# *Distinct Tau Strains:*
# *Exploring Variability in Cell Uptake and Seeding Using Heparinoids*

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## Background

Tauopathies (including Alzheimer's Disease) are incurable, progressive neurodegenerative diseases caused by tau protein aggregation. Evidence suggests that tau aggregates spread pathology as do prions, infectious proteins that transmit a pathologic conformation to native proteins via disease-specific conformers (strains) that propagate indefinitely in living systems. Like prion protein, tau also forms strains.

Strong evidence shows that tau aggregates are taken up by cells through macropinocytosis after binding cell surface heparan sulfate proteoglycans (HSPGs). It has already been observed in animal models that heparin mimetics inhibit prion pathogenesis and that genetic knockdowns within the HSPG pathway reduce tau pathology.

In this project, I explored whether distinct tau strains bind cell surface HSPGs uniquely or generically to trigger uptake. I also used heparinoids to further our understanding of the relative importance of size and sulfation patterns of heparin for tau binding. This information has important implications for our understanding of the pathological variation between tauopathies and for the design of targeted therapies.

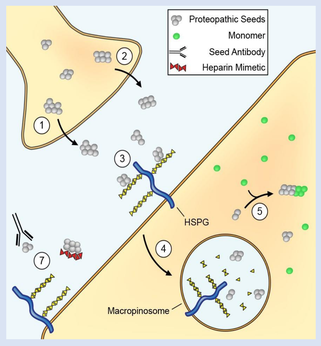



**Figure 1:** Tau aggregates spread pathology by binding cell surface HSPGs. Uptake into cells can be inhibited by heparin and its derivatives which are well known mimetics of HSPG (Holmes et al., JBC 2014).

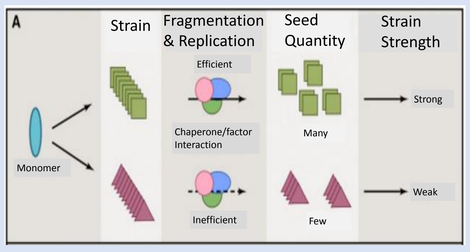



**Figure 2:** Tau strains differ in conformation and behavior (Sanders, Kaufman, et al., Neuron, 2016).

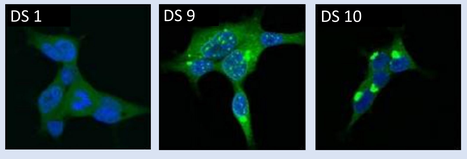



**Figure 3:** The Diamond lab has identified 19 different strains of tau (Sanders, Kaufman, et al., Neuron, 2014).

## Methods

Tau uptake and seeding was measured using a HEK P301S "biosensor" cell line which allowed for intracellular aggregates to be measured using FRET flow cytometry. Experimental methods are as follows:

1. Cell lysate containing tau aggregates was incubated with heparin (0.2, 0.66, 2, 6.66, 20, & 200 ug/mL) for 24-hours prior to addition to cultured cells.
2. After a 48-hour incubation period, cells were washed, dissociated from the tissue culture dish, and fixed in paraformaldehyde.
3. Flow cytometry was used to measure tau seeding.

## Methods

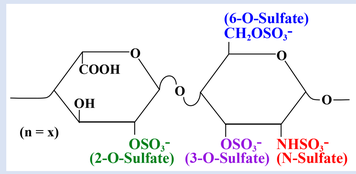



**Figure 4:** Chemical structure of a heparin disaccharide unit. Heparin is composed of approximately 40 such units. HSPGs are composed of 80-100 units. (Image by Brandon Holmes).

(Note on nomenclature below: "dp4" heparinoid contains "4" disaccharides; "De-N" heparinoid is desulfated at the "N" position.)

## Results

1. Tau strains show both similar and unique behavior patterns

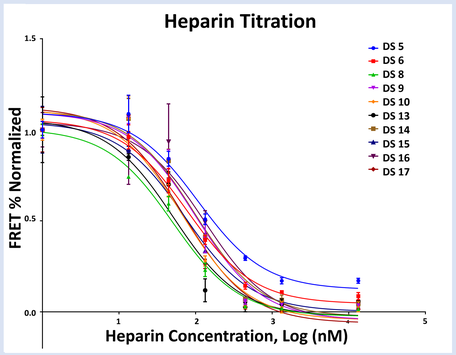



**Figure 5:** All tau strains tested were highly sensitive to heparin inhibition of seeding and most maintained a highly similar dose response.

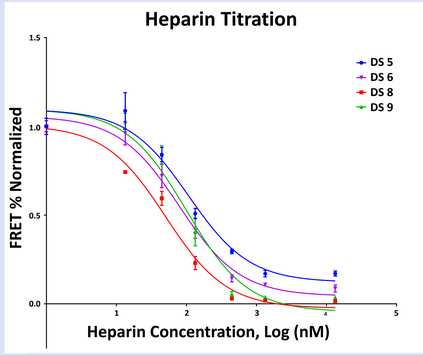



**Figure 6:** These strains show subtle differences. Note the higher seeding in DS 5 & 6 at maximal heparin concentrations.

2. Relative importance of size and sulfation patterns of heparin is consistent between two strains

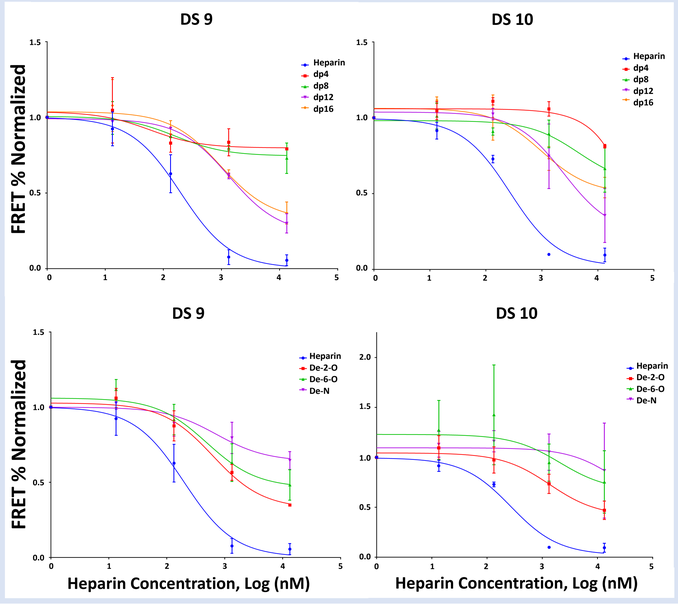


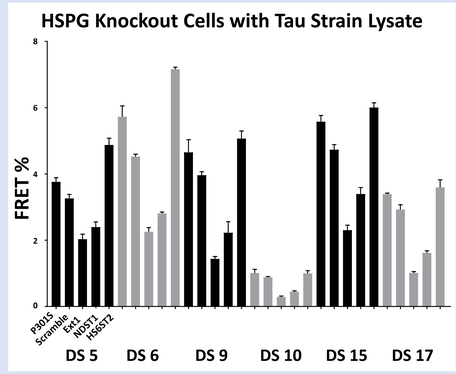

**Figure 7:** Inhibition with *Variably Sized* Heparinoids showed highly similar inhibition patterns between 9 & 10.

**Figure 8:** Inhibition with *Variably Desulfated* Heparinoids showed highly similar inhibition patterns between 9 & 10.

## Results

3. Genetic knockouts in important HSPG enzymes decreased seeding differentially between strains

**Figure 9:** CRISPR Cas9 was used to genetically knockout three important enzymes in the HSPG synthesis pathway to examine the relative important of HSPGs for tau uptake and seeding across different tau strains.




Findings:

- EXT1 (KO): Seeding reduction in DS 5, 6, 15 (38, 50, 51%) was lower than other strains (~65%), suggesting less reliance on HSPG uptake
- NDST1 (KO): N-Sulfation is highly important for tau binding
- HS6ST2 (KO): Results were surprising for 6-O-sulfation knockout and difficult to interpret, seeding increased and we expected a decrease

## Conclusions

Cellular uptake of many tau strains is similarly inhibited by heparin, hinting that the same heparinoid (or small molecule analog) could be used to treat diverse tauopathies. But the unique behavior of some strains suggests a one-size-fits-all treatment approach may not always be sufficient.

Certain size and sulfation patterns on heparin seem to have specific importance for tau binding. Larger heparinoids better inhibit tau seeding (dp16 & dp12 > dp8 & dp4). Regarding sulfation, the "N" position is more important than the "6-O" position which is more important than the "2-O" position. This pattern remains consistent in recombinant tau, DS 9, and DS 10. It is also consistent with the genetic knockout data gathered here (using strains) and by others in the lab (using recombinant tau).

Also, the higher residual seeding observed in some strains at maximal heparin concentrations suggests there may be a route other than HSPG mediated uptake through which tau can enter cells. This finding should be explored further.

This data shows many similarities and some differences between strains of tau. Parsing these differences could have important implications for understanding the diversity of tauopathies and finding unique approaches to diagnosis and treatment.

## Acknowledgements

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