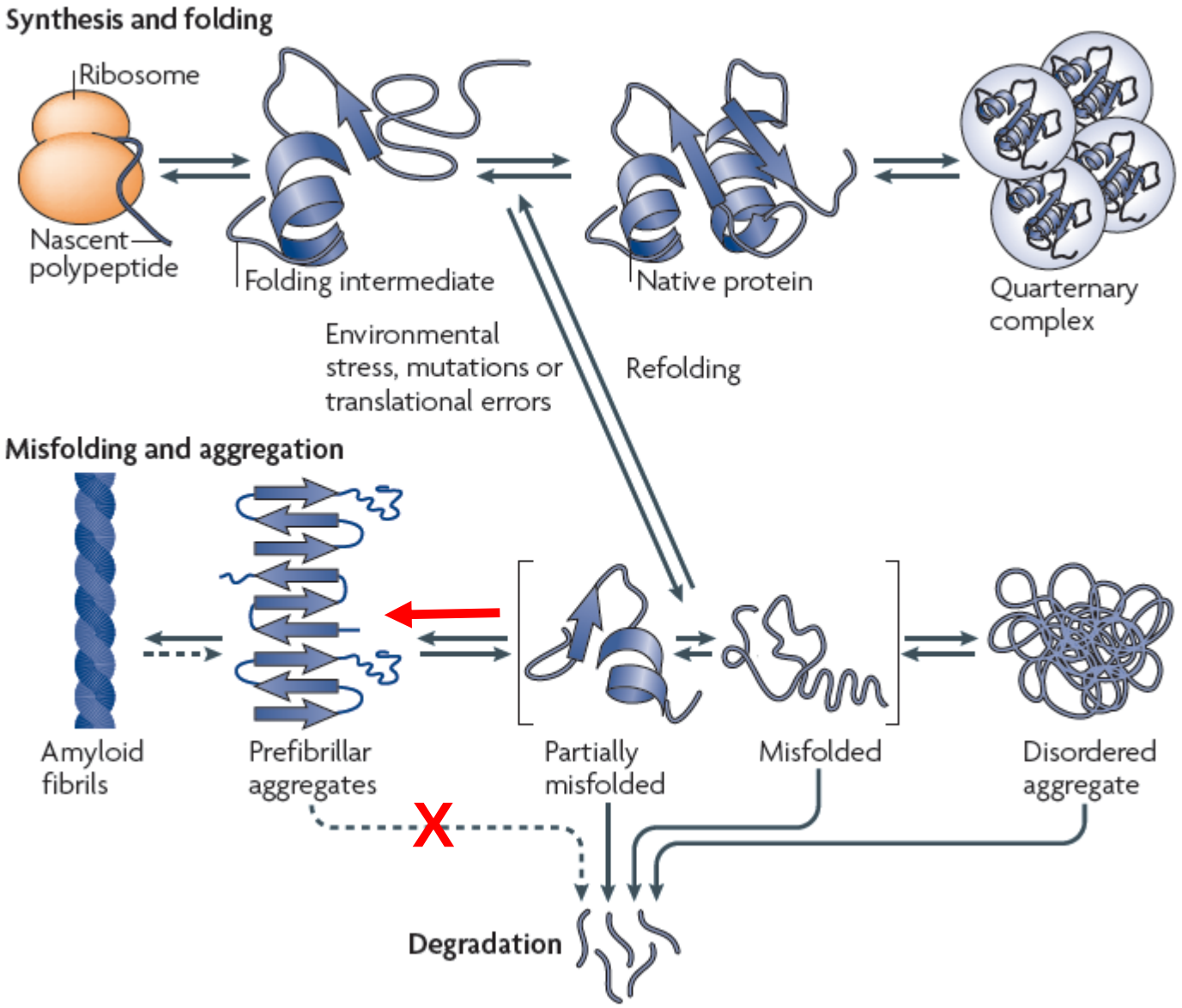
TauRx Pharmaceuticals Ltd

Therapies and Diagnostics for Neurodegenerative Disease

PRION-LIKE PROCESSING OF PATHOLOGICAL PROTEINS AS A UNIFIED BASIS FOR PHARMACEUTICAL DEVELOPMENT IN NEURODEGENERATIVE DISORDERS

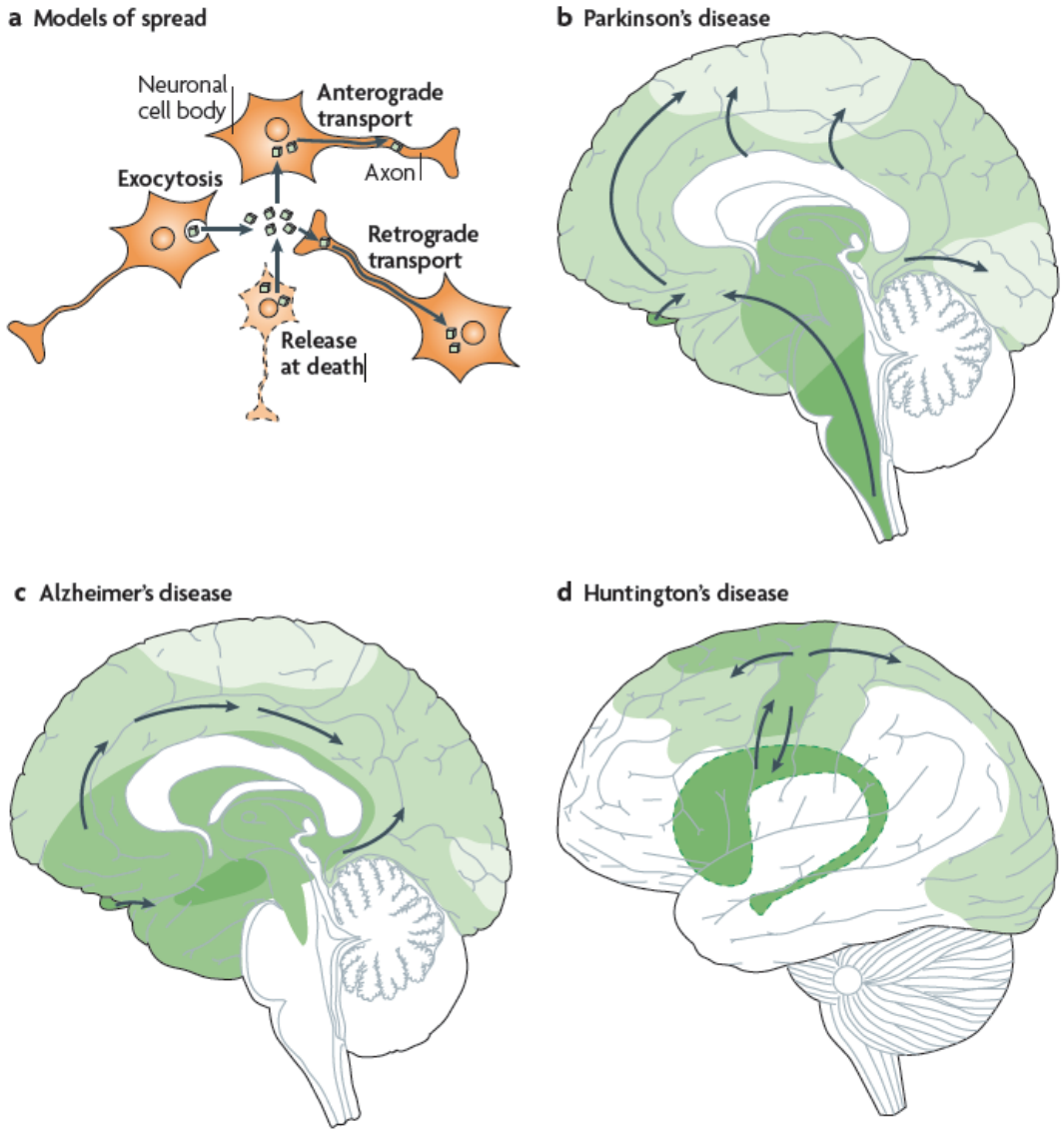
Claude M. Wischik,
Professor of Old Age Psychiatry, University of Aberdeen
& Chairman TauRx Therapeutics

Diseases of protein aggregation



Tyedmers (2010) *Nat Rev Mol Cell Biol 11:777*

Different Proteins – Common General Theme



The spread of disorders may depend on the protein:

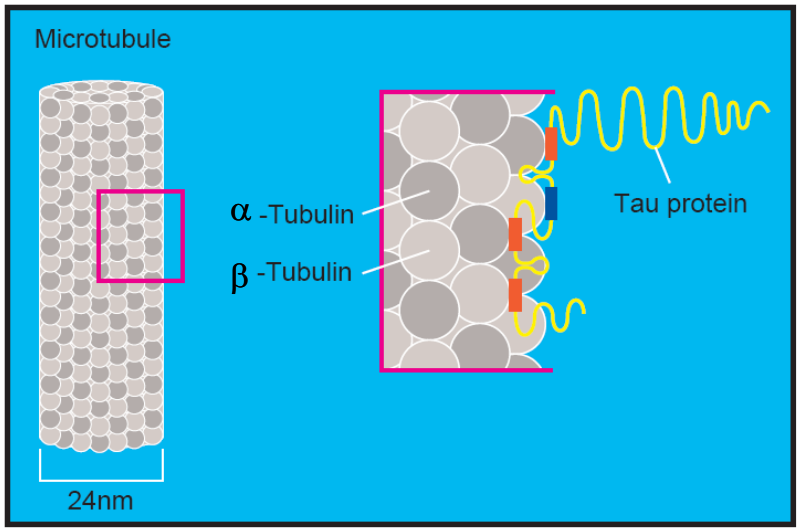
- α-synuclein for PD,
- tau for AD
- huntingtin inclusions (found in nuclei) for HD.

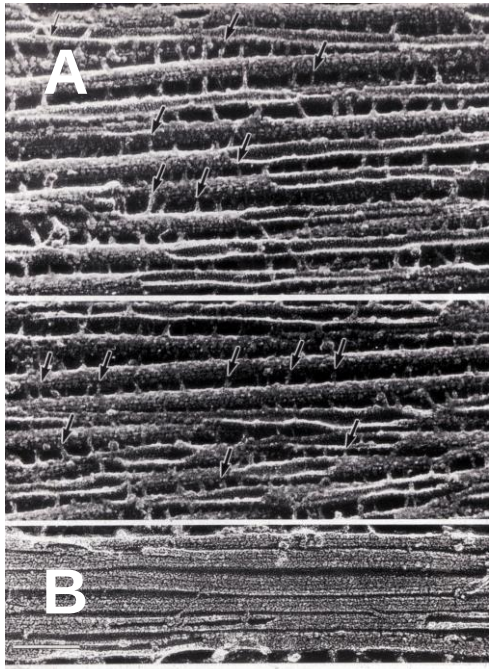
For FTD, where tau or TDP-43 constitute most cases, the regions affected are frontotemporal, but precise staging not determined yet.

Tau Biology and Pathology as an Example

Normal tau function – axonal cytoskeleton

Tau-Tubulin binding via repeat domain

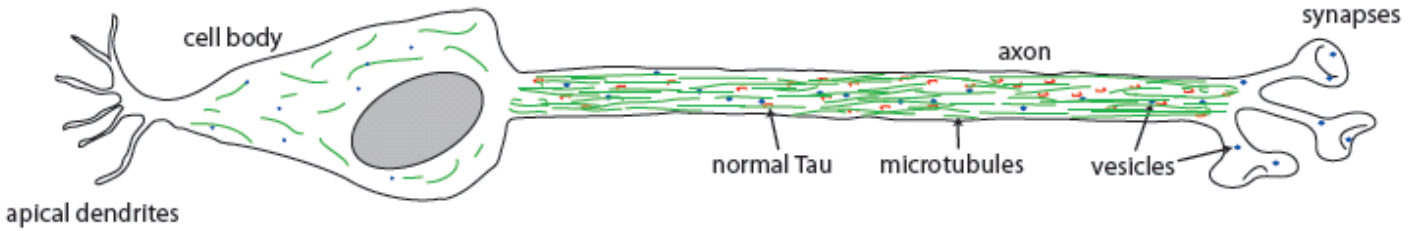




(A) Tau projection arms microtubules assembled with Tau
(Hirokawa et al 1988 J Cell BIol 107: 1449)

(B) Bundling without Tau
But Tau-KO mice are viable - other MAPs can substitute
(Harada et al 1994 Nature 369:489)

Axonal microtubules essential for transport of synaptic vesicles, mitochondria, tubulin recycling



MAPT - the Tau gene

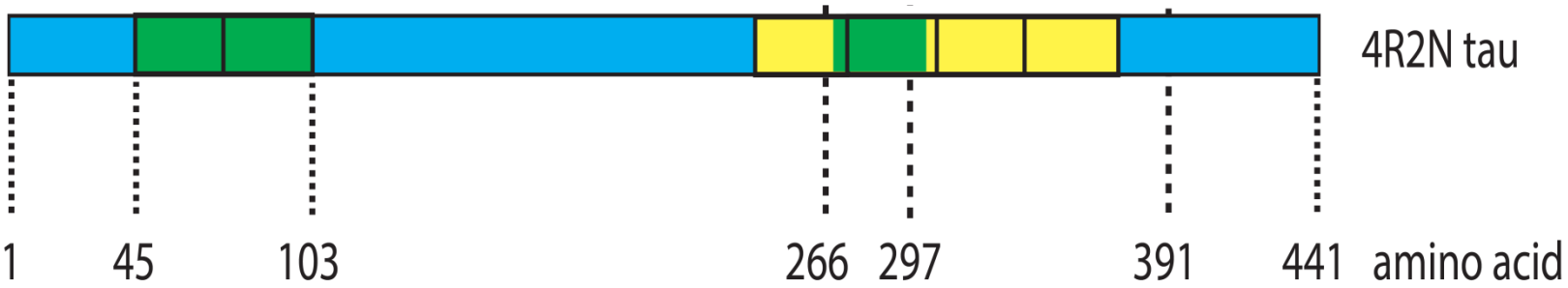
Tau cloned from mouse brain by Gloria Lee (1988). Highly conserved among different species

MAPT is over 100 kb on chromosome 17q21-22 having at least 16 exons

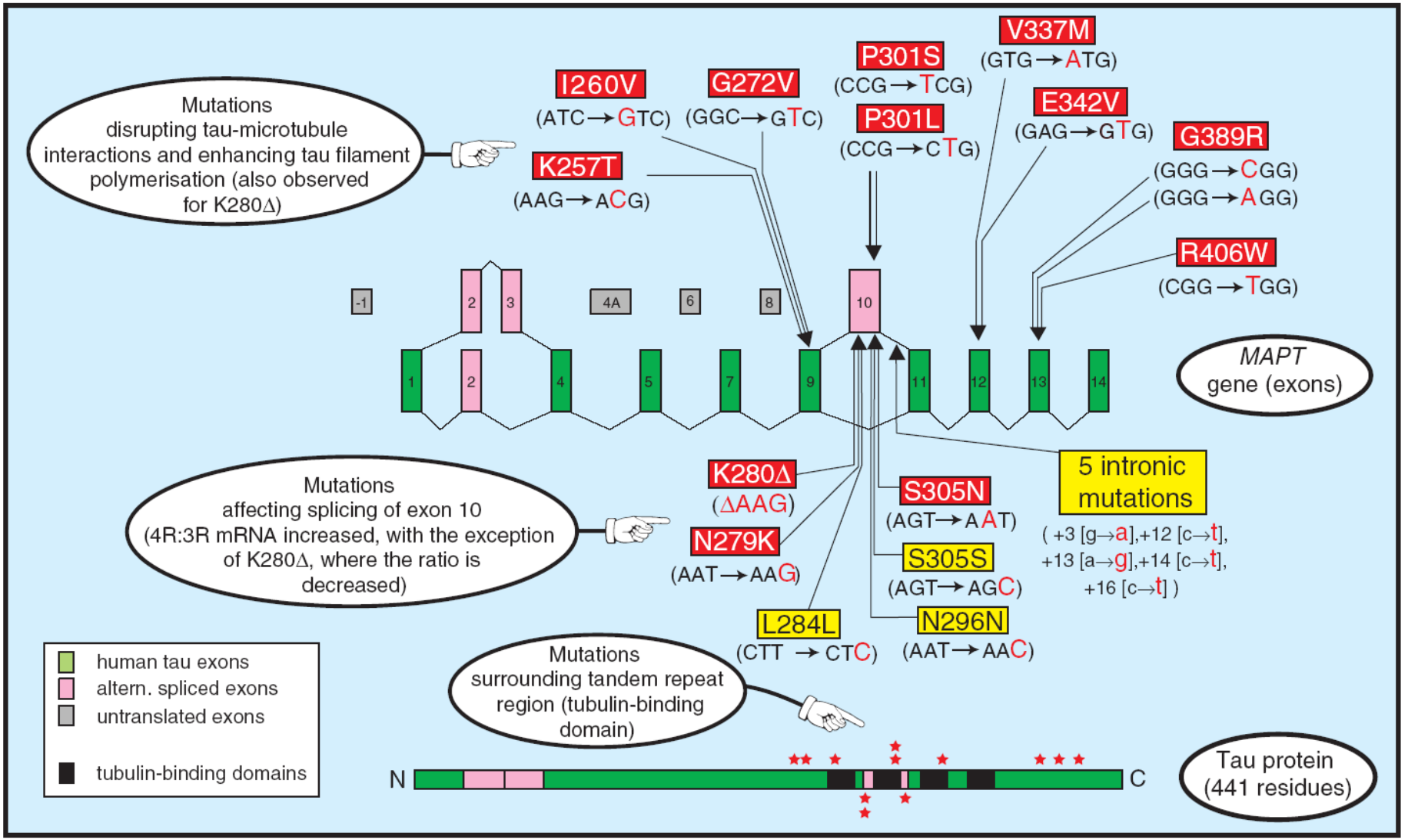
Six tau isoforms by alternative splicing of exons 2, 3 and 10

Isoforms are developmentally regulated, the 3R isoforms alone are expressed in fetus.

Exon 10 encodes one of 4 tandem repeat regions of 31-32 aa; 4R tau bind microtubules better than 3R tau



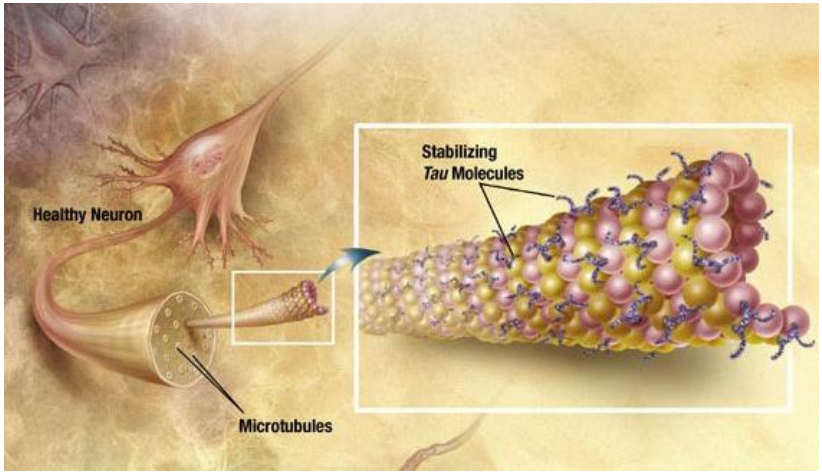
Tau mutations in Frontotemporal dementia with parkinsonism linked with chromosome 17 (FTDP17)

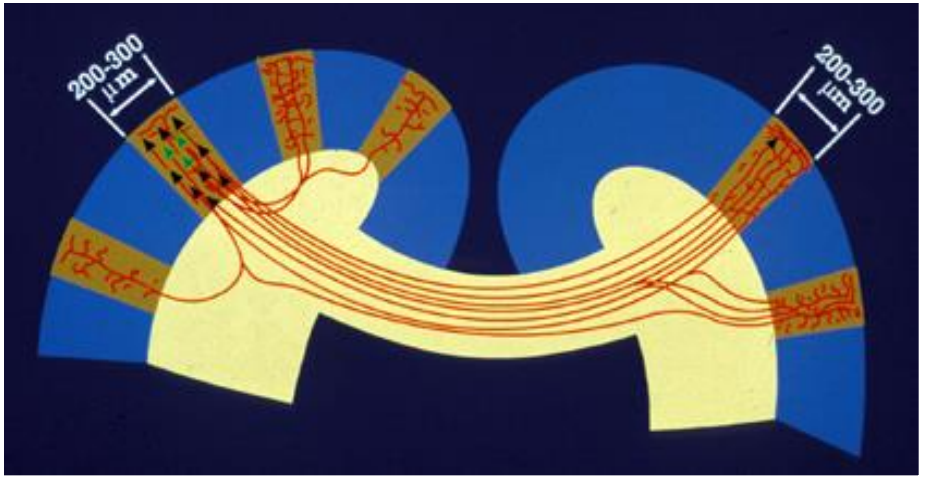


Tau protein normally stabilises axonal microtubules: but polymerises to form Paired Helical Filaments in AD

Tau protein normally functions to stabilize axonal microtubules

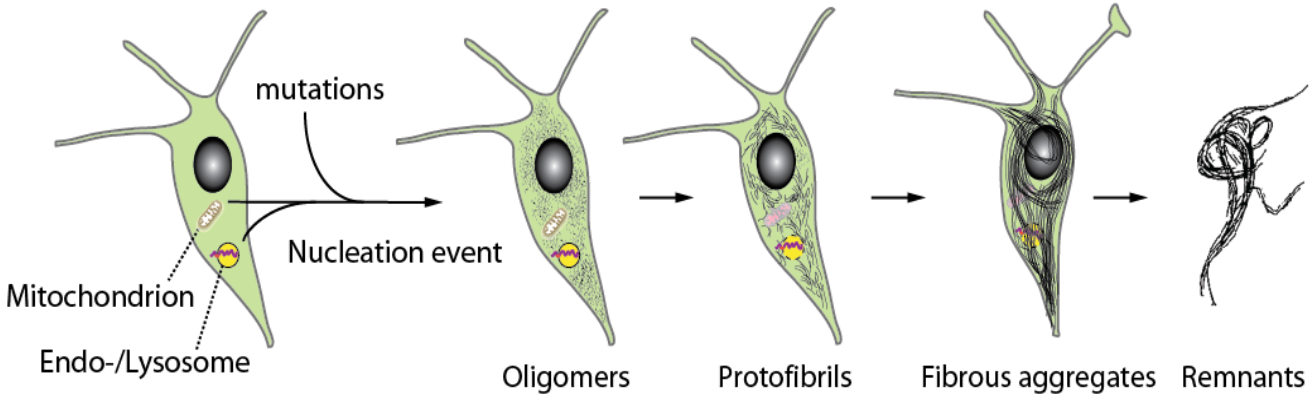
These are critical for axonal transport in cortico-cortical association circuits





In AD Tau aggregates to form first oligomers then fibrous aggregates composed of de novo Tau polymers - Paired Helical Filaments (PHFs)

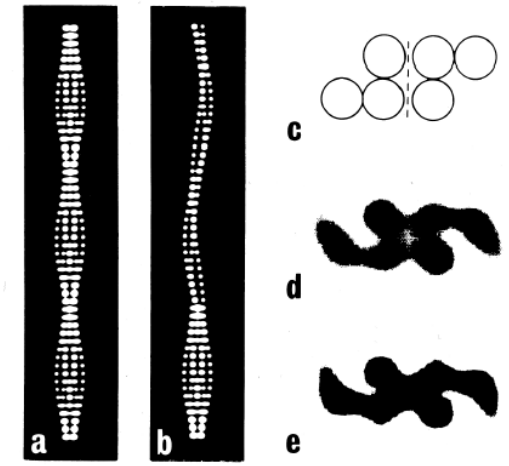
Alzheimer called these fibrous aggregates “neurofibrillay tangles”



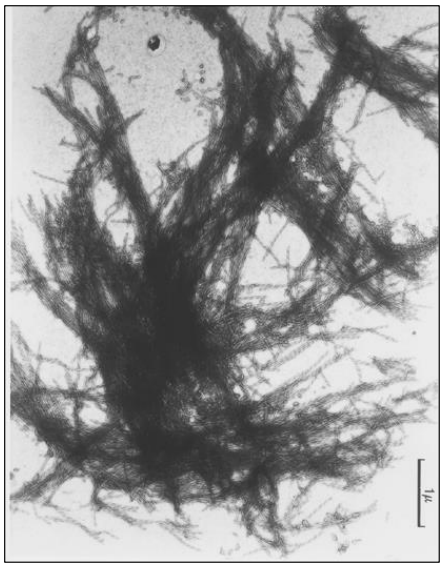
Molecular dissection of Tau pathology of AD
Only 5% of tangle/PHF Tau is phosphorylated pTau

Tau histopathology of AD

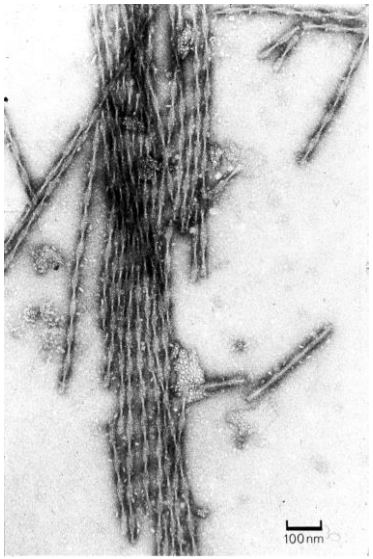
Structure of PHF core [1]



Tangle



PHFs

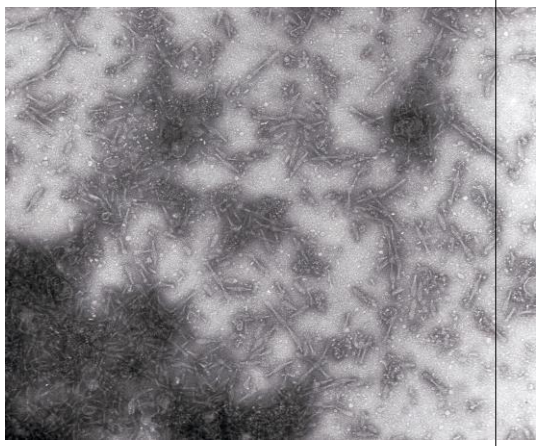


Phosphorylated tau only in outer coat[2], <5% of PHF tau by mass[2] and biochemistry[3]

[1] Wischik & Crowther, EMBO J, 1986, 4:3661-3665;
[2] Wischik et al., PNAS, 1988, 85:4884-4888; [3] Wischik et al., Neurobiol Aging, 1995, 16:409-431

PHF core is composed of 3 Tau repeat domain species, all from phase-shifted repeat domain truncated at Glu-391

90% pure PHFs[1]

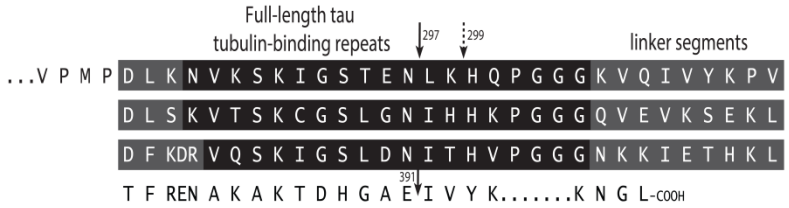


Tau phase-shift in PHF[2]

i PHF-tau



ii Normal tau



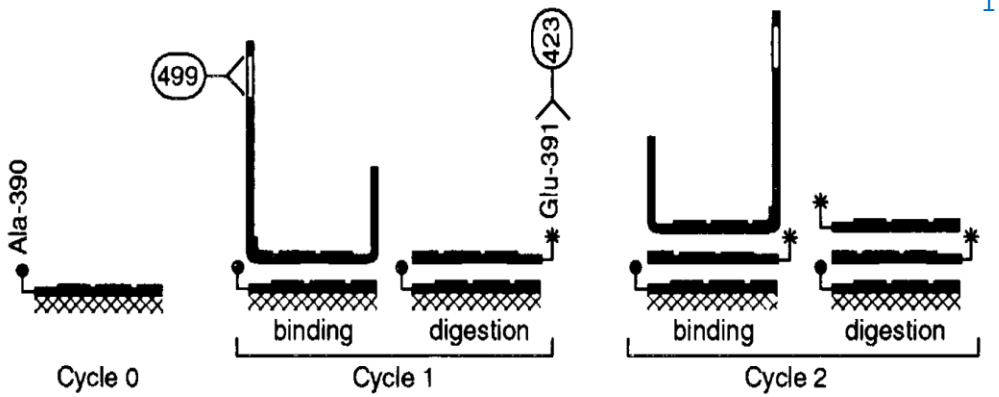
Core Tau units all from repeat domain[2]

Glu-391 truncation due to aggregation[3]

[1] Wischik et al., PNAS, 1988, 85:4506-4510

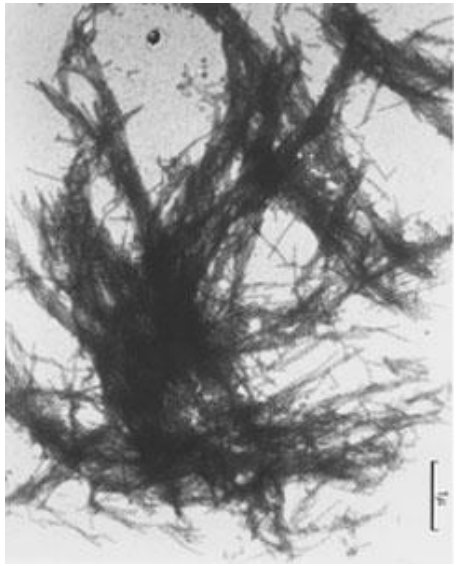
[2] Novak et al., EMBO J, 1991, 88:5837-5841 [3] Wischik et al., PNAS, 1996, 93:11213-11218

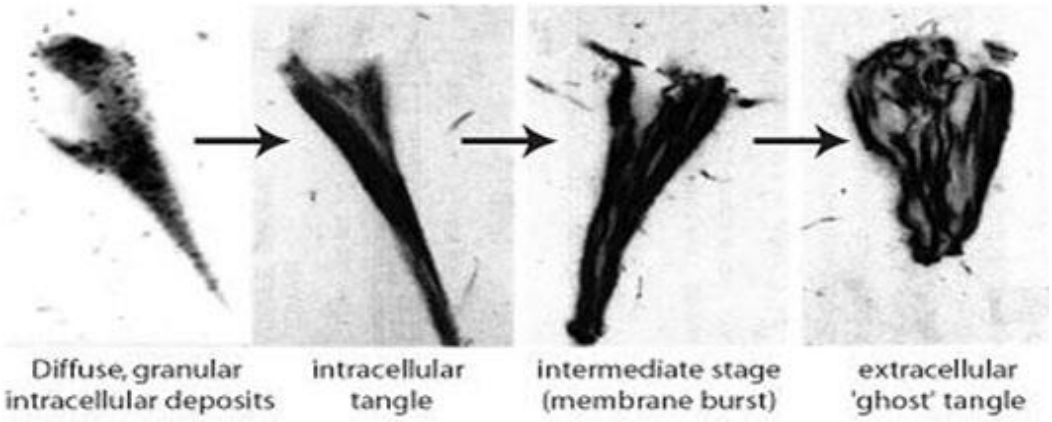
Core-oligomer of the Tangle-PHF Drives Template-Directed Proteolyis: Self-Replicating[1], ie Prion-like[2]



[1] Wischik et al., PNAS, 1996, 93:11213–11218

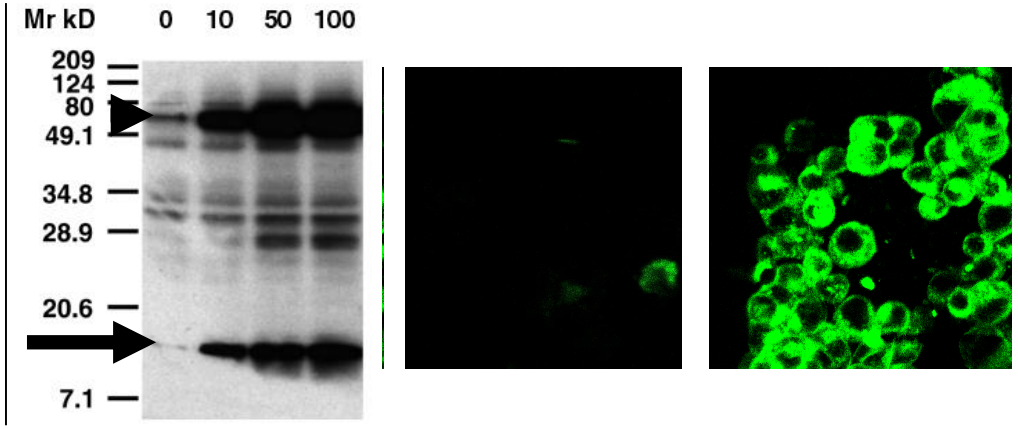


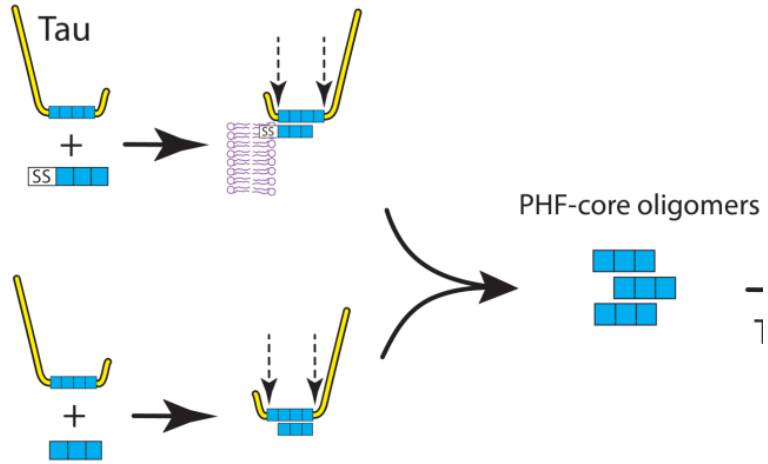


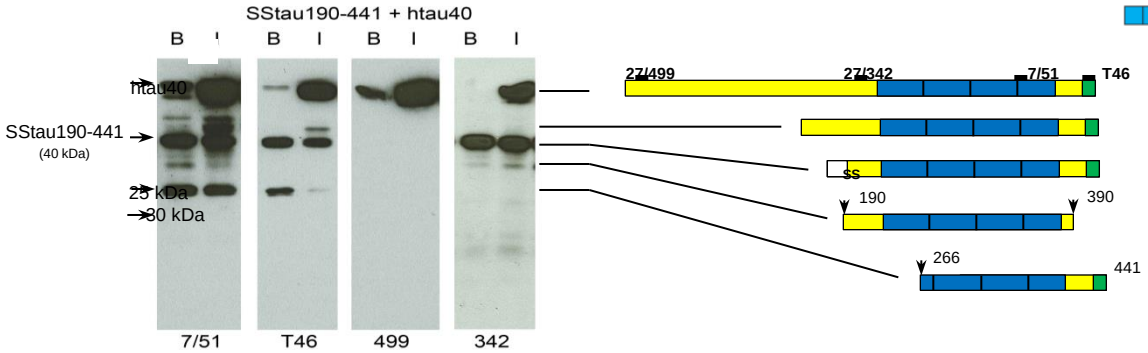


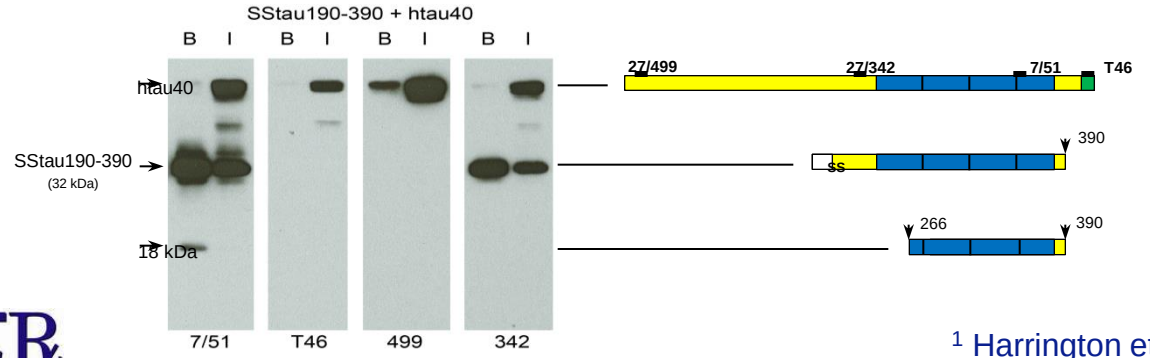
[1] Wischik et al., 1996, PNAS 93:11213-11218;
[2] Wischik et al., 1997, in Microtubule-Associated Proteins: Modifications in Disease., ed. J. Avila, R. Brandt, K. S. Kosik. pp. 185-241

Template-Directed Proteolysis in Cell Models[1]





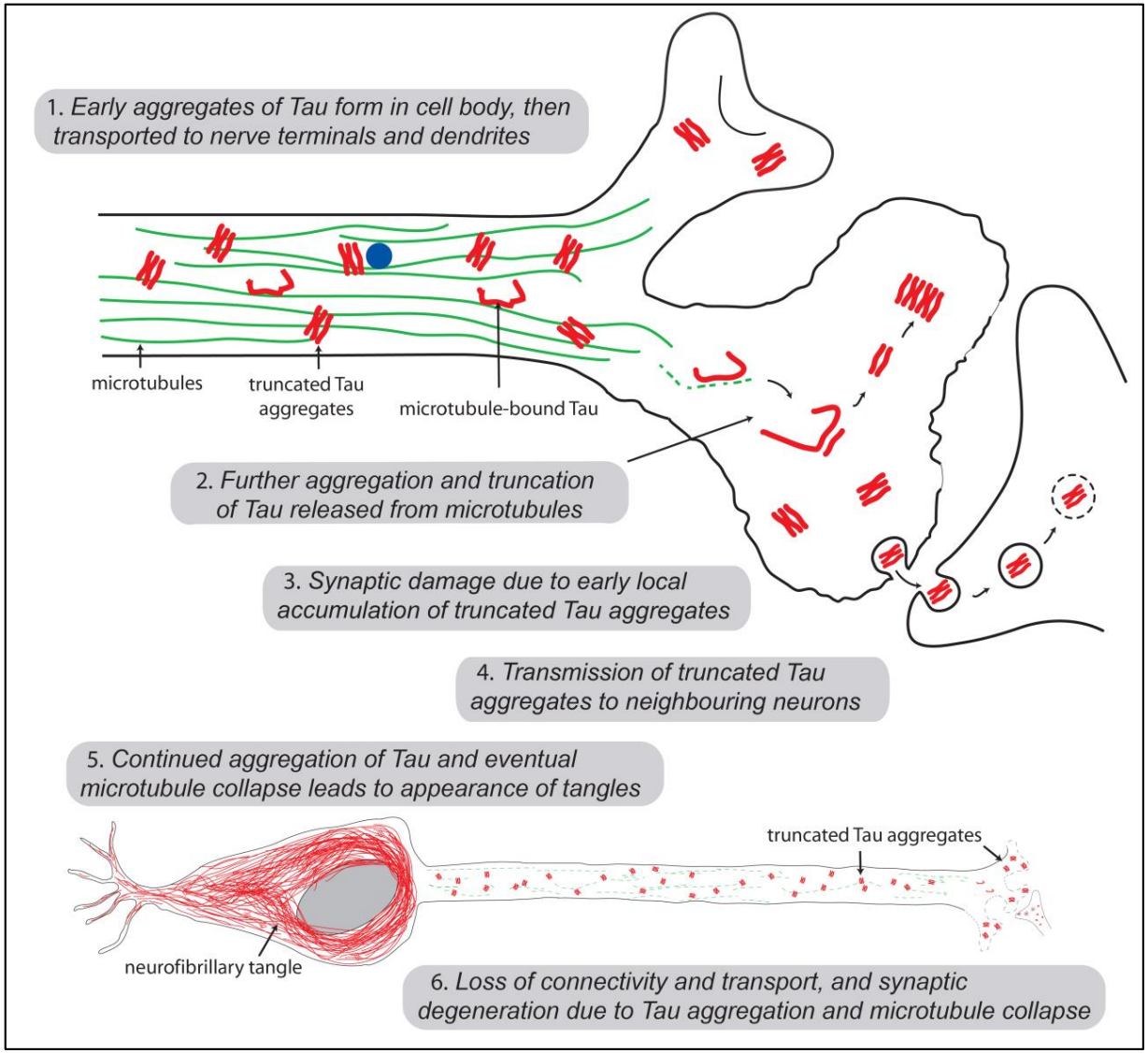




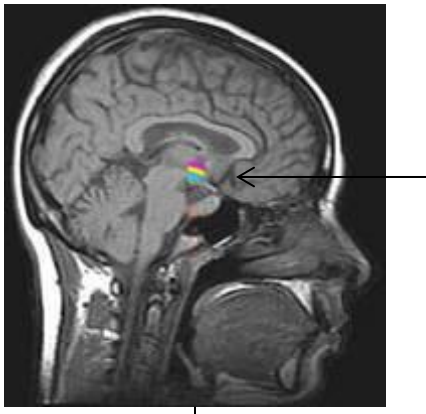
Oligomers produced in cells consist of ~100 units

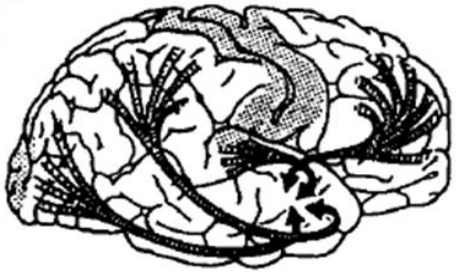
[1] Harrington et al., J Biol Chem, 2015, 290 10862-10875

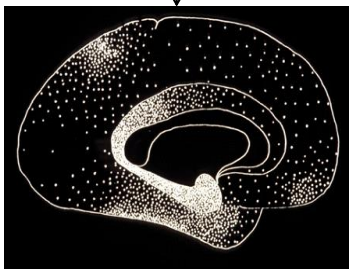
Formation and transmission of toxic oligomers is common feature of several neurodegenerative diseases

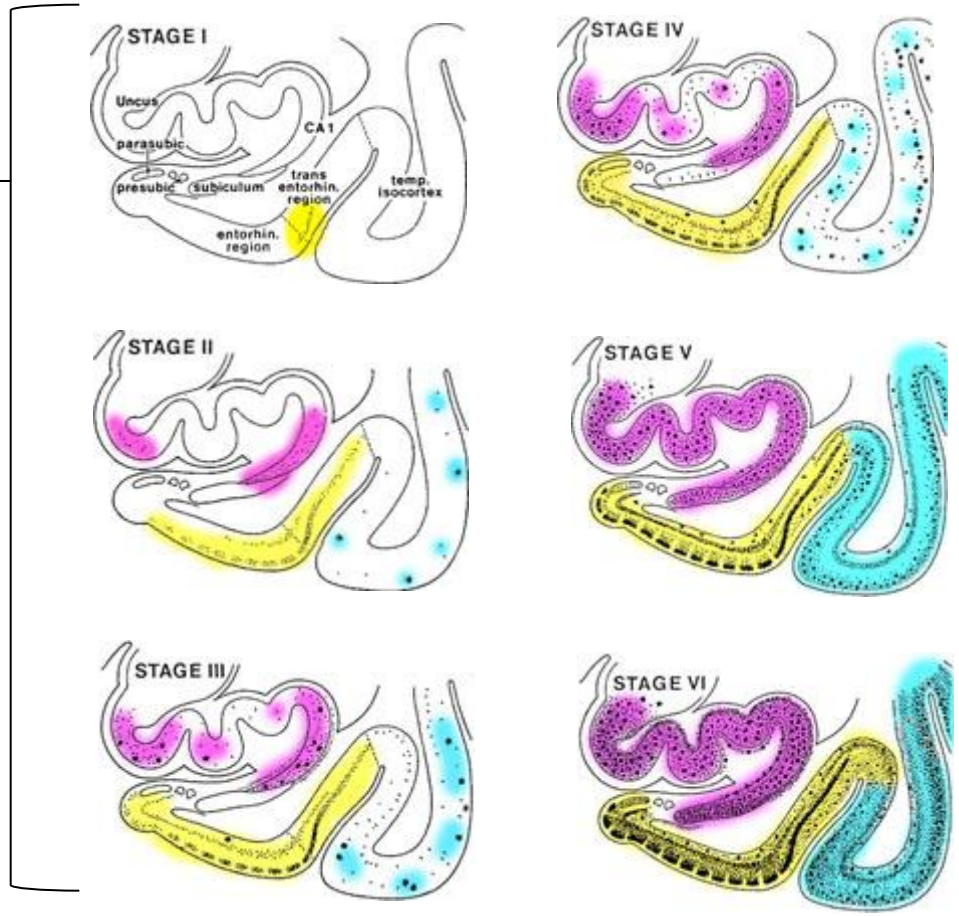


Pattern of spread of Tau pathology in AD = Braak Staging[1]





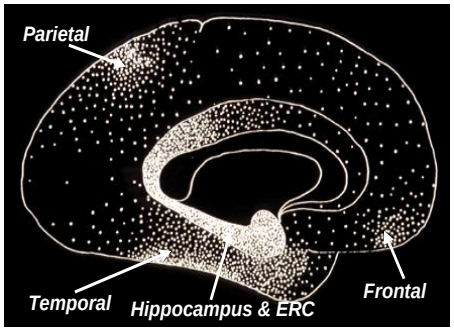




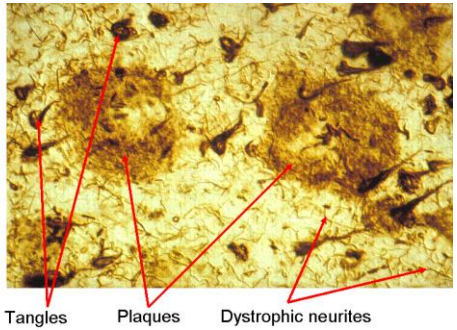
- **Braak stages defined by pattern seen in low-power view of medial temporal lobe**

1 Braak & Braak, 1991, Acta Neuropathol 82:239-259

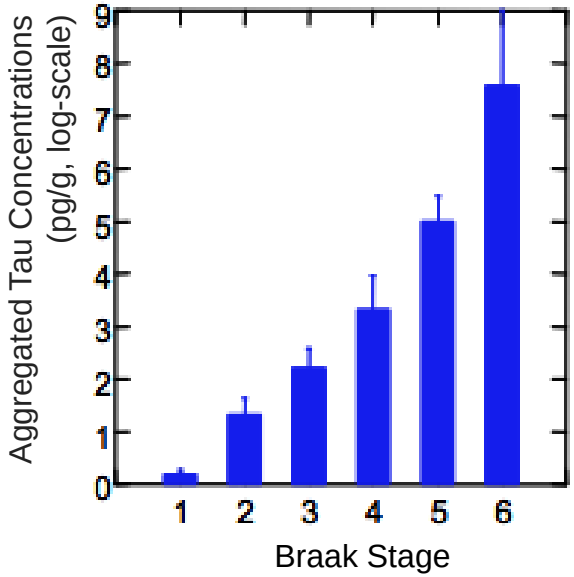
Braak staging[1] measures aggregated Tau load in the brain

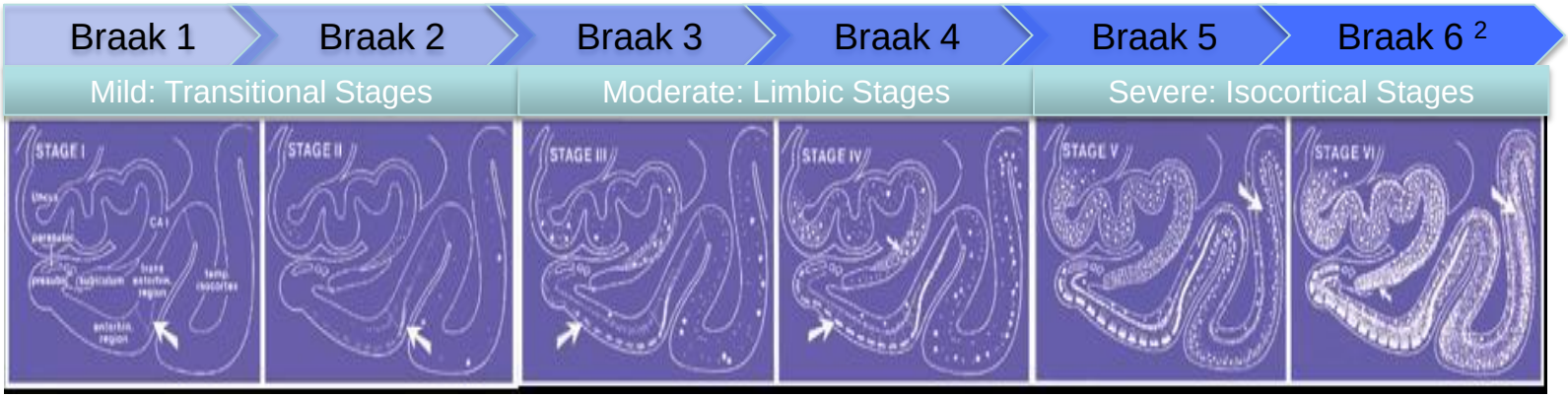


Tau aggregation spreads from medial temporal lobe structures to isocortex



Tau concentrations in neocortex correlated with Braak stage [2]





1 Braak & Braak, 1991, Acta Neuropathol 82:239-259
2 Mukaetova-Ladinska et al. 2000 Am. J. Pathol 157:623–636

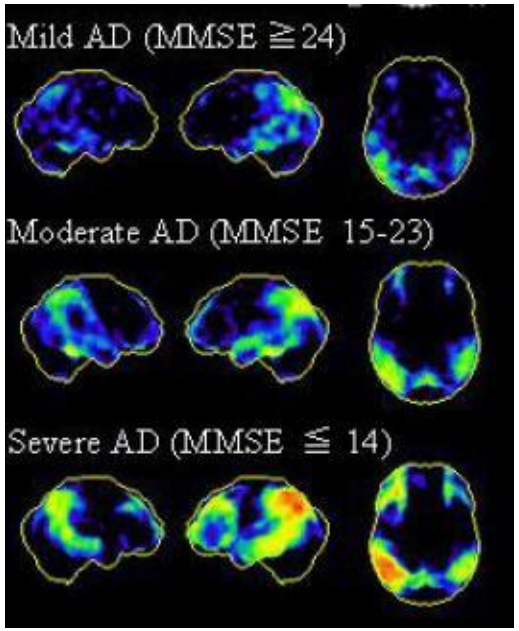
Tau load and distribution correlate with clinical severity and neuroimaging defects (PET & SPECT)

Braak stage and scan deficits[1]

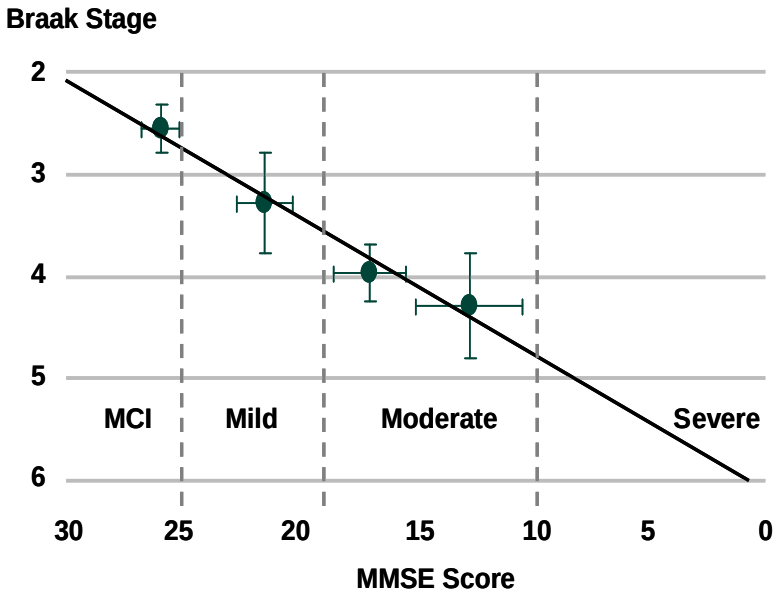
Braak 2-3

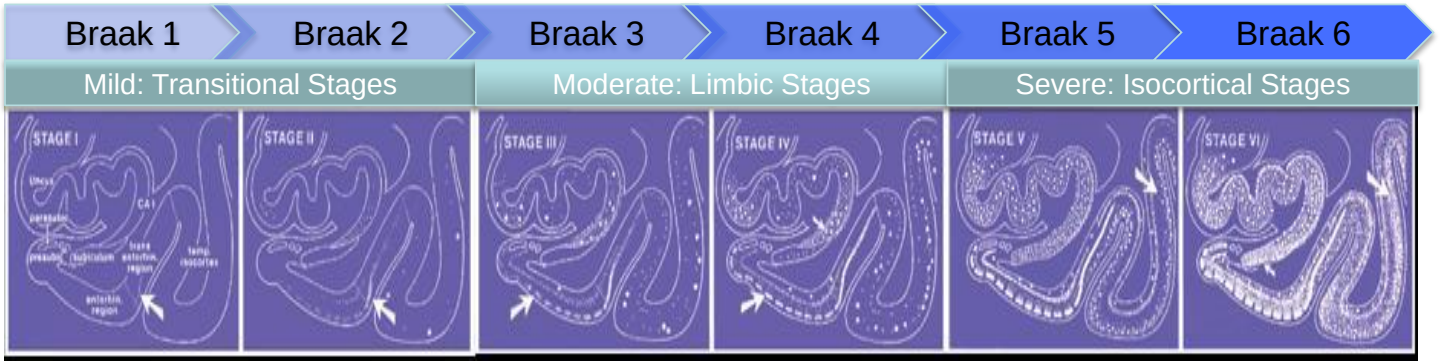
Braak 3-4

Braak 4-6



Braak stage and clinical decline[2]



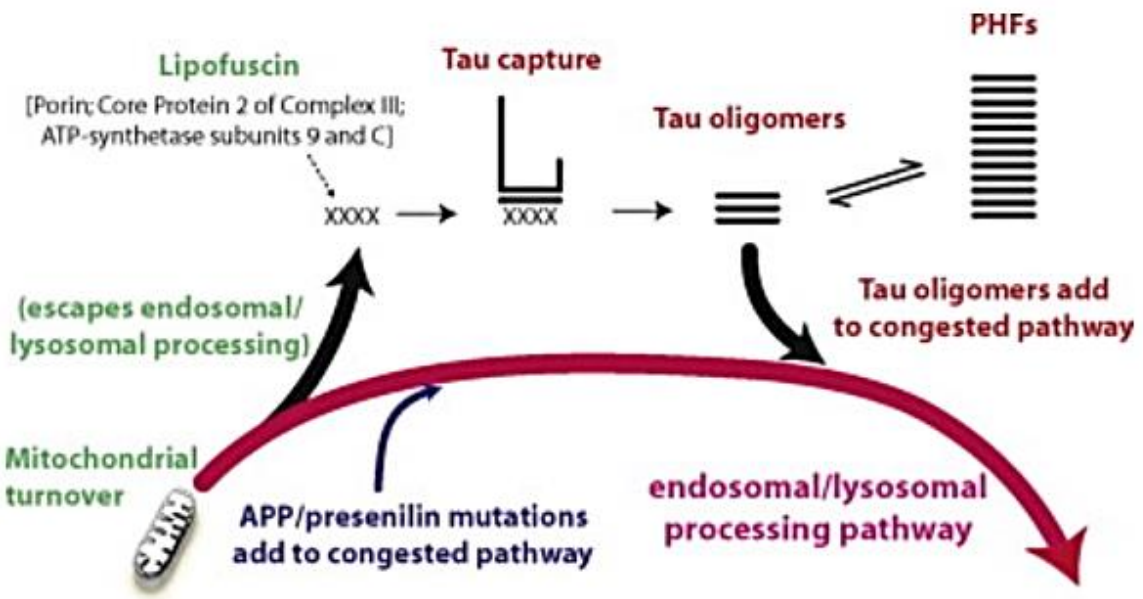


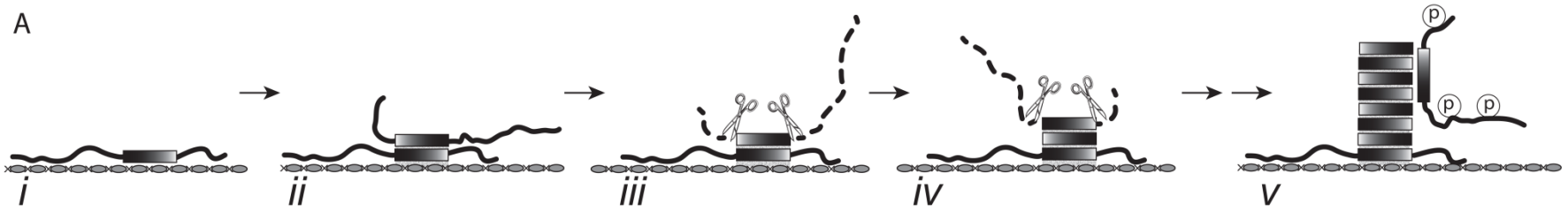
[1] Jobst et al., 1992, J Neurol Neurosurg Psychiat 55, 190-194
Bradley et al., 2002, Br J Radiol 75, 506-513
Nishimura et al. 2007, Ann Nuc Med 21, 15-23

[2] Adapted from Mukaetova-Ladinska et al. 2000 Am. J. Pathol 157:623–636

Importance of age-related impairment of mitochondrial turnover by endosomal-lysosomal pathway

- Potential to identify markers of up-stream endosomal-lysosomal dysfunction



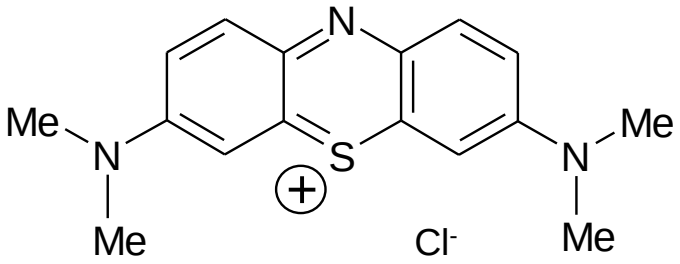


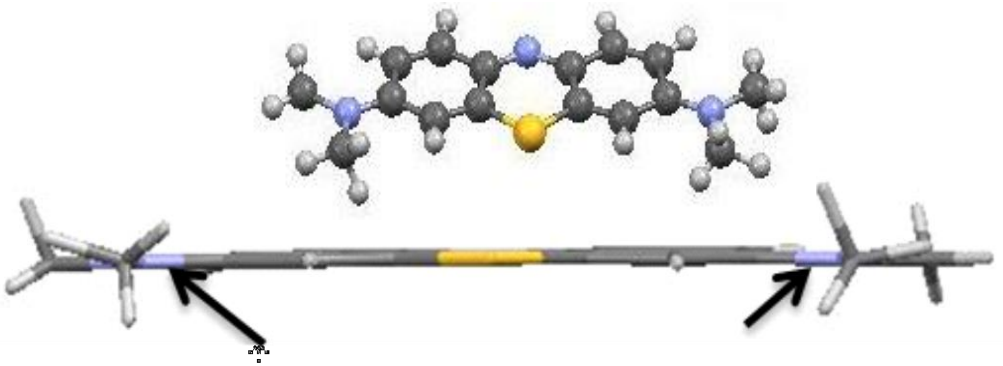
[1] Wischik et al. Tau-aggregation inhibitor therapy for Alzheimer's disease. Biochem Pharmacol (2014), 88:529-539

Utility of Methylthioninium (MT) as Tau Aggregation Inhibitor

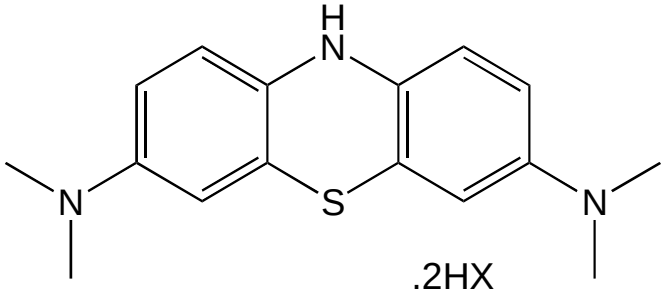
LMTX™ (stable reduced form of MT) overcomes absorption and tolerability limitations of MTC (methylthioninium chloride)[1,2]

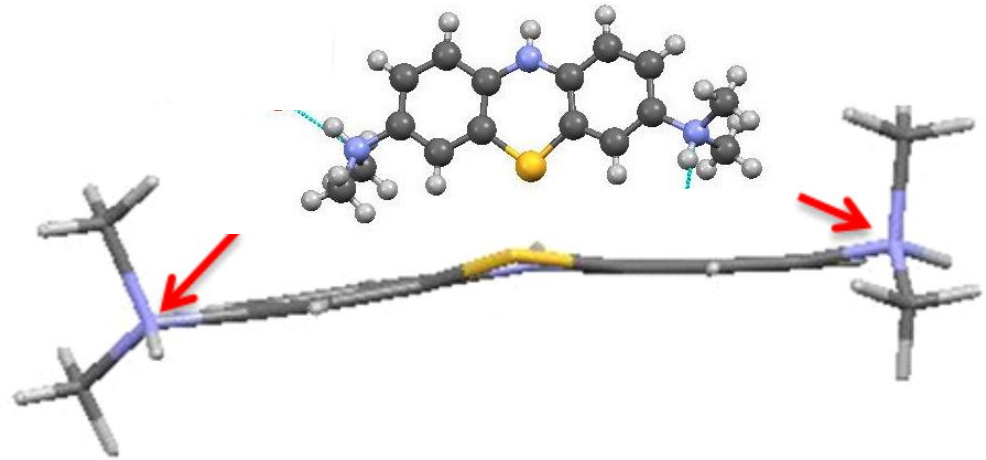
MTC





LMTX™



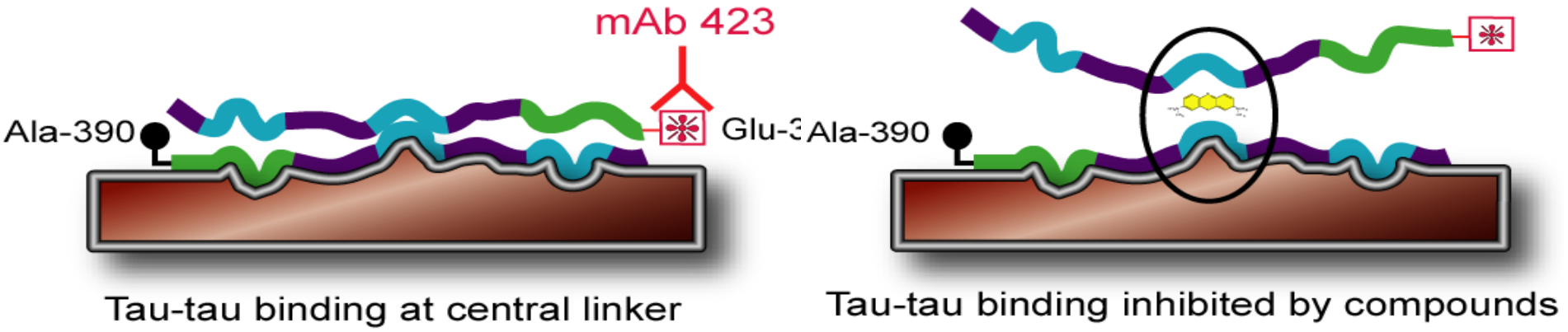


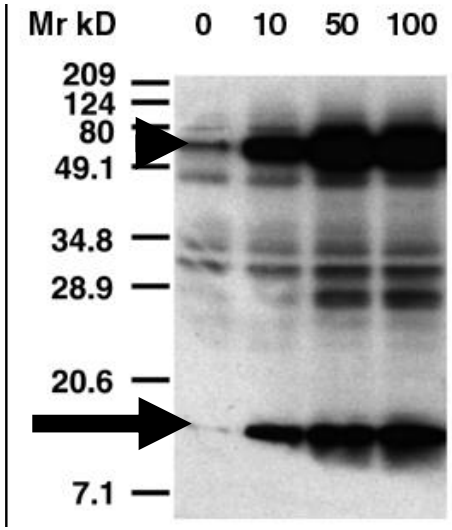
MTC is oxidised MT⁺ and suffers from dose-dependent food interference with absorption, but poorly tolerated in the fasted state

LMTX is a distinct chemical entity which delivers the same active moiety

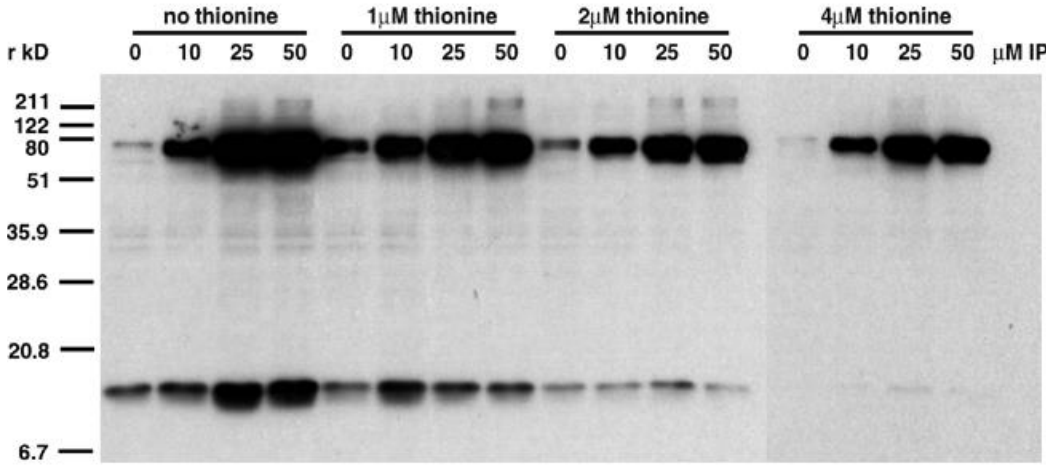
1 Baddeley et al. J Pharmacol Exp Therap (2015) 352:110-118
2 Harrington et al., J Biol Chem, 2015, 290 10862-10875

Cell-free and Cell-based models for identifying Tau Aggregation Inhibitors[1]



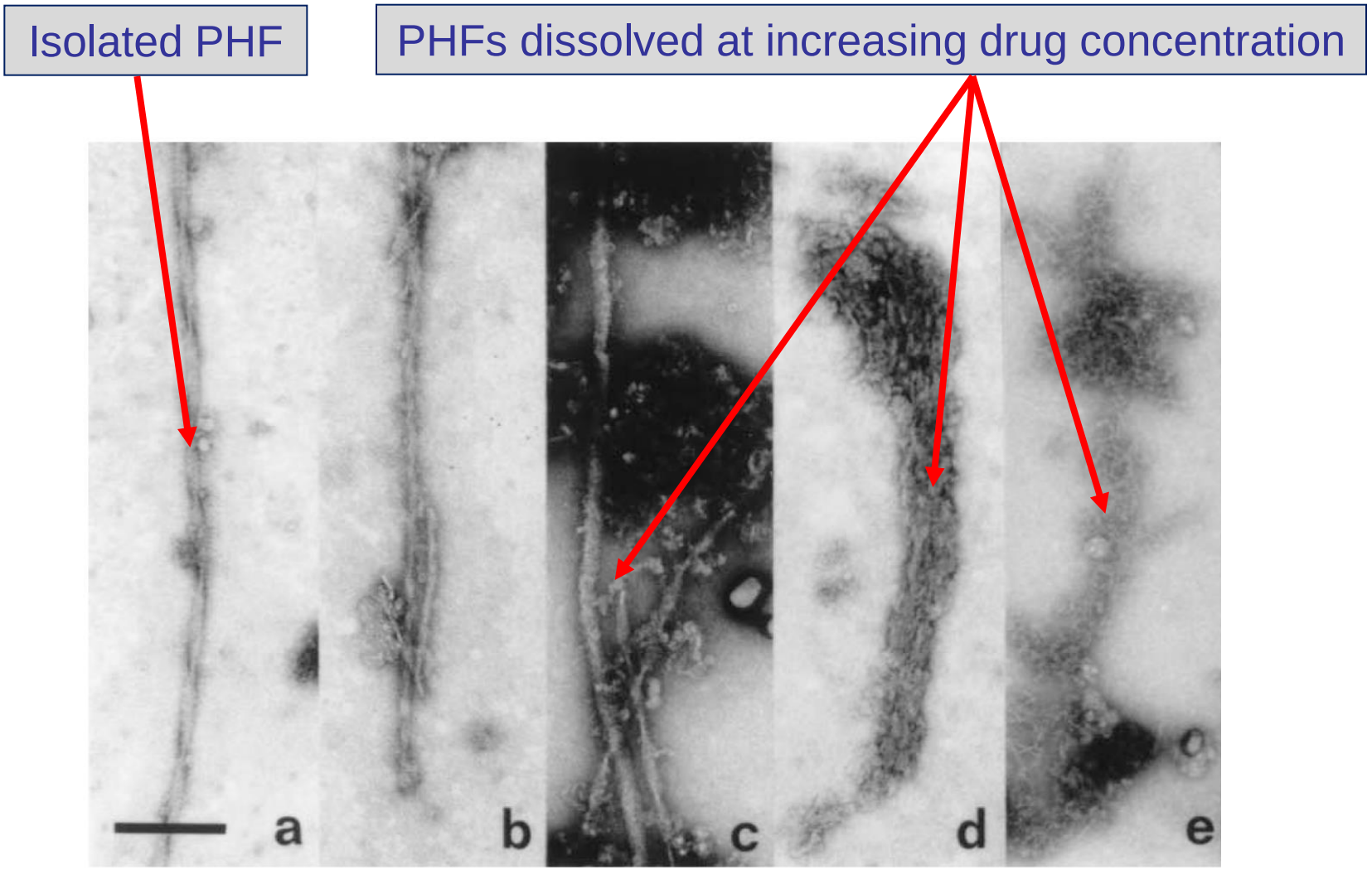


Inhibition of Tau aggregation in cells



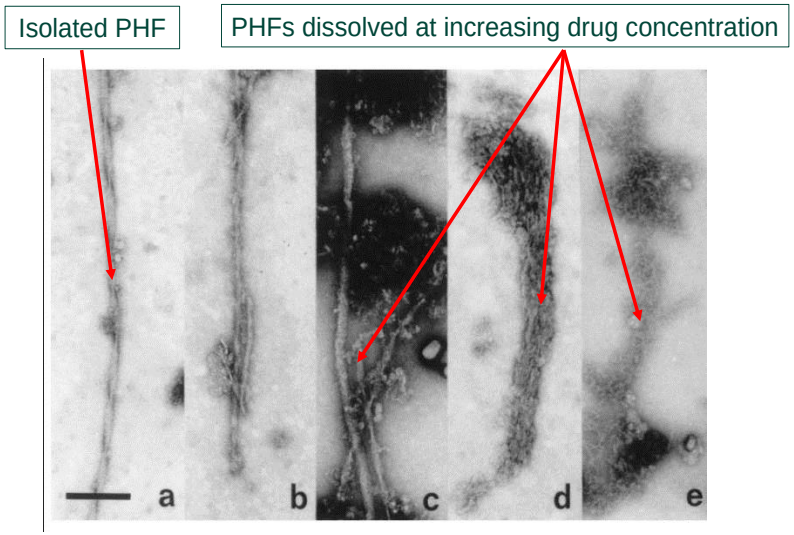
[1] Harrington et al., J Biol Chem, 2015, 290 10862-10875

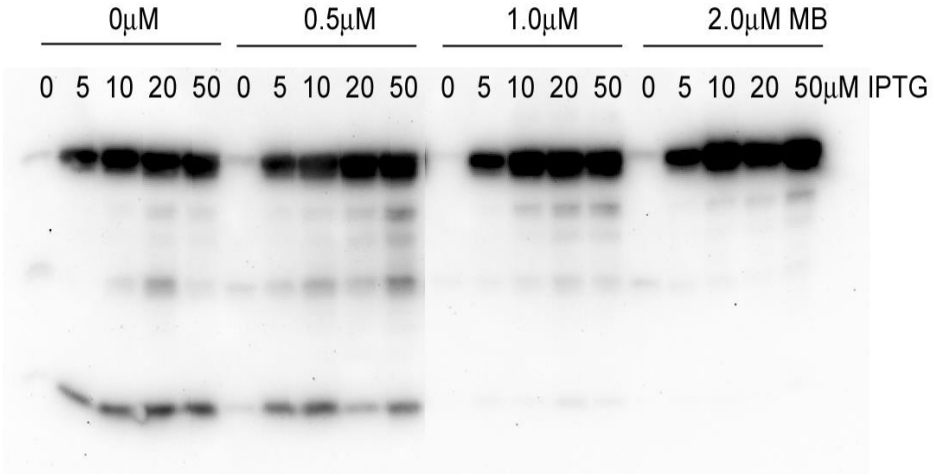
PHFs Isolated from AD Brain are Dissolved by MT and Proteolytic Stability of PHF-core Unit is Reversed[1]

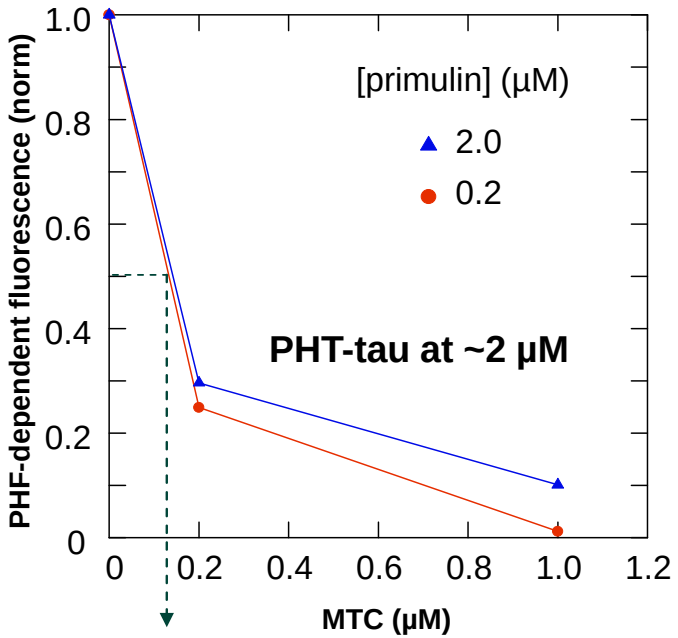


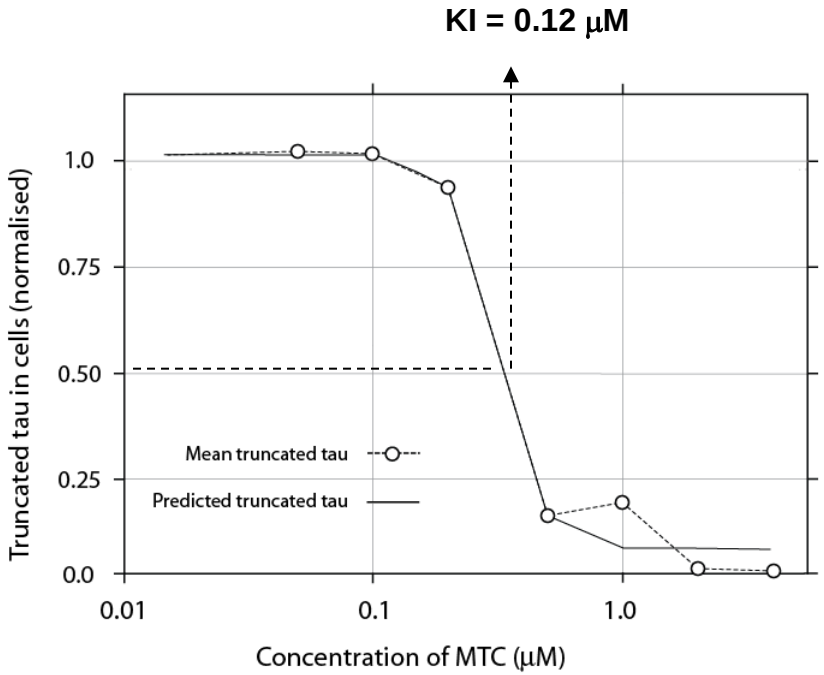
[1] Wischik et al., 1996, PNAS 93:11213-11218

PHF-based and Cell-based Activity of MT[1]



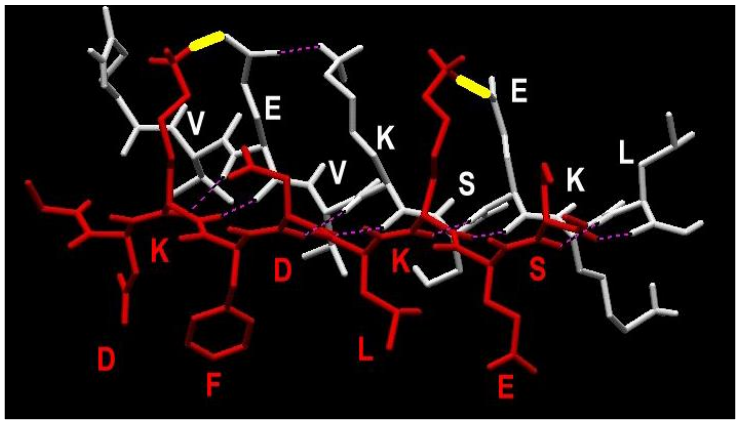




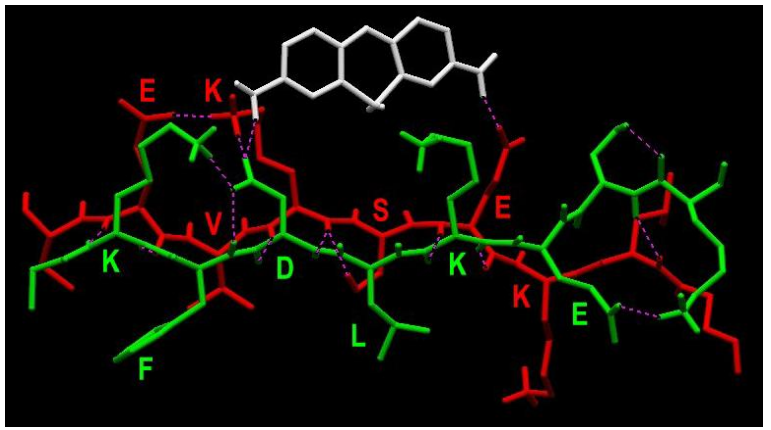


[1] Harrington et al., J Biol Chem, 2015, 290 10862-10875

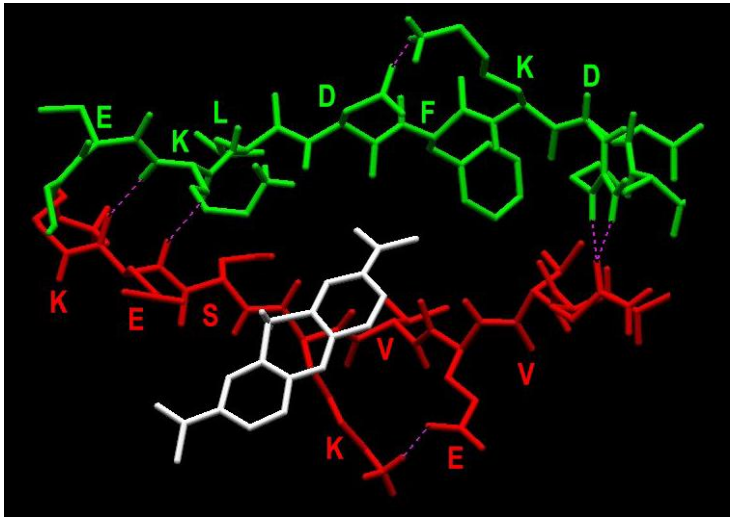
Hypothetical Molecular Dynamic Modelling of MT Action



A. β-sheet stabilised with salt bridges



B. Complexation with MT



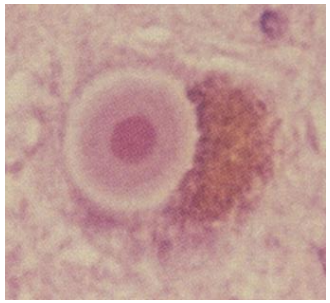
C. Disruption of β-sheet and release of MT

Similar Mechanism for Synuclein In Parkinson's Disease

Lewy bodies

Lewy FH (1912) Paralysis agitans. I. Pathologische Anatomie. Lewandowsky's Handbuch der Neurologie, 3 Band: Spez Neurologie II. Berlin: Springer; p 920–933.

Eosinophilic Lewy body



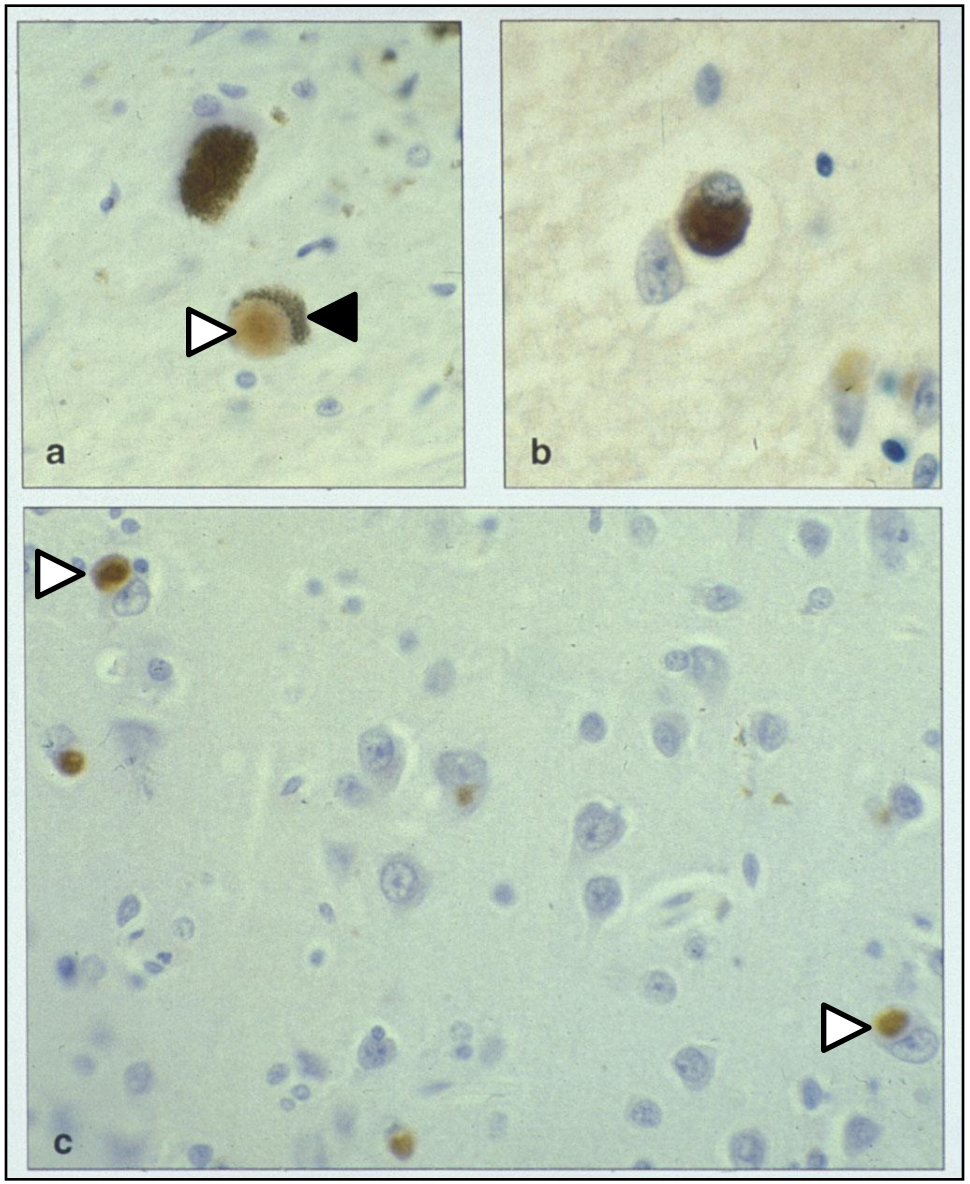
Ubiquitin-immunoreactive Lewy bodies in:

(a) substantia nigra

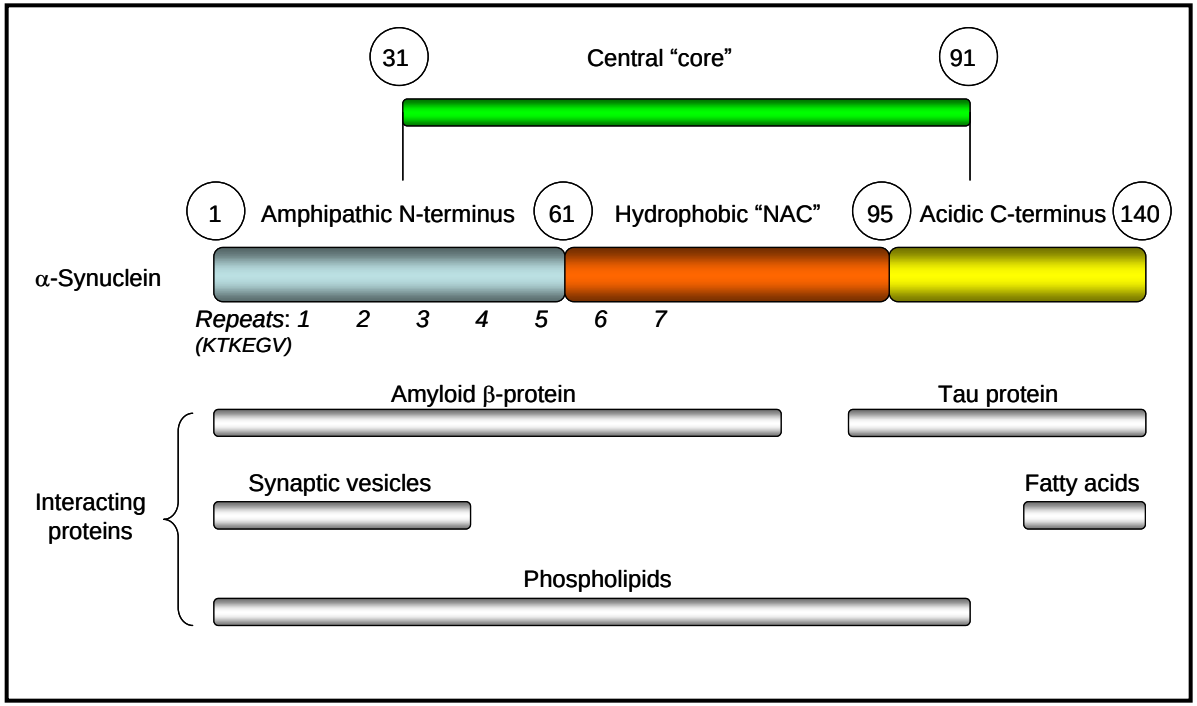
(b,c) cortex.

▷ LB

▶ melanin



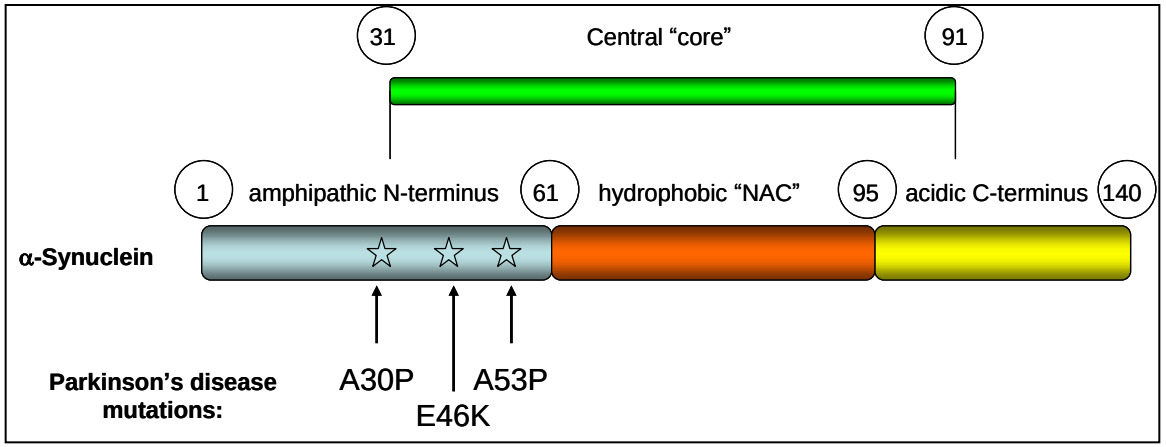
α-Synuclein protein



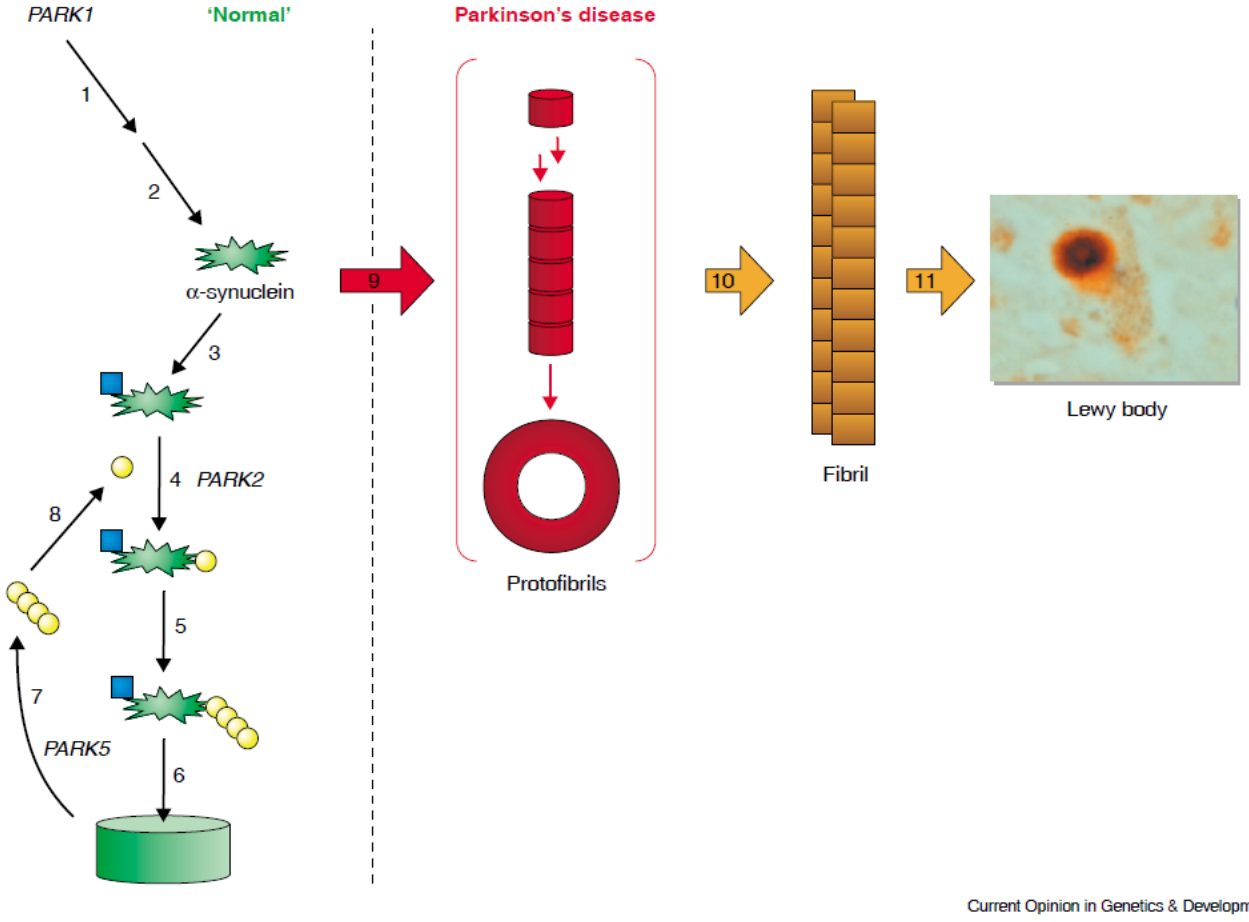
α-Synuclein: small, thermostable protein, lacking secondary or tertiary structure, but acquires a high level of α-helix on binding to membranes and acidic phospholipids.

Divided into three functional domains

Three pathogenic mutations in the N-terminal domain



Similar mechanism in Parkinson's disease: α-Synuclein

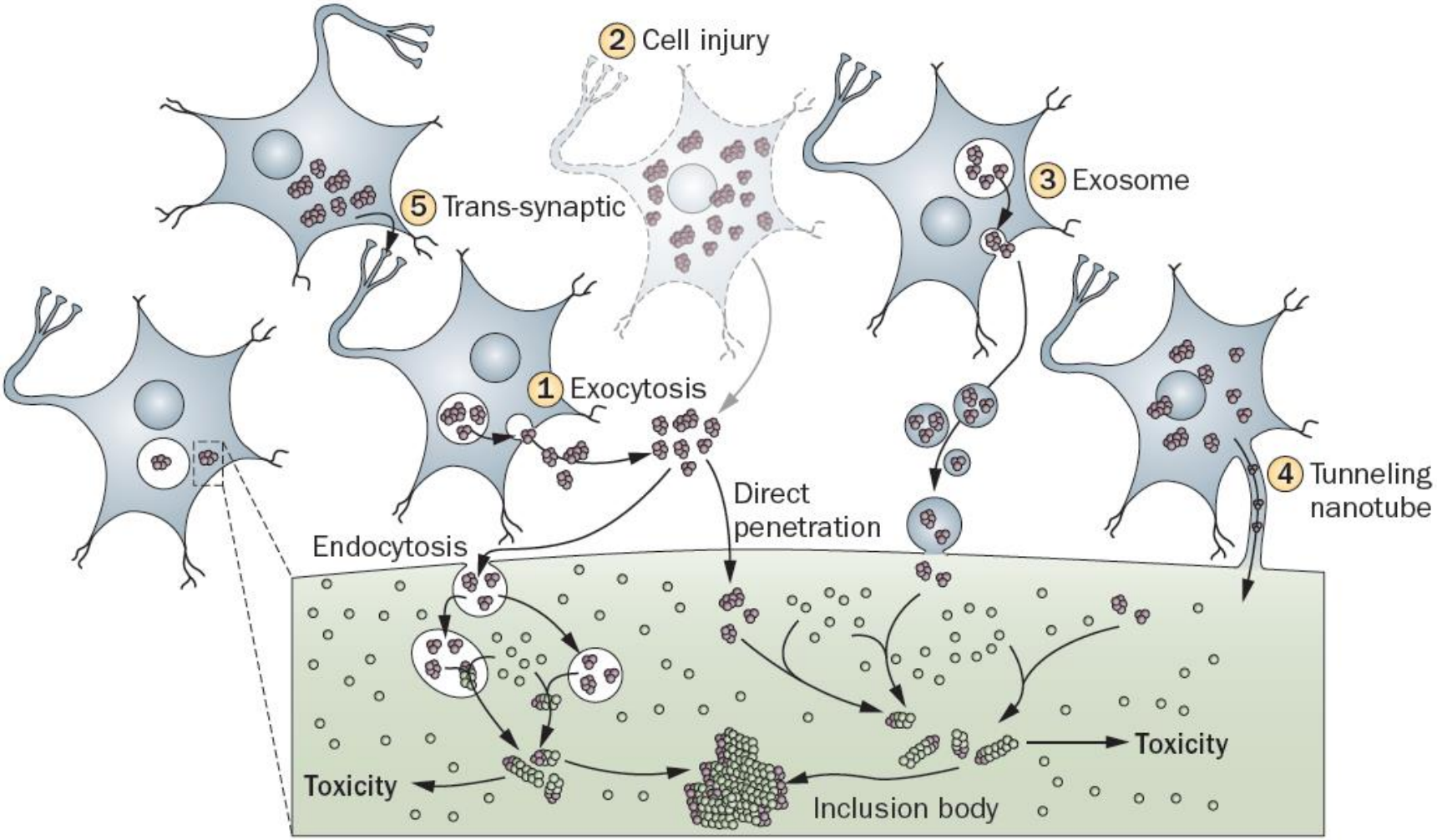


Similarities with tauopathies

- Intracellular lesions
- Aggregation of thermostable protein
- Sporadic PD and rare cases caused by mutations
- Selective neuronal vulnerability

From: Lansbury (2002)

α-Synucleinopathies: neuronal transmission like AD

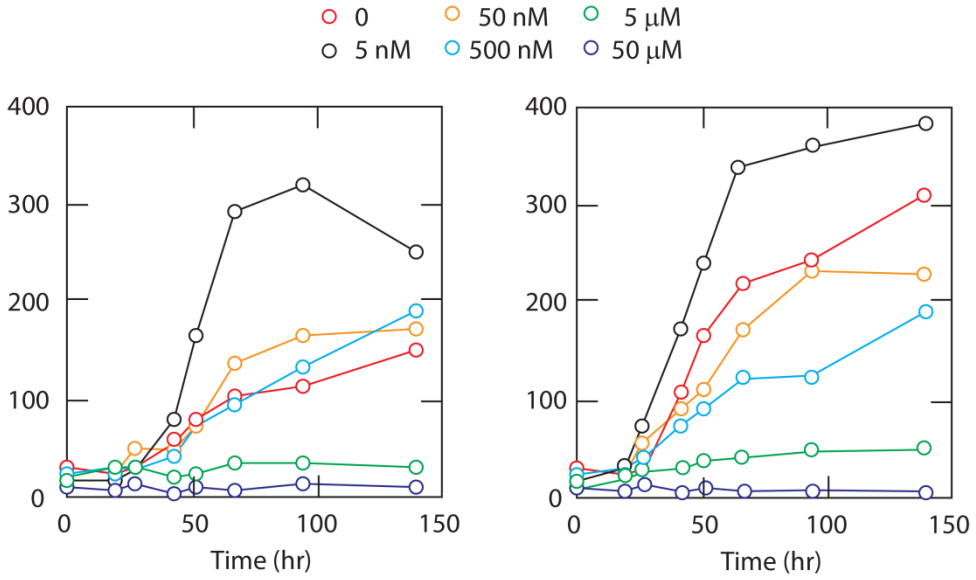


Lee et al. (2010) Nature Rev Neurol. 6_702

Cell-free assay of synuclein aggregation for aggregation-inhibitor activity

- Lewy bodies in PD are composed of synuclein aggregates
- TauRx's lead Synuclein Aggregation Inhibitor (SAI) blocks aggregation *in vitro*

Compound	B_{50} (μM)
LMTX	130.8 ± 15
TRx0018	3.8 ± 0.3

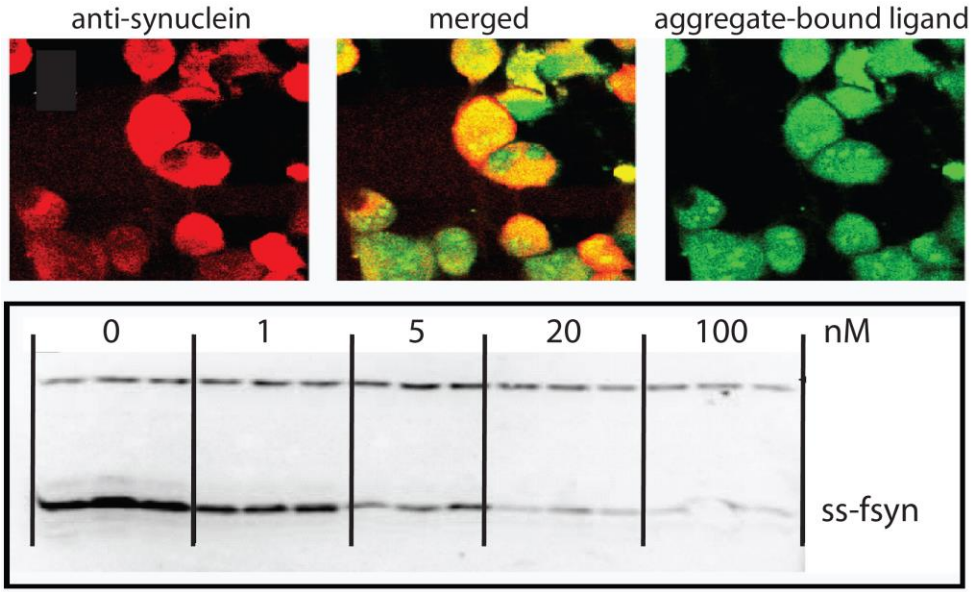


in vitro inhibition of heparin-induced synuclein aggregation

Cell-based model of synuclein aggregation for aggregation-inhibitor activity

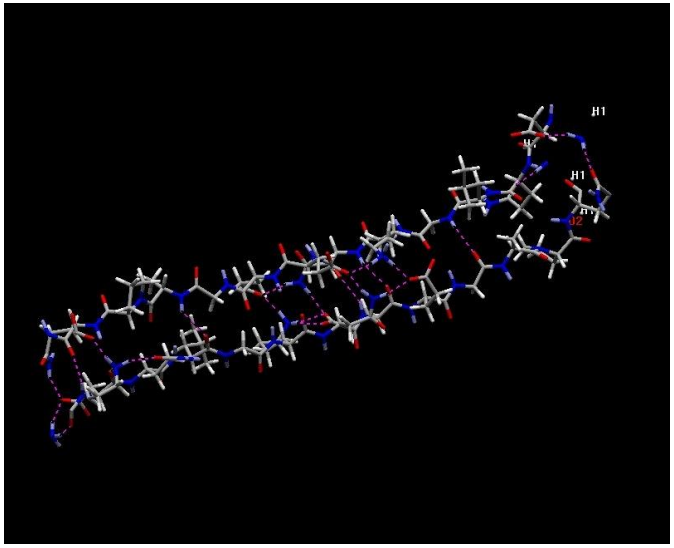
- Lewy bodies in PD are composed of synuclein aggregates
- TauRx's lead Synuclein Aggregation Inhibitor (SAI) blocks aggregation in cell model

Compound	EC_{50} (μM)	LD_{50} (μM)
LMTX	1.17 ± 0.30	62
TRx0018	0.08 ± 0.02	38

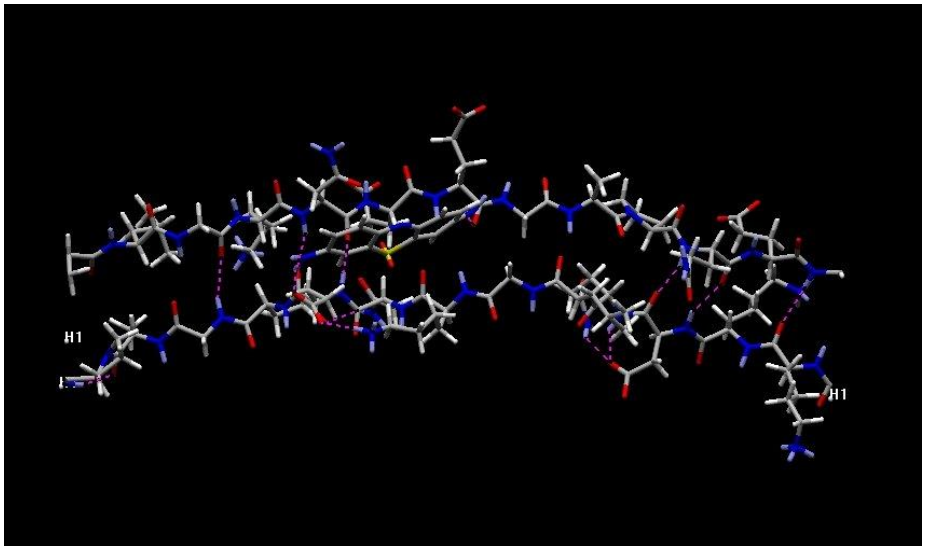


Cell-based inhibition of synuclein aggregation and truncation

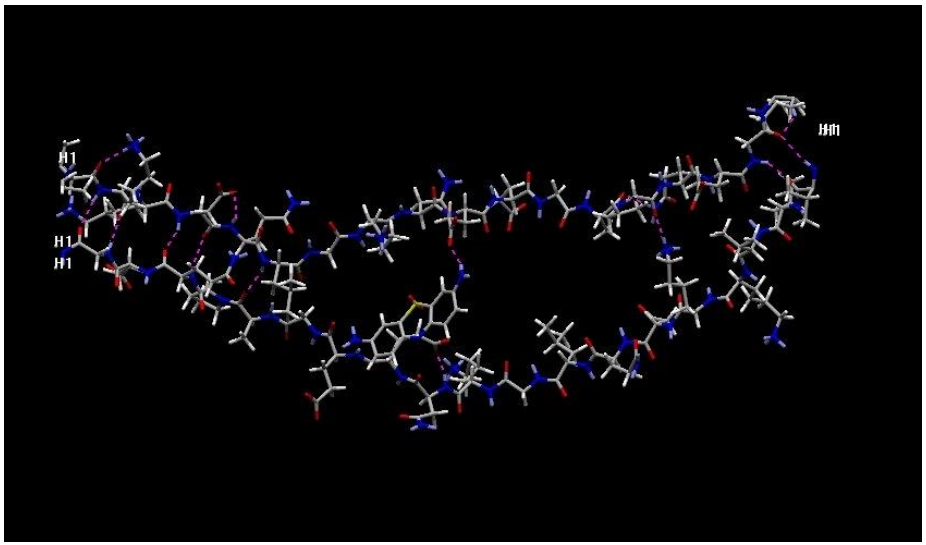
Molecular Dynamic Modelling of Synuclein aggregation: MT disrupts β-sheet structure around aggregation domain



A. High affinity “cage structure” stabilising β-sheet configuration

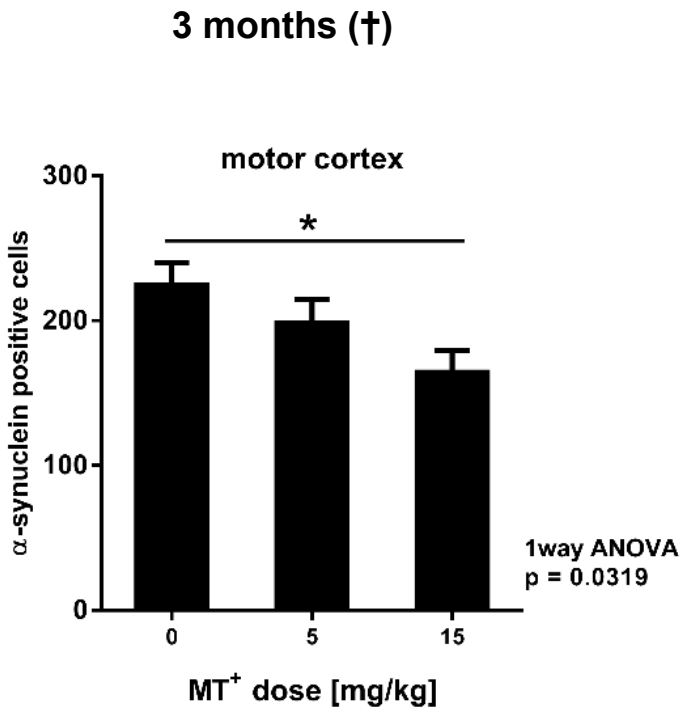


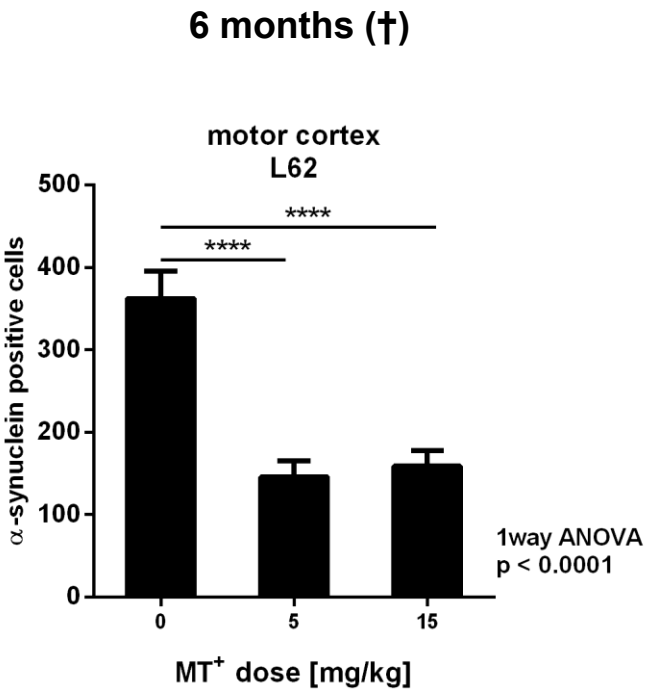
B. MT H-bonds to β-sheet and disrupts β-sheet backbone



C. Unravelling of β-sheet, and MT released

Effect of LMTX™ on counts of syn-positive cells in motor cortex in synuclein L62 mice

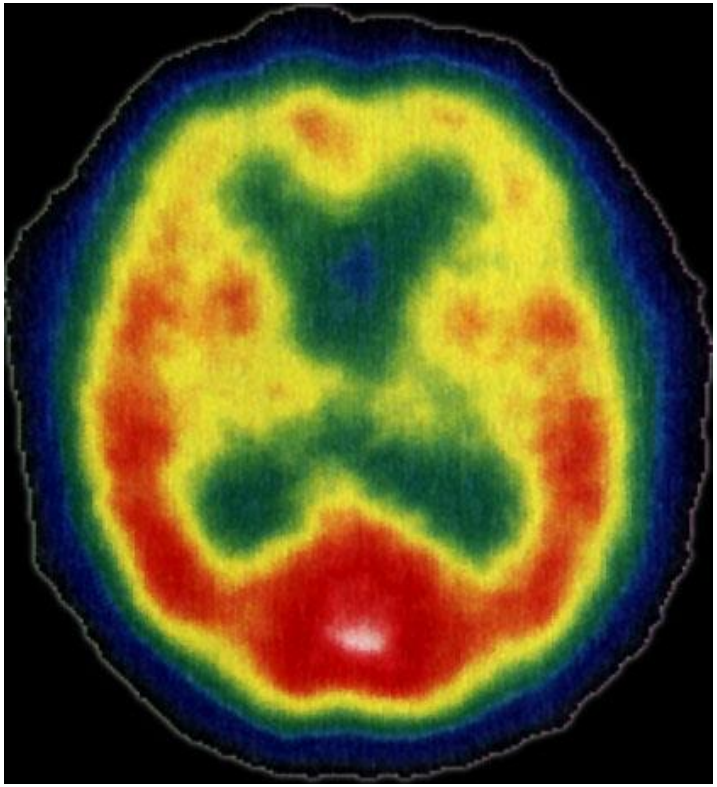




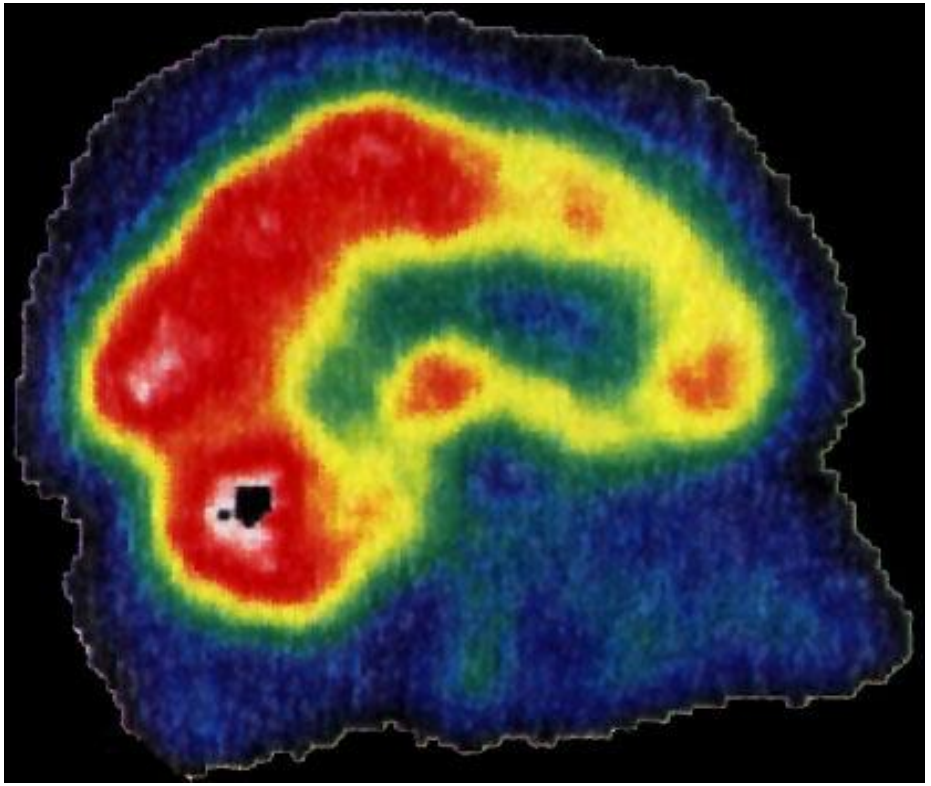
Strong efficacy signal with LMTM at 5 and 15 mg MT/kg/day
Values are represented as mean ± S.E.

Similar Mechanism for TDP43 in Fronto-Temporal Dementia

rCBF SPECT in FTD



Axial



Sagittal

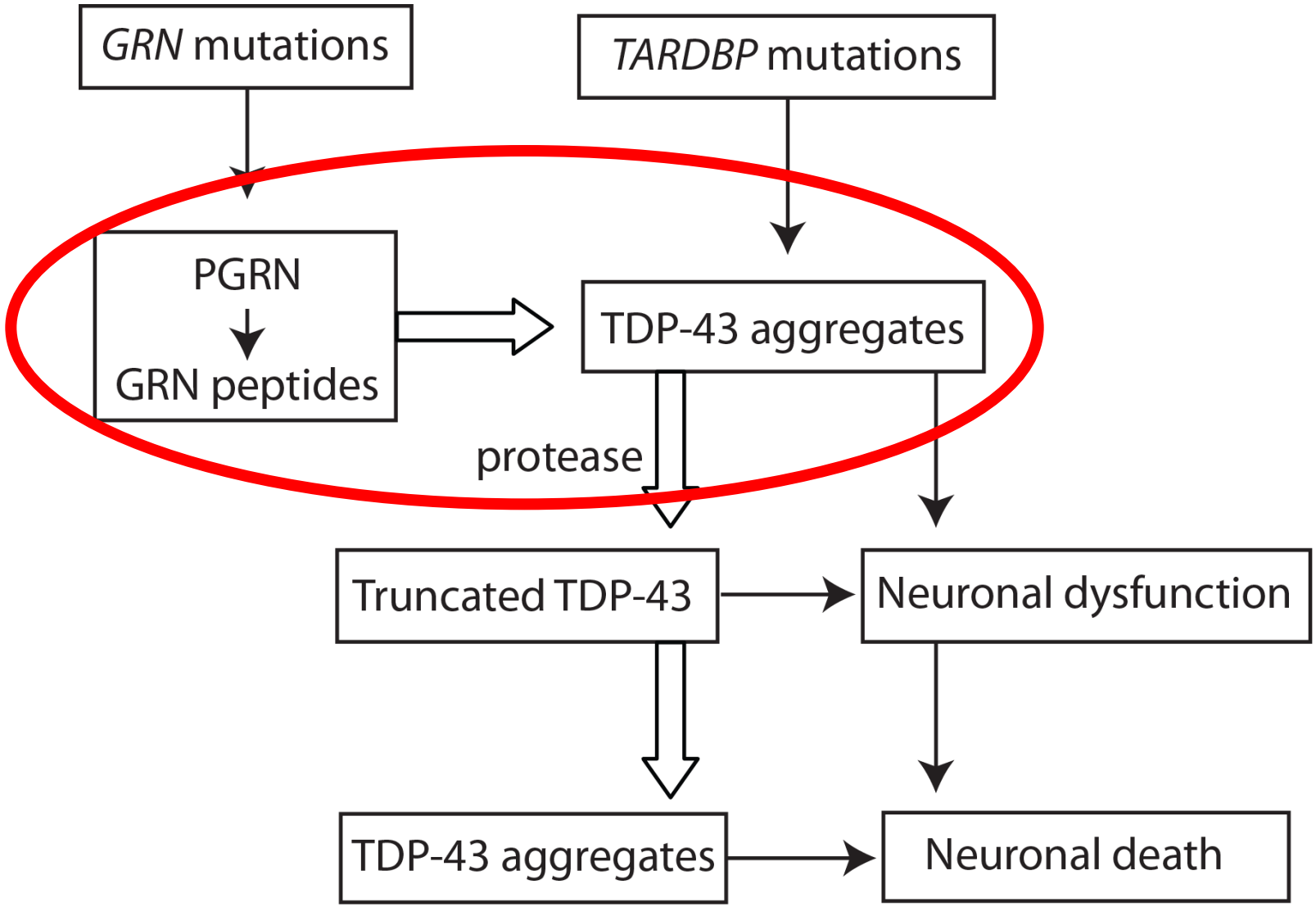
Pathology of FTLD syndromes

Relationship between Clinical Features, Pathology and Proteinopathies in FTLD sub-syndromes

[modified from Cairns et al, 2007 and Josephs 2008]

	Frontotemporal Lobar Degeneration Syndromes							
Sub-syndrome	**bvFTD**		**SD**	**PNFA**		**FTD-MND**	**FTDP-17** [*MAPT* or *GRN* mutations]	
Clinical features	Predominantly behavioural		Predom. language	Predominantly language		Behavioural Language + MND	Behavioural Language +/- EPS	
Histopathology	PiD-CBD-PSP-types	FTLD-U types 1-3	FTLD-U types 1-3	PiD-CBD-PSP-types	FTLD-U types 1-3	FTLD-U types 1-3	Tau +ve neuronal / glial inclusions	FTLD-U type 3
Molecular pathology	**Tau**	**TDP-43**	**TDP-43**	**Tau**	**TDP-43**	**TDP-43**	**Tau**	**TDP-43**

Molecular pathogenesis of TDP-43 proteinopathies: the aggregating species differs from the trigger species

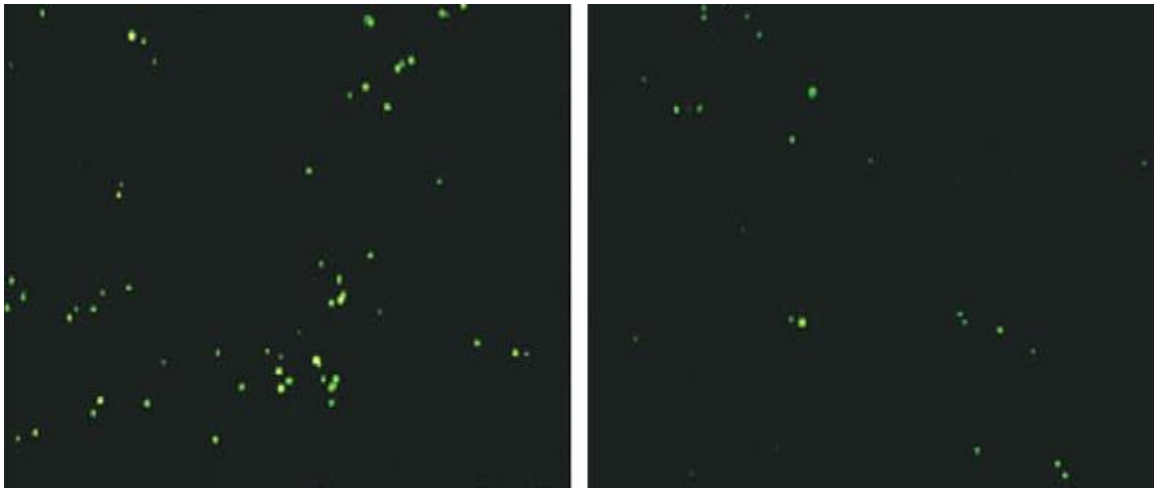


TDP-43 protein aggregation is inhibited by MT

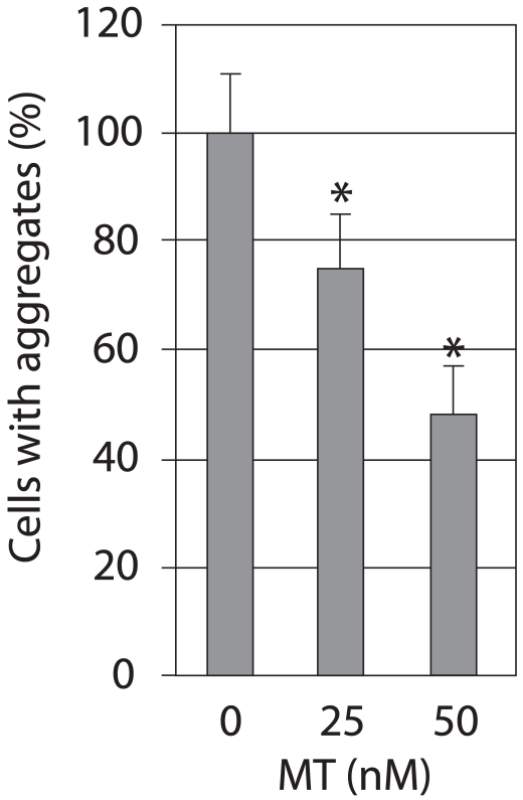
[Yamashita et al. (2009) FEBS Letters 583:2419-2424]

Two SH-SY5Y cell models: a) TDP-43(ΔNLS&187-192)
b) C-terminal TDP-43(162-414)

exhibit phosphorylated/ubiquitinated, TDP-43 aggregate inclusions



Control MT (50nM)



TDP-43 aggregates decreased 50%
by 50nM MT (in both models)

Similar Mechanism for Huntingtin In Huntington's Chorea

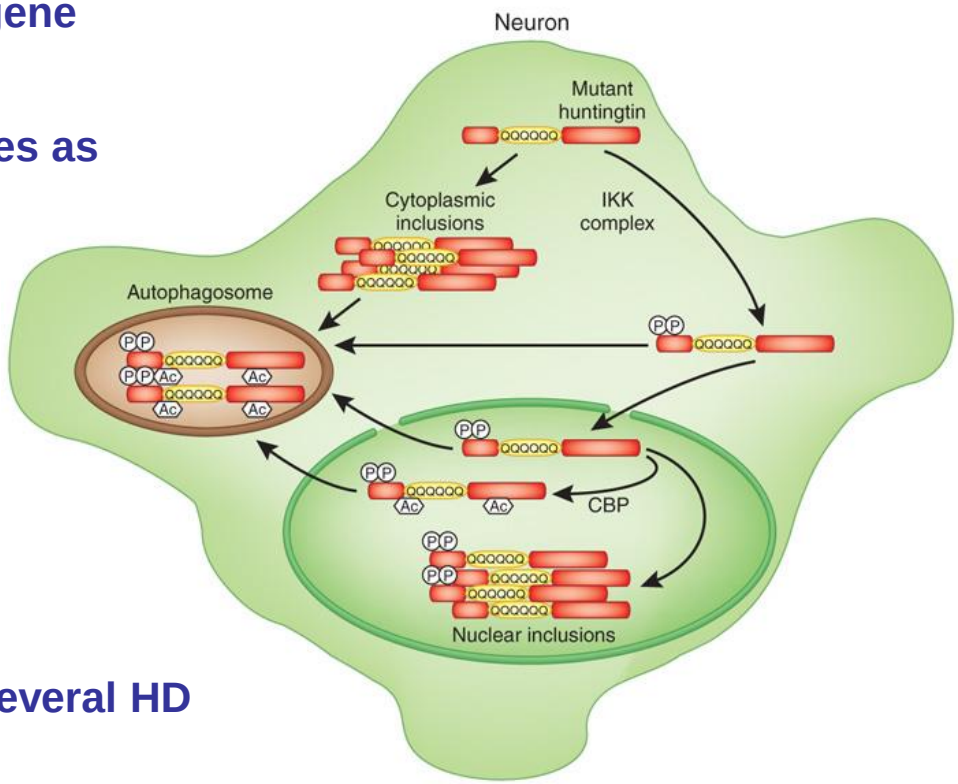
Huntington's Disease

Autosomal dominant disease – 1:10,000

Personality, cognitive and movement disorder

Caused by an expansion of the polyglutamine-coding CAG trinucleotide repeat in the gene encoding huntingtin (htt)

Mutant htt that is not cleared accumulates as inclusions in nucleus and cytoplasm



MT inhibits aggregation *in vitro* and in several HD models

Krainc D (2010) Nature Genet 16:32

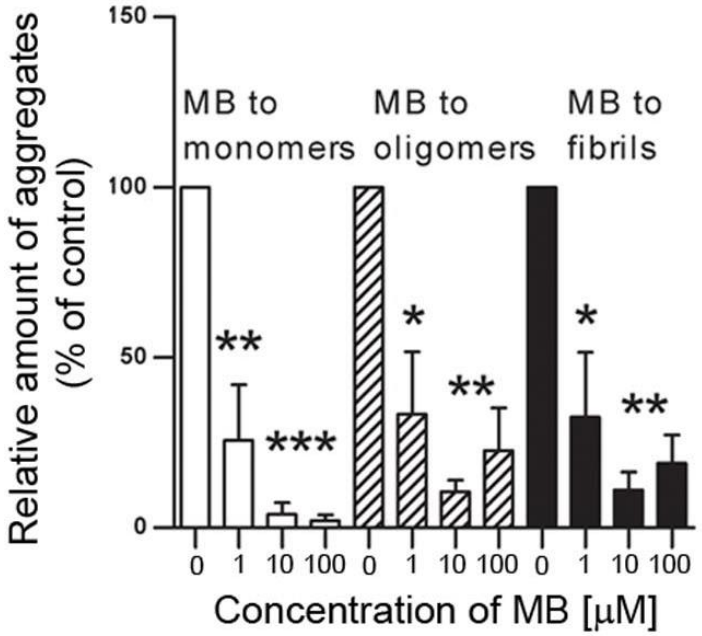
Huntingtin aggregation inhibited by MT (1)

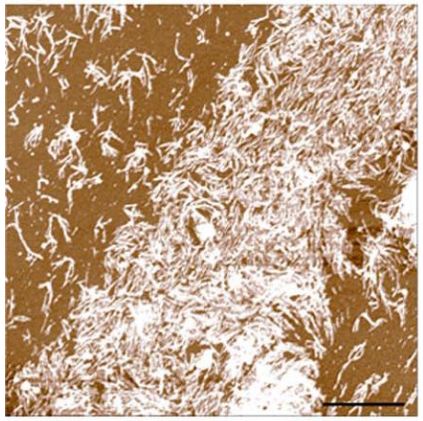
Sontag *et al* (2012) J Neurosci 32:11109

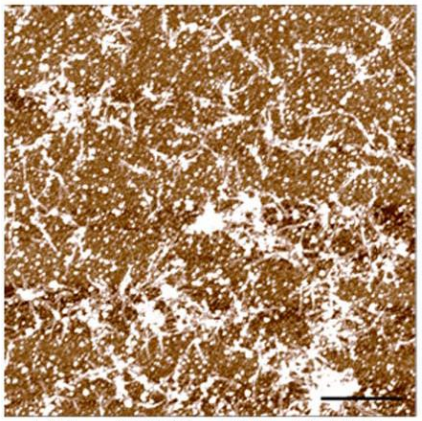
1

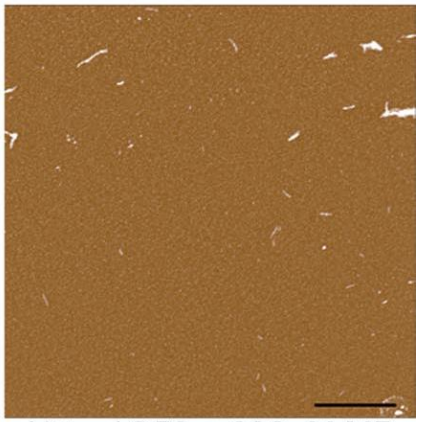
in vitro

MT inhibits aggregation of N-term-httex1Q53 from either monomers or oligomers/fibrils



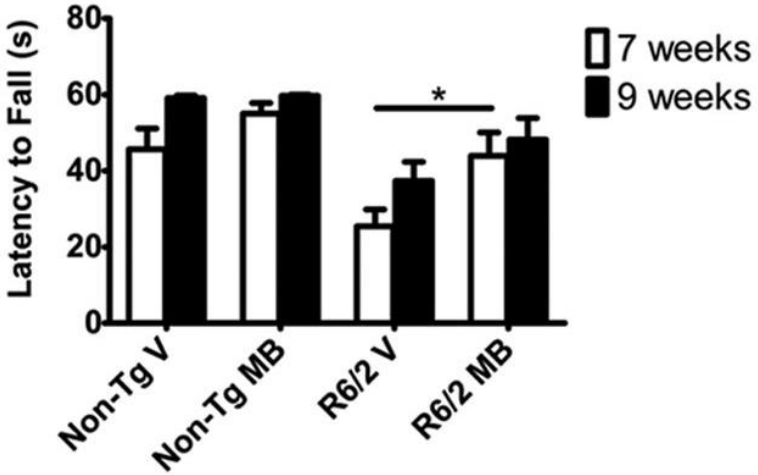

Httex1Q53 + 0µM MB


Httex1Q53 + 20µM MB


Httex1Q53 + 300µM MB

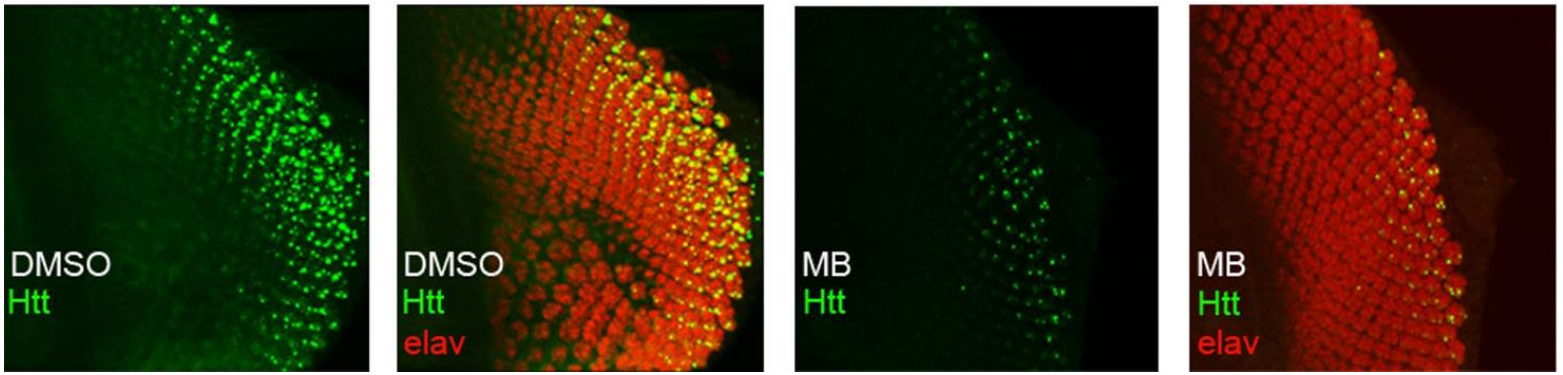
Huntingtin aggregation inhibited by MT (2)

Sontag *et al* (2012) J Neurosci 32:11109



2

MT ameliorates **RotaRod** phenotype in *R6/2* mouse (115 CAG repeats)



3

MT decreases aggregation in ***Drosophila*** expressing Httex1Q93
& increase in number of rhabdomeres per omatidium

Conclusions

- Prion-like processing of aggregating proteins is increasingly recognised as a common underlying mechanism of molecular pathogenesis in a number of progressive neurodegenerative disorders
- This involves a primary conformational change in a critical aggregating domain that becomes self-propagating
- The oligomers produced in this way are able to infect neighbouring neurones, invading previously unaffected brain regions
- The aggregating domain has a distinctive conformation which makes it amenable to pharmaceutical inhibition
- The methylthioninium (MT) moiety has promise for treatment of a range of neurodegenerative disorders of late life

Neurodegenerative disorders potentially treatable with LMTX™

Cognitive disorders:

AD	Alzheimer's disease (Tau)
FTLD	Fronto-temporal dementia (Tau / TDP43)
CTE	Chronic traumatic encephalopathy (Tau)

Movement disorders:

PSP	Progressive supranuclear palsy (Tau)
CBD	Cortico-basal degeneration (Tau)
PD	Parkinson's disease (Synuclein)
HD	Huntington's disease (Huntingtin / Tau)