

## Mechanistic approaches to understand the prion-like propagation of aggregates of the human tau protein

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### ABSTRACT

The dynamic nature of the tau protein under physiological conditions is likely to be critical for it to perform its diverse functions inside a cell. Under some conditions, this intrinsically disordered protein assembles into pathogenic aggregates that are self-perpetuating, toxic and infectious in nature. The role of liquid-liquid phase separation in the initiation of the aggregation reaction remains to be delineated. Depending on the nature of the aggregate, its structure, and its localization, neurodegenerative disorders with diverse clinical features are manifested. The prion-like mechanism by which these aggregates propagate and spread across the brain is not well understood. Various factors (PTMs, mutations) have been strongly associated with the pathological aggregates of tau. However, little is known about how these factors modulate the pathological properties linked to aggregation. This review describes the current progress towards understanding the mechanism of propagation of tau aggregates.

### 1. Introduction

#### 1.1. The tau protein

Tau is an intrinsically disordered protein (IDP) that binds to microtubules (MT) and stabilizes them [1]. The human tau gene, MAPT (microtubule-associated protein tau), is located on chromosome 17q21 [2]. It comprises of 16 exons, and alternative splicing of exons 2, 3 and 10 generates six isoforms of the tau protein [2] (Fig. 1). The isoforms can be grouped into two classes: the tau-3R class members contain three microtubule binding repeats (MTBRs) while the tau-4R class members contain four MTBRs (Fig. 1). Tau is a cytosolic protein which is seen to be mainly expressed in the neurons of the central nervous system, and the six isoforms are equally expressed in a healthy adult brain [2–5]. Changes in the relative amounts of these isoforms have been shown to be linked with various tauopathies [2,3]. Different disease-linked mutations such as R5H, R5L, N279K, ΔK280, L266V, G272V have been shown to alter the relative amounts of tau isoforms, by influencing the alternative splicing of exon 10 [3,6].

#### 1.2. Structure and function of the tau protein

The longest isoform of tau (2N4R) contains 441 amino acid residues. It contains mainly polar and charged amino acid residues, and hence, it is highly soluble in water under physiological conditions. The N-terminal region (~120 residues) contains mainly acidic amino acid residues,

whereas the proline-rich domains, P1 and P2, contain basic amino acid residues. The four MTBRs are basic in nature, and each repeat is made of ~ 31 amino acid residues. The four MTBRs are similar, but not identical, in sequence.

*In-vitro*, purified recombinant human tau is functional and unstructured under physiological conditions as probed by circular dichroism (CD) [7], Fourier transform infrared (FTIR) spectroscopy [7], NMR [8], and small-angle X-ray scattering (SAXS) [9]. An analysis of its sequence has, however, suggested that tau may acquire local residual structures ( $\alpha$ -helices,  $\beta$ -sheets, polyproline-II helices) in various sequence segments [10]. A fluorescence resonance energy transfer (FRET) based study has suggested a 'paperclip' model for tau, wherein the N-terminal, C-terminal and repeat domains are folded in such a manner that these regions approach each other [11].

Many IDPs are known to gain structure after binding to other proteins [12]. Hence, it was hypothesized that tau also might fold or gain structure after binding to MTs, but various studies using cryo-electron microscopy (cryo-EM), NMR and single-molecule FRET have shown that tau remains predominantly unstructured after binding to MTs [13–18]. All MTBRs were found to interact with MTs, and each MTBR acquires an extended conformation [13]. Being an IDP, tau is likely to adopt multiple conformations under various conditions, and to show promiscuous binding [19], unlike a well-folded protein whose structure restricts binding to only one type of ligand.

Tau is known to stabilize MTs and promote their assembly, and hence, to regulate MT dynamics [1,20]. To understand the

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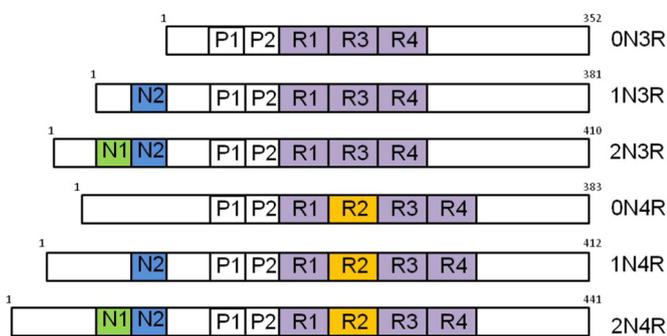
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**Fig. 1.** Different isoforms of tau present in the human brain. The MAPT gene which codes for human tau contains 16 exons. Alternative splicing of exons 2, 3 and 10 results in six isoforms. These isoforms have different numbers of N and R (MTBR) repeats, and are divided into two classes; tau-3R and tau-4R.

physiological role of tau, knock-out studies were carried out in mice. Surprisingly, no developmental defects were observed [21,22]. Hence, it was concluded that tau is not an essential gene. These studies did not, however, conclude that tau is a non-functional protein. It is possible that the expression of a similar other protein might compensate for the loss of tau function in tau knock-out mice [23]. Detailed studies on the tau knock-out mice concluded that tau might be involved in the formation of neuronal circuits [23–26]. Although not an essential protein, tau may have different functions under various conditions, which might help a cell to survive under stress.

### 1.3. Tau aggregation and its role in neurodegenerative diseases

Tau aggregates have been identified in various neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), progressive supranuclear palsy (PSP), Pick's disease, frontotemporal dementia with parkinsonism-17 (FTDP-17), corticobasal degeneration (CBD), argyrophilic grain disease, and chronic traumatic encephalopathy (CTE) [21,27]. Despite a strong association of tau aggregates with various diseases, it is not known whether tau aggregates cause the disease, or are an effect of the disease. In neurodegenerative diseases, other proteins such as  $\alpha$ -synuclein, A $\beta$ , and TDP-43 have also been shown to co-aggregate with tau [28–31]. It is not clear whether these proteins interact to induce the aggregation of each other, or whether they form aggregates independently. Tau and  $\alpha$ -synuclein have been shown to influence the aggregation of each other in a synergistic manner [32–34]. The interaction of tau and A $\beta$  appears to be essential for the *in vivo* formation of aggregates of tau and A $\beta$  [28–30]. A $\beta$  aggregates were found to induce neurotoxicity in mice when tau was expressed with A $\beta$ , whereas neurons were unaffected in tau knockout mice [28]. This study suggested that the interaction of tau with other proteins is essential for the development of the disease pathology. It is, however, not well understood how the interaction of tau with other proteins helps in the development of the pathology.

Tau aggregates isolated from diseased patients, were found to be hyper-phosphorylated [35,36] and acetylated [37]. It seems that phosphorylation and acetylation play a crucial role in determining the fate of tau. Hence, much effort has been put into identifying the role of phosphorylation and acetylation. It has been shown that depending on the site, these post-translation modifications (PTMs) can induce or inhibit the aggregation of tau, as well as its affinity for MTs [37–45]. It therefore becomes important to understand how these modifications alter the binding affinity of tau to MTs, and the aggregation of tau. Tau is positively charged at physiological pH, and phosphorylation reduces the net positive charge, which appears to induce the aggregation of the protein. *In-vitro*, tau remains soluble under physiological conditions, and negatively charged molecules such as glycosaminoglycans, fatty acids, detergents, and nucleic acids, act as inducers of tau aggregation

[46–49]. It is possible that in cells, the binding affinity of tau to MTs (to regulate MT dynamics) is modulated by altering the sites and extent of PTMs. More experiments need to be carried out to generalize the role of PTMs in neurodegenerative diseases.

Other than PTMs, various mutations in the MAPT gene have been shown to be linked to tau pathologies [3,6]. Mutations may be pathogenic because they suppress the function of tau, by reducing the binding affinity of tau for MTs, which can be detrimental to neurons. A few mutations (P301L,  $\Delta$ K280) which have been linked to various tauopathies, promote the aggregation of tau [3,6]. Many mutations influence the alternative splicing of tau pre-mRNA, which perturbs the ratio of tau-3R to tau-4R in neurons [3,6,50]. Changes in the ratio of the two isoforms of tau has been shown to result in pathogenicity [50–52]. Depending on the isoform of tau present in the aggregates, tauopathies have been divided into three categories. In AD, both isoforms of tau, tau-3R, and tau-4R were found in the aggregates [53]. In PSP, only tau-4R was found in the aggregates [54]. In Pick's disease, tau aggregates contained only tau-3R [53]. Aggregates formed by the two isoforms of tau could not cross-seed each other; a cross-seeding barrier appears to be present between tau-3R and tau-4R. This type of barrier appears to be similar to the species barrier observed in the case of the prion, which determines the infectivity of the prion protein [55,56]. *In-vitro*, an asymmetric cross-seeding barrier between the two isoforms of tau has been observed, wherein tau-3R fibrils can seed tau-4R monomers, but the *vice-versa* is not true [57,58]. One of the disease-linked mutations ( $\Delta$ K280) in tau has been shown to affect the cross-seeding barrier [59]. Fibrils of tau-4R having this mutation could seed tau-3R monomers. Thus, no cross-seeding barrier was observed for this mutant variant of tau [59].

### 1.4. Structure of tau aggregates and polymorphism

Proteins such as  $\alpha$ -synuclein, A $\beta$ , prion,  $\beta$ 2-microglobulin, huntingtin, and tau, form amyloid fibrils under various physico-chemical conditions [60–65]. In amyloid fibrils, the protein acquires a mainly ordered cross- $\beta$ -sheet structure in which monomers stack on top of each other and form intermolecular hydrogen bonds, and  $\beta$ -sheets are oriented perpendicular to the fibril axis. In the cross- $\beta$ -sheet structure, the backbone amide and carbonyl groups of the protein interact with each other to form intermolecular hydrogen bonds, which provide sufficient stability to the cross- $\beta$ -sheet structure [66,67]. Hence, irrespective of the amino acid sequence, the exposure of backbone amide groups appears to be essential for amyloid fibril formation. Amyloid fibrils are thermodynamically more stable than the native fold, due to the presence of additional intermolecular hydrogen bonding interactions [66–69]. Not surprisingly then, amyloid fibrils are found to be more resistant to heat, denaturants, extreme pH, pressure and proteases.

Aggregates formed by the tau protein have been shown to have characteristics of amyloid fibrils [60]. Tau fibrils extracted from diseased tissue have been found by electron microscopy (EM) to possess multiple morphologies, including paired helical filaments (PHFs) and straight filaments (SFs) [21,70]. PHFs are twisted double helical ribbons with heights varying between 8 and 20 nm, and with a half periodicity of about 80 nm, whereas SFs have a height of about 15 nm throughout the filament [21,70]. These fibrils have parallel, in-register, cross- $\beta$ -sheets [68,71,72] in which R2, R3, and R4 form the structural core [71,73–76]. Studies utilizing the limited digestion of tau fibrils with proteases in conjunction with SDS-PAGE, have identified the critical peptide stretches that are essential for fibril formation [77]. Two peptide segments, VQIINK (PHF6\*) and VQIVYK (PHF6) (present in R2 and R3, respectively) were found to be part of the structural core of tau aggregates, and appear to be essential for the formation of tau fibrils [78]. The I277P and I308P mutations in these peptide segments completely inhibit the aggregation of tau protein [79,80]. *In-vitro*, the aggregation of recombinant tau protein in the presence of an inducer under physiological pH leads to the formation of amyloid fibrils which

are morphologically similar to those formed *in vivo* [81,82]. Recently, it was found that heparin-induced tau fibrils are polymorphic in nature, and different from those formed in Alzheimer's and Pick's diseases [83,84].

A high-resolution structure of the filaments formed by the peptide VQIVYK, has been solved. It showed that the peptides form a parallel, in-register, cross- $\beta$ -sheet [68]. The structure uncovered new sites for the design of inhibitors of tau aggregation [68,85]. Recently, cryo-EM has been used to solve the structures of the full-length tau filaments extracted from two different diseased patients, in which residues 306–378 form the structural core [86,87]. AD-linked filaments have mainly two types of morphologies: SFs and PHFs [86,88]. The two kinds of filaments have similar structural folds, but the interacting region between the two protofilaments is different for the straight filaments and for the PHFs [86]. Filaments extracted from Pick's disease patients, which contain only the tau-3R isoform, have a structural fold different from that of AD-linked filaments [87]. The demonstration that two different structural folds are associated with two different diseases, suggests that differences in structural folds determine the type of pathology, although it is not clear how this happens.

### 1.5. Prion-like self-propagation and the infectious nature of tau fibrils

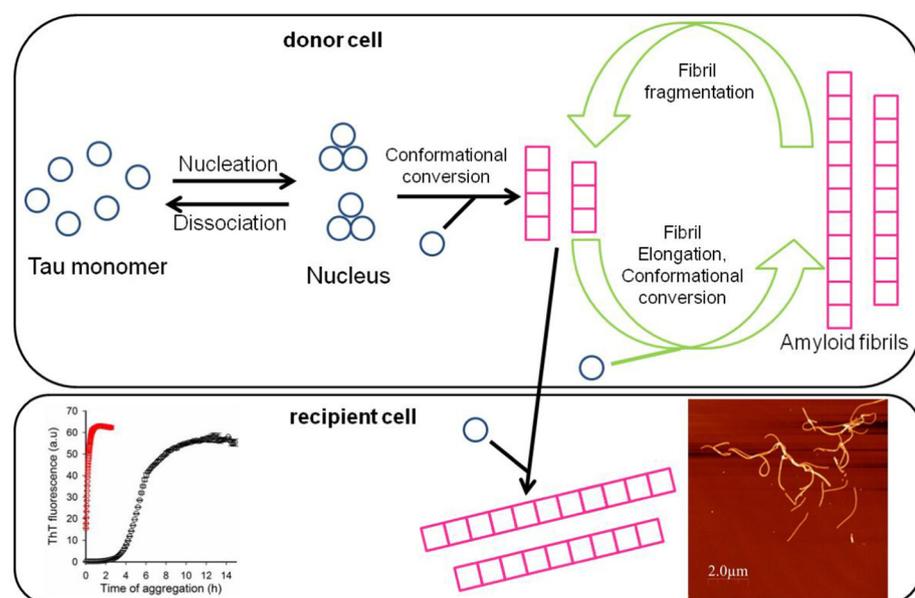
Prions are proteinaceous infectious particles made of the misfolded conformation of the prion protein, and possess a cross- $\beta$ -sheet structure [89,90]. These misfolded aggregates, which are similar to the amyloid fibrils formed by other proteins, can act like seeds, and can convert native protein into misfolded aggregates with similar structural and functional properties [90]. Faithful propagation of different misfolded conformations in such a template-dependent manner is a key feature of prion-like behavior. Prion-like propagation of aggregates occurs *via* several steps, including uptake of aggregates by cells, template-dependent elongation of aggregates by recruiting free monomer protein, fragmentation of aggregates, release of aggregates from the cell, and intercellular transfer of aggregates [91,92]. Propagation could be the unintended consequence of the amyloid fibrillary aggregates being extremely stable and not dissociating easily; cells can get rid of them only by secreting them to their outside. The secreted fibrils appear to be taken up by another cell, and induce the aggregation of soluble native protein in that cell, via a prion-like mechanism (Fig. 2) [93]. Prions are also well known for their ability to stably propagate their misfolded conformation from one organism to other and generate the same

pathology [90].

The ability of the prion protein to undergo template-driven conformation conversion, and the ability of aggregated prion protein to propagate from one cell to another, are shared by the amyloid fibrillar aggregates formed by several other proteins, including  $\alpha$ -synuclein [94], tau [91,95,96], A $\beta$  [97,98], and huntingtin protein [99,100]. These proteins too can acquire alternative self-propagating conformations [91,92]. It is the capability of the amyloid fibrils formed by these proteins to propagate across different cells, tissues, organs, individuals, and species, which makes them infectious particles like the prion. The faithful self-propagation of misfolded aggregates in various biological systems has been linked to multiple protein misfolding diseases [91,92]. The physical and chemical parameters which determine the extent of infectivity and propagation are not well understood quantitatively.

In different tauopathies, a large multitude of neurons are found to contain a variety of inclusions which are composed mainly of tau protein [91,92]. It is possible that these aggregates form autonomously in each of the neuronal cells, but this is unlikely given the stochastic nature of *de novo* aggregation. A more likely possibility is that the formation of inclusion initiates at a specific location in the brain and then propagates to other parts of the brain. The appearance of tau inclusions in different parts of the brain does not occur in a disorderly manner with no pattern. Instead, the manner in which the inclusions appear at various stages during the propagation of disease suggests that tau inclusions spread in a prion-like manner from one cell to adjacent cells [101]. In different tauopathies, inclusions of tau begin to spread from different regions of brains. In Pick's disease, they start spreading from the fronto-temporal cortex and limbic regions [102]; in AD, from the locus coeruleus and transentorhinal cortex [101,103]; and in CTE, from the corticle sulci [104]. The injection of human tau inclusions into the intracerebral region of a mice brain expressing human tau, was found to induce the formation of tau inclusions, which then spread into various distant regions of the brain [95], leading to neuronal loss [105]. The pathology was found to spread to regions of the brain that were synaptically connected [106–109].

Extracellular tau aggregates can be taken up by cells, and once taken up, these aggregates can induce or catalyze the aggregation of the host cytosolic tau monomer [110,111]. As reviewed by Scialo et al. [116], tau aggregates seem to be taken up by cells by multiple mechanisms including macropinocytosis/endocytosis, micropinocytosis, heparan sulfate proteoglycans, and tunneling nanotubes [112–116].



**Fig. 2.** A schematic representation of the cell to cell propagation of tau fibrils. Inside a cell, the formation of tau fibrils follows a NDP mechanism, in which formation of a trimeric nucleus is the rate-limiting step. This nucleus elongates by recruiting and converting the soluble monomer into amyloid fibrils. Fragmentation of tau fibrils generates new catalytic seeds which makes the process autocatalytic in nature. These fibril fragments can be secreted out, and taken up by a recipient cell, where they convert soluble functional tau into pathological tau fibrils. Such a mechanism for the propagation of tau fibrils across cells is similar to a prion-like mechanism. [Reproduced and modified from ref [58]. Copyright 2018 Elsevier].

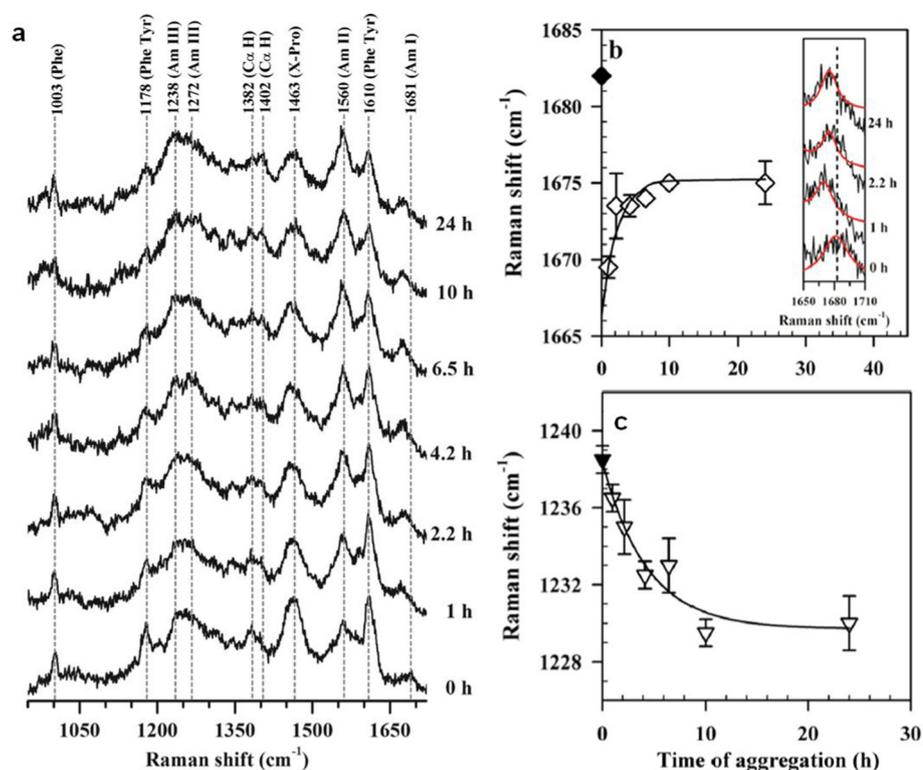


Fig. 3. UVRR monitored kinetics of tau fibril formation. (a) UVRR spectra acquired at different times of tau fibrillation at pH 7. Dashed lines through the spectra indicate important bands which correspond to the different secondary structures. Changes in the position of the (b) Am I band, and (c) Am III band as a function of aggregation time. In plots b and c, the 0 h data point is represented using a closed symbol and the continuous lines through the data points are lines drawn by inspection, intended to serve as guides for the eyes. [Reproduced and modified from ref [136]. Copyright 2014 American Chemical Society].

While the entry of tau monomer into cells was found to occur *via* a rapid dynamin- and actin-dependent macropinocytosis (endocytosis) mechanism, tau aggregates mainly take the dynamin-dependent route to enter the cell [114]. Monomeric tau could enter into cells as efficiently as aggregated tau [114]. Until recently, it appeared that only aggregated tau is able to induce the aggregation of monomeric tau [115]. Nevertheless, a recent study has suggested that a different conformation of monomeric tau can be taken up by cells, and that this monomeric conformation can then seed the aggregation of monomeric tau [117]. Other than endocytosis, a trans-synaptic pathway has also been proposed for the movement of tau between cells [118].

The secretion of tau from cells is crucial for spreading the pathology, but the mechanism by which aggregated tau is secreted is poorly defined. The secretion of tau aggregates by cells to the outside seems to be important for the trans-cellular movement of tau [111]. A recent study suggests that soluble tau can be secreted through direct translocation across the plasma membrane, and that this unconventionally secreted tau is able to spread trans-cellularly and induce the aggregation of tau in the host cell [119].

Structurally different types of fibrils have been observed to be formed by the same protein under the same conditions [120–123]. The populations of the different aggregates appear to be regulated by various factors such as PTMs [124], mutations [125], the presence of small molecules, and solution conditions [123]. The different conformational variants of the aggregates formed by the same protein, which possess different chemical and pathological properties, are known as strains. Depending on the physico-chemical conditions, a particular strain might get selected, and self-propagate from one cell to the another, and determine the type of pathology [92,126]. This type of behavior was first observed for the prion protein, for which each strain were associated with different prion diseases [90,127]. Tau aggregates are also known to propagate by a prion-like mechanism in which various tau strains can propagate from cell to cell, and region to region in the brain [91,92,96,110,111,128–131]. The tau aggregates may recruit free soluble monomer and convert them into fibrils (Fig. 2) [96,110,111,128–130]. These fibrils may further dissociate into small

fragments which can act as seeds and infect other cells, resulting in the propagation of the pathology throughout the entire brain [92,111,130,132].

Since tau is expressed intracellularly in a soluble form, the spread of tau aggregates in cells and tissues, requires the uptake of tau aggregates into a cell, seeding of the aggregation of intracellular tau, and the release of aggregates [92,96,111,131]. Tauopathies, which are associated with tau aggregates, are neurodegenerative disorders with diverse clinical features [21,96,133]. In different tauopathies, inclusions of tau were found to stain differently suggesting that they are present in diverse conformations [134]. Various conformations (different strains with different biochemical properties) of tau inclusions, which behave like prion strains, were identified when cells expressing tau were seeded with homogenates prepared from the brains of 29 patients suffering from five different tauopathies [96,128,129]. Tau inclusions purified from the brains of different patients with tauopathies, including AD, CBD, and PSP seemed to possess different structural folds [135]. The injection of pathological inclusions of tau isolated from different tauopathies, into the mouse brain, seeded the aggregation of tau with different efficiencies [135]. This study suggested that different tau strains might be responsible for different tauopathies [135]. In the case of PSP, a distinct tau strain with about 300-fold higher seeding efficiency than AD, CBD, and other PSP tau strains was identified suggesting that different strains of tau might present in different cases of PSP [135]. Nevertheless, a recent study identified the presence of structurally similar folds of tau filaments in multiple cases of sporadic and inherited AD [88]. Different conformations of tau aggregates appear to be responsible for different tauopathies. However, the link between different types of strains and various tauopathies is not very well understood. An understanding of the mechanism of conversion of soluble tau into structurally and functionally distinct types of aggregates in a template-dependent manner is important for understanding the causes of various tauopathies.

### 1.6. Mechanism of tau fibril formation and propagation

Formation of  $\beta$ -sheet fibrils from an intrinsically disordered tau monomer can be monitored by measuring the increase in  $\beta$ -sheet, or decrease in disordered structure as a function of time. Different types of aggregates populated during the tau fibril formation process have been identified using various structural probes such as NMR [8,73], atomic force microscopy (AFM) [49,136,137], X-ray diffraction [68], FTIR [49], cryo-EM [86,87], hydrogen-exchange mass spectrometry (HXMS) [58,76,138], electron paramagnetic resonance (EPR) spectroscopy [71], and ultraviolet resonance Raman (UVR) spectroscopy [136]. The kinetics of formation of tau aggregates has been monitored using various biophysical methods such as ThT fluorescence [49,132], light scattering [139], HX-MS [138], single molecule FRET [140–143], and UVR spectroscopy [136]. Fig. 3 shows the UVR spectroscopy-monitored conversion of intrinsically disordered tau monomer into  $\beta$ -sheet fibrils as a function of time [136]. It was observed that tau monomer converts into ordered fibrils in two stages. In the first stage, immature fibrils were formed from tau monomer (Fig. 3a and 3b). In the second stage, the immature fibrils converted into mature fibrils with a compact  $\beta$ -sheet core (Fig. 3a and c).

Tau fibril formation is describable as a nucleation-dependent polymerization (NDP) reaction [144]. In a NDP mechanism, the rate-limiting and critical stage is the formation of a nucleus, which is a transient, unstable conformation that may be monomeric or oligomeric. A NDP reaction has three defining characteristics: (1) the kinetics of fibril formation shows a lag phase followed by an elongation phase; (2) the lag phase is abolished by the addition of preformed fibrils, which act like a seed; and, (3) there is a critical monomer concentration below which fibril formation cannot take place. In the case of tau fibril formation, all three criteria are met [49,137,145]. In a NDP mechanism, nucleus formation takes place early on, and the nucleus elongates to form long fibrils upon the addition of monomer (Fig. 2) [49,144,145]. Due to the transient nature of the nucleus, there is a controversy about its size as well as its structure. The size of the tau nucleus was determined by analyzing the dependence of the lag phase on protein concentration, and the nucleus was found to be a trimer [132]. Surprisingly, a stable tau trimer has also been shown to be the minimal propagation unit which can act as a seed, when taken up by cells, can induce the aggregation of cytosolic tau monomer (Fig. 2) [146]. At the present time, it is not known how different the trimer seed and the transient trimer nucleus are in conformation. More recently, it has been reported that there exists a different monomeric conformation of tau, which when taken up by cells, appears to convert free soluble tau into amyloid fibrils [117]. Despite the debate on the size of the seed, the propagation of seeds is thought to spread the pathology across the brain. It is important to note that tau fibril derived from diseased brain have a higher seeding activity than fibrils formed by recombinant protein [115].

Tau fibrils have been shown to elongate by the addition of monomer and not oligomer to the fibril end [58]. The addition of monomer to fibril ends can be describable by three different mechanisms. In a conformation selection mechanism, amyloid fibrils/seeds select and bind to a compatible conformation of monomer from a pool of heterogeneous mixture of monomer conformations, which leads to elongation of the fibrils (Fig. 4a) [147]. It is possible that monomer as well as fibrils exist in different conformations, and depending on the conditions, compatible conformations of monomer and fibril select and bind to each other, leading to the growth of heterogeneous amyloid fibrils [147]. In an induced fit mechanism, monomer first binds to the fibril ends, and then conformation conversion takes place (Fig. 4b) [58,148–151]. In a mixed type mechanism, amyloid fibrils/seeds select and bind to the most compatible conformation of monomer, and then further conformational conversion of monomer into amyloid fibril happens. A detailed analysis of monomer concentration-dependent fibril formation kinetics has shown that the elongation of tau fibrils can

be described minimally as a two-step reaction (Induced fit mechanism) (Fig. 5) [58]. Intrinsically disordered tau monomer first binds to amyloid fibrils, and then converts to amyloid fibrils [58]. The transition of intrinsically disordered protein to  $\beta$ -sheet fibril appears to be similar to the protein folding reaction.

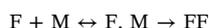
During and after elongation, tau fibrils may break into fragments, which further can act as seeds, and catalyze the process of fibril formation (Fig. 2) [132]. The formation of a new catalyst (seed) during the elongation process makes this process autocatalytic. As reviewed by Cohen et al. [152], a new catalyst can be formed by secondary pathways such as fibril fragmentation, surface catalyzed secondary nucleation and branching [132,152–155]. Analysis of monomer concentration-dependent kinetics of fibril formation of tau suggests that fibril fragmentation is the main secondary pathway, which operates during the fibril formation of tau [132].

Different PTMs and mutations in tau seem to influence the efficiency of an aggregate to act as a seed, as well as the efficiency of a monomer to be seeded. A quantitative description of the interaction between monomer and aggregate is expected to provide a better understanding of molecular parameters determining the pathological properties of aggregates.

### 1.7. The Michaelis-Menten-like mechanism for the growth of tau fibrils and its implication

In enzyme kinetics, the dependence of the initial rate of the reaction on substrate concentration is often describable by a Michaelis-Menten (MM) mechanism. The initial rate shows an apparently first order dependence at a lower concentration of substrate, and does not depend on substrate concentration at high substrate concentrations. When the initial rate of tau fibril elongation was determined over a range of monomer concentrations (Fig. 5a), a non-linear dependence of the initial rate of tau fibril elongation (in the presence of tau seed) on monomer concentration (Fig. 5b) was observed. The initial rate of fibril elongation showed a linear dependence on seed concentration (Fig. 5c and d) as observed for enzyme-catalyzed reactions. The results were suggestive of a MM-like mechanism, in which free monomer serves as the substrate, and the seed plays the role of the enzyme.

Thus, the mechanism is describable as:

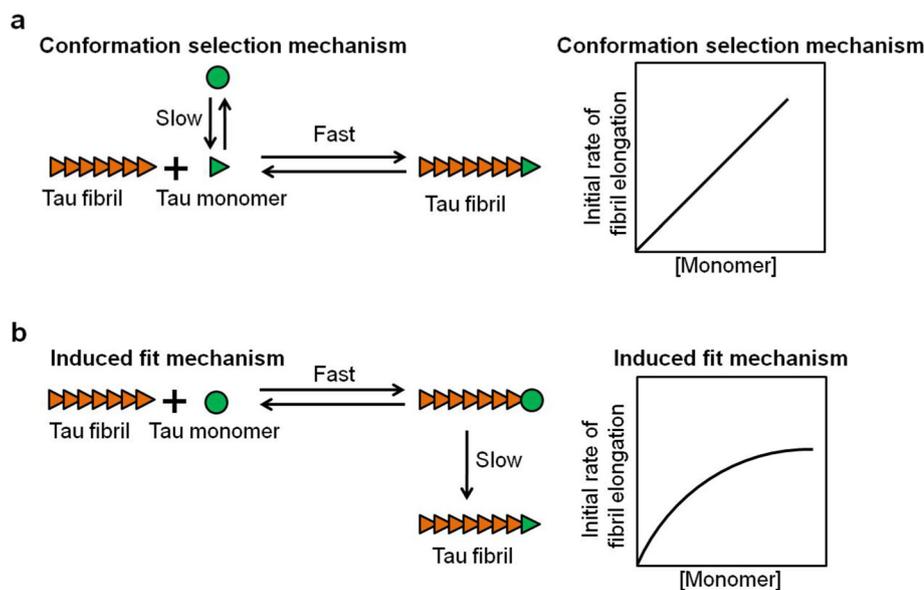


The MM-like model provides quantitative information about the binding affinity of the monomer for the fibril end, and about conformational conversion of the native monomer to an amyloid form. Such a simple quantitative approach is expected to help in understanding the mechanism of various disease-linked mutations, PTMs, cofactors, ligands, inducers, and inhibitors in modulating the pathological properties of tau fibrils.

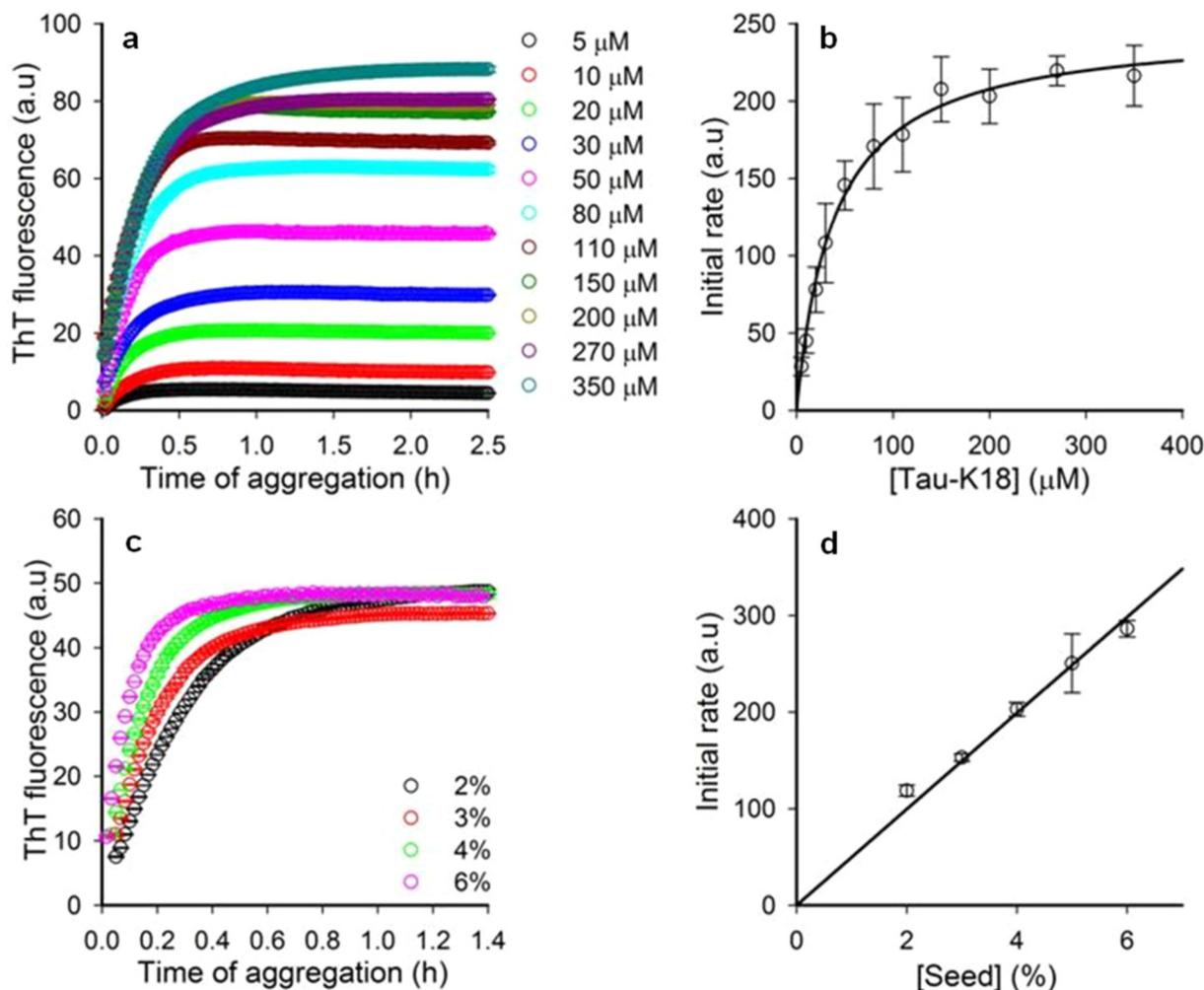
Modeling the tau fibril elongation reaction to a simple MM-like model has provided a mechanistic understanding for the asymmetric cross-seeding barrier which exists between the two isoforms of tau. It was not understood why tau-3R fibrils could seed tau-4R monomer, but the reverse was not possible. It was found that tau-4R fibrils had more catalytic efficiency than tau-3R fibrils [58]. However, tau-3R fibrils had a higher affinity for tau-3R and tau-4R monomer, than did tau-4R fibrils. The absence of R2 in the tau-3R monomer further reduced the affinity of tau-3R monomer for tau-4R fibrils. Structural characterization of tau-3R and tau-4R fibrils showed that two-third of R3 and one-third of R4 was structured in tau-3R fibrils, whereas this region remained mostly unstructured when R2 was present and formed the structural core in tau-4R fibrils [58]. Thus, the absence of R2 in the tau-3R monomer makes it incompatible for binding to tau-4R fibrils.

### 1.8. Strategies to halt the propagation of amyloid fibrils

The observation of the prion-like behavior of tau, has uncovered



**Fig. 4.** Mechanism of fibril growth by monomer addition. Two types of mechanisms can operate for fibril elongation by monomer addition. (a) In the conformation selection mechanism, tau fibrils select a compatible monomer from a pool of monomer conformations, and formation of this fibril compatible monomer is the slow and the rate-limiting step. Hence, the initial rate of fibril elongation shows a linear dependence on monomer concentration. (b) In the induced fit mechanism, binding of unfolded tau monomer to fibril is fast, and the rate-limiting step is the conformation conversion step in which unfolded tau monomer converts into  $\beta$ -sheet rich amyloid fibrils. Hence, at higher monomer concentration, the initial rate of fibril elongation does not change with an increase in monomer concentration, because the rate-limiting step is conformation conversion.



**Fig. 5.** Mechanism of tau fibrils elongation – (a) ThT fluorescence-monitored aggregation kinetics of tau at different monomer concentrations in the presence of 2% seed. (b) Initial rate of fibrillation (obtained by measuring the initial slopes of the kinetic curves shown in panel a) versus the concentration of monomeric tau. (c) Aggregation kinetics of tau at different seed concentrations. (d) Initial rate of fibrillation (determined from the kinetic curves shown in panel c) versus seed concentration. The continuous line through the data points in panel b is a best fit for hyperbolic model. [Reproduced and modified from ref [58]. Copyright 2018 Elsevier].

new steps and states which can be targeted to halt the propagation of tau fibrils. The following approaches can be used to halt the pathological process: (1) The first approach would be to block the binding of tau monomer to fibrils by modulating the properties of the tau monomer, using PTMs, such that a conformation of tau which cannot bind to tau fibrils is stabilized. (2) A second approach would be to shield the tau fibril ends/active sites with designed molecules. Hence, monomers would not be able to bind the fibril ends. (3) A third approach would be to stabilize tau fibrils so that they cannot break down into small fragments, so that the generation of new catalytic seeds, which could infect other cells and spread the pathology, is blocked. (4) A fourth approach would be to make use of the concept that the growth of amyloid fibrils follows a prion-like auto-catalytic mechanism. New amyloid strains can be generated and screened to find the strains with maximum catalytic efficiencies, but which are not toxic to the system. The large pool of free monomer would be consumed and converted into inert amyloid by such catalytically efficient strains. Hence, pathological fibrils would not be able to propagate at lower monomer concentration. In the future, similar approaches can be applied to other amyloid-forming proteins, and the effects of various physiological variables can be understood, which should help in the development of new inhibitors.

### 1.9. The role of liquid-liquid phase separation in tau aggregation

Many proteins such as FUS [156,157], TDP-43 [158–160], and hnRNPs [156,158,161–163], which contain low complexity domains (LCD) or prion domains, undergo the phenomenon of liquid-liquid phase separation (LLPS). LLPS leads to the formation of membrane-less compartments/organelles which serve multiple biological functions inside the cell [164–167]. Membrane-less compartments concentrate various cellular components at a specific location inside the cell, and can assemble and disassemble rapidly in response to a small signal [165].

Recently, it was found that tau, despite the absence of LCD and prion domains, undergoes reversible LLPS and forms condensed liquid droplets *in-vitro* as well as inside the cell [168–172]. Droplets of tau appear to recruit tubulin, which can lead to the regulated assembly of MT bundles [169,173]. A recent phase diagram for tau provides a detailed insight into the forces driving the process of tau LLPS [171]. Phosphorylation, as well as intracellular molecular crowding, facilitate the droplet formation of tau [168–170], whereas acetylation of tau appears to disfavor LLPS [174].

The LLPS of tau, like its aggregation, is facilitated by crowding agents, RNA, and heparin [168,170–172]. Similar to tau aggregation, the LLPS of tau is also regulated by PTMs, and disease-linked mutations [168,170]. These observations suggest that tau aggregation may be linked to its LLPS. As in the case of FUS and hnRNPs [157,158,175], whose droplets initiate aggregation, tau droplets too appear to initiate its aggregation. [168–170].

## 2. Conclusion

The formation and accumulation of tau aggregates in the brain are associated with a variety of neurodegenerative diseases. These misfolded tau aggregates can replicate and propagate in a prion-like manner, and spread the pathogenesis in the whole brain. An understanding of the prion-like propagation mechanism is essential for designing molecules which can halt the propagation of tau aggregates. Although two different structural folds of tau filaments have been associated with different diseases, the parameters that determine the type of pathology remain poorly understood. The current understanding of the propagation mechanism of tau fibrils may uncover new strategies for therapeutic treatment. Drugs that target and inhibit the binding of monomer to the fibril end, are expected to prevent the propagation of tau fibrils as well as disease. Finally, how different cellular conditions affect the LLPS of tau needs to be understood well, and the role played

by LLPS in initiating the pathological aggregation of tau remains to be delineated.

## Conflict of interest

The authors declare that they have no conflict of interest with the contents of this article.

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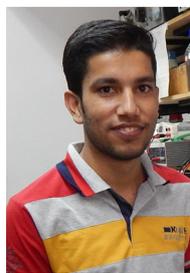
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