Proteome variability of postsynaptic density (PSD) fractions analyzed by quantitative mass spectrometry

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Introduction

The postsynaptic density (PSD) is a specialized protein complex at the synaptic junction of glutamatergic excitatory synapses. The protein components of the PSD, including neurotransmitter receptors, cytoskeletal proteins, and signaling molecules, can be altered by synaptic activity and drug exposure. Therefore, methodologies to quantify the changes in the abundance of PSD proteins should help our understanding of the molecular basis of synaptic plasticity. In this study, we attempted to develop methods for the detection and quantitation of PSD proteins in a reproducible manner using targeted with MRM and SWATH mass spectrometry.

Overview of our studies

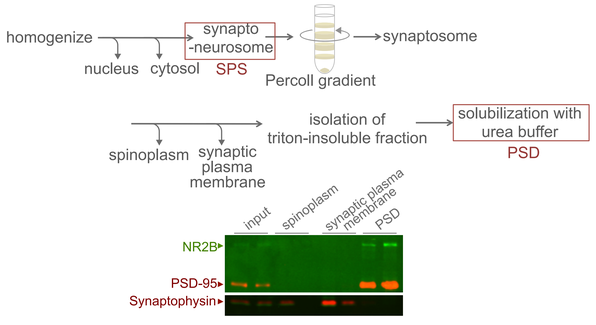
Development of MRM method for PSD proteins

Identification of PSD core proteins
selection of 'appropriate' MRM target

Characterization of the differences in the PSD
proteome following cocaine treatment

We originally started this project to characterize of the differences in the PSD proteome following cocaine treatment. For this purpose, we designed a comprehensive workflow for the development of large scale (>1000 transitions/run) label-free LC-MRM proteome assay. However, the MRM data raised some issues related to sample variability in PSD proteins. To address these issues, we have examined in more detail the basis for altered PSD protein levels that might be caused by preparation conditions.

PSD preparation



Rat brain tissue was homogenized using a Dounce tissue grinder. Nuclear and unhomogenized cell contaminants were removed by low-speed centrifugation, followed by a high-speed centrifugation to obtain the pellet containing synaptoneurosomes (SPS). This was applied to a Percoll gradient and ultracentrifuged. The synaptosome fraction was collected and subjected to hypotonic lysis. Subsequently, the synaptic plasma membrane fraction was collected by ultracentrifugation. Following a Triton extraction, the PSD fraction was collected by ultracentrifugation.

Acknowledgements

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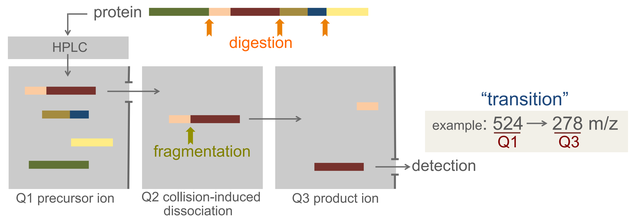
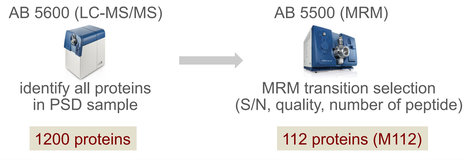
MRM - multiple reaction monitoring



MRM is highly specific assay method for detecting analytes of interest utilizing, most predominantly, a triple quadrupole-based mass spectrometer. Q1 is set to transmit only the parent m/z of the peptide, the fragmentation via collisional induced dissociation occurs in Q2, and Q3 is set to transmit this a single diagnostic fragment. The specific combination of m/z associated with the parent and fragment ions selected is referred to as a "transition".

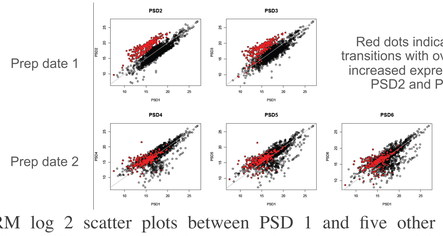
Discovery proteomics to MRM transition selection



	requirement	our study
protein	depend on aim	proteins associated with PSD
peptide	observability - high ionization efficiency - unique sequence	>3 peptides/protein >10 S/N >0.75 quality
transition	observability increased throughput -balance the number of transitions with retention time	3 peptides/protein 5 transitions/peptide

1,200 proteins were identified in the PSD fraction by discovery runs on a TripleTOF 5600 MS. From the protein list, 112 proteins were selected as target analytes (M112) based on the number of peptides detected, peak distribution and signal/noise ratio. The data were translated into a QTRAP 5500 LC-MRM assay enabling rapid quantitation of 112 proteins. 3 peptides per protein were quantified by measuring 5 transitions for each peptide.

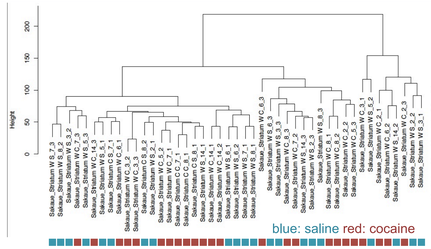
Mitochondria related proteins are susceptible to the prep conditions



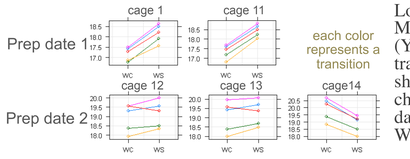
Red dots indicate 218 transitions with over 4 fold-increased expression in PSD2 and PSD3

MRM log 2 scatter plots between PSD 1 and five other PSD biological replicates. The red dots indicate transitions that are four fold up-regulated in PSD 2 vs. PSD 1. These same transitions are then mapped onto the other four scatter plots.

Effect of preparation date and cage compared to cocaine treatment on the M112 proteins

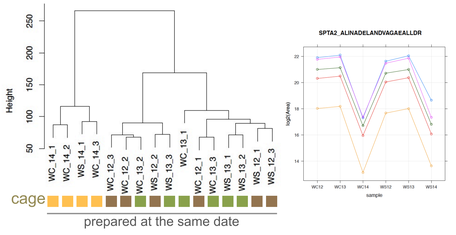


Peak area clustering analysis of PSD from saline or cocaine treated rat. The control (saline) and cocaine treated groups could not be distinguished.



each color represents a transition

Log scale peak area of MTCH1 peptide (YSGVLSSIGK) for 5 transitions. The result showed significant change only for Prep date 1 (WC – cocaine; WS – saline).



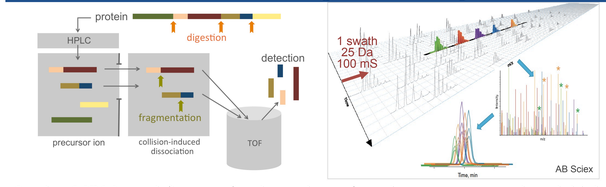
Left: Peak area clustering analysis of 6 PSD samples prepared on the same date. Right: Log scale peak area of SPTA2 peptide for 5 transitions. The peptide decreased only the samples from cage 14 (WC14, WS14).

Evaluation of variability from preparation conditions for the reselection of MRM targets using SWATH

cage	rat ID	tissue	frozen or unfrozen	prep-date	fractions
A	1	half cortex	unfrozen	day 0	SPS and PSD
		half cortex	frozen	day 0	SPS and PSD
	2	half cortex	unfrozen	day 0	SPS and PSD
		half cortex	frozen	day 0	SPS and PSD
B	3	half cortex	frozen	day 1	SPS and PSD
		half cortex	frozen	Day 2	SPS and PSD
	4	half cortex	frozen	day 1	SPS and PSD
		half cortex	frozen	Day 2	SPS and PSD
C	5	half cortex	frozen	day 1	SPS and PSD
		half cortex	frozen	Day 2	SPS and PSD
	6	half cortex	frozen	day 1	SPS and PSD
		half cortex	frozen	Day 2	SPS and PSD

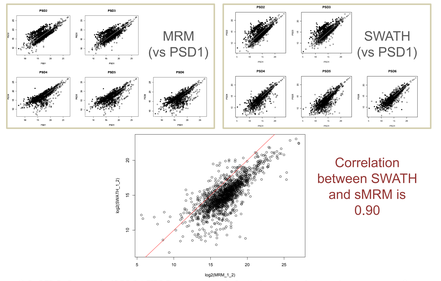
The PSD fraction might be susceptible to variability in preparation condition. To analyze the variability among sample preparations, both the SPS and PSD fractions were prepared in different conditions and compared for ALL proteins by SWATH analysis.

SWATH - sequential windowed acquisition of all theoretical fragment-ion spectra



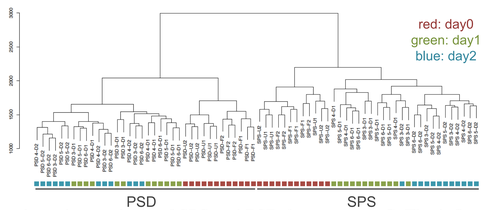
In the MRM workflow, a fixed number of analytes are targeted and high resolution MS/MS spectra are collected across an LC run. On the other hand, in SWATH, a wider Q1 window containing more analytes is passed. This produces a more complex MS/MS spectrum which is a composite of all the analytes within that Q1 m/z window. The Q1 quadrupole is stepped at 25 amu increments across the mass range of interest, passing a 25 amu window through into the collision cell. The transmitted ions are fragmented and the resulting fragments are analyzed in the TOF MS Analyzer at high resolution.

Correlation of MRM and SWATH



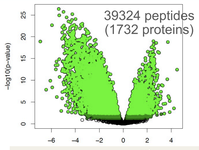
Correlation of MRM and SWATH using 6 biological replicates of cortical PSD samples. Upper panel: log 2 scatter plot between PSD 1 and the other 5 PSD samples. Lower panel: log 2 fold change scatter plot between MRM and SWATH for PSD 1 vs PSD 2. The correlation of 0.90 shows that the two methods give consistent results for the M112 proteins.

Effect of preparation conditions on ALL proteins

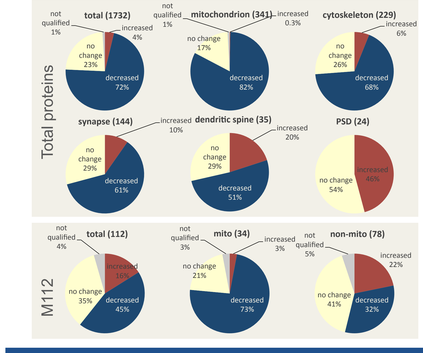


Peak area clustering analysis of SPS and PSD from cage A-C. The influence of prep-date is a big factor for PSD preparation.

Fold change of PSD/SPS by SWATH



Volcano plot for peptides. -log10 scaled p-value (y-axis) against log2 fold change (x axis). According to the adjusted p-values, they are labeled in black, dark green (< 0.1), green (< 0.05), or light green (< 0.01).



PSD/SPS fold change by SWATH classified into cellular component using gene ontology annotations. Increased: >1.5 FC, decreased: <-1.5 FC. Upper panel: total proteins. Only 4% of total proteins are increased in the PSD fraction. Nearly all mito proteins are decreased in the PSD fraction. lower panel: PSD/SPS FC of 112 MRM targets.

Conclusions

- Listed 112 target PSD proteins for MRM assay
- Mitochondria related proteins are decreased in PSD fraction compared with SPS -they might be contaminants
- Proteins that are susceptible to preparation conditions should be excluded from MRM targets