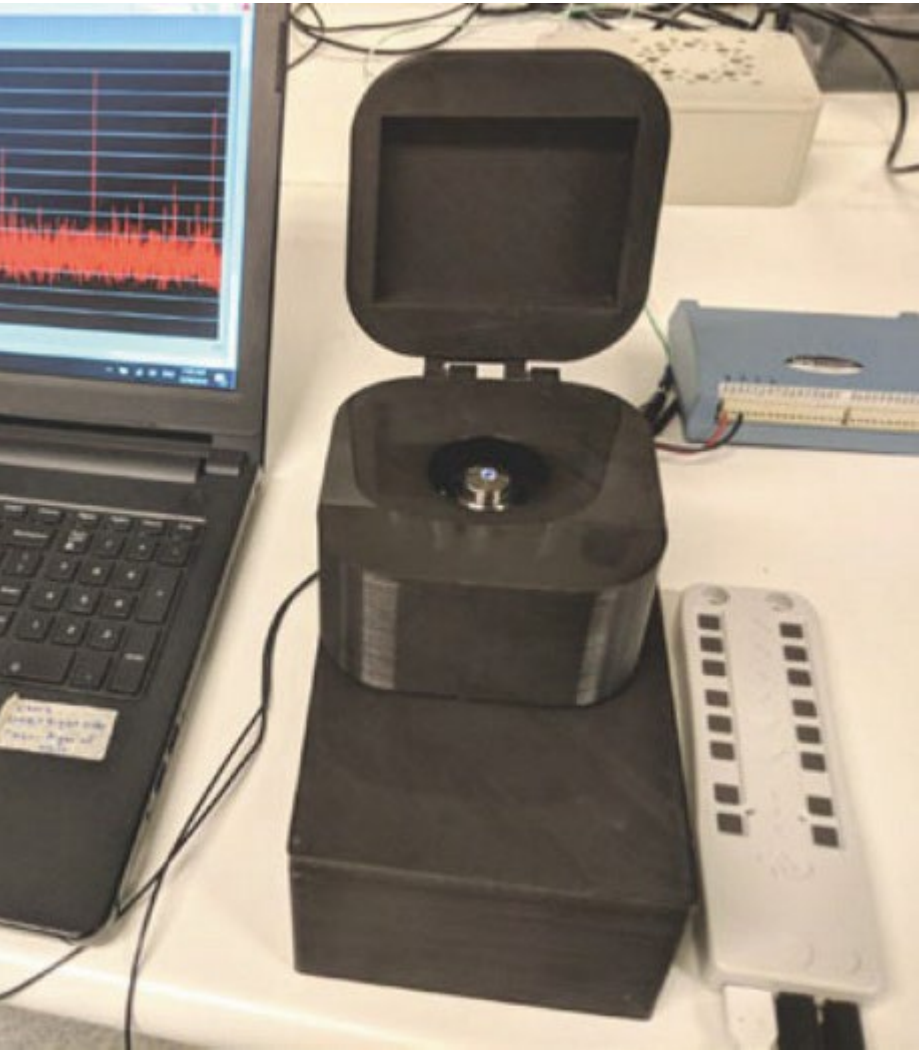


AttoBright:
3D-printed confocal system for detection of single αSynuclein aggregates

Elif Senem Köksal

Journal Club, 10.05.22

nature COMMUNICATIONS

2019

Single-molecule detection on a portable 3D-printed microscope

James W.P. Brown, Arnaud Bauer, Mark E Polinkovsky, Akshay Bhumkar, Dominic J.B. Hunter, Katharina Gaus, Emma Sierecki* & Yann Gambin*

Angewandte International Edition Chemie

2021

Single-Molecule Counting Coupled to Rapid Amplification Enables Detection of α-Synuclein Aggregates in Cerebrospinal Fluid of Parkinson's Disease Patients

Akshay Bhumkar, Chloe Magnan, Derrick Lau, Eugene Soh Wei Jun, Nicolas Dzamko, Yann Gambin,* and Emma Sierecki*

ACS Chemical Neuroscience

2022

Single Molecule Fingerprinting Reveals Different Amplification Properties of α-Synuclein Oligomers and Preformed Fibrils in Seeding Assay

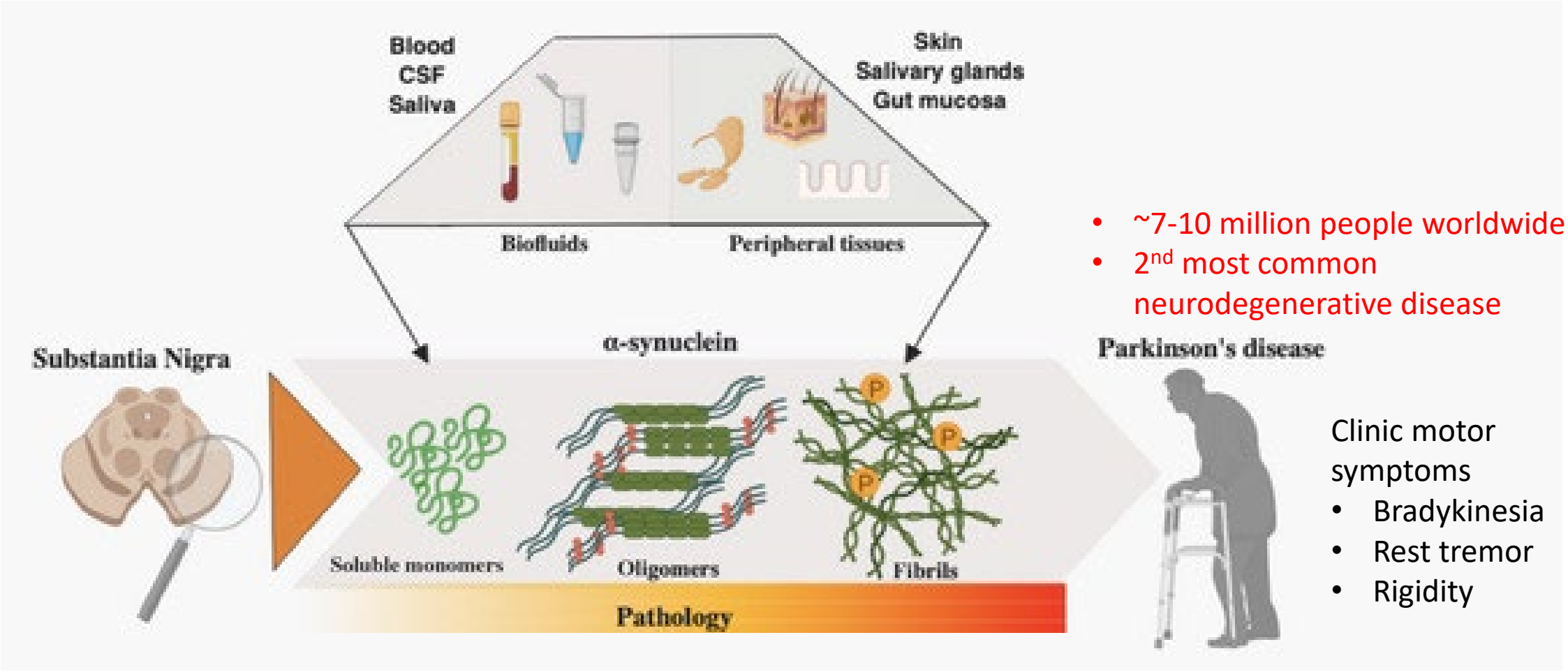
Derrick Lau, Chloé Magnan, Kathryn Hill, Antony Cooper,* Yann Gambin,* and Emma Sierecki*

UNSW THE UNIVERSITY OF NEW SOUTH WALES

Group of researchers led by
Yann Gambin & Emma Sierecki

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

αSynuclein (αSyn) aggregates are a biomarker for Parkinson's disease (PD).



- ~7-10 million people worldwide
- 2nd most common neurodegenerative disease

Clinic motor symptoms

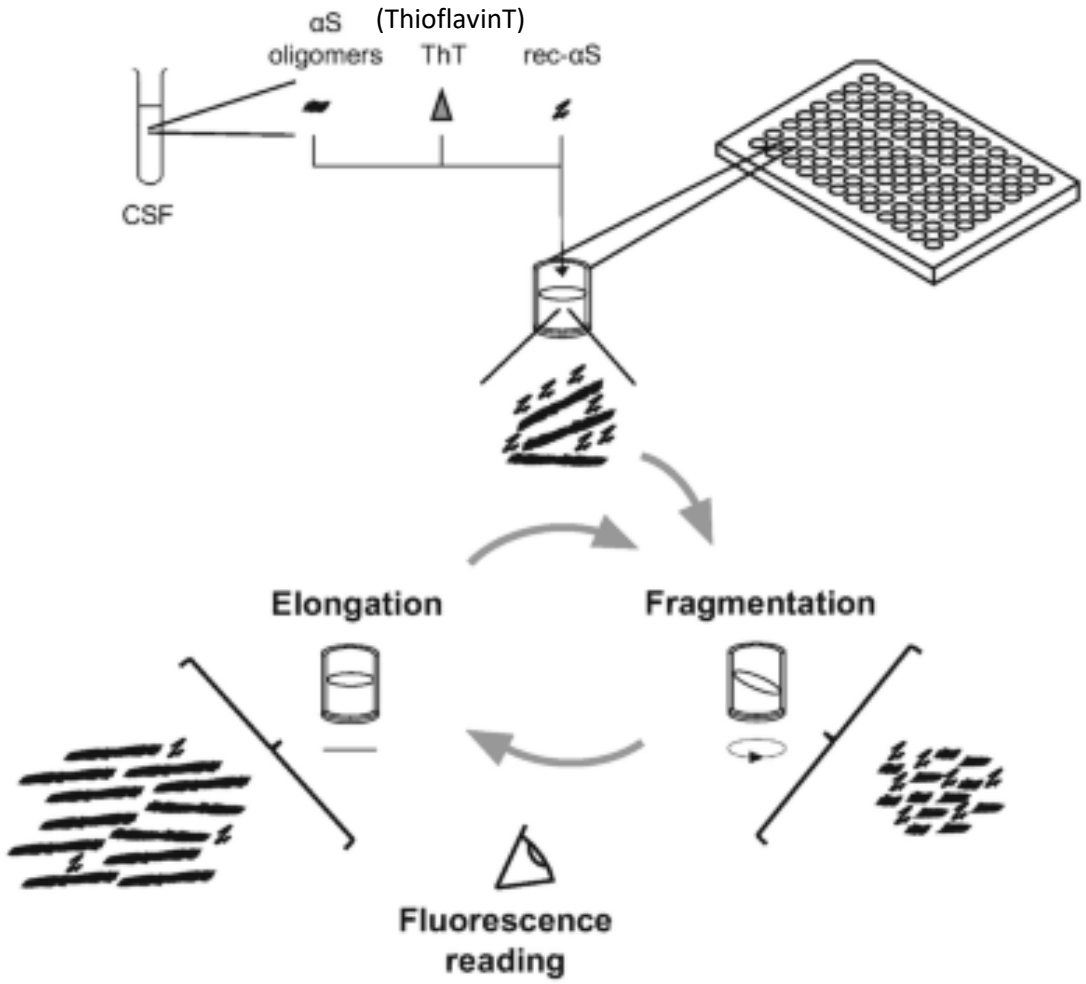
- Bradykinesia
- Rest tremor
- Rigidity

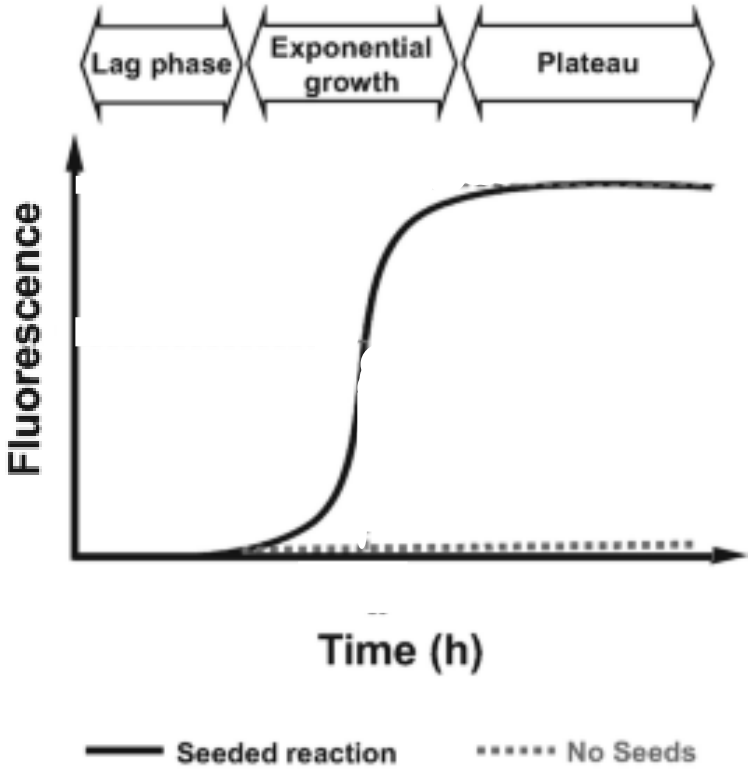
Fayyad et al. *Journal of Neurochemistry* 2019.

Currently available αSyn detection methods

Protein misfolding cycling amplification (PMCA) (Soto Lab)
Real-time quaking-induced conversion (RT-QuIC) (Green Lab)

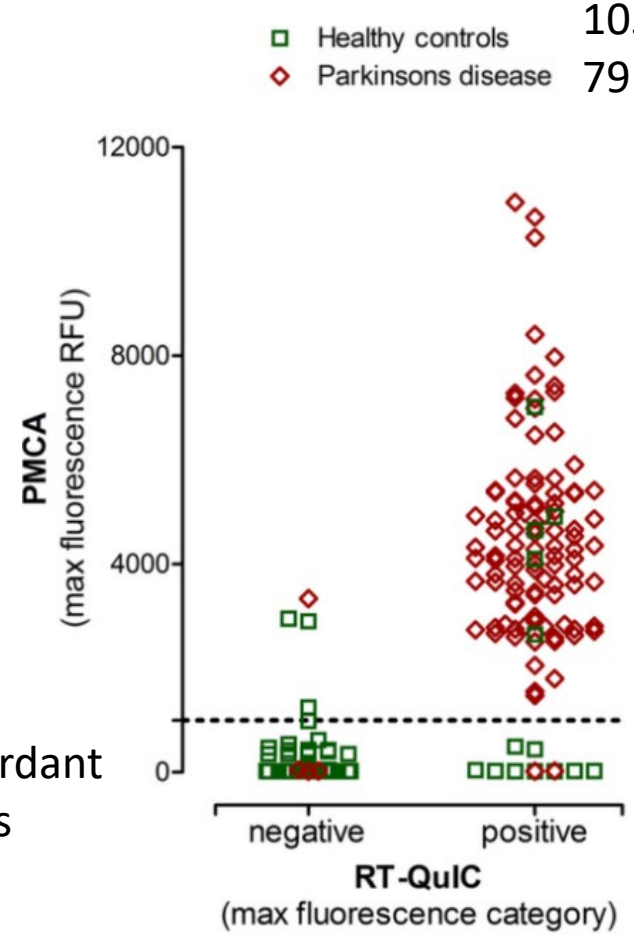
Differences (Buffer, pH and shaking conditions)





Marambio, Shahnawaz, Soto. *Alpha-Synuclein* 2019.

PMCA and RT-QuIC can detect αSyn in CSF samples with >90% selectivity and specificity.



105 PD,
79 healthy controls

92% concordant results

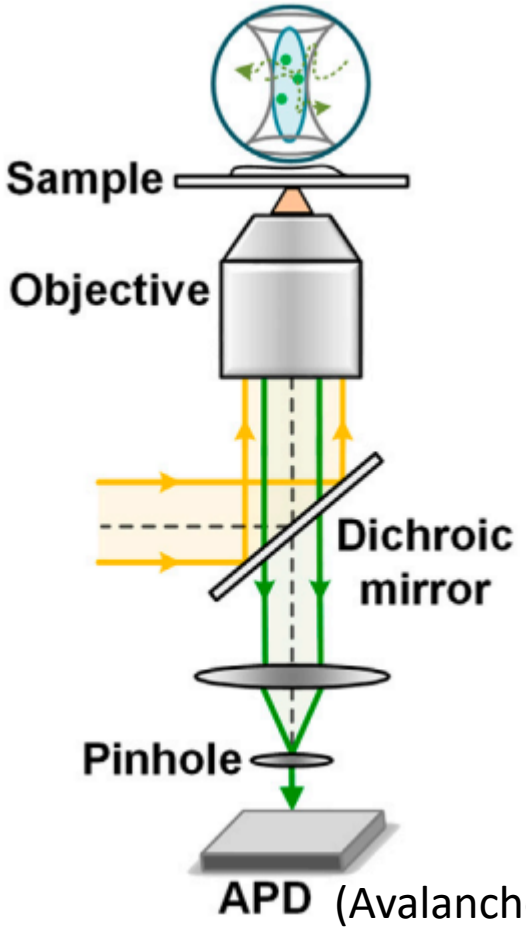
Limitations

- Long assay time (48h-400h)
- Repeated sonication and/or heating steps
- Only works with CSF samples
- Monitoring disease progression

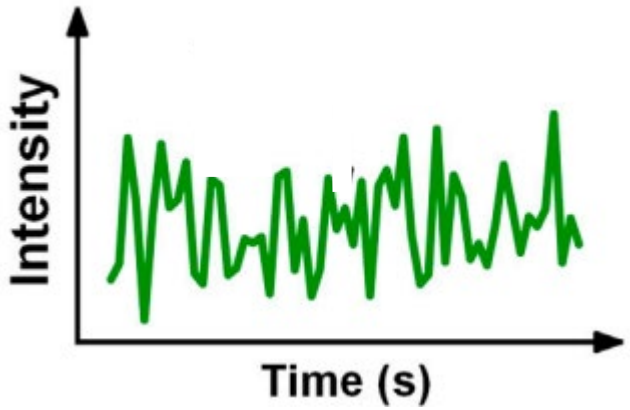
Kang et al. *Movement Disorders* 2019.

Fluorescent fluctuation spectroscopy (FFS) combined with confocal microscopy

Fluorescent molecules freely move in and out of detection volume

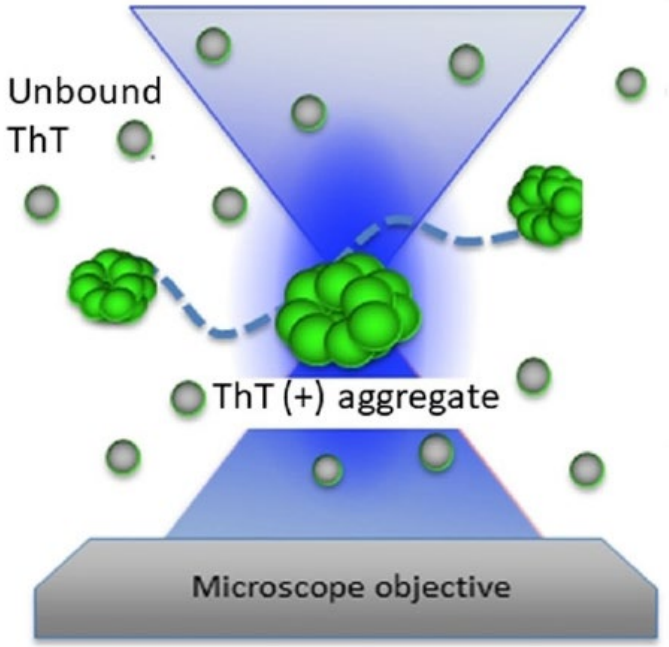


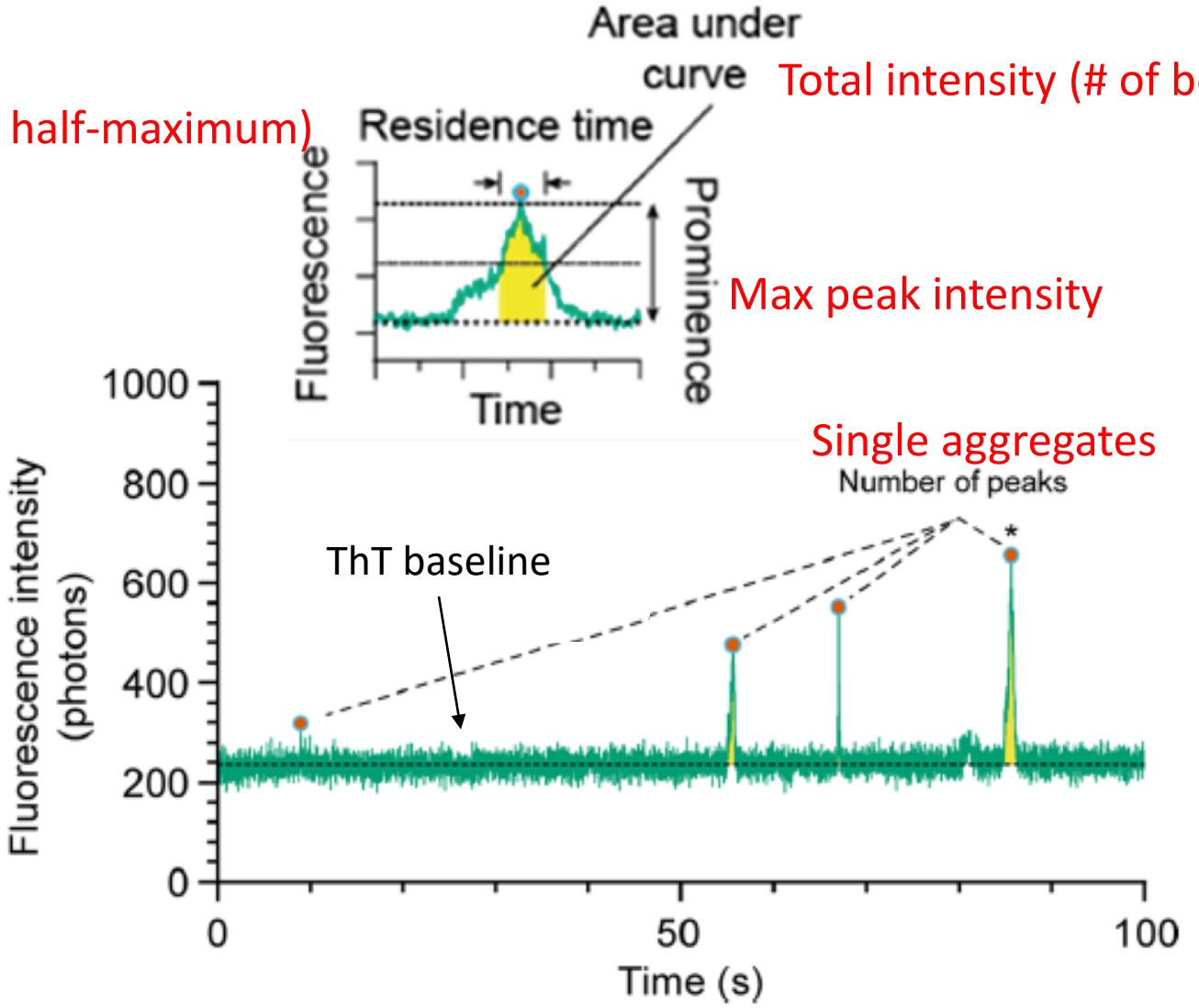
(Avalanche Photodiode - Single photon detector)



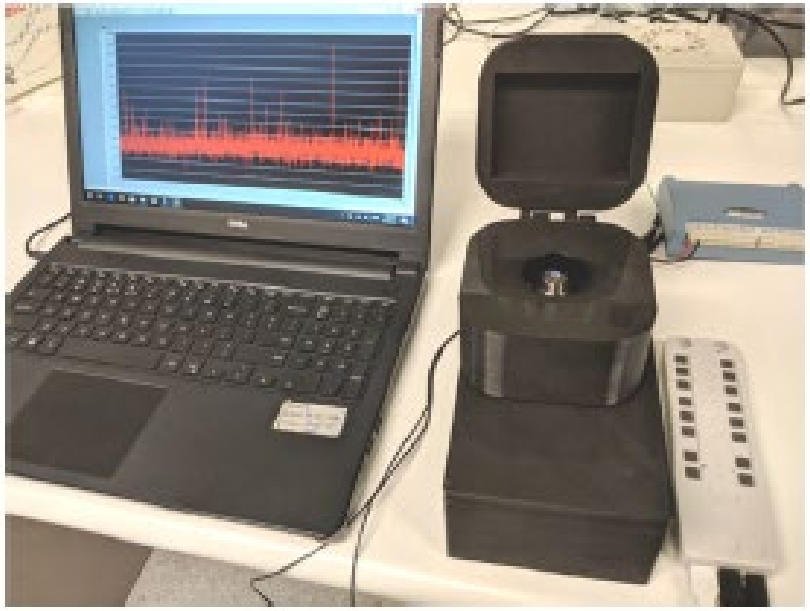
- Detection volume is sub-fL range
- High signal/noise ratio with confocal illumination

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



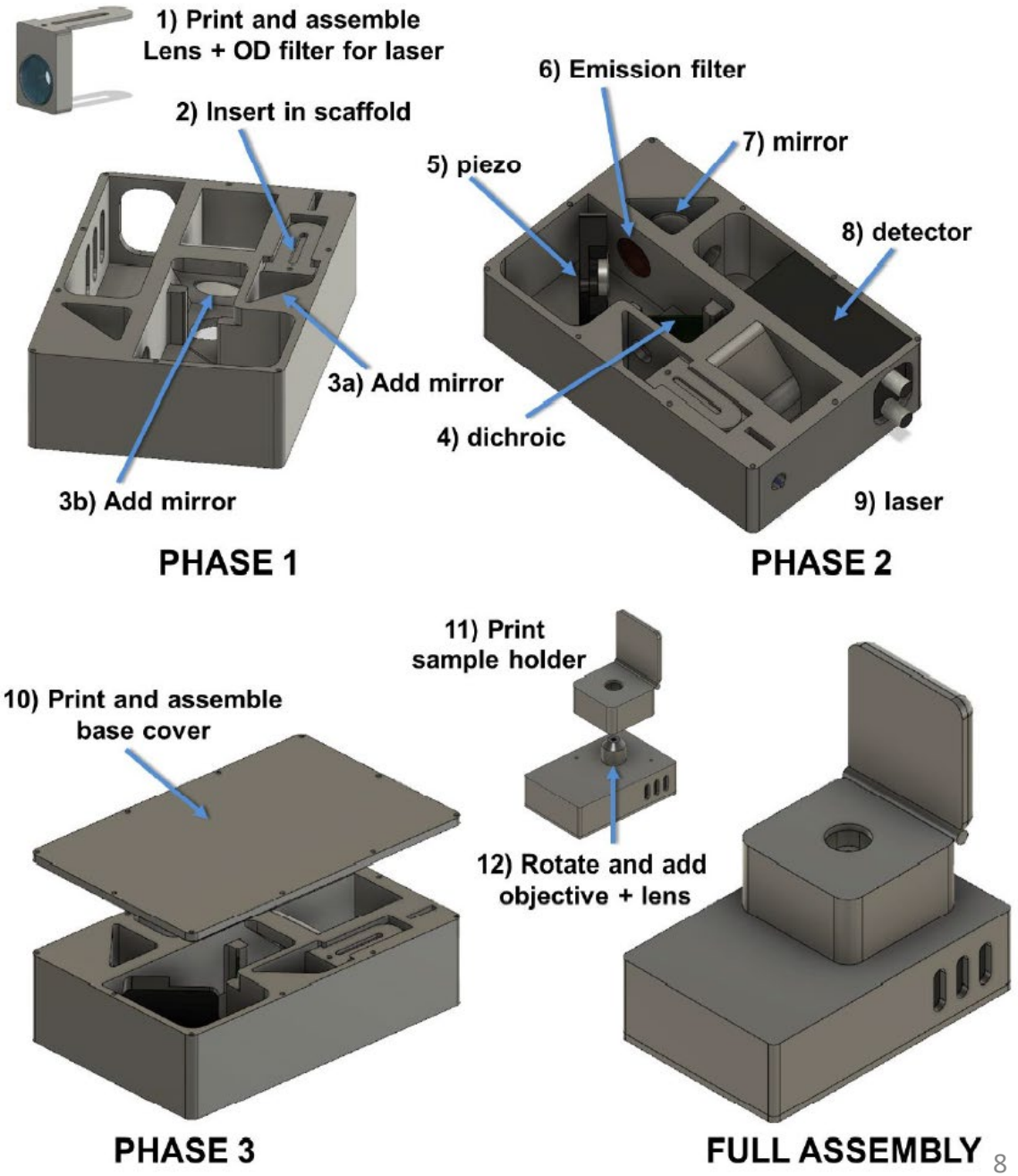


AttoBright: 3D-printed single molecule microscope



Device dimensions:
10 cm x 20 cm

Free design is available online



AttoBright costs ~12k. (2019 prices)

Part name	Price	Link
laser diode 450 nm	$234.82	https://www.thorlabs.com/thorproduct.cfm?partnumber=CPS450
power supply for laser diode	$92.26	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=8861&pn=LDS5#9175
achromatic converging lens	$524.83	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=6083&pn=ACA254-200-A#6784
USB based counter device with times and DIO	$395.00	https://www.mccdaq.com/usb-data-acquisition/USB-CTR-Series.aspx
3 Spools of PLA filament	$120.00	https://www.amazon.com/Polymaker-PolyMax-Printing-Filament-Printer/dp/B07ML69CYZ
3 mirrors	$66.00	https://www.newport.com/p/10D620BD.1
adaptor for objective	$21.86	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=1524&pn=SM1A35#3229
micro-controle controller	$481.00	https://www.newport.com/p/AG-UC2
piezo Agilis	$531.00	https://www.newport.com/p/AG-M100N
objective 40x 1.15NA water immersion	$4300.00	https://www.olympus-lifescience.com/en/objectives/detail/0-DIRECTORY%3A%3ADirFrontend-itemId.511706640.html
BCC diverging lens	$52.00	https://www.newport.com/p/KBC043AR.14
488 dichroic	$445.00	https://www.semrock.com/FilterDetails.aspx?id=Di02-R488-25×36
525 emission filter	$325.00	https://www.semrock.com/filterdetails.aspx?id=ff03-525/50-25
neutral density filter	$52.22	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=6106&pn=NDUV10B#3322
M3 x 0.5 Stainless Steel Setscrew, 6 mm Long, Pack of 50	$10.23	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=1437&pn=SS3M6#10009
photon counting detector	$5,300.00	Quote for MPD Bolzano PD-050-CTC

Optical path of the AttoBright system

Data acquisition and analysis with software written in LabView (National Instruments)

- Acquisition time and frequency

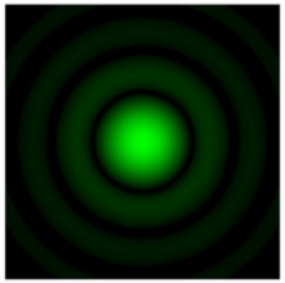
Small number of optical elements

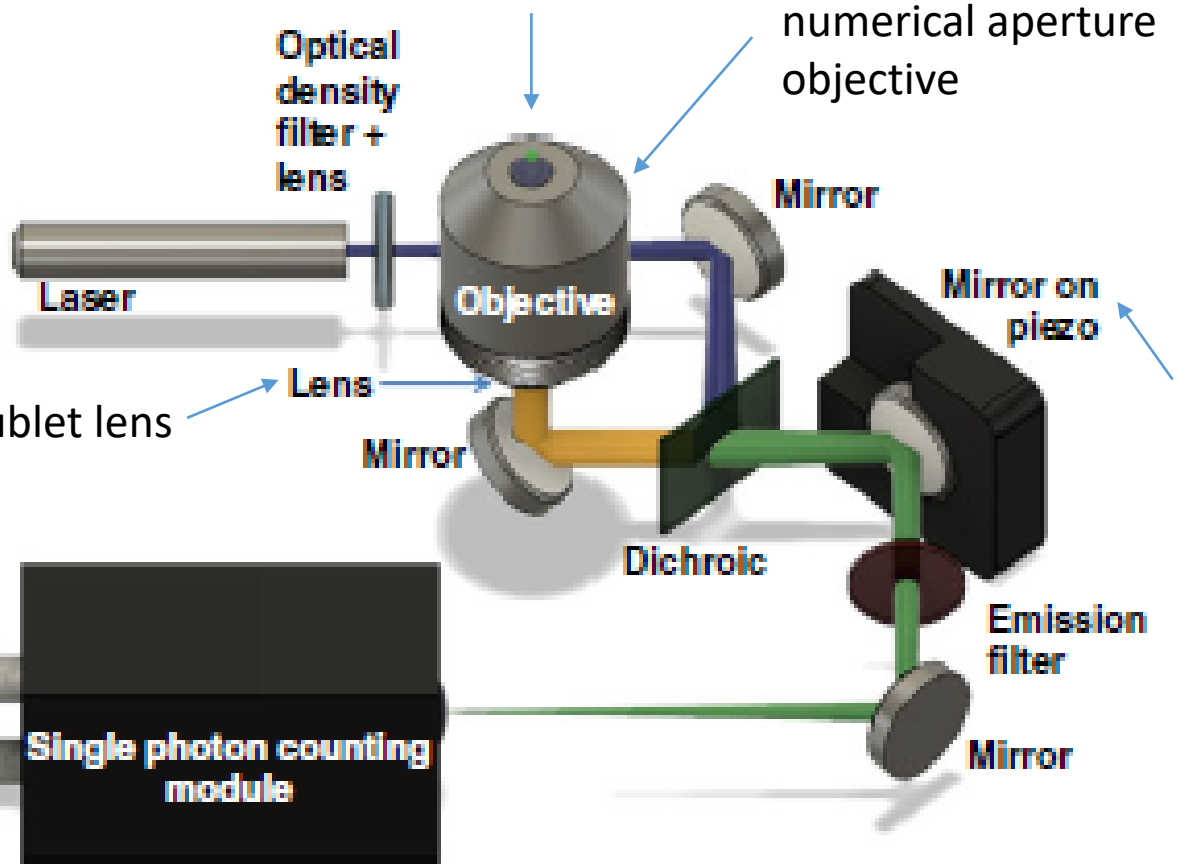
- ✓ To reduce misalignment and chromatic aberration issues
- ✓ To increase ease of use and assembly

20ul sample is in custom made PDMS well on a microscope slide

40X or 63X high numerical aperture objective

$$\text{Airy Disc Diameter} = 1.22\frac{\lambda}{NA}$$



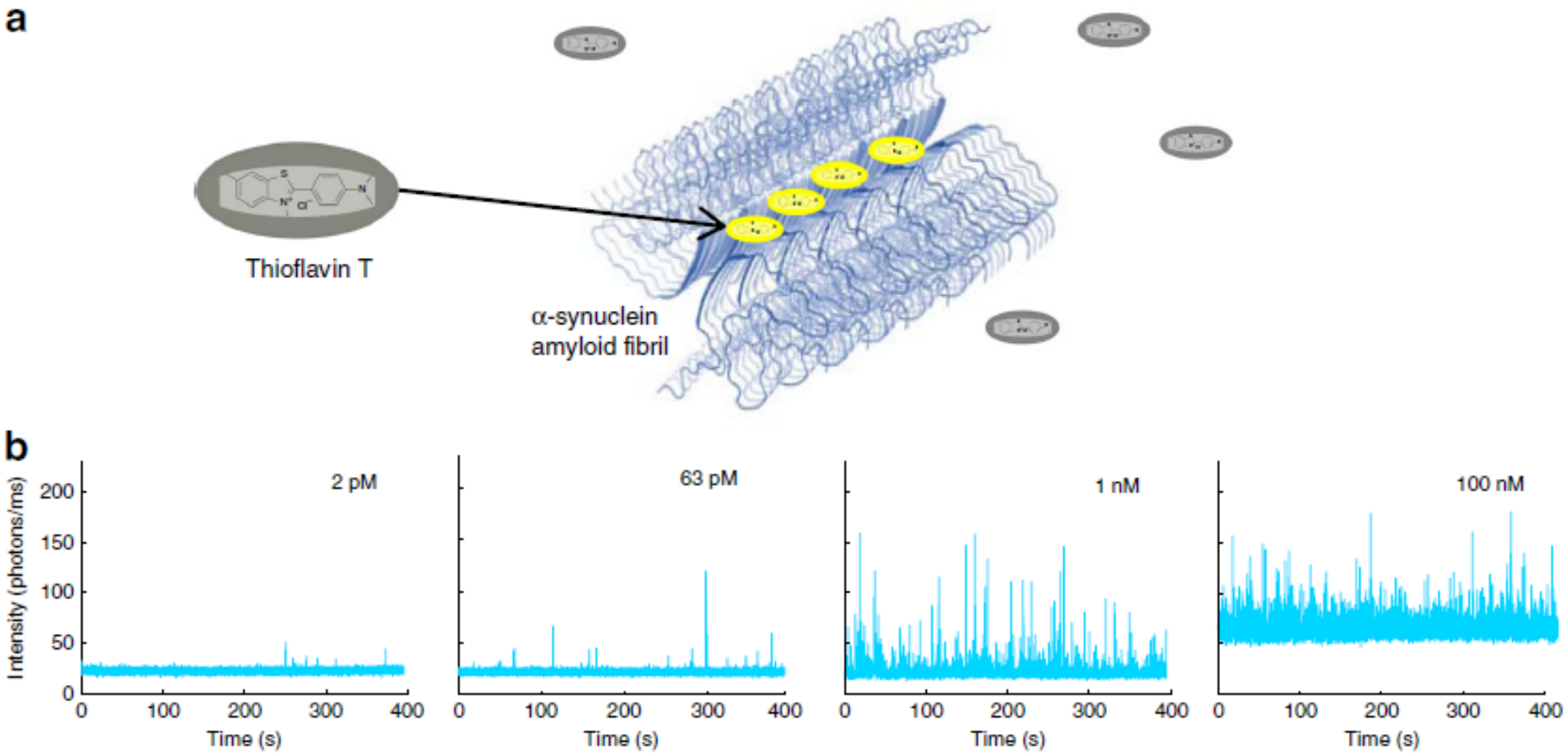


Achromatic doublet lens

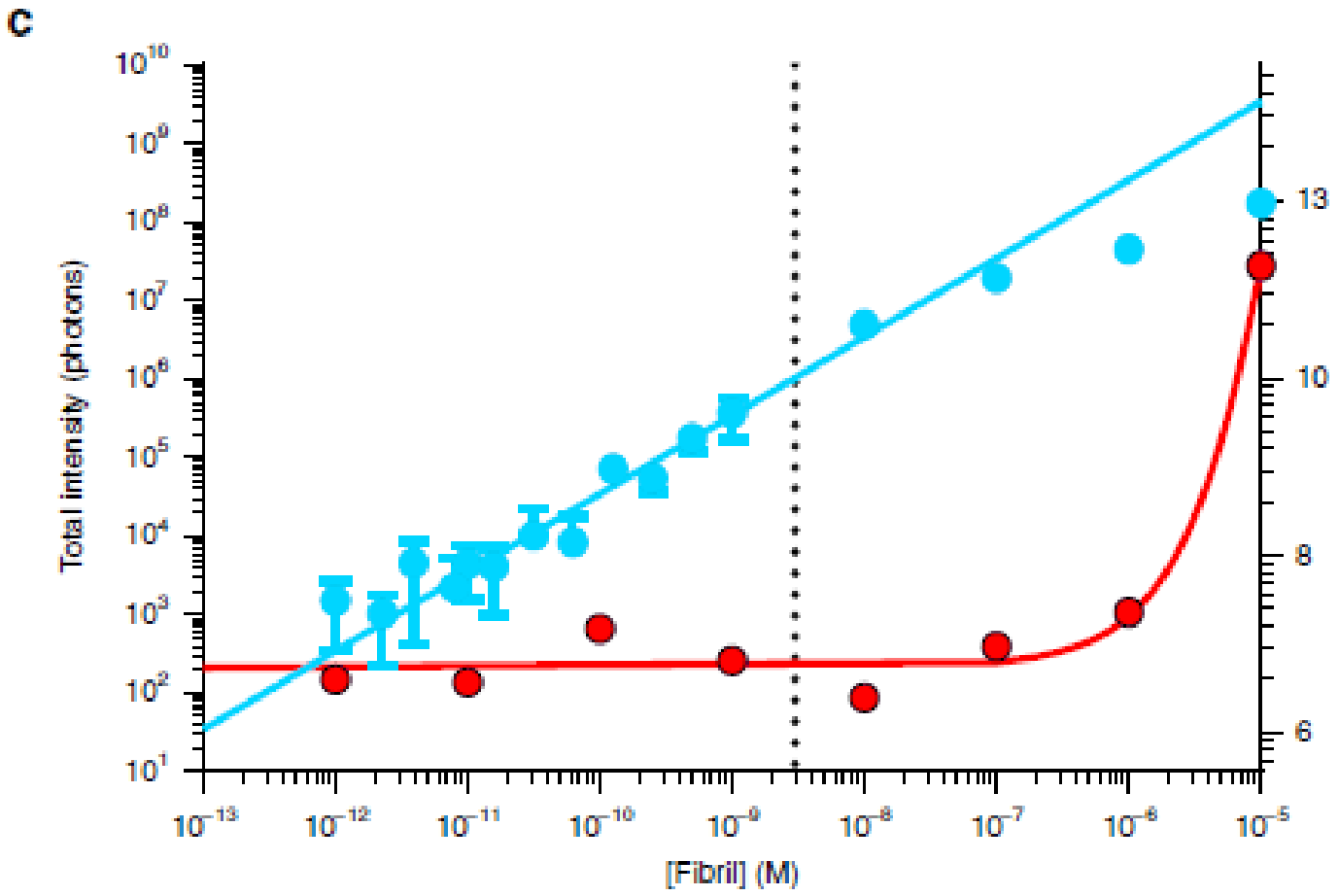
Mirror is aligned with piezo-electric motors to get optimum signal

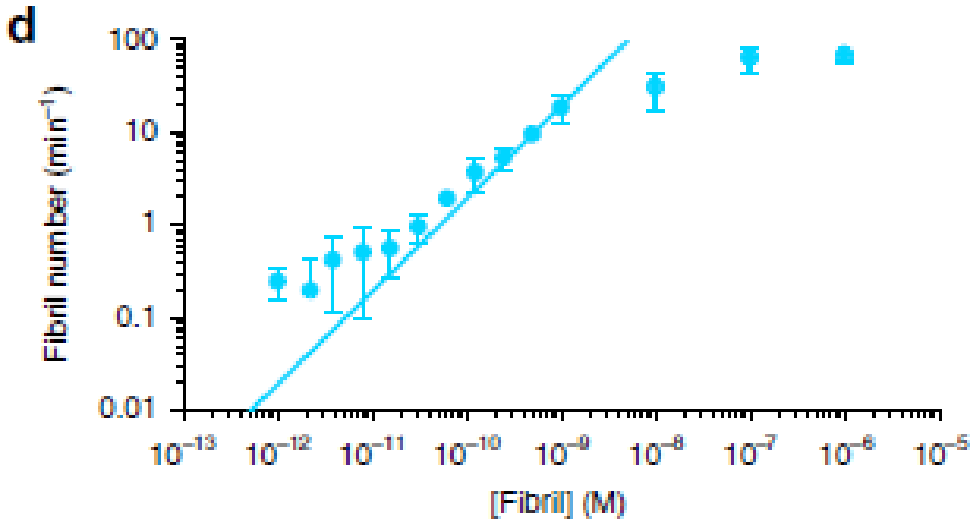
50um diameter active area of single photon avalanche photodiode (APD) is used as pinhole
(Detector can move back and forward)

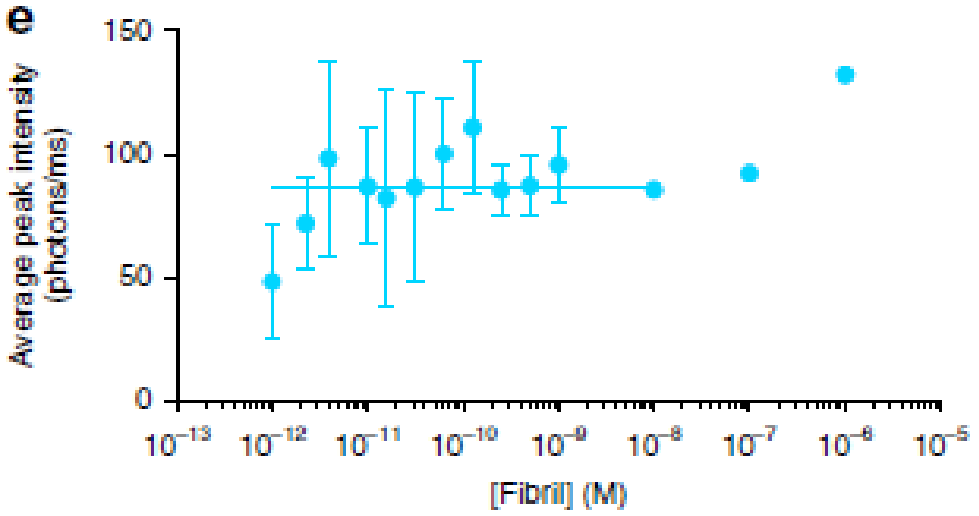
AttoBright detects individual αSyn fibrils in different concentrations.



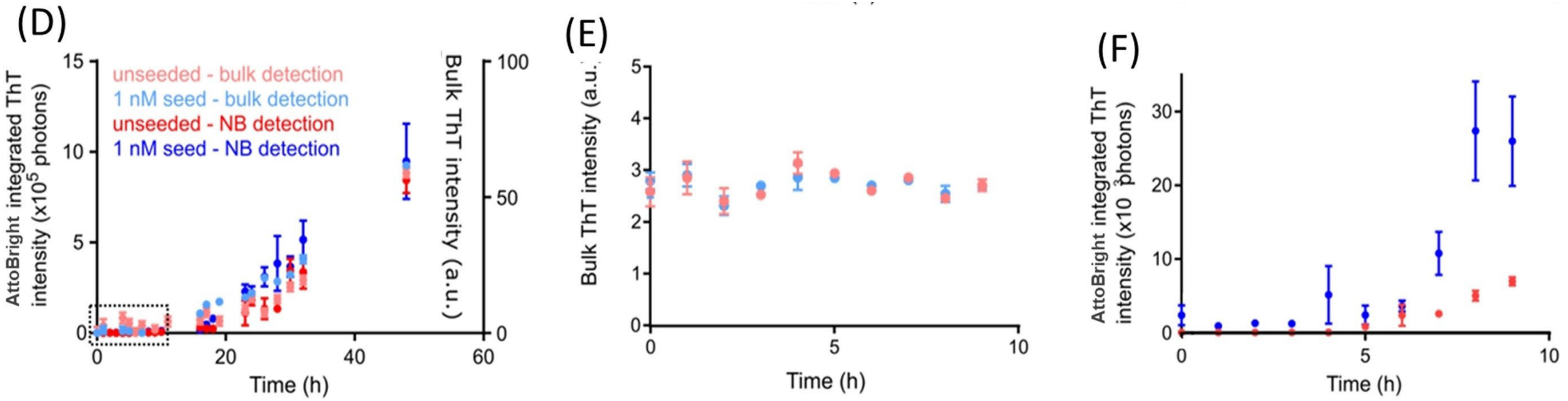
AttoBright is 10^6 times more sensitive than bulk measurement to detect αSyn fibrils.







AttoBright detects αSyn aggregation at earlier time points comparing to bulk seed-amplification assays.



Incubation conditions

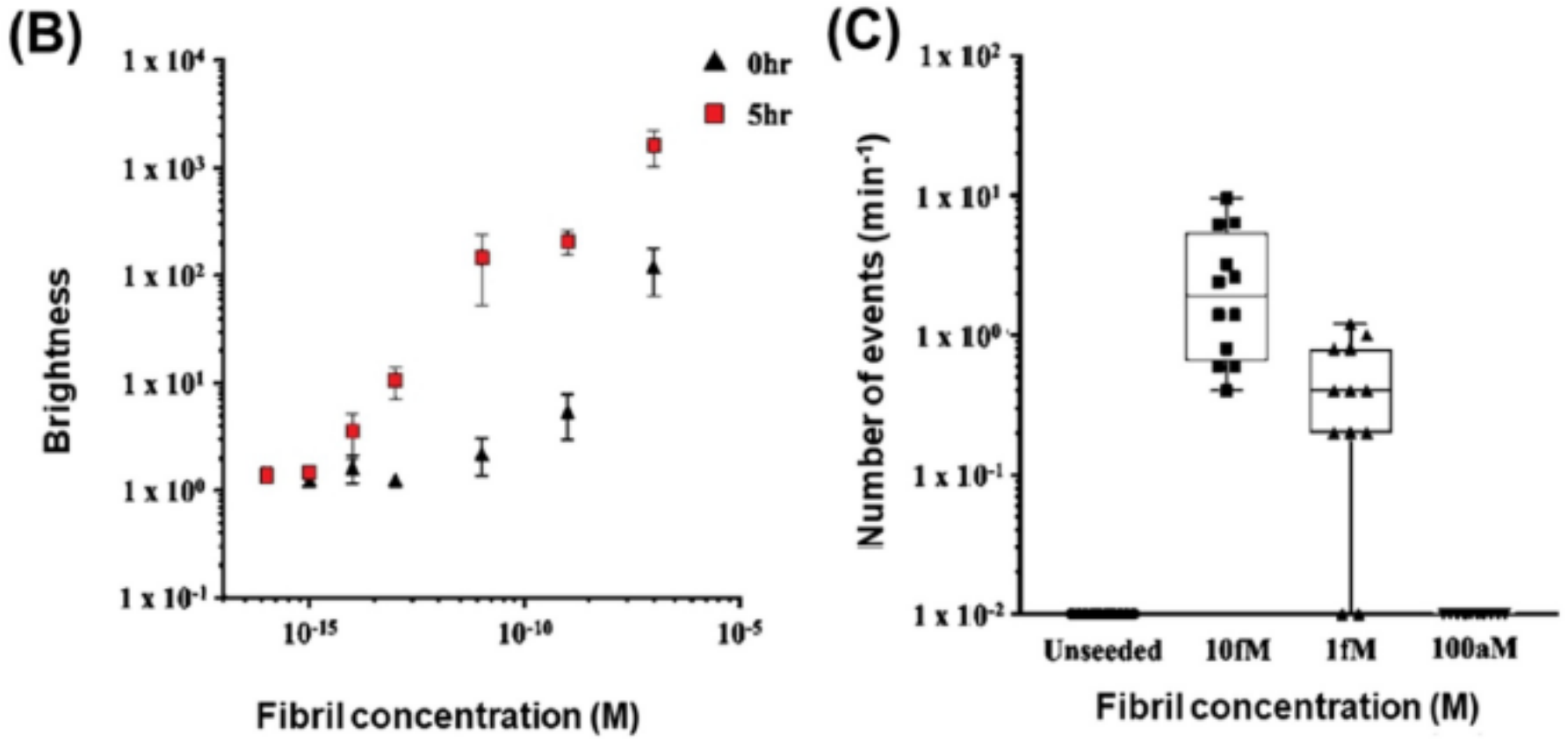
37C with orbital shaking at 500rpm

Seed concentration: 1nM

Monomer concentration: 50uM

AttoBright detects down to 1fM αSyn fibrils after amplification.

Brightness
$=\sigma^2/\mu$



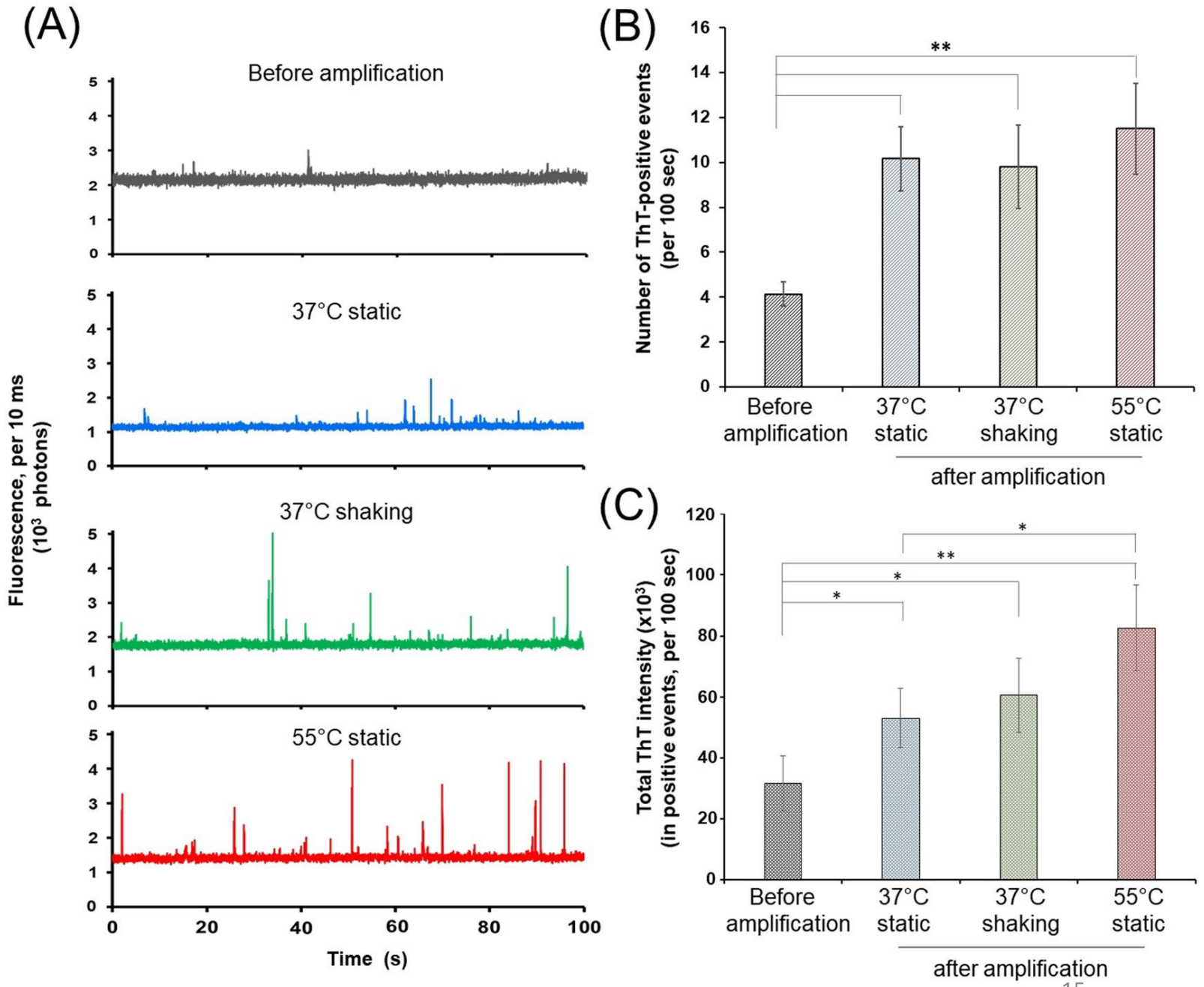
<u>Incubation conditions</u>
5h at 55C without shaking

Monomer concentration: 20uM

Amplification of αSyn aggregates is rapid at high temperatures.

<u>Decided incubation conditions</u>
55C without shaking

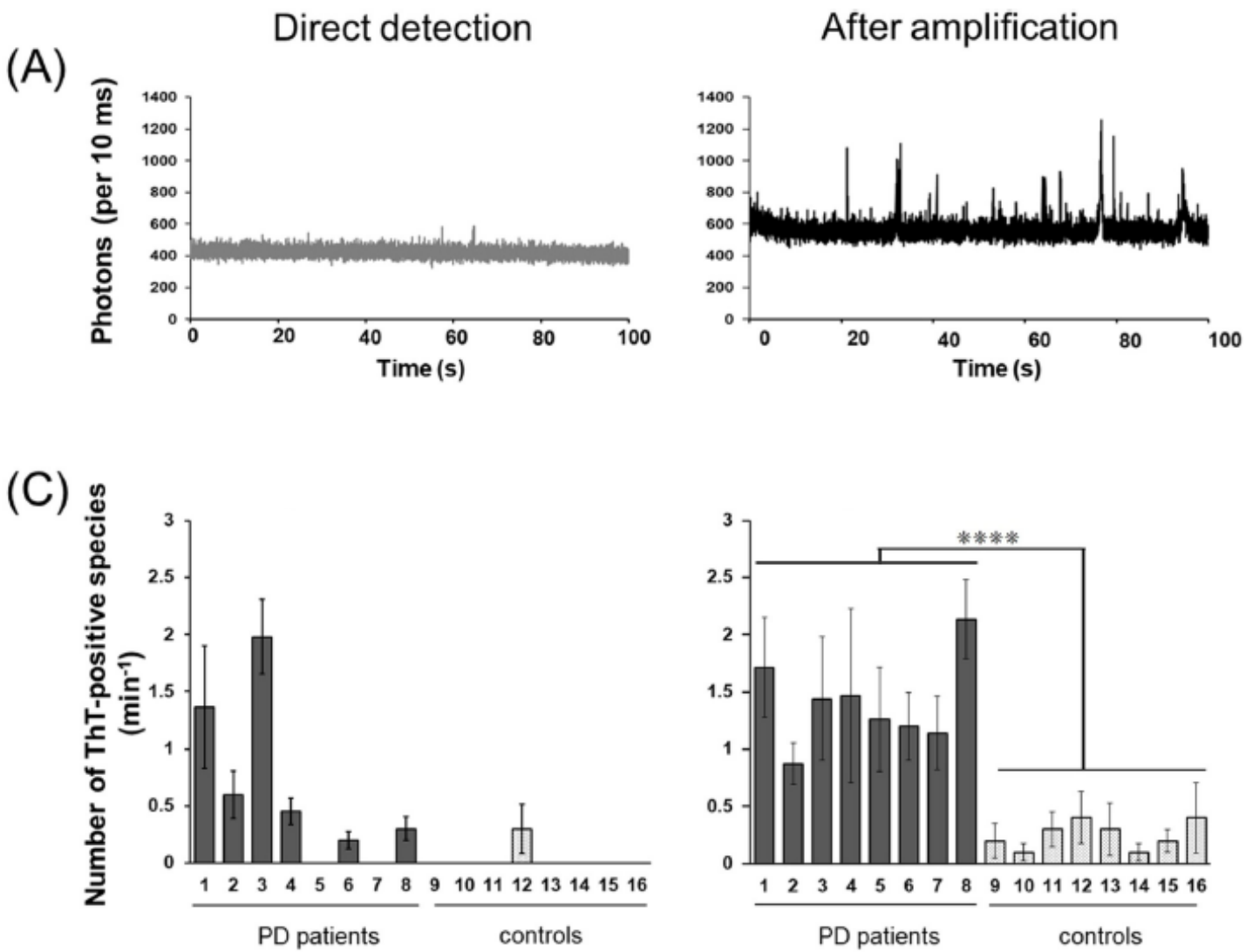
Seed concentration: 2pM
Monomer concentration: 20uM



AttoBright discriminates between CSF of PD patients and healthy samples.

<u>2ul CSF samples</u>

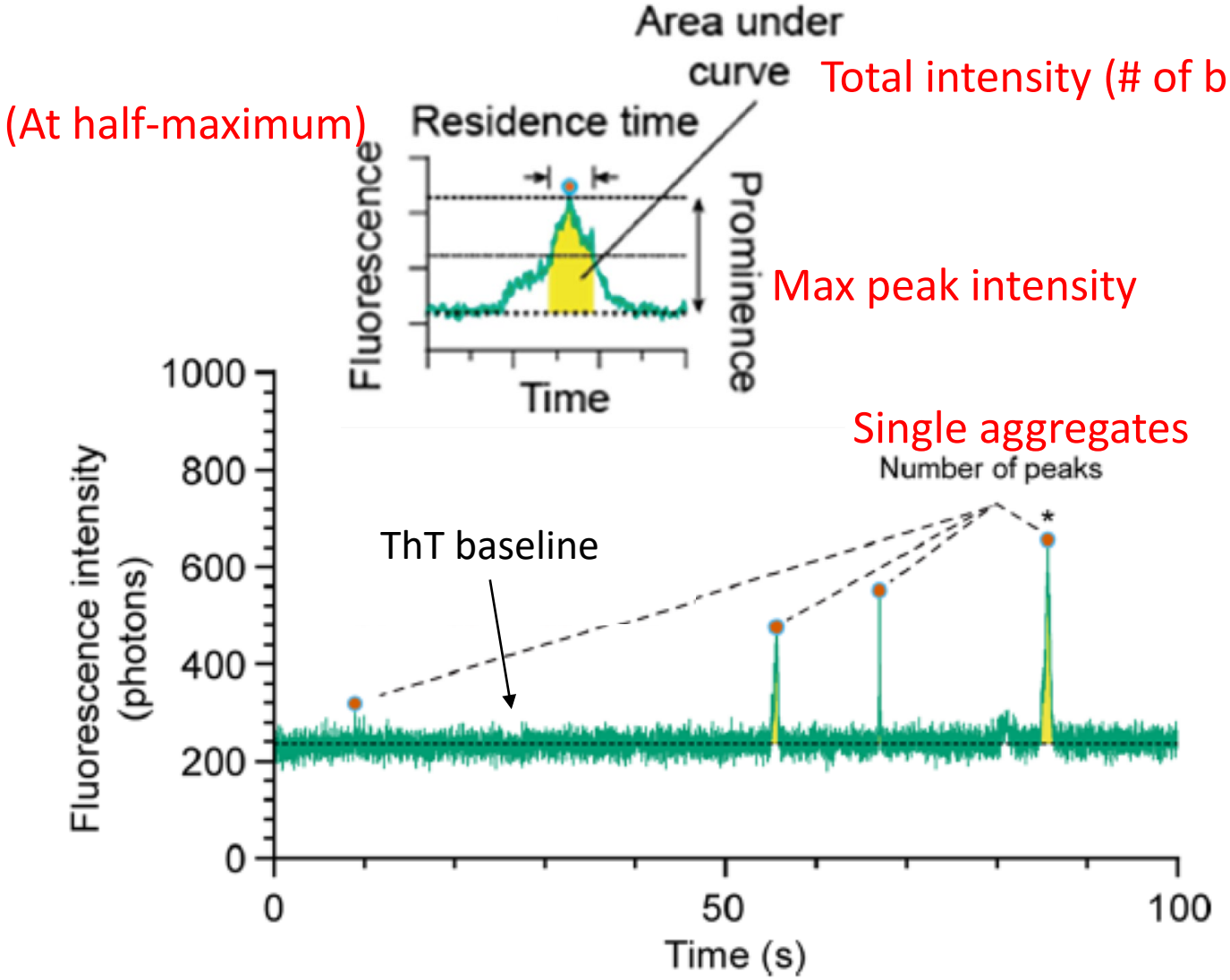
8 PD patients
8 healthy controls

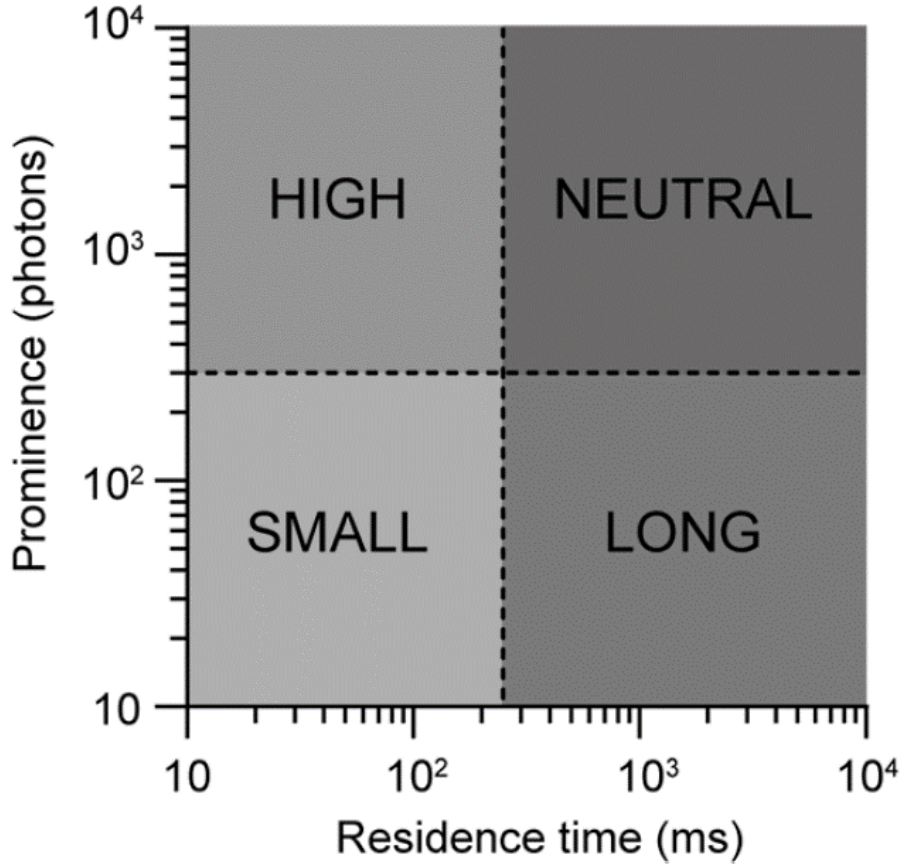


<u>Incubation conditions</u>
5h at 55C without shaking

Monomer concentration: 20uM

Fingerprinting of individual events characterizes αSyn aggregates.

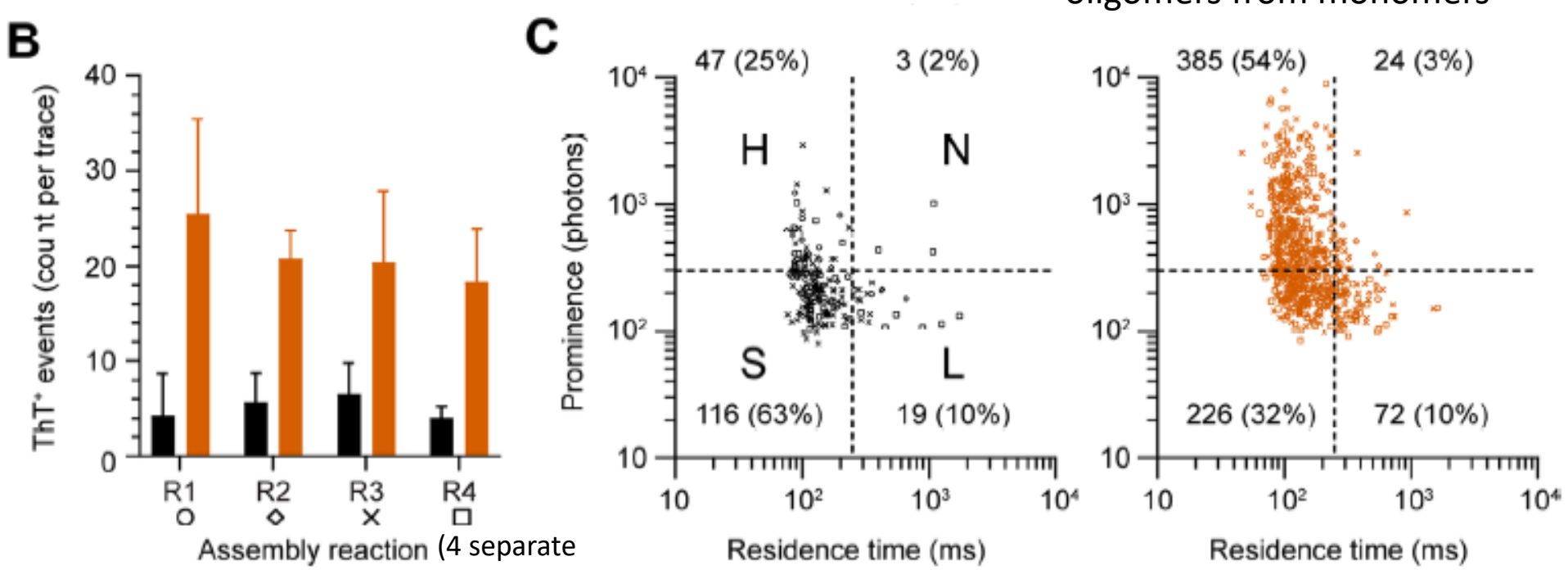




αSyn oligomers has seeding potential.

Preparation of αSyn oligomers

- 830uM αSyn in PBS at pH 7.4, 5h horizontal shaking at 900rpm at 37C
- Centrifugation at 18000g for 10min to remove of large fibrils
- Size exclusion chromatography (SEC) to separate oligomers from monomers



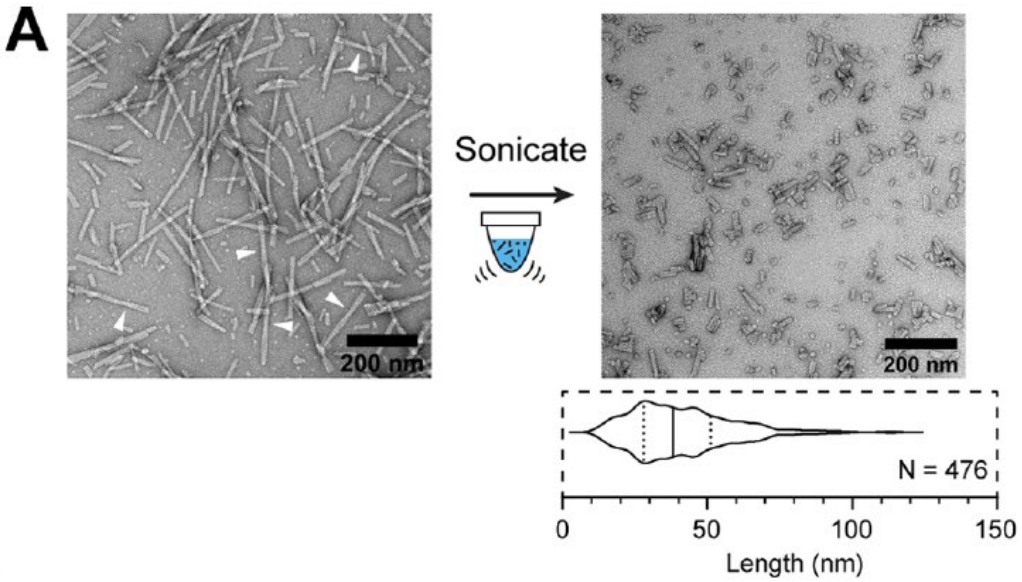
Incubation conditions

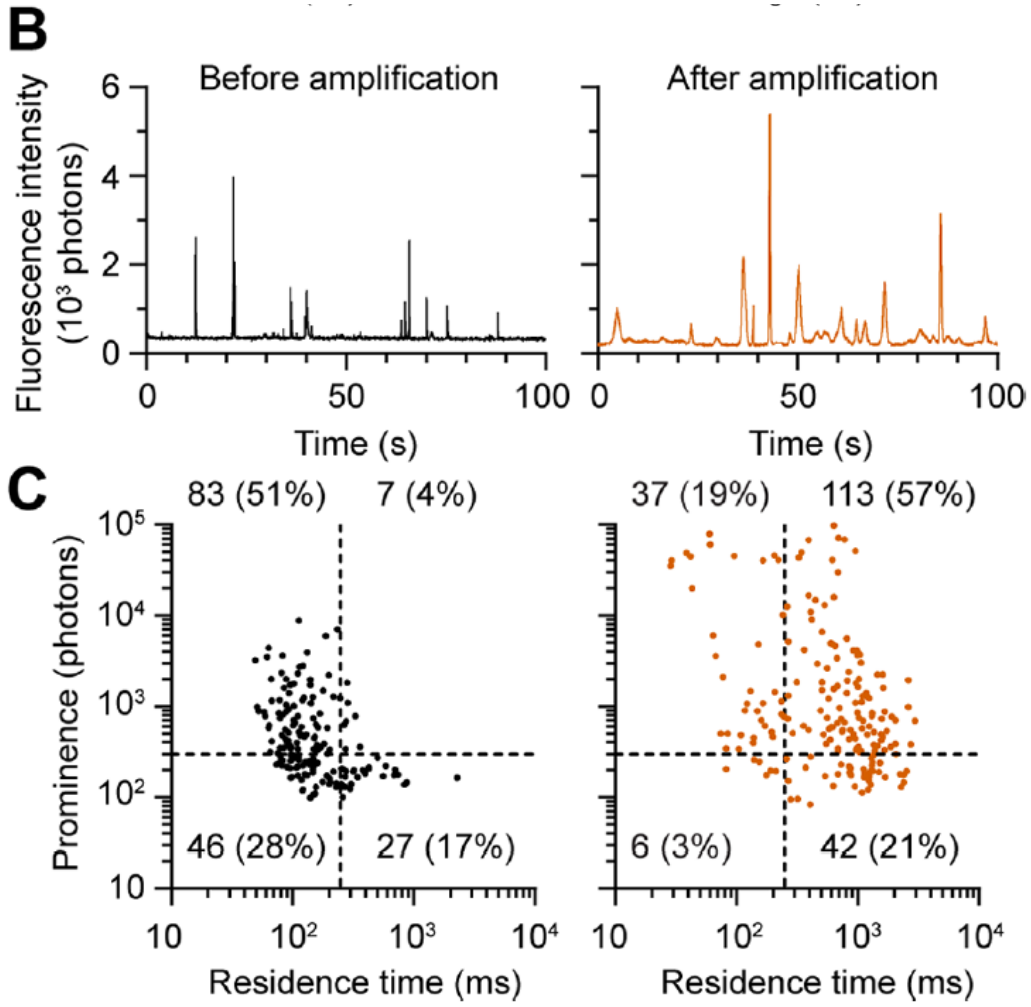
5h at 55C without shaking

Oligomer seed concentration: 0.2-3.1uM

Monomer concentration: 30uM

Sonicated αSyn preformed fibrils has seeding potential.





Preparation of αSyn fibrils

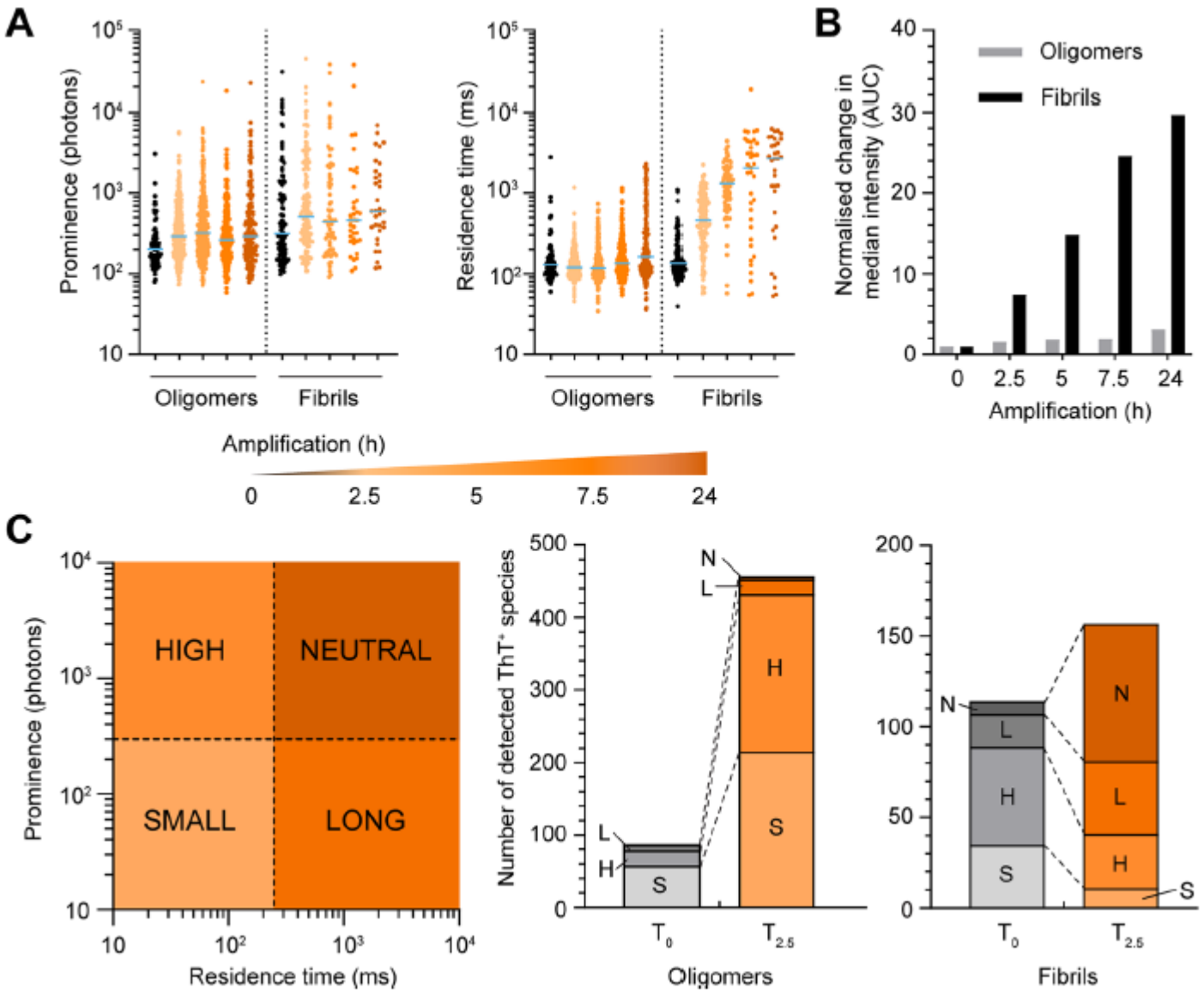
- 208uM αSyn in PBS
  72h shaking at 500rpm at 45C
  15 min sonication every 24h

- 10min sonication before use

Incubation conditions
5h at 55C without shaking

Fibril seed concentration:
Monomer concentration: 30uM

Sonicated αSyn preformed fibrils amplify more efficiently than oligomers.



Conclusions

- AttoBright is a cheap and simple single molecule detecting confocal system.
- AttoBright detects single αSyn amyloid fibrils.
  - 10^6 times more sensitive than bulk measurement.
  - Detection in early phase of amplification.
  - Down to 1fM αSyn fibril detection sensitivity after short, single amplification cycle.
- AttoBright discriminates between CSF samples of PD patients and healthy controls.
- Fingerprinting of size and reactivity of individual aggregates with AttoBright reveals preformed fibrils has higher seeding potential than oligomers.