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REVIEW

Recent studies of atomic-resolution structures of tau protein and structure-based inhibitors

Lili Zhu, Zhenyu Qian*

Key Laboratory of Exercise and Health Sciences (Ministry of Education) and School of Kinesiology, Shanghai University of Sport, Shanghai 200438, China
* Correspondence: qianzhenyu@sus.edu.cn

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***Background*: Alzheimer's disease (AD) is one of the most popular tauopathies. Neurofibrillary tangles and senile plaques are widely recognized as the pathological hallmarks of AD, which are mainly composed of tau and β-amyloid (Aβ) respectively. Recent failures of drugs targeting Aβ have led scientists to scrutinize the crucial impact of tau in neurodegenerative diseases. Mutated or abnormal phosphorylated tau protein loses affinity with microtubules and assembles into pathological accumulations. The aggregation process closely correlates to two amyloidogenic core of PHF6 (^{306}VQIVYK^{311}) and PHF6* (^{275}VQIINK^{280}) fragments. Moreover, tau accumulations display diverse morphological characteristics in different diseases, which increases the difficulty of providing a unifying neuropathological criterion for early diagnosis.**

***Results*: This review mainly summarizes atomic-resolution structures of tau protein in the monomeric, oligomeric and fibrillar states, as well as the promising inhibitors designed to prevent tau aggregation or disaggregate tau accumulations, recently revealed by experimental and computational studies. We also systematically sort tau functions, their relationship with tau structures and the potential pathological processes of tau protein.**

***Conclusion*: The current progress on tau structures at atomic level of detail expands our understanding of tau aggregation and related pathology. We discuss the difficulties in determining the source of neurotoxicity and screening effective inhibitors. We hope this review will inspire new clues for designing medicines against tau aggregation and shed light on AD diagnosis and therapies.**

Keywords: tau; paired helical filaments; inhibitor; cryo-electron microscopy; molecular dynamics simulation

Author summary: The accumulation of tau protein is closely related to the pathological process of Alzheimer's disease (AD). At present, the source of tau neurotoxicity has not been fully clarified. It may come from the misfolding of tau in the early stage, oligomeric intermediates, or the aggregation process itself. Therefore, probing the atomic structures of tau, exploring key interactions, and screening potential inhibitors are crucial to the proposal of effective treatments. We hope this review can expand our understanding of tau pathology to accelerate medicine development for AD therapies.

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disorder with the characteristic of cognitive decline [1]. By 2020, there are more than 50 million dementia patients worldwide, of which AD accounts for 50% to 75% [2]. The incidence rate of AD increases dramatically with age [3]. 11.3% of people over 65 years old suffer from AD and the number comes to 34.6% for people over 85. The patient has a slight memory loss in the early stage of the disease, and the memory continually declines accompanied by cognitive dysfunction, and eventually, dementia occurs as the disease progresses [4,5]. The amyloid cascade hypothesis

proposed in 1992 that the deposition of β-amyloid (Aβ) is the causative event of AD pathology [6]. Toxic aggregates of Aβ may lead to oxidative stress, synaptic dysfunction, cellular membrane damage, telomerase dysfunction, and neuronal apoptosis [7,8]. However, drugs targeting Aβ have not yet succeeded. Emerging preclinical data demonstrate that tau pathology is not driven by Aβ pathology [9]. The accumulation of tau arises independently, and can correlate to Aβ deposits with the transcellular spread of pathological tau. Especially once cognitive deficits occur, the correlation between tau burden and clinical damage is stronger than that of Aβ burden [10]. The development of tau pathology may lead to microtubule instability, defects in microtubule transportation, oxidative stress response, mitochondrial dysfunction, and eventually neuronal apoptosis [11,12]. The synergistic effect of tau and Aβ runs throughout the AD course and fundamentally drives the progression of the disease [13], suggesting tau can serve as an alternative therapeutic target in AD treatment. A few clinical anti-tau strategies have been tried and some have turned out to be a failure, such as the microtubules stabilizer epithilone D, the GSK-3 inhibitor tideglusib, the aggregation inhibitor methylene blue and its derivatives [10], and monoclonal antibody semorinemab [14]. These failures may be attributed to the diversity of tau structure and the complexity of its interaction. As a promising therapeutic strategy, structure-based inhibitors targeting tau aggregation have gained more and more attention in recent years.

TAU DOMAINS AND ISOFORMS

Tau is a highly soluble and unfolded intracellular protein. It belongs to the microtubule-associated protein family and is widely found in the central nervous system [15]. Tau is abundant in axons and less in neuron dendrites, oligodendrocytes, and some non-neuronal tissues [16,17]. Tau is composed of the N-terminal region, proline-rich domain, microtubule-binding domain, and the C-terminal region [18], as shown in Fig. 1. During the transcription and translation of tau, the alternative splicing of exons 2, 3, and 10 generates six 37–46 kDa isoforms which correspond to 352–441 residues respectively. It is worth noting that exon 3 can only be translated in the presence of exon 2 [19,20]. Exon 2 and 3 are accountable for encoding N1 and N2 regions, and exon 10 is responsible for encoding the second repeat region (R2) of the microtubule-binding domain. Tau isoforms can be distinguished by the number of N1/N2, and whether tau includes R2 [19,21]. As displayed in Fig. 1, 4R tau isoforms have four repeat regions, while 3R tau isoforms lack R2.

TAU FUNCTIONS

Major function: binding and stabilizing microtubule

Tau constitutes more than 80% of microtubules-associated protein, and its primary function is to bind and stabilize microtubules [22,23]. The microtubule-binding domain is composed of four repeat regions (R1, R2, R3, and R4) and is the dominant structure of tau binding to microtubules [19]. The binding of tau to microtubules may be attributed to their electrostatic interaction in between [24]. One α-tubulin and one β-tubulin form a tubulin dimer, and then the dimers assemble into protofilaments that associate laterally into hollow microtubules. A recent cryo-electron microscopy (Cryo-EM) study suggested that one single repeat region of tau spans the interface of the intra-dimer and inter-dimer of tubulins takes α-tubulins as the center to

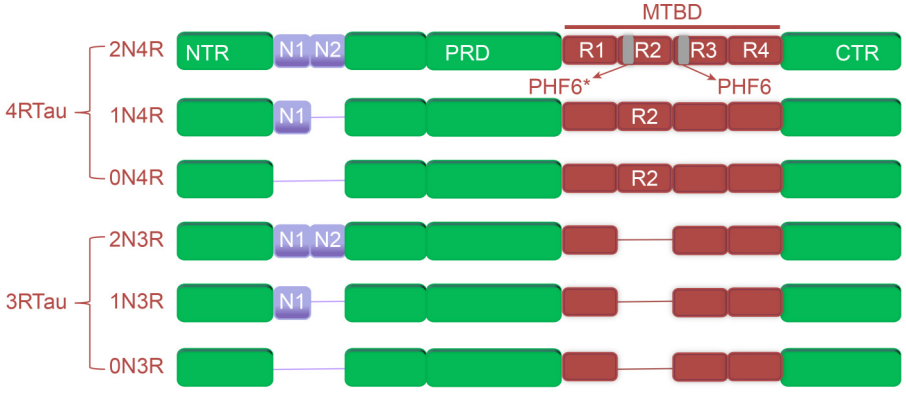


Figure 1. The domains and isoforms of tau. Tau is composed of the N-terminal region (NTR), proline-rich domain (PRD), microtubule-binding domain (MTBD), and the C-terminal region (CTR). MTBD involves four repeat regions named R1, R2, R3, and R4. Six isoforms are formed: 4R2N, 4R1N, and 4R0N for 4Rtau isoforms; 3R2N, 3R1N, and 3R0N for 3Rtau isoforms. Hexapeptides ^{275}VQIINK^{280} (PHF6*) and ^{306}VQIVYK^{311} (PHF6) are respectively located at the beginning of R2 and R3.

connect two β-tubulins along the protofilaments of microtubules [25], as shown in Fig. 2. Peter W. Baas group suggested that the actual stabilizer of axonal microtubules is MAP6 rather than tau. The reduction of MAP6 weakens the stability of the unstable domain of microtubules, while the decrease of tau reduces the unstable domain and increases the stable domain [26,27]. In general, tau is able to initiate the assembly of microtubules [28] and contribute to the stability of microtubules, directly or indirectly. Inspired by this notion, reducing the loss of microtubules through low-dose drugs that stabilize microtubules is a general treatment strategy for tau-related diseases [29].

Minor functions

Tau participates in axon transport, neurite growth regulation, and nutrition signal enhancement [30,31]. Tau may serve as a mediator to link the cytoskeleton and indirectly regulate cell functions. For example, microtubule-bound actin connects to two or more repeat regions of tau, which maintains the cellular coordinative operation and normal function [32]. Tau also participates in the protection and restoration of DNA, and plays an important role in maintaining the integrity of neuronal genomic DNA and RNA [33]. Tau in the nucleus may bind to DNA and protect it from mild heat stress damage [34]. Tau is suggested to help maintain the balance between ions K^+ and Na^+ at the neuron resting state and the intercellular transmitting of the electrical signal [24].

TAU AGGREGATION

Tau pathology based on its aggregation

Neurodegenerative diseases associated with tau self-assembly are known as tauopathies [35], which are related to more than 20 neurodegenerative disorders [36]. In the mutation or abnormal phosphorylation, tau detaches from microtubules, leading to the subsequent pathological process [25]. Paired helical filaments (PHFs) are the pathological aggregates in AD formed by tau self-assembly, which further aggregate into NFTs [37]. This aggregation process of intrinsically disordered tau can be affected by various factors, such as post-translational modifications, polyanion, residue mutation, metal ions, membrane environment, proteolytic cleavage, and cross-seeding with other amyloids [37–39]. For instance, post-translational modifications can regulate the affinity of tau to microtubules, affect the conformational state of tau and trigger the amyloidogenesis of tau monomer [37]. For another instance, heparin, the most frequently studied polyanion, can promote dimerization at the early stage of tau aggregation [37] and overcome the kinetic and thermodynamic barriers for tau fibrillization [40]. Once pathological tau is formed, it would gradually cover neurons, impact cellular basis functions, result in neuron death, and transcellularly spread to the entire brain along with the neuron networks [19,41]. The tau-containing PHFs in AD brain have a robust quantitative and topographical association with neurodegeneration, and the spatial distribution of pathological tau reflects the level and location of cortical atrophy [42].

The formation of tau fibrils involves oligomerization, nucleation, and elongation stages [43,44], as shown in Fig. 2. It begins with the transition of tau monomer from inert to seed-competent type. Compared with inert monomer, seed-competent monomer has the aggregated-prone PHF6 segment exposed to water solution, which provides the structural basis for further oligomerization [45]. Tau oligomers are diverse and reversible [46–48]. The smaller transient oligomers with the cross-β structure may assemble into fibrillar nucleus and are on-pathway to fibrils formation. Otherwise, types of oligomers are formed to be off-pathway to fibril formation [47,49,50]. There also exists unstable oligomers which are in rapid exchange with monomers and held together by electrostatic interaction [47]. Nucleation is a relatively slow but key step in the process of tau fibrillization. Once the nucleus is established, filaments can elongate rapidly with the addition of monomers [43,51]. Interestingly, a very small amount of PHF fibrils may disaggregate into soluble monomers over 1 month incubation, detected in the aggregation of K18 tau (the four repeat regions) *in vitro* using single-molecule fluorescence [46]. The tau fibril also displays a temperature-dependent stability and the formation of tau fibrils at 343 K can be reversed into monomers when cooled, demonstrated by the EM and molecular dynamics simulation (MD) study [52].

Tau fibrils show structural polymorphism in different diseases

In AD, all six tau isoforms are expressed, and the structural diversity increases the difficulty of providing a unifying neuropathological criterion for early diagnosis [53]. Both the straight filaments (SFs) and PHFs in AD brains are formed by two identical protofilaments (Fig. 3) [54]. In PHFs, the two protofilaments have a helical symmetry with an interface formed by the anti-parallel stacking of residues ^{332}PGGGQ^{336}. The C-shaped core of PHFs formed by residues E306 to G378 involves eight β-strands. β1 and β2 are parallel stacking with β8 through face-to-face hydrophobic groups and polar-zipper motifs respectively. A turn is accomplished by the links between β2 and β3 on the outer side of the protofilament and between β8 and β7 on the inner side.

β3 and β7 are arranged in anti-parallel alignment and are stabilized by sidechain hydrogen bonds. β4, β5 and β6 form a triangular conformation, which is stabilized by interior hydrophobic groups, aliphatic stacking and aromatic stacking [54]. Interestingly, the SF and PHF structures resolved by Cryo-EM are identical between sporadic and inherited patients of AD [55].

In Pick's disease (PiD), tau filaments are distinguished by the narrow filament (93%) and wide filament (7%). A recent Cryo-EM study shows that the two kinds of filaments both have an ordered core formed by residues 254−378 involving nine β-strands [56]. The ordered core is in a cross−β and elongated J-shaped conformation. The wide filament consists of two narrow filaments which form strong contacts at their distal tips by van der Waals (vdW) interaction. Besides, fibrils in PiD comprise 3Rtau isoform merely, for the tau sequence adopting the Pick fold is unable to accommodate R2 [57].

In chronic traumatic encephalopathy (CTE) [58], there are two types of tau fibrils due to the relative arrangement

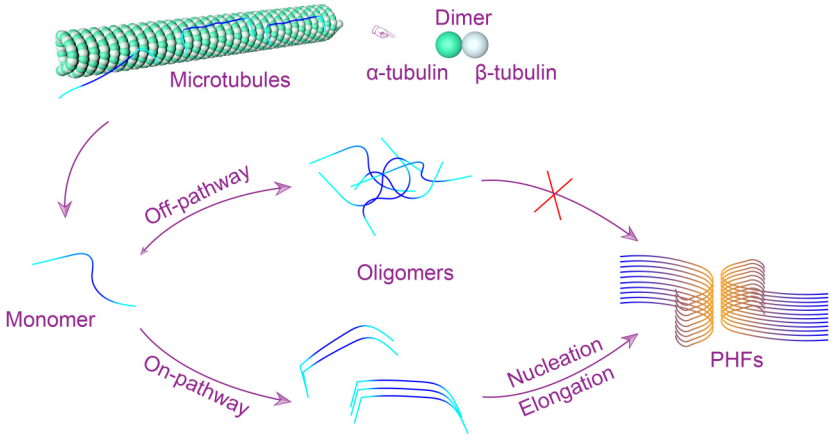


Figure 2. The process of tau aggregation. Tau binds to microtubules by the repeat regions, and one repeat region connects three tubulins along with the protofilaments. Abnormally, tau detaches from microtubules and assembles into on-pathway and off-pathway oligomers.

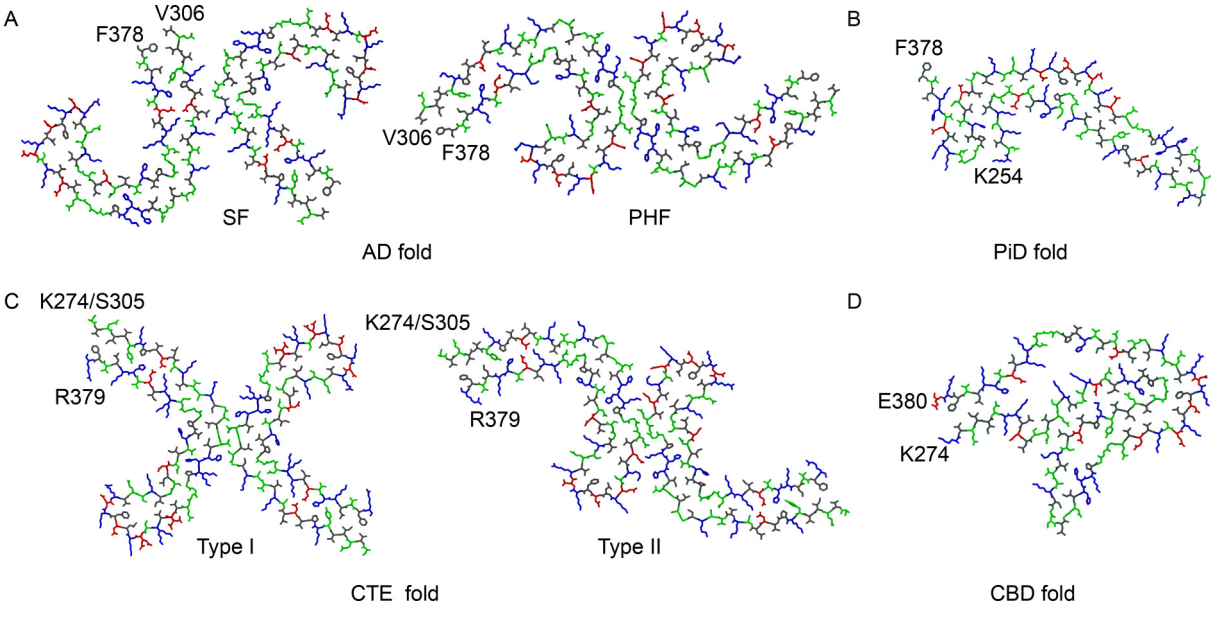


Figure 3. Tau filaments in diseases. These structures are derived by means of Cryo-EM. (A) Straight filament (SF) and paired helical filament (PHF) in AD (PDB ids: 5O3T, 5O3L). (B) Narrow filament (NF) in PiD (PDB id: 6GX5). The wide filament is not shown here, for its structure is limited to 8 Å resolution. (C) Type I and Type II filaments in CTE (PDB ids: 6NWP, 6NWQ). (D) Type I filament in CBD (PDB id: 6TJO). Type II filament in CBD is comprised of two type I filaments, which is not shown here.

of paired protofilaments [59]. Type I comprises about 90% of filaments and type II 10%. Revealed by negative-stain EM, the helical twists of type I filament result in projected widths of 20−25 nm, and the projection width of the type II filament is 15–30 nm. The tau filaments in CTE yield a C-shaped fold formed from residues K274–R379 of 3Rtau and S305–R379 of 4Rtau, more open than the tau fold in AD. The protofilament involves eight β-strands, with the interface of ^{324}SLGNIH^{329} in type I and ^{331}KPGGGQVE^{338} in type II [59].

In corticobasal degeneration (CBD), tau filamentous inclusions are made of 4Rtau [60,61], involving type I and type II filaments [61]. Type I filaments are composed of a single protofilament and adopt a previously unknown four-layered fold. Type II filaments consist of a pair of identical protofilaments of type I and the two protofilaments are related by C2 symmetry. The core of tau filaments in CBD comprises residues 274−380, consisting of eleven β-strands. The interface between protofilaments is formed by antiparallel stacking of ^{343}KLDFKDR^{349} through vdW interaction and hydrogen bonds [61].

Amyloidogenic cores formed by PHF6 and PHF6*

Two β-sheet-prone hexapeptides ^{275}VQIINK^{280} (PHF6*) and ^{306}VQIVYK^{311} (PHF6) are considered as the ordered core of promoting tau aggregation, which are respectively located at the beginning of R2 and R3 [62,63]. Normally, the repeat regions of tau tend to form a β-hairpin structure and failed to propagate in amyloid, where PHF6 is shielded by its upstream flanking region [45,64]. Under abnormal conditions such as disease-related mutation, key proline isomerization, and alternative splicing, this local structure may be destroyed, giving rise to the self-aggregation of tau [64]. The two hexapeptides can assemble into fibrils on their own, and the formed assemblies are rich in β-sheet with 3D zipper conformation of closely packed backbones and interdigitated side chains [36,65,66]. Moreover, PHF6 has a structural similarity with oligomers and neurofibrillary tangles formed by full-length tau, and thus it is broadly applied as the template of screening and exploring candidate drugs for amyloidosis [67]. PHF6-capping inhibitors are highly effective at blocking the aggregation of 3Rtau isoforms *in vitro*, and ineffective at inhibiting 4Rtau isoforms which contain the PHF6* segment [36,68]. In addition, PHF6* inhibitors are able to inhibit 4Rtau seeding, which is impossible for PHF6 inhibitors [36].

INHIBITORS TARGETING TAU AGGREGATION

The assembly process of tau protein from misfolding monomers into highly ordered β-sheet-rich aggregates is a key step in the development of tau pathology [69], and reducing or removing these aggregates is beneficial to alleviate the symptoms of diseases. According to the process of amyloid formation, inhibitors are developed to interfere with tau aggregation in three stages. First, inhibitors can act on the stage of misfolding and oligomerization. They bind to tau monomer and stabilize the monomeric structure, which reduces the misfolding and association of monomers [70,71]. Second, inhibitors can act on the nucleation stage [36,72]. They directly suppress the formation of tau nuclei, or sequester oligomers efficiently to neutralize the ability of seeding further aggregation. Finally, inhibitors can act on the fibrillary elongation or mature fibrils. They block the ends of short fibrils to prevent the addition of tau peptides [36,73], or remodel and disaggregate the preformed fibrils [74,75]. These inhibitors involve small molecules, nanoparticles, and short peptides, etc. The structure of inhibitors mentioned below are shown in Tables 1 and 2.

Small molecules

Polyphenol compounds are potent in the inhibition of tau amyloidosis [35,76]. Curcumin has the ability to inhibit tau aggregation and depolymerize pre-aggregated fibrils *in vitro* [77,78], while the poor absorption and metabolism of curcumin in body limit its biological activity [79]. Therefore, kinds of curcumin derivatives are developed. Curcumin derivatives can promote tau oligomers aggregating into large off-pathway aggregates and reduce the neurotoxicity of products [80]. Curcumin derivative PE859 with the straight conformation and a central pyrazole group has superior pharmacokinetic characteristics and more effective pharmacology of binding to and inhibiting tau [81–83]. Curcumin conjugated on the scaffold of Ru(II) complexes can inhibit the aggregation of tau R3 fragment in both nucleation and elongation stage, demonstrated by Liu *et al.* [84]. Besides, the smaller ancillary ligand bipyridine in curcumin-Ru1 complex can insert into the cave formed by ^{304}GSVQIVY^{310} which is the key segment for R3 aggregation. Resveratrol can impede the formation of neurofibrillary tangles [85], inhibit cellular toxicity induced by tau aggregates, and restrict the spread of tau oligomer across cells [86]. However, rapid metabolism and low bioavailability limit the utility of resveratrol in the human body [87]. Rosmarinic acids

can hinder the oligomerization and fibrillation of tau K18 fragment, and disrupt the oligomer stability of paired hexapeptide ^{306}VQIVYK^{311} by inserting into the cylindrical cavity and forming salt-bridges with residue K311 [88]. Green tea extract EGCG can promote the off-pathway aggregation and dissolve already-exiting tau oligomers or filaments [49]. Long-term treatment with EGCG improves the memory function of mice [89]. Quercetin or enzyme-MNP treated quercetin can effectively inhibit the formation of tau fibrils and decompose pre-formed tau fibrils [90]. Baicalein can inhibit tau aggregation by sequestering tau oligomers and promote the formation of off-pathway tau oligomers. It can also depolymerize pre-formed oligomers or mature fibrils in a concentration-dependent manner [91]. Xanthohumol, a natural phenolic compound xanthohumol, can reduce the aggregation of tau K18, prevent K18 from forming long and compact fibrils, and break the preformed mature tau K18 fibrils, reported by Zhang *et al.* [92].

Quinones can hinder the formation of pathological entities by inhibiting the misfolding and accumulation of tau [93,94]. Parietin, an orange anthraquinone pigment, can inhibit tau aggregation *in vitro* with the concentration range from 3 to 28 μg/mL, and the binding is driven by H-bond interaction with the VQIVYK motif [95]. Purpurin, another natural pigment of anthraquinones, can inhibit tau aggregation in a dose-dependent matter and dissemble pre-assembled tau fibrils. In a study of AD fruit fly, purpurin also reduces the neurotoxicity caused by tau pathology and alleviates the neurodegenerative symptoms [75]. Naphthoquinone-tryptophan (NQTrp) complex can inhibit the assembly of PHF6 *in vitro*. In *Drosophila* model, NQTrp treatment moderates the cytotoxicity induced by tau aggregates and ameliorates the degeneration of the eyes and brain with tauopathies [96]. NQTrp and NQTrp-Cl disassemble pre-formed PHF6 fibrils into non-toxic intermediates and dissolve pre-formed full-length tau fibrils by opening up the ordered core [67].

Tenuiorin moderately holds back the aggregation of tau and reduces the content of β-sheet structure, which may result from the hydrogen bonding and hydrophobic interactions between tenuiorin and the PHF6 motif of tau [97]. The derivative of usnic acid effectively hinders the formation of Ac-PHF6 fibrils and thus Ac-PHF6 peptides adopt small dot structures [98]. Nimbin and Salannin are respectively the intermediate and terminal limonoids, which can hamper the formation of β-sheet structure and enhance the cellular viability. Nimbin is more effective in inhibiting tau aggregation, while Salannin prefers to depolymerize preformed tau fibrils [99].

Isobavachalcone, an ingredient in traditional Chinese medicine psoralea corylifolia, can protect cells from the toxicity of tau oligomer, inhibit the aggregation of tau K18 and dissolve well-formed K18 fibrils by mainly interacting with R2 region, reported by Xiao *et al*. [100]. Vitamin B12 can inhibit the aggregation and fibrillization of tau by binding to the cysteine residues of tau [101]. Folic acid, also known as vitamin B9, helps stabilize the natural conformation of tau. It can effectively hold back the formation of β-sheet structure and large fibrils, and in turn cause misfolded tau to form amorphous oligomers and shorter fibrils [102]. On the contrary, the lack of vitamin B12 and folic acid may increase the risk of AD [103].

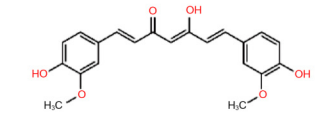
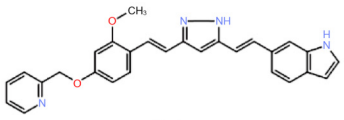
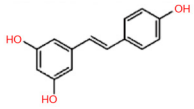
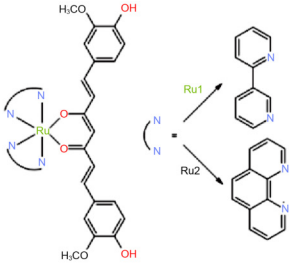
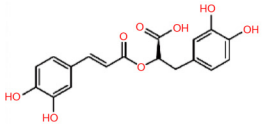
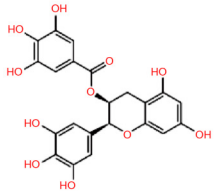
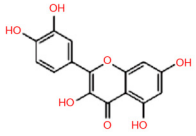
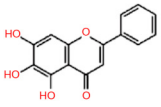
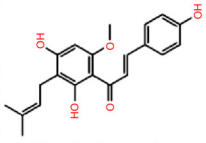
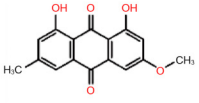
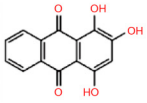
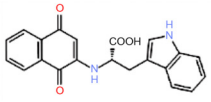
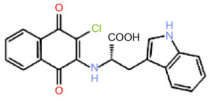
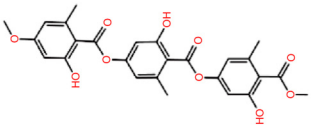
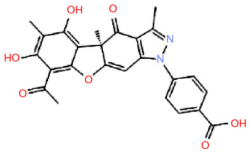
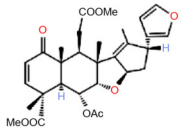
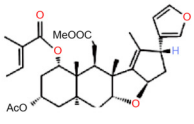
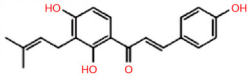
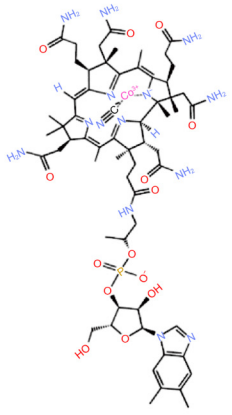
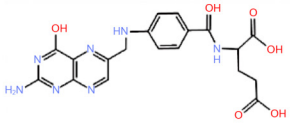
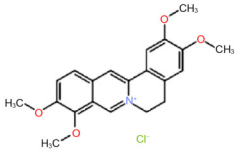
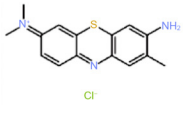
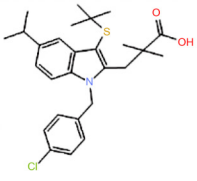
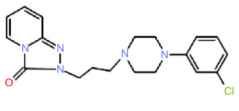
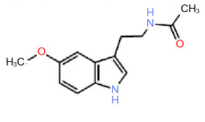
Toluidine blue (TB), a basic dye of the phenothiazine family, can stabilize the coil structure of tau in the brains of fruit flies and prevent the conformational change into aggregates [104]. Moreover, light excited TB can disaggregate mature fibrils and thereby relieve neurodegenerative symptoms of fruit flies such as olfactory disorders, memory loss, and movement disorders [104].

Palmatine chloride can inhibit tau aggregation and dissolve pre-formed tau fibrils into small fragments, and the anti-tau pathological ability of palmatine chloride increases obviously with the increment of concentration [105]. MK-886 can inhibit the formation of tau toxic oligomers and transfer toxic oligomer to nontoxic conformation, via binding to the proline-rich domain and microtubule-binding domain of tau monomer [106].

Trazodone alleviates the burden of tau phosphorylation, recovers memorial injury, lessens hippocampal atrophy, and prevents neurodegeneration [107–110]. Trazodone can inhibit the formation of small toxic oligomers and destruct the β-sheet structure of mature fibrils [111]. Melatonin can reduce the acetylated and hyperphosphorylated tau in mice models, and as a result, it reverses cognitive impairment and dementia-associated behaviors of anxiety and apathy [112]. At the concentration of 5 mM, melatonin can inhibit tau assembly through hydrophobic interaction revealed by an NMR spectroscopy study. Melatonin hinders the formation of the salt bridge between tau molecules and decomposes the already-formed tau aggregates by interacting with histidine [113]. Melatonin reduces the formation of higher-ordered oligomers of full-length tau but has no significant effect on the β-sheet content [114].

There are two points to note here. First, different small molecules have a distinct ability to cross the blood-brain barrier (BBB) [115,116], which may restrict the opportunity to enter the human body in the form of food to exert anti-tau pathology effects. Second, small molecules have to accumulate in the brain to pharmacologically relevant concentration (~μM) so as to achieve effective inhibition [87,117]. The penetration and accumulation, as well as the toxicity, are concluded

Table 1 The structure of small molecular inhibitors

Small molecules	 Curcumin  PE859  Resveratrol  Curcumin Ru1/Ru2 complex  Rosmarinic acid  EGCG  Quercetin  Baicalein  Xanthohumol  Parietin  Purpurin  NQTrp  NQTrp-Cl  Tenuiorin  Usnic acid derivative  Nimbin  Salannin  Isobavachalc  Vitamin B12  Folic acid  Toluidine blue  Palmatine chloride  MK886  Trazodone  Melatonin

to be curial in screening prospective candidate drugs and measuring the effectiveness of inhibitors. Therefore, the combination of nanomaterials and small molecules is employed to overcome the inherent shortcomings of isolated small molecules and improve the effectiveness of inhibitors.

Nanoparticles

During the past few years, nanoparticles have consistently exhibited great value in the diagnose and treatment of amyloid disorders [118,119]. Nanoparticles inhibitors have high large surface areas and strong adsorption capacity, enabling peptides, protein molecules, or aggregates to interact with their surfaces in various forms, generate a large number of conformational changes, and affect the interaction of protein/peptide molecules, the pathway of aggregation, and the structure of the aggregates [120]. Kinds of nanoparticles are designed to prevent tau pathology and tau-related neuronal apoptosis. Iron nanoparticles can stabilize the random coil conformation of tau monomer through vdW interaction and hydrogen bonds and impede the misfolding [121]. Nicotinamide loaded phosphatidylserine-functionalized solid lipid nanoparticles can protect tau from hyperphosphorylation and as a result relieve neuron death and cognitive impairment in the study of AD rat model [122]. Nanogold polyethylene glycol (Au-PEG) conjugate hinders tau aggregation in the early stage by stabilizing the conformation of aggregation-prone tau [123]. Au-PEG is suggested to reduce the burden of phosphorylated tau and improve the learning ability of mice. In addition, Au-PEG treatment has no apparent toxicity and modulate tau function to prevent proteotoxicity. Nanoparticles of protein-capped cadmium sulfide (PC-CdS) and iron oxide (PC-Fe_3O_4) impede the formation of toxic tau fibrils and dissolve already existing PHFs [124]. The smaller size and stronger absorption capacity make PC-CdS nanoparticle an inhibitor of greater potential.

Treatment of methylene blue (MB) alone has failed in Phase 3 of clinical therapeutics [125]. Isolated MB merely inhibits fibrils formation but is invalid to prevent the formation of granular oligomer which is closely related to neuron death. However, combining nano composite CeNC/IONC/MSN-T807 with MB (CeNC/IONC/MSN-T807-MB) can impede the hyperphosphorylation and polymerization of tau effectively by binding to phosphorylated tau with high affinity, demonstrated by Chen *et al.* [1]. CeNC/IONC/MSN-T807-MB can also reduce neuronal apoptosis, rescue memory decline and cognitive impairment, and improve the learning ability of AD rats. Nanomaterial Fe-MIL-88B-NH_2-NOTA-DMK6240 loading MB (Fe-MIL-88B-NH_2-NOTA-DMK6240/MB) can bind to full-length tau to hamper further fibrillization [126]. In AD model rats, it can also lessen neuronal apoptosis and alleviate memory injury as well as cognitive decline.

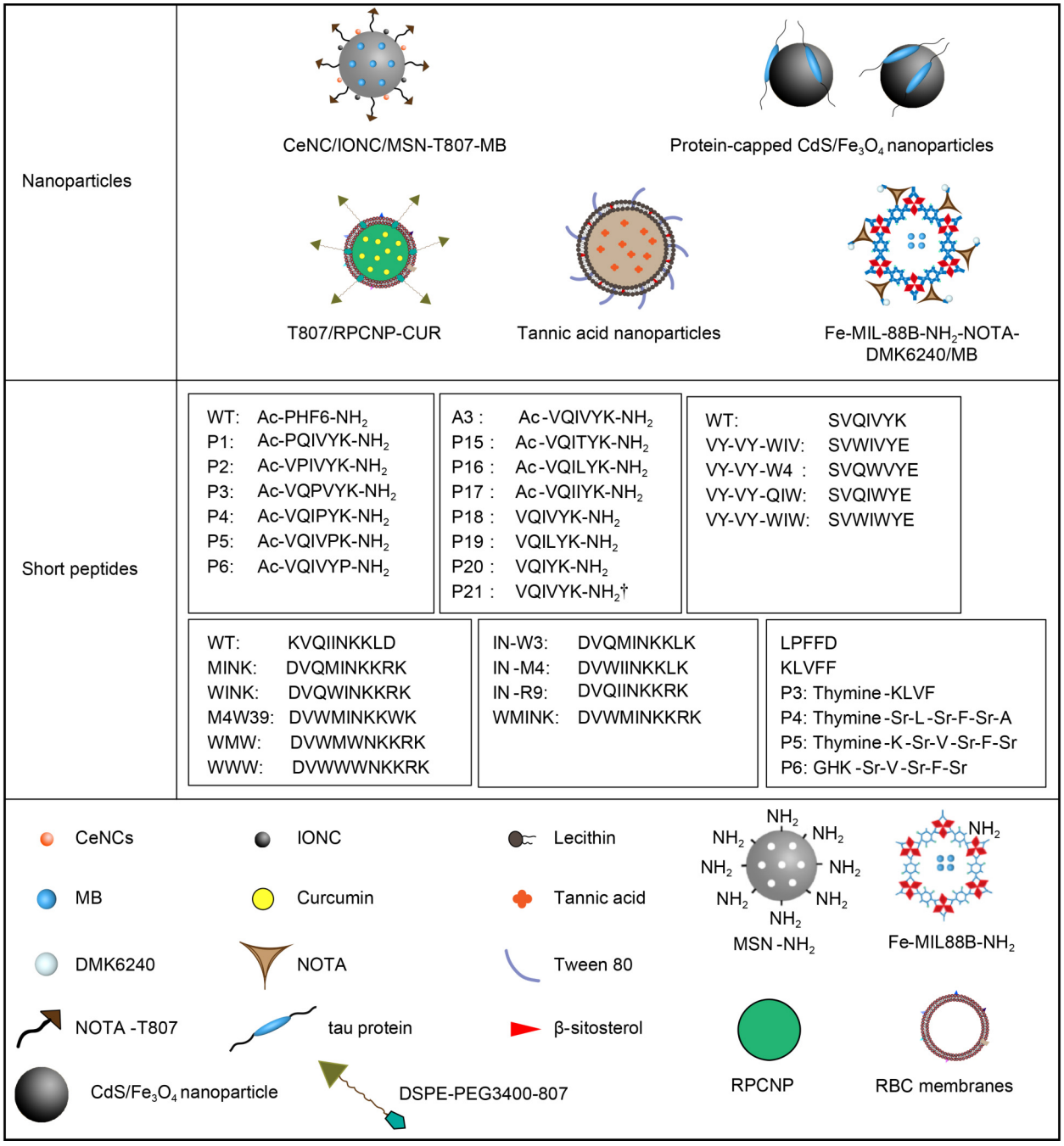
Encapsulating curcumin in nanoparticles can release curcumin slowly and lastingly, thereby increasing its concentration in the central nervous system [127,128] and enhancing the inhibitory effect of curcumin on tau in body [77]. PLGA-PEG-B6 nanoparticles can effectively transport curcumin across BBB. PLGA-PEG-B6/curcumin nanoparticles can reduce the burden of Aβ and inhibit the hyperphosphorylation of tau in APP/PS1 mice [129]. This treatment also improves the learning ability, alleviates the memory deficit, and rescues the cognitive decline of mice. The complexes formed by T807/RPCNP nanoparticles and curcumin (CUR-load T807/RPCNP NPs) can cross BBB, lessen phosphorylated tau and inhibit tau polymerization. CUR-load T807/RPCNP NPs treatment improves the memory impairment of AD mice without obviously affecting cell viability [130]. Tannic acid nanoparticles promote tau R3 fragment forming a hairpin structure to hinder the interaction between tau peptides, which is against the further aggregation [131]. EGCG combined with nanoparticles can reduce Aβ plaque and neurofibrillary tangles of tau in the brain [132]. EGCG nanoparticles inhibit the activity of acetylcholinesterase, moderate oxidative stress, and greatly save the learning disabilities and memory deficits in AD rats induced by aluminum chloride.

Short peptides

Peptide-based inhibitors can interact with tau at different stages of fibrillar formation: monomeric misfolding, primary nucleation, secondary nucleation, fibril elongation, and fibril fragmentation, or remodel, degrade or disassemble already-formed fibrils, mainly by three modes [133]. First, the native sequence of amyloid core and its derivatives with the introduction of β-breaker (*e.g.*, proline), amide bond N-methylation or cyclization can prevent amyloidosis of the protein. Second, the crystal structure of the amyloid core can also be used as the template for designing inhibitors capping the ends of the fibrils to prevent further elongation. Third, short peptides derived from one amyloid core can impede the cross-interaction of two amyloids and isolatedly mediate the self-assembly of another amyloid.

The steric zipper structure of PHF6 fragment is widely used as the template to design tau inhibitors [66,68]. Short peptides targeting PHF6 can inhibit fibril formation not only of the VQIVYK segment, but also of tau K12 and K19 [68]. PHF6 mutants (see Table 2) with one single residue substituted by proline can prevent the

Table 2 The structure of nanoparticles and the sequence of short peptides employed as inhibitors



Different boxes correspond to different studies; †: D-amino acid. Sr: sarcosine.

aggregation and decompose the pre-formed fibril of VQIVYK segment [69], for the high rigidity and lack of amide protons for hydrogen bonding make proline an efficient β-sheet breaker [134,135]. Especially, the inhibitor P2 increases the lag time of aggregation and reduces the cytotoxicity of fibrils. P2 and P3 show the best inhibitory effect, which may be attributed to the sensitivity of the second and third residue positions to the hydrogen bond absence caused by proline substitution. Other forms of PHF6 segment with single residue mutation, acetylation in the N terminus and different chirality are tested [136]. P15 and P16, as accelerators, can reduce cytotoxicity by promoting the aggregation of A3 into nontoxic stable fibrillar aggregates; P18 to P21, as inhibitors, can inhibit the aggregation progress and disrupt the A3 assembly; P17 shows no significant effect on the fibrillization and cytotoxicity. A similar reduction in cytotoxicity by accelerating the fibrillization progress

was observed in the study of Aβ [137], and the employment of accelerators may also be a promising strategy against tau-related pathologies. The SVQIVYK mutants by single tryptophan substitution at each position along the peptide can also serve as inhibitors [73]. Among them, VY-WIW exhibits the best potency to block seeding tau K18 by AD extracts and purified fibrils.

The structure of KVQIINKKLD consisting of PHF6* fragment is another template used to design short peptide inhibitors [36,73]. Substitution of the isoleucine at position 4 along the fragment with methionine or tryptophan, will disrupt the steric zipper interfaces formed in tau fibrils. Inhibitors based on this structure, MINK, WINK and W-MINK, not only cap the fibril ends to block the steric incorporation of additional tau peptide, but also restrict the ability of exogenous full-length tau fibrils to seed tau amyloidogenesis in HEK293 biosensor cells [36]. M4W39 mutant with the substitution of the arginine at position 9 of W-MINK by tryptophan, is more effective in inhibiting tau aggregation. However, WWW and WMW do not exhibit any inhibitory effect. Particularly, IN-M4 is the best inhibitor of seeding tau K18 by AD extracts. The aggregation-prone interface of PHF6* is broadly sensitive to IN-M4, implying that this interface adopts a similar structure despite numerous tau fibril polymorph [73]. Still, short peptides have a high protease sensitivity and low BBB permeability, which needs to overcome before applied in clinical therapy [138].

Aβ-derived peptides can also be used to inhibit tau aggregation, such as KLVFF and its derivative (P4 and P5) peptides [139]. The high-ordered tau aggregates are significantly reduced and more random coil conformation of tau tends to form in the presence of KLVFF, P4, or P5. It can be observed by transmission electron microscopy that heparin-induced tau filaments are broken and form short fractured filaments when coexisting with KLVFF, P4, or P5 peptides.

TAU INTERACTION REVEALED BY MOLECULAR DYNAMICS SIMULATION

MD simulation is a good complement to experimental observations of tau aggregation, especially for tau monomer, oligomer, and interaction mechanisms that are difficult to observe experimentally [140]. MD simulation can provide detailed information about the structure, dynamics, and interaction at atomic level [141].

Tau monomer

The N-terminus and C-terminus of tau bound to the microtubules are too far from each other to form an extended conformation, which allows tau to bind to the tubulin heterodimer through electrostatic interaction and hydrogen bonds [25]. When falling off the microtubules, tau gets compacted in solution and the N-terminus and C-terminus may have a higher chance of contacting each other to form a paperclip conformation [24]. The native structure of tau monomer in solution involves an ensemble of compact globular conformations with transient secondary structure elements [142]. Especially, plenty of β-sheet elements are identified in R3 and R4 regions, which may facilitate the subsequent misfolding and oligomerization. Using replica exchange MD simulations, Larini *et al.* showed that monomeric tau273−284 encompassing PHF6* preferentially adopts compact conformations, while the point mutation of ΔK280 can disrupt the K280-D283 salt-bridge and lead to the extension of backbone [143].

Tau oligomer

R3 is a core component of tau fibrils [144] that can self-aggregate into fibrils in the absence of poly-anion and shares similar characteristics to full-length tau [145]. Liu *et al*. demonstrated that the N-terminal PHF6, middle residues 324−327, and C-terminal residues 331−334 of R3 are critical to its misfolding [146]. These residues undergo a conformational change from disordered structure to β-sheet through a critical intermediate state of turn structure, revealed by the transition path analysis. Li *et al*. showed that R3−R4 repeats of K18 protofilament exhibit a stable C-shaped motif while R1−R2 repeats tend to be linear in shape. The former can be further stabilized by heparin [147].

The aggregation of tau may begin with the local environmental change of PHF6/PHF6*, in which the aggregation-prone hexapeptides become more extended and get exposed to water to enable further intermolecular assembly [45]. A computational study showed that isolated PHF6 can self-aggregate through a two-step nucleation. The hexapeptide first aggregates into a disordered oligomer, and then converts into a fibrillar structure consisting of two parallel β-sheets [148]. Liu *et al.* studied the dimerization of PHF6 and found it occurs by three steps [149]. First, the separated monomers randomly collide, and then form a short β-sheet structure at the N-termini; finally, the β-sheets elongate continuously to form an extended parallel β-sheet dimer. Larini *et al*. showed that dimeric tau273−284 encompassing PHF6* can adopt kinds of conformations without predominant structural preference [143]. The extended dimer in parallel and antiparallel alignments are detected, and these extended conformations probably constitute pre-cursors to fibrils. The end-to-end distances of tau273−284 dimers and fibrils are respectively 1.5−3.0 nm and ~3.5 nm, significantly larger than ~0.8 nm for monomers with the most

populated hairpin structure [150]. The extended β-strand state is readily populated in aggregates, which can be used as a conformational signature of aggregation-prone tau.

Tau and Aβ can form soluble complexes in extracts from AD brain tissue, and the fibrils and prefibrillar oligomers of Aβ can also facilitate tau aggregation [151]. A recent MD simulation indicated that Aβ tetramer mainly interacts with R1 and R4 regions of tau by ^{16}KLVFFA^{21} fragment [152]. Although the number of hydrogen bonds formed between each isolated repeat domain and Aβ is similar, the R2 and R3 regions in a four-repeat tau are shielded in a β-hairpin structure which impedes their interaction with Aβ. The Aβ-tau interaction also increases the β-sheet content, but does not enhance the ability of tau repeat regions to dimerize, which may be attributed to the necessity of R2 or R3 to tau aggregation. Buyong Ma group found that the oligomer formed by isolated tau repeat R3 or R4 is structurally stable while the R2 oligomer is not; however, the R2 oligomer gets stabilized when interacting with Aβ17−42 protofibrillar oligomer. Thus, the R2 region is suggested to be most likely to interact with soluble Aβ oligomers and consequently promote aggregation [153]. Tau273–284 monomer was found to prefer to associate with Aβ25–35 monomer over another tau monomer, for the dry interface and abundant hydrogen bonds in between enabling the heterodimer to be more stable than pure tau273–274 dimer [154]. This heterodimer mainly adopts an extended conformation conducive to further fibrillization. When these two peptides are abundantly mixed, their proportion determines the morphologies of formed aggregates. The hetero-tau oligomers tend to produce granular aggregates with small sizes, limited by the side-chain interdigitation; the hetero-Aβ oligomers have a high content of β-sheet, and can grow into heterofibrils. Aβ oligomer is the most effective form of seeding tau compared to Aβ monomer or fibril [155]. These computational researches have given useful enlight-enment on the interactions involved in tau aggregation.

Interaction of tau with inhibitors

Recent MD studies provide atomistic insights into the interaction of tau with inhibitors and some potential inhibitory mechanisms have been proposed.

Quercetin was found to stabilize the random coil conformation of monomeric tau R2 fragment by hydrogen bonding and hydrophobic interactions [90]. It also disrupts the α-helix structure of tau R2 monomer by forming hydrogen bonds with the residue G273, resulting in the increment of coil content. Especially, quercetin has a strong interaction with tau273−284 encompassing aggregation-prone PHF6*, and the packing of quercetin spatially hinders tau self-association to prevent further aggregation.

Trazodone has six preferential binding sites when interacting with 1N4R tau isoform: Y29 and Y197; residues 118−125; residues 210−215; residues 275−280; residues 306−310; Y394 [111]. The binding of trazodone causes the fluctuation of the six corresponding regions to be significantly reduced. Besides, the decrements of root-mean-square deviation (RMSD), radius of gyration and solvent-accessible surface area indicate that tau becomes more compressed in the presence of trazodone. As a result, the aggregation-prone motifs PHF6 and PHF6* are shielded by surrounding environments, which lessens fibril formation.

EGCG preferentially binds to PHF6 of monomeric tau K18 with a series of short-term interactions, binding and partial dissociation events, revealed by MD simulation [49]. In tau-EGCG interaction, hydrogen bonding is predominant, to which residues N265, L266 and V313 contribute the majority; hydrophobic interaction also plays an important role, to which K267, Y310, P312 and K340 have a great contribution.

Purpurin contacting with PHF6 fibrils leads to the break of fibrillar strands and makes the β-sheets loosely packed [75]. This remarkable conformation change is attributed to the formation of hydrogen bonds and π-π stacking between purpurin and PHF6 residues, which destroys the interpeptide mainchain hydrogen bonds of tau fibrils and facilitates fibril disaggregation.

Baicalein can inhibit the aggregation of repeat tau, which is suggested to be dominated by covalent modification [91]. The simulation showed that baicalein stably binds to tau by hydrogen bonding and hydrophobic interactions, especially with residues Q307, Y310 and K311 of PHF6. The two adjacent hydroxyl groups of Baicalein interact with L266, which might have the ability to modify the tau oligomers and induce the formation of off-pathway oligomers.

NQTrp and NQTrp-Cl can reduce the integrity, stability and β-sheet content of PHF6 fibrils [67]. The interaction of adjacent peptides and neighboring β-strands is disrupted by the formation of hydrogen bonds between tau and inhibitors. Palmatine chloride exhibits the inhibitory effect by converting the β-sheet structure of PHF6 oligomer to the random coil. It can also disassemble PHF6 fibrils by disrupting the inter-peptide hydrogen bonds [105].

CONCLUSIONS

We have reviewed recent studies of tau protein in the monomeric state, their nucleation, and fibril elongation. With the help of new experimental techniques, several atomic structures of tau fibrils have been resolved,

showing the structural polymorphism in different tau-related diseases. This diversity reflects the complexity of pathological aggregation for tau as an intrinsically disordered protein, and increases the difficulty of providing a unifying neuroimage criterion for early diagnosis. The computational simulations mainly focus on the monomeric or oligomeric structures of tau, as well as the kinetics of monomer association to the fibrillar tips. These studies have provided valuable insights into the structures and pathways of tau aggregation.

We also reviewed recent studies of promising tau inhibitors. With continuous failures of medicines targeting Aβ, scientists have turned their attention to tau-based drug development. As mentioned above, kinds of compounds have been applied to inhibit tau misfolding or aggregation. These inhibitors are usually good β-sheet breakers, carrying one or more aromatic rings, hydroxyl or amino groups. The most popular binding site of inhibitors to tau is the highly aggregation-prone PHF6 or certain amino acids in this segment. The binding affinity of most inhibitors is dominated by hydrogen bonds and hydrophobic interactions. Some inhibitors can even remodel or dissolve mature fibril, which is very promising as a new drug candidate.

In spite of intensive efforts from experimental and computational studies, several questions on tau aggregation remain elusive. First, there is still a gap between oligomeric structure and toxic species involved in tau pathology. Does less β-sheet content necessarily correspond to less toxicity, since some off-pathway oligomers may still have neurotoxicity? Second, inhibitors in brain have to accumulate to a pharma cological abundance so as to achieve effective inhibition. They may react with other substances in the human body and be consumed in multiple physiological processes, and their BBB permeability also needs to be taken into considerationbeforeclinicalapplication.Theunderstanding of the current progress at atomic level of detail and potential difficulties may help in designing medicines against tau aggregation. We hope this review will inspire new clues for AD therapies to shed light on the possibility of treatment before symptom onset.

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The authors Lili Zhu and Zhenyu Qian declare that they have no conflict of interest or financial conflicts to disclose.

REFERENCES

1. Chen, Q., Du, Y., Zhang, K., Liang, Z., Li, J., Yu, H., Ren, R., Feng, J., Jin, Z., Li, F., *et al*. (2018) A tau-targeted multifunctional nanocomposite for combinational therapy of Alzheimer's disease. ACS Nano, 12, 1321−1338
2. Zeisel, J., Bennett, K., and Fleming, R. (2020) World Alzheimer report 2020: Design, dignity, dementia: dementia-related design and the built environment. Accessed: February 1, 2021
3. Alzheimer's Association. (2021) 2021 Alzheimer's disease facts and figures. Alzheimers Dement., 17, 327−406
4. Grandy, J. K. (2013) Melatonin: Therapeutic intervention in mild cognitive impairment and Alzheimer disease. J. Neurol. Neurophysiol., 4, 148
5. Uddin, M. S., Kabir, M. T., Al Mamun, A., Abdel-Daim, M. M., Barreto, G. E. and Ashraf, G. M. (2019) APOE and Alzheimer's disease: Evidence mounts that targeting APOE4 may combat Alzheimer's pathogenesis. Mol. Neurobiol., 56, 2450−2465
6. Hardy, J. A. and Higgins, G. A. (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science, 256, 184−185
7. Rajasekhar, K., Chakrabarti, M. and Govindaraju, T. (2015) Function and toxicity of amyloid beta and recent therapeutic interventions targeting amyloid beta in Alzheimer's disease. Chem. Commun. (Camb.), 51, 13434−13450
8. Rajasekhar, K. and Govindaraju, T. (2018) Current progress, challenges and future prospects of diagnostic and therapeutic interventions in Alzheimer's disease. RSC Advances, 8, 23780−23804
9. van der Kant, R., Goldstein, L. S. B. and Ossenkoppele, R. (2020) Amyloid-β-independent regulators of tau pathology in Alzheimer disease. Nat. Rev. Neurosci., 21, 21−35
10. Congdon, E. E. and Sigurdsson, E. M. (2018) Tau-targeting therapies for Alzheimer disease. Nat. Rev. Neurol., 14, 399−415
11. Alavi Naini, S. M. and Soussi-Yanicostas, N. (2015) Tau hyperphosphorylation and oxidative stress, a critical vicious circle in neurodegenerative tauopathies? Oxid Med. Cell. Longev., 2015, 151979
12. Kerr, J. S., Adriaanse, B. A., Greig, N. H., Mattson, M. P.,

Cader, M. Z., Bohr, V. A. and Fang, E. F. (2017) Mitophagy and Alzheimer's disease: Cellular and molecular mechanisms. Trends Neurosci., 40, 151−166
13. Busche, M. A. and Hyman, B. T. (2020) Synergy between amyloid-β and tau in Alzheimer's disease. Nat. Neurosci., 23, 1183−1193
14. Mullard, A. (2021) Failure of first anti-tau antibody in Alzheimer disease highlights risks of history repeating. Nat. Rev. Drug Discov., 20, 3−5
15. Dehmelt, L. and Halpain, S. (2005) The MAP2/Tau family of microtubule-associated proteins. Genome Biol., 6, 204
16. Jameson, L., Frey, T., Zeeberg, B., Dalldorf, F. and Caplow, M. (1980) Inhibition of microtubule assembly by phosphorylation of microtubule-associated proteins. Biochemistry, 19, 2472−2479
17. Gu, Y., Oyama, F. and Ihara, Y. (1996) Tau is widely expressed in rat tissues. J. Neurochem., 67, 1235−1244
18. Bakota, L. and Brandt, R. (2016) Tau biology and tau-directed therapies for Alzheimer's disease. Drugs, 76, 301−313
19. Guo, T., Noble, W. and Hanger, D. P. (2017) Roles of tau protein in health and disease. Acta Neuropathol., 133, 665−704
20. Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D. and Crowther, R. A. (1989) Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron, 3, 519−526
21. Mandelkow, E.-M. and Mandelkow, E. (2012) Biochemistry and cell biology of tau protein in neurofibrillary degeneration. Cold Spring Harb. Perspect. Med., 2, a006247
22. Conde, C. and Cáceres, A. (2009) Microtubule assembly, organization and dynamics in axons and dendrites. Nat. Rev. Neurosci., 10, 319−332
23. Weingarten, M. D., Lockwood, A. H., Hwo, S. Y. and Kirschner, M. W. (1975) A protein factor essential for microtubule assembly. Proc. Natl. Acad. Sci. USA, 72, 1858−1862
24. Castro, T. G., Munteanu, F. D. and Cavaco-Paulo, A. (2019) Electrostatics of tau protein by molecular dynamics. Biomolecules, 9, 116
25. Kellogg, E. H., Hejab, N. M. A., Poepsel, S., Downing, K. H., DiMaio, F. and Nogales, E. (2018) Near-atomic model of microtubule-tau interactions. Science, 360, 1242−1246
26. Qiang, L., Sun, X., Austin, T. O., Muralidharan, H., Jean, D. C., Liu, M., Yu, W., and Baas, P. W. (2018) Tau does not stabilize axonal microtubules but rather enables them to have long labile domains. Curr. Biol., 28, 2181−2189. e4
27. Baas, P. W. and Qiang, L. (2019) Tau: It's not what you think. Trends Cell Biol., 29, 452−461
28. Witman, G. B., Cleveland, D. W., Weingarten, M. D. and Kirschner, M. W. (1976) Tubulin requires tau for growth onto microtubule initiating sites. Proc. Natl. Acad. Sci. USA, 73, 4070−4074
29. Zhang, B., Carroll, J., Trojanowski, J. Q., Yao, Y., Iba, M., Potuzak, J. S., Hogan, A. M., Xie, S. X., Ballatore, C., Smith III, A. B., *et al*. (2012) The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice. J. Neurosci., 32, 3601−3611
30. Mietelska-Porowska, A., Wasik, U., Goras, M., Filipek, A. and Niewiadomska, G. (2014) Tau protein modifications and interactions: their role in function and dysfunction. Int. J. Mol. Sci., 15, 4671−4713
31. Medina, M. and Avila, J. (2014) The role of extracellular tau in the spreading of neurofibrillary pathology. Front. Cell. Neurosci., 8, 113
32. Elie, A., Prezel, E., Guérin, C., Denarier, E., Ramirez-Rios, S., Serre, L., Andrieux, A., Fourest-Lieuvin, A., Blanchoin, L. and Arnal, I. (2015) Tau co-organizes dynamic microtubule and actin networks. Sci. Rep., 5, 9964
33. Violet, M., Delattre, L., Tardivel, M., Sultan, A., Chauderlier, A., Caillierez, R., Talahari, S., Nesslany, F., Lefebvre, B., Bonnefoy, E., *et al*. (2014) A major role for tau in neuronal DNA and RNA protection *in vivo* under physiological and hyperthermic conditions. Front. Cell. Neurosci., 8, 84
34. Sultan, A., Nesslany, F., Violet, M., Bégard, S., Loyens, A., Talahari, S., Mansuroglu, Z., Marzin, D., Sergeant, N., Humez, S., *et al*. (2011) Nuclear tau, a key player in neuronal DNA protection. J. Biol. Chem., 286, 4566−4575
35. Bulic, B., Pickhardt, M., Mandelkow, E. M. and Mandelkow, E. (2010) Tau protein and tau aggregation inhibitors. Neuropharmacology, 59, 276−289
36. Seidler, P. M., Boyer, D. R., Rodriguez, J. A., Sawaya, M. R., Cascio, D., Murray, K., Gonen, T. and Eisenberg, D. S. (2018) Structure-based inhibitors of tau aggregation. Nat. Chem., 10, 170−176
37. Nizynski, B., Dzwolak, W. and Nieznanski, K. (2017) Amyloidogenesis of tau protein. Protein Sci., 26, 2126−2150
38. Liu, Y., Nguyen, M., Robert, A. and Meunier, B. (2019) Metal ions in Alzheimer's disease: A key role or not? Acc Chem. Res., 52, 2026−2035
39. Fanni, A. M., Vander Zanden, C. M., Majewska, P. V., Majewski, J. and Chi, E. Y. (2019) Membrane-mediated fibrillation and toxicity of the tau hexapeptide PHF6. J. Biol. Chem., 294, 15304−15317
40. Zhu, H. L., Fernández, C., Fan, J. B., Shewmaker, F., Chen, J., Minton, A. P. and Liang, Y. (2010) Quantitative characterization of heparin binding to tau protein: implication for inducer-mediated tau filament formation. J. Biol. Chem., 285, 3592−3599
41. Kfoury, N., Holmes, B. B., Jiang, H., Holtzman, D. M. and Diamond, M. I. (2012) Trans-cellular propagation of tau aggregation by fibrillar species. J. Biol. Chem., 287, 19440−19451
42. La Joie, R., Visani, A. V., Baker, S. L., Brown, J. A., Bourakova, V., Cha, J., Chaudhary, K., Edwards, L., Iaccarino, L., Janabi, M., *et al*. (2020) Prospective longitudinal atrophy in Alzheimer's disease correlates with the intensity and topography of baseline tau-PET. Sci. Transl. Med., 12, eaau5732

43. Lee, C. C., Nayak, A., Sethuraman, A., Belfort, G. and McRae, G. J. (2007) A three-stage kinetic model of amyloid fibrillation. Biophys. J., 92, 3448−3458
44. Iannuzzi, C., Borriello, M., Irace, G., Cammarota, M., Di Maro, A. and Sirangelo, I. (2017) Vanillin affects amyloid aggregation and non-enzymatic glycation in human insulin. Sci. Rep., 7, 15086
45. Mirbaha, H., Chen, D., Morazova, O. A., Ruff, K. M., Sharma, A. M., Liu, X., Goodarzi, M., Pappu, R. V., Colby, D. W., Mirzaei, H., *et al*. (2018) Inert and seed-competent tau monomers suggest structural origins of aggregation. eLife, 7, e36584
46. Shammas, S. L., Garcia, G. A., Kumar, S., Kjaergaard, M., Horrocks, M. H., Shivji, N., Mandelkow, E., Knowles, T. P., Mandelkow, E. and Klenerman, D. (2015) A mechanistic model of tau amyloid aggregation based on direct observation of oligomers. Nat. Commun., 6, 7025
47. Kjaergaard, M., Dear, A. J., Kundel, F., Qamar, S., Meisl, G., Knowles, T. P. J. and Klenerman, D. (2018) Oligomer diversity during the aggregation of the repeat region of tau. ACS Chem. Neurosci., 9, 3060−3071
48. Roman, A. Y., Devred, F., Byrne, D., La Rocca, R., Ninkina, N. N., Peyrot, V. and Tsvetkov, P. O. (2019) Zinc induces temperature-dependent reversible self-assembly of tau. J. Mol. Biol., 431, 687−695
49. Sonawane, S. K., Chidambaram, H., Boral, D., Gorantla, N. V., Balmik, A. A., Dangi, A., Ramasamy, S., Marelli, U. K. and Chinnathambi, S. (2020) EGCG impedes human tau aggregation and interacts with Tau. Sci. Rep., 10, 12579
50. Ramachandran, G. and Udgaonkar, J. B. (2011) Understanding the kinetic roles of the inducer heparin and of rod-like protofibrils during amyloid fibril formation by tau protein. J. Biol. Chem., 286, 38948−38959
51. Chirita, C. N., Congdon, E. E., Yin, H. and Kuret, J. (2005) Triggers of full-length tau aggregation: a role for partially folded intermediates. Biochemistry, 44, 5862−5872
52. Luo, Y., Dinkel, P., Yu, X., Margittai, M., Zheng, J., Nussinov, R., Wei, G. and Ma, B. (2013) Molecular insights into the reversible formation of tau protein fibrils. Chem. Commun. (Camb.), 49, 3582−3584
53. Goedert, M., Eisenberg, D. S. and Crowther, R. A. (2017) Propagation of tau aggregates and neurodegeneration. Annu. Rev. Neurosci., 40, 189−210
54. Fitzpatrick, A. W. P., Falcon, B., He, S., Murzin, A. G., Murshudov, G., Garringer, H. J., Crowther, R. A., Ghetti, B., Goedert, M. and Scheres, S. H. W. (2017) Cryo-EM structures of tau filaments from Alzheimer's disease. Nature, 547, 185−190
55. Falcon, B., Zhang, W., Schweighauser, M., Murzin, A. G., Vidal, R., Garringer, H. J., Ghetti, B., Scheres, S. H. W. and Goedert, M. (2018) Tau filaments from multiple cases of sporadic and inherited Alzheimer's disease adopt a common fold. Acta Neuropathol., 136, 699−708
56. Goedert, M., Falcon, B., Zhang, W., Ghetti, B. and Scheres, S. H. W. (2018) Distinct conformers of assembled tau in Alzheimer's and Pick's diseases. Cold Spring Harb. Symp. Quant. Biol., 83, 163−171
57. Falcon, B., Zhang, W., Murzin, A. G., Murshudov, G., Garringer, H. J., Vidal, R., Crowther, R. A., Ghetti, B., Scheres, S. H. W. and Goedert, M. (2018) Structures of filaments from Pick's disease reveal a novel tau protein fold. Nature, 561, 137−140
58. Qi, B., Jin, S., Qian, H. and Zou, Y. (2020) Bibliometric analysis of chronic traumatic encephalopathy research from 1999 to 2019. Int. J. Environ. Res. Public Health, 17, 5411
59. Falcon, B., Zivanov, J., Zhang, W., Murzin, A. G., Garringer, H. J., Vidal, R., Crowther, R. A., Newell, K. L., Ghetti, B., Goedert, M., *et al*. (2019) Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. Nature, 568, 420−423
60. Sergeant, N., Wattez, A. and Delacourte, A. (1999) Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively "exon 10" isoforms. J. Neurochem., 72, 1243−1249
61. Zhang, W., Tarutani, A., Newell, K. L., Murzin, A. G., Matsubara, T., Falcon, B., Vidal, R., Garringer, H. J., Shi, Y., Ikeuchi, T., *et al*. (2020) Novel tau filament fold in corticobasal degeneration. Nature, 580, 283−287
62. Mukrasch, M. D., von Bergen, M., Biernat, J., Fischer, D., Griesinger, C., Mandelkow, E. and Zweckstetter, M. (2007) The "jaws" of the tau-microtubule interaction. J. Biol. Chem., 282, 12230−12239
63. Mukrasch, M. D., Bibow, S., Korukottu, J., Jeganathan, S., Biernat, J., Griesinger, C., Mandelkow, E. and Zweckstetter, M. (2009) Structural polymorphism of 441-residue tau at single residue resolution. PLoS Biol., 7, e34
64. Chen, D., Drombosky, K. W., Hou, Z., Sari, L., Kashmer, O. M., Ryder, B. D., Perez, V. A., Woodard, D. R., Lin, M. M., Diamond, M. I., *et al*. (2019) Tau local structure shields an amyloid-forming motif and controls aggregation propensity. Nat. Commun., 10, 2493
65. Nelson, R., Sawaya, M. R., Balbirnie, M., Madsen, A. O., Riekel, C., Grothe, R. and Eisenberg, D. (2005) Structure of the cross-beta spine of amyloid-like fibrils. Nature, 435, 773−778
66. Sawaya, M. R., Sambashivan, S., Nelson, R., Ivanova, M. I., Sievers, S. A., Apostol, M. I., Thompson, M. J., Balbirnie, M., Wiltzius, J. J., McFarlane, H. T., *et al*. (2007) Atomic structures of amyloid cross-beta spines reveal varied steric zippers. Nature, 447, 453−457
67. KrishnaKumar, V. G., Paul, A., Gazit, E. and Segal, D. (2018) Mechanistic insights into remodeled tau-derived PHF6 peptide fibrils by Naphthoquinone-Tryptophan hybrids. Sci. Rep., 8, 71
68. Sievers, S. A., Karanicolas, J., Chang, H. W., Zhao, A., Jiang, L., Zirafi, O., Stevens, J. T., Münch, J., Baker, D. and Eisenberg, D. (2011) Structure-based design of non-natural amino-acid inhibitors of amyloid fibril formation. Nature, 475, 96−100
69. Chemerovski-Glikman, M., Frenkel-Pinter, M., Mdah, R., Abu-Mokh, A., Gazit, E. and Segal, D. (2017) Inhibition of the aggregation and toxicity of the minimal amyloidogenic fragment of tau by its pro-substituted analogues. Chemistry, 23,

9618−9624
70. Pickhardt, M., Neumann, T., Schwizer, D., Callaway, K., Vendruscolo, M., Schenk, D., St George-Hyslop, P., Mandelkow, E. M., Dobson, C. M., McConlogue, L., *et al*. (2015) Identification of small molecule inhibitors of tau aggregation by targeting monomeric tau as a potential therapeutic approach for tauopathies. Curr. Alzheimer Res., 12, 814−828
71. Davidowitz, E. J., Krishnamurthy, P. K., Lopez, P., Jimenez, H., Adrien, L., Davies, P. and Moe, J. G. (2020) *In vivo* validation of a small molecule inhibitor of tau self-association in htau mice. J. Alzheimers Dis., 73, 147−161
72. Kundel, F., De, S., Flagmeier, P., Horrocks, M. H., Kjaergaard, M., Shammas, S. L., Jackson, S. E., Dobson, C. M. and Klenerman, D. (2018) Hsp70 inhibits the nucleation and elongation of tau and sequesters tau aggregates with high affinity. ACS Chem. Biol., 13, 636−646
73. Seidler, P. M., Boyer, D. R., Murray, K. A., Yang, T. P., Bentzel, M., Sawaya, M. R., Rosenberg, G., Cascio, D., Williams, C. K., Newell, K. L., *et al*. (2019) Structure-based inhibitors halt prion-like seeding by Alzheimer's disease-and tauopathy-derived brain tissue samples. J. Biol. Chem., 294, 16451−16464
74. Larbig, G., Pickhardt, M., Lloyd, D. G., Schmidt, B. and Mandelkow, E. (2007) Screening for inhibitors of tau protein aggregation into Alzheimer paired helical filaments: a ligand based approach results in successful scaffold hopping. Curr. Alzheimer Res., 4, 315−323
75. Viswanathan, G. K., Shwartz, D., Losev, Y., Arad, E., Shemesh, C., Pichinuk, E., Engel, H., Raveh, A., Jelinek, R., Cooper, I., *et al*. (2020) Purpurin modulates tau-derived VQIVYK fibrillization and ameliorates Alzheimer's disease-like symptoms in animal model. Cell. Mol. Life Sci., 77, 2795−2813
76. Porat, Y., Abramowitz, A. and Gazit, E. (2006) Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. Chem. Biol. Drug Des., 67, 27−37
77. Bijari, N., Balalaie, S., Akbari, V., Golmohammadi, F., Moradi, S., Adibi, H. and Khodarahmi, R. (2018) Effective suppression of the modified PHF6 peptide/1N4R Tau amyloid aggregation by intact curcumin, not its degradation products: Another evidence for the pigment as preventive/therapeutic "functional food". Int. J. Biol. Macromol., 120, 1009−1022
78. Rane, J. S., Bhaumik, P. and Panda, D. (2017) Curcumin inhibits tau aggregation and disintegrates preformed tau filaments *in vitro*. J. Alzheimers Dis., 60, 999−1014
79. Anand, P., Kunnumakkara, A. B., Newman, R. A. and Aggarwal, B. B. (2007) Bioavailability of curcumin: problems and promises. Mol. Pharm., 4, 807−818
80. Lo Cascio, F., Puangmalai, N., Ellsworth, A., Bucchieri, F., Pace, A., Palumbo Piccionello, A. and Kayed, R. (2019) Toxic tau oligomers modulated by novel curcumin derivatives. Sci. Rep., 9, 19011
81. Sato, R., Vohra, S., Yamamoto, S., Suzuki, K., Pavel, K., Shulga, S., Blume, Y. and Kurita, N. (2020) Specific interactions between tau protein and curcumin derivatives: Molecular docking and ab initio molecular orbital simulations. J. Mol. Graph. Model., 98, 107611
82. Okuda, M., Hijikuro, I., Fujita, Y., Wu, X., Nakayama, S., Sakata, Y., Noguchi, Y., Ogo, M., Akasofu, S., Ito, Y., *et al*. (2015) PE859, a novel tau aggregation inhibitor, reduces aggregated tau and prevents onset and progression of neural dysfunction *in vivo*. PLoS One, 10, e0117511
83. Okuda, M., Hijikuro, I., Fujita, Y., Teruya, T., Kawakami, H., Takahashi, T. and Sugimoto, H. (2016) Design and synthesis of curcumin derivatives as tau and amyloid β dual aggregation inhibitors. Bioorg. Med. Chem. Lett., 26, 5024−5028
84. Liu, W., Hu, X., Zhou, L., Tu, Y., Shi, S. and Yao, T. (2020) Orientation-inspired perspective on molecular inhibitor of tau aggregation by curcumin conjugated with ruthenium(ii) complex scaffold. J. Phys. Chem. B, 124, 2343−2353
85. Yu, K. C., Kwan, P., Cheung, S. K. K., Ho, A. and Baum, L. (2018) Effects of resveratrol and morin on insoluble tau in tau transgenic mice. Transl. Neurosci., 9, 54−60
86. Sun, X. Y., Dong, Q. X., Zhu, J., Sun, X., Zhang, L. F., Qiu, M., Yu, X. L. and Liu, R. T. (2019) Resveratrol rescues tau-induced cognitive deficits and neuropathology in a mouse model of tauopathy. Curr. Alzheimer Res., 16, 710−722
87. Pasinetti, G. M., Wang, J., Ho, L., Zhao, W. and Dubner, L. (2015) Roles of resveratrol and other grape-derived polyphenols in Alzheimer's disease prevention and treatment. Biochim. Biophys. Acta, 1852, 1202−1208
88. Cornejo, A., Aguilar Sandoval, F., Caballero, L., Machuca, L., Muñoz, P., Caballero, J., Perry, G., Ardiles, A., Areche, C. and Melo, F. (2017) Rosmarinic acid prevents fibrillization and diminishes vibrational modes associated to β sheet in tau protein linked to Alzheimer's disease. J. Enzyme Inhib. Med. Chem., 32, 945−953
89. Guo, Y., Zhao, Y., Nan, Y., Wang, X., Chen, Y. and Wang, S. (2017)(−)-Epigallocatechin-3-gallate ameliorates memory impairment and rescues the abnormal synaptic protein levels in the frontal cortex and hippocampus in a mouse model of Alzheimer's disease. Neuroreport, 28, 590−597
90. Kumar, S., Krishnakumar, V. G., Morya, V., Gupta, S. and Datta, B. (2019) Nanobiocatalyst facilitated aglycosidic quercetin as a potent inhibitor of tau protein aggregation. Int. J. Biol. Macromol., 138, 168−180
91. Sonawane, S. K., Balmik, A. A., Boral, D., Ramasamy, S. and Chinnathambi, S. (2019) Baicalein suppresses repeat tau fibrillization by sequestering oligomers. Arch. Biochem. Biophys., 675, 108119
92. Zhang, M., Wu, Q., Yao, X., Zhao, J., Zhong, W., Liu, Q. and Xiao, S. (2019) Xanthohumol inhibits tau protein aggregation and protects cells against tau aggregates. Food Funct., 10, 7865−7874
93. Pickhardt, M., Gazova, Z., von Bergen, M., Khlistunova, I., Wang, Y., Hascher, A., Mandelkow, E.-M., Biernat, J. and Mandelkow, E. (2005) Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments *in vitro* and in

cells. J. Biol. Chem., 280, 3628−3635
94. Nepovimova, E., Uliassi, E., Korabecny, J., Peña-Altamira, L. E., Samez, S., Pesaresi, A., Garcia, G. E., Bartolini, M., Andrisano, V., Bergamini, C., *et al*. (2014) Multitarget drug design strategy: quinone-tacrine hybrids designed to block amyloid-β aggregation and to exert anticholinesterase and antioxidant effects. J. Med. Chem., 57, 8576−8589
95. Cornejo, A., Salgado, F., Caballero, J., Vargas, R., Simirgiotis, M. and Areche, C. (2016) Secondary metabolites in ramalina terebrata detected by UHPLC/ESI/MS/MS and identification of parietin as tau protein inhibitor. Int. J. Mol. Sci., 17, 1303
96. Frenkel-Pinter, M., Tal, S., Scherzer-Attali, R., Abu-Hussien, M., Alyagor, I., Eisenbaum, T., Gazit, E., Segal, D. and Lichtenthaler, S. (2016) Naphthoquinone-tryptophan hybrid inhibits aggregation of the tau-derived peptide PHF6 and reduces neurotoxicity. J. Alzheimers Dis., 51, 165−178
97. Salgado, F., Caballero, J., Vargas, R., Cornejo, A. and Areche, C. (2020) Continental and antarctic lichens: isolation, identification and molecular modeling of the depside tenuiorin from the Antarctic lichen *Umbilicaria antarctica* as tau protein inhibitor. Nat. Prod. Res., 34, 646−650
98. Shi, C. J., Peng, W., Zhao, J. H., Yang, H. L., Qu, L. L., Wang, C., Kong, L. Y. and Wang, X. B. (2020) Usnic acid derivatives as tau-aggregation and neuroinflammation inhibitors. Eur. J. Med. Chem., 187, 111961
99. Gorantla, N. V., Das, R., Mulani, F. A., Thulasiram, H. V. and Chinnathambi, S. (2019) Neem derivatives inhibits tau aggregation. J. Alzheimers Dis. Rep., 3, 169−178
100. Xiao, S., Wu, Q., Yao, X., Zhang, J., Zhong, W., Zhao, J., Liu, Q. and Zhang, M. (2021) Inhibitory effects of isobavachalcone on tau protein aggregation, tau phosphorylation, and oligomeric tau-induced apoptosis. ACS Chem. Neurosci., 12, 123−132
101. Rafiee, S., Asadollahi, K., Riazi, G., Ahmadian, S. and Saboury, A. A. (2017) Vitamin B12 inhibits tau fibrillization via binding to cysteine residues of tau. ACS Chem. Neurosci., 8, 2676−2682
102. Ghasemzadeh, S. and Riazi, G. H. (2020) Inhibition of tau amyloid fibril formation by folic acid: *In-vitro* and theoretical studies. Int. J. Biol. Macromol., 154, 1505−1516
103. Kifle, L., Ortiz, D. and Shea, T. B. (2009) Deprivation of folate and B12 increases neurodegeneration beyond that accompanying deprivation of either vitamin alone. J. Alzheimers Dis., 16, 533−540
104. Dubey, T., Gorantla, N. V., Chandrashekara, K. T. and Chinnathambi,S. (2019) Photoexcited toluidine blue inhibits tau aggregation in Alzheimer's disease. ACS Omega, 4, 18793−18802
105. Haj, E., Losev, Y. Guru KrishnaKumar, V., Pichinuk, E., Engel, H., Raveh, A., Gazit, E., and Segal, D. (2018) Integrating *in vitro* and *in silico* approaches to evaluate the "dual functionality" of palmatine chloride in inhibiting and disassembling tau-derived VQIVYK peptide fibrils. Biochim. Biophys. Acta Gen. Subj., 1862, 1565−1575
106. Lo, C. H., Lim, C. K., Ding, Z., Wickramasinghe, S. P., Braun, A. R., Ashe, K. H., Rhoades, E., Thomas, D. D. and Sachs, J. N. (2019) Targeting the ensemble of heterogeneous tau oligomers in cells: A novel small molecule screening platform for tauopathies. Alzheimers Dement., 15, 1489−1502
107. Halliday, M., Radford, H., Zents, K. A. M., Molloy, C., Moreno, J. A., Verity, N. C., Smith, E., Ortori, C. A., Barrett, D. A., Bushell, M., *et al*. (2017) Repurposed drugs targeting eIF2α-P-mediated translational repression prevent neurodegeneration in mice. Brain, 140, 1768−1783
108. La, A. L., Walsh, C. M., Neylan, T. C., Vossel, K. A., Yaffe, K., Krystal, A. D., Miller, B. L. and Karageorgiou, E. (2019) Long-term trazodone use and cognition: A potential therapeutic role for slow-wave sleep enhancers. J. Alzheimers Dis., 67, 911−921
109. Kumar, P., Kalonia, H. and Kumar, A. (2011) Novel protective mechanisms of antidepressants against 3-nitropropionic acid induced Huntington's-like symptoms: a comparative study. J. Psychopharmacol., 25, 1399−1411
110. Grippe, T. C., Gonçalves, B. S. B., Louzada, L. L., Quintas, J. L., Naves, J. O. S., Camargos, E. F. and Nóbrega, O. T. (2015) Circadian rhythm in Alzheimer disease after trazodone use. Chronobiol. Int., 32, 1311−1314
111. Akbari, V., Ghobadi, S., Mohammadi, S. and Khodarahmi, R. (2020) The antidepressant drug; trazodone inhibits tau amyloidogenesis: Prospects for prophylaxis and treatment of AD. Arch. Biochem. Biophys., 679, 108218
112. Corpas, R., Griñán-Ferré, C., Palomera-Ávalos, V., Porquet, D., GarcíadeFrutos,P.,FranciscatoCozzolino,S.M.,Rodríguez-Farré, E., Pallàs, M., Sanfeliu, C. and Cardoso, B. R. (2018) Melatonin induces mechanisms of brain resilience against neurodegeneration. J. Pineal Res., 65, e12515
113. Balmik, A. A., Das, R., Dangi, A., Gorantla, N. V., Marelli, U. K. and Chinnathambi, S. (2020) Melatonin interacts with repeat domain of tau to mediate disaggregation of paired helical filaments. Biochim. Biophys. Acta, Gen. Subj., 1864, 129467
114. Das, R., Balmik, A. A. and Chinnathambi, S. (2020) Effect of melatonin on tau aggregation and tau-mediated cell surface morphology. Int. J. Biol. Macromol., 152, 30−39
115. Ren, S. C., Suo, Q. F., Du, W. T., Pan, H., Yang, M. M., Wang, R. H. and Liu, J. (2010) Quercetin permeability across blood-brain barrier and its effect on the viability of U251 cells. Sichuan Da Xue Xue Bao Yi Xue Ban (in Chinese), 41, 751–754, 759
116. Habtemariam, S. (2018) Molecular pharmacology of rosmarinic and salvianolic acids: Potential seeds for Alzheimer's and vascular dementia drugs. Int. J. Mol. Sci., 19, 458
117. Wang, J., Tang, C., Ferruzzi, M. G., Gong, B., Song, B. J., Janle, E. M., Chen, T. Y., Cooper, B., Varghese, M., Cheng, A., *et al*. (2013) Role of standardized grape polyphenol preparation as a novel treatment to improve synaptic plasticity through attenuation of features of metabolic syndrome in a mouse model. Mol. Nutr. Food Res., 57, 2091−2102
118. Rahmani, S., Mogharizadeh, L., Attar, F., Rezayat, S. M., Mousavi, S. E. and Falahati, M. (2018) Probing the interaction of silver nanoparticles with tau protein and neuroblastoma cell line as nervous system models. J. Biomol. Struct. Dyn., 36,

4057−4071
119. Zaman, M., Ahmad, E., Qadeer, A., Rabbani, G. and Khan, R. H. (2014) Nanoparticles in relation to peptide and protein aggregation. Int J Nanomedicine, 9, 899−912
120. Niu, L., Zou, Y., Lin, Y., Zheng, Y., Yang, Y. and Wang, C. (2019) Regulation mechanism of nanobiointerfaces in amyloid peptide assembly and aggregation structures. Sci. Sin. Chim., 49, 500−515
121. Hajsalimi, G., Taheri, S., Shahi, F., Attar, F., Ahmadi, H. and Falahati, M. (2018) Interaction of iron nanoparticles with nervous system: an *in vitro* study. J. Biomol. Struct. Dyn., 36, 928−937
122. Vakilinezhad, M. A., Amini, A., Akbari Javar, H., Baha'addini Beigi Zarandi, B. F., Montaseri, H. and Dinarvand, R. (2018) Nicotinamide loaded functionalized solid lipid nanoparticles improves cognition in Alzheimer's disease animal model by reducing Tau hyperphosphorylation. Daru, 26, 165−177
123. Vimal, S. K., Zuo, H., Wang, Z., Wang, H., Long, Z. and Bhattacharyya, S. (2020) Self-therapeutic nanoparticle that alters tau protein and ameliorates tauopathy toward a functional nanomedicine to tackle Alzheimer's. Small, 16, e1906861
124. Sonawane, S. K., Ahmad, A. and Chinnathambi, S. (2019) Protein-capped metal nanoparticles inhibit tau aggregation in Alzheimer's disease. ACS Omega, 4, 12833−12840
125. Soeda, Y., Saito, M., Maeda, S., Ishida, K., Nakamura, A., Kojima, S. and Takashima, A. (2019) Methylene blue inhibits formation of tau fibrils but not of granular tau oligomers: A plausible key to understanding failure of a clinical trial for Alzheimer's disease. J. Alzheimers Dis., 68, 1677−1686
126. Zhao, J., Yin, F., Ji, L., Wang, C., Shi, C., Liu, X., Yang, H., Wang, X. and Kong, L. (2020) Development of a tau-targeted drug delivery system using a multifunctional nanoscale metal-organic framework for Alzheimer's disease therapy. ACS Appl. Mater. Interfaces, 12, 44447−44458
127. Manju, S. and Sreenivasan, K. (2011) Hollow microcapsules built by layer by layer assembly for the encapsulation and sustained release of curcumin. Colloids Surf. B Biointerfaces, 82, 588−593
128. Chen, X., Zou, L. Q., Niu, J., Liu, W., Peng, S. F. and Liu, C. M. (2015) The stability, sustained release and cellular antioxidant activity of curcumin nanoliposomes. Molecules, 20, 14293−14311
129. Fan, S., Zheng, Y., Liu, X., Fang, W., Chen, X., Liao, W., Jing, X., Lei, M., Tao, E., Ma, Q., *et al*. (2018) Curcumin-loaded PLGA-PEG nanoparticles conjugated with B6 peptide for potential use in Alzheimer's disease. Drug Deliv., 25, 1091−1102
130. Gao, C., Chu, X., Gong, W., Zheng, J., Xie, X., Wang, Y., Yang, M., Li, Z., Gao, C. and Yang, Y. (2020) Neuron tau-targeting biomimetic nanoparticles for curcumin delivery to delay progression of Alzheimer's disease. J. Nanobiotechnology, 18, 71
131. Hu, Y., Hu, X., Lu, Y., Shi, S., Yang, D. and Yao, T. (2020) New strategy for reducing tau aggregation cytologically by a hairpinlike molecular inhibitor, tannic acid encapsulated in liposome. ACS Chem. Neurosci., 11, 3623−3634
132. Singh, N. A., Bhardwaj, V., Ravi, C., Ramesh, N., Mandal, A. K. A. and Khan, Z. A. (2018) EGCG nanoparticles attenuate aluminum chloride induced neurobehavioral deficits, beta amyloid and tau pathology in a rat model of Alzheimer's disease. Front. Aging Neurosci., 10, 244
133. Armiento, V., Spanopoulou, A. and Kapurniotu, A. (2020) Peptide-based molecular strategies to interfere with protein misfolding, aggregation, and cell degeneration. Angew. Chem. Int. Ed. Engl., 59, 3372−3384
134. Rauscher, S., Baud, S., Miao, M., Keeley, F. W. and Pomès, R. (2006) Proline and glycine control protein self-organization into elastomeric or amyloid fibrils. Structure, 14, 1667−1676
135. Li, S. C., Goto, N. K., Williams, K. A. and Deber, C. M. (1996) Alpha-helical, but not β-sheet, propensity of proline is determined by peptide environment. Proc. Natl. Acad. Sci. USA., 93, 6676−6681
136. Pandey, G., Morla, S., Kumar, S. and Ramakrishnan, V. (2020) Modulation of tau protein aggregation using 'Trojan' sequences. Biochim. Biophys. Acta, Gen. Subj., 1864, 129569
137. Cheng, I. H., Scearce-Levie, K., Legleiter, J., Palop, J. J., Gerstein, H., Bien-Ly, N., Puoliväli, J., Lesné, S., Ashe, K. H., Muchowski, P. J., *et al*. (2007) Accelerating amyloid-beta fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. J. Biol. Chem., 282, 23818−23828
138. Dammers, C., Yolcu, D., Kukuk, L., Willbold, D., Pickhardt, M., Mandelkow, E., Horn, A. H., Sticht, H., Malhis, M. N., Will, N., *et al*. (2016) Selection and characterization of tau binding D-enantiomeric peptides with potential for therapy of Alzheimer disease. PLoS One, 11, e0167432
139. Gorantla, N. V., Sunny, L. P., Rajasekhar, K., Nagaraju, P. G., Cg, P. P., Govindaraju, T. and Chinnathambi, S. (2021) Amyloid-β-derived peptidomimetics inhibits tau aggregation. ACS Omega, 6, 11131−11138
140. Best, R. B. (2017) Computational and theoretical advances in studies of intrinsically disordered proteins. Curr. Opin. Struct. Biol., 42, 147−154
141. Meneksedag-Erol, D. and Rauscher, S. (2019) Atomistic simulation tools to study protein self-aggregation. Methods Mol. Biol., 2039, 243−262
142. Popov, K. I., Makepeace, K. A. T., Petrotchenko, E. V., Dokholyan, N. V. and Borchers, C. H. (2019) Insight into the structure of the "unstructured" tau protein. Structure, 27, 1710−1715.e4
143. Larini, L., Gessel, M. M., LaPointe, N. E., Do, T. D., Bowers, M. T., Feinstein, S. C. and Shea, J. E. (2013) Initiation of assembly of tau (273–284) and its ΔK280 mutant: an experimental and computational study. Phys. Chem. Chem. Phys., 15, 8916−8928
144. Daebel, V., Chinnathambi, S., Biernat, J., Schwalbe, M., Habenstein, B., Loquet, A., Akoury, E., Tepper, K., Müller, H., Baldus, M., *et al*. (2012) β-Sheet core of tau paired helical

filaments revealed by solid-state NMR. J. Am. Chem. Soc., 134, 13982−13989

145. Adamcik, J., Sánchez-Ferrer, A., Ait-Bouziad, N., Reynolds, N. P., Lashuel, H. A. and Mezzenga, R. (2016) Microtubule-binding R3 fragment from tau self-assembles into giant multistranded amyloid ribbons. Angew. Chem. Int. Ed. Engl., 55, 618−622
146. Liu, H., Zhong, H., Xu, Z., Zhang, Q., Shah, S. J. A., Liu, H. and Yao, X. (2020) The misfolding mechanism of the key fragment R3 of tau protein: a combined molecular dynamics simulation and Markov state model study. Phys. Chem. Chem. Phys., 22, 10968−10980
147. Li, X., Dong, X., Wei, G., Margittai, M., Nussinov, R. and Ma, B. (2018) The distinct structural preferences of tau protein repeat domains. Chem. Commun. (Camb.), 54, 5700−5703
148. Smit, F. X., Luiken, J. A. and Bolhuis, P. G. (2017) Primary fibril nucleation of aggregation prone tau fragments PHF6 and PHF6*. J. Phys. Chem. B, 121, 3250−3261
149. Liu, H., Zhong, H., Liu, X., Zhou, S., Tan, S., Liu, H. and Yao, X. (2019) Disclosing the mechanism of spontaneous aggregation and template-induced misfolding of the key hexapeptide (PHF6) of tau protein based on molecular dynamics simulation. ACS Chem. Neurosci., 10, 4810−4823
150. Eschmann, N. A., Georgieva, E. R., Ganguly, P., Borbat, P. P., Rappaport, M. D., Akdogan, Y., Freed, J. H., Shea, J. E. and Han, S. (2017) Signature of an aggregation-prone conformation of tau. Sci. Rep., 7, 44739
151. Guo, J. P., Arai, T., Miklossy, J. and McGeer, P. L. (2006) Abeta and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer's disease. Proc. Natl. Acad. Sci. USA, 103, 1953−1958
152. Rojas, A. V., Maisuradze, G. G. and Scheraga, H. A. (2018) Dependence of the formation of tau and Aβ peptide mixed aggregates on the secondary structure of the N-terminal region of Aβ. J. Phys. Chem. B, 122, 7049−7056
153. Miller, Y., Ma, B. and Nussinov, R. (2011) Synergistic interactions between repeats in tau protein and Aβ amyloids may be responsible for accelerated aggregation via polymorphic states. Biochemistry, 50, 5172−5181
154. Do, T. D., Economou, N. J., Chamas, A., Buratto, S. K., Shea, J. E. and Bowers, M. T. (2014) Interactions between amyloid-β and Tau fragments promote aberrant aggregates: implications for amyloid toxicity. J. Phys. Chem. B, 118, 11220−11230
155. Shin, W. S., Di, J., Murray, K. A., Sun, C., Li, B., Bitan, G. and Jiang, L. (2019) Different amyloid-beta self-assemblies have distinct effects on intracellular tau aggregation. Front. Mol. Neurosci., 12, 268