



BASICS OF IN VIVO X-NUCLEAR MRS

Lijing Xin

CIBM EPFL MRI

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C I B M . C H

Lijing.xin@epfl.ch

PROPERTIES OF OTHER NUCLEI BEYOND ^1H

Isotope	Spin I^a	Natural abundance	γ ($10^6 \text{ rad} \cdot \text{s}^{-1} \text{T}^{-1}$)
^1H	1/2	0.99985	267.522
^2H	1	0.00015	41.066
^{12}C	0	0.989	—
^{13}C	1/2	0.01108	67.283
^{14}N	1	0.9963	19.338
^{15}N	1/2	0.0037	-27.126
^{16}O	0	0.99963	—
^{17}O	5/2	0.00037	-36.279
^{23}Na	3/2	1	70.801
^{31}P	1/2	1	108.394

Low
natural abundance

- Metabolite levels
- Physiological parameters
- Kinetics in metabolic pathways
- Endogenous tracer (^{31}P MRS)
- Exogenous tracers (nuclei with low natural abundance)



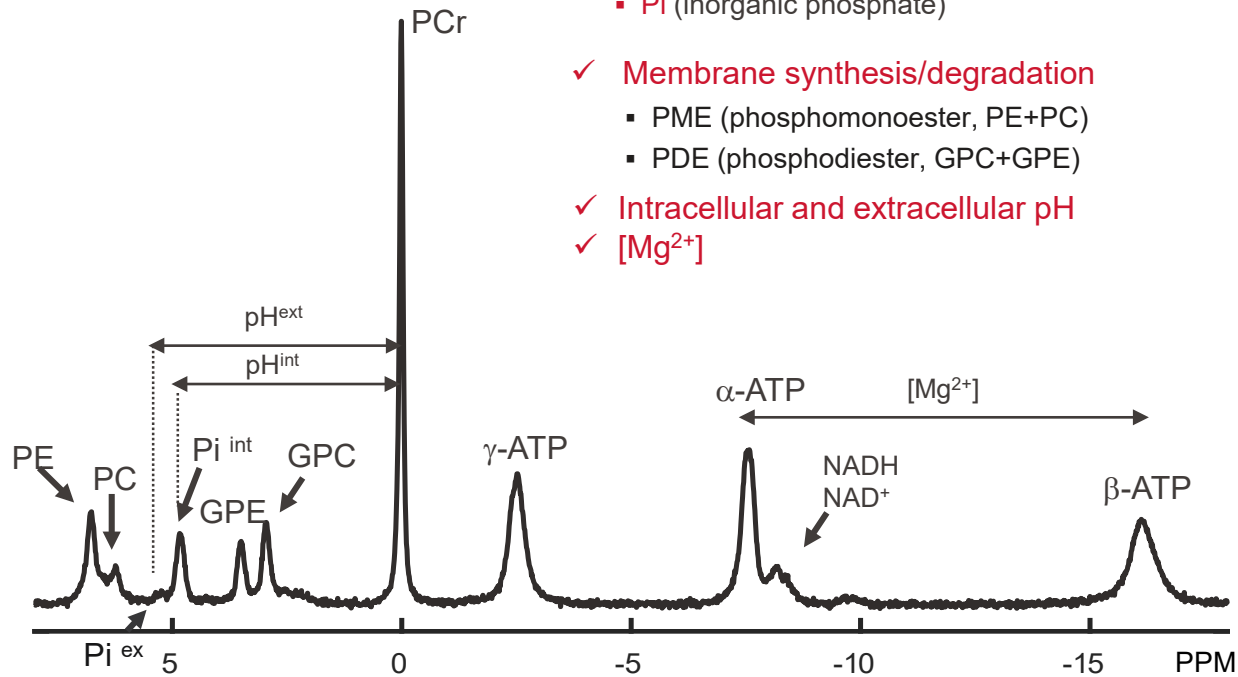
Spin $I > \frac{1}{2}$ (^{23}Na , ^{17}O , ^2H ...) asymmetric charge distribution (elliptical) → a **quadrupole** moment.

The interaction between the nucleus and the asymmetric electric field gradients becomes orientation-dependent and leads to enhanced relaxation → **short relaxation times**

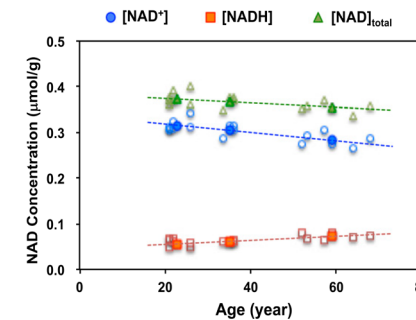
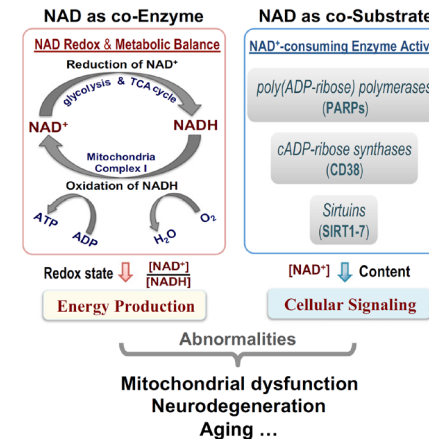
^{31}P MRS

- ✓ Energy metabolites
 - ATP (adenosine triphosphate)
 - PCr (phosphocreatine)
 - Pi (inorganic phosphate)

- ✓ Membrane synthesis/degradation
 - PME (phosphomonoester, PE+PC)
 - PDE (phosphodiester, GPC+GPE)
- ✓ Intracellular and extracellular pH
- ✓ $[\text{Mg}^{2+}]$

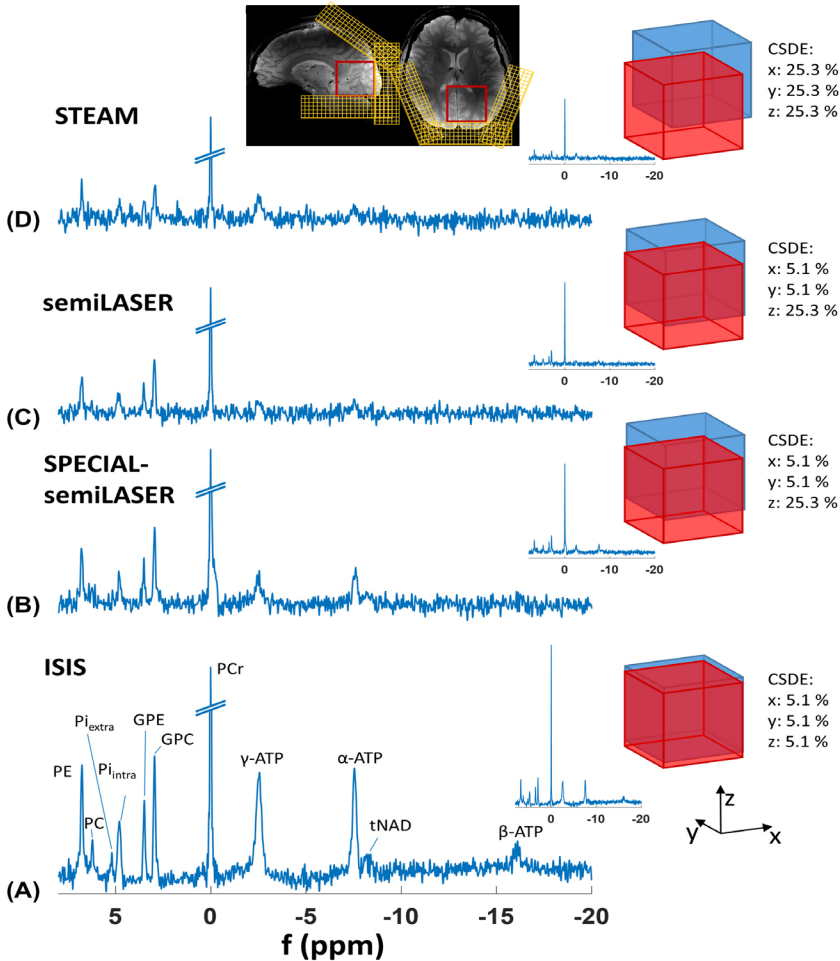


- ✓ Nicotinamide adenine dinucleotide ($\text{NAD}^{+}/\text{NADH}$)



Zhu et al., PNAS, (2015)

LOCALIZATION FOR ³¹P MRS



Comparison of four ³¹P single-voxel MRS sequences in the human brain at 9.4 T

	Meta.	References
T_1 [s]	PCr	3.37-3.39 (6; 7)
	Pi	3.19-3.70 (6; 7)
	γ ATP	1.27-1.70 (6; 7)
	α ATP	1.26-1.35 (6; 7)
	β ATP	1.02-1.13 (6; 7)
T_2 [ms]	PCr	132.0 $\pm$ 12.8 (6)
	Pi	86 $\pm$ 2 (8)
	γ ATP	26.1 $\pm$ 9.6 (6)
	α ATP	25.8 $\pm$ 6.6 (6)
	β ATP	-

T_2 of ¹H metabolites:
60-200ms

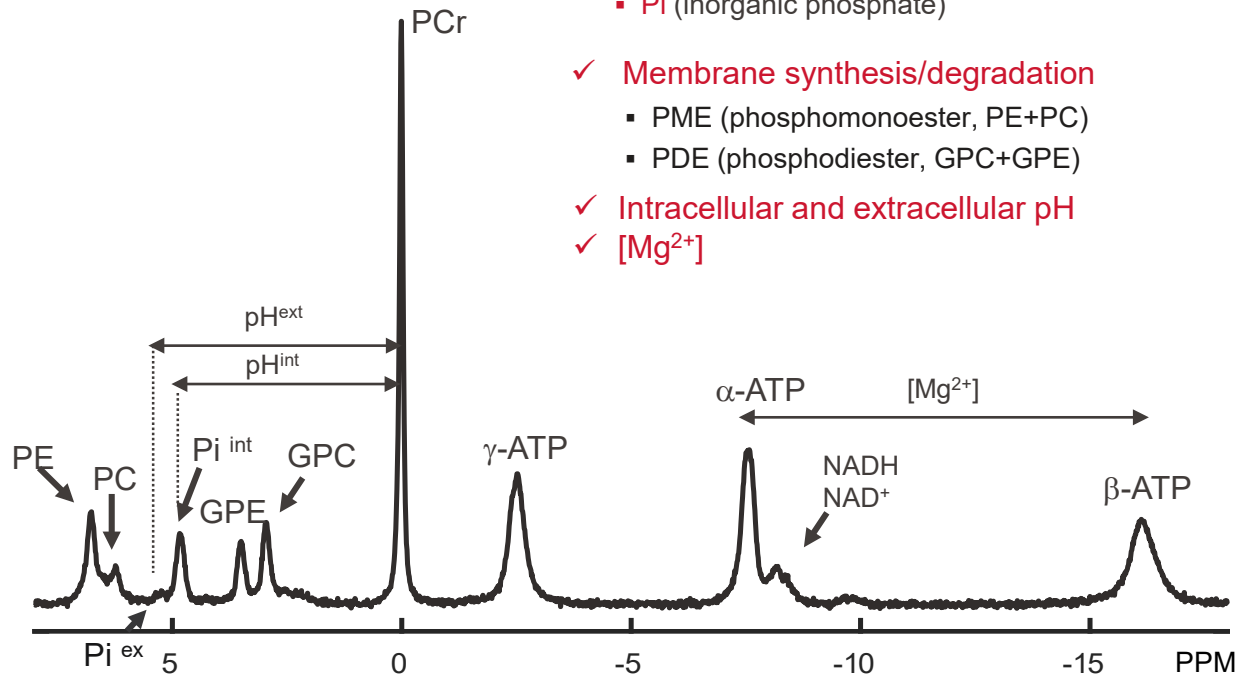
Relaxation times of ³¹P metabolites at 7T

^{31}P MRS

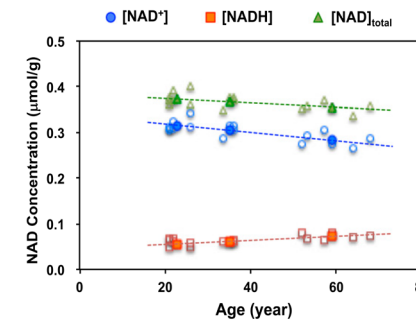
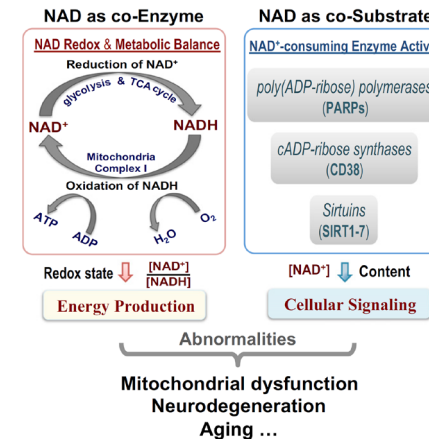
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- ✓ Intracellular and extracellular pH
- ✓ $[\text{Mg}^{2+}]$

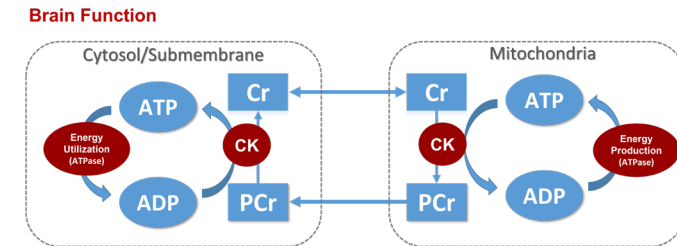


- ✓ Nicotinamide adenine dinucleotide (NAD⁺/NADH)

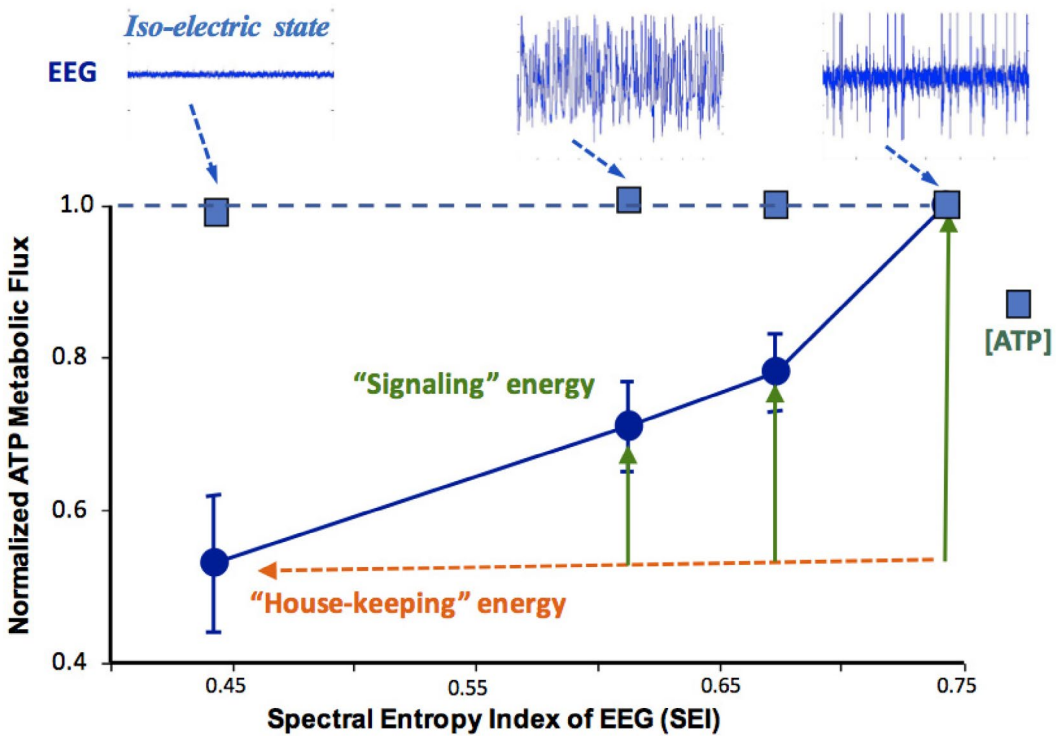


Zhu et al., PNAS, (2015)

- ✓ ATP metabolism



WHY MEASURE ATP METABOLISM



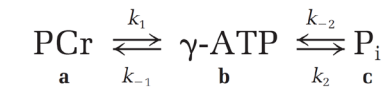
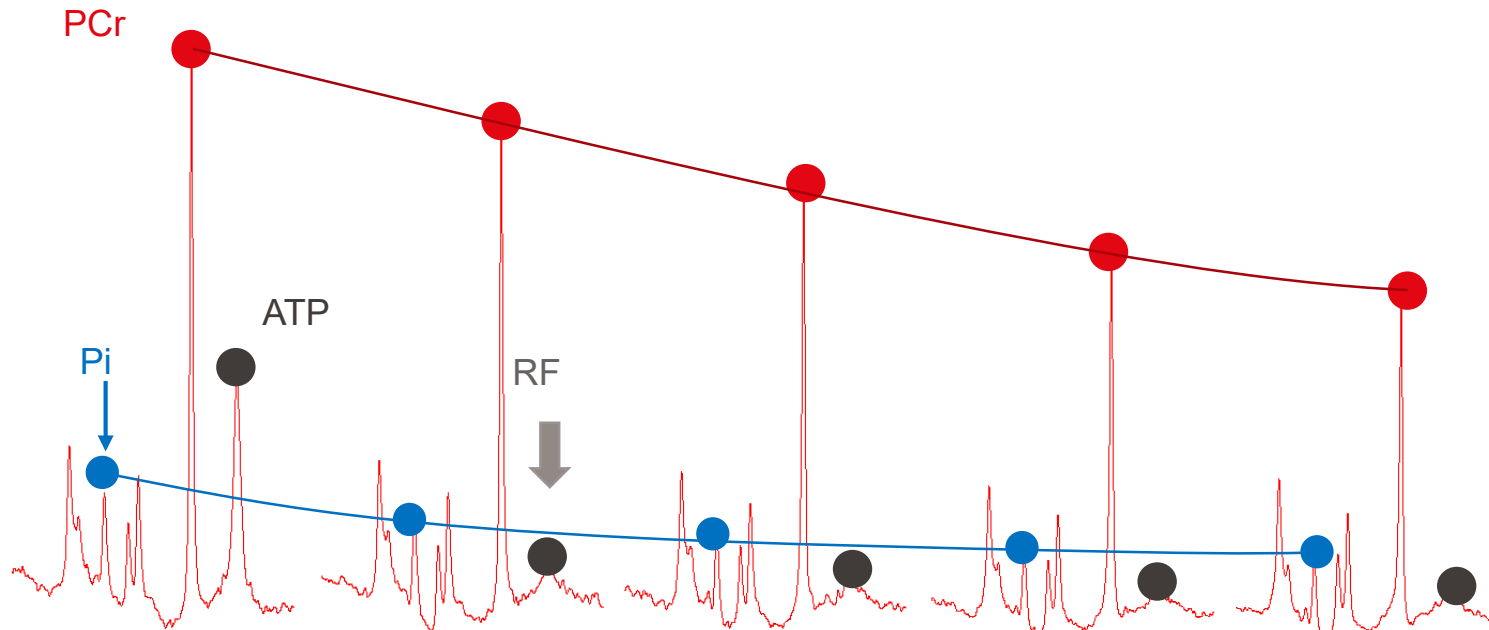
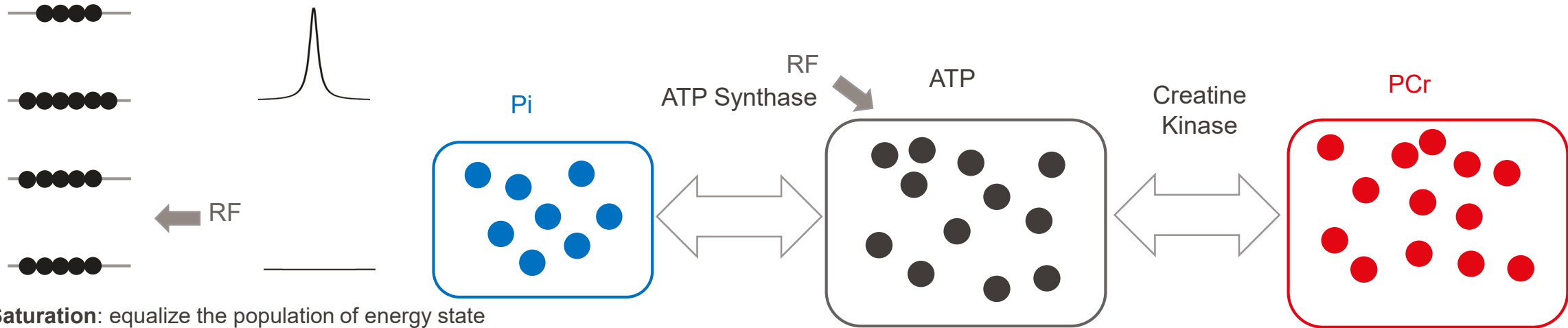
Zhu et al., Frontiers in Aging Neuroscience (2018)

Table 2. Results of ³¹P Magnetization Transfer Spectroscopy Measurements in the Human Frontal Lobe of Patients With Schizophrenia (SZ) and Healthy Control (HC) Subjects

Variable	Mean (SD)		P Value
	SZ (n = 26)	HC (n = 26)	
Magnetization ratio			
Phosphocreatine to β-ATP	1.35 (0.24)	1.36 (0.17)	.71
Inorganic phosphate to β-ATP	0.44 (0.09)	0.42 (0.07)	.47
Phosphodiester to β-ATP	0.91 (0.19)	1.05 (0.19)	.02
Phosphomonoester to β-ATP	1.05 (0.14)	1.09 (0.16)	.54
Intracellular pH	7.00 (0.02)	7.03 (0.01)	.007 ^a
M_z/M_0 ratio of phosphocreatine ^b	0.50 (0.12)	0.43 (0.07)	.01
Rate constant k_f of creatine kinase, s ⁻¹	0.21 (0.07)	0.27 (0.06)	.003
Intrinsic longitudinal T_1 relaxation time of phosphocreatine, s	5.21 (1.24)	5.03 (1.09)	.67
Chemical exchange flux of creatine kinase, μmol/g/min	49.93 (21.95)	61.43 (16.08)	.03
Magnesium ion concentration, mmol/L	0.149 (0.026)	0.145 (0.027)	.59

Du et al., JAMA Psychiatry (2014)

Saturation transfer



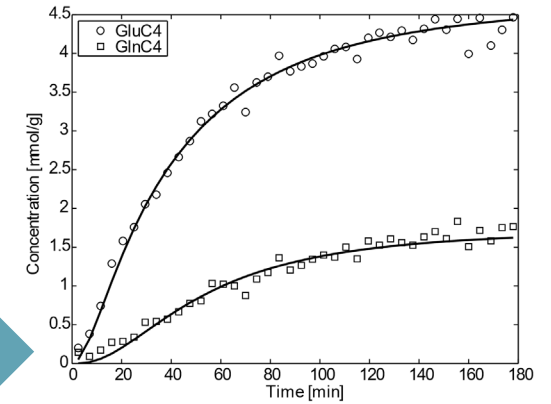
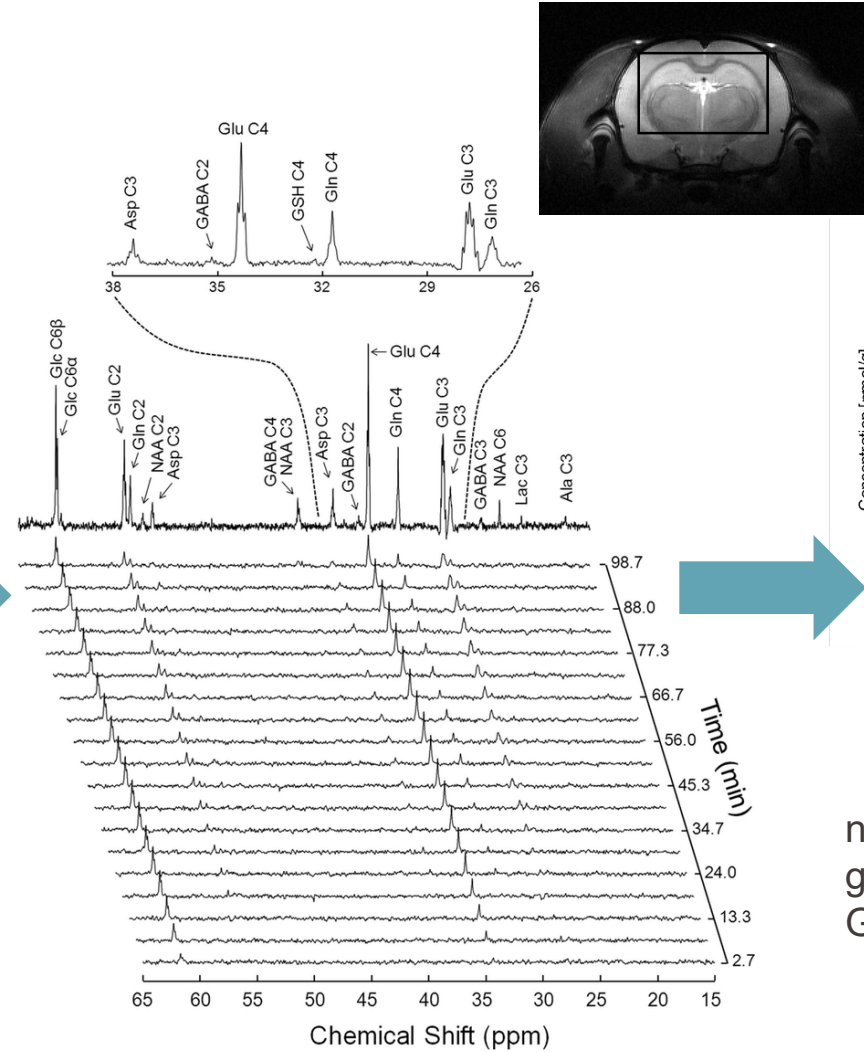
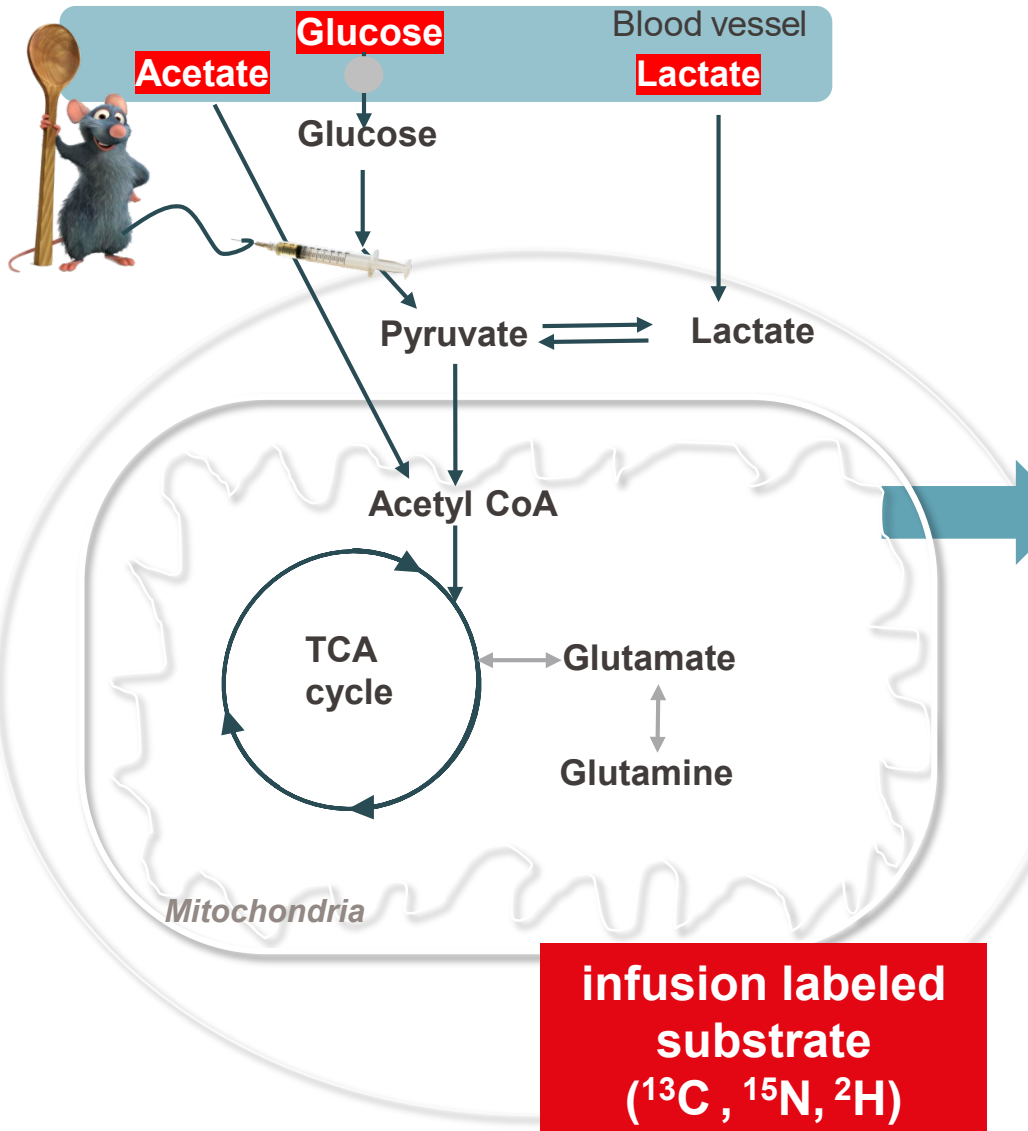
$$\frac{dM_a}{dt} = -\frac{(M_a - M_a^0)}{T_{1a}} - k_1 M_a + k_{-1} M_b \quad [1a]$$

$$\frac{dM_b}{dt} = -\frac{(M_b - M_b^0)}{T_{1b}} - k_{-1} M_b - k_2 M_b + k_1 M_a + k_{-2} M_c \quad [1b]$$

$$\frac{dM_c}{dt} = -\frac{(M_c - M_c^0)}{T_{1c}} - k_{-2} M_c + k_2 M_b \quad [1c]$$

Du et al., MRM, (2007).

DYNAMIC X-NUCLEI MRS: METABOLIC FLUX

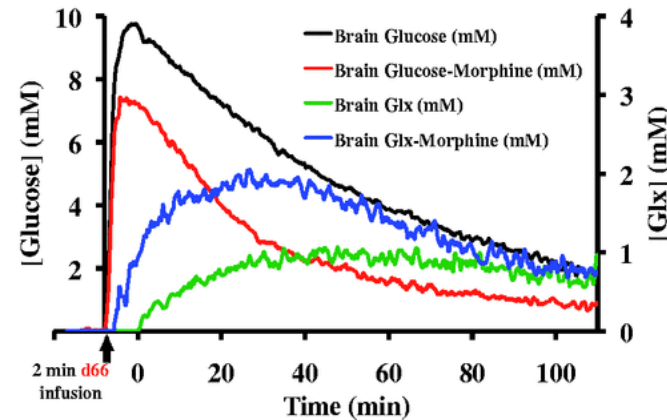
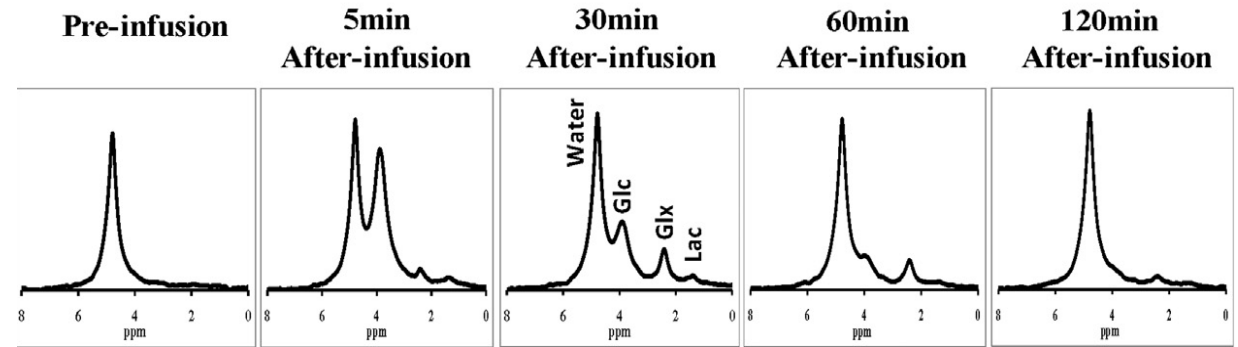
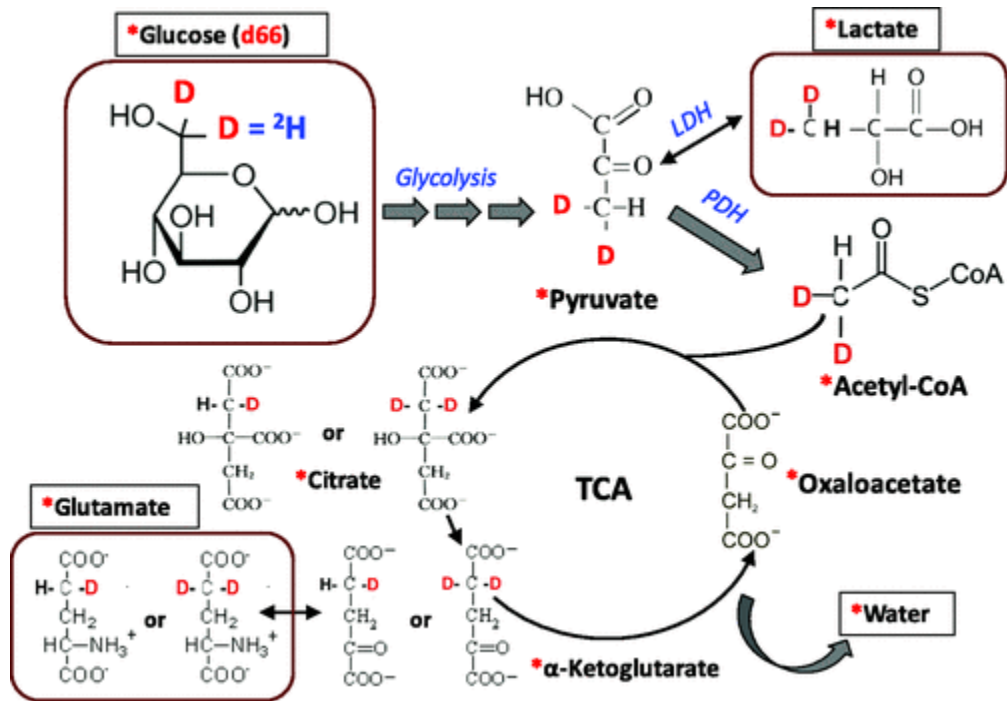


$$\frac{d[P^*]}{dt} = \frac{[S^*]}{[S]} V_1 - \frac{[P^*]}{[P]} V_2$$

neuron TCA flux ($V_{\text{TCA}n}$)
glial TCA flux ($V_{\text{TCA}g}$)
Glu-Gln cycling flux (V_{NT})

mathematic modelling
(metabolic fluxes)

^2H MRS – DYNAMIC METABOLIC STUDY

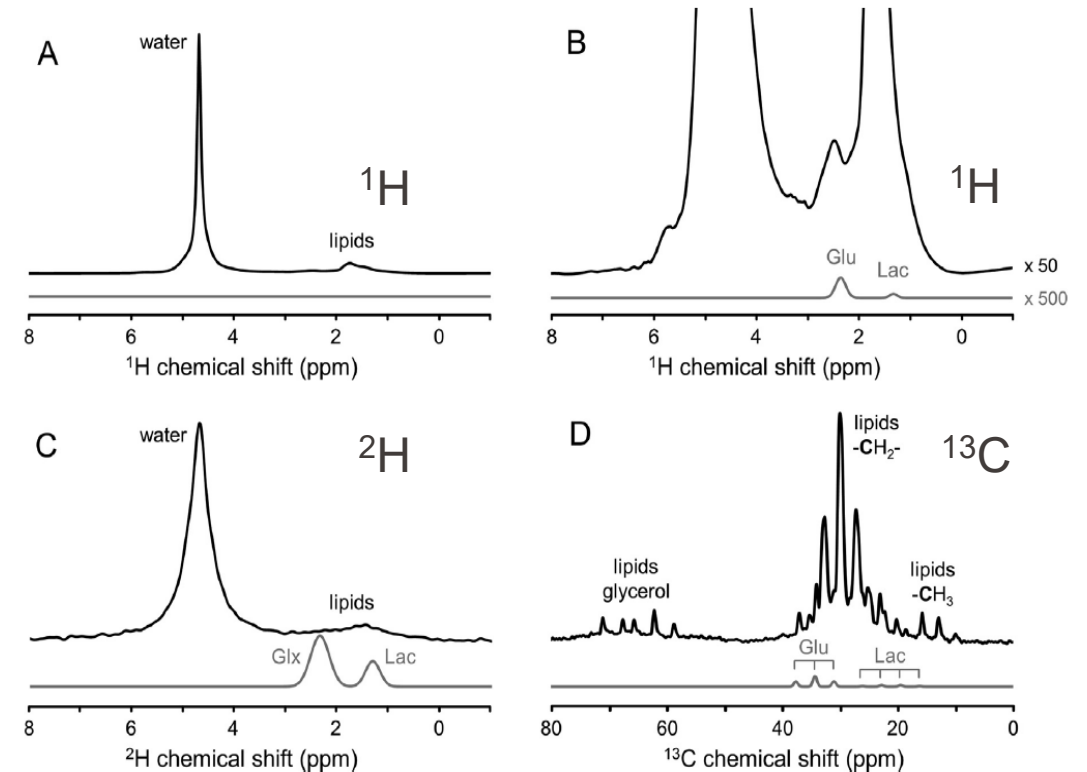
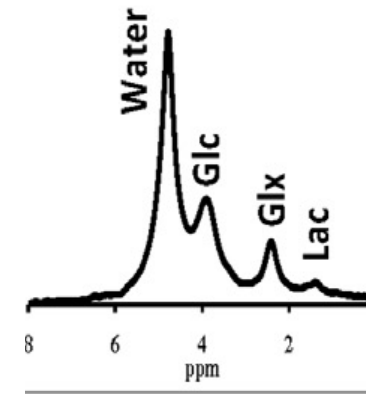


CMR
 V_{TCA}
glc

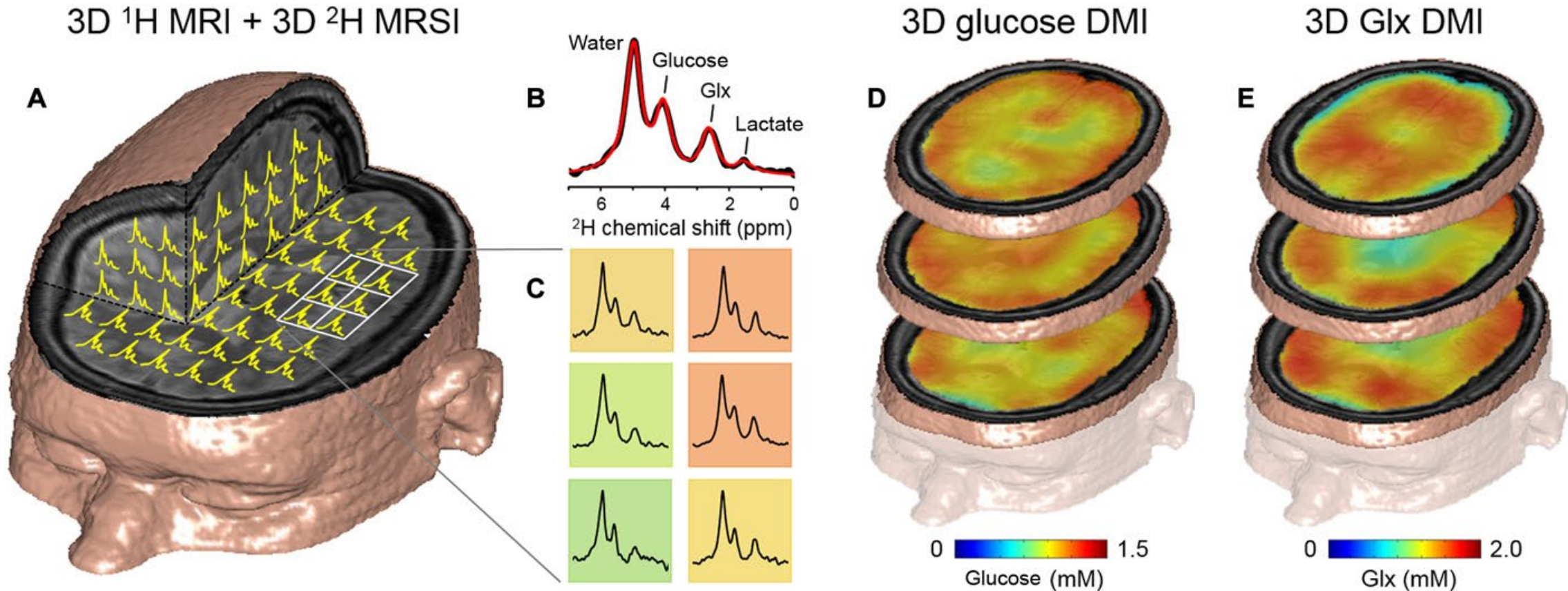
Lu et al., JCBFM (2017)

^2H MRS – SIMPLE AND ROBUST

- Short T_1 (100-400ms): fast averaging, sensitivity gain
- Short T_2 (30-60ms): broad resonances
 - Gln+Glu, problem for Glu-Gln cycling flux
 - Insensitive to B_0 inhomogeneity
- Low natural abundance: no water and fat suppression required



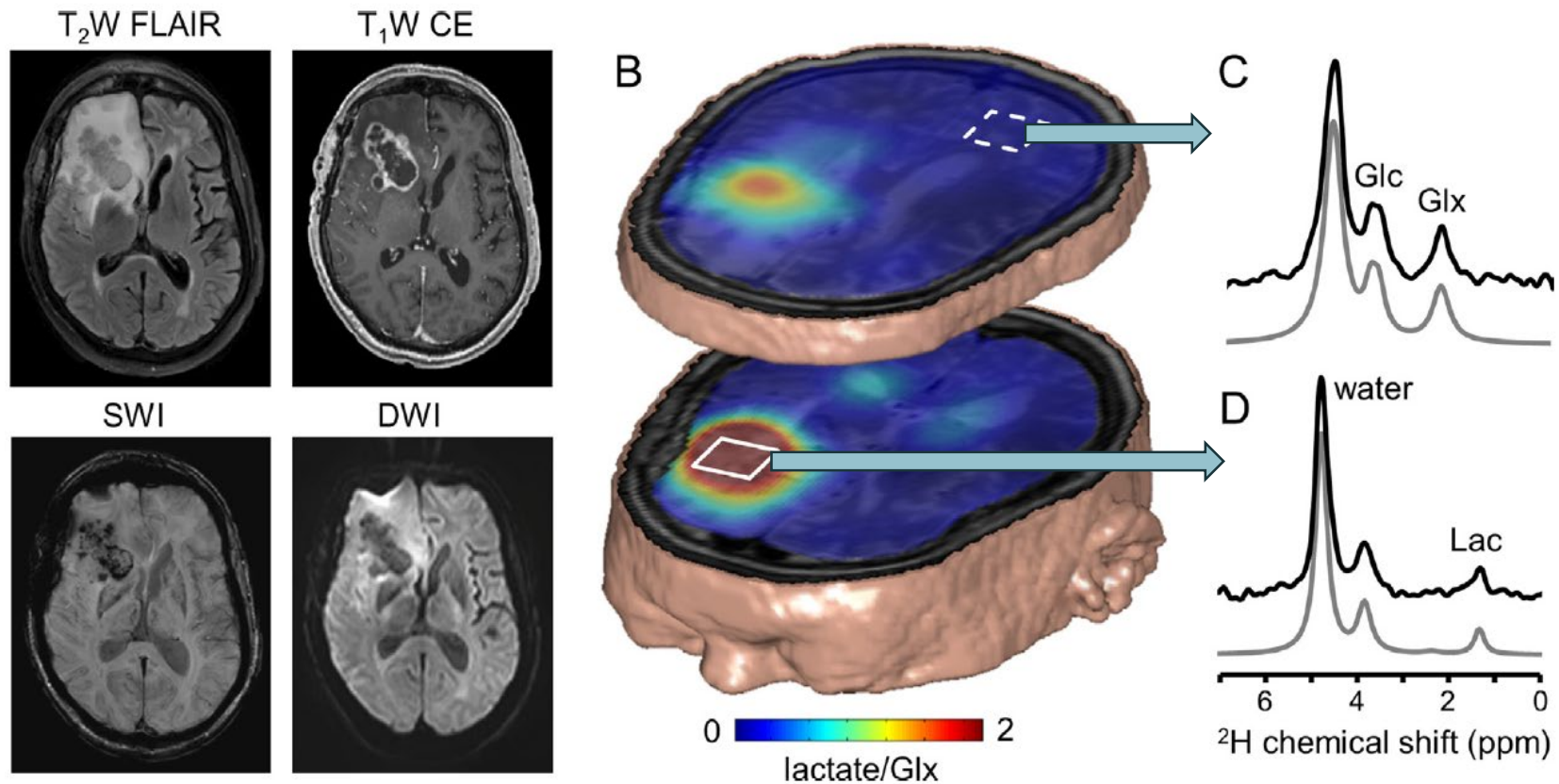
3D ^2H MRSI



3D FID-MRSI data set: $20 \times 20 \times 20 \text{ mm}^3$ nominal spatial resolution, $\text{TR} = 333\text{ms}$, 29min, acquired between 65 and 90 min after oral $[6,6'\text{-}^2\text{H}_2]\text{glucose}$ administration.

De Feyter et al, Sci. Adv. (2018).

2H MRS APPLICATION IN BRAIN TUMOR



De Feyter et al, Sci. Adv. (2018).

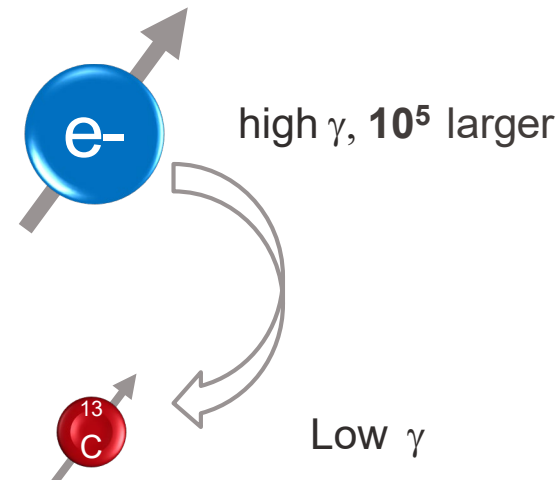
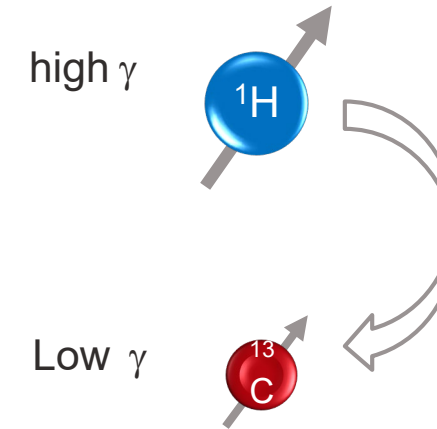
SENSITIVITY ENHANCEMENT

- Decoupling
- NOE: Nuclear Overhauser Enhancement (x 3)
- Polarization transfer (x 4)
- Heteronuclear editing (Indirect detection ^1H - ^{13}C , x 16)

$$M_0 = N_s \frac{\gamma^2 \hbar^2 B_0}{4kT} k$$

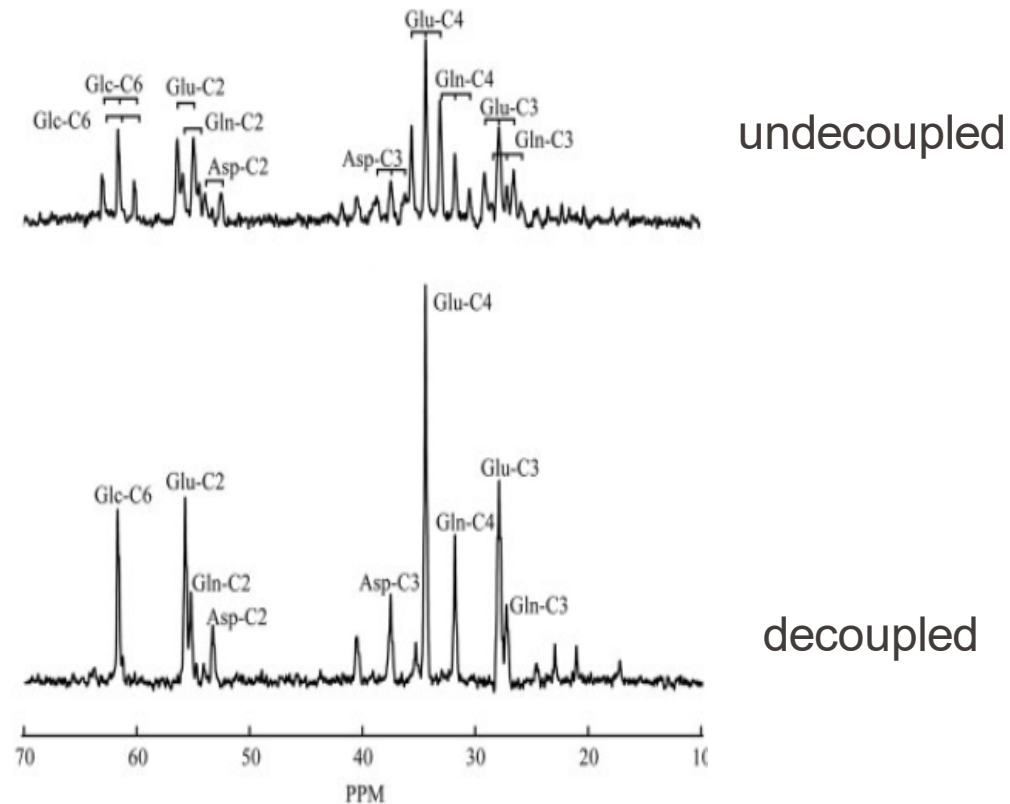
- Hyperpolarization (DNP, x 10000)

$$\gamma(^1\text{H}) \sim 4 \gamma(^{13}\text{C})$$

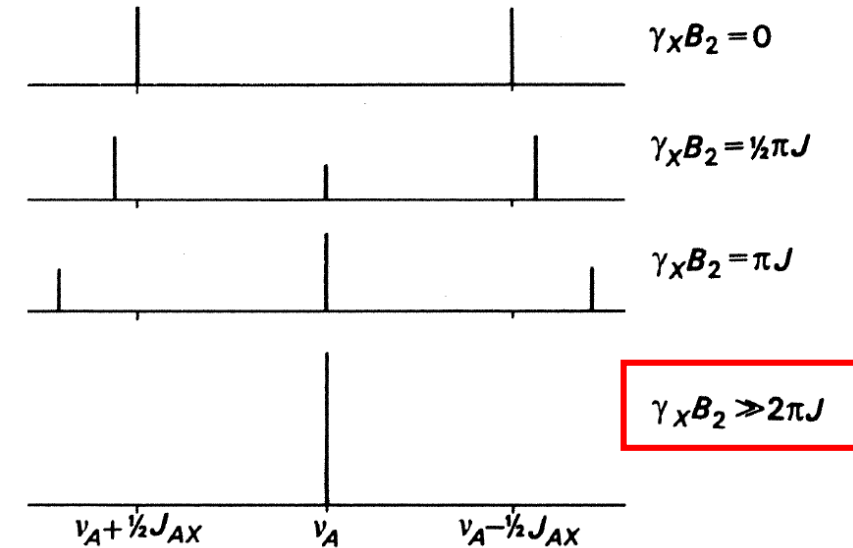
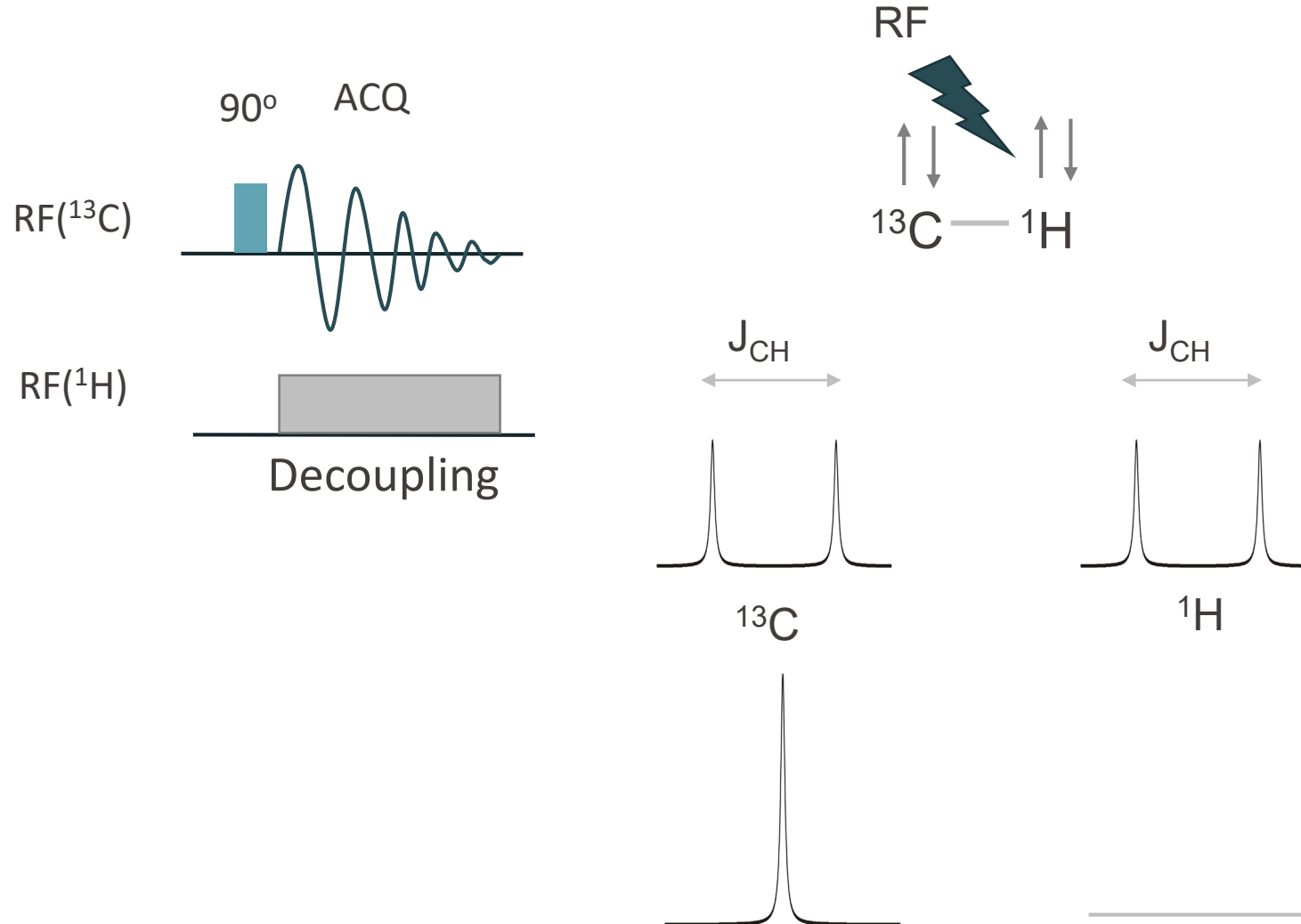


DECOUPLING

- Why decoupling? simplify spectra and enhance SNR

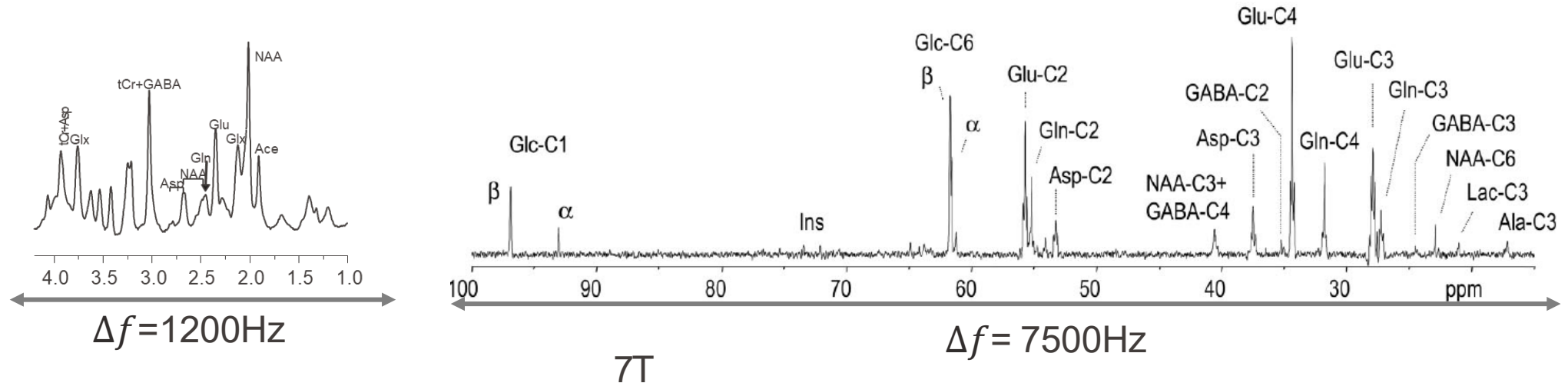
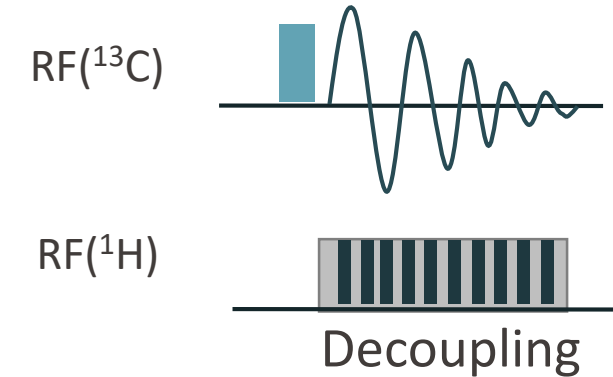


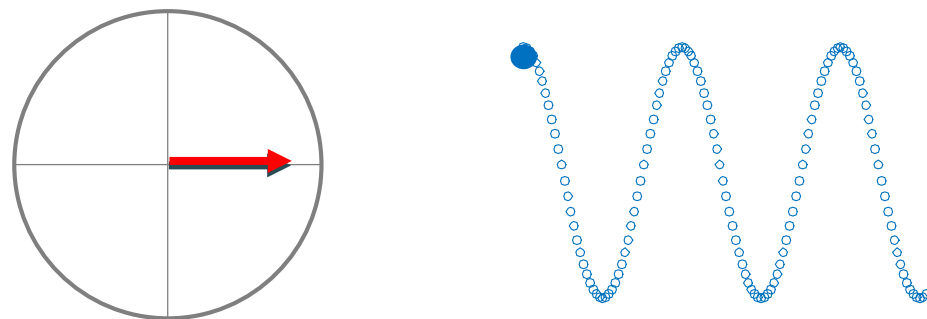
HOW TO DO DECOUPLING?



DECOUPLING SCHEMES

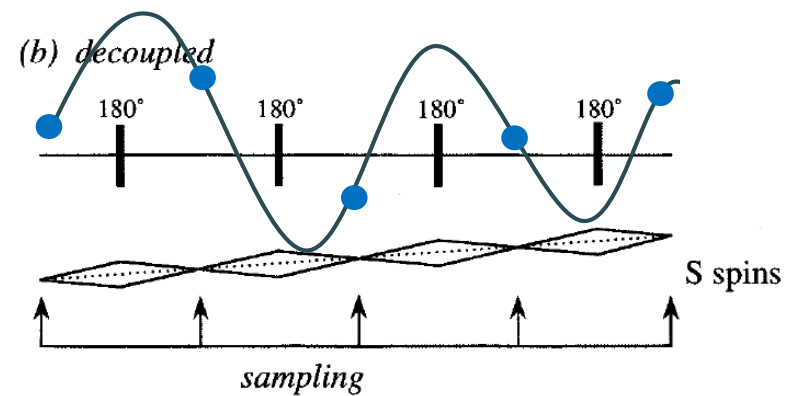
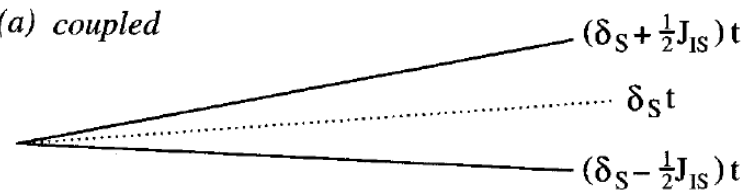
- Continuous wave (narrow band, on resonance decoupling)
- Increase decoupling bandwidth
 - 'spin-flip' decoupling



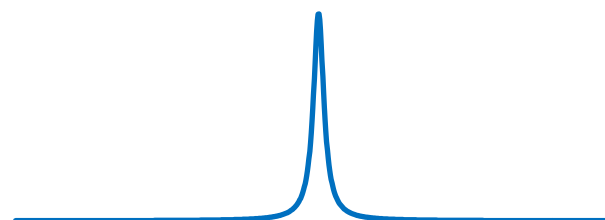
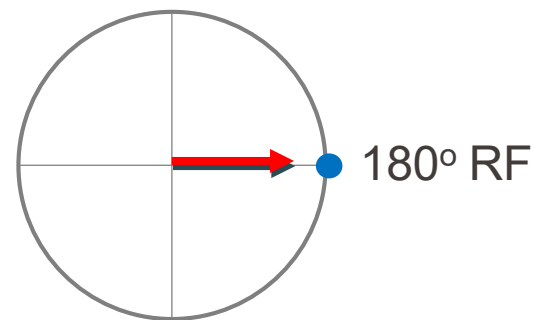


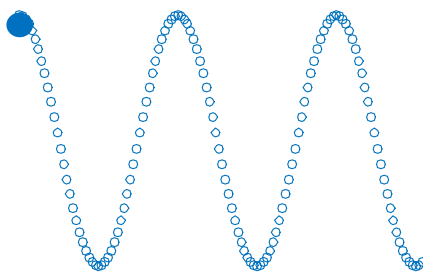
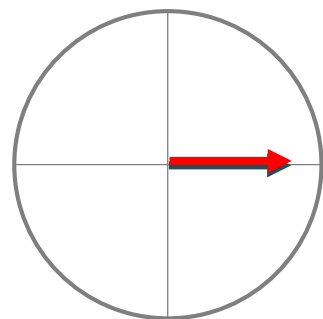
FT

(a) coupled



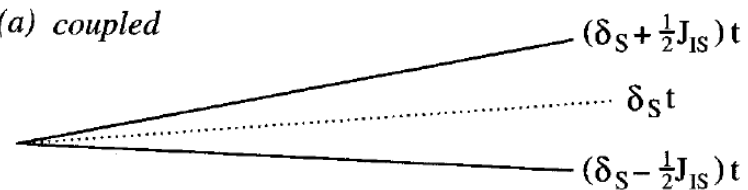
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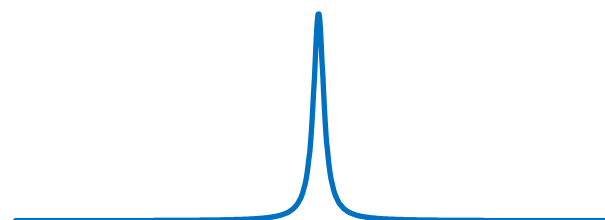
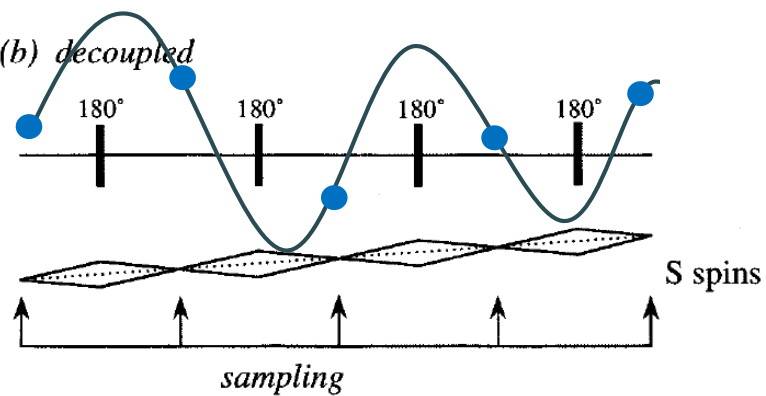


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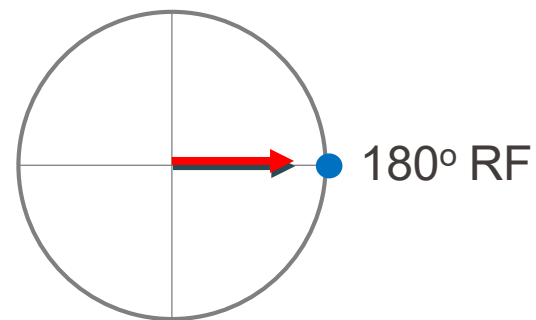
(a) *coupled*

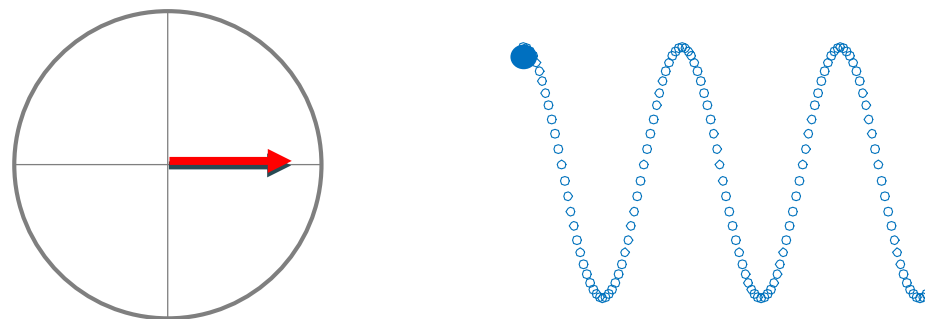


(b) *decoupled*



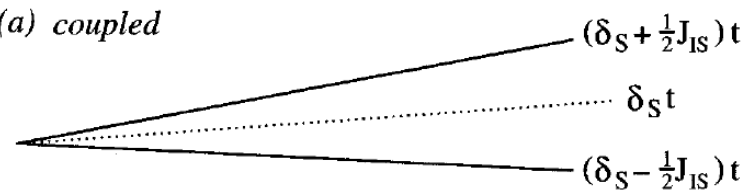
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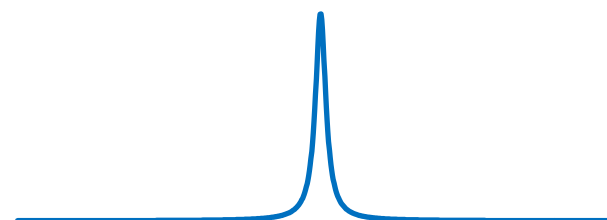
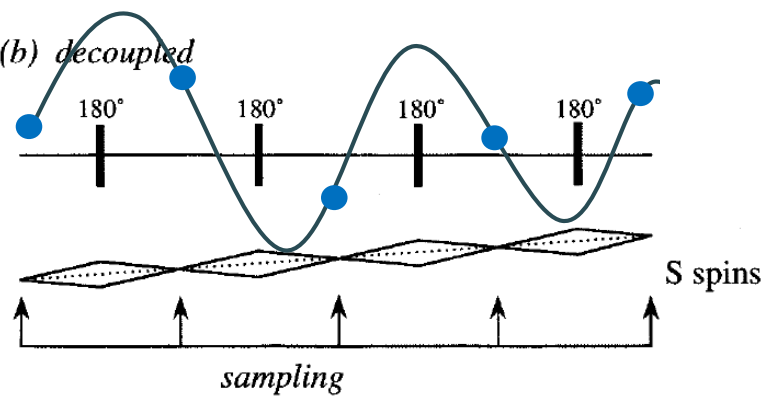


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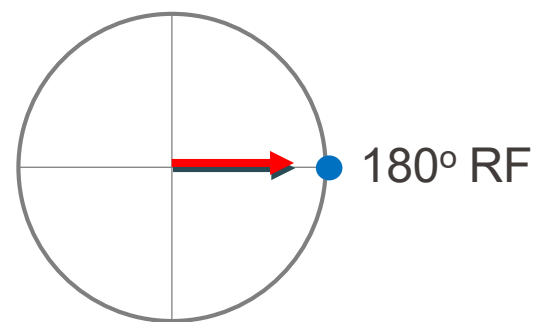
(a) coupled



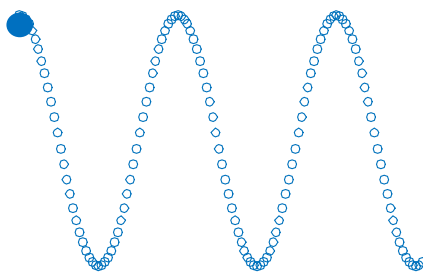
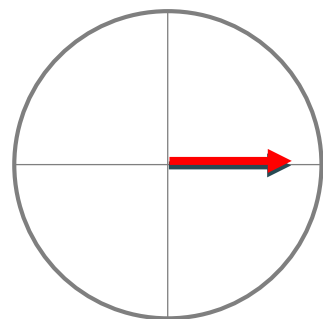
(b) decoupled



FT

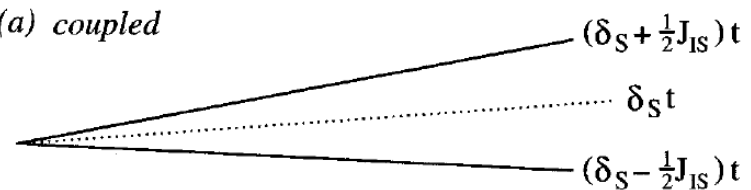


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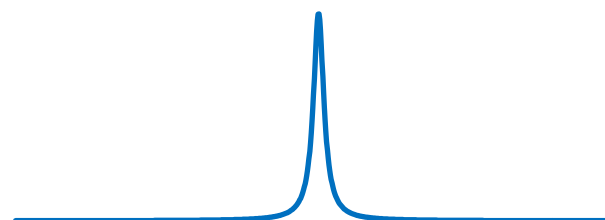
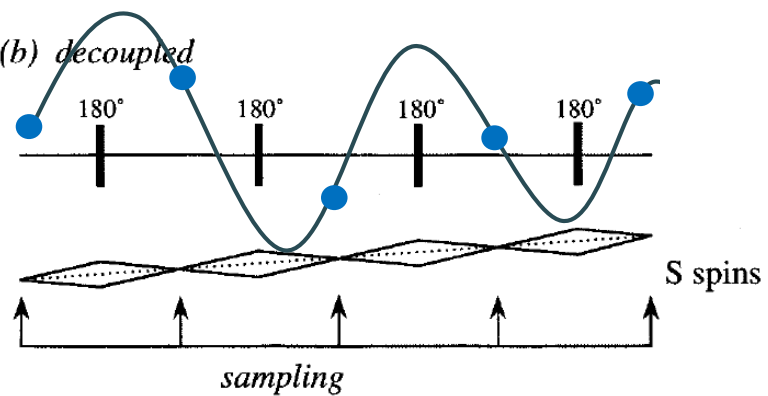


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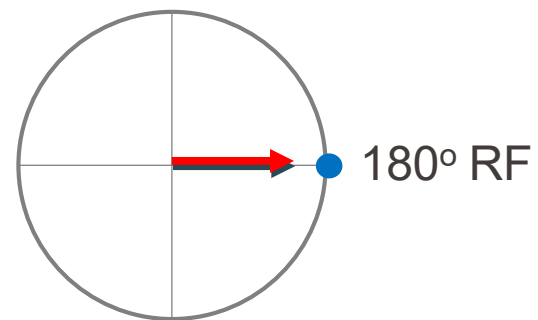
(a) *coupled*



(b) *decoupled*



FT



DECOUPLING SCHEMES

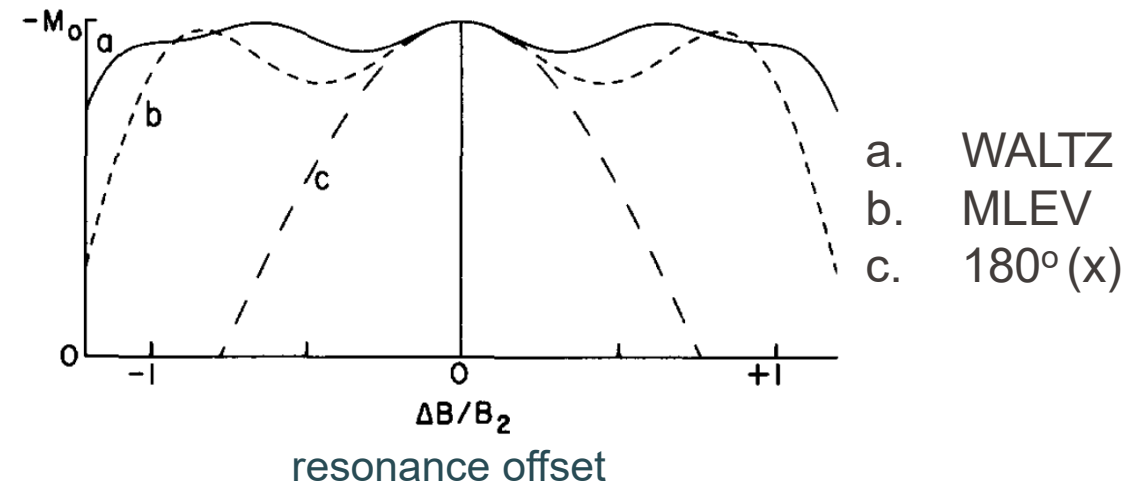
■ Composite pulses

- MLEV (Modulated Low-power Evolution): $90^\circ(x) 180^\circ(y) 90^\circ(x)$
- WALTZ: $90^\circ(x) 180^\circ(-x) 270^\circ(x) = 1\bar{2}3$

■ Supercycle

- WALTZ16:

$\overline{342312423} \quad \overline{342312423} \quad \overline{342312423} \quad \overline{342312423}$

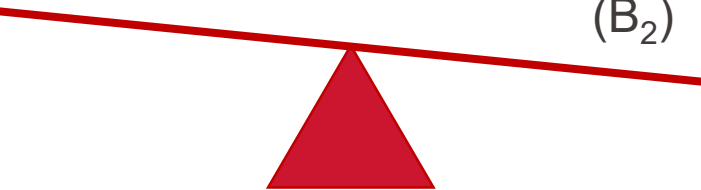


DECOUPLING: BW VS SPECIFIC ABSORPTION RATE

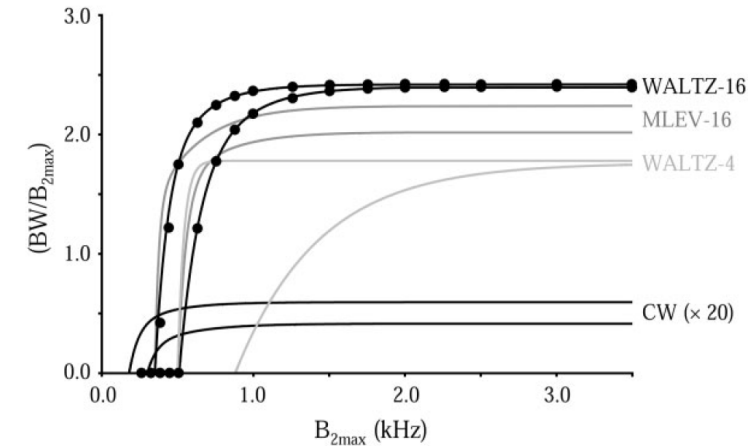
- Efficient decoupling: the desired decoupling bandwidth with the lowest possible RF power

Decoupling
performance
(BW)

SAR
deposition
(B_2)

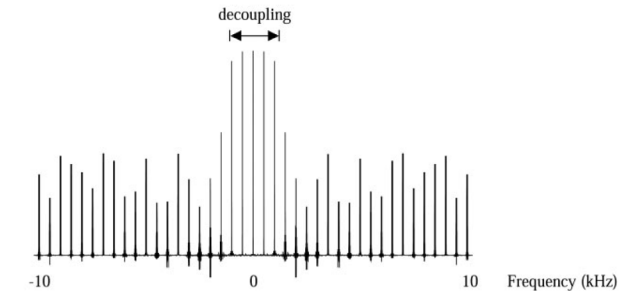


IEC (International Electrotechnical Commission): 3.2 W/kg (head) or 10 W/kg (body, over any 10g)



WALTZ-16
 $B_{2max} = 0.90$ kHz

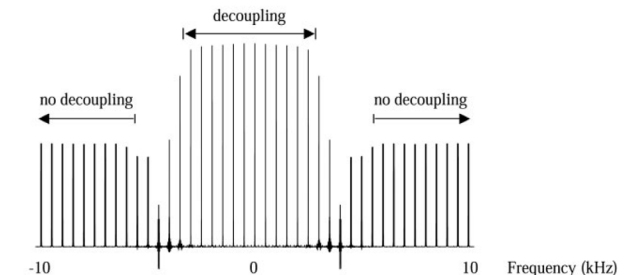
a



$B_{2rms} = 0.90$ kHz

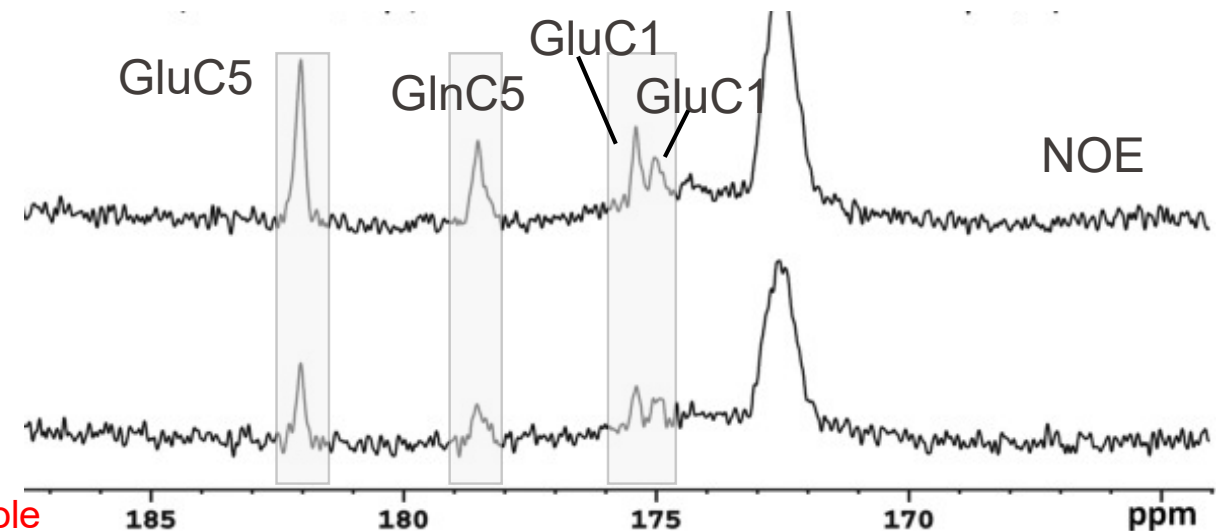
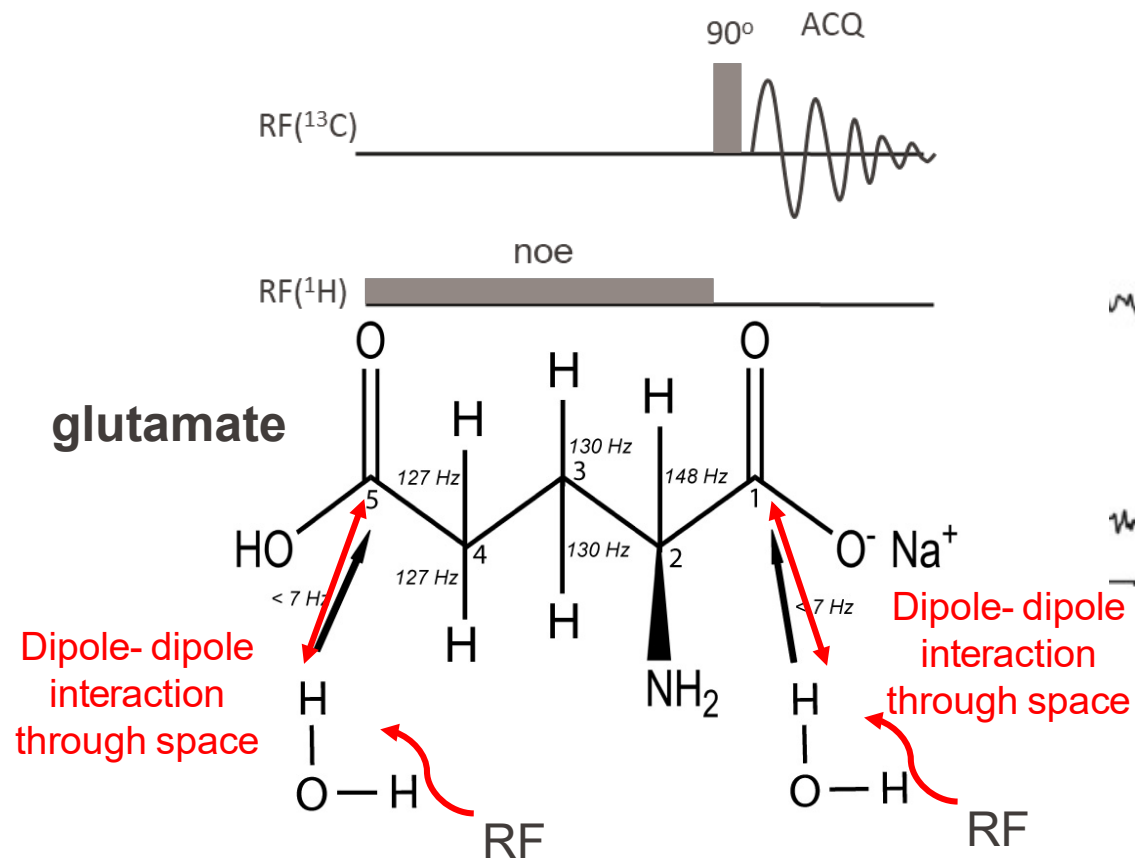
AFPST1, R=10
 $B_{2max} = 3.00$ kHz

b



Nuclear overhauser enhancement (NOE)

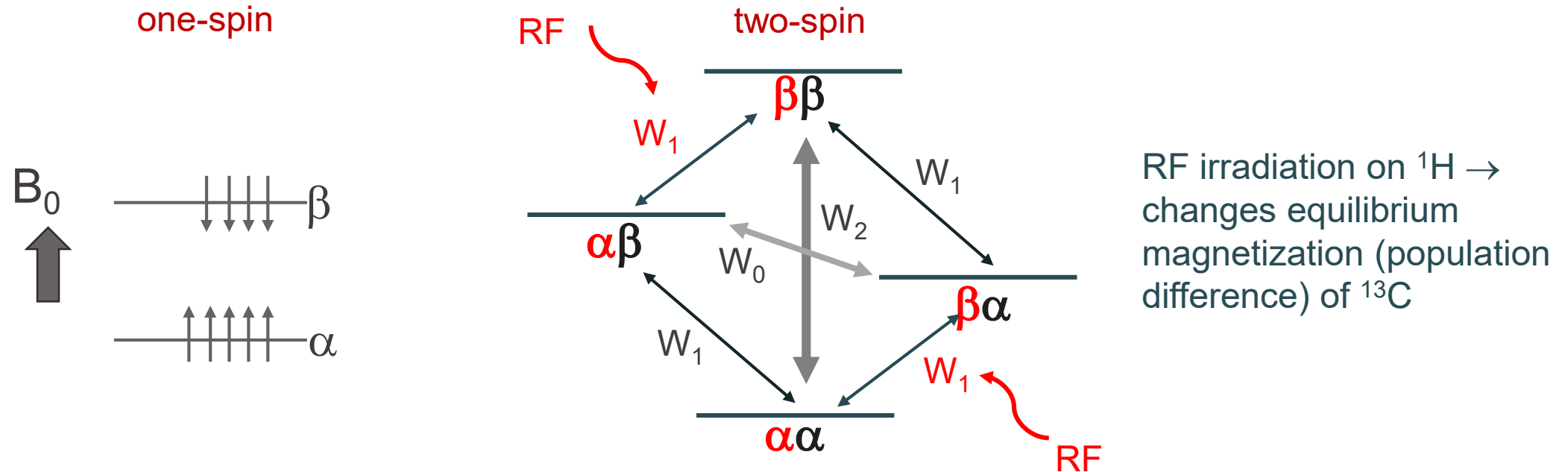
- Albert Overhauser (1953)
- equilibrium magnetization of a given nucleus changes via RF irradiation of neighboring nuclei (induced by relaxation mechanism)



Li et al., MRM, 75:954–961, 2016

Sailasuta et al., J Magn Reson. 195: 219–225. 2008

NOE FOR TWO-SPIN SYSTEM: I (^{13}C), S(^1H)

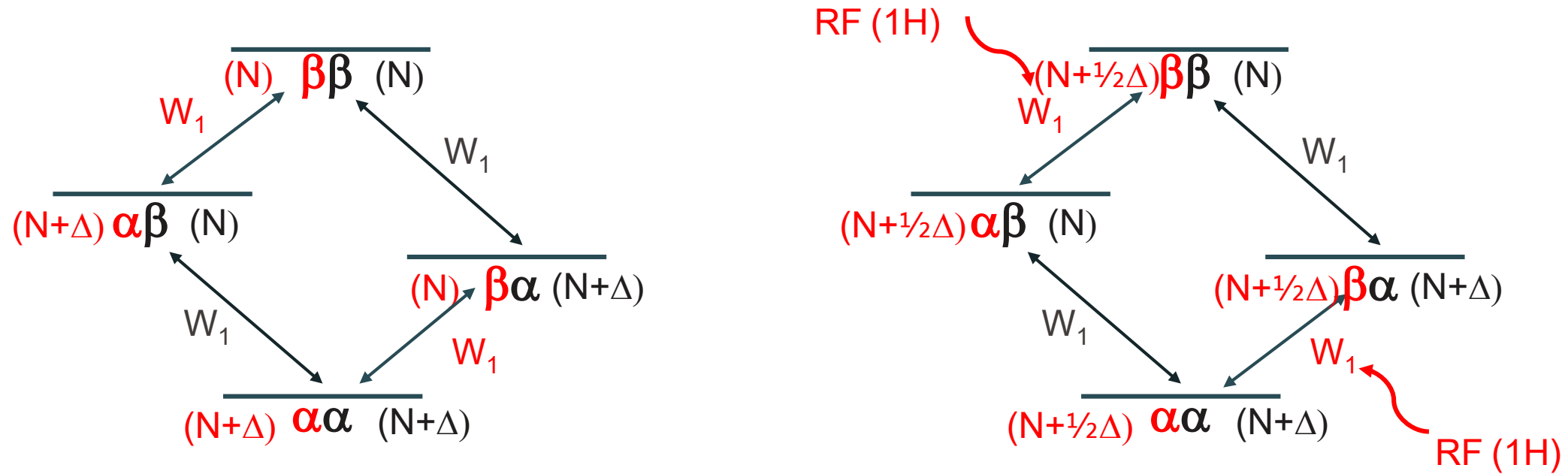


- ✓ **Relaxation** is caused by random fluctuating magnetic fields due to the thermal motion of the molecules
- ✓ The fluctuating field induces **transitions** between the spin energy states

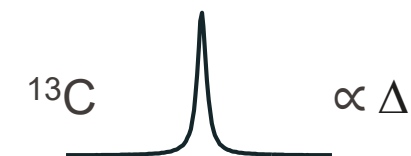
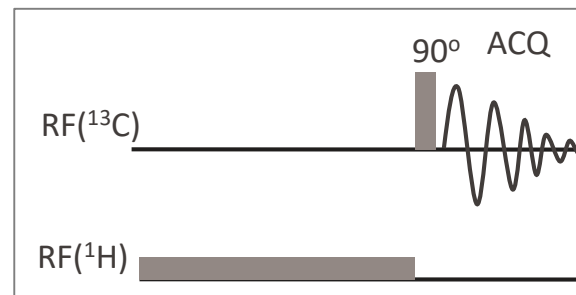
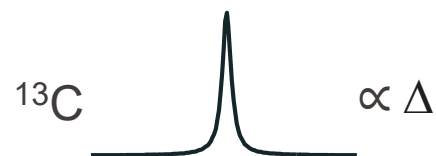
Transition probability W : probabilities/time that spins will change energy states (transition)

- W_1 : single quantum transition (flip of only one spin)
- W_0 : zero quantum transition (simultaneous flip of both spins in the opposite direction)
- W_2 : double quantum transition (simultaneous flip of both spins in the same direction)

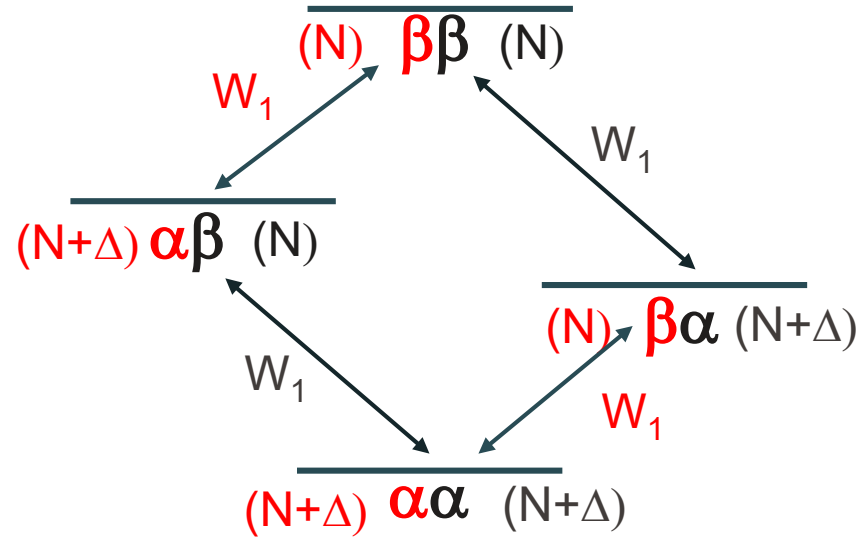
NOE FOR TWO-SPIN SYSTEM: I (^{13}C), S(^1H)



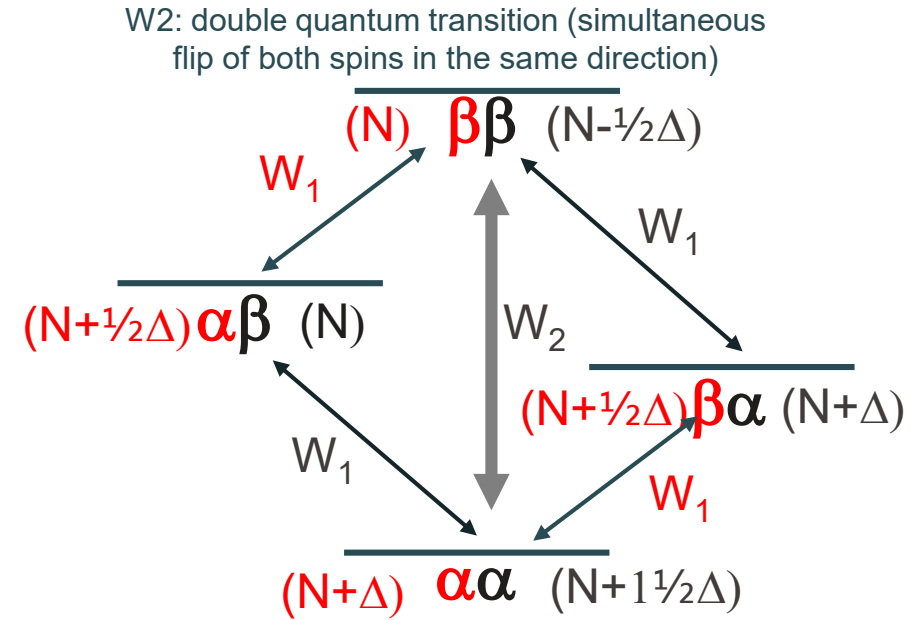
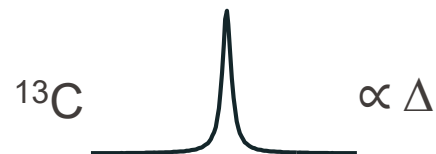
Saturation: equalize the population of energy state



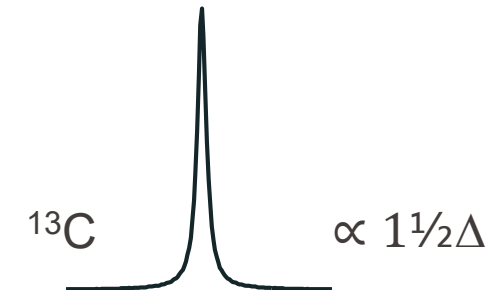
NOE FOR TWO-SPIN SYSTEM: I (^{13}C), S(^1H)



Relaxation will force the distribution back to equilibrium distribution (Boltzmann)

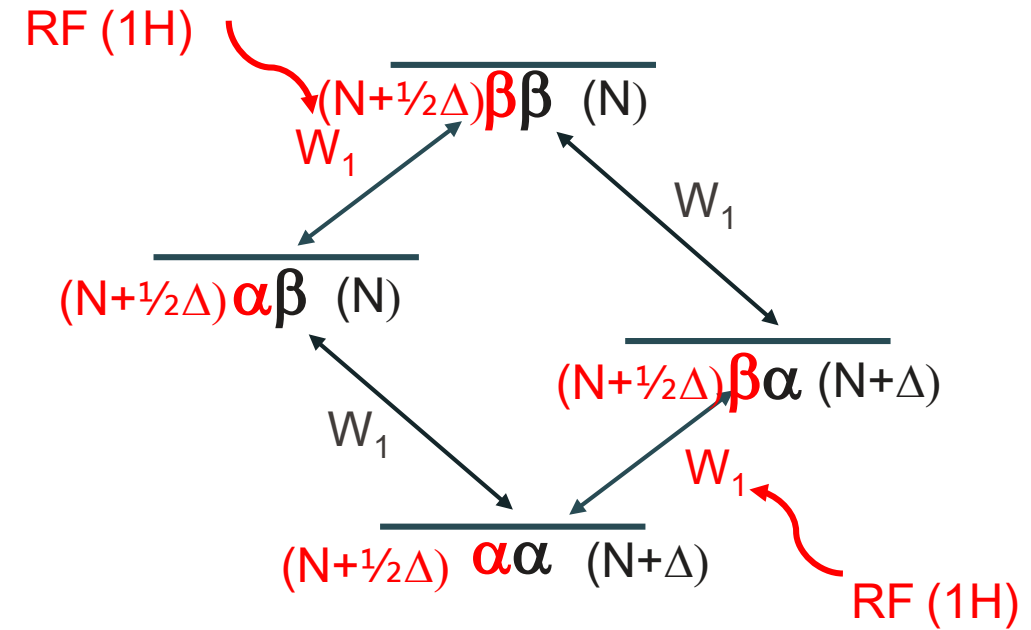
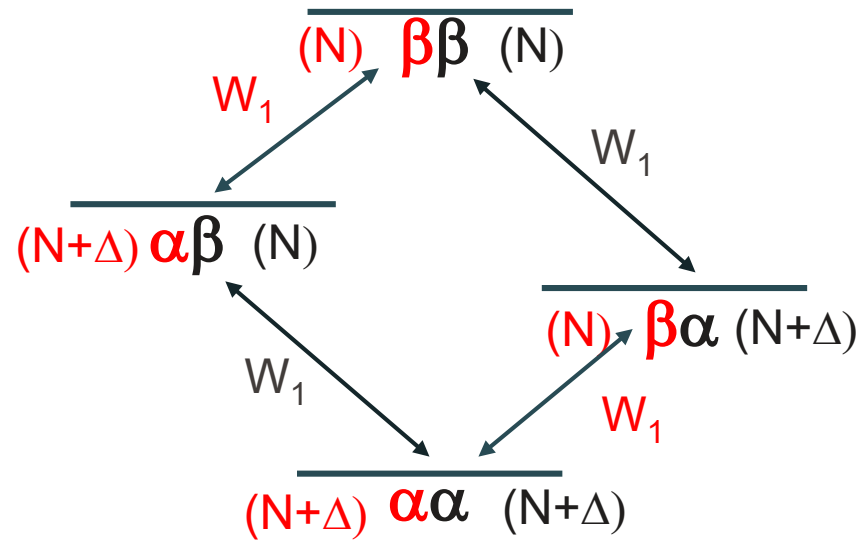


W_2 : double quantum transition (simultaneous flip of both spins in the same direction)

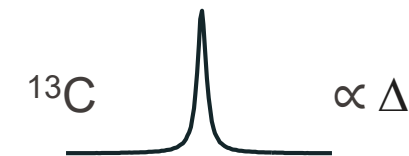
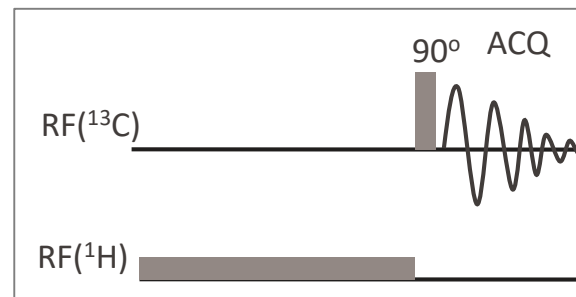
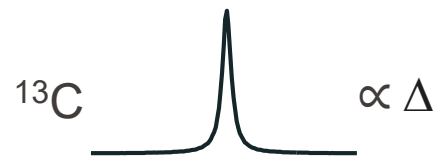


positive enhancement

NOE FOR TWO-SPIN SYSTEM: I (^{13}C), S(^1H)

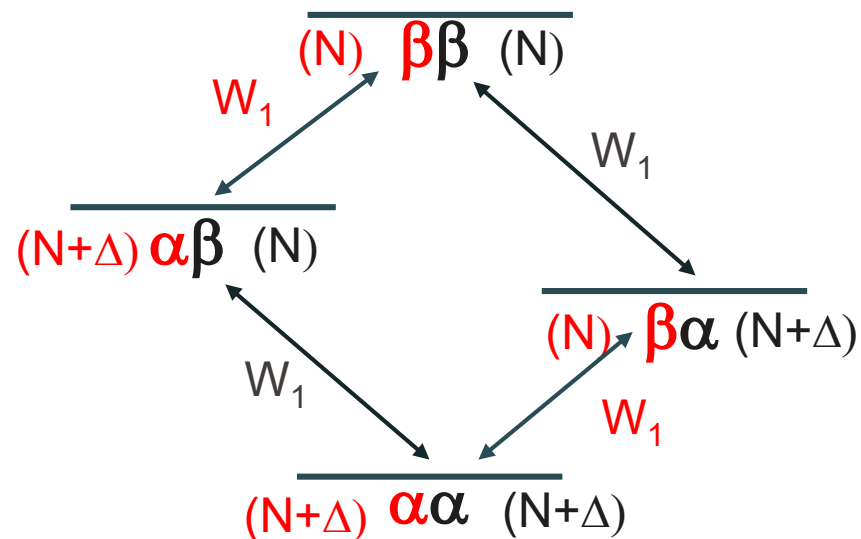


Saturation: equalize the population of energy state

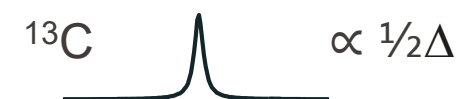
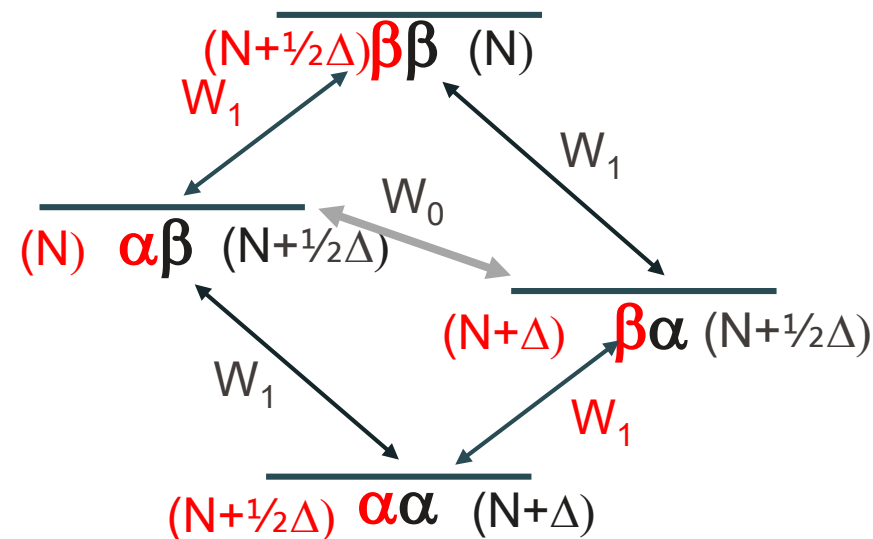
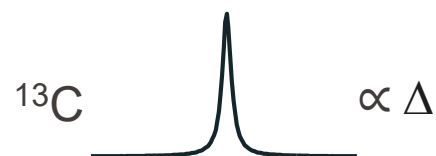


NOE FOR TWO-SPIN SYSTEM: I (^{13}C), S(^1H)

W_0 : zero quantum transition (simultaneous flip of both spins in the opposite direction)

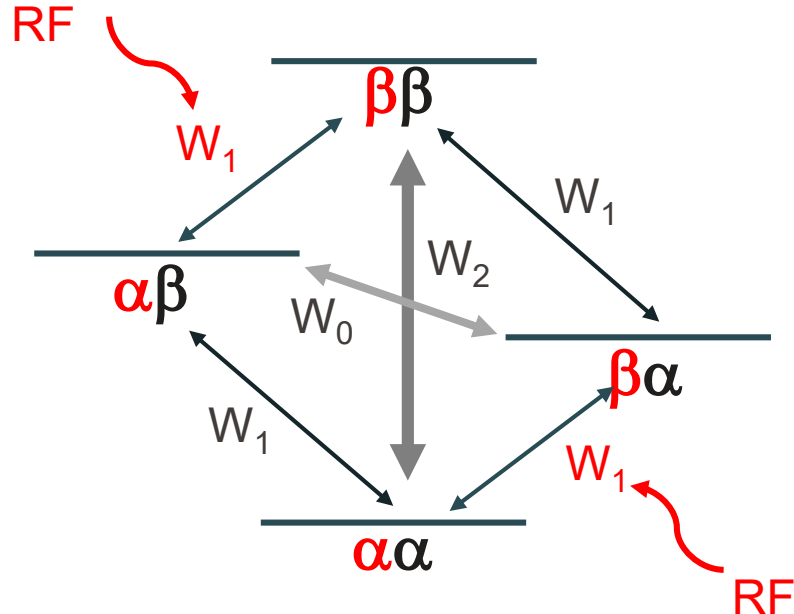


Relaxation will force the distribution back to equilibrium distribution (Boltzmann)



negative enhancement

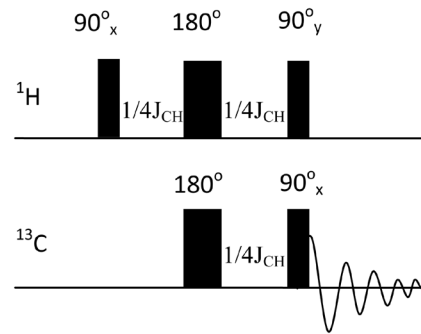
SUMMARY FOR NOE



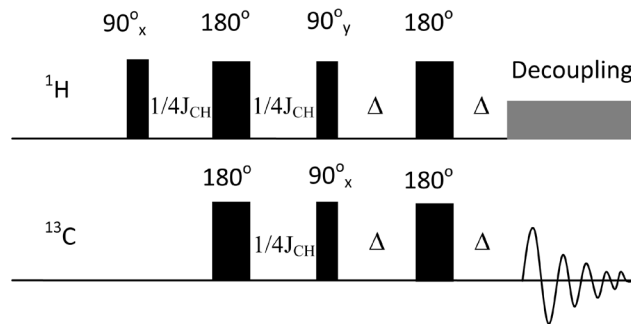
- An RF irradiation $\rightarrow$ saturation for one spin
- Mechanism: relaxation
- The enhancement depends on the contribution of relaxation mechanisms and molecules (e.g. the distance between spins)
 - W_2 : positive enhancement , W_0 : negative enhancement
- Experimental determination for NOE factor in vivo and in vitro
 - ^{31}P - ^1H : 1.4-1.8
 - ^{13}C - ^1H : 1.3-2.9

POLARIZATION TRANSFER (INEPT AND DEPT)

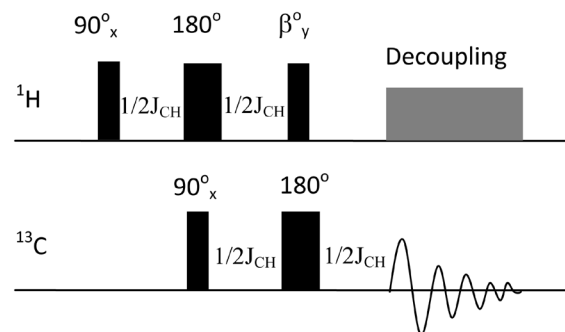
INEPT



Refocused INEPT

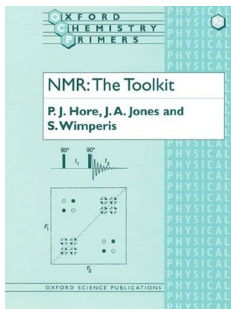


DEPT



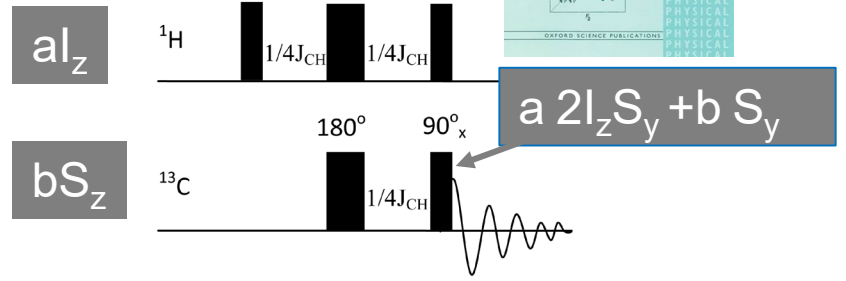
- INEPT: Insensitive Nuclei Enhanced by Polarization Transfer
- DEPT: Distortionless Enhancement by Polarization Transfer
- Polarization is transferred from a high γ nucleus to a low γ nucleus through J-coupling
- Enhancement by a factor of 4 (γ_{1H}/γ_{13C})

INEPT



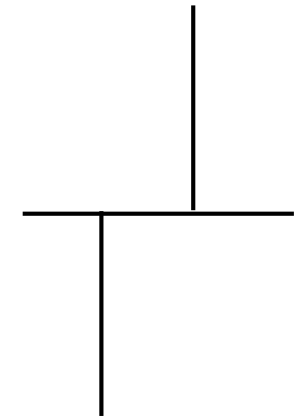
I_z, S_z	z magnetization (population difference)
I_x, I_y, S_x, S_y	in-phase magnetization in the $x' - y'$ plane
$2I_x S_z, 2I_y S_z, 2S_x I_z, 2S_y I_z$	antiphase magnetization in the $x' - y'$ plane
$2I_x S_y, 2I_y S_x, 2I_x S_x, 2I_y S_y$	zero- and double-quantum coherences (not observable)

INEPT



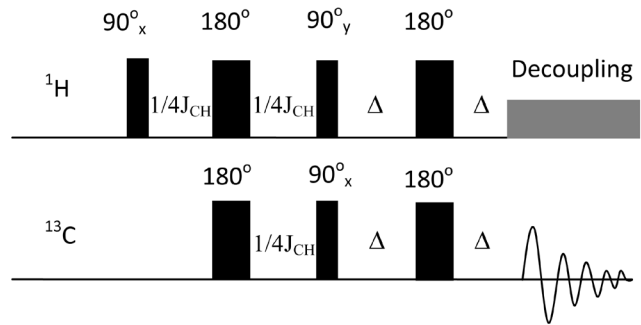
$$a=4b \quad (\gamma_{1H}=4\gamma_{13C})$$

CH

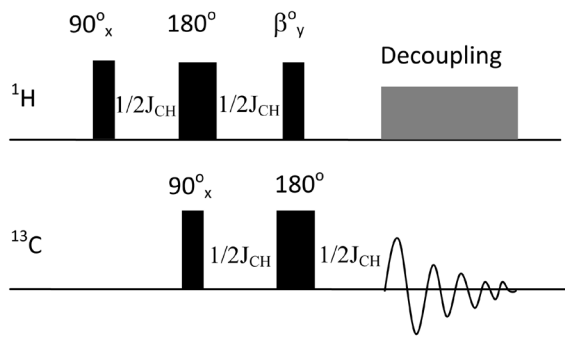


Decoupling
Enhancement
lost

Refocused
INEPT



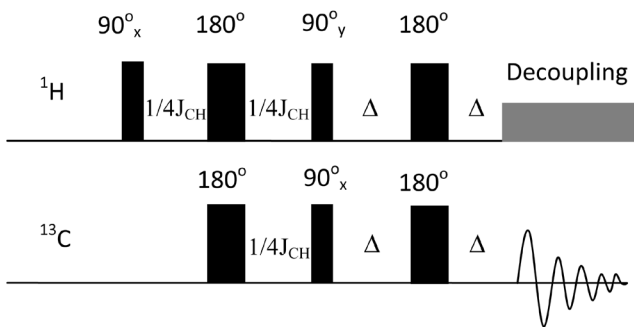
DEPT



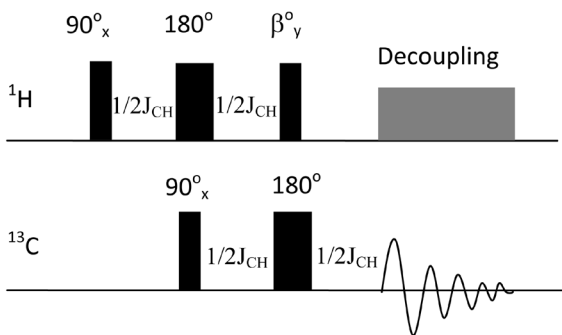
refocused INEPT AND DEPT

I_z, S_z	z magnetization(population difference)
I_x, I_y, S_x, S_y	in-phase magnetization in the $x' - y'$ plane
$2I_xS_z, 2I_yS_z, 2S_xI_z, 2S_yI_z$	antiphase magnetization in the $x' - y'$ plane
$2I_xS_y, 2I_yS_x, 2I_xS_x, 2I_yS_y$	zero-and double-quantum coherences (not observable)

Refocused
INEPT



DEPT



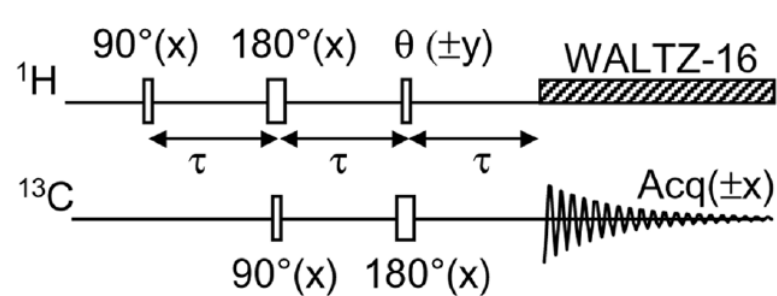
$$a=4b \ (\gamma_{1H}=4\gamma_{13C})$$

	Δ	1/4J	1/2J	3/4J
CH	$S_x \sin(\pi J \Delta)$	0.707	1	0.707
CH ₂	$2S_x \sin(\pi J \Delta) \cos(\pi J \Delta)$	1	0	-1
CH ₃	$3S_x \sin(\pi J \Delta) \cos^2(\pi J \Delta)$	1.061	0	1.061

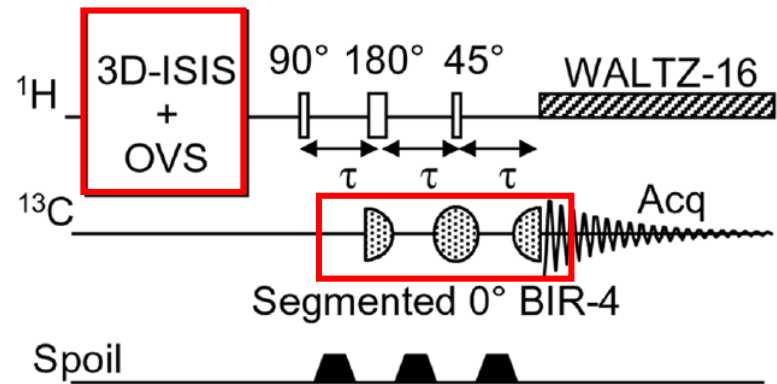
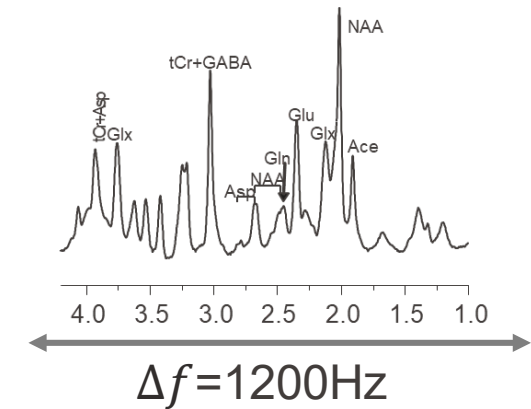
	β	45°	90°	135°
CH	$S_x \sin \beta$	0.707	1	0.707
CH ₂	$2S_x \sin \beta \cos \beta$	1	0	-1
CH ₃	$3S_x \sin \beta \cos^2 \beta$	1.061	0	1.061

PT+¹H LOCALIZATION

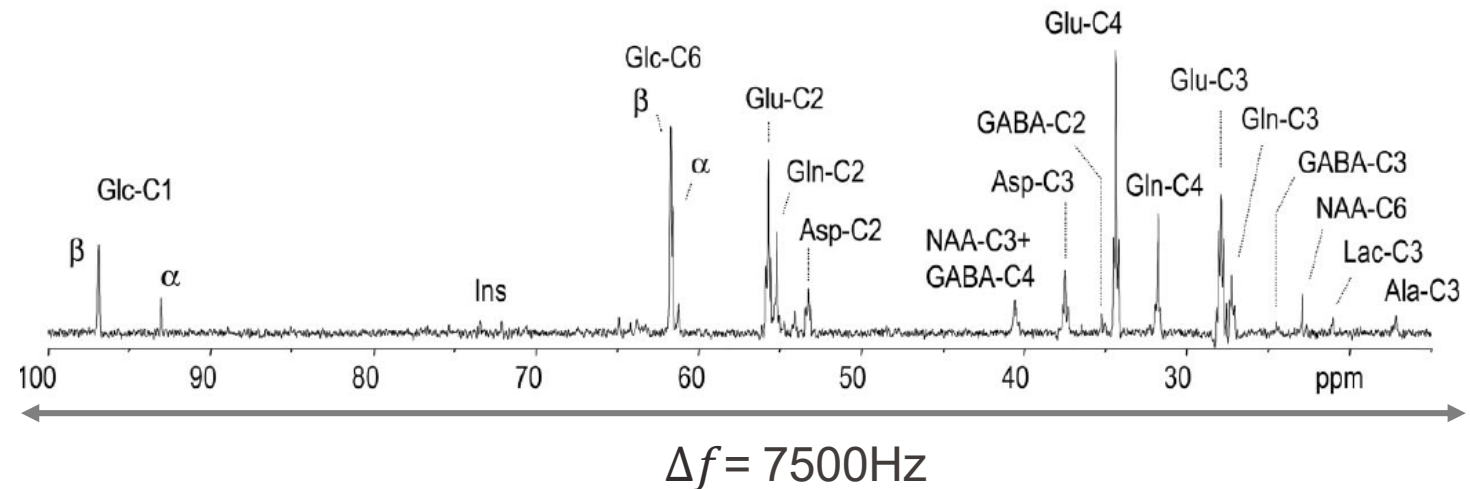
- ¹H localization with PT: long T₂ metabolites
 - Localization with ¹H magnetization: CSDE ↓



$$\text{CSDE (\%)}: \frac{\Delta x}{x} = \frac{\Delta f}{BW}$$

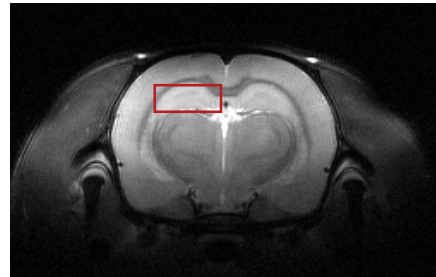
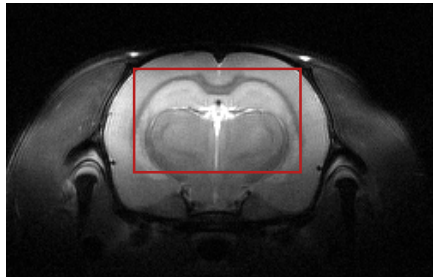


Henry et al., ., Magn Reson Med , 50:684–692 (2003)

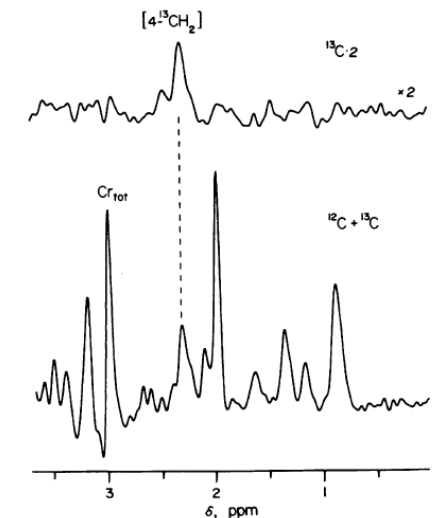
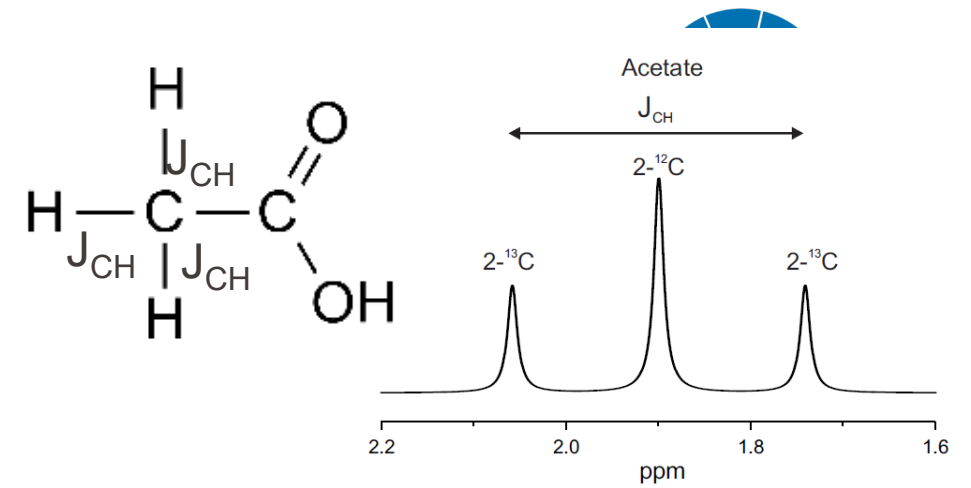


Heteronuclear editing (Indirect detection)

- Detect ^1H resonances bound to ^{13}C
- Higher sensitivity $\times 16$ ($\gamma_{1\text{H}} = 4\gamma_{13\text{C}}$) $M_0 = N_s \frac{\gamma^2 \hbar^2 B_0}{4kT} k$



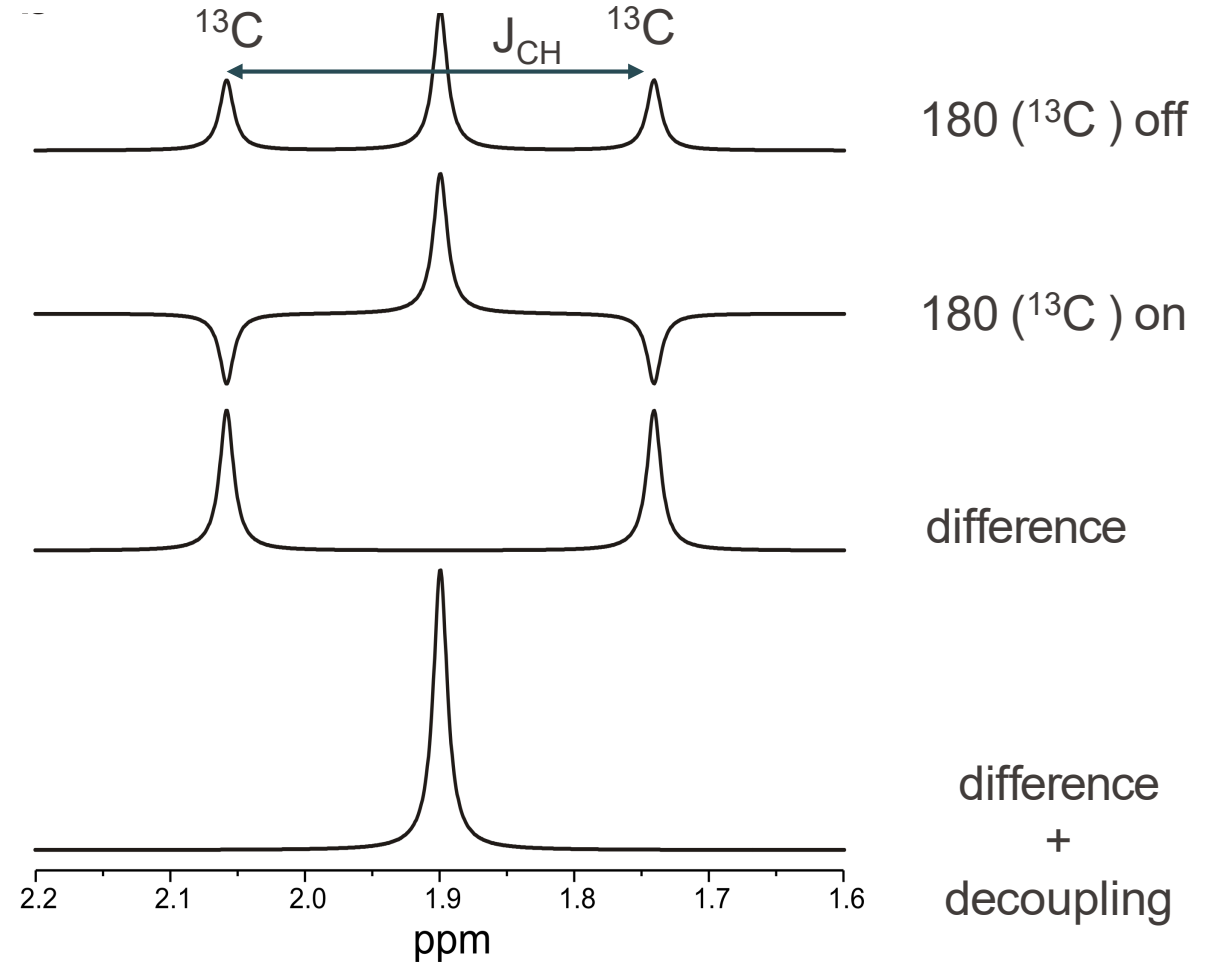
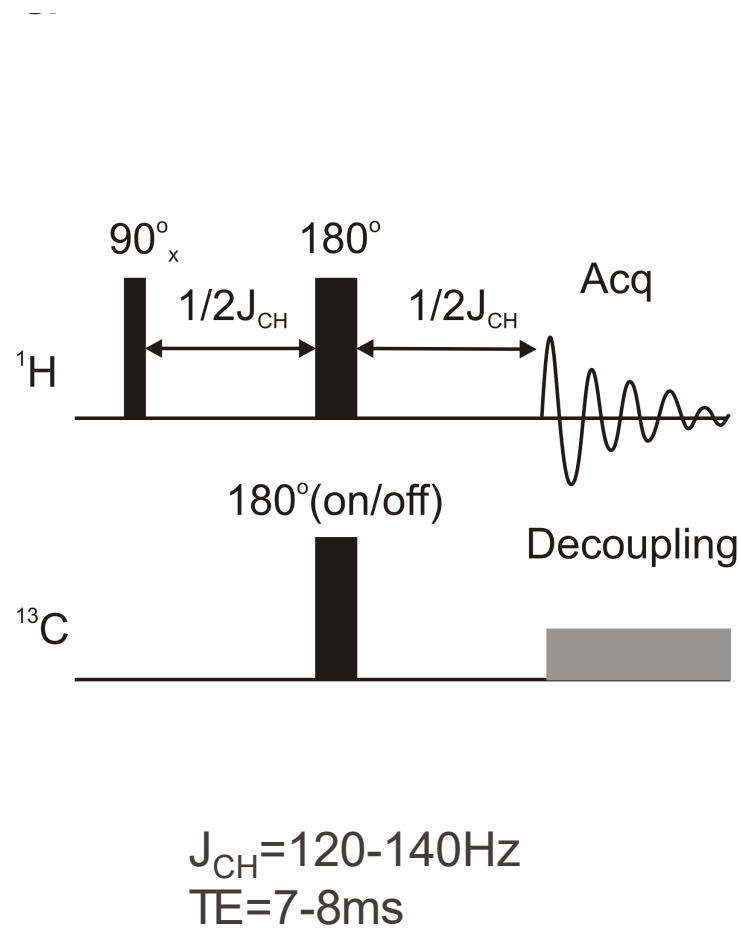
- Direct measurement of fractional enrichment [FE = $^{13}\text{C} / (^{13}\text{C} + ^{12}\text{C})$]
- Heteronuclear editing: J-difference editing
- limitation: narrow spectral resolution in ^1H



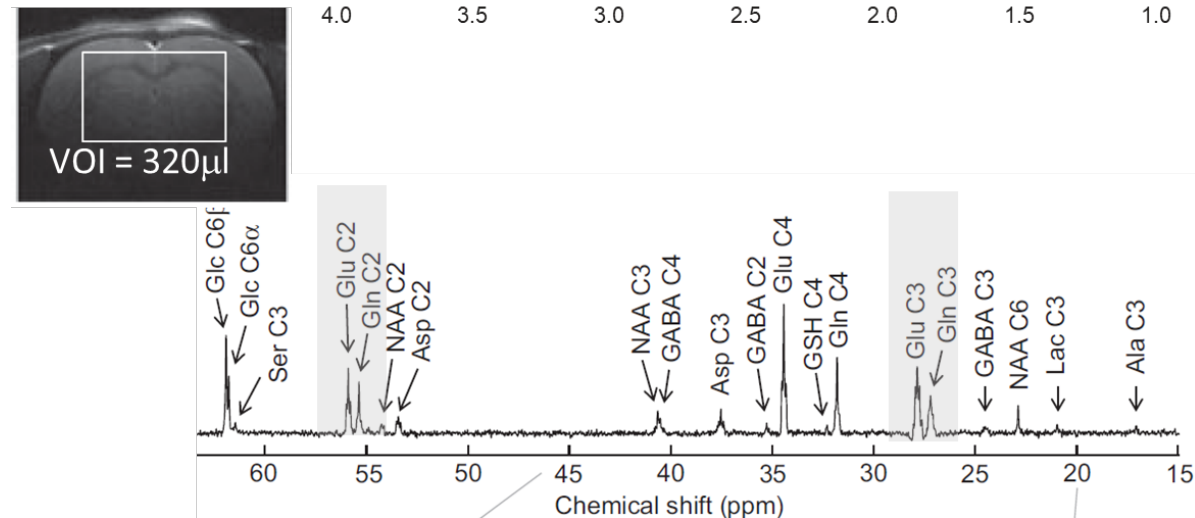
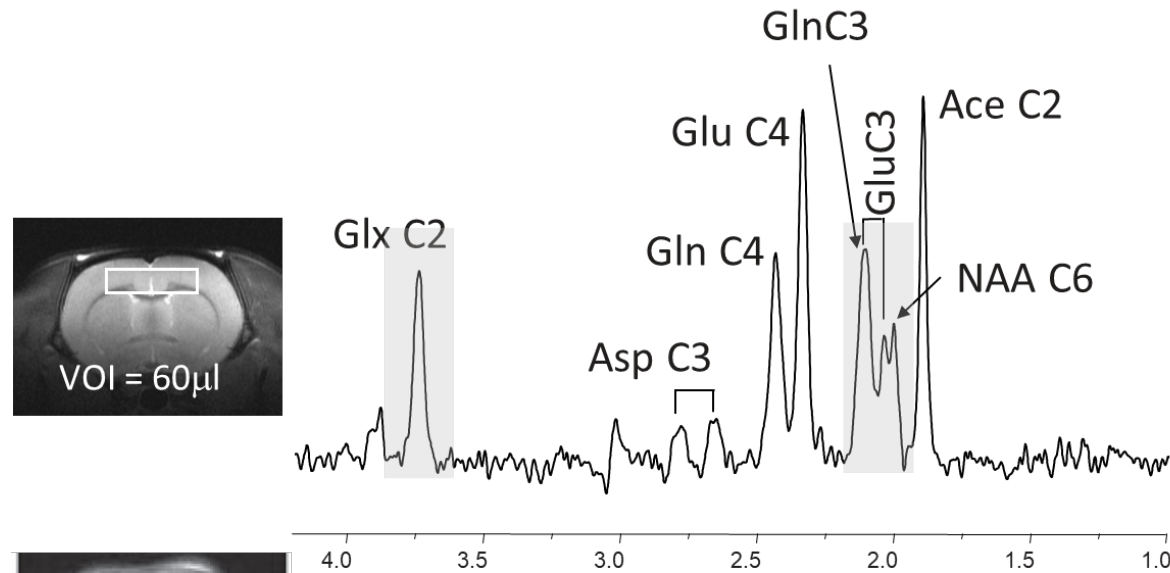
$\{^{13}\text{C}\}$ - ^1H pulse sequence *in vivo*. When corrections were made for concentration differences, ^1H NMR gave a S/N enhancement over ^{13}C NMR of 11-fold for $[3\text{-}^{13}\text{CH}_3]\text{lactate}$ detection (3.6 per proton) and a 6-fold S/N enhancement for $[4\text{-}^{13}\text{CH}_2]\text{glutamate}$ (3.0 per proton). A factor of 2 in poten-

J-DIFFERENCE EDITING

Proton-Observed Carbon-Edited Spectroscopy (POCE) ^{12}C

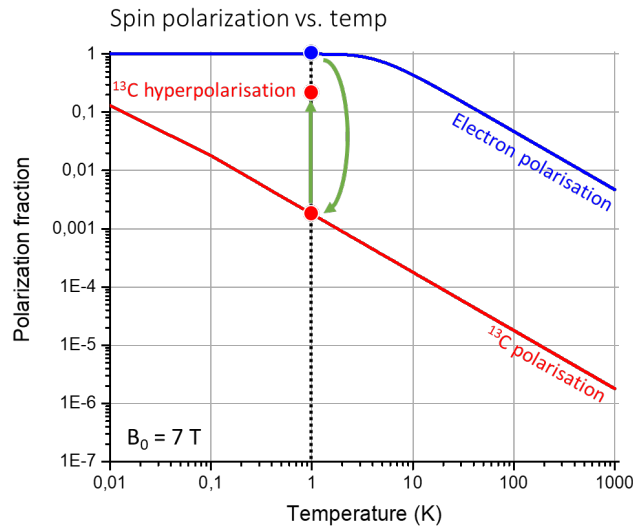


COMPARISON OF DIRECT AND INDIRECT ^{13}C MRS



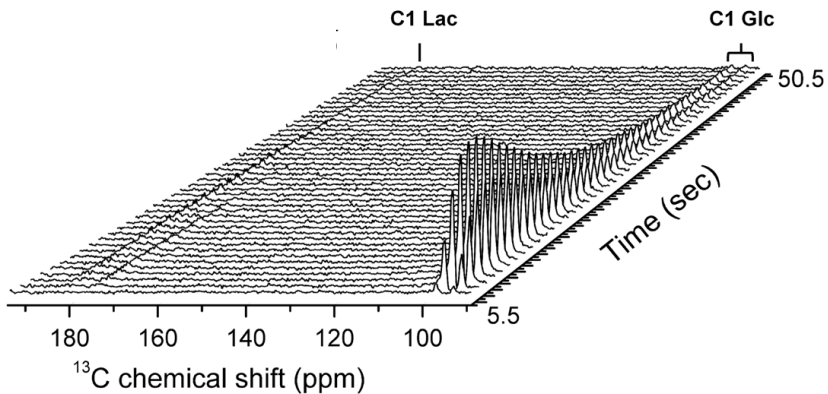
- 😊 High sensitivity (high temporal and spatial resolution)
- 😊 total metabolite concentrations (^{13}C + ^{12}C)
- 😞 Limited spectral resolution $\rightarrow$ limited metabolic info
- 😊 High spectral resolution $\rightarrow$ large metabolic info
- 😞 Low sensitivity (limited temporal and spatial resolution)
- 😞 Additional measurement for metabolite concentration

HYPERPOLARIZED ^{13}C MRS



Carver et al., Phys. Rev. (1953)

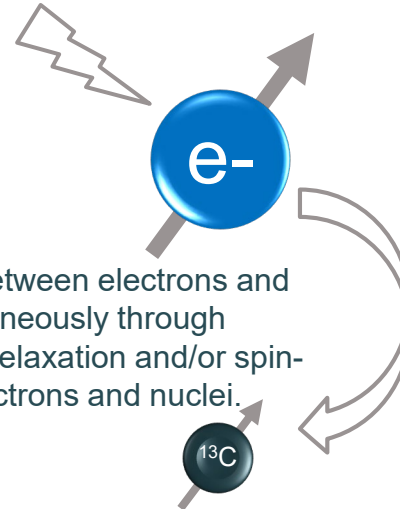
- loss of the polarization back to thermal equilibrium
- short measurement window limited by T_1
- a compound with long T_1 is favorable for DNP experiments



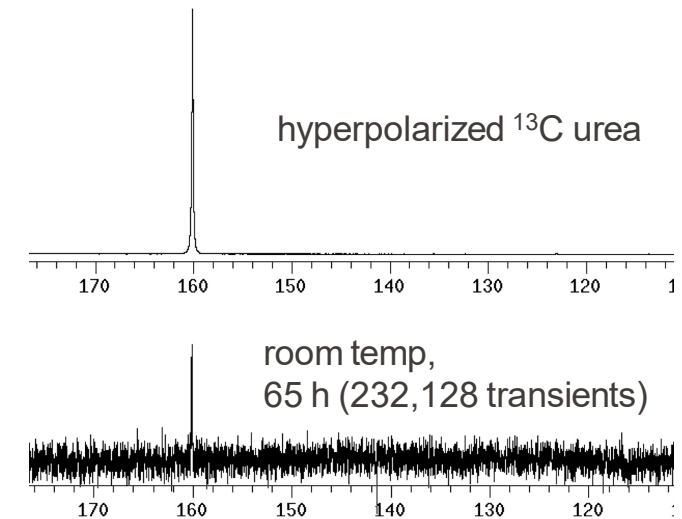
Mishkovsky et al., Sci. Rep. (2017)

microwaves

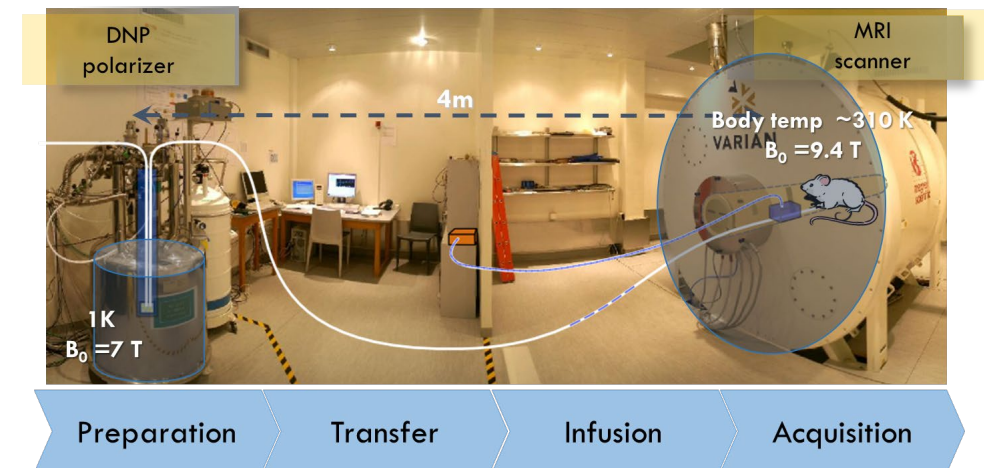
polarization transfers between electrons and nuclei can occur spontaneously through electron-nuclear cross-relaxation and/or spin-state mixing among electrons and nuclei.



Dissolution DNP



Ardenkjær-Larsen et al., PNAS (2003)

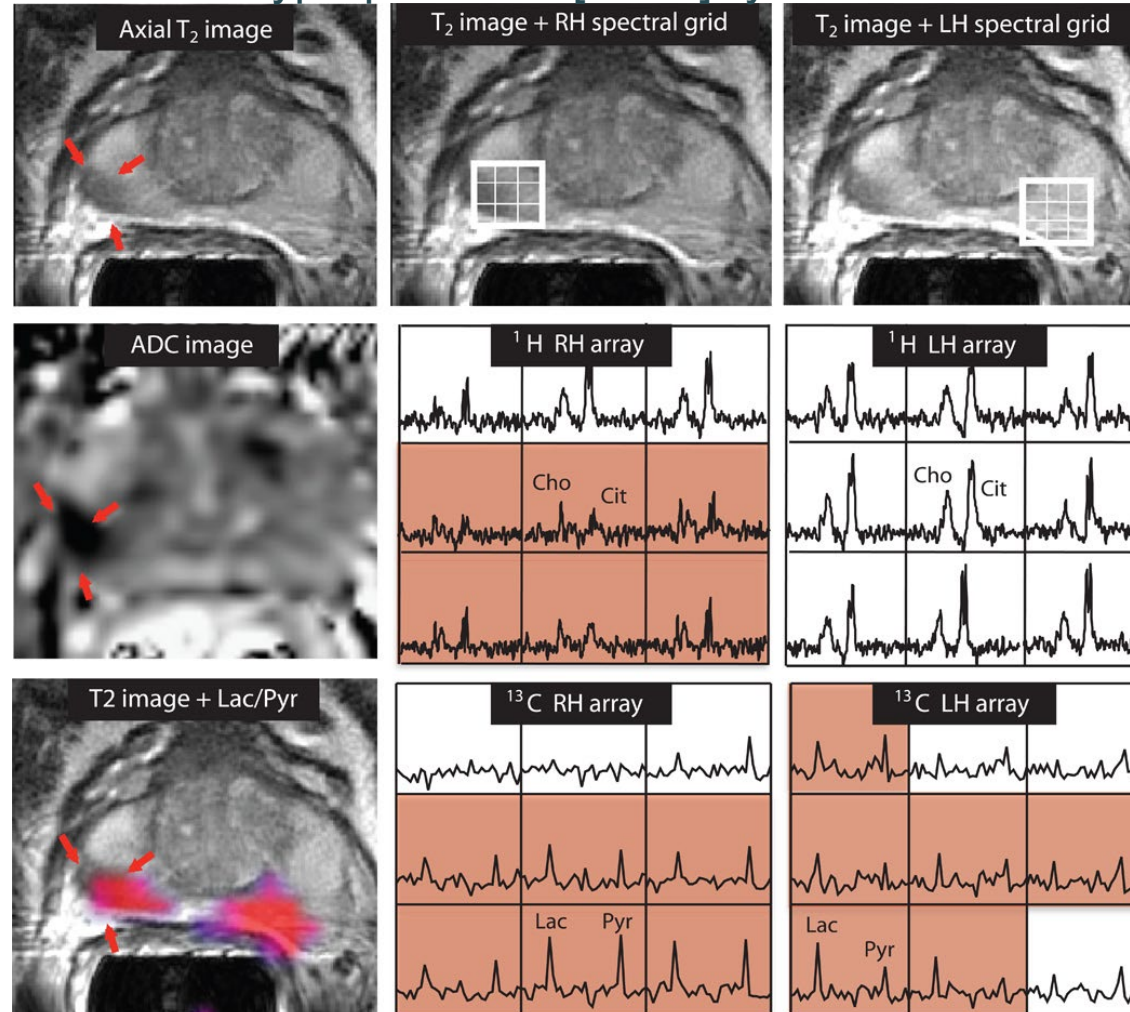


FAST process: minimize loss of polarization

Courtesy of M. Mishkovsky

HP ^{13}C MRS APPLICATION IN PROSTATE CANCER

Hyperpolarized $[1-^{13}\text{C}]$ Pyruvate

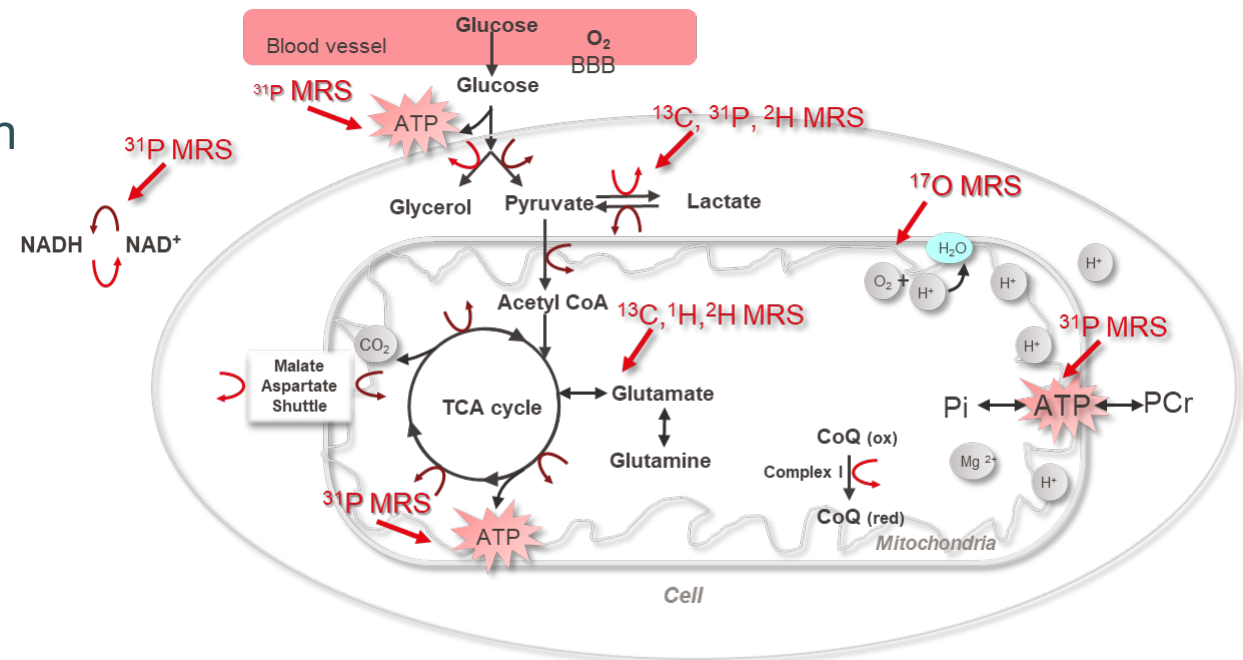


prostate cancer: ^1H MRSI
reduced citrate and
elevated choline/citrate

**HP ^{13}C could detect affected areas
that are negative in MRI images and
also ^1H MRSI.**

SUMMARY

- Multinuclear MRS (^1H , ^2H , ^{13}C , ^{31}P , ^{17}O etc): a valuable tool to study brain metabolism
 - metabolite levels
 - chemical reaction rates
 - kinetics in metabolic pathway (e.g. CMR glc, TCA cycle and neurotransmitter cycling fluxes)
- Challenges in clinical implementation
 - low sensitivity, limited spatial resolution
 - long scanning time
 - stringent RF deposition regulations





THANK YOU FOR YOUR ATTENTION



C I B M . C H