Lecture: Biomolecular NMR, part II

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Prerequisite knowledge:	Description of solution state NMR experiments using the product operator formalism	
	Assignment of protein resonances using	2D homonuclear ^{1}H NMR and nD heteronuclear NMR
	Basic understanding of NOESY experiments	
	Basic understanding of structure calculation	

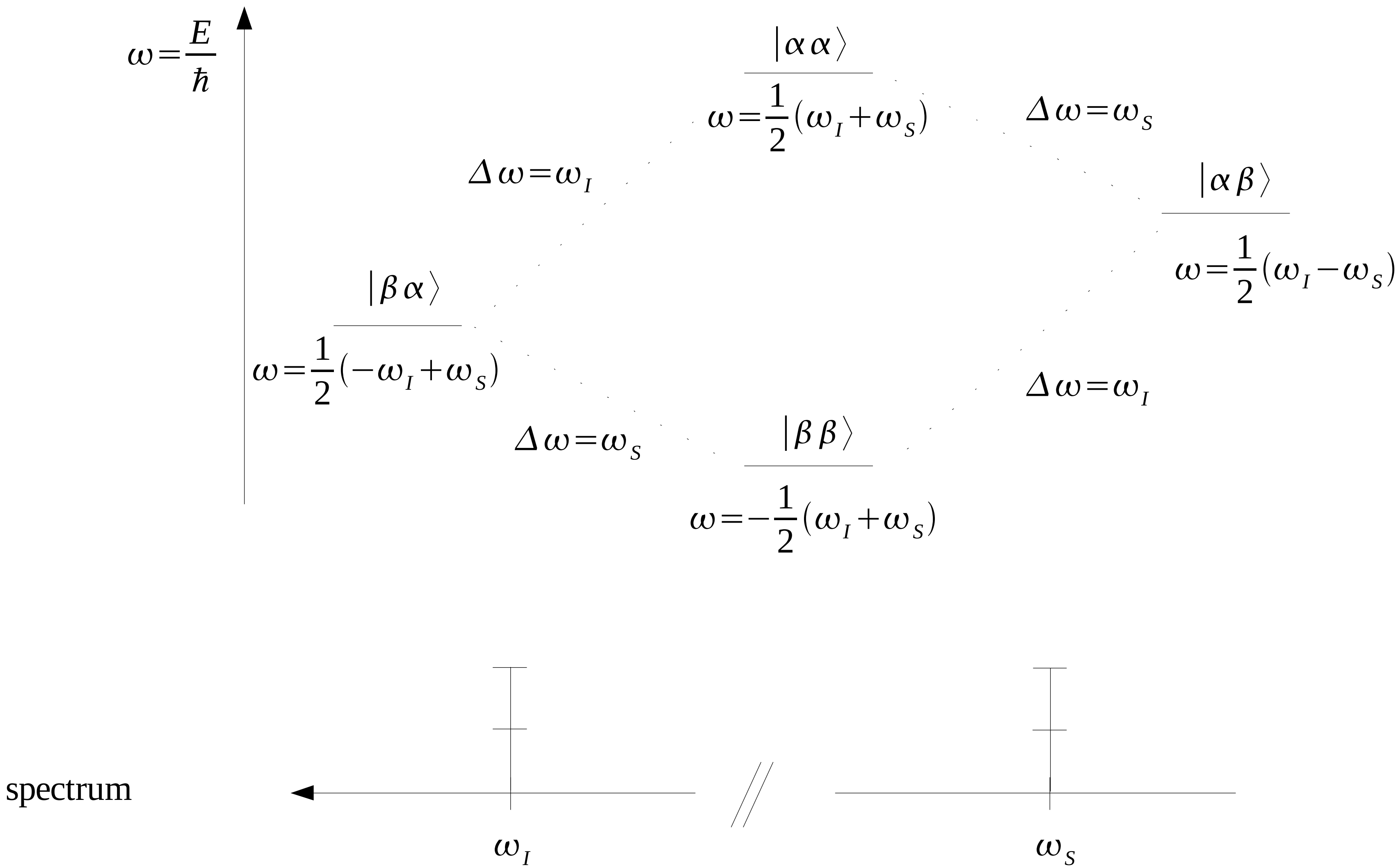
(Topics of lecture Biomolecular NMR I
lecture Biophysical Chemistry I
lab course Biophysical Chemistry

Topics of this lecture

1.	Additional structure defining NMR parameters:	scalar couplings residual dipolar couplings and partially aligned proteins hydrogen bonds and chemical exchange
2.	Studies of larger proteins	TROSY, deuteration
3.	Structure calculation using NMR data:	a more detailled view structure validation
4.	Interaction of proteins with other molecules	Characterization of molecular interactions
5.	Dynamics of proteins	Introduction in spin relaxation

Energy level scheme of a twospin-system (I,S = 1/2) without scalar coupling

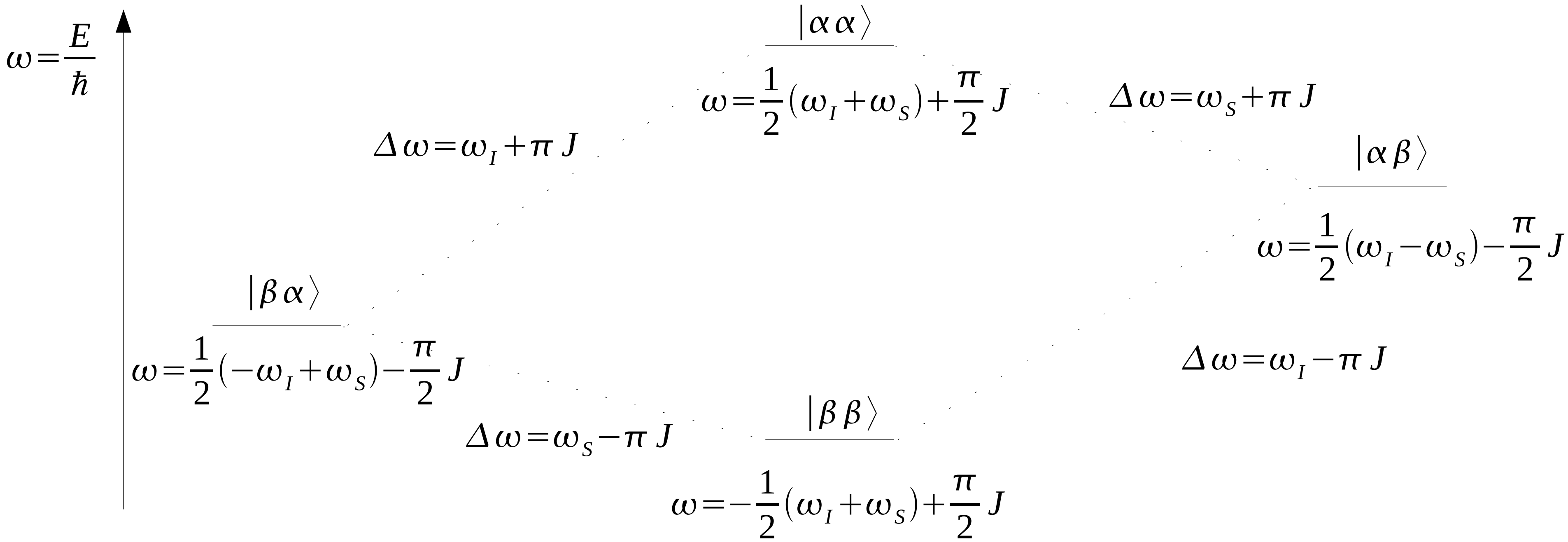
Hamilton operator $\boldsymbol{H} = \boldsymbol{H}_{CS} = \omega_I \boldsymbol{I}_z + \omega_S \boldsymbol{S}_z$

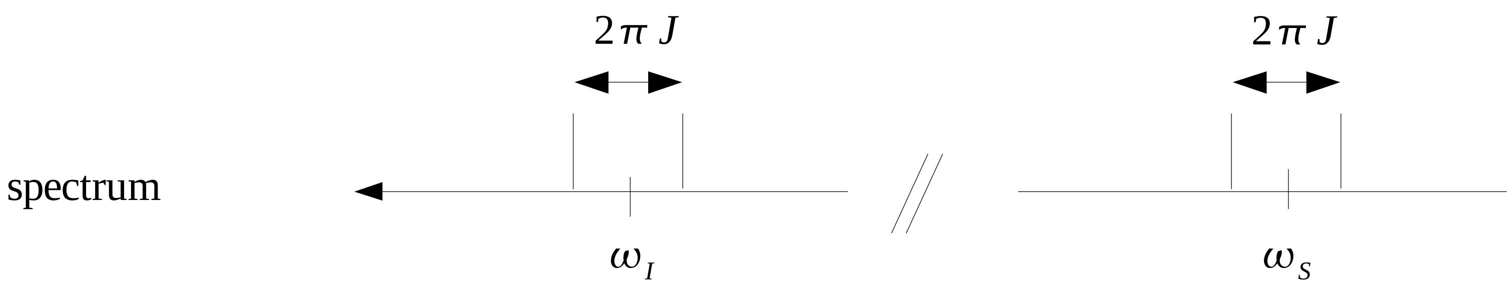


Energy level scheme of a twospin-system (I,S = 1/2) with scalar coupling

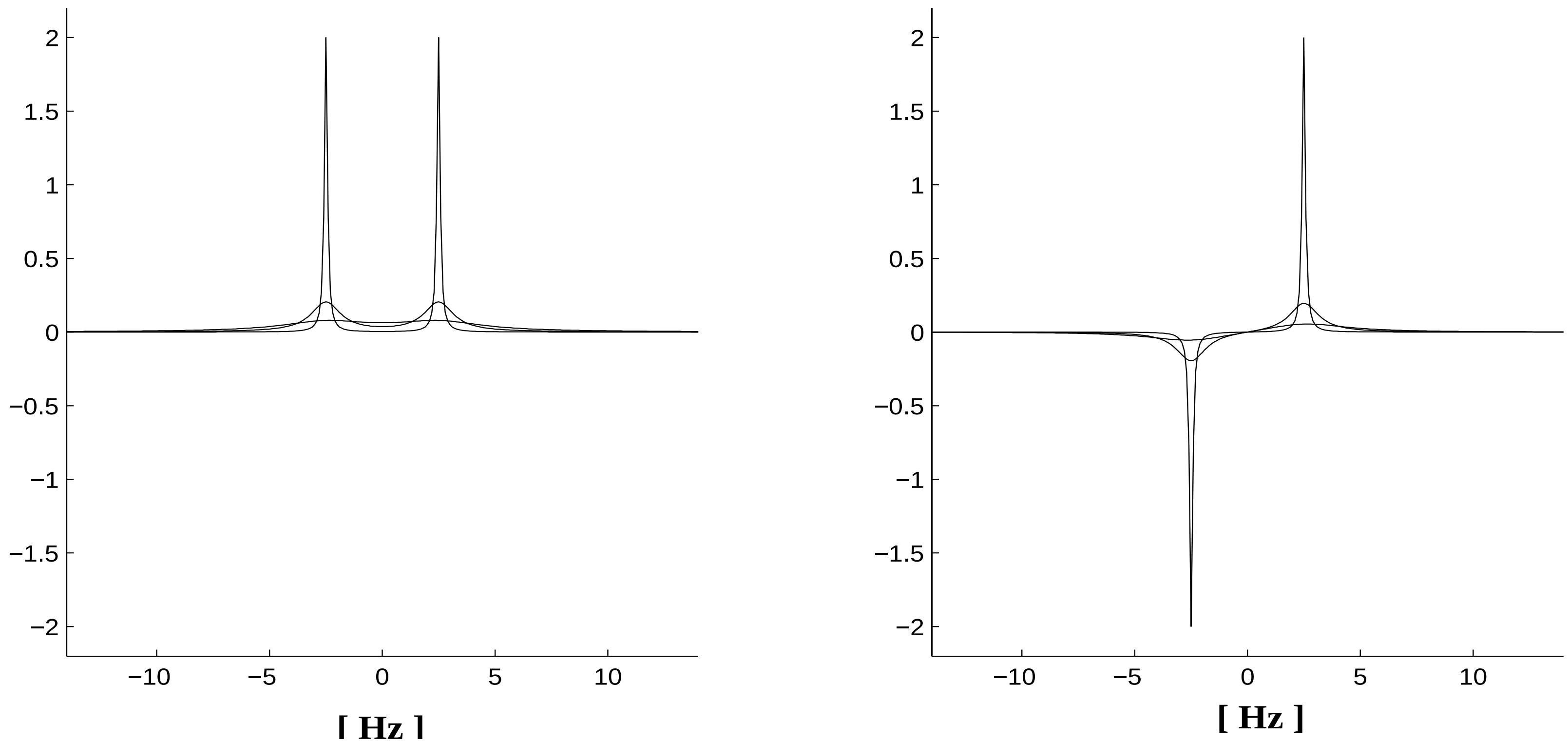
Hamilton operator
approximation of weak coupling

$$\boldsymbol{H} = \boldsymbol{H}_{CS} = \omega_I \boldsymbol{I}_z + \omega_S \boldsymbol{S}_z + \pi J \, \boldsymbol{2} \boldsymbol{I}_z \boldsymbol{S}_z$$





Direct determination of couplings from doublet splitting



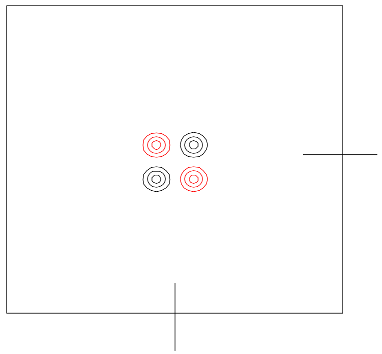
parameters for simulation: J = 5 Hz, R_2 = 0.5 Hz, 5.0 Hz und 15 Hz

Inphase-doublet : underestimation of J at $\Delta\nu_{max}$

Antiphase-doublet : overestimation of J at $\Delta\nu_{max}$

partial overlap of doublet components $\longrightarrow$ nonlinear fit (filterfunction influences lineshape!)

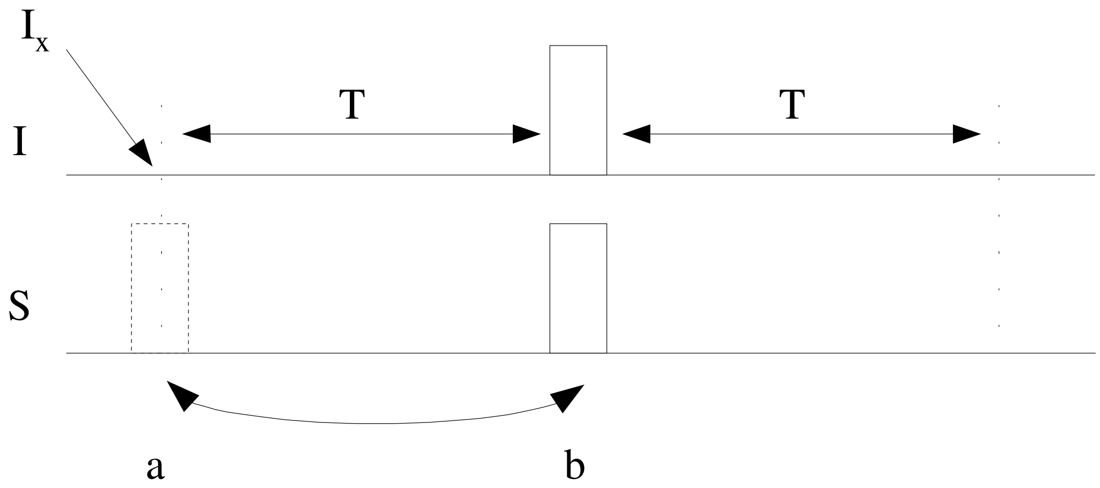
problems: partial signal cancellation of signals in a antiphase doublett
additional not resolved couplings in the multiplet structure



In COSY:

Antiphase $^3J(H^N,H^a)$ at ν_{HN}
Antiphase $^3J(H^N,H^a)$ at ν_{HA}
Inphase $^3J(H^\alpha, H^\beta)$ at ν_{HA}

Basic principle of quantitative J-correlation based on spin-echo difference spectroscopy

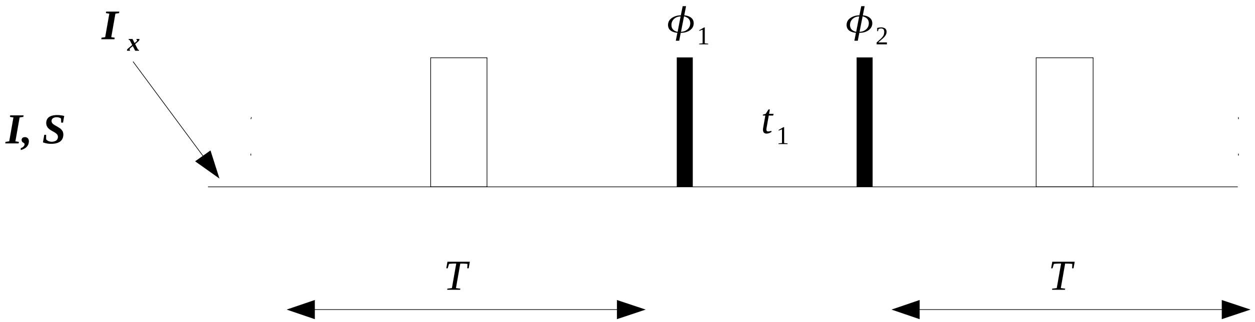


a: J_{IS} inactive (refocusing by 180°(I) pulse)

b: J_{IS} active -> cos (2 π *J T*) factor in the transfer function

$$\longrightarrow \quad \frac{S_b}{S_a} = \cos(2\pi J T)$$

Basic principle of quantitative J correlation based on a COSY-like transfer



$$\phi_1 = x, -x$$
$$\phi_2 = 2x, 2(-x)$$
$$rec. = 4(x)$$

$$\boldsymbol{I}_x \xrightarrow{\pi J T\, 2\boldsymbol{I}_z \boldsymbol{S}_z} \boldsymbol{I}_x \cos(\pi J T) + 2\boldsymbol{I}_y \boldsymbol{S}_z \sin(\pi J T) \xrightarrow{\frac{\pi}{2}(\boldsymbol{I}_x + \boldsymbol{S}_x)} \boldsymbol{I}_x \cos(\pi J T) - 2\boldsymbol{I}_z \boldsymbol{S}_y \sin(\pi J T)$$

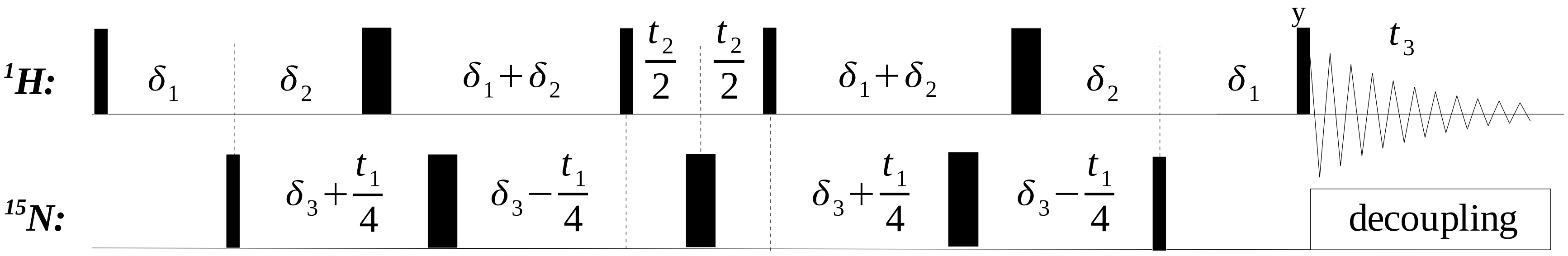
$$\xrightarrow{\omega_I \boldsymbol{I}_z t_1 + \omega_S \boldsymbol{S}_z t_1} \boldsymbol{I}_x \cos(\pi J T)\cos(\omega_I t_1) - 2\boldsymbol{I}_z \boldsymbol{S}_y \sin(\pi J T)\cos(\omega_S t_1)$$

$$\xrightarrow{\frac{\pi}{2}(\boldsymbol{I}_x + \boldsymbol{S}_x)} \boldsymbol{I}_x \cos(\pi J T)\cos(\omega_I t_1) + 2\boldsymbol{I}_y \boldsymbol{S}_z \sin(\pi J T)\cos(\omega_S t_1)$$

$$\xrightarrow{\pi J T\, 2\boldsymbol{I}_z \boldsymbol{S}_z} \boldsymbol{I}_x \cos^2(\pi J T)\cos(\omega_I t_1) - 2\boldsymbol{I}_y \boldsymbol{S}_z \sin^2(\pi J T)\cos(\omega_S t_1)$$

$$\longrightarrow \frac{S_{cross}}{S_{diag}} = -\frac{\sin^2(\pi J T)}{\cos^2(\pi J T)} = -\tan^2(\pi J T)$$

The 3D HNHA experiment for determing $^{3}J(H^{N},H^{\alpha})$ in ^{15}N labeled proteins



$\delta_1 = 5.5\,ms \qquad \delta_2 = 13\,ms \qquad 2\delta_2 - \delta_1 = 2\delta_3$

$$\boldsymbol{H_z^N} \longrightarrow \boldsymbol{2H_x^N N_y}\cos(\omega_N t_1)$$

$$\longrightarrow \begin{array}{c} \boldsymbol{2H_x^N N_y}\cos(\omega_N t_1)\cos(\pi J\, 2(\delta_1+\delta_2)) \\ \boldsymbol{4H_y^N H_z^\alpha N_y}\cos(\omega_N t_1)\sin(\pi J\, 2(\delta_1+\delta_2)) \end{array} \longrightarrow \begin{array}{c} \boldsymbol{2H_x^N N_y}\cos(\omega_N t_1)\cos(\pi J\, 2(\delta_1+\delta_2)) \\ -\boldsymbol{4H_z^N H_y^\alpha N_y}\cos(\omega_N t_1)\sin(\pi J\, 2(\delta_1+\delta_2)) \end{array}$$

$$\longrightarrow \begin{array}{c} \boldsymbol{2H_x^N N_y}\cos(\omega_N t_1)\cos(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{HN} t_2) \\ -\boldsymbol{4H_z^N H_y^\alpha N_y}\cos(\omega_N t_1)\sin(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{H\alpha} t_2) \end{array} \longrightarrow \begin{array}{c} \boldsymbol{2H_x^N N_y}\cos(\omega_N t_1)\cos(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{HN} t_2) \\ \boldsymbol{4H_y^N H_z^\alpha N_y}\cos(\omega_N t_1)\sin(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{H\alpha} t_2) \end{array}$$

$$\longrightarrow \begin{array}{c} \boldsymbol{2H_x^N N_y}\cos(\omega_N t_1)\cos^2(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{HN} t_2) \\ -\boldsymbol{2H_x^N N_y}\cos(\omega_N t_1)\sin^2(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{H\alpha} t_2) \end{array} \longrightarrow \begin{array}{c} \boldsymbol{H_y^N}\cos(\omega_N t_1)\cos^2(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{HN} t_2)\exp\{i\,\omega_{HN} t_3\} \\ -\boldsymbol{H_y^N}\cos(\omega_N t_1)\sin^2(\pi J\, 2(\delta_1+\delta_2))\cos(\omega_{H\alpha} t_2)\exp\{i\,\omega_{HN} t_3\} \end{array}$$

Summary 3D HNHA

The 3D HNHA experiment results in a ^{15}N edited $^{1}H^{N}$, $^{1}H^{\alpha}$ correlation

The $^{3}J(H^{N},H^{\alpha})$ can be extracted from the ratio of diagonal and cross peak

Therefore the diagonal peak must be without overlap in the 3D data cube,

usually not a problem for medium sized well structured proteins

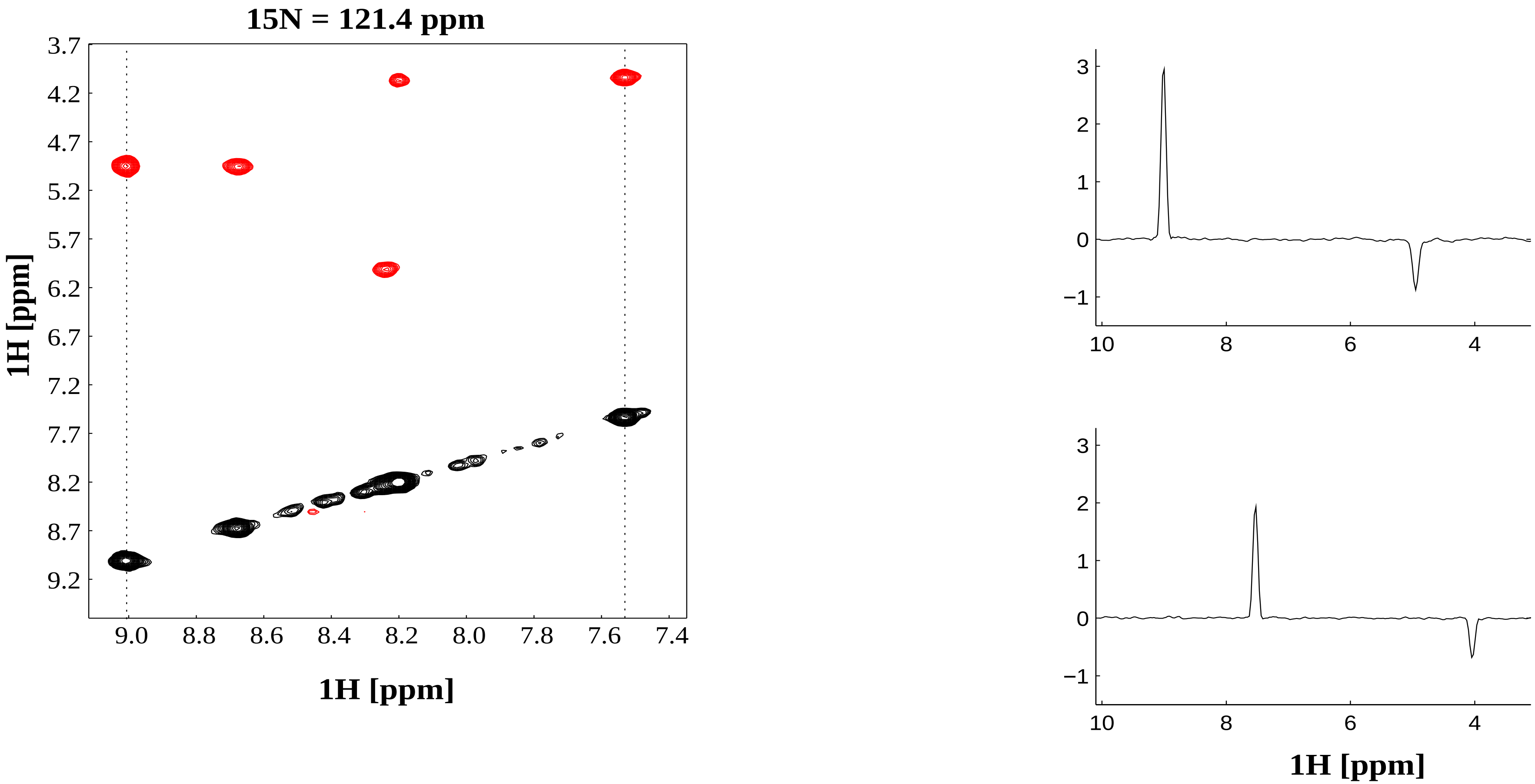
The two relevant spin operators have different relaxation rates:

$$R_2(2H_x^N N_y) < R_2(4H_y^N H_z^\alpha N_y)$$

This results in an underestimation of $^{3}J(H^{N}, H^{\alpha})$

This can be adjusted by applying a correction factor of 1.05 (MW < 10 kDa) or 1.10 (MW > 10kDa)

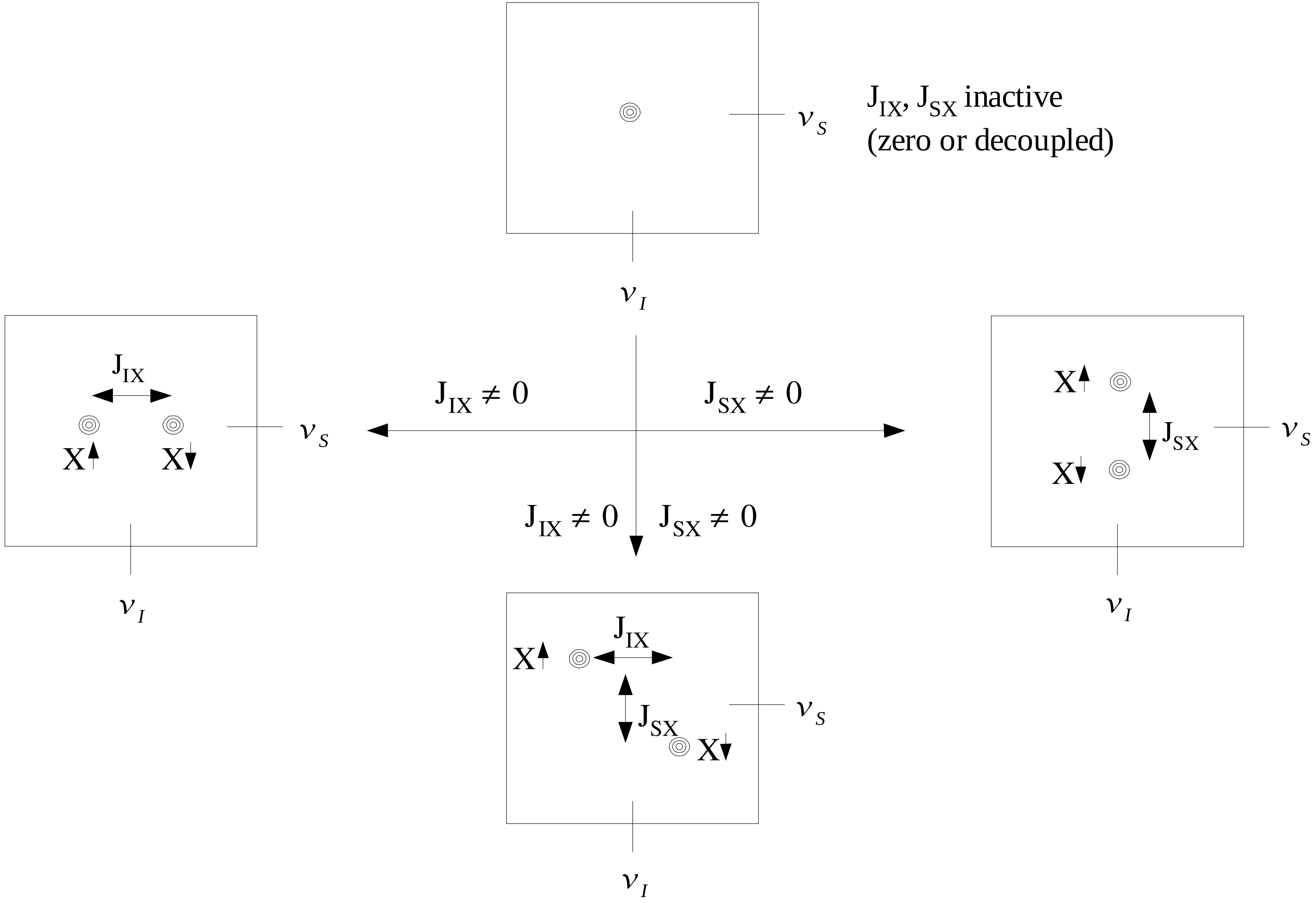
experimental 3D HNHA spectrum



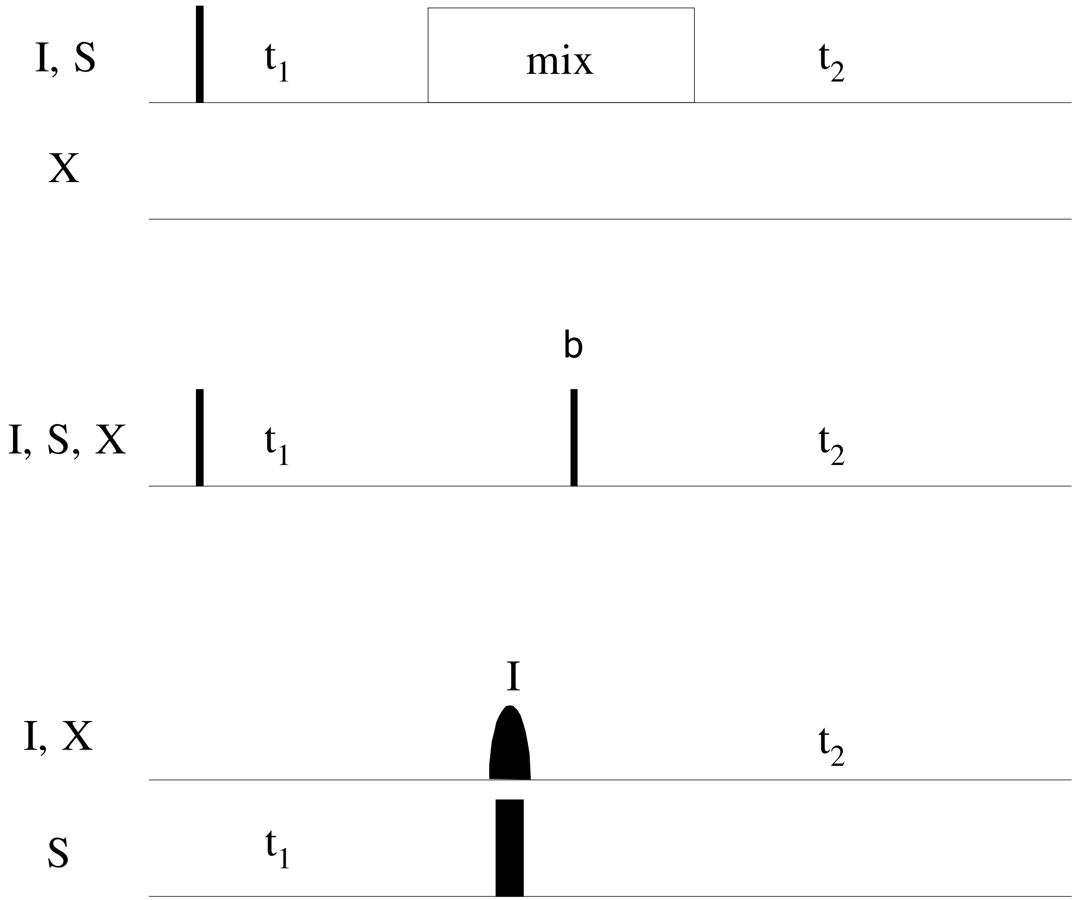
	F1(^{1}H):	80* data points	SW 4901 Hz	
AV700 MHz	F2 (^{15}N)	32* data points	SW 1703 Hz	total duration ca. 60 h
	F3 (^{1}H)	512* data points	SW 9766 Hz	

ca. 2mM of a15 kDa protein

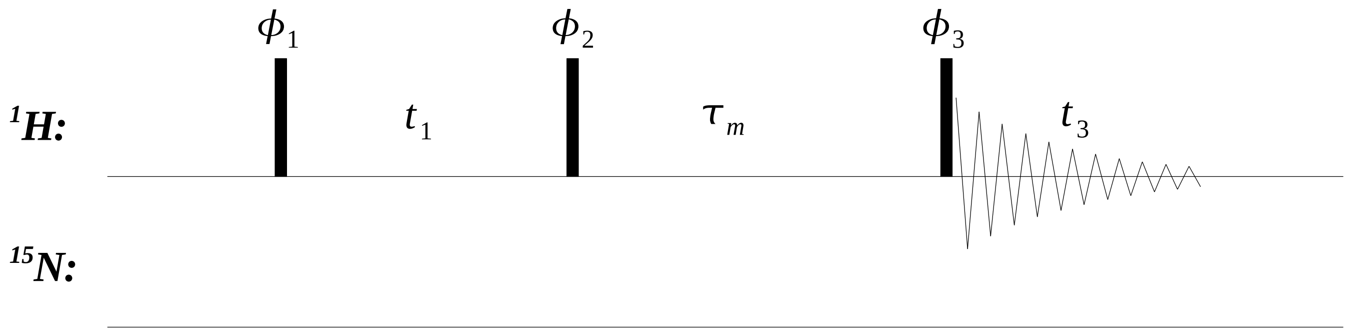
E. COSY principle in a (I, S) correlation



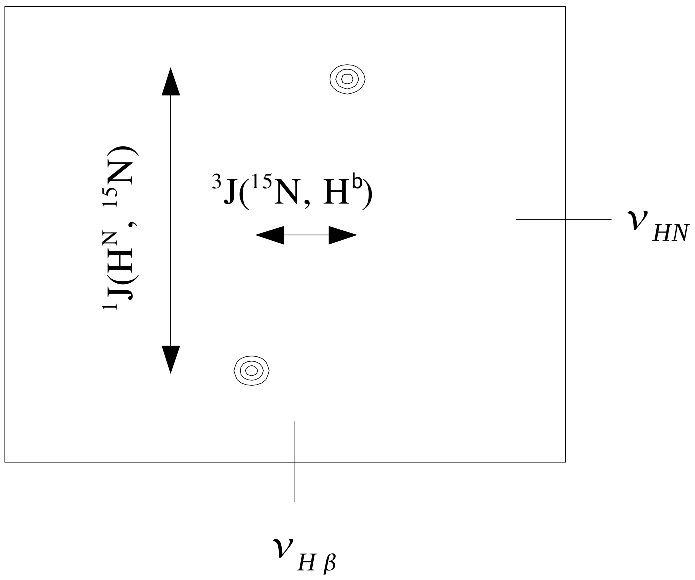
Schematic E. COSY transfers



2D NOESY of a ^{15}N labeled protein without ^{15}N decoupling



missing ^{15}N decoupling (180° pulse during t_1 and cpd during t_2) avoids mixing of ^{15}N spin states



heteronuclear E.COSY in a triple resonance experiment

basic transfer

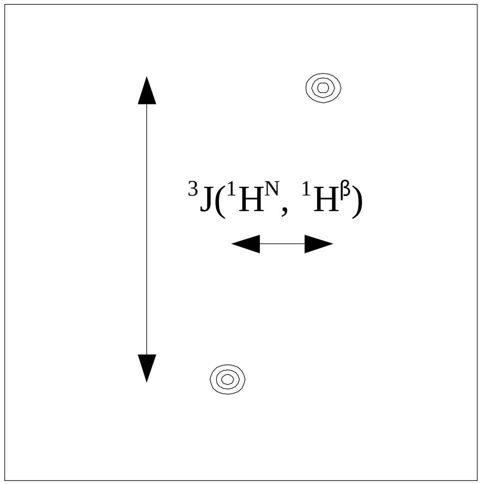
$$\boldsymbol{H_z^N} \longrightarrow \boldsymbol{2 H_z^N N_y} \longrightarrow \boldsymbol{2 N_y C_z^\alpha} \longrightarrow \boldsymbol{2 N_z C_y^\alpha}$$

$$\boldsymbol{2 N_z C_y^\alpha} \longrightarrow \boldsymbol{H^\alpha}(\alpha)\boldsymbol{N_z C_y^\alpha}\cos((\omega_{C\alpha}+\pi J_{C\alpha H\alpha})t_1) + \boldsymbol{H^\alpha}(\beta)\boldsymbol{N_z C_y^\alpha}\cos((\omega_{C\alpha}-\pi J_{C\alpha H\alpha})t_1)$$

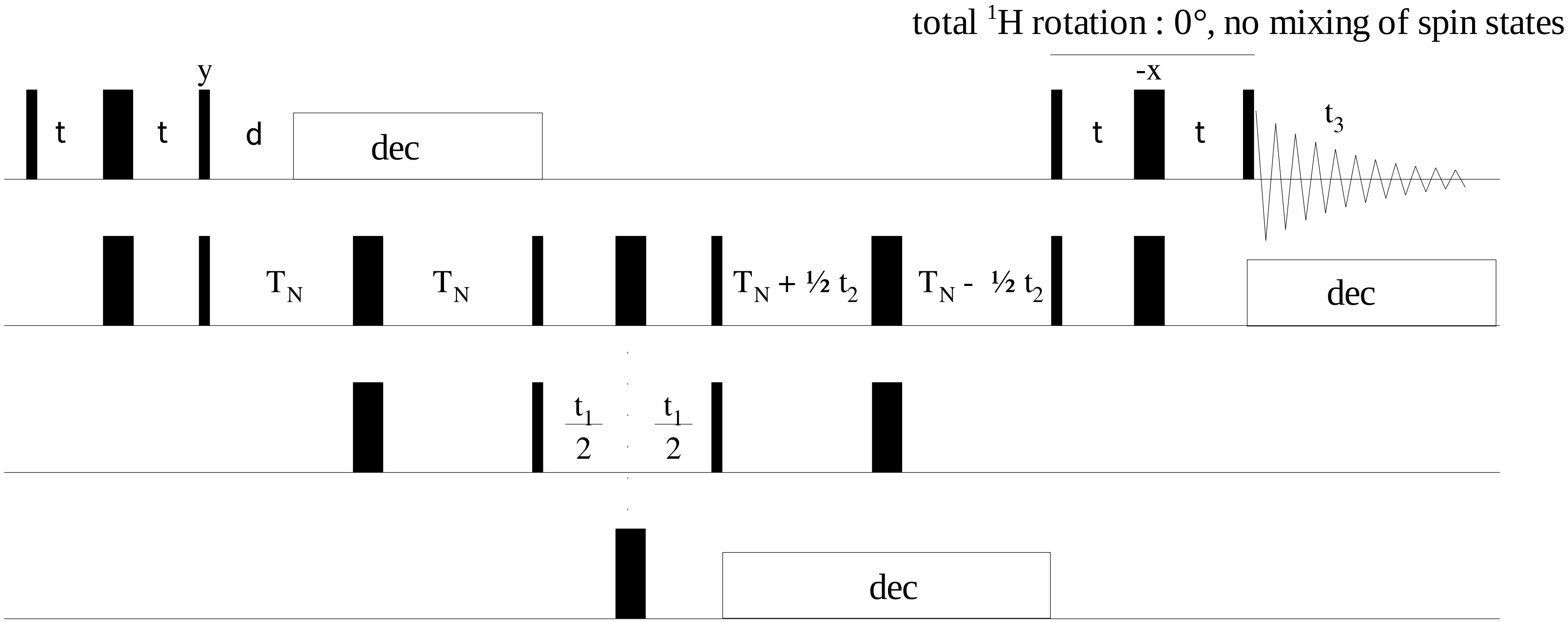
$^{13}C^\alpha$ evolution without $^{1}H^\alpha$ decoupling

$$\boldsymbol{H^\alpha}(\alpha)\boldsymbol{N_z C_y^\alpha}\cos((\omega_{C\alpha}+\pi J_{C\alpha H\alpha})t_1) + \boldsymbol{H^\alpha}(\beta)\boldsymbol{N_z C_y^\alpha}\cos((\omega_{C\alpha}-\pi J_{C\alpha H\alpha})t_1) \dashrightarrow \boldsymbol{H^\alpha}(\alpha)\boldsymbol{H_x^N}\cos((\omega_{C\alpha}+\pi J_{C\alpha H\alpha})t_1)\cos(\omega_N t_2)\cos((\omega_{HN}+\pi J_{HNH\alpha})t_3) + \boldsymbol{H^\alpha}(\beta)\boldsymbol{H_x^N}\cos((\omega_{C\alpha}-\pi J_{C\alpha H\alpha})t_1)\cos(\omega_N t_2)\cos((\omega_{HN}-\pi J_{HNH\alpha})t_3)$$

$F2=\omega_{15N}$

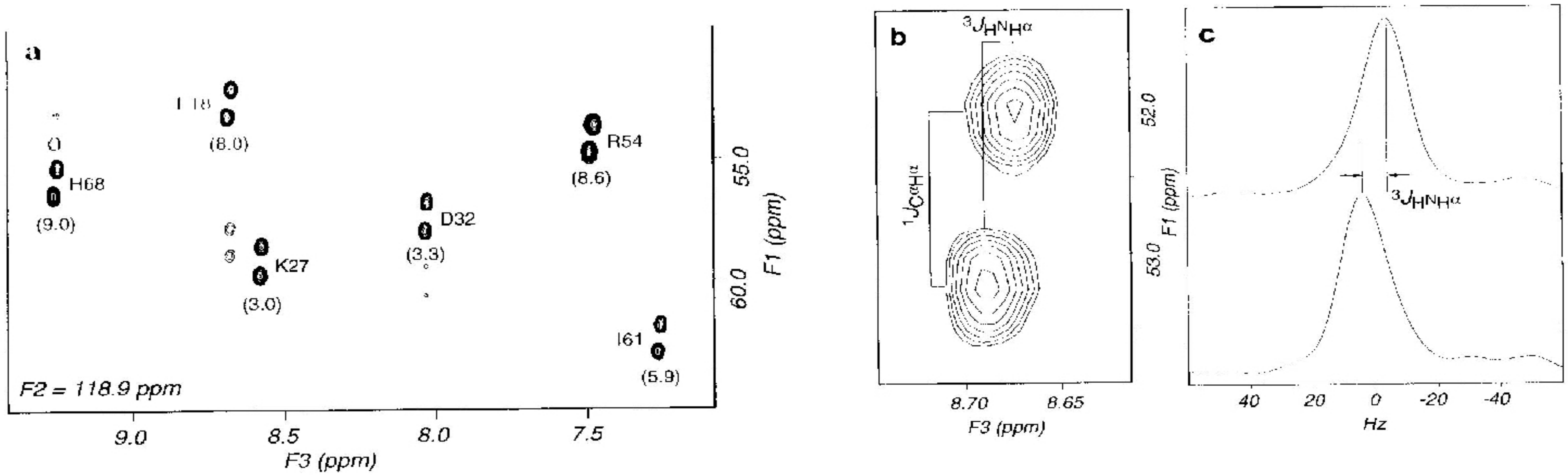


HNCA-J pulse sequence

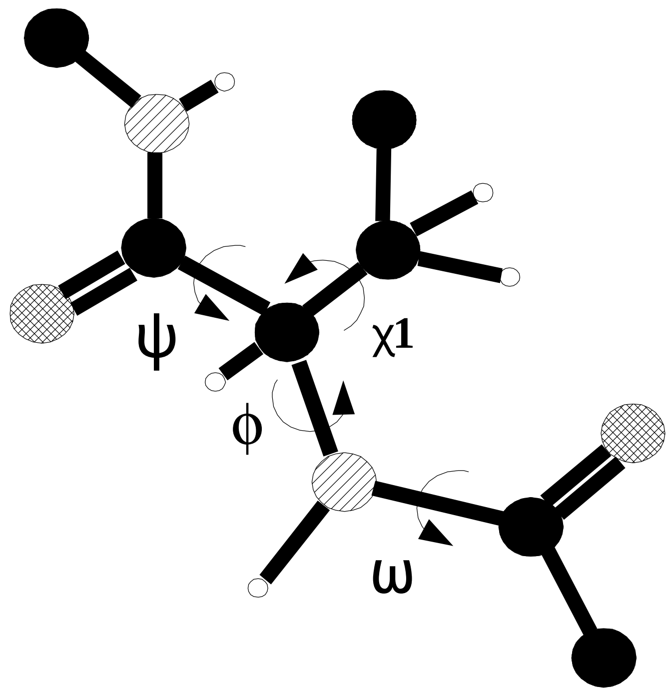


$t = ¼\ ^1J(H^N,N)$ $d = ½\ ^1J(H^N,N)$ $T_N < ¼\ ^1J(C^a,N)$

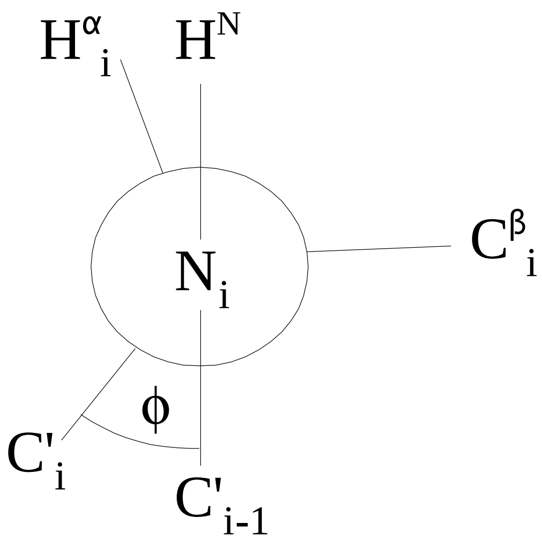
HNCA-J spectrum



Dihedral angles in peptide chains



The dihedral backbone angle ϕ



possible ^3J coupling constants

$^3J(H^N, H^\alpha)$

$^3J(H^\alpha, C'_{(i-1)})$

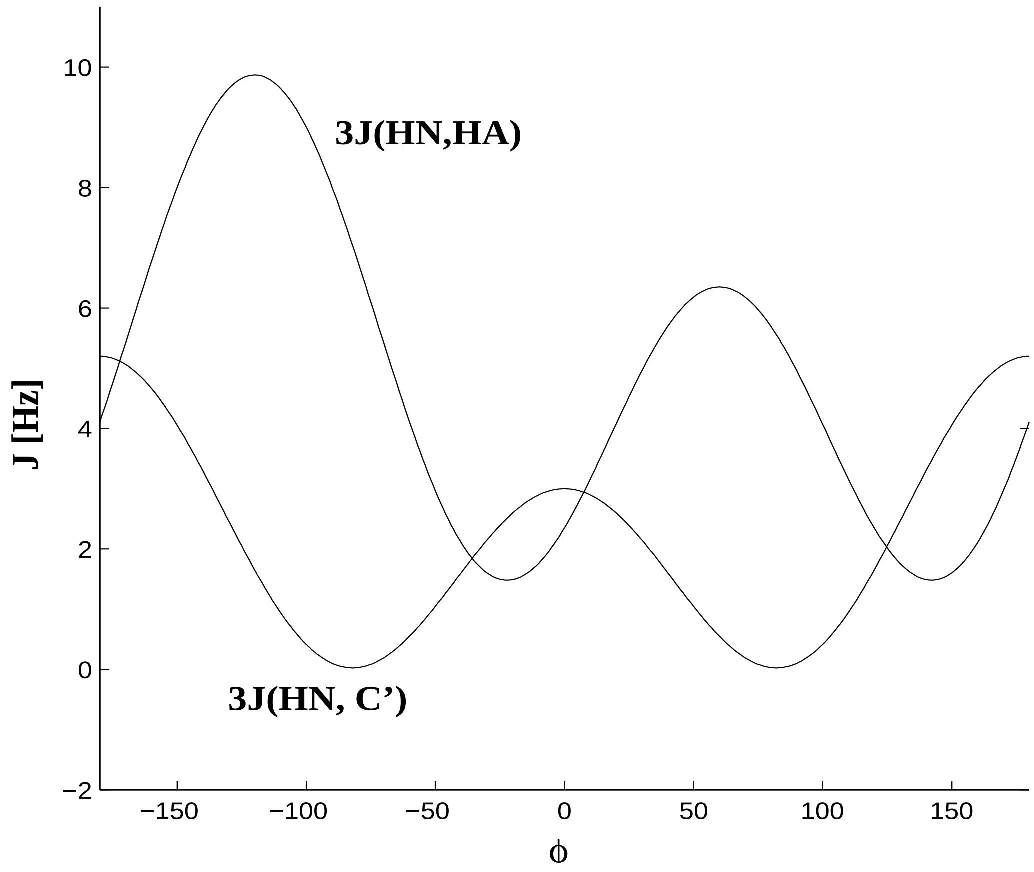
$^3J(H^N, C')$

$^3J(C', C'_{(i-1)})$

$^3J(C^\beta, C'_{(i-1)})$

$^3J(H^N, C^\beta)$

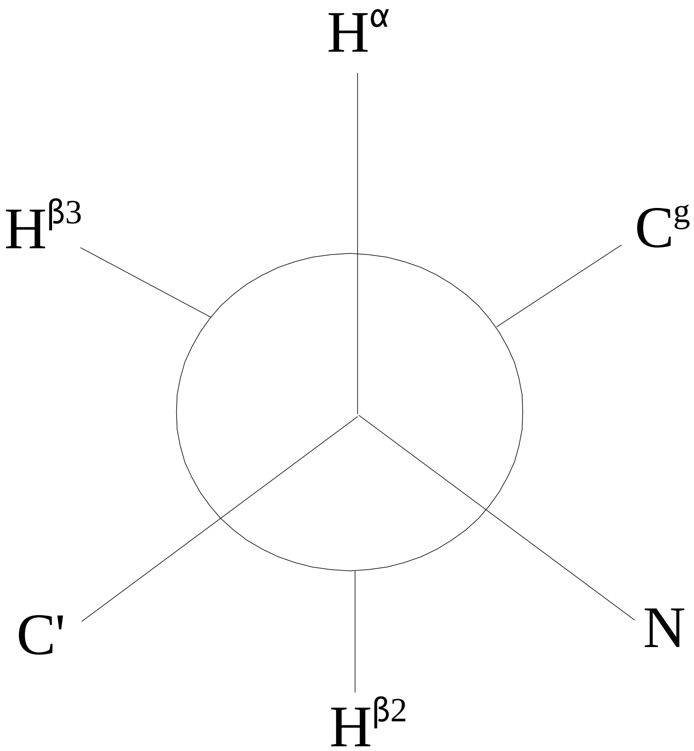
Karplus relations for ϕ



$$^{3}J(H^{N}, H^{\alpha}) = 6.51 \cos^{2} (\phi\text{-}60°) - 1.76 \cos (\phi\text{-}60°) + 1.60$$

$$^{3}J(H^{N}, C') = 4.00 \cos^{2} (\phi) - 1.10 \cos (\phi) + 0.10$$

The dihedral angle χ_1 - definition of side chain orientation

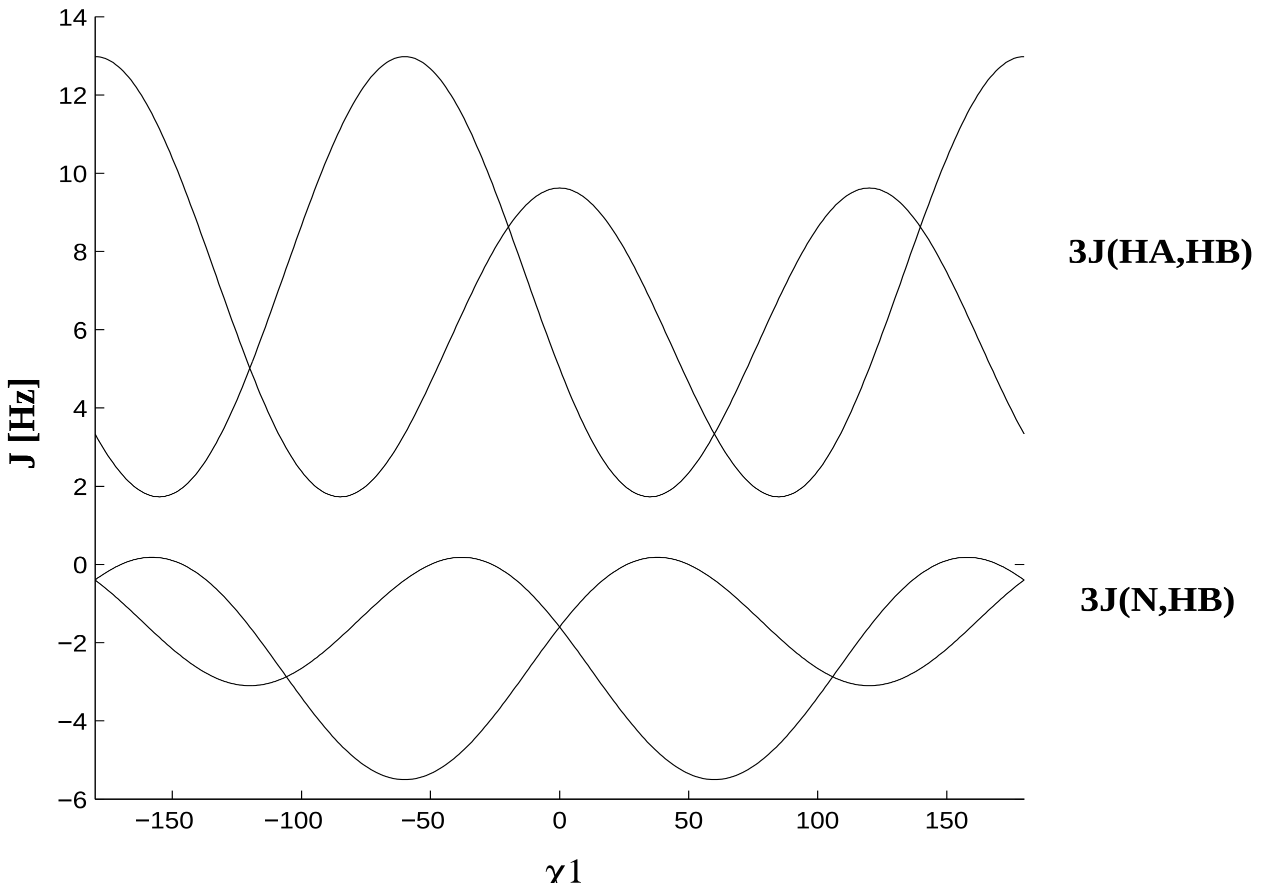


important coupling constants

$^{3}J(H^{\alpha}, H^{\beta})$

$^{3}J(N, H^{\beta})$

Karplus-relations for χ_1



$$^3J(H^{\alpha}, H^{\beta 2}) = 9.5 \cos^2 (\chi_1 -120°) -1.68 \cos (\chi_1 - 120°) + 1.80$$

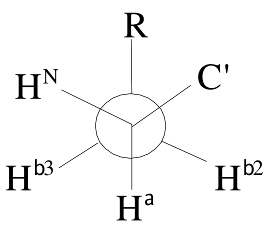
$$^3J(H^{\alpha}, H^{\beta 3}) = 9.5 \cos^2 (\chi_1) -1.68 \cos (\chi_1) + 1.80$$

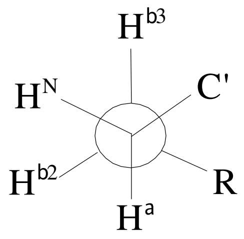
$$^3J(^{15}N, H^{\beta 2}) = -4.4 \cos^2 (\chi_1 + 120°) +1.20 \cos (\chi_1 + 120°) + 0.10$$

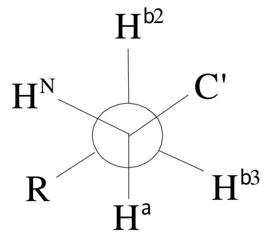
$$^3J(^{15}N, H^{\beta 3}) = -4.4 \cos^2 (\chi_1 - 120°) +1.20 \cos (\chi_1 - 120°) + 0.10$$

stereospecific assignment of β-CH_2 protons

rotamer	g_2g_3	g_2t_3	t_2g_3
$\chi 1$	60°	180°	-60°







$^3J_{\alpha\beta 2}$ (Hz)	3.4	3.4	12.9
$^3J_{\alpha\beta 3}$ (Hz)	3.4	12.9	3.4
NOEs	$H^\alpha, H^{\beta 2} \sim H^\alpha, H^{\beta 3}$ $H^N, H^{\beta 2} < H^N, H^{\beta 3}$	$H^\alpha, H^{\beta 2} > H^\alpha, H^{\beta 3}$ $H^N, H^{\beta 2} \sim H^N, H^{\beta 3}$	$H^\alpha, H^{\beta 2} < H^\alpha, H^{\beta 3}$ $H^N, H^{\beta 2} > H^N, H^{\beta 3}$
$^3J_{N\beta 2}$ (Hz)	~5	~1	~1
$^3J_{N\beta 3}$ (Hz)	~1	~1	~5