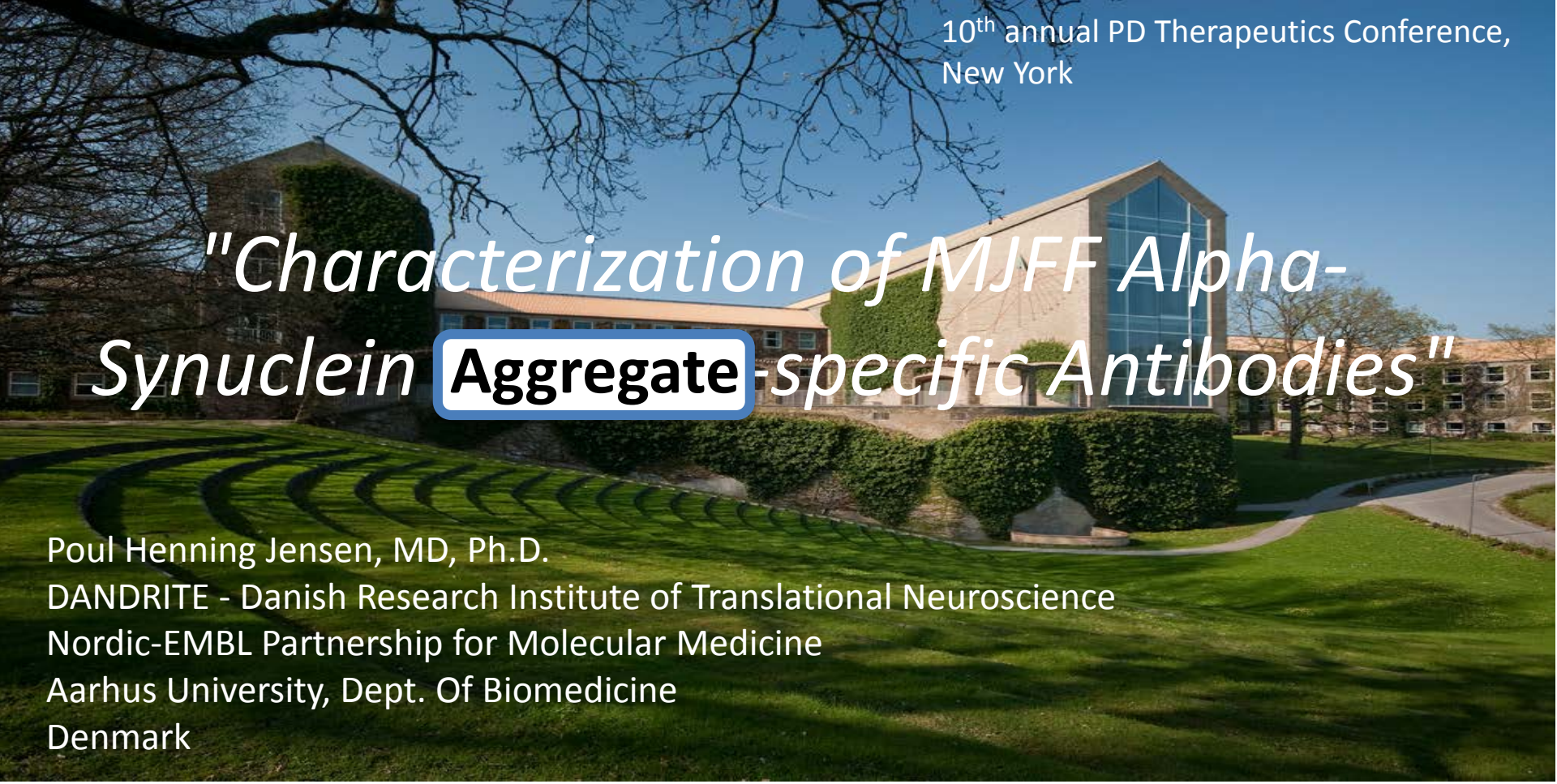
Overview

- This presentation was presented by Dr Poul Henning Jensen at the 10th annual Parkinson's Disease Therapeutics Conference in New York City on October 24, 2016.
- This presentation discusses characterization of the alpha synuclein aggregate-specific antibody MJFR14 that is available at Abcam.
  - A link to the Abcam product page can be found here.
- The alpha synuclein filament protein used to generate and test this antibody is available from Proteos and can be found in the MJFF Tools Catalog under the Proteins tab as "alpha-synuclein filament protein".
  - A link to the Proteos order form for the protein can be found here.



10th annual PD Therapeutics Conference, New York

"Characterization of MJFF Alpha-Synuclein Aggregate-specific Antibodies"

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DANDRITE - Danish Research Institute of Translational Neuroscience
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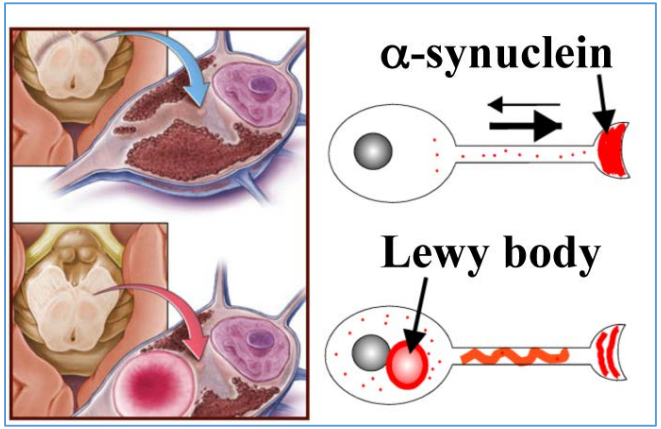
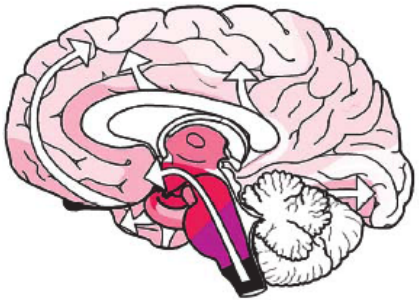
NORDIC EMBL PARTNERSHIP FOR MOLECULAR MEDICINE
EMBL
AARHUS UNIVERSITY
Better lives through new knowledge

Why are alpha-synuclein aggregates interesting?

- a-synuclein aggregates (Lewy bodies) has to be present in brains to confirm the clinical diagnosis PD
- a-synuclein mutations can directly cause PD
- **Hypothesis: a-synuclein aggregates cause neurodegeneration and spread the process within the nervous tissue**





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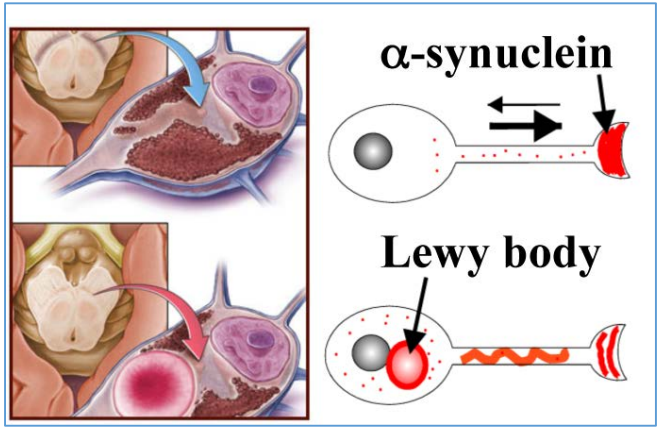
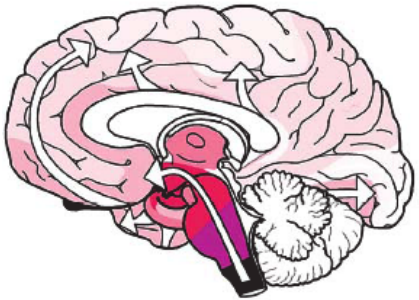
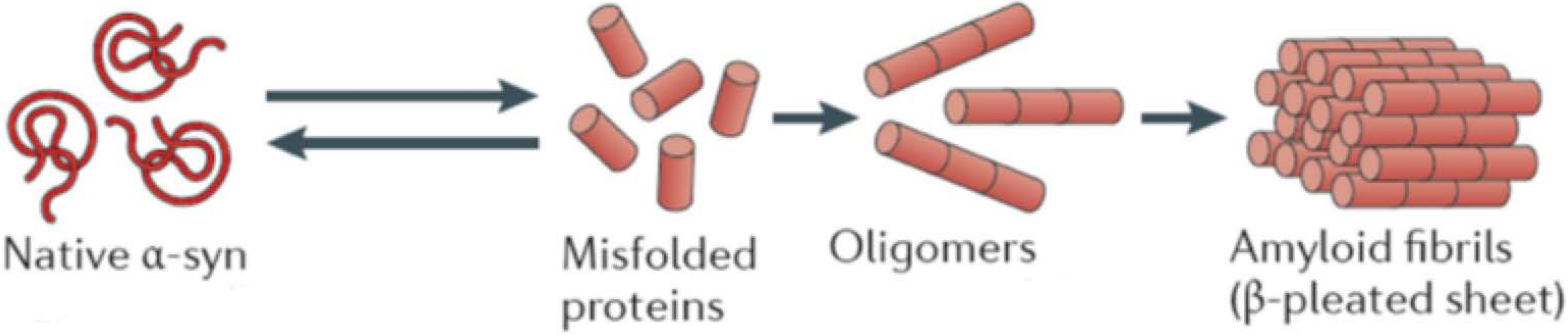




You say, aggregates?

Alpha-synuclein aggregates
A messy field!

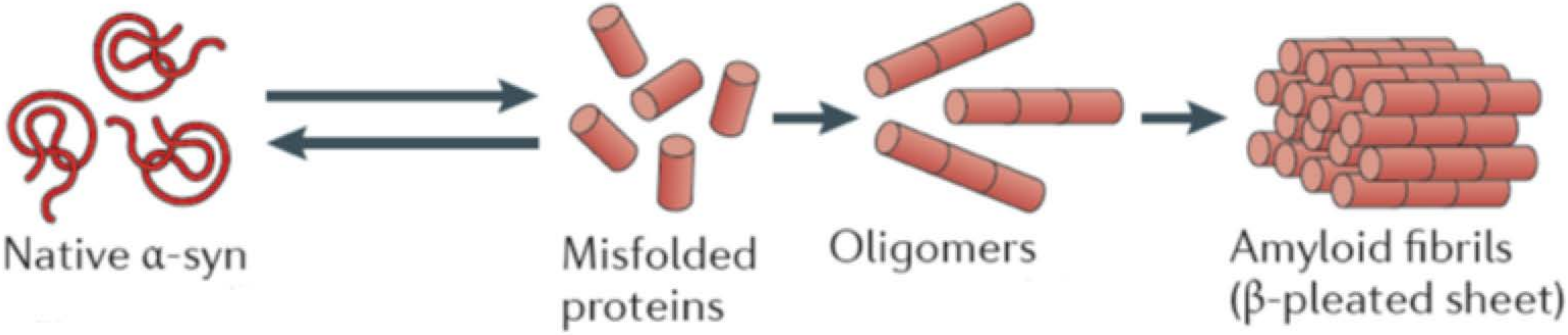


- Can be induced by chemicals, alcohol, acid, detergents, temperature

Our spontaneously forming oligomers and fibrils can be dissociated into monomers. No modifications

Lashuel, J Mol Biol. 2002
Lindersson, J Biol Chem. 2004
Betzer, PLoS One. 2015

Alpha-synuclein aggregates
A messy field!

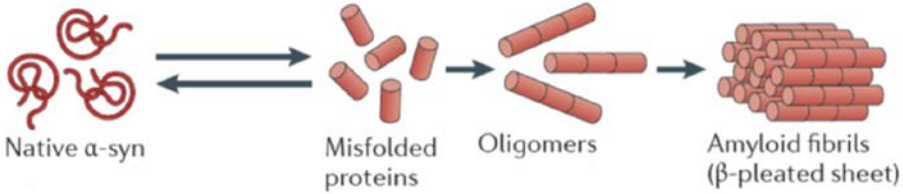


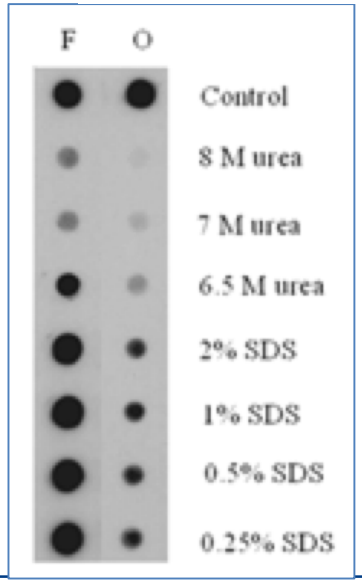
- Can be induced by chemicals, alcohol, acid, detergents, temperature

Aim: Develop antibodies specific for the alpha-synuclein aggregates developing in Parkinson's disease and other synucleinopathies brain that will expand functional, diagnostic and biomarker studies

Can one make an aggregate-specific alpha-synuclein antibody?

- **FILA-1** was raised against a-synuclein filaments in rabbits
- Does not bind monomers
- Recognize filaments >> soluble oligomers
- Epitope is lost upon denaturation of aggregate
- Recognize a-syn in PD brain extracts and in a-syn transgenic mice

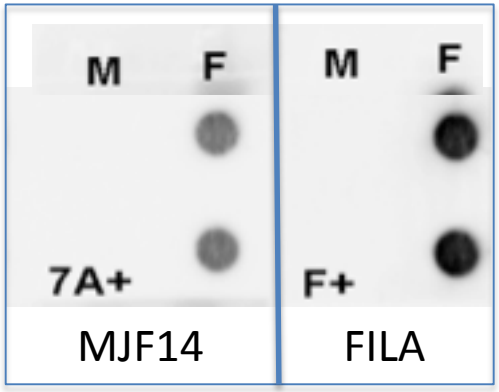




Lindersson, J Biol Chem. 2004
Paleologou, Brain. 2009
Colla, J Neurosci. 2012
Nuber Brain. 2013
Bousset Nat Commun. 2013
Rockenstein, Brain. 2014

Anti-alpha-synuclein filament antibody [MJFR-14-6-4-2]

- Rabbit monoclonal raised against purified recombinant human a-syn filaments in collaboration between Epitomics/Abcam and P.H. Jensen lab
- Does not bind monomeric soluble a-syn



Preabsorption
1 pM MJF14 IgG + 1 µM a-syn bind a-syn aggregates

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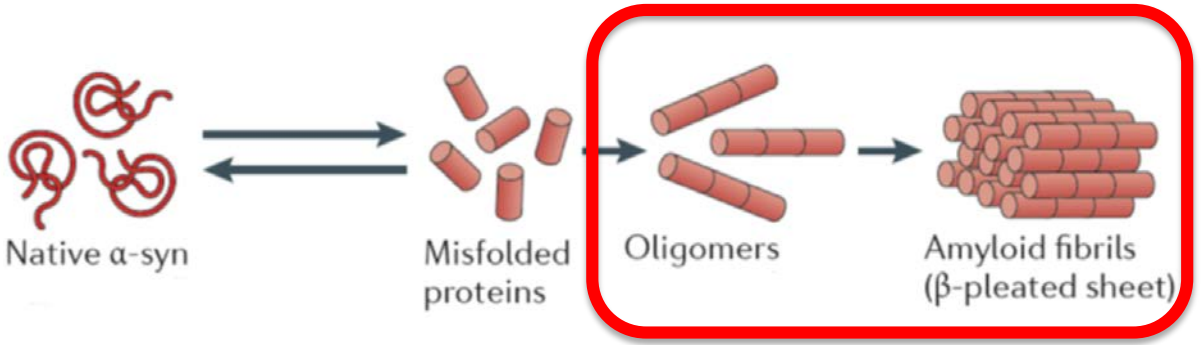
- Strong binding to oligomers
- Does not bind filaments assembled from C-terminal truncated a-syn
- Very high affinity



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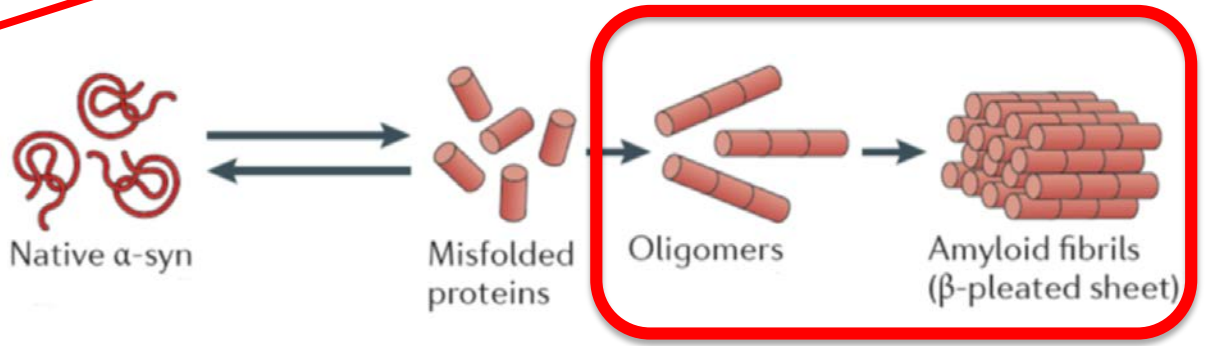
- Ver

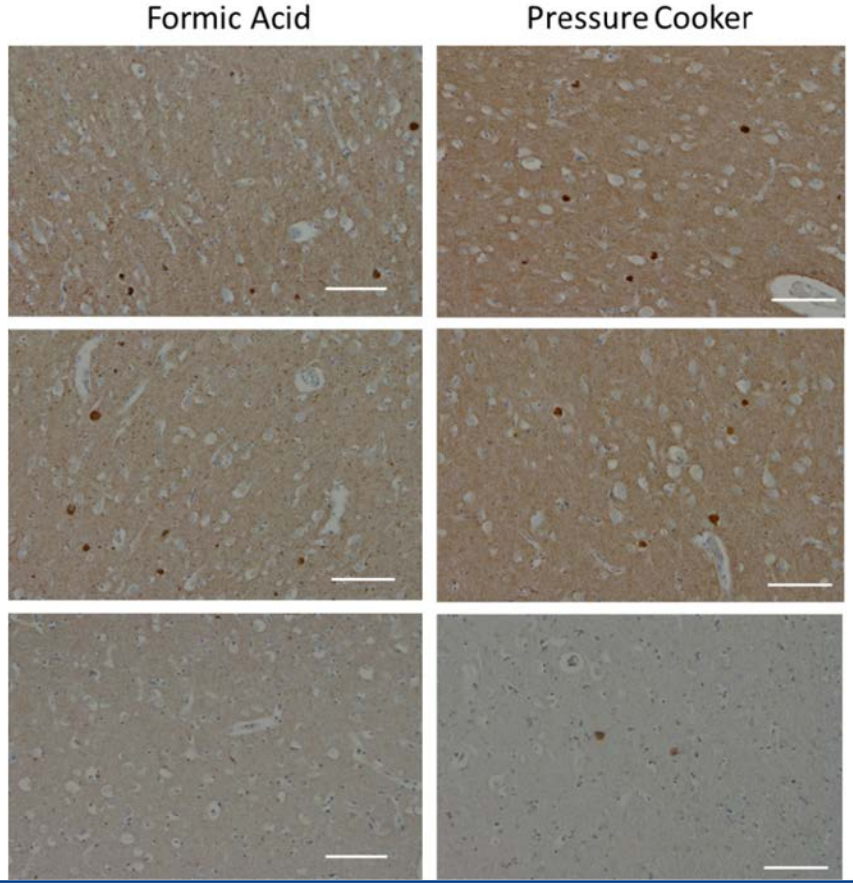
It does not work on Western blotting



MJFF funded usability study

- Biochemistry – P.H. Jensen, Aarhus, e.g. immunoprecipitation
- Immunohistochemistry – Janice Holton, London; Glenda Halliday, Sydney; Benoit Giasson, Gainesville; Laura Parkkinen and Javier Alegre Abarrategui, Oxford
- Fluid-phase/biomarker – Jing Zhang, Washington; P.H. Jensen, Aarhus

Immunohistochemical work-up

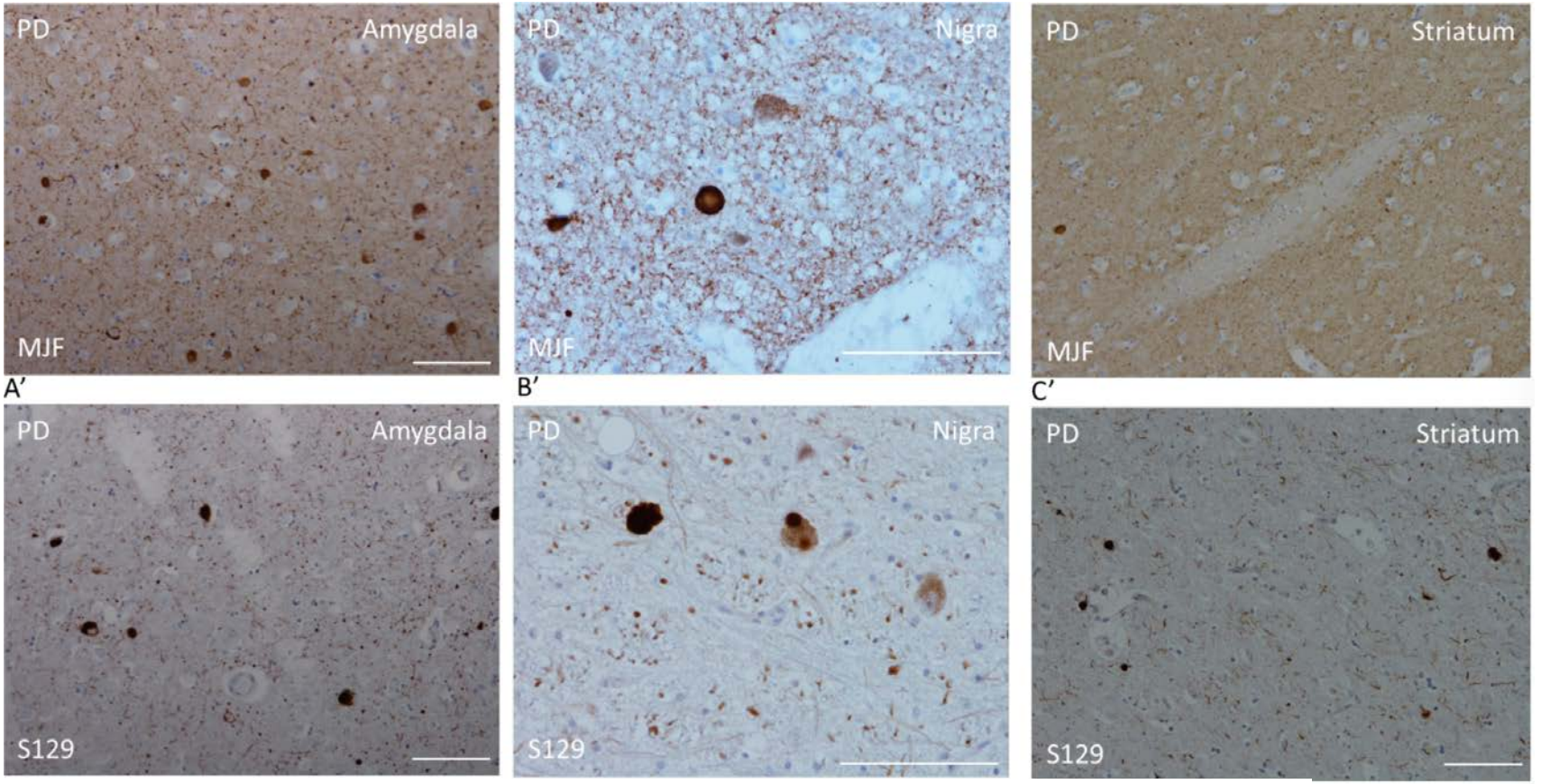


Protocols will be publicly available soon

PD cingulate cortex sections

Holton lab.

Immunohistochemical work-up



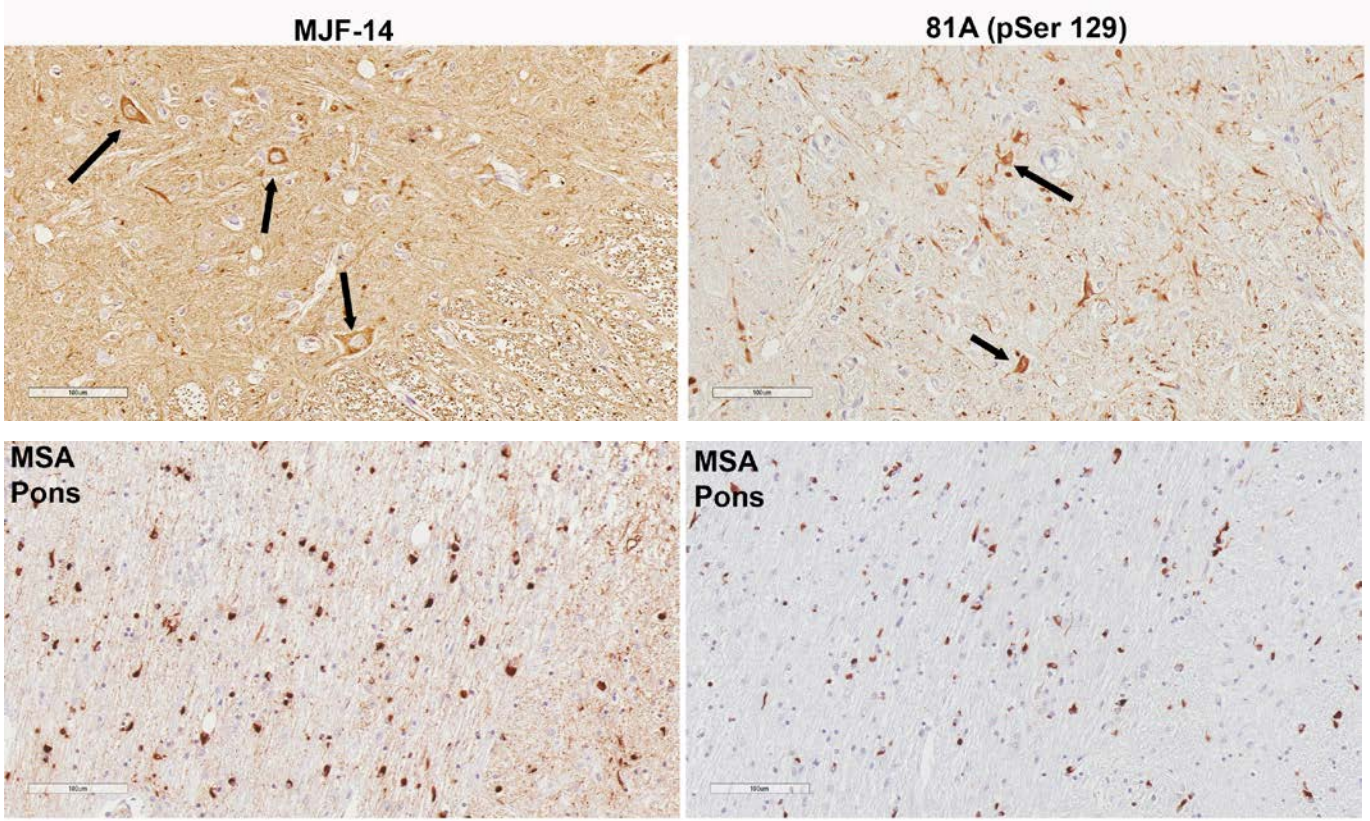
MJF-14 vs pS129 - PD nigra, amygdala and striatum

Holton lab.

Immunohistochemical work-up

M83 mouse tissue

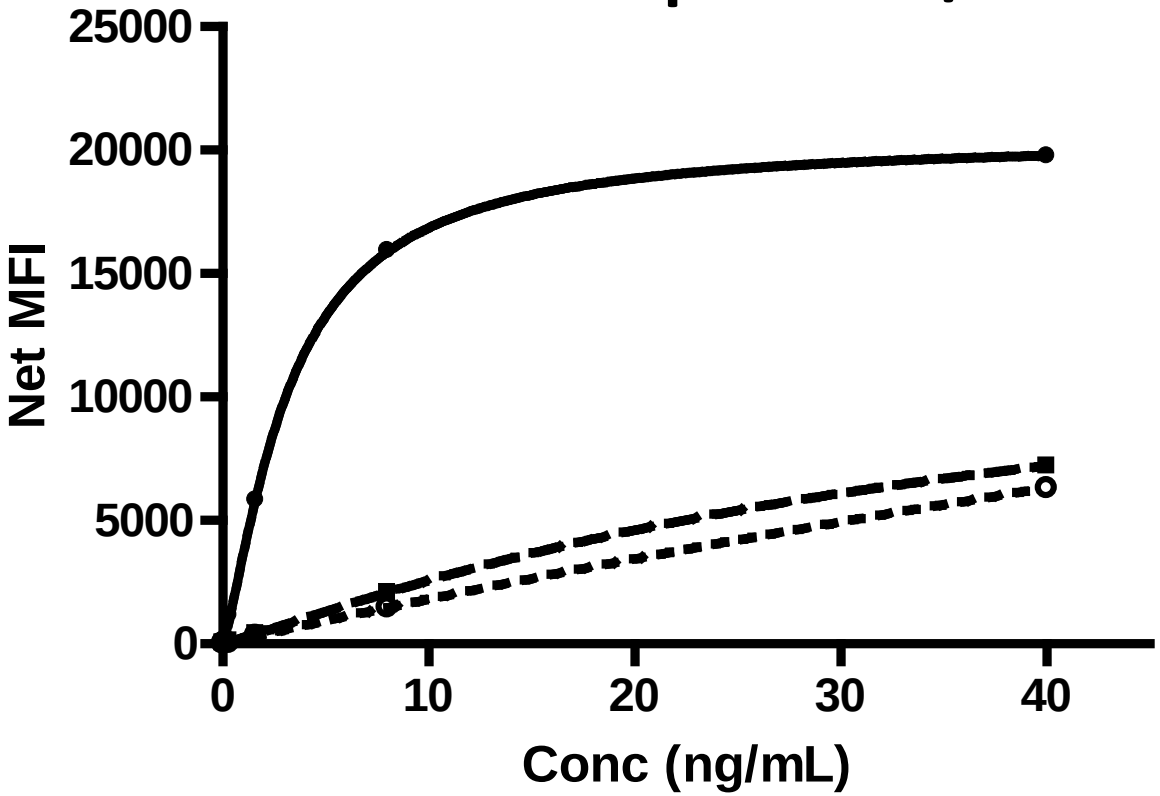
MSA Pons tissue



MJF-14 vs pS129: MSA and M83 a-syn transgenic mouse

Giasson lab.

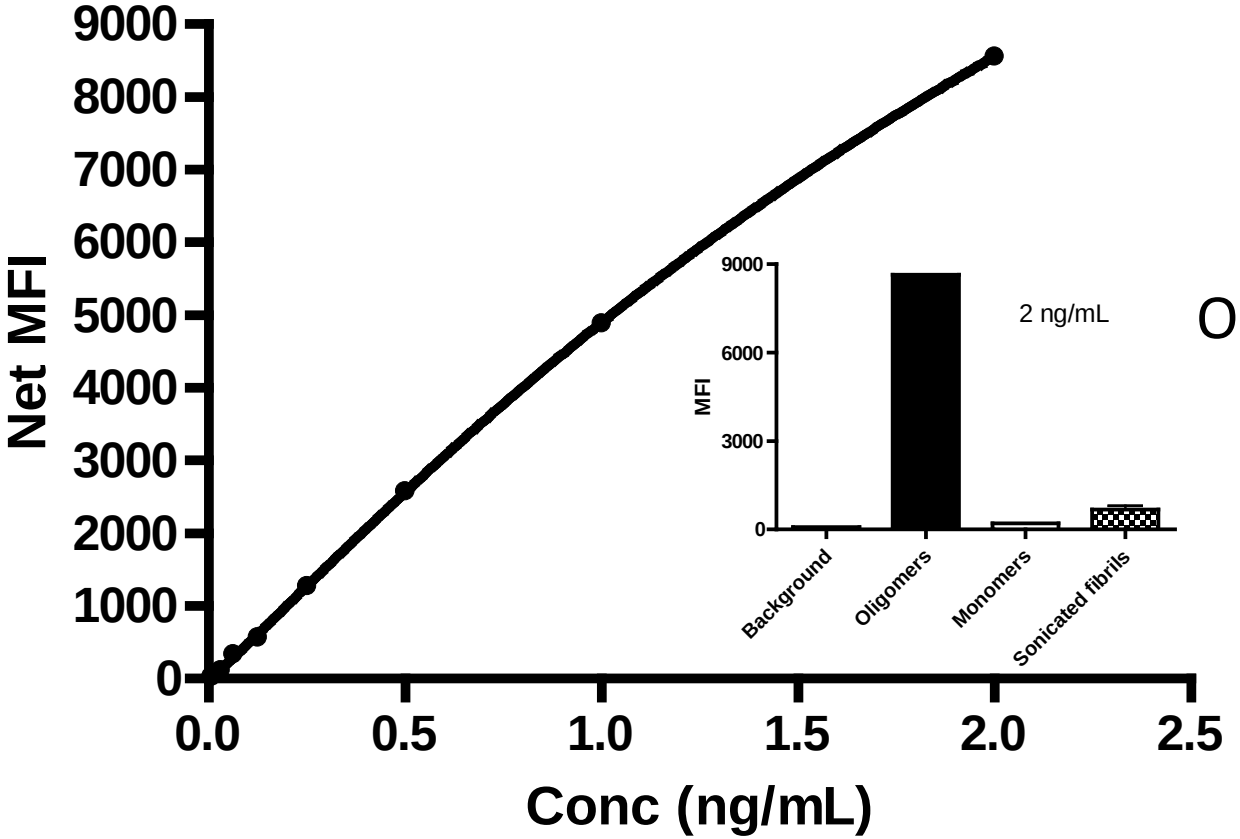
Fluid-phase/biomarker



Oligomeric α-syn Luminex

Jing Zhang lab.

Fluid-phase/biomarker



Oligomeric α-syn Luminex

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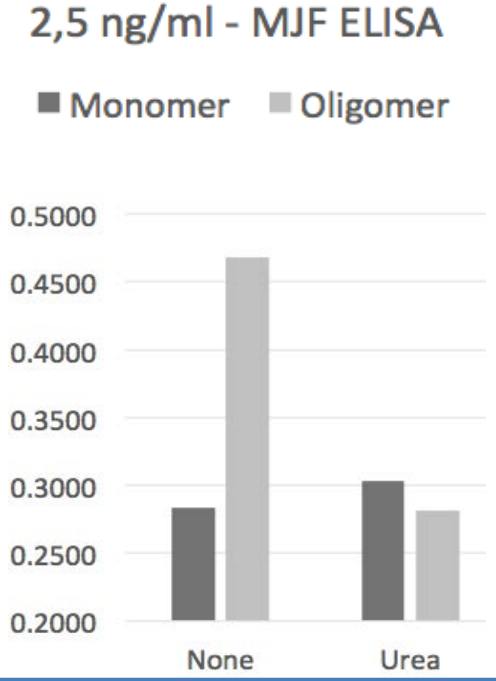
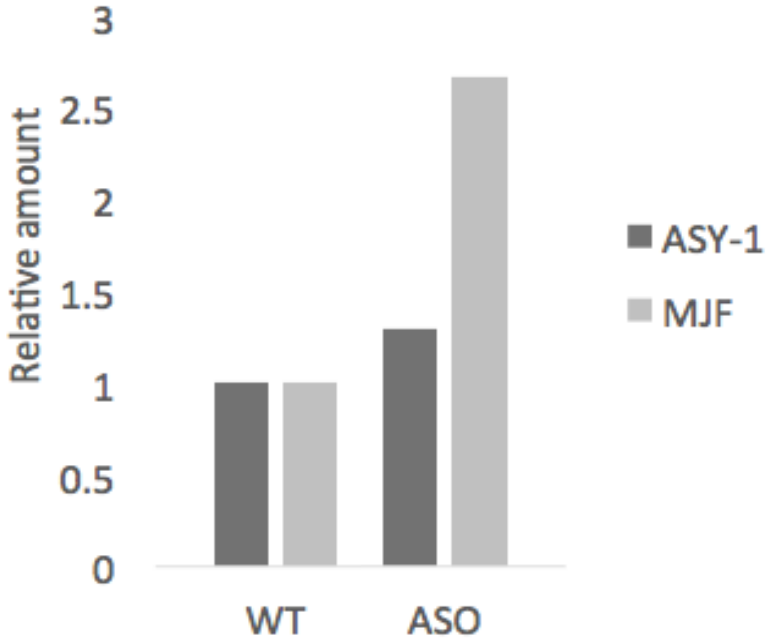
Fluid-phase/biomarker

Conformation specific binding



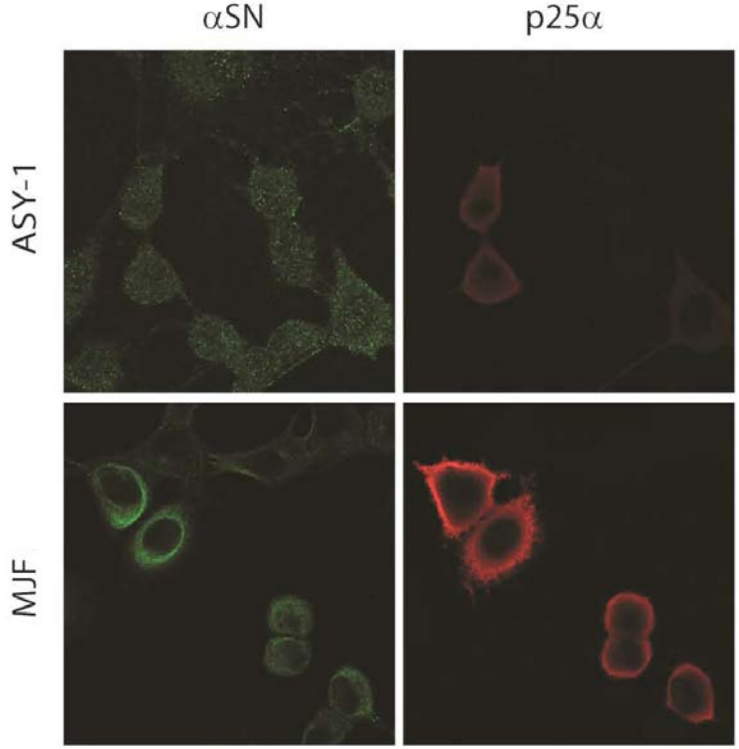
Increased MJF-14 reactivity in soluble fraction of a-synuclein transgenic mouse brain



Jensen lab.

Cell biology

Can we detect subcellular sites of a-synuclein aggregation?



OLN-AS7 Model

All cells express a-synuclein.

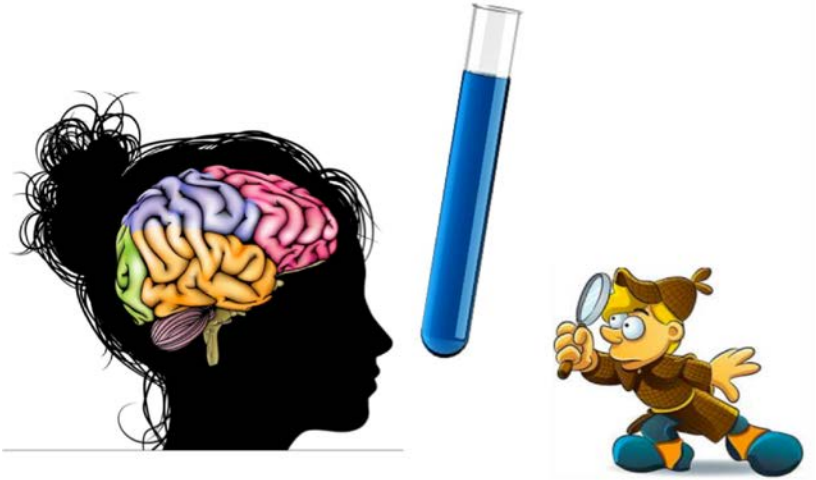
Only cells transiently expression p25a suffers from intracellular aggregation and cytotoxicity

Jensen lab.

Challenges and possibilities?

The high affinity,conformation-specific MJF-14 antibody works on multiple platforms and allows us to ask questions in relation to basic biology, disease modeling and biomarkers

- When is monomeric a-syn a ”monomer”, unfolded or folded?
- What is the native states of a-synuclein in tissue?
- Does distinct structural species exist at subcellular sites?
- Does specific anatomical and subcellular sites contribute to a-synucleinopathies?



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- Benoit Giasson, Univ. Florida
- Laura Parkkinen, Oxford Uni.
- Javier Alegre Abarrategui, Oxford Uni.

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