UTHealth
The University of Texas
Health Science Center at Houston

PMDL
Mitchell Center for
Alzheimer's disease

Protein Misfolding Diseases Lab, UTHealth

Seed Amplification Assays: From Prions to ADRDs and Parkinsonism

Claudio Soto, PhD

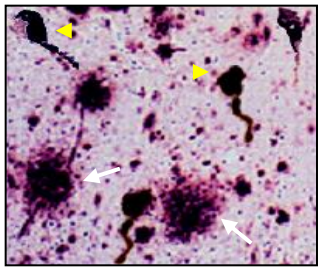
Mitchell Center for Alzheimer's disease and Related Brain Disorders
University of Texas Medical School at Houston
and Amprion Inc

Conflict of Interest Disclosure

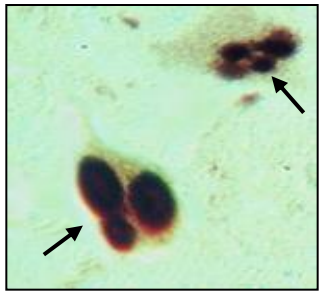
Protein Misfolding Diseases Lab, UTHealth

Dr Soto is Founder, Chief Scientific Officer, and Member of the Board of Directors of Amprion Inc., a Biotech Company which owns the license and is aiming to commercialize seed amplification assays (such as PMCA and RT-QuIC) for the detection of misfolded protein aggregates implicated in Alzheimer's, Parkinson's, Prion diseases and other neurodegenerative disorders.

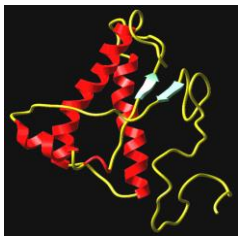
Misfolded Aggregates deposited in the brain



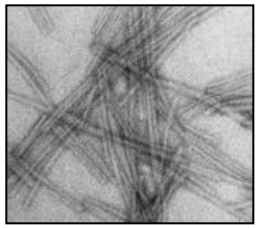
Alzheimer's disease

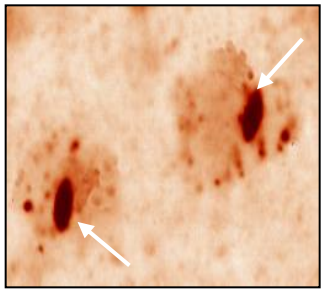


Parkinson's disease

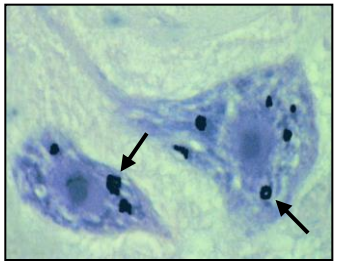


Protein Misfolding and aggregation

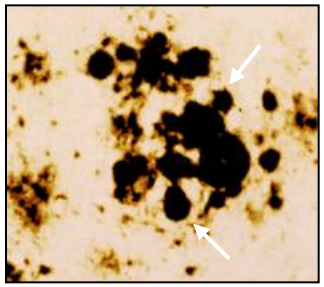




Huntington's disease



Amyothropic lateral sclerosis



Prion diseases

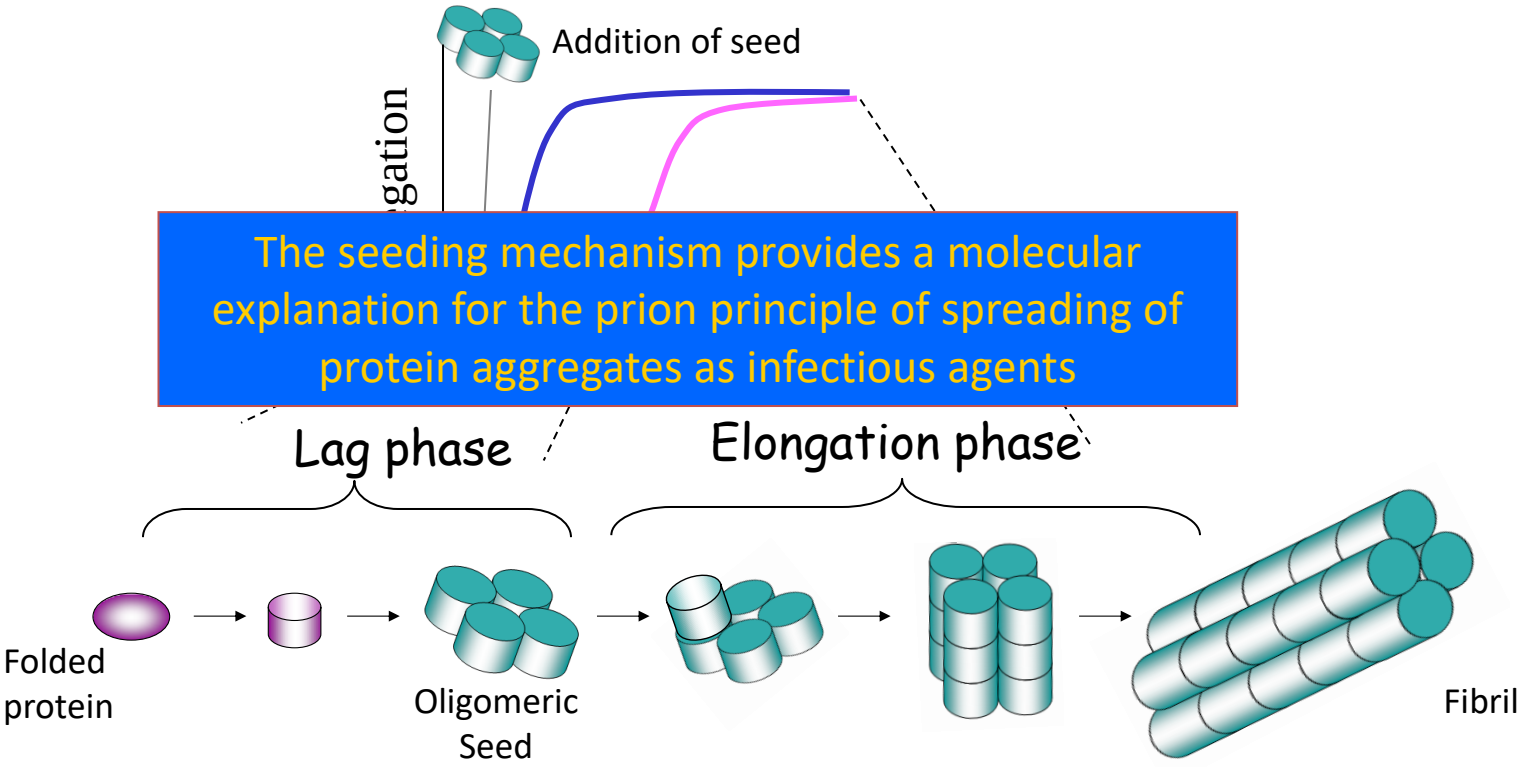
Soto (2003) Nature Rev Neurosci. 4:49-60

How is a Protein Infectious?: The Prion Principle

The prion principle posits that pathological changes can spread faithfully between cells and self-perpetuate by the auto-catalytic propagation of misfolded protein aggregates

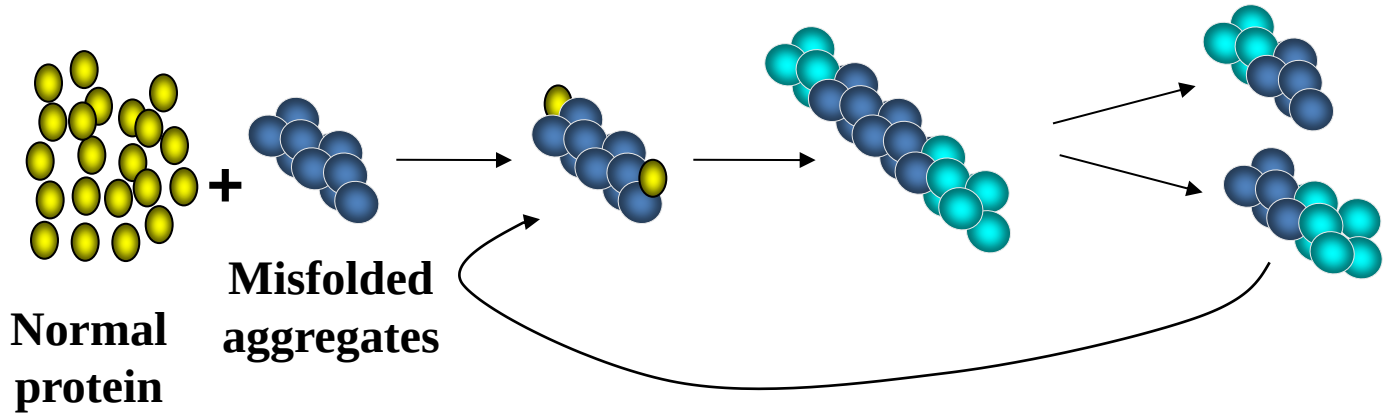
This mechanism operates to transmit diseases in an infectious manner and to spread disease pathogenesis among cells and tissues during the progression of the disease. The prion principle may be at the root of some of the most prevalent and incurable diseases of our time, including Alzheimer's, Parkinson's diseases, diabetes and cancer.

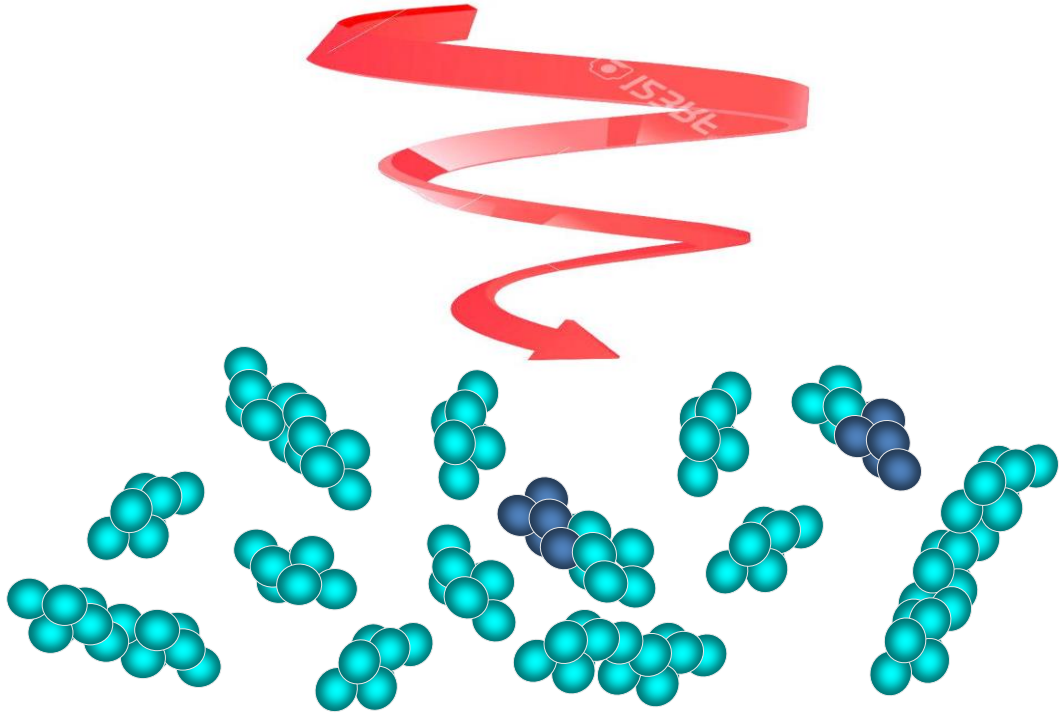
Mechanism of protein misfolding



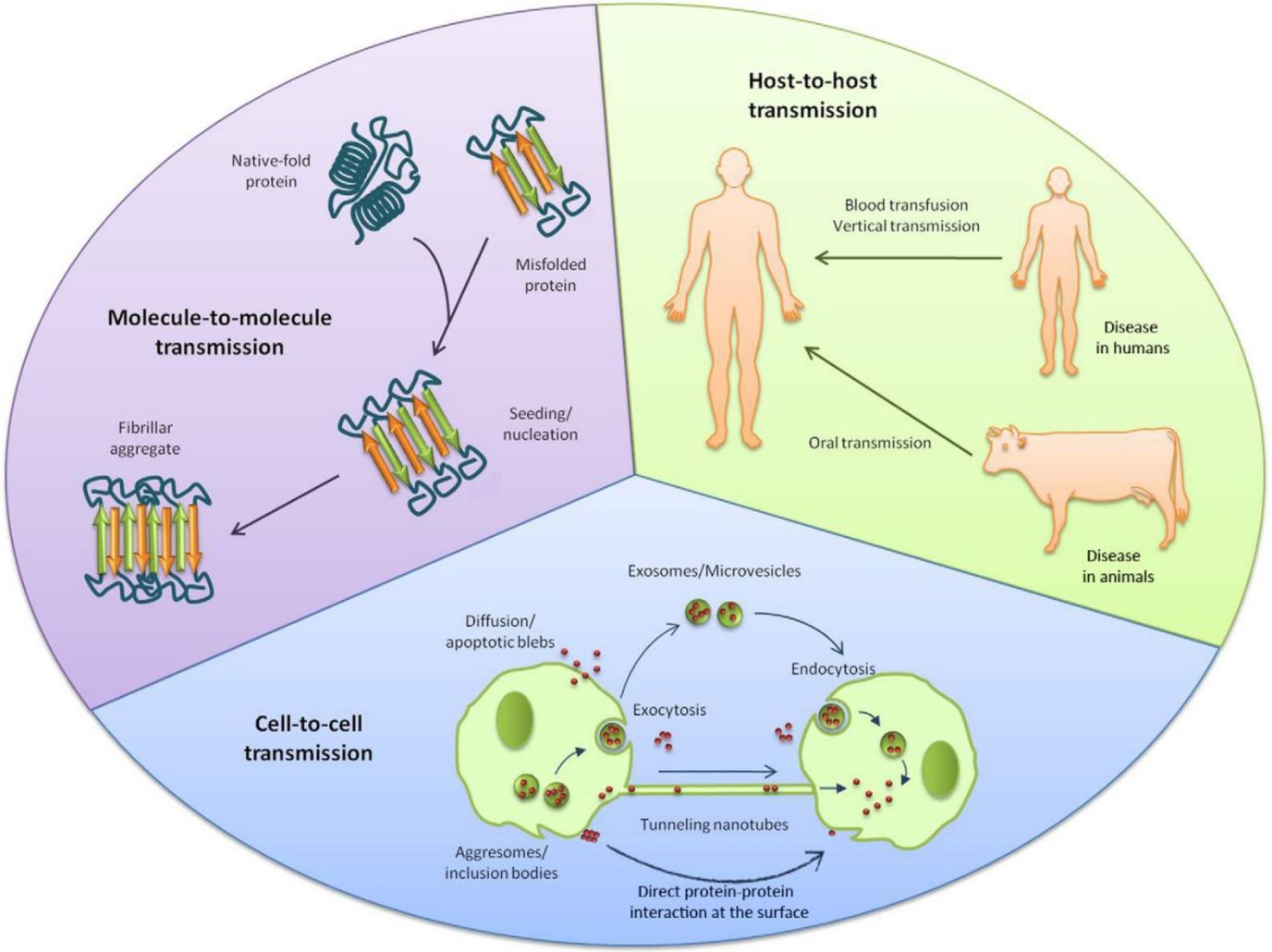
Soto, Estrada, Castilla (2006) TIBS 31: 150-155

Molecular basis of the Prion principle





The Prion Principle Operates at Different Levels



Moreno-Gonzalez and Soto (2011)
Sem Dev Biol 22: 482-487

Reproducing the Prion Principle in vitro

Implementation of a procedure to "cultivate" prions by mimicking in vivo prion replication with high efficiency in the test tube

Availability of such a technology will enable to study the biochemical characteristics of the infectious agent and the mechanism of prion propagation.

letters to nature

NATURE | VOL 411 | 14 JUNE 2001 | www.nature.com

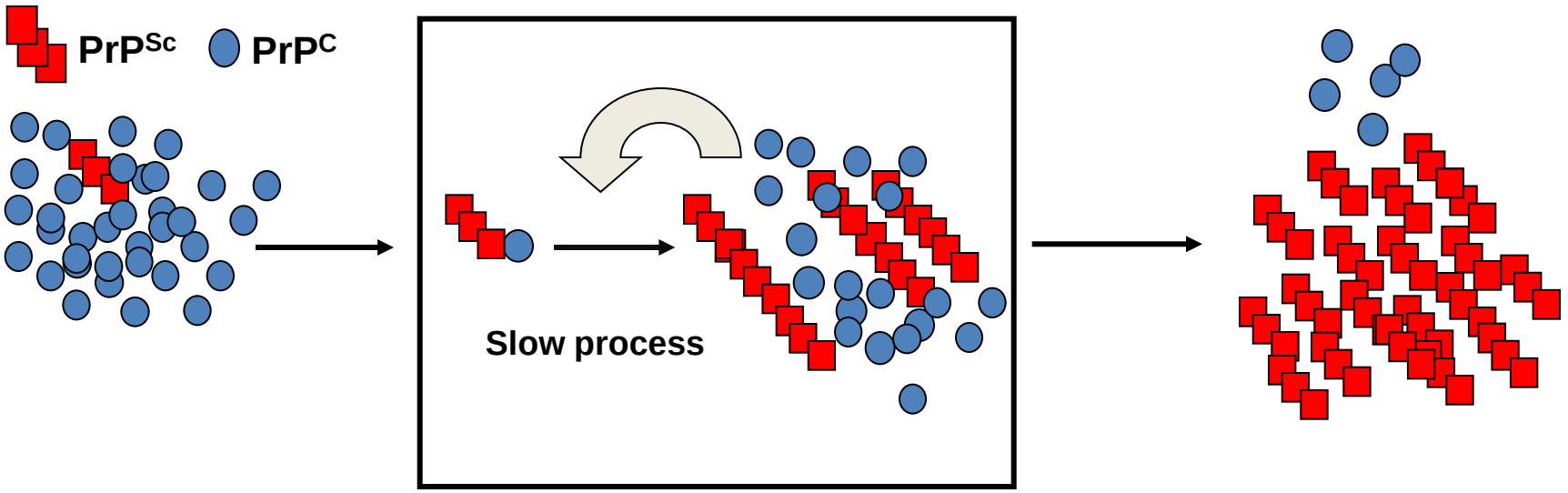
nature

Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding

Gabriela P. Saborio, Bruno Permanne & Claudio Soto

Serono Pharmaceutical Research Institute, CH1228 Geneva, Switzerland

Prion replication during disease propagation



Infection

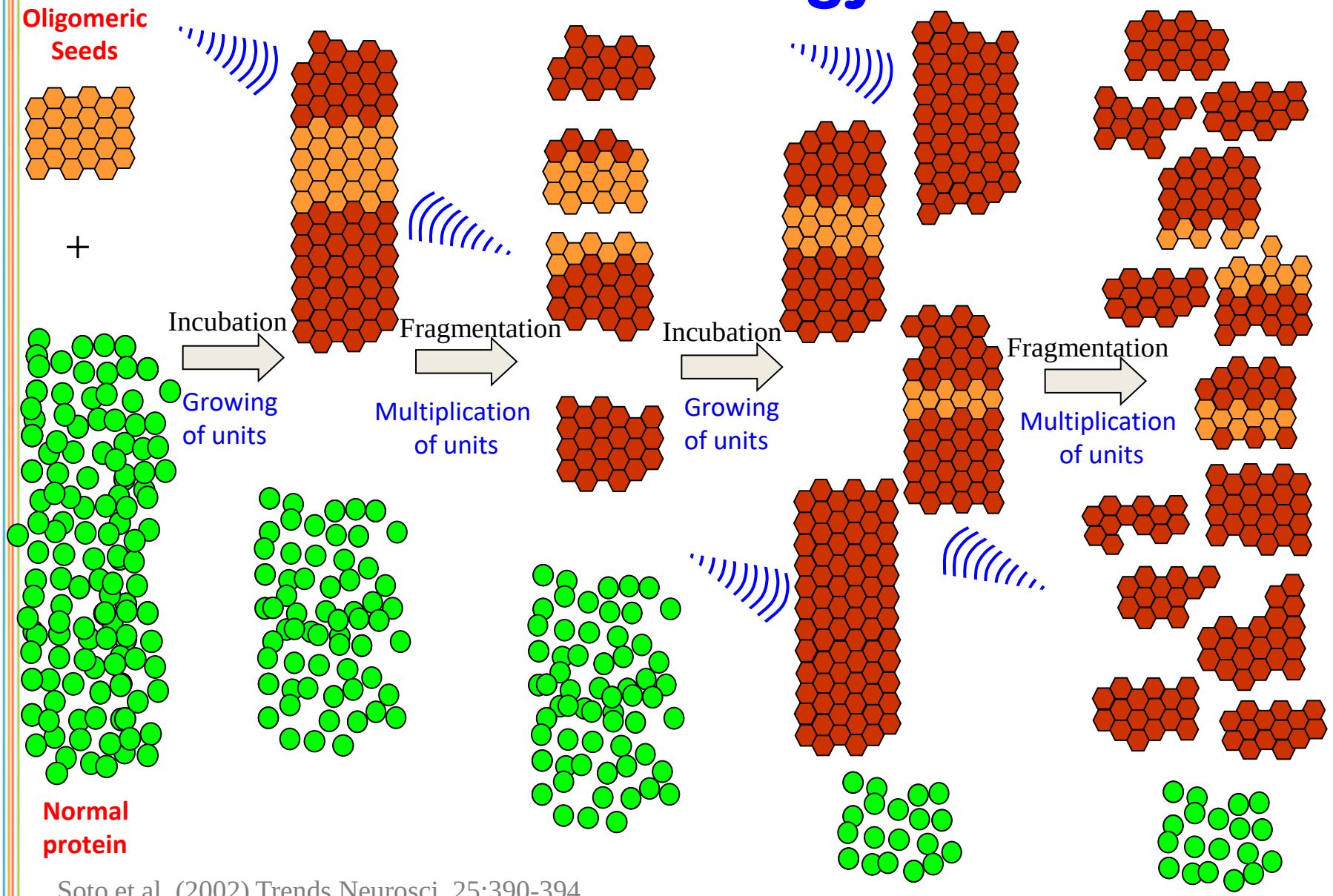
60 - 120 d

3 - 5 y

7 - 40 y

Clinical symptoms

The Protein Misfolding Cyclic Amplification Technology



Soto et al. (2002) Trends Neurosci. 25:390-394

Application of PMCA to understand prion biology

Cell, Vol. 121, 195–206, April 22, 2005, Copyright ©2005 by Elsevier Inc.

Cell

In Vitro Generation of Infectious Scrapie Prions

Joaquín Castilla,[1] Paula Saá,[1,2] Claudio Hetz,[1,3] and Claudio Soto[1,*]

May 2009 | Volume 5 | Issue 5 | e1000421 PLoS PATHOGENS

De Novo Generation of Infectious Prions *In Vitro* Produces a New Disease Phenotype

Marcelo A. Barria[1,2], Abhisek Mukherjee[1,2], Dennisse Gonzalez-Romero[1,2], Rodrigo Morales[1,2,3], Claudio Soto[1,2*]

The EMBO Journal (2008) 27, 2557–2566 | © 2008 European Molecular Biology Organization

Cell-free propagation of prion strains

Joaquín Castilla[1,5], Rodrigo Morales[1,2,5], Paula Saá[1,3], Marcelo Barria[1], Pierluigi Gambetti[4] and Claudio Soto[1,*]

THE EMBO JOURNAL

Cell *134*, 757–768, September 5, 2008 ©2008 Elsevier Inc.

Cell

Crossing the Species Barrier by PrP^{Sc} Replication In Vitro Generates Unique Infectious Prions

Joaquín Castilla,[1] Dennisse Gonzalez-Romero,[1,4] Paula Saá,[1,2,4] Rodrigo Morales,[1,3] Jorge De Castro,[1] and Claudio Soto[1,*]

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 9, pp. 7490–7495, March 4, 2011

Generation of a New Form of Human PrP^{Sc} *in Vitro* by Interspecies Transmission from Cervid Prions*

Received for publication, October 28, 2010, and in revised form, December 27, 2010 Published, JBC Papers in Press, January 5, 2011,

Marcelo A. Barria‡, Glenn C. Telling§, Pierluigi Gambetti¶, James A. Mastrianni‖, and Claudio Soto‡1

FEBS Letters 584 (2010) 2409–2414

ELSEVIER

FEBS Letters

journal homepage: www.FEBSLetters.org

Cellular factors implicated in prion replication

Karim Abid[a,1], Rodrigo Morales[a,b], Claudio Soto[a,b,*]

Cell Reports *11*, 1168–1175, May 26, 2015

Cell Reports

Grass Plants Bind, Retain, Uptake, and Transport Infectious Prions

Sandra Pritzkow,[1] Rodrigo Morales,[1] Fabio Moda,[1,3] Uffaf Khan,[1] Glenn C. Telling,[2] Edward Hoover,[2] and Claudio Soto[1,*]

Sensitive detection of prions by PMCA

letters to nature

NATURE | VOL 411 | 14 JUNE 2001 | www.nature.com nature

Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding

Gabriela P. Saborio, Bruno Permanne & Claudio Soto

LETTERS nature medicine

VOLUME 11 | NUMBER 9 | SEPTEMBER 2005 NATURE MEDICINE

Detection of prions in blood

Joaquín Castilla[1], Paula Saá[1,2] & Claudio Soto[1]

nature methods | VOL.7 NO.7 | JULY 2010

Estimating prion concentration in fluids and tissues by quantitative PMCA

Baian Chen[1,2], Rodrigo Morales[1], Marcelo A Barria[1] & Claudio Soto[1]

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

7 JULY 2006 VOL 313 Science

Presymptomatic Detection of Prions in Blood

Paula Saá,[1,2] Joaquín Castilla,[1] Claudio Soto[1*]

The NEW ENGLAND JOURNAL of MEDICINE AUGUST 7, 2014

ORIGINAL ARTICLE

Prions in the Urine of Patients with Variant Creutzfeldt–Jakob Disease

Fabio Moda, Ph.D., Pierluigi Gambetti, M.D., Silvio Notari, Ph.D., Luis Concha-Marambio, B.Sc., Marcella Catania, Ph.D., Kyung-Won Park, Ph.D., Emanuela Maderna, B.Sc., Silvia Suardi, B.Sc., Stéphane Haïk, M.D., Ph.D., Jean-Philippe Brandel, M.D., James Ironside, M.D., Richard Knight, M.D., Fabrizio Tagliavini, M.D., and Claudio Soto, Ph.D.

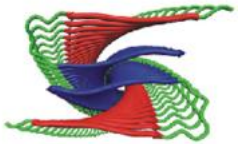
PRION DISEASES Concha-Marambio *et al.*, *Sci. Transl. Med.* **8**, 370ra183 (2016) 21 December 2016

Detection of prions in blood from patients with variant Creutzfeldt-Jakob disease

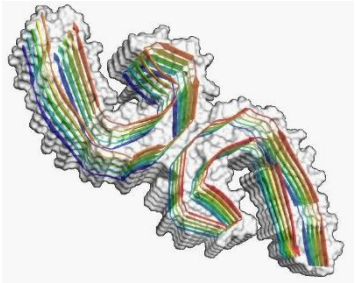
Luis Concha-Marambio,[1,2] Sandra Pritzkow,[1] Fabio Moda,[1,3] Fabrizio Tagliavini,[3] James W. Ironside,[4] Paul E. Schulz,[1] Claudio Soto[1,2]*

Science Translational Medicine AAAS

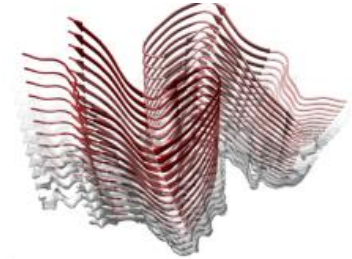
Misfolded proteins in human brains



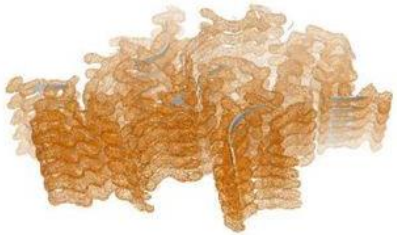
Amyloid-beta (Aβ) — **Alzheimer's disease**



Tau — **Alzheimer's disease and other tauopathies (FTD, PSP, CBD, CTE, Pick's disease)**



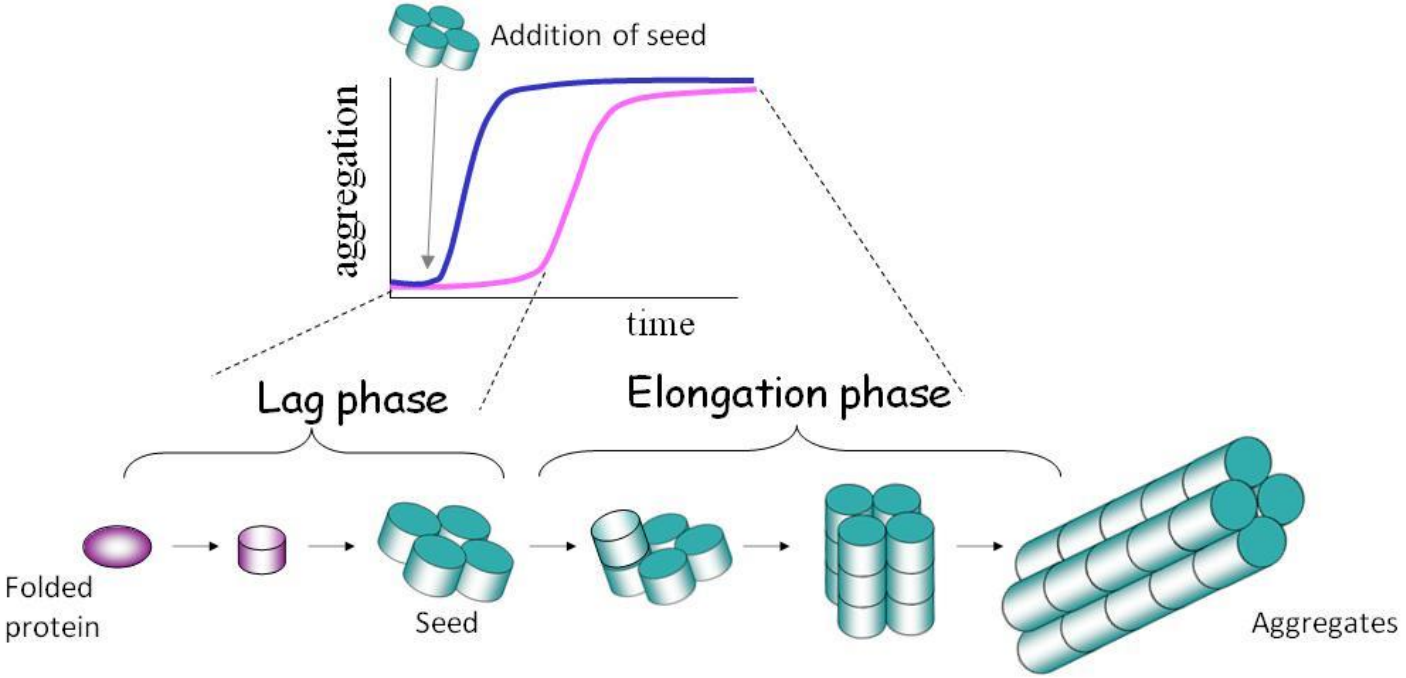
α-synuclein — **Parkinson's disease and other synucleinopathies (DLB, MSA, PDD)**



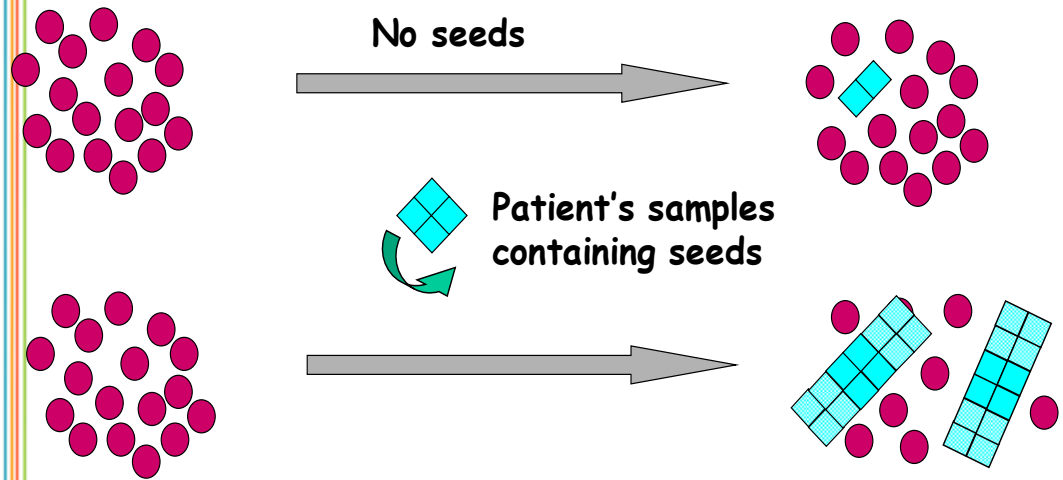
TDP-43 — **Amyothrophic lateral sclerosis, fronto-temporal dementia, limbic-predominant age-related TDP-43 encephalopathy**

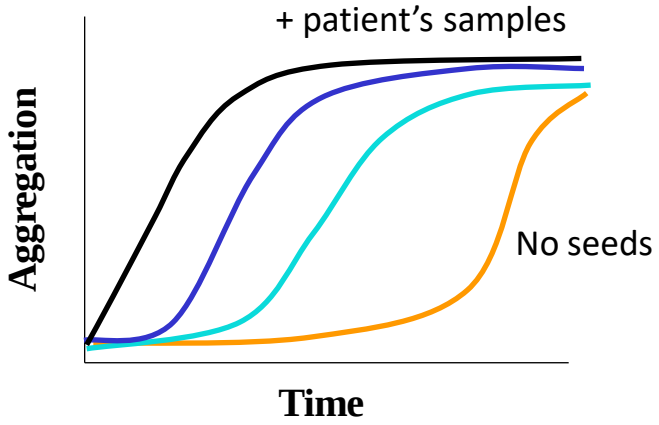
In addition, other misfolded proteins are found in some rare diseases, such as PrP, huntingtin, SOD-1, ataxin, etc

Our strategy for high sensitive detection

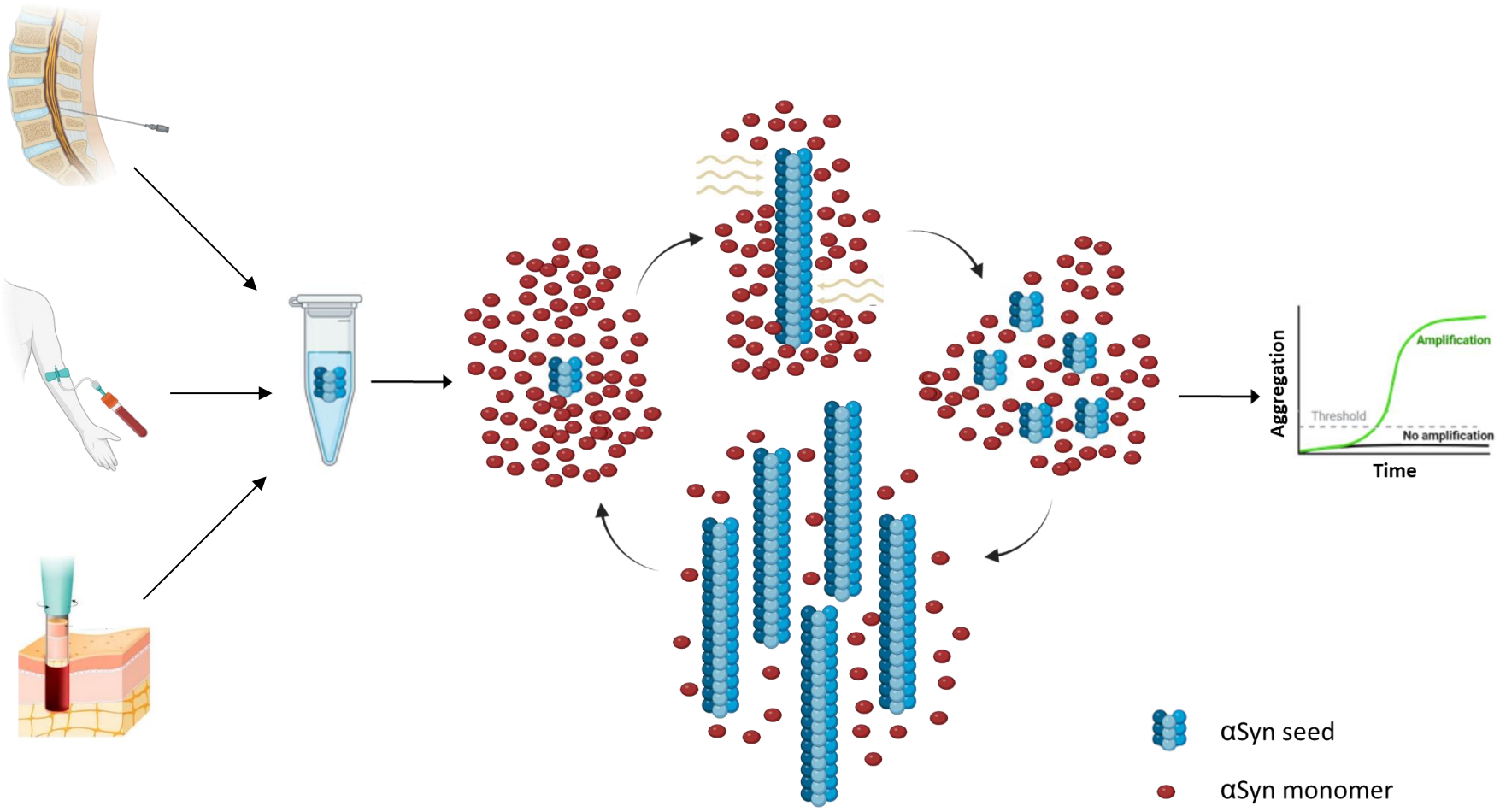


Our strategy is to use the ability of misfolded oligomers to seed polymerization of monomeric protein to enable their high sensitivity detection.





Seed Amplification Assay (aka PMCA or RT-QuIC)



Soto et al. (2024) Trends Biotech. 42(7):829-841

JAMA Neurology

JAMA Neurology | Original Investigation

JAMA Neurol. doi:10.1001/jamaneurol.2016.4547
Published online December 5, 2016.

Development of a Biochemical Diagnosis of Parkinson Disease by Detection of α-Synuclein Misfolded Aggregates in Cerebrospinal Fluid

Mohammad Shahnawaz, PhD; Takahiko Tokuda, MD; Masaaki Waragai, MD; Nicolas Mendez, BSc; Ryotaro Ishii, MD; Claudia Trenkwalder, MD; Brit Mollenhauer, MD; Claudio Soto, PhD

Movement Disorders, 2019

Movement Disorders
Official Journal of the International Parkinson and Movement Disorder Society

Comparative Study of Cerebrospinal Fluid α-Synuclein Seeding Aggregation Assays for Diagnosis of Parkinson's Disease

Un Jung Kang, MD,[1*] Amelia K. Boehme, PhD,[1] Graham Fairfoul, BS,[2] Mohammad Shahnawaz, PhD,[3] Thong Chi Ma, PhD,[1] Samantha J. Hutten, PhD,[4] Alison Green, PhD,[2] and Claudio Soto, PhD[3]

nature

Article

Nature | Vol 578 | 13 February 2020 | 273

Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy

Mohammad Shahnawaz[1], Abhisek Mukherjee[1], Sandra Pritzkow[1,6], Nicolas Mendez[1,6], Prakruti Rabadia[1], Xiangan Liu[2], Bo Hu[2], Ann Schmeichel[3], Wolfgang Singer[3], Gang Wu[4], Ah-Lim Tsai[4], Hamid Shirani[5], K. Peter R. Nilsson[5], Phillip A. Low[3] & Claudio Soto[1*]

Annals of NEUROLOGY

RESEARCH ARTICLE

ANN NEUROL 2020;88:503–512

Alpha-Synuclein Oligomers and Neurofilament Light Chain in Spinal Fluid Differentiate Multiple System Atrophy from Lewy Body Synucleinopathies

Wolfgang Singer, MD,[1] Ann M. Schmeichel,[1] Mohammad Shahnawaz, PhD,[2] James D. Schmelzer,[1] Bradley F. Boeve, MD,[1] David M. Sletten,[1] Tonette L. Gehrking,[1] Jade A. Gehrking,[1] Anita D. Olson,[1] Rodolfo Savica, MD, PhD,[1] Mariana D. Suarez,[1] Claudio Soto, PhD,[2] and Phillip A. Low, MD[1]

Movement Disorders
Official Journal of the International Parkinson and Movement Disorder Society

Movement Disorders, Vol. 38, No. 4, 2023

Accurate Detection of α-Synuclein Seeds in Cerebrospinal Fluid from Isolated Rapid Eye Movement Sleep Behavior Disorder and Patients with Parkinson's Disease in the DeNovo Parkinson (DeNoPa) Cohort

Luis Concha-Marambio, PhD,[1] Sandrina Weber, MD,[2,3] Carly M. Farris, MSc,[1] Mohammed Dakna, PhD,[2] Elisabeth Lang, BSc,[3] Tamara Wicke, MSc,[3] Yihua Ma, MSc,[1] Maritta Starke,[3] Jens Ebentheuer, MD,[3] Friederike Sixel-Döring, MD,[3,4] Maria-Lucia Muntean, MD,[3] Sebastian Schade, MD,[3] Claudia Trenkwalder, MD,[3,5] Claudio Soto, PhD,[1,6] and Brit Mollenhauer, MD[2,3*]

THE LANCET
Neurology

www.thelancet.com/neurology Vol 22 May 2023

nature protocols

NATURE PROTOCOLS | VOL 18 | APRIL 2023 | 1179–1196 | www.nature.com/nprot

Seed amplification assay for the detection of pathologic alpha-synuclein aggregates in cerebrospinal fluid

Luis Concha-Marambio[1], Sandra Pritzkow[2], Mohammad Shahnawaz[2], Carly M. Farris[1] and Claudio Soto[1,2]✉

Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study

Andrew Siderowf, Luis Concha-Marambio*, David-Erick Lafontant, Carly M Farris, Yihua Ma, Paula A Urenia, Hieu Nguyen, Roy N Alcalay, Lana M Chahine, Tatiana Foroud, Douglas Galasko, Karl Kieburtz, Kalpana Merchant, Brit Mollenhauer, Kathleen L Poston, John Seibyl, Tanya Simuni, Caroline M Tanner, Daniel Weintraub, Aleksandar Videnovic, Seung Ho Choi, Ryan Kurth, Chelsea Caspell-Garcia, Christopher S Coffey, Mark Frasier, Luis M A Oliveira, Samantha J Hutten, Todd Sherer, Kenneth Marek, Claudio Soto, on behalf of the Parkinson's Progression Markers Initiative†*

Current status of αSyn-SAA in CSF samples

JAMA Neurology | Original Investigation

JAMA Neurol. doi:10.1001/jamaneurol.2016.4547
Published online December 5, 2016.

Development of a Biochemical Diagnosis of Parkinson Disease by Detection of α-Synuclein Misfolded Aggregates in Cerebrospinal Fluid

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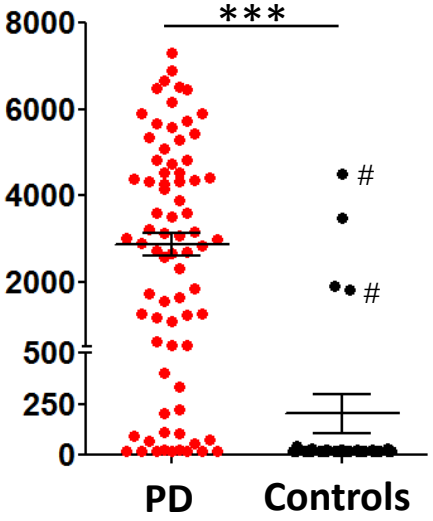

Sensitivity, Specificity and predictive value for αSyn-PMCA in CSF samples

Parameter	Value	95% confidence intervals
Sensitivity for PD	88.5%	79.2 – 94.6%
Sensitivity for DLB	100.0%	94.9-100.0%
Sensitivity for MSA	80%	79.5-94.6%
Specificity against disease controls	96.9%	89.3-99.6%
Specificity against controls and neurodegenerative diseases	94.0%	86.5-98.0%
Positive predictive value	94.7%	88.0-98.3%
Negative predictive value	87.6%	78.7-93.7%

Large study of αSyn-SAA accuracy in CSF

THE LANCET
Neurology

www.thelancet.com/neurology Vol 22 May 2023

Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study

Andrew Siderowf, Luis Concha-Marambio*, David-Erick Lafontant, Carly M Farris, Yihua Ma, Paula A Urenia, Hieu Nguyen, Roy N Alcalay, Lana M Chahine, Tatiana Foroud, Douglas Galasko, Karl Kieburtz, Kalpana Merchant, Brit Mollenhauer, Kathleen L Poston, John Seibyl, Tanya Simuni, Caroline M Tanner, Daniel Weintraub, Aleksandar Videnovic, Seung Ho Choi, Ryan Kurth, Chelsea Caspell-Garcia, Christopher S Coffey, Mark Frasier, Luis M A Oliveira, Samantha J Hutten, Todd Sherer, Kenneth Marek, Claudio Soto, on behalf of the Parkinson's Progression Markers Initiative†*

Groups	Number of patients	Sensitivity (95% CI)
All PD cases	558	87.8% (85.1 – 90.5)
Sporadic PD	374	93.3% (90.8 – 95.8)
LRRK2 PD	123	67.5% (59.2 – 75.8)
LRKK2 PD (hyposmics)	69	89.9% (82.7 – 97.0)
GBA PD	49	95.9% (90.4 – 100)

Specificity for healthy controls (N = 163) was 96.3% (93.4 – 99.2)

Preclinical detection of αSyn aggregates

Movement Disorders
Official Journal of the International Parkinson and Movement Disorder Society

Movement Disorders, Vol. 38, No. 4, 2023

Accurate Detection of α-Synuclein Seeds in Cerebrospinal Fluid from Isolated Rapid Eye Movement Sleep Behavior Disorder and Patients with Parkinson's Disease in the DeNovo Parkinson (DeNoPa) Cohort

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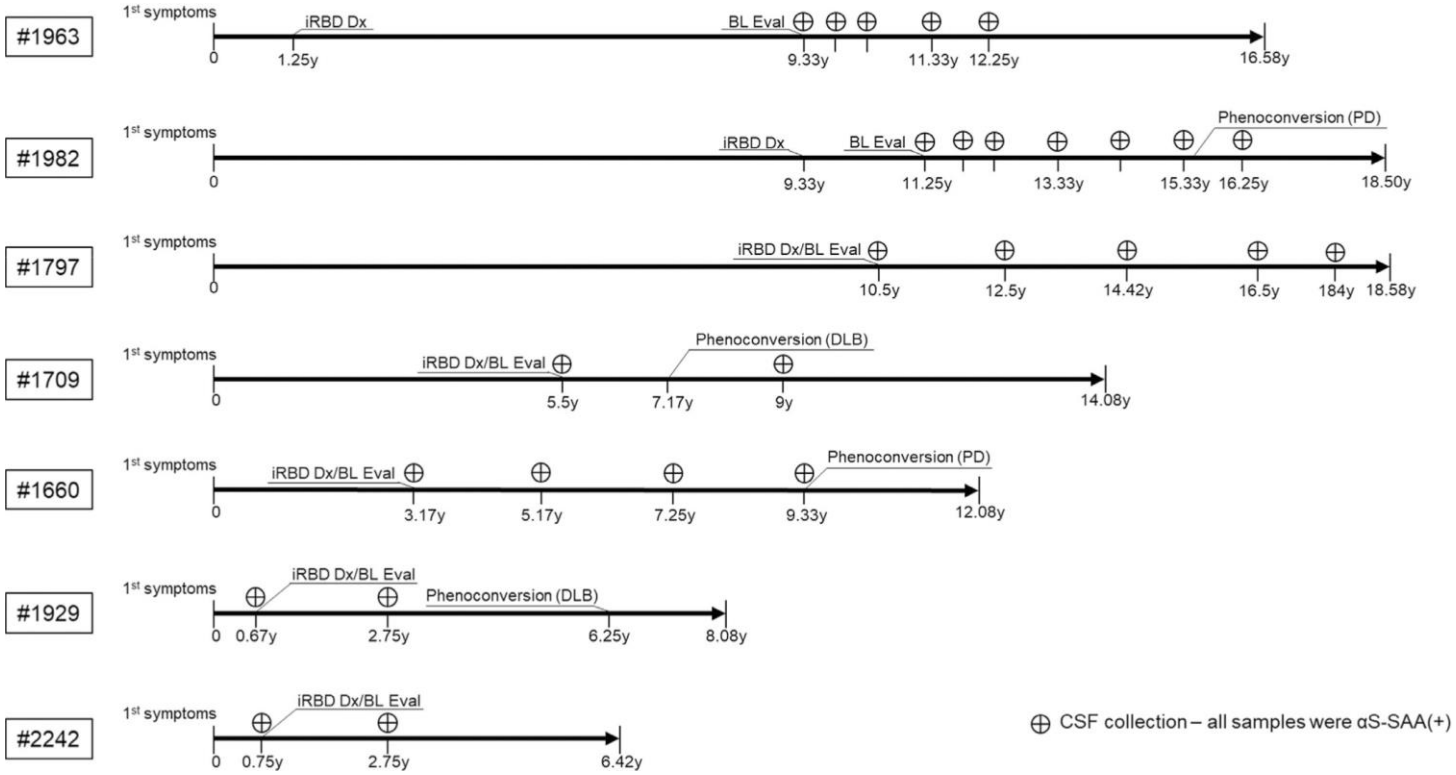
SAA+ before phenoconversion



4.08 years

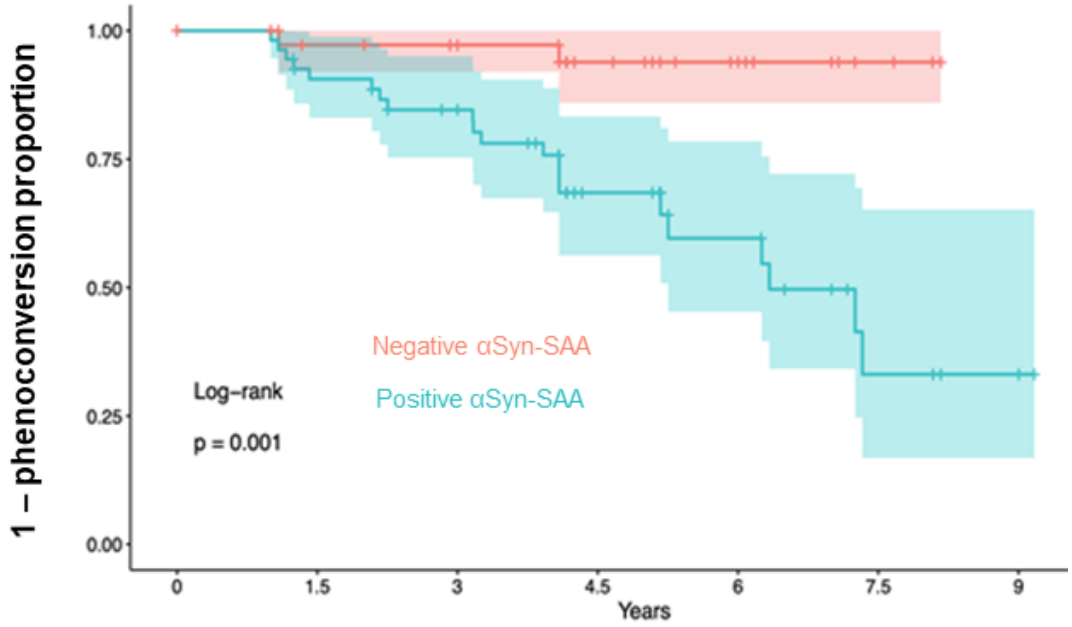
1.67 years

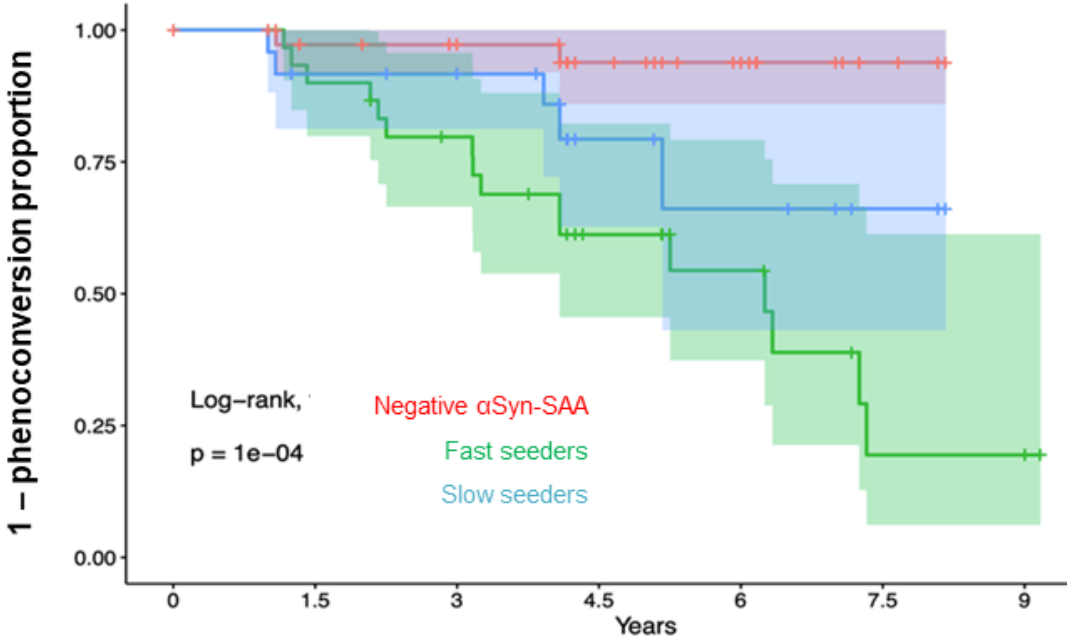
6.16 years

5.58 years

Protein Misfolding Diseases Lab, UTHealth

Predicting prodromal phenoconversion to PD

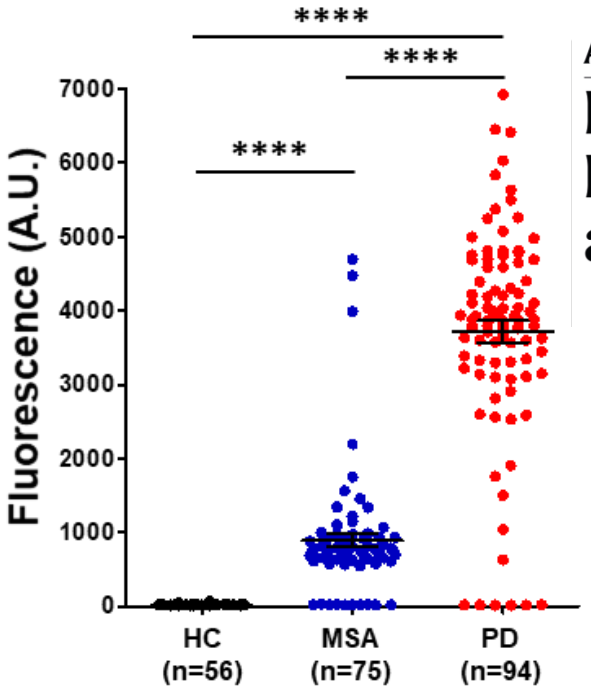




Coughlin et al Neurology (In press)

Protein Misfolding Diseases Lab, UTHealth

Distinguishing PD and MSA by αSyn-SAA



Article

nature

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Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy

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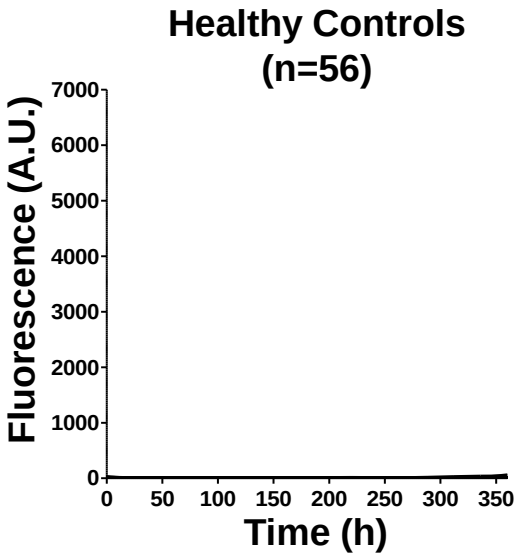
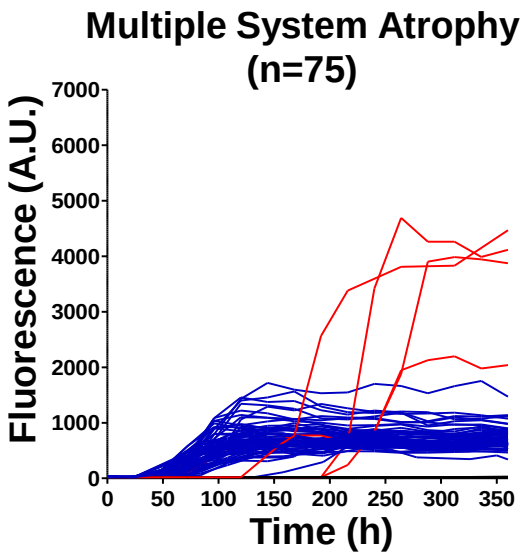
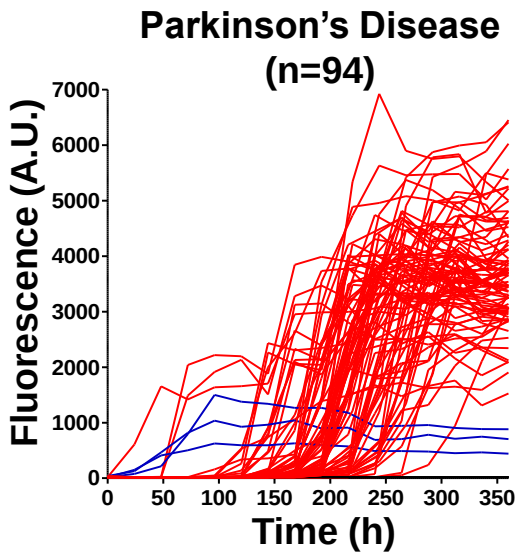
Comparison	Sensitivity (%)
PD vs healthy controls	93.6
MSA vs healthy controls	84.6
PD vs MSA	95.4







Shahnawaz et al. (2020) Nature 578:273-277

Fast αSyn-SAA in CSF samples

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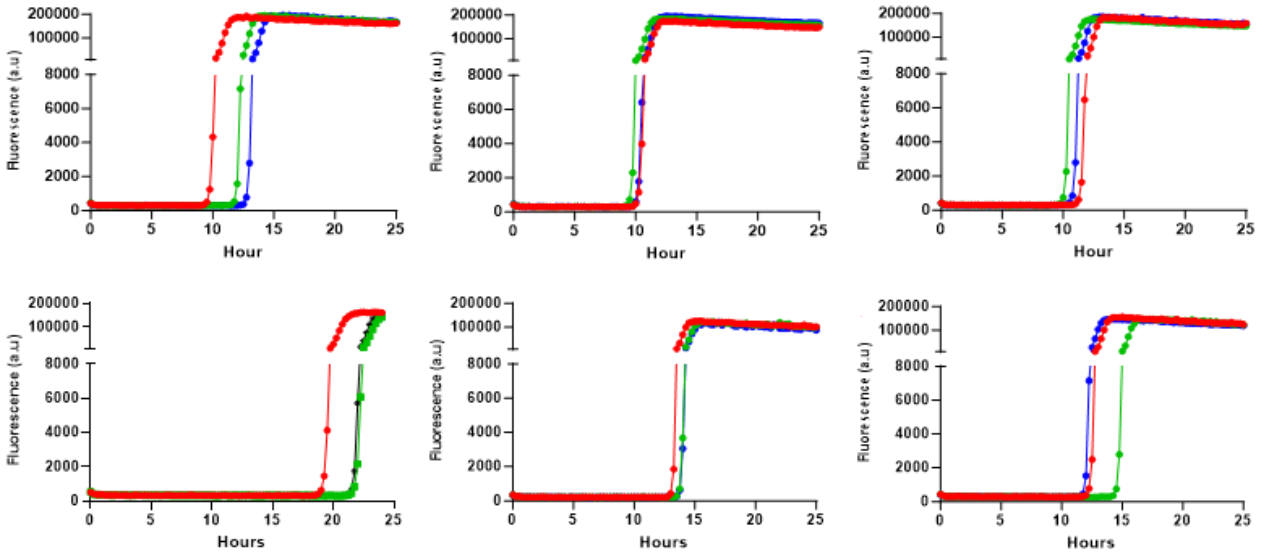
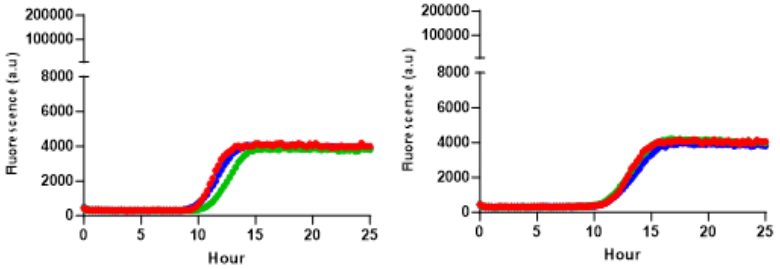
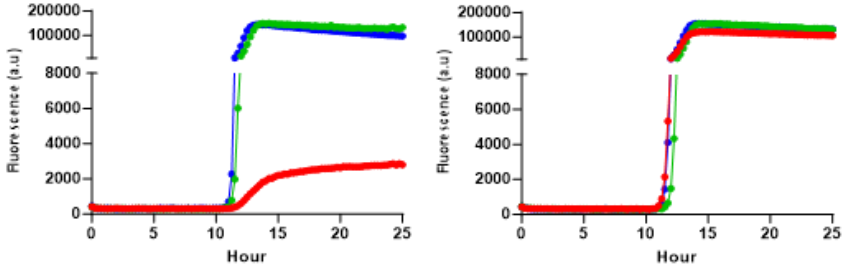
PD



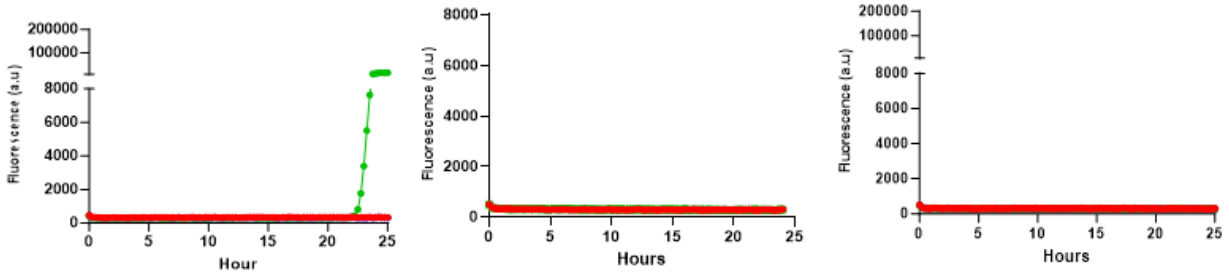
MSA



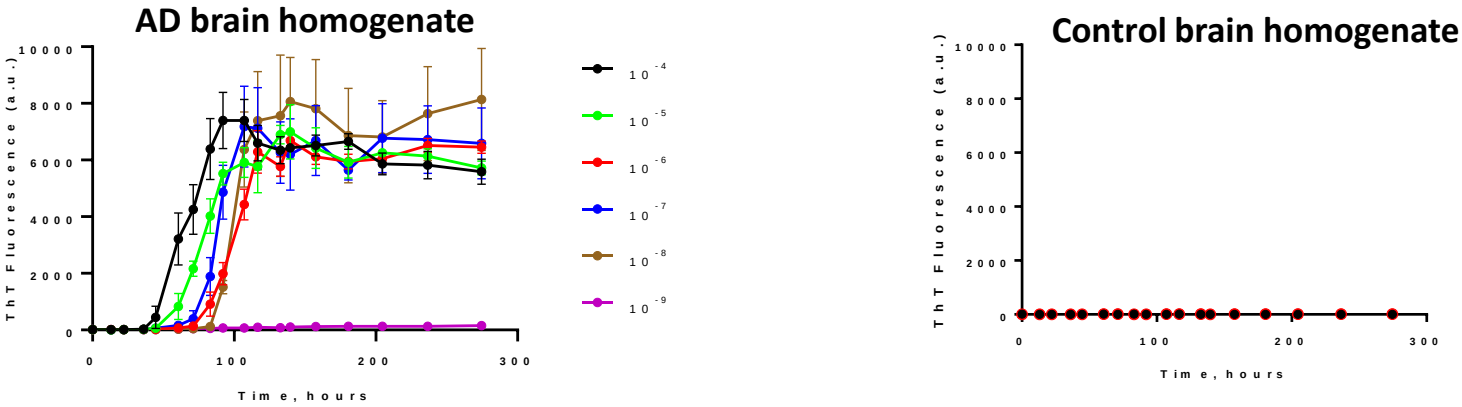
DLB

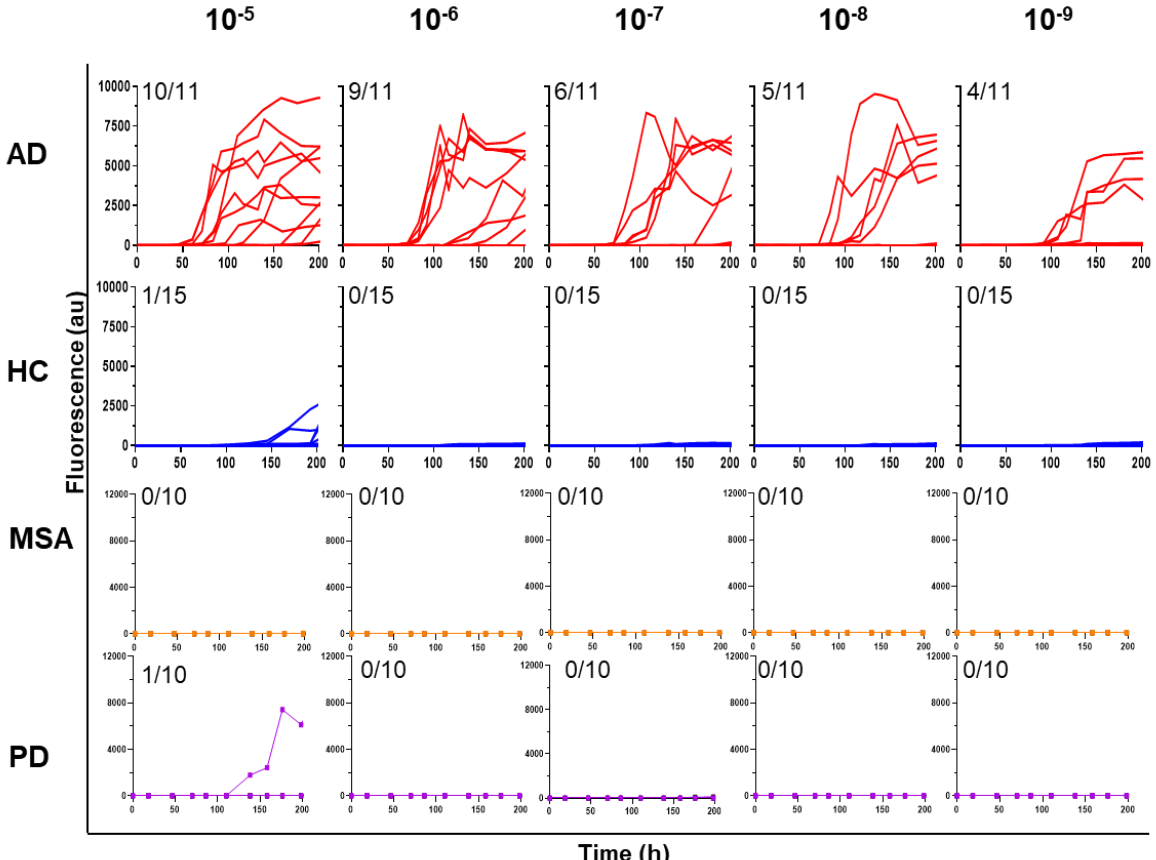


Control



Tau-SAA in brain samples





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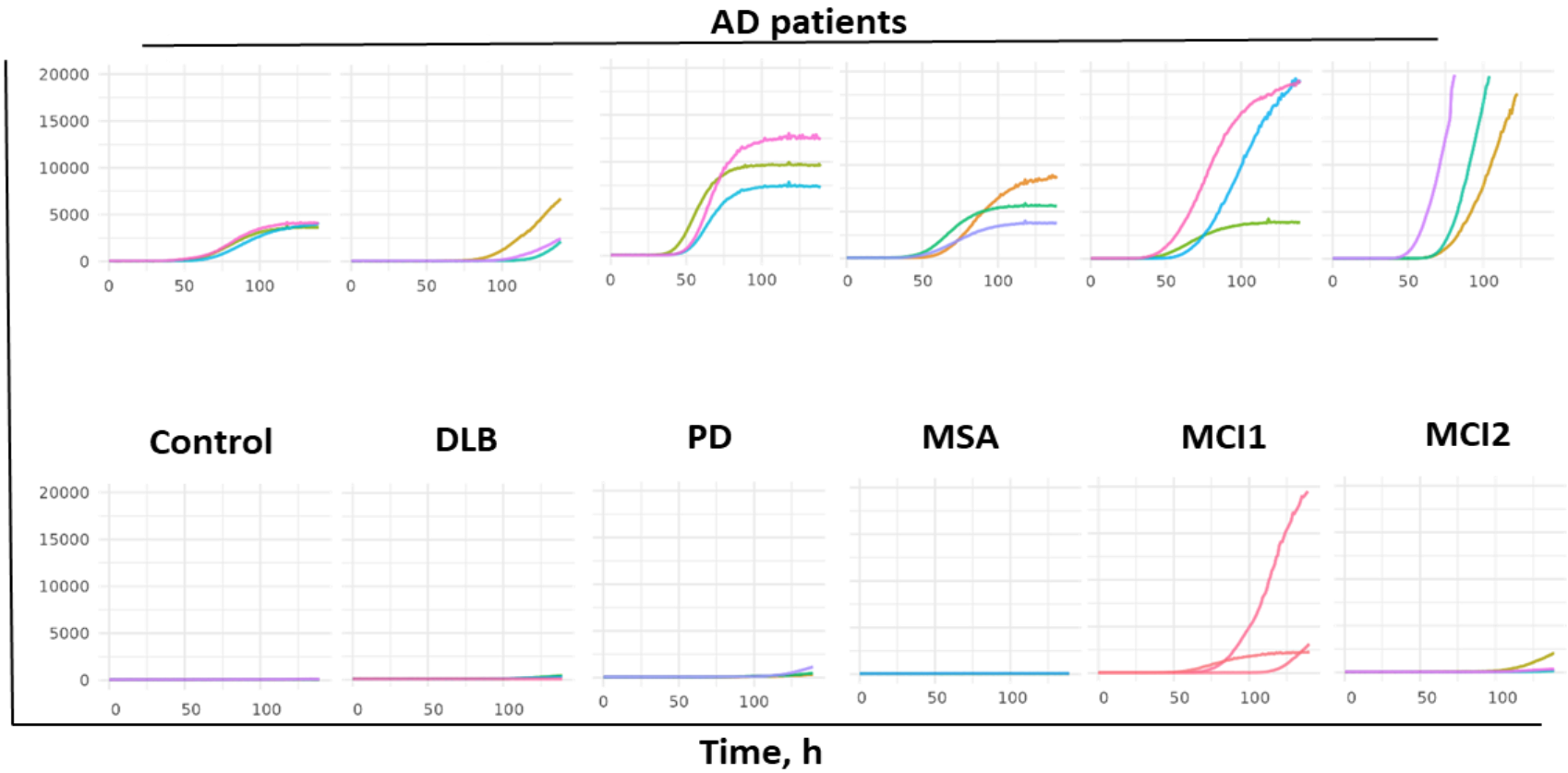
Tau-SAA in CSF samples



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Tau-SAA in CSF in relation to brain AD pathology

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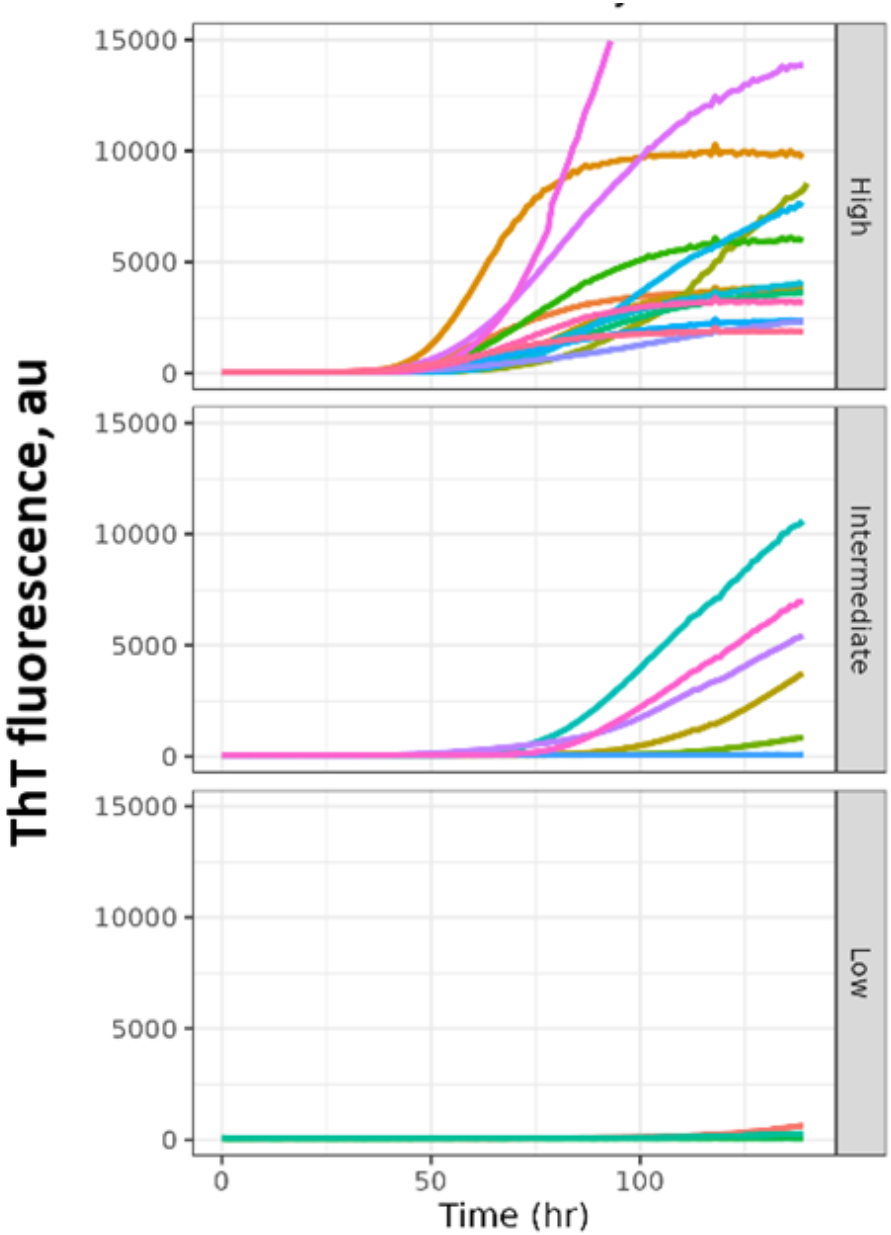

Potential Applications of SAA technology

Patient diagnosis. To increase sensitivity of clinical diagnosis

Identification of mixed pathology. To identify co-pathology and its contribution to disease.

Monitor disease progression. To measure the accumulation of aggregates over time and relate to brain damage and clinical phenotype.

Differentiating conformational strains. To distinguish diseases produced by distinct conformational strains of the same protein (e.g. PD vs MSA).

Pre-clinical detection of disease pathogenesis. To detect the disease process early on, before substantial damage in the brain and clinical symptoms.

Development of new therapeutics. To serve as a screening assay for drugs interfering with protein aggregation and spreading.

Patient enrollment in clinical trials. To help enrollment of the right patients for different trials.

End-point for clinical trials. To monitor the efficacy of drugs under development, especially those targeting protein aggregation.

Personalized medicine. To determine the type of proteinopathies present in distinct patients and help physicians to provide the best treatment available.

Defining disease by underlying biology. To help to define disease by the type and extent of protein pathology rather than by clinical symptoms.

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