



^{17}O quadrupolar chemical exchange saturation transfer (Q-CEST) NMR for investigations of molecular dynamics in solids

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ABSTRACT

We introduce quadrupolar chemical exchange saturation transfer (Q-CEST) for half-integer quadrupolar nuclei such as oxygen-17 as a complementary NMR tool for studies of molecular dynamics in solids. Experiments on the model compounds NaNO_3 and hydration water in fibrils formed by pyro-glutamate E3 Amyloid- β protein are combined with simulations and theoretical approaches to obtain parameters of molecular motions. We determine the rate constants for 3-site jumps of oxygen atoms in NaNO_3 and rate constants and populations of tetrahedral jumps of hydration water in the proximity to protein surface below the bulk freezing point. The detection is focused on the central transition (CT). However, during the saturation the transmitter is swept across a wide range of frequencies reaching the first satellite transitions. A detailed analysis is provided for CT Q-CEST profiles in the presence of molecular dynamics covering two dynamical transitions occurring approximately in the microsecond and nanosecond time scale ranges and paying special attention to correct inclusion of the dynamics averaging of the second order quadrupolar interaction.

1. Introduction

The development of NMR methods for ^{17}O nuclei is a topic of active investigations due to importance of oxygen in a variety of materials and biomolecular compounds [1,2]. Dynamics of ^{17}O nuclei can provide important information on molecular flexibility in strategic molecular locations. ^{17}O is half-integer quadrupolar nuclei with a spin of 5/2 and a wide range of quadrupolar coupling constant (C_q) reaching up to tens of MHz. The spin physics is dominated by the quadrupolar interaction, considered in detail in the seminal work by Pound [3]. The former does not affect the central transition (CT), defined as the transition between the $m_z = +1/2$ and $m_z = -1/2$ states; thus, many studies utilize CT detection schemes taking advantage of the relatively narrow line shape [2].

In this work, we introduce the ^{17}O quadrupolar chemical exchange saturation transfer (Q-CEST) method for investigations of dynamics, primarily under static (non-spinning) conditions. CEST is known in solution NMR toolkit as one of the main techniques for elucidation of minor conformational states [4,5] and has also been used in different applications in solid-state NMR, [6,7] including ^2H Q-CEST [8,9]. A

weak RF irradiation along the transverse axis is applied as a function of broad off-resonance conditions spanning the entire CT or even broader regions, including the satellite transitions (ST). The resulting longitudinal magnetization is then transferred to the transverse plane for detection over the CT spectral region. A similar experiment was proposed by Wimperis and co-workers [10] to observe the evolution of coherent interactions under the off-resonance spin-lock for spin 3/2 and 5/2 nuclei.

We present the details of experimental and computational approaches supplemented by theoretical analysis. In the theory presentation, we pay special attention to the inclusion of the correct averaging of the second order quadrupolar interaction in the fast motional limit, which leads to the second dynamical transition in the ^{17}O line shapes [11]. The experimental results and corresponding computational treatment are presented for two cases. The first case is ^{17}O nuclei in NaNO_3 undergoing 3-site jumps (Fig. 1A), which serves as a model compound for testing the validity of the method. The rate constant were previously assessed with line shape analysis in the 413 to 173 K temperature range [11] and ^{17}O laboratory and rotating frame relaxation times measurements (T_1 , T_2 and $T_{1\rho}$) in the 280 to 195 K temperature range [12]. We

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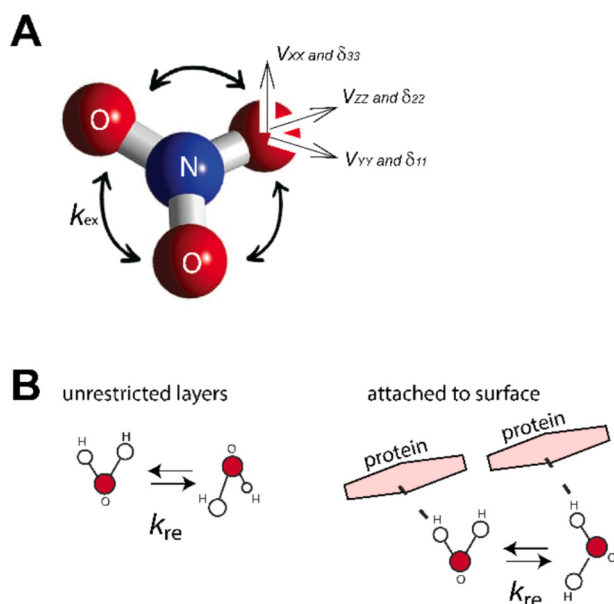


Fig. 1. A) 3-site jump model for NaNO_3 oxygen atoms, with a rate constant of k_{ex} and equal populations. Also shown are quadrupolar and CSA tensor orientations in their corresponding principal axis systems, such that their major axes are aligned along the N—O bond. B) 2-site tetrahedral reorientations models of water in hydration layers of pyro-E3 A β fibrils with the rate constant k_{re} between, in general, non-equal population states. Left: unrestricted 2-site jumps for layers not in direct contact with the protein surface; right: tethered jumps for the water directly attached to the protein surface by hydrogen bonding.

compare the results of the new ^{17}O Q-CEST measurements to show that the rate constants fall within the trends obtained from other types of experiments. The second case is hydration water dynamics in the hydration shell of amyloid- β fibrils with the pathological pyroglutamate-3 post-translational modification (pyro-E3 A β), implicated in Alzheimer's disease [13]. We previously assessed hydration shell dynamics of these fibrils at low temperatures using ^{17}O T_1 and $T_{1\rho}$ measurements [14]. In this work we complement the results with those obtained from the new ^{17}O CT Q-CEST measurements and compare the resulting rate constants of tetrahedral reorientations of water molecules in the proximity of protein surface at low temperatures. The hydration shell dynamics below the bulk freezing point is most sensitive to the tetrahedral reorientations of water molecules (Fig. 1B).

2. Experimental

2.1. Materials

The NaNO_3 sample in the crystalline phase was generously provided by Prof. Gang Wu of Queen's University and prepared as described in reference [11]. Additionally, the degree of ^{17}O isotopic incorporation measured by Beewerth et al. using the same preparation protocol was assessed to be 4.5% [15], thus rendering intra-molecular ^{17}O – ^{17}O coupling a low occurrence. Homonuclear dipolar couplings between ^{17}O nuclei are in general small, on the order of 60 Hz for a pair of intra-molecular oxygens in NaNO_3 .

The pyro-E3 A β peptide was synthesized commercially using the solid-state peptide synthesis, and purified to at least 95%, performed by using Peptide 2.0. The primary sequence is: [E] FRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV, where [E] stand for pyroglutamate. The preparation of the fibrils followed the previously developed protocols [16–18] and the TEM image is shown in Supplementary Material Fig. S1. 27 mg of the lyophilized protein powder was rehydrated with H_2^{17}O with 70% isotopic incorporation, introduced by pipetting the required amount of water, and mixing inside the NMR

container. The samples were equilibrated for at least 36 h after mixing at room temperature. Under these conditions no ^{17}O isotope exchange is expected to occur between hydration water and protein sites. The NMR containers were custom-made 5 mm outer diameter tubes with the ability to seal liquid using caps with O-rings. The water content was 1 μl of water per 1 mg of protein for the fibrils.

2.2. ^{17}O Q-CEST NMR

The measurements were performed using the 18.8 T spectrometer at the National High Magnetic Field Laboratory, equipped with the static low-E probe of 5 mm coil diameter [19]. Cooling was achieved with liquid nitrogen, and the temperature was calibrated using lead nitrate [20]. The number of scans in the Q-CEST measurements was 8 for NaNO_3 and either 8 or 16 for the amyloid fibrils, and 8 dummy scans were used. The inter-scan delay was 1.5–3.5 s, except for cases explicitly noted in the *Results and Discussion* section. The detection block in the Q-CEST measurements consisted of Hahn echo [21] for NaNO_3 and for the fibrils below 220 K. A detection pulse of 30° and a refocusing pulse of 60° were used. For the fibrils in 245 to 220 K temperature range, the TRIP block [22] was used, which involves the series of three 30° pulses phase-cycled to retain the signal from water with C_q values far from zero. For the case of fibrils at 278 K (above the bulk freezing point), the detection element consisted of a single 90° pulse. The strength of the ^{17}O detection pulses was 30–35 kHz. SPINAL64 proton decoupling [23] during the acquisition period was applied with 70 kHz RF strength.

2.3. ^{17}O Q-CEST simulations

For Q-CEST simulations, the initial equilibrium state of longitudinal magnetization was set up in the base of 36 spin 5/2 coherences. They are listed explicitly in Eq. S1 in reference [12]. In the detection period involving generation of transverse coherence, the effect of finite excitation pulse was included explicitly to match those used in the experiment. The echo refocusing pulse was taken as ideal. For the theoretical simulations (Figs. 2–4), an ideal 30° pulse acting on the CT was assumed. The acquisition FID block included a single component of the I_- operator corresponding to the central transition, with the details of the approach specified in reference [12].

Below we describe the details of the simulations during the saturation CEST period, performed in the doubly rotating frame and utilizing the numerical solution to Eq. (3). For each crystalline orientation, evolution during the mixing period is calculated by direct matrix exponentiation in MATLAB. The effect of Q2 averaging (i.e., the second order quadrupolar terms as defined in the *Theory* section) was not included when fitting experimental data, as it is not expected to play a role based on the expected time scales of motions and the Larmor frequency, as discussed in the *Theory* section.

The dynamics is simulated through a matrix of Markovian jumps between different orientations in the molecular frame. In the case of NaNO_3 , these correspond to three equally populated states related by 120° rotations around the z axis, in the principal axis system (PAS) of the electric field gradient (EFG) tensor, with the concomitant transformation of the CSA tensor. In the case of water at sub-freezing temperatures, 2-site jumps with the tetrahedral geometry were employed. The two orientations have w to $1-w$ occupation number (weights) ratios and the orientations are given by Euler angles in the PAS of the EFG tensor: $(0, 0, 0)$ and $(\varphi, 120^\circ, 180^\circ - \varphi)$ where $\varphi = \arccos\sqrt{2/3} \approx 35.3^\circ$. The dynamics affects the CSA and dipole-dipole OH terms in the same fashion. The exchange rate k_{re} is defined as a sum of the forward and backward jump rates $k_{\text{re}} = k_{\text{re},1} + k_{\text{re},-1}$, where the forward and backward rates are related by relative weights of the two states, $k_{\text{re},1} = (1 - w)k_{\text{re},-1}$. As described in prior work [24], modeling diffusion for the case of a highly anisotropic ^{17}O EFG tensor requires a grid with precise coverage of rotations along all three axes. We used a scheme

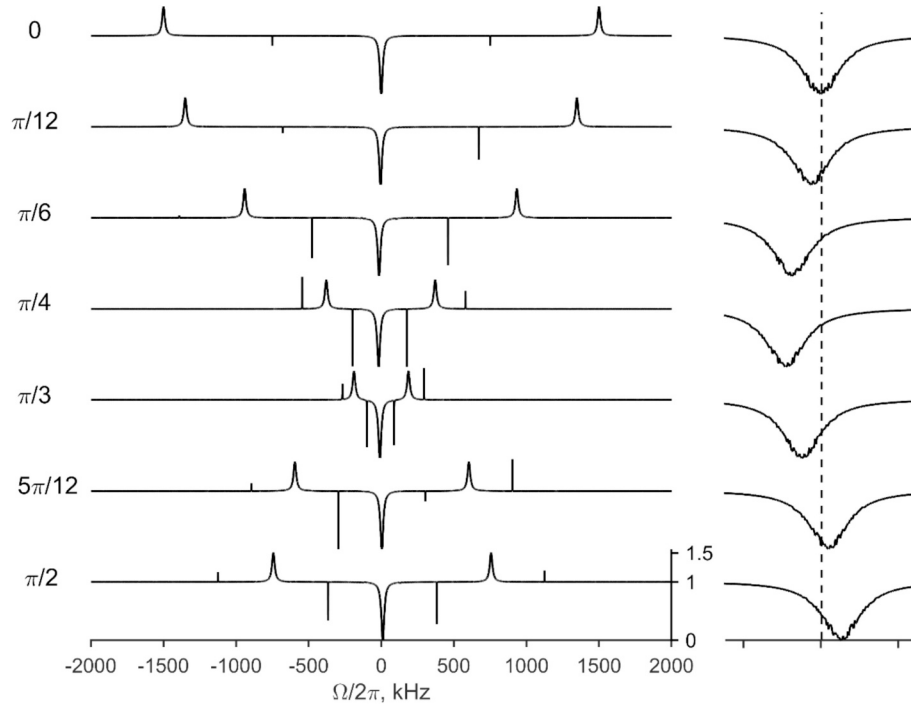


Fig. 2. ^{17}O CT Q-CEST profiles in the absence of motion for different single crystallite orientations relative to the static magnetic field, with the angles indicated to the left of each profile. The EFG tensor parameters are $C_q = 10$ MHz, $\eta = 0$. Other relevant parameters are: $\omega_{RF}/2\pi = 4$ kHz, saturation time 10 ms, and $\omega_0 = 108.4$ MHz (18.8 T magnetic field strength). The range of offsets spanned CT + ST1 regions, and the profiles were normalized to the intensities at the ± 2000 kHz offsets, at which there is no saturation of CT and ST1 magnetization. The vertical scales are between 0 and 1.5 in relative intensities for all panels, as shown explicitly on the lowest panel. The insert to the right shows the expanded region of offsets in the CT range to demonstrate the shift in the resonance position due to the differences in Q2 contributions. The CT resonance position in the absence of the Q2 term is indicated by the dashed vertical line at zero frequency. RF inhomogeneity was taken into account as described in the *Experimental* section. The theoretical expressions of the intensities and widths of the lines are given in SI1.

based on 60 orientations derived from the 600-cell in a space of unit quaternions [25].

In the case of CEST applied to water molecules, the effect of the heteronuclear dipolar interaction on ^{17}O coherences with the two protons, denoted by S_1 and S_2 , was included as an extra terms in the Hamiltonian, $H_{DD} = d_{OH,1}I_zS_{z,1} + d_{OH,2}I_zS_{z,2}$, where $d_{OH,1}$ and $d_{OH,2}$ are the spatial terms [26]. $S_{z,1}$ and $S_{z,2}$ are fixed at the values $\pm 1/2$. This approach neglects separate dynamics contribution to protons; thus, the modification of dynamics due to the dipolar interaction only enters through its effect on ^{17}O nuclei. The homonuclear proton-proton interaction was not included.

Coherent oscillations of longitudinal magnetization, at the frequency $3\omega_{RF}$, are quenched in the experiment due to RF inhomogeneity. To account for this effect in the simulations, we introduced averaging over the period of these oscillations by using a 10-point grid of CEST saturation times, centered at the experimental values, 3 ms saturation time and 2 kHz RF field for NaNO_3 and 0.5–3 ms saturation time and 3.5 kHz RF field for water in the fibrils. For the theoretical calculations (Figs. 2–4), the averaging was performed around 10 ms saturation times. This approach is an alternative to explicit inclusion of RF inhomogeneity employed in the simulations of ^2H CEST profiles [8,9].

For NaNO_3 , we used the following quadrupolar and CSA tensor parameters, reported in Hung et al. [11]: $C_q = 12.5$ MHz, $\eta = 0.8$ for the EFG tensor with major axis perpendicular to NO_3^- plane, and $[\delta_{11}, \delta_{22}, \delta_{33}] = [250, 400, 550]$ ppm for the CSA tensor, with relative orientation $[0, 90^\circ, 90^\circ]$ of the two PAS frames.

For water, the quadrupolar coupling constant of $C_q = 7.5$ MHz and the asymmetry parameter of 0.92 was used. The PAS of the EFG tensor has the z -axis perpendicular to the HOH plane, the y -axis along the H–H direction, and the x -axis as the HOH bisector. The ^{17}O CSA tensor parameters correspond to the anisotropy of 25 ppm and the asymmetry parameter of 0.25 in Haeblerlen convention, [27] with the Euler's angles

between the principal axis of the EFG and CSA tensors given by $[0^\circ, 0^\circ, 90^\circ]$. The heteronuclear OH dipolar interactions strength was taken as 17 kHz [24,28]. The OH bond orientations, which determine the spatial part of the heteronuclear dipolar interactions, are given by $(\sin \frac{\alpha}{2}, \pm \cos \frac{\alpha}{2}, 0)$ in the PAS system of the EFG tensor, where the HOH angle $\alpha = 104.5^\circ$ [24,28].

Powder averaging over crystalline orientations were done as a sum over 420 tiles arranged to maintain H_4 symmetry (the symmetry of four dimensional 600-cell) of three-dimensional rotations parametrized through unit quaternions [25,29]. The high degree of symmetry ensures correct averaging for the highly anisotropic quadrupolar and CSA tensors, while allowing for the use of a relatively small number of tiles. Because of inherent symmetry of the isotropic diffusion mode, there was no need for tiling in that case.

3. Results and discussion

3.1. Theory

In the presence of a transverse field, the secular part of the Hamiltonian in the doubly rotating frame is given by

$$H = -\Omega I_z + H_Q + \omega_{RF} I_x \quad (1)$$

where Ω is the offset frequency of the transverse field, H_Q is the secular part of the quadrupolar Hamiltonian, and ω_{RF} is the strength of the transverse field. The quadrupolar Hamiltonian includes first and second order terms, $H_Q = H_{Q1} + H_{Q2}$, with the second order quadrupolar term stemming from Magnus expansion in average Hamiltonian theory, [30] in which we are following the notation in reference [31]. The averaging is over the period defined by the inverse Larmor frequency. The first order Hamiltonian is given by

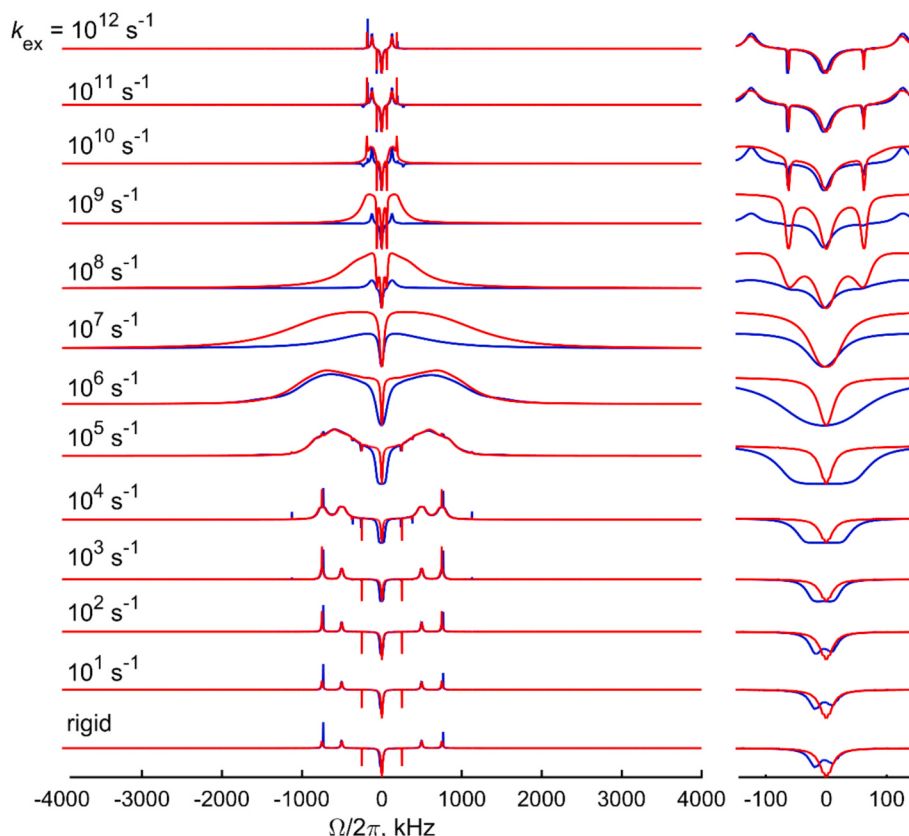


Fig. 3. ^{17}O CT Q-CEST profiles for the dynamical exchange model for a single crystal with two sites at the angles 90° and 41° with respect to magnetic field. The EFG tensor parameters are $C_q = 10$ MHz and $\eta = 0$, and 90° and 41° angles represent the orientations for which Q2 contribution has extreme values. Other relevant parameters are: $\omega_{RF} = 4$ kHz, saturation time 10 ms, and $\omega_0 = 108.4$ MHz (18.8 T magnetic field strength). The range of offsets spanned CT + ST1 regions. Vertical scales are adjusted for better visual comparison, with normalization on the intensities at ± 4000 kHz offsets, at which there is no saturation. Calculations were performed for two cases: without the inclusion of the Q2 interaction (red lines) and with the Q2 interaction included in the rigid limit (blue lines). The insert shows the expanded region of offsets in the CT range to demonstrate the shift in the resonance position due to differences in the Q2 contributions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$H_{Q1} = \frac{\omega_Q}{3} (3I_z^2 - I(I+1)),$$

where $\frac{\omega_Q}{2\pi} = \frac{3C_q}{4I(2I-1)} \left(\frac{3\cos^2\theta-1}{2} + \frac{\eta}{2}\sin^2\theta\cos 2\varphi \right)$ is the orientation-dependent quadrupolar frequency. C_q and η are quadrupolar coupling constant and asymmetry parameter, respectively, and θ and φ are azimuthal and polar angles of transformation from the principal axis system of the quadrupolar interaction to the laboratory frame. The first and second order quadrupolar terms are abbreviated as Q1 and Q2, respectively. Q2 consists of the contributions stemming from the terms fluctuating with ω_0 and $2\omega_0$ frequencies in the rotating frame, respectively

$$H_{Q21} = \frac{1}{\omega_0} \left(\frac{2\pi C_q}{I(2I-1)} \right)^2 16I_z \left(I_z^2 - \frac{I(I+1)}{2} + \frac{1}{8} \right) v_1 v_{-1} \quad (2)$$

and

$$H_{Q22} = \frac{1}{\omega_0} \left(\frac{2\pi C_q}{I(2I-1)} \right)^2 4I_z \left(I_z^2 - \frac{I(I+1)}{2} + \frac{1}{2} \right) v_2 v_{-2},$$

where $v_{\pm 1}$ and $v_{\pm 2}$ are the quadrupolar tensor components corresponding to fluctuating parts of the quadrupolar interaction, in which the dependence on C_q is factored out.

The density matrix for spin $I = 5/2$ consists of $(2I+1)^2 = 36$ basis matrices. Under the Hamiltonian that includes operators I_z , I_x , and quadrupolar interaction (Eq. (1)), virtually all coherences interconvert, making the superoperator approach more practical for calculations. The

initial conditions for the Q-CEST experiment consist of pure longitudinal magnetization $\rho(0) = I_z$. We limit ourselves to the discussion of the detection of the CT transition only.

In the absence of motions, examples of predicted CEST profiles for several single crystallite orientations are shown in Fig. 2. The resonance nature of the simulated profiles is obvious. These resonances stem from the effective coherence transfer between the components of longitudinal magnetization when frequencies of fluctuations for states with different I_z quantum numbers approach each other. To understand these conditions qualitatively, we can first neglect the Q2 and the RF terms contributions to the Hamiltonian. Then, in the basis of eigenstates of the operator I_z , $I_z|m\rangle = m|m\rangle$, the simplified Hamiltonian is diagonal, with the diagonal matrix elements given by $E_m/\hbar = -\Omega m + \omega_Q(m^2 - I(I+1)/3)$. Two levels with the quantum numbers m_1 and m_2 intersect when $-\Omega m_1 + \omega_Q m_1^2 = -\Omega m_2 + \omega_Q m_2^2$, which is equivalent to the condition $\Omega = \omega_Q(m_1 + m_2)$. To make a resonance observable during the CT detection, at least one of the numbers $m_{1,2}$ must be $\pm 1/2$. The single quantum transition due to the RF field imposes the requirement of $|m_1 - m_2| = 1$. Thus, one can expect three resonances contributing to the observable CT signal, $m_{1,2} = \pm 1/2$ and $m_1 = \pm 1/2$ and $m_2 = \pm 3/2$ at offset frequencies $\Omega = 0$ and $\Omega = \pm 2\omega_Q$, respectively. The latter two resonances are observable in ST1 region of the offset frequencies. The ST1 transitions are defined as transitions between $m = +1/2$ and $m = +3/2$ or $m = -1/2$ and $m = -3/2$ states and the Q-CEST experiment makes them observable for the resonance values of $\Omega = \pm 2\omega_Q$. All three resonances are about $3\omega_{RF}$ wide, the first one leads to decreased intensity of the transverse signal and the second and third lead to

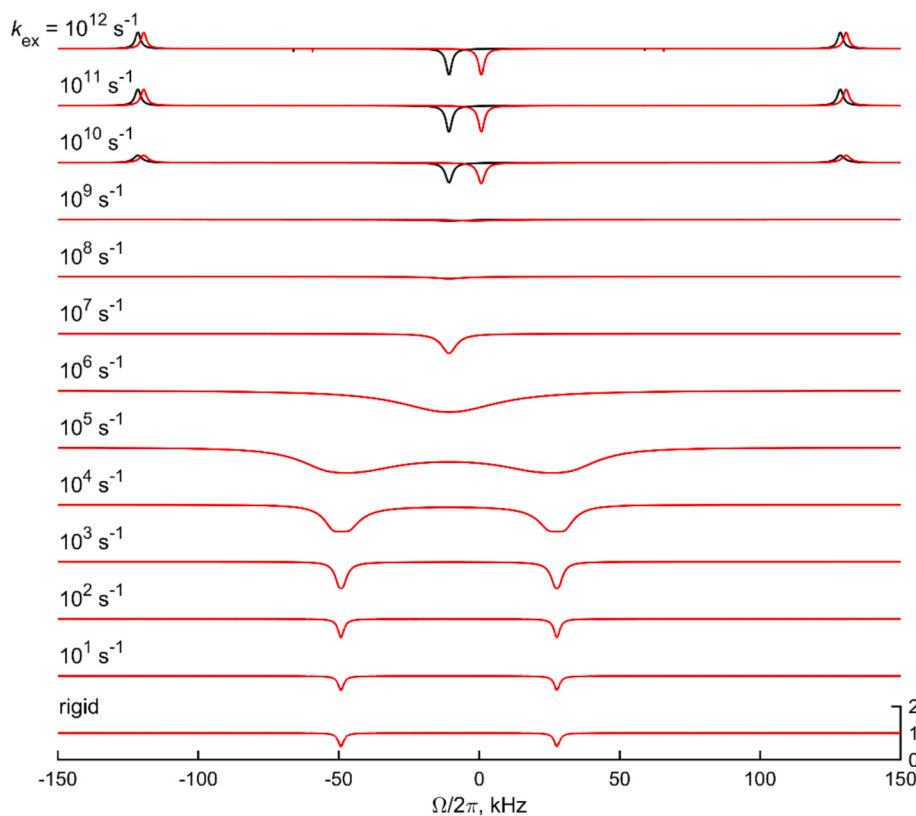


Fig. 4. ^{17}O CT Q-CEST profiles for the dynamical exchange model for a single crystal with two sites at the angles of 90° and 41° with respect to magnetic field. The EFG tensor parameters are $C_q = 10$ MHz and $\eta = 0$. Other relevant parameters are: $\omega_{RF} = 0.4$ kHz, saturation time 10 ms, $\omega_0 = 40.4$ MHz (7 T magnetic field strength). The range of offsets spans CT region only. Intensities are normalized to the intensities at the ± 150 kHz offsets. Vertical scale is between 0 and 2 in relative intensities for all panels, as shown for the lowest panel. Calculations were performed for two cases: with the Q2 interaction included in the rigid limit (black lines) and Q2 interactions included according to Lorentzian mixture model (red lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

increased intensity. There are also narrow, on the order of ω_{RF}^2/ω_Q , multiple-quantum resonances visible in Fig. 2. Supplementary Material S11 presents details of the resonance positions, linewidths, and shapes.

When Q2 is taken into account, the effective positions of resonances shift, as can be seen in the right panel of Fig. 2. For a polycrystalline sample, the Q-CEST signal would trace the CT line shape, which follows the distribution of Q2 for different powder orientations, broadened by a Lorentzian function with width of $3\omega_{RF}$. For resonances involving satellite transitions, the resonance position, occurring at $\Omega = \pm 2\omega_Q$, depends on the crystallite orientation primarily through orientation dependance of ω_Q , with Q2 playing only a minor role.

Motions are included in the calculations through discrete exchanging sites which have different orientations in the molecular frame. This is accomplished by expanding the density matrix, represented in the superoperator approach as vector of coherences, ρ_a , to include a coherence state for each of the sites, ρ_{aj} , where the first index refers to the coherence state and the second one refers to the site number. Each ρ_{aj} is acted upon by the superoperator corresponding to the Hamiltonian of Eq. (1) with quadrupolar interaction parameters (such as ω_Q and Q2) dependent on the orientation of each site in the laboratory frame. In addition to the coherent terms, one has to include relaxation due to the terms of the quadrupolar interaction fluctuating with frequencies ω_0 and $2\omega_0$ in the rotating frame, detailed in reference [12]. Eq. (1) shows only the secular part of the Hamiltonian and does not include them. They can be calculated in the Redfield approximation and included in the Liouville superoperator, L_{ab}^j . The exchange between the sites is given by a matrix of Markovian jumps, K_{jk} . The Liouville – von Neumann equation governing the evolution of the coherence states in all sites is thus given

by

$$\frac{d\rho_{aj}}{dt} = i \sum_b L_{ab}^j \rho_{bj} + \sum_k K_{jk} \rho_{ak} \quad (3)$$

Fig. 3 illustrates effects of motions on the Q-CEST profiles, demonstrated for a single crystallite aligned with the magnetic field and two exchanging sites with the symmetric quadrupolar tensor oriented at 90° and 41° in the molecular frame. Calculations were performed for two cases: with and without the inclusion of Q2 interactions, shown in blue and red lines, respectively. In this case, Q2 was calculated for each of the sites independently of each other, an approximation that will be addressed later.

Two transitions are evident as the exchange rate increases. First, approximately from $k_{ex} \sim 10^4 \text{ s}^{-1}$ to $k_{ex} \sim 10^6 \text{ s}^{-1}$, centered around the “slow” motional transition occurring at $\frac{1}{\omega_0} \left(\frac{2\pi C_q}{I(2I-1)} \right)^2$ as elaborated in the reference [12]. Comparing the two types of calculations, one can see that the Q2 interaction plays a major role in the $\Omega \sim 0$ region, while the Q1 interaction is responsible for the profile broadening in the ST regions. When the exchange rate is on the order of the Larmor frequency, i.e., $k_{ex} \sim \omega_0$, there is a second transition associated with effective averaging of Q1, while at the same time Q2 is responsible for broadening and smoothing of the Q-CEST profile in the ST regions during the transition. For $k_{ex} \gg \omega_0$, the Q-CEST profile returns to its rigid form, but with the components of the quadrupolar tensor averaged over both orientations.

The latter effect is the result of the approximation made in the calculations of the Q2 term and requires special attention. In average Hamiltonian theory, the Q2 term, in the rotating frame with respect to

ω_0 , is derived as the secular part of the fluctuating components of the quadrupolar tensor averaged over $2\pi/\omega_0$ time period. The values of the quadrupolar tensor components are assumed to be constant apart from the regular oscillations with ω_0 and $2\omega_0$ frequencies.

In the presence of motions, this assumption is valid insofar the rate of motions is much smaller than ω_0 . In the opposite limit, the components of the quadrupolar tensors for different sites are effectively averaged by motions and it is the average values that must be used in calculating the Q2 contribution. As evident from Eq. (2), the contribution of the quadrupolar tensor parameters, $\nu_{\pm 1}$ and $\nu_{\pm 2}$, into $H_{Q,21}$ and $H_{Q,22}$ are nonlinear and, therefore, averaging over motions and over the time period of the inverse Larmor frequency are not interchangeable. Thus, there is a transition in Q2 terms associated with the relative order of taking the averages. This transition was demonstrated by Hung et al. for the case of CT half-integer quadrupolar nuclei line shapes [11].

For the calculations of the line shapes in different limits of k_{ex} with respect to ω_0 it is possible to work entirely in the laboratory frame. Then, in the absence of an RF field, the Hamiltonian in the laboratory frame is time independent and there is no need to use the average Hamiltonian theory. This is not possible for Q-CEST (or rotating frame relaxation), where the RF term fluctuates with the sum of Larmor and RF offset frequencies, $H_{RF}^{ab} = \omega_{RF}(I_x \cos(\omega_0 + \Omega)t + iI_y \sin(\omega_0 + \Omega)t)$, which makes numerical solution of the Liouville – von Neumann equations very difficult, because it requires using time intervals much smaller than $1/\omega_0$ in matrix exponentiations. To account for this effect in the Q-CEST experiment, we suggest the phenomenological approach, in which the two limits of rigid Q2 (corresponding to Q2 calculations for individual sites) and fast-limit Q2 are combined using Lorentzian weighing factors. In the simplest ultra-fast case, the fast limit consists of the complete averaging of the tensor components before averaging the Hamiltonian over the of $2\pi/\omega_0$ period and is thus independent of k_{ex} . The Lorentzian weighing factors ensure the convergence between the two limits:

$$f_1\left(\frac{k_{ex}}{\omega_0}\right) = \frac{\omega_0^2}{k_{ex}^2 + \omega_0^2} \text{ and } f_2\left(\frac{k_{ex}}{\omega_0}\right) = \frac{4\omega_0^2}{k_{ex}^2 + 4\omega_0^2} \text{ for } H_{Q,21} \text{ and } H_{Q,22} \text{ respectively.}$$

The applicability of this approach is tested by comparison of the exact line shape calculations in the laboratory frame and the phenomenological calculations in the rotating frame (Figs. S2 and S3), which includes both CT and the full CT + ST spectral regions.

Inclusion of the full Q2 contribution becomes important when it is comparable or greater than the width of the Q-CEST resonance: $\frac{3}{\omega_0} \left(\frac{2\pi C_q}{I(2I-1)} \right)^2 \gtrsim 3\omega_{RF}$. An example of Q-CEST profiles for which this effect is significant is shown in Fig. 4 for a single crystallite orientation: when Q2 is included in the phenomenological manner described above, for $k_{ex} > 10^9 \text{ s}^{-1}$ one observes a distinct shift in the resonance positions of the CT and ST signals (red lines) in comparison to the calculations in the rigid Q2 limit (black lines). Thus, the shift of the resonance profile is visible on the high k_{ex} end of the second dynamical transition outlined above.

The ^{17}O Q-CEST method in combination with relaxation approaches enhances the observation window of motional time scales and can remove ambiguities in k_{ex} values when the motional regime cannot be determined from a single type of relaxation measurement. Recently, Dai et al. [32] presented a theoretical description and experimental measurements of transverse relaxation rates of the satellite transitions, which provide an alternative approach for the extension of the range of the time scale sensitivity.

3.2. Experimental and computational considerations for Q-CEST measurements

The pulse sequence (Fig. 5) starts with application of weak RF irradiation to the equilibrium magnetization. The weak RF field is one of the main practical advantages of the CEST experiment due to minimization of RF-induced heating in comparison to other measurements such as

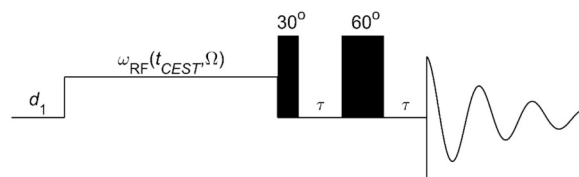


Fig. 5. Pulse sequence for ^{17}O CT Q-CEST measurements. After the interscan delay d_1 , a weak RF field with the strength of ω_{RF} and duration of t_{CEST} is applied along a transverse axis as function of resonance offset Ω , spanning either the CT region or including both the CT and ST1 regions. This is followed by the detection period focused on the CT spectral region, shown as a modified Hahn echo sequence: for the ^{17}O nuclei in the powder sample, the optimal excitation pulse is 30° and refocusing pulse is 60° . Other detection schemes are discussed in the text. Proton decoupling during the acquisition period is an option for molecules with directly attached protons.

rotating frame relaxation.

During the CEST mixing period, the effect of dynamics is seen by mixing of the quadrupolar frequencies associated with different spatial orientations. Since the detection is focused on the CT, for powder samples the condition $\omega_{RF} < \frac{1}{\omega_0} \left(\frac{2\pi C_q}{I(2I-1)} \right)^2$ ensures sensitivity of the measurements to dynamics. The CT linewidth is given approximately by $\frac{3}{\omega_0} \left(\frac{2\pi C_q}{I(2I-1)} \right)^2$. Too low values of RF, while still sampling the dynamics processes, may require large mixing time for sufficient evolution to occur. During large mixing times, the relevant coherences can be quenched due to longitudinal relaxation, implying $\omega_{RF} \gg t_{CEST}$. The evolution time itself is thus also limited by T_1 values, $t_{CEST} < T_1$, as well as probe and sample RF heating limits, which renders times greater than 20–30 ms impractical.

The range of offsets for the RF field may include not only the range of the CT region, but also ST1. As explained in *Theory* section, there are Q-CEST resonances in the range ST1, which are affected by dynamics. This is true even if the detection is restricted to CT region only. To sample the ST1 region, the magnitude of offsets has to reach the order of $\pm C_q/10$, in which the factor of 10 stems from $(I(2I-1))$.

The detection scheme is focused on the narrow CT transition. The experimental details of the detection are governed by sample properties and instrumentation considerations, such as the receiver's dead time. In cases in which the dynamics is close to isotropic reorientations, a simple 90° pulse can be sufficient. In powder samples with anisotropic motions, the Hahn echo sequence (a 30° excitation pulse followed by a 60° refocusing pulses) can provide optimal non-distorted signal retention. Another sequence that suppresses probe ringing is the triple-pulse excitation (TRIP), [22] which has an additional advantage to act as the selection filter in samples with a distribution of mobile and rigid fractions based on the differences in their respective nutation frequencies [33].

The resulting CEST profiles are presented as intensity versus the offset. Intensity can be chosen as integration over the entire CT powder pattern or its selected regions. An important practical consideration is the calibration of the value of the weak RF field, which is difficult to calibrate on a powder sample. Tap water is one standard with a narrow line that can be used for this purpose.

For systems with directly attached protons such as H_2O , dipolar interactions with protons are significant. Proton decoupling can be employed during the detection period to narrow the CT powder pattern. It is also possible to employ proton decoupling during the CEST period itself. However, this leads to the Hartman-Hahn matching [34] transfer and can complicate the CEST pattern due to coherent and incoherent (dynamics) effects from the Hartman-Hahn matching [35].

In order to simulate the CEST measurements, the following factors need to be considered: i) the parameters of the tensors, such as EFG, CSA, and dipolar interactions, as well as their relative orientations must

be known; ii) depending on the expected time scales of motions, one has to decide whether to implement the phenomenological mixture model for Q2 contribution, as discussed in the *Theory* section; iii) if imperfections in the CT detection block are expected, for example due to finite RF field strength in the detection block pulses, these can be explicitly included in the simulations. Specific detection schemes such as the Hahn echo and TRIP sequences can be also modeled explicitly; iv) effects of RF inhomogeneity can be included either by explicit inclusion of the probe RF inhomogeneity profile into the simulations or, alternatively, they can be approximated by averaging of fast coherent oscillations over the period of $2\pi/\omega_{RF}$; v) the range of offsets and the sampling grid of offsets should be carefully matched to experiment; vi) dipolar interactions with external spins (protons) can be included with various extents of precision. As a first approximation, the dynamics of the proton's spin degree of freedom can be neglected, as well as the effects of dipolar network. Homonuclear ^{17}O dipolar coupling is often neglected due to much lower magnitude and at least partial dilution of the ^{17}O isotopic enrichment. When needed, these factors can be accounted for at the expense of a larger basis of matrices; viii) for the cases of highly anisotropic quadrupolar tensors, the tiling algorithm should include averaging over all three Euler angles consistent with equal weights of all crystallite orientations.

3.3. NaNO_3 dynamics

For NaNO_3 , Q-CEST measurements were performed in the 250 to 190 K temperature range, constrained by the magnitude of T_1 values, which range from 2 ms to 2.5 s at 18.8 T. The CT region spans around 28 kHz. The RF field strength during the CEST period was 2 kHz, calibrated from nutation measurements on tap water, and t_{CEST} was 3 ms, taken as a compromise for the broad range of T_1 values. The detection scheme employed was a Hahn echo with details listed in the *Experimental* section. The offset range was from -2000 to 2000 kHz, to cover offsets in

the vicinity of ST1 transitions. The experimental profiles (Fig. 6 and Fig. S4) were obtained via the integration of the CT powder pattern region.

To find the value of the exchange rate constant k_{ex} for the 3-site model (Fig. 1A), simulations were conducted using parameters matching the experimental conditions. The CSA and EFG tensor parameters and their relative orientations were taken from prior studies [11], as provided in the *Experimental* section.

The results of the best fit to k_{ex} rate constant align well with those from prior ^{17}O T_1 and $T_{1\rho}$ measurements, [12] validating the technique (Fig. 7). The errors in the fitted rate constants were estimated from RMSD analysis focused on the central region, which is most sensitive to the value of k_{ex} (Fig. S5). For the highest temperature there are some discrepancies in the shapes of the experimental and fitted profile in the ST1 region.

As discussed above, for more complicated systems it might be impractical to scan such a wide range of offsets, and it is beneficial to focus on the range of offsets within the CT region. To assess how this limitation may affect resulting fitted values of the rate constants, we constrain experimental CEST data sets for NaNO_3 to the -200 to 200 kHz offset region, and perform normalization using $\pm(150$ to 200 kHz) offset regions. We then overlay the experimental results with simulations performed with k_{ex} values obtained from fits over the full offset ranges but renormalized accordingly. A reasonable agreement between experiment and simulation is observed, implying that constraining the measurement to the range of offsets that excludes the ST1 regions may be sufficient for more challenging systems. The minimum of the CEST profile curve does not correspond to zero offset because of the intrinsic asymmetry of the Q2 contribution. There is an agreement between simulated and experimental data in this respect.

When fitting rate constants at individual temperatures from the Q-CEST profiles in the narrow offset range, there might be ambiguity on which branch of the first motional transition with respect to Q2 (defined

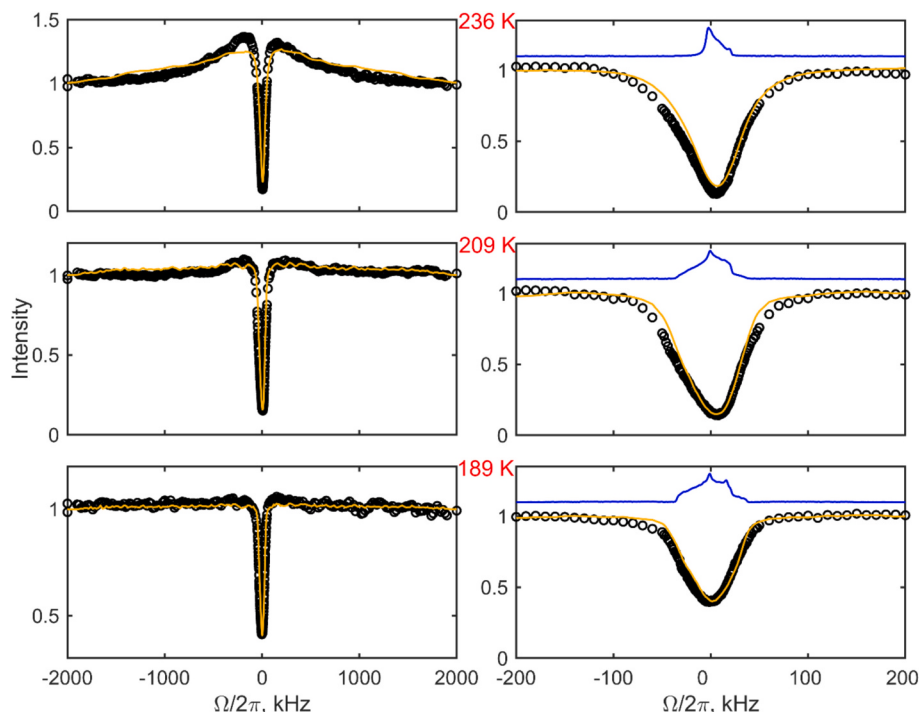


Fig. 6. Examples of ^{17}O CT CEST profiles for NaNO_3 at different temperatures, collected under static conditions at 18.8 T employing $t_{CEST} = 3$ ms and $\omega_{RF}/2\pi = 2$ kHz. A) Profiles spanning offsets from -2 MHz to 2 MHz which include ST1 regions, with normalization performed to the intensities at ± 2 MHz. The light brown solid-lines indicate the best-fit according to the 3-site jump model. B) Profiles constrained to -200 to 200 kHz regions, with normalization performed using intensities in the $\pm(150$ to 200 kHz) ranges of offsets. The solid light brown lines indicate the simulations with the values of k_{ex} which were determined from the fits for the full ranges of offsets. The upper traces (blue lines) show the ^{17}O CT static line shapes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

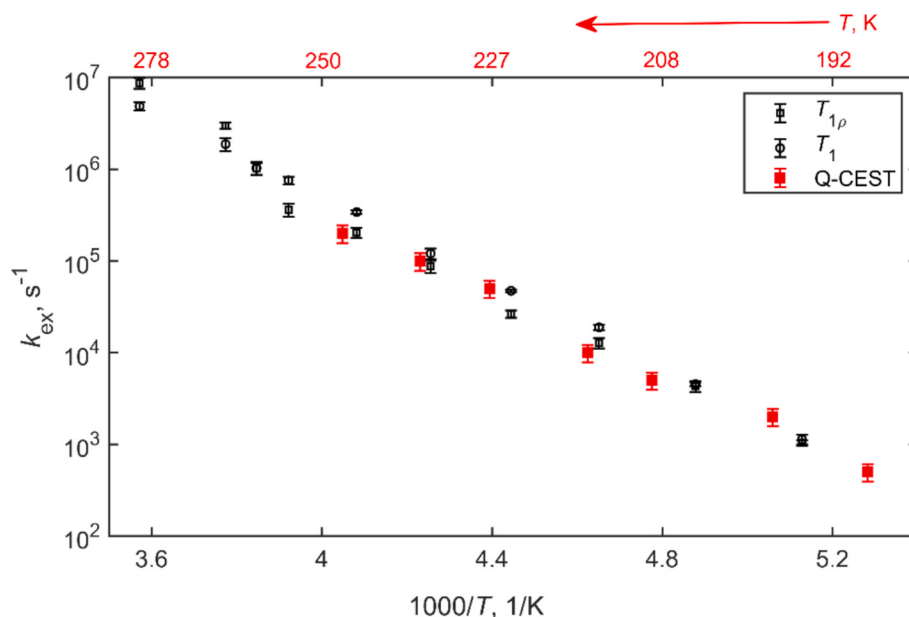


Fig. 7. Rate constants k_{ex} for 3-site jumps of the oxygens atoms in NaNO_3 versus $1000/T$, obtained either from the ^{17}O CT Q-CEST measurements (red) or from prior studies [12] based on ^{17}O T_1 and $T_{1\rho}$ measurements (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

as “low” transition in reference [12]) to choose: fast $k_{ex} > \frac{1}{\omega_0} \left(\frac{2\pi C_Q}{I(2I-1)} \right)^2$

or slow $k_{ex} < \frac{1}{\omega_0} \left(\frac{2\pi C_Q}{I(2I-1)} \right)^2$. The temperature dependence of the data

constrains the fits, by imposing the condition that the rate constant should decrease with temperature (Fig. S6). The wide offset range profiles have additional distinction in the ST regions, which allows for additional constraints when choosing fast versus slow branches of the regimes.

Extension of the Q-CEST experiment is possible for magic-angle spinning (MAS) conditions, if care is taken to consider rotational resonances, [8] expected around the condition $\Omega = n\omega_{MAS}$, in which ω_{MAS} is the spinning rate and n is an integer. Examples of experimental profiles at 10 and 25 kHz MAS rate are shown in Fig. S7.

3.4. Dynamics of the hydration shells in PyroE3-A β fibrils

PyroE3-A β fibrils are insoluble aggregates with the stacked β -sheets secondary structure, spanned primarily by the C-terminal domain of the A β peptide and possessing a relatively flexible solvent accessible N-terminal region [36–38]. The pyro-glutamate post-translational modification reduces the charge of the N-terminal domain and adds to the overall hydrophobicity of the peptide. Transmission electron microscopy images display relatively short straight fibrils (Fig. S1), consistent with prior studies [36,39]. Lyophilized fibrils, prepared as described in the *Experimental* section, were hydrated with 70% ^{17}O isotopically enriched water. The hydration level was 1 μl of water per 1 mg of peptide, except for the hydration dependence study elaborated below.

To assess EFG, CSA, and OH dipolar tensor properties, the spectrum at 180 K (Fig. 8) with and without proton decoupling was compared to the one previously obtained for the globular HP36 protein [24]. The spectra largely coincide, indicating that we can use the same tensor parameters, which are also similar to those used for water in barium chlorate hydrate [28,40] and hexagonal phase of ice [41]. The parameters are listed in the *Experimental* section.

Above the bulk freezing point (Fig. 8A), the spectrum is motionally narrowed, indicating significant large-amplitude motions, as was also found for the globular HP36 protein [24] and other proteins [42]

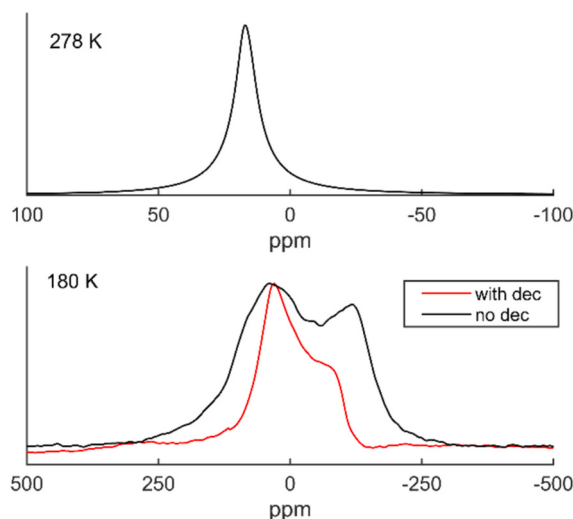


Fig. 8. ^{17}O CT solid-state NMR spectra of hydration water in pyro-E3 A β fibrils at the hydration level of 100% w/w: 27 mg of fibrils were combined with 27 μl of 70% isotopically enriched H_2^{17}O . The spectra were collected under static conditions at 18.8 T and at 278 K (top panel), and 180 K (bottom panel) in the presence and absence of proton decoupling during the acquisition. The chemical shift referencing was performed taking the chemical shift of tap water at 280 K as 0 ppm.

reported in the literature. ^{17}O T_1 relaxation behavior at 278 K is single exponential with the value of T_1 time of 0.91 ± 0.01 ms. As shown in reference [14] for the pyro-E3 A β fibrils, when the temperature is lowered well below the bulk freezing point to around 250 K, one observes clear bi-exponential behavior of ^{17}O CT T_1 relaxation times. For example, at 196 K the fast-relaxing component, whose fraction is 20%, is 110 ms, while the slow-relaxing component is 27 s. Thus, ^{17}O T_1 relaxation times for the two components differ by orders of magnitude. Based on prior analysis of hydration water in globular HP36, we interpreted the fast-relaxing component as the contribution from the water layers in the immediate proximity of the protein surface which undergo

fast reorientations (Fig. 1B). The protein bound layer, which represents approximately 20–30% of the total water content, undergoes tethered 2-site tetrahedral reorientations while the more remote layers have a larger number of degrees of freedom to undergo non-tethered reorientations. Based on the combination of the ^{17}O CT T_1 and $T_{1\rho}$ relaxation times data, the populations of the two sites were shown to be unequal and loosely constrained in a 9:1 ratio. The slow-relaxing component corresponds to the relatively immobile bulk layer in which the tetrahedral reorientations are largely frozen and T_1 relaxation is dominated by small-angle fluctuations. At temperatures below 220 K, the fraction of unfrozen bulk water layer is negligible, [24] as can be assessed using the so-called TRIP filter: setting the excitation pulse-length to 60° along with the appropriate phase cycle selects the states for which the effective C_q value is close to zero [22,33]. The spectra at 180 K (Fig. 8B) were collected with the inter-scan delay of 60 s and, therefore, include contributions from all water layers: the protein-bound layer, layers more remote from the protein surface, as well as frozen bulk.

The Q-CEST profile at 278 K (Fig. 9) indicates significant narrowing due to large-amplitude motions. In this case, the profile was collected using t_{CEST} of 0.5 ms due to the short T_1 relaxation time of 0.9 ms. The profiles can be modeled within isotropic diffusion approximation with the diffusion coefficient of $3 \cdot 10^6 \text{ rad}^2/\text{s}^2$. In the temperature range below the bulk freezing point, between 245 and 165 K, the profiles were collected with the CEST evolution period of either 1 ms (at 245 K) or 3 ms (220 to 165 K) and $\omega_{\text{RF}}/2\pi$ of 3.5 kHz. The T_1 relaxation times of the fast-relaxing component range from 1 to 800 ms between 245 and 165 K. Thus, they guided the choice of the t_{CEST} values. The value of ω_{RF} is much

smaller than the CT linewidth. The detection scheme was chosen as TRIP [22,33] for temperatures between 245 and 220 K to exclude the residual unfrozen bulk component, with details listed in *Experimental* and reference [24]. Below 220 K, Hahn echo detection was used. In the main series (Fig. 9), the inter-scan delay of 0.8–2 s was used, which suppresses the contribution of the frozen bulk fraction below 230 K, where the slow relaxing component of the T_1 build up curve is in the range of 3 to 50 s. The range of offsets focused on detecting the resonance of CT region and was thus constrained to the -200 to 200 kHz region. Normalization of intensities was performed using the $\pm(200 \text{ to } 150 \text{ kHz})$ ranges. To obtain the profiles, integration of the powder pattern signal from the entire CT spectral region was used.

As expected, significant widening is observed below the bulk freezing point with the modest temperature dependence of the profiles in the 245 to 165 K range. The dynamics information is obtained by fitting the profiles to the simulations, with the caveat that dipolar interactions with protons need to be included explicitly in the simulations.

The inclusion of the dipolar interaction with directly attached protons in the simulation is described in the *Experimental* section. It is of course possible to apply proton decoupling during the Q-CEST period itself; however, as explained in the “*Experimental and Computational considerations for Q-CEST measurements*” section, this leads to the Hartman-Hahn matching resonances in the CEST profile that also affect the main CT resonance and complicate the extraction of dynamical information. One such example for water in the fibrils is shown in Fig. S8. In this regard, applying MAS will likely prove to be useful in the future.

Simulated profiles (Fig. 9 solid lines) were generated using the 2-site tetrahedral reorientation model depicted in Fig. 1B and exploring the

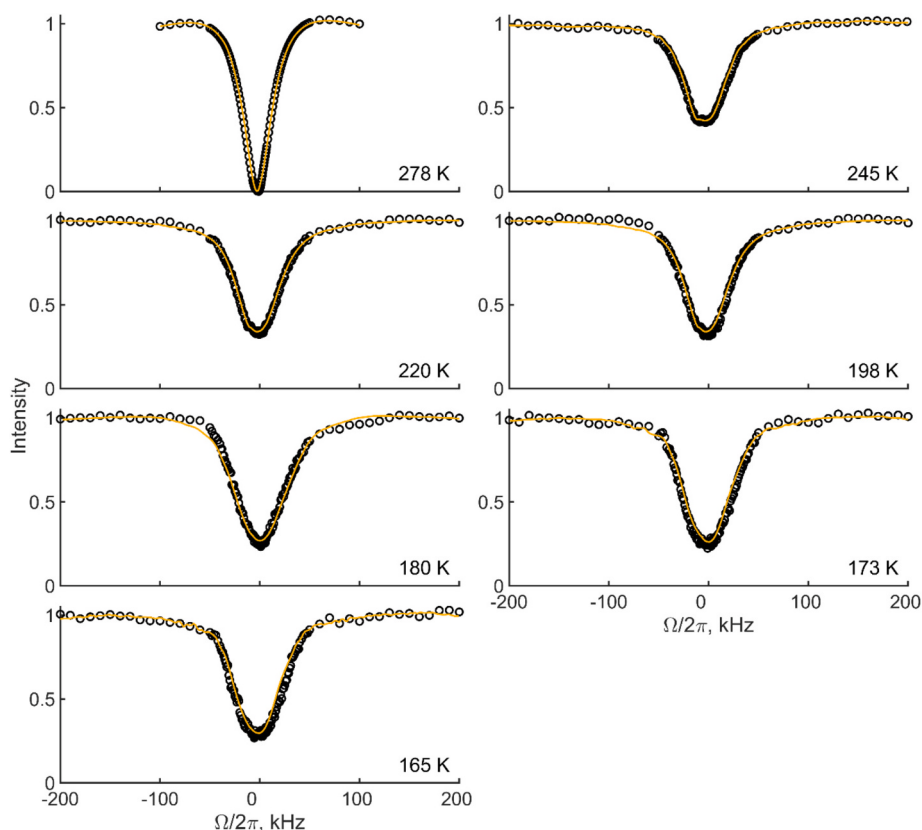


Fig. 9. ^{17}O CT Q-CEST profiles for hydration water in pyro-E3 A β fibrils collected under static conditions at 18.8 T. Above the bulk freezing point at 278 K, $t_{\text{CEST}} = 0.5 \text{ ms}$ and $\omega_{\text{RF}}/2\pi = 2.5 \text{ kHz}$ were employed and normalization was performed using the average of maximum intensity for positive and negative offsets. Below the bulk freezing point in 245 to 165 K temperature range, the data were collected with the 0.8 to 2 s inter-scan delay which selects the layers in proximity to the protein surface. $t_{\text{CEST}} = 1 \text{ ms}$ (245 K) or $t_{\text{CEST}} = 3 \text{ ms}$ (220 to 165 K temperature range) and $\omega_{\text{RF}}/2\pi = 3.5 \text{ kHz}$ were employed. Normalization was performed using the $\pm(200 \text{ to } 150 \text{ kHz})$ ranges. Simulated best-fit profiles (light brown solid lines) were generated either using the isotropic diffusion model for the profile at 278 K or the 2-site tetrahedral reorientations for 245 to 165 K range using the ratio of population of 0.85:0.15, and other details as described in the text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

range of population ratios from 0.7:0.3 to 0.95:0.05. The detailed analysis is shown in Fig. S9 and its legend. The best match is provided by the ratio of 0.85:0.15, based on the RMSD analysis of the experimental and simulated Q-CEST profiles. There is a tendency for even more restricted ratio at low temperatures, but to avoid overfitting the data, we kept the ratio the same for all temperatures. The ratio of populations could also be obtained from the combination of ^{17}O T_1 and $T_{1\rho}$ times, as was done for hydration water in HP36, but CEST-based constraints are more apparent within the precision of the data. For the pyro-E3 A β fibrils, prior T_1 and $T_{1\rho}$ relaxation times data were fitted with the 0.9 to 0.1 ratio within the 2-site reorientations model [14]. There is a good agreement between the values of k_{re} obtained from the combined T_1 and $T_{1\rho}$ relaxation times series and the Q-CEST data (Fig. 10), supporting the validity of this model for the hydration shells in close proximity to the protein surface. The errors in k_{re} at the fixed ratio of the weights of 0.85:0.15 were obtained from RMSD analysis in analogy to what is shown in Fig. S5 for NaNO_3 . The relative errors in k_{re} are about 20–25%.

We then examine the effect of the frozen bulk on the Q-CEST profile. We probed selected offsets across the entire CEST profiles with much higher inter-scan delays, which permitted inclusion of the signal from the frozen bulk (Fig. S10). The absolute intensity increases roughly in proportion to the slow-relaxing T_1 fraction. However, when the profiles are normalized, they are practically identical to those obtained with the short inter-scan delay. This suggests that coherence originating from the bulk layer exchanges rapidly with the coherence of the dynamic layers via spin diffusion, likely driven by the proton dipolar network. To confirm from a different angle that the main effect that we see is due to the protein-bound layers, we examine the Q-CEST profile at two low temperatures for the pyro-E3 A β fibrils sample hydrated not to 100% w/w water content but rather to 20–25% w/w. In this sample, all water resides in the proximity of protein surface. The slow-relaxing T_1 component disappears, in analogy to what was seen for globular HP36 protein (Fig. S11). The normalized Q-CEST profiles for this sample coincide with those obtained at the high hydration state (Fig. S11).

^2H Q-CEST [9] could also be used to probe the dynamics of hydration water as a complementary technique. However, there are several factors limiting its application. First, at temperatures at which there is a significant contribution from the unfrozen bulk layer (around 240 K and higher) [24], the profiles will be significantly modified around the central range of frequencies. This is due to the fact that the unfrozen bulk

layer undergoes fast large-scale rearrangements, which can be approximated by isotropic diffusion [24]. In contrast, in the case of ^{17}O nuclei the TRIP filtering technique or other alternatives based on the differences in the nutation frequencies can be employed to filter out the contribution from unfrozen bulk. Second, the dynamics is expected to be dominated by 2-site 180° deuteron flips, which somewhat limits the insights into the dynamics inherent to the interaction with the protein surface. Fig. S12 present experimental data and simulations of ^2H Q-CEST profiles of hydration layer in the proximity to the protein surface in globular chicken villin headpiece protein [43] at 220 K, when the protein is hydrated with D_2O . The experimental profiles are fitted well with the 2-site deuteron flip model using the value of the rate constant [24] obtained from prior ^2H T_1 relaxation measurements.

4. Conclusion

^{17}O Q-CEST with CT detection can be a sensitive tool for studies of dynamics in molecular solids when the dynamics falls either around microsecond or nanosecond ranges. The motions affect the resulting saturation profiles by modulating the line intensities and their widths. The Q-CEST method is complementary to line shape and relaxation measurements in determining molecular mechanisms and time scales of motions in complicated systems.

One of the main advantages of the method is the application of weak RF irradiation, which is conducive to samples sensitive to RF-induced heating. The range of saturation frequencies can include the ST1 region, when possible. However, constraining the offsets to the CT range is expected to yield a sufficient degree of precision, as we have seen using NaNO_3 as a model compound. We have demonstrated how simulations including the numerical solution to the Liouville – von Neumann equation can be implemented for ^{17}O Q-CEST measurements and suggested a phenomenological inclusion of the Q2 averaging terms in the fast motional limit with respect to the Larmor frequency (i.e., for the second dynamical transition on the nanosecond time scale). The second dynamical transition is distinct for half-integer quadrupolar nuclei [11].

When applied to hydration water layers in pyro-E3 A β fibrils at temperatures below the bulk freezing point in the 245 to 165 K temperature range, the method proved to be useful for characterization of 2-site tetrahedral reorientations of water molecules in the layers in the proximity of the protein surface. The values of rate constants are similar

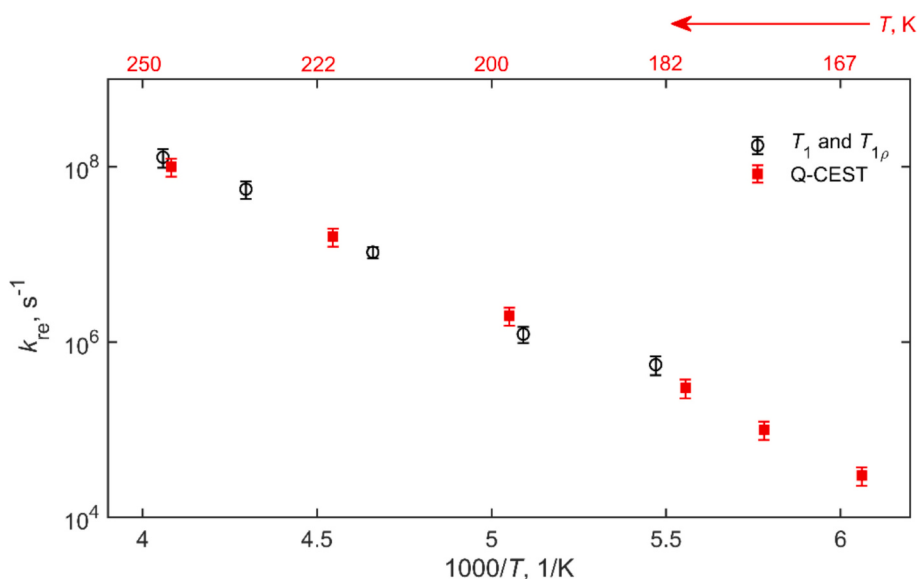


Fig. 10. Rate constants k_{re} versus $1000/T$ obtained either from the ^{17}O Q-CEST measurements (red) or from prior studies based on the T_1 and $T_{1\rho}$ relaxation times measurements [14] (black) for water in the hydration layer in proximity to protein surface in the pyro-E3 A β fibrils. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to those seen with the alternative the T_1 and $T_{1\rho}$ relaxation times measurements from prior studies. Q-CEST provided additional constraints on the relative populations of the two sites. In the case of water, dipolar interactions with the directly attached protons needed to be included explicitly in the simulations.

CRedit authorship contribution statement

Liliya Vugmeyster: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Karen Basaves:** Formal analysis. **Riqiang Fu:** Writing – review & editing, Methodology. **Sean T. Holmes:** Writing – review & editing, Methodology. **Dmitry Ostrovsky:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmr.2026.108045>.

Data availability

Data will be made available on request.

References

- [1] S.E. Ashbrook, Z.H. Davis, R.E. Morris, C.M. Rice, 170 NMR spectroscopy of crystalline microporous materials, *Chem. Sci.* 12 (2021) 5016–5036.
- [2] G. Wu, 170 NMR studies of organic and biological molecules in aqueous solution and in the solid state, *Prog. Nucl. Magn. Reson. Spectrosc.* 114–115 (2019) 135–191.
- [3] R.V. Pound, Nuclear electric quadrupole interactions in crystals, *Phys. Rev.* 79 (1950) 685.
- [4] A.G. Palmer 3rd, Chemical exchange in biomacromolecules: past, present, and future, *J. Magn. Reson.* 241 (2014) 3–17.
- [5] G. Bouvignies, L.E. Kay, Measurement of proton chemical shifts in invisible states of slowly exchanging protein systems by chemical exchange saturation transfer, *J. Phys. Chem. B* 116 (2012) 14311–14317.
- [6] M.J. Jaroszewicz, A.R. Altenhof, R.W. Schurko, L. Frydman, Sensitivity enhancement by progressive saturation of the proton reservoir: a solid-state NMR analogue of chemical exchange saturation transfer, *J. Am. Chem. Soc.* 143 (2021) 19778–19784.
- [7] T. Bauer, J. Gath, A. Hunkeler, M. Ernst, A. Böckmann, B.H. Meier, Hexagonal ice in pure water and biological NMR samples, *J. Biomol. NMR* 67 (2017) 15–22.
- [8] L. Vugmeyster, D. Ostrovsky, A. Greenwood, R. Fu, Deuteron chemical exchange saturation transfer for the detection of slow motions in rotating solids, *Front. Mol. Biosci.* 8 (2021).
- [9] L. Vugmeyster, D. Ostrovsky, R. Fu, Deuteron quadrupolar chemical exchange saturation transfer (Q-CEST) solid-state NMR for static powder samples: approach and applications to amyloid- β fibrils, *ChemPhysChem* 21 (2020) 220–231.
- [10] S. Odedra, S. Wimperis, Spin-locking of half-integer quadrupolar nuclei in NMR of solids: the far off-resonance case, *Solid State Nucl. Magn. Reson.* 84 (2017) 4–13.
- [11] I. Hung, G. Wu, Z. Gan, Second-order quadrupolar line shapes under molecular dynamics: an additional transition in the extremely fast regime, *Solid State Nucl. Magn. Reson.* 84 (2017) 14–19.
- [12] L. Vugmeyster, R. Fu, D. Ostrovsky, 170 NMR relaxation measurements for investigation of molecular dynamics in static solids, using sodium nitrate as a model compound, *Sol. State Nucl. Magn. Reson.* 134 (2024) 101976.
- [13] S. Jawhar, O. Wirths, T.A. Bayer, Pyroglutamate amyloid- β (A β): a hatchet man in Alzheimer disease, *J. Biol. Chem.* 286 (2011) 38825–38832.
- [14] L. Vugmeyster, D. Ostrovsky, Dynamics studies of side-chains and hydration shells in amyloid-beta fibrils using deuterium and oxygen-17 solid-state NMR, in: R. Tycko (Ed.), *Structural Studies of Amyloid-beta (Methods in Enzymology)*, Elsevier, 2026.
- [15] J. Beerwerth, R. Siegel, L. Hoffmann, L.S. Plaga, M. Storek, B. Bojer, J. Senker, W. Hiller, R. Böhmer, From ultraslow to extremely fast dynamics in sodium nitrate: an 170 NMR study, *Appl. Magn. Reson.* 51 (2020) 597–620.
- [16] A.T. Petkova, W.M. Yau, R. Tycko, Experimental constraints on quaternary structure in Alzheimer's beta-amyloid fibrils, *Biochemistry* 45 (2006) 498–512.
- [17] A.T. Petkova, R.D. Leapman, Z.H. Guo, W.M. Yau, M.P. Mattson, R. Tycko, Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils, *Science* 307 (2005) 262–265.
- [18] L. Vugmeyster, M.A. Clark, B.I. Falconer, D. Ostrovsky, D. Gantz, W. Qiang, G. L. Hoatson, Flexibility and solvation of amyloid-beta hydrophobic core, *J. Biol. Chem.* 291 (2016) 18484–18495.
- [19] P.L. Gor'kov, E.Y. Chekmenev, C. Li, M. Cotten, J.J. Buffy, N.J. Traaseth, G. Veglia, W.W. Brey, Using low-E resonators to reduce RF heating in biological samples for static solid-state NMR up to 900 MHz, *J. Magn. Reson.* 185 (2007) 77–93.
- [20] P.A. Beckmann, C. Dybowski, A thermometer for nonspinning solid-state NMR spectroscopy, *J. Magn. Reson.* 146 (2000) 379–380.
- [21] E.L. Hahn, Spin echoes, *Phys. Rev.* 80 (1950) 580–594.
- [22] F. Wang, S.K. Ramakrishna, P. Sun, R. Fu, Triple-pulse excitation: an efficient way for suppressing background signals and eliminating radio-frequency acoustic ringing in direct polarization NMR experiments, *J. Magn. Reson.* 332 (2021) 107067.
- [23] B.M. Fung, A.K. Khitrin, K. Ermolaev, An improved broadband decoupling sequence for liquid crystals and solids, *J. Magn. Reson.* 142 (2000) 97–101.
- [24] L. Vugmeyster, B. Frazier, R. Fu, D. Ostrovsky, Multi-mode water dynamics in hydration shells of villin headpiece subdomain protein in the solid state using deuterium and oxygen-17 NMR spectroscopy, *J. Chem. Phys.* 163 (2025) 025101.
- [25] C.F. Karney, Quaternions in molecular modeling, *J. Mol. Graph. Model.* 25 (2007) 595–604.
- [26] A. Abragam, *Principles of Nuclear Magnetism*, Clarendon Press, Oxford, 1961.
- [27] U. Haeberlen, High-resolution NMR in solids: Selective averaging, in: J.S. Waugh (Ed.), *Advances in Magnetic Resonance*, Academic Press, New York, 1976.
- [28] E.G. Keeler, V.K. Michaelis, R.G. Griffin, 170 NMR investigation of water structure and dynamics, *J. Phys. Chem. B* 120 (2016) 7851–7858.
- [29] H.S.M. Coxeter, *Regular Polytopes*, 3rd ed., Dover, New York, 1973.
- [30] A. Brinkmann, Introduction to average Hamiltonian theory. I. Basics, Concepts *Magn. Reson. Part A* 45A (2016) e21414.
- [31] P.P. Man, Quadrupolar interactions, in: eMagRes, 2011.
- [32] Y. Dai, I. Hung, Z. Gan, G. Wu, Extending 170 transverse relaxation measurement to satellite transitions as a direct probe of molecular dynamics in solids, *Sol. State Nucl. Magn. Reson.* 137 (2025) 102004.
- [33] R. Zhang, T.A. Cross, X. Peng, R. Fu, Surprising rigidity of functionally important water molecules buried in the lipid headgroup region, *J. Am. Chem. Soc.* 144 (2022) 7881–7888.
- [34] S.R. Hartmann, E.L. Hahn, Nuclear double resonance in the rotating frame, *Phys. Rev.* 128 (1962) 2042–2053.
- [35] C. Fülber, D.E. Demco, B. Blümich, The influence of molecular motion on cross-polarization in cross-linked elastomers, *Sol. State Nucl. Magn. Reson.* 6 (1996) 213–223.
- [36] L. Cruceta, Y. Sun, J.M. Kenyaga, D. Ostrovsky, A. Rodgers, L. Vugmeyster, L. Yau, W. Qiang, Modulation of aggregation and structural polymorphisms of beta-amyloid fibrils in cellular environments by pyroglutamate-3 variant cross-seeding, *J. Biol. Chem.* 299 (2023) 105196.
- [37] Z.W. Hu, L. Cruceta, S. Zhang, Y. Sun, W. Qiang, Cross-seeded fibrillation induced by pyroglutamate-3 and truncated A β (40) variants leads to A β (40) structural polymorphism modulation and elevated toxicity, *ACS Chem. Neurosci.* 12 (2021) 3625–3637.
- [38] H.A. Scheidt, A. Korn, B. Schwarze, M. Krueger, D. Huster, Conformation of pyroglutamated amyloid β (3–40) and (11–40) fibrils – extended or hairpin? *J. Phys. Chem. B* 128 (2024) 1647–1655.
- [39] M. Wulff, M. Baumann, A. Thummler, J.K. Yadav, L. Heinrich, U. Knupfer, D. Schlenzig, A. Schierhorn, J.U. Rahfeld, U. Horn, J. Balbach, H.U. Demuth, M. Fandrich, Enhanced fibril fragmentation of N-terminally truncated and pyroglutamyl-modified A β peptides, *Angew. Chem., Int. Ed.* 55 (2016) 5081–5084.
- [40] D. Carnevale, S.E. Ashbrook, G. Bodenhausen, Solid-state NMR measurements and DFT calculations of the magnetic shielding tensors of protons of water trapped in barium chlorate monohydrate, *R. Soc. Chem. Adv.* 4 (2014) 56248–56258.
- [41] M. Adjei-Acheamfour, R. Böhmer, Second-order quadrupole interaction based detection of ultra-slow motions: tensor operator framework for central-transition spectroscopy and the dynamics in hexagonal ice as an experimental example, *J. Magn. Reson.* 249 (2014) 141–149.
- [42] T. Miyatou, T. Araya, R. Ohashi, T. Ida, M. Mizuno, Hydration water dynamics in bovine serum albumin at low temperatures as studied by deuterium solid-state NMR, *J. Mol. Struct.* 1121 (2016) 80–85.
- [43] C.J. McKnight, D.S. Doering, P.T. Matsudaira, P.S. Kim, A thermostable 35-residue subdomain within villin headpiece, *J. Mol. Biol.* 260 (1996) 126–134.