Solid-state NMR reveals a comprehensive view of the dynamics of the flexible, disordered N-terminal domain of amyloid-β fibrils

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ABSTRACT

Amyloid fibril deposits observed in Alzheimer’s disease comprise amyloid-β (Aβ) protein possessing a structured hydrophobic core and a disordered N-terminal domain (residues 1–16). The internal flexibility of the disordered domain is likely essential for Aβ aggregation. Here, we used 2H static solid-state NMR methods to probe the dynamics of selected side chains of the N-terminal domain of Aβ(1–40) fibrils. Line shape and relaxation data suggested a two-state model, in which the domain’s free state undergoes a diffusive motion that is quenched in the bound state, likely because of transient interactions with the structured C-terminal domain. At 37°C, we observed freezing of the dynamics progressively along the Aβ sequence, with the fraction of the bound state increasing and the rate of diffusion decreasing. We also found that without solvation, the diffusive motion is quenched. The solvent acted as a plasticizer reminiscent of its role in the onset of global dynamics in globular proteins. As the temperature was lowered, the fraction of the bound state exhibited sigmoidal behavior. The midpoint of the freezing curve coincided with the bulk solvent freezing for the N-terminal residues and increased further along the sequence. Using 2H R1,ρ measurements, we determined the conformational exchange rate constant between the free and bound states under physiological conditions. Zinc-induced aggregation leads to the enhancement of the dynamics, manifested by the faster conformational exchange, faster diffusion, and lower freezing-curve midpoints.

Amyloid-β protein (Aβ)1 is one of the major components of neurotoxic amyloid plaques in the brains of patients with Alzheimer’s disease. The deposits are made up of Aβ fibrils displaying distinct morphological features.1, 2 Aβ is also present in vivo in oligomeric form, which is believed to be very toxic and capable of disrupting the cell membrane.3, 4 Short fibrillary entities are also capable of membrane disruption and, in general, cellular toxicity might be related to the dynamic properties of all Aβ species.5, 6

Aβ1-40 is most abundant in fibrils, while Aβ1-42 is often considered to be more toxic.7 Aβ comprises a structured C-terminal domain spanning the well-characterized cross-β motif and a mostly unstructured N-terminal domain, corresponding to residues 1–16 of the full length protein. MD simulations suggest that the N-
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The N-terminal domain has been found by several studies to be crucial in regulating the aggregation properties of Aβ. It contains the main binding and regulatory sites for interaction with metals as well as several regulatory sites that have recently been implicated to be controlled via post-translational modifications. For example, the H6R familial mutation associated with the early onset of AD as well as isomerization of aspartate at position #7 to iso-aspartate increase aggregation propensities and zinc-dependent oligomerization. In vitro, this type of isomerization has also been suggested to lead to enhanced insolubility and resistance to enzymatic degradation. Further, molecular dynamics simulations revealed differences in the correlated dynamics between the C-terminal and N-terminal domains upon mutations at positions 6 and 7, which are important for defining polymorphic states and are likely to be involved in altering the aggregation properties between the wild-type and mutants. In general, Hori et al. hypothesized that the region around positions H6–D7 can be a “hot-spot” that facilitates Aβ deposition and the development of AD.

Another important regulatory spot in the N-terminal domain is likely to be related to the post-translational phosphorylation of S8, which has been shown to lead to a distinctly different fibril morphology and suggested to affect the initial nucleation of Aβ fibrillation as well as promote and stabilize the formation of toxic aggregates.

Animal models show that Zn²⁺ coordination can play a crucial role in the formation of plaques in vivo. At concentrations of the order of what is found at synapses, Zn²⁺ specifically binds to Aβ and promotes aggregation. Noy et al. proposed that Zn²⁺ stabilizes transient (non-fibrillary) amyloid forms. The concentration of zinc ions in amyloid plaques reaches 1 mM. Miller et al. suggested that the mechanism of Zn²⁺ binding is due to a population shift of polymorphs, similar to other large zinc-binding proteins. In the NMR structure of Zn-bound Aβ1-16, His6, Glu11, His13, and His14 are coordinated with Zn. It has also been suggested that the aggregation may be governed by the conformational flexibility of His6.

The internal flexibility of the N-terminal domain is likely to be very important for its functionality of controlling the aggregation. Disordered protein regions are known for their control of multiple biological functions including allosteric regulation and enzymatic catalysis. Combined with the high number of existing post-translational modifications and mutations in the N-terminal region, which have the potential to modify structural ensembles, studies of the dynamics of this region can reveal the underlying molecular basis of the aggregation control associated with these modifications.

The general flexibility of non-core regions in Aβ has been demonstrated by multiple techniques such as nuclear magnetic resonance (NMR), electron paramagnetic resonance, hydrogen–deuterium exchange probed by mass spectrometry, X-ray crystallography, and fluorescence spectroscopy. However, experimental site-specific studies of the dynamics of the insoluble aggregates of Aβ in general, and in the N-terminal domain in particular, are relatively sparse due to challenges in obtaining the necessary resolution and sensitivity in the solid non-crystalline state. Of note are the works of Fawzi et al., who utilized solution NMR saturation transfer approaches to probe the binding of monomeric Aβ to the surface of protofibrils.

In fact, the available structures of the wild-type Aβ1-40 fibrils solved by solid-state NMR start at residue number 9, highlighting the enhanced dynamics of the N-terminal region. Further, Hong and coworkers identified several pools of water in the fibrils, such as in the matrix, interfibrillar, peptide-bound, and inside cavities. These different water pools are likely to
be crucial in modulating the Aβ dynamics and transient interactions between the two domains. The structure of fibrils has been shown to be hydration-independent, at least in the structured region, based on chemical shift data. Thus, solvation likely has the largest effect on the properties of the dynamics rather than the overall structure. The motions in the N-terminal region in particular are expected to be highly sensitive to hydration.

The goal of this work is to characterize the site-specific dynamics of selected side chains in the flexible N-terminal domain in the context of hydrated Aβ₁-₄₀ fibrils. We utilize deuterium static solid-state NMR techniques in combination with the selective labeling of the side chains to achieve a comprehensive view of the dynamics of this largely disordered domain. The results allow us to obtain a quantitative picture of the motions of the domain, with the flexibility of the side-chains gradually decreasing along the N-terminal chain in the hydrated fibrils.

Using Aβ₁-₄₀ peptides with deuterium labels incorporated at a single residue along the N-terminal sequence, we generated a number of "singly" labeled samples of Aβ fibrils with morphologies corresponding mostly to the 3-folded symmetric/twisted polymorph. We employed previously developed protocols based on seeding procedures and confirmed the resulting morphologies using transmission electron microscopy (TEM).

The positions of the labeled residues and typical TEM images are shown in Figure 1 and the position of the label in each individual side chain is listed in Table 1. For the H6 side chain, the labeling with the τ-τHis modification was employed, for which the deuteration site is in the methyl group. This modification did not affect the morphology of the resulting fibrils (Figure S1).

Results and Discussion

"Singly" labeled samples for N-terminal domain side chains

Using Aβ₁-₄₀ peptides with deuterium labels incorporated at a single residue along the N-terminal sequence, we generated a number of "singly" labeled samples of Aβ fibrils with morphologies corresponding mostly to the 3-folded symmetric/twisted polymorph. We employed previously developed protocols based on seeding procedures and

Qualitative analysis of the data suggests a hypothesis of free and transiently bound states for the N-terminal domain

The high-temperature line shapes for the N-terminal residues in the hydrated fibrils are significantly narrowed beyond what is expected due to typical local fluctuations such as fast methyl rotations, rotameric jumps, and ring flips. As the temperature is lowered, a widening of the lines is observed with a sharp increase in the wide component appearing around the bulk freezing point of water (~265K) for the A2, F4, and H6 residues. For the G9 and V12 residues, a significant fraction of the wide component is already apparent at much higher temperatures. For V12, a typical relatively wide pattern corresponding to rotameric jumps is observed at 37 °C, while for G9 in addition to the wide component, there is still a narrowed region at the center of the spectrum. In addition, for the F4, H6, and G9 side chains, the longitudinal relaxation data display a striking crossover curve (i.e., an abrupt sigmoidal-like change in $T_1$ with temperature), suggesting two different contributions into relaxation stemming from the
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dramatic differences in the local environments of the two components. At the same time, the relaxation curves remain single exponential even at the middle of the cross-over (Figure S2, S3), pointing to the likelihood of exchange between the two components on the time scale faster than $T_1$. As will be elaborated on later, for A2 and V12, relaxation is dominated by fast local methyl rotations and is not sensitive to hydration.

The temperature dependence behavior of both the line shapes and relaxation does not exhibit any hysteresis behavior, i.e. the same results are obtained independently of whether the experiments are performed by starting at the physiological conditions and freezing the samples or by starting at low temperatures and heating the samples. This points toward the fact that the system is in equilibrium with respect to freezing the bulk water surrounding the immediate hydration layer and cavities; in other words, we do not have a situation in which there are two states of bulk water.

Specific evidence on a dynamic exchange between the two fractions, detected by line shape and longitudinal measurements, is also seen from the rotating frame relaxation measurements, $^2$H $R_{1p}$. These experiments are particularly sensitive to microsecond timescale conformational exchange processes. The existence of the relaxation rate “dispersion curve” – the dependence of the relaxation rate on the radio frequency field strength – serves as an indication of the presence of conformational exchange at the microsecond timescale. As elaborated on below, we observed such a dispersion profile for the fibrils labeled at the A2 methyl position.

Interestingly, for all residues, the dry fibrils lack the features of the narrowing behavior in the line shapes and the cross-over behavior in the relaxation data (Figures 3 and 4), indicating that solvation is crucial in the onset of the global dynamical modes responsible for these features.

Based on all the available data, we propose a hypothesis that in the hydrated fibrils, the N-terminal domain exchanges between the free state in which large-scale concerted motions of the whole domain dominate the dynamics at high temperatures and the “bound” state in which these motions are quenched (Figure 5). The nature of the bound state is likely due to transient interactions with the well-structured C-terminal domain of Ab. Residues located closer to the N-terminus (A2, F4, H6) have a significantly lower fraction of the bound state, probably due to weaker interactions with the C-terminal domain compared with the residues further along the chain. As the temperature is lowered, the large-scale motions of the N-terminal domain diminish and they are very significantly quenched near the bulk freezing point of water, when the frozen bulk solvent no longer acts as a plasticizer. This effect of water is reminiscent of the hydration effect on slow dynamics in globular proteins (i.e., solvent-driven glass transition). The role of solvent on amyloid structure formation has also been probed by explicit molecular dynamics simulations to illustrate how water controls the self-assembly of higher-order structures and plays a role in amyloid polymorphism. The rest of the quantitative data analysis proceeds according to the described hypothesis and the $R_{1p}$ measurements allow us to determine the chemical exchange rate constant between the free and bound states under physiological conditions.

Note that the N-terminal domain in isolation (corresponding to residues 1-16 of the full length Ab) in the hydrated state surprisingly does not show the line narrowing observed in the context of the fibrils. Figure 2 (see the red line in the bottom panel) demonstrates the line shape of this isolated fragment labeled at the F4-ring-$d_5$ position. Thus, it appears that the packing of the fibrils renders enhanced flexibility to the N-terminal domain, which likely is functionally relevant. It is also important to note that the methyl-bearing side chains in the C-terminal core of the fibrils (L17, L34, V36), when probed with the same technique of $^2$H line shape analysis, did
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not show any dependence on solvation, unlike the side chain of M35 spanning a water-accessible cavity. (66, 67, 69) Thus, solvation in conjunction with the environment of the fibrils promotes the flexibility of the N-terminal domain. Without the environment of the fibrils in the lyophilized hydrated state, the side-chains of the N-terminal fragment are free to stack against each other by forming non-native intermolecular contacts, which are likely to lead to the partially aggregated state responsible for the observed restriction in the dynamics.

**Determination of the fraction of free and bound states from the line shape data**

The general strategy for the determination of the fractions of the free and bound states corresponds to the decomposition of the line shapes into a narrow component of the Lorentzian shape and the wide non-Lorentzian components (Figure S4). The former component is assumed to correspond to the free state with motions governed by isotropic diffusion and the latter component to the bound state. For the case of G9, this analysis yielded an unreasonably high fraction of the bound state (75%) at 37°C, which contradicted the existence of the cross-over in the relaxation data. Thus, for this residue, we assumed that a part of the non-Lorentzian line shape is due to deviations from isotropic diffusion. The deviation could arise due to the fact that the probe is at the backbone. The fraction bound in this case was taken as a percentage of the rigid (full powder pattern) line shape. In this regard, it is important to note that for V12, located further along the backbone, all of the non-Lorentzian line shape can be attributed to the bound state, with some extent of rotameric motions of the valine side chains present in the bound state, as detailed in the Materials and Methods.

To check to what extent the location of the probe on the backbone rather than on the side chain affects the freezing of G9 at a higher temperature, we also probed the F4 site at the backbone position labeled as Dα only (Figure S5). The spectrum remains almost entirely unfrozen at 280 K, at which G9 is already over 70% in the bound state. At 263 K, there is still a significant fraction of the mobile phase for the F4-Dα site. Thus, it is clear that the backbone position alone does not cause a significantly higher freezing point. Due to the low sensitivity of this sample (only one deuterium per Aβ molecule), quantitative line shape decomposition was unreliable, and we were not able to assess deviations from the approximation of isotropic tumbling.

The resulting fractions at 37°C (Figure 6A) show that the population of the bound state progressively increases along the N-terminal sequence. The temperature-dependence of the populations (Figure 6B) displays sigmoidal behavior. The data were fitted to the function:

\[
p_{\text{bound}} = a + \frac{b-a}{1+\exp\left(\frac{T-T_m}{\sigma}\right)}
\]

in which \(T_m\) is the midpoint of the freezing curve, \(\sigma\) is the characteristic width of the transition region, and \(a\) and \(b\) are the higher and lower temperature baselines, respectively. The midpoint of the transition is at 268 K for A2, F4, and H6 and at 284 K for G9 (SI5). Thus, the midpoint of the freezing of the first 6–7 residues coincide with the freezing of the bulk point of water, whereas for the residues further along the chain, freezing is observed at higher temperatures.

Additionally, the transition is significantly wider for G9 (the value of \(\sigma\) is 3.5–4 times higher than that of A2–H6). Note that the parameter \(a\) might yield a more accurate overall high-temperature fraction bound projection than just the estimation at a single temperature of +37°C, and we thus display it in the same figure (Figure 6A) for comparison purposes.

**Determination of the timescale of the large-scale motions in the free state assuming an isotropic diffusion model**

We invoke the simplest assumption for the large-scale motions of the N-terminal domain as isotropic diffusion. Obviously, this is an
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approximation, as the environment in the fibrils is not expected to be isotropic. However, this simple model allows us to obtain the relative timescales of the diffusive motions in the free state. The local motional modes were taken as typical modes for the given residues consistent with the temperature dependence of the line shapes. By modeling isotropic diffusion as discrete nearest-neighbor jumps on the surface of a sphere, as described in the Materials and Methods, the diffusion coefficients $D$ were obtained by matching the experimental and simulated line shapes. The resulting values of the diffusion coefficients at 37°C are shown in Figure 6C and they demonstrate a sizeable decrease along the N-terminal sequence.

An alternative model with the sharp temperature dependence of the diffusion coefficient could not reproduce the line shapes at temperatures close to $T_m$, i.e. it is not possible to reproduce the existence of both the central sharp component and the wide powder pattern with a single value of $D$.

The origin of the cross-over in relaxation curves and determination of the timescale of chemical exchange between free and bound states at temperatures close to the midpoint transition

The relaxation behavior (Figure 3) both below and above freezing is governed by mechanisms consistent with the topology of the side chain. Further, the most effective mechanisms are closest in timescale to the Larmor precession frequencies: for the A2 and V12 side chains, these are methyl three-site jumps; for F4, phenylalanine ring flips; and for G9, two-site jumps of the CD$_2$ group. The situation is more complex for H6, as in principle both fast methyl rotations and ring flips can contribute to relaxation. An interesting and important feature is that for the side chains for which there is a considerable hydration dependence of the relaxation (i.e., relaxation times become much smaller upon hydration), there is also a cross-over in the relaxation curves. This is the case for the F4, H6, and G9 side chains.

For the F4 site, relaxation at high temperatures is consistent with ring flips, while at the low temperatures, the flipping motion slows significantly. Note that the diffusion around the ring rotation axis (i.e., around the $\chi_2$ dihedral angle) is ruled out based on the line shape pattern. At low temperatures, one can observe strong relaxation anisotropy: at a frequency of 0 kHz, the contribution is primarily from the free state, while at $\pm 60$ kHz, the contribution is primarily from the more rigid bound state. Around the midpoint of the cross-over, when there is still a significant fraction of the free state even though the intrinsic relaxation rate of the bound state is not very effective, the relaxation of the wide component ($\pm 60$ kHz) is actually governed by the exchange between the free and bound states, as elaborated on at the end of this section.

For H6, at low temperatures, the comparison with the dry sample in which methyl jumps dominate the relaxation as well as the analysis of the temperature dependence of the relaxation indicates that methyl three-site jumps are the main relaxation mechanism. The values of $T_1$ in the low temperature range of the relaxation curve show a decrease in temperature, indicating that the motions are in the fast regime with respect to the Larmor frequency. This behavior would not be expected for the ring-flipping motions of the imidazole ring. On the other hand, at high temperatures, there is the onset of an additional ring-flipping motion of the imidazole ring, giving rise to a major contribution at a frequency of 0 kHz. The ring flipping of imidazole can be functionally relevant for the modulation of the metal coordination properties of the N-terminal domain. Similarly to F4 case, at intermediate temperatures, the relaxation of the bound component is governed by the exchange between the free and bound states. However, unlike the case of F4, the powder pattern spectrum of the bound state is motionally narrowed by 1/3 due to methyl rotations, and thus there is less of a
spectral separation between the bound and free states, which complicates the determination of the exchange rates.

For the case of G9, the two-site jumps of the CD2 group are the major relaxation mechanism at both extremes of the temperature ranges, albeit with different efficiencies for the free and bound states. For the intermediate temperature range of 280 to 260 K, if we assume that the relaxation is determined by the CD2 jumps in the bound state, the resulting rate constants would yield a line shape pattern inconsistent with the experimental results (i.e., these simulations would show over-pronounced horns for the wide component). Thus, the exchange between the bound and free states again dominates the relaxation in this temperature range.

The quantitative details of the modeling of the relaxation rates are elaborated on in the Materials and Methods. The temperature dependence of the rate constants and high and low temperature limits yield values of activation energies for the local motions. They do not provide any major insights relevant to our global picture and we list them in SI6.

Based on the simplified treatment using McConnell equations,(103, 104) the relaxation of the bound state at intermediate temperatures for G9, H6, and F4 is given by \( R_{\text{bound}} = p_{\text{free}} k_{\text{ex}} \). By taking \( p_{\text{free}} \) from the line shape data, we calculate the \( k_{\text{ex}} \) rate for temperatures close to the midpoint of the relaxation cross-over. These temperatures are slightly below the \( T_m \) values corresponding to the \( R_{\text{bound}} \) freezing curves from the line shape analysis (Figure 7). The resulting values of \( k_{\text{ex}} \) are 21±3 s\(^{-1}\) for F4 at 262 K, 9±2 s\(^{-1}\) for H6 at 262 K, and 8±2 s\(^{-1}\) for G9 at 280 K.

**Determination of the conformational exchange rate constant between bound and free states at 37 °C at the A2–CD3 site using \(^2\)H R1\(_p\) measurements**

To determine the rate of conformational exchange between the free and bound states of the N-terminal domain of the fibrils, we utilize \(^2\)H solid-state NMR rotating frame relaxation dispersion measurements under static conditions, which we have previously tested using a model compound.(105) Rotating frame NMR relaxation has yielded a multitude of insights into protein dynamics in solution,(106) and the momentum has picked up to enhance the development of these techniques for solid-state applications.(81-92)

\(^2\)H relaxation rates were measured as a function of the spin-locking field strengths, \( \omega_{\text{SL}} \), between 5 to 35 kHz (Figure 7) for the fibrils labeled at the A2–CD3 position. Because of the significant narrowing of the line shapes due to the diffusive motion (Figure 2), the analysis can be performed within the Redfield limit of NMR relaxation theory, given by the condition that the relaxation rate is much smaller than the rate constant of the underlying motional process (SI8).(107) Experimental relaxation decay curves are not exponential and can be approximated with a double exponential decay (Figure 7D), in which only the fast component (Figure 7A) shows dependence on \( \omega_{\text{SL}} \). The slow component, with a relative contribution to overall signal intensity of 30%, is defined with a much smaller extent of precision within which it does not exhibit any pronounced dependence on \( \omega_{\text{SL}} \) and is of the order of 6–7 ms.

Based on the line shape analysis, the fraction of the bound state is 8% for A2 at 37 °C (Figure 6A) and the dynamics can be approximated by an isotropic diffusion model. In the bound state, the tensor is considered to be static with an effective quadrupolar coupling constant of 55 kHz after narrowing due to methyl rotations taken into account. The diffusion mode alone cannot explain the characteristic dependence of the fast component on \( \omega_{\text{SL}} \) (SI8 and Figure S7). Thus, the mode of the conformational exchange between the free and bound states plays a central role in defining the dispersion curve. The main fitting parameter for this mode is rate constant \( k_{\text{ex}} = k_1 + k_{-1} \). The modeling procedure takes into account all possible relative tensor orientations in the free and bound states. Fitting the fast
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component \( (T_{1p}^{\text{fast}} = 1/R_{1p}^{\text{fast}}) \) of the double-exponential fit in this manner, one can thus extract the values of \( D \) and \( k_{\text{ex}} \). The best-fitted values are \( D = 3.5 \times 10^6 \) rad\(^2\)/s and \( k_{\text{ex}} = 3 \times 10^4 \) s\(^{-1}\) (Figure S8). The diffusion coefficient is larger than the one determined solely from the line shape analysis, \( 1.1 \times 10^6 \) rad/s.

The linewidth is very sensitive to the presence of slight motional anisotropy, and thus this deviation allows us to estimate its extent (SI8, Figure S9, SI10). As elaborated on in SI8 (Figures S7–S12), the presence of the additional slow component points to the need for a more involved motional model with a possibility of including an additional state. It is interesting that an intermediate state was found for the exchange between the monomeric Aβ and its oligomer-bound form, even for the methyl group of A2, based on DEST solution NMR measurements. (70, 71)

To probe the temperature dependence of the conformational exchange process, we also performed the measurements at the lower temperature of 25 °C (Figure 7A). The fitted value of \( k_{\text{ex}} \) is unchanged (3 \times 10^4 s\(^{-1}\)) within the precision of the data, while the diffusion coefficient is smaller, \( D = 2.5 \times 10^6 \) s\(^{-1}\), and the fraction of the bound state is 9%. Thus, the results suggest a relatively low activation energy value for the conformational exchange process between the bound and free states.

Zinc-induced aggregation leads to the enhancement of the dynamics

To determine whether the dynamics are sensitive to different states of Aβ, we investigated the aggregates formed in the presence of equimolar amounts of zinc chloride. These amorphous aggregates have a very wide range of sizes from 1,000 to 10,000 nm, as estimated from dynamic light scattering (DLS) results. The observed distribution is in qualitative agreement with that found by Noy et al.(44) for zinc-containing aggregates based on analytical ultracentrifugation studies. In particular, they emphasized the existence of large molecular-weight aggregates in a very broad range of sizes. The details of the DLS and TEM data are shown in SI11 (Figures S14, S15). Thus, the sample morphology is different to the fibrillar form.

We analyzed these aggregates using the \(^2\)H static line shapes and \( R_{1p} \) measurements employed for the fibrillar form (Figure 8). At 37 °C, there are minor changes in the spectra with an overall moderate extent of narrowing compared with the fibrillar form (Figure 8A), with more pronounced changes toward the end of the N-terminal (H6, G9, and V12). Upon freezing, it is evident that the midpoint of the freezing curves is shifted to lower temperatures (see Figure S13 and the typical spectra close to the midpoint of freezing in Figure 8a). Fitting the freezing curves to Eq. (1) indicates a significant lowering of the midpoint of the freezing curves (see Figure S13) by about 4–5 degrees for A2 and F4 and 7–8 degrees for H6 and G9. Additionally, the value of \( \sigma \) for G9 is about 40% smaller, reflecting the narrowing of the transition. It has previously been suggested that the aggregation may be partially controlled by the conformational flexibility of H6.(19) Our results indicate that the changes in the dynamics in the presence of zinc are more pronounced for the side-chain of H6 and further residues along the backbone of the N-terminal domain, which correlates with the previous study of reference.(19) We note that the use of \( \tau \)-His reduces the coordination efficiency of the histidine ring and, thus, the actual changes in the dynamics upon zinc-induced aggregation may be even more pronounced.

The \(^2\)H \( R_{1p} \) measurements (Figure 8C) at the A2–CD\(_3\) site display a steeper dispersion for the fast component of the relaxation curve \( T_{1p}^{\text{fast}} \). The fraction of the bound state based on the line shape analysis is 6% for this residue compared with 8% for the fibrils. The fitted values of the conformational exchange constant within the model of Figure 5 is 4.5 \times 10^4 s\(^{-1}\), which is 1.5 times larger than that for the fibrillar form, and the fitted value of the diffusion coefficient \( D \) is 5 \times 10^6 rad\(^2\)/s compared with 3.5 \times 10^6 rad\(^2\)/s in the three-fold symmetric fibrils. The combined results of
the line shapes and $R_{1p}$ relaxation analysis indicate the enhanced mobility of the aggregates.

As mentioned in the Introduction, the flexibility of the N-terminal domain of A\(\beta\) and its exposure to solvent has been known for some time.\((52, 54, 56, 57)\) In particular, the works of Olofsson et al.\((56)\) and Whittemore et al.\((57)\) have measured hydrogen-deuterium exchange rates for backbone amide hydrogen in solution using solution NMR spectroscopy. In comparison to the structured regions, the N-terminal domain displayed low protection factors for several of the residues that could be detected. The current work expands these results to the fibrillar state of A\(\beta\) in the 3-fold polymorph and shows a clear progression for the freezing of the dynamics along the sequence in terms of both the fraction of the bound state and the kinetics of the motions approximated by the isotropic diffusion mode. Further, instead of the backbone, the side-chains were tackled, which is expected to be important in the inter-residue contacts (both intra and inter-molecular) that can hinder flexibility. Additionally, the $^2$H $R_{1p}$ experiments allowed us to identify the conformational exchange rate constant, likely due to transient interactions between the unstructured N-terminal and core of the fibrils.

As a first step toward assessing changes in the dynamics in different forms of A\(\beta\), we determined the differences between the 3-fold symmetric fibrillary form and Zn-induced aggregates. To obtain the full biological significance of the role of the dynamics in the potential control of aggregation, further studies will need to focus on discerning the changes in the dynamics between different polymorphs of A\(\beta(1, 2, 77, 108-110)\) as well as in the variants of A\(\beta\) that include post-translational modifications in the N-terminus and mutations mentioned in the Introduction. In particular, it will be important to test the 2-fold native polymorph as well as H6R and D7N mutants\((11, 35, 111)\) and the S8-phosphorylated fibrils that were shown to lead to conformational changes in the N-terminus and accelerate aggregation.\((17, 27, 28)\)

**Conclusion**

Our results demonstrate the quantitative features of the flexibility in the N-terminal domain of A\(\beta\) fibrils probed at several selectively deuterated side-chain sites along the N-terminal sequences. Based on deuterium static solid-state line shape and relaxation data, we proposed the two-state model in which the free state of the N-terminal domain undergoes a diffusive motion; however, in the bound state, this motion is quenched, likely due to transient interaction with the structured C-terminal subdomain. Local motions such as rotameric jumps, methyl rotations, and ring flips dominate the dynamics of the bound state, while they co-exist with the diffusive motion in the free state.

The fraction of the bound state at the physiological temperature was determined to be 8% for the most extreme N-terminal residues (A2, H4, F6), and this increases significantly further along the sequence, with G9 already at 36% and V12 at 87%. We also calculated the rate of the diffusive motion by using the isotropic diffusion approximation that, while an oversimplification, allows us to obtain the relative scale of the diffusive motion along the sequence. Thus, the diffusion coefficient of H6 is 64% of that of A2, but it drops to 30% for G9 and to under 10% for V12.

The temperature dependence of the fractions exhibits sigmoidal behavior, with the midpoint of freezing at 267–268 K for A2, F4, and H6 and at 284 K for G9. Thus, the midpoint of the freezing of the first 6–7 residues is close to the temperature of the freezing of the bulk water, whereas for residues further along the chain, freezing is observed at higher temperatures. The cross-over in the longitudinal relaxation times is also evident for the residues that display strong solvation dependence of the relaxation: F4, H6, and G9. For A2 and V12, the relaxation is dominated by local methyl rotations. Around the midpoint of the cross-over in the relaxation curves, relaxation in the bound state is governed primarily by the exchange process between the two states, and
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thus we can determine the exchange rate constant between the free and bound states at these conditions. The rate constant falls by about 21 s⁻¹ for F4 (at 262 K) and 9 s⁻¹ for H6 (at 262 K) and G9 (at 280 K).

The newly developed ²H R\textsubscript{p} methodology permitted the determination of the conformational exchange rate constant between the free and bound states at the physiological temperature. Probed at the A2–CD\textsubscript{3} site, the conformational exchange rate constant is 3·10⁴ s⁻¹. The effect of zinc-induced aggregation on the dynamics manifests in the overall enhancement of the dynamics. The conformational exchange rate constant is increased by a factor of 1.5 and the line shapes are somewhat narrowed at room temperature, especially for the H6, G9, and V12 residues. Further, the midpoint of the freezing curves is lowered by 4–5 degrees for A2 and F4 and by 7–8 degrees for H6 and G9. Future studies of the different polymorphs of Aβ as well as mutants and post-translational modifications at the N-terminal region will be helpful for revealing the biological importance of the flexibility of this disordered domain.

**Experimental Procedures**

**Preparation of the Aβ\textsubscript{1-40} peptide:** The peptide was prepared by using solid-state peptide synthesis (performed by Thermofisher Scientific Co, Rockford, IL). Fluorenylmethyloxycarbonyl (FMOC)-Phenylalanine-ring-d₅, FMOC-Alanine-d₃, FMOC-Glycine-d₂, and FMOC-Histidine-ring-CD₃ were purchased from Cambridge Isotopes Laboratories (Andover, MA). The native sequence is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV. The peptides were purified by reversed-phase HPLC and their identity and purity were confirmed by mass spectrometry and reversed-phase HPLC. The resulting peptides had isotopic labels in only one chosen residue. The details of the labeling patterns are shown in Table 1.

**Preparation of the fibrils:** Fibrils of the wild-type Aβ\textsubscript{1-40} in the “twisted/3-fold” polymorphs were produced by using established seeded growth protocols under quiescent conditions. For the preparation of the bulk samples, lyophilized peptides were initially dissolved at a 5 mM concentration in dimethyl sulfoxide (DMSO) and immediately transferred to a solution of 10 mM monosodium phosphate buffer at the pH of 7.4, also containing seeds in a 1:10 ratio by weight. The fibrils were allowed to grow for at least 4 days at room temperature with sonication performed at 24 hours during the growth period: aliquots corresponding to 10% of the bulk volume were taken out of solution and sonicated for 2 min by using a probe sonicator, cooled for 15 minutes, and then added back into the bulk solution. The peptide concentration of the bulk samples was 0.5 mg/mL. The fibrils were pelleted by centrifuging at 300,000 g for 12 hours. Fibril pellets were re-suspended in deionized water, rapidly frozen with liquid nitrogen, and lyophilized. The resulting morphologies were confirmed with TEM imaging (Figure 1 and SI1).

**Preparation of amorphous aggregates in the presence of zinc:** lyophilized peptides were dissolved at a 5 mM concentration in DMSO and immediately transferred to a solution of 10 mM monosodium phosphate buffer containing equimolar amounts of zinc chloride at the pH of 7.4 to a total concentration of peptide of 0.5 mg/mL. The aggregates were allowed to grow for at least 3 days at room temperature. The solution underwent dialysis in water using the membrane with a 3 kDa molecular weight cut-off, followed by lyophilization.

**Transmission Electron Microscopy:** Samples were stored at room temperature and diluted to about 0.1 mg/mL with hydro, millipore-filtered water. Samples were negatively stained by using the drop method (112) in the following manner. Four microliter aliquots of the sample, millipore-filtered water, and 2% uranyl acetate aqueous stain were applied sequentially to a freshly glow-discharged, Formvar carbon-coated 300 mesh copper grid for the following incubation times: 2 min, 10 sec, and 1.5 min, respectively. Each 4 µl droplet was removed by wicking with filter paper for 5 sec. The filter paper was blotted prior to the addition of the next droplet. After staining, the grid was air-dried under a vacuum.
for 20 min. Images were collected under low dose conditions of 120,000× using the FEI Tecnai G2 Spirit Biotwin microscope operating at 80 kV.

Hydration and preparation of the NMR samples: A hydrated state with a water content of 200% by weight was achieved by exposing lyophilized powder to water vapor in a sealed chamber at 25°C until the water content reached saturation levels corresponding to about 40% by weight (24–48 hours was sufficient to achieve this), followed by pipetting the remaining water by using deuterium-depleted H2O. The samples were packed in 5 mm NMR tubes (cut to 21 mm length) using Teflon tape to center the sample volume in the coil of the NMR probe. The amount of material packed was 12–15 mg for Ala- and Val-labeled peptides and 20–28 mg for His-, Phe, and Gly-labeled peptides.

Deuteron static solid-state NMR spectroscopy: Experiments were performed on 17.6 T and 14.1 T spectrometers equipped with a static probe operating at temperatures between 160 and 380 K. Line shape experiments were performed with a quadrupole echo pulse sequence based on an eight-step phase cycle(73), with a delay of 31 μs between 90° pulses. A line broadening function of 0.5 to 2 kHz was chosen to enhance the signal. T1 measurements under static conditions were performed at 17.6 T by using the inversion recovery sequence for relaxation times below 80 ms and a saturation recovery sequence for longer times. For the relaxation measurements at low temperatures at which the full width powder pattern was observed, we utilized the multiple echo acquisition detection scheme (QCPMG) for signal enhancement(114). Briefly, QCPMG detection breaks the powder pattern spectrum into a series of spikes that roughly follow the shape of the powder pattern. Unlike magic-angle spinning, QCPMG detection does not suppress relaxation anisotropy(115). The durations of 90° pulses were between 2.0–3.0 μs. Ten to 15 QCPMG echoes were collected with 52 μs pulse spacing, corresponding to QCPMG sidebands spaced at 20 kHz intervals. The number of scans ranged from 128 to 2048 depending on the signal-to-noise ratio in each sample. Five to seven relaxation delays were collected. Relaxation times T1 were obtained by using single-exponential fits to the magnetization build-up curves.

The R1ρ experiment was performed as described in previous work(105) with the pulse sequence (Figure S12) and further details shown in SI8. A 600 MHz NMR spectrometer with a Bruker Neo console equipped with a wide-line low-E probe(116) with a 5-mm inner diameter coil was employed. Spin-lock times varied between 200 μs and 30 ms (12–17 relaxation delays) and powers varied from 5 to 35 kHz. 16–32 dummy scans were utilized.

Motional modeling: The isotropic diffusion of the free state was modeled as discrete nearest-neighbor jumps on the surface of a sphere, using 192 sites with the DistMesh program(117) for the discretization of the Smoluchovski equation. The jump rate was the same for all pairs of sites and was selected to match the second non-zero eigenvalue of the diffusion operator (corresponding to the second-order Legendre polynomial eigenfunction) to be 6D. Supporting Information provides further details of line shape modeling (SI7). The parameters of the quadrupolar tensors, quadrupolar coupling constant Cq, and tensor asymmetry η(73) for each type or residue were taken from the low temperature spectra. The effective values in the fast motional regime, given by the limit at which the rate constant is much greater than Cq, were calculated for the free state by employing the motional models described in the Results section. The values are listed in Table 2. For the case of G9, the averaging of the tensor in the fast limit results in an asymmetry parameter of 1, which yields effective narrowing by itself without the diffusion mode. Diffusion causes further line shape narrowing. In the case of valine-d3, the additional local mode of rotameric jumps had to be added explicitly, as the fast limit approximation was not valid. At 37°C the rotameric exchange rate constant of 1×10^5 s⁻¹ and three rotamers, as well as the corresponding rotations around the χ1 angle, with populations in the 5:1:1 ratio were used. The simulations utilized the EXPRESS program.(118) For the simulated line shapes, an additional line broadening of 1–1.5 kHz was used to approximately account for
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the effect of inhomogeneous dipolar interactions.(119)

To model the longitudinal relaxation rates, static quadrupolar tensor parameters were used (Table 2) and the motional modes listed in Table 1 of the Results and Discussion section were assumed. The values of the rate constants were obtained by matching the experimental and simulated values of $T_1$. The activation energies in the high and low temperature limits were determined by using the Arrhenius Law for the rate constants.

To model the $R_1\rho$ rates, in addition to the isotropic diffusion mode modeled as described for the line shapes, an additional mode of exchange between the free and bound states was included. This was accomplished by the introduction of one additional site with an arbitrary fixed angular position in the crystal-fixed frame. The exchange was modeled by jumps between every site describing the spherical diffusion and bound-state site (SI8). These simulations were performed in the Redfield framework.(107)

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Conflicts of Interest

The authors declare that they have no conflicts of interest with the contents of this article.2

References

1. Paravastu, A. K., Qahwash, I., Leapman, R. D., Meredith, S. C., and Tycko, R. (2009) Seeded growth of beta-amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure, Proc. Natl. Aca. Sci. U.S.A. 106, 7443-7448.
2. Petkova, A. T., Leapman, R. D., Guo, Z. H., Yau, W. M., Mattson, M. P., and Tycko, R. (2005) Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils, Science 307, 262-265.
3. Serra-Vidal, B., Pujadas, L., Rossi, D., Soriano, E., Madurga, S., and Carulla, N. (2014) Hydrogen/deuterium exchange-protected oligomers populated during Abeta fibril formation correlate with neuronal cell death, ACS Chem. Biol. 9, 2678-2685.
4. Laganowsky, A., Liu, C., Sawaya, M. R., Whitelegge, J. P., Park, J., Zhao, M., Pensalfini, A., Sorigia, A. B., Landau, M., Penc, P. K., Cascio, D., Glabe, C., and Eisenberg, D. (2012) Atomic view of a toxic amyloid small oligomer, Science 335, 1228-1231.
5. Delgado, D. A., Doherty, K., Cheng, Q., Kim, H., Xu, D., Dong, H., Greer, C., and Qiang, W. (2016) Distinct membrane disruption pathways are induced by 40-Residue beta-Amyloid peptides, J. Biol. Chem. 291, 12233-12244.
6. Hubin, E., van Nuland, N. A. J., Broersen, K., and Pauwels, K. (2014) Transient dynamics of A beta contribute to toxicity in Alzheimer's disease, Cell. Mol. Life Sci. 71, 3507-3521.
7. Jarrett, J. T., Berger, E. P., and Lansbury, P. T., Jr. (1993) The C-terminus of the beta protein is critical in amyloidogenesis, Annal. N. Y. Acad. Sci. 695, 144-148.
8. Yang, M. F., and Teplow, D. B. (2008) Amyloid beta-protein monomer folding, J. Mol. Biol. 384, 450-464.
9. Xu, L., Chen, Y. G., and Wad, X. J. (2014) Dual effects of familial Alzheimer's disease mutations (D7H, D7N, and H6R) on amyloid beta peptide: Correlation dynamics and zinc binding, Proteins: Struc. Fun. Bioinfo. 82, 3286-3297.
Flexibility of N-terminal domain of Aβ fibrils

10. Sgourakis, N. G., Yan, Y. L., McCallum, S. A., Wang, C. Y., and Garcia, A. E. (2007) The Alzheimer's peptides A beta 40 and 42 adopt distinct conformations in water: A combined MD/NMR study, *J. Mol. Biol.* **368**, 1448-1457.

11. Ono, K., Condron, M. M., and Teplow, D. B. (2010) Effects of the English (H6R) and Tottori (D7N) familial Alzheimer disease mutations on Amyloid beta-protein assembly and toxicity, *J. Biol. Chem.* **285**, 23184-23195.

12. Mazzitelli, S., Filipello, F., Rasile, M., Lauranzano, E., Starvaggi-Cucuzza, C., Tamborini, M., Pozzi, D., Barajon, I., Giorgino, T., Natalelo, L., and Matteoli, M. (2016) Amyloid-β 1–24 C-terminal truncated fragment promotes amyloid-β 1–42 aggregate formation in the healthy brain, *Acta Neuropath Commun* **4**, 110.

13. Brannstrom, K., Ohman, A., Nilsson, L., Pihl, M., Sandblad, L., and Olofsson, A. (2014) The N-terminal region of amyloid beta controls the aggregation rate and fibril stability at low pH through a gain of function mechanism, *J. Am. Chem. Soc.* **136**, 10956-10964.

14. Morris, C., Cupples, S., Kent, T. W., Elbassal, E. A., Wojcikiewicz, E. P., Yi, P., and Du, D. (2018) N-Terminal charged residues of amyloid-beta peptide modulate amyloidogenesis and interaction with lipid membrane, *Chemistry* **24**, 9494-9498.

15. Nussbaum, J. M., Schilling, S., Cynis, H., Silva, A., Swanson, E., Wangsanut, T., Tayler, K., Willgen, B., Hatami, A., Ronicke, R., Reymann, K., Hutter-Paier, B., Alexandru, A., Jagla, W., Graubner, S., Glabe, C. G., Demuth, H. U., and Bloom, G. S. (2012) Prion-like behaviour and tau-dependent cytotoxicity of pyroglutamylated amyloid-beta, *Nature* **485**, 651-655.

16. Kummer, M. P., Hermes, M., Delekarte, A., Hammerschmidt, T., Kumar, S., Terwel, D., Walter, J., Pape, H. C., Konig, S., Roeber, S., Jessen, F., Klockgether, T., Korte, M., and Heneka, M. T. (2011) Nitration of tyrosine 10 critically enhances amyloid beta aggregation and plaque formation, *Neuron* **71**, 833-844.

17. Rezaei-Ghaleh, N., Amininasab, M., Kumar, S., Walter, J., and Zweckstetter, M. (2016) Phosphorylation modifies the molecular stability of beta-amyloid deposits, *Nat. Commun.* **7**, 11359.

18. Xu, L., Nussinov, R., and Ma, B. (2016) Allosteric stabilization of the amyloid-β peptide hairpin by the fluctuating N-terminal, *Chem Comm* **52**, 1733-1736.

19. Istrate, A. N., Kozin, S. A., Zhokhov, S. S., Mantsyzov, A. B., Pastore, A., Makarov, A. A., and Polshakov, V. I. (2016) Interplay of histidine residues of the Alzheimer's disease Aβ peptide governs its Zn-induced oligomerization, *Sci. Rep.* **6**, 21734.

20. Bush, A. I. (2013) The Metal theory of Alzheimer's disease, *J. Alzheim. Dis.* **33**, S277-S281.

21. Miller, Y., Ma, B. Y., and Nussinov, R. (2012) Metal binding sites in amyloid oligomers: Complexes and mechanisms, *Coord. Chem.* **256**, 2245-2252.

22. Tougu, V., Tiiman, A., and Palumaa, P. (2011) Interactions of Zn(II) and Cu(II) ions with Alzheimer's amyloid-beta peptide. Metal ion binding, contribution to fibrillization and toxicity, *Metallomics* **3**, 250-261.

23. Miura, T., Yoda, M., Tsutsumi, C., Murayama, K., and Takeuchi, H. (2010) Conformational regulation of Amyloid beta-peptide by lipid membranes and Metal Ions, *J Pharm. Soc. Japan* **130**, 495-501.

24. Miura, T., Suzuki, K., Kohata, N., and Takeuchi, H. (2000) Metal binding modes of Alzheimer's amyloid beta-peptide in insoluble aggregates and soluble complexes, *Biochemistry* **39**, 7024-7031.

25. Kulikova, A. A., Makarov, A. A., and Kozin, S. A. (2015) Roles of zinc ions and structural polymorphism of beta-amyloid in the development of Alzheimer's disease, *Mol. Biol.* **49**, 217-230.

26. Kummer, M. P., and Heneka, M. T. (2014) Truncated and modified amyloid-beta species, *Alzheim. Reser. Ther.* **6**.

27. Rezaei-Ghaleh, N., Kumar, S., Walter, J., and Zweckstetter, M. (2016) Phosphorylation interferes with maturation of Amyloid-β fibrillar structure in the N-terminus, *J. Biol. Chem.* **291**, 16059-67.

28. Hu, Z.-W., Ma, M.-R., Chen, Y.-X., Zhao, Y.-F., Qiang, W., and Li, Y.-M. (2017) Phosphorylation at Ser8 as an intrinsic regulatory switch to regulate the morphologies and structures of Alzheimer's 40-residue β-Amyloid (Aβ40) Fibrils, *J. Biol. Chem.* **292**, 2611-2623.
Flexibility of N-terminal domain of Aβ fibrils

29. Jawhar, S., Wirths, O., and Bayer, T. A. (2011) Pyroglutamate Amyloid-β (Aβ): A hatchet man in Alzheimer disease, J. Biol. Chem. 286, 38825-38832.

30. Roher, A. E., Lowenson, J. D., Clarke, S., Wolkow, C., Wang, R., Cotter, R. J., Reardon, I. M., Zurchermeely, H. A., Heinrikson, R. L., Ball, M. J., and Greenberg, B. D. (1993) Structural alterations in the peptide backbone of beta-amyloid core protein may account for its deposition and stability in Alzheimer's disease, J. Biol. Chem. 268, 3072-3083.

31. Shimizu, T., Fukuda, H., Murayama, S., Izumiyama, N., and Shirasawa, T. (2002) Isoaspartate formation at position 23 of amyloid beta peptide enhanced fibril formation and deposited onto senile plaques and vascular amyloids in Alzheimer's disease, J. Neurosci. Res. 70, 451-461.

32. Kuo, Y. M., Webster, S., Emmerling, M. R., De Lima, N., and Roher, A. E. (1998) Irreversible dimerization/tetramerization and post-translational modifications inhibit proteolytic degradation of Aβ peptides of Alzheimer's disease, Biochim. Biophys. Acta 1406, 291-298.

33. Fukuda, H., Shimizu, T., Nakajima, M., Mori, H., and Shirasawa, T. (1999) Synthesis, aggregation, and neurotoxicity of the Alzheimer's A beta 1-42 amyloid peptide and its isoaspartyl isomers, Bioorg. Med. Chem. Lett. 9, 953-956.

34. Janssen, J. C., Beck, J. A., Campbell, T. A., Dickinson, A., Fox, N. C., Harvey, R. J., Houlden, H., Rossor, M. N., and Collinge, J. (2003) Early onset familial Alzheimer's disease - Mutation frequency in 31 families, Neurology 60, 235-239.

35. Hori, Y., Hashimoto, T., Wakutani, Y., Urakami, K., Nakashima, K., Condron, M. M., Tsubuki, S., Saida, T. C., Teplow, D. B., and Iwatsubo, T. (2007) The Tottori (D7N) and English (H6R) familial Alzheimer disease mutations accelerate A beta fibril formation without increasing protofibril formation, J. Biol. Chem. 282, 4916-4923.

36. Kumar, S., and Walter, J. (2011) Phosphorylation of amyloid beta (Aβ) peptides – A trigger for formation of toxic aggregates in Alzheimer's disease, Aging 3, 803-812.

37. Kumar, S., Singh, S., Hinze, D., Josten, M., Sahl, H.-G., Siepmann, M., and Walter, J. (2012) Phosphorylation of Amyloid-β peptide at serine 8 attenuates its clearance via insulin-degrading and angiotensin-converting enzymes, J. Biol. Chem. 287, 8641-8651.

38. Rezaei-Ghaleh, N., Amininasab, M., Kumar, S., Walter, J., and Zweckstetter, M. (2016) Phosphorylation modifies the molecular stability of β-amyloid deposits, Nat. Commun. 7, 11359.

39. Hu, Z. W., Vugmeyster, L., Au, D. F., Ostrovsky, D., Sun, Y., and Qiang, W. (2018) The solution structure of a “seeding-prone” N-terminal phosphorylated β-amyloid fibrils, submitted.

40. Hamley, I. W. (2012) The Amyloid beta peptide: A chemist's perspective. Role in Alzheimer's and fibrillization, Chem. Rev. 112, 5147-5192.

41. Ayton, S., Lei, P., and Bush, A. I. (2015) Biometals and their therapeutic implications in Alzheimer's diseases, Neurotherapeutics 12, 109-120.

42. Suh, S. W., Jensen, K. B., Jensen, M. S., Silva, D. S., Kesslak, P. J., Danscher, G., and Frederickson, C. J. (2000) Histochemically-reactive zinc in amyloid plaques, angiopathy, and degenerating neurons of Alzheimer's diseased brains, Brain Res. 852, 274-278.

43. Lovell, M. A., Robertson, J. D., Teesdale, W. J., Campbell, J. L., and Markesbery, W. R. (1998) Copper, iron and zinc in Alzheimer's disease senile plaques, J. Neurosci. 158, 47-52.

44. Noy, D., Solomonov, I., Sinkevich, O., Arad, T., Kjaer, K., and Sagi, I. (2008) Zinc-amyloid-beta interactions on a millisecond time-scale stabilize non-fibrillar Alzheimer-related species, J. Am. Chem. Soc. 130, 1376-1383.

45. Lovell, M. A., Robertson, J. D., Teesdale, W. J., Campbell, J. L., and Markesbery, W. R. (1998) Copper, iron and zinc in Alzheimer's disease senile plaques, J. Neurosci. 158, 47-52.

46. Miller, Y., Ma, B. Y., and Nussinov, R. (2010) Zinc ions promote Alzheimer A beta aggregation via population shift of polymorphic states, Proc. Natl. Acad. Sci. USA 107, 9490-9495.

47. Iannuzzi, C., Adrover, M., Puglisi, R., Yan, R., Temussi, P. A., and Pastore, A. (2014) The role of zinc in the stability of the marginally stable IscU scaffold protein, Prot. Sci. 23, 1208-1219.

48. Tsvetkov, P. O., Kulikova, A. A., Golovin, A. V., Tkachev, Y. V., Archakov, A. I., Koizin, S. A., and Makarov, A. A. (2010) Minimal Zn2+ Binding Site of Amyloid-beta, Biophys. J. 99, L84-L86.
Flexibility of N-terminal domain of Aβ fibrils

49. Wright, P. E., and Dyson, H. J. (2015) Intrinsically disordered proteins in cellular signalling and regulation, *Nat. Rev. Mol. Cell. Bio.* **16**, 18-29.

50. Berlow, R. B., Dyson, H. J., and Wright, P. E. (2018) Expanding the Paradigm: Intrinsically Disordered Proteins and Allosteric Regulation, *J. Mol. Biol.* **430**, 2309-2320.

51. Larion, M., Miller, B., and Brüschweiler, R. (2015) Conformational heterogeneity and intrinsic disorder in enzyme regulation: Glucokinase as a case study, *Intrin. Disord. Prot.* **3**, e1011008.

52. Scheidt, H. A., Morgado, I., Rothemund, S., and Huster, D. (2012) Dynamics of Amyloid beta fibrils revealed by solid-state NMR, *J. Biol. Chem.* **287**, 2017-2021.

53. Luhrs, T., Ritter, C., Adrian, M., Rick-Loher, D., Bohrmann, B., Doeli, H., Schubert, D., and Riek, R. (2005) 3D structure of Alzheimer's amyloid-beta(1-42) fibrils, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 17342-17347.

54. Petkova, A. T., Yau, W. M., and Tycko, R. (2006) Experimental constraints on quaternary structure in Alzheimer's beta-amyloid fibrils, *Biochemistry* **45**, 498-512.

55. Bertini, I., Bonnelli, L., Luchinat, C., Mao, J., and Nesi, A. (2011) A New structural model of A beta(40) fibrils, *J. Am. Chem. Soc.* **133**, 16013-16022.

56. Olofsson, A., Sauer-Eriksson, A. E., and Ohman, A. (2006) The solvent protection of Alzheimer amyloid-beta-(1-42) fibrils as determined by solution NMR spectroscopy, *J. Biol. Chem.* **281**, 477-483.

57. Whittemore, N. A., Mishra, R., Kheterpal, I., Williams, A. D., Wetzel, R., and Serpersu, E. H. (2005) Hydrogen-deuterium (H/D) exchange mapping of Aβ(1-40) amyloid fibril secondary structure using nuclear magnetic resonance spectroscopy, *Biochemistry* **44**, 4434-4441.

58. Torok, M., Milton, S., Kayed, R., Wu, P., McIntire, T., Glabe, C. G., and Langen, R. (2002) Structural and dynamic features of Alzheimer's A beta peptide in amyloid fibrils studied by site-directed spin labeling, *J. Biol. Chem.* **277**, 40810-40815.

59. Kheterpal, I., Zhou, S., Cook, K. D., and Wetzel, R. (2000) A beta amyloid fibrils possess a core structure highly resistant to hydrogen exchange, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13597-13601.

60. Wang, S. S. S., Tobler, S. A., Good, T. A., and Fernandez, E. J. (2003) Hydrogen exchange-mass spectrometry analysis of beta-Amyloid peptide structure, *Biochemistry* **42**, 9507-9514.

61. Kheterpal, I., Chen, M., Cook, K. D., and Wetzel, R. (2006) Structural differences in A beta amyloid protofibrils and fibrils mapped by hydrogen exchange - Mass spectrometry with on-line proteolytic fragmentation, *J. Mol. Biol.* **361**, 785-795.

62. Sawaya, M. R., Sambashivan, S., Nelson, R., Ivanova, M. I., Sievers, S. A., Apostol, M. I., Thompson, M. J., Balbirnie, M., Wiltzius, J. J. W., McFarlane, H. T., Madsen, A. O., Riekel, C., and Eisenberg, D. (2007) Atomic structures of amyloid cross-beta spines reveal varied steric zippers, *Nature* **447**, 453-457.

63. Liu, H., Morris, C., Lantz, R., Kent, T. W., Elbassal, E. A., Wojciechiewicz, E. P., and Du, D. (2018) Residue-specific dynamics and local environmental changes in Abeta40 oligomer and fibril formation, *Angew. Chem. Int. Ed.* **57**, 8017-8021.

64. Linser, R., Sarkar, R., Krushelnitzky, A., Mainz, A., and Reif, B. (2014) Dynamics in the solid-state: perspectives for the investigation of amyloid aggregates, membrane proteins and soluble protein complexes, *J. Biomol. NMR* **59**, 1-14.

65. Vugmeyster, L., Ostrovsky, D., Hoatson, G. L., Qiang, W., and Falconer, B. I. (2017) Phenylalanine side-chain in the hydrophobic core of amyloid fibrils reveals a solvent-driven dynamical transition, *J. Phys. Chem. B* **121**, 7267–7275.

66. Vugmeyster, L., Ostrovsky, D., Clark, M. A., Falconer, B. I., Hoatson, G. L., and Qiang, W. (2016) Fast motions of key methyl groups in Amyloid-beta fibrils, *Biophys. J.* **111**, 2135-2148.

67. Vugmeyster, L., Clark, M. A., Falconer, B. I., Ostrovsky, D., Gantz, D., Qiang, W., and Hoatson, G. L. (2016) Flexibility and solvation of amyloid -beta hydrophobic core, *J. Biol. Chem.* **291**, 18484-18495.

68. Karamanos, T. K., Kalverda, A. P., Thompson, G. S., and Radford, S. E. (2015) Mechanisms of amyloid formation revealed by solution NMR, *Prog. Nucl. Magn. Reson. Spec.* **88-89**, 86-104.
69. Wang, T., Jo, H., DeGrado, W. F., and Hong, M. (2017) Water distribution, dynamics, and interactions with Alzheimer’s β-amyloid fibrils investigated by solid-state NMR, *J. Am. Chem. Soc.* **39**, 6242-6252.

70. Fawzi, N. L., Libich, D. S., Ying, J., Tugarinov, V., and Clore, G. M. (2014) Characterizing methyl-bearing side chain contacts and dynamics mediating Amyloid β protofibril interactions using (13)C(methyl)-DEST and lifetime line broadening, *Angew. Chem.* **53**, 10345-10349.

71. Fawzi, N. L., Ying, J., Ghirlando, R., Torchia, D. A., and Clore, G. M. (2011) Atomic-resolution dynamics on the surface of amyloid-beta protofibrils probed by solution NMR, *Nature* **480**, 268-272.

72. Paravastu, A. K., Petkova, A. T., and Tycko, R. (2006) Polymorphic fibril formation by residues 10–40 of the Alzheimer’s β-Amyloid peptide, *Biophys. J.* **90**, 4618-4629.

73. Vold, R. L., and Vold, R. R. (1991) Deuterium telaxation in molecular solids, in *Advances in Magnetic and Optical Resonance* (Warren, W., Ed.)Eds.), pp 85-171, Academic Press, San Diego.

74. Meints, G. A., Miller, P. A., Pederson, K., Shajani, Z., and Drobny, G. (2008) Solid-state nuclear magnetic resonance spectroscopy studies of furanose ring dynamics in the DNA Hhal binding site, *J. Am. Chem. Soc.* **130**, 7305-7314.

75. Vugmeyster, L., and Ostrovsky, D. (2017) Static Solid-state 2H NMR methods in studies of protein side-chain dynamics, *Prog. Nucl. Magn. Reson. Spec.* **101**, 1-17.

76. Molugu, T. R., Lee, S., and Brown, M. F. (2017) Concepts and methods of solid-state NMR spectroscopy applied to biomembranes, *Chem Rev* **117**, 12087-12132.

77. Paravastu, A. K., Leapman, R. D., Yau, W. M., and Tycko, R. (2008) Molecular structural basis for polymorphism in Alzheimer's beta-amyloid fibrils, *Proc. Natl. Aca. Sci. U.S.A.* **105**, 18349-18354.

78. Vugmeyster, L., Ostrovsky, D., Khadjinova, A., Ellden, J., Hoatson, G. L., and Vold, R. L. (2011) Slow motions in the hydrophobic core of chicken villin headpiece subdomain and their contributions to configurational entropy and heat capacity from solid-state deuteron NMR measurements, *Biochemistry* **50**, 10637–10646.

79. Vugmeyster, L., and Ostrovsky, D. (2018) Basic experiments in 2H static NMR for the characterization of protein side-chain dynamics, doi: 10.1016/j.ymeth.2018.04.030.

80. Palmer, A. G. (2004) NMR characterization of the dynamics of biomacromolecules, *Chem. Rev.* **104**, 3623-3640.

81. Lakomek, N.-A., Penzel, S., Lends, A., Cadalbert, R., Ernst, M., and Meier, B. (2017) Microsecond dynamics in ubiquitin probed by solid-state NMR 15N R1rho relaxation experiments under fast MAS (60-110 kHz), *Chemistry* **23**, 9425 -9433.

82. Krushelnitsky, A., Zinkevich, T., Reif, B., and Saalwächter, K. (2014) Slow motions in microcrystalline proteins as observed by MAS-dependent 15N rotating-frame NMR relaxation, *J. Magn. Reson.* **248**, 8-12.

83. Kurauskas, V., Izmailov, S. A., Rogacheva, O. N., Hessel, A., Ayala, I., Woodhouse, J., Shilova, A., Xue, Y., Yuwen, T., Coquelle, N., Colletier, J.-P., Skrynnikov, N. R., and Schanda, P. (2017) Slow conformational exchange and overall rocking motion in ubiquitin protein crystals, *Nature. Commun.* **8**, 145.

84. Quinn, C., and McDermott, A. (2009) Monitoring conformational dynamics with solid-state R1ρ experiments, *J. Biomol. NMR* **45**, 5-8.

85. Ma, P., Haller, J. D., Zajakala, J., Macek, P., Sivertsen, A. C., Willbold, D., Boisbouvier, J., and Schanda, P. (2014) Probing transient conformational states of proteins by solid-State R(1ρ) relaxation-dispersion NMR spectroscopy, *Angew. Chem. Int. Ed.* **53**, 4312-4317.

86. Quinn, C. M., and McDermott, A. E. (2012) Quantifying conformational dynamics using solid-state R1ρ experiments, *J. Biomol. NMR* **222**, 1-7.

87. Gauto, D. F., Hessel, A., Rovó, P., Kurauskas, V., Linser, R., and Schanda, P. (2017) Protein conformational dynamics studied by 15N and 1H R1ρ relaxation dispersion: Application to wildtype and G53A ubiquitin crystals, *Solid State Nucl Magn Reson* **87**, 86-95.
Flexibility of N-terminal domain of Aβ fibrils

88. Rovó, P., and Linser, R. (2018) Microsecond timescale protein dynamics: a combined solid-state NMR Approach, ChemPhysChem 19, 34-39.
89. Rovó, P., and Linser, R. (2017) Microsecond time scale proton rotating-frame relaxation under magic angle spinning, J. Phys. Chem. B 121, 6117-6130.
90. Krushelnitsky, A., Gauto, D., Rodriguez Camargo, D. C., Schanda, P., and Saalwachter, K. (2018) Microsecond motions probed by near-rotary-resonance R1rho(15)N MAS NMR experiments: the model case of protein overall-rocking in crystals, J. Biomol. NMR 71, 53-67.
91. Shannon, M., Theint, T., Mukhopadhyay, D., Surewicz, K., Surewicz, W., Marion, D., Schanda, P., and Jaroniec, C. P. Conformational dynamics in the core of human Y145Stop prion protein amyloid probed by relaxation dispersion NMR, ChemPhysChem. doi: 10.1002/cphc.201800779
92. Smith, A. A., Testori, E., Cadalbert, R., Meier, B. H., and Ernst, M. (2016) Characterization of fibril dynamics on three timescales by solid-state NMR, J. Biomol. NMR 65, 171-191.
93. Doster, W. (2008) The dynamical transition of proteins, concepts and misconceptions, Eur. Biophys. J. 37, 591-602.
94. Lewandowski, J. R., Halse, M. E., Blackledge, M., and Emsley, L. (2015) Direct observation of hierarchical protein dynamics, Science 348, 578-581.
95. Ringe, D., and Petsko, G. A. (2003) The 'glass transition' in protein dynamics: what it is, why it occurs, and how to exploit it, Biophys. Chem. 105, 667-680.
96. Kussell, E., and Shakhnovich, E. I. (2002) Glassy dynamics of side-chain ordering in a simple model of protein folding, Phys Rev Lett 89:168101-110.
97. Bauer, T., Dotta, C., Balacescu, L., Gath, J., Hunkeler, A., Böckmann, A., and Meier, B. H. (2017) Line-Broadening in Low-Temperature Solid-State NMR Spectra of Fibrils, J. Biomol NMR 67, 51-61.
98. Thirumalai, D., Reddy, G., and Straub, J. E. (2012) Role of water in protein aggregation and amyloid polymorphism, Acc. Chem. Res 45, 83-92.
99. Malinska, M., Dauter, M., Kowiel, M., Jaskolski, M., and Dauter, Z. (2015) Protonation and geometry of histidine rings, Acta crystal. D, 71, 1444-1454.
100. Vold, R. R. (1994) Deuterium NMR studies of dynamics in solids and liquid crystals, in Nuclear Magnetic Resonance Probes of Molecular Dynamics (Tycko, R., Eds.), pp 27-112, Kluwer academic Publishers, Dordrecht.
101. Gall, C. M., Cross, T. A., Diverdi, J. A., and Opella, S. J. (1982) Protein dynamics by solid-state NMR- aromatic rings of the Coat Protein in Fd Bacteriophage, Proc. Natl. Acad. Sci. U. S. A. 79, 101-105.
102. Hiyama, Y., Silverton, J. V., Torchia, D. A., Gerig, J. T., and Hammond, S. J. (1986) Molecular-structure and dynamics of crystalline para-fluoro-D,L-phenylalanine - a combined X-ray NMR investigation, J. Am. Chem. Soc. 108, 2715-2723.
103. McConnell, H. M. (1958) Reaction rates by Nuclear Magnetic Resonance, J. Chem. Phys. 28, 430-431.
104. Vugmeyster, L., Ostrovsky, D., Moses, M., Ford, J. J., Lipton, A. S., Hoatson, G. L., and Vold, R. L. (2010) Comparative dynamics of leucine methyl groups in FMOC-leucine and in a protein hydrophobic core probed by solid-state deuteron NMR over 7-324K temperature range, J. Phys. Chem. 114 15799–15807.
105. Vugmeyster, L., and Ostrovsky, D. (2018) Deuterium rotating frame NMR Relaxation measurements in the solid state under static conditions for quantification of dynamics, Chemphyschem. doi: 10.1002/cphc.201800454.
106. Broersen, K., Rousseau, F., and Schymkowitz, J. (2010) The culprit behind amyloid beta peptide related neurotoxicity in Alzheimer’s disease: oligomer size or conformation?, Alzheimer's Reser. Ther. 2, 12-12.
107. Abragam, A. (1961) Principles of Nuclear Magnetism, Clarendon Press, Oxford.
108. Hubin, E., Deroo, S., Schierle, G. K., Kaminski, C., Serpell, L., Subramaniam, V., van Nuland, N., Broersen, K., Raussens, V., and Sarroukh, R. (2015) Two distinct β-sheet structures in Italian-
Flexibility of N-terminal domain of Aβ fibrils

mutant amyloid-beta fibrils: a potential link to different clinical phenotypes, Cell. Mol. Life Sci. 72, 4899-4913.

109. Kodali, R., Williams, A. D., Chemuru, S., and Wetzel, R. (2010) A beta(1-40) Forms Five Distinct Amyloid Structures whose beta-Sheet Contents and Fibril Stabilities Are Correlated, J. Mol. Biol. 401, 503-517.

110. Meinhardt, J., Sachse, C., Hortschansky, P., Grigorieff, N., and Fandrich, M. (2009) Abeta(1-40) fibril polymorphism implies diverse interaction patterns in amyloid fibrils, J. Mol. Biol. 386, 869-877.

111. Viet, M. H., Nguyen, P. H., Ngo, S. T., Li, M. S., and Derreumaux, P. (2013) Effect of the Tottori familial disease mutation (D7N) on the monomers and dimers of Abeta40 and Abeta42, ACS Chem Neurosci 4, 1446-1457.

112. Harris, J. R., and Horne, J. W. (1991) Negative Staining, in Electron Microscopy in Biology (Harris, J. R., Ed.)Eds.), pp 203-228, Oxford: IRL Press.

113. Dubochet, J., Groom, M., and Mueller-Neuteboom, S. (1082) The mounting of macromolecules for electron microscopy with particular reference to surface phenomena and the treatment of support films by glow discharge., in Adv. Opt. Electron Microsc. (Barrer, R., and Coslett, V. E., Ed.), pp 107-135.

114. Larsen, F. H., Jakobsen, H. J., Ellis, P. D., and Nielsen, N. C. (1998) High-field QCPMG-MAS NMR of half-integer quadrupolar nuclei with large quadrupole couplings, Mol. Phys. 95, 1185-1195.

115. Vold, R. L., Hoatson, G. L., Vugmeyster, L., Ostrovsky, D., and De Castro, P. J. (2009) Solid state deuteron relaxation time anisotropy measured with multiple echo acquisition, Phys. Chem. Chem. Phys. 11, 7008-7012.

116. Gor'kov, P. L., Chekmenev, E. Y., Li, C., Cotten, M., Buffy, J. J., Traaseth, N. J., Veglia, G., and Brey, W. W. (2007) Using low-E resonators to reduce RF heating in biological samples for static solid-state NMR up to 900 MHz, J. Magn. Reson. 185, 77-93.

117. Persson, P.-O., and Strang, G. (2004) A Simple mesh generator in MATLAB, SIAM Rev. 46, 329-345.

118. Vold, R. L., and Hoatson, G. L. (2009) Effects of jump dynamics on solid state nuclear magnetic resonance line shapes and spin relaxation times, J. Magn. Reson. 198, 57-72.

119. Hirschinger, J., Miura, H., Gardner, K. H., and English, A. D. (1990) Segmental dynamics in the crystalline phase of Nylon-66 solid-state H-2 NMR, Macromol. 23, 2153-2169.

Footnotes

1The abbreviations used are: Aβ, amyloid-β; TEM, transmission electron microscopy; FMOC, Fluorenylmethyloxycarbonyl; DMSO, dimethyl sulfoxide; HPLC, high-pressure liquid chromatography; NMR, nuclear magnetic resonance, DLS, dynamic light scattering.

2The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
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Tables

**Table 1.** Residue-specific side-chain labeling patterns and main local motional modes at high temperatures for the free state of the N-terminal domain.

| Residue | Motional mode | Labeling pattern |
|---------|---------------|------------------|
| A2      | Methyl three-site jumps | $\beta\text{D}_3$ |
| F4      | Ring flips | $\beta\text{CH}_2\text{-ring-}\text{D}_3$ |
| H6      | Ring-flip high temperature limit (angle of 30.55° (between $\text{C}^\beta\text{-C}^\gamma$ and $\text{CD}_3\text{-N}^\varepsilon$)) \(99\) and methyl three-site jumps | $\beta\text{CH}_2\text{-ring-N-C}_3\text{D}_3$ (i.e., $\tau\text{-His}$) |
| G9      | Two-site jumps of $\text{CD}_2$ with tetrahedral geometry | $\alpha\text{CD}_2$ |
| V12     | Methyl three-site jumps and rotameric interconversions | $\beta\text{CD}_2\text{−(CD)}_3$ |

**Table 2.** Quadrupolar coupling tensor parameters: quadrupolar coupling constant $C_q$ and asymmetry $\eta$ for the N-terminal residues of Aβ1-40.

| Residue | Effective $C_q$ and $\eta$ in the fast motional limit for line shape simulations | Static $C_q$ and $\eta$ for relaxation simulations |
|---------|---------------------------------------------------------------------------------|-------------------------------------------------|
| A2      | $C_q = 55.5\text{kHz, } \eta = 0$ | $C_q = 165.5\text{kHz, } \eta = 0$ |
| F4      | $C_q = 107\text{kHz, } \eta = 0.7$ | $C_q = 180\text{kHz, } \eta = 0$ |
| H6      | $C_q = 32.6\text{kHz, } \eta = 0.63$ | $C_q = 159\text{kHz, } \eta = 0$ |
| G9      | $C_q = 77.6\text{kHz, } \eta = 1$ | $C_q = 155\text{kHz, } \eta = 0$ |
| V12     | $C_q = 53.3\text{kHz, } \eta = 0$ and rotameric interconversions | $C_q = 160\text{kHz, } \eta = 0$ |
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Figures

**Figure 1.** Schematic representation of Aβ1-40 protein with the amino acid side chains probed in this work labeled. The structure of the monomer for residues 9 and beyond is taken from the protein data bank file 2LMP.pdb, while the rest of the N-terminal domain is shown schematically as a line. B) 3-fold symmetric fibrils structure, top view. (77) C) A typical negatively stained TEM image of the resulting morphology, shown for fibrils labeled at the F4 site.

**Figure 2.** Representative $^2$H static solid-state NMR line shape data in the hydrated fibril samples of Aβ1-40 in the 3-fold symmetric polymorph at several temperatures. Also shown (lowest right panel, in red) is the line shape of the isolated N-terminal domain (residues 1–16) labeled at the F4-ring-$d_5$ position, for which the narrow peak around 0 kHz is attributed to the HOD signal. Note the intrinsic differences in the quadrupolar interactions for different residues, specified in Table 2.
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Figure 3. $^2$H longitudinal relaxation times $T_1$ vs. 1000/$T$ recorded at 17.6 T field strength in the dry (black) and hydrated (blue) fibril samples of Aβ$_{1-40}$ for the major singularities of the powder pattern spectra: 0 kHz (squares), ±20 kHz for A2, H6, and V12 (circles), ±60 kHz for F4 and G9 (circles). Error bars smaller than the size of the symbol are not shown. Note the different $T_1$ scales for the different residues.

Figure 4. Comparison of the normalized $^2$H static solid-state NMR line shapes for the dry (black) and hydrated (blue) states of the Aβ$_{1-40}$ fibrils in the 3-fold symmetric polymorph.
Figure 5. Schematic representation of the model in which the disordered N-terminal domain (curved line) transiently interacts with the structured C-terminal domain (blue rectangle). In the free state, the N-terminal domain is assumed to undergo isotropic diffusion, as represented by the gray sphere, while in the bound state, the interactions quench this mode. The parameters of the models are shown as the corresponding symbols.
Figure 6. A) Fraction of the bound state ($p_{\text{bound}}$) derived from the $^2$H line shape analysis, either determined directly from line shape data at 37°C (blue) or as the upper temperature baseline parameter from the fits to the sigmoidal curves of Eq. (1) (black). Note that the fit to Eq. (1) was not performed for V12. B) $p_{\text{bound}}$ as a function of the temperature. The solid lines represent the fits to the data according to Eq. (1). C) Diffusion coefficient $D$ fitted based on the $^2$H line shapes at 37°C according to the isotropic diffusion approximation.
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Figure 7. Experimental A) $T_{1p}^{\text{fast}} = 1/R_{1p}^{\text{fast}}$ relaxation times (corresponding to the fast component of the double-exponential fit) and B) $T_{1p}^{\text{slow}}$ versus spin-lock field $\omega_{\text{SL}}$ at 37°C (red circles) and 25°C (blue squares) at 14 T for hydrated Aβ1-40 fibrils in the 3-fold symmetric polymorph labeled at the A2–CβD3 position. Solid lines represent the best-fit simulated data to the two-site exchange model described in the text. C) Examples of the partially relaxed line shapes at 37°C for the $R_1$ measurements at several values of spin-lock times for the 15 kHz spin-lock field, indicated on the graph. D) Experimental magnetization decay curves for the 15 kHz field at 37°C. Peak intensities integrated over the −1 to +1 kHz region (which is approximately the width at the half-height of the peak shown in C) in arbitrary units versus time (circles); the solid line shows the double-exponential fit.
Figure 8. Comparison of the dynamics between the hydrated 3-fold Aβ1-40 fibrils (blue) and hydrated Zn²⁺-induced aggregates. A) ²H static solid-state NMR line shapes at 37 °C and representative spectra around the freezing transition region. B) Midpoint melting temperatures $T_m$ derived from fitting the fractions of the bound states (SI10) to Eq. (1). C) ²H $T_{1P}$ relaxation times (corresponding to the fast component of the double-exponential fit) versus the spin-lock field $\omega_{SL}$ at 37°C at 14 T for the A2–C⁰D₃ site. Solid lines represent the best-fit simulated data to the two-site exchange model described in the text.
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