THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

SHAKE IT UP AUSTRALIA FOUNDATION Partnered with The Michael J. Fox Foundation for Parkinson's Research

Putting numbers on Parkinson's disease

Single Molecule Detection of Oligomeric Synuclein in biofluids

PRINCIPAL INVESTIGATORS: Yann Gambin, Emma Sierecki, Antony Cooper

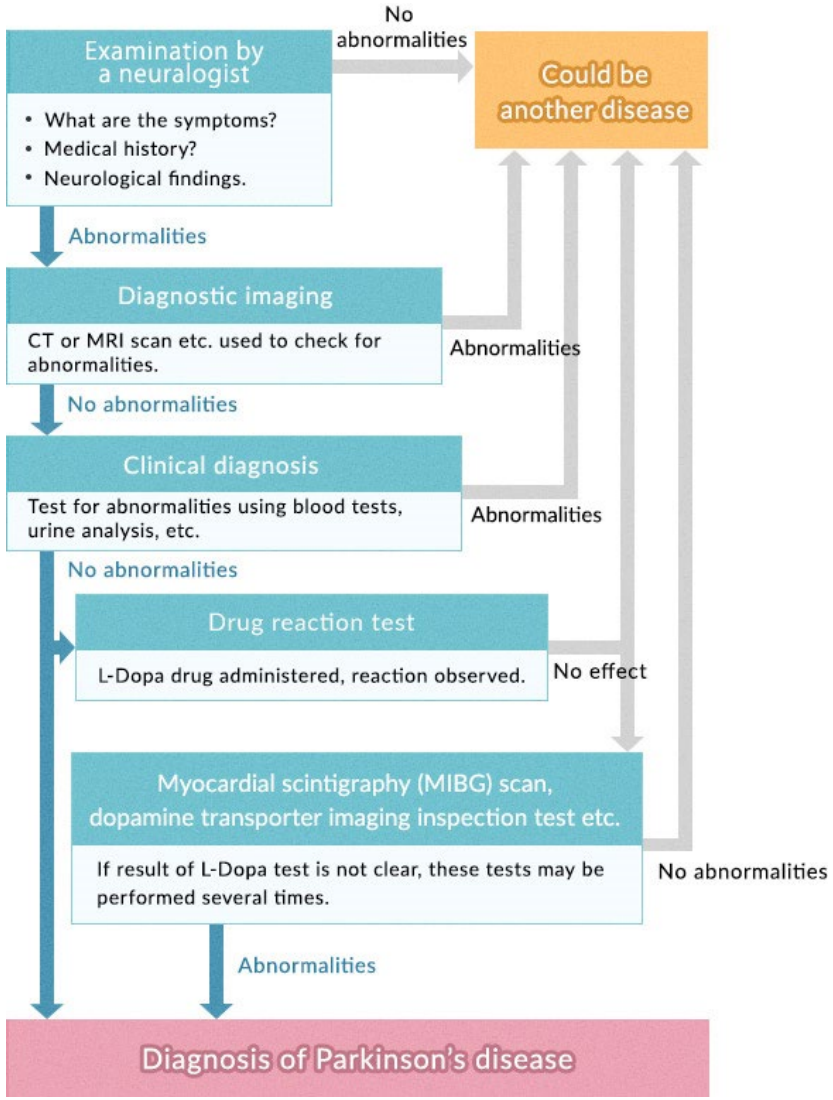
Michael J. Fox Foundation for Parkinson's Research
Shake It Up Australia Foundation
MJFF PROGRAM: Use of PD Biosamples – Spring 2020

UNSW THE UNIVERSITY OF NEW SOUTH WALES

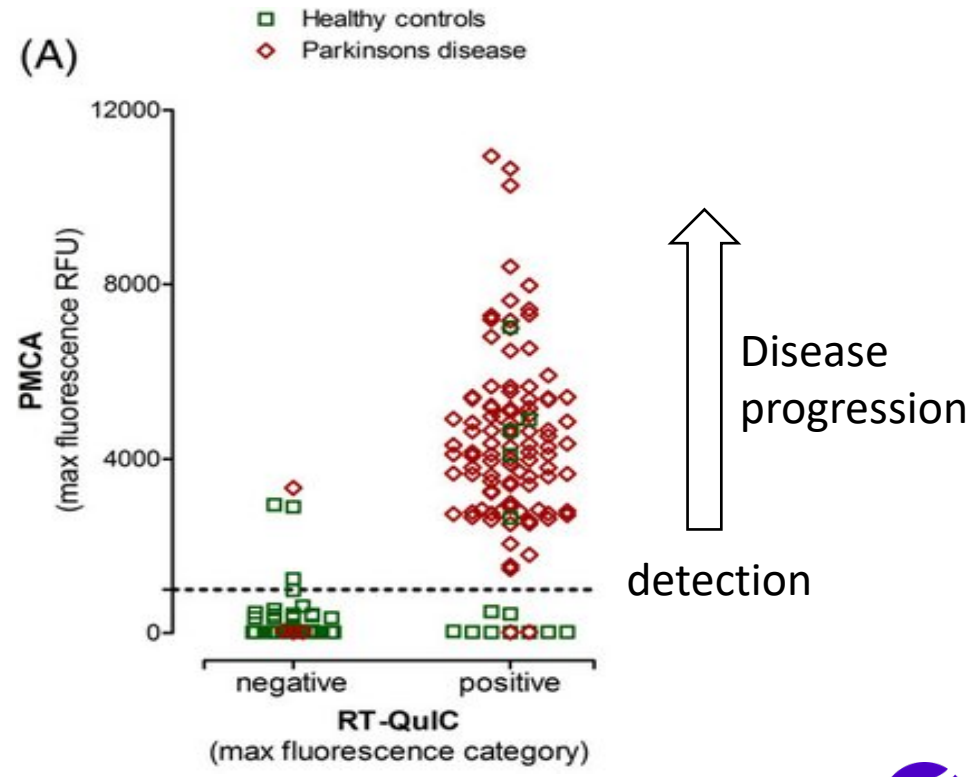
Garvan Institute of Medical Research

The search for biomarkers of Parkinson's disease

What is a biomarker? measurable indicator of the presence or severity of a disease



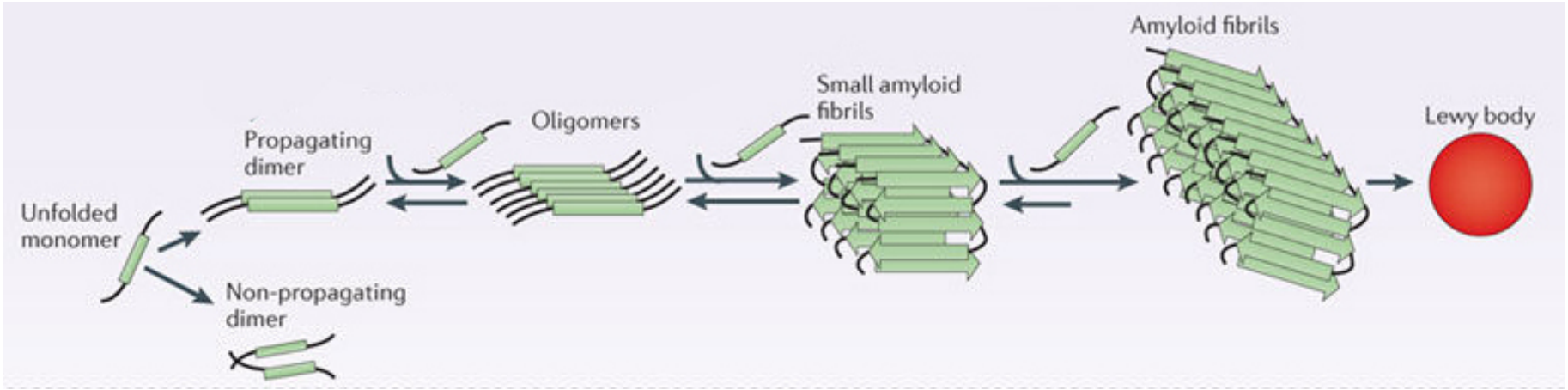
- Improves diagnosis
- Enables earlier diagnosis - before motor symptoms
- Objectively assesses response to therapeutic intervention

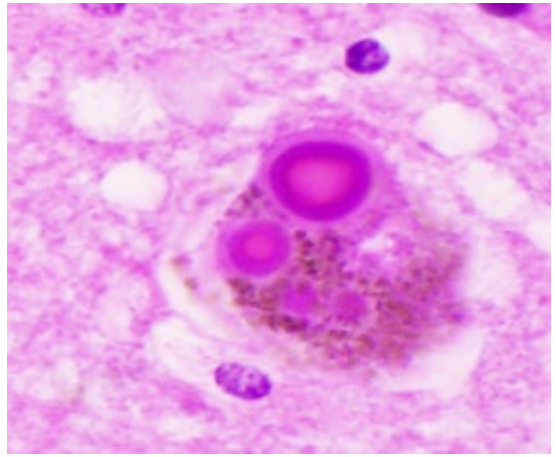


Reference:

α-synuclein aggregates as a biomarker for Parkinson's disease

Parkinson's disease is characterised by **protein misfolding and aggregation**, a recurring feature in neurodegenerative diseases.





First characterization identified large clumps of proteins in the brain

F.H. Lewy - Paralysis agitans In: Lewandowsky M, editor. Handbuch der Neurologie,. Berlin: Julius Springer; 1912.

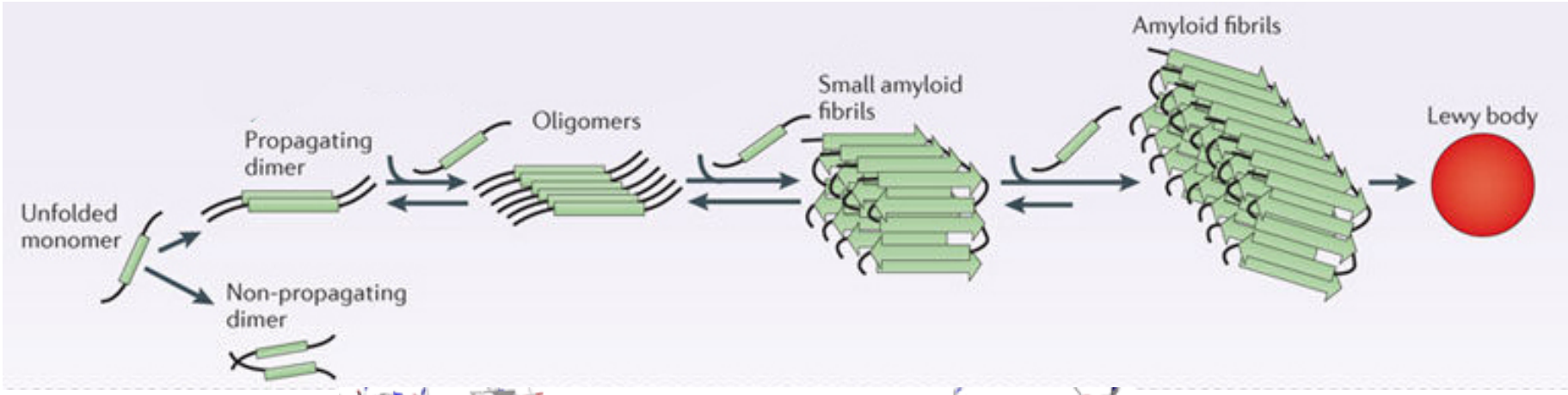
Lewy bodies are mainly composed of alpha-synuclein fibrils

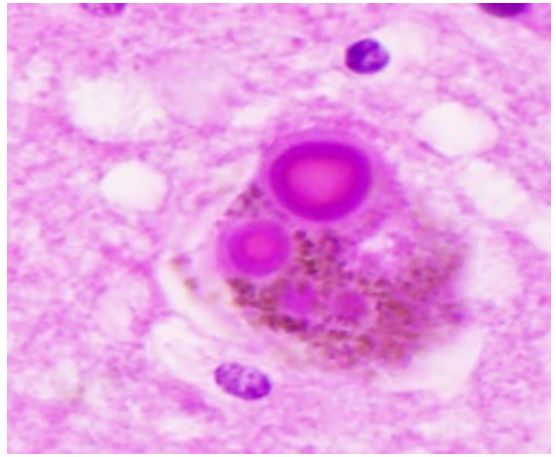
Spillantini et al - Alpha-synuclein in Lewy bodies. Nature. 1997

Processus of alpha-synuclein aggregation has been studied in vitro in the last 20 years.

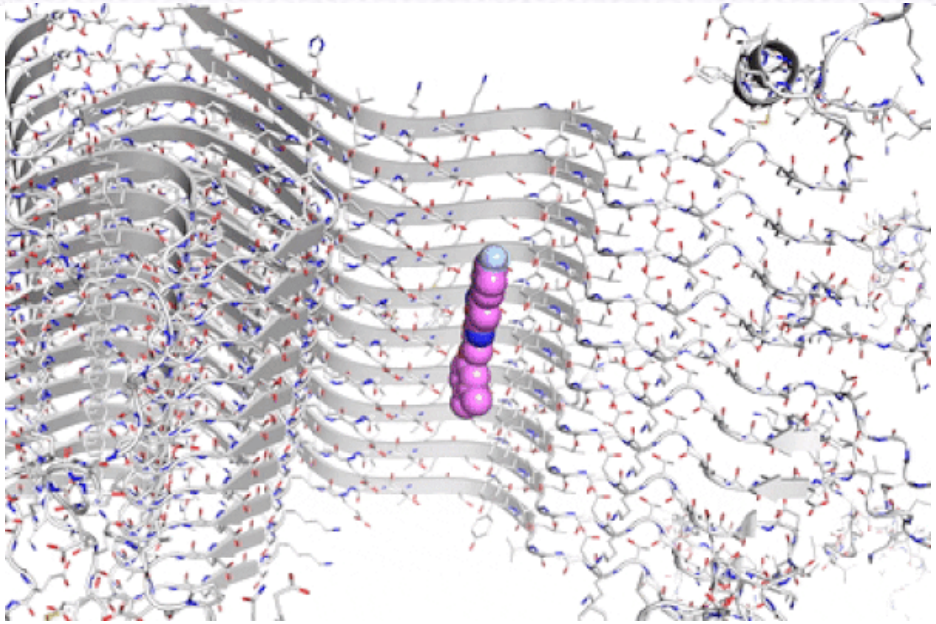
α-synuclein aggregates as a biomarker for Parkinson's disease

Parkinson's disease is characterised by **protein misfolding and aggregation**, a recurring feature in neurodegenerative diseases.





Spillantini et al - Alpha-synuclein in Lewy bodies. Nature. 1997

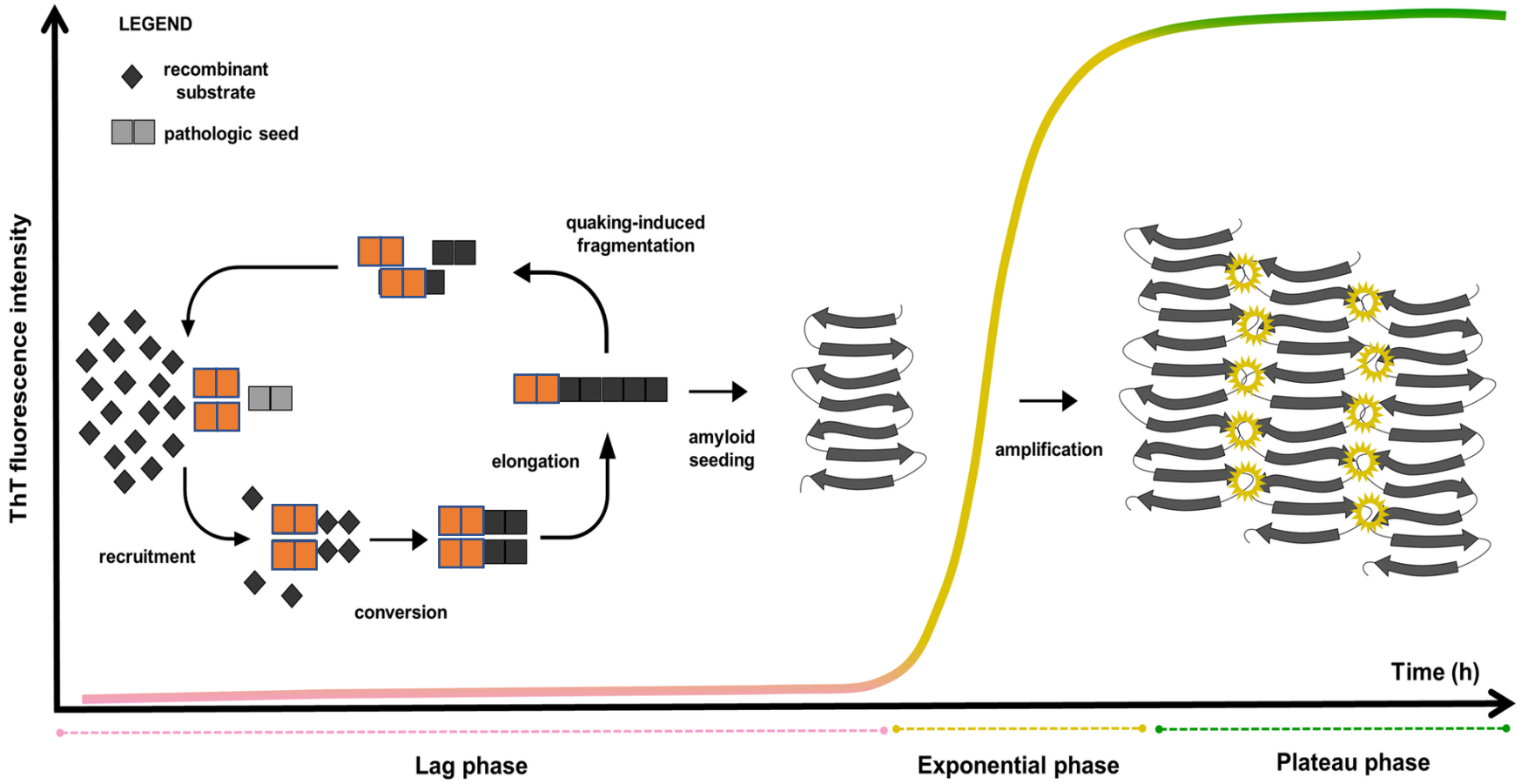


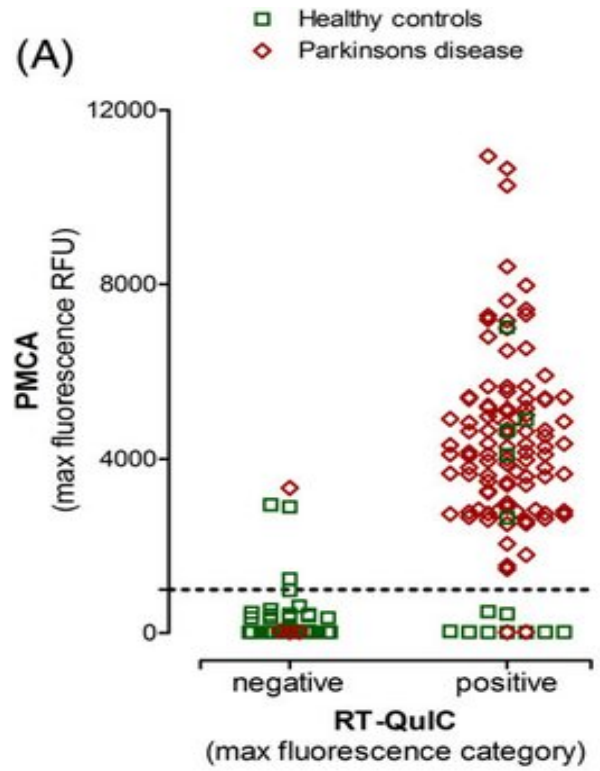
Credit: E. James Petersson

Ferrie et al. Identification of a nanomolar affinity α-synuclein fibril imaging probe by ultra-high throughput in silico screening, *Chemical Science* (2020).

Detecting the presence of α-synuclein aggregates

To detect the presence of aggregates in patients samples, researchers can **accelerate the aggregation process** in a test tube.

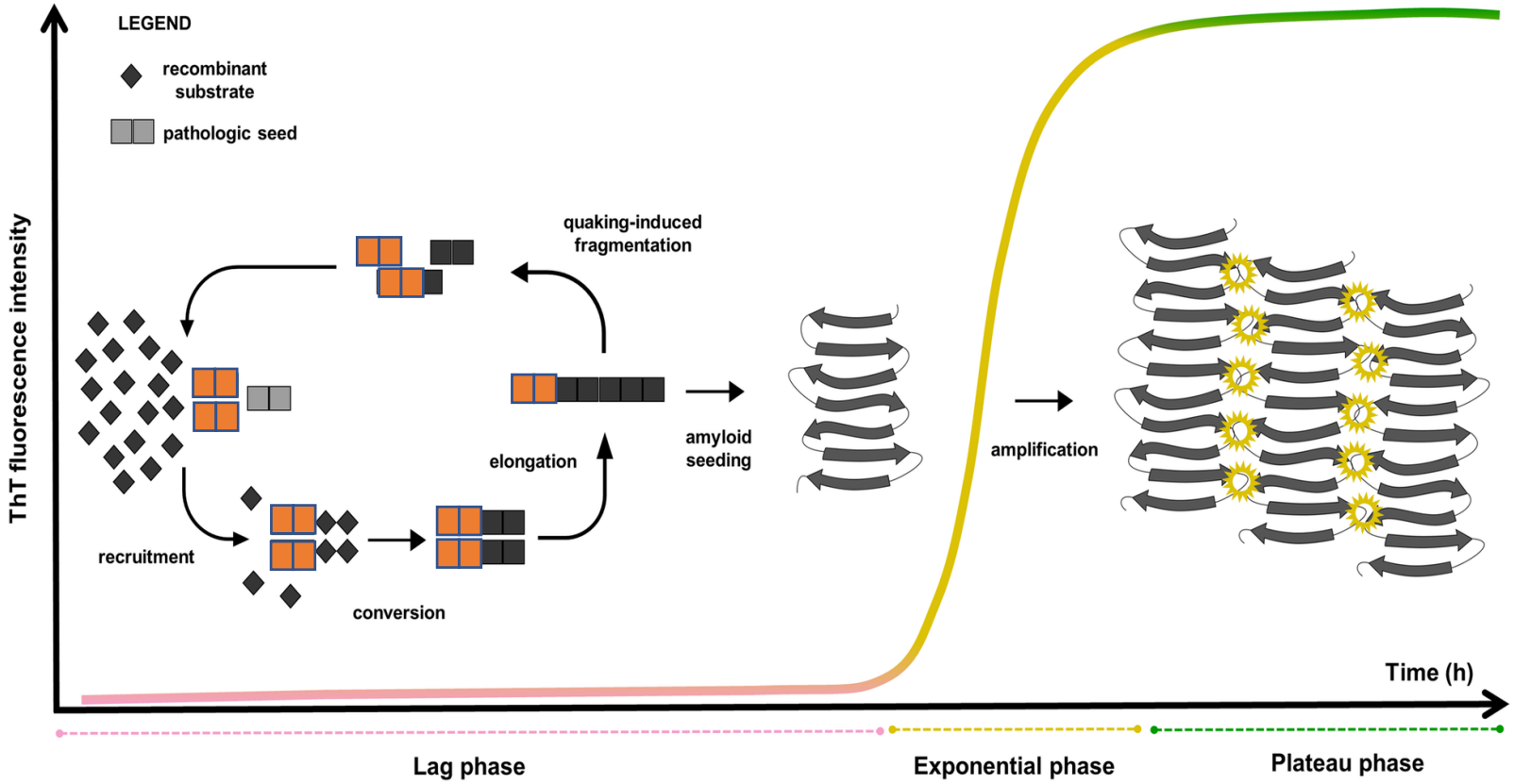


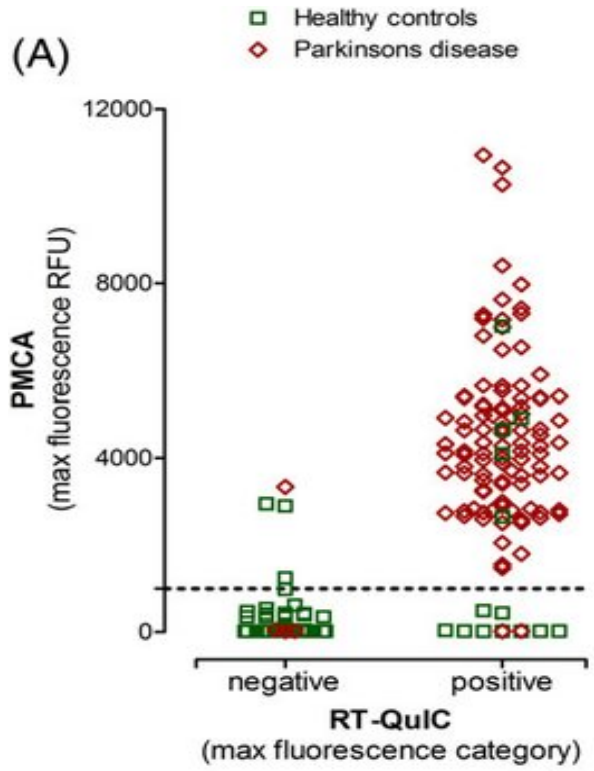


Kang UJ, et al. Comparative study of cerebrospinal fluid α-synuclein seeding aggregation assays for diagnosis of Parkinson's disease. Mov Disord. 2019;34(4).

Current limitations of the detection methods

Practical limitation: currently requiring **cerebrospinal fluid** samples obtained by lumbar puncture.



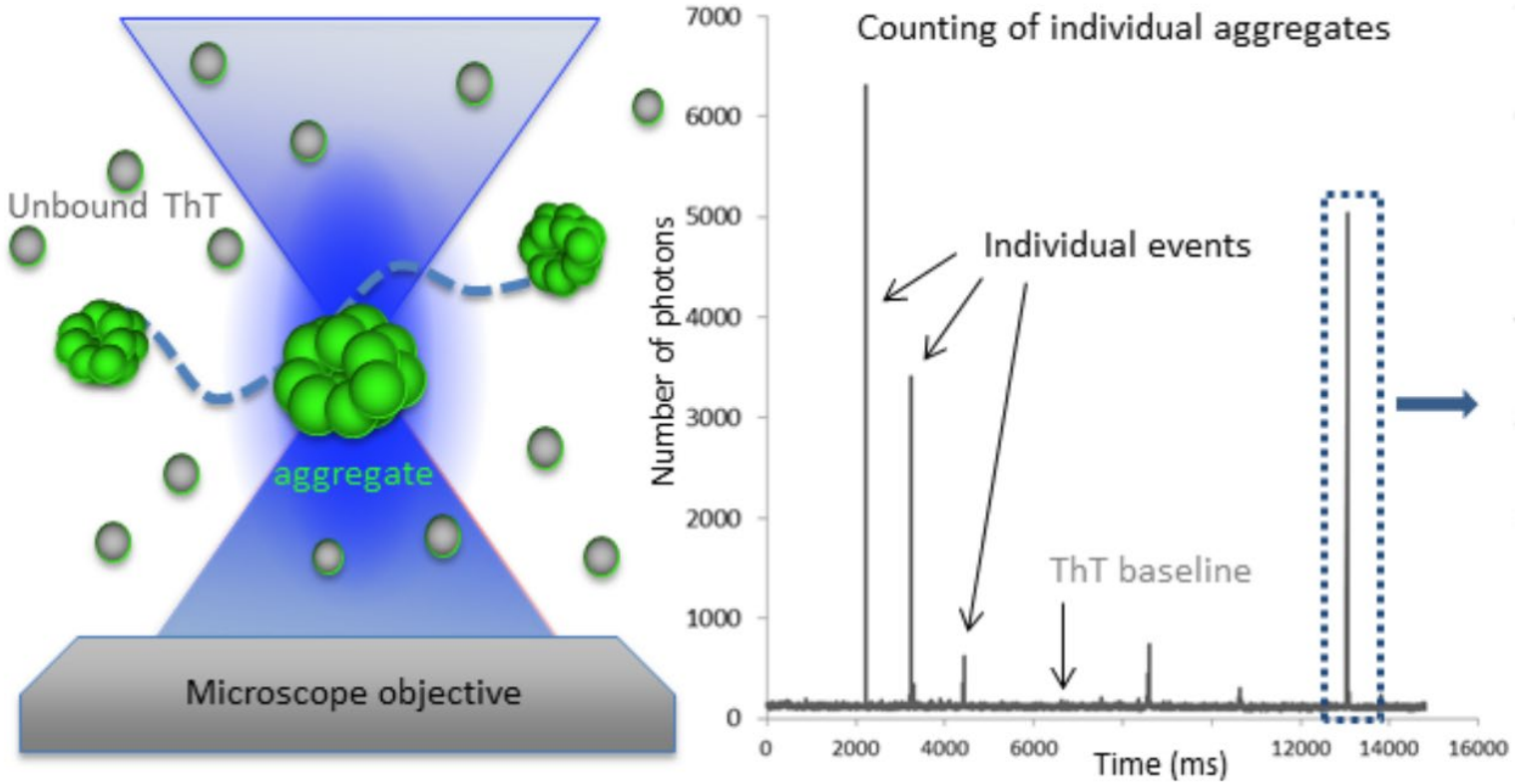


Theoretical limitation :

✓ Improves diagnosis ✓Enables earlier diagnosis ✗ **Objectively assesses response to therapeutic intervention**

One solution: increasing the sensitivity

Our lab specializes in **single molecule microscopy**, detecting individual proteins.

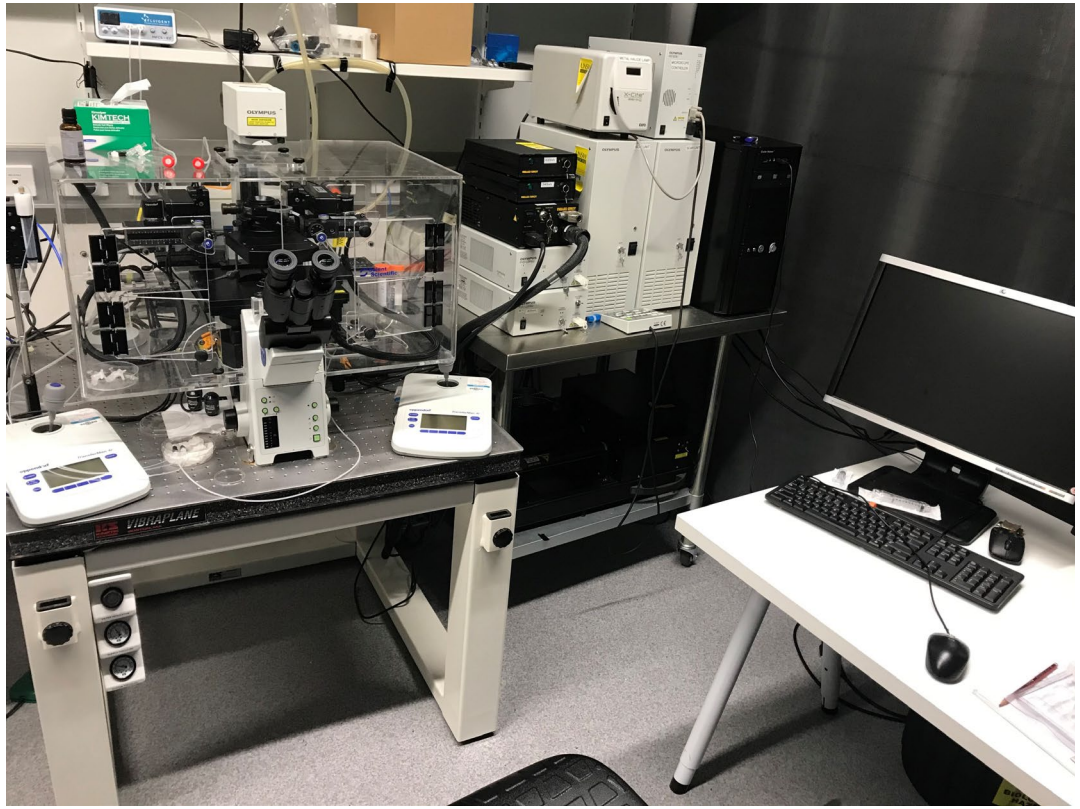


Individual proteins or aggregates appear as peaks on a fluorescence trace.

Reference: Brown, Bauer, … Sierecki, Gambin, *Nature Communications* 2019

Single molecule microscopy is a specialist field

Our lab specializes in **single molecule microscopy**, detecting individual proteins.

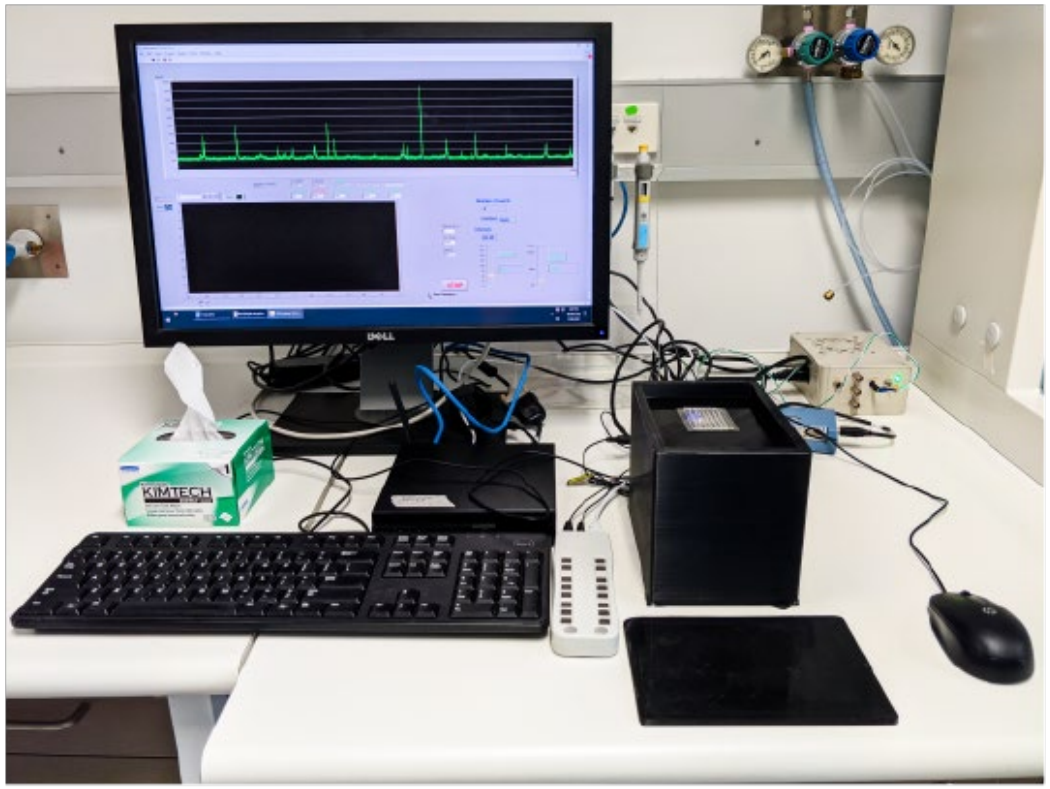


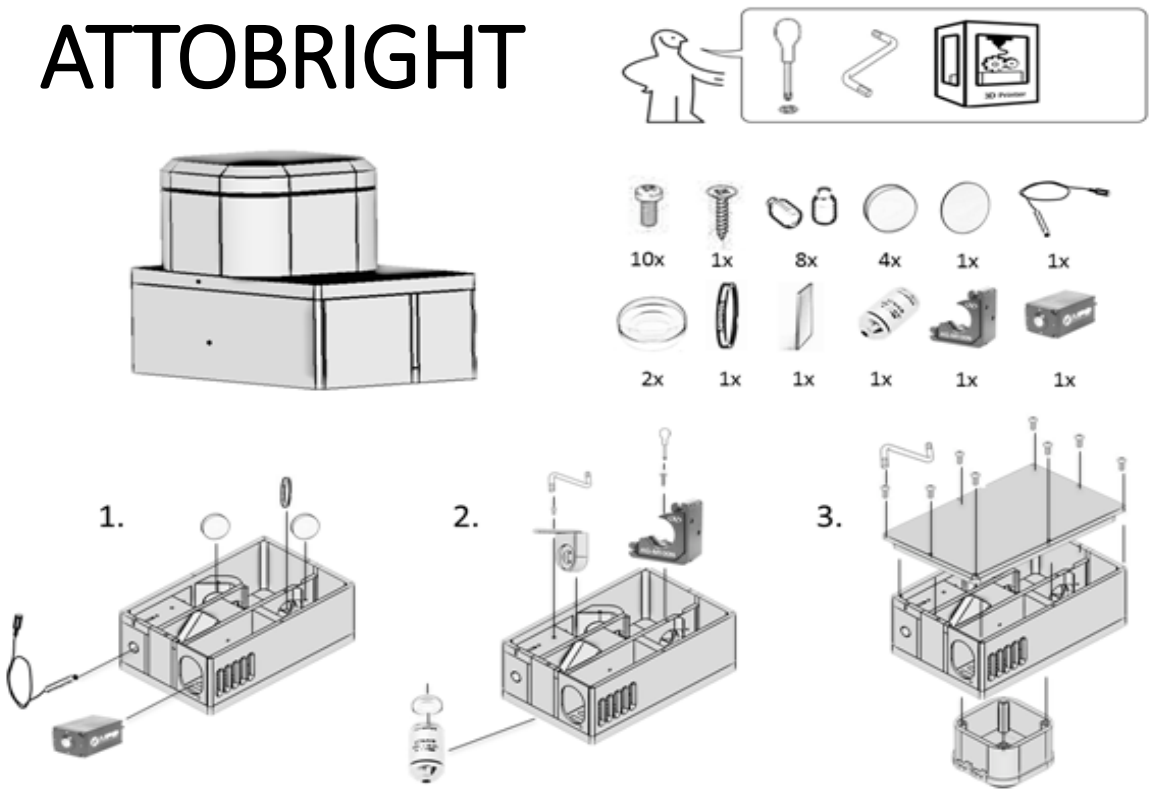
- Large and expensive equipment
- Dedicated dark room
- Vibration controlled table
- Trained operators

=> Difficult to translate to a clinical setting

Single molecule microscopy made simple(r)

We developed a 3D-printed instrument, capable of detecting single α-synuclein fibrils.



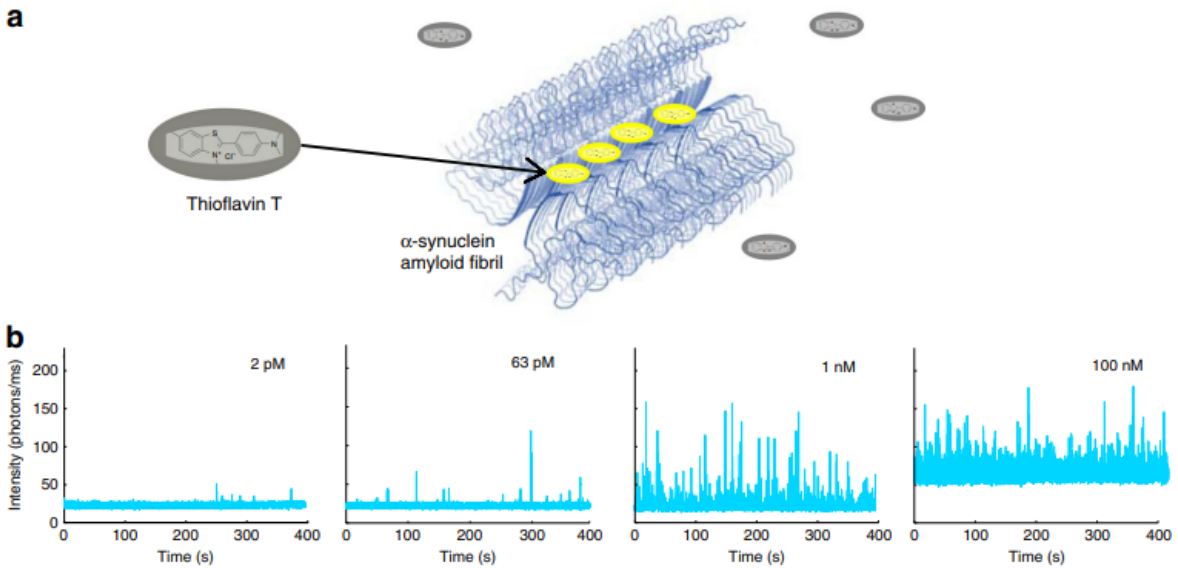


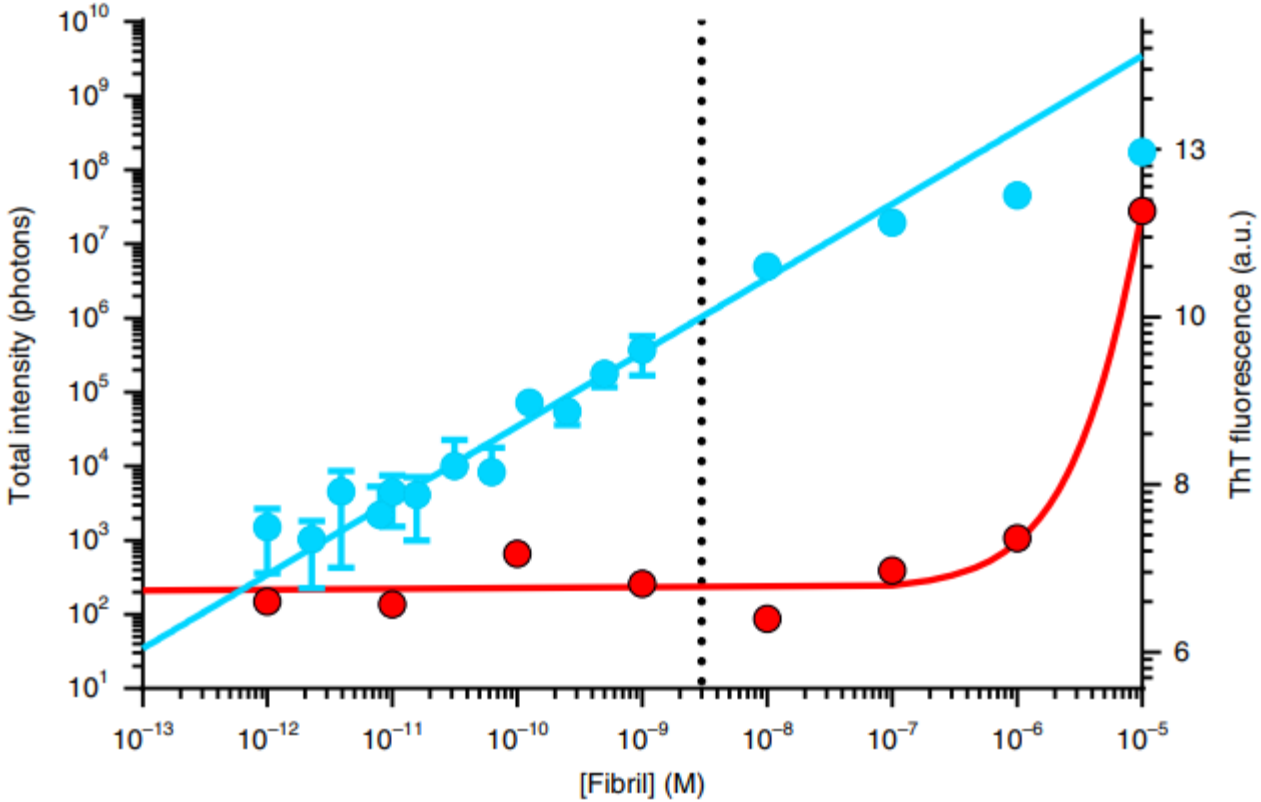
The first goal of the grant was to print a new setup and install it at the Garvan Institute to accelerate research.

Reference: Brown, Bauer, … Sierecki, Gambin, *Nature Communications* 2019

Single molecule microscopy made simple(r)

We developed a 3D-printed instrument, capable of detecting single α-synuclein fibrils.

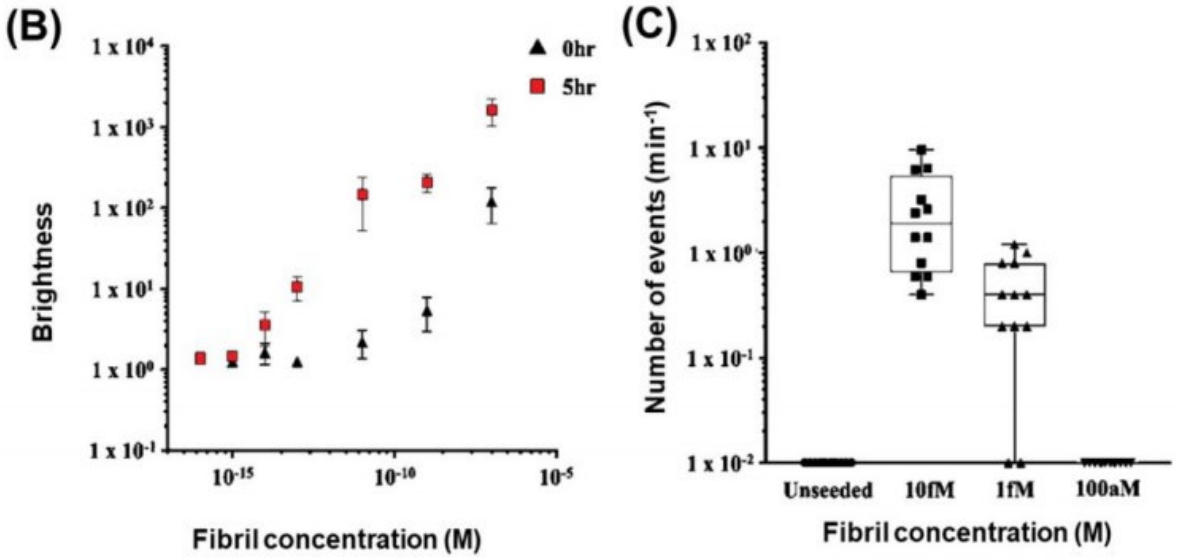


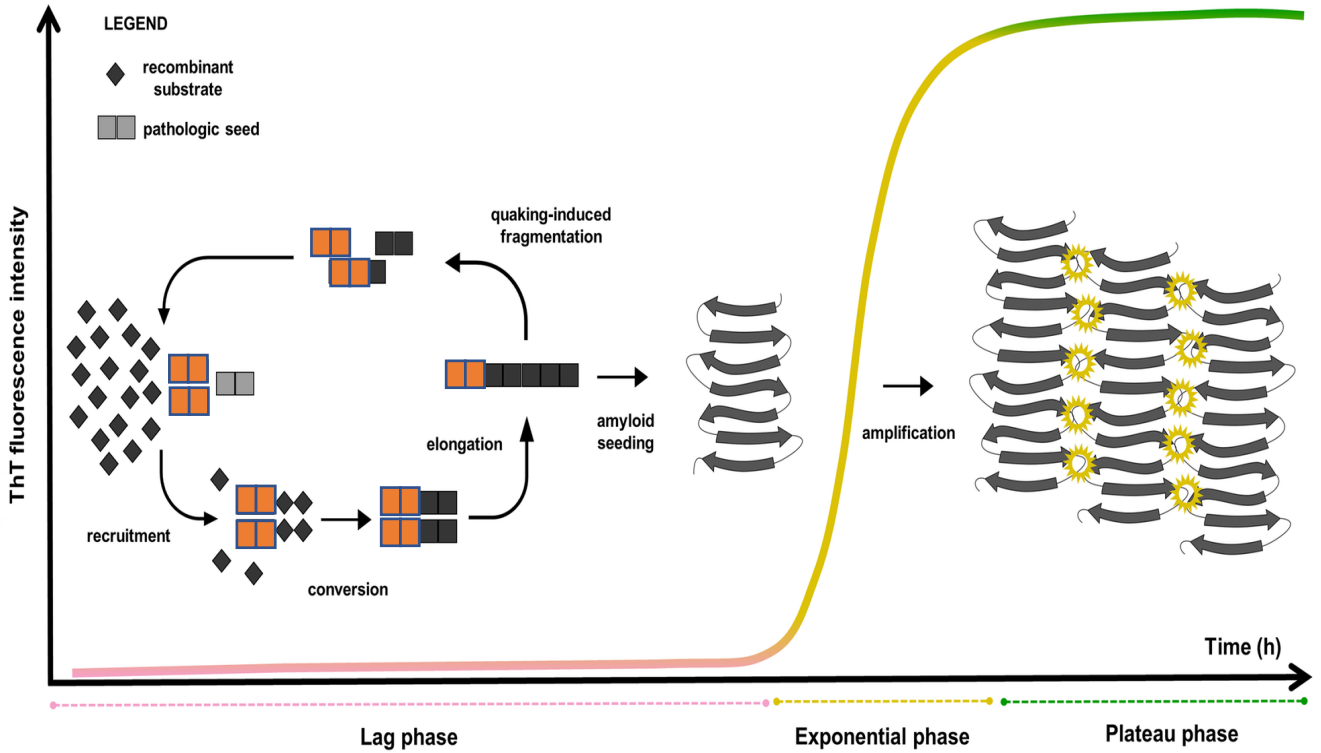


This instrument is 10^6 times more sensitive than the one used for the existing assay.

Reference: Brown, Bauer, … Sierecki, Gambin, *Nature Communications* 2019

Applicability to the detection of α-synuclein aggregates



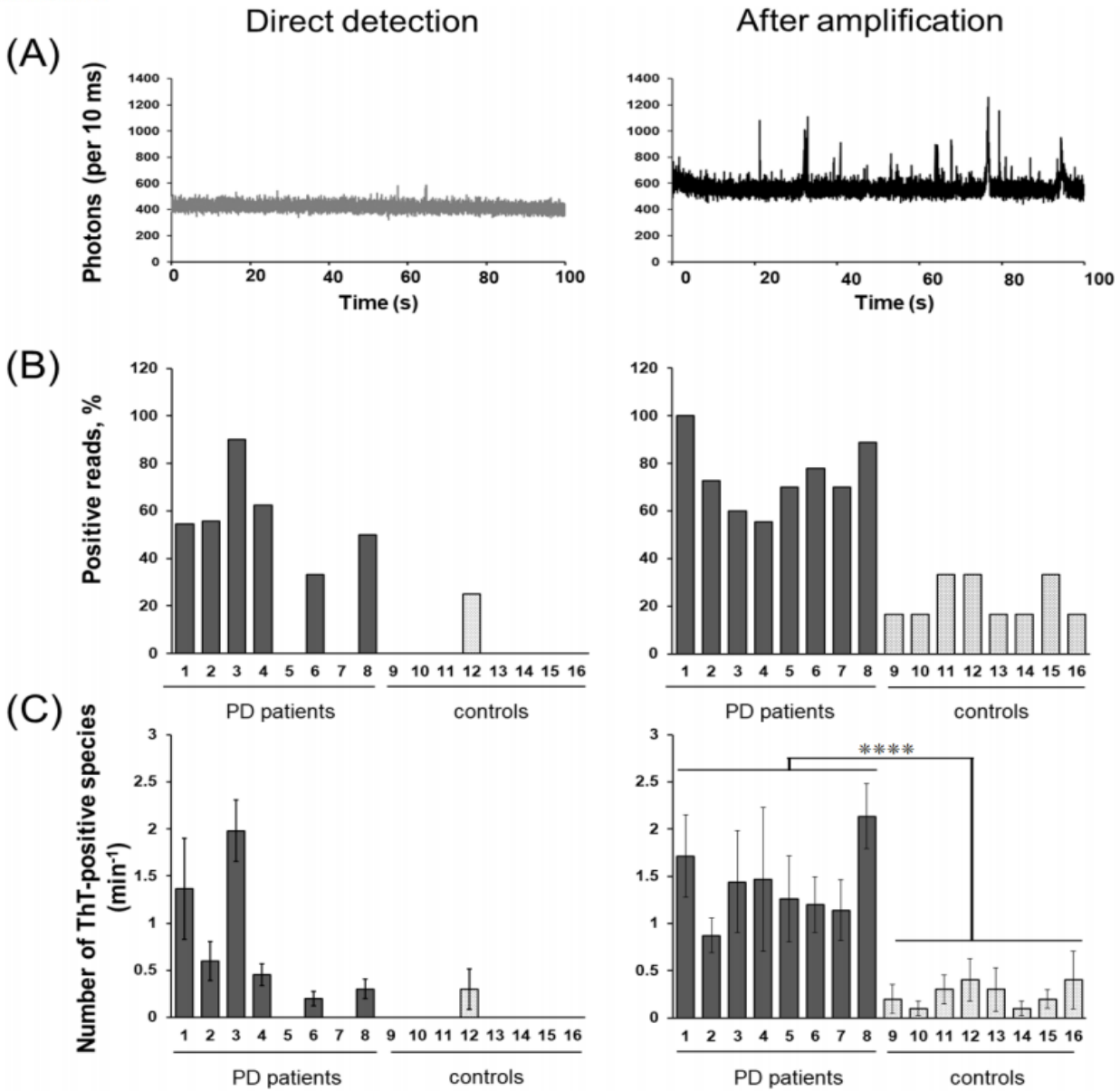


The gain of sensitivity allows to:

- Reduce the duration of the assay (from 300 h to 300 min)
- Eliminate the need for multiple rounds of amplification
- Perform amplification in more “gentle” conditions
- **Count the original numbers of aggregates**

Reference: Bhumkar, Magnan, Lau … Gambin and Sierecki *Angewandte Chemie* 2021

Measuring α-synuclein aggregates in CSF samples



All patients samples reliably show more peaks than control samples.

Some patient samples even show presence of aggregates before amplification.

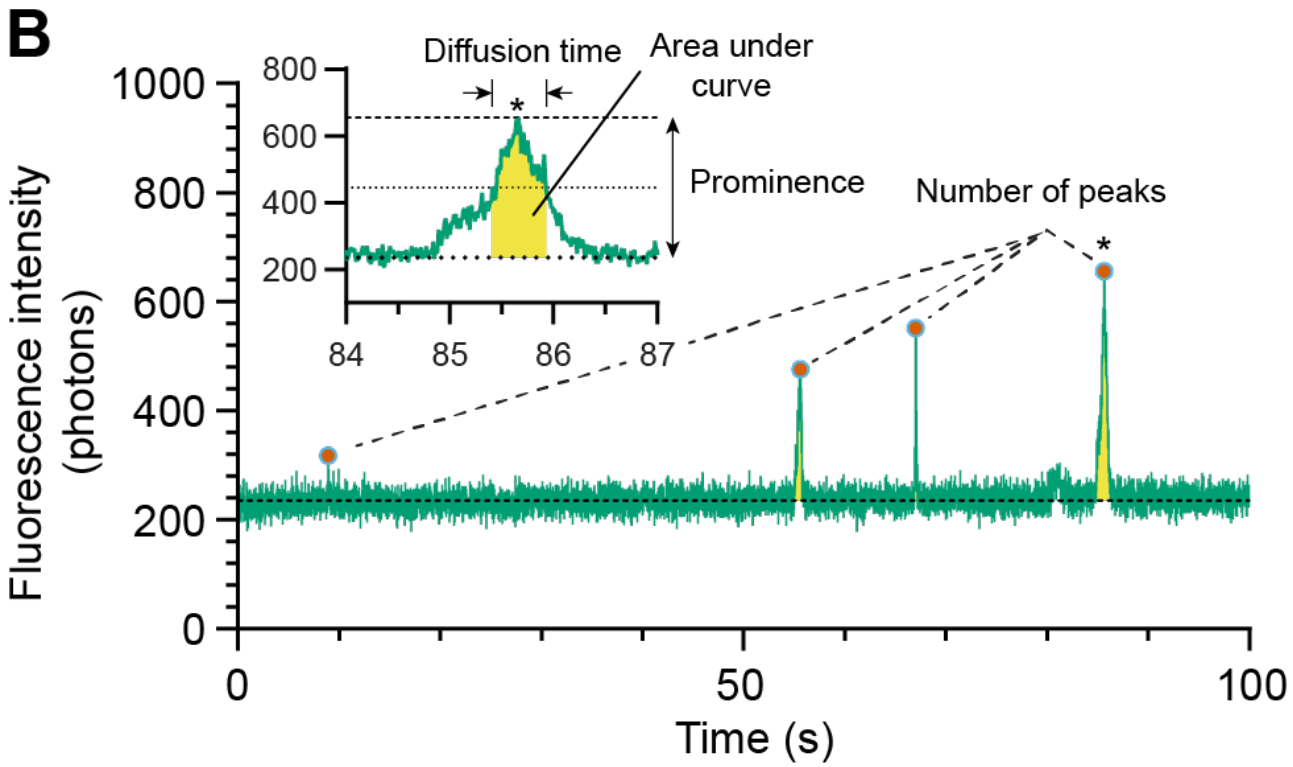
We still need to explore larger cohorts to validate the assay and understand these results.

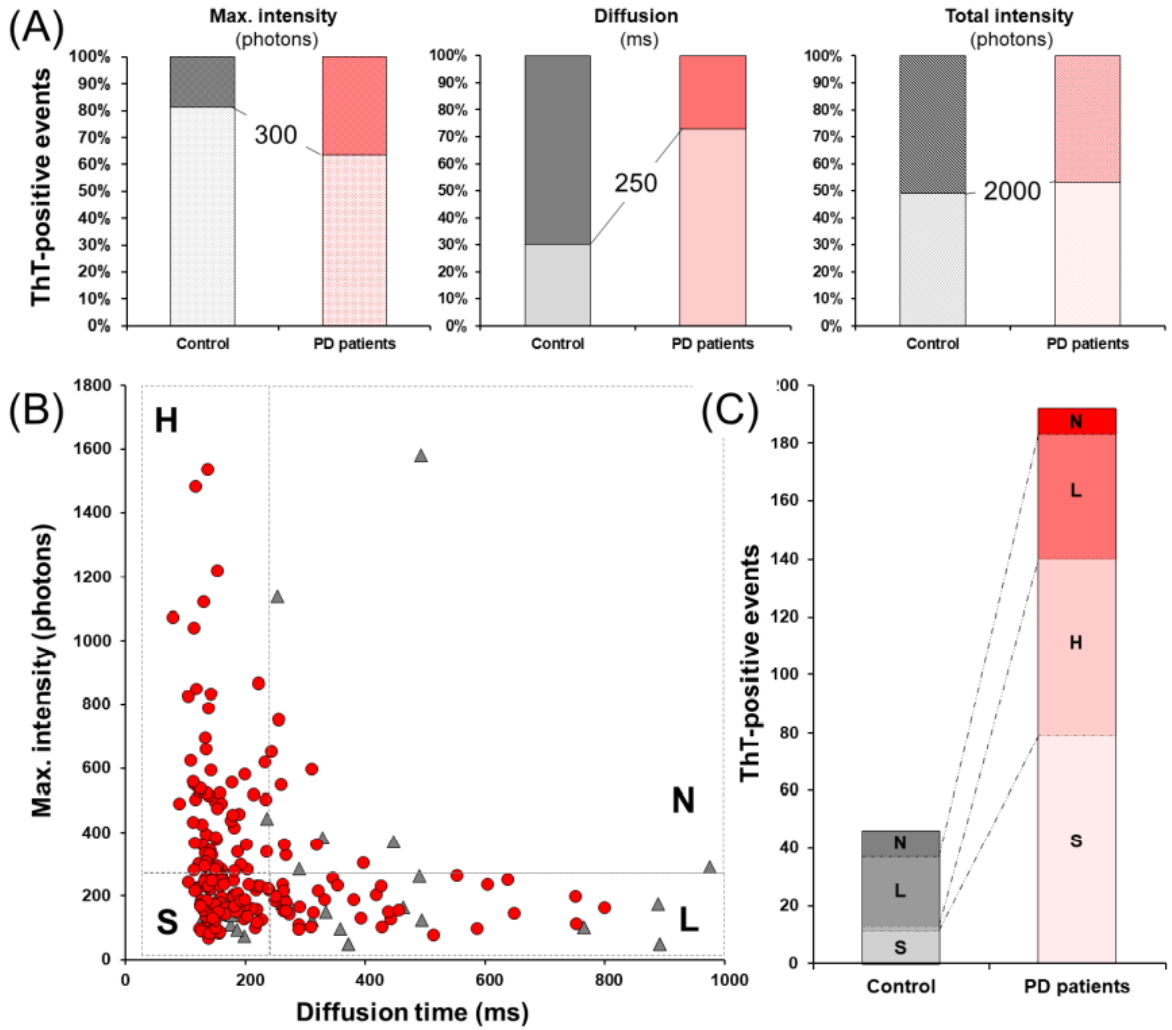
CSF from the LRKK2 cohort was tested for 8 PD patients and 8 healthy controls (age matched)
2µL of CSF was amplified in 18µL of PBS with 20µM Syn monomers, 10µM ThT, measured immediately and after amplification at 55°C for 5h. Experiments were performed 5 times independently; each sample was read for only 100s.

Reference: Bhumkar, Magnan, Lau … Gambin and Sierecki *Angewandte Chemie* 2021

Measuring α-synuclein aggregates in CSF samples

Single molecule detection gives us access to **additional information**.



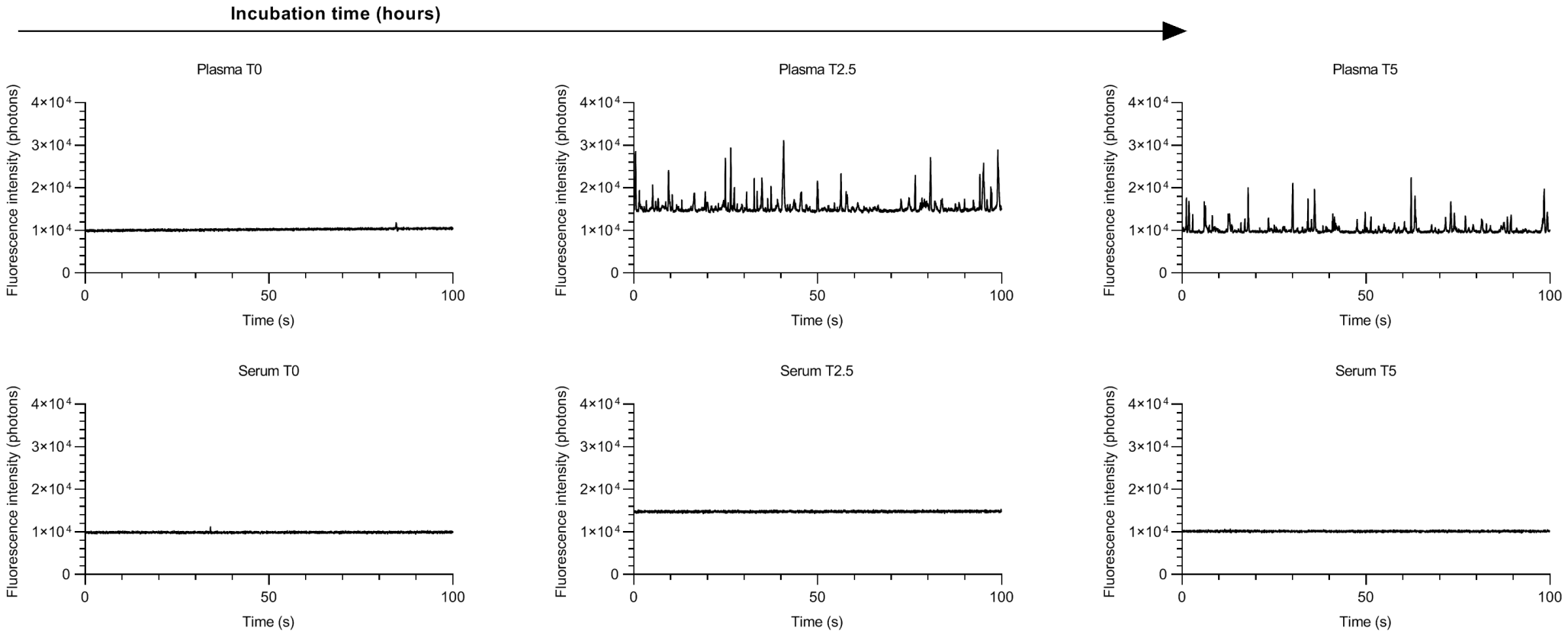


The aggregates from patients samples display a unique signature.
This could enable more precise diagnostics.

Reference: Bhumkar, Magnan, Lau … Gambin and Sierecki *Angewandte Chemie* 2021

Towards a diagnostic from blood

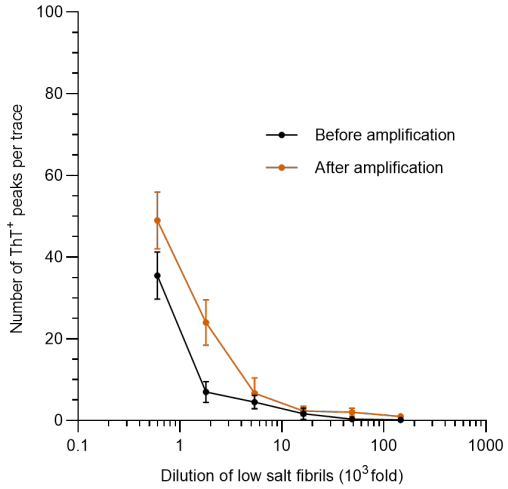
Can we perform the same experiments in plasma or serum ?



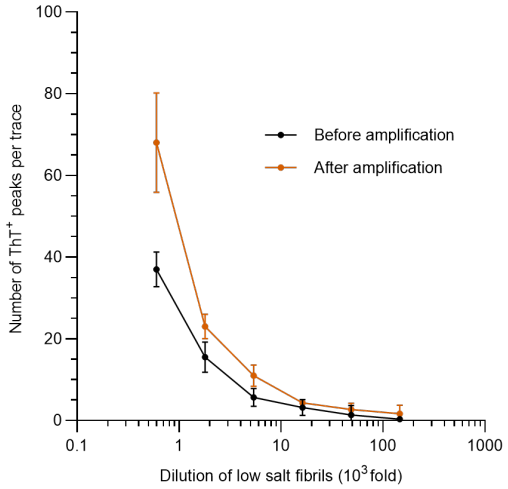
Control plasma (from a healthy donor) creates a false positive, but **serum could be suitable**.

Towards a diagnostic from blood

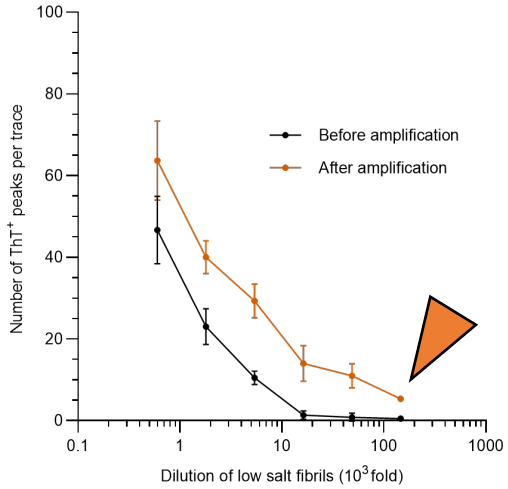
Number of peak in Serum + PBS



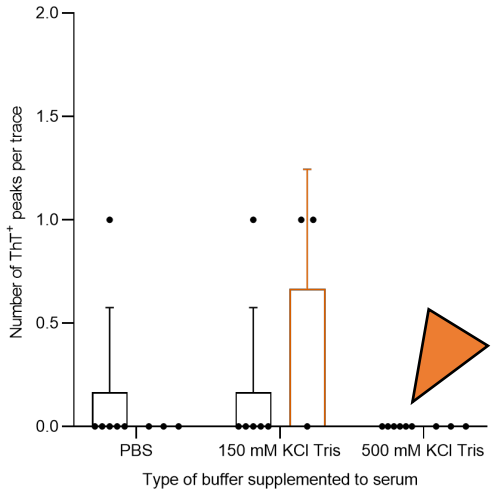
Number of peak in Serum + 150 mM KCl in Tris



Number of peak in Serum + 500 mM KCl in Tris

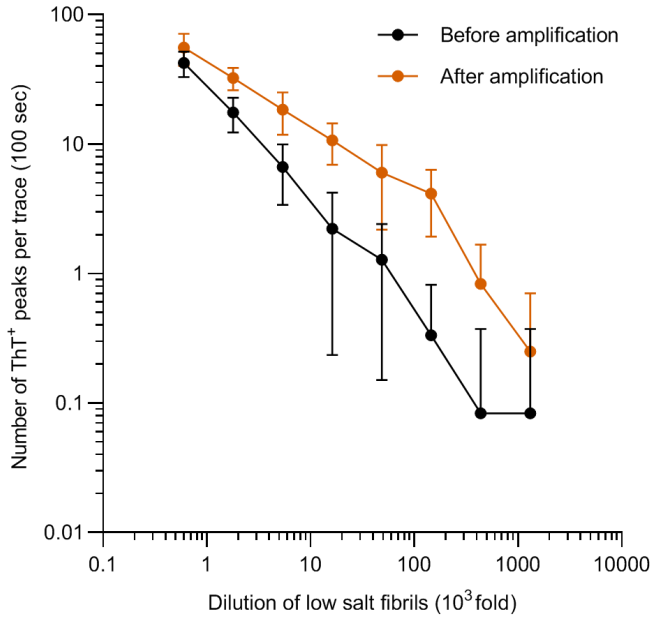


Number of Peak in negative control (no PFF)



Modifying the assay allowed us to **boost detection in serum**, while keeping the negative controls flat.

Effect of amplification in
Ultra-High KCl and serum (combined)



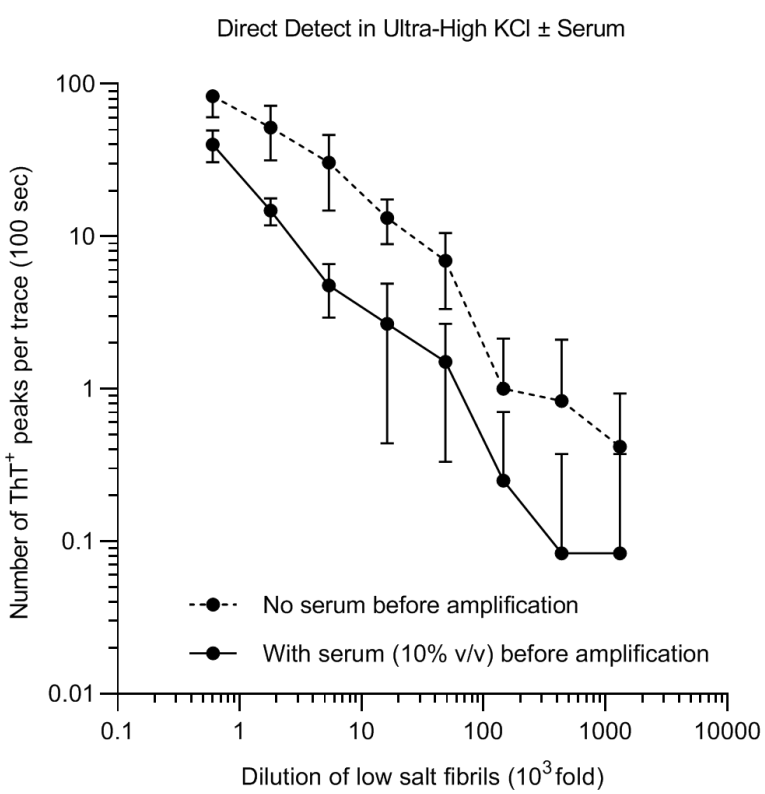
Linearity and reproducibility of the assay allows to **quantify the amount of aggregates**.

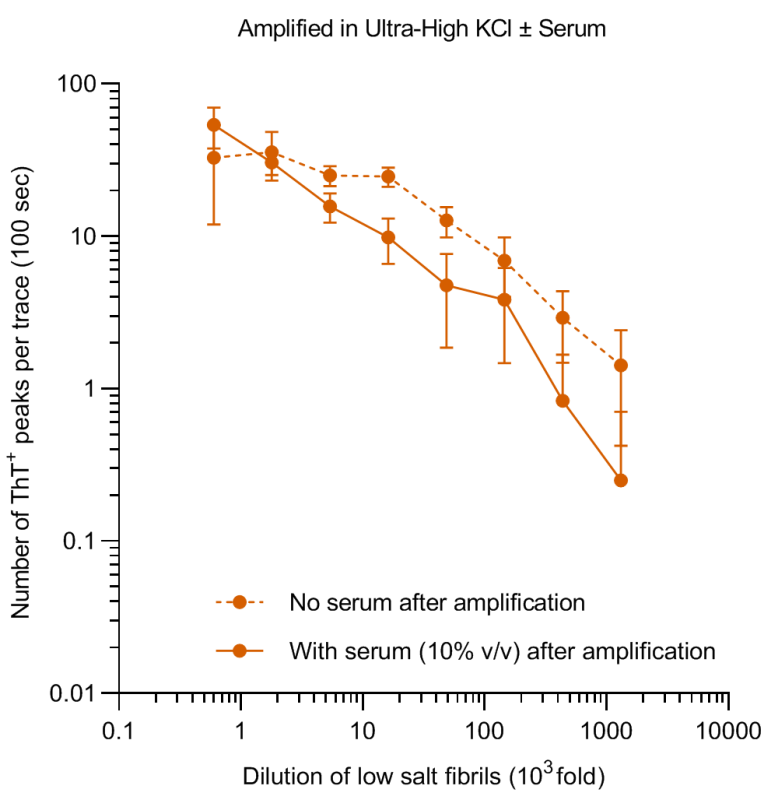
Reference:

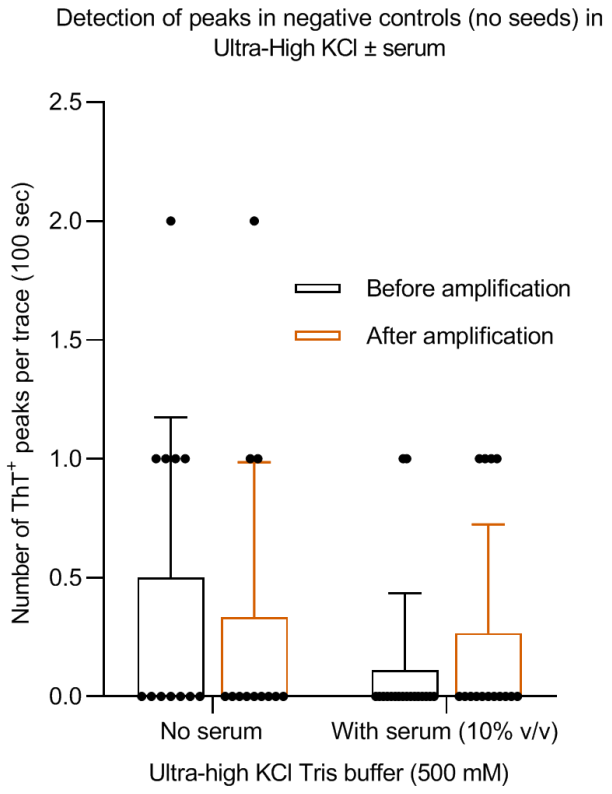
Towards a diagnostic from blood

The detection and amplification of aggregates is almost as good in serum as in PBS (around factor 3).

The good surprise is the fact that the background (number of events) is better in serum !







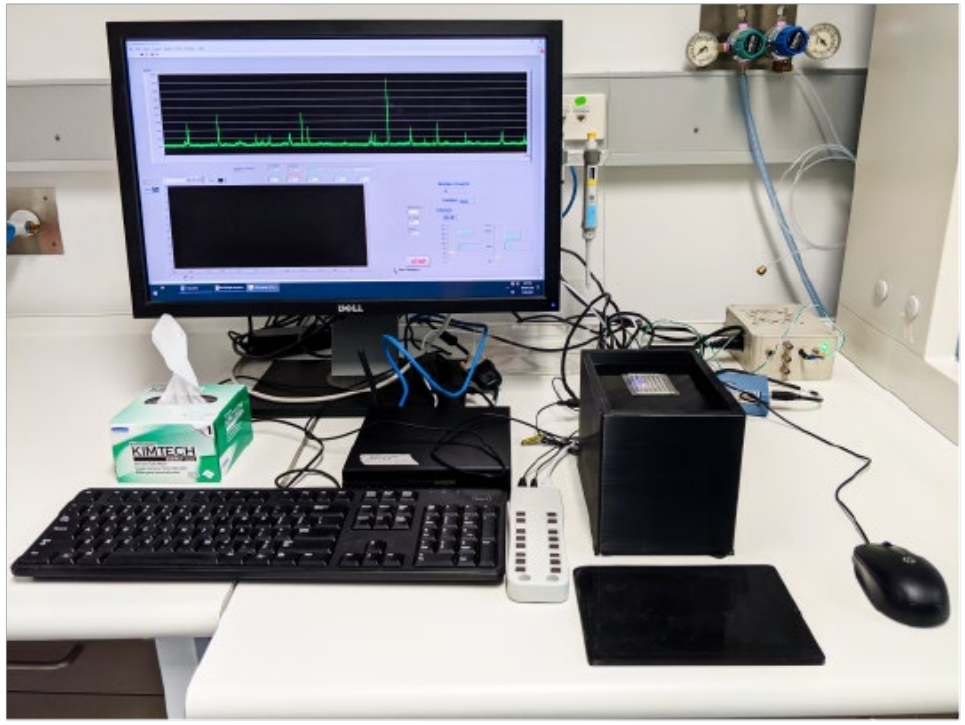
Fibrils were diluted in serum from healthy donor patient, in the range 1,000x dilution to 1,000,000x dilution. Experiments were performed in three independent experiments (different fibrils/ different days)

CONCLUSIONS

- We are one step closer to having a reliable biomarker of Parkinson's disease for earlier detection and quantitative assessment of disease progression.

- Our method shows promise to detect synuclein aggregates not only in CSF but probably in serum.

- There is still a lot of validation to run, using patients samples and larger cohorts, with the support of Michael J. Fox Foundation and Shake It Up.



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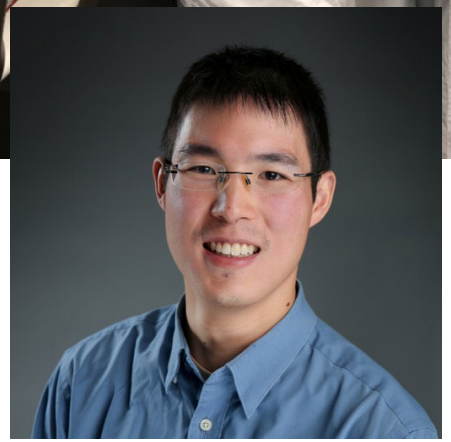

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