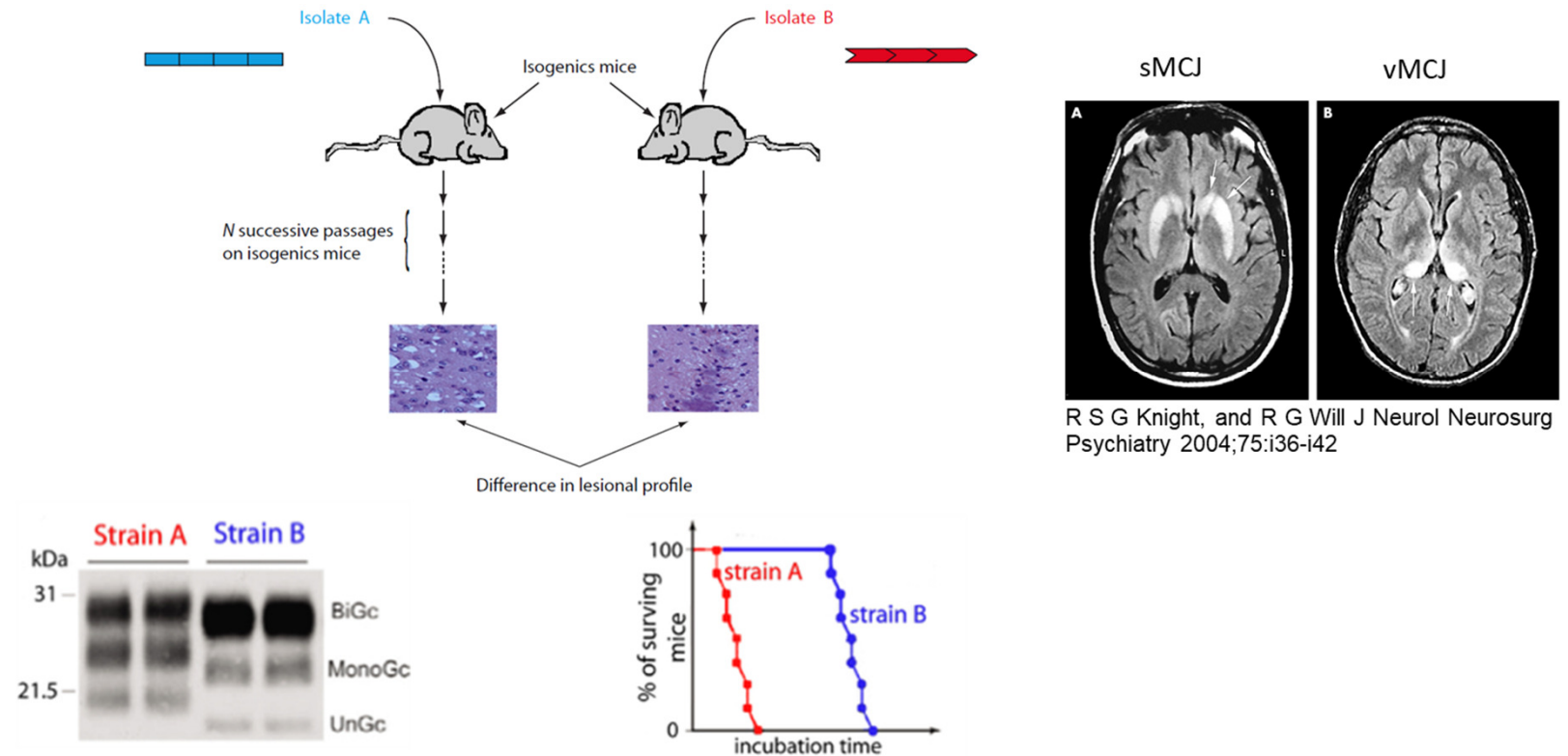


## caractérisation de l'hétérogénéité structurale des prions

Utilisation de banques de protéines ligands synthétiques (AlphaReps) pour l'étude de conformères de la protéine prion cellulaire et scrapie

# ➤ Inter-individual structural diversity of prion assemblies

Prion strains



- ➔ One PrP sequence can produce different **prion strains**
- ➔ Structural variability of Prion assemblies leads to different phenotypes of the disease
- ➔ **Strain phenomom**

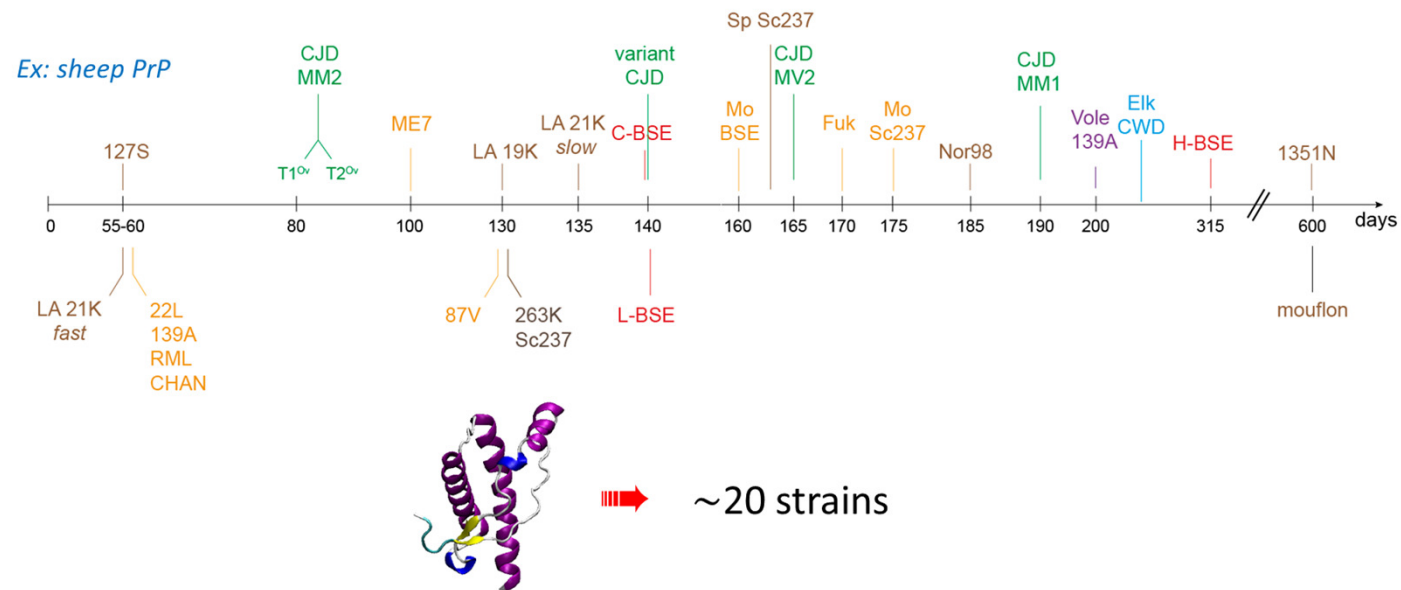


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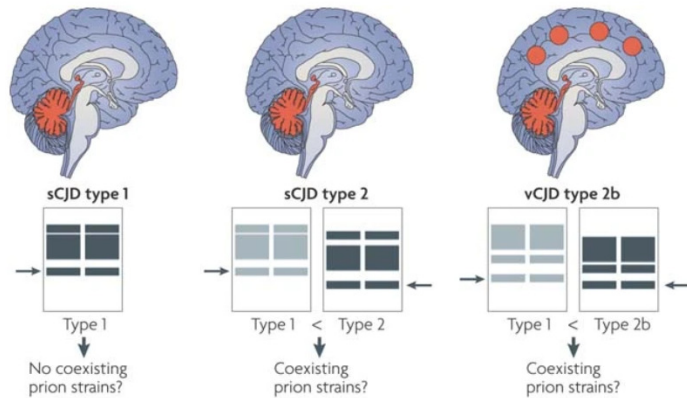
## ➤ Multistability of PrP protein

- Different prion strains can be propagated on a given PrP primary structure without any divergence during passages



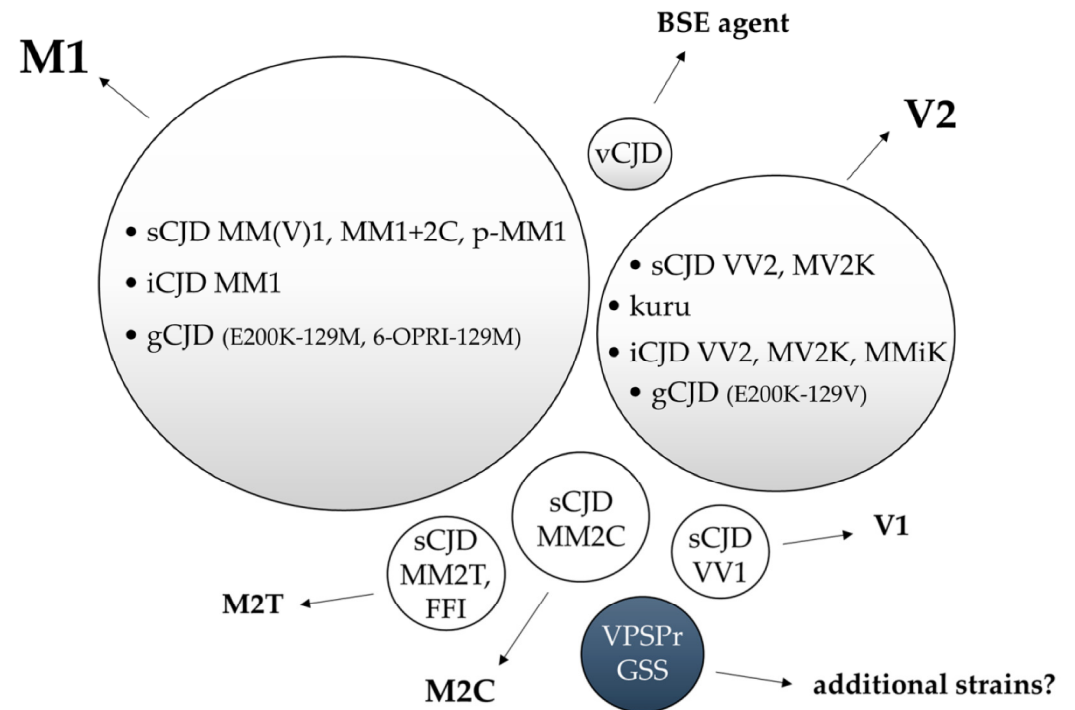
## ➤ Les souches de prion chez l'Homme

- Deux critères pour définir les souches humaines de prion
  - polymorphisme au codon 129 : M / V
  - profil électrophorétique
  - (+ mutation pour les formes génétiques)



Aguzzi et al. 2007

≥ 6 souches de prion chez l'homme



## ➤ Stabilité évolutive des souches de prions

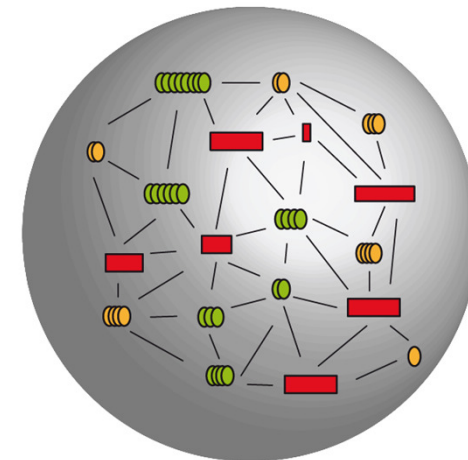
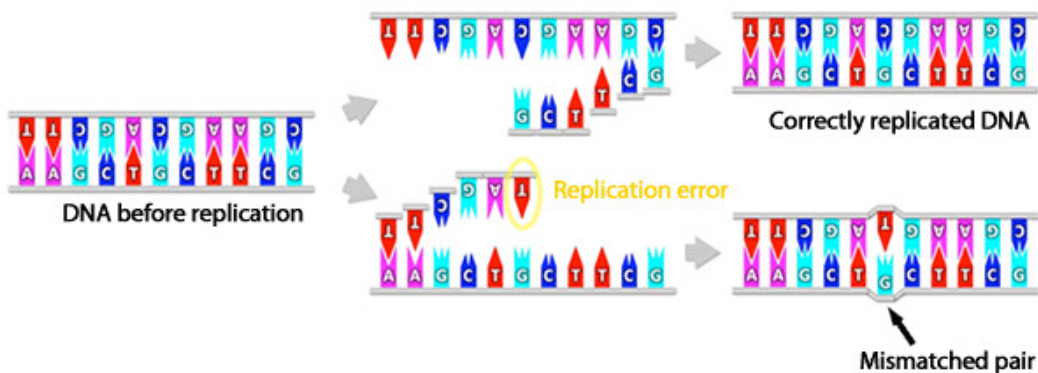
Un système évolutif différent de celui des organismes à acides nucléiques

### • Évolution chez les virus, bactéries

- Impliquée dans le franchissement de barrière d'espèce et le maintien chez l'hôte
- Mécanisme continuellement actif
- Erreurs de copie des RNA/DNApol

### • Évolution chez les prions

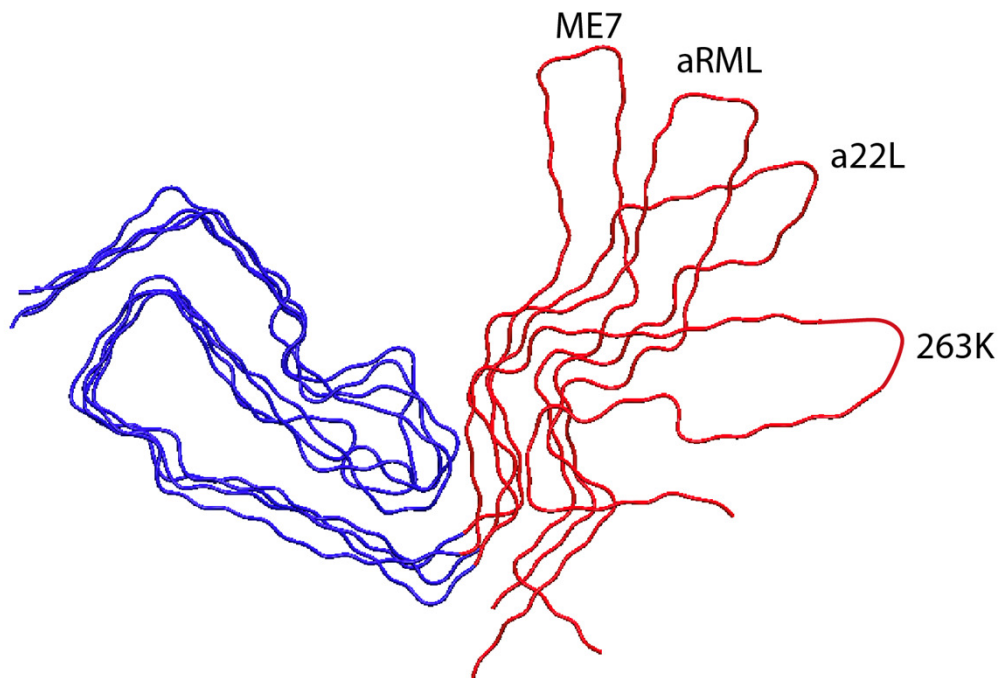
- Seulement pour l'adaptation à un nouvel hôte (franchissement de barrière d'espèce)
- Pas d'évolution, une fois adapté
- Mécanisme inconnu (selection du meilleur replicant ? )



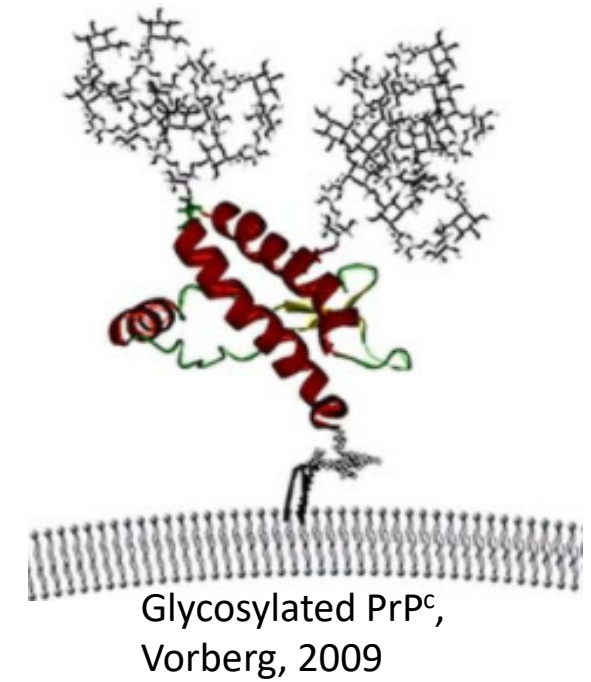
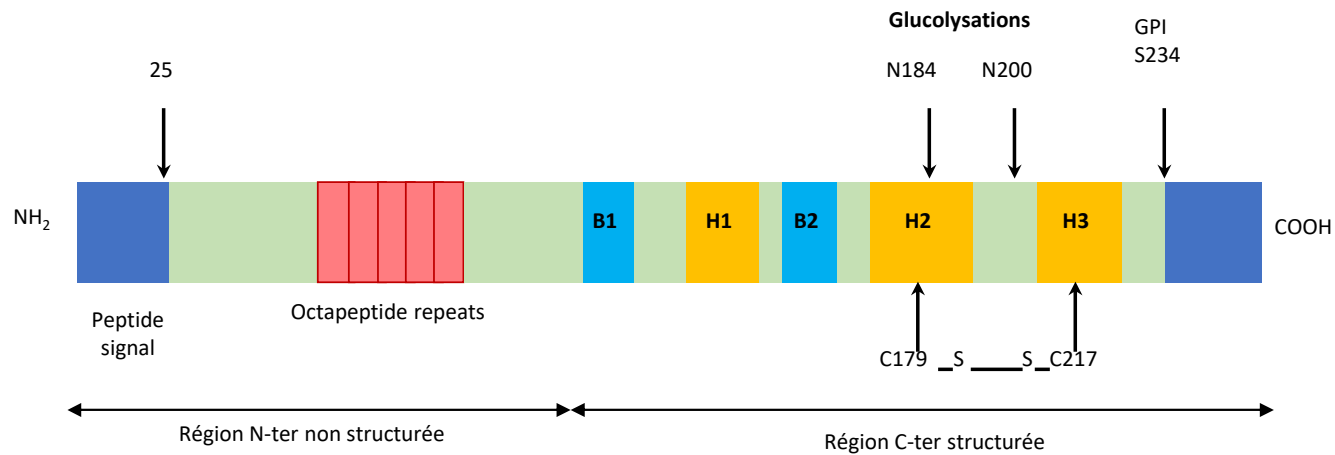
## ➤ Déterminants structuraux de ces caractéristiques de souches ?

Récents apports de la Cryo-microscopie électronique

- Superimposition of 4 prion structures
- Identification of 2 topologically distinct regions
  - “prion determinant” zone
  - “strain determinant” zone



## ➤ Protéine Prion PrP



## ➤ Challenging the selection & identification of prion interactants

### Self protein

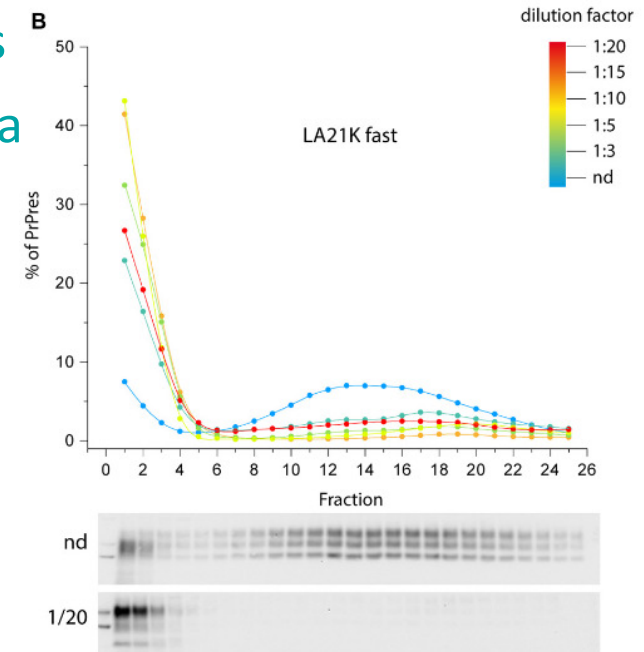
- No/weak immune response
- Immune repertoire bias
- Glycosylated

### Dynamic protein structures

- Labile assemblies
- No structural data (until recently)

⇒ needs for other strategies than classical immunization

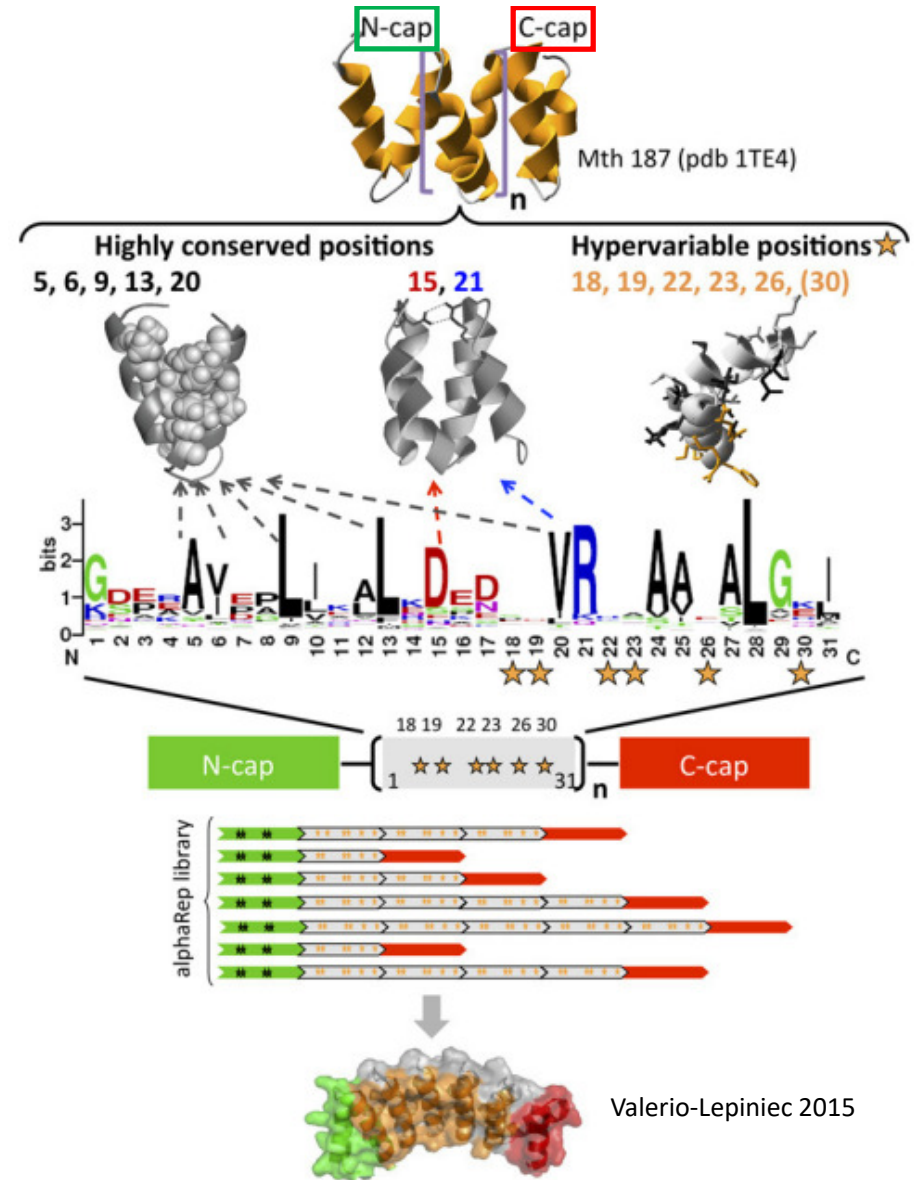
- Use of synthetic libraries
- In vitro selection process





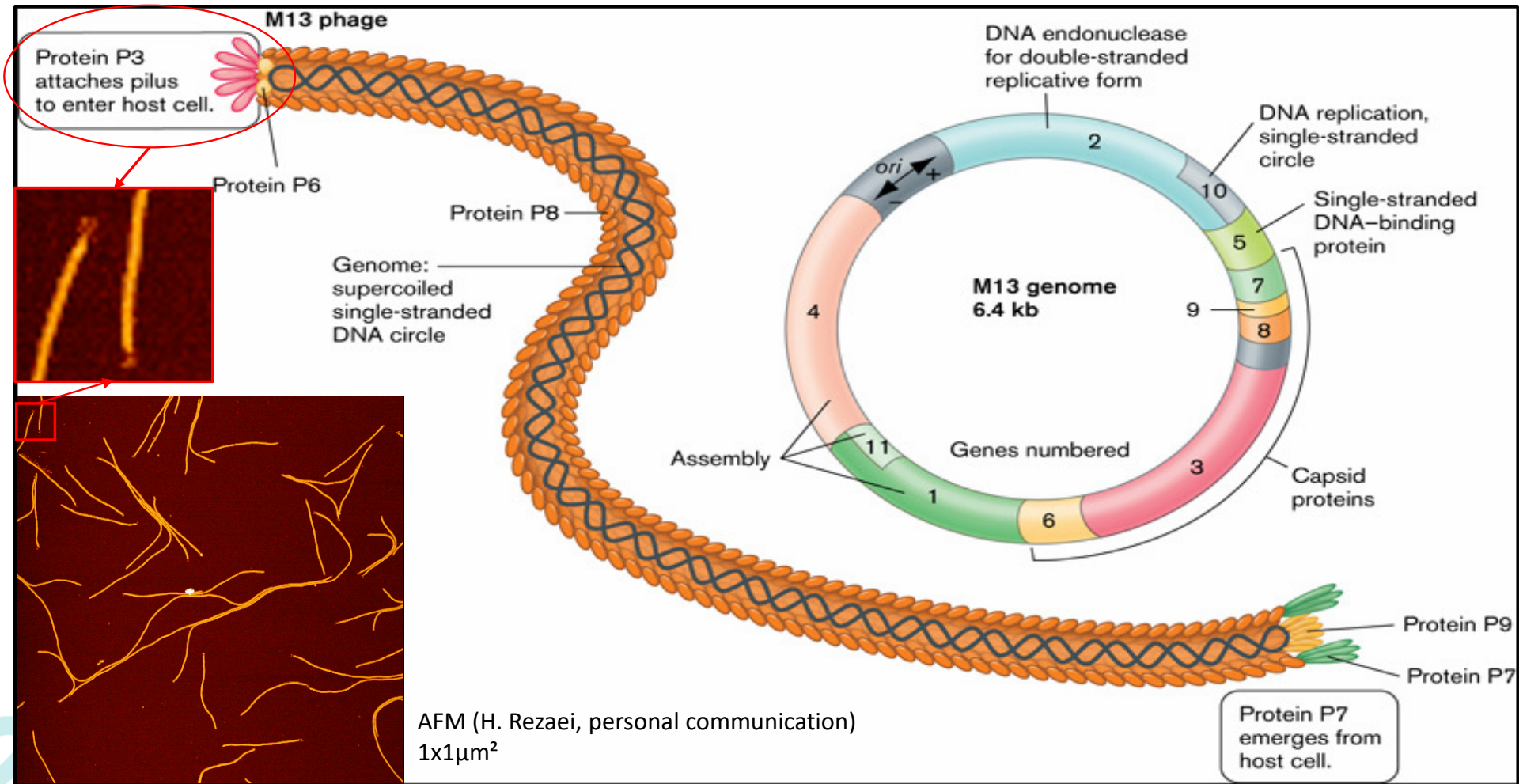
## ➤ Banque αRep

- **N-cap**: 6 positions variables :  
DPEK**V**EM**Y**IK**N**LQ**D**DS☆☆VR☆☆AA☆AL**G**☆I
- HEAT repeats : 6 positions variables /  
domaine (xN):
- GDERAVEPLIKALKDED☆☆VR☆☆AA☆AL**G**☆I  
☆ : localisés à la surface externe de 2<sup>ème</sup> hélice
- **C-cap** : Aucun acide aminé variable :  
GGERVRAAMEKLAETGTGFARKVAVNYLETH
- $\geq 3.10^8$  clones indépendants générés

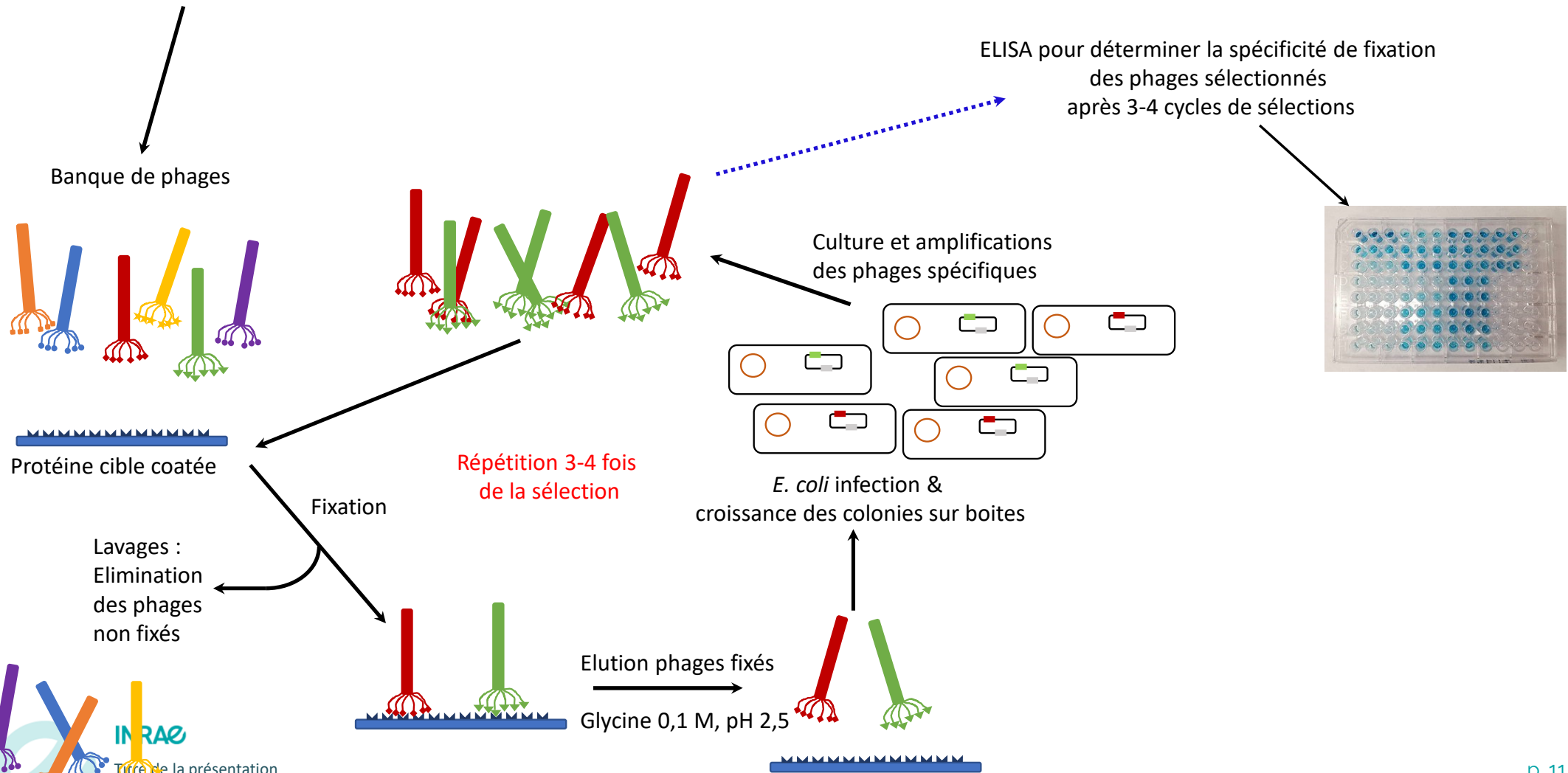


## ➤ Banque aRep

### Le bactériophage M13

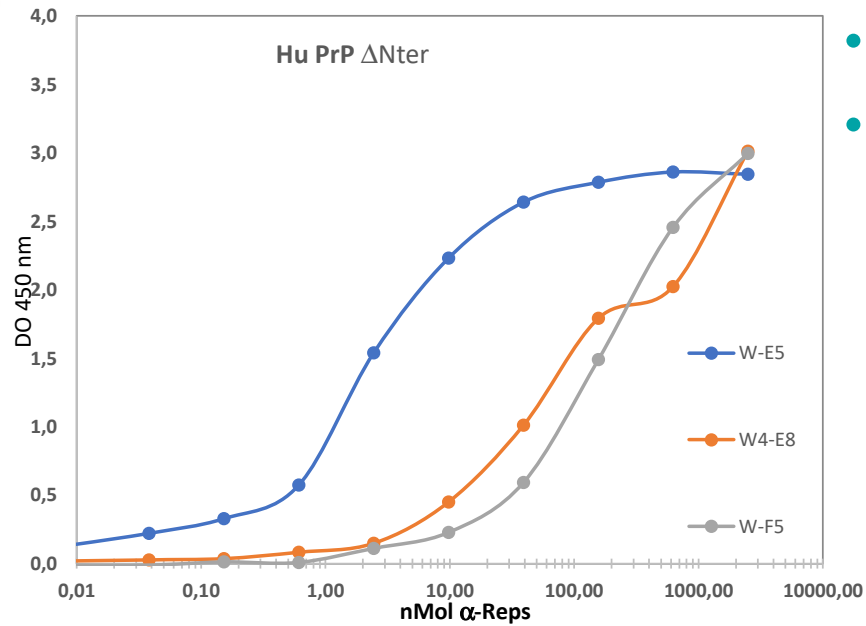
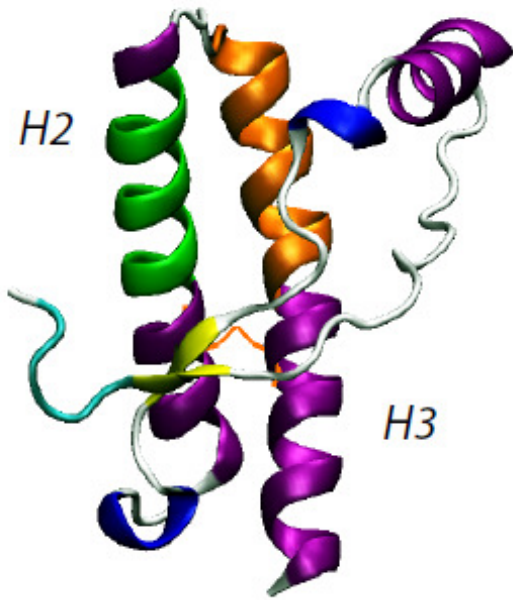
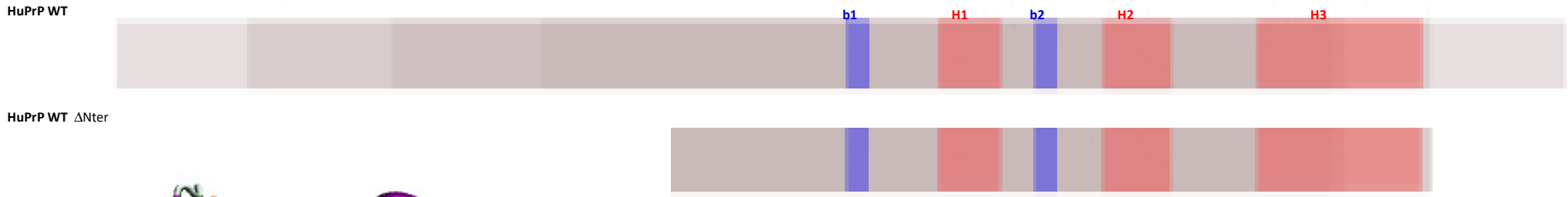


# ➤ Sélection des phages (biopanning)



# ➤ Selecting Alphareps using monomeric Wild Type truncated PrP

(Nter's too sticky...)



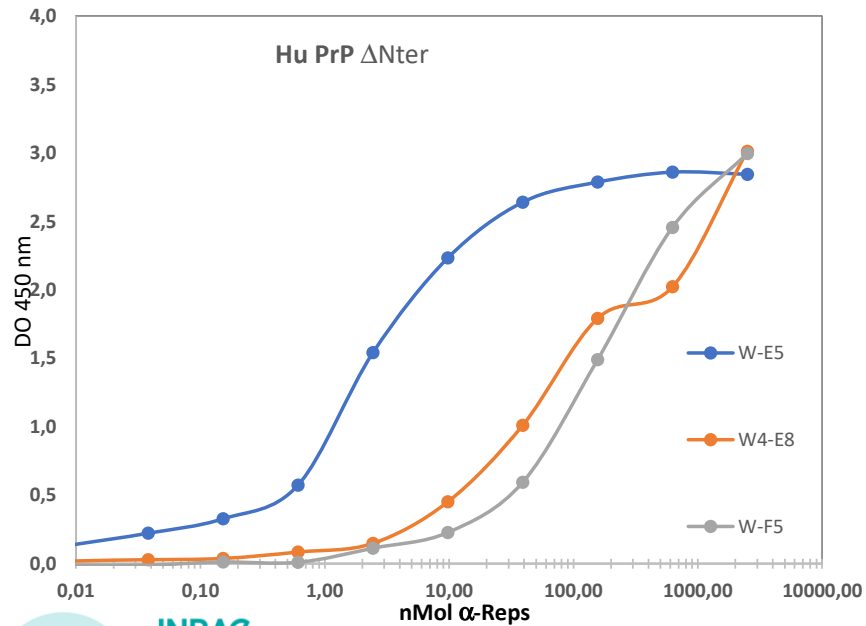
- 5 different Alphareps
- $2\text{nM} < K_D < 200\text{nM}$  (ELISA)

## ➤ Affinity evaluation

Highly dependent on the technology: avidity and steric hindrance

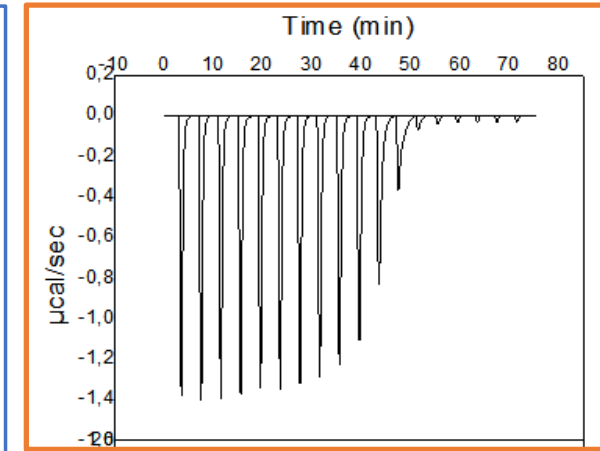
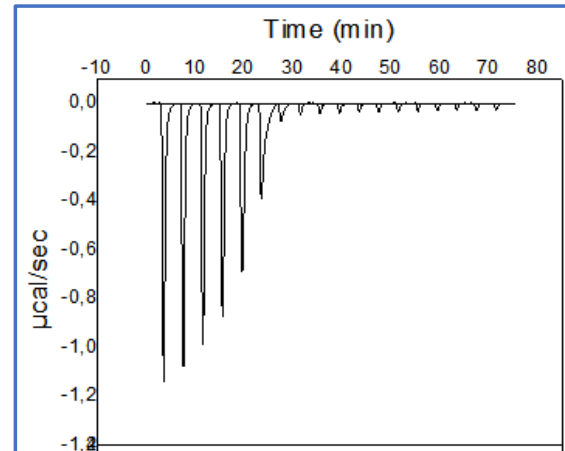
### ELISA : surface interaction

$2\text{nM} < K_D < 200\text{nM}$

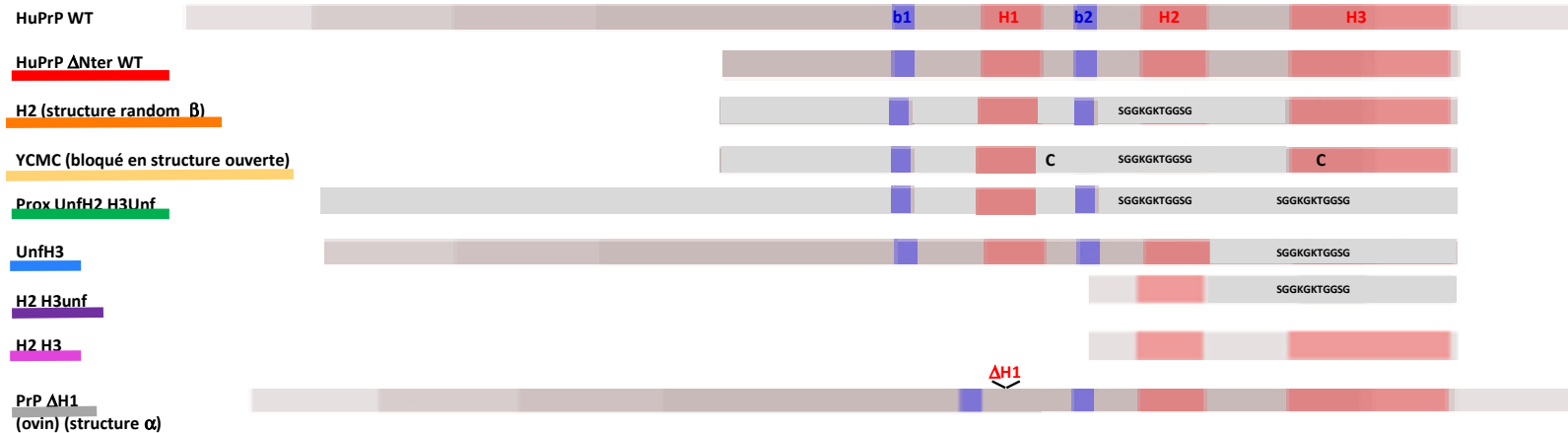


### Isothermal calorimetry (ITC), in solution

- WE5  $K_{eq} = 5,7 \cdot 10^{-7}$
- WE4E8  $K_{eq} = 2,2 \cdot 10^{-9}$

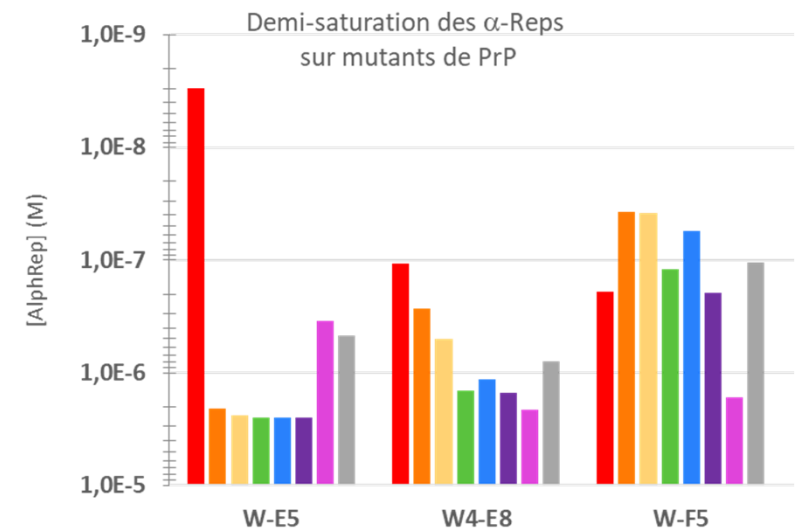


## ➤ Affinity evaluation : Comparison with PrP mutants



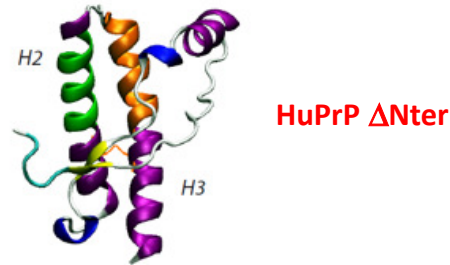
### Topological interaction: no linear epitope

- W-E5 highly specific for WT PrP
- W4-E8 less specific, probably targeted to H1 helix and surrounding H2/H3 topology
- No clear evidence for linear W-F5 binding site



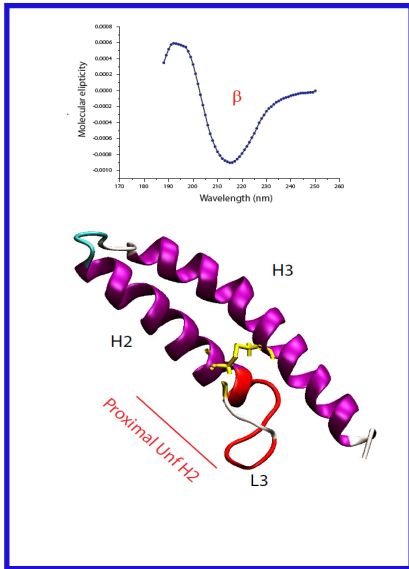
# ➤ Selecting AlphaReps on monomeric PrP conformation mutants

Mutations designed to selectively enhance putative transitional conformations

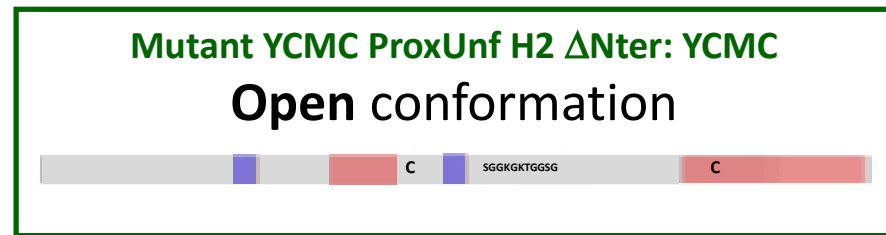
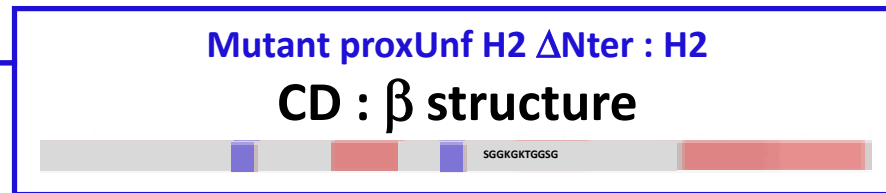
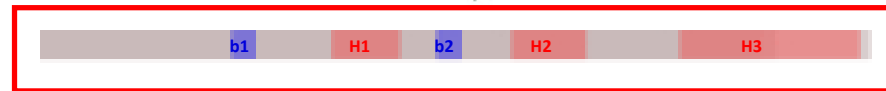


## H2 mutant:

Proximal H2 helix domain unstructured

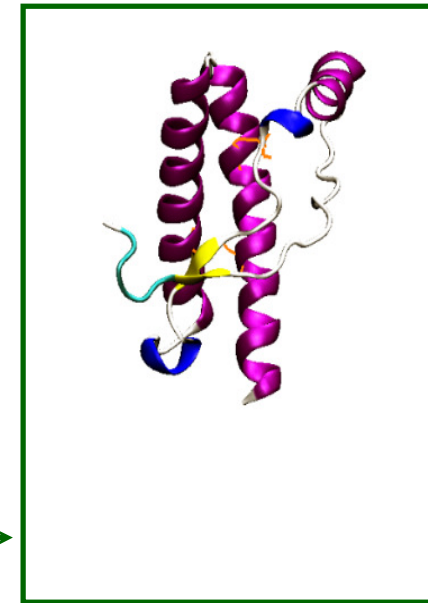


H2



## YCMC mutant:

2 extracysteins stabilizing the proximal H2 mutant



Y



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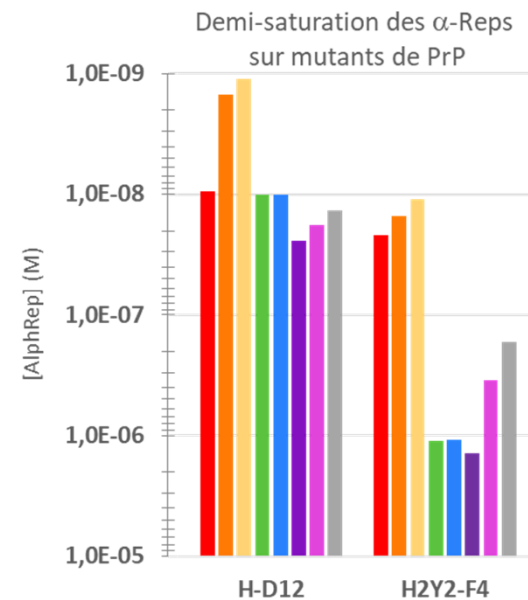
## ➤ Selecting AlphaReps on monomeric PrP conformation mutants

### Results

#### 5 clones retrieved on H2

- Identical clones retrieved on both mutants (2 clones)
- Multireactive alphareps
  - Preference of mutants over WT (H-D12)
  - Some are less selective (H2Y2-F4)
- Probable structural similarity of both mutants on polystyrene coating
- ITC to be performed...

#### 8 clones retrieved on Y

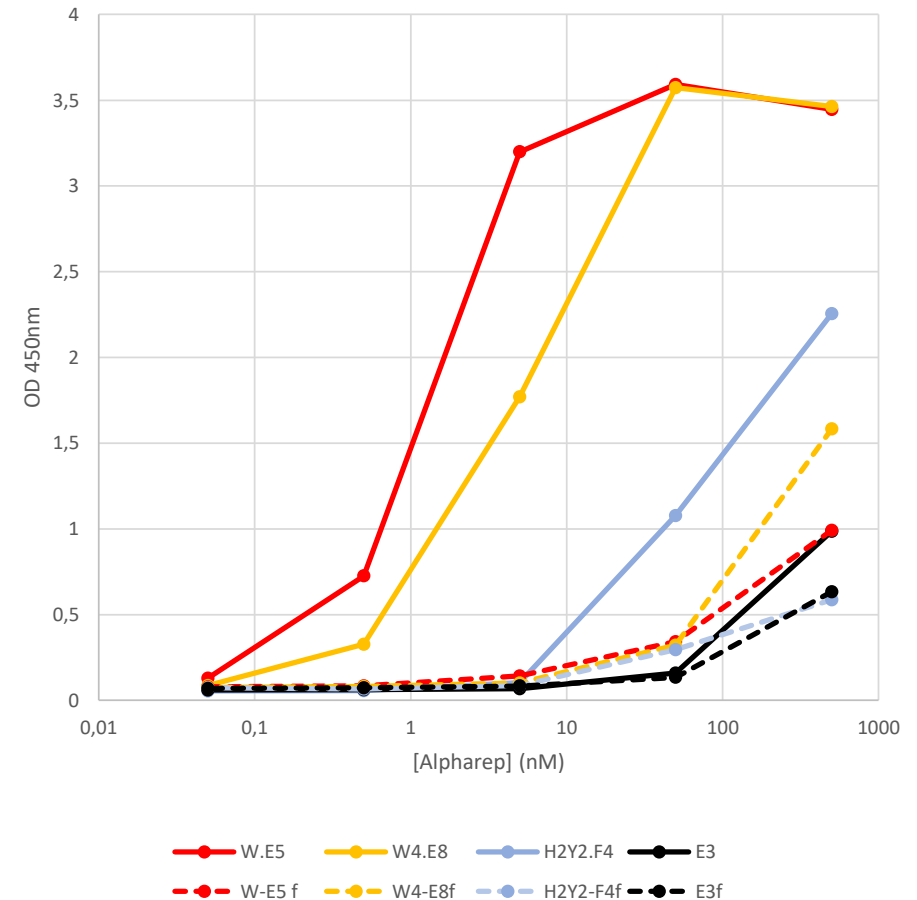




## ➤ Selecting AlphaReps on monomeric PrP

Conclusions on WT & conformation mutants

- Conformational epitopes
- Cross-reactivity between WT and several structural mutants
- bêta structure and open conformation (blocked with 2 extracysteins) behave similarly ( on PS coating)
- No reactivity on extractive PrP (glycosylations...)
- Poor reactivity on fibers
  - Structural reasons
  - bioavailability



## ➤ Selecting AlphaReps on fibrillar PrP

Selection on full length Human or murine Prp fibrills

- Recombinant fibers are infectious

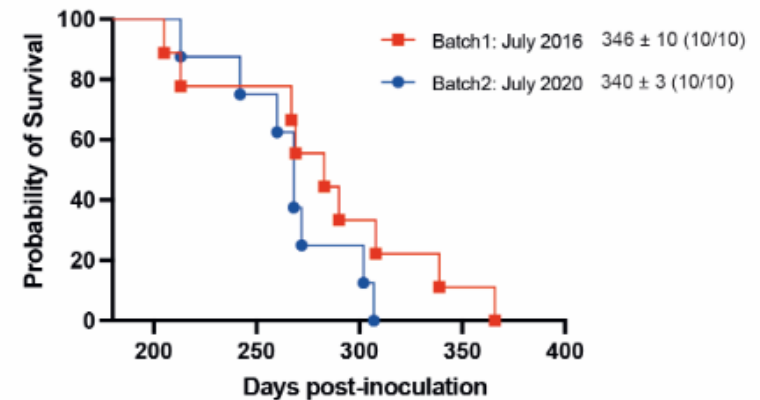
**Table 1. Serial transmission of hamster, mouse and human rPrP assemblies by intracerebral route in tg7 mice**

rPrP assemblies	Mean survival in days $\pm$ SEM (n/n <sub>0</sub> ) <sup>a</sup>		
	Hamster	Mouse	Human
1 <sup>st</sup> passage	282 $\pm$ 17 (9/9)	346 $\pm$ 10 (10/10)	476 $\pm$ 11 (8/9)
2 <sup>nd</sup> passage	273 $\pm$ 12 (8/8)	84 $\pm$ 1 (8/8)	260 $\pm$ 16 (8/8)
3 <sup>rd</sup> passage	244 $\pm$ 13 (8/8)	84 $\pm$ 2 (7/7)	215 $\pm$ 7 (6/6)
4 <sup>th</sup> passage	238 $\pm$ 15 (5/5)	80 $\pm$ 2 (6/6)	219 $\pm$ 7 (8/8)

<sup>a</sup>n/n<sub>0</sub>: number of mice with neurological disease and positive for PrP<sup>Res</sup> in the brain by immunoblotting/number of inoculated mice

Rezaei et al., submitted

**Mouse PrP assemblies**

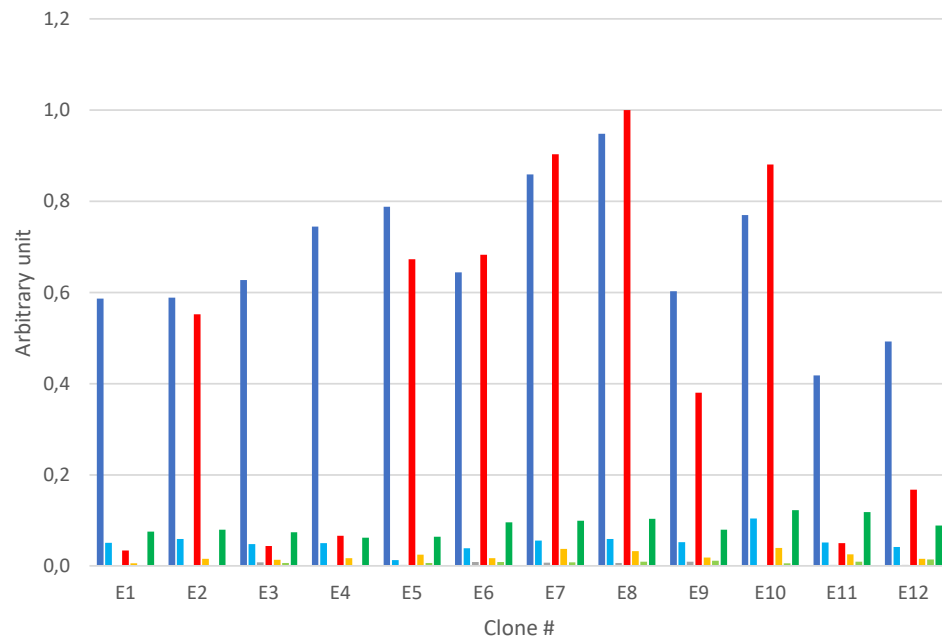


## ➤ Selecting AlphaReps on fibers produce highly reactive clones

High number of polyspecific AlphaReps

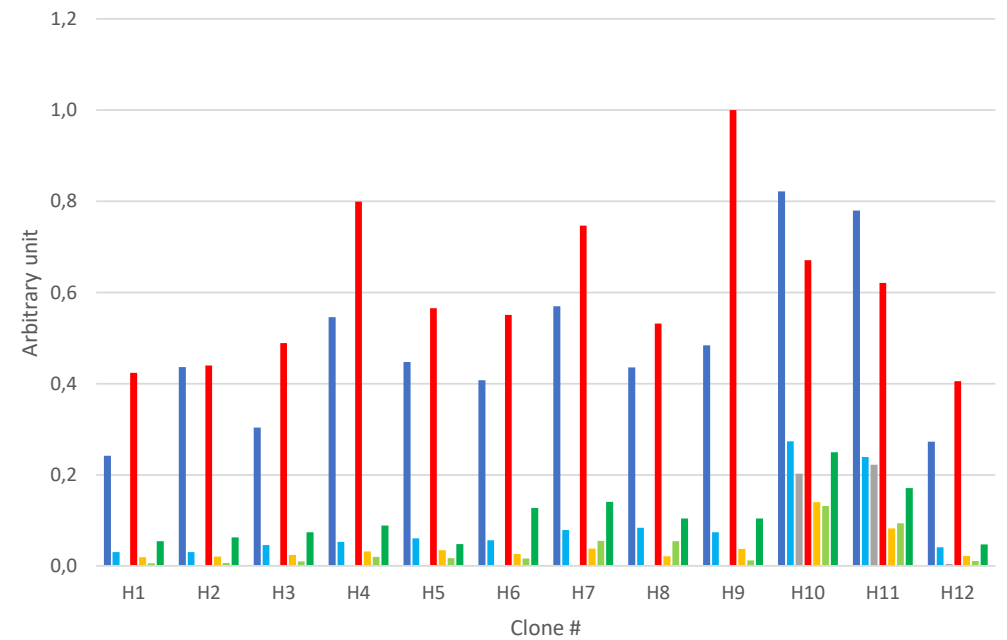
Some monospecific clones

**Phages  $\alpha$ Reps selected on Murine PrP Fibres**



Mo\_fibres Mo\_mono BSA Hu\_fibres Hu\_mono D\_Nter Nter

**Phages  $\alpha$ Reps selected on human PrP fibres**



Mo\_fibres Mo\_mono BSA Hu\_fibres Hu\_mono D\_Nter Nter



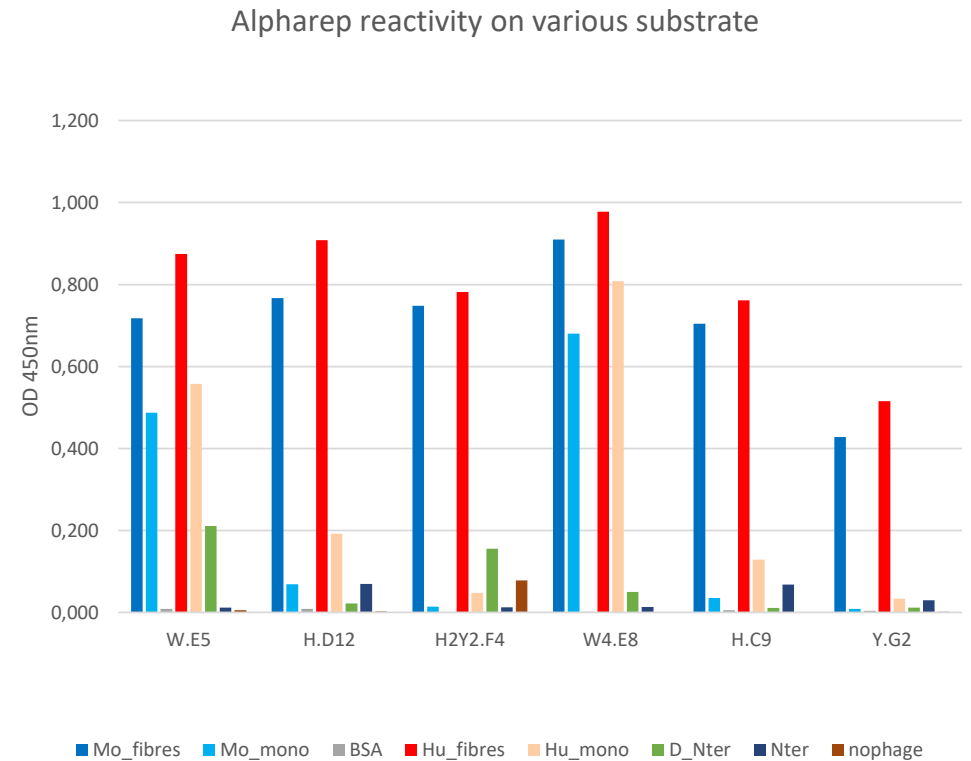
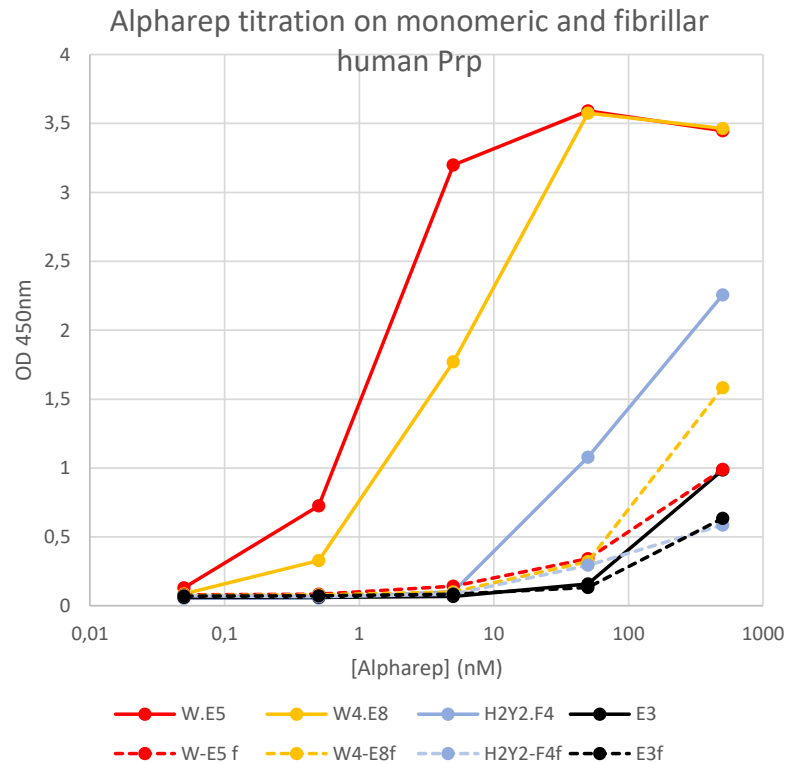
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## ➤ Additional data on monomer-specific Alphareps

Monomer specific alpharep variable reactivity on prion fibrils



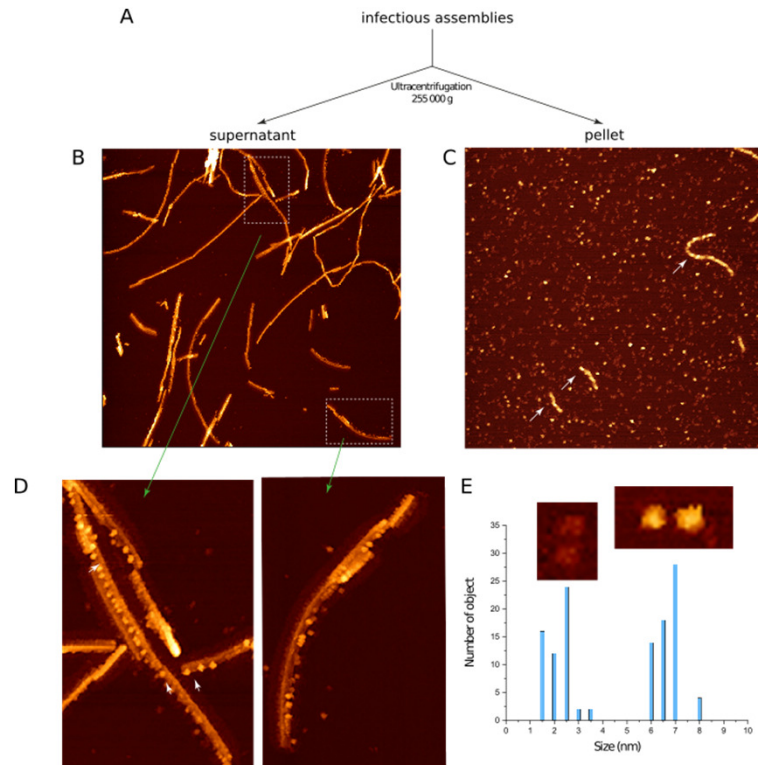
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## ➤ Possible hypotheses

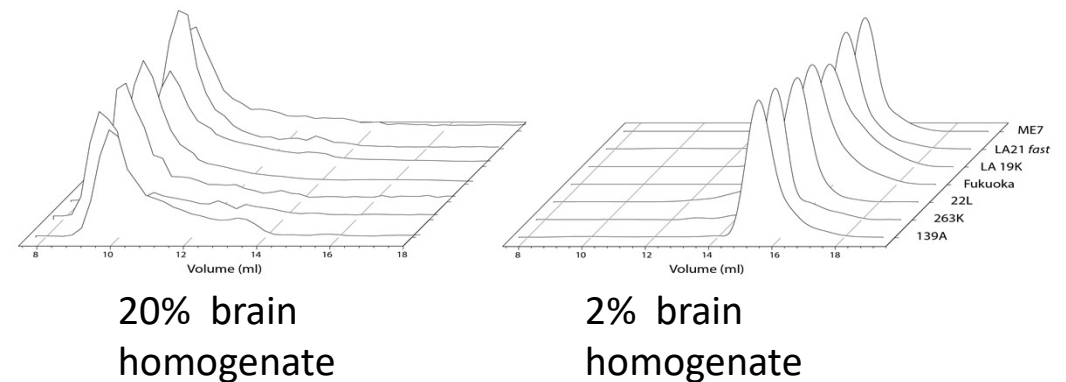
- Recombinant fibers are dynamic



Rezaei, Requena  
et al., unpublished

- Matured fibers produce monomers
  - Reactivity with W- alphareps
  - H- and Y- alpharep differential reactivity
    - Substantial monomer contribution from the fibers
- Diluted fibers produce small objects

Chromatograms of two different dilution preparations of several prion strains separated on SEC



## ➤ Future prospects

Toward a better understanding of the prion fibers dynamics

- Use W- and H/Y alphareps for characterization of Prp preparations over time
  - Progressive loss of W- reactivity,
  - increase in H/Y reactivity.
- Confirm the relative contributions of
  - Fibers and monomers in the fibers preparations and stability
  - Over time
  - Depending on the Prp sequence
- conformer-specific alphareps use for
  - Orientation of the conversion process
  - Modulation of the conversion kinetics



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## > remerciements



- MAP2 (VIM)

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- Fabienne Reine
- Laetitia Herzog

- MIP (I2BC)

- Agathe Urvoas
- Marielle Valério-Lepiniec
- Philippe Minard



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## ➤ Alpharep library

une repetition aléatoire de domaines HEAT

- HEAT : **H**untingtin, **E**longation factor 3, **P**hosphatase **A2**, **T**or2 kinase
- 2 hélices amphiphiles
- une surface riche en acides aminés hydrophobes
- une surface riche en acides aminés hydrophiles
- -> adopte une structure type solénoïde
  
- HEAT repeats bordées par une séquence **N-cap** et **C-cap** de structure semblable
  
- $\alpha$ -Reps :
  - ✓ Structures très souples pouvant changer de conformation suite à des interactions
  - ✓ Thermostables
  - ✓ Ne contiennent pas de ponts disulfures

