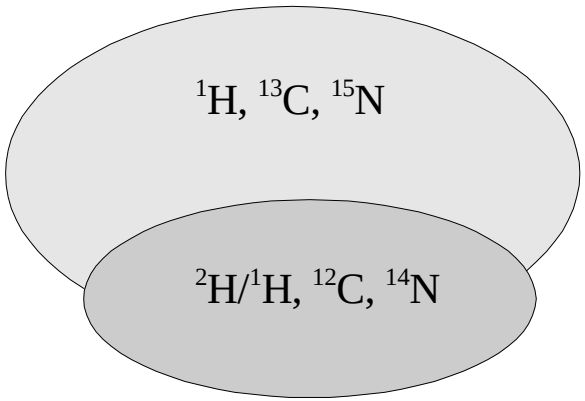
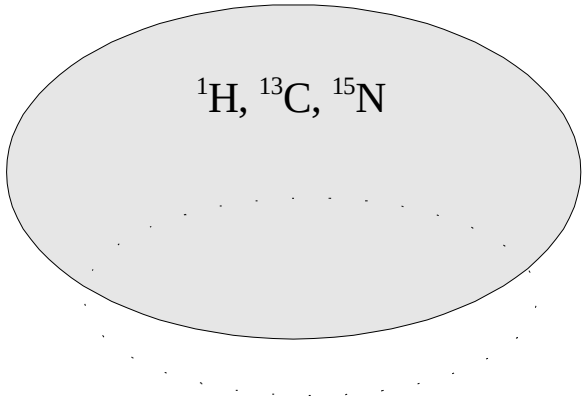
Structural characterization of complexes using NOESY experiments

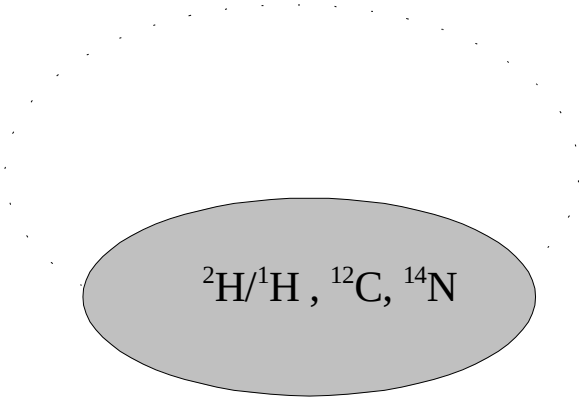
Isotope labeling (^{13}C, ^{15}N) allows observation of individual components of a (binary) complex)



Complex with different labeling of the components



Isotope edited NMR spectra



Isotope filtered NMR spectra

Basic terminology

Isotope editing: selection of protons directly bound to a NMR active heteronucleus (e.g. ^{13}C-^{1}H or ^{15}N-^{1}H)

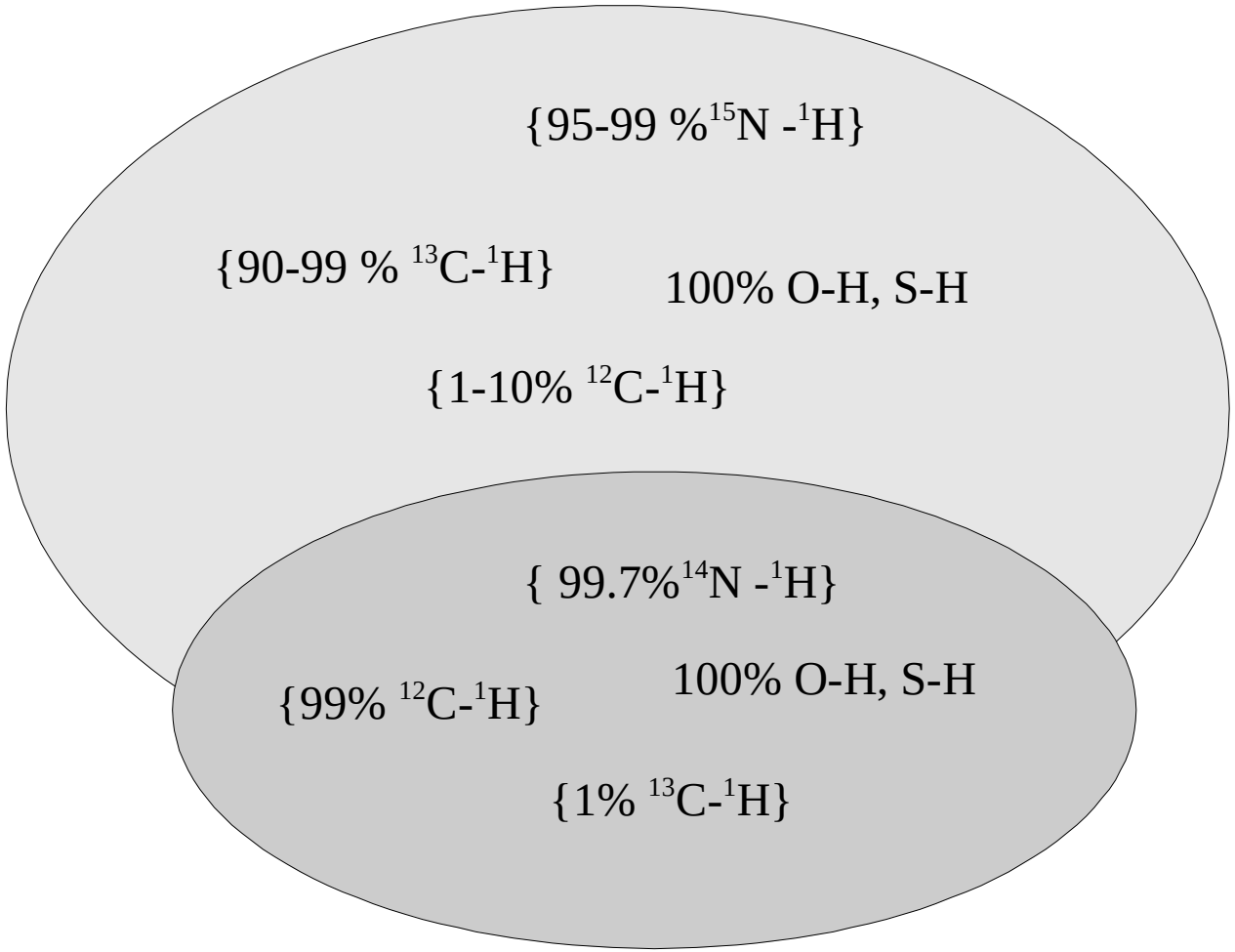
Isotope filtering: suppression of protons directly bound to a NMR active heteronucleus,
ideal suppression corresponds to the 'selection' of protons bound to NMR 'inactive' nuclei
(e.g. ^{12}C-^{1}H, ^{14}N-^{1}H, O-H)

Important isotopes for biomolecular NMR

NMR active isotope	natural abundance	corresponding NMR inactive isotope	natural abundance
^{1}H	99.985 %	^{2}H	0.015 %
^{13}C	1.11 %	^{12}C	98.9 %
^{15}N	0.37 %	^{14}N	99.63%

(^{14}N, ^{2}H are quadrupol nuclei, relaxes very fast in biomacromolecules, nearly NMR inactive)

In practice: real isotope composition of complexes (in H_2O solution)



Selectivity for isotope editing

$$\text{Selectivity} = \frac{\text{Fraction of wanted observed component}}{\text{Fraction of unwanted observed component}}$$

$$\text{Sel.}_{\text{Iso.Ed.}} = \frac{\text{concentration(labeled component)} \ * \ \text{degree of labeling}}{\text{concentration(unlabeled component)} \ * \text{(natural abundance)}}$$

$$\text{Sel.}_{\text{Iso.Filt.}} = \frac{\text{concentration(unlabeled component)}}{\text{concentration(labeled component)} \ * \text{(fraction of protons not bound to a NMR active heteronucleus}}$$

Examples for the selectivity in a 1:1 complex

Degree of ^{13}C labeling	$selektivity_{Iso.Ed.}$	$selektivity_{Iso.Filt.}$
50 %	50	2
90 %	90	10
99 %	99	100

→ Isotope filtering requires a very high degree of isotopic labeling

especially for NOESY experiments, because the relative intensity of different NOESY cross peaks show up factors in the range 10-100 (strong and weak NOE cross peaks !)

Basic principle for isotope filtering and editing

^{1}H - X: $H_x \xrightarrow{\pi\, {}^1J_{HX}\, 2H_zX_z\, t} H_x \cos(\pi\, {}^1J_{HX}\, t) + 2H_yX_z \sin(\pi\, {}^1J_{HX}\, t)$

^{1}H : $H_x \xrightarrow{t} H_x$

Isotope editing: Selection of the term $2H_yX_z$

Isotope filtering: Suppression of the term $2H_yX_z$ and of the term H_x for the ^{1}HX pairs

→ Additional requirements on filtering compared to editing

Basic principle for isotope editing

$$H_x \xrightarrow{\pi\, {}^1J_{HX}\, 2H_zX_z\, t} H_x \cos(\pi\, {}^1J_{HX}\, t) + 2H_yX_z \sin(\pi\, {}^1J_{HX}\, t)$$

Maximizing of the $2H_yX_z$ term by setting $t = 1 / (2\, J_{HX})$

A: $$H_x \cos(\pi\, {}^1J_{HX}\, t) + 2H_yX_z \sin(\pi\, {}^1J_{HX}\, t) \xrightarrow{\pm 90°\, H_x} H_x \cos(\pi\, {}^1J_{HX}\, t) \pm 2H_zX_z \sin(\pi\, {}^1J_{HX}\, t)$$

Alternating of receiver phase eliminates H_x term

B: $$H_x \cos(\pi\, {}^1J_{HX}\, t) \pm 2H_zX_z \sin(\pi\, {}^1J_{HX}\, t) \xrightarrow{\pm 90°\, X_y} H_x \cos(\pi\, {}^1J_{HX}\, t) \pm 2H_zX_x \sin(\pi\, {}^1J_{HX}\, t)$$

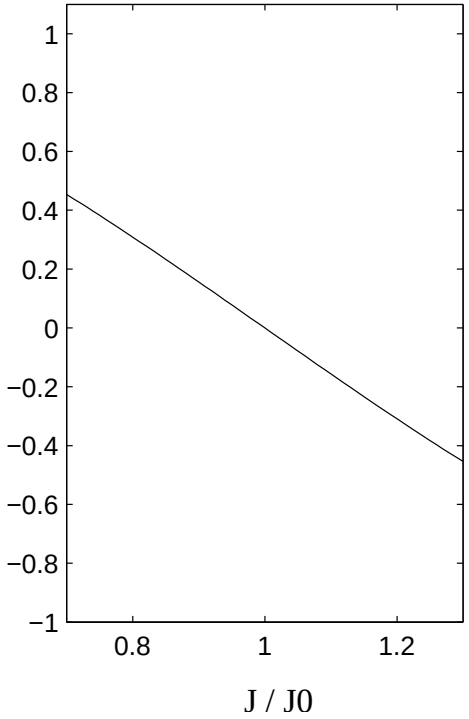
Alternating of receiver phase eliminates H_x term

→ At least one of both phase alternations is found in every phase cycling of an isotope editited experiment (e.g. HSQC) !

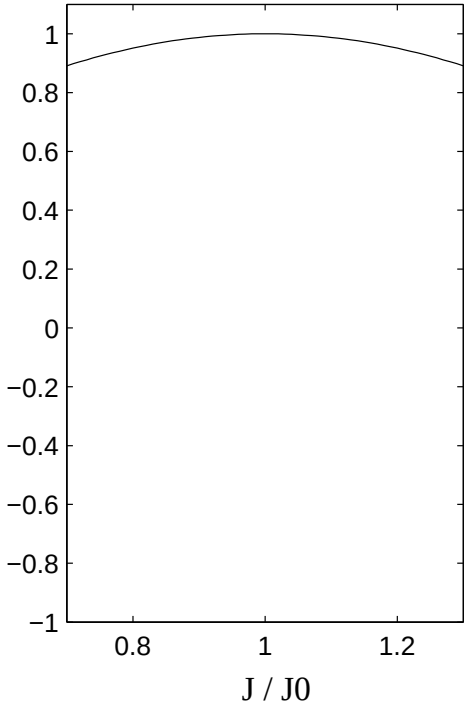
Dependence on the relative size of the J-coupling

$$H_x \xrightarrow{\pi\,{}^1J_{HX}\,2H_zX_z\,t} H_x\cos(\pi\,{}^1J_{HX}\,t) + 2H_yX_z\sin(\pi\,{}^1J_{HX}\,t)$$

$J_0 = 140$ Hz $\longrightarrow$ $t = 3.57$ ms



$y = \cos(\pi\,{}^1J_{HX}\,t)$

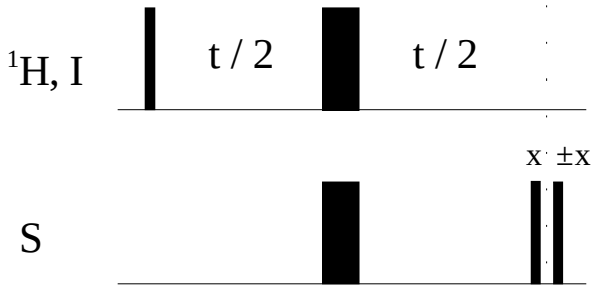


$y = \sin(\pi\,{}^1J_{HX}\,t)$

$\longrightarrow$ Intensity of the H_x-term is more sensitive to variations of the ${}^1J$ couplings

that means: Isotope filtering is more difficult compared to editing

Examples for filtering and editing

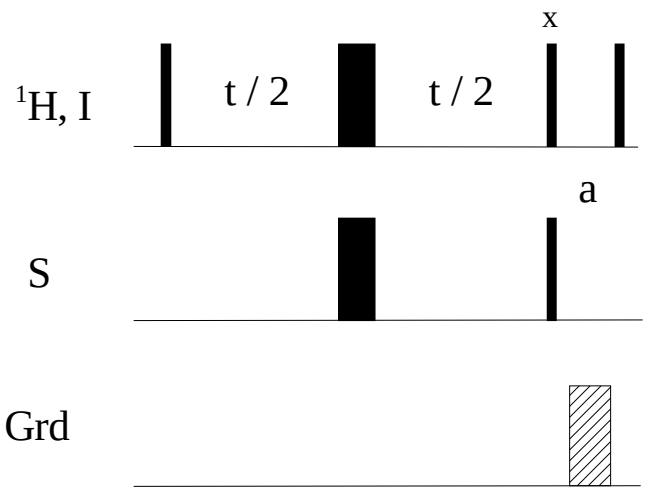


$t = 1 / (2J_{IS})$

$H_z \longrightarrow H_y$

$I_z \longrightarrow I_y \cos(\pi J_{IS} t) \pm 2I_x S_z \sin(\pi J_{IS} t)$

Receiver	+ / +	Filtering
Receiver	+ / -	Editing

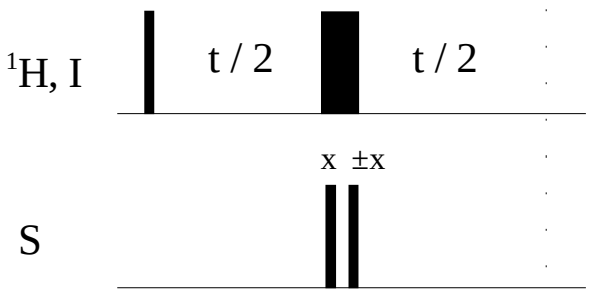


$t = 1 / (2J_{IS})$

$H_z \longrightarrow H_z \longrightarrow -H_y$

$I_z \longrightarrow I_z \cos(\pi J_{IS} t) + 2I_x S_y \sin(\pi J_{IS} t)$

Dephasing by gradients



$$H_z \longrightarrow H_y$$

$$I_z \longrightarrow I_y \cos^2(\pi J_{IS} t/2) \pm (-I_y)\sin^2(\pi J_{IS} t/2)$$

$$t = 1 / (J_{IS})$$

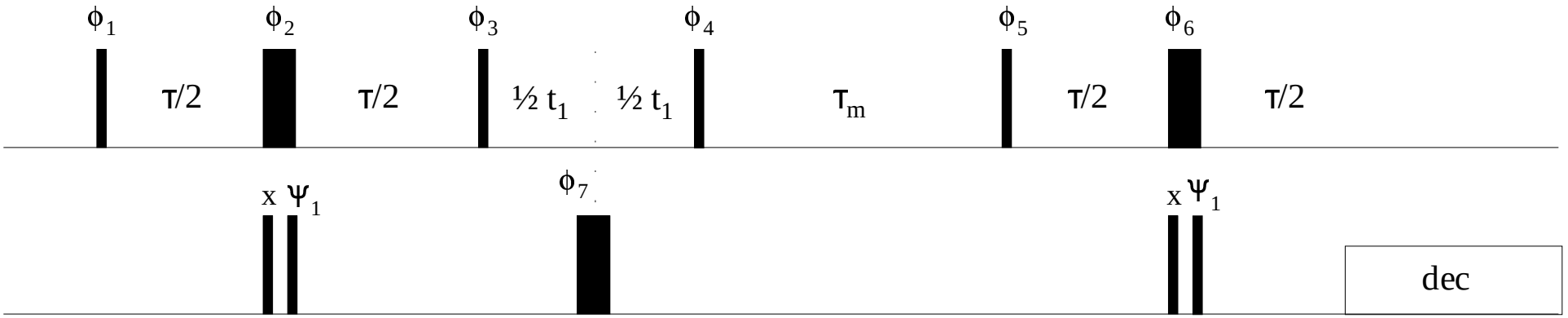
$$H_z \xrightarrow{(90°_x - t/2 - 180°_x - t/2)} H_y$$

$$I_z \xrightarrow{(90°_x - t/2)} - I_y \cos(\pi J_{IS} t/2) + I_x S_z \sin(\pi J_{IS} t/2)$$

$$- I_y \cos(\pi J_{IS} t/2) + I_x S_z \sin(\pi J_{IS} t/2) \xrightarrow{(180°_x (I) + 90°_x (S) + 90°_{\pm x} (S))} I_y \cos(\pi J_{IS} t/2) \pm (- I_x S_z)\sin(\pi J_{IS} t/2)$$

$$I_y \cos(\pi J_{IS} t/2) \pm (- I_x S_z)\sin(\pi J_{IS} t/2) \xrightarrow{(t/2)} I_y \cos^2(\pi J_{IS} t/2) \pm (- I_y)\sin^2(\pi J_{IS} t/2)$$

Inclusion of half filters in a NOESY experiment
(G. Otting, K. Wüthrich (1989) J. Magn. Res. 85, 586-594)



ϕ_1 = x, -x
ϕ_2 = x
ϕ_3 = 2{x}, 2{-x}
ϕ_4 = 4{x}, 4{-x}
ϕ_5 = 8{x}, 8{-x}
ϕ_6 = x
ϕ_7 = x
rec = x, -x, -x, x, -x, x, x, -x
-x, x, x, -x, x, -x, -x, x

Four subspectra

	ψ_1	ψ_2
I	x	x
II	-x	x
III	x	-x
IV	-x	-x

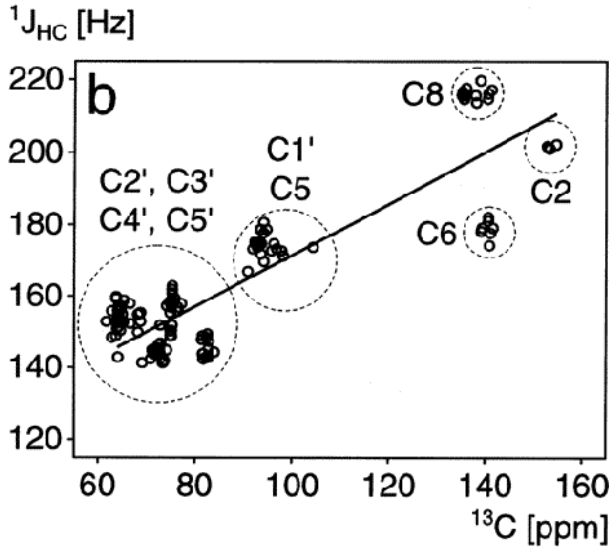
		F1	F2
Subspectrum	I	^{12}C-^{1}H -	^{13}C-$^{1}H$$^{12}C$-$^{1}H$ - ^{13}C-^{1}H
	II	^{12}C-^{1}H +	^{13}C-$^{1}H$$^{12}C$-$^{1}H$ - ^{13}C-^{1}H
	III	^{12}C-^{1}H -	^{13}C-$^{1}H$$^{12}C$-$^{1}H$ + ^{13}C-^{1}H
	IV	^{12}C-^{1}H +	^{13}C-$^{1}H$$^{12}C$-$^{1}H$ + ^{13}C-^{1}H

Linear combinations →

	F1	F2
(I + II) + (III + IV)	^{12}C-^{1}H	^{12}C-^{1}H
(I + II) - (III + IV)	^{12}C-^{1}H	^{13}C-^{1}H
(I - II) + (III - IV)	^{13}C-^{1}H	^{12}C-^{1}H
(I - II) - (III - IV)	^{13}C-^{1}H	^{13}C-^{1}H

$^{1}J(^{1}H, ^{13}C)$ Couplings in nucleic acids and proteins

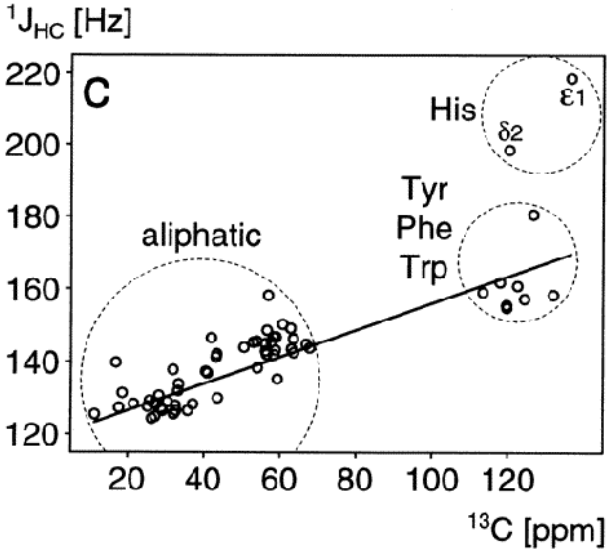
Nucleic acids



linear regression

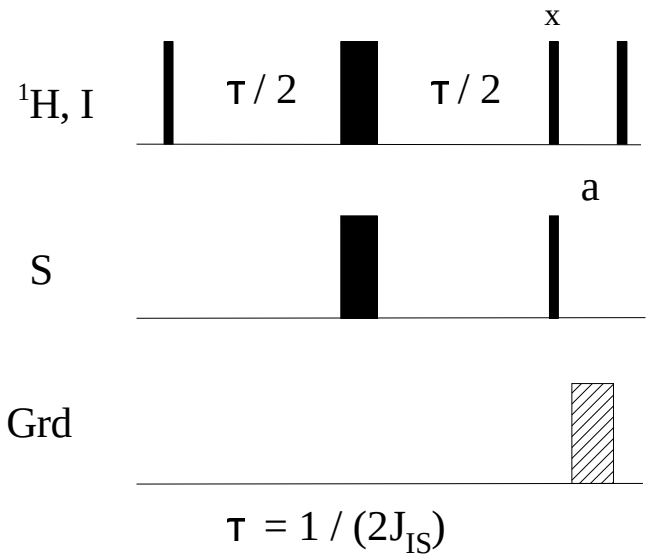
$^{1}J_{CH} = 0.71(\text{Hz/ppm})\ \delta_C + 101\ \text{Hz}$

Proteins

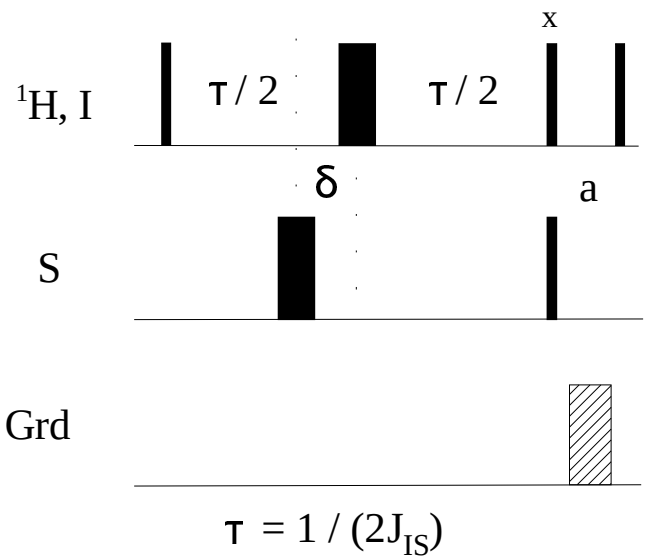


$^{1}J_{CH} = 0.365\ (\text{Hz/ppm})\ \delta_C + 120\ \text{Hz}$

Problem with the variation of the ^1J couplings

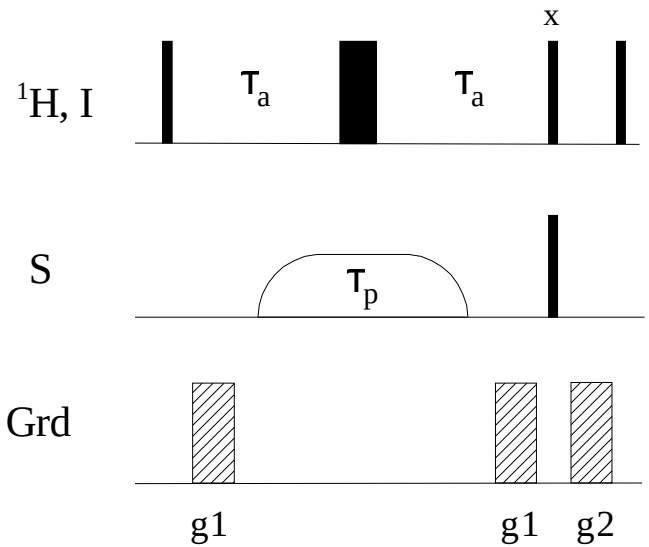


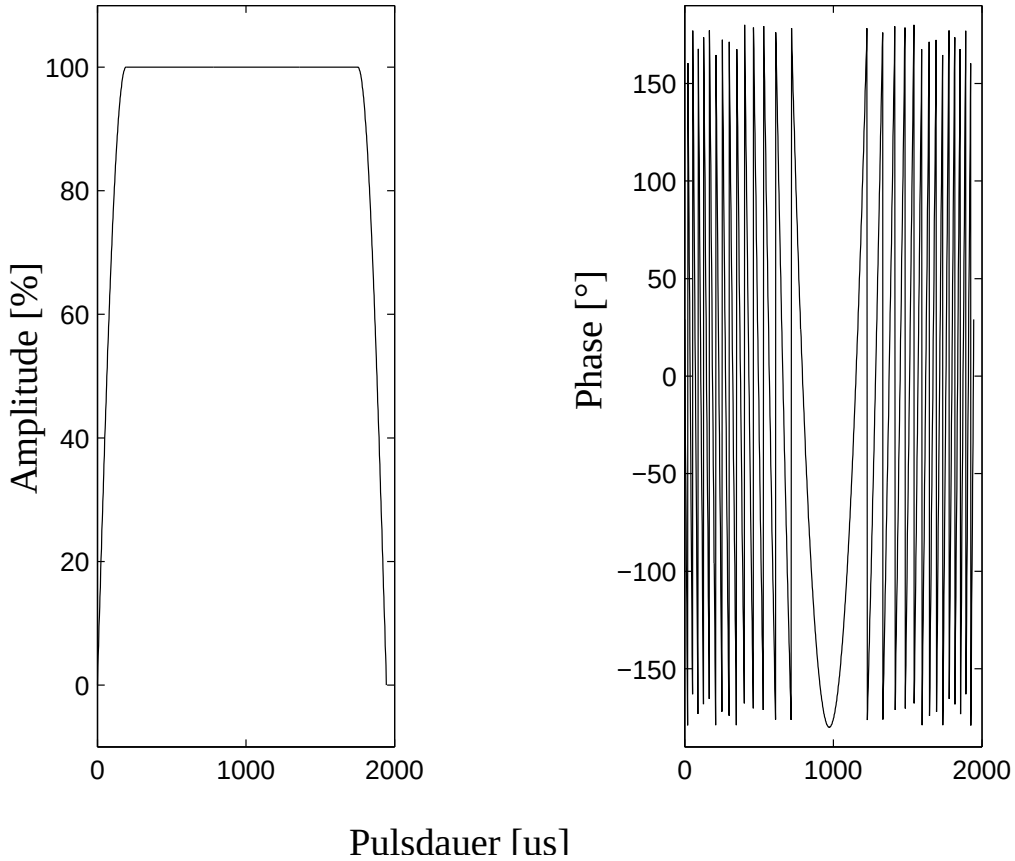
→ no ideal τ can be found



Evolution of the J_{IS}-coupling: τ - 2δ

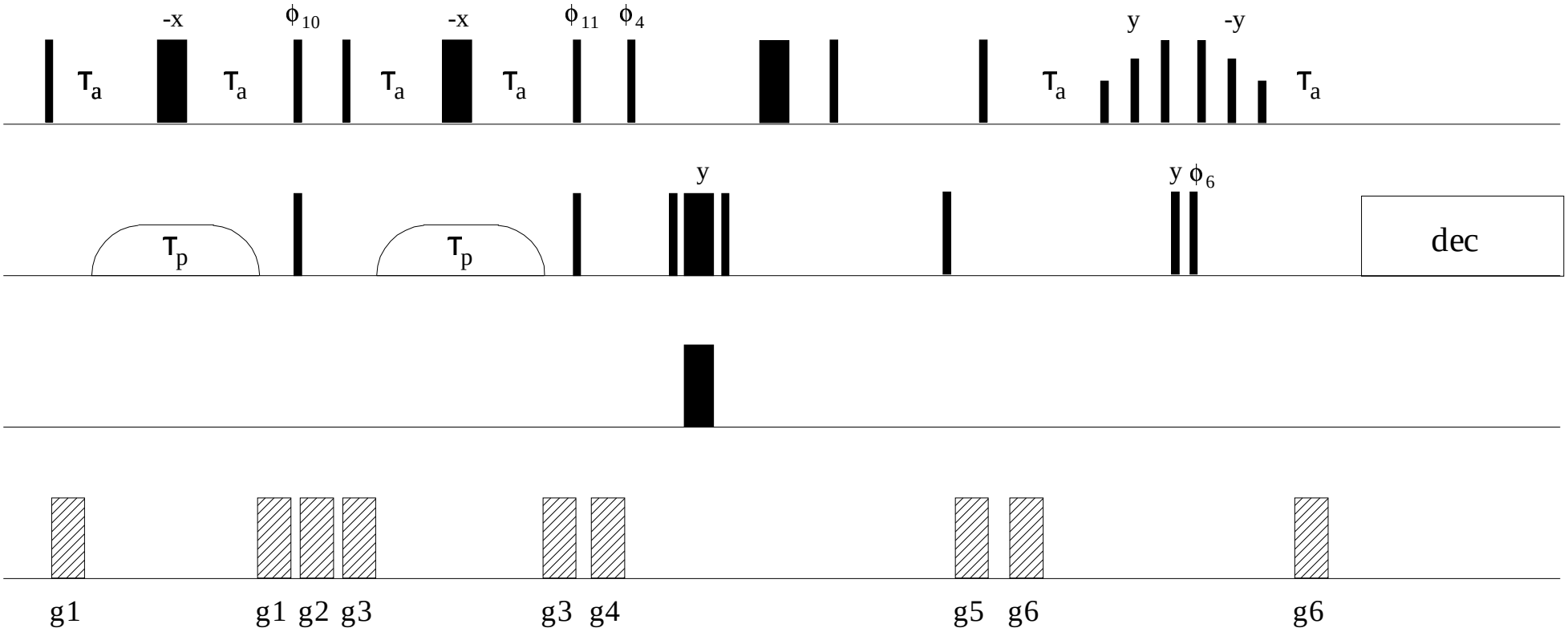
Filterelement with an adiabatic S inversion pulse
(C. Zwahlen et al. (1997), J. Am. Chem. Soc. 119, 6711-6721)





Puls duration 1.948 ms (800 MHz)
smoothed chirp (10 %)
40 kHz sweep (low-field -> high-field)

Pulse sequence for a F1(^{13}C-filtered)-F2(^{13}C-edited) NOESY



ϕ_4 = 2{x}, 2{-x}
ϕ_6 = x, -x
ϕ_{10} = 4{x}, 4{-x}
ϕ_{11} = 8{x}, 8{-x}
rec = x, -x, -x, x, -x, x, x-, -x
-x, x, -x, -x, x, -x, -x, x

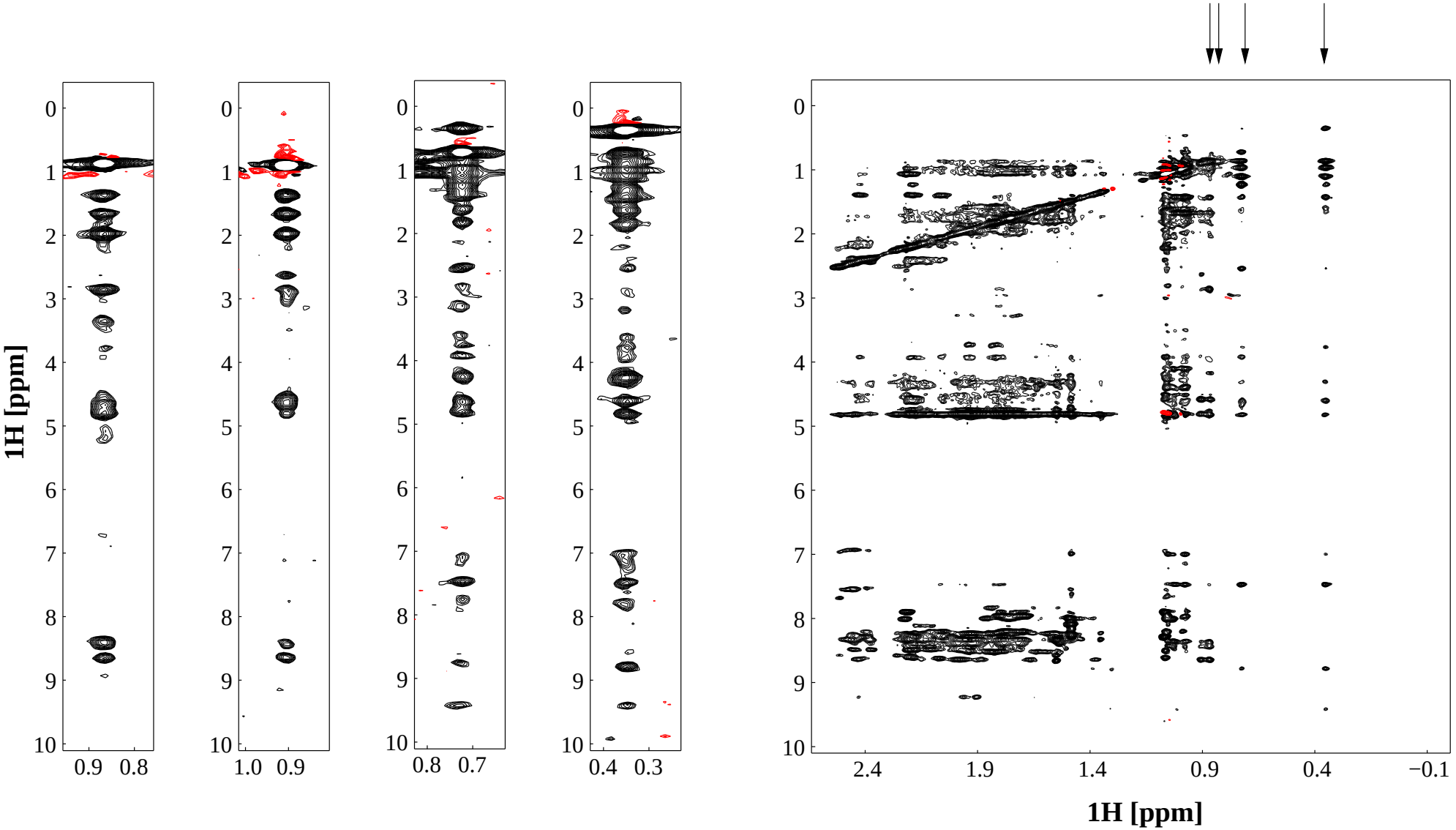
g1 = 13 %	(500 ms)
g2 = 40 %	(1 ms)
g3 = 0.6 %	(500 ms)
g4 = 0.1 %	(1 ms)
g5 = 13 %	(1 ms)
g6 = 50 %	(1 ms)

F1(^{13}C-filtered)-F2(^{13}C-edited) NOESY of a protein-protein complex

^{13}C,^{15}N l N(1-53) in complex with NusA(339-495) (1:2) (l N ca. 1 mM, 800 MHz, 66 h)

Ausschnitte aus dem 13C−editierten NOESY

F1(13C−filtered) − F2 (13C−edited) NOESY



1D slices from the F1(^{13}C-filtered)-F2(^{13}C-edited) NOESY compared with the ^{13}C-NOESYHSQC