

Mechanisms of Protein Seeding in Neurodegenerative Diseases

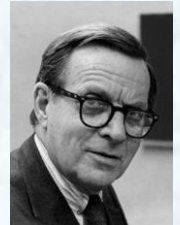
Lary Walker
Emory University

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Jay Rasmussen, Jasmin Mahler, Natalie Beschorner

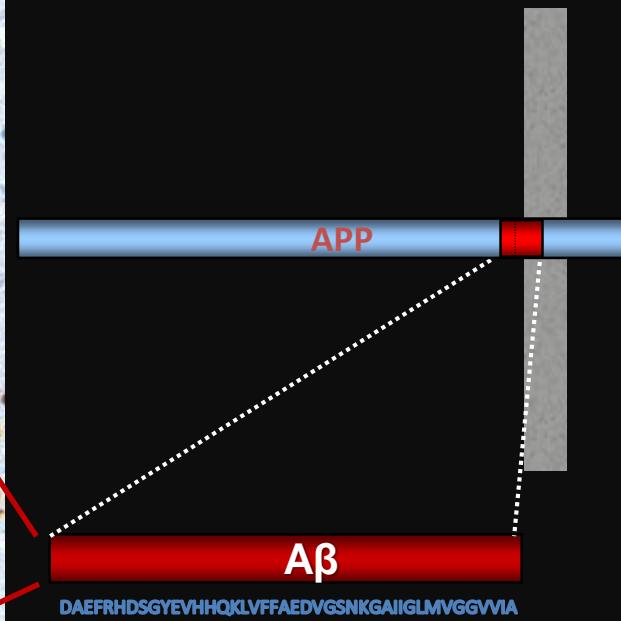
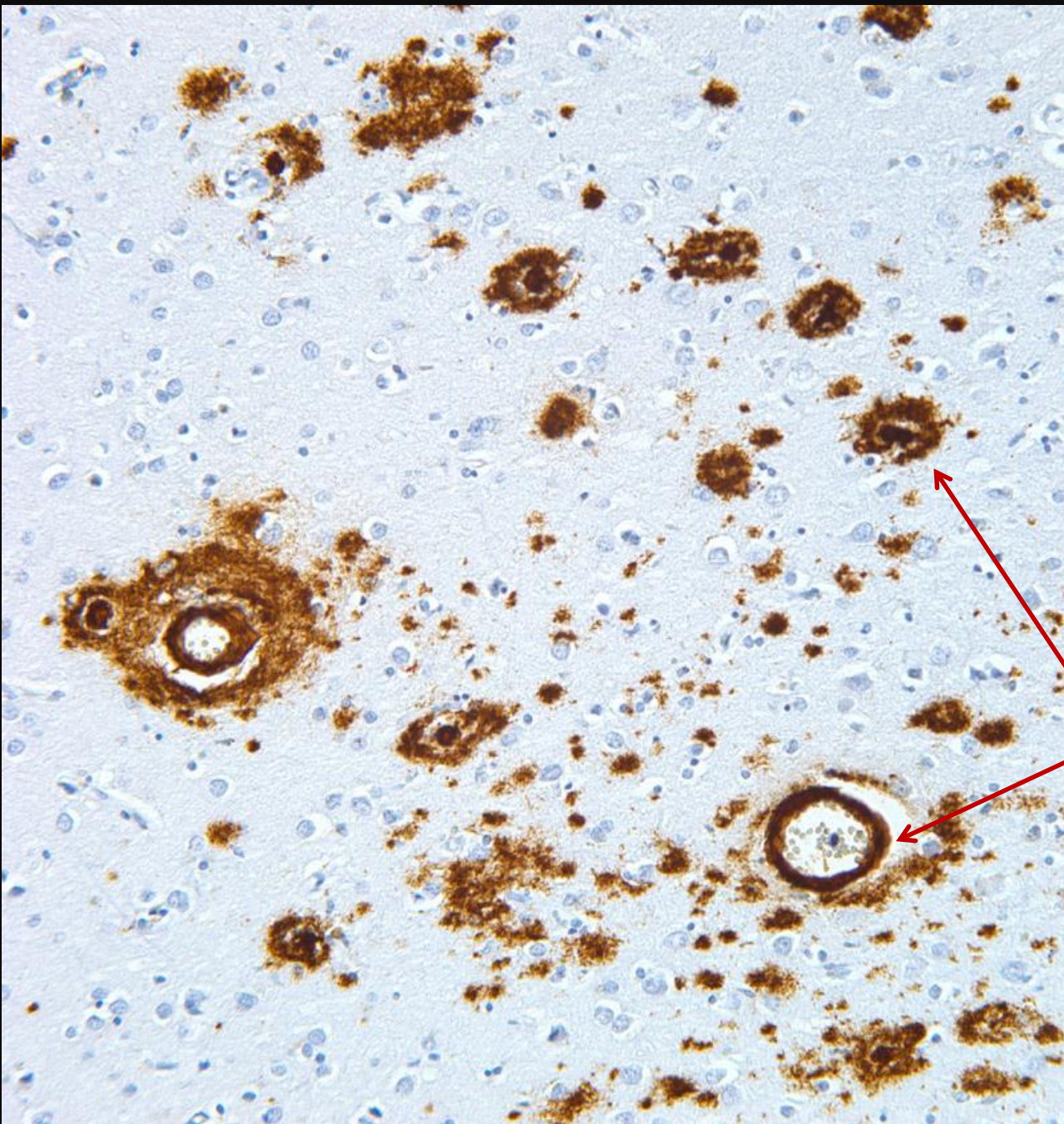
Overview

1. **Alzheimer's disease: The primacy of A β and necessity of tauopathy**
2. **The prion paradigm**
3. **The prion-like properties of A β**
4. **A β strains and the heterogeneity of Alzheimer's disease**
5. **The broad spectrum of prion-like mechanisms**

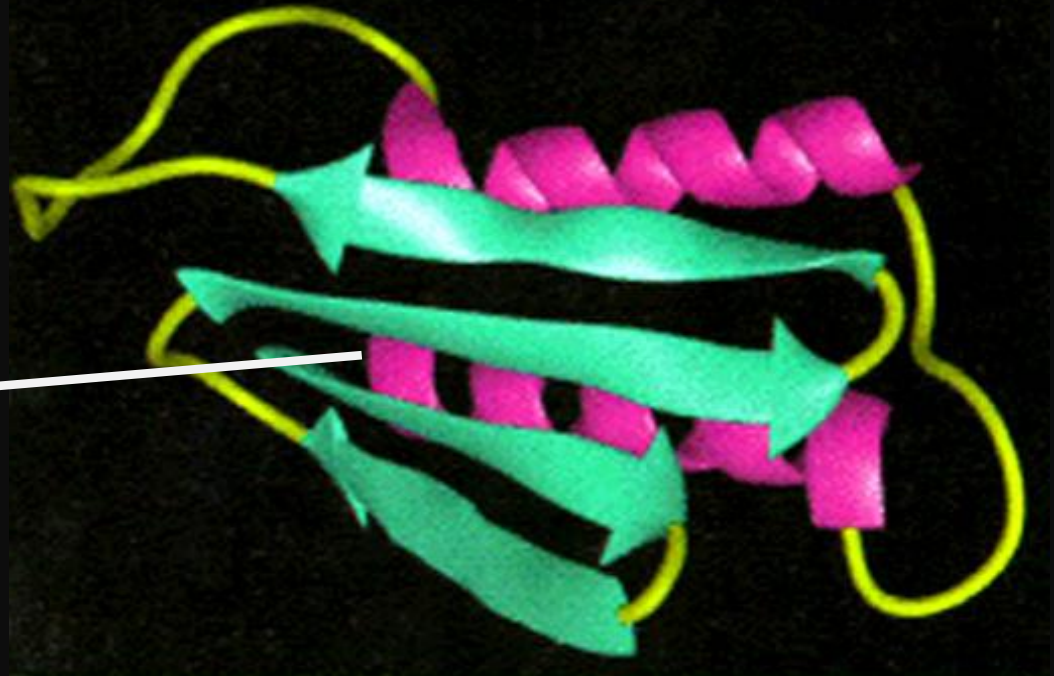
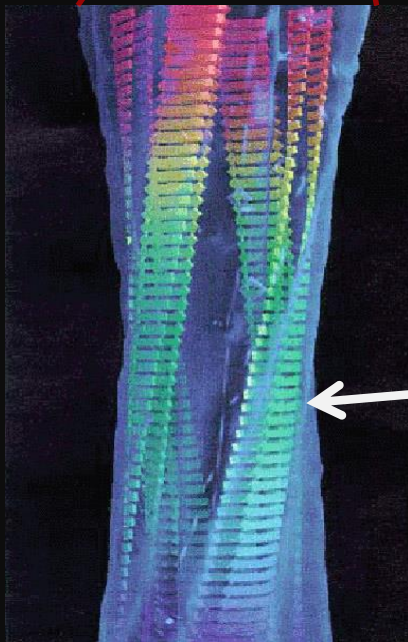
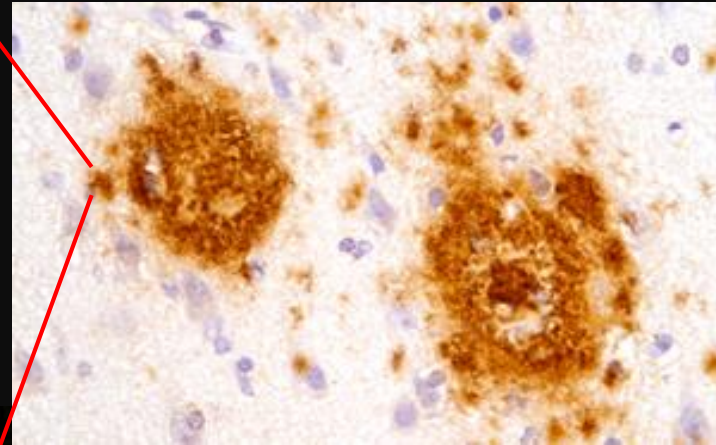
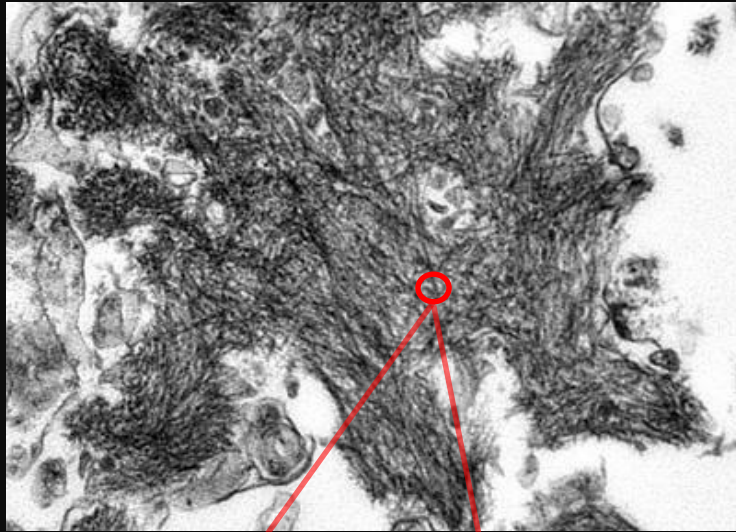
Lewis Thomas' General Principles of Disease:



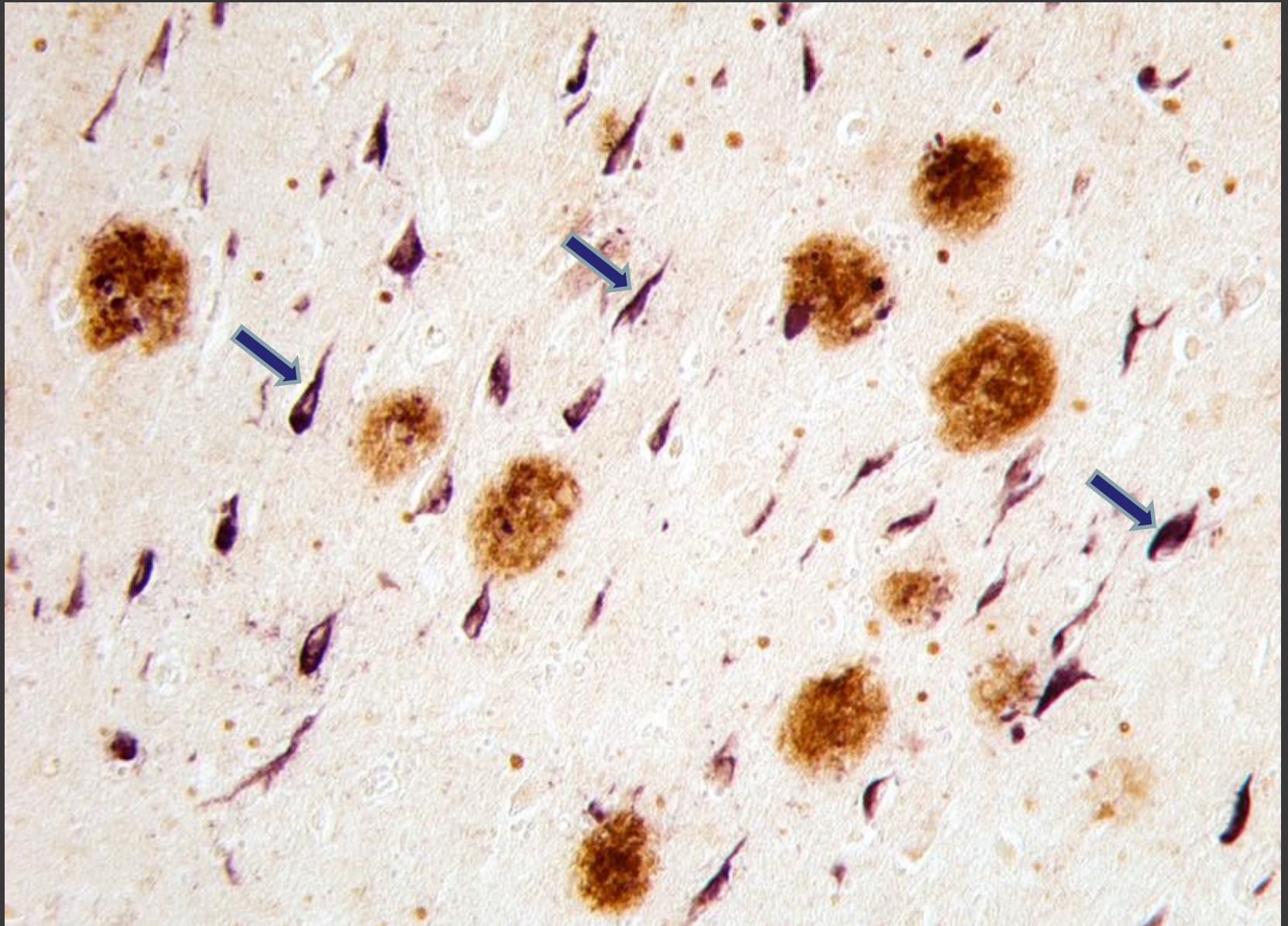
1. “It is necessary to know a great deal about underlying mechanisms before one can really act effectively”
2. “For every disease there is a single key mechanism that dominates all others”

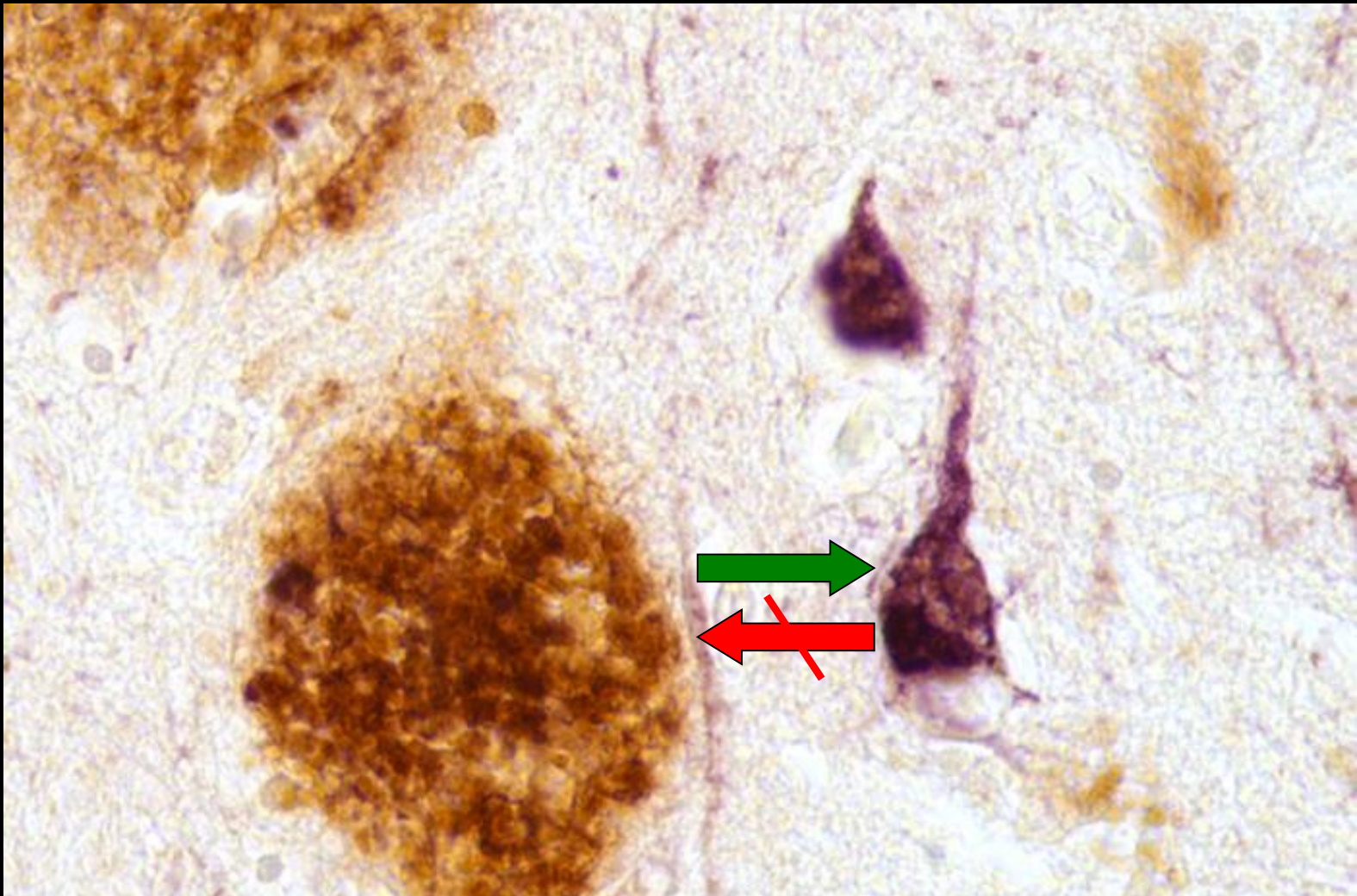


Amyloid: One manifestation of aggregated proteins



Tauopathy in AD





All known genetic and environmental risk factors for AD increase the production, trafficking and/or aggregation of A β

A microscopic image of brain tissue, likely from a mouse model of Alzheimer's disease. The image shows numerous amyloid plaques, which are dense, eosinophilic (pink) deposits of amyloid-beta protein. There are also neurofibrillary tangles, which are dark purple, thread-like structures within the neurons, representing aggregated tau protein. The background shows the general structure of the brain tissue, including neurons and glial cells.

How Does A β Cause Disease?

Clues from the Prion Paradigm

Prion Diseases

Human:

Creutzfeldt-Jakob Disease (CJD)
Gerstmann-Sträussler-Scheinker Disease
Fatal insomnia
Kuru
Variant CJD
Variably protease-sensitive prionopathy



HG Creutzfeldt

AM Jakob

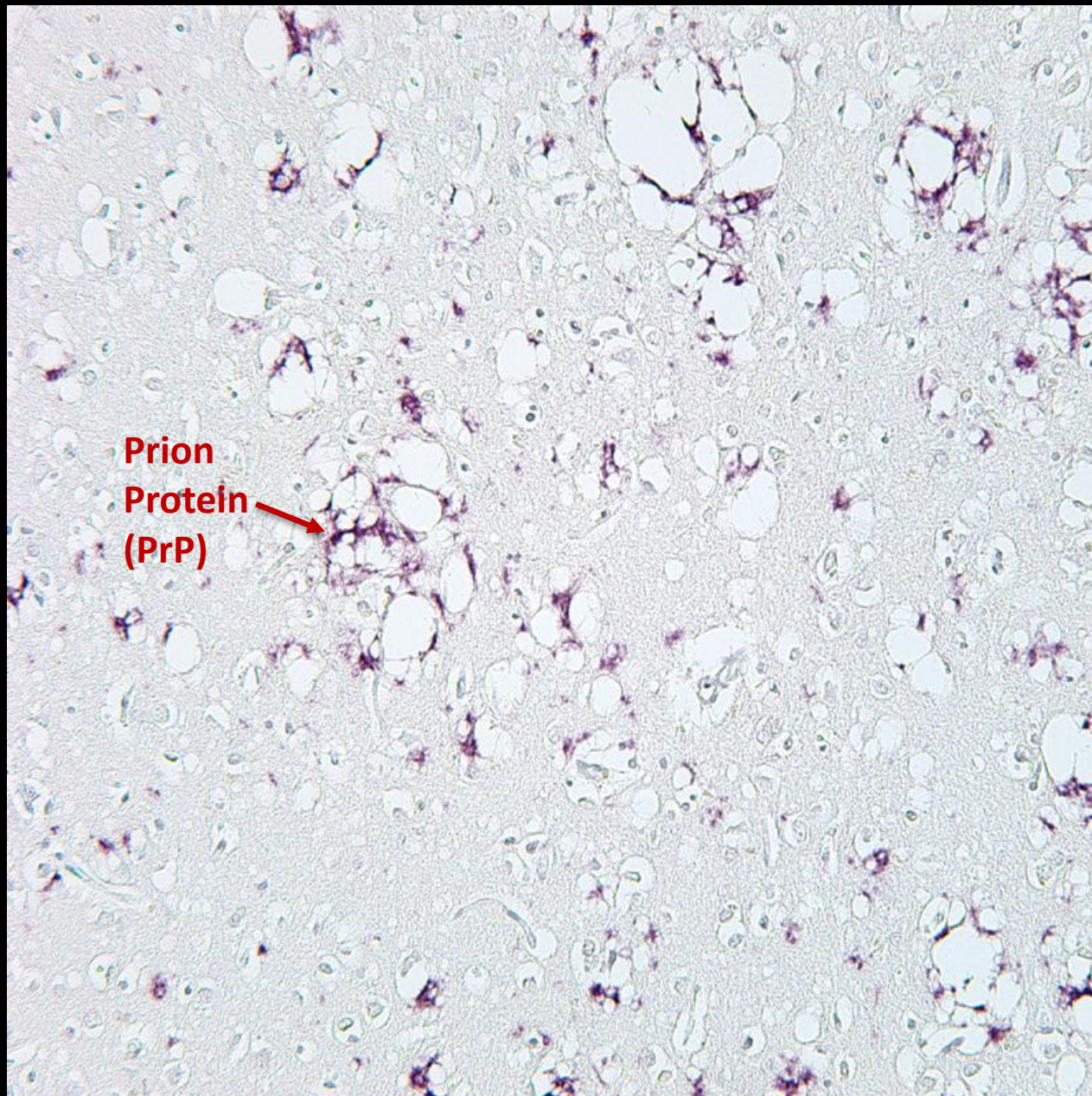
Infectivity?

Nonhuman:

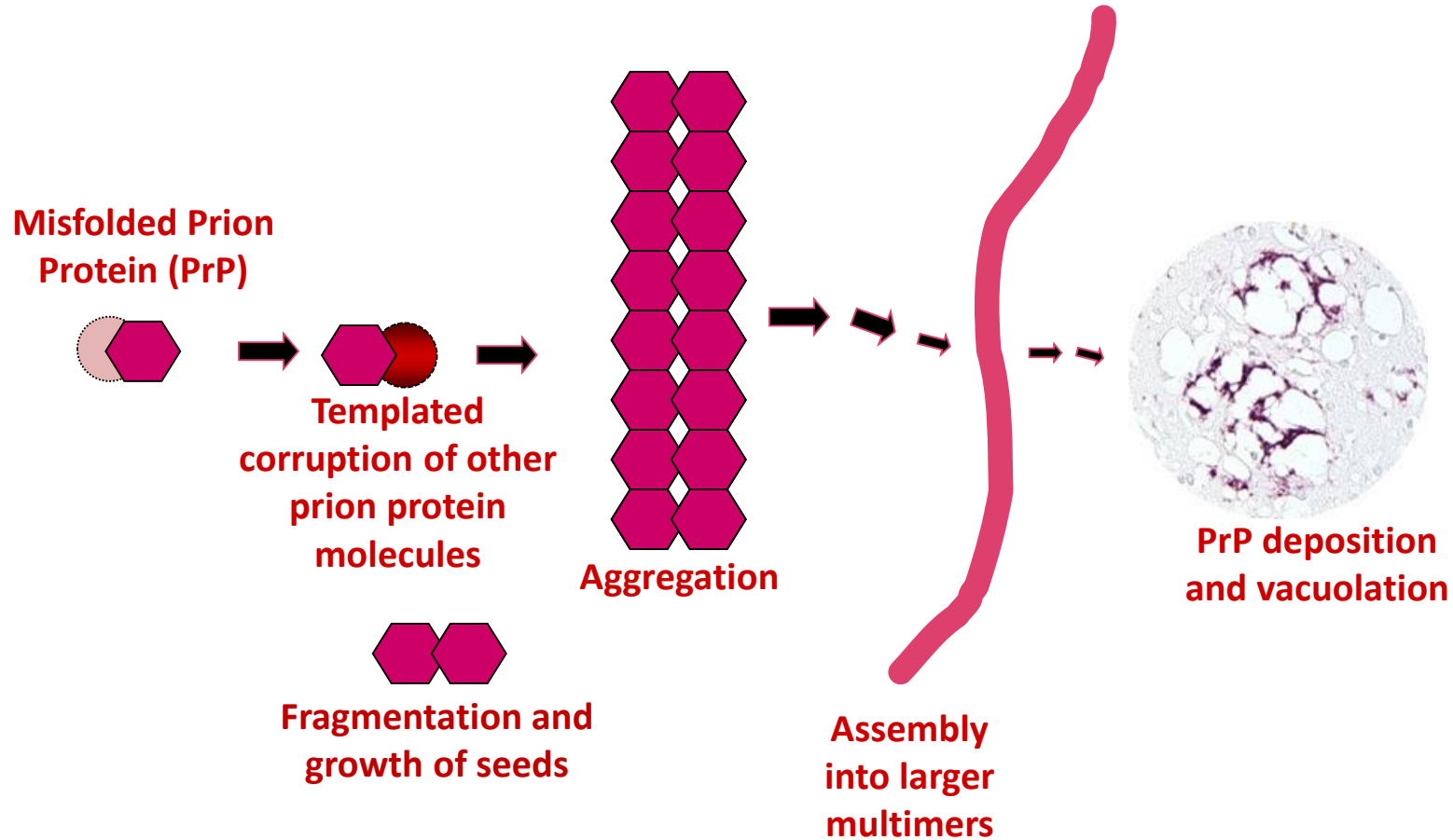
Scrapie
Bovine spongiform encephalopathy
Transmissible mink encephalopathy
Chronic wasting disease
Exotic ungulate spongiform encephalopathy
Feline spongiform encephalopathy
Primate spongiform encephalopathy



Prion Pathology

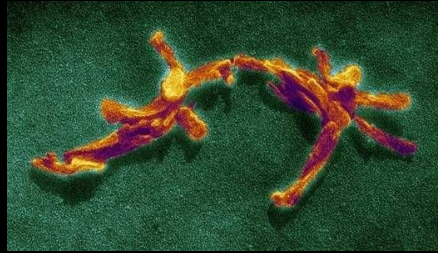


Prion functionality: Fundamentally a molecular mechanism



'Amyloid' is optional

Properties of Prions



Protein-only agents

Induce lesions in susceptible hosts

Spread within brain

Spread to brain

Multiple sizes

Variably sensitive to proteinase K

Resist high temperature

Resist formaldehyde

Exist as *strains*

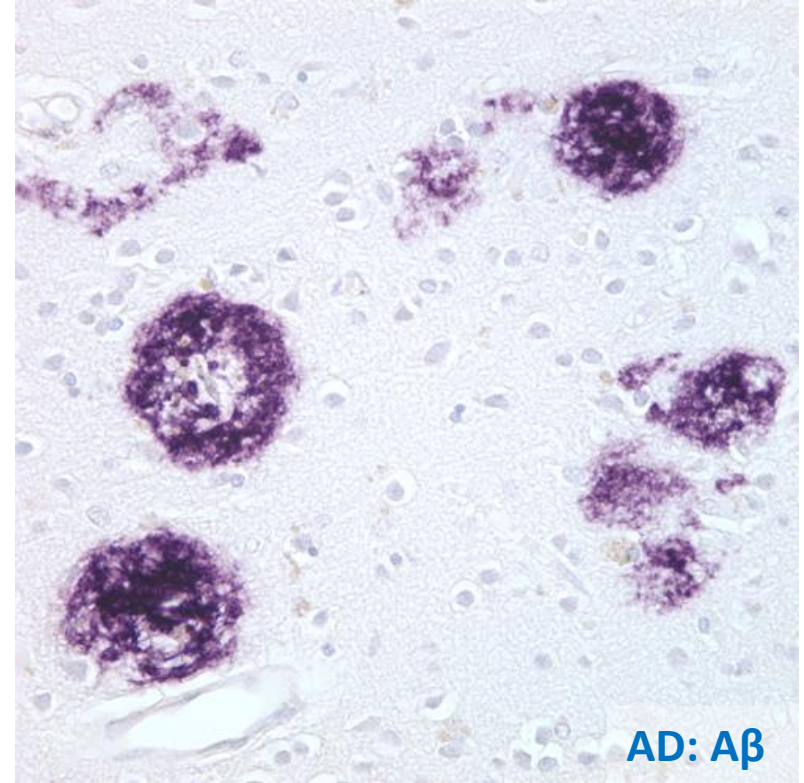
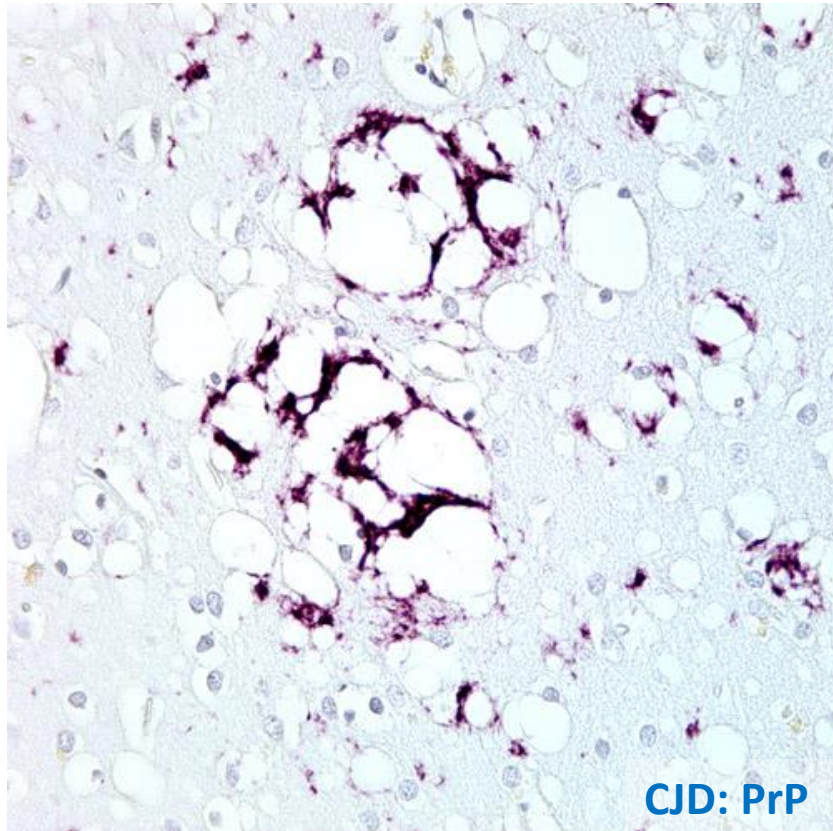
Transmissible

The prion paradigm and Alzheimer's disease

Alzheimer's disease and transmissible virus dementia (Creutzfeldt-Jakob disease). Brown P, Salazar AM, Gibbs CJ Jr, Gajdusek DC. *Ann N Y Acad Sci.* 1982; **396**:131-43

Some speculations about prions, amyloid, and Alzheimer's disease.

Prusiner SB. *N Engl J Med.* 1984; **310**:661-3



NO:

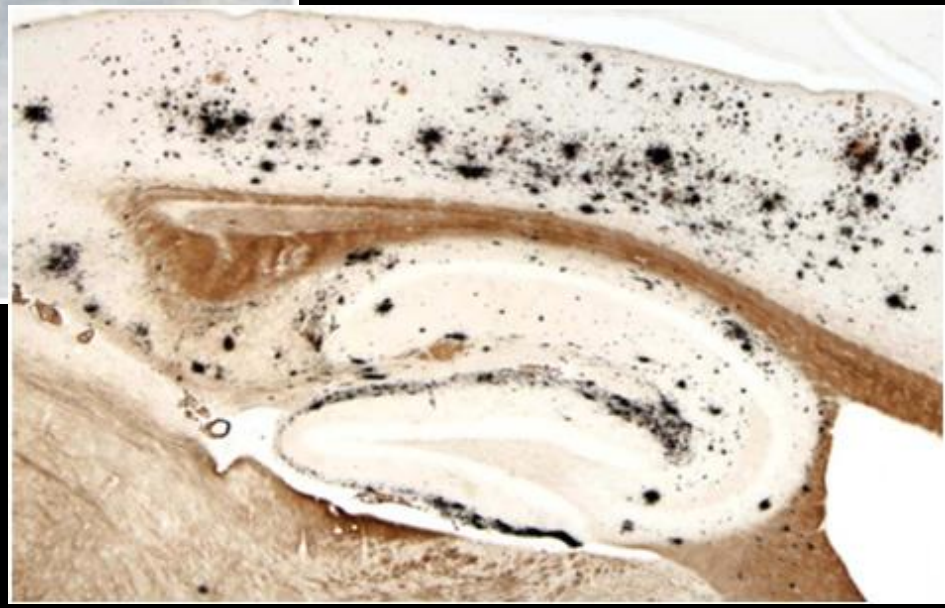
Evidence for and against the transmissibility of Alzheimer disease

Goudsmit J, Morrow CH, Asher DM, Yanagihara RT, Masters CL, Gibbs CJ Jr, Gajdusek DC. *Neurology* 1980; **30**:945-50

MAYBE:

Induction of beta (A4)-amyloid in primates by injection of Alzheimer's disease brain homogenate. Comparison with transmission of spongiform encephalopathy.

Baker HF, Ridley RM, Duchon LW, Crow TJ, Bruton CJ; *Mol Neurobiol.* 1994; **8**:25-39



A β Aggregation and the Prion Paradigm

A β Seeds

Protein-only agents

Induce lesions in susceptible hosts

Spread within brain

Spread to brain

Multiple sizes

Variably sensitive to proteinase K

Resist high temperature

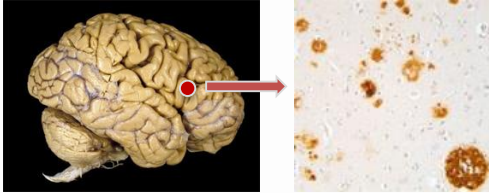
Resist formaldehyde

Exist as *strains*

Transmissible

Seeding A β plaques in APP-transgenic mice

In buffer, a small brain sample containing A β plaques is homogenized



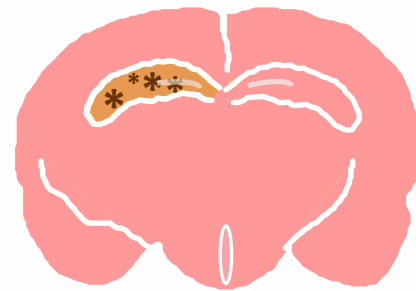
The extract is injected into the hippocampus of an anesthetized APP-transgenic mouse

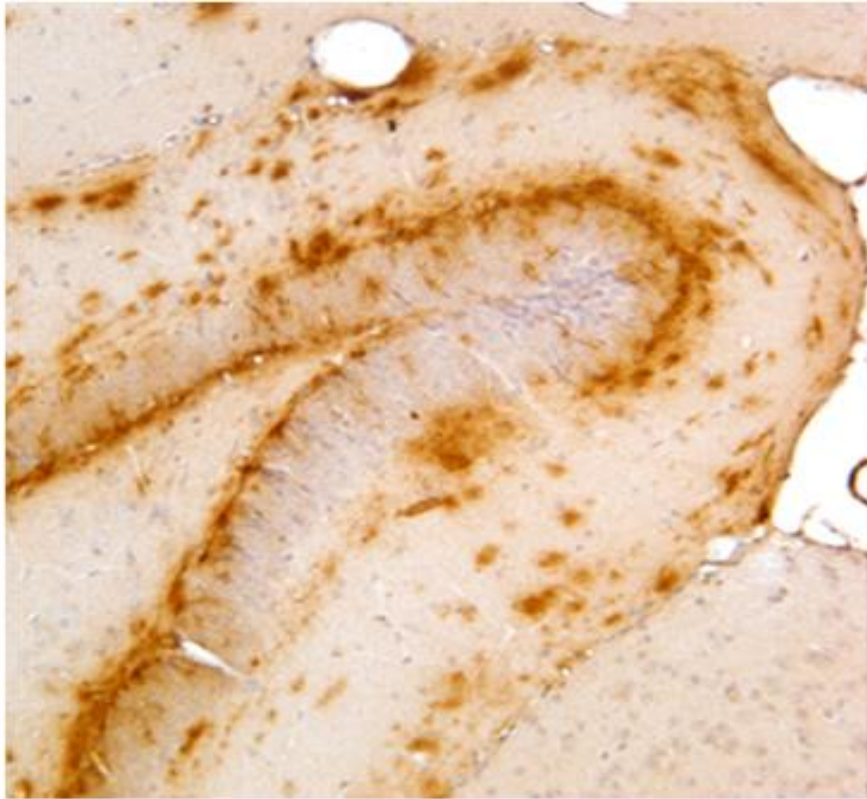
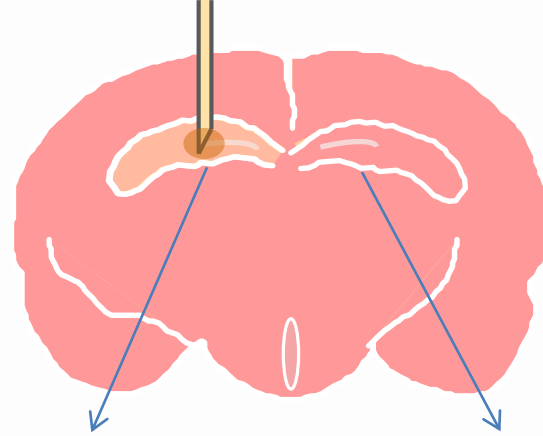


The homogenate is spun briefly in a centrifuge, and the clear liquid (extract) collected

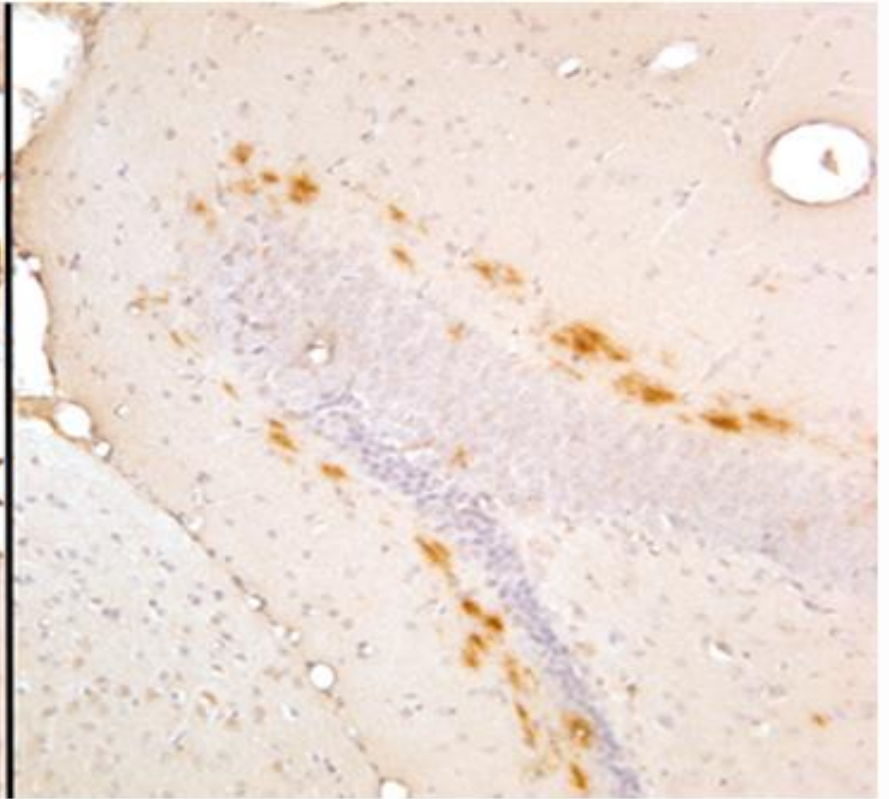


After an incubation period, the mouse brain is examined for seeded A β deposits





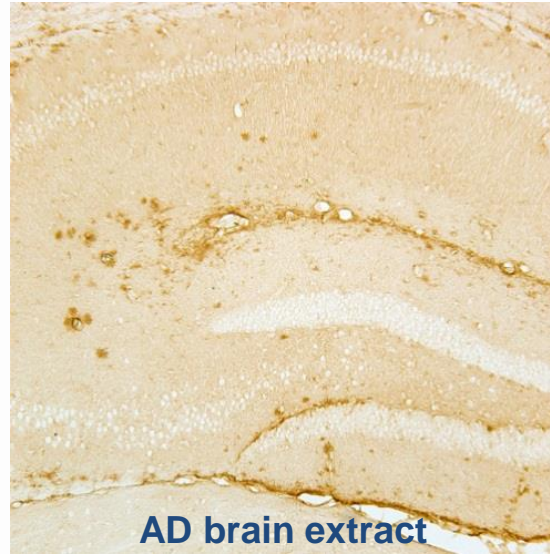
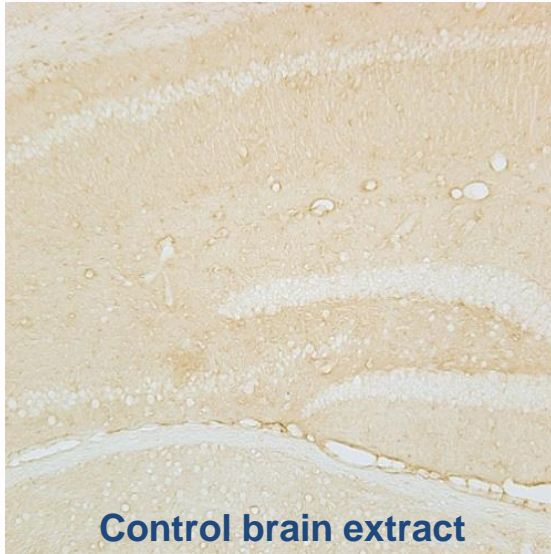
AD-seeded side



Unseeded side

5 months later

The seeding extract must contain aggregated A β



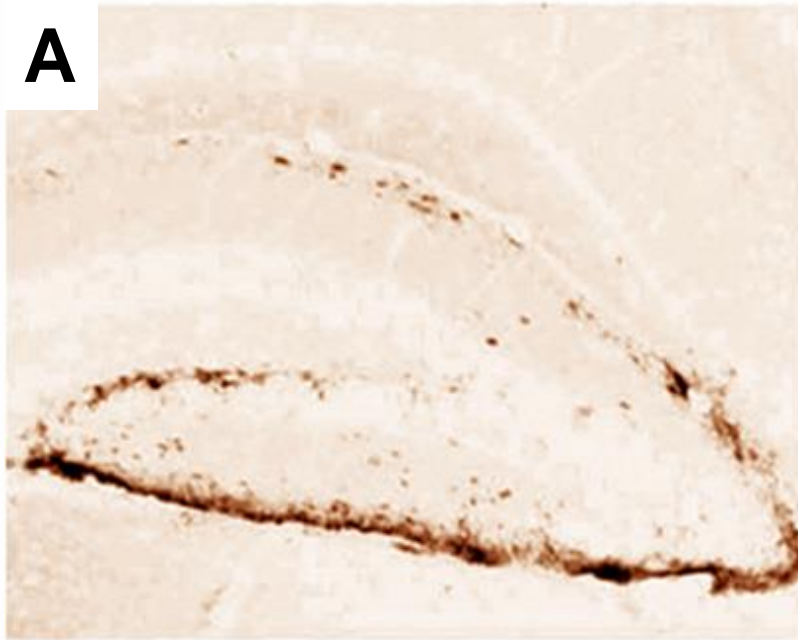
The seeded host must generate human-type A β



DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

DAEFGHDSGF^{EVR}HQKLVFFAEDVGSNKGAIIGLMVGGVVIA

**Seeding is reduced
by immunodepletion of A β**



Whole extract



A β -depleted extract

A β Seeds

Protein-only agents

Induce lesions in susceptible hosts

Spread within brain

Spread to brain

Multiple sizes

Variably sensitive to proteinase K

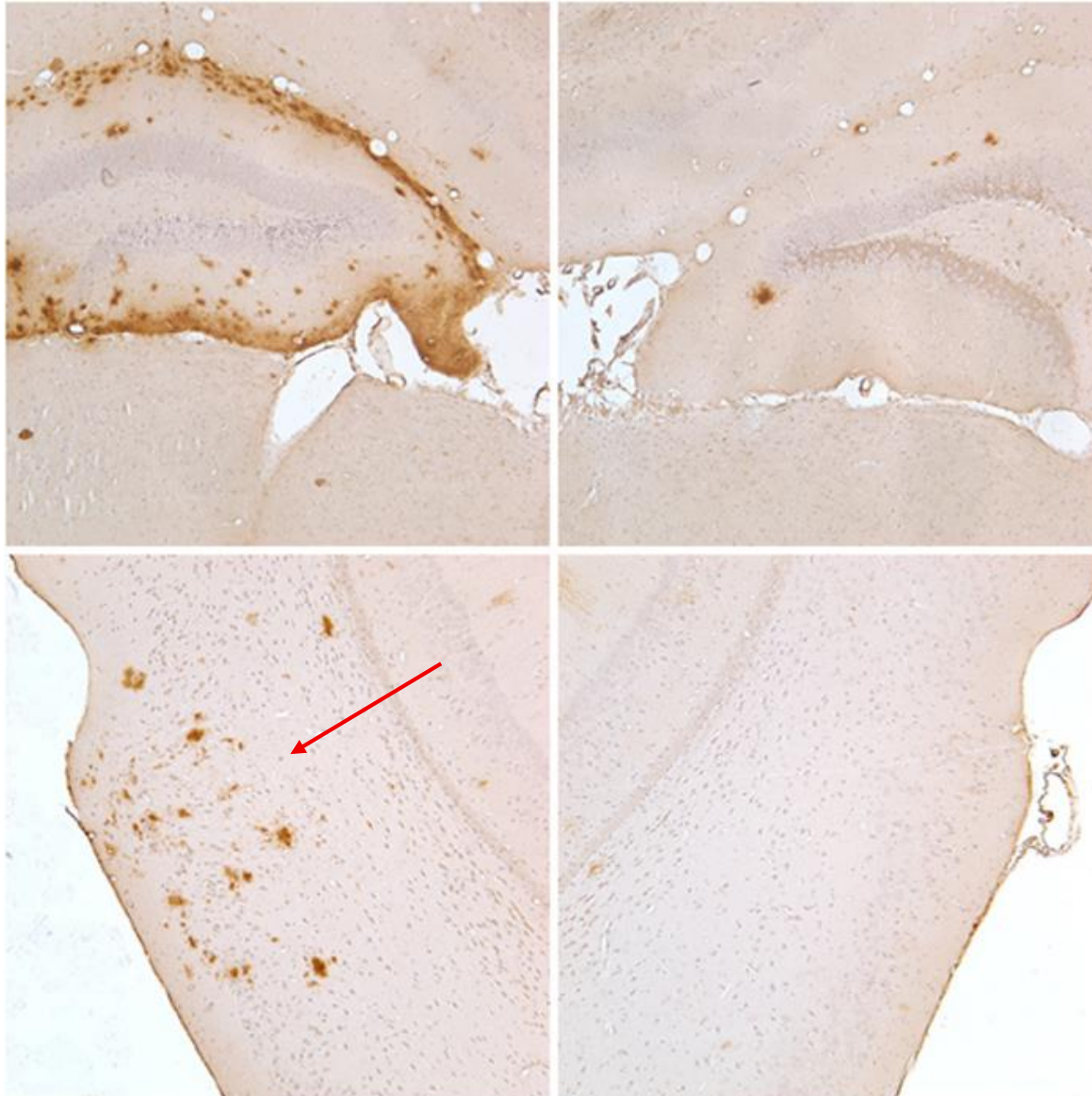
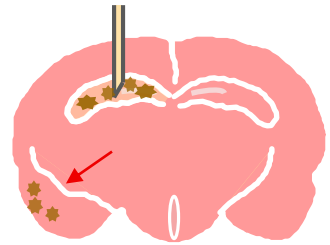
Resist high temperature

Resist formaldehyde

Exist as *strains*

Transmissible

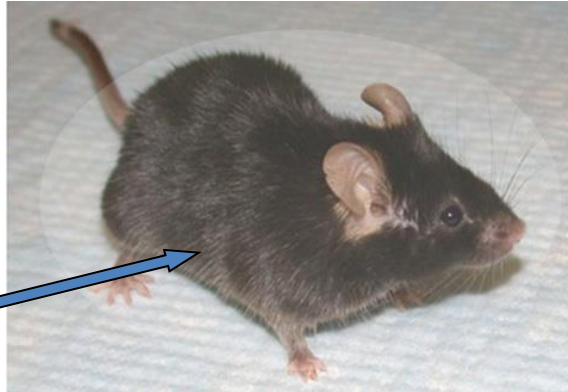
A β seeds spread within the brain



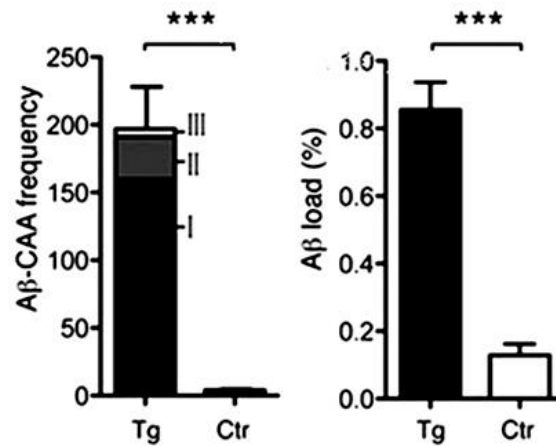
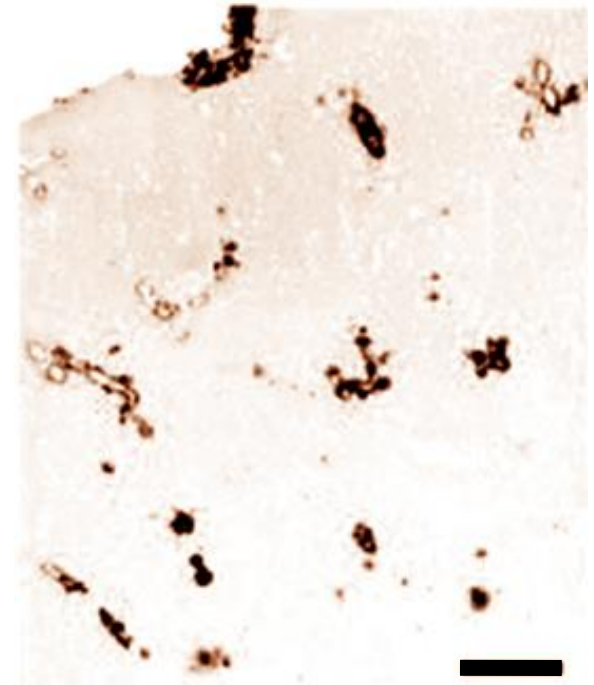
$A\beta$ seeds spread to the brain



Dilute Tg
Mouse Brain
extract



Intraperitoneal injection
6-8 month incubation



A β Seeds

Protein-only agents

Induce lesions in susceptible hosts

Spread within brain

Spread to brain

Multiple sizes

Variably sensitive to proteinase K

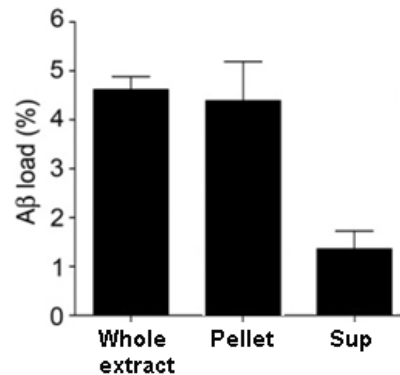
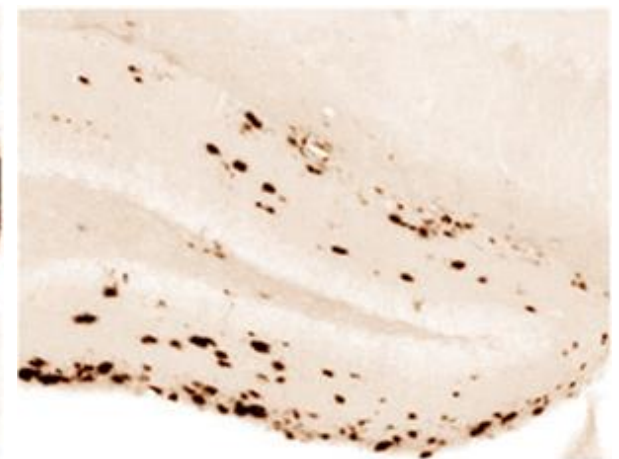
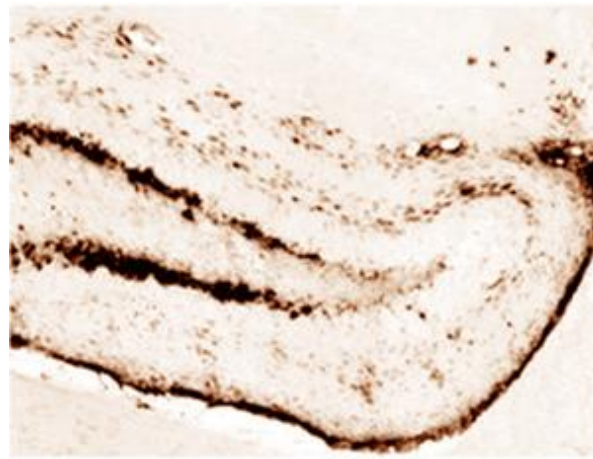
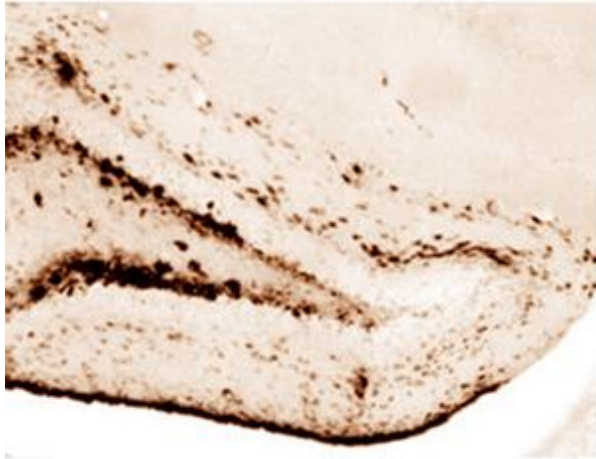
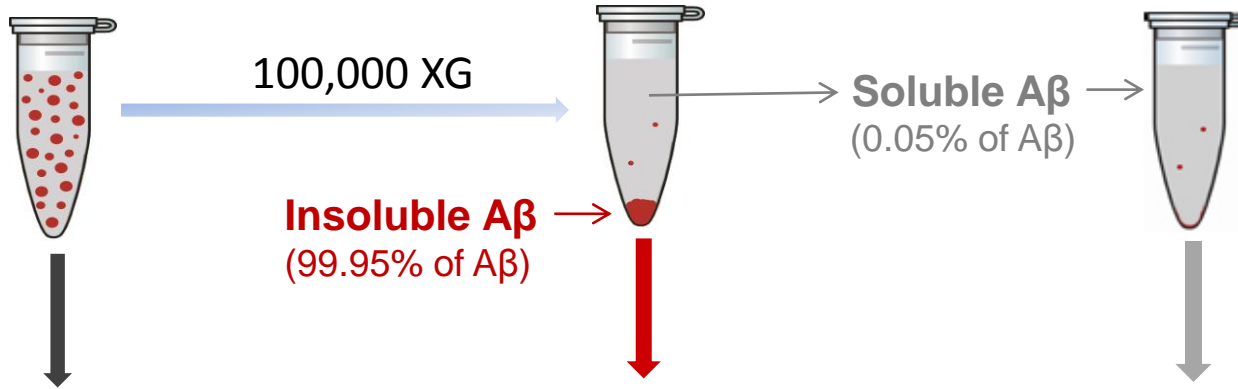
Resist high temperature

Resist formaldehyde

Exist as *strains*

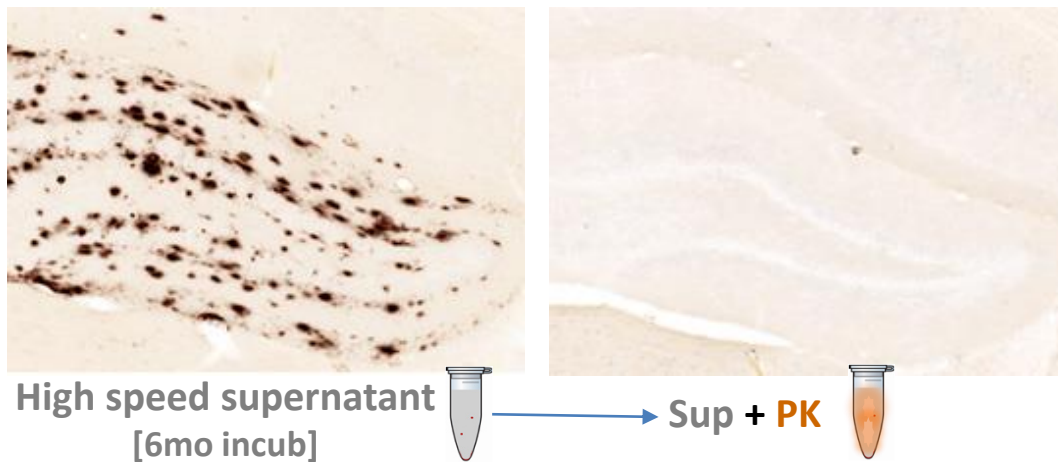
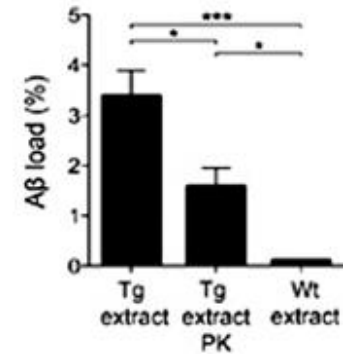
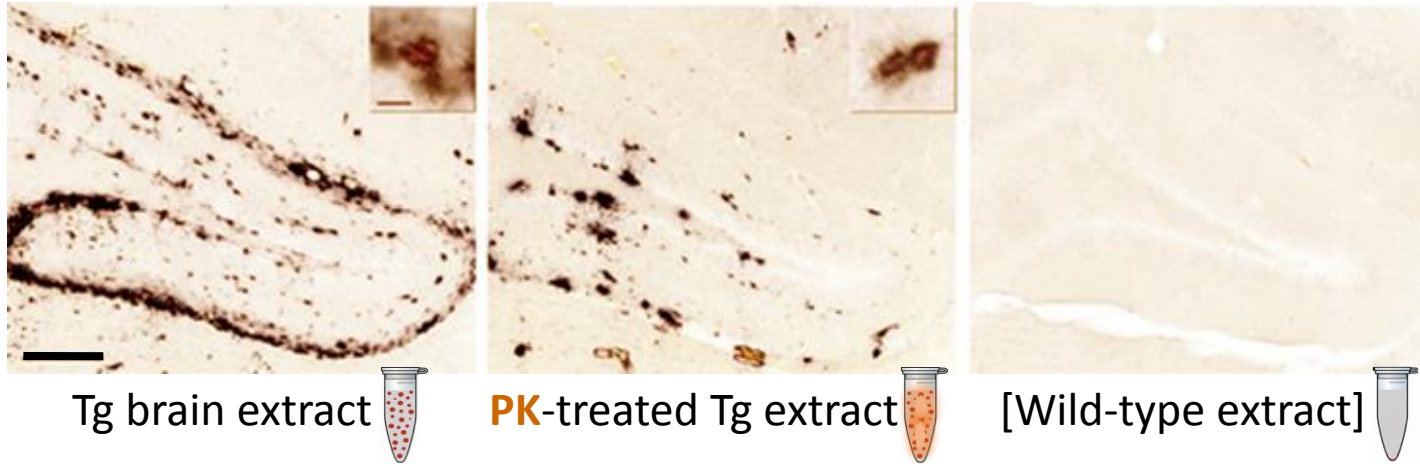
Transmissible

Whole Extract



Aβ seeds can be large or small; Small seeds are potent

Small A β seeds are sensitive to proteinase K



A β Seeds

Protein-only agents

Induce lesions in susceptible hosts

Spread within brain

Spread to brain

Multiple sizes

Variably sensitive to proteinase K

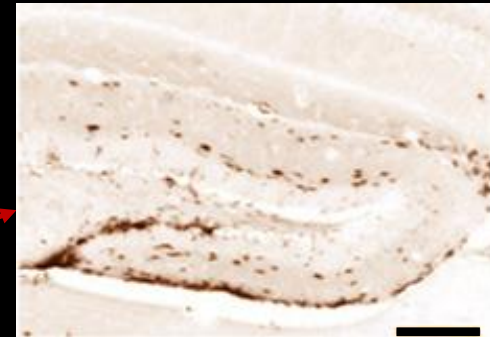
Resist high temperature

Resist formaldehyde

Exist as *strains*

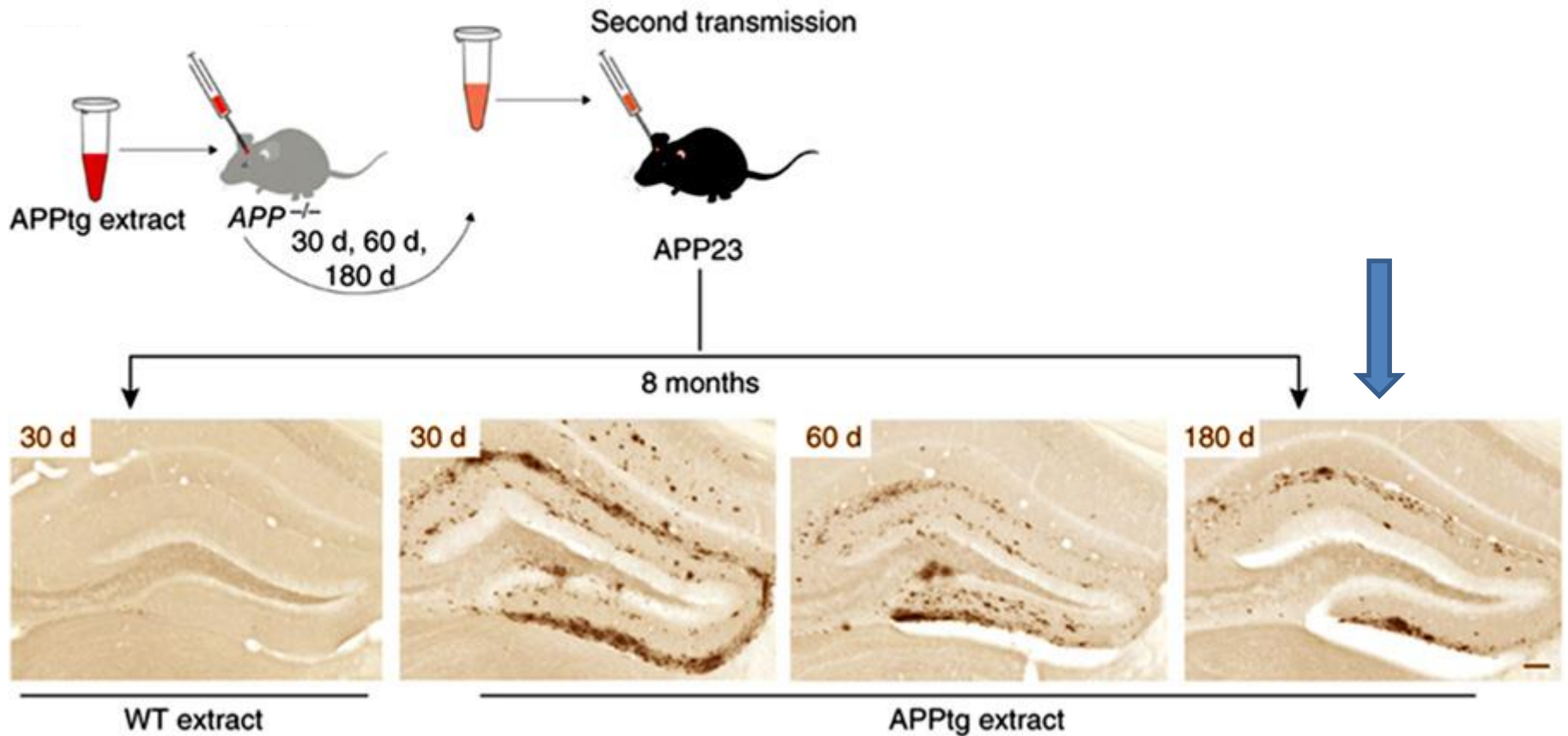
Transmissible

Boiled brain extract



Fixed brain extract

$A\beta$ seeds resist destruction in the living brain



A β Seeds

Protein-only agents

Induce lesions in susceptible hosts

Spread within brain

Spread to brain

Multiple sizes

Variably sensitive to proteinase K

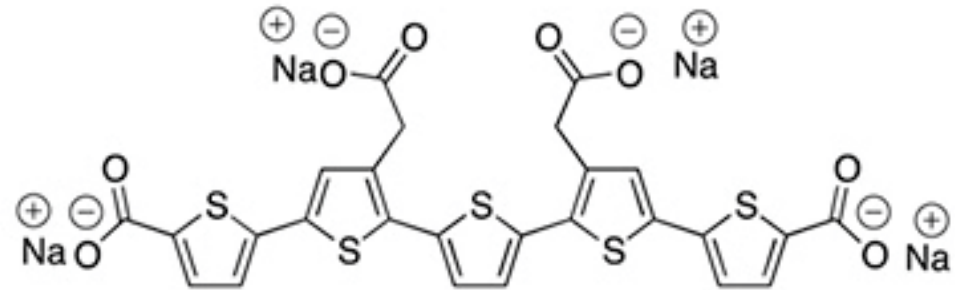
Resist high temperature

Resist formaldehyde

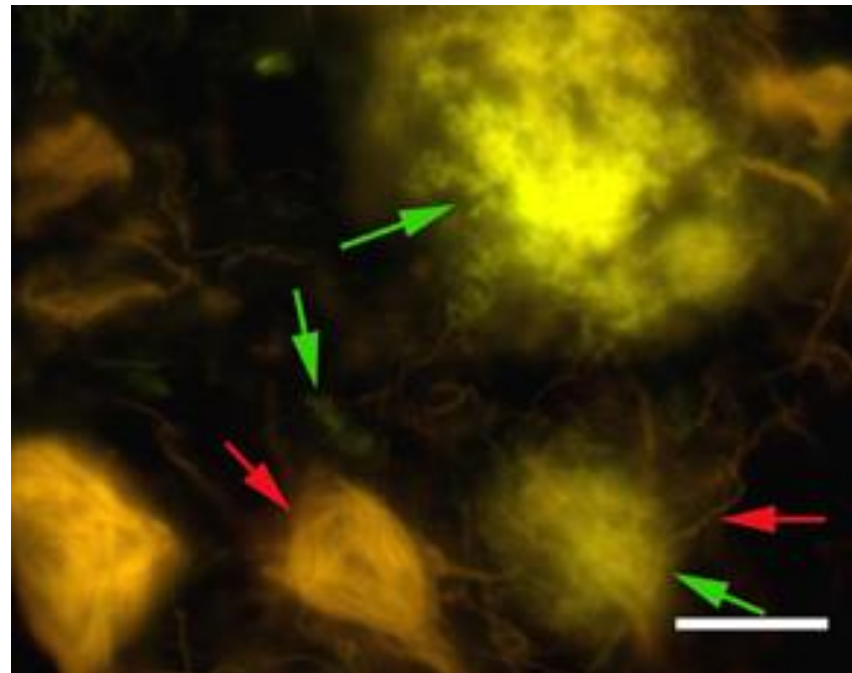
Exist as *strains*

Transmissible

Luminescent Conjugated Oligothiophenes (LCOs): Molecular probes for proteopathic strains

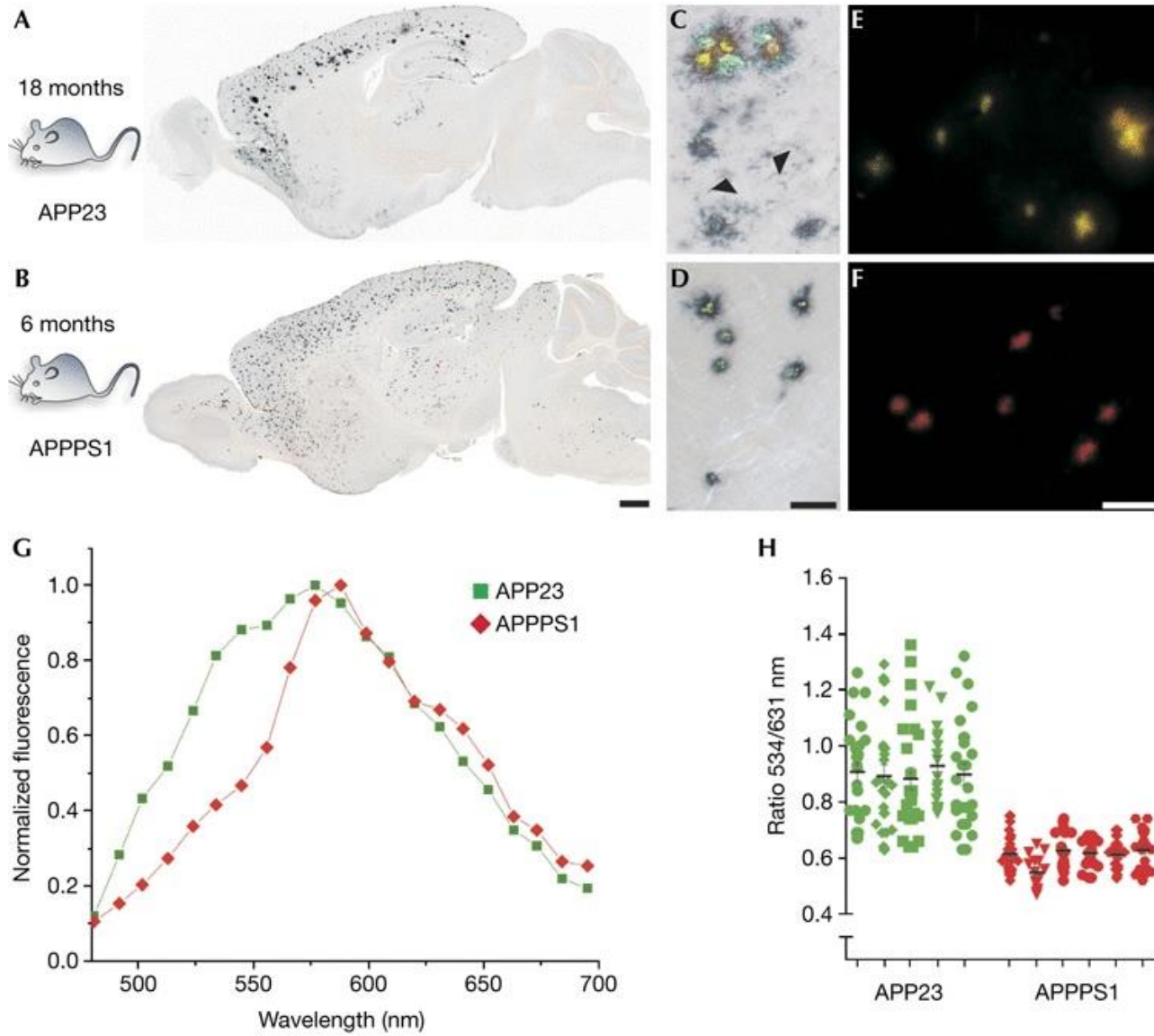


p-FTAA

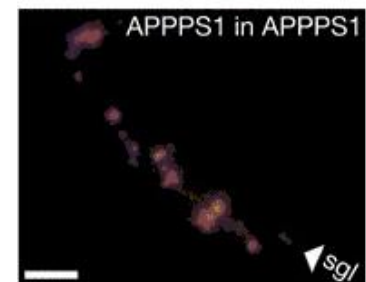
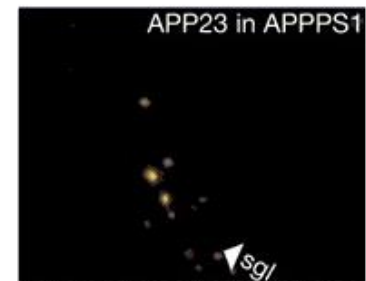
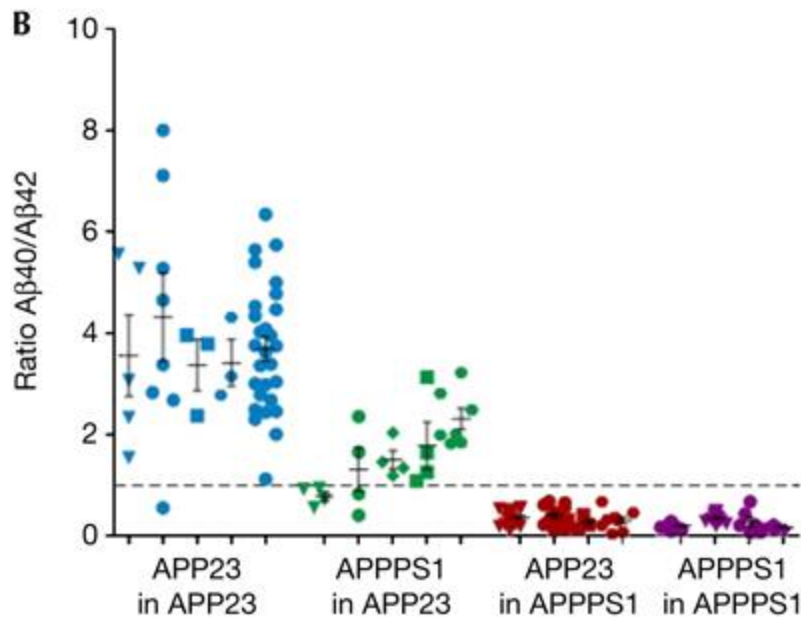
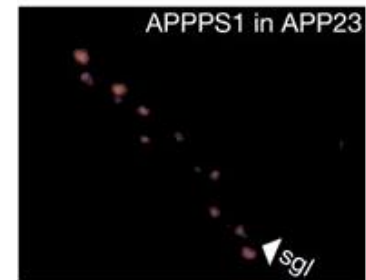
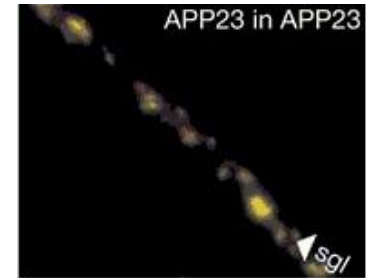
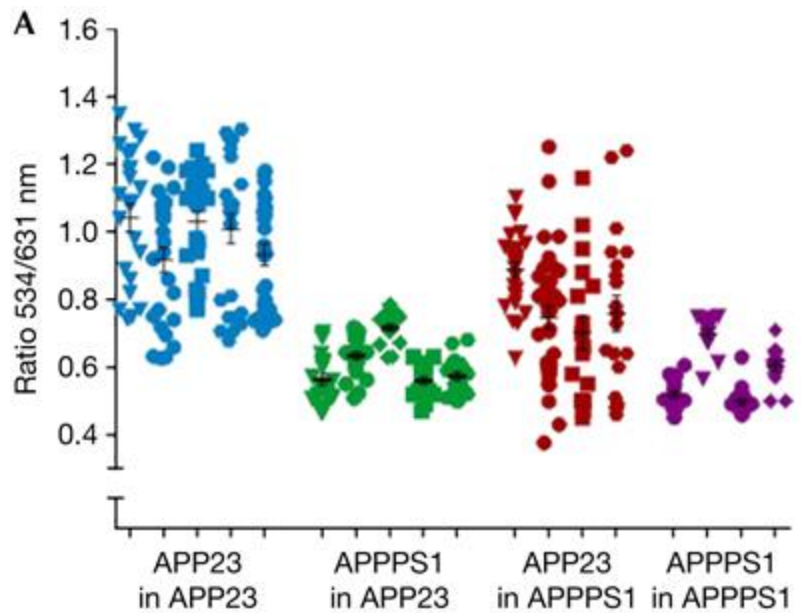


Peter Nilsson, Linköping

Different A β strains in different APP-transgenic mice



Murine strains are seedable



A β strains in sporadic and hereditary AD

Raw Spectral Data LCOs

A β Seeds

Protein-only agents

Induce lesions in susceptible hosts

Spread within brain

Spread to brain

Multiple sizes

Variably sensitive to proteinase K

Resist high temperature

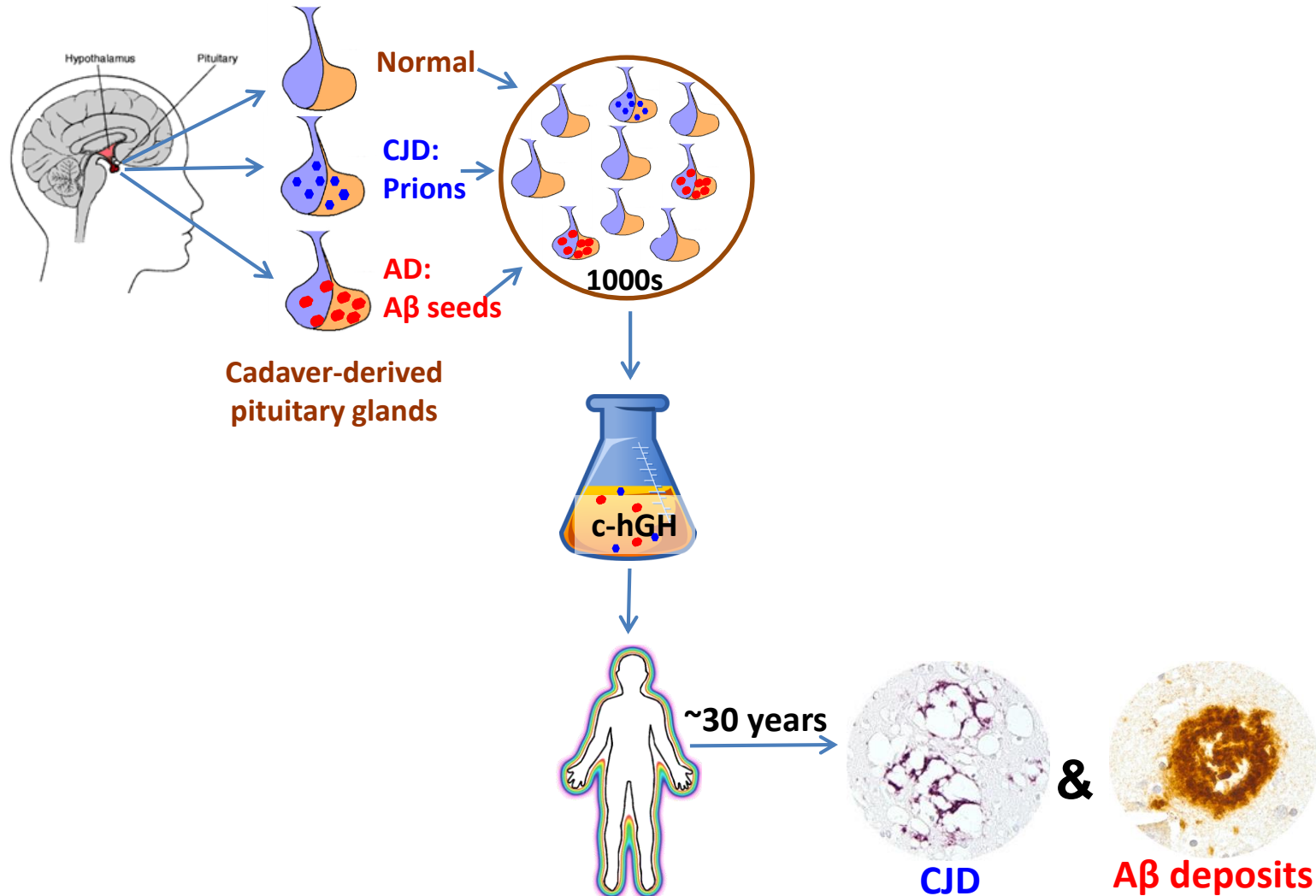
Resist formaldehyde

Exist as *strains*

Transmissible to humans?

Evidence for human transmission of amyloid- β pathology and cerebral amyloid angiopathy

Jaunmuktane et al., *Nature* 2015 525:247-50.



[Dura mater is a potential source of A \$\beta\$ seeds.](#) Kovacs GG, Lutz MI, Ricken G, Ströbel T, Höftberger R, Preusser M, Regelsberger G, Hönigschnabl S, Reiner A, Fischer P, Budka H, Hainfellner JA. *Acta Neuropathol.* 2016 Jun;131(6):911-23.

[Significant association of cadaveric dura mater grafting with subpial A \$\beta\$ deposition and meningeal amyloid angiopathy.](#) Hamaguchi T, Taniguchi Y, Sakai K, Kitamoto T, Takao M, Murayama S, Iwasaki Y, Yoshida M, Shimizu H, Kakita A, Takahashi H, Suzuki H, Naiki H, Sanjo N, Mizusawa H, Yamada M. *Acta Neuropathol.* 2016 Aug;132(2):313-5.

Property	Prions	A β Seeds
Protein-only agents	Yes	Yes
Induce lesions in susceptible hosts	Yes	Yes
Spread within brain	Yes	Yes
Spread to brain	Yes	Yes
Multiple sizes	Yes	Yes
Variably sensitive to proteinase K	Yes	Yes
Resist high temperature	Yes	Yes
Resist formaldehyde	Yes	Yes
Exist as strains	Yes	Yes
Aβ deposition transmissible to humans	Yes	Yes, but...

...no evidence of full-blown Alzheimer's disease in any recipient

The spectrum of proteopathies: Prion-like?

- Alzheimer's disease (A β and tau)
- Prion diseases (PrP)
- Tauopathies (tau)
- Huntington's disease/triplet repeat disorders (polyQ)
- Parkinson's disease/Lewy body disease (α -synuclein)
- Cerebral amyloid angiopathies (A β , cystatin, etc.)
- Amyotrophic lateral sclerosis (SOD, TDP43, FUS, C9ORF72, hnRNPs)
- FTLD ubi+, tau- (TDP43, FUS, C9ORF72, VCP)
- Familial British Dementia (ABri)
- Familial Danish Dementia (ADan)
- Familial Encephalopathy w/ Neuroserpin Inclusion Bodies (neuroserpin)

- Systemic amyloidoses (AA, AL, Transthyretin, etc)
- Type II diabetes (amylin)
- Cirrhosis with hepatocytic inclusions (α 1-antitrypsin)
- ...

Alzheimer's disease, Cerebral β -amyloid angiopathy	Amyloid β peptide (A β)
Prion diseases (multiple)	Prion protein
Parkinson's disease and other synucleinopathies (multiple)	α -Synuclein
Tauopathies (multiple)	Microtubule-associated protein tau
ALS & Frontotemporal lobar degeneration (FTLD)	TDP-43, SOD, FUS, C9ORF72, VCP, hnRNPs
CADASIL	Notch 3
Huntington's disease and other triplet repeat disorders (multiple)	Proteins with tandem amino acid expansions
Familial British dementia / Danish dementia	ABri / ADan
Alexander Disease	GFAP
Familial encephalopathy with neuroserpin inclusion bodies (FENIB)	Neuroserpin
Hereditary cerebral hemorrhage with amyloidosis (Icelandic) (HCHWA-I)	Cystatin C
Type II diabetes	Islet amyloid polypeptide (IAPP; amylin)
Familial amyloidotic neuropathy, Senile systemic amyloidosis	Transthyretin
AL (light chain) amyloidosis	Monoclonal immunoglobulin light chains
AH (heavy chain) amyloidosis	Immunoglobulin heavy chains
AA (secondary) amyloidosis	Amyloid A protein
Aortic medial amyloidosis	Medin (lactadherin)
ApoAI amyloidosis	Apolipoprotein AI
ApoAII amyloidosis	Apolipoprotein AII
ApoAIV amyloidosis	Apolipoprotein AIV
Finnish hereditary amyloidosis	Gelsolin
Lysozyme amyloidosis	Lysozyme
Fibrinogen amyloidosis	Fibrinogen
Dialysis amyloidosis	β 2-microglobulin
Inclusion body myopathy/myositis	Amyloid β peptide (A β), tau, TDP-43, FUS
Cataracts	Crystallins
Medullary thyroid carcinoma	Calcitonin

Disease or disease class	Aggregating protein(s)
Alzheimer's disease	Amyloid β peptide (A β); Tau protein (see tauopathies)
Cerebral β -amyloid angiopathy	Amyloid β peptide (A β)
Retinal ganglion cell degeneration in glaucoma	Amyloid β peptide (A β)
Prion diseases (multiple)	Prion protein
Parkinson's disease and other synucleinopathies (multiple)	α -Synuclein
Tauopathies (multiple)	Microtubule-associated protein tau (Tau protein)
Frontotemporal lobar degeneration (FTLD) (Ubi+, Tau-)	TDP-43, FUS, VCP, C9ORF72
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase, TDP-43, FUS, C9ORF72, hnRNPs
Huntington's disease and other triplet repeat disorders (multiple)	Proteins with tandem glutamine expansions
Familial British dementia	ABri
Familial Danish dementia	ADan
Hereditary cerebral hemorrhage with amyloidosis (Icelandic) (HCHWA-I)	Cystatin C
CADASIL	Notch3
Alexander disease	Glial fibrillary acidic protein (GFAP)
Seipinopathies (multiple)	Seipin
Familial amyloidotic neuropathy, Senile systemic amyloidosis	Transthyretin
Serpinopathies (multiple)	Serpins
AL (light chain) amyloidosis (primary systemic amyloidosis)	Monoclonal immunoglobulin light chains
AH (heavy chain) amyloidosis	Immunoglobulin heavy chains
AA (secondary) amyloidosis	Amyloid A protein
Type II diabetes	Islet amyloid polypeptide (IAPP; amylin)
Aortic medial amyloidosis	Medin (lactadherin)
ApoAI amyloidosis	Apolipoprotein AI
ApoAII amyloidosis	Apolipoprotein AII
ApoAIV amyloidosis	Apolipoprotein AIV
Familial amyloidosis of the Finnish type (FAF)	Gelsolin
Lysozyme amyloidosis	Lysozyme
Fibrinogen amyloidosis	Fibrinogen
Dialysis amyloidosis	Beta-2 microglobulin
Inclusion body myositis/myopathy	Amyloid β peptide (A β), tau, TDP-43, FUS
Cataracts	Crystallins
Medullary thyroid carcinoma	Calcitonin
Cardiac atrial amyloidosis	Atrial natriuretic factor
Pituitary prolactinoma	Prolactin
Hereditary lattice corneal dystrophy	Keratoepithelin
Cutaneous lichen amyloidosis	Keratins
Mallory bodies	Keratin intermediate filament proteins
Corneal lactoferrin amyloidosis	Lactoferrin
Pulmonary alveolar proteinosis	Surfactant protein C (SP-C)
Odontogenic (Pindborg) tumor amyloid	Odontogenic ameloblast-associated protein
Seminal vesicle amyloid	Semenogelin I
Cystic Fibrosis	cystic fibrosis transmembrane conductance regulator (CFTR) protein
Sickle cell disease	Hemoglobin
Critical illness myopathy (CIM)	Hyperproteolytic state of myosin ubiquitination

The Expanded Prion Paradigm:

A basic molecular mechanism driving neurodegenerative disease

Informs a coherent approach to Alzheimer's and many other diseases