Clinical Proteomics: A Technology to shape the future?

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(http://research.med.helsinki.fi/corefacilities/proteinchem)
Over 1200 Researchers in only Medical Research (Cancer, Genetics, Developmental Medicine, Neuroscience etc.)
The Medical Faculty

HYKS-instituutti Oy
Lastentautien laboratorio
Proteomics

What is it all about??
Proteins Rule

Biotech’s latest mantra is “proteomics,” as it focuses on how dynamic networks of human proteins control cells and tissues

By Carol Ezzell  | Wednesday, April 24, 2002 10

Move over, human genome, your day in the spotlight is coming to a close. Researchers are now concentrating
The Post-Genome Project
Could The Human Proteome Will Be Successfully Mapped In Three Years Depends On How You Define "Proteome"
By Karen Hopkin | August 17, 2001 | 0
A Long Way to the Bedside

Despite many breakthroughs, personalized medicine has not translated yet to patient care.
Recent years have seen rapid technological progress in the fields supporting personalized medicine, from biomarker discovery to mapping the genome to pushing back the frontiers of mass spec sensitivity. However, thus far, little of that progress has translated to the clinic where it can benefit patients.
Success rate of biomarker search which would be in clinical use: 0
How can we make Proteomics more suitable to the “real” life?

The technology...

2D gel electrophoresis
2D liquid chromatography
Micro arrays
We need a technology to find changes in Proteome

Administration of a drug known to bind to an orphan receptor

- Changes in expression level of 23 proteins

A protein Array
Two-dimensional gel electrophoresis (2D) could do it?

- 1st dimension, IEF, Proteins are separated according to their isoelectric point (IP)
- 2nd dimension, SDS-PAGE, Proteins are separated according to their molecular mass
- Efficient: More than a thousand proteins resolved in E-Coli cell lysates and ~8000 in brain lysates
1st Dimension - Isoelectric Focusing

pH4  pH5.5  pH7

neutral  gelsurface

pI - isoelectric point

ready made Gel-strips

24 cm Immobiline™ DryStrip
- Highest resolution
- Ready to use, precast gels
- Accurate, stable pH gradients
2nd Dimension - Isoelectric Focusing

2DE
Mw
Protein Fingerprint: 2-DE

About 2000 proteins
Micro gel devices

gel reservoir:
25x32x0.3/0.5 mm
PMMA, silica

electrodes
sample inlet
buffer reservoir
gel reservoir
water cooling

Running time 10 minutes
Buffer reservoir
Sample loading
Micro Pipette
Comb sample applicator
PASGE lid
Gel plate
Resolving gel
Stacking gel

A

Sample loading
O-ring
Buffer reservoir

B

C

D
Automated 2D devices
2-D map of IEF standards

- 3-10 pH gradient

Repeatability of 2-DE runs

<table>
<thead>
<tr>
<th>Rf values (%)*</th>
<th>pI position errors(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>STDV</td>
<td>6.1</td>
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<tr>
<td>max</td>
<td>15</td>
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<tr>
<td>min</td>
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<tr>
<td>max</td>
<td>6</td>
</tr>
<tr>
<td>min</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* comparison of 3 gels

- 2-DE separation completed in **approx. 80 min**
- Limit of detection is **approx. 65 ng**

Running time 20-30 minutes
• Performance
  • Native IEF and native PAGE
    ▪ 5 variants of hemoglobin
    ▪ pH 6.7 - 7.7
  • Native IEF and SDS-PAGE
    ▪ standard IEF proteins
    ▪ pH 3-10
  • Denatured IEF and SDS-PAGE
    ▪ GFAP protein variants expression differences
    ▪ in control and Alzheimer diseased patients
    ▪ pH 4-6
Intra-individual expression differences of Cytokeratin 20 in patient 14.

The left gel segment (a) is zoomed from the normal mucosa, gel b represents the patient's polyp and gel c is the corresponding segment of the same patient's adenocarcinoma.
Also 2D Databases exist!

<table>
<thead>
<tr>
<th>HUMAN Homo sapiens</th>
<th>Colorectal epithelia cells</th>
<th>Cerebrospinal Fluid</th>
<th>Colorectal adenocarcinoma cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroleukemia Cell</td>
<td>HepG2 Secreted Proteins</td>
<td>HepG2</td>
<td></td>
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<td>Promyelocytic leukemia cells</td>
<td>Kidney</td>
<td>Liver</td>
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</tr>
<tr>
<td>Lymphoma</td>
<td>Plasma</td>
<td>Platelet</td>
<td></td>
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</tbody>
</table>
What about chip-based proteomics: DIOS-MS

Desorption Ionization On Silicon (DIOS)
Testing the functionality of various surfaces for proteomics

Signal intensity of unphosphorylated peptide 2423 (left) and phosphorylated peptide 2422 (right) from different surfaces.
Black Silica chip

384 samples (e.g. phosphopeptides)
10800 samples on the chip
High Sample Throughput for the Post-Genomic Era
Schematic diagram of a Reflector MALDI-TOF mass spectrometer.

MALDI-TOF = Matrix Assisted Laser Desorption/Ionization - Time Of Flight
Quadrupole TOF/TOF
Even with the most sophisticated LC-based MS instruments of today we will possibly not be able to use them for high-throughput clinical screening?
Although the time for one analysis is fast...

**UPLC™ increases Speed by 9X (900%)**

**HPLC**
No. of components: 5
Complete Separation: 6.00 min

**UPLC®**
No. Of components: 5
Complete Separation: 0.60 min

Increased sample throughput
And you can analyze thousands of compounds in a few hours...

75 μm x 250 mm, 1.7 μm BEH C_{18}, 45 °C
7% to 40% ACN / in 122 min
Analytical flow rate = 300 nL/ min

Median RT St Dev = 3.2 sec (n=6)
Median RSD intensity = 6.9%
Proteomics chip technology
The whole 2D LC can be done in a chip

- Sub-2-micron chromatographic performance
- Eliminates manual connections
- Low system volumes
- Integrated emitter and electronic components
The whole 2D LC can be done in a chip

- Sub-
- Elimin-
- Inte-

- chip
- control
- electrospray

© 2000 Waters Corporation
High throughput CE

10000 Capillaries

Capillary windows

LED detection (MIT, Forest et al, 2006)

Autosampler
Ultra-high throughput CE chips for proteomics

Geniom chips
- Currently for DNA/RNA analysis
  (Development ongoing for proteome analysis)

Capacity approx. 40000 ligands
A set of thousands of Mass Spectrometers in one chip
Other techniques to help in proteomics?

- Tissue microdissection
- Imaging MS
Why Tissue Microdissection?

You would like to isolate only the targeted diseased tissue.
Laser-based microdissection:
Capturing of the vessels in the control brain slide

Cap
Slide before capture
Slide after capture
Cap with the selected tissue
MALDI MS analysis directly from the tissue captured on the cap membrane
Whole Cell and Protein Microarray Chip structures
Cell trapping and - lysis Chip structures for single Cell Proteomics?

Development of Integrated Nanoliter Analysis Devices (DDTC-Viikki, Microtechnology Center-HUT, Biomedicum Helsinki)

The TIME component!
MALDI mass spectrometric imaging of biological tissue sections for protein imaging
A whole mouse

Penetration of the drug into the tissue
1 DIRECT IMMUNOHISTOCHEMISTRY

MALDI UV LASER

2 LASER DESORPTION

3 SPECIFIC MASS SPECTROMETRY IMAGING
The main objectives today for clinical and general Proteomics:

- **Quantification** of all the proteins expressed in a cell or tissue proteome, body fluids e.g. blood, CSF etc. Searching for Biomarkers!

- **Functional study** of thousands of proteins in parallel, which protein is in contact to another protein and where? Searching for functionality!

For quantification purposes, standard **method** is 2DE electrophoresis or MudPIT separation followed by MS identification

For protein function studies, microarray based assays are used to study protein-protein and protein-ligand interactions
Synopsis of the genome-wide screen of complexes in M. pneumoniae

S Kühner et al. Science 2009;326:1235-1240

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