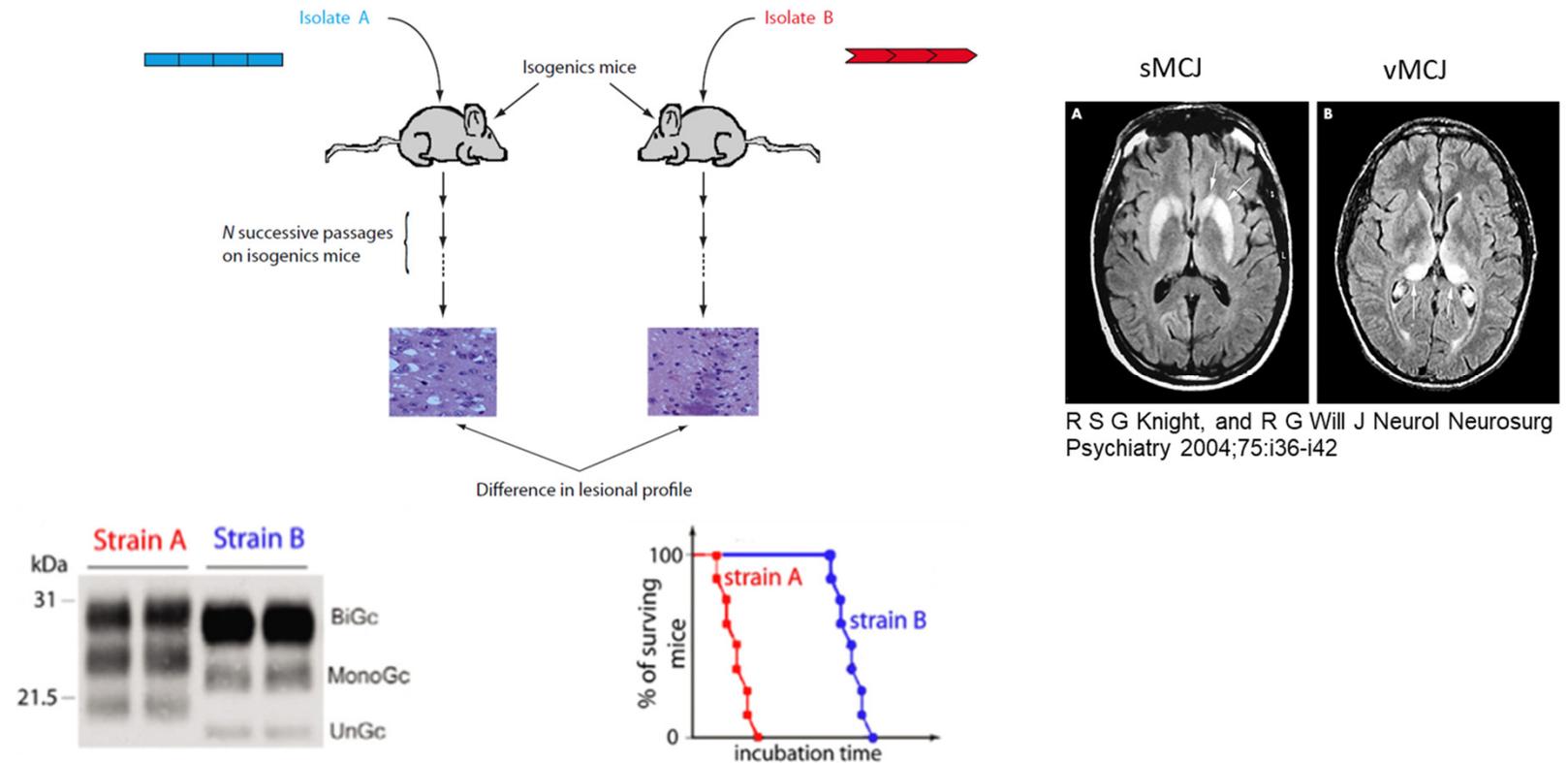


caractérisation de l'hétérogénéité structurale des prions

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**

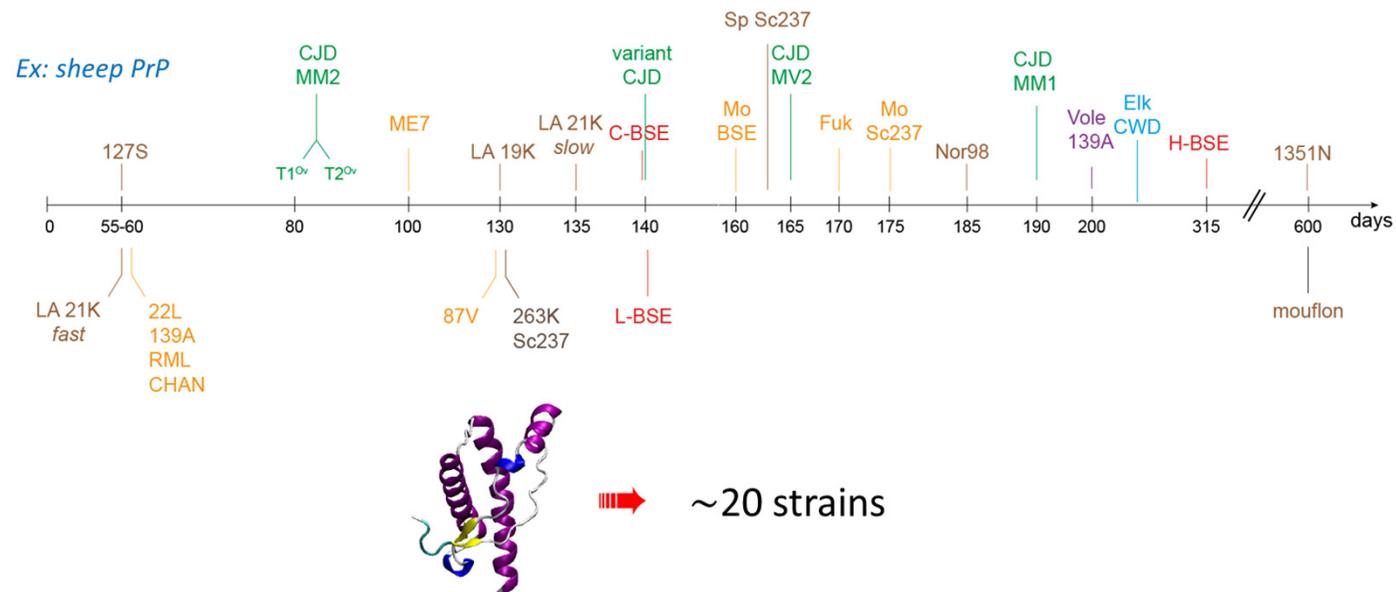


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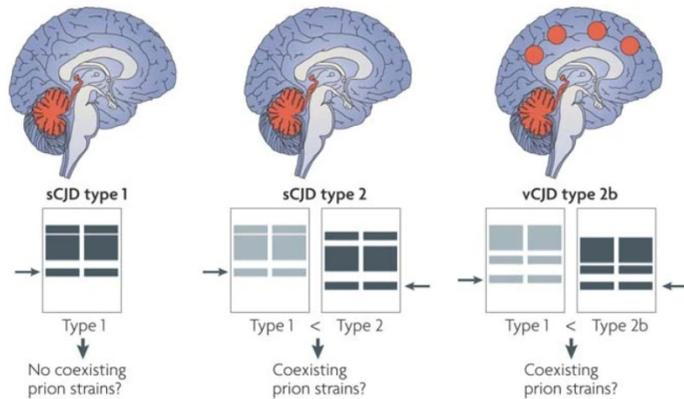
➤ Multistability of PrP protein

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



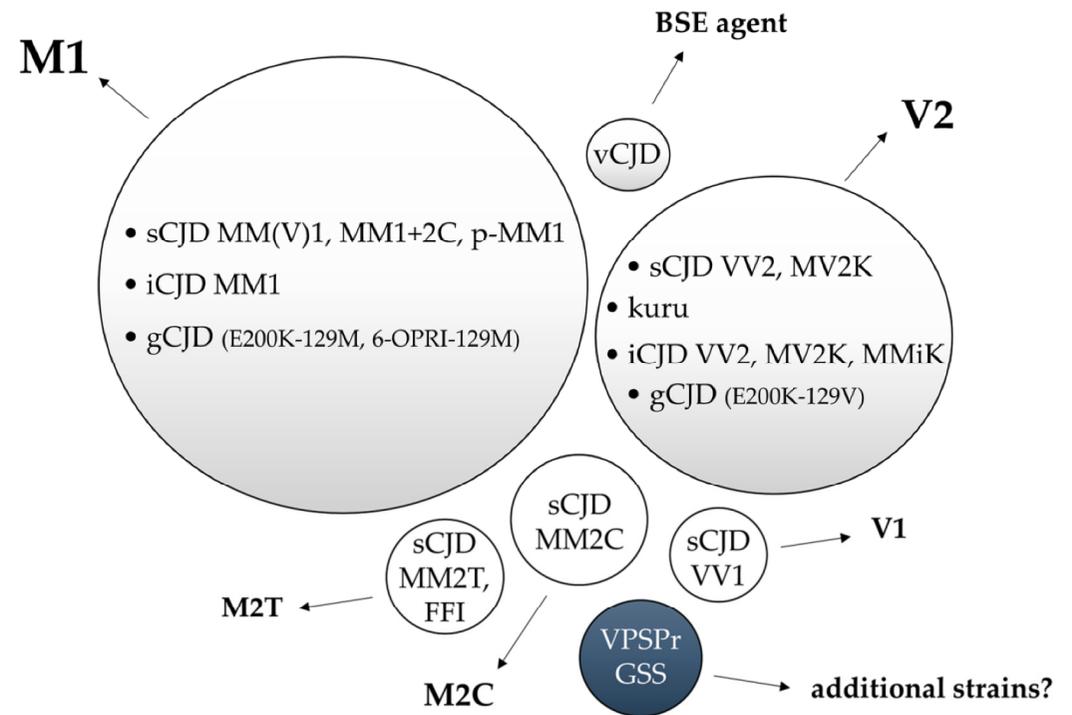
➤ Les souches de prion chez l'Homme

- 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

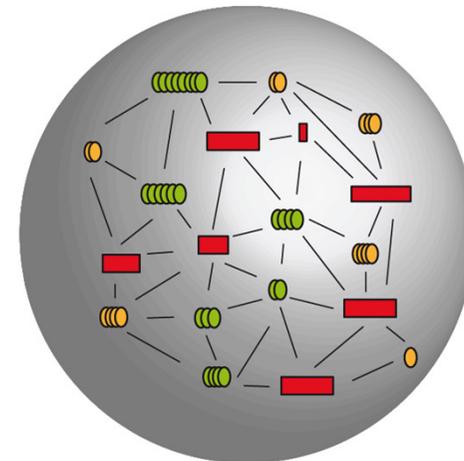
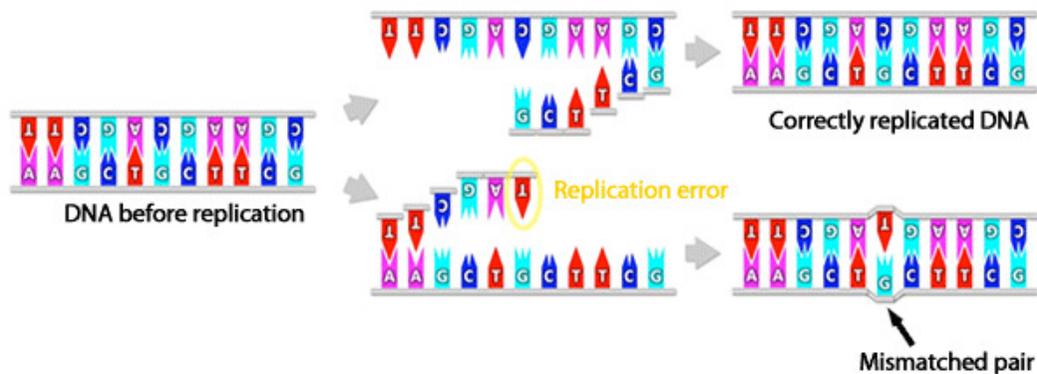
Un système évolutif différent de celui des organismes à acides nucléiques

• 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

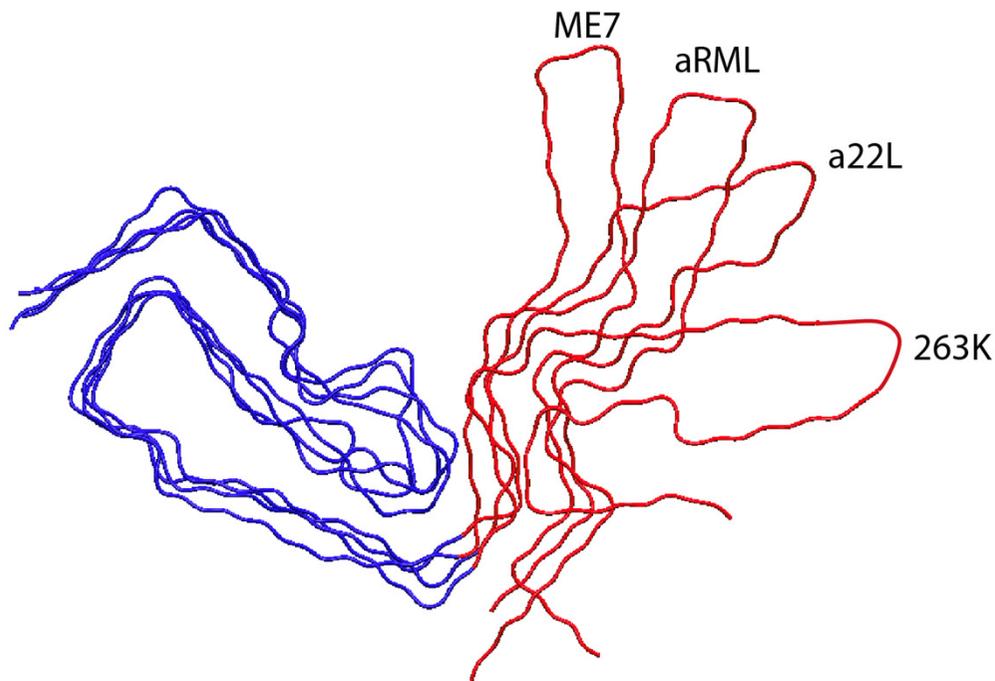
- Seulement pour l'adaptation à un nouvel hôte (franchissement de barrière d'espèce)
- Pas d'évolution, une fois adapté
- Mécanisme inconnu (selection du meilleur replicant ?)



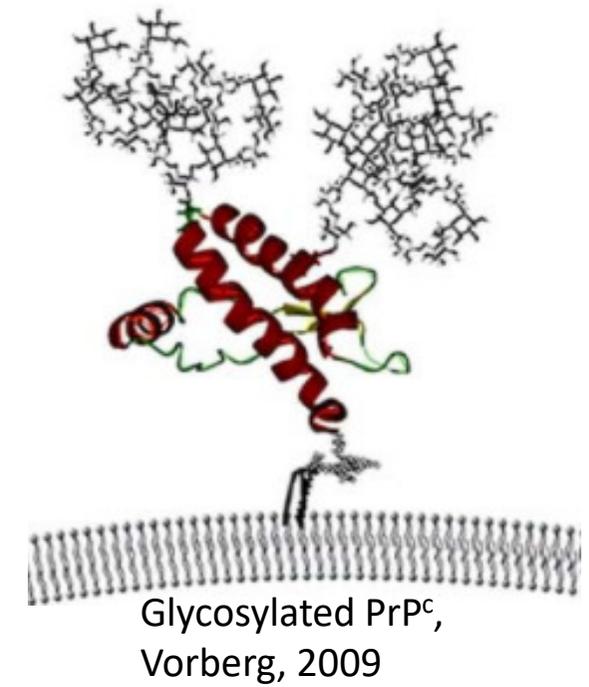
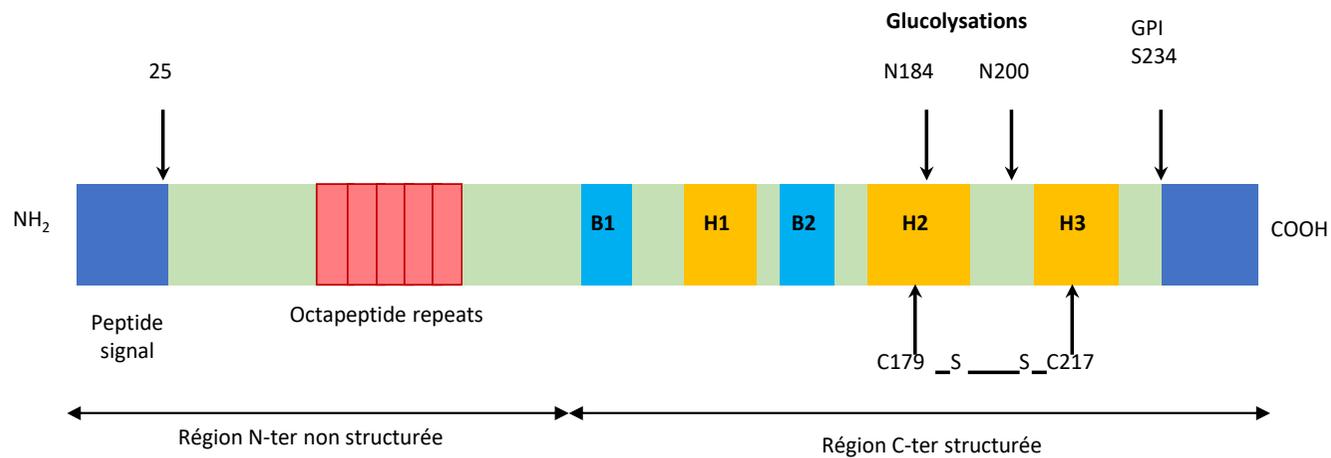
➤ Déterminants structuraux de ces caractéristiques de souches ?

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

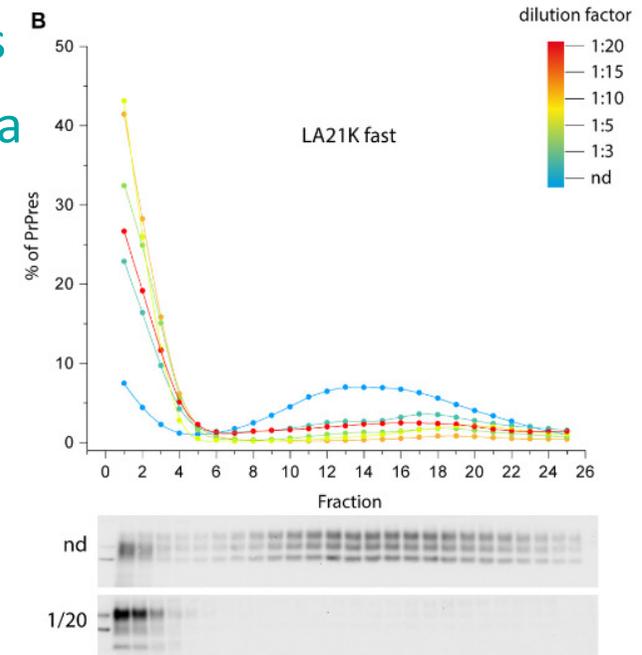
- No/weak immune response
- Immune repertoire bias
- Glycosylated

Dynamic protein structures

- Labile assemblies
- No structural data (until recently)

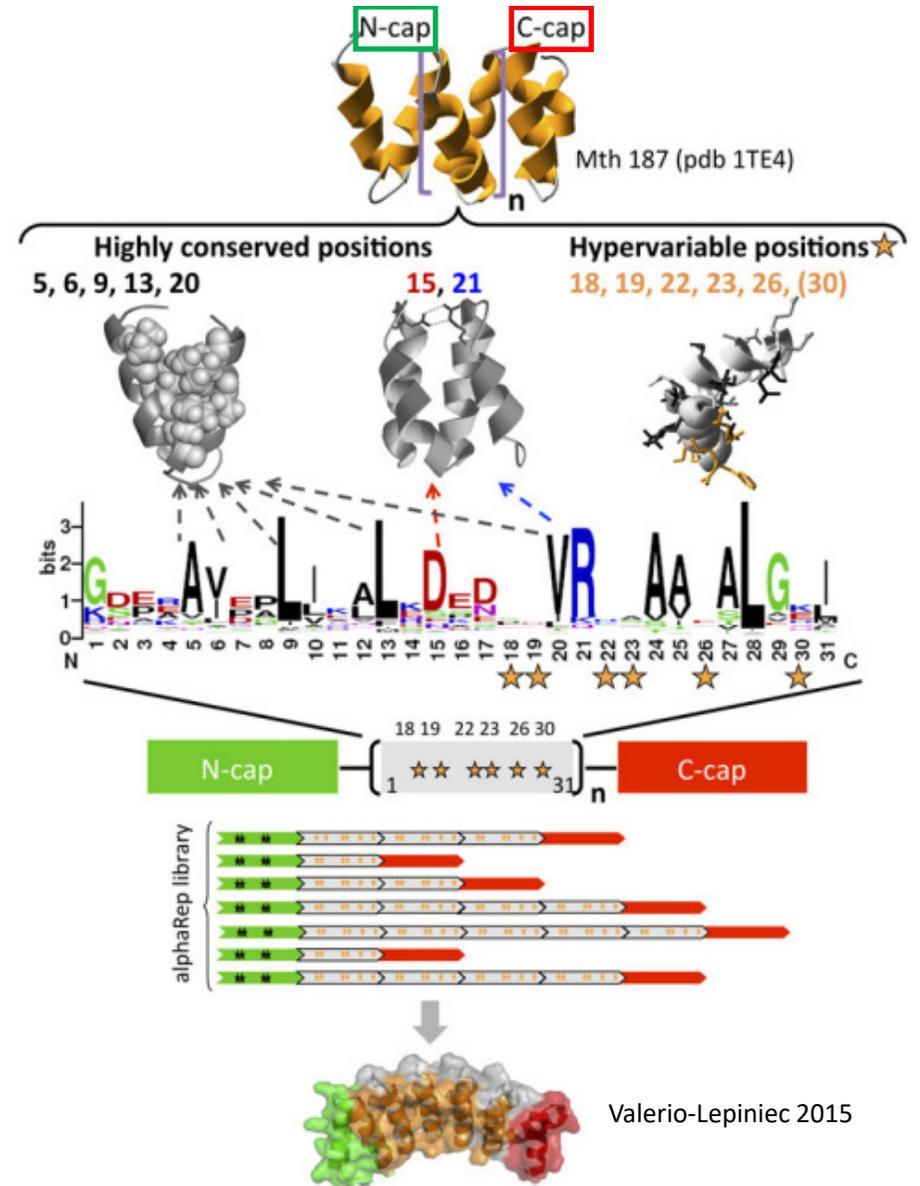
⇒ needs for other strategies than classical immunization

- Use of synthetic libraries
- In vitro selection process



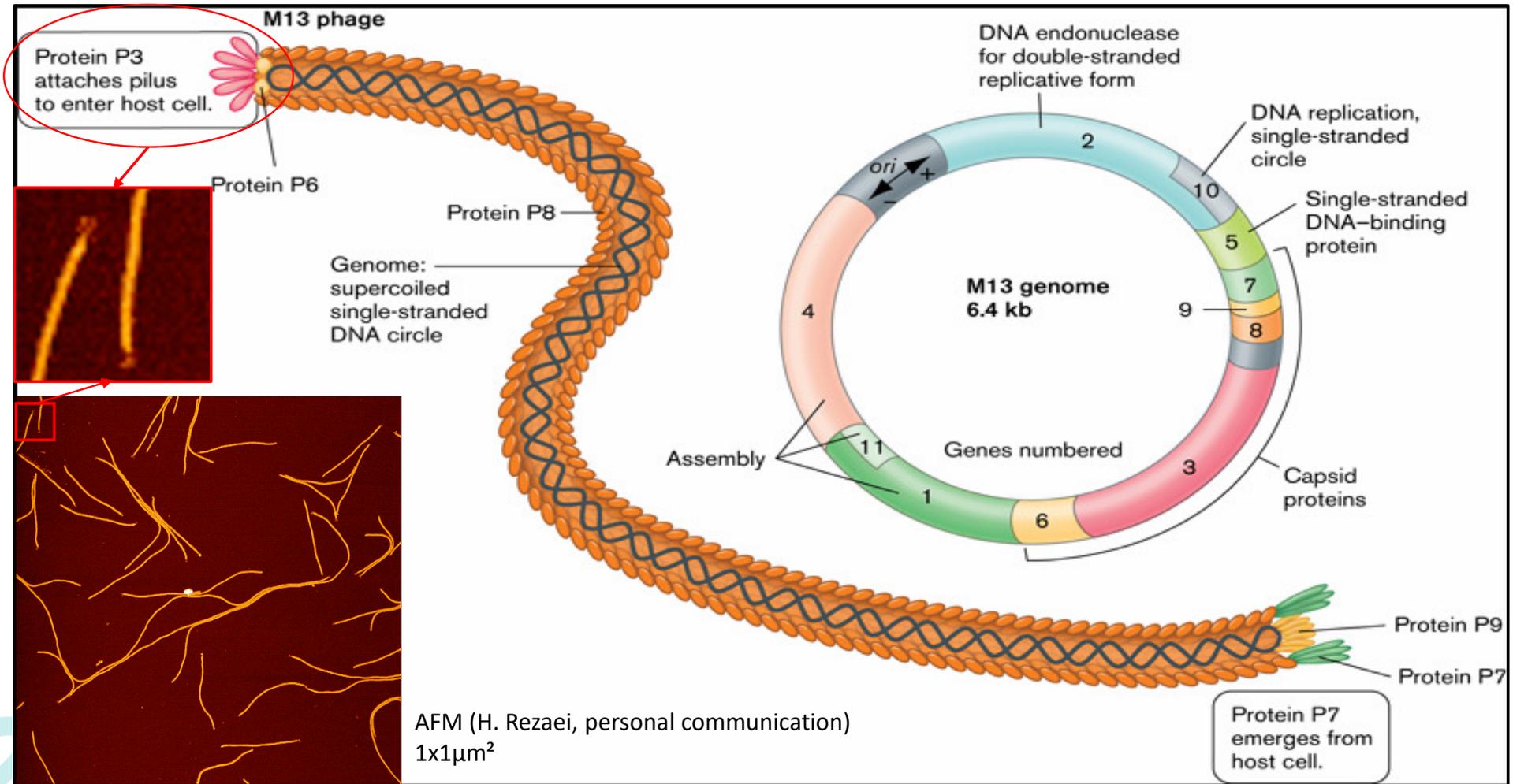
➤ Banque αRep

- **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^{ème} hélice
- **C-cap** : Aucun acide aminé variable :
GGERVRAAMEKLAETGTGFARKVAVNYLETH
- $\geq 3.10^8$ clones indépendants générés

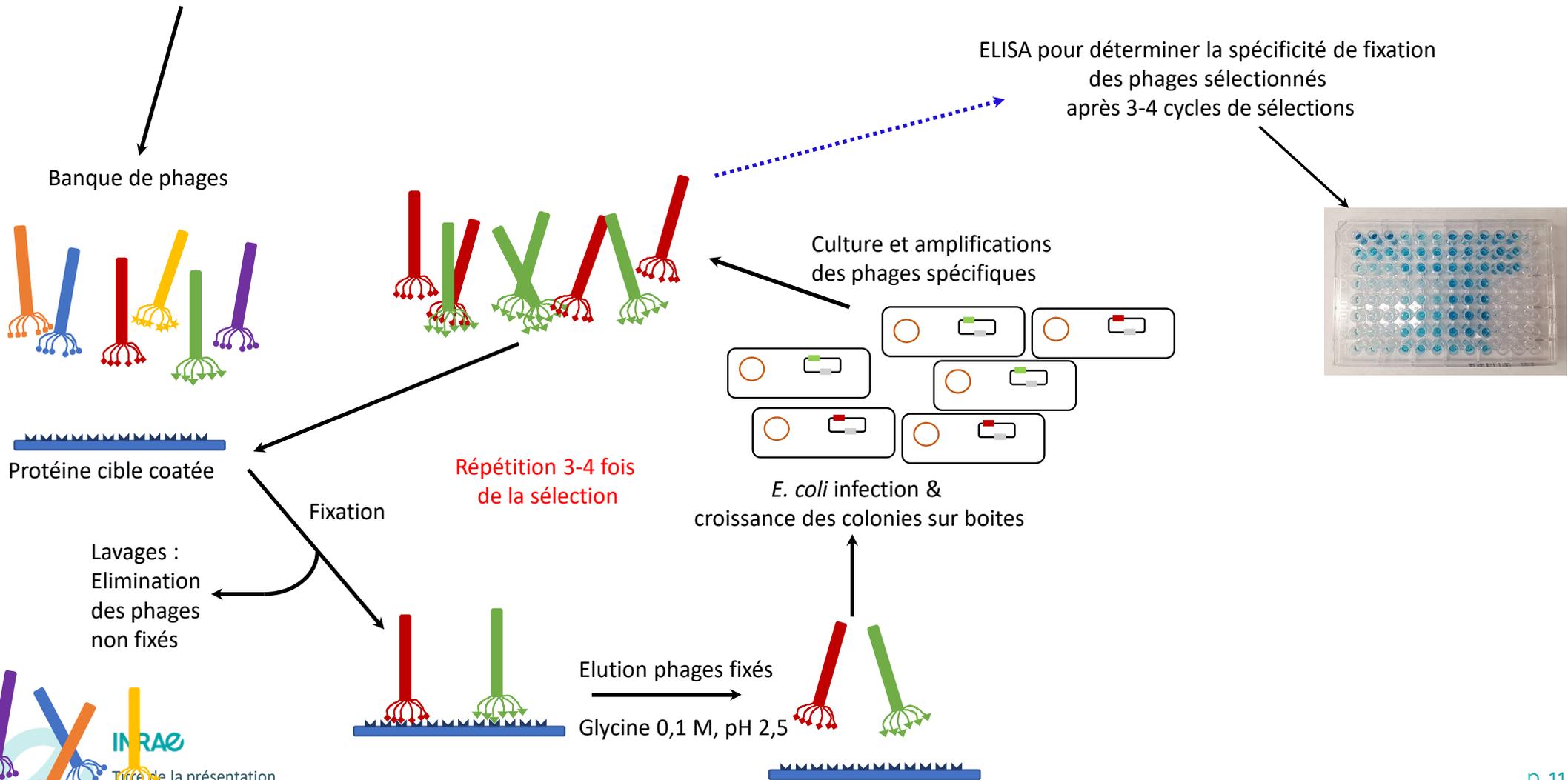


➤ Banque aRep

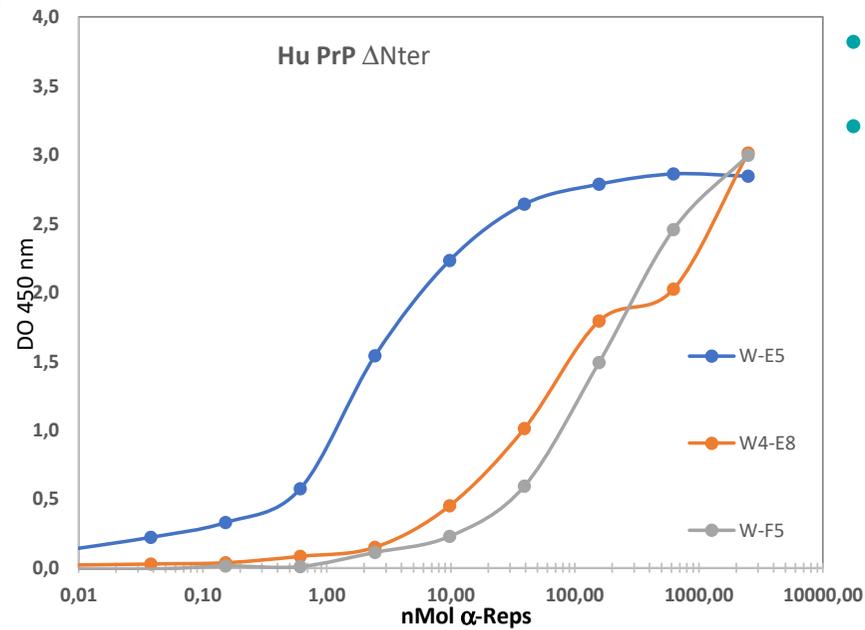
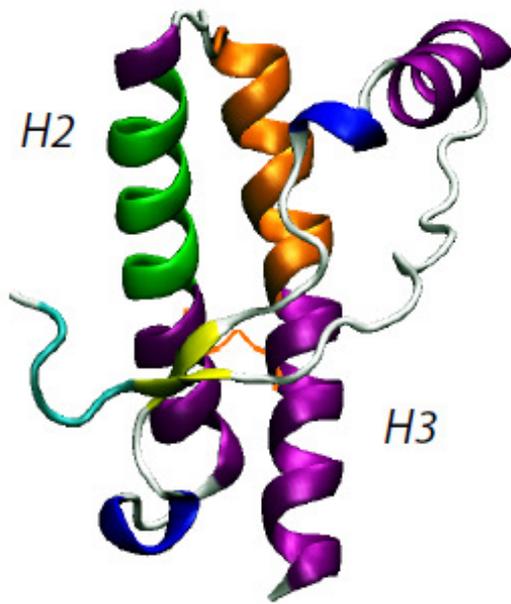
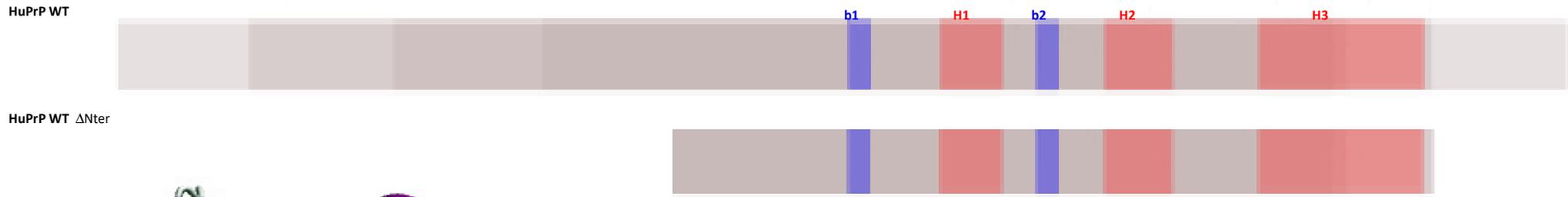
Le bactériophage M13



➤ Sélection des phages (biopanning)



➤ Selecting Alphareps using monomeric Wild Type truncated PrP (Nter's too sticky...)



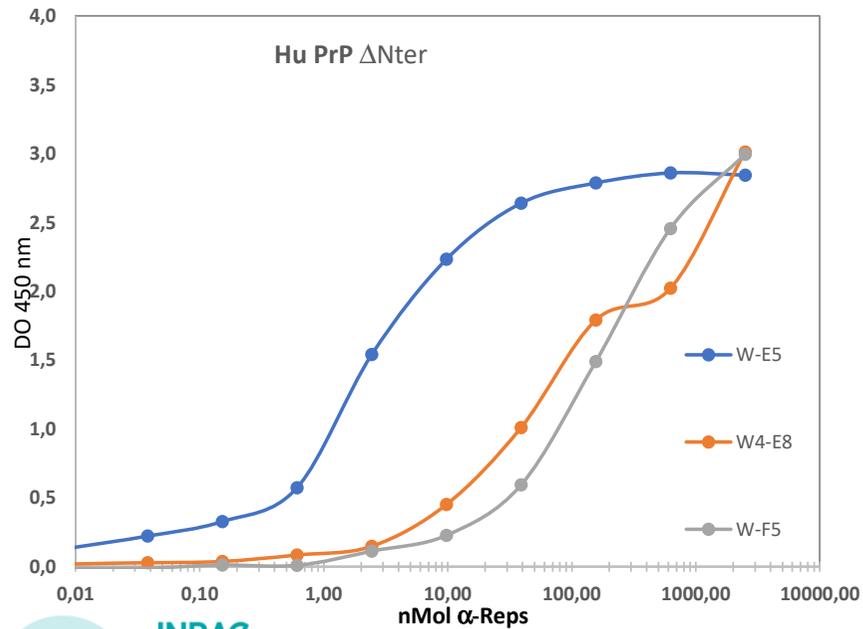
- 5 different Alphareps
- $2\text{nM} < K_D < 200\text{nM}$ (ELISA)

➤ Affinity evaluation

Highly dependent on the technology: avidity and steric hindrance

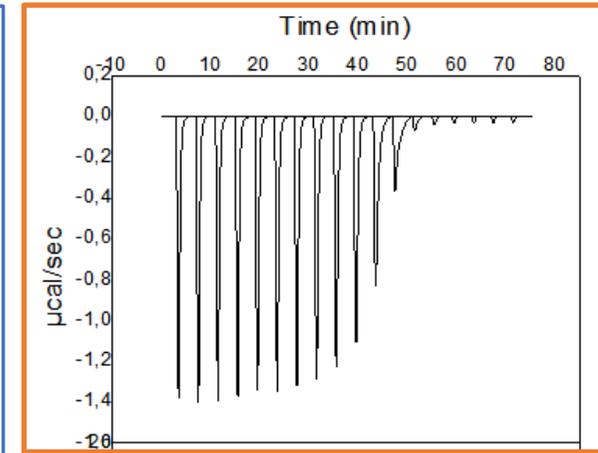
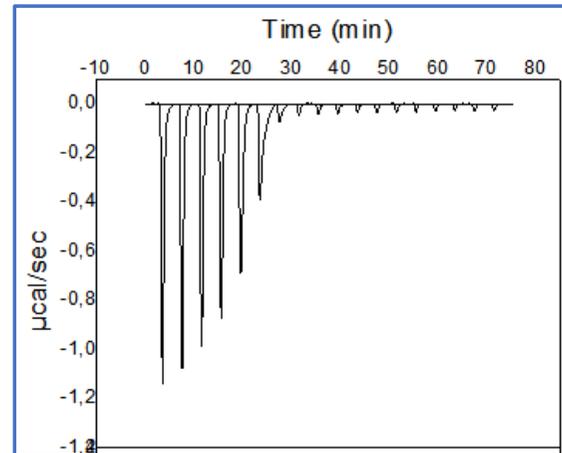
ELISA : surface interaction

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

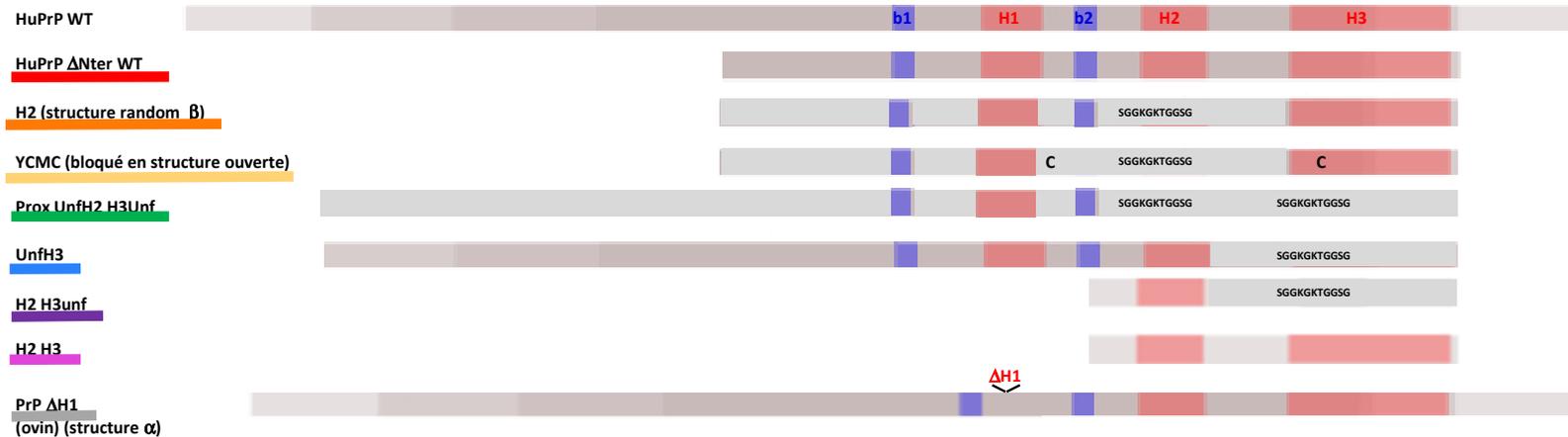


Isothermal calorimetry (ITC), in solution

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

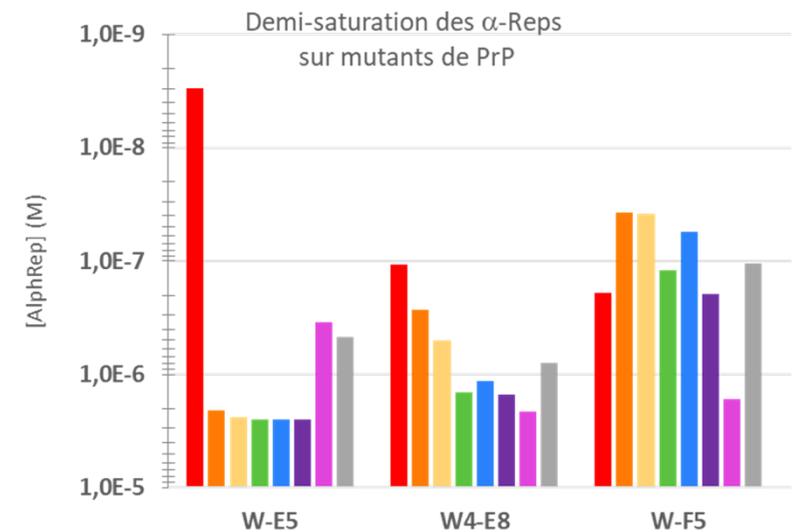


➤ Affinity evaluation : Comparison with PrP mutants



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



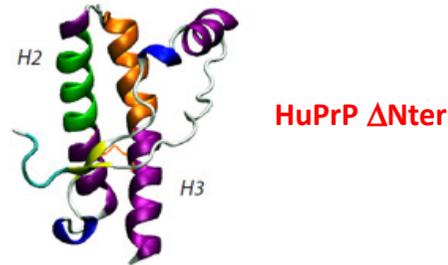
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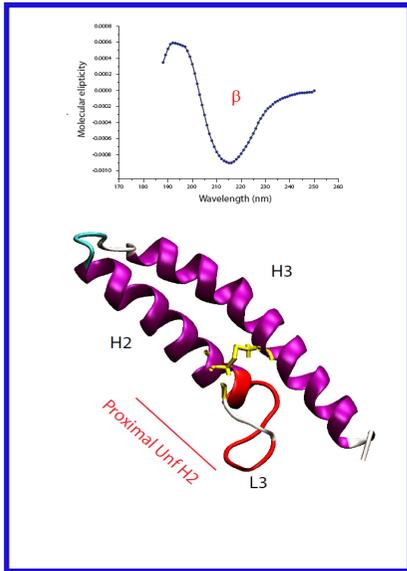
➤ Selecting AlphaReps on monomeric PrP conformation mutants

Mutations designed to selectively enhance putative transitional conformations

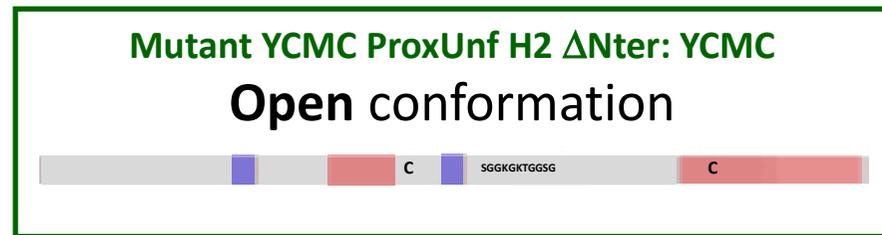
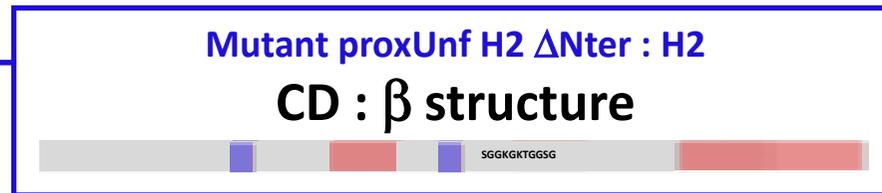
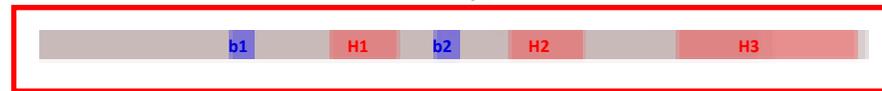


H2 mutant:

Proximal H2 helix domain unstructured

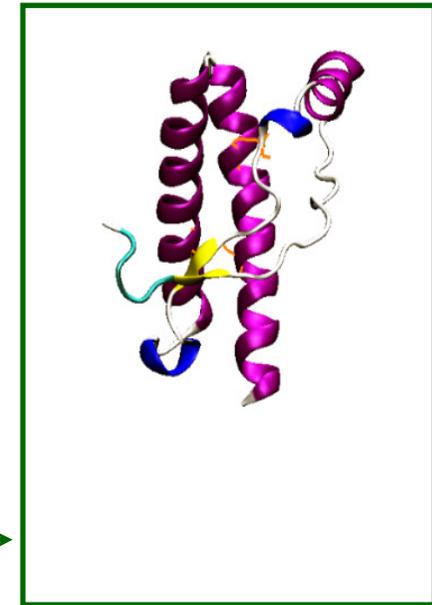


H2



YCMC mutant:

2 extracysteins stabilizing the proxunfold H2 mutant



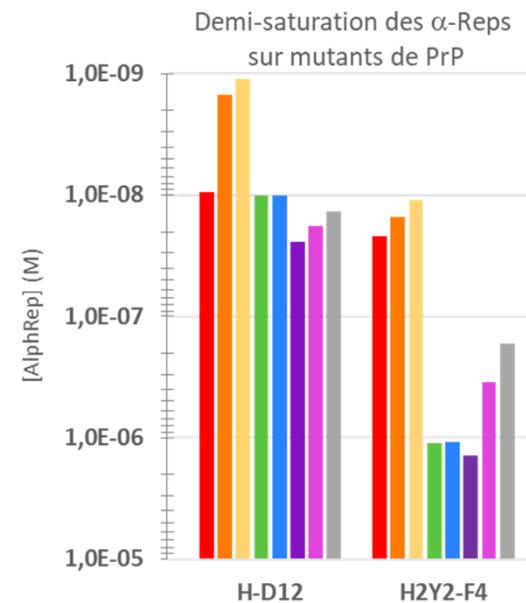
➤ Selecting AlphaReps on monomeric PrP conformation mutants

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...

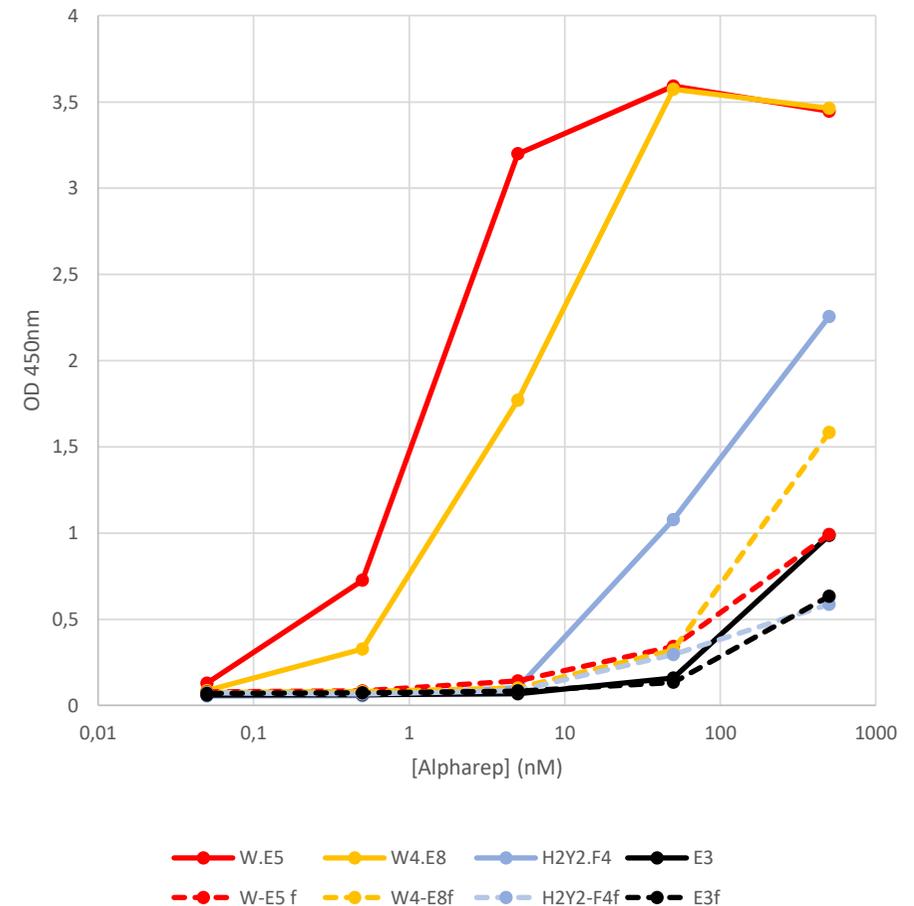
8 clones retrieved on Y



➤ 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

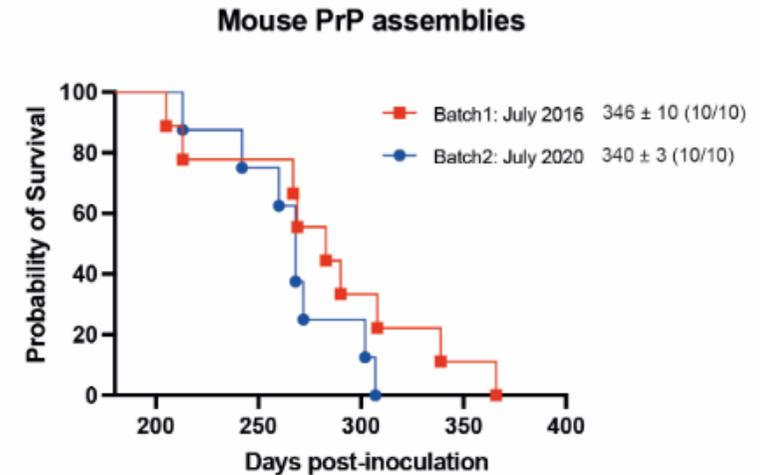
- Recombinant fibers are infectious

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 ₀) ^a		
	Hamster	Mouse	Human
1 st passage	282 \pm 17 (9/9)	346 \pm 10 (10/10)	476 \pm 11 (8/9)
2 nd passage	273 \pm 12 (8/8)	84 \pm 1 (8/8)	260 \pm 16 (8/8)
3 rd passage	244 \pm 13 (8/8)	84 \pm 2 (7/7)	215 \pm 7 (6/6)
4 th passage	238 \pm 15 (5/5)	80 \pm 2 (6/6)	219 \pm 7 (8/8)

^an/n₀: number of mice with neurological disease and positive for PrP^{Res} in the brain by immunoblotting/number of inoculated mice

Rezaei et al., submitted

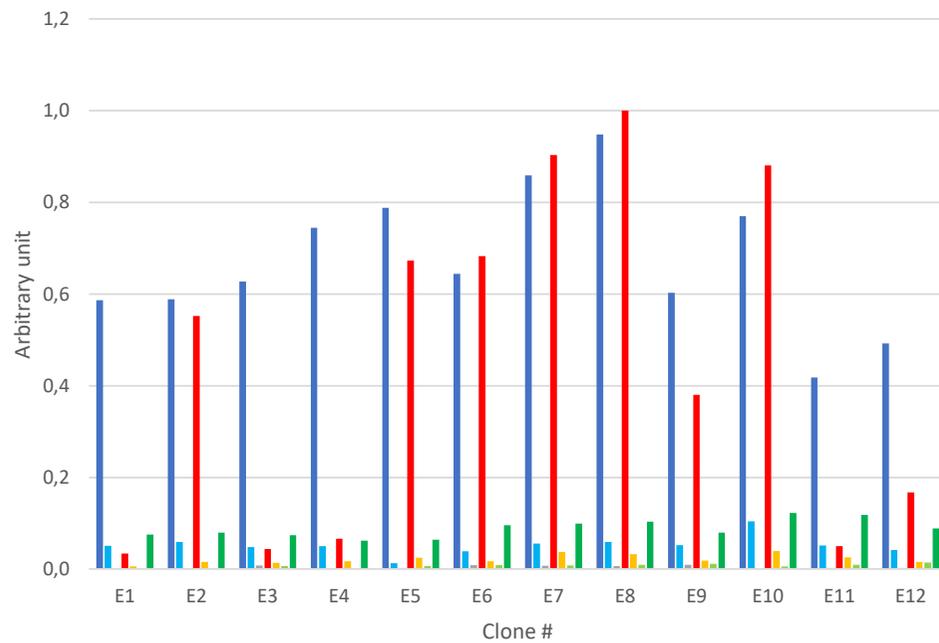


➤ Selecting AlphaReps on fibers produce highly reactive clones

High number of polyspecific AlphaReps

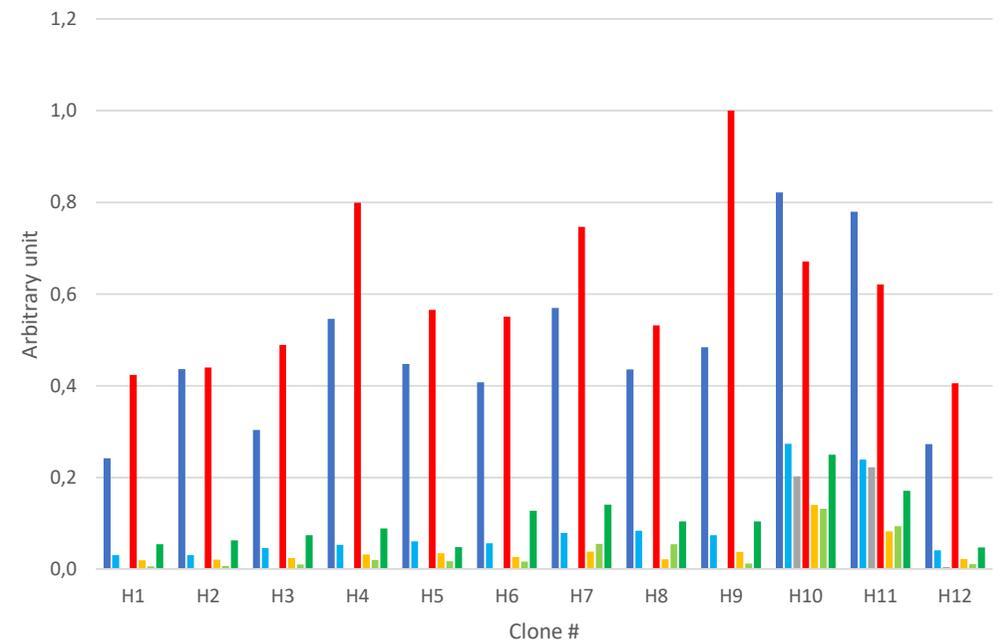
Some monospecific clones

Phages α Reps selected on Murine PrP Fibres



Mo_fibres Mo_mono BSA Hu_fibres Hu_mono D_Nter Nter

Phages α Reps selected on human PrP fibres



Mo_fibres Mo_mono BSA Hu_fibres Hu_mono D_Nter Nter



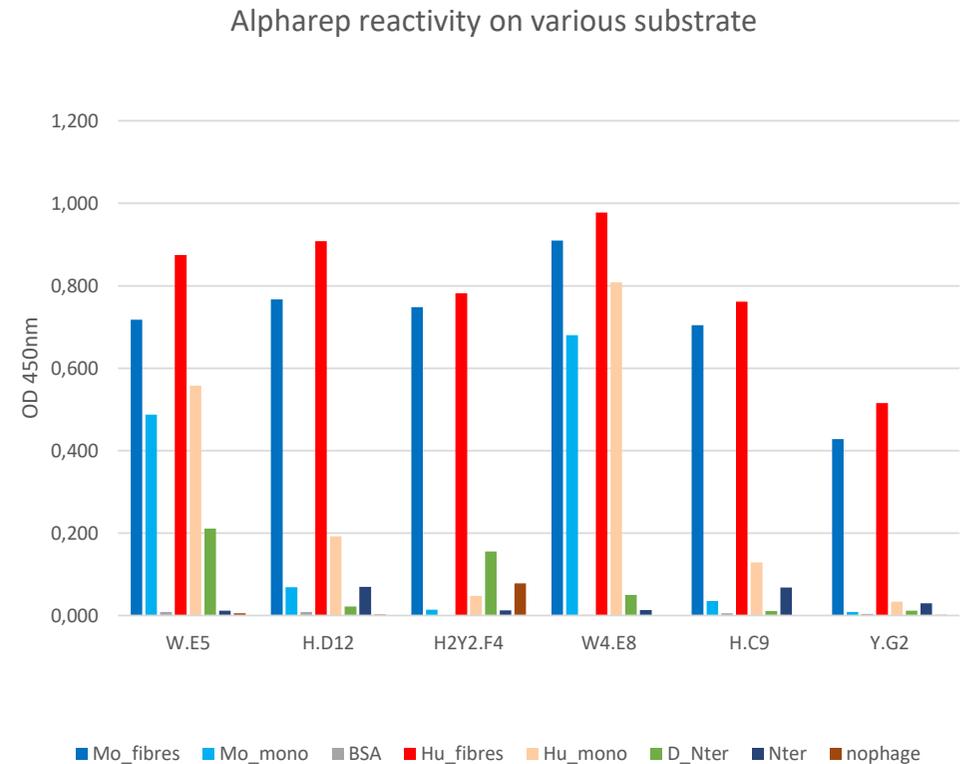
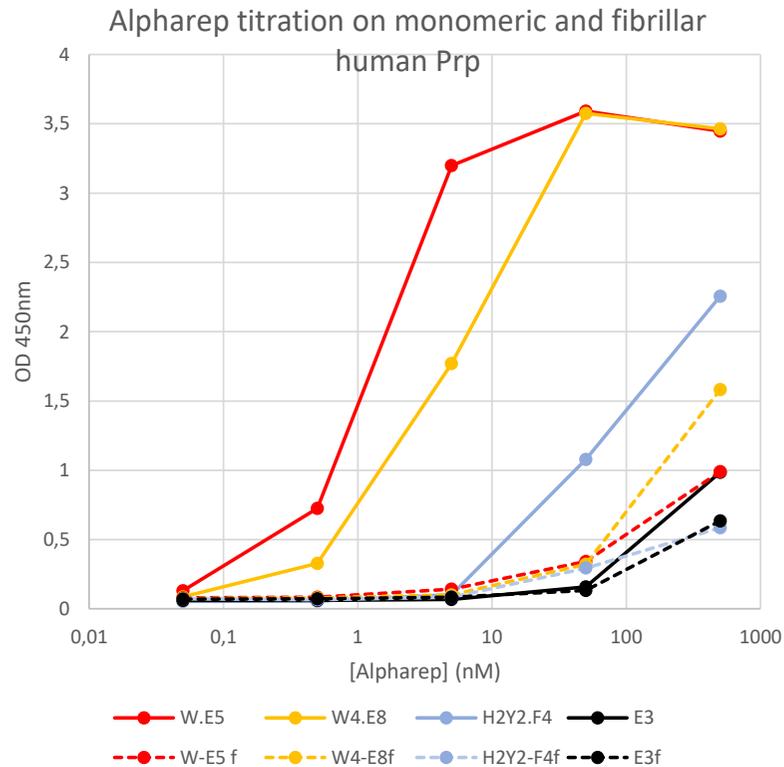
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➤ Additional data on monomer-specific Alphareps

Monomer specific alpharep variable reactivity on prion fibrils



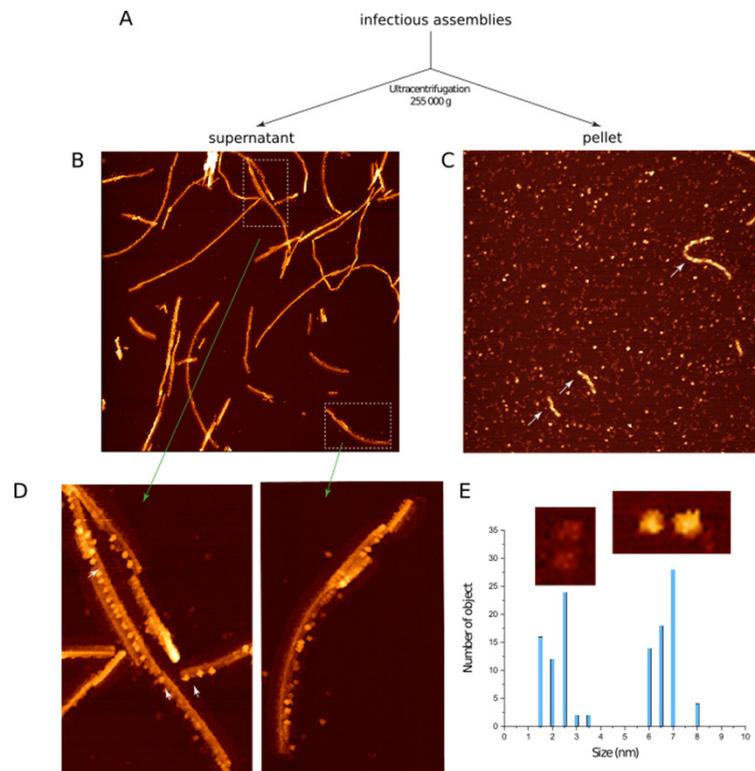
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➤ Possible hypotheses

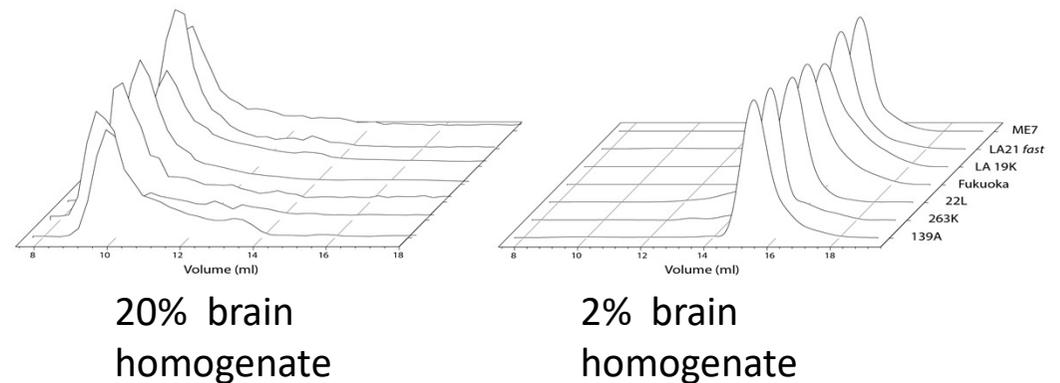
- Recombinant fibers are dynamic



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



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➤ 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



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- Angélique Igel
- Fabienne Reine
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- 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

- α -Reps :
 - ✓ Structures très souples pouvant changer de conformation suite à des interactions
 - ✓ Thermostables
 - ✓ Ne contiennent pas de ponts disulfures

