Title
Limits of the quantum cognition hypothesis: $^{31}$P singlet order lifetimes of pyrophosphate from experiment and simulation

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Abstract
A proposal of quantum cognition advances the hypothesis that quantum entanglement between $^{31}$P nuclei could serve as a means of information storage in the brain. Testing this hypothesis requires an understanding of how long-lived these quantum effects may be. We used NMR spectroscopy and molecular dynamics simulations to study the mechanisms that limit these quantum processes in $^{18}$O-enriched molecules of pyrophosphate, the simplest biomolecule that can sustain quantum-entangled $^{31}$P nuclear spin singlet states. We confirmed that chemical shift anisotropy limits the singlet magnetization order lifetimes in high magnetic fields, and we discovered that rapid rotation of the phosphate groups limits the lifetime in low magnetic fields. These findings represent an important starting point in studying whether quantum cognition can be a true biological phenomenon.

Keywords: nuclear spin singlets • relaxation mechanism • spin-rotation interaction • chemical shift anisotropy • NMR spectroscopy

Introduction
It has been proposed that $^{31}$P nuclear spin entanglement may play a role in physiology and biological information storage and transmission (1, 2). Although the notion of quantum processes involving entangled nuclear spin states may appear far-fetched, the hypothesis has not been easy to directly prove or refute. The quantum cognition proposal involves the existence of so-called Posner clusters with the stoichiometric formula Ca$_9$(PO$_4$)$_6$, which through an interplay between rotational and nuclear spin states may exhibit symmetry-constrained quantized states labeled by what was called ‘pseudospin’. Such clusters have yet to be detected experimentally. Smaller fragments, such as pyrophosphate, have also been considered as potential actors for $^{31}$P nuclear singlet order (SO) modulation of reaction rates, in particular enzymatic ones (1). In this work, we therefore set out to determine the underlying limitations that would have to be considered in support of such claims, in particular regarding the lifetime of $^{31}$P SO, represented by an exponential decay time constant $T_S$, describing the quantum entanglement memory loss.

Nuclear SO in $^1$H and $^{13}$C spin pairs has been observed to have very long $T_S$ values compared to the spin-lattice relaxation time constant $T_1$ in a variety of compounds, in some cases one to two orders of magnitude higher (3-7). Very recently, SO between $^{31}$P nuclei has been observed and characterized in large diphosphate compounds (8, 9). For the $^{31}$P spins in the
compounds studied, however, singlet relaxation has been found to be much more rapid than spin-lattice relaxation, with a major reason being the anticorrelation between the chemical shift anisotropy (CSA) tensors of the two spins \((8)\).

Two aspects of this prior work motivated us to examine \(^{31}\)P-spin SO further. The compounds used previously were particularly bulky and contained large asymmetries between the two spins (either transient or constant). We therefore sought to study the small, highly symmetric molecule pyrophosphate, modified to have slight asymmetry, thereby enabling access to SO. Since the main mechanism in prior work on substituted phosphates appeared to be due to CSA, we wished to perform field-dependent studies. We present here Zeeman and SO relaxation studies over a large field range (2 \(\mu\)T to 9.4 T) to investigate the major relaxation mechanisms as a function of magnetic field, and to determine, in particular, the underlying low-field limit to the SO relaxation time. We further identify the mechanistic contributions to SO relaxation by molecular dynamics (MD) and \textit{ab initio} computation.

**Results**

**Synthesis and NMR characterization of unsymmetrically \(^{18}\)O-labeled pyrophosphate**

One challenge in the study of SO in the pyrophosphate (PP$_i$) molecule is the lack of inequivalence (either chemical or magnetic), which is needed for creating and reading out SO of the \(^{31}\)P spins. To overcome this challenge, we unsymmetrically labeled PP$_i$ with the \(^{18}\)O isotope. The increased mass of the \(^{18}\)O nuclei relative to the abundant \(^{16}\)O isotope was expected to induce a small chemical shift difference between the neighboring \(^{31}\)P nuclei, sufficiently large to allow creation and read-out of SO. This strategy was used previously for pairs of \(^{13}\)C nuclei \((10)\). The tetrasodium salt of the unsymmetrically labelled \(^{18}\)O-PP$_i$ (uPP$_i$) was synthesized as described in the Materials and Methods section below and prepared in D$_2$O under highly alkaline conditions to avoid potentially interfering effects due to proton exchange, which can accelerate SO relaxation \((11)\). An excess of potassium cations, relative to the sodium cations from the tetrasodium salt, was found to promote longer SO lifetimes than if sodium ions alone were present. Similar results were obtained by adding ethylenediaminetetraacetate (EDTA) instead (Fig. S1, Supplementary Materials).

![Fig. 1. \(^{31}\)P NMR spectrum and fitting results of fully deprotonated unsymmetrical pyrophosphate in KOH and D$_2$O. Additional unidentified peaks arising from the synthesis besides the inner and outer doublet of doublet peaks were excluded from fitting. Fitted \(^{31}\)P chemical shift difference and homonuclear \(J\)-coupling values are displayed to the right of the spectra. The structure of the unsymmetric pyrophosphate is shown on the top left.](image-url)
The NMR properties of the synthesized uPP$_i$ $^{31}$P spin system were extracted from a $^{31}$P pulse-acquire spectrum acquired at 9.4 T by multiplet simulation and fitting using the Spinach MATLAB package (http://spindynamics.org/group/) (12). Fig. 1 displays the fitting results. The unsymmetrical isotopic labelling of the uPP$_i$ induces a slight chemical shift difference $\Delta \delta_{PP}$ between the two $^{31}$P nuclei of 0.0663 ppm, or 10.7 Hz at 9.4 T. The $^{31}$P nuclei share a homonuclear $J$-coupling of magnitude $^{2}J_{PP} = 21.5$ Hz. Thus, the uPP$_i$ $^{31}$P spin system is in a strongly coupled regime at 9.4 T. Singlet-triplet mixing can occur at high fields, but this mechanism of SO decay is eliminated when the sample is moved to lower fields. Additional peaks are observed which likely stem from partial labelling of the molecule. We could not fully identify these, but products with partial labelling should not affect the results, since the triplet-singlet transfer is tailored to a particular chemical shift / coupling combination. The isotope composition should not affect relaxation rates due to the small differences in mass. The $^{31}$P $R_1$ values of the unlabeled PP$_i$ and the $^{18}$O-labeled uPP$_i$ were measured at 9.4 T as 0.107 s$^{-1}$ and 0.102 s$^{-1}$, respectively, with identical solution conditions (pD 14.4, 25 °C).

**NMR field-cycling relaxation measurements of uPP$_i$**

We then performed field-dependent measurements of both SO relaxation and spin-lattice relaxation, in order to compare and contrast known relaxation mechanisms. We chose to utilize the spin-lock induced crossing (SLIC) pulse sequence (13) for preparing and reading out SO for NMR spectroscopic relaxation measurements. The SLIC pulse sequence used for field-dependent measurements of $R_S$ ($= 1/T_S$) is displayed in Fig. 2. Optimization of the power and duration of the SLIC spin-lock pulse confirmed the spin system parameters determined via spectral fitting: the optimal pulse amplitude and duration corresponded with $^{2}J_{PP}$ of 20.3 Hz and a $\Delta \delta_{PP}$ of 12.3 Hz (Fig. S2, Supplementary Materials). We performed $^{31}$P relaxation measurements on ~300 mL of the uPP$_i$ solution in a 5 mm NMR tube using a 9.4 T Bruker NMR spectrometer equipped with a home-built field shuttling system, to transport the sample rapidly between regions of different magnetic field. The shuttling system included a shielded region above the magnet and therefore enabled access to magnetic field strengths as low as 2 $\mu$T. An inversion-recovery sequence was used with the same sample shuttling setup in order to measure $R_1$ ($= 1/T_1$).

**Fig. 2.** Pulse sequence diagram for field-shuttling singlet relaxation measurements using SLIC pulses. Conversions between detectable magnetization and singlet order were performed at 9.4 T, and the sample was shuttled to low field for the incremented relaxation delay, then shuttled back for detection. A $T_{100}$ filter prior to singlet readout was used with two-step phase cycling on the first 90° pulse and receiver to remove undesired coherence pathways.
The relaxation measurements are shown in Fig. 3. Generally, the $R_1$ and $R_S$ values tracked each other, with $R_1$ experiencing a slight increase in the 2 µT to 200 mT range. $R_S$ also tended to be smaller than $R_1$ in the high-field regime, above 4.5 T. Both $R_1$ and $R_S$ approached a constant relaxation rate offset of approximately 0.018 s$^{-1}$ at the lowest field values measured. The measured relaxation trends with magnetic field were well approximated using MD simulations and ab initio calculation (Fig. 3, dashed lines), as described below.

Fig. 3. $^{31}$P NMR relaxation measurements of fully deprotonated 30 mM unsymmetrical pyrophosphate as a function of magnetic field strength. (A) spin-lattice relaxation; (B) SO relaxation. Open circles indicate experimental measurements, and dashed lines indicate the total simulated relaxation curve. For each plot, time constant values are shown on the right y-axis which correspond with the relaxation rate values on the left y-axis.

**Molecular dynamics simulation and ab initio calculation of relaxation rate curves**

In order to study the CSA tensors in uPP$i$ and their contributions to longitudinal and SO relaxation, MD simulations were performed using Gaussian 16 and Amber20 (14) software, as described in the Materials and Methods section. Briefly, the uPP$i$ electronic structure was modeled in Gaussian, then energy minimization was performed over 5000 steps, followed by 20,000 steps of 1 fs to reach the desired temperature and pressure (300 K, 1 bar), and the final production run was performed with an isothermal/isobaric ensemble (NPT, 300 K, 1 bar) with $10^7$ steps. The CSA tensors at each $^{31}$P nucleus were then calculated ab initio in Gaussian from 100 randomly selected conformations. Fig. 4 shows average and multiple-snapshot representations of the symmetric portion of the CSA tensors experienced by the $^{31}$P nuclei. As is seen here, the principal component appears almost completely aligned with the bond between phosphorus and the bridging oxygen. Because the -PO$_3$ groups experience fast intramolecular rotation about the bridging P-O bond (see Fig. 4B), the CSA tensors were averaged across the 100 conformations, following molecular alignment along the P-P vector. A more detailed justification for this averaging procedure can be found in the Materials and Methods section. The difference between the average tensors at each $^{31}$P nucleus was computed, and the average and difference tensors were separated into their symmetric and antisymmetric components. The (Frobenius) norms of the tensor components are summarized in Table 1 and were used to calculate the CSA contributions to $R_1$ and $R_S$ using the expressions
In the equations above, $\omega_0$ is the Larmor frequency, $\|\sigma\|_F$ and $\|\Delta\sigma\|_F$ indicate the Frobenius norms of the average and difference tensors, respectively, and $\tau_1$ and $\tau_2$ are the first- and second-rank correlation times, respectively, where $\tau_1 = 3\tau_2$ assuming isotropic motion. The second-rank correlation time was determined to be 48.6 ps, based upon MD simulation following adjustment using the NMR-measured PP$_1$ diffusion coefficient, as described in the Materials and Methods section.

Fig. 4. Graphical representations of uPP$_1$ molecular dynamics. (A) Ovaloid representation of the symmetric CSA tensor components experienced by each $^{31}$P nucleus. (B) Combined snapshots of conformations sampled by the uPP$_1$ molecule within the molecular dynamics simulation, aligned along the P-P vector.

It is seen that CSA accounts for the major relaxation effect at high magnetic fields. The symmetric CSA component (Fig. 5, solid lines) contributes the most to $R_1$ and $R_S$ at high field strengths, whereas the antisymmetric contribution (Fig. 5, dotted lines) is relatively small for both but much larger for $R_S$ than it is for $R_1$. Other smaller yet significant relaxation contributions, largely field-independent, are discussed further below.
Table 1. Frobenius norms of $uPP_i$ chemical shift anisotropy tensor averages from \textit{ab initio} calculation on snapshots from MD simulations.

The spin-rotation contribution to $R_1$ was calculated as follows: From MD simulations, the correlation function $\overline{\omega(0)\omega(t)}$ for the angular rotation frequency of the -PO$_3$ entity about the bridging P-O bond of PP$_i$ was calculated. An exponential fit was performed to this function, which yielded $\omega(0)^2$ and the correlation time $\tau_f$. These values were determined as 3.1 rad$^2$/ps$^2$ and 0.0255 ps, respectively. Gaussian 16 was used to compute the spin-rotation tensor for $^{31}$P in PP$_i$ at the B3LYP/aug-cc-pVTZ level, which produced the value for $C_{\parallel}/2\pi = 4.424$ kHz, for rotation around the bridging P-O vector, and roughly two equivalent values for the perpendicular rotation $C_{\perp}/2\pi = 1.095$ kHz. The spin-rotation tensors are visualized in Fig. S3 in the Supplementary Materials, which indicates that the major component of this tensor also points along the bridging P-O bond similar to the CSA tensor. Given that the motion perpendicular to the P-O bond can be assumed to be very small by comparison (see Fig. 4B, showing the superposition of conformers obtained from MD trajectories), we neglect this portion and calculate the spin-rotation relaxation rate constant by the expression

$$R_{1,SR} = \frac{2}{3\hbar^2} \overline{\omega(0)^2} I_{\parallel}^2 C_{\parallel}^2 \tau_f,$$

where $I_{\parallel} = 1.758\cdot10^{-45}$ kg m$^2$ is the moment of inertia for the -PO$_3$ entity with respect to the bridging P-O axis. This expression can be derived by combining Eq. (22) from McClung (15) with Eq. (4.83) from Spiess (16). The spin-rotation relaxation rate constant then becomes $R_{1,SR} = 0.0113$ s$^{-1}$. The rate is essentially independent of the magnetic field due to the extremely short correlation time for the angular frequency correlation function.

Spin-rotation is also expected to affect the relaxation of SO in uPP$_i$. We made the following considerations: were the spin-rotation field fluctuations produced by each rotating -PO$_3$ group fully uncorrelated, we would predict $R_{S,SR}$ to be twice as large as $R_{1,SR}$. However, in this case $R_S$ would be larger than $R_1$ at low field strengths, whereas experimentally we observed similar low-field values of $R_1$ and $R_S$. We therefore determined the correlation coefficient $\alpha$ for the spin-rotation interaction at each $^{31}$P spin following the discussion about correlated mechanisms of Tayler et al (17), in particular Eqs. (1) and (2). From these considerations, once can obtain $R_{S,SR}/R_{1,SR} = 2(1 - \alpha)$, and when using the experimental values for $R_{S,SR}$ and $R_{1,SR}$ we obtain the correlation coefficient $\alpha = 0.5$. Modeling the spin-rotation contribution to $R_S$ in this manner produced an excellent fit to the experimental data (Fig. 3, dashed line). Other known relaxation contributions to $R_1$ and $R_S$ are described below.
MD simulations following the procedure of Kharkov et al (18) gave the contribution of intermolecular dipolar relaxation between $^{31}$P and $^2$D solvent spins as $5.14 \times 10^{-3}$ s$^{-1}$. The $^{31}$P-$^{31}$P dipolar relaxation contribution, relevant only for $R_1$, was determined to be $1.60 \times 10^{-3}$ s$^{-1}$. The correlation times for these processes range from 20-40 ps, and therefore their contributions are likewise almost completely independent of the magnetic field. The singlet-triplet leakage (STL) contribution to SO relaxation cannot easily be determined in closed form, since it depends on the specifics of the relaxation mechanism. This effect was therefore estimated using the Spinach NMR simulation package in MATLAB (12), by simulating SO relaxation with and without the chemical shift difference included and calculating the difference. The contribution is field-dependent but relatively minor, as seen in Fig. 5. Finally, the $^1$H-$^{31}$P dipolar relaxation contribution arising from the added KOH was estimated from the $^2$D-$^{31}$P contribution as $0.00025$ s$^{-1}$, which is negligible compared to other relaxation contributions.

![Fig. 5. Breakdown of total simulated relaxation curve into components by relaxation mechanism. (A) calculated spin-lattice relaxation contributions; (B) calculated SO relaxation contributions. CSA = chemical shift anisotropy; SR = spin-rotation; DD = dipole-dipole relaxation; STL = singlet-triplet leakage.](image)

**Discussion**

The quantum cognition proposal advanced by Fisher (1) involves multiple components requiring independent validation. One of the most prominent challenges of the proposal in its current form involves assessing whether quantum entanglement can survive for an appreciable duration in a “wet” biological environment, even though some level of protection from the environment may be provided by symmetry in a Posner cluster (1). Our approach to studying entangled spin order in unsymmetrically labeled pyrophosphate represents an important initial step in studying what phenomena most strongly limit the lifetime of quantum entanglement between $^{31}$P nuclei.

Our $R_1$ and $R_S$ measurements show that uPP, high-field relaxation is dominated by the CSA mechanism, similar to the case in other reported diphosphates (8, 9). In contrast to previous studies, the $R_S$ values observed in the high field regime are slightly lower compared with $R_1$, which correspond well with the symmetric CSA tensor norm being somewhat lower for the difference tensor (Table 1). The norm of the antisymmetric component, however, is significantly larger for the difference tensors than for the individual tensors, with the result being a larger antisymmetric CSA contribution to $R_S$. Still, the antisymmetric contribution to $R_S$ is smaller than one fifth of the symmetric contribution.
Importantly, we observed that towards low fields, a constant offset in relaxation rate constants is approached for the experimentally measured values of both $R_1$ and $R_S$. The offset at the lowest field, 2 $\mu$T, was found to be approximately 0.018 s$^{-1}$ for both. The same trend and a similar, albeit slightly higher $R_1$ and $R_S$ offset were observed from measurements on a 30 mM uPP$_1$ sample with 10 mM EDTA added (Fig. S4, Supplementary Materials). We believe this constant contribution at the lowest field to be primarily comprised of spin-rotation relaxation, as shown in Fig. 5. Furthermore, at very low field strengths (2 $\mu$T to 100 mT), $R_1$ showed a peculiar increase in the rate that was consistently observed across different sample formulations (Fig. S4A). This effect is not understood at this time.

The largest values of the $T_1$ and $T_S$ time constants appear to be approximately 65 s for uPP$_1$ under our experimental setup (in the low field range). We note, however, that many of the experimental conditions used for our relaxation measurements are different from those that would be encountered in a biological system. First, our experiments were performed at a relatively high pH, to limit deuteron exchange, whereas faster exchange at physiological pH values would be expected to reduce the $T_S$ and possibly $T_1$ relaxation times (11). In addition, the nature of the counterion played a role in the relaxation measurements, and the longest $T_1$ times were observed with an excess of K$^+$ ions relative to the Na$^+$ ions from the synthesized uPP$_1$ tetrasodium salt. It is worth noting that the intracellular K$^+$ concentration tends to be approximately fourfold higher than Na$^+$, whereas Na$^+$ is much more abundant in the extracellular space (19). This finding may suggest that the intracellular environment is more conducive to long-lived quantum entanglement, at least for free pyrophosphate. Furthermore, D$_2$O was used as a solvent rather than H$_2$O. We note that if H$_2$O were used as a solvent, this limit would be significantly smaller. We measured an increase in $R_1$ of 0.028 s$^{-1}$ at 9.4 T when we replaced D$_2$O with 90% H$_2$O plus 10% D$_2$O. Assuming this increase to be field-independent, we would therefore expect a $T_1$ and $T_S$ maximum of approximately 26 s if we were to repeat the field-dependent measurements with this solvent. Finally, certain paramagnetic species are abundant within cells and tissues and can contribute to relaxation. Comparison of rates observed in degassed and non-degassed samples, however, showed approximately the same rate constants in the low field region, suggesting that the effect of paramagnetic relaxation due to oxygen is low (Fig. S4, Supplementary Materials). Other paramagnetic impurities were considered, but careful and extensive cleaning of glassware with KOH/iPrOH and HCl did not produce significant changes. Examination of relaxation in the presence of EDTA (to potentially capture paramagnetic impurities) likewise did not show significant changes in the observed rate constants (Fig. S4, Supplementary Materials).

In summary, we report measurements of $^{31}$P SO decay in isotope labeling-induced unsymmetric PP$_1$ over a wide range of field strengths. We demonstrate that CSA dominates both $R_1$ and $R_S$ relaxation at high fields but diminishes at low fields, and that the two rates have similar values from 2 $\mu$T to 9.4 T. We observe that both $R_1$ and $R_S$ approach a constant value at low field strengths, and that this relaxation appears to be primarily explained by spin-rotation relaxation, with minor (but non-negligible) contributions from intermolecular $^{31}$P-2D dipolar coupling and intramolecular $^{31}$P-$^{31}$P dipolar coupling. The magnitude of the spin-rotation relaxation contribution in this molecular system was an unexpected discovery.

Our main experimental finding is that the relaxation rates for $^{31}$P longitudinal magnetization and for $^{31}$P nuclear SO are similar for pyrophosphate in solution, with multiple mechanisms contributing to both relaxation processes. Both relaxation times are of the order of 1 minute in low magnetic field under our experimental conditions, and they decrease rapidly as the
magnetic field is increased. In low magnetic fields the $^{31}$P singlet lifetime of pyrophosphate is possibly long enough to sustain the hypothesis that such entangled spin pairs might play a role in quantum cognition (1, 2). As far as the authors know, there is no evidence that cognition is significantly disturbed by high magnetic fields, as would be anticipated from the experimental results described here.

**Materials and Methods**

**Unsymmetrically $^{18}$O-labeled pyrophosphate synthesis and formulation**

The synthesis of $^{18}$O/$^{16}$O unsymmetrical pyrophosphate tetrasodium salt 6, henceforth referred to as uPP, is shown in Fig. 6. Light sensitive silver phosphate salt 1 was prepared from $^{18}$O phosphoric acid by a simple precipitation method (20). Subsequent benzylation in the presence of excess benzyl chloride provided the triester 2 in 75% yield (21). Heating triester 2 in the presence of one equivalent of sodium iodide in acetone accomplished selective mono-deprotection (21), and the resulting dibenzyl phosphate sodium salt 3 was converted to the tetrabenzyl $^{18}$O/$^{16}$O pyrophosphate 4 by reaction with dibenzyl phosphoryl chloride ($^{16}$O, obtained by the chlorination of dibenzyl phosphate with NCS in benzene and used directly (22)) in the presence of triethylamine (23). Global debenzylation of the tetrabenzyl pyrophosphate using hydrogen over Pd required prolonged reaction times and was inefficient due to accompanying partial hydrolysis to the orthophosphate. Ultimately, a two-step procedure via the dibenzyl pyrophosphate disodium salt 5 was optimised, with the remaining two benzyl groups removed by hydrogenolysis over Pd in the presence of sodium bicarbonate in 5 hours. This six-step sequence afforded the regioselectively $^{18}$O/$^{16}$O labelled pyrophosphate tetrasodium salt 6 as a white crystalline solid. Isotopic incorporation was confirmed by mass spectrometry to be 96% $^{18}$O$_4$, 96% $^{18}$O$_3$.

![Fig. 6. Synthesis of $^{18}$O/$^{16}$O unsymmetrical pyrophosphate tetrasodium salt 6.](image_url)

For NMR experiments, the tetrabasic sodium uPP was formulated as a 30 mM solution in deuterium oxide plus 10 equivalents of potassium hydroxide, in order to minimize proton exchange, which can accelerate singlet relaxation (11), and minimize interactions with sodium ions in solution, which our results seem to indicate also accelerates relaxation of both longitudinal and spin order (Fig. S1, Supplementary Materials). The final concentrations of Na$^+$ and K$^+$ counterions were 120 mM and 300 mM, respectively. The pH of the solution was expected to be about 14.4, based upon room-temperature pH electrode measurements of a sample prepared identically but with unlabelled tetrabasic sodium pyrophosphate. The NMR tubes used with the samples were carefully cleaned to avoid any paramagnetic impurities by immersing in a KOH/iPrOH bath overnight followed by HCl.
immersion overnight, rinsing several times with acetone, and drying with argon gas. More details on sample preparation can be found in the Supplementary Materials.

**Field-dependent NMR spectroscopy**

All field-dependent NMR measurements were performed at the University of Southampton. For measurements of $R_1$ via inversion-recovery, the uPP, $^{31}$P populations were inverted with a 180° pulse, the sample was shuttled to a region with the desired magnetic field strength, and then the sample was returned to the bore for excitation with a 90° pulse followed by acquisition. SO was prepared with a SLIC spin-lock pulse at 9.4 T within the bore, the sample was shuttled to a region above the magnet for singlet relaxation at the desired field strength, and then returned to the magnet bore for singlet order readout via SLIC. The sample shuttling speed to and from the low field for all measurements was about 1 m/s, and the shuttling time (one-way) was no greater than 1 second. The sensitivity of singlet-triplet conversion due to transmitter offset during SLIC was mitigated by turning off the temperature regulation within the NMR scanner, in order to minimize the change in temperature between the bore and the shuttling region above the magnet. The probe temperature within the bore was measured to be about 22 °C with the temperature regulation off, and the temperature during sample shuttling was not expected to vary more than ±5 °C from the probe temperature.

**Simulation methods**

MD simulations in Amber20 were performed with the following modifications: PP, was parametrized using ESP charges obtained from Gaussian 16 with B3LYP/6-31G(d), the polyphosphate parameters described by Meagher et al (24), with the missing parameters provided by the GAFF2 force field. Minimization was performed in 5000 steps, Timesteps were 1 fs throughout, and the final isothermal/isobaric ensemble (NPT, 300 K, 1 bar) production run contained $10^7$ steps. The simulation was performed at 300 K. 100 snapshots were selected randomly to perform *ab initio* calculations of CSA tensors with the B3LYP/aug-cc-pVTZ combination and the GIAO method. Fig. S5 in the Supplementary Materials shows the individual tensor norms and eigenvalues of the tensor components for all conformers. To calculate the average CSA tensors across all selected conformations, the molecules were aligned along the P-P vector (i.e. along the x coordinate) with the bridging P-O vector pointing upwards in the x-z plane, as shown in Fig. 4B. The CSA tensors were rotated into this frame and averaged. For the $R_1$ calculation, the Frobenius norms were taken of the symmetric and antisymmetric components of the average tensors. For the $R_S$ calculation, the Frobenius norm was calculated for the difference between the average tensors of each $^{31}$P. Tensor visualizations were generated using the Ovaloid function from SpinDynamica v3.6 (25) in Mathematica, as described previously (26, 27), and displaying with the MoleculePlot3D function.

The CSA tensor averaging procedure described above is strictly valid only in the limit where the internal motion is much faster than the overall tumbling rate. We justify its use as follows: from the MD trajectories the root mean square (rms) angular frequency of the -PO₃ rotation around the bridging P-O bond is determined as 1.76 rad/ps. From this value, we can calculate the root-mean square rotation of -PO₃ within the reorientation correlation time period determined above (48.6 ps) as 13.6σ. We therefore can assume that the -PO₃ rotation is much faster than the molecular reorientation, so that averaging the tensors for the two $^{31}$P spins prior to taking the differences between them is the correct approach.
The second-rank correlation time was extracted from the MD runs for the reorientation of the P-P, which was 65.4 ps. The diffusion of the pyrophosphate molecule was calculated from the MD trajectory as $0.215 \times 10^{-9}$ m$^2$/s. The experimental diffusion coefficient determined by pulsed-field gradient NMR was $0.37 \times 10^{-9}$ m$^2$/s (Fig. S6, Supplementary Materials). Given the known relationships between rotational correlation times, diffusion coefficients, and viscosities, we therefore adjusted the correlation time obtained from computation by the factor $0.215/0.37$, which resulted in a correlation time of 48.6 ps. This correlation time was further used in the spin dynamics simulations to obtain the relaxation rates.

References


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**Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. The data can be provided by Alexej Jerschow pending scientific review and a completed material transfer agreement. Requests for the data should be submitted to: alexej.jerschow@nyu.edu.