CHEM / BCMB 4190/6190/8189

Introductory NMR

Lecture 18

+ 45%

Cl H^B H^A O—C—CH$_3$ (O above)

Cl

Cl

Cl

Cl

Cl Cl

1

+ 18%

H$_3$C H

N—C

H$_3$C O

− 2%

2

+ 17%

H$_3$C H

C=C

H$_3$C COOH

− 4%

3
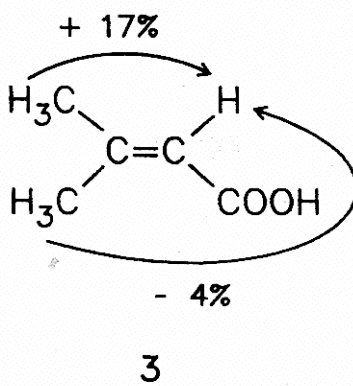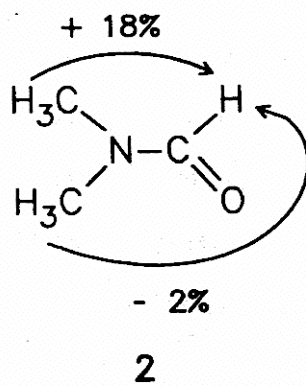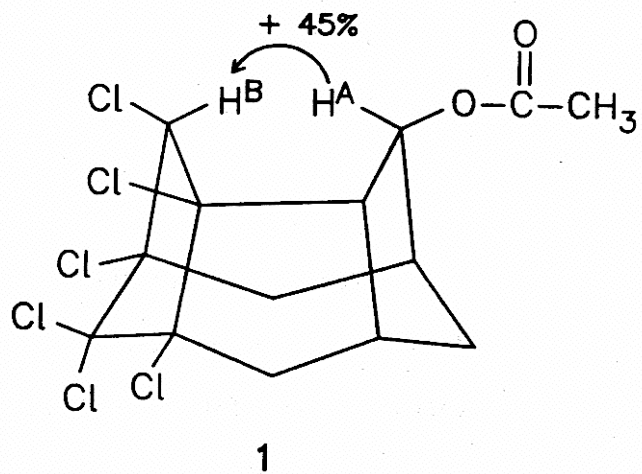
The Nuclear Overhauser Effect

**NOE: Nuclear Overhauser Effect or Nuclear Overhauser Enhancement**

- Depends on dipole-dipole relaxation (an important mechanism of T_1 relaxation)
- Is based on through-space interactions that change M_z
- The NOE changes the intensities of particular signals
- The magnitude of the NOE is related to nuclear distances and molecular motions

1. 1D steady-state NOE experiment:

    Consider a two-spin system AX:

        -Assume the homonuclear case (A and X are both ^{1}H, although they need not be)
        -Assume no scalar coupling between A and X ($J = 0$)
        -Assume the internuclear A-X distance is $\leq \sim 5$Å (NOE enhancement depends on $1/r^6$ where r is internuclear distance)

    So, the simple 1D spectrum gives 2 signals with no splitting (J=0):
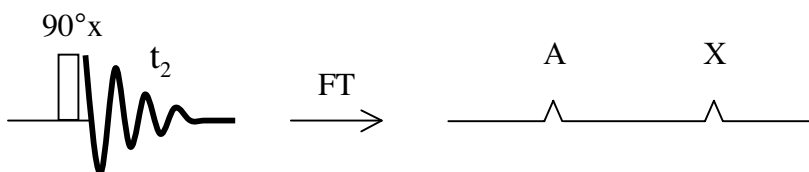
    To establish the NOE, we will selectively saturate the 'A' transition with an RF field to equilibrate the spin populations:
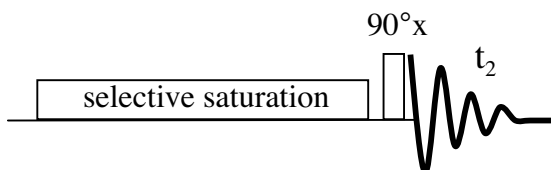
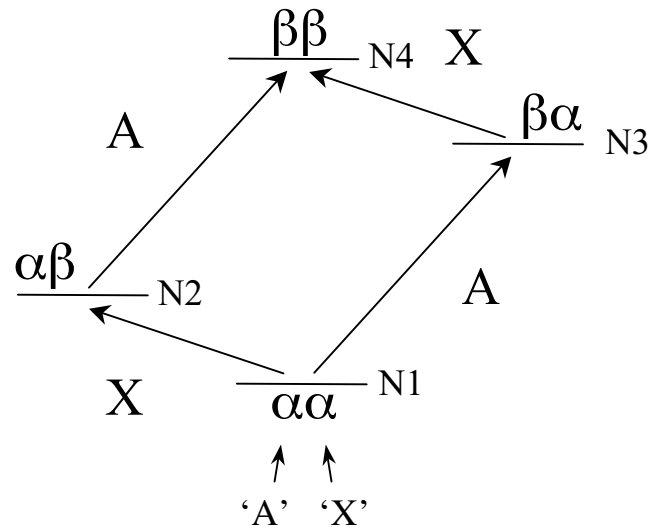**What happens to the population levels when we saturate one of the signals, and what happens afterwards?**
       -Let's look at the energy level diagrams:
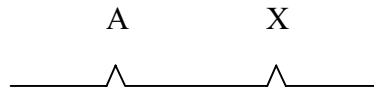
A). At equilibrium:

   N4 = N
   N3 = N + ΔH
   N2 = N + ΔH
   N1 = N + 2ΔH

$$\beta\beta \quad \text{N4} \quad X$$
$$\beta\alpha \quad \text{N3}$$
$$A$$
$$\alpha\beta \quad \text{N2}$$
$$A$$
$$X \qquad \alpha\alpha \quad \text{N1}$$

'A' transitions: N1 – N3 = N2 – N4 = ΔH
'X' transitions: N1 – N2 = N3 – N4 = ΔH

               'A'   'X'

- **'A'** and **'X'** transitions are <u>allowed</u> and <u>observable</u>
- **'A'** and **'X'** transitions give singlets in 1D spectrum
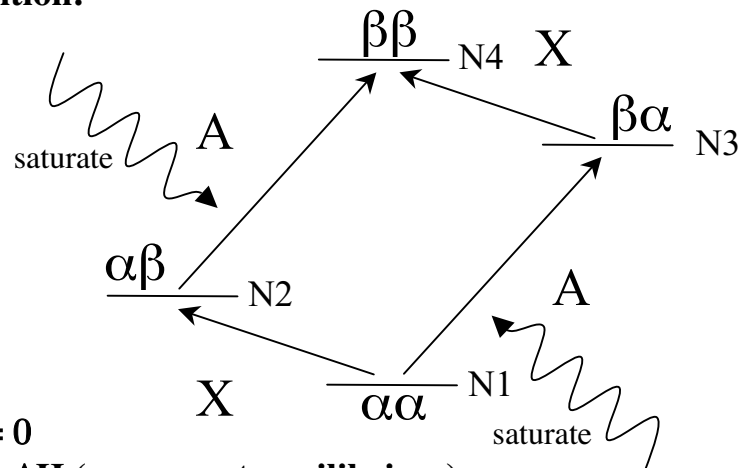
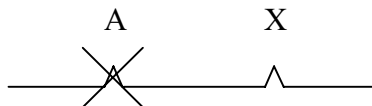   A   X

B). After saturation of the 'A' transition:

   N4 = N + ΔH/2
   N3 = N + 3ΔH/2
   N2 = N + ΔH/2
   N1 = N + 3ΔH/2

$$\beta\beta \quad \text{N4} \quad X$$
$$\text{saturate} \quad A \qquad \beta\alpha \quad \text{N3}$$
$$\alpha\beta \quad \text{N2}$$
$$A$$
$$X \qquad \alpha\alpha \quad \text{N1}$$
               saturate

'A' transitions: N1 – N3 = N2 – N4 = 0
'X' transitions: N1 – N2 = N3 – N4 = ΔH (same as at equilibrium)
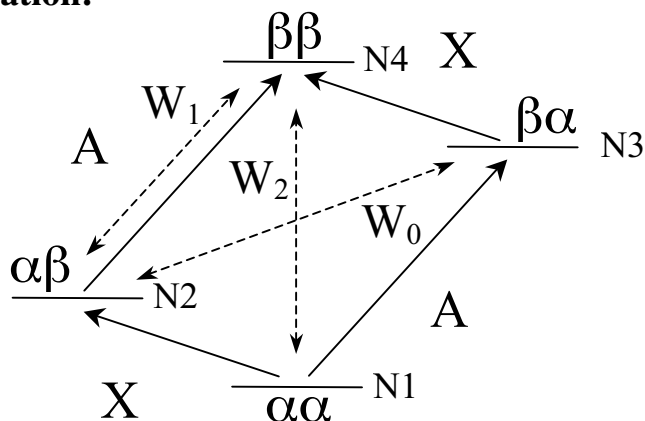
-populations of N1, N2, N3, and N4 are now different than they were at equilibrium

               A   X

C). Now, allow relaxation during saturation:

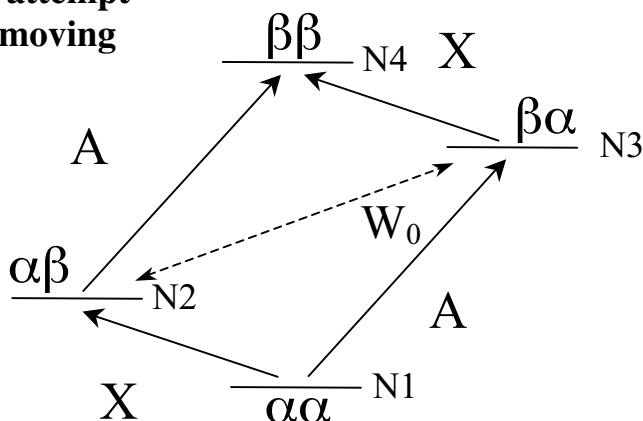|	at equilibrium	after saturation
N4 =	N	N + ΔH/2
N3 =	N + ΔH	N + 3ΔH/2
N2 =	N + ΔH	N + ΔH/2
N1 =	N + 2ΔH	N + 3ΔH/2

- W_0, W_1, and W2 are *rate constants* or *transition probabilities*
- W_1 is a one-spin transition or single quantum transition
- W_0 and W_2 are two-spin transitions
- W_0 is a zero quantum transition
- W_2 is a double quantum transition
- W_0 and W_2 are spectroscopically **FORBIDDEN** and cannot be observed
    -**HOWEVER, they are ALLOWED for relaxation**

Let's suppose W_0 is more efficient than W_2 (<u>large molecule case</u>):

-**in this case, the system will attempt to return to equilibrium by moving spins from N3 to N2**

after saturation
and W_0 relaxation
N4 = N + ΔH/2
N3 = N + 3ΔH/2 - δ
N2 = N + ΔH/2 + δ
N1 = N + 3ΔH/2

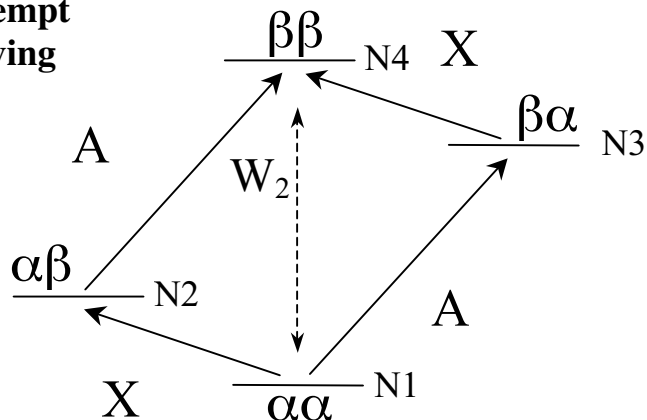**'X' transitions: N1 – N2 = N3 – N4 = ΔH - δ: intensity of 'X' reduced !!**
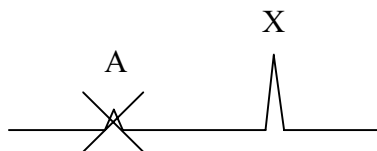
Let's suppose W_2 is more efficient than W_0 (<u>small molecule case</u>):

-**in this case, the system will attempt to return to equilibrium by moving spins from N4 to N1**

after saturation
and W_0 relaxation

N4 = N + ΔH/2 - δ
N3 = N + 3ΔH/2
N2 = N + ΔH/2
N1 = N + 3ΔH/2 + δ

$$\beta\beta \quad \text{N4} \quad X$$
$$A \qquad W_2 \qquad \beta\alpha \quad \text{N3}$$
$$\alpha\beta \quad \text{N2} \qquad A$$
$$X \qquad \alpha\alpha \quad \text{N1}$$

'X' transitions: N1 – N2 = N3 – N4 = ΔH + δ: intensity of 'X' increased !!

X

A

Summary:

(reminder: τ_c is the correlation time of the molecule (sec))

- for small molecules (short τ_c) W_2 dominates
- for large molecules (large τ_c) W_0 dominates
- because W_0 and W_2 depend on B_0, the NOE also depends on B_0

2. <u>**Enhancement Factor for Small Molecules:**</u>

Maximal enhancement for small molecules (η):

$$\eta = \gamma_a / 2\gamma_x \qquad \text{A: saturated} \qquad \text{X: observed}$$
$$(\gamma \text{ is gyromagnetic ratio, of course})$$

Total Intensity (I):

$$I = (1 + \eta) I_0 \qquad I_0 \equiv \text{original intensity}$$

Examples:

a. Fractional increase in ^{13}C NMR signal intensity with BB ^{1}H decoupling:

$$\eta = \gamma_a / 2\gamma_x$$
$$\eta = 4 / (2 \times 1) = 2 \quad \text{i.e. signal increased by a factor of 2 (200\%)}$$

b. Fractional increase in ^{1}H NMR signal intensity with BB ^{13}C decoupling:

$$\eta = 1 / 8 = 0.125 \quad \text{i.e. signal increased by a maximum of 12.5\%}$$
$$I = (1 + 0.125) I_0 = 1.125 \times I_0 \quad \text{(signal is slightly more intense)}$$

3. <u>**Multi-Spin Systems:**</u>

Consider a homonuclear 3-spin system A-B-C
       - assume no *J* coupling
       - assume the molecule is a small molecule

-If A is saturated, the intensity of the signal from B is increased
-The saturation of A affects the populations of the B-C system, which causes an indirect NOE to C.
-Thus, this indirect NOE is negative

4. **Dependence of Maximum ^{1}H-^{1}H Homonuclear NOE on B$_0$ and molecular mass:**

The magnitude and sign of the ^{1}H-^{1}H NOE are dependent on:

- the Larmor frequency, ν (sometimes ω), which depends on B$_0$
- the correlation time, τ_c, which depends on molecular size (mass)
    - VERY crudely, $\tau_c \approx 10^{-12}$ MW (where MW is molecular mass in Daltons)
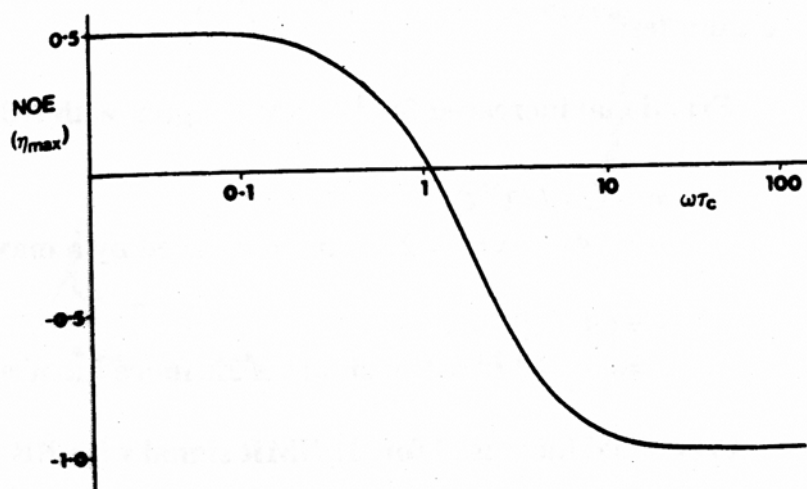   - example: $\tau_c \approx 10$ ns for 10 kDa molecule

FIGURE 2.6
Dependence of maximum homonuclear NOE enhancement on $\omega\tau_c$. Note the log scale of $\omega\tau_c$.

Note:

- NOE is *positive* for *small* molecules
- NOE is *negative* for *large* molecules
- NOE is zero at $\omega\tau_c \approx 1$
    - examples: -at 200 MHz, NOE is zero for molecules with $\tau_c \approx 5$ ns (*approximately* 5 kDa)
    - at 800 MHz, NOE is zero for molecules with $\tau_c \approx 1.25$ ns (*approximately* 1.25 kDa)

5. Dependence of the magnitude of the NOE on Internuclear Distance:

In the absence of external relaxation:
- the maximal enhancement in achieved
- the NOE is distance independent

In the presence of external relaxation:
- two-spin steady-state NOE depends on internuclear distance

$$f = \text{NOE enhancement} \propto 1/r^6$$
(r = internuclear distance)
-this effect is usually observed only for ^1H-^1H distances ≤ 5 Å

Examples of external relaxation:

a). intermolecular relaxation caused by paramagnetic agents
- field at a nucleus depends on the presence of unpaired electrons
- examples: dissolved O_2, manganese and other metal impurities

b). quadrupolar relaxation
- energy of quadrupole nuclei varies with the fluctuating electric field gradient
- dominant mechanism for nuclei with $I > 1/2$ (^2H and ^{14}N)

c). chemical shift anisotropy (CSA)
- anisotropy produces a different field at the nucleus depending on the orientation of the bond relative to the applied field
- significant for nuclei with large CSA (^{19}F, ^{31}P)