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Challenges of preparing, preserving and detecting para-water in bulk: overcoming proton exchange and other hurdles.

Daniele Mammoli\textsuperscript{a}, Nicola Salvi\textsuperscript{1}, Jonas Milani\textsuperscript{2}, Roberto Buratto\textsuperscript{3}, Aurélien Bornet\textsuperscript{4}, Akansha Ashvani Sehgal\textsuperscript{b, c, d}, Estel Canet\textsuperscript{b, c, d}, Philippe Pelupessy\textsuperscript{b, c, d}, Diego Carnevale\textsuperscript{e}, Sami Jannin\textsuperscript{a, f} and Geoffrey Bodenhausen\textsuperscript{a, b, c, d}.

Para-water is an analogue of para-hydrogen, where the two proton spins are in a quantum state that is antisymmetric under permutation, also known as singlet state. The populations of the nuclear spin states in para-water are believed to have long lifetimes just like other Long-Lived States (LLS). This hypothesis can be verified by measuring the relaxation of an excess or a deficiency of para-water, also known as a "Triplet-Singlet Imbalance" (TSI), i.e., a difference between the average population of the three triplet states T (that are symmetric under permutation) and the population of the singlet state S. In analogy with our recent findings on ethanol and fumarate, we propose to adapt the procedure for Dissolution Dynamic Nuclear Polarization (D-DNP) to prepare such a TSI in frozen water at very low temperatures in the vicinity of 1.2 K. After rapid heating and dissolution using an aprotic solvent, the TSI should be largely preserved. To assess this hypothesis, we studied the lifetime of water as a molecular entity when diluted in various solvents. In neat liquid H\textsubscript{2}O, proton exchange rates have been characterized by spin-echo experiments on oxygen-17 in natural abundance, with and without proton decoupling. One-dimensional exchange spectroscopy (EXSY) has been used to study proton exchange rates in H\textsubscript{2}O, HDO and D\textsubscript{2}O mixtures diluted in various aprotic solvents. In the case of H\textsubscript{2}O 50 mM in dioxane-d8, the proton exchange lifetime is about 20 s. After dissolution, one can observe this TSI by monitoring intensities in oxygen-17 spectra of para-water, by analogy to para-hydrogen\textsuperscript{1,2}. The following linear combination of populations:

\begin{equation}
\frac{1}{3} |T_+\rangle |T_+\rangle + |T_0\rangle |T_0\rangle + |T_-\rangle |T_-\rangle - |S_0\rangle |S_0\rangle
\end{equation}

where:

|T_+\rangle = |\alpha\alpha\rangle \quad |T_0\rangle = \frac{1}{\sqrt{2}} (|\alpha\beta\rangle + |\beta\alpha\rangle) \\
|T_-\rangle = |\beta\beta\rangle \quad |S_0\rangle = \frac{1}{\sqrt{2}} (|\alpha\beta\rangle - |\beta\alpha\rangle)

is equivalent to a "Triplet-Singlet Imbalance" (henceforth TSI), in analogy to the expression coined by Meier et al.\textsuperscript{3} for the "A/E imbalance" in \textsuperscript{13}CH\textsubscript{3} groups, which refers to a population imbalance between symmetric and antisymmetric states.
belonging to the irreducible representations A and E of methyl groups. An A/E imbalance can be induced by cooling down to ca. 1 K, molecules such as γ-picoline that contain methyl groups characterized by very low rotational barriers. In the high-temperature approximation, i.e., at spin temperatures above a few degrees Kelvin, the distribution between para- and ortho-water is given by the ratio 1:3. If this ratio is perturbed, as we shall demonstrate in this paper, the resulting scattering and cryo-MAS NMR spectroscopy by a combination of infrared spectroscopy, inelastic neutron scattering and cryo-MAS NMR spectroscopy can be improved by cooling down to very low temperatures in a high magnetic field, DNP can be used to achieve a TSI. The ESR transitions of the radical can be saturated by microwave irradiation to populate mostly the |aα⟩ = |T_{+1}⟩ state (Fig. 1A). During dissolution, the magnetic equivalence of the two protons in each H2O molecule is broken by the anisotropy of the chemical shifts, except for some particular orientations, so that the singlet state |S⟩ is mixed with the central triplet state |T⟩<sup>TSI</sup>. Depending on the coupling between the rotational and Zeeman energy levels, two situations can occur. If the energy levels are primarily determined by the rotational quantum numbers, as in the gas phase or in C60 cages (where the rotational levels typical of the gas phase are not a good approximation), the singlet state |S⟩ has the lowest energy. On the other hand, if rotational quantization can be neglected, the ground state |aα⟩ = |T_{+1}⟩ belongs to the triplet manifold. In our samples, the rotation of water is believed to be hindered by hydrogen bonding with other water and/or solvent molecules. Thus, when H2O molecules are diluted in a deuterated aprotic solvent, doped with a radical and frozen at low temperatures in a high magnetic field, DNP can be used to achieve a TSI. The ESR transitions of the radical can be saturated by microwave irradiation to populate mostly the |aα⟩ = |T_{+1}⟩ state (Fig. 1A). During dissolution, the magnetic equivalence of the two protons in each H2O molecule is restored. Hence, our strategy should lead to an excess of the average population of the three triplet states compared to the population of the singlet state (Fig. 1B). This amounts to a TSI. If its life-time is longer than T<sub>1</sub>, this may be considered as the hallmark of para-water.

Step 2 – Protecting the TSI during transfer

After rapid heating of the sample by injection of a hot aprotic solvent, the sample can be transferred to an NMR or MRI system. Longitudinal T<sub>1</sub> relaxation leads in a few seconds to the return of the triplet manifold to Boltzmann equilibrium at room temperature (Fig. 1C). However, T<sub>1</sub> relaxation does not affect the TSI, so that the singlet state remains depleted. On a longer time-scale T<sub>TSI</sub>, the populations of the triplet and singlet states will return to their Boltzmann equilibrium (Fig. 1D). In the dissolution step, all relaxation mechanisms that could reduce T<sub>TSI</sub> must be kept under control. The radicals can be eliminated by chemical reduction with scavengers such as ascorbate. Alternatively, porous solids that carry radicals can...
other words, the lifetime of proton exchange has been assessed to be much longer than $T_s$. This mechanism could cause losses of the TSI. SR is due to the relaxation times were found to be on the order of $T_s$, which is believed to be much longer than $T_s$.

Spin Rotation (SR) is another mechanism that could cause losses of the TSI. SR is due to the coupling between the nuclear spins and the molecular magnetic dipole induced by collisions on the atomic scale. Indeed, in gas phase, the longitudinal relaxation time was found to be on the order of $T_s = 20$ ms near 0.1 MPa and 373 K at 800 MHz. We believe however that SR is not an efficient mechanism for H$_2$O in condensed phase.

Step 3 – Detecting TSI relaxation

To detect the relaxation of the TSI, at least four distinct approaches can be used: (i) Direct detection by infrared (IR) spectroscopy relies on the fact that ortho- and para-water give rise to distinct IR absorption bands in gas phase. In condensed phase however, in particular when water is diluted in aprotic solvents, we found that the IR signatures are difficult to identify. (ii) Direct detection by multiplet effects in oxygen-17 NMR. In thermal equilibrium, the oxygen-17 signal of H$_2$O shows a normal binomial 1:2:1 triplet. If, however, one is able to populate a TSI, non-binomial multiplets could be observed like in para-ethanol. (iii) If the proton signals relax back to equilibrium with two different time constants this would be a hallmark of ortho-para conversion. (iv) Long-Lived water can be revealed indirectly by chemical addition onto a suitable substrate such as an activated aldehyde or ketone, monitored by $^1$H or $^{13}$C NMR. For this strategy to be successful, the reactivity of water molecules has to fulfill three requirements that are partly contradictory: (a) the water must be sufficiently diluted so that intermolecular $^1$H exchange is slowed down; (b) the rate of the chemical addition that is used to reveal the presence of para-water must be faster than the relaxation of the TSI; (c) the two protons that are added onto the substrate must stem from the same water molecule. Our kinetic measurements show that, under suitable conditions, these requirements may indeed be satisfied.

Results and Discussion

Step 1 – Preparing a TSI

Unlike Jeener and coworkers, who postulated that the two protons of an H$_2$O molecule can have different environments because of intermolecular dipole-dipole interactions in a magnetic field of 0.7 T, we shall assume that also the proton chemical shift anisotropy (CSA) contributes to break the symmetry at 6.7 T. We diluted water in deuterated dimethylsulfoxide (DMSO-d$_6$), doped it with ca. 50 mM TEMPOL radicals and froze it into a glassy state at ca. 1.2 K and 6.7 T. In Fig. 2 we can see proton spectra before (A) and...
Simulations of HDO protons oriented according to 36.75°}, with the dipolar coupling tensor between the two fractions ρ(0) = ½[E + I] and water molecules in barium chlorate monohydrate. These parameters have been calculated in previous work on proton exchange and proton relaxation of high-temperature approximation (Fig. 2C) or to describe the high-temperature approximation (Fig. 2C) or to describe the high-temperature approximation (Fig. 2C) or to describe the high-temperature approximation (Fig. 2C) or to describe the high-temperature approximation (Fig. 2C).

Table 1. Proton exchange rates for mixtures of H2O + HDO diluted in aprotic deuterated organic solvents at 800 MHz and 300 K. The concentrations, protonation fractions α = [H] / ([H] + [D]) and chemical shifts ν of H2O and HDO in the liquid phase are reported. The parameters α, Rex and kex were estimated by global fitting of the four curves in Fig. 5. In order to compare exchange lifetimes, normalized τex have been calculated for [H2O] + [HDO] = 50 mM, assuming a linear dependence of the exchange rate on the concentration of the solvent.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Experiments @ 800 MHz</th>
<th>Fitting @ 800 MHz</th>
<th>τex (s) normalized to [H2O]+[HDO]=50 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioxane-d6</td>
<td>0.28</td>
<td>~ 2.71</td>
<td>189</td>
</tr>
<tr>
<td>Nitromethane-d3</td>
<td>0.26</td>
<td>~ 2.13</td>
<td>50</td>
</tr>
<tr>
<td>Acetone-d6</td>
<td>0.22</td>
<td>~ 2.88</td>
<td>14</td>
</tr>
<tr>
<td>DMSO-d6</td>
<td>0.06</td>
<td>~ 3.33</td>
<td>11</td>
</tr>
<tr>
<td>Acetone-d8</td>
<td>0.73</td>
<td>~ 1.63</td>
<td>21</td>
</tr>
</tbody>
</table>

After (B) saturation of the ESR transitions by microwave irradiation at 187.9 GHz with frequency modulation\(^{40}\). Simulations of H2O powder spectra have been carried out by using the SIMPSON program\(^{48}\) on a spin system made up of two protons with shielding anisotropy ΔCS = –16.19 ppm, asymmetry ηCS = 0.17 and a dipolar coupling d = ~30.314 kHz. 4180 crystalite orientations were considered. The relative orientations of the relevant shielding tensors, expressed in a common crystal frame, were given by the Euler angles Ω\(^{H_{2}O}\) = {–165.11°, 115.33°, 36.75°} and Ω\(^{HDO}\) = {–14.89°, 115.33°, –36.75°}, with the dipolar coupling tensor between the two protons oriented according to Ω\(^{H{\text{HDO}}}_{\text{CS}}\) = (180.00°, 58.83°, 0.00°). These parameters have been calculated in previous work on water molecules in barium chloride monohydrate\(^{45}\) by means of DFT and plane-wave-pseudopotential methods as implemented in CASTEP code\(^{51}\). The shielding ellipsoids have their main components aligned along the OH bonds. Realistic pulse were used, with an rf-field strength V\(_{1}\) = 50 kHz and nutation angles β = 90° and 1.8°, in Fig. 2C and 2D, respectively. The initial density operator was either p(0) = I\(_{2}\) + S\(_{2}\) to describe the high-temperature approximation (Fig. 2C) or p(0) = ½[½E + I\(_{2}\) + S\(_{2}\) + 2I\(_{2}\)S\(_{2}\)] which corresponds to a pure state where only the lowest-lying state is populated at very low spin temperatures (Fig. 2D). The experimental spectra in Fig. 2A and B Fig. have larger linewidths with respect to the simulated Fig. 2C and Fig. 2D: this can be ascribed to the presence of paramagnetic species in solution (TEMPOL) and to intermolecular dipolar couplings that were not considered in the simulations. Finally this evidence suggests that DNP indeed allows one to drive the populations towards the lowest-lying state: since there is some mixing between the central triplet state and the singlet state, a very low spin temperature (on the order of 10 mK in our experiments) is sufficient to generate a TSI.

**Step 2 – Protecting the TSI during transfer**

Proton exchange in water could represent the major source of losses of TSI in our experiment. Hence, we studied exchange in water in bulk and in dilute solutions. The combined effects of proton exchange and proton T\(_{1}\) relaxation on the transverse T\(_{2}\) relaxation of \(^{17}\)O nuclei in H\(_{2}\)\(^{17}\)O can be characterized by using multiple refocusing of transverse \(^{17}\)O magnetization in the manner of Carr, Purcell, Meiboom and Gill (CPMG). One may compare \(^{17}\)O echo decays in the presence or absence of proton decoupling, in analogy with similar work carried out on \(^{15}\)N\(^{45,46}\). Our observations shown in Fig. 3 are in agreement with pioneering studies by Meiboom\(^{44}\). We measured lifetimes τ\(_{\text{ex}}\) = 1/k\(_{\text{ex}}\) of a few milliseconds in pure water at pH 5.93 and different temperatures. The lifetime of pure water as a molecular entity is clearly too short for our purposes but, as mentioned above, dilution in aprotic solvents can be used to extend this lifetime. In dilute solutions, the proton spectra of mixtures of H\(_{2}\)O and HDO feature two distinct resonances. In order to observe HDO triplets due to J\(_{\text{HDO}}\)(H\(_{2}\)O) = 80 Hz, the lifetime of HDO has to be τ\(_{\text{ex}}\) > 1/J\(_{\text{HDO}}\)(H\(_{2}\)O) = 12.5 ms. Slow exchange rates can be quantified using selective 1D or 2D exchange spectroscopy (EXSY)\(^{47}\). The pulse sequence used is shown in Fig. 5. Our samples consisted of a mixture of H\(_{2}\)O, HDO and D\(_{2}\)O diluted in various organic solvents at different concentrations (see Table 1). The concentrations [H\(_{2}\)O] and [HDO] have been determined by NMR within ±10%, by scaling their peak intensities to an external reference with concentration known \(a\ priori\). In nitromethane and dioxane, [H\(_{2}\)O] and [HDO] were increased with respect to the other solvents since, at low concentrations, the proton exchange.

![Figure 3](https://example.com/figure3.png)

Figure 3. Measurement of fast proton exchange rates k\(_{\text{ex}}\) in pure water. Proton exchange rates as a function of the temperature at pH = 5.93 were extracted from the ratio of peak heights\(^{45,46}\). The lifetimes τ\(_{\text{ex}}\) = 1/k\(_{\text{ex}}\) range from 3.6 to 0.9 ms between 275 and 310 K.
The parameters $\alpha$, $R_{\text{eff}}$ and $k_{\text{ex}}$ have been determined by global fitting to the curves in Fig. 5 and are shown in Table 1. The rates $R_i = 1/T_i$ of H$_2$O have been determined experimentally by inversion recovery (see Table 2). $R_{\text{eff}}$ is an average of $R_i$(H$_2$O) and $R_i$(HDO) weighted by their concentrations. Hence, a comparison between $R_{\text{eff}}$ in Table 1 and $R_i$ in Table 2 can only be qualitative (also because of different experimental conditions). However, in most cases the values are similar. In order to facilitate comparisons, in Table 1 we estimated the lifetimes for a concentration $[\text{H}_2\text{O}] + [\text{HDO}] = 50$ mM, assuming that the rates vary linearly with concentration$^{39}$. It is evident that, at concentrations below 50 mM, dioxane allows one to extend the lifetime of water as a molecular entity up to a few minutes. Dioxane, therefore, seems a good solvent for dissolution DNP. However, it has a much higher viscosity and lower heat capacity than water, so that our dissolution apparatus has to be re-designed thoroughly.

**Step 3 –Detecting TSI relaxation**

To observe a TSI in H$_2$O at room temperature after dissolution, we have resorted to a chemical reaction, inspired by the PASadena$^1$ and ALTADena$^{52}$ methods. We have shown recently$^{12}$ that one can lift the degeneracy of the two protons in fumarate (COOC=CHCOO) by addition of D$_2$O to produce malate (COOCHDCHODCOO), a reaction that is catalyzed by fumarase. In our work on para-ethanol$^{31}$, instead, the detection was possible, without any chemical reactions, by monitoring non-binomial multiplets in an $AX_2$ system. Following a suggestion by Jean-Maurice Mallet we have explored the addition of water (ultimately of para-water) on aldehydes, i.e., $\text{RCHO} + \text{H}_2\text{O} \rightarrow \text{RCH(OH)}_2$. The double C=O bond of the aldehyde can be activated by substituents such as $R = \text{CCl}_3$, as in chloral (CCl$_3$CHO, see Fig. 6). When the reaction is carried out in a dilute solution in acetonitrile, the two water protons (highlighted by stars in Fig. 6) may be assumed to end up on the same hydrate molecule. The two OH protons that stem from para-water give rise to degenerate doublets in the vicinity of 6.4 ppm. The lone proton near 5.3 ppm that stems from the aldehyde gives rise to a triplet due to two equal long-range couplings $J_{\text{HH}} = 6$ Hz to the two OH protons. Clearly, as discussed above, deviations of the binomial 1:2:1 distribution of this triplet can be used for a quantitative determination of the TSI$^{31}$.

**Table 2.** Longitudinal relaxation rates $R_i$ of H$_2$O diluted in aprotic deuterated organic solvents at 600 MHz and 300 K. $R_i$ were determined by inversion recovery. Their values can be compared to $R_{\text{eff}}$ in Table 1 but just in a qualitative way since $R_{\text{eff}}$ depends on $[\text{H}_2\text{O}], [\text{HDO}]$ and $k_{\text{ex}}$ and because of different experimental conditions.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$R_{\text{HDO}}$ @ 600 MHz</th>
<th>$[\text{H}_2\text{O}]$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioxane-d8</td>
<td>0.26</td>
<td>358</td>
</tr>
<tr>
<td>Nitromethane-d3</td>
<td>0.08</td>
<td>91</td>
</tr>
<tr>
<td>Acetonitrile-d3</td>
<td>0.15</td>
<td>9</td>
</tr>
<tr>
<td>DMSO-d6</td>
<td>0.30</td>
<td>42</td>
</tr>
<tr>
<td>Acetone-d6</td>
<td>0.17</td>
<td>10</td>
</tr>
<tr>
<td>Dichloromethane-d2</td>
<td>0.11</td>
<td>6</td>
</tr>
</tbody>
</table>

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The relative intensities of the lines in these multiplets can give a measure of the TSI in H₂O, i.e., of the relative populations of ortho- and para-water, much as in the ¹⁷O spectrum of H₂¹⁷O, or in the ¹³C spectrum of the ¹³CH₂ group in partially deuterated ethanol CD₃CH(OH)₂. We therefore explored the possibility of using reactions like in Fig. 6 to observe the binomial distribution of the triplet of the CH₂ protons, initially without DNP and thus without TSI. We explored the kinetics of the addition of H₂O onto two different substrates: chloral diluted in acetonitrile, and 1,3 dichlorotetrafluoroacetone diluted in dioxane. The main requirement is that the reaction must be faster than the relaxation of the TSI and its decay due to proton exchange. The rate constants found using pseudo-first-order kinetic equations have been reported in Table 3, albeit without DNP and thus without TSI. In acetonitrile-d₃, with a 5-fold excess of chloral with respect to H₂O, a pseudo first-order rate constant kₚ₆₀ = 0.002 s⁻¹ was observed. This reaction is too slow to be useful for detecting the TSI characteristic of para-water. However, the addition of water onto the more reactive compound (1,3 dichlorotetrafluoroacetone, in dioxane-d₈) was so fast that an accurate determination of the rate was difficult by NMR. A reaction rate kₚ₆₀ > 0.1 s⁻¹ makes it a good candidate for a "revealing agent" of TSI in para-water. As an alternative method for detection, one can simply measure the ¹⁷O spectrum in 0.037% natural abundance or with partial isotopic enrichment to improve sensitivity. The ¹⁷O spectrum in Fig. 7 shows a triplet due to $J(J, H, ¹⁷O) = 80$ Hz of H₂O 55 mM in dioxane-d₈ at 298 K and 400 MHz. Using water enriched to 20% ¹⁷O, we were able to acquire an ¹⁷O spectrum in few seconds, making this method a valid alternative to the use of a chemical reaction. Again, deviations from the binomial distribution of the intensities of the spectral lines should provide the information needed to assess the lifetime of the TSI and hence of para-water.

![Figure 6](image)

Figure 6. The addition of H₂O to chloral gives chloral hydrate CCl₃CH(OH)₂, where the two protons highlighted by stars can be assumed to stem from one and the same water molecule if the solution is sufficiently dilute.

Table 3. Kinetic pseudo-first-order rate kₚ₆₀ for hydration of activated C=O bonds observed at 800 MHz and 300 K.

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Solvent</th>
<th>[reactant] (mM)</th>
<th>[H₂O] (mM)</th>
<th>kₚ₆₀ (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloral</td>
<td>Acetonitrile-d₃</td>
<td>1000</td>
<td>200</td>
<td>0.002</td>
</tr>
<tr>
<td>1,3 Dichlorotetrafluoroacetone</td>
<td>Dioxane-d₈</td>
<td>275</td>
<td>55</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

We have optimized the most critical aspects of the experimental scheme suggested in this paper. However, we were not yet able to perform complete experiments since our setup needs to be largely adapted in order to support the dissolution with dioxane.

Conclusions

We have proposed an experimental strategy to produce para-water on a macroscopic scale (i.e. with concentrations in the mM range). Provided that the CSA of the protons is sufficient to lift the degeneracy of the two spins in a frozen sample, we demonstrated that one can use DNP to enhance the population of the ground state of water molecules, thus generating a Triplet-Singlet Imbalance (TSI) that is expected to be a long-lived state analogous to para-water. We proved that the lifetime of water as a molecular entity can be extended up to a few minutes by dilution in aprotic solvents. Several detection strategies can be used either by performing a "revealing" reaction such as the addition of water to an aldehyde or another suitable substrate, or by observing the ¹⁷O NMR spectrum of water itself. It is also possible to monitor proton magnetization of water relaxing to equilibrium with two clearly distinct time constants to assess the lifetime of the TSI and hence the amount of para-water in the sample. Similar information can, in principle, be obtained from infrared spectroscopy. The detection of para-water signal with long lifetimes may open the way to study slow transport phenomena such as flow, diffusion, and electrophoretic mobility.
Keywords

Para-water, ortho-water, water isomers, proton exchange, long-lived states, Triplet/Singlet Imbalance, A/E imbalance.

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Notes and references

Appendix

Let consider a solution of pure H$_2$O. Let $p$ be the frequency that a proton is exchanged with another proton belonging to a different water molecule:

$$p \propto [H_2O]$$

Hence, the proton exchange rate $k_{ex}$ can be defined as:

$$k_{ex} = 2p$$

The factor 2 reflects the fact that a proton belonging to a H$_2$O molecule can exchange with either of the two of another H$_2$O. Let now consider a mixture of H$_2$O, HDO and D$_2$O. With the ratio $\alpha = [H]/([H]+[D])$, their concentrations can be expressed as:

$$[H_2O] = \alpha^2 C_{TOT}$$
$$[D_2O] = (1-\alpha)^2 C_{TOT}$$
$$[HDO] = 2\alpha(1-\alpha)C_{TOT}$$

where:

$$C_{TOT} = [H_2O] + [HDO] + [D_2O]$$

The equations for the exchange of proton magnetization between two distinct sites can be written in the general form:

$$\frac{d\vec{M}(t)}{dt} = \overline{R} \vec{M}(t)$$

where:

$$\vec{M}(t) = \begin{pmatrix} M_{H_2O}(t) \\ M_{HDO}(t) \end{pmatrix}$$
$$\overline{R} = \begin{pmatrix} k & k' \\ k & -k' \end{pmatrix}$$

$$k' = k \frac{M_{H_2O}^{EQ}}{M_{HDO}^{EQ}}$$

Let us consider the following reactions involving the exchange of a proton:

$$H_2O + D_2O \leftrightarrow 2HDO$$
$$HDO + D_2O \leftrightarrow D_2O + HDO$$
$$H_2O + HDO \leftrightarrow HDO + H_2O$$

If we focus attention on the magnetization transfer from H$_2$O to HDO, there are three possibilities:

1. H$_2$O meets another H$_2$O. The exchange of two protons does not lead to any transfer of magnetization between different environments with distinct chemical shifts.
2. H$_2$O meets D$_2$O. There are four possible exchange processes. Each process leads to the creation of two HD0 molecules, and there are two protons that transfer their magnetization between different environments:

$$k_2 = 8p[D_2O] = 8p\alpha^2$$

3. H$_2$O meets HDO. Again, four possible exchange processes can take place, but there is only one proton that transfers its magnetization between different environments:

$$k_3 = 4p[HDO] = 8p\alpha(1-\alpha)$$

If we consider that we should count an exchange process not only for the proton that hops but also for its neighbor, the total exchange rate is:

$$k = \frac{k_2 + k_3}{2} = 4\alpha p = 2\alpha k_{ex}$$

If we now consider magnetization transfer from HDO to H$_2$O, there are again three possibilities:

4. HDO meets H$_2$O. This is symmetric to case (3) above:

$$k_1 = 4p[H$_2$O] = 4p(1-\alpha)$$

5. HDO meets D$_2$O. Four possible exchange processes can again occur but none of them leads to any magnetization transfer between different environments.

6. HDO meets HDO. Again, four possible exchange processes are possible, two of which swap a proton with another proton but do not lead to any magnetization transfer. Two processes swap a proton and a deuteron to create two H$_2$O molecules, which is accompanied by a transfer of magnetization between different environments:

$$k_3 = 4p[HDO] = 8p\alpha(1-\alpha)$$

In this case we have counted the exchange processes twice: when molecule $i$ meets molecule $j$ is of course the same case as when $j$ meets $i$. Hence the total rate is:

$$k' = k_1 + \frac{k_3}{2} = 4p(1-\alpha) = 2(1-\alpha)k_{ex}$$

By including longitudinal relaxation, we find:

$$\overline{R} = \begin{pmatrix} R_{H_2O}^{HDO} + 2ak_{ex} & -(2-2a)k_{ex} \\ -2ak_{ex} & R_{HDO}^{HDO} + (2-2a)k_{ex} \end{pmatrix}$$

This matrix can be diagonalized:

$$\overline{M}(t) = \overline{U}^{-1}e^{-\overline{R}t}\overline{U}\overline{M}(0)$$

so that

$$\overline{M}(t) = \begin{pmatrix} D_{H_2O} & C_{HDO} \\ C_{H_2O} & D_{HDO} \end{pmatrix} \overline{M}(0)$$

With the assumption that $R_{H_2O}^{HDO} = R_{HDO}^{HDO} = R_{1}^{eff}$ we finally find:

$$D_{H_2O} = e^{-(k_{ex}+R_{1}^{eff})t}[\cosh(k_{ex}t) + (1-2a)\sinh(k_{ex}t)]$$
$$C_{H_2O} = e^{-(k_{ex}+R_{1}^{eff})t}[(2-2a)\sinh(k_{ex}t)]$$
$$D_{HDO} = e^{-(k_{ex}+R_{1}^{eff})t}[\cosh(k_{ex}t) + (2a-1)\sinh(k_{ex}t)]$$
$$C_{HDO} = e^{-(k_{ex}+R_{1}^{eff})t}2a\sinh(k_{ex}t)$$

In these calculations we have neglected possible kinetic isotope effects on the rates.