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Insights into Molecular Mechanisms of Polyphenols' Inhibition of Amyloid β Aggregation in Alzheimer's Disease

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Abstract. Alzheimer's Disease (AD) is characterized by the aggregation of amyloid beta (Aβ) peptides, particularly Aβ42, into toxic oligomers and fibrils. Polyphenols, natural compounds with low toxicity and good permeability across the blood-brain barrier, have shown promise in inhibiting Aβ aggregation; however, their mechanisms remain unclear. Using molecular dynamics simulations, we investigated the interactions of six polyphenols —apigenin, catechin, curcumin, EGCG, nobiletin, and resveratrol—with the Aβ42 pentamer, analyzing binding positions, residue contacts, and binding affinities. Binding free energy calculations revealed EGCG and nobiletin as the most effective, with strong binding affinities and the ability to disrupt Aβ aggregates. Detailed structural analyses identified hydrogen bonding with the peptide backbone and hydrophobic interactions with side-chain residues as key mechanisms driving the stable binding. EGCG, with its high hydroxyl group content and strong hydrophobic interactions, emerged as the top candidate. Direct dimer assembly simulations confirmed EGCG's ability to inhibit Aβ42 aggregation by reducing inter-peptide hydrogen bonding and suppressing secondary structure formation. These findings provide insights for designing optimized polyphenol-based therapeutics to combat AD, advancing the development of effective treatments for neurodegenerative diseases.

Keywords: Alzheimer's disease, polyphenols, drug discovery, molecular simulations

1. Introduction

Alzheimer's Disease (AD) is the most common form of neurodegenerative disease and has become a major public health problem due to the increasing aging population [1,2]. It is estimated that over 6.7 million people in the United States (US) are living with AD dementia, and that the number is projected to rise to 13.8 million by 2060 [1]. The disease is characterized by symptoms such as memory loss and inability to think and communicate, severely impacting the quality of life of AD patients [3]. Over the decades, many therapeutic strategies have been explored, with the majority focusing on symptomatic mitigation and prevention [4]. Thus far, no curative treatment has been found. The development of curative treatments requires a deeper understanding of the pathological and physiological changes that occur during disease progression.

The most pronounced pathological features of AD are the amyloid deposits that are aggregated from amyloid beta (Aβ) peptides in the milieu of nerve cells [5,6]. Aβ peptides are produced from amyloid precursor protein (APP) by β- and γ-secretase enzymes in neurons. Among Aβ peptides, the

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most aggressive type has been found to be Aβ42, which forms ordered aggregates with repeated β-sheet secondary structures [7]. Under different concentrations, Aβ42 forms oligomers, protofibrils, or mature fibrils that deposit in brain tissues and induce neurodegeneration [5]. Importantly, it has been reported that the ordered structures in Aβ42 possess higher neurotoxicity compared with amorphous aggregates [8]. Therefore, inhibition of the aggregation of Aβ or disruption of the ordered structures in aggregates represents a potential and promising treatment strategy for AD.

To date, a variety of inhibitors have been developed, including peptides [9], antibodies [10], nanoparticles [11], and polyphenols [12,13]. Among these, polyphenols emerge as promising candidates due to their deep blood−brain barrier permeability [14,15]. In addition, polyphenols are abundant in daily food and products, and have the advantages of high availability, low toxicity, and nutritional value. For example, epigallocatechin-3-gallate (EGCG) has been reported to effectively inhibit the aggregation of Aβ and reduce cytotoxicity to neurons [16,17]. However, the mechanisms of inhibition arising from polyphenols remain underexplored. Computational studies have been performed to shed light on the atomic interactions between Aβ and polyphenols [18]. Han et al. [19] revealed the potential interaction between polyphenols and the hydrophobic core of Aβ peptides through conventional MD simulations. Nonetheless, most studies have been limited to certain molecules, and few general principles or rules governing these interactions have been established. This highlights the need for further research to uncover broader insights into how polyphenols inhibit Aβ aggregation and to identify common mechanisms that could guide the development of more effective therapeutic strategies.

In this work, we leveraged computer simulations and correlation studies to probe the mechanisms of polyphenols interacting with Aβ peptides and infer general rules for optimizing polyphenols for future drug design. We performed molecular dynamics (MD) simulations for six polyphenols, analyzed their interaction with Aβ42 pentamer, and computed the binding free energy using the theory of molecular mechanics Poisson-Boltzmann surface area to provide a quantitative scheme for identifying the most potent polyphenol as a potential treatment for AD. The visualization of the process is shown in Figure 1. The polyphenols were simulated to bind to the fibril, disrupting its secondary structure and reducing its neurotoxicity. In alignment with previous experimental observations, EGCG and nobiletin have low binding free energies and thus superior binding affinity to the peptides compared to other polyphenols. Through the detailed structural analysis, we observed that the hydrogen bonding with the peptide backbone and hydrophobic interaction with the side chain residues are the primary molecular forces driving the binding events and potential destabilization of Aβ fibrils. A subsequent correlation study shows that the binding free energy is highly associated with molecular features that represent the hydrophobicity and local chemical environment (e.g., the hydrophobic moieties or hydroxyl groups). In a dimer assembly simulation, we further confirmed EGCG's ability to inhibit Aβ42 dynamic aggregation through the reduction of inter-peptide hydrogen bonding and suppression of secondary structure formation compared with other polyphenols. Overall, we identified EGCG as the most effective candidate for binding to and destabilizing Aβ aggregates. Furthermore, we uncovered key molecular features that enhance a polyphenol's ability to perturb Aβ aggregates. These insights provide valuable guidelines for the design and optimization of polyphenol-based molecules, offering a promising avenue for future drug discovery in the treatment of AD.

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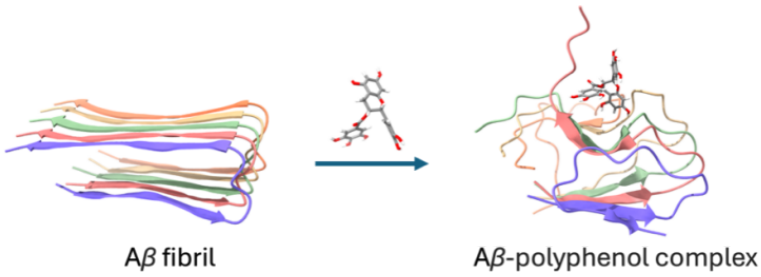


Figure 1. Polyphenols disrupt Aβ β-sheet structures by forming stable complexes that interfere with inter-peptide interactions, offering a molecular basis for anti-amyloid therapeutic design

2. Methods

2.1. Parameterization of Aβ peptide and polyphenols

Our first step in the MD simulation was to establish an equilibrated all-atom model of Aβ42 to accurately capture molecular details and properties of Aβ42 fibrils. The initial structure was obtained from the RCSB Protein Data Bank (ID: 2BEG), which represents a pentameric Aβ42 structure stabilized by beta sheets. We selected this model because it is considered the minimal unit capable of simulating and recapitulating the properties of aggregated Aβ fibrils, as supported by previous studies [19]. Different force fields have been explored in simulations, including Amber, GROMOS, and CHARMM, all possessing different parameters for the description of the energy of the protein as a function of its atomic coordinates. In this work, we employed CHARMM36 [20] as it yields more promising results, as shown by Samantray et al. [21] The parameterization of the initial pentamer structure was performed using the gmx pdb2gmx function to assign atom types, charges, and energy terms. The protonation states of the residues were determined at a physiological pH of 7. The resulting configuration was then placed in a cubic water box with a length of 6.5 nm, and five sodium ions were added to balance the charge of the simulation box.

Regarding the polyphenols, the CHARMM36 force field does not contain parameters for the small molecules. Therefore, the CHARMM General force field [22] (CGenFF) was utilized to parameterize the polyphenols for the simulation. The initial structures of the polyphenols were constructed using openbabel [23], which converted the simplified molecular input line entry system (SMILES) strings into mol2 files encoding the 3D structure information of each molecule. After passing these files through the CGenFF engine, the small molecules were introduced into the water box containing the pre-equilibrated Aβ42 pentamer. The polyphenols were initially placed at a non-interacting distance from the peptide to avoid biasing the simulation. In Figure 2, we provide a comprehensive overview of the chemical structures and 3D representations of the six polyphenols studied in this project: apigenin, catechin, curcumin, epigallocatechin-3-gallate (EGCG), nobiletin, and resveratrol.

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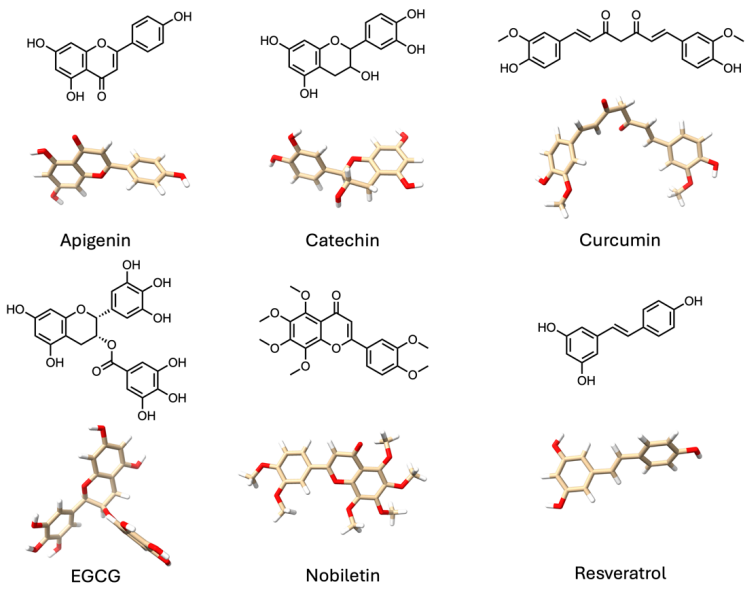


Figure 2. The chemical structures and 3D visualizations of the polyphenols investigated in this work. The chemical structure and molecular configuration are rendered with ChemDraw and ChimeraX [24], respectively

2.2. MD simulation details

All simulations were performed using the Gromacs 2023.1 version [25]. For the Aβ42 simulations, an energy minimization step was first performed to eliminate any forces exceeding 1000 kJ/mol/nm^2 that may arise from high potential energy configurations, such as atomic overlap and restrained structures. Following this, the system was equilibrated to achieve stable and relaxed conformations. During equilibration, the temperature was maintained at 310 K by the velocity-rescale [26] algorithm, with a coupling constant of 1 ps. The pressure was controlled at 1 bar by a stochastic cell-rescale scheme [27], with a coupling constant of 2 ps. These conditions were chosen to replicate the biological settings. The NPT equilibration consisted of 10 ns to converge the thermodynamic quantities, including the potential energy of the system and the root mean squared deviation (RMSD) of the peptide backbone. The equilibrated structure of the Aβ42 pentamer retained a β-sheet structure with minor rearrangements of the chains Figure 3 (A). Both the potential energy of the systems and the RMSD have converged, oscillating around a constant value. Figure 3 (B) and Figure 3 (C), The results indicate that the Aβ42 pentamer has been well equilibrated and prepared for subsequent simulations.

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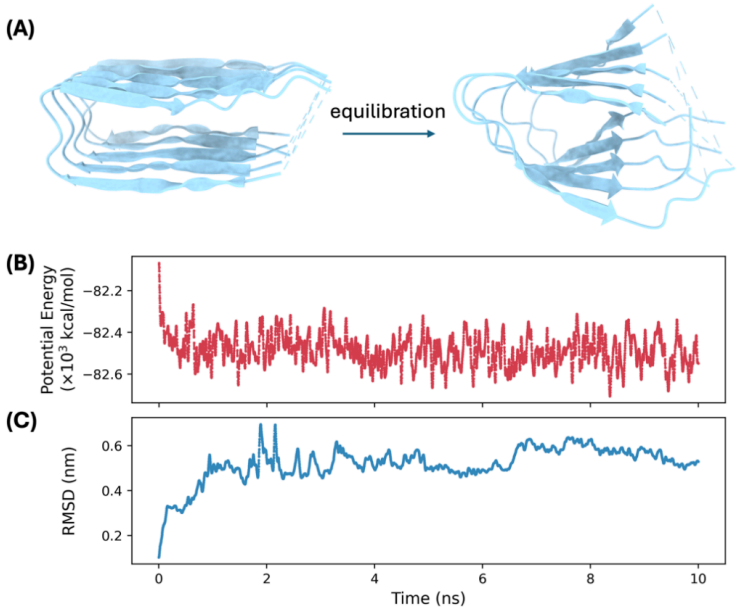


Figure 3. The equilibration of the Aβ42 peptide pentamer at 310 K. (A) Initial and equilibrated structure of the pentamer visualized by ChimeraX [24]. (B-C) The profiles of potential energy (B) and the RMSD of the peptide backbone (C) along the simulation time

After obtaining the equilibrated Aβ42 pentamer structures, we proceeded to simulate their interaction with the six polyphenols. Each polyphenol was initially placed in the water box at a sufficient distance from the Aβ42 pentamer to ensure no direct interactions at the start of the simulation. An energy minimization step was then implemented to remove potential high atomic forces. Following this, a long and unbiased simulation under the NPT conditions was carried out until a stable adduct between polyphenols and Aβ42 pentamer was formed. Empirically, a simulation of 150 ns proved sufficient for all to stably bind to the Aβ42 pentamer.

To analyze the binding mechanisms, structural analyses were performed on the final 5 ns of the trajectory, including calculating hydrogen bonds and residue contact frequencies using MDAnalysis [28], a Python library designed for MD trajectory analysis. In total, six independent simulations were prepared and conducted, each focusing on one specific polyphenol. These simulations formed the basis for subsequent calculations and analyses, enabling us to compare the binding behaviors and affinities of the different polyphenols to the Aβ42 pentamer.

2.3. Free energy calculation

Free energy calculation provides insight into how the binding of a small molecule disrupts the stability of Aβ42 pentamer. Free energy is a thermodynamic quantity that reflects the stability of a system, with lower values indicating high thermodynamic stability. This allows us to quantitatively compare the binding affinity of different polyphenols to the peptide. To compute the binding free energy, we employed the tool gmx_MMPBSA [29] , which relies on the theory of molecular mechanics Poisson–Boltzmann surface area [30] (MM/PBSA). The method calculates free energy in an implicit solvent environment, simplifying the computational process while still providing accurate results. To improve the statistics, we utilized the multi-frame approach by computing the free energy for 250 frames in the final 5 ns and obtained the averaged values with an entropic correction accounting for the loss of freedom of the polyphenol upon binding [31]. By incorporating

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entropy correction, we improve the accuracy of the binding free energy estimation to provide a more realistic representation of the molecular interactions involved. The force field parameters used for the molecular mechanics calculations were consistent with those used in the simulations: the CHARMM36 force field for the protein and the CHARMM General Force Field (CGenFF) for the polyphenols. This consistency ensures that the free energy calculations align with the physical models used throughout the study, providing reliable and comparable results for the binding affinities of the six polyphenols.

2.4. Structure-property relationship analysis

Correlating molecular structure with binding free energy is a non-trivial task that aims to provide design principles for optimizing and engineering polyphenol-based molecules for Alzheimer's disease (AD) treatment. To this end, we first employed an unsupervised clustering algorithm, Uniform Manifold Approximation and Projection (UMAP) [32], to visualize the structural diversity and distribution of the polyphenol candidates in a reduced-dimensional space. The molecules were featurized using Morgan fingerprints with a radius of 3 and a bit length of 2048 using RDKit [33], which captures both local and extended topological features relevant for molecular recognition. The projection onto reduced dimensions enabled us to identify similar clusters of molecules.

Next, we implemented ridge regression (with regularization parameter $\alpha = 0.1$) using the same fingerprint representation to identify the most informative molecular features responsible for variations in binding free energy across the polyphenols. The fingerprint bit assigned the highest regression coefficient, indicating its strong correlation with binding affinity, was mapped back onto the molecular structures and visualized using RDKit [34]. This provided interpretable structure-activity relationships and highlighted specific substructures that may be critical for enhancing binding performance.

2.5. Self-assembly of Aβ42 dimers with polyphenols

The nucleation and assembly of Aβ peptides are primarily driven by inter-peptide hydrogen bonding and the formation of ordered secondary structures. In our previous simulations, we started with a preassembled Aβ42 pentamer and investigated its interaction with various polyphenols under the assumption that binding affinity can be a proxy for the polyphenols' ability to inhibit Aβ peptide nucleation and fibril formation. These ordered fibrillar aggregates have been implicated as the primary neurotoxic species in Alzheimer's disease, in contrast to amorphous or disordered aggregates, which are generally considered less harmful [8]. In this context, we aim to demonstrate that polyphenols with higher binding affinity (e.g., EGCG) can more effectively disrupt or prevent the formation of ordered structures between Aβ peptides, compared to lower-affinity polyphenols (e.g., apigenin). This comparative analysis provides a mechanistic basis for evaluating the inhibitory potential of polyphenols in Aβ aggregation and could inform the rational design of anti-amyloid therapeutics.

As a proof of concept, we conducted simulations involving two Aβ42 peptide chains in the presence of each type of the polyphenols in this study, along with a control system containing no polyphenols. The peptide chains were initially separated in a cubic simulation box with a side length of 8 nm, ensuring no direct interaction between the two peptides. Twelve molecules of each polyphenol were separately inserted into the simulation box, yielding six distinct systems (one for each polyphenol type). Each system was then solvated and neutralized with sodium and chloride ions to achieve a physiological ionic strength of 0.15 M In parallel, a control system without any

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polyphenols was prepared, totaling seven independent simulation systems. Each system was energy-minimized using the steepest descent algorithm and subsequently subjected to a 200 ns NPT simulation to allow for the formation and stabilization of secondary structures, using the same simulation parameters described in Section 2.2. After the simulations, we analyzed the entire trajectories using MDAnalysis to quantify both the number of hydrogen bonds and the extent of secondary structure formation within the peptides. These metrics were used to evaluate the impact of polyphenols on inhibiting the structural ordering associated with Aβ aggregation.

3. Results

3.1. Polyphenols interact distinctly with Aβ42 pentamer

Six polyphenols—apigenin, catechin, curcumin, epigallocatechin-3-gallate (EGCG), nobiletin, and resveratrol—were selected for this study due to their established roles in modulating Aβ fibril aggregation [35] Figure 2. While these polyphenols exhibit distinct topological structures, they share common substructural motifs, such as benzene rings and hydroxyl groups, which may enable them to interact with the Aβ42 pentamer through hydrophobic interactions and hydrogen bonding. For each polyphenol, we conducted extensive molecular dynamics simulations to model the binding processes and obtain their final bound states.

The final configurations of the bound states of each polyphenol are illustrated in Figure 4. Despite sharing common molecular features, the polyphenols exhibit distinct interaction patterns with the Aβ42 pentamer peptide. We observed that apigenin, catechin, and curcumin primarily interact with the pentamer at a surface level, causing minimal disruption to the overall structure of the peptide aggregation. This is evidenced by the relatively preserved secondary structures of the peptide. In contrast, EGCG, nobiletin, and resveratrol demonstrate stronger interactions, with peptide chains wrapping around these molecules. Notably, the unique structural configuration of EGCG enables it to integrate more effectively into the peptide pentamer. These qualitative findings suggest that EGCG, nobiletin, and resveratrol may bind to the peptide with higher affinity and could potentially be more effective than apigenin, catechin, and curcumin in destabilizing the peptide structure. We were then motivated to provide a quantitative perspective on the binding strength of each polyphenol to the peptide and to explore the underlying mechanisms driving these binding interactions.

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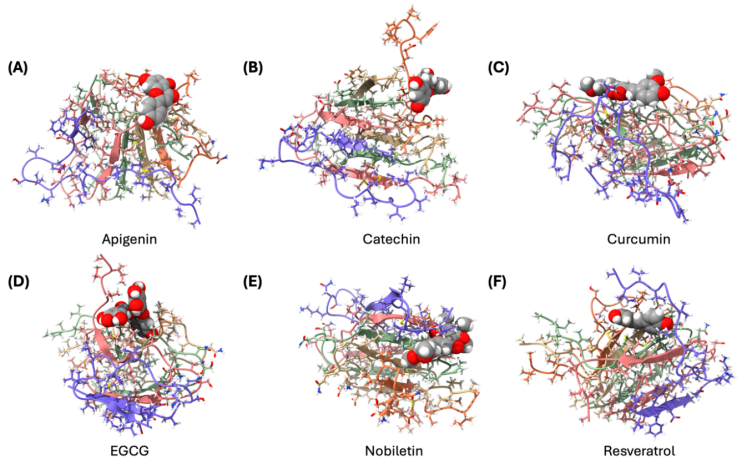


Figure 4. Visualization of the polyphenol-Aβ peptide complexes including (A) Apigenin, (B) Catechin, (C) Curcumin, (D) EGCG, (E) Nobiletin, and (F) Resveratrol. The snapshots were taken from the final frame at 150 ns and rendered using ChimeraX [24]. The polyphenol is represented as a space-filling model. The backbone and side chain of the peptide pentamer are shown as ribbons and sticks, respectively. The water and ions are omitted for clarity

3.2. Binding affinity of polyphenols to Aβ42 pentamer

In this section, we aim to provide a quantitative scheme to quantify the binding affinity and gain insights into the potential mechanisms of action for each polyphenol. We first computed the free energy of binding to quantitatively assess their interactions with the Aβ42 pentamer. A stable binding mode is characterized by a low binding free energy, and we present the computed free energy in Figure 5 (A). It is observed that EGCG and nobiletin exhibit the lowest binding free energies, with values of -20.24 kcal/mol and -18.88 kcal/mol, respectively. These large negative values indicate a significant decrease in free energy upon binding, suggesting that the binding of EGCG and nobiletin to the Aβ42 pentamer is spontaneous and thermodynamically favorable. Following this, resveratrol also showed a strong negative free energy of -13.11 kcal/mol. In contrast, curcumin and catechin exhibited less negative binding free energies, while apigenin showed a positive binding free energy, indicating weak or unfavorable interactions with the Aβ42 pentamer. The results of our study are in excellent agreement with previous computational and experimental findings, which demonstrated that EGCG effectively inhibited Aβ aggregation [36,37].

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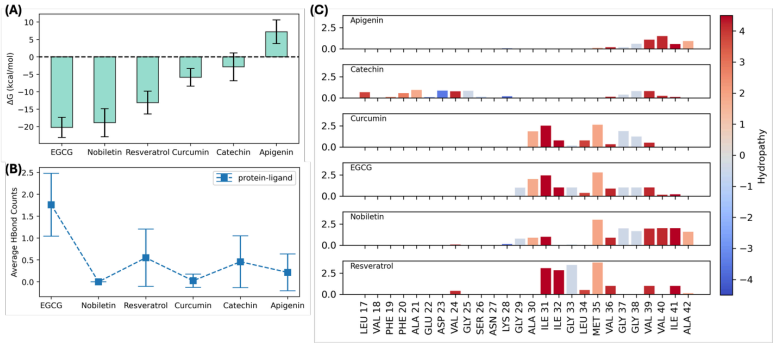


Figure 5. Quantitative analyses of the interaction between polyphenols and Aβ pentamer. (A) The binding free energy and (B) average hydrogen bond counts are plotted as a function of polyphenol identity. (C) The average contact number between the polyphenol and each amino acid residue, where red and blue bars represent hydrophobic and hydrophilic residues, respectively. The statistics are obtained from the final 250 frames (5 ns) of the simulation

We next sought to investigate the molecular mechanisms underlying the observed free energy differences among the polyphenols. As shown in Figure 5 (B), EGCG exhibits the highest average number of hydrogen bonds with the peptide backbone, which may contribute to its ability to disrupt the ordered β-sheet structure of Aβ peptides. This observation aligns with the fact that EGCG possesses the greatest number of hydroxyl groups among the tested compounds, supporting our initial hypothesis that hydrogen bonding by hydroxyl groups plays a critical role in determining the extent of pentamer disruption. In contrast, polyphenols with fewer hydroxyl groups, such as resveratrol, curcumin, catechin, and apigenin, demonstrated more moderate hydrogen bonding interactions. These findings suggest that the abundance of hydroxyl groups and their capacity to form hydrogen bonds are key factors influencing the destabilization of Aβ peptide structures.

Interestingly, although nobiletin lacks hydroxyl groups and does not form hydrogen bonds, it still exhibited a binding free energy comparable to that of EGCG. This observation suggests that hydrogen bonding is not the sole molecular mechanism driving stable binding between polyphenols and the Aβ42 pentamer. To further investigate the underlying interactions, we computed the contact frequency between each polyphenol and the amino acid residues of the peptide pentamer. This analysis revealed that nobiletin engages in extensive hydrophobic interactions with key residues of the Aβ42 pentamer, such as isoleucine, valine, and methionine. The strong hydrophobic interaction may compensate for the absence of hydrogen bonding. We observed that EGCG, curcumin, and resveratrol also show localized and extensive hydrophobic interaction with the Aβ42 pentamer, ranging from residue 31 to 41. These interactions likely contribute to their relatively low binding free energies and enhanced destabilization effects on the Aβ42 structure. In contrast, catechin and apigenin displayed weaker hydrophobic interactions, which aligns with their higher binding free energies and reduced efficacy in disrupting the Aβ42 pentamer.

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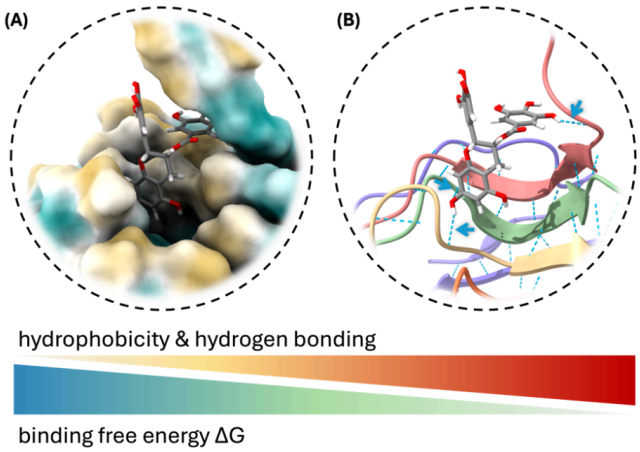


Figure 6. Visualization of the top-performing polyphenol, EGCG, in the bound state. (A) The peptide is represented as a molecular surface, on which yellow indicates the hydrophobic core of the peptide, and cyan indicates the hydrophilic environment. (B) The visualization of the hydrogen bond network in the complex. The blue arrows highlight the presence of hydrogen bonds between the peptide and the EGCG molecule. The remaining dashed lines show the hydrogen bonds within the Aβ pentamer

In Figure 6, we show that EGCG both interacts with the hydrophobic regions of the peptide and forms hydrogen bonds with the Aβ42 pentamer backbone, further demonstrating the interplay between hydrogen bonding and hydrophobic interactions. All these results highlight the multifaceted nature of polyphenol-Aβ42 interactions and suggest that both hydrogen bonding and hydrophobic forces are critical determinants of polyphenol activity in destabilizing Aβ42 aggregates. This insight may inform the design of more effective therapeutic agents targeting amyloid aggregation in neurodegenerative diseases.

3.3. Structure-property relationship

Next, we sought to further elucidate the relationship between molecular structure and binding affinity by employing dimensionality reduction and regression techniques. Specifically, we used Uniform Manifold Approximation and Projection (UMAP) [32], a nonlinear technique that reduces high-dimensional molecular representations to lower dimensions to facilitate visual interpretation. Molecules were encoded using Morgan fingerprints [33], which represent each compound as a bit vector corresponding to the presence or absence of specific submolecular fragments. Despite the limited data, the UMAP projection in Figure 7 (A) reveals a clear separation among the polyphenol compounds, with a notable correlation between their spatial distribution and binding free energy. Compounds exhibiting lower binding free energies, such as resveratrol, nobiletin, and EGCG, are clustered on the right side of the plot, while those with weaker binding affinities are on the left. This spatial pattern suggests that molecular structure is closely associated with binding behavior, highlighting the presence of an underlying structure-property relationship among the polyphenol candidates.

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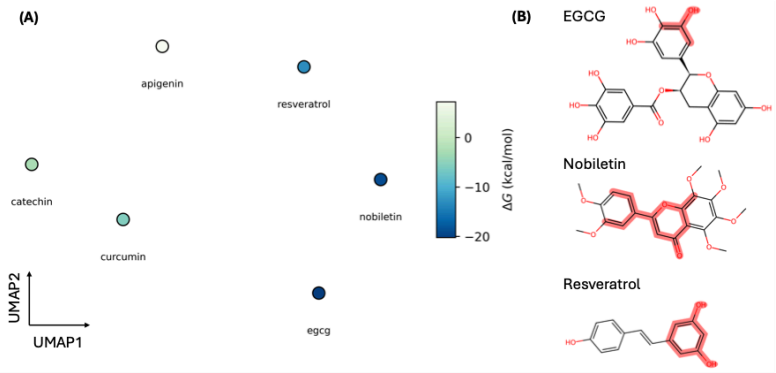


Figure 7. Structure-property analysis between polyphenols and binding free energy. (A) A low-dimensional representation of six polyphenols using UMAP. Each point represents a compound and is colored by its binding free energy to the Aβ42 pentamer, with darker colors indicating stronger binding. (B) Visualization of the most influential molecular substructures in EGCG, nobiletin, and resveratrol, as identified by ridge regression analysis. Highlighted regions (in red) correspond to fingerprint bits most strongly associated with low binding free energy, revealing key functional groups and scaffolds responsible for binding affinity

To further dissect the molecular features responsible for these differences, we applied ridge regression to model the relationship between fingerprint vectors and binding free energy, using a regularization parameter of $\alpha = 0.1$ to mitigate overfitting. From the trained model, we identified the fingerprint bit with the highest absolute regression coefficient, indicating the most influential substructure. This feature was visualized in red in Figure 7 (B). Our analysis revealed that on one hand, hydroxyl groups play a critical role in driving strong binding affinity, as evidenced in EGCG and resveratrol, which exhibited the highest affinity among all compounds. On the other hand, possessing a highly hydrophobic aromatic ring system makes up for the absence of hydroxyl groups in nobiletin, making it the second-best in binding affinity. In agreement with the conclusion in Section 3.2, the finding underscores that both polar interactions (e.g., hydrogen bonding via hydroxyl groups) and hydrophobic interactions contribute to binding strength, depending on the structural context of the molecule.

3.4. EGCG effectively inhibits amyloid β oligomerization

In our previous simulations, we adopted the binding affinity of polyphenols to Aβ peptides as a proxy for their ability to inhibit peptide aggregation and the formation of ordered fibrillar structures. This assumption was based on the understanding that fibril formation is driven by inter-peptide hydrogen bonding and the stabilization of secondary structures, and that polyphenols are capable of forming hydrogen bonds with peptides and thus inhibit the inter-peptide hydrogen bonding [8]. However, the direct correlation between binding free energy and the inhibitory effect on fibril formation has not been explicitly validated. To this end, we conducted additional simulations to directly assess whether polyphenols with higher binding affinity also exhibit greater efficacy in disrupting the inter-peptide hydrogens and delaying the formation of ordered Aβ structures.

We first set up seven water boxes, each with two protein peptides separated with no direct interaction. Next, we added twelve molecules of each polyphenol into a water box, with the seventh box containing no polyphenols serving as the control group.. It is anticipated that polyphenols like EGCG, with the lowest binding free energy, would be able to interrupt the protein assembly the most by inhibiting protein hydrogen bonding and secondary structure for fibril formation. Polyphenols

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like apigenin, on the contrary, would not work nearly as well due to their high binding free energy and weak interaction with Aβ peptides.

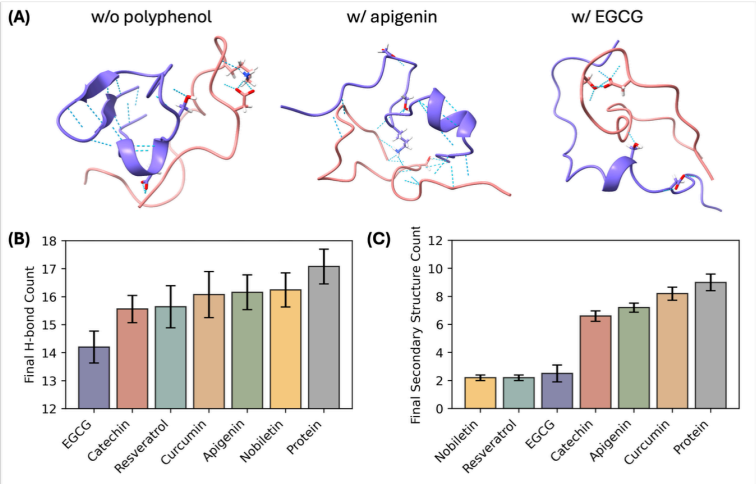


Figure 8. Polyphenol-mediated inhibition of Aβ42 aggregation through disruption of hydrogen bonding and secondary structure formation. (A) Representative simulation snapshots at the final frame (1 μs) show the conformation and hydrogen bonding between two Aβ42 peptides in three systems: without polyphenol (left), with apigenin (middle), and with EGCG (right). Hydrogen bonds are shown as cyan dashed lines. EGCG treatment results in visibly fewer inter-peptide hydrogen bonds compared to the apigenin and control groups. (B) The hydrogen bond counts in the final 5 ns between the two Aβ42 peptides in each system. EGCG significantly reduces hydrogen bonding throughout the simulation, while others exhibit minimal difference from the control protein group. (C) The count of secondary structure elements formed within and between the peptides. EGCG, along with resveratrol and nobiletin, markedly suppresses the emergence of ordered structures compared to others and the control. These findings confirm EGCG's strong inhibitory effect on Aβ42 aggregation, consistent with its high binding affinity and its ability to disrupt inter-peptide interactions critical for nucleation and fibril formation

We monitored the formation of secondary structure and inter-peptide hydrogen bonds throughout the simulation since they serve as a reliable indicator of aggregate toxicity in Aβ peptides. Previous studies have demonstrated that the ordered fibrillar structures possess higher neurotoxicity compared with amorphous aggregates [8]. In this context, tracking secondary structure formation provides a mechanistic link between molecular interactions and functional outcomes. In addition, hydrogen bonding plays a crucial role in stabilizing these ordered conformations and is a key driving force in the nucleation and propagation of fibrils.

Final simulation snapshots revealed that the potent binder EGCG substantially reduced the number of hydrogen bonds formed between Aβ42 peptides compared to both the blank control and the weak-binder apigenin-treated system during the assembly process Figure 8 (A). Quantitative analyses of inter-peptide hydrogen bonding and secondary structure formation confirmed that EGCG is the most effective polyphenol in inhibiting Aβ42 aggregation, as it markedly reduces both protein hydrogen bonds and the emergence of ordered secondary structures Figure 8 (B) (C). Importantly, low free energy candidates -- EGCG, resveratrol, and nobiletin -- strongly suppress secondary structure formation, albeit with distinct effects on hydrogen bonding Figure 8 (C). This inhibitory effect is likely attributed to EGCG's strong capacity to form competing hydrogen bonds with the peptide backbone, thereby disrupting the inter-peptide interactions necessary for nucleation.

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While we acknowledge the limited time scales for the assembly simulations, the consistent and significant differences across systems provide confidence in these observations. The findings are in strong agreement with the binding free energy data and provide direct mechanistic evidence supporting EGCG's ability to interfere with early-stage Aβ aggregation and potentially prevent the formation of toxic fibrillar assemblies.

4. Discussions

While the present study yields meaningful findings on polyphenols' role in inhibiting Aβ42 aggregation, it is important to acknowledge its limitations. The scope of our study was constrained by the computational cost of all-atom molecular dynamics simulations, limiting the number and diversity of polyphenol candidates analyzed. Additionally, the accuracy of free energy calculations could be improved by employing enhanced sampling techniques [38] to provide more precise estimates of binding affinities. In the future, expanding the dataset and refining computational methods will enable data-driven optimization of polyphenol structures using machine learning [39]. Such approaches could identify or design compounds with maximized binding affinity and minimized binding free energy, offering a pathway to develop more effective anti-amyloid peptide agents. Future experimental testing can potentially further furnish the understanding of polyphenols' inhibition on Aβ fibrils.

5. Conclusion

This project aimed to evaluate the ability of various dietary polyphenols to disrupt Aβ42 aggregation, a key pathological hallmark of Alzheimer's disease, and to identify structural features that enhance their inhibitory efficacy. Using molecular dynamics simulations combined with quantitative structural analysis, we demonstrate that both hydrogen bonding and hydrophobic interactions are critical determinants of polyphenol binding affinity and their capacity to interfere with Aβ42 peptide assembly. Among the tested polyphenols, EGCG emerged as the most potent inhibitor with the lowest binding free energy to Aβ42 pentamer. It formed the highest number of hydrogen bonds with the Aβ42 pentamer and engaged in hydrophobic interactions, effectively destabilizing peptide-peptide contacts essential for fibril formation. Furthermore, in a peptide assembly simulation, EGCG indeed substantially reduced inter-peptide hydrogen bonding and suppressed the emergence of secondary structures, both of which are linked to toxic fibril formation. These findings are consistent with the structure-activity relationship uncovered through UMAP clustering and fingerprint-based regression, which revealed that the presence of multiple hydroxyl groups and extended aromatic systems, both characteristic of EGCG, are associated with stronger binding affinity and greater inhibitory activity.

In summary, this work advances understanding of how polyphenols modulate Aβ42 aggregation at the molecular level. It reinforces the therapeutic potential of natural compounds like EGCG while laying the groundwork for the design of novel inhibitors targeting amyloid-driven neurodegenerative diseases. By bridging structure, dynamics, and function, this study contributes valuable insight into the development of effective, mechanism-based interventions for Alzheimer's disease.

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