Inhibiting aggregation of amyloidogenic proteins as a strategy for treating neurodegeneration

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Aufzien Center Conference
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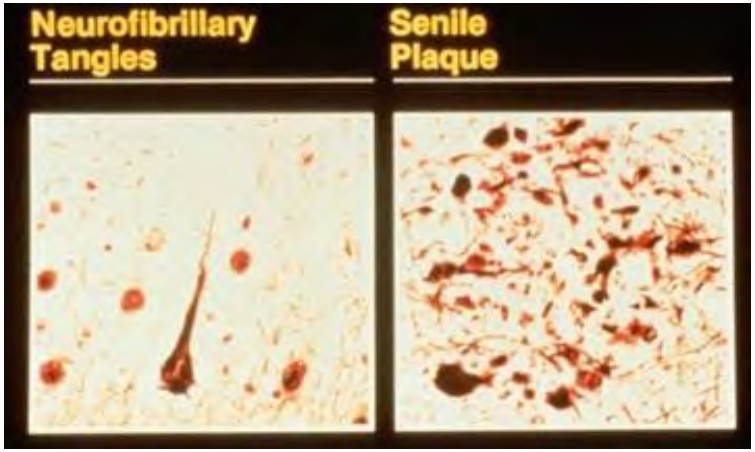
Amyloid deposits are key factors in various neurodegenerative diseases

Alzheimer's disease

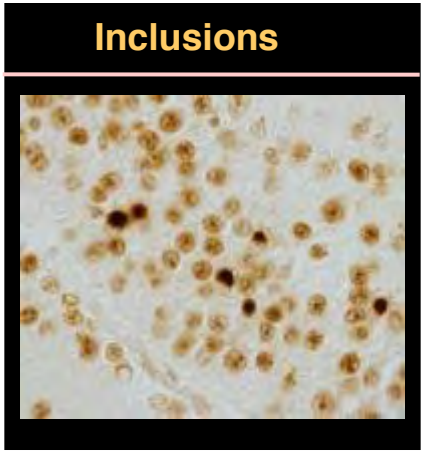


Tau Amyloid β

Parkinson

α-synuclein

ALS

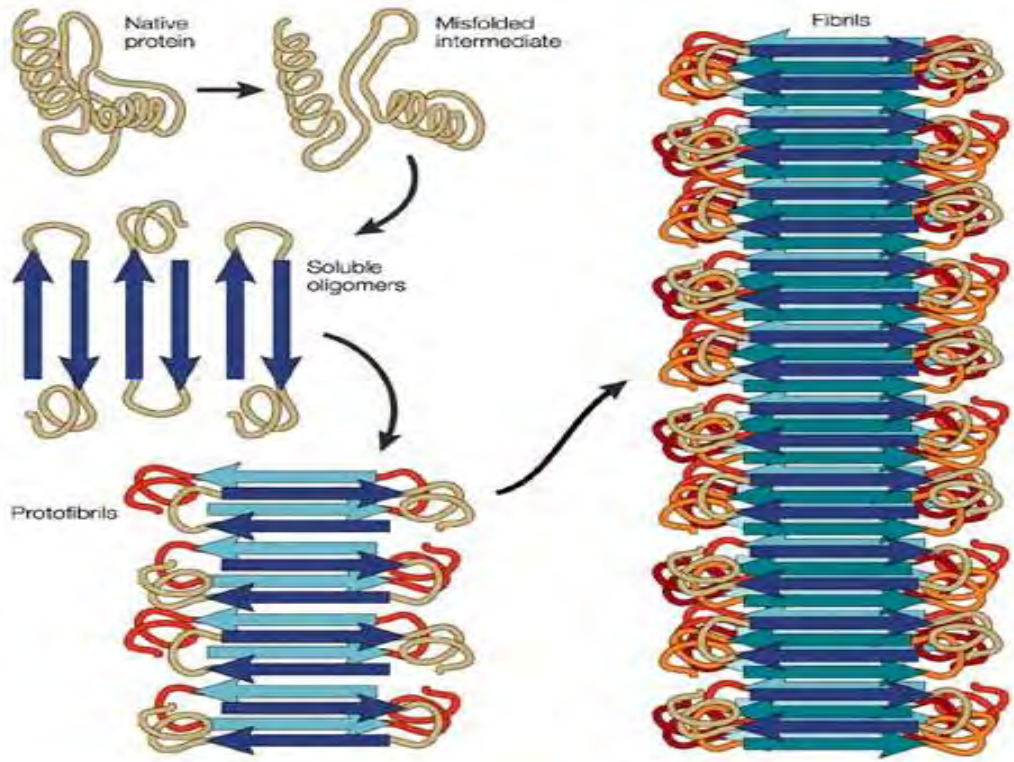


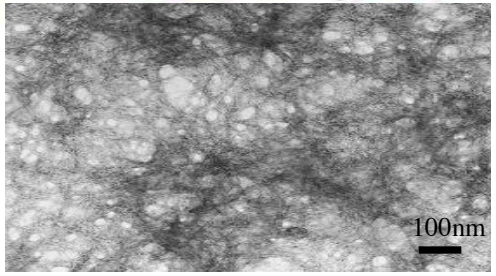
TDP-43

Amyloids of different origins have common characteristics despite no similarity in amino acid sequence

Amyloids:

- Protein filaments (size: nM-µM)
- Composed of aggregated peptide β-sheets.
- Formed by self-assembly of a misfolded protein





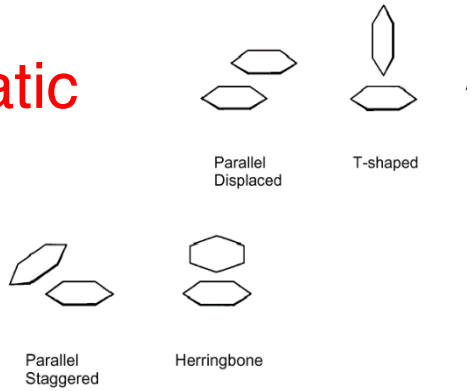
Aromatic interactions play a key role in the self assembly of amyloids

Table 1. Typical amyloid fibril formation by remarkably short aromatic peptide fragments[a].

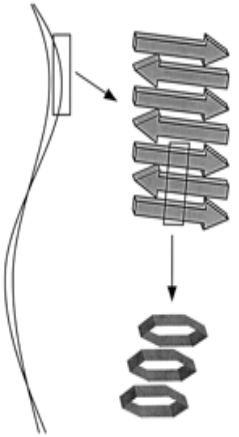
Name of parent peptide	Pathological or physiological condition	Amyloidogenic sequence
Islet amyloid polypeptide	Type II diabetes	NFGAIL
		NFLVH
Amyloid β-peptide	Alzheimer's disease	KLVFFAE
Medin	Aortic medial amyloid	NFGSVQ
Calcitonin	Thyroid carcinoma	DFNKF
Gelsolin	Finnish hereditary amyloidosis	SFNNGDCCFILD[b]
Serum amyloid A	Chronic inflammation amyloidosis	SFFSFLGEAFD[b]
β2-microglobulin	Dialysis-associated renal amyloidosis	DWSFYLLYTEFT[b]
Designed peptide	None	KFFE

[a] Aromatic residues are underlined. [b] The minimal active fragment may be shorter.

π- π aromatic stacking



Gazit, *Bioinformatics*, (2002)



Gazit, *FASEB J.*, (2002)

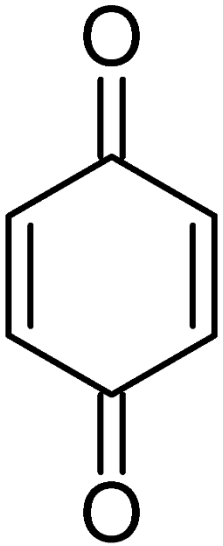
Strategy #1

Prevent self assembly of amyloids

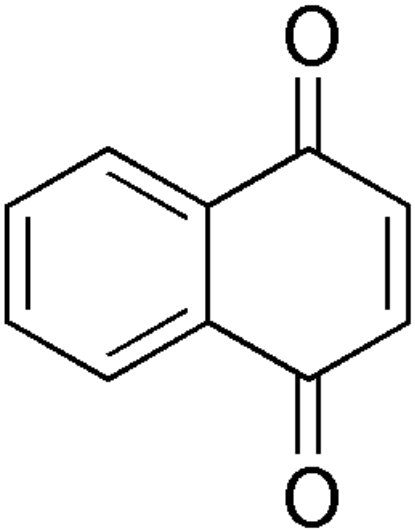
Using small molecules composed of:

1) **Aromatic core**
2) **Moiety that will confer steric hindrance on interacting amyloid monomers**

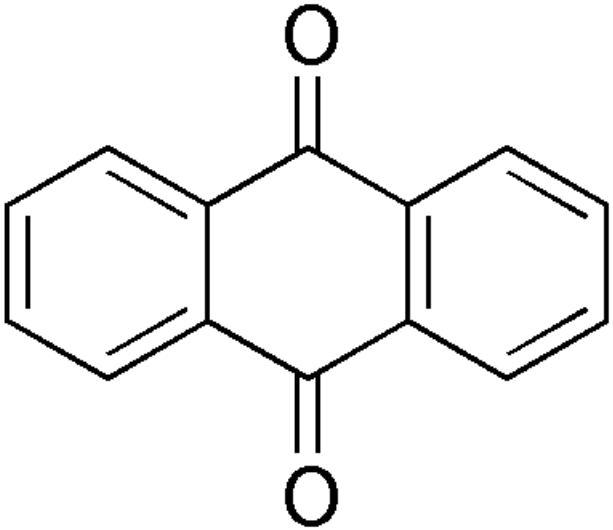
Example for small molecules: quinones



1,4- Benzoquinone



1,4- naphthoquinone

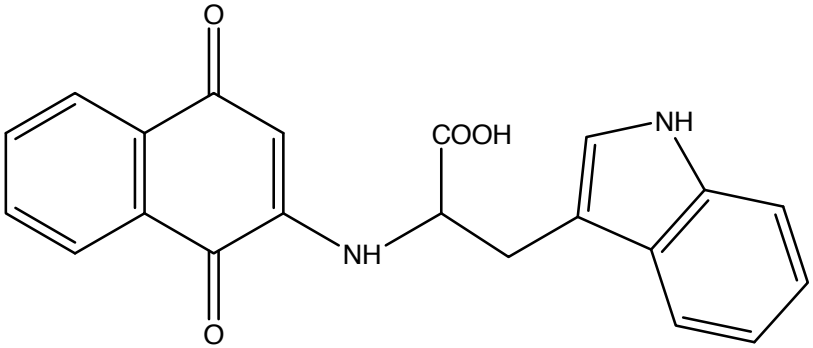


9,10-Anthaquinone

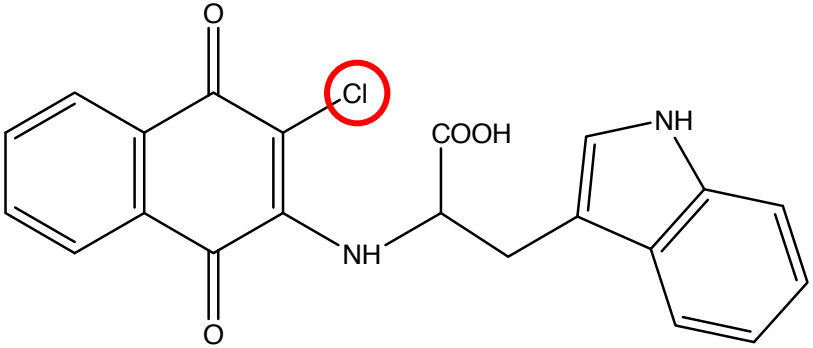
Why quinones?

- Small aromatic molecules.
- Easy and inexpensive to synthesize.
- Known as inhibitors of various metabolic pathways.
- Natural and synthetic quinones serve as anti-bacterial, anti-viral, and anti-cancer agents.
- Danthron (1,8-dihydroxyanthraquinone) reduces neurotoxicity related to β-amyloid.

Tryptophan-Naphthoquinone hybrids



NQTrp

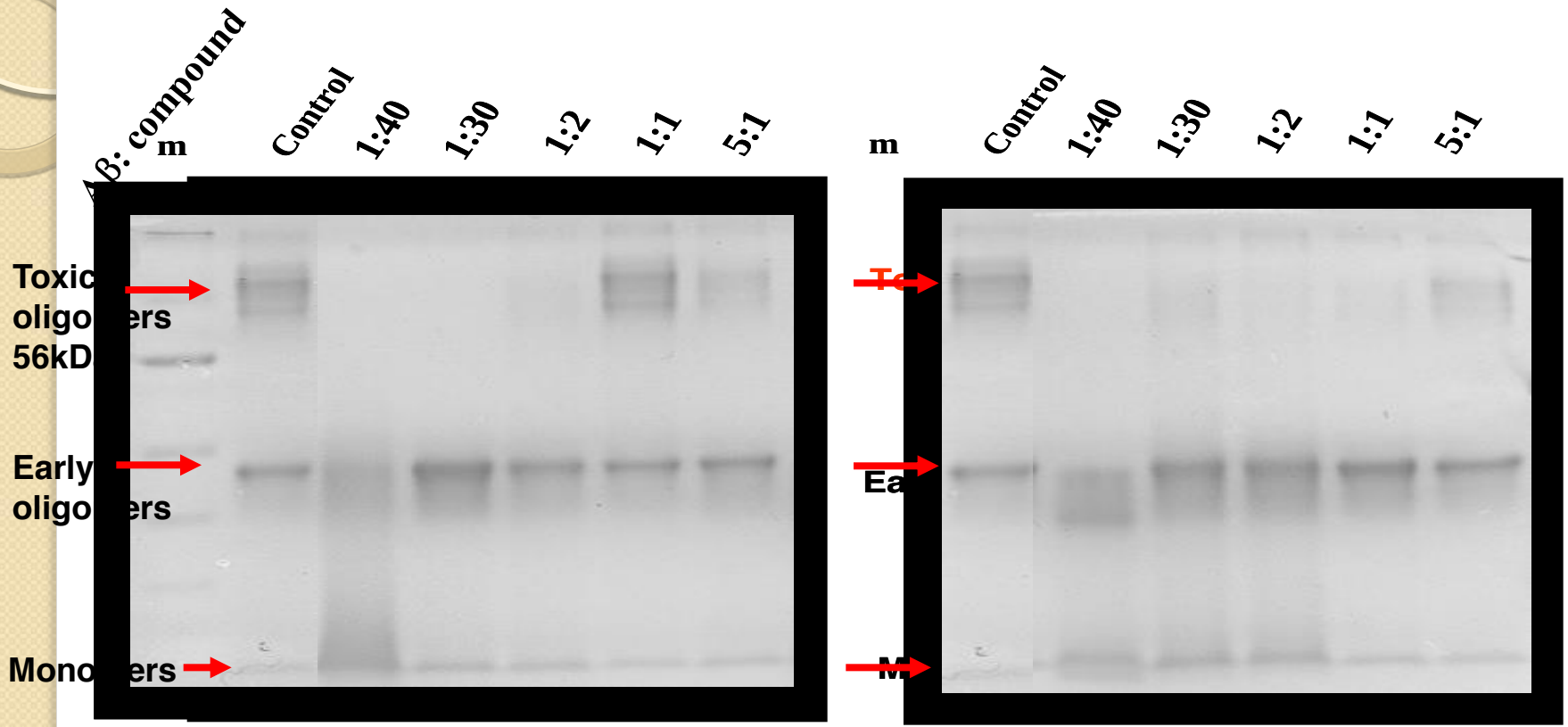


Cl-NQTrp

MW: 360, 395

Trp is the most amyloidogenic-prone amino acid (Pawar et al, JMB 2005)

Both compounds inhibit formation of toxic $A\beta_{1-42}$ oligomers



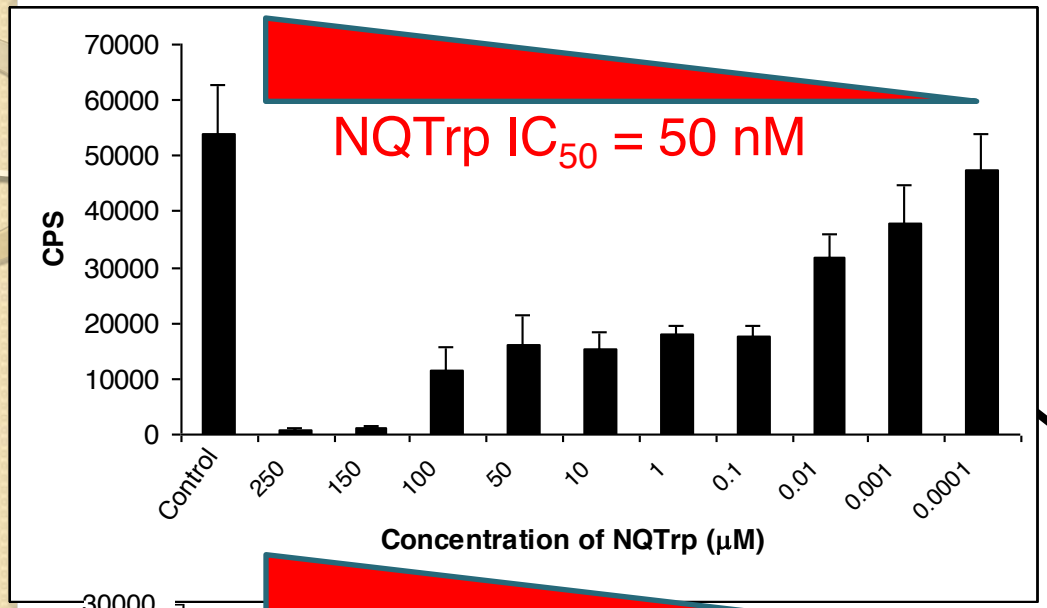
NQTrp **Cl-NQTrp**

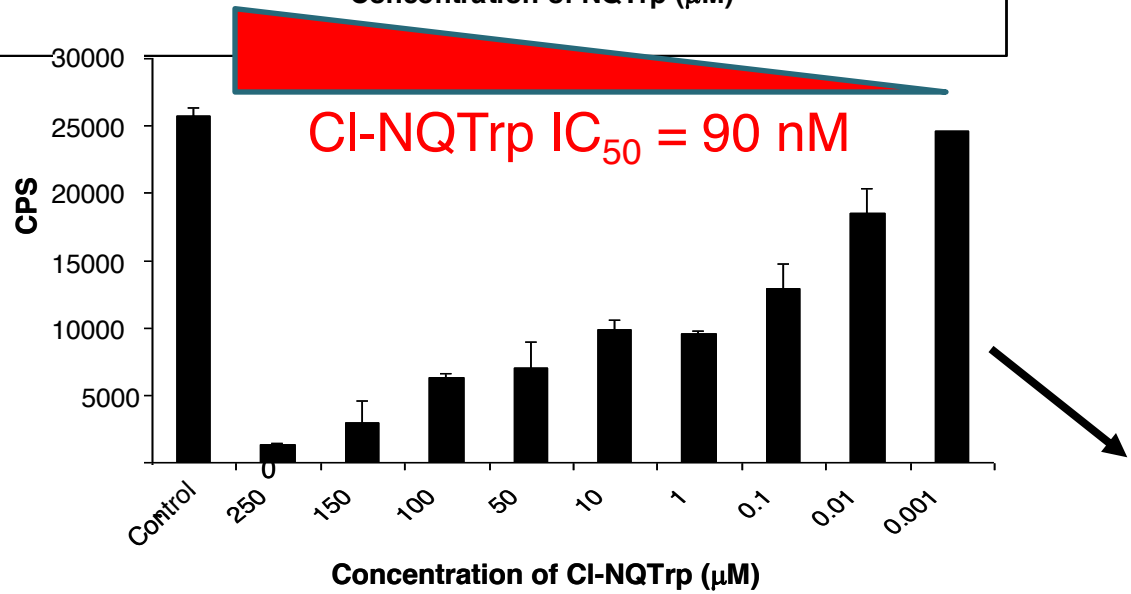
SDS tris-tricine gel

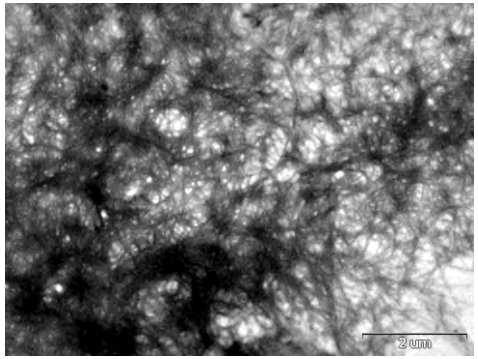
Scherzer-Attali *et al.* PloS ONE 2010

Scherzer-Attali *et al.*, Neurobiology of Disease 2012

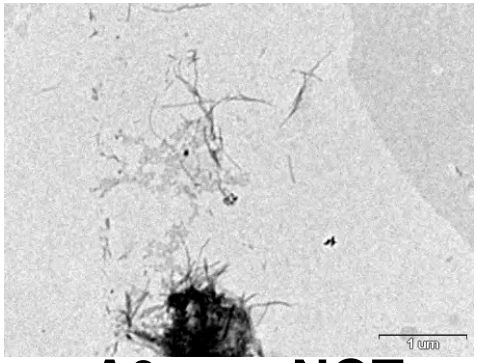
Both compounds inhibit $A\beta_{1\text{-}42}$ fibril formation (ThT fluorescence assay)



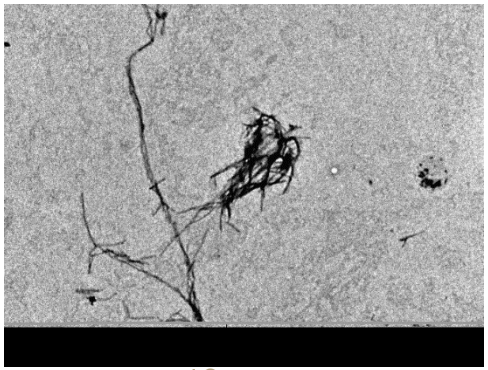




$A\beta_{1-42}$ only



$A\beta_{1-42}$ + NQTrp

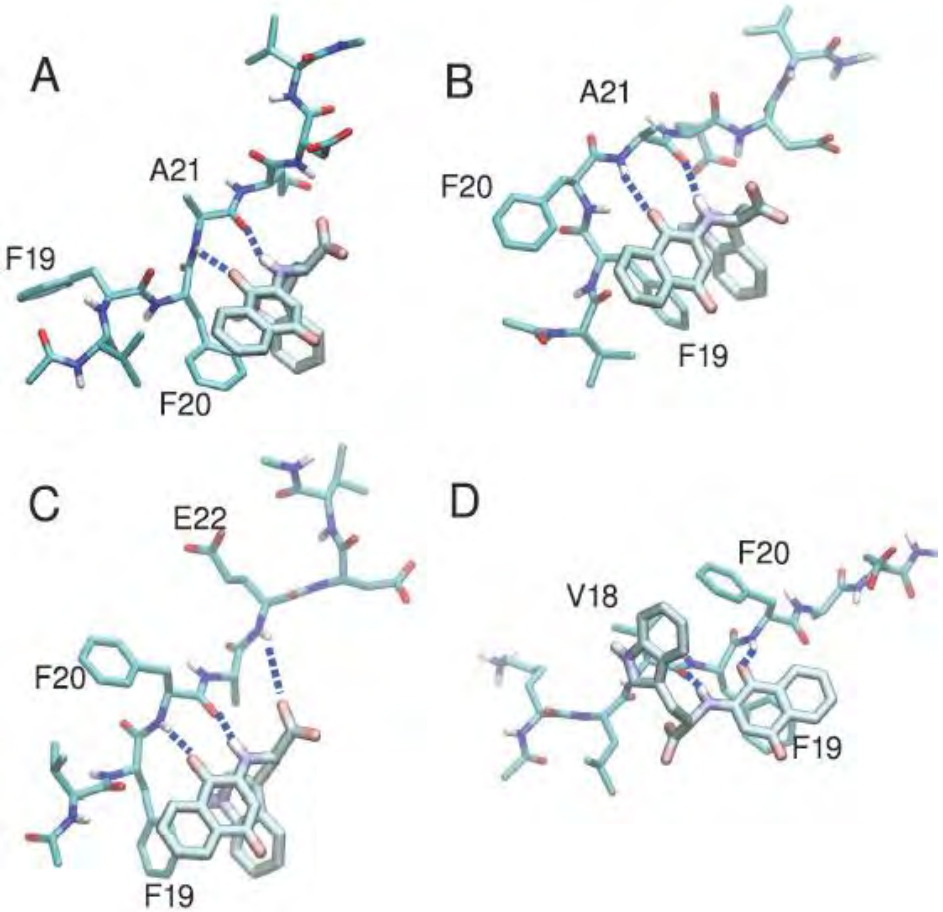


$A\beta_{1-42}$ + Cl-NQTrp

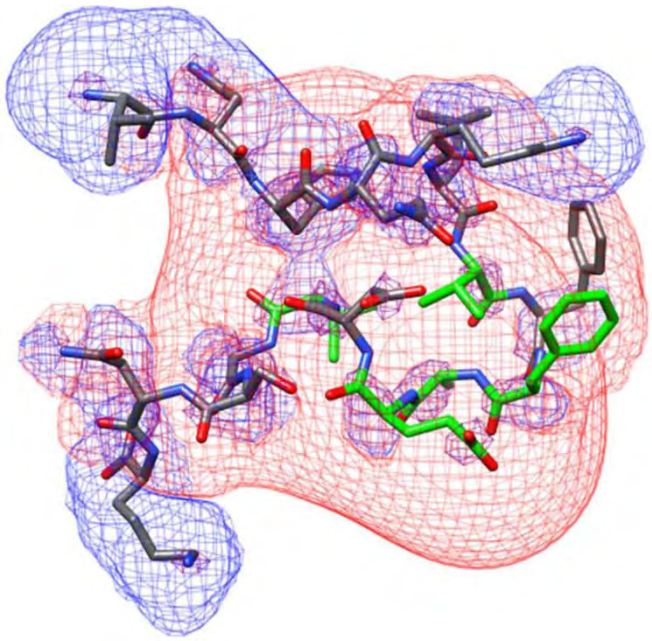
Scherzer-Attali *et al.* 2010; 2012

Compounds indeed bind the aromatic residues in Aβ

Molecular modeling

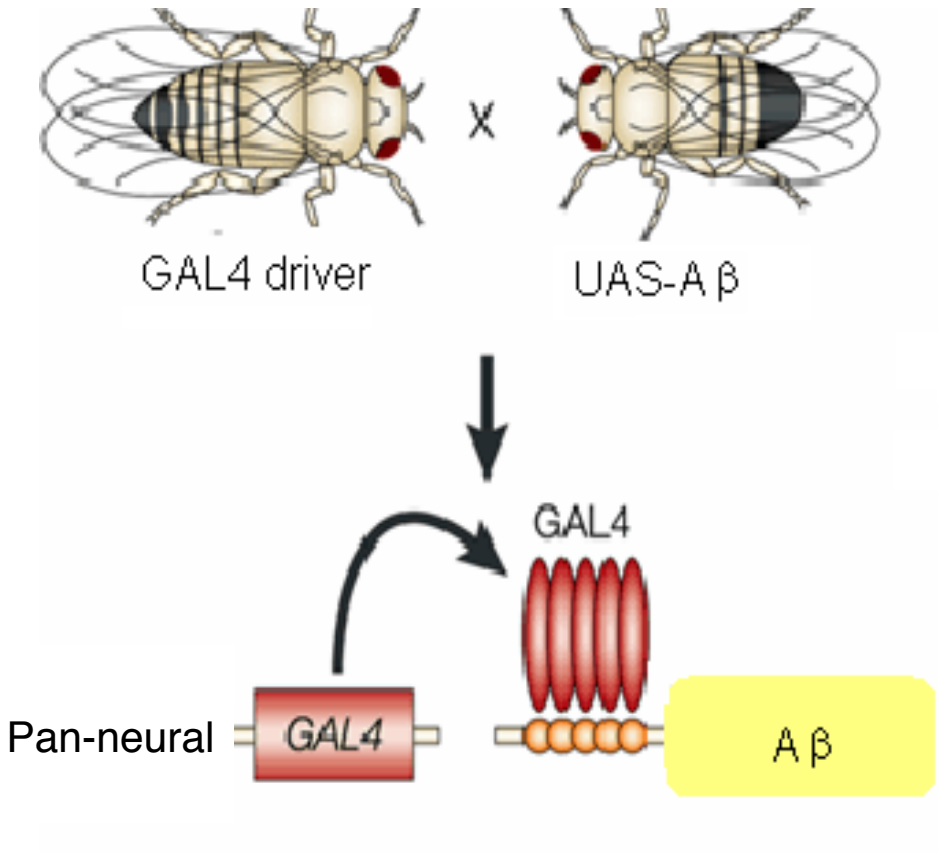


NMR



Scherzer-Attali *et al.* PloS ONE 2010

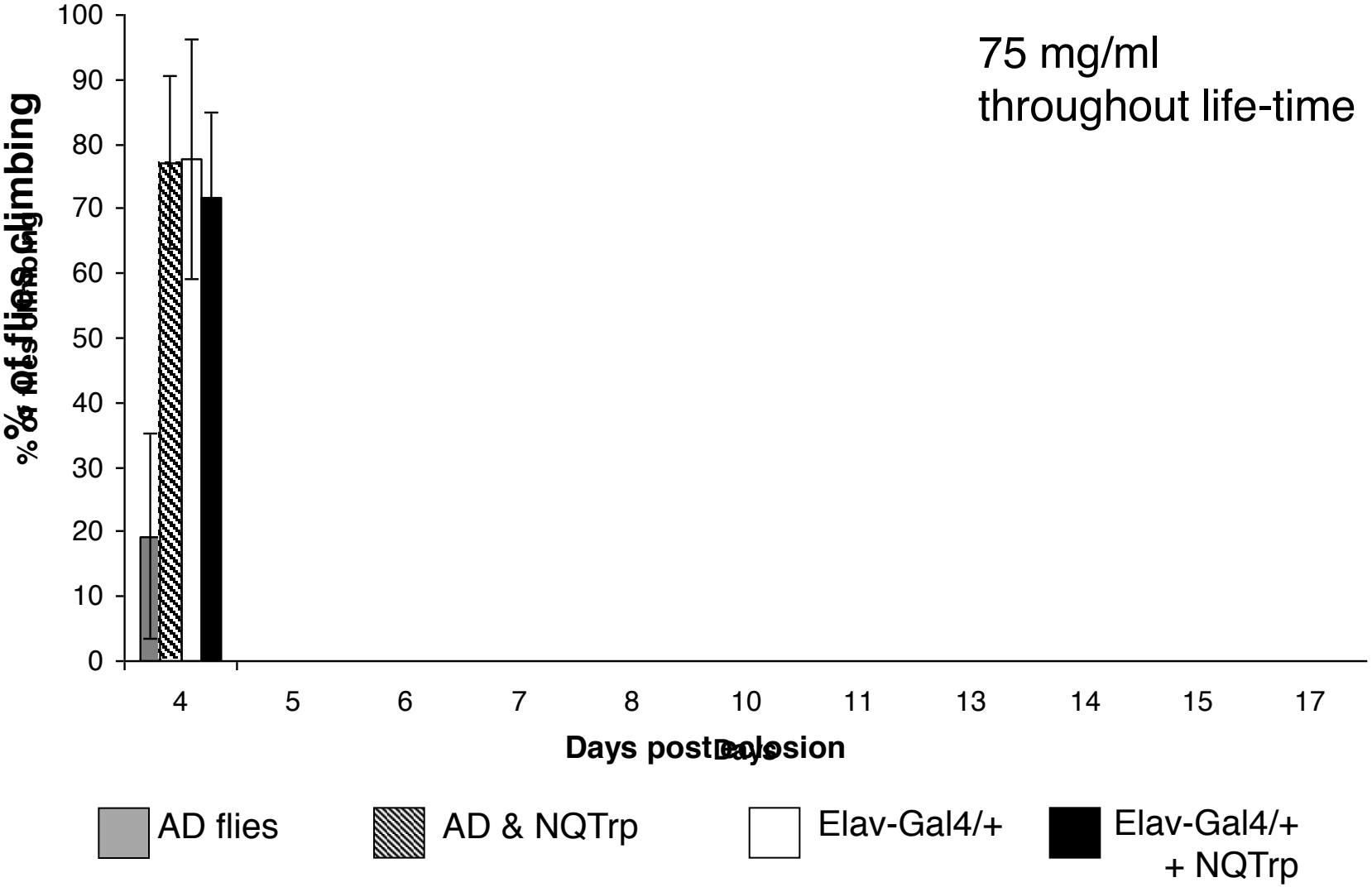
Targeting expression of Aβ pan-neuronally (elav-GAL4) causes brain pathology and abnormal behavior



- Declining memory
- Declining motility
- Reduced life span
- Brain pathology
- Aβ aggregates

e.g. Finelli *et al*., Mol Cell Neurosci, 2004

Compounds fed to Aβ_{1-42}-expressing Drosophila restore climbing ability



Scherzer-Attali *et al.* PloS ONE 2010; 2012

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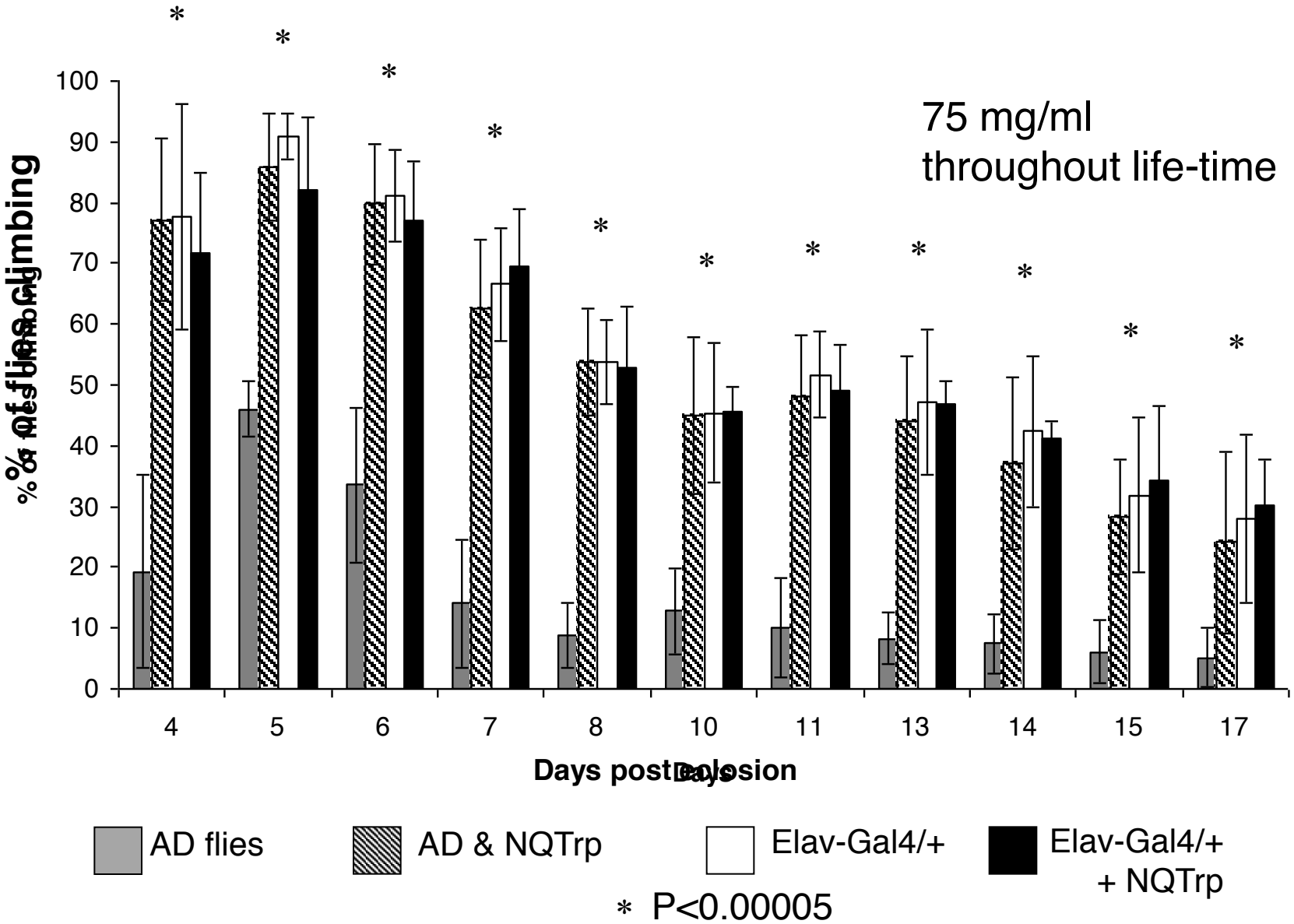
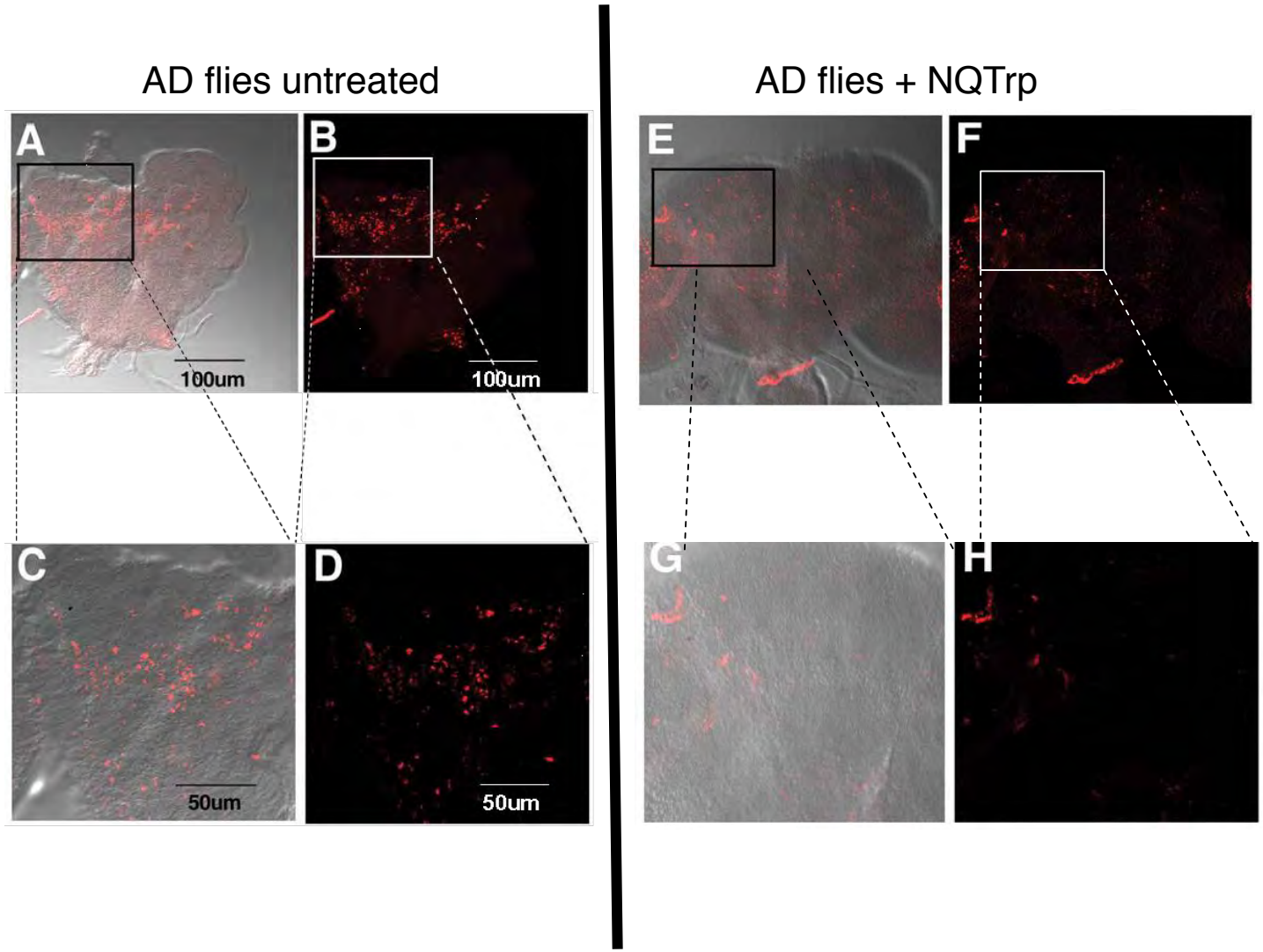


Scherzer-Attali *et al.* PloS ONE 2010; 2012

Compounds reduce $A\beta_{1-42}$ load in fly brain

Congo red staining

Verified using anti Aβ antibody (6E10)

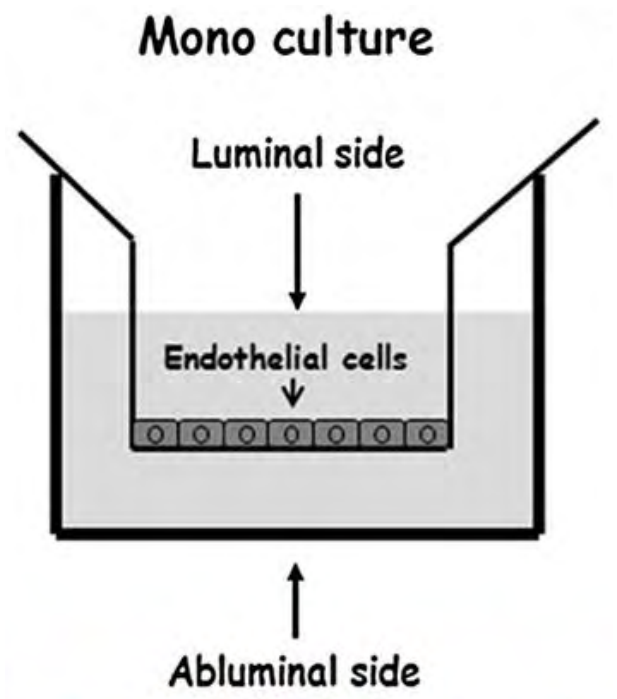


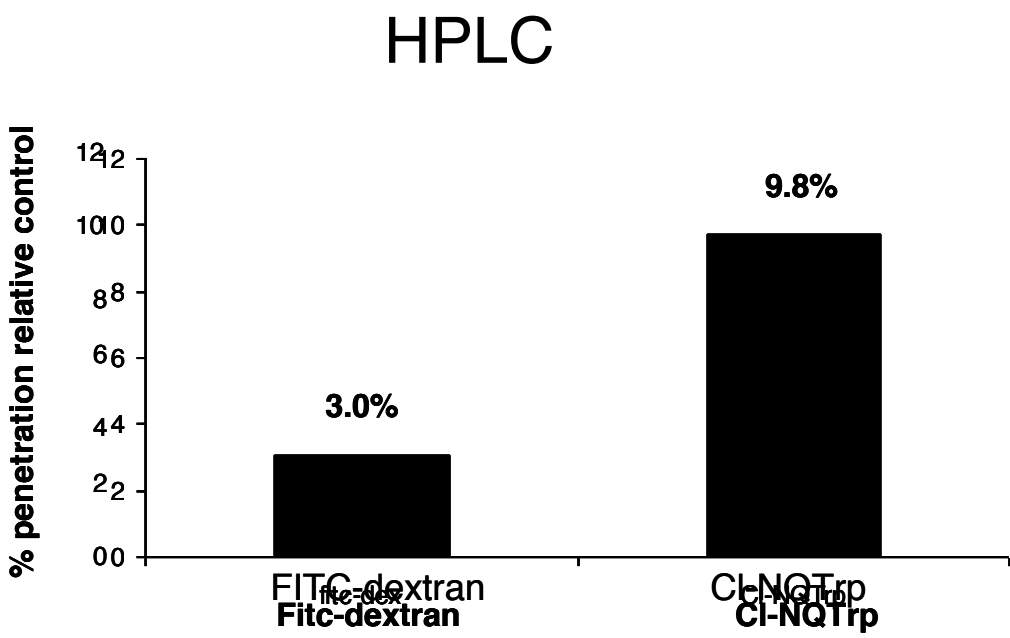
Scherzer-Attali *et al.* PloS ONE 2010

Compounds readily pass through the BBB

(Ex-vivo BBB penetration assay)

Cohen-Kashi Malina *et al*. 2009





Scherzer-Attali *et al*., Neurobiology of Disease 2012

Efficacy tests in AD model mice

Acute model: Transgenics carrying 5 familial Alzheimer's disease mutations, under Thy1 promoter (Oakley et al, 2006)

Injected IP, 50mg/kg, every other day
From age 2 months - before symptoms start

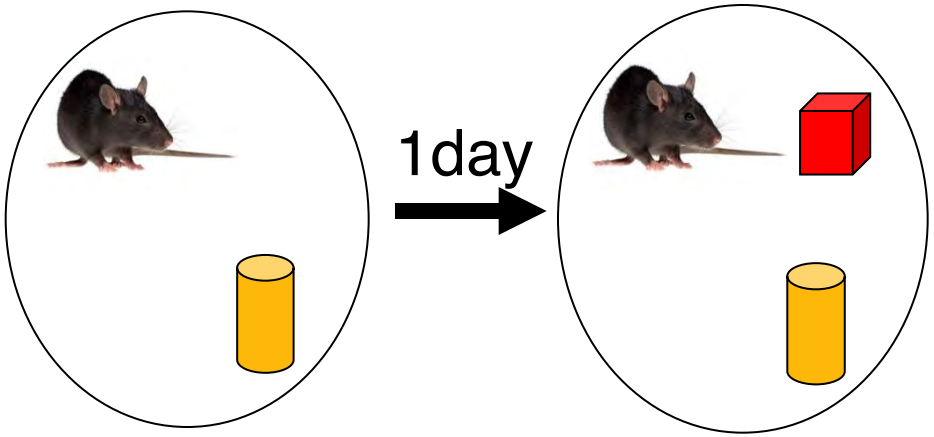
Assayed at age 6 months, when symptoms are severe

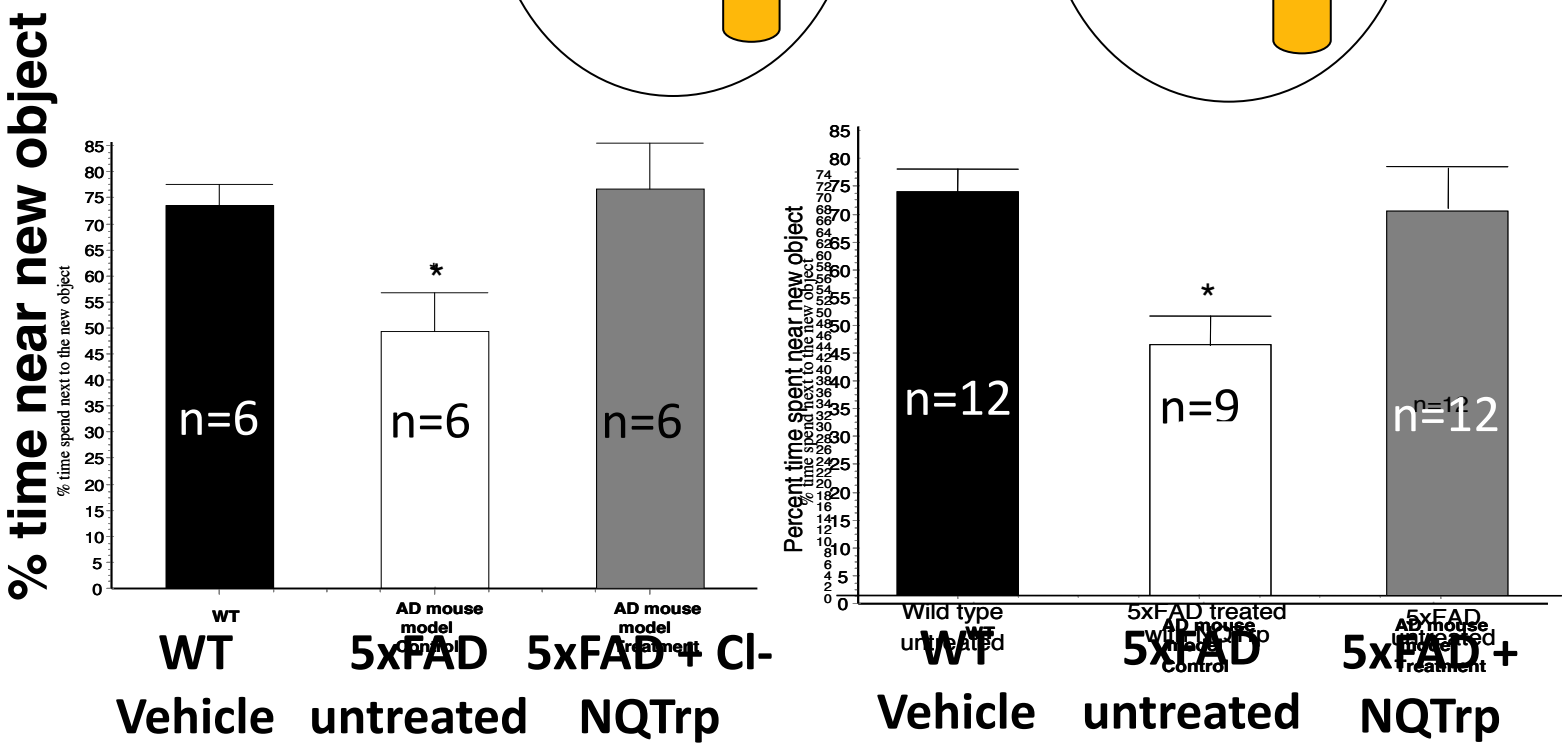
No apparent adverse effects

Scherzer-Attali *et al*., Neurobiology of Disease 2012

Compounds improve cognitive functions of AD model mice

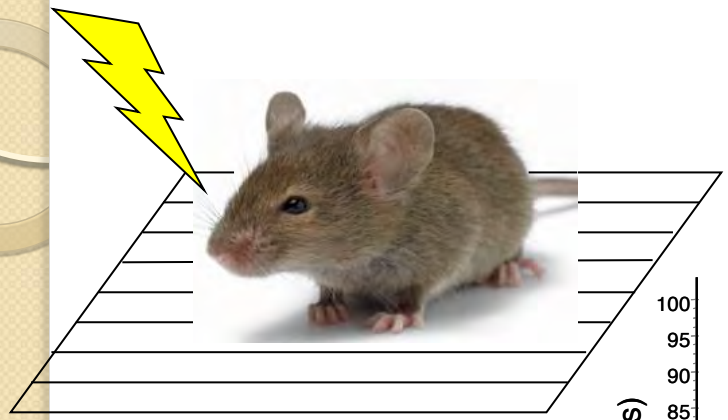
New object recognition test



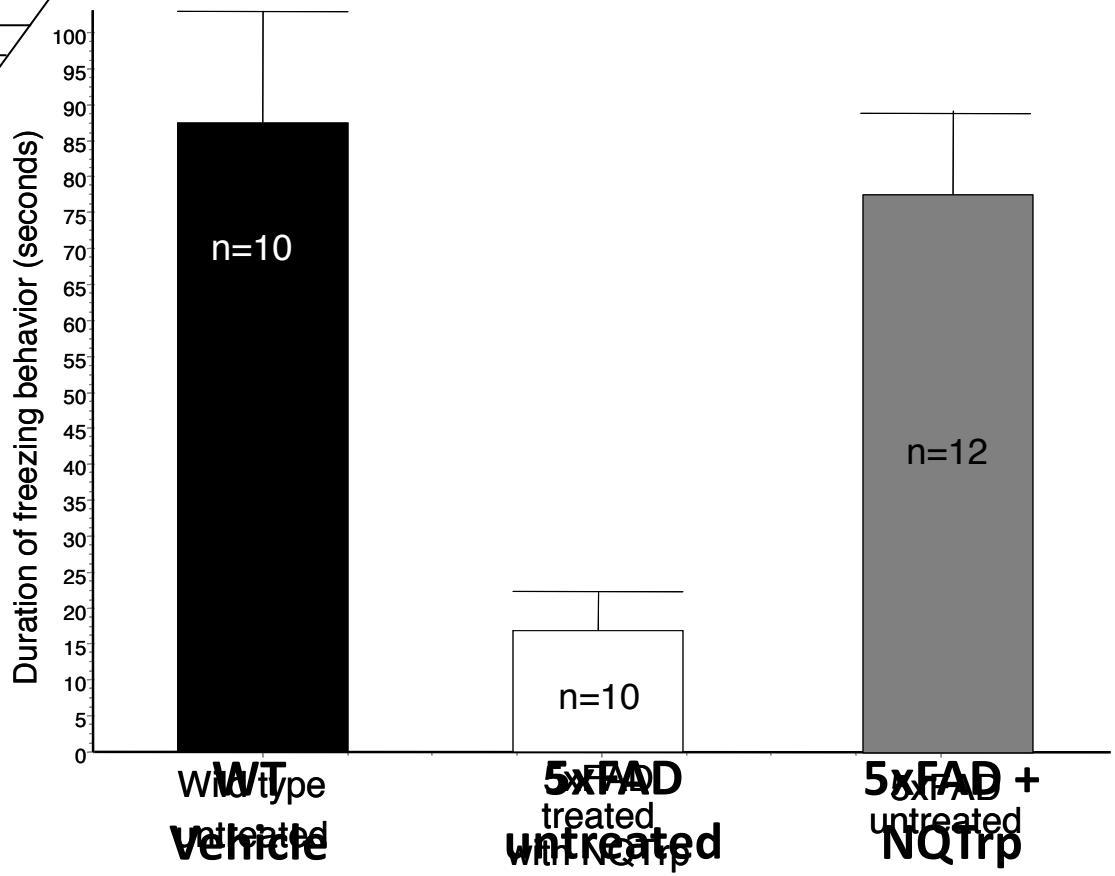


Scherzer-Attali *et al*., Neurobiology of Disease 2012

Compounds improve cognitive functions of AD model mice



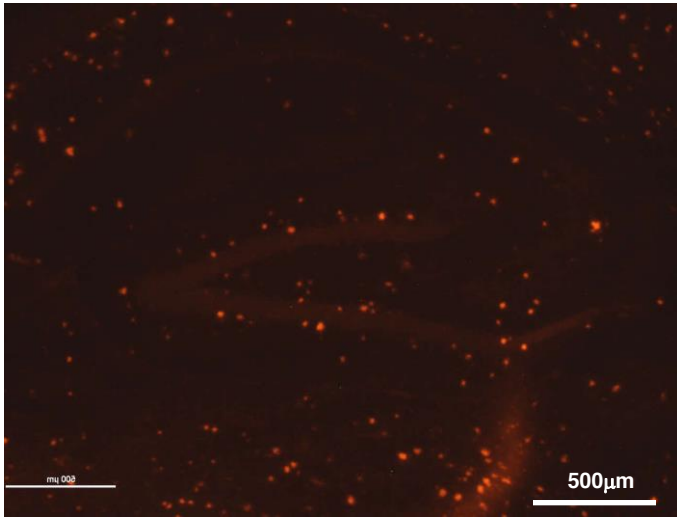
Fear conditioning test



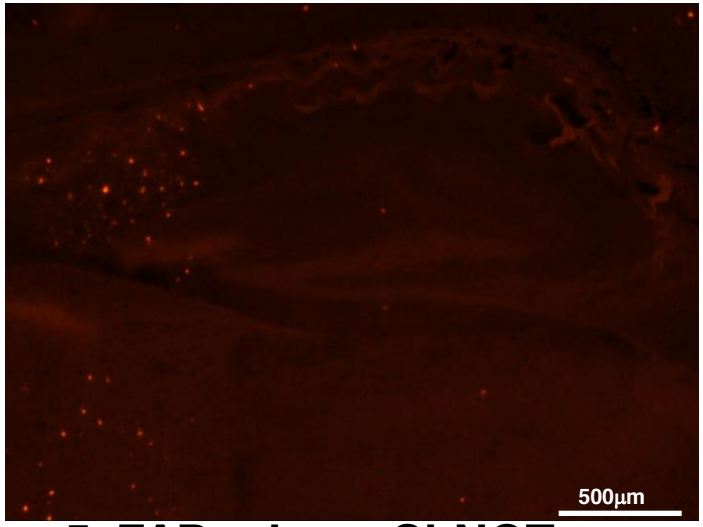
Scherzer-Attali *et al*., Neurobiology of Disease 2012

Compounds reduce by ~40% Aβ deposits in brain sections of AD model mice

Congo red staining

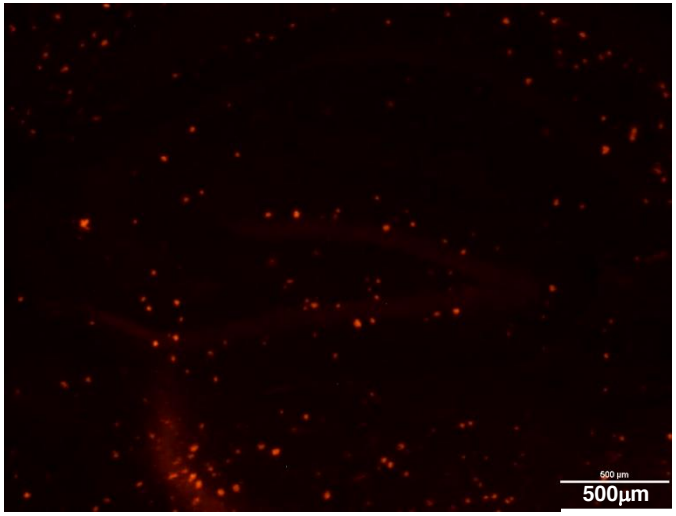


5xFAD mice + vehicle

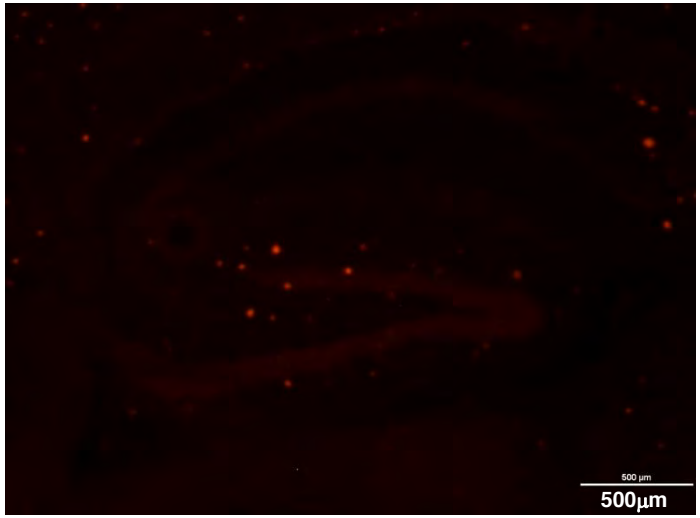


5xFAD mice + Cl-NQTrp

Verified using anti Aβ antibody (6E10)



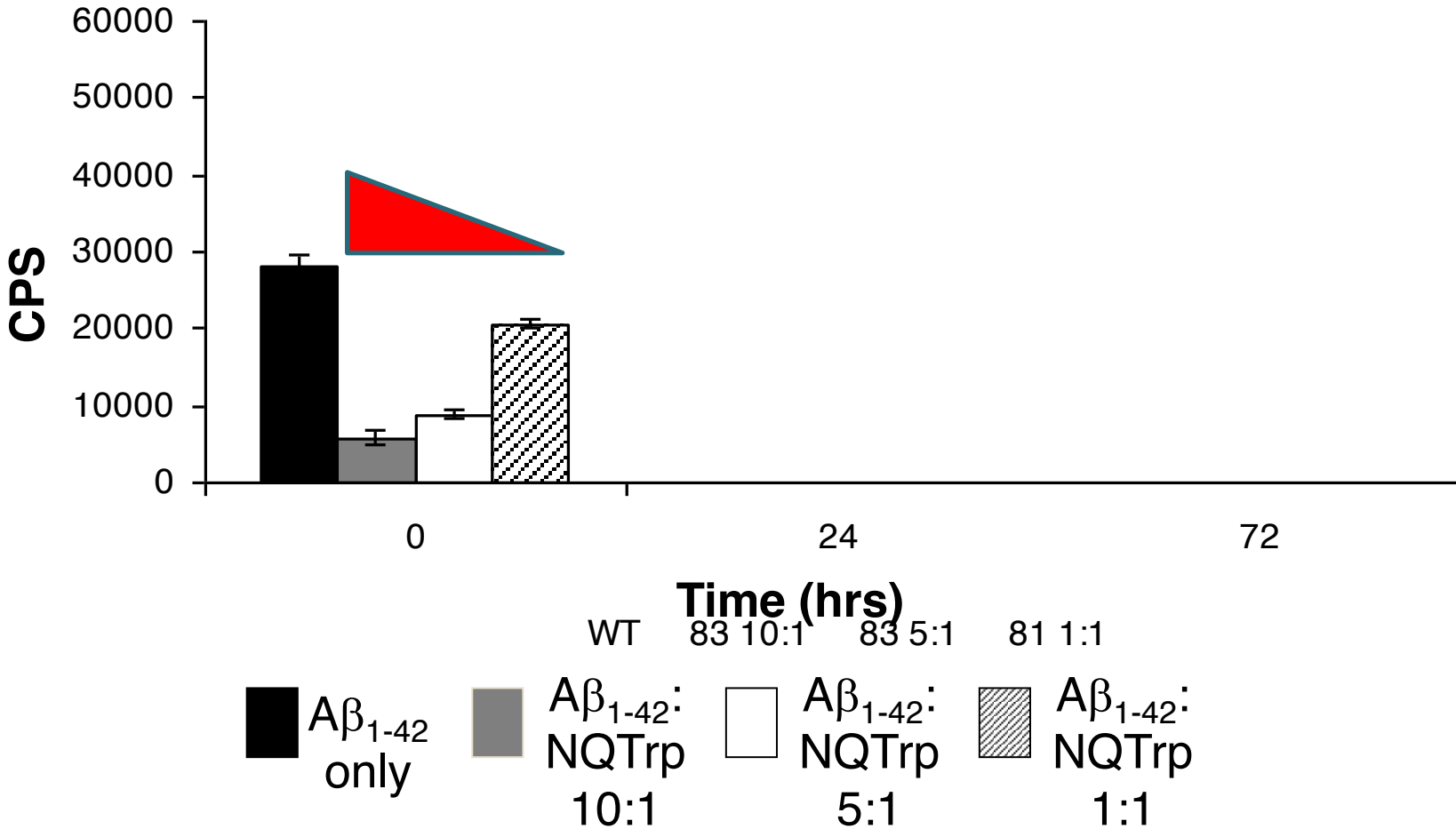
5xFAD mice + vehicle



5xFAD mice + NQTrp

Scherzer-Attali *et al*., Neurobiology of Disease 2012

Both compounds can disassemble pre-formed $A\beta_{1-42}$ fibrils (ThT fluorescence assay)



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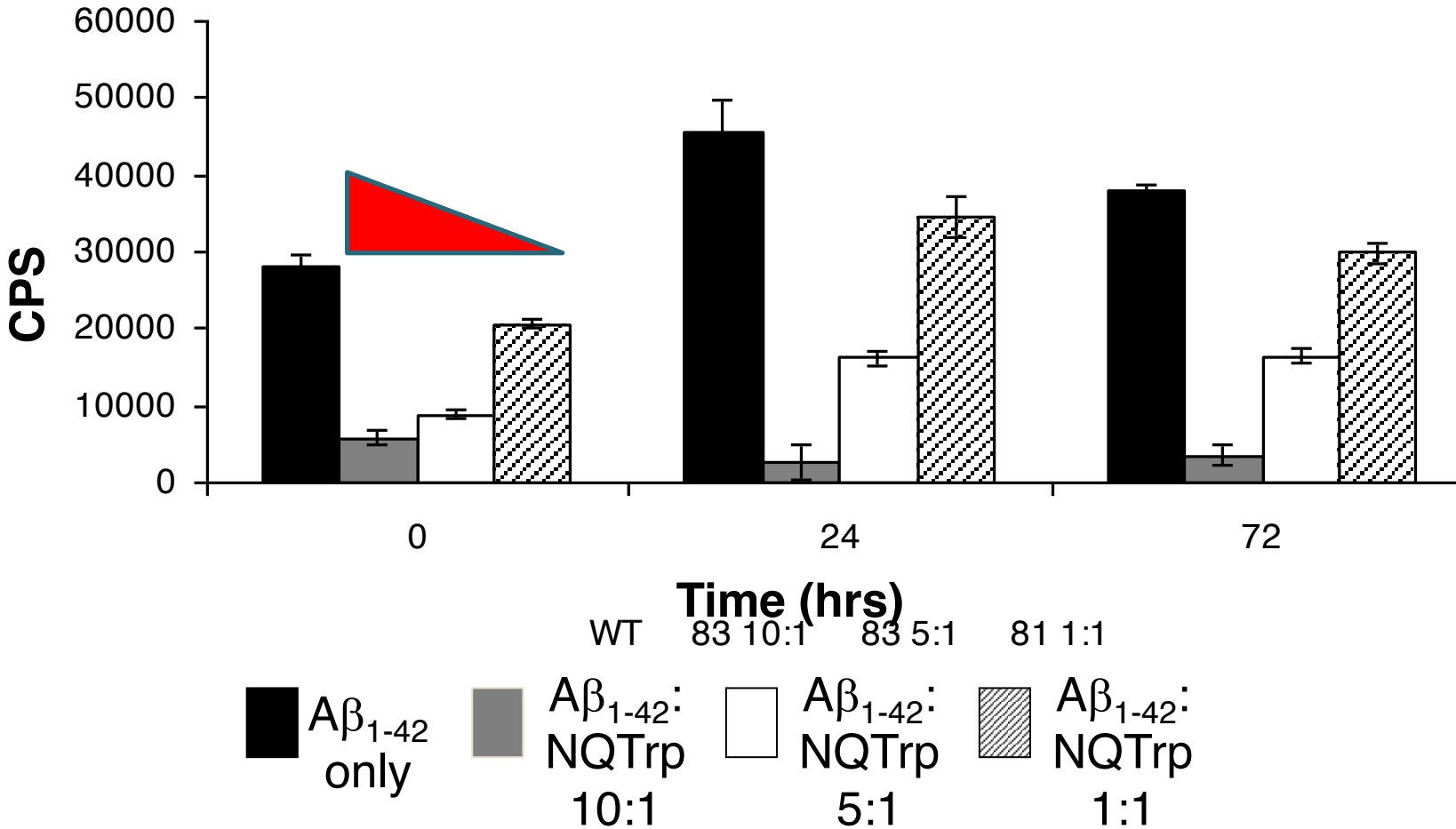


Scherzer-Attali *et al*., Neurobiology of Disease 2012

PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

Generic inhibition of amyloidogenic proteins by two naphthoquinone–tryptophan hybrid molecules

Roni Scherzer-Attali,[1] Ronit Shaltiel-Karyo,[1] Yonatan H. Adalist,[1]
Daniel Segal,[1,2] and Ehud Gazit[1*]

Compounds inhibit in vitro amyloid aggregation also of:

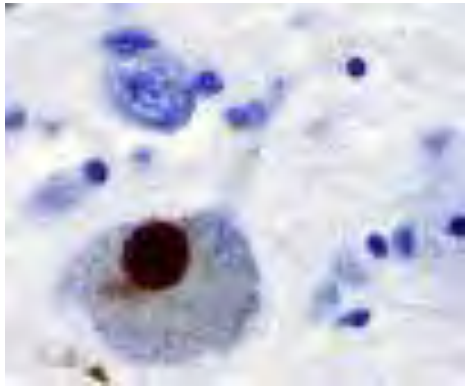
α-synuclein
IAPP (Amylin)
Lysozyme
Calcitonin
Insulin
Tau

Strategy #2

Modulate protein misfolding

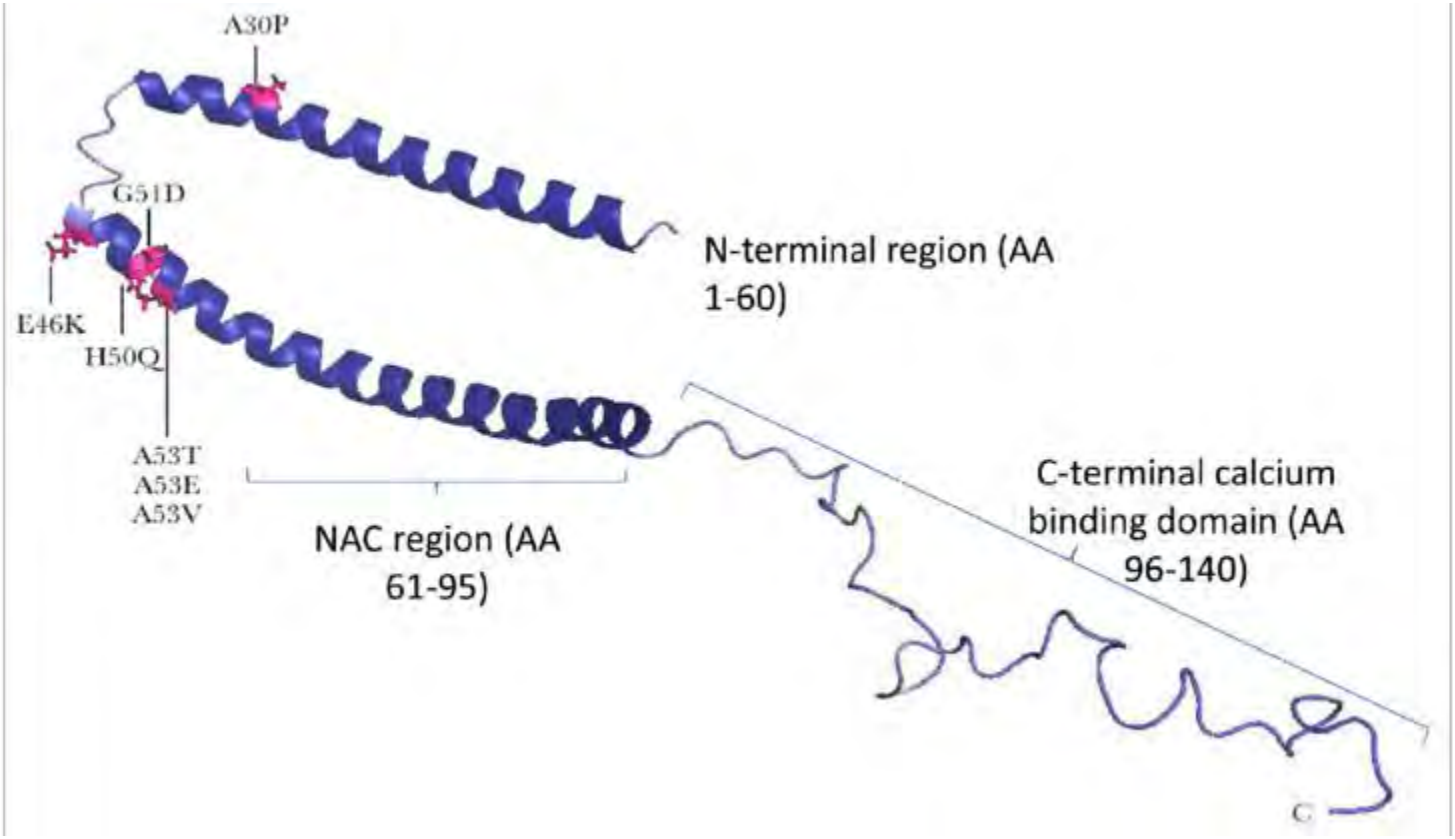
Using chemical chaperones

Parkinson's disease

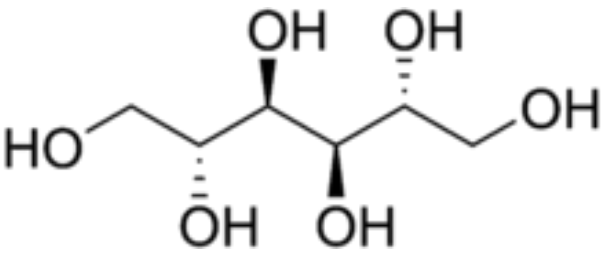


α-synuclein

Misfolds and forms amyloid aggregates in dopaminergic neurons

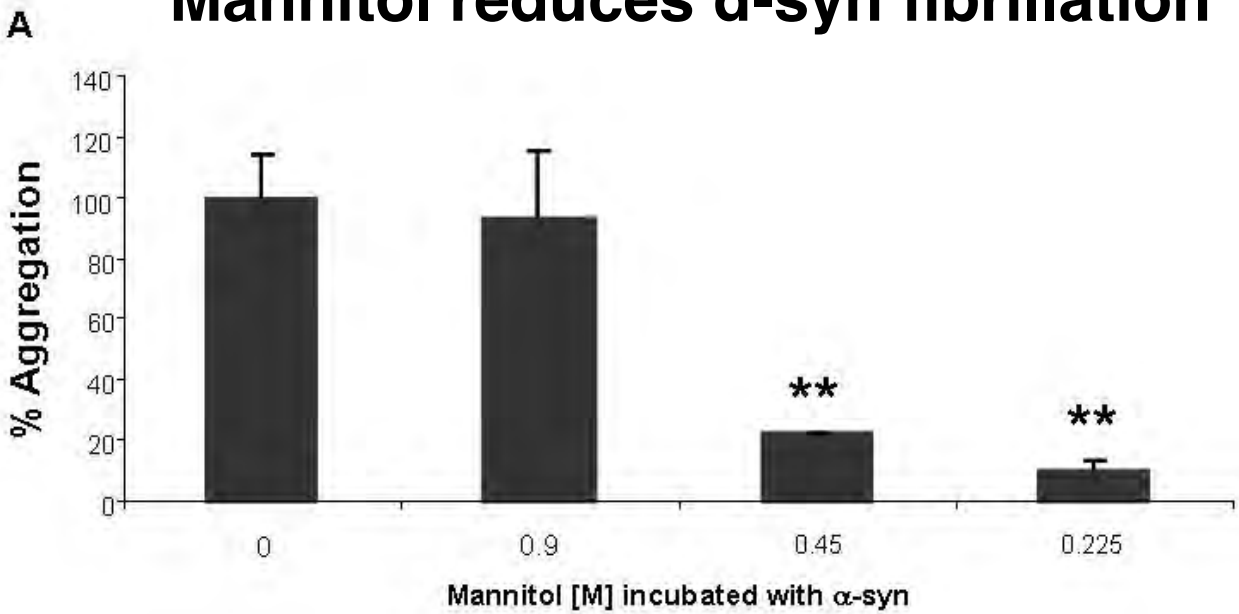


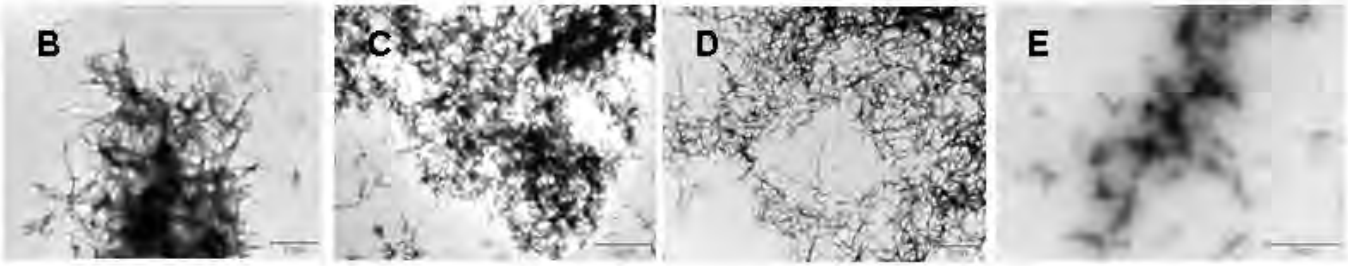
Mannitol



- 6-carbon polyol
- Non-metabolized
- FDA-approved (osmotic diuretic agent)
- Used as BBB disruptor
- Shown to have chemical chaperon activity in vitro (e.g. methallothionein β-glucosidase, Hmg2P)

Mannitol reduces α-syn fibrillation



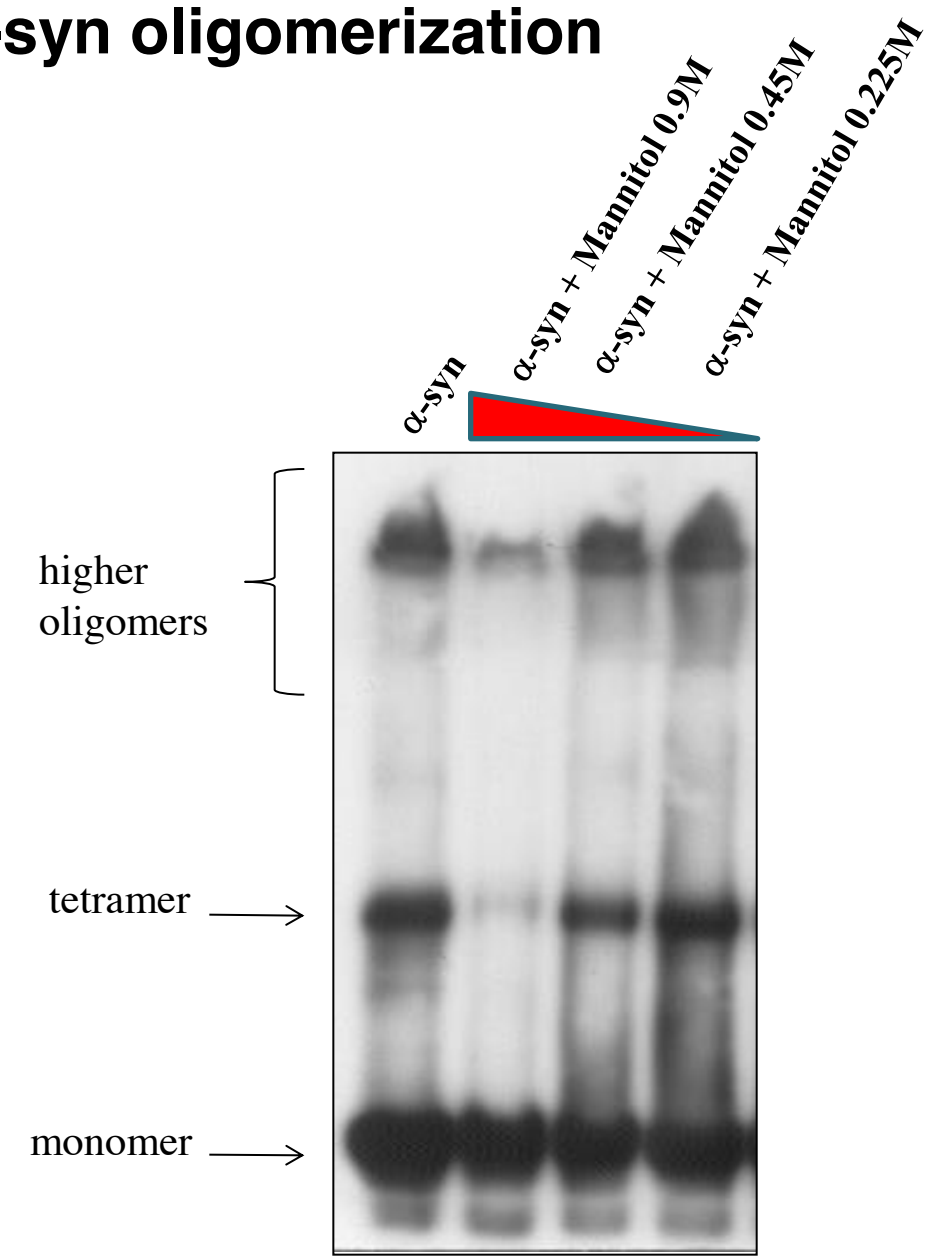


α-syn | α-syn with Mannitol 0.9M | α-syn with Mannitol 0.45M | α-syn with Mannitol 0.225M

Shaltiel-Karyo *et al.*, J Biol Chem 2013

Mannitol inhibits α-syn oligomerization

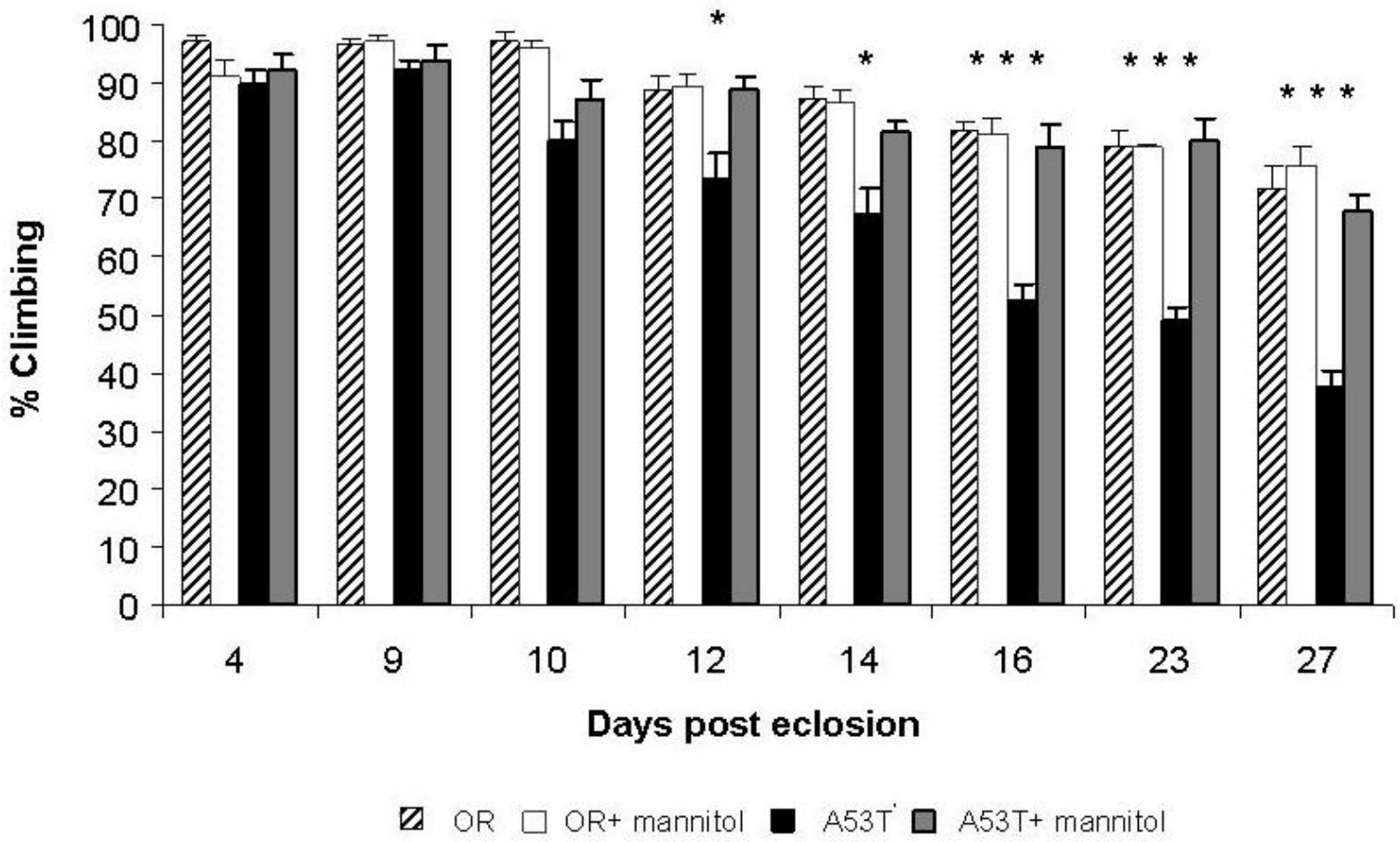
Western blot:
Sc-7011-R
(α-syn antibody)



Shaltiel-Karyo *et al.*, J Biol Chem 2013

Feeding Mannitol rescues defective locomotion of PD flies

Mannitol: 75mM



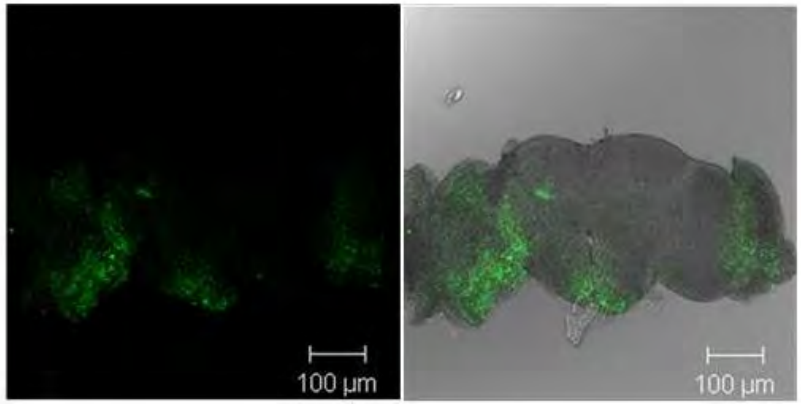
Shaltiel-Karyo *et al.*, J Biol Chem 2013

Mannitol reduces α-syn deposits in the brain of PD flies

Untreated

A

Anti α-syn antibodies



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Anti α-syn antibodies

Untreated

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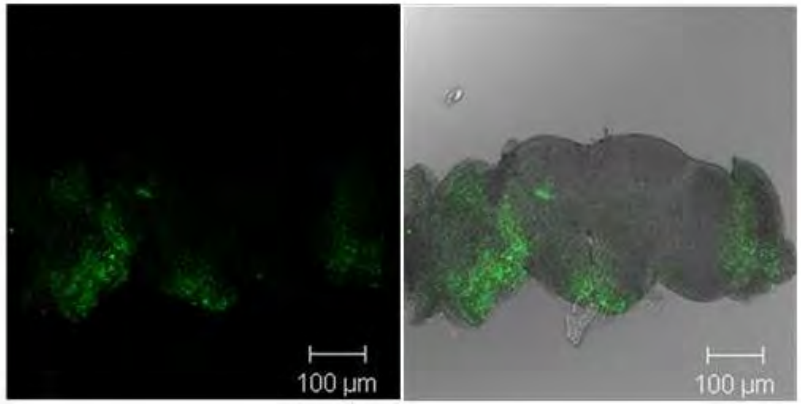
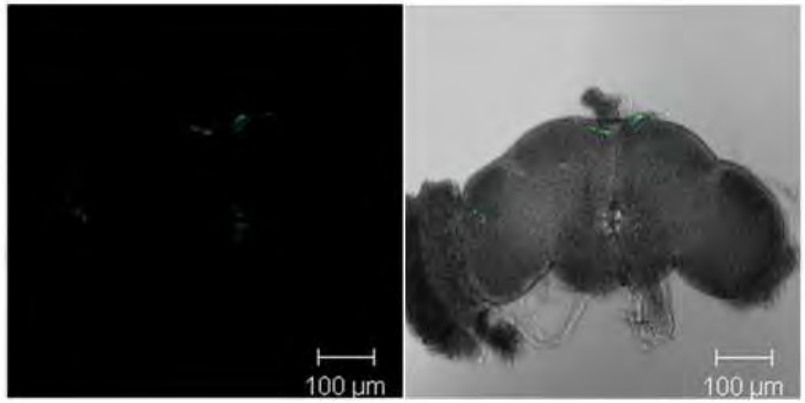


Treated with Mannitol

B



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Mannitol reduces α-syn deposits in the brain of PD flies

Anti α-syn antibodies

Untreated

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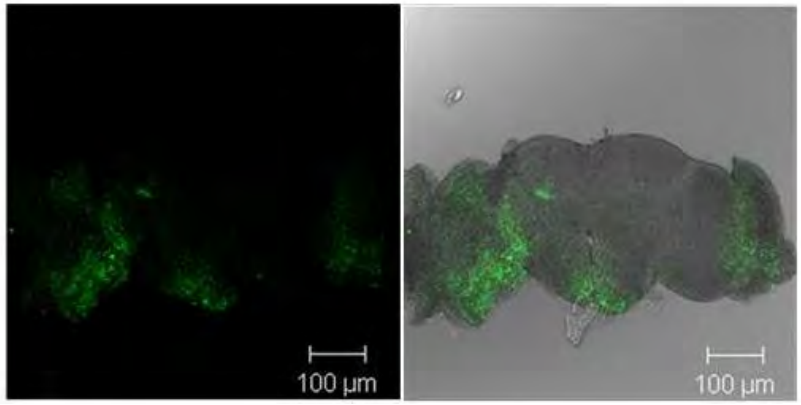
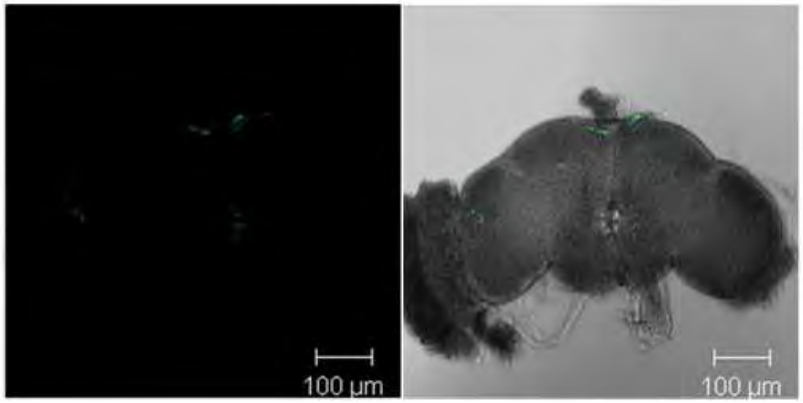
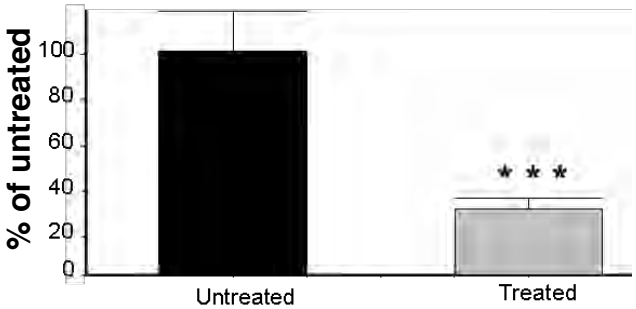


Treated with Mannitol

B

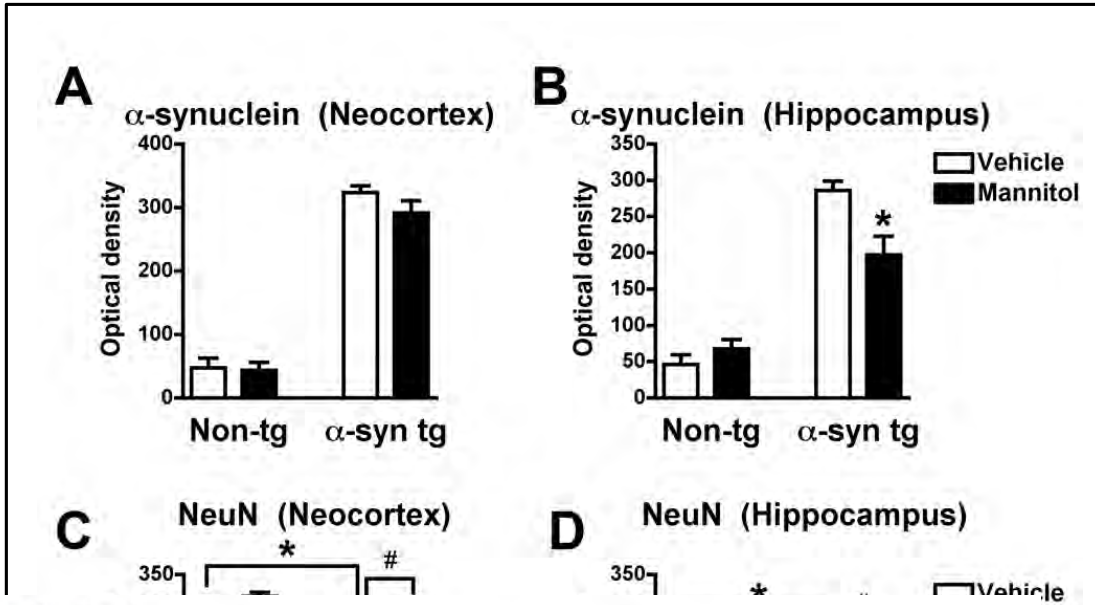


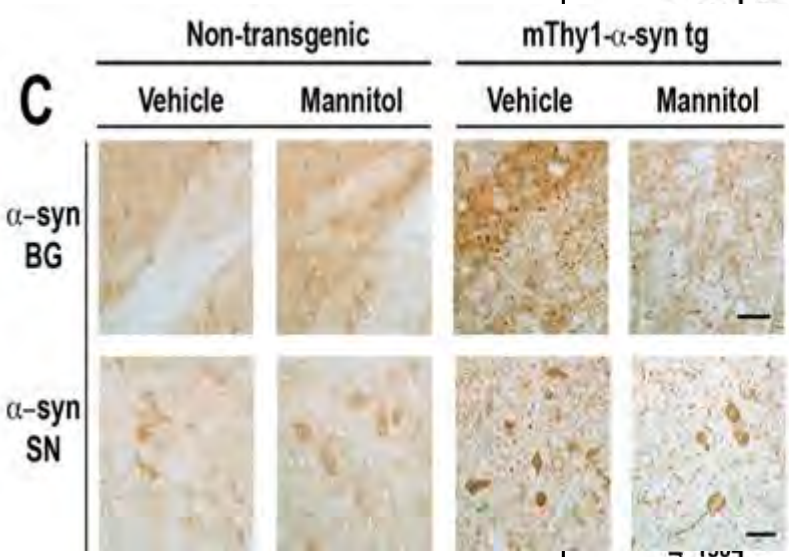


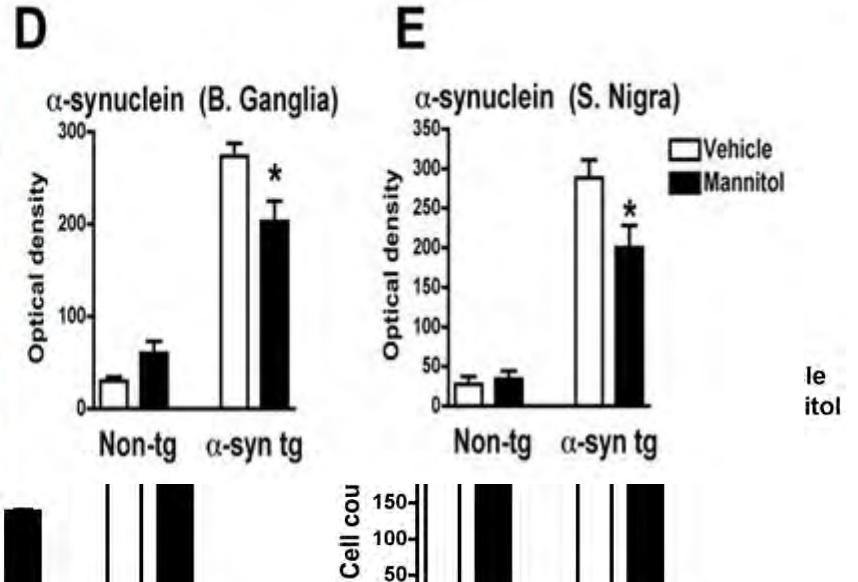
Shaltiel-Karyo *et al.*, J Biol Chem 2013

Mannitol reduces α-syn deposits in the brain of PD mice

mThy1-α-syn tg mice and non-tg age matched littermates received daily IP injections with mannitol (1 g/kg) (n=8 mice per group)

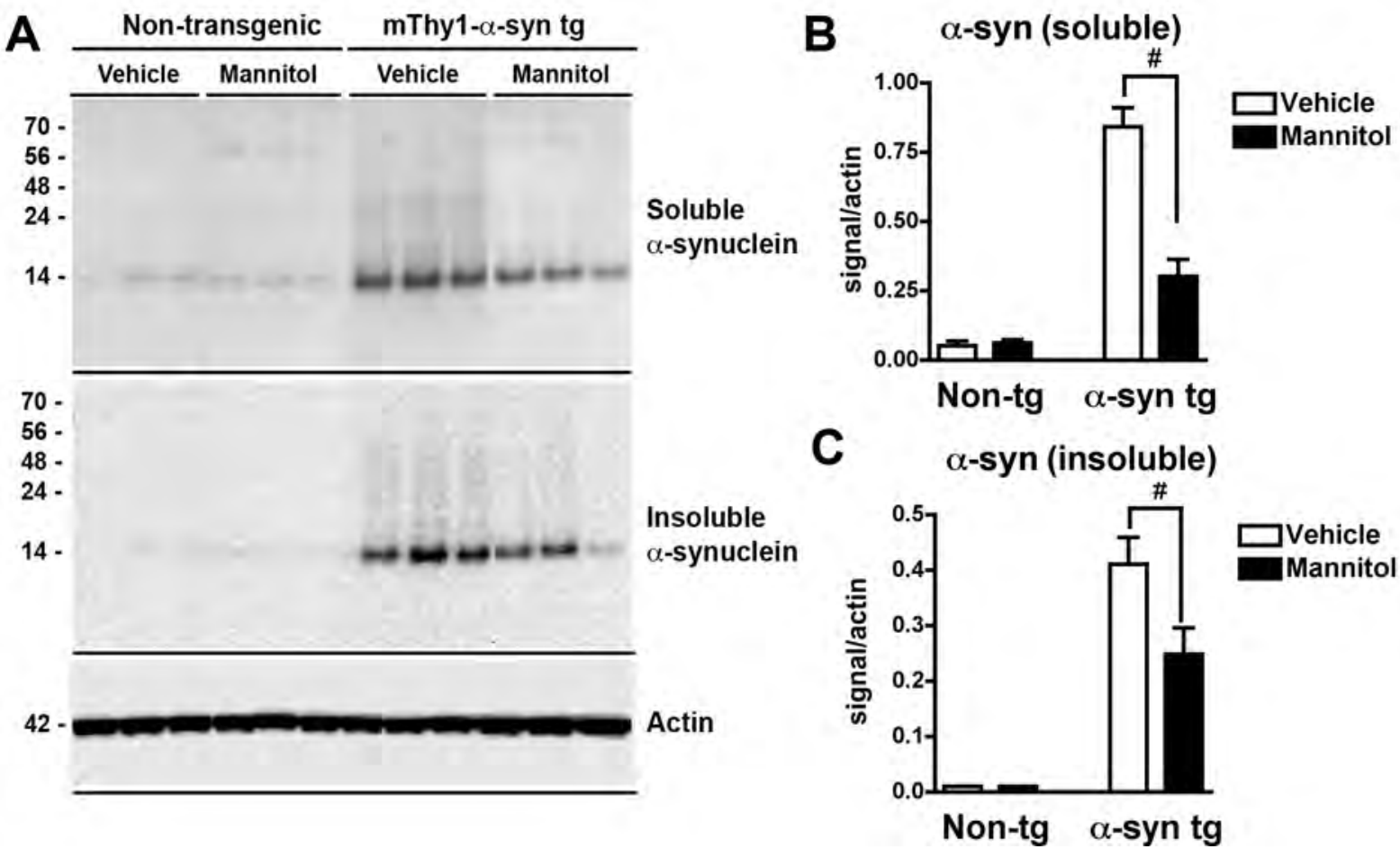






Shaltiel-Karyo *et al.*, J Biol Chem 2013

Mannitol reduces α-syn levels in brain extracts of PD mice



Shaltiel-Karyo *et al.*, J Biol Chem 2013

Mannitol ameliorated additional aspects of neuronal pathology in PD mice

Reduced loss of dopaminergic neurons

Decreased astrogliosis

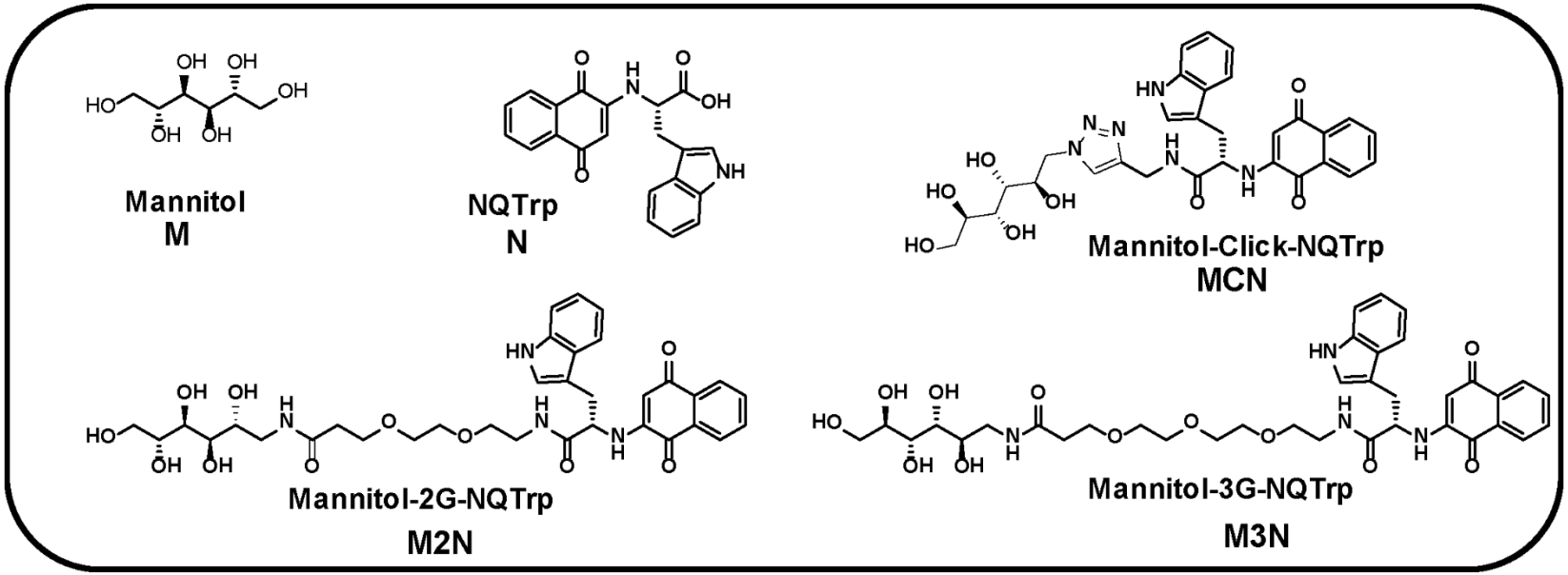
Increased Tyrosine hydroxylase immuno-reactivity

No such effects on normal littermates treated with Mannitol

➡ specificity

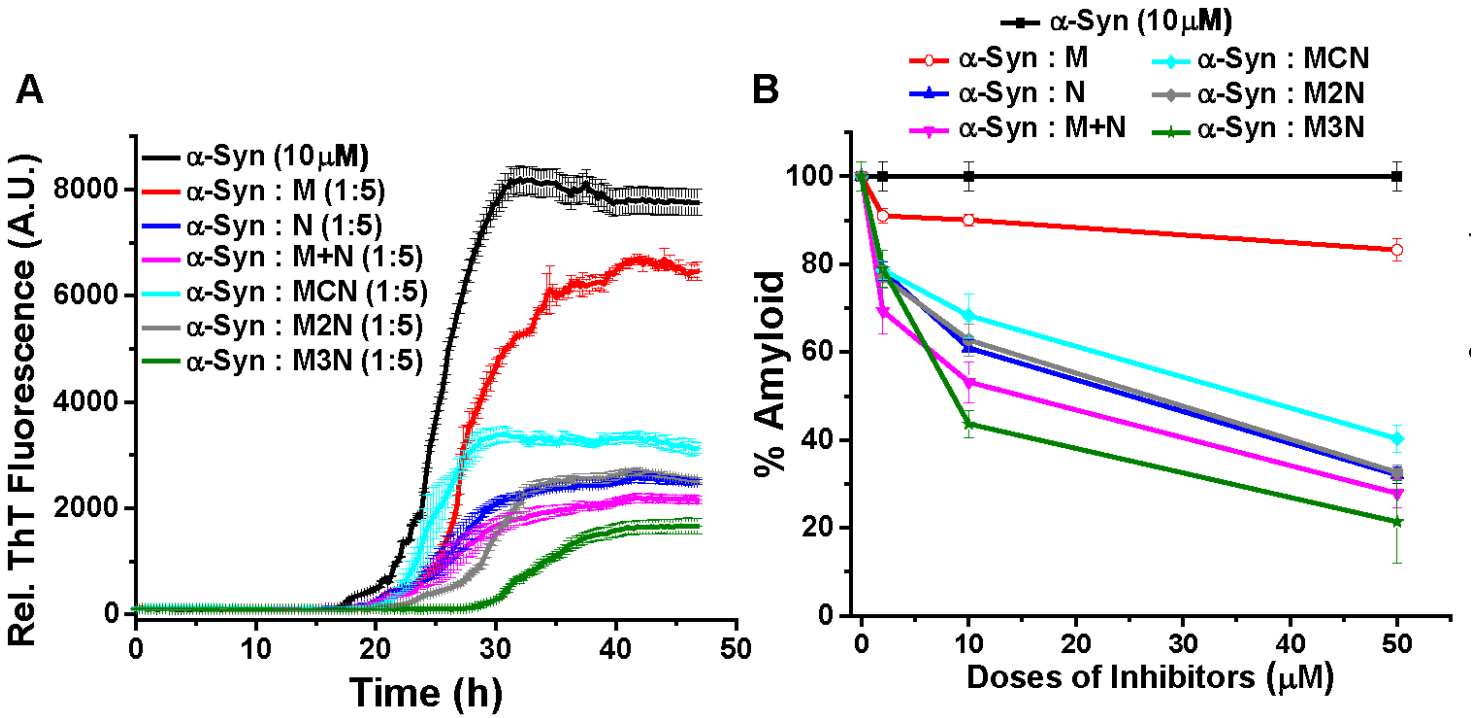
Strategy #3

Conjugate NQTrp and Mannitol



Paul *et al*., Front. Mol. Biosci. 2019

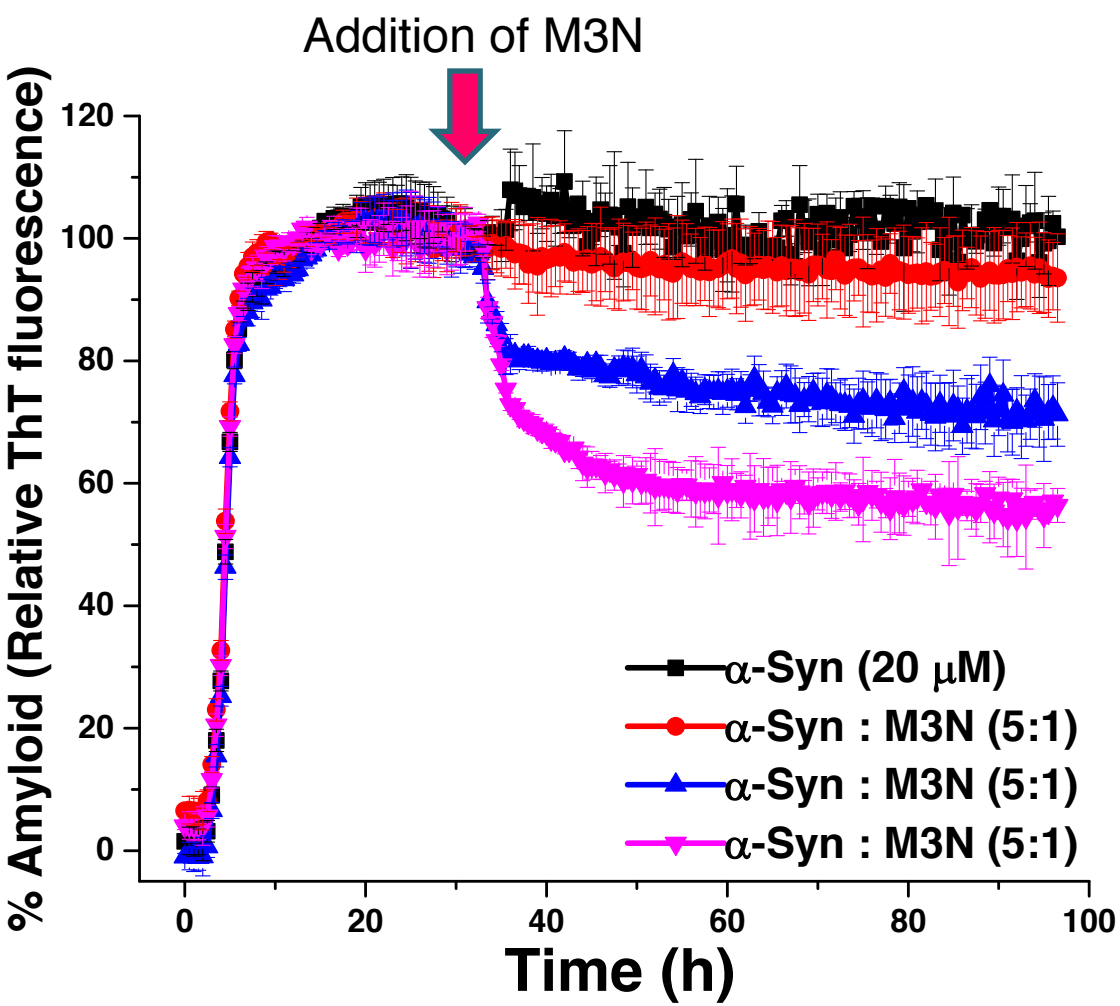
Conjugates inhibit α-syn aggregation in vitro



Paul *et al*., Front. Mol. Biosci. 2019

Conjugates disassemble pre-formed α-syn fibrils

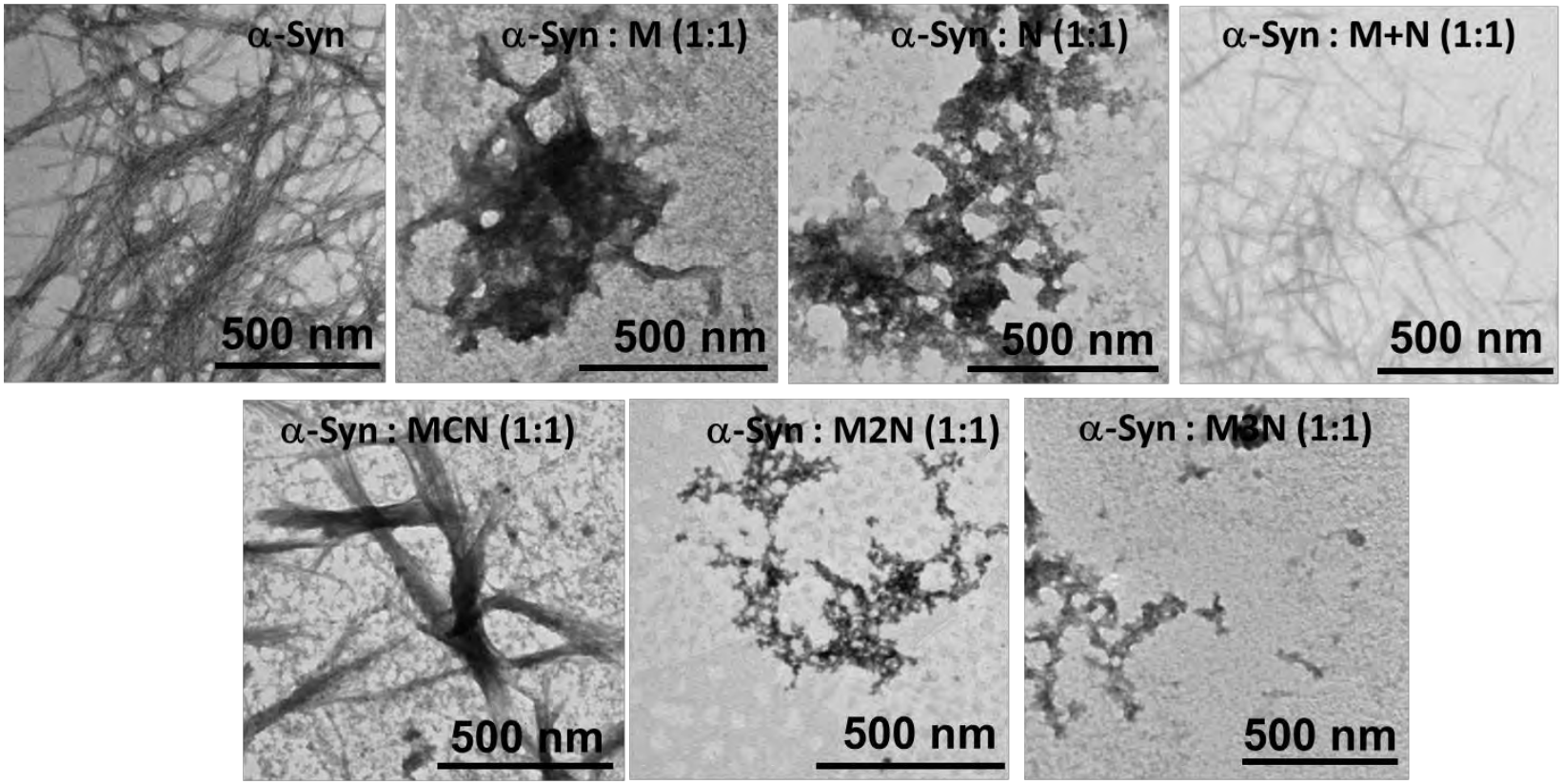
ThT assay



Paul *et al*., Front. Mol. Biosci. 2019

Conjugates disassemble pre-formed α-syn fibrils

TEM images



Paul *et al*., Front. Mol. Biosci. 2019

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