Active Targeting of Nanoparticles to the Brain for Treating Parkinson's Disease

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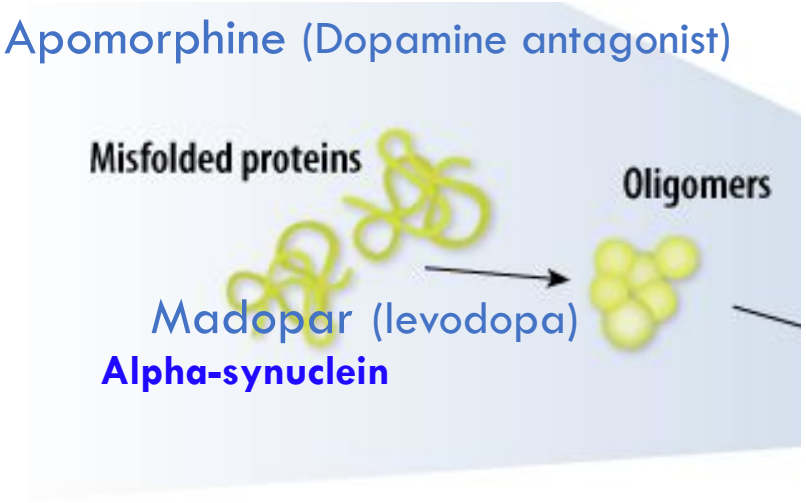
Technion – Israel Institute of Technology

July 2022

Parkinson's Disease

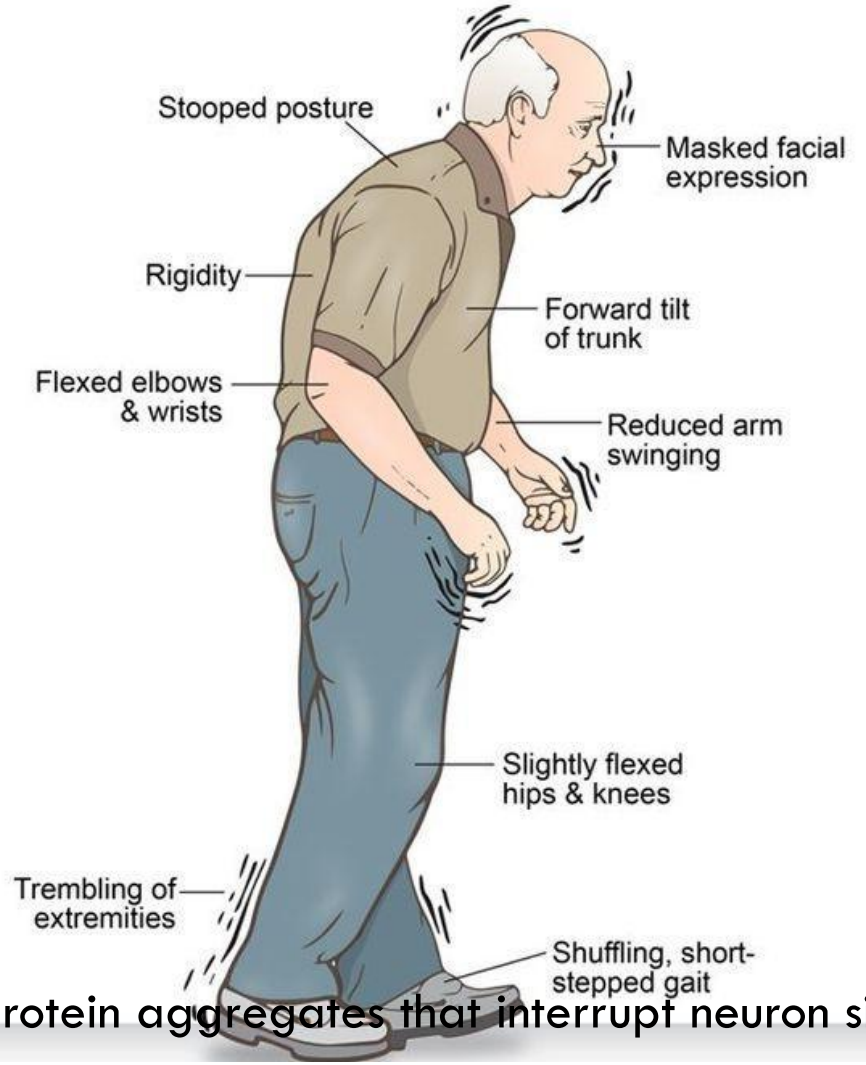
Apomorphine (Dopamine antagonist)



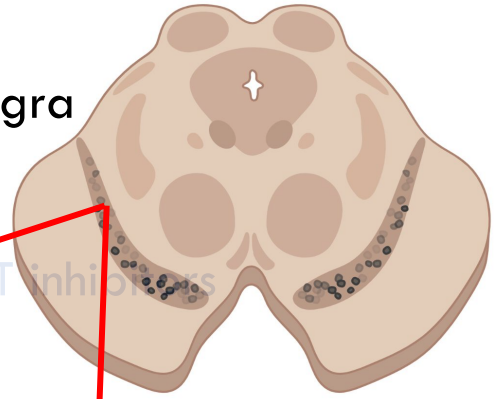
Madopar (levodopa)

Alpha-synuclein

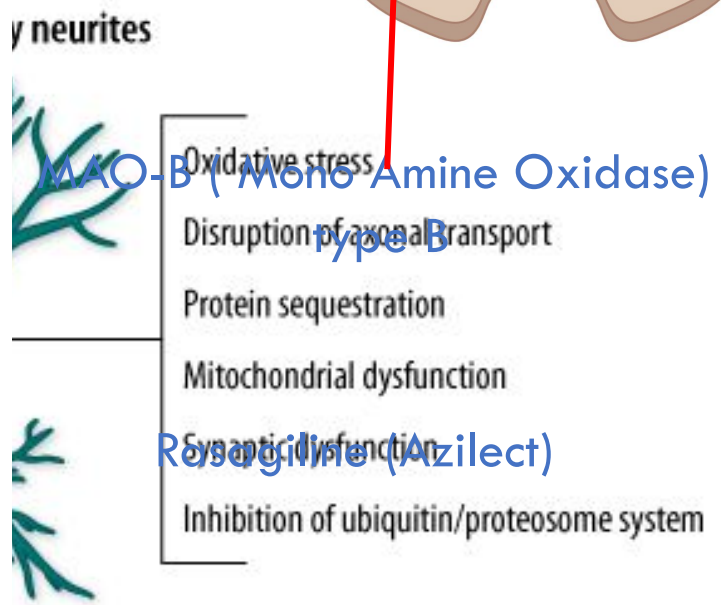
Sinemet (levodopa)



Substantia Nigra (SN)



COMT inhibitors



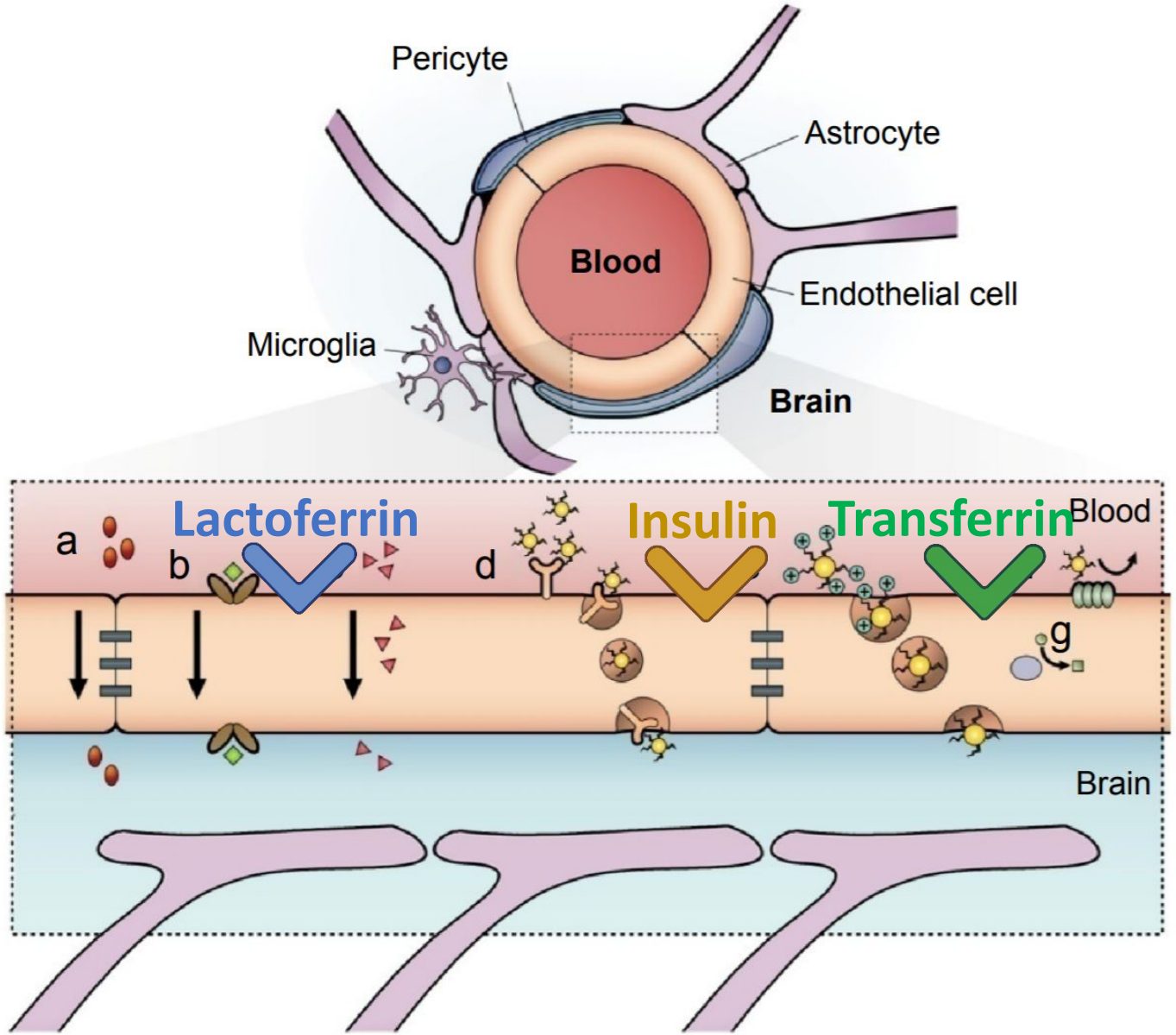
MAO-B ("Mono Amine Oxidase) type B

Rasagiline (Azilect)

Lewy bodies – alpha-synuclein protein aggregates that interrupt neuron signaling thus resulting in neuronal degradation.

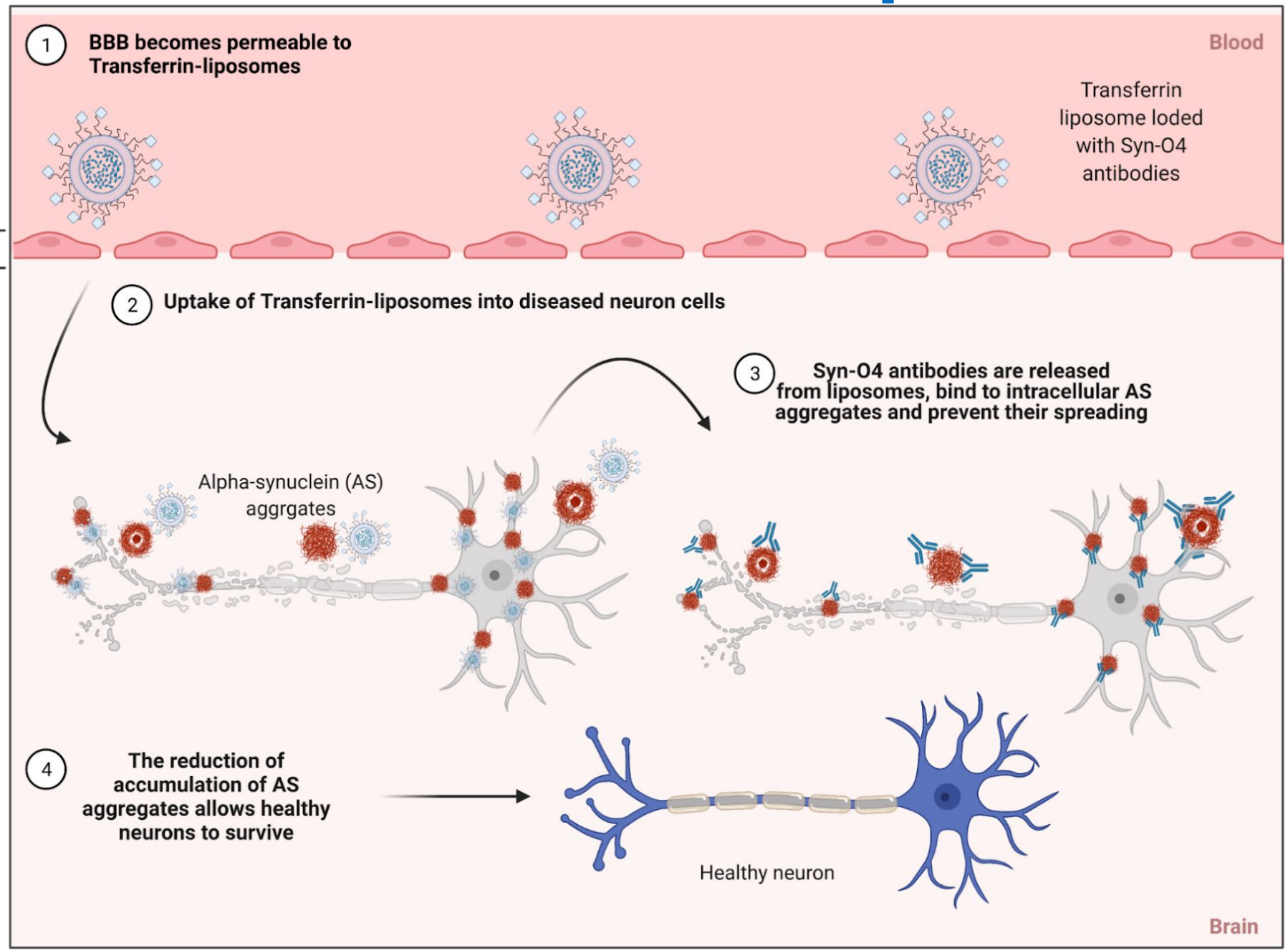
Lee, V. M.-Y. and J. Q. Trojanowski (2006). "Mechanisms of Parkinson's disease linked to pathological α-synuclein: new targets for drug discovery." Neuron **52**(1): 33-38.

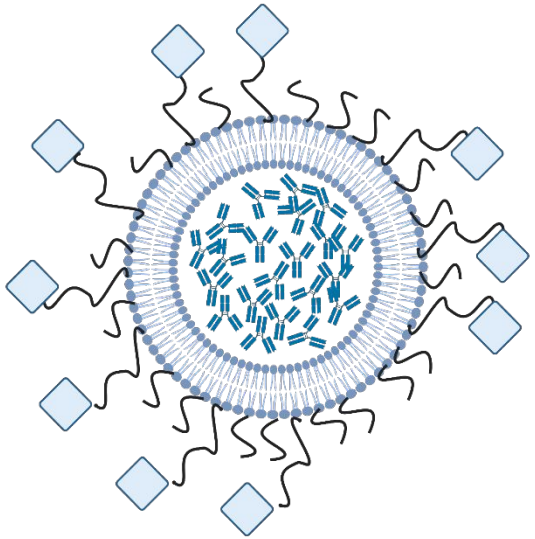
Blood – Brain – Barrier (BBB)



Velasco-Aguirre, C., et al. (2015). "Peptides and proteins used to enhance gold nanoparticle delivery to the brain: preclinical approaches." International journal of nanomedicine **10**: 4919.

Research Objective





DSPE-PEG2000-NH2

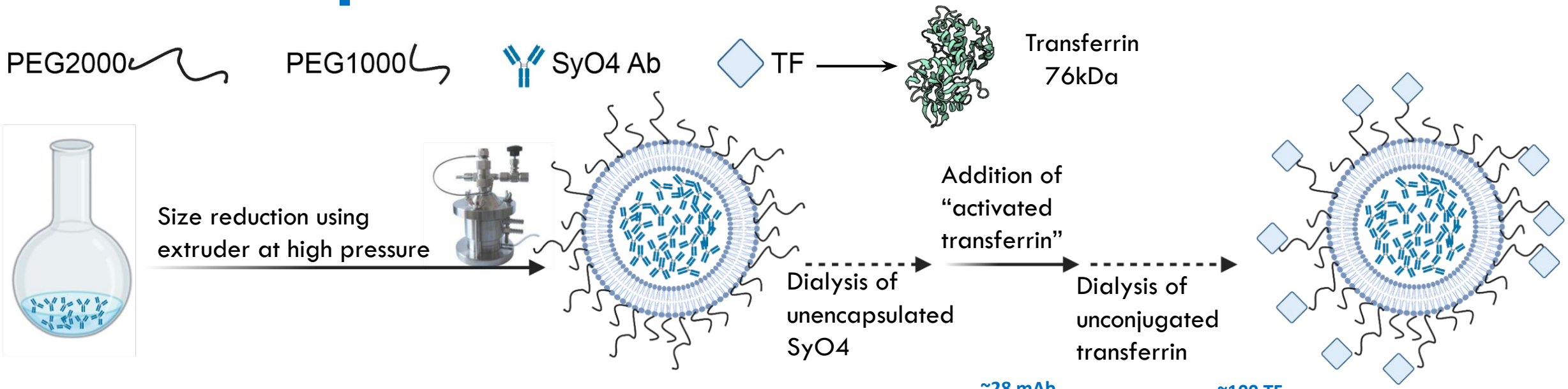
DSPE-PEG1000-Methyl

DPPC, Cholesterol

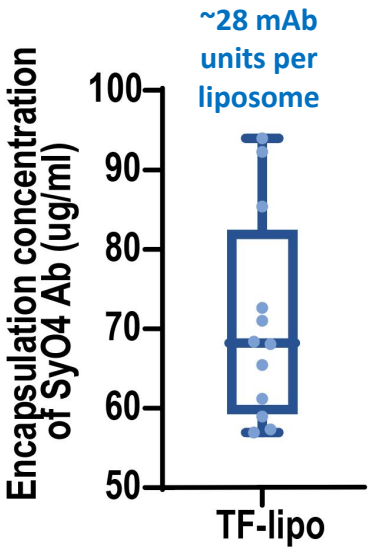
Transferrin (TF) protein

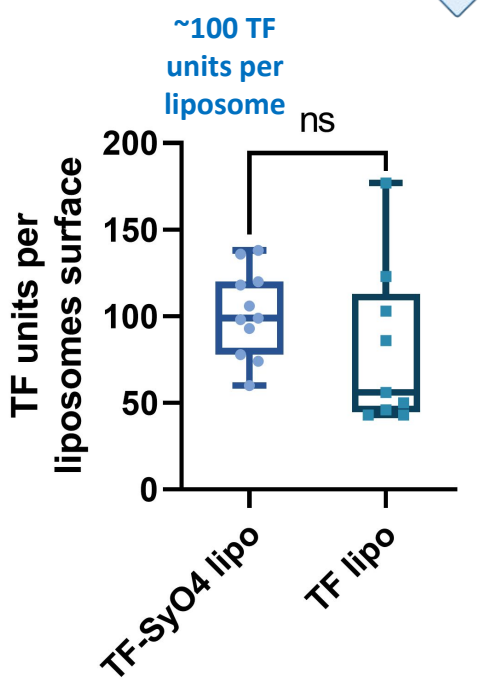
Syn-O4 mAb

Liposomes' Fabrication Process



	Average diameter [nm]	PDI	Zeta potential [mV]
TF-SyO4-lipo	113.3±1.5	0.162±0.010	-28.2±0.1
SyO4-lipo	114.3±0.3	0.165±0.025	-29.1±1.3





* TF = transferrin

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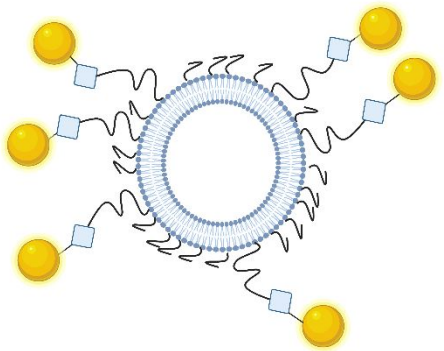
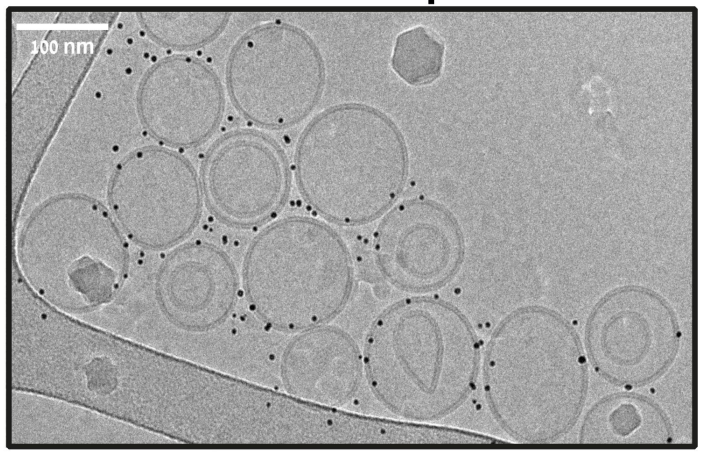
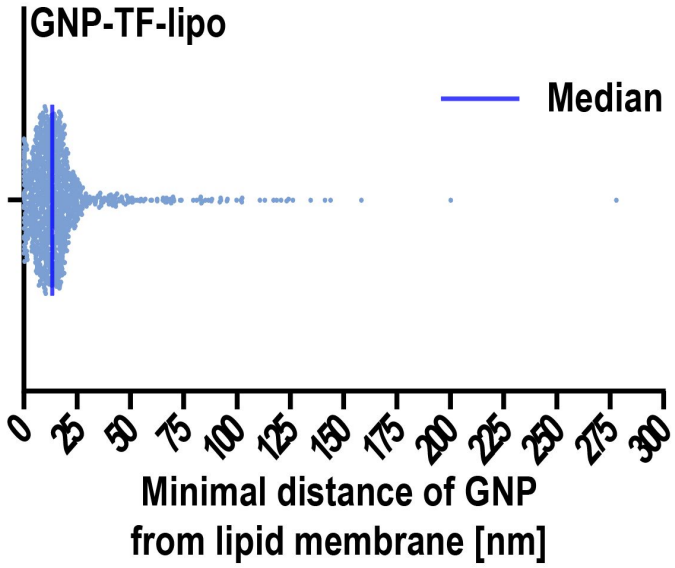
Liposomes' Fabrication Process



GNP-TF-liposome

GNP-TF-lipo





Cryo-TEM Imaging of Transferrin-liposome by conjugation of 5nm NHS-activated gold nanoparticles (GNP) to the amine groups in the transferrin units.

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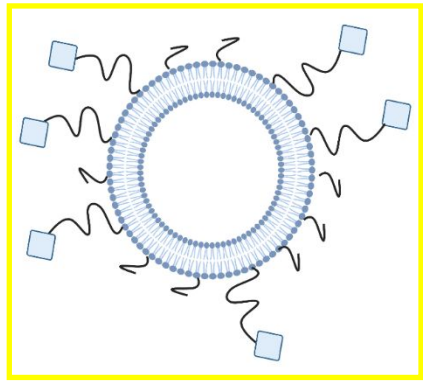
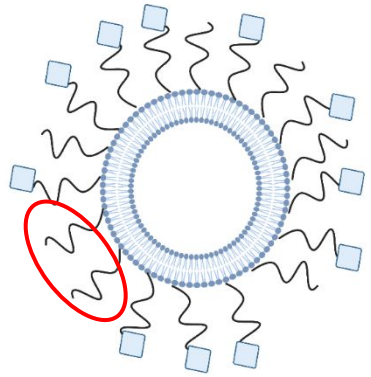
FACS analysis of TF-liposomes uptake in brain endothelial cells-hCMEC/D3:



2.5% molarity of PEG2000-NH2
2.5% molarity of PEG1000-Methyl



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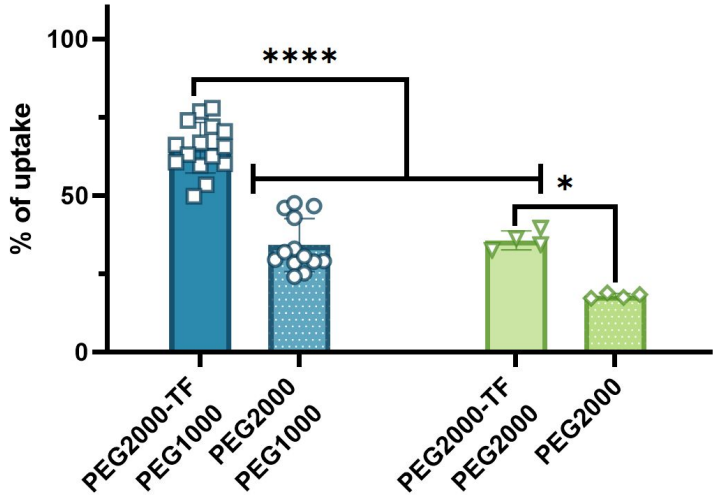
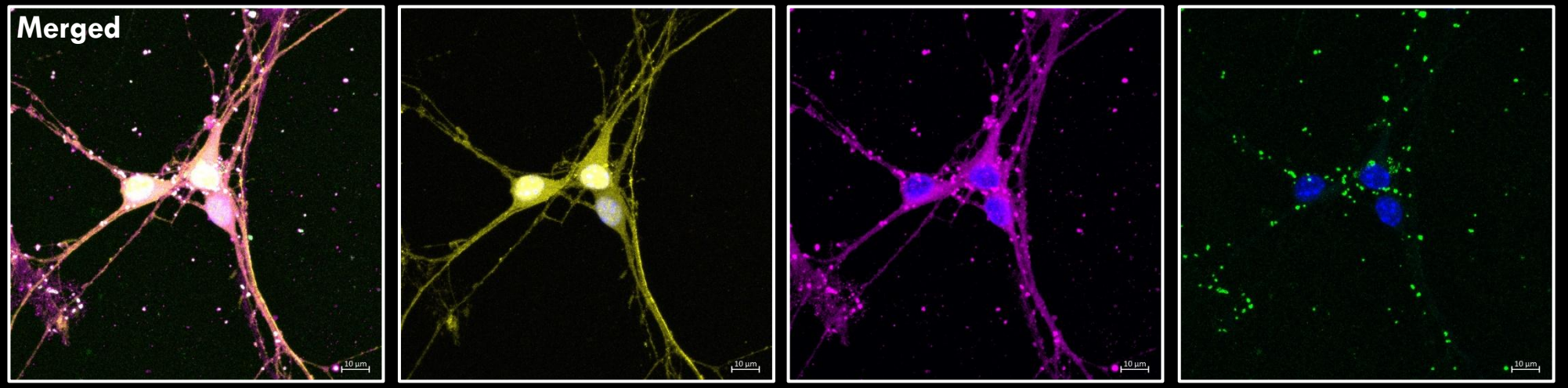


Uptake of TF-SyO4-Liposomes Compared to Free SyO4 mAb *in Primary Cortical Neurons* Cells

TF-SyO4 liposome uptake



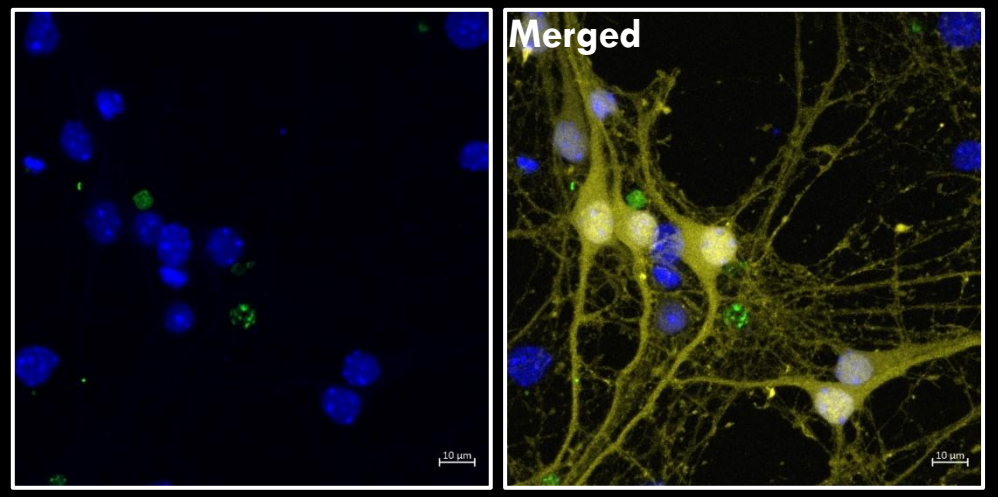
Yellow – alpha synuclein aggregates (GFP)

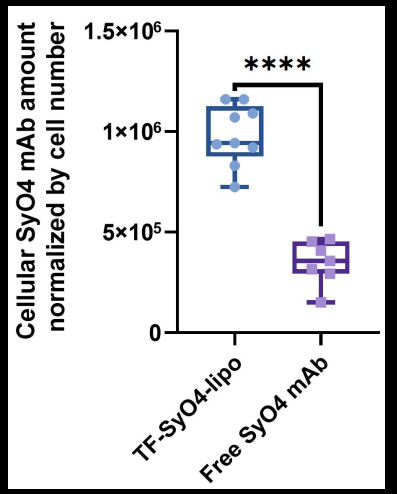
Pink – Cy5-TF-liposomes

Green – Cy3-SyO4

Blue – nuclei

Free SyO4 mAb uptake





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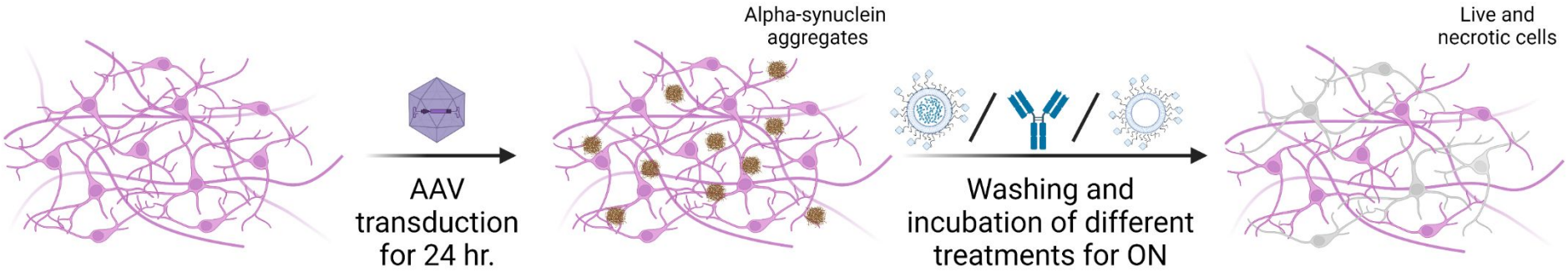
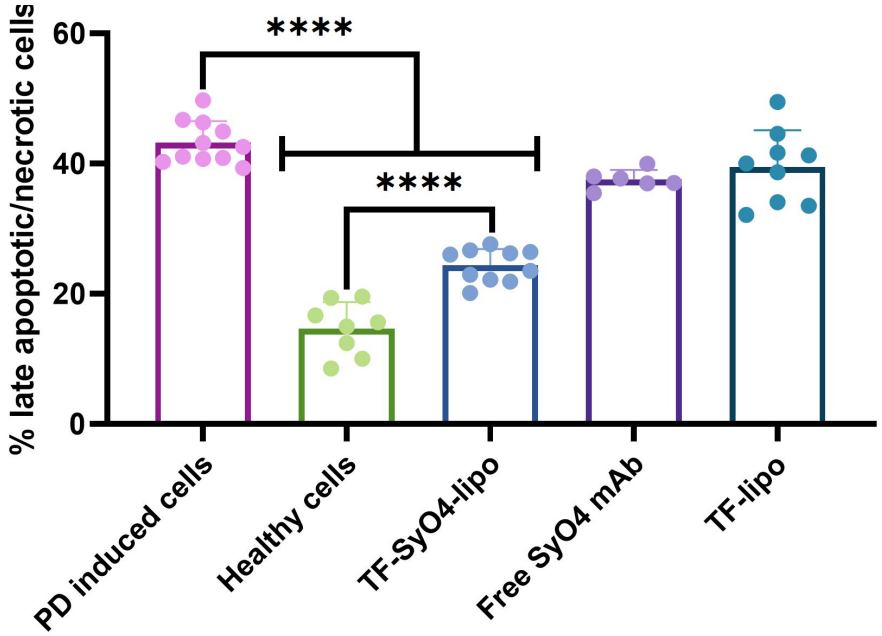
Collaboration with Prof. Uri Ashery - Laboratory of Neuroscience

TEL AVIV UNIVERSITY

Therapeutic Efficacy in *Differentiated PD* SH-SY5Y Cells

FACS analysis of apoptosis assay in PD SH-SY5Y cells:





TF-SyO4-liposomes treatment reduces the level of apoptotic cells by ~ 2-fold

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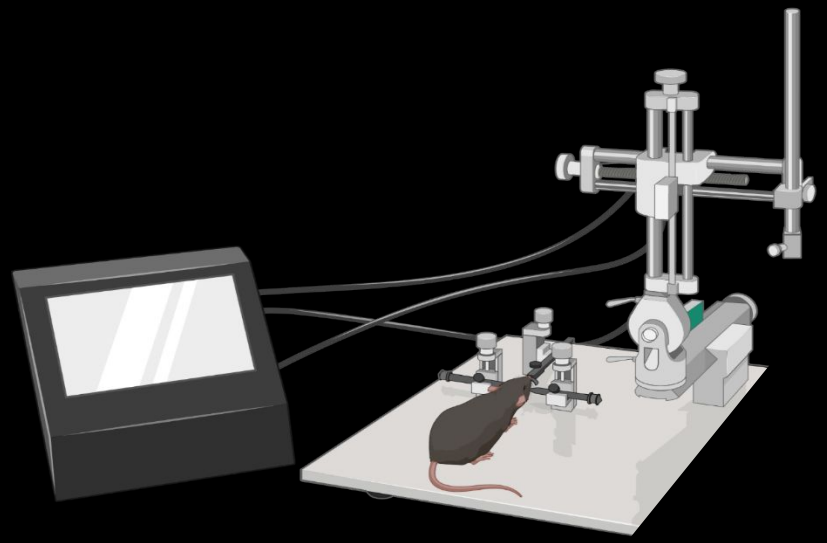
Parkinson's Disease Mice Model Establishment

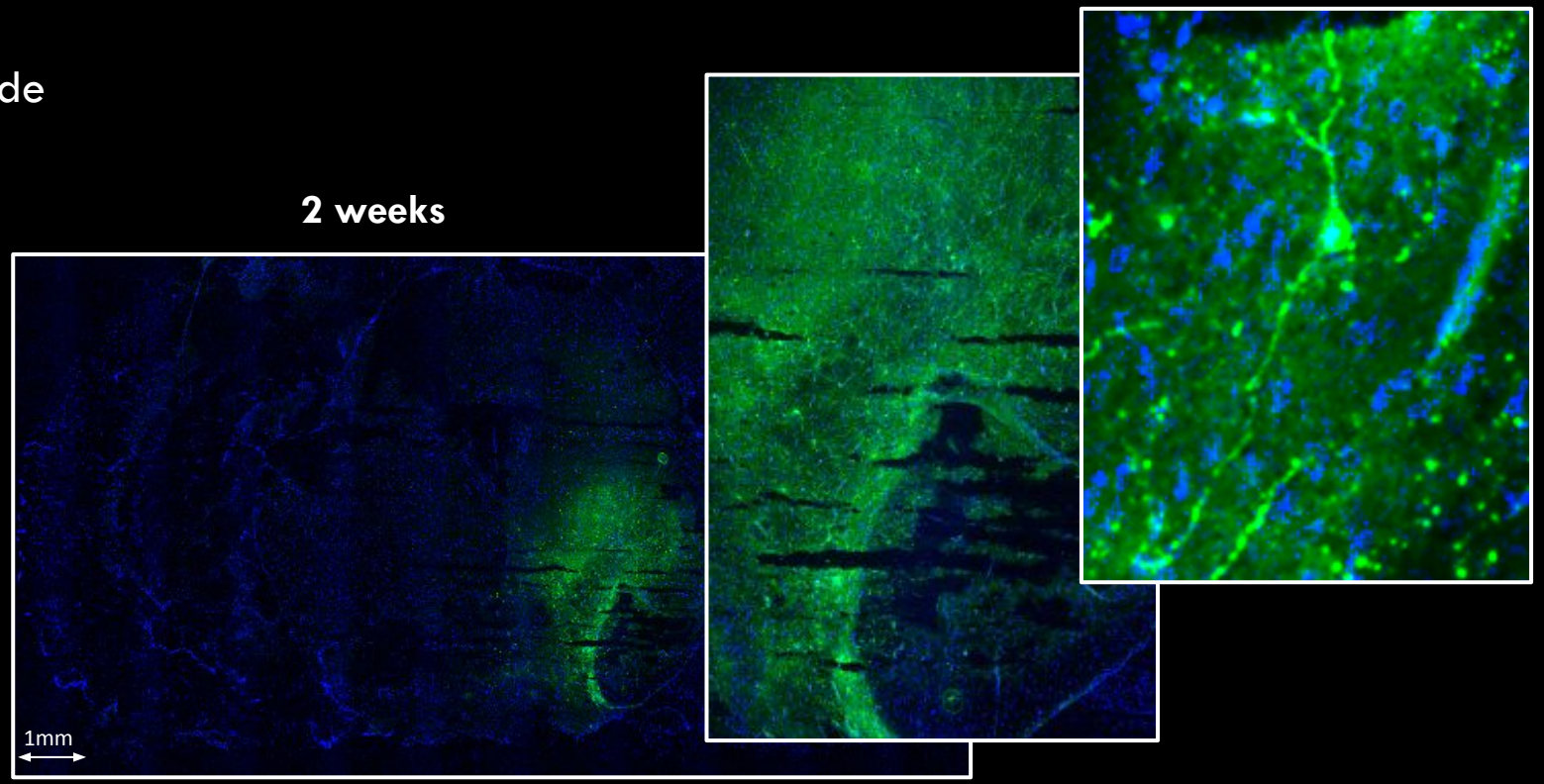
Model characteristics:

- A stereotactic injection of a viral vector expressing the human alpha-synuclein protein.
- The injection is preformed directly to the right side substantial nigra (SN).



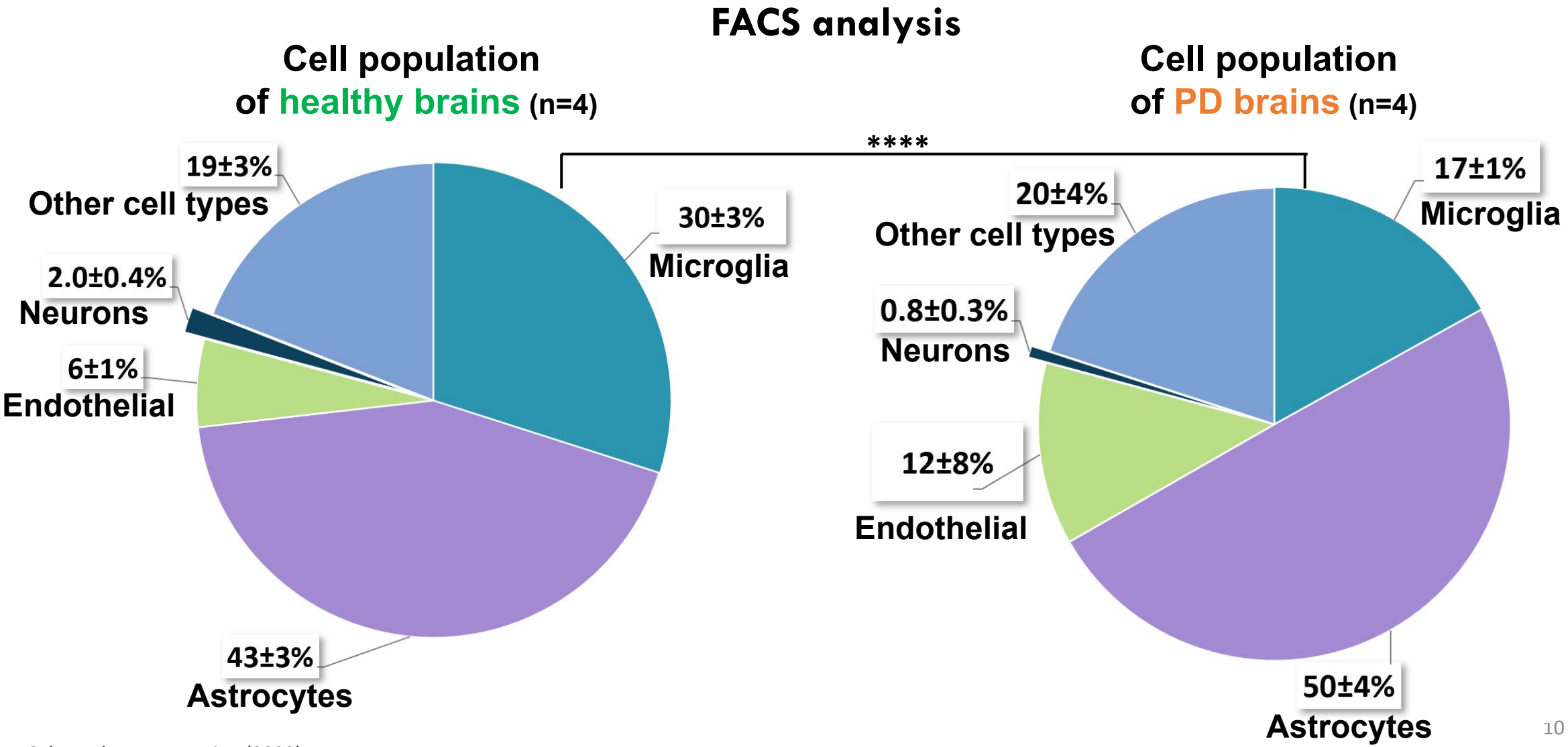
Immunohistochemistry image of brain cuts in the Substantia-nigra (SN) after viral injection:

2 weeks



Green - anti-human alpha synuclein (in all its forms)
Blue – nuclei

Cell Population in Mice Brains



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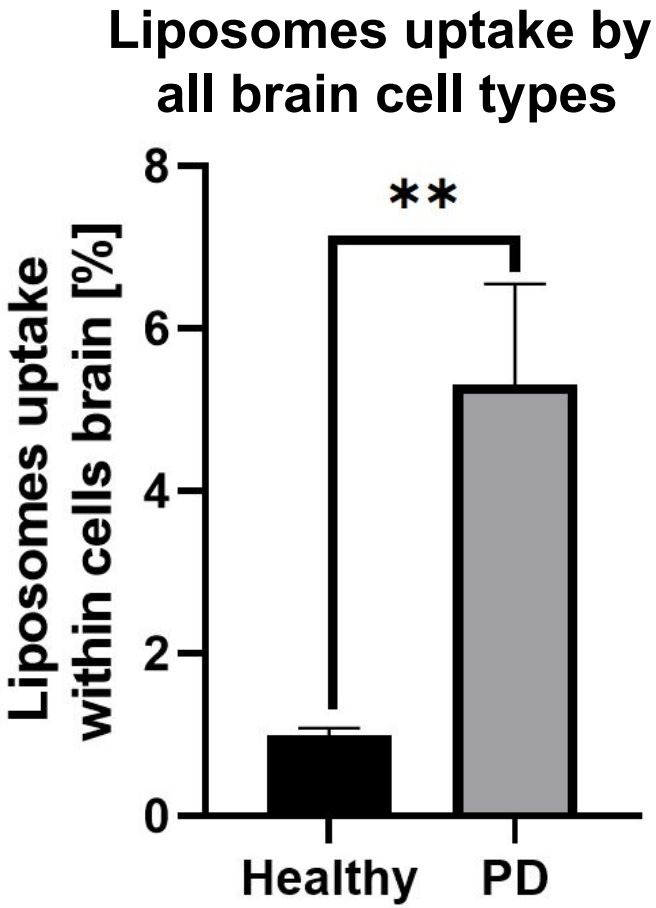
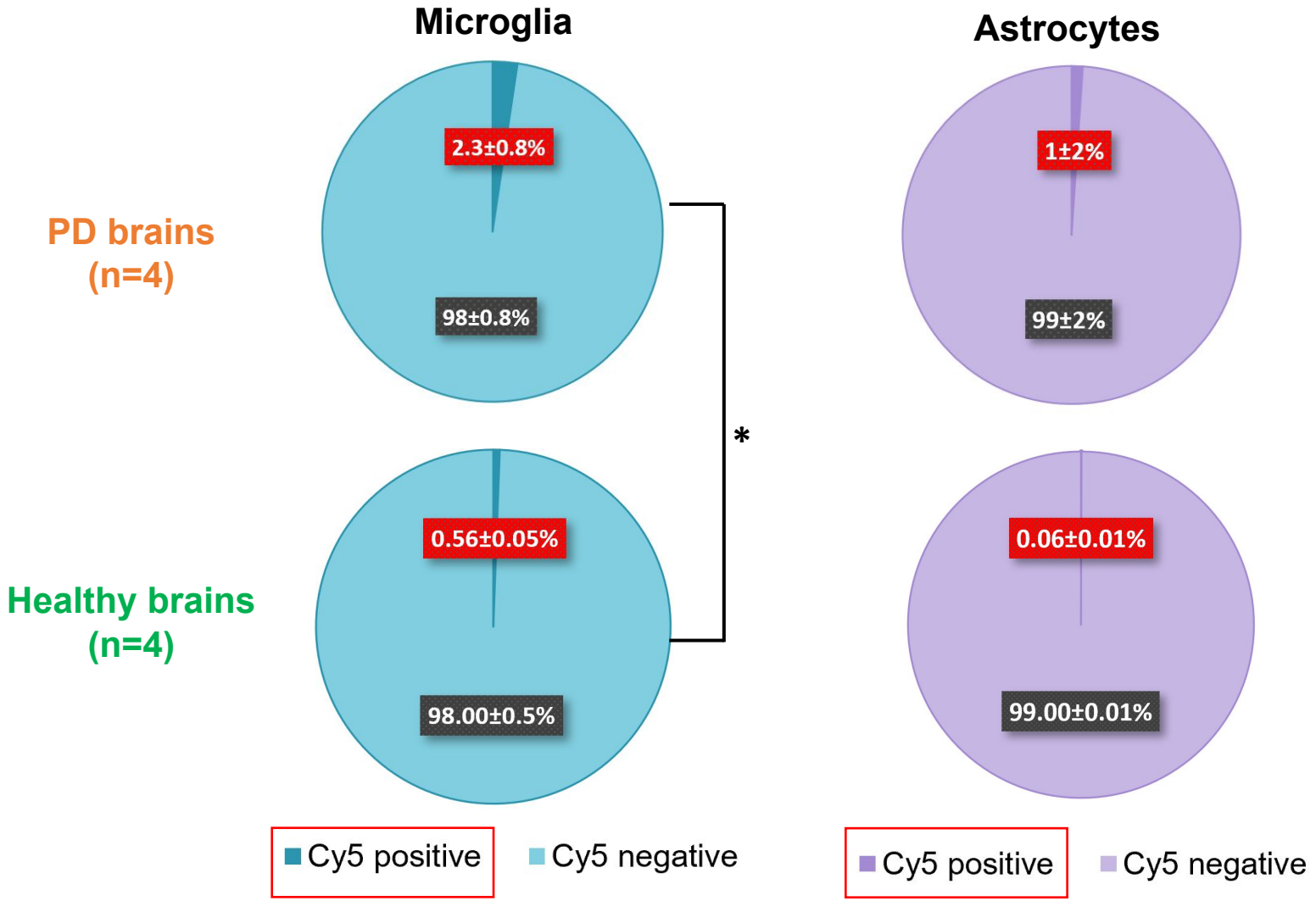
Liposomes Biodistribution in PD Brains Compared to Healthy Brains





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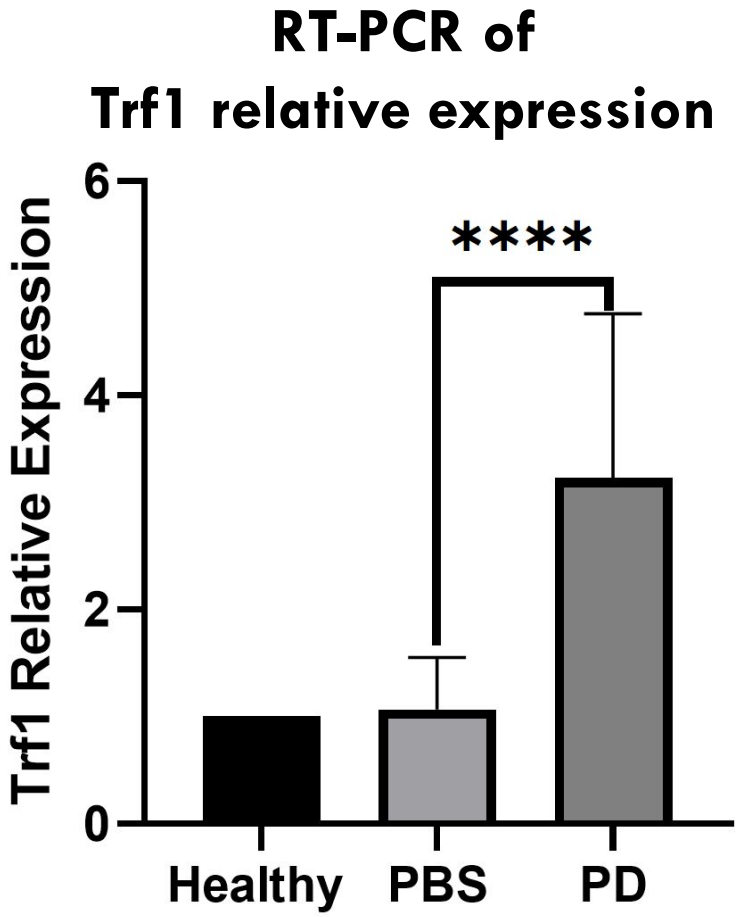
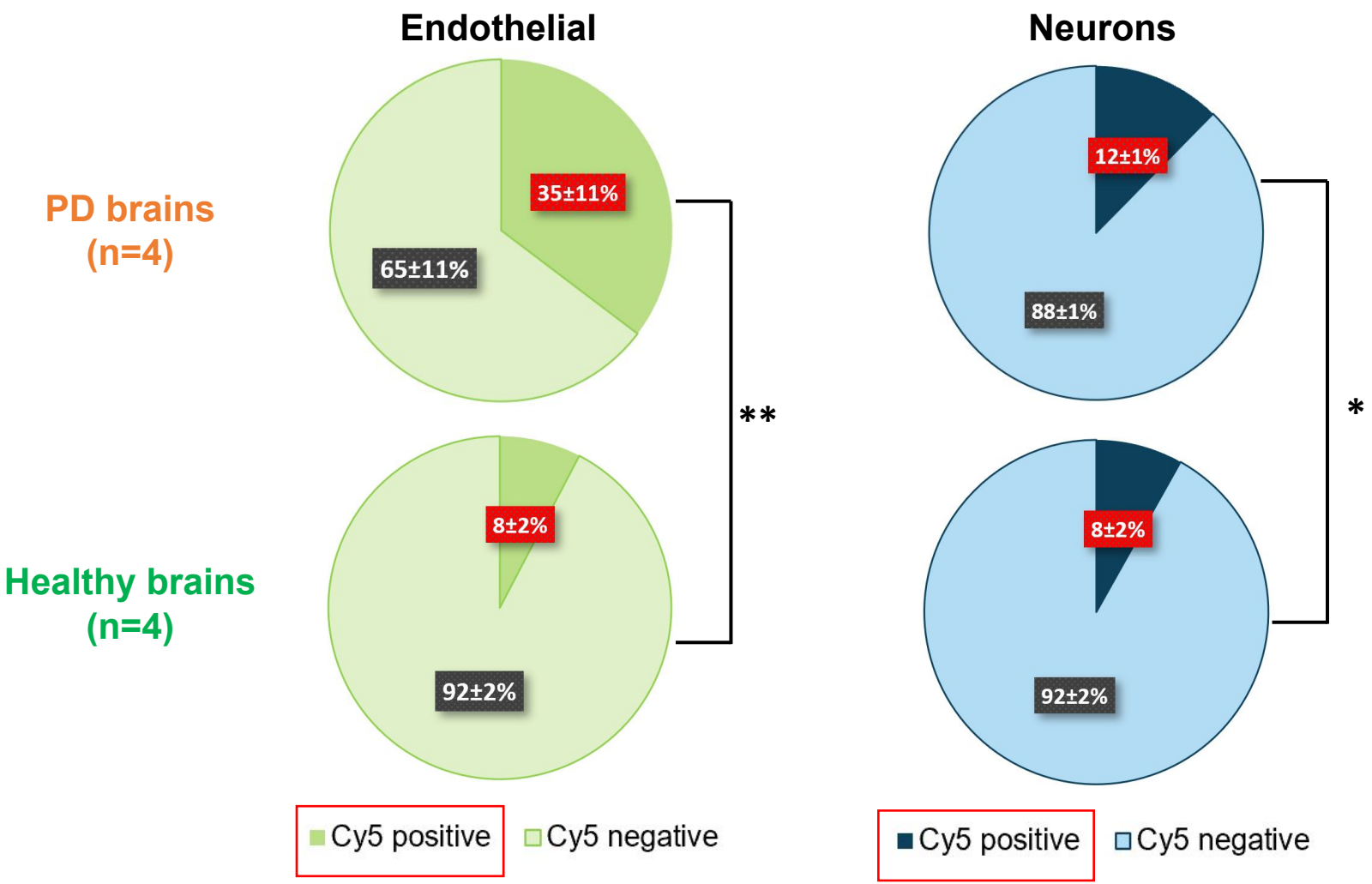
Liposomes Biodistribution in PD Brains Compared to Healthy Brains





Trf = transferrin receptor

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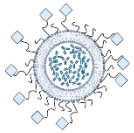
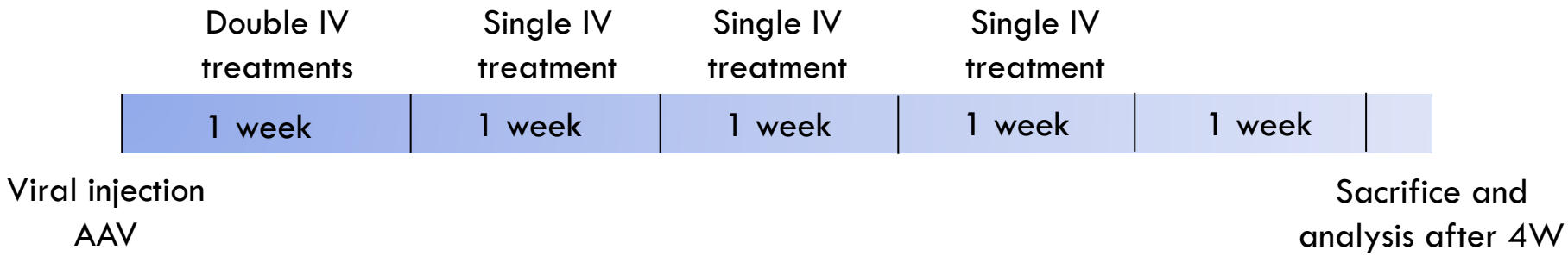
Preliminary Results of Efficacy Experiment

Experiment treatments:

- TF-SyO4-lipo 
- Free SyO4 mAb 
- Control – PD mice
- Healthy mice

Timeline setup:



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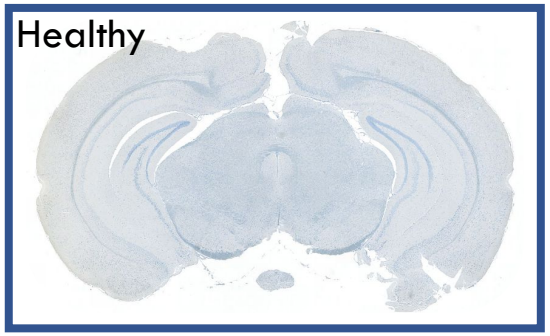
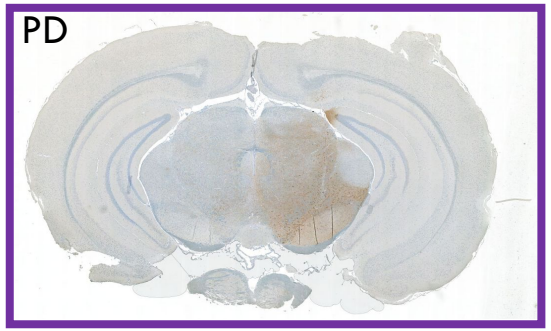
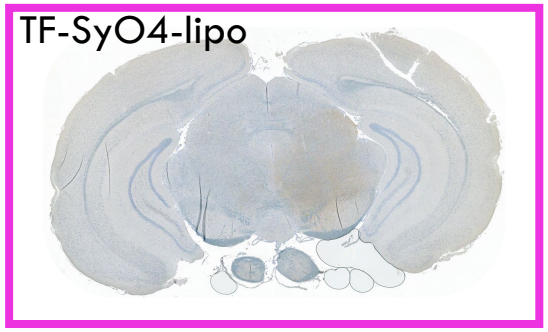
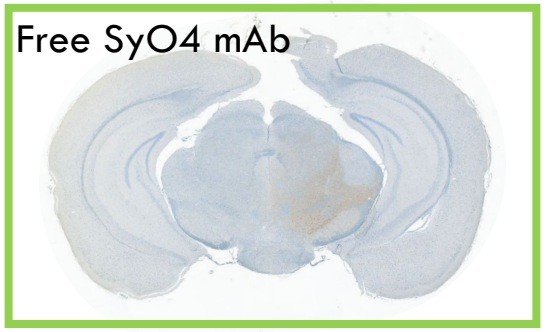
Preliminary Results of Efficacy Experiment



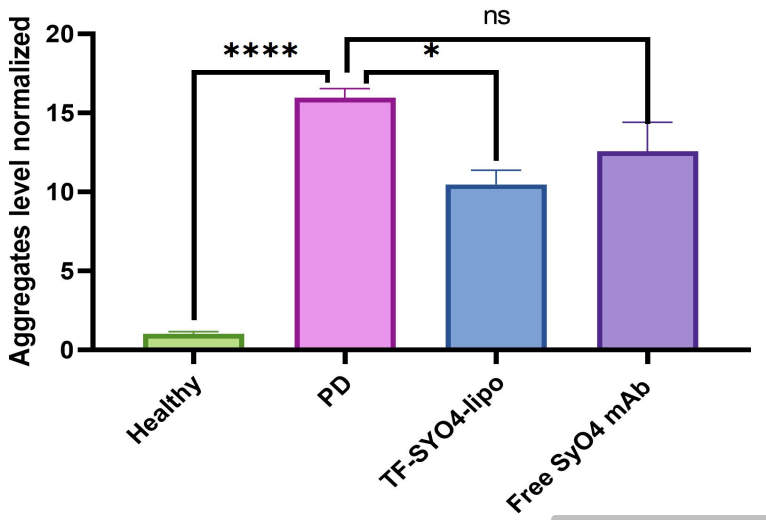






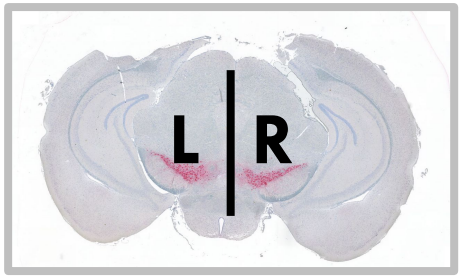
DAB staining of alpha-synuclein aggregates

TF-SyO4-lipo treatment reduces the aggregation level by ~1.5-fold.



The quantification analysis was done by normalization ot the threshold value between the right side (R) to the left side (L).
The columns in the graphs represent a mean of R/L values.

Right side (R) – virus injected
Left side (L) – not injected



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Summary

- TF-SyO4-liposomes successfully reduced the level of apoptotic cells.
- TF-SyO4-liposomes successfully crossed the BBB and taken up by neurons cells.
- 4W post viral injection, there was seen a reduction at the aggregation level due to the liposomal treatment.

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Thank you!

The Schroeder Lab

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Yousef Mansour

רשות החדשנות
Israel Innovation Authority

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TEVA's Bio-Innovators Forum Scholarship