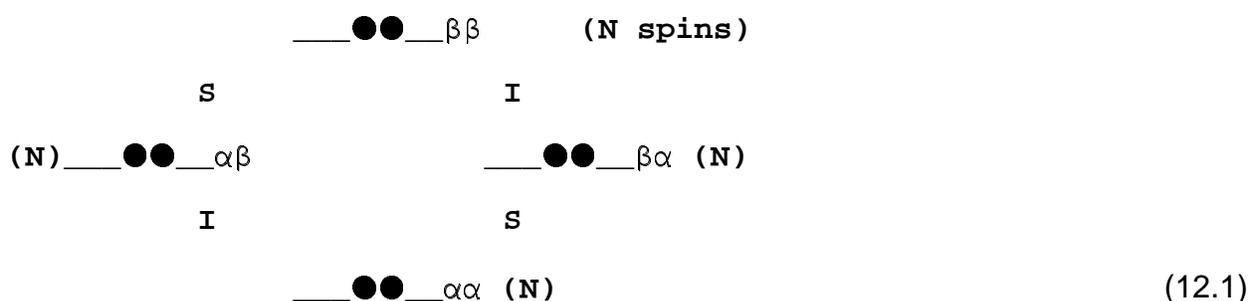


## 12. The Nuclear Overhauser Effect

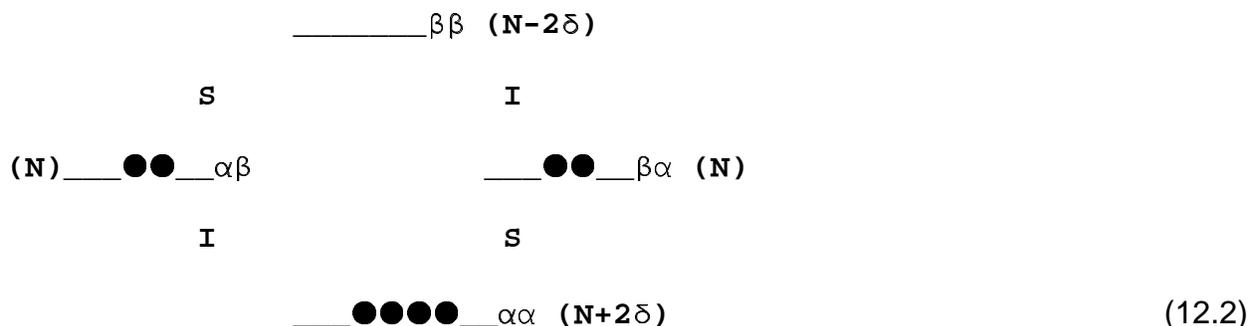
### 12.1 Saturation

Consider two spins, which have a dipolar interaction, placed in a magnetic field. At a time short compared to the longitudinal relaxation time the spins are equally distributed among the energy levels. This state, which corresponds to an infinite spin temperature, is known as the saturated state. Figure 12.1 is an energy level diagram for two interacting spins. The dots, ●, represent the spin populations of the energy levels. In the saturated state there are  $N$  spins in each level.



### 12.2 Relaxation to Thermal Equilibrium

After a time that is long with respect to the longitudinal relaxation time, the spin system reaches thermal equilibrium with the lattice. Figure (12.2) represents the spin populations at equilibrium. The amount of spin population that is transferred from one level to another is indicated as  $\delta$ . The magnitude of  $\delta$  is much less than  $N$ . Consequently, the lack of "dots" in the  $\beta\beta$  state is does not imply that there are no remaining spins in that energy level.



The intensity of S transitions is equal to the population differences,

$$|\alpha\beta\rangle - |\beta\beta\rangle = N - (N-2\delta) = 2\delta$$

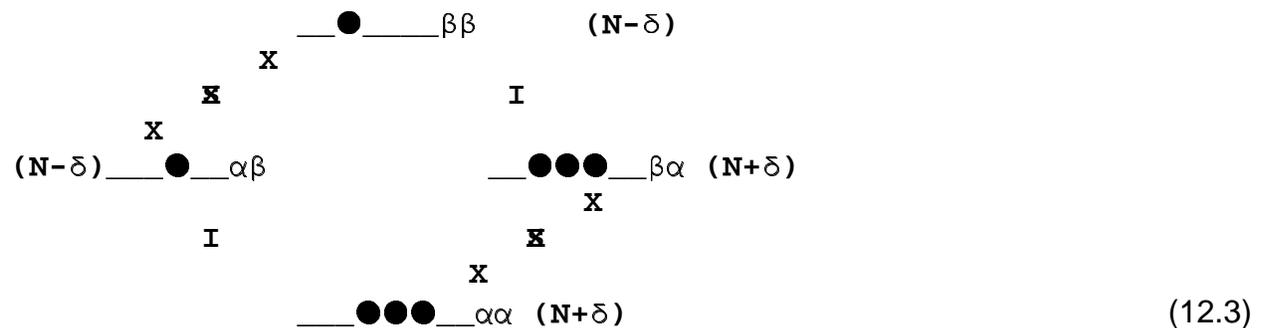
and

$$|\alpha\alpha\rangle - |\beta\alpha\rangle = (N+2\delta) - N = 2\delta$$

Likewise, the differences across the two I transitions yield  $2\delta$ .

### 12.3 Irradiation of the S Spin Transitions

If the spin system is irradiated at the frequency of the **S** transitions for with sufficient power and for a time long compared to the longitudinal relaxation time, the population differences across the **S** transitions will become equal and the transition will be saturated. In Figure 12.3, the X's represent irradiation at the **S** frequency. The dots have been rearranged to equalize them across the **S** levels.



The S intensity is again given by the population differences

$$(N-\delta)-(N-\delta) = (N+\delta)-(N+\delta) = 0.$$

These transitions have no amplitude; they are saturated.

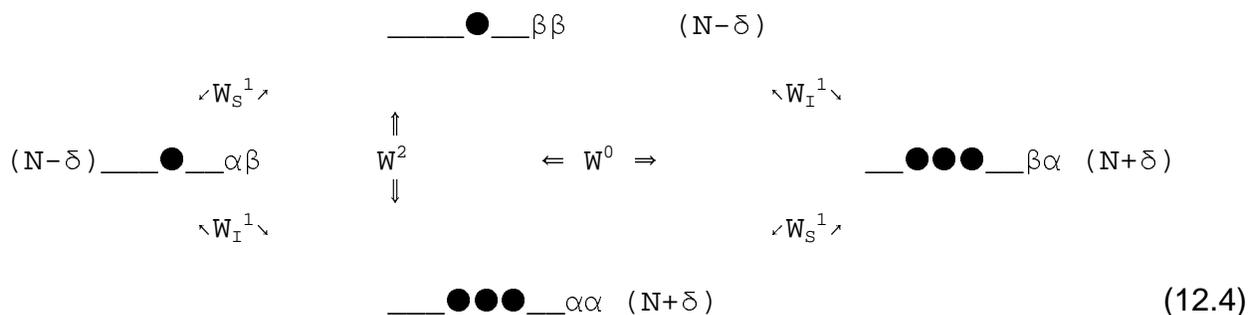
For the I intensity there is no change.

$$(N+\delta)-(N-\delta) = 2\delta$$

### 12.4 Transition Probabilities

The rate of relaxation of nuclear spins toward equilibrium can be described using transition probabilities,  $W_X^n$ . The subscript X is the nucleus to which the transition probability is assigned and the superscript n is the order of the transition. If the total magnetic quantum number remains unchanged in the transition then the order is zero. If the change is plus or minus one then the transition is of order 1. Likewise, if both

spins change such that the magnetic quantum number is different by two, then the order is two. The various transitions are shown in Figure 12.4.



In Figure 12.4,  $W_I^1$  and  $W_S^1$  are the **I** and **S** single quantum transitions.

$$|\alpha\alpha\rangle \Leftrightarrow |\beta\alpha\rangle$$

for **S** and

$$|\beta\alpha\rangle \Leftrightarrow |\beta\beta\rangle$$

for **I**.

The double spin flip,  $W^0$  is the zero quantum transition

$$|\alpha\beta\rangle \Leftrightarrow |\beta\alpha\rangle.$$

This transition involves the spin flip of two spins and is a multiple quantum transition, but since the total magnetic quantum number does not change it is referred to as a zero quantum transition.

The other transition involving the flipping of two spins is  $W^2$ , the double quantum transition

$$|\alpha\alpha\rangle \Leftrightarrow |\beta\beta\rangle.$$

In the presence of a saturating field for the **S** transitions, the transition probabilities have various effects, which are listed below.

$W_S^1$  - No effect since the transition is being irradiated.

$W_I^1$  - No effect since I transitions already at equilibrium.

$W^2$  - Double quantum transition probability:

At equilibrium =  $(N+2\delta)-(N-2\delta) = 4\delta$

Upon saturation of S transition =  $(N+\delta)-(N-\delta) = 2\delta$

The spin system is not at equilibrium across the 2 quantum transition.

If  $W^2 \neq 0$  then the double quantum transition will relax the system.

$W^0$  - Zero quantum transition probability:

At equilibrium =  $(N)-(N) = 0$

Upon saturation of S transition =  $(N+\delta)-(N-\delta) = 2\delta$

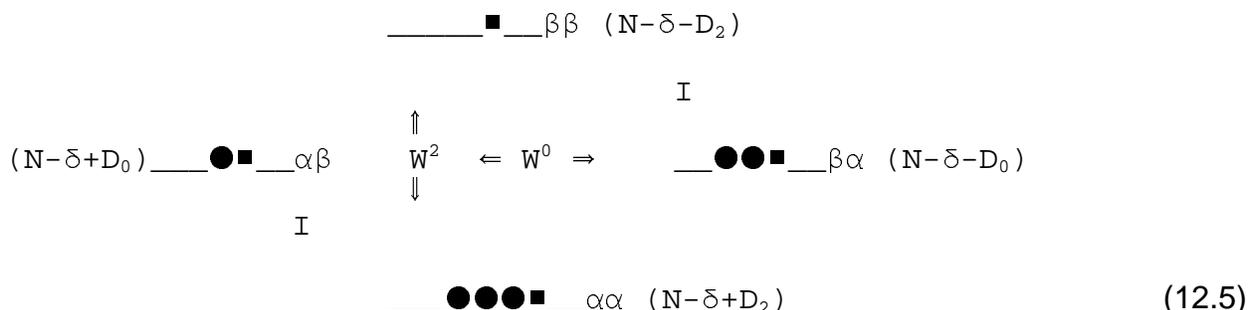
The spin system is not at equilibrium across the 0 quantum transition.

If  $W^0 \neq 0$  then the zero quantum transition will relax the system.

The non-equilibrium condition across the zero- and double-quantum transitions in the presence of the irradiating field drives the redistribution of the spin populations to achieve a new equilibrium.

### 12.5. Redistribution of Spin Populations Due to the NOE

Figure 12.5 shows the state of the spin system after the NOE has redistributed the populations. The square boxes,  $\blacksquare$ , represent an different quantity of spins than the dots.  $D_2$  and  $D_0$  represent the quantity of spins that under go the zero- and double-quantum transitions, respectively.



The magnitude of the I transitions are now

$$(N+\delta-D_0)-(N-\delta-D_2) = 2\delta - D_0 + D_2$$

and

$$(N+\delta+D_2)-(N-\delta+D_0) = 2\delta - D_0 + D_2.$$

The NOE effects the system in two opposite ways. The zero-quantum transition  $W_0$  decreases the intensity of I by an amount  $D_0$ , whereas the double-quantum transition  $W_2$  increases the intensity of I by an amount  $D_2$ .

## 12.6 Transition Probabilities and Spectral Density

For a homonuclear, proton spin system the single quantum transition probabilities,  $W_H^1$ , depend on  $J(\omega_H)$ , the spectral density at the  $^1\text{H}$  Larmor precession frequency. Motion in the spin system that has a finite amplitude at a frequency  $\omega_H$  causes  $W_H^1$  to become non-zero and therefore causes relaxation. In a similar manner the zero quantum,  $W^0$ , and double quantum,  $W^2$ , transition probabilities depend on the spectral density at zero frequency,  $J(0)$ , and at twice the  $^1\text{H}$  frequency,  $J(2\omega)$ , respectively. The  $2\omega$  frequency for  $^1\text{H}$  spins in a 11.75 T magnetic field is about 1 GHz, which corresponds to motion with a correlation time of about  $10^{-9}$  second. From the Stoke's-Einstein equation, a rule-of-thumb calculation for the rotational correlation time in nanoseconds of a rigid, spherical macromolecule, dissolved in  $\text{H}_2\text{O}$  at  $300^\circ\text{K}$ , that undergoing isotropic reorientation is

$$\tau_c = M_r / 3$$

where  $M_r$  is the molecular weight of the molecule in thousands and  $\tau_c$  is expressed in nanoseconds. For a small protein with a molecular weight of 6000 daltons, the rotational correlation time is about 2 nanoseconds. This corresponds to a frequency of 500 MHz. Motions with higher frequencies will be absent for a perfectly rigid molecule. The spectral density at the  $2\omega$  frequency will typically be small for proteins and other macromolecules. The zero frequency component of the spectral density function increases with increasing molecular weight, since the rotational frequencies become lower. The NOE is characterized by the cross relaxation rate,  $\sigma$ , which is given by the difference in the double-quantum and zero-quantum transition probabilities.

$$\sigma = W_2 - W_0$$

The important parameter for the classification of a molecule as small or large is given by  $\omega\tau_c$ , where  $\omega$  is the Larmor frequency and  $\tau_c$  is the correlation time. For small molecules,  $\omega\tau_c \ll 1$ , the  $W^2$  transition probability is larger than the  $W^0$  probability and the cross relaxation rate constant is positive yielding a positive NOE. For large molecules,  $\omega\tau_c \gg 1$ , the  $W^2$  probability approaches zero and only the  $W^0$  is effective in causing cross relaxation. This leads to negative a NOE. When  $\omega\tau_c = \sqrt{5}/2$ , then  $W^0 = W^2$  and no cross relaxation occurs.

In one dimensional NOE experiments of small molecules, the non-saturated peak grows in amplitude due to the NOE, however, in 2D NOESY experiments the NOE cross peaks have the opposite sign as the diagonal and appear to be negative. In large molecules, the 1D experiment, causes the peak receiving the NOE to decrease in amplitude, whereas in 2D NOESY the cross peaks are of the same sign as the diagonal peaks. This apparent discrepancy between the 1D and 2D NOE experiments is due to

the passing of negative (saturated) magnetization in the 1D case and positive magnetization in the 2D case.

## 12.7 NOE General Solution (Solomon, 1955)

$$\frac{W_S}{W_I} \beta\beta \quad (1)$$

$$(2) \frac{W_S}{W_I} \alpha\beta \quad \frac{W_I}{W_S} \beta\alpha \quad (3)$$

$$\frac{W_I}{W_S} \alpha\alpha \quad (4)$$

$$\begin{aligned} \langle I_z \rangle &= 1/2 [P_1 - P_3 + P_2 - P_4] \\ \langle S_z \rangle &= 1/2 [P_1 - P_2 + P_3 - P_4] \\ \frac{d\langle I_z \rangle}{dt} &= 1/2 \left[ \frac{dP_1}{dt} - \frac{dP_3}{dt} + \frac{dP_2}{dt} - \frac{dP_4}{dt} \right] \end{aligned}$$

$$\frac{dP_1}{dt} = -(W_{12} + W_{14} + W_{13})P_1 + W_{21}P_2 + W_{31}P_3 + W_{41}P_4 + \text{Constant}$$

at equilibrium

$$\frac{dP_1}{dt} = 0 \implies \text{Constant} = (W_{12} + W_{14} + W_{13})P_1^0 - W_{21}P_2^0 - W_{31}P_3^0 - W_{41}P_4^0$$

$$\frac{dP_1}{dt} = -(W_{12} + W_{14} + W_{13})\Delta P_1 + W_{21}\Delta P_2 + W_{31}\Delta P_3 + W_{41}\Delta P_4$$

where  $\Delta P_i = P_i - P_i^0$

$$\frac{dP_2}{dt} = -(W_{21} + W_{23} + W_{24})\Delta P_2 + W_{12}\Delta P_1 + W_{32}\Delta P_3 + W_{42}\Delta P_4$$

$$\frac{dP_3}{dt} = -(W_{31} + W_{32} + W_{34})\Delta P_3 + W_{13}\Delta P_1 + W_{23}\Delta P_2 + W_{43}\Delta P_4$$

$$\frac{dP_4}{dt} = -(W_{42} + W_{41} + W_{43})\Delta P_4 + W_{24}\Delta P_2 + W_{14}\Delta P_1 + W_{34}\Delta P_3$$

Combining these to obtain the rate of change of  $I_z$ .

$$\frac{d\langle I_z \rangle}{dt} = -(W_{14} + W_{13})\Delta P_1 - (W_{23} + W_{24})\Delta P_2 + (W_{31} + W_{32})\Delta P_3 + (W_{42} + W_{41})\Delta P_4$$

From the energy diagram:

$$W_{12} = W_{34} = W^S; \quad W_{13} = W_{24} = W^I; \quad W_{14} = W^2; \quad W_{23} = W^0$$

$$\frac{d\langle I_z \rangle}{dt} = -(W^2 + W^I)\Delta P_1 - (W^0 + W^I)\Delta P_2 + (W^I + W^0)\Delta P_3 + (W^I + W^2)\Delta P_4$$

$$\frac{d\langle I_z \rangle}{dt} = -W^I(\Delta P_1 - \Delta P_3) - W^I(\Delta P_2 - \Delta P_4) - W^0(\Delta P_2 - \Delta P_3) - W^2(\Delta P_1 - \Delta P_4)$$

$$\frac{d\langle I_z \rangle}{dt} = -W^I(I_z - I_z^0) - W^I(I_z - I_z^0) - W^0(\Delta P_2 - \Delta P_3) - W^2(\Delta P_1 - \Delta P_4)$$

From the definitions:

$$\langle I_z \rangle + \langle S_z \rangle = (\Delta P_1 - \Delta P_4)$$

and

$$\langle I_z \rangle - \langle S_z \rangle = (\Delta P_2 - \Delta P_3)$$

$$\frac{d\langle I_z \rangle}{dt} = -2W^I(I_z - I_z^0) - W^0((I_z - I_z^0) - (S_z - S_z^0)) - W^2((I_z - I_z^0) - (S_z - S_z^0))$$

Finally,

$$\frac{d\langle I_z \rangle}{dt} = -(2W^I + W^0 + W^2)(I_z - I_z^0) - (W^2 - W^0)(S_z - S_z^0)$$

$$\frac{d\langle I_z \rangle}{dt} = -\rho\Delta I_z - \sigma\Delta S_z$$

$$\frac{d\langle S_z \rangle}{dt} = -\sigma\Delta I_z - \rho\Delta S_z$$

$\rho = 1/T_1$  in absence of cross relaxation

$$\text{NOE} = \sigma/\rho$$

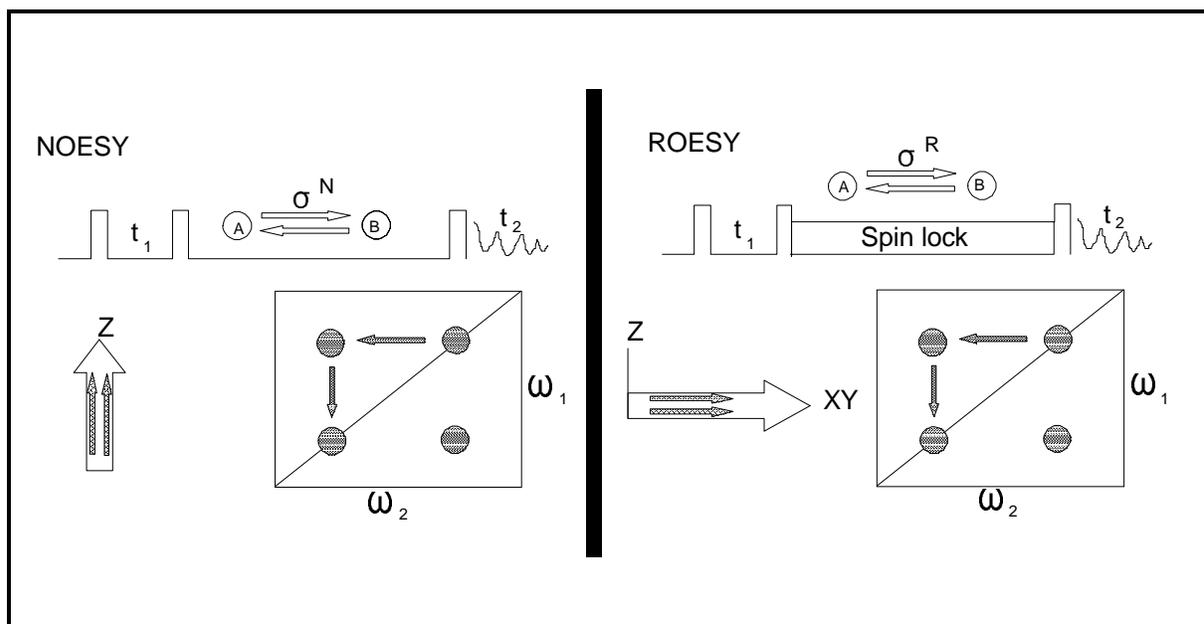
In the presence of multiple spins:

$$\frac{d\langle I_z \rangle}{dt} = -\rho \Delta I_z - \sum_{i \langle j} \sigma_{ij} \Delta J_z$$

Bottom line: In the presence of more than one proton spin, the measurement of the "T<sub>1</sub>" is contaminated by cross-relaxation.

### ROESY (Rotating frame Overhauser Effect Spectroscopy)

In the NOE experiment the interaction of the dipoles is along the Z axis which is collinear with the external magnetic field (Figure 1). The external magnetic field can



**Figure 1.** NOESY and ROESY experiments.

obviously be supplemented by a magnetic field arising from a RF field. If an on-resonance RF magnetic field is applied to spins that have been prepared (by a 90° pulse) to lie along the rotation axis of the RF field, then the magnetic field seen by the spins is only that of the RF field. This technique is called a spin-lock. Essentially a "new" Z axis is created with a field strength much less than the main static field. Under these conditions cross-relaxation can occur between spins that are spatially close. Since the conditions for cross relaxation in the rotating frame are significantly different

than in the NOE experiment, the detailed equations governing cross relaxation are different.

Without going into detail, the cross-relaxation equations for NOE and ROE are:

$$\sigma^{\text{NOE}} = K * r^{-6} * [ -1/10 * \tau_c + 3/5 * \tau_c / (1 + (2\omega\tau_c)^2) ]$$

$$\sigma^{\text{ROE}} = K * r^{-6} * [ 1/5 * \tau_c + 3/10 * \tau_c / (1 + (\omega\tau_c)^2) ]$$

Where K is a product of constants including Plank's constant and the magnetogyric ratio of the proton; r is the distance between the interacting protons;  $\tau_c$  is the correlation time; and  $\omega$  is the proton Larmor frequency.

The salient points of comparison between these equations are:

1). Since the terms in the ROE equation are of the same sign, the ROE is always positive, whereas when  $\omega\tau_c = \sqrt{5}/2$  the NOE vanishes.

2). For small molecules,  $\omega\tau_c \ll 1$ :

$$\begin{aligned} \sigma^{\text{NOE}} &= 3/5 \tau_c \\ \sigma^{\text{ROE}} &= 3/10 \tau_c \\ \sigma^{\text{NOE}}/\sigma^{\text{ROE}} &= 2 \end{aligned}$$

3). For large molecules,  $\omega\tau_c \gg 1$ :

$$\begin{aligned} \sigma^{\text{NOE}} &= -1/10 \tau_c \\ \sigma^{\text{ROE}} &= 1/5 \tau_c \\ \sigma^{\text{NOE}}/\sigma^{\text{ROE}} &= -1/2 \end{aligned}$$