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Title

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Differences in β−strand Populations of Monomeric Aβ40 and Aβ42

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Using homonuclear ^{1}H NOESY spectra, with chemical shifts, $^3J_{H^N H^\alpha}$ scalar couplings, residual dipolar couplings, and ^{1}H-^{15}N NOEs, we have optimized and validated the conformational ensembles of the amyloid-β 1-40 (Aβ40) and amyloid-β 1-42 (Aβ42) peptides generated by molecular dynamics simulations. We find that both peptides have a diverse set of secondary structure elements including turns, helices, and anti-parallel and parallel β−strands. The most significant difference in the structural ensembles of the two peptides is the type of β−hairpins and β−strands they populate. We find that Aβ42 forms a major anti-parallel β-hairpin involving the central hydrophobic cluster (CHC) residues (16-21) with residues 29-36, compatible with known amyloid fibril forming regions, while Aβ40 forms an alternative but less populated anti-parallel β-hairpin between the CHC and residues 9-13, that sometimes forms a β−sheet by association with residues 35-37. Furthermore, we show that the two additional C-terminal residues of Aβ42, in particular Ile41, directly control the differences in the β−strand content found between the Aβ40 and Aβ42 structural ensembles. Integrating the experimental and theoretical evidence accumulated over the last decade, it is now possible to present monomeric structural ensembles of Aβ40 and Aβ42 consistent with available information that produce a plausible molecular basis for why Aβ42 exhibits greater fibrillization rates than Aβ40.

INTRODUCTION

Alzheimer's Disease (AD) is characterized by insoluble fibrils and plaques in the extra-cellular space within the brain that are largely composed of the two cleaved products of the amyloid precursor protein[1], amyloid-β 1-40 (Aβ40) and amyloid-β 1-42 (Aβ42)[2, 3]. Although these two peptides differ only in Aβ42 having two additional hydrophobic residues at its C-terminus, Ile41 and Ala42, Aβ42 has been shown to be more significant in disease development. Aβ42 is more prevalent in the insoluble aggregates and causes more extensive damage to neuronal cell cultures than Aβ40[4-9], and Aβ42 aggregates and fibrillizes much more quickly *in vitro* than Aβ40[10, 11], demonstrating that the addition of these two C-terminal residues has a significant effect on the physiological and biophysical behavior of the two peptides.

Monomeric forms of Aβ40 and Aβ42 have been classified as intrinsically disordered peptides (IDPs), meaning that they populate a diverse set of conformational states as opposed to a single dominant folded conformation[12-14]. However, when part of the ordered fibril state, both peptides adopt highly similar morphologies, with β-strands running orthogonal to the fibril axis, which organize further into intermolecular β-sheets that can extend to microns in length[15-20]. Since Aβ40 and Aβ42 adopt similar structures when part of the fibril, differences in the monomeric conformational ensembles could provide a starting point for understanding the greater predisposition of the Aβ42 peptide for faster fibrillization, aberrant oligomerization, or disease outcomes compared to Aβ40. In particular, does the addition of the two hydrophobic residues Ile41 and Ala42 produce any changes in the monomeric conformational ensemble for Aβ42 with respect to Aβ40?

We have collected ^{1}H NOESY spectra for the Aβ40 and Aβ42 monomers that in fact show differences in their structural ensembles, which are not evident from previous circular dichroism (CD) spectra or NMR chemical shift and J-coupling experiments[12, 21-23]. While the NOESY data yield important differences in residue contacts observed for Aβ40 vs. Aβ42, these NMR experiments can only provide an ensemble-averaged picture of the tertiary contacts that occur, and not in what combinations they are present in specific, significantly populated conformers. As we have shown previously in our comparison study of Aβ21-30[24] and Aβ42[25], and more recently in a review of different computational approaches for generating

IDP conformational ensembles*(14)*, *de novo* molecular dynamics (MD) simulations provide one of the best approaches for most reliably characterizing the structural ensembles sampled by Aβ42 and Aβ40 as monomers.

We find that the MD simulation data, further refined with the ENSEMBLE method*(26-29)* and validated against a range of experimental NMR data including ^{1}H NOEs, show that both peptides have a diverse set of secondary structure elements including turns, helices, and parallel and anti-parallel β−strands in ~99% of the ensemble conformers. However, the most significant difference in the structural ensembles of the two peptides is the type of β−structures they populate. We find that Aβ42 forms a major anti-parallel β-hairpin involving the central hydrophobic cluster (CHC) residues 16-21 and residues 29-36, typically forming with a turn at residues 26-27 which is rarely present in the Aβ40 ensemble*(25)*. This dominant sub-population is consistent with the β−strands and β−turn that form an intermolecular β−sheet steric zipper*(30, 31)* in models of the Aβ40 and Aβ42 fibril structures based on solid state NMR*(15, 16)*. Instead, Aβ40 forms an alternative but less populated anti-parallel β-hairpin between the CHC and residues 9-13, that sometimes forms a β-sheet when the CHC associates with a third β−strand comprising residues 35-37.

We find that the two additional C-terminal hydrophobic residues of Aβ42 sharply increases the hydrophobic clustering between residues 39-40 and 31-36 for Aβ42 compared to Aβ40, and when Ile41 is included the number of structures with hydrophobic contacts with 31-36, it increases to a decisive majority of the Aβ42 ensemble. This hydrophobic clustering is directly responsible for the differences in the populations of secondary structure, and β−strand content in particular, of the two amyloid peptides. When our new experimental NOEs and simulated ensemble results are placed in the context of experimental and theoretical evidence accumulated over the last decade*(21, 22, 32-40)*, we believe that a good consensus has been reached on the monomeric structural ensembles of amyloid-β and the differences that exist between Aβ40 and Aβ42. Based on this consensus, the underlying structural differences between the two Aβ monomeric ensembles are in themselves sufficient to provide clear and testable

hypotheses for why the nucleation step for fibrillization may be more difficult for Aβ40 compared to Aβ42.

METHODS

NMR Experiments. The Aβ40 and Aβ42 monomers were purchased and prepared according to the protocol in [(*25*)]. A more detailed description is provided in a previous publication*(14*) and extensively in the Supplementary Information. In this work we also corrected the experimental $^3J_H{}^N{}_H{}^\alpha$ values by Wang et al using the method described by Vuister and Bax*(41)*, also described in Supplementary Information. This correction accounts for the effect of selective T1 relaxation so that the resulting J-coupling constants are comparable to those measured more accurately from COSY splittings.

Molecular Dynamics Simulations. We computed equilibrium ensembles of Aβ40 and Aβ42 peptide conformations at 287 K and 311 K using Multi-Reservoir Replica Exchange (MRRE)*(42*) and AMBER 11*(43)*. The peptides were modeled with the Amber ff99SB force field*(44, 45*) and solvated with TIP4P-Ew water*(46*), which is currently the best force field combination for reproducing NMR observables of flexible peptides*(47*) and ^1H-^1H NOE data*(24, 25*). Two independent MRRE simulations for each peptide generated final ensembles of 70,000 – 90,000 structures pulled from 0.1 µs of replica exchange simulation time at each temperature. We also performed 100 separate 20 ns microcanonical ensemble simulations for each peptide in order to calculate time-correlation data. Further details are presented in the Supplementary Material.

ENSEMBLE Refinements. We also consider the ENSEMBLE software package*(26-29*), which selects from a large starting pool or "basis set" of structures a subset of conformations that best conform to various NMR experimental data supplied to it. We performed an ENSEMBLE optimization using the *de novo* MD ensemble as the starting 'soup' of structures, and we supplied chemical shifts, J-coupling constants, and RDCs for both Aβ40 and Aβ42. We used default values of the ENSEMBLE program for the experimental observable target energies and selected ensembles of 100 structures from the soup to best match the NMR data, combining the 20 best

ensembles for a total of 2,000 structures in the final MD-ENS ensembles. Further details are given in the Supplementary Material.

$\quad\quad$ ***Calculation of NMR Observables***. We back-calculated chemical shifts, J-coupling constants, Residual Dipolar Couplings (RDCs) based on local*(27)* and global alignments*(48)*, and ^{1}H-^{1}H NOEs from our 287 K Aβ40 and Aβ42 refined ensembles with the same procedure used for Aβ42*(25)* and for IDPs*(14)*. All details of how the procedure differed in the case of Aβ40, as well as a correction to a typographical error in the NOE relaxation matrix equation are available in the Supplementary Material. Finally, we calculated ^{1}H-^{15}N NOEs for the Aβ40 and Aβ42 backbone N-H atoms from the 287 K refined ensembles and MD correlation times. We use the same method as for the ^{1}H-^{1}H NOEs (described in the Supplementary Material) to calculate the spectral density function for each pair of H-N atoms from the short NVE simulations. We then calculate the steady state NOE enhancement factor of the ^{15}N spin by the ^{1}H NOE from our structural ensemble and dynamical trajectories as described elsewhere*(14)*, and which we recapitulate in the Supplementary Material.

RESULTS

Summary of experimental NMR data for Aβ40 and Aβ42. We find that the hydrogen and carbon chemical shifts for both Aβ40 and Aβ42 do not differ significantly from random coil values (Figure S1), and based on analysis of chemical shifts using webserver http://www-vendruscolo.ch.cam.ac.uk/d2D/ *(49, 5*0*)*, both peptides have significant β-strand content if backbone nitrogen chemical shifts are considered*(51)*. However if nitrogen chemical shifts (which have larger experimental uncertainty compared to hydrogen and carbon chemical shifts) are not included in the webserver calculation, then the absolute probabilities of β strand structure were found to be very low for both peptides.

$\quad\quad$ J-coupling values also provide no strong evidence of structural differences between the Aβ peptides (Figure S2), i.e. there are no secondary structure "blocks" at different points in the peptide sequence that would be consistent with a dominant population of α-helical or β strand secondary structure (as in a folded protein) that is different between the two peptides. However, the scalar couplings for both peptides are shifted upward from random coil to yield values mostly between 6.0-8.5 Hz, consistent with an extended random coil ensemble or the presence of β-

strand structure, and there are certainly quantitative differences in the scalar couplings between the peptides. While these highly averaged data may imply that the two peptides do not have substantially different structural ensembles, ^{1}H-^{15}N, and ^{1}H NOE data do provide more information about important structural differences between the conformational ensembles of IDPs Aβ40 and Aβ42.

Experimental RDCs (Figure S3 and S4) are also difficult to interpret structurally because the timescale of interconversion between IDP conformational states may be on the same order as the timescale of the protein realignment in the anisotropic medium. However, RDCs for Aβ40 and Aβ42 vary along the peptide sequences and show differences between the two peptides. Thus, these RDC data contain information about Aβ40 and Aβ42 structural differences, which can help to determine the correct ensembles.

Our high field ^{1}H NOE data identifies 707 crosspeaks for Aβ42 and 1108 crosspeaks for the Aβ40 peptide, but only 382 and 196 of these crosspeaks, respectively, can be uniquely assigned from experimental information alone. This is due to the fact that the NOESY spectra are crowded, different ^{1}H atoms have the same chemical shift, and many NOE peaks have multiple possible assignments (Figure S5). In the case of a strong NOE where only one of the possible assignments is a short-range interaction, we can confidently assign the peak. Most of the assigned cross peaks are intra-residue or sequential peaks, and 147 of them are a result of the same ^{1}H-^{1}H contacts occurring in the Aβ40 and Aβ42 ensembles. However Table 1 shows that 235 of the crosspeaks are unique to Aβ40 and 49 are uniquely present for Aβ42. Therefore, the NOEs which can be assigned from experimental information alone already indicate that the structural ensembles are different between the two peptides.

We cannot assign the longer-range NOE peaks uniquely to one ^{1}H-^{1}H contact because all possible assignments are long-range and therefore it is unclear which is correct. However, we can see that the Aβ40 and Aβ42 spectra are different (Figure S5) and many of the longer-range NOEs present in the Aβ40 ensemble are not present for Aβ42 and vice versa. For example, if we look at NOE peaks that because of the chemical shifts (which restrict residue types involved) cannot be a result of any contact between residues closer than i to i+5, we see that 19 out of 40 of the Aβ40 NOEs are not present in the Aβ42 spectrum and 28 out of 46 of the Aβ42 NOEs are not present

in the Aβ40 spectrum (Table 2). This indicates that there are several long-range NOEs for each peptide that are unique to its structural ensemble, and therefore the two amyloid peptide ensembles have distinct structurally features. Further details on the ^{1}H NOE's are presented in Supplementary Information in Tables S1 and S2. For further interpretation a computational model is needed that is validated by the available NMR data and yet expands upon the molecular structure information that would explain the NOE differences found between the two peptides.

Experimental validation of theoretically derived structural ensembles for Aβ40 and Aβ42. In a recent review we considered the effectiveness of *de novo* MD for generating IDP structural ensembles for Aβ40 and Aβ42*(14)*, as compared to random coil or statistically biased secondary structure ensembles, or selecting a subset of structures from the random or statistical ensembles that give a best fit to experimental NMR data, such as developed in the ENSEMBLE*(26-29)* and ASTEROIDS*(52-54)* approaches. The performance of a given computational method was judged by the ability of a given model to reproduce chemical shifts, J couplings, and RDCs based on local (L-RDC) and global alignments, and experimentally assignable ^{1}H-^{1}H NOEs, as averages over the entirety of their conformational ensembles.

We showed that ensembles of structures based on random coil or statistical conformational distributions perform poorly, and there were no subset of structures from these ensembles that could substantially optimize their agreement with the NMR data for Aβ40 and Aβ42. Instead, ensembles incorporating structural members from the *de novo* MD calculations that contain significant amounts of *cooperative* secondary structure content gave much better agreement with all NMR data. Table 3 summarizes the quality of the *de novo* MD ensemble compared to an additional step of refining the *de novo* MD ensemble using knowledge from NMR experiments conducted on the Aβ40 and Aβ42 peptides using the ENSEMBLE software package (MD-ENS). We note that the MD and MD-ENS ensembles reproduce the chemical shift data equally well, although the chi-squared statistic is lower for the MD-ENS calculation. It is evident from Table 3 that while the MD-ENS structural ensemble is better optimized against scalar couplings and L-RDCs by construction, RDCs based on global alignments are improved (Figure S3) but not to the same extent as L-RDCs (Figure S4). NOE's based on experimental assignment are predicted equally well by MD-ENS when the correlation times from the *de novo* MD simulations are used.

We found that the Aβ40 de novo MD ensemble is more extensively optimized using the ENSEMBLE method compared to the Aβ42 peptide. For Aβ40 nearly half the residues across the sequence exhibit a decrease in the percentage of the ensemble where they are involved in β-strand structure. By contrast for Aβ42 there are fewer changes in the qualitative features of the ensemble and the ENSEMBLE optimization amplifies the primary β–hairpin that is discussed in more detail below. Because the optimization of J-coupling and RDC data results in changes in each type of secondary structure at the Aβ42 C-terminus, it is difficult to draw a direct connection between the change in the observable value and difference in the structures present. However Table S5 gives more detailed changes observed in the calculated MD vs. MD-ENS for each peptide.

Overall, the quantitative agreement between experiment and back-calculations with structures from MD-ENS for chemical shifts (Figure S1), scalar couplings (Figure S2), and L-RDCs evaluated with ENSEMBLE(27), and the ^{1}H-^{15}N and assignable NOEs are very good (Table 3), although the Aβ40 ^{2}H$_2$O spectrum agreement is less good than the other NOEs. Furthermore, given the *de novo* or MD-ENS ensemble of ^{1}H-^{1}H contacts, and using the corresponding timescales given by *de novo* MD simulations to calculate NOEs with the MD-ENS, we can also predict the assignments of the unknown experimental cross peaks (Tables S1 and S2). We also provide the experimentally assignable cross-peaks not due to intra-residue or sequential contacts and agreement with MD-ENS (Tables S3 and S4). We therefore choose to analyze the MD-ENS structural populations for the Aβ40 and Aβ42 peptides given its consistent high quality performance against all available experimental NMR data.

Structural Ensembles of Aβ40 and Aβ42. Given the strong validation against a range of experimental NMR data, we now use the MD-ENS structural ensembles to determine what differences there are between the Aβ40 and Aβ42 IDPs. The MD-ENS structural ensembles of Aβ40 and Aβ42 show stark differences between the two peptides. Figure 1 shows the propensities of each peptide to form β-turns, helical structure, or intramolecular β-bridges, β-hairpins or β-sheets by residue, as averages over their conformational ensembles. As we found for Aβ42(25), Aβ40 is a highly heterogeneous tertiary ensemble, which samples conformations reflecting all possible secondary structure categories and is composed of a range of collapsed structured states to highly extended conformations. Although Aβ40 samples some conformations

very similar to ones seen in the Aβ42 ensemble, such as a highly populated turn centered at residues 7-8 or a helix near Ser26, the two peptides have substantially different secondary structure profiles overall. The simulated structural ensembles show that most of the long-range NOEs produced by each peptide are a result of hydrogen-bonded β-structure, however, different β-strand associations are formed in the Aβ40 ensemble than in the Aβ42 ensemble.

Figure 2 is a contact map from the MD-ENS simulated ensemble for each peptide (the corresponding de novo MD simulated contact map is given in Figure S6). The long-range contacts are clearly different in the Aβ40 and Aβ42 ensembles, and many of these long-range contacts are due to β-strand formation. Clearly visible in the Aβ42 contact map are two β-hairpin sub-populations between the CHC residues 16-21 and 29-36 in ~34% of the ensemble (Figure 3a and 3b); one is defined by β-strand pairing of residues 16-17 with 35-36 (~16%) and the other by β-strand pairing of residues 17-21 with 29-34 (~18%). Furthermore, residues 26-27 form a β-turn in ~22% of the population, half of which also occurs with the dominant anti-parallel β-hairpin, consistent with the same 26-27 β-turn and the 16-21 and 29-36 β–strands that ultimately adopt the intermolecular arrangement of the stable mature fibril state. This feature is also consistent with a number of MD simulations that highlight the importance of residues 23-28 for nucleating monomer folding[55] and supported by detailed structural characterization of the amyloid-β fragments Aβ21-30[24, 25, 56, 57] and Aβ10-35[58, 59], as well as the importance of residues 16-22 that promote β–sheet structure as discussed in [[60, 61]].

In contrast, Aβ40 forms an alternative, less populated anti-parallel β-hairpin between the CHC and residues 9-13 (Figure 4a) in ~10% of its ensemble, that sometimes includes CHC association with a third β–strand comprising residues 35-37 to define a β–sheet (Figure 4b). We note that Val18, at the center of the CHC, is in the middle of this Aβ40 β-hairpin and β–sheet. In fact, previous work by Yan and co-workers[37] examined side-chain methyl groups, showing that Val18 is more ordered in Aβ40 compared to Aβ42. Our simulations provide an explanation for this experimental observation since we find that Val18 participates in more β-bridge or strand contacts within the Aβ40 ensemble (~14%) than in the Aβ42 ensemble (~3%). In the Aβ42 ensemble Val18 is found near the ends of the two β–strands involving the CHC and is less ordered as a result, due to fraying.

However, each peptide exhibits small additional sub-populations of anti-parallel and parallel β–strand associations, although most are defined by two hydrogen bonds only (i.e. a β-bridge). Figure 5a-5c provides three additional populations of β-strand structure for the Aβ42 ensemble that are worth mentioning. The first is an increased amount of anti-parallel β-strand association between residues 3-6 and 10-13 which comprises ~10% of the Aβ42 ensemble, although we emphasize that 7% of the Aβ42 conformers are only stabilized by a single β-bridge. The second is a parallel β-strand association between residues 21-23 and 36-38 in ~8% of the ensemble, half the time exhibiting only one β-bridge. Finally there is an anti-parallel β-hairpin formed by residues 34-36 and 39-40 in 6% of the ensemble that is negligibly populated in the Aβ40 ensemble. This last β-hairpin is consistent with that found in previous MD studies on Aβ42*(47, 62, 63)*, but it is not significantly populated and actually is subsumed into a larger sub-population involving hydrophobic clustering in the C-terminus that is a direct result of Ile41 and Ala42.

   The Role of Ile41 and Ala42. Based on characterization of our simulated ensembles, we have found that the very different populations of β-strand structure for Aβ40 and Aβ42 are consequences of the two additional hydrophobic residues in Aβ42, Ile41 and Ala42, which can form inter-residue contacts not available to Aβ40. These two residues do not form hydrogen bonds in a significant portion of the ensemble, but they are able to form hydrophobic interactions. We observe increased hydrophobic clustering between residues 39-41 and 31-36 for Aβ42, and this C-terminus clustering occurs frequently with contacts between residues 37-38 and residues 12-16. These interactions are visible in the contact map (Figure 2b). The new intramolecular contacts in the Aβ42 ensemble isolate the CHC from the N-terminus and the C-terminus to preclude β–hairpin formation with either the 9-13 or 35-37 β–strands observed in the Aβ40 ensemble. Instead the CHC of Aβ42 is most frequently encased in a loop defined by residues 15-38 or 16-36 that promote the 26-27 β–turn and/or β–hairpin that are compatible with known amyloid fibril forming regions. Furthermore, the more isolated N-terminus of the Aβ42 ensemble forms some type of β–bridge or β–strand association between residues 3-6 and 10-13 in ~10% of the ensemble, while Aβ40 forms it in only 3% of its ensemble. Parallel β-strand

association between residues 21-23 and 36-38 also always occurs with the hydrophobic contacts between 39-41 and 31-36, which is why it never occurs in the Aβ40 ensemble.

Our simulated ensembles are also consistent with the slower relaxation rates and increased ^{1}H-^{15}N NOE intensities seen experimentally that indicate that the Aβ42 backbone is more ordered at the C-terminus than Aβ40(*36, 38*). However, it is not known experimentally if order in the C-terminus arises from a populated helix or β–strand or from hydrophobic clustering often observed in disordered or unfolded states of proteins(*64, 65*). In Figure 6 we provide a comparison of the simulated ^{1}H-^{15}N NOE intensities for Aβ42 and Aβ40, which are in excellent agreement(*14*) with the experimentally measured values by Yan and Wang(*37*) (Table 3). We find there is an increase in ^{1}H-^{15}N NOE intensities calculated from simulation for residues 35-40 for Aβ42 compared to Aβ40 (the same seen experimentally), indicating that the longer peptide is more ordered at the C-terminus. We attribute this to the many hydrophobic interactions involving the Val40 side chain with residues 31-36 that make up 45% of the Aβ42 ensemble compared to 13% of the Aβ40 ensemble, and when Ile41 is included the hydrophobic clustering increases to close to 60%. Example Aβ42 structures in which the dominant β-hairpin and 26-27 β-turn form along with a C-terminal hydrophobic contact between 39-41 and 31-36 are shown in Figure 3a and 3b.

When we analyze the *de novo* MD Aβ40 and Aβ42 ensembles derived at 311 K, near physiological temperature, we find that both peptides exhibit a decrease in population of the major turns and helices at the increased temperature, while Aβ40's most populated β-strands at 287 K melt out to yield significantly reduced percentages at the higher temperature. By contrast, the β-strands present in the Aβ42 ensemble are more stable and persist as the temperature increases, strengthened by the increase in hydrophobic clustering of the C-terminal residues which is expected to become more prominent with increasing temperature. This is consistent with the experimental finding from circular dichroism that the Aβ42 β-strand content is more stable than that of Aβ40 as the temperature of the sample is increased(*22*).

DISCUSSION

For the past decade, Alzheimer's researchers have been interested in understanding why Aβ42 is much more aggregation prone than Aβ40, despite their nearly identical sequences. Since fibrillization of Aβ has been shown to follow a nucleation-dependent polymerization mechanism(66), the kinetic data imply that the nucleation barrier is smaller for Aβ42 than for Aβ40. We suggest that the underlying structural differences between the two Aβ monomeric ensembles identify three possible factors for why the nucleation step is more difficult for Aβ40 compared to Aβ42.

The first is that in order to oligomerize or aggregate into a fibril-forming conformation containing intermolecular β-sheets at CHC residues 16-21 and 29-36, the Aβ peptide must overcome the free energy cost of breaking up any competing β-strand alignments. We have found that the CHC region of Aβ40 forms a different set of β-strand pairings than Aβ42. The fact that in the *de novo* MD ensemble the alternative β-strands for Aβ40 are less populated at higher temperatures means that the rate of fibrillization would increase with temperature, consistent with what is seen in fibrillization experiments(67, 68). The second is the presence of the two additional C-terminal residues Ile41 and Ala42 of Aβ42 that prevents the longer peptide from forming the intramolecular β-sheet seen in the Aβ40 ensemble. Finally, the increased hydrophobic clustering at the Aβ42 C-terminus isolates the CHC within a loop comprising residues 15-38, placing it in register with the 29-36 β–strand to form an intramolecular β–hairpin. These same β–strands are also aligned in the intermolecular β–sheets exhibited in the insoluble fibrillar states.

Thus, while the data do not make a direct connection between the Aβ40 and Aβ42 monomer conformational ensemble data and oligomer and fibril energetics or formation kinetics, our new data allows us to comment on other proposed oligomerization pathways based on assumptions about the monomeric starting point. Several previous NMR studies have observed that the Aβ42 C-terminal residues are less flexible than those of Aβ40, leading some groups to surmise that the C-terminus is preordered in a β-sheet conformation similar to that occupied by fibrils and oligomers, and this contributes to Aβ42's increased aggregation propensity(22, 36-39). Our data, however, indicate that this reduced motility of the Aβ42 C-terminus is a result of

an extensive network of hydrophobic contacts in ~60% of the ensemble rather than β-hairpin hydrogen bonds involving residues 34-36 and 39-40, which occur in only 6% of the ensemble.

Mutational studies have argued that extended conformations at residues 41 and 42 of Aβ42 and a turn at residues 38-39 are important for aggregation*(32, 3*4), leading Irie *et al.* to conclude that Aβ42 forms an intramolecular, anti-parallel β-hairpin between residues 40-42 and 35-37 with turns at residues 33-34 and 38-39*(3*5). Again, our data contradict this picture in that we observe primarily hydrophobic contacts between these regions in the monomer ensemble, although we do find a small population of β-hairpin in this region of the sequence. Other mutation studies show that Aβ42's aggregation propensity is related to the hydrophobicity of Ile41 and Ala42*(21, 32, 3*3), supporting our picture of a C-terminal hydrophobic cluster. We also observe that the C-terminal hydrophobic cluster contacts the central hydrophobic cluster at residues 16-21, which often accompanies the formation of the residue 26-27 β-turn, in a very similar conformation to that seen by Maji and coworkers via photo-induced cross-linking*(4*0).

CONCLUSIONS

Previous all-atom simulation studies*(47, 55, 60, 62, 69-7*6) and experimental CD and NMR spectroscopy*(12, 21, 23, 3*8) have sought to characterize the differences between the monomer ensembles of the Aβ40 and Aβ42 peptides. The CD studies indicated that both peptides should be primarily classified as random coil*(21, 2*2), consistent with the same classification that was derived from chemical shift and J-coupling measurements*(12, 23, 3*8). We showed previously that a random coil ensemble for Aβ40 and Aβ42 does not agree well with the available experimental NMR data*(1*4). Even when we considered the assumption of an ensemble that uses direct secondary structure prediction algorithms for Aβ40 and Aβ42 (but with no cooperative secondary structure), the resulting ensembles did not agree with the experimental data. Further optimization using the ENSEMBLE method to refine the random or statistical ensembles of conformational sub-populations, an accepted procedure for generating IDP structural ensembles*(26-29, 7*7), showed no improvement.

Here we have taken a different approach and used the *de novo* MD results to provide a different "basis set" for selection of conformational states using the ENSEMBLE method. In this case the monomer ensembles of Aβ40 and Aβ42 have heterogeneous structure, presenting a

diverse set of α–helix, β–turns, and β–strands. Based on the optimized MD-ENS structural ensemble of Aβ40 and Aβ42, which show very good agreement with the available NMR data, back-calculations of chemical shifts were also found to be consistent with random coils or ensembles with statistical or predictive assignments of secondary structure(*14*).

We have demonstrated that homonuclear ^{1}H-^{1}H NOE intensities and ^{15}N-^{1}H heteronuclear NOEs are more discriminating with regard to the tertiary contacts that define the important structural differences between the two Aβ peptides. J-coupling constants and RDCs provide additional quantitative information about the differences between Aβ40 and Aβ42 ensembles when combined with simulation data. Our study is further distinguished by the productive interplay of molecular simulation to first simulate the NOE observables and thus validate the theory, which in turn can be used to further refine and interpret the NMR data for Aβ42 and Aβ40. It is important to emphasize that developing a structural model of the Aβ monomer ensembles based on the experimental NOE data, which are averaged by rapid exchange among conformers, would not be possible without the MD simulations providing details of individual structures.

Our data reveal how the addition of residues 41 and 42 drastically changes the conformational landscape of the Aβ42 peptide by increasing hydrophobic interactions within the C-terminus that exclude the formation of intramolecular β-hairpins formed frequently in the Aβ40 ensemble. The major β-hairpin populated in the Aβ42 ensemble is a consequence of the increased hydrophobic interactions, resulting in increased propensity for a β-turn at residues 26-27 and increasing the proximity of β-strands involving CHC residues 16-21 and 29-36, compatible with a stable pre-fibrillar oligomeric species known as the globulomer(*78, 7*9) and various polymorphs of the fibril structure.(*40*)

The results presented here, along with experimental and theoretical evidence accumulated over the last decade, now provide a fairly consistent picture of the monomeric ensembles of amyloid-β and the differences between Aβ40 and Aβ42. The combination of studies unifies our understanding that the hydrophobicity of residues 41 and 42 is crucial to the behavioral difference between Aβ40 and Aβ42(*21, 32, 3*3), and that the Aβ42 C-terminus folds in on itself(*32, 34, 3*5), reducing its flexibility compared to the Aβ40 C-terminus(*22, 36-3*9). Our data

contradict only the hypothesis that the Aβ42 monomer C-terminal structure is significantly populated by a β-hairpin involving residues 34-36 and 39-40. Instead, the Aβ42 C-terminus forms primarily hydrophobic contacts, a classic feature of the disordered or unfolded state *(64, 65)*, which indirectly promotes β–hairpin structure that is compatible with known fibril forming regions of the Aβ sequence.

Finally, the results here emphasize that the disease associated amyloid-β peptides, although clearly classified as IDPs, do not necessarily conform to the standard computational model assumptions or experimental expectations that have been so useful in characterization of functional IDPs *(80)* or IDPs with simpler helical structure motifs *(77)*. In particular, successful use of NMR optimization approaches such as ENSEMBLE required the diverse cooperative secondary structure populations derived from *de novo* MD to achieve good agreement with the NMR data, rather than the commonly assumed random coil or statistical ensembles incorporating secondary structure as the possible conformational populations.

REFERENCES

1. Goedert, M., and M. G. Spillantini. 2006. A century of Alzheimer's disease. Science 314:777-781.
2. Glenner, G. G., and C. W. Wong. 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochemical and Biophysical Research Comm 120:885-890.

3.	Masters, C., G. Simms, N. Weinman, G. Multhaup, B. McDonald, and K. Beyreuther. 1985. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc. Natl. Acad. Sci. U S A 82:4245-4249.

4.	Dahlgren, K., A. Manelli, W. J. Stine, L. Baker, G. Krafft, and M. LaDu. 2002. Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. J. Biol. Chem. 277:32046-32053.

5.	Roher, A. E., J. D. Lowenson, S. Clarke, A. S. Woods, R. J. Cotter, E. Gowing, and M. J. Ball. 1993. beta-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. Proc. Natl. Acad. Sci. U S A 90:10836-10840.

6.	Gravina, S., L. Ho, C. Eckman, K. Long, L. J. Otvos, L. Younkin, N. Suzuki, and S. Younkin. 1995. Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). J. Biol. Chem. 270:7013-7016.

7.	Suzuki, N., T. T. Cheung, X. D. Cai, A. Odaka, L. Otvos, J. C. Eckman, T. E. Golde, and S. G. Younkin. 1994. An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. Science 264:1336-1340.

8.	Iwatsubo, T., A. Odaka, N. Suzuki, H. Mizusawa, N. Nukina, and Y. Ihara. 1994. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). Neuron 13:45-53.

9.	Younkin, S. G. 1995. Evidence that Aβ42 is the real culprit in Alzheimer's disease. Annals of Neurology 37:287-288.

10.	Jarrett, J. T., E. P. Berger, and P. T. L. Jr. 1993. The carboxy terminus of the. beta. amyloid protein is critical for the seeding of amyloid formation: Implications for the pathogenesis of Alzheimer's disease. Biochem. 32:4693-4697.

11.	Hasegawa, K., I. Yamaguchi, S. Omata, F. Gejyo, and H. Naiki. 1999. Interaction between A beta(1-42) and A beta(1-40) in Alzheimer's beta-amyloid fibril formation in vitro. Biochem. 38:15514-15521.

12.	Hou, L., H. Shao, Y. Zhang, H. Li, N. K. Menon, E. B. Neuhaus, J. M. Brewer, I. J. Byeon, D. G. Ray, M. P. Vitek, T. Iwashita, R. A. Makula, A. B. Przybyla, and M. G. Zagorski. 2004. Solution NMR studies of the A beta(1-40) and A beta(1-42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. J. Am. Chem. Soc. 126:1992-2005.

13.	Uversky, V. N. 2009. Intrinsic disorder in proteins associated with neurodegenerative diseases. Front. Biosci. 14:5188-5238.

14.	Ball, K. A., D. E. Wemmer, and T. Head-Gordon. 2012. Determining the Structural Ensemble of Intrinsically Disordered Proteins using Computation and Experiment. J. Am. Chem. Soc. (Perspective).

15.	Tycko, R. 2011. Solid-State NMR Studies of Amyloid Fibril Structure. Annu Rev Phys Chem 62:279-299.

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

17.   Petkova, A. T., Y. Ishii, J. J. Balbach, O. N. Antzutkin, R. D. Leapman, F. Delaglio, and R. Tycko. 2002. A structural model for Alzheimer's beta -amyloid fibrils based on experimental constraints from solid state NMR. Proc. Natl. Acad. Sci. U S A 99:16742-16747.

18.   Antzutkin, O., R. Leapman, J. Balbach, and T. R. 2002. Supramolecular structural constraints on Alzheimer's beta-amyloid fibrils from electron microscopy and solid-state nuclear magnetic resonance. Biochem. 41:15436-15450.

19.   Malinchik, S. B., H. Inouye, K. E. Szumowski, and D. A. Kirschner. 1998. Structural analysis of Alzheimer's beta(1-40) amyloid: protofilament assembly of tubular fibrils. Biophysical Journal 74:537-545.

20.   Stromer, T., and L. C. Serpell. 2005. Structure and morphology of the Alzheimer's amyloid fibril. Microsc. Res. Tech. 67:210-217.

21.   Bitan, G., M. Kirkitadze, A. Lomakin, S. Vollers, G. Benedek, and D. Teplow. 2003. Amyloid beta -protein (Abeta) assembly: Abeta 40 and Abeta 42 oligomerize through distinct pathways. Proc. Natl. Acad. Sci. U S A 100:330-335.

22.   Lim, K., H. Collver, Y. Le, P. Nagchowdhuri, and J. Kenney. 2007. Characterizations of distinct amyloidogenic conformations of the Abeta (1-40) and (1-42) peptides. Biochemical and Biophysical Research Comm 353:443-449.

23.   Yan, Y., S. A. McCallum, and C. Wang. 2008. M35 oxidation induces Abeta40-like structural and dynamical changes in Abeta42. J. Am. Chem. Soc. 130:5394-5395.

24.   Fawzi, N. L., A. H. Phillips, J. Z. Ruscio, M. Doucleff, D. E. Wemmer, and T. Head-Gordon. 2008. Structure and dynamics of the Abeta(21-30) peptide from the interplay of NMR experiments and molecular simulations. J. Am. Chem. Soc. 130:6145-6158.

25.   Ball, K. A., A. H. Phillips, P. S. Nerenberg, N. L. Fawzi, D. E. Wemmer, and T. L. Head-Gordon. 2011. Homogeneous and heterogeneous tertiary structure ensembles of amyloid-β peptides. Biochem. 50:7612-7628.

26.   Choy, W. Y., and J. D. Forman-Kay. 2001. Calculation of ensembles of structures representing the unfolded state of an SH3 domain. J. Mol. Biol. 308:1011-1032.

27.   Marsh, J. A., J. M. R. Baker, M. Tollinger, and J. D. Forman-Kay. 2008. Calculation of residual dipolar couplings from disordered state ensembles using local alignment. J. Am. Chem. Soc. 130:7804-7805.

28.   Marsh, J. A., and J. D. Forman-Kay. 2009. Structure and disorder in an unfolded state under nondenaturing conditions from ensemble models consistent with a large number of experimental restraints. J. Mol. Biol. 391:359-374.

29.   Marsh, J. A., C. Neale, F. E. Jack, W.-Y. Choy, A. Y. Lee, K. A. Crowhurst, and J. D. Forman-Kay. 2007. Improved structural characterizations of the drkN SH3 domain unfolded state suggest a compact ensemble with native-like and non-native structure. J. Mol. Biol. 367:1494-1510.

30.   Goldschmidt, L., P. K. Teng, R. Riek, and D. Eisenberg. 2010. Identifying the amylome, proteins capable of forming amyloid-like fibrils. Proc. Natl. Acad. Sci. U S A 107:3487-3492.

31.   Sawaya, M., S. Sambashivan, R. Nelson, M. Ivanova, S. Sievers, M. Apostol, M. Thompson, M. Balbirnie, J. Wiltzius, H. McFarlane, A. Madsen, C. Riekel, and D.

Eisenberg. 2007. Atomic structures of amyloid cross-beta spines reveal varied steric zippers. Nature 447:453-457.

32. Kim, W., and M. H. Hecht. 2005. Sequence determinants of enhanced amyloidogenicity of Alzheimer A{beta}42 peptide relative to A{beta}40. J Biol. Chem. 280:35069-35076.

33. Weinreb, P. H., J. T. Jarrett, and P. T. Lansbury Jr. 1994. Peptide models of a hydrophobic cluster at the c-terminus of the beta-amyloid protein. J. Am. Chem. Soc. 116:10835-10836.

34. Morimoto, A., K. Irie, K. Murakami, Y. Masuda, H. Ohigashi, M. Nagao, H. Fukuda, T. Shimizu, and T. Shirasawa. 2004. Analysis of the secondary structure of beta-amyloid (Abeta42) fibrils by systematic proline replacement. J Biol. Chem. 279:52781-52788.

35. Irie, K., K. Murakami, Y. Masuda, A. Morimoto, H. Ohigashi, R. Ohashi, K. Takegoshi, M. Nagao, T. Shimizu, and T. Shirasawa. 2005. Structure of beta-amyloid fibrils and its relevance to their neurotoxicity: implications for the pathogenesis of Alzheimer's disease. J. Biosci. Bioeng. 99:437-447.

36. Lim, K., G. Henderson, A. Jha, and M. Louhivuori. 2007. Structural, dynamic properties of key residues in Abeta amyloidogenesis: implications of an important role of nanosecond timescale dynamics. Chembiochem 8:1251-1254.

37. Yan, Y., J. Liu, S. McCallum, D. Yang, and C. Wang. 2007. Methyl dynamics of the amyloid-beta peptides Abeta40 and Abeta42. Biochemical and Biophysical Research Comm 362:410-414.

38. Yan, Y., and C. Wang. 2006. Abeta42 is more rigid than Abeta40 at the C terminus: implications for Abeta aggregation and toxicity. J. Mol. Biol. 364:853-862.

39. Riek, R., P. Güntert, H. Döbeli, B. Wipf, and K. Wüthrich. 2001. NMR studies in aqueous solution fail to identify significant conformational differences between the monomeric forms of two Alzheimer peptides with widely different plaque-competence, A beta(1-40)(ox) and A beta(1-42)(ox). Eur. J. Biochem. 268:5930-5936.

40. Maji, S. K., R. R. Ogorzalek Loo, M. Inayathullah, S. M. Spring, S. S. Vollers, M. M. Condron, G. Bitan, J. A. Loo, and D. B. Teplow. 2009. Amino acid position-specific contributions to amyloid beta-protein oligomerization. J Biol. Chem. 284:23580-23591.

41. Vuister, G. W., and A. Bax. 1993. Quantitative J Correlation - A New Approach For Measuring Homonuclear 3-Bond J(H(N)H(Alpha) Coupling-Constants In N-15-Enriched Proteins. J. Am. Chem. Soc. 115:7772-7777.

42. Ruscio, J., N. Fawzi, and T. Head-Gordon. 2010. How hot? Systematic convergence of the replica exchange method using multiple reservoirs. J. Comput. Chem. 31: 620-627.

43. Case, D. A., T. A. Darden, T. E. C. III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, R. C. Walker, W. Zhang, K. M. Merz, B. Roberts, B. Wang, S. Hayik, A. Roitberg, G. Seabra, I. Kolossváry, K. F. Wong, F. Paesani, J. Vanicek, J. Liu, X. Wu, S. R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M. J. Hsieh, G. Cui, D. R. Roe, D. H. Mathews, M. G. Seetin, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, and P. A. Kollman. 2010. AMBER 11.

44. Wickstrom, L., A. Okur, and C. Simmerling. 2009. Evaluating the Performance of the ff99SB Force Field Based on NMR Scalar and Coupling Data. Biophysical Journal 97:853-856.

45. Hornak, V., R. Abel, A. Okur, B. Strockbine, A. Roitberg, and C. Simmerling. 2006. Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins 65:712-725.

46. Horn, H. W., W. C. Swope, J. W. Pitera, J. D. Madura, T. J. Dick, G. L. Hura, and T. Head-Gordon. 2004. Development of an improved four-site water model for biomolecular simulations: TIP4P-Ew. J. Chem. Phys. 120:9665-9678.

47. Sgourakis, N. G., M. Merced-Serrano, C. Boutsidis, P. Drineas, Z. Du, C. Wang, and A. Garcia. 2011. Atomic-Level Characterization of the Ensemble of the Aβ(1-42) Monomer in Water Using Unbiased Molecular Dynamics Simulations and Spectral Algorithms. J. Mol. Biol. 405:570-583

48. Zweckstetter, M., and A. Bax. 2000. Prediction of sterically induced alignment in a dilute liquid crystalline phase: Aid to protein structure determination by NMR. J. Am. Chem. Soc. 122:3791-3792.

49. Wishart, D. S., and B. D. Sykes. 1994. Chemical shifts as a tool for structure determination. Meth. Enzymol. 239:363-392.

50. Wishart, D. S., and B. D. Sykes. 1994. The 13C chemical-shift index: a simple method for the identification of protein secondary structure using 13C chemical-shift data. J. Biomol. NMR 4:171-180.

51. Camilloni, C., A. De Simone, W. F. Vranken, and M. Vendruscolo. 2012. Determination of Secondary Structure Populations in Disordered States of Proteins Using Nuclear Magnetic Resonance Chemical Shifts. Biochem. 51:2224-2231.

52. Jensen, M. R., L. Salmon, G. Nodet, and M. Blackledge. 2010. Defining Conformational Ensembles of Intrinsically Disordered and Partially Folded Proteins Directly from Chemical Shifts. J. Am. Chem. Soc. 132:1270-.

53. Salmon, L., G. Nodet, V. Ozenne, G. Yin, M. R. Jensen, M. Zweckstetter, and M. Blackledge. 2010. NMR characterization of long-range order in intrinsically disordered proteins. J. Am. Chem. Soc. 132:8407-8418.

54. Schneider, R., J.-R. Huang, M. Yao, G. Communie, V. Ozenne, L. Mollica, L. Salmon, M. R. Jensen, and M. Blackledge. 2012. Towards a robust description of intrinsic protein disorder using nuclear magnetic resonance spectroscopy. Mol. BioSys. 8:56-68.

55. Melquiond, A., X. Dong, N. Mousseau, and P. Derreumaux. 2008. Role of the Region 23-28 in Aβ Fibril Formation: Insights from Simulations of the Monomers and Dimers of Alzheimer's Peptides Aβ40 and Aβ42. Curr. Alz. Res. 5:244-250.

56. Baumketner, A., S. L. Bernstein, T. Wyttenbach, N. D. Lazo, D. B. Teplow, M. T. Bowers, and J. E. Shea. 2006. Structure of the 21-30 fragment of amyloid beta-protein. Prot. Sci. 15:1239-1247.

57. Teplow, D. B., N. D. Lazo, G. Bitan, S. Bernstein, T. Wyttenbach, M. T. Bowers, A. Baumketner, J. E. Shea, B. Urbanc, L. Cruz, J. Borreguero, and H. E. Stanley. 2006. Elucidating amyloid beta-protein folding and assembly: A multidisciplinary approach. Acc. Chem. Res. 39:635-645.

58. Tarus, B., J. E. Straub, and D. Thirumalai. 2007. Dynamics of Asp23-Lys28 salt-bridge formation in Aβ(10-35) monomers. J. Am. Chem. Soc. 128:16159-16168.

59.   Massi, F., J. W. Peng, J. P. Lee, and J. E. Straub. 2001. Simulation study of the structure and dynamics of the Alzheimer's amyloid peptide congener in solution. Biophysical Journal 80:31-44.

60.   Yang, M., and D. Teplow. 2008. Amyloid beta-protein monomer folding: free-energy surfaces reveal alloform-specific differences. J. Mol. Biol. 384:450-464.

61.   Ma, B., and R. Nussinov. 2002. Stabilities and conformations of Alzheimer's β-amyloid peptide oligomers (Aβ16–22, Aβ16–35, and Aβ10–35): Sequence effects. Proc. Natl. Acad. Sci. U S A 99:14126-14131.

62.   Wu, C., and J.-E. Shea. 2012. The structure of intrinsically disordered peptides implicated in amyloid diseases: Insights from fully atomistic simulations. In Computational Modeling of Biological Systems: From Molecules to Pathways. N. Dokholyan, editor. Springer, Berlin. 215-227.

63.   Lin, Y. S., G. R. Bowman, K. A. Beauchamp, and V. S. Pande. 2012. Investigating how peptide length and a pathogenic mutation modify the structural ensemble of amyloid beta monomer. Biophys. J. 102:315-324.

64.   Felitsky, D. J., M. A. Lietzow, H. J. Dyson, and P. E. Wright. 2008. Modeling transient collapsed states of an unfolded protein to provide insights into early folding events. Proc. Natl. Acad. Sci. USA 105:6278-6283.

65.   Dyson, H. J., and P. E. Wright. 2002. Coupling of folding and binding for unstructured proteins. Curr Opin Struct Biol 12:54-60.

66.   Ferrone, F. 1999. Analysis of protein aggregation kinetics. Meth. Enzymol. 309:256-274.

67.   Naiki, H., and K. Nakakuki. 1996. First-order kinetic model of Alzheimer's beta-amyloid fibril extension in vitro. Lab. Invest. 74:374-383.

68.   Kusumoto, Y., A. Lomakin, D. B. Teplow, and G. B. Benedek. 1998. Temperature dependence of amyloid beta-protein fibrillization. Proc. Natl. Acad. Sci. U S A 95:12277-12282.

69.   Sgourakis, N. G., Y. Yan, S. A. McCallum, C. Wang, and A. E. Garcia. 2007. The Alzheimer's peptides Abeta40 and 42 adopt distinct conformations in water: a combined MD / NMR study. J. Mol. Biol. 368:1448-1457.

70.   Urbanc, B., L. Cruz, S. Yun, S. V. Buldyrev, G. Bitan, D. B. Teplow, and H. E. Stanley. 2004. In silico study of amyloid beta-protein folding and oligomerization. Proc. Natl. Acad. Sci. U S A 101:17345-17350.

71.   Olubiyi, O. O., and B. Strodel. 2012. Structures of the Amyloid β-Peptides Aβ(1-40) and Aβ(1-42) as Influenced by pH and a d-Peptide. J. Phys. Chem. B 116:3280-3291.

72.   Yun, S., B. Urbanc, L. Cruz, G. Bitan, D. Teplow, and H. Stanley. 2007. Role of electrostatic interactions in amyloid beta-protein (A beta) oligomer formation: a discrete molecular dynamics study. Biophysical Journal 92:4064-4077.

73.   Triguero, L., R. Singh, and R. Prabhakar. 2008. Comparative Molecular Dynamics Studies of Wild-Type and Oxidized Forms of Full-Length Alzheimer Amyloid beta-Peptides Abeta(1-40) and Abeta(1-42). J. Phys. Chem. B 112:7123-7131.

74.   Vitalis, A., and A. Caflisch. 2010. Micelle-Like Architecture of the Monomer Ensemble of Alzheimer's Amyloid-β Peptide in Aqueous Solution and Its Implications for Aβ Aggregation. J. Mol. Biol. 403:148-165.

75. Lee, C., and S. Ham. 2011. Characterizing amyloid-beta protein misfolding from molecular dynamics simulations with explicit water. J. Comput. Chem. 32:349-355.

76. Straub, J. E., and D. Thirumalai. 2011. Toward a molecular theory of early and late events in monomer to amyloid fibril formation. Ann. Rev. Phys. Chem. 62:437-463.

77. Marsh, J. A., V. K. Singh, Z. Jia, and J. D. Forman-Kay. 2006. Sensitivity of secondary structure propensities to sequence differences between α- and γ-synuclein: Implications for fibrillation. Prot. Sci. 15:2795-2804.

78. Barghorn, S., V. Nimmrich, A. Striebinger, C. Krantz, P. Keller, B. Janson, M. Bahr, M. Schmidt, R. S. Bitner, J. Harlan, E. Barlow, U. Ebert, and H. Hillen. 2005. Globular amyloid beta-peptide(1-42) oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. J. Neurochem. 95:834-847.

79. Yu, L., R. Edalji, J. E. Harlan, T. F. Holzman, A. P. Lopez, B. Labkovsky, H. Hillen, S. Barghorn, U. Ebert, P. L. Richardson, L. Miesbauer, L. Solomon, D. Bartley, K. Walter, R. W. Johnson, P. J. Hajduk, and E. T. Olejniczak. 2009. Structural Characterization of a Soluble Amyloid beta-Peptide Oligomer. Biochem. 48:1870-1877.

80. Uversky, V., J. Gillespie, and A. Fink. 2000. Why are "natively unfolded" proteins unstructured under physiologic conditions? Proteins 41:415-427.

81. Neal, S., A. M. Nip, H. Zhang, and D. S. Wishart. 2003. Rapid and accurate calculation of protein 1H, 13C and 15N chemical shifts. J Biomol NMR 26:215-240.

TABLES

Table 1. *Summary of experimentally assignable NOEs determined for Aβ40 and Aβ42.* Only ~25% of peaks for each peptide are assignable from experiment alone, and the third column indicates the assigned peaks that are present in both the Aβ40 and Aβ42 spectra.

	Aβ40	**Aβ42**	**Both peptides**
Total Assigned NOE peaks	382	196	147
Intra-residue and sequential	362	185	142
i to i+ 2	20	9	5
i to i+ 3		1	
i to i+ 4		1	

Table 2. *Summary of remaining experimental NOEs determined for Aβ40 and Aβ42.* For the remaining ~75% peaks that cannot be assigned, peak intensities may be composed of a single pair contact of which several pair contacts are possible assignments, or may be composed of multiple, fractional contact pairs. In either case we provide the *experimental* lower bound, *n,* for *i* to *i+n* contacts for the unassigned peaks for each peptide. The value in parentheses indicates the number of these peaks that are present in the other peptide spectra as well. This value is given only for contacts from i to i+5 or greater. Some data reproduced from (14).

	Aβ40	**Aβ42**
Total NOE peaks	1108	707
Intra-residue and sequential	828	537
i to i+ 2	142	68
i to i+ 3	58	36
i to i+ 4	40	20
i to i+ 5	11 (2)	13 (8)
i to i+ 6	7 (3)	4 (1)
i to i+ 7	10 (8)	9 (6)
i to i+ 8	2 (2)	3 (0)
i to i+9	2 (0)	6 (1)
i to i+10		1 (0)
i to i+11	2 (2)	
i to i+12	1 (0)	3 (0)
i to i+13	2 (2)	
i to i+14	2 (1)	3 (1)
i to i+20	1 (1)	2 (1)
i to i+21		1 (0)
i to i+24		1 (0)

Table 3. *Comparison between* de novo *MD (MD) and experimentally optimized MD (MD-ENS) structural ensembles for Aβ40 and Aβ42.* For chemical shifts we report that measures agreement between the computational ensembles and the experimentally measured chemical shifts within SHIFTX*(81)* calculator error. We also provide the RMSD between experiment and calculated ensembles for $^3J_H^N{}_H$ and ( ). RMSDs for RDCs are based on either global alignments using PALES*(48)* or on local alignments (L-RDCs) evaluated with ENSEMBLE*(27)*. For NOEs we consider the simulated agreement with experiment for NOEs that can be assigned from the spectrum alone. The RMSD is normalized by the NOE intensity value for each peak to yield the N-RMSD and (correlation coefficient, r) for the H_2O and D_2O experiments. In this work we have used the decay timescales of specific proton pairs from the *de novo* MD simulation to inform the calculation of the MD-ENS NOEs. Finally we provide the RMSD between experiment and *de novo* MD for the heteronuclear NOE's; these are a purely dynamical phenomena and hence can only be derived from the MD simulation. Some data reproduced from (14).

Average Property	Aβ40 MD	Aβ40 MD-ENS	Aβ42 MD	Aβ42 MD-ENS
Hα χ_δ^2	0.58	0.30	0.54	0.33
HN χ_δ^2	0.36	0.34	0.48	0.37
Cα χ_δ^2	0.69	0.46	0.98	0.51
Cβ χ_δ^2	0.70	0.36	0.52	0.34
$^3J_H^N{}_H^\alpha$	0.99 (1.82)	0.62 (0.72)	0.99 (1.83)	0.54 (0.56)
RDC (Hz)	2.22	1.69	2.25	2.13
L-RDC (Hz)	1.88	0.18	2.14	0.33
H_2O NOEs (assigned)	1.15 (0.74)	1.12 (0.74)	1.25 (0.67)	1.21 (0.68)
D_2O NOEs (assigned)	3.22 (0.55)	3.19 (0.55)	0.58 (0.80)	0.57 (0.80)
^1H-^{15}N NOEs	0.17		0.21	

FIGURE LEGENDS

Figure 1. *Percentage of (a) Aβ40 and (b) Aβ42 simulated ensemble in different types of secondary structure by residue.* The dashed red line represents α-helix, the solid blue line for β−bridges or β−strands, and the dotted black line for β−turns.

Figure 2. *Contact map of the simulated ensembles of (a) Aβ40 and (b) Aβ42.* This contact map gives the frequency of interaction between each pair of residues in the peptide MD-ENS simulated ensembles. White indicates contacts present in 100% of the ensemble and black indicates contacts never seen in the ensemble. We define two residues to be in contact if any of their heavy atoms are within 5 Å of each other.

Figure 3. *The dominant 16-21 and 29-36 β-hairpin population for Aβ42.* (a) Aβ42 forms a β-turn at residues 26-27 with the 16-17 and 35-36 β-hairpin. The hydrophobic side-chains of residues 39-41 (brown) also fold back to contact the side-chains of residues 34-35 (yellow). The side chain of Gln15 (pink) caps the end of the β−hairpin by contacting residues 37-38. (b) Aβ42 forms the 26-27 β-turn and 17-21 and 29-33 β-hairpin with a C-terminal hydrophobic side-chain interaction between 39-40 (brown) and 32-34 (yellow). Residues 12-13 (pink) also interact with the C-terminus around residue 38. Turns (blue), Helix (red), β−strands (green arrow).

Figure 4. *The dominant β-hairpin and β-sheet population for Aβ40.* (a) Aβ40 forms the 9-13 and 16-21 β-hairpin. (b) The hairpin interaction between 17-20 and 35-37 occurs simultaneously to form a β-sheet with 3 strands. β−strands (green arrow).

Figure 5. *Small sub-populations of Aβ42 conformations containing β-strands.* (a) Aβ42 forms a β-turn at residues 7-8 nucleating β-strand pairing of residues 3-6 and 10-13, along with a helix from residue 14-17 and at 32-35. (b) Aβ42 forms a parallel β-strand association between residues 21-23 and 36-38 while the 39-40 side-chains (brown) contact Ile32 (yellow). (c) A C-terminal β-hairpin formed by residues 34-36 and 39-40. Turns (blue), Helix (red), β−strands (green arrow).

Figure 6. *Comparison of simulated Aβ40 and Aβ42 ^{1}H-^{15}N NOEs.* The MD results show that the C-terminus is more ordered for Aβ42 when compared to Aβ40.