

Acknowledgements

Collaborators

(Rocky Mountain Laboratories)

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Funding

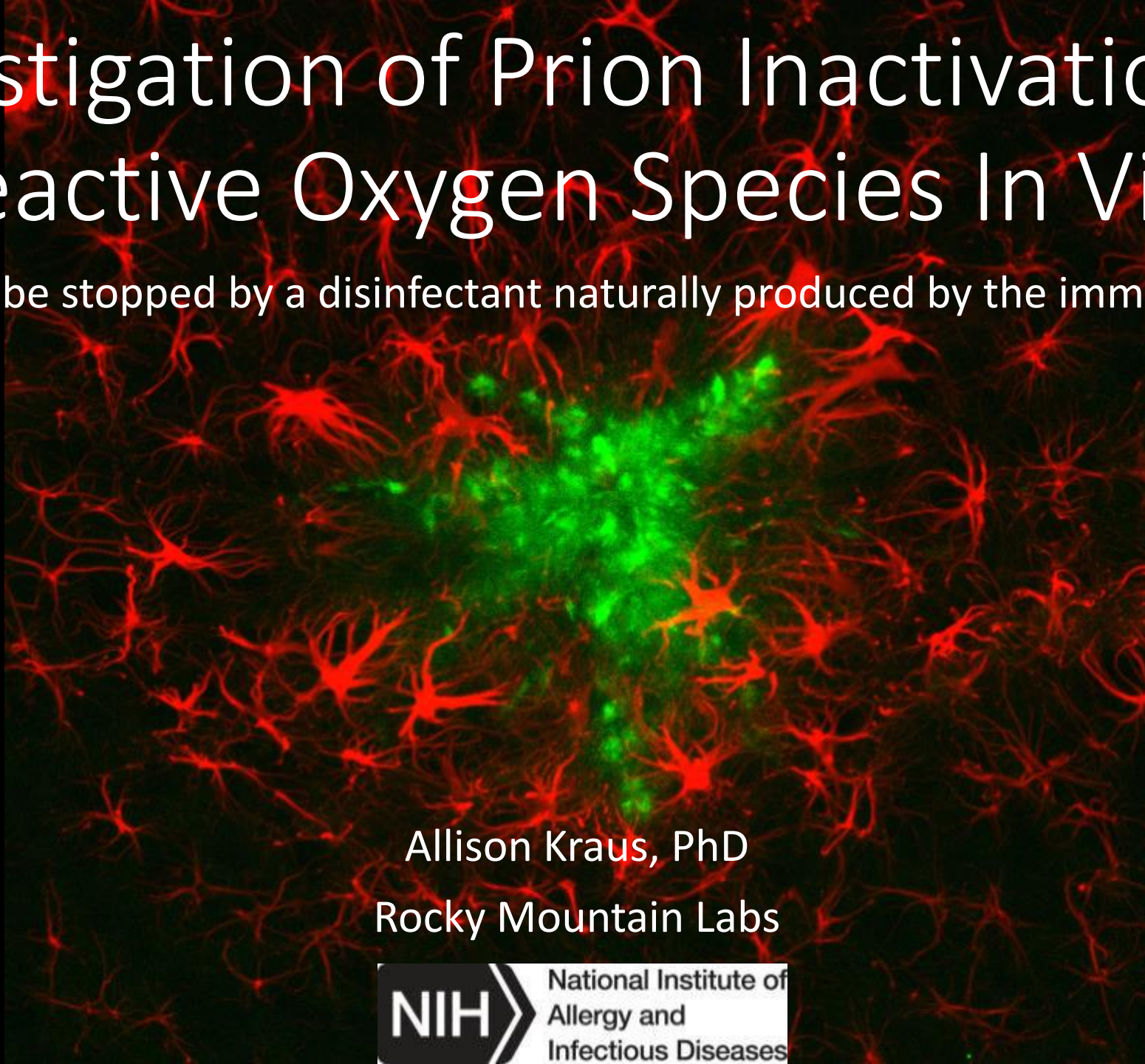
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Investigation of Prion Inactivation by Reactive Oxygen Species In Vivo

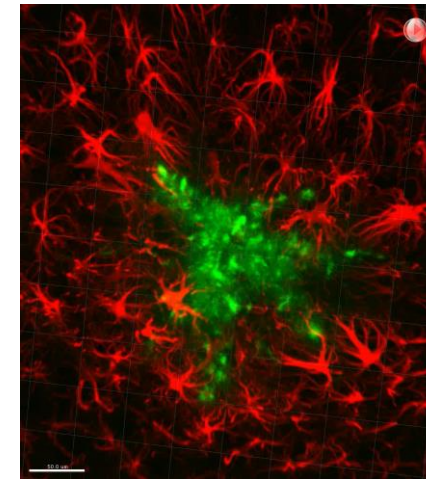
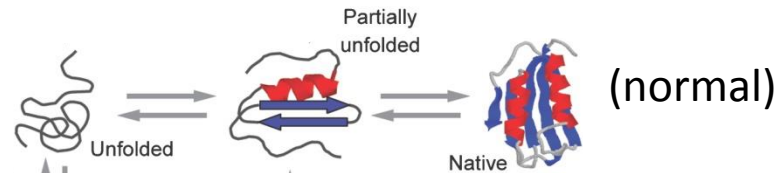
(Can prions be stopped by a disinfectant naturally produced by the immune system?)



Allison Kraus, PhD
Rocky Mountain Labs



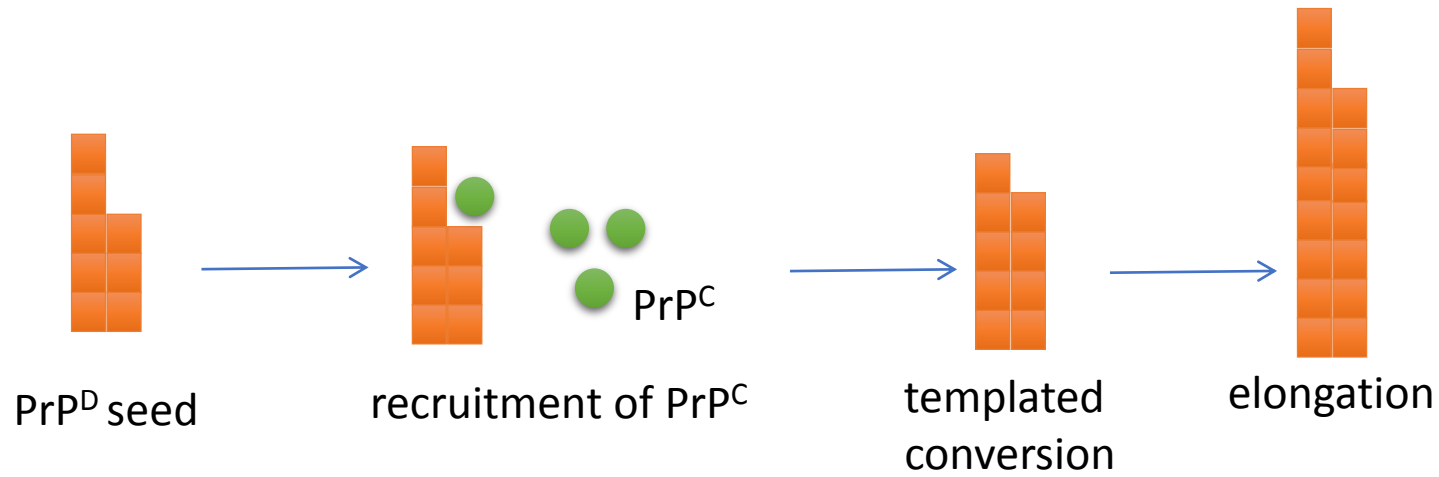
Protein folding & misfolding



Prion plaques
Astrocytes

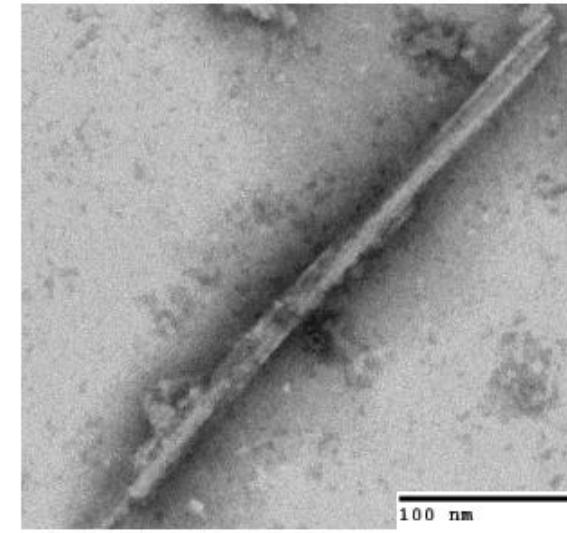
Prion deposits (green) surrounded by support cells (red) in brain tissue from an animal with clinical prion disease

Protein (PrP)-based self-propagation mechanism



PrP^D (*disease associated prions*) - infectious prions

PrP^C (*native prion protein*) – the normal protein

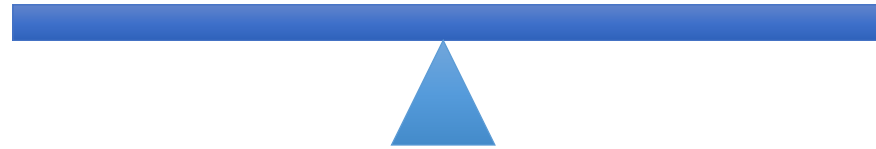


Actual prion fibril

Transmission of a misfolded protein

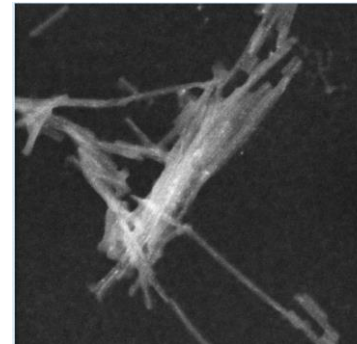
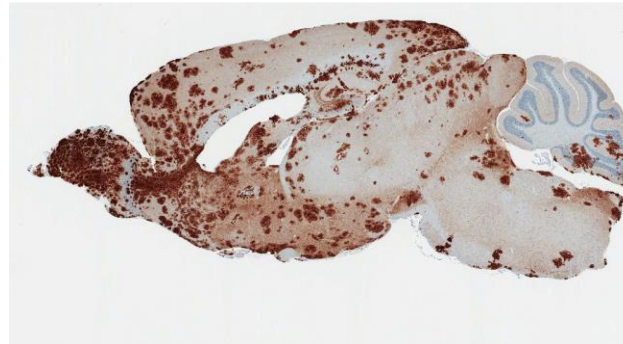
Prion formation
+
accumulation

Prion clearance?



- Genetic mutations
- Cofactors
- ??

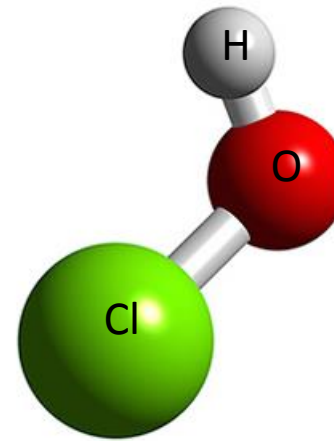
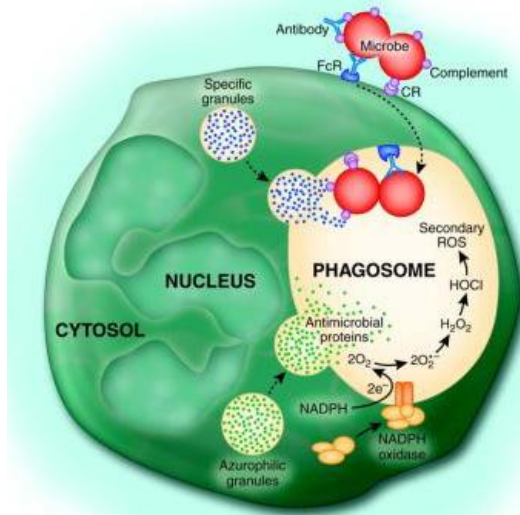
?



Reactive oxygen species (ROS) are produced by the immune system and can act as natural disinfectants

- Immune cells produce ROS when they encounter a pathogen
- This includes **hypochlorous acid (HOCl)**, a potent antimicrobial that is highly reactive with a number of biomolecules including DNA, RNA, fatty acid groups, cholesterol and proteins

Human neutrophils
*The immune system's
"first responders"*



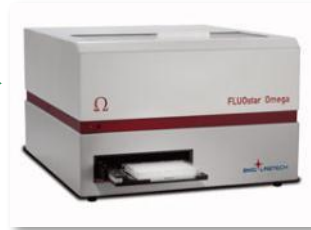
How do we detect prions?

Plate-based fluorescence detection of prion seeding activity (Real-time Quaking-Induced Conversion: RT-QuIC)

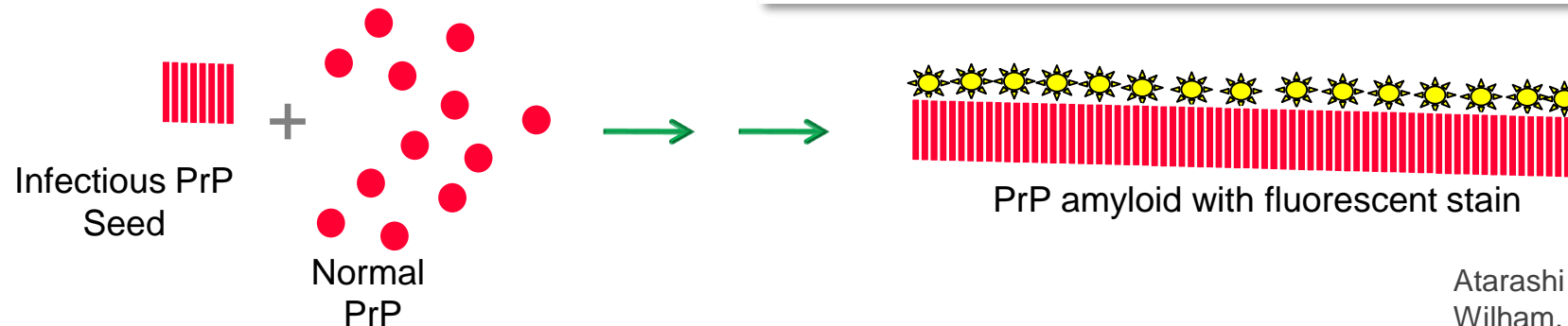
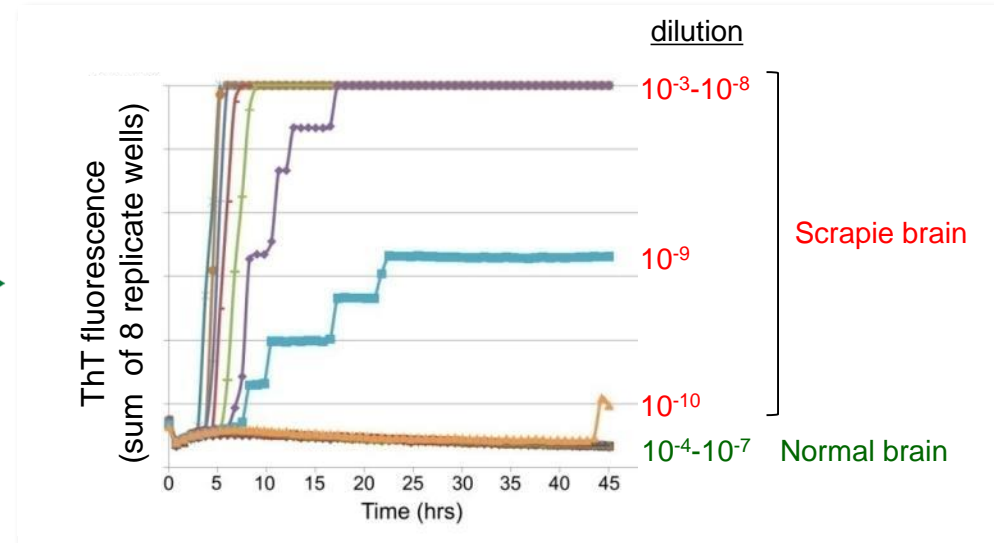
Sample + Recombinant PrP^{sen} + Thioflavin T



96-well plate



Shaking fluorescence plate reader

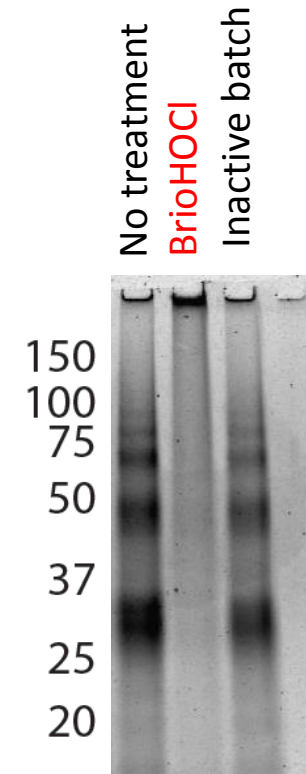
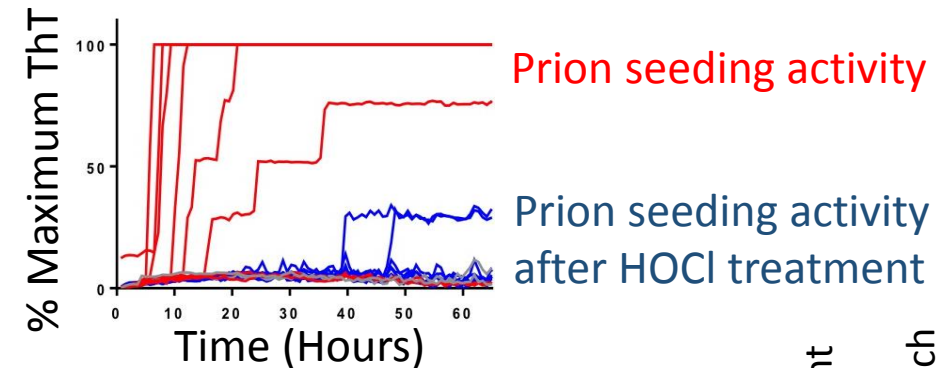


Atarashi et al, *Nature Medicine* 2011
Wilham, et al, *PLoS Pathogens* 2010
Orrù et al, *mBio* 2011

Treatment with HOCl solutions changes prions and prevents their ability to cause prion disease

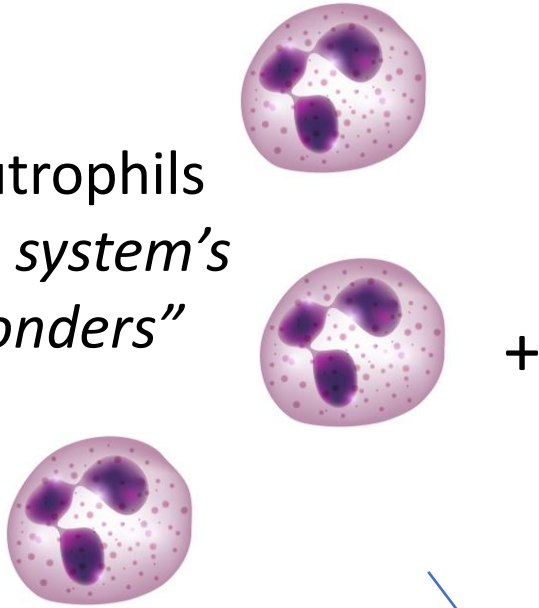
- Prions are a robust pathogen, and require harsh decontamination protocols in surgical and laboratory settings
- Need for less toxic/caustic disinfectants
- BrioHOCl (produced by BrioTech) tested for efficacy against prions
 - BrioHOCl can be applied directly to skin or other surfaces; much less caustic than bleach
 - Non-toxic
 - Environmentally friendly, breaking down into saline
- Treatment of prions with HOCl solutions totally eliminated the ability of those prions to cause disease in mice

(Inactivation of Prions and Amyloid Seeds with Hypochlorous Acid. PLoS Pathogens 2016. Hughson, Race, Kraus et al.)



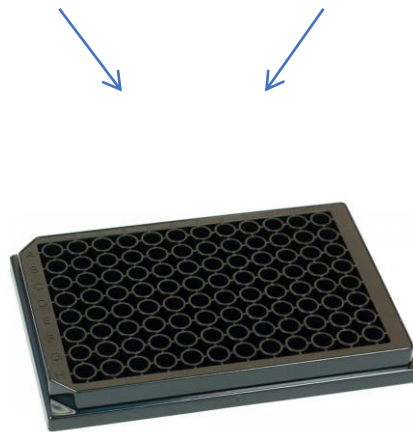
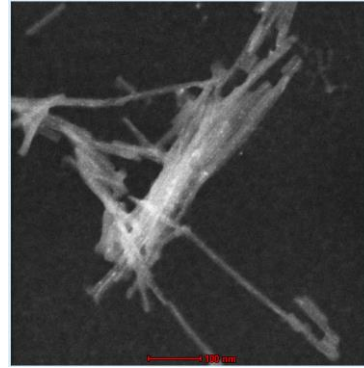
How does the immune system respond to prion exposure?

Human neutrophils
*The immune system's
"first responders"*



+

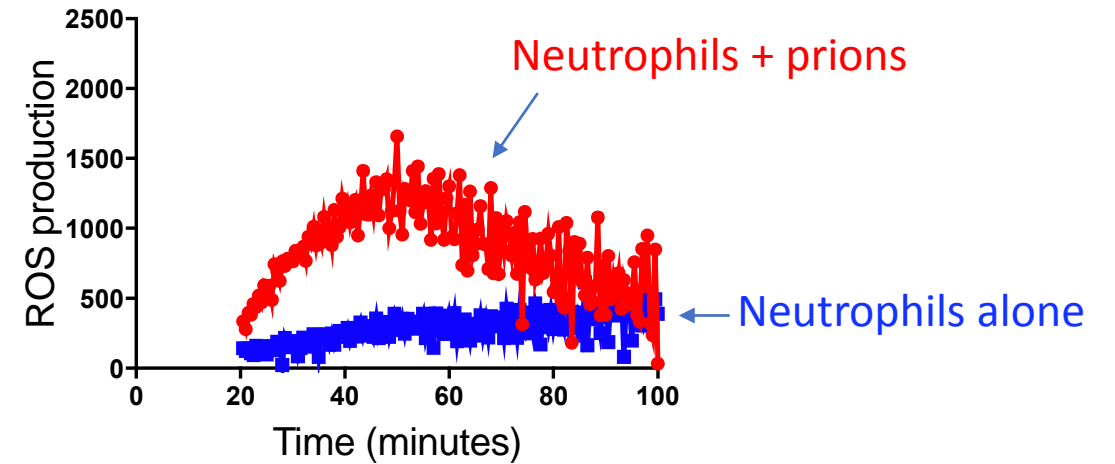
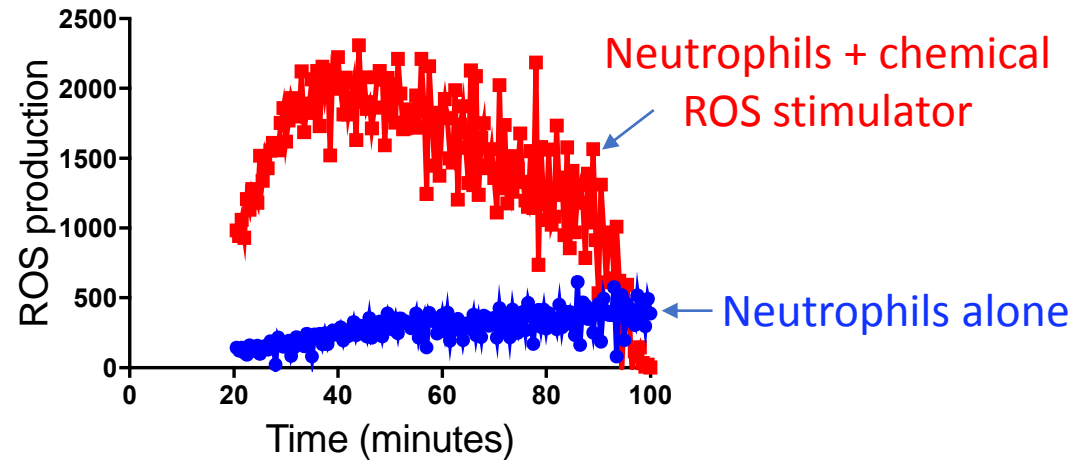
Prions



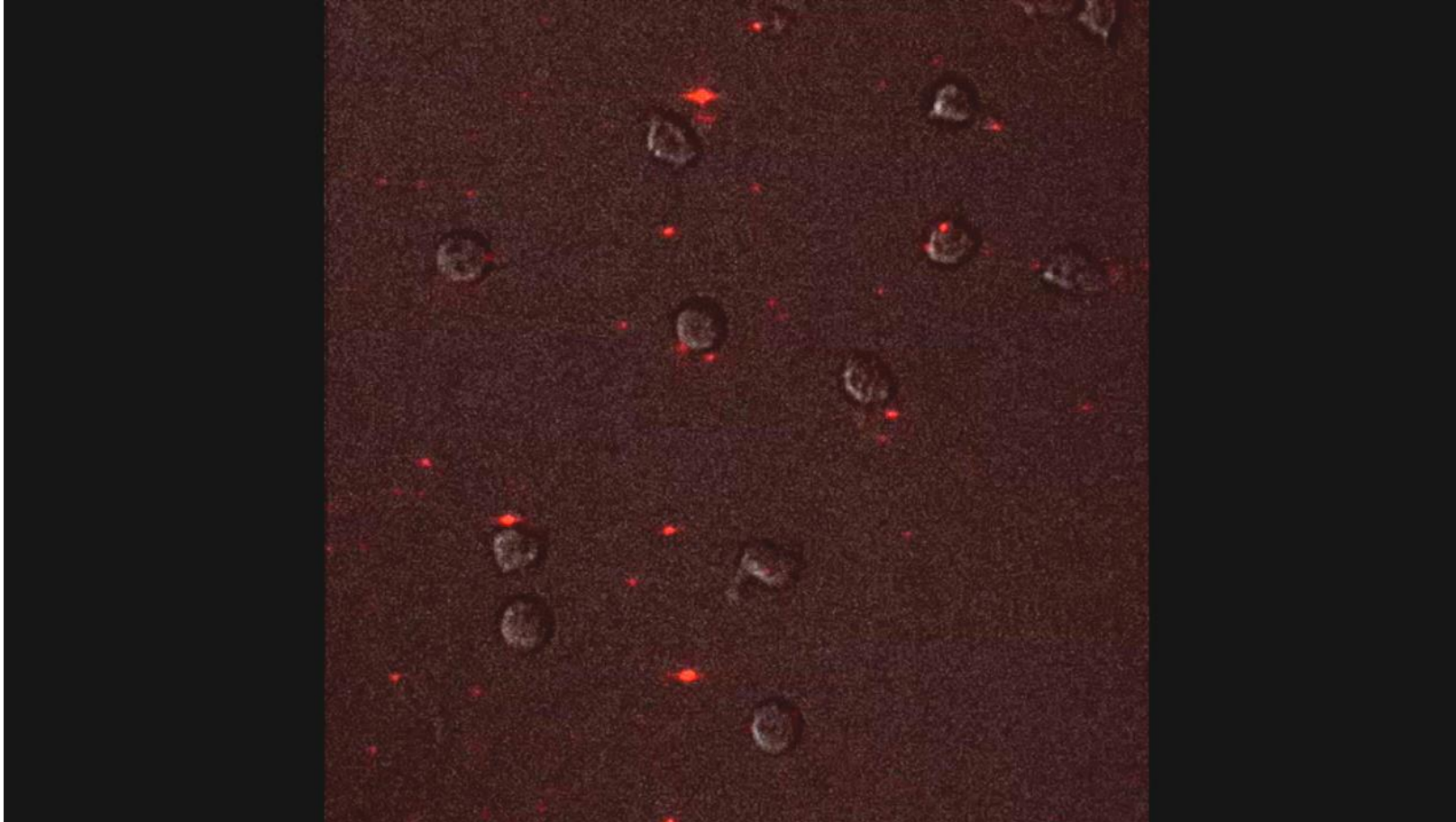
Measure:

- ROS production
- RT-QuIC to look at prion seeding activity
- Protein gel analysis to measure prions

Prions can stimulate ROS production in human neutrophils



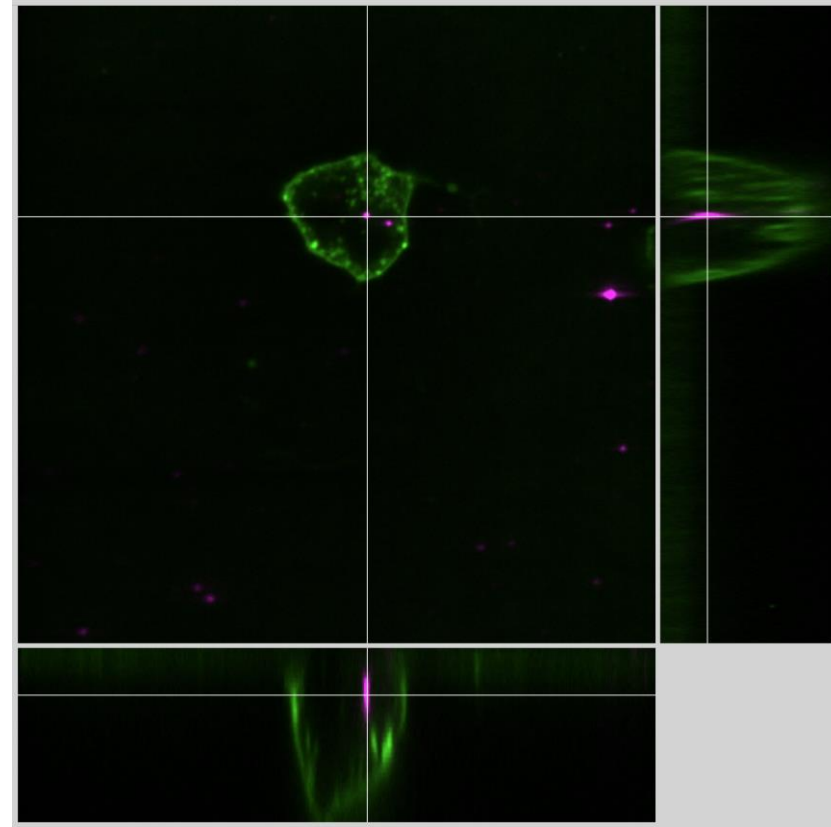
Prions rapidly become neutrophil associated



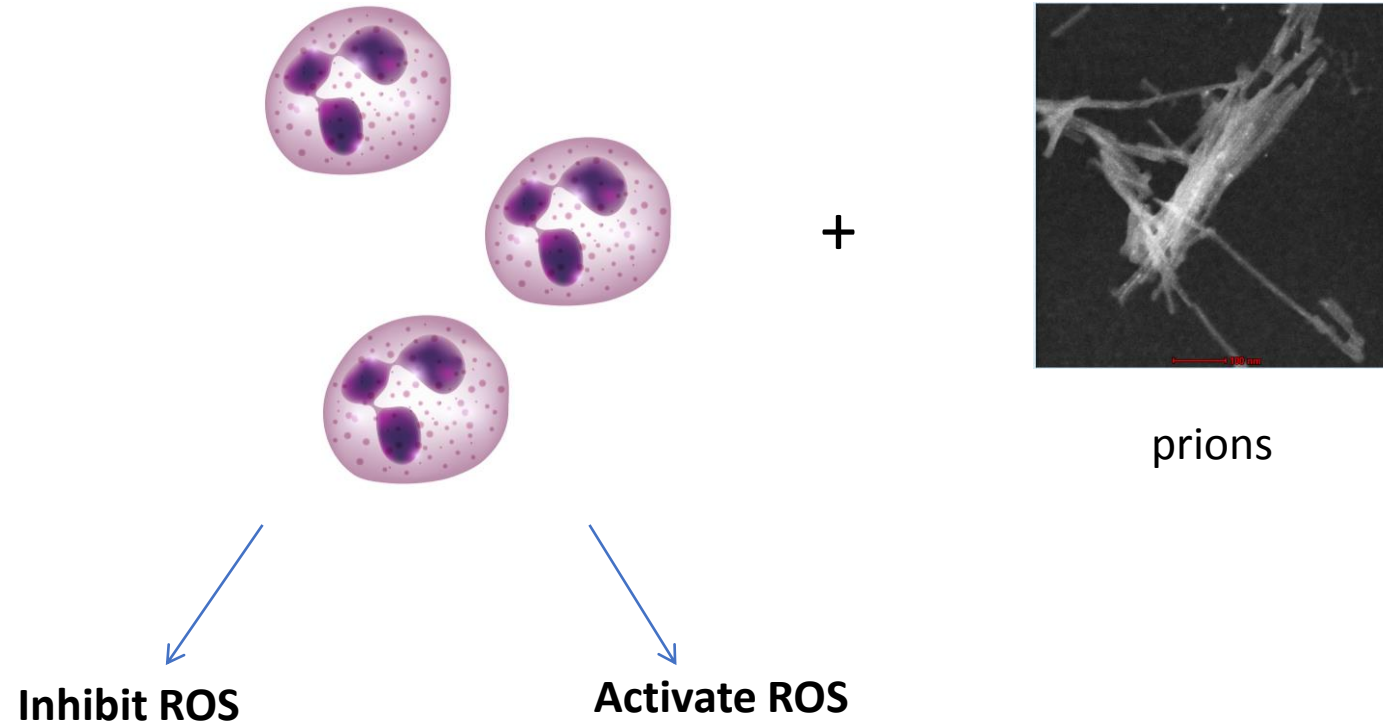
Movie showing human neutrophils rapidly coming to pick up prions (red dots)

Prions are taken up by human neutrophils within minutes

- Cell surface labelling (green) of human neutrophils demonstrates prions (in magenta) are taken up by human neutrophils within minutes.

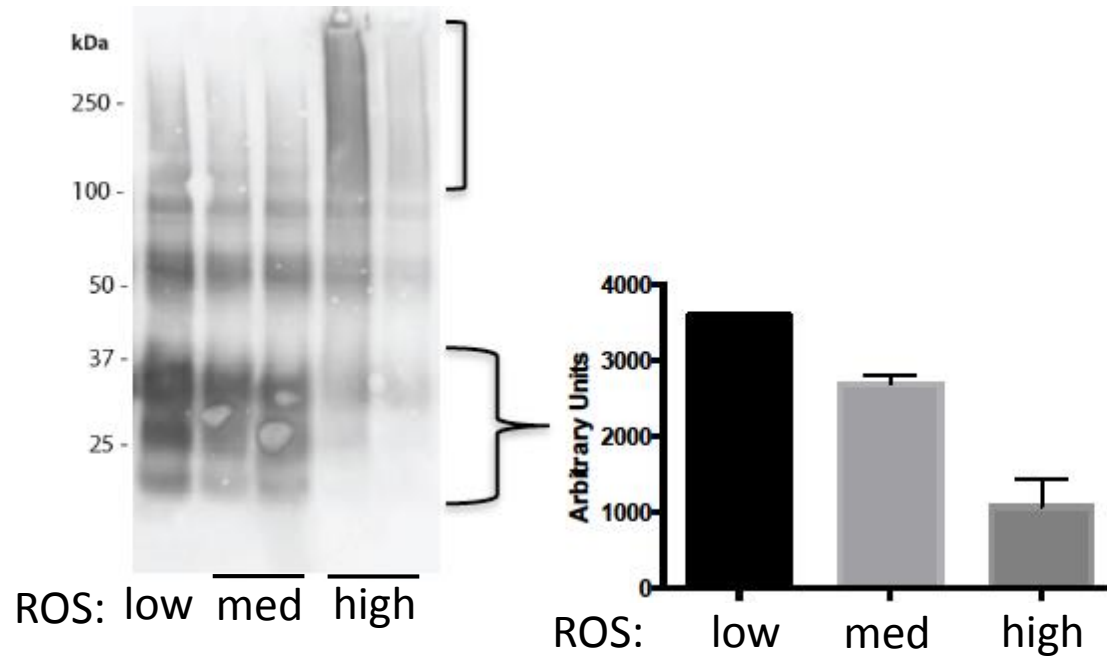


How do neutrophils with different ROS production levels respond to prion exposure?

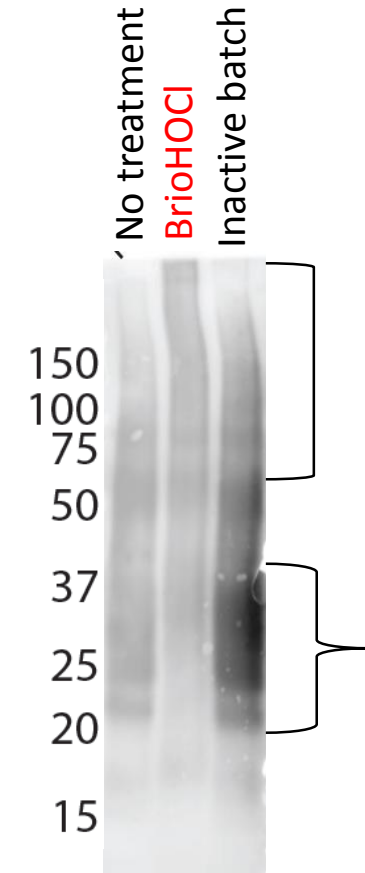


Prions after exposure to neutrophils that have different levels of ROS production

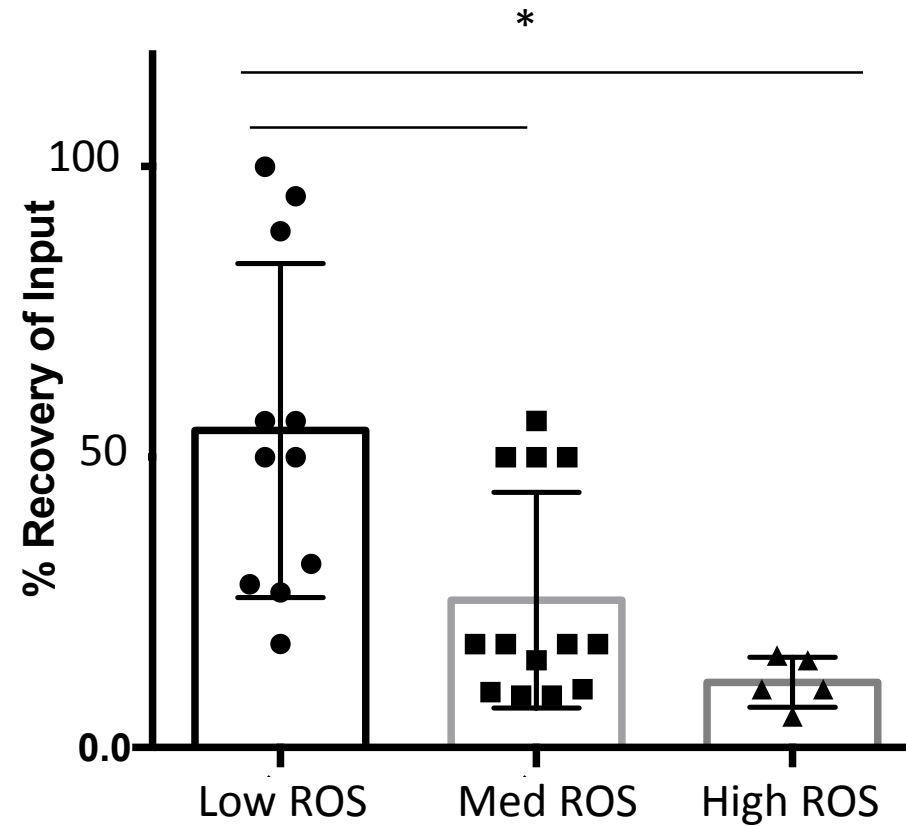
Prions with neutrophils



Prions after HOCl treatment in a test tube

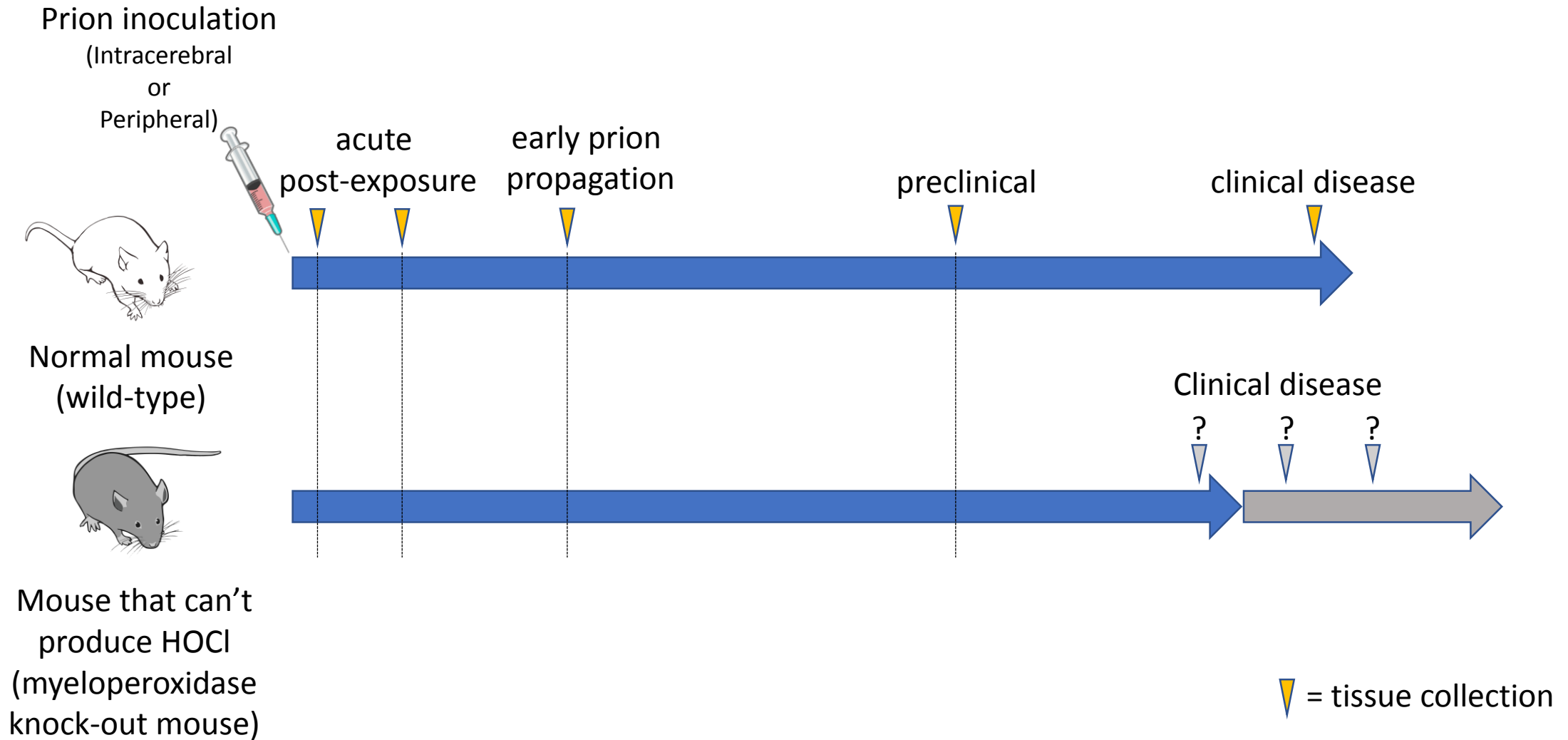


ROS-producing neutrophils reduce prion-seeding activity measured by RT-QuIC



Neutrophils with higher ROS production are better at reducing prions

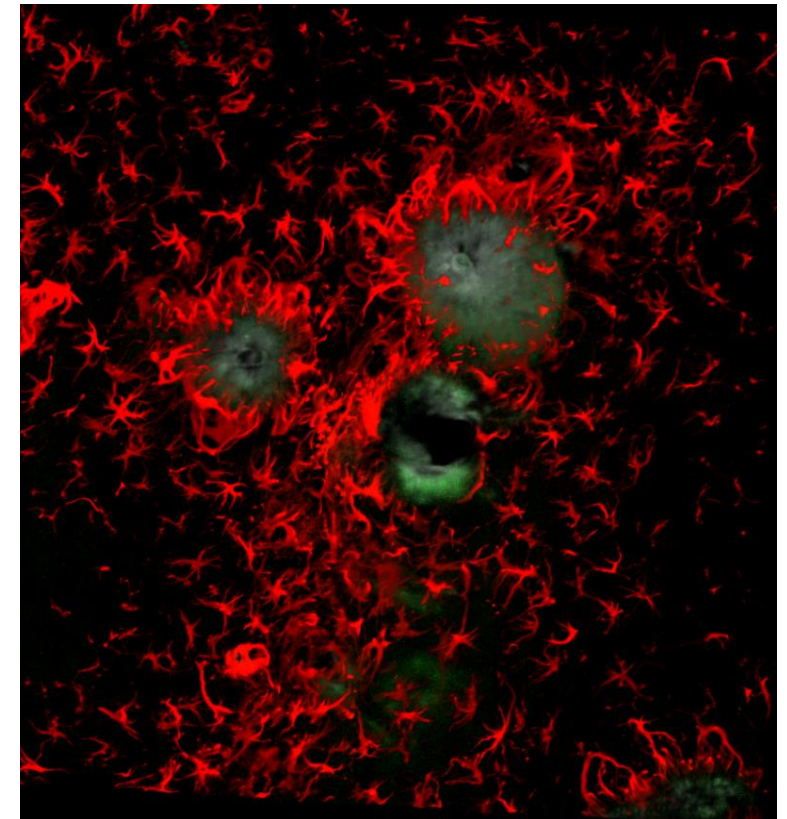
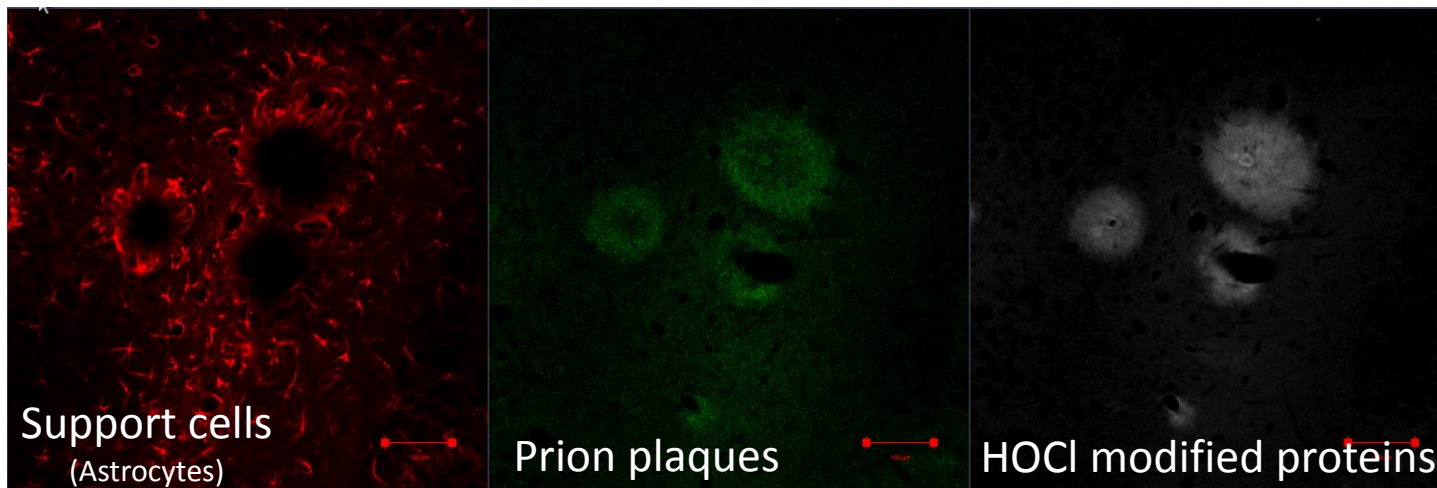
Does ROS production influence prion accumulation and clinical disease?



Evidence for HOCl-modified prions in brain tissue of clinical prion-infected mice

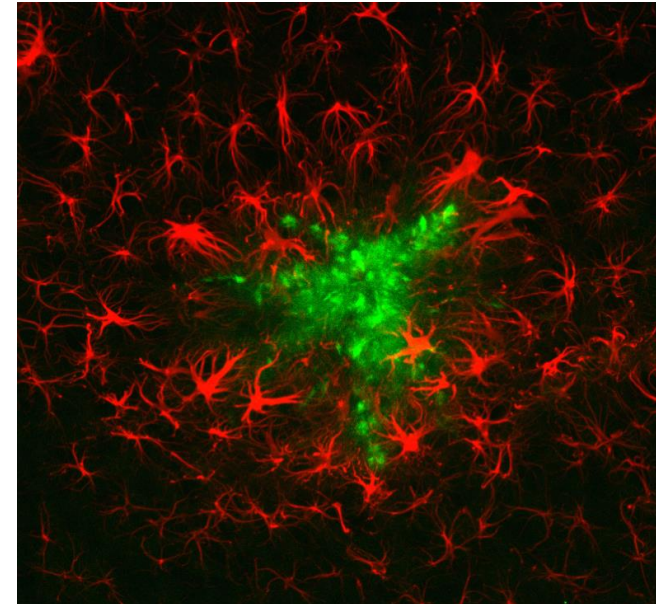
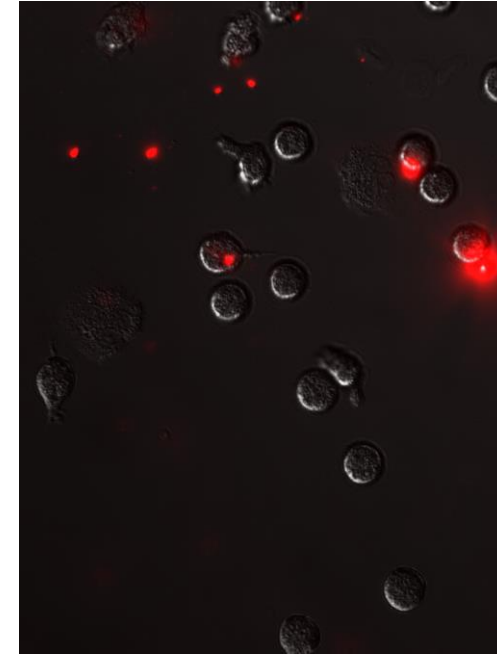
Preliminary evidence indicates prion plaques (green) in mice with clinical prion disease are found with a marker of HOCl-modified proteins (white). Cells in red are brain support cells that are activated in prion disease.

Does this indicate an attempt to block the propagation of accumulating prions?



Summary

- Prions induce ROS in human neutrophils
- Neutrophils that can make ROS reduce detectable prions and prion-seeding activity
- Evidence for the occurrence of HOCl modified prions in the brains of prion-diseased mice.
- Ongoing experiments suggest lack of HOCl production may result in higher prion levels early after infection, but it does not influence early prion propagation. Experiments are in progress to determine the effect of HOCl later in the prion disease course.



Ongoing experiments

- Do animals with different ROS levels have different survival times after prion infection?
- Cells in different parts of the body have different abilities to produce ROS. Do different routes of prion infection (directly into the brain, exposure outside of the nervous system) change the effects of ROS on prions?
- I am using the RT-QuIC to determine where prions go after different routes of infection. Does this change when the host has different ROS production levels?
- Not all neutrophils respond equally to pathogens. Do different types of human neutrophils respond equally to prion infection?
- Can we find evidence of HOCl (or other ROS) dependent modifications on prions in human samples?

Research summary

Hypochlorous acid (HOCl) is a reactive oxygen species (ROS) produced naturally by the immune system as a defense response to invading pathogens. Recently, we have shown that hypochlorous acid solutions are potent inactivators of prions and I have evidence that human immune cells can inactivate prions through production of HOCl and related compounds. The objectives of my research include investigating how HOCl and ROS species produced in a host can influence prion inactivation and removal following prion infection.