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# The prion paradigm and structural information transfer

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► **To cite this version:**

Pierre Sibille. The prion paradigm and structural information transfer. Master. UE d'épigénétique, Paris, France. 2020. hal-04236436

**HAL Id: hal-04236436**

**<https://hal.science/hal-04236436v1>**

Submitted on 10 Oct 2023

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# The prion paradigm and structural information transfer: Structural Epigenetic and prion pathologies

How to propagate a biological information without  
any support of DNA or RNA??

## From a cellular point of view...

There are two transmission types for biological information:

### **Classical Mendelian transmission:**

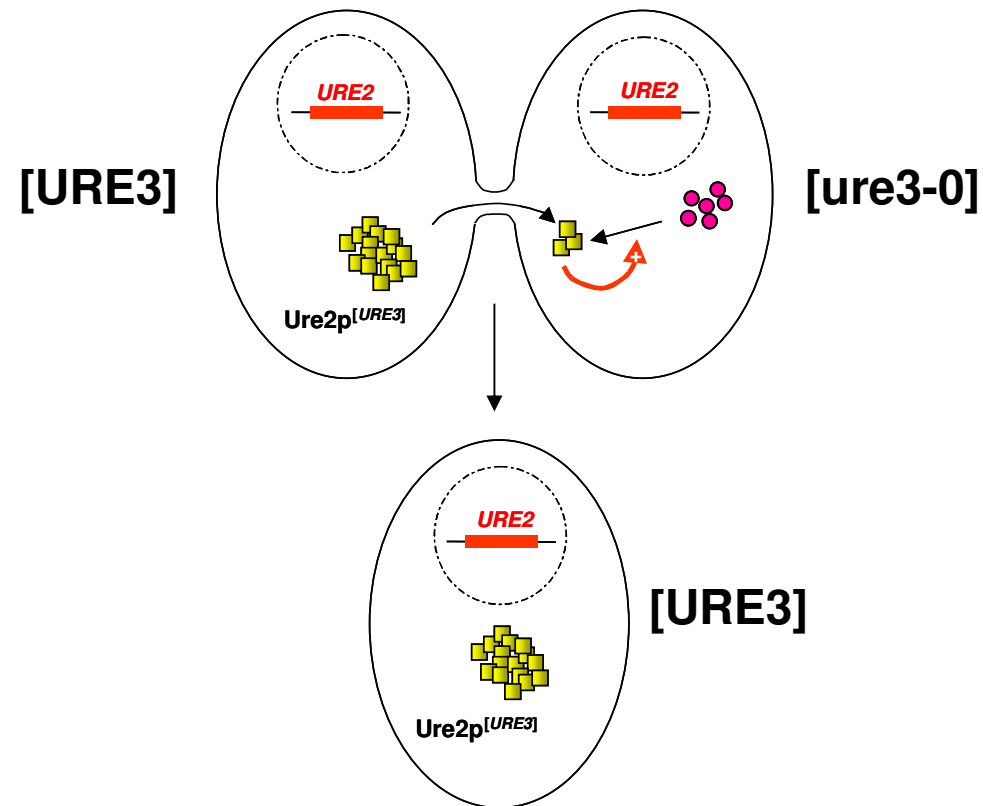
- Gain of function
- Loss of function
- Long-range transmission

### **Non-Mendelian transmission:**

- Dominant
  - Loss/gain of function
- Cytoplasmic inheritance  
(Cytoduction)

Non-Chromosomal transmission!

## Example of cytoduction transmission



### Recommended lecture:

Curr Biol. 1994 Aug 1;4(8):744-8.

**Cytoplasmic inheritance. Prion-like factors in yeast.**

Cox B<sup>1</sup>.



What is the support of the information  
?

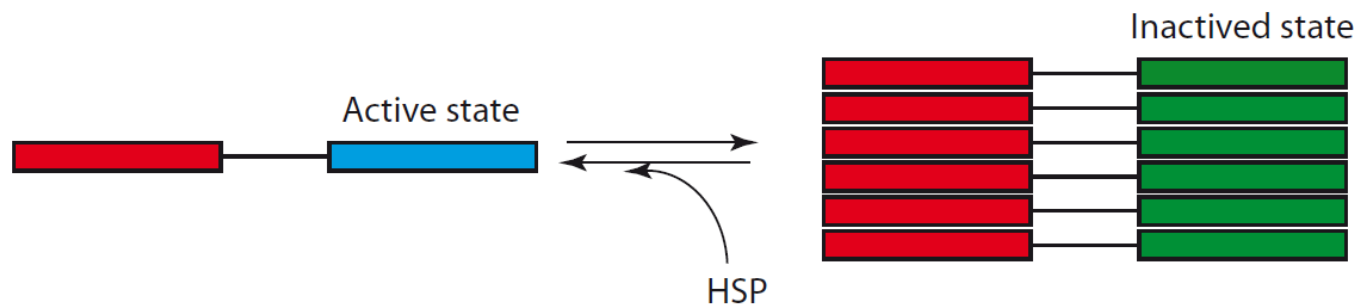
# Propagation of information by structural modification: Concept of alternative folding

Simple mechanism mainly based on the modulation of aggregation propensity of a protein :

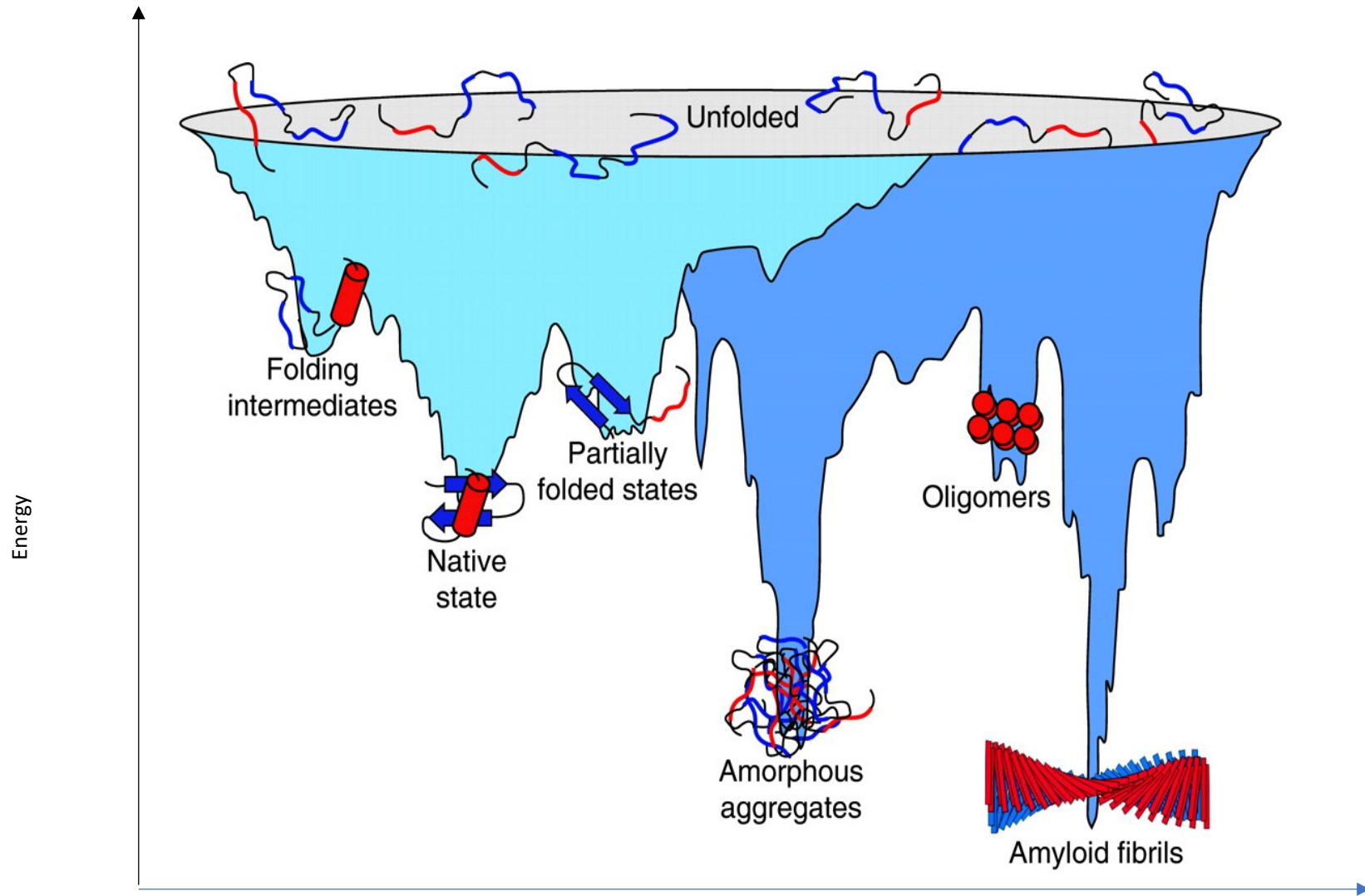
Aggregation state could generate :

- Gain of function
- Inactivation

The system should be reversible but robust!



# Alternative folding pathways

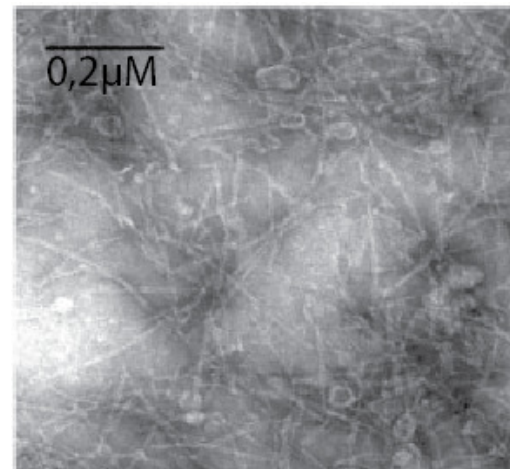
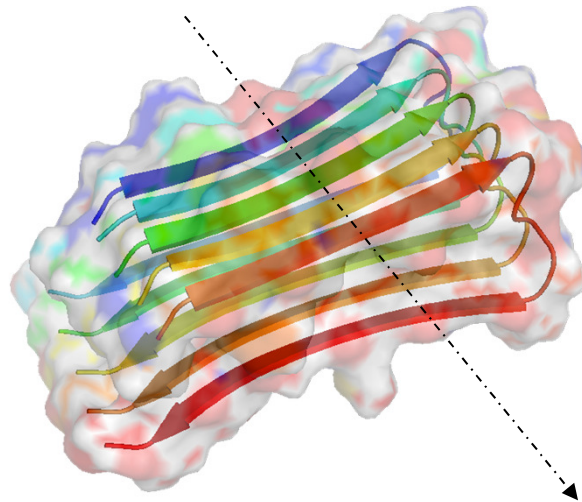




## Les assemblages de type amyloïde

- Assemblages périodiques, non-covalents de type fibrillaire non-anastomosé
- Structure secondaire très riche en feuillets- $\beta$
- Feuillet- $\beta$  orientés perpendiculairement à l'axe des fibres
- Structures thermodynamiquement très stables

Axe de la fibre



Amyloïde A murin

# Amyloïdes pathologiques

**Localisation non-neuronale**  
diabète type II (amylin or IAPP)

**Systémique**  
Amyloïdose AA (SAA)

Amyloïdose ApoAI (ApoAI)

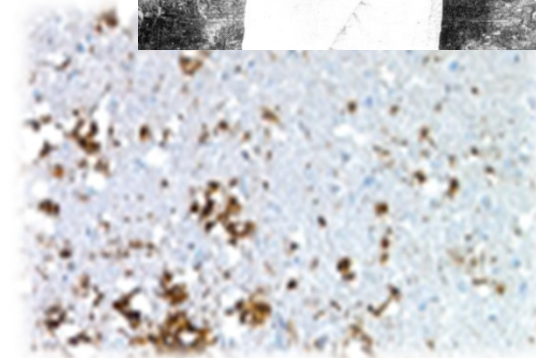
**Neurodégénérative**

Les EST (PrP)

Alzheimer ( $A\beta$ )

Parkinson ( $\alpha$ -synuclein)

...

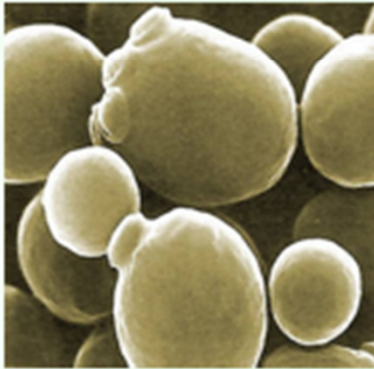




# Amyloïdes fonctionnels

## Éléments épigénétiques

Sup35 (Levures)



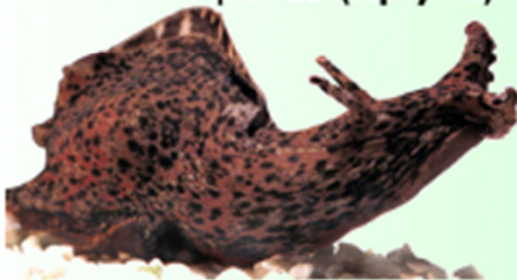
## Nanomatériaux

Fibre de la toile  
(*Nephila edulis*)

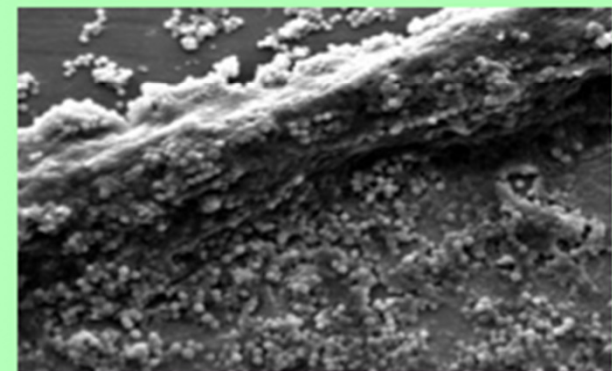
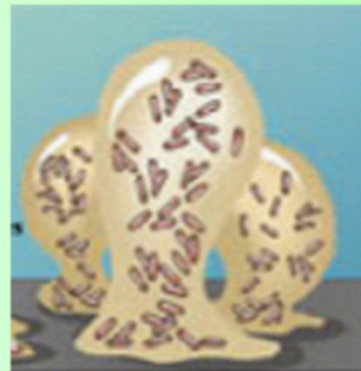


## Facilitation synaptique

ApCPEB (*Aplysia*)



Biofilm bactérien: Curli (*E. Coli*)



# Relation between protein misfolding and protein aggregation

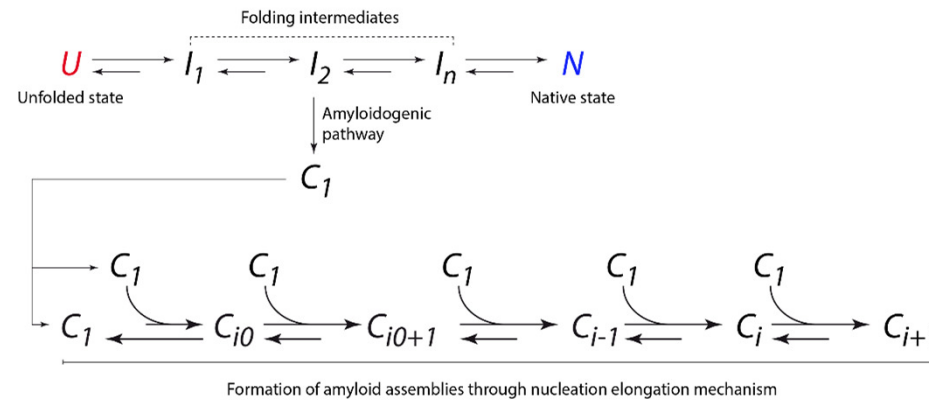
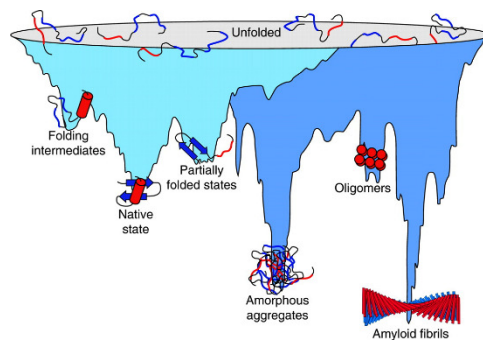
- Protein folding is dictated by the general laws of thermodynamic.

For  $t \rightarrow \infty$  a protein reaches its maximum of stability (i.e. lowest  $\Delta G$ ) through a pathway called “folding pathway”.

The search for this minimum does not correspond to a random exploration of all possible configuration.

- All along the folding pathway there exist different folding intermediates with specific properties.

Amyloidogenic pathway is one of them.

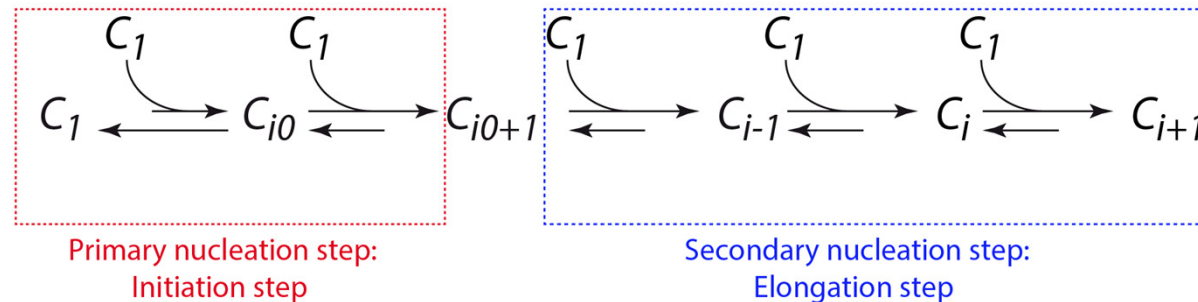




# Introduction of prion concept in protein misfolding disease

The nucleation elongation process can be roughly divided in two process:

- A primary nucleation step responsible for the ignition
- A secondary nucleation responsible for elongation



From a biochemical point of view, in the secondary nucleation pathway if  $C_{i>1}$  is structurally different from  $C_1$  then process become a Templated Assisted Conversion :

## Prion-like propagation

↳ from a molecular point of view  $C_{i>1}$  transfer its structural information to  $C_1$

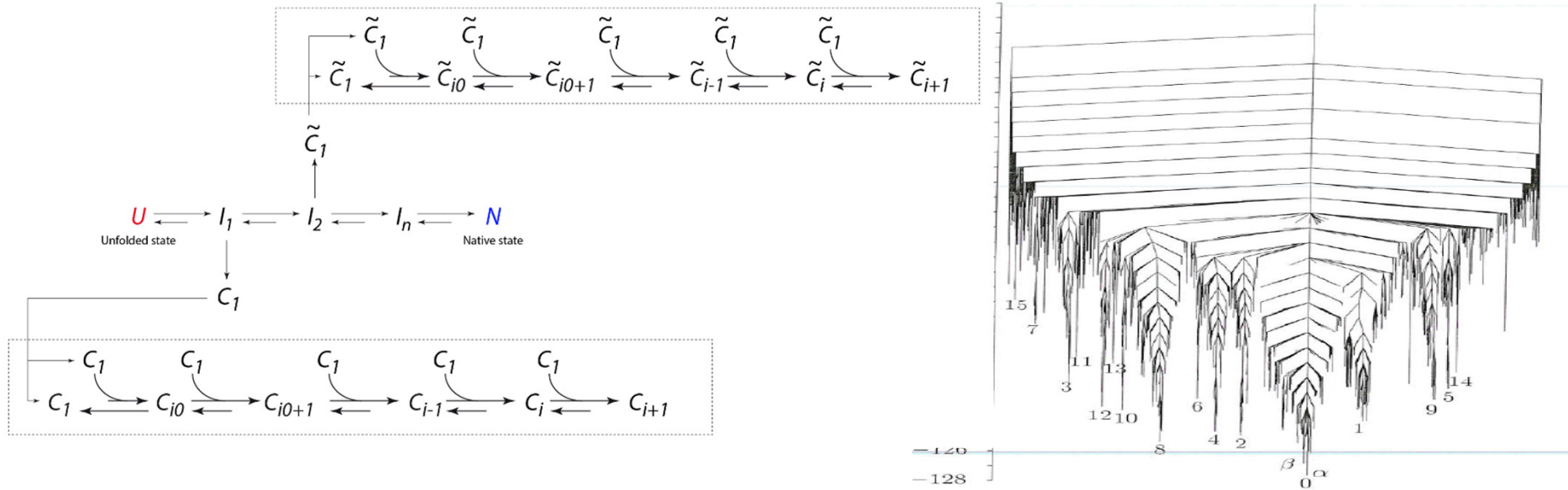
# Existence of multiple misfolding pathways

Things are more complicated than they appear!

For aggregation process there are several pathways in competition.

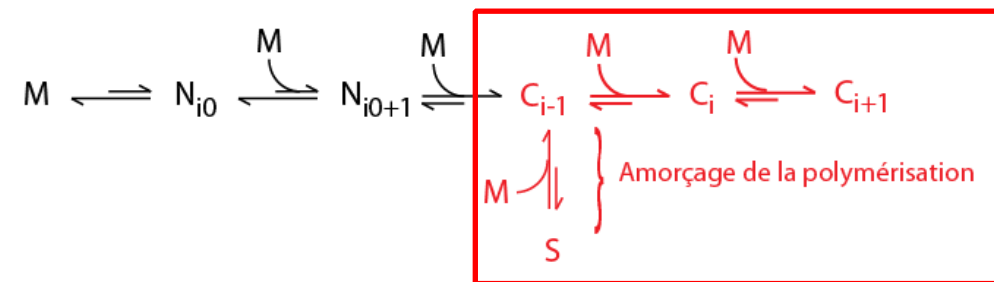
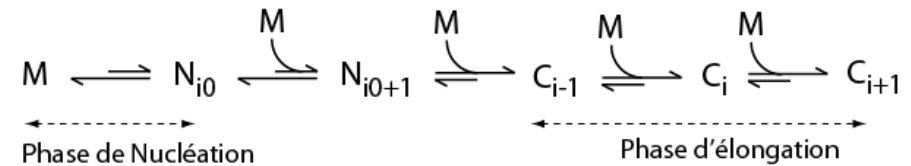
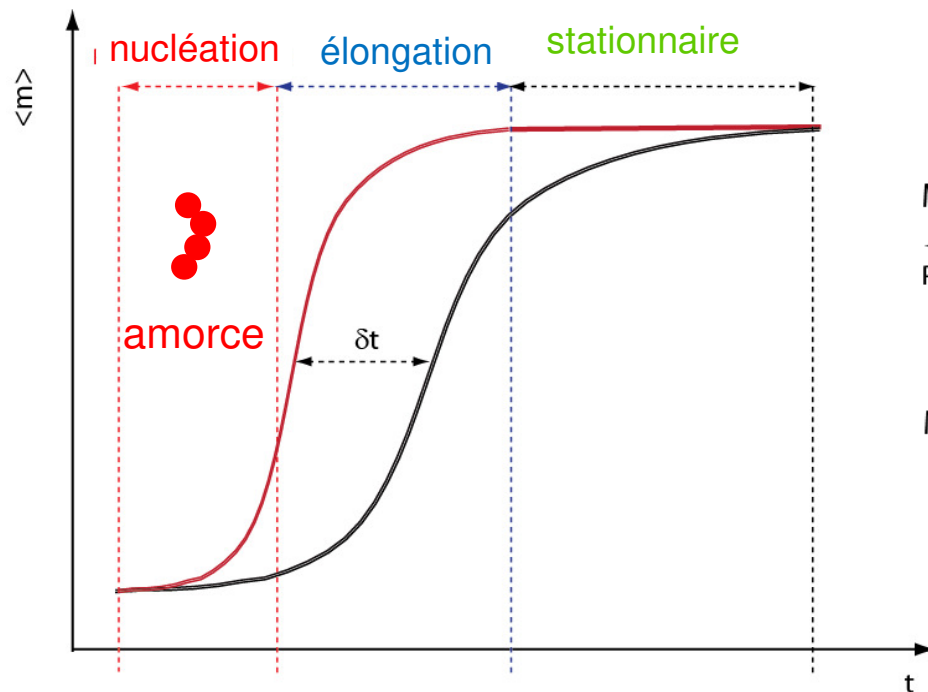
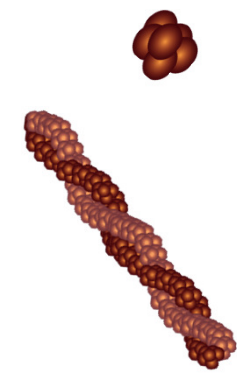
How are these pathways entangled ?

Identification of “relevant” intermediates, etc...



# Formation des fibres amyloïdes : mécanisme de type nucléation polymérisation

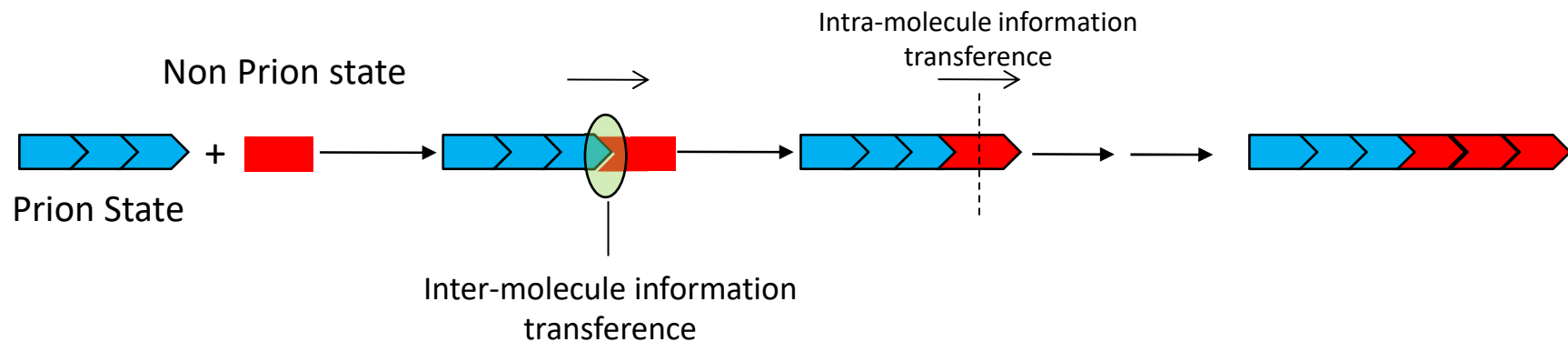
- 1 - phase de **nucléation** : phase initiale, création d'un nucleus  
peut être court-circuitée par l'ajout d'amorce (oligomères ou protofibrilles)
- 2 - phase d'**élongation** (rapide) : déterminée par la concentration en substrat
- 3 - phase **stationnaire** déterminée par l'épuisement du substrat



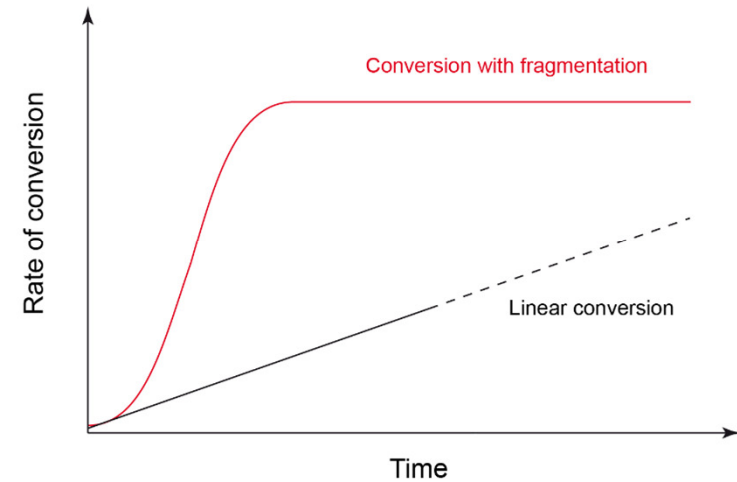
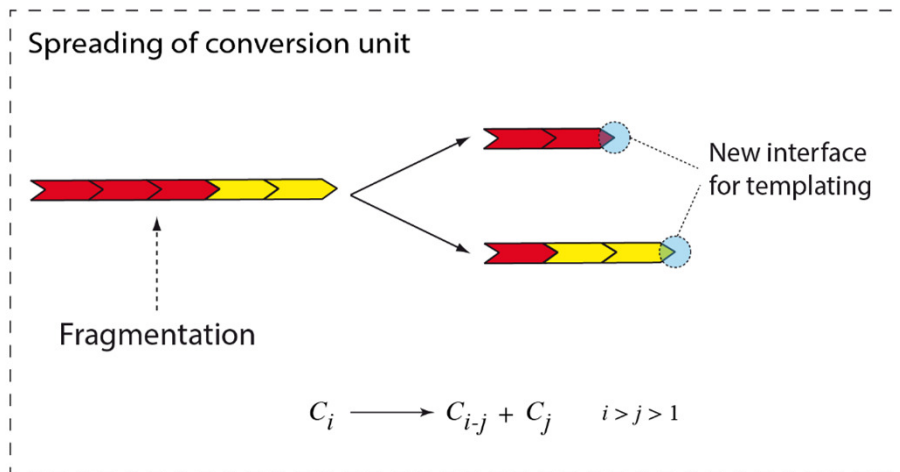
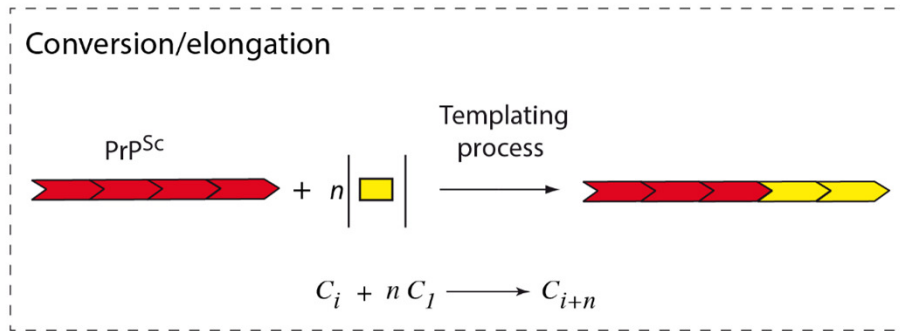
# The Prion theory

## The prion theory implies the perpetuation of a structural information

1. The existence of a Prion object that constitutes the support of the structural information
2. A non-Prion state that constitutes the “receiver”
3. A segment of interaction between the Prion and the receiver: This segment constitutes a zone through which the structural information transits



# The process of prion spreading/amplification by fragmentation



→ The spreading process which is a part of prion propagation theory implicitly needs a fragmentation process to take place

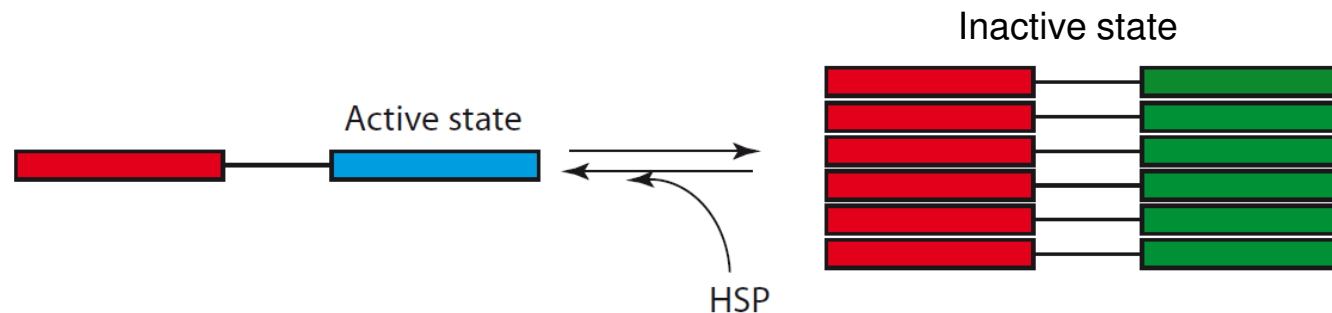
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Simple mechanism mainly based on the modulation of aggregation propensity of a protein :

Aggregation state could generate :

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- Inactivation

The system should be reversible-but robust → **Pathology**



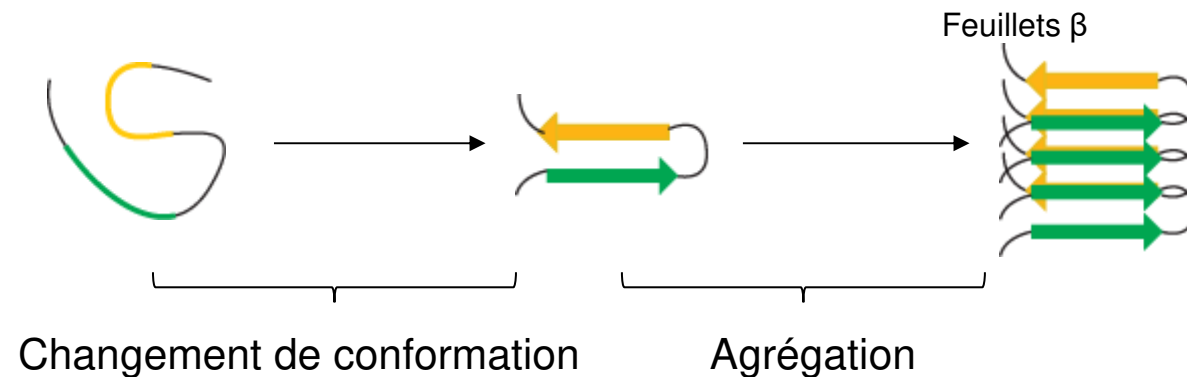


## Prion pathology

- 1 – The concept of protein misfolding and aggregation
- 2 – PrP<sup>C</sup> protein : the “good” prion conformer
- 3 – Prion pathology and the “bad” conformer
- 4 – Validation of Prion hypothesis
- 5 - Molecular basis of the Prion strains

# Les pathologies liées au mauvais repliement des protéines

- De nombreuses pathologies sont dues au mauvais repliement et à l'agrégation des protéines



- Toute perturbation de l'état natif peut favoriser le processus de conversion

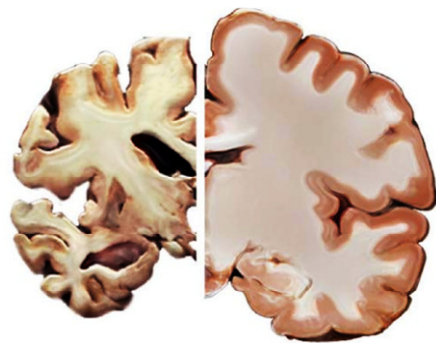
➔ Une des conséquences du mauvais repliement des protéines est la formation **d'assemblages de type amyloïde**



# How protein misfolding could induce physiological disorder?

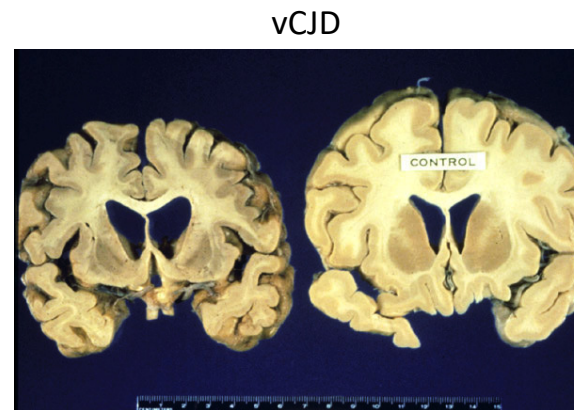
To date what we are sure of is that physiological disorder is the consequence of misfolding  
However the nature of misfolded species involved is uncertain!

In terms of physiology the consequence of misfolding could result from either:  
Loss of function (neuroprotective role, antioxidizing function...)  
Gain of function. Toxicity could be considered as a gain of function



Alz

Control



CONTROL



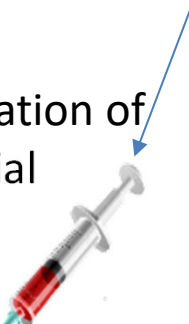
# The prion pathology

# Proof of transmissibility of the scrapie TSE agent

scrapie



intraocular inoculation of  
spinal cord material



1 year incubation



1<sup>st</sup> experimental transmission in 1936



Cuillé



Chelle

**TSE due to a  
slow virus???**

# kuru: EST acquired human disease



**En 1956**



**Gajdusek**

Fore tribe in Papuaia with endocannibalism rites

- Kuru mainly affects children and women (65%)
- Spongiosis of the brain at necropsy but no inflammation (excluding infectious disease ?)

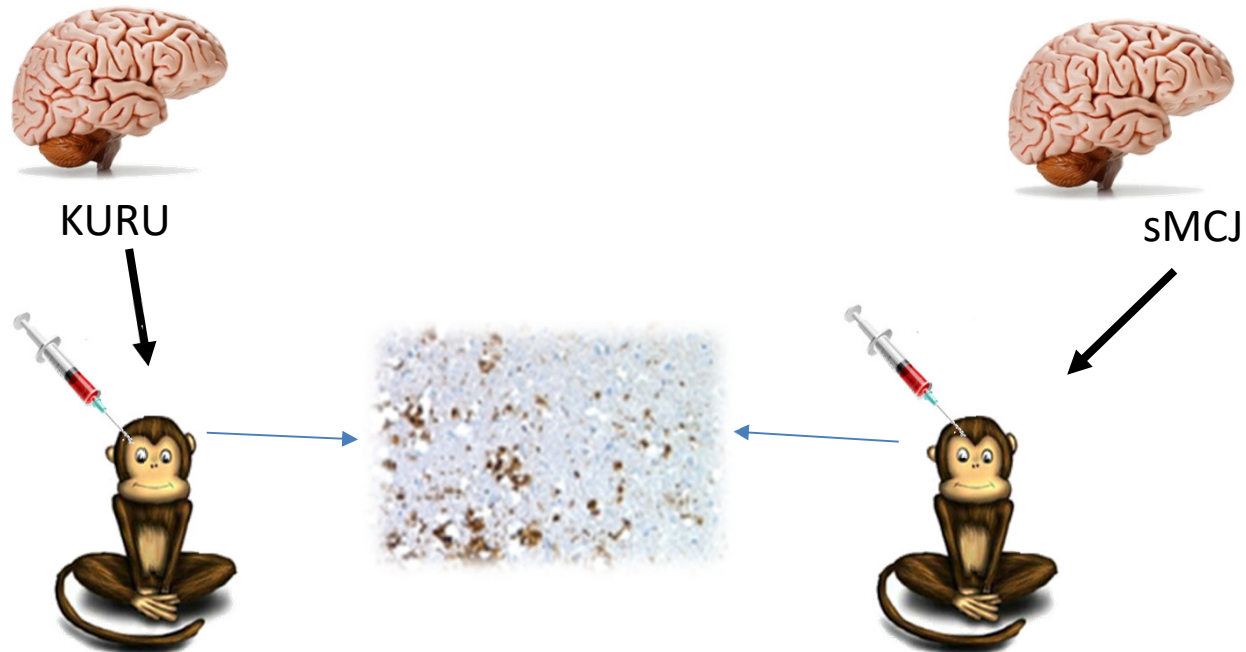
Gajdusek suspects that the disease spreads through mortuar cannibal ceremonies. But concludes with a slow neurotropic virus (Medicine Nobel Prize in 1976)

# Preuve de transmissibilité des EST humaines

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Gadjusek



Il démontre:

- la transmissibilité du kuru et de sCJD
- Que ces 2 maladies provoquent les mêmes lésions cérébrales chez le chimpanzé
- Conclut à un virus lent

Ces travaux lui ont valu le Prix Nobel de Médecine en 1976

# The protein only hypothesis

scrapie due to a slow virus???



**BUT:** In 1967 Alper demonstrated that the agent responsible of TSE is **resistant** to all techniques used in sterilisation



**No RNA & DNA!**

In 1967



J. S. Griffith

scrapie agent was a **proteinaceous infectious particle**

=

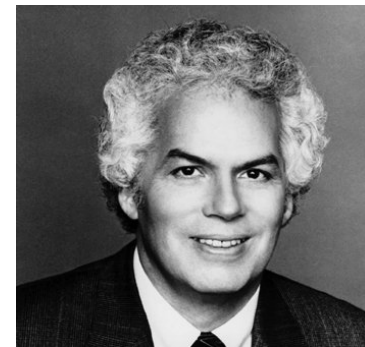
**Prion**

A protein that could exist under two conformations: one physiologic and one pathogenic



The **PrP protein** found in amyloid plaque

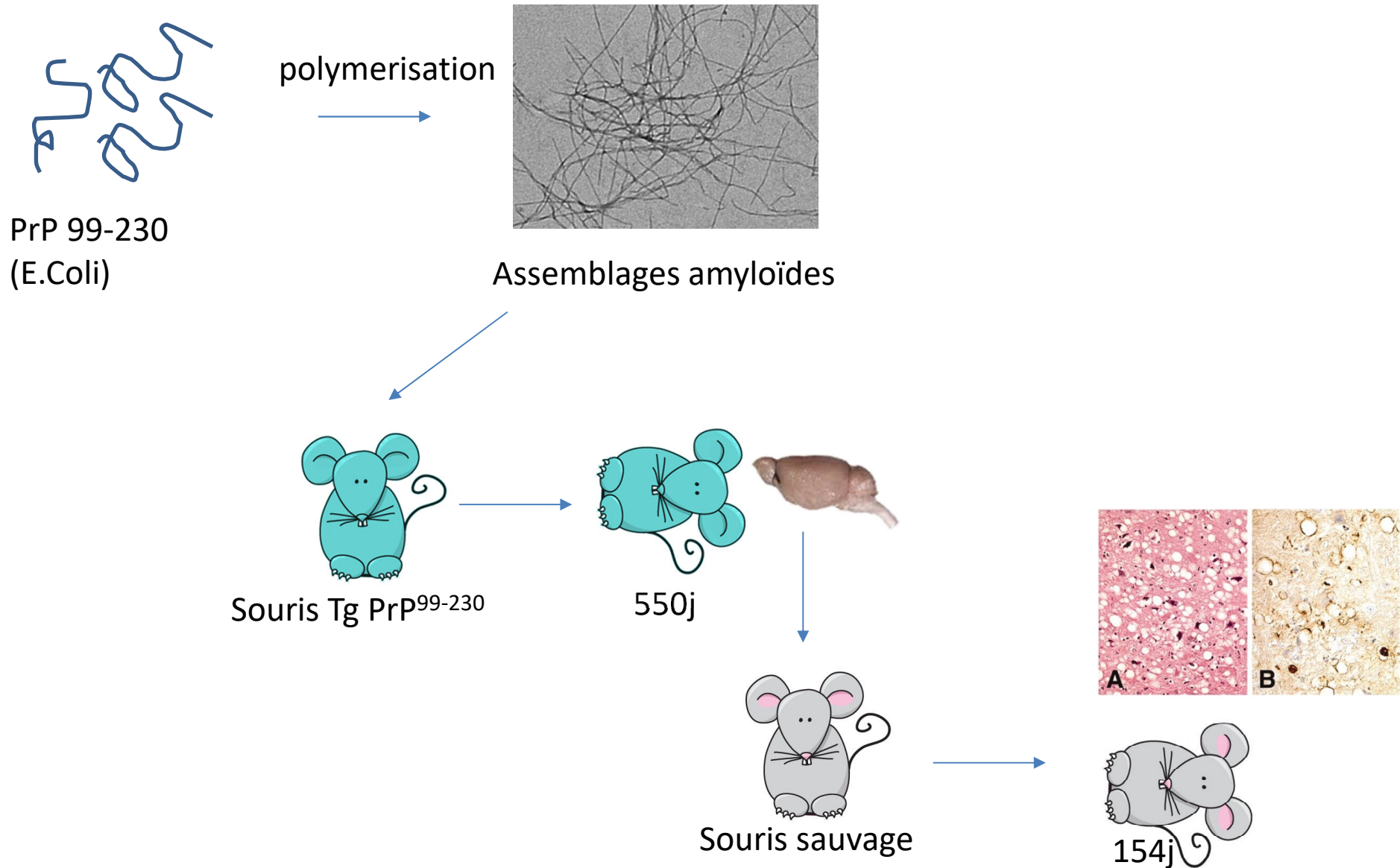
In 1982



S. Prusiner



# Le prion synthétique: dernière preuve irréfutable de l'hypothèse « protein-only »





## Prion pathology

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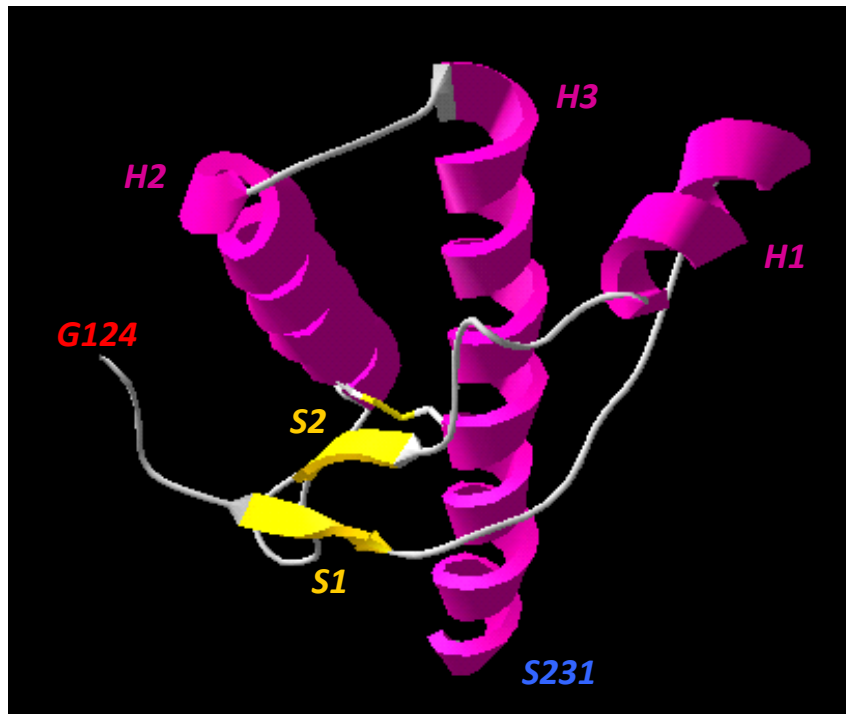
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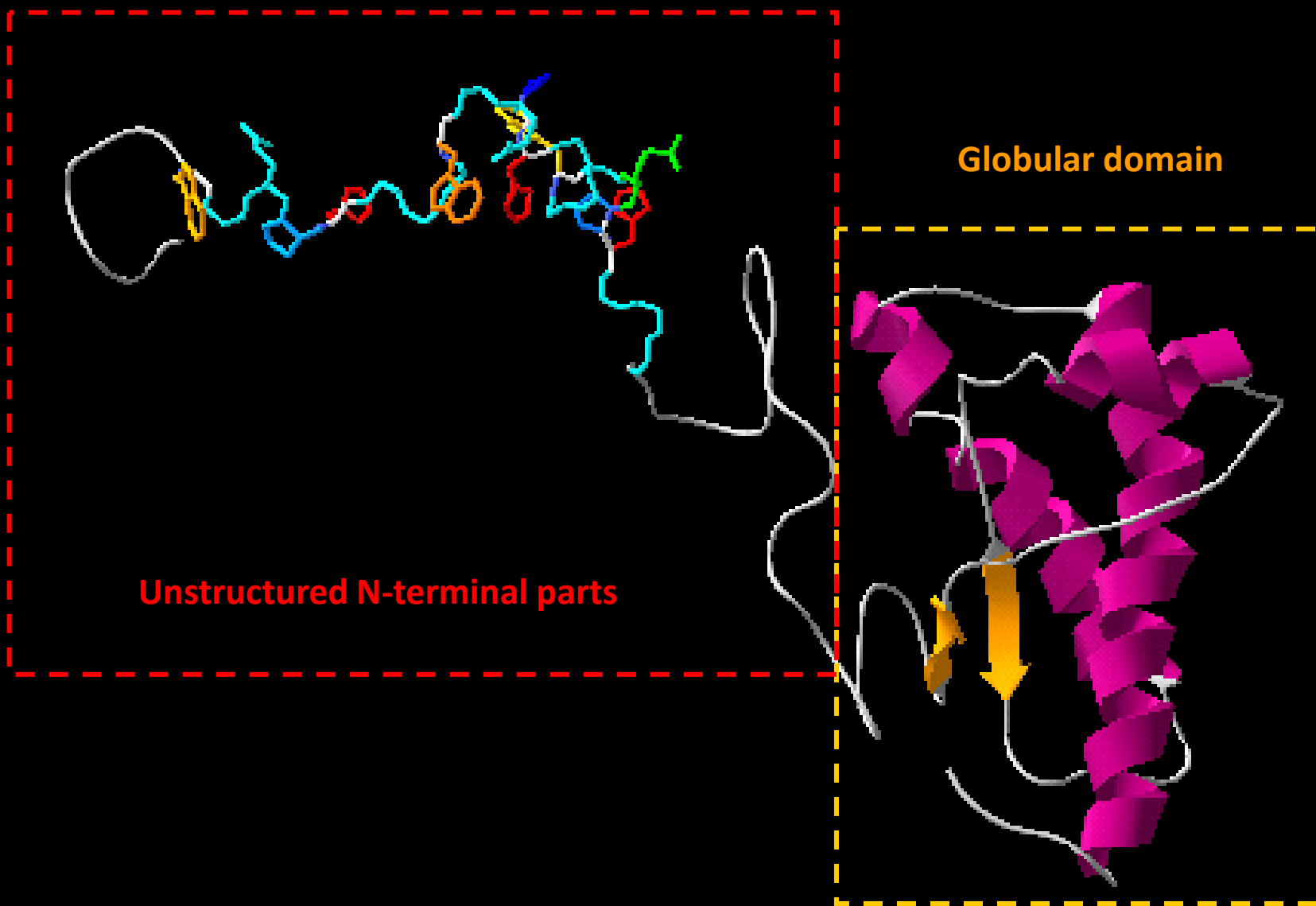


# The PrP<sup>C</sup> structure

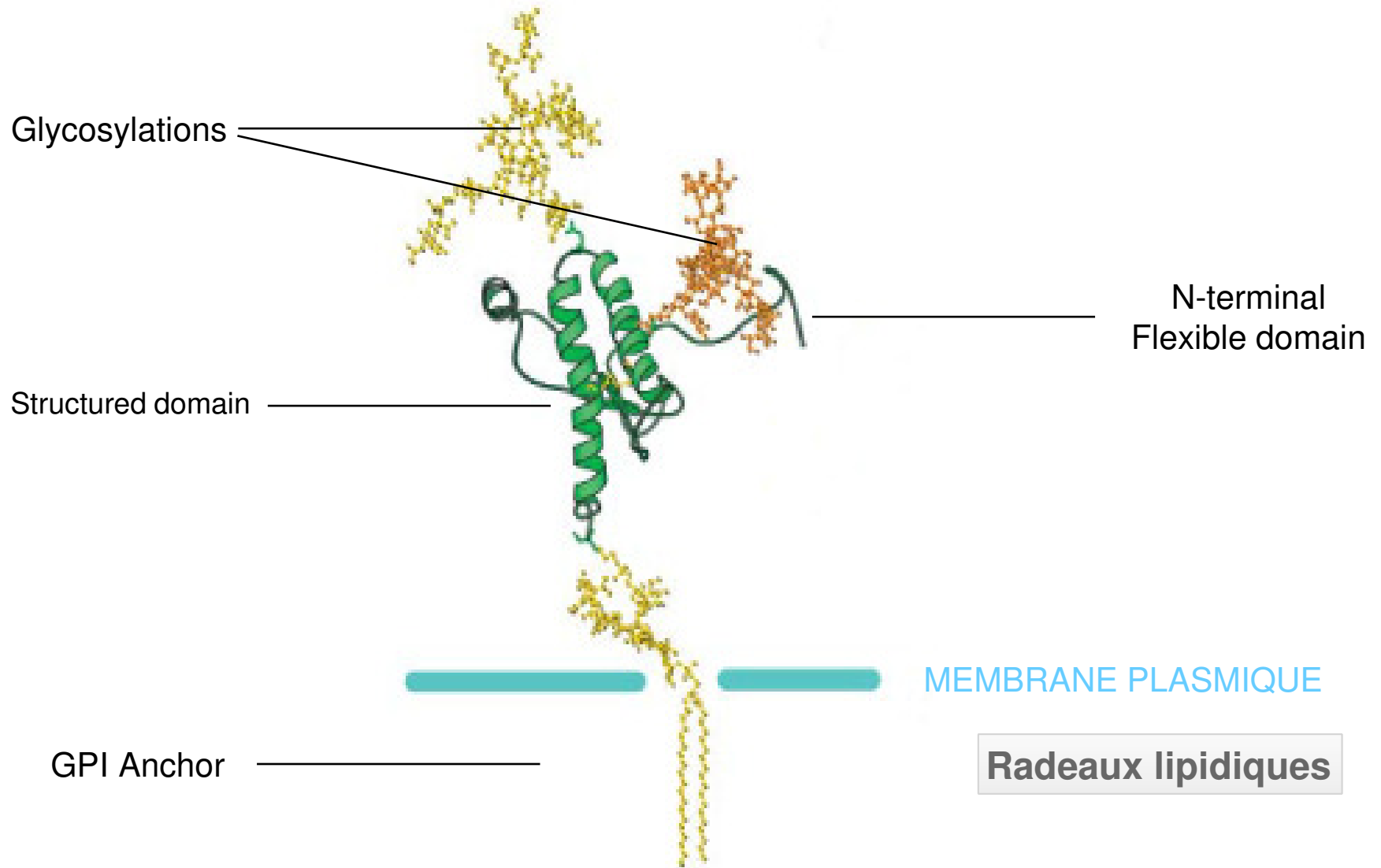
← Signal peptide →  
 1 MVKSHIGSWI LVLVAMWSD VGLCKKRPKP GGGWNTGGSR YPGQGSPGGN  
 51 RYPPQGGGGW GQPHGGGWGQ PHGGGWGQPH GGGWGQPHGG GGWGQGGSHS  
 101 QWNKPSKPKT NMKHVAGAAA AGAVVGGGLGG YMLGSAMSRP LIHFGNDYED  
 151 RYYRENMYRY PNQVYYRPVD QYSNQNNFVH DCV↓ NITVKQH ↓ TVTTTTKGEN↓  
 201 FTETDIKIME RVVEQMCITQ YQRESQAYYQ RGASVILFSS PPVILLISFL  
 251 IFLIVG ↑ GPI



# recPrP 3D NMR structure



# PrP<sup>C</sup> is a membrane anchored glycoprotein

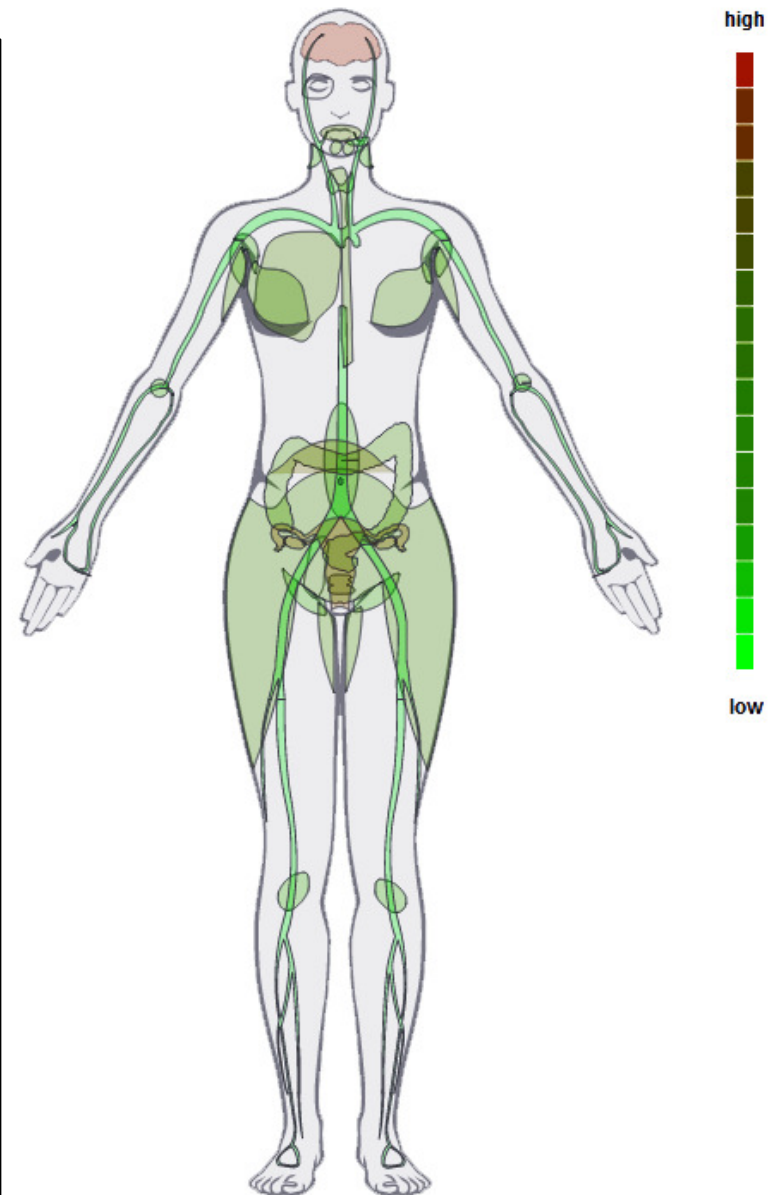


(Rudd, 2001)

## The physiology of PrP protein

- PrP<sup>C</sup> protein is an **ubiquitous** protein. Mainly expressed in the CNS.
- In mammalian PrP is **highly conserved**
- The PrP knock out mice present not any relevant physiopathology.
- PrP<sup>C</sup> is proposed to be involved in metals homeostasis through the octarepeat domain. Transporter, SOD activity, antiapoptotic effect etc...

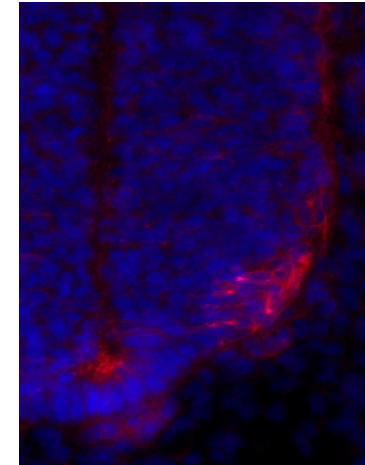
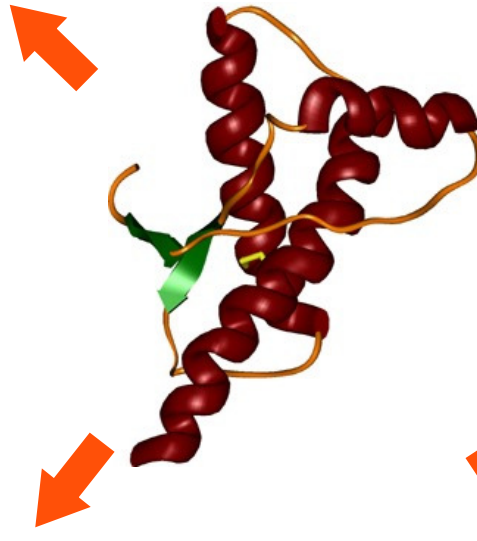
**In one sentence: the physiological role of PrP<sup>C</sup> is still unknown !!**



# Rôle physiologique de la PrP<sup>C</sup> ?

- Homéostasie du Cu<sup>2+</sup>
- Activité SOD ?
- Balance Redox

- Développement embryonnaire
- Biologie des cellules souches
- Adhérence cellulaire



(Halliez et al., 2015)

- Activité neuronale
- Neuritogénèse & différenciation
- Transmission synaptique
- Neuroprotection

- Cancers (pronostic -)
- Récepteur du peptide A $\beta$



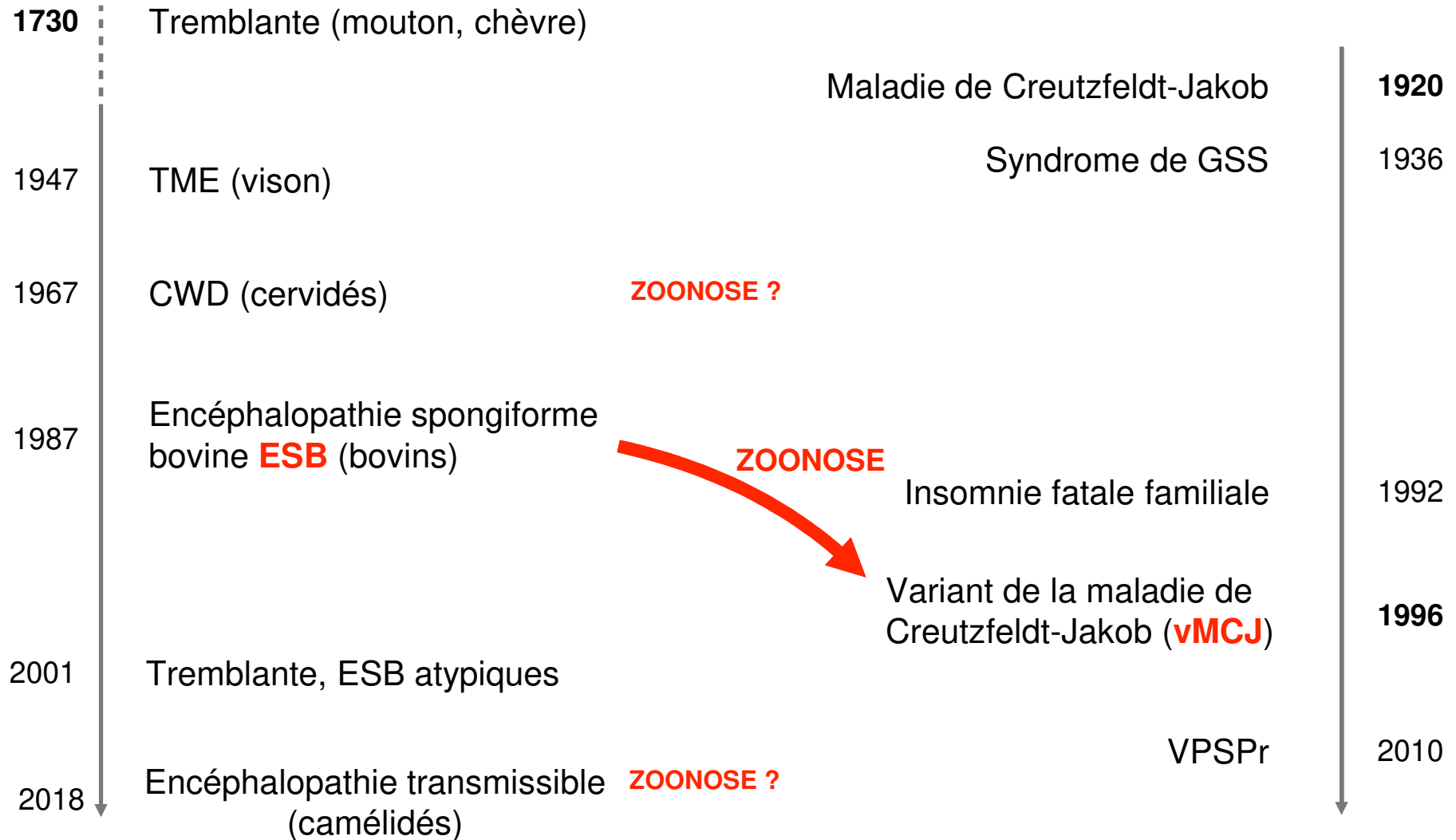
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# Les maladies à prion

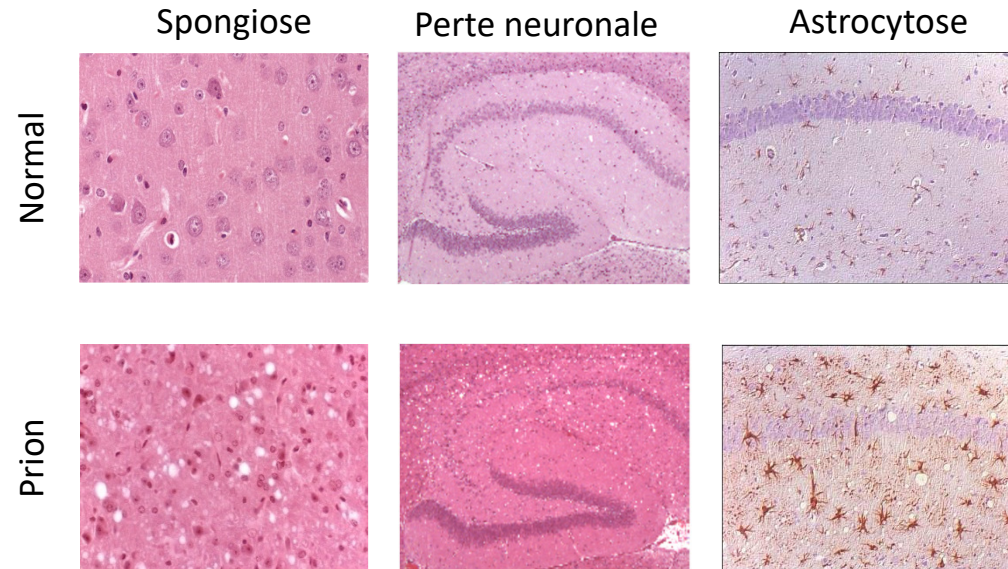
Animaux

Homme

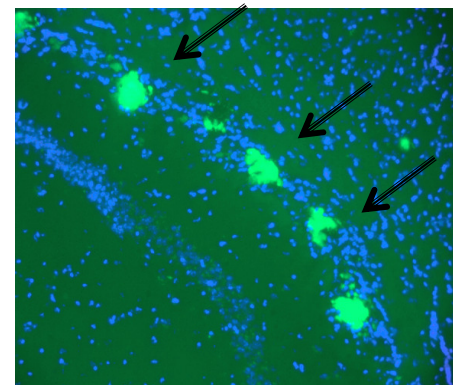


# Prion pathology

- Neurodegenerative disease
- Motor and sensitive dysfunction
- Neuron loss, spongiosis and astrocytosis
- **Accumulation of misfolded PrP**
- Usually a long asymptomatic incubation period
- Fatal issue
- without conventional immune and inflammatory reaction
- Absence of serological response



- **Transmissible/infectious**





# The triple determinism of human Prion disease

**Sporadic** (90 %) : 1-2 case / year / million  
Creutzfeldt-Jakob disease (*CJD*)

## **Infectious**

- Kuru : by cannibalism
- variant of Creutzfeldt-Jakob disease (*vCJD*) : ~ 200 case
- iatrogenic (ex. hGH etc.)

## **Genetic** (> 30 mutations in PrP)

- familial CJD
- Gerstmann-Straüssler-Scheinker (GSS)
- fatal familial insomnia (IFF)



# Maladies à prion : une triple étiologie

## SPORADIQUE



— ?

- Mutation somatique ?
- Défaut conformationnel ?
- Facteur non identifié ?

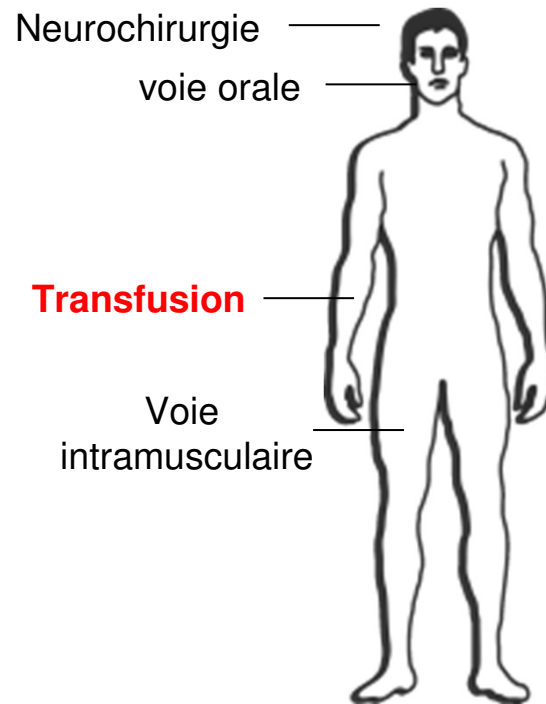


**SNC infectieux**

**> 65 ans**

# Maladies à prion : une triple étiologie

## ACCIDENTELLE



- Kuru (rites anthropophages)
- forme variante de la MCJ
- Hormone de croissance



**incubation : 5-50 ans**

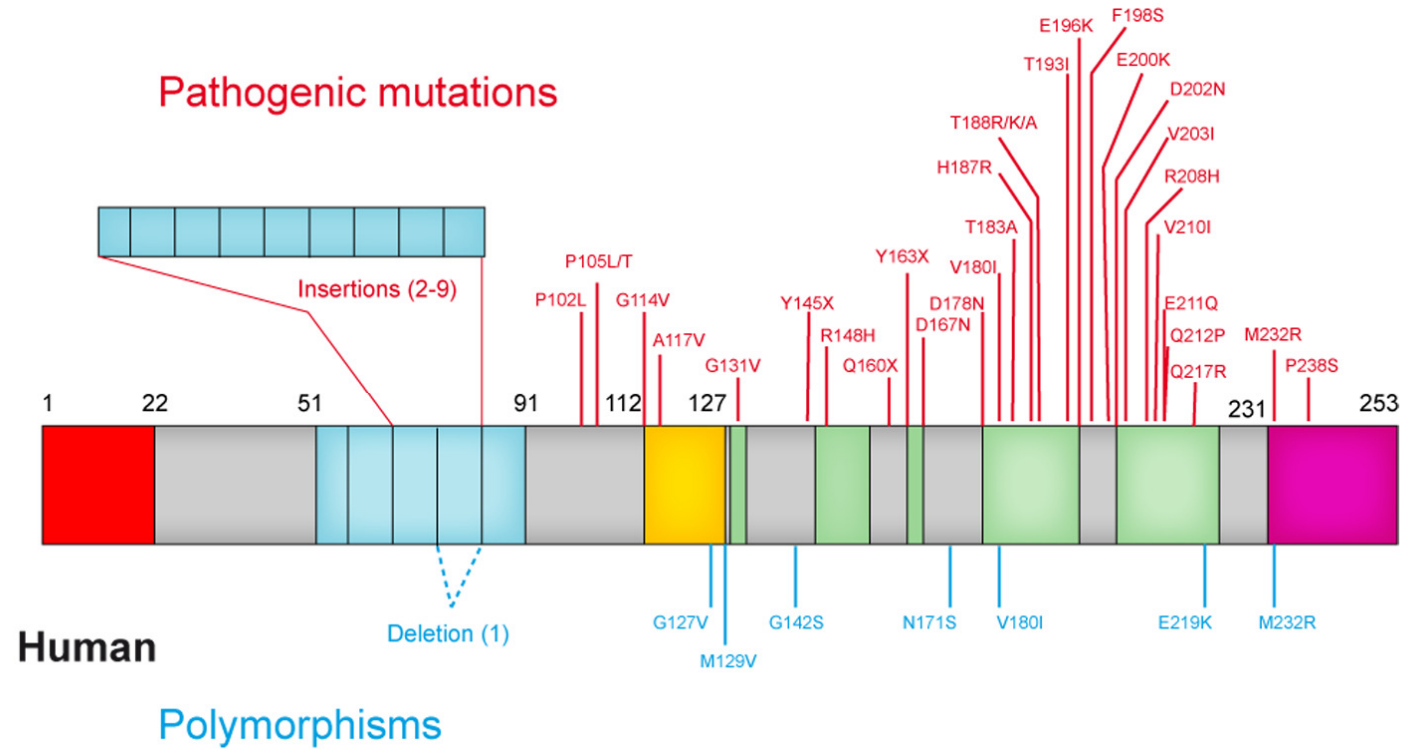
# Maladies à prion : une triple étiologie

## GENETIQUE

- MCJ familiale
- Gerstmann-Straüssler-Scheinker (GSS)
- Insomnie fatale familiale (IFF)

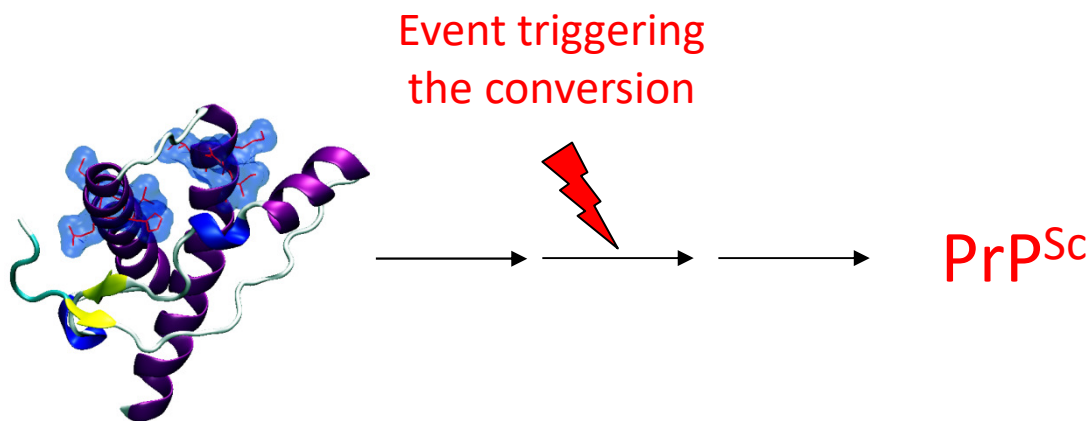


20-60 ans



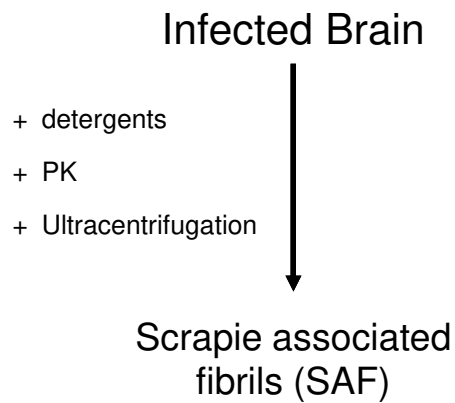
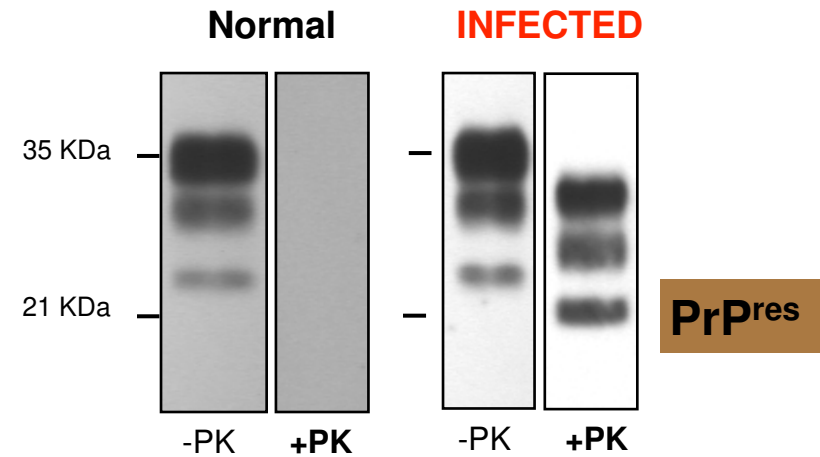
# Molecular basis of prion disease

- Prion disease belongs to pathologies due to proteins misassemblies as Alzheimer, and Parkinson etc ...
- It results from autocatalytic misfolding of an ubiquitous protein : **PrP<sup>C</sup>**
- The misfolded conformer of PrP<sup>C</sup> is named: **PrP<sup>Sc</sup>**
- The evolution of the prion pathology is always associated to the misfolding process

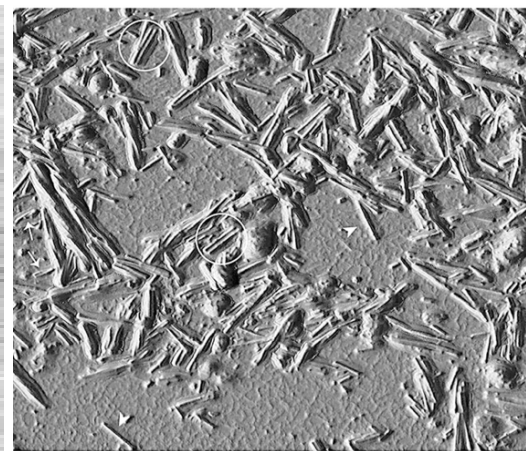


# Biochemical characteristic of PrP<sup>Sc</sup>

Characteristics	PrP <sup>c</sup>	PrP <sup>Sc</sup>
% helix $\alpha$	42 %	30 %
% $\beta$ sheet	3 %	42 %
PK resistance	-	++
Solubility	++	-
Digestion PIPLC	+++	-



(Prusiner et al., 1982)



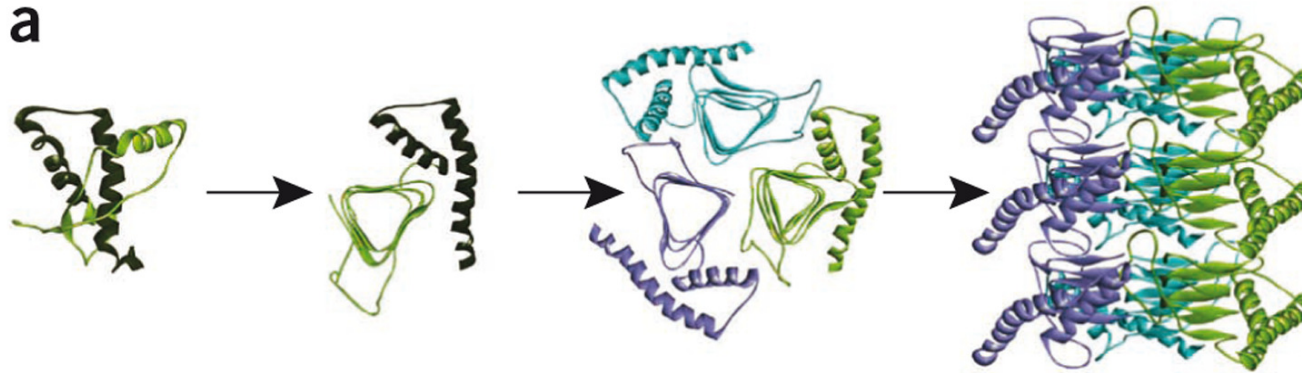
(Sim et al., 2007)





# PrP<sup>Sc</sup> structural models

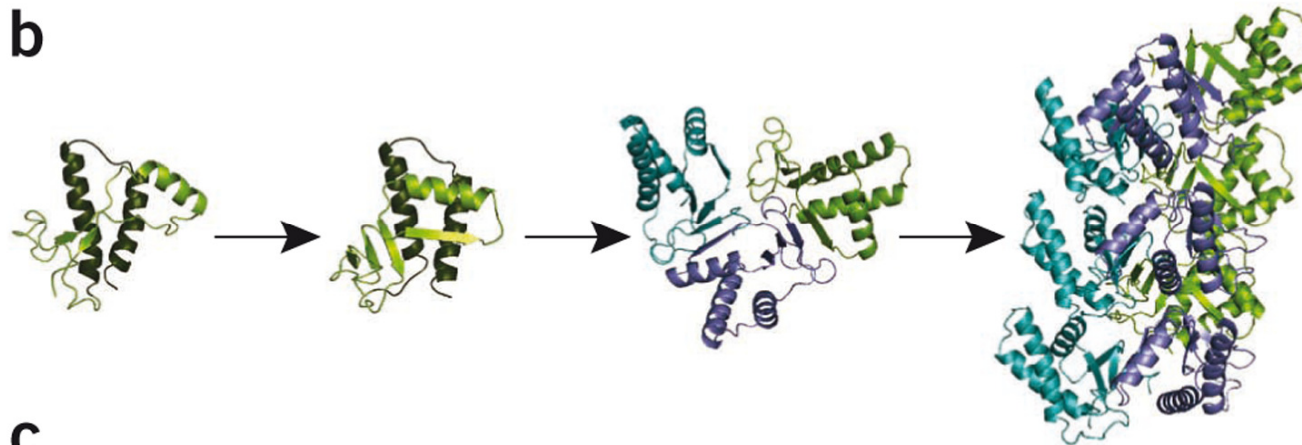
a



**Modèle en hélice- $\beta$  senestre**

*Govaerts et al 2004*

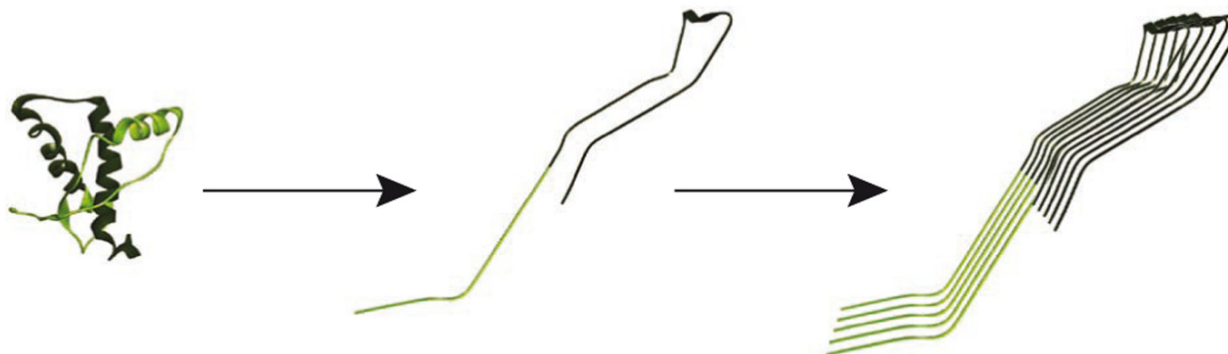
b



**Modèle  $\beta$ -spirale**

*DeMarco & Daggett, 2004*

c



**Modèle en feuillets- $\beta$  parallèles**

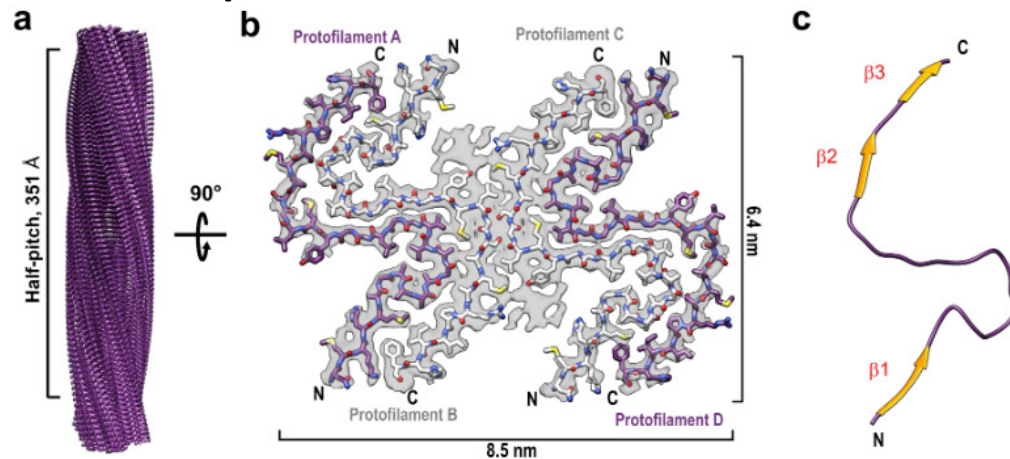
*Cobb et al 2007*

# La cryo microscopie électronique au secours des structuralistes

- Agencement parallèle In register des protomères

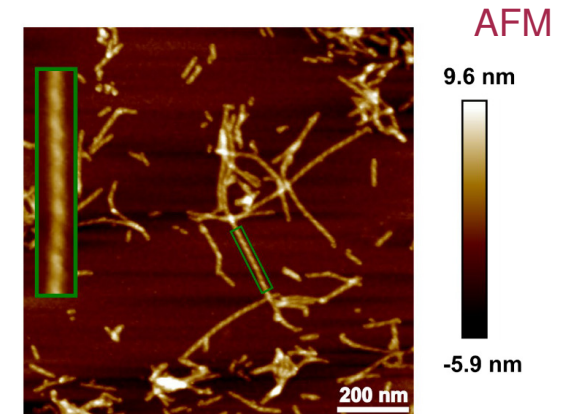
fibres de prp (23-144) (Li, 2021)

– 4 protofilaments associés

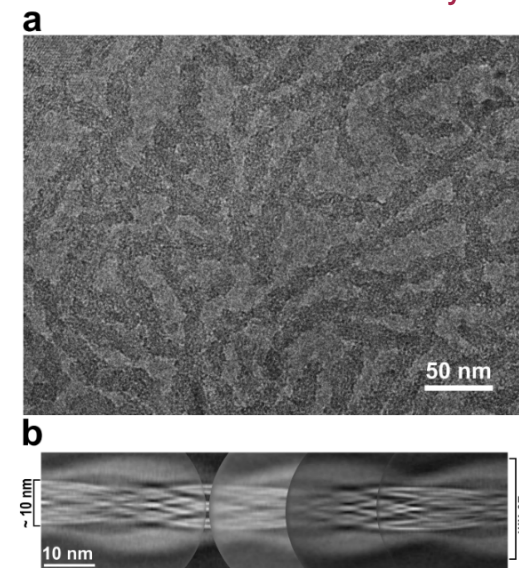


– Implication du core de la protéine (110-140)

– D'autres structures, avec des différences...

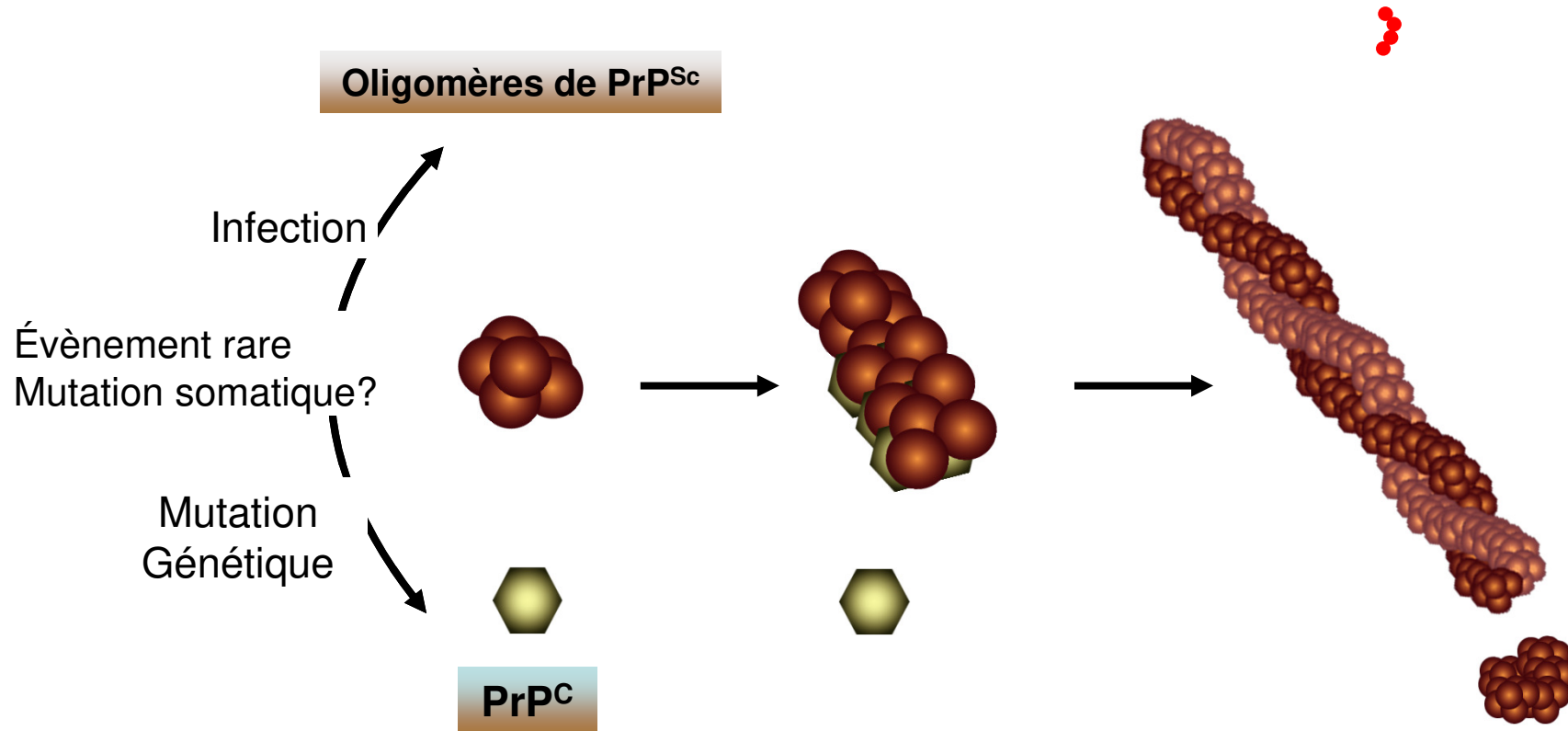


Cryo-EM





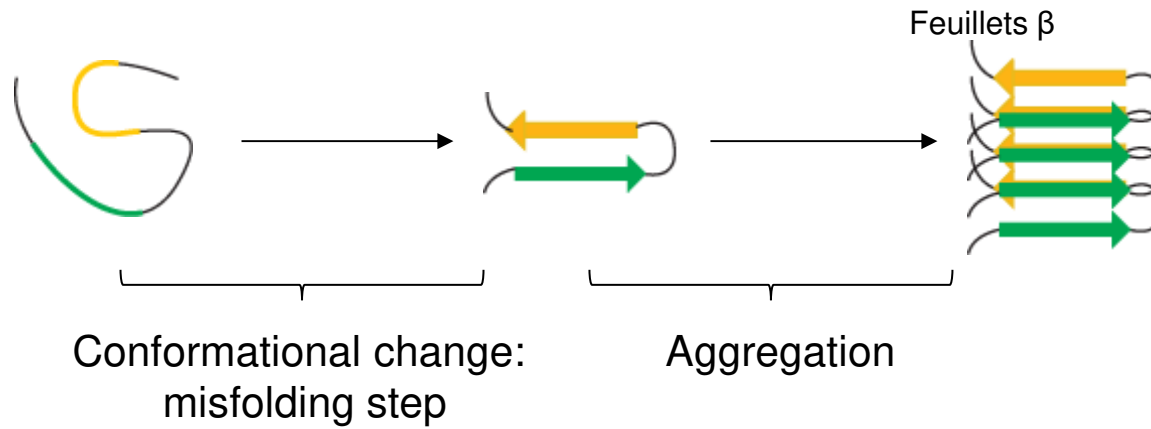
# Replication model: *seeding-polymerization* ?



Site(s) de conversion subcellulaires : surface / ERC / Lysosomes ?

# Mechanisms of cell death ?

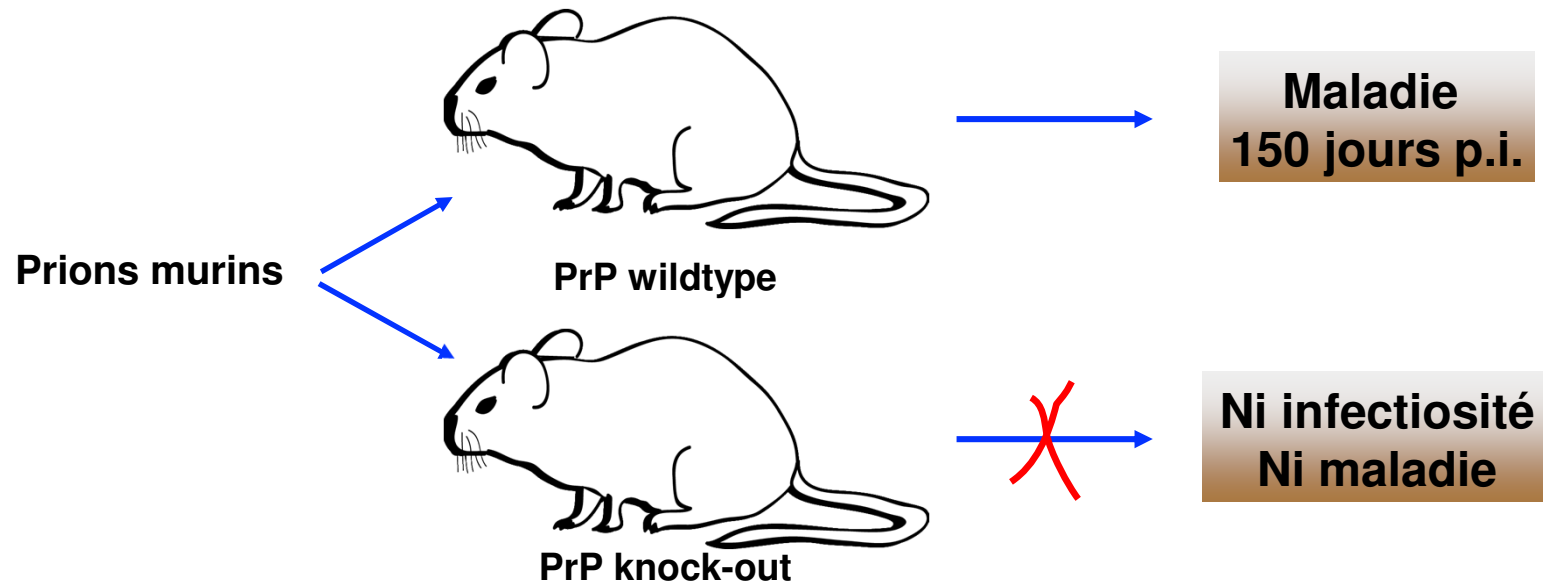
Loss of function



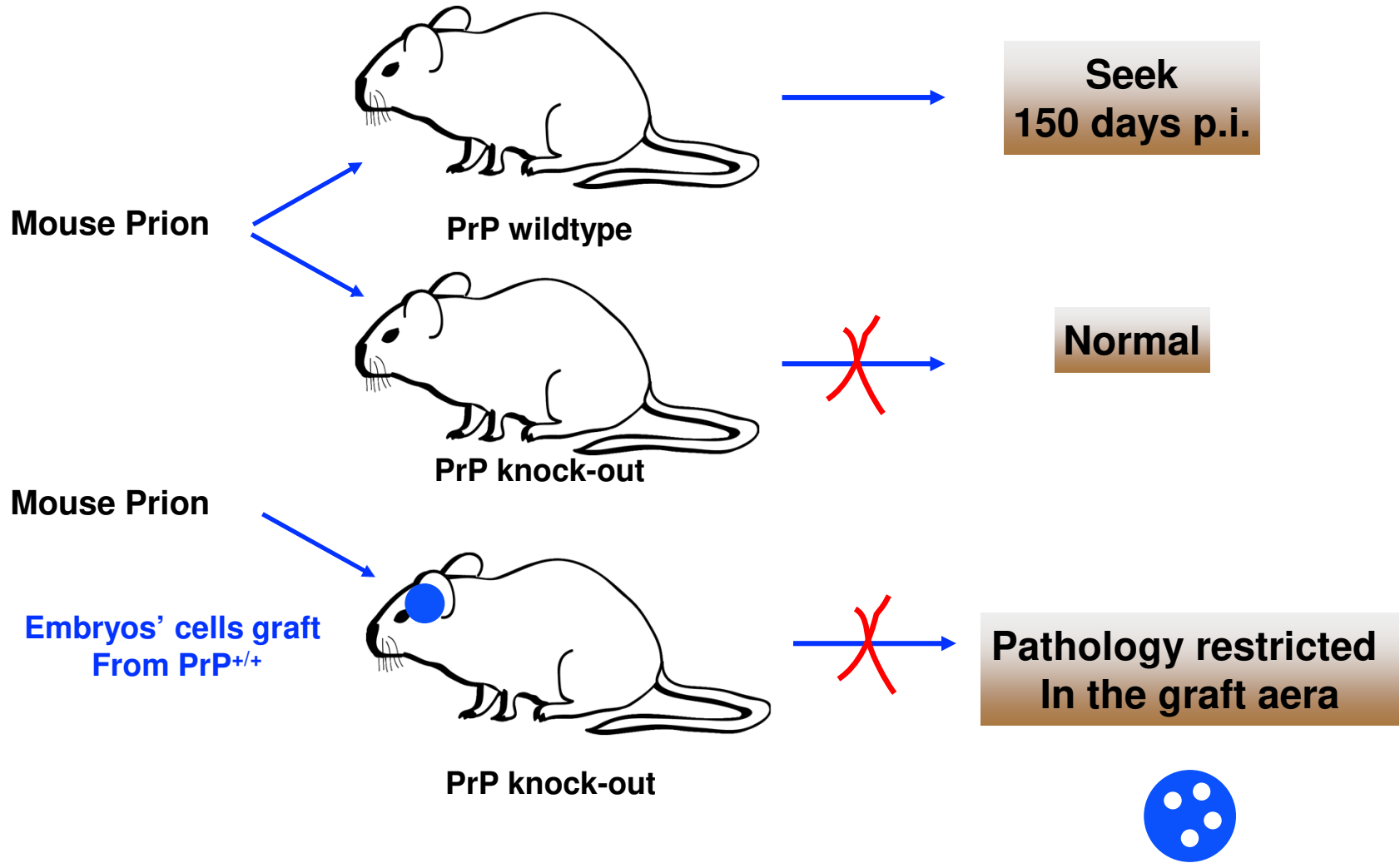
Intrinsic toxicity

Gain of function

# La PrP est essentielle à la réplication des prions et à leur toxicité

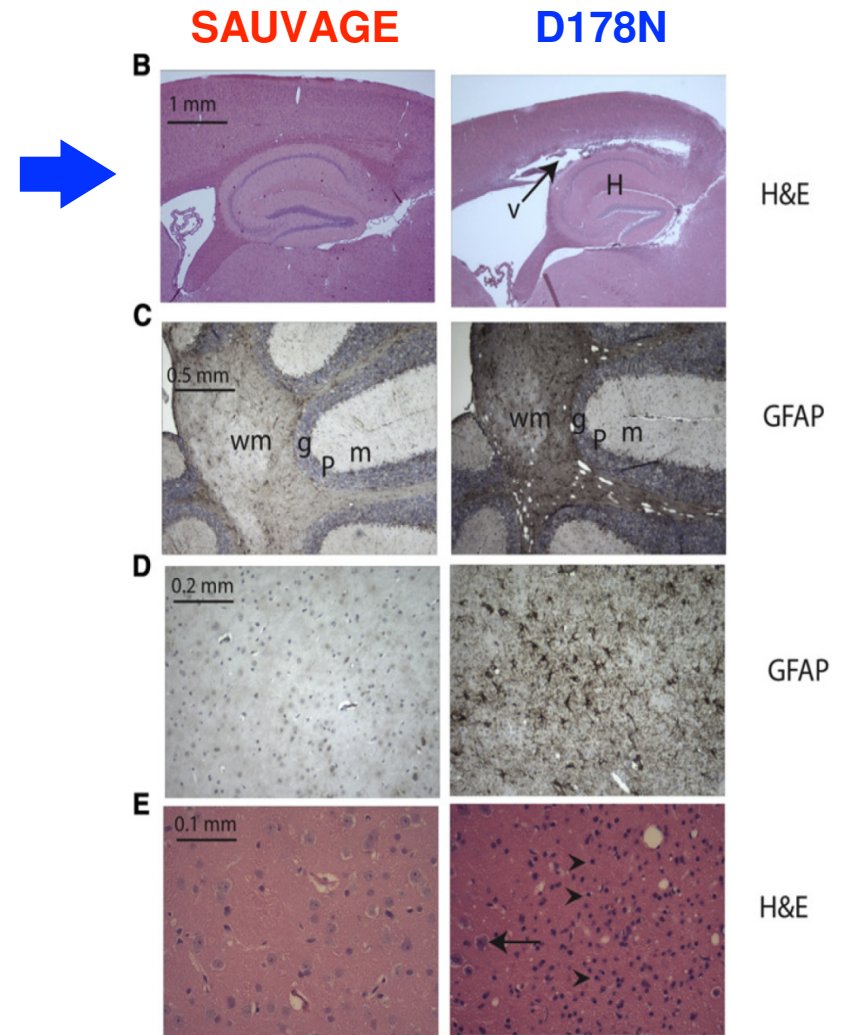
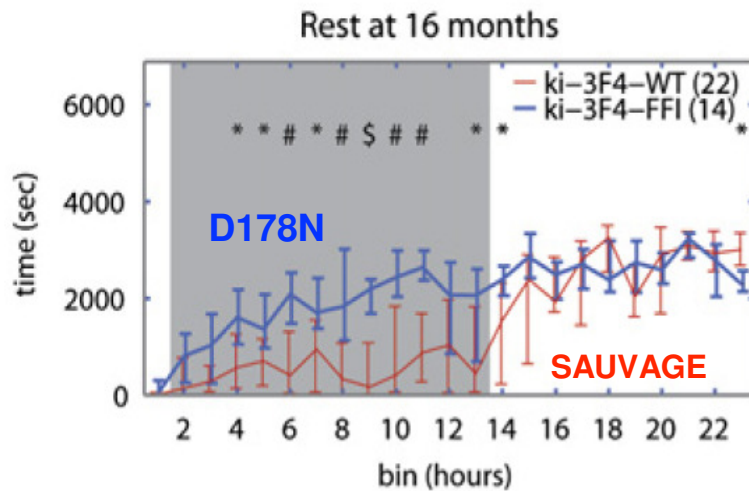


# PrP expression is essential for the infectivity

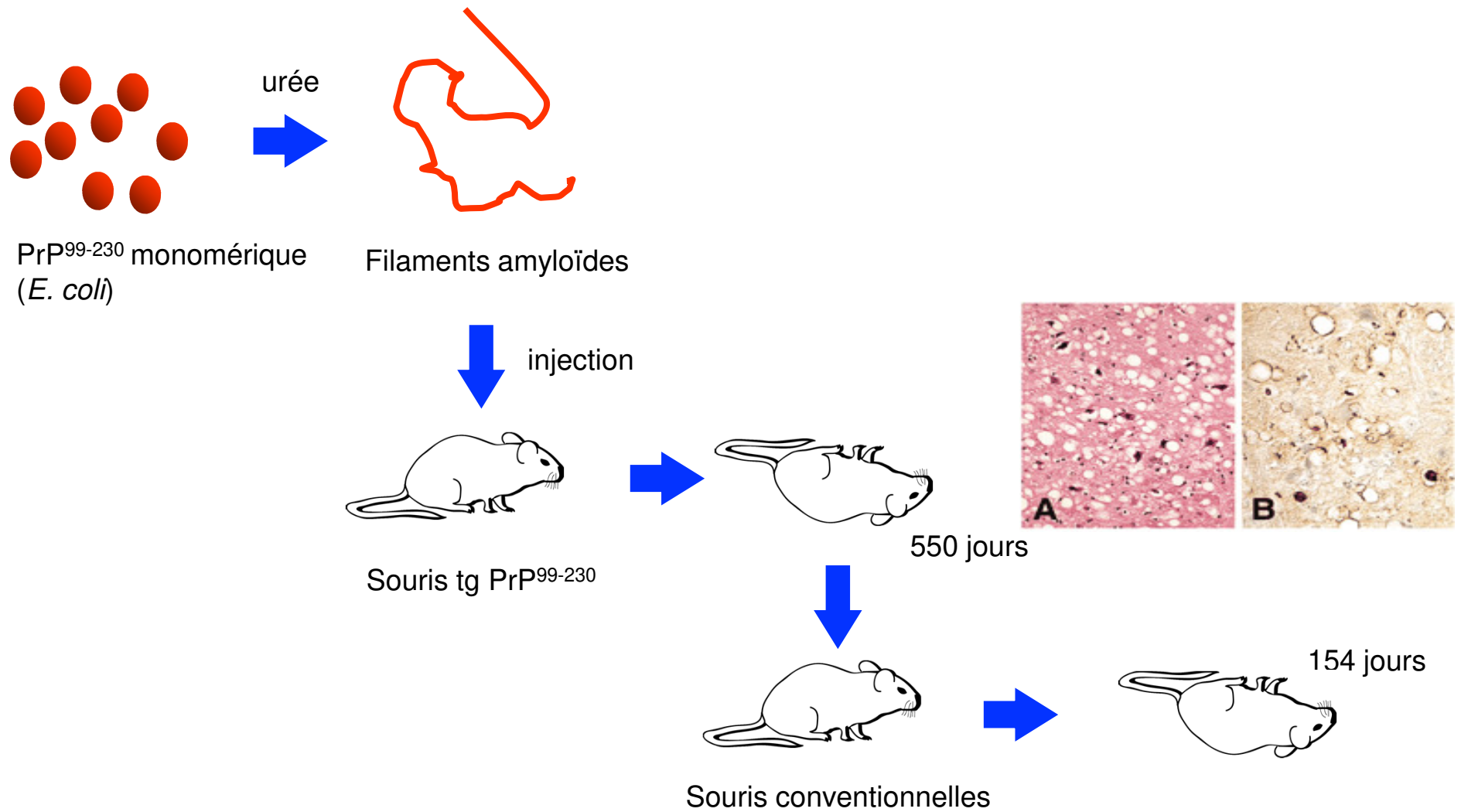


# Des souris exprimant une PrP mutée tombent spontanément malades - D178N (IFF)

- Développement d'une maladie spontanée (400J)
- Symptomatologie évocatrice de l'IFF humaine
- Désordres transmissibles à des souris *wt*



# Des prions synthétiques authentiquement infectieux



(Legname et al., 2004)



## Prion pathology

- 1 – Pathologies due to protein misfolding and aggregation
- 2 – PrP<sup>C</sup> protein : the “good” prion conformer
- 3 – Prion pathology and the “bad” conformer
- 4 – Validation of Prion hypothesis
- 5 - Molecular basis of the Prion strains

# Historical arguments for prion hypothesis

- Absence of nucleic acids (RNA, DNA) + UV- resistance
- PrP<sup>Sc</sup> and infectivity co-purified
- Relation between PrP mutation and the genetic form
- Absence of pathology in knock-out PrP mice

## Definitive proof: synthetic prion

Science. 2004 Jul 30;305(5684):673-6.

### **Synthetic mammalian prions.**

Legname G<sup>1</sup>, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, DeArmond SJ, Prusiner SB.



# Résistance des prions aux procédés de décontamination

- **Résistance à la chaleur**

Pas d'inactivation à 160°C / 24h ou 360°C / 1h (chaleur sèche)

3,5-7,5 log réduction à 134-138°C / 3bar / 20 min (chaleur humide)

- **Résistance aux ultrasons**

- **Résistance aux rayonnements ultraviolets**

dose inactivante 37% = 40 000 J/m<sup>2</sup>

(15 pour le VIH)

- **Résistance aux radiations ionisantes**

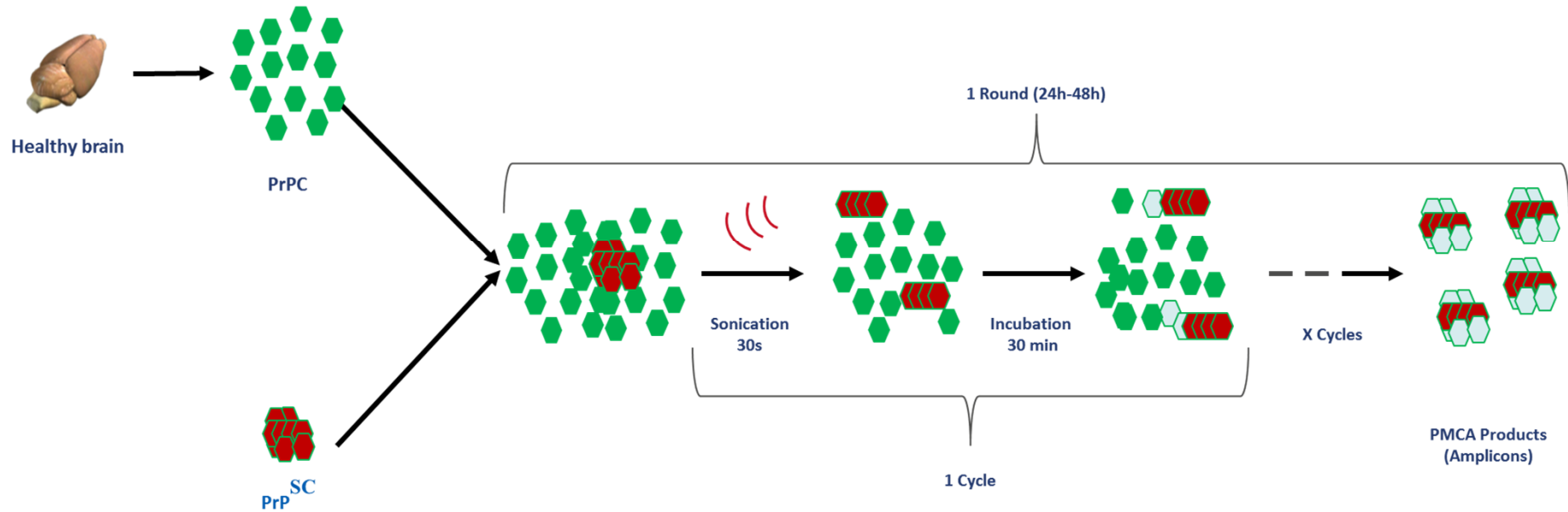
dose inactivante 37% = 10 000 grays

(100 pour l'herpès virus, 5000 pour le VIH)

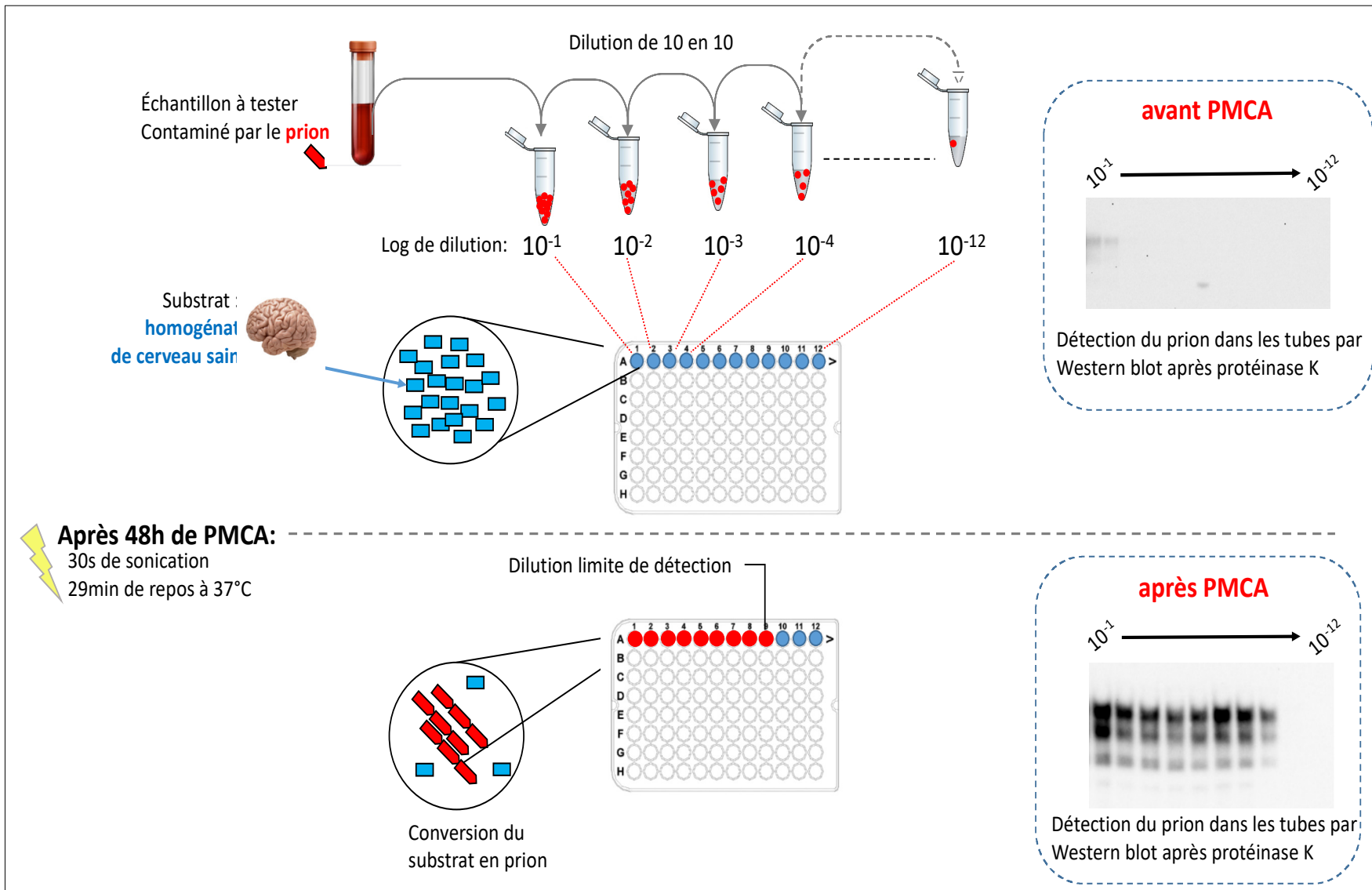
- **Résistance aux procédés chimiques d'inactivation**

Inactivation totale avec la soude 2N, l'eau de Javel 20 000 ppm

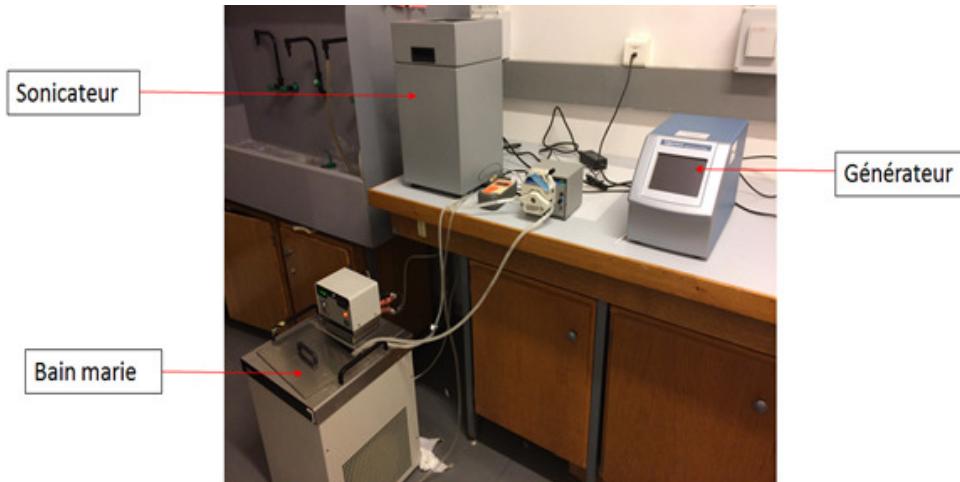
# Amplification acellulaire des prions par PMCA : *protein misfolding cyclic amplification*



# L'amplification in-vitro des prions par PMCA (protein misfolding cyclic amplification)

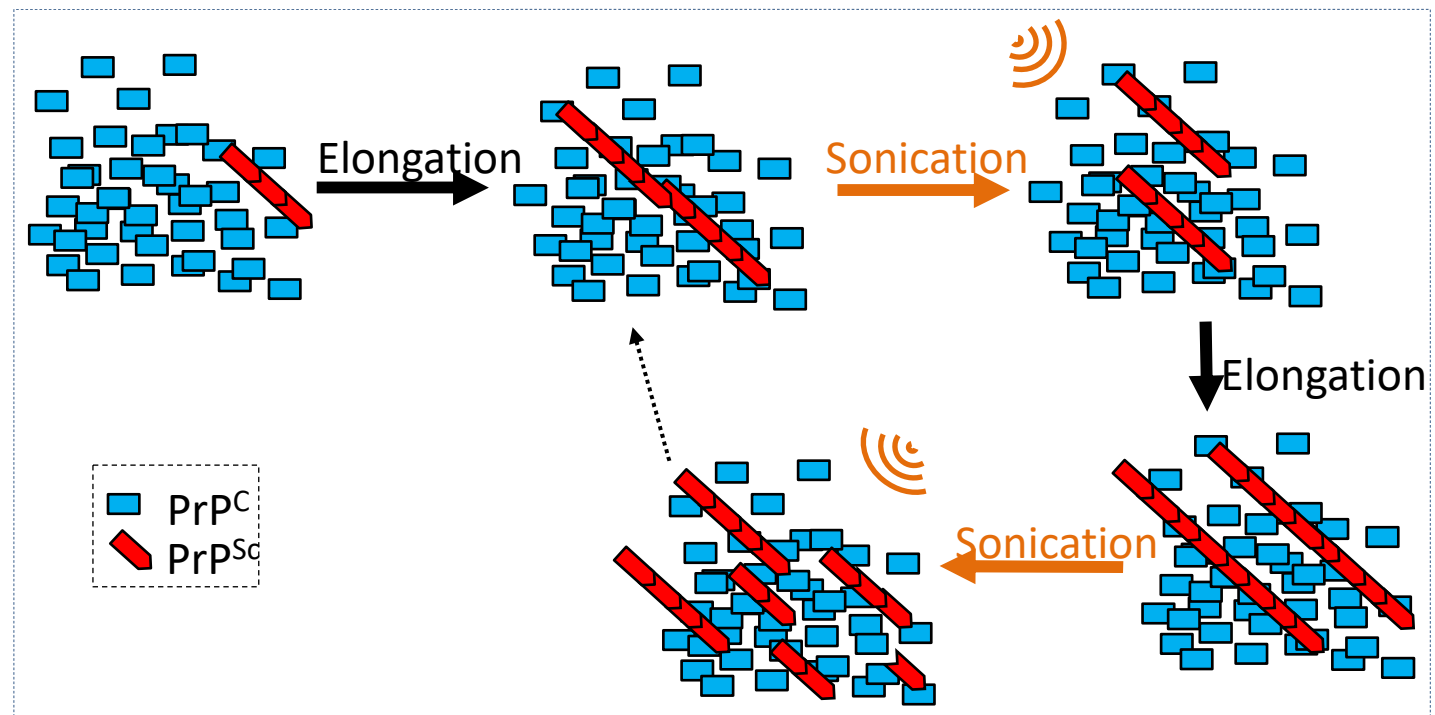


# L'amplification in-vitro des prions par PMCA (proteic misfolding cyclic amplification)



Comment agit la sonication???

- Fragmentation des fibres?
- Dénaturation de la PrP qui favorise la réplication?

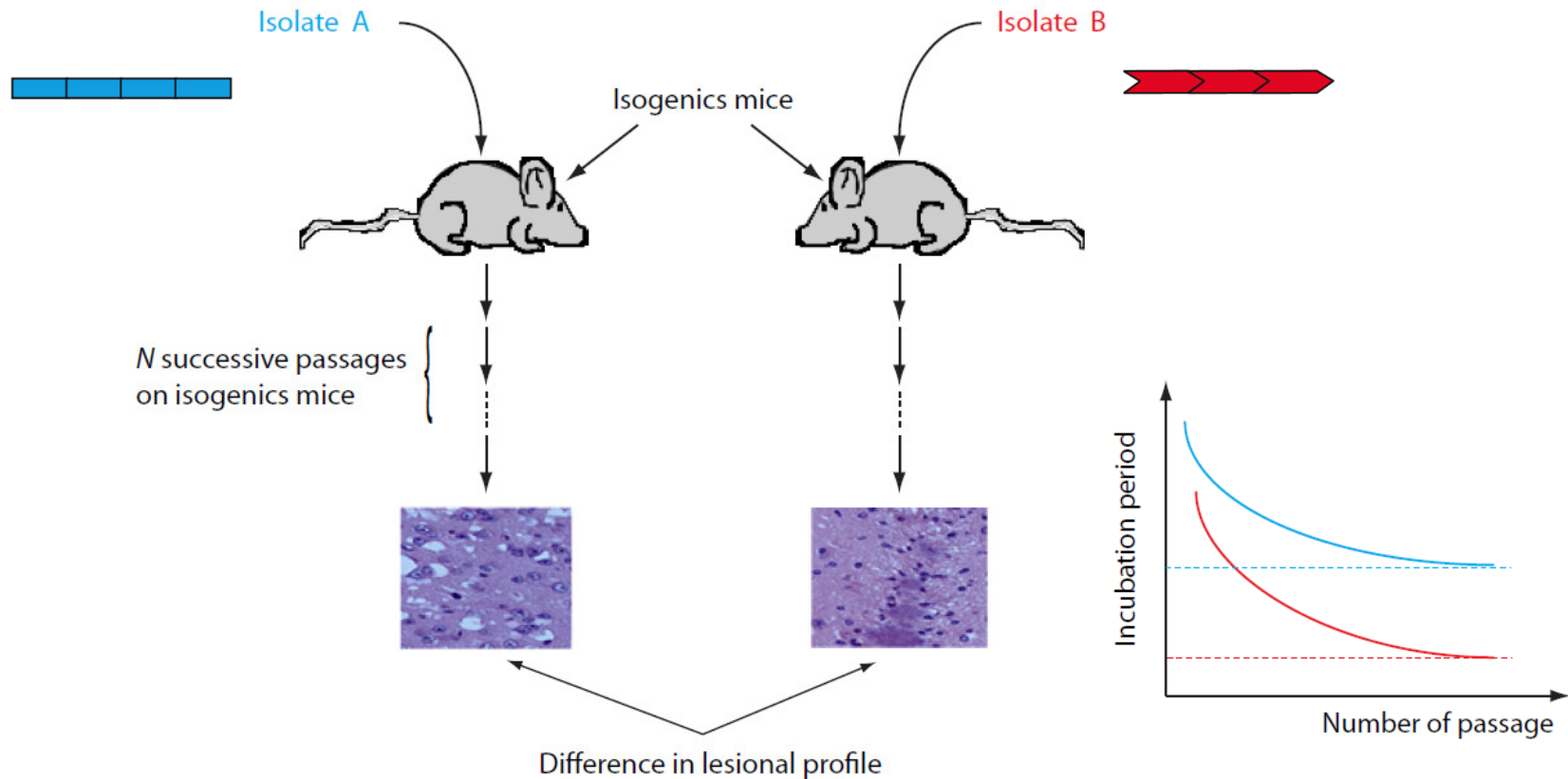




## Prion pathology

- 1 – Pathologies due to protein misfolding and aggregation
- 2 – PrP<sup>C</sup> protein : the “good” prion conformer
- 3 – Prion pathology and the “bad” conformer
- 4 – Validation of Prion hypothesis
- 5 - Molecular basis of the Prion strains

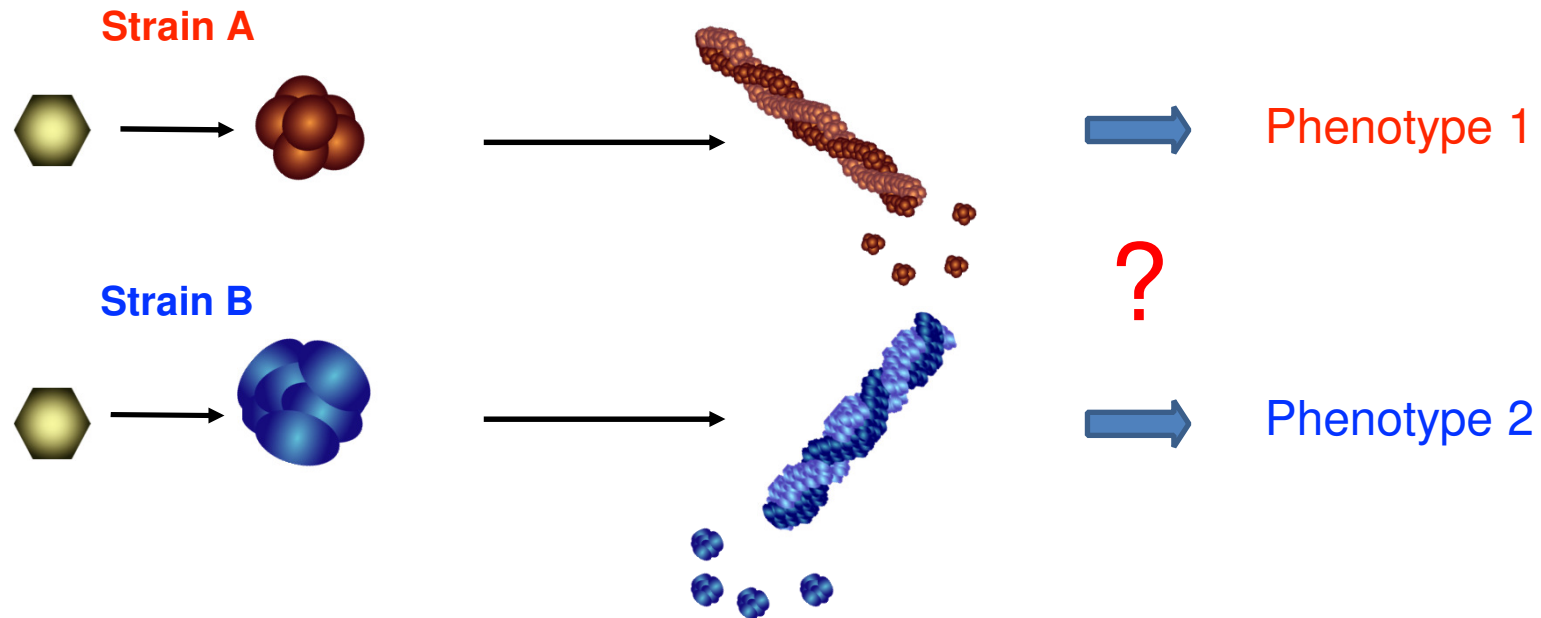
## Prion strains: structural diversity of prion assemblies



In prion diseases, the **pathological information** or **phenotype** is encoded in the structure of PrP<sup>Sc</sup> assemblies .

→ Existence of strains in prion suggests the existence of a PrP<sup>Sc</sup> structural diversity

## Prion strains and conformational diversity of PrP<sup>Sc</sup>

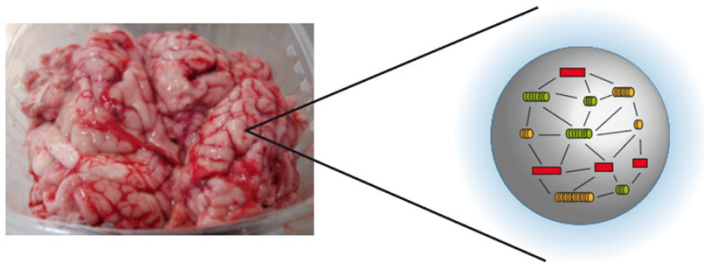


*The strain information is encoded in the structure of PrP<sup>Sc</sup> assemblies*



Prion replication = transfer of structural information and adaptation/evolution

# Evolution of the structural information : Prion evolution and adaptation



	Host A ●	Host B ●	Host C ■
●●●●●	●●●●●		
●●●●●		●●●●●	
■			■

→ Best replicator selection

→ Question : how does this structural diversity occur?



# State of the Art (2000)

- Anatomic Site of Prion replication :
  - CNS, and throughout !!!!
- Cellular Site of Prion replication :
  - Not yet established
- Mechanisms of PrP conversion :
  - Griffiths (1967) and Lansbury (1993) Model
- Molecular basis of Prion Strain :
  - Hypothetic PrP<sup>Sc</sup> structural polymorphisms.
- Mechanisms of Neurotoxicity :
  - An open question
- in vitro neosyntheses of infectivity :
  - Not yet!

# State of the Art (2020)

-Anatomic Site of Prion replication :

→ CNS, and throughout !!!!

- Cellular Site of Prion replication :

→ Sometime in cell surface and sometime in the endocytosis root.

-Mechanisms of PrP conversion :

→ Griffiths (1967) and Lansbury (1993) Model

- Molecular basis of Prion Strain :

→ Hypothetic PrPSc structural polymorphisms.

- Mechanisms of Neurotoxicity :

→ open questions, but converged to the mechanisms of other amyloidopathy.

- in vitro neosyntheses of infectivity :

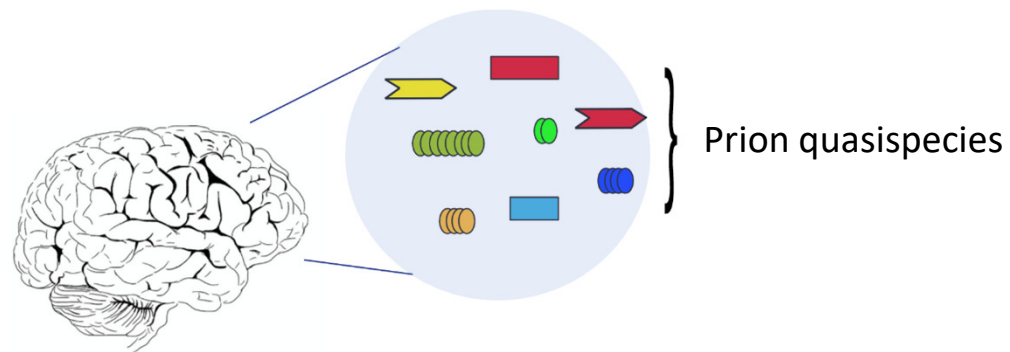
→ Yes.....



## structural diversity of prion assemblies within an individual: Prion quasispecies

→ Different Biochemical and biological outcome suggest the existence of structural diversity within a given strain

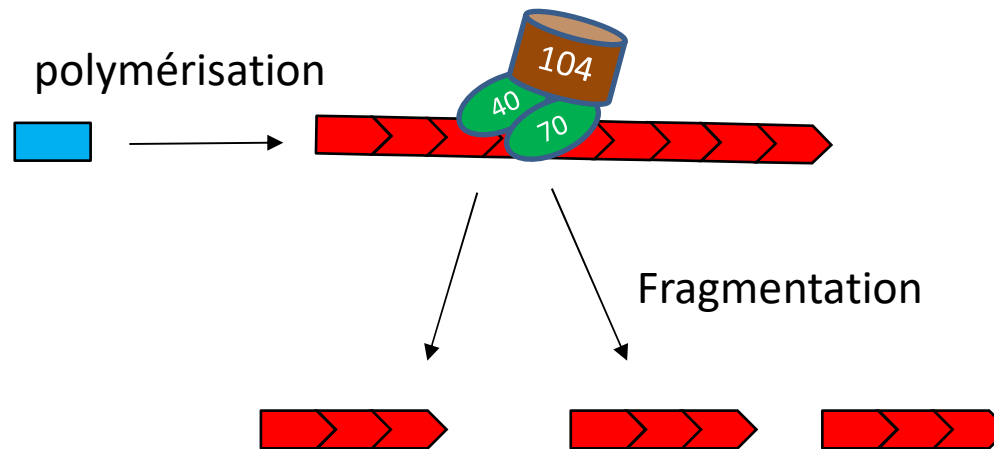
→ Introduction of quasispecies concept in prion paradigm (*Li et al. 2010, Science*).



↳ Prion adaptation based on the best replicator selection within the quasispecies

# Les mécanismes de propagations des prions restent à ce jour inconnus

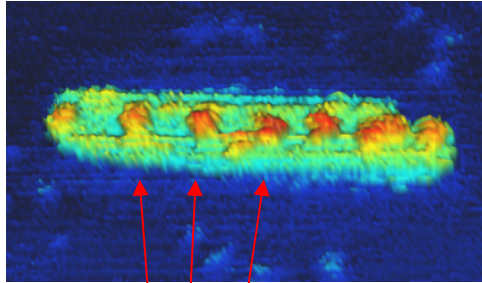
Le modèle actuel de la propagation des prions de mammifères repose sur l'observation des prions fonctionnels de **levures**:



**Aucune preuve** de l'existence de la **fragmentation** chez les prions de **mammifères**

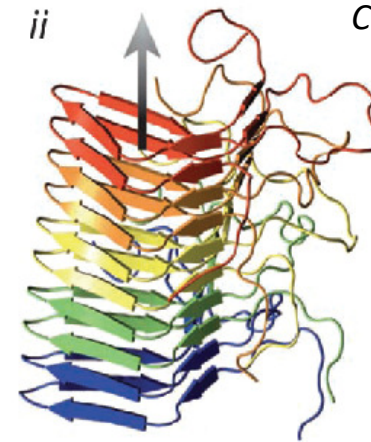
# Les fibres de PrP sont constituées d'une sous-unité

AFM (atomic force microscopy)



*A. Igel-Egalon et D. Martin*

Répétition de motif



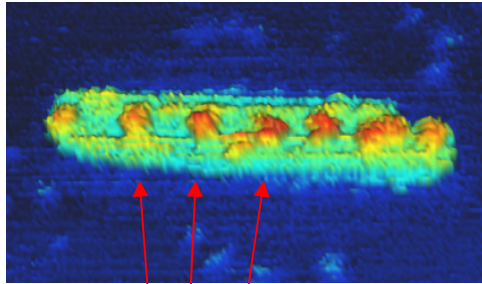
*Cobb et al. 2009*

HET-s amyloid fibril

Contrairement aux prions de levures, les assemblages de mammifères ne sont pas homogènes et présentent des **motifs répétés** le long de la fibre.

# Les fibres de PrP sont constituées d'une sous-unité

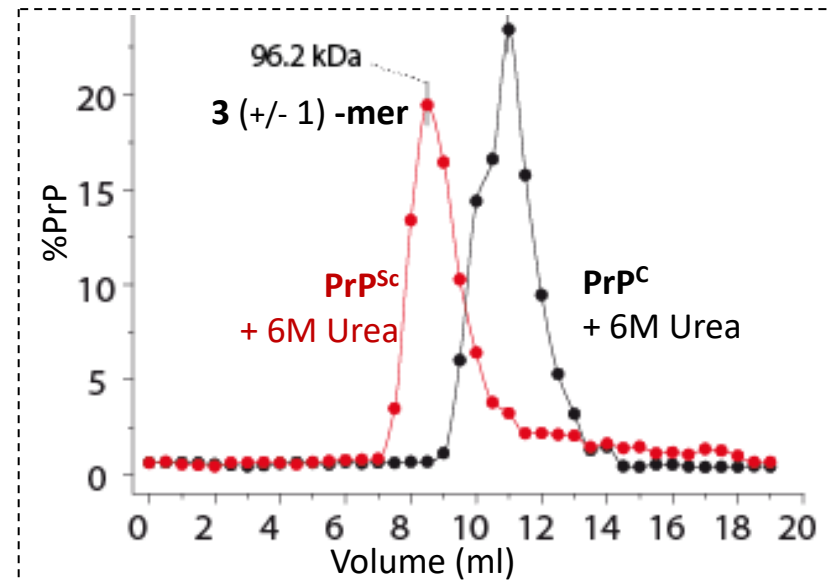
AFM (atomic force microscopy)



A. Igel-Egalon et D. Martin

Répétition de motif

Igel-Egalon et al. 2017



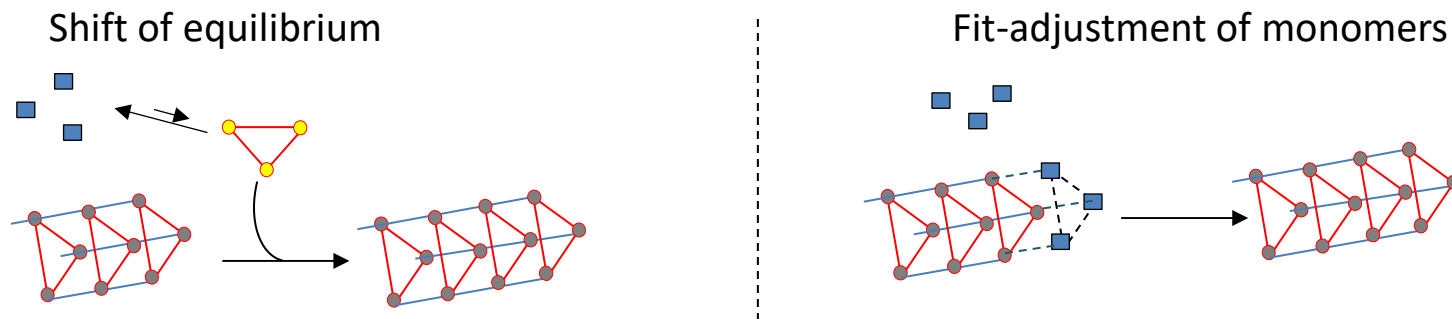
La dénaturation de PrP<sup>Sc</sup> → formation d'un oligomère ( trimère) PrP

L'existence de ce motif remet en cause la séquence d'évènement conduisant à l'élongation de la fibre

# L'existence d'une sous unité change la vision des prions!

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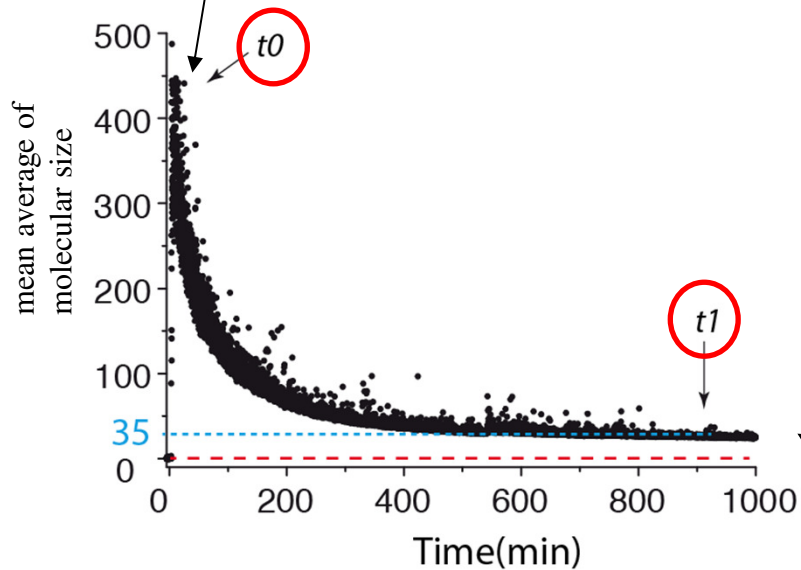
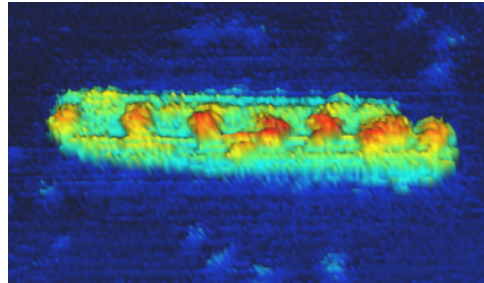
- The existence **multi-scale organization** imposes immediately the question of **sequence of events** during the template assisted **conversion**.
  - We propose two models of templating process:



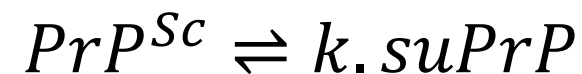
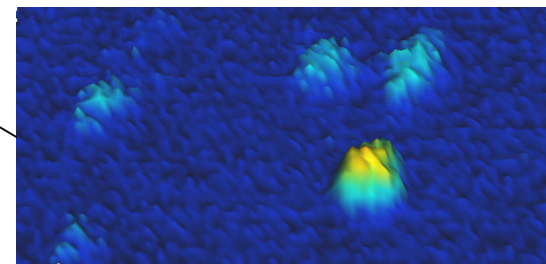
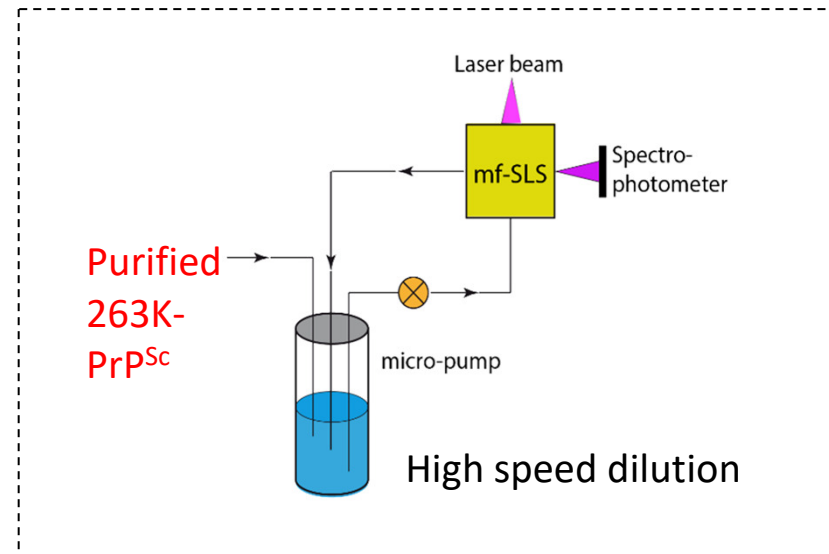


# Les fibres de PrP sont constituées d'une sous-unité qui existe en équilibre avec la forme fibrillaire

AFM (atomic force microscopy)

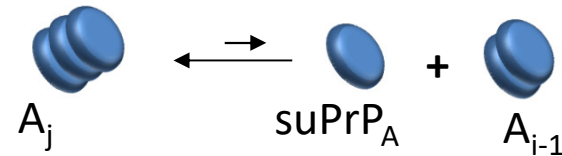
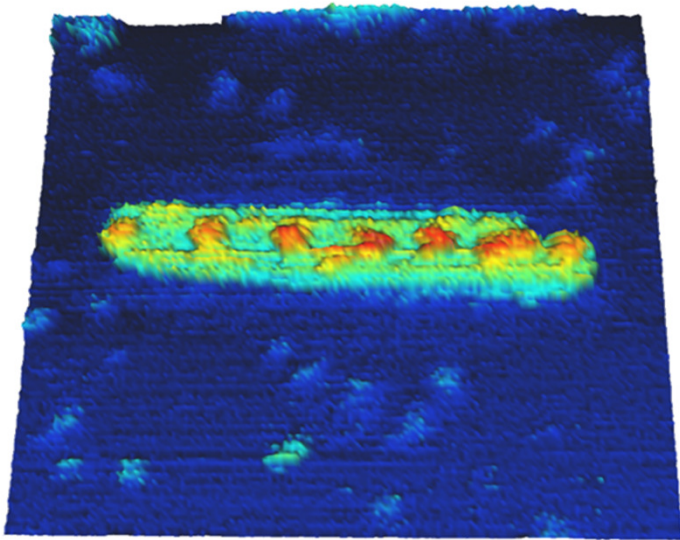


Igel-Egalon et al. 2017



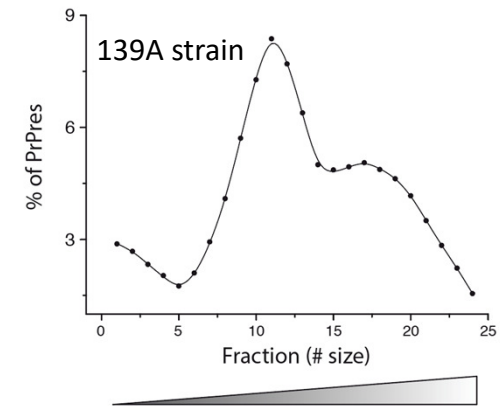
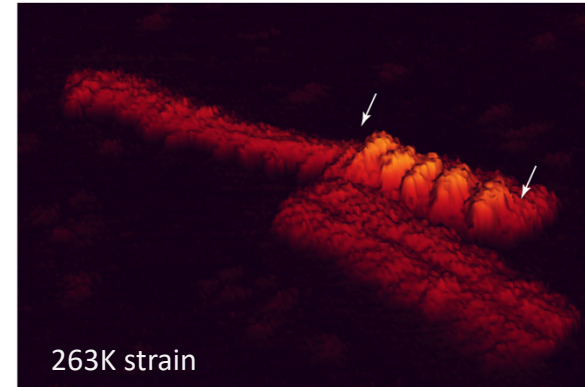
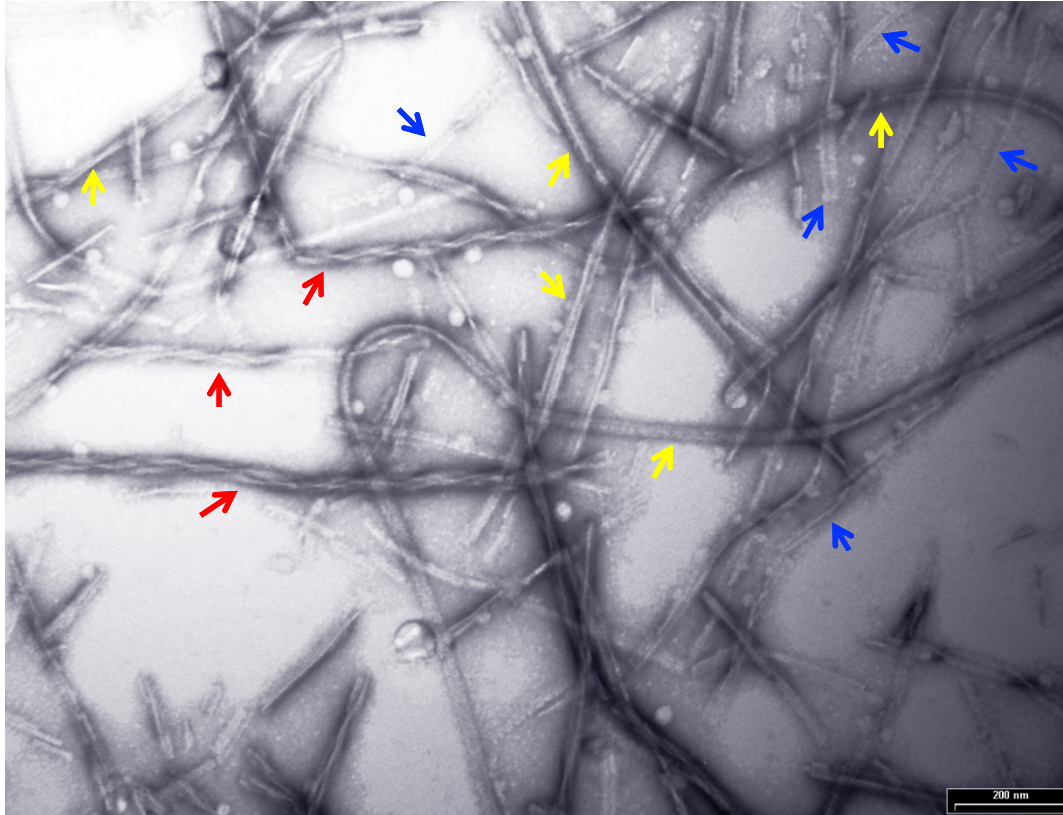
# Une nouvelle vision du mode de replication des prions

*Igel-Egalon et al. 2017*



- Les assemblages de Prion sont des **entités dynamiques** constitués de **sous-unités** existant en **équilibre** avec la forme fibrillaire.
- La **dépolymérisation**, au lieu de la fragmentation, serait à l'origine de la **réplication** des prions.

## Prion structural heterogeneity

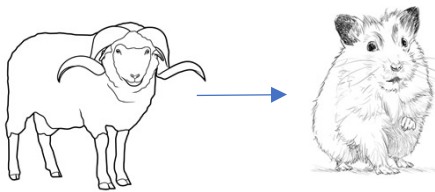


Structural heterogeneity of prion assemblies contributes to its adaptation and evolution

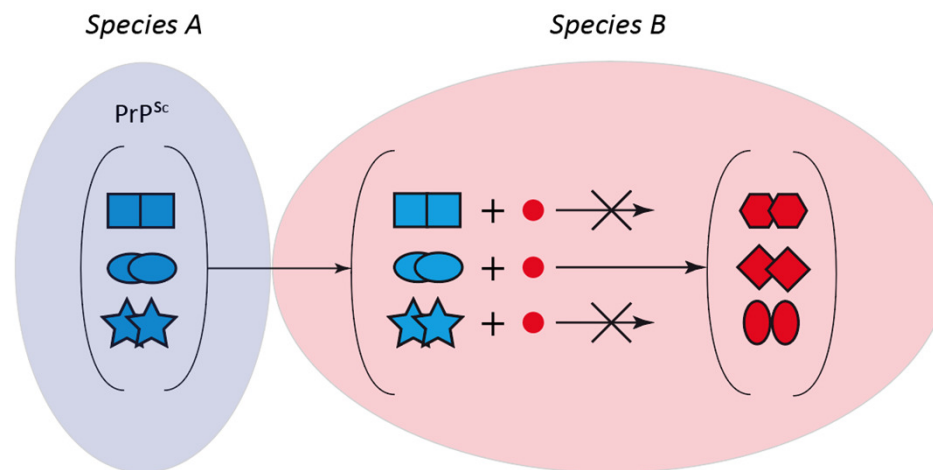
## Prion host-adaptation and evolution

### Questions:

- As a pathogen how prion evolves and adapts to a new host ?



- What is the prion adaptation mechanism?



- The actual dogma is based on replicator dynamic:
  - *Best mutator*
  - *Best response*
  - *Adaptative dynamic*
  - *Brown-von Neumann-Nash (BNN) dynamic*
  - ***Best replicator***

## Open questions

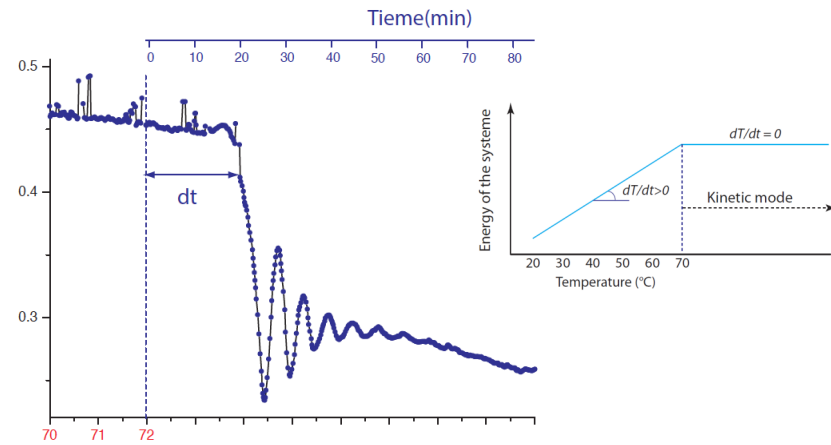
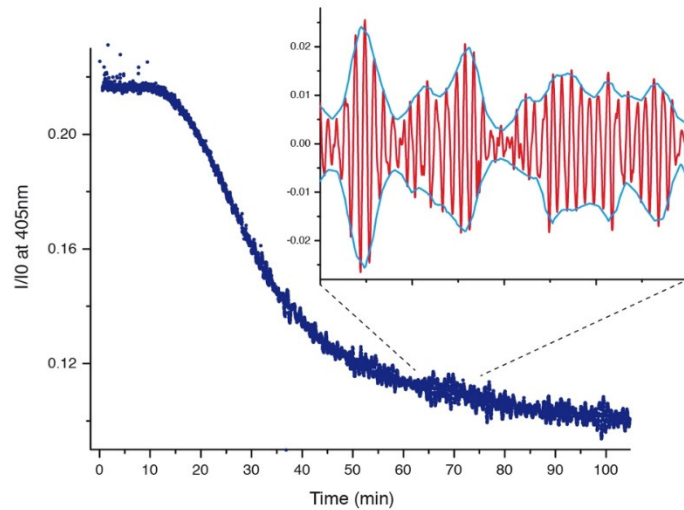
→ What is the origin of prion structural diversity?

Is it deterministic or stochastic ?

→ How structurally distinct assemblies could coevolve within the same environment?

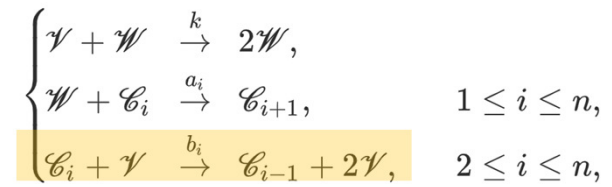
How this diversity is maintained ?

## Unusual depolymerization behaviour of Human Prion fibrils: Existence of oscillatory phenomenon



- **Existence of oscillation :**

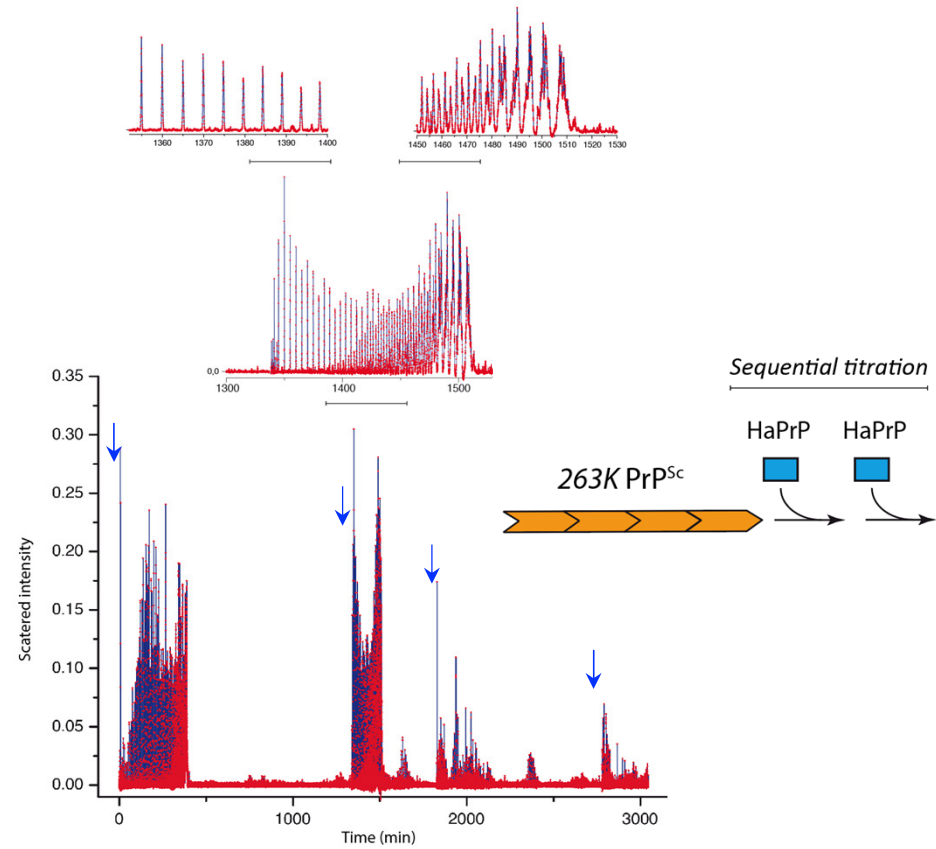
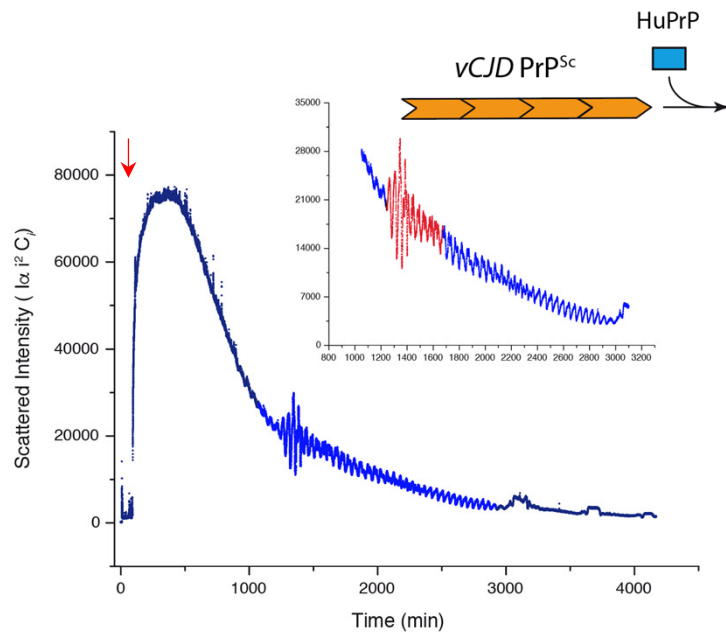
- System far from the equilibrium
- **Multi-compound system with a constitutional exchange**
- Dissipative process
- Existence on non-linear process as autocatalysis



*Mezache et al. 2019.*

# Prion replication is a complex process

*D.Martin and J.Torrent et al. in progress*



→ Prion replication is a dissipative process involving a network of exchange between structural distinct set PrP<sup>Sc</sup> assemblies