



# Regulation of tau by peptidyl-prolyl isomerases

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## Abstract

Tau is an intrinsically disordered protein found abundantly in axons, where it binds to microtubules. Since tau is a central player in the dynamic microtubule network, it is highly regulated by post-translational modifications. Abnormal hyperphosphorylation and aggregation of tau characterize a group of diseases called tauopathies. A specific protein family of cis/trans peptidyl-prolyl isomerases (PPIases) can interact with tau to regulate its aggregation and neuronal resilience. Structural interactions between tau and specific PPIases have been determined, establishing possible mechanisms for tau regulation and modification. While there have been numerous *in vivo* studies evaluating the impact of PPIase expression on tau biology/pathology, the direct roles of PPIases have yet to be fully characterized. Different PPIases correlate to either increased or decreased levels of tau-associated degeneration. Therefore, the ability of PPIases to structurally modify and regulate tau should be further investigated due to its potential therapeutic implications for Alzheimer's disease and other tauopathies.

## Addresses

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## Introduction

The microtubule-associated protein tau is intrinsically disordered and predominantly expressed in the neurons of the central nervous system. An important function of

tau is to regulate the dynamic instability of microtubules [1]. However, in disease, hyperphosphorylation and other post-translational modifications lead to the detachment of tau from microtubules, forming oligomers, condensates, and tangles that are key features of several neurodegenerative disorders referred to as tauopathies [2,3]. Neurodegeneration and cognitive decline in Alzheimer's disease (AD) and other tauopathies correlate with the progression of tau pathology [4,5].

The intrinsically disordered nature of tau is due to the low complexity of its amino acid sequence and the abundance of proline residues. The longest 441-residue isoform of tau comprises 43 proline residues in its sequence, among which 22 prolines cluster in the proline-rich domain (PRD) (Figure 1a). The proline-rich domain is conserved in all six isoforms of tau. The abundance of proline residues influences the ability of tau to undergo structural changes [6]. Peptidyl-prolyl cis/trans isomerases (PPIases) are a group of proteins that can control this process, thereby modulating the structural and functional diversity of tau. Different members of this group have been shown to bind to tau; some PPIases bind broadly across different tau domains, while others bind to specific sites on the tau sequence (Figure 1a and b).

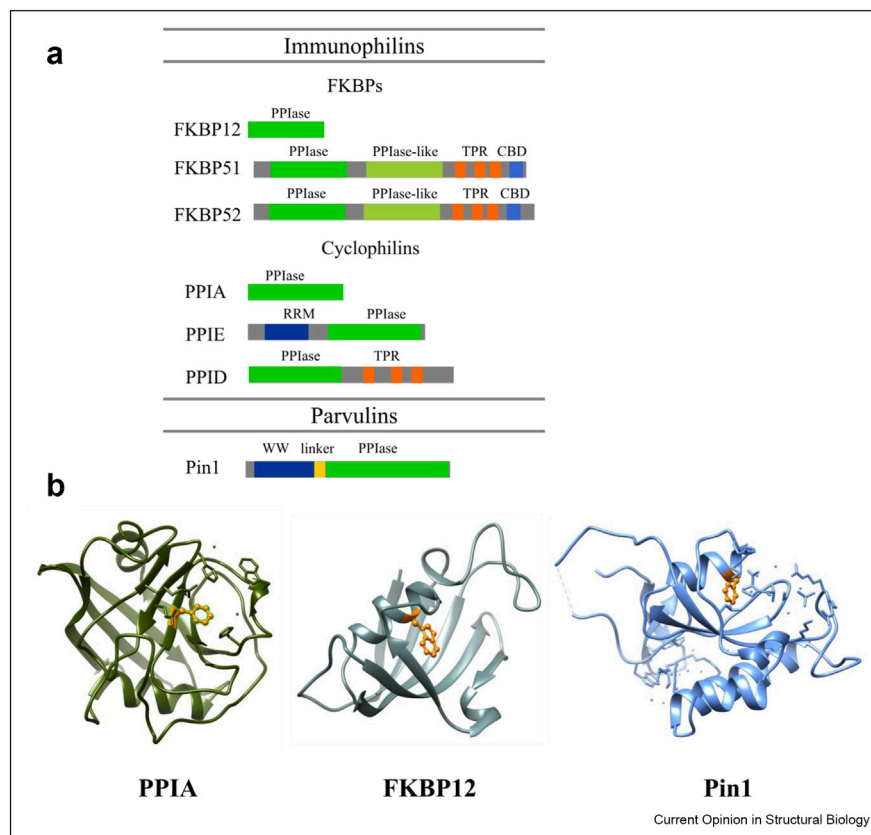
As molecular chaperones, PPIases uniquely catalyze the rotation of the peptide bonds prior to the proline residues from trans to cis conformation or vice versa [7]. The proteins containing the PPIase domain can be divided into two major classes: immunophilins (which are further divided into FK506-binding proteins (FKBPs) and cyclophilins) and parvulins (Figure 2a and Table 1). In this mini-review, we will discuss the current understanding of the regulation of tau by selected proteins containing PPIase domains. We will focus on the structural aspects of the tau-PPIase interactions and how these interactions may regulate tau-mediated neurotoxicity in tauopathies.

## Immunophilins

Immunophilins consist of two different groups of proteins having PPIase domains: FKBPs and cyclophilins (Figure 2a) [8]. Almost all immunophilins bind to specific immunosuppressive drugs, leading to the inhibition



Figure 2



**Structural organization of PPIases. (a)** Domain organization of the PPIases discussed in this review. **(b)** Three-dimensional structures from each of the three main PPIase groups: CypA, FKBP12, and Pin1. Protein Data Bank (PDB) accession codes are 3K0N, 1FKB, and 1PIN, respectively. Each of the PPIase domains features a solvent-exposed active site, formed between the  $\alpha$ -helix and  $\beta$ -sheets, and a key aromatic residue that binds to the target proline (highlighted in orange). CypA and FKBP12 only consist of a PPIase domain.

of calcineurin. FKBP12 binds to FK506 and rapamycin, while cyclophilins bind to cyclosporine [9,10]. FKBP12 holds a wide variety of functions, serving in the regulation of cellular trafficking, gene transcription, protein translation, and intracellular calcium release [11]. FKBP12 proteins also contain a basic domain and drug-binding pocket in addition to their PPIase catalytic domain [12]. For cyclophilins, the PPIase catalytic domain is more commonly referred to as the cyclophilin-like domain (Figure 2a), which comprises 109 amino acids. Members of the cyclophilin subfamily differ in their additional specialized domains [13].

Across all immunophilins, the PPIase catalytic domain forms a similar binding pocket between an  $\alpha$ -helix and antiparallel  $\beta$ -sheets (Figure 2b). An aromatic residue on the floor of this pocket positions the target proline, working together with a hydrogen-bonding network to stabilize client interactions (Figure 2b) [14]. Humans contain 18 FKBP12s and 24 cyclophilins in total [15]. In the following sections, we will be focusing on five immunophilins implicated in tau pathology: FKBP12, FKBP51, FKBP52, Peptidylprolyl

isomerase D (PPID), and Peptidylprolyl isomerase A (PPIA) (Table 1).

### FKBP12

FKBP12 is the smallest FKBP-associated protein that is predominantly expressed in neurites and neuronal cell bodies and colocalizes with tau in neurons and neurofibrillary tangles. It is the sole member of the FKBP family of proteins that is necessary for the immunosuppressant action of FK506 [16]. The expression of FKBP12 decreases in AD patients' brains, which is supported by recent genetic and proteomics studies with AD brains [17,18]. Also, in the AD brain, FKBP12 is redistributed to reactive astrocytes, dystrophic neurites, and neuropil threads [16]. The depletion of FKBP12 levels along with the cellular reorganization may result in the hyperphosphorylation of tau, leading to the formation of tangles.

In our recent work, we provided high-resolution insights into the tau-FKBP12 interaction. Using nuclear magnetic resonance (NMR) spectroscopy, we demonstrated that FKBP12 preferentially binds to two short

**Table 1**  
**PPases involved in tau biology and pathophysiology.**

Class	Group	Protein	Gene	Cell location (main)	Tau binding region	Effect on native tau	Effect on tau aggregation	In vivo behavior
Parvulin	Pin1	Pin1	Pin1	Nucleus [21]	Phosphorylated Thr-212 and Thr-231 [46]	Restores tau binding to microtubules [55]	Protects against aggregation by preventing hyperphosphorylation [48]	Restores tau binding to microtubules [55]
Immunophilin	FK506-Binding	FKBP52 FKBP4	FKBP4	Nucleus, mitochondrion [21]	Repeat domains	Prevents tau binding to tubulin [29]	Promotes P301L tau aggregation in heparin- seeded assays [56]	High levels promote neuronal loss in mouse models [30]
		FKBP51 FKBP5	FKBP5	Nucleus, extracellular [21]	Proline-rich and repeat domains [25]	Co- chaperones tau with Hsp90	Promotes aggregation by protecting neurotoxic tau [22]	Higher levels detected in diagnosed AD patients [22]
		FKBP12 FKBP1A	FKBP1A	Cytosol [21]	307-311 and 391–395 (xIVY motif) [19]	Colocalizes with tau in neurons and NFTs [17]	Delays aggregation in heparin- free assays [20]	FKBP12 is downregulated in AD brains [17]
	Cyclophilin	PPID	PPID	Nucleus [21]	Proline-rich containing region [35]	Higher CypD levels are induced by more tau [33]	Inhibits aggregation in heparin- seeded assays [35]	Dissolves tau aggregates in mice models [33]
		(CypD or Cyp40)						
		PP1A	PP1A (CypA)	Cytosol, nucleus [21]	N-terminus, repeat domains, C-terminus [39]		Protects against aggregation by reducing tau LLPS [39]	Recruited into phase-separated tau droplets [39]

sequences 307–311 and 391–395 in 4R tau (Figure 1b) [19]. These sequences form the unique motif xIVYK, while no similar sequence motifs are found elsewhere in tau. The xIVYK motif forms a common structural motif with distinct side-chain orientations. AlphaFold2 modeling revealed that this structural motif specifically binds to the hydrophobic pocket of FKBP12 (Figure 3) [19]. Notably, tau's binding to FKBP12 depends on its tyrosine residues, as phosphorylation of Y310 and Y394 inhibited FKBP12 binding. On the other hand, tau's binding to FKBP12 does not require phosphorylation, giving FKBP12 the potential to prevent tau aggregation before the initial tau hyperphosphorylation [19,20].

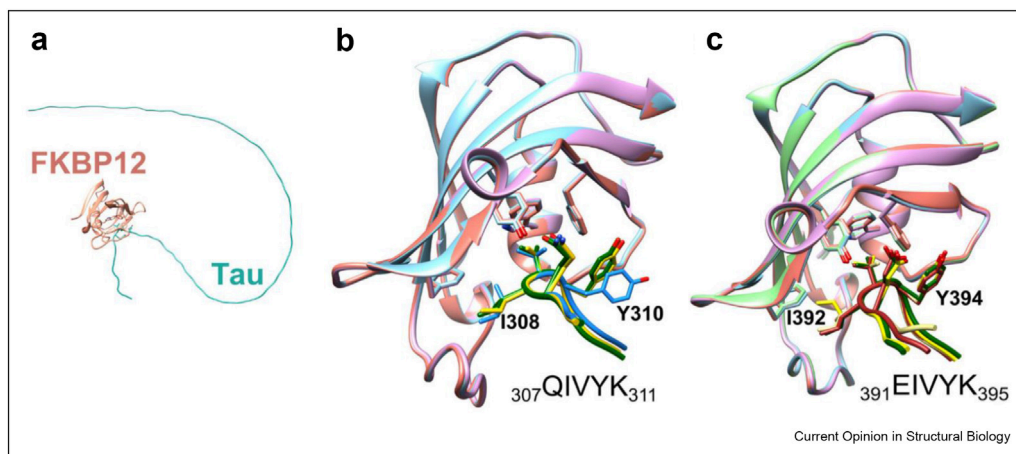
Mouse and cell experiments supported the in vitro binding studies. Mice models overexpressing human P301S tau indicated a correlation between tauopathy progression and decreased FKBP12 levels. The human co-culture AstTau system, created by the Wolozin laboratory to recapitulate neurons and astrocytes in tauopathies, demonstrated a reduction in tau pathology through increased FKBP12 levels [19]. Altogether, these findings support FKBP12 as a promising therapeutic target for tau-mediated neurodegeneration.

### FKBP51

FKBP51 is a 51 kDa molecular chaperone that is abundant in neurons [21]. The predominant function of FKBP51 is to regulate the activity of glucocorticoid receptors (GR), and thus it plays an important role in stress response [22]. FKBP51 comprises three domains: FK1, FK2, and a tetratricopeptide repeat (TPR) domain. The FK1 domain possesses PPIase activity, whereas the FK2 domain, which shares 34% sequence homology with the FK1 domain, bears an Adenosine Triphosphate (ATP)-binding site [23]. The TPR domain is made of several  $\alpha$ -helices, is the binding site of tubulin and different heat-shock proteins (HSPs), and is responsible for the chaperone function of FKBP51 [23]. Increasing evidence suggests that stress-related disorders or chronic stress enhance the probability of developing AD, thus suggesting a potential role for FKBP51 in AD [24].

FKBP51 may also play an important role in AD and other tauopathies by directly regulating tau biology. Characterization of the tau-FKBP51 interaction by NMR spectroscopy revealed that FKBP51 interacts mainly with the proline-rich domain and the repeat-domain of tau with micromolar affinity (Figure 1b and 4a) [25]. However, phosphorylation of tau significantly weakens the interaction with FKBP51, suggesting that the tau-FKBP51 interaction might vary at different stages of AD. Also, FKBP51 is a co-chaperone of Hsp90, and along with other co-chaperones, influences the Hsp90/co-chaperone-mediated maturation of client proteins including tau [26]. NMR spectroscopy revealed that the

Figure 3



**Tau binding to FKBP12.** (a) A schematic representation of FKBP12 (orange) interacting with the disordered tau protein (cyan). (b and c) Ensemble of complex structures predicted by AlphaFold2 for the interaction of FKBP12 with <sup>307</sup>QIVYK<sup>311</sup> (b) and <sup>391</sup>EIVYK<sup>395</sup> (c) of tau. The three (b) and four (c) most similar peptide conformations (from five calculated models) are shown. This entire figure is reprinted from *Chaperoning of specific tau structure by immunophilin FKBP12 regulates neuronal resilience to extracellular stress*, *Science Advances*, Vol. 9, No. 5, Jiang, Chakraborty *et al.*, 2023.

presence of FKBP51 in an Hsp90/FKBP51/tau ternary complex introduces an orientational bias and restricts the interaction of tau ensembles to the N-terminal domain of Hsp90 (Figure 4) [25]. The orientational bias arises due to the interaction of the proline-rich domain of tau with the catalytic pocket present in the FK1 domain of FKBP51 [25].

The overexpression of FKBP51 in HeLa cells has been shown to strongly increase the levels of tau. This may occur because FKBP51 overexpression could impair the ubiquitination of tau, leading to preservation of tau levels [8,27]. Indeed, low levels of tau were detected in FKBP51 knockout mice [28]. Also, it has been reported that FKBP51 impacts microtubule formation and function, and higher levels of FKBP51 have been detected in aged individuals diagnosed with AD [27,28]. In the presence of Hsp90, FKBP51 was reported to protect neurotoxic tau by preventing tau degradation and stimulating its oligomerization [21]. Therefore, the determined NMR interaction profiles between HSp90, FKBP51, and 2N4R tau [Figure 4] [25] may contribute towards a basis for future therapeutic targets.

#### FKBP52

FKBP52 shares around 70 % sequence homology with FKBP51, is present in both the cytoplasm and nucleus, and is detected in all regions of the brain and spinal cord [23]. FKBP52 is associated with the microtubule network in different cell types, and it has been shown that FKBP52 interacts with neuronal tubulin and prevents microtubule formation in vitro [23]. FKBP52 also directly interacts with tau, but the structural details of the tau-FKBP52 interaction are currently unknown [29].

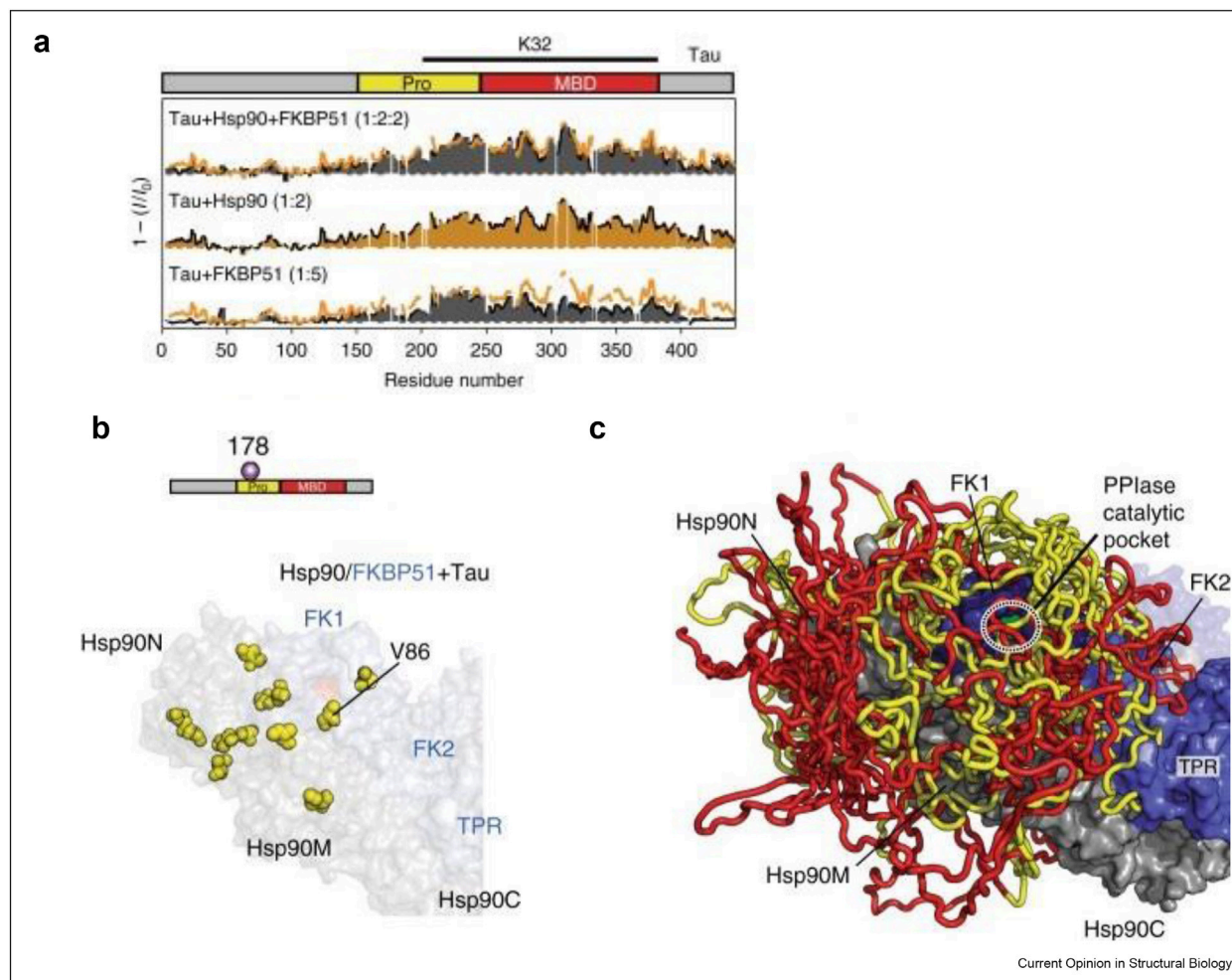
Despite this lack of structural data, FKBP52 has been shown to have relevant interactions with tau. A recent study reported that FKBP52 promotes the aggregation and oligomerization of P301L tau in vitro [30]. This in vitro aggregation assay of tau has been performed in the presence of heparin, which might influence the tau-FKBP52 interaction, suggesting the need for future experiments using a recently developed cofactor-free aggregation assay of tau [31].

The overexpression of FKBP52 in the hippocampus of tau transgenic mice (rTg4510) was shown to increase neurotoxicity, impair spatial learning and working memory, and accelerate tau-mediated reductions in the capability to induce and maintain long-term potentiation (LTP) [30,32]. The same study also reported that FKBP52 overexpression increased the levels of total tau but didn't affect the levels of phosphorylated tau and tau tangles in transgenic mice. Another recent study reported that high levels of FKBP52 in aged wild-type mice impair spatial reversal learning, trigger the accumulation of phosphorylated tau, and promote neuronal loss. Specifically, FKBP52 overexpression was correlated with high levels of AT8 and pS396 phospho-tau [32]. Perhaps gaining structural information between FKBP52 and tau would elucidate the mechanisms behind these findings.

#### Cyclophilins

Cyclophilins are named for their binding to cyclosporin A, thus suppressing the immune system through the deactivation of calcineurin [21]. The cyclophilin PPID (also named CyP40 or CypD) dissolves tau aggregates in vivo, as supported by its ability to lower silver-positive

Figure 4



Tau interaction with the FKBP51/Hsp90 complex. **(a)** 2D NMR interaction profiles of the 441-residue tau (2N4R tau) with Hsp90 (orange; tau/Hsp90 M ratio of 1:2), FKBP51 (bottom; tau/FKBP51 M ratio of 1:5), and the Hsp90/FKBP51 complex (top).  $I_0$  and  $I$  are intensities of H-N cross-peaks of tau in the absence and presence of the binding partner, respectively. **(b)** Isoleucine residues (yellow spheres) in Hsp90 experience paramagnetic broadening by tau tagged with S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate (MTSL) at position 178 in the Hsp90/FKBP51/tau complex. **(c)** Representative ensembles of tau conformations bound to the Hsp90/FKBP51 complex. This entire figure is reprinted from *Structure and protoxic mechanism of the human Hsp90/PPIase/Tau complex*, *Nature Communications*, Vol. 9, No. 4532, Oroz et al., 2018.

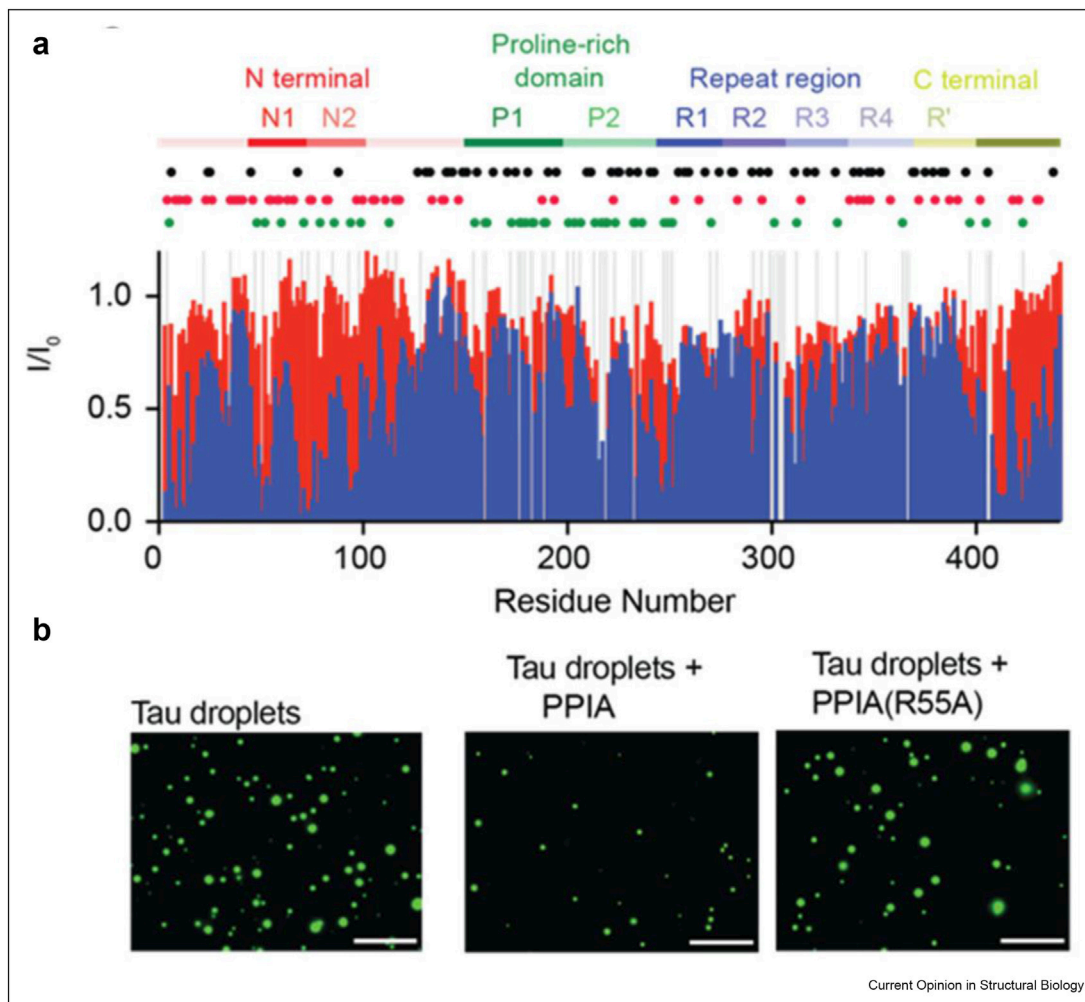
and oligomeric tau species in mouse models [33]. PPID isomerase activity is downregulated by Hsp90 binding. When cellular stress occurs, PPID is released from Hsp90, and its isomerase activity is increased [34]. PPID demonstrates tau disaggregation activity, independent from cofactors but dependent on PPIase activity. PPID selectively binds the proline-containing regions of tau, as shown by NMR spectroscopy. A proposed mechanism is that the PPID's PPIase domain binds to key residues within the amyloid substrate, followed by a conformational switch in the TPR domain [33]. However, the precise mechanism remains elusive, and further experiments are required. It is also worth noting that PPID's disaggregation abilities were tested with heparin-seeded tau fibrils.

Another study concerning PPID's ability to prevent tau aggregation indicated that PPID, along with other cyclophilins, competes for heparin *in vitro*. While PPID and PPIB are the strongest inhibitors of tau aggregation in noncellular experiments, this likely occurs through their competition with heparin binding. More promising candidates for reducing tau aggregation may be Peptidylprolyl isomerase C (PPIC), Peptidylprolyl isomerase E (PPIE), and Peptidylprolyl isomerase F (PPIF). These cyclophilins only moderately bind heparin while reducing soluble and insoluble tau inside the cell [35]. Future experiments are therefore required to investigate the ability of PPID to disaggregate patient-derived tau fibrils or to disaggregate *in vitro*-prepared tau fibrils that have the same structure as patient-derived fibrils.

The ability of cyclophilins to reduce pathogenic aggregation may lie in their modulation of liquid-liquid phase separation (LLPS). LLPS refers to the unmixing of a macromolecular solution into a condensed phase and a dilute phase. Growing evidence points to LLPS as a trigger for pathogenic aggregation [36], and phase-separated tau droplets have been characterized as intermediates towards tau aggregation [37]. The cyclophilin PPIA is the most abundant PPIase in cells [38] and has been shown to modulate tau LLPS [39]. Using 2D NMR spectroscopy, we characterized the possible binding of PPIA to tau's N-terminus, near N-terminal inserts N1/N2, and the C-terminal region (Figure 1a, Figure 5). Upon the addition of PPIA to preformed tau droplets, we found an enrichment of PPIA inside these droplets and a decrease in tau droplet numbers (Figure 5), suggesting

that the recruitment of PPIA triggers the dissolution of tau LLPS [39]. PPIA's ability to modulate tau LLPS likely depends in turn on its PPIase activity. R55 is crucial for PPIA's catalytic activity [40]. In our mutant PPIA (R55A), in which R55 was mutated to alanine, we found reduced PPIA/tau binding (Figure 5). Furthermore, we found that PPIA (R55A) led to a weak decrease in tau droplet numbers, in contrast to wild-type PPIA [39]. For further studies, it will be important to structurally determine and characterize other cyclophilins and tau, especially in their ability to modulate condensation kinetics in tau LLPS. It will be interesting to compare whether cyclophilin chaperoning of tau depends on enzymatic activity and determine if the interplay between enzymatic activity and tau LLPS may regulate tau aggregation and pathogenesis.

Figure 5



**PPIA modulates tau LLPS.** (a) NMR interaction profiles of wild-type PPIA and mutant PPIA (R55A) binding to  $^1\text{H}$ - $^{15}\text{N}$  labeled tau. Interaction is measured by intensity changes of 2D tau peaks upon addition of a 10-fold excess of PPIA (blue) and PPIA (R55A) (red).  $I$  and  $I_0$  are the intensities of tau Heteronuclear Single Quantum Coherence (HSQC) peaks in the presence and absence of PPIA, respectively. (b) Fluorescence images of Alexa488-labeled LLPS droplets for the tau control (left) and tau droplets with PPIA (middle) and PPIA (R55A) (right). Scale bar, 30  $\mu\text{m}$ . This entire figure is reprinted from *Peptidyl Prolyl Isomerase A Modulates the Liquid-Liquid Phase Separation of Proline-Rich IDPs*, *Journal of the American Chemical Society*, Vol. 144, No. 35, Babu *et al.*, 2022. NMR, nuclear magnetic resonance.

## Parvulins

Parvulins differ from other PPIase families through their N-terminal WW (Tryp-Tryp) domain (Figure 2a). This domain is responsible for client-binding, with a reported ten-fold higher affinity compared to its C-terminal PPIase domain for binding phosphorylated peptides *in vitro* [41]. Parvulin homologs are present in both prokaryotes and eukaryotes [42,43]. Eukaryotic parvulins can be divided according to substrate specificity: either phosphorylated Ser/Thr-Pro bonds or non-specific moieties.

Pin1 is the most characterized member of the first group, as it is the only PPIase that specifically recognizes phosphorylated Ser/Thr-Pro bonds [44]. The only other human parvulins, hPar14 and hPar17, belong to the second group, binding to substrates in a phosphorylation-independent manner and lacking the WW-domain [45]. As the cellular functions of hPar14 and hPar17 remain undefined, we will focus more on Pin1, an established player in cell proliferation and neuronal function [46].

## Pin1

Pin1 plays an important role in the inverse correlation between Alzheimer's disease and cancer. The overexpression of Pin1 is implicated in cancer, while the inhibition of Pin1 activity is associated with tau and A $\beta$ -related pathologies [47]. Pin1 is proposed to facilitate a cis ('unhealthy' tau) to trans ('healthy' tau) isomerization of tau, restoring tau's ability to bind to microtubules [48]. In the absence of Pin1 in mice (Pin1 KO), the stability of the wild-type tau protein was increased, while P301L tau protein stability decreased [49]. Pin1 KO mice also exhibited age-dependent neuropathy characterized by tau hyperphosphorylation and neuronal degeneration [41]. Interestingly, high Pin1 expression was associated with the surviving neurons in degenerative brains [41].

The binding of Pin1 to tau is most characterized at two tau phosphorylation sites, Thr-212 and Thr-231, with Pin1 binding to other possible tau phosphorylation sites being less studied. Pin1 also has a widely accepted binding model: the WW domain first binds to a specific pS/T-P motif, allowing the PPIase domain to subsequently bind to another pS/T-P motif on the substrate. Then, the PPIase displaces the binding of the WW domain, causing isomeric activity to occur [50]. Thus, based on this binding model, Pin1's substrate specificity is dictated by its WW domain.

Since the WW domain has a higher affinity for the motif at Thr212, when compared to the pThr231-Pro232 site on tau [50], Pin1 may overall prefer Thr212 over pThr231-Pro232. This challenges the proposed mechanism of how Pin1 "restores" tau's ability to bind to

microtubules. It is Thr231 phosphorylation, not Thr212, that correlates with the degree of tau oligomerization [51]. A previous study exposed this inconsistency, as their results actually refuted the previously established claims that Pin1 promotes phosphorylated-tau-induced microtubule formation. Through turbidity assays and small-angle X-ray scattering (SAXS), they demonstrated that phosphorylation of Thr231/Ser235 regulated microtubule formation, independent of Pin1 catalysis [52]. Their time-resolved SAXS data further indicated Pin1's inability to restore tubulin polymerization with phosphorylated tau [52]. These *in vitro* findings contrast with the *in vivo* studies demonstrating Pin1 expression's positive role in neuron health [41,49]. Thus, further studies determining the structure of the tau-Pin1 complex would be crucial, as pinning down the specific binding behavior between Pin1 and tau would help corroborate Pin1's unclear role in tauopathy.

## Outlook

The proline-rich domains of tau hold significant potential for PPIase targeting. As PPIases catalyze the cis-trans isomerization of proline, they can serve as crucial regulatory switches in protein behavior and pathology. Therefore, it is important to consider how various members of the PPIase family differ in their abilities to manipulate tau biology. *In vivo* studies including cell and animal models, imply positive roles for Pin1, FKBP12, PPIA, PPIC, PPIE, and PPIF, as they may delay or prevent tau aggregation. On the other hand, FKBP52 and FKBP51 are associated with promoting tau aggregation, with high levels linked to neuronal loss and AD development.

Taken together PPIases seem to play a dual role to either protect against or promote tau-associated neurodegeneration. The explanation for these contrasting effects can likely be found in the varying structural interactions between the specific PPIase and tau (Figure 1a and 1b). To date, there have been several advancements in defining the specific binding of tau and PPIase structures. Our previous studies have established FKBP12's binding to a unique xIVYK motif in tau, and we have solved a pro-toxic complex structure for FKBP51/Hsp90/tau. Pin1 targets two tau phosphorylation sites (Thr-212 and Thr-231), with inconsistent claims on its preferred phosphorylation site and subsequent impact. Various cyclophilins have been shown to directly influence tau biology in mouse models and other *in vivo* assays, although detailed structural information remains to be revealed.

Available NMR data on PPIases FKBP12, FKBP51, and PPIA indicate varying tau binding behavior (Figure 1b). FKBP51 binds broadly across the proline-rich and repeat domains and is associated with promoting tau pathogenicity. On the other hand, PPIA and

FKBP12 bind specifically to the N-terminal region and small sequence-specific motifs, respectively, and are associated with protecting roles. Gaining structural information for other PPIases would be crucial for determining trends and differences across all tau-PPIase interactions.

Additionally, determining structural trends between PPIases and tau could provide insight into the disease progression for pathogenic tau. Proposed disease-relevant mechanisms include chaperoning healthy tau to prevent tau pathogenicity, restoring the function of already pathogenic tau, or inhibiting mechanisms that promote tau pathogenicity. In other words, PPIases have the potential to be important drug targets in tauopathies. Currently, designing drugs to selectively target PPIases has proven challenging due to their well-conserved and solvent-exposed active sites [53]. Yet in recent developments, FKBP inhibitors have improved in strength, as a study increased binding affinities up to 10-fold after adding a single water-displacing methyl group [54]. The creation of strong picomolar FKBP inhibitors now places a greater emphasis on increasing their selectivity. Overall, the determination of distinct PPIase-tau structures could prove fundamental for differentiating between various targets, enabling the design of selective and potent therapeutics.

## Author contributions

All authors contributed to the preparation of the review.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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