Separation and identification of mouse liver membrane proteins using a gel-based approach in combination with 2DnanoLC-Q-TOF-MS/MS

The Thanh Tran and Van Chi Phan

Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam
E-mail: chi@ibt.ac.vn

Received 21 February 2010
Accepted for publication 28 March 2010
Published 6 July 2010
Online at stacks.iop.org/ANSN/1/015015

Abstract
In this work, we present results of membrane proteome profiling from mouse liver tissues using a gel-based approach in combination with 2DnanoLC-Q-TOF-MS/MS. Following purification of the membrane fraction, SDS-PAGE was carried out as a useful separation step. After staining, gels with protein bands were cut, reduced, alkylated and trypsin-digested. The peptide mixtures extracted from each gel slice were fractionated by two-dimensional nano liquid chromatography (2DnanoLC) coupled online with tandem mass spectrometry analysis (NanoESI-Q-TOF-MS/MS). The proteins were identified by MASCOT search against a mouse protein database using a peptide and fragment mass tolerance of ±0.5 Da. Protein identification was carried out using a Mouse scoring algorithm with a confidence level of 95% and processed by MSQuant v1.5 software for further validation. In total, 318 verified membrane proteins from mouse liver tissues were identified; 66.67% of them (212 proteins) contained at least one or more transmembrane domains predicted by the SOSUI program and 43 were found to be unique microsome membranes. Furthermore, GRAVY values of membrane proteins varied in the range $-1.1276$ to $0.9016$ and only 31 (9.76%) membrane proteins had positive values. The functions and subcellular locations of the identified proteins were categorized as well, according to universal GO annotations.

Keywords: 2DnanoLC-ESI-Q-TOF-MS/MS, mouse liver, membrane proteins, membrane proteome, proteomics

Classification numbers: 2.00, 2.04

1. Introduction
Membrane proteins (MPs) are proteins associated with cellular membranes [2, 17] and are important biological and pharmacological targets involved in intercellular communication, cellular development, cell migration and drug resistance [1, 8, 9, 15]. The importance of MPs is highlighted by the fact that about 20–30% of all the genes in various organisms code for this class of protein [20, 23], over two-thirds of all medications take effect through MPs and numerous human diseases result from malfunctions of membrane proteins [10, 24]. As membrane proteins play a key role in cellular processes, being involved in energy metabolism, response to environmental stimuli, and transport processes, the analysis of MPs is highly relevant, not only to our understanding of life and diseases but also to the possibility of profiling cell surface MPs for vaccination or drug targets [7, 21].

Although proteomics technologies have made rapid progress in the characterization and investigation of soluble proteins in recent years, MPs have lagged behind. The major challenge of membrane proteome study is the low solubility due to the complex structure and hydrophobic nature of the membrane proteins and their low abundance...
Therefore, new strategies for the identification and characterization of these special kinds of proteins are of great interest in modern proteomic research.

Despite their important functions, relatively few MPs have been identified due to the lack of standard profiling techniques. In this work a gel-based approach combined with NanoLC-MS/MS has been developed to establish a throughput profiling platform for MPs. Firstly, SDS-PAGE was used to separate the mouse liver MPs. After Coomassie-Blue staining the gels were excised, reduced/alkylated and then analyzed by 2DnanoLC coupled online to NanoESI-Q-TOF-MS/MS. Proteins were identified and validated by MASCOT search engine and MSQuant software. Our goal is identification and characterization of the membrane proteome of mouse liver tissue, including that with low abundance and hydrophobicity. This was achieved by a combination of MP extraction methods, integral membrane enrichment, and protein separation and identification techniques. Our data showed that gel-based methods combined with 2DnanoLC and NanoESI-Q-TOF-MS/MS gives a straightforward tool for proteomic analysis of multiprotein complexes, and especially for the identification of very hydrophobic MP constituents.

2. Materials and methods

2.1. Materials
Calbiochem Protease Inhibitor Cocktail Set 111, Cat #39134, contains AEBSF, aprotinin, bestatin, E-64, leupeptin, pepstatin A. Dithiothreitol (DTT), iodoacetamide (IAA), ammonium bicarbonate, ammonium acetate, trypsin (proteomics sequencing grade), sodium bicarbonate and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid (FA) and triflouracetate (TFA) were obtained from Fluka (Fluka Chemie GmbH, Buchs, Switzerland). Acetonitrile (ACN, chromatogram grade) and other chemicals (analytical grade) were obtained from Barker (Pittsburgh, USA). The Bradford assay kit, acrylamide, bis-acrylamide, urea, glycine, Tris, CHAPS, and SDS were purchased from Bio-Rad (Hercules, CA, USA). All equipment and standard reagents used directly should be clean as necessary.

2.2. Preparation of mouse liver MPs

The membrane fraction from mouse livers was mainly obtained by the protocol provided by Professor Bill Jordan from Victoria University of Wellington (New Zealand) used for the preparation of mouse liver microsomal fractions as a ‘membrane standard’. Briefly, Swiss mouse livers were collected as soon as possible after the animals were killed. The livers were excised into 1–2 mm size pieces and washed with 10 ml of ice cold PBS buffer (0.2 g KCl, 8 g NaCl, 1.44 NaHPO4, 0.24 g KH2PO4) and then resuspended in 2–3 volumes of the Homogenization Buffer (0.25 M sucrose in 5 mM Tris-HCl pH 7.4 with 1 mM tetrasiomde EGTA, 1 mM sodium orthovanadate and 2 mM sodium fluoride) containing protease inhibitors (Protease Inhibitor Cocktail Set III, Cat #539134, contains AEBSF, aprotinin, bestatin, E-64, leupeptin, pepstatin A). Completely homogenized samples were centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was collected and centrifuged at 40000 rpm at 4°C for 1 h. After discarding the clear supernatant, the membrane pellet was retained and washed by resuspending in ice-cold 0.1 M Na2CO3 containing protease inhibitors for 1 h. The mouse liver membrane fraction was obtained by centrifugation again at 40000 rpm for 1 h at 4°C. The sample was divided and stored at −80°C until use. The protein concentration was assessed using a Bio-Rad Bradford assay.

2.3. Electrophoresis and in-gel digestion

The membrane fraction was solubilized in lysis buffer containing 3% SDS. Equal volumes (50 µg of MP) of the mouse liver membrane fraction were separated by 10% SDS-PAGE and were visualized by staining with Coomassie Brilliant Blue G-250.

The stained protein bands were excised from gels and placed into 1.5 ml eppendorf tubes. The proteins were digested in gel with trypsin as described in our previous study [22]. Briefly, Coomassie blue-stained bands were destained with 50% ACN in 25 mM NH4HCO3 pH 8.0. The gel pieces were then reduced by incubating with 5 mM DTT solution at 56°C for 45 min and alkylated for 1 h with 20 mM IAA solution in darkness at room temperature. The MPs were digested by adding trypsin (0.03 µg µl−1) and incubating overnight at 37°C. Finally, the resulting peptide mixture was extracted with 60% ACN in 1% TFA (v/v). All extracts were saved and dried, and re-dissolved in 0.1% TFA for mass spectrometry.

2.4. 2DnanoLC-ESI-Q-TOF-MS/MS

An online 2DnanoLC system (Dionex, The Netherlands) in which samples were fractionated into 17 fractions was developed for improved separation and hydrophobic peptide recovery. As the first step, the peptide mixture was re-dissolved in 30 µl of 0.1% FA and directly loaded onto a strong cation exchange (SCX) column (500 µm ID × 15 mm, 5 µm, 300 Å) at a flow rate of 30 µl min−1. Bound peptides were eluted by ammonium acetate gradient from 0 mM to 2 M. Peptides were then desalted and concentrated on a C18 TRAP column (300 µm ID × 5 mm, 5 µm, 100 Å), and further separated onto a Vydac reverse phase C18 column (75 µm × 150 mm, 5 µm, 300 Å), for the second step. The flow rate was maintained at 0.2 µl min−1 with solvent A containing 0.1% FA. After 12 min washing, peptides were eluted from a reverse phase C18 column using the solvent B (85% ACN, 0.1% FA) gradient: from 5 to 20% of solvent B in 25 min, 20 to 70% in 28 min, 70 to 100% in 10 min and maintaining 100% B in 10 min, and back to 5% B in 5 min. After 2DnanoLC separation, peptides were independently analyzed by a QSTAR®XL MS/MS mass spectrometer (MDS SCIEX/Applied Biosystems) equipped with a nanoESI source. MS and MS/MS spectra were recorded and processed in IDA mode (Information Dependent Acquisition) controlled by Analyst QS software. The range of the MS full scan was from 200 to 1500 amu followed by MS/MS fragmentation of the three most intense precursor ions. The dynamic ion selection threshold for MS/MS experiments was set to 45 counts.
2.5. Protein identification and validation

For identification, data were searched against the NCBInr and Swiss-Prot mouse protein database using Mascot v1.8 software in which the criteria were based on the manufacturer’s definitions (Matrix Science Ltd, London, UK) [16]. The parameters were set as follows: enzymatic cleavage with trypsin; 1 potential missed cleavage; a peptide and fragment mass tolerance of ±0.5, and fixed modification: carbamidomethyl (cysteine); variable modification: oxidation (methionine). Protein identifications were performed using a Mowse scoring algorithm with a confidence level of 95% and at least two peptides matched, showing a score higher than 43. For further verification, proteins were validated by MSQuant v1.5 software [3, 5, 18] available at http://msquant.sourceforge.net. The MSQuant software is used as a validation and quantitation tool that produces the Mascot peptide identifications (HTLM files) and allows manual verification against the raw MS data (QSTAR®-XL raw files).

2.6. Data processing and bioinformatics

The MSQuant software will pick up significant and verified hits from the Mascot output file and export information of identified proteins into an .xls file, including the GI number and molecular-mass values. The FASTA formatted protein sequence from NCBInr and Swiss-Prot databases is collected for proteins identified by each MS experiment. The average hydrophobicity and transmembrane domains of the identified proteins were calculated using the SOSUI system that is available at http://bp.nuap.nagoya-u.ac.jp/sosui/. The proteins exhibiting positive GRAVY values were recognized as hydrophobic and those with negative values were hydrophylic [11]. Also, the mapping of putative transmembrane domains in the identified proteins could be predicted by using a TMHMM algorithm, available at http://www.cbs.dtu.dk/services/TMHMM-2.0/ [13]. The subcellular location and functions of the identified proteins were processed by gene ontology (GO) annotations, text-based annotation files of which were available for download from the GO database ftp site: ftp://ftp.geneontology.org/pub/go/ [4].

3. Results and discussion

3.1. Enrichment and separation of MPs

The enrichment and purification of MPs remains challenging to membrane proteomics due to their complexity and hydrophobic properties. In order to overcome the abundance problem, several methods have been reported to isolate and purify MPs, including density gradient centrifugation, biotinylation and density perturbation approaches. In this study, ultracentrifugation was used as an important step to enrich MPs. Several previous studies showed that hydrophilic proteins are routinely extracted by nonionic detergents like Triton X-100 or by alkaline treatments such as Na₂CO₃ and they are easily obtained in the pellet after centrifugation [12, 14]. In our work, MPs were extracted and re-dissolved by using lysis buffer containing SDS, urea and DTT after Na₂CO₃ treatment. After enrichment, SDS-PAGE was used to separate MPs in order to maximize MP solubility and recovery prior to identification (figure 1).

A large number of protein bands which mainly focused on an above 45 kDa region were observed in the gel image. In a lower molecular weight area, fewer protein bands could be detected. This issue reflects the possible nature of MPs with high molecular weight and complexity. MPs solubilized in lysis buffer containing 3% SDS and CHAPS, DTT or β-mercaptoethanol, were well separated by SDS-PAGE. Traditionally, proteomics analysis of complex proteins involves the resolution of proteins using two-dimensional electrophoresis (2DE) followed by mass spectrometry identification. The limitations of this method for MPs are well documented [17]. Thus, many investigators have returned to SDS-PAGE as a suitable step for solubility and pre-separation, and coupled it with mass spectrometry [4, 26]. The limitation of this strategy is the increased complexity in each SDS-PAGE gel band. This problem can be easily overcome by the use of 2DnanoLC to resolve the extracted peptides. In our identification strategy, the gel bands were excised into 10 slices after gel electrophoresis and trypsin-digested before 2DnanoLC separation and ESI-Q-TOF-MS/MS analysis, which reduced the complication of the sample and facilitated the protein identification as represented in figure 2.

3.2. Identification and characterization of MPs

The spectra, obtained from 2DnanoLC-ESI-Q-TOF-MS/MS analysis, were searched against the NCBInr and Swiss-Prot database for protein identification. More than 32,000 MS/MS spectra acquired by Analyst QS software from 10 running batches were analyzed using the Mascot search engine. To restrict false-positive hits, a stricter criterion for peptide/protein identification and limited taxonomy for house mouse/rat data were applied. MSQuant, a well-known validation software [3, 5, 18] was also used to avoid...
strategy. As shown in table (13.52%) were found to be unique microsome membranes. Additionally, 88 were integral MPs with one or more transmembrane domains, and their molecular mass ranged from 25 to 168 kDa. The GRAVY values of those proteins were negative and varied from −0.765 to −0.081, suggesting that they are hydrophobic.

If compared to the most extensive characterization and cataloging of MPs presented by Zhang et al [26], it should be noted that the number of MPs identified (457) was the sum of three different methods including 0.1 M Na₂CO₃ treatment, chloroform/methanol extraction and Triton X-100 fraction with two gel-based methods. In our study, 318 MPs were isolated by only using sucrose gradient centrifugation, and resolved by 1D SDS-PAGE. Importantly, of 457 identified MPs, only 197 were from the 0.1 M Na₂CO₃ treatment strategy, much lower than our results. In addition, a detailed comparison of the subcellular locations of the MPs identified between the two studies showed that there were 9% and 3% of microsome and Golgi MPs, respectively, in our research. By contrast, no microsome and no Golgi MPs were found in the 457 MPs from Zhang et al [26]. In their earlier study only 175 MPs were characterized from mouse liver plasma membrane, in which 88 were integral MPs with one or more transmembrane domains [25].

Several physicochemical characteristics of the 318 identified proteins were evaluated according to their molecular weight (MW), the number of transmembrane domains (TMs), and hydrophobicity (GRAVY value) by using bioinformatics software (figure 3). In our work 151 and 72 MPs, with a MW above 100 kDa and 200 kDa, respectively, were observed in the SDS-PAGE image. Figure 3(a) shows that the identified MPs are distributed on three regions with MW of 40–60 kDa, 60–80 kDa and ≥200 kDa. The range of

| NCBInr a | Description                  | MW     | GRAVY b | TM c | Process d | Function e | Location f                      |
|---------|------------------------------|--------|---------|------|-----------|------------|----------------------------------|
| gil12840895 | Unnamed protein product       | 25026  | −0.081  | 12   | Regulation| Binding    | Plasma membrane/Lyosomal membrane |
| gil26341426 | Unnamed protein product       | 44016  | −0.353  | 2    | Cell adhesion | Binding    | Plasma membrane                  |
| gil26341186 | Unnamed protein product       | 52512  | −0.168  | 1    | Oxidation reduction | Enzyme | Plasma membrane                  |
| gil12836645 | Unnamed protein product       | 56250  | −0.213  | 1    | Oxidation reduction | Enzyme | ER membrane/plasma and microsome membrane |
| gil26343437 | Unnamed protein product       | 57673  | −0.246  | 6    | Transport | Receptor | ER membrane                      |
| gil21758069 | Unnamed protein product       | 58690  | −0.378  | 0    | Regulation | Binding | Plasma membrane                  |
| gil12841692 | Unnamed protein product       | 60430  | −0.988  | 0    | Transport | Enzyme | Plasma membrane                  |
| gil12833101 | Unnamed protein product       | 74879  | −0.645  | 1    | Cell differentiation | Receptor | Plasma membrane                  |
| gil26337305 | Unnamed protein product       | 80561  | −1.128  | 0    | Unknown       | Binding | Plasma membrane                  |
| gil26336877 | Unnamed protein product       | 85878  | −0.657  | 1    | Unknown       | Binding | Plasma membrane                  |
| gil12855383 | Unnamed protein product       | 89210  | −0.910  | 0    | Unknown       | Binding | Plasma membrane                  |
| gil26349775 | Unnamed protein product       | 92544  | −0.122  | 8    | Homeostasis/transport Channel/receptor | Plasma membrane |
| gil26327363 | Unnamed protein product       | 97302  | −0.021  | 2    | Metabolism | Enzyme | Plasma membrane                  |
| gil26335845 | Unnamed protein product       | 100564 | −0.007  | 2    | Metabolism | Enzyme | ER membrane/plasma membrane      |
| gil26336330 | Unnamed protein product       | 149228 | −0.445  | 0    | Signaling     | Binding | Myosin complex                   |
| gil26342298 | Unnamed protein product       | 168512 | −0.765  | 0    | Unknown       | Motor/binding | Golgi apparatus membrane |

a Accession number in the NCBInr mouse database.
b The average values of hydrophobicity, expressed as GRAVY, predicted by SOSUI.
c Number of transmembrane domains predicted by SOSUI.
d,e GO annotations of MPs categorized as process, function and subcellular location groups.

Figure 2. Schematic representation of the MP identification strategy.

unconfident hits. These analyses resulted in the identification of 318 verified MPs in the liver membrane faction from C57BL/6J mice. It should be noted that beside many high-abundant proteins such as sodium/potassium-transporting ATPase, families of cytochrome P450 and several families of UDP glucuronosyltransferase and so on, a large number of low-abundant proteins were detected. Additionally, 23 were proteins with unknown functions and 43 proteins (13.52%) were found to be unique microsome membranes.

Interestingly, 16 proteins were also identified as unnamed products using this approach. As shown in table 1, the majority of 16 poorly characterized proteins displayed one or more transmembrane domains, and their molecular mass ranged from 25 to 168 kDa. The GRAVY values of those proteins were negative and varied from −0.765 to −0.081, suggesting that they are hydrophobic.
the calculated MW of our data was from 20.4 to 611.2 kDa, in which no proteins had a MW < 20 kDa.

For low MW proteins, a different electrophoresis system such as Tricine-SDS-PAGE or higher concentration of polyacrylamide gel might be chosen for analysis. In the region above 500 kDa, 13 MPs were also detected, revealing that our methods could be used to investigate complex and insoluble proteins with high MW, especially in the identification of proteins with mass > 100 kDa. Of 318 verified proteins, 212 (66.67%) were integral MPs with at least one or more TM domains (as predicted using SOSUI software), and there were 16 MPs with TM regions > 10, indicating that our procedure is ideally suited for the detection of highly hydrophobic and complex proteins. Besides, 106 MPs in our study do not display any TM domains and are therefore unlikely to be inserted in the membrane. These proteins are probably peripherally attached to the membrane (called peripheral membrane proteins) and are resistant to the alkali treatment. The TM domain distribution showed that 89 and 75 proteins, 77.36% out of a total of 212 integral MPs, had one and two TM domains, respectively (figure 3(b)). These theoretical TM domains are higher than those 88 TMs from Zhang et al [25] and 105 TMs from Zhang et al [26]. This might be due to the enrichment of low-abundant highly hydrophobic proteins in the microdomain fractionation strategy.

The average hydrophobicity of MPs is usually termed the GRAVY value. Our data revealed that the GRAVY values changed from $-1.1276$ to 0.9016 and only 31 (9.76%) of MPs had positive values (figure 3(c)). The majority of the analyzed proteins (75.79%) have a GRAVY value between
Table 2. Top ten proteins with the lowest GRAVY values.

| NCBInr  | Protein name                              | MW (Da) | Process | Function | GRAVY | TM |
|---------|-------------------------------------------|---------|---------|----------|-------|----|
| gi|200381 Mouse protein kinase C delta mRNA, complete cds | 78951   | Unknown | Binding   | −1.127755 | 0  |
| gi|6752966 A disintegrin and metalloprotease domain 19 preproprotein | 103703  | Unknown | Unknown   | −0.991061 | 0  |
| gi|11990231 ABC transporter                  | 272866  | Unknown | Motor     | −0.987533 | 0  |
| gi|31560705 acyl-CoA synthetase long-chain family member 1 | 78928   | Transport | Enzyme   | −0.982901 | 0  |
| gi|75992917 acyl-CoA synthetase long-chain family member 6 isoform 4 | 79003   | Unknown | Structure | −0.97355 | 0  |
| gi|34783870 Adenylate cyclase 3              | 130159  | Unknown | Binding   | −0.90977 | 0   |
| gi|119372300 ADP-riboseylation factor-like 6 interacting protein 2 isoform 1 | 66809   | Unknown | Binding   | −0.89732 | 0   |
| gi|10798999 Anion exchanger 2 type b1       | 135922  | Unknown | Binding   | −0.896847 | 0  |
| gi|28892815 Ankyrin and armadillo repeat containing | 167010  | Signaling | Binding  | −0.891756 | 0  |
| gi|56206474 Ankyrin-repeat and fibronectin type III domain containing 1 | 87896   | Unknown | Unknown   | −0.869029 | 1  |

−0.6 and 0. Interestingly, the top ten proteins with the lowest GRAVY had no TM domains, except ankyrin-repeat and fibronectin type III domain containing 1 (gi|56206474) with one TM segment (table 2). It is not always easy to distinguish the hydrophobic and hydrophilic nature of proteins. For example, the ATP-binding cassette transporter (gi|116297244) with 9 TM domains, considered to be a hydrophobic protein, has a negative GRAVY value (−0.046), while annexin A1 (gi|124517663) without a predicted TM segment, also has a negative GRAVY value of −0.435. And UDP glycosyltransferase 1 family polypeptide A12 (gi|31324702) had a GRAVY value of only 0.061, but displayed two TM regions.

According to Gene Ontology (GO) annotations, subcellular locations of verified MPs were calculated and categorized as indicated in figure 4(a). Of 318 MPs from mouse liver membrane fractions, 49% and 27% were proteins with plasma membrane and ER membrane subgroups, respectively. This is particularly appropriate because plasma and ER occupy the largest areas on the cellular membranes. Approximately 9% and 4% were involved in the microsomal membrane and mitochondrion membrane. Other subgroups were also classified, including the membranes of peroxisome, endosome, the Golgi apparatus, vesicle, nuclear and so on. It should be noted that one kind of MP could be located in one or several sites on the cellular membrane. Therefore, two subgroups might share one or more proteins. Figure 4(b) illustrates the annotated proteins by their known function. 93.77% identified proteins had a GO annotation, whereas there were 6.23% proteins with unknown function. The GO function distribution showed that 38% and 25% were proteins with catalytic and binding activities. Our results are consistent with those presented in the studies by Zhang’s group [4, 25, 26] who also found a large percent of enzyme and binding distributions. Besides, other proteins which had special functions were also analyzed including receptor (11%), structure (4%), immune response (2%) transporter (5%), motor (4%), channel (4%) and transducer (1%), as described in figure 4(b). Usually, one protein might have one or more relative functions and also one subgroup could be from many proteins. Thus, MPs should be categorized in the form of percentage other than number.

The biological process distribution of the 318 identified proteins was also analyzed using GO annotation. There were 16 subgroups classified such as regulation, signaling, transport, biosynthesis, catabolism, cell adhesion, cell proliferation, immune response, homeostasis, oxidation reduction, phosphorylation, proteolysis, cell differentiation, apoptosis, metabolism and unknown. The results suggested that the identified MPs diversify and take part in various important processes in the cell, of which 19% were proteins with unknown processes and about 10% of proteins were related to signaling in the cell. Recently, classified proteomics approaches were carried out to analyze the relative distribution of MPs in different biological processes for drug targets. The strategy presented here might be applied for these purposes due to its throughput, high sensitivity and accurate properties.

4. Conclusions
A total of 318 MPs from mouse liver tissues were confidently identified and 66.67% of them contained at least one or more TM domain. Interestingly, 16 unnamed proteins were found among the identified MPs. Several physicochemical characteristics of those proteins were also assessed in terms of MW, TM segments, and hydrophobic GRAVY values by using bioinformatics analysis. The biological processes, functions and subcellular locations of the identified proteins were categorized as well according to universal GO annotations, and about 10% were proteins involved in cell signaling pathways. Our results suggested that membrane proteomics will allow more pathway study related to cancers or other diseases in the cell and will enable a better understanding of drug targets.

Acknowledgments
We would like to thank Professor Bill Jordan from Victoria University of Wellington (New Zealand), Director of AOHUPO MPI, for providing the protocol for preparation of the mouse liver microsomal fraction and C57BL/6J mouse liver microsomal membrane fraction as a AOHUPO
MPI ‘membrane standard’ sample. The work has been carried out at the Key Laboratory of Gene Technology, Institute of Biotechnology, Vietnam Academy of Science and Technology.

References

[1] Adam P J et al 2003 Comprehensive proteomic analysis of breast cancer cell membranes reveals unique proteins with potential roles in clinical cancer J. Biol. Chem. 278 6482–9

[2] Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P 2002 Molecular Biology Of The Cell, 4th edn (New York: Garland Science)

[3] Andersen J S, Wilkinson C J, Mayor T, Mortensen P, Nigg E A and Mann M 2003 Proteomic characterization of the human centrosome by protein correlation profiling Nature 426 570–4

[4] Ashburner M et al 2000 Gene ontology: tool for the unification of biology The Gene Ontology Consortium, Nat. Genet. 25 25–9

[5] Blagoev B, Ong S E, Kratchmarova I and Mann M 2004 Temporal analysis of phosphotyrosine-dependent signaling networks by quantitative proteomics Nat. Biotechnol. 22 1139–45

[6] Chen P et al 2007 Evaluation of strategy for analyzing mouse liver plasma membrane proteome Sci. China C: Life Sci. 50 731–8

[7] Dailey M M, Hait C, Holt P A, Maguire J M, Meier J B, Miller M C, Petraccone L and Trent J O 2009 Structure-based drug design: from nucleic acid to membrane protein targets Exp. Mol. Pathol. 86 141–50

[8] Goette A, Lendeckel U and Klein H U 2002 Signal transduction systems and atrial fibrillation Cardiovasc. Res. 54 247–58

[9] Gottesman M M, Fojo T and Bates S E 2002 Multidrug resistance in cancer: role of ATP-dependent transporters Nat. Rev. Cancer 2 48–58

[10] Hopkins A L and Groom C R 2003 The druggable genome Nat. Rev. Drug. Discov. 1 727–30

[11] Kyte J and Doolittle R F 1982 A simple method for displaying the hydropathic character of a protein J. Mol. Biol. 157 105–32

[12] Marmagne A, Rouet M A, Ferro M, Rolland N, Alcon C, Joyard J, Garin J, Barbier-Brygoo H and Ephritikhine G 2004 Identification of new intrinsinc proteins in Arabidopsis plasma membrane proteome Mol. Cell Proteomics 3 675–91

[13] Melen K, Krogh A and von Heijne G 2003 Reliability measures for membrane protein topology prediction algorithms J. Mol. Biol. 327 735–44

[14] Molloy M P, Herbert B R, Walsh B J, Tyler M J, Traini M, Sanchez J C, Hochstrasser D F, Williams K L and Gooley A A 1998 Extraction of membrane proteins by differential solubilization for separation using two-dimensional gel electrophoresis Electrophoresis 19 837–44

[15] Oh P, Li Y, Yu J, Durr E, Krasinska K M, Carver L A, Testa J E and Schnitzer J E 2004 Subtractive proteomic mapping of the endothelial surface in lung and solid tumors for tissue-specific therapy Nature 429 629–35

[16] Perkins D N, Pappin D J, Creasy D M and Cottrell J S 1999 Probability-based protein identification by searching sequence databases using mass spectrometry data Electrophoresis 20 3551–67

[17] Santoni V, Molloy M and Rabilloud T 2000 Membrane proteins and proteomics: un amour impossible? Electrophoresis 21 1054–70

[18] Schulze W X and Mann M 2004 A novel proteomic screen for peptide-protein interactions J. Biol. Chem. 279 10756–64

[19] Seddon A M, Curnow P and Booth P J 2004 Membrane proteins, lipids and detergents: not just a soap opera Biochim. Biophys. Acta 1666 105–17

[20] Stevens T J and Arkin I T 2000 Do more complex organisms have a greater proportion of membrane proteins in their genomes? Proteins 39 417–20

[21] Svirnovski A I, Shimian T V, Serhiyenka T F, Savitski V P, Smolnikova V V and Fedasenka U U 2009 ABCB1 and ABCG2 proteins, their functional activity and gene expression in concert with drug sensitivity of leukemia cells Hematology 14 204–12

[22] Thanh T T, Phuong N T M, Nhi N B and Chi P V 2008 Changes of serum glycoproteins patients with lung cancer J. Proteomics Bioinformatics 1 15–22

[23] Wallin E and von Heijne G 1998 Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms Protein Sci. 7 1029–38

[24] Wu C C and Yates J R 2003 The application of mass spectrometry to membrane proteomics Nat. Biotechnol. 21 262–7

[25] Zhang L, Xie J, Wang X, Liu X, Tang X, Cao R, Hu W, Nie S, Fan C and Liang S 2005 Proteomic analysis of mouse liver plasma membrane: use of differential extraction to enrich hydrophobic membrane proteins Proteomics 5 4510–24

[26] Zhang L, Wang X E, Peng X, Wei Y JR, Cao, Liu Z, Xiong J X, Yin X F, Ping C and Liang S 2006 Proteomic analysis of low-abundant integral plasma membrane proteins based on gels Cell. Mol. Life Sci. 63 1790–804