

# Proteomic analysis of the rat brain postsynaptic density<sup>1</sup>

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## The Postsynaptic Density

The majority of excitatory neurotransmission in the brain occurs via glutamatergic synapses. In the pre-synaptic element of the synapse, specialized secretion machinery determines the activity-dependent membrane-fusion of glutamate-containing vesicles and the release of transmitter into the synaptic cleft. In the post-synaptic element, glutamate receptors and downstream signal transduction are organized by the protein assembly of the postsynaptic density (PSD). Both the pre-synaptic release machinery and the PSD are electron dense assemblies (1, 2), in which proteins are thought to be organized into distinct functional complexes (3-5) that may be dynamically regulated by neuronal activity (6-8). The modulation of this molecular architecture of the synapse is at the basis of synaptic plasticity (6-8), and aberrations thereof may underlie neuronal disorders. In view of the importance of the PSD in glutamatergic neurotransmission and its involvement in neuroplasticity, considerable efforts have been made to identify its protein constituents as a prelude to understanding the molecular basis of PSD functioning.

One of the first applications of proteome research in neuroscience was aimed at the characterization of novel PSD proteins (9). The PSD fraction was isolated using a standard protocol developed by Carlin et al (10). The PSD proteins were partially separated on an SDS electrophoresis gel, trypsinized and then characterized based on their peptide mass fingerprint (PMF). About thirty proteins, including “classic” and novel PSD proteins, were successfully

elucidated. Overall, the number of proteins identified was lower than that expected, which may amount to above a hundred (4, 11,12). This suggested that a considerable number of PSD proteins remained to be characterized.

In the present study we took multiple, independent proteome approaches for the characterization of the protein complement of the PSD. Indeed, the combination of proteomics workflows resulted in better and more protein identification allowing a better understanding of the functioning of the PSD. A large number of previously identified PSD proteins, as well as novel groups of proteins were detected. Grouping of the identified proteins based on their functions indicated the presence of protein complexes that are involved in diverse physiological activities and indicates that the PSD is a structurally and functionally complex organelle that may be involved in a broad range of synaptic activities. These include the receptors and ion channels for glutamate neurotransmission, proteins for maintenance and modulation of synaptic architecture, sorting and trafficking of membrane proteins, generation of anaerobic energy, scaffolding and signalling, local protein synthesis, and correct protein folding and breakdown of synaptic proteins. Together, these results imply that the PSD may have the ability to function (semi-) autonomously and may direct various cellular functions in order to integrate synaptic physiology.

The goals of this work were two-fold, (1) to better understand the composition of the PSD and from that better understand PSD function and (2) to study the effect on protein identification of different proteomics workflows (separation and mass spectrometry) in an attempt to define their efficacy and complementarity.

## **Workflows**

2D gel electrophoresis    In-gel digestion of all visible spots and extraction of peptides    PMF by MALDI TOF MS    Database searching    MALDI TOF/TOF MS/MS    Database searching

Isotope coded affinity tag (ICAT®) labelling    Avidin affinity separation of cys-ICAT labelled peptides    LC-MS/MS (MALDI or ESI)    Database searching

ICAT labelling    SCX Chromatography    Avidin affinity separation of cys-ICAT labelled peptides    LC-MS/MS (MALDI or ESI)    Database searching

1D gel gel is cut into pieces from high to low molecular weight in-gel digestion of bands and extraction of peptides LC-MS/MS Database searching

### **Advantages and Disadvantages**

Each workflow has advantages but combining the workflows produces the greatest amount of mass spectral information and the greatest number of confident protein identifications, which allows more confident analysis of the way that the PSD proteins interact with each other and with proteins external to the PSD. Therefore a better understanding of the functioning of the PSD is possible which is our ultimate goal. In short, proteins that were water-soluble, typically between 15 and 100 kDa, with a pI between 4 and 10, not hydrophobic and reasonably abundant could be readily identified using the 2D gel electrophoresis based workflow. Some major, known PSD proteins however were under-represented. Using the other workflows resulted in additional identification. For example, the non-2D gel electrophoresis based workflows allowed the detection of membrane proteins such as the ionotropic glutamate receptors and ion channels, large proteins (plectin > 500 kDa) and basic proteins (ribosomal proteins > pH 10) that were not detected by the 2-D gel electrophoresis approach. The advantages of the 2D gel electrophoresis approach in terms of protein identification were the additional information available for identification purposes, that is, the protein molecular weight and pI and the ability to perform a peptide mass fingerprint as well as tandem MS analysis on the same MALDI MS platform thus saving time. In addition, it is possible to observe post-translational modifications (multiple spots per protein). The advantages of the ICAT- non-2D gel based approaches were the ability to reduce the complexity of the sample by affinity selecting only peptides containing cysteine and also the extra information provided for database searching, that is, the knowledge that each peptide must contain a cysteine. However, PMF database searching and protein molecular weight and pI are lacking. Some of the major advantages of the 1D gel electrophoresis approach are the simplicity of the workflow and the fact that protein molecular weight information is retained. PMF and pI information are lost.

### **Summary**

The brief answer to the question ‘Which workflow is best?’ is that all the workflows have advantages and disadvantages and must be chosen on the basis of instrumentation availability and the nature of the sample. For the PSD, the

correct choice was to choose multiple workflows. It may well be the case that this is the correct answer for most complex proteomic samples in order to achieve the most complete characterization possible.

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