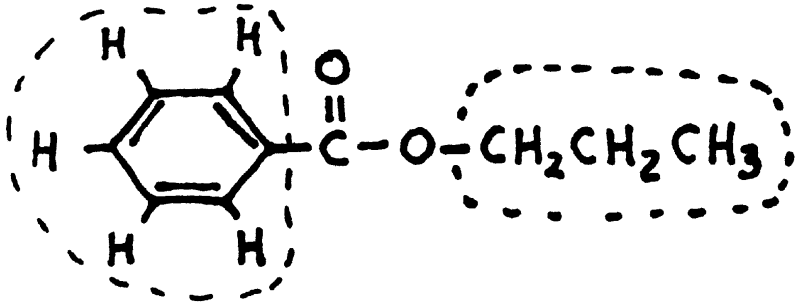
Selective 1D TOCSY

The 1D TOCSY is a variant of the 2D-TOCSY, an experiment which is used to identify entire **spin systems**. A spin system is a group of spins (usually ^1H) in a molecule which are connected together by J-couplings. Specifically, each member of the group has at least one J-coupling with another member of the group. For example, n-propyl benzoate has two proton spin systems: the CH_3 and two CH_2 groups form one spin



system and the 5 aromatic protons form the other. Since there is no J-coupling between the CH_3 or CH_2 protons and any of the aromatic protons, these are two distinct spin systems. TOCSY (total correlation spectroscopy) is a technique which spreads NMR magnetization from any one member of a spin system to all the other members of a spin system. Thus if you excite one member of the spin system (e.g., the CH_3 protons), the TOCSY mixing sequence will transfer that magnetization first to its J-coupled partners (the next CH_2 group) and then to *their* J-coupled partners (the CH_2 group next to oxygen) until all of the members of the spin system are excited in the same way. If the FID is recorded at this time a spectrum will be observed which contains only the peaks due to the spin system which was excited (in this case the CH_3-CH_2-CH_2 group). Peaks from other spin systems in the molecule (the aromatic protons) would not appear in this selective spectrum.

TOCSY mixing is acheived by a long series of pulses at "medium" power (90^o ^1H pulse about 25-30 μs long). A single 90^o pulse is sufficient to transfer magnetization between J-coupled protons (e.g., in the INEPT experiment), so it is not surprising that a series of pulses can result in multiple jumps which eventually spread magnetization throughout a spin system. The exact duration and phase of these pulses is the subject of recent experimentation and improvement. The goal is to achieve good magnetization transfer, only through bonds and not through space (NOE), with minimal power and minimal dependence on chemical shift. A commonly used sequence is called MLEV-17, named after its inventor Malcolm Levitt. It consists of a series of 16 composite 180^o pulses:

ABBA BBAA BAAB AABB

where A is a composite 180^o pulse about the x axis (90^o_{-y} - 180^o_x - 90^o_{-y}) and B is the same thing with opposite phase (90^o_y - 180^o_{-x} - 90^o_y). A final 180^o pulse about the x axis completes the set of 17 pulses. This sequence is then repeated over and over again in a computer-driven loop for the complete duration of the mixing time, which usually ranges

from 30 ms to 100 ms. That's a lot of pulses for a long time, so the sample actually heats up a bit and you can usually see the lock signal go down a bit when you start the experiment. But because the power level is lower than that used for "hard" pulses (about one-tenth of the power) we can get away with this without burning up the amplifiers or the probe. A more efficient sequence than MLEV-17 is the DIPSI family of mixing sequences. The basic repeating unit of DIPSI is:

$$320^{o}_{x} - 420^{o}_{-x} - 290^{o}_{x} - 285^{o}_{-x} - 30^{o}_{x} - 245^{o}_{-x} - 375^{o}_{x} - 265^{o}_{-x} - 370^{o}_{x}$$

There is no clear theoretical basis for this sequence; it was arrived at by computer simulation and found to give the most efficient transfer of magnetization with the least sample heating.

These sequences all have one thing in common: they rotate the sample magnetization continuously about the x axis. This has the effect of "locking" the magnetization on the x axis and holding it there. For this reason it is called a "spin-lock". Evolution of the spins, due to chemical shifts or J-couplings, does not take place because the rotations about the x axis, like a very rapid series of spin-echoes, keeps bringing the magnetization back to the x axis. Any sample magnetization which is not on the x axis at the start of the mixing sequence rotates wildly about the spin-lock axis (x axis) and eventually fans out due to the inhomogeneity of the B_1 field and decays to nothing.

To design a 1D TOCSY experiment, we need to selectively excite only one peak in the spectrum, corresponding to a particular proton in the molecule, place this magnetization on the x-axis, and then apply the TOCSY spin-lock on the x-axis for a period of time (the mixing time) to transfer this magnetization via J-couplings to the other protons in the spin system. Then we simply turn off the spin-lock (i.e., stop pulsing) and start recording the FID. The FID signal will contain all the frequencies of the spin system, and after the Fourier transform the spectrum will include the selectively excited peak as well as the other peaks which belong to that spin system, but no peaks will be observed for protons in other spin systems of the molecule. These steps can be summarized as follows:

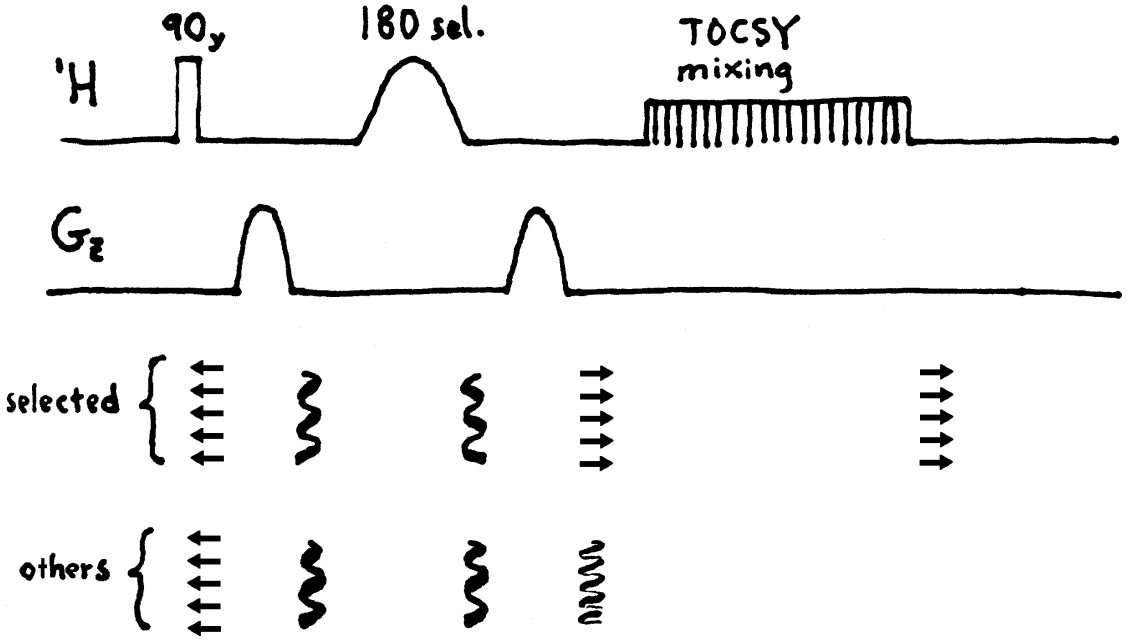
1. **Preparation.** Selectively excite the peak of interest to place its magnetization on the x-axis.

2. **Mixing.** Apply the TOCSY mixing sequence (spin-lock) on the x-axis for a period of time between 30 and 100 ms.

3. **Detection.** Record the FID.

How can we accomplish step 1? This is very similar to the selective 1D transient NOE, where we used a combination of hard pulses, shaped pulses and pulsed field gradients to invert one peak in the spectrum while destroying all magnetization from the other peaks. In that experiment, a 90^{o} hard pulse puts all magnetization on the -x axis and then a gradient "twists" this magnetization into a helix along the axis of the NMR tube. A 180^{o} selective (shaped) pulse reverses the sense of this helix for the selected peak only, leaving

all the other spins in the molecule unaffected. Then a second gradient of the same sign and duration as the first "untwists" the magnetization for the selected peak, while doubling the twist for the peaks which were not affected by the selective 180° pulse. At this point we have magnetization for the peak of interest aligned along the x axis, regardless of the position of the molecule within the tube. All other protons have no magnetization at all, not even z-magnetizaion. In the selective 1D transient NOE experiment we continue on with a hard 90° pulse to further rotate the selected magnetization to the -z axis, equivalent to a reversal of the Boltzmann population difference. But for the selective TOCSY we want the magnetization on the x-axis, so we just leave out the second hard 90° pulse and go immediately to the mixing step. The selected magnetization is exactly where we want it now, on the x axis.

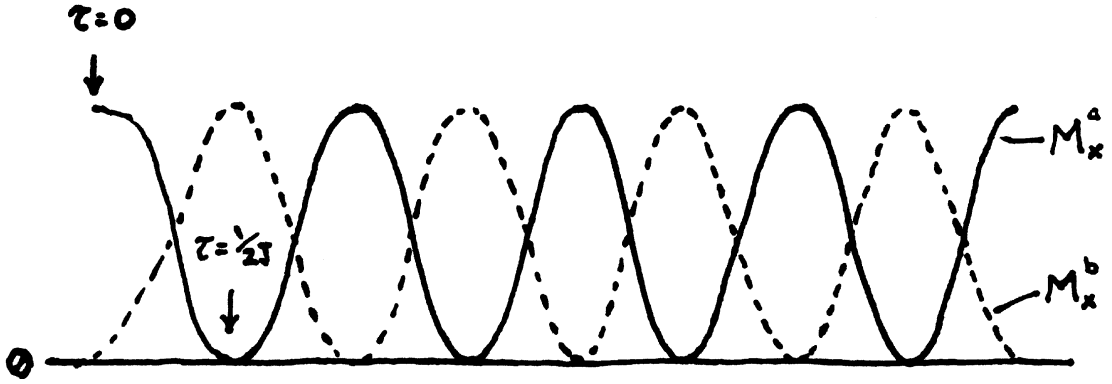


The spin-lock sequence is then started (MLEV-17 is used in this pulse program) and the MLEV loop is repeated 30 times. This leads to a mixing time of 62.2 ms, which generally gives good TOCSY mixing in small spin systems. At the end of the TOCSY mixing, the FID is detected, digitized and saved.

Factors which effect TOCSY mixing efficiency. In the real world, complete mixing throughout the spin system is not observed. Protons near the selected proton usually give more intense signals, and protons farther away give weaker peaks in the 1D spectrum. The simplest case, where there are only two protons (H_a and H_b coupled with J_{ab}) in the spin system, has been described precisely. If we start with magnetization on H_a

along the x axis at the beginning of the TOCSY mixing sequence, the magnetization will oscillate between H_a and H_b:

$$\sigma = I_x^a(1+\cos(2\pi J\tau))*0.5 \; + \; I_x^b(1-\cos(2\pi J\tau))*0.5$$



where τ is the mixing time. Note that at time zero we have pure I_x^a, at time $\tau = 1/(2J_{ab})$ we have pure I_x^b and none of the starting magnetization (100% transfer) and at time $\tau = 1/J_{ab}$ we are back to pure I_x^a (no transfer). So we can conclude that when magnetization hits the end of a spin system, it "bounces back" and we see oscillatory behavior as a function of mixing time. With our mixing time of 62.2 ms, complete transfer would occur for J = 8.0 Hz, not far from a typical vicinal coupling. For a small coupling constant such as J_{ab} = 2.0 Hz, you would get only 4% transfer and with J_{ab} = 0.5 (very small) you would get 0.24% transfer. Thus when coupling constants are small, we hit a "bottleneck" in transfer of magnetization and most of the magnetization remains on the starting nucleus.

In long spin systems such as flexible chains of CH_2 groups, magnetization transfers in a diffusion-like process. The signal is strongest on the starting spin and weaker as you move farther away along the chain. With longer mixing times, magnetization spreads farther along the chain. For example, with a mixing time of 70 ms it is usually possible to reach the ε position of the Lysine side-chain starting with magnetization on the amide NH in a peptide or protein:

$$HN\text{-}CH^{\alpha}\text{-}CH_2^{\beta}\text{-}CH_2^{\gamma}\text{-}CH_2^{\delta}\text{-}CH_2^{\varepsilon}\text{-}NH_3^{+}$$

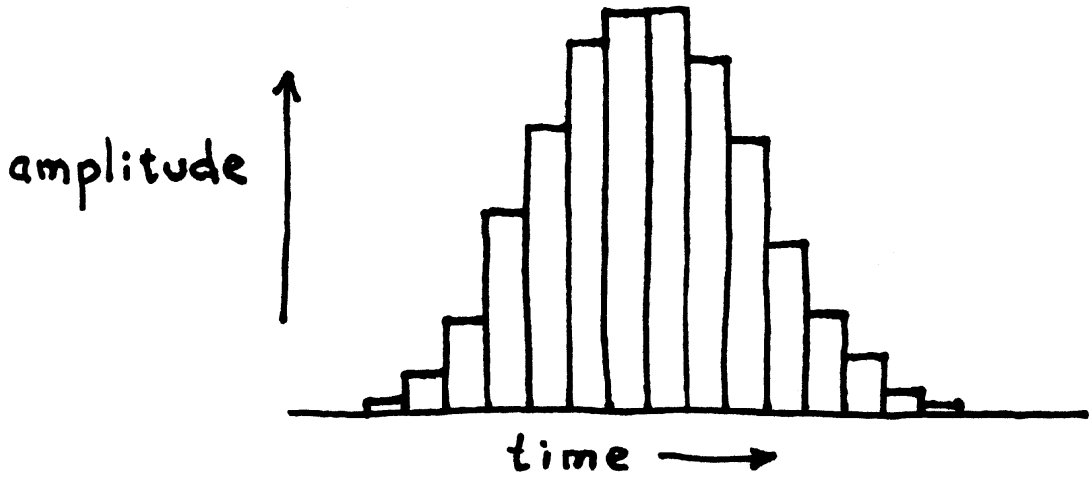
Short mixing times (e.g., 30 ms) lead to INEPT-type spectra (or COSY-type 2D spectra) where transfer is mostly limited to a single jump over one J coupling. Unlike INEPT and COSY, however, the transfer results in an in-phase rather than antiphase signal. This is a significant advantage since the peaks have the same shape and pattern as they do in a 1D spectrum.

In the selective 1D TOCSY we need a starting point for the magnetization, and this is usually chosen as a peak in the spin system which is well separated from other peaks. This is often a downfield-shifted peak such as the amide NH in a peptide or the anomeric proton in a carbohydrate. Selection of this peak can then reveal the whole spin

system (e.g., amino acid or monosaccharide unit) even if the normal 1D spectrum is heavily overlapped in the region of the other peaks of the spin system. But without a "handle" which is separated from other peaks, this method is not very useful.

Biselective 1D TOCSY. A variant of this technique is to excite two peaks in the spectrum, one with positive phase and the other upside-down (negative phase). Since transfer of magnetization during TOCSY mixing preserves the phase (in-phase transfer), all of the other peaks of the spin system will be either normal phase ("up") if they in the spin system which was originally excited with positive phase, or negative phase ("down") if they belong to the spin system which was originally excited with negative phase. In this way we can get two experiments for the price of one! This is reminiscent of the APT and DEPT (^{13}C) experiments where the phase information ("up" or "down") is used to "edit" the spectrum to give additional information.

When a single frequency is excited by a selective pulse, the excitation profile will be centered on the pulse frequency, which is the center of the spectral window. If the peak we want to select is not in the center of the spectral window, we can always move the spectral window to put it there, but we may have to increase the spectral width to keep the whole spectrum within the spectral window. An easier way to selectively excite a peak which is not in the center of the spectral window is to use a frequency-shifted shaped pulse. The frequency shift is relative to the center of the spectral window, so that a peak which is 200 Hz upfield of the spectral window would be excited by a shaped pulse with a frequency shift of -200 Hz. The frequency shift is accomplished by shifting the phase of each element (rectangular pulse) which makes up the shaped pulse. For example, a 35 ms gaussian pulse might be made up of 1,000 rectangular pulses, one right after the other, with each one having a duration of 35 μs and an amplitude set by the value



of the gaussian function at that time during the pulse. Instead of executing all these pulses with the same phase, suppose that we shift the phase of each one by 10^o relative to the phase of the pulse before it in the sequence, so that the pulses complete a full cycle

from 0^o to 360^o after 36 of the pulses have been executed. This has the effect of speeding up the oscillation of the radio frequency by one oscillation every 36 pulses (36*35ms = 1.26 ms). One extra cycle every 1.26 ms corresponds to an increase in the radio frequency by 1/(1.26 ms) or 793.7 Hz. Thus we have shifted the frequency of the shaped pulse from the center of the spectral window to a position 793.7 Hz (1.59 ppm for ^{1}H on the DRX-500) downfield of the center. In time domain we have "squeezed" the sine wave of the radio frequency signal making up the pulse to increase its frequency. This technique is called "frequency shifted laminar pulses" to emphasize the layered (laminar) nature of shaped pulses.

We can view the RF pulse as a vector in the rotating frame: a normal RF pulse would be stationary in the x'-y' plane, at a position (e.g., on the x' axis) corresponding to its phase. The frequency-shifted pulse would be represented by a vector sitting at one place in the x'-y' plane for the duration of one pulse element, then jumping ahead or behind in phase to a new position in the x'-y' plane for the next element. At the start of each new element, the vector jumps by the same angle of rotation in the x'-y' plane. Although its motion over the time of the full shaped pulse is "jerky", the overall motion is a rotation in the x'-y' plane, corresponding to a frequency which is not at the center of the spectral window.

Suppose we wanted to excite two peaks in the spectrum at the same time, without affecting any of the other peaks. All we need to do is visualize each of the two desired frequencies as a vector rotating in the x'-y' plane in the rotating frame. Each vector rotates at the frequency (relative to the center of the spectral window) desired to excite that peak in the spectrum. At the time of each of the elements (rectangular pulses) of the shaped pulse the vector sum of the two vectors is calculated and its magnitude is used to modify the magnitude of that pulse and its phase is used to modify the phase. This might sound like a lot of work, but the computer does it rapidly for a few thousand elements and stores the results (amplitudes and phases) in a table. Every time the spectrometer needs to execute the shaped pulse, it just reads the phase and amplitude from the table to execute each element (rectangular pulse) in the shaped pulse. The shaped pulse now contains the sum of the two desired frequencies, and both peaks are excited simultaneously. The overall phase of each frequency can be set independently, so that you could apply a 90^o_{+x} pulse to one peak in the spectrum and a 90^o_{-x} pulse to another peak, resulting in one peak being "right-side up" and the other being "upside-down" in the spectrum. This techniques makes the biselective 1D TOCSY possible.

On the Bruker DRX-500, the offset frequency for a single excitation is set by setting the parameters **SPOFFS1** (Shaped Pulse Offset 1) and **SPOFFS2** to the frequency (in Hz) of the center of the selected peak, relative to the center of the spectral window. An easy way to measure this is to set the parameter **SR** (Spectral Reference) to the value of **O1** (Transmitter Offset). This puts 0 ppm at the center of the spectral window so that the shaped pulse offset can be read directly from the spectrum in Hz. For the biselective 1D TOCSY you have to generate a customized shaped pulse which contains the two frequency. **SPOFFS1** and **SPOFFS2** are set to zero and the desired frequencies are put into a frequency list, which is a simple text table listing the two frequencies. The **stdisp** command starts the ShapeTool program which allows you to set the overall shape and the phases of each frequency. This program calculates the phase and amplitude for each of

the 4096 elements of the gaussian pulse and saves them in a text file table which is used by the spectrometer every time the pulse program executes that shaped pulse.

A closer look at TOCSY transfer. For liquid samples, there are two interactions which define the energy of a pair of spins in NMR: the interaction of each spin's magnet with the external magnetic field (the "Zeeman" energy) and the interaction of the two spins' magnets with each other (J-coupling). The first term is dependent on the strength of the external field and small, structure-dependent differences in this energy define the chemical shift. The second term is independent of field strength and so we measure it in units of Hz. If both spins are aligned the same way with respect to the external field ($\alpha\alpha$ or $\beta\beta$ state) the energy is slightly higher (if J is positive) and if they are aligned opposite to each other ($\alpha\beta$ or $\beta\alpha$ state) the energy is slightly lower. The goal of the TOCSY mixing sequence is to eliminate the chemical shift (Zeeman) term and leave only the J-coupling interaction. We know that a simple homonuclear spin-echo can accomplish this, eliminating the chemical shift evolution and allowing the J-coupling evolution, so it's not surprising that the right kind of spin-lock can also do it, since the spin lock is similar to a very rapid series of spin-echoes. This ideal situation where the only interaction left is J-coupling is called "isotropic mixing".

Why would this lead to transfer of magnetization throughout a spin system? Consider an intermediate case where chemical shift differences are not eliminated completely, but are small compared to the J-couplings. This is the so called "non-first order" or "strong coupling" situation which is commonly encountered in low-field (e.g., 60 MHz) instruments but can show up at any field strength. A common phenomenon occurs at lower magnetic fields when one nucleus (H_a) is coupled to another (H_b) which is far away from it in chemical shift. We expect for H_a a simple doublet, but suppose that H_b is coupled to a third spin (H_c) which is very close to H_b in chemical shift. We say that H_b and H_c are **strongly coupled**, which will distort the multiplet patterns of these two spins. The odd thing, however, is that the H_a multiplet becomes more complex, as if it were coupled to H_c as well as to H_b. This is called "virtual coupling" because the H_a nucleus, which has no J coupling to H_c, appears to be coupled to it because of the strong coupling between H_b and H_c. A general way of stating this is that *any nucleus which is coupled to one member of a strongly-coupled group of nuclei will behave as if it is J-coupled to all of the members of the group*. This phenomenon can lead to some very baffling results if you don't take it into account. A classic case occurs with a long, straight alkyl chain such as a fatty acid: the methyl group should appear as a simple triplet since it is only coupled to the CH_2 group next to it, but since most of the CH_2 groups in the chain have nearly the same chemical shift and are therefore strongly coupled, the CH_3 group is very broad because of these virtual couplings.

Now consider what happens during the ideal TOCSY mixing scheme. There are no chemical shift differences but the J couplings are still active, so all protons in a spin system are strongly coupled. Any one proton behaves as if it is coupled to all protons of the spin system since it is coupled to at least one member of the group and all the other protons are strongly coupled to each other. This way of understanding the TOCSY scheme comes closest to the actual theoretical explanation, so hopefully it will give you a feeling for what goes on in the TOCSY experiment.