Determining the Therapeutic Potential of Anti-PrP Nanobodies

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Humans

Sporadic

Sporadic Creutzfeldt-Jakob disease (**sCJD**)

Sporadic fatal insomnia

Variably Protease-sensitive prionopathy (**VPSPr**)

Inherited

Gerstmann-Sträussler-Scheinker syndrome (**GSS**)

Fatal familial insomnia (**FFI**)

Familial Creutzfeldt-Jakob disease (**fCJD**)

Acquired

Iatrogenic Creutzfeldt-Jakob disease (**iCJD**)

Kuru

Variant Creutzfeldt-Jakob disease (**vCJD**)

Animals

Scrapie − sheep, goats

Chronic Wasting Disease (CWD)
− deer, elk

TME − mink

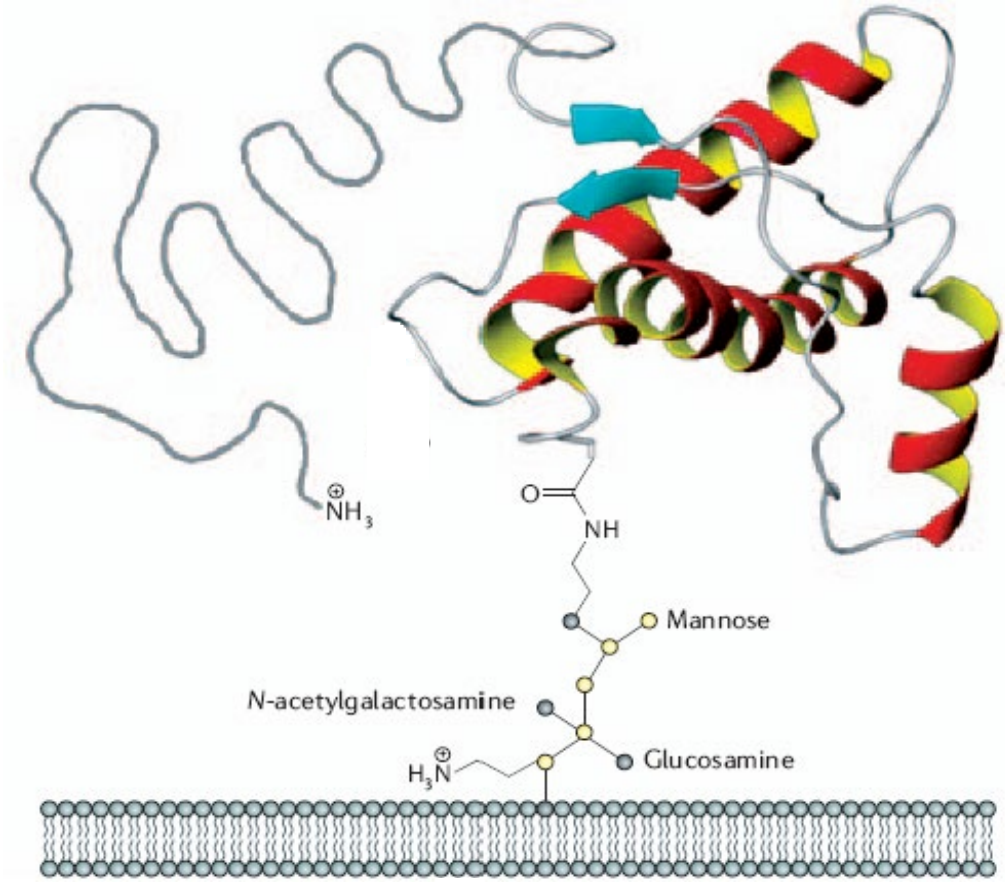
BSE − cattle

FSE − domestic cats, captive wild cats

Exotic Ungulate Spongiform Encephalopathy
− exotic zoo ruminants of the family Bovidae (kudu, elands, etc)

TSE in non-human primates − captive
lemurs, Rhesus macaque

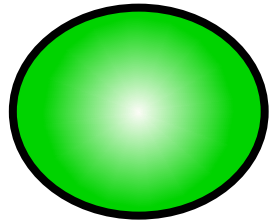
Prion Protein (PrP)



Aguzzi and Heikenwalder *Nature Reviews Microbiology* **4**, 765–775

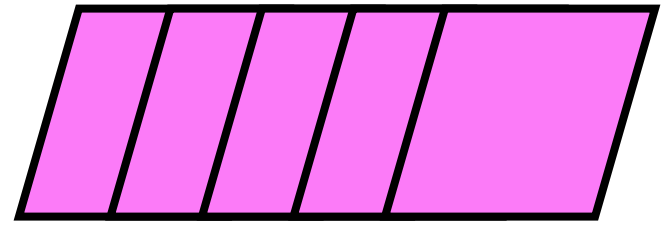
PrP^{C}

- 42% α-helix, 3% β-sheet
- Soluble in mild detergents
- Sensitive to protease digestion
- Sensitive to PI-PLC digestion

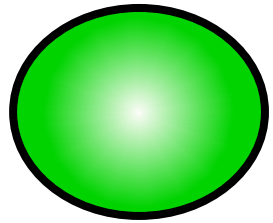


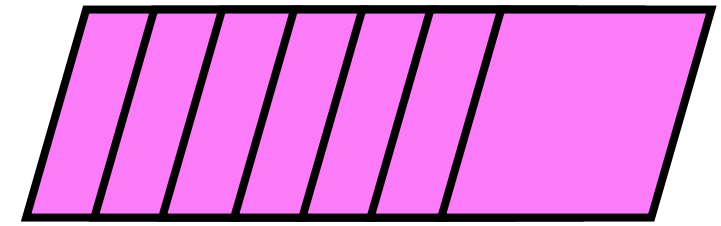
PrP^{Sc}

- Almost all β-sheet
- Insoluble in mild detergents
- Resistant to protease digestion
- Resistant to PI-PLC digestion

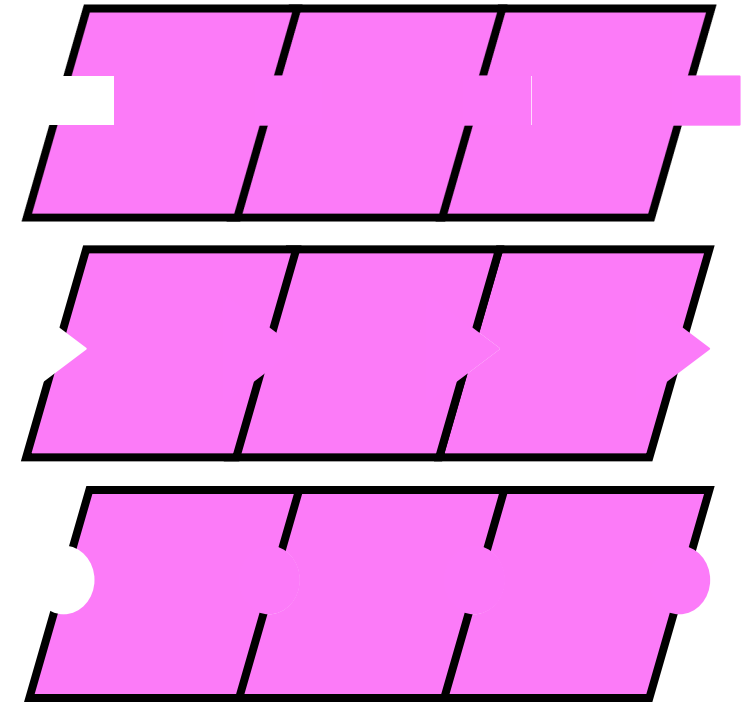


Prion infectivity: Seeded conversion





Prion strains

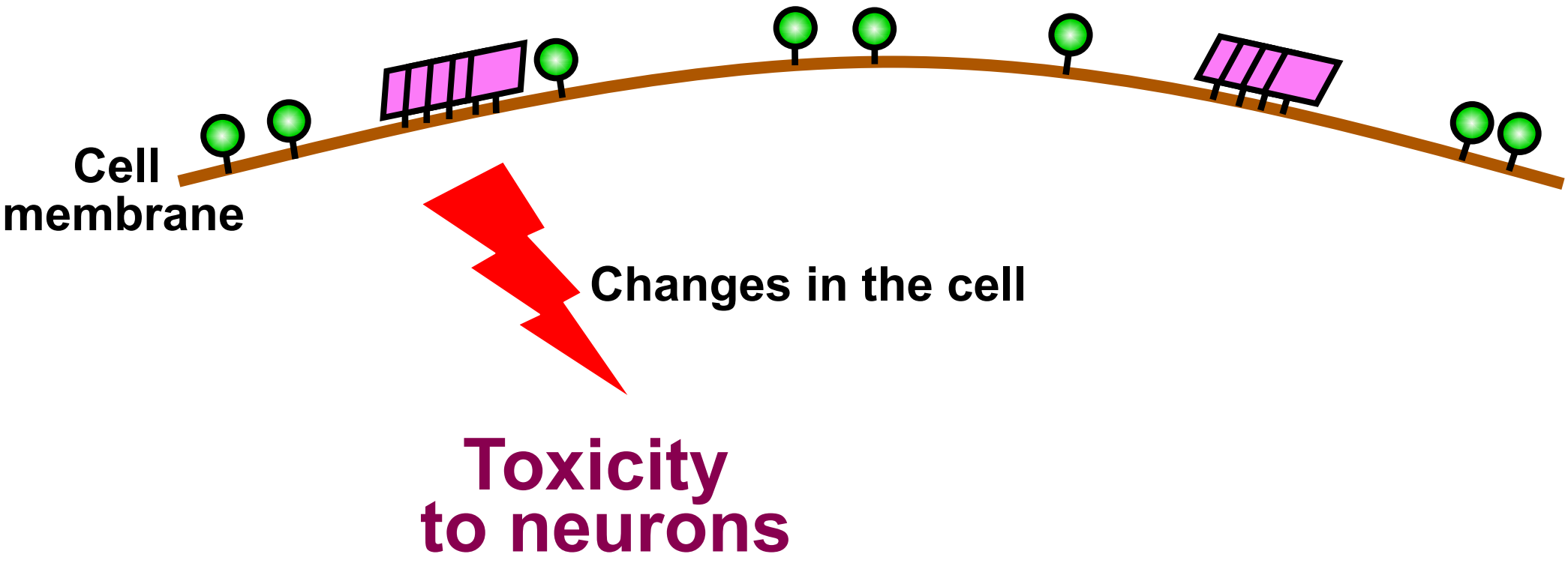


Targets

- PrP^{Sc}
- PrP^{C}
- Cellular changes

Agents

- Small molecules
- Antibodies



PrP^{Sc}

Advantage

- Disease specific

Potential pitfalls

- Prion strains
- Difficult to generate PrP^{Sc}-specific reagents

Developing Therapeutics for PrP Prion Diseases

Kurt Giles,[1,2] Steven H. Olson,[1,2] and Stanley B. Prusiner[1,2,3]

Cold Spring Harb Perspect Med. 2017 Apr 3;7(4). doi: 10.1101/cshperspect.a023747.

IND24

- Significantly increase the life span of mouse prion infected mice
- Ineffective against human prions

Cellular changes

Advantage

- Independent of strains

Potential pitfalls

- Affecting other cellular processes

RESEARCH ARTICLE

PRION DISEASE

Oral Treatment Targeting the Unfolded Protein Response Prevents Neurodegeneration and Clinical Disease in Prion-Infected Mice

Julie A. Moreno,[1] Mark Halliday,[1] Colin Molloy,[1] Helois Radford,[1] Nicholas Verity,[1] Jeffrey M. Axten,[2] Catharine A. Ortori,[3] Anne E. Willis,[1] Peter M. Fischer,[4] David A. Barrett,[3] Giovanna R. Mallucci[1]*

Sci Transl Med. 2013 Oct 9;5(206):206ra138.

GSK2606414

- penetrates the blood-brain barrier
- prevents clinical disease in prion-infected mice
- but severe side effects (toxicity)

PrP^C

Advantage

- Independent of strains
- Bind to a larger region of a protein

Potential pitfalls

- Potential toxicity due to PrP binding
- Difficult to cross the blood brain barrier (BBB).

Monoclonal antibodies inhibit prion replication and delay the development of prion disease

Anthony R. White*, Perry Enever*, Mourad Tayebi*, Rosey Mushens†, Jackie Linehan‡, Sebastian Brandner‡, David Anstee†, John Collinge*‡ & Simon Hawke*

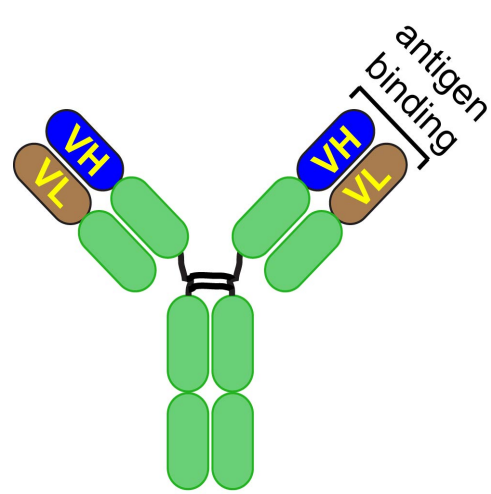
Nature. 2003 Mar 6;422(6927):80-3.

i.p. injection of anti-PrP antibody

- Prevents prion disease in mice received intraperitoneal prion infection (> 500 dpi)
- No effect against mice received intracerebral prion infection.

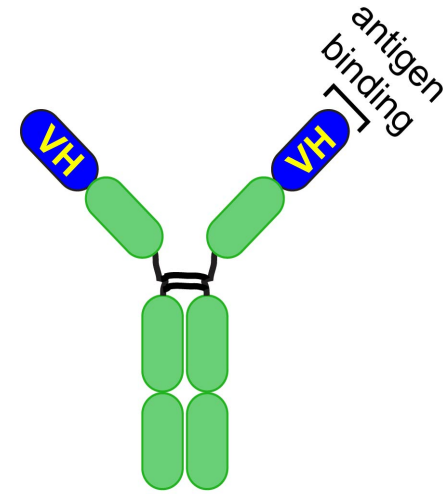
Thus far, PrP^C appears to be a good target and the therapeutic effects of anti-PrP antibodies are better than small molecules.

But, crossing the blood brain barrier appears to be a major obstacle for diseases already reached central nervous system.



Conventional
antibody

Encoded by
two different genes



Heavy Chain antibody
(camelid)

Encoded by
one genes

Nanobody

Adeno-associated Virus (AAV)

- A replication-defective virus found in humans (It requires co-infection of other virus, such as adenovirus or herpesviruses, for its replication).
- ~ 80 - 90% of adults are positive with AAV, but it is not associated with any symptoms or disease.
- In human cells, it preferentially integrate into the AAVS1 region, ~ 2Kb region on the long arm of human chromosome 19.
- Gene therapy treatment of spinal muscular atrophy (SMA) with AAV vector has been approved by FDA.
- Scientists are actively searching for AAVs that can cross the BBB. In C57BL mice, the newly identified AAV-PHP.eB is able to cross BBB.

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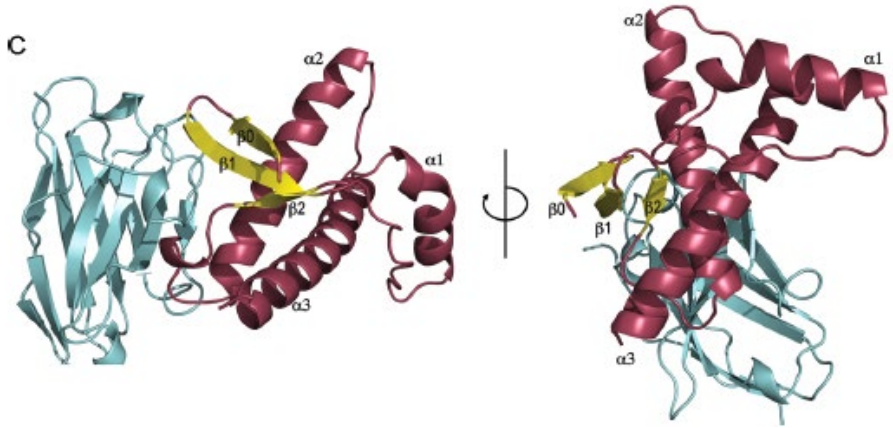
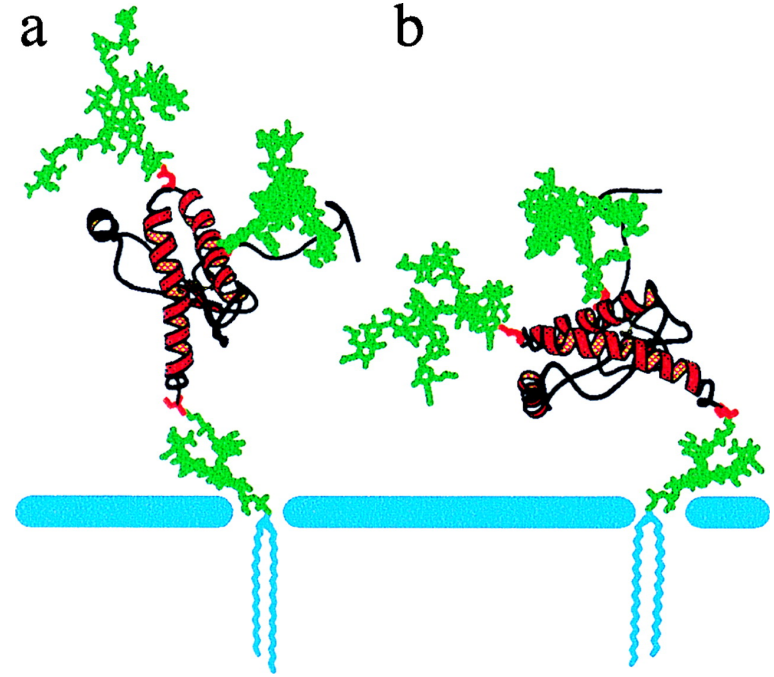
Probing the N-Terminal β-Sheet Conversion in the Crystal Structure of the Human Prion Protein Bound to a Nanobody

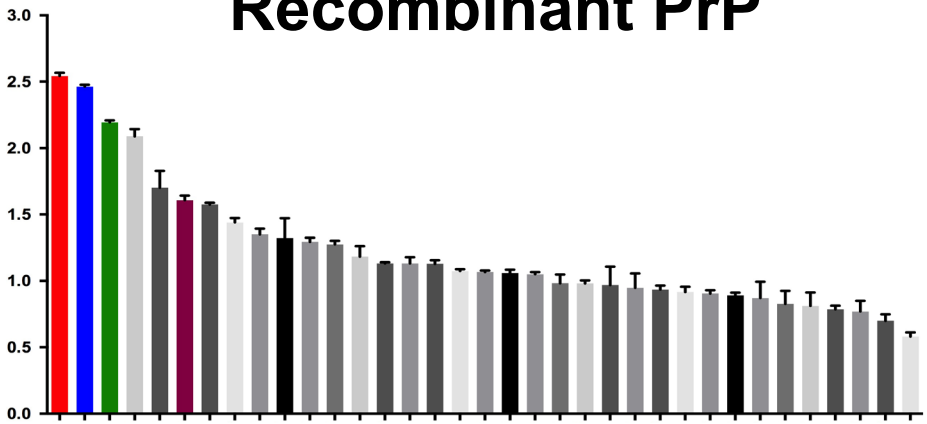
Romany N. N. Abskharon,[†,‡,∥,#] Gabriele Giachin,[§,#] Alexandre Wohlkonig,[†,‡,#] Sameh H. Soror,[†,‡,¶] Els Pardon,[†,‡] Giuseppe Legname,[*,§] and Jan Steyaert[*,†,‡]

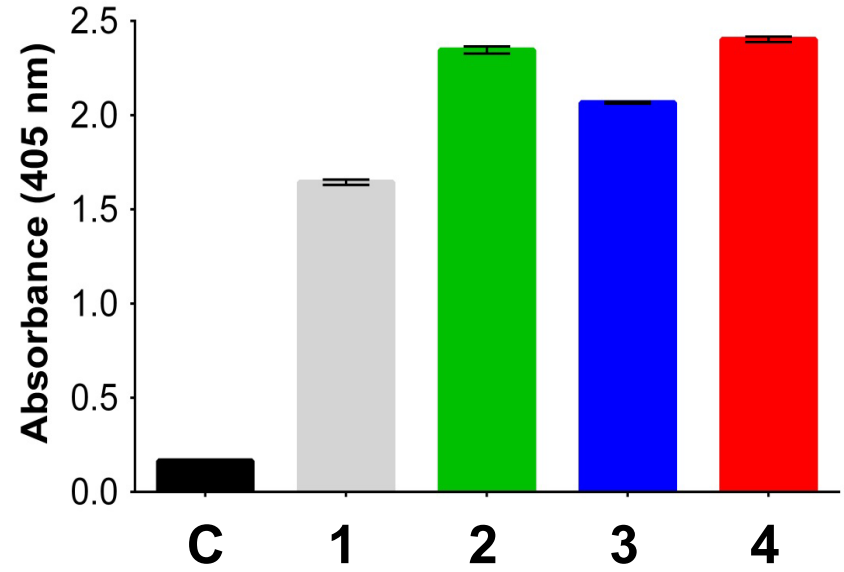




Pauline M. Rudd et al. PNAS 1999;96:23:13044-13049

Recombinant PrP





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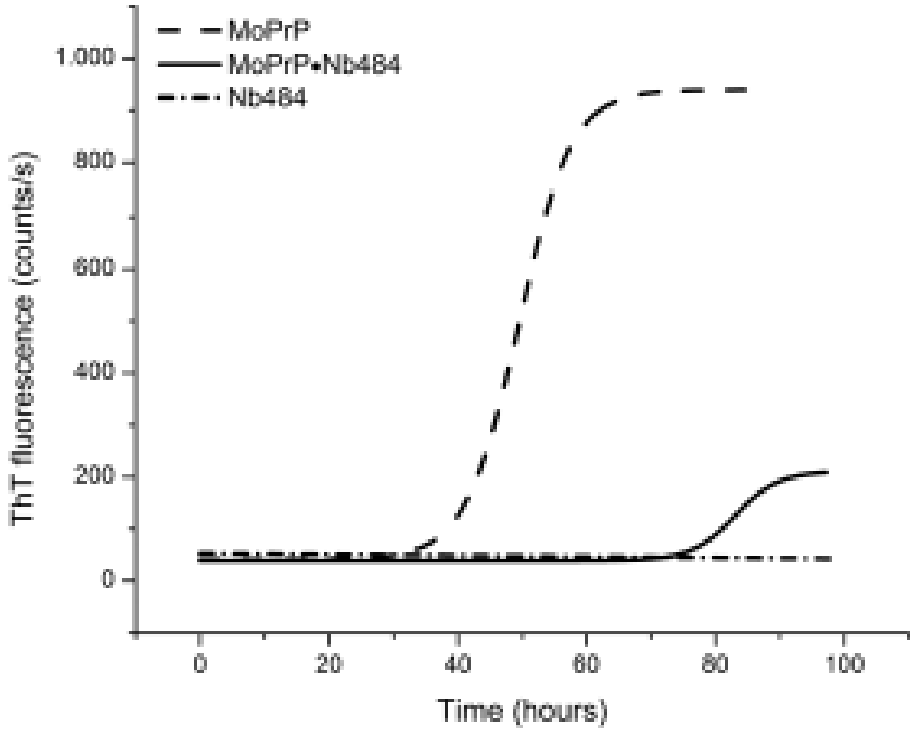
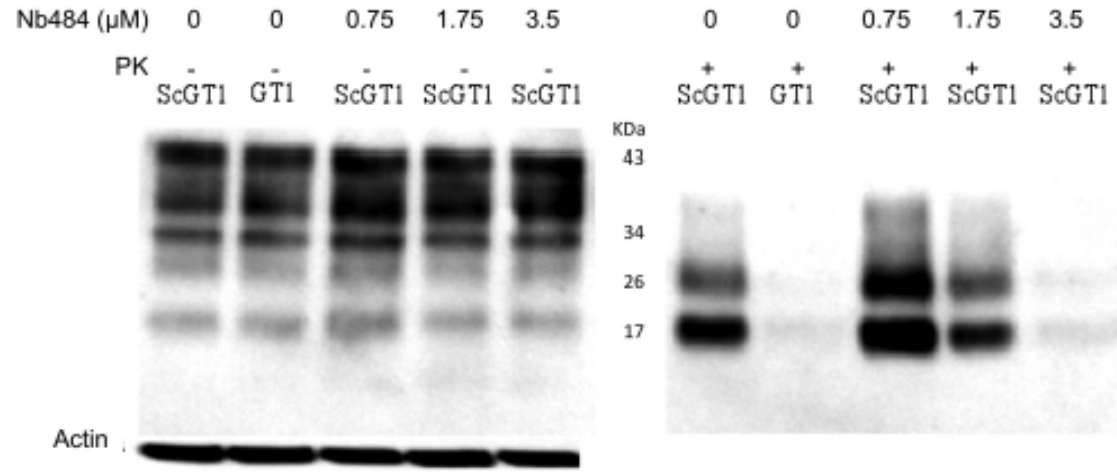
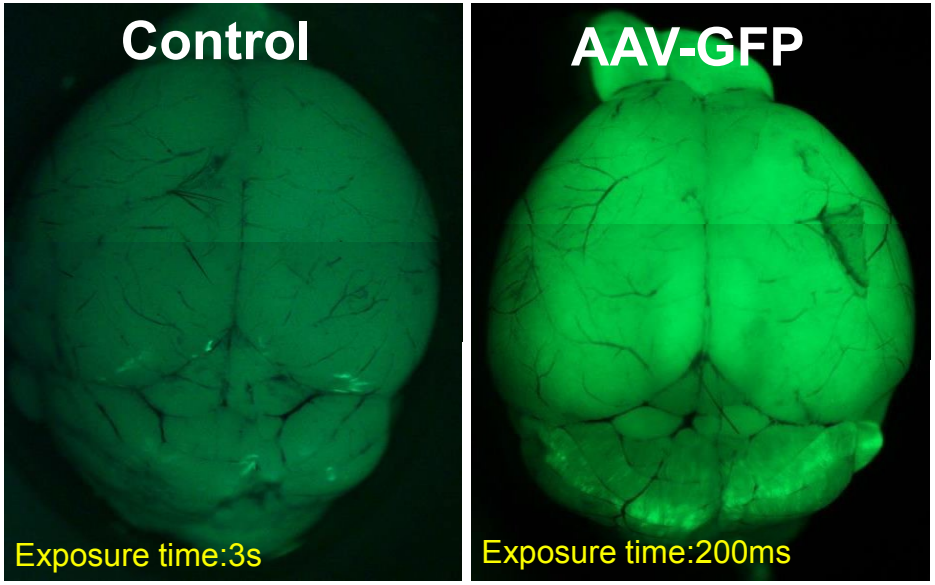
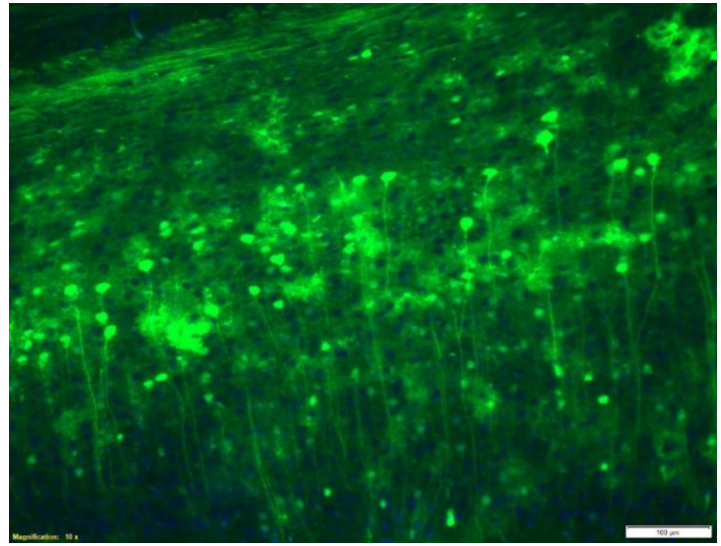


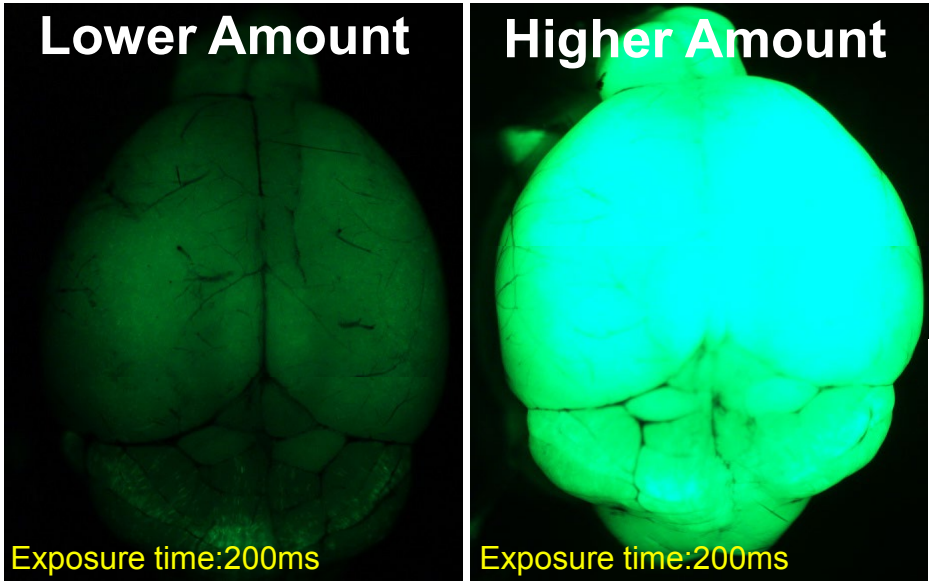


AAV delivery to central nervous system

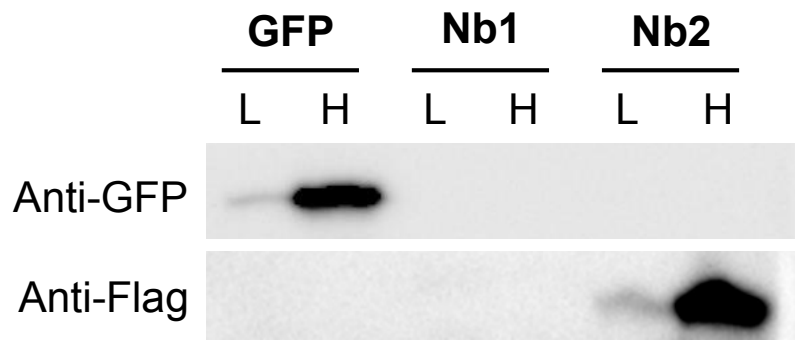


14 days





21 days



Summary

- We identified nanobodies that bind to not only PrP expressed in bacteria, but also the fully modified PrP expressed in mammalian cells.
- These anti-PrP nanobodies are able to inhibit prion replication *in vitro,* and do not show any neurotoxicity.
- The anti-PrP nanobodies have been packaged into AAV and successfully expressed in the central nervous system.
- The study of the potential therapeutic effect of expressing anti-PrP nanobodies by AAV in mice is underway.

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