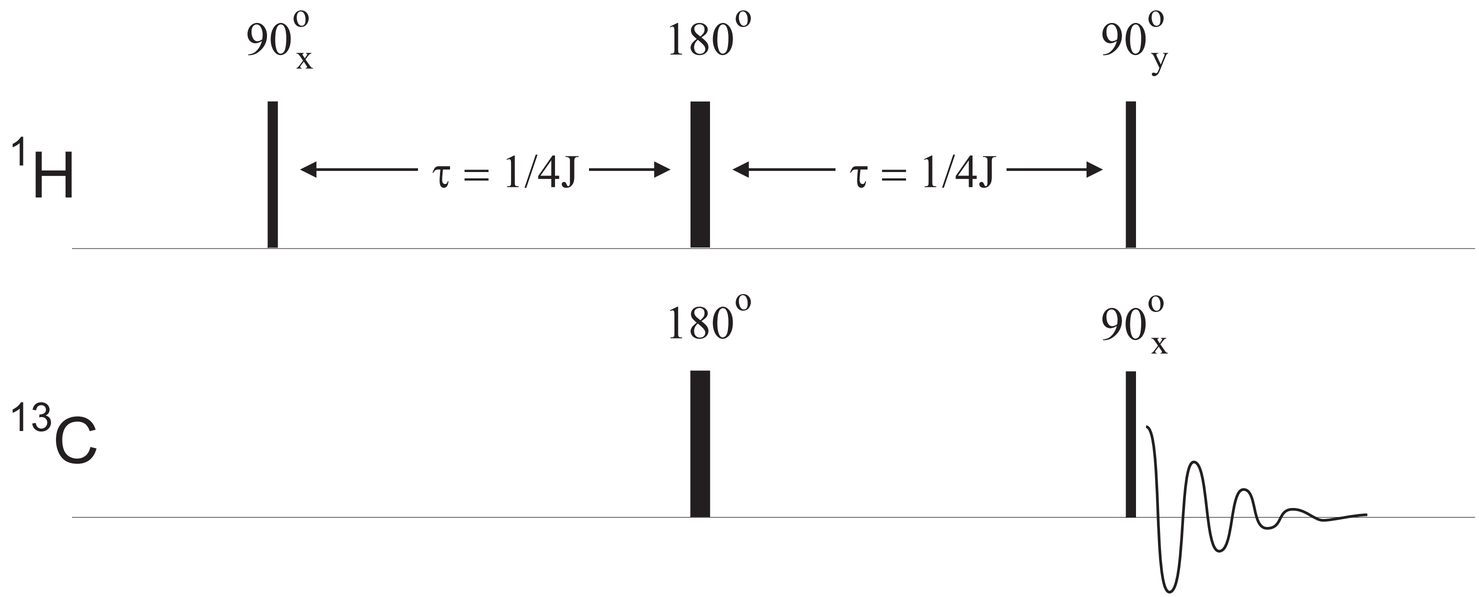
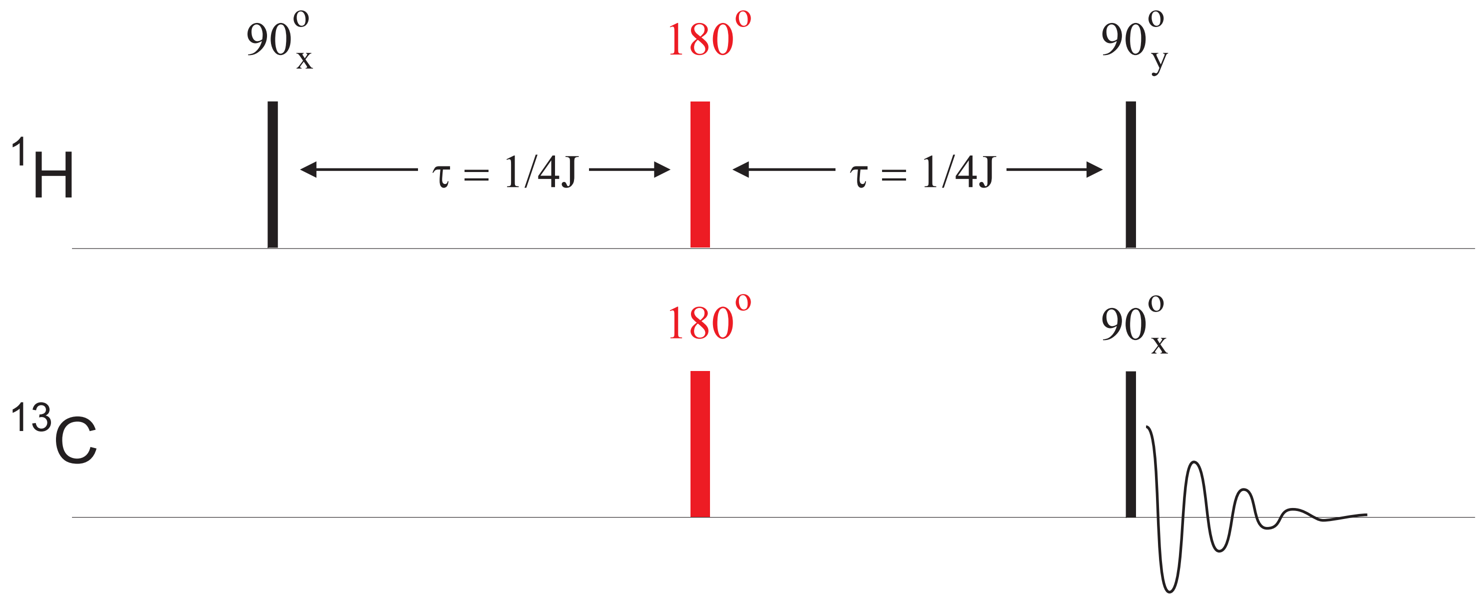
The INEPT Experiment – Front Section



For more, see Claridge's excellent discussion in section 4.4, pgs 114-126.

Biochem 800 goes into more detail on Product Operators.

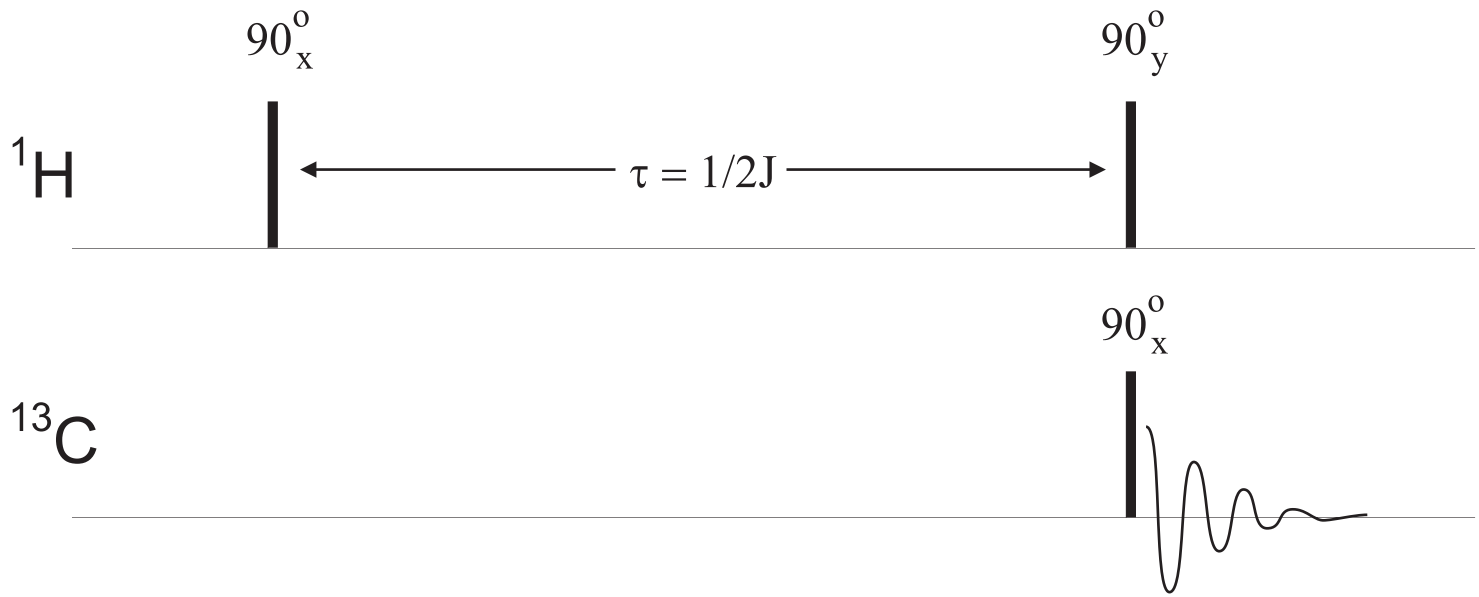
The INEPT Experiment



The ^1H 180^o pulse refocuses ^1H chemical shift.

The ^{13}C 180^o pulse, in combination with the ^1H 180^o pulse, prevents J_{CH} from being refocused; i.e., J_{CH} evolves throughout the 1/2J period.

The INEPT Experiment



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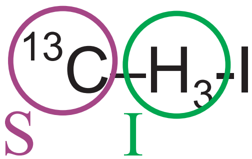
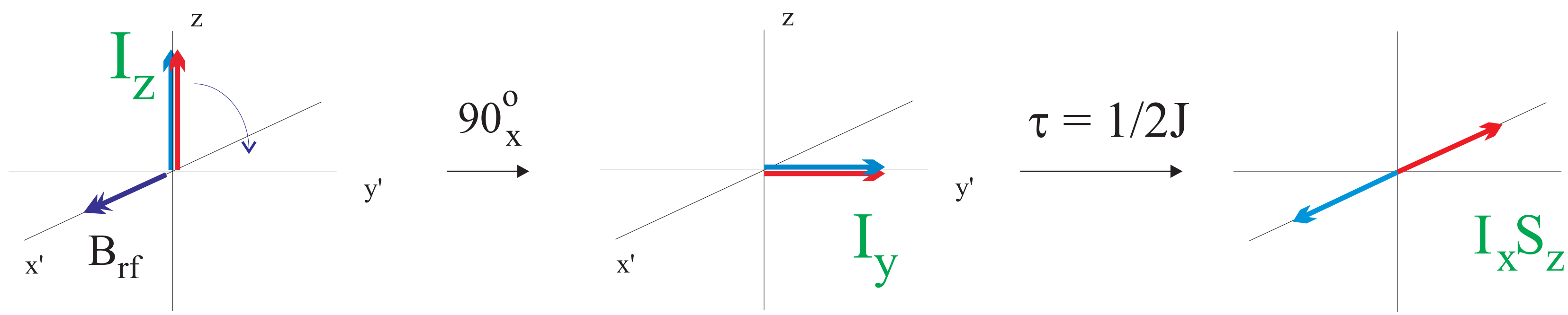
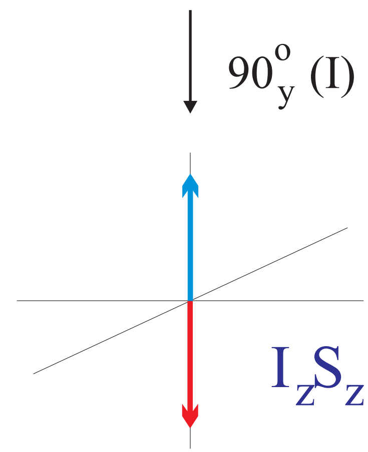
Chemical shift can therefore be ignored in further analysis. Only J_{CH} evolution is important, using the simplified schematic above.



Vector Description of the INEPT Experiment

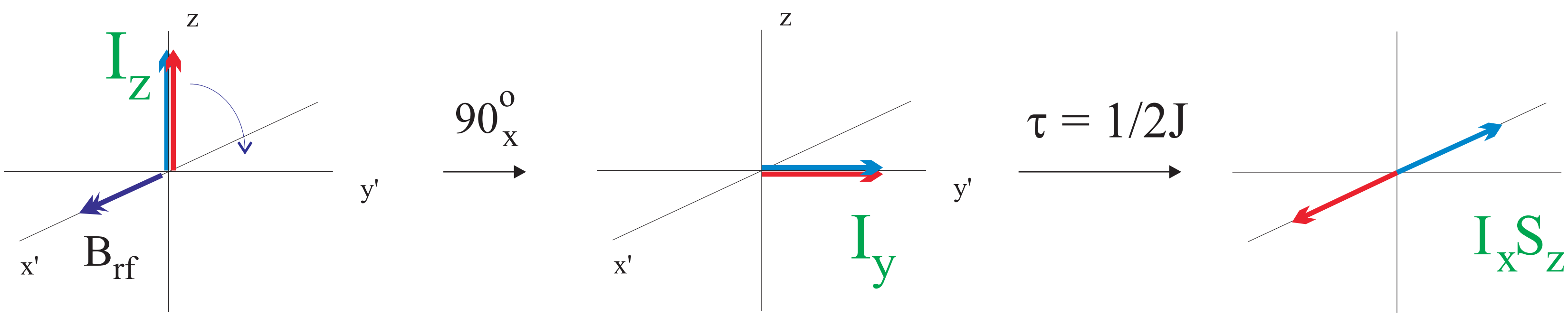


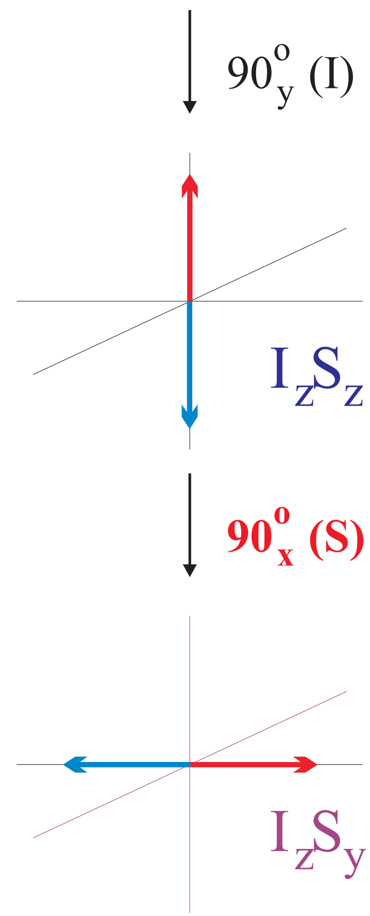


The key is that the proton population is inverted along only one of the two transitions.

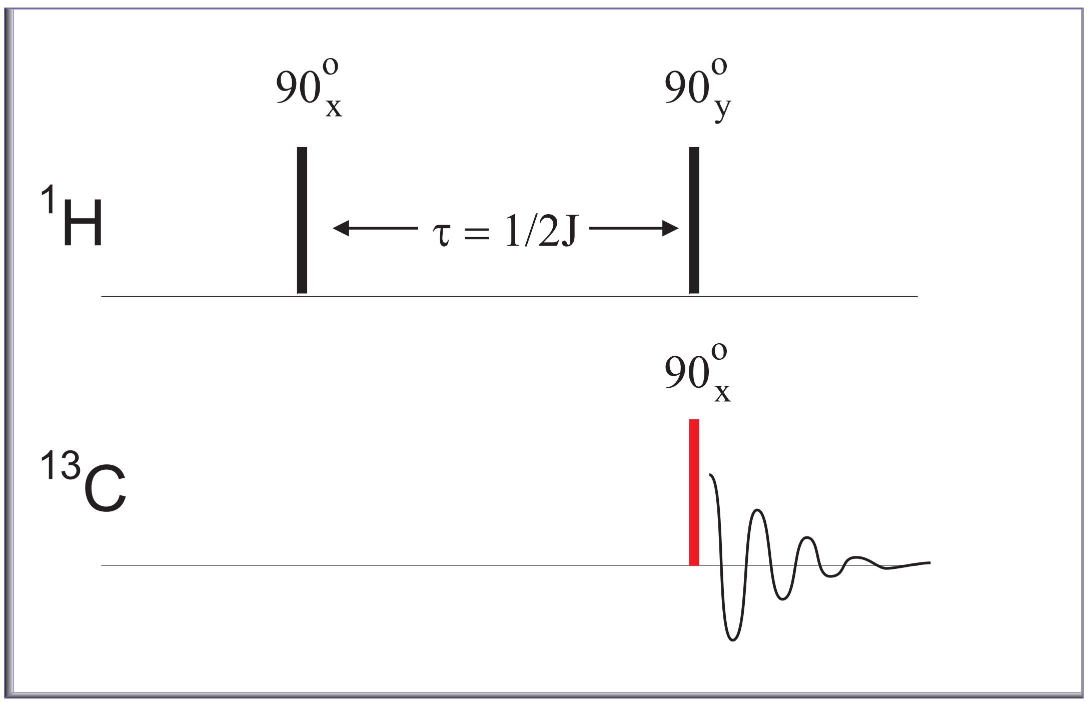
We are on a slippery slope with the vector description: it appears to be correct, but it works *only* for on-resonance magnetization (and is not a proper description even there). The vector presentation is used in introductory textbooks as it provides the simplest method of conceptualizing polarization transfer.

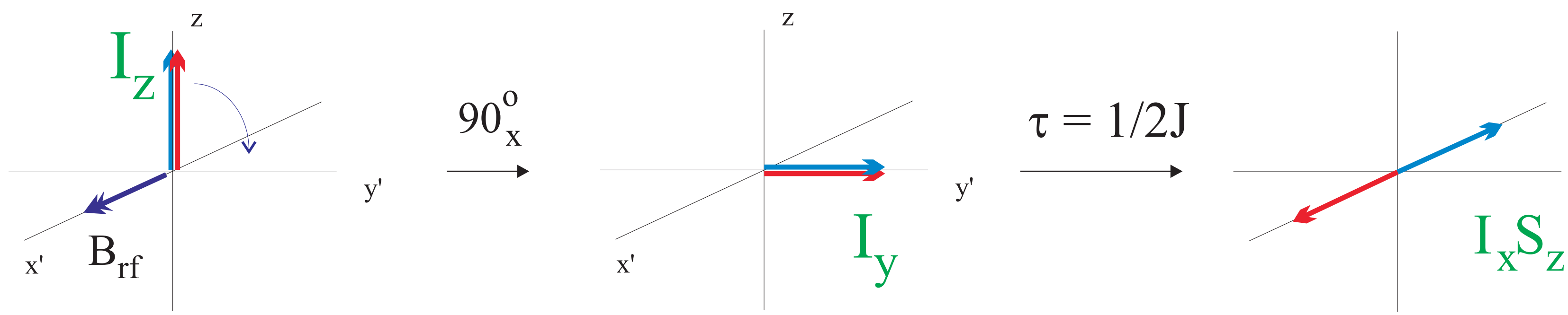
A product operator description is carried along — a few more details are given in Chem 637; see Biochem 800 for a fuller description — that properly describes the quantum mechanics of *antiphase magnetization* $\equiv I_zS_z$

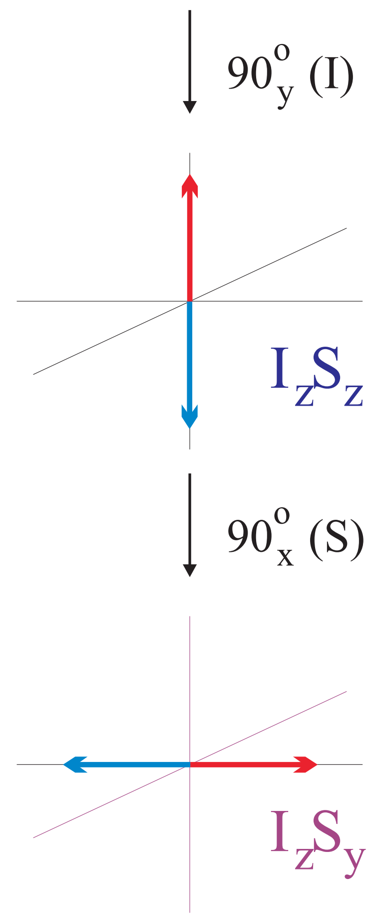




The final ^{13}C 90^o pulse transfers the magnetization to the carbon spin. Although the axes look the same, we have transformed into the carbon spin system (**S, not I, is now transverse!**).



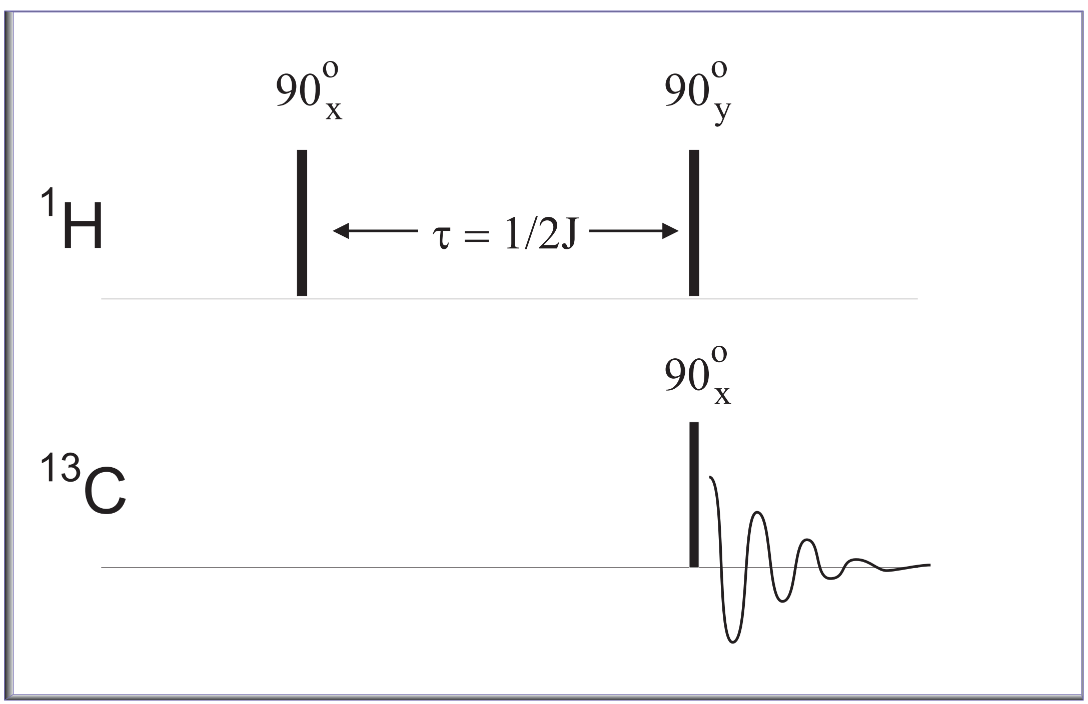


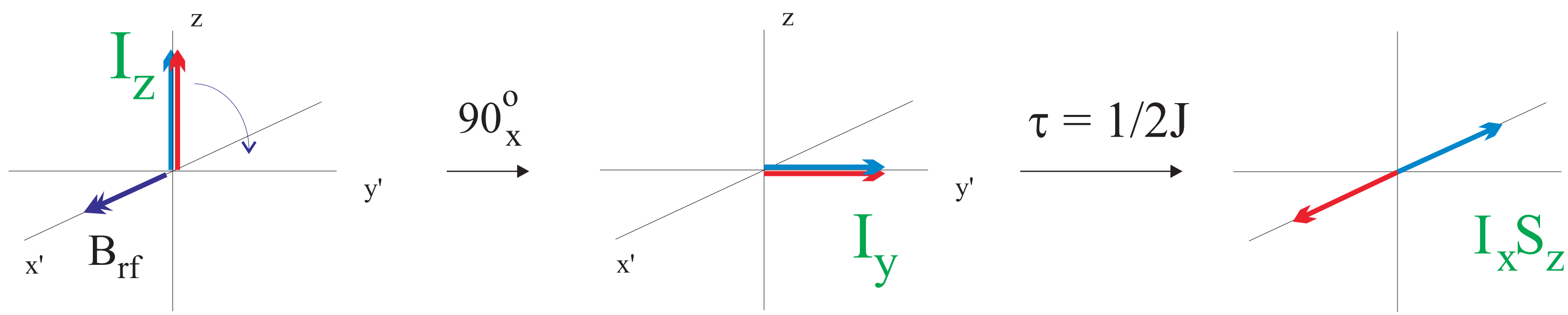


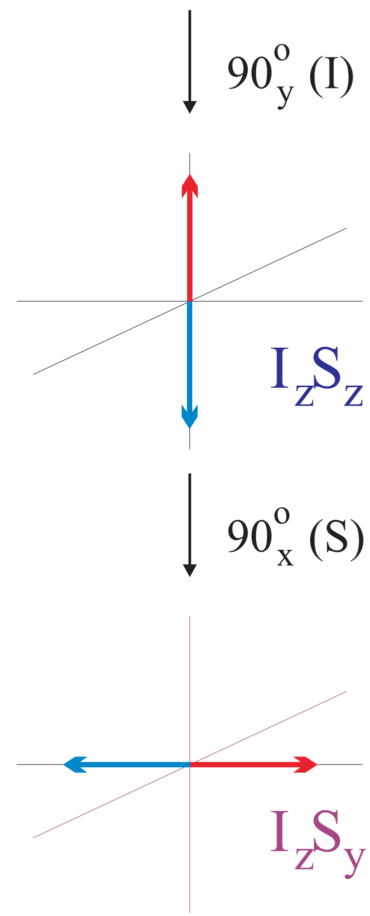
This completes the front end of the INEPT experiment:

I magnetization is transferred to **S** via J_{IS} coupling.

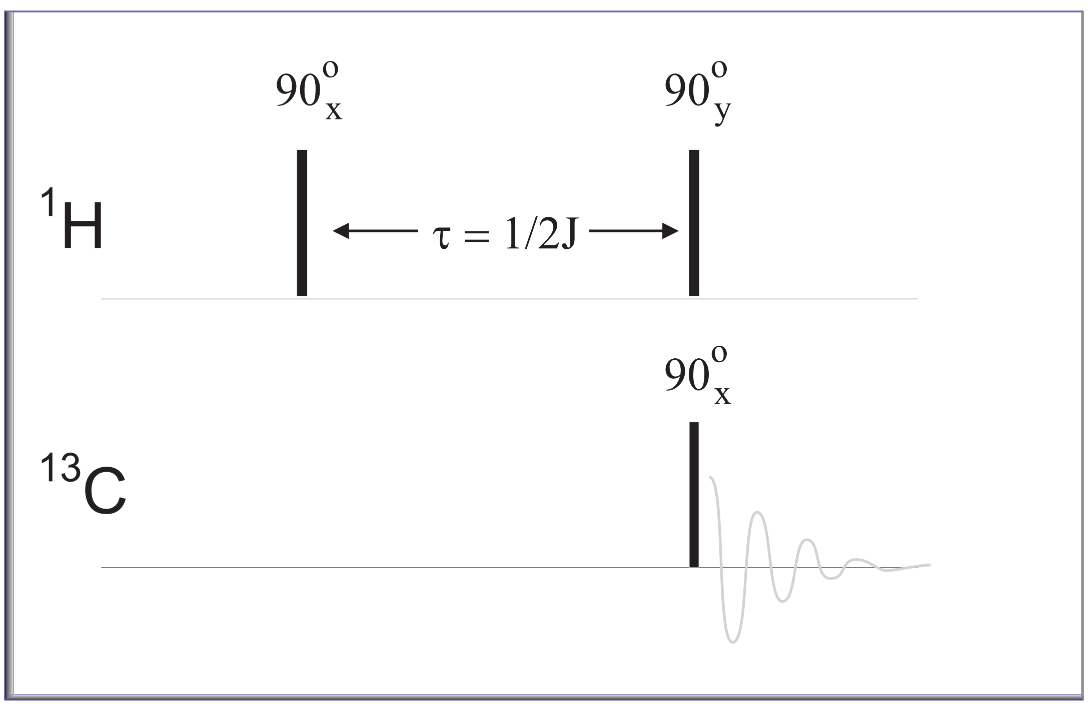
This basic building block (usually including 180° pulses) is used in many heteronuclear 2D and nD experiments (e.g., HSQC).

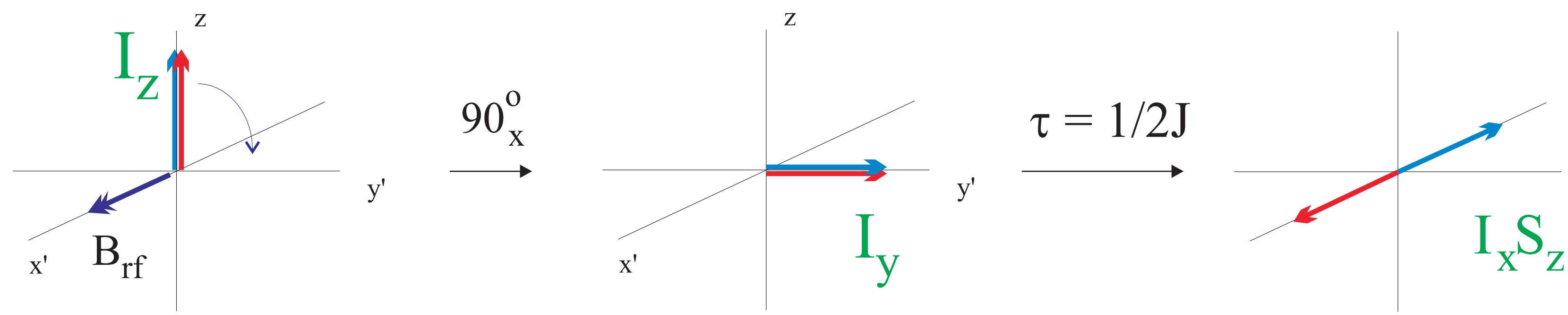


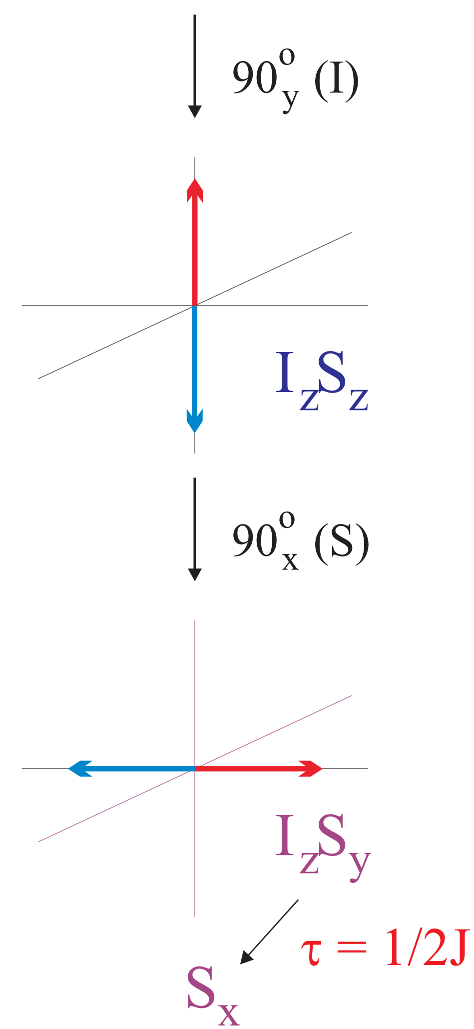




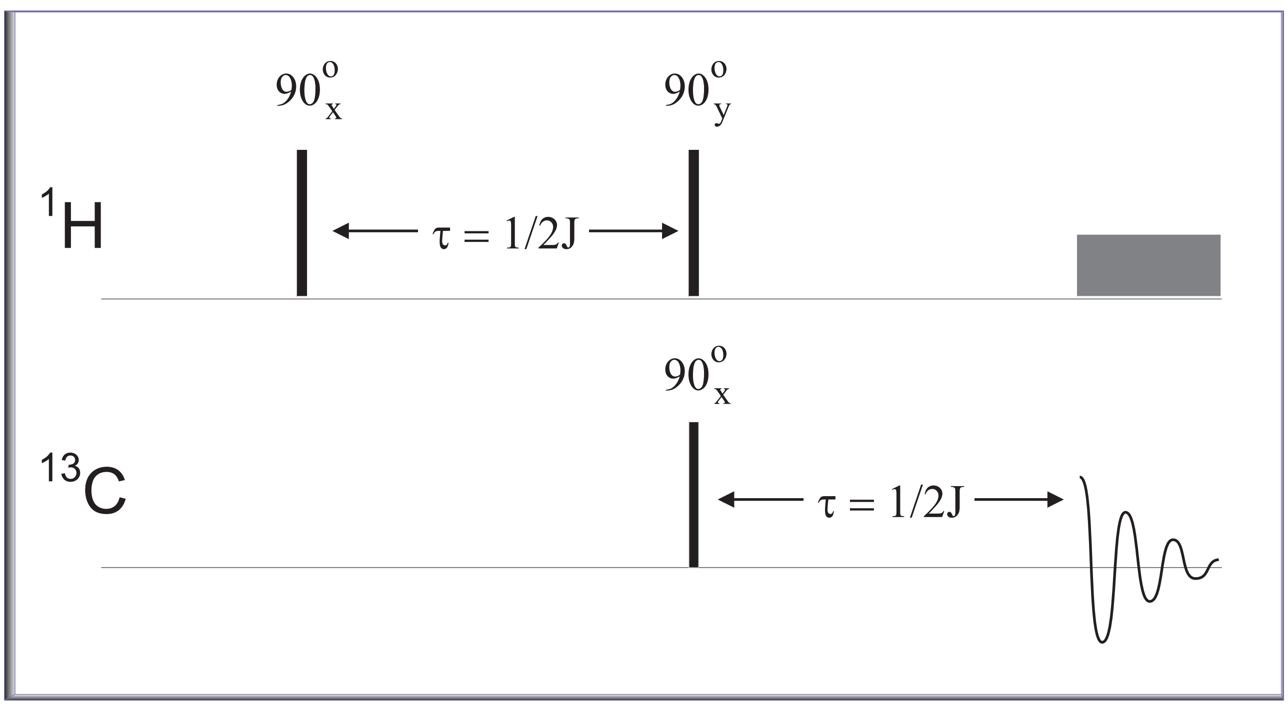
For INEPT, we could acquire data now, but the magnetization is antiphase. Decoupling can***not*** be applied at this point, as the antiphase signals are not observable (it's not correct to say they cancel, although it looks that way) under decoupling.

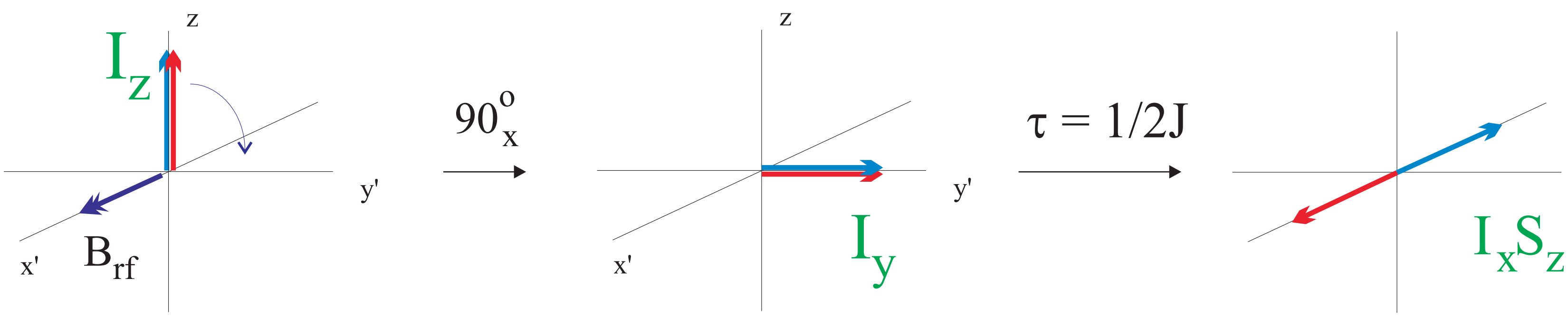


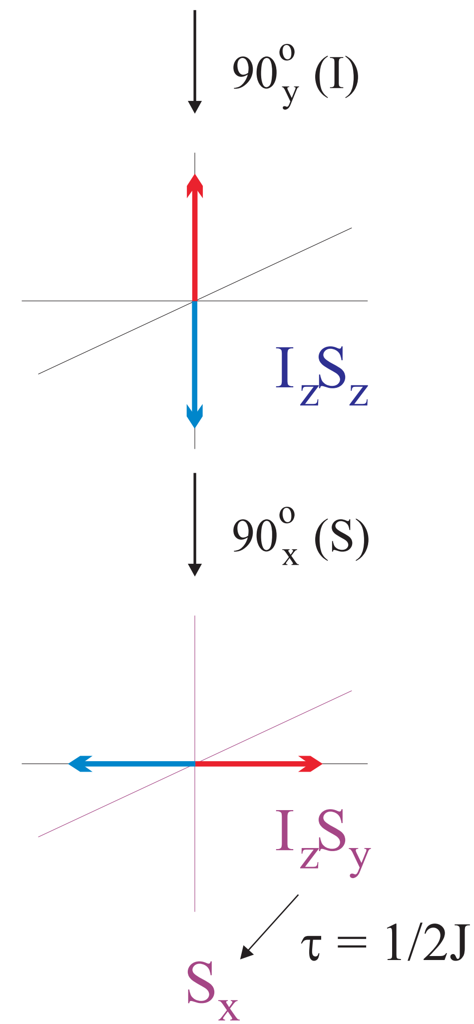




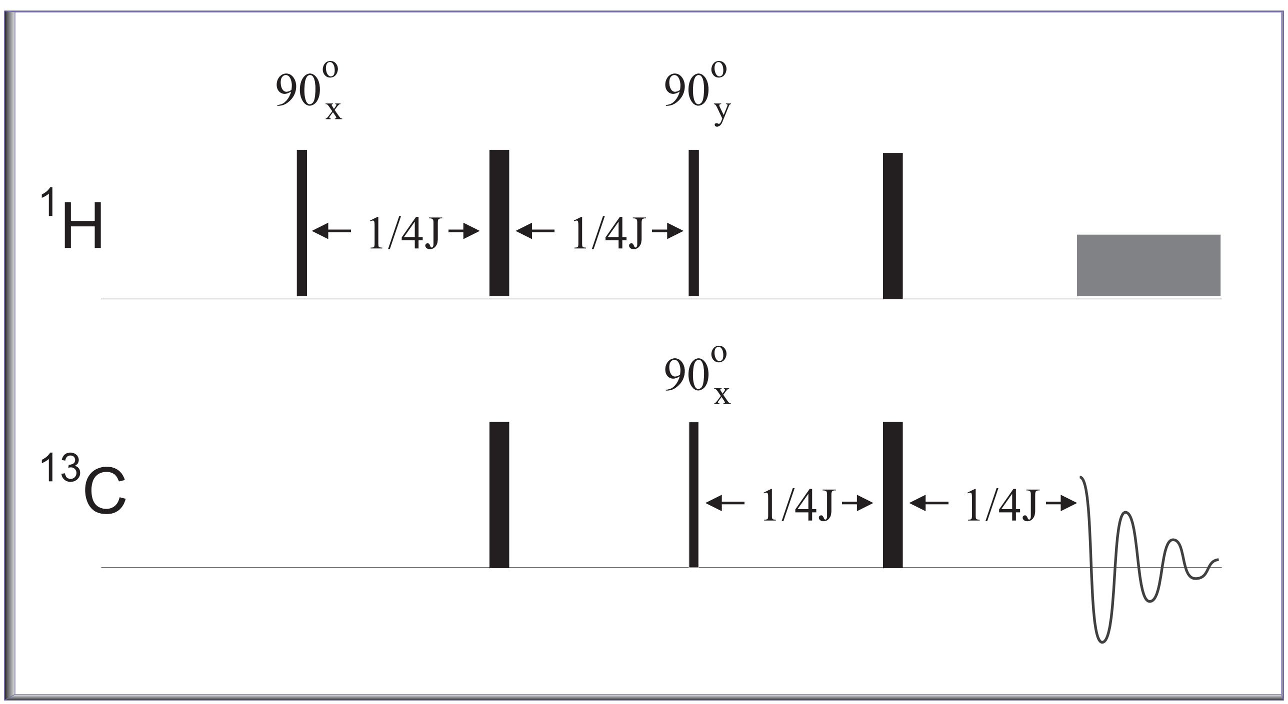
Addition of another 1/2J period allows I_zS_y to evolve into S_x, at which point ^{1}H decoupling can be applied, and ^{13}C signals can be observed.



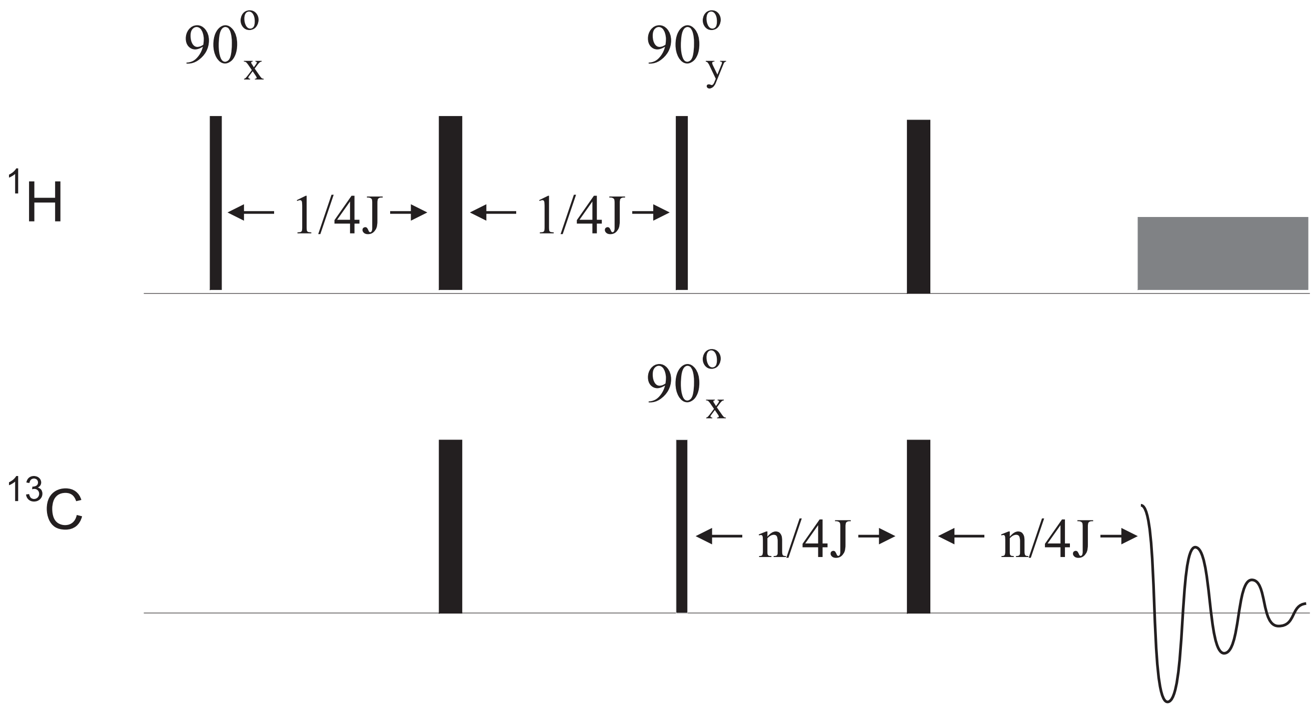




More generally (not ignoring chemical shift), two more 180° pulses are inserted midway in the last period, to refocus chemical shift. The final delays are modified to provide for multiplicity analysis (# of attached protons).

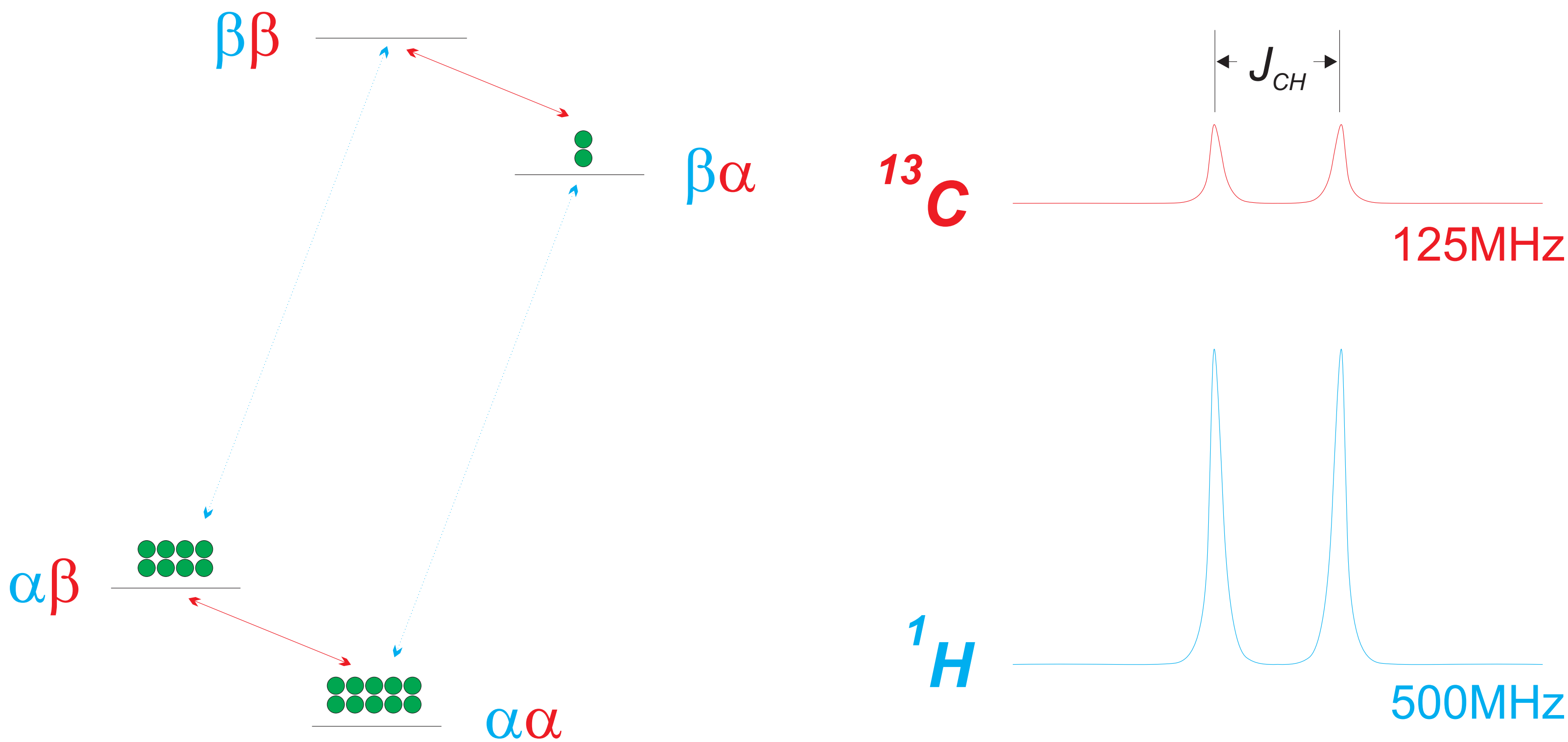


The INEPT Experiment

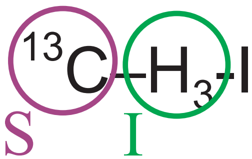


The previous vector and product operator discussion completely avoided the crucial issue of sensitivity in the INEPT experiment. The simplest approach for this is to consider populations, or polarization.

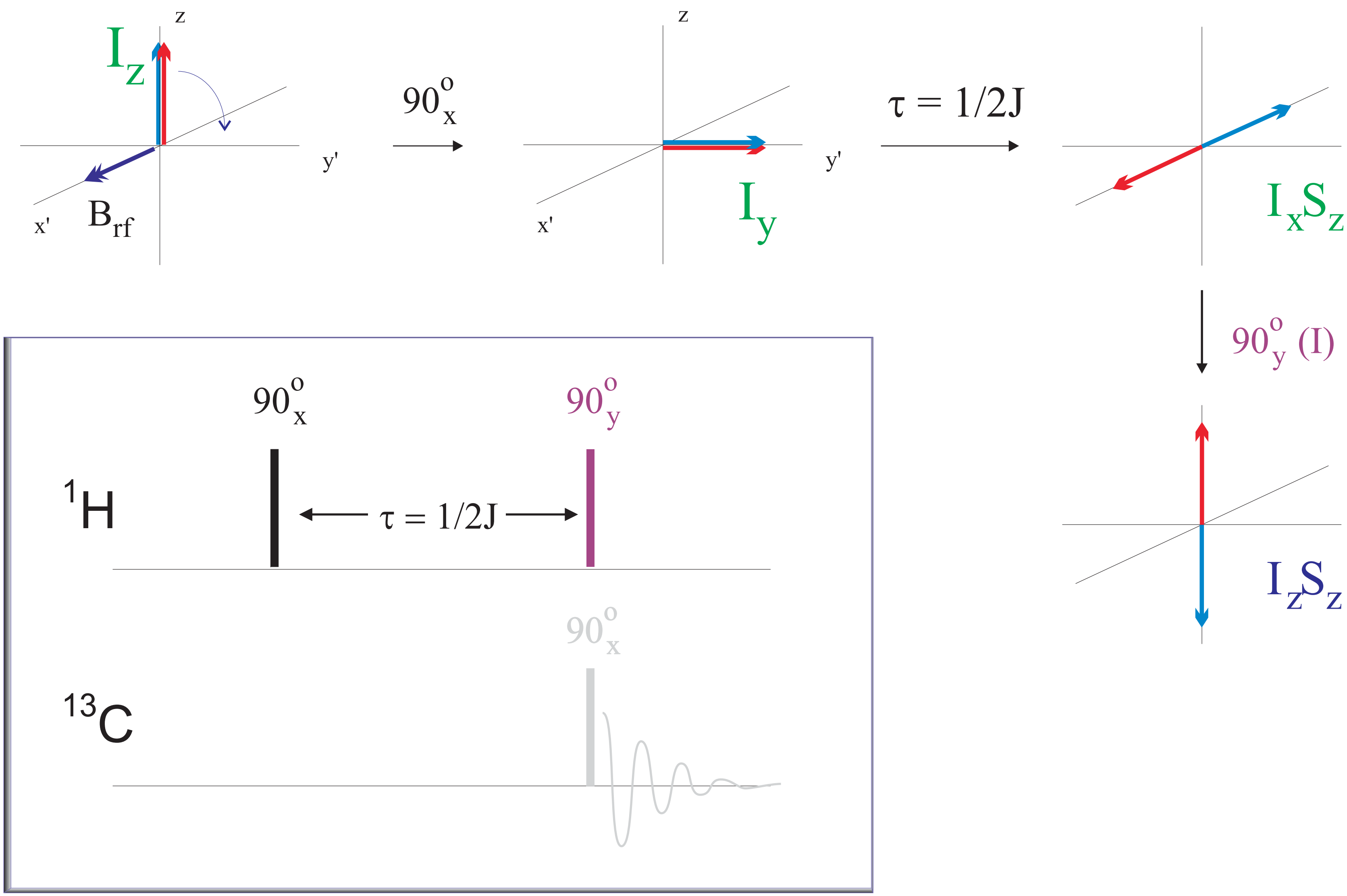
Population Description for a Two-Spin Heteronuclear System



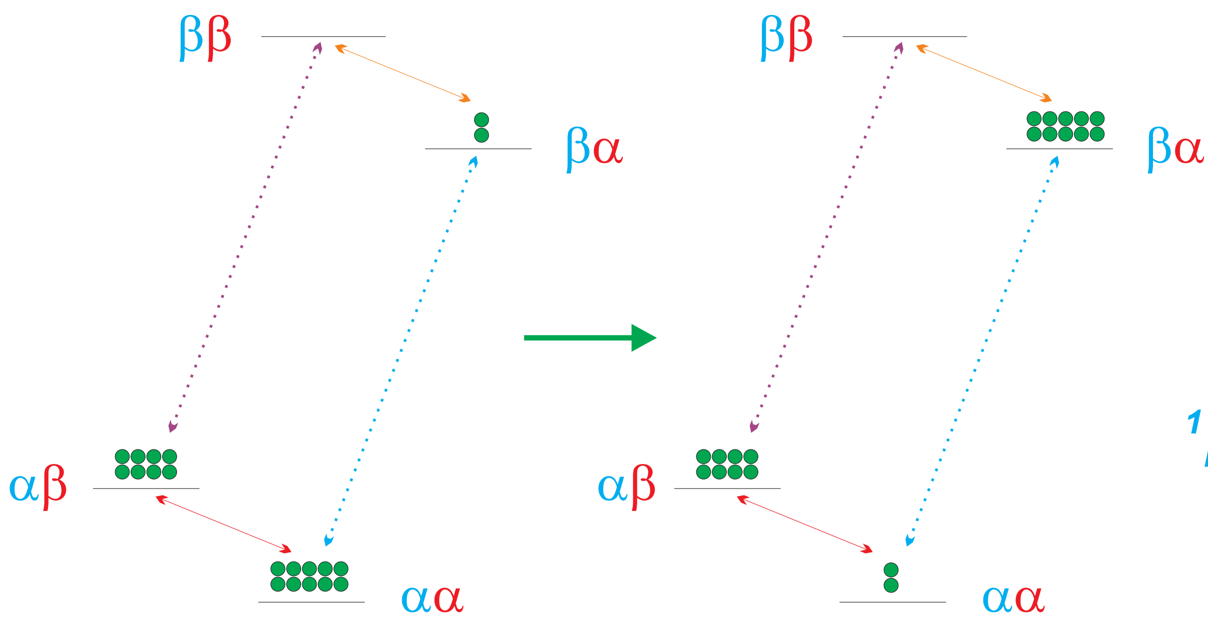
Here is a representation of the populations at equilibrium. Consider what happens in the INEPT sequence at the point of the I_zS_z state.

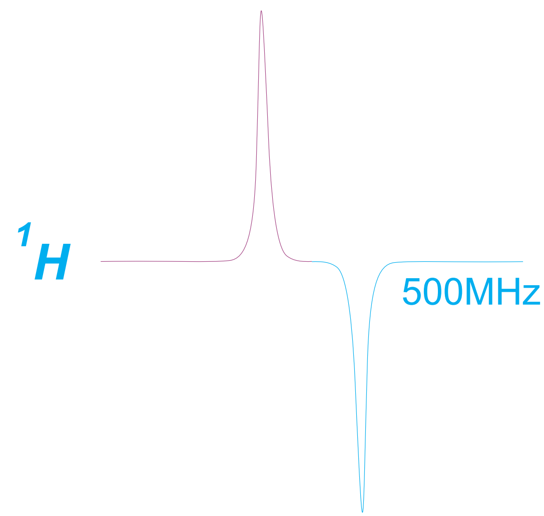


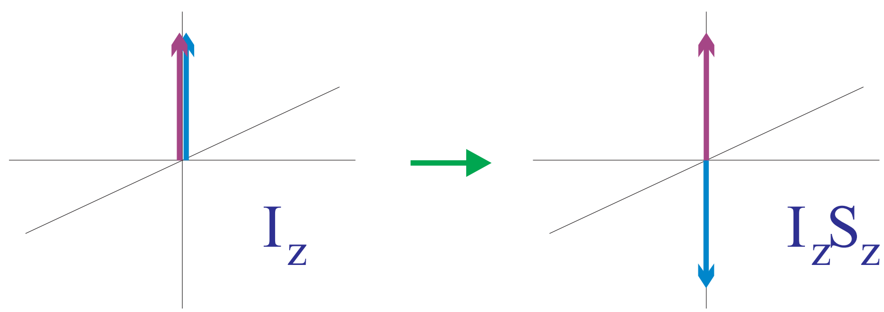
Vector Description of the INEPT Experiment

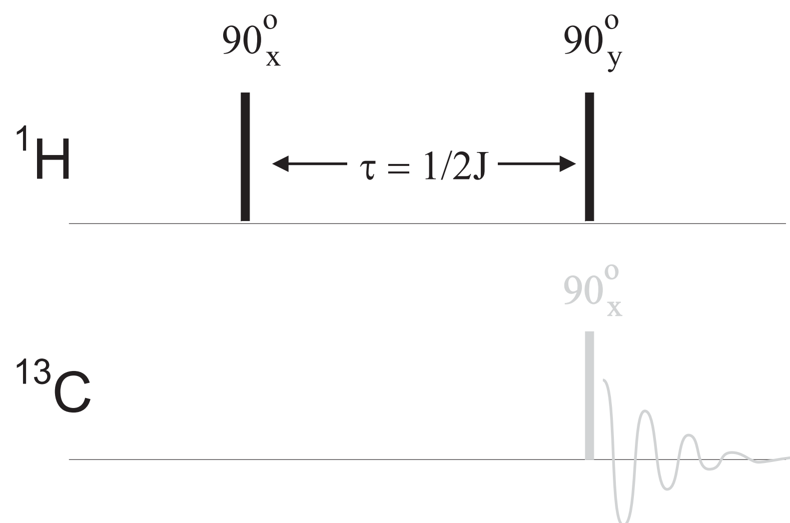


The INEPT starts with a selective population inversion of the protons.

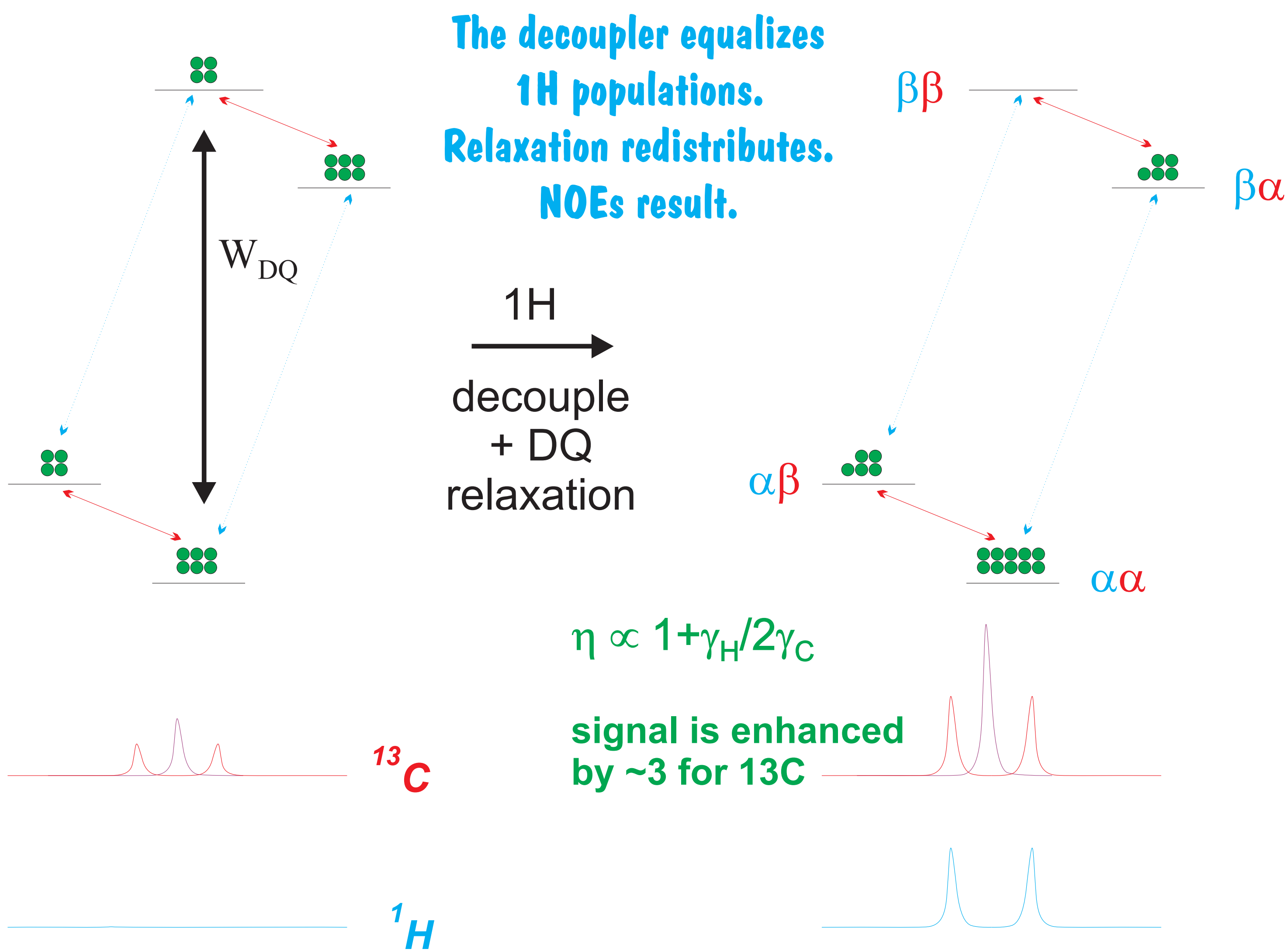


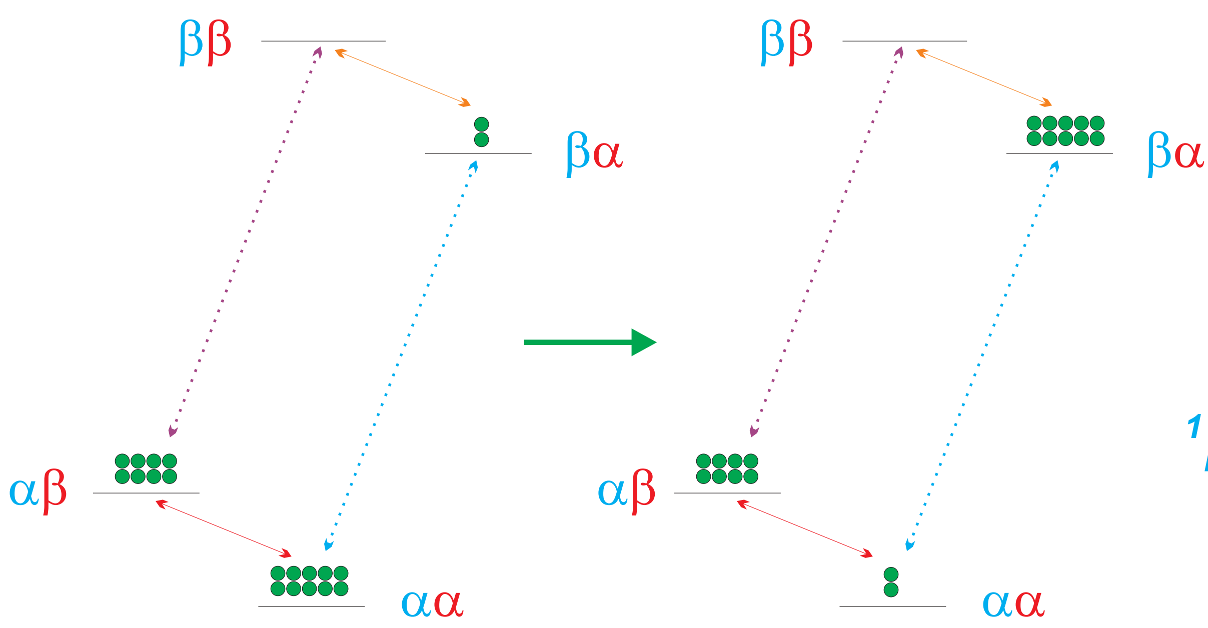




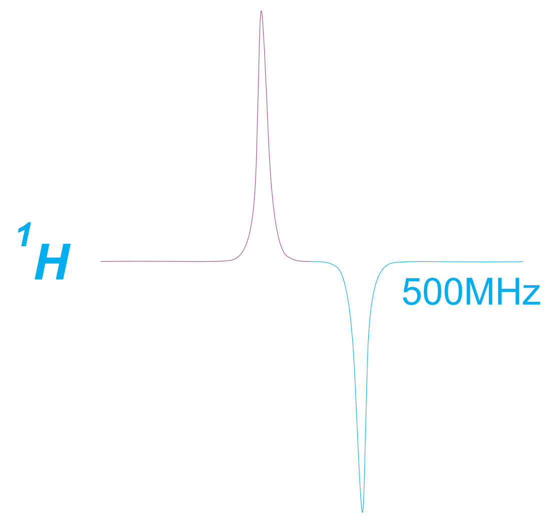


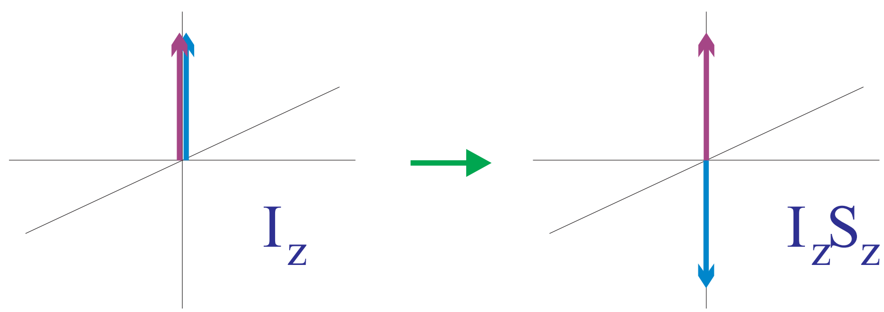
Population Description of NOE

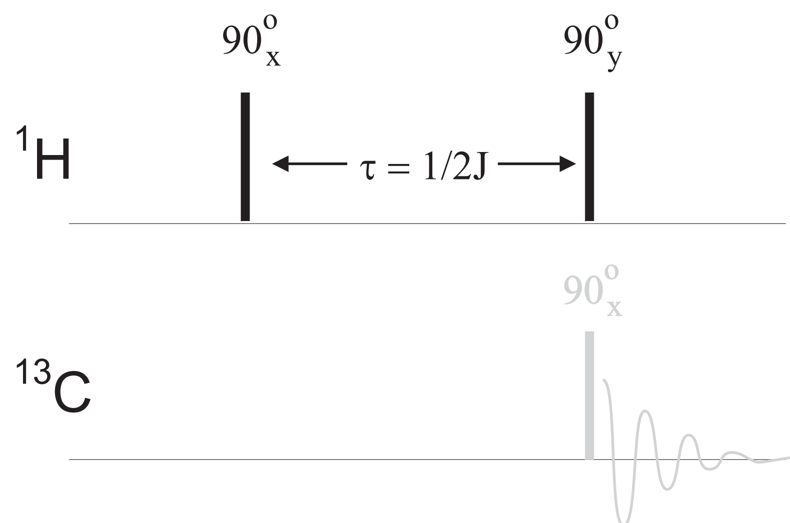




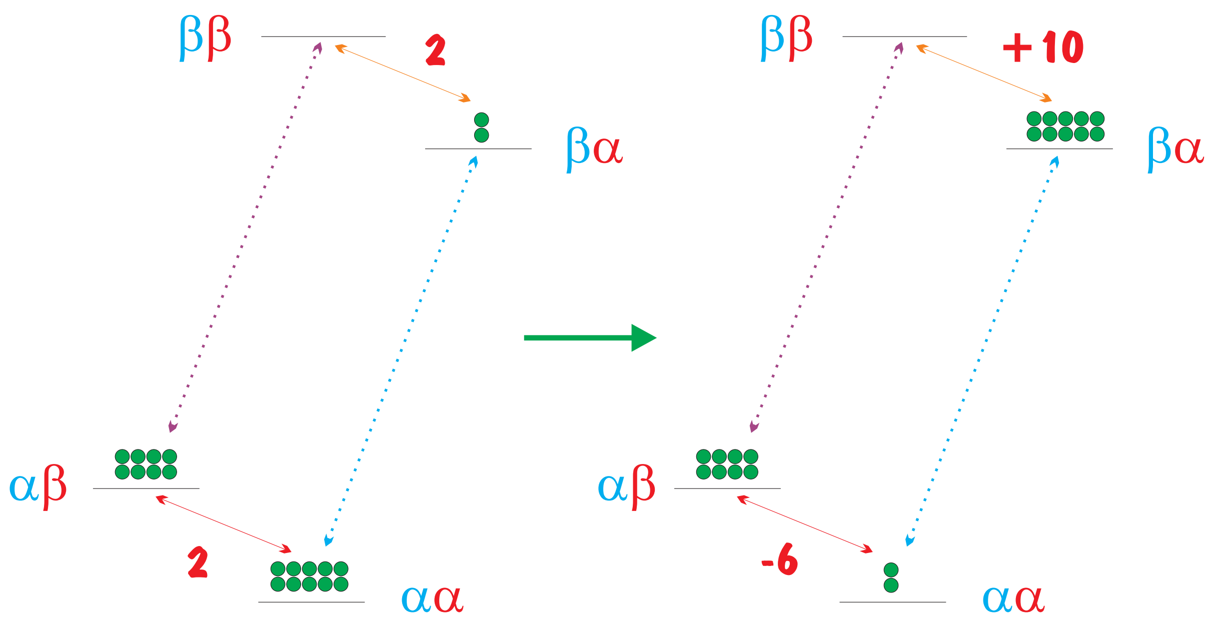
INEPT inverts one side of the proton populations.

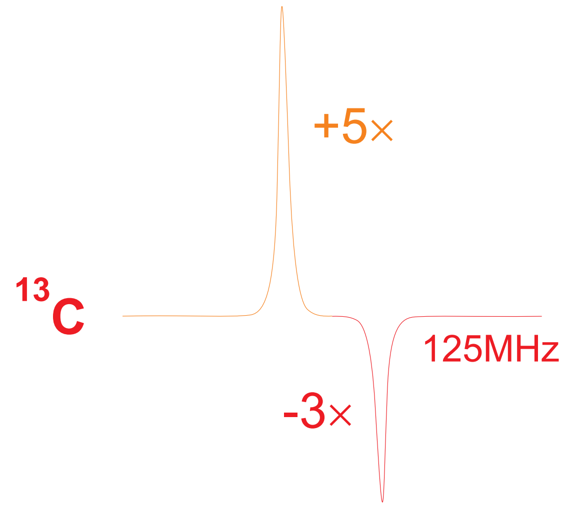


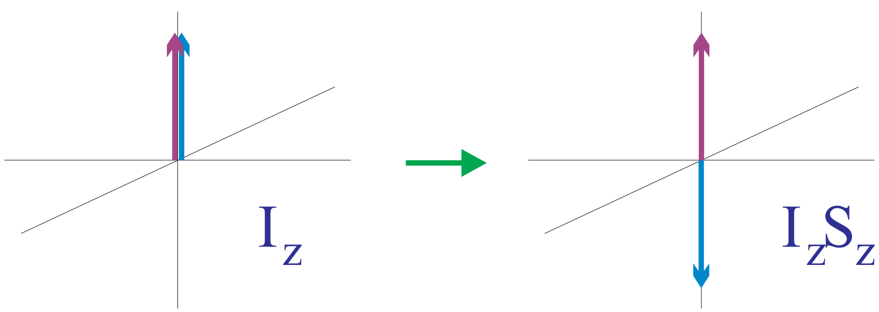


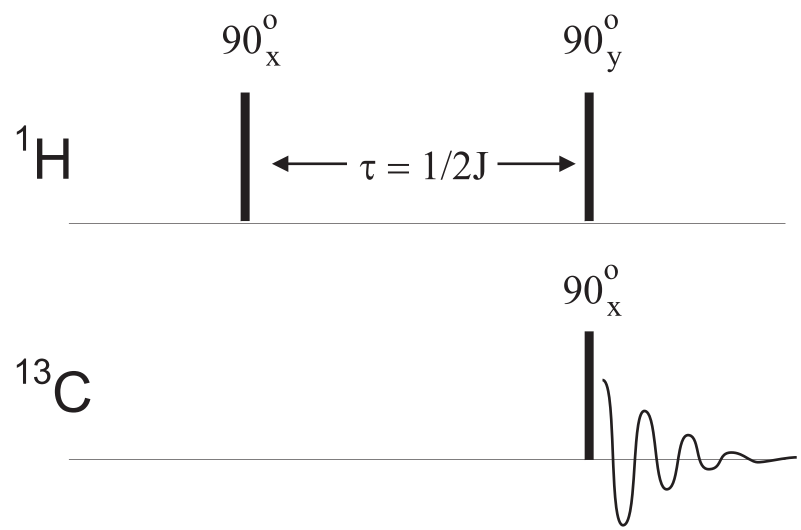


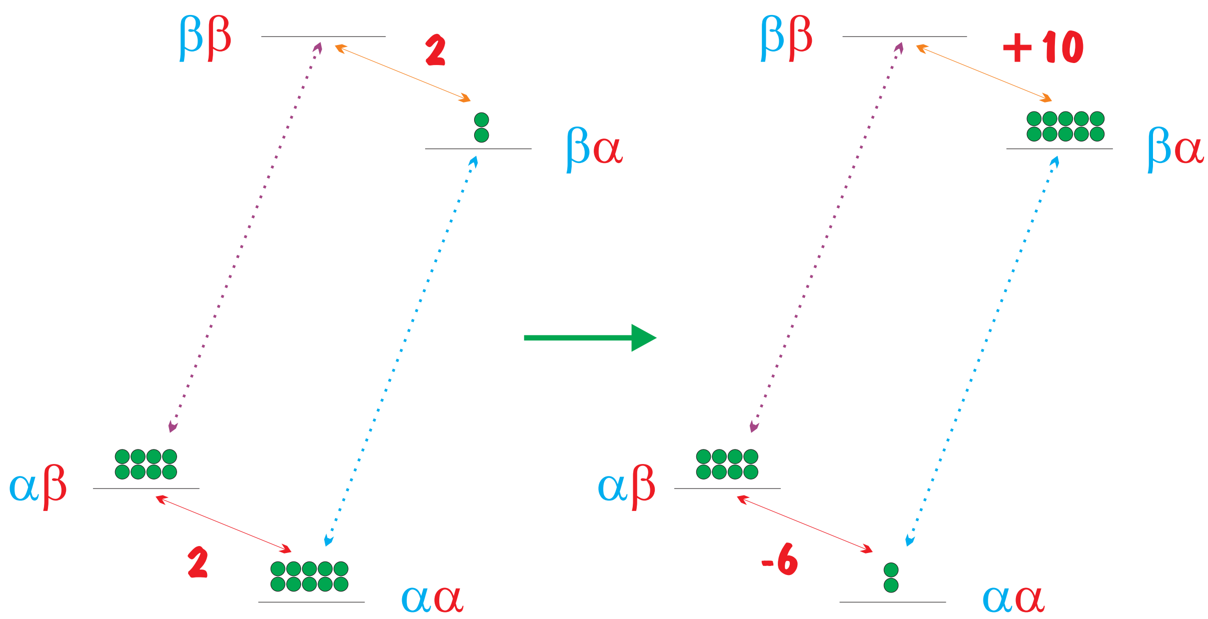
and transfers that proton polarization into enhancement of the ^{13}C signal.



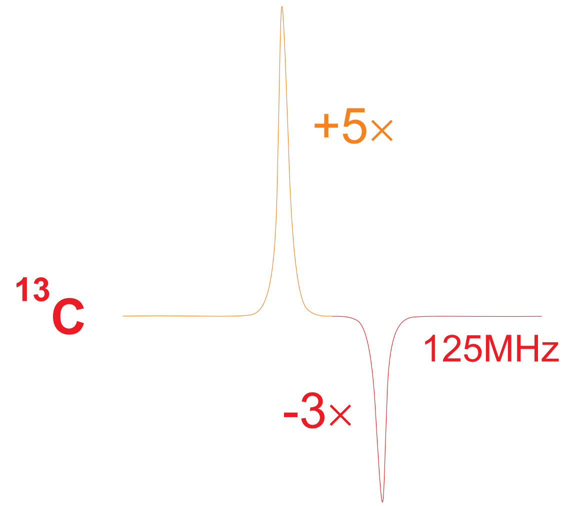


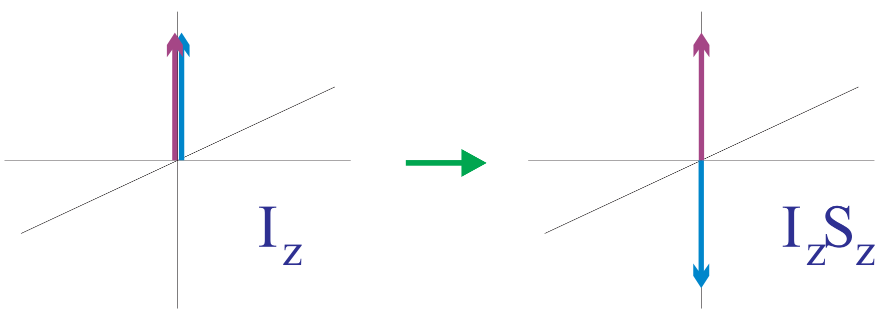


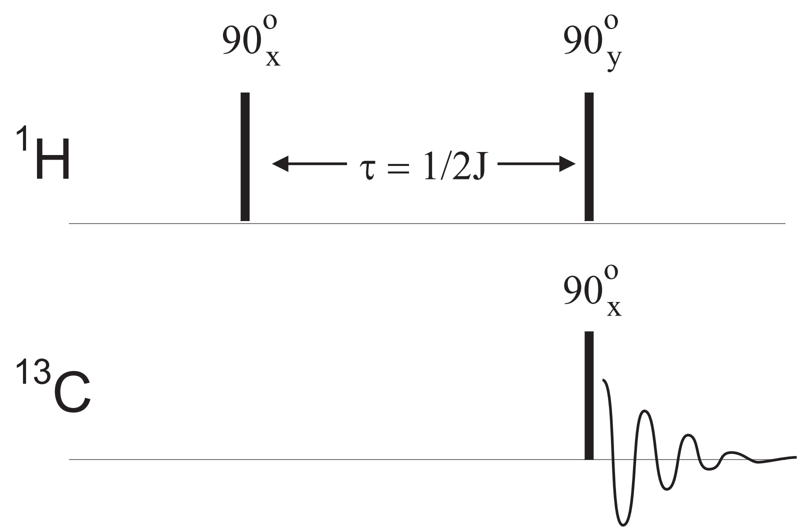


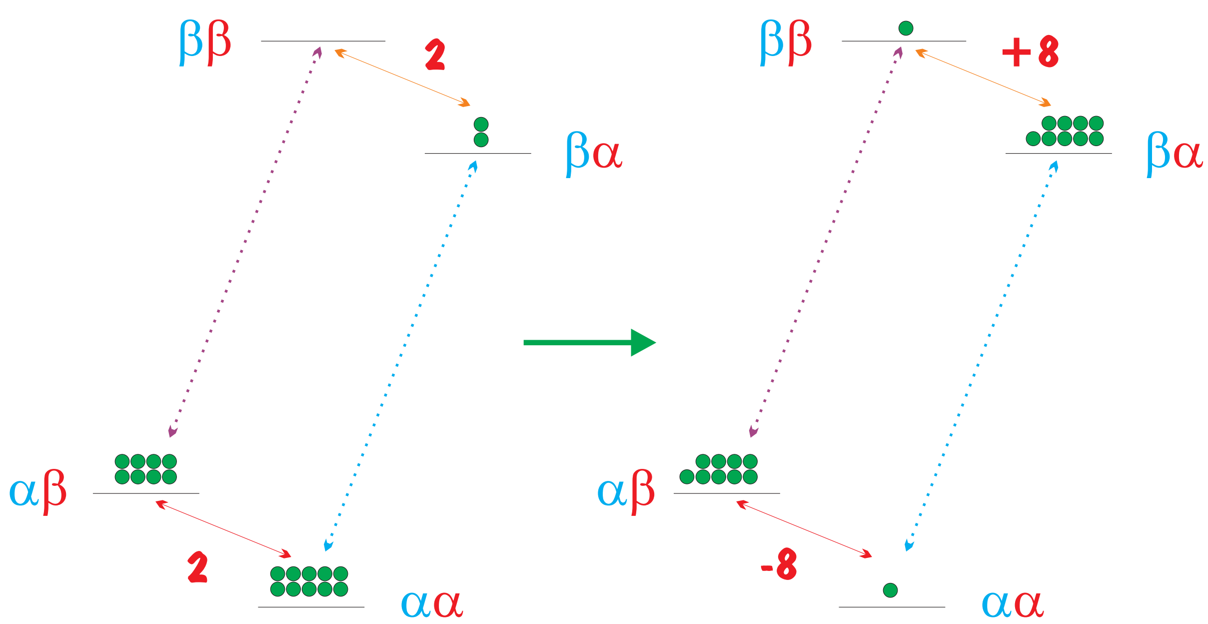


Polarization enhancement is immediate, whereas NOEs took ~ T_1 times to grow in.

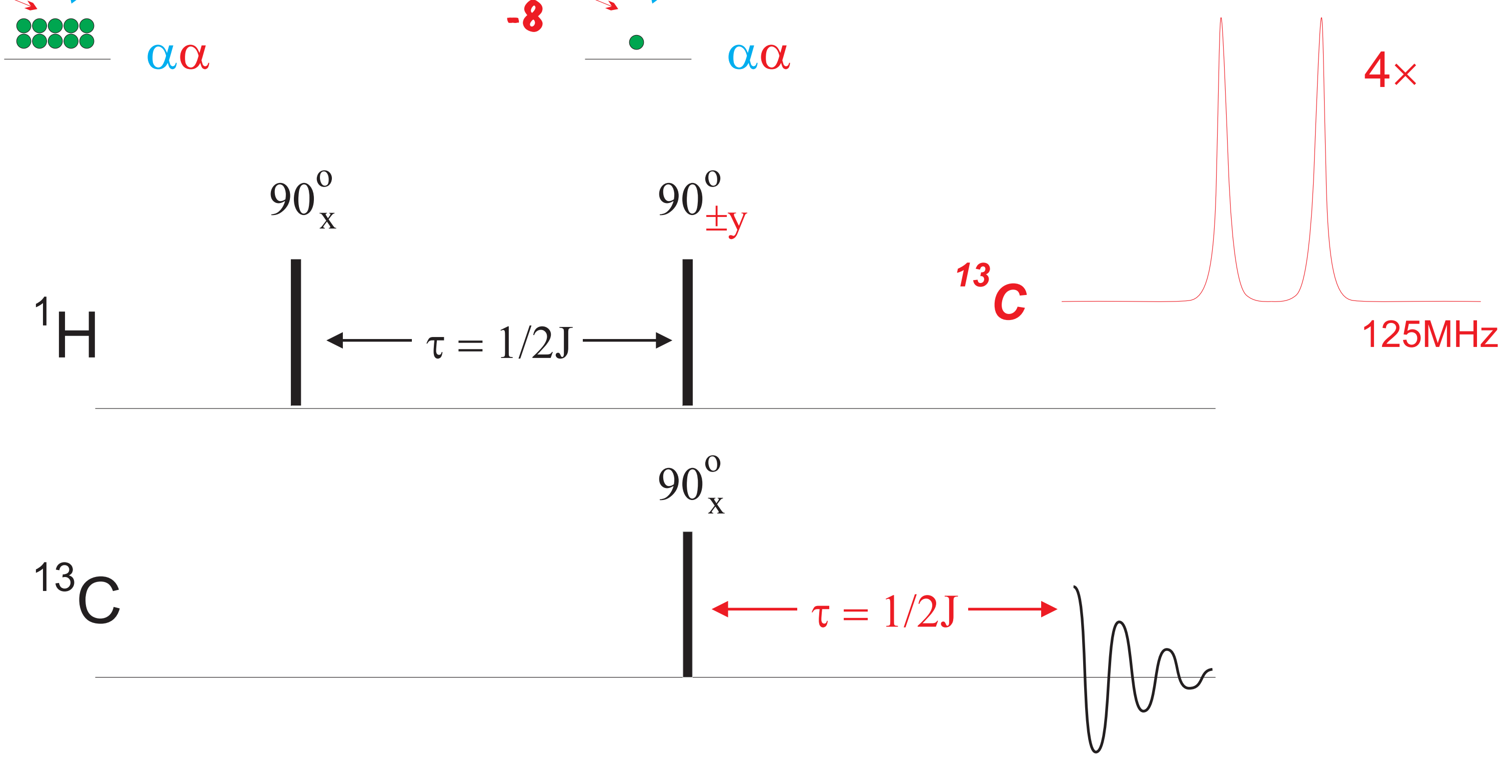




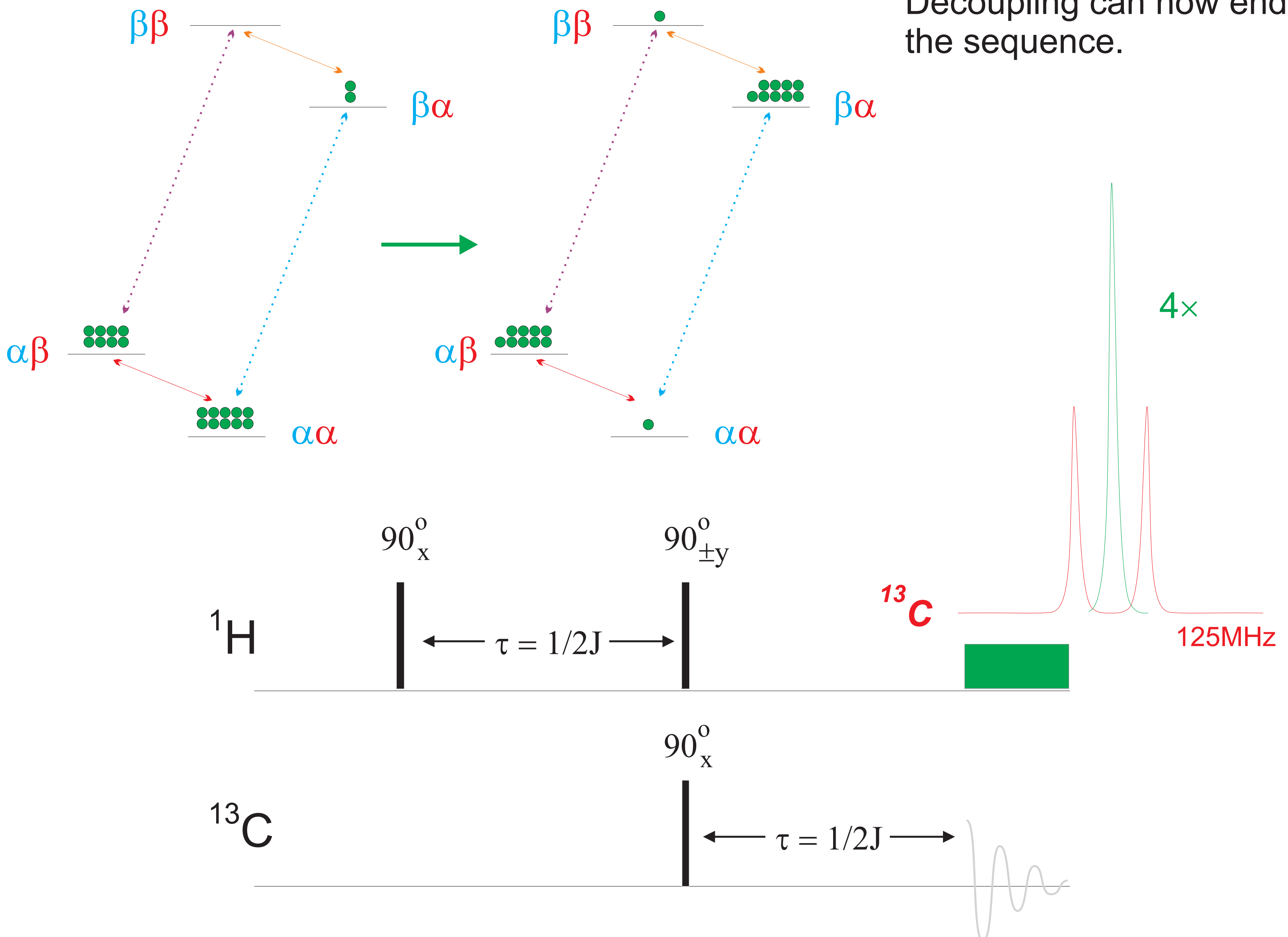


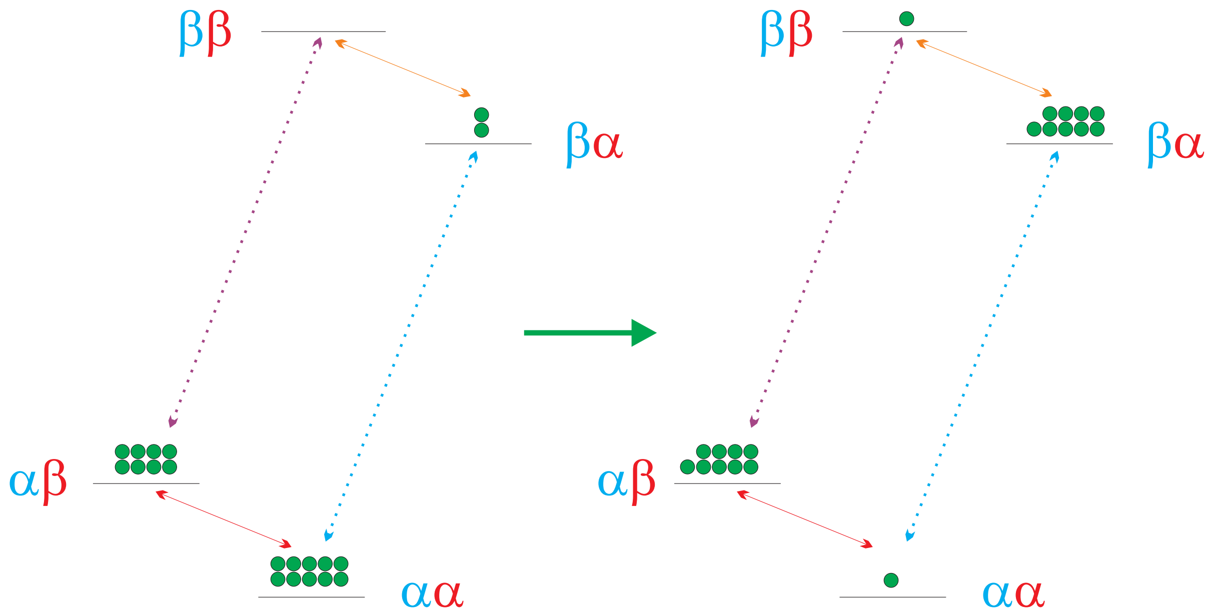


Sign alternation of the 2nd proton pulse removes the natural ^{13}C intensity, equalizing the intensity of the doublet. Addition of a 2nd 1/2J delay lets the antiphase state evolve into in-phase, magnetization.

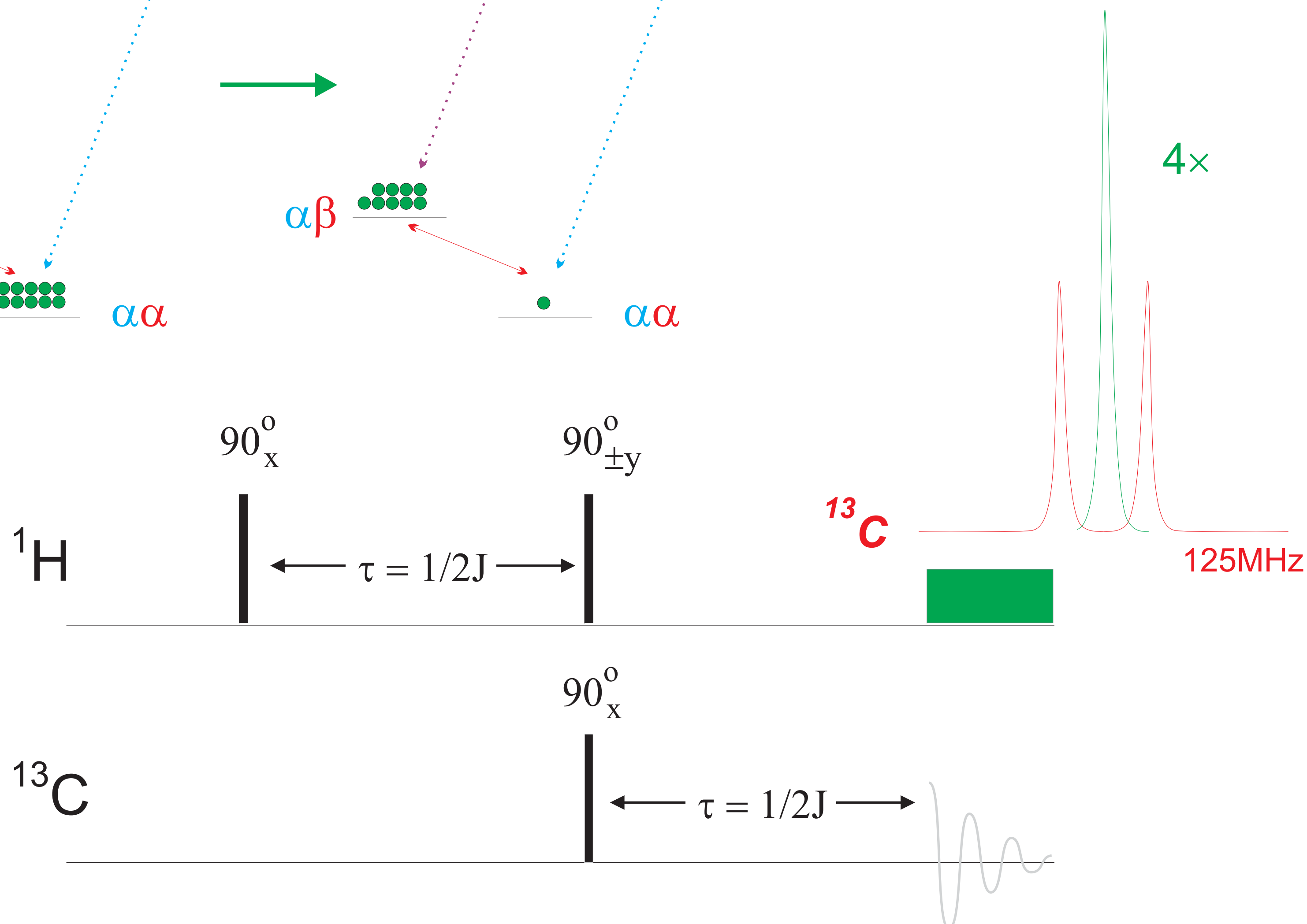


Decoupling can now end the sequence.





The key to the INEPT (and DEPT) experiment is the enhancement of the ^{13}C signal by γ_H/γ_C = 4×.



Summary Comments – Polarization Transfer (PT)

- **INEPT is the front half of all HSQC 2D experiments, and most ^{1}H-X experiments rely on similar PT effects for signal enhancement.**
- **PT involves J-coupling. Knowledge about, and uniformity of J_{HX} is critical to the experiment.**
- **PT enhancement does not require relaxation delays, but the 1/2J delay + relaxation imposes lower bounds on J-couplings that can be accessed (depends on experiment!).**
- **PT is not impacted by negative γ_X, as are NOE enhancements.**
- $\eta_{PT} \propto \gamma_H/\gamma_X$ $\qquad\qquad$ $\eta_{NOE} \propto 1 + \gamma_H/2\gamma_X$

  PT is critical for nuclei with small γ (e.g., ^{15}N, most metals).

  $\eta_{PT}(^{15}N) \approx 10$ $\qquad\qquad$ $\eta_{NOE}(^{15}N) \leq 6$