Introduction to BioMEMS & Medical Microdevices

Proteomics and Protein Microarrays

Companion lecture to the textbook: Fundamentals of BioMEMS and Medical Microdevices, by Prof. Steven S. Saliterman, http://saliterman.umn.edu/
Proteomics

The study of all proteins, including their relative abundance, distribution, post-translational modifications, functions, and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.
Areas of Interest

1) Abundance proteomics:
   - Relative abundance of specific proteins in a given tissue under different conditions of health and disease.

2) Cell-mapping:
   - Intracellular signaling pathways and regulatory networks

3) Structural proteomics:
   - Study of active sites and functional domains.
Eukaryotic Gene Regulation

The entire collection of proteins, estimated to be more than 100,000.

More proteins comprise a proteome than genes a genome.

- Alternative *gene splicing* of mRNA,
- *Posttranslational modification* (PTM).

There is neither a one to one correlation of gene to protein, nor mRNA levels to proteins levels.

PTM and signal transduction play a major role in cell transformation, such as tumor cells.
Post-translational Modification

- Post-translational modification (PTM):
  - Phosphorylation, glycosylation, acetylation, ubiquitination, methylation etc.
  - PTM of proteins, not detected through RNA analysis, may occur at different stages of tumor development indicative of early or late events of transformation.
  - High throughput techniques may be useful for screening and surveillance.
Amino Acids to Proteins

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Tertiary Structure

Polypeptide chain

Hydrogen bond

Ionic bond

van der Waals forces

Covalent (disulfide) bond

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Identification of Proteins

Western Blot

(A) Protein transfer on membrane
- Performing SDS-PAGE
- Proteins on membrane
- Detection with a secondary antibody

ELISA

(B) Adding sample
- Antigens coated on plate
- Antibodies bind to antigens
- Detection with a secondary antibody

Bead-based Method

(C) Adding sample
- Beads coated on surface
- Antibodies bind to beads
- Detection with a secondary antibody

Using Mass Spectrometry

Protein Purification → 2-D Gels → Digest single protein to peptides → HPLC → Mass Spectrometer → Data Analysis

Electrophoresis Approaches

1-D Gels → Digest protein mixture to peptides

Non-electrophoresis Approaches

Digest protein mixture to Peptides

Mass Spectrometers

Left: University of Maryland
Right: NASA Martian Rover

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Protein Microarrays

- Useful for study of studying protein expression, interaction, function and post-translational modifications.
- High-throughput, high sensitivity, low sample volumes, and efficient sample-to-result time.
- Forward-phase microarrays:
  - Proteins and peptides are immobilized for capturing antibodies.
  - Antibodies, sugars or aptamers are immobilized and labeled proteins are captured.
  - Sandwich mode – a labeled secondary antibody is used for detection.
- Reverse phase microarrays:
  - Complex samples such as serum, plasma, or even tissues are immobilized in an array format and probed with antibodies to determine the differential amount of protein molecules in the screened samples.
Additional Classification

- Differential profiling and screening protein arrays.
- Functional protein arrays.
- Arrays from biological samples.
- Cell-based protein arrays.
- Cell-free protein arrays.
- Antibody arrays.
Array Formats

Analytical protein (detecting) microarrays

a) Protein/Peptide microarrays for antibody detection
b) Antibody/sugar/aptamer microarrays for protein detection (direct mode)
c) Antibody/sugar/aptamer microarrays for protein detection (sandwich mode)
d) Reverse phase microarrays

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Protein, Peptide and Small Molecule Array

# Capture Molecules

<table>
<thead>
<tr>
<th>Type</th>
<th>Principle</th>
<th>Application</th>
</tr>
</thead>
</table>
| Protein-Protein    | ![Diagram](image1.png)                        | - Disease progression  
                        |                                                | - Signal-pathway studies                      |
| Enzyme-Substrate   | ![Diagram](image2.png)                        | - Substrate binding analyses                    |
| Receptor-Ligand    | ![Diagram](image3.png)                        | - Drug discovery                                |
| Antigen-Antibody   | ![Diagram](image4.png)                        | - Biomarker identification in auto-immune diseases |
| Aptamers           | ![Diagram](image5.png)                        | - Protein-protein interaction analyses          |

Pin Printing

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. Analyst, 2014, 139, 1303-1326,
Microstamping

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. Analyst, 2014, 139, 1303-1326,
Photolithographic


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E-Beam Lithography

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. * Analyst, 2014, 139, 1303-1326,  

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Dip Pen Nanolithography

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. Analyst, 2014, 139, 1303-1326,
Single Droplet Noncontact Printing

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. Analyst, 2014, 139, 1303-1326,
A Microfluidic-Interfaced Printer

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. Analyst, 2014, 139, 1303-1326,
Nucleic Acid Programmable Protein Array

In-Situ Puromycin Capture

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. Analyst, 2014, 139, 1303-1326,
Protein In-Situ Array

Romanov, V. et al. A critical comparison of protein microarray fabrication technologies. Analyst, 2014, 139, 1303-1326,
## Technology Comparison

<table>
<thead>
<tr>
<th>Throughput</th>
<th>Spot quality</th>
<th>Array fabrication flexibility (ability to print different biomolecules: cells, antibodies, proteins, lipids etc.)</th>
<th>Maintenance</th>
<th>Special requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pin printing</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++, n, τ, δ</td>
</tr>
<tr>
<td>Microstamping</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++, δ, τ, ε</td>
</tr>
<tr>
<td>Photolithography</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++, δ, τ, ε</td>
</tr>
<tr>
<td>DPN</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++, τ, δ, ε</td>
</tr>
<tr>
<td>E-beam</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++, δ, τ, ε</td>
</tr>
<tr>
<td>Thermal InkJet</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++, n, τ, δ</td>
</tr>
<tr>
<td>Pico Actuation</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++, n, τ, δ</td>
</tr>
<tr>
<td>Valve jet</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++, n, τ, δ</td>
</tr>
<tr>
<td>Microfluidics</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>BSA cell free</td>
<td>++</td>
<td>++</td>
<td>N/A</td>
<td>+++</td>
</tr>
<tr>
<td>NAPPA cell free</td>
<td>++</td>
<td>++</td>
<td>N/A</td>
<td>+++</td>
</tr>
</tbody>
</table>

### Ratings criteria

- **++++**: 1,000’s spots per second. Consistent inter and intra spot morphology. Can be used to generate arrays employing a very large and diverse number of biomolecules. Very little maintenance, can be performed by the user. Very few external and internal factors need to be controlled. Samples can be printed straight out of the box or as directed. Some variables with need to be controlled. Some modification of protocols is necessary to insure effective printing.

- **+++**: 100’s spots per second. Usually consistent requiring very little adjustment. Can be used to generate arrays using a number of different biomolecules. Maintenance is required however user can be guided to fix most issues. A few variables with need to be controlled. A large number of external or internal factors will need to be accounted for.

- **++**: 10’s spots per second. Some inconsistency in morphology, requiring consistent user readjustment. Is limited to printing a small amount of biomolecules. Maintenance requires outside expertise. Samples need to be modified in order to be used with the system. Most variables will need to be controlled.

- **+**: <1 spot per second. Inconsistent spot morphology. Can effectively array a certain type of biomolecule. Intricate maintenance, requires outside expertise, with potentially long machine downtimes. Samples need to be modified in order to be used with the system. Most variables will need to be controlled.

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Microarray Immobilization

<table>
<thead>
<tr>
<th>Immobilization Methods</th>
<th>Peptides</th>
<th>Small Molecules</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncovalent</td>
<td>biotin/avidin</td>
<td>fluorous/fluorous</td>
<td>His tag/Ni-NTA</td>
</tr>
<tr>
<td>DNA/DNA</td>
<td>biotin/avidin</td>
<td></td>
<td>ZR/ZE domain</td>
</tr>
<tr>
<td>DNA/PNA</td>
<td></td>
<td></td>
<td>GST/anti-GST</td>
</tr>
<tr>
<td>DNA/DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random covalent</td>
<td>amine/NHS</td>
<td>photocrosslink</td>
<td>amine/NHS</td>
</tr>
<tr>
<td>amine/epoxy</td>
<td>isocyanate/variou</td>
<td></td>
<td>amine/epoxy</td>
</tr>
<tr>
<td>amine/aldehyde</td>
<td>silyl chloride/alcohol</td>
<td></td>
<td>amine/aldehyde</td>
</tr>
<tr>
<td>Site-specific covalent</td>
<td>Diels-alder</td>
<td>staudinger ligation</td>
<td>native chemical ligation</td>
</tr>
<tr>
<td>native chemical ligation</td>
<td>glyoxylyl/aminooxy</td>
<td></td>
<td>click chemistry</td>
</tr>
<tr>
<td>Staudinger ligation</td>
<td>tetrazine/dienophile</td>
<td></td>
<td>staudinger ligation</td>
</tr>
<tr>
<td>glyoxylyl/semicarbazide</td>
<td>thiol/quinone methide</td>
<td></td>
<td>oxime ligation</td>
</tr>
<tr>
<td>thiol-ene</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Selected Examples of Immobilization Strategy in Microarray Fabrication

Immobilization Strategy Continued

Protein Microarray Uses

- Protein expression profiling.
- Studying posttranslational modifications,
- Protein-protein binding,
- Drug interaction,
- Protein folding,
- Substrate specificity,
- Enzymatic activity and
- Interaction between proteins and nucleic acids.
Recent Application Examples

Applications Continued

D

Hairpin RNA

E

Amyloid peptide

F

Lectin

Antigen Array

To Study Autoimmune disease

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Factors Affecting Performance

- Appropriate surface for the immobilization of either protein or antibody samples.
- Microarray patterning technique.
- Protein conformational changes with expression, purification or immobilization may alter their function or render them inactive.
- Charged surfaces, temperature, pH and solvents may denature some proteins, and therefore surfaces must be biocompatible to minimize denaturation.
- Protein instability may lessen shelf-life.
Summary

- **Proteomics** - The study of all proteins, including their relative abundance, distribution, post-translational modifications, functions, and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.

- **Proteome** - The entire collection of proteins, estimated to be more than 100,000,

- **Gene Expression and Regulation.**

- **Identification of Proteins**
  - Western Blot, ELISA, and Bead Methods.
  - Mass Spectrometry
Protein Microarrays

- Array Formats
- Capture Molecules
- Fabrication Technologies
- Immobilization Methods
- Applications
- Factors Affecting Performance