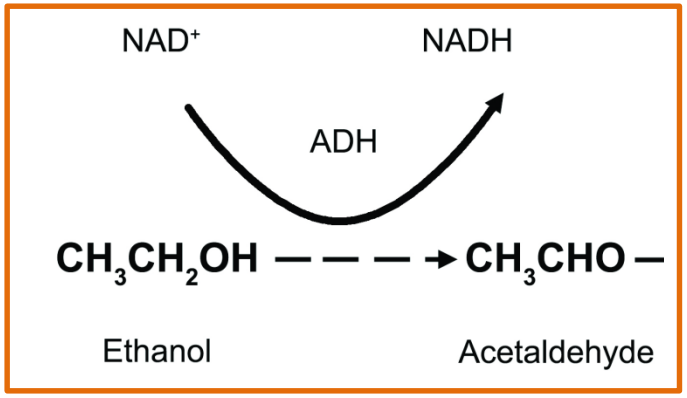
Enzyme Biosensors

- Most common catalytical biosensors
- Their performances are affected from:
  - Enzyme loading
  - Temperature and pH conditions
  - Inhibitors
  - Denaturing agents
  - Immobilization method
- Response is determined by enzyme activity and mass transport:
  - If enzyme activity << mass transfer rate, the response is determined by biocatalytical reaction, and a biosensor acts in “kinetically controlled”. ☺ quick response ☹ narrow linear working range
  - If overall process is determined by mass transport, “diffusion controlled”

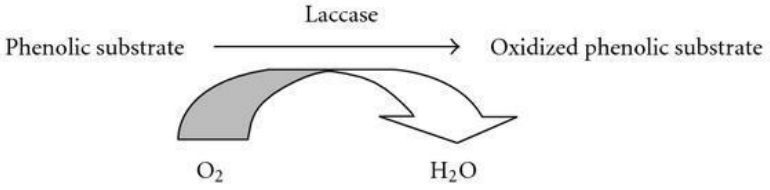
Oxidoreductase Enzyme Biosensors

Enzymes used are generally oxidoreductases responsible for oxidation-reduction reactions, often using coenzyme as NAD+/FAD

- ***Dehydrogenases:*** oxidizes a substrate by a reduction reaction that transfers H to an electron acceptor (NAD+/NADP+ or a flavin coenzyme such as FAD or FMN)
  - e.g. Alcohol dehydrogenase (ADH)
- ***Oxidases:*** catalyzes an redox reaction involving molecular oxygen (O_2) as the electron acceptor. In these reactions, oxygen is reduced to water (H_2O) or hydrogen peroxide (H_2O_2).
  - E.g. Glucose oxidase (GOX) and laccase
- ***Peroxidases:*** mostly horseradish peroxidase; ZH_2 is oxidized to Z. Z indicates that the enzyme can use several different molecules as the source of the hydrogen atoms



Glucose + O_2 → H_2O_2 + gluconic acid



$H_2O_2 + ZH_2 \rightarrow 2H_2O + Z$

Oxidoreductase Enzyme Biosensors

- Dehydrogenases:
  - Glucose dehydrogenase*
  - Alcohol dehydrogenase
  - Lactate dehydrogenase
  - Alanine dehydrogenase (NH_4^+)
  - Sulfite Dehydrogenase (sulfite)
- Oxidases:
  - Glucose oxidase*
  - Alcohol oxidase
  - Cholesterol oxidase
  - Laccase
  - Glutamine oxidase
- Peroxidases:
  - Horseradish peroxidase

*90 % of global biosensor market (*Monosik, 2012*)

Hydrolase Enzyme Biosensors

- Hydrolases: responsible from hydrolysis of a chamical bond:

$$A–B + H_2O \rightarrow A–OH + B–H$$

- Examples:
  - Penicilinase (Penicillin → H^+)
  - Lipase (fats → fatty acids)
  - Urease (Urea → NH_4)
  - Aspartase (Aspartam → NH_3)

Enzyme Biosensors

- Based on
  - Analyte consumption/product formation
  - Inhibition
- Most widely used detection methods:
  - Electrochemical
  - Optical

Electrochemical Enzyme Biosensors

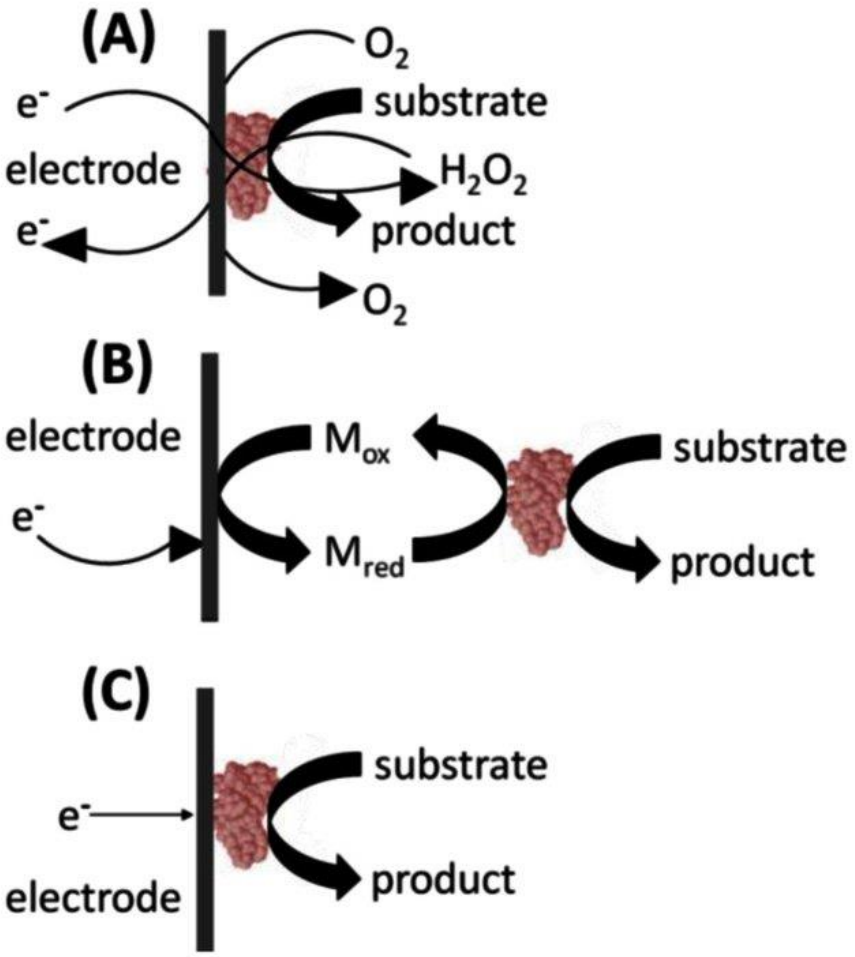
How an electrical communication is established between the enzyme active site and the electrode?

Three types of enzyme-electrode reactions can allow for this:

A. Use of a Natural Substrate

B. Artificial Redox Mediators

C. Transfer of Electrons between the Enzyme and the Electrode



Electrochemical Enzyme Biosensors

A. Use of a Natural Substrate

- One of the most common types of electrochemical bio-sensing reactions involves natural substrates that are oxidized to transfer electrons to molecular oxygen (O_2) resulting in the production of hydrogen peroxide (H_2O_2).
- Both oxygen and H_2O_2 are electrochemically active and can transfer electrons into the electrode to generate an electrical current.

☺ Simple→ the normal product of the reaction diffuses to the transducer and causes electrical response

☹ Slow response rate (diffusion of O_2 from the active site(buried deep within the enzyme), limited solubility of O_2)

☹ Electron transfer decays exponentially with distance

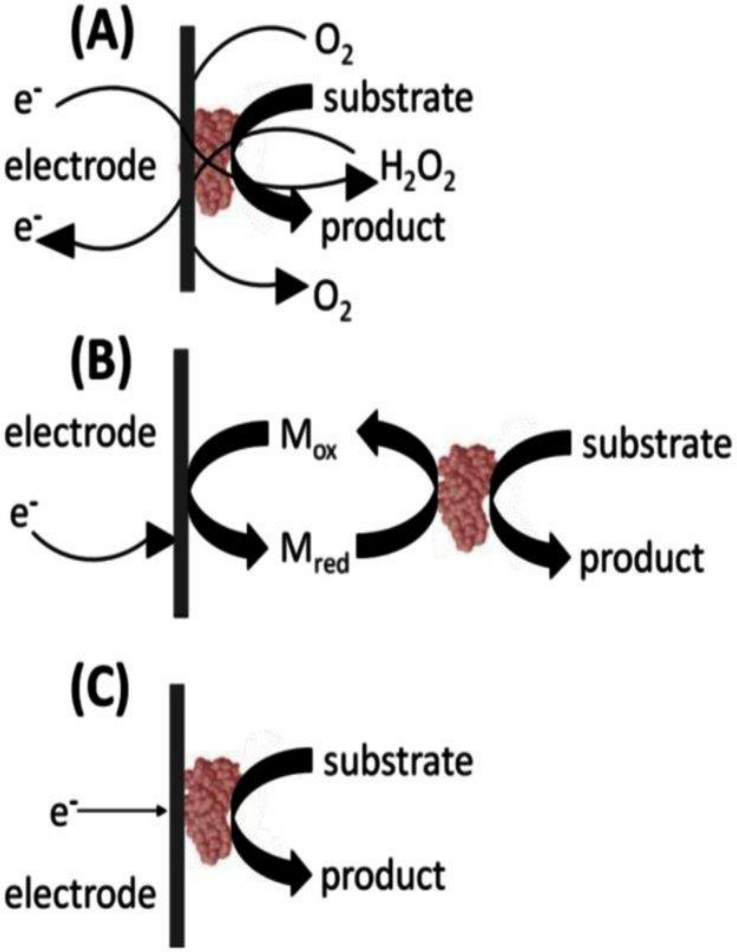
☹ Oxidation of O_2 to H_2O_2 does require a high voltage (one of the biggest drawbacks) so prone to interferences from easily oxidized substances such as ascorbic acid, paracetamol and uric acid

➢ To deal with the interferants:

- the use of semipermeable selective membranes like nafion, cellulose acetate
- preliminary oxidation of interferants

Electrochemical Enzyme Biosensors

B. Artificial Redox Mediators



The enzyme either generates or consumes a redox-active compound (biologically active charge carriers) during conversion of the analyte.

A mediator (e- transfer shuttle) transfer electrons from the enzyme to the working electrode surface.

☺ Redox mediator enhances the electron transfer between the redox center of enzyme and the electrode surface

☺ Usage of redox mediator eliminate the need of oxygen for electron transfer at the electrode surface, thus overcoming the drawback of limited oxygen pressure

☺ The mediator requires less operating voltage to power the catalytic reaction and results in no interference from other electroactive species

- ferrocene derivatives, ruthenium, or osmium complexes
- Other small redox proteins (e.g. horse heart cytochrome c)

It should be non-toxic, chemically stable and react rapidly with the enzyme

B. Artificial Redox Mediators

☹ 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.

Electrochemical Enzyme Biosensors

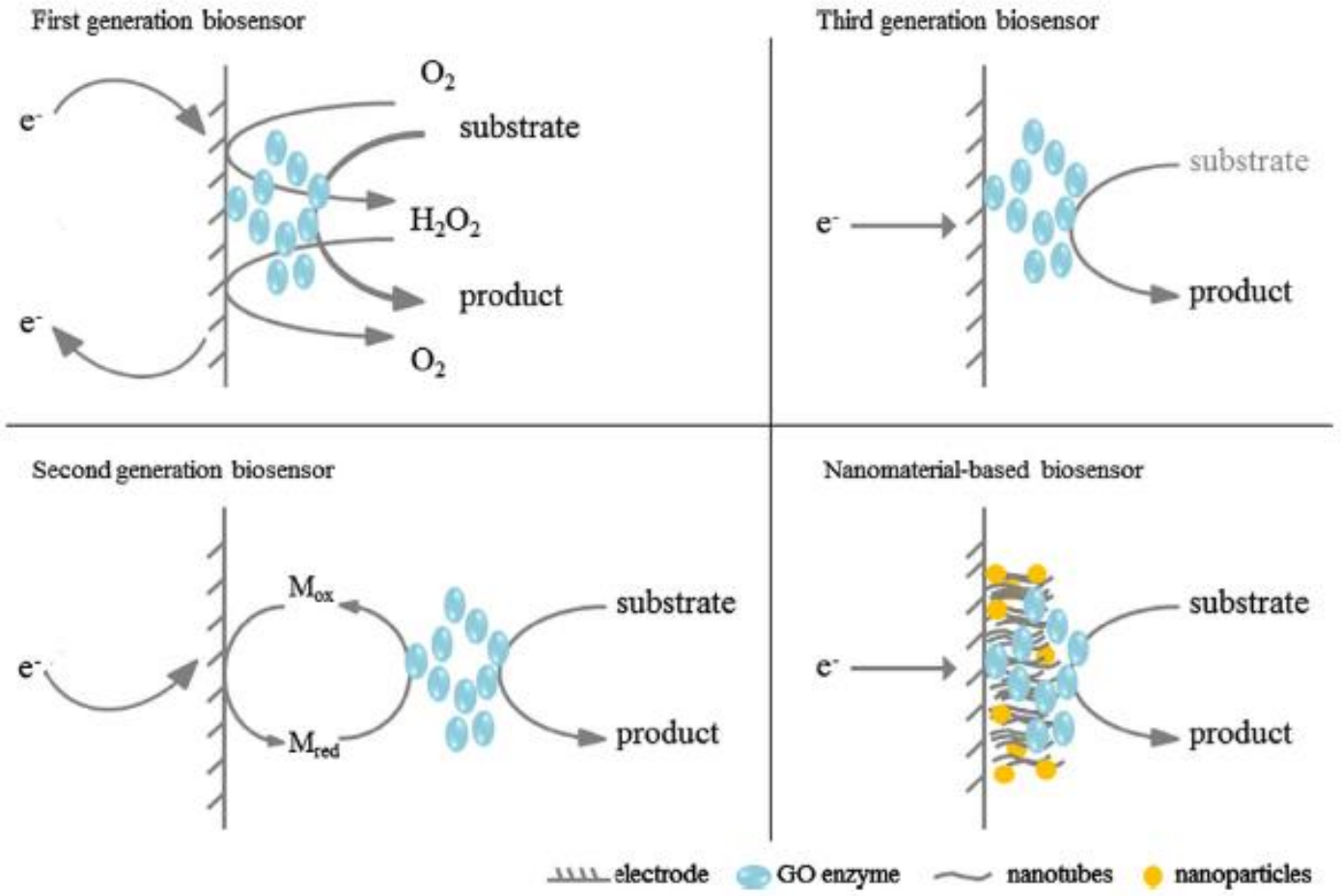
C. Transfer of Electrons between the Enzyme and the Electrode

Direct electrical communication between the electrode and the enzyme component.

- It involves wiring an enzyme to the electrode by co-immobilizing the enzyme and mediator directly onto the electrode surface or into an adjacent matrix such as a conductive polymer film.
- The immobilized mediators act as non-diffusion redox relay stations, effectively facilitating the transport of electrons from the enzyme active site to the electrode
- In some cases, direct electrical contact can be established between the enzyme and the electrode thus greatly increasing the efficiency of the electron transfer.
- immobilized mediators allow efficient electron transfer, resulting in a higher current density.
- Close proximity of the enzyme and the mediator to the transducer surface minimizes the electron transfer distance thereby resulting in faster response times.
- Because they are immobilized, mediators cannot escape the biosensor film and leach into the surroundings thereby allowing sensor use for in vivo measurements.
- The applied electrode can be operated at the desired voltage, eliminating background interference.

Electrochemical Enzyme Biosensors

Impact of Nanotechnology (Scognamiglio, 2013)



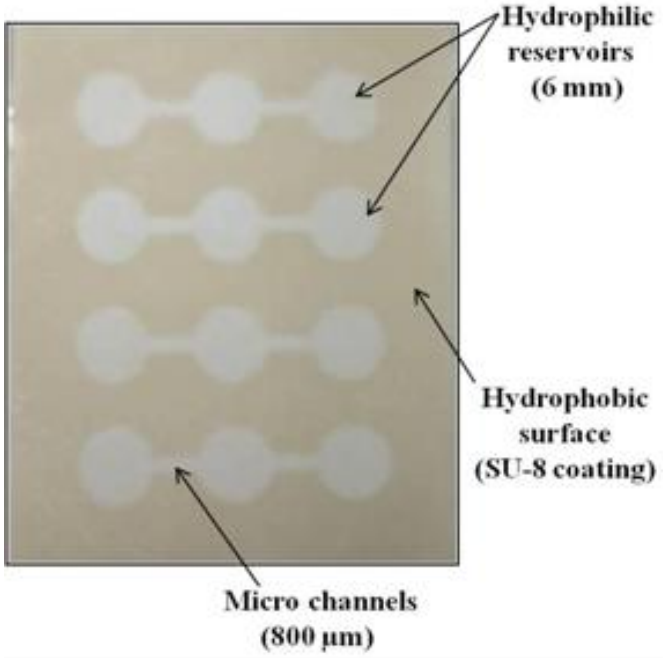
Optical Enzyme Biosensors

Example: Textile based biosensor for the determination of lactate in sweat *(Baysal G, 2014)*

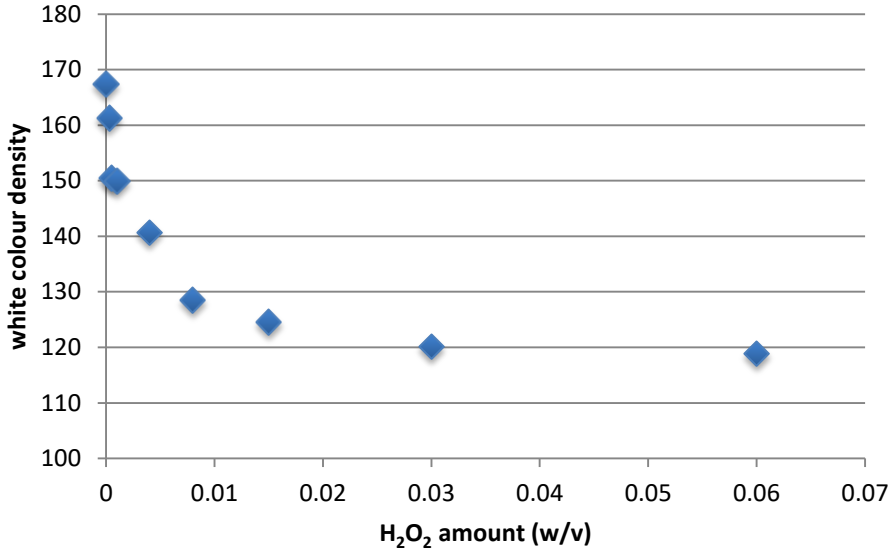
- Coupled reaction

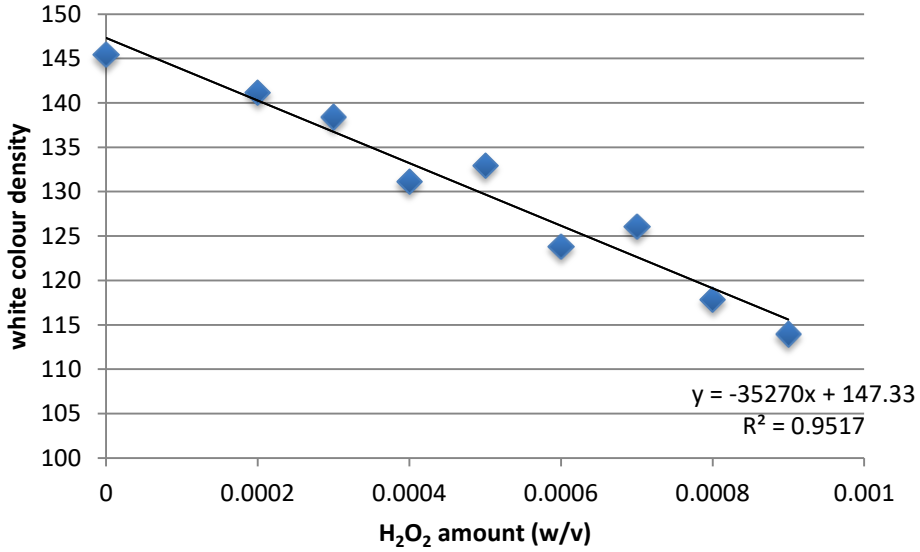
LOX: lactate oxidase, POX: peroxidase, ABTS: gives color when it is oxidized

- Reservoirs were made on textile surface by photolitography
- Color formation based on analyte concentration was determined

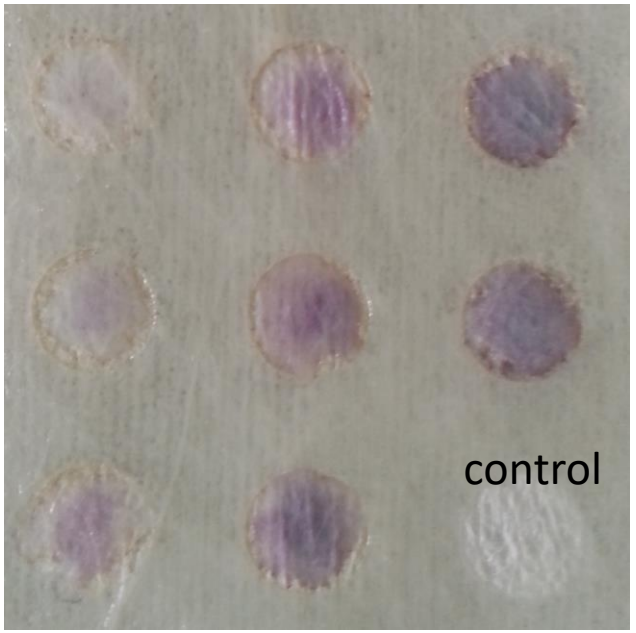


Optical Enzyme Biosensors





Color intensity increased with increasing analyte concentration



Glucose Biosensors

- Two main type (in enzyme-based):
  - Many glucose meters employ the oxidation of glucose catalyzed by glucose oxidase (GOX).
  - Others use a similar reaction catalysed by glucose dehydrogenase (GDH). This has the advantage of sensitivity over GOX but is more susceptible to interfering reactions

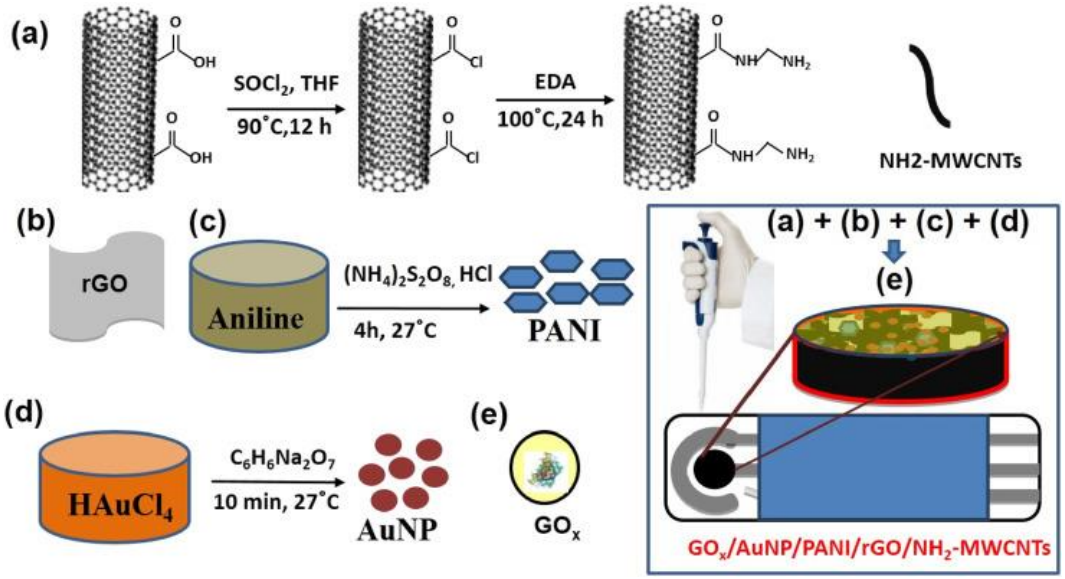
Glucose Biosensors

Impact of Nanotechnology (Scognamiglio, 2013)

- Nanobiosensors, provide to tune level of sensitivity to allow different detection limits for dermal interstitial fluids, urine, capillary or venous blood glucose monitoring.
- The use of nanomaterials functionalized with biocomponents can dramatically improve the stability and specificity of the detection system, yielding also reproducibility and reliability.
- Integration of nanoparticles, nano- and microfluidics, nanstructured surfaces improve performance and make construction of implantable sensors possible

Impact of Nanotechnology- Example:1

Glucose oxidase immobilized amine terminated multiwall carbon nanotubes/reduced graphene oxide/polyaniline/gold nanoparticles modified screen-printed carbon electrode for highly sensitive amperometric glucose detection / https://doi.org/10.1016/j.msec.2019.110075



- The current has enhanced 13.43 times using modified SPCE when compared to bare SPCE. Furthermore:

  - ✓ glucose biosensor has exhibited good reproducibility (0.9%, *n* = 7),
  - ✓ high stability (after 30 days 96% at −20 °C storage, 2 week 74.5% at −4 °C storage),
  - ✓ wide linear range (1–10 mM)
  - ✓ low detection limit (64 µM)

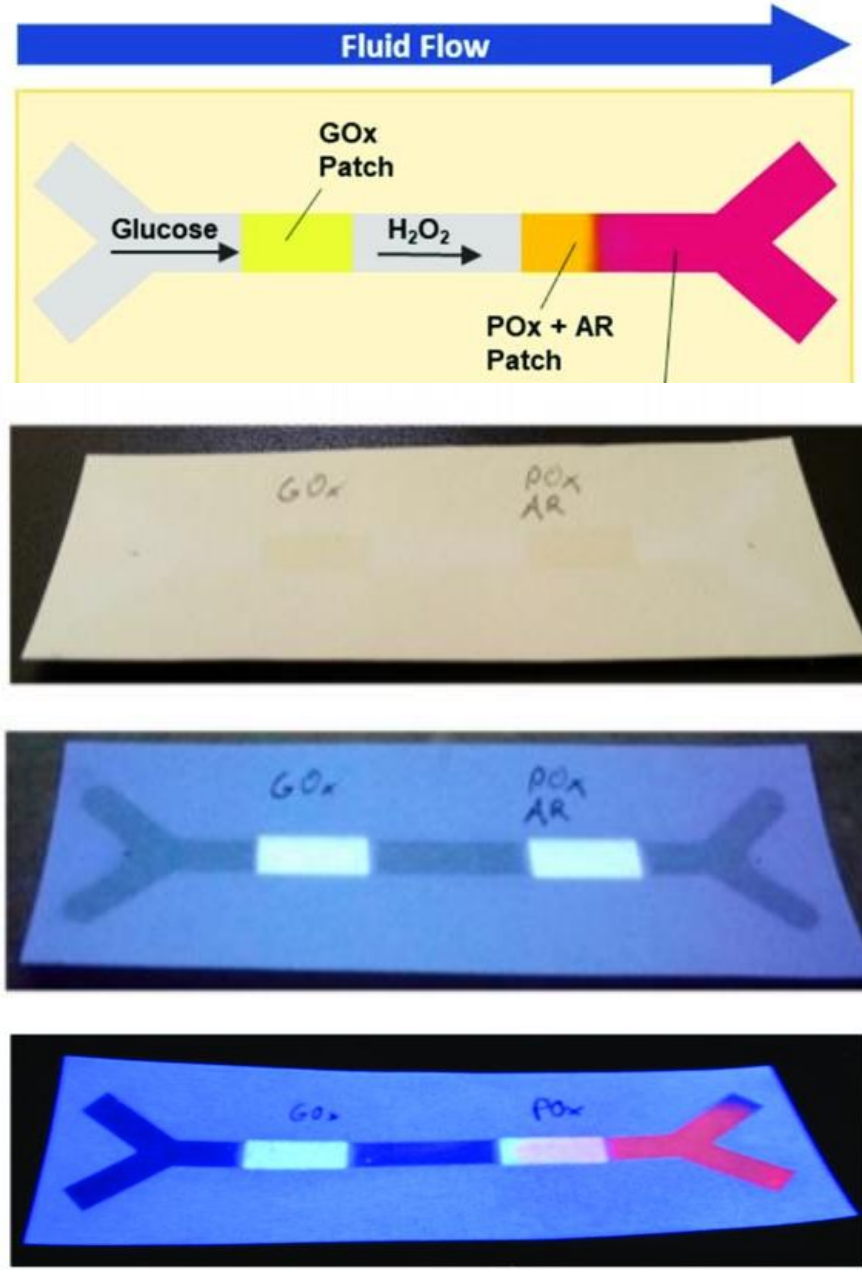
Impact of Nanotechnology- Example:2

Covalent Attachment of Enzymes to Paper Fibers for Paper-Based Analytical Devices

/Front. Chem., 27 June 2018 | https://doi.org/10.3389/fchem.2018.00214

Microfluidic paper-based glucose sensor as a proof-of-concept application:

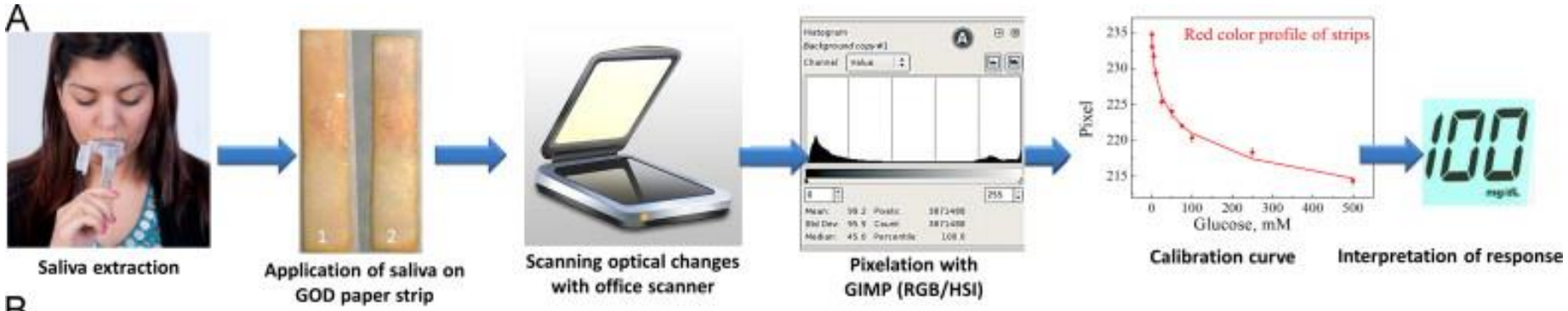
- Schematic illustration of the work principle: A solution of glucose is transported through the microfluidic channel by capillary forces. The glucose passes the GOx patch, where it is oxidized to a gluconolactone concomitant with the formation of H 2 O 2. The latter species oxidizes the non-fluorescent Amplex Red dye present within the POx patch to red-fluorescent resorufin.
- photograph of the microfluidic sensor under visible light.
- photograph of the microfluidic sensor under UV light.
- photograph of the microfluidic sensor after use.



A paper strip based non-invasive glucose biosensor for salivary analysis

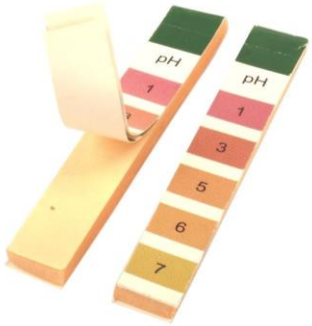
(Biosensors and Bioelectronics, 2015)

- This work presents investigation on the possibilities of using saliva as alternate body fluid for non-invasive glucose monitoring
- The biosensor was developed by immobilising glucose oxidase onto a filter paper along with a pH color indicator and then reacting it with synthetic glucose samples.
- The filter paper changed color based on concentration of glucose in reaction media and hence, by RGB profiling through an office scanner the glucose concentration in the reaction medium was deduced.
- The biosensor was validated on clinical samples and correlation between SGL (salivary glucose) and BGL (blood glucose) levels can enable low cost mass diagnostic sensor for rapid screening of diabetes.



B

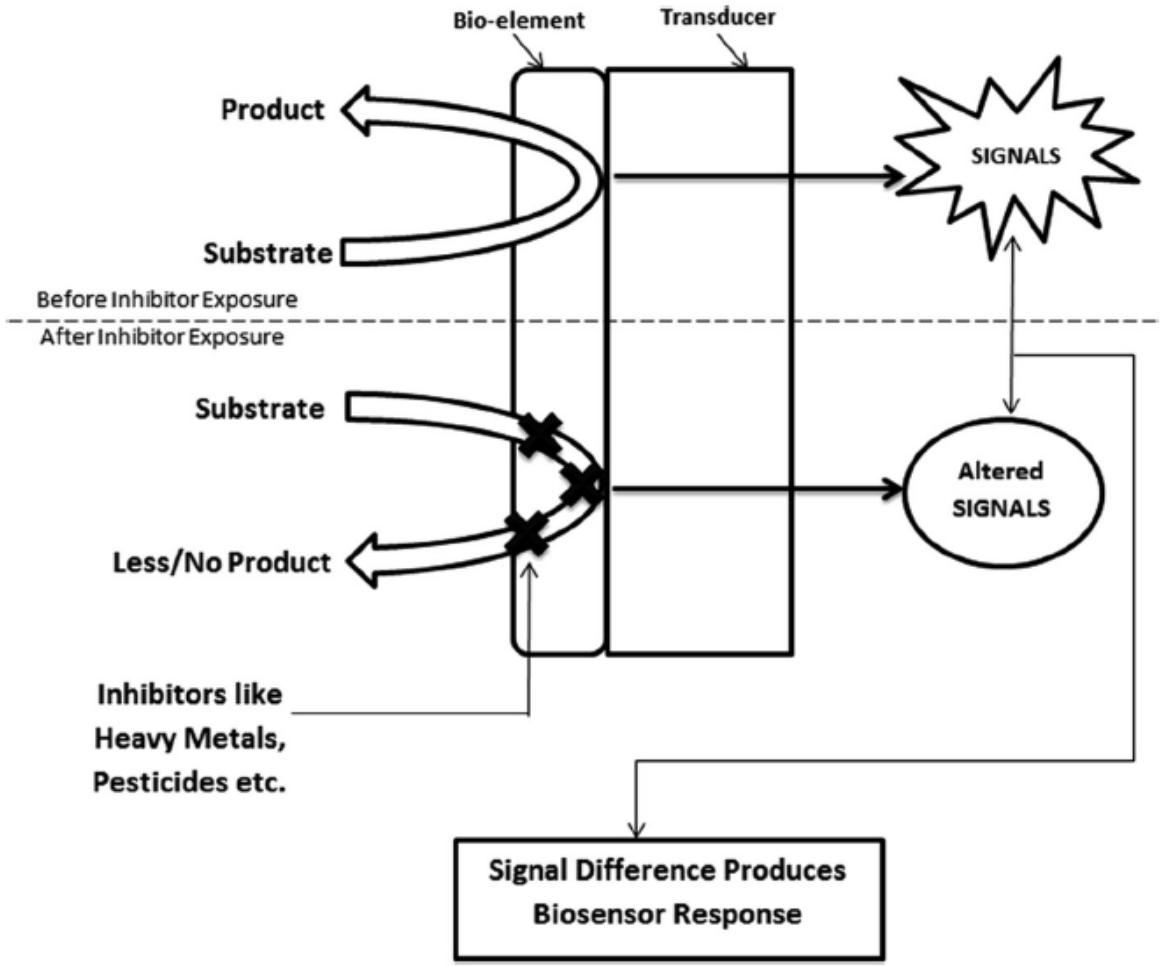
(A) Schematics of salivary glucose detection using paper strip based biosensor. (B) Color of filter paper strips used in the study at the time of application of sample (1) and after 45 s (2).



Inhibition-based Enzyme Biosensors

Inhibition can be:

- Reversible
  - Reusable biosensors
  - heavy metal, pesticic food preservative, nicotine, etc
- Irreversible
  - Disposible
  - Mercury ions, organophosphate pesticides, etc



Inhibition-based Enzyme Biosensors

Cholinesterase Biosensors

BASIC FACTS:

- acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)
- AChE is responsible from the ending the signal in synaptic cleft

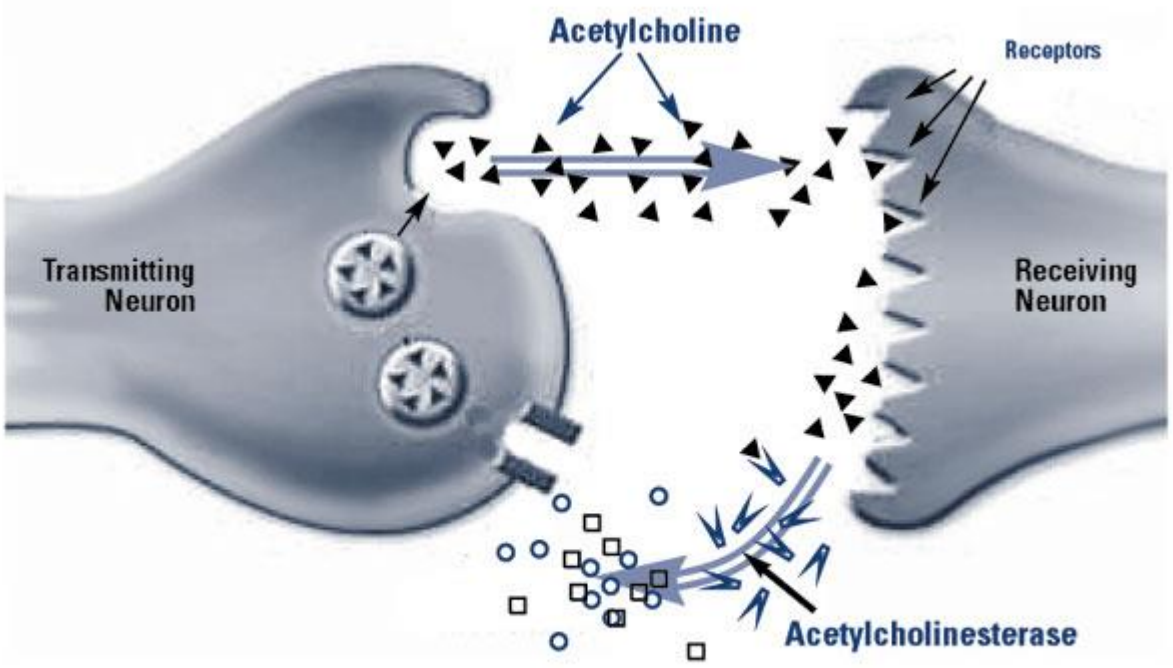


Fig. 1. After signalling, acetylcholine is released from receptors and broken down by acetylcholinesterase to be recycled in a continuous process.

Inhibition-based Enzyme Biosensors

Cholinesterase Biosensors

- Inhibitors:
  - Drugs (neostigmine, tacrine, etc)
  - Insecticides (aldicarb, carbofuran, etc)
  - Chemical weapons (Soman)
- Repeated and unchecked firing of electrical signals can cause uncontrolled, rapid twitching of some muscles, paralyzed breathing, convulsions, and in extreme cases, death

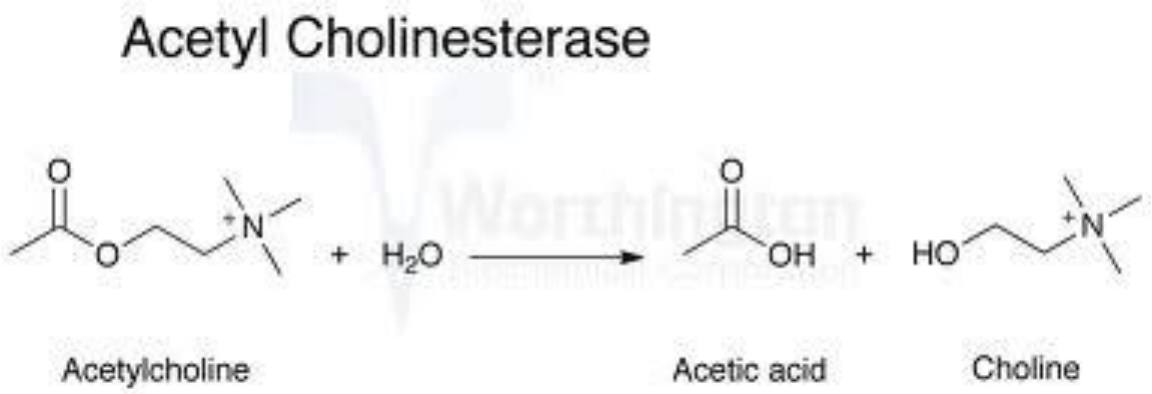
☺ Most of the enzymes are susceptible to a very low concentration of inhibitors, thus increase the sensitivity of biosensor.

☹ presence of some other types of inhibitors in the assay sample may inhibit the enzyme activity and therefore produces unexpected results.

Inhibition-based Enzyme Biosensors

Cholinesterase Biosensors

- Inhibition-based assay has a major advantage in an amplification of the measured signal.
- One molecule of an inhibitor can inhibit the production of thousands molecules of substrate. For example, human AChE can convert 6,500 molecules of ACh/s
- Irreversible inhibitors kill the enzyme; therefore devices based on cholinesterases are typically considered as disposable.
- Reaction:



- When inhibitor is present, the reaction will slow down…….

Inhibition-based Enzyme Biosensors

Cholinesterase Biosensors

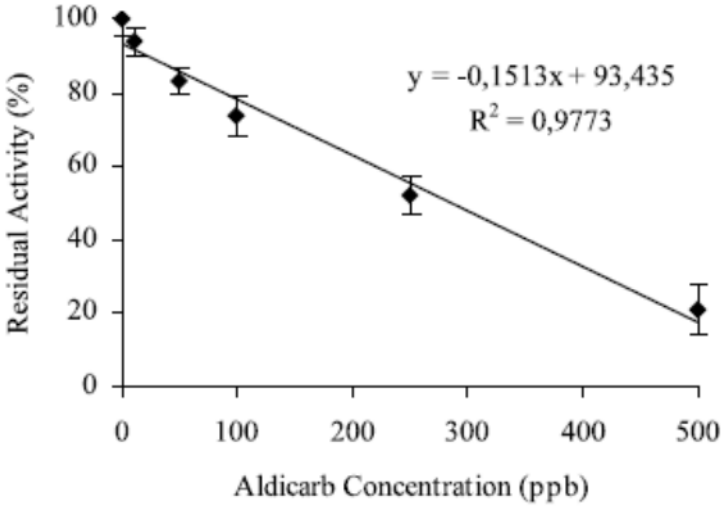
Example: Aldicarb (pesticide)

- Coupled reaction:

I. $Ach + H_2O \xrightarrow{AchE} choline + acetate$

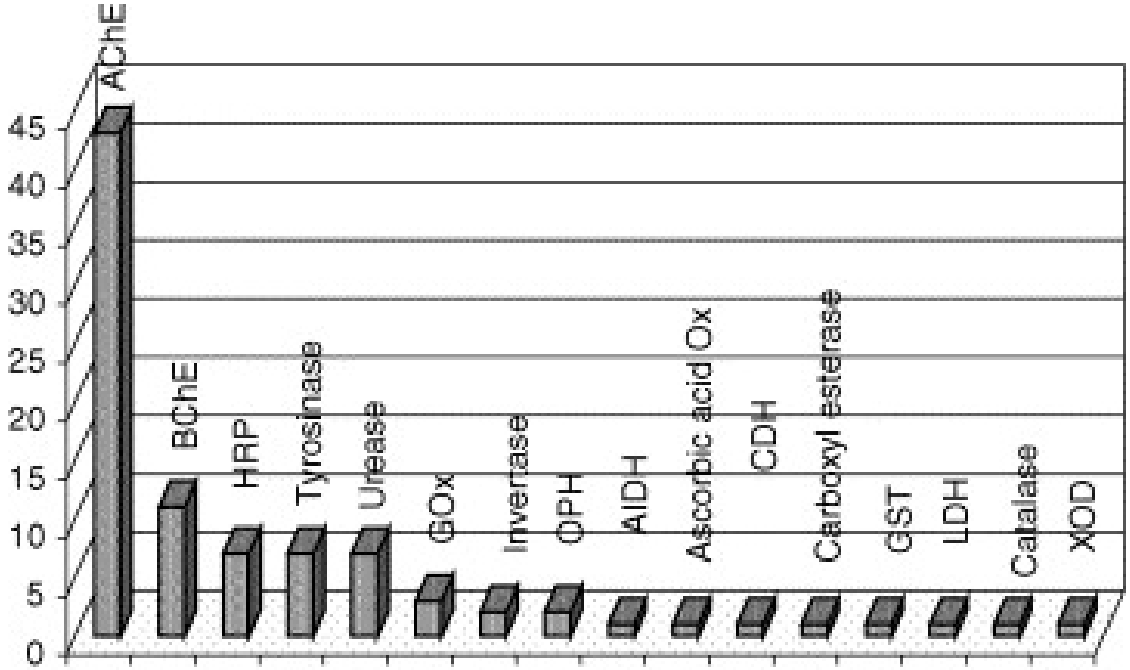
II. $Choline + 2O_2 + H_2O \xrightarrow{choline\ oxidase} betaine + peroxide\ (H_2O_2)$

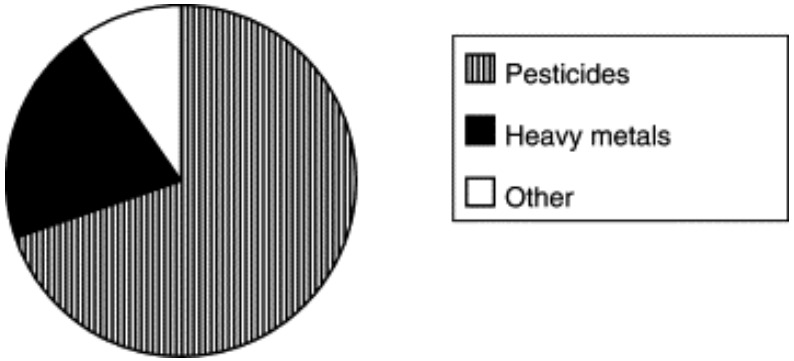
- Rxn could be follow with an O_2 or H_2O_2 electrode (electrochemical)



Inhibition-based Enzyme Biosensors

Amine, 2006



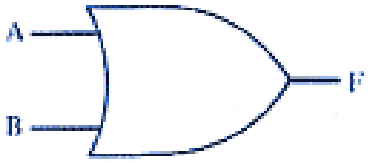
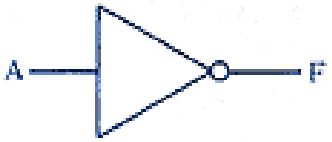
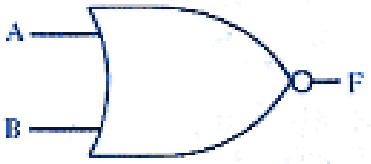


Logic gates

↓

Enzyme Logic Gates:

Biochemical Computing

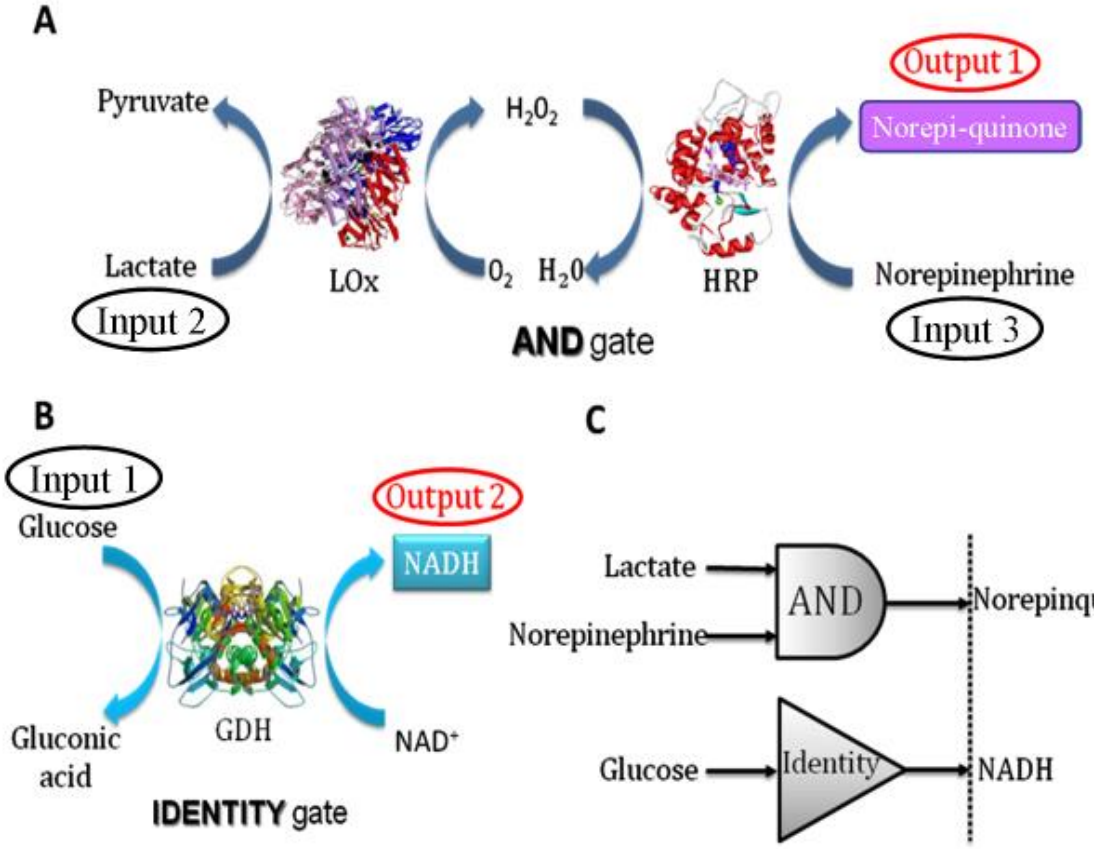
Name	Graphic Symbol	Algebraic Function	Truth Table
AND		$F = A \cdot B$ or $F = AB$	A B | F 0 0 | 0 0 1 | 0 1 0 | 0 1 1 | 1
OR		$F = A + B$	A B | F 0 0 | 0 0 1 | 1 1 0 | 1 1 1 | 1
NOT		$F = \overline{A}$ or $F = A'$	A | F 0 | 1 1 | 0
NAND		$F = (\overline{AB})$	A B | F 0 0 | 1 0 1 | 1 1 0 | 1 1 1 | 0
NOR		$F = (\overline{A + B})$	A B | F 0 0 | 1 0 1 | 0 1 0 | 0 1 1 | 0

Use of Biochemical Computing

http://people.clarkson.edu/projects/nanophys/

- A sensor for decision-making for monitoring and treatment of injured civilians and soldiers.
- An integrated therapeutic feedback-loop system
- INPUT: lactate, norepinephrine and glucose
- OUTPUT: norepi-quinone and NADH
  - Physiologically normal concentrations: 0
  - Abnormally increased concentrations: 1
    - indicative of various injury conditions such as traumatic brain injury (TBI) and hemorrhagic shock (HS)

http://people.clarkson.edu/projects/nanophys/



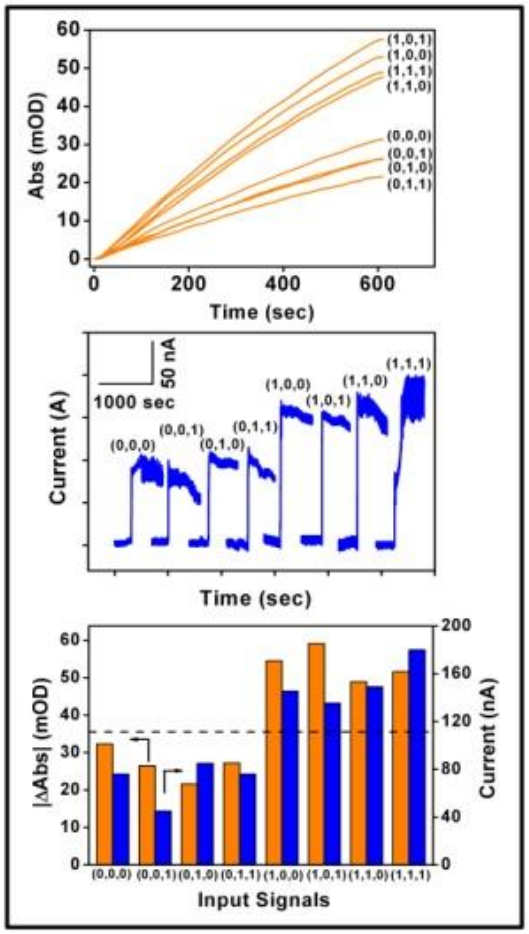


Figure 13. (left panel) Biochemical reactions catalyzed by LOx and HRP (A) and GDH (B) being used to perform AND / IDENTITY logic operation and the equivalent logic system used for processing the lactate, norepinephrine and glucose input signals (C). (right panel) Time-dependent signals corresponding to the formation of NADH generated by the combined AND-IDENTITY logic system upon application of various combinations of the input signals (glucose, lactate and NE) measured by optical means (top) and amperometrically (middle). (Bottom) Bar diagram featuring the combined AND-IDENTITY logic operation of the optical and electrochemical systems. Absorbance measurement were performed at wavelength 340 nm. Electrochemical measurements were performed at +0.75 V. Dash line shows the threshold values separating digital 0 and 1 output signals produced by the both systems.

Table 1
The truth table for the combination of AND/IDENTITY logic gates.

Glucose input signal	Lactate input signal	NE input signal	NQ output signal	NADH output signal	Biomedical conclusions
0	0	0	0	0	Normal physiological conditions
1	0	0	0	1	Abnormal level of glucose—not related to injuries
0	0	1	0	0	Stress—not related to injuries
1	0	1	0	1	Physiologically not applicable
0	1	0	0	0	Hard physical exercise—not related to injuries
1	1	0	0	1	Physiologically not applicable
0	1	1	1	0	TBI
1	1	1	1	1	HS