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Dissolving capability difference based sequential extraction: A versatile tool for in-depth membrane proteome analysis

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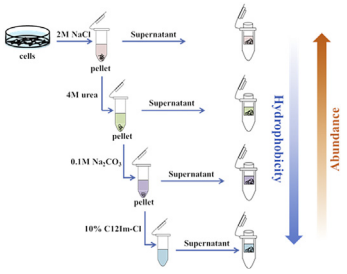
HIGHLIGHTS

- A sample preparation method was developed for in-depth membrane proteome profiling.
- Our strategy utilized the differential dissolving capability of extraction solvents.
- A most comprehensive membrane proteome dataset of HeLa was achieved by our study.
- Referred to neXtProt database, 358 missing proteins were discovered in our dataset.
- 110 of the identified 358 missing proteins were annotated to be membrane proteins.

GRAPHICAL ABSTRACT



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ABSTRACT

Profiling membrane proteins would facilitate revealing disease mechanism and discovering new drug targets as they play essential roles in cellular signaling, substrate transport, and cell adhesion. However, the analysis of membrane proteins still remains a challenge due to their high hydrophobicity, as well as the suppression effect of high abundant soluble proteins. In this work, to achieve a membrane proteome profiling, a sample preparation strategy based on sequential extraction at the protein level assisted by a range of extraction reagents with different dissolving capabilities, followed by nano-RPLC-ESI-MS/MS analysis was developed and applied for HeLa cell line analysis. It was found that with progressively harsher extraction reagents (i.e., 2 M NaCl, 4 M urea, 0.1 M Na_2CO_3, and 10% 1-dodecyl-3- methylimidazolium chloride (C12ImCl) performed, much more high hydrophobic proteins and low abundant proteins were identified. With our developed strategy, 5553 of the identified proteins (4419 gene products) were annotated to be membrane proteins and 2573 proteins (2183 gene products) have at least one transmembrane domain, to our best knowledge, which is the most comprehensive membrane proteome dataset for HeLa cell line. Notably, 110 of the identified membrane proteins were discovered in the "missing proteins" list referred to those in the neXtProt database. All above results indicated that our strategy has great potential to tackle the difficult but relevant task of identifying and profiling membrane proteins.

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1. Introduction

Membrane proteins lie at the critical junctions between intracellular compartments and cells and their environment to mediate a host of cellular processes, including cell communication, substances transportation, and immune response recognition dictation [1–3]. Furthermore, the membrane proteins are considered to be the most popular drug targets, accounting for over 50% of all modern medicinal drug targets [4]. As such, profiling membrane proteins would facilitate revealing disease mechanism and discovering new drug targets. However, different from the rapid progress in the analysis of soluble proteins, membrane proteins have lagged behind and are typically under-represented in datasets due to their low abundance and high hydrophobicity [5].

Subcellular fractionation, including differential centrifugation and density gradient centrifugation, was frequently used to isolate the membrane protein fraction [6]. Lai's group [7] employed a differential centrifugation method for membrane proteins enrichment, and 49% of the proteins with Gene Ontology (GO) compartment annotation were annotated as "membrane proteins". Besides, Triton X-114 phase partitioning was proposed to separate the membrane and aqueous proteins by heating the Triton X-114 until it reaches its cloud point to yield aqueous phase soluble materials and detergent phase soluble materials [8]. This strategy was applied by Mobasheri et al. [9] for membrane proteome analysis of articular chondrocytes, and the proportion increased from 20% to 55%.

Moreover, to improve the identification efficiency of integral membrane proteins (IMPs), the strategy of washing respectively with high-salt and high-pH buffers for the removal of non-membrane proteins and membrane-associated proteins was employed [10,11]. With this strategy, Petrak et al. identified 1224 proteins, 65% of them were predicted to have at least one transmembrane domain [12]. Wu et al. employed discontinuous sucrose gradient centrifugation and 0.2 M sodium carbonate (Na_2CO_3) buffer (pH 11.0) to obtain an enriched plasma membrane fraction, followed by protease-accessible peptides remove by proteinase K digestion [13]. With this strategy, 98% of identified proteins were predicted to have at least one transmembrane domain.

However, with all the above mentioned methods, though the suppression effect of high abundant proteins on low abundant membrane proteins was significantly decreased, the loss of membrane proteins occurred during the soluble proteins removal, detrimental to the mapping of membrane proteome. To improve the proteome coverage, sequential protein solubilization in solvents with different solubilizing strength was utilized by many groups [14–16]. However, there was still some pelleted material remained due to the low solubilization ability of the used solubilizers, which was a barrier for membrane proteome profiling [17].

In our strategy, considering both the ability and bias of different solvents on membrane proteins dissolving, proteins sequential extraction with a series of reagents was employed for membrane proteome profiling. Additionally, the extracted proteins were efficiently digested with our previously developed in-situ filter-aided sample pretreatment method, imFASP [18] with minor modification, followed by two dimensional-reverse phase/reverse phase-liquid chromatography-tandem mass spectrometry (2D-RP/RP-LC-MS/MS) analysis. By this strategy, not only the suppression effect of high abundant proteins on low abundant membrane proteins could be significantly decreased, but also the loss of membrane proteins occurred in pretreatment was reduced, achieving a more comprehensive membrane proteome dataset of HeLa cell line than those that have been reported. Given the crucial cellular functions of membrane proteins, the information obtained by our strategy could help to map signaling pathways, further to elucidate the molecular function and discover new drug targets.

2. Experimental procedure

2.1. Reagents and materials

The ionic liquid 1-dodecyl-3-methylimidazolium chloride (C12Im-Cl) was obtained from Shanghai Cheng Jie Chemical (Shanghai, China). Urea was purchased from Fluka (Buchs, Switzerland) and sequencing-grade modified trypsin was purchased from Promega (Madison, WI, USA). Tris(hydroxymethyl) aminomethane (Tris), dithiothreitol (DTT), iodoacetamide (IAA), formic acid (FA) and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). Acetonitrile (ACN, HPLC grade) and methanol were purchased from Merck (Darmstadt, Germany). The Microcon filtration device with a relative molecular mass cut-off of 10,000 (10k filter) was from Sartorius AG (Goettingen, Germany). Deionized water was purified using a Milli-Q system from Millipore (Milford, MA, Germany). Other chemicals were of analytical grade.

Luna C18 particles (5 μm, 100 Å pore) were obtained from Phenomenex (Torrance, CA, USA) and Durashell C18 particles (5 μm, 100 Å) were obtained from Agela Technologies (Tianjin, China). Fused-silica capillaries (75 μm i.d./360 μm o.d.) were purchased from Sino Sumtech (Handan, China).

2.2. Solubility measurement for bacteriorhodopsin

Equal aliquots of bacteriorhodopsin (25 μg) were solubilized in 20 μL four extractants, including 2 M NaCl, 4 M urea, 0.1 M Na_2CO_3 and 1% C12Im-Cl in phosphate-buffered saline (PBS) buffer (1.4 mM sodium chloride (NaCl), 0.27 mM potassium chloride (KCl), 1 mM disodium hydrogen phosphate (Na_2HPO_4), 0.18 mM potassium dihydrogen phosphate (KH_2PO_4), pH 7.4). The samples were mixed using a vortex mixer for 1 min and sonicated for 15 min in a water bath at room temperature. Afterward, the samples were centrifuged to remove insoluble materials, and the supernatants were collected. The samples were then quantified by the BCA method at 562 nm with BSA as the standard protein. All the measurements were repeated twice in parallel.

2.3. Sequential protein extraction

HeLa cells (2.5×10^8) were washed with PBS thrice, centrifuged at 250*g* for 5 min at 4 °C to collect and stored at −80 °C. The pellets were thawed on ice and subjected to the following extraction procedure as shown in Fig. 1. (i) NaCl extraction: the pellet was lysed and extracted with 9 mL of 2 M NaCl in PBS (pH 8.0) and 1% (v/v, 1 mL of cocktail solution per 100 mL of extraction buffer) final concentration of cocktail, and processed under sonication with 15 cycles of 10 s on/off. After centrifugation at 1000*g* for 5 min at 4 °C, the supernatant was collected and under sonication in a water bath for 20 min. Then, the sample was centrifuged at 100,000*g* (SW40Ti rotor, Beckman Coulter, Fullerton, CA, USA) for 60 min at 4 °C and the collected supernatant was named as 'NaCl fraction'. (ii) Urea extraction: the obtained pellet was resuspended with 9 mL of 4 M urea in PBS and 1% cocktail, and processed under sonication with 9 cycles of 10 s on/off. Then, the sample was centrifuged at 100,000*g* for 60 min at 4 °C and the collected supernatant was named as '4 M urea fraction'. (iii) Na_2CO_3 extraction: the obtained pellet was resuspended with 9 mL of 0.1 M Na_2CO_3 in PBS and 1% cocktail, and processed under sonication with 9 cycles of 10 s on/off. Then, the sample was centrifuged at 100,000*g* for 60 min at 4 °C and the collected supernatant was named as 'Na_2CO_3 fraction'. (iv) C12Im-Cl extraction: the residual pellet was further extracted with 1.5 mL 10% C12Im-Cl (m/v) in PBS and 1% cocktail and named as