Engineered Biosensors for Improved Prion Detection

Joel Watts, PhD

Associate Professor

Canada Research Chair in Protein Misfolding Disorders

Tanz Centre for Research in Neurodegenerative Diseases

Department of Biochemistry, University of Toronto

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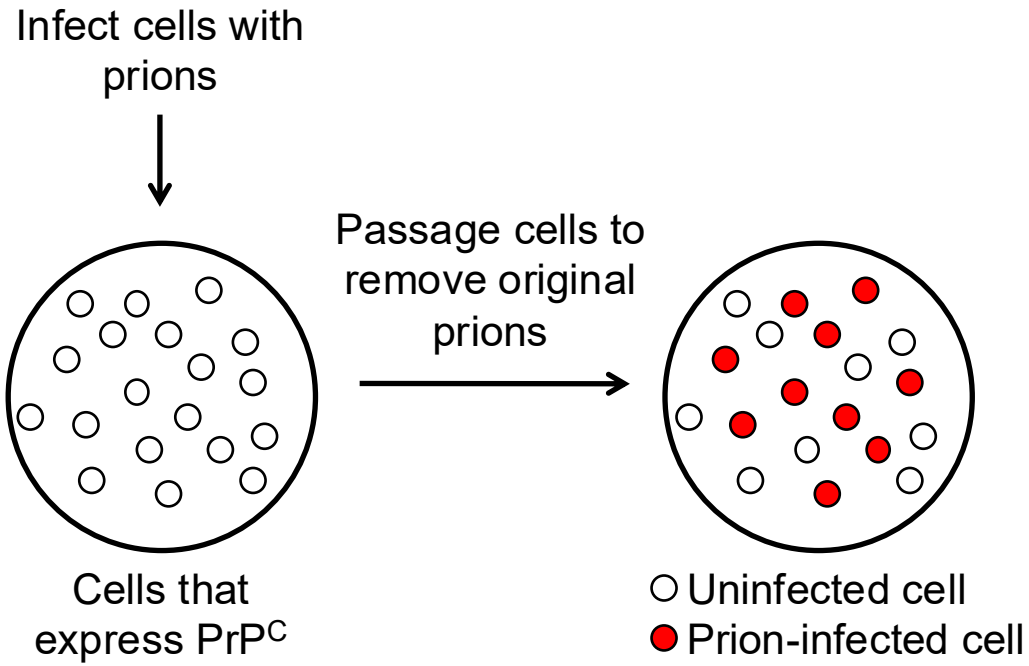
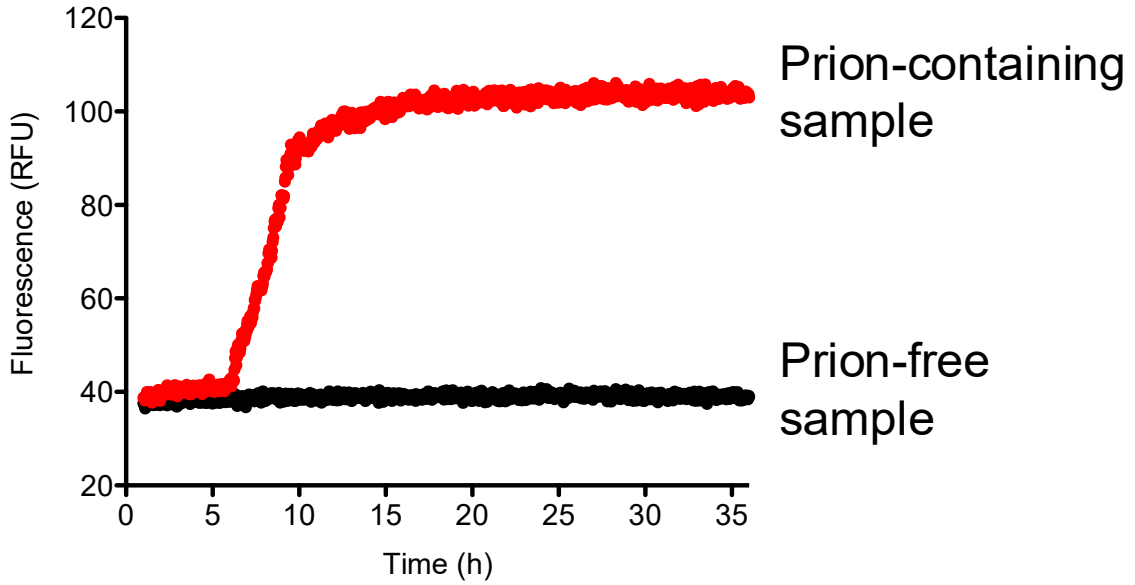
The Problem: Some Types of Prions are Difficult to Study

Replication of prions in cultured cells



Prion detection using RT-QuIC

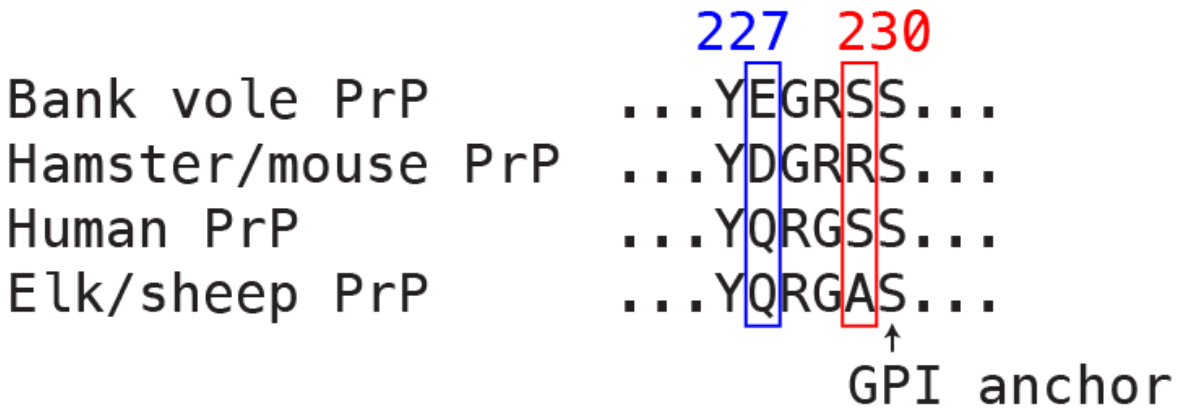


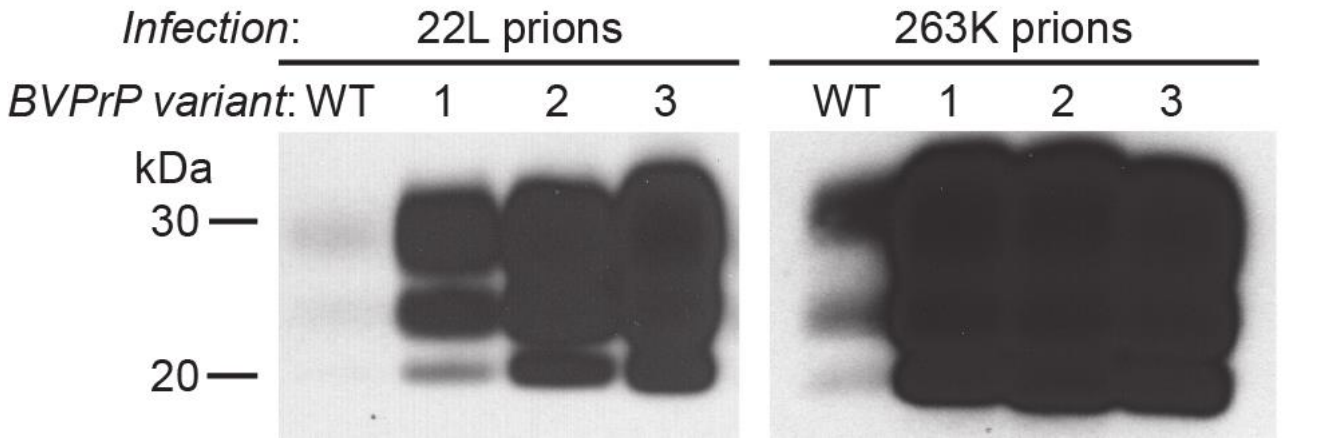
Several types of prions cannot be readily detected using RT-QuIC or replicated in cultured cells. A ‘universal’ prion substrate is desirable.

Our Approach: Exploit the Unique Properties of Bank Voles

	Mice	Bank voles
Mouse prions	✓	✓
Hamster prions	✗	✓
Human prions	✗	✓
Deer prions	✗	✓
Sheep prions	✗	✓
Cow prions	✗	✓

Bank vole prion protein functions as a permissive prion replication substrate in animals, cultured cells, and the RT-QuIC assay

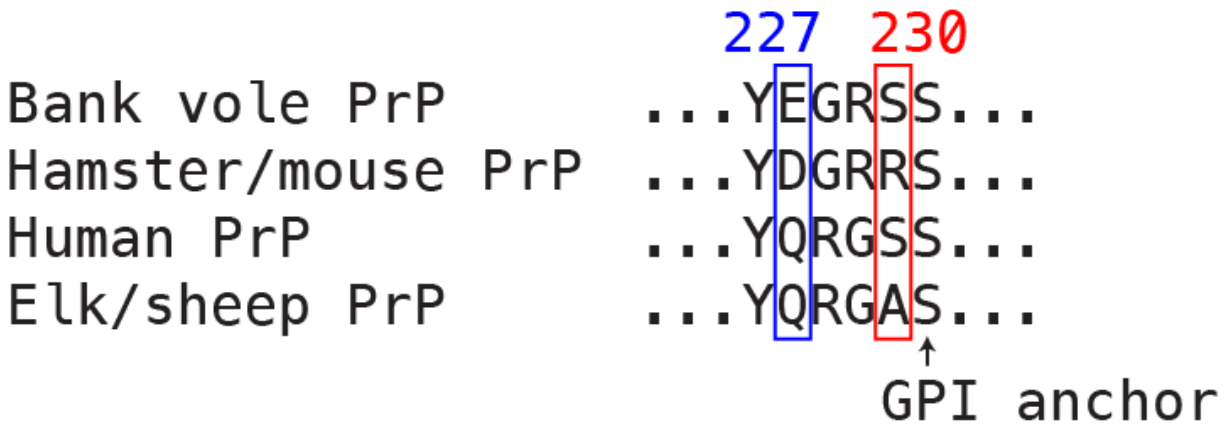




Bank vole PrP Variants: 1 = Hamster/mouse; 2 = human; 3 = elk/sheep

Arshad *et al.*, *PLoS Pathogens*, 2024

Our Hypothesis: Bank vole PrP can be Engineered to Become Even Better at Replicating Prions



	Position 227		Position 228/229		Position 230		
	E, D, or Q		GR or RG		S, R, or A		
Number of possibilities:	3	X	2	X	3	=	18

Our approach: Test all 18 bank vole PrP variants for their relative abilities to replicate/detect diverse prion types in cultured cells and RT-QuIC

Progress: Cell Lines and Recombinant Protein Generation

***Step 1*: Generate the 18 bank vole PrP variants**

- 18 variants for expression in cultured cells for prion infection experiments
  - *Status*: complete
- 18 variants for expression in bacteria to generate recombinant proteins for RT-QuIC experiments
  - *Status*: complete

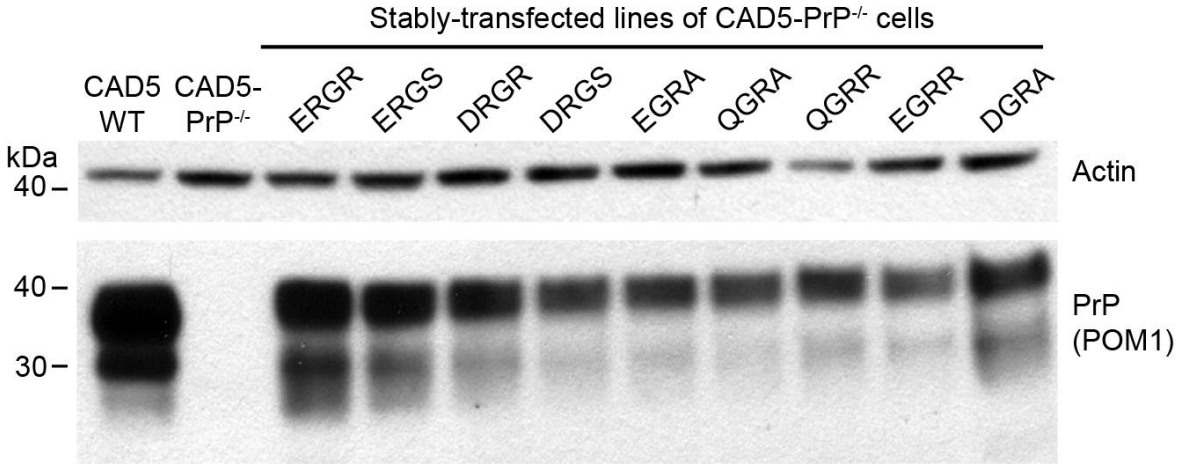
***Step 2*: Generate cell lines expressing bank vole PrP variants**

- CAD5-PrP$^{-/-}$ cells were stably transfected with each of the 18 variants
  - *Status*: complete

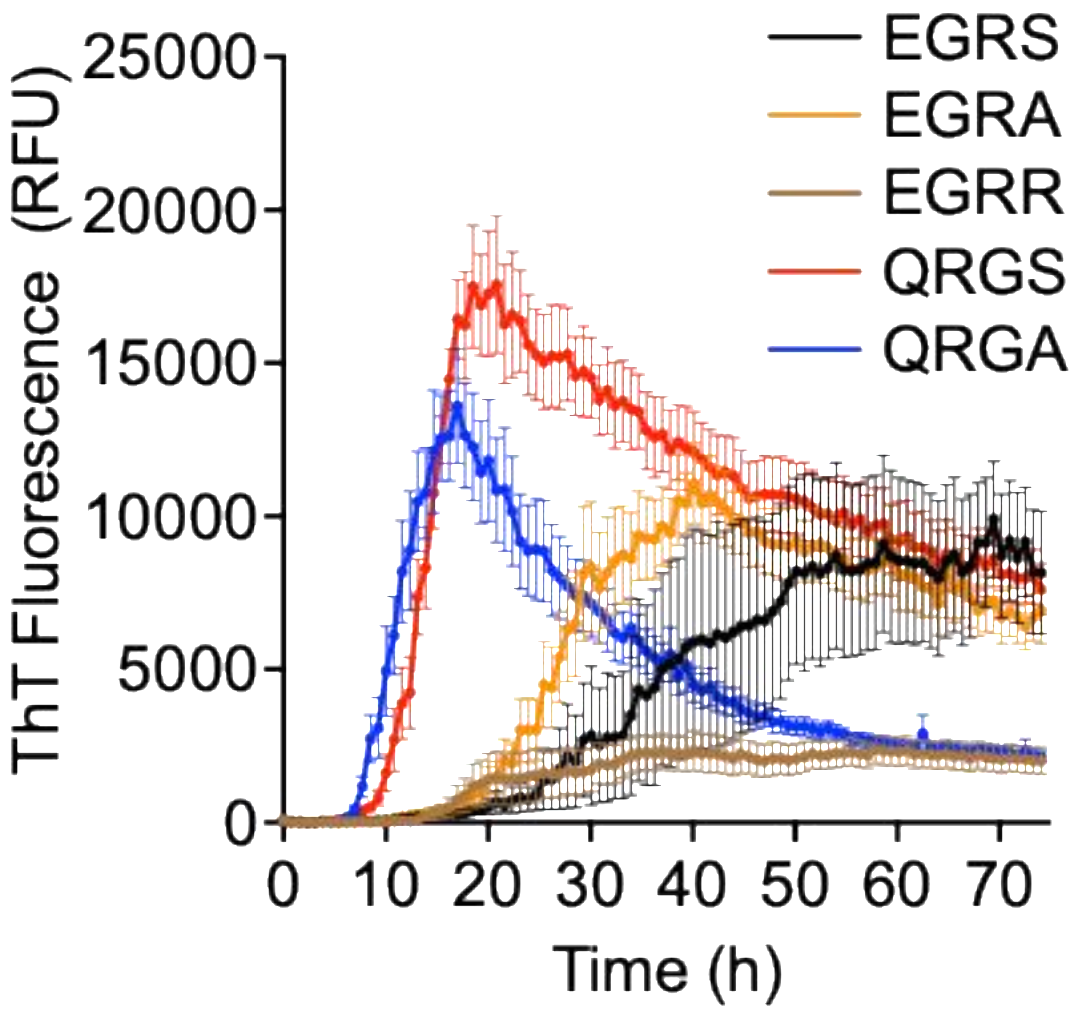
***Step 3*: Generate recombinant bank vole PrP variants**

- Purification of each of the 18 variants after expression in bacteria
  - *Status*: 9/18 variants have been produced so far

Tom Morningstar



Progress: Recombinant Bank Vole PrP Variants



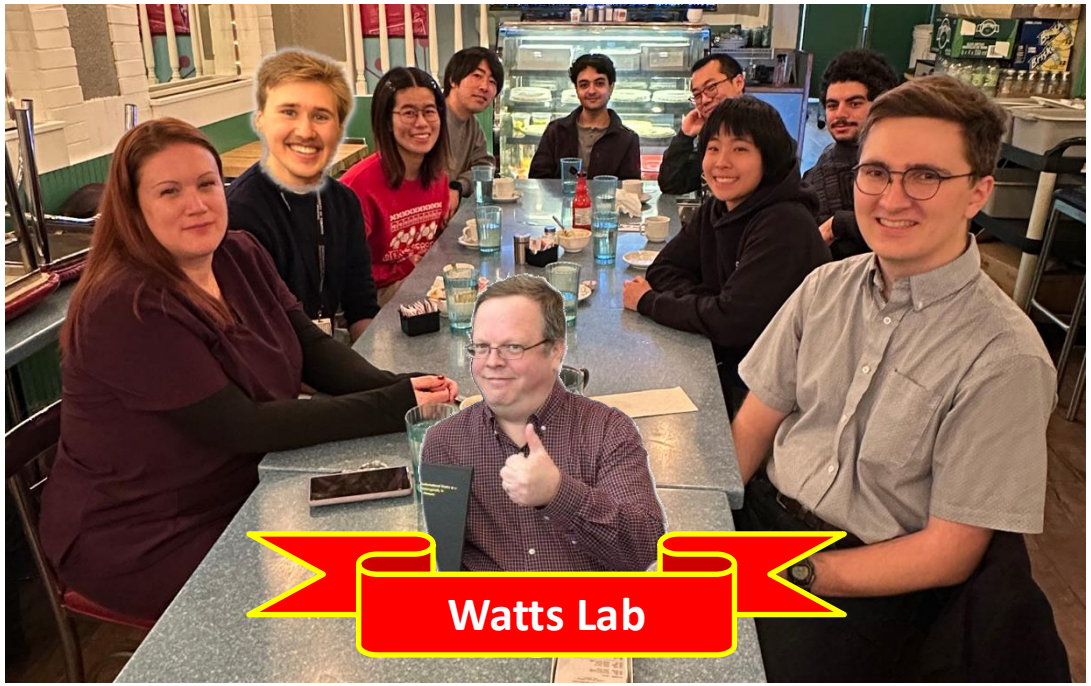
- Continuous shaking of recombinant PrP at 37 °C causes it to form aggregates that react with the dye Thioflavin T (ThT)
- In our experience, the faster a recombinant PrP aggregates, the better is it at replicating diverse types of prions in cells
- Tentative conclusions (based on 9/18 variants analyzed so far):
  - The identity of the amino acid at position 230 (S, R, or A) doesn't matter
  - RG is better than GR at position 228/229
  - Q or D is better than E at position 227
  - The best variants we've found (QRGS and QRGA) come from natural PrP sequences

Implications and Next Steps

- A natural segment within the prion protein from the bank vole helps to prevent the protein from turning into a prion
- Swapping this segment with the corresponding segment from mouse, human, or elk prion protein allows the bank vole prion protein to more readily become a prion
- We are currently optimizing this segment within the bank vole prion protein to generate cells with an enhanced ability to replicate prions as well as an RT-QuIC test with improved diagnostic sensitivity
- Enhanced variants will ideally exhibit the following properties:
  - Enable a greater diversity of prions to be replicated/detected (*i.e.*, act as a 'universal prion substrate')
  - Enable lower amounts of prions in a sample to be detected

The SuperVole

Acknowledgements



Watts Lab (Team Prion)

Erica Stuart
Tom Morningstar
Genki Amano
Surabhi Mehra
Leo Li
Hamza Arshad
Matthew Bourkas
Zeel Patel
Zaid Al-Azzawi

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