Effect of small molecule inhibitors on the aggregation mechanism of mutant Huntingtin Exon1

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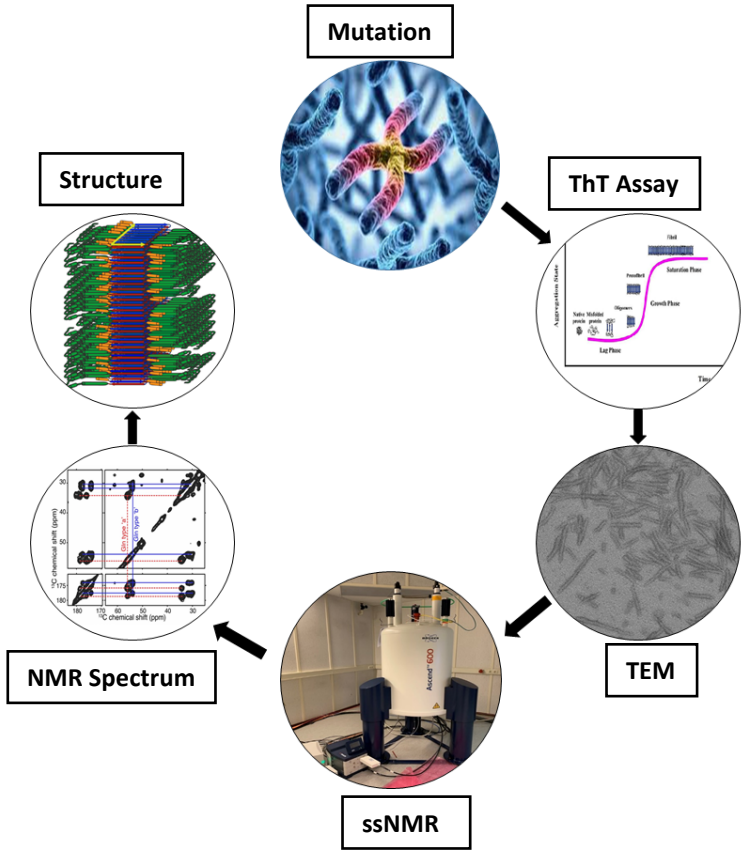
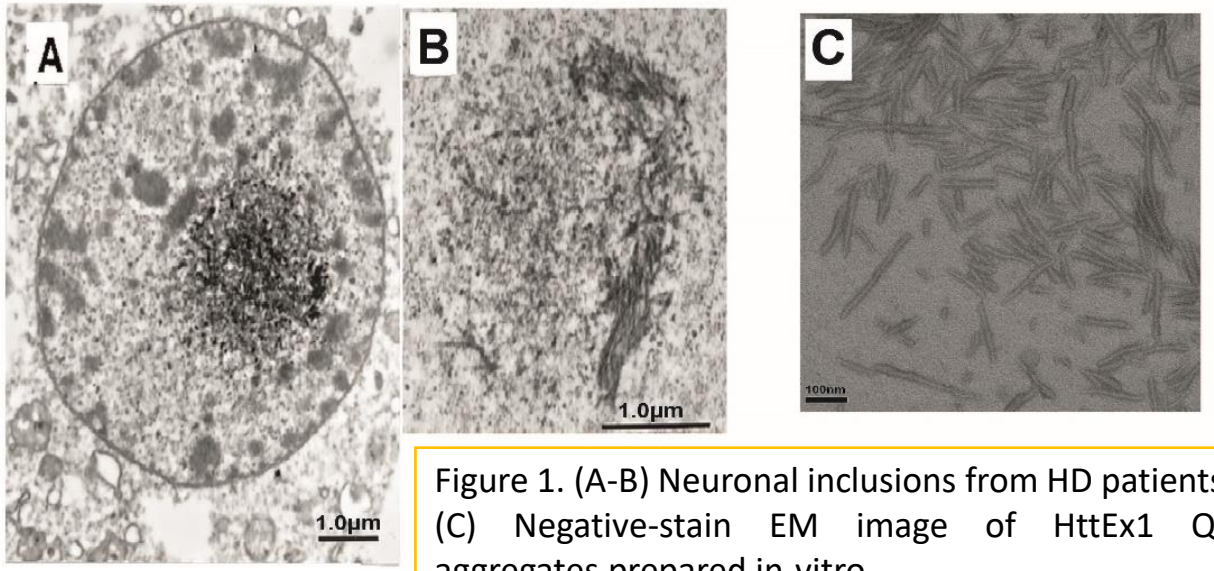
ABSTRACT

Huntington's Disease [HD] is a neurodegenerative disease caused by the expansion of the polyQ domain in the exon 1 region of the huntingtin protein. This expansion of the polyQ domain leads to protein misfolding and the formation of β-sheet-rich fibrillar aggregates. Several studies have indicated that these fibrils can cause cytotoxicity, whereas others argue that certain types of aggregates would be non-toxic. In the current study, we probe the conformational features of the fibrillar protein and examine how small molecule inhibitors can modulate the pathogenic aggregation process. We perform Thioflavin T (ThT) assays to study the aggregation kinetics of Huntingtin Exon 1 (HttEx1) along with high-resolution imaging technique like transmission electron microscopy to understand the fibril morphology. We used small molecule inhibitors to perturb the aggregation process, seeing effective inhibition of aggregation even at sub stoichiometric ratios of inhibitor relative to the mutant protein. The mechanisms by which the inhibitors modulate aggregation are dissected via kinetic analysis of the ThT aggregation curves, distinguishing, e.g., impacts on de-novo nucleation versus secondary nucleation processes. The mechanistic analysis is combined with measurements of aggregate cytotoxicity performed with cultured neuronal cells. These mechanistic and cytotoxicity studies are combined with morphological and structural studies of the HttEx1 fibril species, based on a combination of electron microscopy and solid-state nuclear magnetic resonance spectroscopy. This integrated multidisciplinary approach provides a novel perspective on the ability of small molecule inhibitors to modulate the misfolding landscape of mutant huntingtin exon 1, with potential implications for future treatment strategies.



Introduction



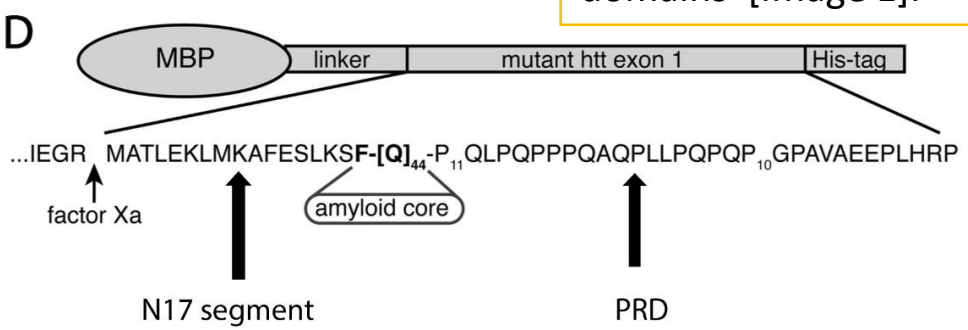
DiFiglia et al.
Science (1997) 277:1990-3

Figure 1. (A-B) Neuronal inclusions from HD patients. (C) Negative-stain EM image of HttEx1 Q44 aggregates prepared in-vitro

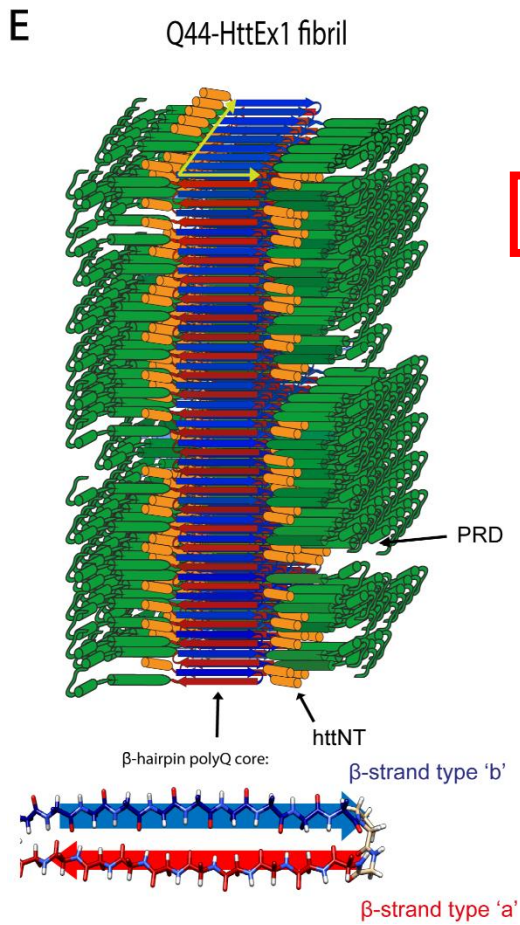
- HD is a rare autosomal dominant neuro-degenerative disease caused by the mutation in Htt gene leading to expansion of the polyQ domain in the exon1 region of the huntingtin protein.
- This mutation results in protein misfolding and formation of aggregates. These aggregates were also observed in the brains of the HD patients [Figure 1].

Huntingtin Exon1 construct

With help of ssNMR a model was proposed where the amyloid core consists of two types of β-strand: β-strand 'a' (red) and β-strand 'b' (blue), clustered with immoblized flanking domains [Image E].



Hoop et al. PNAS (2016) 113:1546- 1551



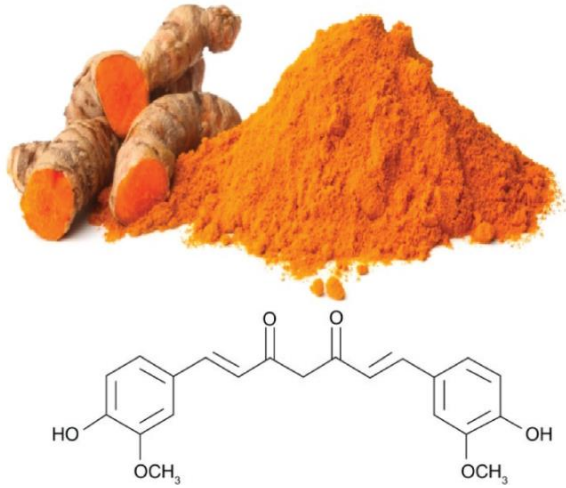
Why structure is important???

- To understand the aggregation mechanism and cytotoxicity of these fibrils
- To work towards the therapeutic approach to halter or delay or inhibit aggregate formation

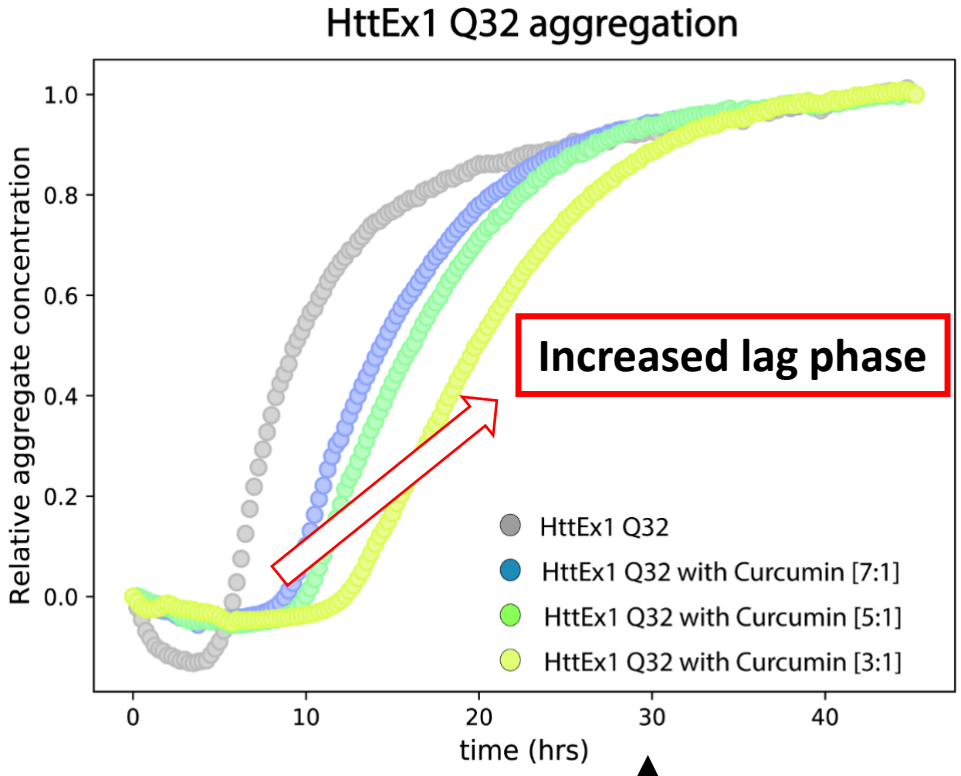
Boatz et al. JMB (2020) 432:4722- 4744

Effect of small molecule inhibitor on the aggregation mechanism

- We study the aggregation mechanism of HttEx1 by Thioflavin T assay.
- We used ''Curcumin'' as a small molecule inhibitor to perturb the aggregation mechanism of HttExon1. We observed effective delay in aggregation at sub stoichiometric ratios of curcumin.
- This aggregation mechanism study is combined with the cytotoxicity and morphological studies of HttEx1 fibrils.

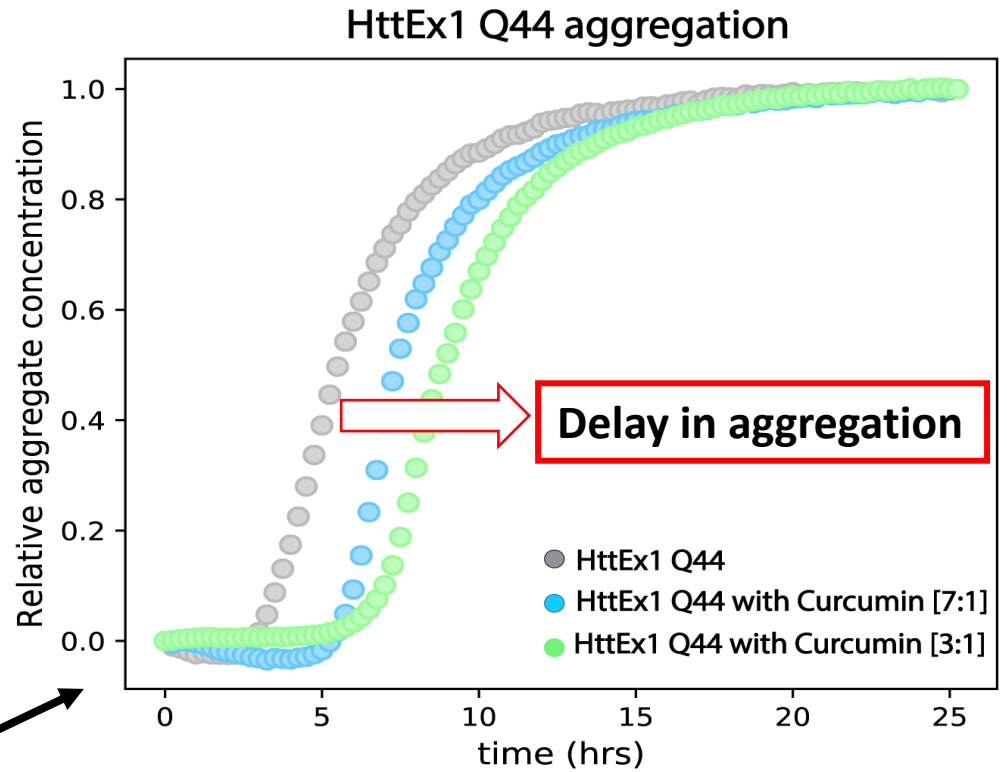


- Natural polyphenol found in turmeric
- Has anti-oxidative and anti-inflammatory properties



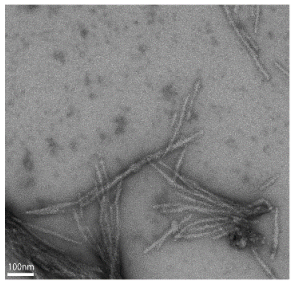
ThT assay of HttEx1 Q32 [expressed in-vitro] with and without Curcumin

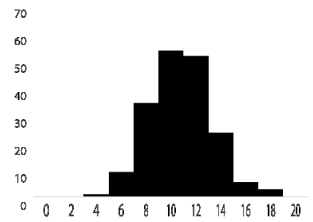
ThT assay of HttEx1 Q44 [expressed in-vitro] with and without Curcumin



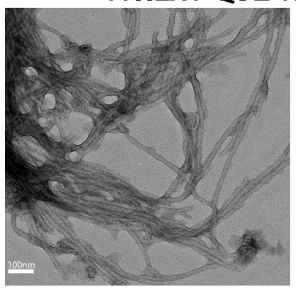
Effect of curcumin on the morphology and toxicity of the aggregates

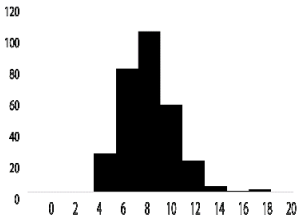
HttEx1 Q32



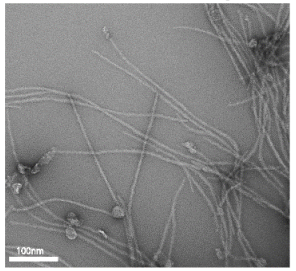


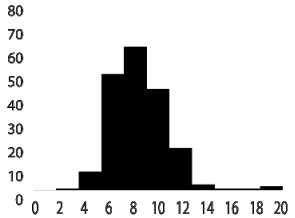
HttEx1 Q32 with Curcumin [7:1]



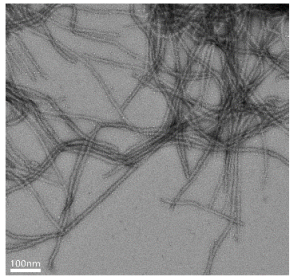


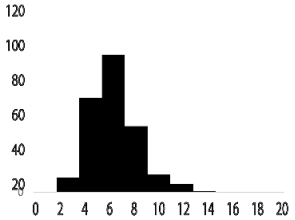
HttEx1 Q32 with Curcumin [5:1]





HttEx1 Q32 with Curcumin [3:1]

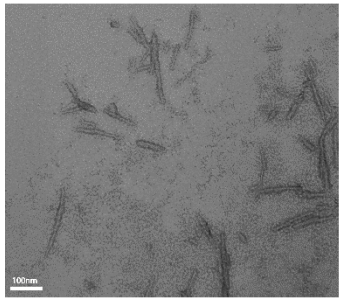


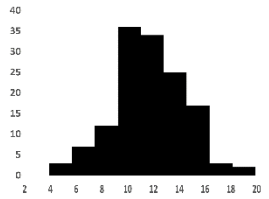


Width in nm

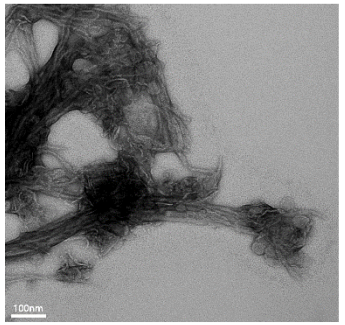
Number of Fibrils

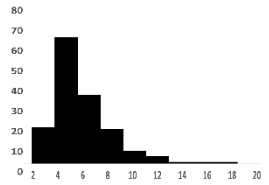
HttEx1 Q44





HttEx1 Q44 with 10uM Curcumin [5:1]





Width in nm

Number of Fibrils

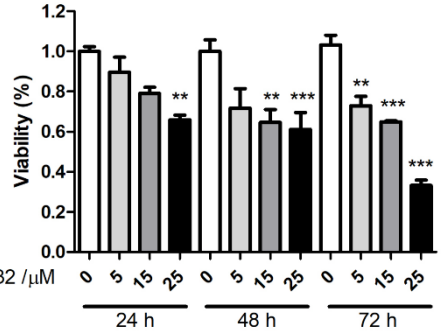
Aggregates formed in presence of Curcumin appears to be thinner

- Image G and H shows the effect of curcumin formed HttEx1Q32 aggregates on the cell toxicity.

- **HttEx1 Q32 aggregates shows cytotoxic effect on neuronal cells.**
- **Cell viability % decreases with increasing concentration of protein aggregates**

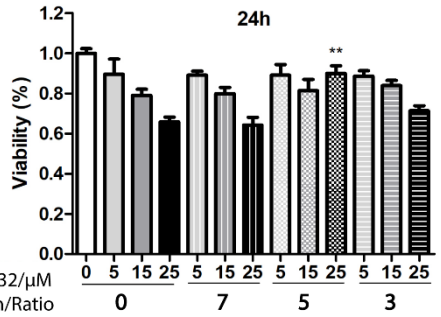
Neuronal Toxicity Assay

F



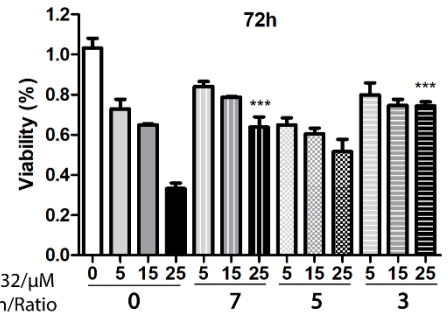
- Mouse hippocampal HT22 cells were treated for 24, 48 and 72 hours with different concentration of pre-formed HttEx1Q32 aggregates [Image F].

G



The aggregates formed in presence of curcumin shows less cell toxicity than aggregates made in absence of curcumin

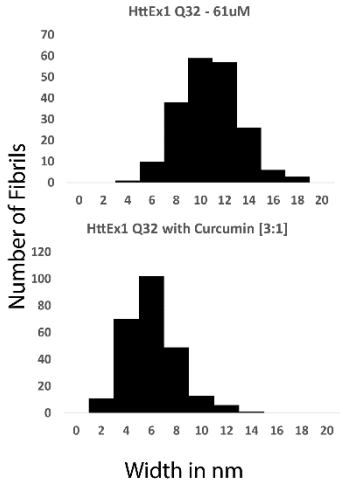
H



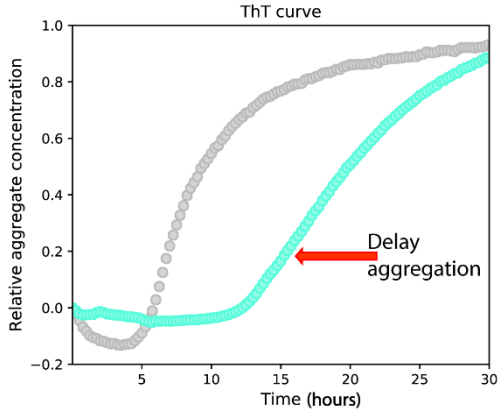
TEM images of HttEx1 Q32 and HttEx1 Q44 aggregates in presence and absence of Curcumin

Conclusions

- Curcumin causes delay in the lag phase of the protein aggregation. The increase in the lag phase is directly proportional to the curcumin concentration i.e. more the curcumin concentration, more delay in the lag phase.

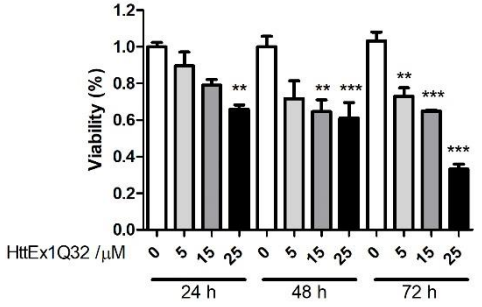


- Although delayed, the protein still forms aggregates, but after an increasing lag.
- The aggregates formed in presence curcumin have different morphology and appear to be thinner than the aggregates formed in absence of curcumin.



- Toxicity assays shows that with increasing concentration of HttEx1 Q32 aggregates, we observe decrease in cell viability %. Also, increase in the incubation time decreases increases the cell toxicity.

- The protein aggregates formed in presence of curcumin cause less cell toxicity, compared to those formed under normal conditions.



- Thus, curcumin has dual mode of action, causing two beneficial effects:
  - it delays aggregation and the aggregates that do form, have reduced toxicity.

Future Directions

➢ Study the molecular / structural factors that explain the differential toxicity of the distinct aggregates formed in presence and absence of inhibitors, using ssNMR and other techniques.

Acknowledgments

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Campagneteam Huntington

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