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Kevin Pounot, Clara Piersson, Andrew Goring, Frédéric Rosu, Valérie Gabelica, et al.. Mutations in tau protein promote aggregation by favoring extended conformations. 2023. hal-04255772

**HAL Id: hal-04255772**

**<https://hal.science/hal-04255772v1>**

Preprint submitted on 24 Oct 2023

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## Mutations in tau protein promote aggregation by favoring extended conformations

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### Keywords

Intrinsically disordered protein, SAXS, tau protein, amyloid, IDP conformation

### Abstract

Amyloid aggregation of the intrinsically disordered protein (IDP) tau is involved in several diseases, called tauopathies. Some tauopathies can be inherited due to mutations in the gene encoding tau, which might favor the formation of tau amyloid fibrils. This work aims at deciphering the mechanisms through which the diseases-associated single point mutations promote amyloid formation. We combined biochemical and biophysical characterization, notably small angle X-ray scattering (SAXS), to study six different FTDP-17 derived mutations. We found that the mutations promote aggregation to different degrees and can modulate tau conformational ensembles, intermolecular interactions and liquid-liquid phase separation propensity. In particular, we found a good correlation between the aggregation lag time of the mutants and their radius of gyration. We show that mutations disfavor intramolecular protein interactions, which in turn favor extended conformations and promote amyloid aggregation. This work proposes a new connection between the structural features of tau monomers and their propensity to aggregate, providing a novel assay to evaluate aggregation propensity of IDPs.

### 1. Introduction

Tau pathologies form a class of neurodegenerative diseases in which deleterious deposits enriched in a protein called tau are present in the brain. The tau protein accumulates in these deposits in the form of amyloid filaments, which are highly ordered protein aggregates in which each protein stacks in a cross-beta structure. Strikingly, recent structural work pointed toward a correlation between the conformation of tau within these amyloid aggregates and the associated pathology phenotype<sup>1</sup>.

Several disease-associated mutations have been identified in the tau proteins, in particular in frontotemporal dementia and parkinsonism (FTDP) linked to chromosome 17 (FTDP-17) (see review by Goedert and Jakes <sup>2</sup>). Most of the mutations are present in the repeat domains, i.e. in the region that both binds to microtubule and forms the core of amyloid filaments. Accordingly, mutations can exhibit a dual effect on the protein activity : they hinder microtubule binding <sup>3-5</sup>, and they promote amyloid assembly <sup>5-8</sup>. The latter findings result from studies conducted in different conditions – inducers, incubation, buffer – and are thus not amenable to a quantitative comparison across mutants. The lack of consistency makes the evaluation of the effect of a specific mutation difficult.

Tau is an intrinsically disordered protein (IDP), meaning that it does not possess a well-defined 3D structure, but rather coexists as many different conformations.

The conformational landscape of IDPs is highly complex leading to multiple aggregation pathways<sup>9</sup>. Therefore, linking structural properties with aggregation is not trivial. Using different aggregation enhancers (pH, cofactors), experimental studies showed that aggregation prone states of IDPs can be characterized by global collapse for protein prothymosin- $\alpha$  <sup>10</sup> and by local compaction and extension for  $\alpha$ -synuclein and tau <sup>11,12</sup>. In solution state, tau lacks any stable secondary structure elements and is mostly in random coil conformation <sup>13</sup>. This limits the applicable biophysical methods and has hindered the understanding of the relationship between tau conformations and tau aggregation propensity. The mutation P301L was first shown by NMR to have a small but significant effect on the local conformation with no increased beta-sheet propensity <sup>14</sup>. Early work suggested that mutations modify the conformation of the flanking regions of aggregation prone regions PHF6 (306-311) and PHF6\*(275-280) <sup>6</sup>. More details were provided by Chen *et al.* showing that unshielding the PH6 region can explain mutation-induced aggregation enhancement <sup>15</sup>. More generally, long-range intramolecular interactions seem to play an important role in aggregation modulation as shown by FRET and cross-linking mass spectrometry <sup>15-17</sup>. Yet, there is no quantitative work linking the modulation of aggregation propensity by mutations and their structural properties.

Here we studied the effect of six different disease-associated mutations on aggregation propensity and structural features of tau. We used a fragment of tau, referred to as tau187 (residues 255-441 of full length 2N4R, Fig. 1), onto which we engineered the following single point mutations: I260V, G272V, P301L, P301S, Q336R and V337M (Fig. 1). Tau187 contains most of the four repeat domains and the C terminal region, thus encompassing the known amyloid cores, while being more prone to aggregate as compared to full-length 2N4R. Using small-angle X-ray scattering (SAXS), native mass spectrometry (MS) and biochemical characterization methods, we show that a single point mutation modulates tau monomer conformation, intra- and inter-protein interactions and aggregation propensity. We find a good correlation between aggregation lag time and the radius of gyration.

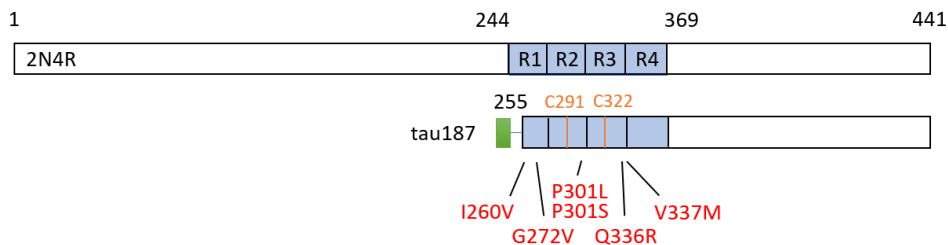


Figure 1: The longest human tau isoform 2N4R contains 441 amino acids. In this work we have used a fragment of 2N4R that starts at residue 255, referred to as tau187, onto which were added single point mutations. Each mutation (red) is located in one of the repeat domains R1 to R4. The green region indicates a poly-histidine tag.

## 2. Results

From the construct tau187, referred to as tau187 WT, we made six mutants that each contained a disease-associated single-point mutation: tau187-I260V, tau187-G272V, tau187-P301L, tau187-P301S, tau187-Q336R, tau187-V337M (Fig. 1).

### 2.1. Single point mutations have an important effect on aggregation kinetics

Amyloid aggregation for all mutants was assessed by Thioflavin T (ThT) fluorescence and transmission electron microscopy (TEM). All mutants were stable over 4 days at 37 °C under shaking (Fig. S1). The addition of a cofactor, the RNA homonucleotide polycytosine (polyC), was used to favor the formation of amyloid fibrils on the experimental timescale. Aggregates were observed by TEM for all mutants (Fig. S2).

We assessed the aggregation propensity by recording the ThT fluorescence as a function of time where the RNA was added at time  $t = 0$  h (Fig. 2A). All ThT curves were fitted with a sigmoid function (equation (2)) from which we extracted the aggregation half time (Fig. 2B). The fitting functions are plotted in figure S3. The single-point mutations show a drastic effect on aggregation kinetics. All mutations enabled aggregation to occur over 110 h, which was not seen in the WT version. The P301S and P301L mutations lead to the fastest aggregation. The same experiments were performed with other nucleotides (polyA and polyU) and lead to the same qualitative trend (Fig. S4A-D)

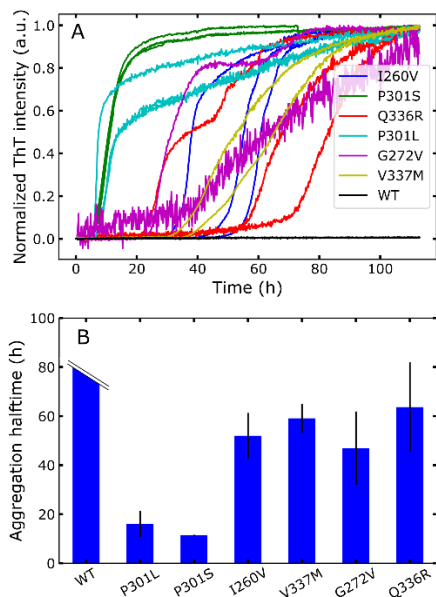


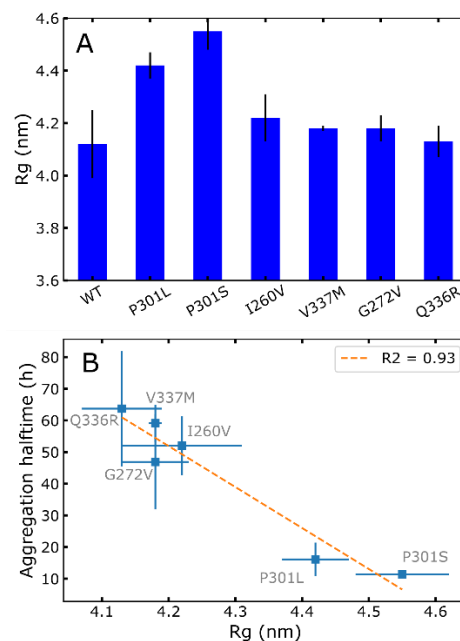
Figure 2: ThT fluorescence as a function of time for different mutants of tau187 incubated with RNA polyC (A). The curves are normalized between 0 and 1. Aggregation half time for each mutant (B) extracted from a fit of each ThT curve. Error bars represent the standard deviation over the half times obtained from the different replicates. Protein and RNA concentrations were 20  $\mu$ M and 200  $\mu$ M, respectively.

## 2.2. Tau expansion, due to mutations, correlates with aggregation propensity

We then characterized the structural properties of each mutant by small angle X-ray scattering (SAXS). We measured SAXS of all tau187 mutants and extracted their radius of gyration (Fig. 3A). Tau187-WT exhibits the smallest Rg of 4.12 +/- 0.13 nm and tau187-P301S presents the highest Rg of 4.55 +/- 0.07 nm. Other mutants exhibit intermediate Rg. These data show that a single-point mutation can significantly change the Rg of the protein, reflecting a change in the conformation ensemble of the different mutants.

In order to confirm that single point mutations can trigger global conformation rearrangement, we performed native electrospray ion mobility mass spectrometry on tau187-WT and tau187-P301S, which showed the highest difference in Rg from SAXS experiments (Fig. 3A). Proteins with intrinsically disordered regions typically show broad and multimodal charge state distributions, with the relative intensity of higher charge states reflecting the proportion of extended conformations<sup>18</sup>. Fig. S4 shows that the charge state distribution of tau187-P301S is slightly shifted towards the higher charge states. We confirmed by ion mobility spectrometry that, in addition, the collision cross section distribution of those higher charge states is shifted towards higher values. This primarily indicates more extended gas-phase conformations, but it has been shown that these reflect solution compactness (collision cross sections are correlated to Rg<sup>19</sup>), and thus indicate the presence of more extended conformations in the P301S mutants.

We further investigated whether the aggregation propensity could be linked to structural features of the different mutants. We plotted the aggregation half time as a function of the radius of gyration Rg (Fig. 3B). Strikingly, we found an excellent correlation ( $R^2=0.93$ ) for the two parameters, demonstrating that the more extended a mutant is, the more aggregation prone it is. The same trend was found when aggregation was triggered with other homonucleotides polyA or polyU (Fig. S5E-F). Because the Rg vs. aggregation half time plots are clustered, we performed additional statistical analysis and determine the minimum number of clusters that can be obtained from the data, using the elbow method (Fig. S6). It results that at least 3 to 4 clusters are necessary to properly explain the data, confirming the validity of the regression used in figure 3B.



*Figure 3: A single point mutation significantly changes tau conformational ensembles, as shown by different radius of gyration  $R_g$  (A). The  $R_g$  correlates with aggregation half time (B). Error bars on  $R_g$  originates from the covariance matrix generated by the fitting procedure. Tau187-WT does not aggregate on the observed timescale and thus has no aggregation half time. Hence it is not plotted here in panel B.*

### **2.3. Interactions between tau molecules are repulsive and modulated by single point mutation.**

Aggregation involves the assembly of many molecules together. Thus, we investigated the nature of interactions between tau molecules in solution. Light scattering from a particle solution intrinsically contains information on the interactions between the particles. Here, we extracted from SAXS experiments the second virial coefficient  $A_2$ , a parameter that reflects interparticle interactions<sup>20</sup>. The scattering intensities extrapolated at scattering angle  $q=0$  were plotted against protein concentration (Fig. S7) and fitted with equation 1 (see Materials & Methods). Positive values of  $A_2$  originate from repulsive interactions (the higher  $A_2$  is, the more repulsion exists) and negative  $A_2$  originate from attractive interactions (the more negative  $A_2$  is, the more attraction exists). Figure 4A presents  $A_2$  for each mutant.  $A_2$  is significantly different for all mutants, revealing that a single point mutation is sufficient to significantly modulate intermolecular protein interactions. In addition,  $A_2$  are positive for all mutants, except Q336R where it is close to 0, pointing to the fact that interactions are overall repulsive between tau molecules. This is in good agreement with the observation that recombinant tau is extremely stable in solution and did not aggregate in the absence of RNA (Fig. S1). Moreover, we evaluated if the interaction between tau monomers is linked to aggregation propensity triggered by RNA. We found no correlation between  $A_2$  and aggregation halftime (Fig. 4B), confirming that aggregation induced by RNA is not directly related to intermolecular protein interactions in solution.

Next, we evaluated the capacity to participate in liquid-liquid phase separation (LLPS), as another parameter reflecting inter-molecule interactions. LLPS is a physical process where protein molecules can spontaneously form a high concentration phase relying on a network of interaction between the different molecules<sup>21</sup>. Tau was previously shown to form LLPS under high salt conditions, where hydrophobic interactions are enhanced<sup>22</sup>. In a buffer containing 3 M NaCl, we measured the saturation concentration ( $C_{sat}$ ), which is the minimum protein concentration at which the formation of LLPS is observed (Fig. S8). Figure 4C shows the  $C_{sat}$  for all mutants. All mutants but I260V exhibited LLPS in the explored range of 0-40  $\mu$ M. We found that  $C_{sat}$  is modulated by the single-point mutations. Tau187-P301L, tau187-P301S and tau187-V337M are more prone than tau187-WT to form LLPS, suggesting that these mutations favor hydrophobic intermolecular protein interactions. This observation is consistent with MS data where the preferred formation of slightly higher charge states might result from a higher hydrophobicity in the P301S mutant. Indeed, according to the known electrospray ionization mechanisms, higher charge states require some degree of chain ejection from the parent droplets<sup>23,24</sup>, which can only occur if the protein can sit on the droplet surface, at the water/air interface, and thus if the protein exposes hydrophobic residues<sup>25</sup>.

In contrast, tau187-I260V (which did not form LLPS up to 40  $\mu$ M), tau187-G272V and tau187-Q336R are less prone than tau187-WT to form LLPS. Furthermore, we found a good correlation between the propensity to form LLPS, as shown by the saturation concentration, and  $R_g$  (Fig 4D). This correlation indicate that more extended conformations facilitate hydrophobic interactions, which are responsible for high-salt LLPS.

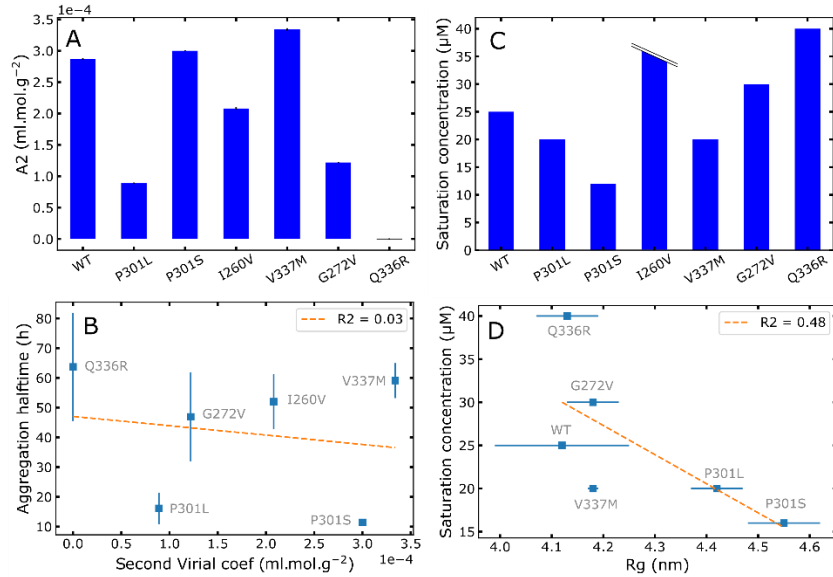


Figure 4: Inter-protein interactions modulated by mutations. (A) The second virial coefficient  $A_2$  shows overall repulsive interactions. Error bars are from the fitting procedure. (B) Aggregation half time is not linked to  $A_2$ . (C) LLPS saturation concentration  $C_{sat}$  for different mutants (I260V exceeded the tested maximum concentration of 40 μM).  $C_{sat}$  is defined as the lowest concentration giving a significant absorption over 3 independent replicates ( $p$ -value < 0.05; see methods) (D) LLPS saturation concentration correlates with  $R_g$ . For correlation plots (B) and (D), WT and I260V data points are not shown because their aggregation half time and saturation concentration, respectively, are not defined.

#### 2.4. Extended conformations originate from enhanced protein-water interactions

Then we further investigated the origins of the variation of tau conformations in the different mutants. To do so, we evaluated the contributions of intramolecular protein-protein interactions and protein-solvent interactions, we treated the SAXS data with a previously developed approach aiming at assessing the hydration quality of disordered proteins<sup>26,27</sup>. From polymer theory, the radius of gyration typically follows the relation  $R_g \sim N^\nu$ , where  $N$  is the length of the polymer and  $\nu$  the Flory exponent. Riback et al. used simulations on model polymers to obtain the relation between  $R_g$  and  $\nu$  for various solvent conditions<sup>26</sup>. The polymer molecular form factor and the Flory exponent  $\nu$  were extracted from the SAXS data using the web server made available by Riback et al. (Fig. S9). The  $\nu$  parameter reflects the protein solvation quality for disordered polymers: in the case of an ideal polymer,  $\nu$  is greater or smaller than 0.5 (theta-solvent condition) for favored or disfavored protein-solvent interactions, respectively. The self-avoiding polymer model would typically present a value of  $\nu=0.59$  for theta-solvent conditions. Recent works have unveiled the presence of localized secondary structure in disordered proteins that tends to increase the Flory coefficient<sup>28,29</sup>. Conversely, disordered proteins also have long-range interactions that tends to make the structure more compact and lower the Flory coefficient<sup>30,31</sup>. Hence, the notion of good and poor solvent should be taken with care with proteins and a comparison of relative differences between similar sequences in the same buffer is more robust than an interpretation of absolute values. The use of the simulations by Riback et al.<sup>26</sup> allows to avoid the pitfalls of making too many assumptions

on a polymer model and protein-solvent interaction strength. Figure 5A shows  $\nu$  for the different mutants. Note that tau187-WT data produced a poor-quality fit and therefore its Flory exponent was considered not reliable and was not analyzed (see Fig. S10). We observe in Fig. 5A that the quality of hydration is significantly modulated by the single point mutations. P301L/S show the highest  $\nu$ , which suggests from polymer theory that these mutations favor protein-solvent interactions over protein-protein interactions. The value of  $\nu$  is furthermore positively correlated with  $R_g$  (Fig. 5B). This correlation shows that weaker intramolecular protein interactions (i. e. higher Flory exponent) promote extended conformations, and in turn leads to increased aggregation propensity (Fig. 5C).

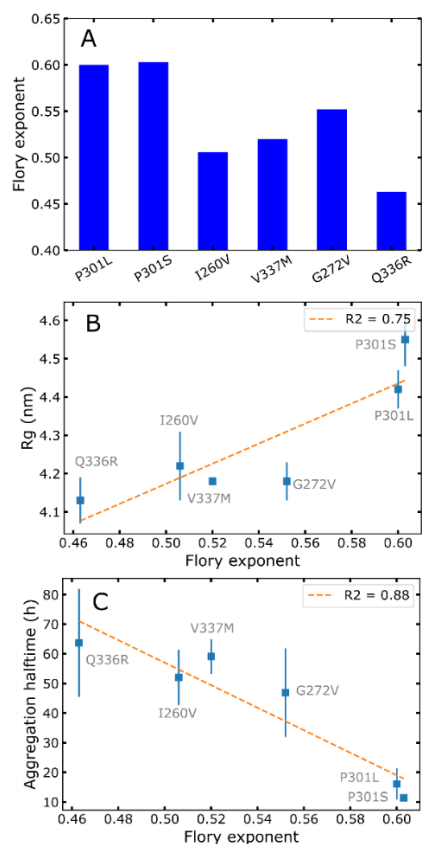


Figure 5: (A) Flory exponent  $\nu$  of tau187 mutants reveal that single point mutations modulate tau-solvent interactions. (B)  $R_g$  is positively correlated with  $\nu$  and (C) aggregation half time is negatively correlated with  $\nu$ .

### 3. Discussion

We analyzed the aggregation propensity and structural features of different disease-associated mutants of the tau187 protein fragment. We showed that single point mutations significantly change the aggregation propensity, as observed by different aggregation half time in the presence of RNA as an inducer. We further found that single point mutations change tau conformations as well as the interactions between monomers. Strikingly we found that the measured  $R_g$  correlates with aggregation half time.

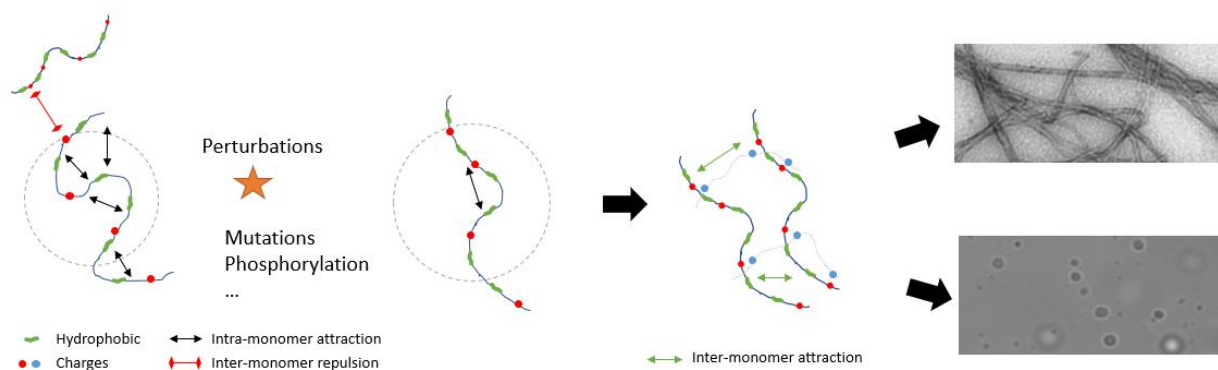
FTD-related mutations exhibit little similarities in terms of chemical modifications, some mutations add charges (Q336R) some other remove charges ( $\Delta$ K280), some change hydrophobicity (P301L) while some



are chemically similar (I260V). Thus the mechanisms of action must rely on subtle disruptions of local or global intramolecular interactions. Early studies suggested that structural effects of disease-associated mutations on tau monomers were small or nonexistent<sup>6,7,14</sup>. NMR showed subtle local structural changes that were hard to interpret in terms of conformational changes<sup>14</sup>. A study of the P301L mutation on tau fragments by Chen et al. provided a more comprehensive view of the structural effect of the mutation based on cross-linking mass spectrometry and simulations. They showed that the P301L mutation induces a local opening of the protein, thereby exposing a specific amyloidogenic region of tau. In agreement with this report, here we establish a more general and quantitative link between the differential aggregation propensities of tau mutants and their soluble conformation as probed by its  $R_g$ , (Fig. 6). This finding is in good agreement with the model where extended conformers are overall favorable for intermolecular contacts of the amyloidogenic region such as PHF6 and PHF6\*.

The analysis of the full SAXS curve using a molecular form factor for disordered polymer<sup>26</sup> (Fig. 5 and S6) allowed us to extract the Flory exponent, reflecting the quality of the protein solvation. We show that mutations modulate this parameter. Specifically, some mutants (most drastically P301L/S) exhibit enhanced protein-solvent interactions over intramolecular protein interactions. Although one might intuitively think that favoring solvation quality would stabilize the protein, here we highlight a different mechanism (Fig. 6). Rather, P301L/S mutations disrupt protective intramolecular interactions observed before<sup>15</sup>, thereby leading to increased radius of gyration and higher aggregation propensities. This mechanism is furthermore consistent with the observation that an increased  $R_g$  correlates with high-salt LLPS propensity (Fig 4D) which relies on hydrophobic interprotein contacts<sup>22</sup>. Notably, a similar mechanism has been highlighted for  $\alpha$ -synuclein,<sup>30</sup> another amyloid-forming IDP. More generally, this work demonstrates that tau aggregation propensity is encoded, at least partially, in the monomer structure, in agreement with reports on other IDPs such as  $\alpha$ -synuclein<sup>30</sup> and Huntingtin<sup>32</sup>.

Analysis of  $A_2$  showed that overall the tau monomers exhibit repulsive interactions (Fig. 4A), which was not expected for an amyloid-forming protein. This observation might explain why recombinant tau hardly aggregates *in vitro* and often requires the use of inducers such as heparin or RNA. The observation that the aggregation half time induced by RNA does not correlate with  $A_2$  shows that RNA completely rewrites this repulsion, likely by compensating the numerous charges present in the tau protein. One can speculate that cofactor-free aggregation seen for smaller fragments<sup>15,33</sup> would be more related to this  $A_2$  parameter.



*Figure 6: Aggregation model based on the presented data. In solution, the tau proteins exhibit intermolecular repulsions and does not readily aggregate. Upon modifications, such as mutations,*

*protective intramolecular interactions are released, leading to an increased radius of gyration. These extended conformations are more prone to intermolecular hydrophobic interactions, which in turn drive the formation of amyloid aggregates and LLPS.*

Aggregation cofactors induce specific pathways of fibrillization for tau<sup>34</sup>. RNA acts as a “mild” cofactor<sup>35</sup> so that the protein still needs to overcome a significant energy barrier to form amyloid, leading to a significant lag time (Fig. 2A). Herein we show that increasing the population of aggregation-prone conformers, characterized by lower intramolecular affinity and increased Rg, reduces this lag time and therefore the energy barrier to form ThT active species. The impact of mutations is not expected to be similar for other conditions or cofactors, such as heparin, that completely suppress this lag time. Indeed, we verified that there is no significant lag time for the different mutants incubated with heparin and that aggregation half time does not correlate with Rg (Fig S11). In this fast kinetics without lag phase, the aggregation half time is dominated by the growth rate, which we conjecture to be very dependent on the detailed properties of the tau-cofactor interactions. This view is in line with a recent report showing that the effect of mutations on aggregation is inducer dependent<sup>36</sup>.

A direct correlation between aggregation half time and Rg lays the ground for a rapid and convenient way to evaluate tau aggregation propensity. From SAXS measurements, one can obtain Rg, A2 and  $\nu$  of different tau variants, e.g. carrying different post-translation modifications or mutations, to obtain an idea of the aggregation propensity of the variant. This is particularly useful as many different combinations of modifications such as phosphorylation are possible and have been shown to have non-trivial effect on aggregation propensity<sup>37</sup>.

## **4. Material and methods**

### **4.1. Protein expression and purification**

Tau187, a truncated version of 2N4R (residues 255-441) was engineered with a poly-histidine tag at the N-terminal end. Mutants of tau187 were prepared using site-directed mutagenesis.

The expression and purification of tau187 variants have been previously reported<sup>38,39</sup>. Genes were transformed into E. Coli BL21(DE3) that grew at 37 °C, 200 rpm, with addition of 10 µg/mL kanamycin, until reaching optical density (600nm) of 0.6. Expression was induced by incubation with 1 mM isopropyl-β-D-thiogalactoside for 2–3 h. Cells were harvested with centrifugation at 5000 g for 20 min. Cell pellets were resuspended in lysis buffer (Tris-HCl, pH = 7.4, 100 mM NaCl, 0.5 mM DTT, 0.1 mM EDTA) added with 1 Pierce protease inhibitor tablet (Thermo Scientific, A32965), 1 mM PMSF, 2 mg/mL lysozyme, 20 µg/mL DNase and 10 mM MgCl<sub>2</sub> (10 mM), and incubated on ice for 30 min. Samples were then frozen and thawed for 3 times using liquid nitrogen, then centrifuged at 10,000 rpm for 10 min. 1 mM PMSF was added again and samples were heated at 65 °C for 12 min and cooled on ice for 20 min. Cooled samples were then centrifuged at 10,000 rpm for 10 min to remove the precipitant. The resulting supernatant was loaded onto a column pre-packed with 5ml Ni-NTA resins (cytivia HisTrap HP) using an Akta pure system. The column was washed with 25 mL of buffer A (20 mM sodium phosphate, pH = 7.0, 500 mM NaCl, 10 mM imidazole, 100 µM EDTA), 25 mL of buffer B (20 mM sodium phosphate, pH = 7.0, 1 M NaCl, 20 mM imidazole, 0.5 mM DTT, 100 µM EDTA) and 25 ml of buffer A. Protein was eluted with 0-100% gradient over 50 ml of buffer C (20 mM sodium phosphate, pH = 7.0, 0.5 mM DTT, 100 mM NaCl, 300 mM imidazole). Eluents were analyzed by SDS-PAGE to collect the pure fractions. Proteins were then buffer exchanged into working buffer of 20 mM HEPES, 100mM NaCl, pH 7.0.

## 4.2. SAXS experiments

The SAXS experiments were conducted on the BM29 beamline at the European synchrotron radiation facility (ESRF)<sup>40,41</sup>. Following two different procedures.

The first experiment was performed in batch, by loading 50  $\mu\text{L}$  of sample in the dedicated BM29 sample changer after centrifugation at 10,000 rpm for 10 minutes. For each sample measured, a series of 20 frames was acquired with an integration time of 1 s for each frame. The azimuthal integration of the images was done automatically from the beamline control software and the 1D scattering curves were subsequently used for data analysis. These datasets were used to extract the radius of gyration and the Flory exponent.

In a second experiment, a size-exclusion chromatography (SEC) S200 column of 3 mL volume was used to separate possible contaminants and aggregates from the monomers. The buffer used was 20mM ammonium acetate, 100mM NaCl and 5mM TCEP. The samples were spin downed at 10,000 rpm for 10 minutes before the injection on the column. The flow rate was set to 0.3 mL/min and the injected volume was 50  $\mu\text{L}$ . The images were acquired on a Pilatus 2M detector at a distance of 2.869 m from the sample. Each image is obtained from a 0.5 s exposure of the sample to the X-ray beam. These data were used to extract  $A_2$  parameter using the online UV-VIS spectrophotometer to extract protein concentration and directly obtain  $I(c,0)$ .

## 4.3. SAXS data treatment and analysis

The radius of gyration ( $R_g$ ) was obtained from the linearized batch SAXS data,  $\ln(I(q))$  vs.  $q^2$ , fitted using the following relation  $\ln(I(q^2)) = \ln(I_0) - q^2 R_g^2 / 3$ . To obtain a reliable value of the  $R_g$ , the fit is performed on multiple subdivisions of the fitting region. The subdivisions size ranges from 6 points to the full  $q$ -region used for the fit. The obtained  $q$ -values are then plotted on a frequency histogram and the final  $R_g$  is obtained by taking the weighted-average of the histogram. The quality of the  $R_g$  can be assessed by inspection of the histogram, where a proper linear Guinier region should give a narrow distribution of  $R_g$  around the mean value. The obtained  $R_g$  values were compared with values obtained from other software (ATSAS and Riback and Sosnick's web server<sup>26,27</sup>) and showed good agreement.

The Flory exponent was obtained by fitting the frames using a  $\chi^2$ -type distance from the average of all frames. All the frames that deviated from  $2\sigma$ ,  $\sigma$  being the standard deviation of  $\chi^2$  distances, were eliminated. The buffer subtraction was then performed and the subtracted data were used as input for the Riback and Sosnick's web server<sup>26,27</sup>.

The SEC-SAXS data contain the UV measurement as the function of time along with the X-ray scattering images. The frames that pertain to the UV peak were manually selected, as well as the frames that contain only the buffer. The buffer frames are chosen such that they are positioned in time just before the sample frames, which gives the best buffer subtraction (Fig. S12). The SEC-SAXS data were used to compute the second virial coefficient  $A_2$  according to the following:

$$I(0, c) = \frac{I(0)}{1 + 2M_w c A_2} \quad (1)$$

where  $I(0, c)$  is the extrapolated SAXS intensity at  $q = 0 \text{ \AA}^{-1}$  (obtained from a guinier fit at low  $q$ ) and at protein concentration  $c$ , and  $M_w = 20,570 \text{ Da}$  is the protein molecular weight. The concentration was

obtained from the UV signal recorded after the SEC by dividing the absorbance at 280 nm by the protein molar extinction coefficient  $\epsilon = 2,800 \text{ M}^{-1}\text{cm}^{-1}$ . The equation 1 was fitted to the data using the Python Scipy's *curve\_fit* routine.

Scripts for SAXS data analysis are deposited and available on Github (DOI 10.5281/zenodo.7893438).

#### 4.4. LLPS experiments

Different concentrations (0-40  $\mu\text{M}$ ) of Tau187 mutants were incubated with 3 M NaCl in a 384-well low-volume microplate. Total volume was 30  $\mu\text{l}$  in each well. Each condition was prepared independently in three different wells. Standard deviations over the three wells are presented as error bars. Absorbance at 500 nm was measured in a BMG fluoroStar Omega after 10min of incubation. LLPS was not detected in the range 0-12  $\mu\text{M}$  protein so the absorbances measured at 0, 4, 8 and 12  $\mu\text{M}$  protein were used to define an absorption baseline. A t-test was performed between the absorption of these 4 concentrations and each of the upper concentration. The lowest protein concentration giving  $p < 0.05$  was reported as the saturation concentration. Raw data are shown in Fig. S8 for each mutants.

#### 4.5. ThT experiments and data fitting

The tau protein was incubated at 20  $\mu\text{M}$  in 384-well low-volume microplate with 20  $\mu\text{M}$  ThT. The RNA polyC (Sigma P4903), polyA (Roche 10108626001) or polyU (Sigma P9528) were added at 200  $\mu\text{M}$ . Heparin (Sigma H6279) was added at a concentration of 5  $\mu\text{M}$  in supplementary experiments (Fig. S11). The working volume was 20  $\mu\text{l}$ . The fluorescence was bottom read in a BMG fluoroStar Omega with an excitation and emission wavelength of 440 and 480 nm, respectively. Each condition was prepared independently in three or two different wells.

ThT kinetic curves were first normalized between 0 and 1. They were then fitted using the *curve\_fit* python function with the following equation:

$$y = \frac{F}{1 + \exp(-k(t - t_{1/2}))} \quad (2)$$

Where F represent the final fluorescent intensity, k represents the growth rate and  $t_{1/2}$  represent the aggregation half time. Each replicate was fitted independently. The presented error bars on the aggregation halftimes represent the standard deviation over the output  $t_{1/2}$  obtained from the fit of each replicate. The fitting functions are shown in figure S3. Tau187-WT signal was normalized with the maximum intensity of tau187-P301L for visualization in Fig. 2A, since it did not increase.

#### 4.6. Electron microscopy

The carbon film 300 mesh copper grids are hydrophilised by UV light for 10 min. 4  $\mu\text{L}$  of samples were applied on the grid for 2 min before applying the staining solution. 4  $\mu\text{L}$  of 2% Uranyl Acetate filtered were applied on grid twice for 1 min. Samples were imaged by Philips CM 120 transmission electron microscope.

#### 4.7. Native electrospray ion mobility mass spectrometry

The proteins were buffer exchanged against 100 mM ammonium acetate (prepared with water from Biosolve (UPLC-MS grade)) using Zeba column (Thermo Scientific). The protein was then diluted to 7  $\mu\text{M}$  in 100 mM  $\text{NH}_4\text{OAc}$  for the mass spectrometry analysis. Experiments were performed on an Agilent 6560 DTIMS-Q-TOF instrument (Agilent Technologies, Santa Clara, CA), with the dual-ESI source operated in the positive ion mode. A syringe pump flow rate of 190  $\mu\text{L}/\text{h}$  was used. Capacitance diaphragm gauges are connected to the funnel vacuum chamber and to the drift tube. An in-house modification to the pumping system allows better equilibration of the pressures: an Edwards E2M40 vacuum pump (Edwards, UK) is

connected to the source region with two Edwards SP16K diaphragm valves connected to the front pumping lines, while an Edwards nXR40i vacuum pump is connected to the Q-TOF region. The helium pressure in the drift tube was  $3.89 \pm 0.01$  Torr, and the pressure in the trapping funnel was  $3.80 \pm 0.01$  Torr. The pressure differential between the drift tube and the trapping funnel ensures only helium is present in the drift tube. The acquisition software version was B.09.00. All spectra were recorded using soft source conditions. The tuning parameters of the instrument (electrospray source, trapping region and post-IMS region (QTOF region)) are optimized for softness as described elsewhere<sup>42</sup>. The source temperature was set at 220 °C and the source fragmentor voltage was set to 320 V. The trapping time was 1000  $\mu$ s and release time 200  $\mu$ s. Trap entrance grid delta was set to 2 V.

Step-field experiments (five drift tube voltages for each sample) were performed to determine the collision cross sections (CCS). The arrival time  $t_A$  is related to  $\Delta V$  (voltage difference between the entrance and the exit of the drift tube region) by:

$$t_A = \frac{L^2 T_0 p}{K_0 p_0 T} \cdot \left( \frac{1}{\Delta V} \right) + t_0$$

$t_0$  is the time spent outside the drift tube region and before detection. A graph of  $t_A$  vs.  $1/\Delta V$  provides  $K_0$  from the slope and  $t_0$  as the intercept. The drift tube length is  $L = 78.1 \pm 0.2$  cm, the temperature is measured accurately by a thermocouple ( $T = 297 \pm 1$  K), and the pressure is measured by a capacitance gauge ( $p = 3.89 \pm 0.01$  Torr). The CCS is determined using:

$$CCS = \frac{3ze}{16N_0} \cdot \sqrt{\frac{2\pi}{\mu k_B T}} \cdot \frac{1}{K_0}$$

The relative combined standard uncertainty on the CCS of the peak center is  $\sim 2.0\%$ <sup>43</sup>. The reconstruction of the experimental CCS distributions from the arrival time distributions at the lowest voltage is then performed using equation<sup>44</sup>:

$$CCS = a \cdot \frac{z}{\sqrt{\mu}} \times t_A$$

where the factor  $a$  is determined from the  $t_A$  of the peak center at the lowest voltage and the CCS calculated from the regression described above, from the peak centers.

## 5. Associated content

Supporting Information available. Supplementary figures S1 to S12 present additional data and complementary plots detailing the data treatment procedures. SAXS data acquired at the European Synchrotron Radiation Facility (ESRF) are stored under the DOI 10.15151/ESRF-ES-771376723. Scripts for SAXS data analysis are deposited and available on Github (DOI 10.5281/zenodo.7893438). All other data, data analysis scripts and figure-making scripts are available under 10.5281/zenodo.7986281.

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## 6. Acknowledgements

The author would like to thank Dr. Loquet to provide access to his lab to prepare sample and perform experiments. The authors would like to thank Dr. Riback for discussion about his approach and the

webserver. YF would like to thank the European Research Council (Grant 101040138) and the Federation of European Biochemical societies (FEBS) for their financial support.

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TOC graphic

