Decoupling: Homonuclear and Heteronuclear

Theory. Decoupling is the process of removing specific kinds of J-coupling interactions in order to simplify a spectrum or identify which pairs of nuclei are involved in the J-coupling. In order to understand how decoupling works, you must first understand what causes J-coupling in the first place. Consider two protons (^1H_aC-C^1H_b) with different

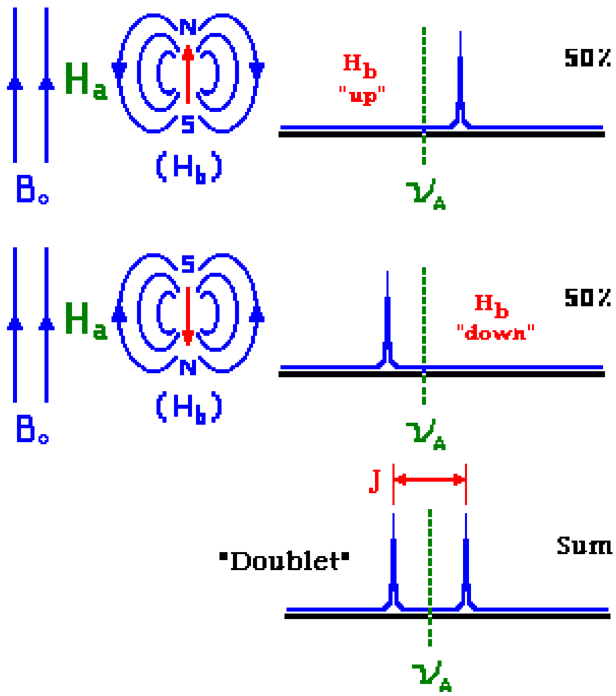

Figure 4. Effect of the nuclear magnet of a nearby (2-3 bonds) proton H_b on the resonant frequency of the observed proton H_a. In about 50% of the molecules in the sample, H_b is oriented "up" with respect to the external magnetic field B_o, and this causes a slight reduction in the field experienced by H_a, shifting its resonant frequency upfield by J/2 Hz. In the remaining 50% of the sample molecules, H_b is oriented "down" and its magnetic field adds to the external field in the vicinity of H_a, leading to a downfield shift of J/2 Hz. The observed resonance signal, which is the sum of all molecules in solution, is a "doublet" centered at the chemical shift position ν_a of proton H_a, with a peak separation of J Hz.

chemical shifts on two adjacent carbon atoms in an organic molecule. The magnetic nucleus of H_b can be either aligned with ("up") or against ("down") the magnetic field of the spectrometer. From the point of view of H_a, the H_b nucleus magnetic field perturbs the external magnetic field, adding a slight amount to it or subtracting a slight amount from it, depending on the orientation of the H_b nucleus ("up" or "down"). This changes the H_a chemical shift so that it now resonates at one of two frequencies very close together. Since roughly 50% of the H_b nuclei are in the "up" state and roughly 50% are in the "down" state, the H_a resonance is "split" by H_b into a pair of resonance peaks of equal intensity (a "doublet"). The relationship is mutual, so that H_b experiences the same splitting effect from H_a. This effect is transmitted through bonds and operates only when the two nuclei are very close (three bonds or less) in the bonding network.

Decoupling is accomplished by irradiating at the frequency of one nucleus (H_b) with continuous, low-power RF. This irradiation causes the H_b nucleus to "flip" from the lower energy (aligned) to the higher-energy (opposed) state and back again very rapidly. Because the NMR "time scale" or "shutter speed" is relatively slow (in this case on the order of 1/J or more than 100 milliseconds), the other nucleus (H_a) sees only an average magnetic environment, which is not perturbed at all by the presence of H_b's magnetic field. The two components of the H_a doublet are averaged to a single peak in the center as long as the H_b spins are "flipping" back and forth rapidly enough. If the RF power is not enough to create perfect averaging, the H_b spins will flip back and forth more slowly and we will see a doublet for H_a with a reduced separation or J-value. The RF irradiation must go on during the entire process of recording the FID (the acquisition time) in order to eliminate the coupling. If the frequency of the irradiation is not exactly at the resonant frequency of the nucleus (H_b), there will still be some decoupling but it depends on the power of the RF signal and the frequency difference. The larger the frequency difference between the RF signal and the resonant frequency of H_b the greater the power required to achieve decoupling. Another way of saying that is that a high-power RF signal will decouple a wider range or band of frequencies (chemical shifts) around the frequency of the RF signal. In some cases this is desirable and in other cases, where we want to irradiate a specific peak in the spectrum and no others, higher power is undesirable because it reduces the selectivity of decoupling.

Decoupling Applications. There are two main reasons to decouple. The first is to remove the one-bond ^{1}H-^{13}C couplings from ^{13}C spectra. This is so routine that most users forget that these couplings (J ~ 150 Hz!) even exist. In fact, without ^{1}H decoupling all ^{13}C spectra would show very wide quartets for CH_3 carbons, triplets for CH_2 carbons, and doublets for CH carbons. This can be useful information, but for molecules of any size and complexity it leads to a tangled forest of absorbance peaks. ^{1}H decoupling gives ^{13}C spectra in which there is only one (singlet) peak for each unique carbon in the molecule. Normally in ^{13}C spectra we want to decouple **all** of the protons from their attached ^{13}C atoms. This means that we cannot irradiate exactly at the frequency of each proton simultaneously. We need "broad-band" decoupling which will "cover" the entire range of ^{1}H chemical shifts, which typically range from 0 ppm to 10 ppm, a width of 3000 Hz on the Unity-300 instrument. One solution is to place the decoupler frequency at the center of the proton spectrum (e.g., 5.0 ppm) and turn up the decoupler power until a wide enough band of frequencies is effectively decoupled. Unfortunately, this would require power levels that would fry the amplifiers, fry the probe, and boil your sample! An early solution was to "sweep" the decoupler frequency rapidly back and forth over the 0-10 ppm range so that each peak is "hit" over and over again. The best solution available today is a sequence of 16 RF pulses at the center frequency of the proton spectrum which is repeated over and over. The phases and durations of the pulses is specifically designed so that a wide range of chemical shifts can be decoupled with minimal RF power and minimal sample heating. This sequence is called "WALTZ-16".

The second reason for decoupling is to identify the coupling "partner" of a particular peak in the spectrum. Irradiation of that peak at its exact frequency using low-power (for selectivity), continuous RF during the acquisition time will "collapse" any multiplet patterns which result from the protons in the irradiated peak. For example, you might irradiate a doublet at 4.68 ppm and find that a double-doublet at 3.24 ppm "collapses" to a doublet. This means that the doublet at 4.68 ppm was the source of one of the couplings in the double-doublet at 3.24 ppm. This technique can also be used for heteronuclear couplings, so that irradiating a particular proton resonance results in the collapse of a ^{13}C multiplet to a sharp singlet in the ^{13}C spectrum. This is called *selective* heteronuclear decoupling, to distinguish it from the broad-band non-selective ^{1}H decoupling which is normally used during the acquisition of ^{13}C spectra. Selective heteronuclear decoupling is rarely used, however, since the 2D HETCOR and related inverse 2D experiments give the same information with far less ambiguity. In fact, selective *homonuclear* decoupling has been all but replaced by 2D COSY and related variants such as DQF-COSY and COSY-35. There are instances, however, where only one or two couplings are ambiguous and a 1D selective decoupling experiment can sort it out quickly.

Decoupling Hardware. How does a spectrometer deliver this RF irradiation to the probe? Compared to normal excitation pulses, which are very high power and short (~ 10 μs) duration, decoupling requires low-power irradiation for the entire acquisition time (~ 2 s). This is usually accomplished by having two separate sources of RF power, a **transmitter** which can be operated at a wide range of frequencies (e.g., ^{15}N is 30.4 MHz, ^{13}C is 75.4 MHz, and ^{1}H is 300.0 MHz on the Unity-300) and a proton **decoupler** which can only produce the proton frequency (300.0 MHz on the Unity). The transmitter is set to the frequency of the nucleus to be observed with a high power level for pulses, and the decoupler is set to a low power level for proton decoupling. As soon as the pulse sequence is over and acquisition of the FID begins, the decoupler is turned on for the duration of the acquisition time. Even when ^{1}H is being observed, the proton (high power) pulses come from the transmitter, and the decoupler is used to deliver the low power ^{1}H irradiation during the acquisition time. This is necessary because it takes time (milliseconds!) to switch the power level of the transmitter, so you can't just use a single source of RF to supply high-power pulses and decoupling irradiation.

The Unity-300 is a bit more modern than this basic system in that the decoupler is not fixed at the ^{1}H frequency. Instead, it is identical to the transmitter in that it can be set to the frequency of any nucleus. Thus, you could decouple ^{13}C while observing ^{1}H, for example (an "inverse" experiment). Even more modern instruments than the Unity can switch power levels in a few microseconds, so that a single "box" can be used for all proton RF, whether it be for high-power pulses or for low-power decoupling. This feature makes the whole concept of "transmitter" and "decoupler" a matter of language rather than real hardware differences. Figures 5-7 show the configuration of the Unity-300 hardware for routine ^{1}H, for ^{13}C with ^{1}H decoupling, and for ^{1}H with homonuclear decoupling. Note that the *inner* coil of the Four-Nucleus Probe is tuned to ^{13}C (and ^{31}P) to maximize the sensitivity of detection of these nuclei. The *outer* coil, which is farther from the sample and therefore less sensitive, is tuned to ^{1}H (and ^{19}F) since these nuclei are much easier to detect. Changing from one of these configurations to another simply involves changing the frequency settings of the two channels ("transmitter" and "decoupler") and re-routing the outputs to the probe inputs. This is done by re-setting electrical relays, which give a "click" when you execute the **su** or **go** command after changing the experimental parameters in VNMR. Figure 8 shows the configuration for an "inverse-mode" experiment (see p. 10), in which ^{1}H is detected and pulses are delivered to the probe on both the ^{1}H and ^{13}C channels (e.g., HMQC). In this case the

Inverse Probe is used, which has the *inner* coil tuned to ^{1}H (the "observe" nucleus) and the *outer* coil tuned to ^{13}C.

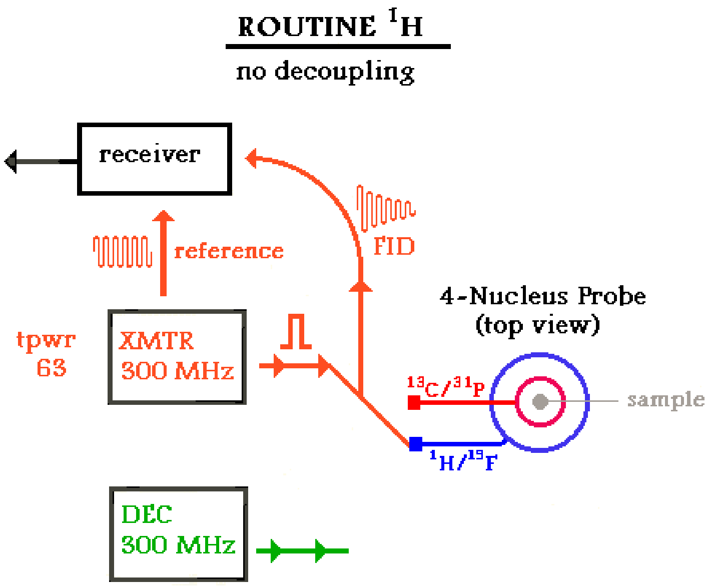

Figure 5. Hardware configuration of the Unity-300 for routine ^{1}H acquisition. The transmitter is set to the ^{1}H frequency (300 MHz) and pulses from the transmitter are directed to the outer coil of the probe, which is double-tuned to the ^{1}H and ^{19}F frequencies. The decoupler is not used. After the exciting pulse, the ^{1}H FID is detected on the outer coil of the probe and directed to the receiver, which uses a continuous signal from the transmitter (300 MHz) as a reference frequency which is "mixed" with (i.e. subtracted from) the FID frequency.

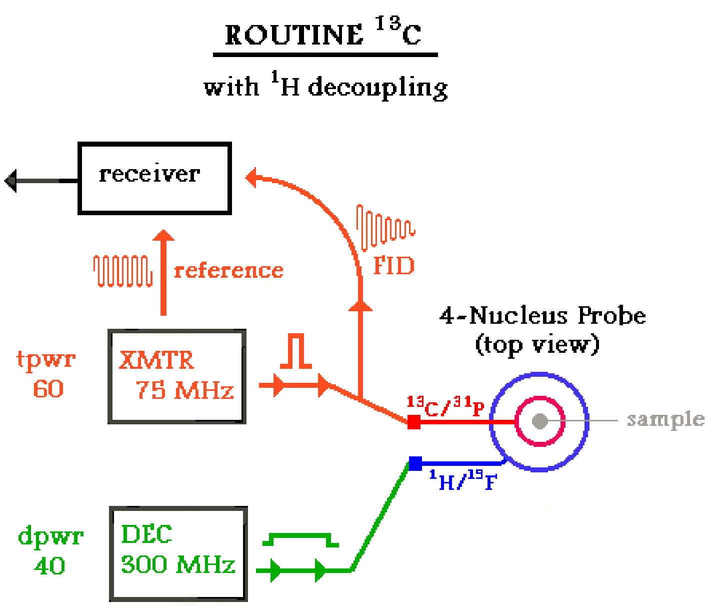

Figure 6. Hardware configuration of the Unity-300 for routine ^{13}C acquisition with ^{1}H decoupling. The transmitter is set to the ^{13}C frequency (75 MHz) and pulses from the transmitter are directed to the inner coil of the probe, which is double-tuned to the ^{13}C and ^{31}P frequencies. The decoupler operates continuously at low power and its output is directed to the outer coil of the probe, which is double-tuned to the ^{1}H and ^{19}F frequencies. After the exciting pulse, the ^{13}C FID is detected on the inner coil of the probe and directed to the receiver, which uses a

ontinuous signal from the transmitter (75 MHz) as a reference frequency which is "mixed" with (i.e. subtracted from) the FID frequency.

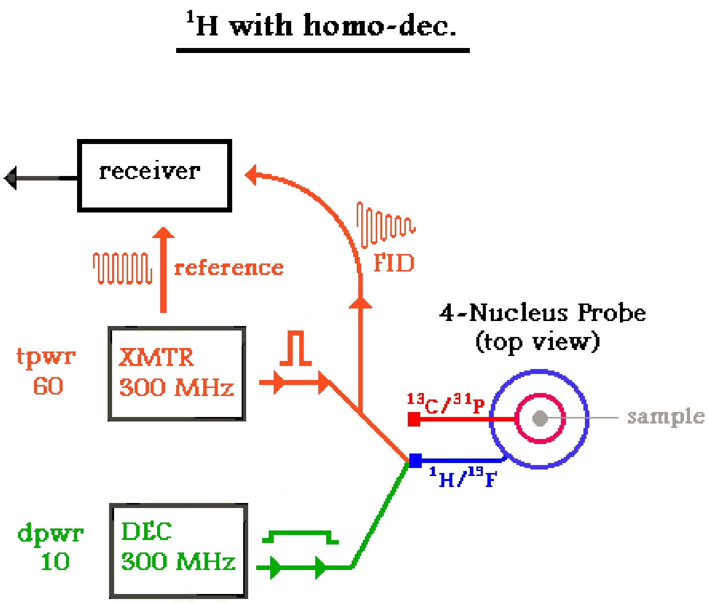

Figure 7. Hardware configuration of the Unity-300 for ^{1}H acquisition with selective ^{1}H decoupling. The transmitter is set to the ^{1}H frequency (300 MHz) and pulses from the transmitter are directed to the outer coil of the probe, which is double-tuned to the ^{1}H and ^{19}F frequencies. The decoupler operates continuously at very low power during acquisition of the FID and its output is also directed to the outer (^{1}H) coil of the probe. After the exciting pulse, the ^{1}H FID is detected on the outer coil of the probe and directed to the receiver, which uses a continuous signal from the transmitter (300 MHz) as a reference frequency which is "mixed" with (i.e. subtracted from) the FID frequency.

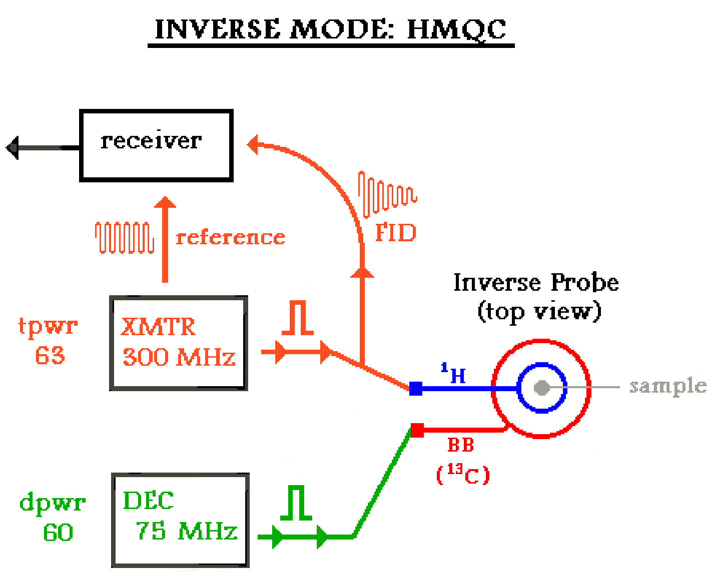

Figure 8. Hardware configuration of the Unity-300 for ^{1}H acquisition with both ^{1}H and ^{13}C pulses. The transmitter is set to the ^{1}H frequency (300 MHz) and pulses from the transmitter are directed to the inner coil of the probe, which is tuned to the ^{1}H frequency. The decoupler is set to the ^{13}C frequency (75 MHz) and pulses from the decoupler are directed to the outer coil of the probe, which is tuned to the ^{13}C frequency. After the HMQC pulse sequence, the ^{1}H FID is detected on the inner coil of the probe and directed to the receiver, which uses a continuous signal from the transmitter (300 MHz) as a reference frequency which is

"mixed" with (i.e. subtracted from) the FID frequency.

Decoupling Parameters. Most decoupling parameters on the Unity start with the letter "**d**" to distinguish them from the transmitter parameters, which start with a "**t**". Thus the transmitter power level is **tpwr** and the decoupler power level is **dpwr**. The following parameters can be examined by entering **dg** in VNMR and looking under the heading "DECOUPLING" in the parameter list:

dn	*decoupler nucleus*: H1, C13, N15, etc. This determines the basic frequency of the decoupler irradiation (300.0, 75.4, 30.4 MHz)
dof	*decoupler offset*: This sets the exact frequency (chemical shift) of the decoupler irradiation in Hz.
dm	*decoupler mode*: This determines when the decoupler is on or off during the pulse sequence.
dmm	*decoupler modulation*: This sets the decoupler to either continuous irradiation ('**c**') or the waltz-16 repeated pulse sequence ('**w**')
dmf	*decoupler modulation frequency*. This sets the duration of individual pulses used in the waltz-16 sequence.
dpwr	*decoupler power*: This sets the power level of the decoupler irradiation in decibel (dB) steps from 0 to 63.
homo	*homonuclear*: Set to '**y**' for homonuclear (^{1}H-^{1}H) decoupling, or '**n**' for heteronuclear (e.g., ^{1}H-^{13}C) decoupling.

The decoupler offset can be determined by placing the cursor on a peak in the spectrum and entering **sd** (set decoupler). The appropriate offset frequency in Hz will be displayed on the screen, and this value can be entered manually as **dof**. The decoupler mode (**dm**) can be used to determine when the decoupler should be turned on and off during the pulse sequence. VNMR defines time periods A, B, C... during any pulse sequence, and the definition of these time periods can be observed by entering **dps** (display pulse sequence). For the simple 1D sequence called "s2pul" (simple 2-pulse), the periods A, B and C are defined as follows:

D1 ------ P1 ------- D2 ------ PW -------FID

|<--------- A -------->|<----------- B ---------->|<--C-->|

In other words, the "A" period consists of the relaxation delay (**d1**) and the first (**p1**) pulse, the "B" period consists of the **d2** delay and the second pulse (**pw**), and the "C" period consists of the entire period of data collection (acquisition) of the FID. This skeleton pulse sequence can be used for a number of purposes, but normally **p1** and **d2** are set to zero and it is simply a 1D single pulse experiment: **d1**, **pw**, FID. For decoupling it is only necessary to have the decoupler on during the acquisition time, so that **dm='nny'** would give effective decoupling. This means that the decoupler is off ('**n**') during the A and B periods and on ('**y**') during the acquisition (C) period. In practice, the decoupler can also be used to generate a nuclear Overhauser effect (NOE). The NOE is an enhancement of the signal of any nearby (within 5 Angstroms) nucleus when a given nucleus is irradiated continuously long enough to equalize the populations in its two spin states (aligned and opposed). It takes less power to equalize population levels than it does to decouple; saturation (equalization of populations) is sort of like a simmer while decoupling (rapid flipping of all spins between the two levels) is more of a rolling boil. In the NOE experiment, irradiation occurs *before* the acquisition period so that you might use a decoupler modulation of **dm='yyn'**. In the standard 1D ^{13}C experiment, you want the NOE (enhanced ^{13}C peak intensities) as well as the decoupling (a singlet for each unique carbon in the molecule), so you would set **dm='yyy'**.

The parameter **dmm** determines whether the decoupler output is a simple continuous irradiation (**dmm='c'**) or a pulsed waltz-16 modulation (**dmm='w'**). For non-selective ("broad-band") decoupling such as that desired for a 1D ^{13}C spectrum, the waltz-16 mode is used to minimize power requirements and maximize the range ("bandwidth") of chemical shifts decoupled. For selective decoupling the continuous mode is used to minimize the range of chemical shifts affected. The **dmf** parameter is used to determine the duration of pulses (e.g., 90^o pulse, 180^o pulse) in the waltz-16 sequence. It is determined by calibration of the 90^o pulse width at the power level **dpwr**. The value is set by facility personnel in the standard ^{13}C parameter set, so you should never have to worry about it. The decoupler power (**dpwr**) is set according to the desired effect of the decoupler irradiation. For homonuclear NOE experiments, a very low power (~ 5) is used to maximize selectivity - only a "simmer" is required to equalize populations. For selective decoupling, values of 10-15 are typical - small enough to be selective but powerful enough to maintain the "rolling boil" necessary for decoupling. For broad-band (non-selective) decoupling (e.g., waltz-16), a power level of 40 is typical, with **dmf** set appropriately to obtain good decoupling over the entire range (-1 to 11 ppm) of proton chemical shifts. There are experiments such as DEPT which observe ^{13}C and use the decoupler to supply high-power, short duration pulses at the ^{1}H frequency. This requires full power from the decoupler, but VNMR avoids using the parameter **dpwr** for these pulses. Setting **dpwr** to the maximum decoupler power might lead to disastrous mistakes, since the decoupler can only deliver full power for short (~ 10 µs) periods of time without burning up the decoupler, the probe, and the sample. Instead, the parameters **pp** (90^o pulse width for decoupler pulses) and **pplvl** (power level for short-duration high-power decoupler pulses) are used for these applications. The parameter **homo** must be set to **'y'** for all homonuclear decoupling experiments, including presaturation, NOE difference, and selective decoupling.