Introduction to BioMEMS & Medical Microdevices

**Protein Micro Total Analysis Systems (Protein μTAS)**

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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 mediated through protein-protein interactions.

3) Structural proteomics:
   ● Study of active sites and functional domains of proteins to better understand processes such as enzyme catalysis, protein stability and interaction with multi-molecular complexes.
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.
- Specific genes are turned on or off at the onset of initiation, development, and progression of diseases such as cancer.
- Signal transduction, via growth factors, proteins, and peptides, plays a major role in cell transformation (e.g. carcinogenesis).
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.
• Proteins are made from the linkage of *amino acids* by peptide bonds to form a polypeptide.
• If there are less than 50 amino acids it is called a peptide, and if greater than 50 amino acids it is called a protein.
• The position of each type of amino acid in a polypeptide chain and the total number of amino acids in the chain distinguish one polypeptide from another.
Bonding and Tertiary Structure…

Myoglobin

Identification of Proteins

Western Blot - separation of proteins according to their length and isoelectric point using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) followed by transfer to a membrane.

ELISA – wet samples incubated with specific antibodies in a microtiter plate. Detection is in wells.

Bead-based Method – e.g. Luminex® is a bead-based method that can analyze multiple samples in one experiment by utilizing different bead types.

a) Protein purification may be performed by affinity chromatography, ion exchange, or subcellular fractionation. Then, 2-dimensional gel electrophoresis allows isolation of single proteins which may be digested for MS.

b) Use of 1-dimensional gel allows partial electrophoretic separation, requiring further separation such as high-performance liquid chromatography (HPLC).

c) The entire mixture is digested to peptides and the peptide mixture is resolved by multi-dimensional chromatography.
Mass Spectrometers…

Left: University of Maryland
Right: NASA Martian Rover

Steven S. Saliterman
Separation is Based on Mass-to-Charge Ratio…

Mass Spectrometer

1. Sample enters chamber
2. If necessary, heater vaporizes sample
3. Electron beam knocks electrons from atoms
4. Electric field accelerates particles toward magnetic region
5. Magnetic field separates particles according to their mass-to-charge ratio

Lightest particles in sample
Heaviest particles in sample

Detector
X^+
Y
Z^+
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.
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.
Antibodies for Antigen Identification...

**Direct immunoassay**

- Antigen affixed to substrate.
- Dye, nanoparticle, enzyme etc.
- Binding to the exposed epitope of the immobilized antigen.

**Indirect immunoassay**

- Multiple secondary antibodies bind to the original antibody, amplifying the signal.

Sequences of 110 amino acids.

The sample is prepared and incubated with the microarray. Previously, a corresponding binding partner for the molecule of interest was immobilized on the surface of the microarray. Here, an interacting antibody and antigen are depicted. Detection is performed by labeling a secondary antibody, which results in intensive signals if the molecule of interest is present.
Array Formats...

a) Proteins and peptides are immobilized as capturing agents for antibody detection, whereas an anti-immunoglobulin, a common labelled antibody, is used for detection.

b) Antibodies, sugars, or aptamers are immobilized, and labelled proteins are captured.

c) A labelled secondary antibody is used for detection.

d) Complex samples such as serum, plasma or tissues are immobilized in an array format and probed with antibodies to determine the differential amount of protein molecules in the screened samples.

Three General Protein Microarray Types…

a) Antibodies may be probed with cell lysate for determining protein expression levels as well as the specificity of the resultant interaction.

b) Can be used to study the biochemical properties and activities of target proteins.

c) Can be used for investigating post-translational modifications and biomarker identification.
a) Outline of the general experimental workflow for protein array and antibody arrays analysis.
b) Overview of different array configurations showing increasing number of features/spots printed and detected as round fluorescent spot signals.
Protein, Peptide and Small Molecule Array...

Surface functionalization

Biomolecule immobilization

Microarray Construction

Peptides
Small molecules
Proteins

Function
Annotation
Substrate Fingerprinting
Ligand/Inhibitor Binding
Other Applications

Microarray
Application

Most common microarray interaction partners and their application possibilities.

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<th>Type</th>
<th>Principle</th>
<th>Application</th>
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</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…

a) A robotic print head with multiple printing pins is loaded with print solutions from a source plate and then contacts the substrate surface to deposit protein solution in

b) Various types of pins: C1 is a solid pin. C2 is a slotted pin. C3 is a quill pin, distinguished from the split pin by the inclusion of a reservoir.
Microstamp Fabrication and Printing…

Photolithographic…

a) Undeveloped photoresist (red).
b) Photoresist is exposed to light (yellow) through the photomask.
c) Development removes the exposed, softened photoresist and a nano-patterned photoresist is generated.
d) Microarray is generated by attachment of proteins to patterned photoresist.
E-Beam Lithography

a) Undeveloped photoresist.
b) E-beam ablates photoresist.
c) Protein/antibodies attach to photoresist to generate array.
Dip Pen Nanolithography

a) Patterned microarray generated by AFM.
b) Previously dipped AFM tip transferring protein solution to surface. A meniscus of protein solution on the AFM tip transports molecules to the surface in the desired pattern.

a) Thermal Inkjet. A heating element rapidly creates a bubble within the chamber. As the bubble propagates, liquid is further squeezed out of the orifice. Upon bubble collapse, sample is ejected,
b) Piezo actuation. A diaphragm is used to displace the sample within.
c) Pressure valve.
a) CFM print head is docked against the surface.
b) Close-up of the flow cells within the print head.
c) Close-up of one channel. Solution can be cycled back and forth over the surface, ensuring total coverage of the surface.
a) Expression plasmids encoding the proteins, as glutathione s-transferase (GST) fusions are biotinylated and immobilized onto a glass slide that has been coated with avidin and an anti-GST antibody which acts as the protein capture reagent.
b) Plasmid array is then used for in situ expression of the proteins using rabbit reticulocyte cell lysate or a similar cell-free expression system.
c) The protein is synthesized.
d) The protein is immediately captured by the immobilized antibody within each spot. This process generates a protein array where every protein is co-localized with its analogous expression plasmid.
In-Situ Puromycin Capture…

a) A streptavidin surface,
b) mRNA is hybridized with a single-stranded DNA oligonucleotide that has been modified with biotin and puromycin,
c) The ribosome interacts with the RNA/DNA section of the molecule, where DNA is cross-linked to the nascent polypeptide through the puromycin moiety,
d) mRNA is digested with added RNase, leaving a protein array immobilized through the C-termini to the DNA linker, which is in turn immobilized through a biotin/streptavidin interaction to the surface.
Protein In-Situ Array...

a) Protein capture tags are array on the surface
b) DNA and cell-free extract is added to the slide
c) mRNA is produced via the cDNA template
d) Newly synthesized protein is captured by the capture agent via a tag
e) Slide is washed to remove any non-specific binding and is ready for quantification.
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
  ● Uses
  ● Array Formats
  ● Capture Molecules
  ● Fabrication Technologies
  ● Factors Affecting Performance
● Addendum – Technology comparison
# Technology Comparison

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<tr>
<th></th>
<th>Throughput</th>
<th>Spot quality</th>
<th>Array fabrication flexibility</th>
<th>Maintenance</th>
<th>Special requirements</th>
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Ratings criteria:

- +++ 1,000 spots per second
- +++ 100 spots per second
- ++ 10 spots per second
- + <1 spot per second

Array fabrication flexibility:
- Ability to print different biomolecules: cells, antibodies, proteins, lipids, etc.

Notes:
- ✪ = viscosity, ✤ = ongoing expenses, ✧ = humidity, ✩ = temperature, ✫ = buffers, ✬ = clean room conditions.