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

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Keck MS & Proteomics Resource
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

<table>
<thead>
<tr>
<th>PSD protein</th>
<th>Associated disorder</th>
<th>Supporting literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaMKII</td>
<td>Learning and memory formation, <strong>Drug addiction</strong></td>
<td>Elgersma et al. 2012, Lisman et al., 2002, Mayford et al., 1996, Anderson et al., 2008, Robinson et al., 2013, Loweth et al., 2010</td>
</tr>
<tr>
<td>Shank1</td>
<td>Autism spectrum disorder (ASD), <strong>Drug addiction</strong></td>
<td>Sato et al., 2012, Sungur et al., 2014, Gong et al., 2015, Pal et al., 2013, Sungur et al., 2018</td>
</tr>
<tr>
<td>Shank3</td>
<td>Autism spectrum disorder (ASD)</td>
<td>Durand et al., 2007, Peça et al., 2011, Gauthier et al., 2009, Moessner et al., 2007</td>
</tr>
<tr>
<td>PSD-95</td>
<td>Intellectual disorders, ASD, Schizophrenia, Williams’ Syndrome, <strong>Drug addiction</strong></td>
<td>Toro et al., 2005, Feyder et al., 2010, Xing et al., 2016, Yao et al., 2004, Wang et al., 2014</td>
</tr>
<tr>
<td>SynGAP1</td>
<td>Intellectual disorders, ASD, Epilepsy</td>
<td>Hamdan et al., 2009, Hamdan et al., 2011, Berryer et al., 2013</td>
</tr>
<tr>
<td>DLGAP1</td>
<td>Schizophrenia, ADHD, OCD</td>
<td>Li et al., 2013, Fan et al., 2018, Soreq et al., 2017, Gazzellone et al., 2016</td>
</tr>
<tr>
<td>Homer1</td>
<td>Schizophrenia, Depression, <strong>Drug addiction</strong></td>
<td>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</td>
</tr>
<tr>
<td>Grin2A</td>
<td>Depression, <strong>Drug addiction</strong></td>
<td>Taniguchi et al., 2009, Domart et al., 2012, Karpyak et al., 2011</td>
</tr>
<tr>
<td>Gria2/3</td>
<td><strong>Drug addiction</strong>, Depression</td>
<td>Baptista et al., 2004, Bowers et al., 2004, Steinberg et al., 2006</td>
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</tbody>
</table>
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

![Diagram of m/z bars with highest intensity highlighted]

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

![Diagram of m/z bars with multiple windows highlighted]

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

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

![Diagram of m/z bars with highlighted m/z of interest]

“Targeted”
**Parallel reaction monitoring (PRM)**

Isolate and fragment m/z of interest

![Diagram with highlighted m/z of interest]
## Comparison of quantitative LC-MS/MS methods

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

### 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

- Homogenize tissue
- Centrifuge 1,000 xg (1 min) → discard pellet
- Centrifuge 2,000 xg (10 min) → discard pellet (transs)
- Centrifuge 15,000 xg (10 min) → discard supernatant (cytosol)
- Resuspend pellet → "P2" → Triton-insoluble fraction → "PSD"
- Detergent extraction (Triton X-100)
- Synaptosomal lysis
- Collect interface (synaptosomes)
- Density centrifugation 25,000 xg (12 min)

2) Immunoblot validation of PSD enrichment

<table>
<thead>
<tr>
<th>Replicate:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td>Wild-type</td>
<td>P2</td>
<td>PSD</td>
<td>P2</td>
<td>PSD</td>
<td>P2</td>
<td>PSD</td>
<td>P2</td>
<td>PSD</td>
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<tr>
<td>Shank3B KO</td>
<td>P2</td>
<td>PSD</td>
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- PSD-95
- GAPDH
- Prohibitin

3) Tryptic digestion of PSD protein

- Trypsin (R/K)
- PSD protein → PSD tryptic peptides

4) LC-MS/MS
DIA analysis results: Pre-fractionation vs PSD-enriched

1721 proteins were differentially expressed between the two groups

Wilson et al., 2019
DIA analysis results: Pre-fractionation vs PSD-enriched

Wilson et al., 2019
DIA analysis results: WT vs Shank3B KO

140 proteins were differentially expressed between the two groups
DIA analysis results: WT vs Shank3B KO

Wilson et al., 2019
PRM assay development for quantitation of PSD proteins

<table>
<thead>
<tr>
<th>Target PSD proteins for PRM analysis</th>
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<tbody>
<tr>
<td>Anks1b</td>
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<tr>
<td>Arc</td>
</tr>
<tr>
<td>Baiap2</td>
</tr>
<tr>
<td>Bsn</td>
</tr>
<tr>
<td>Camk2a</td>
</tr>
<tr>
<td>Camk2b</td>
</tr>
<tr>
<td>Camk2d</td>
</tr>
<tr>
<td>Camk2g</td>
</tr>
<tr>
<td>Cldn11</td>
</tr>
<tr>
<td>Csnk2a1</td>
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</table>

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

31 proteins were differentially expressed between the two groups

- Shank3: 12-fold decrease in KO samples (p=0.005)

Wilson et al., 2019
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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Weiwei Wang
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JPT Peptide Technologies

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