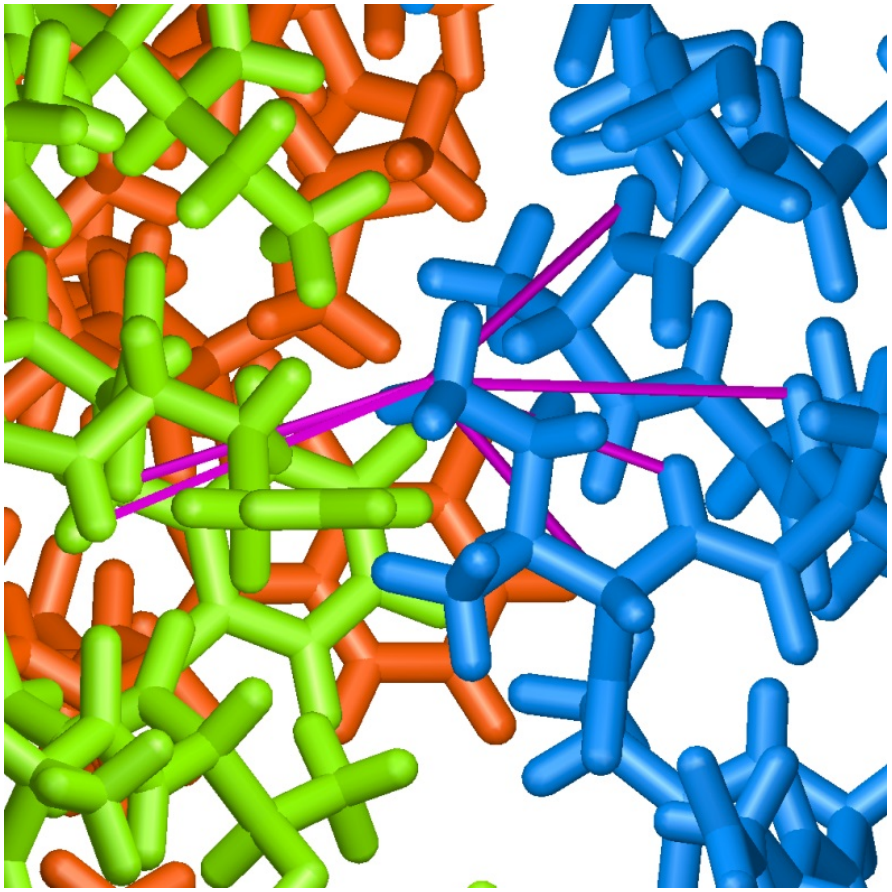


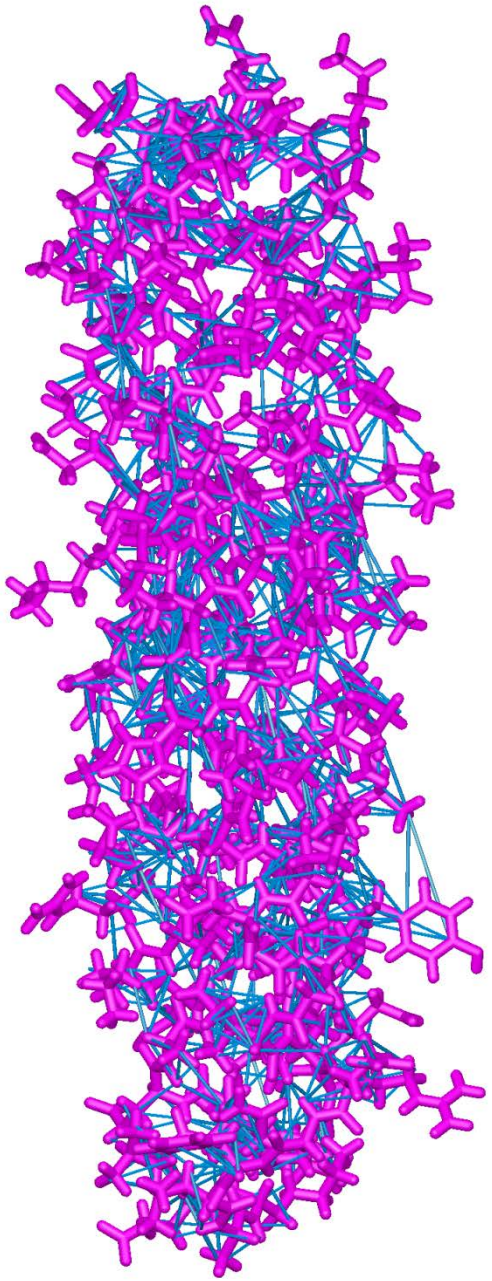
3D ^{15}N-edited NOESY: Distance information



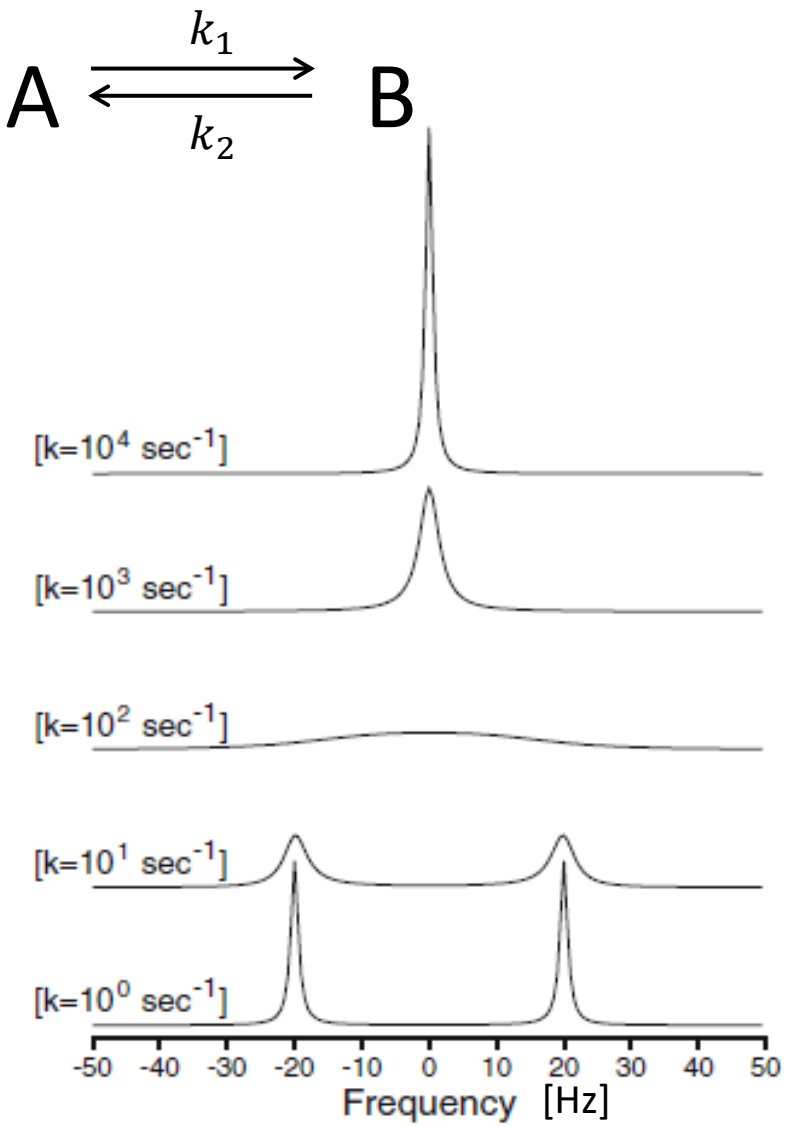
Structure calculation:

Molecular dynamics simulation using distance (and other) restraints





Chemical Exchange



$k_{ex} = k_1 + k_2$

Exchange rate

$k_{ex} \gg \Delta\nu$: fast exchange

Single line: $\omega = p_A\ \omega_A + p_B\ \omega_B$

Population (fraction) of A

$k_{ex} \approx \Delta\nu$: intermediate exchange

broad line(s)

$k_{ex} \ll \Delta\nu$: slow exchange

Two separate lines at ω_A and ω_B

Integrated intensities $\propto\ p_A$ and p_B

Rule, G.S. and Hitchens, T.K. Fundamentals of Protein NMR Spectroscopy, 2006, Springer

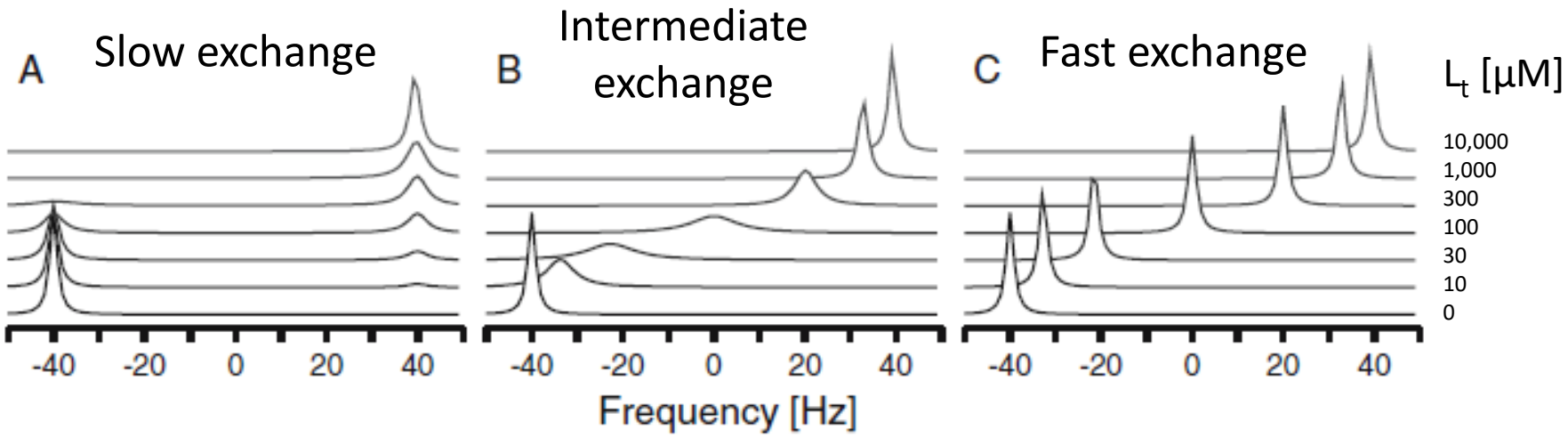
Ligand binding

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

$$k_{ex} = k_{on}[L] + k_{off}$$

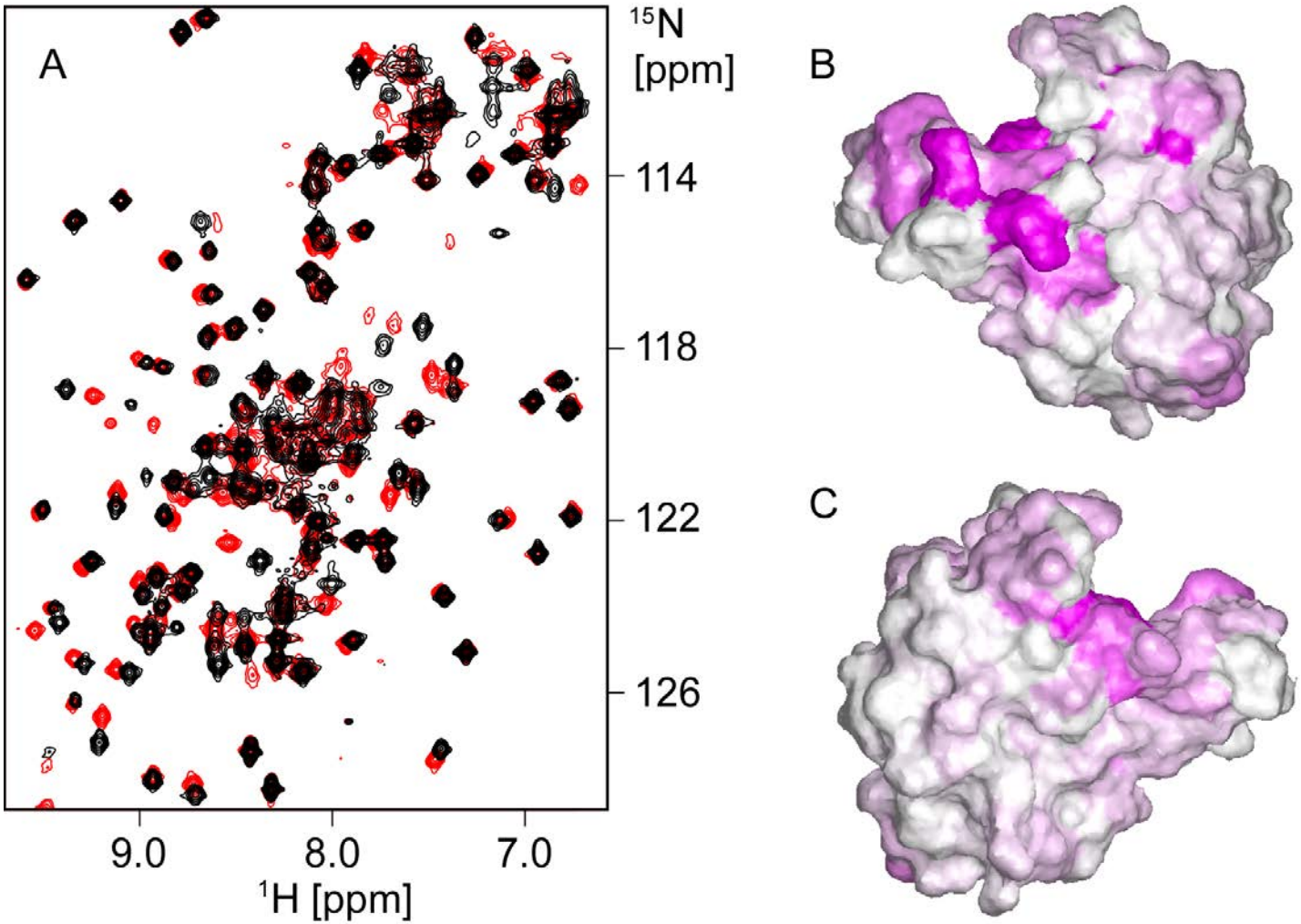
Exchange rate

$$K_d = \frac{k_{off}}{k_{on}} = 100\mu M$$



Rule, G.S. and Hitchens, T.K. Fundamentals of Protein NMR Spectroscopy, 2006, Springer

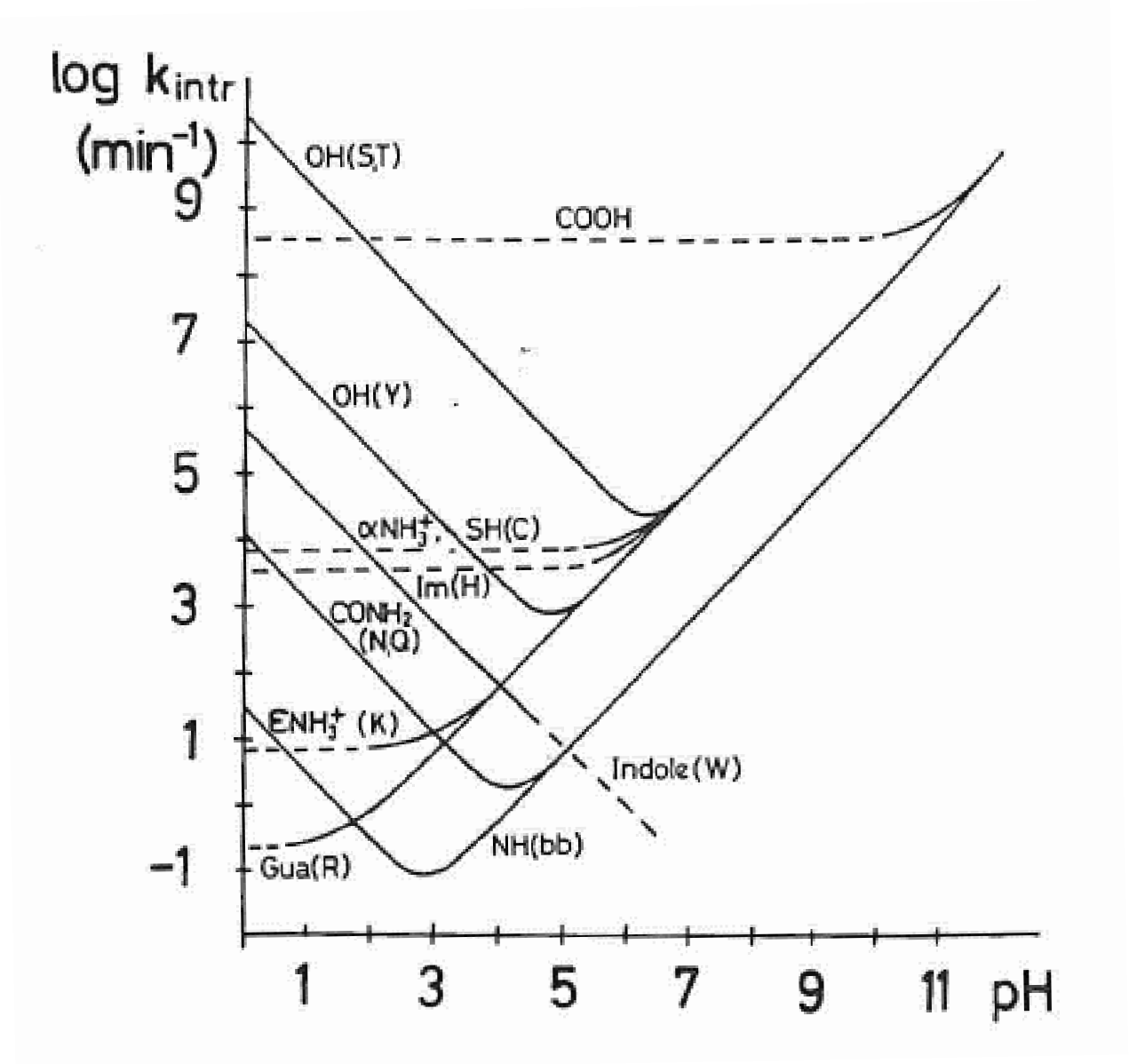
Mapping ligand binding using chemical shift perturbations



Briknarová K, Zhou X, Satterthwait A, Hoyt DW, Ely KR, Huang S. Structural studies of the SET domain from RIZ1 tumor suppressor. Biochem Biophys Res Commun. 2008 Feb 15;366(3):807-13.

Hydrogen exchange

- Hydrogen atoms attached to N, O and S exchange with hydrogens in H_2O
- Hydrogens that exchange fast are not observable
- When the protein is dissolved in D_2O (heavy water) instead of H_2O, exchangeable hydrogens will be replaced by D (= deuteron = ^2H)
- Exchangeable hydrogens that participate in hydrogen bonding are (somewhat) protected from exchange with solvent



Wüthrich, K. NMR of Proteins and Nucleic Acids, 1986, John Wiley & Sons