1

INEPT and Signal Enhancement

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The purpose of the INEPT sequence is to transfer the large magnetization from high-γ nuclei, like ^{1}H or ^{19}F, to low-γ nuclei (labeled 'X'), like ^{13}C and ^{15}N. This transfer significantly improves the signal intensity of 'X' nuclei when measuring their chemical shifts. For example, the magnetic moment and magnetization of ^{1}H nuclei is roughly 4 times greater than ^{13}C and 10 times greater than ^{15}N. Additionally, the ^{1}H T_1 is typically much shorter than the T_1 for ^{13}C or ^{15}N, which allows a shorter recycle delay and the collection of a greater number of spectra.

Morris GA, Freeman R. Enhancement of Nuclear Magnetic Resonance Signals by Polarization Transfer. J Am Chem Soc. 1979;101(3):760–762.

1.1. Theory

The INEPT sequence (Fig. 1.1) transfers magnetization from ^{1}H nuclei bonded the 'X' nuclei. The two nuclei must be bonded, or at least connected through intermediary bonds with other atoms, because the nuclei must share a J-coupling.

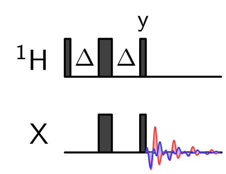

Fig. 1.1. The basic INEPT sequence between nuclei ^{1}H and X.

The INEPT sequence comprises a set of 90° pulses (thin bars), 180° pulses (thick bars) and delays (Δ).

1.1.1. Methine, Amide and the AX Spin Systems

The first 90°_{x} (^{1}H) pulse creates $-\boldsymbol{H_y}$ ^{1}H magnetization. Thereafter, the ^{1}H 180° pulse in the middle of the two Δ delays acts as a Hahn-echo to refocus the ^{1}H chemical shifts. Without the accompanying 180° pulse on the 'X' channel, the J-coupling between the ^{1}H and X nucleus would be refocused as well—and nothing would be accomplished. The 180° pulse on the 'X' channel acts to *cancel* the refocusing effect of the ^{1}H 180° pulse for the J-coupling.

A 90°_{x} pulse rotates the magnetization by 90° with a phase of 'x'.

During the first delta period, the $-\boldsymbol{H_y}$ magnetization evolves under the ^{1}H chemical shift (ω_H) and the ^{1}H-X J-coupling. Since the chemical shift and J-coupling Hamiltonians commute, the rotations of each can be conducted sequentially.

In the first step, the operators are propagated for the 90°_{x} (^{1}H) pulse and the first 'Δ' period. We'll use a ^{15}N nucleus as an example of an 'X' nucleus.

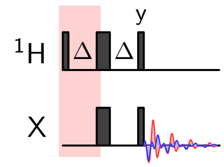

Fig. 1.2. The first step of the INEPT sequence highlighted in red.

$$
\begin{aligned}
\boldsymbol{H_z} &\xrightarrow{90^\circ_x(^1H)} -\boldsymbol{H_y}\\
-\boldsymbol{H_y} &\xrightarrow{\omega_H\Delta} -\boldsymbol{H_y}\cos(\omega_H\Delta)+\boldsymbol{H_x}\sin(\omega_H\Delta)\\
&\xrightarrow{J_{NH}\Delta} -\boldsymbol{H_y}\cos(\omega_H\Delta)\cos(\pi J_{NH}\Delta)+\\
&\qquad \boldsymbol{2H_xN_z}\cos(\omega_H\Delta)\sin(\pi J_{NH}\Delta)+\\
&\qquad \boldsymbol{H_x}\sin(\omega_H\Delta)\cos(\pi J_{NH}\Delta)+\\
&\qquad \boldsymbol{2H_yN_z}\sin(\omega_H\Delta)\sin(\pi J_{NH}\Delta)
\end{aligned}
$$

In the second step, the 180°_x pulses on the ^{1}H and 'X' channels inverts $\boldsymbol{H_y}$ and $\boldsymbol{2H_xN_z}$ terms because these are orthogonal to the x-phase of the pulse.

The many cosine and sine terms can be abbreviated to simplify their notation in equations.

$$
\begin{aligned}
\cos(\omega_H\Delta) &= C_{CS\Delta}\\
\cos(\pi J_{NH}\Delta) &= C_{J_{NH}\Delta}\\
\sin(\omega_H\Delta) &= S_{CS\Delta}\\
\sin(\pi J_{NH}\Delta) &= S_{J_{NH}\Delta}
\end{aligned}
$$

$$
\begin{aligned}
\xrightarrow{180^\circ_x(^1H),\ 180^\circ_x(X)}\ & \boldsymbol{H_y}C_{CS\Delta}C_{J_{NH}\Delta}-\boldsymbol{2H_xN_z}C_{CS\Delta}S_{J_{NH}\Delta}\\
&+\boldsymbol{H_x}C_{CS\Delta}C_{J_{NH}\Delta}+\boldsymbol{2H_yN_z}S_{CS\Delta}S_{J_{NH}\Delta}
\end{aligned}
$$

In the third step, we'll propagate the 'Δ' delay for the chemical shift first.

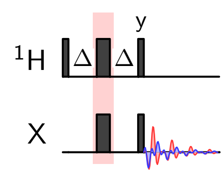

Fig. 1.3. The second step of the INEPT sequence highlighted in red.

$$
\begin{aligned}
\xrightarrow{\omega_H\Delta}\ & \boldsymbol{H_y}C_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}-H_xC_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta}\\
&-\boldsymbol{2H_xN_z}C_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}-\boldsymbol{2H_yN_z}C_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta}\\
&\boldsymbol{H_x}S_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}+\boldsymbol{H_y}S_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta}+\\
&\boldsymbol{2H_yN_z}S_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}+-\boldsymbol{2H_xN_z}S_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta}
\end{aligned}
$$

These terms can be grouped.

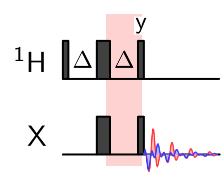

Fig. 1.4. The third step of the INEPT sequence highlighted in red.

$$
\begin{aligned}
=\ & \boldsymbol{H_x}(S_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}-C_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta})\\
&+\boldsymbol{H_y}(C_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}+S_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta})\\
&-\boldsymbol{2H_xN_z}(C_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}+S_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta})\\
&+\boldsymbol{2H_yN_z}(S_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}-C_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta})
\end{aligned}
$$

Thereafter, we propagate the 'Δ' delay for the J_{NH}-coupling and simplify the expression.

$$
\begin{aligned}
\xrightarrow{J_{NH}\Delta}\ & \boldsymbol{H_x}(S_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}-C_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta})C_{J_{NH}\Delta}\\
&+\boldsymbol{2H_yN_z}(S_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}-C_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta})S_{J_{NH}\Delta}\\
&+\boldsymbol{H_y}(C_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}+S_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta})C_{J_{NH}\Delta}\\
&-\boldsymbol{2H_xN_z}(C_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}+S_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta})S_{J_{NH}\Delta}\\
&-\boldsymbol{2H_xN_z}(C_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}+S_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta})C_{J_{NH}\Delta}\\
&-\boldsymbol{H_y}(C_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}+S_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta})S_{J_{NH}\Delta}\\
&+\boldsymbol{2H_yN_z}(S_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}-C_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta})C_{J_{NH}\Delta}\\
&-\boldsymbol{H_x}(S_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}-C_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta})S_{J_{NH}\Delta}
\end{aligned}
$$

$$
\begin{aligned}
= \quad & \boldsymbol{H_x}(S_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}C_{J_{NH}\Delta} - C_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta}C_{J_{NH}\Delta} \\
& - S_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}S_{J_{NH}\Delta} + C_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta}S_{J_{NH}\Delta}) \\
& \boldsymbol{H_y}(C_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}C_{J_{NH}\Delta} + S_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta}C_{J_{NH}\Delta} \\
& - C_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}S_{J_{NH}\Delta} - S_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta}S_{J_{NH}\Delta}) \\
& \boldsymbol{- 2H_xN_z}(C_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}S_{J_{NH}\Delta} + S_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta}S_{J_{NH}\Delta} \\
& + C_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}C_{J_{NH}\Delta} + S_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta}C_{J_{NH}\Delta}) \\
& \boldsymbol{2H_yN_z}(S_{CS\Delta}C_{J_{NH}\Delta}C_{CS\Delta}S_{J_{NH}\Delta} - C_{CS\Delta}C_{J_{NH}\Delta}S_{CS\Delta}S_{J_{NH}\Delta} \\
& + S_{CS\Delta}S_{J_{NH}\Delta}C_{CS\Delta}C_{J_{NH}\Delta} - C_{CS\Delta}S_{J_{NH}\Delta}S_{CS\Delta}C_{J_{NH}\Delta}) \\
= & \boldsymbol{- 2H_xN_z}\sin(2\pi J_{NH}\Delta) + \boldsymbol{H_y}\cos(2\pi J_{NH}\Delta)
\end{aligned}
$$