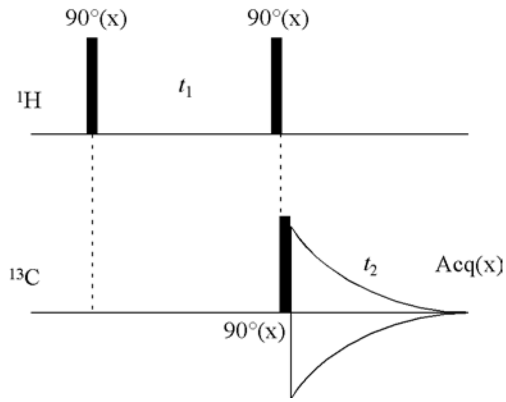
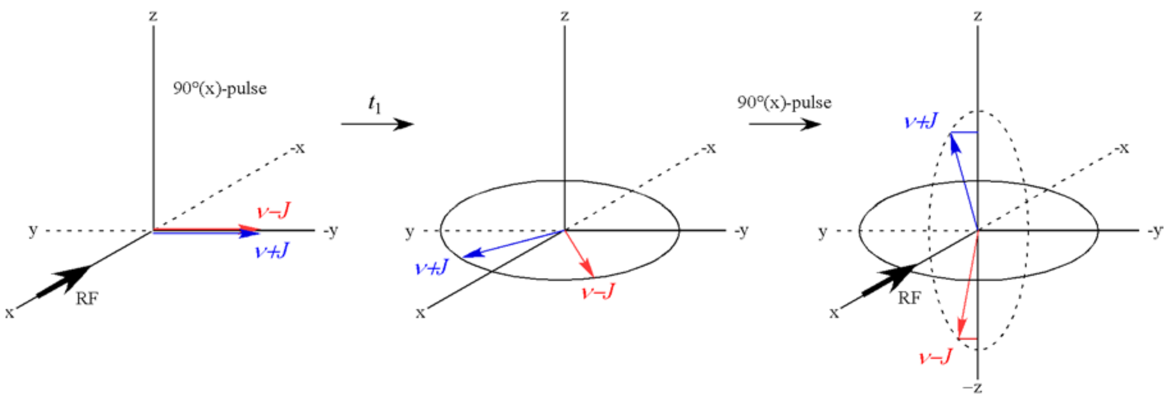
Inverse Detection in Multinuclear NMR

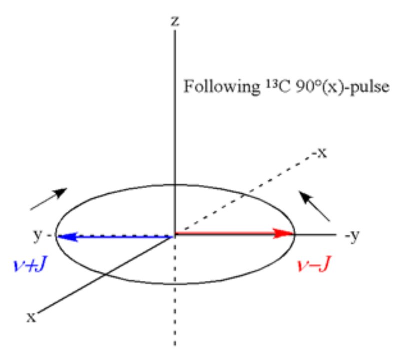
The HETCOR experiment is an example of a *directly-detected* heteronuclear experiment. The timing diagram for the most basic form of the HETCOR pulse sequence is shown below:



The vector analysis of the pulse sequence is very similar to the ^{1}H COSY experiment:



and then following the ^{13}C 90° (x) pulse we obtain



This is reminiscent of an INEPT type transfer, but since evolution of the ^{1}H vector is variable, there can be no proper optimization of the τ delay.

Sensitivity

Recall that the sensitivity of a 1D NMR experiment depends upon three factors.: The initial polarization (population difference between α and β states for spin-1/2 particles); the magnetization (product of the polarization and gyromagnetic ratio) and the actual voltage measured in the coil of the NMR probe divided by the noise in the coil.

Recall that the energies of spins in the α and β states are given by:

$$E_\alpha = -\hbar\gamma B_0/2 \quad ; \qquad E_\beta = +\hbar\gamma B_0/2$$

The probability of finding a spin-1/2 nucleus in the α-state is given by:

$$P_\alpha \propto e^{-E_\alpha/k_b T}$$

and in the β-state:

$$P_\beta \propto e^{-E_\beta/k_b T}$$

and the polarization is the difference in the probabilities as shown below:

$$I_z = P_\alpha - P_\beta = e^{-E_\alpha/k_b T} - e^{-E_\beta/k_b T} \approx \frac{\hbar\gamma B_0}{k_b T}$$

The magnetization is given by the following expression:

$$M_z = \hbar\gamma I_z$$

The voltage produced in the RF receiver coil depends upon the Larmor frequency:

$$V_c \propto \omega = \gamma B_0$$

while the noise in the coil depends upon the square root of the Larmor frequency:

$$N_c \propto \sqrt{\omega} = \left(\gamma B_0\right)^{1/2}$$

We may summarize these statements as shown below:

$$S \propto M_z\left(\frac{V_c}{N_c}\right) = \hbar\gamma I_z\left(\frac{V_c}{N_c}\right) = \hbar\gamma\left(\frac{\hbar\gamma B_0}{k_b T}\right)\left(\frac{\gamma B_0}{\left(\gamma B_0\right)^{1/2}}\right)$$

or exclusively in terms of the γ:

$$S \propto \gamma_p \gamma_d \gamma_d^{1/2} B_0^{3/2}$$

wherein the subscript *p* and *d* correspond to contributions from polarization and detection respectively.

The relative sensitivity of direct detection of ^{13}C nuclei versus detection using the INEPT ^{1}H-^{13}C polarization transfer experiment is thus given by the following:

$$\frac{S_{\text{INEPT}}}{S_{\text{Direct}}} = \frac{\gamma_{\text{H}}\gamma_{\text{C}}\gamma_{\text{C}}^{1/2}}{\gamma_{\text{C}}\gamma_{\text{C}}\gamma_{\text{C}}^{1/2}} \approx \frac{\left(4\gamma_{\text{C}}\right)\gamma_{\text{C}}\gamma_{\text{C}}^{1/2}}{\gamma_{\text{C}}\gamma_{\text{C}}\gamma_{\text{C}}^{1/2}} = 4$$

The sensitivity of NMR experiments may be summarized in the following table:

	Relative Sensitivity		
Transfer	Dependence	I = ^{1}H; S = ^{13}C	I = ^{1}H; S = ^{15}N
S	$\gamma_S \gamma_S \gamma_S^{1/2}$	1	1
$I \rightarrow S$	$\gamma_I \gamma_S \gamma_S^{1/2}$	4	10
$S \rightarrow I$	$\gamma_S \gamma_I \gamma_I^{1/2}$	8	30
$I \rightarrow S \rightarrow I$	$\gamma_I \gamma_I \gamma_I^{1/2}$	32	300

After Becker, E.D. (1998) *High Resolution NMR*, Academic Press, New York, Table 12.2

Note that the values derived above neglect important contributions from NMR relaxation, which alters the effective relative sensitivities.

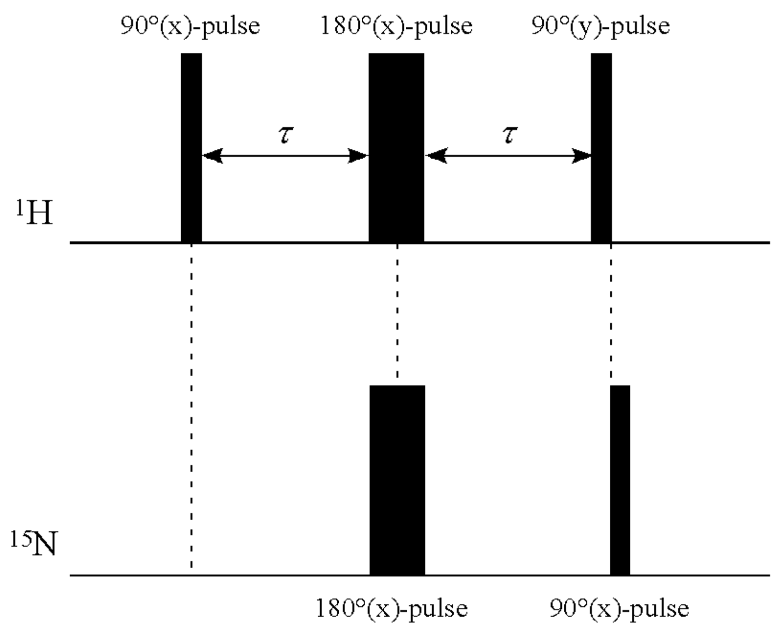
Heteronuclear Single Quantum Correlation Spectroscopy: HSQC

Bodenhausen, G.; Ruben, D.J. (1980) Natural Abundance Nitrogen-15 NMR by Enhanced Heteronuclear Spectroscopy. *Chem. Phys. Lett.* **69**, 185-189.

Although the HETCOR experiment provides information about ^{1}H-^{13}C *J*-coupling interactions, even with fully developed NOE enhancement, the sensitivity of the experiment is relatively low compared with multidimensional ^{1}H experiments since $\gamma_{H} \approx 4\gamma_{C}$ (see sensitivity table).

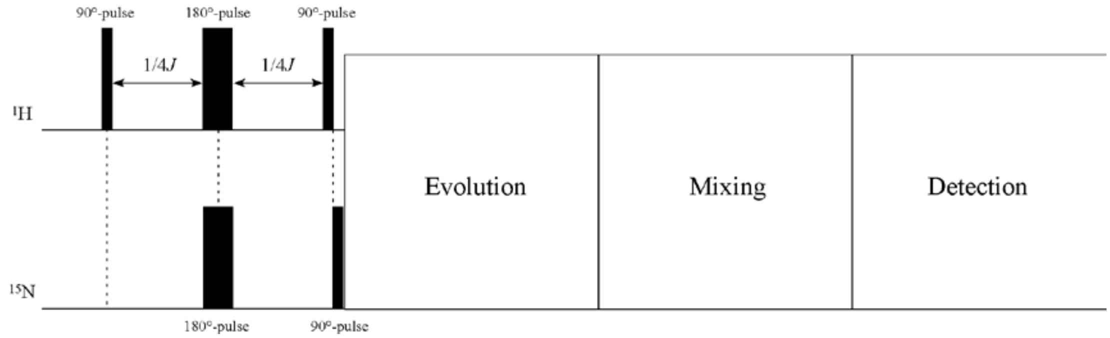
A substantial improvement in this sequence may be achieved by combining the HETCOR sequence with a sensitivity enhancement sequence.

Recall the INEPT sequence:

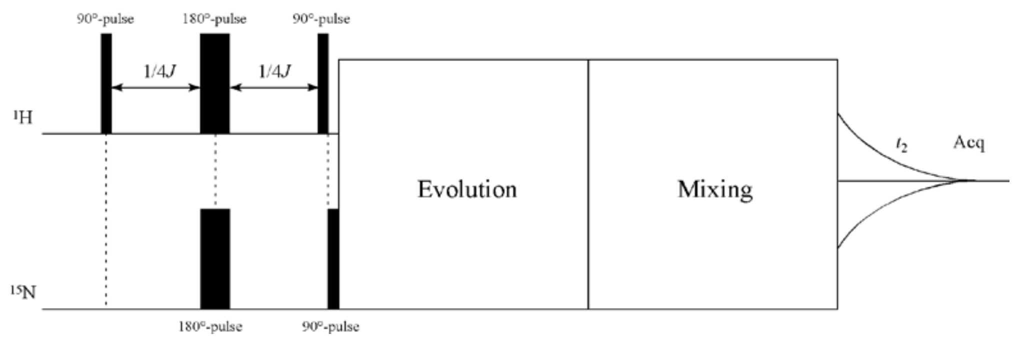


We demonstrated that the enhancement of sensivity gained using this sequence was equal to the ratio of the gyromagnetic rations, i.e., approximately a factor 4 for ^{13}C and a factor of 10 for ^{15}N.

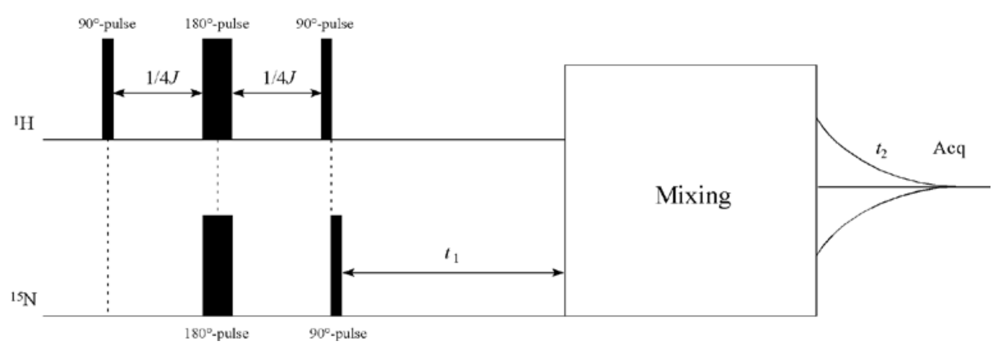
We can incorporate the INEPT sequence in a modular fashion as a part of an enhanced heteronuclear correlation experiment:



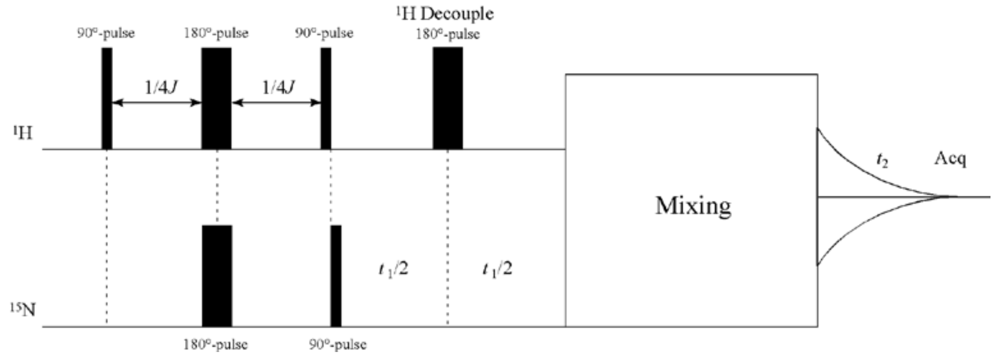
We can assume that the detection interval will look like the previous experimental examples:



Likewise, following the INEPT sequence we could simply allow the system to evolve:



Alternatively, we could install a ^1H 180°-pulse midway through the incrementable interval:

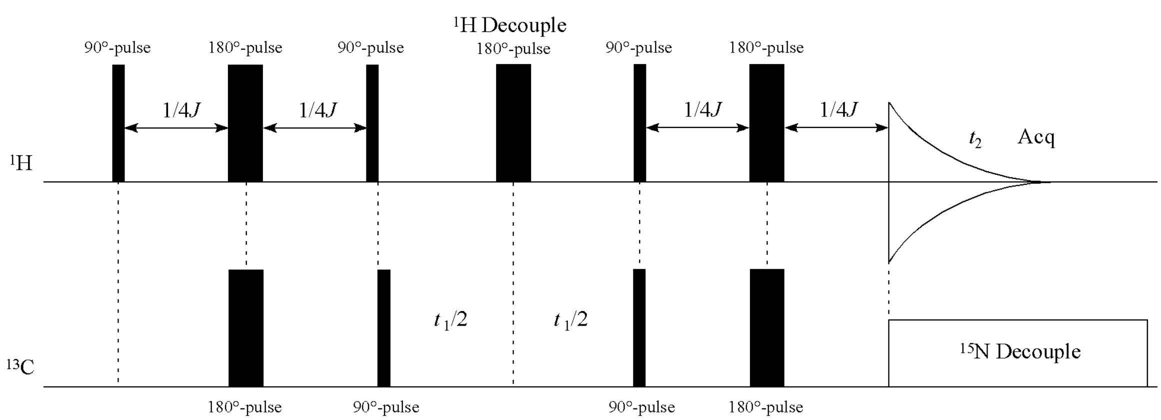


Since the ^{15}N magnetization already lies in the xy-plane, we might imagine that we could simple gate on the receiver and proceed through t_2…

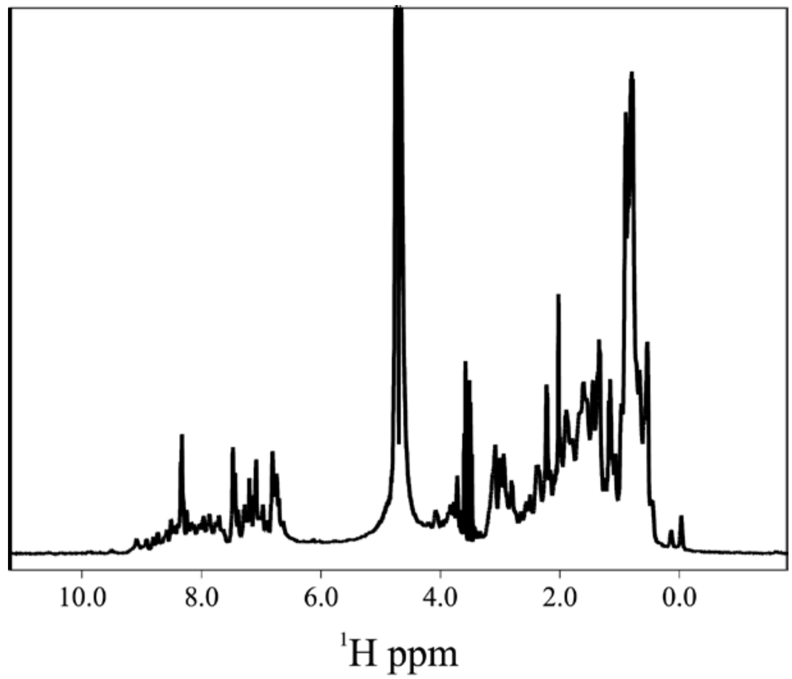
We have an even better idea. Based on our sensitivity calculations, we can generate a substantial sensitivity enhancement by transferring the magnetization back to ^1H for the detection interval.

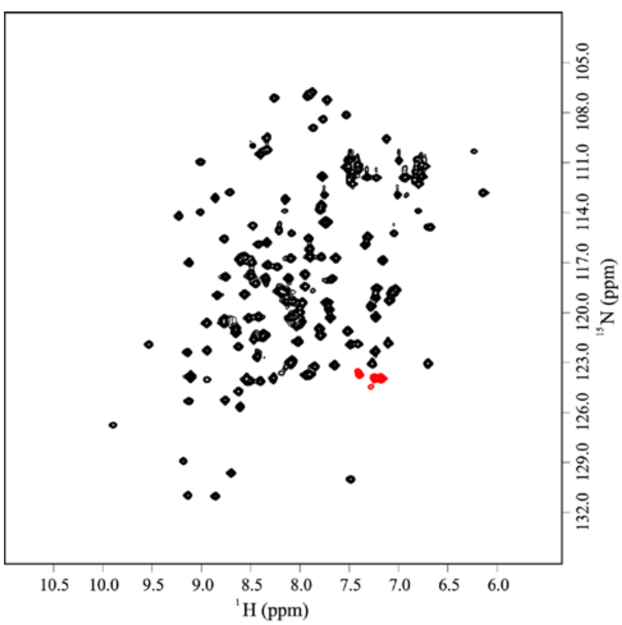
Thus the mixing interval used in the HSQC is essentially the reverse of the INEPT sequence.

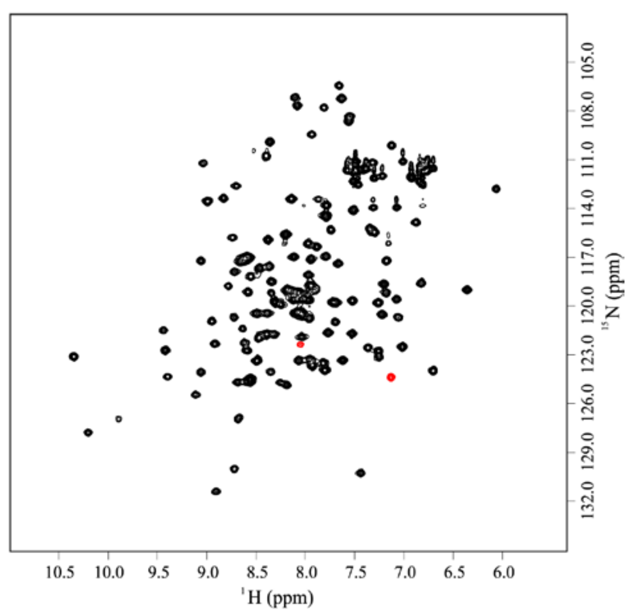
The HSQC experiment with H decoupling during t_1 and heteronuclear decoupling during t_2 is shown below:



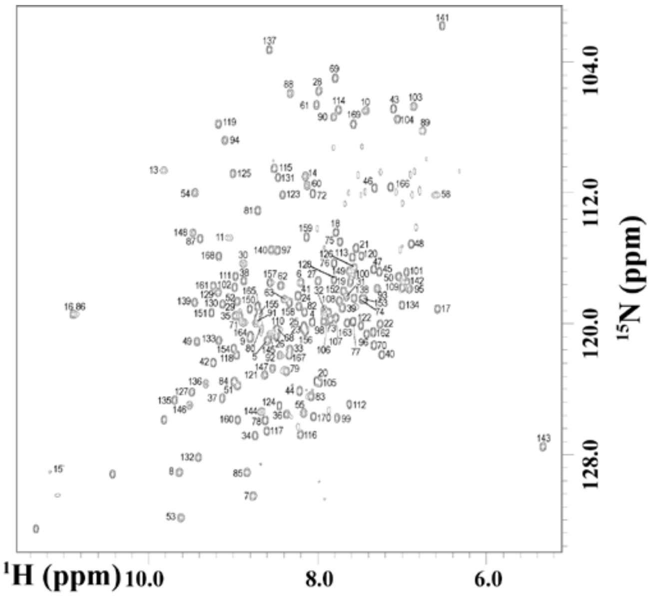
^{1}H 1D and ^{15}N-HSQC spectra of NHPX, a small protein composed of 114 amino acids:

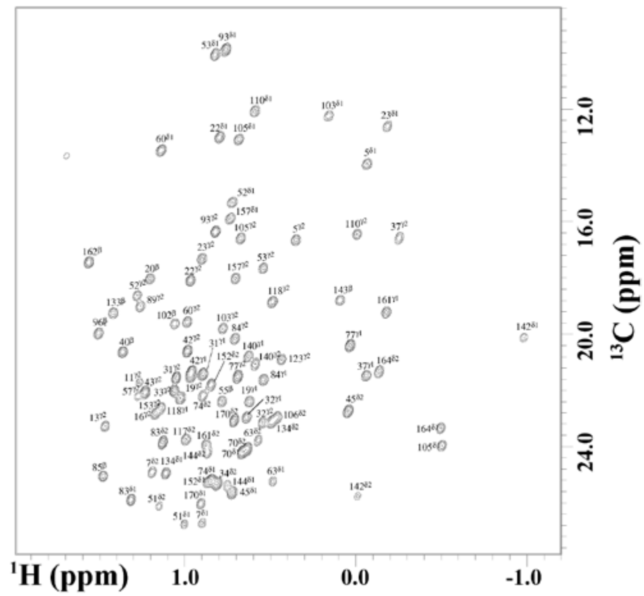




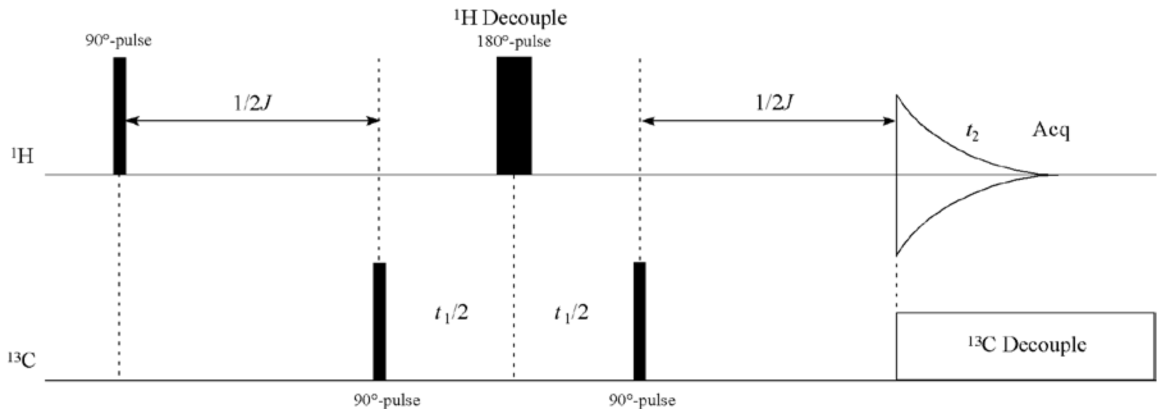


^{15}N-HSQC and Methyl Region of ^{13}C-HSQC for Anabaena Flavodoxin, a 179 amino acid protein:





Heteronuclear Multiple Quantum Correlation Spectroscopy: HMQC



This sequence produces correlations similar to those obtained in the HSQC experiment, however the sequence makes use of multiple-quantum coherence transfer, which produces intrinsic resolution that is somewhat lower than for the HSQC experiment.

A major historical advantage of the HMQC sequence over the HSQC sequence was the ease of implementation, i.e., fewer RF pulses.

The following figure shows a series of ^{1}H-^{15}N 2D heterocorrelation spectra recorded using difference pulse sequences: a) HMQC, b) HSQC with ^{1}H 180°-pulse decoupling during t_1, c) HSQC with CPD ^{1}H decoupling during t_1, d) constant-time HSQC:

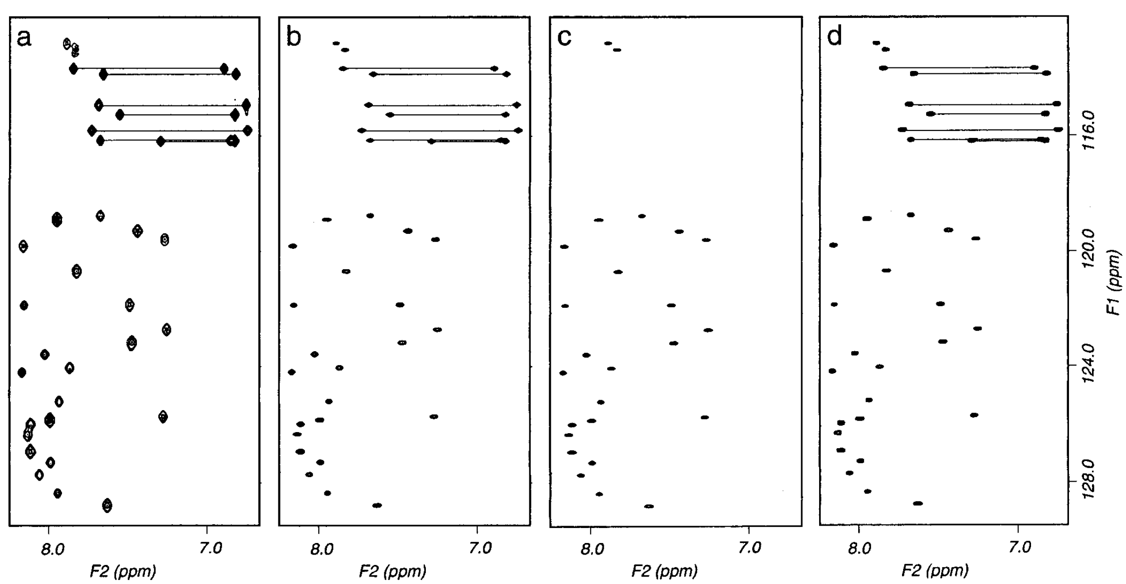
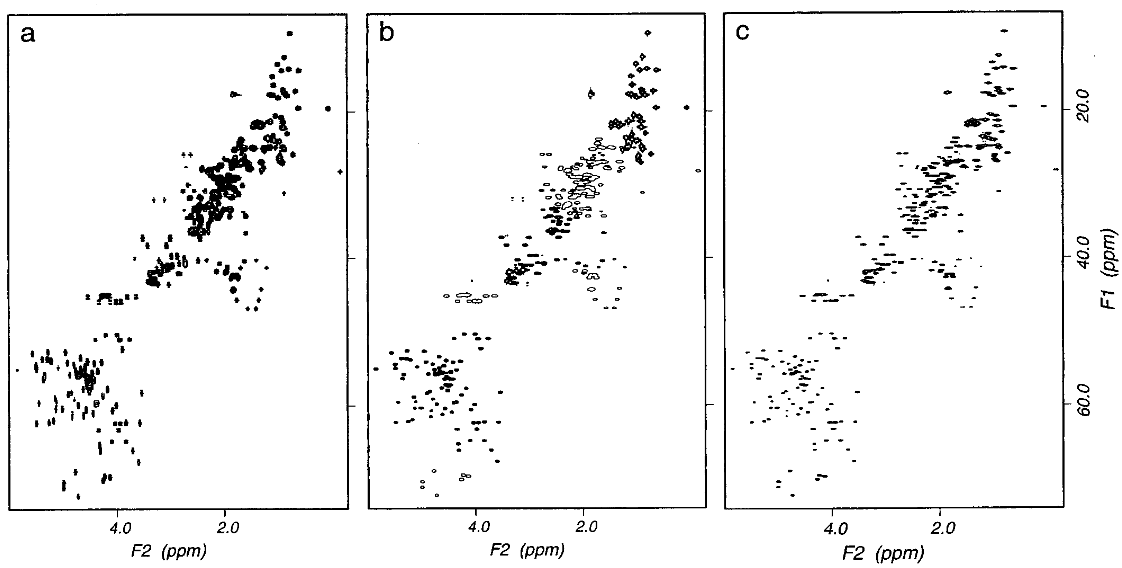
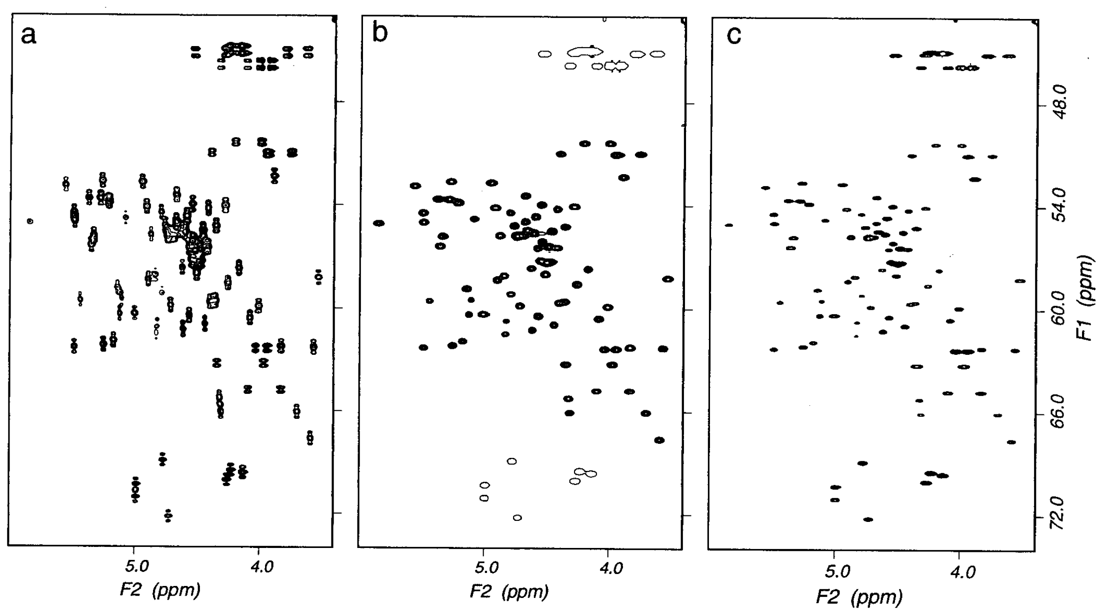


Figure 7.3 from Cavanagh, J., Fairbrother, W.J., Palmer, A.G. and Skelton, N.J. (1996) Protein NMR Spectroscopy, Academic Press, New York.

Representative ^{13}C HSQC spectra are shown below: a) ^{13}C HSQC with ^{1}H 180°-pulse during t_1 and ^{13}C decoupling during t_2, b) constant time (CT) HSQC with CT interval set to 27 ms, c) as in b with CT delay set to 54 ms:





Figures 7.8 and 7.9 from Cavanagh, J., Fairbrother, W.J., Palmer, A.G. and Skelton, N.J. (1996) Protein NMR Spectroscopy, Academic Press, New York.