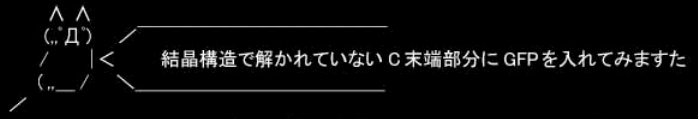
Protein NMR

Part III

(let's start by reviewing some of the things we have learned already)

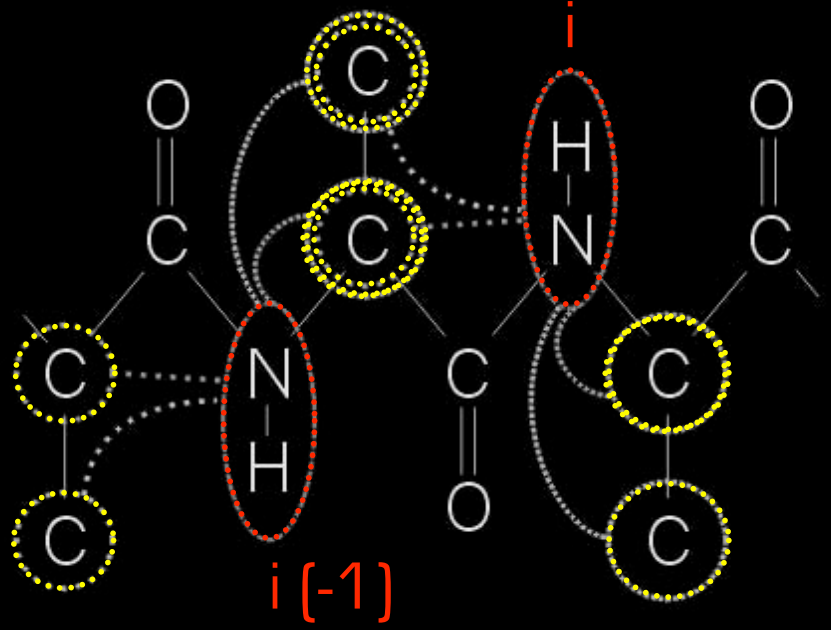


GroEL/GroES (Xu et al. *Nature* **388**: 720)
GFP (Ormo et al. *Science* **273**: 1392)

1. Magnetization Transfer

Magnetization transfer through space > NOE

Magnetization transfer through bonds > J-coupling

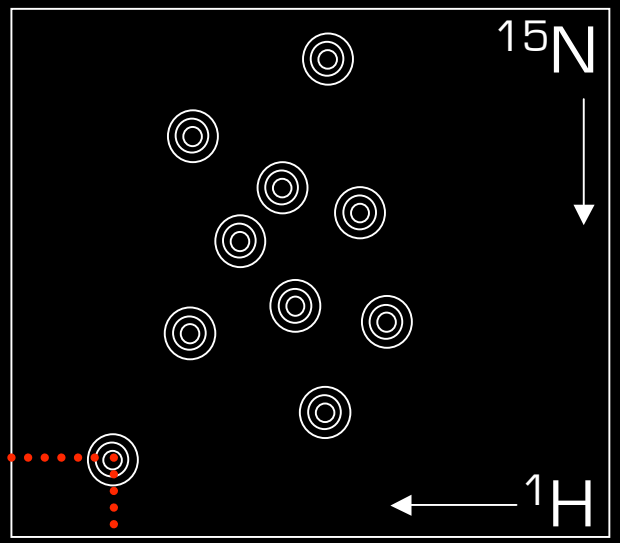


$^{1}H/^{15}N$ Correlation (2D)

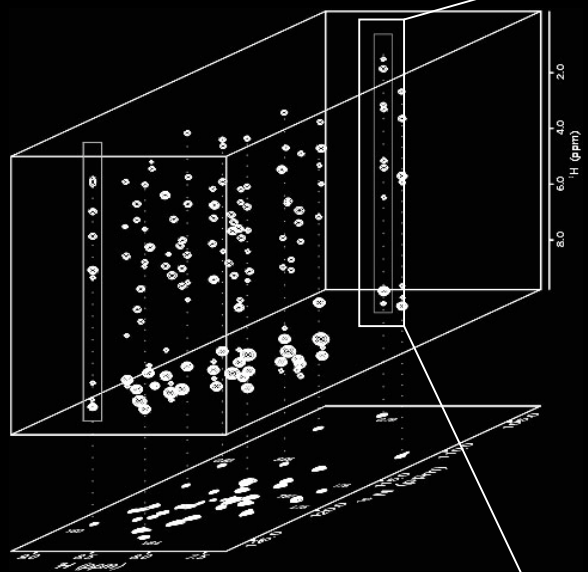
HSQC or HMQC-type

HNCA Experiment (3D)

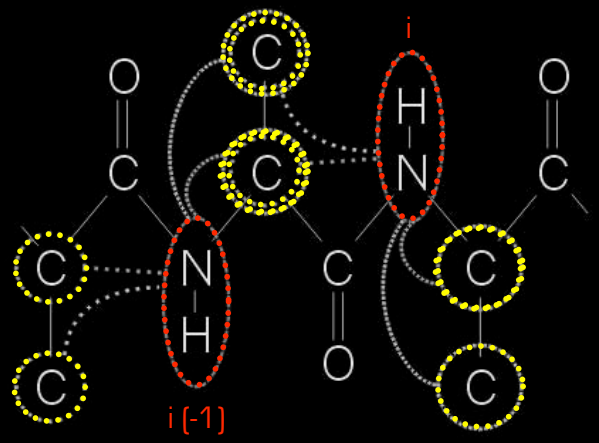
HNCACB Experiment (3D)

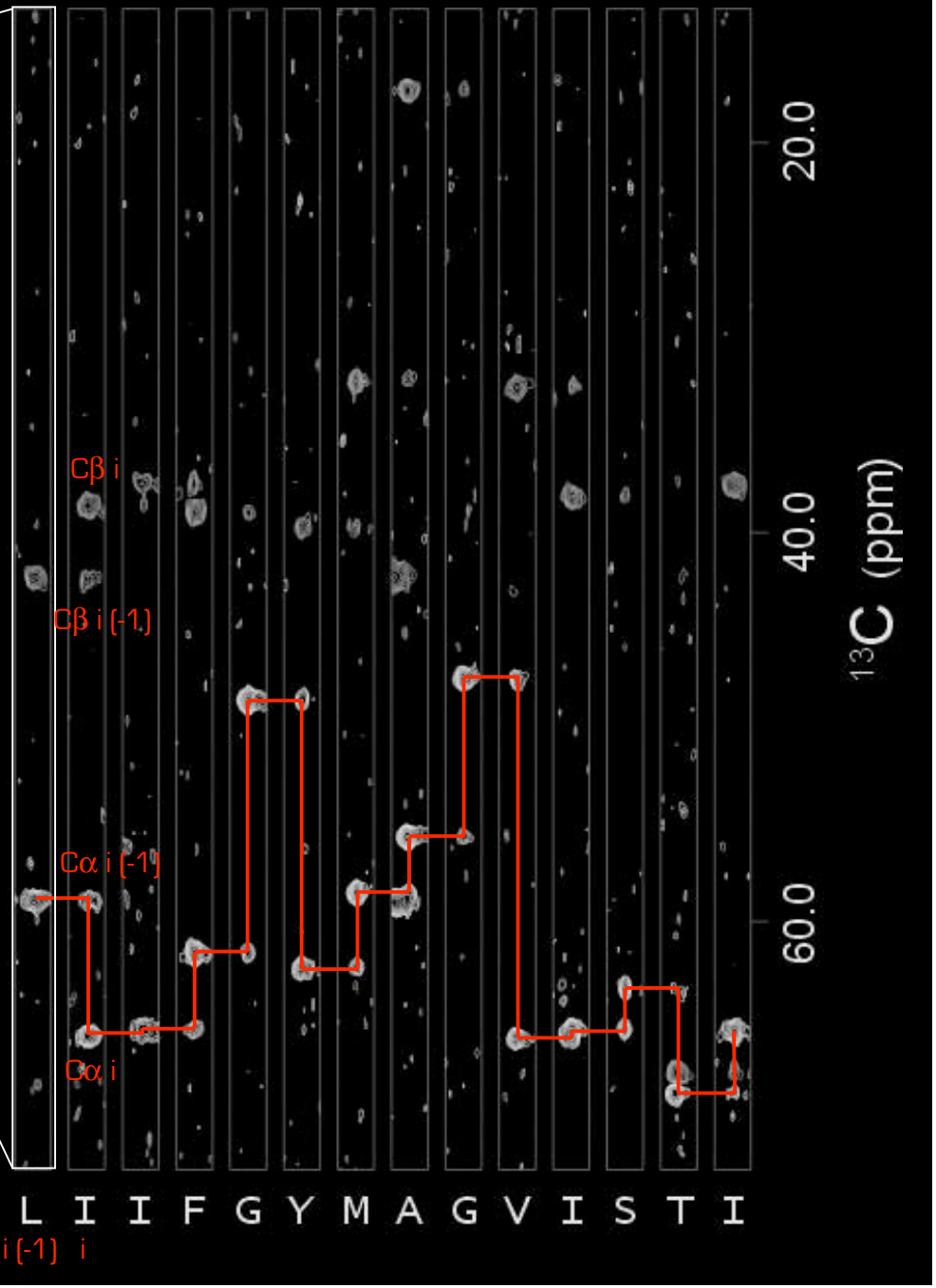


2. Assignment



HNCACB Experiment
(as an example)



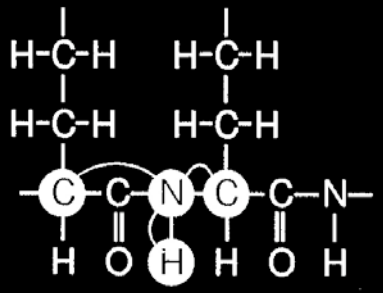


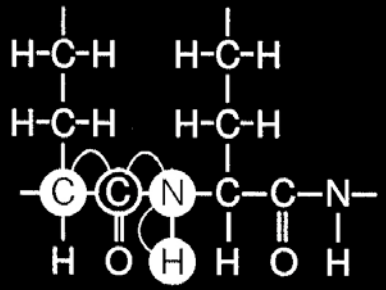
3. NMR Experiments

Types of experiments and nomenclature

BACKBONE EXPERIMENTS

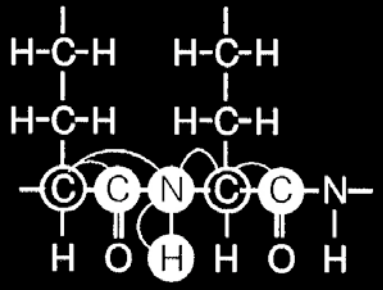
HNCA

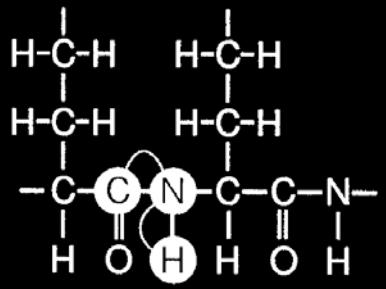




HN(CO)CA

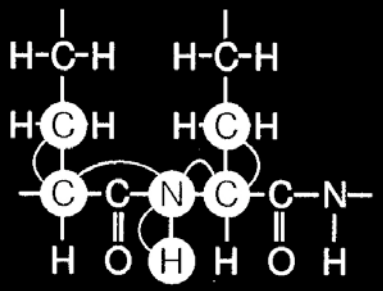
HN(CA)CO

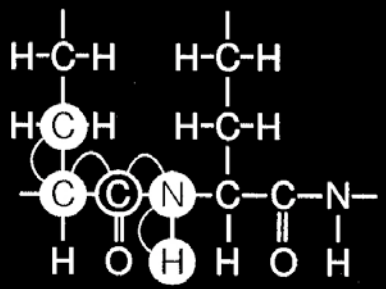




HNCO

HNCACB



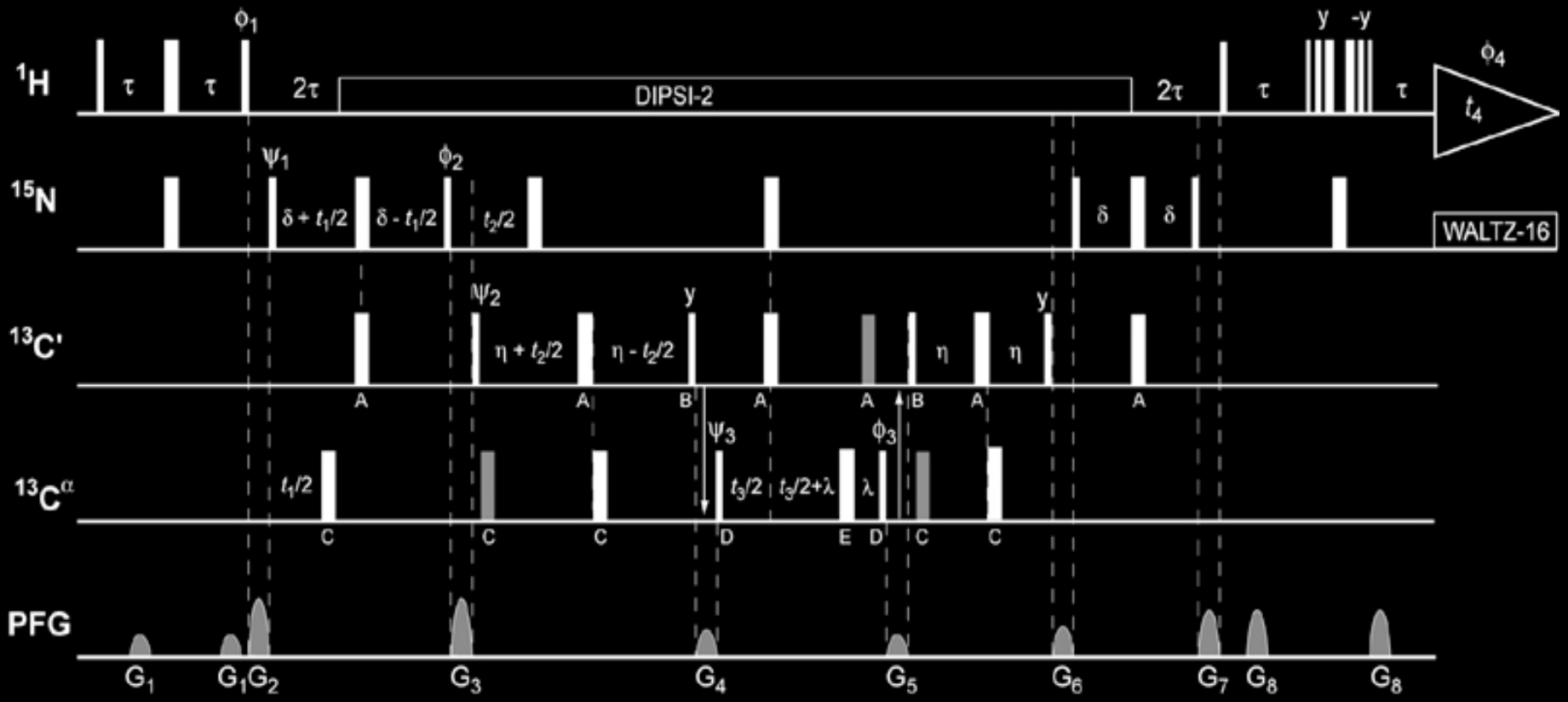


HN(CO)CACB

4. NMR Pulse-Sequences

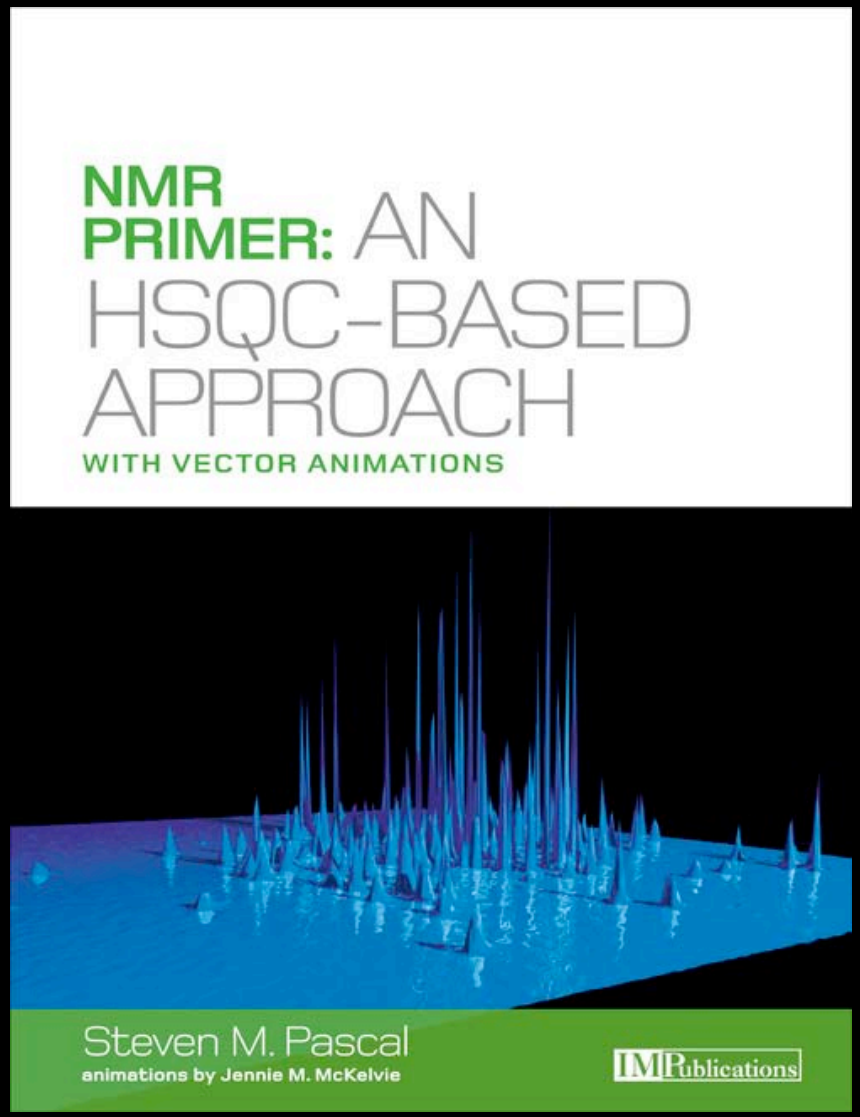
How do we READ them?

What do they MEAN and how do they WORK?



5. How to best get started ...

> Read a SMART (but easy to understand) BOOK !



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Scope of this lecture:

What is J-coupling (from a qualitative perspective)?

How can J-coupling be exploited to transfer magnetization between different atomic nuclei in a protein?

How does the evolution of J-coupling lead to basic building blocks in NMR pulse sequences?

How do we go from an INEPT building block to a 2D HSQC?

What types of 2D HSQCs are there, why are they so important (and when do we use them)?

Now that we understand the 2D HSQC, why is it so easy to understand 3D triple resonance NMR experiments?

6. J-coupling revisited

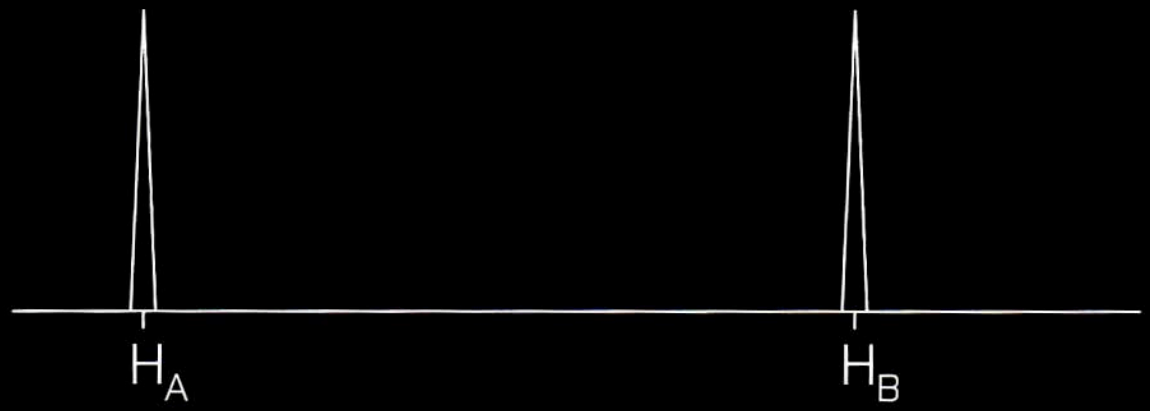


Figure 3.1. Schematic 1D ^{1}H NMR spectrum. A molecule with two non-identical protons that are isolated from each other.

Figure 3.2. Schematic 1D ^{1}H NMR spectra. A molecule with two non-identical protons that are *J*-coupled to each other.

6. J-coupling to other nuclei

After a 90deg. pulse on PROTONS, there are
2 POPULATIONS of NET ^{1}H MAGNETIZATION along y.

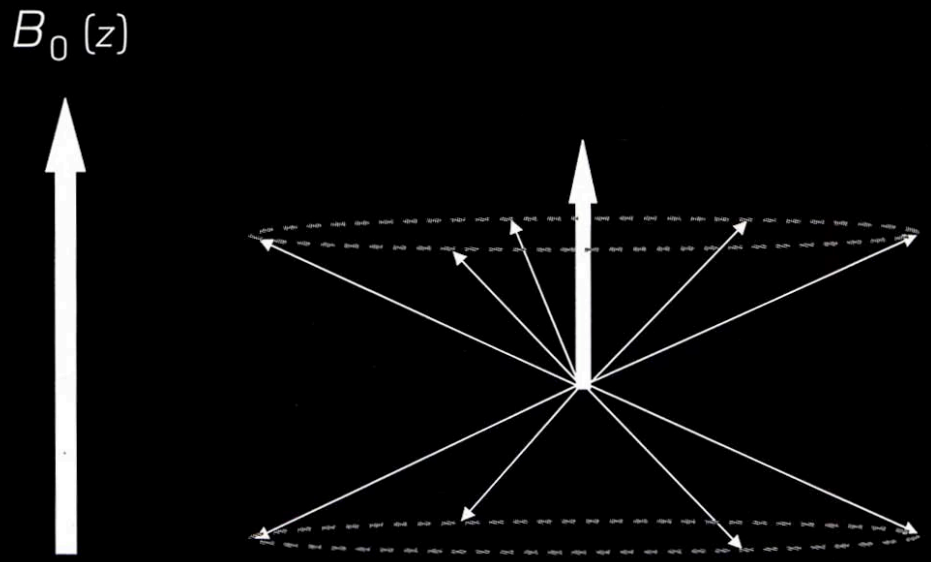


Figure 1.4. Cone of precession. Due to quantum mechanical considerations, the proton spin orientations can only precess in one of two cones, oriented at 54.7° from either the +*z* or the –*z* axis. As the energy difference between these two orientations is very small, at room temperature the population of the two cones is nearly equal. Of the 10^{17} protons in the sample, the upper cone contains an excess of only about 10^{11} spins relative to the lower cone. All *xy* spin components cancel, leaving a net magnetisation along +*z* (arrow in centre of upper cone).

6. J-coupling to other nuclei

After a 90deg. pulse on PROTONS, there are
2 POPULATIONS of NET ^{1}H MAGNETIZATION along y.

[1] that associates with ^{15}N NUCLEI from the UPPER CONE (spin up)
[2] that associates with ^{15}N NUCLEI from the LOWER CONE (spin down)

[1] INCREASES the effective spectrometer field B_0
> attached ^{1}H experience a STRONGER MAGNETIC FIELD + precess faster

[2] DECREASES the effective spectrometer field B_0
> attached ^{1}H experience a WEAKER MAGNETIC FIELD + precess slower

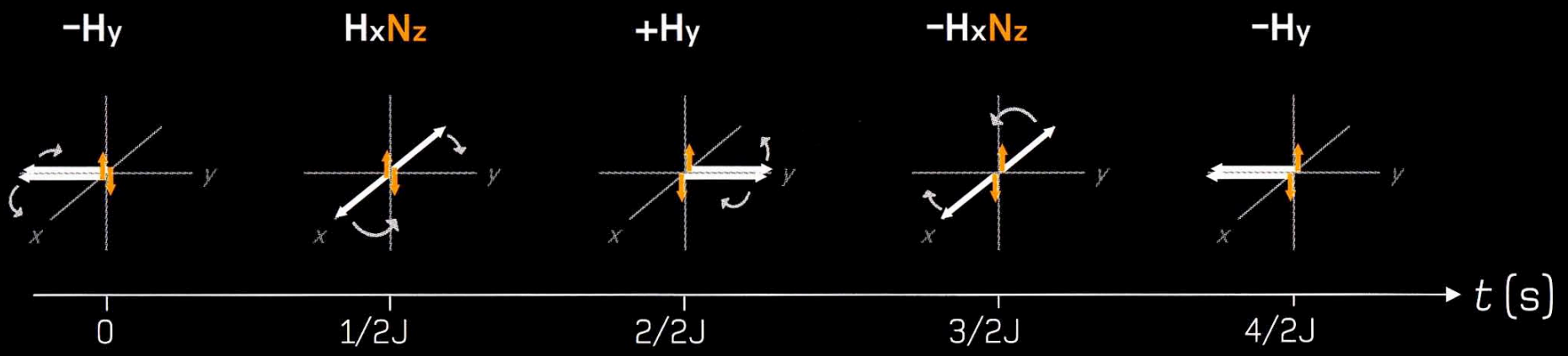
1-bond ^{1}H-^{15}N J-coupling ($^{1}J_{NH}$) is about 92Hz (+/- 46Hz)
J-coupling changes the precession rate of the 2 populations by +/- J/2Hz

7. J-coupling refocuses itself

[1] population 1 precesses ANTI-CLOCKWISE (+$J/2$Hz)
[2] population 2 precesses CLOCKWISE (-$J/2$Hz)

Each population returns to the -y axis after ONE PERIOD, which is defined as the INVERSE OF THE FREQUENCY i.e. ($1/(J/2)$) = $2/J$ sec.

After 1/2 of a full PERIOD ($1/J$) both populations lie along + y (in-phase)
After 1/4 of a full PERIOD ($1/2J$) they are along +/- x (anti-phase)



8. Magnetization transfer via J-coupling requires anti-phase terms

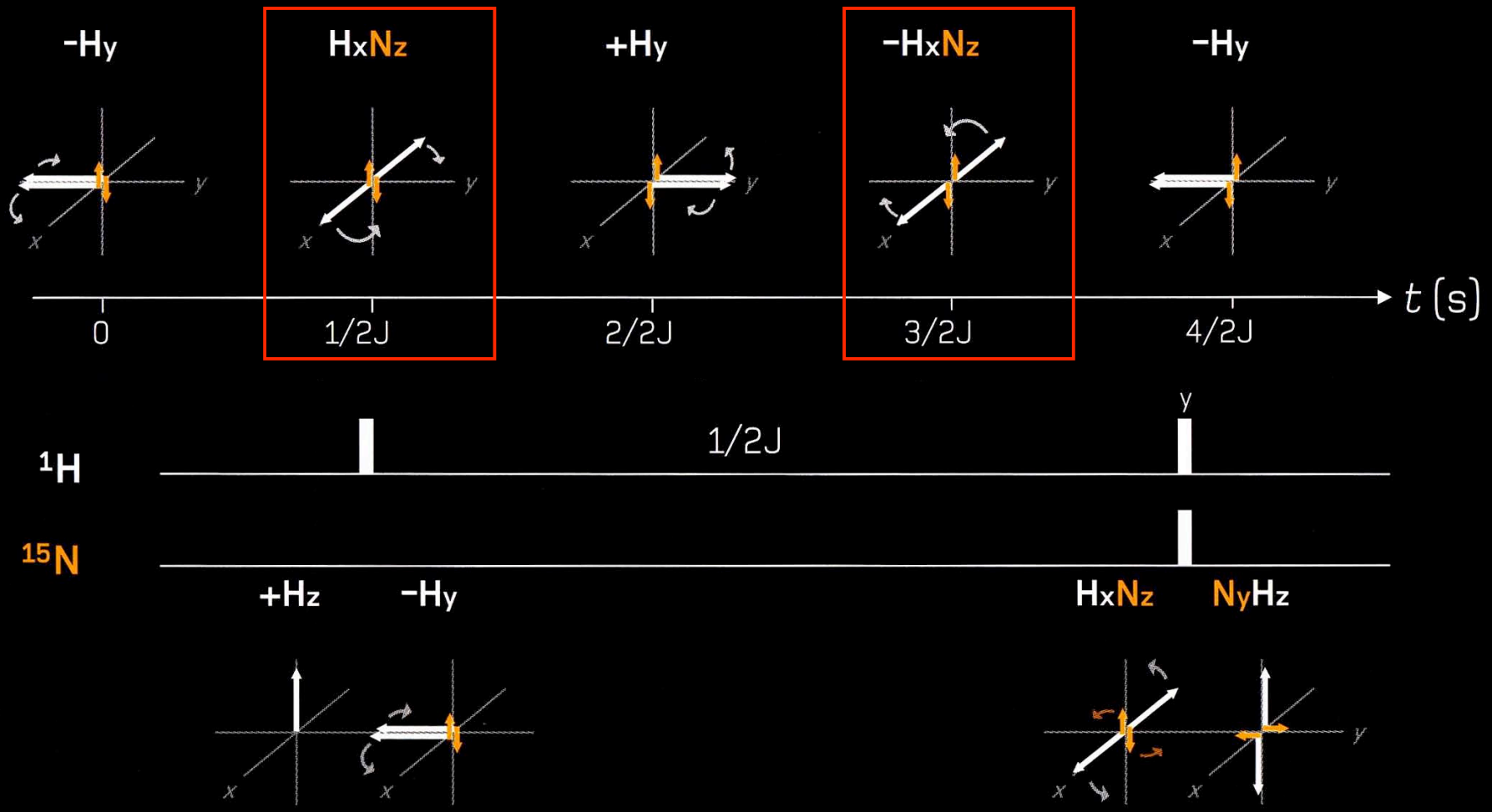
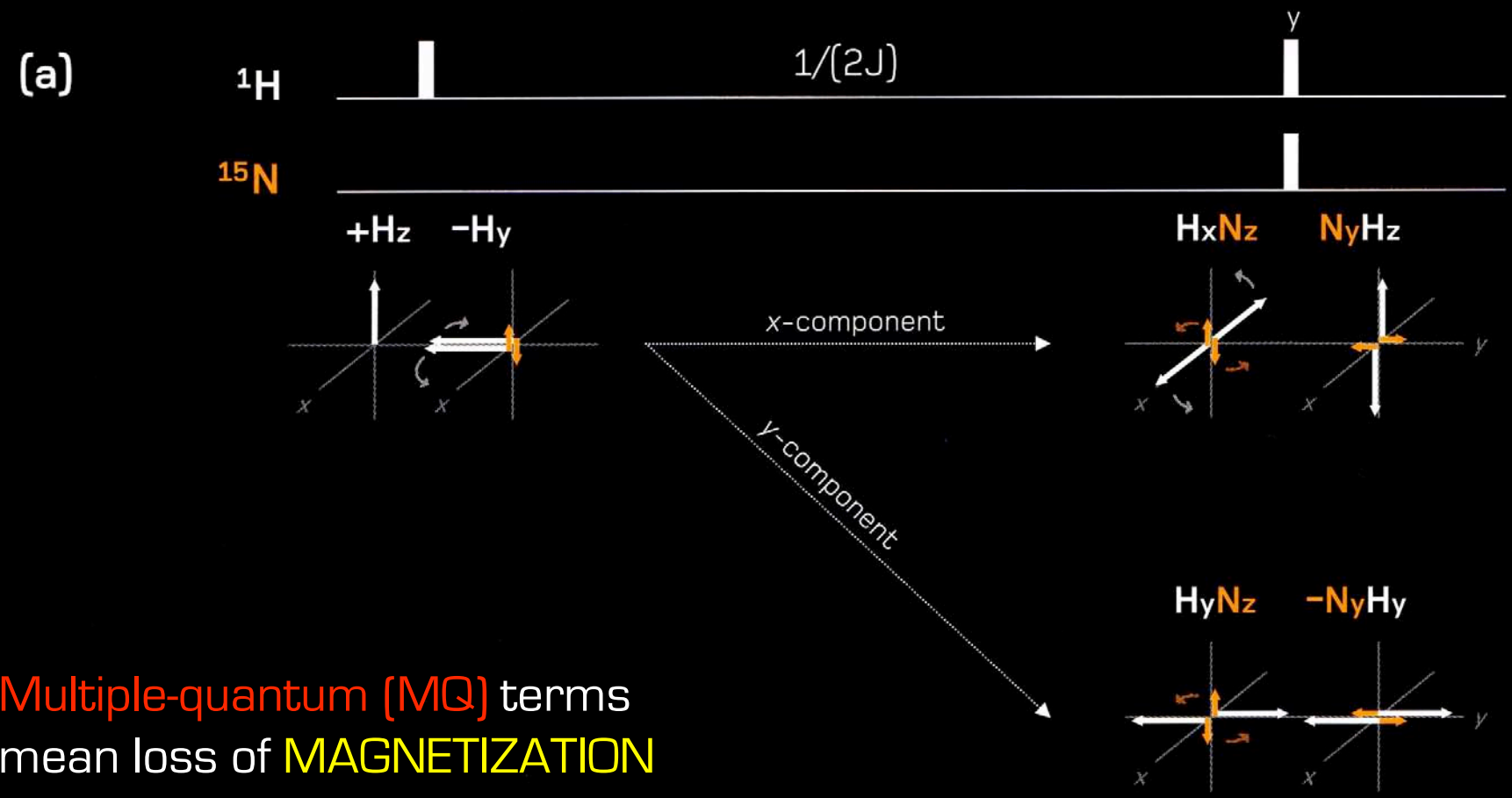


Figure 3.4. Magnetisation transfer: zero chemical shift. Evolution is due to $^{1}J_{HN}$ alone. After an initial 90° ^{1}H pulse, transverse ^{1}H magnetisation evolves for a period $1/(2\times{}^{1}J_{NH})$ and is then subject to simultaneous ^{1}H and ^{15}N 90° pulses. All available ^{1}H magnetisation is converted to transverse ^{15}N magnetisation (N_yH_z).

Animation 1+2

9. Effect of chemical shift evolution

Multiple-quantum (MQ) terms are generated (bad!)

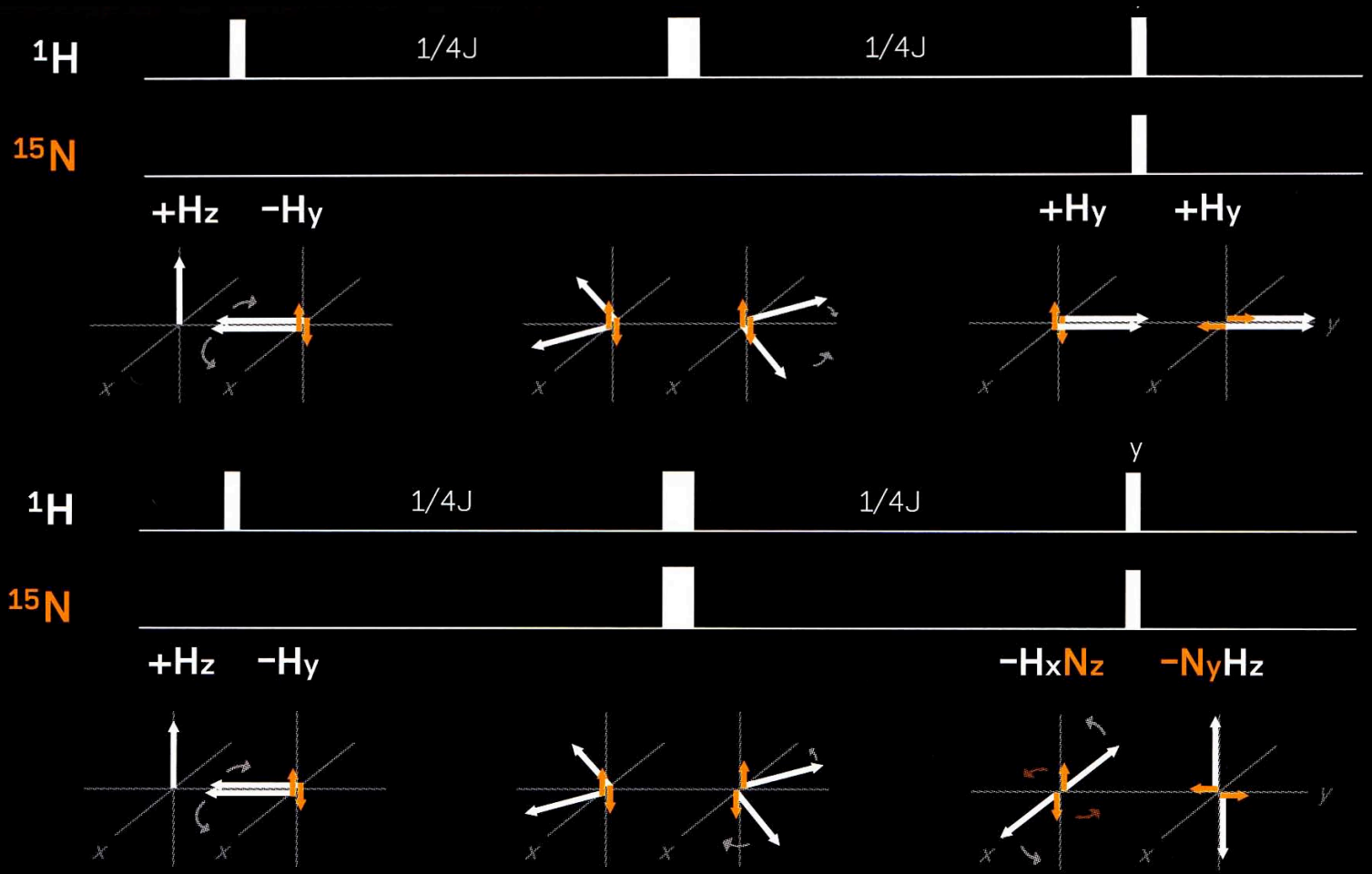


Multiple-quantum (MQ) terms
mean loss of MAGNETIZATION

Figure 3.5. Magnetisation transfer: non-zero chemical shift. Evolution is due to $^1J_{HN}$ and ^{1}H chemical shift. (a) A ^{1}H chemical shift sufficient to cause a 45° precession during the 1/(2J) period results in equal amounts of the desired N_yH_z term and the multiple quantum (MQ) $-N_yH_y$ term. The second term essentially represents lost magnetisation, reducing the sensitivity of the experiment.

10. How to solve the problem ...

In NMR, almost all unwanted things that happen during pulse-sequences can be 'REMOVED' by 180deg. PULSES!



Animation 3+4+5

11. Why use $^{1}J_{NH}$ to create ^{15}N excitation at all?

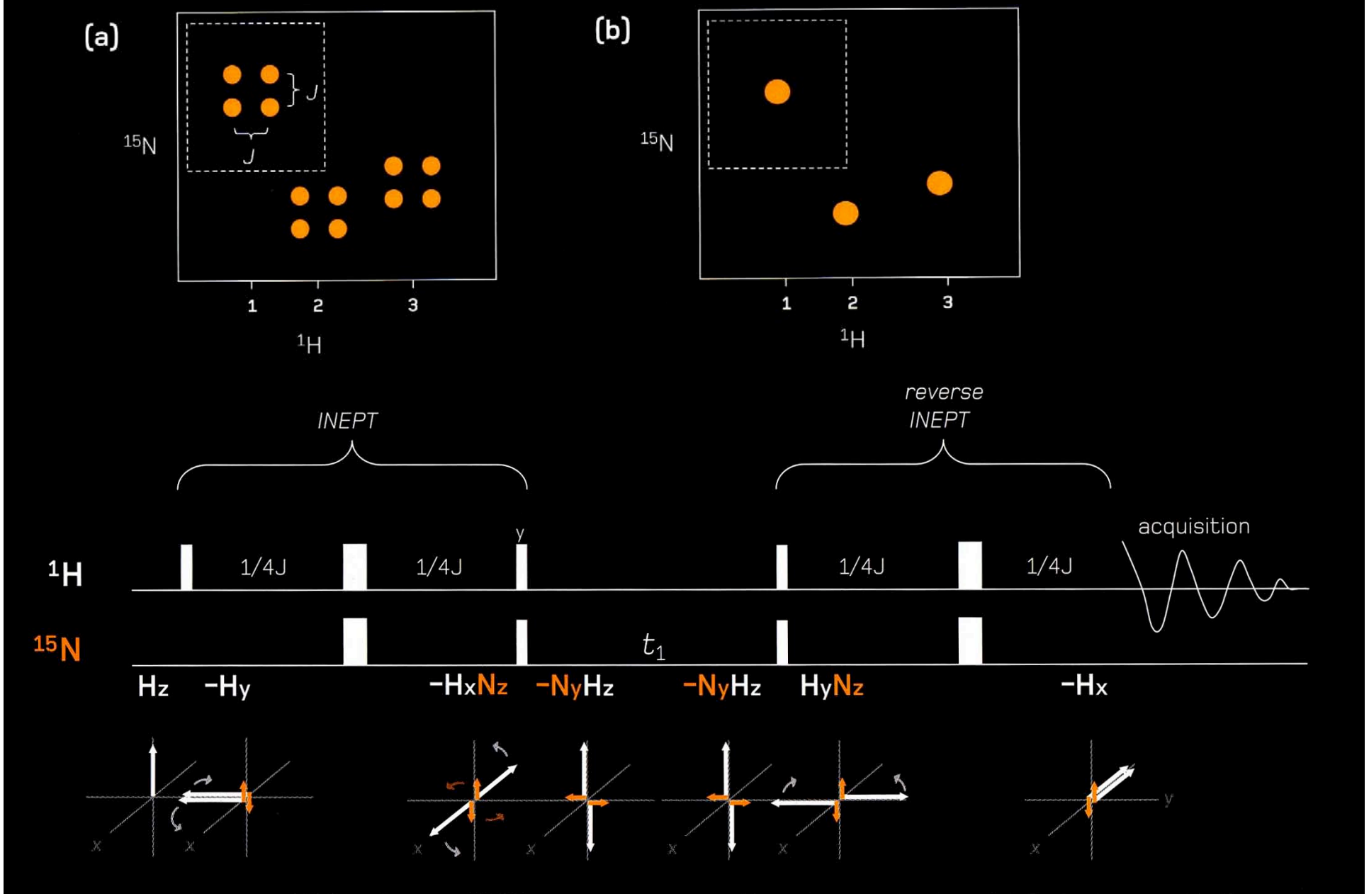
(... and not directly pulse on nitrogen?)

The advantages are 2-fold:

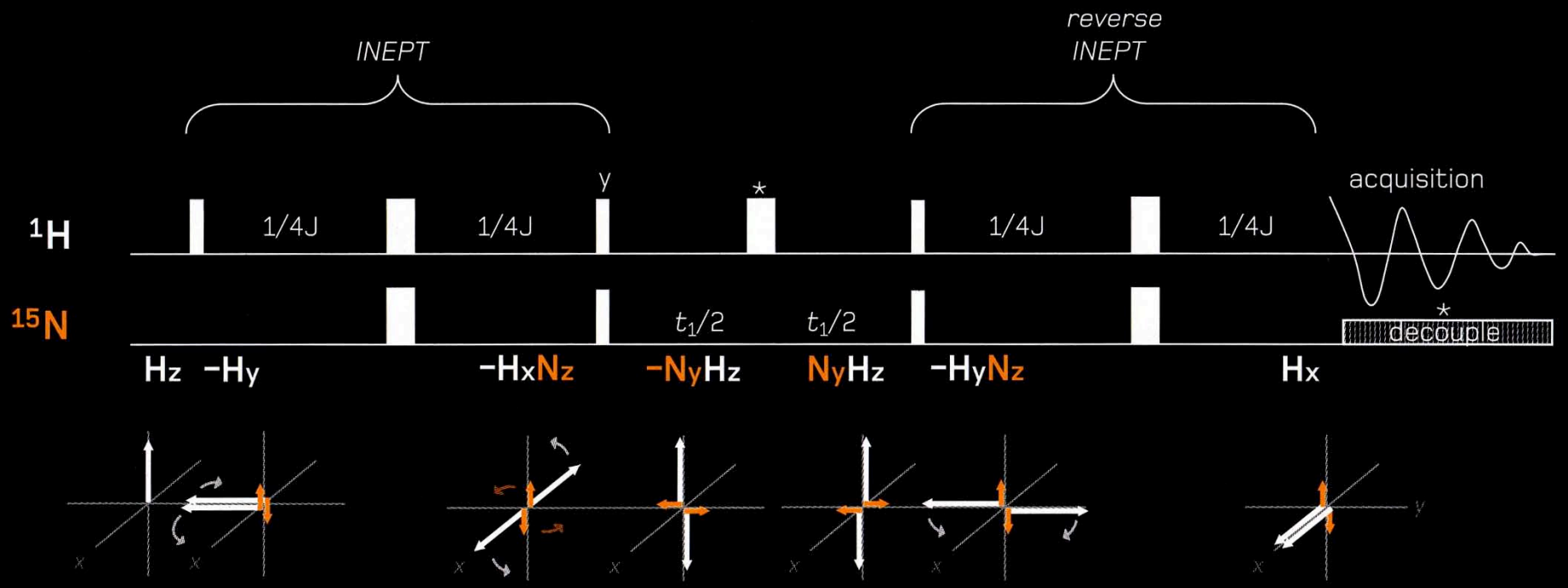
[1] Amount of ^{15}N EXCITATION produced via J-coupling is 10x GREATER compared to direct pulsing on nitrogen. (This is due to the differences in gyromagentic ratios between ^{1}H and ^{15}N and leads to the sequence name 'INEPT': Insensitive Nucleus Enhanced by Polarization Transfer)

[2] INEPT sequences allow to transfer magnetization TO- AND FROM- (back and forth) hetero-nuclei (hence the chemical shift information of correlated nuclei remains preserved!)

12. The 2D HSQC experiment



13. Decoupled 2D HSQC



Animation 6+7

14. Pulsed field gradients (PFGs)

Magnetization is de-phased along z > net magnetization = 0

Magnetization can be recovered by inverse gradients

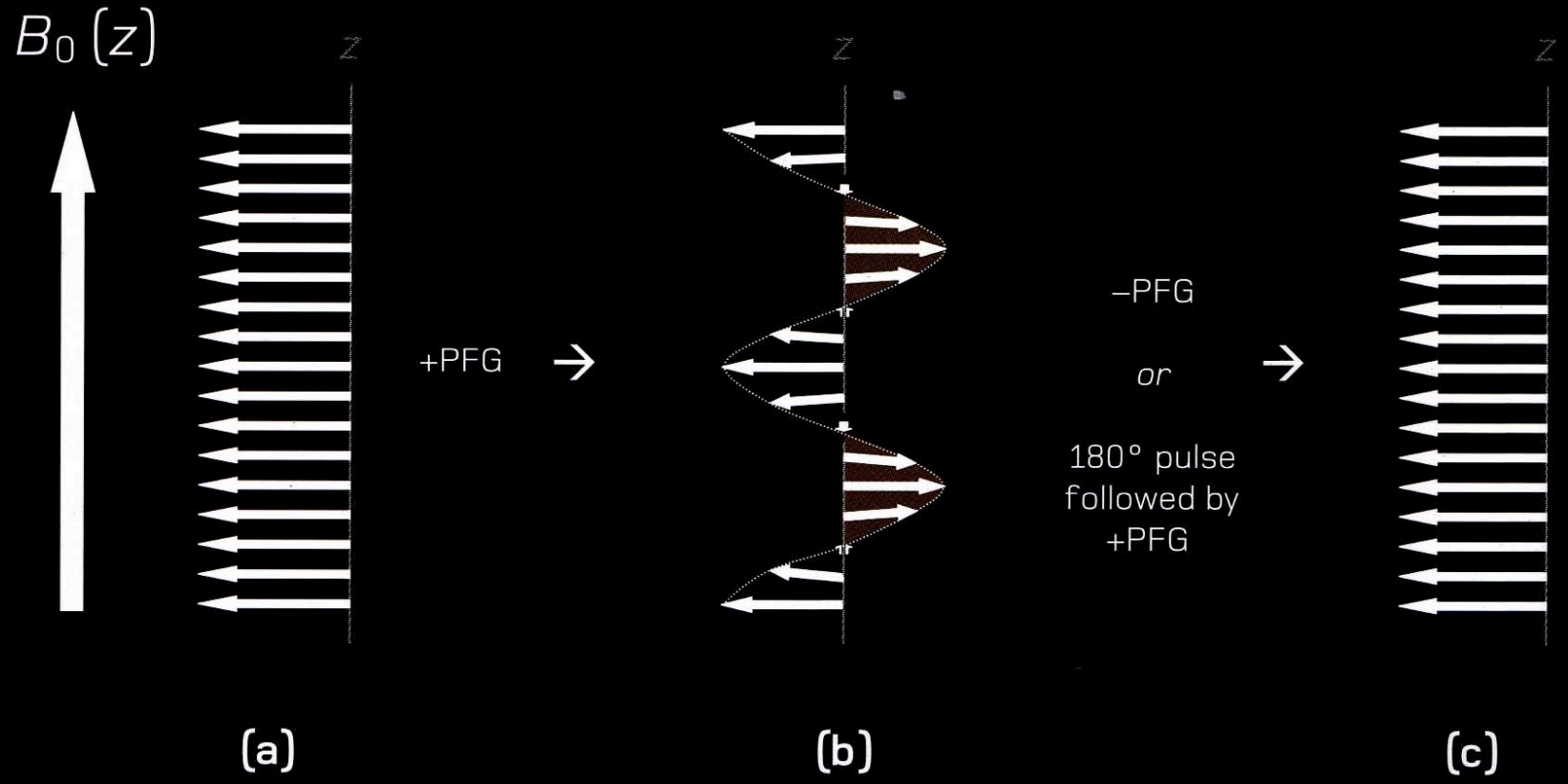
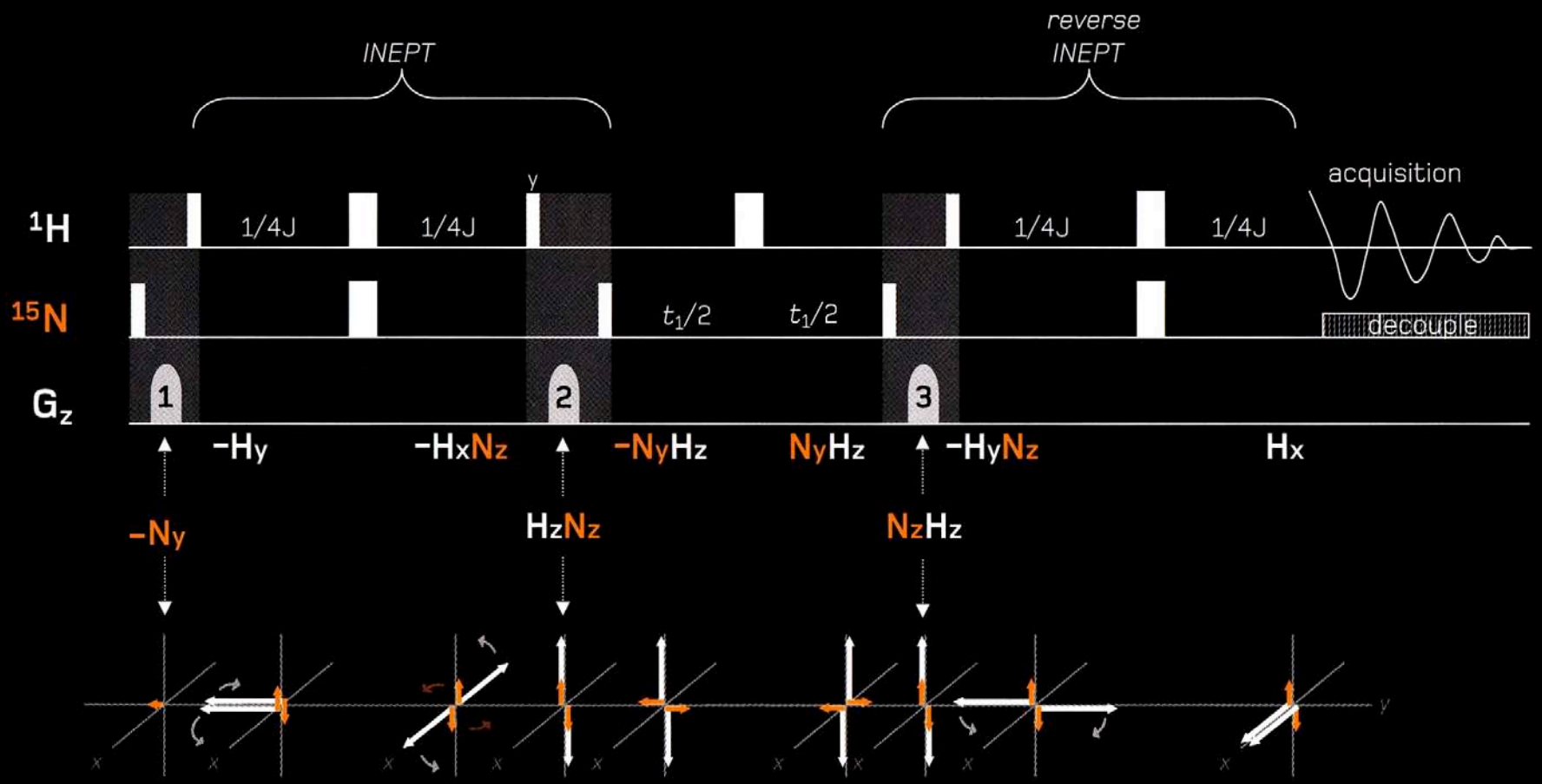


Figure 4.4. Pulsed field gradients. (a) Magnetisation from equivalent spins precessing in concert. (b) A PFG causes the magnetisation to fan out in the *xy*-plane as a linear function of *z*-position within the sample. The magnetisation vectors resemble a spiral staircase, and no net magnetisation is present. (c) An equal and negative PFG, or alternatively a 180° pulse followed by an equal PFG of the same sign, can be used to refocus the spins if desired.

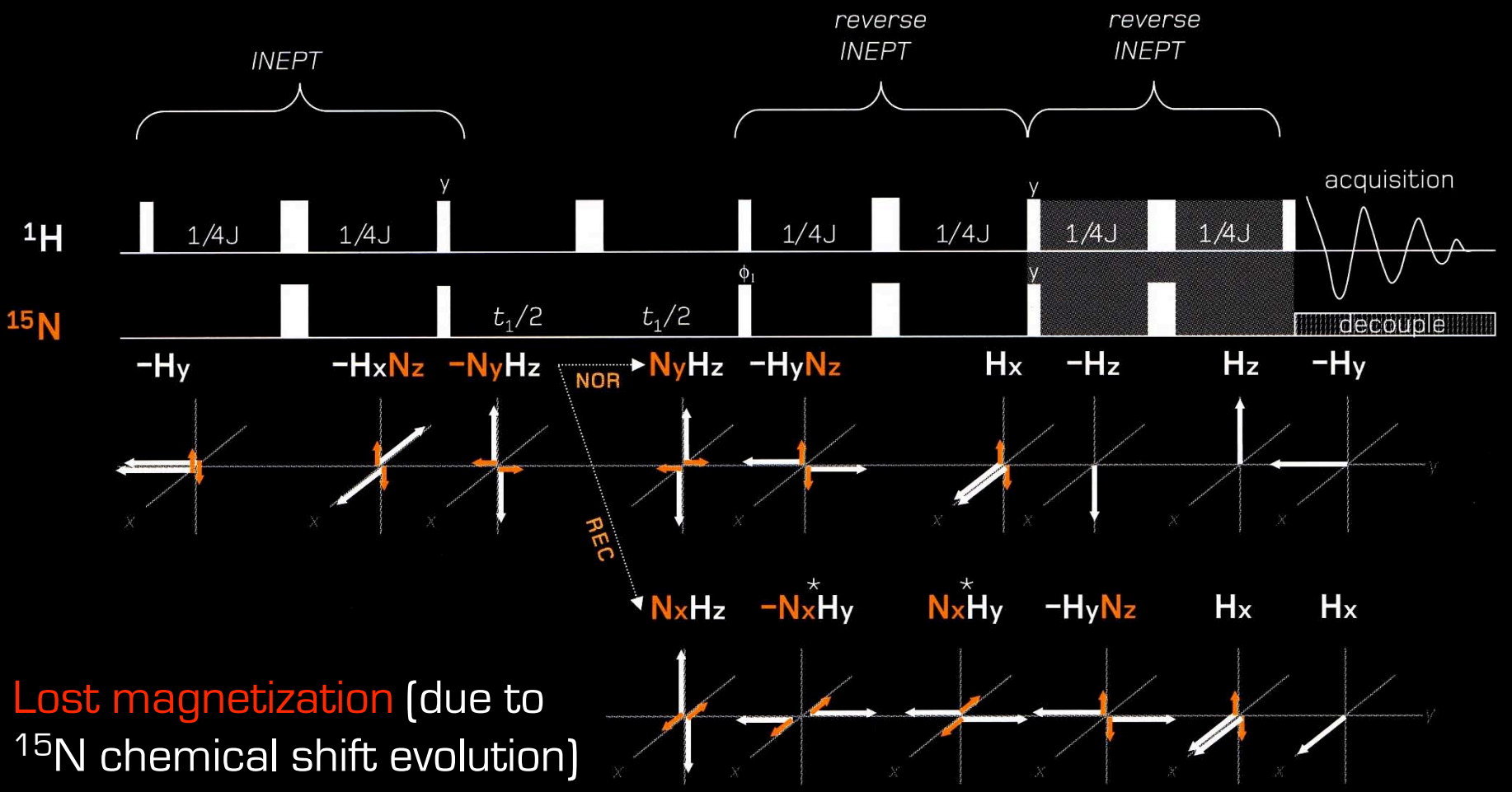
15. PFG-HSQC (decoupled)



Magnetization is briefly stored as MQ along z while SPOIL (or PURGE) GRADIENTS remove any UNWANTED MAGNETIZATION that might still be present along x and y.

16. SE-HSQC (decoupled)

Sensitivity Enhanced HSQC

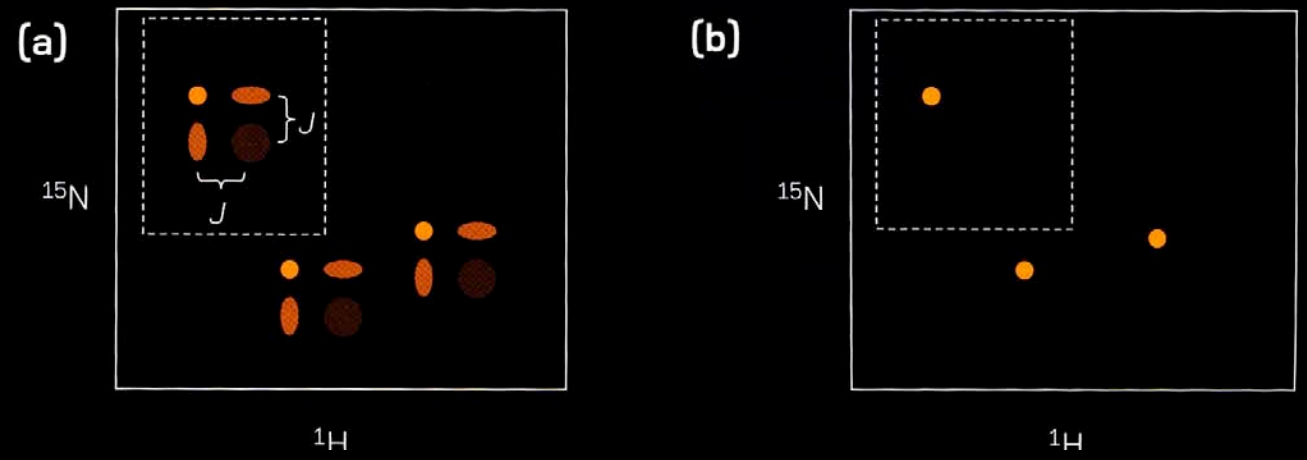


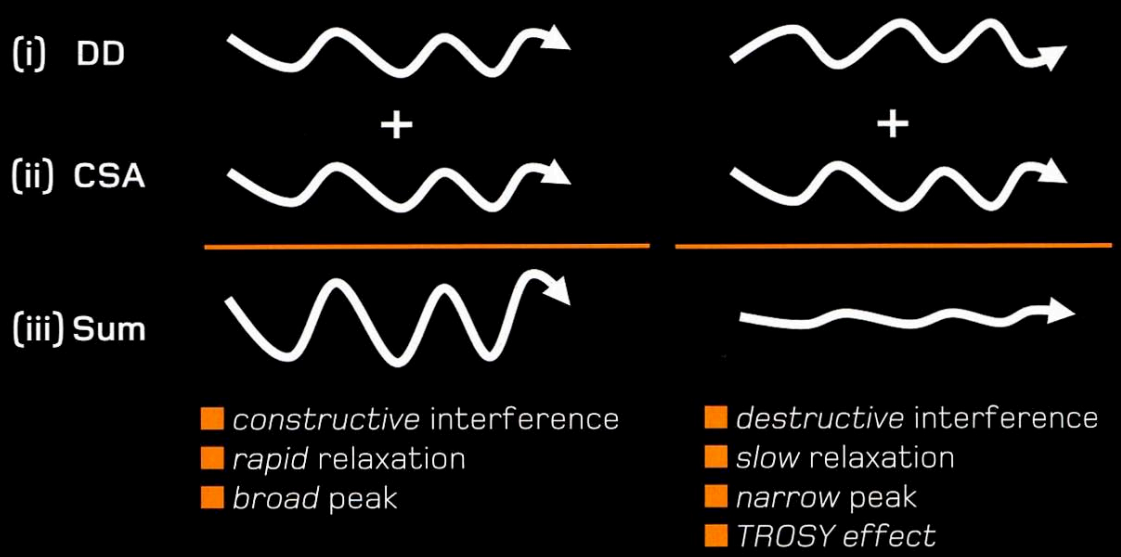
Lost magnetization (due to ^{15}N chemical shift evolution) is recovered via a second INEPT sequence.

Animation 8+9

17. TROSY-HSQC (non decoupled)

Transverse Relaxation Optimized Spectroscopy HSQC





The goal of a TROSY experiment is to design the pulse-sequence in a way to only select for the DD, CSA destructive interference peak (also called the TROSY peak).

17. TROSY-HSQC (non decoupled)

Transverse Relaxation Optimized Spectroscopy HSQC

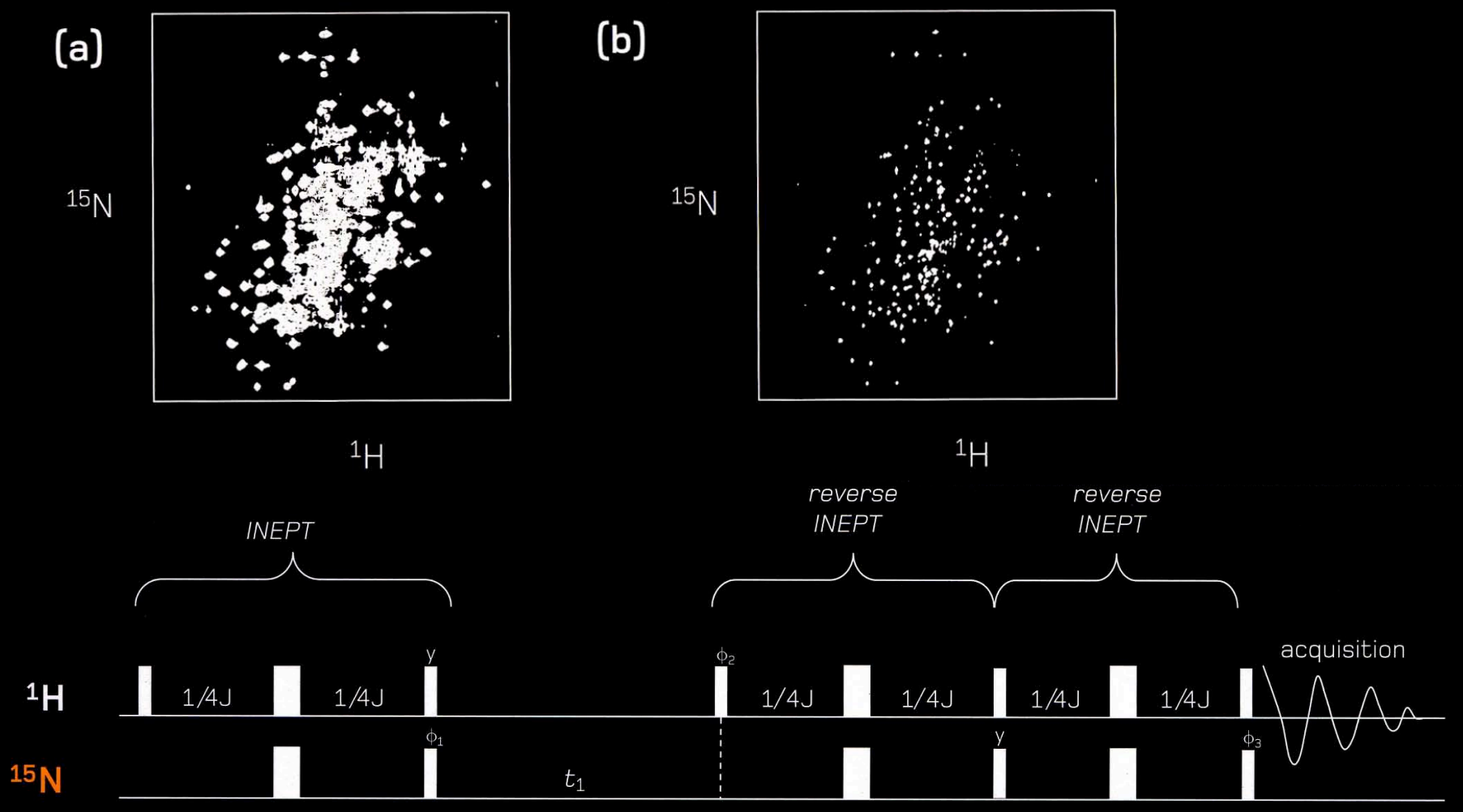
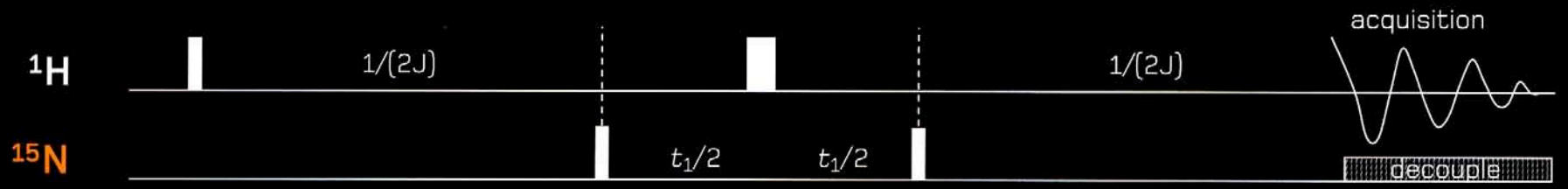


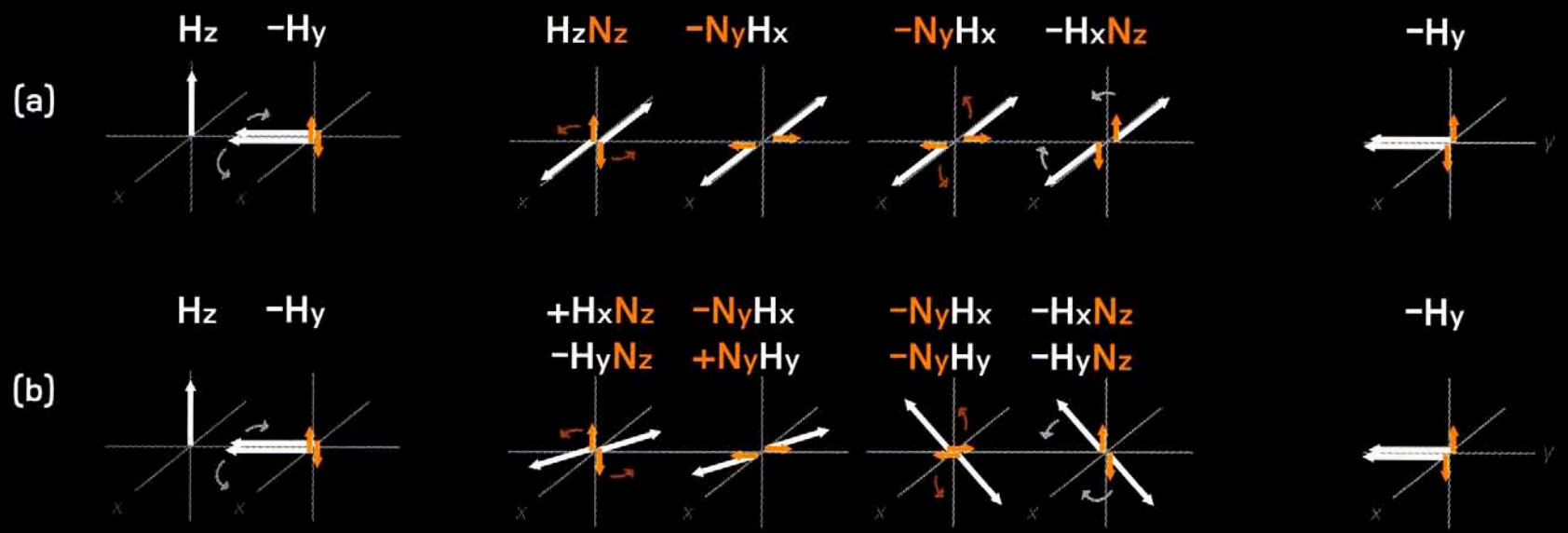
Figure C.4. TROSY HSQC. Decoupling is omitted in each dimension to prevent mixing of the broad and narrow multiplet components. Selection of the narrow component is performed via phase cycling (pulses marked with ϕ_1, ϕ_2 and ϕ_3 are phase cycled).

18. 2D HMQC

Hetero-nuclear Multiple Quantum Coherence

A short 4-pulse only (!) sequence often used for ^{1}H-^{13}C correlations





Why then do we bother using mostly HSQC sequences?

18. 2D HMQC

Hetero-nuclear Multiple Quantum Coherence

A short 4-pulse only (!) sequence often used for ^{1}H-^{13}C correlations

Why bother with HSQC sequences?

(1) During t_1 the $-N_yH_x$ and other MQ terms relax more rapidly than the $-N_yH_z$ and the other anti-phase terms present during t_1 in the HSQC sequence.

Why?

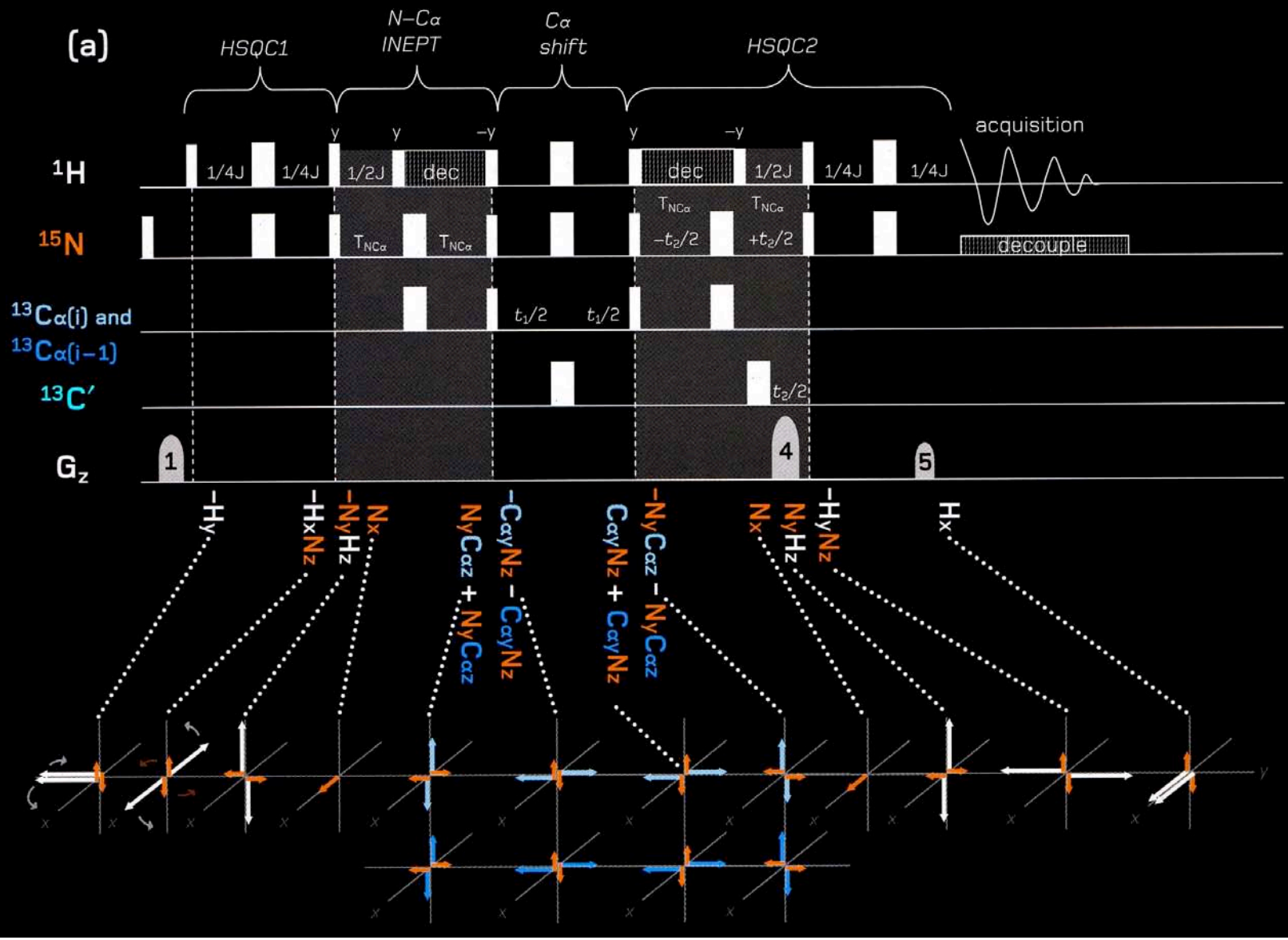
H_x relaxation is due to ^{1}H T_2 while H_z relaxation is due to ^{1}H T_1 and $T_2 < T_1$
(this is a bit oversimplified but will suffice as an explanation for now)

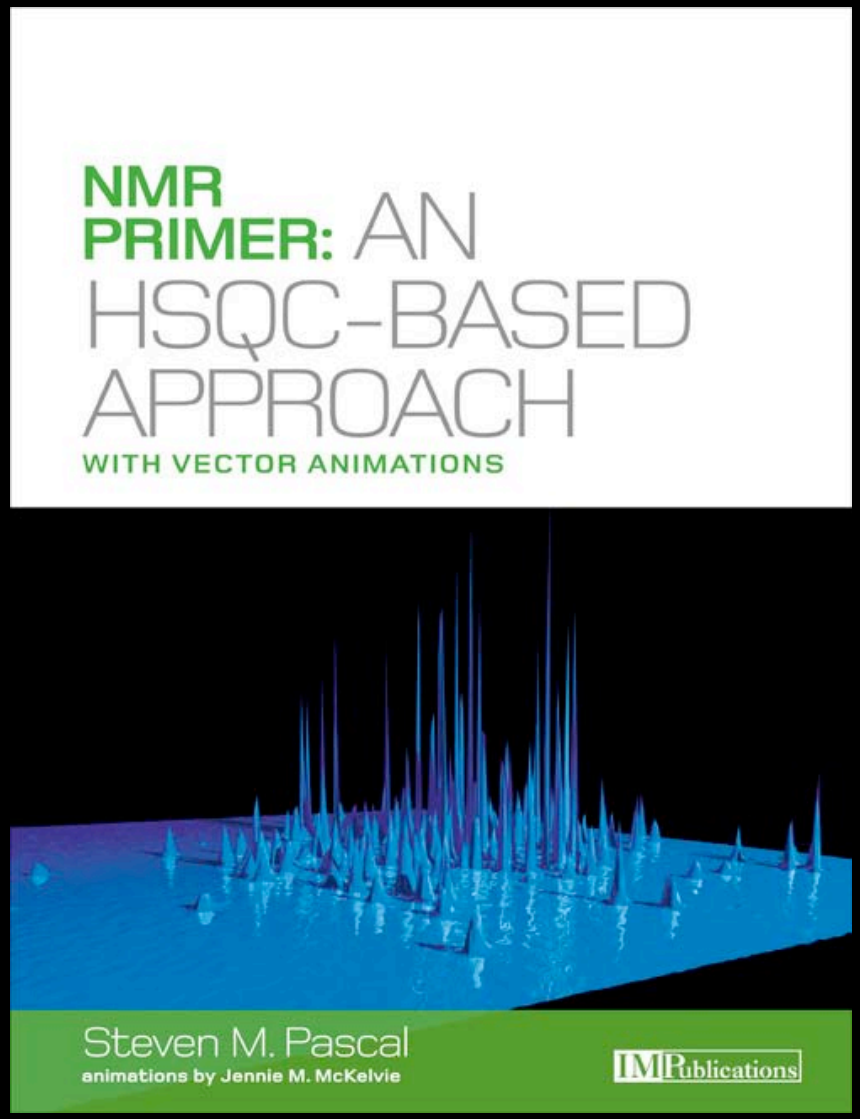
(2) In the HMQC the amid ^{1}H is transverse during t_1 and subject to $^{3}J_{HNH\alpha}$ coupling to the Hα proton (this is not refocused during the 180deg. pulse). Thus, the order of 10Hz (the value of $^{3}J_{HNH\alpha}$) is added to the ^{15}N line width.

Hence, NMR signals are usually more narrow in HSQCs!

18. … coming up next:

3D triple-resonance experiments (for backbone assignment)





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