

ND Nuclear Overhauser Experiments

Earlier we considered the effects of steady-state irradiation of one member of a $I-S$ spin pair. Analysis of the effect led to the development of a quantitative expression for the nuclear Overhauser effect, which we named the nuclear Overhauser enhancement:

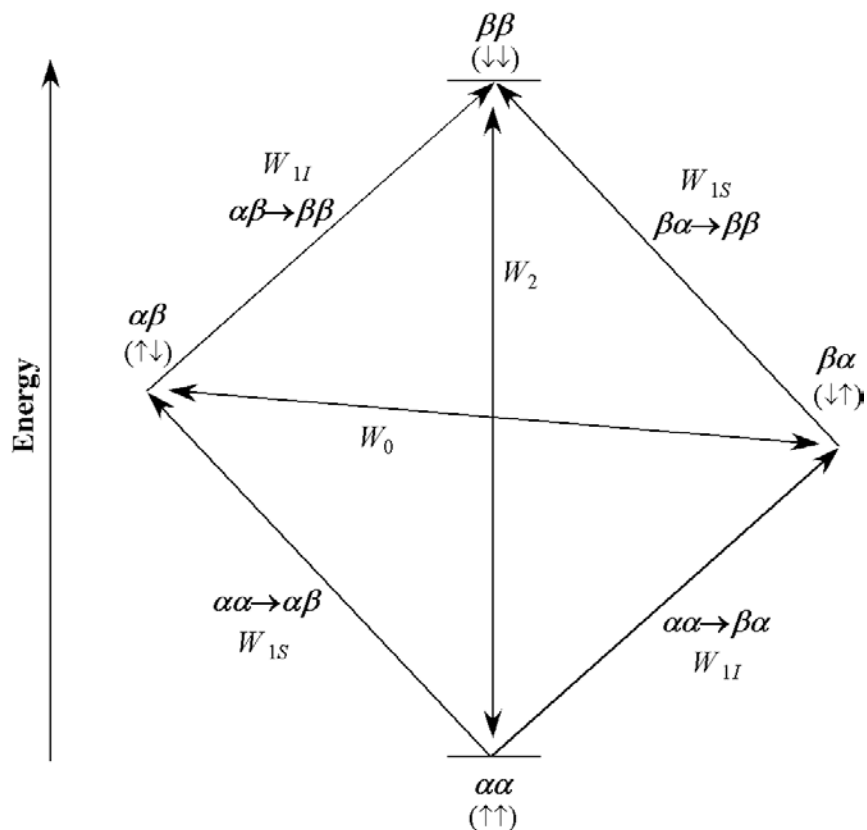
$$\eta = \frac{\gamma_I}{\gamma_S} \left(\frac{\sigma}{\rho'} \right)$$

$$\rho = W_0 + 2W_{1I} + W_2$$

$$\rho' = W_0 + 2W_{1S} + W_2$$

$$\sigma = W_2 - W_0$$

and the W are transition rates as defined in the energy-level diagram below:



If the autocorrelation function of the reorientational motion of the molecule may be accurately described as an exponential, then the transition rates are given as shown below:

$$\eta = \left(\frac{\sigma}{\rho'} \right) = \frac{\gamma_I}{\gamma_S} \frac{W_2 - W_0}{W_0 + 2W_{1S} + W_2}$$

$$W_0 = W(\alpha\beta \leftrightarrow \beta\alpha) = W(\beta\alpha \leftrightarrow \alpha\beta) = \frac{\hbar^2 \gamma_I \gamma_S}{10r_{IS}^6} \left(\frac{\tau}{1 + (\omega_I - \omega_S)^2 \tau^2} \right)$$

$$W_{1I} = W(\alpha\alpha \leftrightarrow \beta\alpha) = W(\alpha\beta \leftrightarrow \beta\beta) = \frac{3\hbar^2 \gamma_I \gamma_S}{20r_{IS}^6} \left(\frac{\tau}{1 + \omega_I^2 \tau^2} \right)$$

$$W_{1S} = W(\alpha\alpha \leftrightarrow \alpha\beta) = W(\beta\alpha \leftrightarrow \beta\beta) = \frac{3\hbar^2 \gamma_I \gamma_S}{20r_{IS}^6} \left(\frac{\tau}{1 + \omega_S^2 \tau^2} \right)$$

$$W_2 = W(\alpha\alpha \leftrightarrow \beta\beta) = \frac{6\hbar^2 \gamma_I \gamma_S}{10r_{IS}^6} \left(\frac{\tau}{1 + (\omega_I + \omega_S)^2 \tau^2} \right)$$

Fast Motion Limit

For small molecules, in simple aqueous or organic solution the *fast motion limit* applies:

$$\omega^2 \tau^2 \ll 1$$

In which case our expression become:

$$W_0 = W(\alpha\beta \leftrightarrow \beta\alpha) = W(\beta\alpha \leftrightarrow \alpha\beta) = \frac{\hbar^2 \gamma_I \gamma_S}{10r_{IS}^6} \tau$$

$$W_{1I} = W(\alpha\alpha \leftrightarrow \beta\alpha) = W(\alpha\beta \leftrightarrow \beta\beta) = \frac{3\hbar^2 \gamma_I \gamma_S}{20r_{IS}^6} \tau$$

$$W_{1S} = W(\alpha\alpha \leftrightarrow \alpha\beta) = W(\beta\alpha \leftrightarrow \beta\beta) = \frac{3\hbar^2 \gamma_I \gamma_S}{20r_{IS}^6} \tau$$

$$W_2 = W(\alpha\alpha \leftrightarrow \beta\beta) = \frac{6\hbar^2 \gamma_I \gamma_S}{10r_{IS}^6} \tau$$

Under these circumstances η becomes:

$$\eta = \left(\frac{\sigma}{\rho'} \right) = \frac{W_2 - W_0}{W_0 + 2W_{1S} + W_2} = \frac{\gamma_I}{2\gamma_S}$$

If our I -spins are ^1H nuclei and our S -spins are ^{13}C nuclei, then we obtain:

$$\eta = \left(\frac{\sigma}{\rho'} \right) = \frac{\gamma_I}{2\gamma_S} = \frac{\gamma_{\text{H}}}{2\gamma_{\text{C}}} = \frac{267.522 \times 10^{-6} \text{ rad s}^{-1} \text{ T}^{-1}}{2(67.283 \times 10^{-6} \text{ rad s}^{-1} \text{ T}^{-1})} = 1.988$$

whereas if the nuclei are both protons we obtain:

$$\eta = \left(\frac{\sigma}{\rho'} \right) = \frac{\gamma_I}{2\gamma_I} = \frac{1}{2}$$

Slow Motion Limit

For larger molecules a new set of approximations apply. For a pair of dipolar-coupled ^1H nuclei ($\gamma_I = \gamma_S$) in the limit as

$$\omega^2 \tau^2 \gg 1$$

the transition rates assume the following form:

$$W_0 = W(\alpha\beta \leftrightarrow \beta\alpha) = W(\beta\alpha \leftrightarrow \alpha\beta) = \frac{\hbar^2 \gamma_I \gamma_S}{10r_{IS}^6} \left(\frac{\tau}{1 + (\omega_I - \omega_S)^2 \tau^2} \right) = \frac{\hbar^2 \gamma_I \gamma_S}{10r_{IS}^6} \tau$$

$$W_{1I} = W(\alpha\alpha \leftrightarrow \beta\alpha) = W(\alpha\beta \leftrightarrow \beta\beta) = \frac{3\hbar^2 \gamma_I \gamma_S}{20r_{IS}^6} \left(\frac{\tau}{1 + \omega_I^2 \tau^2} \right) \approx 0$$

$$W_{1S} = W_{1I} = W(\alpha\alpha \leftrightarrow \alpha\beta) = W(\beta\alpha \leftrightarrow \beta\beta) = \frac{3\hbar^2 \gamma_I \gamma_S}{20r_{IS}^6} \left(\frac{\tau}{1 + \omega_S^2 \tau^2} \right) \approx 0$$

$$W_2 = W(\alpha\alpha \leftrightarrow \beta\beta) = \frac{6\hbar^2 \gamma_I \gamma_S}{10r_{IS}^6} \left(\frac{\tau}{1 + (\omega_I + \omega_S)^2 \tau^2} \right) \approx 0$$

The NOE for ^1H nuclei in the slow motion limits is thus given by:

$$\eta = \left(\frac{\sigma}{\rho'} \right) = \frac{W_2 - W_0}{W_0 + 2W_{1s} + W_2} = \frac{W_2 - W_0}{W_0 + 2W_{1l} + W_2} = \frac{-W_0 \gamma_I}{W_0 \gamma_I} = -1$$

i.e., the enhancement produces a negative value.

In practice, since the gyromagnetic ratio for ^{13}C nuclei is lower than that of protons the maximum heteronuclear enhancement is only achieved for large biological macromolecules at high applied magnetic field strength, i.e., at 11.74 (500 MHz ^1H), the Larmor frequency of ^{13}C is approximately 125 MHz and given a correlation time of 10^{-8} seconds we observe that

$$\omega\tau = 2\pi(125 \times 10^6 \text{ s}^{-1})(1 \times 10^{-8} \text{ s}) \approx 8$$

Thus for dipolar-coupled ^1H spins ($\gamma_I = \gamma_S$) the enhancement is -1, while for a ^1H - ^{13}C pair the maximum enhancement is -4.

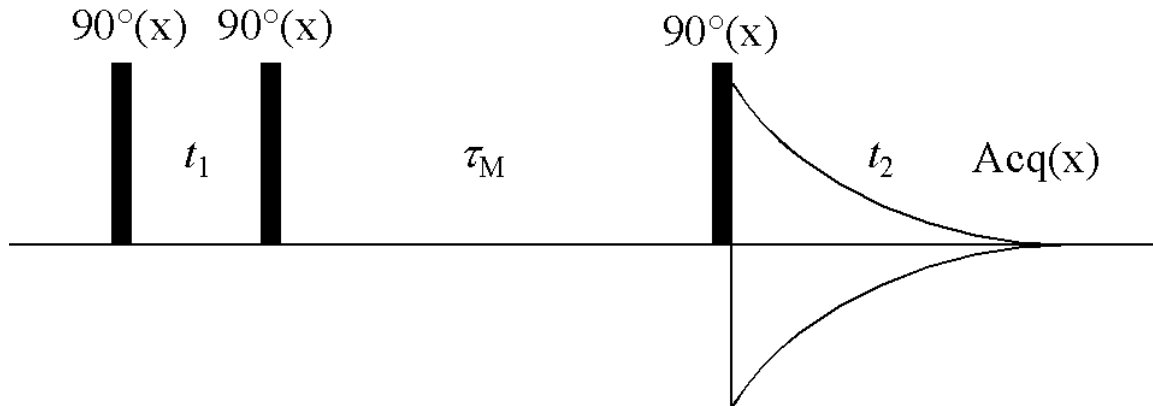
We have already seen that the NOE can be an effective approach to sensitivity enhancement. The utility of the NOE as a structural tool far surpasses its value as a spectral enhancement tool.

If NOE effects can be monitored at a numbers of molecular sites, the information obtained, ^1H - ^1H distances, can be used to reconstruct the molecule of interest.

The most effective applications of the NOE make use of multidimensional techniques and may also involve resolution of the heteronuclear chemical shifts.

NOESY: Nuclear Overhauser Effect Spectroscopy

The pulse sequence of the 2D ^1H - ^1H NOESY experiment is shown below:



The first part of the NOESY experiment looks like the COSY experiment, and the initial ^1H 90-pulse generates transverse magnetization that evolves under chemical shift and J-coupling influences.

Instead of gating on the receiver after the second ^1H 90-pulse however, a delay is inserted. This interval is known as the mixing time, since it formally occupies the mixing component of the 2D experiment.

The length of the mixing time is adjusted to allow nuclear Overhauser enhancement to develop. Unlike the steady-state case we previously considered, the value of the NOE will depend upon the length of the mixing time.

Transient Nuclear Overhauser Effect

$$\frac{d}{dt} I_z^I = -\rho(I_z^I - I_{z0}^I) - \sigma(I_z^S - I_{z0}^S)$$

$$\frac{d}{dt} I_z^S = -\rho'(I_z^S - I_{z0}^S) - \sigma(I_z^I - I_{z0}^I)$$

In our previous discussion of the NOE we considered the effect on S -spins when we supplied RF irradiation at the I -spin frequency.

We now reconsider these effects before a steady-state condition is established.

As before, RF-irradiation causes the number of spins in the α and β states of the I -spins to become equal. This means that I_z^I is equal to zero, and in the pre-steady-state regime we may write that:

$$\frac{d}{dt} I_z^S = -\rho' (I_z^S - I_{z0}^S) - \sigma \left(\underbrace{I_z^I}_{=0} - I_{z0}^I \right) = -\rho' (I_z^S - I_{z0}^S) + \sigma (I_{z0}^I)$$

If we focus on ^1H - ^1H NOE effects then we may assume that $I_{z0}^I \cong I_{z0}^S = I_{z0}$,

and then rewrite the expression above as shown below:

$$\begin{aligned} \frac{d}{dt} I_z^S &= -\rho' (I_z^S - I_{z0}^S) + \sigma (I_{z0}^I) = -\rho' (I_z^S - I_{z0}) + \sigma (I_{z0}) \\ &= -\rho' \left[I_z^S - I_{z0} - \frac{\sigma}{\rho'} I_{z0} \right] = -\rho' \left[I_z^S - \left(1 + \frac{\sigma}{\rho'} \right) I_{z0} \right] \end{aligned}$$

We define a parameter, $\eta(t)$, that represents the transient nuclear Overhauser effect:

$$\eta_S(t) = \frac{I_z^S - I_{z0}}{I_{z0}}$$

and using this expression we may then write the following

$$I_z^S = \eta_S(t) I_{z0} + I_{z0} = I_{z0} (\eta_S(t) + 1)$$

and finally

$$\begin{aligned} \frac{d\eta_S(t)}{dt} &= \frac{1}{I_{z0}} \frac{dI_z^S}{dt} \\ &= -\frac{\rho'}{I_{z0}} \left[I_z^S - \left(1 + \frac{\sigma}{\rho'} \right) I_{z0} \right] \\ &= -\rho' \left[\eta_S(t) - \frac{\sigma}{\rho'} \right] \\ &= -(\rho' \eta_S(t) + \sigma) \end{aligned}$$

Separation of variables and integration leads to

$$\frac{1}{\rho'} \ln \left(\frac{\rho' \eta_s(t) - \sigma}{\rho' \eta_s(0) - \sigma} \right) = -t$$

since

$$\eta_s(0) = \frac{I_{z0}^s - I_{z0}}{I_{z0}} = 0$$

thus

$$\frac{1}{\rho'} \ln \left(\frac{\rho' \eta_s(t) - \sigma}{-\sigma} \right) = -\frac{1}{\rho'} \ln \left(\frac{\rho' \eta_s(t) - \sigma}{\sigma} \right) = -t$$

$$-\frac{\rho' \eta_s(t) - \sigma}{\sigma} = e^{-\rho' t}$$

$$\eta_s(t) = \frac{\sigma}{\rho'} (1 - e^{-\rho' t})$$

Notice that as $t \rightarrow 0$, $\eta_s \rightarrow 0$ as we would expect and furthermore as $t \rightarrow \infty$, we find that

$$\eta_s(\infty) = \frac{\sigma}{\rho'} \left(1 - \frac{1}{\infty} \right)$$

$$\eta_s(\infty) = \frac{\sigma}{\rho'}$$

which confirms that in the limit of long time, the transient and steady-state nuclear Overhauser expressions predict the exact same behavior.

In practice, for optimal accuracy one should record a series of experiments in which the irradiation interval of NOESY mixing time are incremented. This procedure is formally equivalent to calculating the time derivative $\eta(t)$, evaluated in the limit as $t \rightarrow 0$:

$$\frac{d\eta(t)}{dt} \Big|_{t \rightarrow 0} = -\sigma$$

Since the initial rate of increase is proportional to σ , i.e.,

$$\frac{d\eta(t)}{dt} \Big|_{t \rightarrow 0} = -\sigma$$

and in the slow-motion regime we know that

$$\sigma \approx W_0 = \frac{\gamma_I^2 \gamma_S^2}{10r_{IS}^6} \tau_c$$

wherein r_{IS} is the internuclear distance and τ_c is the time constant for reorientational (tumbling) motion.

The distance between two protons may be calculated based on a known distance ($r_{IS} = r_0$) say the distance between two geminal protons, ($\sim 1.8\text{\AA}$), by measuring the initial rate of the NOE increase and comparing that rate to the rate of another pair of protons

$$\frac{\sigma}{\sigma_0} = \frac{\frac{\gamma_I^2 \gamma_S^2}{10r^6} \tau_c}{\frac{\gamma_I^2 \gamma_S^2}{10r_0^6} \tau_c} = \left(\frac{r_0}{r} \right)^6$$

are more usefully, we write that

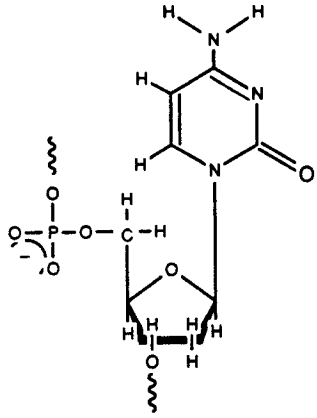
$$r = r_0 \left(\frac{\sigma}{\sigma_0} \right)^{1/6}$$

This last expression is the master equation of NMR-based structural methods.

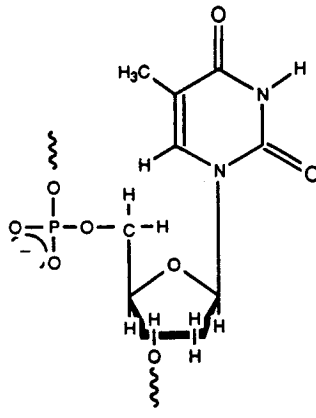
The application of the NOE as a structural tool revolutionized the way in which chemists and structural biologists think about and solve structure. Prior to the developments outlined above, the only comprehensive structural method was crystallography.

From the structural biology perspective, clearly the analysis of NOESY data is of central significance. Although J-coupling structural restraints are an important component of the overall structure determining effort, analysis of ^1H - ^1H distances remains the major contributor to structural information in biological macromolecules.

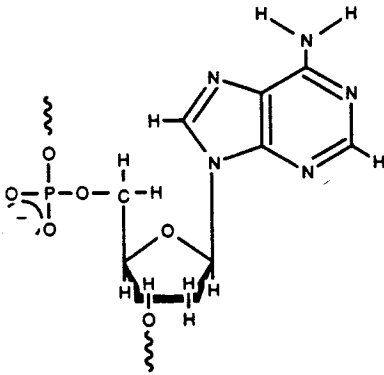
Chain form of DNA nucleotides:



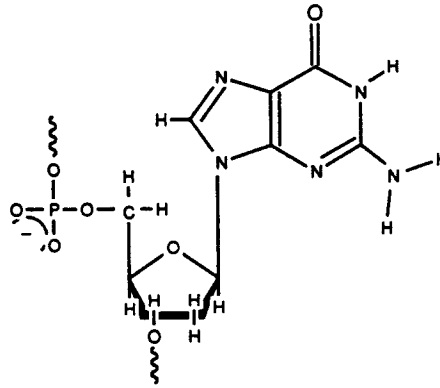
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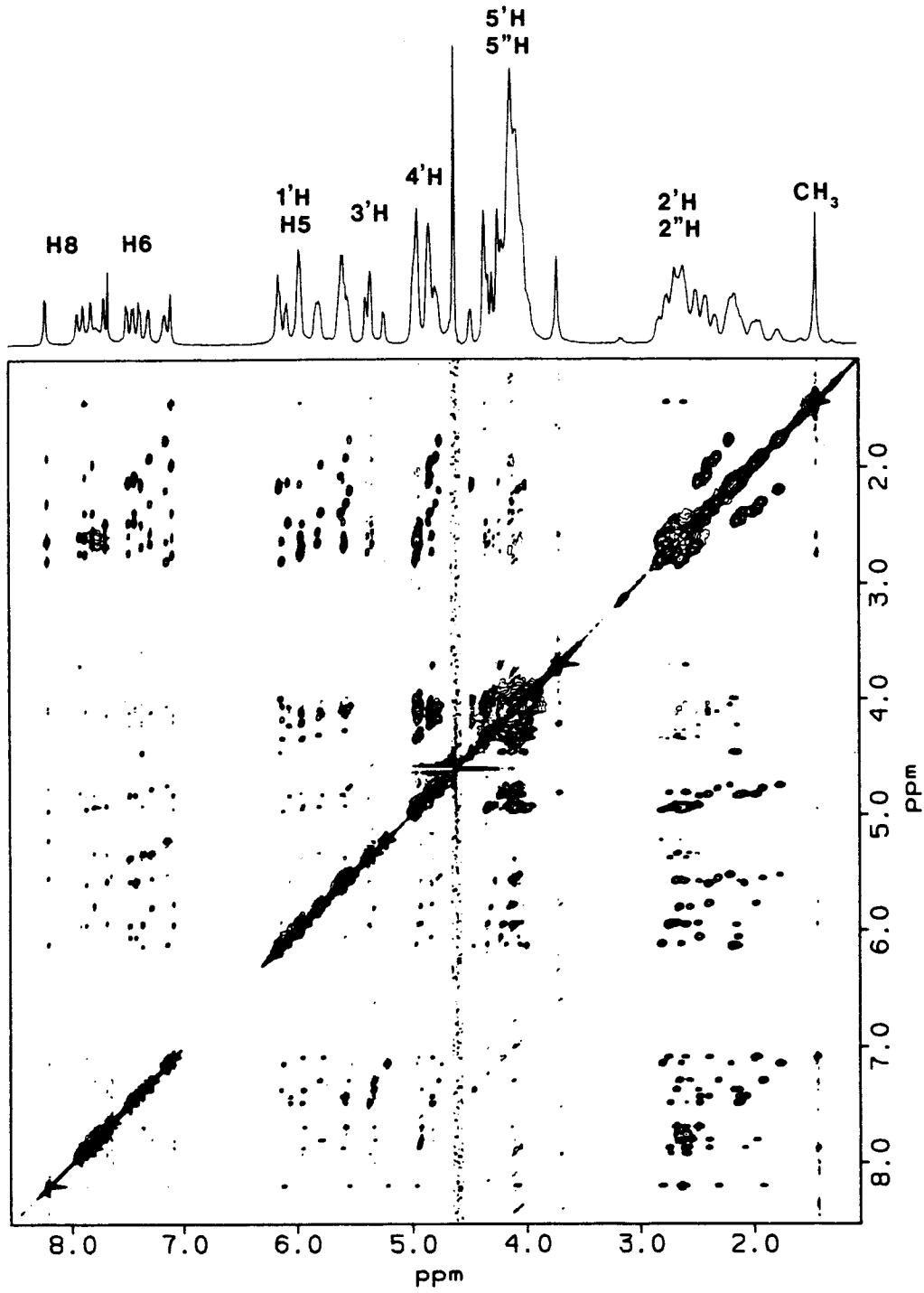


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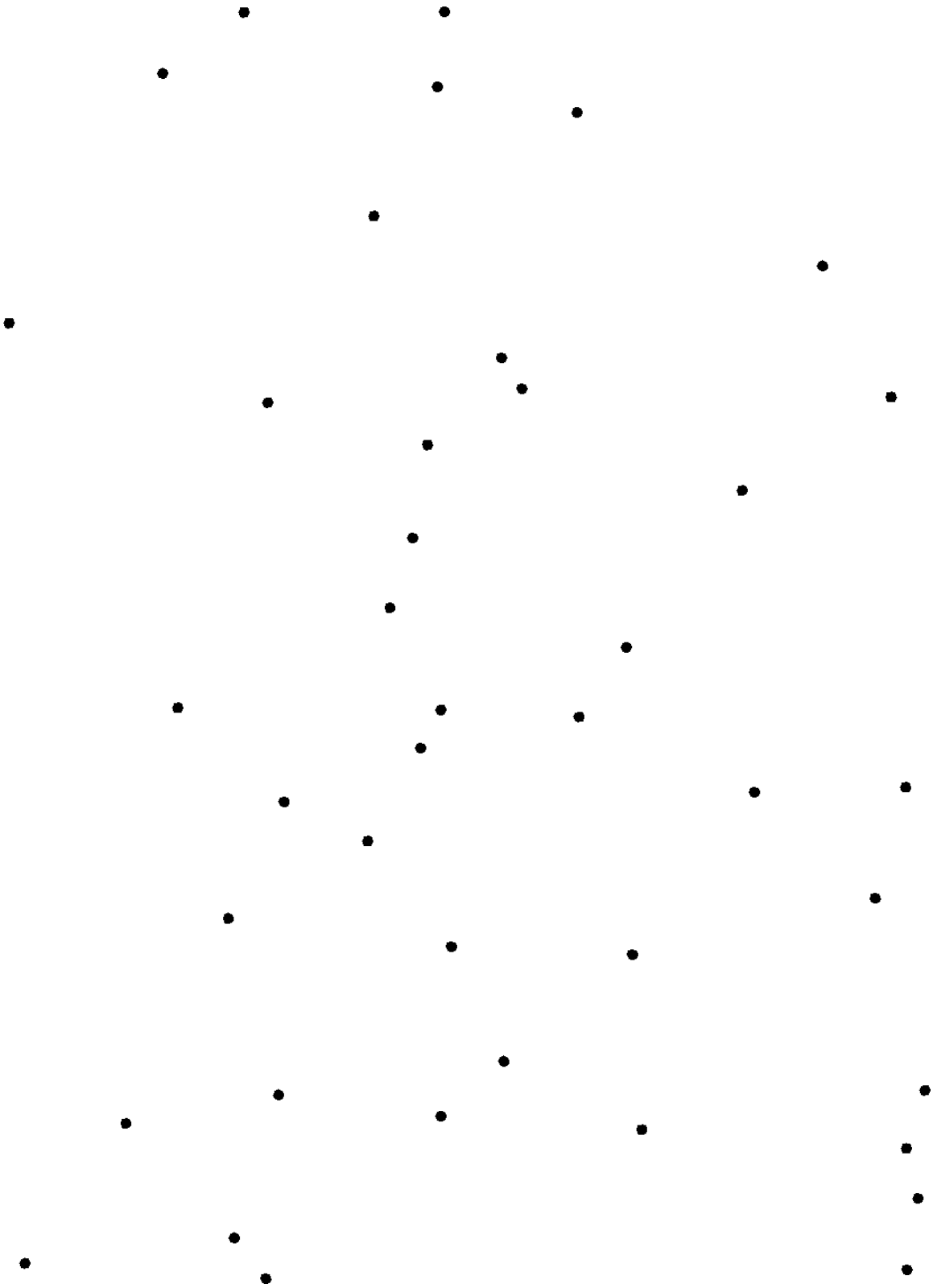


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2D NOESY of [d(GCCGTGGCCACGGC)]₂:



City	City	Distance (miles)
Big Water	Ticaboo	66
Blanding	Bluff	25
Bluff	Mexican Hat	18
Brigham City	Logan	18
Brigham City	Tremonton	16
Delta	Deseret	7
Delta	Richfield	48
Delta	Nephi	50
Delta	Manti	50
Green River	Cisco	46
Green River	Hanksville	53
Hanksville	Loa	50
Hanksville	Ticaboo	48
Heber City	Provo	21
Manti	Price	48
Moab	Blanding	75
Moab	Cisco	30
Moab	Green River	42
Moab	La Sal	24
Park City	Heber City	11
Price	Duchesne	42
Provo	Nephi	38
Provo	Payson	13
Richfield	Loa	48
Roosevelt	Duchesne	25
SLC	Lehi	34
SLC	Magna	13
SLC	Ogden	33
SLC	Provo	36
SLC	Tooele	30
Tremonton	Stone	35
Vernal	Roosevelt	28
Wendover	Lucin	53



Zero NOE

The fact that the NOE ranges from a positive value (+1/2) in the fast-motion limit to a negative value in the slow-motion limit implies that for some range of correlation times the NOE will be close to zero.

The range of correlation times that produce low NOE values will of course vary with the viscosity of the solution, but the consensus problem range appears to be consistent with molecules with molecular weights in the range between 700 and 2000.

For molecules that fall in the zero-NOE range, conventional NOE experiments fail to generate ^1H - ^1H crosspeaks.

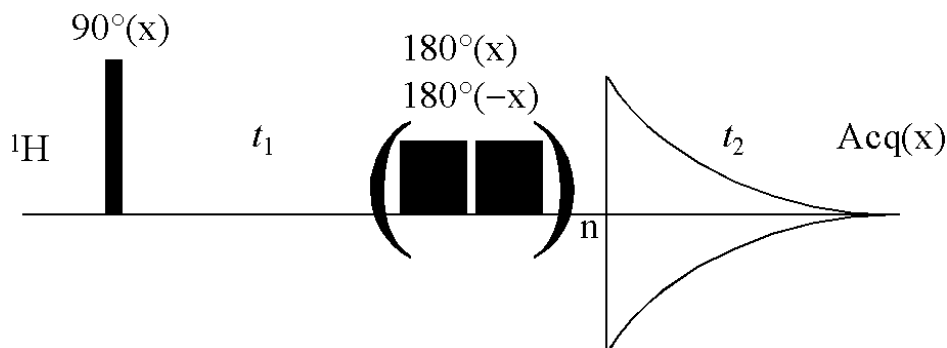
Of course we have a solution – ROESY: Rotating Frame Overhauser Effect Spectroscopy.

The approach recognizes the fact that it is the magnitude of the polarization versus the tumbling rate of the molecule that leads to a low or zero NOE effect. The solution is simply to requantize the spins along a weaker magnetic field.

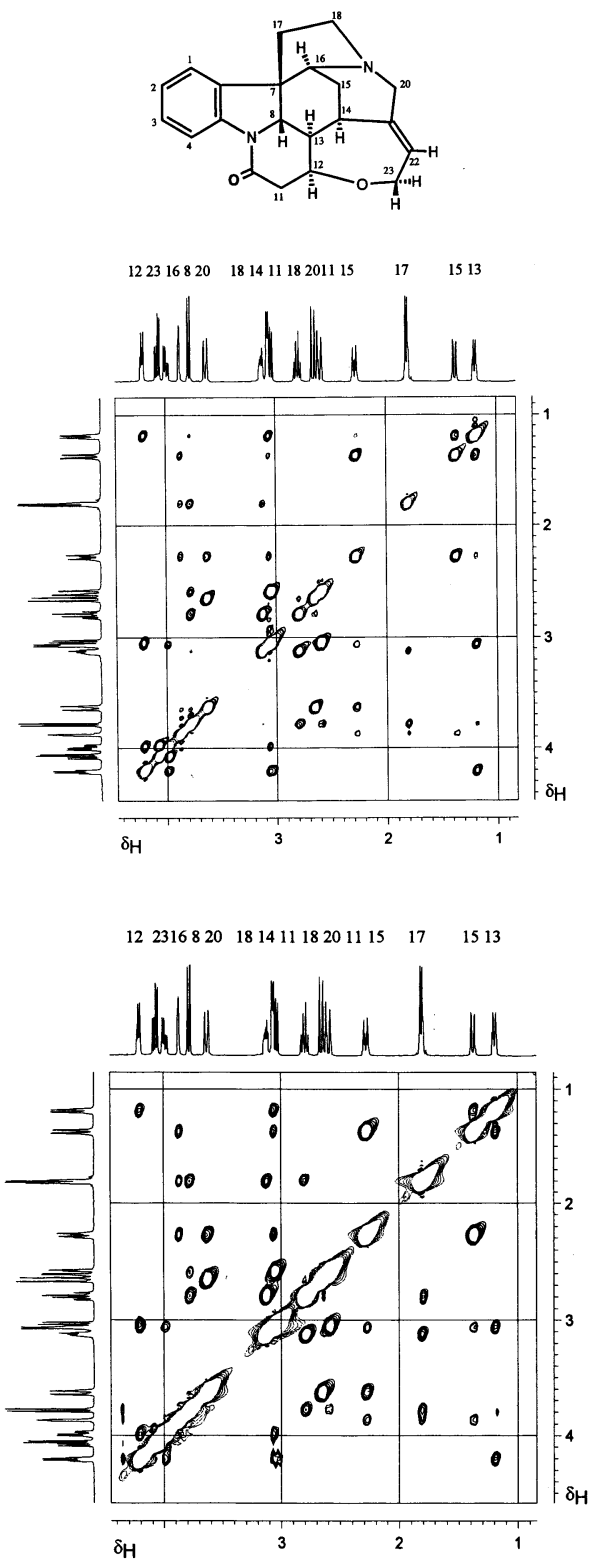
This sounds a little complicated, or even unlikely, but in fact we have already been using this technology all along. Since the applied RF is resonant, the oscillating magnetic field component is dominant during an RF pulse. We can requantize the spin system using the resonant magnetic field generated by the applied RF pulse.

The spin physics present under these circumstances mean that the fast-motion limit always exists, i.e., all NOE have the same sign and there is no zero-NOE (actually ROE) regime.

The experiment used to conduct this experiment is shown below:



Comparison of NOESY and ROESY spectra:



Figures 10.19 and 10.20 from Braun, S., Kalinowski, H.O. and Berger, S. (1998) *150 and More Basic NMR Experiments*. Wiley-VCH, New York.