Cellular Biochemistry (Part I)

18.12.17

Protein aggregation

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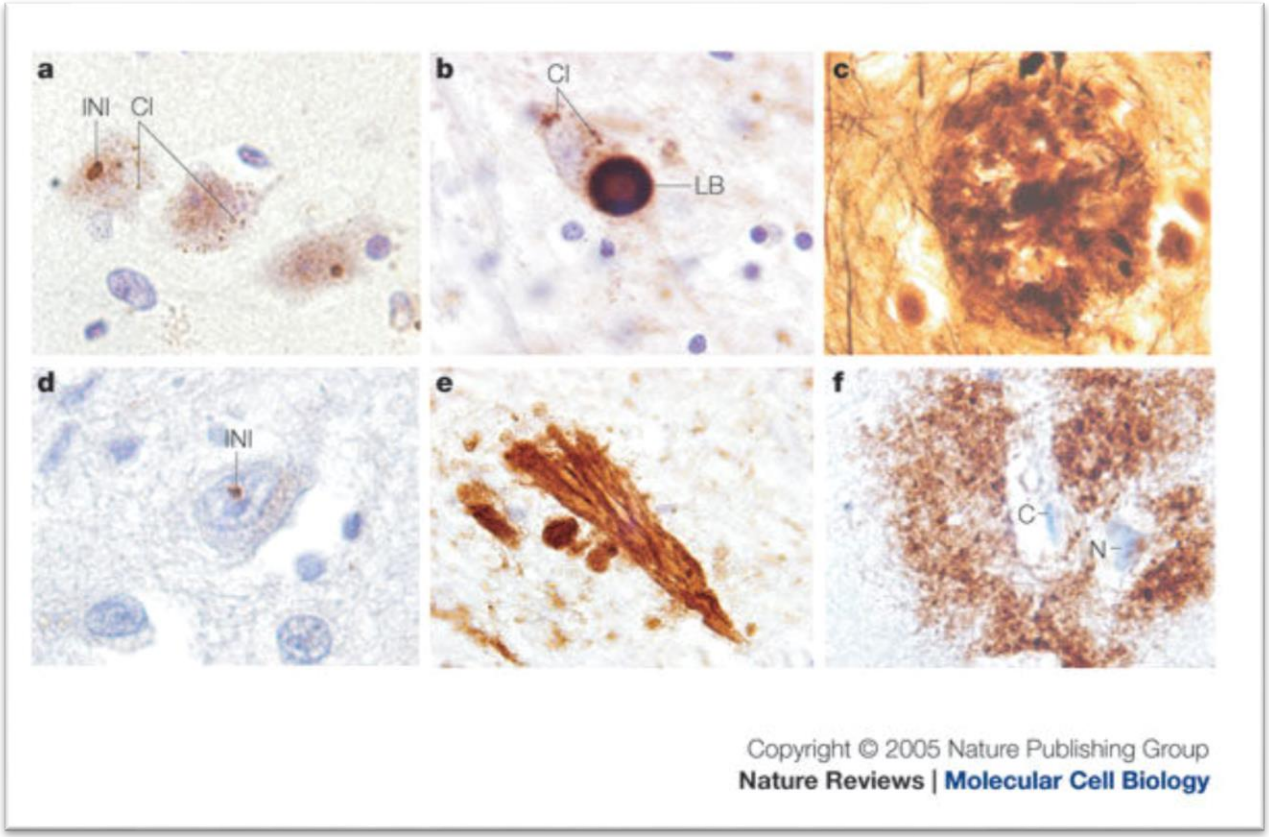
Protein aggregation diseases

Diseases characterized by abnormal protein interactions leading to the deposition of cytosolic, nuclear or extracellular protein aggregates

Other names: Protein conformational diseases, protein deposition diseases, protein misfolding diseases, amyloidosis.



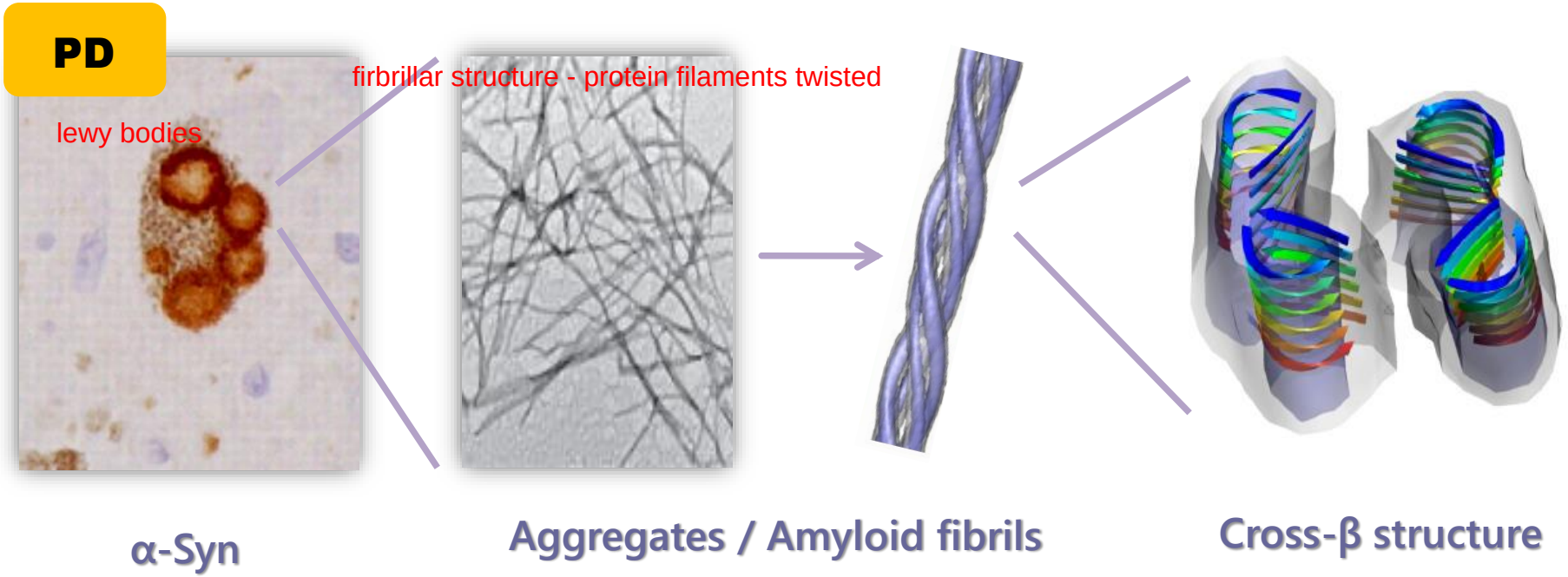
Protein aggregation diseases

- > 40 different proteins have been implicated in protein aggregation diseases

Disease	Protein
Parkinson's disease	**α-Synuclein**
Alzheimer's disease	**Aβ, Tau**
Prion Diseases (e.g. Kuru, CJD, BSE)	**PrP**
Amytrophic lateral sclerosis	**SOD1**
Huntington's disease	**Huntingtin**
Frontotemporal lobar degeneration	**TDP-43, Tau**
Spinocerebellar ataxias	**Ataxin-1,2,3**
Amyloid polyneuropathy I	**Transthyretin**
(...)	(...)

- Diseases can be classified based on the main protein component of the deposits

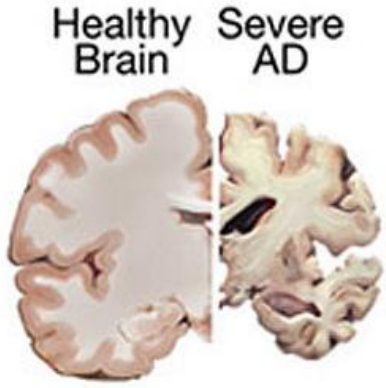
Protein aggregation diseases



"Toxic Proteins in Neurodegenerative Disease", JP Taylor et al., Science, **296**, 1991-1995, 2002

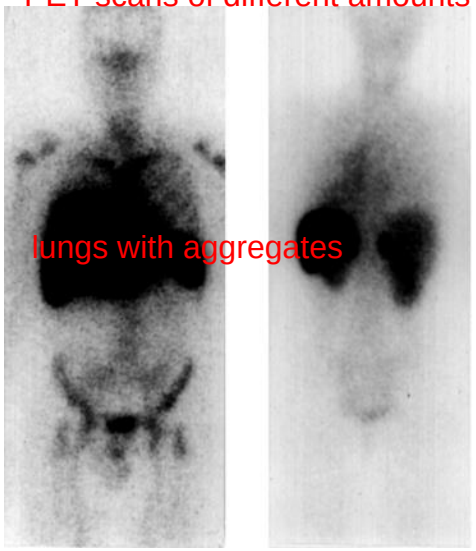
Protein aggregation diseases

Localized - Aggregates arise from proteins expressed by cells at the deposition site. Examples: neurodegenerative diseases like Alzheimer's or Parkinson's disease

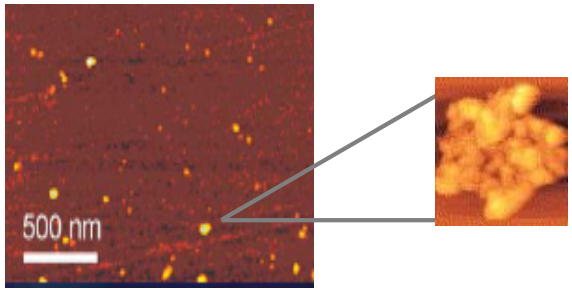


Systemic - Aggregates are deposited systemically after production at a local site, affecting specific or multiple organs. Examples: Transthyretin amyloidosis

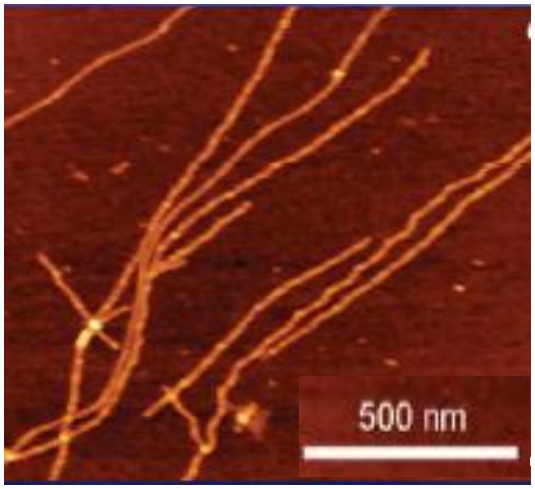
PET scans of different amounts of alymoids



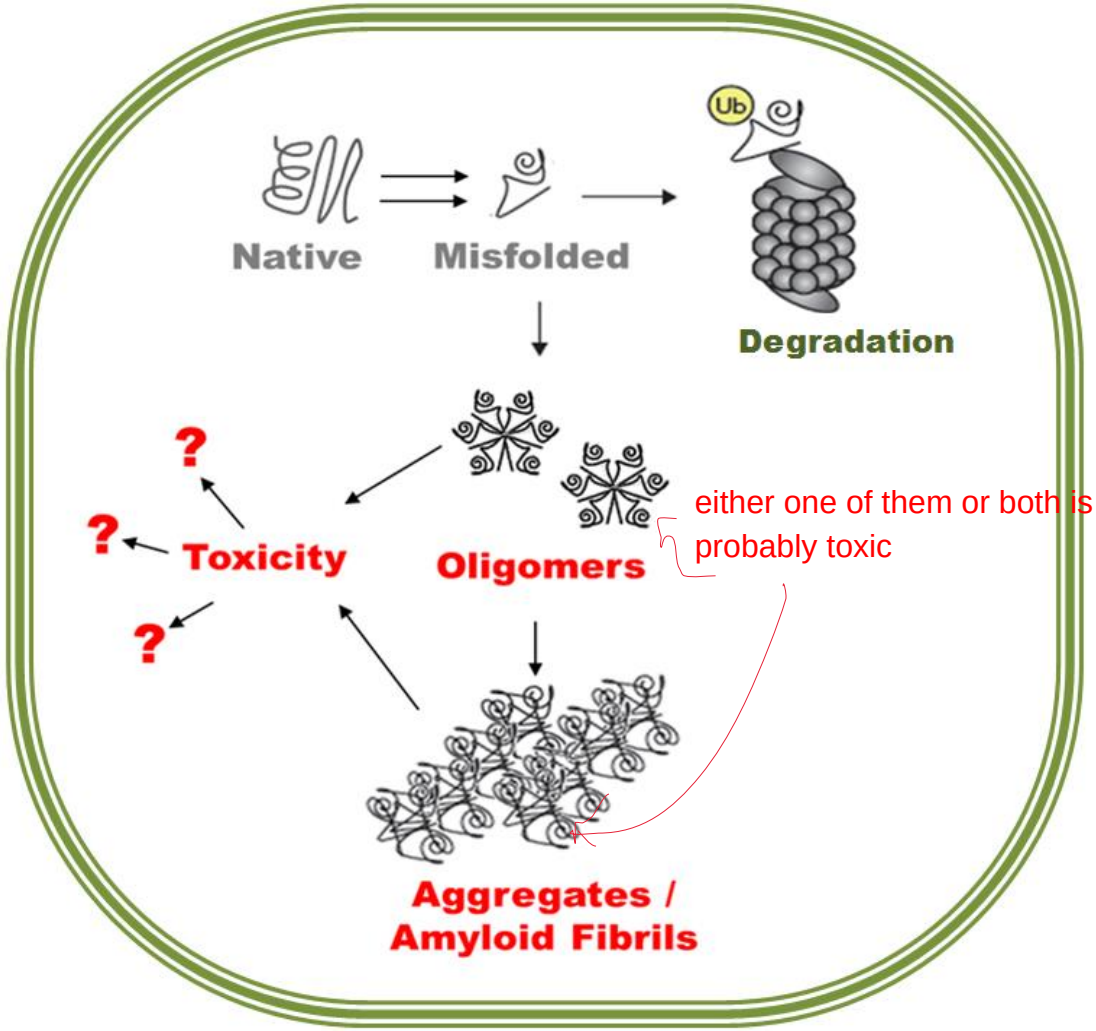
Protein aggregation diseases



Amyloid oligomers

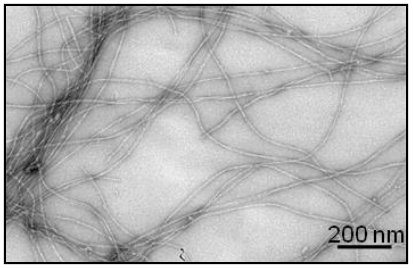


Amyloid fibrils



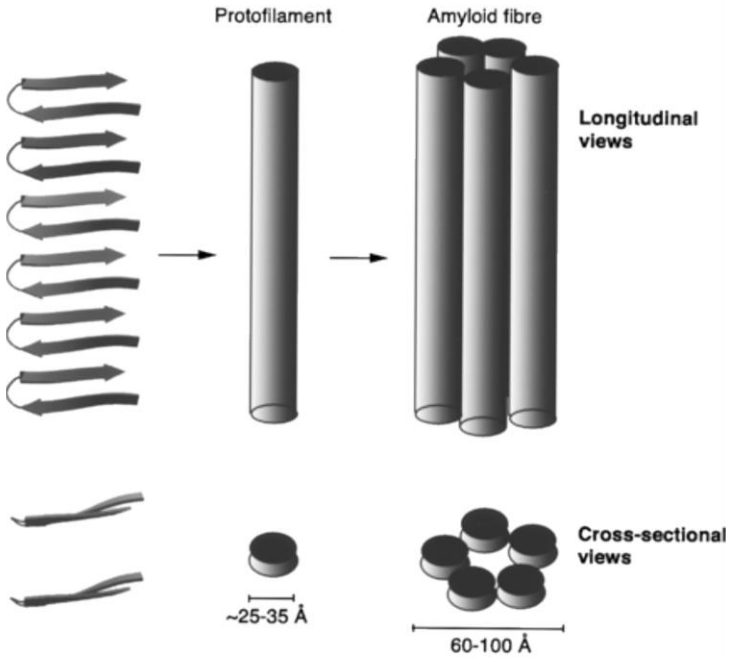
Amyloid: features

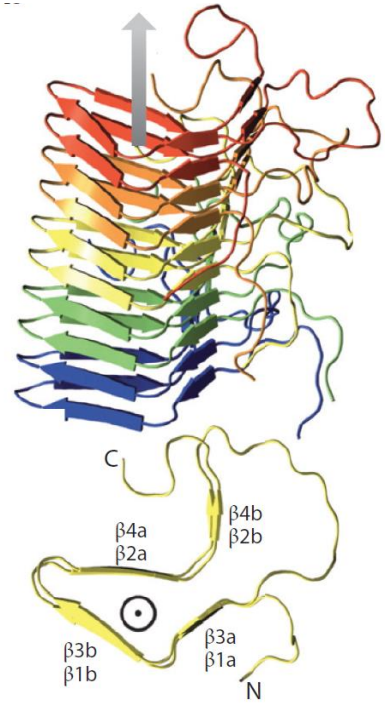
Morphological – Fibrillar appearance on EM, 7-13 nm in diameter, 2-8 filaments, 2-7 nm in diameter, often twisted or associated laterally as ribbons up to 30 nm wide



Structural - Cross-β structure:

β-Strands oriented perpendicularly to the fibril axis and assembled into β-sheets that run the length of the fibril.





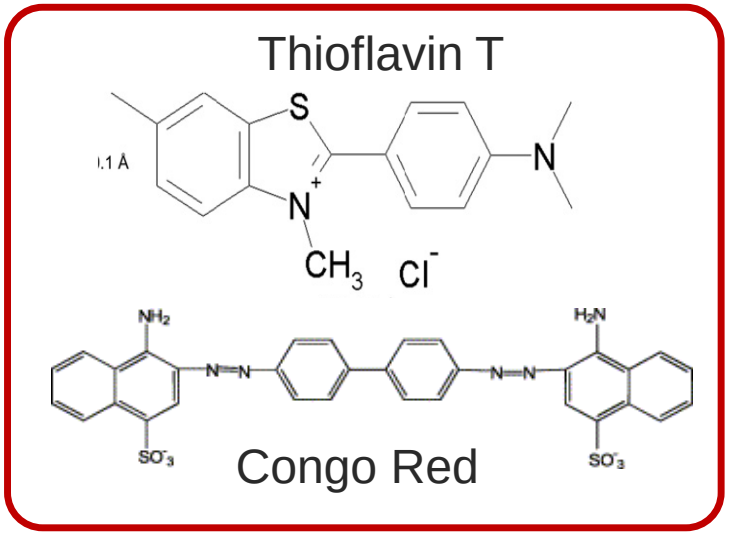
those bind amyloids in general

Tinctorial properties:

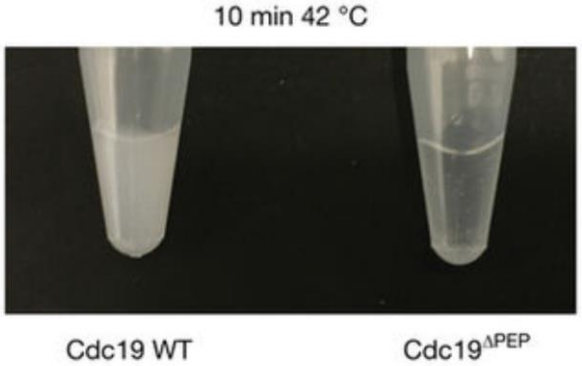
Binding of specific dyes:

Congo Red and **Thioflavin T**

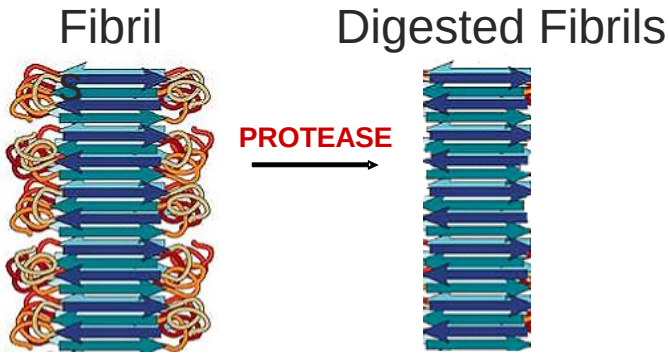
those dyes can enter the amyloid structure, changing their spectoscopic structure



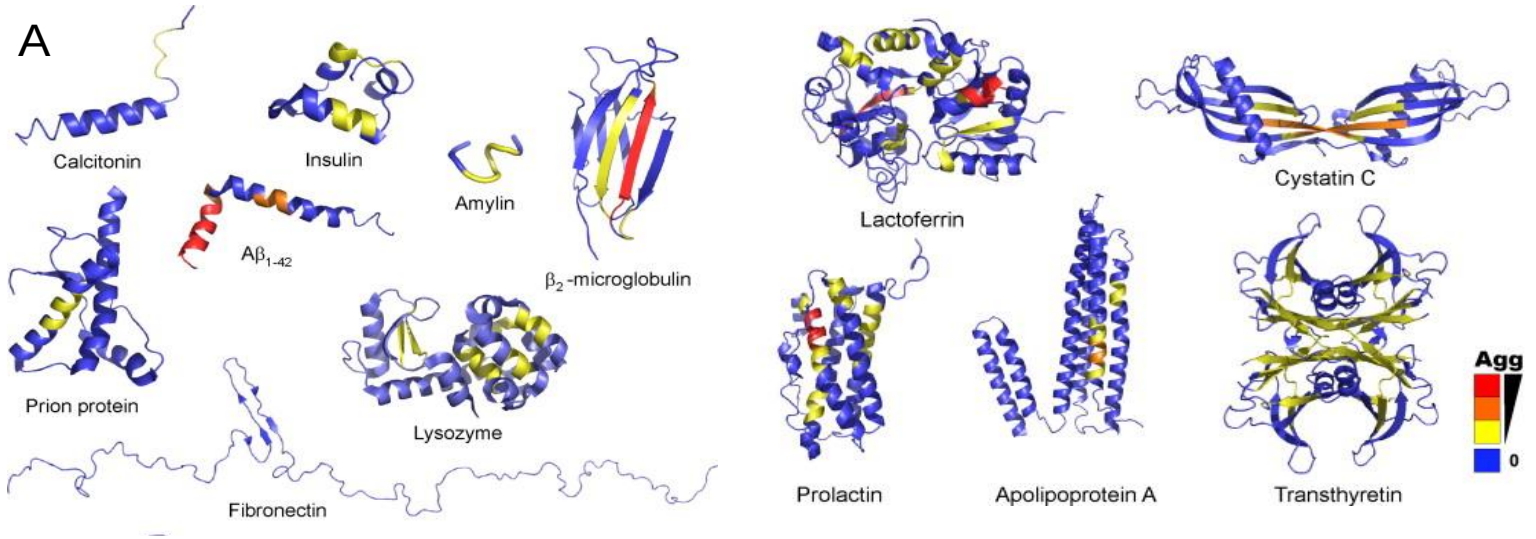
Insolubility in water and buffer in the absence of detergents

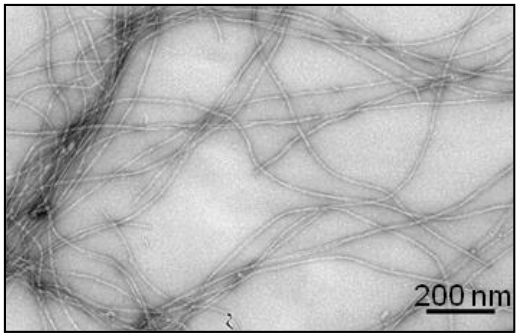


Extreme stability to heat, proteolysis and chemical denaturants. Due to H-bonds between sheets and between protofilaments.

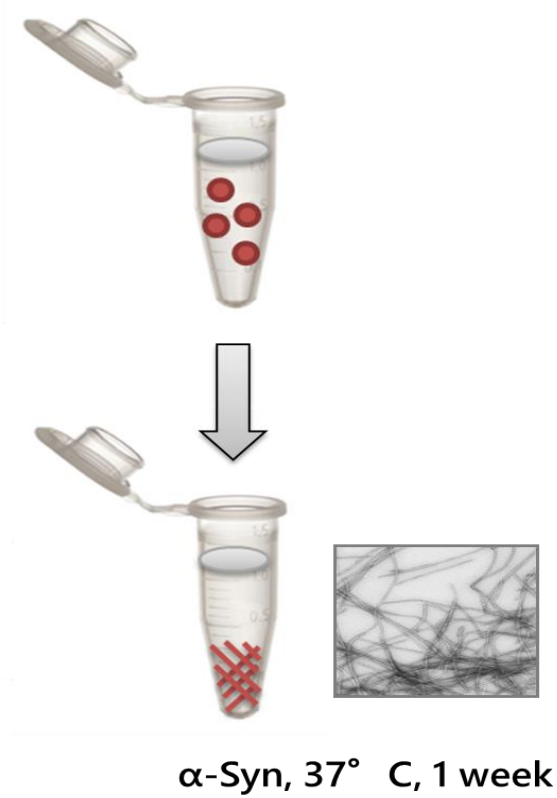


«Generic» nature: Fibrils from different proteins are morphologically and tinctorially similar. Generic nature (in which "generic" indicates common but not identical features) can be attributed to dominance of main chain interactions, with variations resulting from differences in AA seq, chain lengths and incubation conds.

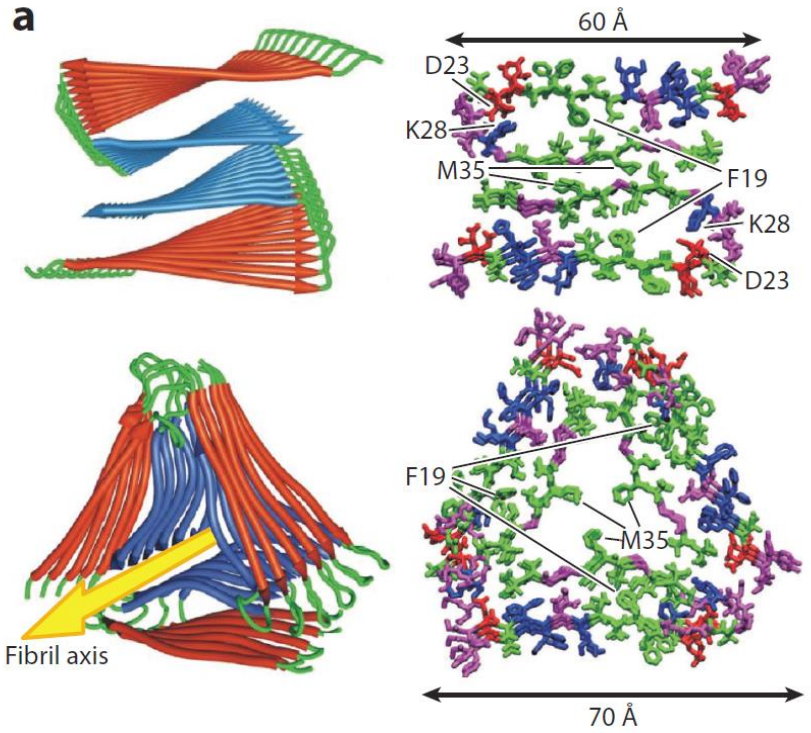




Polymorphism: The same protein sequence can generate fibrils that differ in terms of **molecular structures of protofilaments** and **overall fibril morphology**

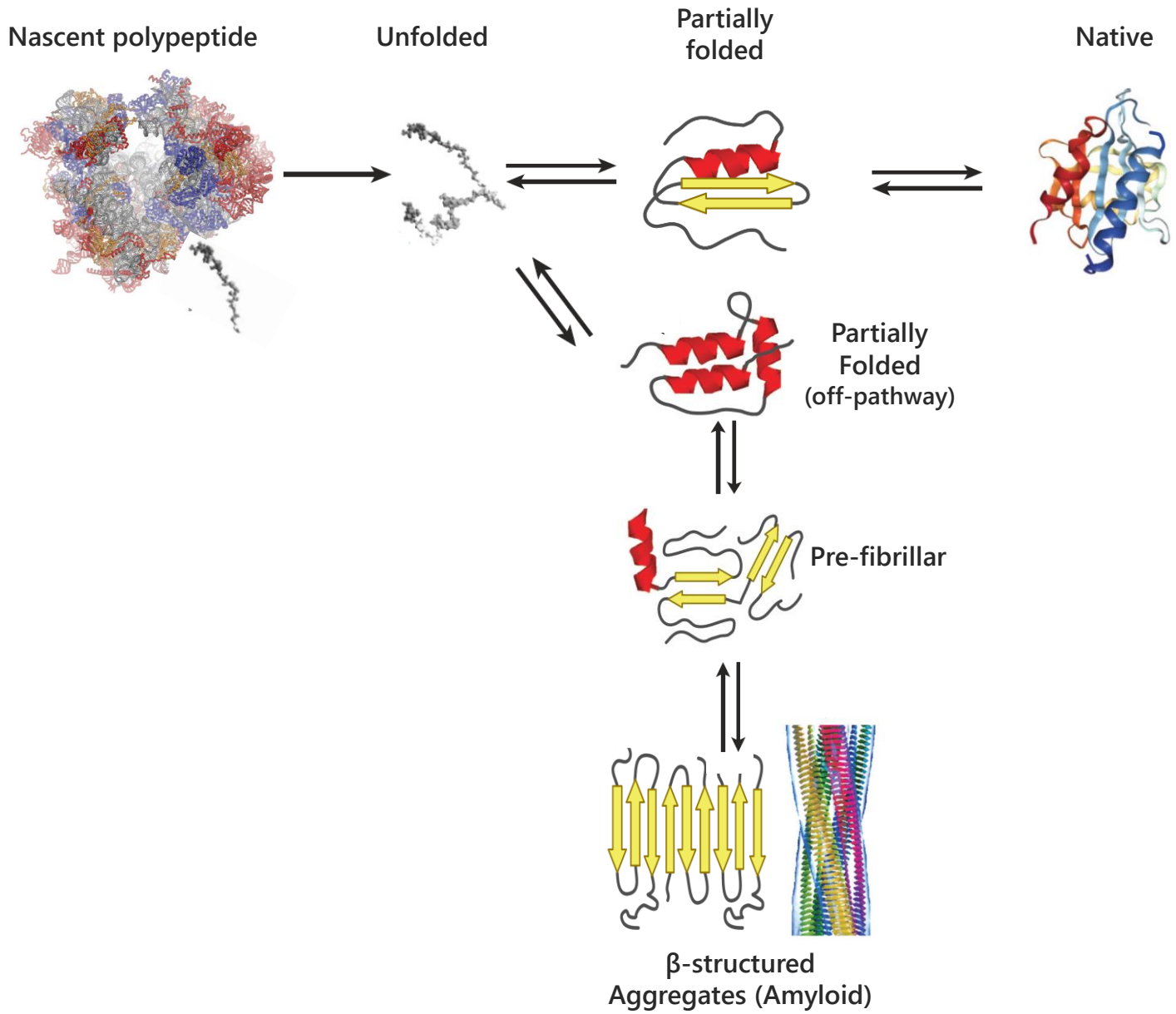


α-Syn, 37° C, 1 week



• Native folds have been selected through evolution. Amyloid architecture may be a consequence of the phys-chem properties of a polypeptide chain.

Protein aggregation: molecular bases



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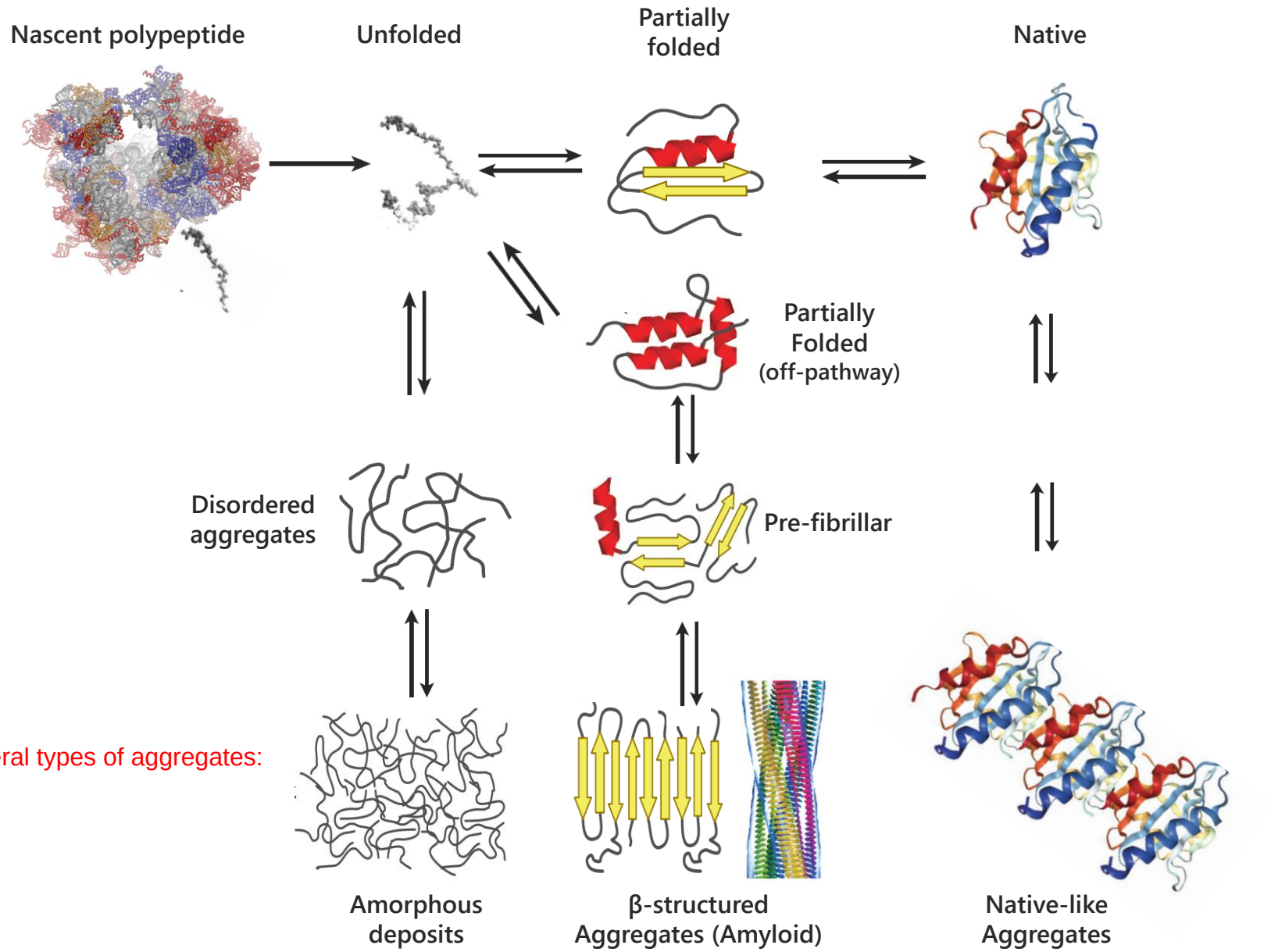


several types of aggregates:

Triggers of protein aggregation

overexpression

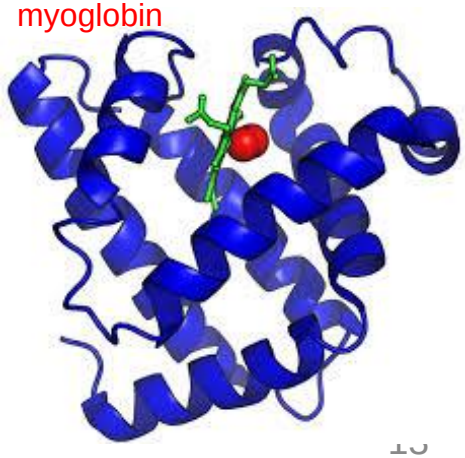
1. Genetic alteration (Mutations, gene multiplication)
2. Proteolysis (or introduction of stop codon)
3. Increase in concentration

prinicipally, every protein can be pushed to form amyloid fibers

4. PTMs (e.g. phosphorylation)
5. Binding of other molecules (e.g. RNA, metabolites, lipids)
6. Alterations of quality control systems
7. Environmental changes (pH, T, oxid. stress…)

→ the myoglobin example

myoglobin



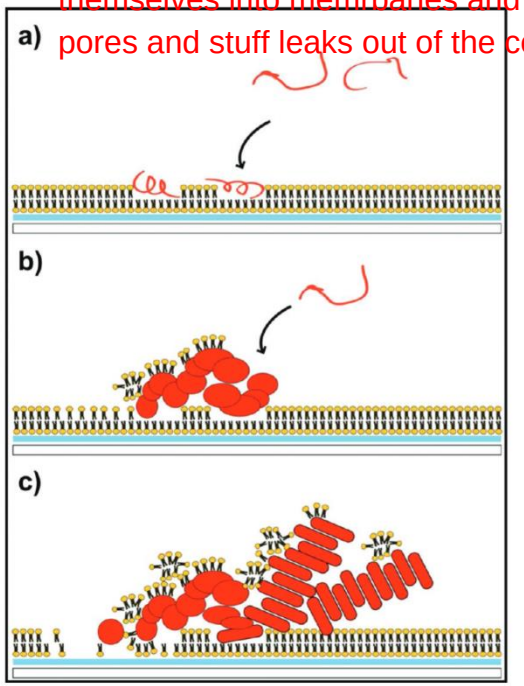
tau when found in aggregates is highly phosphorylated

Faendrich et al., *Nature*, 2001

Cellular effects of protein aggregation (Mechanisms of toxicity)

1. **Loss of function**
2. **Gain of toxicity**:
   - Co-sequestration of crucial cellular factors
   - Membrane damage (pores)
   - Aggregation process itself is toxic
   - Aberrant intracellular interactions
   - Saturation of QC systems
   - Physical damage: replacement of substantial cell and organ mass / volume

membrane damage, some can insert themselves into memrbanes and form pores and stuff leaks out of the cell



Reynolds et al. JACS, 2011

Protein aggregation diseases

- **Familial** → the disorder is genetically inherited and symptoms appear as early as during childhood
- **Sporadic** → patternless, randomly occurring, typically characterized by a later onset
- iatrogenic - induced through medical treatment
- **Iatrogenic** → induced inadvertently by medical treatment or diagnostic procedures
- **Transmissible** → (e.g., prion disease, spongiform encephalopathies and fatal familial insomnia)

how to check if amyloid: drop in congo red and see if it is colored red afterwards: if yes, then dealing with a amyloid aggregate

Protein aggregation diseases

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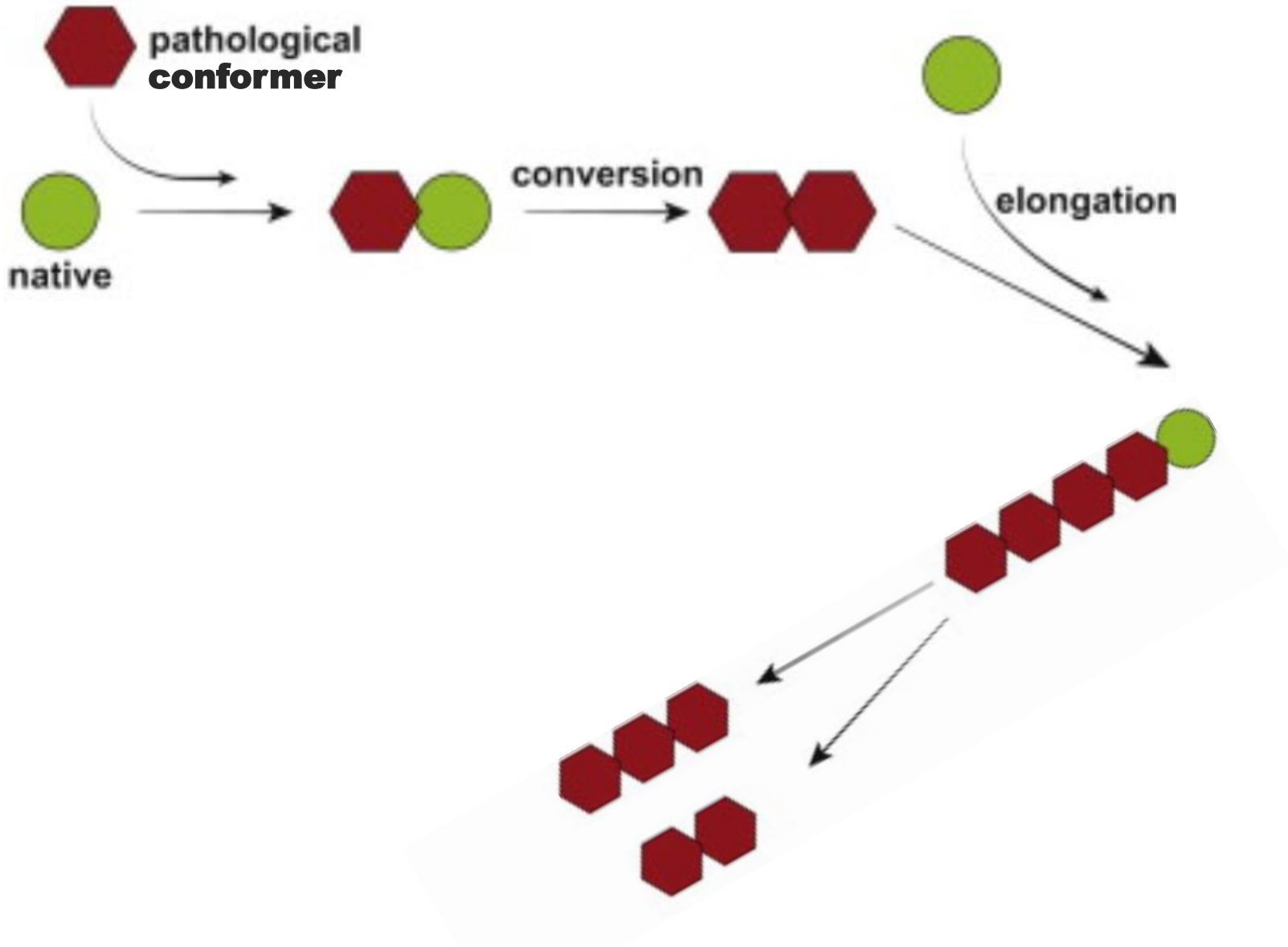
The seeding process



Transmissibility of PADs amyloids

- Population of sheep inoculated against a virus with formalin extract of brain tissue from animal with scrapie. 2 years later: 10% of population developed scrapie
- In humans, kuru propagated among the cannibalistic tribes of New Guinea they ate the brains of dead people as death ritual
- Kuru and scrapie subsequently experimentally transmitted to sheep, mice and monkeys
- Is it a virus?

Protein-only hypothesis

- Material responsible for transmission of TSEs might be a protein that has the surprising ability to replicate in the body

- Prion: new proteinaceous infectious particle

(Clarke S, Griffith LS, Prusiner S, Weissman C)

Supporting evidence

- Agent is resistant to UV and ionizing radiation (nucl. acids are not)

  MW : >2 × 10^5 Da
- Isolation of protease-resistant infectious material (PrP^{res})
- Highly purified preps of PrP^{res}, in which no other component was detectable, retained infectivity
- Infectivity reduced by agents that destroy protein structure and by antiPrP antibodies
- Most familial cases of TSEs: mutations in PrP gene
- Mice lacking PrP: resistant to scrapie
- PrP^{res} catalyzed conversion of PrP^{C} into PrP^{res} in vitro

  (…) pathology induced by incoulation of in vitro preformed firbils in animals overexpressing precursos protein

Criticism

- TSEs occur in multiple 'strains' (e.g. Incubation period, clinical features). In infectious diseases, strains arise from mutations in the genetic code of infectious agent

  → Alternative conformational states of amyloids

- Quantity of PrP^{res} correlates with infectivity but infectivity can be propagated in the absence of detectable PrP^{res}

  → Oligomers

- Small quantities of nucleic acids detected in infectious samples
- Lack of infectious origins for other neurodegenerative but…

Prionoids: Aß in AD

- **Alzheimer's Disease**: Injection of the Aß peptide from human AD brains induced aggregation of Aß in transgenic mice overexpressing precursor protein

- No evidence of transmission between individuals.

Prionoids: α-Syn in PD

- **Parkinson's disease:** Exogenous α-synuclein fibrils induced the formation of intracellular inclusions in cell culture (Luk et al., 2009)
- Inoculation into transgenic mice and wild-type animals induces pathology

- No evidence of transmission between individuals.

Prions and prionoids

- **Prions**: Infectious agents, transmissible between individuals, and tractable with microbiological techniques—including, e.g., titer determinations.

(both are amyloids obviously)

- **Prionoids**: Infect neighboring molecules and neighboring cells, propagate within an organ, but do not propagate within communities. Not found to cause macroepidemics.

A. Aguzzi, L. Rajendran