Membrane Proteome Analysis Using Lipid-Based Protein Immobilization (LPI™) Technology

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Objectives

To evaluate the capability of the Nanoxis LPI™ technology using an erythrocyte membrane preparation as the model system.

Introduction

Plasma membrane proteins are fundamental in defining the physiological state of the cell by performing functions such as signal transduction, cell-cell contact, the selective transport of molecules and other essential functions. Elucidating the profile of integral membrane proteins on live cells is vital for uncovering diagnostic biomarkers, therapeutic agents and drug receptor candidates. Moreover, the significance of plasma membrane proteins in drug discovery and drug development is evident by the fact that about 70% of all drug targets are directed towards these proteins [1]. Here, the LPI™ technology was used to perform a global expression profiling of membrane proteins in erythrocytes.

Materials and Methods

LPI™ SamplePrep Kit

LPI™ FlowCell is a single use device with a proprietary surface that allows for immobilization of intact proteoliposomes directly produced from membrane preparations of a wide variety of cells and tissues. Proteins are kept in their native cell membrane with retained structure and function.

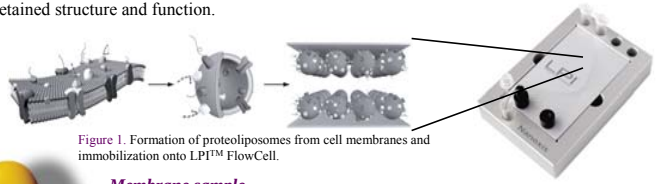


Figure 1. Formation of proteoliposomes from cell membranes and immobilization onto LPI™ FlowCell.

Membrane sample

Membranes prepared from red blood cells were diluted with buffer (10 mM Tris-HCl, 300 mM NaCl, pH 8) to a total lipid/protein concentration of approximately 1 mg/mL. Proteoliposomes (approximately 150 nm in diameter) were created using a tip sonication protocol. 350 µL of the resulting proteoliposome solution was injected into the LPI™ FlowCell where the proteoliposomes were immobilized. Any contaminating debris was removed by rinsing the flow cell with buffer (20 mM ammoniumbicarbonate (AMBIC), pH 8).

LPI™ flow cell trypsination

The membrane proteins in the immobilized proteoliposomes were digested by incubating the sample with trypsin (0.005 mg/mL in 20 mM AMBIC, pH 8) for 2 hours at 37°C. The resulting peptides were eluted with 700 µL buffer (20 mM AMBIC, pH8). The sample was vacuum centrifuged to dryness and reconstituted in 20 µL 0.1 % FA in water.

Nano HPLC Chip ESI-MS/MS analysis

- HPLC chip: 150 x 0.075 mm analytical column and 160 nL enrichment column (operated in forward flush mode), packed with 5 µm Zorbax C18 material.
- Sample load: 1 µL of the 20 µL reconstituted sample after drying in a SpeedVac.
- Flow: 200 nL/min analytical pump, 4 µL/min loading pump.

Figure 2. The **Agilent 1200 HPLC-Chip MS/MS system** equipped with an **6510 QTOF** was used for the nano reversed-phase HPLC-ESI-MS/MS analysis.

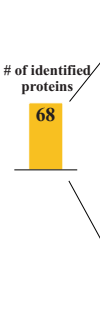
- Mobile phases A: 0.1% FA, B: 90% acetonitrile, 0.1% FA.
- Gradient: 3%B to 50%B during 40 min.
- MS parameters: capillary voltage 1850 V, mass range 300-2000 m/z, fragmentor voltage, skimmer voltage and octopole RF were set to 175 V, 65 V and 750 V, respectively.
- Data acquisition: positive ionization profile mode, data dependent MS/MS, acquisition rate 5 spectra/s, max 3 precursors per cycle were selected for MS/MS, active exclusion was enabled (exclusion after 2 spectra, released after 0.16 min), ref. mass correction was enabled.
- The MassHunter Workstation Acquisition B.01.02 software was used for system control

Protein identification by database searching

The peak list from the acquired MS/MS data was generated by Mascot Distiller and submitted to database searching using Mascot (MatrixScience). The search was performed against the human subset of AstraZeneca in-house protein sequence databases (Genseq P, RefSeqP, PDB, PIR, SwissProt and TREMBL). The Mascot searching was performed using the following settings for the Agilent 6510 QTOF instrument: peptide mass tolerance of 50 ppm and a fragment mass tolerance of 100ppm). Oxidation (M) was allowed as variable modification. The criteria for a positive protein identification was the at least two tryptic peptides where the total Mascot ion score was ≥ 50. After manual validation, roughly 70 distinct integral membrane proteins belonging to human erythrocytes were identified.

Results

The results from the global membrane protein expression analysis of the red blood cells (RBC) indicated a large portion of integral membrane proteins. This is correlated with the sample preparation where high salt and and high pH washes were used to diminish the amount of associated proteins. About 70 membrane proteins were identified, with abundance levels from millions of copies per cell down to a couple of hundred copies per cell as depicted in figure 3.



Integral Membrane Protein	Copies/cell	# Peptides	Coverage(%)
High abundance:			
Band 3 anion transport protein	1000000	25	24
Glycophorin A	1000000	8	15
Erythrocyte band 7 integral mp	300000	14	48
Facilitated glucose transp. member 1	300000	9	20
Blood group Rh(CE) polypeptide	200000	10	14
Rhesus blood-group-assoc. glycoprot.	200000	7	18
Aquaporin-1	200000	6	12
Glycophorin C	50000	5	19
Low abundance:			
Urea transporter, erythrocyte	14000	3	6
Equilibrative nucleoside transporter 1	10000	4	8
Kell blood group glycoprotein	6000	6	15
Membrane transport protein XK	6000	3	5
Plasma membrane Ca-transp. ATPase 1	4000	4	4
Plasma membrane Ca-transp. ATPase 4	4000	5	8
T-cell surface glycoprot. E2 precursor	4000	2	3
Sodium/potassium-transp. ATPase	400	2	4

Figure 3. Abundance level and sequence coverage of identified integral membrane proteins.

References

1. Wiles, T., Drug Discovery World, 2006.

Conclusions

It has been demonstrated that the LPI™ technology is a powerful tool for global protein expression analysis of integral membrane proteins. It enabled identification of about 70 distinct membrane proteins using stringent Mascot dB search criteria. Furthermore, the dynamic range of the identified membrane proteins was from one million copies per cell down to a couple of hundred copies per cell.

Acknowledgements

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