Development of Targeted Mass Spectrometry-Based Approaches for Quantitation of Proteins Enriched in the Postsynaptic Density (PSD)

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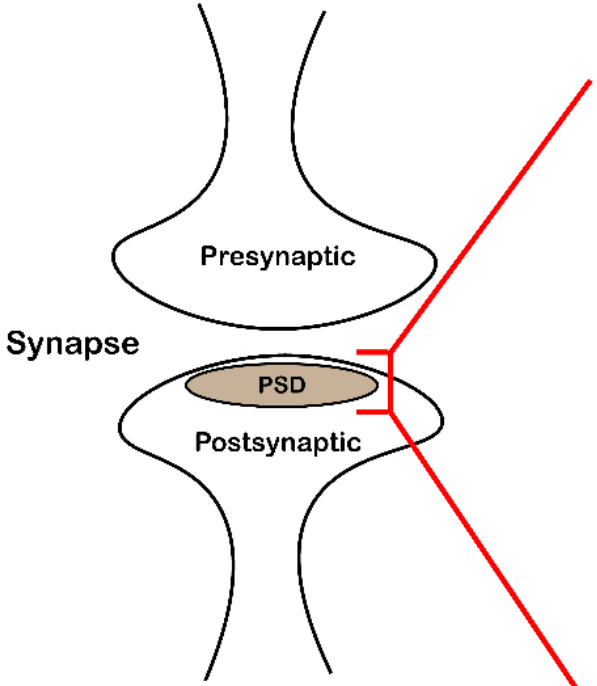
Postsynaptic density (PSD): Overview

Electron-dense region of excitatory glutamatergic synapses



MAGUK

- PSD-95 (DLG4)
- PSD-93 (DLG2)
- SAP-102 (DLG3)
- SAP-97 (DLG1)

GKAP

- Disks large-associated protein (DLGAP1,2,3,4)

Kinases/Signaling

- Casein kinase II
- CaMKII (alpha, beta, delta, gamma)
- Arc
- Erc2
- Rims1

Shank/Homer

- SH3 and multiple ankyrin repeat domains protein (SHANK1, 2, 3)
- Homer1, Homer2, Homer3

Receptors

- Glutamate receptor (Gria1, Gria2, Gria3)
- Glutamate receptor ionotropic (Grin1, Grin2a, Grin2b)

Translation

- 60S Rpl3
- 60S Rpl7a
- 60S Rpl10
- 60S Rpl18a
- 40S Rps20

MAGUK: Involved in **structural maintenance and signaling** through interactions with integral membrane proteins and receptors, protein complexes, and other structural proteins within the PSD

GKAP: Enable the **formation of protein complexes** with MAGUKs and proteins found in the pallial layer of the PSD

Shank: Implicated in **scaffolding and organization of signaling complexes** at glutamatergic synapses

Homer: Create a **scaffolding structure** that is involved in excitatory **signal transduction** as well as in **receptor plasticity**

PSD proteins have been linked to many neurological and behavioral disorders

PSD protein	Associated disorder	Supporting literature
CaMKII	Learning and memory formation, **Drug addiction**	*Elgersma et al. 2012, Lisman et al., 2002, Mayford et al., 1996, Anderson et al., 2008, Robinson et al., 2013, Loweth et al., 2010*
Shank1	Autism spectrum disorder (ASD), **Drug addiction**	*Sato et al.,2012, Sungur et al., 2014, Gong et al., 2015, Pal et al., 2013, Sungur et al., 2018*
Shank3	Autism spectrum disorder (ASD)	*Durand et al., 2007, Peça et al., 2011, Gauthier et al., 2009, Moessner et al., 2007*
PSD-95	Intellectual disorders, ASD, Schizophrenia, Williams' Syndrome, **Drug addiction**	*Toro et al., 2005, Feyder et al., 2010, Xing et al., 2016, Yao et al., 2004, Wang et al., 2014*
SynGAP1	Intellectual disorders, ASD, Epilepsy	*Hamdan et al., 2009, Hamdan et al., 2011, Berryer et al., 2013*
DLGAP1	Schizoprenia, ADHD, OCD	*Li et al., 2013, Fan et al., 2018, Soreq et al., 2017, Gazzellone et al., 2016*
Homer1	Schizophrenia, Depression, **Drug addiction**	*Szumlinski et al., 2005, Rietschel et al., 2010, Spellmann et al., 2011, Sartor et al., 2017, Brakeman et al., 1997, Zhang et al., 2007, Ghasemzadeh et al., 2006*
Grin2A	Depression, **Drug addiction**	*Taniguchi et al., 2009, Domart et al., 2012, Karpyak et al., 2011*
Gria2/3	**Drug addiction**, Depression	*Baptista et al., 2004, Bowers et al., 2004, Steinberg et al., 2006*
Gria1	**Drug addiction**, Alzheimer's	*Churchill et al., 1999, Fitzgerald et al., 1996, Almeida et al. 2005, Chen et al., 2010, Das et al., 2008*

Challenges associated with PSD proteomics

- The PSD is not enclosed in a bilayer, which makes it challenging to minimize contamination of the PSD fraction with other subcellular proteins.

- Synapses differ significantly from one another and can change their composition rapidly, making reproducibility and accuracy of the analysis important.

Previous studies have identified proteins using LC-MS/MS analysis:

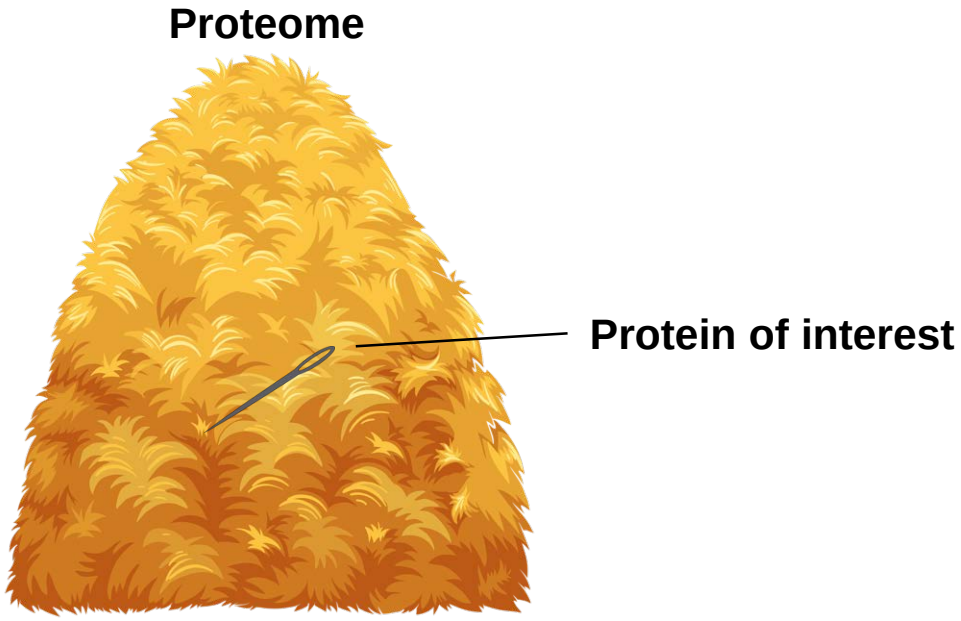
Discovery analysis

- *Bayés et al., 2012:* Identified over **1500 proteins** from **mouse and human cortical PSD fractions**
- *Li et al., 2017:* Identified **2876 PSD-associated proteins** from **mouse brain tissue** immunoprecipitation (IP) samples
- *Roy et al., 2018:* Identified **1213 proteins** in PSD fractions from **12 human neocortical brain regions**

Targeted analysis

- *Colangelo et al., 2015:* Used multiple reaction monitoring (MRM) coupled with stable-isotope peptide standards (SIS) to quantify 112 rat synaptic proteins

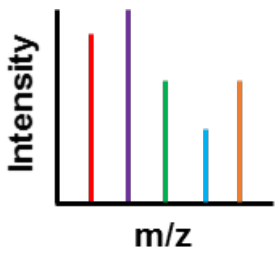
An accurate, reproducible assay is necessary for robust quantitation of PSD proteins

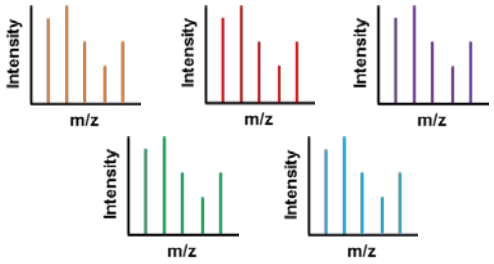


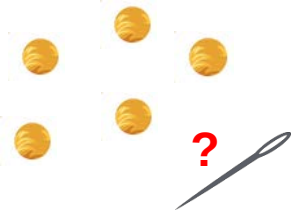
How can we identify and reproducibly quantify our protein(s) of interest using mass spectrometry analysis?

“Discovery”
Data-dependent acquisition (DDA)

Isolate and fragment most abundant ions

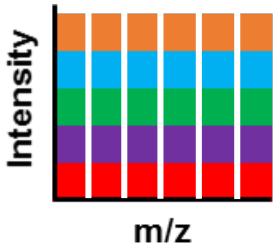


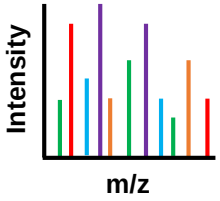




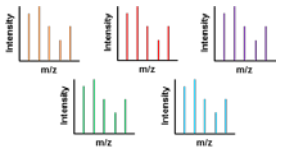
“Semi-targeted”
Data-independent acquisition (DIA)

Isolate and fragment in consecutive mass/charge (m/z) windows



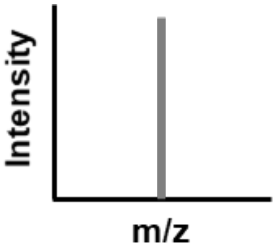


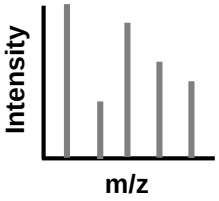
Spectral library

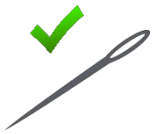


“Targeted”
Parallel reaction monitoring (PRM)

Isolate and fragment m/z of interest







Comparison of quantitative LC-MS/MS methods

	DDA	DIA	PRM
Advantages	• Simplified data analysis • No spectral library required	• m/z windows increase coverage of proteome • High sensitivity and reproducibility	• Peptide of interest isolated and fragmented • High sensitivity and reproducibility • Can be multiplexed
Disadvantages	• Low sensitivity and reproducibility	• Challenging data analysis • Limited by spectral library	• Lose information about the rest of the proteome • Requires more optimization than DDA and DIA methods

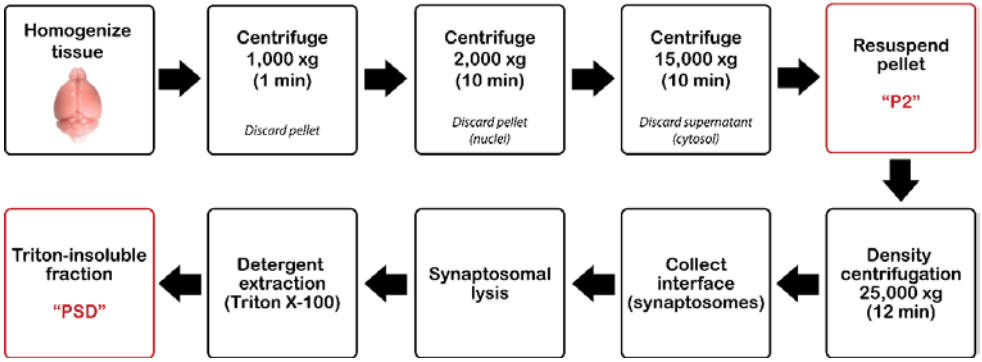
Sample sets used for PSD DIA analysis:

1) Pre-fractionation vs. PSD-enriched
   (Mouse cortical tissue, 3 biological replicates per group)

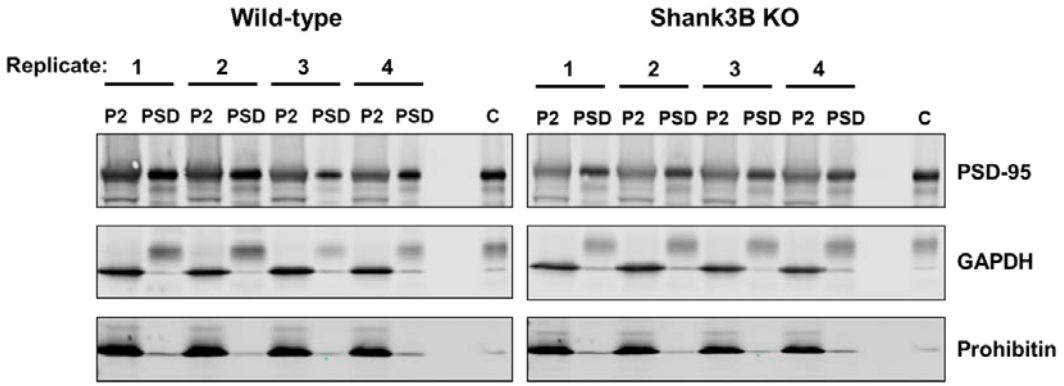
2) Wild-type (WT) vs. Shank3B knockout (KO)
   (Mouse cortical tissue, 4 biological replicates per group)

Experimental design for PSD DIA analysis

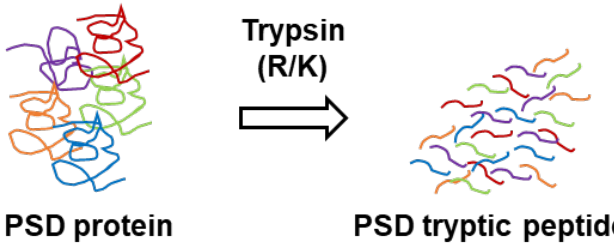
1) PSD enrichment from brain tissue



2) Immunoblot validation of PSD enrichment



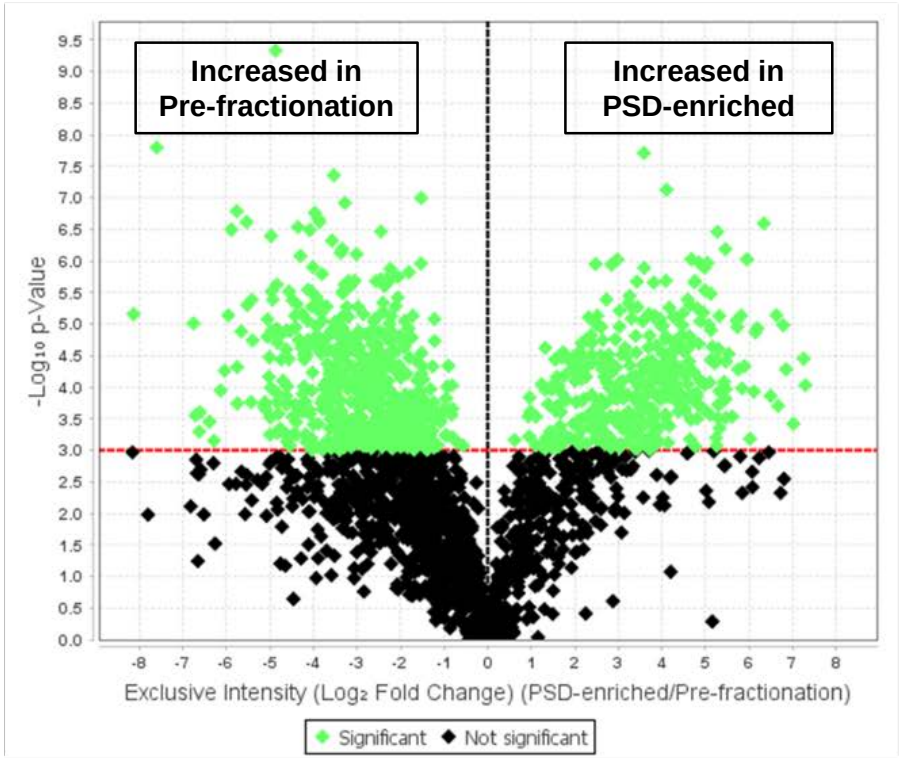
3) Tryptic digestion of PSD protein

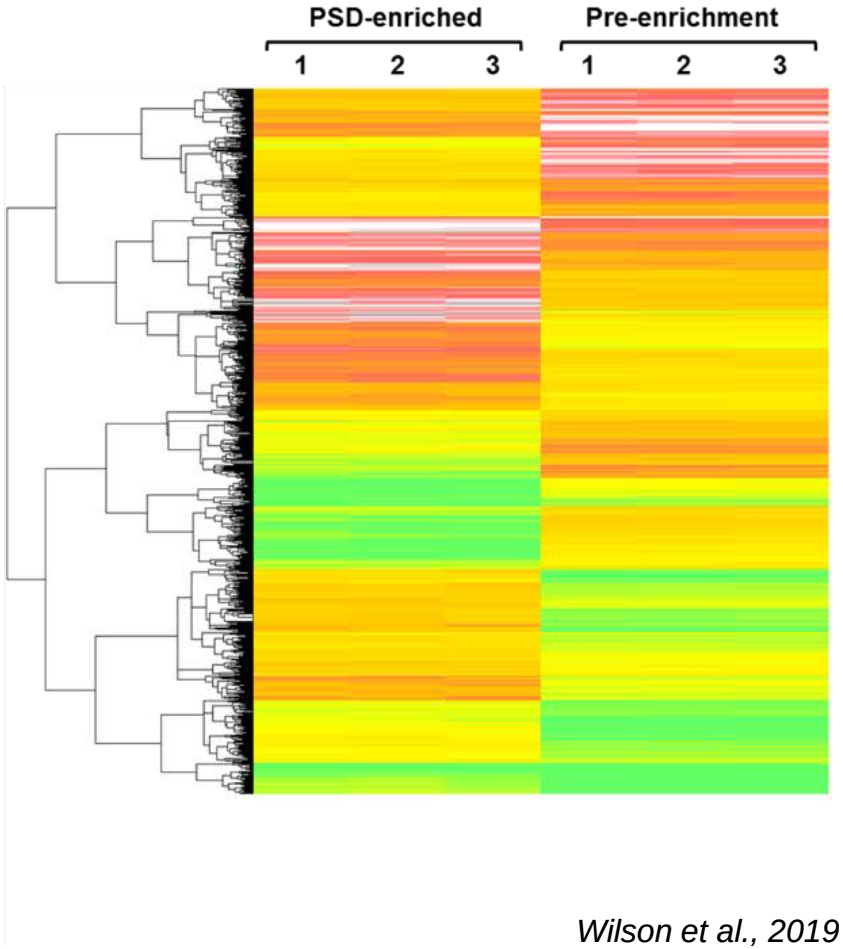


4) LC-MS/MS

DIA analysis results: Pre-fractionation vs PSD-enriched

1721 proteins were differentially expressed between the two groups





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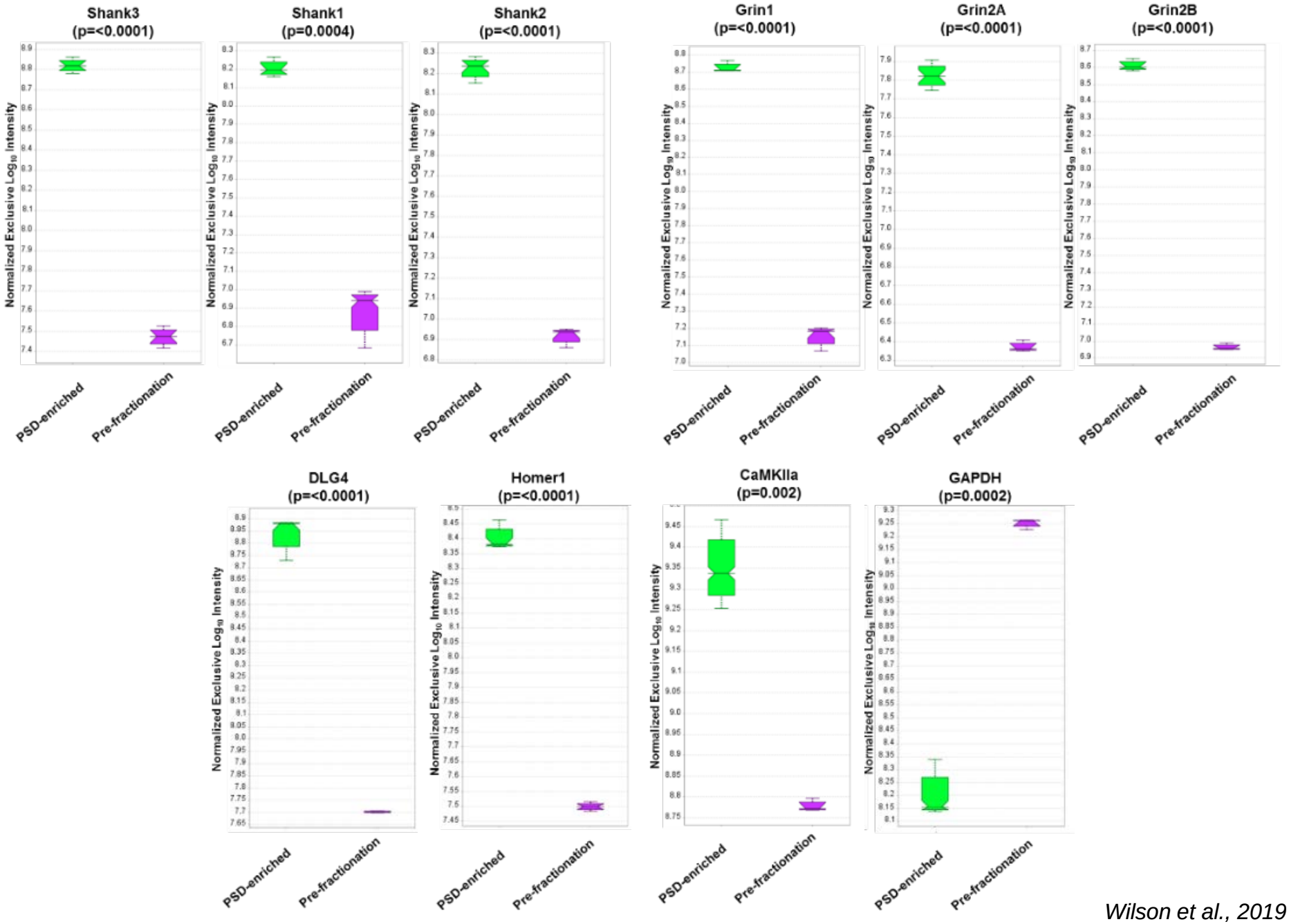
DIA analysis results: Pre-fractionation vs PSD-enriched



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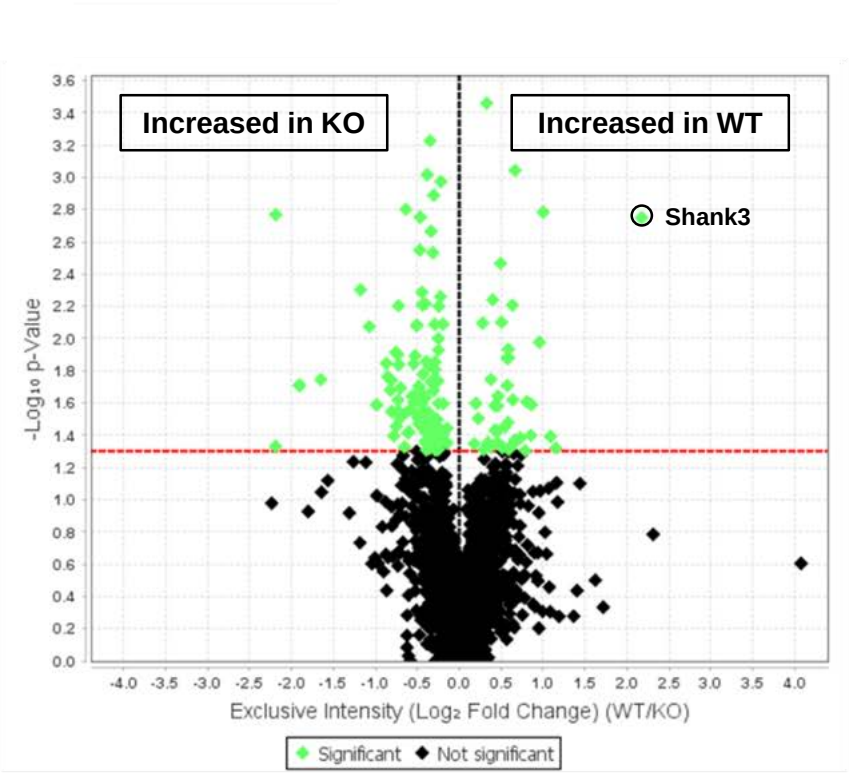
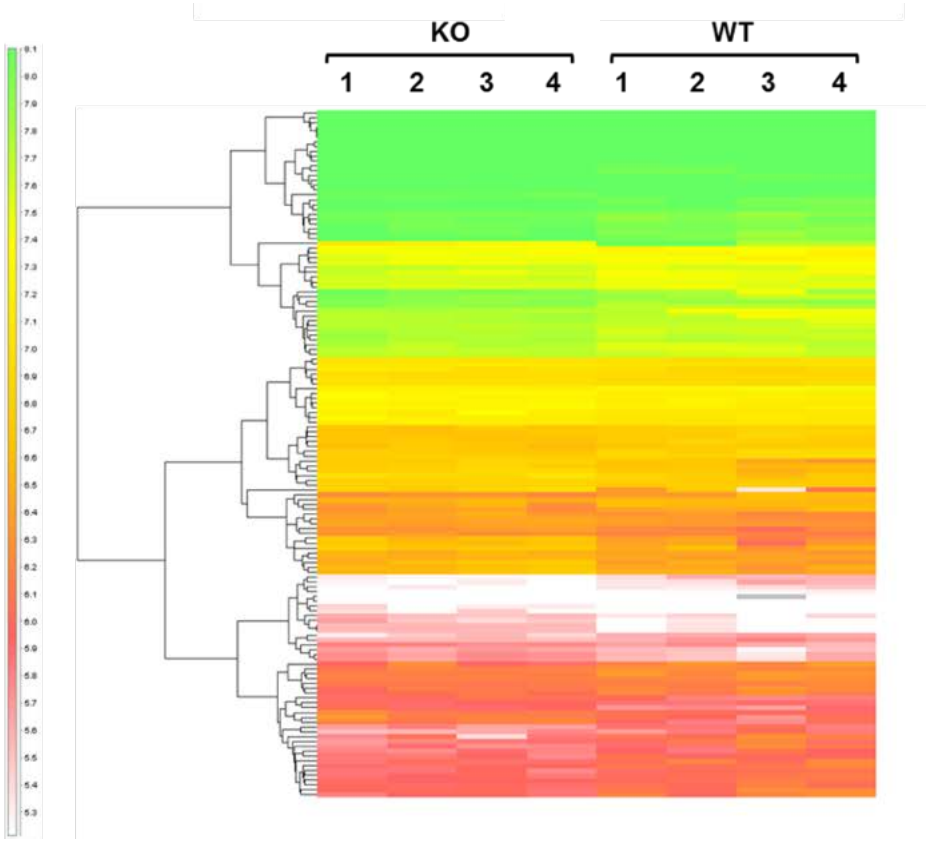
DIA analysis results: WT vs Shank3B KO

140 proteins were differentially expressed between the two groups





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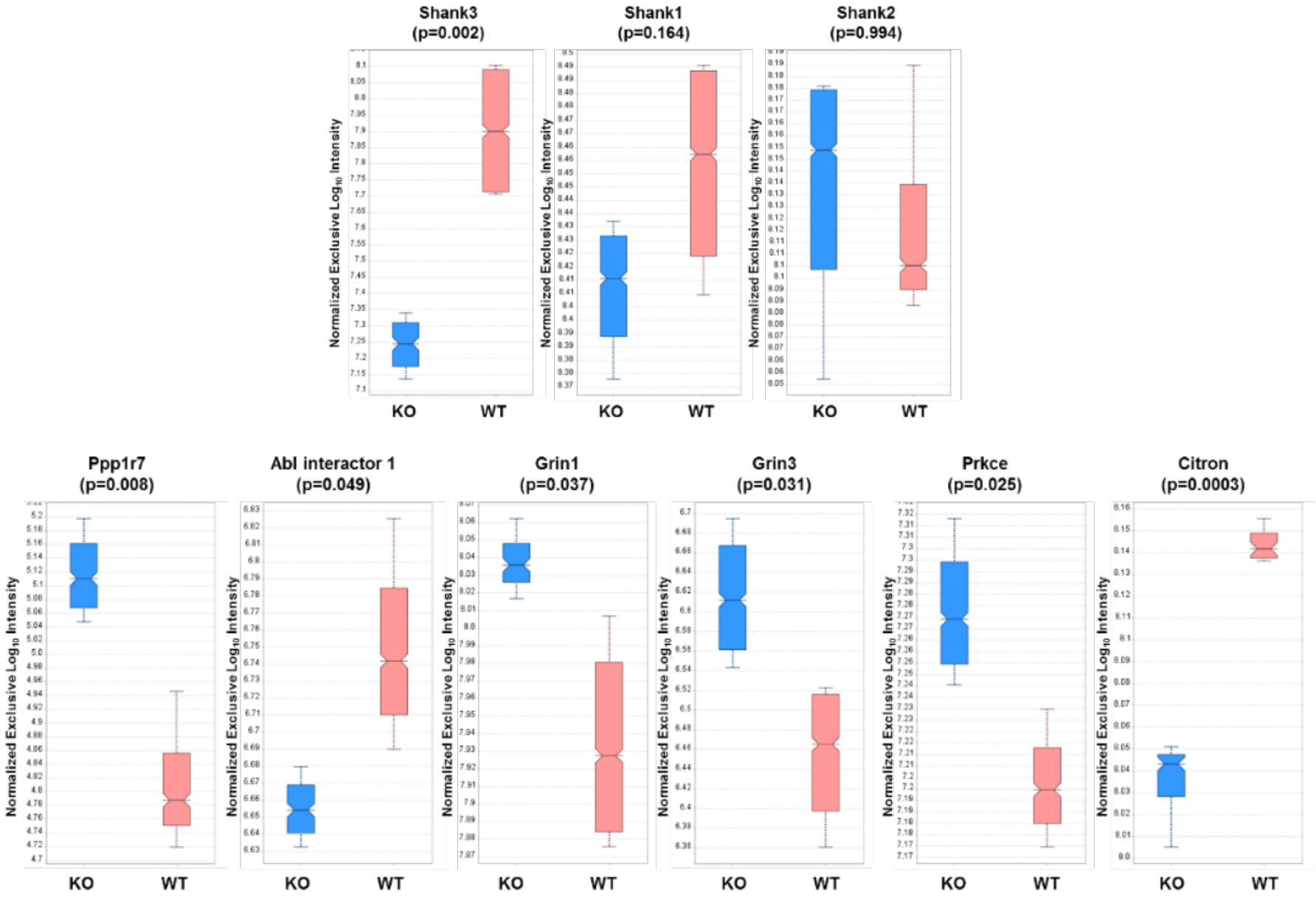
DIA analysis results: WT vs Shank3B KO



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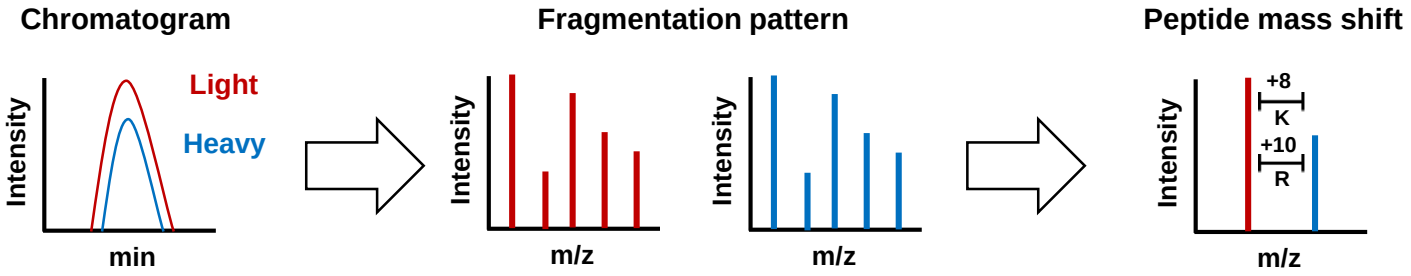
PRM assay development for quantitation of PSD proteins

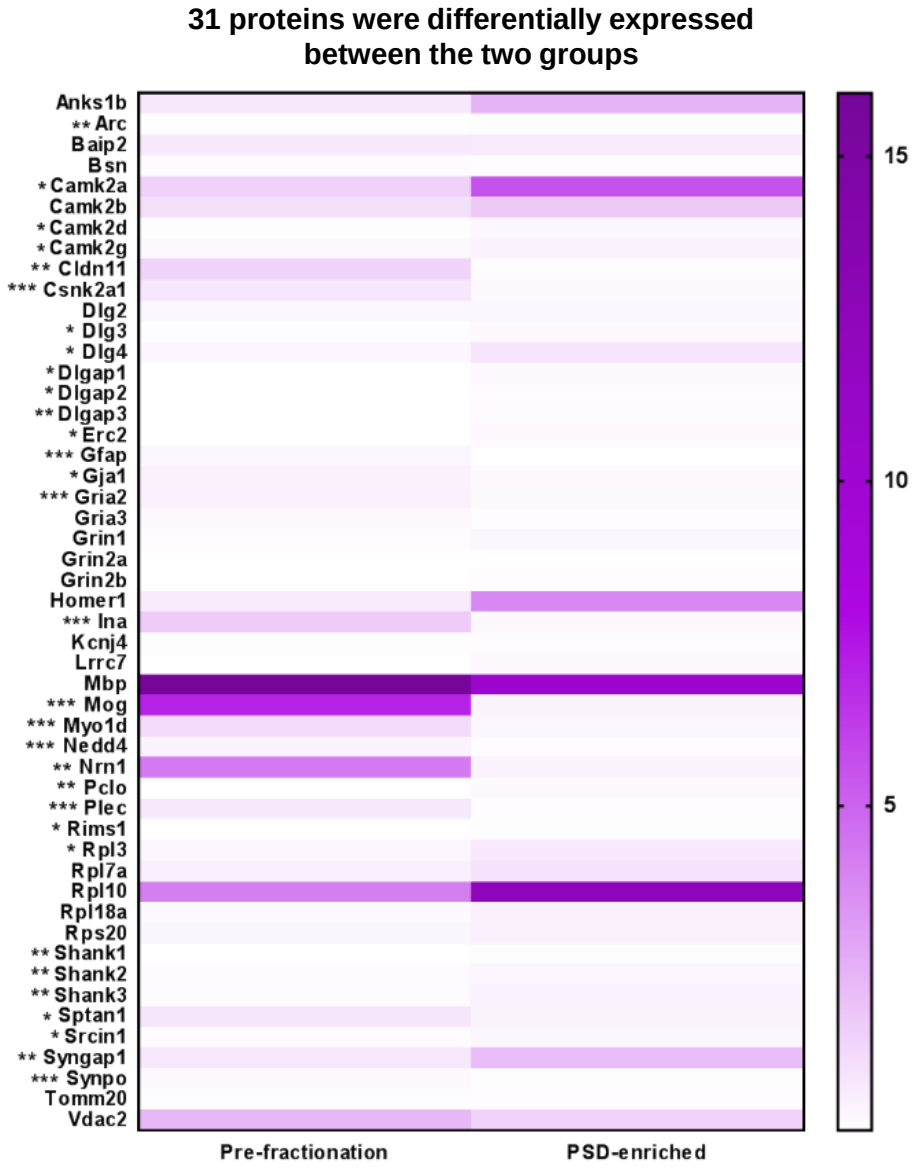
Target PSD proteins for PRM analysis				
Anks1b	Dlg2	Gria3	Myo1d	Rps20
Arc	Dlg3	Grin1	Nedd4	Shank1
Baiap2	Dlg4	Grin2a	Nrn1	Shank2
Bsn	Dlgap1	Grin2b	Pclo	Shank3
Camk2a	Dlgap2	Homer1	Plec	Sptan1
Camk2b	Dlgap3	Ina	Rims1	Srcin1
Camk2d	Erc2	Kcnj4	Rpl10	Syngap1
Camk2g	Gfap	Lrrc7	Rpl18a	Synpo
Cldn11	Gja1	Mbp	Rpl3	Tomm20
Csnk2a1	Gria2	Mog	Rpl7a	Vdac2

Selected 1-3 peptides/protein for heavy, stable isotope-labeled (SIL) synthesis (50 proteins/138 peptides total)

SIL peptides are added to sample in a fixed amount and act as an internal standard for peptide quantitation



PRM analysis of PSD proteins



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Future applications for PSD targeted proteomics assays

Key advantages for future applications:

- Assays are compatible with both mouse and rat tissue
- Can create new DIA libraries to “target” and quantify specific proteins of interest

For investigators interested in PSD proteomics:

- Assays are now available at the Yale/NIDA Neuroproteomics Center for investigator use
- Contact me at **rashaun.wilson@yale.edu**

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