Project Title: Microenvironment mapping of the PrP^{Sc} Interactome

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Project Objective:

The main objective of this work is to apply a novel proximity labeling technique (µMap) to the discovery of proteins and other cellular factors that interact with prions. The elucidation of the PrP^{Sc} interactome will revolutionize our understanding of prion infection and propagation, and identify new therapeutic targets for the treatment of prion disease.

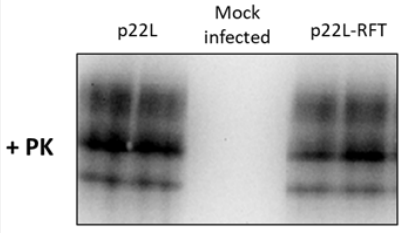
Summary of accomplishments to date and key findings:

1- Preliminary experiments targeting PrP^{C} by µMap have identified PrP^{C} interacting proteins determined using other methods, demonstrating the utility of µMap for use with our cellular systems.

2- These experiments require large amounts of PrP^{Sc} starting material. To achieve this, we have successfully "scaled up" established purification methods.

3- We have performed experiments to determine that RFT catalyst-labeled PrP^{Sc} (p22L-RFT) remains infectious after the labeling procedure, as revealed by positive signal on western blots following proteinase K (PK) digestion (Figure 1).

Figure 1: Catalyst labeled 22L prions remain infectious



Cells were inoculated with purified 22L prions (p22L), mock purified material (mock: negative control), or catalyst labeled purified 22L prions (p22L-RFT). Cells were passaged three times before lysis, PK digestion, and western blot analysis.

4- Previous iterations of the µMap method have used antibodies to deliver the catalyst to the protein of interest to induce the biotinylation of interacting proteins. We have determined that p22L-RFT is able to catalyze the biotinylation of cellular proteins at a level comparable to that of antibody-based labeling methods (Figure 2).

5- We have established a collaboration with Dr. Gerold Schmitt-Ulms at the University of Toronto for the analysis of µMap samples by mass spectrometry. Dr. Schmitt-Ulms is an expert in the field of mass spectrometry with a long-standing interest in PrP interactomics. His lab has performed experiments to refine the methods used for the recovery of

biotinylated proteins after purification with magnetic beads.

Next steps:

Our important preliminary experimentation is now complete and we are beginning µMap experiments of p22L-RFT using N2a and CAD5 cells. Following this, our investigations will expand to include the use of 1) RML and ME7 prions and 2) cultured neurons and brain slices.

We will then examine the role of identified PrP^{Sc} interacting proteins in prion infection/propagation using genetic manipulations to change their expression levels. These experiments will use well-established methods and cell systems.

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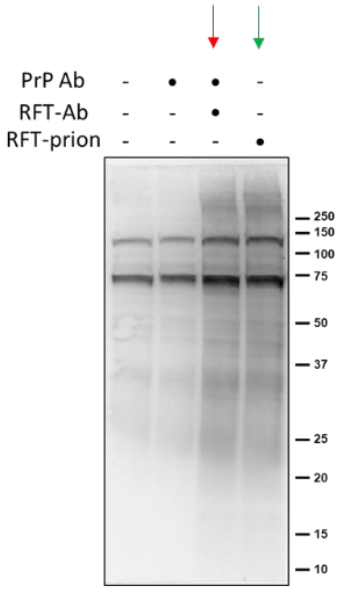
Figure 2: p22L-RFT efficiently labels cellular proteins



Cells were treated with the indicated µMap components and exposed to blue light to induce labeling. Western blots of cellular lysates using an antibody recognizing the biotin label show comparable levels of labeling between the two methods. Antibody based method (red arrow); p22L-RFT based method (green arrow).