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A proteomic survey of rat cerebral cortical synaptosomes

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Abstract

Previous findings from our laboratory and others indicate that two-dimensional gel electrophoresis (2-DE) can be used to study protein expression in defined brain regions, but mainly the proteins which are present in high abundance in glia are readily detected. The current study was undertaken to determine the protein profile in a synaptosomal subcellular fraction isolated from the cerebral cortex of the rat. Both 2-DE and liquid chromatography – tandem mass spectrometry (LC-MS/MS) procedures were used to isolate and identify proteins in the synaptosomal fraction and accordingly >900 proteins were detected using 2-DE; the 167 most intense gel spots were isolated and identified with matrix-assisted laser desorption/ionization – time of flight peptide mass fingerprinting or LC-MS/MS. In addition, over 200 proteins were separated and identified with the LC-MS/MS "shotgun proteomics" technique, some in post-translationally modified form. The following classes of proteins associated with synaptic function were detected: (a) proteins involved in synaptic vesicle trafficking-docking (*e.g.*, SNAP-25, synapsin I and II, synaptotagmin I, II, and V, VAMP-2, syntaxin 1A and 1B, *etc.*); (b) proteins that function as transporters or receptors (*e.g.*, excitatory amino acid transporters 1 and 2, GABA transporter 1); (c) proteins that are associated with the synaptic plasma membrane (*e.g.*, post-synaptic density-95/synapse-associated protein-90 complex, neuromodulin (GAP-43), voltage-dependent anion-selective channel protein (VDACs), sodium-potassium ATPase subunits, alpha 2 spectrin, septin 7, *etc.*); and (d) proteins that mediate intracellular signaling cascades that modulate synaptic function (*e.g.*, calmodulin, calcium-calmodulin-dependent protein kinase subunits, *etc.*). Other identified proteins are associated with mitochondrial or general cytosolic function. Of the two proteins identified as endoplasmic reticular, both interact with the synaptic SNARE complex to regulate vesicle trafficking. Taken together, these results suggest that the integrity of the synaptosomes was maintained during the isolation procedure and that this subcellular fractionation technique enables the enrichment of proteins associated with synaptic function. The results also suggest that this experimental approach can be used to study the differential expression of multiple proteins involved in alterations of synaptic function.

Keywords

Cerebral cortex; Mass spectrometry; Proteome; Rat; Synaptic proteins; Synaptosomes; Two-dimensional gel electrophoresis

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1 Introduction

The advent of genomics, which includes the mapping of gene sequences and the development of functional genomics, has contributed insights to many physiological and pathophysiological conditions. Despite these contributions, however, genomics is limited in its ability to address such important issues as levels of protein expression. In this regard, the proteome is dictated by more factors than simply the level of mRNA, *e.g.*, post-transcriptional events such as alternative splicing and PTMs of proteins. These deficiencies in genomics have led to an increased interest in proteomics, the analysis of the profile of proteins expressed and/or modified by an organism, tissue, cell type, or sub-cellular compartment. Recently, evolving technical advances have yielded the capability to perform such complex analyses.

One discipline in which proteomics promises to have significant impact is neuroscience. Many neurodegenerative diseases, such as Alzheimer's, are thought to be due to altered functional levels of structural or metabolic proteins. Other conditions, such as addiction and mood disorders, are likely to be secondary to altered expression of proteins, which are involved in neurotransmission or neuroplasticity. Reference proteome databases have been constructed for whole rat brain [1], whole mouse brain [2], mouse cerebellum [3], human parietal cortex [4], and human hippocampus [5]. Our laboratories have recently demonstrated that the expressed proteome can vary in various brain regions based on genetic selection for alcohol preference, and, within these genetic lines, by functional nuclei [6]. Interesting as these documented changes in whole brain tissue are, we are aware that 90–95% of the cells in such tissue are not neurons but glia, which provide support or insulation for neurons [7], and that the majority of these glia are astrocytes [8]. It is likely, therefore, that many of the proteins previously identified by us and by others in whole brain tissue preparations are of glial, not neuronal origin.

We wished to improve our ability to resolve the proteome of neurons and in doing so turned to a well-established procedure for isolating the sub-cellular fraction containing the inter-cellular communication junction between nerves, the synapse [9,10]. Preparations of these "synaptosome" fractions should be greatly enriched in proteins involved in synaptic transmission and reception, the genetic or pathologic alterations of which may underlie many neurologic and psychiatric disorders. There is precedence behind the assumption that sub-cellular fractionation can improve resolution of brain proteins. In rat brain, fractionation of whole tissue into cytosolic, mitochondrial, and microsomal fractions before 2-DE separation and MS identification has led to the identification of hundreds of additional proteins that were not identified in a high-speed supernatant of total rat forebrain [11]. Comprehensive studies on the synaptic proteome, however, have been scarce. This is due in part to the fact that many synaptic proteins, such as receptor, transporter, and channel proteins, are hydrophobic and membrane-bound, characteristics that can lead to poor protein resolution by 2-DE. Some studies have used limited versions of various proteomics approaches such as SDS-PAGE combined with MALDI-TOF MS [12], where 31 individual proteins were identified from resolved bands from post-synaptic densities of whole rat brain. Efforts have also been made to identify proteins from membrane-enriched fractions from pig cerebellum [13], and squid optic lobe synaptosomes [14]. Most recently, using LC/ESI-IT/MS, over a hundred proteins were identified from the tryptic digests of rat forebrain synaptic plasma membranes [15].

As suggested in the prior paragraph, the methods chosen for the analysis of synaptosomal preparations are of critical importance. Because our goals included both reliable quantitation of relative protein levels under different experimental conditions, and detection of PTM of detected proteins, we chose to analyze our synaptosome samples with several techniques. One of the most effective tools for differential protein expression analysis is 2-DE [16,17]. When combined with MALDI-TOF MS, the electrophoretically separated proteins can be identified and characterized [18]. In-line HPLC separation followed by IT MS/MS, so-called "shotgun

proteomics", can also be used to detect individual proteins in the expressed proteome and is a valuable tool in detecting PTMs in detected proteins. Glycoproteins exhibit both functional and structural importance in the synapse [19] and can be concentrated using lectin affinity columns prior to analysis with tandem MS.

In summary, the current study was undertaken to focus on the more behaviorally and functionally relevant neuronal elements by determining the protein profile of synaptosomes isolated from the cerebral cortex of the rat. Techniques used to resolve the expressed proteome of synaptosomes included 2-DE and LC-MS/MS procedures, the latter with and without prior application of a lectin affinity column that binds glycoproteins. Proteins resolved by 2-DE were subsequently identified by MALDI-TOF and LC-MS/MS.

2 Materials and methods

2.1 Materials

Acrylamide for slab gels and IPG strips were purchased from Bio-Rad (Richmond, CA, USA). Other ultrapure electrophoretic reagents were obtained from Bio-Rad, Sigma (St. Louis, MO, USA), or BDH (Poole, UK). Sequence grade trypsin was obtained from Promega (Madison, WI, USA). Ammonium bicarbonate was purchased from Mallinckrodt (Paris, KY, USA). Proteomics grade trypsin, formic acid, iodoethanol, and triethylphosphine were obtained from Sigma-Aldrich (St. Louis, MO, USA). ACN and hydrochloric acid solution N/10 were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Con A Sepharose was obtained from Amersham Biosciences (Piscataway, NJ, USA). All other chemicals used were of the highest grade obtainable.

2.2 Animals

Adult male Wistar rats (n = 3, for 2-DE and LC-MS/MS studies) were used in this study, and were singly housed in standard animal colony rooms under normal 12 h light cycle conditions (lights on at 700 h). Rats were sacrificed by decapitation, the brain rapidly removed, and placed on a chilled glass plate on ice. All subsequent procedures involved in the tissue preparation were performed at 4°C. Animals used in this study were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, and all experimental procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, and the Guide for the Care and Use of Laboratory Animals of the National Research Council, 1996.

2.3 Preparation of synaptosomes

The cerebral cortex (frontal) was dissected and the adhering white matter was removed. Cortical samples were weighed and homogenized in 10 volumes of 0.32 M sucrose buffered to pH 7.4 with 20 mM HEPES, and containing 1 mM EDTA, 5 mM dithioerythritol, 1 mM PmsF, 0.2 mM sodium vanadate, and 1 mM sodium fluoride [11]. Standard homogenization and ultracentrifugation procedures were used to isolate synaptosomes [20,21]. Homogenization was performed using a glass homogenizer and a teflon pestle. Homogenates were centrifuged at 1000 × g for 10 min to obtain the crude nuclear pellet (P1) and the S1 supernatant. The S1 fraction was centrifuged at 17 000 × g for 15 min to obtain the crude mitochondrial fraction (P2 pellet), which was used for the preparation of synaptosomal fractions. The P2 pellet was resuspended in the same homogenizing buffer used for initial homogenization of the tissue, and layered on top of a discontinuous sucrose density gradient consisting of 1.2 M sucrose and 0.8 M sucrose. The gradient was centrifuged at 54 000 × g for 90 min, and the synaptosomal fraction was removed from the 0.8 M sucrose and 1.2 M sucrose interface. This fraction was

slowly diluted with 10 volumes of ice-cold 0.32 M sucrose, centrifuged at 20 000 × *g* for 15 min, and the resulting synaptosomal pellet frozen at −80°C until used for protein extraction.

2.4 2-DE and image analysis

Frozen synaptosomes were solubilized in 500 μL of a solution containing 9 M urea, 4% Igepal CA-630 ((octylphenoxy) polyethoxyethanol), 1% DTT, and 2% carrier ampholytes (pH 3–10). Each sample was sonicated with a Fisher® Sonic Dismembranator using 3 × 2 s bursts at instrument setting no. 3. Sonication was carried out every 15 min for 1 h at room temperature. The protein concentration of each sample was determined using the RC DC Protein Assay kit (Bio-Rad) according to the manufacturer's protocol. After solubilization, the samples were stored at −45°C. 2-DE was performed on synaptosomal protein samples as follows. Aliquots (180 μL each) containing ~200 μg of protein from the solubilized synaptosomes were diluted with 320 μL of rehydration buffer (8 M urea, 2% CHAPS, 15 mM DTT, 0.2% carrier ampholytes pH 3–10, and 0.001% orange G). The resulting 500 μL protein dilutions were loaded onto IPG strips (24 cm, linear pH 3–10) by overnight, passive rehydration at room temperature. Isoelectric focusing was performed simultaneously on all IPG strips using the Protean IEF Cell (Bio-Rad), by a program of progressively increasing voltage (150 V for 2 h, 300 V for 4 h, 1500 V for 1 h, 5000 V for 5 h, 7000 V for 6 h, and 10 000 V for 3 h) for a total of 100 000 Vh. A computer-controlled gradient casting system was used to prepare second dimension SDS gradient slab gels (20 × 25 × 0.15 cm) in which the acrylamide concentration varied linearly from 11 to 17%T. First dimension IPG strips were loaded directly onto the slab gels following equilibration for 10 min in Equilibration Buffer I and 10 min in Equilibration Buffer II (Equilibration Buffer I: 6 M urea, 2% SDS, 0.375 M Tris-HCl pH 8.8, 20% glycerol, 130 mM DTT; Equilibration Buffer II: 6 M urea, 2% SDS, 0.375 M Tris-HCl pH 8.8, 20% glycerol, 135 mM iodoacetamide). Second dimension slab gels were run in parallel at 8°C for 18 h at 160 V. Slab gels were stained using a colloidal CBB G-250 procedure [22]. Gels were fixed in 1.5 L of 50% ethanol/2% phosphoric acid overnight followed by three 30 min washes in 2 L of deionized water. Gels were transferred to 1.5 L of 30% methanol/17% ammonium sulfate/3% phosphoric acid for 1 h followed by an addition of 1 g of powdered CBB G-250 stain. After 96 h, gels were washed several times with water and scanned at 95.3 μm *per* pixel resolution using a GS-800 Calibrated Imaging Densitometer (Bio-Rad). The resulting 12-bit images were analyzed using PDQuest™ software (Bio-Rad, v.7.1). Background was subtracted and peaks for the protein spots located and counted. The most abundant spots (190) were selected for MS identification.

2.5 In-gel tryptic digestion and PMF

Ninety-four protein spots with the highest intensity were cut from the gel by hand using a 1.5 mm gel cutting tool and placed in each of 94 wells of a 96-well plate, along with an grp78 standard and one gel blank, and processed using the Multiprobe II (Perkin-Elmer, Boston MA, USA). The remaining 96 gel cutouts were placed in a second 96-well plate and processed for LC-MS/MS analysis (see below). In this automated system, the 94 excised protein spots were first destained with 50 mM ammonium bicarbonate-50% ACN followed by 100% ACN. Reduction with 10 mM DTT and alkylation with 55 mM iodoacetamide was carried out prior to overnight tryptic digestion using modified trypsin at 6 ng·μL^{-1}. The grp78 (StressGen, Victoria, BC Canada) calibrant and a gel blank were digested in the additional two wells using identical conditions. The resulting peptides were extracted by the addition of 25 μL 0.2% formic acid (aqueous) and 7 μL of ACN solution to the wells, and plates were shaken at 37°C for 1 h. The resulting solution was placed in a separate 96-well plate and dried using a Speed-Vac. The dehydrated peptides were then reconstituted in 5 μL of 0.2% formic acid and 1 μL of ACN with continuous shaking of the plate for 5 min. Aliquots from peptide extracts (in 3 μL volumes) were then placed onto a MALDI target plate, air dried, and the application repeated until all

the extraction solution was used up. Just before the spots finished drying, 0.8 μL of matrix (2 $mg \cdot mL^{-1}$ CHCA in 50% ACN) was added to each peptide spot and allowed to dry completely.

Peptide masses were analyzed by MALDI-TOF MS using a Waters Micromass M@LDI SYSTEM (Micromass, Milford, MA, USA). Prior to data collection, the instrument was calibrated externally using a mixture of peptide standards, digested standard (grp78), and experiment artifact peaks based on tryptic autolysis. Twenty-five to thirty-five peaks were used in conjunction with a fifth-order curve to produce the external calibration plot. After data collection, each spectrum was processed (background subtracted, smoothed, and centroid determined), recalibrated (for MALDI plate topology using trypsin autolysis peaks 1045.56 or 2211.10 as internal calibrants), and the data exported to mass-only text files. Proteins were identified by manual ProFound™ (Proteometrics LLC) database searches using the mass lists obtained from exported MALDI spectra of the excised 94 spots. A *Z*-score of 1.30, corresponding to the 90th percentile, was the threshold for what was considered a positive identification.

2.6 In-gel tryptic digestion for LC-MS/MS analysis

The next 96 most abundant protein spots on the 2-D gel (95–190) were excised, placed in an Eppendorf tube, cut into smaller (less than 1 mM in each dimension) pieces, and destained with 200 μL of 200 mM ammonium bicarbonate in 40% ACN at 37°C for 30 min. This destaining step was repeated once and the gel pieces were completely dehydrated in a Vacufuge concentrator (Eppendorf, Westburg, NY, USA) for 20 min followed by rehydration with 20 μL of 20 $g \cdot mL^{-1}$ trypsin solution (in 36 mM ammonium bicarbonate, 8% ACN). An aliquot of 50 μL of 40 mM ammonium bicarbonate in 9% ACN was added to each sample before the digestion was carried out at 37°C for 18 h. The tryptic digests were extracted from the gel pieces, dried in a Vacufuge concentrator, and rehydrated with 10 μL of 1% formic acid. The extract solution was kept frozen until LC-MS/MS analysis.

2.7 In-solution tryptic digestion for LC-MS/MS analysis

Synaptosomal proteins were resuspended in water to produce 1 $mg \cdot mL^{-1}$ sample concentrations. Forty-five microgram of total synaptosomal protein were mixed with 5 μL of 1 M ammonium bicarbonate (final concentration 50 mM). Reduction and alkylation were carried out for 1 h at 37°C by adding an equal volume of a cocktail containing 2% iodoethanol, 0.5% triethylphosphine, and 97.5% ACN [23]. The reaction mixture was evaporated to dryness in a Vacufuge concentrator. The dried sample was digested in 20 μL of 10 mM, pH 7.85, ammonium bicarbonate containing 1 μg of trypsin for 18 h at 37°C. The digested protein mixture was subsequently subjected to LC-MS/MS analysis.

2.8 Isolation of glycoproteins for LC-MS/MS analysis

Frozen synaptosomes were diluted with 150 μL of the binding buffer consisting of 50 mM Tris, 500 mM NaCl, pH 6.5. The sample was loaded onto a Con A Sepharose column (1 mL bed volume) and unbound proteins were eluted with 5 bed-volumes of binding buffer. The glycoproteins, which were expected to bind to the Con A Sepharose column, were eluted with 5 bed-volumes of elution buffer that was identical to the binding buffer but contained 300 mM 1-O-methyl-β-D-glucopyranoside. The fraction enriched in glycoprotein was desalted overnight using 1000 MW cut-off dialysis membrane. The dialyzed sample was concentrated to dryness using a Vacufuge concentrator, and the dried sample was resuspended in 50 μL of 1 M ammonium bicarbonate and subjected to trypsin digestion as described above.

2.9 LC-MS/MS and "shotgun" proteomic analysis

The nano-LC separations were performed using an LC Packings system (Dionex, Sunnyvale, CA, USA) consisting of a Famos™ autosampler, Switchos™ switching valve and pump (used for sample trapping and washing), and UltiMate gradient pump. Aliquots of the tryptic digests (3 μL for solution digestion and 6 μL for in-gel digestion) were loaded onto a trapping column (15 mm × 100 mm) in-house packed with 5 μm, 200 Å Magic C18AQ packing media. The trapping column was then washed to remove any salts and unretainable materials prior to elution and separation of the retained peptides on a pulled-tip capillary column (150 mm × 75 mm) in-house packed with the same packing materials used for the trapping column, but with 100 Å pore size. In-gel digested peptide samples were separated by a gradient in which solvent B was increased linearly from 10 to 35% in 15 min at a flow rate of 250 nL·min^{-1}. Solvent B consisted of ACN with 0.1% formic acid, while solvent A consisted of 3% ACN and 97% water with 0.1% formic acid. A much longer gradient was used for the separation of the tryptic digests of the total proteome or isolated glycoproteins. In this case, a 3 h gradient was utilized in which solvent B was first increased linearly from 6 to 20% in 120 min, followed by another linear increase from 20 to 40% in 45 min, both at a flow rate of 250 nL·min^{-1}. The ions were directly sprayed from the separation column into an LCQ Deca XP ion-trap mass spectrometer (Thermo-Finnigan, San Jose, CA, USA). The mass spectra of the separated peptide ions and data-dependent tandem mass spectra of product ions from precursor ions were recorded. The acquired MS/MS spectra were searched against protein sequences for *Rattus* in the Swiss-Prot database using MASCOT for peptide recognition and consequent protein identification.

Except for glycosylation, PTM identification was based on the use of MASCOT with selecting the identified PTMs as variable modifications. MS/MS data with an ion score of >35 were then manually inspected to confirm the identified PTM. For glycosylation, the identification was based on the LC-MS/MS analysis of the tryptic digest of the lectin bound fraction. The amino acid sequence of the identified proteins was then checked to account for the presence or absence of the *N*-glycan motif.

2.10 Bioinformatic analysis of identified proteins

Functions and sub-cellular locations of identified proteins were analyzed by both manual and automated methods. PubMed was used to search for abstracts pertaining to synapse-specific proteins; the remaining proteins were categorized by Pandora [24] (http://www.pandora.cs.huji.ac.il/) according to the gene ontology (GO) sub-cellular location schema [25].

3 Results

3.1 2-DE and MS

Figure 1 illustrates a representative image of the synaptosomal fraction separated by 2-DE and stained with colloidal CBB. Distinct spots identified by PMF or LC-MS/MS have been assigned a number ranging from 1 to 163 for the convenience of the reader. These proteins are listed in Table 1 where they are accompanied by their respective unique PDQuest spot numbers, which were assigned automatically during creation of the matchset. A total of 968 protein spots were detected and matched by PDQuest; the 190 most abundant spots were cut from the gel and subjected to tryptic digestion. The resulting peptides were analyzed by one of the two mass spectrometric methods.

The 94 most abundant spots were subjected to MALDI-based PMF resulting in the identification of 85 spots, representing 61 unique proteins. The peptides from the remaining 96 spot digests were analyzed by LC-MS/MS, which yielded 79 identifications, representing 46 unique proteins. The proteins identified using the combination of 2-DE and the two MS

techniques derived from synapse-specific structures such as synaptic vesicles and the synaptic membrane as well as from the cytoplasmic and mitochondrial compartments. Identified proteins that are specific to the synapse and function in neurotransmission are of particular interest; these include: calmodulin (CaM), $Ca^{(2+)}$-dependent ganglio-side-binding protein (fragment), cAMP-dependent protein kinase inhibitor beta, chain B of complex between *N*-terminus of SNAP25 and SNARE region of Syntaxin 1a, chain B, neuronal synaptic fusion complex, SNAP 25 synaptosomal-associated protein, 25 kDa, synapsin II, synaptotagmin I, transducin beta, and synaptobrevin 3.

3.2 Shotgun proteomics and post-translational modification analysis

Although 2-DE coupled with MS or LC-MS is a powerful approach to differential expression analysis, the number of proteins that can be resolved and identified in 2-DE gel is limited. Highly hydrophobic proteins and those with extremes of p*I*, particularly basic proteins, are poorly resolved by this technique. In addition, 2-DEs relatively high concentration threshold for detection makes the analysis of low abundance proteins, many of which are physiologically relevant, a major challenge. To augment our 2-DE approach, proteins from synaptosomal fractions were analyzed directly after solution tryptic digestion using an LC-MS/MS approach.

Using this approach, 201 distinct proteins were identified (Table 2). Of these, ~20–30 proteins are known to be involved in synaptic vesicle trafficking/docking (*e.g.*, Syntaxin 1A, Synapsin I, II, Synaptophysin, and Synaptotagmin I, II, V, protein kinase C and kinase substrate (PACSIN1), and calcium/CaM-dependent protein kinase type II) and synaptic plasma membrane structure and function (*e.g.*, sodium-potassium ATPase, clathrin, channel-associated protein of synapse-110, pre-synaptic density protein-95, Dynamin 1–3, glutamate-aspartate transporter 2, neural cell adhesive molecule 1, GAP-43, opioid binding protein B, regulating synaptic membrane exocytosis protein 1, GABAB transporter, Septin 7, and Synaptojanin 1).

Regarding PTM of the 201 proteins identified by LC-MS/MS, 47 proteins were found to be glycosylated, five proteins were methylated, 11 proteins were acetylated, two were oxidized, and one was phosphorylated (Table 2). An additional 71 proteins that were not identified during the initial database search were found to be post-translationally modified in some way (glycosylated, methylated, acetylated, oxidized, or phosphorylated, Table 3). The majority of glycosylated proteins, which were determined by LC/MSMS analysis of lectin trapped proteins, possessed the *N*-glycan motif, suggesting several proteins involved in synaptic vesicle trafficking/docking underwent PTM (*e.g.*, Synapsin I, Synaptotagmin, Synaptophysin, Syntaxin 1B, syntaxin binding protein 1 (Unc-18A), CaM, actin, protein kinase C, and casein kinase substrate (PACSIN1 or syndapin1)) as did several with synaptic membrane function (*e.g.*, excitatory amino acid transporter, clathrin, Septin 7, Dynamin).

When the two sets of identified proteins are compared, as expected, there is some overlap in the proteins identified by either 2-DE/MS or LC-MS/MS. Of the 91 unique proteins identified by the former and the 201 unique proteins identified by the latter, 46 were found in both sets. Accounting for this intersection, the total number of unique proteins identified by the combined methods was 246. Of these 246 proteins, 61 were identified by PubMed literature search as having synapse-specific function (Fig. 2a and b). Nineteen identified proteins are involved in synaptic vesicle trafficking or docking, nine serve receptor or transporter functions, nine are involved in intra-cellular signaling cascades that affect synaptic transmission, and 24 have other synapse-specific functions.

The remaining 185 proteins were categorized in a semiautomated manner. Swiss-Prot accession numbers of these proteins were uploaded to Pandora and were categorized by sub-cellular compartment according to the GO. Twenty-four proteins were categorized in this

fashion. Assuming that this subset of 24 proteins is representative of the larger set of 185, extrapolation leads to an estimate that 65 of the 185 non-synapse-specific proteins are mitochondrial, 48 are cytoskeletal, and 40 are cytoplasmic.

4 Discussion

In the present study, multiple protein separation and identification approaches were used in conjunction to analyze the synaptosomes isolated from rat cerebral cortex, providing both confirmatory and complementary proteomic information. The identified proteins confirm that the primary objective of the study was accomplished – perhaps the single most important functional portion of the CNS, the synapse, has been isolated for proteomic analysis, providing for significant enrichment of synaptic proteins when compared to prior techniques.

Application of 2-DE to rat cerebral cortex synaptosome fractions resulted in the separation and detection of >900 protein spots, among which 163 of those with the highest abundance were identified by either MALDI-TOF or LC/MS-MS. These 163 spots represent various forms of 91 distinct proteins. Among these, a number of synaptic vesicle proteins were detected including vesicle-associated membrane protein (VAMP, synaptobrevin, no. 147 in Table 1, also listed as VAMP-3). VAMP is a synaptic vesicle docking protein (v-SNARE) that plays a fundamental role in synaptic vesicle exocytotic fusion, initiated by the binding of v-SNARES and t-SNARES. Another integral vesicle membrane protein, synaptotagmin, was detected as spot 25 (synaptotagmin I). Synaptotagmin serves as a calcium sensor for exocytosis, yet may also be considered a v-SNARE due to its interaction with t-SNARE syntaxin.

Synapsin II, a vesicle-associated protein was shown as spots 26, 29, and 32. Synapsins are anchor proteins, which tether the synaptic vesicles to the actin filaments of the nerve terminal in a Ca^{2+}/phosphorylation-dependent manner, regulating the distribution of the vesicles between the reserved pool and the active zone for exocytotic release [26]. Vacuolar ATP synthase (V-ATPase) F subunit shown as spot 155, is a part of vacuolar proton pump present on all acidic cellular organelles, such as clathrin-coated vesicles, endosomes, lysosomes, and Golgi membranes. The acidification of the synaptic vesicle's lumen is critical to the packaging and processing of the contents of synaptic vesicles [27]. Since synaptosomes are pinched-off nerve endings, containing both pre and post-synaptic structures, it was not surprising to detect proteins associated with the post-synaptic membranes. For example, spots 93, 94, 95, and 111 were identified as the β-subunit of G protein (GBB1 or GBB2), a membrane-associated protein that mediates the effects of numerous G protein-coupled receptors (GPCRs). In the brain, neurotransmitter receptors can be classified as two distinct super-families: ligand-gated channels (LGCs) and GPCRs. The receptors belonging to the GPCR family include muscarinic ACh receptors, DA receptors, adrenergic receptors, most 5-HT receptors, metabotropic glutamate receptors, $GABA_B$ receptors, histamine receptors, cannabinoid receptors, and neuropeptide receptors. While most of these receptors can be either a post-synaptic component or a pre-synaptic autoreceptor depending on the receptor sub-type, some of them, such as $GABA_B$, can be found both pre- and post-synaptically [28,29]. GPCRs can also be pre-synaptic, as has been reported in the regulation of voltage-dependent Ca^{++} channels during neurotransmitter release [30].

2-DE also successfully displayed numerous non-membrane bound and cytosolic proteins, some of which play an important role in synaptic and neuronal function. For instance, protein kinase C and casein kinase substrate in neurons (PACSIN1, spots 40 and 41), also named Syndapin I, is a cytoplasmic protein. Its interaction with dynamin (a GTPase implicated in clathrin-mediated endocytosis of synaptic vesicle membranes) and neural Wiskott-Aldrich syndrome protein (an actin-depolymerizing protein), suggests its role in cytoskeletal dynamics and synaptic vesicle formation, and transport and recycling at the pre-synaptic nerve terminal

[31]. Glutamine synthetase (GS, GLNA, no. 60) is a key enzyme in the brain's glutamate-glutamine cycle. It also plays an important role in protecting neurons against excitotoxicity by converting excess ammonia and glutamate into glutamine [32]. Though commonly found in astrocytes, the detection of GS in cortical synaptosomes should not be surprising. This is because of the extreme anatomical and communicational proximity of astrocytes and neuron, and the enrichment of glutamatergic neurons in the cortex.

Neuron-specific enolase (NSE) is an enzyme of the glycolytic pathway, which is found in numerous isomeric forms. Alpha (ENOA) and gamma enolases (ENOG), enzymes of the glycolytic pathway, are present specifically in neuronal cell cytoplasm and dendrites [33] and constitute the so-called NSE. ENOG has been shown to be located in cells of neuroectodermal origin and constitutes approximately 1.5% of the total soluble protein in the brain. Both ENOA and ENOG are also found in the synaptic membrane as homo- and hetero-dimers [34]. On the 2-D map, spots 51–53 were identified as ENOG and spots 47–50 as ENOA. Beyond being a neuronal marker, NSE can be released from distressed neurons into the cerebrospinal fluid and peripheral blood, serving as a biomarker of parenchymal brain injury [35]. Neuronal protein NP25 (no. 133) is also a neuron-specific protein present in highly differentiated neural cells [36].

In addition to the proteins that serve specific neuronal or synaptic structure and function, some of the proteins resolved by 2-DE are present universally in various cell types, but still play a crucial role in neurotransmission. For instance, actin (ACTB, nos. 54 and 55) is a cytoplasmic cytoskeleton protein that can be found in all cell types. At the synapse, actin filaments harbor some of the synaptic vesicles, forming a reserve pool. As mentioned above, synapsins serve as anchors for the vesicles. CaM (nos. 136, 139, 142, and 143) is a universal acidic calcium-binding protein, in virtually all eukaryotic cells, which regulates the activity of target molecules such as protein kinases, adenylyl cyclase, and nitric oxide (NO) synthase. Binding of CaM to various cytoskeletal proteins, such as the tubulins (nos. 11–15, nos. 18–24), microtubule-associated protein-2 (MAP-2), tau, and fodrin, appears to affect the cell shape, motility, secretion, and transport [37]. The activity of CaM is regulated by a variety of covalent modifications, such as methylation, phosphorylation, ubiquitinylation [38,39] and glycoslylation (see Table 2), and these likely account for its heterogeneous appearance on the 2-D gel pattern. While methylation and phosphorylation only cause slight mass alterations, ubiquitylation can increase the mass of CaM by 50% or more [40]. In Fig. 1, CaM appears as a group of unique spots with similar p*I*, but different molecular weights. Whether the heterogeneities in CaM migration (mass and charge) observed here are the result of the above modifications or proteolysis, as suggested by the ID of spot 143 as a CaM fragment, remains to be determined.

Intact synaptosomal preparations are expected to contain mitochondria that reside near the synapse, and several mitochondrial proteins are found in Table 1. For example, ATP synthase is a mitochondrial protein that catalyzes ATP production in the presence of proton gradient [41]. Several subunits of the ATP synthase complex were resolved on the 2-D map, including the α-chain (ATPA, nos. 33–39), β-chain (ATPB, nos. 43–46), D-chain (ATPQ, no. 132), and E-chain (ATPJ, no. 160). The presence of these ATP synthase components is essential to synaptic function because they are involved in the synthesis of ATP. The packaging of neurotransmitters into the synaptic vesicles through vesicle transporters [42] and the transportation of Ca^{++} from the cytoplasm into the ER or extracellular fluid via the Ca^{++} pump are fueled by the hydrolysis of ATP [43]. Another mitochondrial protein detected by 2-DE and shotgun proteomics is VDAC. VDAC1 (POR1) was resolved as a complex charge train (~p*I* 8.4) (nos. 101, 103–108), suggesting possible PTMs or heterogeneous isoforms. VDAC2 (POR2) also appears on the 2-D map (nos. 99 and 102) with both VDACs resolved at or near their predicted p*I*. VDACs are outer mitochondrial membrane proteins with weak anion selectivity in the open

state, producing anion fluxes, including ATP, which regulate mitochondrial function. Several reports have confirmed their multi-topological localization, particularly in post-synaptic membrane structures [44,45]. Interestingly, it has been shown that certain isoforms of these channel proteins can be up- or down-regulated in a certain cortical area in pathological conditions.

In comparison to 2-DE which identified two t-SNARE proteins, VAMP-3, and the Ca^{++} sensor synaptotagmin I, LC-MS/MS identified VAMP-2 in its glycosylated form (Table 3) and three isoforms of synaptotagmin (I, II, V, Table 2). The detection of two of the three forms of the VAMPs is supported by their tissue-specific expression, because VAMP-1 is more abundant in the spinal cord, while VAMP-2 is highly expressed in the brain, and VAMP-3 has ubiquitous tissue distribution [46,47]. Although all three forms of synaptotagmin are abundantly expressed in the brain, synaptotagmin I is preferentially expressed in rostral, phylogenetically younger brain regions; synaptotagmin II is predominant in caudal, phylogenetically older brain regions, and synaptotagmin V has a wider peripheral tissue distribution [48,49]. In addition to synapsin II, also identified by 2-DE, LC-MS/MS detected synapsin I. Three forms of free syntaxins, 1A, 1B, and seven were also identified, as was a syntaxin binding protein 1 (n-Sec1/Unc-18–1). As either cytoplasmic or membrane-associated, n-Sec1/Unc-18–1 binds to syntaxin, thereby regulating synaptic transmission.

Two subunits of clathrin were identified by LC-MS/MS, light chain B and heavy chain. The latter was also found to be modified by acetylation and glycosylation (Table 2). Clathrin is the major protein of polyhedral coat of coated pits and vesicles, playing an important role in the endocytotic retrieval and transport (recycling process) of vesicle membrane components from the pre-synaptic membrane [50]. Two additional proteins related to clathrin that were identified include clathrin coat assembly protein (AP180) and subunits of clathrin-associated adaptor protein complexes.

While most proteomic platforms have an inherent and variable bias in identifying certain types of proteins (*e.g.*, hydrophilic, ionizable peptides, *etc.*), the combination of several proteomic techniques in the present study has offered complementary approaches. Figure 3 summarizes the major pre-synaptic proteins identified by 2-DE/MS and/or shotgun proteomics. Interestingly, most of the neuro-transmission regulating proteins were identified either by one or both the technique(s). The application of the PTM-detection option in the sequence database search greatly increased the likelihood of protein identification and suggests that PTM is common in synaptosomal proteins. Several proteins, such as neurexins, vesicular neurotransmitter reuptake transporters, and some components of SNAPs were left unidentified, as were neurotransmitter receptor proteins. Their absence is likely due to their unique biochemical properties (hydrophobicity, low abundance, *etc.*), which are unfavorable for identification in this proteomic approach.

Several adjacent spots on the 2-D map were assigned identical protein IDs, but by different MS analysis. In such cases, the LC-MS/MS results provide confirmatory evidence for the accuracy of the PMF ID. More importantly these “charge trains” on a 2-D map typically represent a single protein resolved at varying p*I*, due to PTM. For example, spot nos. 20–24 were all identified as tubulin β-chain (TBB1), with spot 20 identified by LC-MS and the rest by PMF. It has been shown that brain tubulins exhibit a significant charge heterogeneity, with up to 21 charge variants (for both α- and β-subunits) observed in different studies [51,52]. Phospho-rylation [53] and polyglycosylation [54] have been reported for tubulin β. As indicated in Tables 2 and 3, tubulin β was detected by LC-MS/MS with methylation, M and H oxidation, and glycosylation of various peptides. It has been shown that reductive methylation of the tubulin dimer with formaldehyde and sodium cyanoborohydride greatly inhibits the microtubule assembly [55], with the β-subunit being more susceptible to methylation than the

α-subunit [56], although we observed methylation in both. The ability to observe and determine modifications in this way will be of great importance in future studies using this synaptosomal preparation in assessing the effects of alcohol ingestion, neurotoxins, *etc.*

Other modified proteins identified by LC-MS/MS include glyceraldehyde-3-phosphate dehydrogenase (GAPDH, G3P, nos. 82, 84, 86–90, 92, and 97), creatine kinase B chain (KCRB, nos. 56 and 57), triosephosphate isomerase (nos. 117, 120, 122–124), ubiquitin carboxy-terminal hydrolase isozyme L1 (nos. 112 and 113), ATP synthase β-subunit (ATPB, nos. 43–46), actin (nos. 54 and 55), and protein kinase C and casein kinase substrate in neurons protein 1 (PAC1, nos. 40 and 41). Though the chemical nature and the physiological relevance of these PTMs is beyond the scope of this manuscript, these results demonstrate the unique power of 2-DE in resolving the differentially modified protein charge forms and quantifying the extent of modification [57] established by mass spectrometric techniques.

Overall, the results of the current study indicate that a sample preparation incorporating pre-fractionation and enrichment of specific cell components can improve the capability of proteomics techniques to detect important synaptic proteins from brain tissues. In the present study, the proteome profile of cerebral cortical synaptosomes indicates that major protein components involved in synaptic vesicle trafficking and docking, post-synaptic densities, transporters and receptors, mitochondrial function and the glycolytic pathway can be detected and their relative expression studied. Because these are the proteins normally present in intact nerve endings, an approach that uses sub-cellular fractionation to produce synaptosomes may prove to be useful in proteomic studies of brain function where neural, not glial proteins, are of interest.

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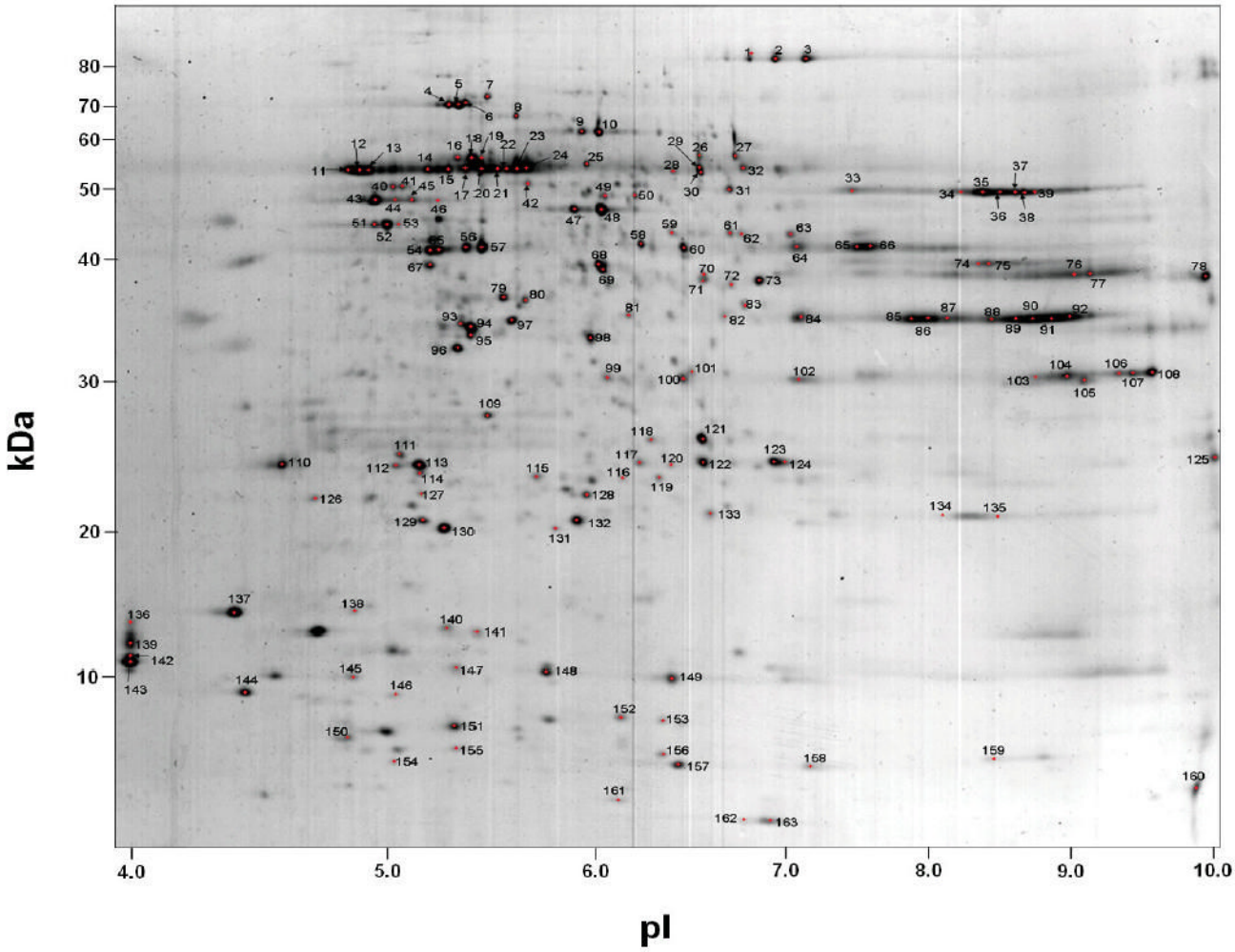

Figure 1.

Representative 2-DE pattern of synaptosomal proteins stained with colloidal CBB. Proteins (200 μg) were focused on 24 cm IPG strips pH 3–10, followed by SDS-PAGE in a linear acrylamide gradient. Protein spots were cut from the gel, tryptically digested, and identified either by MALDI-MS or LC-MS/MS. These are numbered arbitrarily 1–163 and appear in Table 1 along with their PDQuest spot number assignments and other pertinent information. Axes were calibrated based on calculated p*I* and mass from identified proteins, using the Compute p*I*/Mw Tool (<http://us.expasy.org/tools/pi_tool.html>).

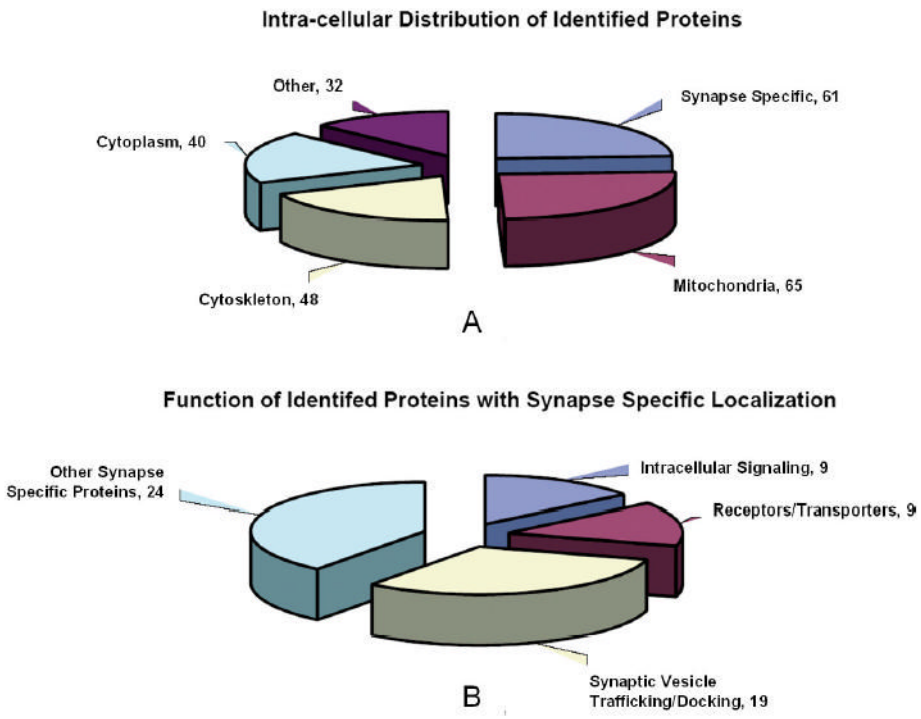

Figure 2.

A: Intracellular distribution of all 254 unique synaptosomal proteins identified by either 2-DE/MS or LC-MS/MS. The synapse-specific fraction was determined using a manual search of PubMed™. Automated categorization of the remaining 193 proteins was performed using the GO *via* the webtool Pandora. Of these 193 proteins, 24 were successfully categorized. Estimated fractions were then extrapolated from the distribution of proteins in this subset. B: Distribution by synaptic function of those 61 proteins identified by manual PubMed search as having a synapse-specific function.

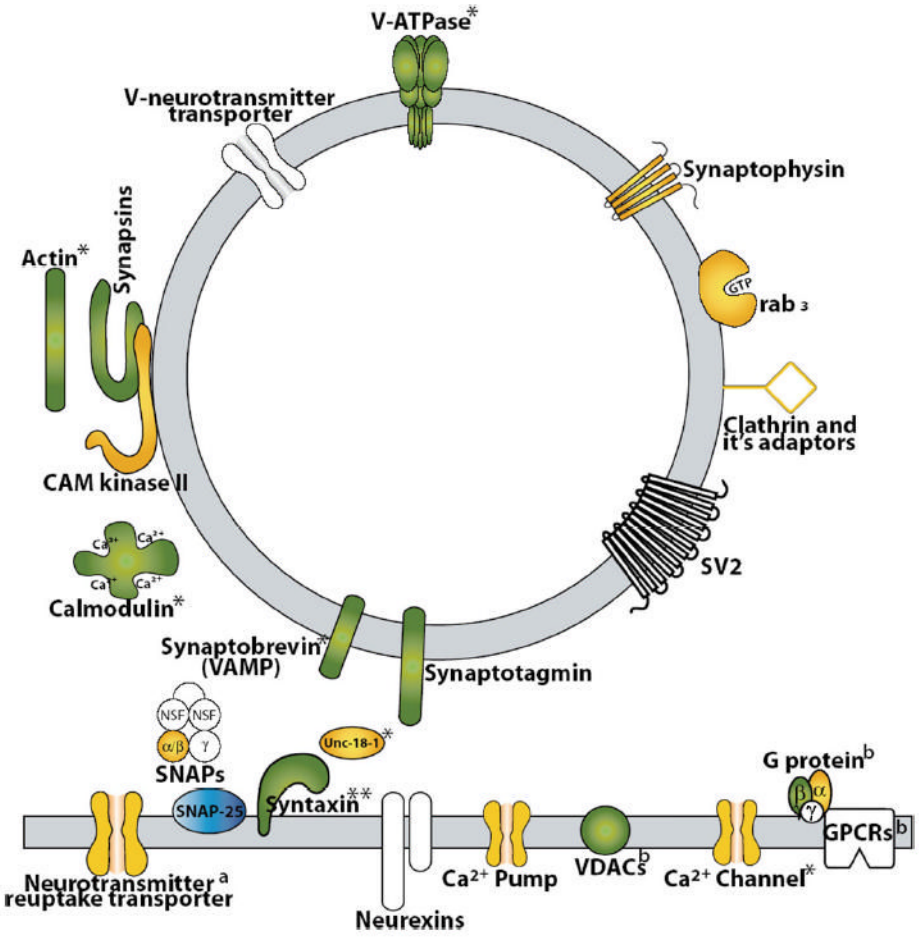

Figure 3.

Diagrammatic illustration of the major pre- and post-synaptic proteins identified by 2-DE/MS and/or shotgun proteomics, and normally expected as constituent in synaptosomal preparations. Blue: The proteins that were identified by 2-DE/MS; yellow, the proteins that were identified by shotgun proteomics; green, the proteins that were identified by both 2-DE/MS and shotgun proteomics; and blank (white), those major constituents expected but *not* identified. *Proteins were identified by shotgun proteomics only after the PTM analysis; **Proteins were identified as a complex with other proteins by 2-DE/MS; (a) EAA1, EAA2, and GABA transporter. b: Post-synaptic proteins.

Table 1

Synaptosomal proteins cut from the 2-D gel and identified by either PMF or peptide sequencing *via* LC-MS/MS

#	SSP	NCBI accession	Swiss-Prot entry name	Protein ID	*Z-score*	p*I*	Mass (kDa)	%c	MS/MS (sequence data)
1	6813	NP_077374.1	Q99KI0	Mitochondrial aconitase (nuclear aco2 gene)	1.07	8.2	86.2	13	—
2	6815	NP_077374.1	Q99KI0	Mitochondrial aconitase (nuclear aco2 gene)	2.4	8.2	86.2	20	—
3	7801	NP_077374.1	Q99KI0	Mitochondrial aconitase (nuclear aco2 gene)	2.39	8.2	86.2	23	—
4	2807	S31716	BQ078983*	DNAk-type molecular chaperone hsp72-ps1	2.43	5.4	71.1	34	—
5	3801	S31716	BQ078983*	DNAk-type molecular chaperone hsp72-ps1	2.43	5.4	71.1	38	—
6	3802	P08109	HS7C_ MOUSE	Heat shock cognatete 71 kDa protein	—	5.4	70.8	—	LLQDFFNGK; FEELNADLFR; IINEPTAAAIAYGLDK
7	3811	P48721	GR75_RAT	DNAk-type molecular chaperone grp75 precursor	2.43	5.9	74.0	35	—
8	3815	P08461	ODP2_RAT	Dihydrolipoamide acetyl-transferase component of pyruvate dehydrogenase	—	−5.7	58.7	—	ISVNDFIIK; YLEKPVTMLL + oxidation (M)
9	4813	P47942	DPY2_RAT	Dihydropyrimidinase related protein-2 (DRP-2) (collapsin response mediator protein 2)	2.3	6.0	62.7	20	—
10	4815	P47942	DPY2_RAT	DRP-2 (collapsin response mediator protein°2)	2.37	6.0	62.7	24	—
11	1704	P04691	TBB1_RAT	Tubulin beta chain 15	2.43	4.8	50.4	31	—
12	1705	P05218	TBB5_HUMAN	Tubulin, beta 5	2.39	4.8	50.1	31	—
13	1708	P04691	TBB1_RAT	Tubulin beta chain	—	4.8	49.9	—	FPGQLNADLR; INVYYNEAAGNK; NSSFBYVEWIPNNVK + 8 additional peptides
14	2713	P04691	TBB1_RAT	Tubulin beta chain	—	4.8	49.9	—	AIL VDLEPGTMDSVR + oxidation (M); NSSYFVEWIPNNVK; GHYTEGAELVDSVLDVVR + 2 additional peptides
15	2717	P05218	TB B5_ HUMAN	Tubulin, beta 5	2.43	4.8	50.1	23	—
16	2718	P19226	CH60_MOUSE	60 kDa heat shock protein	—	5.9	60.9	—	DIGNIISDAMK + oxidation (M); GYISPBYFINTSK; TLNDELE TIEGMK + oxidation (M); TAL LDAAGVASLLTTAEAVVTEIPK
17	3703	P02571	ACTG_HUMAN	Actin, cytoplasmic 2, gamma	—	5.3	41.8	—	EITALAPSTMK; DSYVGDEAQSK; SYELPDGQVITIGNER; VAPEEHPVLLTEALNPK + 1 additional peptide
18	3704	P05218	TBB5_HUMAN	Tubulin, beta 5	2.43	4.8	50.1	27	—
19	3705	P05218	TBB5_HUMAN	Tubulin, beta 5	2.43	4.8	50.1	27	—
20	3708	P04691	TBB1_RAT	Tubulin beta chain	—	4.8	49.9	—	FPGQLNADLR; NSSFYFVEWIPN NK; ALTVPELTQQMFDSK + oxidation (M) + 3 additional peptides
21	3712	P04691	TBB1_RAT	Tubulin beta chain 15	2.43	4.8	50.4	36	—
22	3716	P04691	TBB1_RAT	Tubulin beta chain 15	2.43	4.8	50.4	36	—
23	3719	P04691	TBB1_RAT	Tubulin beta chain 15	2.43	4.8	50.4	36	—
24	3720	P04691	TBB1_RAT	Tubulin beta chain 15	2.43	4.8	50.4	36	—
25	4716	P21707	SYT1_RAT	Synaptotagmin I	—	—	8.4	47.4	TLNPVFNEQFTFK
26	6703	Q63537	SYN2_RAT	Synapsin II	—	8.7	63.4	—	MNQLLSR + oxidation (M); ILGDYDIK; QLITDLVISK; EMLTLPTFPVVVK + 2 additional peptides
27	6709	NP_445749.1	KPY2_MOUSE	Pyruvate kinase 3	2.39	6.6	58.3	20	—
28	5716	P15999	ATPA_RAT	ATP synthase alpha chain	—	9.2	58.8	—	IMNVIGEPIDER + oxidation (M); VALTGLTVAEYFR; EGNDLYHE MIESGVINLK + oxidation (HW)

#	SSP	NCBI accession	Swiss-Prot entry name	Protein ID	*Z-score*	p*I*	Mass (kDa)	%c	MS/MS (sequence data)
29	6702	Q63537	SYN2_RAT	Synapsin II	—	8.7	63.4	—	TPALSPQR; ILGDYDIK; QLITDL VISK; SFRPDFVLIR; FPLIEQ TYYPNHR; TNTGSAMLE QIAMSDR + 2 oxidation (M)
30	6704	NP_476554.1	Q8TAU2	Pancreatic lipase-related protein 2	2.43	6.0	54.8	10	—
31	6605	AAA61256	PYR5_HUMAN	Orotidine 5′-monophosphate decarboxylase (EC 4.1.1.23)	2.43	6.6	51.5	15	—
32	6710	Q63537	SYN2_RAT	Synapsin II	—	8.7	63.4	—	ILGDYDIK; SFRPDFVLIR; EMLTLPTFPVVVK + oxidation (M); VLLVVDEPHTDWAK
33	7601	P15999	ATPA_RAT	ATP synthase alpha chain	—	9.2	58.8	—	QAVAYR; HALIIYDDSK; ILGADTSVDLEETGR; TGAIVDVPVGDELLGR
34	8601	P15999	ATPA_RAT	ATP synthase alpha chain	—	9.2	58.8	—	QAVAYR; LTELLK; APGIIPR; ELIIGDR; VLSIGDGIAR; AVDSLVPIGR; HALIIYDDLSK + 6 additional peptides
35	8603	1MAB	—	Chain A, rat liver F-1 Atpase	2.23	8.4	55.4	22	—
36	8604	1MAB	—	Chain A, rat liver F1-Atpase	2.23	8.4	55.4	22	—
37	8605	P1599	ATPA_RAT	ATP synthase alpha chain	—	9.2	58.8	—	QAVAYR; APGIIPR; VGSAAQTR; STVAQLVK; FNDGTDEK; VLSIGDGIAR; AVDSLV PIGR + 9 additional peptides
38	8606	P15999	ATPA_RAT	ATP synthase alpha chain	—	9.2	58.8	—	QAVAYR; LTELLK; QMSLLLR + oxidation (M); FNDGTDEK; VLSIGDGIAR; AVDSLVPIGR + 7 additional peptides
39	8608	P15999	ATPA_RAT	ATP synthase alpha chain	—	9.2	58.8	—	LTELLK; APGIIPR; ELIIGDR; STVAQLVK; OMSLLLR + oxidation (M); EPMQTGIK; VLSIGDGIAR + 8 additional peptides
40	2701	Q9Z0W5	PAC1_RAT	Protein kinase C and casein kinase substrate in neurones protein 1	—	5.2	50.4	—	QLIEK; VLEDVGK; ELEQAIR; GSVSSYDR; GADAQEDLR + 9 additional peptides
41	2707	Q9Z0W5	PAC1_RAT	Protein kinase C and casein kinase substrate in neurons protein 2	—	5.2	50.4	—	VLEDVGK; VSELHQEVK; NSLLNEDLEK; TEQSVTPEQQK + 3 additional peptides
42	4702	P08461	ODP2_RAT	Dihydroliponamide succinyltransferase component of 2-oxoglutarate dehydrogenase	—	8.2	47.4	—	EAVTFLR; GLVVPVIR; TINELGEK
43	1610	P10719	ATPB_RAT	ATP synthase beta subunit	2.43	4.9	51.2	28	—
44	2601	P10719	ATPB_RAT	ATP synthase beta chain	—	5.2	56.3	—	VLDSGAPIK; IGLFGGAGVGK; IPVGPETLGR; VVDLLAPYAK + 7 additional peptides
45	2604	P10719	ATPB_RAT	Chain B, rat liver F1-ATPase	2.43	4.9	51.3	19	—
46	2608	P10719	ATPB_RAT	ATP synthase beta chain	—	5.2	56.3	—	VVDLLAPYAK; VALTGLTVAEYFR; DQEGQDVLLFIDNIFR + 2 additional peptides
47	4607	P04764	ENOA_RAT	Alpha enolase (2-phospho-D-glycerate hydrolyase) (non-neural enolase, NNE	2.43	5.8	47.5	23	—
48	4613	P04764	ENOA_RAT	Alpha enolase (2-phospho-D-glycerate hydrolyase) NNE (enolase 1)	2.43	6.2	47.4	50	—
49	5602	P04764	ENOA_RAT	Alpha enolase (2-phospho-D-glycerate hydrolyase) NNE (enolase 1)	—	5.8	47.0	—	EALELLK; IEEELGSK; LNVVEQEK; KLNVVEQEK; GNPTVEVDLY TAK + 3 additional peptides
50	5605	P04764	ENOA_RAT	Alpha enolase (2-phospho-D-glycerate hydrolyase) NNE (enolase 1)	—	5.8	47.0	—	YITPDQLADLYK; AAVPSGAST GIYEALELR; LAMQEFMILPV GASSFR + 2oxidation (M)
51	1611	NP_647541	Q922A0	Enolase 2, gamma; enolase 2, gamma, neuronal	2.43	5.0	47.5	25	—

#	SSP	NCBI accession	Swiss-Prot entry name	Protein ID	*Z-score*	p*I*	Mass (kDa)	%c	MS/MS (sequence data)
52	1612	NP_647541	Q922A0	Enolase 2, gamma; enolase 2, gamma, neuronal	2.43	5.0	47.5	25	—
53	2602	P07323	ENOG_RAT	Enolase, gamma	—	5.0	47.0	—	LGAEVYHTLK; GNPTVEDLHTAK; AAVPSGASTGIYEALELR; AVD HINSTIAPALISSGLSWEQEK
54	2508	NP_112406	ACTB_RAT	Cytoplasmic beta-actin	2.43	5.3	42.1	28	—
55	2513	P02570	ACTB_HUMAN	Actin beta	2.43	5.3	42.1	32	—
56	3502	P07335	KCRB_RAT	Creatine kinase-B	2.43	5.3	40.9	29	—
57	3504	P07335	KCRB_RAT	Creatine kinase, brain	2.43	5.3	43.0	48	—
58	5505	NP_653134.2	—	Tribbles homolog 2	2.43	5.8	39.4	11	—
59	5508	P02551	TBA1_MOUSE	Tubulin alpha-1 chain	—	4.9	50.1	—	LIGQIVSSITASLR
60	6502	1717354A	GL-_RAT	Glutamine synthetase	2.43	6.4	41.2	19	—
61	6507	P26284	ODPA_RAT	Pyruvate dehydrogenase E1 component alpha subunit	—	8.5	43.2	—	SDPIMLLK + oxidation (M); AAASTDYYK; RGDFIPGLR; LEEGPPVTTVLTR + 3 additional peptides
62	6509	P14408	FUMH_RAT	Fumarate hydratase	—	9.1	54.4	—	LHDALSAK; IEYDTFGELK; VAALTGLPFVTAPNK
63	7501	NP_080215.1	PUR6_MOUSE	Phosphoribosylaminoimidazole carboxylase [*Mus musculus*]	2.43	7.0	47.7	17	—
64	7502	P25809	KCRU_RAT	Creatine kinase	—	8.9	47.3	—	LPLLSK; SGYFDER; HTTDLDASK; VVVDALSGLK; GWEFMWNER + oxidation (M) + 2 additional peptides
65	7505	XP_215806.1	KCRU_RAT	Creatine kinase, mitochondrial 1, ubiquitous	2.38	8.9	47.3	—	—
66	7508	XP_215806.1	KCRU_RAT	Creatine kinase, mitochondrial 1, ubiquitous	2.26	8.9	47.3	29	—
67	2511	Q99963	SH33_HUMAN	SH3-domain GRB2-like protein 2	2.43	5.3	40.1	37	—
68	4511	NP_446383.1	—	Glycoprotein lb (platelet), beta polypeptide	2.43	6.3	44.4	40	—
69	4512	NP_446383.1	—	Glycoprotein lb (platelet), beta polypeptide	2.43	6.3	44.4	15	—
70	6403	P09117	ALFC_RAT	Fructose-bisphosphate aldolase C	—	6.8	39.1	—	DNAGAATEEFIK; GILAA DESVGSMAK + oxidation (M); LSQIGVENTEENR; YSPEEIA MATVTALR + oxidation (M)
71	6404	P09117	ALFC_RAT	Fructose-bisphosphate aldolase C	—	6.8	39.1	—	QVLFSADDR; TPSALAILENAN-VLAR; YSPEEIAMATV-TALR + oxidation (M)
72	6406	P09117	ALFC_RAT	Fructose-bisphosphate aldotase C	—	6.8	39.1	—	TPSALAILENANVLAR; YSPEEIA MATVTALR + oxidation (M)
73	6409	CAA30044.1	ALFC_RAT	Brain-specific rat aldolase C	2.43	6.8	39.6	23	—
74	8506	P16617	PGK2_RAT	Phosphoglycerate kinase	—	7.5	44.4	—	YSLEPVAAELK
75	8508	P16617	PGK2_RAT	Phosphoglycerate kinase	—	7.5	44.4	—	DVLFLK, YAEAVAR; KYAEAVAR; YSLEPVAAELK; LGDVYVN DAFGTAHR + 2 additional peptides
76	8418	AAL99984.1	Q8R4B4	Down syndrome cell adhesion molecule-like protein	2.08	9.6	40.7	22	—
77	8419	P05065	ALFA_RAT	Fructose-bisphosphate aldolase A	—	8.4	39.2	—	PFPQVIK, ELADIAHR; AAQEEYIK; QLLLTADDR; GILAA DESTGSIAK; LQSIGTEN TEENR + 2 additional peptides
78	9408	NP_037309.1	AATM_RAT	Glutamate oxaloacetate transaminase 2	2.43	9.4	47.7	33	—
79	3408	NP_446090	AB047541*	Isocitrate dehydrogenase 3(-D +) alpha	2.43	6.5	40.1	33	—
80	4401	NP_620266	Y15068*	Stress-induced phosphoprotein 1	1.56	6.1	40.7	31	—
81	5402	P26284	ODPA_RAT	Pyruvate dehydrogenase E1 component alpha subunit	—	8.5	43.2	—	EEIQEVR; AAASTDYYK; LEEGPPVTTVLTR; YGMGTS VER + oxidation (M) + 2 additional peptides

#	SSP	NCBI accession	Swiss-Prot entry name	Protein ID	*Z-score*	p*I*	Mass (kDa)	%c	MS/MS (sequence data)
82	6405	P04797	G3P_RAT	Glyceraldehyde-3-phosphate dehydrogenase	—	8.4	35.7	—	GAAQNIIPASTGAAK; VPTPNVS VVDLTCR + carbamidomethyl (C); LISWYDNEYGYSNR + 1 additional peptide
83	6408	P51635	AKA1_RAT	Alcohol dehydrogenase	—	6.8	36.4	—	YIVPMITVDGK + oxidation (M); QIDDVLSVASVR; GLEVTAYS PLGSSDR; HPDEPVLLEEPVV LALAEK
84	7403	NP_058704.1	Q8K4T7	Glyceraldehyde-3-phosphate dehydrogenase	1.45	8.4	36.1	20	—
85	7409	NP_034342.1	Q8VDP9	Four and a half LIM domains 2	2.43	7.8	34.1	40	—
86	7410	XP_214333.1	G3P_RAT	Glyceraldehyde-3-phosphate dehydrogenase	2.43	7.8	36.1	29	—
87	7413	P04797	G3P_RAT	Glyceraldehyde-3-phosphate dehydrogenase	—	8.4	35.7	—	LVINGK; PITIFQER; VVDLMAY MASK + 2 oxidation (M); GAAQNIIPASTGAAK; LISWYDNEYGYSNR + 1 additional peptide
88	8407	NP_058704.1	Q8K4T7	Glyceraldehyde-3-phosphate dehydrogenase	2.4	8.4	36.1	44	—
89	8410	NP_058704.1	Q8K4T7	Glyceraldehyde-3-phosphate dehydrogenase	2.4	8.4	36.1	44	—
90	8412	NP_058704.1	Q8K4T7	Glyceraldehyde-3-phosphate dehydrogenase	2.4	8.4	36.1	44	—
91	8415	NP_058704.1	Q8K4T7	Glyceraldehyde-3-phosphate dehydrogenase	2.4	8.4	36.1	44	—
92	8417	NP_058704.1	Q8K4T7	Glyceraldehyde-3-phosphate dehydrogenase	2.4	8.4	36.1	44	—
93	3303	P54313	GBB2_RAT	Guanine nucleotide-binding protein G(1)/G(S)/G(T)	—	5.6	37.5	—	LIIWDSYTTNK
94	3306	P54311	GBB1_RAT	Transducin beta (guanine nucleotide-binding protein beta subunit 1)	2.4	5.5	38.2	24	—
95	3305	P54313	GBB2_RAT	Guanine nucleotide-binding protein G(I)/G(S)/G(T)	—	5.6	37.5	—	LIIWDSYTTNK
96	3301	ODPB_RAT	ODPB_RAT	Pyruvate dehydrogenase E1 component beta subunit, mitochondrial precursor (PDHE1B)	2.43	5.9	39.3	34	—
97	3312	P42123	LDHB_RAT	Lactate dehydrogenase B	2.29	5.7	36.9	25	—
98	4309	NP_150238	O88989	Malate dehydrogenase 1; malate dehydrogenase, soluble	2.21	6.2	36.6	28	—
99	5301	P81155	POR2_RAT	Voltage-dependent anion-selective channel protein 2	—	7.4	31.7	—	YQLDPTASISAK; LTFDTTFSPNTGK
100	6301	NP_058927.1	TPIS_RAT	Phosphatidylinositol transfer protein	2.43	6.0	32.2	22	—
101	6304	Q9Z2L0	POR1_RAT	Voltage-dependent anion-selective channel protein 1	—	8.4	32.4	—	DVFTK; VTQSNFAVGYK; LTFDSSFSPNTGK
102	7301	P81155	POR2_RAT	Voltage-dependent anion-selective channel protein 2	—	7.4	31.7	—	GFGFGLVK; LTLSALVDGK; YQLDPTASISAK; LTFDTTFSPNIGK; TGDFQLHTNVNNGTEFGG SIYQK + 1 additional
103	8306	Q9Z2L0	POR1_RAT	Voltage-dependent anion-selective channel protein 1	—	8.4	32.4	—	WTEYGLTFTEK; LTFDSSFSPNTGK
104	8309	NP_112643.1	POR1_RAT	Voltage-dependent anion channel 1	2.43	8.8	30.9	24	—
105	8311	NP_112643.1	POR1_RAT	Voltage-dependent anion channel 1	2.43	8.8	30.9	24	—
106	8312	Q9Z2L0	POR1_RAT	Voltage-dependent anion-selective channel protein 1	—	8.4	32.4	—	DVFTK; LTLSALLDGK; VTQSNFAVGYK; WTEYGLTF TEK; LTFDSSFSPNTGK + 2 additional peptides

#	SSP	NCBI accession	Swiss-Prot entry name	Protein ID	*Z-score*	p*I*	Mass (kDa)	%c	MS/MS (sequence data)
107	9301	NP_112643.1	POR1_RAT	Voltage-dependent anion channel 1	2.43	8.8	30.9	43	—
108	9304	NP_112643.1	POR1_RAT	Voltage-dependent anion channel 1	2.43	8.8	30.9	43	—
109	3204	P24142	PHB_MOUSE	Prohibitin	2.43	5.4	27.8	30	—
110	210	NP_112253	SN25_RAT	SNAP 25 synaptosomal-associated protein, 25 kDa	2.43	4.7	23.5	33	—
111	2202	P54313	GBB2_RAT	Guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 2	—	6.5	37.5	—	GHLAK; AGVLAGHDNR; TFVSGACDASIK + carbamidomethyl (C); LIIWDSYTTNK
112	2201	Q00981	UBL1_RAT	Ubiquitin carboxyl-terminal hydrolase isozyme L1	—	5.1	24.8	—	QIEELK; QFLSETEK; MPFPVNHGASSEDSLLQ DAAK + oxidation + P29(M)
113	2207	Q00981	UBL1_RAT	Ubiquitin carboxy-terminal hydrolase L1 (cerebral protein-6)	2.43	5.1	25.1	51	—
114	2208	P19234	NUHM_RAT	NADH-ubiquinone oxidoreductase 24 kDa subunit	—	6.0	26.5	—	DSDSILETLQR; AAAVLPVLDLAQR
115	4201	O35244	PDX6_RAT	Peroxiredoxin 6	2.43	5.6	24.9	23	—
116	5204	P22062	PIMT_RAT	Protein-L-isoaspartate (D-aspartate) *O*-methyltransferase	—	7.3	24.5	—	LVVGDGR; VFEVMLATDR + oxidation (M); ELVDDSITNVK; SGGASHSELIHNLR + 1 additional peptide
117	5208	P48500	TPIS_RAT	Triosephosphate isomerase	—	6.5	26.8	—	VVFEQTK; FFVGGNWK; TATPQQAQEVHEK; HIFGESDELIGQK + 2 additional peptides
118	5210	P25113	PMG1_RAT	Phosphoglycerate mutase 1	—	6.2	28.5	—	FSGWYDADLSPAGHEEAK
119	5211	Q9Z2L0	POR1_RAT	Voltage-dependent anion-selective channel protein 1	—	8.4	32.4	—	DVFTK; GYGFGLIK; VTQSNFAV GYK; WTEYGLTFTEK; LTFDSSFSPNTGK + 2 additional peptides
120	5213	P48500	TPIS_RAT	Triosephosphate isomerase	—	6.5	26.8	—	TATPQQAQEVHEK; HIFGE SDELIGQK; VVLAYEPV WAIGTGK + 3 additional peptides
121	6202	JC1132	PMG2_RAT	Phosphoglycerate mutase (EC 5.4.2.1) B chain	2.43	6.7	28.9	65	—
122	6204	NP_075211.1	TPIS_RAT	Triosephosphate isomerase 1	2.43	6.5	27.4	62	—
123	6212	NP_075211.1	TPIS_RAT	Triosephosphate isomerase 1	2.43	6.5	27.4	39	—
124	6213	NP_075211.1	TPIS_RAT	Triosephosphate isomerase 1	2.43	6.5	27.4	43	—
125	9204	NP_032996.1	PTHR_RAT	Parathyroid hormone-related protein; PTH-related peptide	2.43	10.7	20.1	61	—
126	1101	P14701	TCTP_MOUSE	Translationally controlled tumor protein	—	4.8	19.5	—	DLISHDELFSDIYK
127	2106	P14701	TCTP_MOUSE	Translationally controlled tumor protein	—	4.8	19.5	—	DLISHDELFSDIYK
128	4110	NP_476484	AF157511*	SP22 (fertility protein)	2.43	6.3	20.2	39	—
129	2107	P35704	PDX2_RAT	Peroxiredoxin 2; thioredoxin peroxidase 1	2.43	5.3	21.9	34	—
130	2111	P31044	PEBP_RAT	Phosphatidylethanolamine binding protein; hippocampal cholinergic neurostimulating peptide	2.4	5.5	20.9	60	—

#	SSP	NCBI accession	Swiss-Prot entry name	Protein ID	*Z-score*	p*I*	Mass (kDa)	%c	MS/MS (sequence data)
131	4106	P04631	S10B_RAT	S-100 protein, beta chain	—	4.5	10.6	—	AMVALIDVFHQYSGR + oxidation (M)
132	4109	P31399	ATPQ_RAT	ATP synthase subunit d	2.3	6.2	18.8	42	—
133	6101	P37805	NP25_RAT	Neuronal protein NP25	—	6.5	24.7	—	DMAAVQR; GPSYGLSR; AAE VYGVR; GFSEEQLR; YDAD LENK + 7 additional peptides
134	7108	P07895	SODM_RAT	Superoxide dismutase	—	9.0	24.7	—	DFGSFEEK; YHEALAK; GELLEAIK; NVRPDYLK; GDVTTQVALQ PALK; AIWNVINWENVSQR
135	8106	P07895	SODM_RAT	Superoxide dismutase	—	9.0	24.7	—	GELLEAIK; NVRPDYLK; GDVTTQVALQPALK
136	107	P02593	CALM_HUMAN	Calmodulin	—	4.1	16.7	—	ELGTVMR + oxidation (M); DTDSEEEIR
137	111	Q63754	SYUB_RAT	Synuclein, beta	1.51	4.5	14.5	26	—
138	1108	P01946	HBA_RAT	Hemoglobin alpha-1 and alpha-2 chains	—	7.9	15.2	—	FLASVSTVLTSK
139	105	P02593	CALM_HUMAN	Calmodulin	—	4.1	16.7	—	ELGTVMR + oxidation (M); EAFSLFDK; DTDSEEEIR; DGNGYISAAELR; EAFSLFDKDGDGTITTK
140	2112	Q63228	GLMB_RAT	Glia maturation factor beta	—	5.3	16.6	—	LVQTAELTK; LVVLDEE LEGVSPDELK
140	2112	P13668	STN1_RAT	Stathmin	—	5.8	17.1	—	SHEAVLK; DLSLEEIQK; ASGQA FELILSPR
141	3104	P13668	STN1_RAT	Stathmin	—	5.8	17.1	—	ASGQAFELILSPR
142	104	P02593	CALM_HUMAN	Calmodulin	—	4.1	16.7	—	EAFSLFDK
143	103	NP_036645	Q9QWC5	Calmodulin, Ca(2+)-dependent ganglioside-binding protein (fragment)	1.34	4.0	11.7	47	—
144	6	P10639	THIO_MOUSE	Thioredoxin 1; thioredoxin	1.43	4.8	12.0	40	—
145	1107	Q04758	IPKB_MOUSE	cAMP-dependent protein kinase inhibitor beta	2.43	4.7	9.7	53	—
146	2001	Q9CQI6	COAC_MOUSE	Coactosin-like protein	—	5.3	15.9	—	EVVQNFAK
147	2114	Q64271	VAM3_MOUSE	Vesicle-associated membrane protein 3	—	8.7	11.5	—	LSELDDR; ADALQAGAS QFETSAAK
148	4103	P13795	SN25_HUMAN	Chain B of complex between *N*-terminus of SNAP25 and SNARE region of syntaxin 1a	2.38	5.9	9.1	19	—
149	5109	1SFC	—	Chain B, neuronal synaptic fusion complex	2.43	5.1	9.6	35	—
150	1005	P11232	THIO_RAT	Thioredoxin	—	4.8	11.5	—	VGEFSGANK; EAFQEALAAAGDK
151	2005	NP_067710	Q9Z2N6	CaM-KII inhibitory protein	2.43	5.3	8.7	38	—
152	5004	XP_220432.1	HNT1_MOUSE	Similar to histidine triad nucleotide binding protein	2.43	6.2	11.6	28	—
153	5013	Q63362	NUFM_RAT	NADH-ubiquinone oxidoreductase 13 kDa-B subunit	—	7.1	13.3	—	KYTEQITSEK; TTGLVGLAVCDT PHER + carbamidomethyl (C); KLENLLQGGEVEEVILQAEK
154	2002	P80144	MTPN_MOUSE	Myotropin	—	5.3	12.7	—	GPDGLTALEATDNQAIK
155	2006	P50408	VATF_RAT	Vacuolar ATP synthase subunit F	—	5.5	13.4	—	SIPAVLEIPSK; DTTINEIEDTFR

#	SSP	NCBI accession	Swiss-Prot entry name	Protein ID	*Z-score*	p*I*	Mass (kDa)	%c	MS/MS (sequence data)
156	5012	Q63362	NUFM_RAT	NADH-ubiquinone oxidoreductase 13 kDa-B subunit	—	7.1	13.3	—	ILDLLK; YTEQITSEK; KYTEQITSEK; PWEPLVEEPPANQWK + 3 additional peptides
157	5014	1JTH	—	Chain B of complex between *N*-terminus of SNAP25 and SNARE region of syntaxin 1a	2.43	5.9	9.1	30	—
158	7010	P30904	MIF_RAT	Macrophage migration inhibitory factor	—	7.3	12.3	—	LLCGLLSDR + carbamidomethyl (C); PMFIVNTNVPR + oxidation (M)
159	8001	Q62658	FKB1_RAT	FK506-binding protein 1A	—	8.1	11.8	—	GVQVETISSGDGR
160	9006	P26772	CH10_RAT	10 kDa heat shock protein, mitochondrial (Hsp 10) (10 kDa chaperonin) (CPN10)	—	9.3	8.1	—	GGEIQPVSVK; VLLPEYGGTK; VLQATVVAVGSGGLK; VVLDDKDYFLFR
161	5003	P17074	RS19_RAT	40S ribosomal protein S19	—	10.4	15.9	—	IAGQVAAANK
162	6002	P02248	UBIQ_HUMAN	Ubiquitin	—	6.6	8.6	—	TITLEVEPSDTIENVK
163	6003	NP_006189.1	PE19_MOUSE	Purkinje cell protein 4; brain specific polypeptide PEP-19	1.81	6.2	6.8	44	—

Protein spot number (arbitrarily assigned) from Fig. 1; SSP, PDQuest assigned spot number;%C, percent sequence coverage by measured masses; *Z*-score from ProFound database.

Table 2

Synaptosomal proteins identified by shotgun LC-MS/MS Analysis

No.	Abbreviation	NCBI Accession	Protein	1 coverage [%] *a)*	2 coverage [%] *a)*	3 coverage [%] *a)*	PTM
1	143B	P35213	14-3-3 protein beta/alpha(Protein kinase C inhibitor protein-1)	18.40	14.80	19.60	g
2	A180	Q05140	Clathrin coat assembly protein AP180	3.97	2.69	3.97	
3	A1A1	P06685	Sodium/potassium-transporting ATPase alpha-1 chain	10.57	11.82	11.05	G
4	A1A2	P06686	Sodium/potassium-transporting ATPase alpha-2 chain	10.32	10.70	10.03	
5	A1A3	P06687	Sodium/potassium-transporting ATPase alpha-3 chain	11.36	9.61	10.10	G
6	A1A4	Q64541	Sodium/potassium-transporting ATPase alpha-4 chain	4.02	2.58		
7	A1B1	P52303	Adapter-related protein complex 1 beta 1 subunit	2.8	2.80	2.8	
8	A2A2	P18484	Adaptor-related protein complex 2 alpha 2 subunit	2.94	9.86	8.5	G
9	AATC	P13221	Aspartate aminotransferase, cytoplasmic	20.05	20.05	20.05	
10	AATM	P00507	Aspartate aminotransferase, mitochondrial precursor	25.34	13.24	13.24	
11	ACLY	P16638	ATP-citrate synthase (EC 2.3.3.8)			1.7	
12	ADT1	Q05962	ADP,ATP carrier protein, heart/skeletal muscle isoform	19.47	20.46	11.55	A, g
13	ADT2	Q09073	ADP,ATP carrier protein, fibroblast isoform	15.18	12.54	10.23	A, G
14	ALFA	P05065	Fructose-bisphosphate aldolase A (EC 4.1.2.13)	35.68	27.57	21.98	G
15	ALFC	P09117	Fructose-bisphosphate aldolase C (EC 4.1.2.13)	15.72	26.02	18.16	
16	AMPH	O08838	Amphiphysin	3.45	2.88	2.88	G
17	ANX5	P14668	Annexin A5 (Annexin V) (Lipocortin V) (Endonexin I)		4.94	4.94	
18	ANX6	P48037	Annexin A6 (Annexin VI) (Lipocortin VI) (P68) (P70)		2.34		
19	AOFA	P21396	Amine oxidase [flavin-containing] A (EC 1.4.3.4)		2.62		
20	ATB1	P11505	Plasma membrane calcium-transporting ATPase 1		2.74		
21	ATB2	P11506	Plasma membrane calcium-transporting ATPase 2	1.34		2.77	
22	ATB3	Q64568	Plasma membrane calcium-transporting ATPase 3	1.33		1.33	
23	ATB4	Q64542	Plasma membrane calcium-transporting ATPase 4	1.39	2.86	2.86	
24	ATHA	P09626	Potassium-transporting ATPase alpha chain 1	2.67			
25	ATHL	P54708	Potassium-transporting ATPase alpha chain 2		0.85		
26	ATNB	P07340	Sodium/potassium-transporting ATPase beta-1 chain	8.71	8.71	4.19	
27	ATPA	P15999	ATP synthase alpha chain, mitochondrial precursor	34.54	35.26	36.35	G
28	ATPB	P10719	ATP synthase beta chain, mitochondrial precursor	43.49	49.81	56.51	g
29	ATPD	P35434	ATP synthase delta chain, mitochondrial precursor	5.26			
30	ATPF	P19511	ATP synthase B chain, mitochondrial precursor		5.75	5.75	
31	ATPG	P35435	ATP synthase gamma chain, mitochondrial	4.32		3.60	G
32	ATPJ	P29419	ATP synthase e chain, mitochondrial (EC 3.6.3.14)	16.67	16.67	31.94	
33	ATPO	Q06647	ATP synthase oligomycin sensitivity conferral protein	5.07	17.98	17.97	
34	ATPQ	P31399	ATP synthase D chain, mitochondrial (EC 3.6.3.14)	30.06	33.13	23.31	
35	ATPR	P21571	ATP synthase coupling factor 6, mitochondrial precursor		17.27		
36	BASP	Q05175	Brain acid soluble protein 1 (BASP1 protein)	12.56	12.56	12.56	G
37	BIN1	O08839	Myc box dependent interacting protein 1		2.34	2.34	
38	CAH2	P27139	Carbonic anhydrase II (EC 4.2.1.1)			9.85	
39	CAP1	Q08163	Adenylyl cyclase-associated protein 1 (CAP 1)	4.57	8.52	3.95	A
40	CAP2	P52481	Adenylyl cyclase-associated protein 2 (CAP 2)		3.09		
41	CATD	P24268	Cathepsin D precursor (EC 3.4.23.5).	4.35	4.35	4.35	
42	CH10	P26772	10 kDa heat shock protein, mitochondrial (Hsp10)	13.59	25.24	13.59	
43	CLCB	P08082	Clathrin light chain B (Lcb)	4.29	4.29		
44	CLH	P11442	Clathrin heavy chain	13.51	18.44	17.56	A, G
45	CN37	P13233	2′,3′-cyclic nucleotide 3′-phosphodiesterase	13.38	3.41	9.00	
46	COA1	P11497	Acetyl-CoA carboxylase 1 (EC 6.4.1.2) (ACC-alpha)		0.67	0.67	
47	COF1	P45592	Cofilin, non-muscle isoform	6.51		6.51	A
48	COX2	P00406	Cytochrome c oxidase polypeptide II (EC 1.9.3.1)	4.33	4.33	4.33	
49	COXA	P11240	Cytochrome c oxidase polypeptide Va, mitochondrial	30.20	10.07	20.13	
50	CPV1	P22443	Cytochrome P450 19A1 (Aromatase) (EC 1.14.14.1)			3.68	
51	CRP2	P36201	Cysteine-rich protein 2 (CRP2) (ESP1 protein)	15.09	15.09		

No.	Abbreviation	NCBI Accession	Protein	1 coverage [%] *a)*	2 coverage [%] *a)*	3 coverage [%] *a)*	PTM
52	CX41	P10888	Cytochrome c oxidase subunit IV isoform 1, mitochondrial	6.98		6.40	
53	DCE2	Q05683	Glutamate decarboxylase, 65 kDa isoform	3.70			
54	DDH1	O08557	NG,NG-dimethylarginine dimethylaminohydrolase 1		9.00		A
55	DLG2	Q63622	Channel associated protein of synapse-110		1.61		
56	DLG4	P31016	Presynaptic density protein 95 (PSD-95)			2.04	
57	DOPD	P80254	D-dopachrome tautomerase		10.08		G
58	DPY1	Q62950	Dihydropyrimidinase related protein-1 (DRP-1)	3.95	6.70	12.89	
59	DPY2	P47942	Dihydropyrimidinase related protein-2 (DRP-2)	15.98	34.36	37.11	G
60	DPY4	Q62951	Dihydropyrimidinase related protein-4 (DRP-4)	2.79	2.79		
61	DPY5	Q9JHU0	Dihydropyrimidinase related protein-5 (DRP-5) ULIP6 protein			4.88	
62	DYN1	P21575	Dynamin-1 (EC 3.6.1.50) (D100) (Dynamin, brain)	20.44	11.09	13.28	G
63	DYN2	P39052	Dynamin 2 (EC 3.6.1.50)	3.16	4.18	3.84	
64	DYN3	Q08877	Dynamin 3 (EC 3.6.1.50) (Dynamin, testicular)	3.82	1.97	2.78	
65	EAA1	P24942	Sodium-dependent glutamate/aspartate transporter 2		6.33	3.62	
66	EAA2	P31596	Sodium-dependent glutamate/aspartate transporter 2	4.46	6.35	6.00	A, G
67	ECHM	P14604	Enoyl-CoA hydratase, mitochondrial precursor		5.76	5.76	
68	ENOA	P04764	Alpha enolase (EC 4.2.1.11) (2-phospho-D-glycerate)	10.19	27.66	32.20	
69	ENOB	P15429	Beta enolase (EC 4.2.1.11) (2-phospho-D-glycerate)			10.43	G
70	ENOG	P07323	Gamma enolase (EC 4.2.1.11) (2-phospho-D-glycerate)	35.15	32.88	36.51	
71	FKB1	Q62658	FK506-binding protein 1A (EC 5.2.1.8)	24.77			
72	FRAP	P42346	FKBP-rapamycin associated protein (FRAP)	0.27			
73	FUMH	P14408	Fumarate hydratase, mitochondrial precursor		4.07		
74	G3P	P04797	Glyceraldehyde 3-phosphate dehydrogenase	36.98	30.77	40.53	G
75	GABT	P50554	4-aminobutyrate aminotransferase, mitochondrial precursor	5.70	7.86	2.95	
76	GB01	P59215	Guanine nucleotide-binding protein G(O), alpha subunit	27.86			
77	GB02	P30033	Guanine nucleotide-binding protein G(O), alpha subunit		15.88	24.79	
78	GB12	Q63210	Guanine nucleotide-binding protein, alpha-12 subunit	2.86	2.86		
79	GBAK	P08753	Guanine nucleotide-binding protein G(k), alpha subunit		3.06		
80	GBB1	P54311	Guanine nucleotide-binding protein G(I)/G(S)/G(T) beta subunit 1	10.69	8.96	10.69	A
81	GDIA	P50398	Rab GDP dissociation inhibitor alpha (Rab GDI alpha)	40.66	28.35	29.45	
82	GDIC	P50399	Rab GDP dissociation inhibitor beta-2 (Rab GDI beta)			4.19	
83	GLNA	P09606	Glutamine synthetase (EC 6.3.1.2)	4.21	11.58	11.58	
84	GLSK	P13264	Glutaminase, kidney isoform, mitochondrial precursor	5.10	5.10	3.06	
85	GR75	P48721	Stress-70 protein, mitochondrial precursor (GRP 75)	1.74	4.49	1.59	M
86	GR78	P06761	78 kDa glucose-regulated protein precursor (GRP 78)		2.41		
87	GTM2	P08010	Glutathione S-transferase Yb-2 (EC 2.5.1.18)		7.69		
88	GTP	P04906	Glutathione S-transferase P (EC 2.5.1.18)		7.51	7.51	
89	GUAD	Q9WTT6	Guanine deaminase (EC 3.5.4.3) (Guanase)			3.03	
90	HCD2	O70351	3-hydroxyacyl-CoA dehydrogenase type II		9.81		
91	HEM0	Q63147	5-aminolevulinic acid synthase, erythroid-specific			1.84	
92	HES2	P35429	Transcription factor HES-2			6.25	
93	HS1A	P55063	Heat shock protein 1A (Heat shock 70 kDa protein 3)	4.45	4.45	1.99	
94	HS72	P14659	Heat shock-related 70 kDa protein 2		6.52	6.52	G
95	HS9B	P34058	Heat shock protein HSP 90-beta (HSP 84)	4.76	3.53	3.53	M
96	HXK1	P05708	Hexokinase, type I (EC 2.7.1.1) (HK I) (Brain form)	8.89	6.96	4.60	A, G
97	HXK2	P27881	Hexokinase type II (EC 2.7.1.1) (HK II)	1.18			
98	IDHG	P41565	Isocitrate dehydrogenase [NAD] subunit gamma	5.00		5.00	
99	JAG2	P97607	Jagged 2 (Jagged2) (Fragment)			1.06	
100	K6PF	P47858	6-phosphofructokinase, muscle type (EC 2.7.1.11)		1.39		
101	K6PL	P30835	6-phosphofructokinase, liver type (EC 2.7.1.11)			2.40	
102	K6PP	P47860	6-phosphofructokinase, type C (EC 2.7.1.11)	3.00	6.00	6.00	

No.	Abbreviation	NCBI Accession	Protein	1 coverage [%] *a)*	2 coverage [%] *a)*	3 coverage [%] *a)*	PTM
103	KAD1	P39069	Adenylate kinase isoenzyme 1 (EC 2.7.4.3)		7.07	7.07	
104	KCCA	P11275	Calcium/calmodulin-dependent protein kinase type II	19.55	22.84	22.84	G
105	KCCB	P08413	Calcium/calmodulin-dependent protein kinase type II	18.84	16.67	21.20	M, O
106	KCCD	P15791	Calcium/calmodulin-dependent protein kinase type II	10.15	7.75	7.75	
107	KCCG	P11730	Calcium/calmodulin-dependent protein kinase type II	12.87	8.21	12.87	
108	KCRB	P07335	Creatine kinase, B chain (EC 2.7.3.2) (B-CK)	30.93	38.92	34.79	G, M
109	KCRS	P09605	Creatine kinase, sarcomeric mitochondrial precursor	2.11	3.76		
110	KCRU	P25809	Creatine kinase, ubiquitous mitochondrial precursor	23.06	11.06	13.18	G
111	KILO	Q9Z0J8	Kilon protein precursor (Kindred of IgLON)			3.67	
112	KPRB	O08618	Phosphoribosyl pyrophosphate synthetase-associated protein 2		6.12		
113	KPYM	P11980	Pyruvate kinase, M1/M2 isozyme (EC 2.7.1.40)	44.90	35.62	31.91	
114	KPYR	P12928	Pyruvate kinase, isozymes R/L (EC 2.7.1.40) (L-PK)	1.88	1.88		
115	LDHA	P04642	L-lactate dehydrogenase A chain (EC 1.1.1.27) (LDH)	12.72	14.20	8.28	G
116	LDHB	P42123	L-lactate dehydrogenase B chain (EC 1.1.1.27) (LDH)	13.86	12.98	4.72	
117	MA32	O35796	Complement component 1, Q subcomponent binding protein	15.55		10.60	G
118	MAPB	P15205	Microtubule-associated protein 1B (MAP 1B)		0.72		
119	MBP	P02688	Myelin basic protein S (MBP S)	17.53	8.25	11.34	G
120	MDHM	P04636	Malate dehydrogenase, mitochondrial precursor	32.56	38.08	34.59	G
121	MDR1	P43245	Multidrug resistance protein 1 (P-glycoprotein 1)	1.00			
122	MDR2	Q08201	Multidrug resistance protein 2 (P-glycoprotein 2)	1.00			
123	MIF	P30904	Macrophage migration inhibitory factor (MIF)		25.86	18.10	
124	MPCP	P16036	Phosphate carrier protein, mitochondrial precursor	3.31			
125	MYHA	Q9JLT0	Myosin heavy chain, nonmuscle type B	1.34			
126	MYOG	P20428	Myogenin	3.08		3.08	
127	NCA1	P13596	Neural cell adhesion molecule 1, 140 kDa isoform		3.44	2.06	
128	NCP1	P55161	Nck-associated protein 1 (NAP 1) (p125Nap1)		1.22	1.74	
129	NDKB	P19804	Nucleoside diphosphate kinase B (EC 2.7.4.6)	5.81			
130	NEUM	P07936	Neuromodulin (Axonal membrane protein GAP-43)	10.00	10.00	10.00	
131	NP25	P37805	Neuronal protein NP25	15.25	8.97	8.97	
132	NPX1	P47971	Neuronal pentraxin I precursor (NP-I) (NP1)		4.09		
133	NTRI	Q62718	Neurotrimin precursor (GP65)	6.29			
134	NUHM	P19234	NADH-ubiquinone oxidoreductase 24 kDa subunit	5.28	4.47	9.76	
135	ODO2	Q01205	Dihydrolipoamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex	2.67	4.67		
136	ODP2	P08461	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex	8.14	12.74	9.56	G
137	ODPA	P26284	Pyruvate dehydrogenase E1 component alpha subunit	14.61	17.13	8.56	P
138	ODPB	P49432	Pyruvate dehydrogenase E1 component beta subunit	7.40	20.27	11.78	G
139	OPCM	P32736	Opioid binding protein/cell adhesion molecule precursor	11.11		5.41	
140	OPLA	P97608	5-oxoprolinase (EC 3.5.2.9) (5-oxo-L-prolinase)			0.46	
141	PAC1	Q9Z0W5	Protein kinase C and casein kinase substrate	9.58	12.69	15.37	G
142	PDX5	Q9R063	Peroxiredoxin 5, mitochondrial precursor (Prx-V)	24.42	16.59	5.99	
143	PDX6	O35244	Peroxiredoxin 6 (EC 1.11.1.-)	8.81	10.13	7.49	
144	PEBP	P31044	Phosphatidylethanolamine-binding protein (PEBP)	36.32	48.42	36.84	A, G
145	PGK2	P16617	Phosphoglycerate kinase, testis specific (EC 2.7.2)	18.68	18.44	24.59	g
146	PHS3	P53534	Glycogen phosphorylase, brain form (EC 2.4.1.1)	1.41			
147	PIMT	P22062	Protein-L-isoaspartate(D-aspartate) O-methyltransferase		8.26		
148	PMG1	P25113	Phosphoglycerate mutase 1 (EC 5.4.2.1)		15.12	8.14	
149	POR1	Q9Z2L0	Voltage-dependent anion-selective channel protein	18.69	17.38	25.25	
150	POR2	P81155	Voltage-dependent anion-selective channel protein	10.00	10.00	9.67	
151	POR3	Q9R1Z0	Voltage-dependent anion-selective channel protein		14.93	10.42	
152	PPIA	P10111	Peptidyl-prolyl cis-trans isomerase A (EC 5.2.1.8)	45.78	42.17	34.34	
153	RB10	P35281	Ras-related protein Rab-10	5.39			

No.	Abbreviation	NCBI Accession	Protein	1 coverage [%] *a)*	2 coverage [%] *a)*	3 coverage [%] *a)*	PTM
154	RB1A	Q6NYB7	Ras-related protein Rab-1A	5.26		5.26	
155	RB2A	P05712	Ras-related protein Rab-2A	7.41		7.41	
156	RB3A	P63012	Ras-related protein Rab-3A	40.45	40.45	36.82	
157	RB3C	P62824	Ras-related protein Rab-3C		16.74	16.74	
158	RIM1	Q9JIR4	Regulating synaptic membrane exocytosis protein 1	0.85	0.85		G
159	RPA1	O54889	DNA-directed RNA polymerase I largest subunit	0.52			
160	RTN1	Q64548	Reticulon 1 (Neuroendocrine-specific protein)			3.67	
161	RUN1	Q63046	Runt-related transcription factor 1			2.62	
162	S109	P50116	Calgranulin B		10.53		
163	S6A1	P23978	Sodium- and chloride-dependent GABAb transporter		1.97		
164	SAP	P10960	Sulfated glycoprotein 1 precursor (SGP-1)	2.66	4.61	2.66	G
165	SEP7	Q9WVC0	Septin 7 (CDC10 protein homolog)	5.18	5.86	3.83	
166	SFX1	Q63965	Sideroflexin 1 (Tricarboxylate carrier protein)		4.57		
167	SH31	O35964	SH3-containing GRB2-like protein 1	3.20	3.20		
168	SH32	O35179	SH3-containing GRB2-like protein 2	11.07	6.32	6.32	G
169	SNAA	P54921	Alpha-soluble NSF attachment protein (SNAP-alpha)		3.67	3.67	
170	SNGP	Q9QUH6	Ras GTPase-activating protein SynGAP	1.14	1.37	1.37	
171	SODC	P07632	Superoxide dismutase [Cu-Zn] (EC 1.15.1.1)	7.05	8.33	8.33	G
172	SODM	P07895	Superoxide dismutase [Mn], mitochondrial precursor	12.39	12.39	12.39	
173	SPCN	P16086	Spectrin alpha chain, brain	2.74	5.53	5.45	G
174	SSDH	P51650	Succinate semialdehyde dehydrogenase (EC 1.2.1.24)	3.62			
175	ST1A	P32851	Syntaxin 1A(Synaptotagmin associated 35 kDa protein)	9.22	9.22	13.65	
176	SUCA	P13086	Succinyl-CoA ligase [GDP-forming] alpha-chain		4.72		G
177	SX10	0O55170	Transcription factor SOX-10	1.48			
178	SYJ1	Q62910	Synaptojanin 1 (EC 3.1.3.36)			1.25	G
179	SYN1	P09951	Synapsin I	25.98	24.02	27.65	G
180	SYN2	Q63537	Synapsin II	22.48	22.15	19.97	
181	SYPH	P07825	Synaptophysin (Major synaptic vesicle protein p38)	3.19	7.35	3.19	A
182	SYT1	P21707	Synaptotagmin I (SytI) (p65)	20.51	19.11	15.15	G
183	SYT2	P29101	Synaptotagmin II (SytII)	5.12	5.12	5.12	
184	SYT5	P47861	Synaptotagmin V (SytV)	3.05		3.05	
185	SYUA	P37377	Alpha-synuclein	25.17	26.57	26.57	g
186	SYUB	Q63754	Beta-synuclein (Phosphoneuroprotein 14) (PNP 14)	28.57	28.57	28.57	g, M
187	TAU	P19332	Microtubule-associated protein tau	6.28	11.52	1.57	
188	TBB1	P04691	Tubulin beta chain (T beta-15)	50.55	56.95	57.40	G, O
189	TERA	P46462	Transitional endoplasmic reticulum ATPase (TER ATPase)		4.76		
190	THIL	P17764	Acetyl-CoA acetyltransferase, mitochondrial precursor		3.94	8.33	
191	THIO	P11232	Thioredoxin	12.26	12.26	12.26	
192	THY1	P01830	Thy-1 membrane glycoprotein precursor	10.37	8.54	8.54	G
193	TKT	P50137	Transketolase (EC 2.2.1.1) (TK)		3.00	3.00	
194	TMO2	P70566	Neuronal tropomodulin (N-Tmod) (Tropomodulin 2)		10.08	10.08	
195	TPIS	P48500	Triosephosphate isomerase (EC 5.3.1.1) (TIM)	40.71	42.29	42.29	
196	TPM2	P58775	Tropomyosin beta chain (Tropomyosin 2)		3.46		
197	UBL1	Q00981	Ubiquitin carboxyl-terminal hydrolase isozyme L1	6.61	7.93	7.93	
198	UCR2	P32551	Ubiquinol-cytochrome C reductase complex core protein	17.17	16.09	13.70	
199	UCRI	P20788	Ubiquinol-cytochrome C reductase iron-sulfur subunit		3.07	13.03	
200	VP3B	Q63616	Vacuolar protein sorting 33B (r-vps33b)	4.30			
201	VPP1	P25286	Vacuolar proton translocating ATPase 116 kDa subunit	4.93	5.05	3.76	G

a) Absence of sequence coverage indicates identification was based on the observation of peptides in the chromatogram without MS/MS data A: acetylation; G, g: glycosylation and G indicates the presence of N-glycosylation motif; M: methylation; O: oxidation; P: phos-phorylation.

Table 3

protein PTM of synaptosomal proteins determined by LC-MS/MS

s- Entry	NCBI Accession	Protein	Acetylation[a)]	Glycosylation[b)]	Methylation Methylester (DE)/ Methylester (C-term)	Oxidation (HW)	Phosphorylation (ST)
HUMAN	P62258	14-3-3 protein epsilon	MDDREDLVYQAK*				
HUMAN	P42655	14-3-3 protein epsilon (Mitochondrial import stimulation factor L subunit)		G			
MOUSE	P35216	14-3-3 protein tau (14-3-3 protein theta)		G			
MOUSE	P35215	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein-1) (KCIP-1)	MDKNELVQK*	G			
1 HUMAN	P21851	Adapter-related protein complex 2 beta 1 subunit (Beta-adaptin)		G			
M RAT	P00507	Aspartate aminotransferase, mitochondrial precursor (EC 2.6.1.1) (Transaminase A)		G			
B HUMAN	P60709	Actin, cytoplasmic 1 (Beta-actin)				YPIEHGIVTNWDDMEK*	HQGVMVGMGQK*
G HUMAN	P02571	Actin, cytoplasmic 2 (Gamma-actin) actin 1)		G		HQGVMVGMGQK*	
S HUMAN	P02538	Actin, alpha skeletal muscle (Alpha-		G			
1 RAT	P51635	Alcohol dehydrogenase [NADP +]	TASSVLLHTGQK*				
U RAT	P02770	Serum albumin precursor [Contains: Neurotensin-related peptide (NRP)]		q			
D RAT	Q00982	3-methyl-2-oxobutanoate dehydrogenase [lipoamide]] kinase, mitochondrial precursor		G			
B HUMAN	P06795	Calcineurin B subunit isoform 1 (Protein phosphatase 2B regulatory subunit 1)		q			
M HUMAN	P02593	Calmodulin	ADQLTEEQIAEFKG*	G			
H RAT	Q9EQ60	Voltage-dependent T-type calcium channel alpha-1H subunit (Cav3.2)		G			
0 MOUSE	P19226	60 kDa heat shock protein, mitochondrial precursor (Hsp60) (60 kDa chaperonin) (CPN60)		G			
MOUSE	P00009	Cytochrome c, somatic		q			
R RAT	P11348	Dihydropteridine reductase				SMPEADFSSWTPLEFLVETFHDWITGNK*	
5 MOUSE	P60904	DnaJ homolog subfamily C member 5	SLS*TSGESLYHVLGLDK				
C RAT	P38650	Dynein heavy chain, cytosolic (DYHC) (Cytoplasmic dynein heavy chain) (MAP 1C)		G			
RAT	Q9JJH5	6-phosphofructo-2-kinase/ fructose- 2,6-biphosphatase 2		G			

s- Entry	NCBI Accession	Protein	Acetylation[a)]	Glycosylation[b)]	Methylation Methylester (DE)/ Methylester (C-term)	Oxidation (HW)	Phosphorylation (ST)
H RAT	P19132	Ferritin heavy chain (Ferritin H sub-unit)		G			
RAT	Q9Z272	ARF GTPase-activating protein GIT1 (G protein-coupled receptor kinase-interactor 1)		G			
2 RAT	Q64232	Synaptic glycoprotein SC2		G			
M1 RAT	P04905	Glutathione S-transferase Yb-1 (EC 2.5.1.18) (Chain 3) (GSTYb1) (GST M1-1) (GST class-mu)		q			
1 or 2 RAT	P02091	Hemoglobin beta chain, major-form		q			
C MOUSE	P08109	Heat shock cognate 71 kDa protein		G			
HUMAN	P62256	Mitochondrial import inner membrane translocase subunit TIM13 A	MDSGFGSDFGGTGGGK*				
RAT	P97686	Interleukin-18 precursor (IL-18) (Interferon-gamma inducing factor)		G			
RAT	P29995	Inositol 1,4,5-trisphosphate receptor type 2		G			
S RAT	Q63279	Keratin, type I cytoskeletal 19 (Cytokeratin 19) (K19) (CK 19) (Fragment)		G			
3 RAT	P12369	cAMP-dependent protein kinase type II-beta regulatory chain	SIEIPAGLTEL-LQGFTVEVLR*				
A RAT	P97633	Casein kinase I, alpha isoform (EC 2.7.1.-) (CKI-alpha) (CK1)		G			
1 RAT	P19139	Casein kinase II, alpha chain (CK II) (EC 2.7.1.37)		G			
1 or 2 RAT	P11981	Pyruvate kinase, M2 isozyme (EC 2.7.1.40)		G			
1 RAT	P11981	Pyruvate kinase, M2 isozyme (EC 2.7.1.40)				FGVEQD**	
1 MOUSE	P27703	Mitogen-activated protein kinase 1	AAAAAAGPEMVR*				
2 RAT	Q63120	Canalicular multispecific organic anion transporter 1; Multidrug resistance-associated protein 2			IMNEILSGIKILK*		
1 RAT	Q01728	Sodium/calcium exchanger 1 precursor (Na(+)/Ca(2+)-exchange protein 1)		G			
2 RAT	P48768	Sodium/calcium exchanger 2 precursor (Na(+)/Ca(2+)-exchange protein 2)		G			
A MOUSE	P20652	Serine/threonine protein phosphatase 2B catalytic subunit, alpha isoform (EC 3.1.3.16)		G			
L RAT	P20611	Lysosomal acid phosphatase precursor (EC 3.1.3.2) (LAP)		G			

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s-Entry	NCBI Accession	Protein	Acetylation[a)]	Glycosylation[b)]	Methylation Methylester (DE)/ Methylester (C-term)	Oxidation (HW)	Phosphorylation (ST)
A RAT	P10111	Peptidyl-prolyl cis-trans isomerase A (EC 5.2.1.8) (PPIase) (Rotamase) (Cyclophilin A)		q			
A RAT	P09320	Placental prolactin-like protein A precursor (PLP-A)		G			
RAT	Q00438	Polypyrimidine tract-binding protein 1			IDFSKLTSLNVK*		
RAT	Q9R1A7	Orphan nuclear receptor PXR (Pregnane X receptor)		G			
A MOUSE	P05713	Ras-related protein Rab-3A		G			
A RAT	P49242	40S ribosomal protein S3a	FKLITEDVQGK*				
1 RAT	P50914	Ras GTPase-activating protein 1 (GTPase-activating protein) (GAP)				WPTNNTMR*	
1 RAT	P50914	Ras GTPase-activating protein 1 (GTPase-activating protein) (GAP)		G			
6 RAT	Q9EQ23	28S ribosomal protein S26, mitochondrial precursor (MRP-S26) (5′OT-EST protein)		G			
A RAT	P35467	S-100 protein, alpha chain	GSELETA-METLINVFHAHSGK*				
B RAT	P04631	S-100 protein, beta chain	SELEKAMVA-LIDVFHQYSGRG*	G			
3 RAT	Q9JHY2	Sideroflexin 3		G			
1 RAT	P59622	SRC-like-adapter (Src-like-adapter protein 1)		G			
3 MOUSE	P32853	Syntaxin 1B (P35B) SAKDS*DDEEEVVHVDR		G			
1 HUMAN	Q64320	Syntaxin binding protein 1 (Unc-18 homolog) (Unc-18A) (Unc-18-1) (N-Sec1) (rbSec1) (p67)		G			
7 RAT	O70257	Syntaxin 7	SYTPGIGGDPAQLAQR*				
1 MOUSE	P02551	Tubulin alpha-1 chain		G	AVFVDLEPTVIDEVR*		
5 HUMAN	P05218	Tubulin beta-5 chain		G	GFWE-VISDEHGIDPTG-TYHGDSDLQLDR*	EVDEQMLNVQNK*	
RAT	P48500	Triosephosphate isomerase (EC 5.3.1.1) (TIM)		G			
2 RAT	P00763	Trypsin II, anionic precursor (EC 3.4.21.4) (Pretrypsinogen II)		q			
3 RAT	P08426	Trypsin III, cationic precursor (EC 3.4.21.4) (Pretrypsinogen III)		q			
R RAT	P21463	Thyrotropin receptor precursor (TSH-R) (Thyroid stimulating hormone receptor)		G			
0_ HUMAN	P13472	Thymosin beta-10	ADKPDMGEIASFDK*				
4 HUMAN	P62328	Thymosin beta-4	SDKPDMAEIEK*				
Q HUMAN	P02248	Ubiquitin		g			

s- Entry	NCBI Accession	Protein	Acetylation[a)]	Glycosylation[b)]	Methylation Methylester (DE)/ Methylester (C-term)	Oxidation (HW)	Phosphorylation (ST)
2 RAT	P56500	Mitochondrial uncoupling protein 2					SLYNGLVAGLQR*
2 RAT	P32551	Ubiquinol-cytochrome C reductase complex core protein 2, mitochondrial precursor		G			
2 MOUSE	P50517	Vacuolar ATP synthase subunit B, brain isoform (EC 3.6.3.14) (V-ATPase B2 subunit)		G			
M2 MOUSE	Q64357	Vesicle-associated membrane protein 2 (VAMP-2) (Synaptobrevin 2)		G			

terminal acetylat ion was observed

tes the presence of N-glycosylation motif, while g indicates the absence of such a motif

amino acid