



The Development of [^{11}C]M503-1619 As a PET Tracer for Imaging α -Synucleinopathies in Parkinson's Disease (PD)

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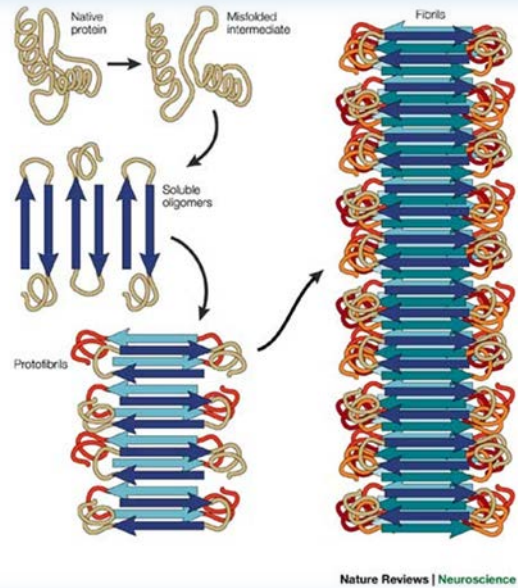
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⁶ Department of Radiology, University of Pittsburgh, Pittsburgh, PA, USA.

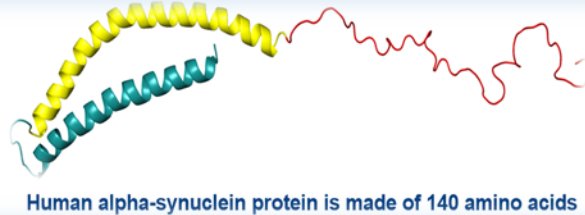
The 25th iSRS - Honolulu, Hawaii, USA. May 26, 2023

Features of Neurodegenerative Diseases

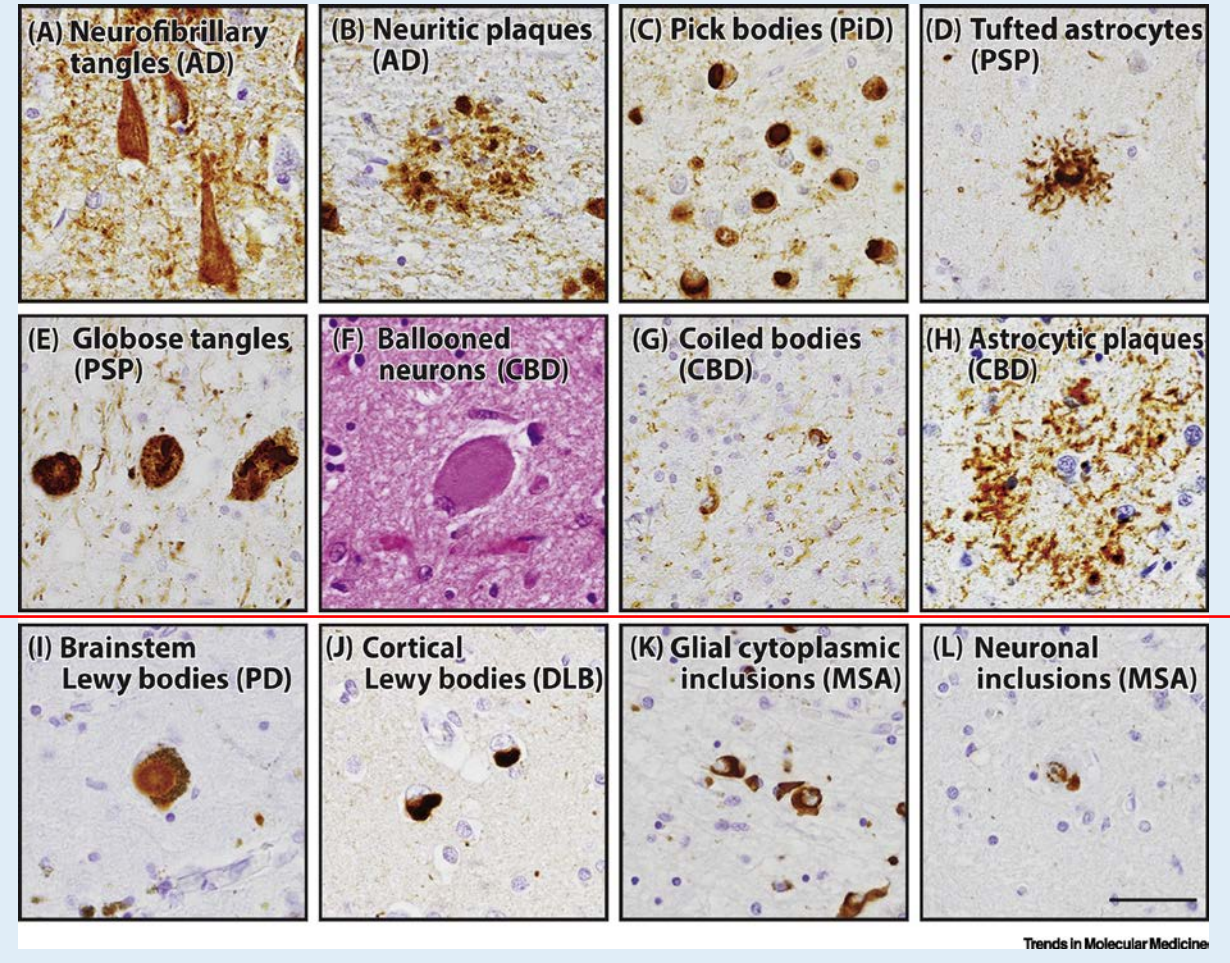


Abnormal protein assemblies

(Protein misfolding)



α -Synuclein aggregates



Charan Ranganath and Gregor Rainer *Nature Rev. Neurosci.* **2003**, 4, 193; Norihito Uemura *et al. Trends in Molecular Medicine* **2020**, 26, 936.

α -Synuclein aggregates are a hallmark of Parkinson's disease (PD) and multiple system atrophy (MSA)

Challenges for Developing α -Synuclein PET Tracers

- The absolute concentration of α -syn aggregates vs A β and tau (10 to 50-fold lower);



- High affinity for α -synuclein ~ 1 nM;

Jamie L. Eberling. *et al. J. Park. Dis.* **2013**, 3, 565; Chester A. Mathis *et al. Semin. Nucl. Med.* **2017**, 47, 553; Devika P. Bagchi *et al. PLoS ONE* **2013**, 8, e55031.

- Co-existence and co-localization of α -synuclein aggregates with A β and tau fibrils;



- Good selectivity Vs A β and tau ~ 30-50;

Maliha Shah *et al. J. Nucl. Med.* **2014**, 55, 1397; Elina T. L'Estrade *et al. Neuropharmacology* **2020**, 172, 107830.

- Most of α -synuclein inclusions are found intracellularly.



- Pass the BBB *plus* cell membranes.

Chester A. Mathis *et al. Semin. Nucl. Med.* **2017**, 47, 553; Maliha Shah *et al. J. Nucl. Med.* **2014**, 55, 1397.

Various structural forms:
Oligomers, fibrils, misfolded proteins

.....

The limited ligands number of ligands and data

Mohammad Shahnawaz *et al. Nature* **2020**, 578, 273.

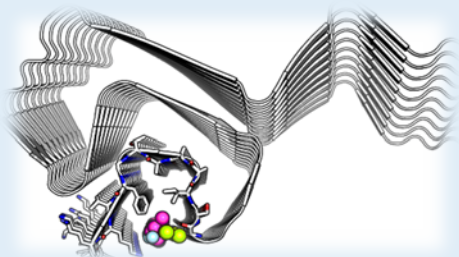
in vitro assays or *in vivo* models

Marcus D Tuttle *et al. Nat. Struct. Mol. Biol.* **2016**, 23, 409.

Timo Strohäker *et al. Nat. Commun.* **2019**, 10, 5535.

The Process of Finding the Ligand: *In silico* Methods and *In Vitro* Screening

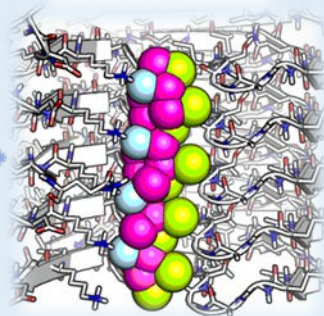
Identify Binding Site in Protein



α -Syn ssNMR Site 9 (2NOA)

Chia-Ju Hsieh *et al.* *ACS Chem. Neurosci.* **2018**, 9, 2521;
John J. Ferrie *et al.* *Chem. Sci.* **2020**, 11, 12746.

Exemplar



In Silico Ultrahigh Throughput Search

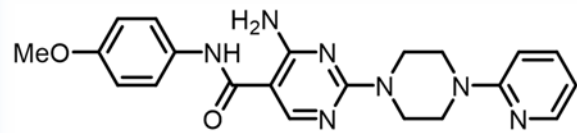
Large Database
47 M Compounds

Molecular Filter
To identify "in silico hits"

~6 M compounds

~50000 compounds

Chia-Ju Hsieh *et al.* *Pharmaceuticals* **2023**, 16, 317.

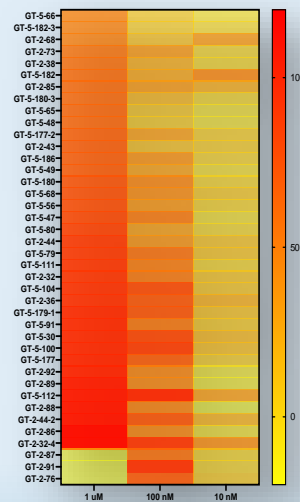


M503-1619

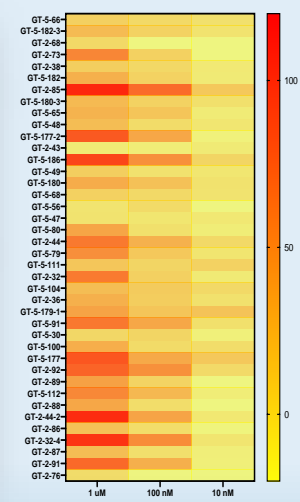
One of the α -Synuclein ligands we confirmed

Confirmed ligands

α -Syn

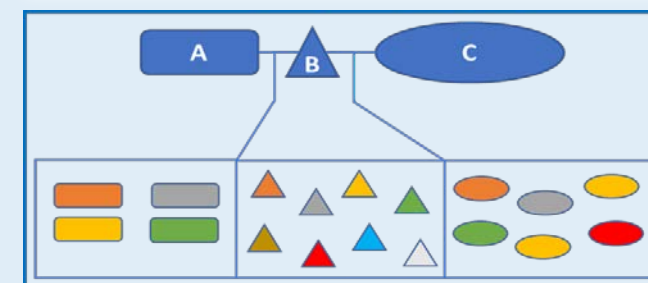


A β



High
Throughput
Screen

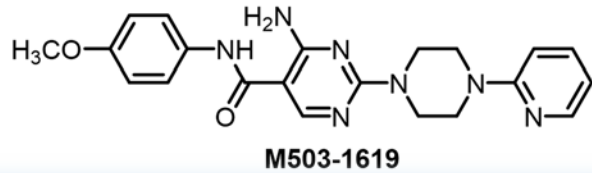
Buy compounds from commercial source



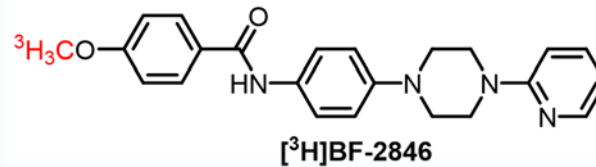
M503-1619 was identified as a suitable hit for further study

In Vitro Affinity Evaluation Toward Synthetic α -Synuclein Fibrils and AD Tissues

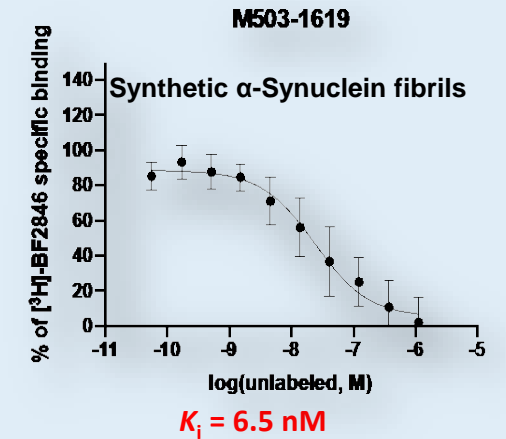
α -Synuclein competition assay



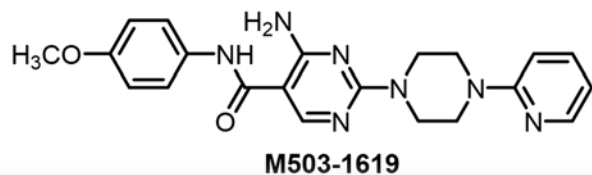
VS



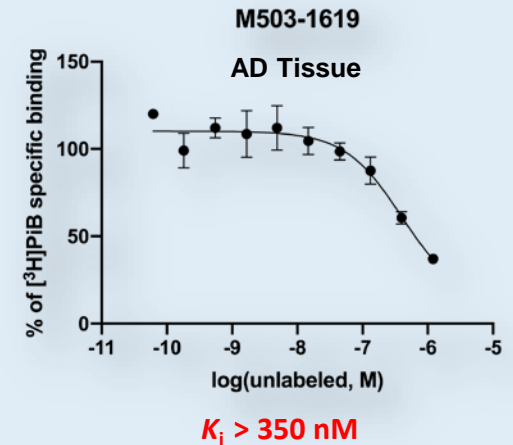
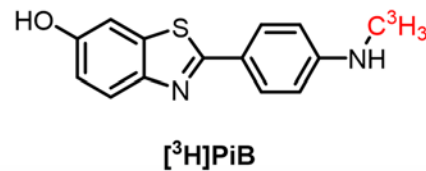
Chia-Ju Hsieh *et al.* ACS Chem. Neurosci. **2018**, 9, 2521.



A β -competition assay

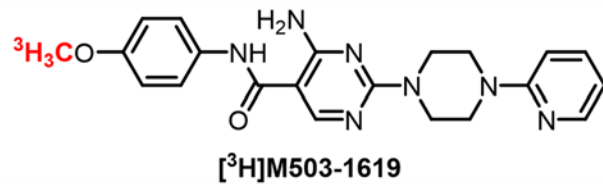
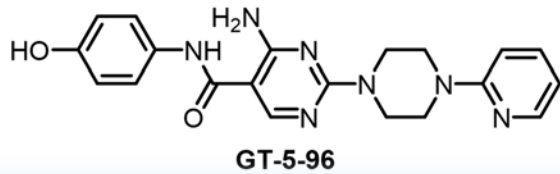


VS



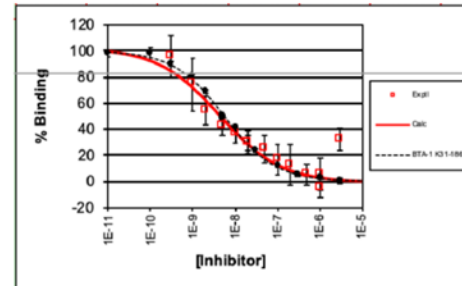
M503-1619 has selectivity to α -synuclein aggregates vs A β

Saturated Binding with PD Tissues

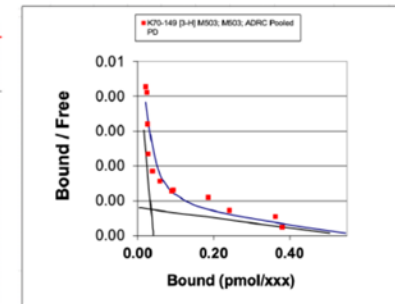


Saturation Assays with Human PD Tissues

0.1% BSA + Tris



$K_i = 4.2 \text{ nM}$



$K_d = 2.5 \text{ nM}$
 $B_{max} = 60 \text{ nM}$

Ligand	K_i (nM) α -Synuclein fibrils vs [³H]BF2846	K_i (nM) AD Tissue vs [³H]PIB	K_i (nM) PD Tissue	K_d (nM) PD Tissue	K_d (nM) AD Tissue	K_d (nM) PSP Tissue
M503-1619	6.5	>350				
[³H]-M503-1619			4.2	2.5	32	53

M503-1619 has selectivity to α -synuclein aggregates vs $A\beta$

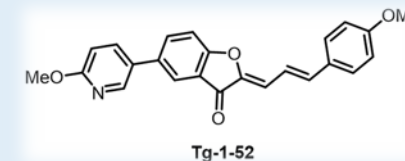
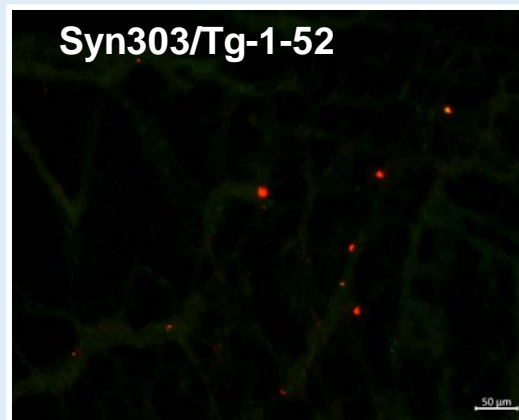
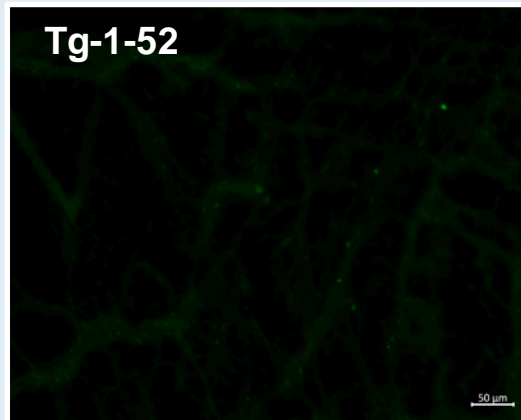
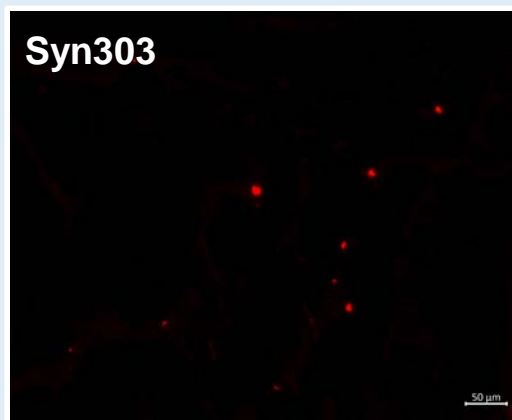
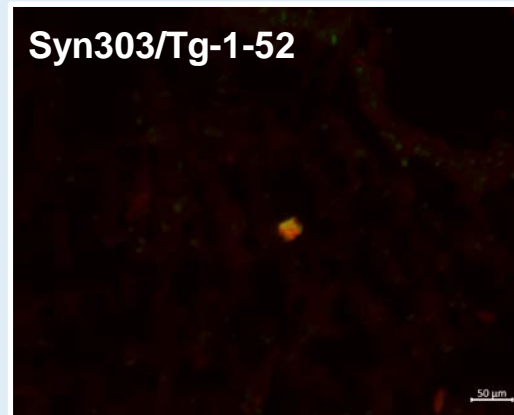
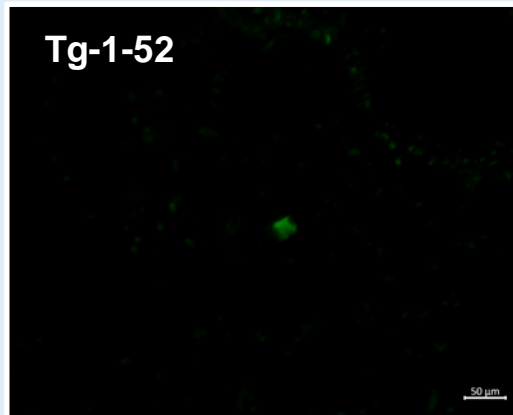
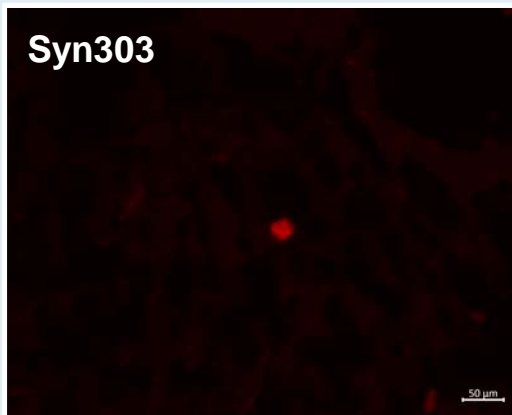
In Vitro Off Target Binding: Screening Against 44 G Protein-Coupled Receptor (GPCRs)

GPCPs	K _i (nM) or % inhibition	GPCPs	K _i (nM) or % inhibition	GPCPs	K _i (nM) or % inhibition
Dopamine D1	NA	Muscarinic M1	NA	BZP Rat Brain Site	NA
D2	NA	M2	NA	GABAA	NA
D3	NA	M3	NA	SERT	NA
D4	NA	M4	NA	DAT	NA
D5	NA	M5	NA	NET	NA
Serotonin 5-HT1A	NA	Adrenergic α1A	NA	PBR	NA
5-HT1B	NA	α1B	NA	Opioid μ	NA
5-HT1D	NA	α1D	NA	κ	NA
5-HT1E	NA	α2A	NA	δ	NA
5-HT2A	NA	α2B	NA	Histamine H1	NA
5-HT2B	NA	α2C	NA	H2	NA
5-HT2C	NA	β1	NA	H3	NA
5-HT3	NA	β2	NA	H4	NA
5-HT5A	NA	β3	NA		
5-HT6	NA				
5-HT7A	NA	Sigma σ 1	NA	NA = not active (no binding at 10 μM)	
		σ 2	NA		

M503-1619 doesn't bind to the tested GPCRs

Structure of Insoluble α -Synuclein Aggregates is Different in Different Pathologies

Tg-1-52: Microscopy Studies in Human Brain



Parkinson's disease (PD)

- Lewy body pathology
- Middle Frontal Gyrus
- α Syn 2+
- Neuron loss 1+
- A β , tau 0

Multiple System Atrophy (MSA)

- Glial cell inclusions
- Cerebellum
- α Syn 3+
- Neuron loss 3+
- A β , tau 0

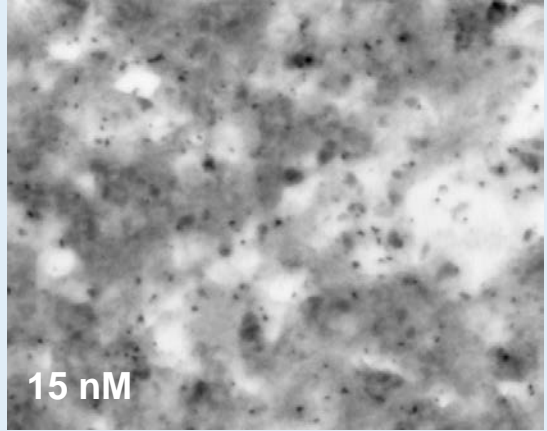
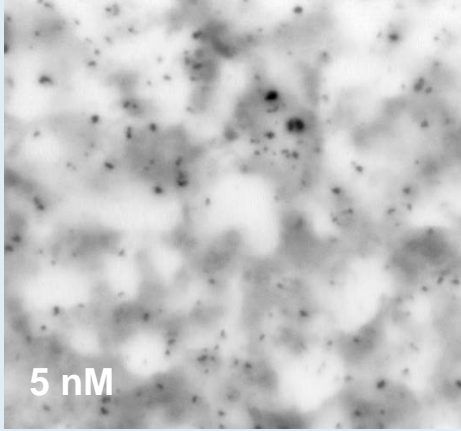
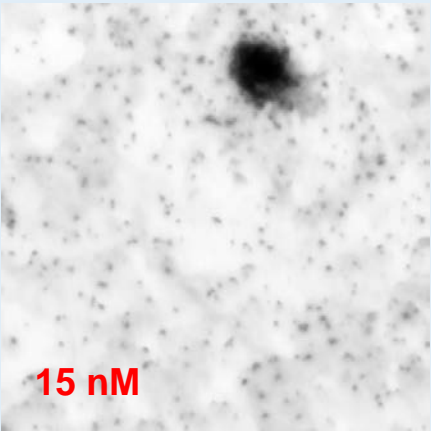
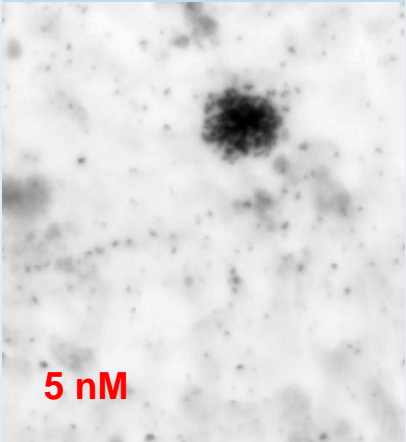
Zsofia Lengyel-Zhand *et al.* *Chem. Commun.* **2020**, 56, 3567.

[³H]M503-1619: Nuclear Emulsion Autoradiography Studies

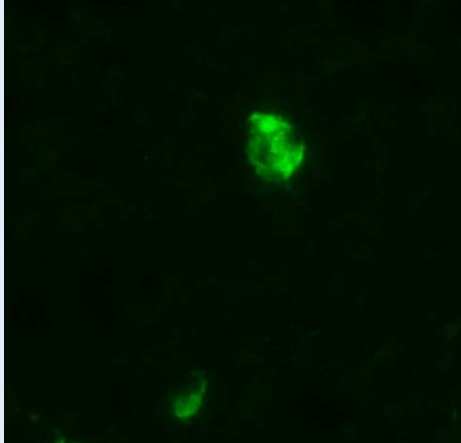
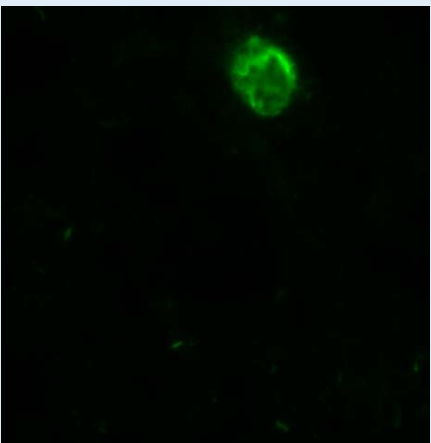
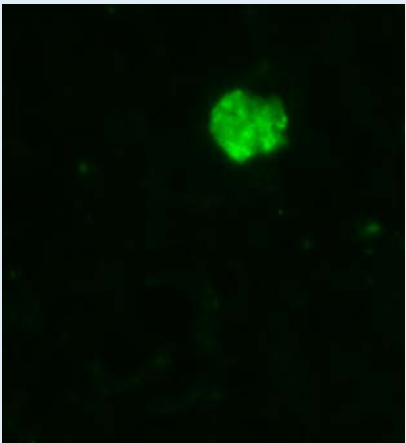
PD Brain

MSA Brain

[³H]M503-1619



α -Syn Antibody

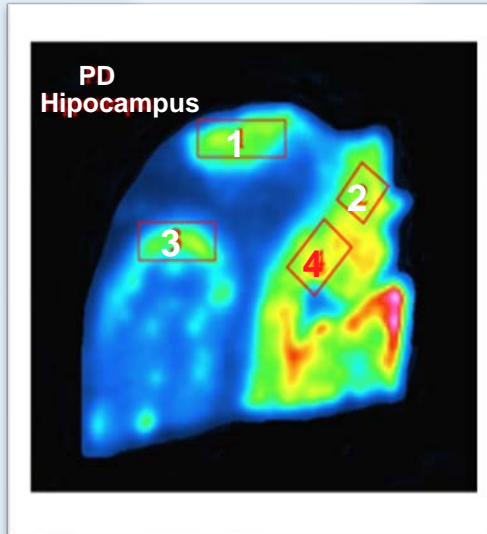


[³H]M503-1619 co-localized with α -synuclein antibody in PD not MSA

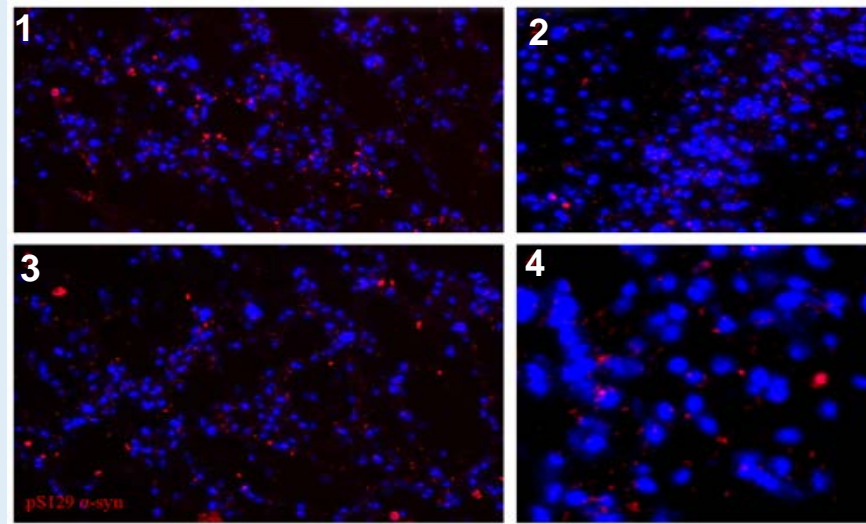
[³H]M503-1619: Autoradiography Studies

PD Brain

[³H]M503-1619



α-Syn Antibody

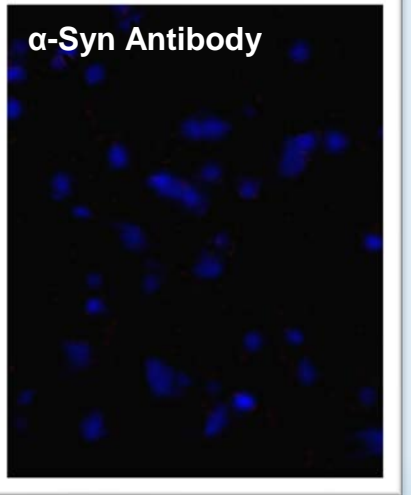
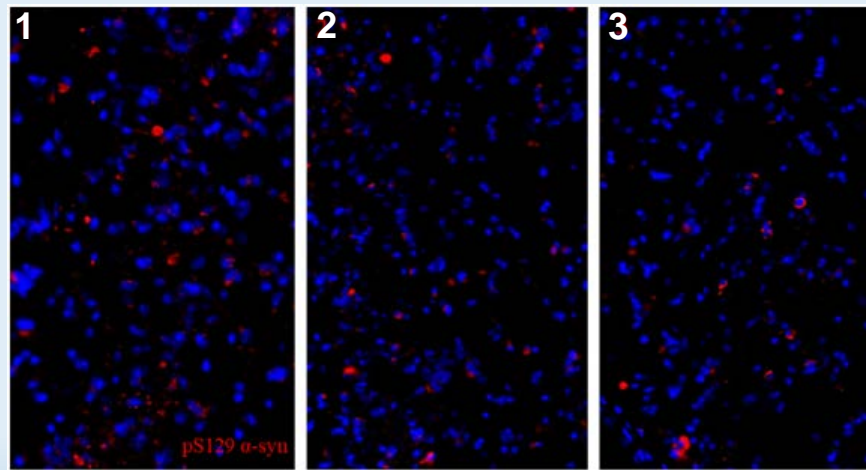
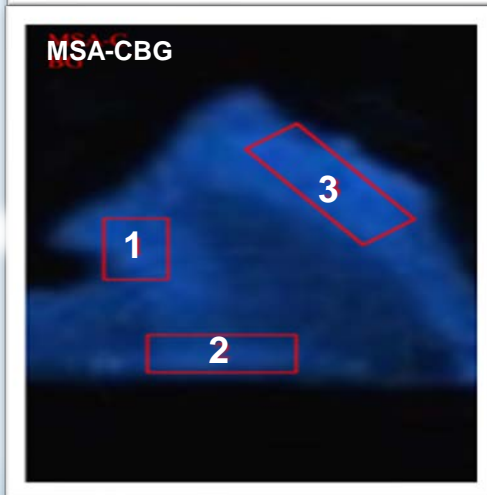


CT Frontal



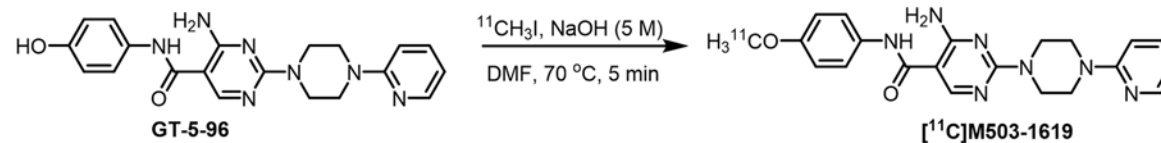
MSA Brain

MSA-CBG

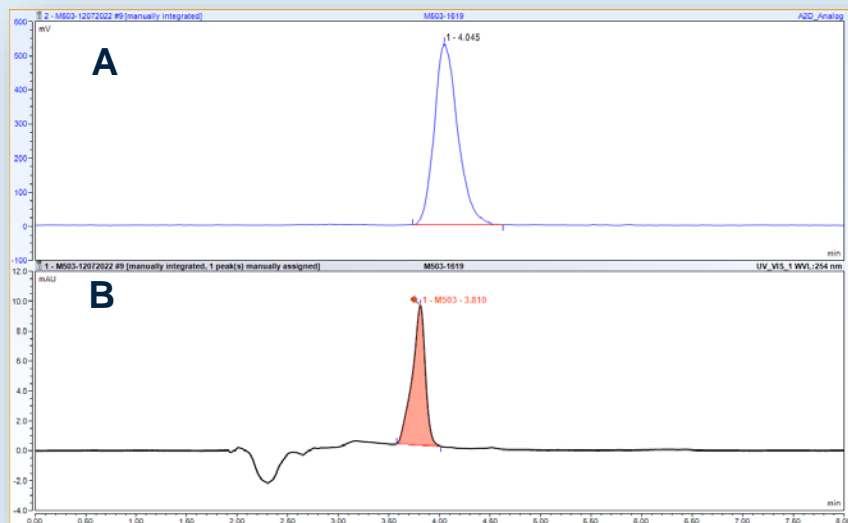


[³H]M503 binds to α-synuclein aggregates in PD not MSA

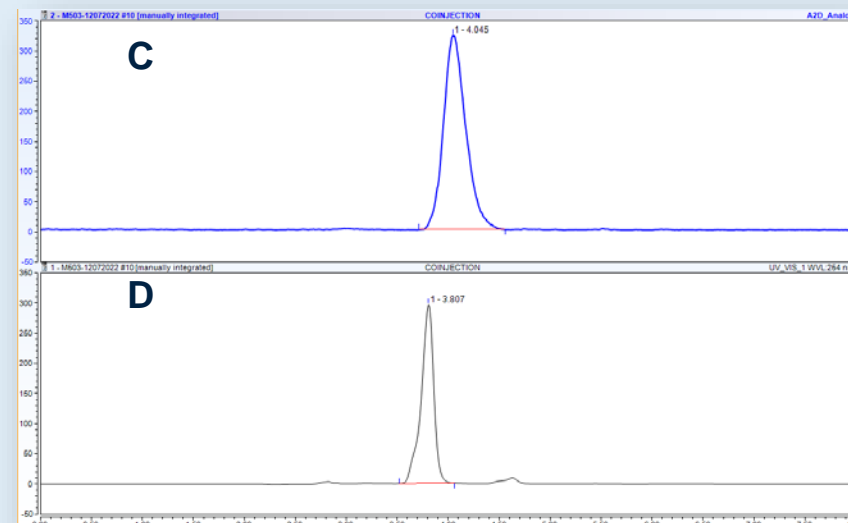
Radiosynthesis of [¹¹C]M503-1619 and HPLC Analysis



- ♣ 56-63% radiochemical yield; ♥ >1,187 GBq/μmol molar activity;
- ♣ >99% radiochemical purity; ♦ radiochemical yield and mol molar activity: decay corrected to EOB.



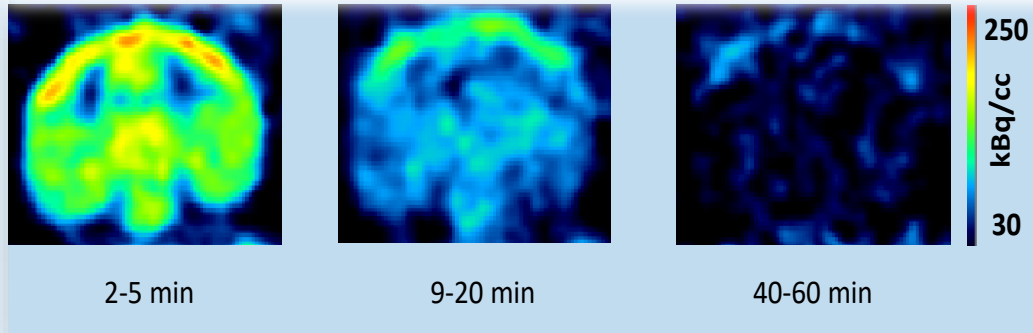
A: [¹¹C]M503-1619 radiochemical trace;
B: UV trace for [¹¹C]M503-1619 with 10% ethanol in 0.9% saline;



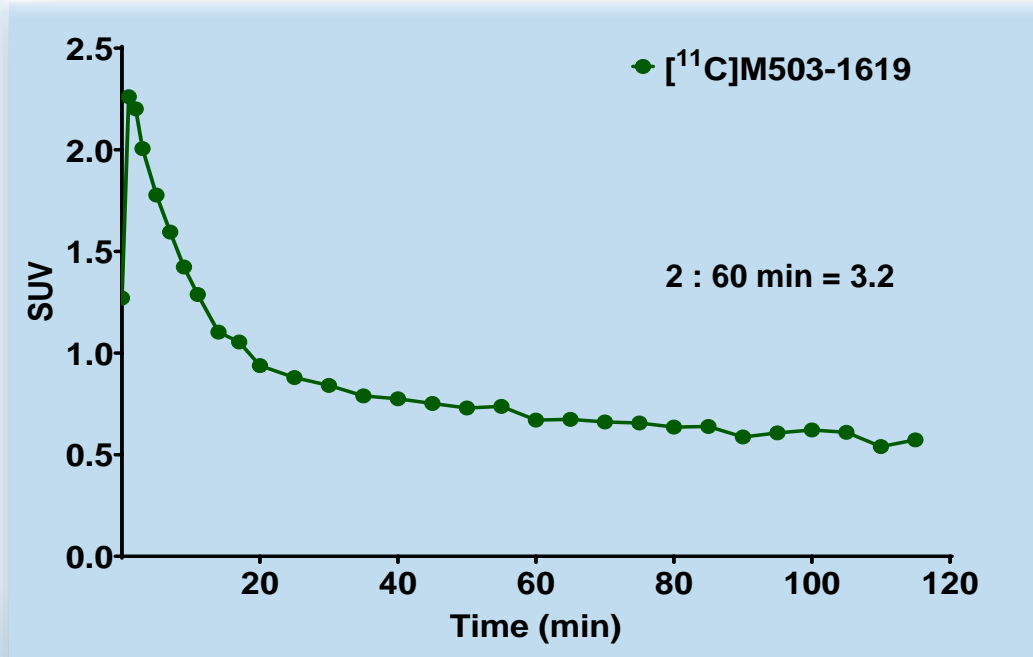
C: Radiochemical trace for co-injection of [¹¹C]M503-1619 and M503-1619;
D: UV trace for co-injection of [¹¹C]M503-1619 and M503-1619.

[¹¹C]M503-1619: PET Imaging in Non-Human Primates (NHPs) and Radiometabolite Studies

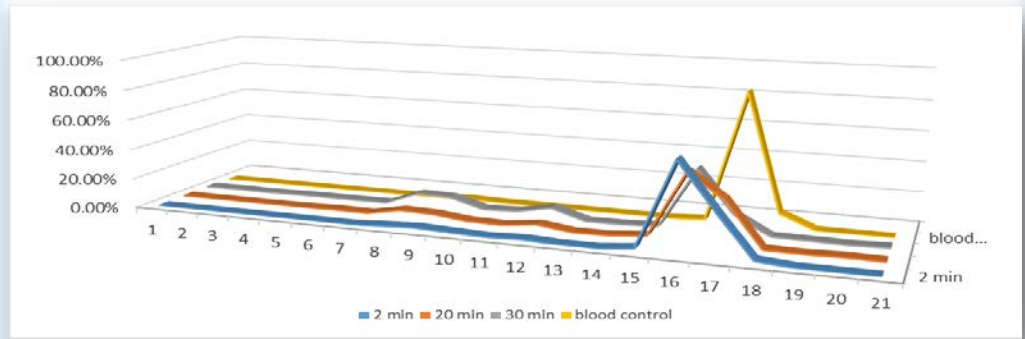
PET imaging studies in NHPs



Time-activity curves



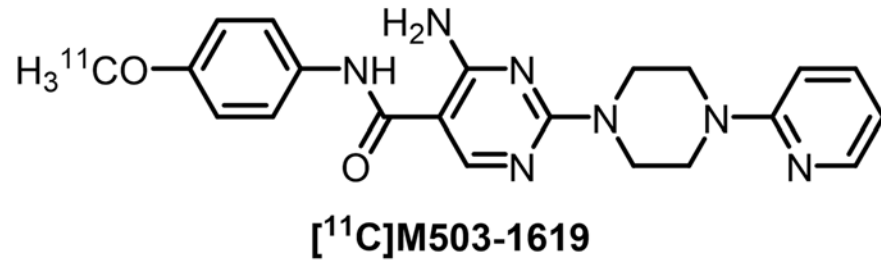
Radiometabolite Analysis



Time HPLC	2 min	20 min	30 min	blood control
1	0.11%	0.52%	0.85%	0.04%
2	0.04%	0.32%	0.97%	0.07%
3	0.05%	0.33%	0.57%	0.05%
4	0.05%	0.22%	0.82%	0.06%
5	0.06%	0.34%	0.89%	0.06%
6	0.05%	0.73%	0.76%	0.05%
7	0.06%	0.48%	0.94%	0.07%
8	1.25%	4.60%	9.30%	0.11%
9	2.18%	3.97%	8.59%	0.95%
10	1.29%	1.52%	2.43%	0.32%
11	0.41%	1.09%	3.30%	0.30%
12	1.28%	3.63%	7.48%	0.12%
13	0.18%	0.63%	0.73%	0.11%
14	0.16%	0.78%	0.87%	0.07%
15	2.39%	2.67%	1.92%	0.07%
16	58.50%	45.31%	40.89%	1.41%
17	29.50%	29.56%	13.90%	86.15%
18	2.05%	0.90%	1.51%	9.50%
19	0.20%	0.60%	1.13%	0.27%
20	0.12%	1.08%	0.72%	0.14%
21	0.08%	0.72%	1.41%	0.09%

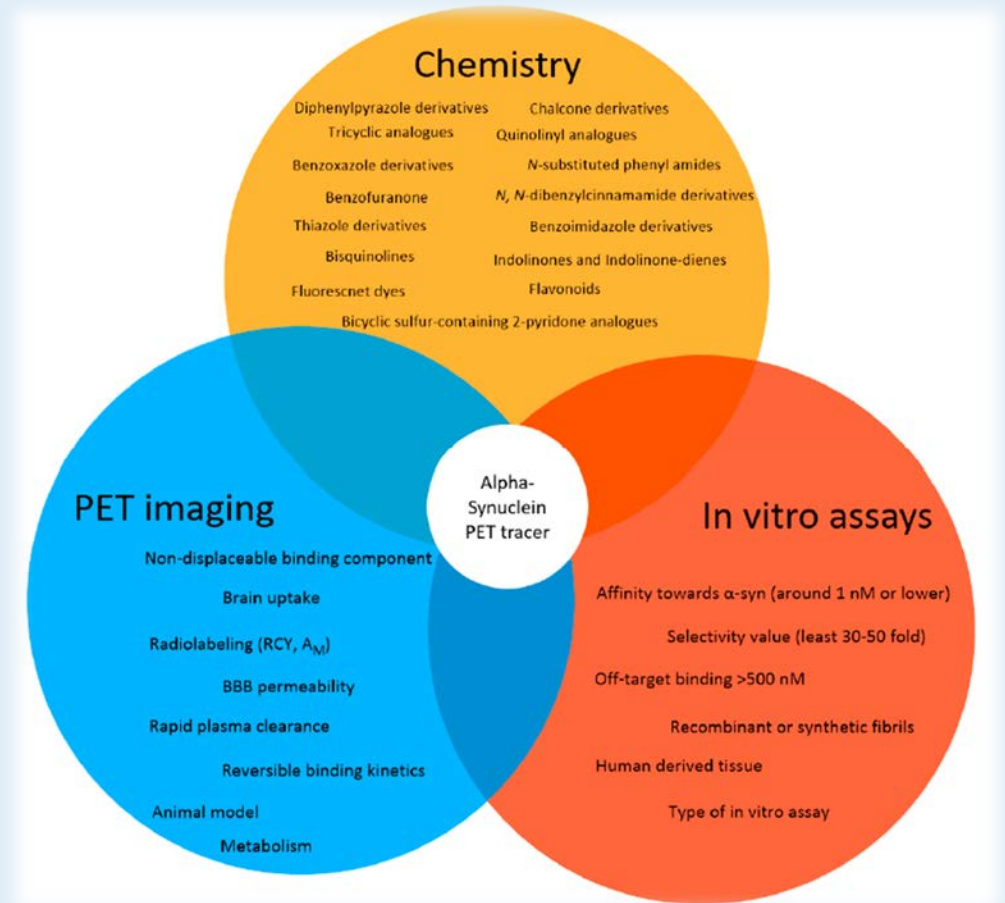
Pharmacokinetic and imaging properties are quite suitable for a PET tracer

Conclusions



- High affinity for α -synuclein, low affinity for $\text{A}\beta$;
- Good radiochemical yield, high molar activity and radiochemical purity;
- High specificity as demonstrated by *in vitro* off-target studies;
- Nuclear emulsion and ARG studies: α -Syn aggregates in PD not in MSA;
- High brain uptake and rapid washout.

Our data suggest that [^{11}C]M503-1619 has suitable properties for a PET radiotracer for translational imaging studies in PD subjects.



Špela Korat *et al. Pharmaceuticals* **2021**, *14*, 847.

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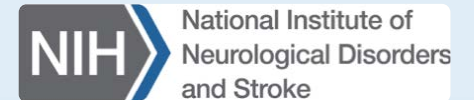
Gil Rabinovici, M.D.



<https://www.youtube.com/watch?v=IUA6XBHDPI0>



Thank you very much!



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