ELECTROCHEMICAL BIOSENSORS

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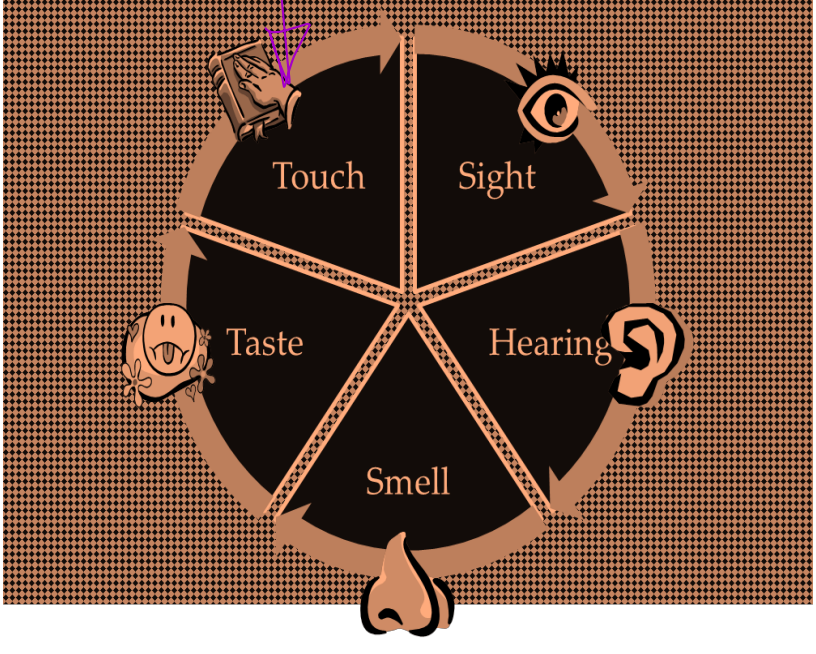
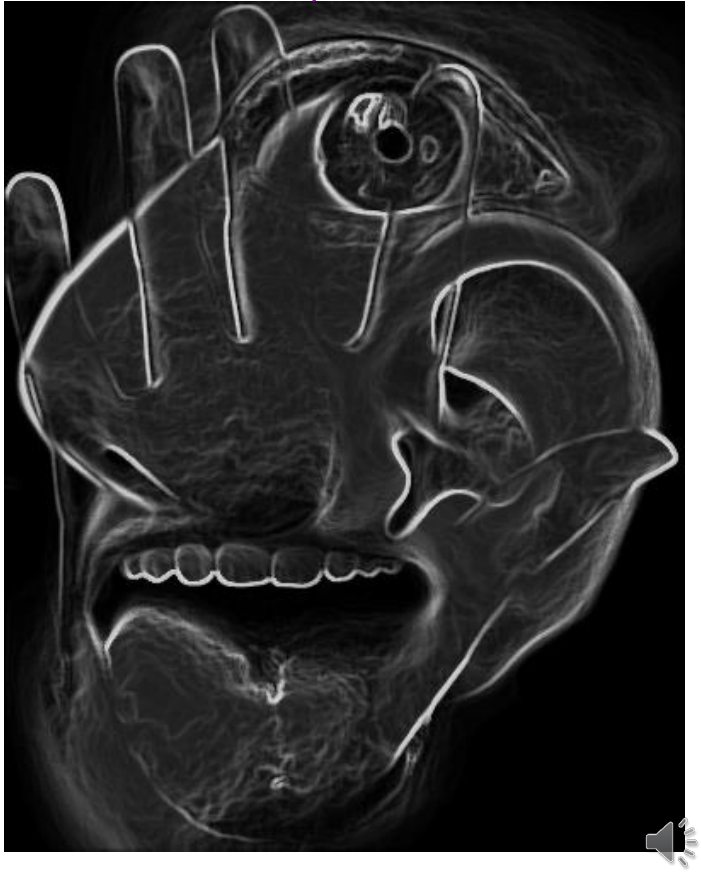
What is sensor?

A converter that measures a physical quantity and converts it into a signal that can be read by an observer or by an instrument

hot / cold



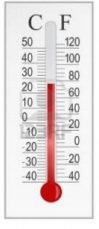
Combination!!



Type of sensors

Physical sensors

- Distance
- Mass
- Temperature
- Pressure



Chemical sensors

- Chemical substances
- Chemical or physical responses

pH meter one way for detector

Biosensors

DNA, tissue, protein.

- Chemical substances by a biological sensing component
- Subset of chemical sensor

Definition of biosensor

A biosensor is defined by the **International Union of Pure & Applied Chemistry (IUPAC)** as a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal or optical signals.

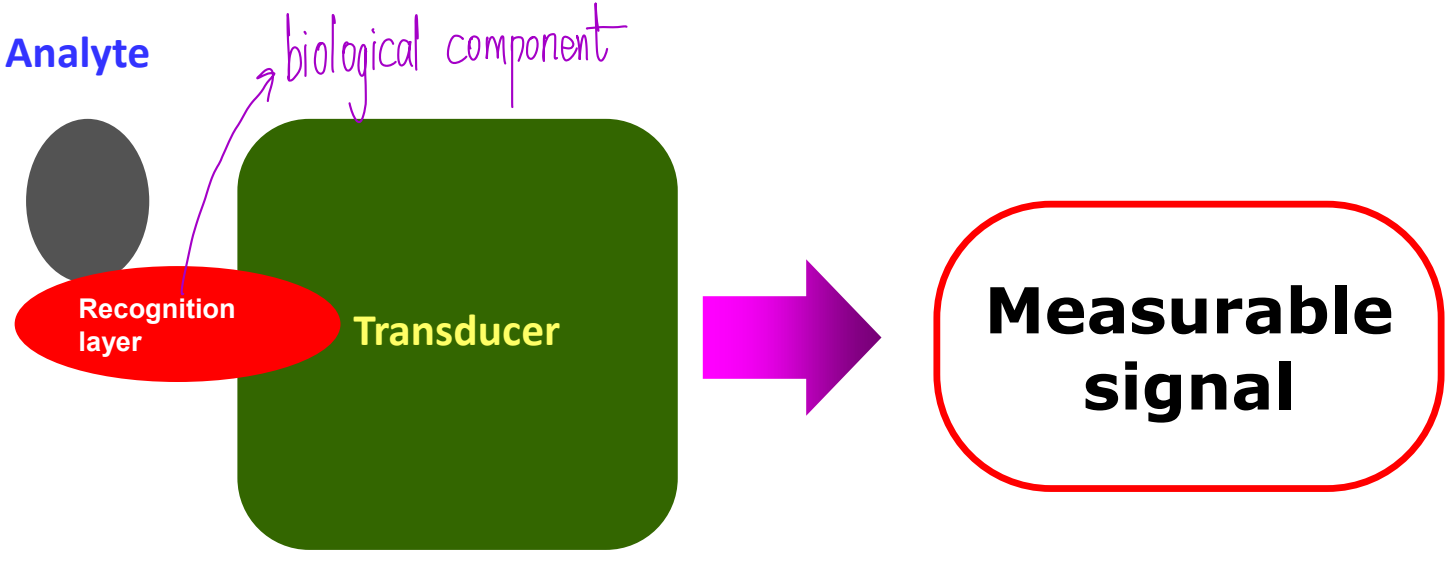
→ Other signals.

https://www.iupac.org/goldbook/B00663.pdf

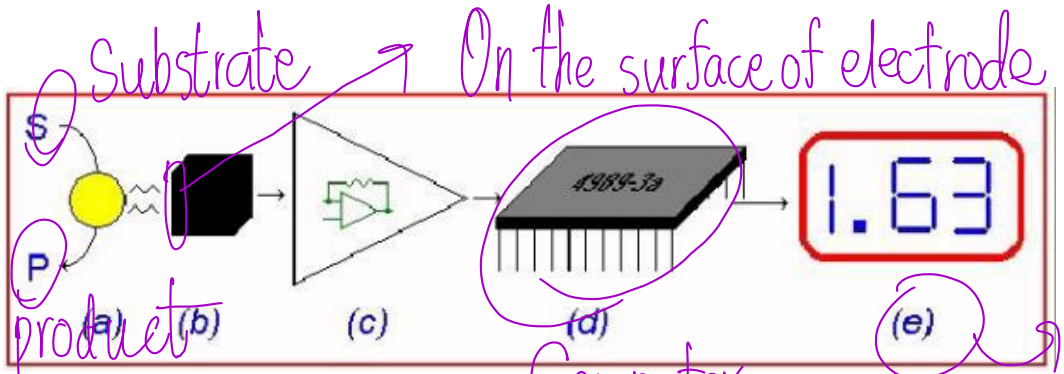
An analytical device composed of a biological recognition element directly interfaced to a signal transducer, which relates the concentration of an analyte (or group of related analytes) to a measurable response.

Components of a Biosensor

Schematic diagram showing the main components of a biosensor. The bio-reaction (a) converts the substrate to product. This reaction is determined by the *transducer* (b) which converts it to an signal. The output from the transducer is amplified (c), processed (d) and displayed (e).



Overview



a) Bioreaction – converts the analyte into product

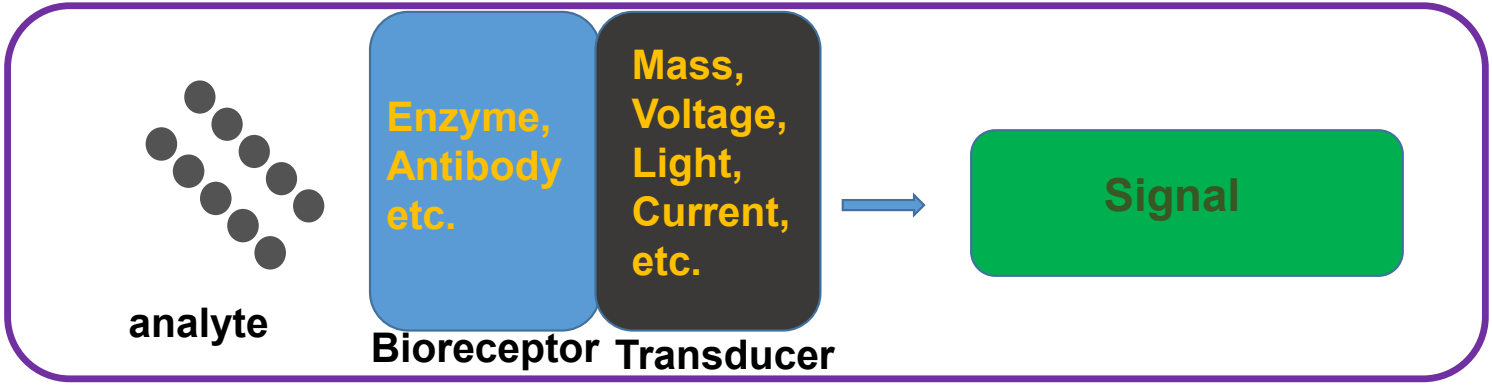
b) Transducer – detects the bioreaction and converts it into an electrical signal

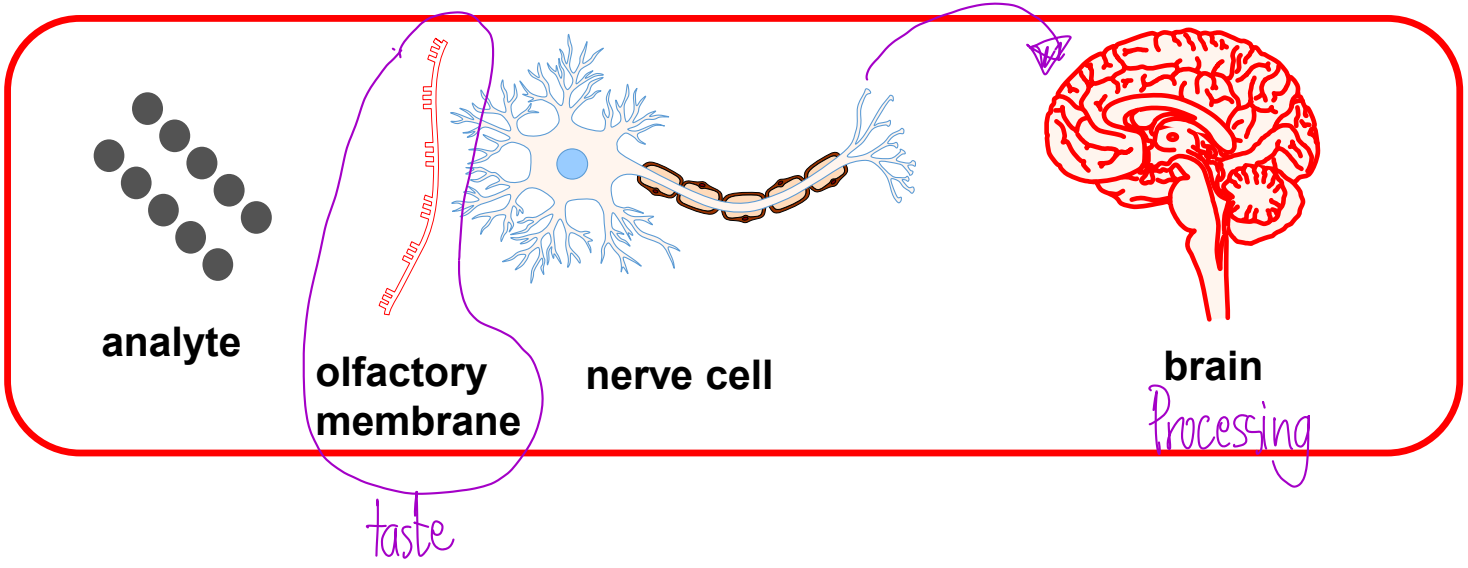
c) Amplifier – amplifies the usually tiny signal to useable level

d) Microprocessor – signal is digitalized and stored for further processing e.g integration, derivatization

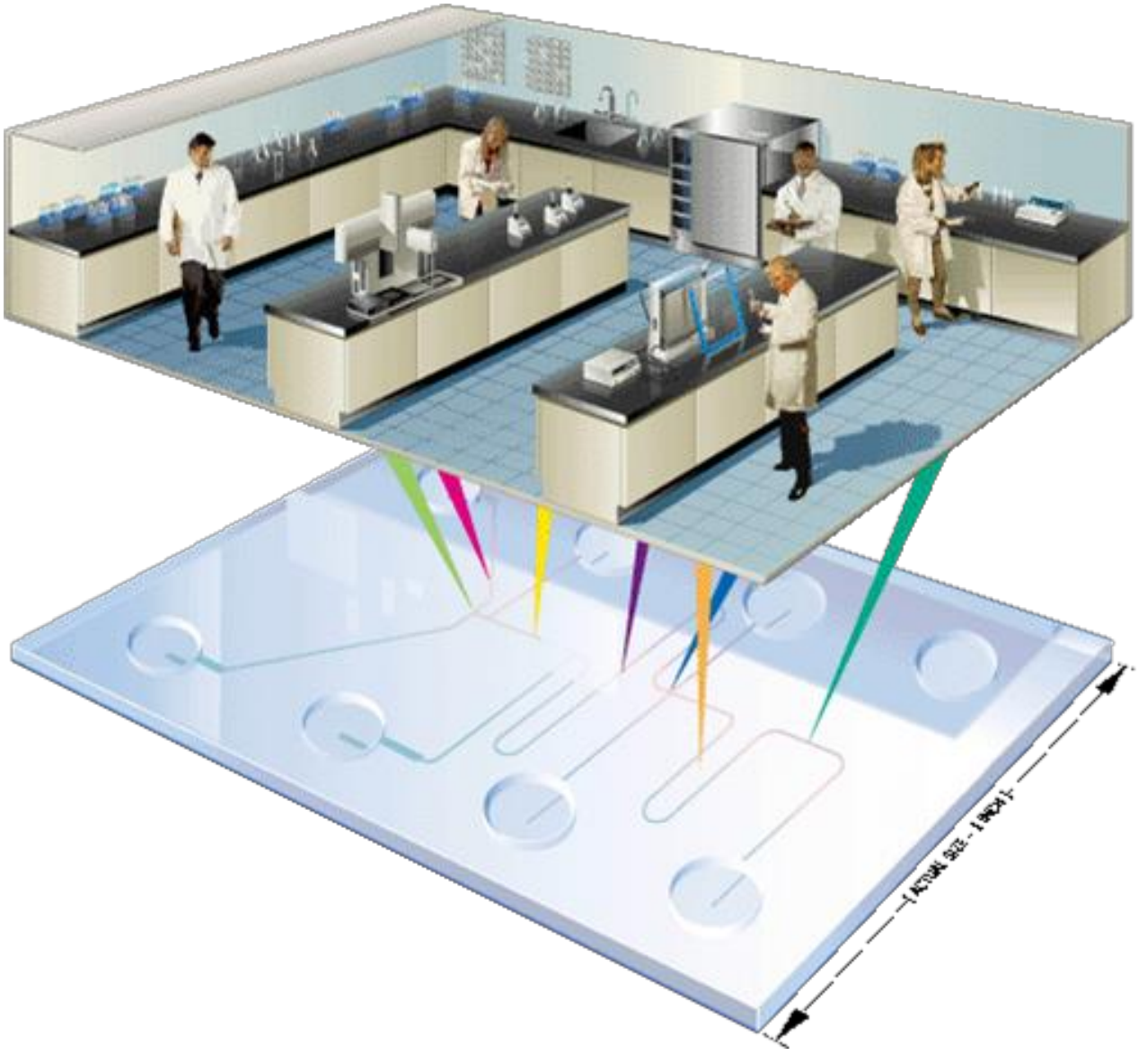
e) Display – usually need a real-time display of the analyte concentration

Human vs. Biosensors





Why do we need BIOSENSOR ??

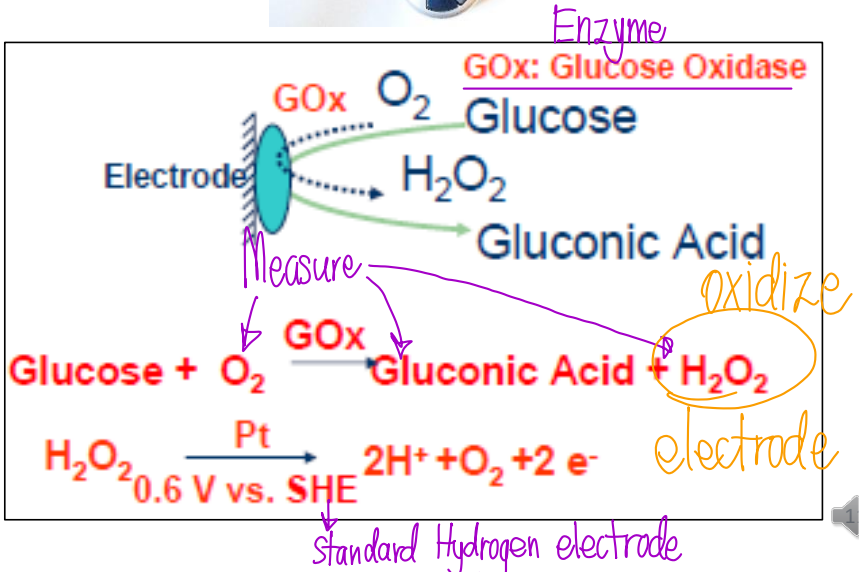

ACTUAL SIZE - 25% REDUCTION

Father of Biosensors

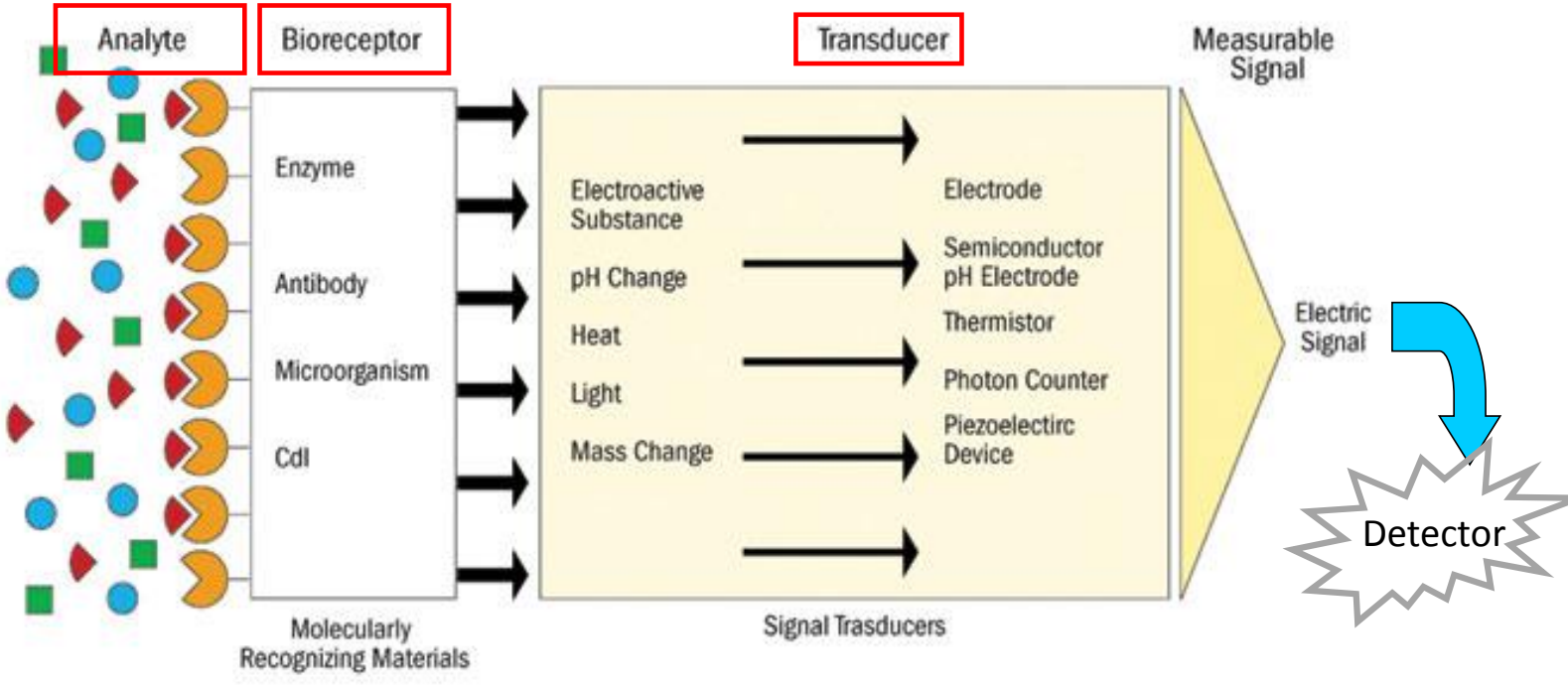
Professor Leland C Clark

1918–2005

The first and the most widely used commercial biosensor: **the blood glucose biosensor -developed by *Leland C. Clark in* 1962**



Components of a Biosensor



http://www.dddmag.com/images/0409/HTS1_lrg.jpg

1st component: The analyte

1. The Analyte *(What do you want to detect)*

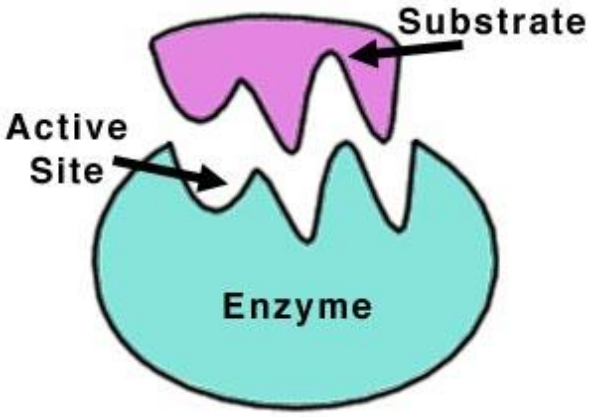
Molecule - Protein, toxin, peptide, vitamin, sugar, metal ion

2nd Component: Biological element

The component used to bind the target molecule.

Must be highly specific, stable under storage conditions, and immobilized.

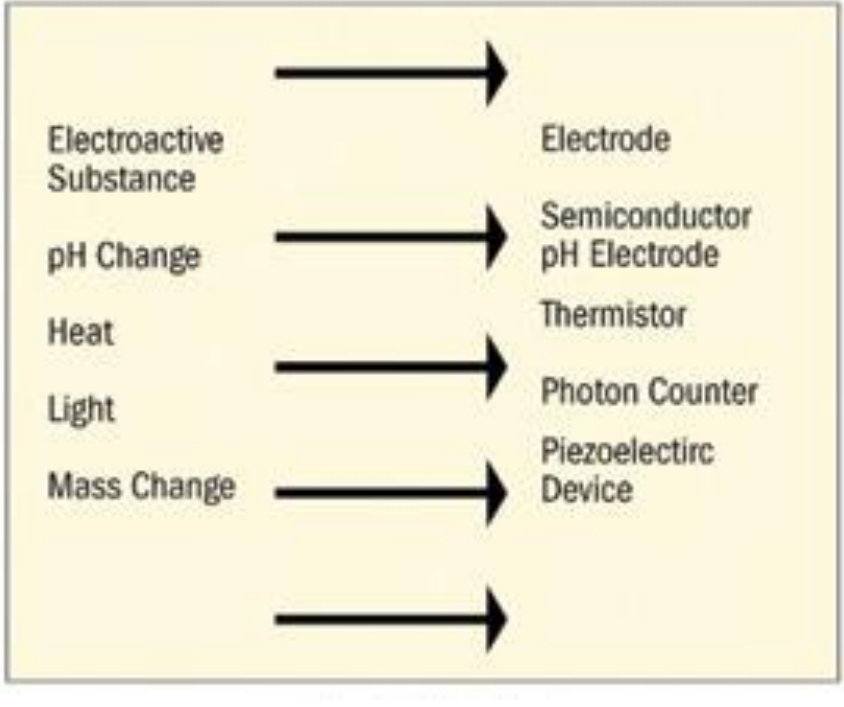
Microorganism
Tissue
Cell
Organelle
Nucleic Acid
Enzyme
Enzyme
Component
Receptor
Antibody

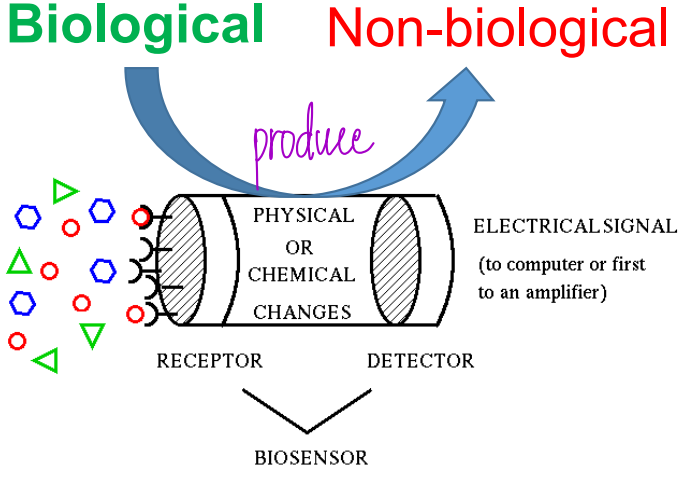


http://www.chemistry.wustl.edu/~edudev/LabTutorials/HIV/DrugStrategies.html

3rd Component: Physico-chemical Transducer

Acts as an interface, measuring the physical change that occurs with the reaction at the bioreceptor then transforming that change into measurable output.





4th Component: Detector

- **Signals from the transducer are passed to a microprocessor where they are amplified and analyzed.**

Quantitative

Qualitative

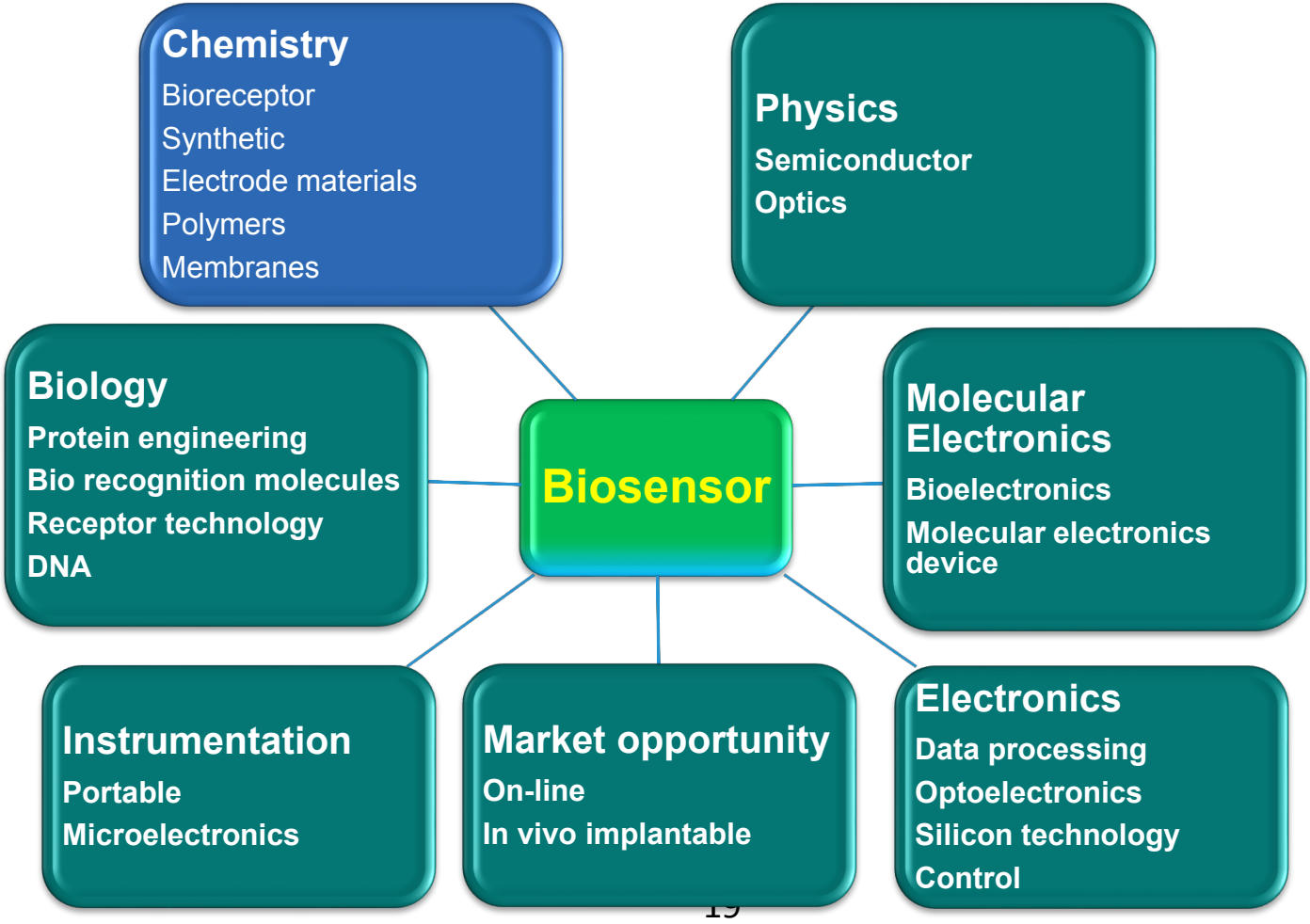
- **The data is then converted to concentration units and transferred to a display or/and data storage device.**

History of Biosensors

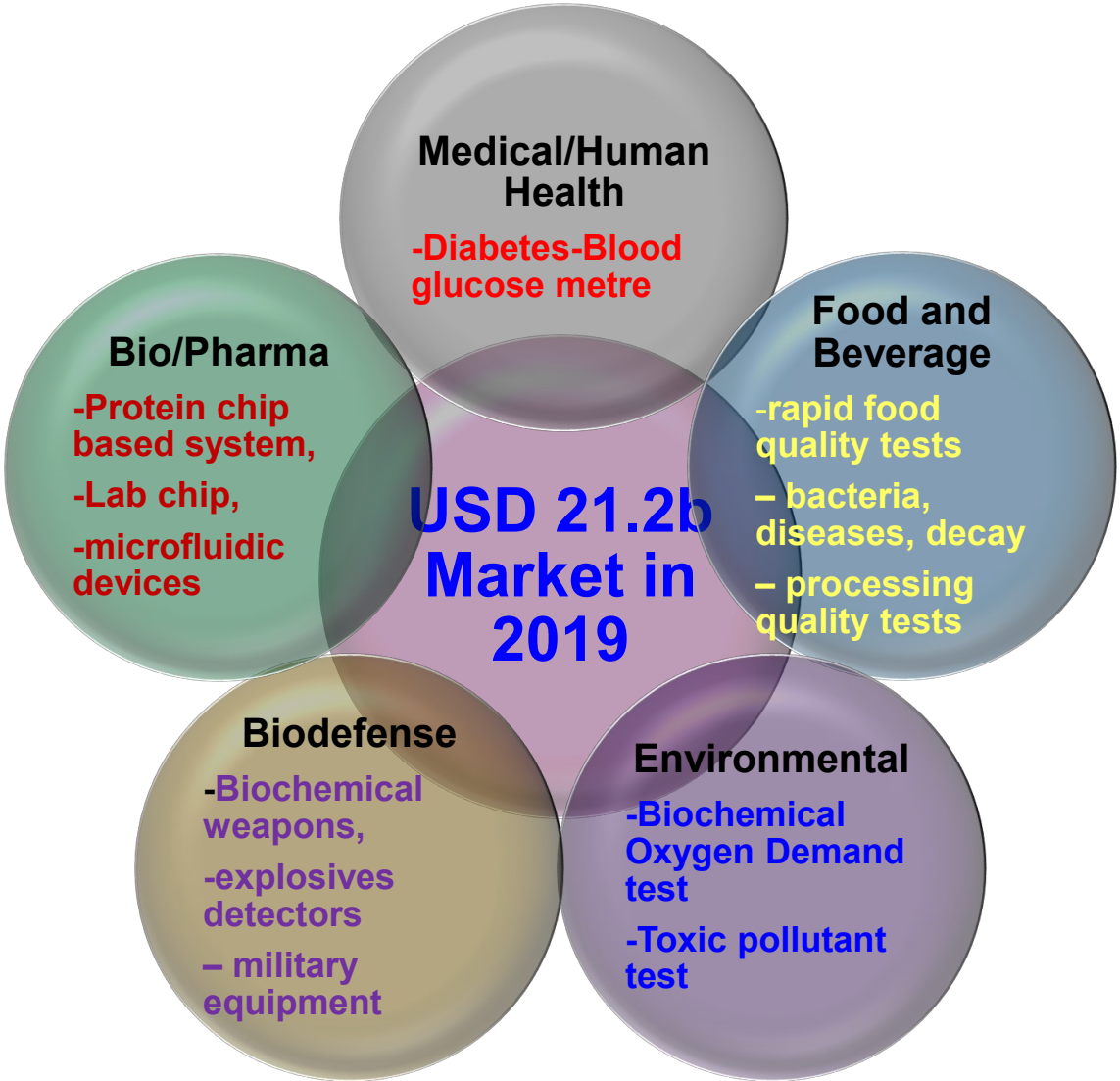
1916	First report on immobilization of proteins : adsorption of invertase on activated charcoal
1922	First glass pH electrode
1956	Clark published his definitive paper on the oxygen electrode.
1962	First description of a biosensor: an amperometric enzyme electrode for glucose (Clark)
1969	Guilbault and Montalvo – First potentiometric biosensor:urease immobilized on an ammonia electrode to detect urea
1970	Bergveld – ion selective Field Effect Transistor (ISFET)
1971	Lubbers and Opitz described a fibre-optic sensor with immobilised indicator to measure carbon dioxide or oxygen.

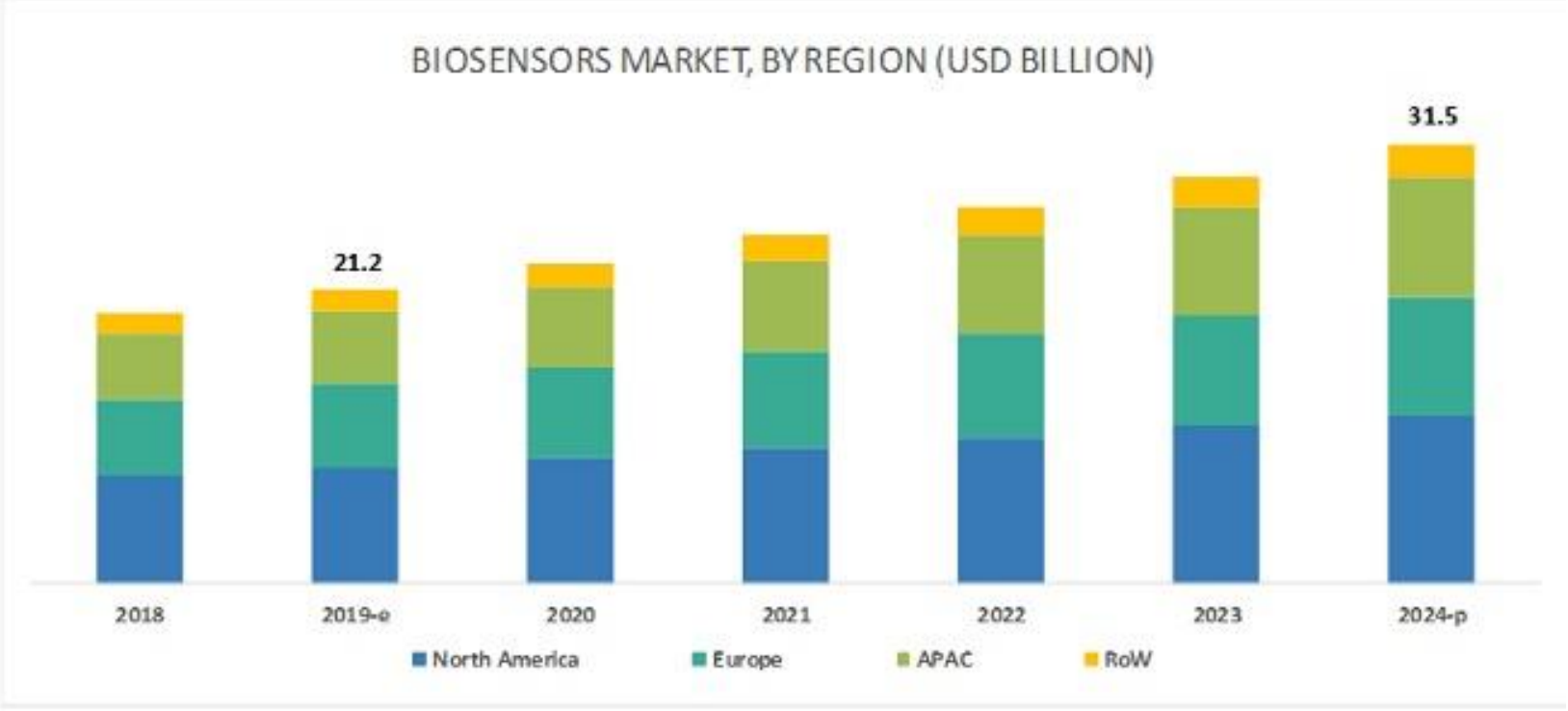
1975	First commercial biosensor (Yellow springs Instruments glucose biosensor)
1975	First microbe based biosensor, First immunosensor
1980	First fibre optic pH sensor for in vivo blood gases (Peterson)
1982	First fibre optic-based biosensor for glucose
1983	First surface plasmon resonance (SPR) immunosensor
1984	First mediated amperometric biosensor: ferrocene used with glucose oxidase for glucose detection
1987	Blood-glucose biosensor launched by MediSense ExacTech

Biosensors : combines multi disciplines



Application and market of Biosensors




BIOSENSORS MARKET, BY REGION (USD BILLION)
21.2
31.5
2018
2019-e
2020
2021
2022
2023
2024-p
North America
Europe
APAC
RoW

BIOSENSORS MARKET

Global Market Insights
Insights to innovation

$18.6 BN (2018) → 2019 → 2020 → 2021 → 2022 → 2023 → 2024 → >$31.5 BN (2025)

CAGR (2019-25): **8%**

Wearable biosensors market CAGR (2019-25): **9.3%**

Non-wearable biosensors market share (2018): **>52%**

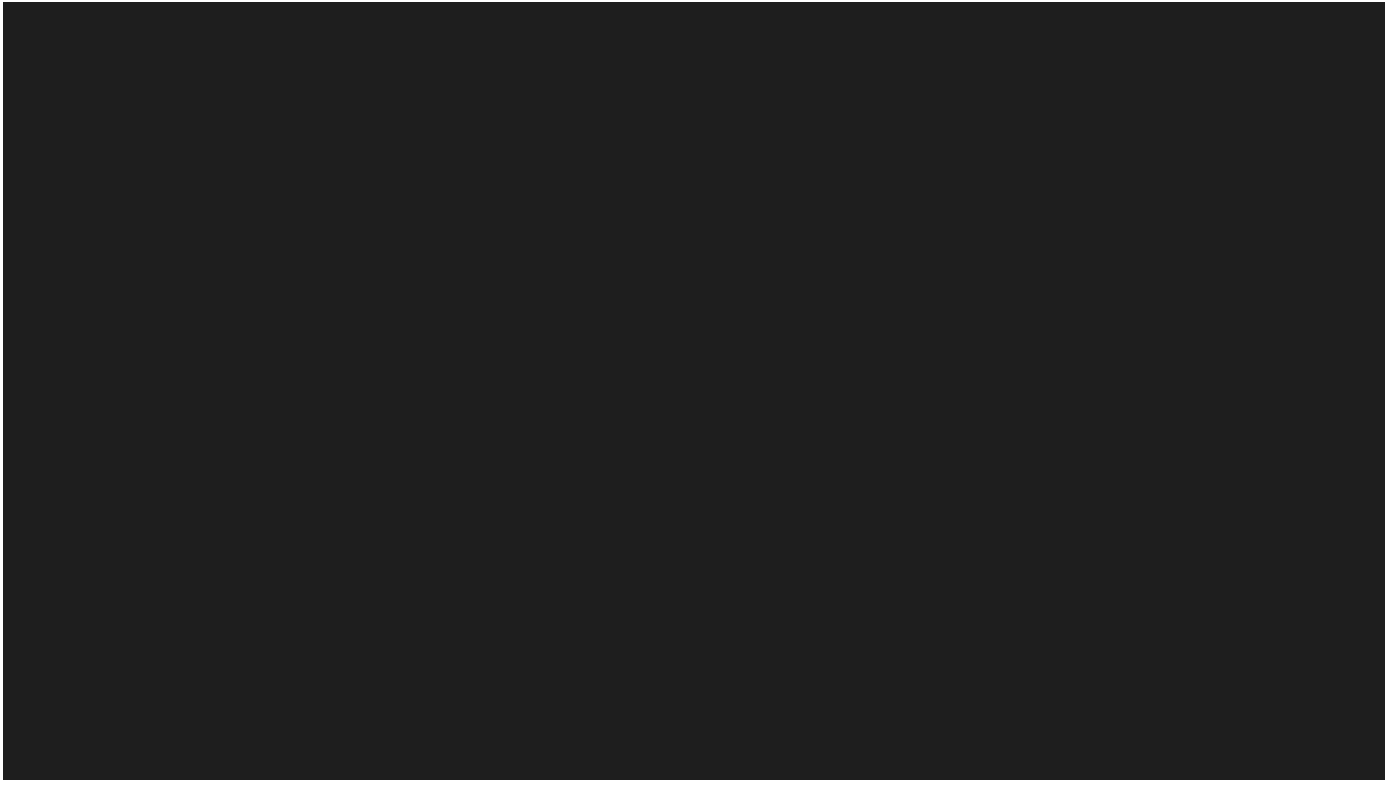
U.S. regional share (2018): **81%**

Germany regional share (2018): **>20%**

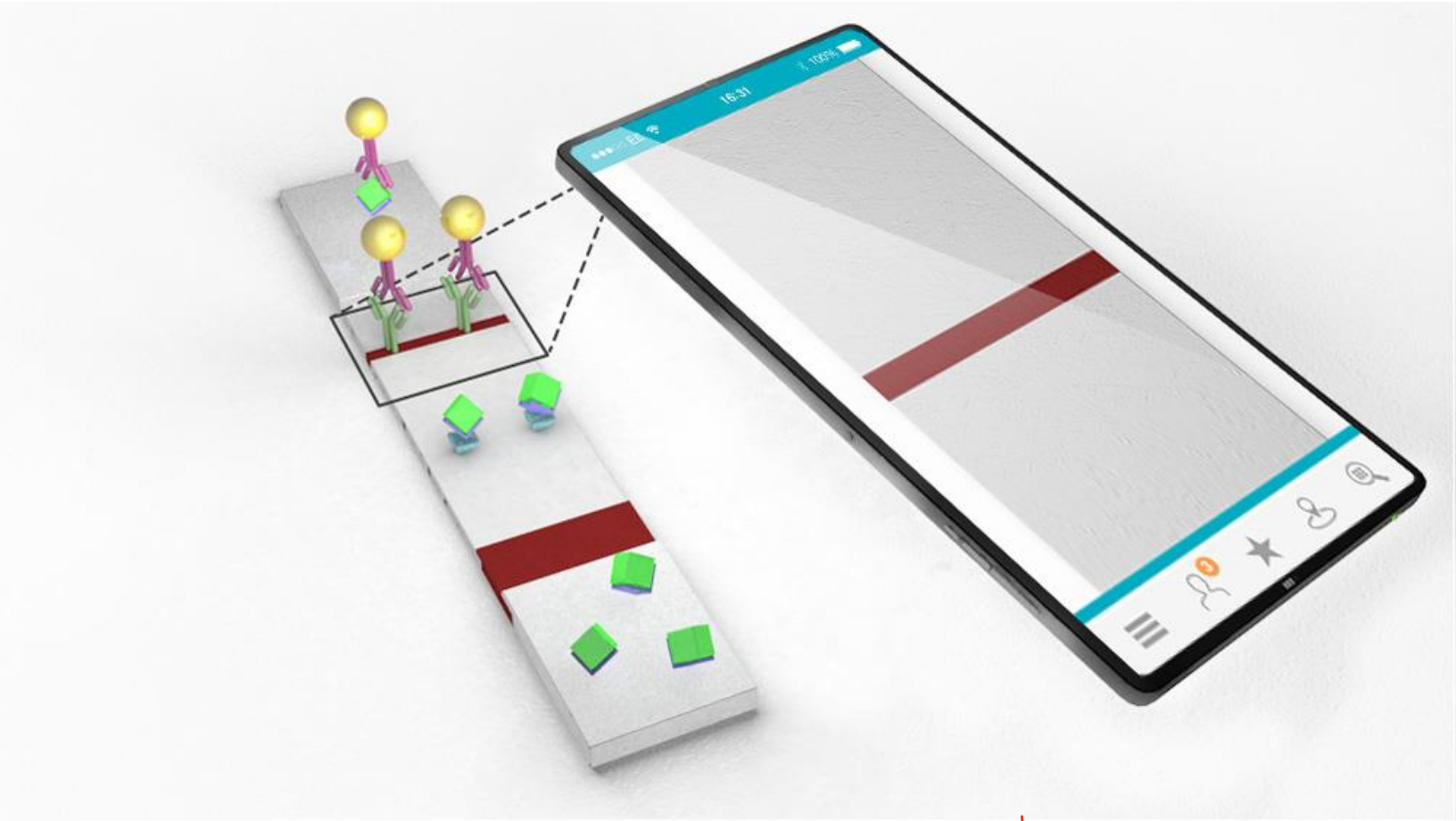
China market CAGR (2019-25): **>9.5%**

Examples of Biosensors

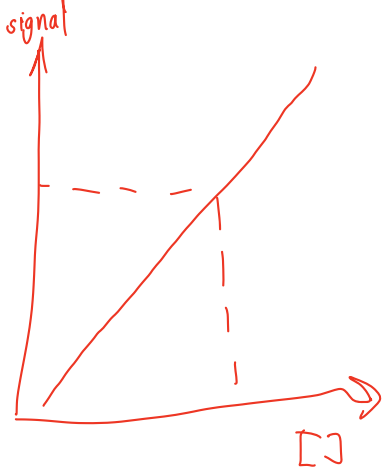
Company	Analyte	Technology Basis
Autoteam GmbH (Berlin, Germany)	Biological oxygen demand (BOD)	Microbial electrode
Bio-Technical Resources (Manitowoc, WI, USA)	As, Pb, Cd, polychlorinated benzenes (PCB's)	Fluorescence from luminescent bacteria
Life Scan (Milpitas, CA, USA)	Blood Glucose	Enzyme (glucose oxidase/peroxidase)
MediSense Inc. (Waltham, MA, USA)	Blood glucose (ExacTech®, Companion 2®)	Amplified enzyme electrode
Pharmacia Biosensor AB (Uppsala, Sweden)	Multiple antigens (BIAcore®)	Antibody-coated surface plasmon resonance-based instrument
YSI (Yellow Springs, OH, USA)	Sugars, alcohol, starch	Enzyme electrodes
Molecular Devices Corp (Menlo Park, CA, USA)	Total DNA (Threshold®); drugs, toxins (Cytosensor®)	Binding protein/antibody-based assay on a light addressable potentiometric transducer; pH changes in whole cells
Oriental Electric Co., LTD (Tokyo, Japan)	ATP degradation (fish freshness indicator)	Enzyme electrode
Ohmicron Corp. (Newtown, PA, USA)	Herbicides, PCBs, polyaromatic hydrocarbons (PAHs) (SmartSense®)	Enzyme immunoassays on chemiresist (capacitance) transducers



Component in Biosensors

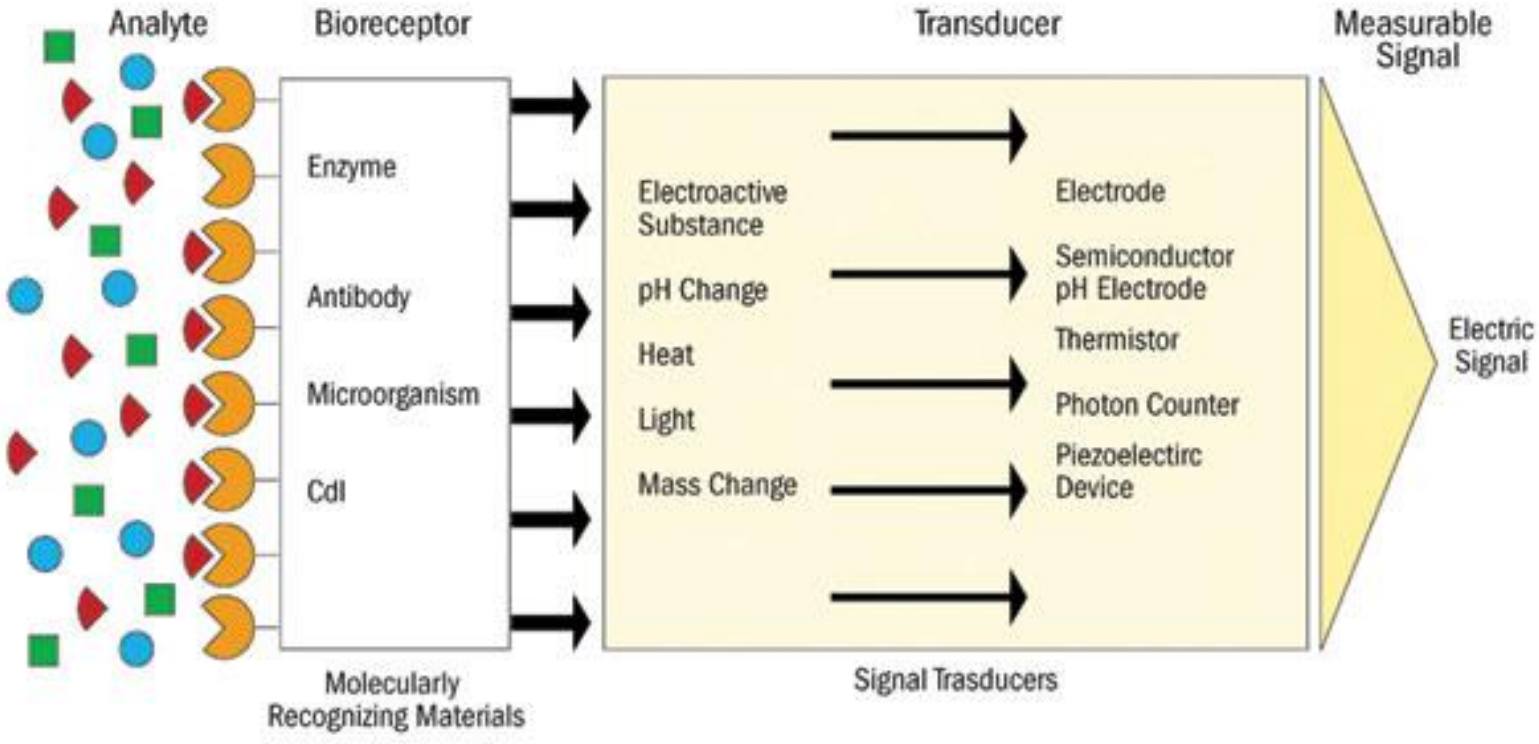


Ideal Biosensor:

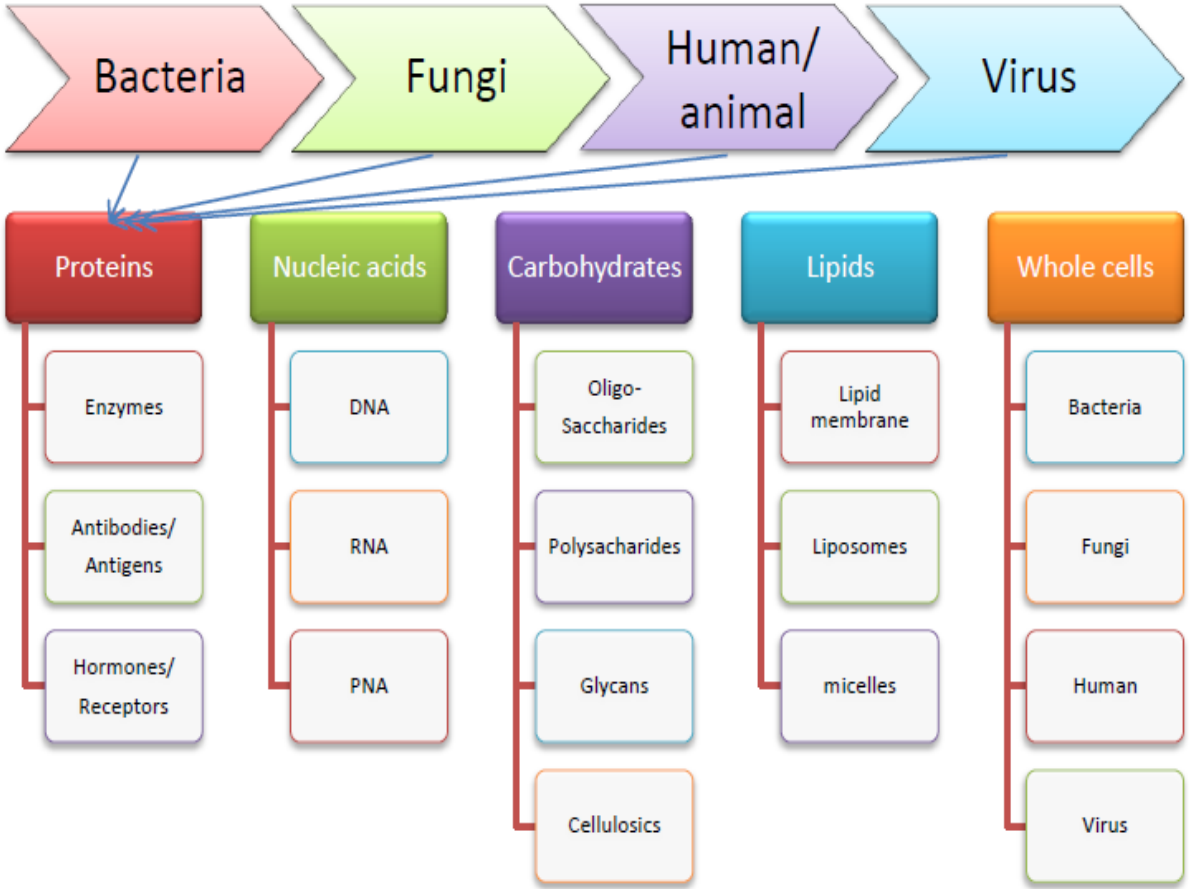


- Wide **applicability** to many sample matrices;
- High **accuracy** and **precision**;
- Excellent **sensitivity** and **specificity**;
- Wide **linearity range**;
- **Rapid** response time for real-time monitoring;
- High operational and physical **robustness** (i.e. insensitivity to variations of pH, ionic strength, temperature, pressure etc);
- Long-term **stability**, lifetime and reliability;
- Amenability to testing and **calibration**;
- **Low service requirements**, running and capital costs;
- Product **safety** (biocompatibility if the biosensor is to be used for invasive monitoring in clinical situations, and in environmental applications the host system must not be contaminated by the sensor);
- Small size, **portability** and low power requirements.

Components of biosensors



Sources of Biological recognition elements



Classification Biosensors:

Biocatalytic type

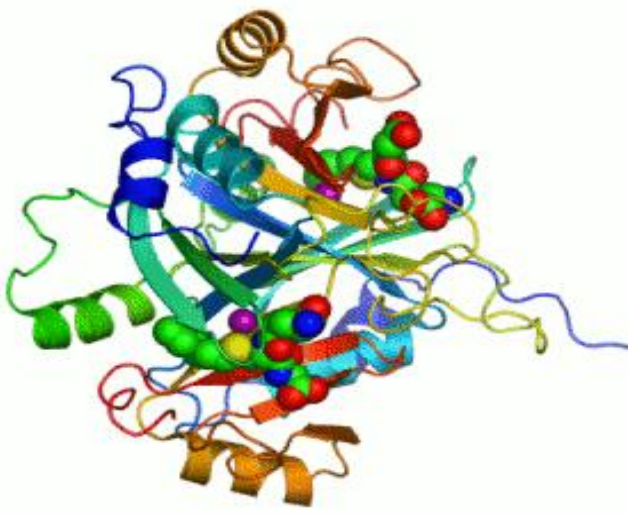
- Biological elements recognize the target analyte for catalytic reaction (e.g. ~~Eznyme~~ Enzyme)

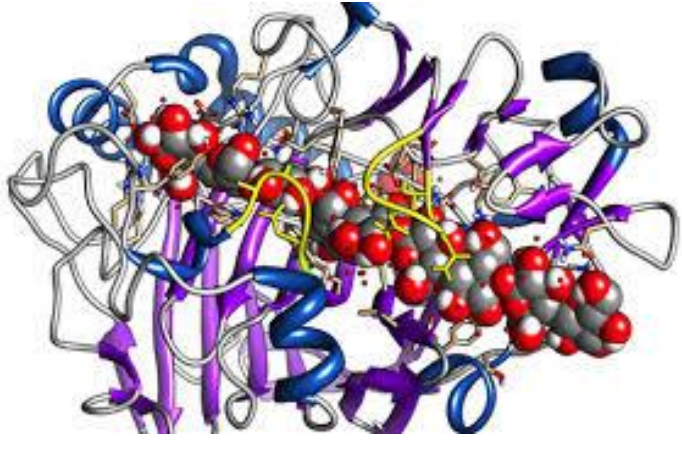
Bioaffinity type

- Biological elements selectively bind to the target analyte through surface-restricted ligand partner (e.g. antibody, DNA)

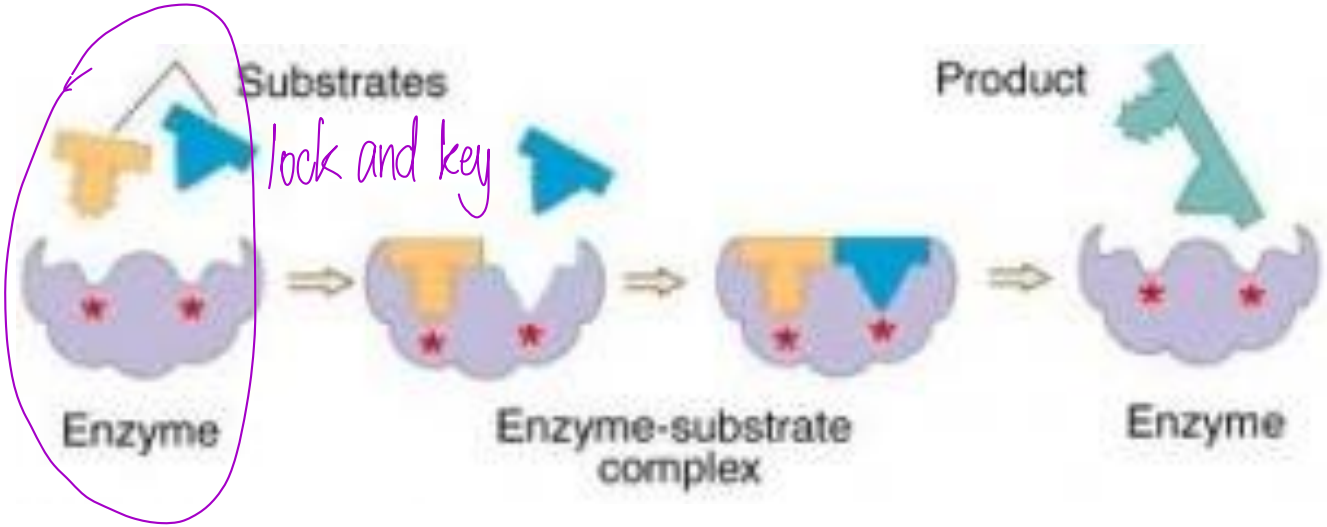
Biocatalytic – Enzymes:

- Proteins that act as catalysts for biochemical reactions
- Activity is determined by their chemical composition and 3D structure



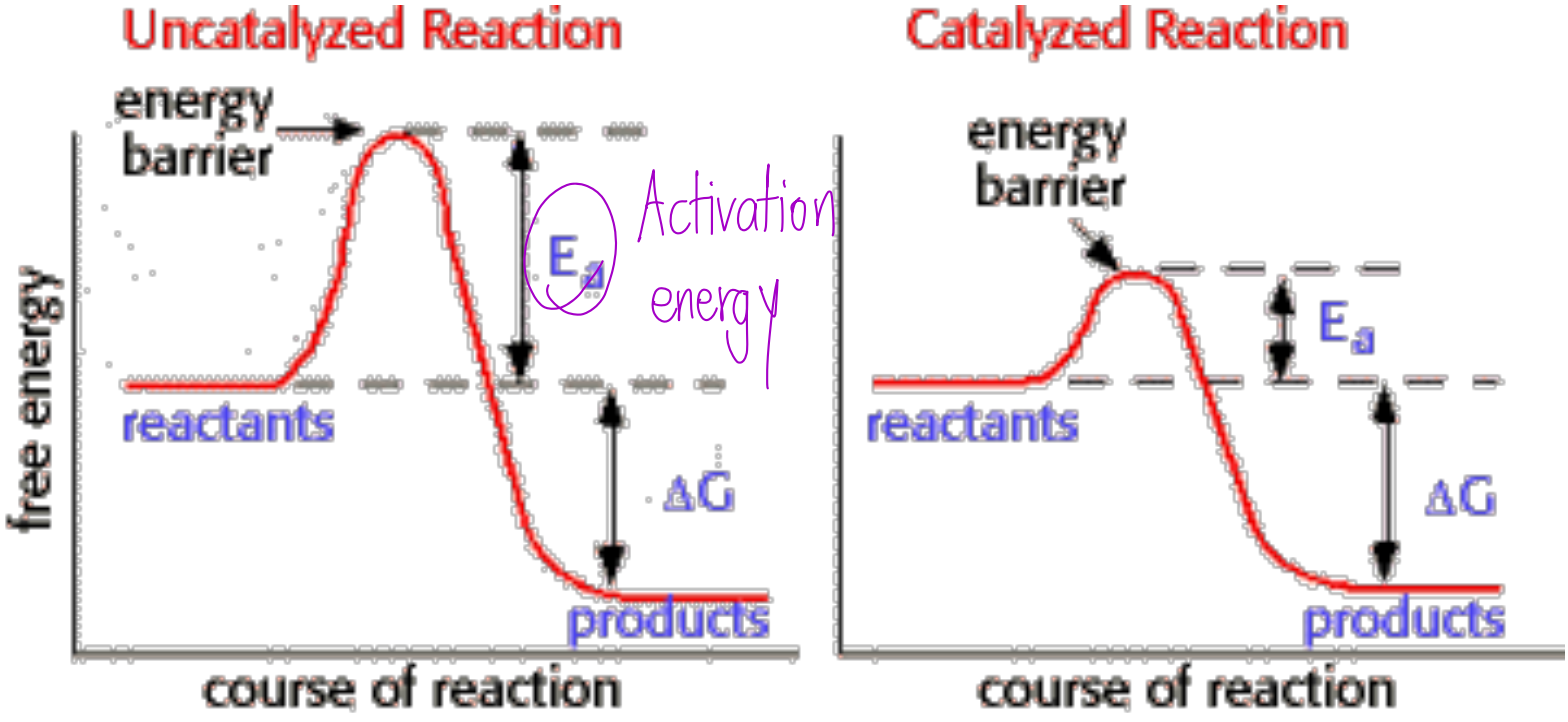


How enzyme works?



- **Substrate – Enzyme interaction = specific (key & lock mechanism)**
- **Enzyme-substrate complex is formed before the conversion into final product**
- **Enzyme remains unchanged after the reaction**

Enzyme works as catalyst



- Enzyme (catalyst) offers the lower activation energy (Ea)
- Allows reaction occurs at faster rate

Six Classes of Enzyme:

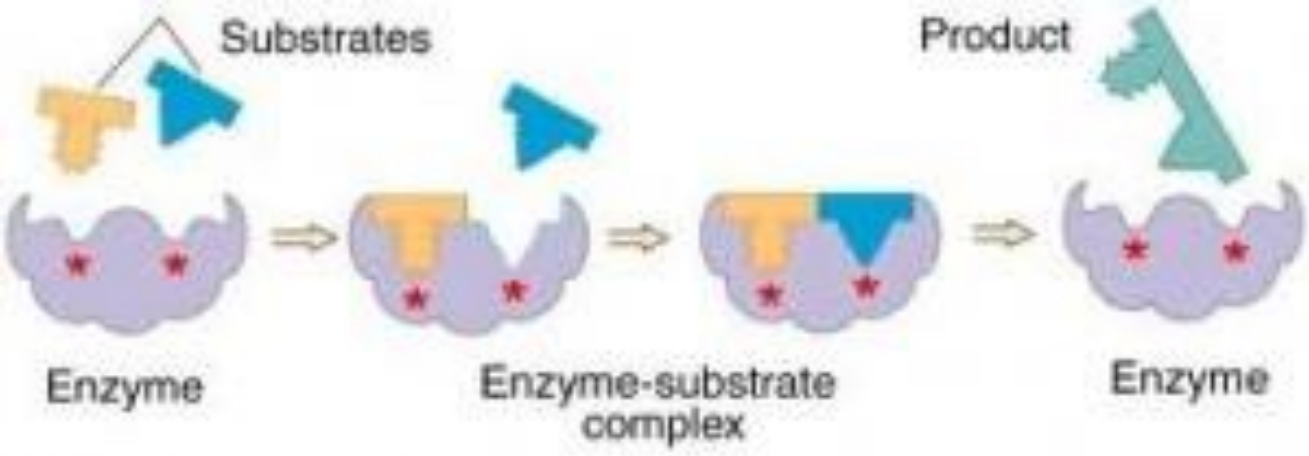
No.	Enzyme category	Function
1	Oxidoreductases	Oxidation-reduction reactions
2	Transferases	Group transfer
3	Hydrolases	Hydrolysis reaction (transfer of functional group to water)
4	Lyases	Addition or removal of groups to form double bonds
5	Isomerases	Izomerization (intramolecular group transfer)
6	Ligases	Joining of two molecules

Oxidoreductase Enzyme and its Reaction

what kind of signal for detect?

Enzyme	Enzymatic reaction
alcohol oxidase	ethanol + O_2 → acetaldehyde + H_2O_2
glucose oxidase	glucose + O_2 → gluconic acid + H_2O_2
lactose oxidase	lactose+ O_2 → pyruvate + H_2O_2
alcohol dehydrogenase	ethanol + NAD^+ → acetaldehyde + NADH + H^+ acetaldehyde + NAD^+ → acetic acid + NADH + H^+
lactate dehydrogenase	lactate + NAD^+ → pyruvate + NADH
Acetylcholinesterase	acetylcholine + H_2O → choline + acetic acid
cholineoxidase	choline + O_2 → betaine aldehyde + H_2O_2
horseradish peroxidase	H_2O_2+ electron donor → $2H_2O$ + oxidized donor (electron donor = phenols, aromatic amines, luminol etc)
tyrosinase	phenol + $1/2O_2$ → quinone + H_2O
lipase	triglycerides + $3H_2O$ → glycerol + 3 fatty acids
nitrate reductase	NO_3^- + NADH + H^+→ NO_2^- + NAD^+ + H_2O

How enzyme works in biosensor



Measure:

- **Substrate consumption**
- **Product generation**

Advantages of Enzyme Biosensor:

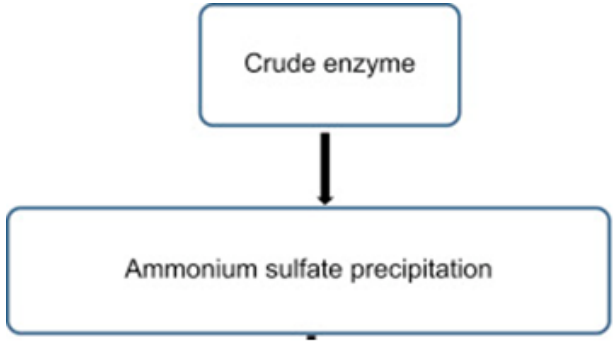
- Large number of **enzymatic reactions** can be used
- Wide array of **detectable species** (substrate, product, inhibitor, mediator)
- **Flexibility** (different type of transducer can be used)
- **Low consumption** of enzyme (not being consumed during sensing)
- High **selectivity** of enzymatic reactions
- Commercial availability in high **purity**

Disadvantages of Enzyme Biosensor:

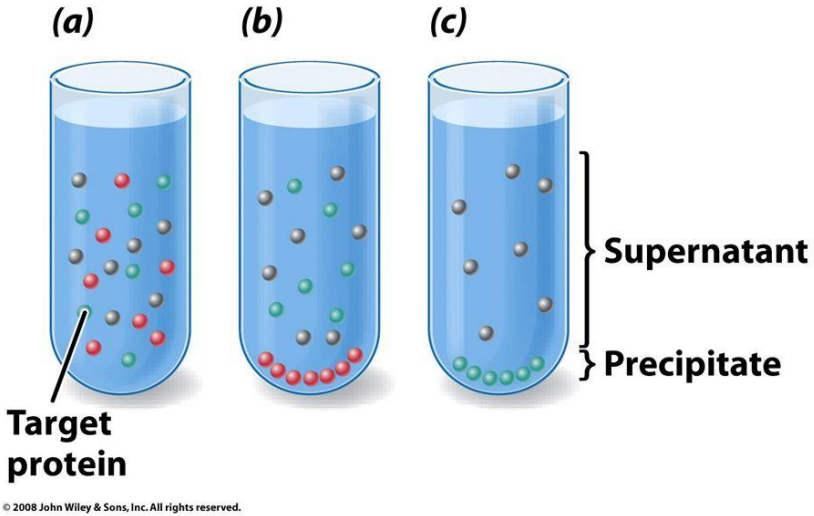
3D structure

- Enzymes are **bulky protein** (active sites maybe blocked and activity is reduced)
- **Limited lifetime** (deactivation by sample, conditions)
- Activity **depends** on pH, ionic strength, chemical inhibitors and temperature)
- **Cost** of commercial enzyme is high

Enzyme Extraction:

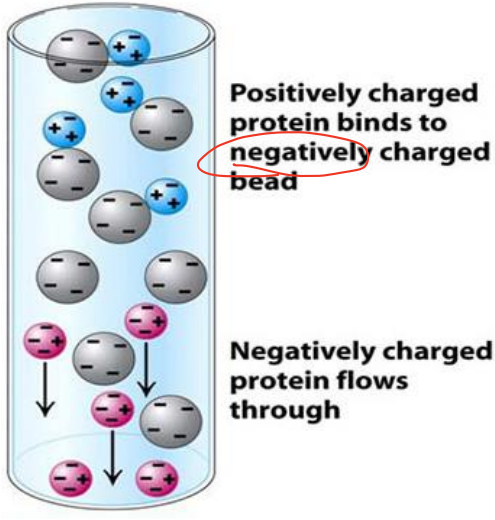


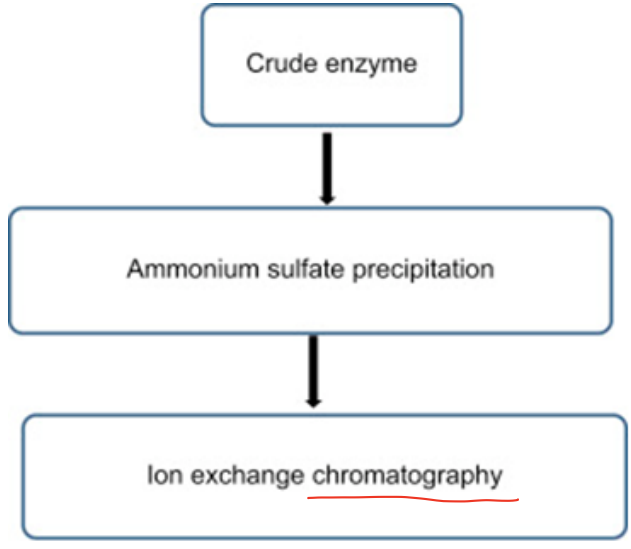
Purifying proteins: “salting out”



- Enzyme can be from extracellular or intracellular
- Digestion is done by lysate
- Precipitation of enzyme by ammonium sulfate

Enzyme Extraction:

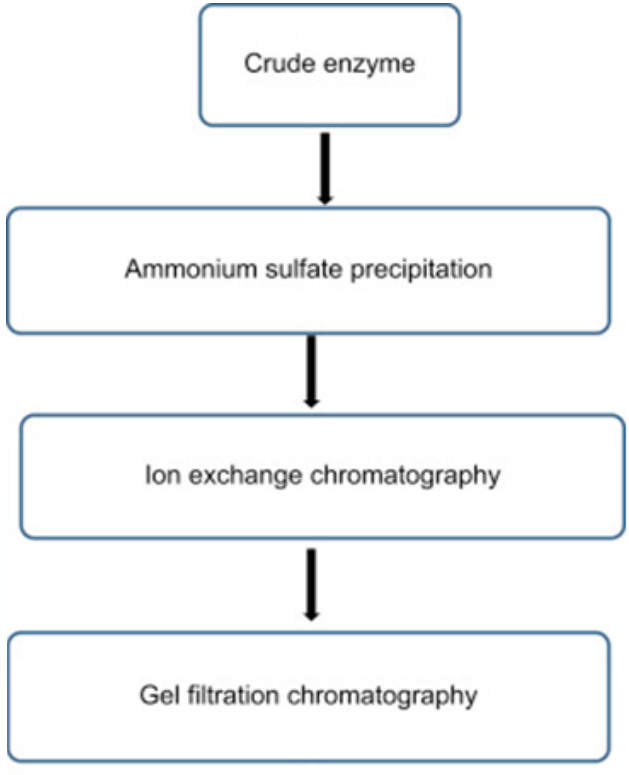


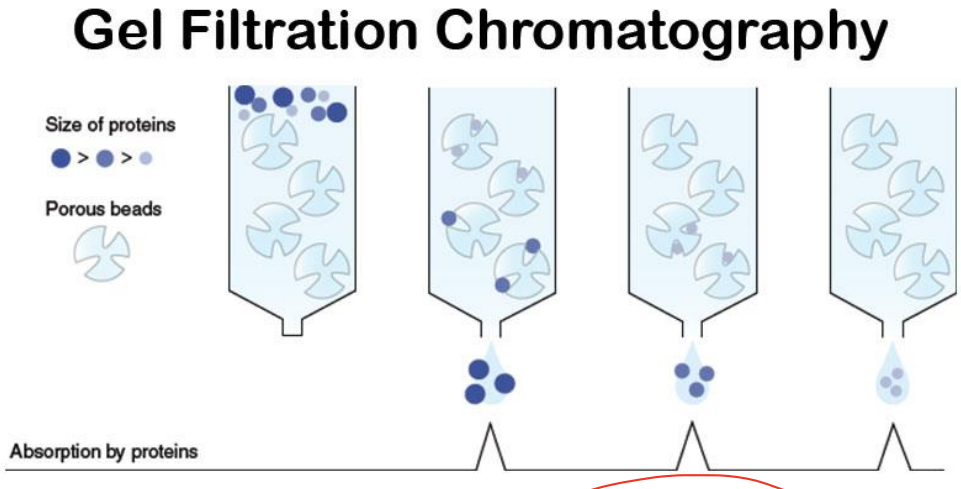


Ion Exchange Chromatography

- Separate based on different charges

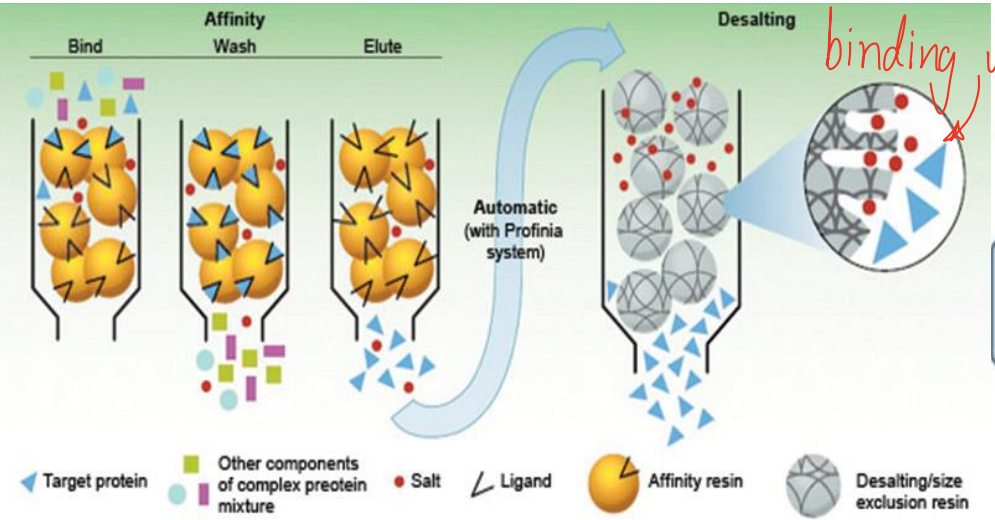
Enzyme Extraction:





- Separate based on different in sizes

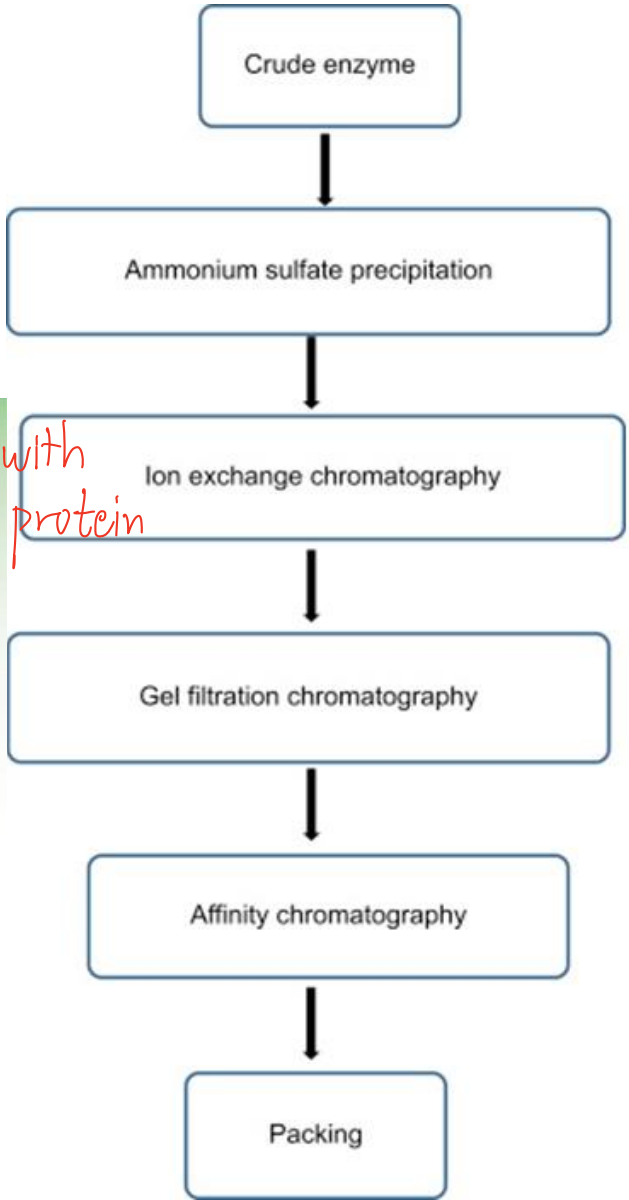
Enzyme Extraction:

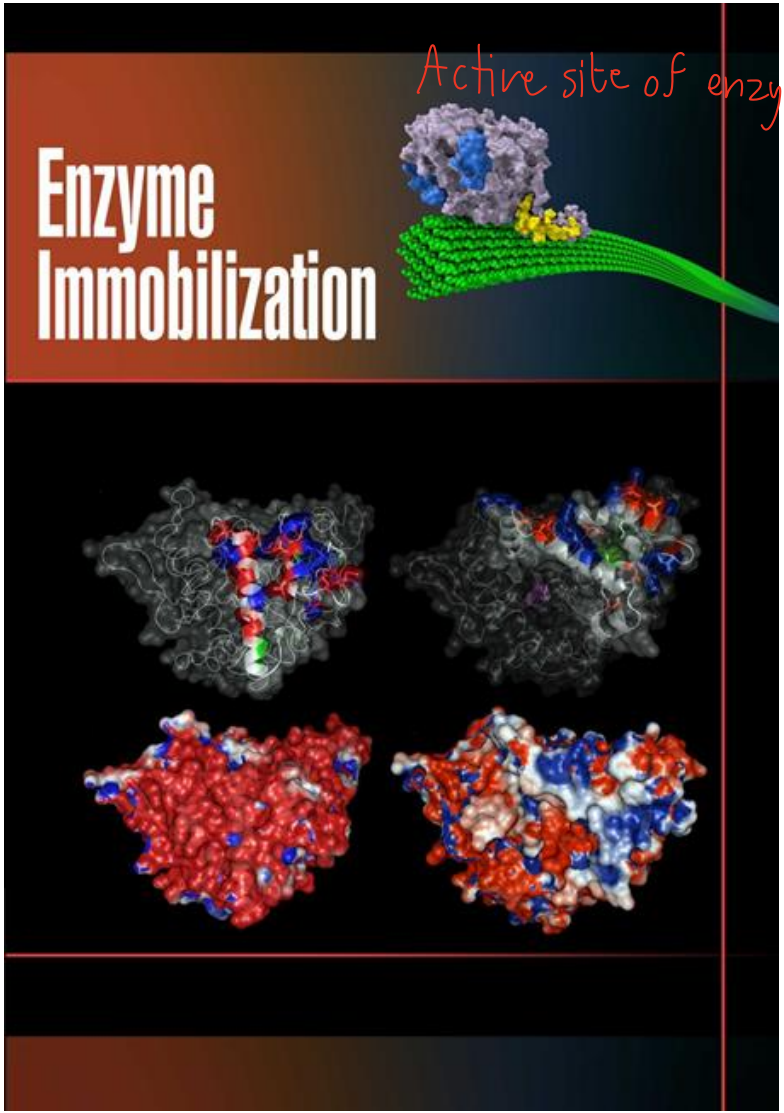


binding with protein

Affinity Chromatography

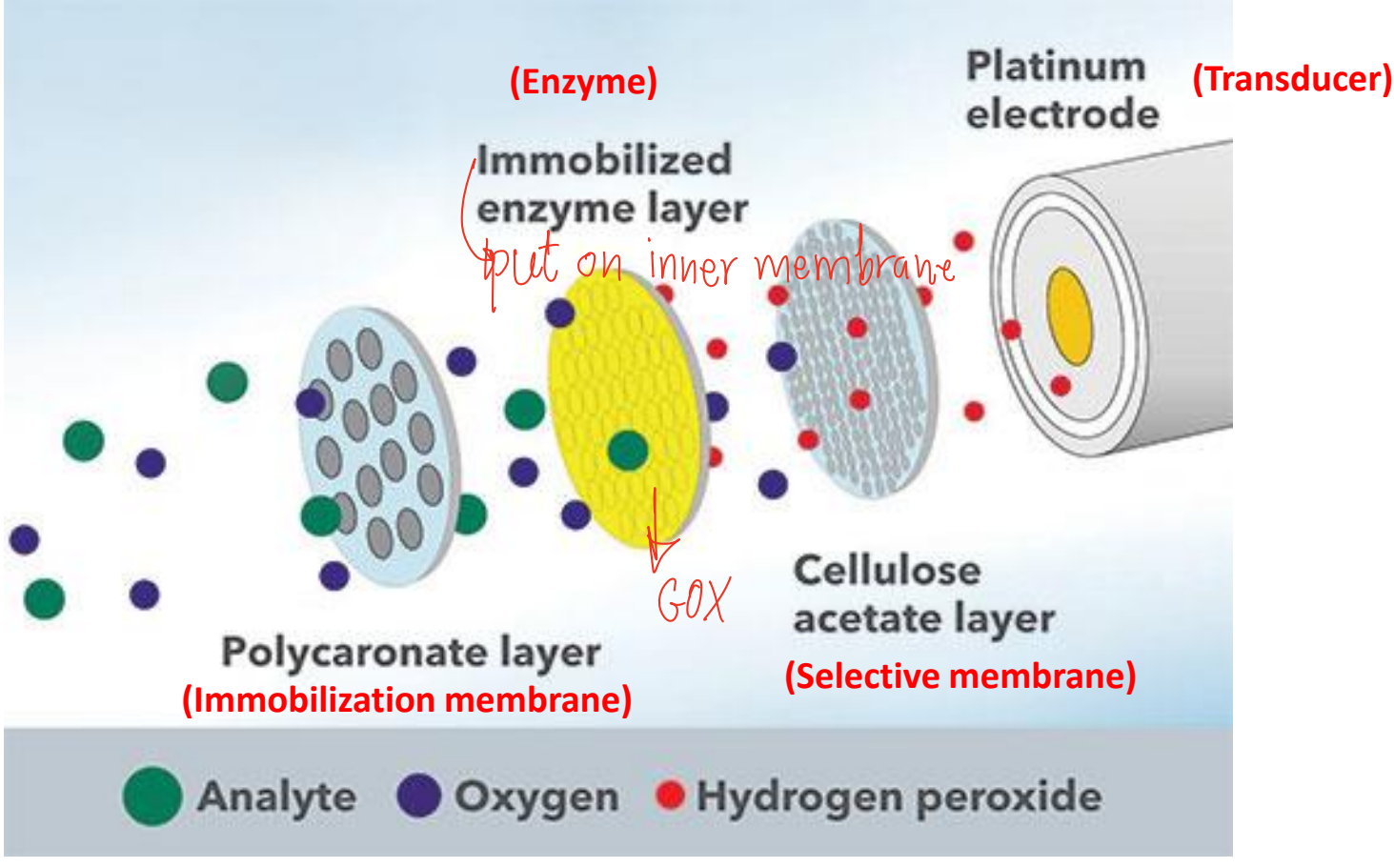
- Separate based on specific binding





- Extracted enzyme will be immobilized onto transducer
- Active sites must be close to transducer
- Enzyme activity is retained

Example of Immobilized Glucose Oxidase for Glucose Biosensor



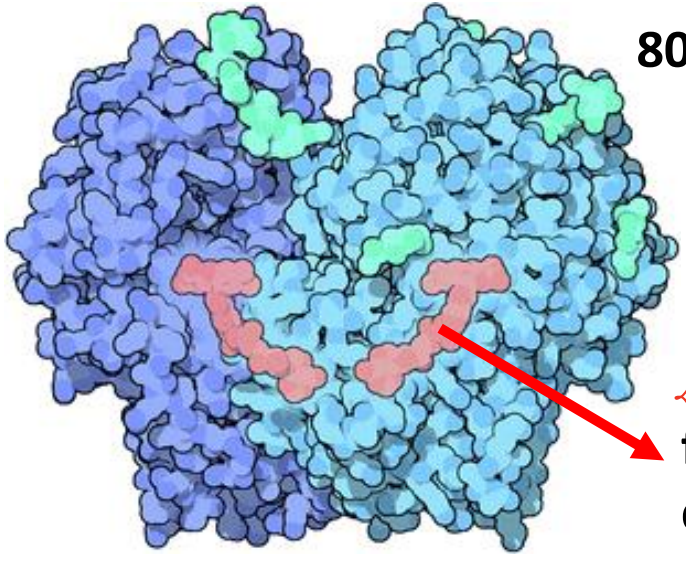
Glucose Biosensor

History of Glucose Biosensor

Year	Event
1962	First description of a biosensor by Clark and Lyons
1967	First practical enzyme electrode by Updike and Hicks
1973	Glucose enzyme electrode based on detection of hydrogen peroxide
1975	Relaunch of first commercial biosensor, *i.e.*, YSI analyzer
1976	First bedside artificial pancreas (Miles)
1982	First needle-type enzyme electrode for subcutaneous implantation by Shichiri
1984	First ferrocene mediated amperometric glucose biosensor by Cass
1987	Launch of the MediSense ExacTech blood glucose biosensor
1999	Launch of a commercial *in vivo* glucose sensor (MiniMed)
2000	Introduction of a wearable noninvasive glucose monitor (GlucoWatch)

↗ Clark electrode

Glucose Oxidase (GOx)



- 1.9 Å homodimer made up of two identical 80-kDa subunits and two non-covalently bound flacin adenine dinucleotides (FAD)
- FAD coenzyme acts as an electron carrier during catalysis
- First discovered in 1928 by Muller in *Aspergillus niger* extracts

Source of GOx

- Main function as anti-bacterial and anti-fungal agent through the production of hydrogen peroxide

	Natural occurrence	Functions
Insect	Honey bee (*Apis mellifera* L.)—salivary secretion from hypopharyngeal gland (Ohashi et al. 1999; Santos et al. 2005; AbuSara 2006)	Honey preservation (AbuSara 2006)
	Larva (*Helicoverpa zea* (Eichenseer et al. 1999), *Helicoverpa armigera*, *Helicoverpa assulta* (Zong and Wang 2004)—salivary secretion from labial glands	Disinfect ingested food and gastrointestinal function (Eichenseer et al. 1999), suppress plant defence (Musser et al. 2002; Zong and Wang 2004; Musser et al. 2005)
	Grass hopper (*Schistocerca americana*)—cuticle (Candy 1979)	Antibacterial, hardening of cuticle (Candy 1979)
	Larva (*Spodoptera exigua*, *Mamestra configurata*)—salivary secretion from labial glands (Merkx-Jacques and Bede 2004, 2005)	NA
Fungi	*Talaromyces flavus* (Kim et al. 1990; Murray et al. 1997)	Anti-fungal, i.e. antibiosis (Kim et al. 1990; Murray et al. 1997)
	Pleurotus ostreatus (Shin et al. 1993)	Generate H_2O_2 for H_2O_2 dependent lignin degradation by extracellular peroxidase (Kang et al. 1993; Shin et al. 1993)
	Aspergillus niger (Muller 1928; Kriechbaum et al. 1989; Hatzinikolaou et al. 1996)—mycelium cell wall (Witteveen et al. 1992)	Lower pH of environment (Magnuson and Lasure 2004)
	Penicillium spp.—*Penicillium amagasakiense* (Wohlfahrt et al. 1999), *Penicillium canescens* (Simpson 2006), *Penicillium notatum* (Coulthard et al. 1945; Bright and Appleby 1969), *Penicillium pinophilum* (Rando et al. 1997), *Penicillium funiculosum* (Sukhacheva et al. 2004), *Penicillium variabile* (Pulci et al. 2004), *Penicillium chrysogenum* (Leiter et al. 2004), *Penicillium adametzii* (Eremin et al. 2006)	Antibacterial and anti-fungal (Leiter et al. 2004)
	Phanerochaete chrysosporium (Ramasamy et al. 1985)	Assist lignin degradation (Ramasamy et al. 1985)
	Botrytis cinerea (Liu et al. 1998)	Assist in infection of plants (Edlich et al. 1989)

Reaction by GOx

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-gluconolactone} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{spontaneous/catalase}} 2H_2O + O_2$$

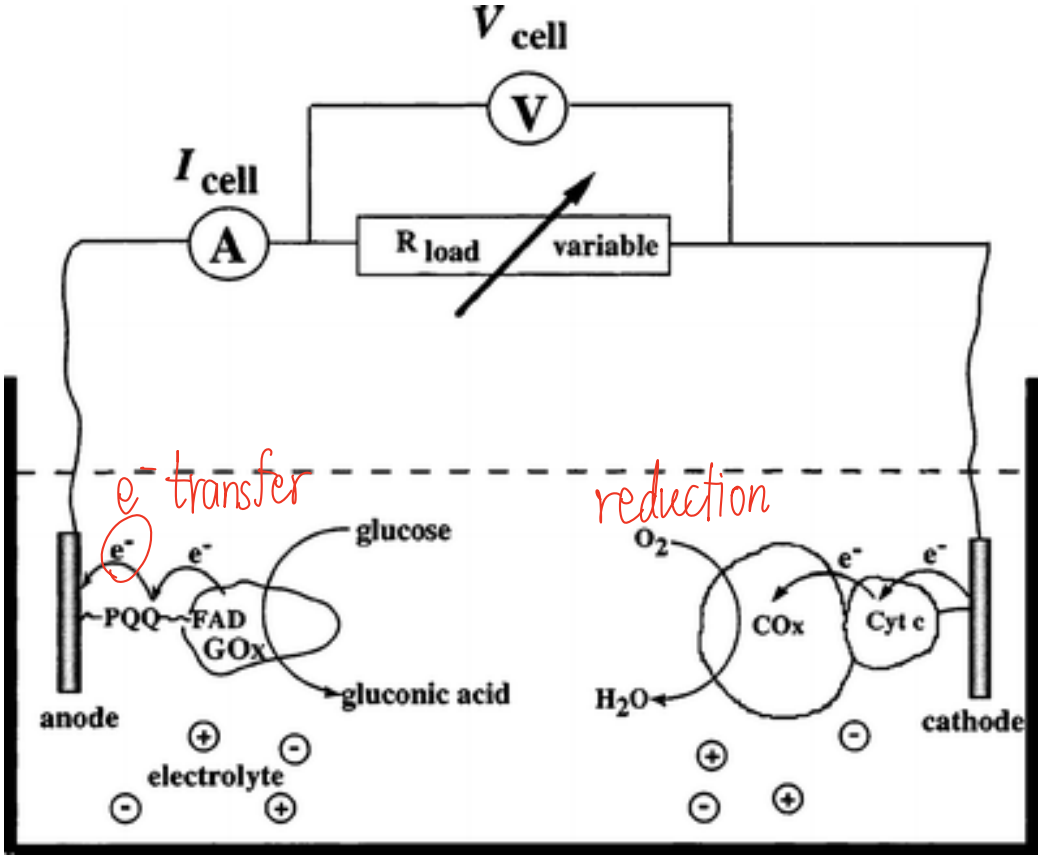
$$\text{D-gluconolactone} + H_2O \xrightarrow{\text{spontaneous/lactonase}} \text{gluconic acid}$$

- The presence of too much H_2O_2 and gluconic acid (lower pH) could deactivate the enzyme GOx

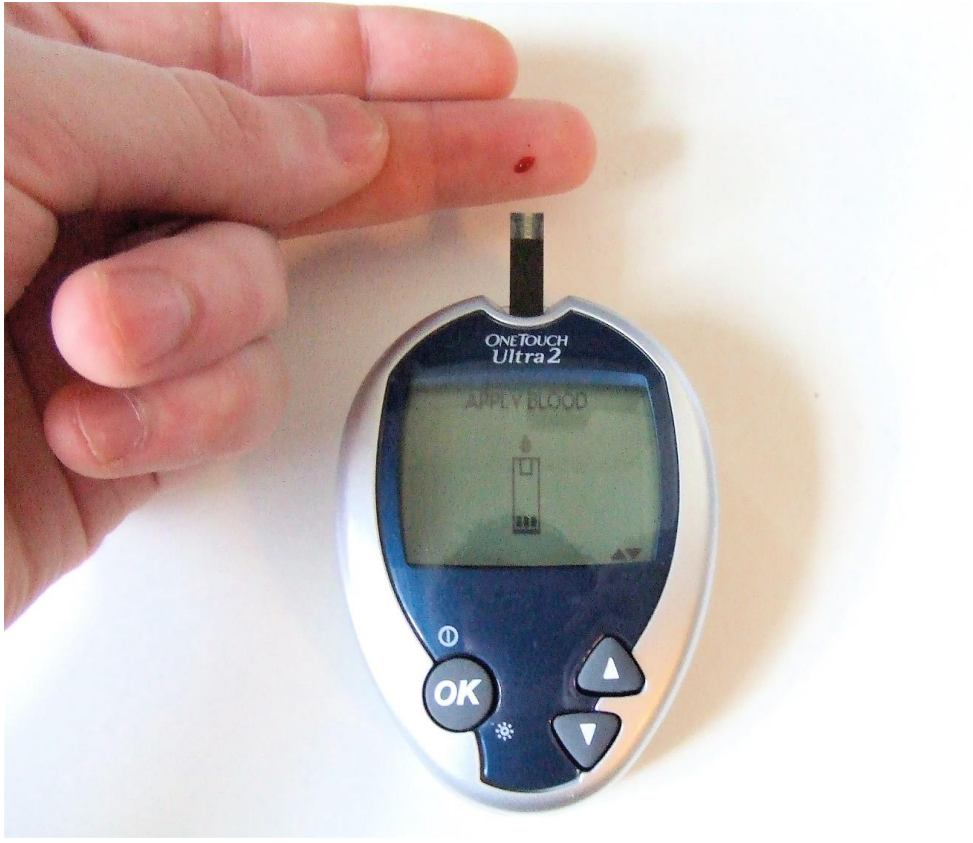
Application of GOx

- Gluconic acid production
  - Used in textile dying, metal surface cleaning, food additives, detergents, concrete, cosmetics and pharmaceuticals
- Reduced alcohol wine
  - GOx reduces sugar content in alcohol fermentation, produces wine with lower % alcohol
- Antioxidant/preservative
  - GOx removes the oxygen in food
- Dry egg powder
  - Glucose in liquid egg is removed by GOx to avoid browning, increase shelf life
  - GOx inhibits microorganism growth by hydrogen peroxide
- Breadmaking
  - GOx as oxidant
- Food preservation – Lactoperoxidase system (LP-GOx)
  - Lactoperoxidase + thiocyanate + hydrogen peroxide as antimicrobial agent (human safe)
  - GOx provides hydrogen peroxide with glucose
  - Used in transportation of raw milk
  - Cheese production (gluconic acid for direct acidification)
- Food processing – additive
  - Antioxidant, preservative and stabilizer
  - Often being used together with catalase

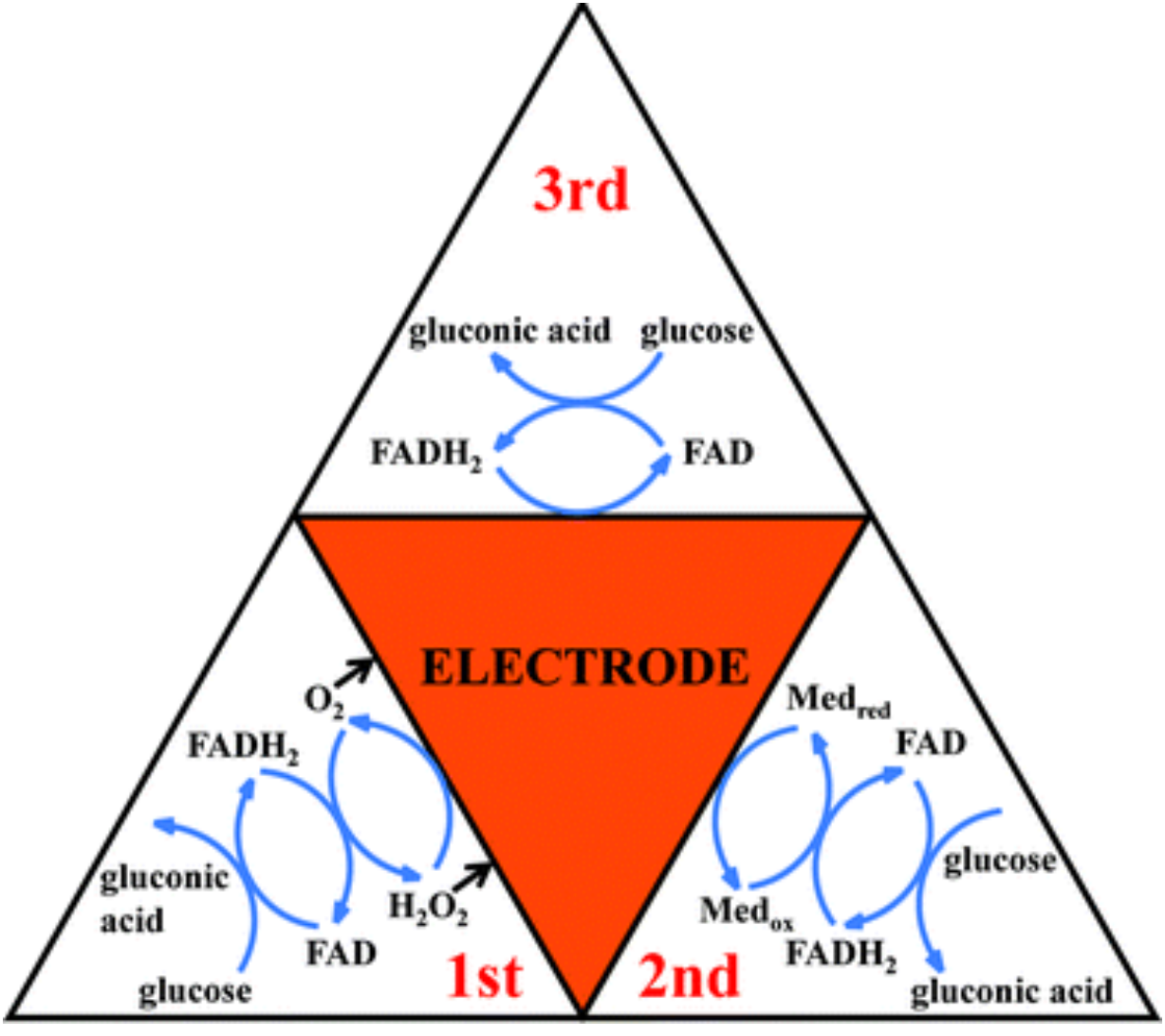
- Fuel cell immobilize
  - GOx in anode of biofuel cells to oxidise glucose (electron flows from anode to cathode to reduce oxygen to water)



- Biosensor
  - GOx is immobilized onto transducer to monitor glucose level

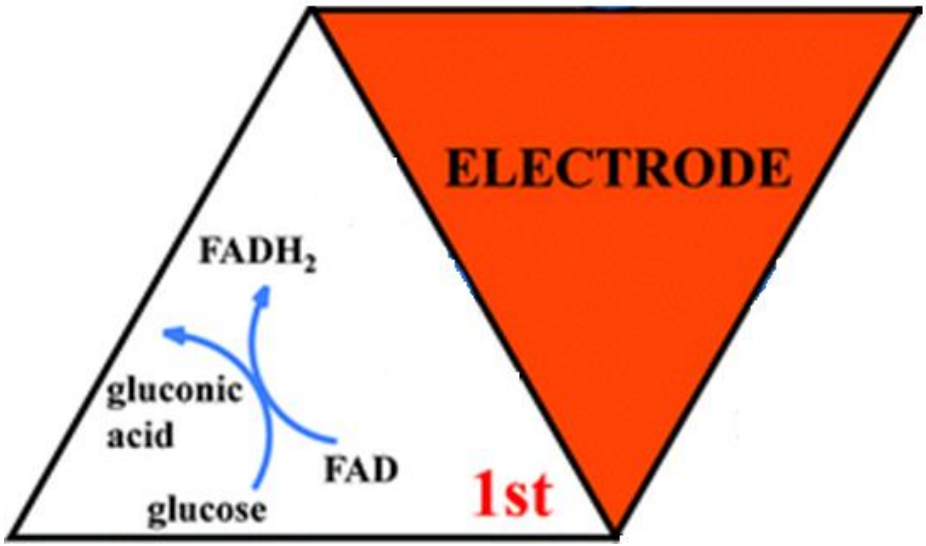


Glucose Biosensor



Glucose Biosensor (1st Gen)

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

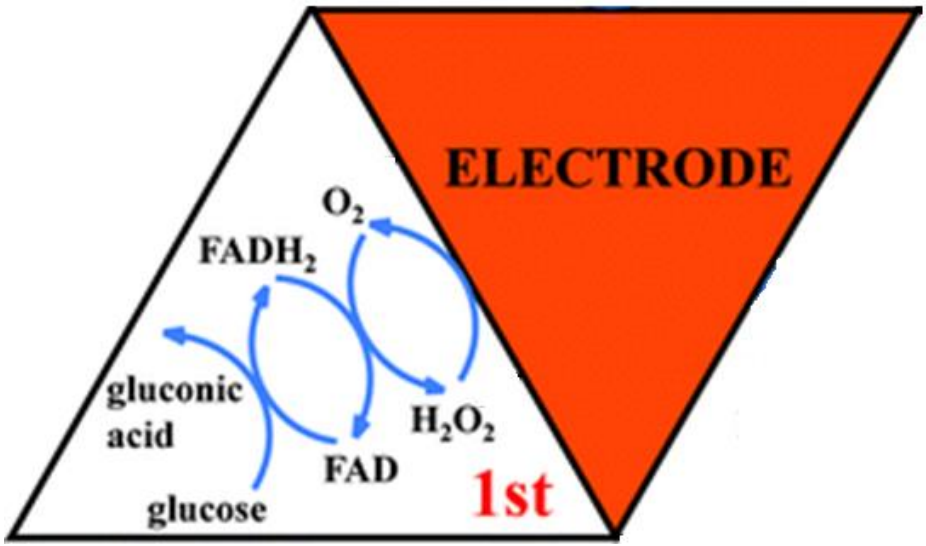


Glucose Biosensor (1st Gen)

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

Combye product

$$\text{GOx} - \text{FADH}_2 + \mathbf{O}_2 \rightarrow \text{GOx} - \text{FAD} + \mathbf{H}_2\,\mathbf{O}_2$$



Glucose Biosensor (1st Gen)

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

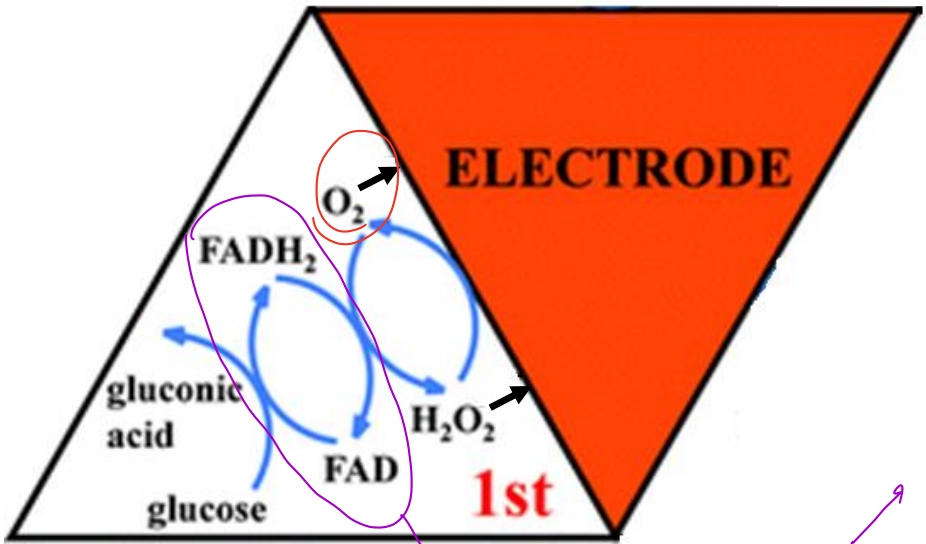
Measure O_2 & H_2O_2

$$\text{GOx} - \text{FADH}_2 + \mathbf{O}_2 \rightarrow \text{GOx} - \text{FAD} + \mathbf{H}_2\,\mathbf{O}_2$$

Glucose concentration determination by oxygen consumption or hydrogen peroxide production

$$H_2O_2 \rightarrow 2H^{+} + O_2 + 2e$$

E = ? V



How much H production?

read back glucose concentration.

- **First glucose biosensor** was developed by Clark and Lyons of the Cincinnati Children's Hospital in 1962
- Their sensor used **glucose oxidase** (GOx) entrapped over an oxygen electrode by a semipermeable membrane to delect for D-glucose in the presence of oxygen
- The oxygen consumption was measured by the electrode as a change in potential
- A decrease in measured O_2 was proportional to the glucose concentration

limitation

$$\text{glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2$$

ELECTRODE SYSTEMS FOR CONTINUOUS MONITORING IN CARDIOVASCULAR SURGERY

Leland C. Clark, Jr., and Champ Lyons
Medical College of Alabama, Birmingham, Ala.

Instruments capable of continuously indicating the chemical composition of blood have proved to be useful in controlling heart-lung machines, in regulating operative and postoperative management of patients, and in teaching and research. At first, such instruments were used with sensors mounted directly in the extracorporeal blood circuit that is used for perfusion of open-heart surgery patients.[1] Later, continuous monitoring of both machine and patients was conducted by means of continuous withdrawal of blood pumped into external cuvettes equipped with appropriate sensors.

Clark, L.C.; Lyons, C. Electrode systems for continuous monitoring in cardiovascular surgery. Ann. N.Y. Acad. Sci. 1962, 102, 29–45.

Glucose Biosensor (1st Gen)

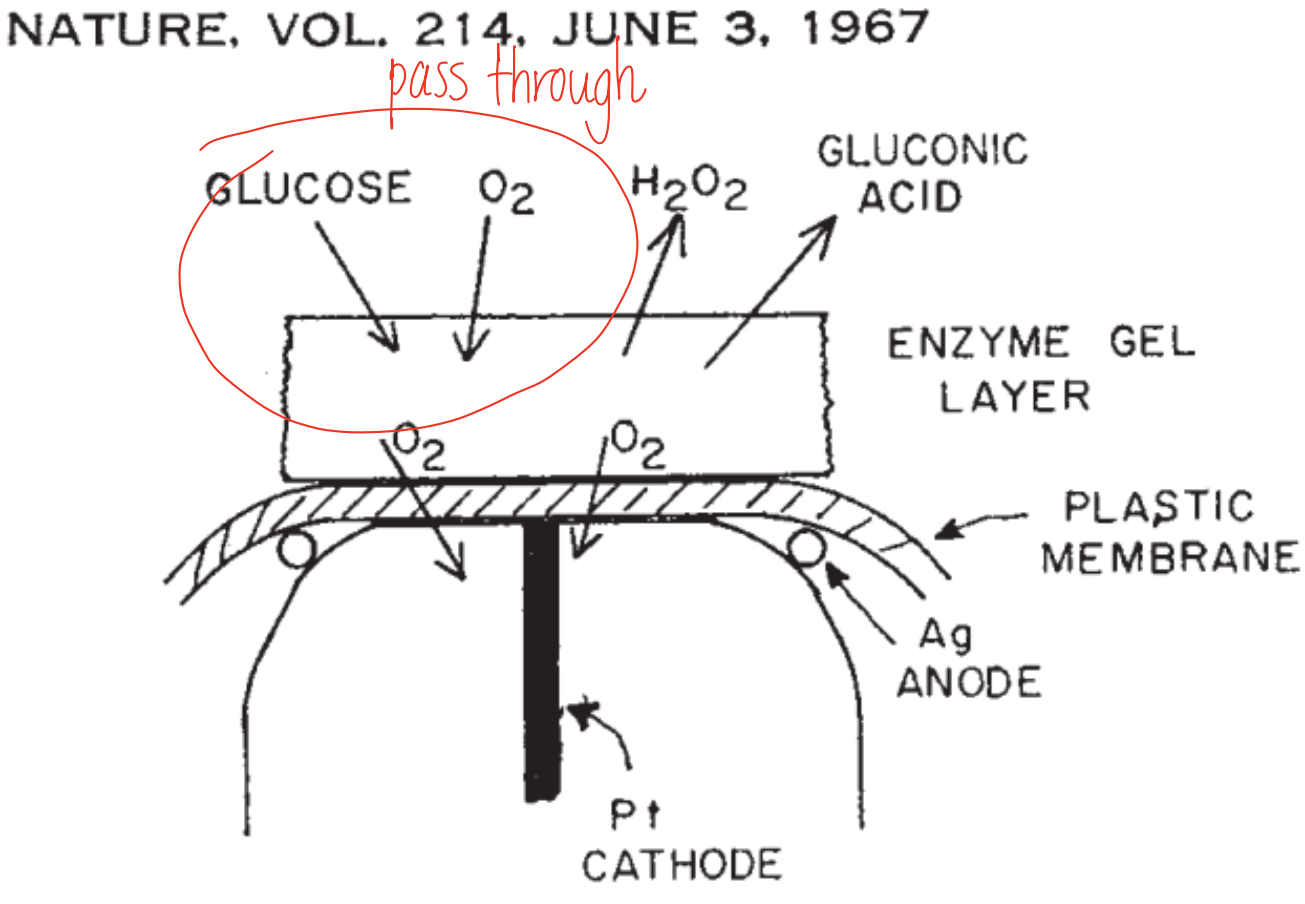


Fig. 1. Principle of enzyme electrode.

Glucose Biosensor (1st Gen)

- **First commercial** glucose biosensor using Clark's technology by Yellow Springs Instrument (Model 23A YSI analyzer) in 1975
- Based on the amperomeric detection of hydrogen peroxide
- Exclusively used in clinical laboratories due to high cost of platinum electrode

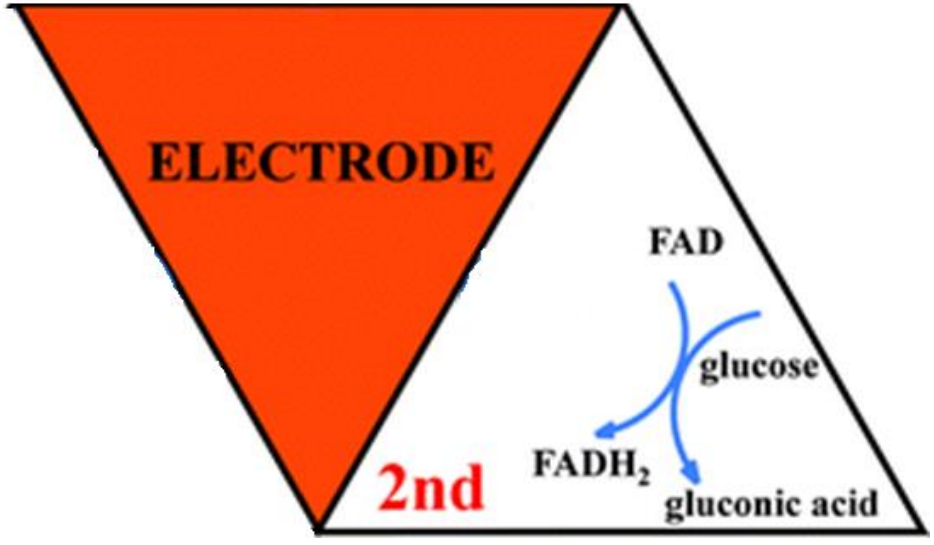
Problems:

- Amperometric detection of hydrogen peroxide requires high potential for high selectivity
- Interference of endogeneous electroactive species (ascorbic acid, uric acid, drugs)
- Restricted solubility of oxygen in biological fluids

  $[O_2]$ << [glucose] => oxygen deficit => limit linearity

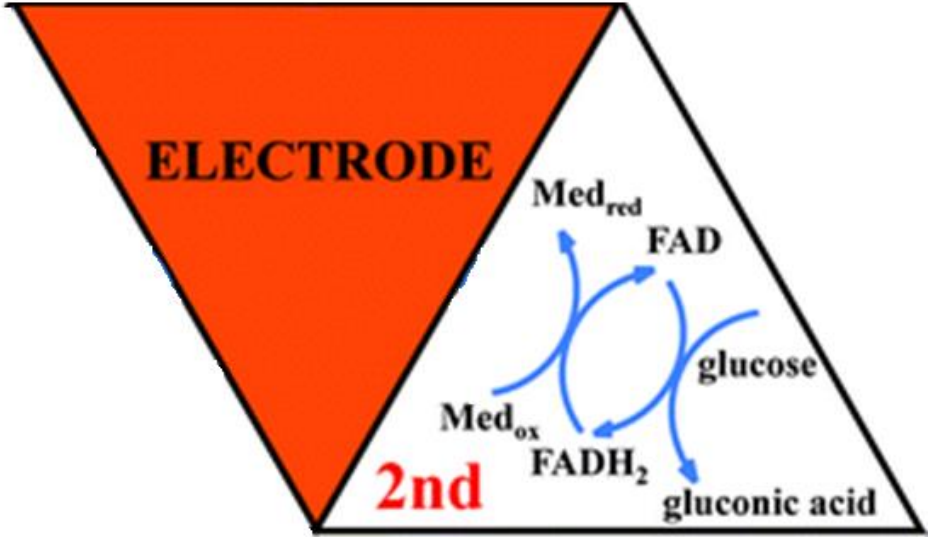
Glucose Biosensor (2nd Gen)

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$



Glucose Biosensor (2nd Gen)

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$



Glucose Biosensor (2nd Gen)

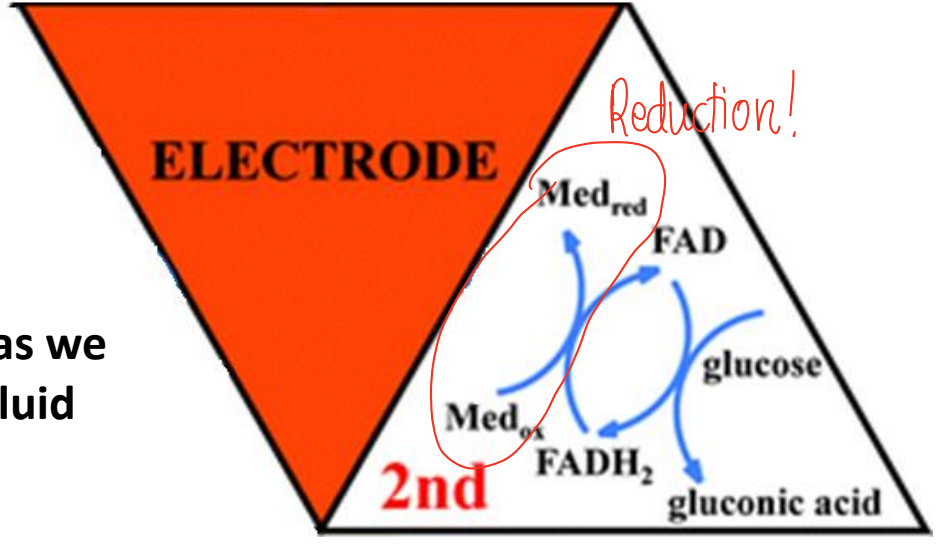
$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

$$\text{GOx} - \text{FADH}_2 + \text{Med}_{ox} \rightarrow \text{GOx} - \text{FAD} + \text{Med}_{red}$$

Measure

reduce enzyme

oxidize



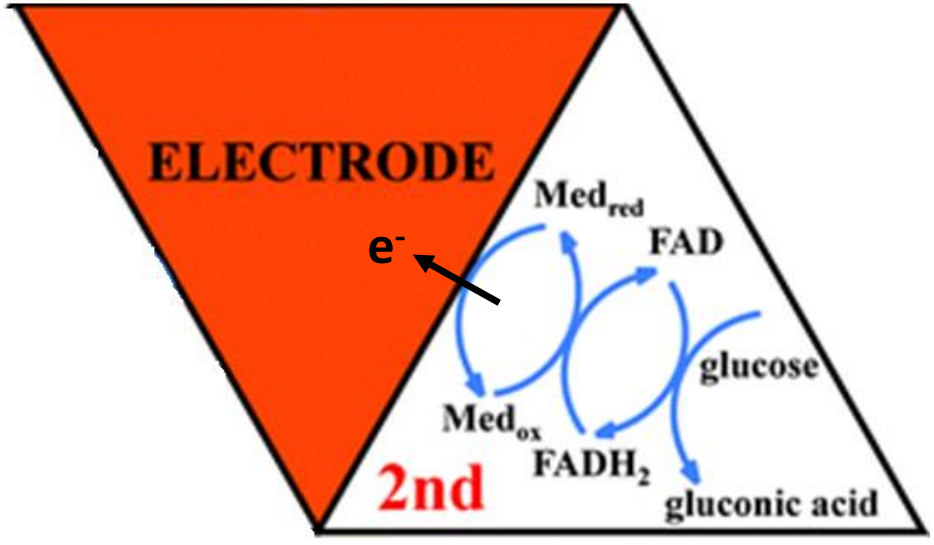
Replace O_2 by Med_{ox} as we cannot change body fluid

Glucose Biosensor (2nd Gen)

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

$$\text{GOx} - \text{FADH}_2 + \text{Med}_{ox} \rightarrow \text{GOx} - \text{FAD} + \text{Med}_{red}$$

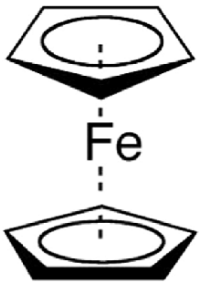
Oxidation by electrode: $\text{Med}_{red} \rightarrow \text{Med}_{ox} + e^-$



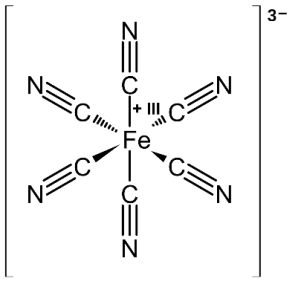
Glucose Biosensor (2nd Gen)

- Replacing oxygen with non-physiological electron acceptors or redox mediators that are able to carry electrons from enzyme to the surface of the working electrode
- Reduced mediator is formed instead of hydrogen peroxide and reoxidized at the electrode, providing an amperometric signal, regenerating oxidized form of mediator

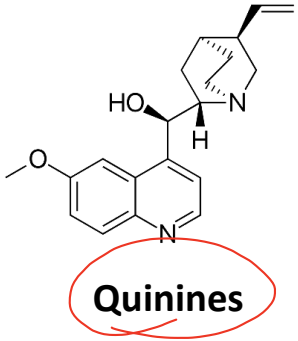
Mediators:



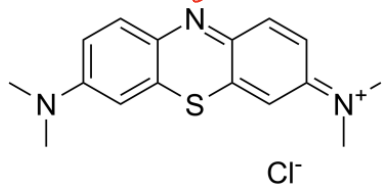
Ferrocene



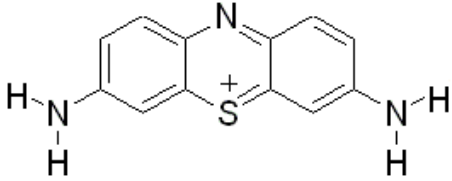
Ferricyanide



Quinines



Methylene Blue

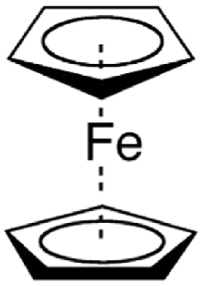


Thionine

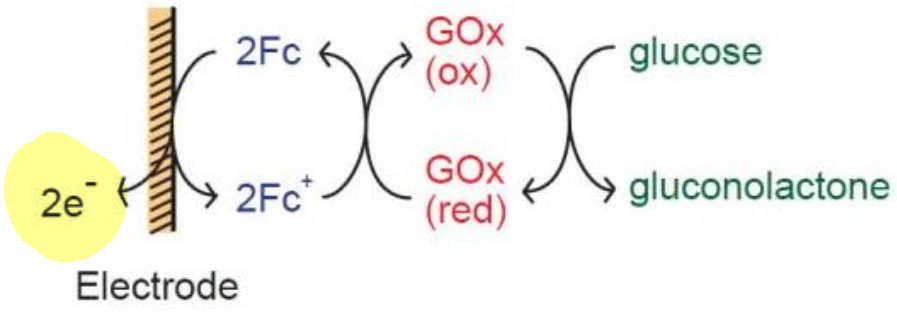
Glucose Biosensor (2nd Gen)

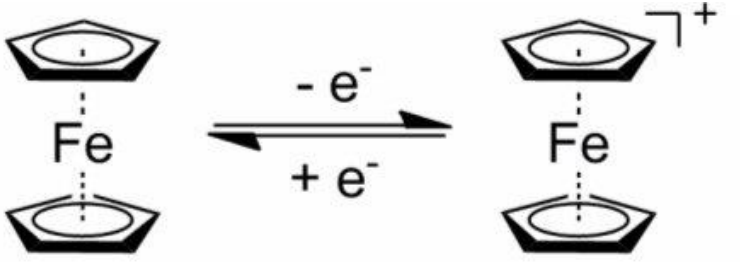
Mediators:

- Not reacting with oxygen
- Stable in both oxidized and reduced forms
- Independent of pH
- Reversible electron transfer kinetics
- React rapidly with enzyme



Ferrocene





Glucose Biosensor (2nd Gen)

- The first electrochemical blood glucose monitor for self-monitoring of diabetic patients in pen-sized was launch in 1987 as Exactech by Medisense Inc.
- Based on GDH-PQQ (glucose-1-dehydrogenase - pyrroquinolinequinone) and ferrocene derivative
- Current commercial glucose biosensor is based on ferrocene or ferricyanide mediators

remove interference, O_2

Methods and Devices

PEN-SIZED DIGITAL 30-SECOND BLOOD GLUCOSE METER

D. R. MATTHEWS, R. R. HOLMAN, E. BOWN, J. STEEMSON, A. WATSON, S. HUGHES, D. SCOTT

Diabetes Research Laboratories, Radcliffe Infirmary, Oxford OX2 6HE; and Genetics International, Abingdon, Oxford

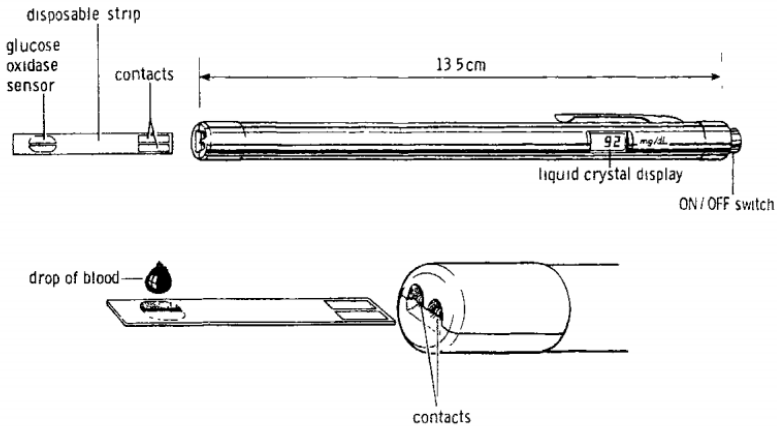
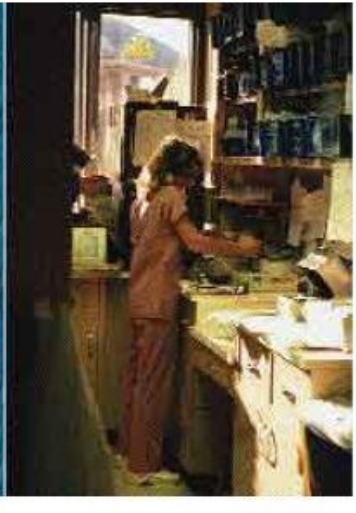


Diagram of the pen-sized glucose meter.

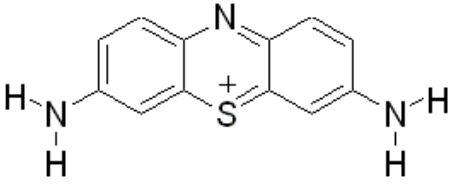
Glucose Biosensor (2nd Gen)

THE MEDISENSE* EXACTECH™
GLUCOSE biocapteur pour le sang

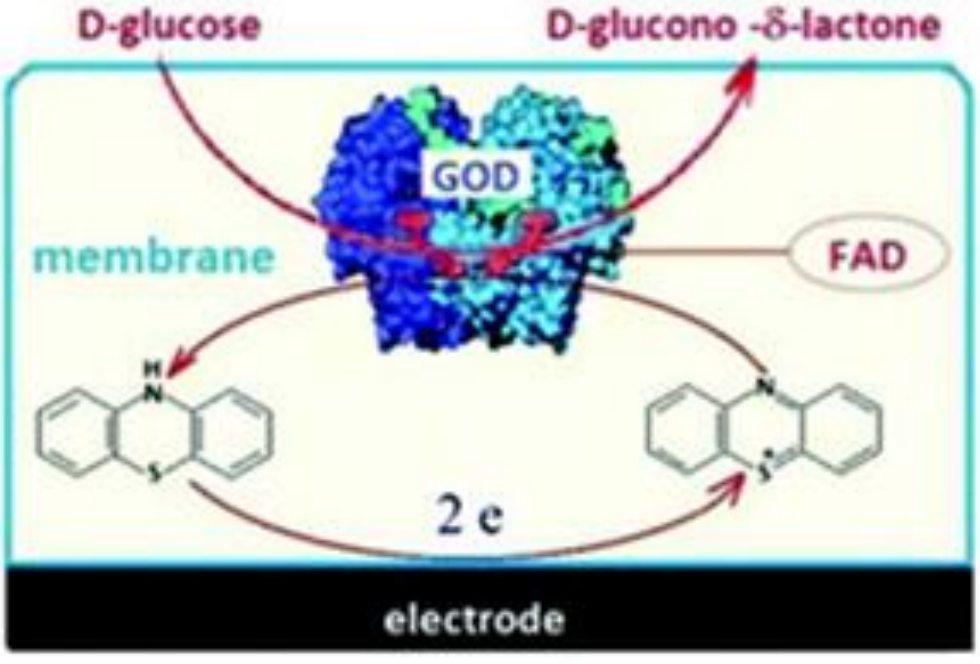


Glucose Biosensor (2nd Gen)

Mediators:



Thionine



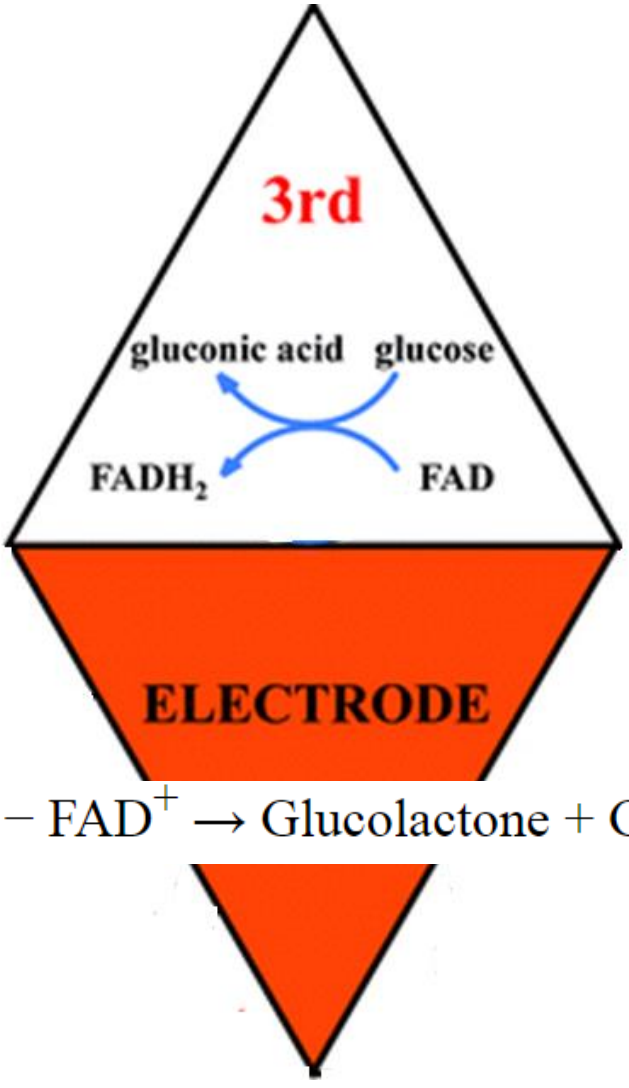
Anal. Chem., **2012**, *84* (3), pp 1220–1223

Glucose Biosensor (2nd Gen)

Drawbacks:

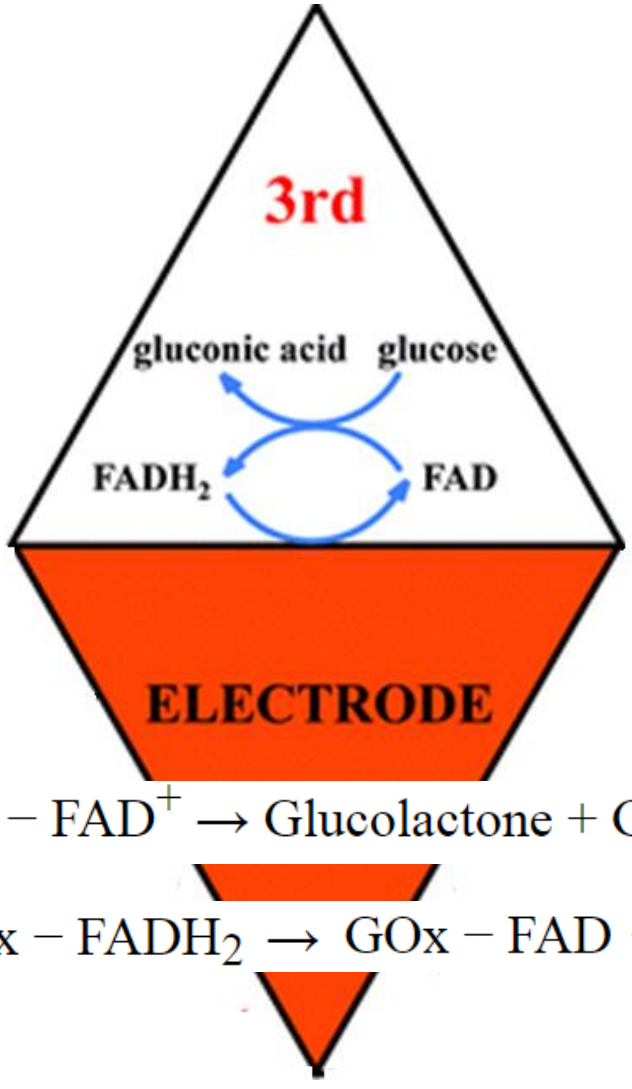
- High competition between redox mediator and oxygen.
- Interference of other electroactive species lead to false and inaccurate results.
- Small size and highly diffusive nature of mediators poses problem of leaching of mediator from intermediate region between enzyme and electrode surface.
- Some mediators are toxic

Glucose Biosensor (3rd Gen)



$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

Glucose Biosensor (3rd Gen)



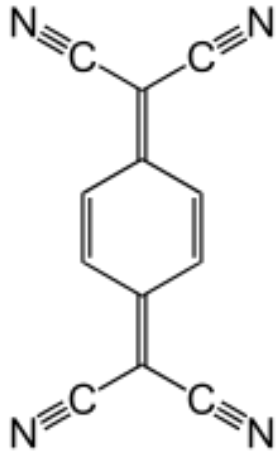
$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

$$\text{GOx} - \text{FADH}_2 \rightarrow \text{GOx} - \text{FAD} + e^{-}$$

Glucose Biosensor (3rd Gen)

- Reagentless
- Direct electron transfer between enzyme and electrode without mediators
- Connection by organic conducting materials
- Implantable, needle-type devices for continuous *in vivo* monitoring of blood glucose
- Offers superior selectivity without the usage of mediators
- The intrinsic barrier to electron flow is the globular structure of glucose oxidase with the active site, containing FAD/$FADH_2$ redox cofactor, buried deep inside a cavity of ~ 13 A° is a major hindrance for direct electron transfer.
- Only few enzymes such as GOx and horseradish peroxidase exhibit direct electron transfer at normal electrode (electrode modification is required most of the time)

Organic Conducting Material:



Tetracyanoquinodimethane (TNCQ)

"polymerization"

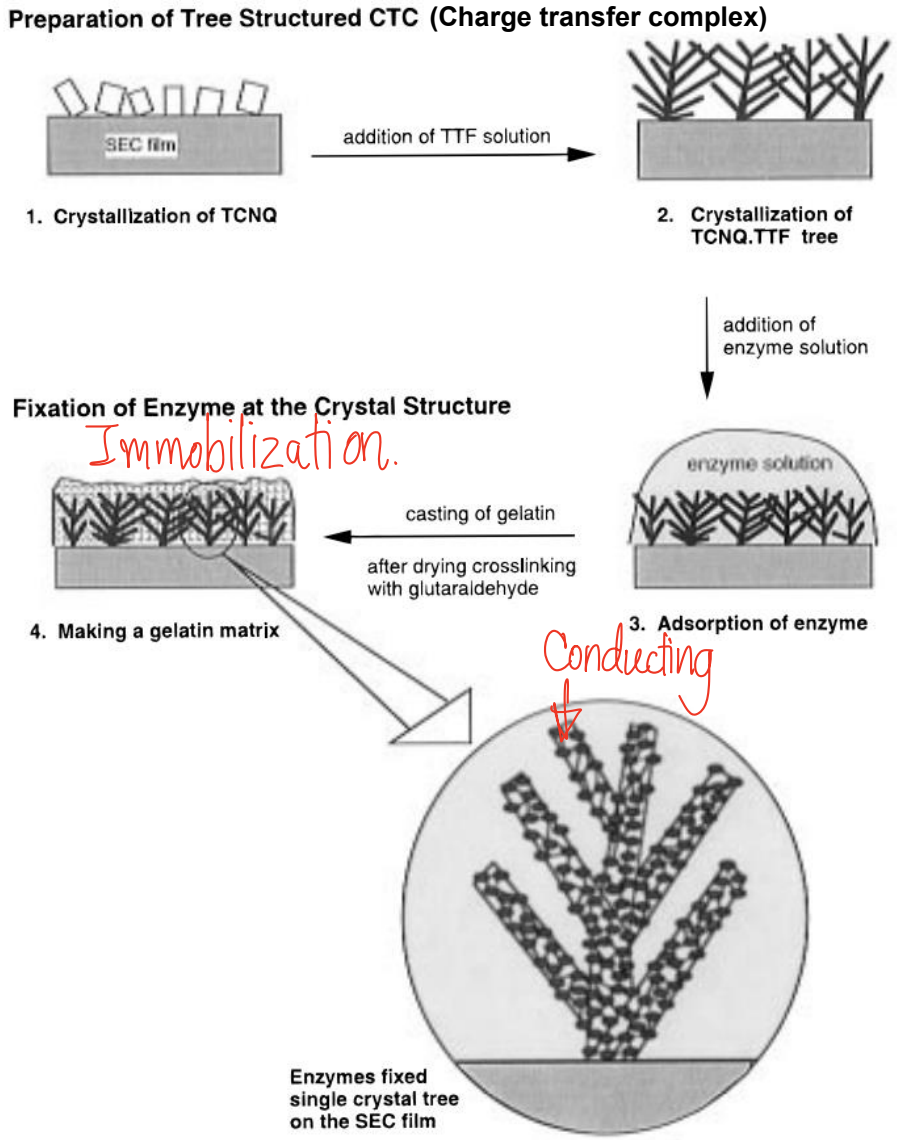
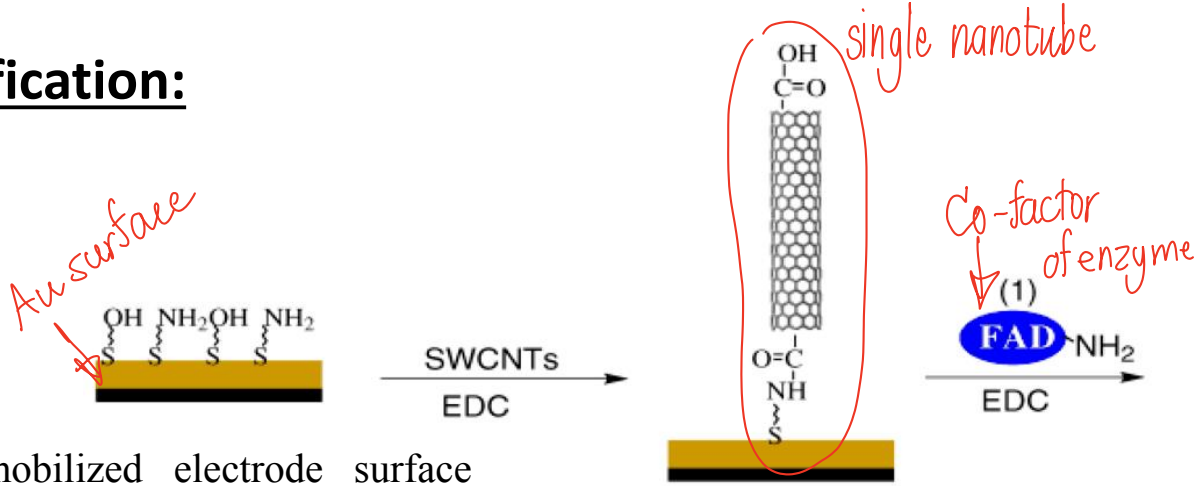


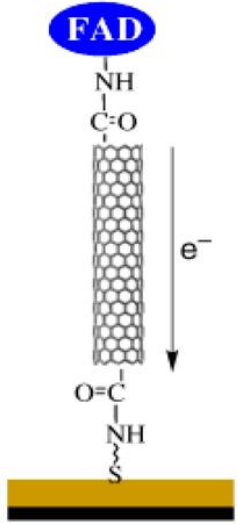
Figure 1. Schematic illustration of the preparation steps and the cross-sectional view of a single CTC tree of the sensor.

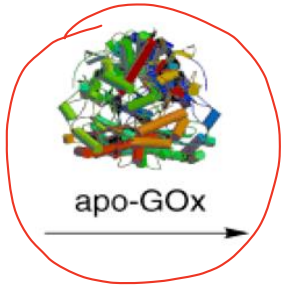
Anal. Chem. **1996,** *68,* 2939−2945

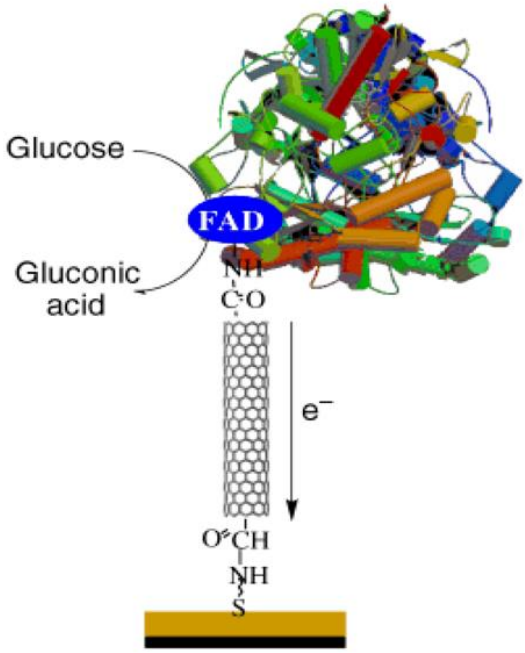
Electrode modification:



Carbon nanotubes immobilized electrode surface provide suitable orientation for enzyme immobilization and establish connection between electrode surface and deeply buried active site of enzyme.

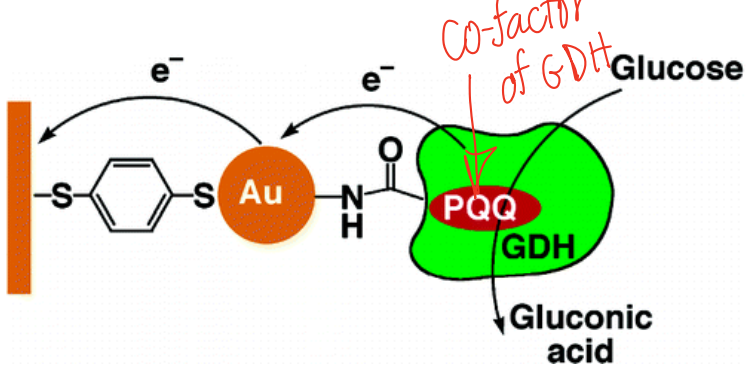




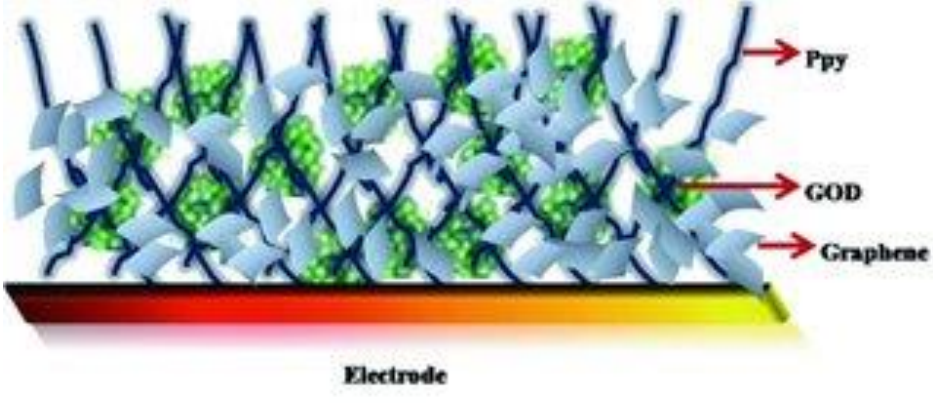


Angew. Chem.Int. Ed. **2004**, *43*, 2113–2117.

An electrically contacted glucose dehydrogenase (GDH) enzyme electrode is fabricated by the reconstitution of the apo-GDH on pyrroloquinoline quinone (PQQ)-functionalized Au nanoparticles (Au-NPs), 1.4 nm, associated with a Au electrode.



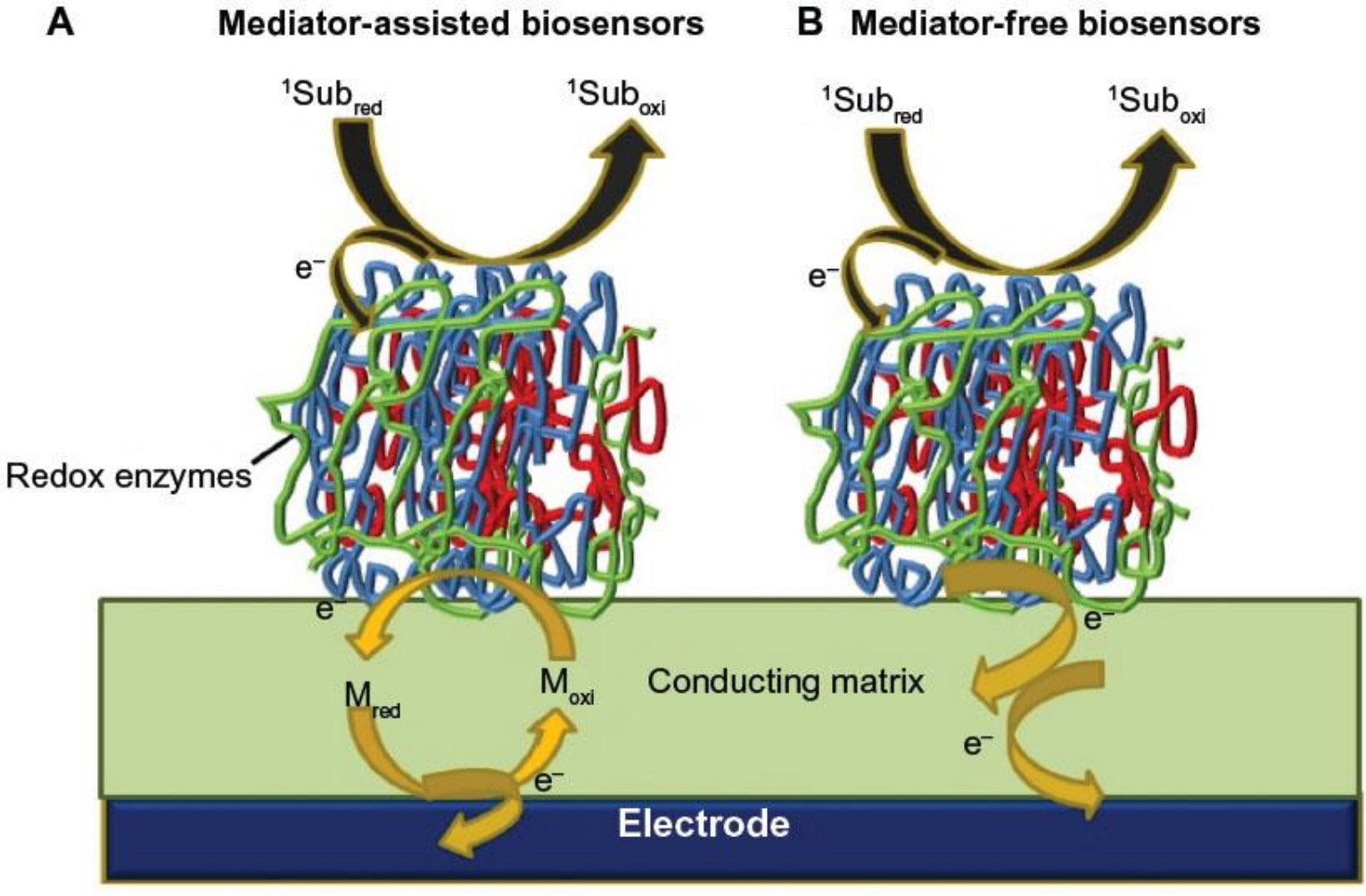
J. Am. Chem. Soc., **2005**, *127* (35), pp 12400–12406



Wang et al. detected glucose based on the direct electron transfer reaction of GOx immobilized on highly ordered PANI nanotubes.

Polymer aniline

Anal. Chem., **2009**, 81, 1638–1645.


A
Mediator-assisted biosensors
B
Mediator-free biosensors
$^1Sub_{red}$
$^1Sub_{oxi}$
e⁻
Redox enzymes
M_{red}
M_{oxi}
Conducting matrix
Electrode

Antibody / Antigen

B. Affinity Biosensors

- Based on selective binding of certain biomolecules towards specific species that triggers electrical signals

- Measures electrochemical signals resulting from the binding process

- Highly sensitive and selective

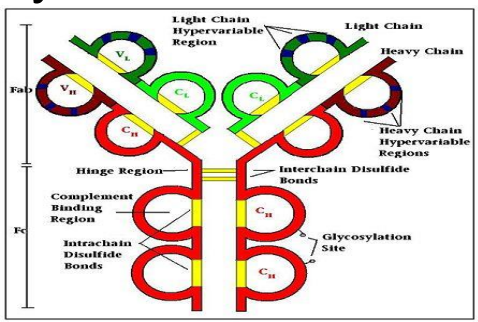
BI. Immunosensors

- Based on immunological reactions

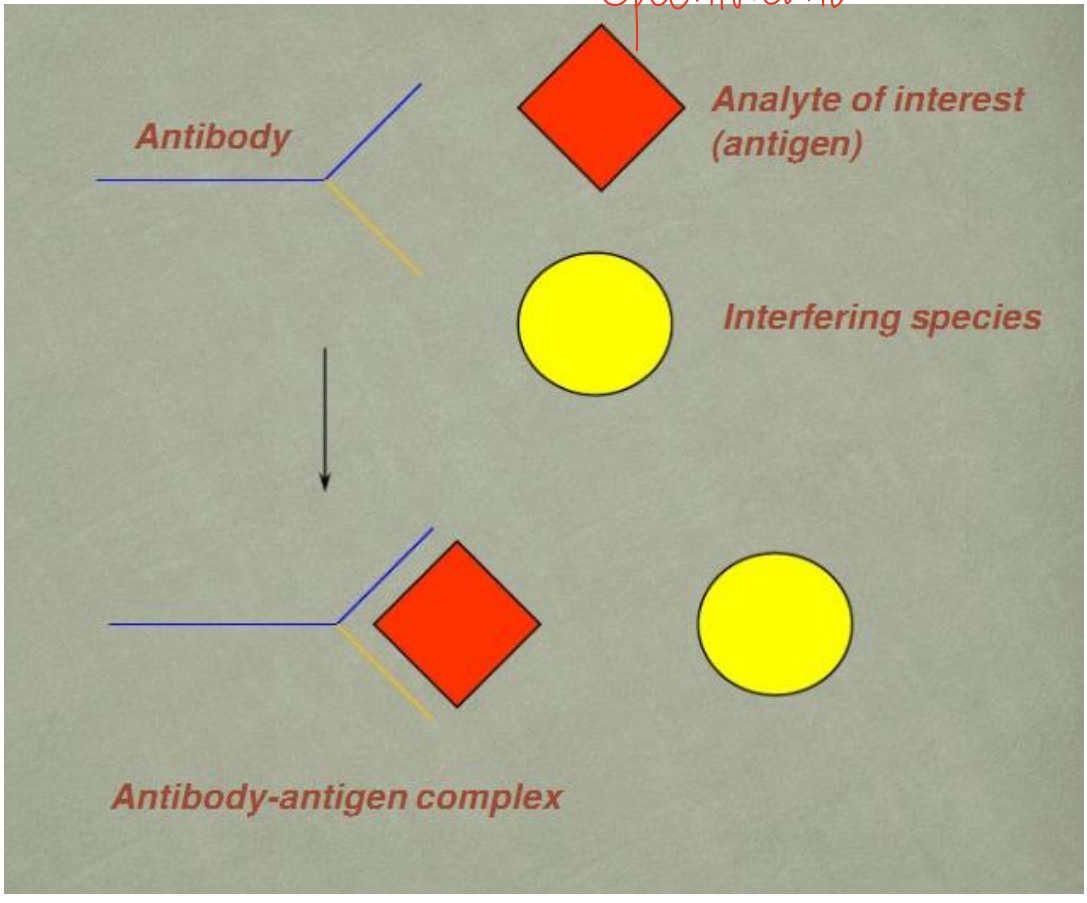
- Useful for identifying and quantifying proteins

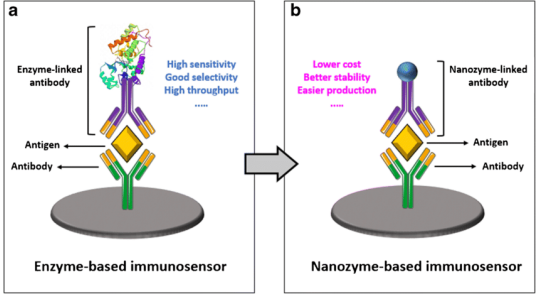
Antibody.

- Antibodies represent one of the major class of protein;
- They constitute about 20% of the total plasma protein and are collectively called immunoglobulins (Ig).
- The simplest antibodies are usually described as Y-shaped molecules with two identical binding sites for antigen.
- An antigen can be almost any macromolecule that is capable of inducing an immune response.
- The antibody binds reversibly with a specific antigen.
- Unlike the enzyme proteins, the antibody do not act as catalysts. Their purpose is to bind foreign substances - antigens - so as to remove them from the system.



Specificant





BII. DNA Hybridization Biosensors

§ Bacteria single-strand.

- Nucleic acid recognition layers are combined with electrochemical transducers

- Used to obtain DNA sequence information

- Electrochemical response of DNA is strongly dependent on DNA structure

BII. DNA Hybridization Biosensors

Applications

- For chemical diagnosis of infectious diseases

- For environmental monitoring

- For detecting drugs, carcinogens, food containing organisms

- For criminal investigations

BIII. Receptor-Based Sensors

- Make use of chemoreceptors as biological recognition elements

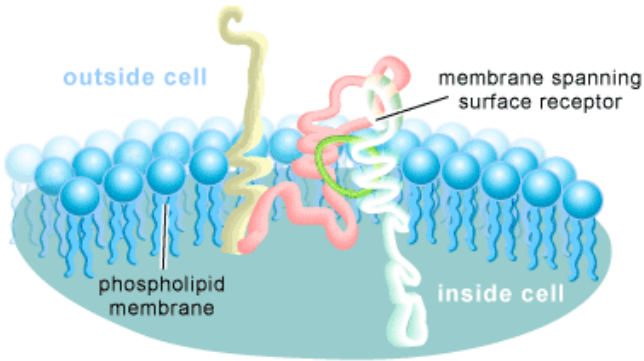
- Class-specific device (binds specific classes of substances that possess similar chemical properties)

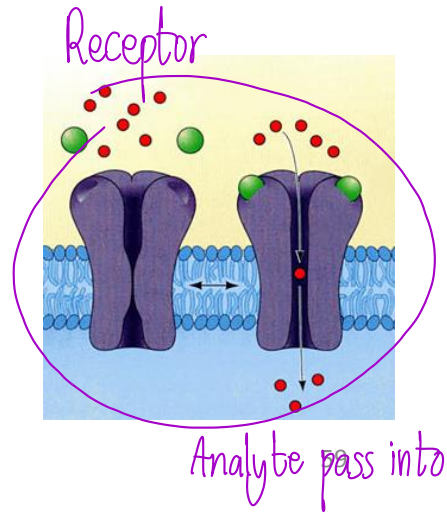
Receptors ; only bind analyte
- Protein molecules embedded in the cellular membrane to which target analytes specifically bind

Chemoreceptor (chemosensor)
- Converts chemical signal into potential

Receptor Protein

- Receptor proteins are protein molecules having specific affinity for hormones, antibodies, enzymes, and other biologically active compound.
- These proteins are mostly bound to membrane (Figure).
- There are hormone receptors, taste receptors, olfactory receptors for smelling, photoreceptors for eyes, etc.
- Receptor proteins are responsible for opening and closing of membrane channels for transport of specific metabolites.





Other Possibilities

recognizing a target

- In principle, any biomolecules and molecular assembly that have the capability of recognizing a target substrate (= the analyte) can be used as a bioreceptor.

- In fact, membrane slices or whole cells have been used in biosensors.

- Note that the bioreceptors require suitable environment for maintaining their structural integrity and biorecognition activity.

What are the advantages of integrating biological elements as opposed to purely chemical recognition elements into the design of sensor?

❖ provide overall **superior properties** compared with chemical systems developed to carry out equivalent functions

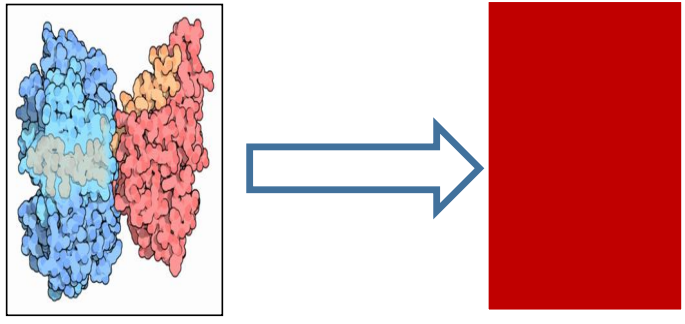
❖ Nature has designed **far better systems** for tasks such as recognition specificity, catalytic efficiency, electron transfer and other complex integrated functions than the talented bench scientist is capable of creating with current design and synthesis approach.

- BUT…
- They tend to be functionally less robust than chemical systems to specific factors in their environment (extremes of pH, temperature, the presence of oxidizing agents, enzymatic degradation etc)

Otimized condition!!!

Immobilisation

Immobilisation is the process of attaching (or associating) the biological recognition agent to the transducer



Criteria
- Retention of biological recognition agent activity
- Tight association with transducer surface
- Long term stability and durability
- Reproducible methodology

Enzyme Immobilization:

- Easy separation from reaction mixture, providing the ability to control reaction times and minimize the enzymes lost in the product.

- Re-use of enzymes for many reaction cycles, lowering the total production cost of enzyme mediated reactions.

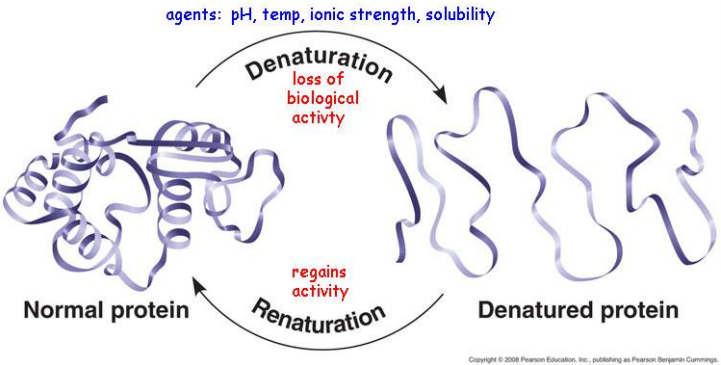
- Ability of enzymes to provide pure products.

- Possible provision of a better environment for enzyme activity

- Diffusional limitation

- Enzyme immobilization: key factor to develop efficient biosensors with appropriate performances such as
  - good operational and storage stability,
  - high sensitivity,
  - high selectivity,
  - short response time and
  - high reproducibility.
- Immobilized biomolecules have to maintain their structure, their function, to retain their biological activity after immobilization, to remain tightly bound to the surface and not to be desorbed during the use of the biosensor, stable for long-term application.
- The type of immobilization method affects activity and stability of enzymatic biosensors.
- Factors such as accuracy of measurements, the sensor-to-sensor reproducibility and operational life times are drastically influenced by enzyme stability.

- Sensitivity decreases if immobilization causes enzyme denaturation or conformational changes or if the enzyme has been modified, especially on its active site.
- A better sensitivity is obtained with oriented immobilization of enzymes on the transducer surface or by selecting the nature of the spacer arm between the enzyme, and the support under covalent binding.



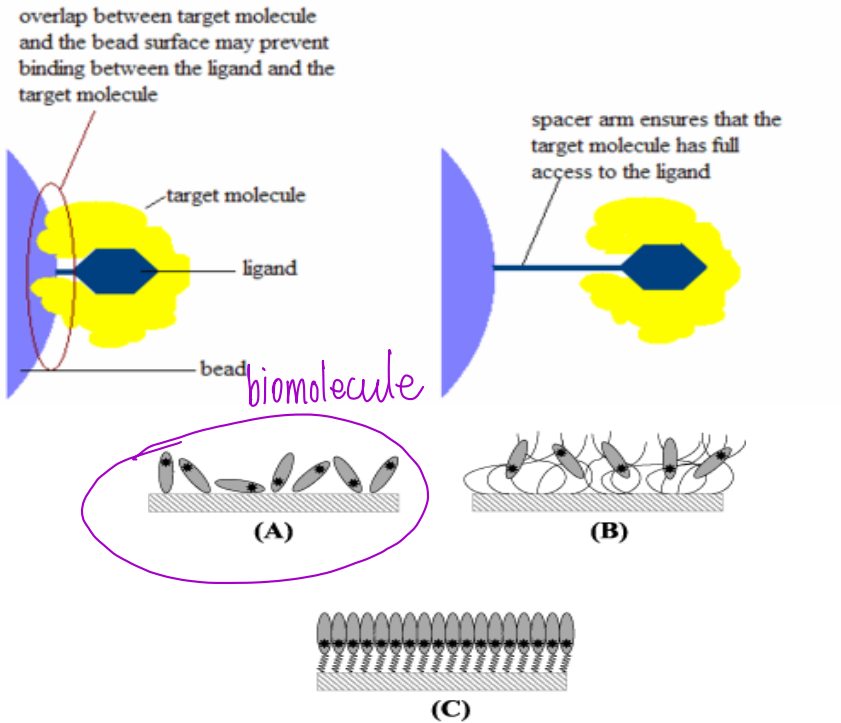


Figure 4. Representation of some methods used to achieve enzyme immobilization. (A) physical adsorption; (B) cross linking or inclusion in polyelectrolytes/conducting polymers; (C) oriented attachment to self-assembled monolayers.

Immobilisation Methods

- Adsorption
- Membrane microencapsulation
- Gel entrapment
- Gel cross-linking
- Covalent bonding
- Self-assembled

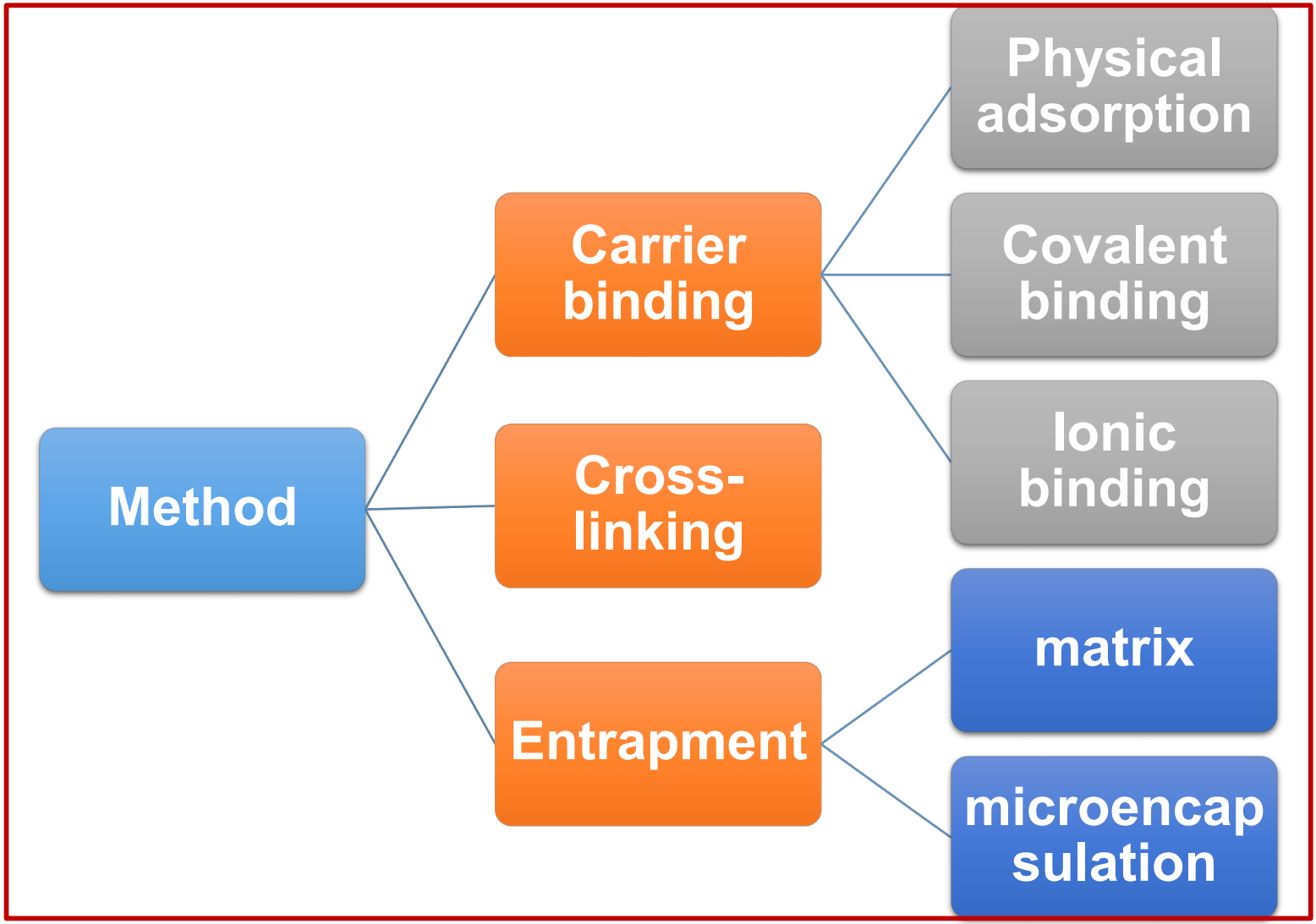
The advantages of long-term stability also typically require more complicated immobilisation procedures

Immobilisation	Method Stability
adsorption	1 day
gel entrapment	1 week
gel cross-linking	3-4 weeks
covalent bonding	4-14 months

Why it is important to choose a method of attachment prevent loss of enzyme activity?

- To avoid reaction
- The structure is retained in the enzyme through hydrogen bonding or the formation of electron transition complexes: prevent vibration of the enzyme and increase thermal stability

Immobilization Methods



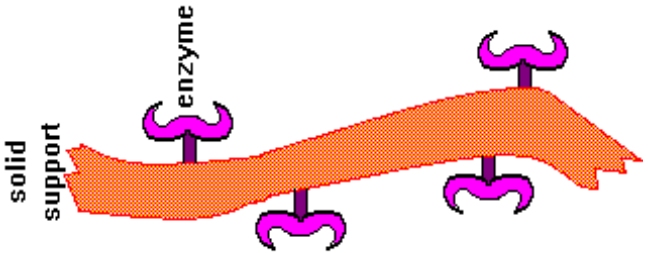
The different methods of are:

- **Carrier-Binding:** the binding of enzymes to water-insoluble carriers
- **Cross-linking:** intermolecular cross-linking of enzymes by bi-functional or multi-functional reagents.
- **Entrapping:** incorporating enzymes into the lattices of a semi-permeable gel or enclosing the enzymes in a semi-permeable polymer membrane

Carrier-Binding:

- The oldest immobilization technique for enzymes

- the carrier-binding method can be further sub-classified into:
  - **Physical Adsorption**
  - **Covalent Binding**
  - **Ionic Binding**



Physical Adsorption

Immobilisation of enzyme protein on the surface of water-insoluble carriers.

-**Advantages:** Easy; spontaneous; no reagent required

-**Disadvantages**: The adsorbed enzyme may leak from the carrier during use due to a weak binding force between the enzyme and the carrier. Moreover, the adsorption is non-specific, further adsorption of other proteins or other substances

Adsorption

Many substances adsorb enzymes on their surfaces, e.g. alumina, charcoal, clay, cellulose, kaolin, silica gel, glass and collagen.

No reagents are required, there is no clean-up step and there is less disruption to the enzymes.

Adsorption : not specific

- Drawbacks include:
  - enzymes are loosely bound to the support
  - desorption of the enzyme resulting from changes in temperature, pH and ionic strength.
  - poor operational and storage stability.
  - the non-specific adsorption of other proteins or substances
- Divided into:
  - Physical adsorption
  - Electrostatic interactions
  - Retention in a lipidic microenvironment

Covalent Binding

Covalent binding: the attachment of biological recognition agent onto the transducer surface using a chemical reaction (e.g. peptide bond formation) or linkage to activated surface groups (e.g. thiol, epoxy, amino, carboxyl)

› **Advantages:** - High stability with respect to pH and ionic strength, as well as temperature
- Free diffusion of analyte – faster reaction time

› **Disadvantage:**
- Potential loss of activity upon immobilization (*covalent binding may alter the conformational structure and active center of the enzyme, resulting in major loss of activity and/or changes of the substrate*)

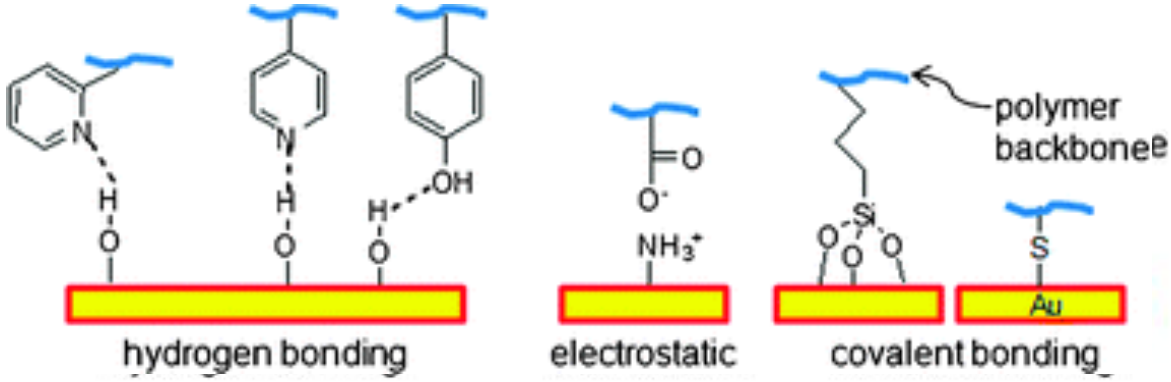
Covalent modification and activation of surfaces using surface chemistry

Chemical modifications of surfaces and their applications

❖ *Chemical modification* - ***two major reasons***

1. T**o attach** selective groups (binding sites or catalysts) to the sensor surface in order to recognize target species in the sample.

2. **To increase** the selectivity of the sensor by reduction of interferences arising from non-specific interactions.



❖Surface modification - Covalent and non-covalent strategies

1. Covalent approaches- a chemical bond is made between the surface and the attached species - irreversible process.

2. Non-covalent approaches- weaker, non-bonding, and the adsorbed species are utilized – reversible

e.g; charge-charge, charge-dipole, dipole-dipole, dipole-induced dipole, and induced dipole-induced dipole interactions (Van der Waals interactions) -these are less surface specific than the covalent approaches - can be more readily achieved.

Covalent Immobilisation

› Reagents

- Glutaraldehyde; carbodiimide; succinimide; esters; periodate

› Biomolecule residues

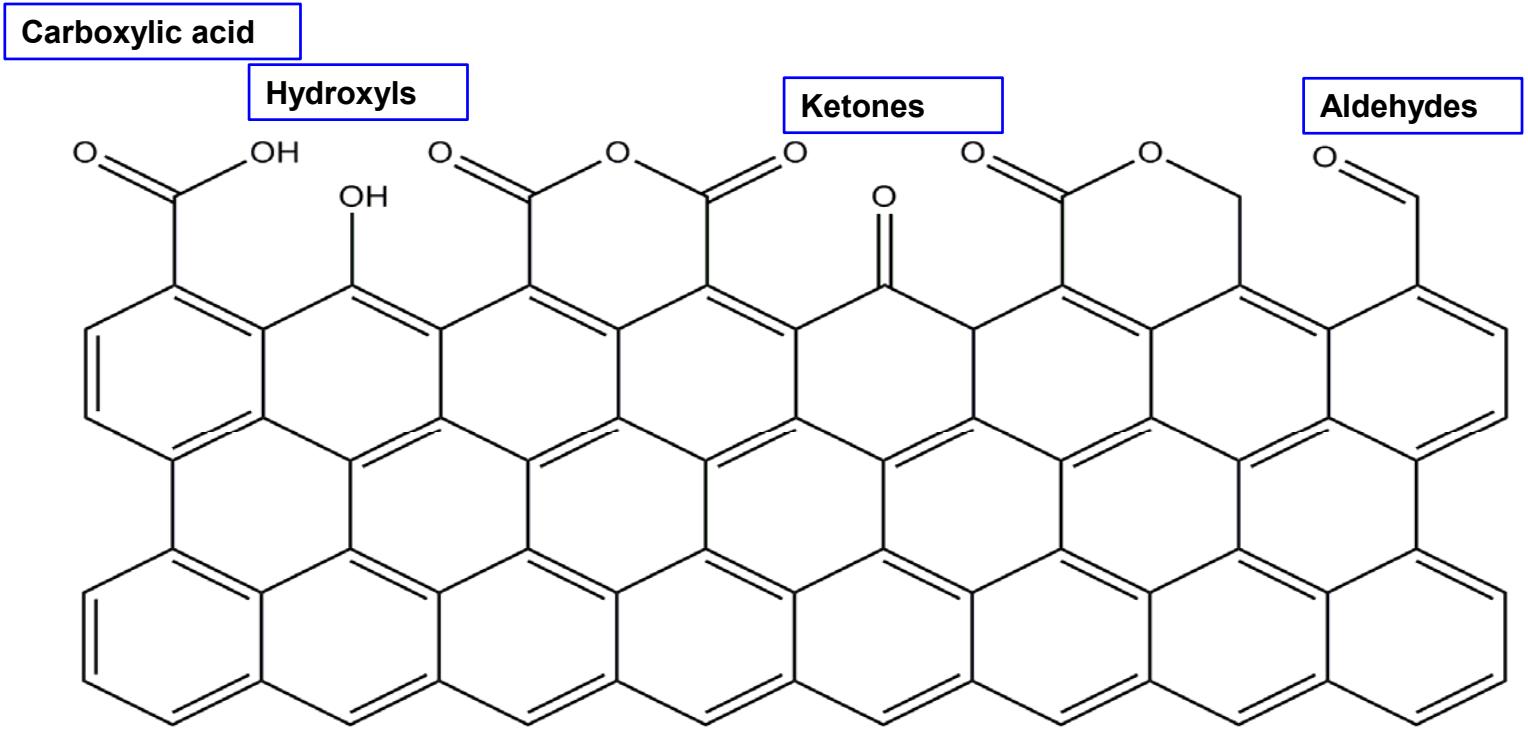
- Sulfhydryl (thiol); carboxyl; amine; hydroxyl

› Transducer surfaces

- Silica (hydroxyl); titanium dioxide; polysaccharides; polymers; graphite; gold

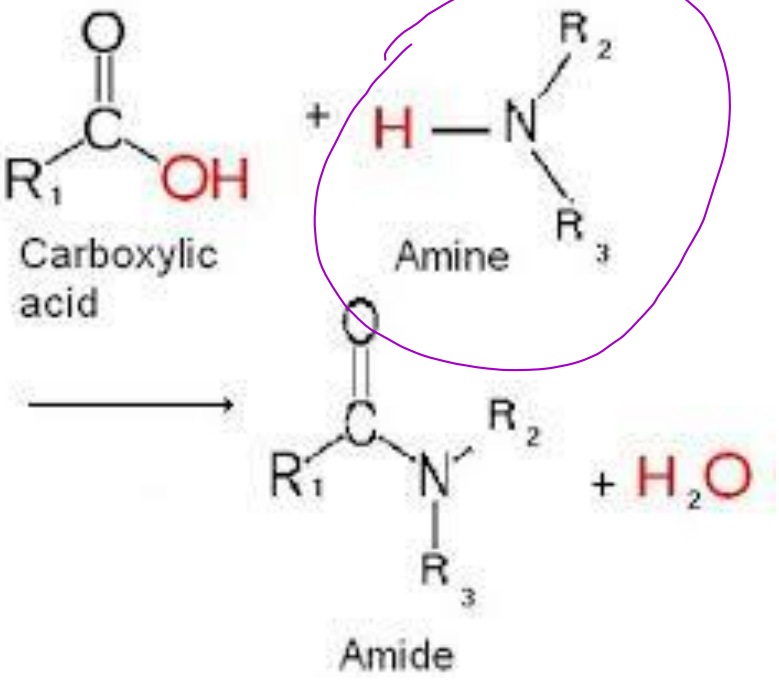
Activation of Transducer Surface

Following oxidation, carbon surfaces (i.e. graphite) have a variety of surface groups



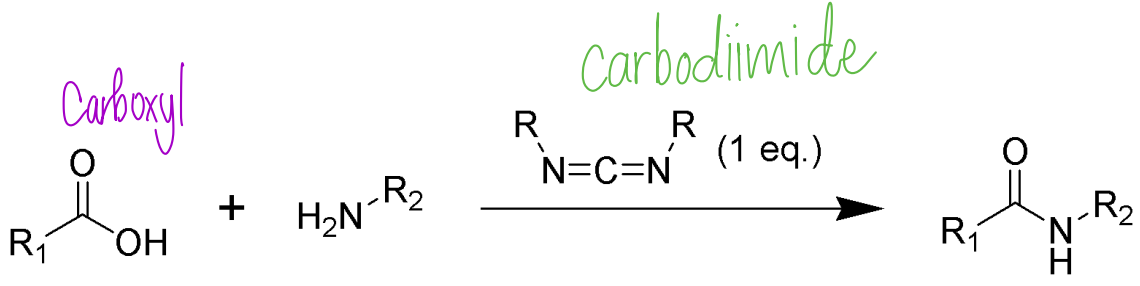
Amines are organic compounds and functional groups that contain a basic nitrogen atom with a lone pair.

common molecules



A carbodiimide or a methanediimine is a functional group consisting of the formula RN=C=NR. Carbodiimides hydrolyze to form ureas, which makes them uncommon in nature.

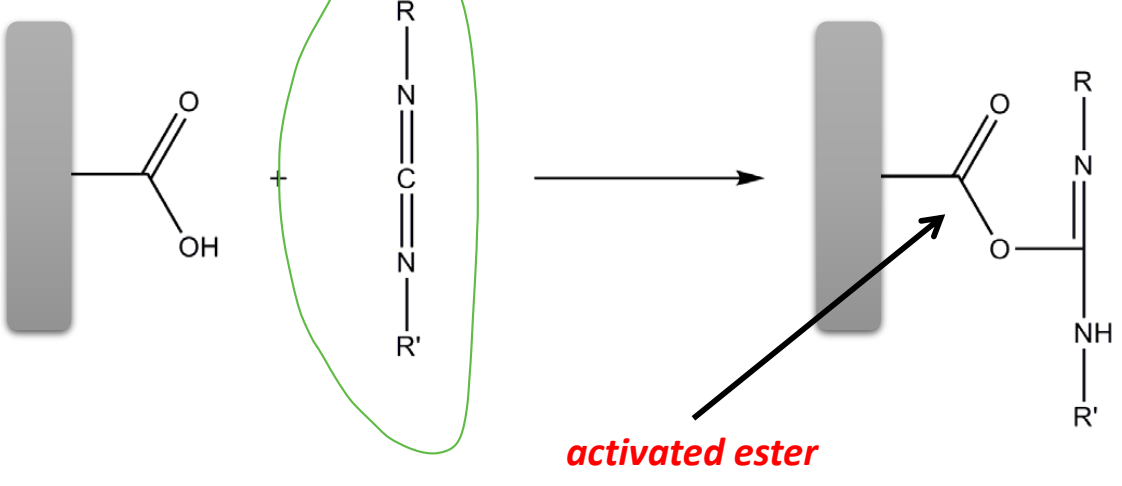
In synthetic organic chemistry, compounds containing the carbodiimide functionality are dehydration agents and are often used to activate carboxylic acids towards amide or ester formation.



Additives, such as N-hydroxybenzotriazole or N-hydroxysuccinimide, are often added to increase yields and decrease side reactions.

Amide coupling utilizing a carbodiimide

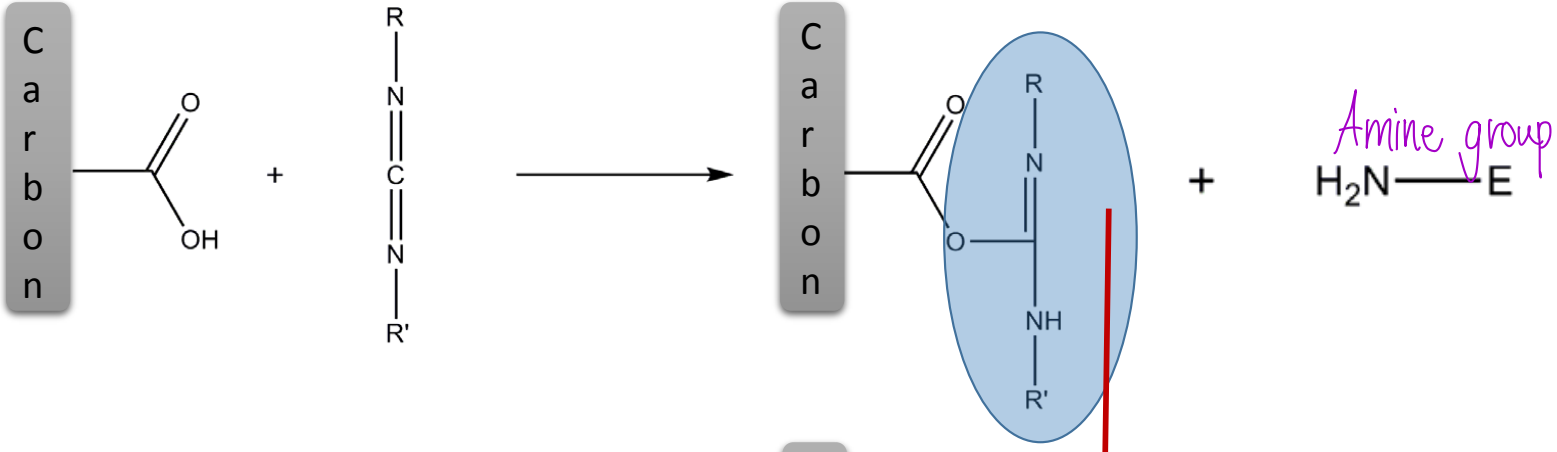
› A carbodiimide is a compound containing a –N=C=N- group
- They are used to attach amine-containing compounds (proteins!) to acid groups
› Allows for functionalisation of transducer
- Note: not all amino acids are suitable



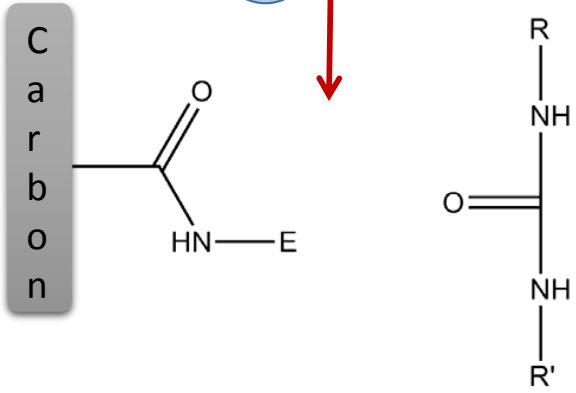
Brain storming

What is an activated ester?

- An ester that contains a **good leaving group**
- The ester is now more susceptible to further reaction



In the absence of an activating agent such as a carbodiimide, carboxylic acids and amines may only form a salt rather than an amide



E-Glucose Oxidase

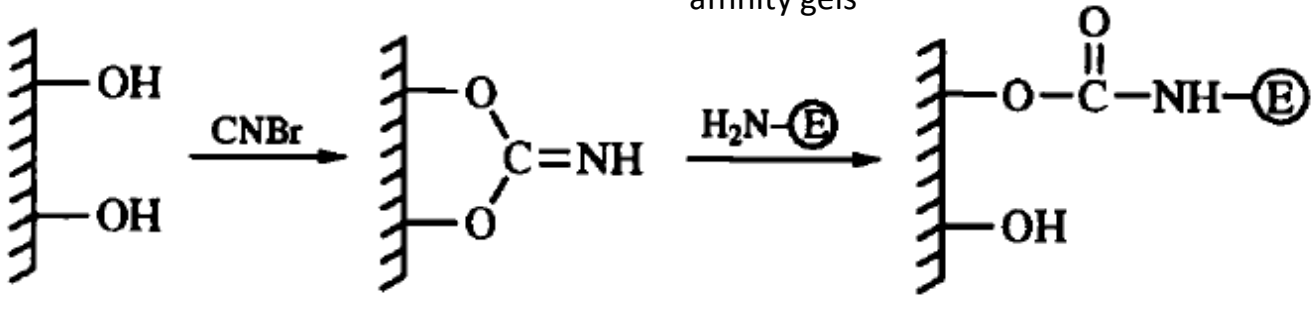
Covalent Bonding

Some functional groups, which are not essential for the catalytic activity of an enzyme, can be covalently bonded to the support matrix (transducer or membrane).

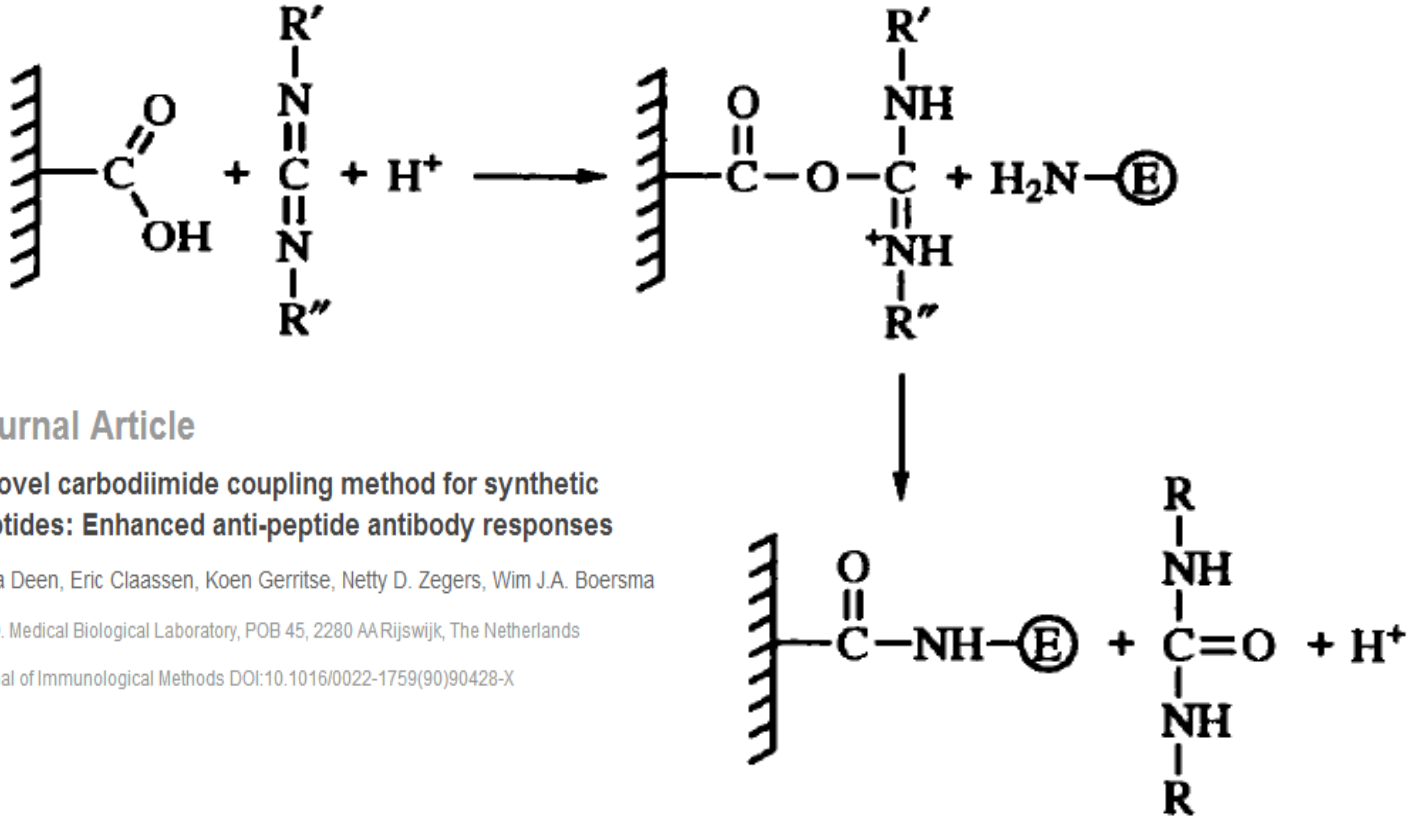
This method uses nucleophilic groups for coupling, such as NH_2, $C0_2H$, OH, C_6H_40H and SH, as well as imidazole.

1. (Simple, mild pH)
2. The most common method for preparing affinity gels

(a) The cyanogen bromide technique



(b) The carbodiimide method



Journal Article

A novel carbodiimide coupling method for synthetic peptides: Enhanced anti-peptide antibody responses

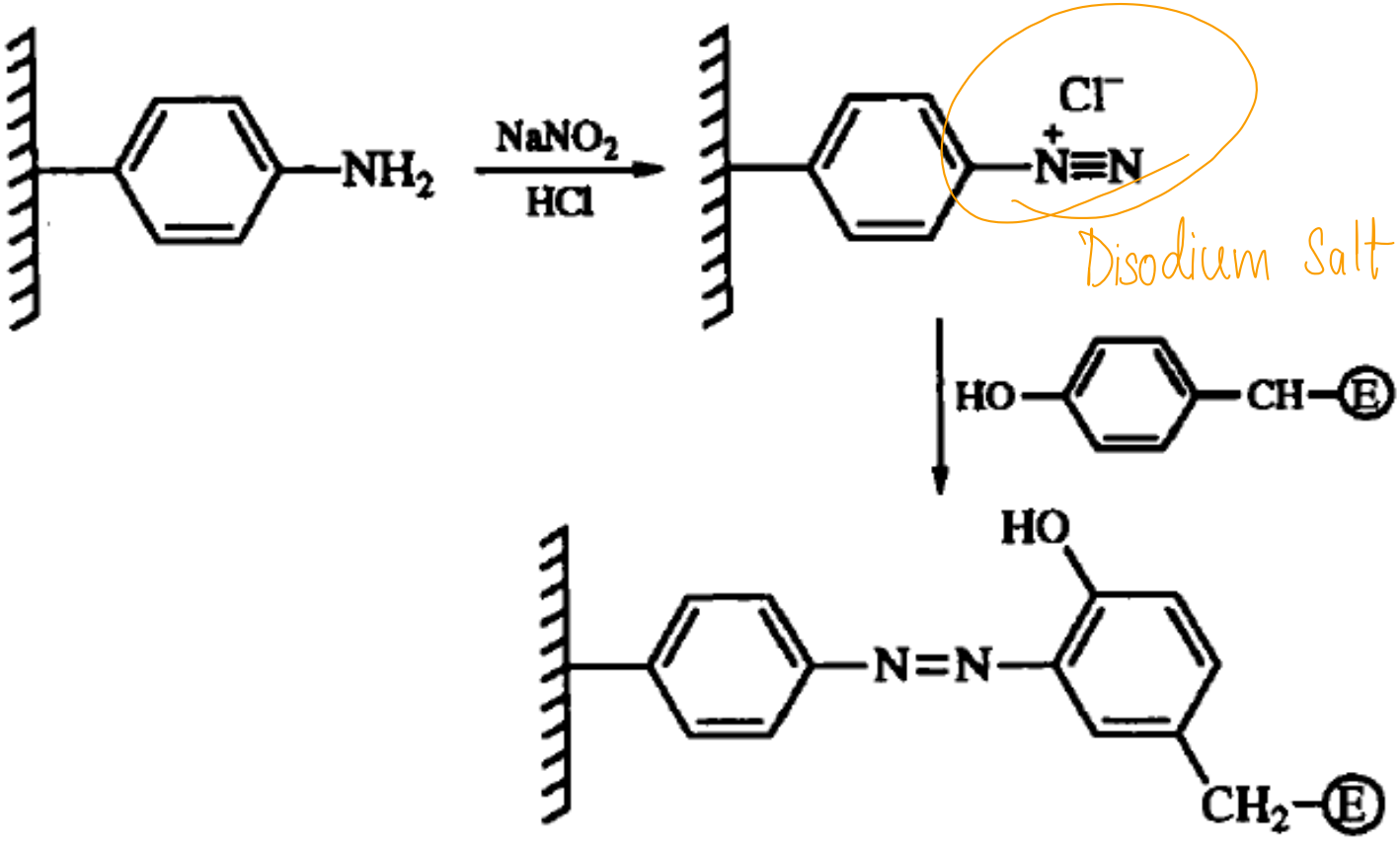
Carla Deen, Eric Claassen, Koen Gerritse, Netty D. Zegers, Wim J.A. Boersma

T.N.O. Medical Biological Laboratory, POB 45, 2280 AA Rijswijk, The Netherlands

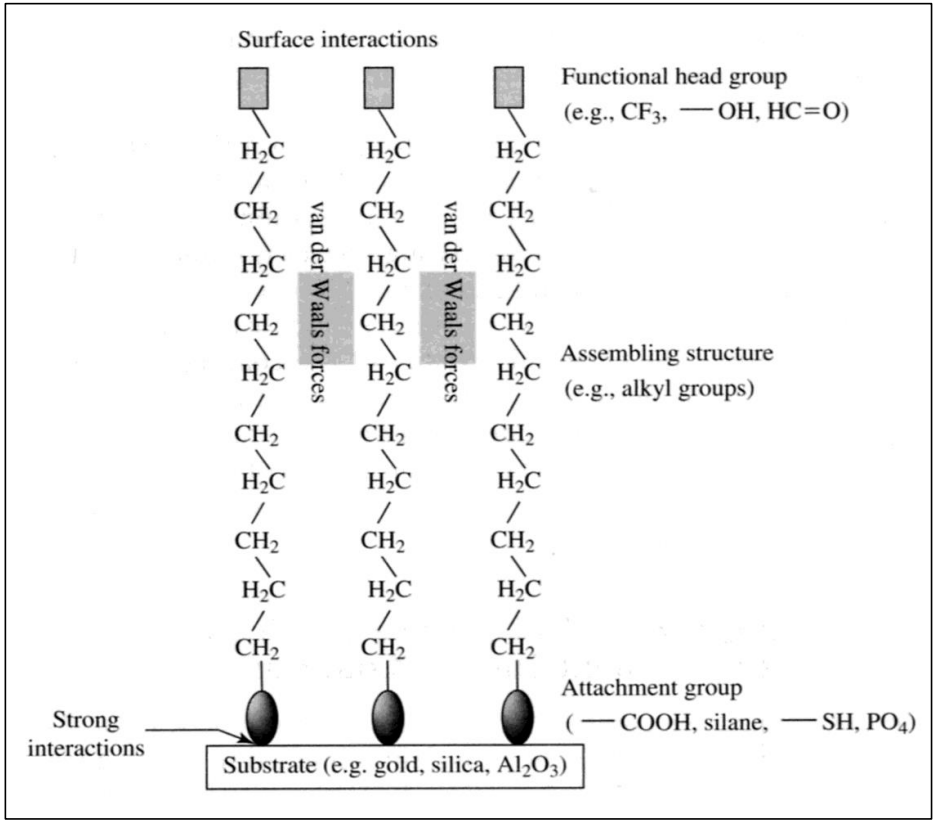
Journal of Immunological Methods DOI:10.1016/0022-1759(90)90428-X

Carbodiimides like EDC crosslink -COOH groups to hydrazides or amines. They are zero length crosslinkers that do not incorporate a cross-bridge when linking.

(e) Coupling through diazonium groups from aromatic amino groups

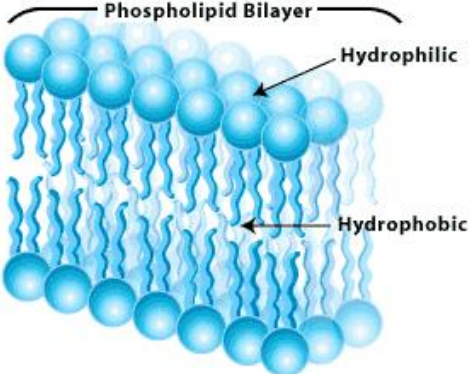


Self-Assembled Monolayers (SAMs)



SAMs are ***amphiphilic***, having both hydrophilic (polar) and hydrophobic (nonpolar) parts. They are made up of 3 parts:

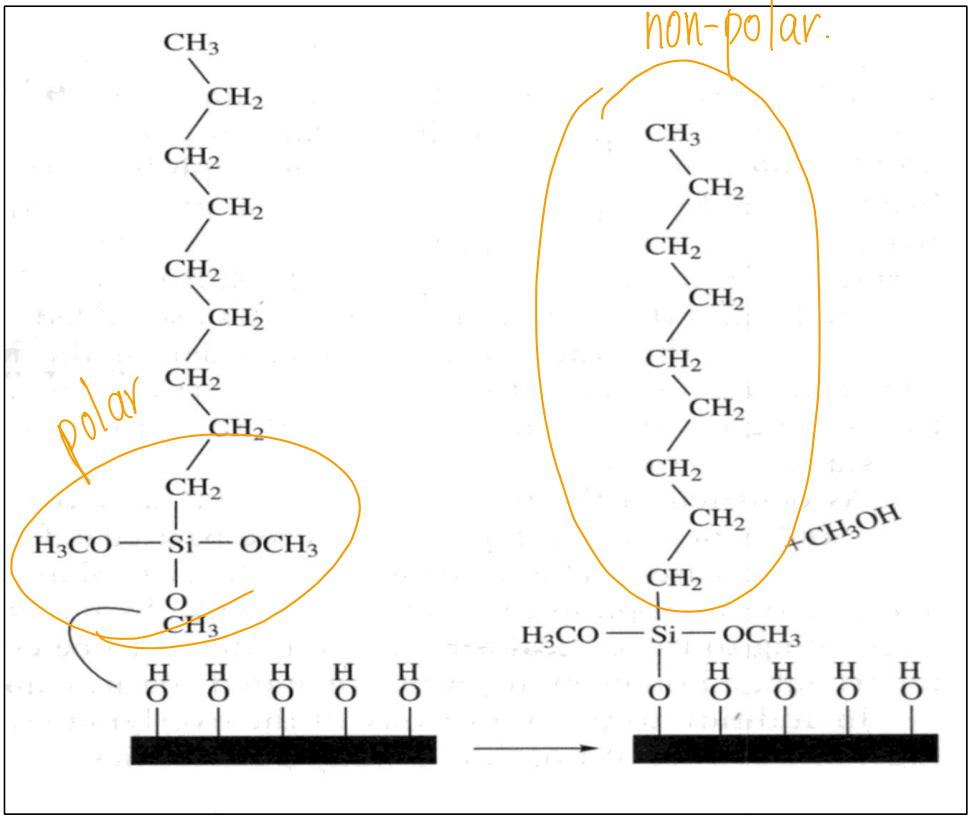
- The attachment group
- A long hydrocarbon chain
- The functional (polar) head group



A phospholipid bilayer with hydrophobic and hydrophilic sections indicated.

In the picture, hydroxyl groups form a strong attachment to the substrate.

A strong exothermic reaction attaches the Silane to the OH



Immobilization

- Molecules may be immobilized either passively through;
  - Hydrophobic
  - Ionic interactions
  - Covalently by attachment to activated surface groups.
- Noncovalent surfaces are effective for many applications; however, passive adsorption fails in many cases.
- Covalent immobilization is often necessary for binding of molecules that do not adsorb, adsorb very weakly, or adsorb with improper orientation and conformation to noncovalent surfaces.
- Covalent immobilization may result in better biomolecule activity, reduced nonspecific adsorption, and greater stability.

Immobilization

- The immobilization process should occur selectively in the presence of common functional groups, including amines, thiols, carboxylic acids, and alcohols.

Amino-**NH2**,
Carboxy-**COOH**,
Aldehyde-**CHO**,
Thiol-**SH**,
Hydroxyl-**OH**

Immobilization

- Immobilization reaction should have several characteristics;

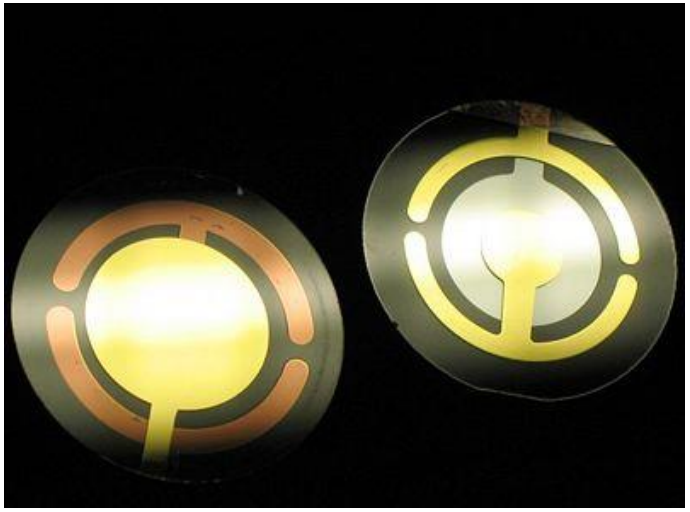
1. Firstly, the reaction should occur rapidly and therefore allow the use of low concentrations of reagents for immobilization.

2. The chemistry should require little, if any, post-synthetic modification of ligands before immobilization to maximize the number of compounds that can be generated by solution or solid-phase synthesis and minimize the cost of these reagents.

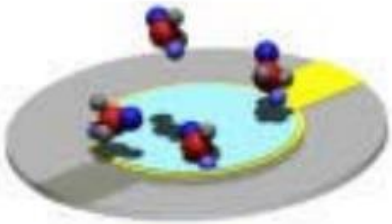
3. Immobilized ligands must be in an oriented and homogeneous manner.

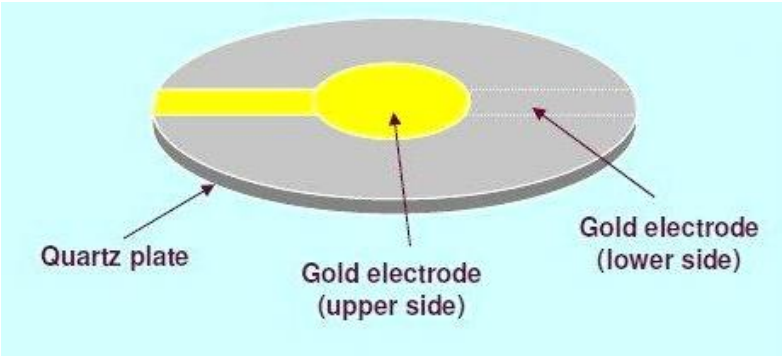
Preparation of Surface for Biomolecule Immobilization

- Modification of the gold electrode surface to create functional groups.
- Modification of biomolecules for covalent attachment to the surface.



Gold electrode surface



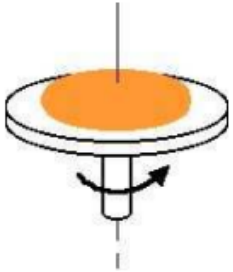


Preparation of Surface for Biomolecule Immobilization

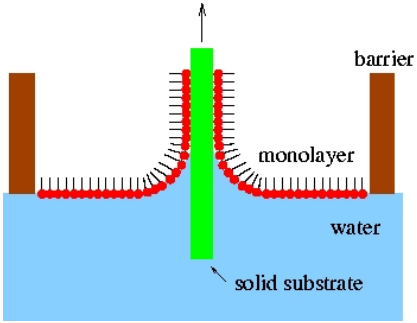
- Cleaning the surface electrode
- Modification of the surface to create functional groups by:

➢ Polymer coating:

- Glow discharge
- Dipping
- Spin coating
- Electrochemical deposition

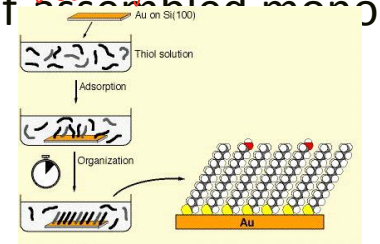


➢ Langmuir & Langmuir-Blodgett films



➢ Chemical modification

- Self assembled monolayers (SAMs)



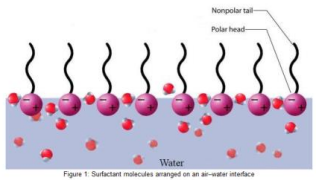
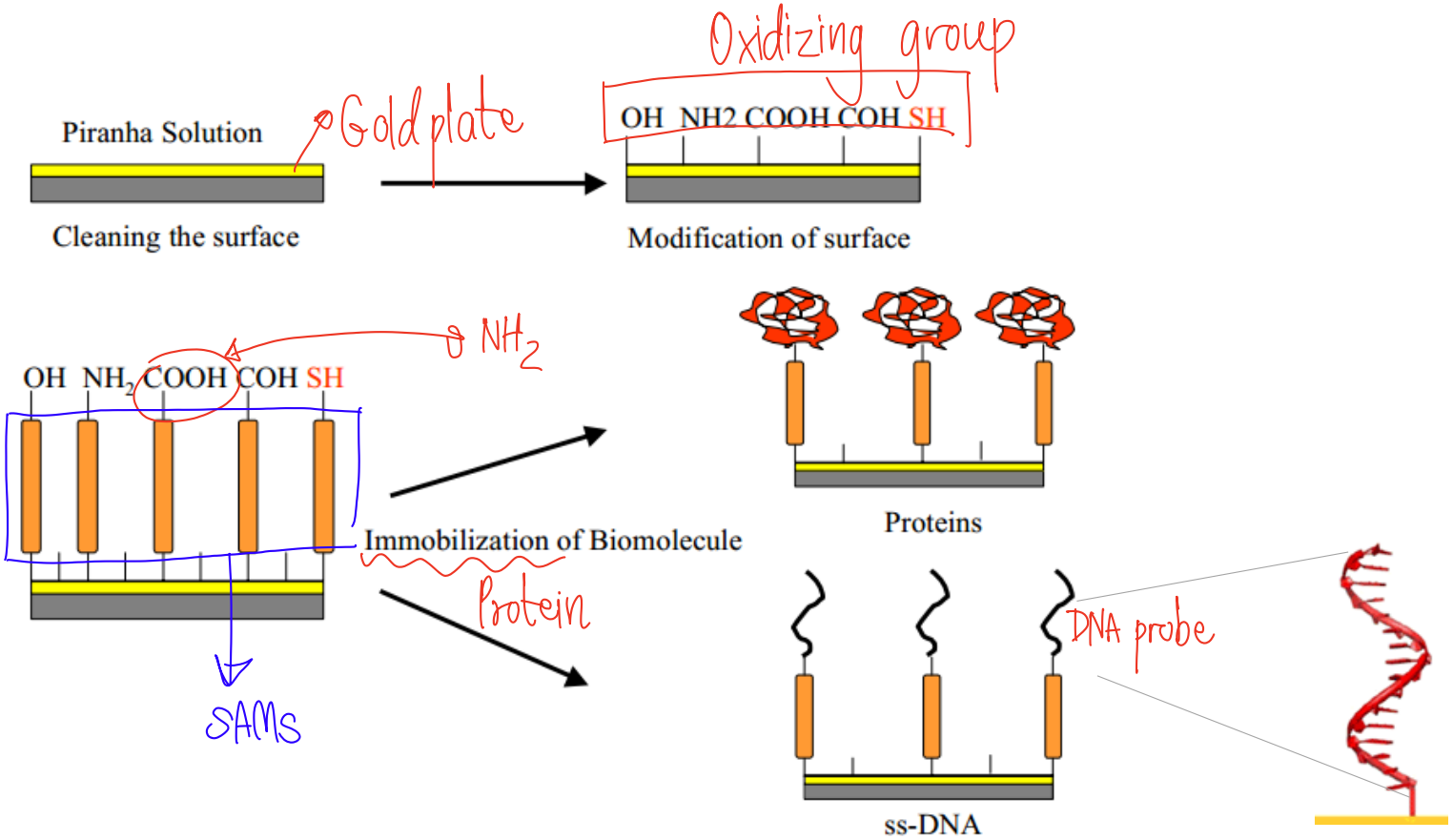
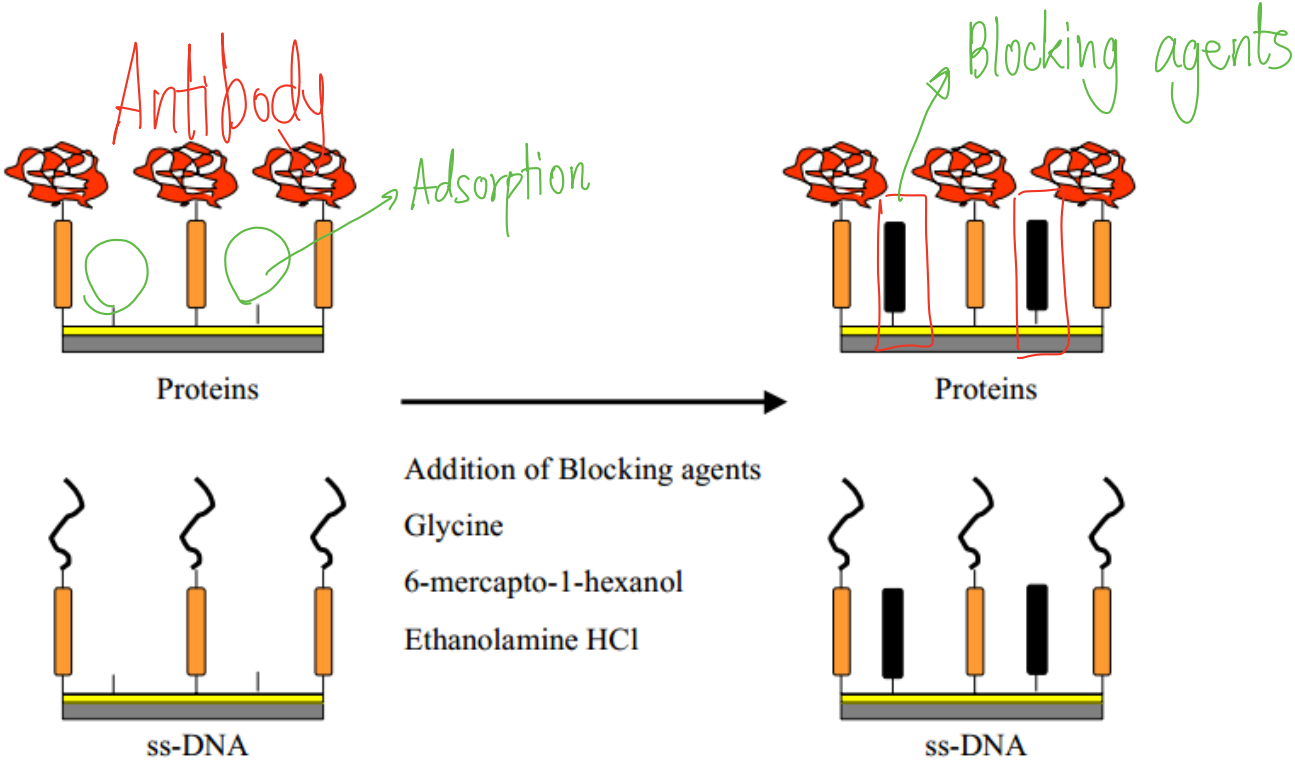


Figure 1: Surfactant molecules arranged on an air–water interface

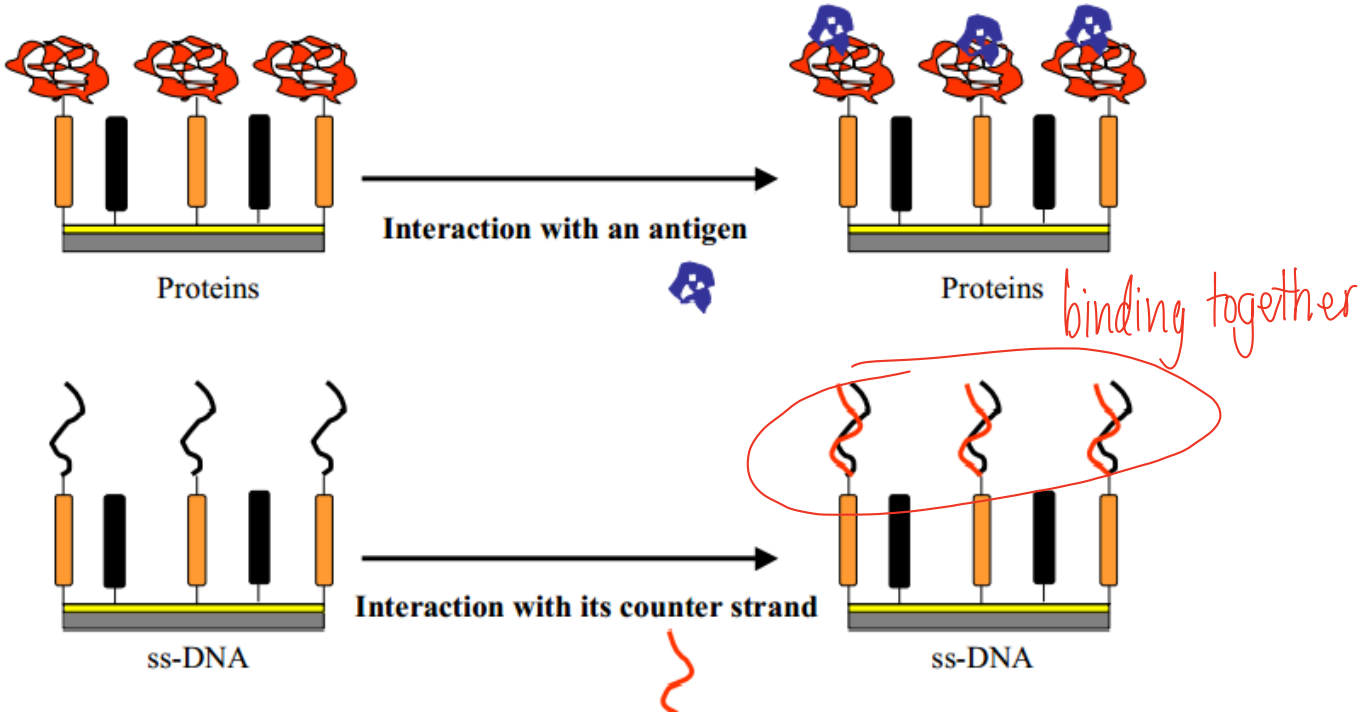
General route for immobilization



General route for immobilization



General route for immobilization



Ionic Binding

Of the enzyme protein to water-insoluble carriers containing ion-exchange residues
(Polysaccharides and synthetic polymers having ion-exchange centers are usually used as carriers)

Advantages : the enzyme to carrier linkages is much stronger for ionic binding

Disadvantages : the binding forces between enzyme proteins and carriers are weaker than those in covalent binding

Cross-linking:

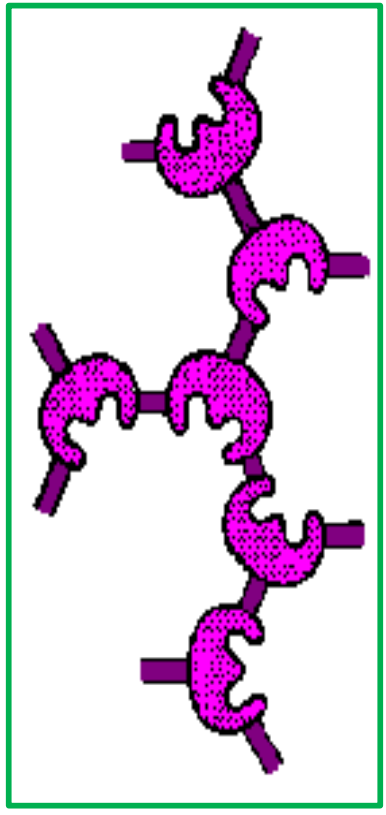
Features: similar to covalent binding.

Either to other protein molecules or to functional groups on an insoluble support matrix

It is used mostly as a means of stabilizing adsorbed enzymes and also for preventing leakage from polyacrylamide gels.

The most common reagent used for cross-linking is Glutaraldehyde

Disadvantages : Cross-linking reactions are carried out under relatively severe conditions. These harsh conditions can change the conformation of active center of the enzyme; and so may lead to significant loss of activity.



Cross-linking

- Immobilization of enzymes by cross-linking with glutaraldehyde or other bifunctional agents such as glyoxal is another well-known approach to develop biosensors
- The enzyme can either be cross-linked with each other or in the presence of a functionally inert protein such as bovine serum albumin.
- Simple and the strong chemical binding can be achieved between biomolecules.
- The main drawback is the possibility of activity losses due to the distortion of the active enzyme conformation and the chemical alterations of the active site during crosslinking

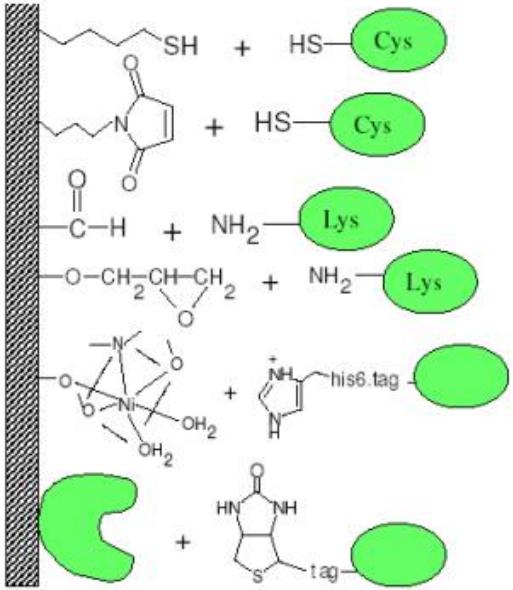
Cross-linking

Cross-linking agents:

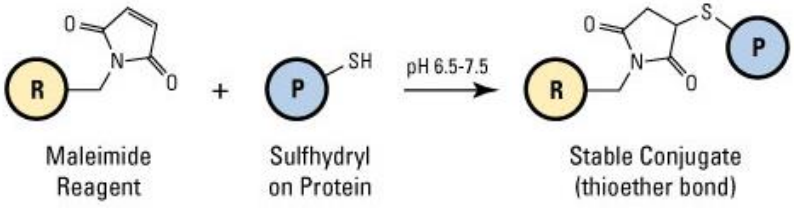
Types	comments
• homo-bifunctional	same active group
• hetero-bifunctional	different active group
• NHS ester	reacts with primary amines
• maleimides	reacts with sulfhydryls (cys)
• water soluble	may be partially memb. soluble
• membrane permeable	may be totally water insol.
• cleavable	usually with thiols (e.g., DTT)
• photo-activated	normally hetero-bifunctional

Crosslinking Strategies for Protein Immobilization

- Thiol surface
- Maleimide surface
- Aldehyde surface
- Epoxy surface
- Nickel Chelate surface
- Streptavidin surface
- Biotin surface



e.g



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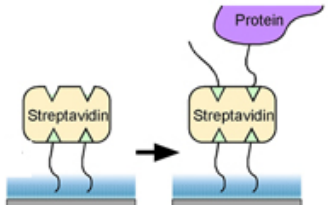

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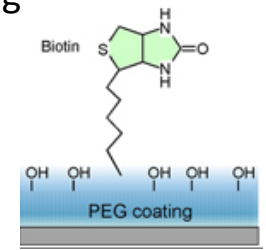
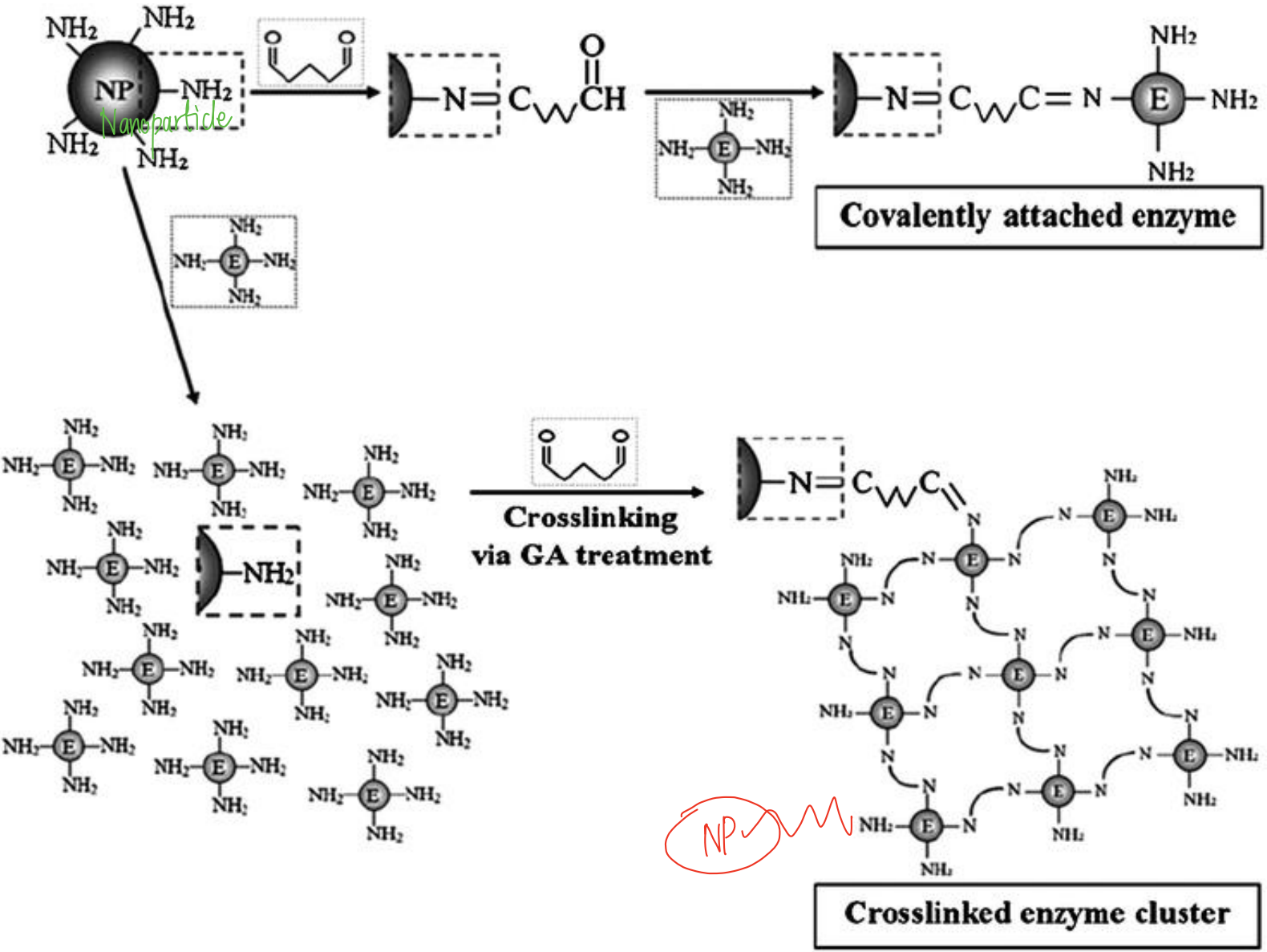



NP
Nanoparticle
Covalently attached enzyme
Crosslinking
via GA treatment
NP
Crosslinked enzyme cluster

Entrapping:

Based on the localization of an enzyme within the lattice of a polymer matrix or membrane

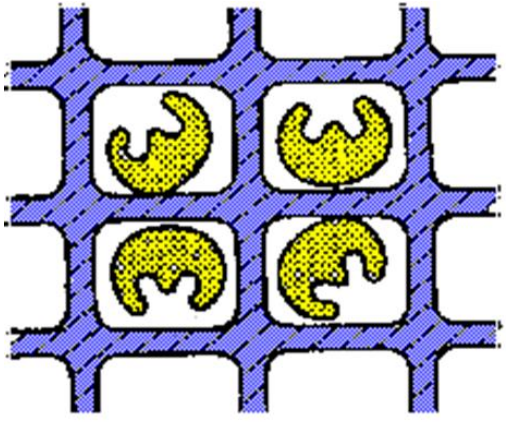
It can be classified into **matrix** and **micro capsule** types.

This method differs from the covalent binding and cross linking in that the enzyme itself does not bind to the gel matrix or membrane. This results in a wide applicability

Disadvantages **:** The conditions used in the chemical polymerization reaction are relatively severe and result in the loss of enzyme activity.

Entrapping:

- Matrix Entrapment



entrapped in a matrix

- Membrane Entrapment (microencapsulation)

entrapped in droplets

Matrix Materials:

Organics: polysaccharides, proteins, carbon, vinyl and allyl polymers, and polyamides. e.g. Ca-alginate, agar, K-carrageenin, collagen

Immobilization procedures:

Enzyme + polymer solution → polymerization
→ extrusion/shape the particles

Inorganics: activated carbon, porous ceramic.

Shapes: particle, membrane, fiber

Entrapment

- Enzymes can be immobilized in 3D matrices such as in

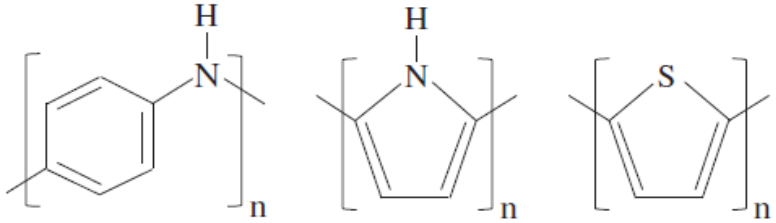
  - Electropolymerized film
  - Poly dimethyl siloxane (PDMS),
  - Photopolymer
  - Silica gel
  - Polysaccharide
  - Carbon paste

Entrapment

- Easy to perform.
- Enzyme, mediators and additives can be simultaneously deposited in the same sensing layer.
- No modification of the biological element so that the activity of the enzyme is preserved during the immobilization process.
- Biosensors based on physically entrapped enzymes are often characterized by increased operational and storage stability.
- However, limitations such as leaching of biocomponent and possible diffusion barriers can restrict the performances of the systems

Entrapment – Electropolymerized Film

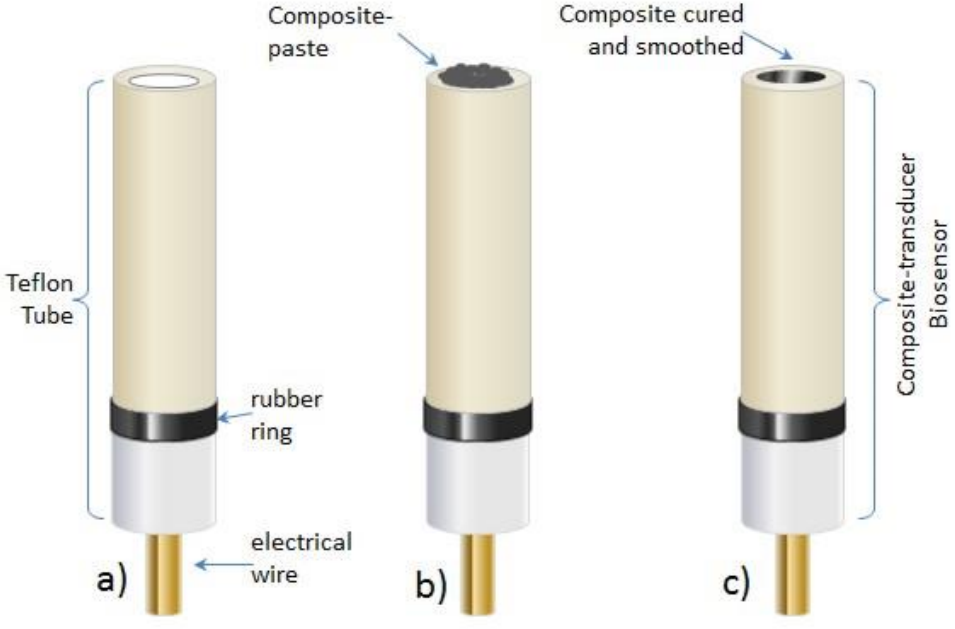
- one-step method consists in applying an appropriate potential or current to the transducer soaked in an aqueous solution containing both enzyme and monomer molecules
- Monomer oxidation gives rise to a radical cation which can either react with a second radical cation or with a neutral monomer in order to obtain a dimer that is then oxidized.
- Finally, a polymer is formed at the electrode surface.
- Enzyme molecules that are present in the immediate vicinity of the electrode surface are physically incorporated within the growing polymer network
- Most are conducting polymers such as polyaniline, polypyrrole or polythiophene



Polyaniline Polypyrrole Polythiophene

Entrapment – in a carbon paste

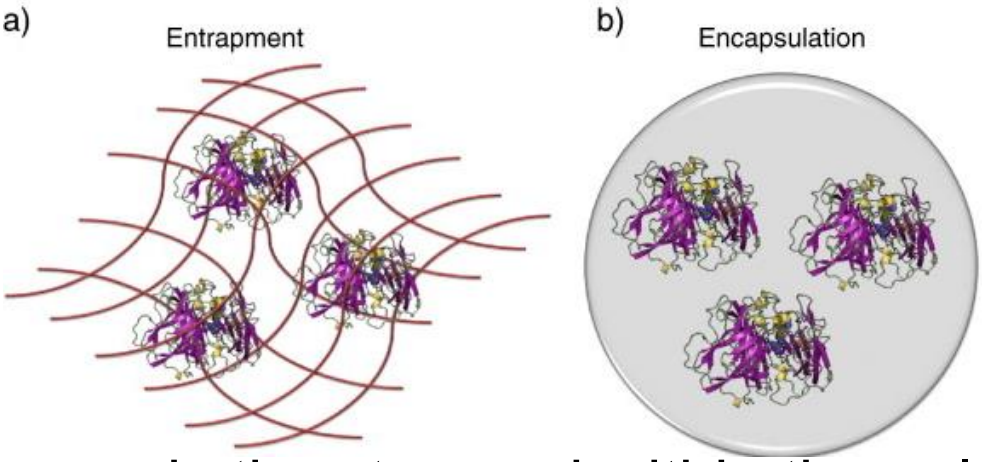
- Carbon paste, a mixture of carbon (graphite) powder and a binder (pasting liquid) is a popular electrodematerial used for the preparation of various electrodes, sensors and detectors



Entrapment – in a carbon paste

- Allows a close or direct contact between incorporated enzymes, mediator and sensing sites permitting a fast electron transfer.
- It is versatile, stable and the surface is easily renewed with good reproducibility.
- Carbon paste electrodes modified with enzyme are prepared by first mixing enzyme solution and graphite powder. Then, the resulting enzymatic powder is mixed with mineral oil (e.g. paraffin).
- The final paste is filled into a plastic cylindrical cartridge

Entrapment



The enzyme is thus trapped within the gel matrix.

The commonly used polymer is polyacrylamide, copolymerization of acrylamide with N,N'-methylenebisacrylamide.

Other materials : starch gels, nylon, silastic gels and conducting polymers (such as polypyrrole).

The problems encountered with this method :

(i) Large barriers are created, thus inhibiting the diffusion of the substrate, which slows down the reaction, and hence the response time of the sensor.

(ii) There is loss of enzyme activity through the pores in the gel, although this may be overcome by cross-linking, e.g. with glutaraldehyde.

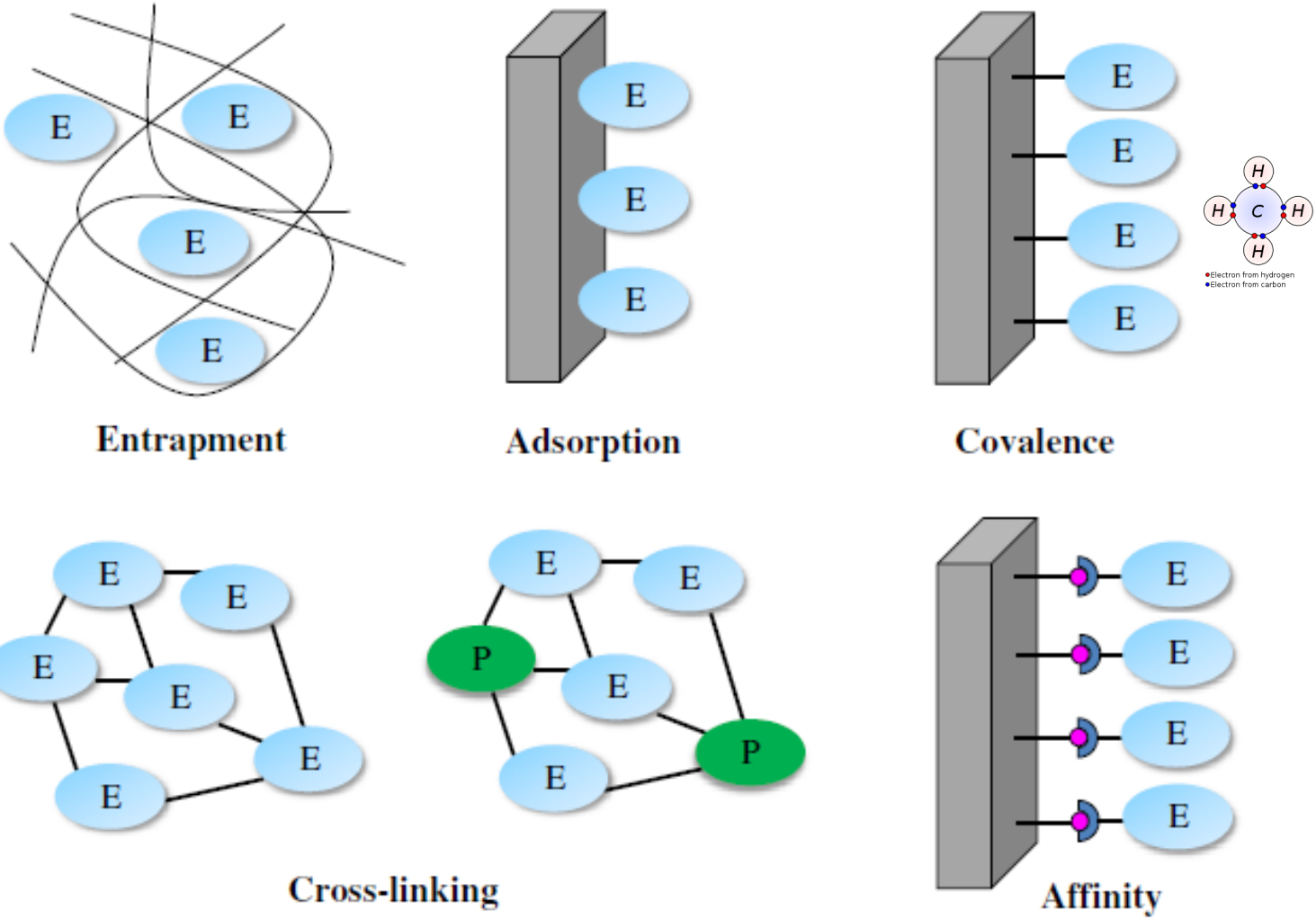
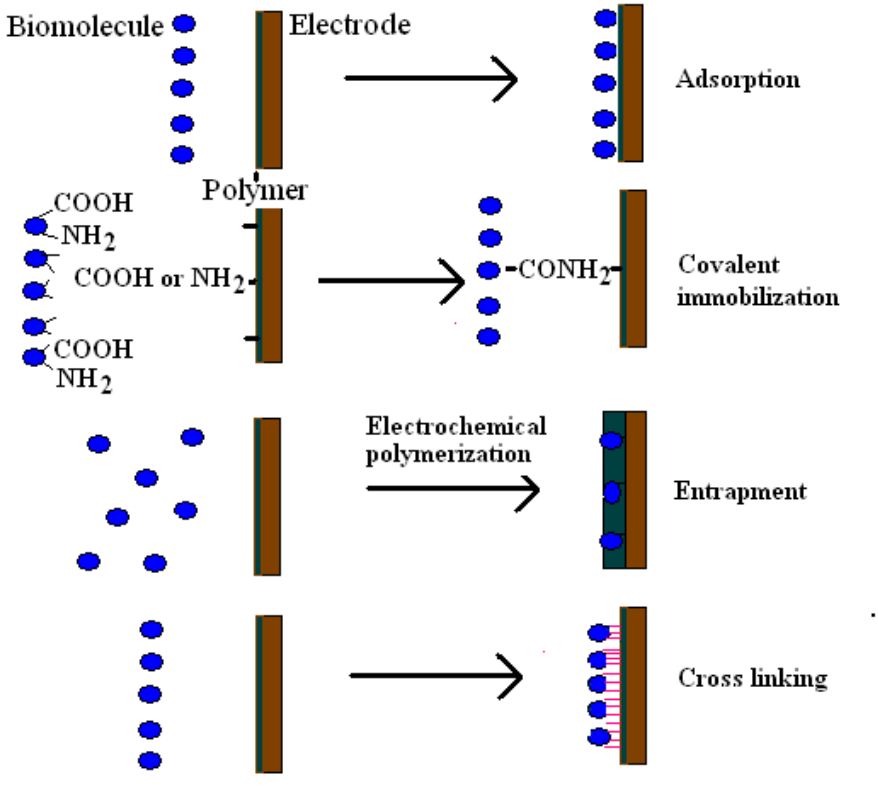


Fig. 2. Schematic representation of the main different methods of enzyme immobilization. E: enzyme, P: inert protein.


Biomolecule
Electrode
Adsorption
Polymer
COOH
NH2
COOH or NH2
COOH
NH2
CONH2
Covalent
immobilization
Electrochemical
polymerization
Entrapment
Cross linking

What is ‘Transducer’?

A transducer is a device that converts one form of energy to another.

Different type of transducer

Electrochemical	Optical	Other
Potentiometric Amperometric Conductometric	Luminescense Ultra Violet/ Visible (UV-VIS) Fiber Optic	Colorimetric Piezoelectric Thermal Acoustic

Electrochemical Transducers

- **Produces an electrical signal that is related to the concentration of an analyte**
- **Biological recognition processes are converted into quantitative amperometric or potentiometric response**

› **Potentiometry**

- Measurement of voltage (cell potential) at zero current

Voltage (potential difference) = measure of the energy of electricity (stored or dynamic)

›**Amperometry**

- Measurement of current at an applied voltage

Current = the flow of electric charge through a medium

› **Conductimetry**

- Measurement of conductance (reciprocal of resistance)

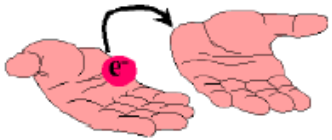
Resistance = measure of opposition to the passage of electric current

Electrochemical Biosensor

Electrochemistry -

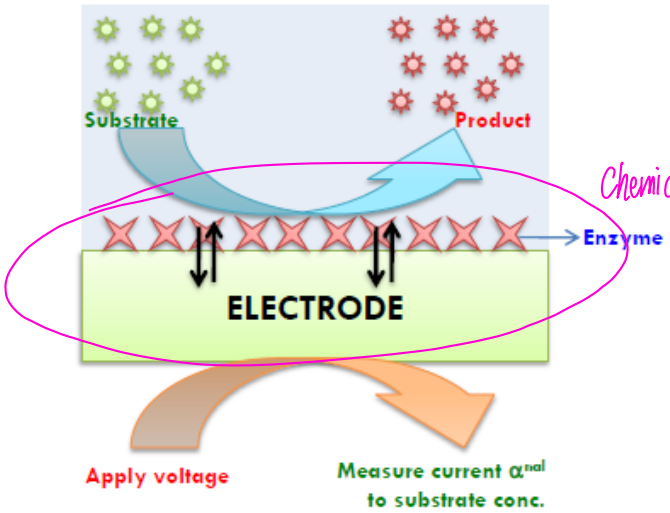
a. branch of chemistry that studies chemical reactions and processes in which electric charges are involved
b. Transfer of an electron from a species in solution to an electrode, or vice versa
c. Common in the analytical field and has resulted in the development of:
   - Potentiometry
   - Voltammetry (amperometry)
   - Coulometry - an electrolysis reaction by measuring the amount of electricity (in coulombs) consumed or produced

Where there is oxidation, there is reduction



Substance oxidized loses electron(s)

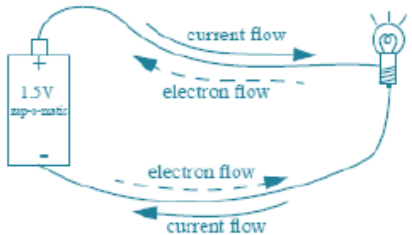
Substance reduced gains electron(s)



Chemical reaction.

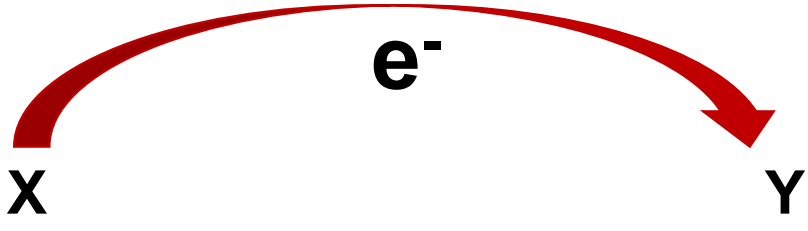
Conventional current flow is opposite to electron flow

eecs.oregonstate.edu/~traylor/ece112/.../elect_flow_vs_conv_I.pdf



Electrochemistry

Electrochemistry **is the study of the relationship between chemical change and electrical work.**



transfer of electrons

X loses electrons	Y gains electrons
X is **oxidised**	Y is **reduced**
X is the **reducing agent**	Y is the **oxidising agent**
X **increases oxidation #**	Y **decreases oxidation #**

Reduction-Oxidation (REDOX)

$$Zn(s) + 2H^{+}(aq) \rightleftharpoons Zn^{2+} + H^{2}(g)$$

Oxidation

- Electrons are lost
- Oxidation number increases
- Reducting agent is oxidised

zinc loses electrons
Zn(O) to Zn(II)
zinc becomes oxidised

Reduction

- Electrons are gained
- Oxidation number decreases
- Oxidising agent is reduced

H+ gains electrons
H(I) to H(O)
H+ becomes reduced

Cell Notation

According to IUPAC† recommendations, oxidation is assumed to occur in the left half-cell and reduction in the right half-cell.

1 cell

Anode

Double

Cathode

$$Zn_{(s)} \mid Zn^{2+}_{(aq)} \parallel Cu^{2+}_{(aq)} \mid Cu_{(s)}$$

Oxidize

reduce

A vertical line represent a phase boundary (e.g.; solid; aqueous) and a double vertical line separates the two half cell reactions

› **Direction of electron movement?**

- Reduction potentials show that Cu^{2+} gains electrons more readily than Zn^{2+} does
- Oxidation occurs at the ANODE while reduction occurs at the CATHODE

$Zn \rightarrow Zn^{2+} + 2e^-$

Stop and think

› What reaction is described by this cell notation?

$$Pb_{(s)} | Pb^{2+}_{(aq)} || Fe^{3+}_{(aq)} | Fe_{(s)}$$

Answer:
- Oxidation on the left, reduction on the right

$Fe3^{+}_{(aq)} + 3e^{-} \rightleftharpoons Fe_{(s)}$

coupled to the oxidation of Zn(s) to $Zn^{2+}_{(aq)}$.

$Pb_{(s)} \rightleftharpoons Pb^{2+}_{(aq)} + 2e^{-}$

Overall, the reaction is ***(don't forget to balance!):***:

$2Fe^{3+}_{(aq)} + 3Pb_{(s)} \rightleftharpoons {}_2Fe_{(s)} + 3Pb^{2+}_{(aq)}$

Electrochemical Cell

› **Galvanic cell:** one type of electrical cell exploits a spontaneous reaction to produce electricity (e.g., a battery).

- Galvanic cells do work by releasing free energy from a spontaneous reaction ($\Delta G^\circ < 0$).

› **Electrolytic cell:** another type of electrochemical cell, uses electrical energy to drive a non-spontaneous reaction ($\Delta G^\circ > 0$).

- Electrolytic cells do work by absorbing free energy from a source of electricity to drive a non-spontaneous reaction (e.g., electroplating) .

Whether an electrical cell releases or consumes free energy, it always involves the movement of electrons from one species to another in a oxidation-reduction (redox) reaction

Electrochemical Cell

If a strip of zinc metal is placed in a beaker of copper(II) ions, the blue colour of the solution fades as a brown-black crust of Cu metal forms on the Zn strip.

The reaction involves the reduction of $Cu^{2+}_{(aq)}$ to $Cu_{(s)}$

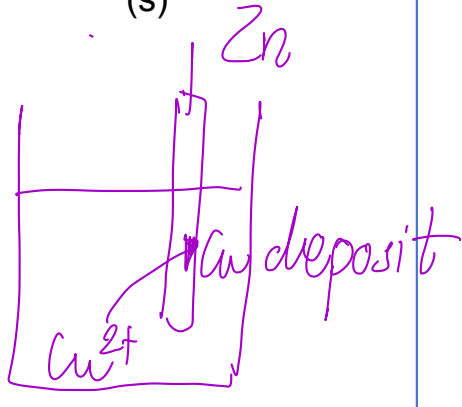
$$Cu^{2+}_{(aq)} + 2e^- \rightleftharpoons Cu_{(s)}$$

coupled to the oxidation of Zn(s) to $Zn^{2+}_{(aq)}$.

$$Zn_{(s)} \rightleftharpoons Zn^{2+}_{(aq)} + 2e^-$$

Overall, the reaction is:

$$Cu^{2+}_{(aq)} + Zn_{(s)} \rightleftharpoons Cu_{(s)} + Zn^{2+}_{(aq)}$$

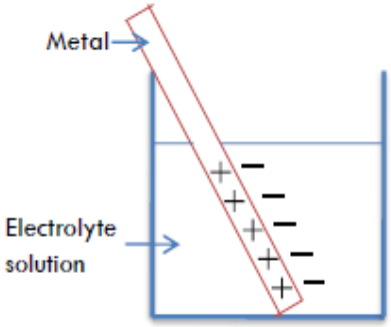


Although electrons are being transferred in the Zn strip in Cu^{2+}solution, the system does not generate electrical energy, as the oxidizing agent (Cu^{2+}) and the reducing agent (Zn) are in contact in the same beaker.

Potentiometry

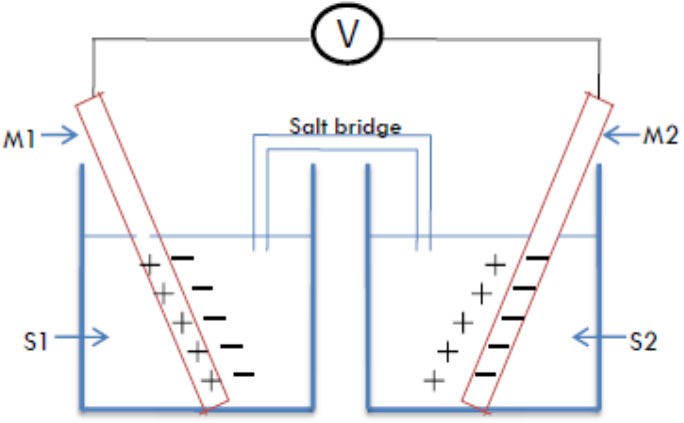
1. Potentiometry - principle

A metal electrode dipped in electrolyte solution (one half cell)



- ✓ If a piece of metal is placed in an electrolyte solution, there is charge separation b/w metal (electrode) and the solution
- ✓ Sets up an electron pressure, usually called a **potential**.
- ✓ It cannot be measured directly - requires a combination of two such electrode-electrolyte solution combinations.
- ✓ Each is called a **half-cell**.

Two half-cell electrodes combined, making a complete cell



- ✓ Two half cells -connected by means of an electrically **conducting bridge or membrane**
- ✓ Two electrodes are connected externally by a potential measuring device (digital voltmeter, DVM).
- ✓ DVM has a very high internal impedance (~$10^{12}\Omega$) - such that very little current will flow through it.
- ✓ If the voltage to be measured is 1V, then the Ohm's law (V=IR), current $I = 10^{-12}$ A (1pA)
- ✓ The electrical circuit is now complete and the e.m.f. of the cell can be measured.
- ✓ This value is the difference between the electrode potentials of the two half-cells.

emf value is the difference between two half cell-electrode and its magnitude depends on

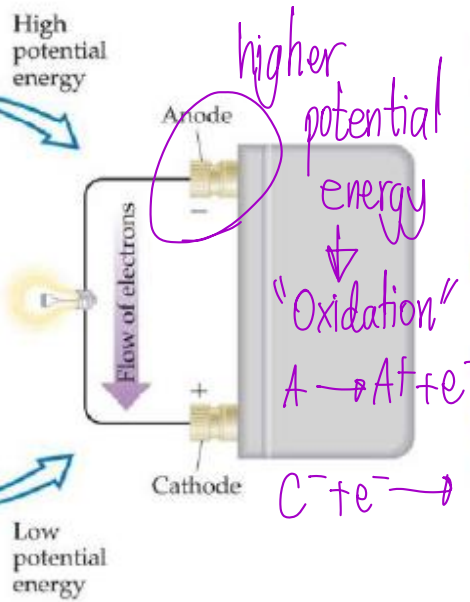
1. **The nature of electrodes**

2. **The nature and concentrations of solutions in each half-cell**

3. **The liquid junction potential across the membrane or salt bridge**

Electromotive force (e.m.f)

- Water only spontaneously flows one way in a waterfall.
- Likewise, electrons only spontaneously flow one way in a redox reaction : from higher to lower potential energy.



The potential difference between the anode and cathode in a cell is called the electromotive force (emf).

It is also called the cell potential, and is designated E_{cell}.

lectromotive force, also called **emf** (denoted and measured in volt), is the voltage eveloped by any source of electrical energy such as a battery or dynamo. It is generally efined as the electrical potential for a source in a circuit.

Ohm's law

To make a current flow through a resistance there must be a voltage across that resistance. Ohm's Law shows the relationship between the voltage (V), current (I) and resistance (R). It can be written in three ways:

$$V=IxR \quad \text{(or)} \quad I=V/R \quad \text{(or)} \quad R=V/I$$

where: **V** = voltage in volts (V)

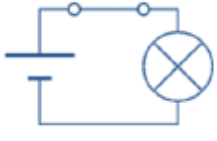
I = current in amps (A)

R = resistance in ohms (Ω)

Voltage can be thought of as the pressure pushing charges along a conductor, while the electrical resistance of a conductor is a measure of how difficult it is to push the charges along.

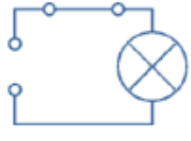
Voltage is the Cause, Current is the Effect

Voltage attempts to make a current flow, and current will flow if the circuit is complete. Voltage is sometimes described as the 'push' or 'force' of the electricity, it isn't really a force but this may help you to imagine what is happening. It is possible to have voltage without current, but current cannot flow without voltage.



Voltage and Current
The switch is closed making a complete circuit so current can flow.

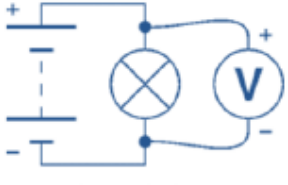
Voltage but No Current
The switch is open so the circuit is broken and current cannot flow.



No Voltage and No Current
Without the cell there is no source of voltage so current cannot flow.

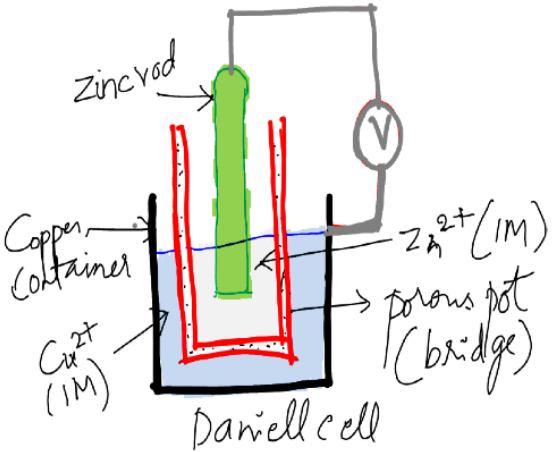
Voltage, V

- Voltage is a measure of the **energy carried by the charge**.
  Strictly: voltage is the "energy per unit charge".
- The proper name for voltage is **potential difference** or p.d. for short, but this term is rarely used in electronics.
- Voltage is **supplied by the battery** (or power supply).
- Voltage is **used up in components**, but not in wires.
- We say **voltage across** a component.
- Voltage is measured in **volts**, V.
- Voltage is measured with a **voltmeter**, connected in **parallel**.
- The symbol **V** is used for voltage in equations.



Connecting a voltmeter in parallel

Daniell cell - an example for potentiometry



Observed electrode volt

E_{obs} = 1.10 V

Gibbs free energy, the energy that can be converted into work at a uniform temperature and pressure throughout a system

✓If we consider each half-cell ->reaction for each half-cell is:

$Cu^{2+} + 2e^- = Cu$ (half-cell electrode reaction 1)

$Zn^{2+} + 2e^- = Zn$ (another reaction 2)

If we subtract reaction equation 2 from 1 we obtain

$$Cu^{2+} + Zn = Cu + Zn^{2+}$$

✓The Gibbs free energy (ΔG) for this reaction is negative (spontaneous in the direction shown).

✓The ΔG is simply related to the e.m.f of the cell:

$$\Delta G = -nFE$$

n-No. of electrons transferred (here n = 2)

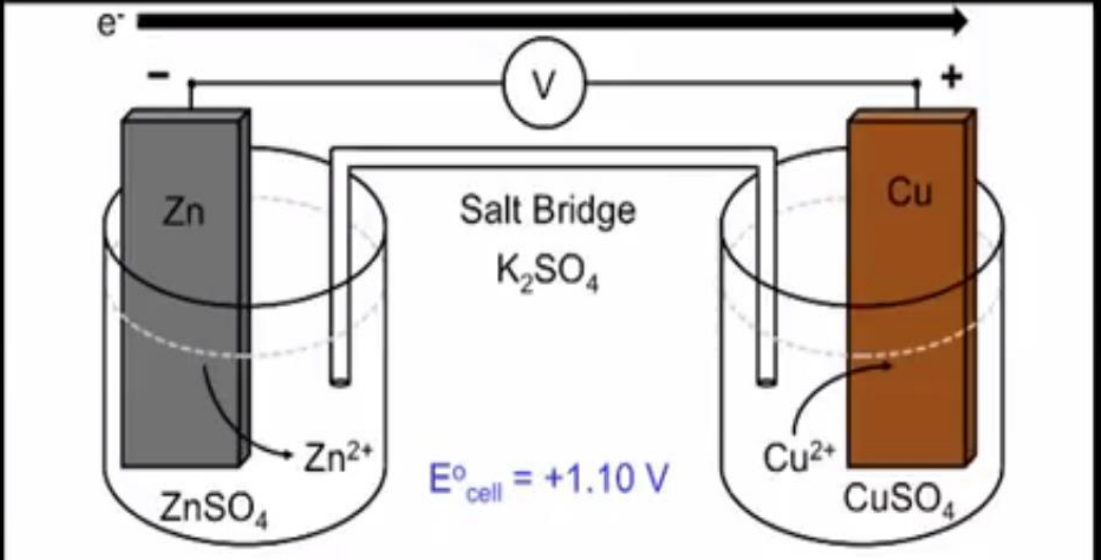
F - is Faraday constant = 96,487 C/mol

E - is the e.m.f of the cell

What the ΔG values are for reactions 1 and 2 separately ?

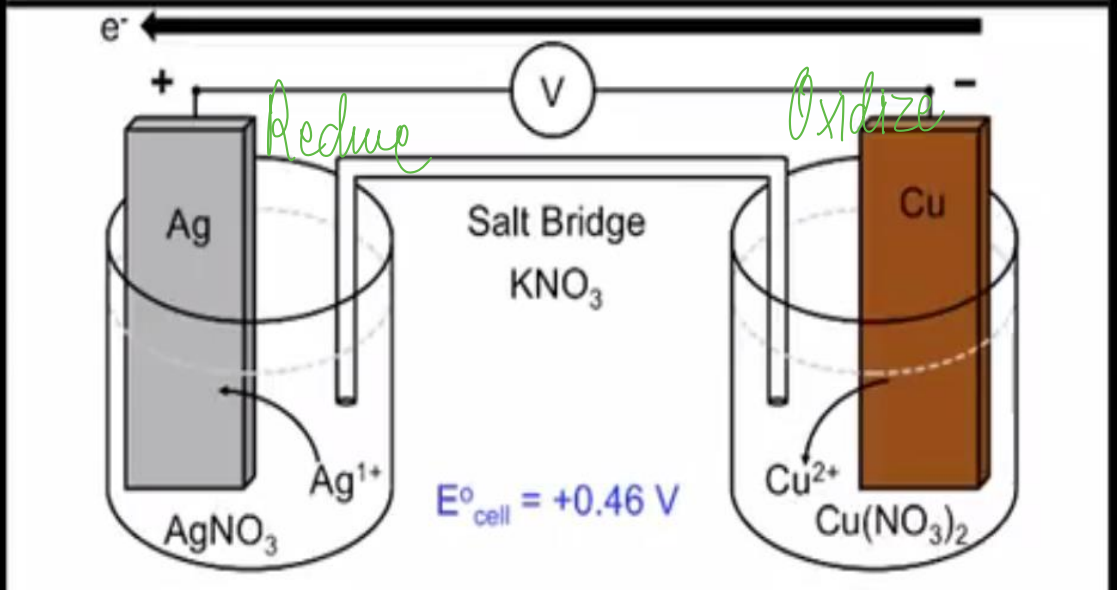
Electrochemistry

A Copper-Zinc Galvanic Cell



Electrochemistry

A Copper-Silver Galvanic Cell

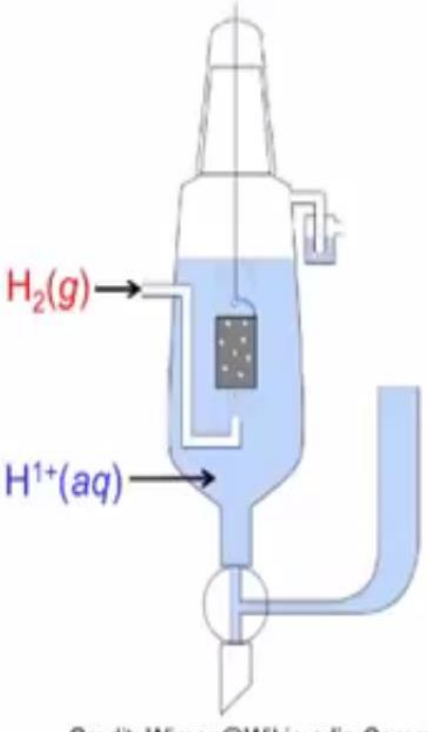


$Ag^{1+}(aq) + 1e^- \longrightarrow Ag(s)$ $\qquad$ $Cu(s) \longrightarrow Cu^{2+}(aq) + 2e^-$

Cathode $\qquad$ $2Ag^{1+}(aq) + Cu(s) \longrightarrow 2Ag(s) + Cu^{2+}(aq)$ $\qquad$ Anode

Electrochemistry

The Standard Hydrogen Electrode



Anode: $H_2(g) \longrightarrow 2H^{1+}(aq) + 2e^-$

Cathode: $2H^{1+}(aq) + 2e^- \longrightarrow H_2(g)$

Credit: Wimox@Wikimedia Commons

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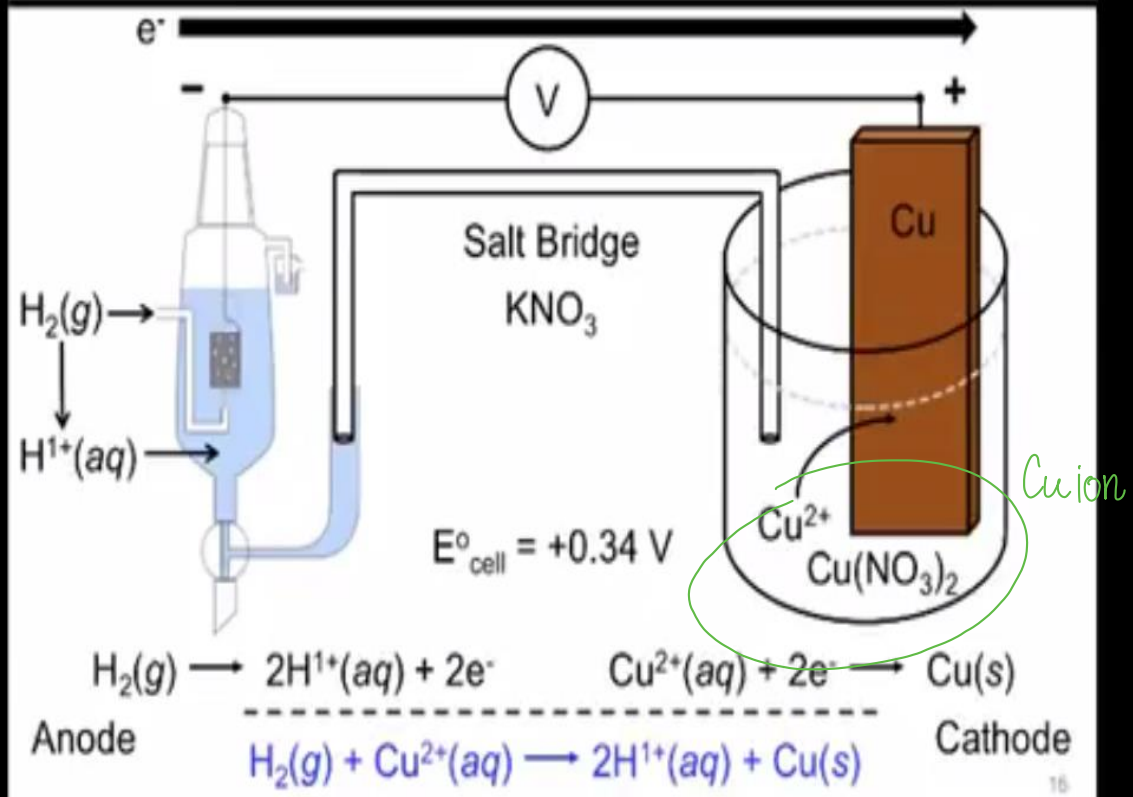
Electrochemistry

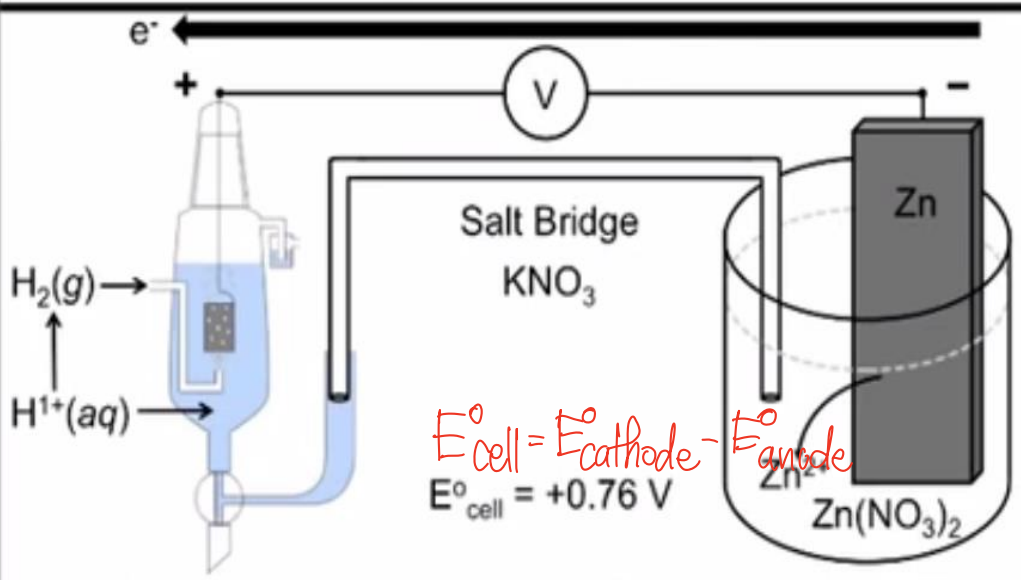
The Standard Hydrogen Electrode



Cu ion

Electrochemistry

The Standard Hydrogen Electrode



$E^\circ_{cell} = E^\circ_{cathode} - E^\circ_{anode}$

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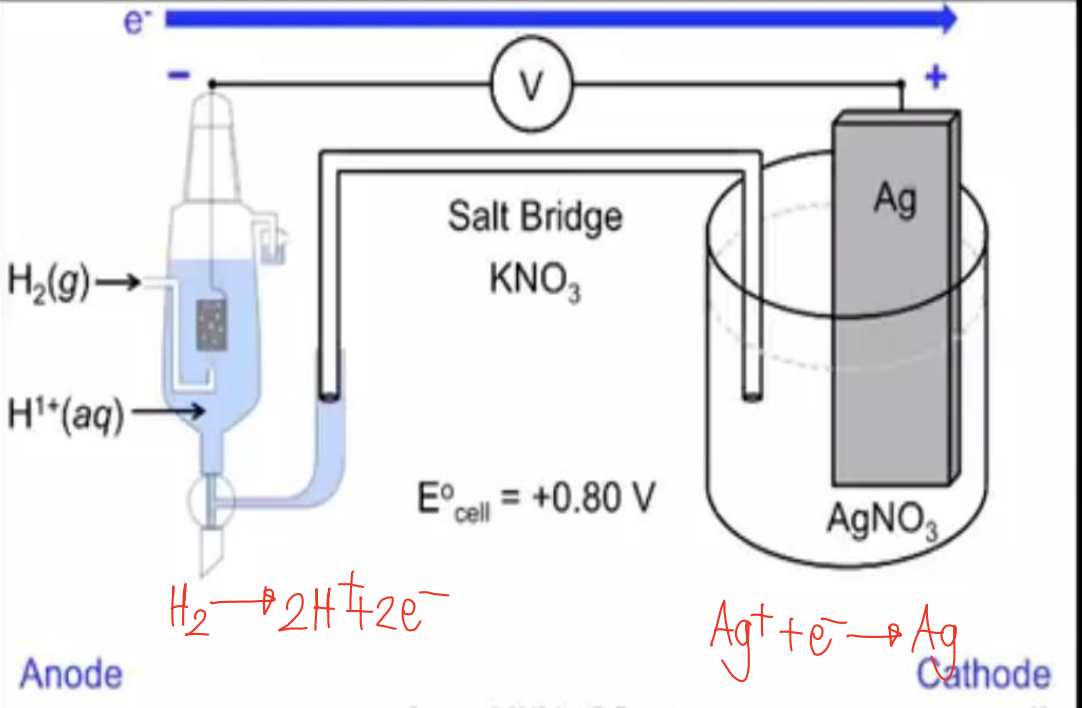
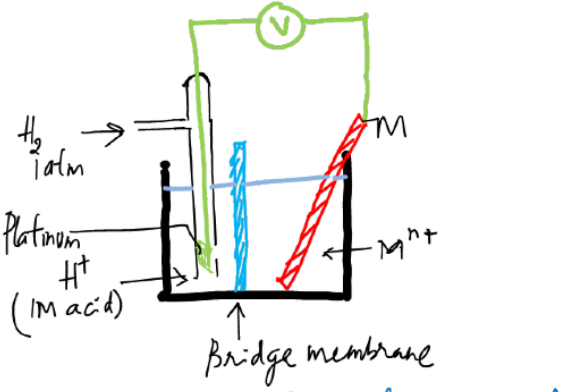
Electrochemistry

The Standard Hydrogen Electrode



Hydrogen electrode (separate measurement of std. **electrode potential** of one half-cell)



Hydrogen electrode connected to another half-cell

- If ΔG_1 and ΔG_2, we could find E_1 and E_2 separately.
- Hydrogen electrode provides separation of E_1 and E_2.

Hydrogen is not a metal but it can be oxidized to H^+ by the removal of an electron:

$$H - e^- = H^+$$

Also written as : $H^+ + e^- = \frac{1}{2}H_2$

ΔG for this reaction is ZERO

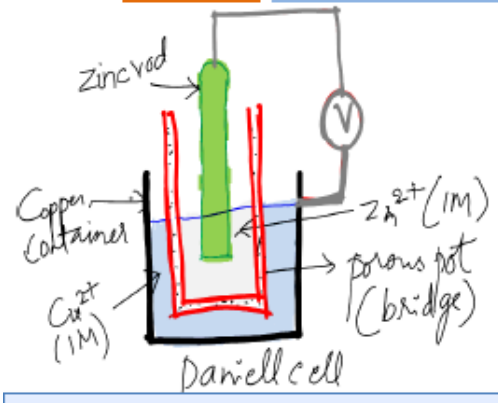
The std. state being with $[H^+]$ - 1M, partial pressure of H_2=1 and temp = 298 K (25 °C).

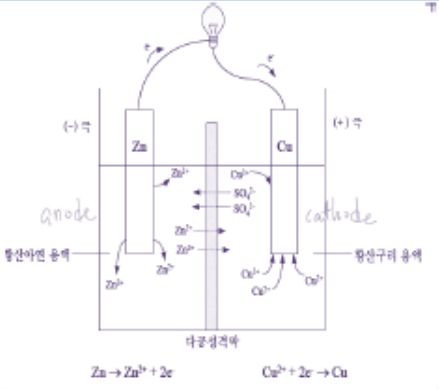
The Gibbs free energy is designated ΔG^0

The std. electrode potential for hydrogen is therefore:

$$E^0_{H^+/H2} = 0$$

Half-cell Potential (single electrode)





Practical- half-cell of **hydrogen** electrode: *this can be combined with any other half-cell electrode*

Eg., $Cu_2^+ + 2e^- = Cu$ E_1 3

$2H^+ + 2e^- = H_2$ $E_H\ (=0)$ 4

Subtracting eqn. 4 from eqn. 3:

$Cu_2^+ + H_2 = Cu + 2H^+$

Thus $E_{cell} = E_1 - E_H = +0.34$ V

Therefore, $E^0_{Cu} = +0.34$ V (one half)

The other half of the Daniel Cell, the **zinc** electrode:

$Zn_2^+ + 2e^- = Zn$ E_{Zn} 5

$2H^+ + 2e^- = H_2$ $E_H\ (=0)$ 6

Subtracting eqn. 6 from eqn. 5:

$Zn_2^+ + H_2 = Zn + 2H^+$

Thus $E_{cell} = E_{Zn} - E_H = -0.76$ V

Therefore, $E^0_{Zn} = -0.76$ V (other half)

Combining two half-cell e.m.f.s for copper and zinc gives the cell e.m.f. for the Daniel cell

$E_{cell} = +0.34 - (-0.76) = \mathbf{1.10\ V}$

Standard Reduction Potentials

Standard potentials are ALWAYS written for the reduction half reaction

$$Ox + ne^- \rightleftharpoons RED$$

Standard Potential, E^o, (V)	Reduction half-reaction
0.34	$Cu^{2+}_{(aq)} + 2e^- \rightarrow Cu_{(s)}$
0	$H^+_{(aq)} + e^- \rightarrow \frac{1}{2} H_{2(g)}$
−0.44	$Fe^{2+}_{(aq)} + 2e^- \rightarrow Fe_{(s)}$

The reference potential for standard electrode potentials, E^o, is the standard hydrogen electrode (SHE): $H^+ + e^- \rightleftharpoons \frac{1}{2}H_2$

Electrode Potentials – Worked Example

- The total cell potential is: $E^0_{total} = E^0_{cathode} - E^0_{anode}$
- Let's look at the Daniell Cell:

Cathode: $Cu^{2+}_{(aq)} + 2e^- \rightleftharpoons Cu_{(s)}$ $E^0 = 0.34$ V

Anode: $Zn_{(s)} \rightleftharpoons Zn^{2+}_{(aq)} + 2e^-$ $E^0 = -0.76$ V

The MINUS sign is because the reaction at the anode is an OXIDATION reaction

- For this reaction: $E^0_{total} = 0.34 - (-0.76) = 1.1$ V

- This reaction is spontaneous
  - Spontaneous reactions always have a POSITIVE CELL POTENITAL
  - Spontaneous reactions always ALSO have a NEGATIVE ΔG°

- Therefore, $\Delta G^\circ \propto -E^0_{cell}$

Measure of 'available' energy at constant temperature/pressure

Cell Potential

Cell potential is measured in volts (V).

$$1\ V = 1\frac{J}{C}$$

Standard Cell Potentials - The cell potential at standard conditions can be found through this equation:

$$E^\circ_{cell} = E^\circ_{red}\ (cathode) - E^\circ_{red}\ (anode)$$

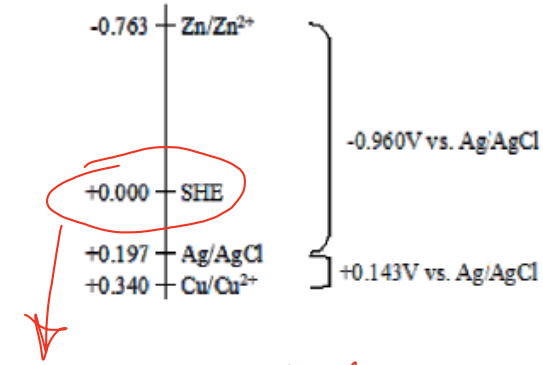
Because cell potential is based on the potential energy per unit of charge, it is an intensive property.

e.m.f (V) = Work/energy (Joule, J)/Charge (Columb, C)

$$E^\circ_{cell} = E^\circ_{red}\ (cathode) - E^\circ_{red}\ (anode)$$
$$= +0.34\ V - (-0.76\ V)$$
$$= +1.10\ V$$

		E°
	$Cu^{2+} + 2e^- \rightleftharpoons Cu(s)$	+0.340
−	$Zn^{2+} + 2e^- \rightleftharpoons Zn(s)$	-0.763
	$Cu^{2+} + Zn(s) \rightleftharpoons Cu(s) + Zn^{2+}$	$E^\circ_{cell} = +1.103\ V$

Standard Electrode Potential (E^0) for few reactions

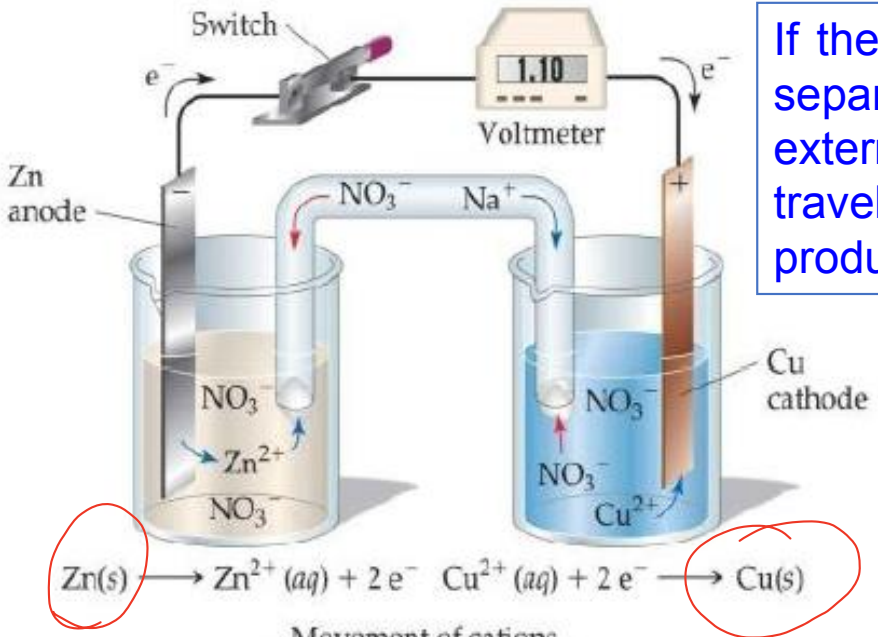


anode

Cathode

Half-reaction	E°, V
$Li^+(aq) + e^- \rightarrow Li(s)$	-3.040
$K^+(aq) + e^- \rightarrow K(s)$	-2.924
$Na^+(aq) + e^- \rightarrow Na(s)$	-2.713
$Al^{3+}(aq) + 3e^- \rightarrow Al(s)$	-1.676
$Zn^{2+}(aq) + 2e^- \rightarrow Zn(s)$	-0.763
$Fe^{2+}(aq) + 2e^- \rightarrow Fe(s)$	-0.440
$2H^+(aq) + 2e^- \rightarrow H_2(g)$	**0.000**
$Cu^{2+}(aq) + e^- \rightarrow Cu(s)$	+0.340
$I_2(s) + 2e \rightarrow 2I^-$	+0.535
$Ag^+(aq) + e^- \rightarrow Ag(s)$	+0.800
$Br_2(l) + 2e^- \rightarrow 2Br^-(aq)$	+1.065
$Cl_2(g) + 2e^- \rightarrow 2Cl^-(aq)$	+1.358
$Au^+ + e^- \rightarrow Au(s)$	+1.680
$F_2(g) + 2e^- \rightarrow 2F^-(aq)$	+2.866

Electron transfer through electrode/solution interface - A schematic representation



Oxidation −0.76V

Reduction. +0.34V

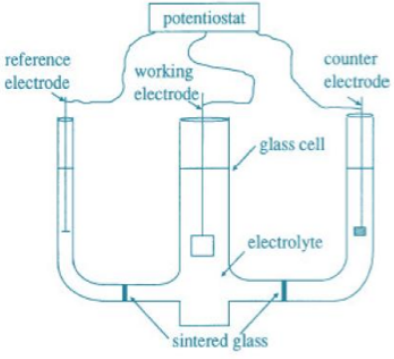
$Zn \rightarrow Zn^{2+} + 2e^-$

$Cu^{2+} + 2e^- \rightarrow Cu$

If the half reactions are physically separated and connected by an external circuit, the electrons try to travel around the circuit, thus producing a voltage.

- **Cu will be deposited on the Cu electrode (mass will increase)**
- **Zn electrode will weigh less, since the Zn metal will be transferred into the solution**.

Three electrode system



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Components of Electrochemical cell

Three electrodes:
Working, Counter, and Reference

An electrolyte solution:
Solvent, supporting

Membrane: O or X

Potentiostat: e^- delivery of, or redox reactions
Involves the transmission of ions

The potential difference between the anode and cathode in a cell is called the electromotive force (emf).

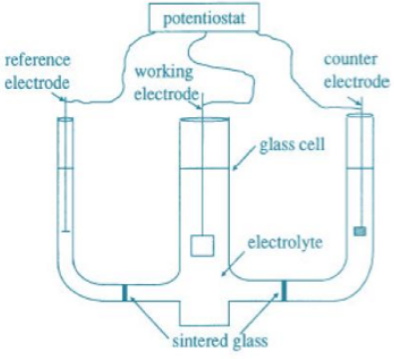
It is also called the cell potential, and is designated E_{cell}.

Standard Reduction Potentials of Half-Cells

(Ionic concentrations are at 1M in water @ 25^0 C)

			E^0 (Volts)
$F_2(g) + 2e^-$	$\rightarrow$	$2F^-(aq)$	+2.87
$PbO_2(s) + SO_4^{2-}(aq) + 4H^+(aq) + 2e^-$	$\rightarrow$	$PbSO_4(s) + 2H_2O(\ell)$	+1.69
$MnO_4^-(aq) + 8H^+(aq) + 5e^-$	$\rightarrow$	$Mn^{2+}(aq) + 4H_2O(\ell)$	+1.51
$Au^{3+}(aq) + 3e^-$	$\rightarrow$	$Au(s)$	+1.50
$ClO_4^-(aq) + 8H^+(aq) + 8e^-$	$\rightarrow$	$Cl^-(aq) + 4H_2O(\ell)$	+1.39
$Cl_2(g) + 2e^-$	$\rightarrow$	$2Cl^-(aq)$	+1.36
$Cr_2O_7^{2-}(aq) + 14H^+(aq) + 6e^-$	$\rightarrow$	$2Cr^{3+}(aq) + 7H_2O(\ell)$	+1.33
$2HNO_2(aq) + 4H^+(aq) + 4e^-$	$\rightarrow$	$N_2O(g) + 3H_2O(\ell)$	+1.30
$O_2(g) + 4H^+(aq) + 4e^-$	$\rightarrow$	$2H_2O(\ell)$	+1.23
$MnO_2(s) + 4H^+(aq) + 2e^-$	$\rightarrow$	$Mn^{2+}(aq) + 2H_2O(\ell)$	+1.22
$Br_2(aq) + 2e^-$	$\rightarrow$	$2Br^-(aq)$	+1.07
$Hg^{2+}(aq) + 2e^-$	$\rightarrow$	$Hg(\ell)$	+0.85
$ClO^-(aq) + H_2O(\ell) + 2e^-$	$\rightarrow$	$Cl^-(aq) + 2OH^-(aq)$	+0.84
$Ag^+(aq) + e^-$	$\rightarrow$	$Ag(s)$	+0.80
$NO_3^-(aq) + 2H^+(aq) + e^-$	$\rightarrow$	$NO_2(g) + H_2O(\ell)$	+0.80
$Fe^{3+}(aq) + e^-$	$\rightarrow$	$Fe^{2+}(aq)$	+0.77
$O_2(g) + 2H^+(aq) + 2e^-$	$\rightarrow$	$H_2O_2(\ell)$	+0.70
$I_2(s) + 2e^-$	$\rightarrow$	$2I^-(aq)$	+0.54
$O_2(g) + 2H_2O(\ell) + 4e^-$	$\rightarrow$	$4OH^-(aq)$	+0.40
$Cu^{2+}(aq) + 2e^-$	$\rightarrow$	$Cu(s)$	+0.34
$SO_4^{2-}(aq) + 4H^+(aq) + 2e^-$	$\rightarrow$	$H_2SO_3(aq) + H_2O(\ell)$	+0.17
$Sn^{4+}(aq) + 2e^-$	$\rightarrow$	$Sn^{2+}(aq)$	+0.15
$S(s) + 2H^+(aq) + 2e^-$	$\rightarrow$	$H_2S(aq)$	+0.14
$AgBr(s) + e^-$	$\rightarrow$	$Ag(s) + Br^-(aq)$	+0.07
$2H^+(aq) + 2e^-$	$\rightarrow$	$H_{2(g)}$	0.00
$Pb^{2+}(aq) + 2e^-$	$\rightarrow$	$Pb(s)$	-0.13
$Sn^{2+}(aq) + 2e^-$	$\rightarrow$	$Sn(s)$	-0.14
$AgI(s) + e^-$	$\rightarrow$	$Ag(s) + I^-(aq)$	-0.15
$Ni^{2+}(aq) + 2e^-$	$\rightarrow$	$Ni(s)$	-0.26
$Co^{2+}(aq) + 2e^-$	$\rightarrow$	$Co(s)$	-0.28
$PbSO_4(s) + 2e^-$	$\rightarrow$	$Pb(s) + SO_4^{2-}(aq)$	-0.36
$Se(s) + 2H^+(aq) + 2e^-$	$\rightarrow$	$H_2Se(aq)$	-0.40
$Cd^{2+}(aq) + 2e^-$	$\rightarrow$	$Cd(s)$	-0.40
$Cr^{3+}(aq) + e^-$	$\rightarrow$	$Cr^{2+}(aq)$	-0.41
$Fe^{2+}(aq) + 2e^-$	$\rightarrow$	$Fe(s)$	-0.45
$NO_2^-(aq) + H_2O(\ell) + e^-$	$\rightarrow$	$NO(g) + 2OH^-(aq)$	-0.46
$Ag_2S(s) + 2e^-$	$\rightarrow$	$2Ag(s) + S^{2-}(aq)$	-0.69
$Zn^{2+}(aq) + 2e^-$	$\rightarrow$	$Zn(s)$	-0.76
$2H_2O(\ell) + 2e^-$	$\rightarrow$	$H_2(g) + 2OH^-(aq)$	-0.83
$Cr^{2+}(aq) + 2e^-$	$\rightarrow$	$Cr(s)$	-0.91
$Se(s) + 2e^-$	$\rightarrow$	$Se^{2-}(aq)$	-0.92
$SO_4^{2-}(aq) + H_2O(\ell) + 2e^-$	$\rightarrow$	$SO_3^{2-}(aq) + 2OH^-(aq)$	-0.93
$Al^{3+}(aq) + 3e^-$	$\rightarrow$	$Al(s)$	-1.66
$Mg^{2+}(aq) + 2e^-$	$\rightarrow$	$Mg(s)$	-2.37
$Na^+(aq) + e^-$	$\rightarrow$	$Na(s)$	-2.71
$Ca^{2+}(aq) + 2e^-$	$\rightarrow$	$Ca(s)$	-2.87
$Ba^{2+}(aq) + 2e^-$	$\rightarrow$	$Ba(s)$	-2.91
$Li^+(aq) + e^-$	$\rightarrow$	$Li(s)$	-3.04

Three electrode system



Schematic of an electrochemical cell

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Components of Electrochemical cell

Three electrodes:
Working, Counter, and Reference

An electrolyte solution:
Solvent, supporting

Membrane: O or X

Potentiostat: e^- delivery of, or redox reactions
Involves the transmission of ions

The potential difference between the anode and cathode in a cell is called the electromotive force (emf).

It is also called the cell potential, and is designated E_{cell}.

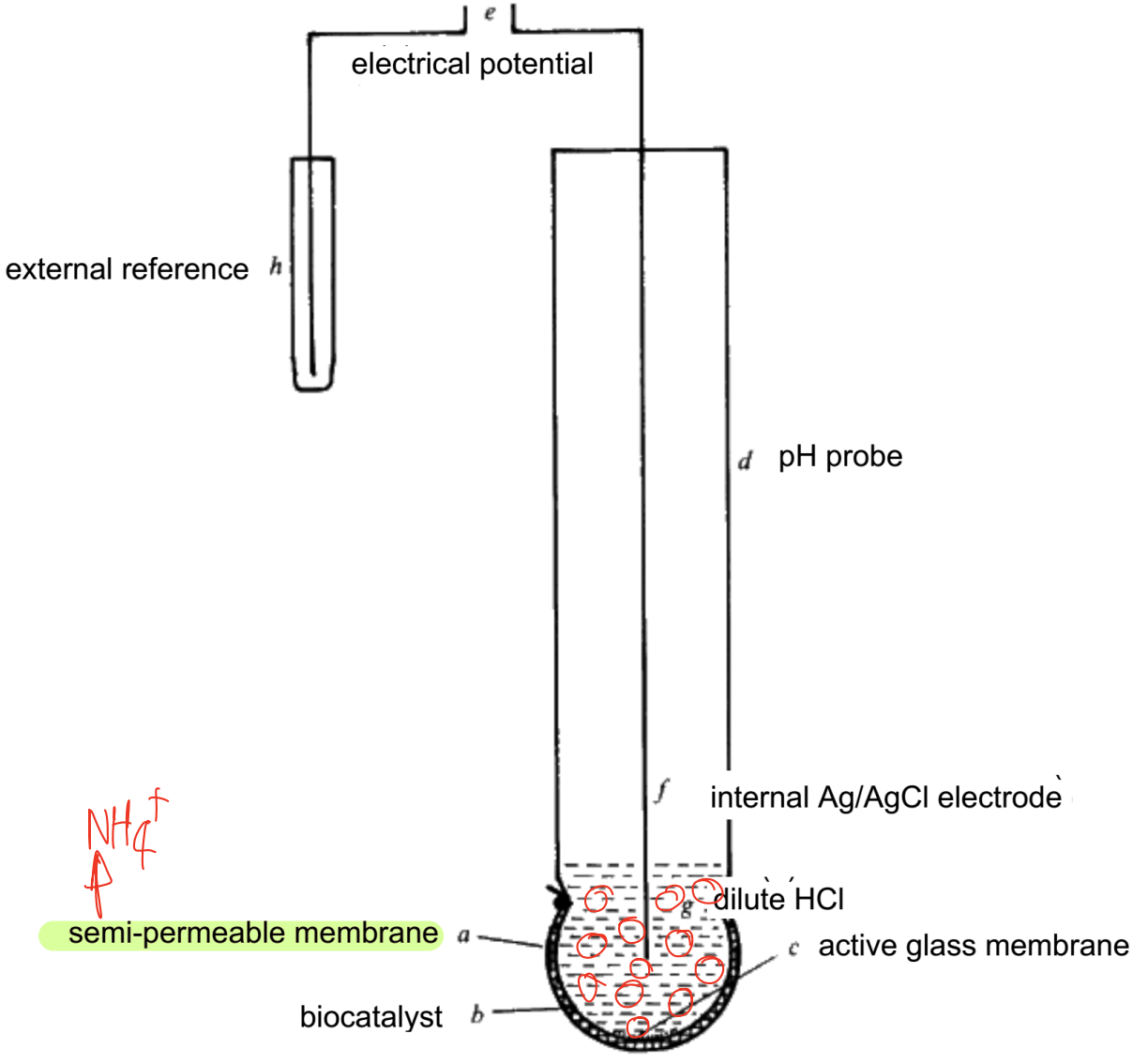
Potentiometer

- A device for measuring the potential of an electrochemical cell without drawing a current or altering the cell's composition.

Potentiometric measurements:

Potentiometric measurements are made using a potentiometer to determine the difference in potential between a working (an indicator) electrode and a counter (a reference) electrode.

- Cathode is the working/indicator electrode. (right half-cell)
- Anode is the counter/reference electrode. (left half-cell)


e
electrical potential
external reference
h
d
pH probe
f
internal Ag/AgCl electrode
NH4+
semi-permeable membrane
a
g
dilute HCl
c
active glass membrane
biocatalyst
b

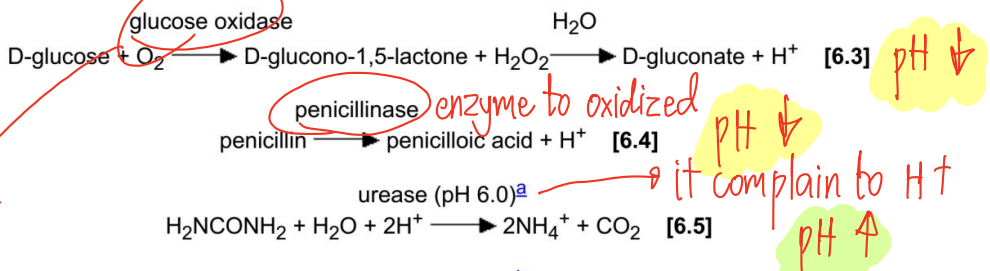
POTENTIOMETRIC BIOSENSORS [I]

- In potentiometric sensors, the zero-current potential (relative to a reference) developed at a selective membrane or electrode surface in contact with a sample solution is related to analyte concentration (logarithmic relationship between measured potential and analyte concentration).
- The main use of potentiometric transducers in biosensors: a pH-stat (the reactions consume or produce protons).
- The biosensor consists of an immobilized enzyme membrane surrounding the probe from a pH-meter:

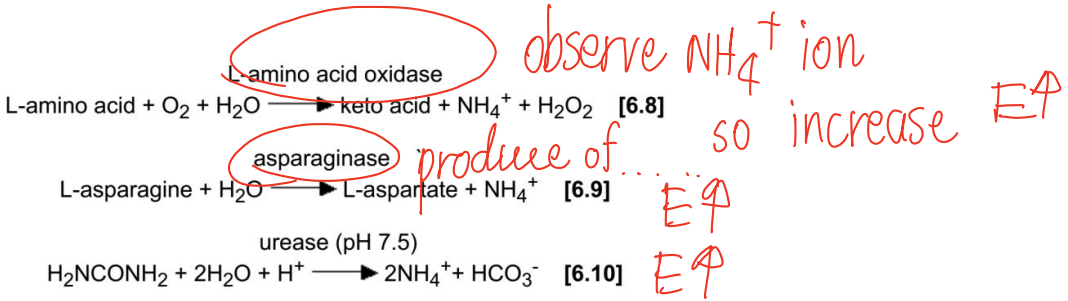
- **Three types of ion-selective electrodes** which are of use in biosensors:

- Glass electrodes for cations (typical potentiometric biosensors)
- Glass pH electrodes coated with a gas-permeable membrane selective for CO_2, NH_3 or H_2S.
- The iodide electrode is useful for the determination of I^- or CN^- in the peroxidase reaction in penicillinase reaction mediated with I^- or CN^- .

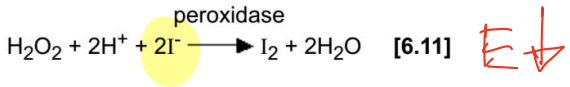
(a) H^+ cation,



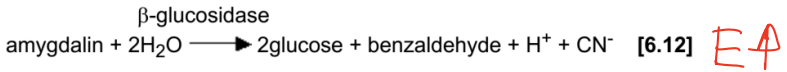
(b) NH_4^+ cation,



(c) I^- anion,



(d) CN^- anion,



$$E_{cell} = E^{\ominus}_{cell} - \frac{RT}{nF}\ln(Q)$$

$A + B \rightarrow C + D$

$$Q = \frac{[C][D]}{[A][B]}$$

Sensors and Actuators B 140 (2009) 260–266

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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Potentiometric biosensor for urea determination in milk

U.B. Trivedi[a], D. Lakshminarayana[a], I.L. Kothari[b], N.G. Patel[c], H.N. Kapse[d], K.K. Makhija[a], P.B. Patel[a], C.J. Panchal[e,*]

The urea sensor operation is based on the enzymatic decomposition of urea by urease:

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3$$

In the pH region where the enzyme is active (around pH 7), the products of the above enzymatic reaction dissociate as:

Produce to

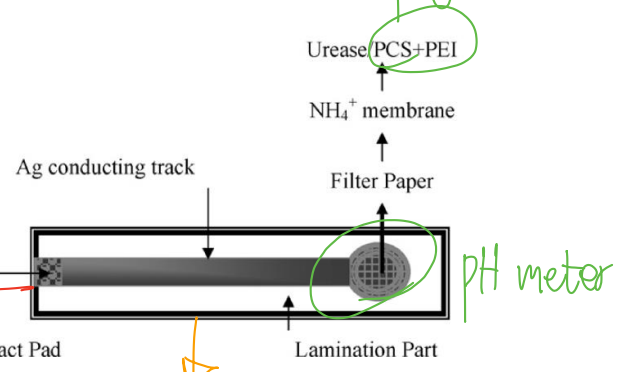
$$CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$$

$$NH_3 + H_2O \rightleftarrows NH_4^+ + OH^-$$

Detect Ammonium. pH ↓

For urea detection, the urease enzyme was immobilized by entrapping it in a PCS + PEI polymer matrix on the surface of the NH_4^+ ion sensitive membrane electrode.

poly matrix



pH meter

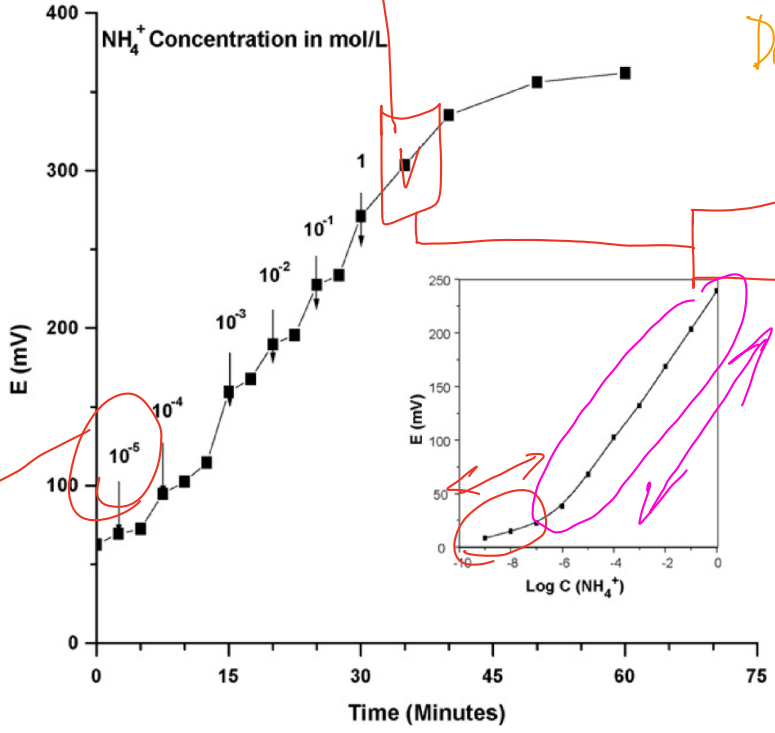
entrapment

transducer

Detection of potential

ref

NH_4^+



Amperometric biosensors

With amperometric biosensors, the electrode potential is maintained at a constant level sufficient for oxidation or reduction of the species of interest (or a substance electrochemically coupled to it).

The current that flows is proportional to the analyte concentration.

Amperometric biosensors

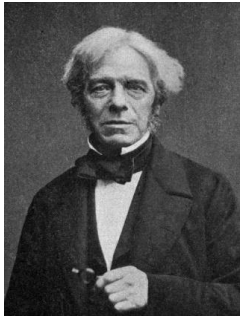
The reactions produce a current when a potential is applied between two electrodes.

The current measured from the redox process is proportional to the concentration of the analyte

Pt anode $H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$

Ag cathode $2AgCl + 2e^- \longrightarrow 2Ag^0 + 2Cl^-$

Faraday's Law



Relationship between the quantity of current (charge) passed through a system, and the quantity of (electro) chemical change that occurs due to the passage of the current

- Number of electrons cross the electrode/solution interface = total charge, Q (unit of coulombs, C)
- 1 C = 6.24 x 10^{18} electrons
- Current, i = Q/t [rate of flow of coulombs] (C/s)
- Faraday's law – passage of 96475 C causes 1 equivalent of reaction

m = M I t /n F

$Q \propto N$

$Q = nNF$

$It = nNF$

$It = n \frac{M}{M} F$

m - mass of substance

M - molecular weight of the substance

I - current passed (A)

t - time for which the current is passed (s)

n - number of electrons transferred

F - Faraday constant (96475 C / eqv)

All processes that obey Faraday's law are termed faradaic processes

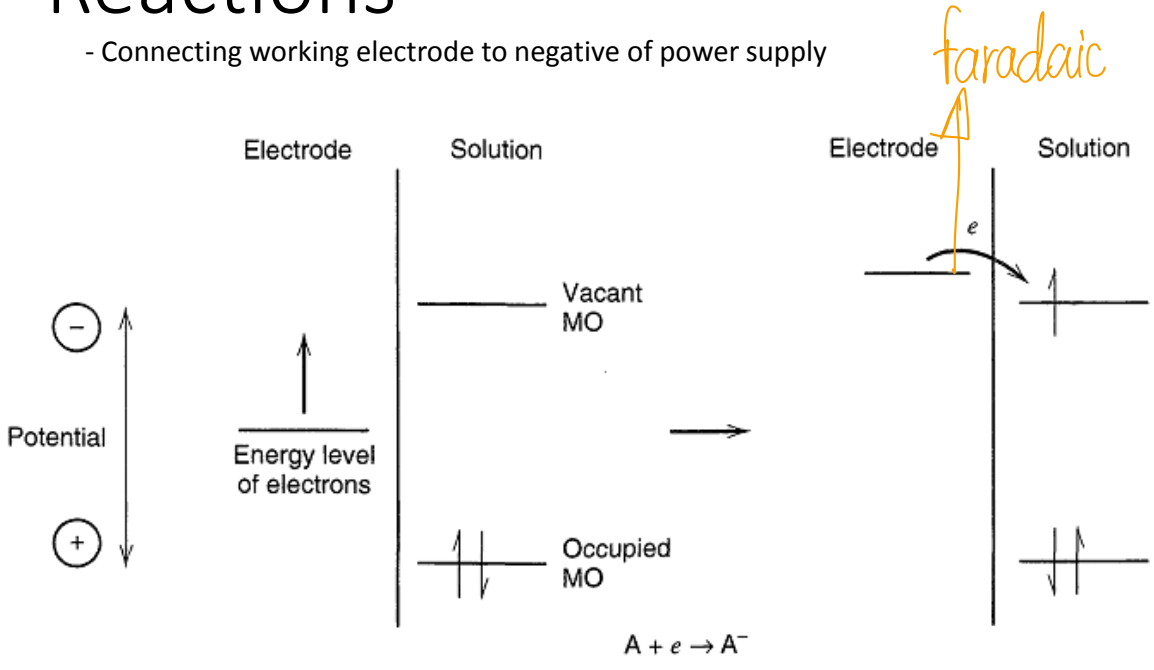
All these processes involve electron transfer at an electrode / electrolyte interface

These reactions are also called electron / charge transfer reactions

Electrodes at which these processes occur are called charge transfer electrodes

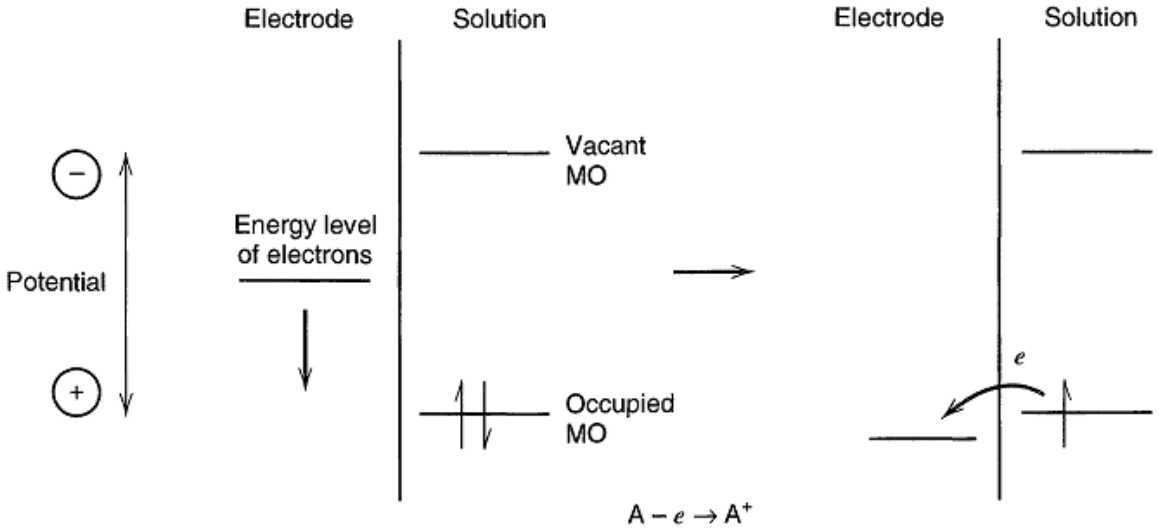
Electrochemical Cells & Reactions

- Connecting working electrode to negative of power supply



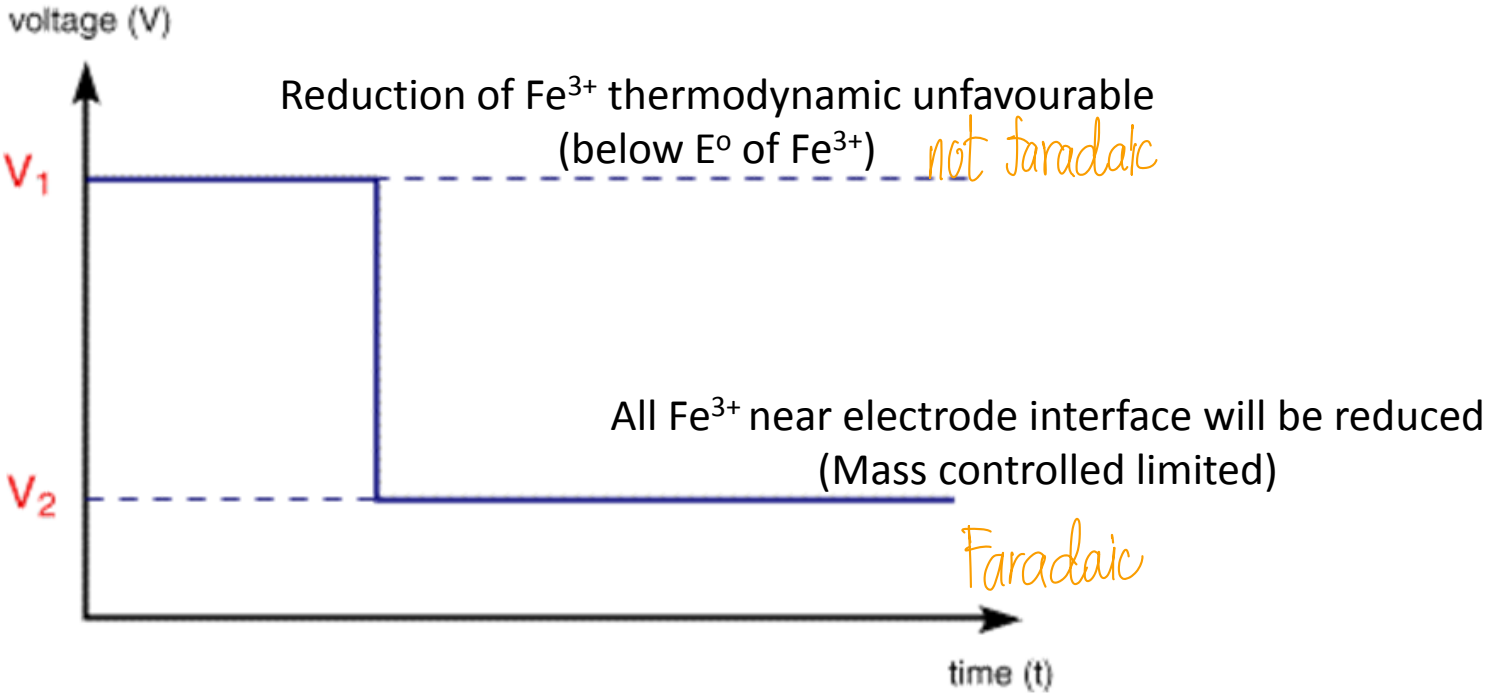
Electrochemical Cells & Reactions

- Connecting working electrode to positive of power supply

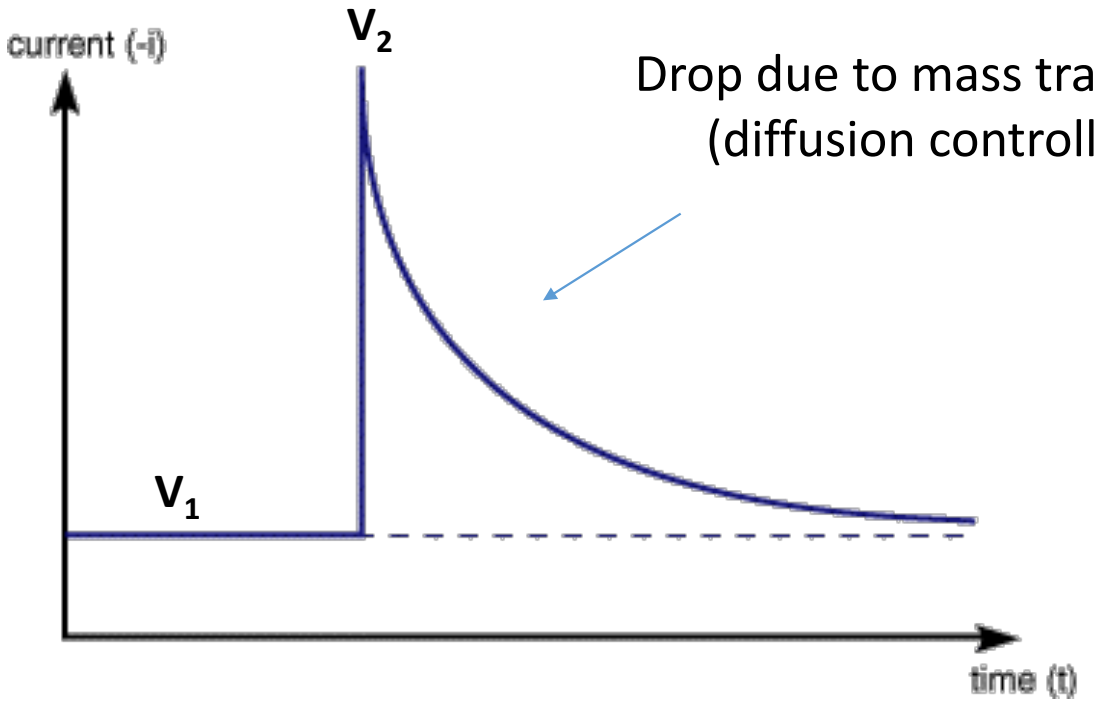


Potential Step Voltammetry

$$Fe^{3+} + e^- \longrightarrow Fe^{2+}$$



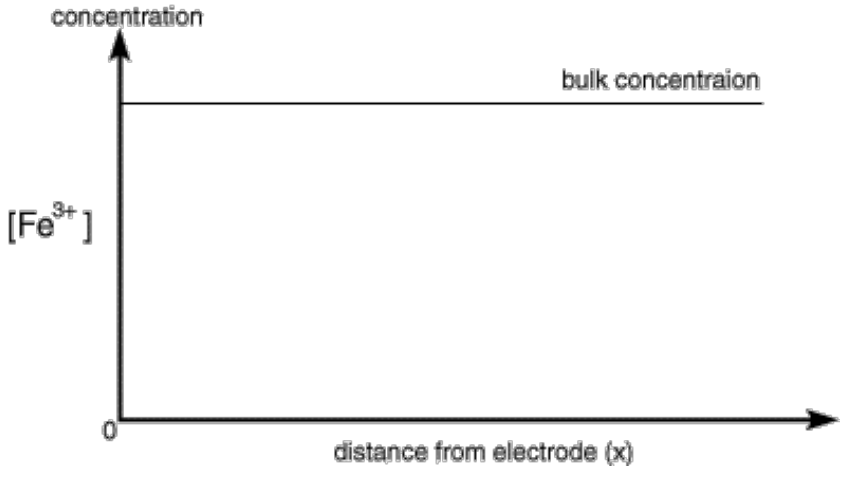
Potential Step Voltammetry

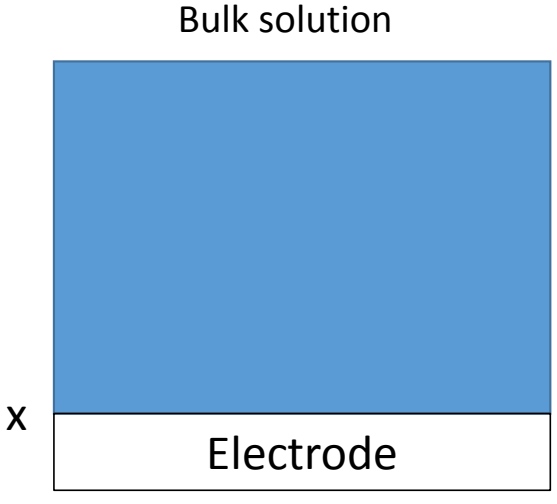


Drop due to mass transport
(diffusion controlled)

Potential Step Voltammetry

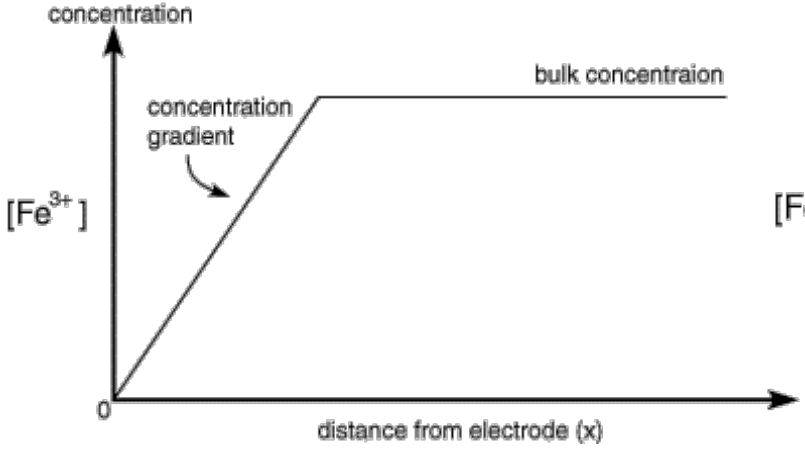
From V_1 to V_2



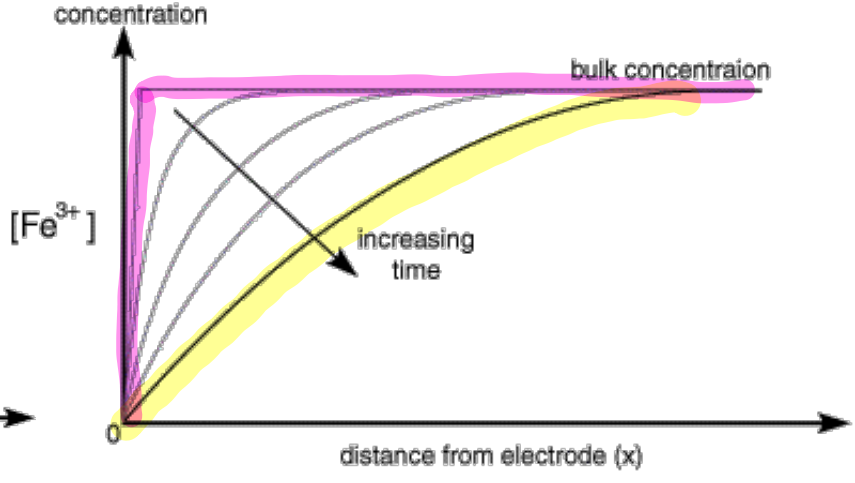


Potential Step Voltammetry

From V_2 onwards

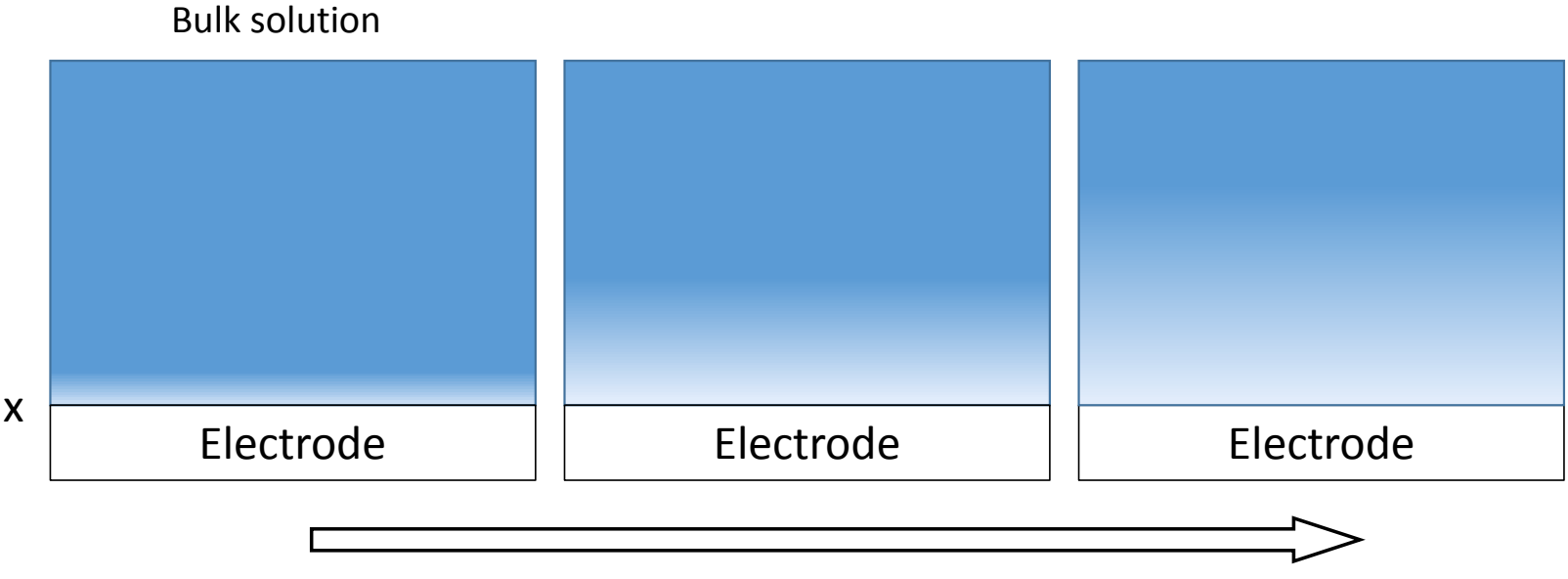


Concentration gradient is developed due to Fe^{3+} is consumed at electrode interface



Concentration gradient is decreasing over time as more Fe^{3+} is consumed at electrode interface

Potential Step Voltammetry



Over time, amount of Fe3+ available for reduction at electrode surface is decreasing

Potential Step Voltammetry

Mass transport equation (diffusion mode)

$$\frac{\partial[Fe^{3+}]}{\partial t} = D_{Fe^{3+}}\left(\frac{\partial^2[Fe^{3+}]}{\partial x^2}\right)$$

Assumption only

Butler Volmer theory

$$i_c = -nFAk_{red}[Fe^{3+}]_0$$

Diffusion not being considered

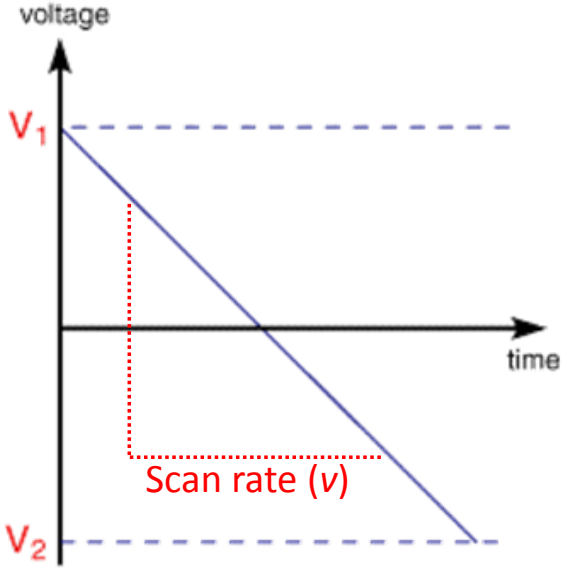
Cottrell equation

$$|i| = \frac{nFA[Reactant]_{Bulk}\sqrt{D}}{\sqrt{\pi}\sqrt{t}}$$

Linear Sweep Voltammetry

Potential is scanned from V_1 to V_2

Current is recorded and plotted as I vs. E (Linear sweep voltammogram)

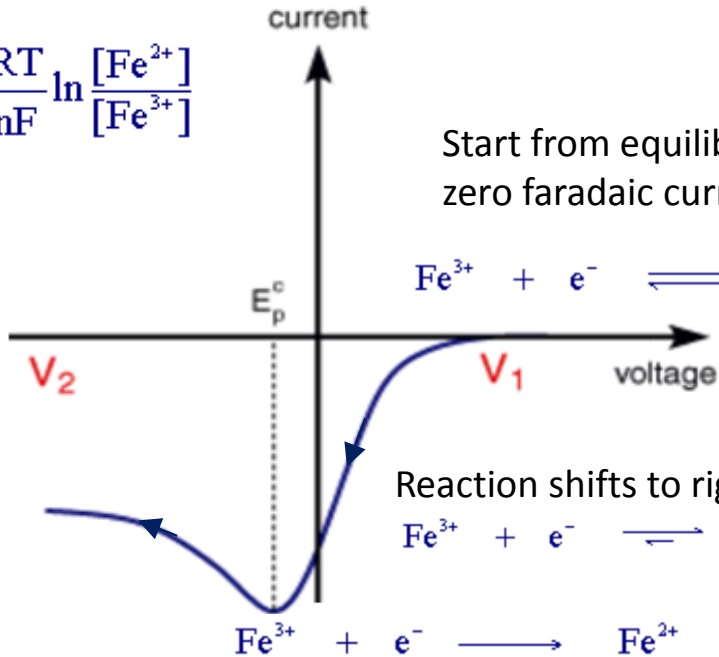


The shape of LSV depends:

- Electron transfer rate, k
- Chemical reactivity of electroactive species
- Scan rate

Linear Sweep Voltammetry

$$E = E^{\theta} - \frac{RT}{nF}\ln\frac{[Fe^{2+}]}{[Fe^{3+}]}$$



Start from equilibrium, zero faradaic current

$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$

Reaction shifts to right, faradaic current rises

$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$

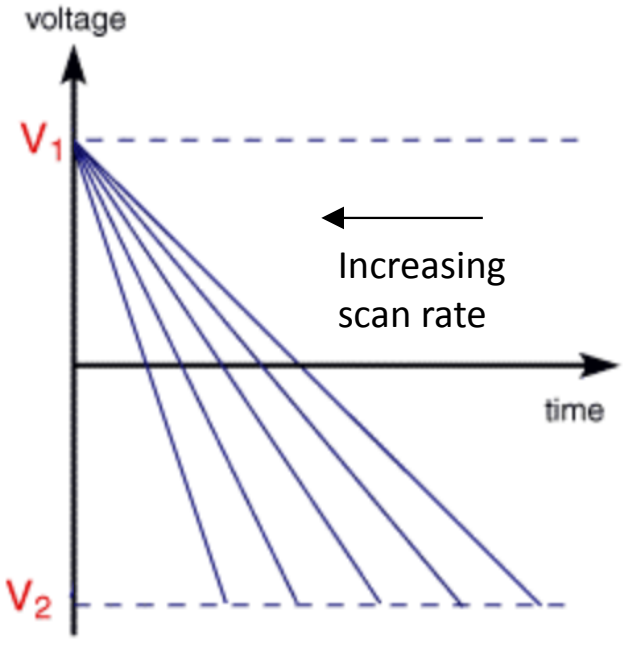
Limit by diffusion of Fe3+ to electrode interface (Cottrell eq.)

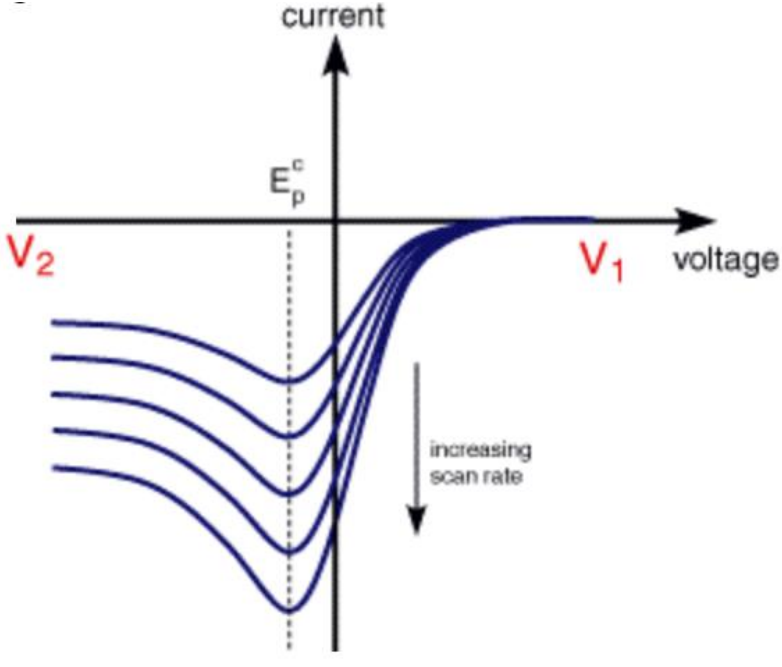
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$

Faradaic current highest, all Fe3+ at interface will be converted to Fe2+ instantaneously

Linear Sweep Voltammetry

Scan rate effect

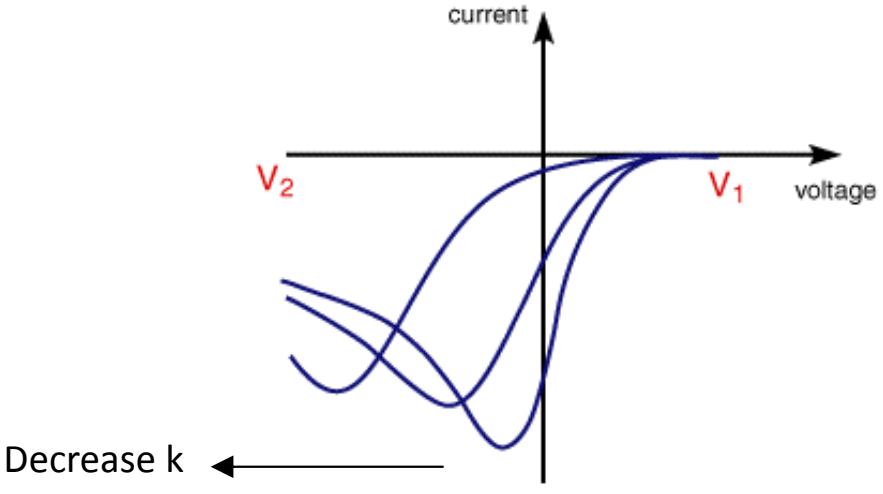




Linear Sweep Voltammetry

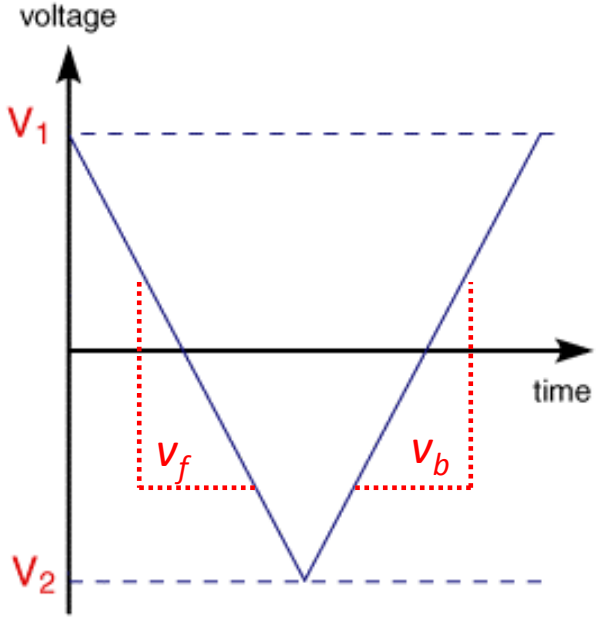
- At different scan rate, LSV shape is the same, only peak current different
- For high k (electron transfer rate constant), LSV peak voltage same at different scan rate,
- Due to the diffusion layer and time to record scan
- Low scan rate, diffusion layer grows further from electrode interface, flux is smaller, current is also smaller (Cottrell eq.)
- Low scan rate -> Low peak current
- High scan rate -> High peak current

Linear Sweep Voltammetry



- For slow electron transfer (k) at electrode interface, the more energy is required for electron transfer, peak potential occurs at larger voltage (more energy) -> does not obey to Nernst Eq. (not equilibrium)
- Peak current also drops

Cyclic Voltammetry



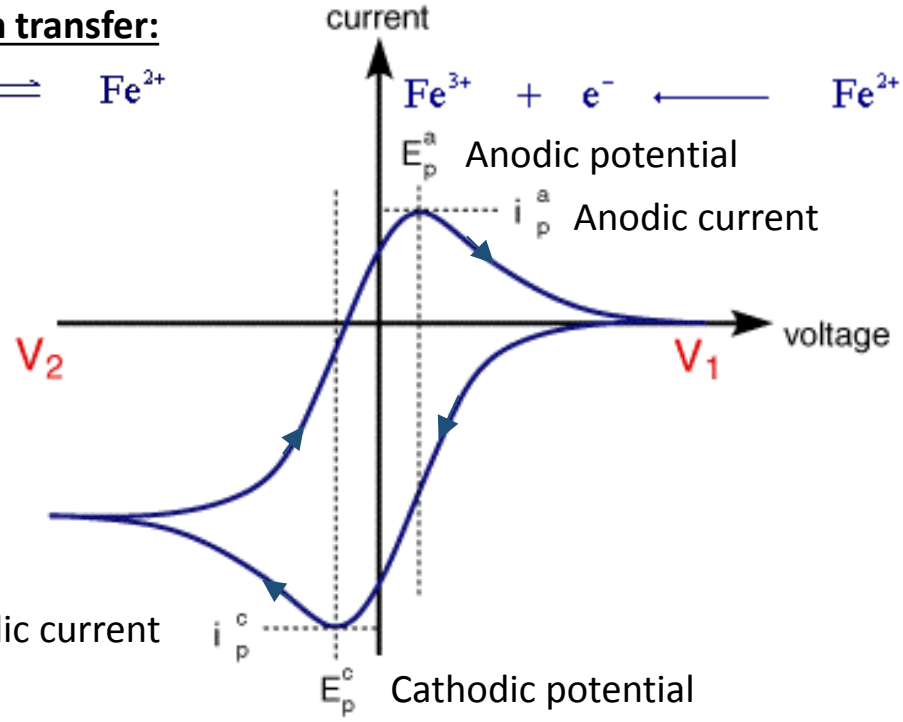
$v_f = v_b$

- Voltage is scanned from V1 to V2 and from V2 back to V1, at similar scan rate

Cyclic Voltammetry

Reversible electron transfer:

$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$



$Fe^{3+} + e^- \longrightarrow Fe^{2+}$

Cyclic Voltammetry

- Voltage separation between peaks is

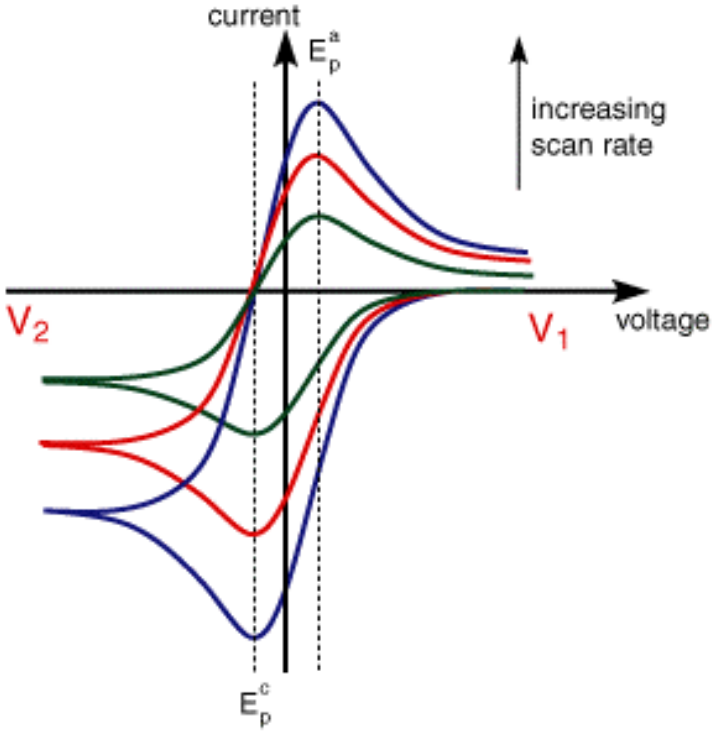
$$\Delta E = E_p^a - E_p^c = \frac{59}{n} mV$$

- Scan rate does not affect the peak voltage
- Scan rate affects the peak current (Higher scan rate, higher peak current)
- Peak currents are proportional to square root scan rate ($I \alpha v^{-1/2}$)

$$|i| = \frac{n\,FA[\text{Reactant}]_{\text{Bulk}}\sqrt{D}}{\sqrt{\pi}\sqrt{t}} \qquad i \text{ vs } \frac{1}{\sqrt{t}}$$

- The peak ratio $\left|\frac{i_p^a}{i_p^c}\right| = 1$

Cyclic Voltammetry



Diffusion layer increase:

- Peak in CV
- Peak current increase at increasing scan rate

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

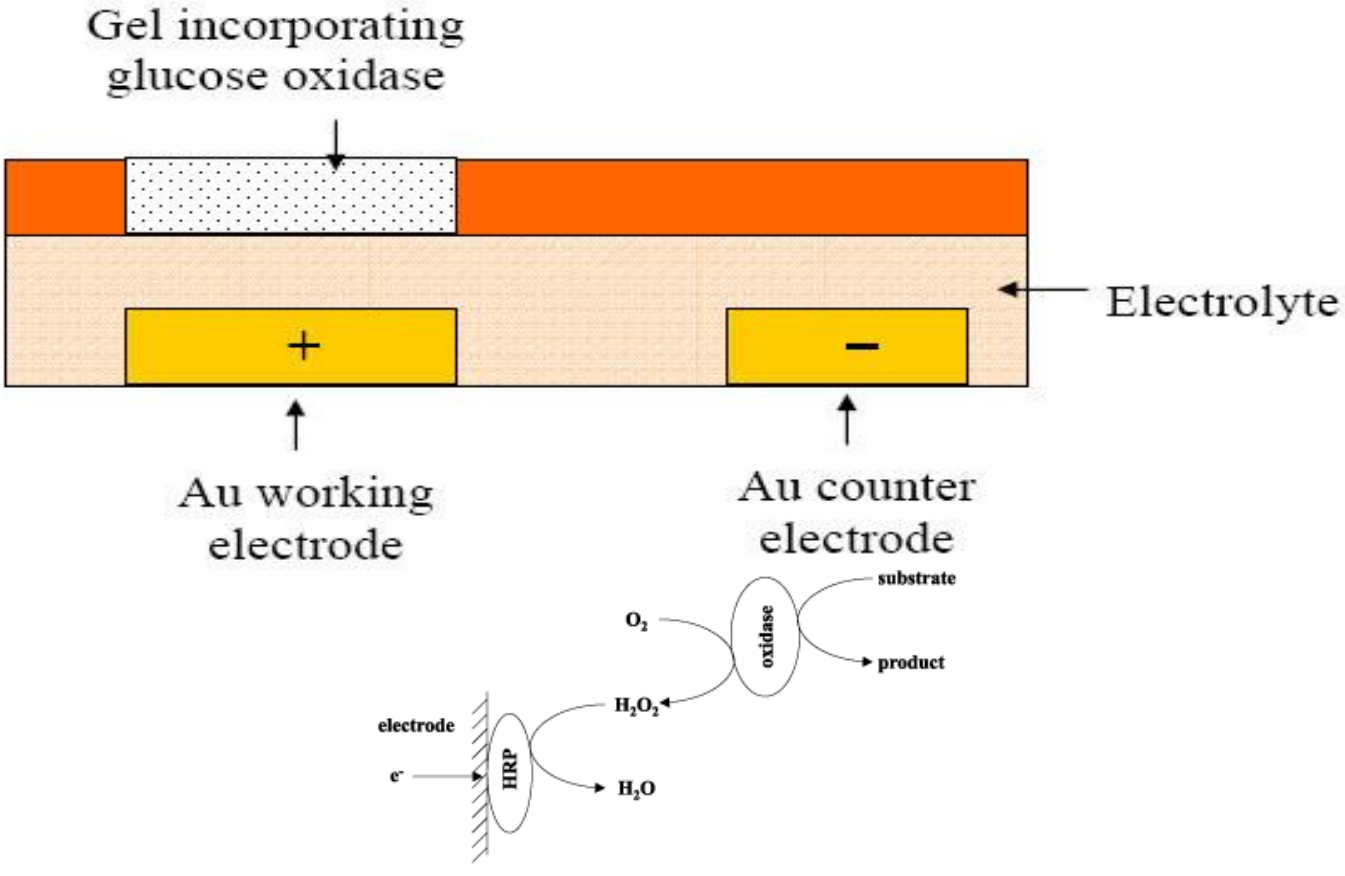


Figure 6. Schematics of coupling of two enzymes.

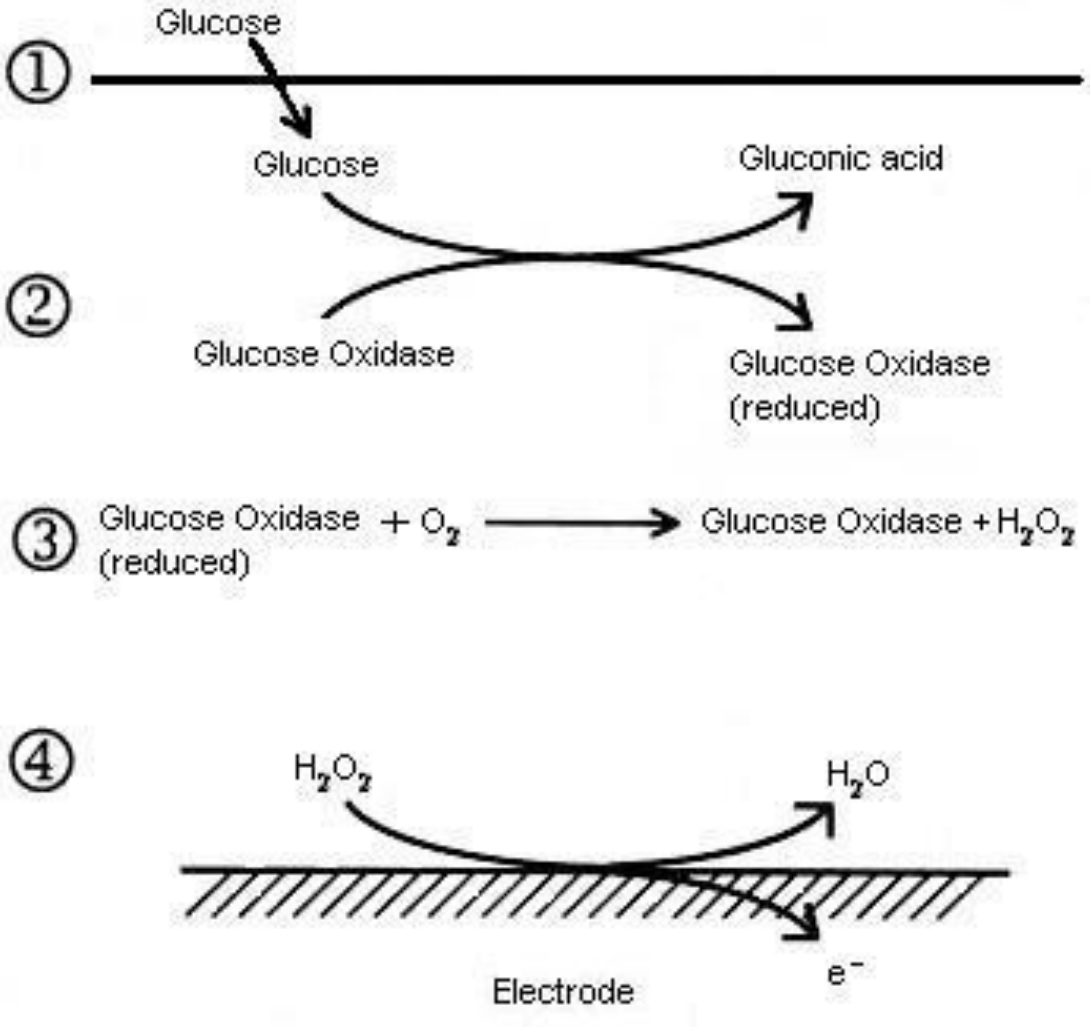
Example

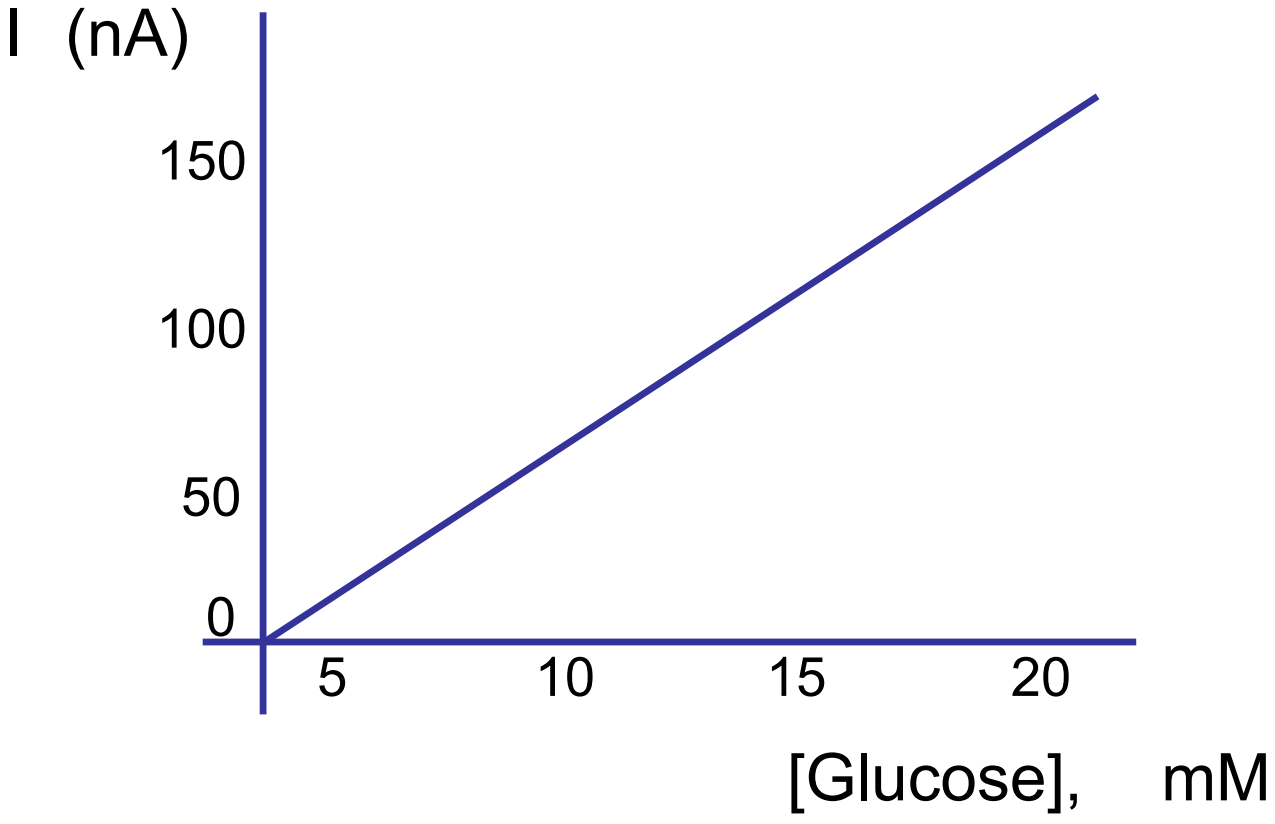
$$\text{Glucose} + O_2 \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconic Acid} + H_2O_2$$

The product, H_2O_2, is oxidised at +650mV vs a Ag/AgCl reference electrode.

Thus, a potential of +650mV is applied and the oxidation of H_2O_2 measured.

This current is directly proportional to the concentration of glucose.


①
Glucose
Glucose
②
Gluconic acid
Glucose Oxidase
Glucose Oxidase
(reduced)
③
Glucose Oxidase (reduced) + O_2
Glucose Oxidase + H_2O_2
④
H_2O_2
H_2O
Electrode
e^-


I (nA)
150
100
50
0
5
10
15
20
[Glucose], mM

AMPEROMETRIC BIOSENSORS

- Various approaches have been taken to increase the selectivity of the detecting electrode by chemically modifying it by the use of:

  - membranes
  - mediators
  - metallised electrodes
  - polymers

AMPEROMETRIC BIOSENSORS

1. Membranes.

Various permselective membranes have been developed which controlled species reaching the electrode on the basis of charge and size.

Examples include cellulose acetate (charge and size), Nafion (charge) and polycarbonate (size).

The disadvantage of using membranes is, however, their effect on diffusion.

2. Mediators

Many oxidase enzymes can utilise artificial electron acceptor molecules, called mediators.

A mediator is a low molecular weight redox couple which can transfer electrons from the active site of the enzyme to the surface of the electrode, thereby establishing electrical contact between the two.

These mediators have a wide range of structures and hence properties, including a range of redox potentials.

- Examples of mediators commonly used are:
    - Ferrocene (insoluble)
    - Ferrocene dicarboxylic acid (soluble)
    - Dichloro-indophenol (DCIP)
    - Tetramethylphenylenediamine (TMPD)
    - Ferricyanide
    - Ruthenium chloride
    - Methylene Blue (MB)

3. Metallised electrodes

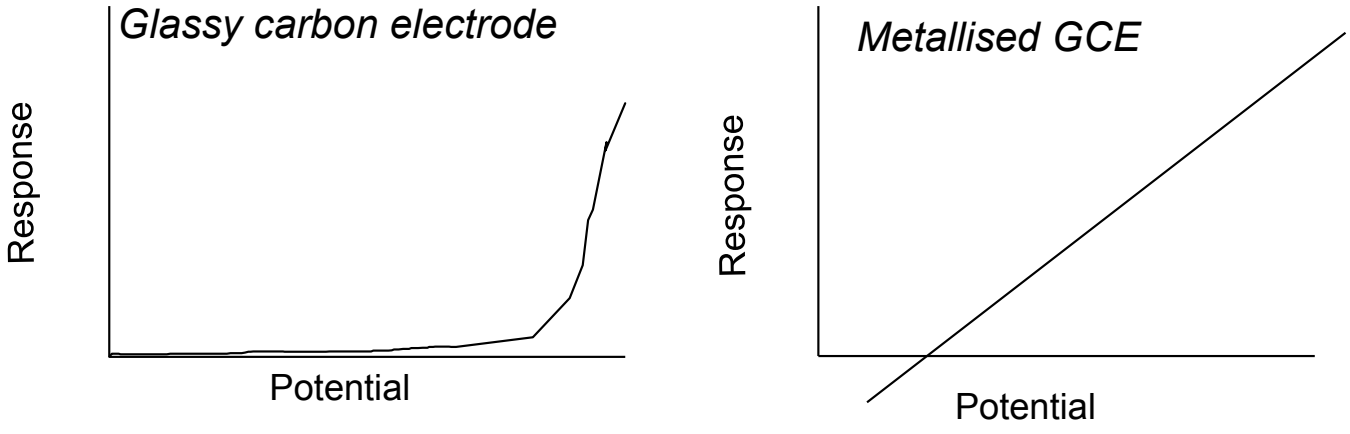
The purpose of using metallised electrodes is to create conditions in which the oxidation of enzymatically generated H_2O_2 can be achieved at a lower applied potential, by creating a highly catalytic surface.

In addition to reducing the effect of interferents, due to the lower applied potential, the signal-to-noise ratio is increased due to an increased electrochemically active area.

AMPEROMETRIC BIOSENSORS

Metallisation is achieved by electrodepositing the relevant noble metal onto a glassy carbon electrode using cyclic voltammetry.

Successful results have been obtained from a few noble metals - platinum, palladium, rhodium and ruthenium being the most promising.



Glassy carbon electrodes do not catalyse the oxidation of hydrogen peroxide.

GCEs metallised with ruthenium, rhodium, palladium or platinum do.

AMPEROMETRIC BIOSENSORS

4. Conductive Polymers

As with membranes, polymers are used to prevent interfering species from reaching the electrode surface. Polymers differentiate on the basis of size and charge.

An example is that of polypyrrole. A polypyrrole film has to be in the reduced state to become permeable for anions. If the film is oxidised, no anion can permeate.

AMPEROMETRIC BIOSENSORS

- **Examples of commonly used polymers are:**
  - **polypyrrole**
  - **polythiophene**
  - **polyaniline**
  - **diaminobenzene**
  - **polyphenol**

Chronoamperometry

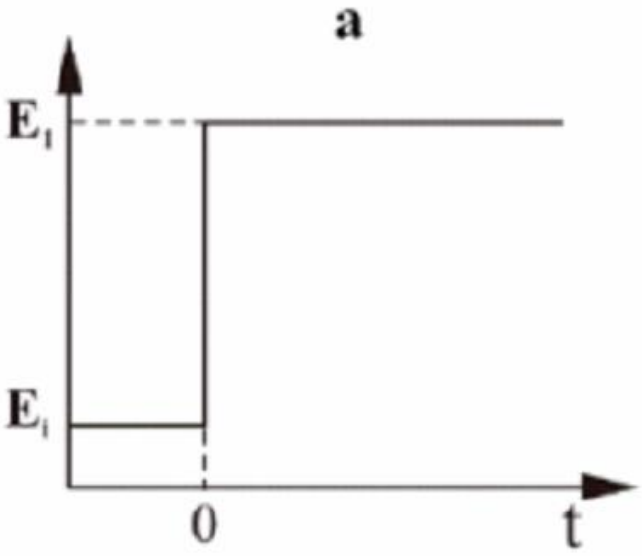
- Most widely used method in biosensor
- Powerful method for quantitative analysis
- Current is measured vs. time as a response to a potential pulse. Normally, the potential is set in order to oxidize all diffused analyte instantaneously.

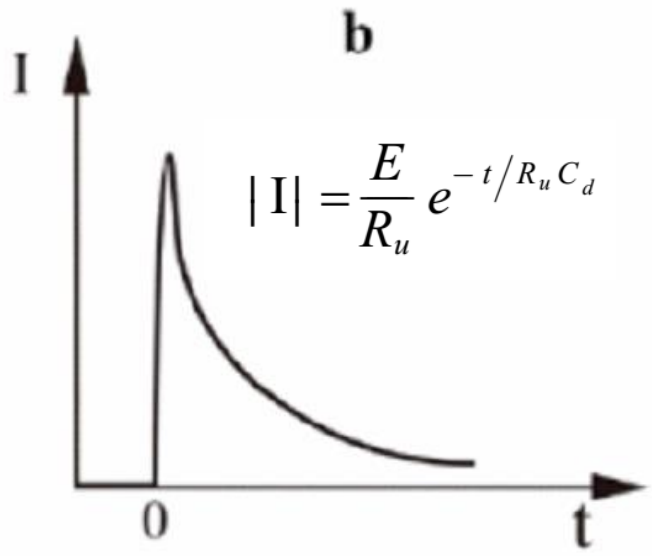
- At short time, capacitive current is dominant

$$(\propto e^{-t/RC}; \text{ with R= solution resistance and C=capacitance})$$

- At longer time, diffusion limited faradaic current is dominant

$$(\propto t^{-1/2})$$





$$|\mathrm{I}| = \frac{E}{R_u} e^{-t/R_u C_d}$$

$$|\mathrm{I}| = \frac{nF\sqrt{D_O}\; c_O^{\infty}}{\sqrt{\pi t}}$$