The regulatory role of PrP^c at glutamatergic synapses

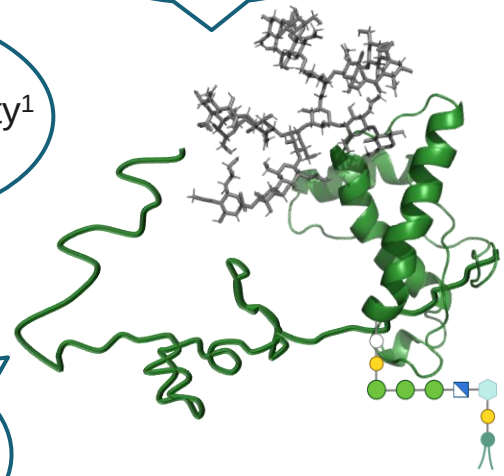
Mahsa Pourhamzeh, Ph.D.

Sigurdson Laboratory
Department of Pathology, School of Medicine
University of California, San Diego

Mechanisms of Neurodegeneration in Human Prion Diseases
and Their Intersection with AD/ADRD

November 12-14, 2024

Functions attributed to *PrP*^{C}



Memory formation[2]

Neuronal excitability[1]

Myelin homeostasis[5]

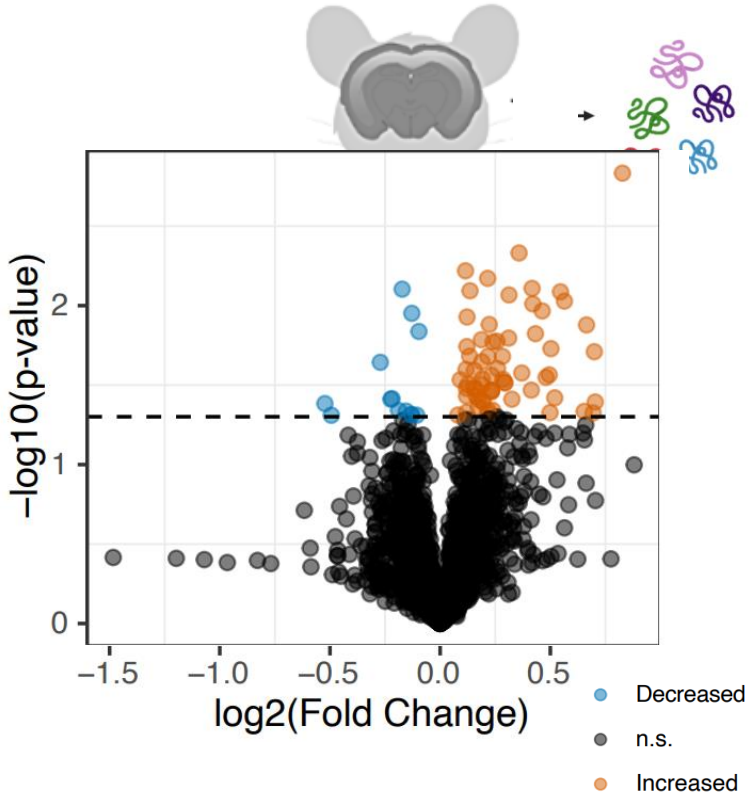
Synaptic function[2,3,4]

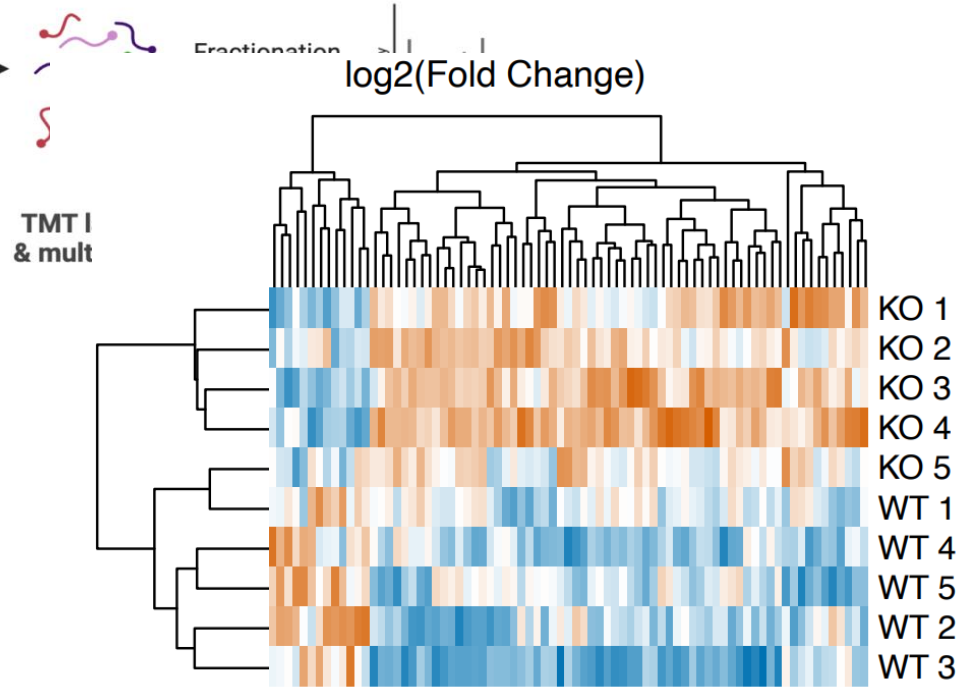
Mitochondrial homeostasis[6]

How does PrP^{C} function in intercellular signaling?

1- Khosravani et al., 2008, Cell Biol.
2- Matamoros-Angles et al., 2022, BMC Biol.
3- Carulla et al., 2011, Mol Biol Cell.
4- Ratte et al., 2011, Neuroscience.
5- Kuffer et al., 2016, Nature.
6- Faris et al., 2017, Sci. Rep.

Phosphoproteomic analysis of *Prnp*$^{-/-}$ (ZH3) and *Prnp*^{WT} cortex





2107 phosphopeptides detected

78 significantly different

- 65 upregulated phosphopeptides
- 12 downregulated phosphopeptides

83% of proteins were increased

Prnp$^{-/-}$ mice showed enhanced phosphorylation of

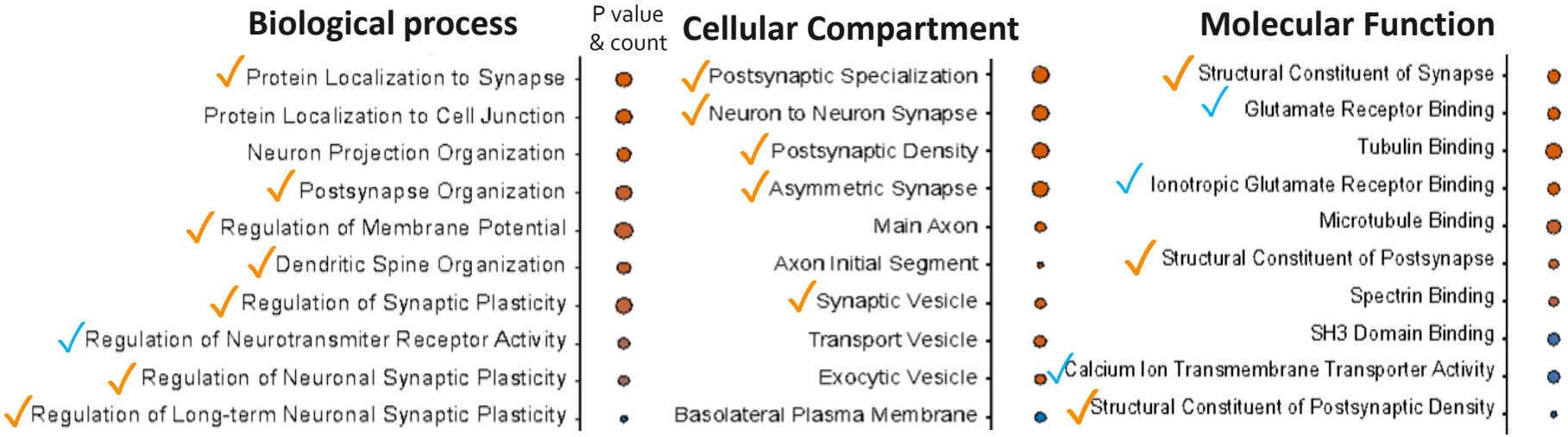
- ✓ GluN2B (S929;S930)
- ✓ Glun2A (S1198,S1201)
- ✓ CaMKII-B

John R. Yates III, Daniel McClatchy, Joshua Mayfield

Gene ontology enrichment analysis of identified phosphoproteins

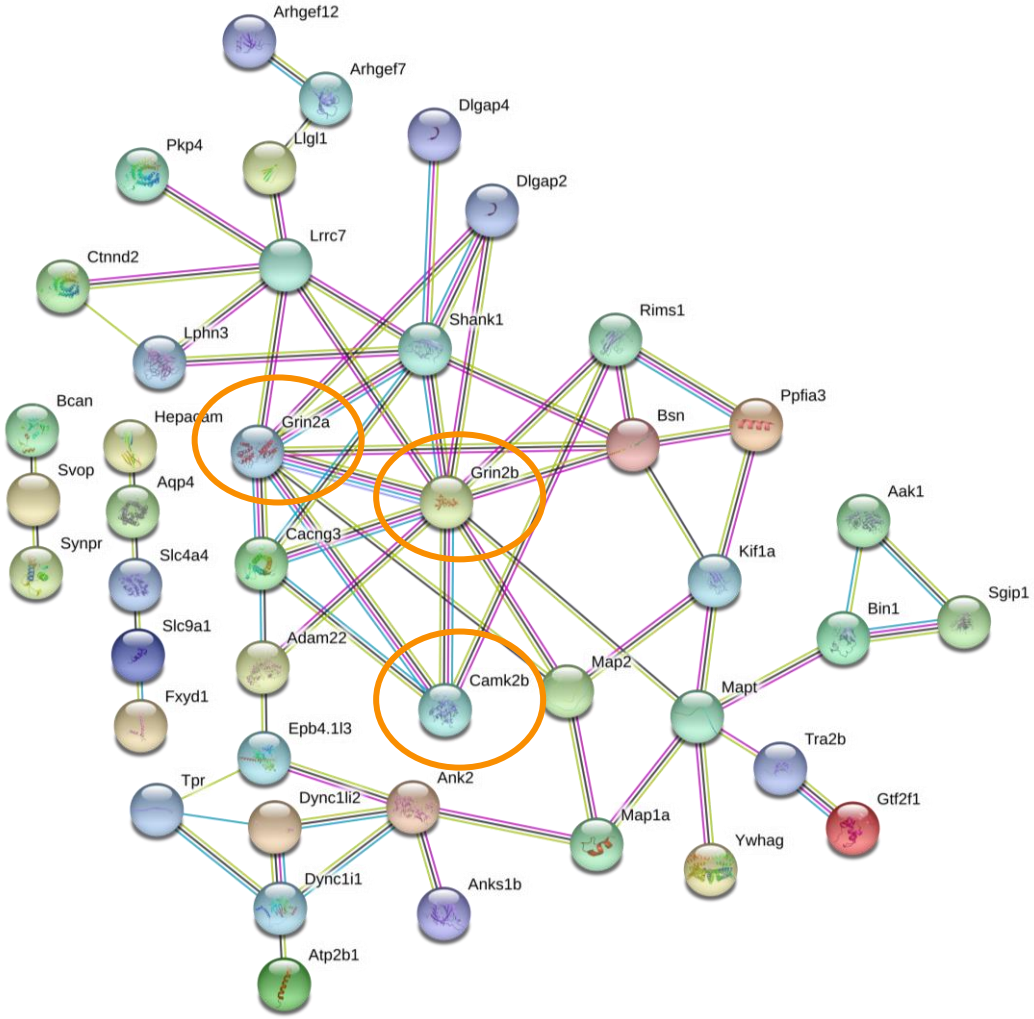
✓ **Synapse**

✓ **Glutamate receptor specific**



***Prnp$^{-/-}$ vs. Prnp^{WT}* cortex**

Interaction network map of identified phosphoproteins

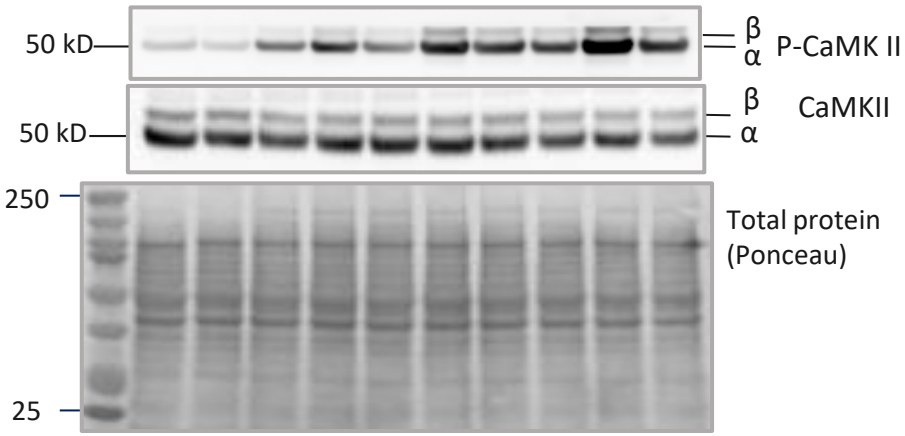


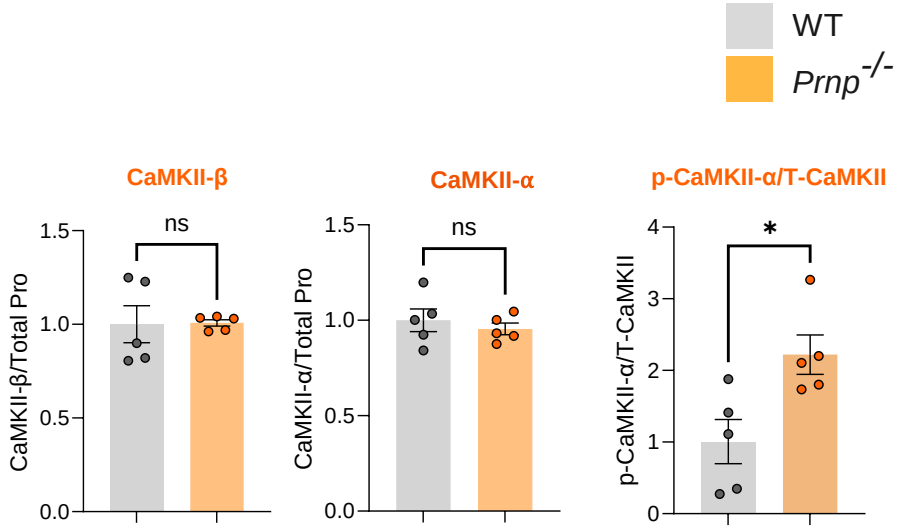
Core molecules within the network include:

- ✓ GluN2B (S929;S930)
- ✓ Glun2A S1198,S1201)
- ✓ CaMKII-B

The network displays only significant altered phosphopeptides, with disconnected nodes hidden for clarity.
https://string-db.org/

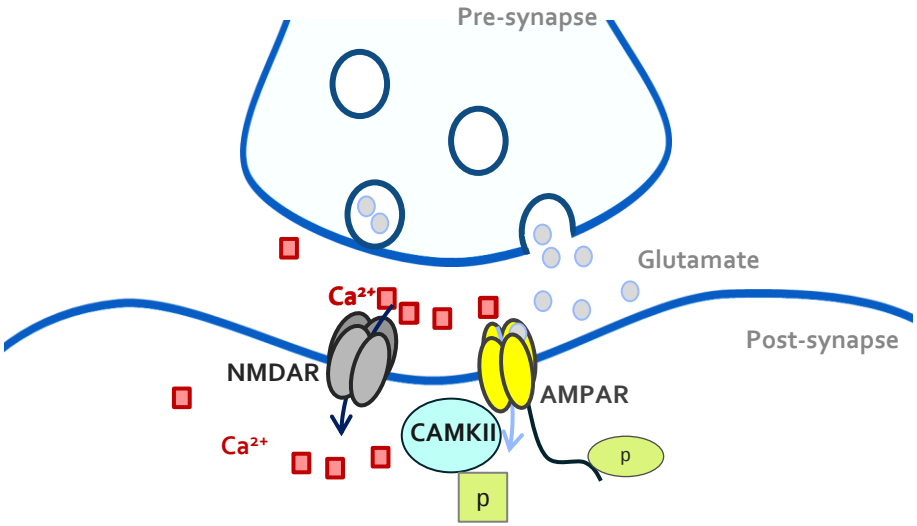
Prnp$^{-/-}$ mice showed increased CaMKII-α phosphorylation

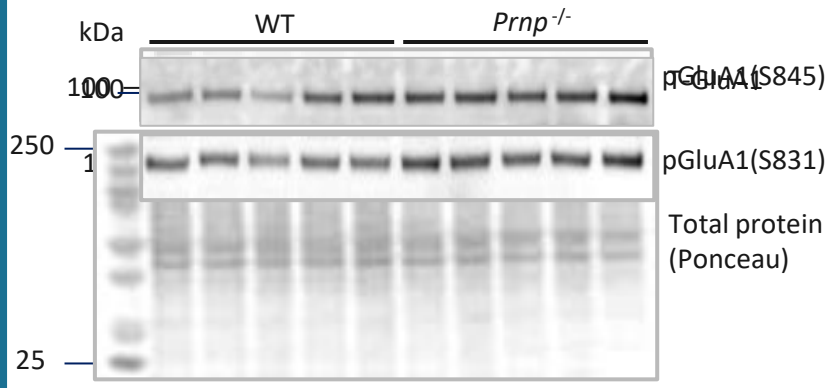


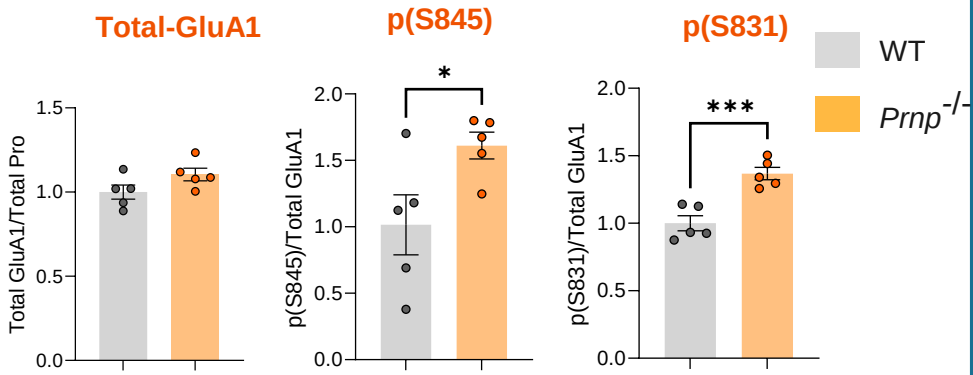


Prnp$^{-/-}$ mice showed increased phosphorylation of AMPA receptors

GluN2A and GluN2B levels were unaffected

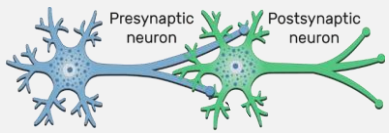


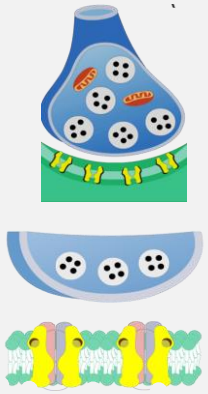


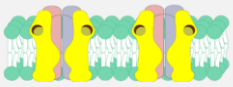


Is PrP^{C} localized to the pre- or post-synapse?

PrP^c is localized to the post-synaptic density (PSD)



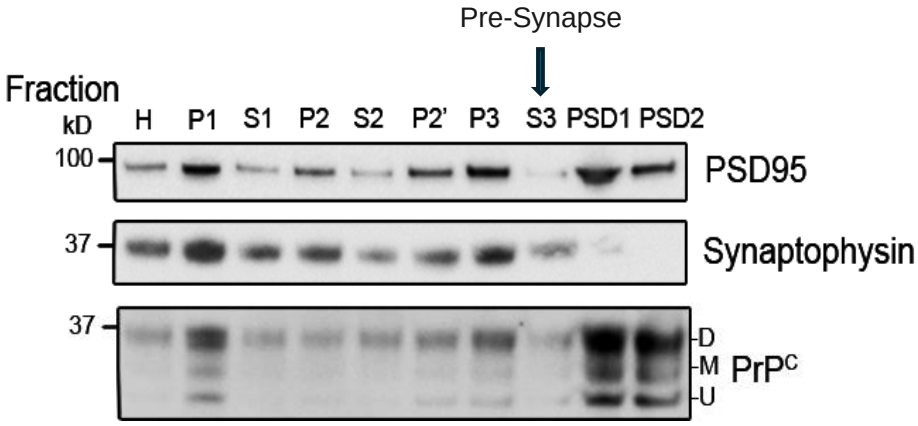


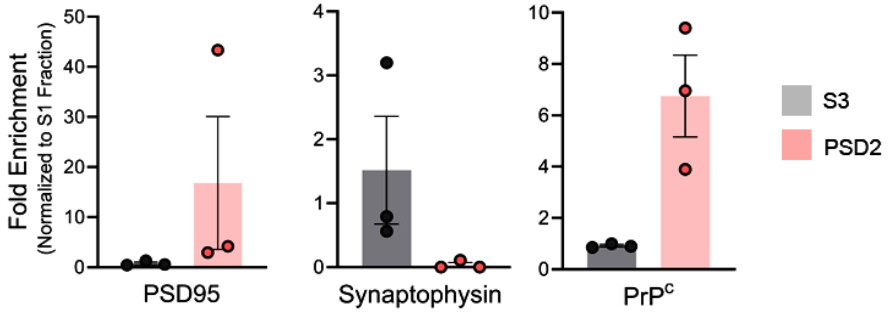


Postsynaptic density

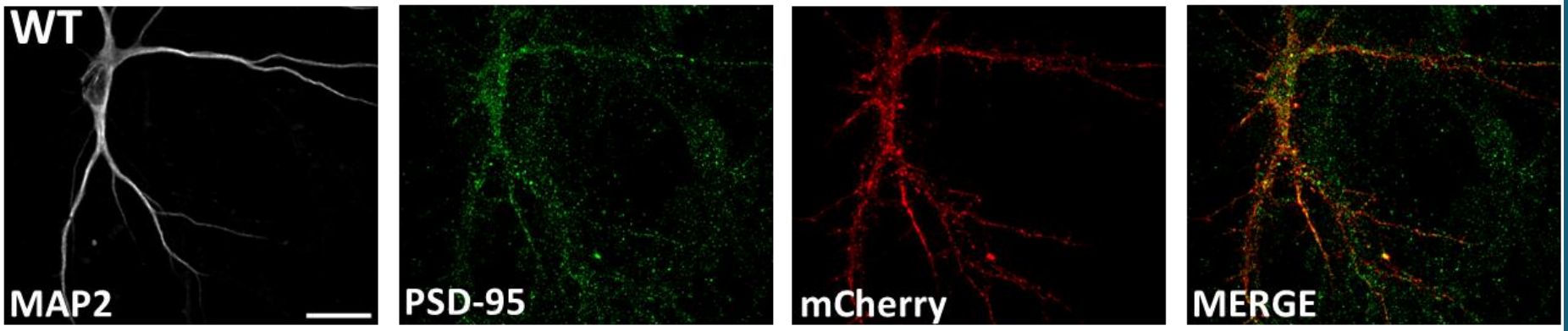
Brain Homogenates

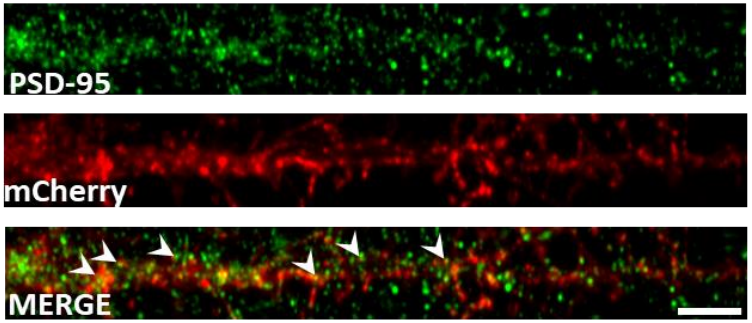
1000 x g
P 1
S 1
10000 x g
P 2
(Crude Synaptosomes)
S 2
P2'
25000 x g
P 3
(Synaptosomal Membrane)
S 3
TX-100
32000 x g
PSD-1T
(Postsynaptic Densities)
TX-100
200000 x g
PSD-2T





Synaptic PrP^C co-localizes with PSD-95



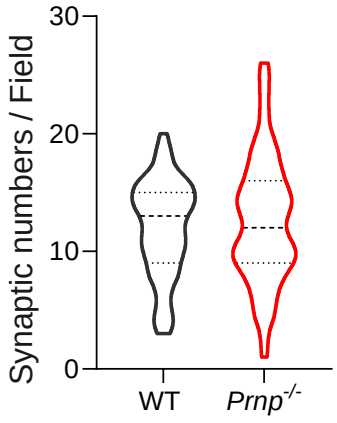


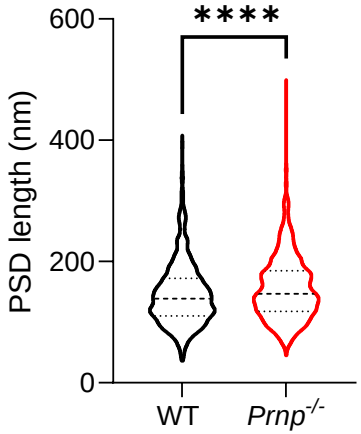
PrP-WT-mCherry, Cortical primary neurons

How does synaptic PrP^C affect synapse structure?

Gentry Patrick & Lara Dozier

Post-synaptic density (PSD) is longer in *Prnp*$^{-/-}$ mice





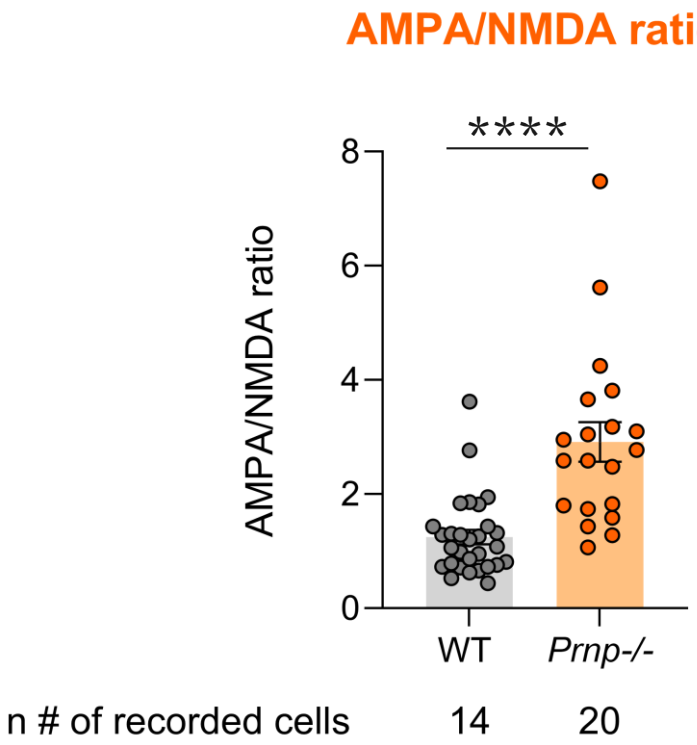
N = 3 mice / group

Synapses counted:
n = 1034 (WT)
n = 1083 (*Prnp*$^{-/-}$)

WT = 146 ± 1.7 nm
Prnp$^{-/-}$ = 156 ± 1.6 nm

How does synaptic PrP^{C} affect neuronal function?

Prnp$^{-/-}$ neurons showed functional differences in neuronal activity, with increased AMPA/NMDA-evoked responses



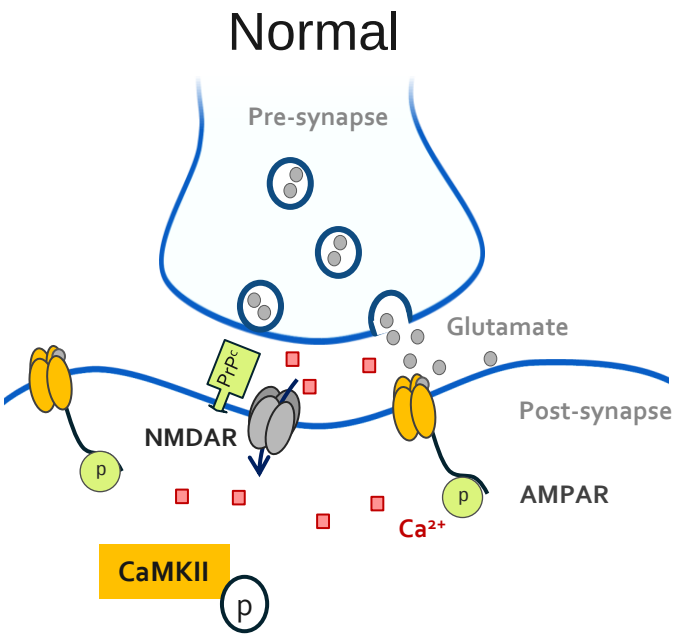
Suggests that magnitude of synaptic AMPA or NMDA receptor function is markedly altered by PrP^{C} deficiency

Electrophysiology experiments on organotypic brain slices from *Prnp*$^{-/-}$ and *Prnp*^{WT} mice

Yixing Du & Kim Dore

Summary

Signaling model



PrP^{c} plays a role in intercellular signaling by regulating CaMKII pathway and modulating synaptic activity, which can affect synaptic plasticity and structural changes at glutamatergic synapses.

Acknowledgements

Sigurdson Lab

Christina Sigurdson
Alexander Rajic
Daniel Ojeda-Juárez
Joshua E. Mayfield
Amanda Wilpitz
Emily Richards
Paige Sumowski
Samantha Flores
Kika Funk
Jin Wang
Katrin Soldau
Garrett Danque

UC San Diego

Kim Dore
Yixing Du
Gentry Patrick
Lara Dozier

The Scripps Research Institute

John R Yates
Daniel McClatchy

National Institutes of Health

National Institute of Neurological Disorders and Stroke