ADVANCED ENZYME TECHNOLOGY

ENZYMES IN BIOSENSOR

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Chapter Description

- Expected Outcomes
  - To describe the application of enzymes in biosensor
  - To differentiate the element of biosensor

Content

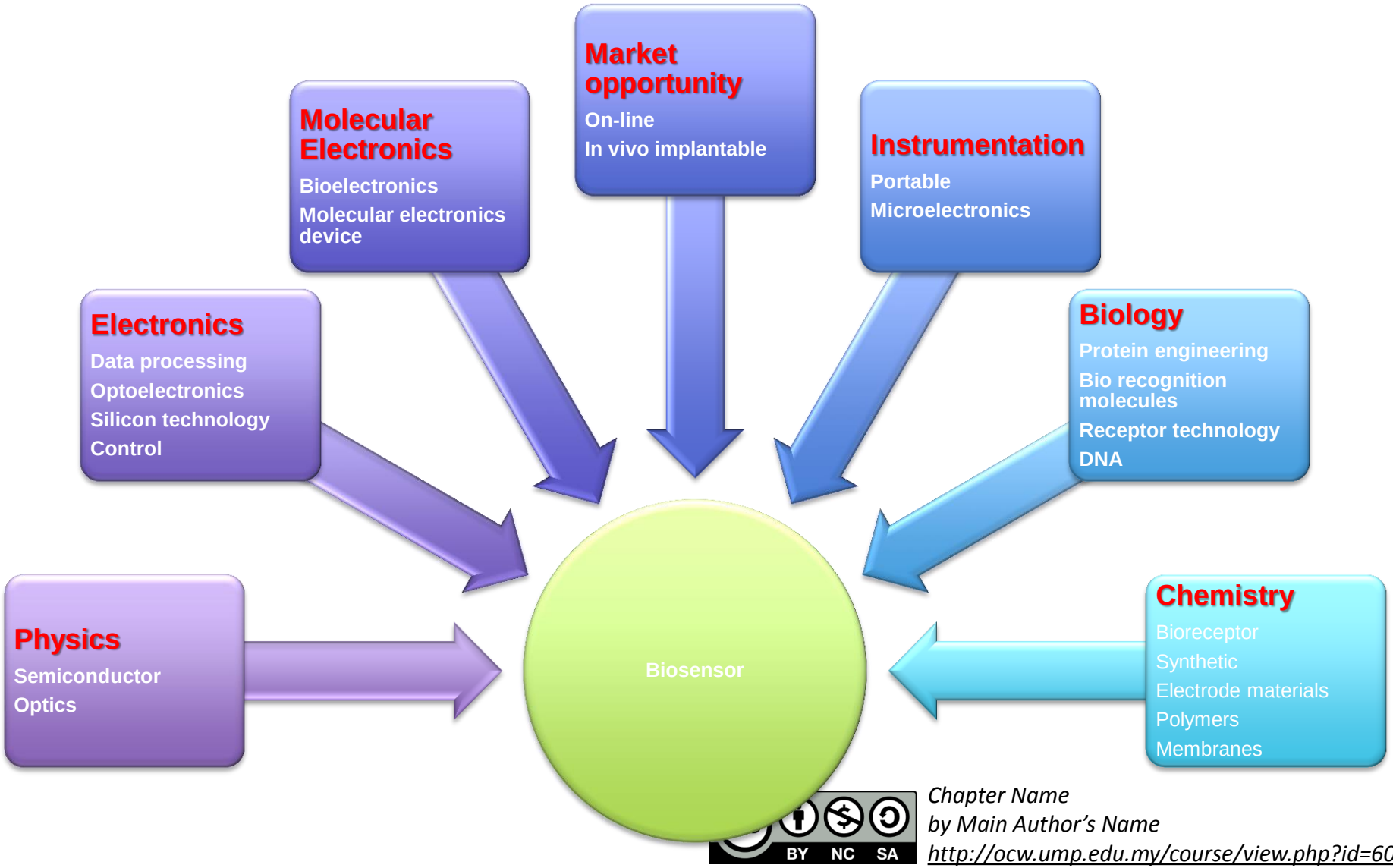
- Introduction
- Type of sensors
- Biosensor element
- Enzyme as a biorecognition elements

INTRODUCTION

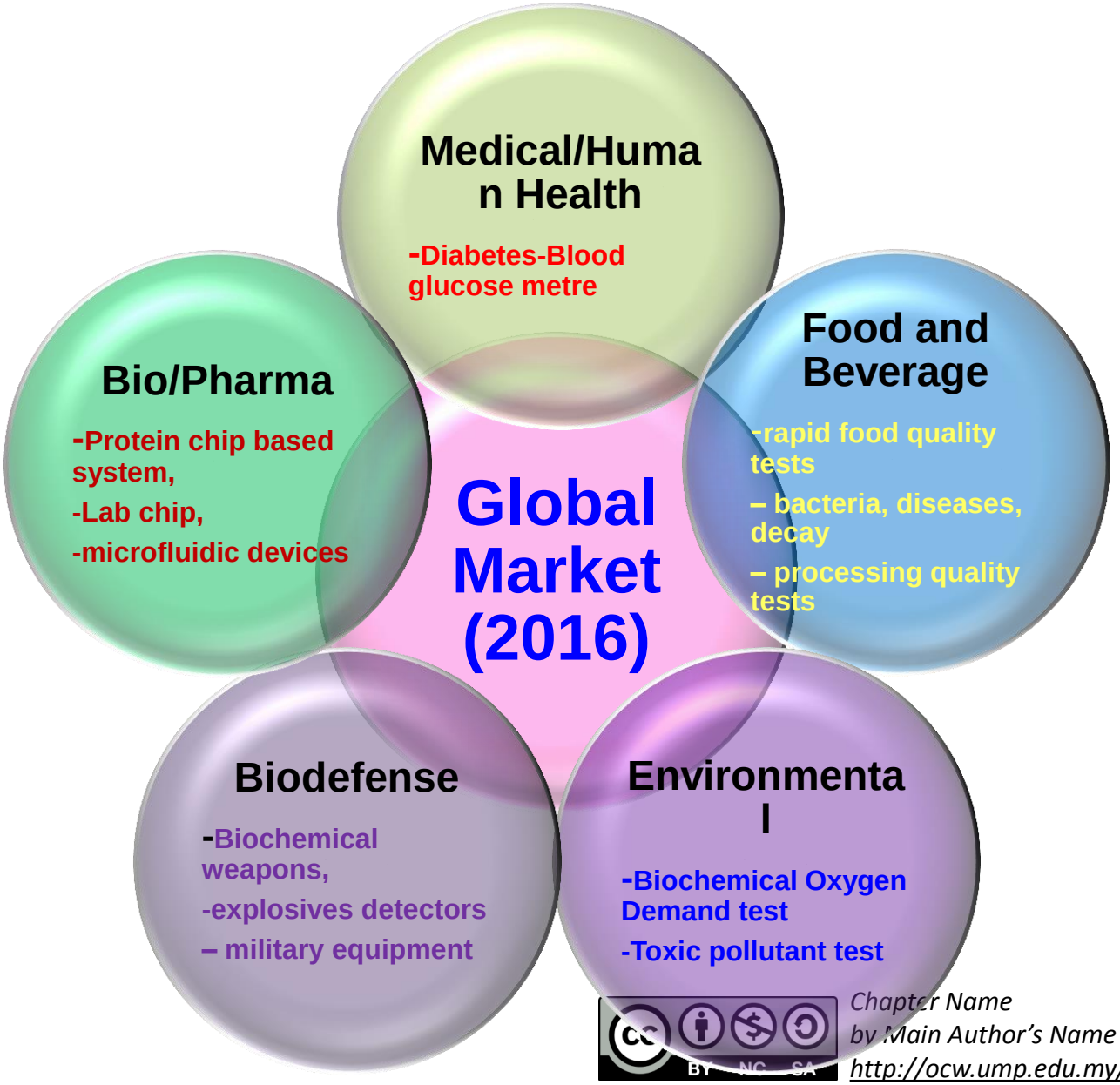
- What is Biosensor?

a device that employs definite reactions of biochemical mediated by particular components such as isolated enzymes, immunosystems, tissues, organelles, or whole cells to identify chemical compounds typically by electrical, thermal or optical signals **(IUPAC)** .

Biosensors as a multidisciplinary area of technology



Application and Global Market of Biosensors



Type of sensors

Physical sensors

- Distance
- Temperature
- Pressure
- Mass

Chemical sensors

- Substances
- Physical responses or chemical

Biosensors

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

Biosensor Example: Glucometer

Glucometer:

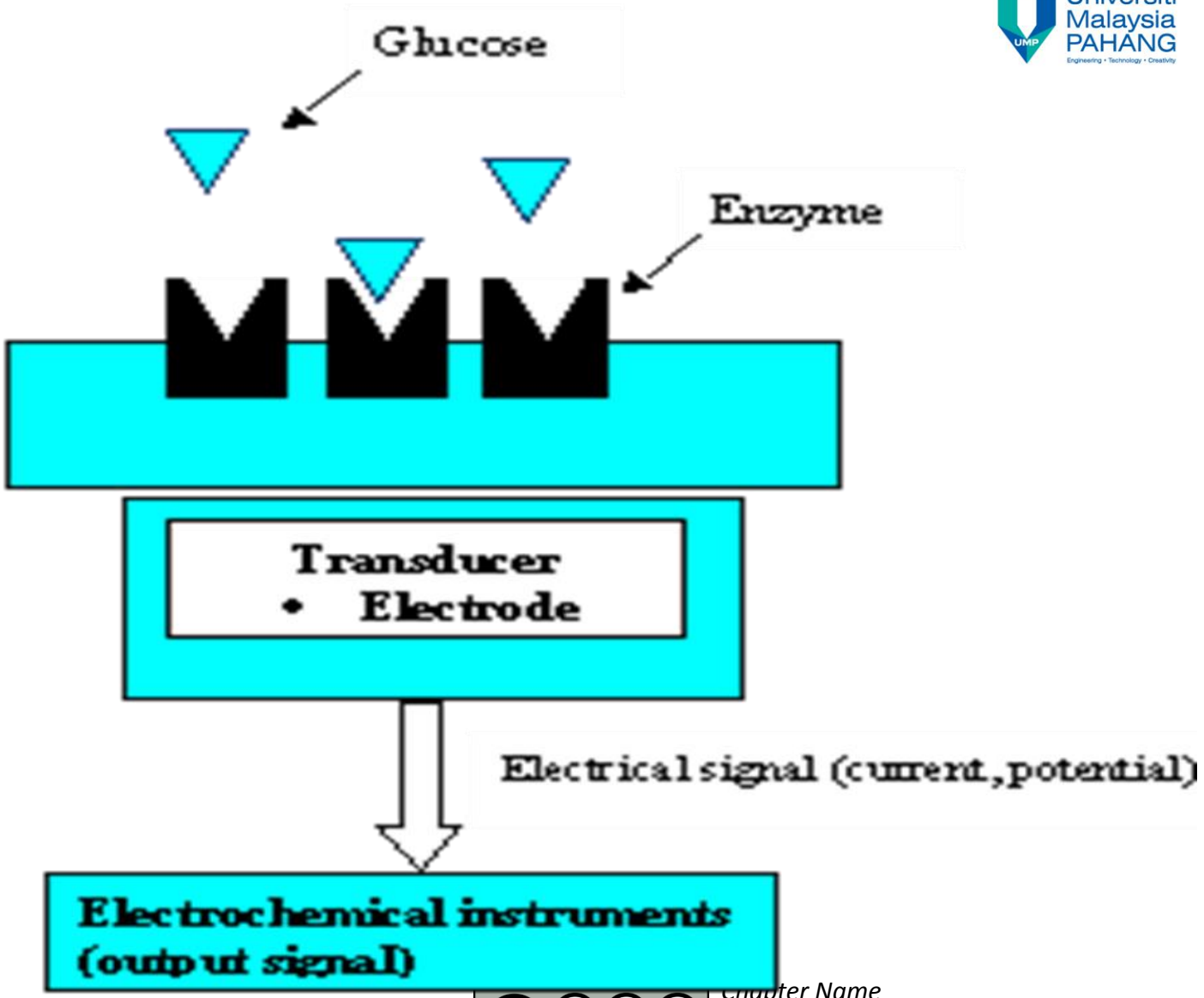
- A good biosensor model.
- Used to examine blood sugar level of diabetics patient .
- Composed of strips and a display unit component.
  - **Glucose oxidase enzyme** is immobilised in the strips which responsible as the biosensor bioreceptor / biorecnition part.
  - The physical part or the display unit is the transducer which will record any reaction occur.

How glucometer works?

1. Firstly, biosensor assembly is performed by inserting the strip into the display part.
2. Put a drop of blood on the strip and allow it to move up the strip towards the enzyme by capillary action mechanism.
3. Once the blood reaches the biorecognition part (glucose oxidase) the device begines to count down from 30 seconds

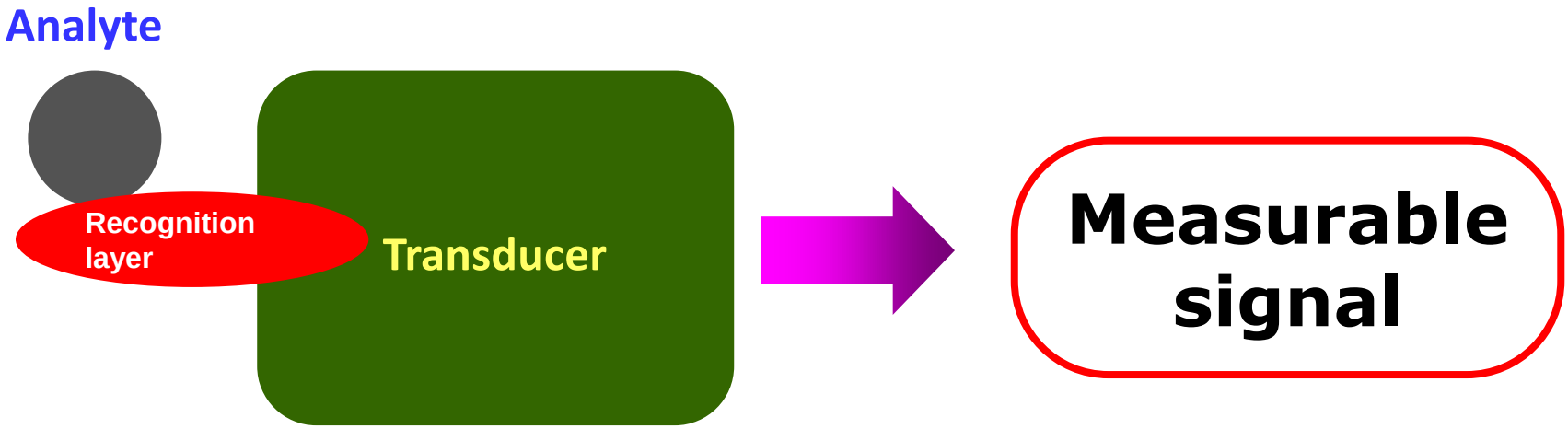
so **what's happenning?**

- Glucose oxidase will convert or hydrolyse any glucose contain in the blood sample into gluconic acid.
- This hydrolisys occurred will be detected by **the transducer** (physical part) and the value displayed will determine the amount of glucose contain in the blood sample.



Biosensor elements

Schematic diagram of a biosensor element.

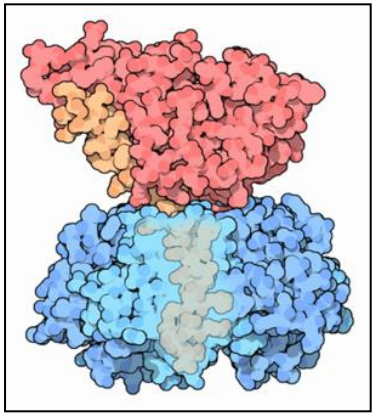


- **The bio-reaction between analyte and biorecognition part - converts the substrate to product.**
- **This reaction is detected by the transducer - converts reaction into an electrical signal.**
- **The output from the transducer is amplified, processed and displayed.**

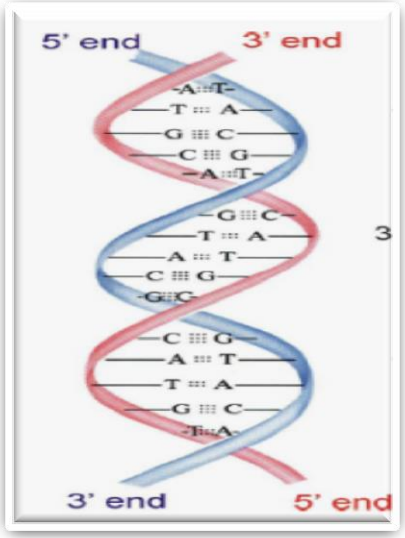
Target Analytes

What can be the analyte?

DNA, Protein, Vitamin, metal ion, Glucose, Heavy metal, Sugar,

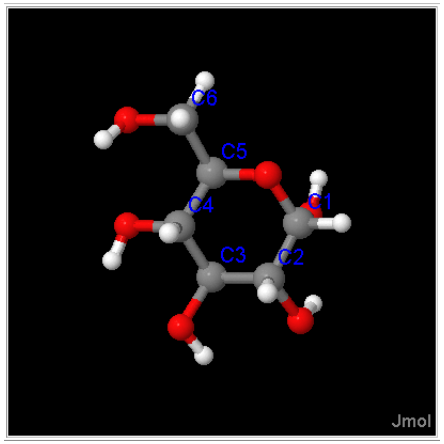


Protein



DNA

Bacteria



Glucose

1st element: The analyte

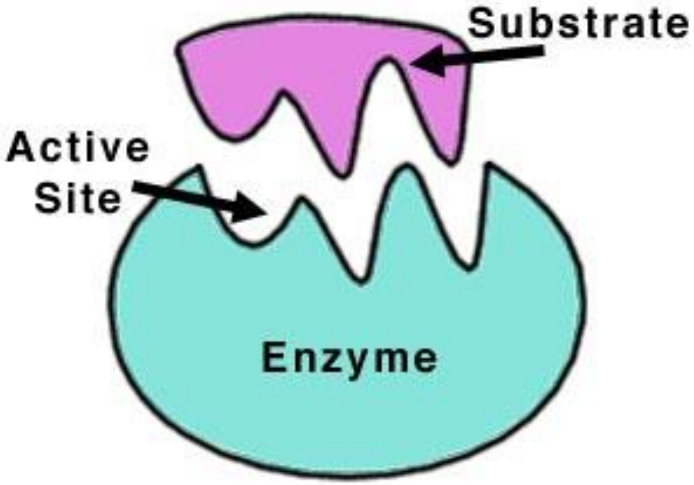
1. The Analyte (What do you want to detect)

- the things that need to be detected
  - ***Molecule - Protein, toxin, peptide, vitamin, sugar, metal ion***

2nd element: Bioreceptor

- The important element for analyte binding.
- Need to be immobilized, stable under storage conditions and extremely specific.
- Example of bioreceptor:

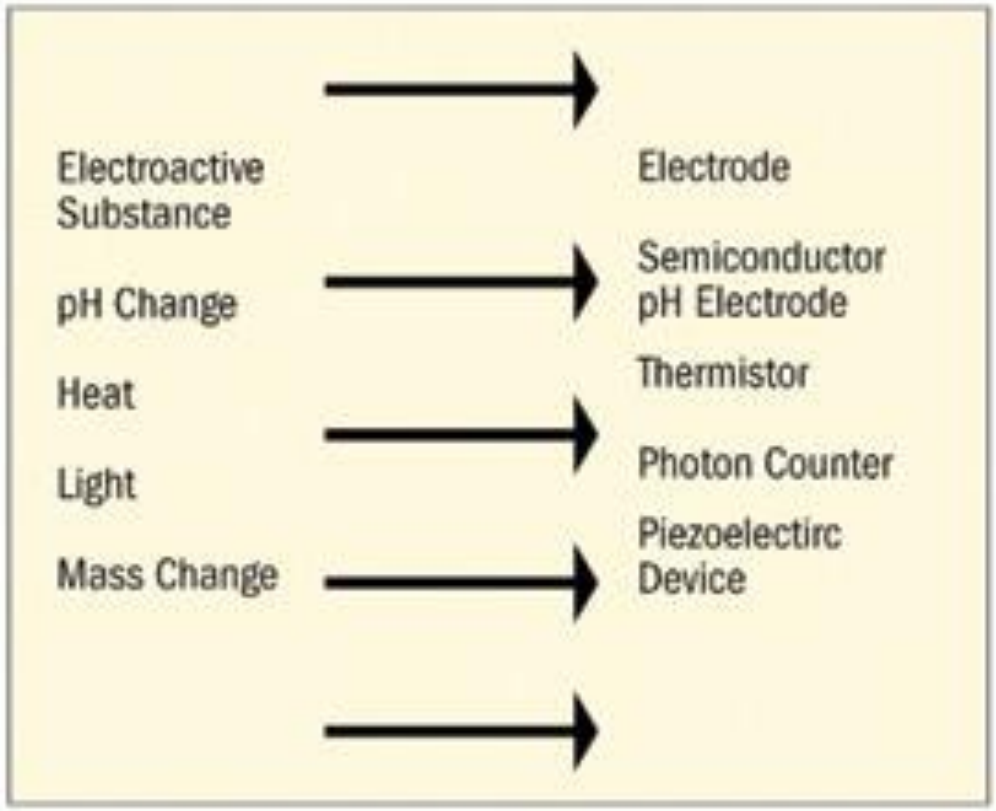
Microorganism
Tissue
Cell
Organelle
Nucleic Acid
Enzyme
Component
Receptor
Antibody

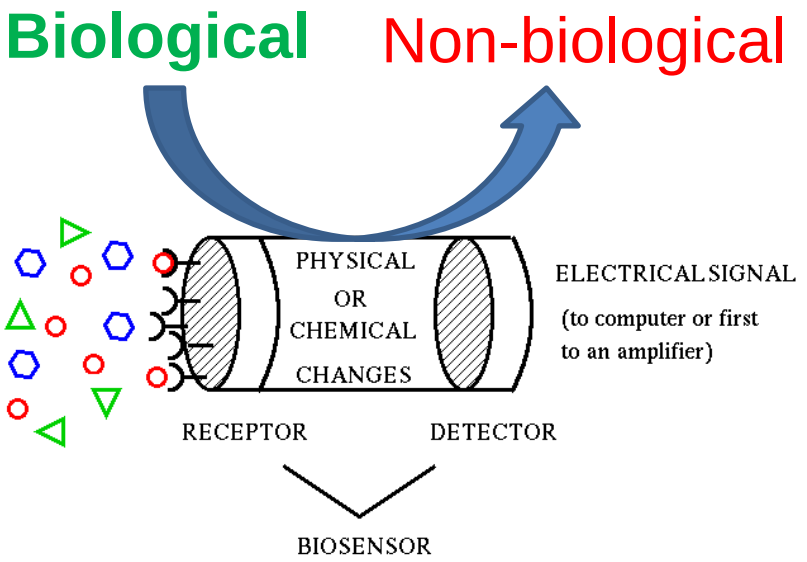


http://www.chemistry.wustl.edu/~edudev/LabTutorials/HIVDrugStrategies.html

3rd element: Physico-chemical Transducer

- **Measure the physical change that happens with the reaction at the bioreceptor part – interface**
  - **converting that signal into measurable electrical output.**





4th element: Detector

- **Microprocessor will received any signals from the transducer that will then amplified and analyzed.**

Quantitative

Qualitative

- **The data will then converted to concentration units then display or/and data storage device will received the data.**

Two categories depending on the nature of the biological recognition process

A. Biocatalytic Devices

- Use of enzymes, cells or tissues as immobilized bioreceptor

B. Affinity Sensors

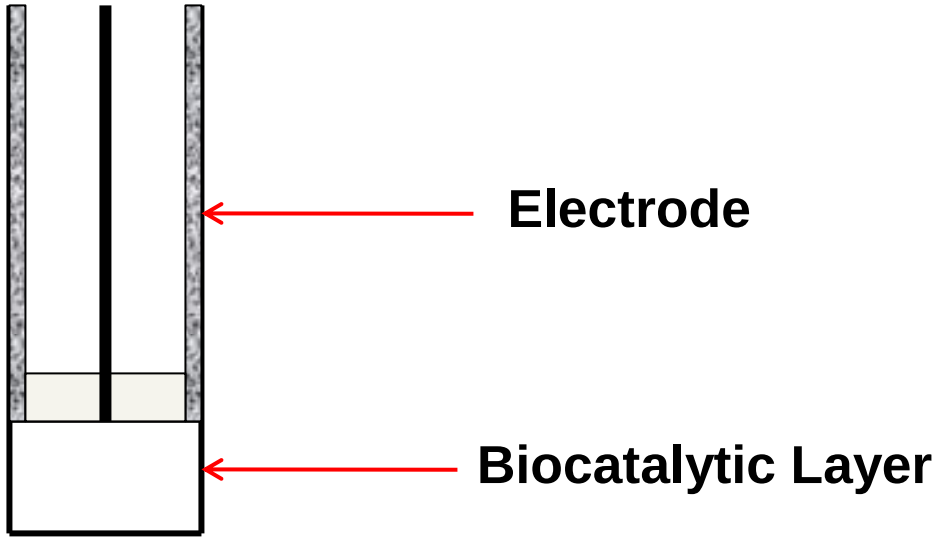
- Use antibodies, membrane receptors or nucleic acids

Enzyme as a biorecognition elements

- Enzymes are proteins that can perform chemical catalysis reactions in living cells.
- Use of enzyme as biosensor is based on combination immobilization of enzyme on an electrode that will work as a transducer.
- Use of enzyme is very extremely efficient and specific.

Enzyme as a biorecognition elements

Enzymes (biocatalytic) layer immobilized on an electrode



- Normally, polymeric films, for example nafion, polypyrrole, are used for enzyme entrapment purpose.
- Enzyme may be trapped
    - between electrode and a dialysis membrane
    - by mixing with carbon paste
    - by surface adsorption
    - by covalent binding
- Convenient for clinical, environmental and food samples monitorings.

Enzymes

Analyte	Enzyme
Amygdalin	β-Glucosidase
Asparagine	Asparaginase
Cholesterol	Cholesterol oxidase
Esters	Chymotrypsin
Glucose	Glucose oxidase
H_2O_2	Catalase
Lipids	Lipase
Penicillin G	Penicillinase
Peptides	Trypsin
Starch	Amylase
Sucrose	Invertase
Urea	Urease
Uric acid	Uricase

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