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Lecture 17
Biosensors

1. What are biosensors?

The term is used in the literature in many ways. Some definitions:

a) A device used to measure biologically-derived signals
b) A device that "senses" using "biomimetic" (imitative of life) strategies
ex.,"artificial nose"
c) A device that detects the presence of biomolecules

We will adopt a recent IUPAC definition:

"A self-contained integrated device which [sic] is capable of providing specific quantitative or semi-quantitative analytical information ***using a biological recognition element*** which is in direct spatial contact with a transducer element."

2. Uses of biosensors

- Quality assurance in agriculture, food and pharmaceutical industries
ex. *E. Coli, Salmonella*

- Monitoring environmental pollutants & biological warfare agents
ex., *Bacillus anthracis* (anthrax) spores

- Medical diagnostics
ex., glucose

- Biological assays
ex., DNA microarrays

3. Classes of biosensors

A) Catalytic biosensors: kinetic devices that measure steady-state concentration of a transducer-detectable species formed/lost due to a biocatalytic reaction

Monitored quantities:
i) rate of product formation
ii) disappearance of a reactant
iii) inhibition of a reaction

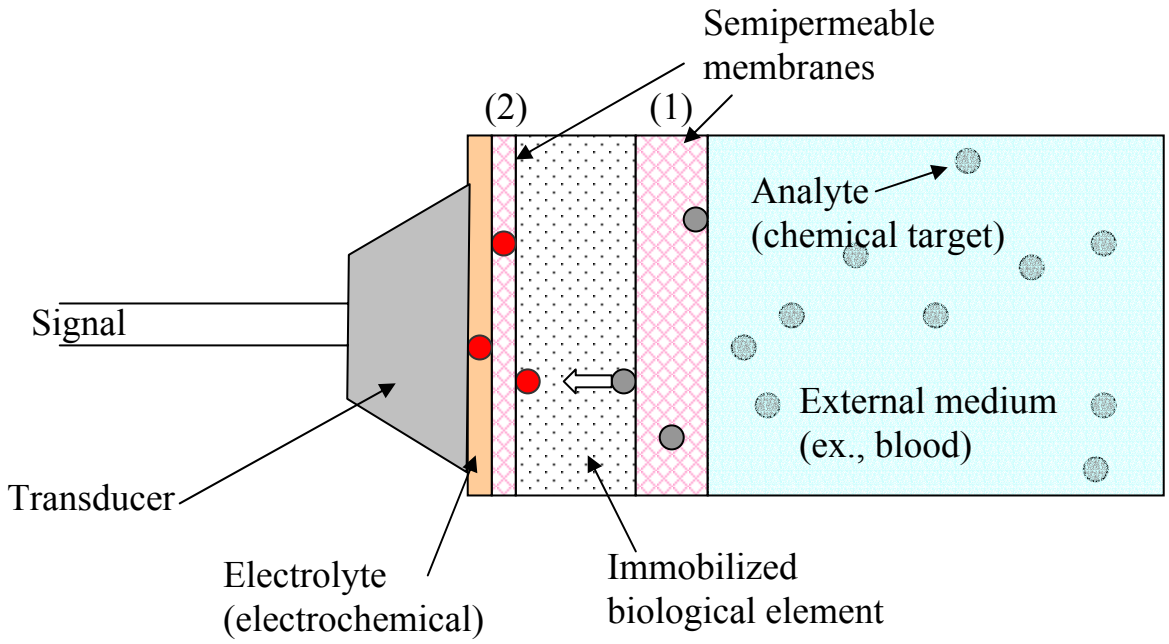
Biocatalysts used:
i) enzymes
ii) microorganisms
ii) organelles
iv) tissue samples

B) Affinity biosensors: devices in which receptor molecules bind analyte molecules "irreversibly", causing a physicochemical change that is detected by a transducer

Receptor molecules:
i) antibodies
ii) nucleic acids
iii) hormone receptors

Biosensors are most often used to detect molecules of biological origin, based on specific interactions.

4. Biosensor Components



Analyte: chemical/biological target

Semipermeable Membrane (1): allows preferential passage of analyte (limits fouling)

Detection Element (Biological): provides specific recognition/detection of analyte

Semipermeable Membrane (2): (some designs) preferential passage of by-product of recognition event

Electrolyte: (electrochemical-based) ion conduction medium between electrodes

Transducer: converts detection event into a measurable signal

A) Detection Elements

1) Catalysis Strategies: enzymes most common
ex., glucose oxidase, urease (catalyzes urea hydrolysis), alcohol oxidase, etc.

Commercial Example: glucose sensor using glucose oxidase (GOD)

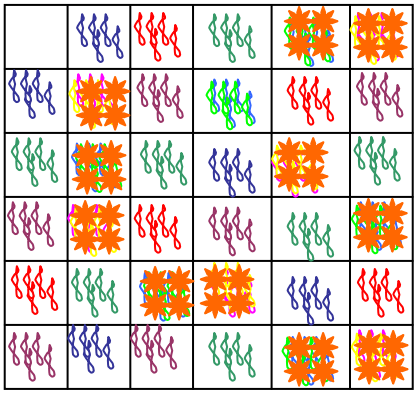
$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{GOD}]{} \text{Gluconic acid} + H_2O_2$$

3 potential measurement routes:
1. pH change (acid production)
2. O_2 consumption (fluorophore monitor)
3. H_2O_2 production (electrochemical)

Commercially Available Biosensors: glucose, lactate, alcohol, sucrose, galactose, uric acid, alpha amylase, choline, L-lysine—all amperometric based (O_2 /H_2O_2)

2) Affinity Binding strategies: antibodies & nucleic acid fragments most common

Commercial Example: DNA chip



B) Transducers

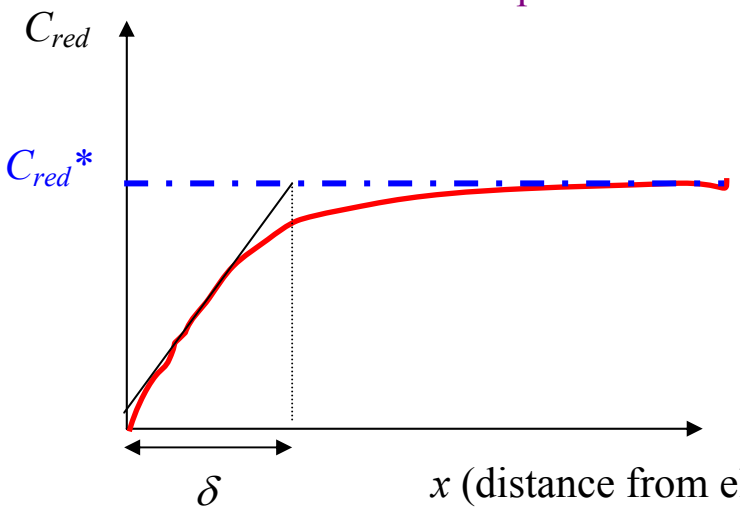
1) Electrochemical: translate a chemical event to an electrical event by measuring current passed (amperometric = most common), potential change between electrodes, etc.

Oxidation reaction of the reduced chemical species C_{red}:

$$C_{red} \rightarrow C_{ox} + ne^-$$

Amperometric Devices

Measured current is mass transport limited



$$i = i_{\lim} = -n\Im AJ$$

$\Im$ = 96,487 coulombs (Faraday const.)

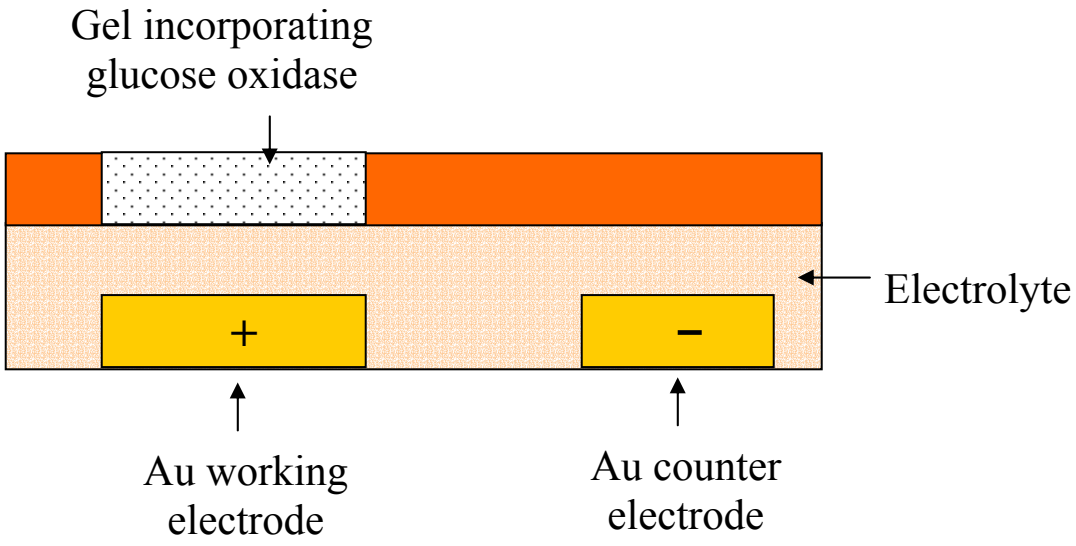
A = electrode area

δ = boundary layer width

where J is the flux:

$$J = -D\frac{dC_{red}}{dx} \approx -D\frac{C^*_{red} - 0}{\delta} \quad \Rightarrow \quad i \approx \frac{n\Im ADC^*_{red}}{\delta}$$

Example: Glucose sensor based on oxidation of peroxide
(most commercial devices)



$$\text{Glucose} + O_2 + H_2O \xrightarrow[\text{GOD}]{} \text{Gluconic acid} + H_2O_2$$

Anodic: $H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$

current passed thru
working electrode

(Recall: oxidation occurs at anode;
here, $O^{-1} \rightarrow O^0$)

2) Photochemical: translate chemical event to a photochemical event, measure light intensity and wavelength (λ)

a) Colorimetric: measure absorption intensity

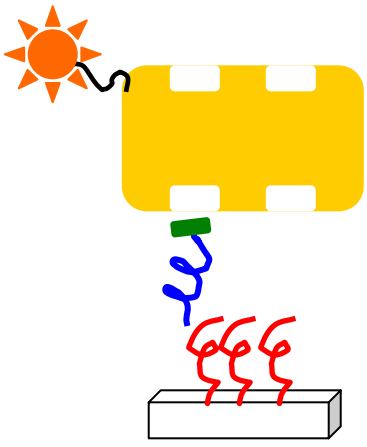
Examples

Indirect: H_2O_2 + Dye Precursor $\xrightarrow[\text{enzyme}]{\text{peroxidase}}$ Colored Dye

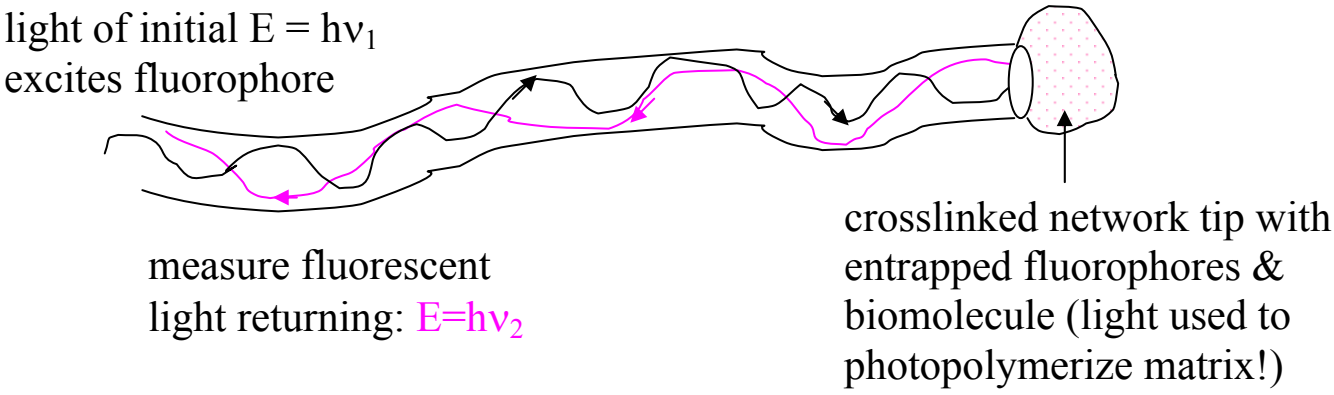
Direct: flavin adenine dinucleotide (FAD) bound cofactors (redox sites on GOD) absorption at 377nm & 455nm disappears in presence of glucose

b) Fluorescence

Example 1: DNA microarrays– fluorophores selectively bound to detected molecule via avidin-biotin complex; commercialized by Affymetrix (S. Fodor)



Example 2: fiber optic sensors: fluorophores incorporated into tip change fluorescence level depending on level of target present



Typically:

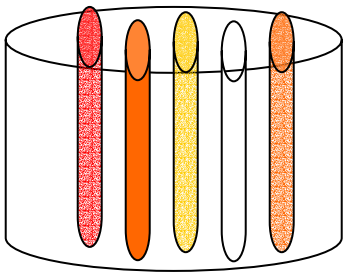
- Oxygen present at tip quenches fluorescence from trapped fluorophore (ex., tris(4,7-diphenyl-1,10 phenantroline) Ru(II) dichloride = $Ru(dpp)_3^{2+}$ Cl_2)

- Action of trapped oxidase (biological element, ex., GOD) depletes O_2, causing ↑ fluorophore emission

$$\text{Glucose} + O_2 + H_2O \xrightarrow[\uparrow]{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

How can we account for natural O_2 fluctuations?

Multichannel fiber optic: 1. enhancing selectivity and/or
2. multianalyte detection

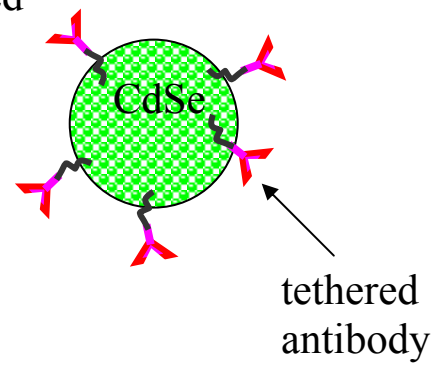


How can we measure multiple analytes?

MD. Marazuela et al., "Fiber-optic biosensors- an overview", *Anal. Bioanal. Chem.* **372**, 664 (2002).

Example 3: Semiconductor nanoparticles (quantum dots)
currently in development, ex., Quantum Dot Corp. (P. Alivasatos)

Typically affinity binding-based

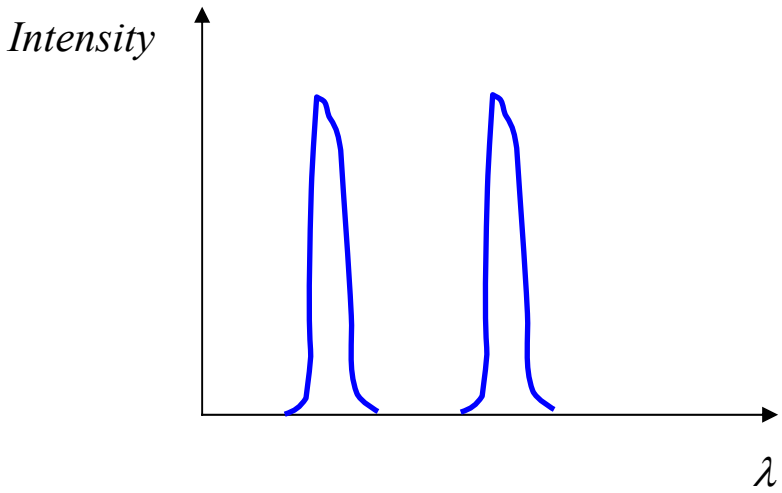


ADVANTAGES:

i) QD band gap (and hence emission)
varies with size ⇒ multiple analyte capability

2 nm CdSe⇒ green
5 nm CdSe⇒ red

ii) sharp, intense emission spectra (higher signal/noise)



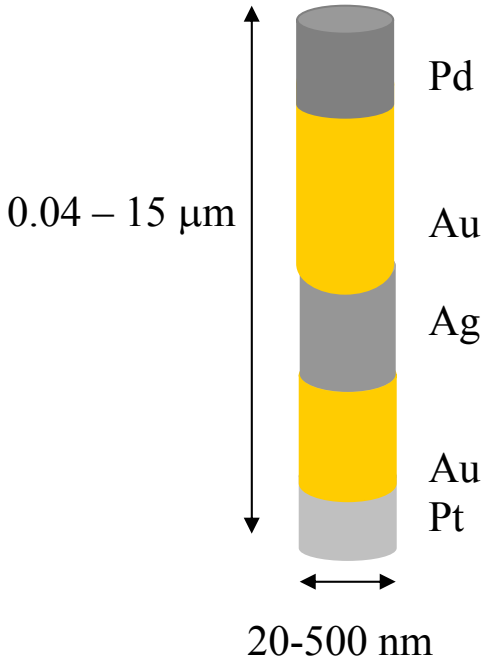
iii) can be used for surface or solution-based approaches

A.P. Alivisatos, *Science* **271**, 2013 (1998).

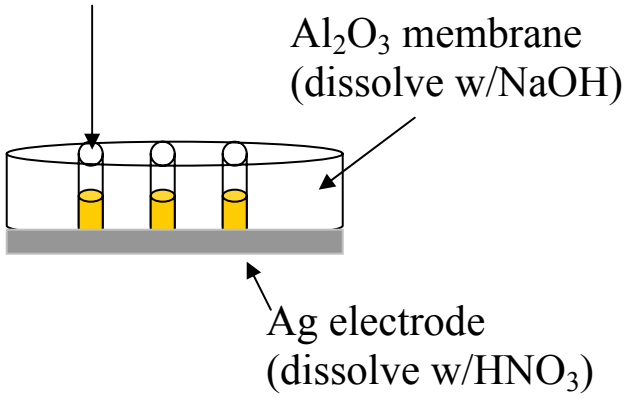
c) Reflectance

Example 1: "Nanobarcodes" – reflection from surface of multilayer metallic rods provides optical signature; being developed by Surromed, Inc. (M. Natan)

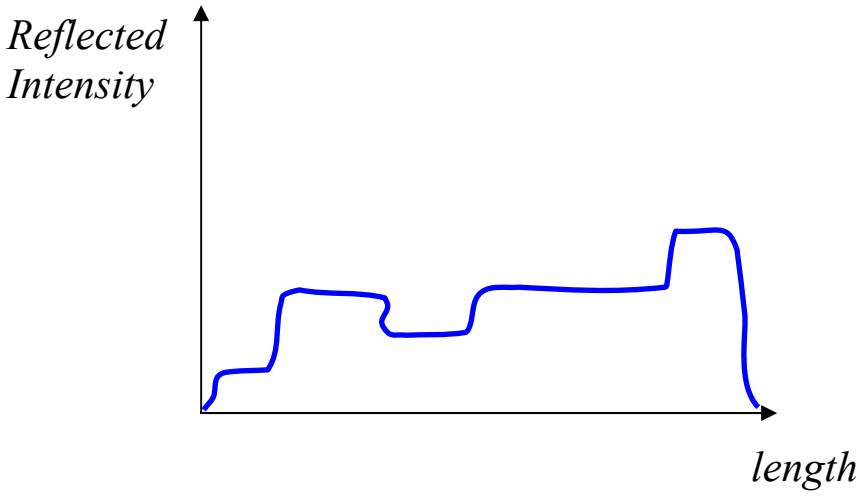
Affinity-binding based



Made by electrochemical reduction of a series of metal salts into template pores



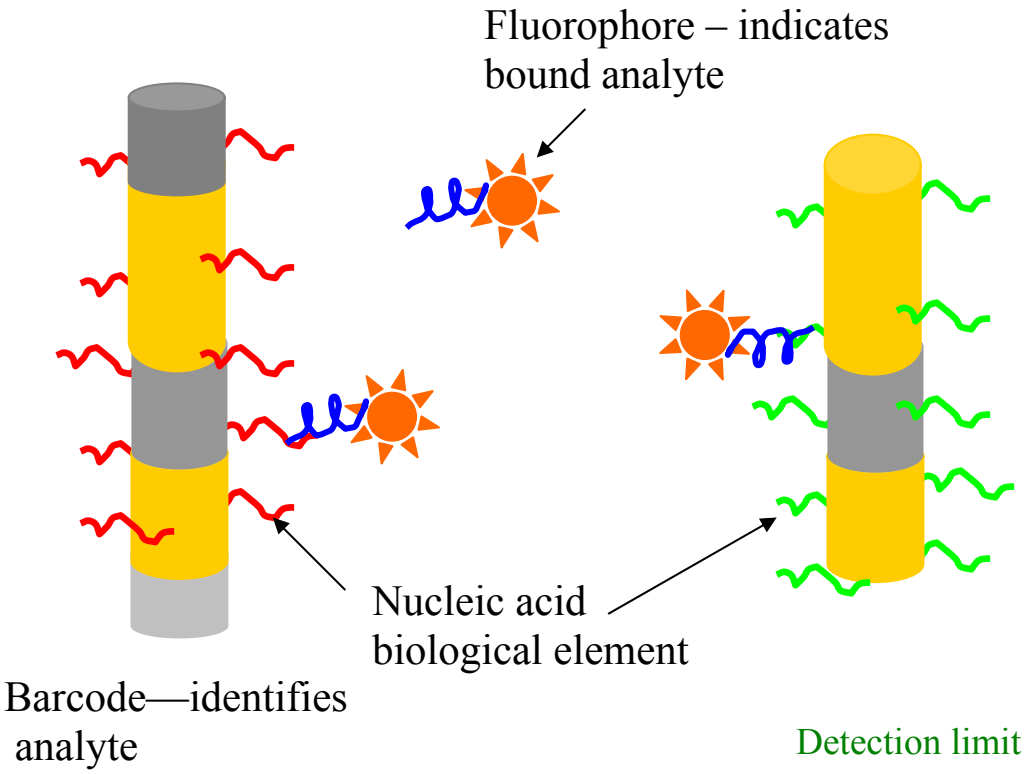
Reflectance microscopy gives unique signature for each rod



ADVANTAGES:

i) solution based (not limited by surface area)
ii) many combinations of lengths/sequences ⇒ multiple analyte capability

Multianalyte transduction uses a single fluorophore



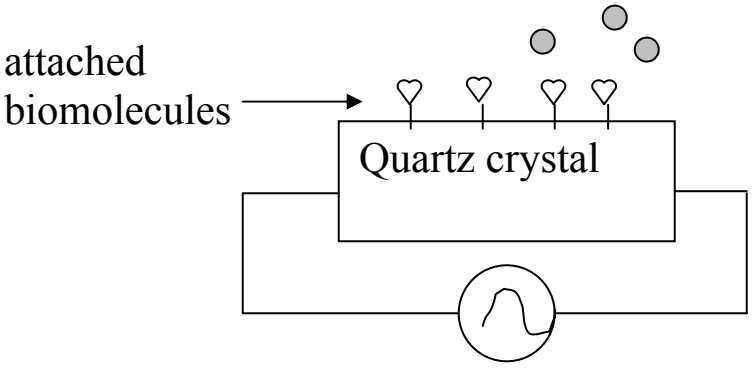
Detection limit: 1-10 ng/ml

Challenges: will require high-throughput readout mechanism

S.R. Nicewarner-Pena et al., *Science* **294**, 137 (2001).

3) Piezoelectric: translate a mass change from a chemical adsorption event to electrical signal

Example: Quartz Crystal Microbalance



- Crystal vibrates at resonant frequency parallel to applied field:
$\nu = (k/m)^{1/2}$

typical: 5 MHz;
research grade: 100-200MHz

- A change in quartz mass (due to adsorption) changes ν.

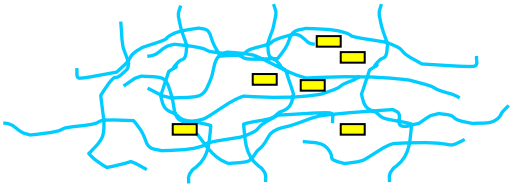
Advantage: high sensitivity-- 10's of nanograms/cm^2
Disadvantage: highly sensitive to nonspecific adsorption

C.K. O'Sullivan and GG. Guilbault, *Biosensors & Bioelectronics* **14**, 663 (1999).

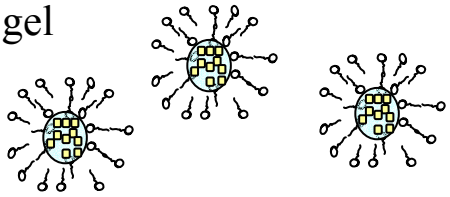
5. Detection Element Immobilization Methods

Physical entrapment—viscous aqueous soln trapped by membrane permeable to analyte

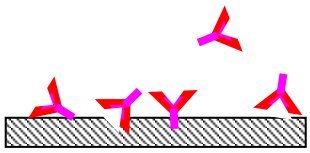
Membranes: cellophane, cellulose acetate, PVA, polyurethane
Entrapment Gels: agarose, gelatin, polyacrylamide, poly(N-methyl pyrrolidone)

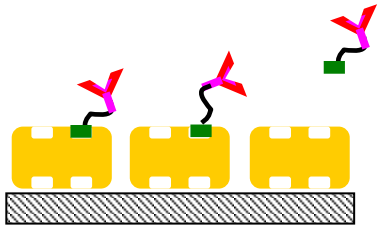


Microencapsulation: inside liposomes, or absorbed in fine carbon particles that are incorporated in a gel or membrane

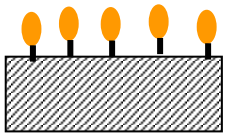


Adsorption: direct adsorption onto membrane or transducer; can also be adsorbed onto pre-adsorbed proteins, e.g., albumin; avidin (via biotin linker)





Covalent binding (via –COOH, -NH_2, -OH chemistries) or crosslinking (ex., via glutaraldehyde) to transducer or membrane surface



6. Ideal Biosensor Characteristics

1. Sensitivity: high $\Delta S/ \Delta c_{analyte}$ (S = signal)

2. Simple calibration (with standards)

3. Linear Response: $\Delta S/ \Delta c_{analyte}$ constant over large concentration range

4. Background Signal: low noise, with ability for correction (ex., 2^{nd} fiber sensor head lacking biological species to measure background O_2 changes)

5. No hysteresis—signal independent of prior history of measurements

6. Selectivity—response only to changes in target analyte concentration

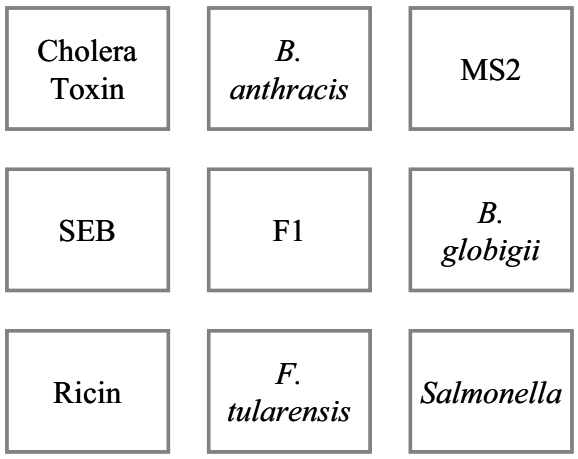
7. Long-term Stability—not subject to fouling, poisoning, or oxide formation that interferes with signal; prolonged stability of biological molecule

8. Dynamic Response—rapid response to variation in analyte concentration

9. Biocompatibility—minimize clotting, platelet interactions, activation of complement when in direct contact with bloodstream

7. Future Directions

1. Multianalyte capability (proteins, biowarfare agents, pathogens, etc.)



Naval Research Lab biowarfare agent multianalyte antibody array

- Bacteria
- Bacteriophage
- Toxic protein

C.R.Taitt et al, *Anal. Chem.* **74**, 6114 (2002)

Figure by MIT OCW.

2. Integration/Miniaturization (microfluidic "lab on a chip" devices)

Motorola Labs prototype microfluidic biochip for full DNA analysis from blood samples ($60\times100\times2$ mm^3)

- cell separation
- cell lysis
- DNA amplification
- DNA detection

photo removed due to copyright reasons.

R.H. Liu et al, *Anal. Chem.* **76**, 1824 (2004)

3. Implantable Devices

ex., Medtronic glucose sensor implant in major vein of heart—shear from blood flow inhibits cell attachment

Photos removed for copyright reasons.

R.F. Service, *Science* **297**, 962 (2002).

4. Living cells/tissues as biological element

Figures removed for copyright reasons.

BioImage screening platform for protein translocations (e.g., cytoplasm→ nucleus) associated with the activation of signaling pathways (from www.bioimage.com)