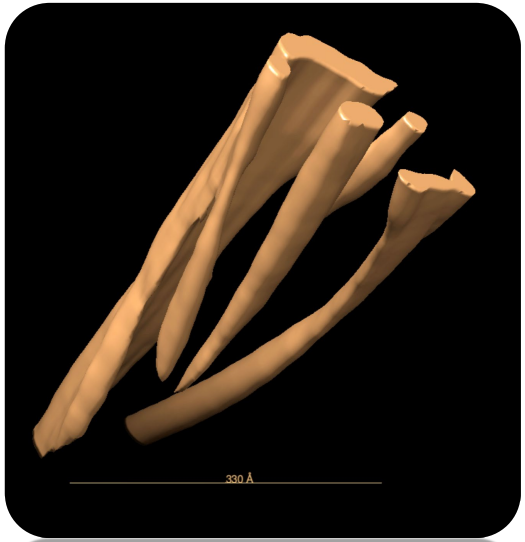
New approaches to CWD prevention

A conversation on possibilities



Ontario Federation of Anglers and Hunters

March 15, 2019

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Canada Research Chairs
www.chairs-chaires.gc.ca

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How can one molecule give different diseases? How does sporadic disease start? What are the early misfolding events? How do we stop prion diseases?

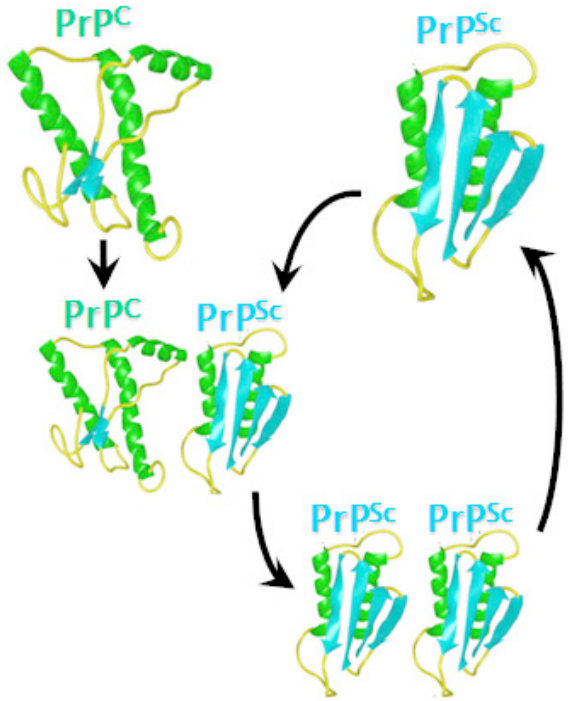
- key points in prion replication and diversity
- A new approach to CWD vaccines
- Natural genetic effects in prion diseases
- A genetic approach to CWD eradication

- *strengths/weaknesses*
- *shapes at the forefront*
- *lessons from kuru and scrapie*
- *Gene drive*

1. Prions and prion replication – their strengths and frailties

…prions are all about three-dimensional shape

Prion ("pree-on") propagation involves templated refolding



http://www.bio.davidson.edu

PrP^C

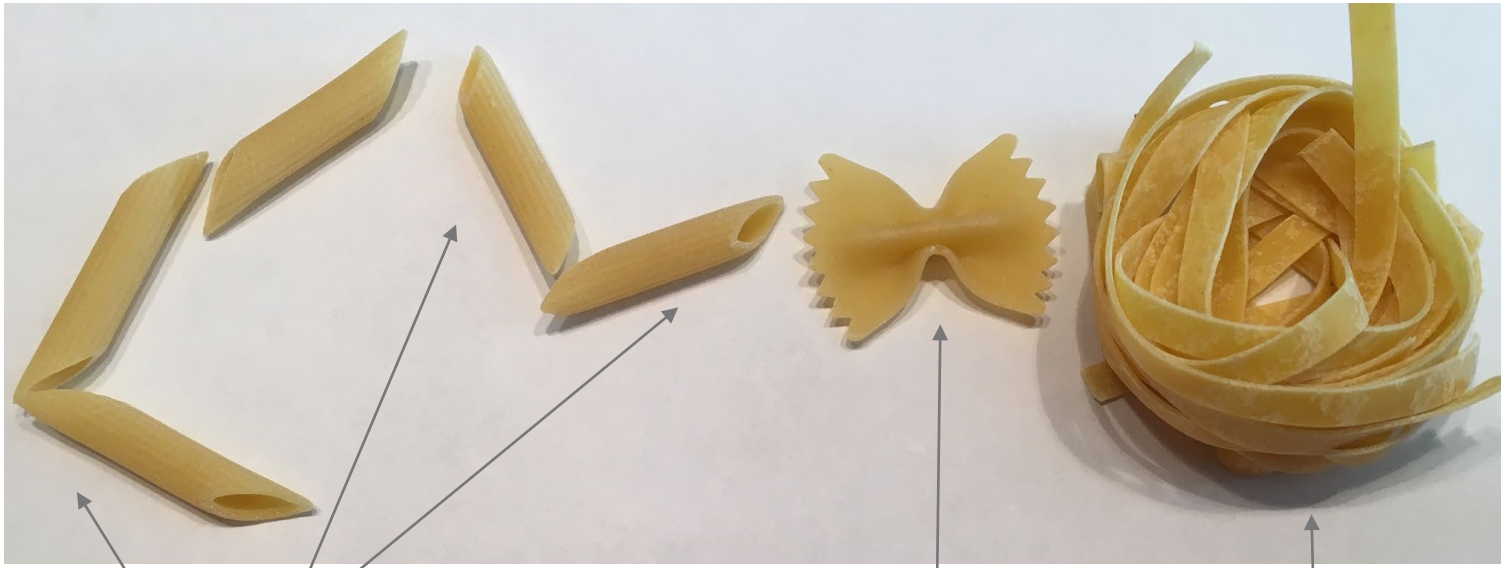
- Normal protein abundant in the brain – we all make PrP^C, every day

PrP^{Sc}

- Only known component of the infectious prion particle.
- Partially resistant to processes that normally get rid of proteins

Prions are not bacteria, nor are they viruses

How to understand PrP^C

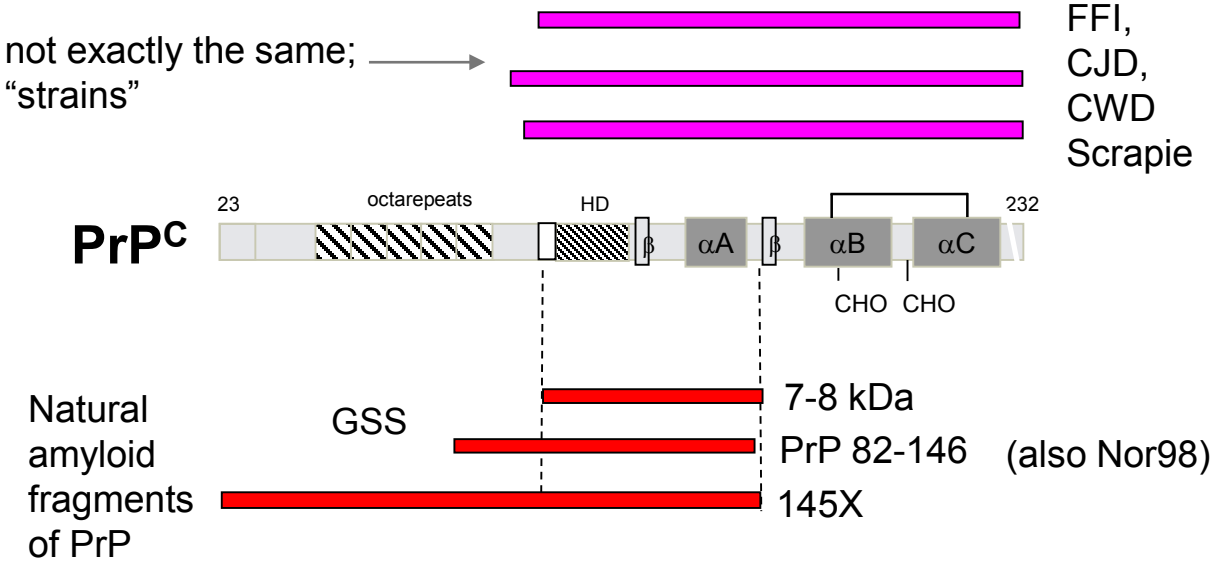


front region
(floppy)

middle “switch” region
(pretty interesting)

back-end
(folded ball)

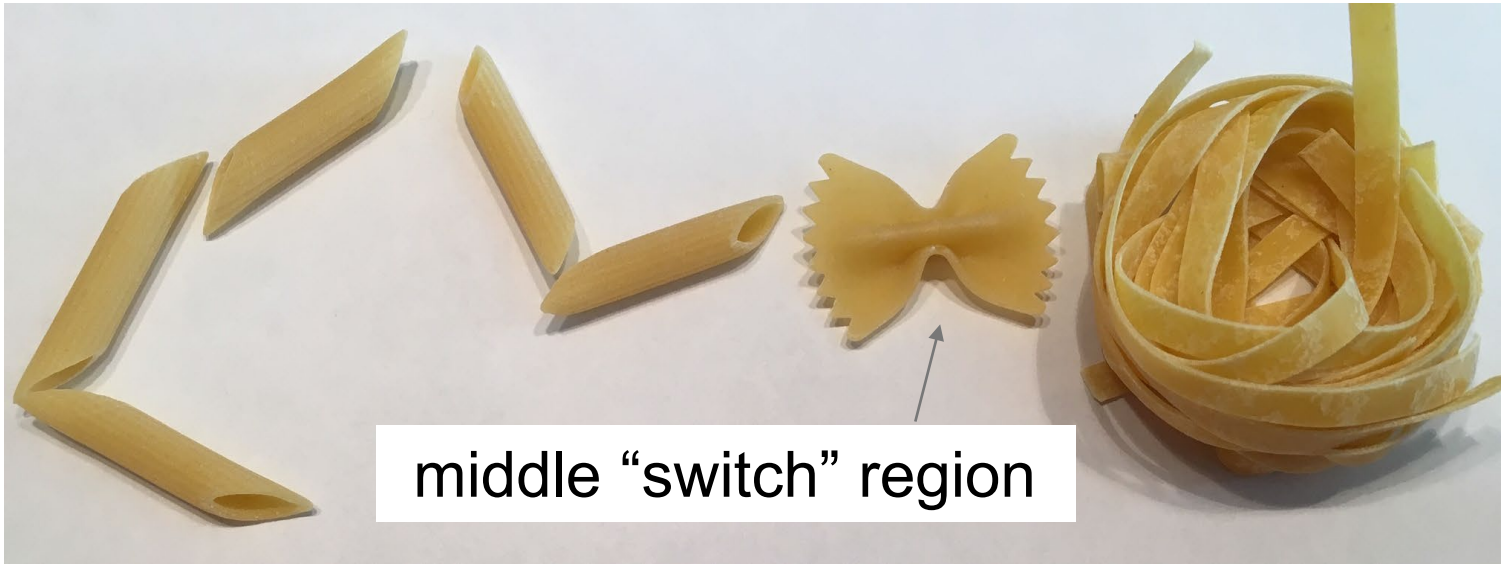
What have human and animal prion diseases told us about the misfolding possibilities for PrP^C ?



- Protease resistant fragments of PrP differ amongst different diseases
- "back-end"
- "middle and front"

*Measure the length after removing N-linked sugars with PNGaseF

How to understand PrP^C



Options: a) stay healthy b) Get CWD c) Get Nor98

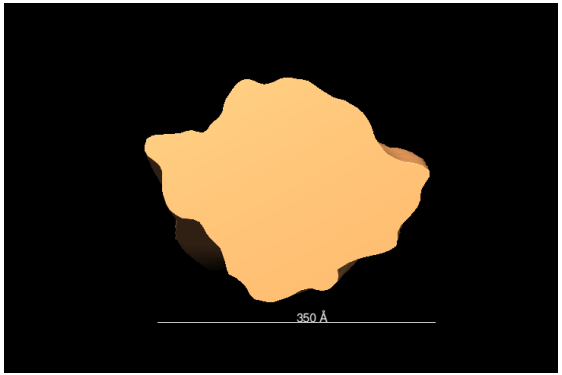
(get CJD) (get GSS)

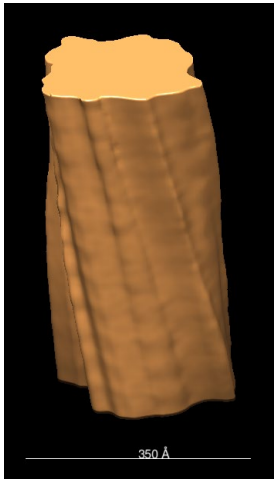
2. A new approach to prion disease vaccines

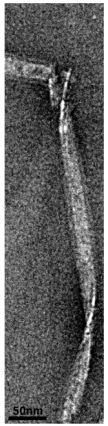
How can we stop the disease process in CWD?

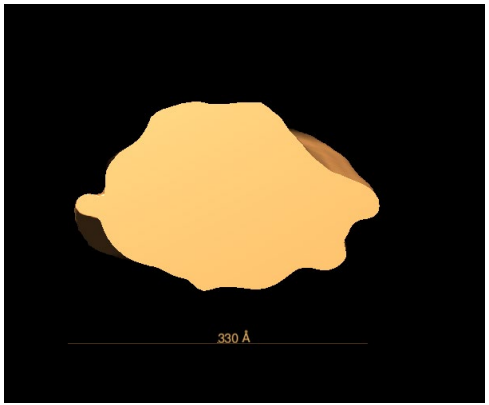
Target	Treatment	what can go wrong?
PrP protein	antibody against PrP^{Sc}	strain evasion ?
PrP protein	small molecule against PrP^{Sc}	strain evasion ?
PrP protein	target the switch region	a bit theoretical right now
Gene that codes for PrP protein	delete it; no gene, no PrP	the deleted gene gets diluted by breeding, slow
Gene that codes for PrP protein	propagate a natural variation by gene drive and control it with gene brake	slow (-ish), too cutting-edge

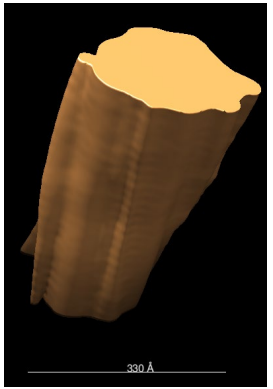
CWD Morphology I: Twisted Ribbon–Like Fibrils







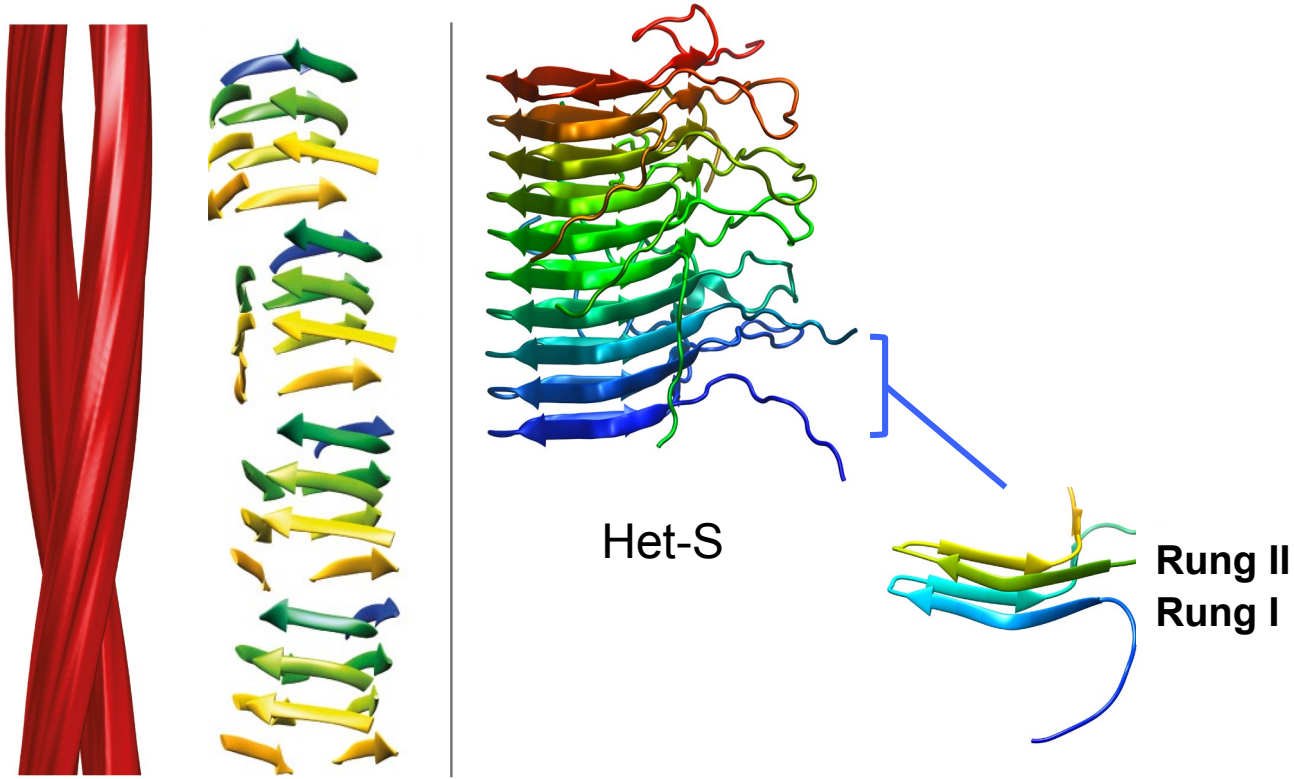




c/o Sara Amidian

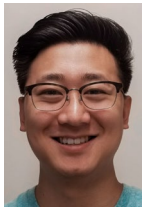
Centre for Prions and Protein Folding Diseases

Structure of PrP^{Sc} fibrils - similarity to a fungal prion called Het-S



PrP^{Sc} fibril

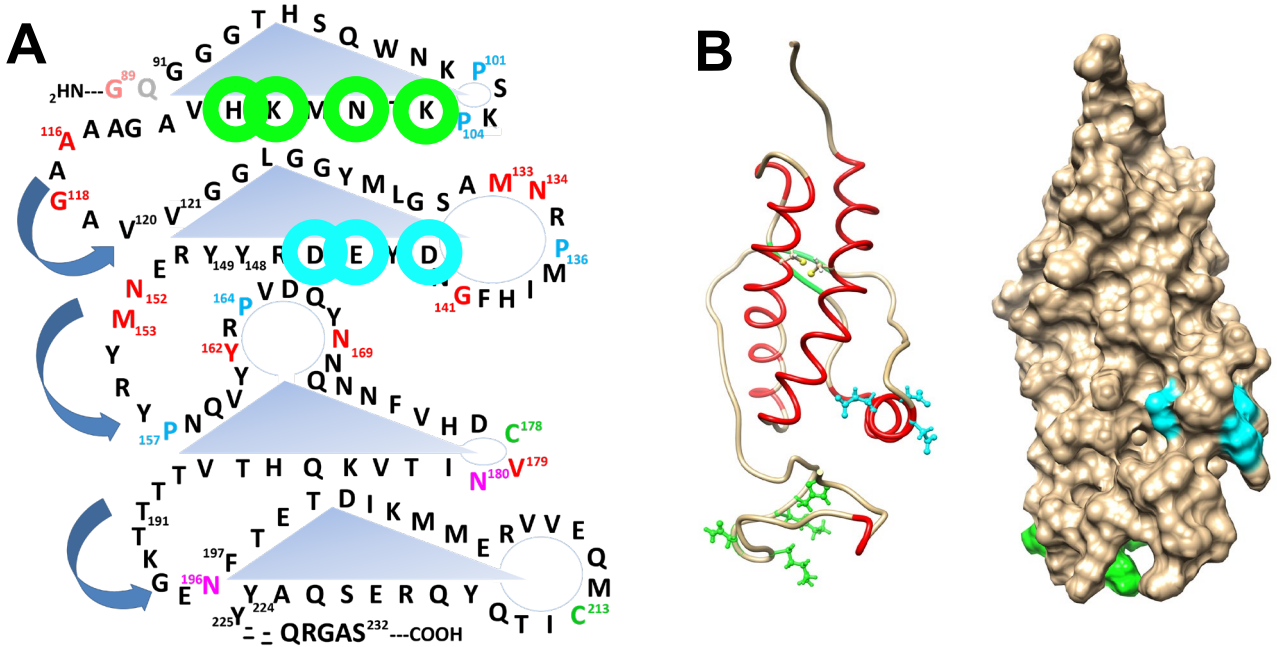
Adapted from Vázquez-Fernández, E., et al. (2016). PLoS Pathogens **12**(9): e1005835.



light-bulb idea

Andrew Fang and Holger Wille, University of Alberta

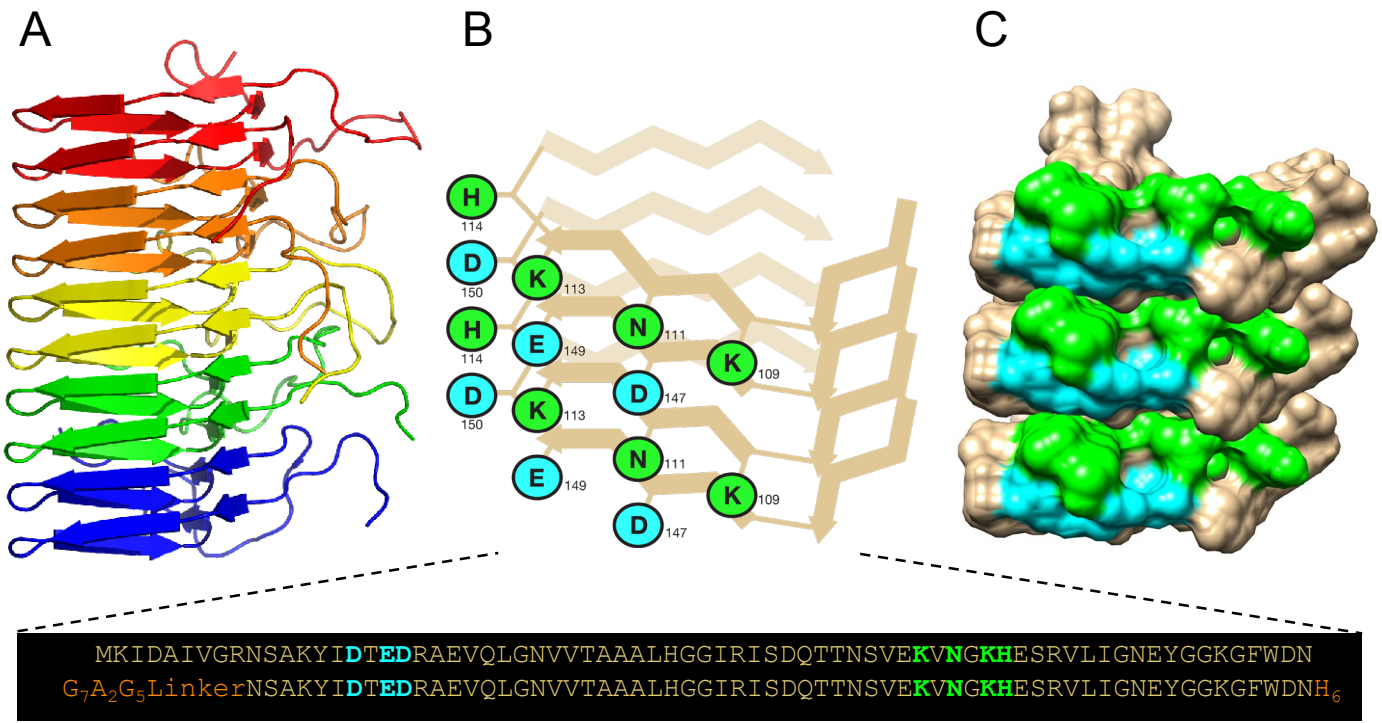
A rationally designed, structure-based vaccine candidate targeting CWD prions



Adapted from (left) Silva et al., 2015; Virus Research and (right) 4YXH Baral et al., 2015; J. Struct. Biol.

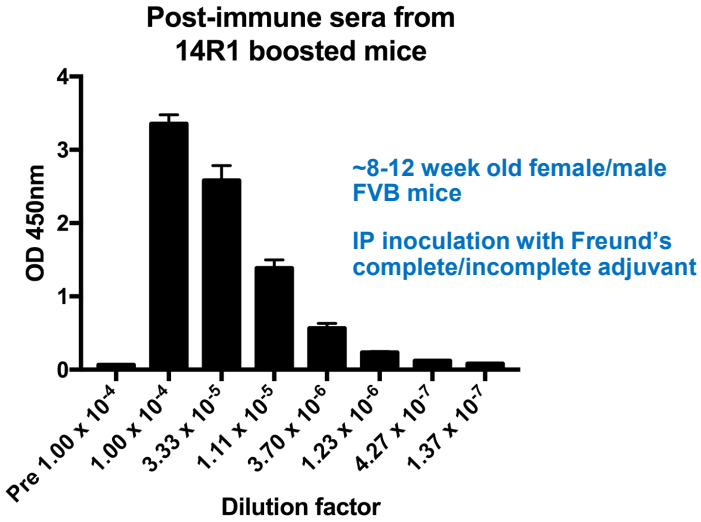
United States provisional patent application US 62/674,916 "An innocuous, structured scaffold for structure-based amyloid disease vaccines and antigens" Co-inventors: Fang, A., Flores-Fernández, J. M., Wille, H. Filed May 22, 2018

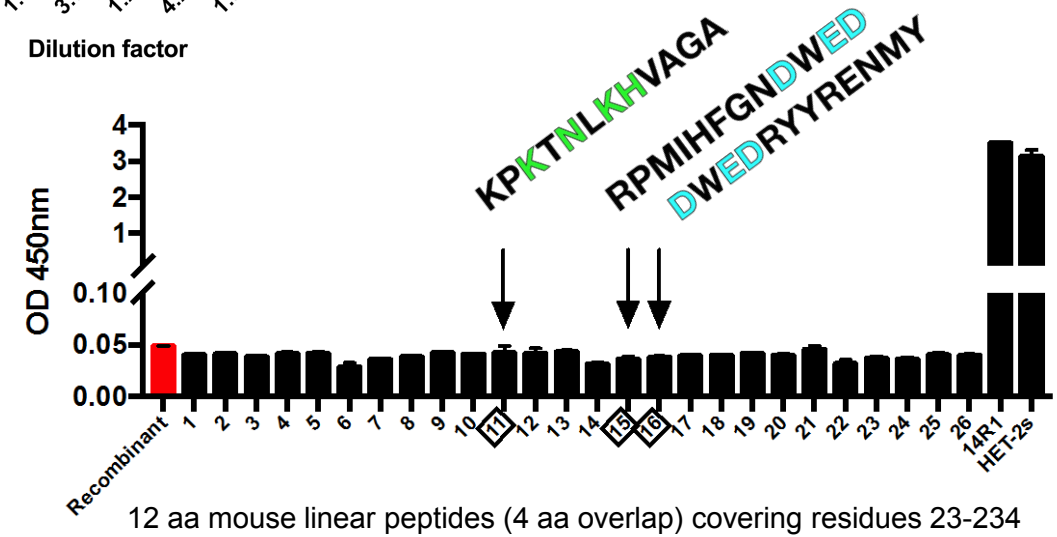
Exposing selected residues on the surface of a 4-rung HET-s scaffold (“Het2S”) – making a mimic



Structure in (A) adapted from: 2RNM Wasmer et al., 2008; Science.
K-N-KH is in charged patch 2 in the flexible region of deer PrP^{C} and D-ED is in helix 1 of the globular domain

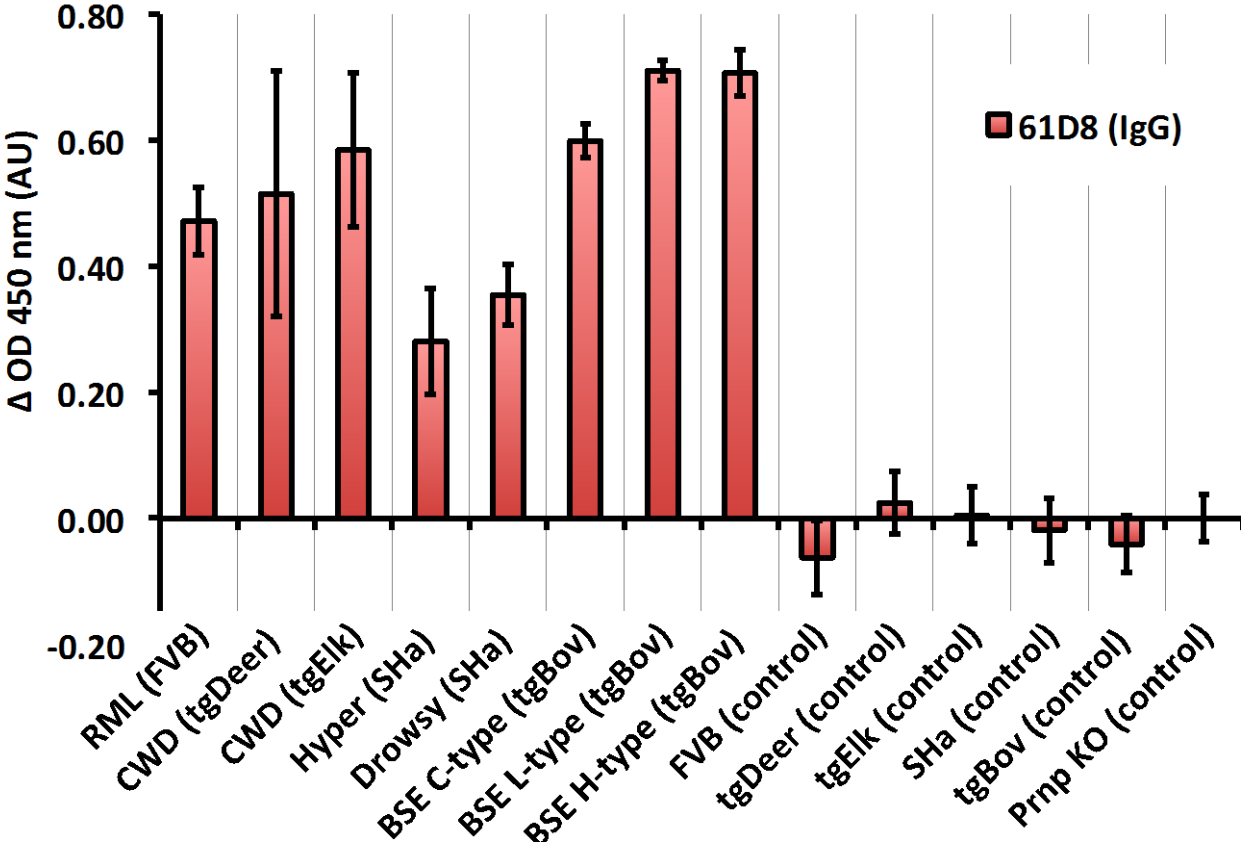
Characteristics of 14R1 sera





12 aa mouse linear peptides (4 aa overlap) covering residues 23-234

PrP^{Sc}-specific monoclonal antibody



Lili Tang, Andrew Fang, Holger Wille

3. Natural genetic variation and prion disease susceptibility

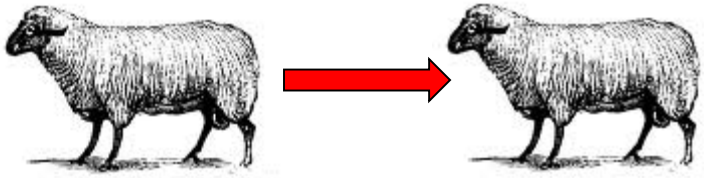
- scrapie in sheep
- Kuru and emergence of natural resistance

Was the first recognized prion disease, scrapie, infectious or genetic?

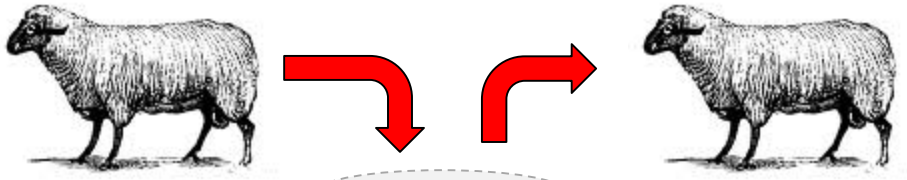
- HB “James” Parry, University of Oxford: a genetic disease controlled by the recessive “s” gene
- Alan Dickinson, University of Edinburgh: a naturally infectious disease modulated by host genetics

Scrapie of sheep – a transmissible disease

Natural scrapie



Affected Affected

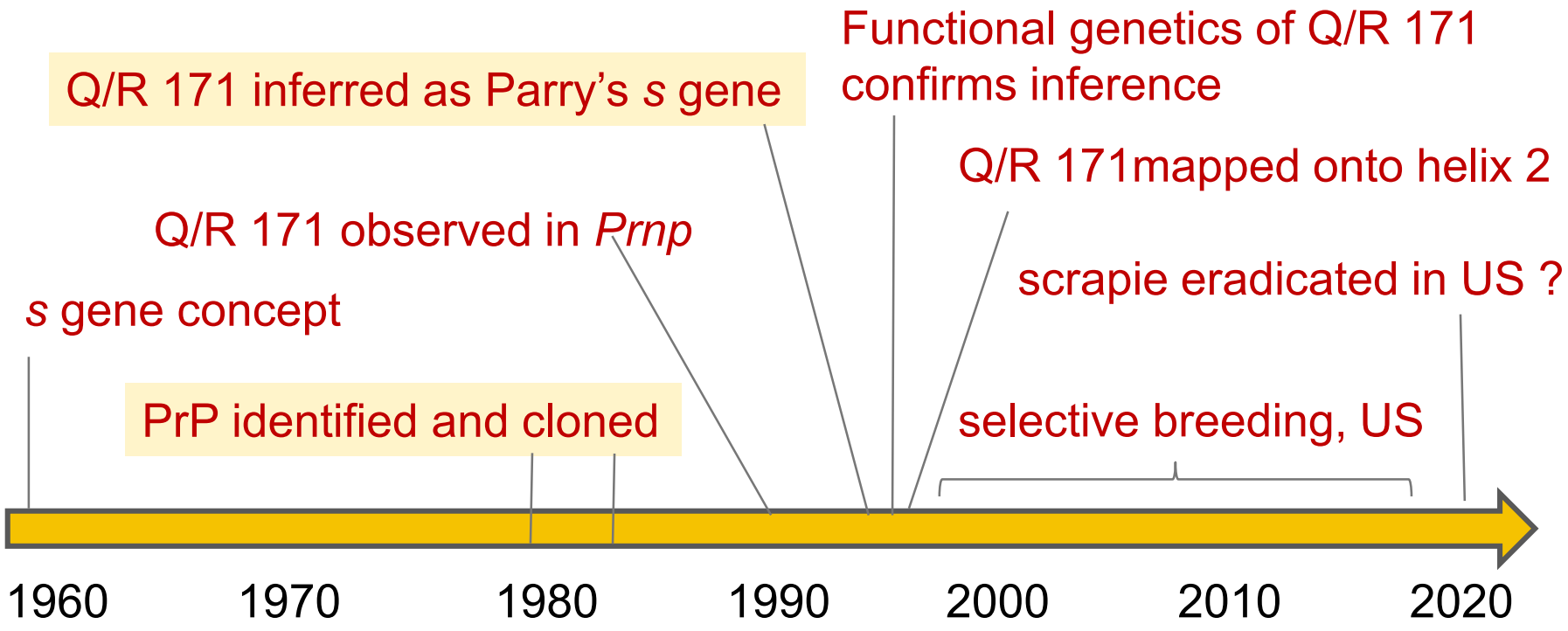


Affected Contaminated Environment Affected

Experimental scrapie

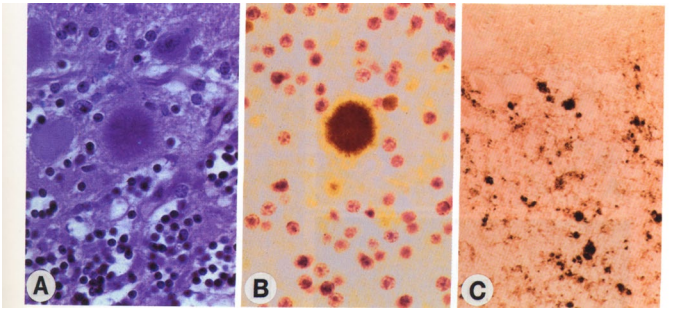
The scrapie ‘*s*’ gene was a real susceptibility gene

timeline over 6 decades



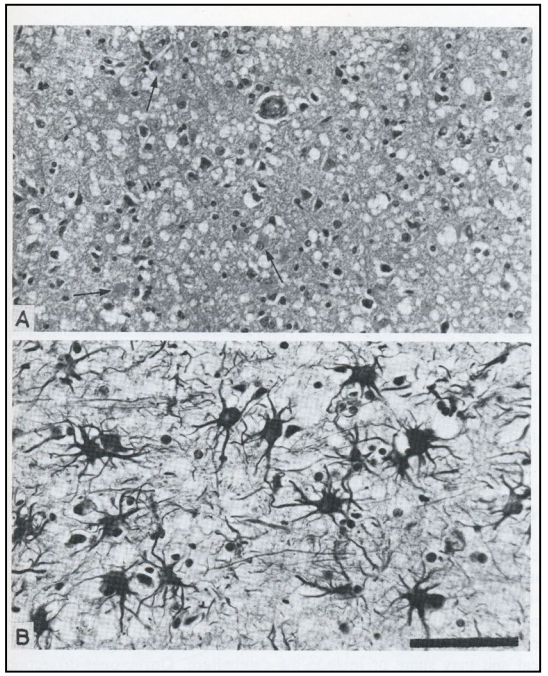
Kuru

Story from the 60's and 70's Illustrates the following concepts



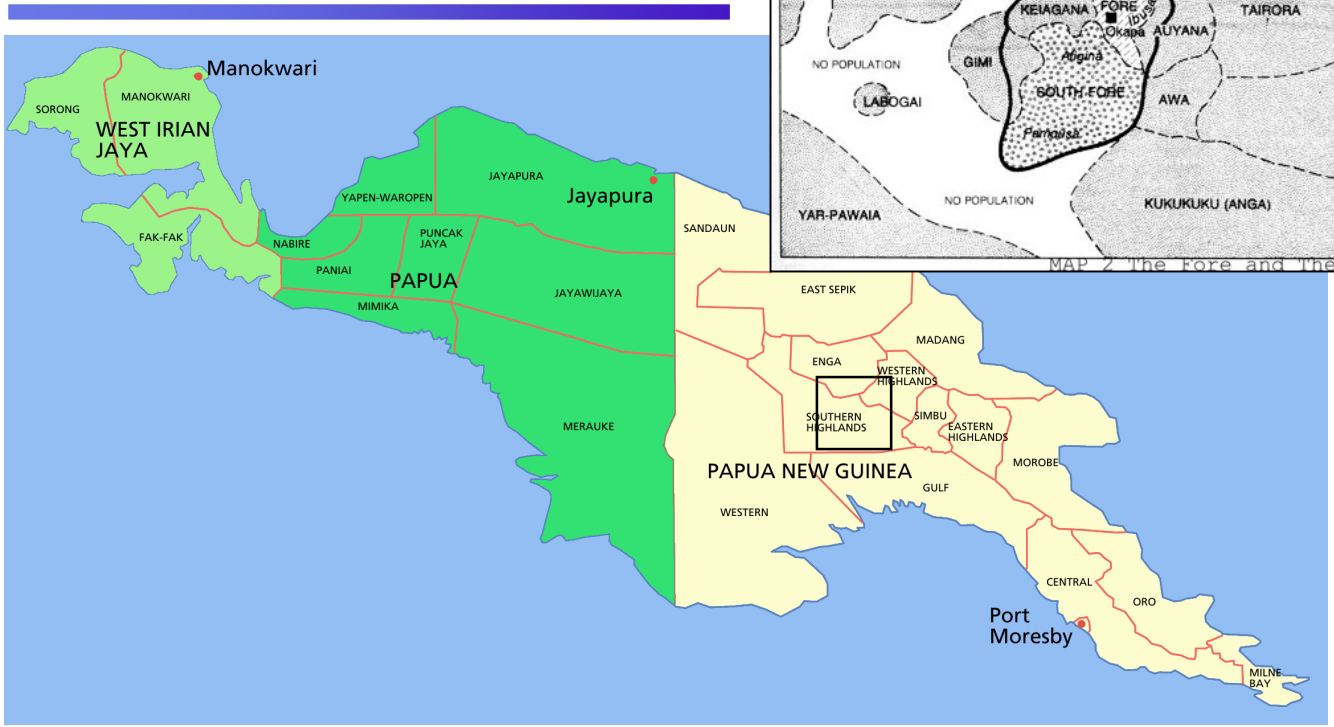
- Transmissible
- Infectious disease spread by oral route
- Extended incubation period lasting decades
- (Cannibalism a no-no)

D. Carleton Gajdusek



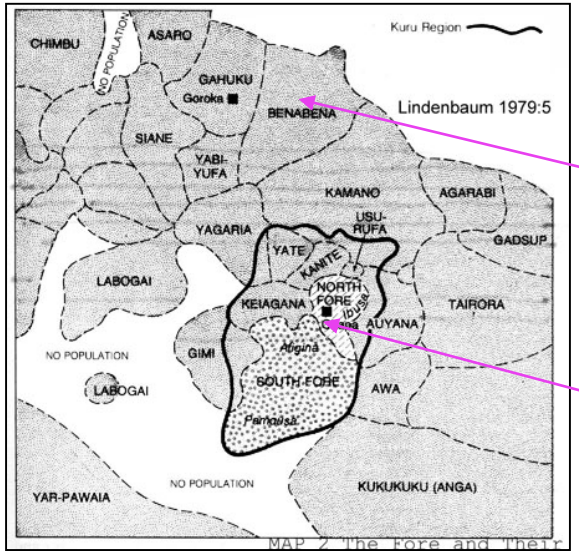
Kuru

Geography



KURU

A spontaneous gene variant conferring protection



PrP's switch region

```
                    100       110       120       130
                     |         |         |         |
…WGQPHGGGWGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYVLGS… regular Human PrP

                    100       110       120       130
                     |         |         |         |
…WGQPHGGGWGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGVYVLGS… Human PrP
                                                        in kuru epicentre
```

(Work of Simon Mead, John Collinge and colleagues)

4. Sexually-transmitted *prophylaxis*

4. Taking aim at CWD with genetics

- animals have about 30,000 genes
- we are interested in just one called *Prnp* that makes PrP^C
  - (i.e., just one part in 30,000)
- variations in *Prnp* genes can attenuate prion disease
- *but*, we only provide half our genes to each generation
  - so useful genes can get diluted out with breeding, 50%, 25%, 12.5% etc, etc

Genetic engineering

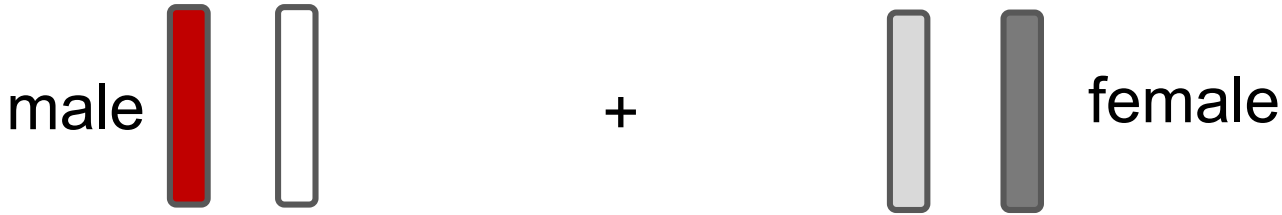
“I don’t like genetically engineered organisms because I don’t want to eat DNA”

In fact, genetic engineeing is all around us (and has been around us for hundreds of millions of years)

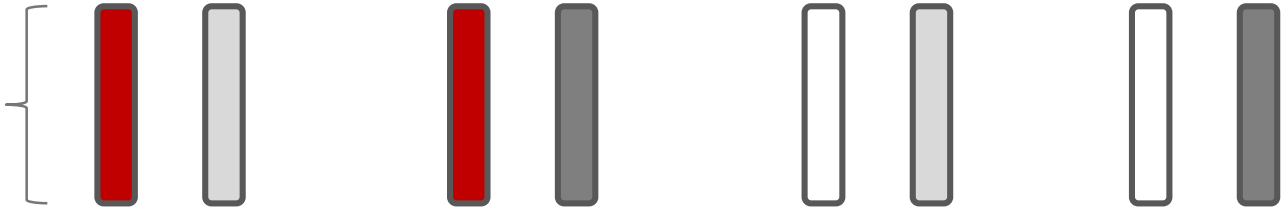
Organisms encode their own DNA engineering tools to help them survive

How do we stop useful genes being diluted out?

Normal inheritance = Mendelian

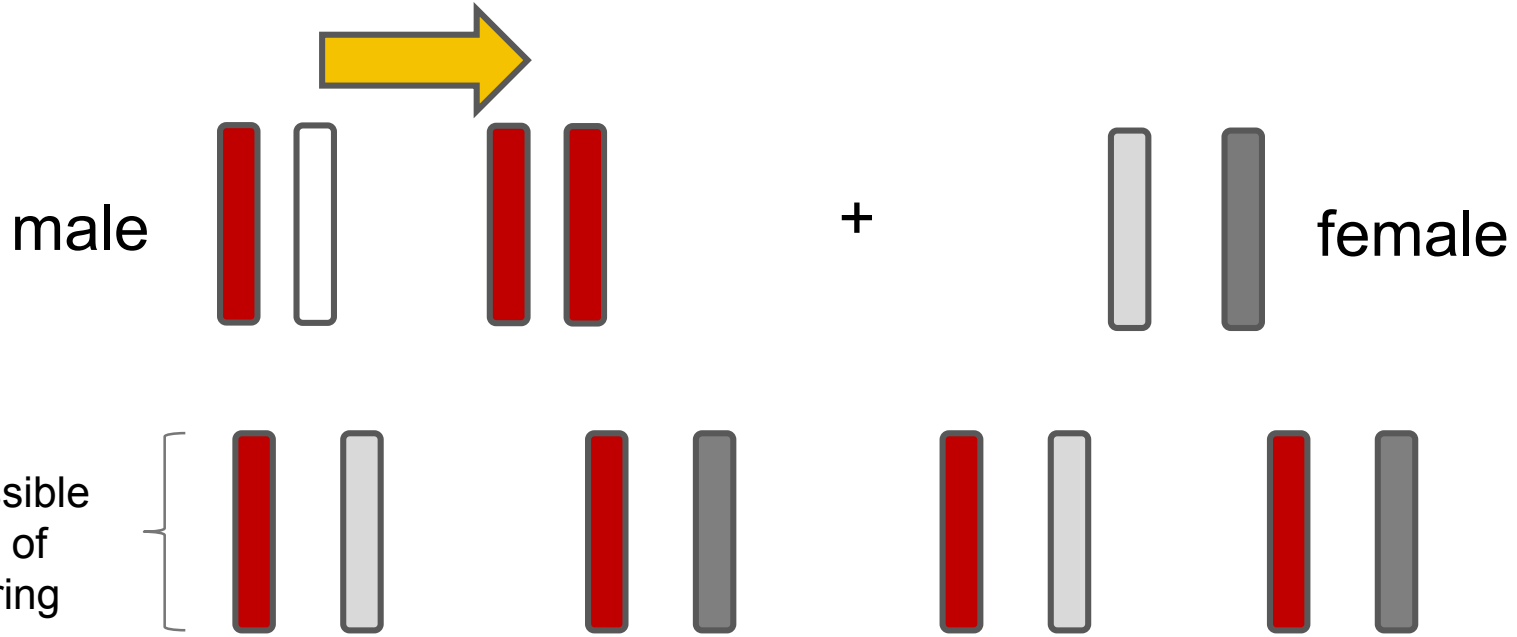


4 possible types of offspring



Gene Drive by gene conversion

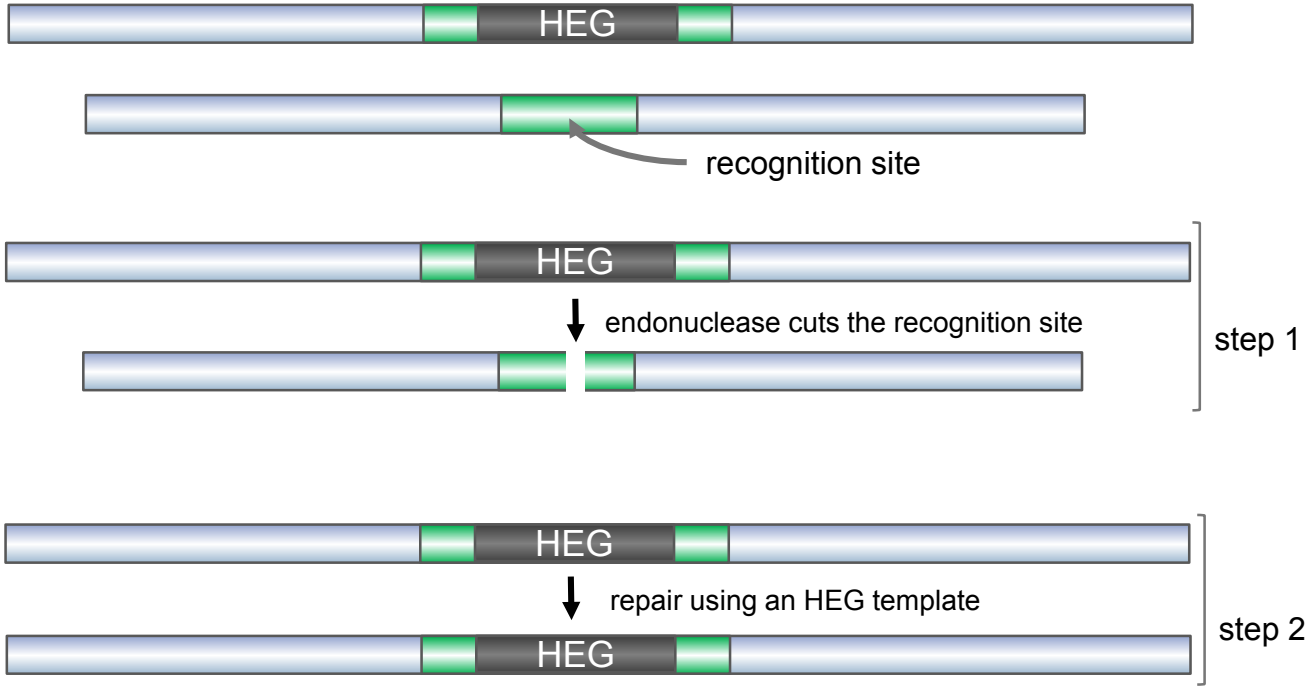
Super Mendelian inheritance*



*only one gene though; 29,999 genes are not altered

Gene drive

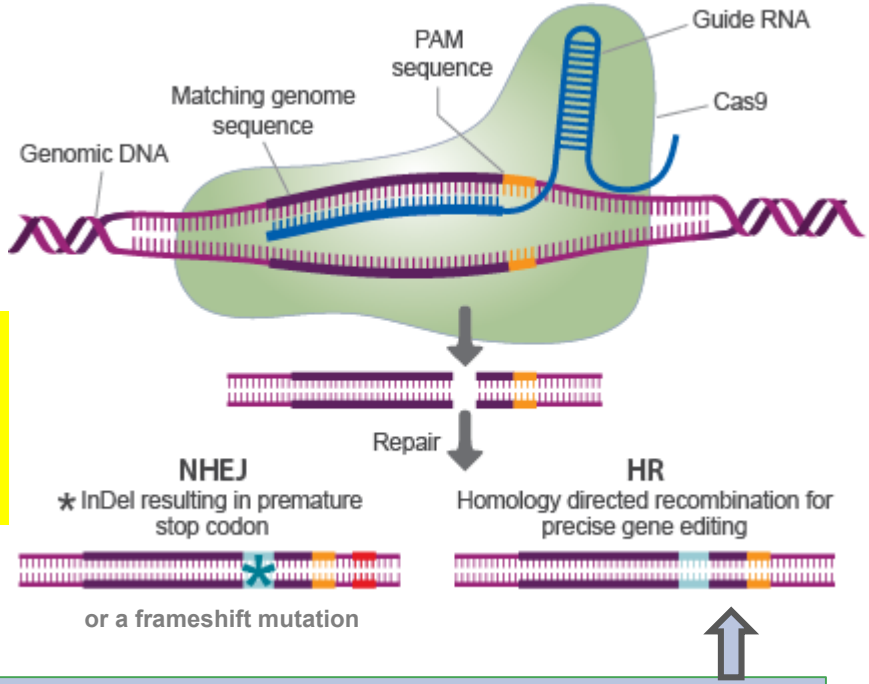
Homing endonucleases and selfish DNA



Site-specific endonucleases, like homing endonucleases, can drive through populations by promoting their duplication within the host genome; using gene conversion, they can “improve upon” Mendelian gene segregation ratios

CRISPR/Cas9

Using a designed DNA-cutting enzyme for gene drive

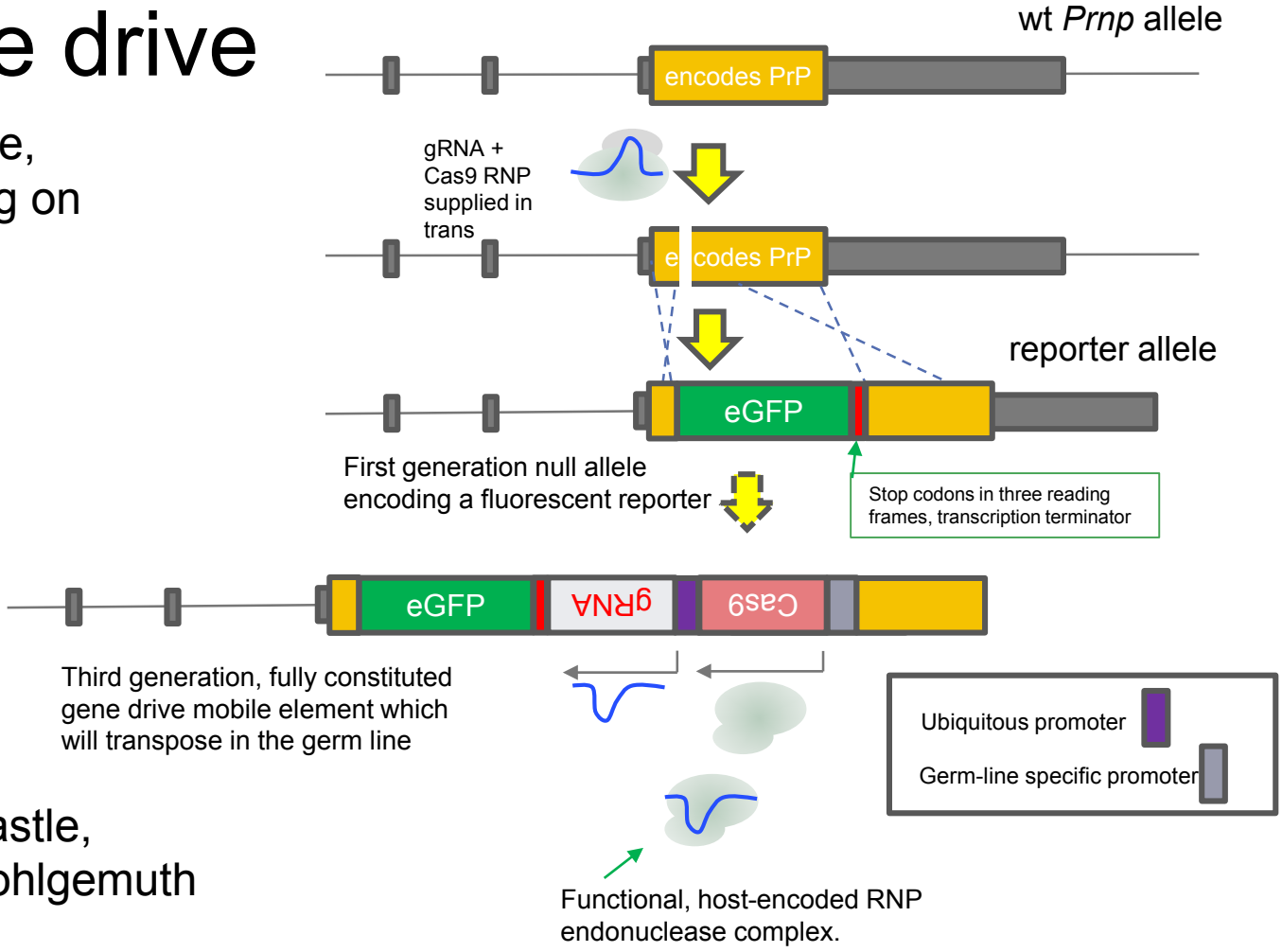


Using Cas9/gRNA as the key endonuclease: two classes of recombination events that can happen to repair the chromosomal DNA break

Recombinant DNA including additional sequences flanking the cut site can be integrated via this homologous recombination repair pathway (see Fig. 2). The insertion site for the inserted DNA in the large construct is such that the cut site is separated from the PAM sequence in the gRNA after HR – this can preempt any attempted re-cutting by Cas9/gRNA after HR has occurred (Henao-Mejia, Cold Spring Harbor Protocols in Mol. Biol., 2016)

Gene drive

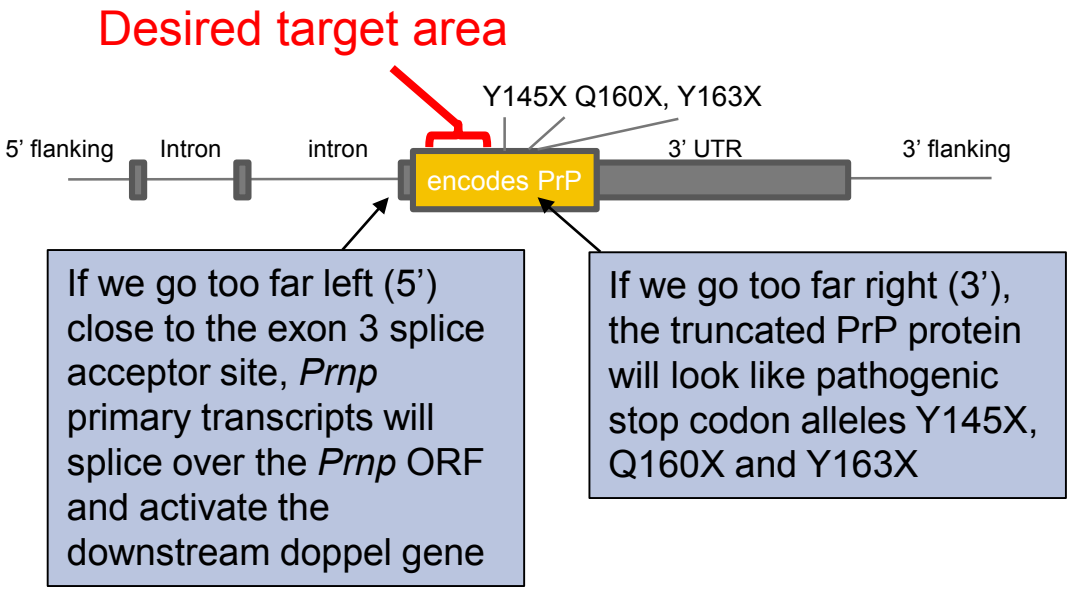
A scheme, practising on mice



Andrew Castle,
Serene Wohlgemuth

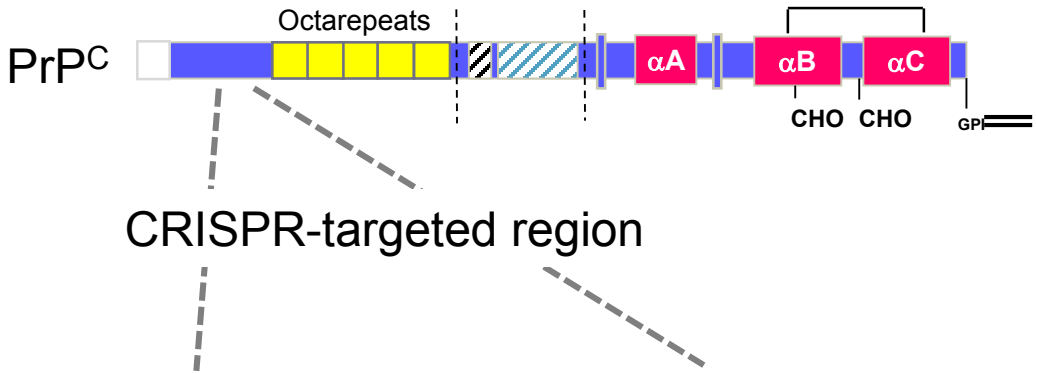
Putting a directed cut into the *Prnp* gene

Where to put the double-stranded cut in the chromosomal *Prnp* locus to make a safe, non-functional version?



CRISPR-induced inactivating mutations in germline *Prnp*

Guide RNA #9



Line 33 (1 bp insertion):	WNTGGSRY…../…..stop	frameshift
Line 34 (21 bp deletion):	WNTGGSR~~YPGQGSP~~GGNRYP	Δ38-44
Line 36 (6 bp deletion):	WNTGGS~~RY~~PGQGSPGGNRYP	Δ37, 38

Task List

- Making Cas9 complexes against wt *Prnp* genomic targets
  - murine
  - cervid

- Assessment of null alleles made by NHEJ from Cas9 complexes

- Making tagged *Prnp* alleles to track gene drive

- Making CWD-resistant *Prnp* alleles for HR pathway
- Expressing chromosomally-encoded Cas9 complexes

- Gene brake system

Thanks

Westaway Lab
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Luis Arce

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Andrew Fang
Razieh Kamali-Jamil

Dept of Biochemistry UofA
Brian Sykes
Olivier Julien

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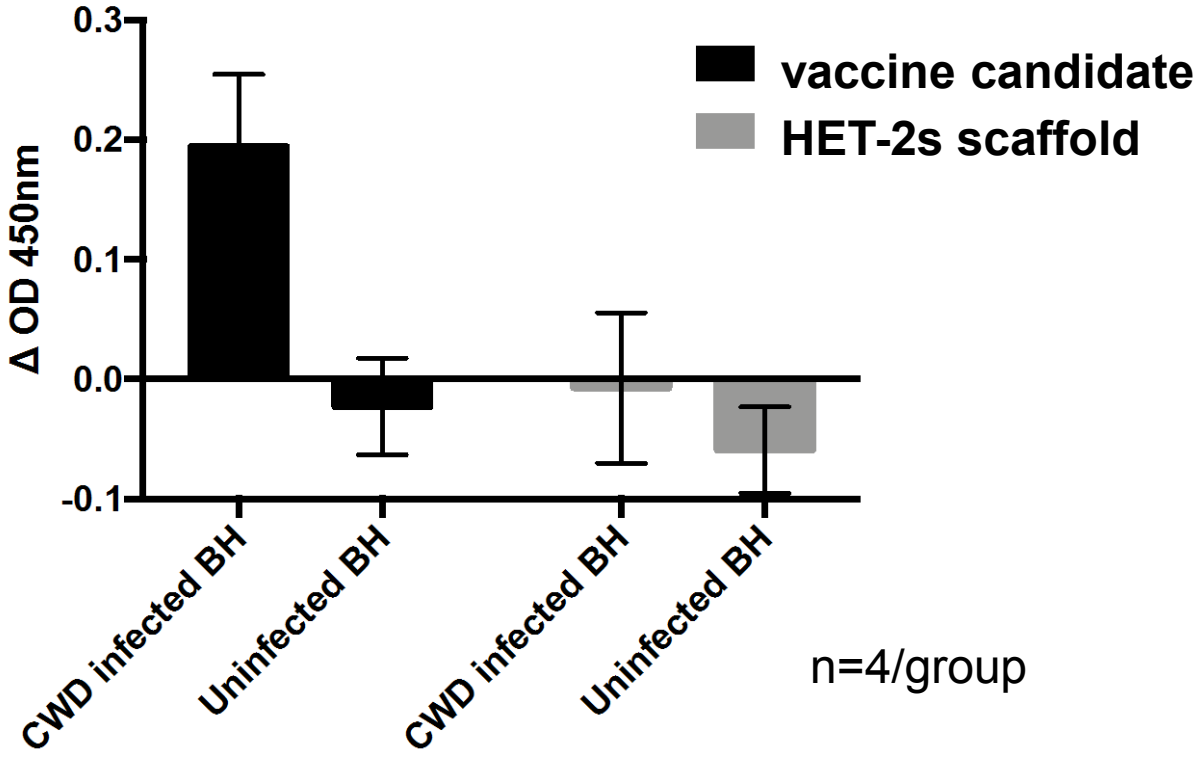
…and conclusions

5. Conclusions

- Thanks to molecular biology, there will be directed strategies for tackling CWD (conversely, inaction is not an option)
- Eradication measures will likely take a while – we need to think in decades
- thank you for your attention !
- open to new suggestions

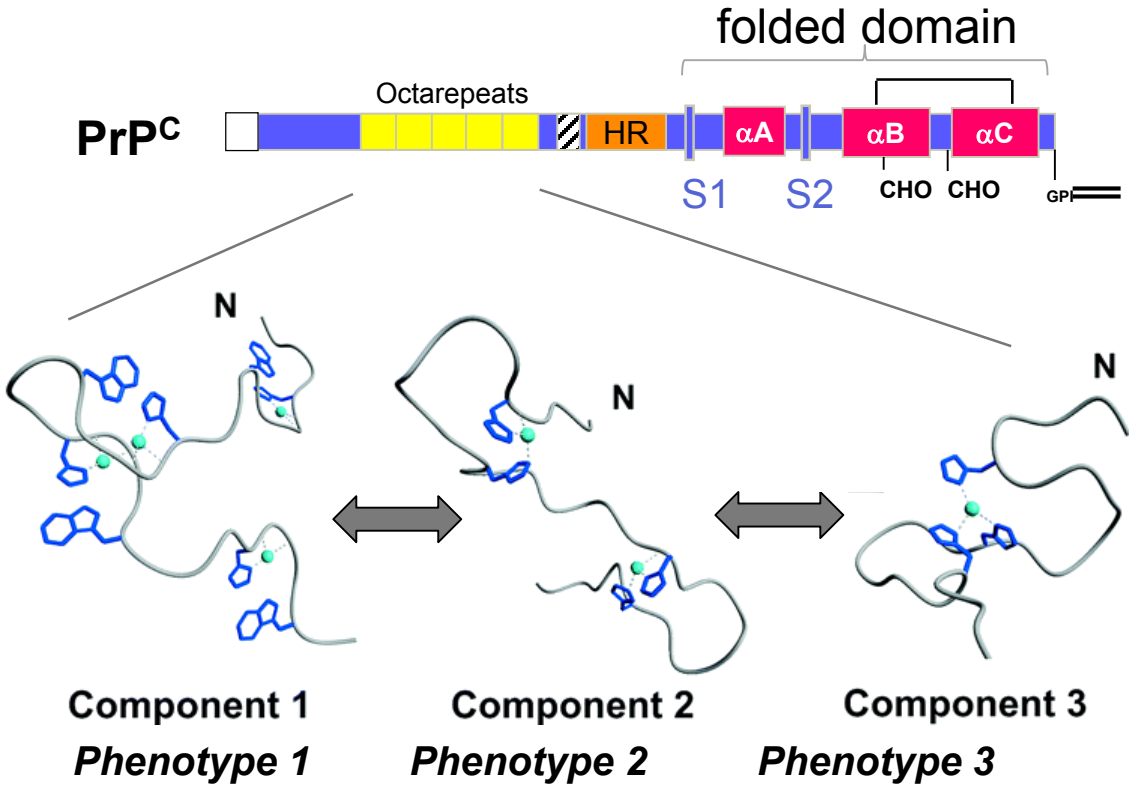
Testing the vaccine candidate *in vivo* indicates a specific response against PrP^{Sc}

Competition ELISA: **Immune response elicited against the vaccine candidate and the unmodified HET-2s scaffold**

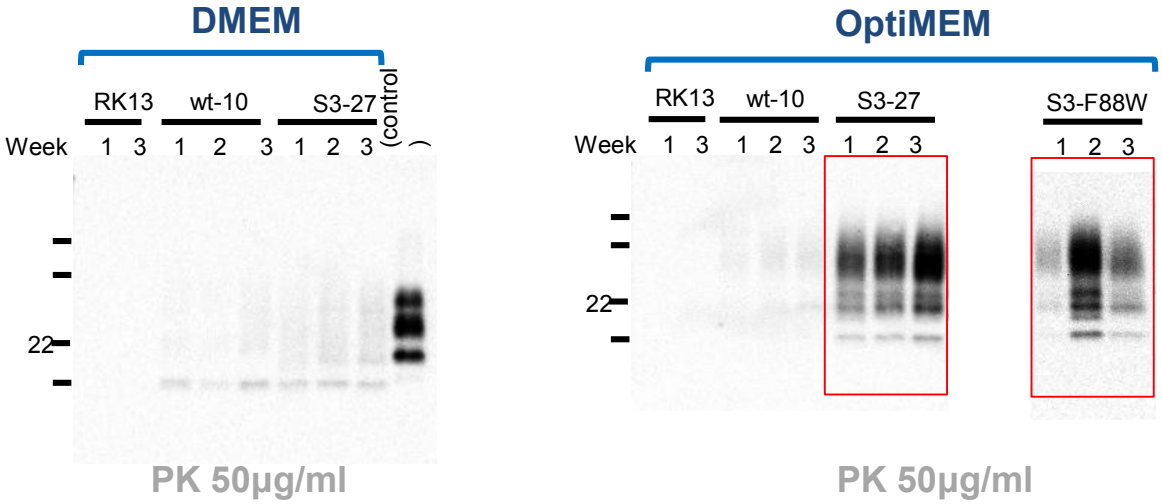


n=4/group

Hypothesis: interchangeable geometries in WT PrP^C blur phenotypic attributes



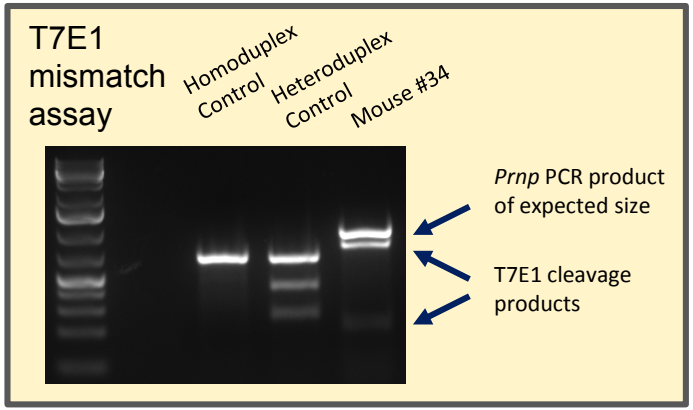
“Spontaneous” accumulation of PK-resistant material in S3 expressing RK13 cells: effect of DMEM vs OptiMEM



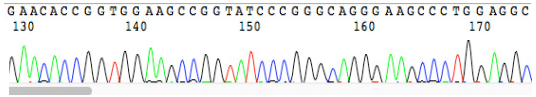
- Interesting because OptiMEM medium is commonly used for establishing *de novo* prion infections
- The effect is absent for wt and S1 PrP alleles assessed in parallel

Gene drive

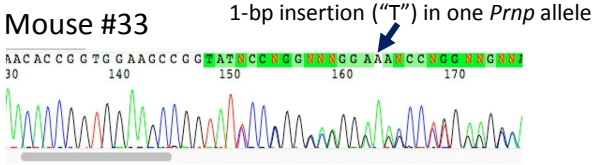
Germline genome editing



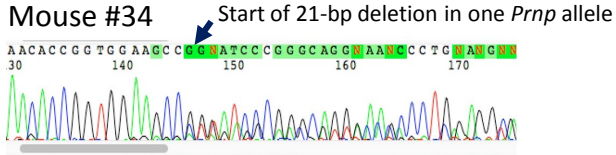
WT *Prnp*



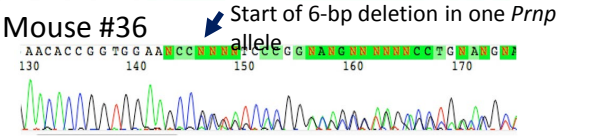
Mouse #33



Mouse #34

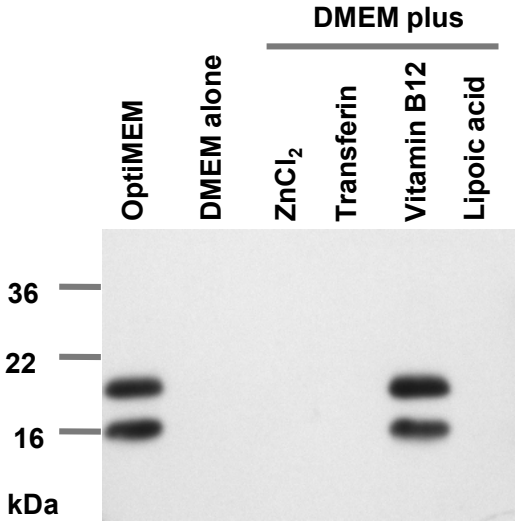


Mouse #36

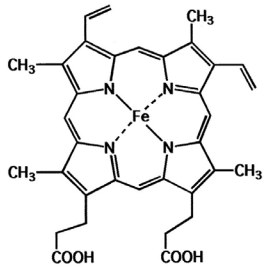


- In parallel with cell experiments, gRNAs were tested for generation of NHEJ-induced indels *in vivo.*
- Initially, Cas9/gRNA RNPs were delivered into fertilised FVB oocytes by microinjection.
- Another technique produced more success – in one attempt, 3 out of 5 viable pups had disruptions to their *Prnp* coding sequence (see left). This suggests that the particular gRNA used is effective at promoting Cas9 cleavage *in vivo*.

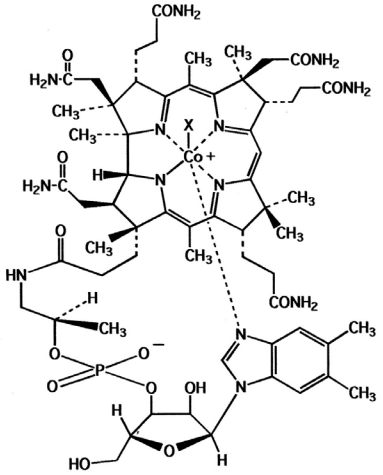
Addition of OptiMEM components to DMEM - effect on S3-27 cells



All samples are treated with PK (50µg/ml) and PNGase

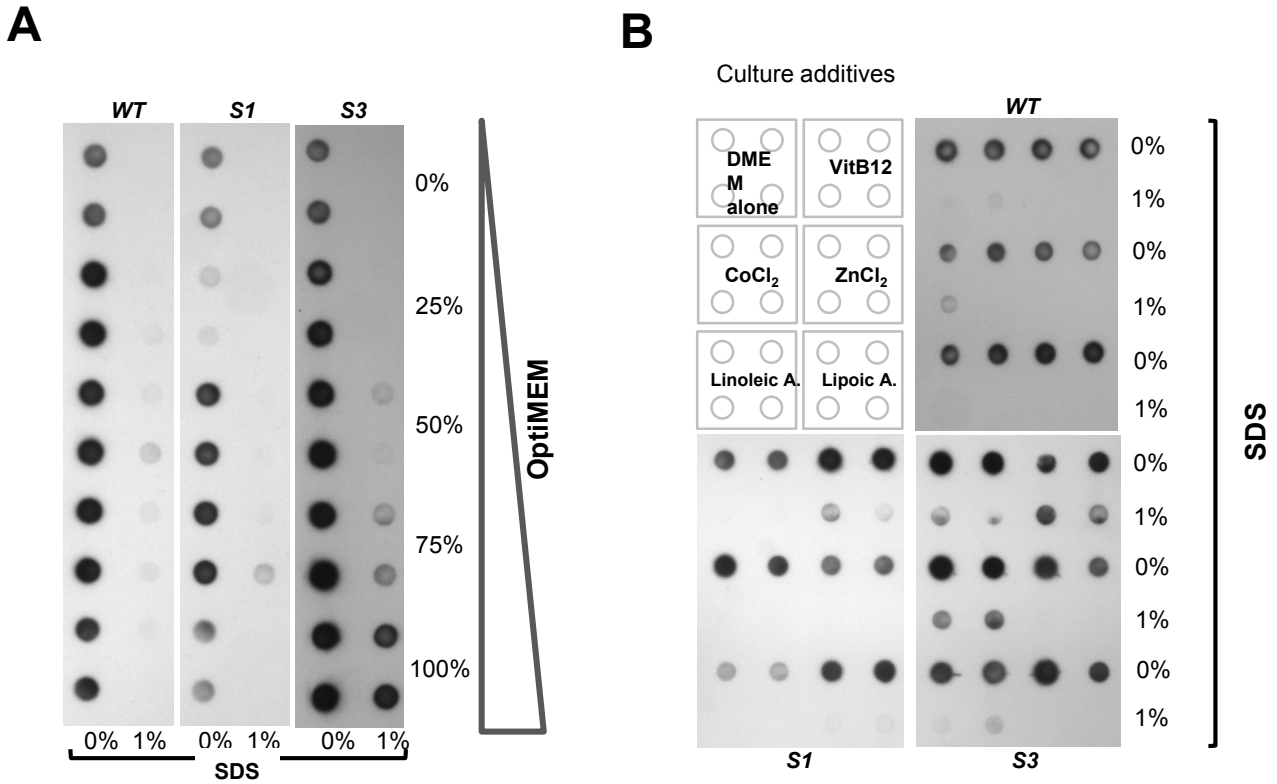


Heme

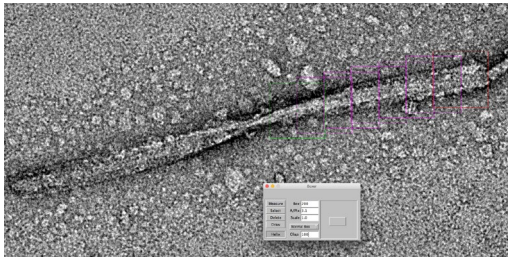


Vitamin B_{12}

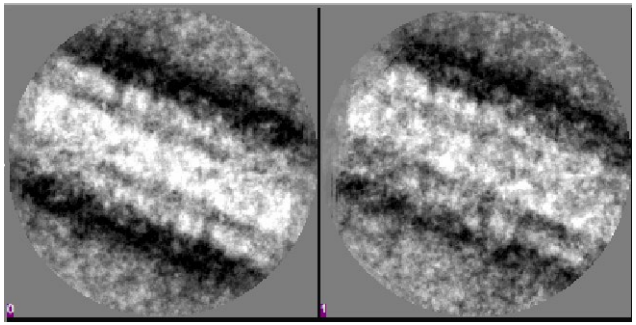
OptiMEM effects on S3 PrP are associated with SDS insolubility

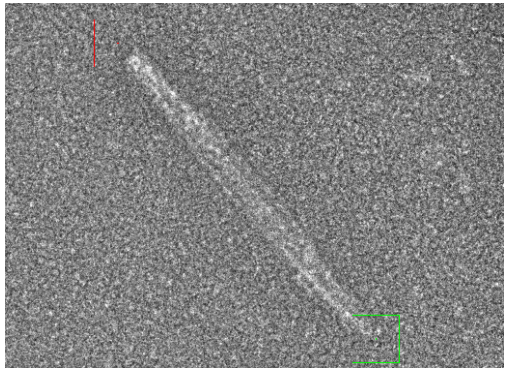


Morphology I: Twisted Ribbon–Like Fibrils

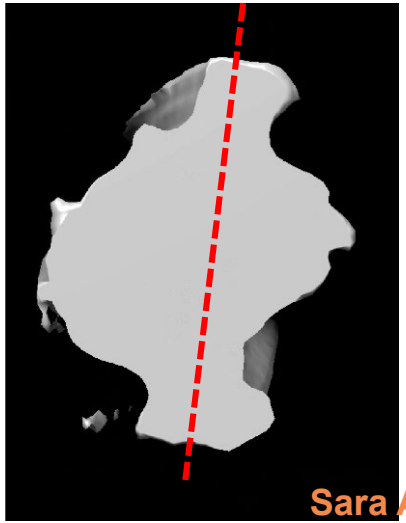


2D class average





Reconstruction



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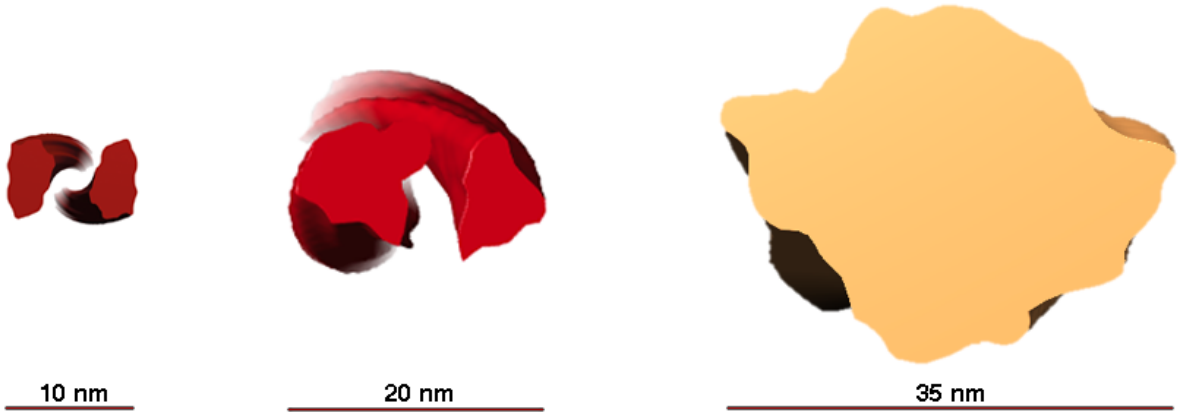
Scrapie vs BSE vs CWD fibrils



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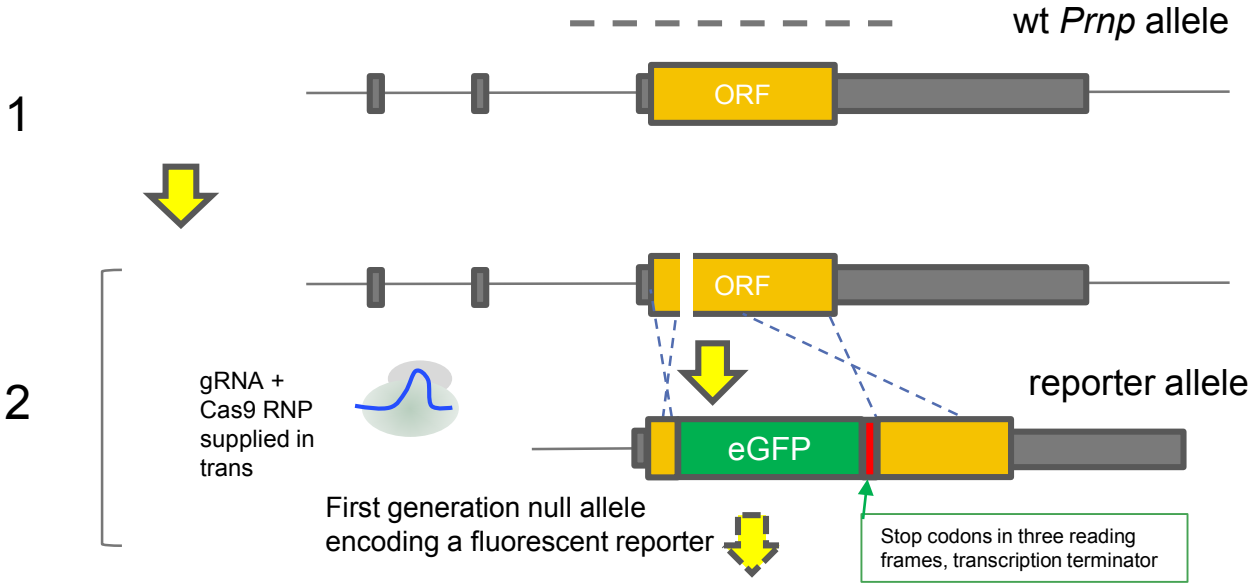
BSE reconstruction provided by razieh Kamali-jamil
Scrapie reconstruction provided by Dr. Ester Vázquez-Fernández

Gene drive

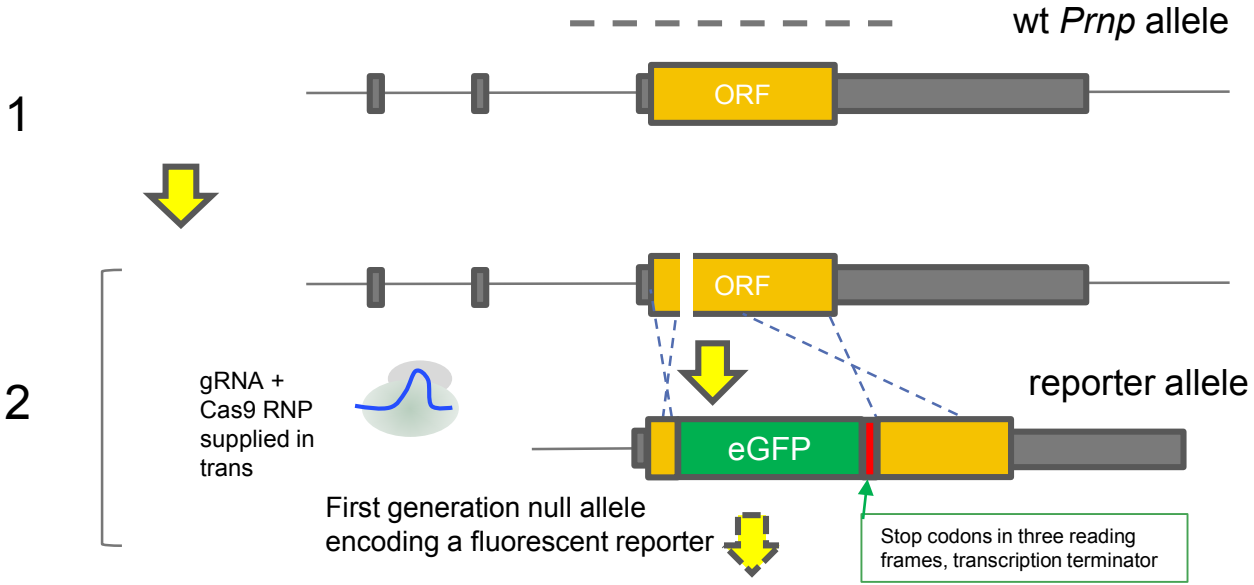
Making a tagged target



Andrew Castle,
Serene Wohlgemuth

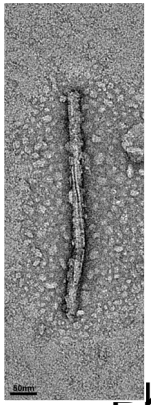
Gene drive

Making a tagged target

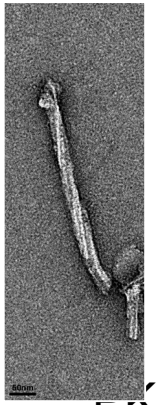


Andrew Castle,
Serene Wohlgemuth

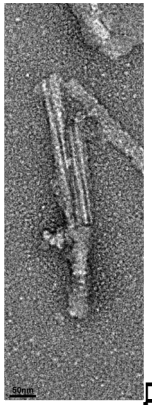
Morphology II:



PK

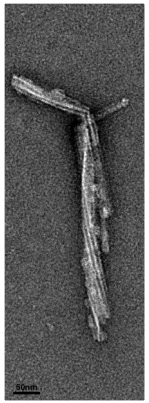


PK

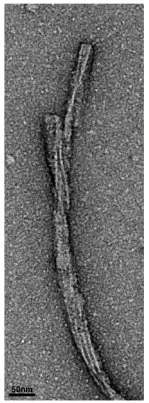


PE

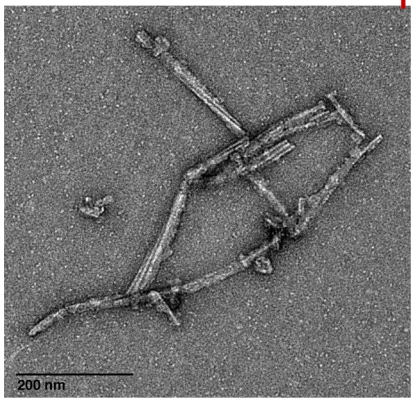
- Second dominant morphology
- Seen in Both PE and PK treated samples



PK



PE



PK

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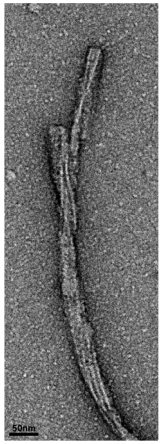
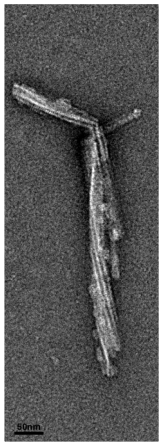
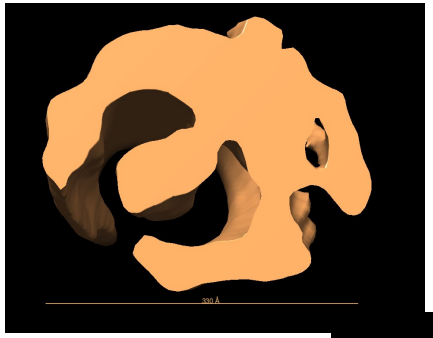
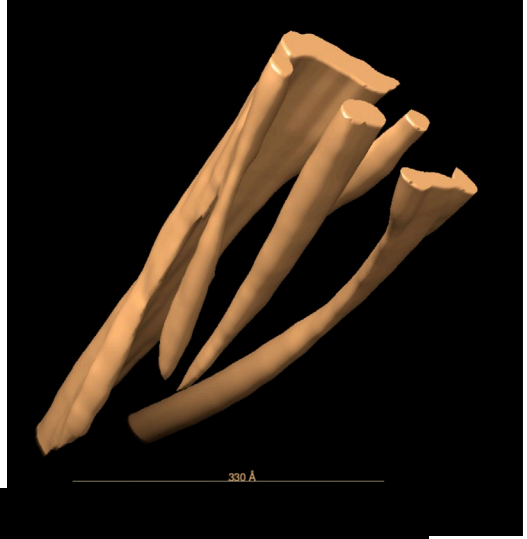
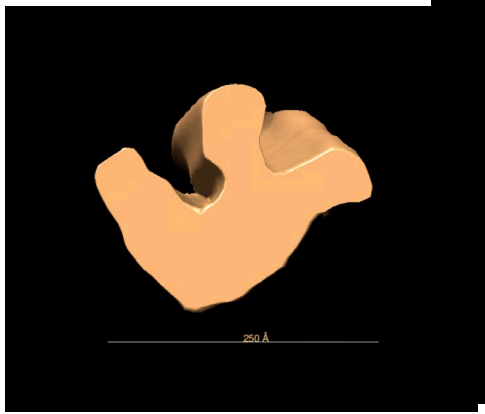
Morphology II:

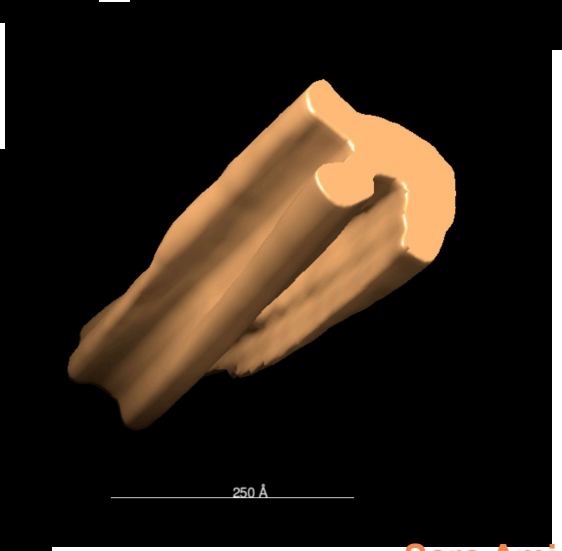












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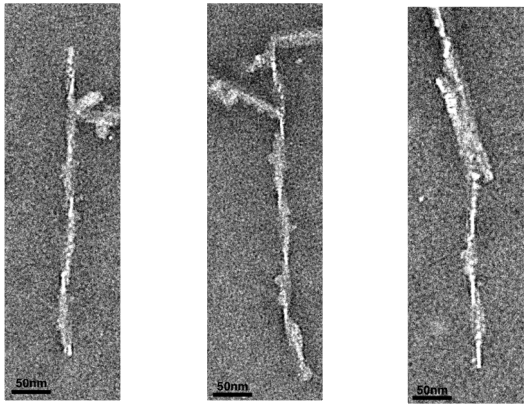
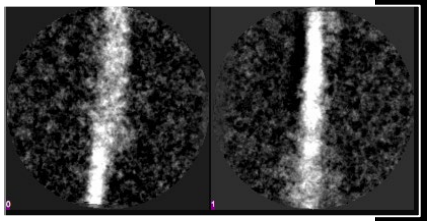
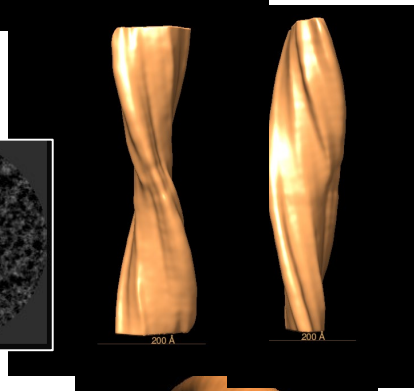
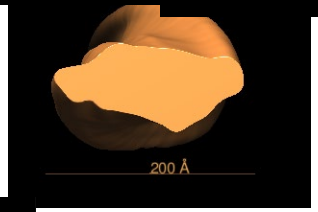
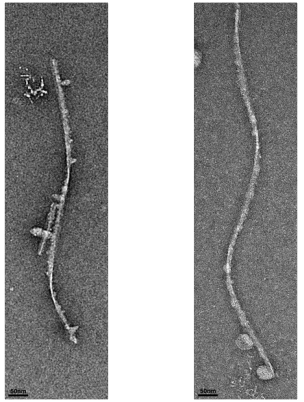
Morphology III:

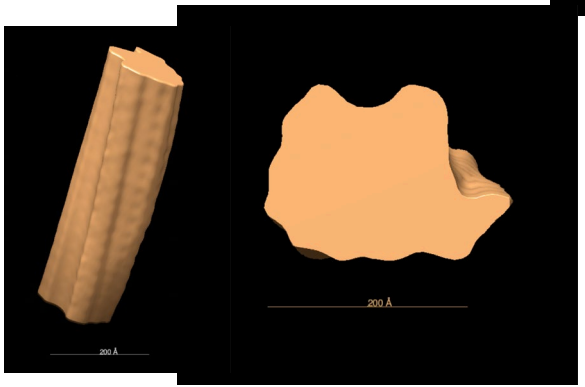












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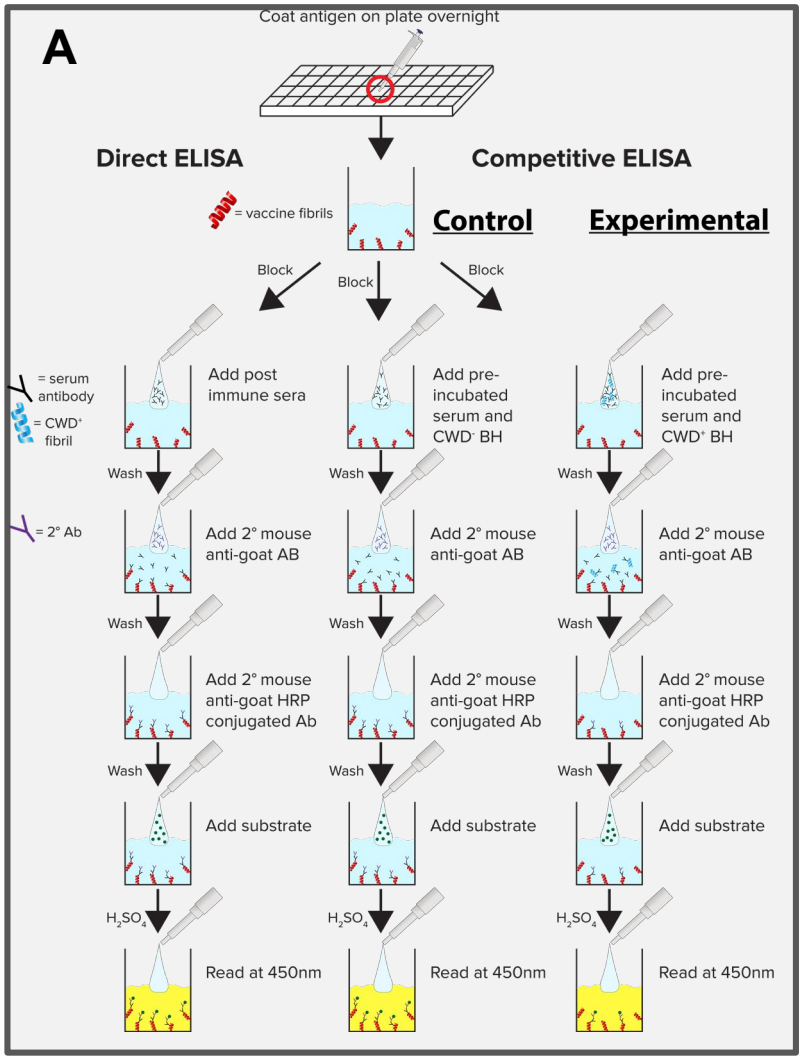

Testing the vaccine candidate *in vivo* indicates a specific response against PrP^{Sc}

B