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Zinc Induces Temperature-Dependent Reversible Self-Assembly of Tau

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Abstract

Tau is an intrinsically disordered microtubule-associated protein that is implicated in several neurodegenerative disorders called tauopathies. In these diseases, Tau is found in the form of intracellular inclusions that consist of aggregated paired helical filaments (PHFs) in neurons. Given the importance of this irreversible PHF formation in neurodegenerative disease, Tau aggregation has been extensively studied. Several different factors, such as mutations or post translational modifications, have been shown to influence the formation of late-stage non-reversible Tau aggregates. It was recently shown that zinc ions accelerated heparin-induced oligomerization of Tau constructs. Indeed, in vitro studies of PHFs have usually been performed in the presence of additional co-factors, such as heparin, in order to accelerate their formation. Using turbidimetry, we investigated the impact of zinc ions on Tau in the absence of heparin and found that zinc is able to induce a temperature-dependent reversible oligomerization of Tau. The obtained oligomers were not amyloid-like and dissociated instantly following zinc chelation or a temperature decrease. Finally, a combination of isothermal titration calorimetry and dynamic light scattering experiments showed zinc binding to a high-affinity binding site and three low-affinity sites on Tau, accompanied by a change in Tau folding. Altogether, our findings stress the importance of zinc in Tau oligomerization. This newly identified Zn-induced oligomerization mechanism may be a part of a pathway different of and concurrent to Tau aggregation cascade leading to PHF formation.

Introduction

Microtubule (MT)-associated protein Tau is known to play a vital role in cytoskeleton regulation by binding tubulin and thus controlling essential MT functions, from the necessary dynamic instability in dividing cells to axonal stability in neurons [1,2]. Tau is also associated with a number of neurodegenerative diseases (NDs) including Alzheimer's disease (AD), Parkinson's disease (PD), Pick's disease, frontotemporal lobar degeneration, corticobasal degeneration and progressive supranuclear palsy, where it forms intracellular aggregates [3–6]. These aggregates would spread in the brain through prion-like propagation [3,7]. In the case of AD, Tau is believed to be the main factor underlying the development and progression of the pathology [8]. Different types of inclusions made of abnormal forms of Tau are found in different areas of the nervous system mostly in neuronal cells and, in some cases, in glial cells. The most common form of inclusions is called neurofibrillary tangles. They are found in AD, PD and in other tauopathies and consist of stacked paired helical filaments (PHFs) of hyper-phosphorylated Tau molecules [9]. In some diseases, Tau-mediated neuronal death can occur even in the absence of tangle formation [10], and it often aggregates with other teammates such as amyloid-β or α-synuclein [11,12].
Despite the extensive study of Tau aggregation over the last two decades, the exact causes of this process as well as its molecular mechanism are still not completely elucidated. PHF remains the most studied form of Tau aggregates. Among the endogenous factors that have been shown or suggested to favor Tau aggregation are post-transitional modifications (in particularly hyper-phosphorylation), mutations and the presence of zinc ions [13–16]. Interestingly, zinc ions were found to play a critical role in the development of several different NDs including AD and PD. Zinc can bind not only to a number of prone-to-aggregate proteins implicated in neurodegeneration (such as amyloid-β [17], FUS/TL [18], α-synuclein [19], TDP-43 [20,21], etc.) and favor their aggregation, but also induce aggregation of stable proteins [22].

The potential impact of zinc ions on Tau aggregation/oligomerization was first discovered in 2009 [23]. It was shown that low micromolar concentrations of zinc are able to accelerate the fibrillation of the 244- to 372-aa fragment of human Tau40 (hTau40), which consists of the four MTBR binding repeats R1, R2, R3 and R4 [23]. Similar results have been recently obtained on this same fragment lacking R3 repeat [24]. Nevertheless, both studies were carried out in the presence of heparin, which induces Tau fibrillation into thioflavin T (ThT)-positive PHFs. Recently, it was demonstrated that zinc not only accelerates aggregation of a pathological mutant ΔK280 of hTau40 induced by Congo red in vitro, but also significantly increases its toxicity in neuronal cells [25]. This study also demonstrated the importance of Cys-291 and Cys-322 residues both for zinc binding and Tau aggregation [23,25]. While using non-physiological specific inductors of Tau aggregation such as heparin or Congo red is the most common way to induce PHFs structure and test inhibitors, it might not be the most pertinent model to study PHF formation itself, especially in the early stages where the process has already been hypothesized to be reversible [26]. Also, it should be noted that in these studies the pathological aggregation in the presence of zinc were demonstrated for Tau mutants and fragments that could have higher propensity to aggregate in comparison with wild type human full-length Tau (hTau40).

Here, we have investigated aggregation of full-length human hTau40 isoform in the absence of heparin but in the presence of zinc ions under a wide range of temperature conditions using turbidimetry, isothermal titration calorimetry (ITC), dynamic light scattering (DLS) and transmission electron microscopy. We found that, contrary to its aggregation in the presence of heparin or with other inducers such as arachidonic acid or congo red [25,27,28], self-assembly of Tau is a reversible process that depends on temperature and is induced by zinc ions. Moreover, our findings point to an important role of low-affinity auxiliary zinc-binding sites in this process.

Results

Zinc ions induce Tau oligomerization

Turbidimetry was used to monitor the impact of zinc on Tau self-assembly in the absence of heparin at different temperatures. The absorbance (∆A 350 nm) of 30 μM Tau samples at different ZnCl₂ concentrations (from 0 to 240 μM) in 50 mM Tris buffer and 1 mM TCEP at pH 7.5 was measured over a wide range of temperatures from 15 to 95 °C (Fig. 1a). In the absence of zinc ions as well as in the presence of low ZnCl₂ concentrations (15 μM), Tau protein did not demonstrate any propensity to aggregate. Increasing zinc concentration, starting from a Zn/Tau molar ratio of 1, led to a pronounced rise in the turbidity upon sample heating, which could indicate the formation of large oligomers of Tau. We found that Tau protein started to form oligomers at lower temperatures when higher zinc concentrations were used. Indeed, a 2-fold excess zinc induced Tau...
assembly at near physiological temperatures, while in the presence of an 8-fold zinc excess, Tau was already partially assembled at 15 °C. In parallel, to investigate the reversibility of this observed assembly, Tau sample was heated to 37 °C and cooled down to 13 °C in the presence of 4-fold zinc excess (120 μM of ZnCl₂). Each time, absorbance increased upon heating and dropped after cooling demonstrating near 100% reversibility of the oligomerization (Fig. 1b).

**Thermodynamics of Tau–zinc interaction**

To verify if Tau oligomerization is directly linked to zinc binding, we investigated Tau–zinc interaction using ITC at different temperatures. In the turbidimetry experiments, at low temperatures, we observed Tau aggregation only at high zinc concentrations (above 120 μM), but at high temperatures, Tau aggregation started at an equimolar zinc concentration. ITC titration of 30 μM Tau solution by zinc at 10 and 45 °C did not reveal significant differences in thermodynamic parameters of Tau–zinc interaction (Fig. 2), indicating that zinc binds to Tau just as well at 10 °C than at 45 °C. In both cases, fitting the binding isothersms using a two-sets-of-sites model revealed the existence of one high-affinity site for zinc ($N = 1.0 \pm 0.1; K_a = 2.0 \pm 0.5 \times 10^6 \text{ M}^{-1}$) and three low-affinity sites ($N = 3.2 \pm 0.3; K_a = 5.9 \pm 1.7 \times 10^4 \text{ M}^{-1}$). The high-affinity site was enthalpy driven but entropy unfavorable ($\Delta H = -12.1 \pm 0.2 \text{ kcal M}^{-1}; \Delta S = -13.8 \text{ cal M}^{-1} \text{ K}^{-1}$) probably due to partial folding of initially unstructured Tau molecule. Meanwhile, the three low-affinity sites were both enthalpy and entropy favorable ($\Delta H = -1.8 \pm 0.3 \text{ kcal M}^{-1}; \Delta S = 15.4 \text{ cal M}^{-1} \text{ K}^{-1}$) pointing to the burying of hydrophobic surfaces upon interactions during Tau aggregation.

**Characterization of Tau oligomers**

To characterize the oligomers of Tau that form in the presence of zinc ions, we used DLS, transmission electron microscopy and ThT assay. DLS analysis of 30 μM Tau solution showed that, in the absence of zinc, at 10 °C, Tau is mostly represented in solution as a species with a median hydrodynamic diameter of 9.6 ± 1.4 nm by mass (Dv50; Table 1). Given that Tau is an intrinsically disordered protein, this size would correspond to the unfolded monomer [29]. Heating up to 40 °C did not reveal significant conformational changes of the monomeric state of Tau (Table 1), in agreement with our turbidimetry data.

**Table 1. Characteristics of Tau polymers in presence of different zinc concentrations by DLS**

<table>
<thead>
<tr>
<th>$T$, °C</th>
<th>[ZnCl₂], μM</th>
<th>Z-average, nm</th>
<th>Pdi</th>
<th>Dv50, nm</th>
<th>Di50, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
<td>14.3 ± 0.7</td>
<td>0.31 ± 0.02</td>
<td>9.6 ± 1.4</td>
<td>13.7 ± 1.1</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>20.0 ± 2.1</td>
<td>0.50 ± 0.07</td>
<td>12.2 ± 0.6</td>
<td>13.0 ± 0.5</td>
</tr>
<tr>
<td>40</td>
<td>30</td>
<td>55.1 ± 63.7</td>
<td>0.30 ± 0.13</td>
<td>10.5 ± 0.4</td>
<td>12.2 ± 0.2</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>193.3 ± 46.1</td>
<td>0.21 ± 0.02</td>
<td>11.4 ± 0.2</td>
<td>11.4 ± 0.2</td>
</tr>
<tr>
<td>40</td>
<td>90</td>
<td>3158.0 ± 1462.0</td>
<td>0.99 ± 0.02</td>
<td>8.8 ± 1.1</td>
<td>8.9 ± 1.2</td>
</tr>
<tr>
<td>40</td>
<td>120</td>
<td>99,000.0 ± 20,000.0</td>
<td>OOR</td>
<td>OOR</td>
<td>OOR</td>
</tr>
<tr>
<td>10φ</td>
<td>120</td>
<td>26.0 ± 9.6</td>
<td>0.27 ± 0.08</td>
<td>11.3 ± 2.5</td>
<td>13.2 ± 0.3</td>
</tr>
<tr>
<td>40φ</td>
<td>90φ</td>
<td>55.2 ± 30.2</td>
<td>0.41 ± 0.05</td>
<td>11.3 ± 0.3</td>
<td>12.2 ± 0.4</td>
</tr>
</tbody>
</table>

Z-average, mean intensity size (diameter) of sample; Pdi, polydispersity index; Dv50, volume-based diameter median; Di50, intensity-based diameter median in nm; OOR, out of range.

φ Sample cooled from 40 to 10 °C.

φ 2 mM EDTA added.
To investigate the impact of zinc ions on the oligomeric state of Tau, 30 μM of Tau was titrated with up to a 4-fold molar excess of zinc at 40 °C and analyzed by DLS (Fig. 3a–c). Within this range of zinc concentration, the main Tau species represented by mass had diameters ranging from 10 to 12 nm. Furthermore, below the molar ratio Zn/Tau of 2.3, the polydispersity index (Pdi) remained low, while Z-average (mean particle size) gradually increased (Fig. 3c; Table 1). Above this Zn/Tau ratio, Z-average started to increase together with the polydispersity indicating protein aggregation (Fig. 3c). Further increases in zinc concentration, up to excess of 4, led to a complete disappearance of low size.

Fig. 3. Size distribution by volume (a) and by intensity (b) of 30 μM tau in the absence of zinc (orange curve) and in the presence of 30 and 60 μM zinc (in red and burgundy respectively) at 40 °C. (c) Correlation coefficient versus time in μs for Tau 30 μM in the presence of 0, 10, 60 and 100 μM ZnCl₂ (shown in colors orange, red, burgundy and black, respectively). (d) Median volume-based diameter (DV50) in nm (blue), Pdi (orange) and Z-average mean intensity size in (black) for different zinc/Tau molar ratio, with Tau 30 μM. (e) and (f) Representative electron micrographs of negatively stained Tau aggregates at 37 °C in the presence and in the absence of zinc ions respectively. (g) Representative electron micrographs of negatively stained sample of Tau in the presence cooled down from 37 to 10 °C. (h) Fluorescence spectra of ThT at 60 μM in buffer (dotted curve), in the presence of zinc-induced Tau aggregates (black curve) and in the presence of heparin-induced Tau aggregates (blue line) used as positive control.
species (Fig. 3a, b) and the formation of oligomers too large to be properly monitored by DLS. Further investigation of formed structures by electron microscopy revealed the existence of large (~10 μm) aggregates (Fig. 3e), which are in a good agreement with our DLS experiment. Indeed, as shown in the correlograms (Fig. 3d), as the concentration of zinc increases, the decay time increases, which indicates an increase in Tau size. Characterization of Tau oligomerization using ThT assay (Fig. 3g) did not reveal the presence of amyloid-like structures.

When the temperature was then decreased from 40 to 10 °C, we observed the reappearance of low-size species with a Dv50 of 11.3 ± 2.5 nm in the presence of 120 μM zinc (Table 1), which indicates that the oligomerization induced by zinc at 40 °C is reversible. Moreover, chelation of zinc ions with high excess of EDTA (2 mM) at 40 °C also led to complete dissociation of aggregates, which returned to the same size distribution as it was before addition of zinc ions (Table 1). We have thus shown that reducing temperature can reverse the effect of zinc oligomerization of Tau and that removal of zinc at high temperatures can also reverse the oligomerization of Tau.

**Discussion**

Zinc, which plays an important role in many physiological processes, has been implicated in the molecular mechanisms of many NDs by favoring aggregation of proteins such as amyloid beta, FUS, TDP-43 [21,30]. Recent in vitro studies showed that zinc could also impact Tau aggregation into PHF, a hallmark of several tauopathies. Nevertheless, the conclusions from these studies were limited by the fact that the oligomerization of Tau into PHFs was induced by heparin. Here, for the first time, we demonstrated that the presence of the zinc ions combined with increased temperature is sufficient to induce a fast oligomerization of Tau, and that it is reversible. Indeed, we have found that in the presence of equimolar zinc concentration, Tau oligomerizes at 50 °C, while the increase of molar ratio of Zn/Tau to 2 decreases the temperature of oligomerization to 32 °C (Fig. 1), thus potentially impacting physiological function of Tau. Similar interplay between ions and temperature of polymerization has already been observed in other important physiological systems such as MgATP/tubulin wherein tubulin polymerization depends both on temperature and MgATP concentration [31].

Characterization of Zn/Tau binding by ITC at different temperatures allowed us to demonstrate that the thermodynamics of the interaction is the same whether Tau oligomerizes or not. Indeed, the binding isotherms registered at 10 °C (where oligomerization is only triggered by high zinc concentrations) and 45 °C (where oligomerization starts at the beginning of titration) are almost identical. At both temperatures, ITC demonstrates the existence of two types of sites, one site with a high affinity ($K_a = 2.0 \pm 0.5 \times 10^9$ M$^{-1}$) and three sites with a low affinity ($K_a = 5.9 \pm 1.7 \times 10^4$ M$^{-1}$), which could have different functions. DLS showed that upon saturation of the high-affinity site, the hydrodynamic diameter of Tau species dropped from 12.2 to 10.8 nm and remained stable at 10.5 ± 0.22 nm until a molar ratio Zn/Tau of 2.3 (Fig. 3b). This suggests a partial folding of Tau with an increase in homogeneity as shown by the Pdi. This is also in agreement with the unfavorable entropy of zinc binding found by ITC for this site. This first high-affinity site likely corresponds to the one localized in the R2–R3 Tau region in which zinc is chelated by two Cys and two His amino acids, as previously proposed [23,25]. Such binding would be accompanied by the folding of Tau in R2–R3 as a hairpin, which is in good agreement with our DLS data that showed a compaction of the molecule. The three low-affinity sites may be responsible for the oligomerization process. Indeed, at 40 °C, we start to detect oligomerization by DLS at Zn/Tau around 2, when zinc starts to bind to these low-affinity sites, as it can be seen from ITC titration curve (Fig. 2). Interestingly, the hydrodynamic size of Tau monomer species continues to decrease (down to 8.9 ± 1.2 nm) as zinc binds to auxiliary sites, pointing to a further Tau folding (Fig. 3c). This leads us to propose that zinc binding to both types of sites induces a structural change that may have a consequence on Tau physiological functions.

It should be stressed that, contrary to heparin-induced fibrillization of Tau in most PHF models, zinc-induced oligomerization of Tau is a reversible process with fast kinetics. Indeed, chelation of zinc ions using EDTA, or a decrease of temperature instantly led to dissociation of oligomers. Contrary to ThT-positive irreversible aggregates formed in the presence of heparin, oligomers induced by zinc ions were not only reversible but also ThT-negative which indicates that the oligomers in the absence of heparin formed are not amyloid like. Most of the previous studies of Tau self-association in vitro were carried out in the presence of agents such as heparin or arachidonic acid [27,28] and led to generation of irreversible structures. It has been hypothesized, and widely accepted, that irreversible Tau aggregation leading to PHFs found in neurons of patients affected with ND would be preceded by a stage of reversible Tau multimers formation [26]. In our study, for the first time, we were able to reproduce a reversible oligomerization of Tau proteins in the presence of Zn without any additional non-physiological additives. Thus, our experimental conditions could constitute a useful model to study Tau multimerization in vitro.

The reversibility of Tau structures observed in our experiments also allows us to hypothesize that...
oligomers could be induced in cells by zinc signals under physiological conditions and play a role in Tau regulation, modulating its numerous functions [1]. For example, zinc was found to be involved in regulation of Tau interaction with DNA [32]. More generally, zinc is abundantly present in the brain where it plays an important role in axonal and synaptic transmission [33,34]. It is highly concentrated in the synaptic vesicles of several types of neurons [35]. The majority of zinc ions in cell are bound to metalloproteins, which have metal binding affinities in the nM and even in pM range. The physiological concentration of free Zn$^{2+}$ in cells is considered to be between around 1 and 10 nM [36,37], which locally would be sufficient to bind to Tau and modulate its activity. Thus, the observed binding constants that we determined are consistent with the possible physiological role of Zn in Tau regulation.

Conclusion

In conclusion, we demonstrated for the first time that zinc can induce a reversible polymerization of Tau. This process could have a significant function in the brain and nervous system where zinc is known to be abundant. Our data, which demonstrate that zinc favors Tau self-assembly at near physiological temperatures, support the idea that zinc could be implicated both in the pathological aggregation of Tau and in tau regulation. As summarized in Fig. 4, while the right part depicts the irreversible cascade leading to the formation of neurofibrillary tangles, the left part depicts a pathway that would ultimately lead to the formation reversible granular aggregates of Tau, which could have a sequestration purpose. Since tau phosphorylation is known to have physiological and pathological consequences [38], impact of tau phosphorylation on this pathway will need to be addressed. We have also shown the existence of two types of sites (one main and three auxiliary ones) that gradually modulate Tau structure enabling zinc to be a subtle modulator of Tau activity. In order to understand how zinc ions can impact physiological Tau oligomerization, it will be important to identify the specific amino acids responsible for binding of those auxiliary zinc ions and the structure of such oligomers. Likewise, confirming that this reversible oligomerization of Tau is part of a concurrent pathway will have important consequences in ND research.

Fig. 4. Model. The right part of the scheme corresponds to the pathway leading to PHFs and neurofibrillary tangle formation. This pathway is globally irreversible and favored by post-translational modifications such as (hyper) phosphorylation of Tau and Zn$^{2+}$. The left part of the scheme summarizes our findings: in the presence of low Zn$^{2+}$ concentrations, Tau undergoes compaction; in the presence of high Zn$^{2+}$ concentrations, it leads to the formation of granular Tau oligomers. This pathway is fully reversible.
Materials and Methods

Protein purification

Human Tau (hTau40) was expressed in *Escherichia coli* and purified as described previously [39]. Tau could thus be directly resuspended before use in the appropriate buffer. Protein was passed through the Zeba Spin Desalting Columns (Thermo Scientific) to equilibration in Tris 50 mM and TCEP 1 mM (pH 7.5) buffer before being used for experiments. TCEP was used to maintain Tau in a reduced state. Its concentration was measured at 280 nm using extinction a coefficient of 7700 M\(^{-1}\) cm\(^{-1}\) [31].

ITC

Binding of zinc to Tau was analyzed by ITC using MicroCal iTC200 as described previously [40,41]. Experiments were performed at 10 and 45 °C and in 50 mM Tris buffer in the presence of 1 mM TCEP at pH 7.5. Tau concentration in the calorimetric cell was 30 μM, whereas the ZnCl\(_2\) concentration in the syringe was 600 μM. Tau was titrated by repeated injections of 2 μL aliquots of zinc solution. At the end of titration, the cell contained the mixture of zinc and Tau with the molar ratio equal to 4:1. The syringe was refilled with the same zinc solution without cell refilling, and the titration was continued until the final ratio of 8:1. Each resulting titration peak was integrated and plotted as a function of the Tau/tubulin molar ratio. The baseline was measured by injecting ZnCl\(_2\) into the protein-free buffer solution. Data were analyzed using Origin software and were fitted with a “two set of sites” model via a non-linear least squares minimization method and led to the determination of affinity constants (\(K_a\)) and enthalpy changes (\(\Delta H\)). Thermodynamic values are an average of at least three different experiments.

Turbidimetry

Tau polymerization was monitored by turbidimetry (A 350 nm) using a PerkinElmer spectrophotometer Lambda 800 (PerkinElmer) and a Prometheus NT. Plex (Nanotemper) instrument equipped with absorbance module with a heating rate of 1 K/min. Purified Tau (30 μM) was equilibrated in 50 mM Tris and 1 mM TCEP (pH 7.5) buffer in the absence or in the presence of zinc and incubated at 5 °C. Polymerization was initiated by temperature increase to 40 °C and reversed by cooling sample to 13 °C.

DLS

DLS experiments were carried out to determine the hydrodynamic diameter of Tau in the presence and absence of zinc using a Zetasizer Nano ZS (Malvern Instruments) with a scattering angle of 173°. Particles in solution are in constant random motion and the intensity of their scattered light fluctuates with time. To determine the hydrodynamic diameter (\(D_h\)), the provided software uses the Stokes–Einstein relation to obtain the intensity averaged size distribution from the raw correlation data. The correlogram displays much information about the sample. The time at which the correlation starts to decay is an indication of the mean size of the sample. Smaller samples fluctuate quicker than larger samples in solution. The steeper the exponential decay, the more monodisperse (single population) the sample, and the more the decay is extended the greater the sample polydispersity (several populations) which is indicated by the Pdi. To analyze Tau, DLS was performed at temperatures ranging from 10 to 40 °C. For each assay, three measurements were performed; each one consisting in 10–15 runs of 10 s. Tau was analyzed at 30 μM in 50 mM Tris and 1 mM TCEP (pH 7.5) following centrifugation for 15 min at 14,000 rpm at 6 °C. The overall thermostability and aggregation of Tau was tested in the absence and presence of 10–120 μM of ZnCl\(_2\). To determine the hydrodynamic diameter (\(D_h\)), the viscosity and refractive index values of the dispersion medium were used in the Stokes–Einstein equation. In our case, we used a viscosity of 1.3198 cP and a refractive index of 1.331 (at 10 °C). Results were displayed as volume and intensity size distributions including the overall mean size (Z-average) using the Zetasizer software 7.12 with a detailed high-resolution spectrum of 300 classes. All experiments were performed at least in triplicates.

Fluorescence ThT assay

ThT assay, which is a common marker of amyloid fibrils, was performed as described before [42] with some modifications. Briefly, samples of 30 μM Tau alone or Tau with 8-fold excess of ZnCl\(_2\) were mixed with ThT. Fluorescence spectra were acquired in 0.2 (excitation direction) × 1 cm (emission) cells (Hellma) thermostated at 37 °C ± 0.5 °C without pre-incubation. Excitation was at 440 nm and emission spectra were recorded from 460 to 520 nm with slit widths of 15/15 nm using a PerkinElmer LS 55 fluorescence spectrometer operating at a PM of 800 V.

Transmission electron microscopy

Four microliters of incubated Tau samples (30 μM) was placed on carbon-coated copper grids (300 mesh) for 1 min. After blotting, grids were washed with distilled water and blotted again, and negatively stained for 30 s with 2% (wt/vol) uranyl acetate as previously described [21]. The grids were then dried and observed with a JEOL 2200FS transmission electron microscope (Tokyo, Japan) operating at
200 kV. Images were recorded using a 4k x 4k slow-scan CCD camera (Gatan, Inc., Pleasanton, USA).

CRediT authorship contribution statement

Andrei Yu. Roman: Formal analysis. François Devred: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - review & editing. Deborah Byrne: Methodology, Writing - review & editing. Romain La Rocca: Formal analysis. Natalia N. Ninkina: Writing - review & editing. Vincent Peyrot: Conceptualization, Data curation, Formal analysis, Supervision, Writing - original draft, Writing - review & editing. Philipp O. Tsvetkov: Conceptualization, Data curation, Formal analysis, Supervision, Writing - review & editing.

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Consent for Publication: Not applicable.

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

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Authors Contributions: A.Y.R. purified the recombinant protein and performed all experiments; F.D. supervised the experiments and wrote the manuscript; D.B. performed DLS data analysis and wrote the manuscript; R.L.R. performed experiments; V.P. and N.N.N. supervised the research; P.O.T. designed the research, performed data analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Abbreviations used: MT, microtubule; ND, neurodegenerative diseases; AD, Alzheimer’s disease; PD, Parkinson’s disease; PHF, paired helical filaments; ThT, thioflavin T; ITC, isothermal titration calorimetry; DLS, dynamic light scattering.

References

Zinc-Induced Reversible Tau Self-assembly


