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Tracking the Mechanism of Fibril Assembly by Simulated Two-Dimensional Ultraviolet Spectroscopy

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of plaque deposits in the human brain. The main component of these plaques consists of highly ordered structures called amyloid fibrils, formed by the amyloid β -peptide ($A\beta$). The mechanism connecting $A\beta$ and AD is yet undetermined. In a previous study, a coarse-grained united-residue model and molecular dynamics simulations were used to model the growth mechanism of $A\beta$ amyloid fibrils. Based on these simulations, a dock/lock mechanism was proposed, in which $A\beta$ fibrils grow by adding monomers at either end of an amyloid fibril template. To examine the structures in the early time-scale formation and growth of amyloid fibrils, simulated two-dimensional ultraviolet spectroscopy is used. These early structures are monitored in the far ultraviolet regime ($\lambda = 190\text{--}250\text{ nm}$) in which the computed signals originate from the backbone $n\pi^*$ and $\pi\pi^*$ transitions. These signals show distinct cross-peak patterns that can be used, in combination with molecular dynamics, to monitor local dynamics and conformational changes in the secondary structure of $A\beta$ -peptides. The protein geometry-correlated chiral $xyxy$ signal and the non-chiral combined signal $xyxy$ - $xyyx$ were found to be sensitive to, and in agreement with, a dock/lock pathway.

Keywords

$A\beta$ -peptide; Alzheimer's disease; Dock/Lock process; Elongation assembly; 2DUV signals; Chirality factor

1 Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder whose pathology is associated with the formation and deposition of amyloid plaques and fibrils in the brain^{1,2}. Amyloid plaques contain highly-ordered forms of a protein known as the β -amyloid peptide ($A\beta$) which originates by cleavage of a large and multi-functional membrane protein called amyloid precursor protein (APP). $A\beta$ peptides are normally present in the body

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6 Supporting Information

Additional Figures corresponding to Configuration 2 are displayed in this section. These Figures are available free of charge via the Internet at <http://pub.acs.org>

predominantly in two alloforms, $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$, with the latter having two additional amino-acid residues at the C-terminus. The neurotoxicity of the $A\beta$ peptides has been linked to their ability to form β -sheet structures and aggregates rather than to the amyloid plaques and fibrils themselves³⁻⁷.

It has been reported that small fragments derived from $A\beta$ -peptide also form amyloid fibrils and can be crystallized⁸, revealing important features that are believed to also be present in the fibrils formed by the parent peptides⁹. Colletier and co-workers¹⁰ used a set of amyloid fragments composed of 6–7 residues and found that these fragments have the tendency to crystallize in different polymorphic oligomers with a variety of lifetimes and toxicity levels, which reveals that $A\beta$ -peptides may have a repertoire of accessible fibril structures. However, neither of the full length peptides, $A\beta_{(1-40)}$ or $A\beta_{(1-42)}$, has been crystallized.

Because of their rapid aggregation and their propensity to assume different geometrical shapes, it is difficult to reveal the conformations of the $A\beta$ -peptides in aqueous solution. Crescenzi and co-workers¹¹ determined a three-dimensional structure of the $A\beta_{(1-42)}$ monomer by using CD and 2D NMR techniques in different media that mimic the lipid phase of membranes. The reported structure consists of two α -helices in which one of the helices, $A\beta_{(28-38)}$, corresponds to the transmembrane region of APP. Tycko *et al.* used solid state NMR techniques to study $A\beta_{(1-40)}$ fibrils and determined that these fibrils can assume different geometrical arrangements¹²⁻¹⁶. Based on the constraints imposed by their solid state NMR data, they proposed geometrical models for wild-type $A\beta_{(1-40)}$ and Iowa mutant $A\beta$ (D23N). According to these models, $A\beta_{(1-40)}$ fibrils are formed by the stacking of U-shaped monomers held together by intermolecular hydrogen bonds. A quenched hydrogen/deuterium exchange NMR study by Lührs and co-workers¹⁷ resulted in a three-dimensional structure of the $A\beta_{(1-42)}$ amyloid fibrils that is similar to that reported by Tycko and co-workers.

In addition to the efforts made to understand the stability of $A\beta$ oligomers and fibrillar or non-fibrillar aggregates, considerable attention has been paid to the different pathways leading to the formation of $A\beta$ oligomers and their evolution into highly-ordered amyloid fibrils¹⁸⁻²⁸. Bitan *et al.*²² determined that $A\beta_{(1-42)}$ peptides have a higher propensity to form larger oligomers than $A\beta_{(1-40)}$ peptides. Lim and co-workers²⁶ found that $A\beta_{(1-42)}$ monomers have a higher tendency to form β -strand conformations than $A\beta_{(1-40)}$ monomers. Jarret *et al.*^{30, 31} reported that the C-terminus plays a critical role in the amyloidogenesis process. Despite these results, the growth mechanism of amyloid fibrils and the stage in which the oligomers become neurotoxic leading to AD is still an open issue.

Simulating fibril formation at the all-atom level is not possible with the computational resources available today. To reduce the computational cost, studies usually focus on fragments of $A\beta$ -peptides³²⁻³⁵. Simulations of the full-length $A\beta$ -peptides have also been reported³⁶⁻⁴⁰, but they are usually combined with coarse-grained models^{36, 37, 39}, implicit solvent^{38, 40}, or the replica exchange method⁴¹ to speed up the computations. These studies provide some understanding of the conformational changes that $A\beta$ -peptides undergo when transitioning from a disordered to a highly-ordered structure, but do not fully reflect the true dynamics of the full-length $A\beta$ -peptide folding and aggregation.

Esler *et al.*⁴² used radiolabeled $A\beta$ -peptides to model the amyloid growth and concluded that the deposition of soluble $A\beta$ onto amyloid fibrils has two distinguishable kinetic steps: A reversible step in which an $A\beta$ monomer docks onto an amyloid template and an irreversible lock step in which an $A\beta$ monomer fully associates with the amyloid fibril. Cannon *et al.*⁴³ used surface plasmon resonance biosensors to study the kinetics associated with the early stage of β -amyloid fibril elongation and concluded that a peptide association/dissociation

mechanism occurred in multiple steps. The soluble peptide first binds reversibly to the growing amyloid fibril and, in subsequent steps, the bound A β peptide is stabilized into the growing fibril through post-binding transitional events. This step-by-step mechanism observed in fibril elongation was described theoretically by Massi and Straub⁴⁴ by using a schematic energy landscape with loosely defined reaction coordinates and transition states for peptide/fibril association and reorganization. Their model incorporated different possible channels for peptide deposition, which included fast deposition from solution through an activation/nucleation event, and deposition of peptide from solution onto existing fibrils followed by reorganization of the peptide/fibril deposit. Nguyen *et al.*⁴⁵ proposed a dock/lock mechanism using the A β _(16–22) fragment. Initially, in the first stage (docking), the A β _(16–22) monomer increases its β -strand content while it docks onto a preformed oligomer. Subsequently, a slow lock-phase occurs, and the monomer rearranges to form in-register anti-parallel structures. O'Brien *et al.*⁴⁶ used simulations with an implicit solvent model and all-atom molecular dynamics in explicit water to study the thermodynamics of the dock-lock mechanism and showed that a locked full length monomer exhibited large conformational fluctuations when interacting with the underlying fibril.

Rojas *et al.*⁴⁷ used a coarse-grained united-residue (UNRES) model and replica-exchange molecular dynamics (REMD) simulations to model the assembly of a free and unrestrained A β _(9–40) monomer onto a fibril template based on Tycko's A β wild-type fibril model. They simulated the A β _(9–40) assembly process and found that hydrophobic interactions and hydrogen bonds play a leading role in stabilizing the structures. The entire process follows a common dock/lock mechanism reaching two-locking states at the molecular level that agree with experiments on A β monomer deposition^{42, 43}.

Two-dimensional infrared (2DIR) optical spectroscopy has recently been applied to characterize protein structure and monitor fast folding processes^{48–53}. Sequences of laser pulses are used to excite vibrational states and the correlations between evolution in two controlled time intervals are determined. These are displayed in a two-dimensional map that reveals electronic and vibrational fluctuations in the ultrafast regime providing information about the molecular dynamics (MD). Two-dimensional ultraviolet spectroscopy (2DUV) is a natural extension of 2DIR. In recent years, the development of 2DUV experimental protocols for application to fast biochemical processes such as the interaction between DNA bases has proven that this novel technique is suitable for characterization of dynamical properties not observed with traditional techniques^{54,55}. Simulations of applied 2DUV spectroscopy showed that this technique can be used to characterize and distinguish secondary structures of proteins^{56,57} and highly ordered structures such as A β -amyloids fibrils^{58,59}.

The purpose of the present study is to demonstrate how the 2DUV signals, calculated from the conformational changes in the protein geometry, obtained from the simulated UNRES trajectories⁴⁷, may be used to monitor the assembly mechanism of an A β -peptide monomer into an amyloid fibril template, and obtain structural information at early events (over a time scale of 20 ns). Correlation between these calculated signals and the simulated conformational changes that the A β -peptide monomer exhibits during the assembly process are discussed.

2 Theoretical Methods

Rojas *et al.* performed REMD simulations of the binding of an A β peptide to an amyloid fibril template. Technical details of the force field and protein model used in the simulations are given in refs. 47 and 60. The simulation set up is described as follows: A free and unrestrained single extended A β -peptide monomer is initially placed 20Å away from the

amyloid fibril template. Due to an asymmetry in the configuration adopted by the A β peptides, the opposite ends of a fibril are different. At one end, the N-terminal region is more exposed, while at the other, it is the C-terminal region that is left exposed. Therefore, in order to explore the possibility that the two ends may differ in binding modes, two initial Configurations were prepared: in Configuration 1, the extended monomer faces the N-terminal strands of the amyloid fibril template, while in Configuration 2, it faces the C-terminal strands (See Fig. 1). The time evolution of Configurations 1 and 2 was shown in Figures 7 and 8, respectively, of ref. 47. Each of these two Figures displays six representative conformations at different times at which a single A β monomer starts to bind and finally docks onto a template. For each of the six representative conformations, a set of 100 snapshots were recorded at intervals of 121 fs. These coarse-grained snapshots were converted into all-atom conformations, and were used to simulate the corresponding 2DUV signals.

The 2DUV simulation protocol was described elsewhere⁶¹. It uses a Quantum Mechanics/Molecular Mechanics computational approach based on the Frenkel Exciton Hamiltonian with the Electrostatic Fluctuations algorithm (EHEF). The Hamiltonian that describes the interactions among all the electronic transitions has the form:

$$\hat{H}_{EHEF} = \sum_{m_e} \varepsilon_{m_e}^{(i)} \hat{B}_{m_e}^\dagger \hat{B}_{m_e} + \sum_{m_e, n_f}^{m_e \neq n_f} J_{m_e, n_f}^{(i)} \hat{B}_{m_e}^\dagger \hat{B}_{n_f}, \quad (1)$$

where m and n denote the amino-acid residues, e and f denote the excited and the final states of the transition (either $n\pi^*$ or $\pi\pi^*$) of the amino-acid unit, i denotes the snapshot index, $\varepsilon_{m_e}^{(i)}$ is the energy of the transition state for the m th residue in snapshot i . $\hat{B}_{m_e}^\dagger$ and $\hat{B}_{m_e}$ are the exciton creation and annihilation operators, respectively, with the commutation relation $[\hat{B}_{m_e}, \hat{B}_{n_f}^\dagger] = \delta_{mn}(1 - 2\hat{B}_{m_f}^\dagger \hat{B}_{m_e})$. $J_{m_e, n_f}^{(i)}$ denotes the resonant coupling of the transitions in two amino-acid residues in the approximate form:

$$J_{m_e, n_f}^{(i)} = \frac{1}{4\pi\epsilon_0} \sum_{m_e} \sum_{n_f}^{n \neq m} \frac{Q_{m_e}^{eg} Q_{n_f}^{gf}}{|\mathbf{r}_{m_e} - \mathbf{r}_{n_f}|}, \quad (2)$$

where ϵ_0 is the electric permittivity in vacuo, g denotes the ground state, Q_m and r_m denote the charges and position of the m th residue. Diagonalization of the Hamiltonian of Eq. 1 yields the exciton eigenstates, and the nonlinear optical signals of the A β assembly are computed by using the procedure given in ref. 61 and implemented in the SPECTRON software package.

The UV linear absorption (LA), circular dichroism (CD) and 2DUV photon echo of the assembly are obtained by averaging over a set of MD snapshots. With the aid of the Hamiltonian of Eq. 1, the LA signal contains the eigenvalues and eigenstates of the protein, but not the geometry. The CD signal provides the distribution of the relative orientations of the dipole moments, i.e., the relative orientations of the amino-acid residues, and hence can identify secondary structures. 2DUV signals provide the information from LA and CD signals, and also the interactions between specific i and j dipole moments, i.e., between specific amino-acid residues. The DichroCalc software package⁶² was used to compute the LA and CD signals from the trajectories of ref. 47.

The 2DUV photon echo signal technique uses three laser pulses with wave vectors $\mathbf{k}_1$, $\mathbf{k}_2$ and $\mathbf{k}_3$ (in their respective chronological order, t_1 , t_2 and t_3) that interact with the target (a peptide or complex of peptides). A coherent signal field, $\mathbf{k}_f = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$, is also

generated with a time-interval-dependent signal $S(t_3, t_2, t_1)$ that carries information about the interactions and intensity changes of the oscillator fields of the system. The signals are displayed as the two-dimensional Fourier transform of the time delays $t_1 \rightarrow \Omega_1$ and $t_3 \rightarrow \Omega_3$ (See Fig. 2). The time delay t_2 is fixed to zero, avoiding energy transfer processes between excited states. Depending on the polarization of the lasers (x,y,z), signals can be non-chiral and chiral⁵⁶. Chiral signals are much more sensitive to the geometry of the protein than the non-chiral signals, but the non-chiral signals are larger in magnitude.

Three non-chiral signals are independent in the dipole approximation: $xyxy$, $xyyx$, and $xyyx$ (x and y are the polarizations of the lasers). The linear combination, $xxxx = xyxy + xyyx + xxyy$, gives an absorptive signal with all four lasers polarizations in the same direction. Other combinations can be obtained such as $xyxy - xyyx$, which eliminates the excited-state population pathways and reveals the pathways for coherent quantum dynamics arising from the off-diagonal crosspeaks in the $(-\Omega_1, \Omega_3)$ signal diagrams. The combination $xxyy - xyxy$ suppresses the pathways with coherence in stimulated emission and highlights the excited-state population dynamics. For $t_2=0$, $xyxy = xxyy$ and the combination $xyxy - xyyx$ displays only excited-state absorption. In addition, nine independent chiral signals are possible. The chiral $xxxy$ signal was computed and used earlier in the characterization of secondary structure of proteins⁶¹.

In the current study, three sets of signals are computed: the absorptive non-chiral $xxxx$, the non-chiral combination $xyxy - xyyx$, and the chiral $xxxy$. These signals are most easily related to protein conformation. To achieve these signals and compute the 2DUV spectra, the four lasers are set to pulses of 3754 cm^{-1} bandwidth centered at $52,000 \text{ cm}^{-1}$ (190 nm)⁵⁶.

For comparison purposes, all 2D spectra are normalized in a color scale to rank the weak (in blue) and strong (in red) peaks, and plotted on a nonlinear scale that interpolates between logarithmic, for small values, and linear for large values, of the signal intensity. The nonlinear scale is defined as⁶³

$$\text{arcsinh}(cS) = \ln(cS + \sqrt{(1+c^2S^2)}). \quad (3)$$

S is the 2D signal and c is a constant factor defined in such a way that, for larger cS , the scale becomes logarithmic, and weak features are amplified and their resolution enhanced. For $cS < 1$, the scale is linear. In each set of computed signals, the constant factor is fixed.

3 Results

The dock/lock mechanism of the A β amyloid fibrils described in ref. 47 has three-stages, and the transition between stages coincides with abrupt changes in the number of native hydrogen bonds formed between the monomer and the amyloid fibril (See Fig. 9 in ref. 47). The three distinguishable stages can be described as follows: (a) The monomer initially interacts freely with the amyloid fibril template. (b) The monomer partially locks into the amyloid fibril template by making intermolecular hydrogen bonds along one of its strands, while the remaining strand is allowed to move freely. (c) The monomer is fully locked in the fibril conformation and can serve as a template for addition of subsequent monomers.

The simulations suggested that the monomers have no preference for binding to either of the ends of Configuration 1 or 2 and the dock/lock mechanism does not depend on the size of the template. However, the simulations presented here show that the formation of hydrogen bonds during the conformational changes of the monomer depends on the initial configuration. The description of this dock/lock mechanism is consistent with experiments on deposition of A β -peptide monomers^{42, 43}. Molecular dynamics simulation studies of the

oligomerization of the $A\beta_{(16-22)}$ monomers also indicate that a monomer binds onto a pre-formed oligomer by forming anti-parallel U-shape sheets via a dock/lock mechanism similar to what experiments reported⁴⁴.

3.1 The 2DUV signals of the Amyloid $A\beta$ -Peptide Dock/Lock Mechanism at Different Simulation Times

The 2DUV signals at different simulation times provide an avenue to assess the conclusions in ref. 47, in terms of the geometry of the dipole-dipole interactions. The simulated 1D and 2DFUV (2D far-ultraviolet) spectra for Configurations 1 and 2 are presented here, and Figures S1-S5 are displayed in the Supporting Information. The FUV signals (190–250 nm) originate from the amino-acid residues of peptide backbone $n\pi^*$ and $\pi\pi^*$ transitions and are sensitive to secondary structure. The aromatic residues also contribute to the FUV signal.

The LA signals of Configurations 1 and 2 are displayed in Figs. 3 and S1 (top panels) at six simulation times. All signals share a common peak centered around $52,000\text{ cm}^{-1}$ with modest variations in their profile due to the different stages in the dock/lock process of the $A\beta_{(9-40)}$ monomer onto the amyloid template. The $xxxx$ signals (Figs. 3 and S1, middle panels) show a symmetric and elongated blue peak around $52,000\text{ cm}^{-1}$ along the diagonal of the $(-\Omega_1, \Omega_3)$ plots. The diagonal elements correspond to the excitation energies, and their lineshapes show lifetimes and couplings to the dipole-dipole interactions. Off-diagonal elements on the $(-\Omega_1, \Omega_3)$ maps are cross-peak signals that carry information about couplings and correlations at different states. Other non-chiral signal combinations enhance some cross-peak signals not observed in the $xxxx$ signals. The combination $xyxy-xyyx$ displayed at the bottom of Figs. 3 and S1 shows some off-diagonal asymmetric shapes that indicate weak correlations between modes. Since we set $t_2 = 0$, all the signals display excited states absorption. The combination $xyxy-xyyx$ for both configurations displays changes in their cross-peaks in time. The signal in Configuration 2 displays a split in the peak at $52,000\text{ cm}^{-1}$ at all times.

The circular dichroism (CD) and the $xxxy$ signals, which reveal the secondary structure chirality, are described below. The CD signal of Configurations 1 and 2 at six simulation times are shown in Figs. 4 and S2 (middle panels), respectively. Absorptions at 240 nm and below are due mainly to two backbone transitions in the peptide groups: A strong peak around 190 nm from $\pi\pi^*$ transitions and a weak but broad plateau due to the $n\pi^*$ transition around 220 nm. The $A\beta_{(9-40)}$ monomers in the amyloid template in Configuration 1 are predominantly U-shape hairpins and β -strands. The contribution to the changes in the CD signals comes from the conformational changes of the free $A\beta_{(9-40)}$ monomer during the dock/lock process onto the amyloid template. The changes in the intensity around 190 nm ($52,000\text{ cm}^{-1}$) and 220 nm ($46,000\text{ cm}^{-1}$) come from the interaction between the monomer and the template by hydrogen-bond formation, electrostatic contacts between charged residues, and transitions from aromatic residues⁵⁶.

An extension of the CD signals are the $xxxy$ signals, shown in Figs. 4 and S2 (bottom panels). Signals in both Configurations show two common peaks along the diagonal of the $(-\Omega_1, \Omega_3)$ map: A strong peak around $52,000\text{ cm}^{-1}$ and a weaker one at $54,000\text{ cm}^{-1}$, which are characteristic of a fibrillar structure⁵¹. Off-diagonal peaks describe the local dynamics of the system and the coupling between amide groups of the backbone.

For Configuration 1, the $xxxy$ signal at $t \sim 0.76\text{ ns}$ (Fig. 4, first left column, bottom panel) shows an asymmetric butterfly-shape with peaks at $48,000\text{ cm}^{-1}$ and $56,000\text{ cm}^{-1}$ that indicate aromatic coupling transitions originating mainly from Phe residues of the monomers in the amyloid stacking of the template (top of Fig. 4). From $t \sim 0.76\text{ ns}$ to $t \sim 6.89\text{ ns}$, the single monomer interacts weakly with the template and can still move and freely

change its position. The 2DFUV $xxxy$ signals (bottom of Fig. 4) reflect these changes. The peak at $56,000\text{ cm}^{-1}$ observed at $t \sim 0.76\text{ ns}$ disappears at $t \sim 2.62\text{ ns}$, but reappears at $t \sim 4.77\text{ ns}$. Similarly, the peak at $48,000\text{ cm}^{-1}$ almost disappears at $t \sim 2.62\text{ ns}$ but reappears, although weakened, at $t \sim 4.77\text{ ns}$ and $t \sim 6.89\text{ ns}$. During these times, the coupling interactions, Phe-Tyr and Tyr-Tyr, between the free monomer and the amyloid template occur (See top of Fig. 4). The CD spectrum at $t \sim 6.89\text{ ns}$ (Fig. 4, fourth left column, middle panel) has a lower peak at $52,000\text{ cm}^{-1}$ and a plateau around 220 nm which indicates that the free monomer has formed a U-shape hairpin and is partially bound onto one of the stacks in the amyloid template making Phe-Tyr coupling interactions. This occurs similarly at $t \sim 13.01\text{ ns}$. Accordingly, the signal displays strong peaks at $48,000\text{ cm}^{-1}$ and $56,000\text{ cm}^{-1}$ (See Fig. 4, bottom of fifth column from the left). The free segment, Gly9-Asp23, is still unbound and free to move. At $t \sim 20\text{ ns}$, this free segment finally binds to the template with the fibrillar conformation, locking the monomer and allowing it to form Phe-Phe and Tyr-Tyr interactions with the template.

For Configuration 2, the $xxxy$ signals display a different profile. At $t \sim 0.05\text{ ns}$ (Fig. S2, first column from the left), the main contribution to the signal comes from the template since the free monomer has an extended shape and is far from the template. The signal displays peaks at $48,000\text{ cm}^{-1}$ and $56,000\text{ cm}^{-1}$ due to the interactions between the aromatic residues. Within the times $t \sim 0.27\text{ ns}$ and $t \sim 3.76\text{ ns}$ (Fig. S2, second to fourth columns from the left), conformational changes in the monomer are reflected in the $xxxy$ signal. At $t \sim 0.27\text{ ns}$, the Ala30-Val40 segment of the free monomer interacts with the template allowing Phe-Tyr coupling interactions that are reflected in the $xxxy$ signal as several cross-peaks along the diagonal. At $t \sim 1.45\text{ ns}$, the monomer binds partially to the template, and the signal displays peaks at $48,000\text{ cm}^{-1}$, $52,000\text{ cm}^{-1}$ and $56,000\text{ cm}^{-1}$. At $t \sim 3.76\text{ ns}$, the signal exhibits a butterfly-like shape and the monomer forms a bulky structure and interacts with the amyloid template. At $t \sim 16.75\text{ ns}$, the monomer starts to bind to the template. Phe-Phe, and Tyr-Tyr couplings are present, and the butterfly-like shape appears in the signal. At $t \sim 20\text{ ns}$, the monomer binds and locks fully to the template, and the $xxxy$ signal displays diffused peaks along the diagonal. The signal still preserves the characteristic peaks at $48,000\text{ cm}^{-1}$, $52,000\text{ cm}^{-1}$ and $56,000\text{ cm}^{-1}$, but the profile of the signal differs from that in Configuration 1.

3.2 Chirality Factor and the Secondary Structure at Different Simulation Times

From the 2DUV spectroscopy point of view, changes in the conformation of the monomer are associated with changes in the relative orientations of the transition dipole moments in the residues. These can be used to characterize the secondary structure by measuring the interactions between the transition dipole moments during different orientations. For example, the contribution of pairs of amino-acid residues to the chiral signals can be characterized quantitatively in terms of the average chirality factor $\langle CF(m,n) \rangle$ ⁵²:

$$\langle CF(m,n) \rangle = \sum_{i=1}^N |\Psi_{m_e}^i \Psi_{n_e}^i \mathbf{R}_{mn}^i \bullet (\mu_m^i \times \mu_n^i)| \quad (4)$$

where $i=1 \dots N$ are the indices of the snapshots of a set in a simulation time interval. m and n are the indices of the local amino-acid residue mode ($n\pi^*$ and $\pi\pi^*$), $\Psi_{m_e}^i$ is the eigenstate wavefunction e of the localized amino-acid residue m , $\mathbf{R}_{mn}^i$ is the distance between the positions of the two transition electric dipole moments μ_m and μ_n . The CD signal is obtained by summing over all the eigenstates at one frequency,

$S(e) = \sum_{i=1}^N \Psi_{m_e}^i \Psi_{n_e}^i \mathbf{R}_{mn}^i \bullet (\mu_m^i \times \mu_n^i)$. In the UV regime, each amino-acid residue undergoes two transitions, $n\pi^*$ and $\pi\pi^*$ each of which carries the same conformational information.

For this study, the $\pi\pi^*$ transitions of each amide group were used to compute the $\langle CR(m,n) \rangle$ maps, and each pair of amino acids is associated with formation of secondary structure in the $A\beta_{(9-40)}$ monomer.

The $\langle CR(m,n) \rangle$ maps of the monomer for Configuration 1 at different simulation times are displayed in Fig. 5. At $t \sim 0.76$ ns (Fig. 5A), a long anti-diagonal structure is displayed on the map, characteristic of anti-parallel β -sheets (as in contact distance maps). This indicates the presence of a turn around Asn27 which brings the Gln15-Gly25 and Ala30-Val36 segments close to each other and in anti-parallel orientation. This structure persists at all times, with only the turn region shifting slightly. Accordingly, the anti-diagonal structure on the maps also persists at all times, except that it moves toward right or left depending on the position of the turn.

Another persistent feature is the region along the diagonal in the lower left corner of all the maps between $t \sim 0.76$ ns and $t \sim 13.01$ ns (Fig. 5A–E). It indicates that the dipole moment vectors are parallel, typical of α -helices. This is consistent with the presence of an α -helix formed by the Gly9-Asp23 segment whose length changes in time as observed in each panel in Fig. 5.

At $t \sim 20$ ns (Fig. 5F), the monomer finally docks adopting the fibrillar conformation. The chirality factor map clearly shows the geometrical configuration of the fibril. The α -helix has disappeared and so has the diagonal structure in the lower left corner of the map. The Gly9-Asp23 and Ala30-Val40 segments lie in anti-parallel orientation, resembling a β -hairpin. Their interaction is seen in the signal from the turn region as a high-chirality region (blue) intersecting the diagonal around residue Asn27.

The chirality factor maps for Configuration 2 at different simulation times are shown in Fig. S3. Between $t \sim 0.05$ and $t \sim 1.45$ ns (Fig. S3A–C), the segment Gly9-Asp23 of the free monomer exhibits an α -helical-shape, which is consistent with the red region along the diagonal in the lower left corner of the maps. At $t \sim 1.45$ ns, the segment Ala30-Val40 of the monomer interacts with one monomer from the template and gradually starts bending to form a turn around residue Leu34, which is reflected in the map by the region intersecting the diagonal around residue Leu34 (Fig. S3C). At $t \sim 3.76$ ns (Fig. S3D), the chirality map displays an irregular profile since the free monomer forms a bulky structure, and π -stacking interactions between aromatic residues from the free monomer and the template are present. At $t \sim 16.75$ ns, the segment Gly9-Asp23 of the free monomer binds partially to the template and the remaining portion (Val24-Val40) moves freely with all its transition dipole moments interacting with each other in all directions (Fig. S3E). At $t \sim 20.0$ ns (Fig. S3F), the monomer finally docks to the template, and the profile of the $\langle CR(m,n) \rangle$ map is similar to that in Fig. 5F. A red segment intersects the diagonal around residue Ser26, indicating the formation of a turn at this location and interactions between the Gly9-Asp23 and Ala30-Val40 segments.

3.3 Sensitivity Analysis of the 2DUV Spectra

The 2DUV signals reported in section 3.1 depend strongly on the initial configurations of the incoming monomer. These signals arise mainly from the complex (monomer + amyloid template). For Configurations 1 and 2, a sensitivity analysis was performed to determine which component (monomer or amyloid template) is more sensitive in making the larger changes in the signals.

Sensitivity analysis of the signals consists of dissecting the 2DUV signal into the following parts:

$$S_{Complex} = S_{Template} + S_{Monomer} + \Delta S, \quad (5)$$

where S_A is the 2DUV signal of component A labeled as Complex, Template and Monomer, respectively, in Eq. 5. Because of the coupling elements in the Hamiltonian, the total signal, $S_{Complex}$, is not the addition of the signal of each component. Therefore, a factor ΔS is included and used to weight the contribution of each component to the total spectrum. The non-chiral signals $S^{(xxxx)}$ of each component for Configurations 1 and 2 are shown in Figs. S4, and S5, respectively. The $S^{(xxxx)}$ signals at different simulation times (See the columns of Figs. S4 and S5) coming from the amyloid template and the monomer for both Configurations show that the main contribution to the $S^{(xxxx)}$ Complex comes strongly from the amyloid template rather than from the monomer, where the latter is the component with the largest conformational changes over time. The dynamics of these signals for both Configurations (Figs. S4 and S5) can be observed in the changes of ΔS at different simulation times.

For Configuration 1 (Fig. S4), at $t \sim 0.76$ ns, the monomer is initially far from the template, and the coupling terms of the monomer and the template display two well-separated positive (yellow) and negative (blue) cross-peaks around $52,000 \text{ cm}^{-1}$ and $54,000 \text{ cm}^{-1}$ (Fig. S4, ΔS). At $t \sim 2.76$ ns, the negative peak elongates sharply along the diagonal, which indicates that the coupling terms have a broader range in frequency due to the fact that the monomer binds partially as a β -sheet to the template with the hairpin in the opposite orientation. The positive cross-peak signals remain unaltered.

Later at $t \sim 4.77$ ns, ΔS displays both cross-peaks elongated uniformly along the diagonal. Here, the monomer forms a hairpin shape with some helical structure. At $t \sim 6.89$ ns, a segment of the monomer binds to one of the already-stacked monomer in the template, and the cross-peaks spread out broadly around $52,000 \text{ cm}^{-1}$. At $t \sim 13.01$ ns, the monomer has a hairpin shape and partially unlocks from the original stack, and ΔS exhibits a similar behavior as at early times, with the negative cross-peak signals spreading out broader than the positive cross-peak signals. At $t \sim 20.0$ ns, when the monomer binds fully to the template, ΔS displays its cross-peaks in the entire FUV range.

For Configuration 2 (Fig. S5), ΔS at different times reveals a distinct pathway. At early times, $t \sim 0.05$ ns and $t \sim 0.27$ ns, two cross-peaks are displayed around $52,000 \text{ cm}^{-1}$ and $54,000 \text{ cm}^{-1}$ as in Configuration 1. The monomer binds partially with one of the stacks at $t \sim 1.45$ ns and $t \sim 3.76$ ns. At that time interval, the negative cross-peak spreads out and becomes broader and more delocalized than the positive peak. Later in time, at $t \sim 16.75$ ns the monomer binds to the template as a U-shape hairpin, and ΔS exhibits an elongated negative cross-peak along the diagonal, which persists once the monomer binds fully to the template at $t \sim 20.0$ ns.

Thus, the sensitivity analysis has helped to extract information from the non-chiral signals about the changes in the cross-peaks at different times that are usually seen in the chiral signals.

4 Discussion and Conclusions

2DUV spectra at specific times during the amyloid assembly reveal that the local dynamics depend on the initial condition of the monomer-template Configuration. Rojas *et al.*⁴⁷ showed that both initial Configurations are valid for the amyloid assembly and did not find evidence of preference of one Configuration over the other. Although both Configurations share a common two-stage dock/lock mechanism as indicated in ref. 47, the 2DFUV chiral $xxxx$ signals at various times indicate distinct signal pathways (See Figs. 4 and S2). While in

Configuration 1 the free monomer seems to follow a highly and steadily cooperative folding process, in Configuration 2 the *xxxxy* signals display abundant cross-peaks indicating local strong coupling in certain pairs of residues in the monomer.

In silico studies of fragments or full-length A β -peptides have been reported^{32–41}. These studies used intramolecular average residue-contact maps or atom-distance-based plots that usually provide a general overview of the secondary structure and critical contacts formed during folding at a given temperature. Here, the characteristics of the local dynamics in the amino-acid backbone residues are well represented by the average chirality factor maps that preserve the general description of the secondary structure and reveal that the free monomer displays a more localized dynamics in Configuration 1 than in Configuration 2. In Configuration 1, segments Gly9-Gln15, Leu17-Ala21, and Ile31-Val36 of the monomer are dominant portions of the sequence that lead to monomer folding and cooperate with each other in the formation of secondary structure. In Configuration 2, the formation of helical and β -strand structures seems to be slower, less cooperative and with more delocalized dynamics than in Configuration 1, at least at early times.

In the final locking step, both Configurations display similar profiles in the chirality factor map. The Leu17-Ala21 and Ile31-Val36 segments form β -strands connected by a hairpin loop. The Leu17-Ala21 and Ile31-Val36 segments of the free monomer in Configuration 1 display a uniform contribution to the chirality while, in Configuration 2, some pairs of residues, especially the aromatics Phe19 and Phe20, make the strongest contribution to the chirality.

These results agree with experimental studies of A $\beta_{(10-35)}$ fragments. Studies by Lee *et al.*⁶⁴ demonstrated that A $\beta_{(10-35)}$ fragments populate collapsed coil conformations under physiological conditions, under which the hydrophobic amino-acid residues L17-A21 formed a well-structured core. Benzinger *et al.*^{65,66} used solid state NMR on fibrils of A $\beta_{(10-35)}$ peptides and found that the full length A β peptides adopt the structure of an extended parallel in-register β -sheet at pH 7.4. Also, they demonstrated that structural transitions from α -helix to β -sheet were observed at residues Phe19 and Phe20 by using peptides with ¹³C incorporated at the carbonyl position of adjacent amino acids, Val18 and Phe19, respectively.

Finally, the analyzed 2DUV spectra can be used to characterize the mechanism of fibril elongation of the A β amyloid fibrils in great detail, and clearly discriminate the different dock/lock pathways which a single monomer takes to bind to a fibril template. The results shown here indicate that it is possible to correlate the different conformations, that the monomer adopts, with the 2DFUV signals, especially with the chiral signals (Figs. 4 and S2) and the chirality factors (Fig. 5 and S3). The 2DUV spectra analyzed here have provided confirming details of the structures in the resulting docking events described in ref. 47. Recent applications of experimental and simulated spectroscopy in the infrared regime such as 2DIR and FTIR have been found to be promising for proteins and higher-order structures such as amyloid fibrils, Ras proteins and the photosystem. They exhibit ultrafast chemical processes that are sensitive in the infrared regime such as proton transport, vibrational dynamics, secondary structure stability, and protein activation, and their detailed characterization has helped to understand macroscopical processes in proteins^{67–72}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Lorenzo A, Yuan M, Zhang Z, Paganetti PA, Sturchler-Pierrat C, Staufenbiel M, Mautino J, Vigo FS, Sommer B, Yankner BA. *Nature Neurosci.* 2000; 3:460–464. [PubMed: 10769385]
2. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. *Nature.* 2002; 416:535–539. [PubMed: 11932745]
3. Selkoe DJ. *Physio Rev.* 2001; 81:741–766.
4. Kirkitadze MD, Bitan G, Teplow DBJ. *Neurosci Res.* 2002; 69:567–577.
5. Hardy J, Selkoe DJ. *Science.* 2002; 297:353–356. [PubMed: 12130773]
6. Hardy J. *Neurobiol Aging.* 2002; 23:1073–1074. [PubMed: 12470803]
7. Haass C, Selkoe DJ. *Nat Rev Mol Cell Biol.* 2007;101–112. [PubMed: 17245412]
8. Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R, Eisenberg D. *Nature.* 2005; 435:773–778. [PubMed: 15944695]
9. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJW, McFarlane HT, et al. *Nature.* 2007; 447:453–457. [PubMed: 17468747]
10. Colletier JP, Laganowsky A, Landau M, Zhao ML, Soriaga AB, Goldschmidt L, Flot D, Cascio D, Sawaya MR, Eisenberg D. *Proc Natl Acad Sci USA.* 2011; 108:16938–16943. [PubMed: 21949245]
11. Crescenzi O, Tomaselli S, Guerrini R, Salvatori S, D'Ursi AM, Temussi PA, Picone D. *Eur J Biochem.* 2002; 269:5642–5648. [PubMed: 12423364]
12. Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F, Tycko R. *Proc Natl Acad Sci USA.* 2002; 99:16742–16747. [PubMed: 12481027]
13. Petkova AT, Leapman RD, Guo Z, Yau WM, Mattson MP, Tycko R. *Science.* 2005; 307:262–265. [PubMed: 15653506]
14. Petkova AT, Yau WM, Tycko R. *Biochemistry.* 2006; 45:498–512. [PubMed: 16401079]
15. Paravastu A, Leapman RD, Yau W, Tycko R. *Proc Natl Acad Sci USA.* 2008; 105:18349–18354. [PubMed: 19015532]
16. Tycko R, Sciarretta KL, Orgel JPRO, Meredith SC. *Biochemistry.* 2009; 48:6072–6084. [PubMed: 19358576]
17. Lührs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Döbeli H, Schubert D, Riek R. *Proc Natl Acad Sci USA.* 2005; 102:17342–17347. [PubMed: 16293696]
18. Walsh DM, Lomakin A, Benedek GB, Condron MM, Teplow D. *J Biol Chem.* 1997; 272:22364–22372. [PubMed: 9268388]
19. Lazo ND, Downing DT. *J Pept Res.* 1999; 53:633–640. [PubMed: 10408337]
20. Kirkitadze MD, Condron MM, Teplow DB. *J Mol Biol.* 2001; 312:1103–1119. [PubMed: 11580253]
21. Kaye R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. *Science.* 2003; 300:486–489. [PubMed: 12702875]
22. Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB, Teplow DB. *Proc Natl Acad Sci USA.* 2003; 100:330–335. [PubMed: 12506200]
23. Bitan G, Vollers S, Teplow DB. *J Biol Chem.* 2003; 278:34882–34889. [PubMed: 12840029]
24. Bitan G, Tarus B, Vollers SS, Lashuel HA, Condron MM, Straub JE, Teplow DB. *J Am Chem Soc.* 2003; 125:15359–15365. [PubMed: 14664580]
25. Klein WL, Stine WB, Teplow DB. *Neurobiol Aging.* 2004; 25:569–580. [PubMed: 15172732]
26. Lazo ND, Grant MA, Condron MC, Rigby AC, Teplow DB. *Prot Sci.* 2005; 14:1581–1596.

27. Glabe CG. *Subcell Biochem.* 2005; 38:167–177. [PubMed: 15709478]
28. Cheng IH, Searce-Levie K, Legleiter J, Palop JJ, Gerstein H, Bien-Ly N, Puolivli J, Lesne S, Ashe KH, Muchowski PJ, et al. *J Biol Chem.* 2007; 282:23818–23828. [PubMed: 17548355]
29. Lim KH, Collver HH, Le YTH, Nagchowdhuri P, Kenney JM. *Biochem and Biophys Res Com.* 2006; 353:443–449. [PubMed: 17184733]
30. Jarret JT, Berger EP, Lansbury PT. *J Ann N Y Acad Sci.* 1993; 695:144–148.
31. Jarret JT, Berger EP, Lansbury PT. *Biochemistry.* 1993; 32:4693–4697. [PubMed: 8490014]
32. Massi F, Peng JW, Lee JP, Straub JE. *Biophys J.* 2001; 80:31–44. [PubMed: 11159381]
33. Borreguero JM, Urbanc B, Lazo ND, Buldyrev SV, Teplow DB, Stanley HE. *Proc Natl Acad Sci USA.* 2005; 102:6015–6020. [PubMed: 15837927]
34. Wei G, Jewett AI, Shea JE. *Phys Chem Chem Phys.* 2010; 12:3622–3629. [PubMed: 20336261]
35. Nguyen PH, Li MS, Derreumaux P. *Phys Chem Chem Phys.* 2011; 13:9778–9788. [PubMed: 21487594]
36. Urbanc B, Cruz L, Yun S, Buldyrev SV, Bitan G, Teplow DB, Stanley HE. *Proc Natl Acad Sci USA.* 2004; 101:17345–17350. [PubMed: 15583128]
37. Lam A, Urbanc B, Borreguero J, Lazo ND, Teplow DB, Stanley HE. *Proceedings of 2006 International Conference on Bioinformatics Computational Biology.* 2006; 1:322–328.
38. Baumketner A, Bernstein SL, Wyttenbach T, Bitan G, Teplow DB, Bowers MT, Shea JE. *Prot Sci.* 2006; 15:420–428.
39. Lam AR, Teplow DB, Stanley HE, Urbanc B. *J Am Chem Soc.* 2008; 130:17413–17422. [PubMed: 19053400]
40. Kent A, Jha AK, Fitzgerald JE, Freed KF. *J Phys Chem B.* 2008; 112:6175–6186. [PubMed: 18348560]
41. Sgourakis NG, Yan Y, McCallum SA, Wang C, Garcia AE. *J Mol Biol.* 2007; 368:1448–1457. [PubMed: 17397862]
42. Esler WP, Stimson ER, Jennings JM, Vinters HV, Ghilardi JR, Lee JP, Mantyh PW, Maggio JE. *Biochemistry.* 2000; 39:6288–6295. [PubMed: 10828941]
43. Cannon MJ, Williams ADRW, Myska DG. *Anal Biochem.* 2004; 328:67–75. [PubMed: 15081909]
44. Massi F, Straub JE. *Proteins: Struc, Func and Gen.* 2001; 42:217–229.
45. Nguyen PH, Li MS, Stock G, Straub JE, Thirumalai D. *Proc Natl Acad Sci USA.* 2007; 104:111–116. [PubMed: 17190811]
46. O'Brien EP, Okamoto Y, Straub JE, Brooks BR, Thirumalai D. *J Phys Chem B.* 2009; 113:14421–14430. [PubMed: 19813700]
47. Rojas A, Liwo A, Browne D, Scheraga HA. *J Mol Biol.* 2010; 404:537–552. [PubMed: 20888834]
48. Smith AW, Tokmakoff A. *J Chem Phys.* 2007; 126:045109-1-10. [PubMed: 17286519]
49. Kim YS, Liu L, Axelsen PH, Hochstrasser RM. *Proc Natl Acad Sci USA.* 2008; 105:7720–7725. [PubMed: 18499799]
50. Strasfeld DB, Ling YL, Shim SH, Zanni MT. *J Am Chem Soc.* 2008; 130:6698–6699. [PubMed: 18459774]
51. Shim SH, Gupta R, Ling YL, Strasfeld DB, Raleigh DP, Zanni MT. *Proc Natl Acad Sci USA.* 2009; 106:6614–6619. [PubMed: 19346479]
52. Zhuang W, Sgourakis NK, Zhenyu L, Garcia A, Mukamel S. *Proc Natl Acad Sci USA.* 2010; 107:15687–15692. [PubMed: 20798063]
53. Remorino A, Korendovych IV, Wu YB, DeGrado WF, Hochstrasser RM. *Science.* 2011; 332:1206–1209. [PubMed: 21636774]
54. West BA, Womick JM, Moran AM. *J Phys Chem A.* 2011; 115:8630–8637. [PubMed: 21756005]
55. Tseng C-H, Sándor P, Kotur M, Weinacht TC, Matsika S. *J Phys Chem A.* 2012; 116:2654–2661. [PubMed: 22074393]
56. Jiang J, Abramavicius D, Bulheller BM, Hirst JD, Mukamel S. *J Phys Chem B.* 2010; 114:8270–8277. [PubMed: 20503991]

57. Abramavicius D, Jiang J, Bulheller BM, Hirst JD, Mukamel S. *J Am Chem Soc.* 2010; 132:7769–7775. [PubMed: 20481498]
58. Jiang J, Abramavicius D, Falvo C, Bulheller BM, Hirst JD, Mukamel S. *J Phys Chem B.* 2010; 114:12150–12156. [PubMed: 20795695]
59. Lam A, Jiang J, Mukamel S. *Biochemistry.* 2011; 50:9809–9816. [PubMed: 21961527]
60. Liwo A, Czaplewski C, Pillardy J, Scheraga HA. *J Chem Phys.* 2001; 115:2323–2347.
61. Abramavicius D, Palmieri B, Voronine DV, Sanda F, Mukamel S. *Chemical Review.* 2009; 109:2350–2408.
62. Bulheller BM, Rodger A, Hirst JD. *Phys Chem Chem Phys.* 2007; 9:2020–2035. [PubMed: 17464384]
63. Read EL, Engel GS, Calhoun TR, Mancal T, Ahn TK, Blankenship RE, Fleming GR. *Proc Natl Acad Sci USA.* 2007; 104:14203–14208. [PubMed: 17548830]
64. Lee JP, Stimson ER, Ghilardi JR, Mantyh PW, Lu YA, Felix AM, Llanos W, Behbin A, Cummings M, Crieke MV, et al. *Biochemistry.* 1995; 34:5191–5200. [PubMed: 7711039]
65. Benzinger TL, Gregory DM, Burkoth TS, Miller-Auer H, Lynn DG, Botto RE, Meredith SC. *Proc Natl Acad Sci USA.* 1998; 95:13407–13412. [PubMed: 9811813]
66. Benzinger TL, Gregory DM, Burkoth TS, Miller-Auer H, Lynn DG, Botto RE, Meredith SC. *Biochemistry.* 2000; 39:3491–3499. [PubMed: 10727245]
67. Smith AW, Lessing J, Ganim Z, Peng CS, Tokmakoff A, Roy S, Jansen TLC, Knoester J. *J Phys Chem B.* 2010; 114:10913–10924. [PubMed: 20690697]
68. Kobus M, Nguyen PH, Stock G. *J Chem Phys.* 2010; 133:034512. [PubMed: 20649342]
69. Liang CW, Jansen TLC, Knoester J. *J Chem Phys.* 2011; 134:044502. [PubMed: 21280743]
70. Wang L, Middleton CT, Singh S, Reddy AS, Woys AM, Strasfeld DB, Marek P, Raleigh DP, de Pablo JJ, Zanni MT, et al. *J Amer Chem Soc.* 2011; 133:16062–16071. [PubMed: 21916515]
71. Rudack T, Xia F, Schlitter J, Kotting C, Gerwert K. *Proc Nat Acad Sci USA.* 2012; 109:15295–15300. [PubMed: 22949691]
72. Suzuki H, Sugiura M, Noguchi T. *Biochemistry.* 2012; 51:6776–6785. [PubMed: 22880689]

Figure 1.

Initial configurations taken from Rojas *et al.*⁴⁷. $\text{A}\beta(9-40)$ monomer (ribbon in red) is 20 Å away from the amyloid fibril template composed of two β -sheets of two $\text{A}\beta$ monomers each (in navy and blue, respectively). The primary sequence of $\text{A}\beta(9-40)$ is displayed below the Figures with the aromatic residues Tyr and Phe indicated in orange. Each monomer has one Tyr (Tyr10) and two Phe (Phe19 and Phe20) residues.

Figure 2.

Configuration of pulses for a multidimensional four-laser mixing experiment. Three laser pulses with wave vectors $\mathbf{k}_1$, $\mathbf{k}_2$ and $\mathbf{k}_3$ (in their respective chronological order, t_1 , t_2 and t_3) interact with a target (a peptide or complex of peptides). A coherent signal field, $\mathbf{k}_I = -\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$, is also generated to enhance the output of the time-interval dependent signal $S(t_3, t_2, t_1)$ that carries information about the interactions and intensity changes and is collected by a detector. The signal is displayed as the two-dimensional Fourier transform of the times $t_1 \rightarrow \Omega_1$ and $t_3 \rightarrow \Omega_3$.

Figure 3.

1D and 2D spectra in the FUV regime for Configuration 1 at different simulation times.

Top: Linear Absorption (1D). **Middle:** The 2D non-chiral xxxx spectra. **Bottom:** The 2D non-chiral combination xyxy-xyyx signal. The non-chiral xxxx spectra have extended blue peaks at $52,000 \text{ cm}^{-1}$, while the non-chiral combination xyxy-xyyx spectra have extended red peaks at that center. These peaks centered at $52,000 \text{ cm}^{-1}$ correspond to the maxima in the LA signal displayed at the top row. The yellow cross-peaks in both non-chiral spectra correspond to the pairs of dipole-dipole interactions.

Figure 4. 1D and 2D chiral signals in the FUV for Configuration 1 at different simulation times. **Top Row:** Representative snapshots (from ref. 47) of $A\beta_{(9-40)}$ monomer (red) interacting with the amyloid template (blue) at different simulation times. Aromatic residues, Tyr and Phe, are colored in orange and green, respectively. **Middle Panels:** circular dichroism. **Bottom Panels:** 2DFUV chiral xxxxy spectra.

Figure 5.

Maps A to F show the average chirality factor $\langle CR(m,n) \rangle$ (see Eq. 4) for $\pi\pi^*$ transitions for Configuration 1 at different simulation times, indicated at the left of each map. A representative snapshot (obtained from ref. 47) of the A $\beta_{(9-40)}$ monomer (red) interacting with the amyloid template (blue) for the corresponding time, is also shown at the left of each map. Axes in each map correspond to the amino-acid residue index. Eq. 3 was used with a fixed value for c to highlight the contribution of a pair of amino-acid residues to the chirality factor.