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Development of PET tracers for aggregated α-synuclein - towards imaging of Parkinson's disease

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

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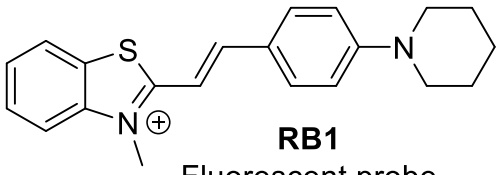
WSIC Werner Siemens Imaging Center

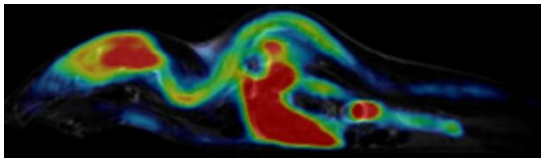
Development of PET tracers for aggregated α-synuclein - towards imaging of Parkinson's disease



RB1
Fluorescent probe
Gaur et al. 2021

^{3}H labeling,
Fibril binding assays/SAR,
Brain autoradiography,

^{11}C/^{18}F labeling,
Characterization

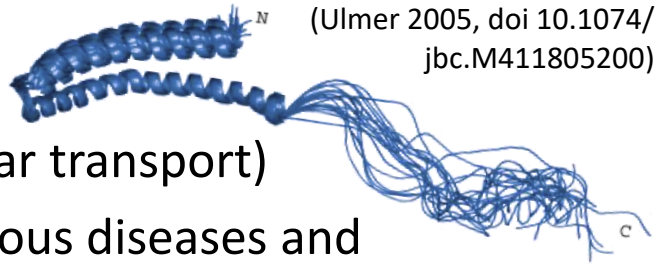


PET tracer candidate
for in vivo imaging
of synucleinopathies

Abstract:
Aggregation of α-synuclein into fibrils and their deposition in Lewy-bodies is a characteristic hallmark of Parkinson's disease, multiple system atrophy and various other neurodegenerative diseases. Better understanding of the spatiotemporal development of these aggregates would greatly facilitate diagnosis and therapy development and is thus desperately awaited. However, despite great efforts from different research groups in academia and industry no PET tracer for synuclein has reached clinical application yet. The need for high selectivity and optimal pharmacokinetic properties due to frequent presence of Aß and Tau co-pathologies, the intracellular localization and the low abundance of the target are critical challenges in the development of synuclein PET tracers.
We are currently pursuing several libraries on independent scaffolds and assessing their binding to recombinant synuclein, Aβ and Tau in direct or competitive assays. Automated radiolabeling procedures with ^{18}F or ^{11}C at high specific radioactivity are developed for promising tracer candidates. Furthermore, the compounds' binding to human brain slices harboring defined pathology is measured using *in vitro* autoradiography. Blood-brain barrier penetration, pharmacokinetics and metabolism are assessed in healthy mice to ensure suitability for brain imaging with low non-specific retention.
Using this strategy, we were able to achieve a diverse set of radiotracers with promising characteristics. While most of the compounds in literature suffer from suboptimal selectivity, we were able to identify a candidate with virtually absent Aß binding in our *in vitro* competition assay. While the pharmacokinetics of this candidate will need further optimization, the obtained data represent a promising starting point for future work.

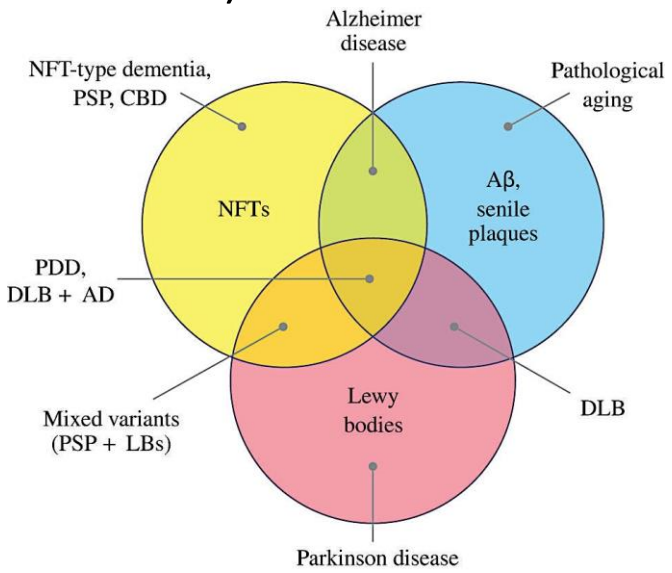
Keywords: Radiotracer; PET imaging; Alpha-Synuclein; Parkinson's disease

α-Synuclein:



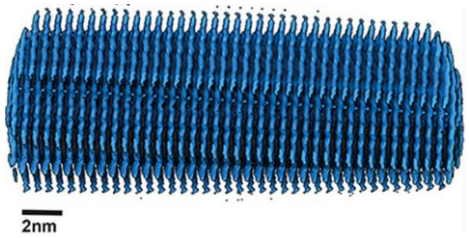
(Ulmer 2005, doi 10.1074/jbc.M411805200)

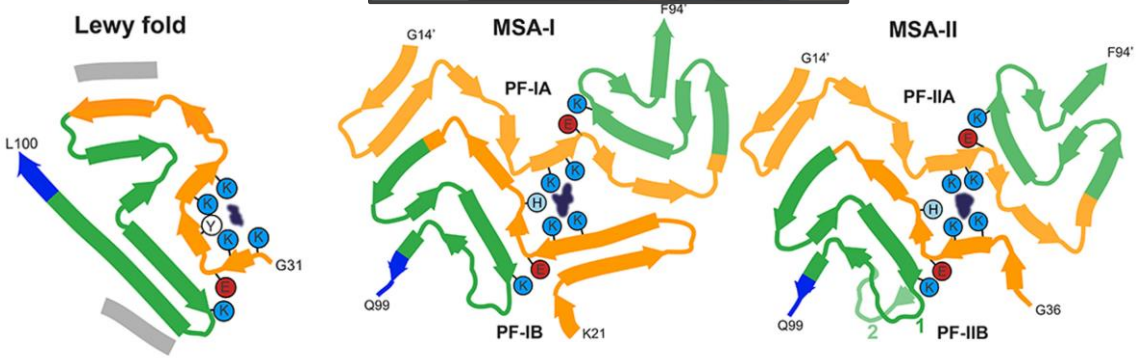
- 14.5 kDa protein
- Physiological role not precisely defined (vesicular transport)
- Aggregation (Lewy Bodies) is biomarker for various diseases and accompanied by neurodegeneration.
  - Parkinson's disease (genetic and environmental factors)
  - Multiple System Atrophy (MSA)
  - Dementia with Lewy Bodies (DLB)
- Often overlapping pathologies (proteins)
- Spatiotemporal interplay?
- Differential diagnosis?



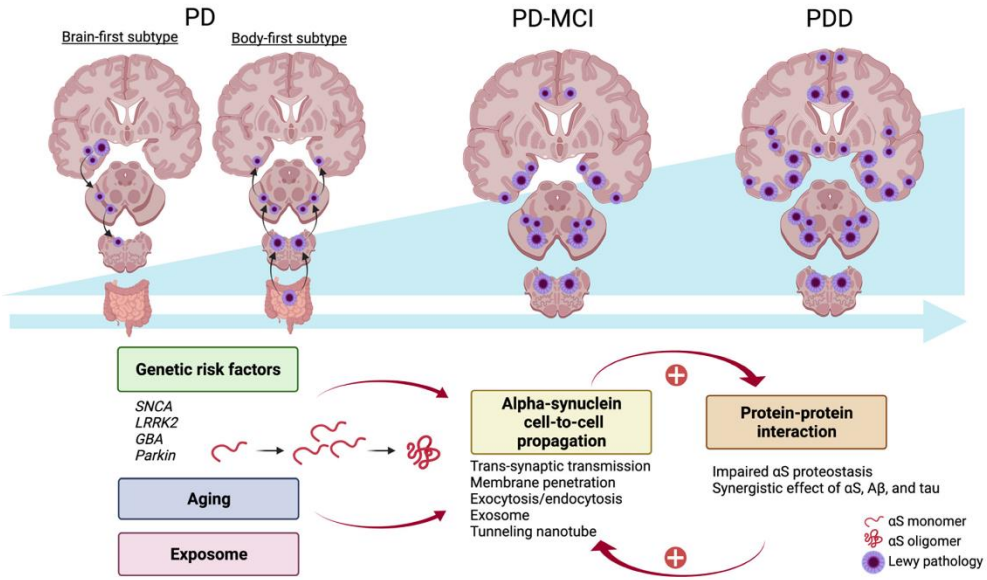
(Jellinger 2011, doi 10.1100/2011/371893)

Fibril types and structures





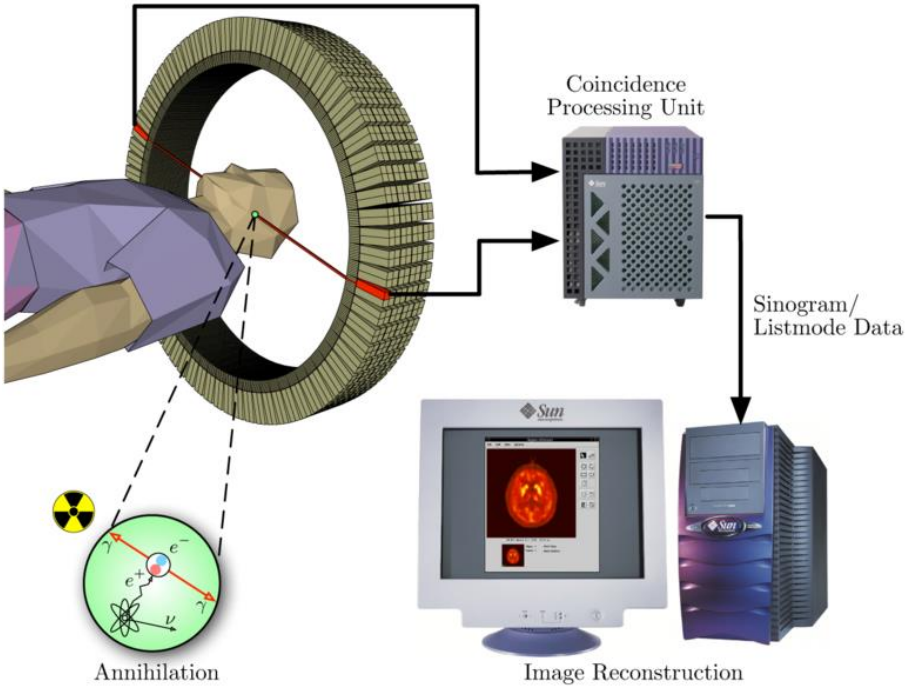
(Yang et al. 2022, doi 10.1038/s41586-022-05319-3)



(Fan et al. 2021, doi 10.3390/life11111239)

MDPI

Positron Emission Tomography



(Jens Maus)

- Sensitive and quantitative
- Clinical scanners and radiopharmacy infrastructure available in many places
- Proven for other many diseases, e.g. Alzheimer's
- ^{18}F ($T_{1/2}$=109.7 min, cyclotron-produced) is favorable for small molecules
- Only trace amounts need to be applied → imaging of scarce binding sites

ACS Chemical Neuroscience

pubs.acs.org/chemneuro Letter

Fluorescent Probe for Selective Imaging of α-Synuclein Fibrils in Living Cells

Pankaj Gaur,* Maksym Galkin, Andrii Kurochka, Subrata Ghosh, Dmytro A. Yushchenko, and Volodymyr V. Shvadchak*

Cite This: *ACS Chem. Neurosci.* 2021, 12, 1293–1298 Read Online

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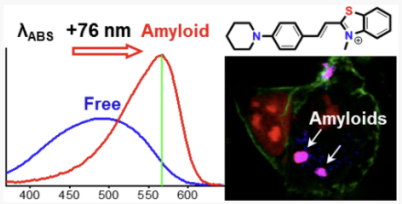
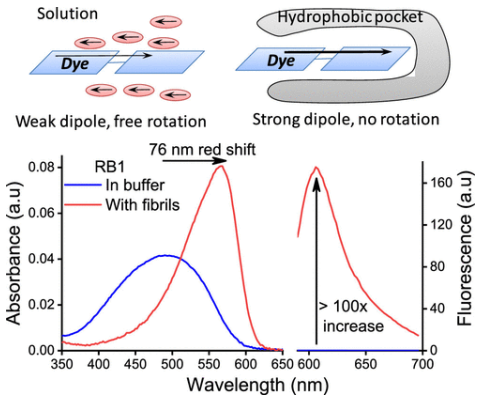
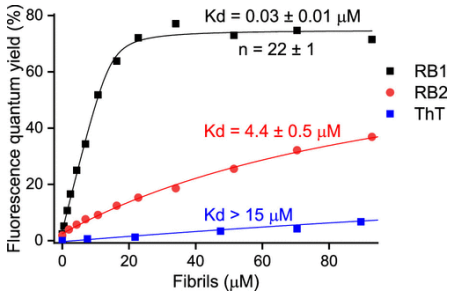
ABSTRACT: Plaques of amyloid fibrils composed of neuronal protein α-synuclein are one of the hallmarks of Parkinson's disease, and their selective imaging is crucial to study the mechanism of its pathogenesis. However, the existing fluorescent probes for amyloids are efficient only in solution and tissue systems, and they are not selective enough for the visualization of amyloid fibrils in living cells. In this study, we present two molecular rotor-based probes RB1 and RB2. These thiazolium probes show affinity to α-synuclein fibrils and turn-on fluorescence response upon interactions. Because of its extended π-conjugation and high rotational degree of freedom, RB1 exhibits a 76 nm red-shift of absorption maxima and 112-fold fluorescence enhancement upon



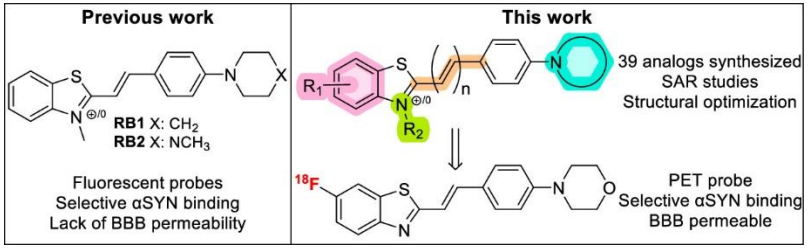


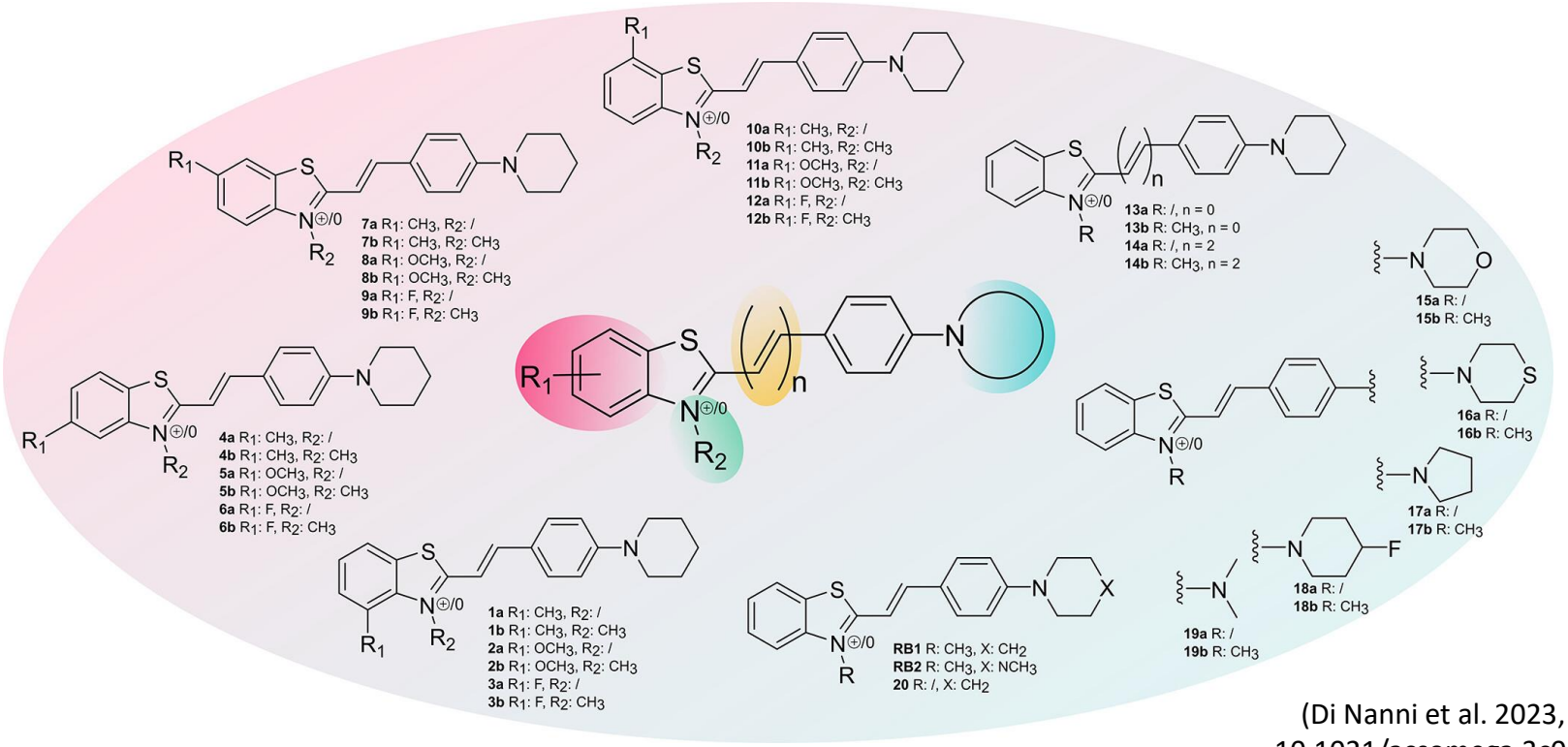


(Gaur et al. 2021, doi 10.1021/acschemneuro.1c00090)

- Fluorescent *in vitro* probes
- Selective binding (affinity unclear)
- Charged, presumably no BBB permeability
- Still a good starting point for PET tracer development?

A. Di Nanni



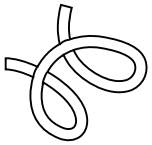


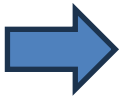
(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

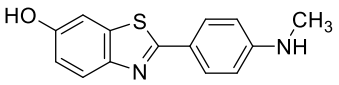

RS Saw


Prof. K. Herfert

Building the tools…

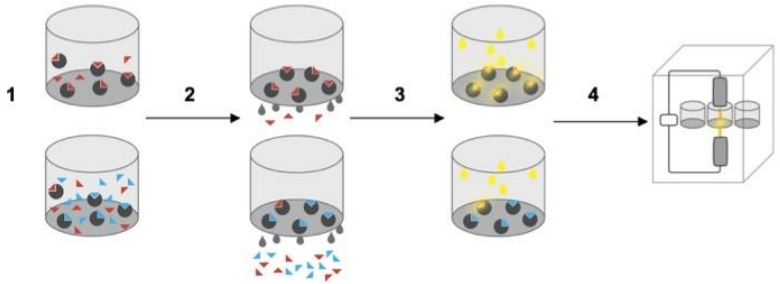

Recombinant protein fibrils





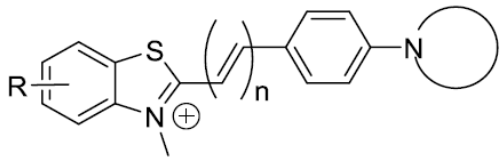
^{3}H-labeled PiB

Filter Binding Assay



Herfert *et al.*, 2019, Radiopharmaceutical Chemistry (Springer, 2019)

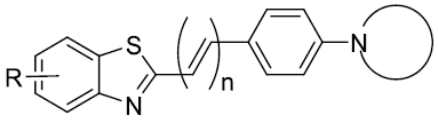
A. Di Nanni



#	R	n	*N*-substitution	K_i (nM)
RB1	H	1	*N*-piperidine	>400
RB2	H	1	*N*-(*N*-methyl)piperazine	>400
1b	4-CH_3	1	*N*-piperidine	36.1; 83.9
2b	4-OCH_3	1	*N*-piperidine	70.8; 34.3
3b	4-F	1	*N*-piperidine	>400
4b	5-CH_3	1	*N*-piperidine	84.6; 59.9
5b	5-OCH_3	1	*N*-piperidine	27.5; 162.9
6b	5-F	1	*N*-piperidine	435.2; 268.2
7b	6-CH_3	1	*N*-piperidine	16.8; 23.8
8b	6-OCH_3	1	*N*-piperidine	19.7; 9.6
9b	6-F	1	*N*-piperidine	335.4; 43.6
10b	7-CH_3	1	*N*-piperidine	231.3; 63.9
11b	7-OCH_3	1	*N*-piperidine	233.7; 177.3
12b	7-F	1	*N*-piperidine	>400
13b	H	0	*N*-piperidine	>400
14b	H	2	*N*-piperidine	23.0; 16.8
15b	H	1	*N*-morpholine	>400
16b	H	1	*N*-thiomorpholine	>400
17b	H	1	*N*-pyrrolidine	144.7; 58.6
18b	H	1	*N*-fluoropiperidine	>400
19b	H	1	*N*-dimethylamine	>400

(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

- 6-Fluorination is tolerated
- Longer conjugated system seemed desirable (not confirmed in next generation)
- *N*-methylation (+) might be an issue for BBB penetration



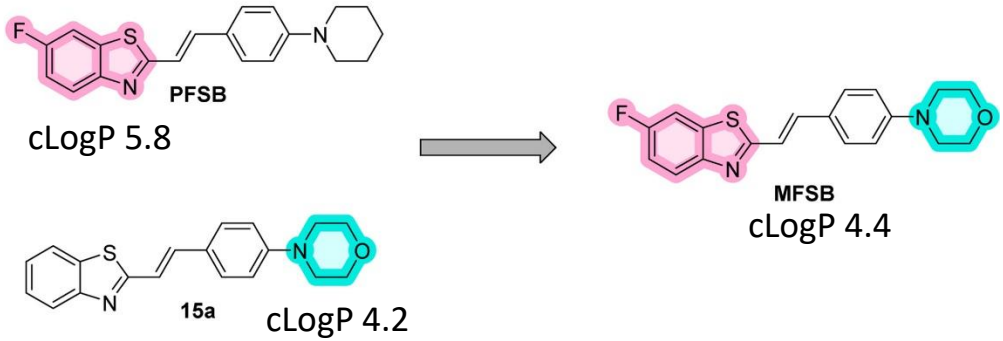
#	R	n	*N*-substitution	K_i (nM)	BBB score	CNS MPO
1a	4-CH3	1	*N*-piperidine	71.2; 198.3	4.79	3.0
2a	4-OCH3	1	*N*-piperidine	>400	4.90	3.3
3a	4-F	1	*N*-piperidine	186.6; 110.5	4.78	3.0
4a	5-CH3	1	*N*-piperidine	233.4; 88.1	4.79	3.0
5a	5-OCH3	1	*N*-piperidine	128.4; 187.8	4.90	3.3
6a	5-F	1	*N*-piperidine	169.1; 124.5	4.78	3.0
7a	6-CH3	1	*N*-piperidine	230.0; 217.8	4.79	3.0
8a	6-OCH3	1	*N*-piperidine	118.1; 102.9	4.90	3.3
9a (PFSB)	6-F	1	*N*-piperidine	25.4 ± 2.3[a]	4.78	3.0
10a	7-CH3	1	*N*-piperidine	>400	4.79	3.0
11a	7-OCH3	1	*N*-piperidine	>400	4.90	3.3
12a	7-F	1	*N*-piperidine	283.3[b]	4.78	3.0
13a	H	0	*N*-piperidine	134.5; 12.1	4.77	3.0
14a	H	2	*N*-piperidine	81.9 ± 15.6[a]	4.76	3.0
15a	H	1	*N*-morpholine	92.0 ± 30.3[a]	4.76	3.5
16a	H	1	*N*-thiomorpholine	219.1; 58.6	4.62	3.0
17a	H	1	*N*-pyrrolidine	73.4 ± 19.0[a]	4.81	3.0
18a	H	1	*N*-fluoropiperidine	99.8 ± 32.6[a]	4.68	3.0
19a	H	1	*N*-dimethylamine	>400	4.84	3.1
20	H	1	*N*-piperidine	170.5; 33.5	4.83	3.0

[a]Three data points available (mean K_i ± SEM). [b]Single data point available.

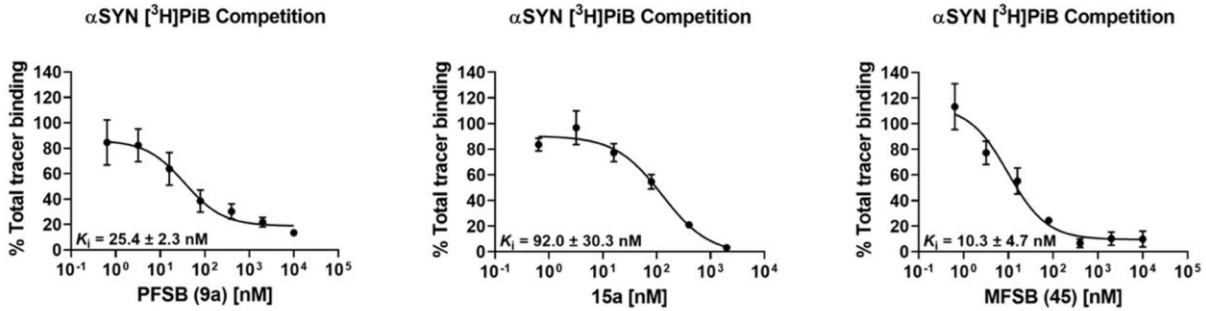
Unmethylated compounds have good BBB scores and promising binding

(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

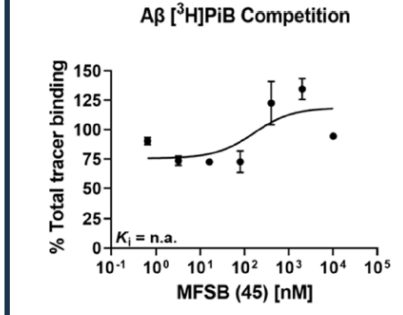
Quite hydrophobic molecules - Can the polarity be further balanced by combining 6-fluorination and morpholine?



Target binding

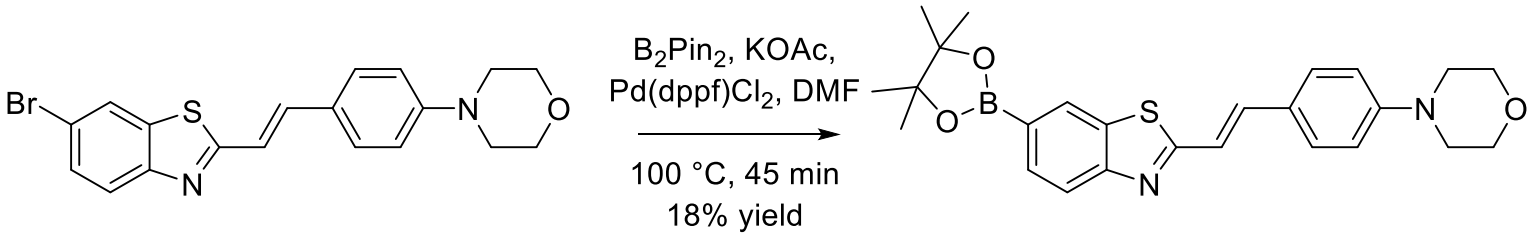


Selectivity

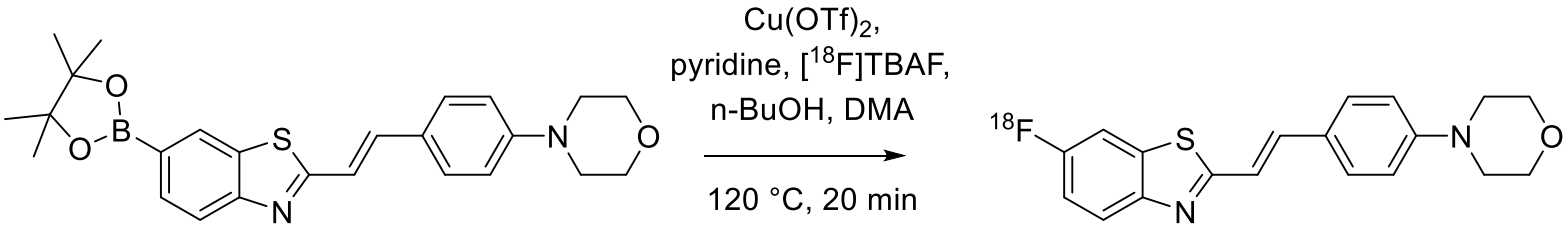


(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

Precursor synthesis

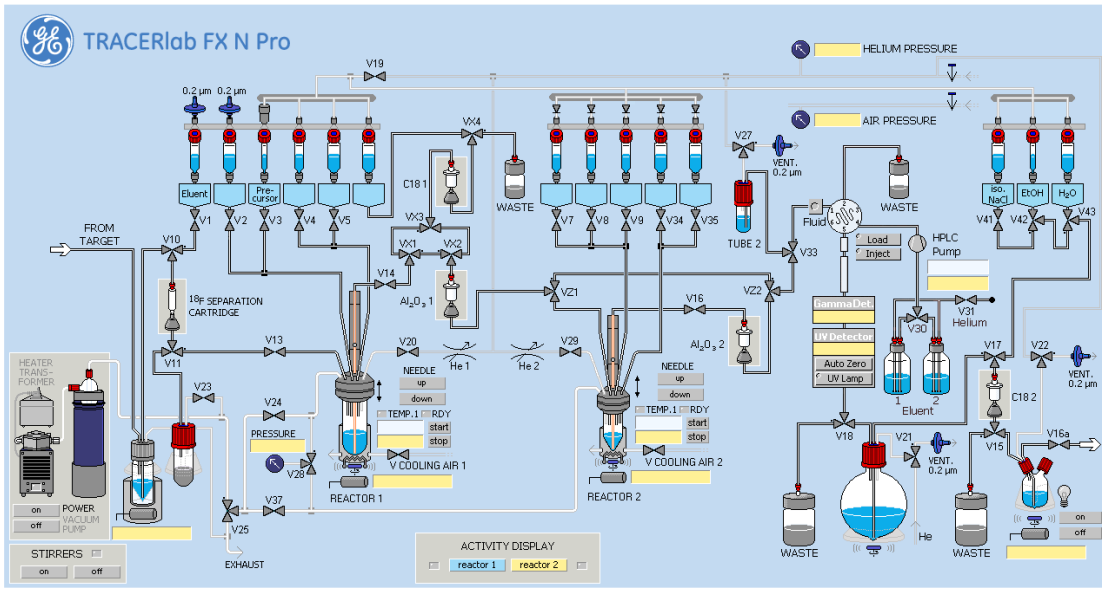


Copper-mediated radiofluorination

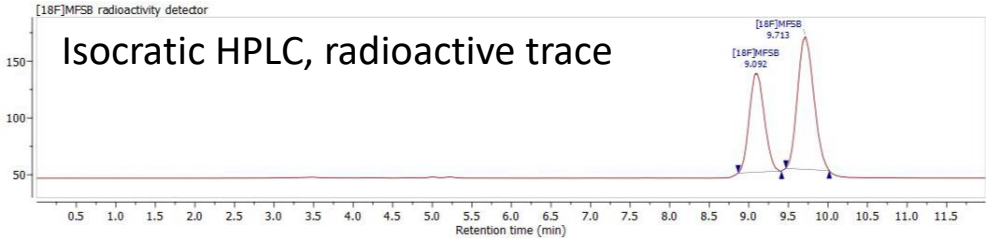


(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

Automation on GE FX N Pro synthesizer

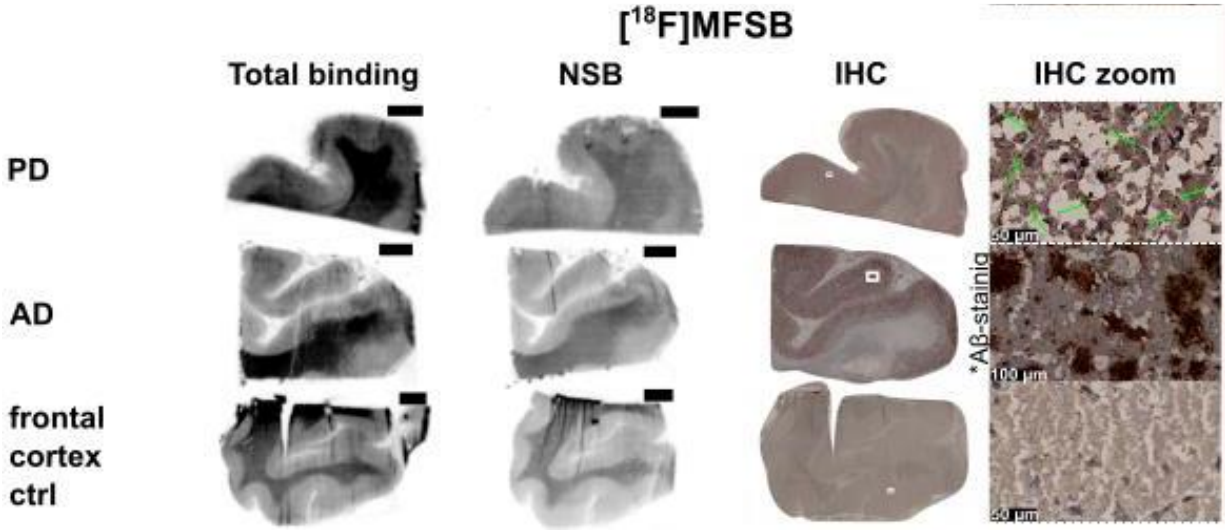


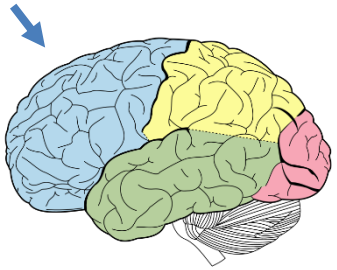
- RCY 11.6 ± 2.9% (decay-corrected)
- A_m 41.2 ± 12.0 GBq/μmol (n = 3)
- RCP >95% (combined, E/Z isomer)



(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

Frontal cortex, autoradiography of clinical cases:

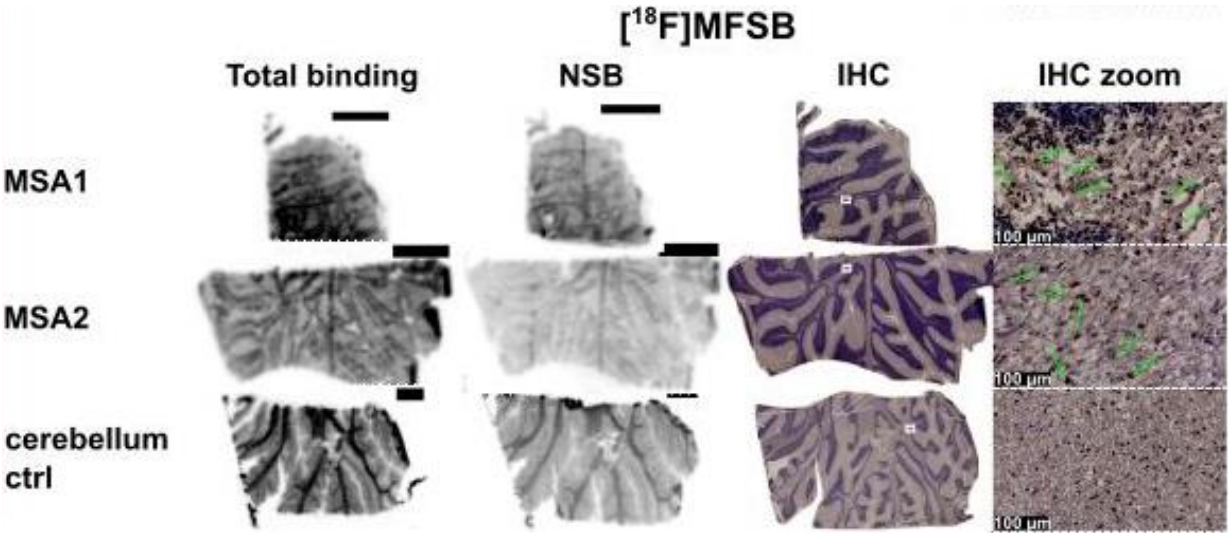


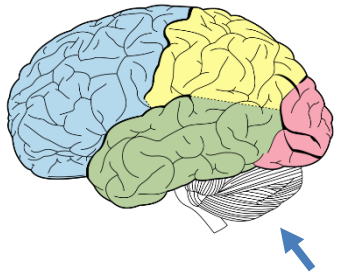


- Strong binding to white matter
- Makes it hard to detect specific binding (PD: gray matter)
- AD plaques (gray matter) are not stained!

(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

Cerebellum, autoradiography of clinical cases:



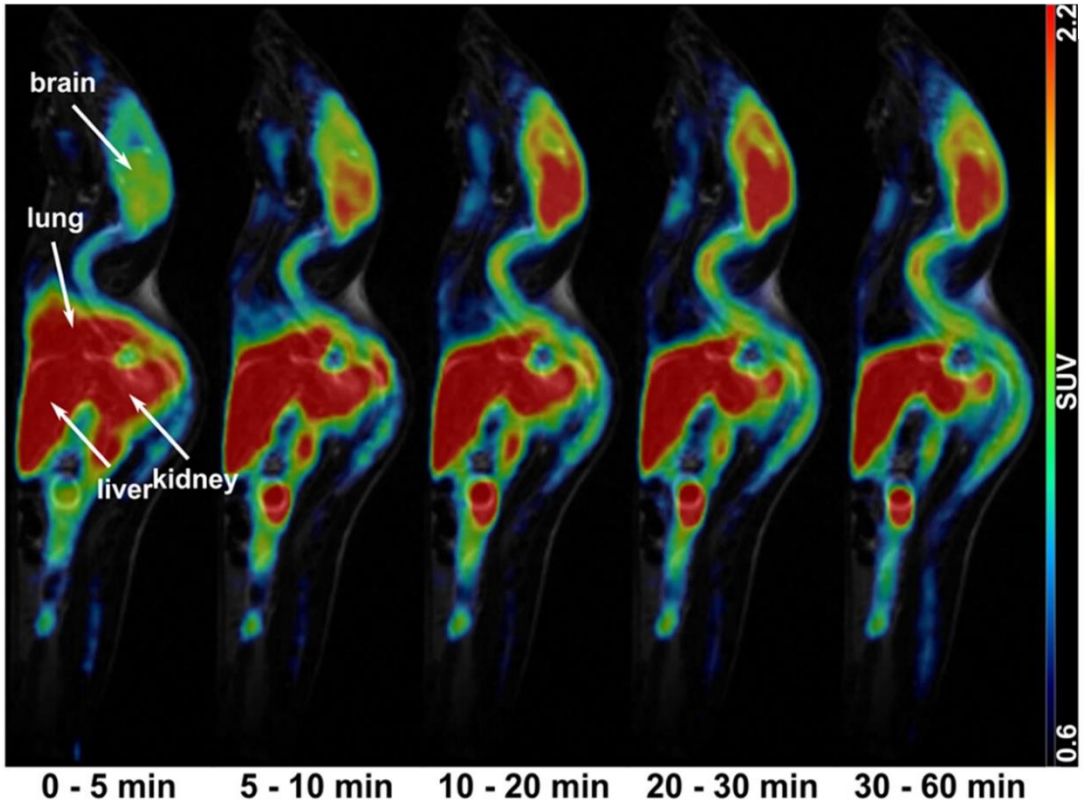


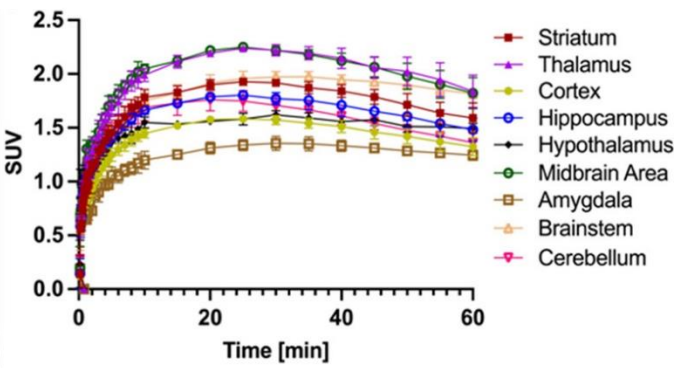
- Cerebellum white matter shows strong non-specific signal
- Hard to detect specific binding (MSA: white matter)

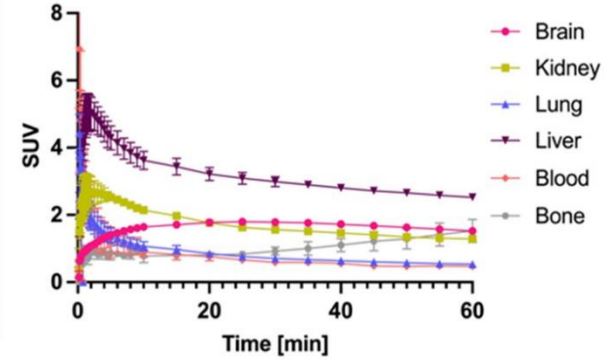
(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

MDPI

Dynamic imaging of naïve C57BL/6 mice after tail vein injection:







BBB penetration, but slow clearance. Hydrophobicity? Metabolism?

(Di Nanni et al. 2023, doi 10.1021/acsomega.3c04292)

Summary

- Identification of PET tracer candidates
- Good affinity *in vitro*, virtually absent Aß binding in competition assay and direct autoradiography
- Autoradiography indicated non-displacable binding
- BBB penetration but slow clearance
- Suboptimal PK
- Further improvement and more extensive analysis necessary
- Aims: Better properties with retained selectivity

Conclusions

- This class of compounds indeed shows promising binding to aSyn
- It can be efficiently radiolabeled with F-18
- It crosses the blood-brain barrier
- Future work will address PK optimization
- Rapid *in vitro* screening is important
- Early evaluation pipeline should also include clinical tissue and *in vivo*

Acknowledgments

WSS
WERNER SIEMENS-STIFTUNG

THE MICHAEL J. FOX FOUNDATION
FOR PARKINSON'S RESEARCH

erc European Research Council

PET-AlphaSy

MODAG
Neuroscience Solutions

Department of Preclinical Imaging and Radiopharmacy
Prof. Kristina Herfert
Ran Sing Saw

Prof. Bernd Pichler
Prof. Gerald Reischl
Sabrina Buss
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