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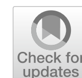
REVIEW



The structure and phase of tau: from monomer to amyloid filament

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Abstract

Tau is a microtubule-associated protein involved in regulation of assembly and spatial organization of microtubule in neurons. However, in pathological conditions, tau monomers assemble into amyloid filaments characterized by the cross-β structures in a number of neurodegenerative diseases known as tauopathies. In this review, we summarize recent progression on the characterization of structures of tau monomer and filament, as well as the dynamic liquid droplet assembly. Our aim is to reveal how post-translational modifications, amino acid mutations, and interacting molecules modulate the conformational ensemble of tau monomer, and how they accelerate or inhibit tau assembly into aggregates. Structure-based aggregation inhibitor design is also discussed in the context of dynamics and heterogeneity of tau structures.

Keywords Protein aggregation · Liquid–liquid phase separation · Drug design · Conformation transition · Intrinsically disordered protein

Introduction

Tau protein is encoded by the *MAPT* gene which is located on chromosome 17. Six different tau isoforms are generated by alternative splicing, containing zero, one or two N-terminal inserts and three or four microtubule-binding repeats [1]. The constructs of 2N4R and 2N3R are illustrated in Fig. 1a. Tau protein can be divided into four distinct portions: the N-terminal domain (NTD), the proline-rich domain (PRD), the microtubule-binding domain (MTBD), and the C-terminal domain (CTD). The two aggregation-prone hexapeptide motifs, PHF6* (^{275}VQIINK^{280}) and PHF6 (^{306}VQIVYK^{311}), are located on the R2 repeat and R3 repeat of MTBD, respectively. Consequently, the 4R tau isoform contains two hexapeptide motifs, and the 3R tau isoform has only one hexapeptide motif.

Tau is mainly expressed in central and peripheral nerve systems, where it is largely distributed in axons. As a microtubule-associated protein, tau regulates assembly and spatial organization of microtubule, thus playing a critical role in axon development and navigation (Fig. 1b) [2, 3]. Tau monomer is intrinsically disordered, with some transient secondary structure elements populated. However, tau monomers assemble into amyloid filaments characterized by the cross-β structures in a number of neurodegenerative diseases known as tauopathies, including Alzheimer's disease (AD), Pick's disease (PiD), chronic traumatic encephalopathy (CTE), and corticobasal degeneration (CBD) [4–7]. Consequently, tau protein is widely believed to be a major target for treatment of tauopathies [8–11]. In addition to its standard function as a microtubule regulating protein, tau has numerous binding partners and is distributed into various cell compartments. Recent studies show that tau also plays roles in signaling, cytoskeletal organization, and chromosome stability [12, 13].

In this review, we summarize recent structural characterization on tau monomer and filaments, aiming to reveal how post-translational modifications (PTMs), amino acid mutations, and interacting molecules modulate the conformational ensemble of tau monomer, and how they accelerate or inhibit tau aggregation. We pay special attention to the liquid–liquid phase separation (LLPS) of tau monomer and structure-based screening/designing of tau aggregation inhibitors.

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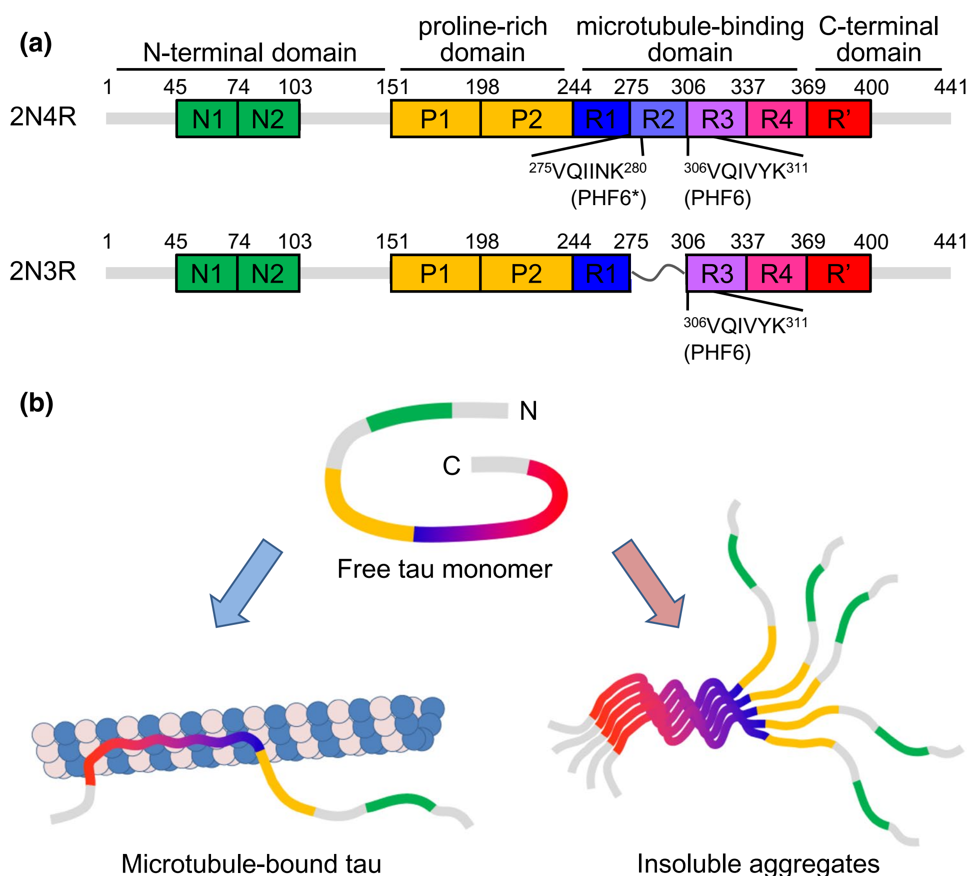

Fig. 1 Domain organization and conformation states of tau. **a** Illustrations of two tau isoforms, 2N4R and 2N3R. The 2N4R isoform contains two N-terminal inserts (N1 and N2), two proline-rich regions (P1 and P2), and four microtubule-binding repeats (R1, R2, R3, and R4). The repeat-like segment R' in the C-terminus is also indicated. The microtubule-binding repeat R2 is not present in the 2N3R isoform. **b** Schematic illustrations of the three conformation states of tau. The disordered while compact tau monomers bind to microtubule, forming elongated conformations. Under certain conditions, tau monomers aggregate into filaments characterized by the presence of cross-β structures. The length of individual domain is not to scale

Structure of tau monomer

Tau is a polyelectrolyte. The NTD and CTD are negatively charged, whereas the PRD and MTBD are positively charged (Fig. 2a). Disordered propensity predictions suggest that tau is highly disordered except the MTBD (Fig. 2b). Consistently, experimental characterization has shown that tau does not have well-defined three-dimensional structures [14–20]. Circular dichroism (CD), Fourier transform infrared spectroscopy, and nuclear magnetic resonance (NMR) have revealed that dynamic and residual secondary structures are present in tau monomer [14, 18, 21, 22]. In particular, the PHF6* and PHF6 motifs adopt β-strand conformations [20, 22, 23]. The mean radius of gyration (R_g) of tau (5.1 ± 0.5 nm from single molecule Förster resonance energy transfer (smFRET) measurement or 6.5 ± 0.3 nm from small-angle X-ray scattering (SAXS) measurement) is smaller than that of random coil of equal length [16, 17]. Therefore, tau is globally compact in solution. Further characterization shows that tau can form long-range contacts, some of which are mediated by electrostatic interactions [18, 22, 24, 25]. For example, the C-terminus folds on to the MTBD and the N-terminus is in contact with the C-terminus. Such an overall structure of tau can be described by the "paperclip-like" model [26]. Although tau is globally compact, different domains have distinct conformational characteristics and the conformation of MTBD can be altered by the flanking domains. For example, MTBD becomes more compact when it is isolated from the full-length protein [27]. Furthermore, backbone dynamics analysis revealed that the NTD is highly mobile while the MTBD shows increased rigidity [22].

Several atomic conformational ensembles of tau monomer have been constructed based on paramagnetic relaxation enhancement, residual dipolar coupling, cross-linking data, and computational sampling, further demonstrating the globular conformations of tau with distinct topology and variable secondary-structure elements (Fig. 2c) [17, 18, 22, 25, 28]. The free energy landscape of tau monomer may have several minima separated by free energy barriers. Therefore, distinct tau conformer species can be detected and isolated. Single-molecule fluorescence anisotropy combined with an anti-Brownian electrokinetic trap has revealed that tau resides in two groups of conformations, i.e., a more compact family and a less compact family [29, 30]. In another study, after sonication treatment, two tau monomeric species were isolated from tau fibrils, i.e., the inert monomer "Mi" and the seed-competent monomer "Ms" [31]. Although CD showed no observable difference between Mi and Ms, conformation

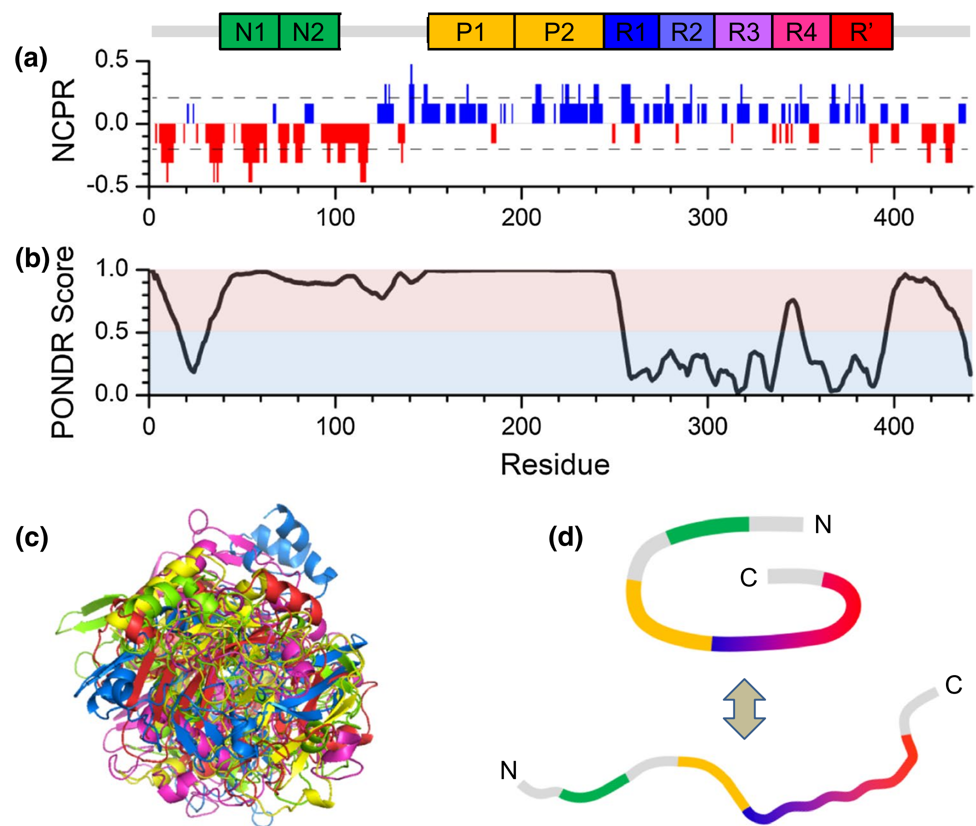

Fig. 2 Sequence and structure properties of tau monomer. **a** Net charge per residue (NCPR) distribution of 2N4R tau analyzed by CIDER [174]. **b** Disorder prediction of 2N4R tau using the PONDR VLXT [175, 176]. **c** Conformational ensemble of tau constructed based on cross-linking data and computational sampling [28]. Five conformations are shown by different colors. **d** Schematic illustration of conformation remodeling of tau

modeling using restrains from cross-linking data revealed that the PHF6* and PHF6 are buried in Mi but are relatively exposed in Ms. Furthermore, the Ms group itself is heterogeneous as Ms from AD patient brain and CBD patient brain encodes one tau prion strain and three tau prion strains, respectively [32]. Other observations also suggest that tau can reside in different conformational states, some of which are called "pathological conformation" and can be recognized by specific antibodies [33–35]. By monitoring the end-to-end distance distribution of PHF6 and PHF6* through pulsed double electron–electron resonance measurements, Eschmann et al. found that the extended β-strand conformational states of PHF6 and PHF6* constitute a defining signature of aggregation-prone tau [36]. Since different tau conformational states could exhibit distinct rigidity, structure propensity, and steric hindrance surrounding PHF6* and PHF6 motifs, they may possess different aggregation propensity and contribute differently to tau-related diseases.

Mutations, PTMs as well as intermolecular interactions can remodel the conformational ensemble of tau monomer (Fig. 2d), thus promoting or suppressing its aggregation. The pro-aggregant mutant ΔK280 is found to suppress long-range intermolecular contacts and stabilize the β-strand conformations [24]. The aggregation-prone PHF6 motif forms metastable compact structures with its upstream sequence and is shielded by a β-turn structure in the inert monomer state [31]. P301 mutations destabilize this local structure and trigger spontaneous aggregation [37]. Depending on the phosphorylation state, the overall conformation of tau monomer and the transient intramolecular interactions could be modified [38]. Phosphorylation mimic in the epitopes recognized by the AT8 antibody (S199E, S202E, T205E) enhances tau aggregation by reducing the electrostatic attraction between NTD and PRD or by moving NTD away from CTD [39, 40]. Phosphorylation at Ser202 and Thr205 induces formation of a turn-like structure, protecting tau against aggregation [41]. On the contrary, hyperphosphorylation by glycogen synthase kinase-3β results in global expansion of tau and increased exposure of PHF6 [38]. SmFRET characterization showed that binding of aggregation enhancers (e.g., heparin and cytoplasmic polyphosphates) eliminate long-range contacts and induce expansion of tau [17, 27, 42]. The mean R_g of tau is increased from 5.1 ± 0.5 to 6.0 ± 0.6 nm upon addition of heparin [17]. NMR titration showed that the segment at the beginning of R2 exhibits the largest chemical shift displacements [23]. Although the MTBD becomes more compact upon heparin binding [27], the PHF6 and PHF6* motifs remain extended [36]. A similar chemical shift pattern is observed when tau is bound to polyglutamic acid [23]. Binding of polyglutamic acid also compacts the MTBD and tightens the interactions between PHF6 and PHF6* [43].

In contrast to 4R tau isoform, structural investigations on 3R tau are very limited. Molecular dynamics simulation and NMR characterization have been applied to compare the structural difference between K18 (the repeat region of 4R tau) and K19 (the repeat region of 3R tau) constructs of tau [23, 25]. K18 and K19 show similar β-structure propensity at the beginning of repeats [23]. In K18, the R1 repeat interacts with R2 but not R3 or R4. In contrast, in K19, R1 interacts with both R3 and R4 [25]. Therefore, although the removal of R2 does not affect the local β-structure propensity of MTBD, it remodels the global intramolecular interactions of tau.

Liquid–liquid phase separation and structure of tau in the droplet-like assembly

When the solution conditions favor formation of massive dynamic intermolecular interactions, some intrinsically disordered proteins (IDPs) will demix into a light phase and a dense phase. Such a process has been termed as liquid–liquid phase separation, which underlies the formation of the membraneless compartments in cells [44–49]. Importantly, some IDP droplets can convert into solid states, suggesting that the liquid condensates are on pathway to fibers and LLPS can be related to the progression of some neurodegenerative diseases [50–53].

Recent studies have revealed that tau readily undergoes LLPS in the presence of macromolecular crowding agents or in cells [54–58]. The concentration of tau can be increased more than ten-fold upon LLPS [56, 59]. Fusion, fission, fluorescence recovery after photobleaching and electron paramagnetic resonance spectroscopy of droplets indicate that tau droplets are in liquid state [57, 60, 61].

LLPS of tau is sensitive to the concentration of salt. Decreasing salt concentration promotes LLPS while increasing salt concentration suppresses droplets formation (Fig. 3a) [54, 55, 57, 62]. The sensitivity of LLPS to salt concentration suggests that electrostatic interactions are critical for the formation of tau droplets [55, 57]. The charges are clustered along the tau sequence (Fig. 2a). Truncation experiments suggest that interactions between the positively charged domains and the negatively charged domains are the main driving force of tau LLPS (Fig. 3c) [57]. Although the K18 construct of tau is also able to undergo LLPS under very high concentration, its LLPS is sensitive to 1,6-hexanediol rather than salt concentration, indicating that the main driving force of K18 LLPS is hydrophobic interactions rather than electrostatic interactions [55, 59]. Similarly, under high salt conditions where electrostatic interactions are screened, droplets formed by N-terminal truncated tau are dissolved with 1,6-hexanediol [63]. PTMs also regulate LLPS of tau (Fig. 3b). Acetylation neutralizes the positive charges on lysine residues, thereby reducing electrostatic attractions that drive intramolecular and intermolecular contacts. Consequently, acetylation suppresses LLPS of tau

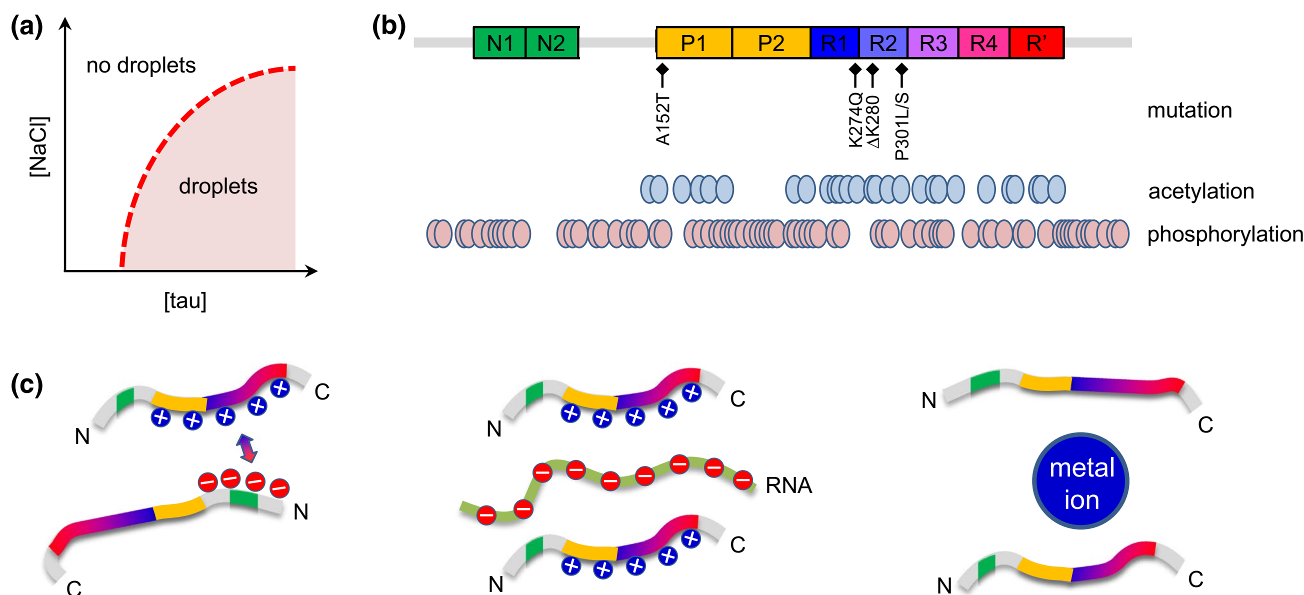

Fig. 3 Liquid–liquid phase separation of tau. **a** A phase diagram illustrating the effect of salt concentration on LLPS of tau. **b** The mutations, acetylation sites, and phosphorylation sites that influence tau LLPS. **c** Intermolecular interactions that may be critical for tau LLPS, such as electrostatic interactions between the negatively charged NTD and the positively charged PRD and MTBD of tau, electrostatic interactions between tau and RNA, and metal ions mediated interactions

[64]. By introducing negatively charged groups into PRD and MTBD, phosphorylation also reduces electrostatic attractions. However, phosphorylation of tau, either introduced by MARK2 or SF9 insect cells, promotes LLPS of tau [55, 56]. Interestingly, phosphorylation reverses the sensitivity of tau LLPS to salt and 1,6-hexanediol, where LLPS of phosphorylated tau is insensitive to increasing salt concentration but sensitive to the addition of 1,6-hexanediol [56]. It seems that phosphorylation suppresses tau LLPS driven by electrostatic interactions but promotes tau LLPS driven by hydrophobic interactions. It is possible that phosphorylation modulates the conformations of PRD and MTBD and induces exposure of hydrophobic segments. Structural investigations on phosphorylated tau will be valuable for further understanding the influence of phosphorylation on tau LLPS. Taken together, these data show that tau LLPS is driven by a complex combination of electrostatic and hydrophobic interactions.

Tau forms droplets with various types of RNA, which may result from the complex coacervation effect [60–62]. Lysine/RNA and arginine/RNA interactions are critical in forming the complex coacervate phase [61]. Phosphorylation reduces the propensity of tau/RNA LLPS, likely due to electrostatic repulsion between the phosphorylated residues and the negatively charged RNA [60]. Acetylation also suppresses LLPS of tau with RNA, probably due to the removal of positive charges on lysine sidechains [61].

Metal ions play important roles in regulating the function of tau. Rane et al. showed that Al^{3+} and Zn^{2+} enhance tau LLPS [65]. Singh et al. further showed that the two cysteines within the MTBD are required for Zn^{2+} to induce tau droplet formation [66]. Metal ions may promote LLPS of tau through a conformational change favorable for intermolecular electrostatic attractions or mediating intermolecular contacts [65].

Structural characterization of tau droplets remains challenging. However, progress has been made. Double electron–electron resonance spectroscopy characterization indicated that the mean distance flanking the PHF6* region remains unchanged when tau undergoes LLPS with RNA [62]. However, the entire polypeptide chain of tau adopts more extended conformations in the droplet state [67]. Secondary structure characterization of tau K18 via CD and NMR indicated that the level of β-structure content and the propensity for β-hairpin conformation are increased upon LLPS [55, 59]. Although the thioflavin T fluorescence of tau droplets gradually increases over incubation, its intensity is much weaker than what is observed in the presence of heparin [55, 62]. Therefore, the β-structure content of tau in the droplets is much smaller than that in the amyloid fibrils.

Whether LLPS of tau is related to fibril formation remains controversial. Some studies suggest that LLPS of tau mediates and facilitates aggregation. Aggregation enhancing factors, including heparin, pro-aggregation mutations, and K274 acetylation, promote LLPS of tau [56, 64, 68]. Protein disulfide isomerase directly interacts with tau and suppresses the formation of tau droplets and aggregates [69]. EFhd2 is associated with aggregated tau species in AD brains. Recent results showed that EFhd2 regulates tau aggregation and LLPS in a calcium dependent manner [58]. Furthermore, droplets formed by phosphorylated tau rapidly transition from a liquid state to a gel-like state, and finally transition into large aggregates containing β-structures [56]. Therefore, tau concentrated in the droplets is postulated to be on pathway to fibril formation. On the contrary, other studies point out that LLPS and amyloid aggregation of tau are independent processes although they occur in overlapping conditions [63]. Lin et al. systematically investigated the impacts of LLPS on tau aggregation by evaluating the conformation of tau, kinetics of aggregation and fibril quantity [63]. They found that none of these properties are influenced directly by LLPS. The presence of extended β-strand conformation of PHF6 and PHF6* has been postulated as a defining signature of aggregation-prone tau [36]. However, structural investigation discussed above shows that the β-structure content of PHF6 and PHF6* in the droplets is almost indistinguishable from that in the dilute state. Though LLPS may not directly promote aggregation of tau, Kanaan et al. showed that phase separation of tau could facilitate the formation of non-filamentous pathogenic tau oligomers in vitro [70]. It is noted that all tau phase separation studies have been carried out in vitro or in cells. So far, no study shows a direct connection between LLPS of tau and neurodegenerative diseases in vivo or tau undergoing LLPS in neurons in situ in brain. Taken together, LLPS of tau is promoted by a variety of factors. Tau concentration is increased inside the droplets and tau molecules adopt conformations that are slightly different from those in dilute state. The connection between LLPS and tau aggregation remains elusive and requires further investigation.

Structures of soluble tau oligomers

Soluble tau oligomers with various molecular weights have been identified and they have been suggested as the toxic species in vivo [35, 71–86]. Once formed, tau oligomers can be released and taken up by cells [87]. The conformations of oligomeric tau could be different from those of monomeric tau as monoclonal antibodies raised against tau oligomers show no reactivity toward monomeric tau [73, 88, 89]. Since some tau oligomers can be converted to amyloid fibrils, hydrophobic segments in the MTBD may become exposed upon oligomerization [31, 75, 90]. While some studies show that tau dimers can be stabilized by intermolecular disulfide bonds [84, 85], non-disulfide linked tau dimers are also

observed [91–93]. Via force measurement, Rosenberg et al. showed that two tau monomers can associate in an antiparallel configuration stabilized by complementary electrostatic interactions between the negatively-charged NTDs and the positively-charged PRDs [91]. Moreover, a tau dimer can be formed via bridging by a heparin molecule [72, 94, 95]. Although tau oligomers have been recognized as important players in tau pathogenesis for a long time, structural characterization on tau oligomers is very limited and requires more studies in the future.

Structures of tau filaments and aggregation mechanism

It's challenging to characterize the structures of tau aggregates because they are partially disordered and heterogeneous. Nevertheless, accumulated evidence from studies using various constructs of recombinant tau shows that the amyloid core is dominantly formed by the MTBD, ranging from the second half of R1 to the first half of R4 [96]. Jakes et al. further revealed that the core of paired helical filament (PHF) in AD is restricted to the repeat regions of 3R and 4R tau isoforms [97]. The repeats pack against each other parallel and form β-sheets [98–100]. Except the amyloid core, the remainder of tau protein remains highly mobile, forming the fuzzy coat [4, 20, 101–103].

Since six tau isoforms are expressed in adult human brain, different neurodegenerative diseases can have different tau isoform compositions and filament structures [104, 105]. Limited proteolysis applied on aggregates extracted from patient brains shows different banding patterns in immunoblot analyses for PiD, AD, CBD, and progressive supranuclear palsy [106]. However, the atomic structures of tau fibrils in pathological conditions are not available until the last 3 years when structures of tau aggregates from various sources were determined by cryo-EM [107–111].

In AD, tau filaments are made of PHFs and straight filaments (SFs), where all six tau isoforms are present [107]. Early electron microscopy images indicated that PHFs and SFs have a common C-shaped morphology [112, 113]. Recently, atomic cryo-EM structures revealed that the filament core is made of amino acids V306-F378, comprising R3 and R4, as well as part of the C-terminal domain (Fig. 4a) [107]. Similar to AD, all six tau isoforms assemble into filaments in CTE, and residues K274-R379 of 3R tau and S305-R379 of 4R tau form the enlarged C-shaped filament core (Fig. 4b) [110]. Importantly, a hydrophobic cavity which is not present in the AD filament is observed within the filament core from CTE. Hydrophobic cofactors may be incorporated during tau aggregation in CTE. In PiD, two filament forms (narrow and wide) have been identified [109]. Narrow filaments are composed of a single protofilament while wide filaments are made of two narrow filaments. Different from the C-shape core of AD, the filament core of PiD is an elongated structure, which comprises amino acids K254-F378 of 3R tau (Fig. 4c). The latest tau filament structure is from CBD [111]. The CBD filaments are made of 4R tau exclusively. Two types of filaments are observed depending on the numbers of protofilaments. The CBD filament core comprises K274-E380 and adopts a four-layered fold (Fig. 4d). An additional uncharacterized density surrounded by the sidechains of K290, K294, and K370 is found within the filament core.

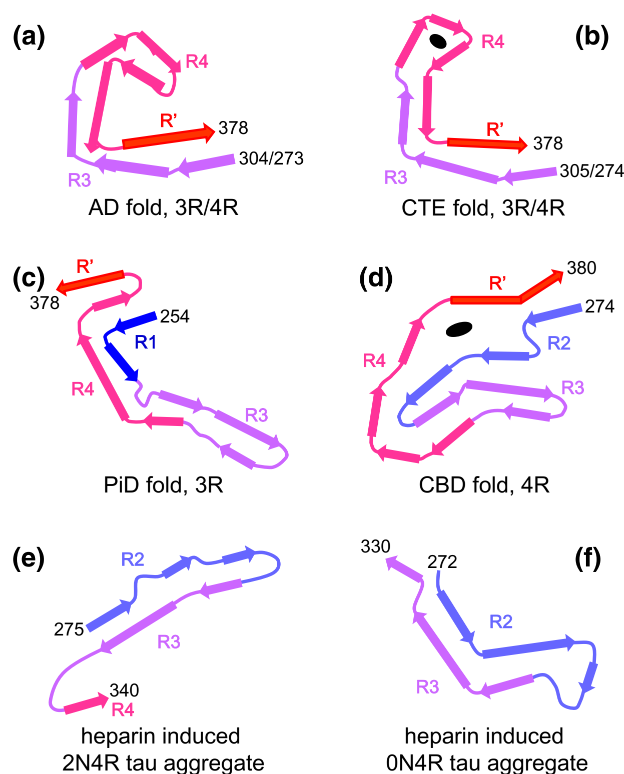

Fig. 4 Structure models of tau from various filaments. **a** 3R/4R tau filaments from AD patients. **b** 3R/4R tau filaments from CTE patients. **c** 3R tau filaments from PiD patients. **d** 4R tau filaments from CBD patients. **e** Heparin induced 2N4R tau filaments. **f** Heparin induced 0N4R tau filaments. Additional uncharacterized density found within the filament core of the CTE and CBD folds are indicated the by the black dots

The structures of fibers derived from different patients are identical, indicating that they are disease- rather than patient-specific. So far, it remains unknown what factors drive tau into specific conformations in tauopathies. PTMs, mutations, and cofactors may be critical. The structural heterogeneity of tau filaments suggests that different factors or tau segments may play different roles in the aggregation process. On one hand, the cellular environment is much more complicated than what we can mimic in a test tube. It is not surprising that the structures of heparin-induced

tau filaments are different from those in diseases (Fig. 4e, f) [114, 115]. On the other hand, the marked difference in the structures of tau filaments suggests the existence of distinct seeds of tau in different tauopathies [116–118]. Since stable seed-competent tau monomers can be isolated from patient brains [32], it is important to determine whether these seeds will serve as templates to drive tau assembling into filaments identical to those observed in patients.

In vitro, the aggregation process of tau is generally described by the classic nucleation-elongation model, with the nucleating species thought to be the assembly-competent monomer [119] or soluble oligomer [75, 85]. Recent studies have demonstrated that monomeric tau species derived from heparin-induced aggregates or isolated from patient brains are capable of seeding tau aggregation both in vitro and in cultured cells [31, 32]. Through a photochemical cross-linking technique, Patterson et al. demonstrated that dimerization is an early step in the aggregation process of tau and these dimers self-associate to form larger aggregates [73]. Tau aggregation induced by heparin further suggests that the aggregation-competent tau species is a tau dimer which may be bridged by a heparin molecule [72, 94, 95]. Exposure of hydrophobic segments in the assembly-competent monomer and dimer may be critical for the subsequent self-assembling process [31, 75].

Tau is stable under normal conditions. However, factors inducing formation of oligomers or enhancing exposure of hydrophobic segments will promote tau aggregation. Previous analysis has revealed that tau is often mutated or hyperphosphorylated in tauopathies, suggesting that mutation or phosphorylation regulates tau aggregation. Indeed, mutations found in frontotemporal dementias promote tau aggregation [19, 120–123]. Although these mutations generate marginal change on the overall structure of tau monomer, they enhance local β-structure propensity [19, 124]. On the contrary, proline mutations within the two hexapeptide motifs (I277P and I308P) disrupt β-structure and abrogate aggregation [20]. The influence of phosphorylation on tau aggregation is also complicated. The abnormally hyperphosphorylated tau from AD brain or tau phosphorylated by glycogen synthase kinase-3β has been found to self-aggregate into PHF-like structures [125, 126]. Specific phosphorylation patterns promote tau aggregation can be recognized [41, 127]. However, hyperphosphorylated tau obtained by in vitro phosphorylation with recombinant extracellular-regulated kinase or rat brain extract, or obtained from recombinant expression in Sf9 cells shows no significant increased susceptibility to in vitro aggregation than unphosphorylated tau [83, 128]. Furthermore, phosphorylation has also been found to protect tau against aggregation [129, 130]. Importantly, kinetics studies suggest that even when phosphorylated tau is aggregated, phosphorylation enhances but not triggers tau aggregation [131, 132].

Interactions with surrounding molecules can modulate the conformational ensemble of tau monomer or shift the equilibrium between tau monomer and oligomer. The tau fibril core is mainly made of the positively charged MTBD which interacts with various polyanions, including DNA, RNA, heparin, polyphosphates and polyglutamic acid. Structural and kinetic characterization suggests that polyanions enhance tau aggregation by remodeling the conformational ensemble of monomeric tau and noncovalent cross-linking of multiple tau monomers [42, 43, 94, 95, 133, 134]. Tau contains multiple metal ion binding sites. Binding to metal ions has been showed to enhance tau aggregation although the mechanism remains unclear [9, 65, 135–137]. Furthermore, crowded cell-like environments can significantly promote tau aggregation by accelerating the nucleation step [138–140].

Structure-based design of tau aggregation inhibitors

Tau aggregation can be inhibited by binding to various molecules. Molecular chaperones suppress tau aggregation efficiently. Hsp70 suppresses the formation of tau nuclei [141]. Hsp27 delays tau fibril formation by weakly interacting with early species in the aggregation process, whereas HspA8 is highly efficient at preventing tau fibril elongation, possibly by capping the ends of tau fibrils [142]. A number of chaperones bind tau at or around the PHF6* and PHF6 motifs [143–145]. Thus, a major mechanism of anti-aggregation activity of molecular chaperones seems to be the direct binding to tau at the aggregation-prone regions [144]. Antibodies and protein disulfide isomerase may also adopt similar mechanism to inhibit tau aggregation [69, 146].

Small molecules can inhibit tau aggregation, although the mechanism remains elusive [9, 147–158]. Based on the K18 conformational ensembles, Kiss et al. analyzed the potential hot spots and small molecule binding sites using FTMap [159]. They found that the PHF6 and PHF6* motifs have the highest probability of forming the hot spots. Chong et al. also identified nine druggable cavities from the K18 conformational ensembles [160]. Docking of methylene blue with various tau conformations revealed that methylene blue binds in close proximity of C291/C322 [159] and NMR characterization showed that the molecular tweezer CLR01 binds preferentially to Lys residues in the MTBD [161]. Recently, through molecular dynamics simulations and ensemble docking, Baggett and Nath identified novel tau aggregation inhibitors [162]. Since the structure of tau monomer is highly dynamic, small molecules may bind to tau in a fuzzy way [163, 164].

PHF6 and PHF6* are critical for tau aggregation. Tau molecules lacking these two hexapeptide motifs cannot

aggregate [165]. Consequently, it is possible to block tau aggregation by shielding these two motifs. Based on the atomic structures of amyloid fibrils formed by PHF6 and PHF6*, molecules have been designed to cap the ends of tau fibrils and they are found to efficiently inhibit the aggregation of 3R and 4R tau isoforms [166–170].

Conclusions and perspectives

Tau is a major target for tauopathies treatment, the structures of whose monomer and fiber have been studied for decades. It turns out that the conformational ensemble of tau monomer is very dynamic and can be remodeled by a variety of factors. Up to date, atomic models of unmodified free tau monomer are available. For better understanding the conversion of tau from inert state to aggregation-prone state, it is urgent to determine the structures of tau monomer upon phosphorylation, acetylation, or binding to other molecules.

As indicated above, the cryo-EM structures of tau fibers in distinct diseases are different. It remains unclear what factors induce or determine the heterogeneity of tau fiber structures. It is noted that the PRD is absent in the cores of available fibril structures. However, PRD is subjected to extensive PTMs. One possibility is that PTMs on PRD induce formation of various aggregation-prone tau species, which act as templates in the subsequent aggregation process. Furthermore, unknown densities are present in the cryo-EM structures of tau fibers. Clarifying their identities will be also valuable for understanding the heterogeneity of tau structures in the future.

Inhibiting the aggregation of tau has been widely accepted as a therapeutic strategy for tauopathies. It is appearing that inhibitors can be designed to bind tau monomers to block their seeding or to cap tau fibrils to block their propagation. The distinct structures of filaments from different tauopathies and the difference between structures of heparin-induced tau aggregates and those of filaments isolated from diseased brains indicate the complexity of tau assembling process. Reconstruction of disease specific tau filaments will be valuable to test the efficacy of inhibitors in this regard. The LLPS of tau seems to be related to tau aggregation. Therefore, molecules designed to suppress the formation of tau droplets may be also able to inhibit tau aggregation. LMTX is a potent tau aggregation inhibitor. While LMTX failed to show effects on the primary cognitive endpoints in two phase 3 trials [171, 172], pharmacological activity has been demonstrated on brain structure and function at the 8 mg/day dose [173]. Further clinical trials in mild/moderate AD will be required to confirm efficacy at this dose. Due to the dynamics of tau monomer, heterogeneity of fibril structures, and enrichment of PTMs, a variety of tau aggregation inhibitors are expected to be designed in the future.

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Compliance with ethical standards

Conflict of interest All authors declare that there are no conflicts.

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