Biosensor Design Considerations
Outline

- What do you want to detect?
- How will you fabricate your sensor?
- How will you ensure specificity?
- What is your preferred detection method?
- How will you avoid false signals?
- How will you improve sensitivity?
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The Analyte: What do you want to measure?

Target Molecule:

- Protein, toxin, peptide, vitamin, sugar, metal ion

Glucose

Cholera Toxin
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Sample Handling

(How do you deliver the analyte to the sensitive region?)

- (Micro)fluidics
- Concentration (increase/decrease)
- Filtration/Selection
Biosensor Design
Design & Fabrication
(How do you make the device?)
Biosensor Design

Design & Fabrication

(How do you make the device?)

Biosensor Design
Detection/Recognition
(How do you specifically detect the analyte?)
Antibody/Antigen Interaction

- Antibody: Consists of four polypeptides - two heavy chains and two light chains joined to form a "Y" shaped molecule.

- The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. This variable region, composed of 110-130 amino acid residues, give the antibody its specificity for binding antigen.
Peptides and Polypeptides

Structure of an Amino Acid

Side chain

Amino Group

Peptide group 1

Peptide group 2

Carboxyl Group

A side chain:
Glutamine (Gln)

\[ \text{CH}_2 - \text{CH}_2 - \text{C} = \text{O} \]
\[ \text{NH}_2 \]

Dipeptide

Polypeptide

N-terminus

+H\textsubscript{3}N−

Cys

Ar

Gly

Leu

C-terminus
Proteins and Antibodies

Secondary Structure

Tertiary Structure

Quaternary Structure
Antibody/Antigen Interaction

In hen egg white lysozyme, a glutamine at position 121 (Gln 121) protrudes away from the antigen surface.

Hydrogen bonds (yellow) stabilize the antibody-antigen interaction. Van der Waals, hydrophobic and electrostatic forces also improve the binding specificity between antibody and antigen.
Immunoassay: Competitive Binding 1

- Antibody
- Immobilisation surface
- Antigen
- Antigen-enzyme complex

Coating → Incubation

Affinity Reaction

Enzyme Reaction

Product Measurement
I. No analyte - high detection signal

II. Analyte present - detection signal reduced
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Signal (How do you know there was a detection?)

Specific Recognition?

The antibody is usually immobilized on a solid support/sensor

- Common Signalling Principles:
  - Optical (Surface Plasmon Resonance, Total Internal Reflectance Fluorescence)
  - Electrical (Voltammetry, Potentiometry, Conductivity)
  - Electromechanical (Piezoelectric crystal)
  - Thermal
  - Magnetic (Beads)
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Avoiding False Signals

Specific Recognition

Non Specific Signal

False Specific Recognition?
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Improving Performance

Secondary Signal Amplifier

Highly Specific Detection

Inert Background

Magnetic bead, fluorescent dye, enzyme, etc.
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Regeneration or Single Use?

Break Binding

Low and high pH buffers
pH~1 and pH~13
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Data Analysis

- Response: Variable vs Time
- Example of Response

Variables:
- Fluorescence
- Refractive index
- Potential
- Current
- Frequency
- Mass
- Temperature
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Baseline

Should be stable when there is no binding

Quantifying Noise:
Root mean square (RMS) of a sample of data points for a given time

\[ x_{\text{rms}} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} x_i^2} = \sqrt{\frac{x_1^2 + x_2^2 + \ldots + x_n^2}{n}} \]

Quantifying Drift:
Shift in the baseline (RMS) shown as response units per time

Stable Baseline

Drifting Baseline
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Sensitivity

Signal-to-Noise Ratio:
Per time unit

Spikes:
Rapid (1 datapoint) shift in signal

Baseline Shift:
Rapid (1 datapoint) shift in baseline (offset)
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Identify Signal Error Sources

- Inhomogenous sample
- Bubbles/flow artifacts
- Temperature
- Electromagnetic interference
- Electronic unstability
- Unstable chip/detection layer
Improving Sensitivity

Active Sensor: Detects the analyte.

Reference Sensor: Coated with inert material does not detect the analyte.

Output Signal $S$:

$S = R_1 - R_2$, or $S = \frac{R_1}{R_2}$

The reference is exposed to the same kind of disturbances as the active sensor. These effects are cancelled out by taking the difference or ratio between the two sensor outputs.