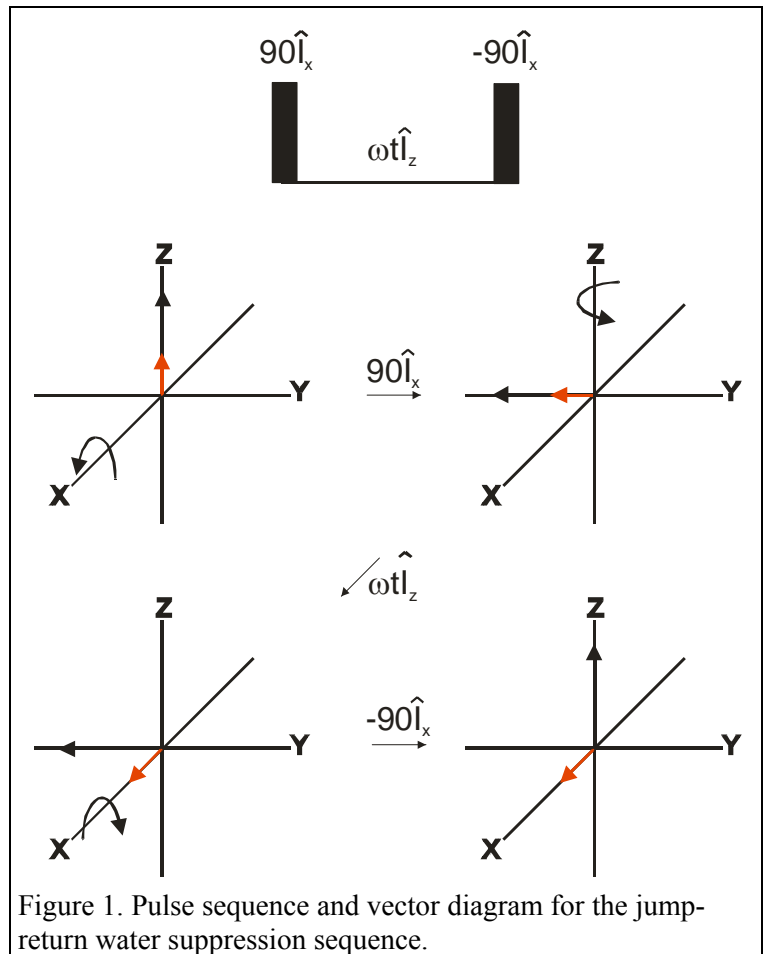


## Water suppression

In a typical biological sample the concentration of the solute is 1 mM or less. In many situations, the signals of interest are those of amide protons that exchange with the solvent water. In order to observe these signals the sample must be dissolved in  $^1\text{H}_2\text{O}$  with perhaps a few percent  $^2\text{H}_2\text{O}$  that serves as a lock signal. The concentration of protons in pure  $^1\text{H}_2\text{O}$  is 110 M. The dynamic range of such a sample is greater than 100,000:1. It is a spectroscopic rule-of-thumb that one should never try to detect a small signal in the presence of a large signal (large dynamic range). One way of eliminating reducing this problem is to dissolve the sample in  $^2\text{H}_2\text{O}$ , which significantly lowers the concentration of  $^1\text{H}$  nuclei,

but as noted above exchangeable protons sites then will be populated with deuterium atoms and will not be visible by  $^1\text{H}$  NMR. There have been many techniques developed for suppressing the  $^1\text{H}_2\text{O}$  signal in order to observe low concentration samples. One of the simplest methods is to presaturate the  $^1\text{H}_2\text{O}$  signal by irradiating only the  $^1\text{H}_2\text{O}$  resonance with a weak RF field in order to equalize the  $\alpha$  and  $\beta$  spin states (saturation). With the energy



levels saturated no signal is detected upon exciting the system, i.e. no Z (or X or Y) vector is present to be detected. However, if the  $^1\text{H}_2\text{O}$  signal is saturated and there is exchange with protons, the saturation can be transferred to the exchange site and causing the signal intensity to decrease or disappear. There are other problems with the presaturation method such as unsuppressed residual signals due to poor lineshape (shimming) and the loss of signal due to spin diffusion from saturated solute resonances that have frequencies near the water resonance. Early attempts to suppress water without saturation used the off-resonance behavior of NMR signals to low power rf pulses (long pulse [*Rev. Sci. Inst.* **32** 1066 (1961)], 2̄14̄12 [*J. Magn. Res.* **19** 114-117 (1975)]). The frequency was placed at that of the solute and the pulse widths and amplitude were adjusted to minimize the excitation of water. These sequences have rather poor water suppression and lead to large frequency dependent phase shifts resulting in poor baselines. They are used little in modern experiments. The majority of pulse sequences contain multiple pulses and delays. One of the simplest multipulse sequences for water suppression is that of the "jump-return" sequence. It consists of a  $90^\circ_x$  pulse followed by a specified delay and then another  $90^\circ_x$  pulse with an opposite rotation (Fig. 1 and Eqn.1).

$\mathbf{I}_Z \xrightarrow{\frac{\pi}{2} \hat{I}_X} ? \xrightarrow{\omega t \hat{I}_Z} ? \xrightarrow{-\frac{\pi}{2} \hat{I}_X} ?$	(1)
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The initial magnetization is at thermal equilibrium along the Z axis ( $\mathbf{I}_Z$ ). The magnetization for both the  $^1\text{H}_2\text{O}$  and the solute is first tipped by an angle of  $90^\circ$  around the X-axis, leaving the resultant vectors along the  $-Y$  axis (Eqn. 2).

$\mathbf{I}_z \xrightarrow{\frac{\pi}{2} \hat{\mathbf{I}}_X} \mathbf{I}_z \cos\left(\frac{\pi}{2}\right) - \mathbf{I}_y \sin\left(\frac{\pi}{2}\right) = -\mathbf{I}_y$	(2)
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During the delay time, the spin vectors of the solute and  $^1\text{H}_2\text{O}$  will precess at their own chemical shift frequencies (here we assume that the solute has a chemical shift different from that of  $^1\text{H}_2\text{O}$ ). We adjust the spectrometer such that the  $^1\text{H}_2\text{O}$  signal is on-resonance ( $\omega=0$ ) and the delay time is adjusted such that the vector arising from the solute precesses around the Z axis by  $90^\circ$  ( $\omega t = \pi/2$ ). This delay is calculated by measuring the frequency difference between the water and the solute signals, multiplying by 4 and taking the reciprocal. The  $^1\text{H}_2\text{O}$  signal will remain along the  $-Y$  axis during the delay (Eqn. 3) and the solute resonance will precess to the X axis (Eqn. 4).

$-I_y \xrightarrow{(\omega=0)t\hat{\mathbf{I}}_Z} -I_y$	(3)
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$-I_y \xrightarrow{\left(\omega t = \frac{\pi}{2}\right)\hat{\mathbf{I}}_Z} -I_y \cos\left(\frac{\pi}{2}\right) + I_x \sin\left(\frac{\pi}{2}\right) = I_x$	(4)
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The final  $-90^\circ$  pulse final along the X axis will rotate the  $^1\text{H}_2\text{O}$  signal back to the Z axis and will not affect the solute resonance which is parallel to the rotation axis (Eqn. 5).

$-I_y \xrightarrow{-\frac{\pi}{2} \hat{\mathbf{I}}_X} I_z$	(4)
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$I_x \xrightarrow{-\frac{\pi}{2} \hat{\mathbf{I}}_X} I_x$	(5)
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Ideally, the vector of the  $^1\text{H}_2\text{O}$  will not have a projection into the X,Y plane and will not give rise to a signal, whereas the solute vector remains in the XY plane and will be detected. If there are imperfections in the act of returning the water signal to the Z axis, a projection of the signal will remain in the XY plane and will be detected as a large unwanted signal. Obviously, if there is more than one solute resonance with different chemical shifts, the delay will not align all of the vectors along the X axis and only the projections of the vectors

$\mathbf{I}_Z \xrightarrow{\frac{\pi}{4} \hat{I}_X} ? \xrightarrow{\omega t \hat{I}_Z} ? \xrightarrow{\frac{\pi}{4} \hat{I}_X} ?$	(6)
---	-----

onto the X axis will remain as the Y component of the vector will be rotated to the Z axis. This leads to a non-uniform excitation profile. Other effects such as  $B_0$  field inhomogeneities (poor shimming), finite linewidths, and radiation damping degrades the performance of this sequence in providing

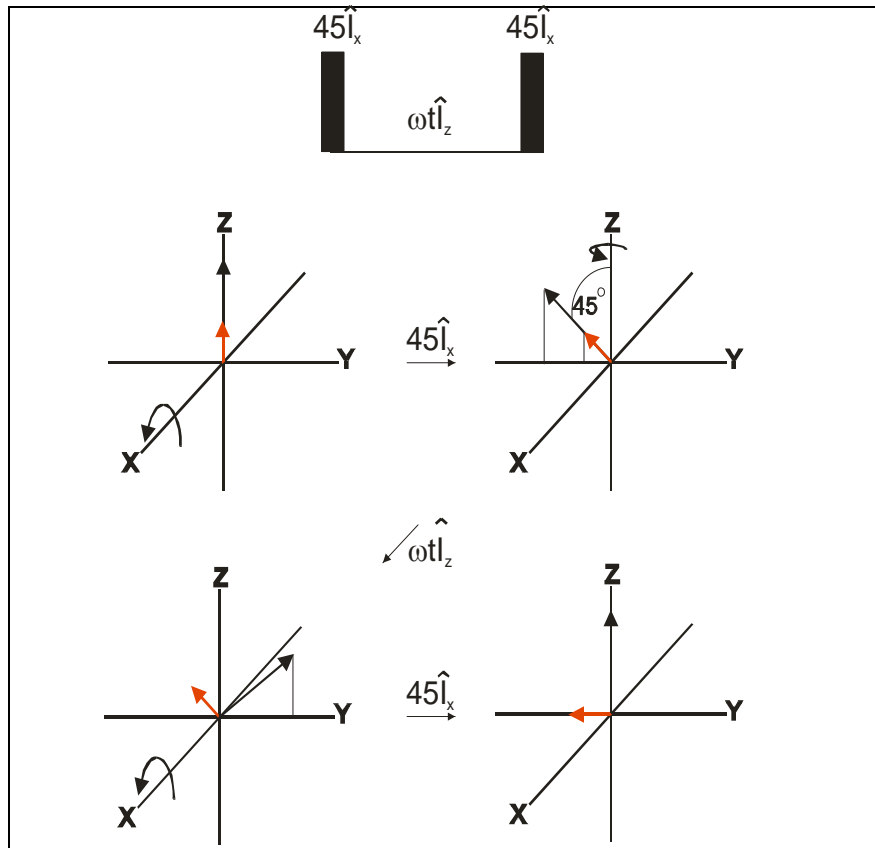


Figure 6. Pulse sequence and vector picture of the one-one pulse sequence.

suppression of the water signal. This sequence also gives rise to severe baseline distortions. One good quality of this sequence is that is very short. The delay time is typically 200-300  $\mu\text{s}$ , allowing for the observation of very wide lines in the presence of water. As an example see

$\mathbf{I}_Z \xrightarrow{\frac{\pi}{4} \hat{\mathbf{I}}_X} \mathbf{I}_Z \cos\left(\frac{\pi}{4}\right) - \mathbf{I}_Y \sin\left(\frac{\pi}{4}\right) = \frac{\sqrt{2}}{2} (\mathbf{I}_Z - \mathbf{I}_Y)$	(7)
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Westler and Markley (*Biochemistry*, **35** (34), 11092 -11097, 1996) where the broad signal from the hydrogen-bonded proton at the active site of chymotrypsinogen is observed.

An equally simple pulse sequence with similar performance characteristics as the jump-return is the one-one sequence. The carrier frequency is placed at the frequency of the signals to be

$\frac{\sqrt{2}}{2} (\mathbf{I}_Z - \mathbf{I}_Y) \xrightarrow{(\omega t = \pi) \hat{\mathbf{I}}_Z} \frac{\sqrt{2}}{2} (\mathbf{I}_Z + \mathbf{I}_Y)$	(8)
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observed. The water resonance is off-resonance and now has a non-zero frequency. The  $45^\circ_x$  pulse rotates both the water signal and the solute signal halfway to the  $-Y$  axis (Eqn. 7). The delay  $t$  is set to allow the water signal to precess by  $180^\circ$  around the  $Z$  axis (Eqn. 8), while the solute signal with a zero frequency will remain perched over the  $-Y$  axis (Eqn. 9).

$\frac{\sqrt{2}}{2} (\mathbf{I}_Z - \mathbf{I}_Y) \xrightarrow{(\omega t = 0) \hat{\mathbf{I}}_Z} \frac{\sqrt{2}}{2} (\mathbf{I}_Z - \mathbf{I}_Y)$	(9)
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The delay is calculated by measuring the frequency difference perched between the water and the solute signals, multiplying by 2, and inverting. The final  $45^\circ$  pulse around the  $X$  axis will rotate the water signal

$\frac{\sqrt{2}}{2} (\mathbf{I}_Z + \mathbf{I}_Y) \xrightarrow{\pi/4 \hat{\mathbf{I}}_X} \frac{\sqrt{2}}{2} \left( \mathbf{I}_Z \cos\left(\frac{\pi}{4}\right) - \mathbf{I}_Y \sin\left(\frac{\pi}{4}\right) + \mathbf{I}_Y \cos\left(\frac{\pi}{4}\right) + \mathbf{I}_Z \sin\left(\frac{\pi}{4}\right) \right)$ $= \mathbf{I}_Z$	(10)
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$$\frac{\sqrt{2}}{2} (\mathbf{I}_Z - \mathbf{I}_Y) \xrightarrow{\pi/4 \widehat{I}_x} \frac{\sqrt{2}}{2} \left( \mathbf{I}_Z \cos\left(\frac{\pi}{4}\right) + \mathbf{I}_Y \sin\left(\frac{\pi}{4}\right) + \mathbf{I}_Y \cos\left(\frac{\pi}{4}\right) - \mathbf{I}_Z \sin\left(\frac{\pi}{4}\right) \right) \quad (11)$$

$$= -\mathbf{I}_Y$$

to along the Z axis, while the solute signal will end up along the  $-Y$  axis (Eqns. 10 and 11). The utility of this pulse sequence is that if broad resonances are far off-resonance from a large solvent signal, this sequence can effectively suppress the water signal while maintaining a beneficial on-resonance condition with the broad line.

Extensions of the jump-return ( $1\bar{1}$ ) sequence leads to a series of binomial water suppression sequences such as  $1\bar{2}1, 1\bar{3}3\bar{1}, 1\bar{4}6\bar{4}1$ , and  $1\bar{5}10\bar{1}0\bar{5}\bar{1}$  [*J. Mag.Res.* **55** 283-300 (1983)]. The numbers are the relative lengths of the pulses in the sequence, which generally add up to the length of a  $90^\circ$  pulse (or  $180^\circ$ ), there are implicit specified delays between the pulses given by  $1/(2*\text{offset})$ , and the bars indicate that the phase of the pulse is inverted. These longer binomial sequences can give quite good water suppression, but the required linear phase correction becomes quite large and can cause baseline problems.

The most popular, current method of water suppression in biological samples is the watergate sequence. This pulse sequence consists of a spin echo sequence containing symmetric pulsed field gradients around a

"special"  $180^\circ$  pulse (Fig 3.

and Eqn 11). The  $180^\circ$  pulse is constructed such

that the solute spins are

inverted and the water peak

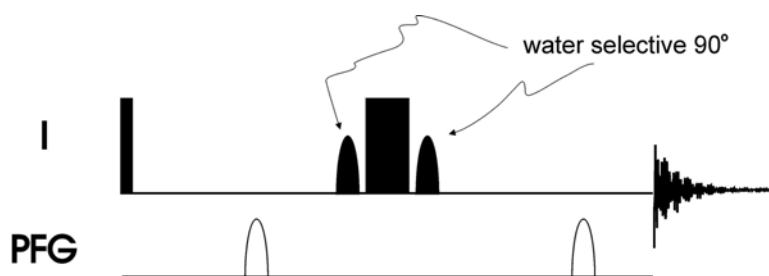


Figure 3. Watergate pulse sequence: two shaped rf pulses that selectively rotate the water resonance by  $90^\circ$  sandwich a non-selective  $180^\circ$ .

is not. The 180° pulse on the solute signal refocuses the influence of the gradients, whereas the water is dephased since it is not inverted. The behavior of the solute is described in the **Spin echoes and pulsed field gradients** section of paradigm\_I. For the net zero degree rotation of the

$$\begin{aligned} & \xrightarrow{\pi/2\widehat{I}_x} \xrightarrow{\gamma_I B_G(r)t\widehat{I}_z} \xrightarrow{0\widehat{I}_x} \xrightarrow{\gamma_I B_G(r)t\widehat{I}_z} \\ & = \xrightarrow{\pi/2\widehat{I}_x} \xrightarrow{2\gamma_I B_G(r)t\widehat{I}_z} \end{aligned} \quad (12)$$

water resonance the equivalent rotation is shown in Eqn 12. The water resonance will be completely dephased by the gradient and therefore not observed. The water suppression of this sequence is uniform across the frequency range and very good. The setup of this experiment is a bit longer because the selective 90° pulses must be calibrated for pulse width, amplitude and phase relative to the non-selective 180° pulse and in reality the calibration for the two individual selective pulses is different because of radiation damping effects on water. A simple modification of this sequence is made by replacing the special 180° with 6 pulses and delays known as the 3-9-19 sequence. The full sequence is 3-t-9-t-19-t-19-t-9-t-3 where the t value is 1/(2\*offset). This sequence is

very good at water suppression and is simple to set up, but it does not have as uniform frequency coverage as the sequence with the selective 90° pulses.

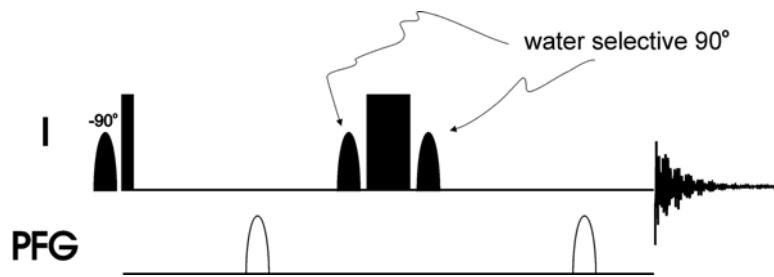


Fig 4. Watergate pulse sequence with water flip-down pulse that precedes the initial excitation 90° pulse.

The skeptical reader will note that the dephasing of water in the watergate sequence has the same

effect as saturation; there is zero remaining magnetization associated with water. Chemical exchange between the "saturated" water and exchangeable solute protons can cause loss of solute

$$\begin{aligned}
 & \xrightarrow{-\pi/2\widehat{I}_x} \xrightarrow{\pi/2\widehat{I}_x} \xrightarrow{\omega_1 t \widehat{I}_z} \xrightarrow{\gamma_1 B_G(r) t \widehat{I}_z} \xrightarrow{0\widehat{I}_x} \xrightarrow{\gamma_1 B_G(r) t \widehat{I}_z} \xrightarrow{\omega_1 t \widehat{I}_z} \\
 & = \xrightarrow{2\omega_1 t \widehat{I}_z} \xrightarrow{2\gamma_1 B_G(r) t \widehat{I}_z} \\
 & I_z \xrightarrow{2\omega_1 t \widehat{I}_z} \xrightarrow{2\gamma_1 B_G(r) t \widehat{I}_z} I_z
 \end{aligned} \tag{13}$$

signals. The pulse sequence can be modified with a flip-down pulse selective for water that precedes the initial  $-90^\circ$  pulse (Fig. 4). The initial  $-90^\circ$  selective pulse rotates the water magnetization to the +Y axis. The non-selective  $90^\circ$  that follows then rotates the water magnetization to the +Z axis at the same time as all other resonances are rotated to the  $-Y$  axis. The "special"  $180^\circ$  pulse acts as described above except that the water signal remains along the +Z axis. Since the water magnetization is along the Z axis when the gradients are applied, it is not affected and remains close to thermal equilibrium (it is not saturated). Equation 13 shows the equivalent rotation and the effect on  $I_z$  magnetization.