Electron transfer pathways in electrochemical biosensors and in some other biodevices

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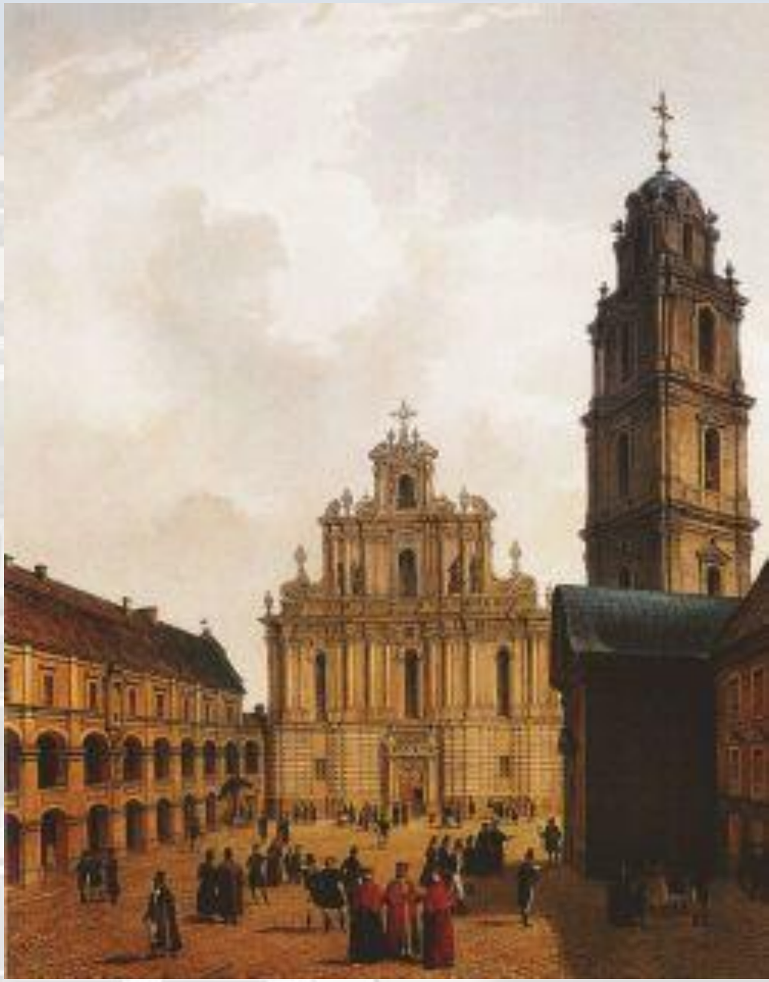
Lithuania (Lietuva)

Capital: Vilnius

3.0 Mln. People.

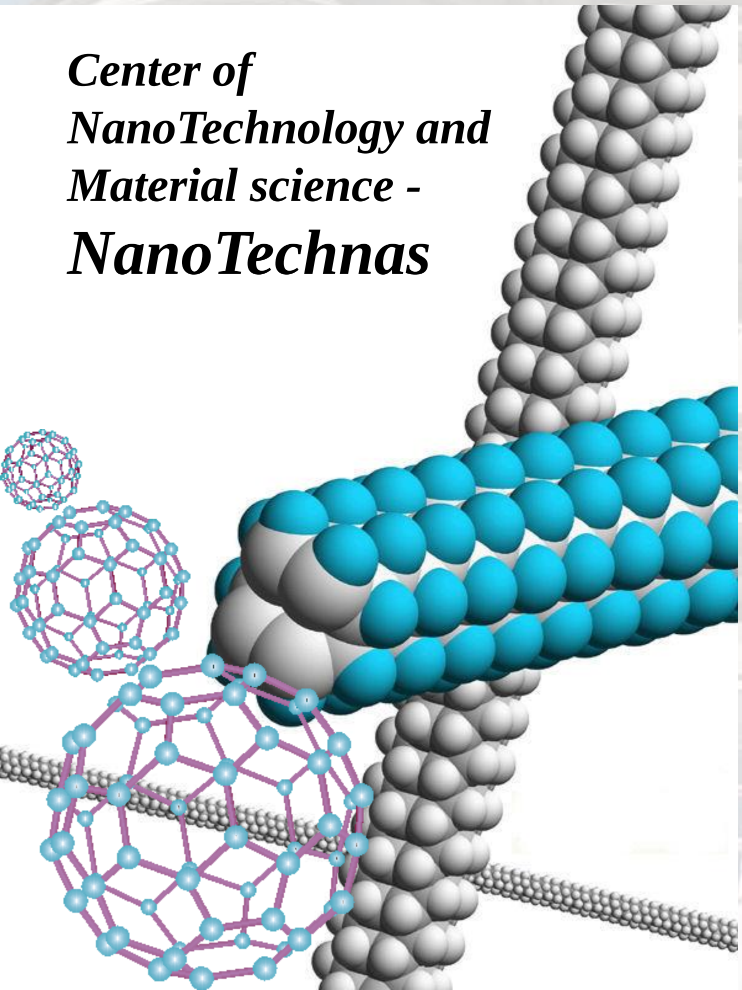
Vilnius University

Was founded in 1579



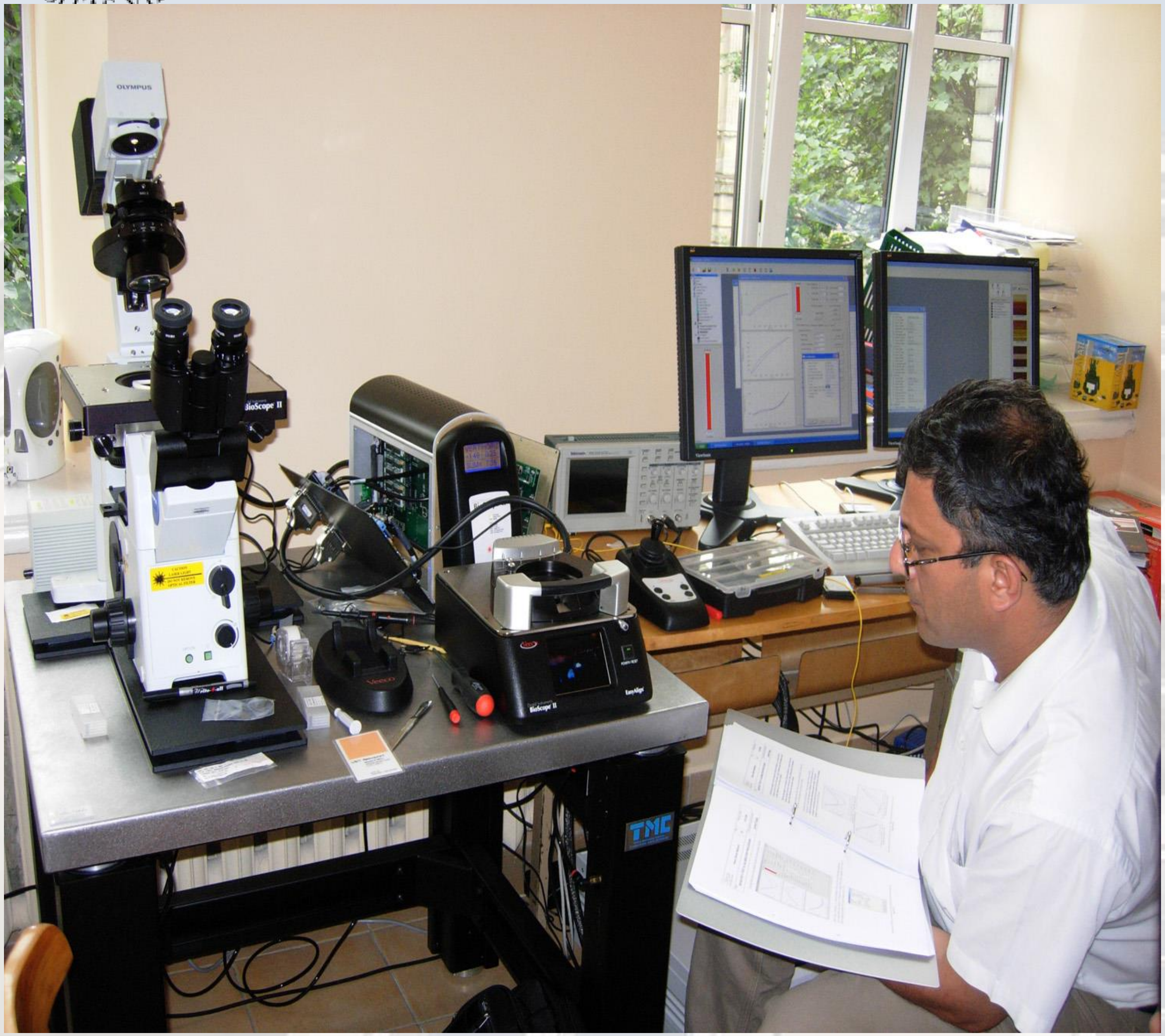
Center of NanoTechnology and Materials science - NanoTechnas

Faculty of Chemistry, Vilnius University

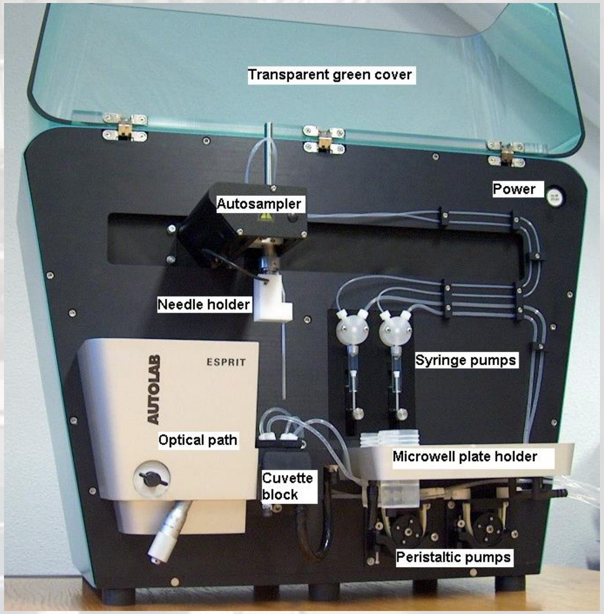


Center of NanoTechnology and Material science – NanoTechnas
established within 2005-2008

Some Research Directions in NanoTechnas



- **Bio – AFM (Veeco)**
- **SPR device (ECO-Chemie);**
- **QCM+EQCM (MacTex); Potentiostat/Galvanostat (ECO-Chemie);**



Some Research Directions in NanoTechnas

Sensors and Biosensors

Catalytic Enzymatic Sensors

DNA Sensors

Immuno- Sensors

Molecularly imprinted polymer based Sensors

Fuel cells and Biofuel cells

Treatment and Modification of Living cells

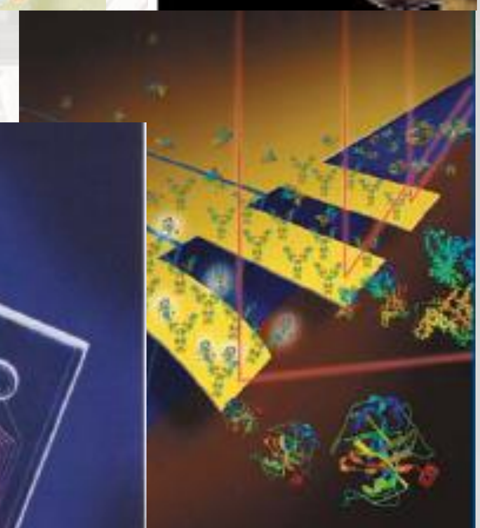
Biomedicine: Implants; Drug delivery systems

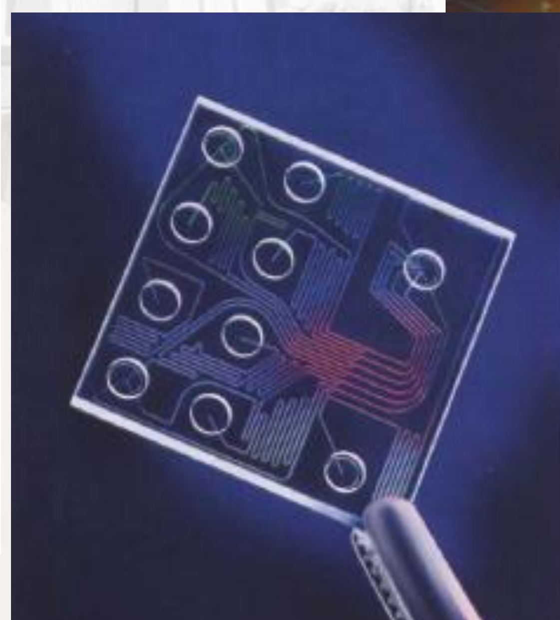
Introduction // Sensors // Lab on the chip

Do we need sensors?

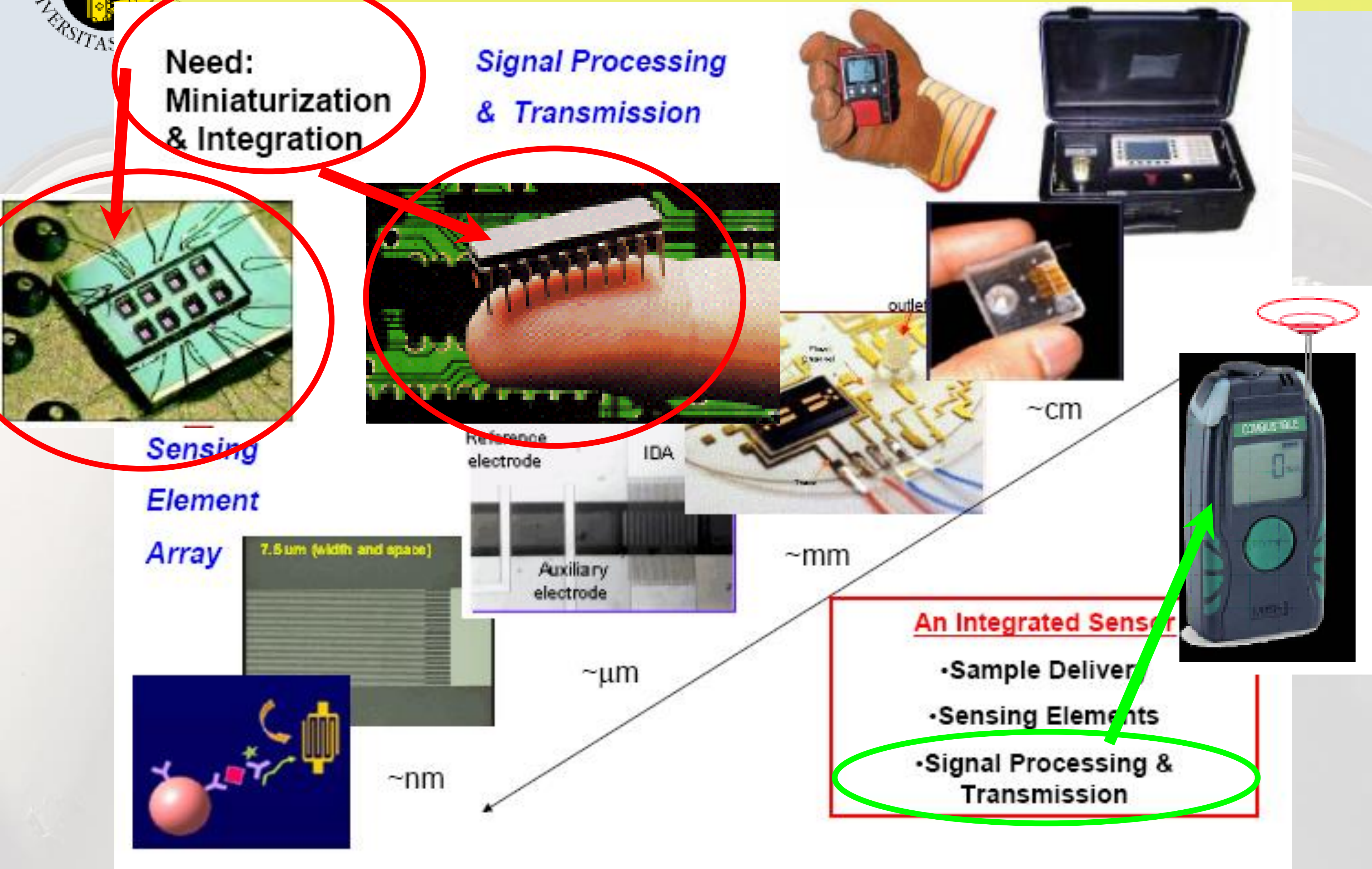
Why do we need sensors?

Sensors // What they are??

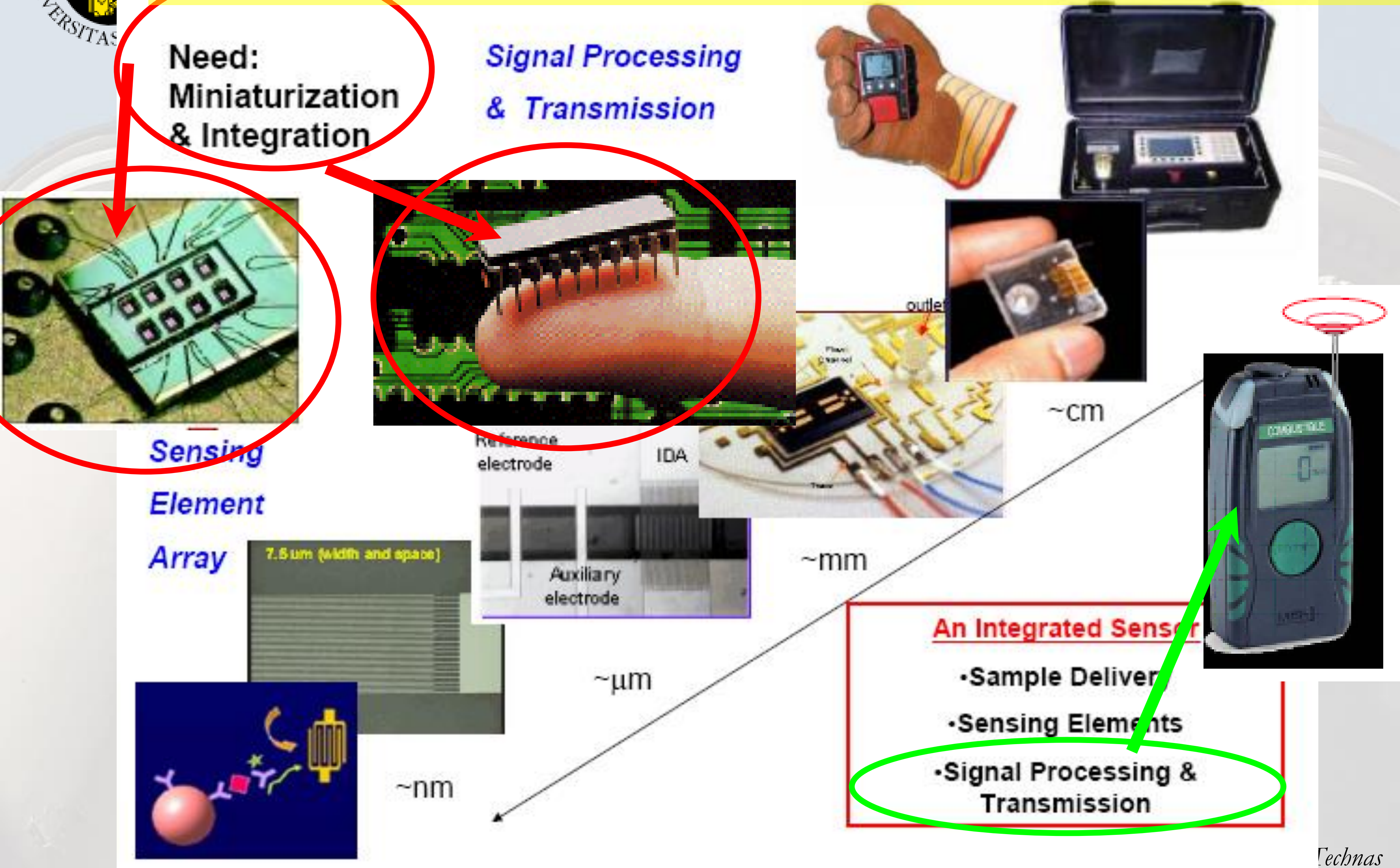




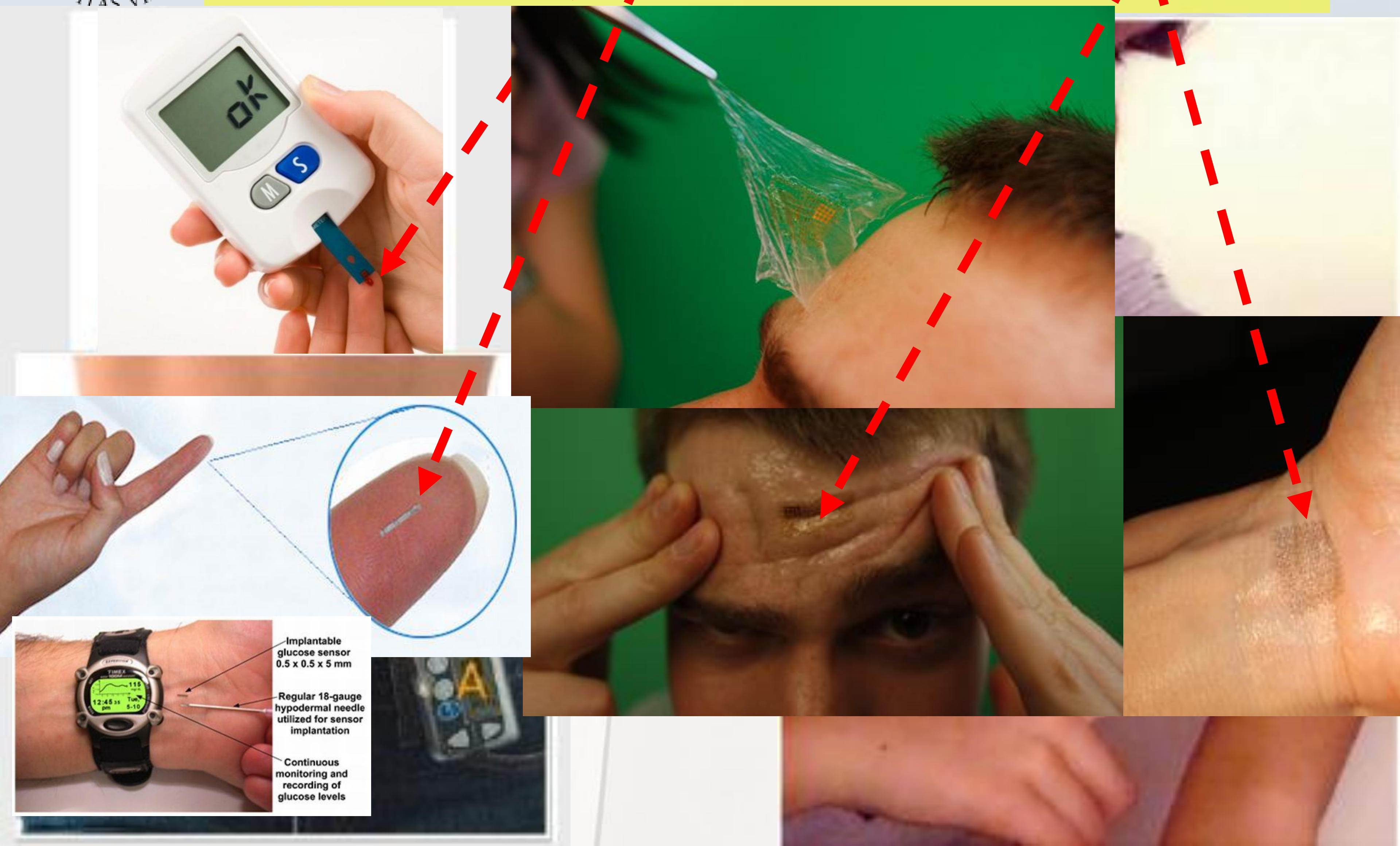
Introduction // Some Challenges in Sensorics and Biosensorics // Biofuel Cells in Biosensors

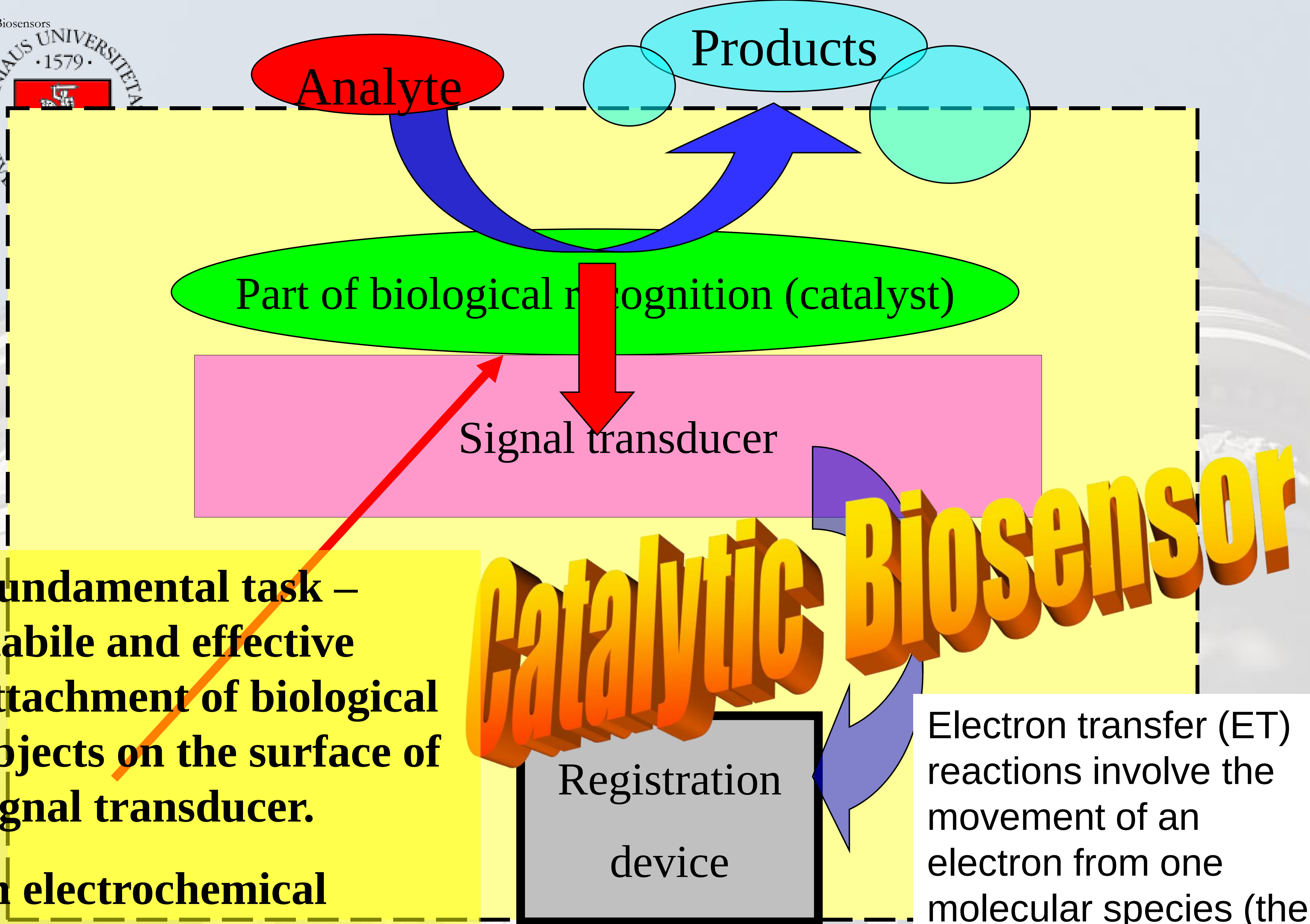


Application of Biofuel Cells in Biosensorics



Sensors Reality and Dreams





Fundamental task – stabile and effective attachment of biological objects on the surface of signal transducer.

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Electron transfer (ET) reactions involve the movement of an electron from one molecular species (the donor) to another (the acceptor)

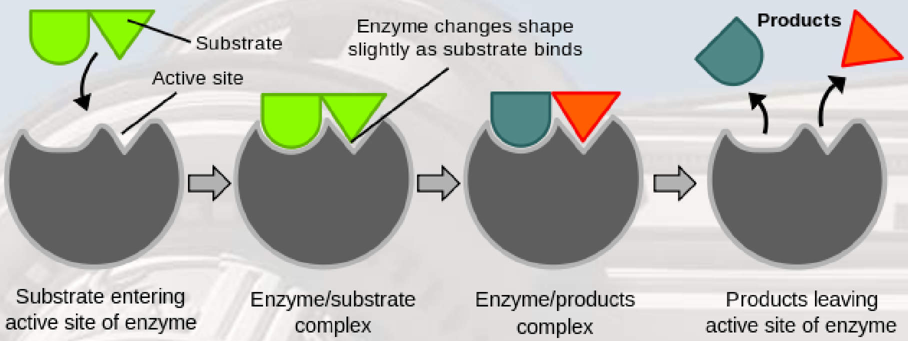
Enzyme catalyzed reactions

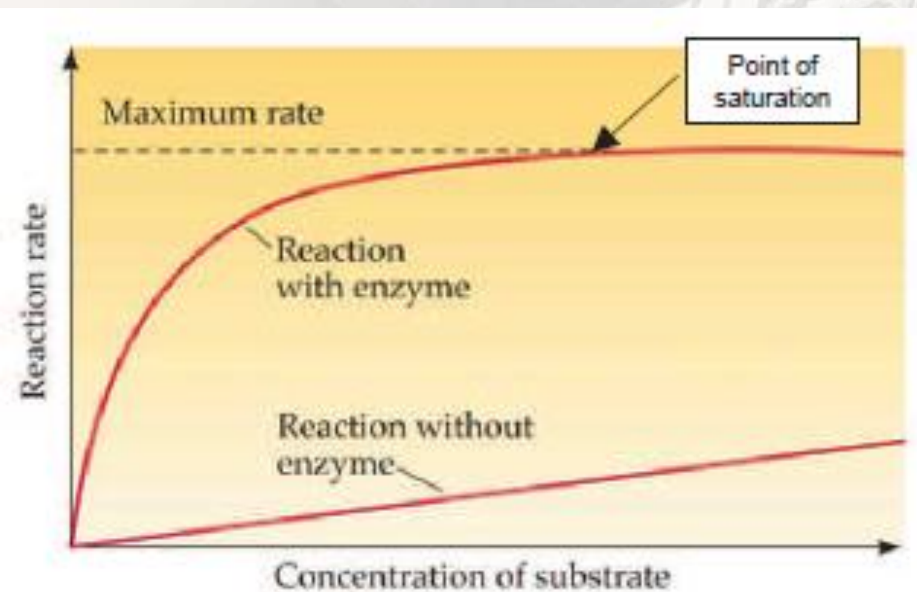
- In addition to many other types of catalysis biochemical catalysis also has number of advantages, e.g.:
- Enzymes are "green catalysts" that are produced by all living cells and/or microorganisms. **Life is impossible without enzymes…**
- **COST Action TD1102**

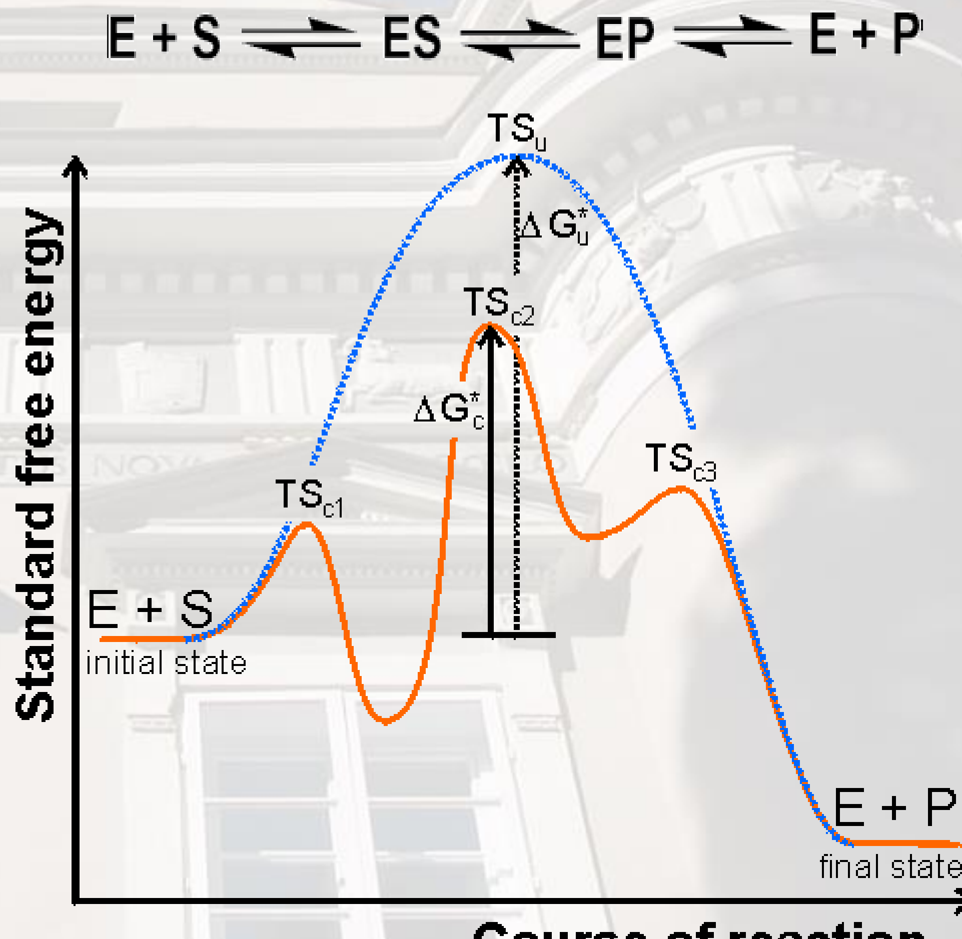
Enzymes are biological catalysts

- There are known about **40,000** different enzymes alone **in human cells**, each controlling a different chemical reaction. They **increase the rate of reactions** by a factor of between **10 to 10^{12} times**, allowing the chemical reactions that make life possible to take place at normal temperatures. They were discovered in fermenting **yeast in 1900 by Buchner**, and the name enzyme means "in yeast". As well as catalysing all the metabolic reactions of cells (such as respiration, photosynthesis and digestion), they may also act as motors, membrane pumps and receptors.

Enzymatic catalysis



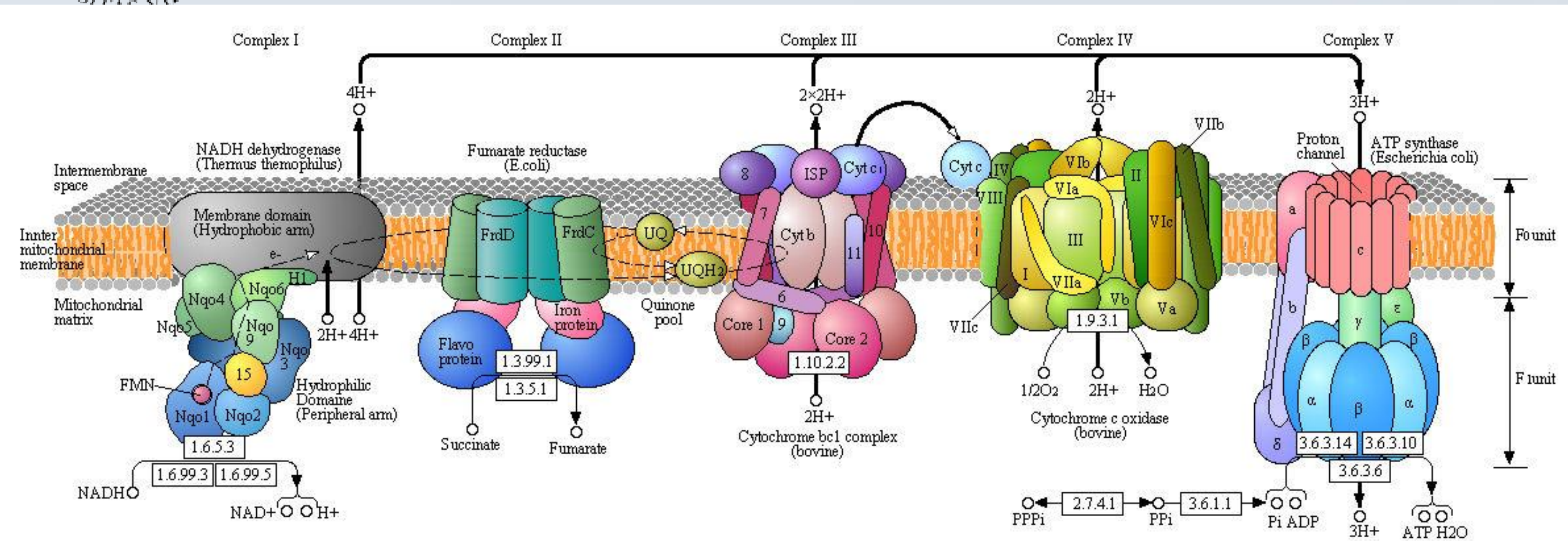


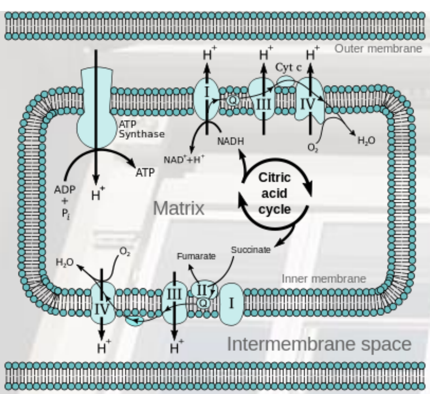


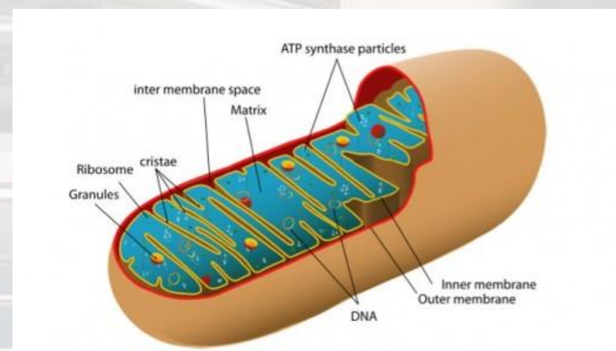
Classes of Enzymes

Class	Chemical Reaction Catalyzed	Sample Enzymes
Oxidoreductase	Oxidation-reduction in which oxygen and hydrogen are gained or lost	Cytochrome oxidase, lactate dehydrogenase
Transferase	Transfer of functional groups, such as an amino group, acetyl group, or phosphate group	Acetate kinase, alanine deaminase
Hydrolase	Hydrolysis (addition of water)	Lipase, sucrase
Lyase	Removal of groups of atoms without hydrolysis	Oxalate decarboxylase, isocitrate lyase
Isomerase	Rearrangement of atoms within a molecule	Glucose-phosphate isomerase, alanine racemase
Ligase	Joining of two molecules (using energy usually derived from the breakdown of ATP)	Acetyl-CoA synthetase, DNA ligase

Some Examples of Enzymatic Redox Reactions



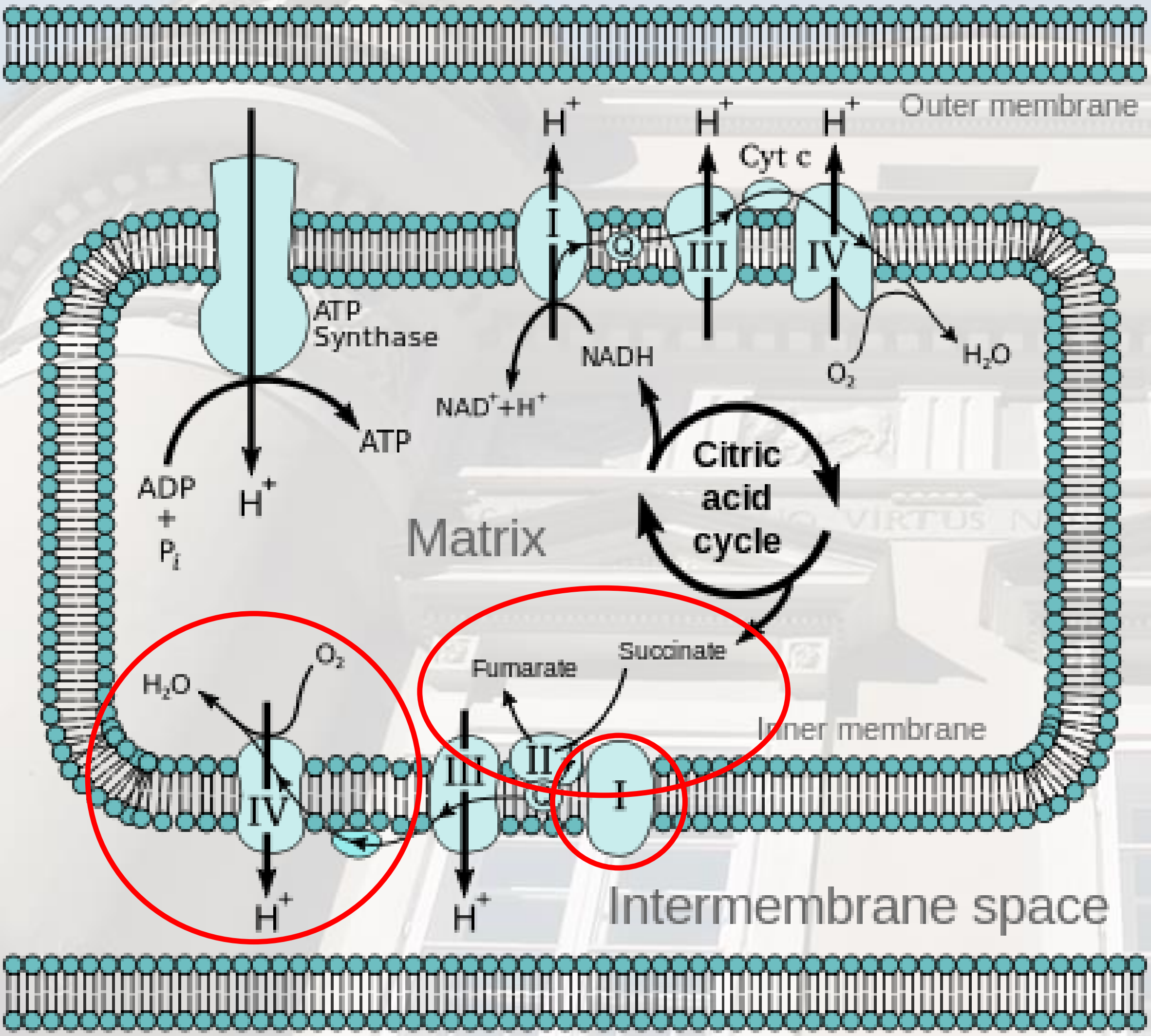




https://www.google.lt/search?q=sensors+today+and+tomorrow&source=lnms&tbm=isch&sa=X&ei=HA5GU6vlB4eoO6OwgZAC&ved=0CAYQ_AUoAQ&biw=1536&bih=762#q=oxidative+phosphorylation+mitochondria&tbm=isch&facrc=_&imgdii=_&imgrc=CF_NdLb9ECRi8M%253A%3BIt4Rm2ZstkPiaM%3Bhttp%253A%252F%252Femployees.csbsju.edu%252Fhjakubowski%252Fclasses%252Fch331%252Foxphos%252Foxphos2.jpg%3Bhttp%253A%252F%252Femployees.csbsju.edu%252Fhjakubowski%252Fclasses%252Fch331%252Foxphos%252Folcouplingoxphos.html%3B1204%3B411

Some Examples of Enzymatic Redox Reactions

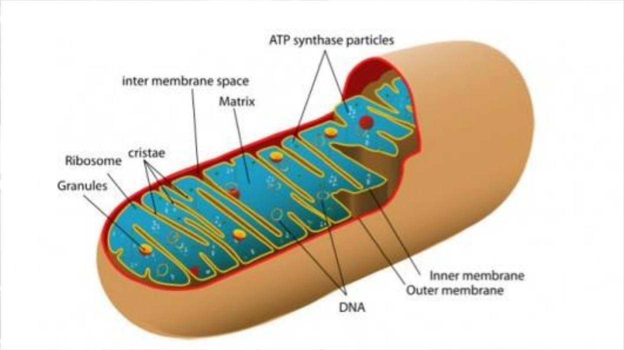
(Oxidative phosphorylation)



Complex I or [NADH-Q oxidoreductase](http://en.wikipedia.org/wiki/NADH-Q_oxidoreductase).

Complex II: [Succinate-Q oxidoreductase](http://en.wikipedia.org/wiki/Succinate-Q_oxidoreductase).

Complex IV: [cytochrome c oxidase](http://en.wikipedia.org/wiki/Cytochrome_c_oxidase).



http://en.wikipedia.org/wiki/Oxidative_phosphorylation

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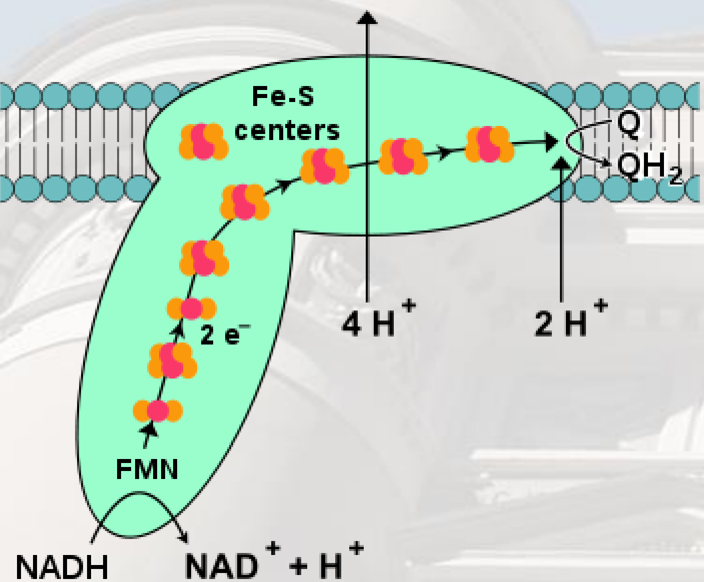
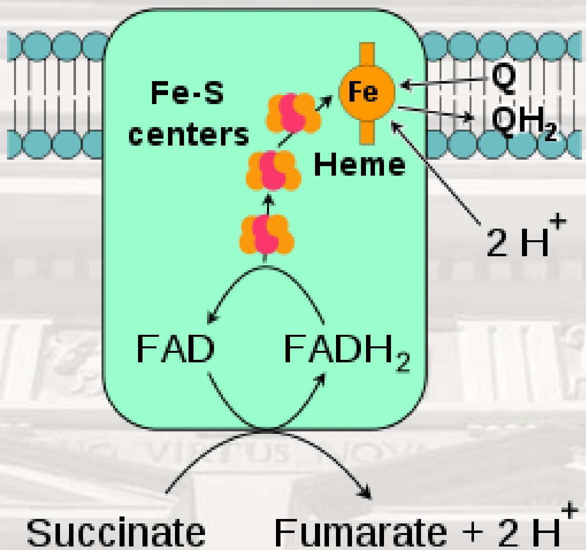
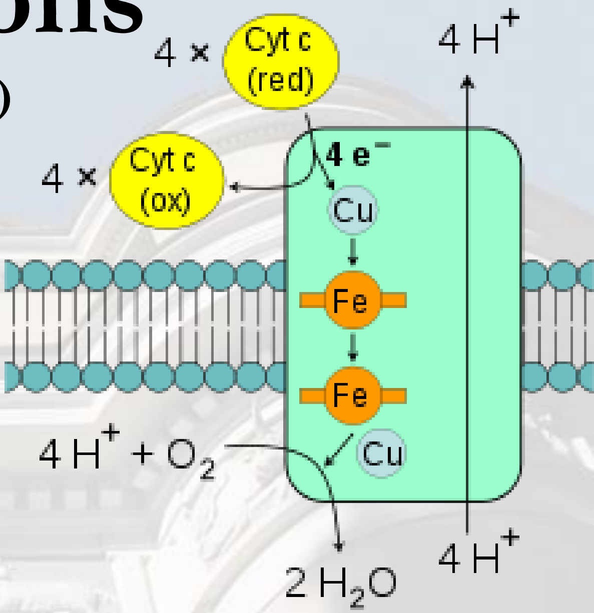


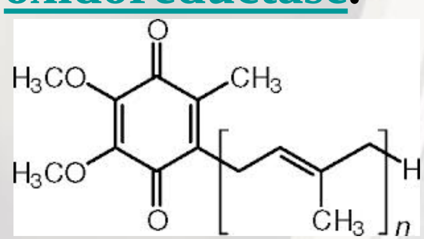




Complex I or [NADH-Q oxidoreductase](#).

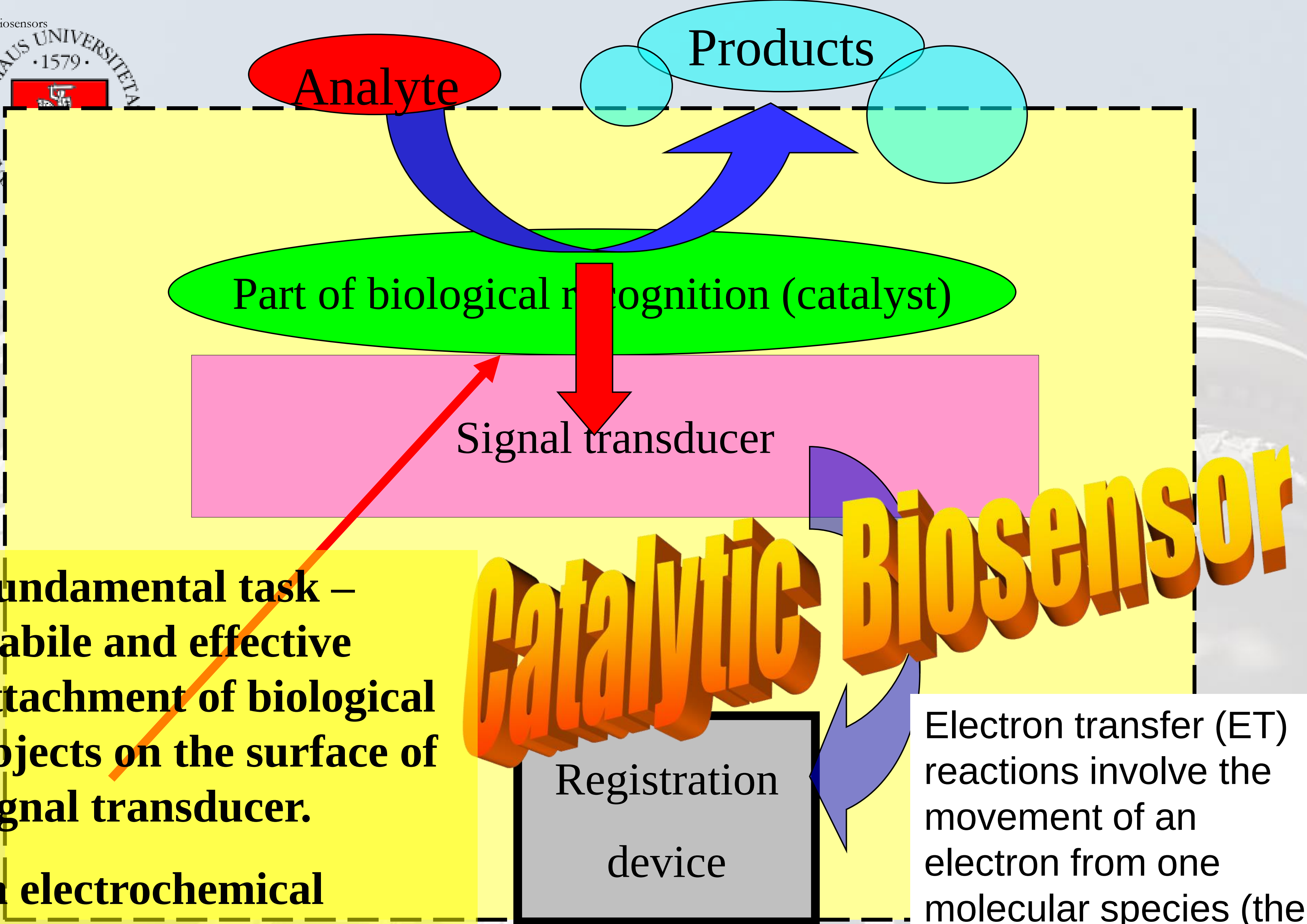
Complex II: [Succinate-Q oxidoreductase](#).

Complex IV: [cytochrome c oxidase](#).



Ubiquinone

http://en.wikipedia.org/wiki/Oxidative_phosphorylation



Fundamental task – stabile and effective attachment of biological objects on the surface of signal transducer.

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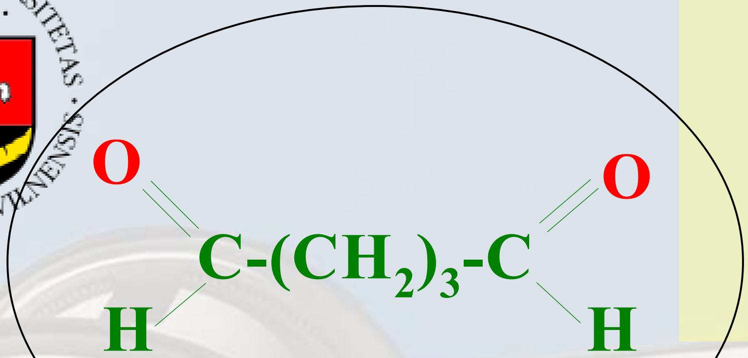
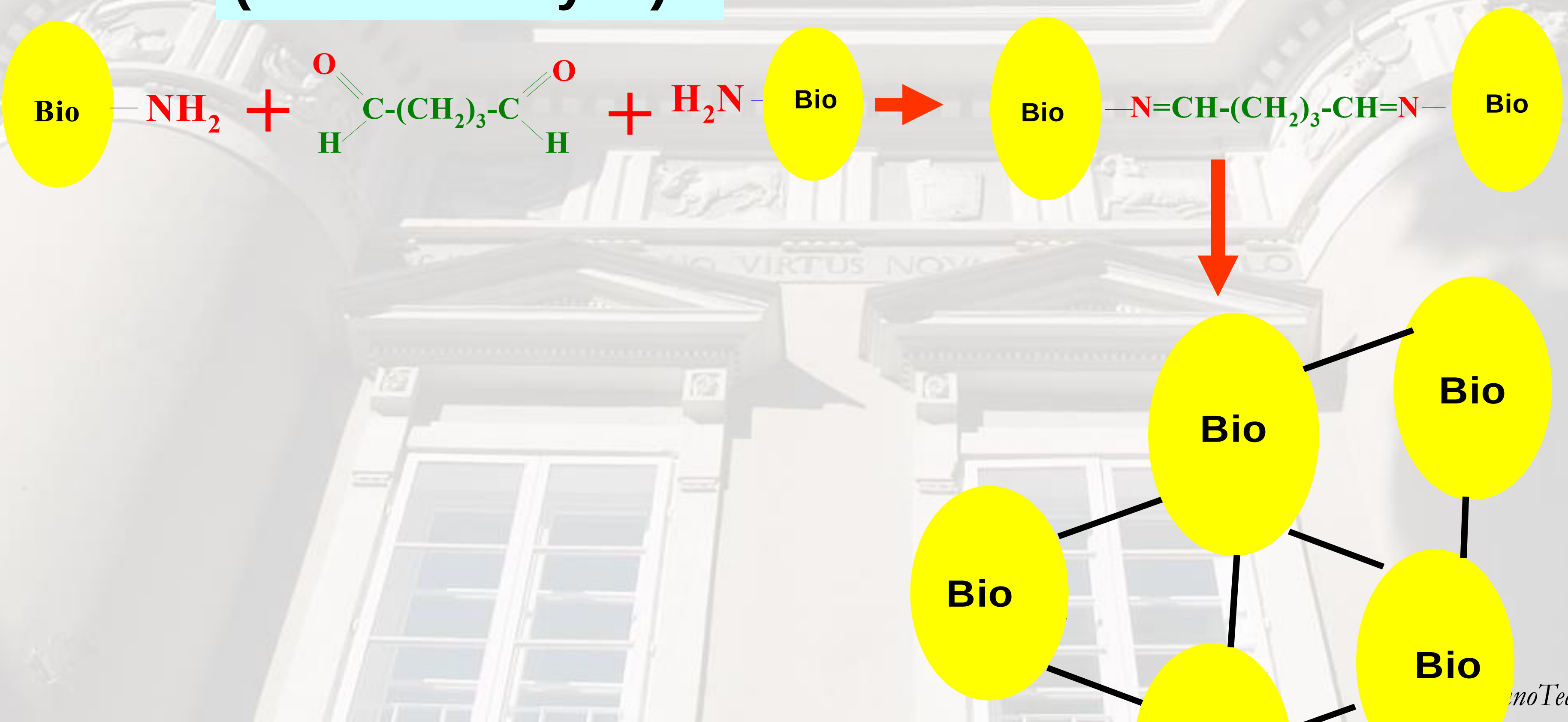
Major immobilization methods used for design of biosensors

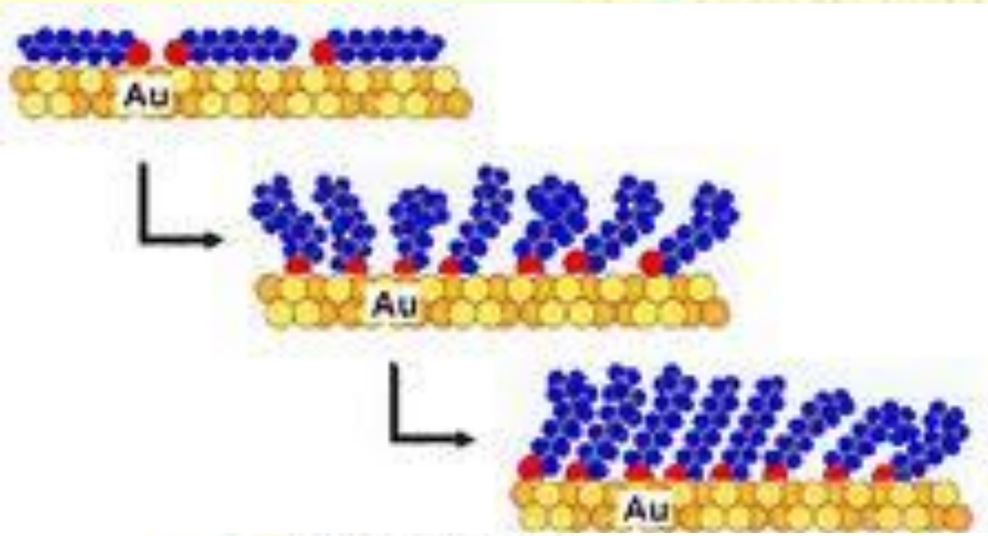
- **Adsorption;**
- **Covalent attachment;**
- **Cross-linking with chemical agents;**
- **Application of SAM's followed by covalent attachment;**
- **Entrapment within polymers.**

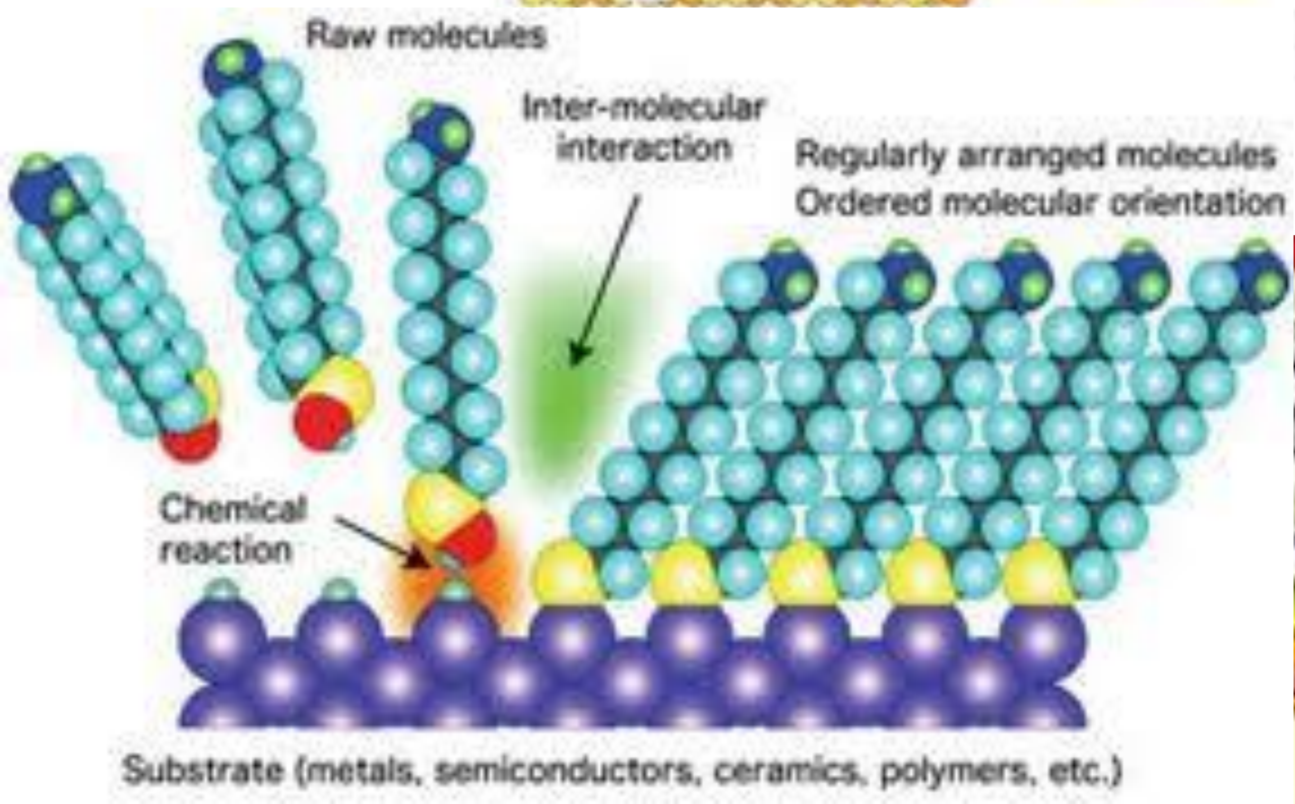
Immobilization methods cross-linking

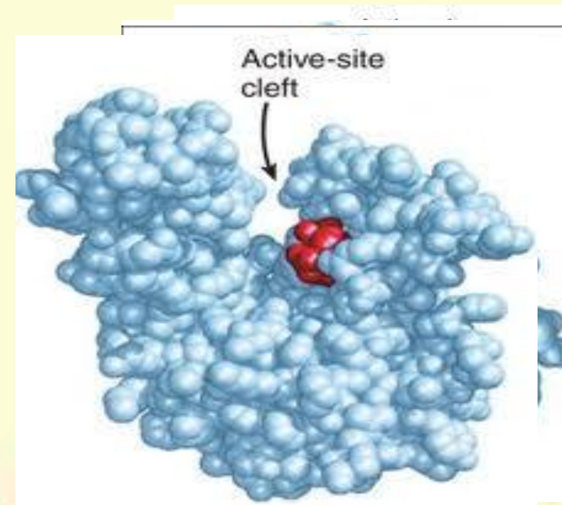


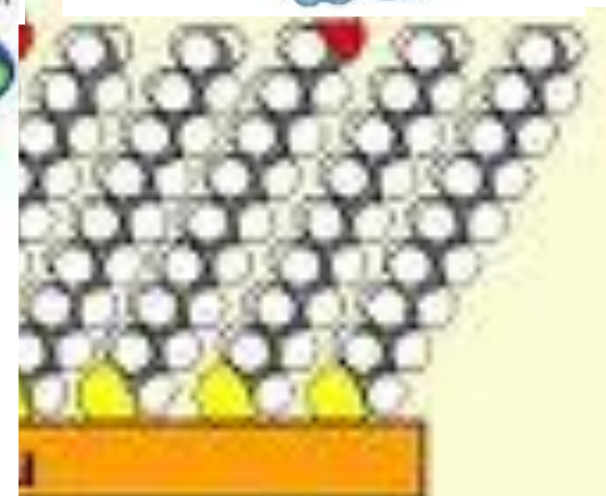
Pentandiol (Glutaraldehyde)




Au
Au
Au

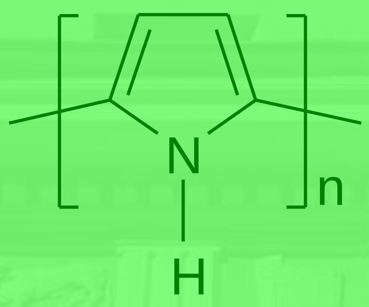

Raw molecules
Inter-molecular interaction
Regularly arranged molecules
Ordered molecular orientation
Chemical reaction
Substrate (metals, semiconductors, ceramics, polymers, etc.)


Active-site cleft



Entrapment within polymers / Chemical structures of common conducting polymers / Selection principles

Can be electrochemically synthesized on the conducting surfaces.



Polypyrrole

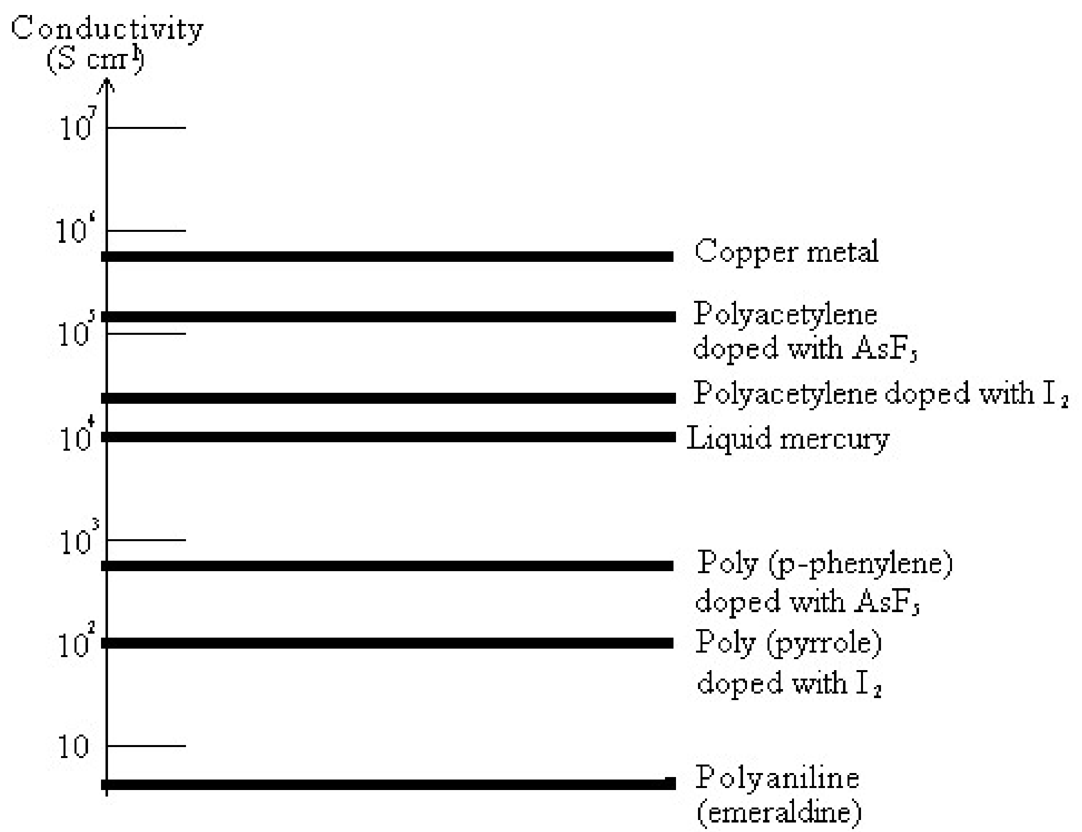
Can be doped by biological objects e.g.: proteins.

The layer is stabile and elastic

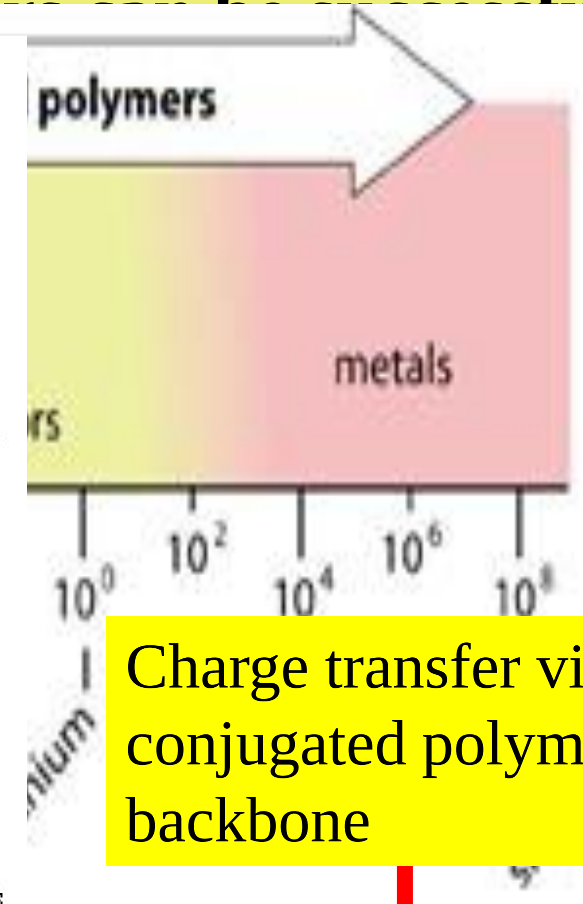
Can by synthesized at neutral pH. (It is especially important during immobilization of biological objects)

.

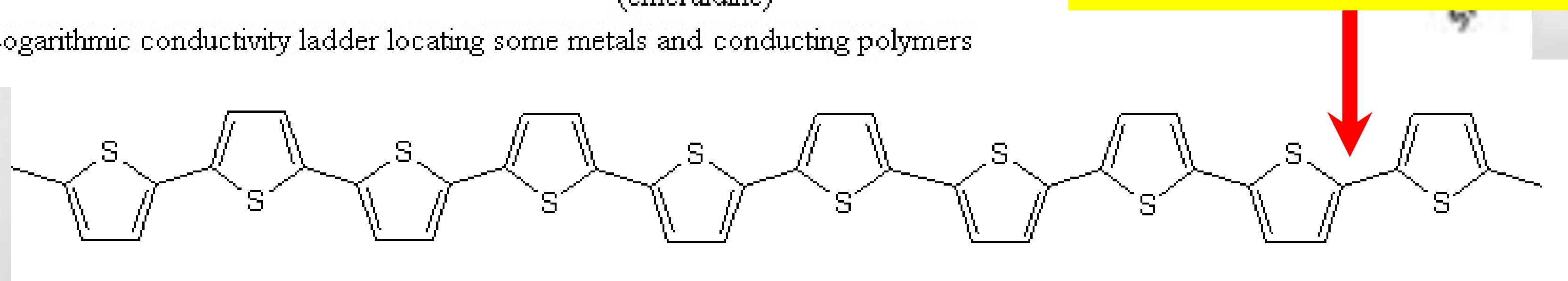
Co… …lly ap… …es



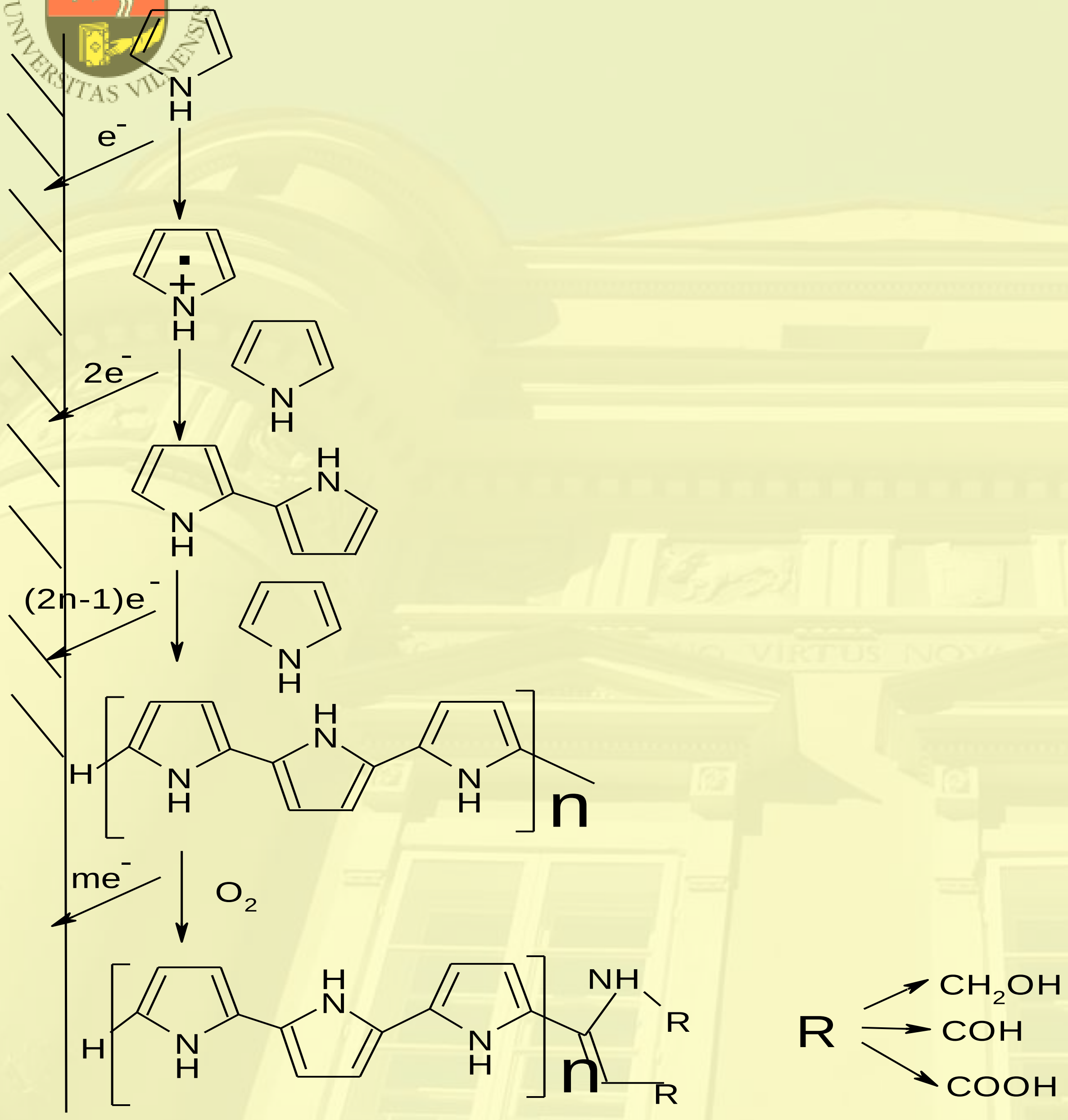
Logarithmic conductivity ladder locating some metals and conducting polymers

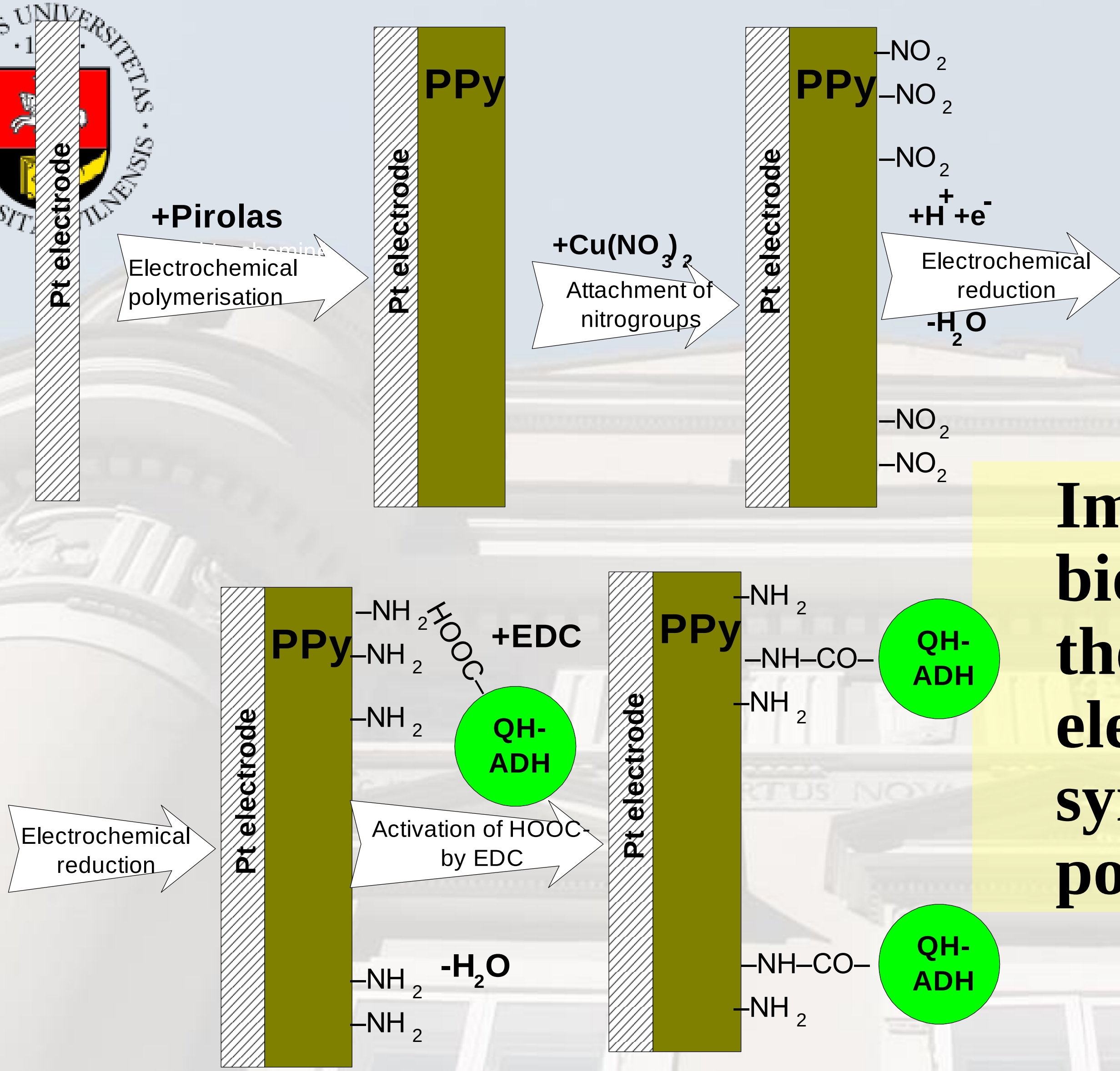


Charge transfer via conjugated polymeric backbone



The Electrochemical synthesis of conducting polymers (e.g. polypyrrole)

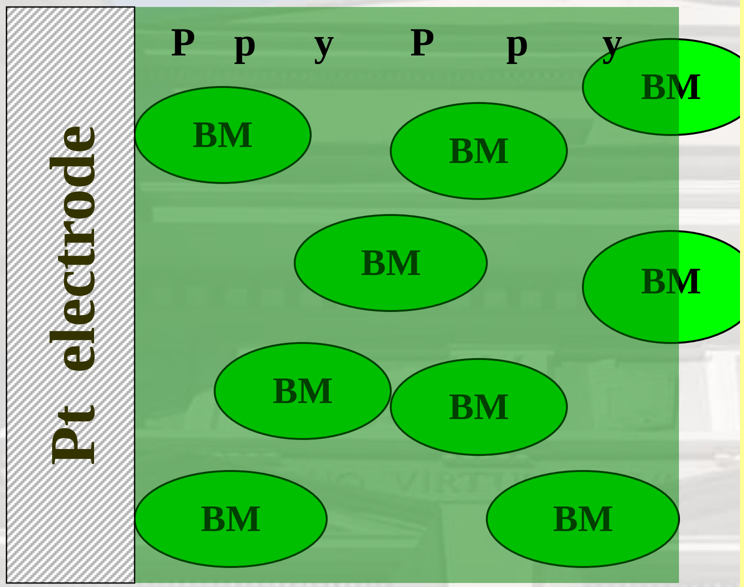


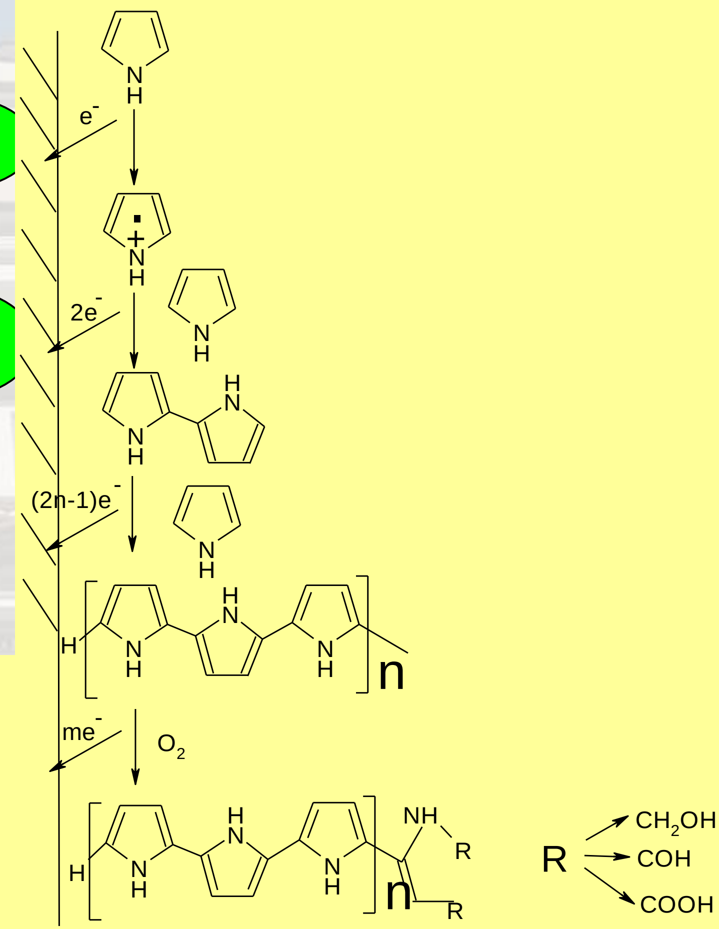


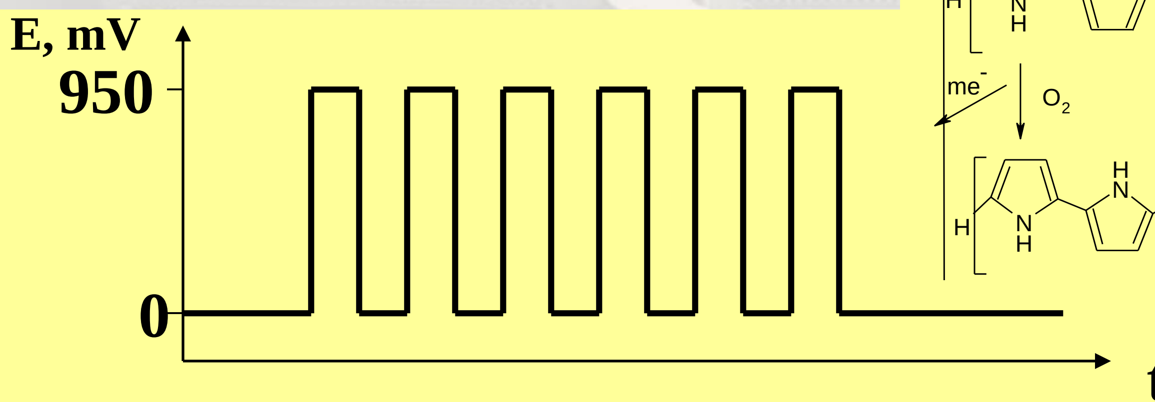
Immobilization of bio-molecules on the surface of electrochemically synthesized polypyrrole

Ramanavicius A., Habermüller K., Razumiene J., Meskys R., Marcinkeviciene L., Bachmatova I., Csöregi E., Laurinavicius V., and Schuhmann W. (2000) **An oxygen-independent ethanol sensor based on quinohemoprotein alcohol dehydrogenase covalently bound to a functionalized polypyrrole film**, *Progress in Colloid and Polymer Science*, 116, 143-148.

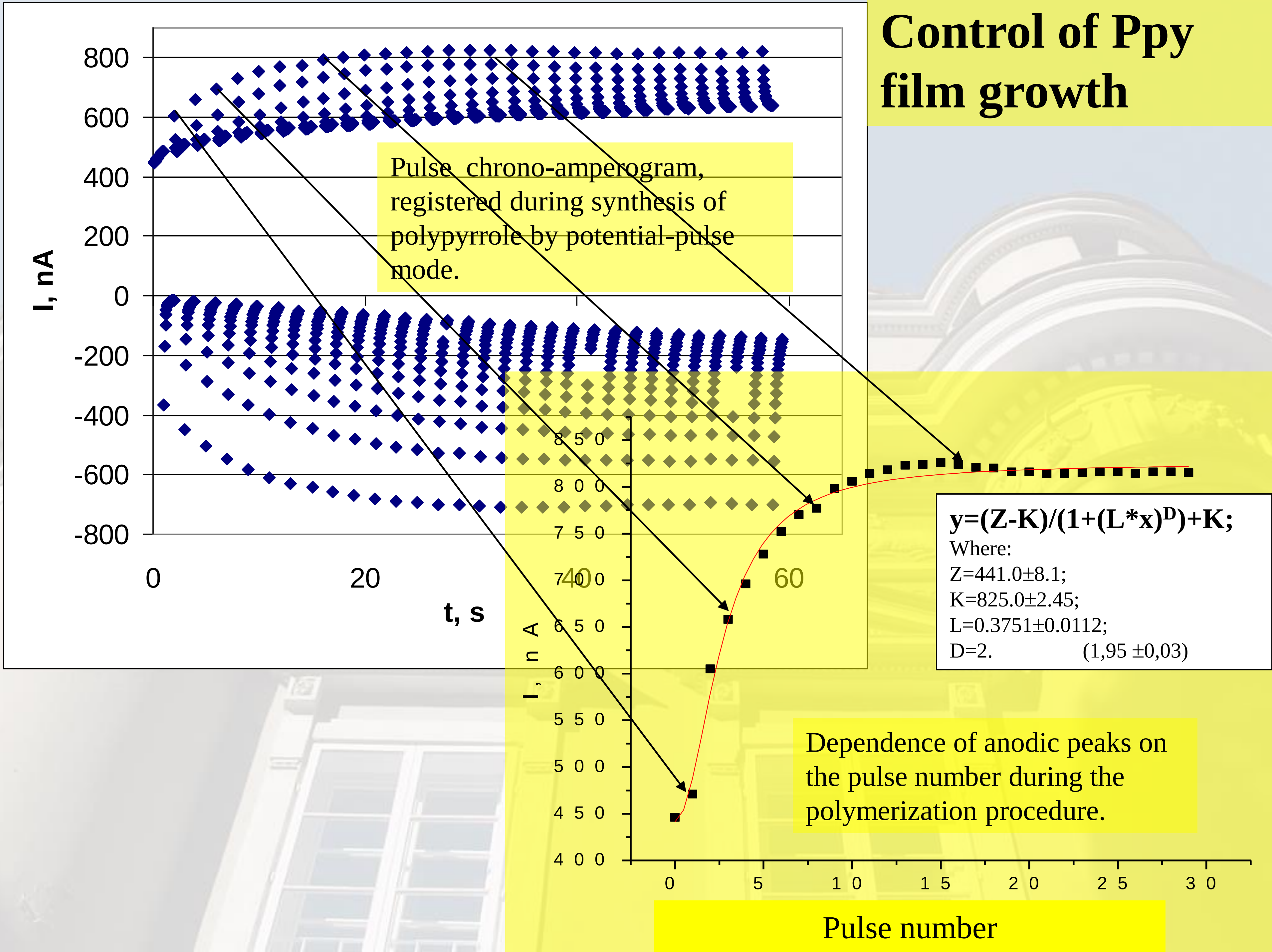
Immobilization of bio-molecules within electrochemically formed polypyrrole (Entrapment of bio-molecules within Ppy)



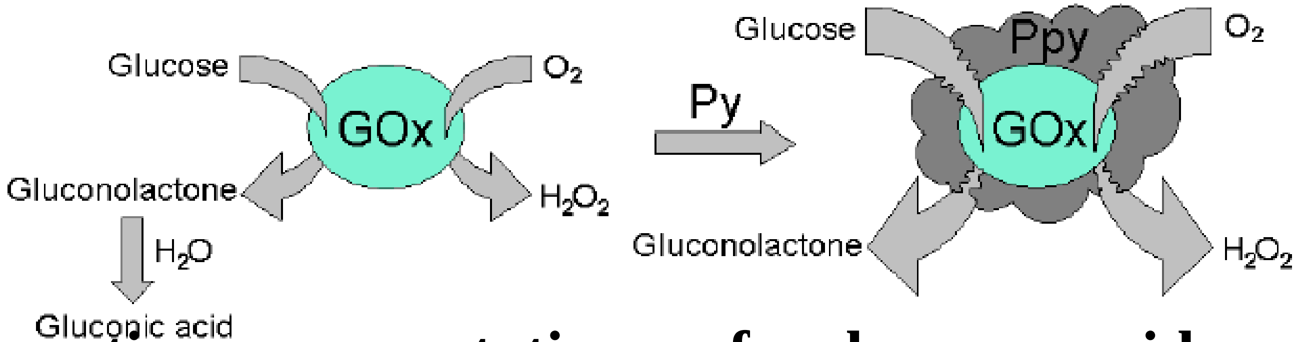




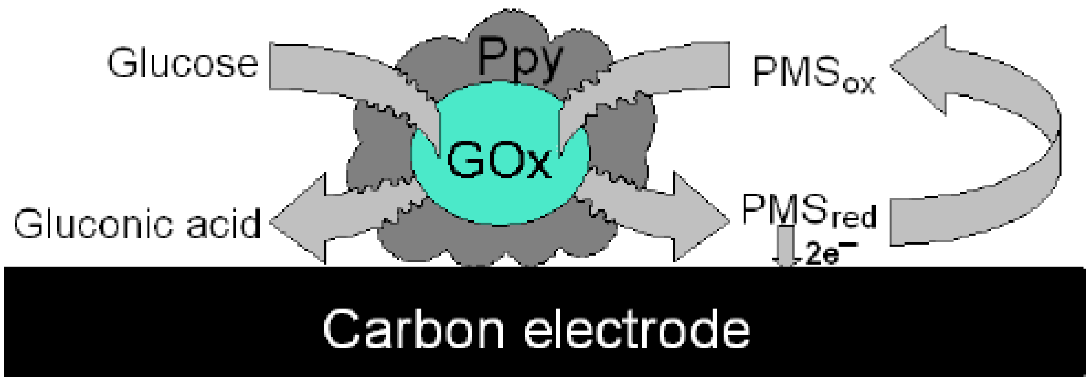
Control of Ppy film growth



Polypyrrole Coated Glucose Oxidase Nanoparticles



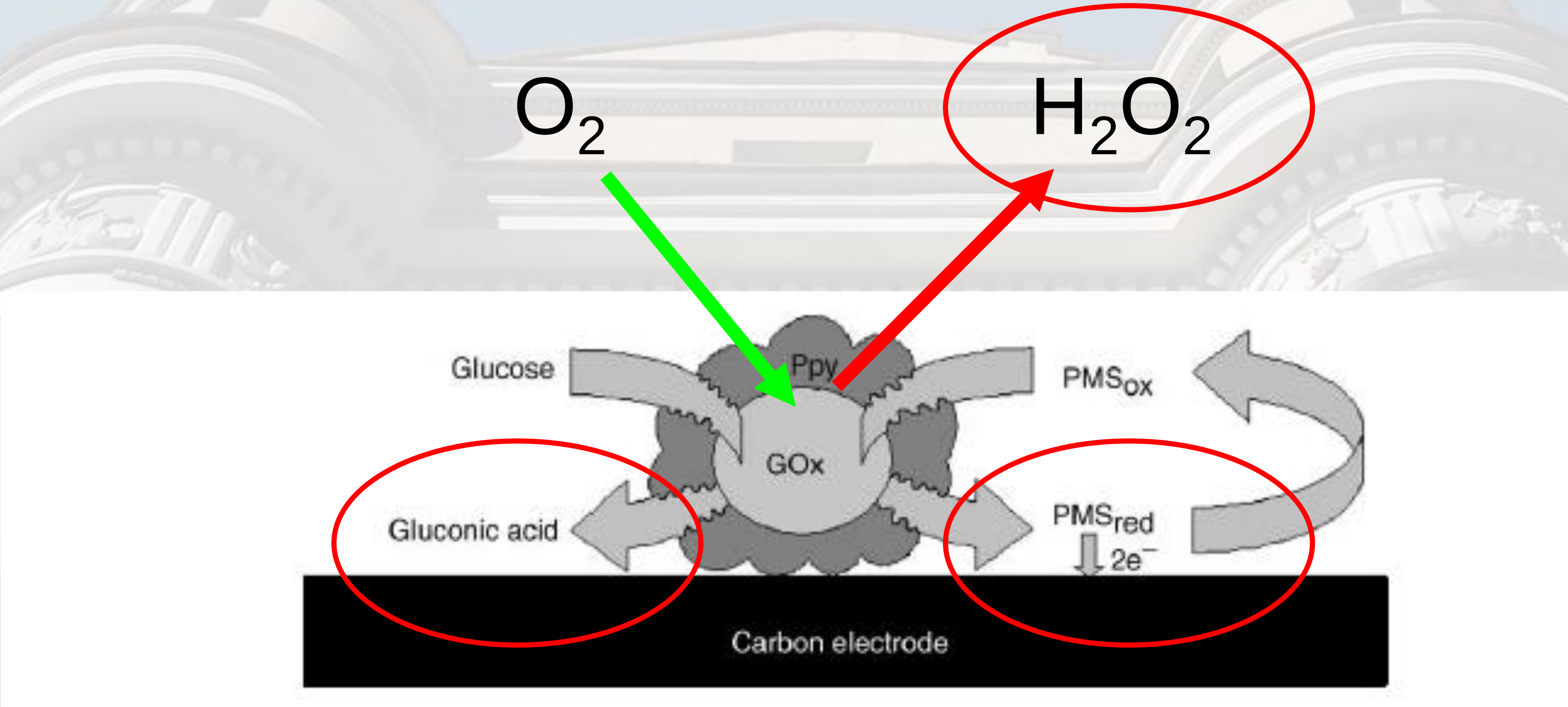
Schematic representation of glucose oxidase coating by polypyrrole initiated by catalytic action of this enzyme.



Schematic representation of application of polypyrrole coated glucose oxidase nanoparticles in PMS mediated biosensor design.

Ramanavicius A., Kausaite A., Ramanaviciene A. (2005) **Polypyrrole Coated Glucose Oxidase Nanoparticles for Biosensor Design**, *Sensors and Actuators B-Chemical 111-112*, 532-539. /// Ramanavicius A., Kausaite A., Ramanaviciene A., Acaite J., Malinauskas A., (2006) **Redox enzyme – glucose oxidase – initiated synthesis of polypyrrole** *Synthetic Metals,* 156, 409-413 ///

Activity of entrapped enzymes



Ramanavicius A., Kausaite A., Ramanaviciene A. (**2005**) Polypyrrole Coated Glucose Oxidase Nanoparticles for Biosensor Design, ***Sensors and Actuators B-Chemical*** *111-112*, 532-539.

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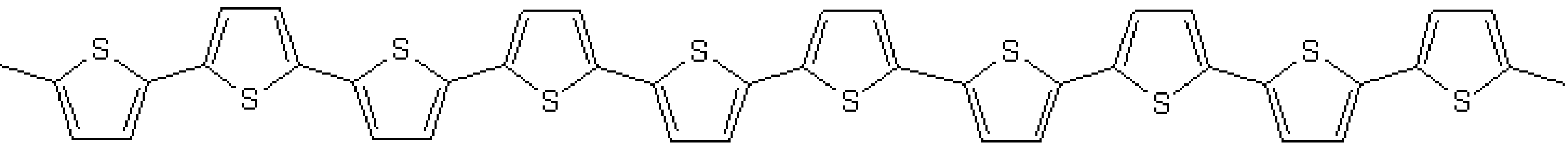
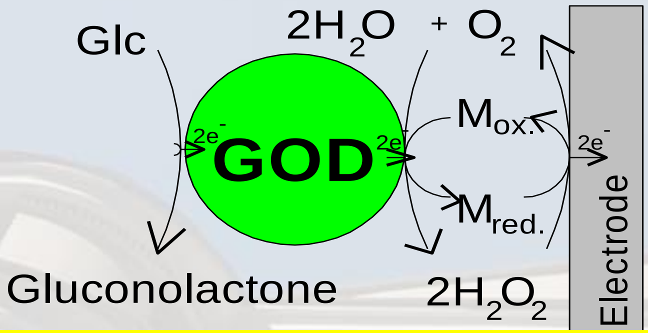
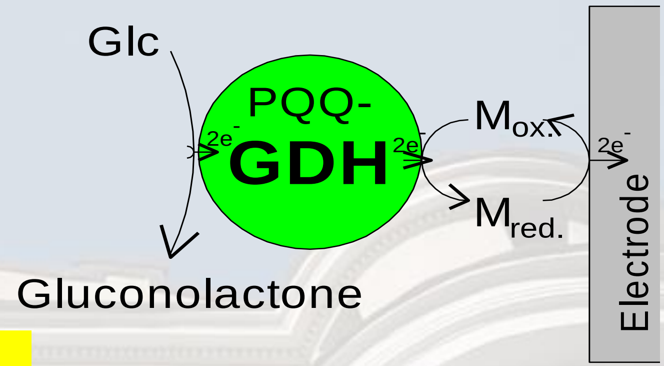


Conducting polymers might be very useful: (i) for modification, (ii) and for protection of biosensor surfaces. (2nd lecture)

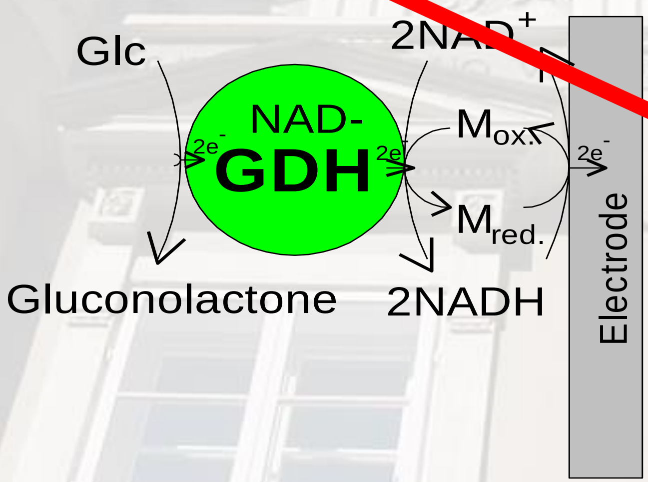
Enzymes in design of catalytic biosensors

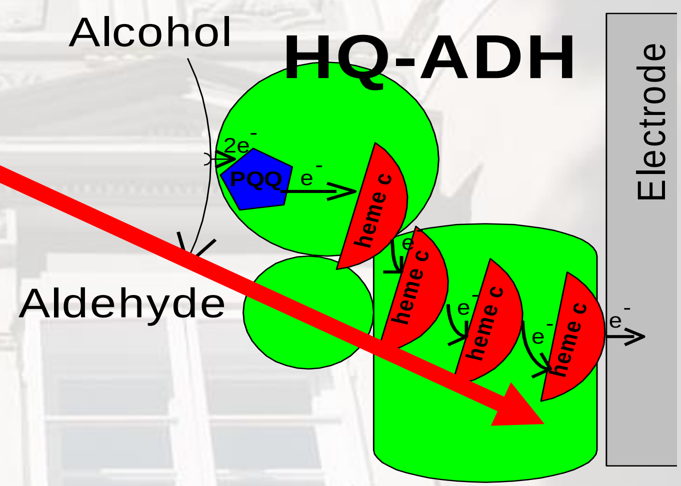




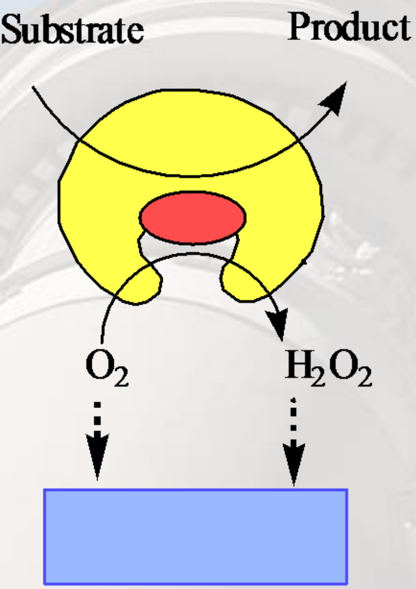
The most promising Enzymes, since has "well established intrinsic electrical circuit" and are able to transfer electrons directly to conducting surfaces.

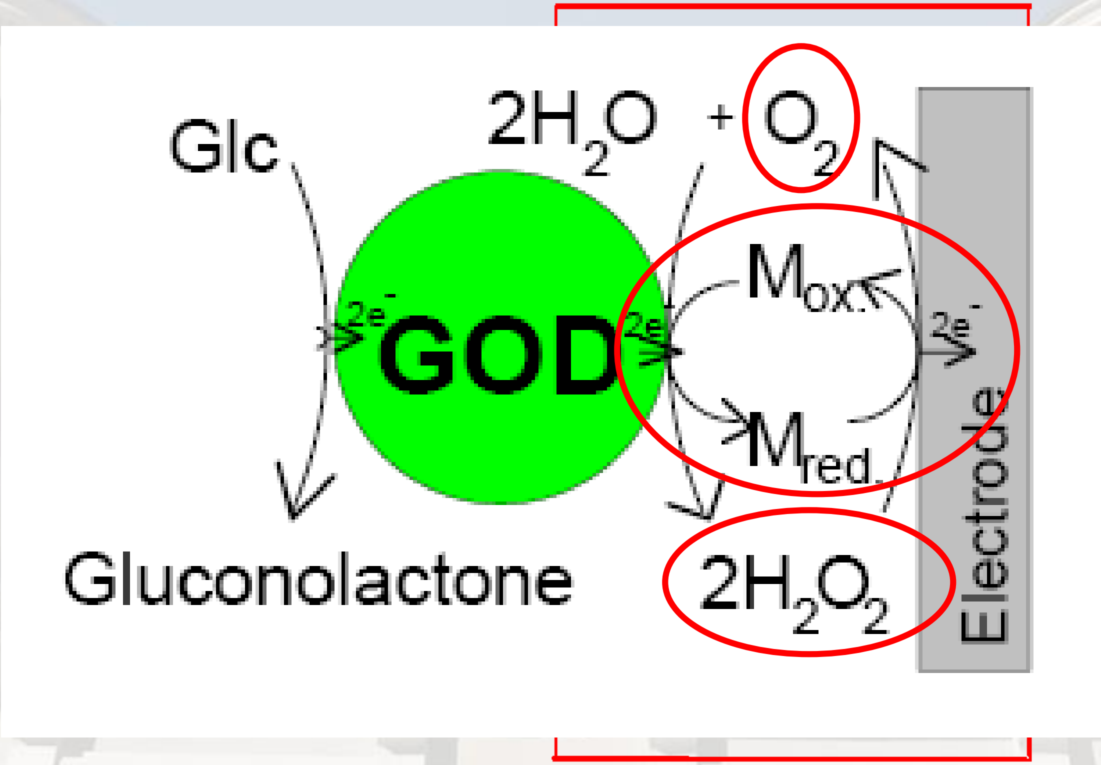
M=PMS; DCPIP; BQ

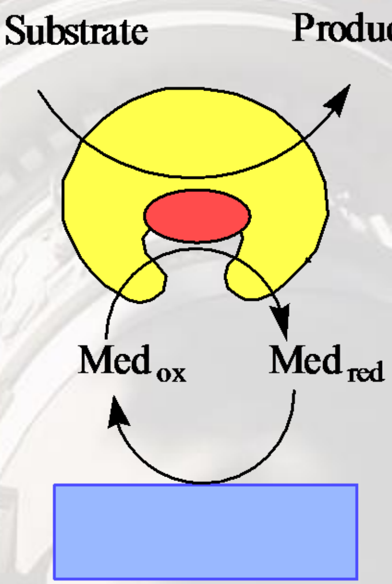




Oxidases – mostly used enzymes in catalytic biosensor design

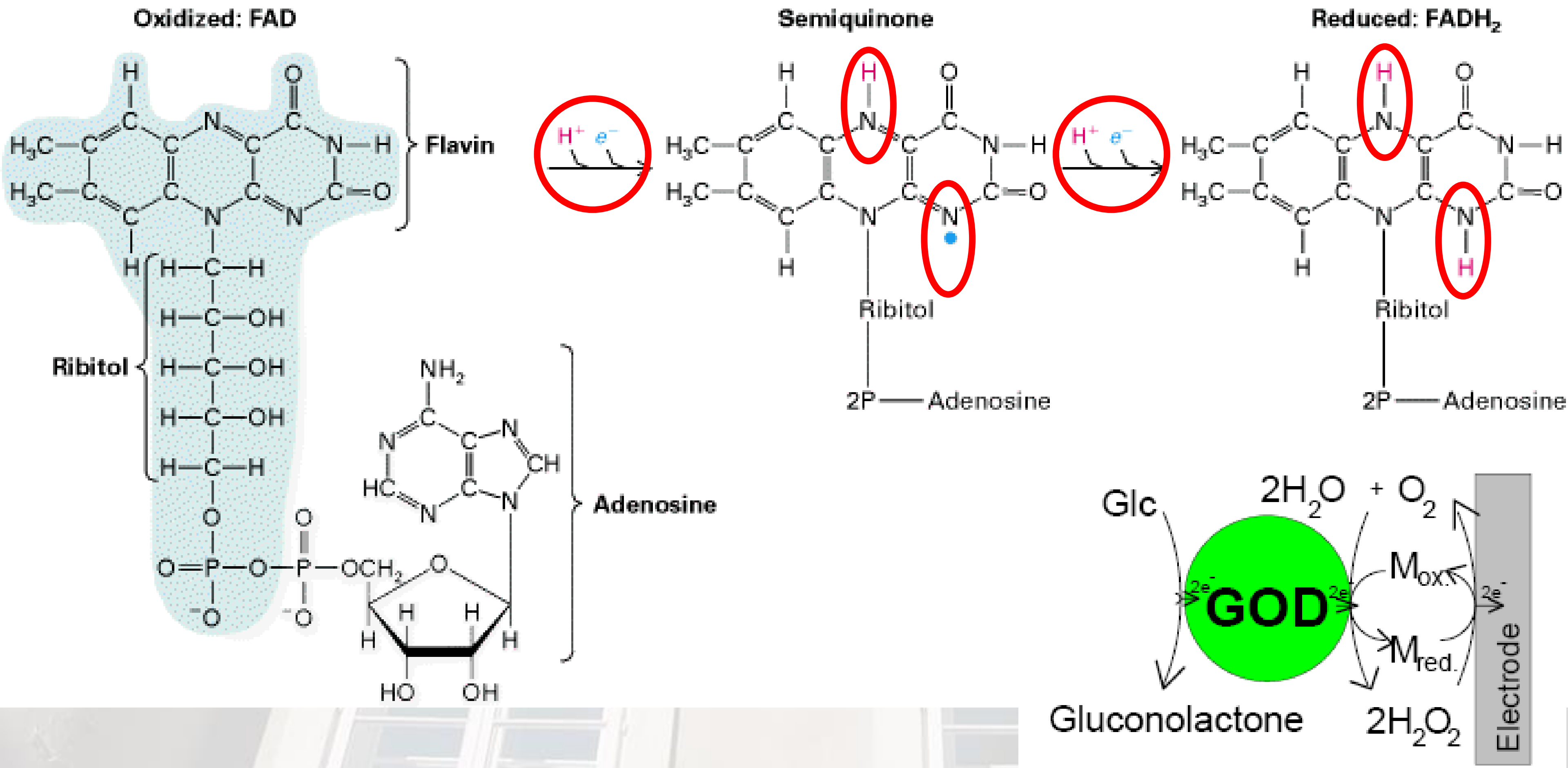




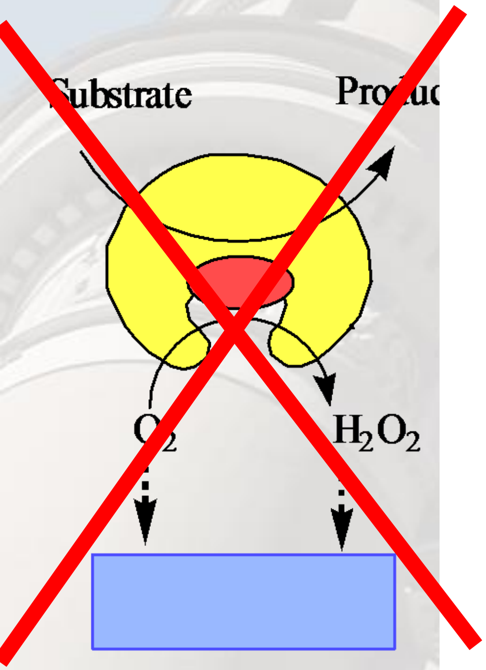


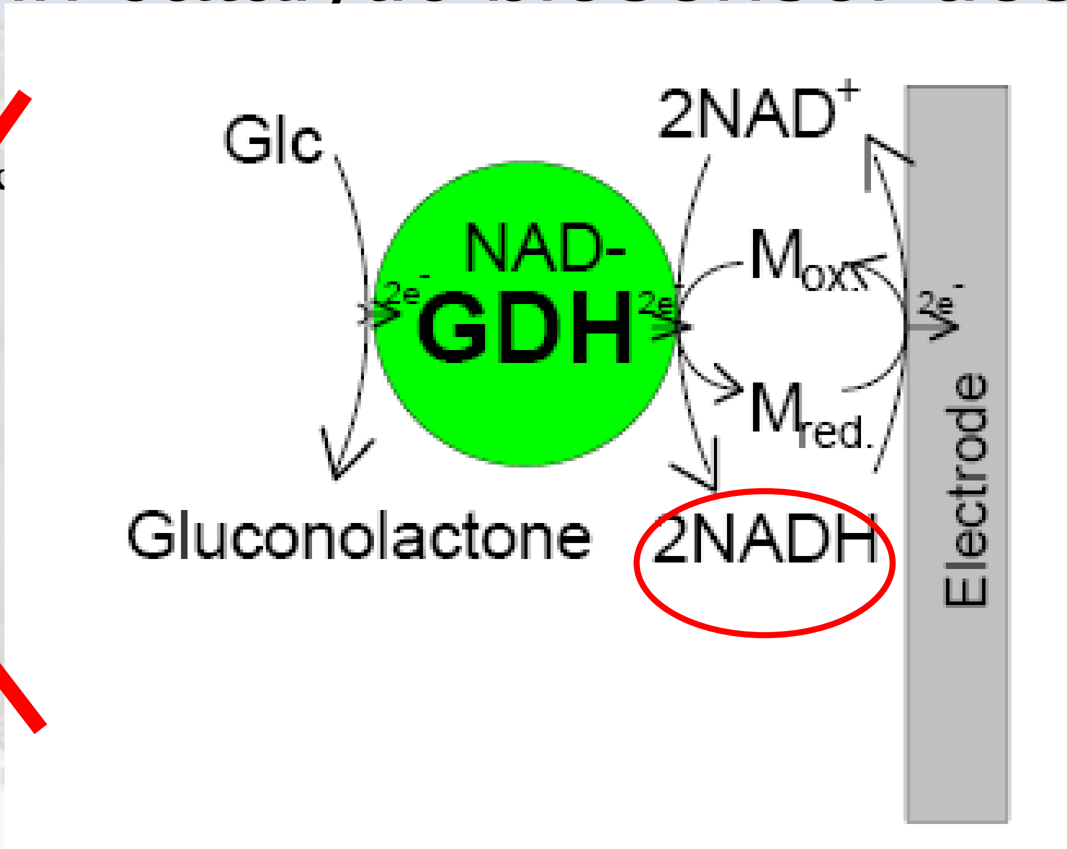
FAD cofactor

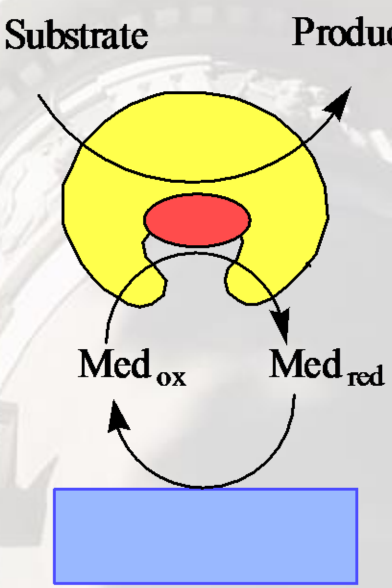
- Flavin adenine dinucleotide (FAD) is a redox cofactor involved in several important reactions in metabolism



NAD-dependent dehydrogenases in catalytic biosensor design

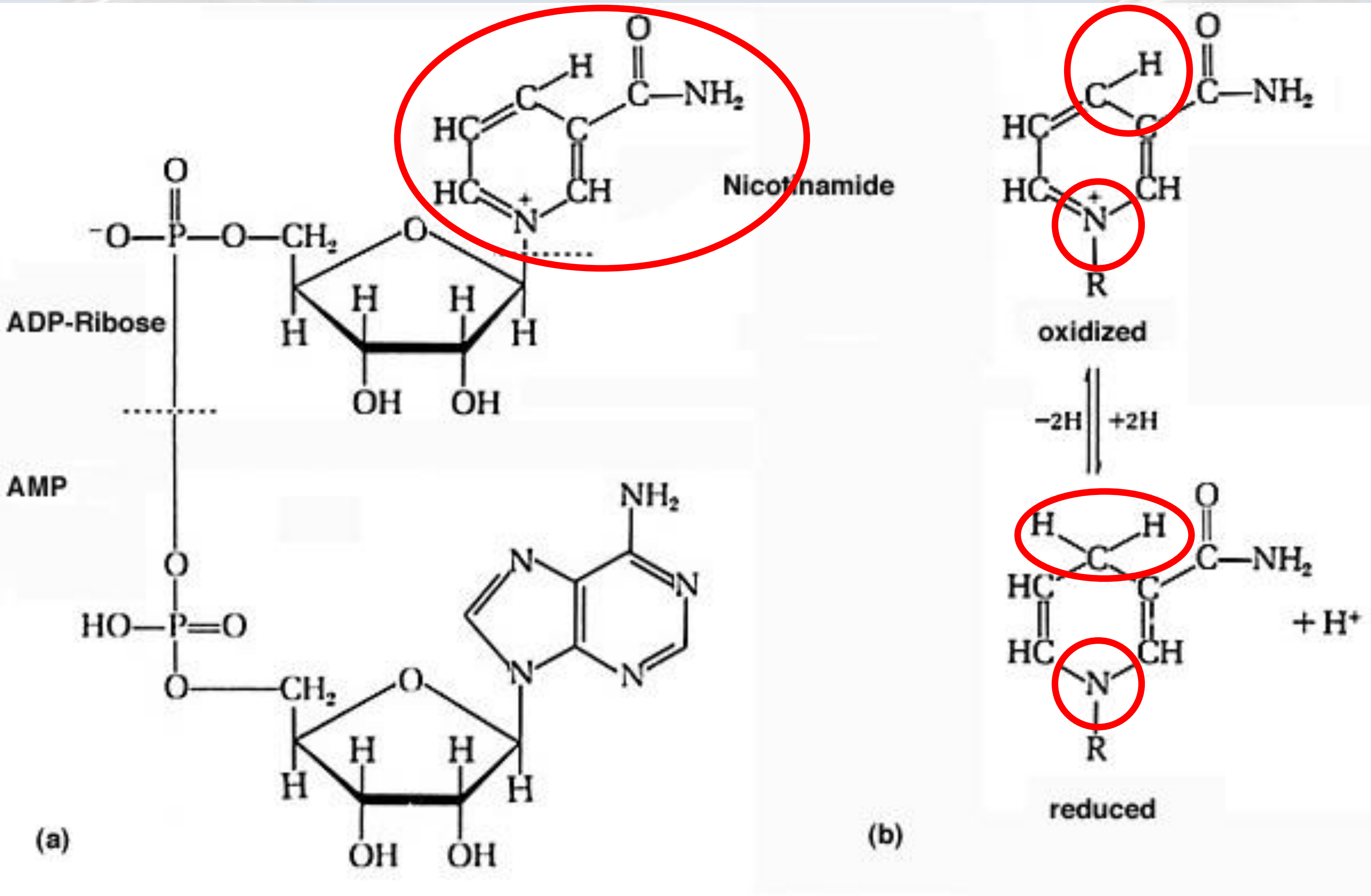


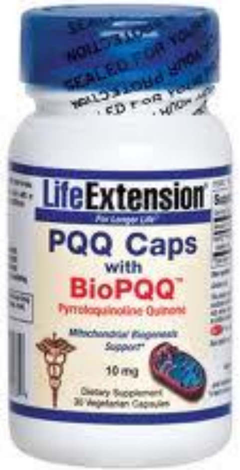


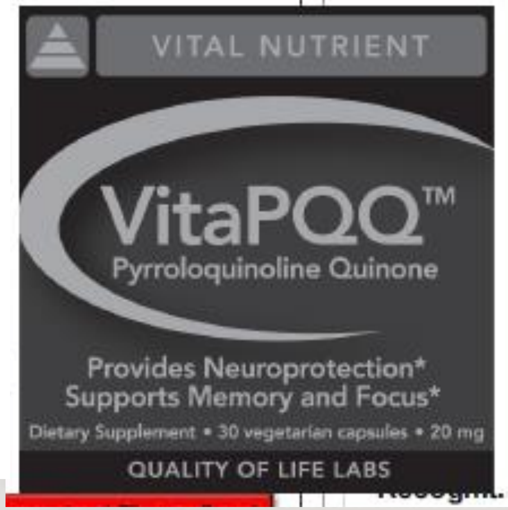


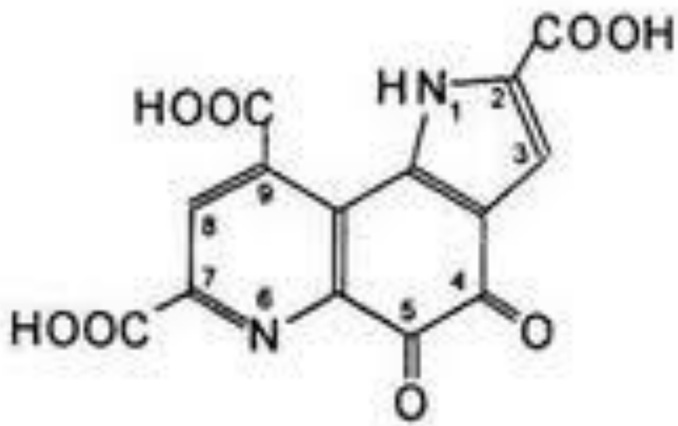
NAD+/NADH cofactor

- ***Nicotinamide adenine dinucleotide* (*NAD*)**



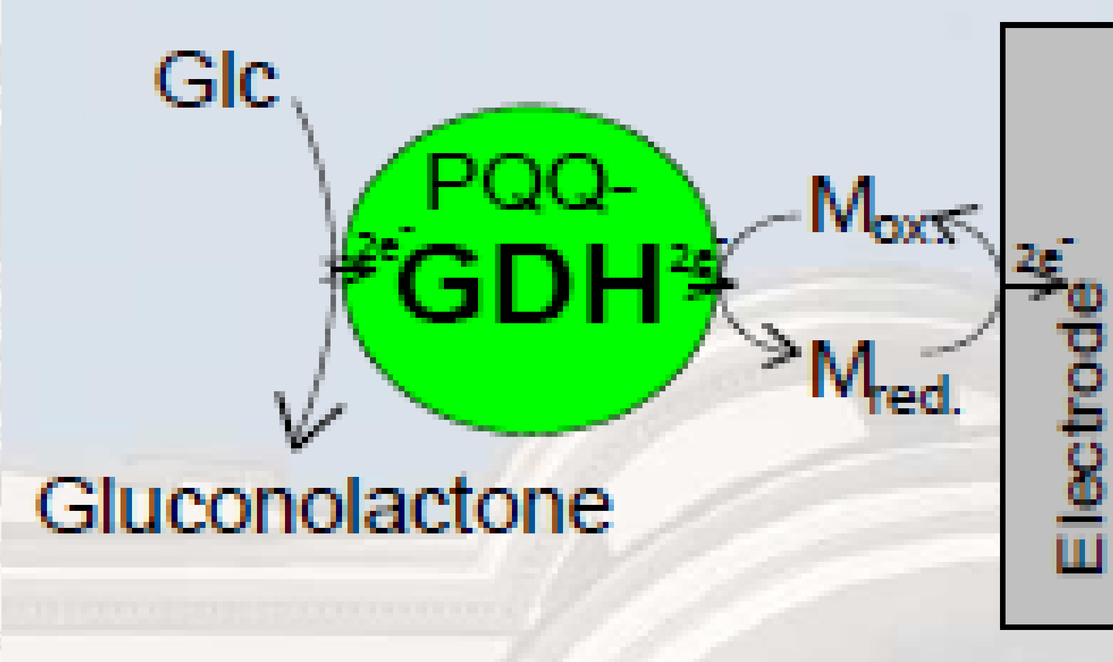






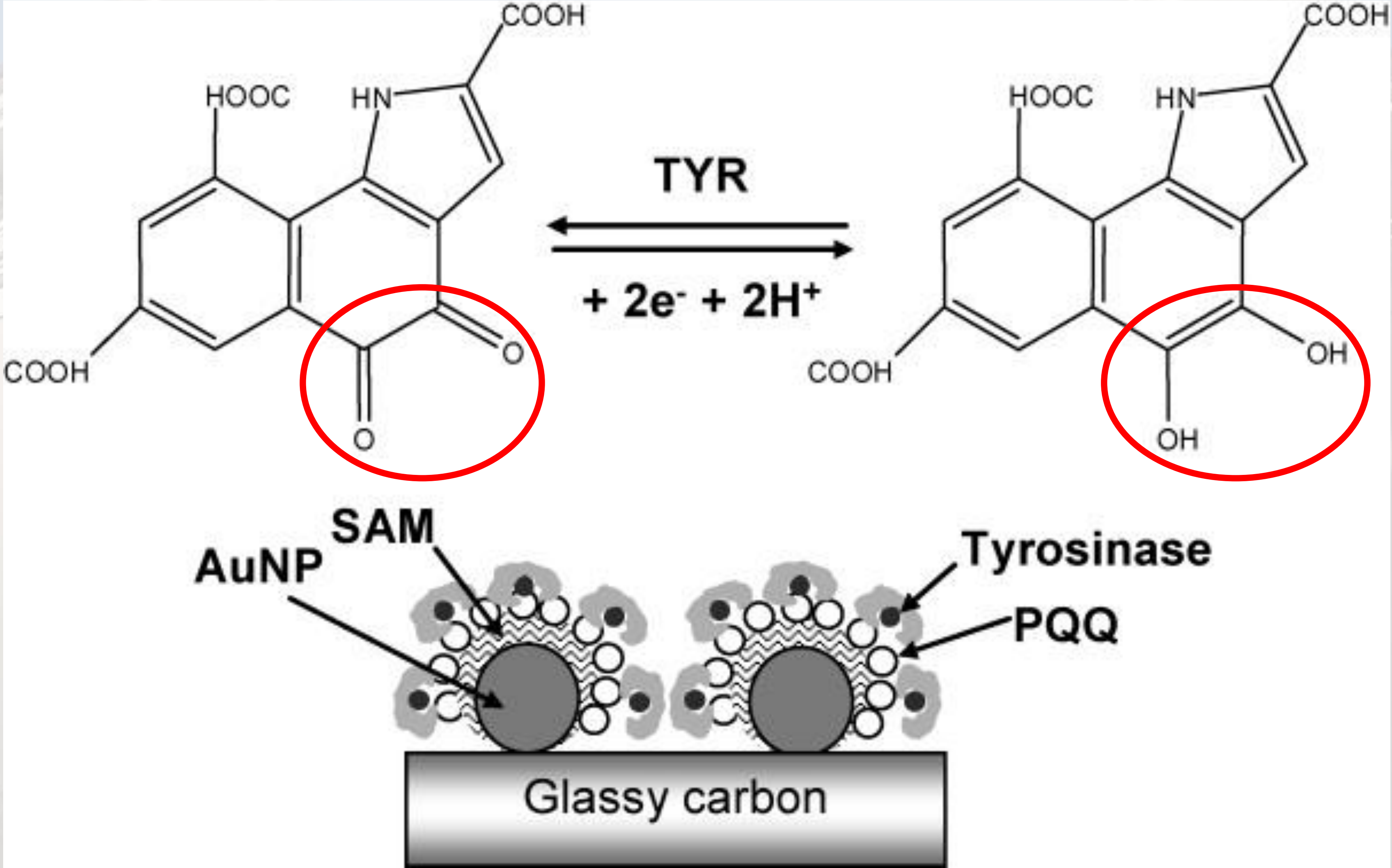
Pyrroloquinoline quinone [PQQ]

Prosthetic group of bacterial quinoprotein dehydrogenases

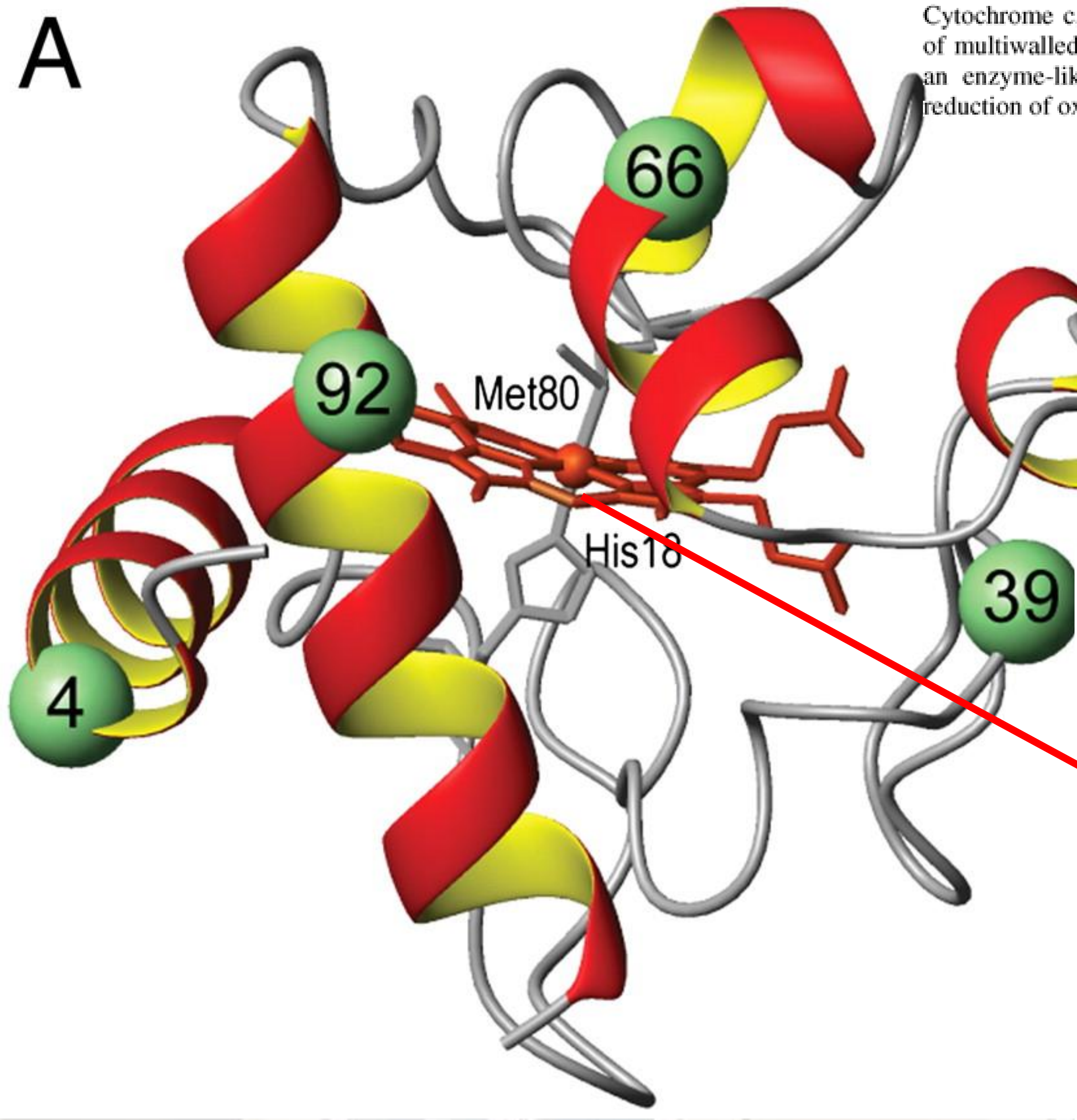


PQQ Cofactor

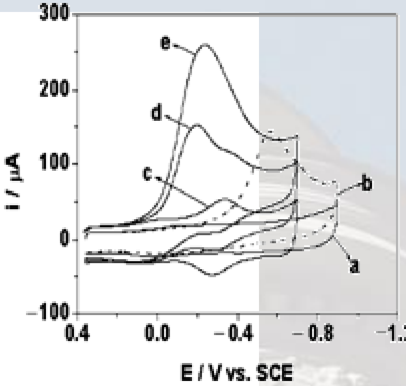
Pyrroloquinoline quinone (PQQ)

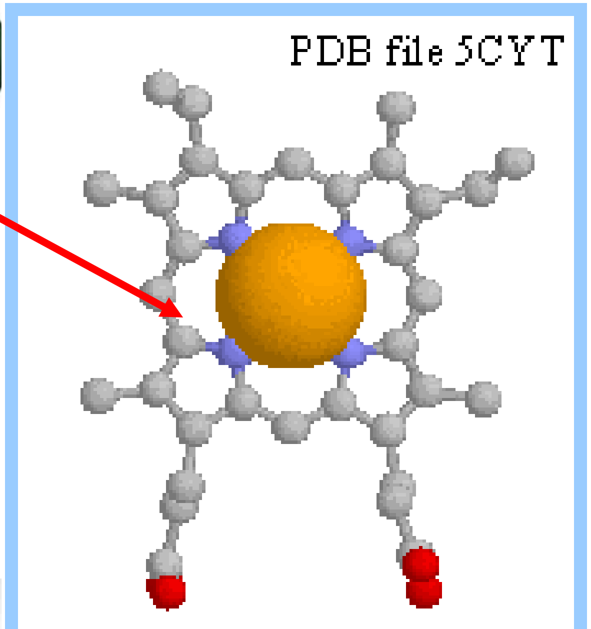


Cytochrome c

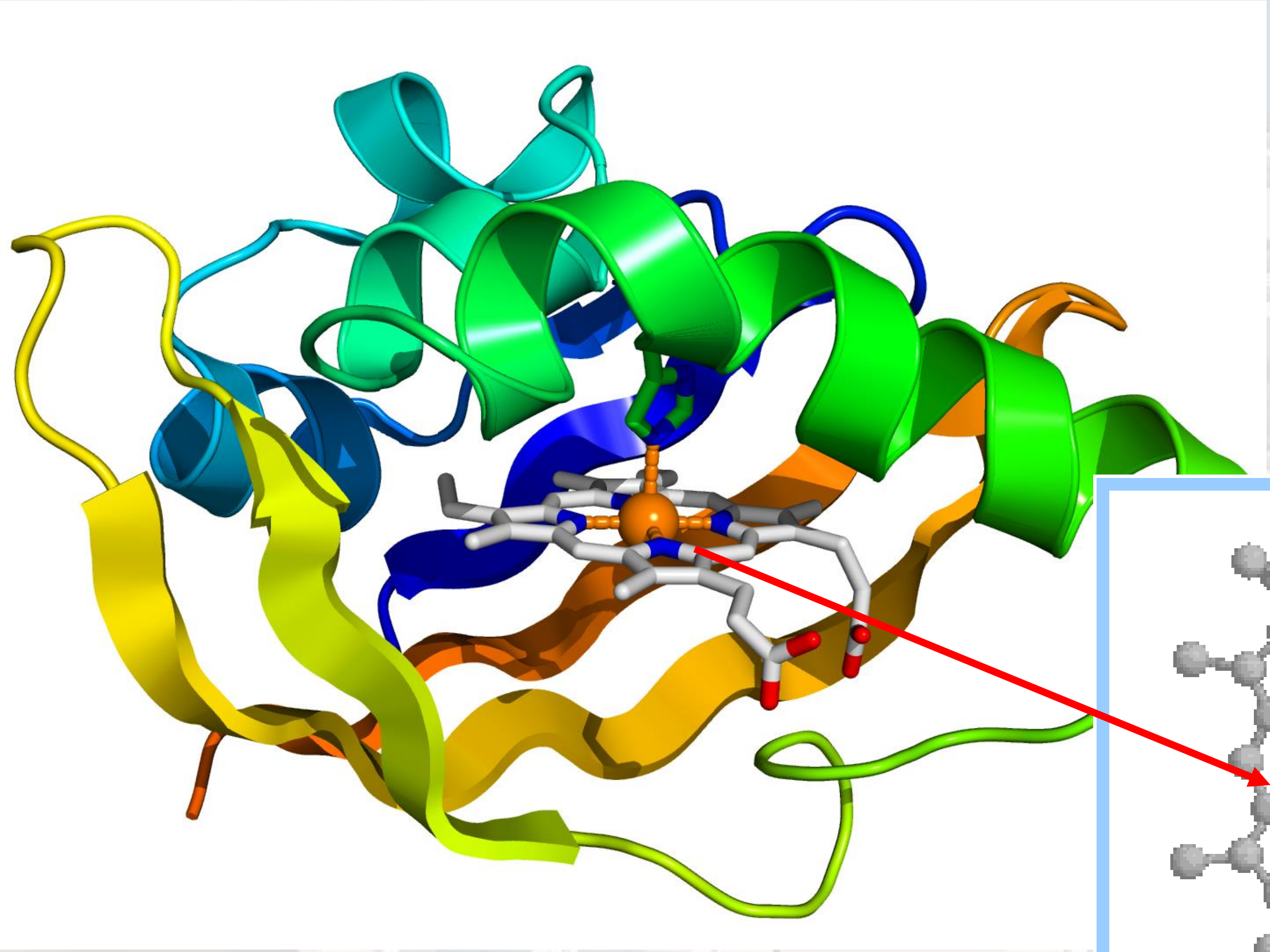


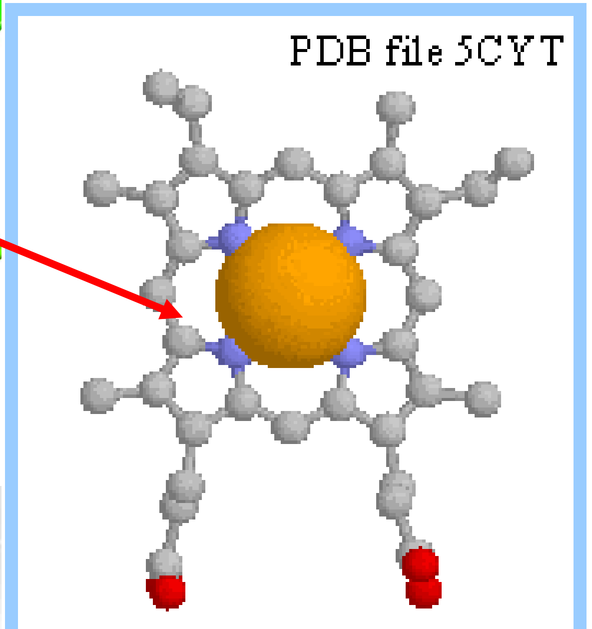
Cytochrome c, adsorbed onto the surface of multiwalled carbon nanotubes, showed an enzyme-like activity to catalyze the reduction of oxygen.



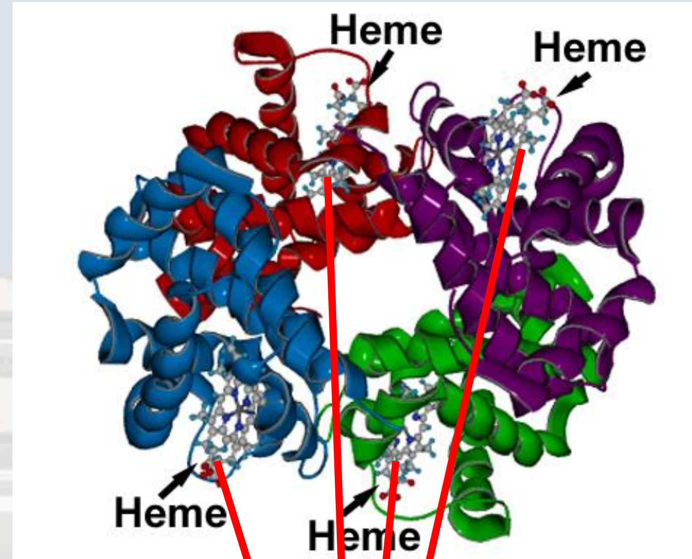


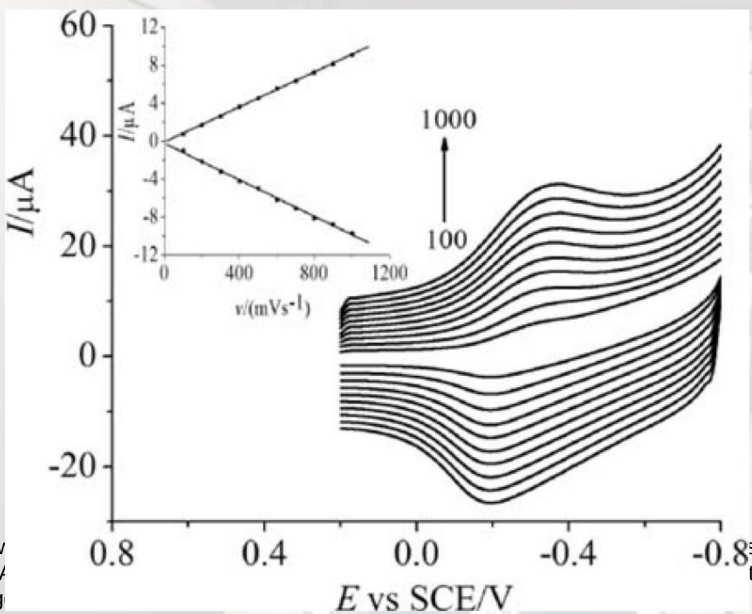
Horseradish peroxidase

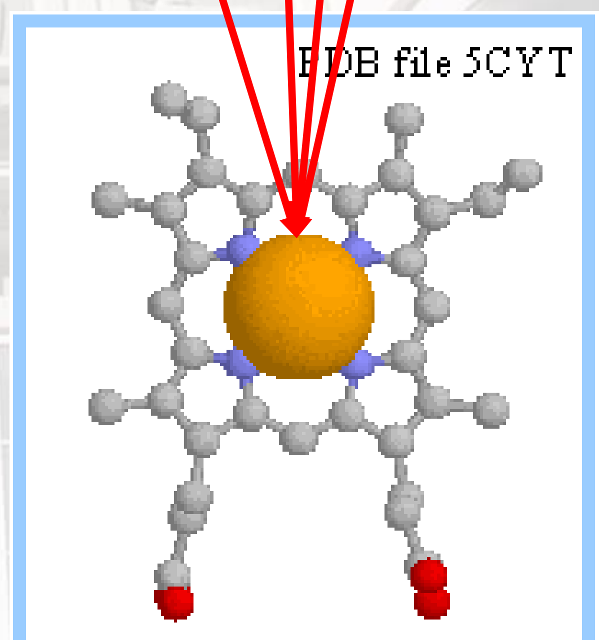




Hemoglobin







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Heme-c dependent proteins

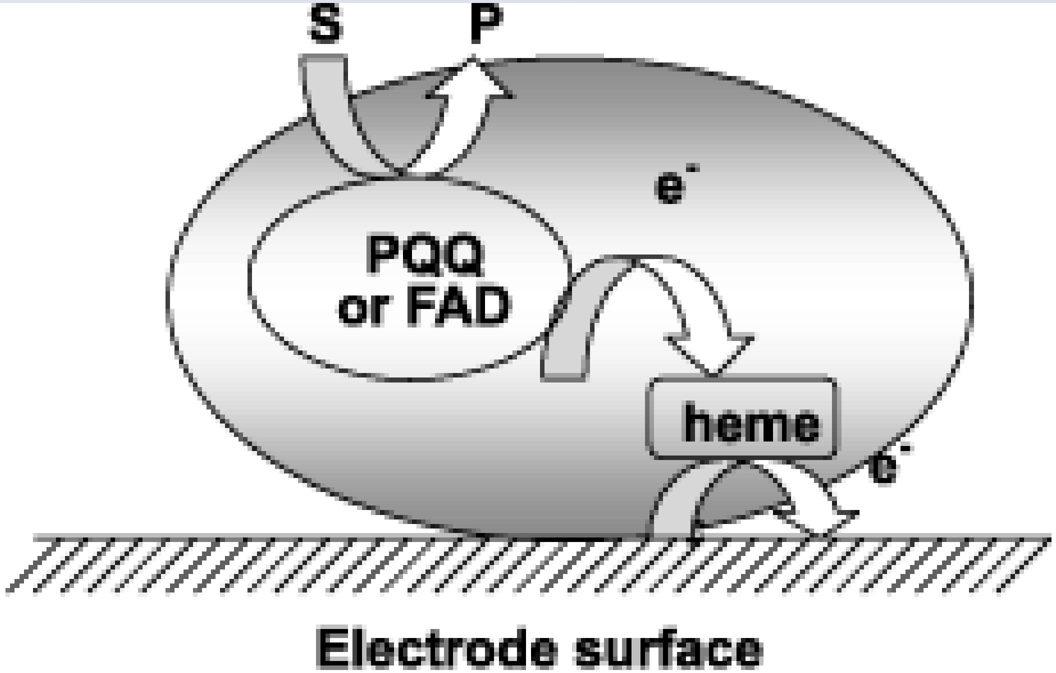
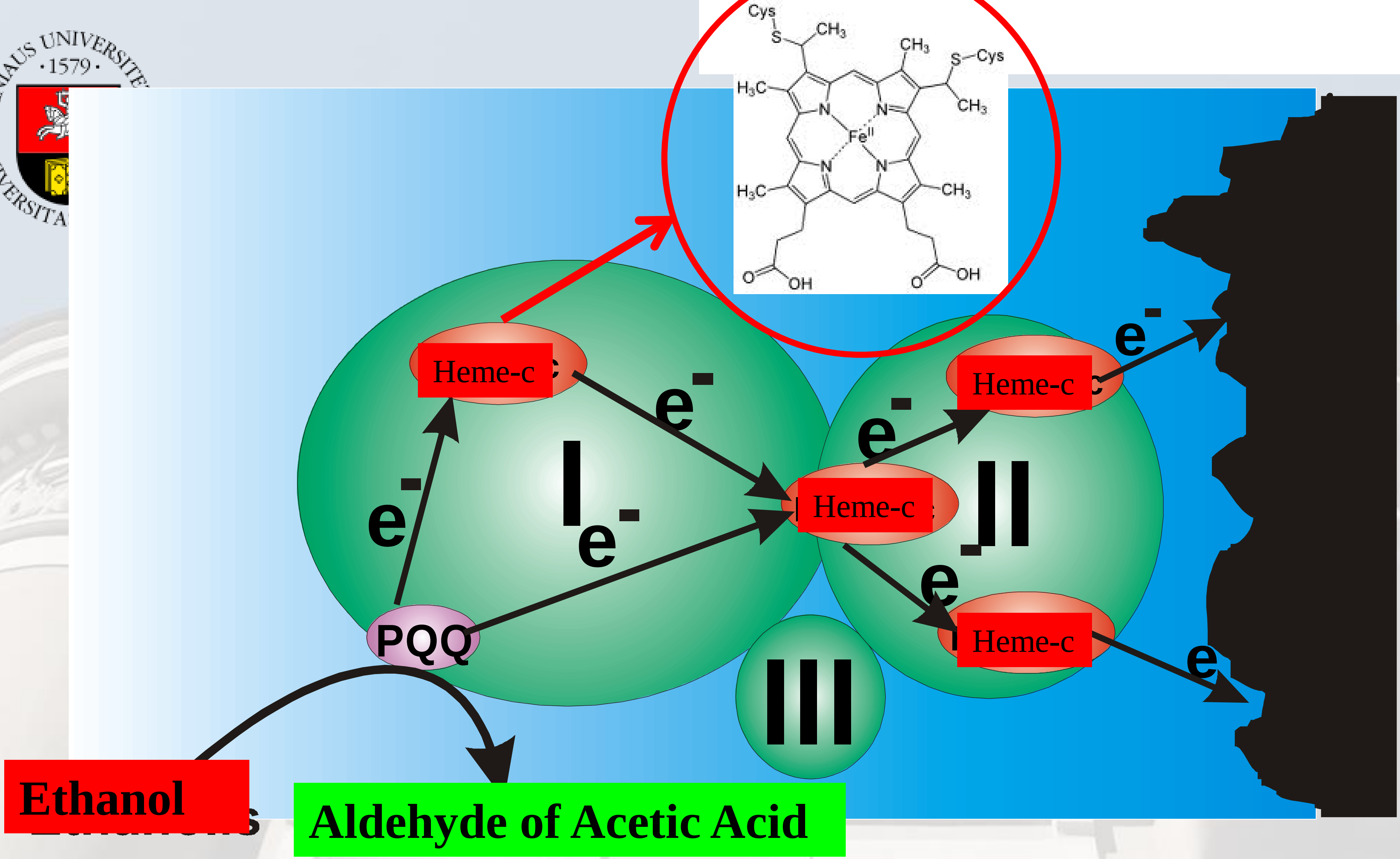


Figure 5. Schematic picture of multi-factor enzymes and the electron transfer pathway.

https://www.google.lt/search?q=sensors+today+and+tomorrow&source=lnms&tbm=isch&sa=X&ei=HA5GU6vlB4eoO6OwgZAC&ved=0CAYQ_AUoAQ&biw=1536&bih=762#q=Biosensor+electron+transfer+cytochrome+c+&tbm=isch&facrc=_&imgdii=_&imgrc=YhYGvo8RQamrEM%253A%3B0i3fb-iaPxfbzM%3Bhttp%253A%252F%252Fwww.scielo.br%252Fimg%252Frevistas%252Fjbchs%252Fv14n2%252F15600f5.gif%3Bhttp%253A%252F%252Fwww.scielo.br%252Fscielo.php%253Fscript%253Dsci_arttext%2526pid%253DS0103-50532003000200008%3B351%3B194

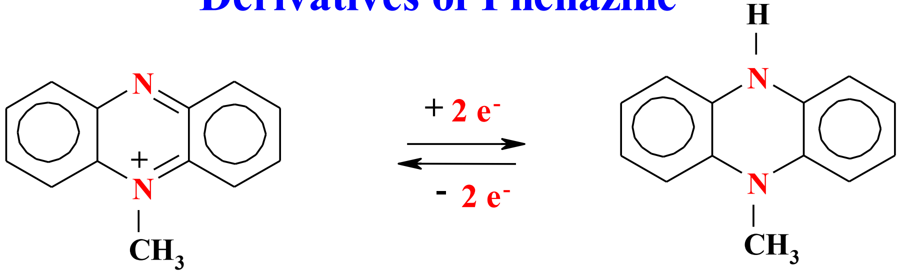


Ramanavičius A., Habermüller K. Csöregi, E., Laurinavičius V., Schuhmann. W. (1999) Polypyrrole entrapped quinohemoprotein alcohol dehydrogenase. evidence for direct electron transfer via conducting polymer chains, ***Analytical Chemistry***, 71, 3581-3586.

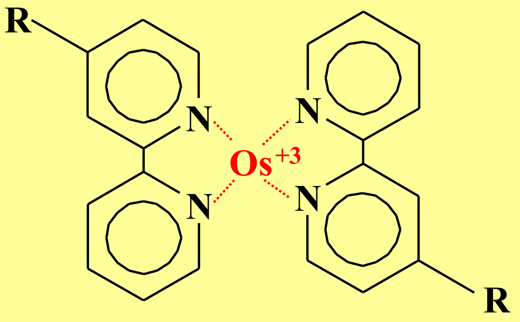
Razumienė J., Niculescu M., Ramanavičius A., Laurinavičius V., Csöregi E. (2002) Direct Bioelectrocatalysis at Carbon Electrodes Modified with Quinohemoprotein Alcohol Dehydrogenase from *Gluconobacter* sp. 33, ***Electroanalysis***, 14, 43-49.

Some Red-Ox Mediators

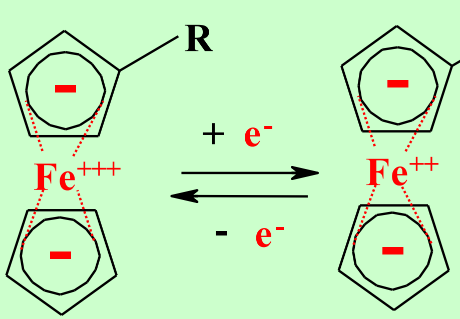
Derivatives of Phenazine



Derivatives of Os-bipyridine complex



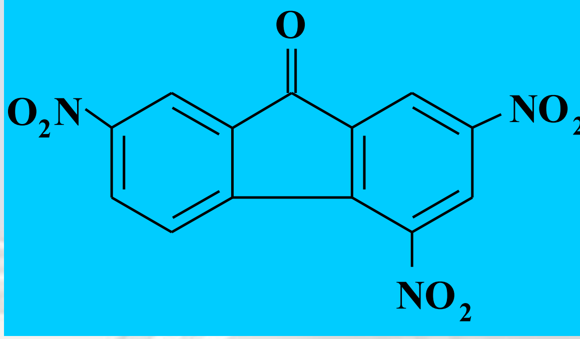
Derivatives of Ferrocene



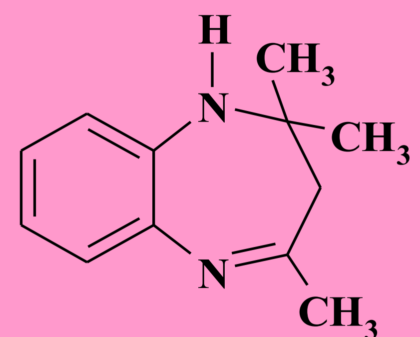
Prussian Blue

$$Fe^{+3}{}_4[Fe^{+2}(CN)_6]_3$$

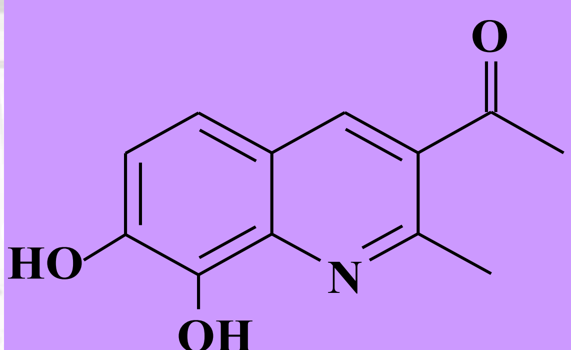
Fluorenone



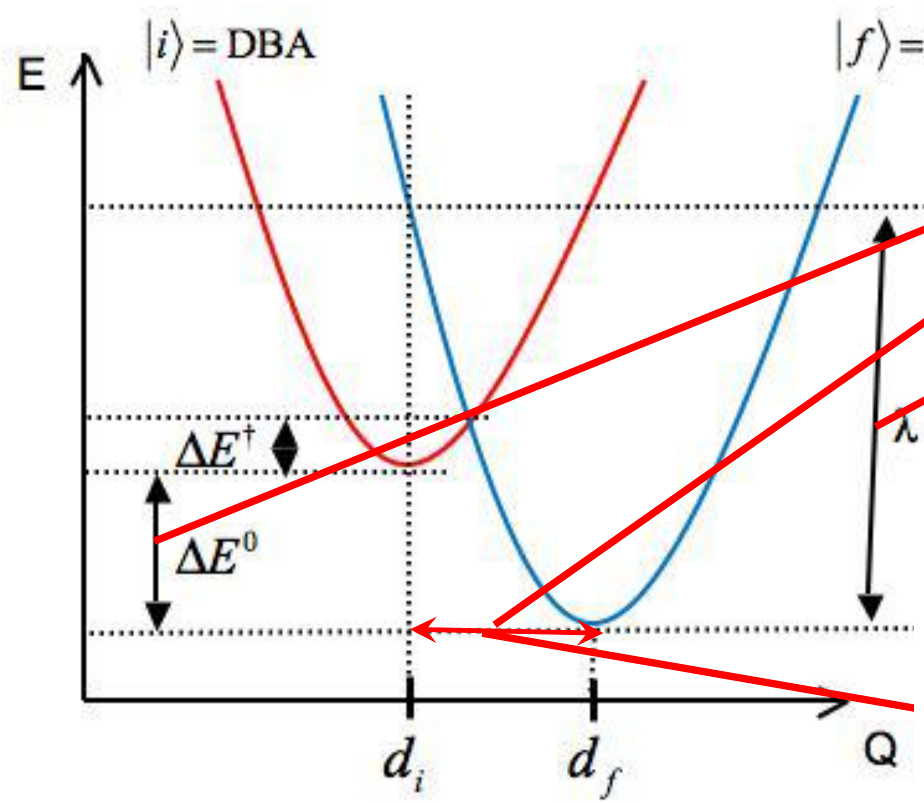
Derivatives of benzodiazepine



Derivatives of quinones



Electron transfer of efficiency // Marcus-Hush theory



thermodynamic driving force

$$k_s \propto e^{-\beta(d-d_0)} e^{\frac{-(\Delta G^{\circ}+\lambda)^2}{4RT\lambda}}$$

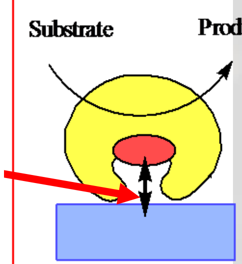
distance

reorganisation energy

λ: reorganization energy

ΔE^{0} : energy of reaction or driving force

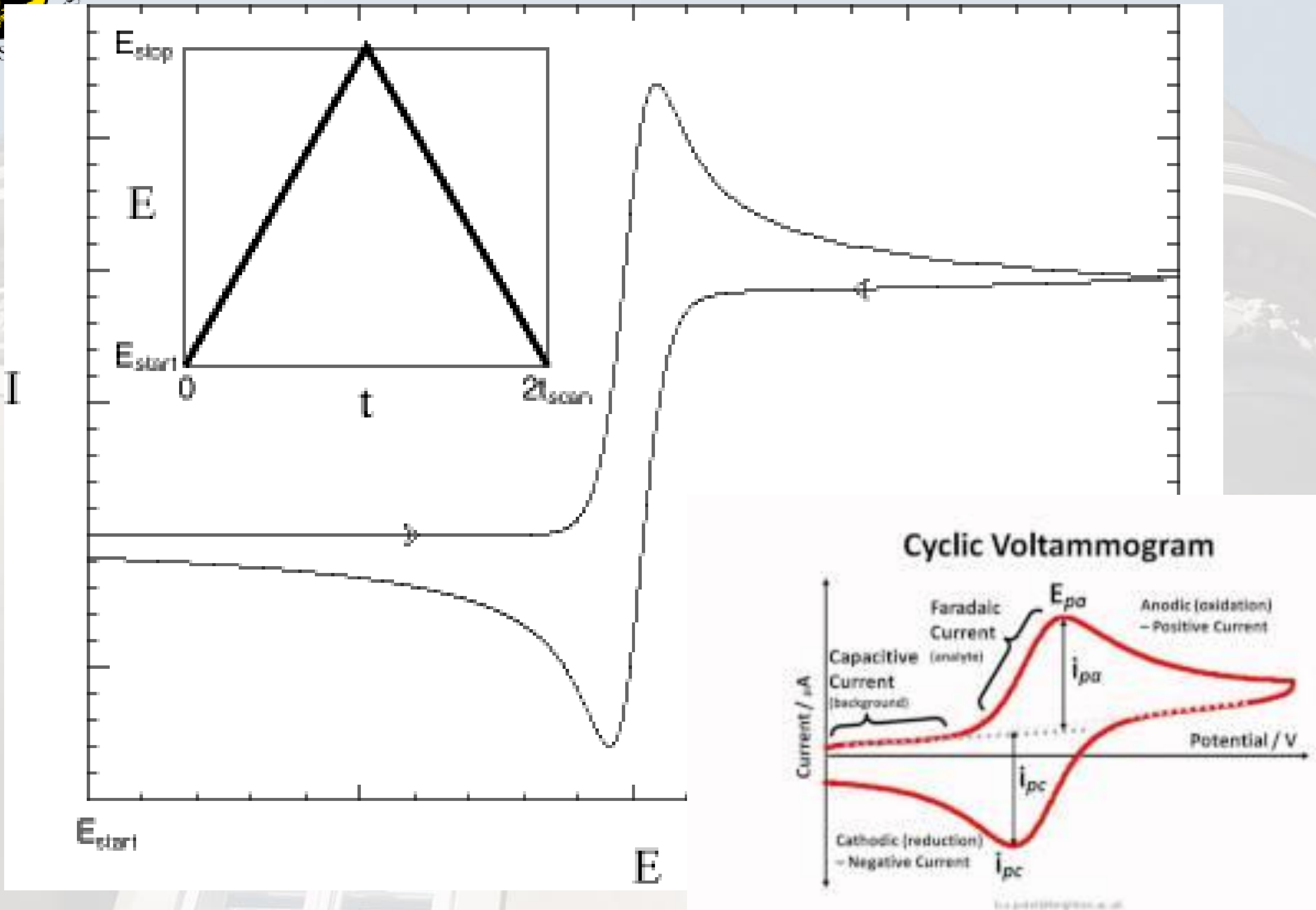
$\Delta E^{\dagger}$: activation energy



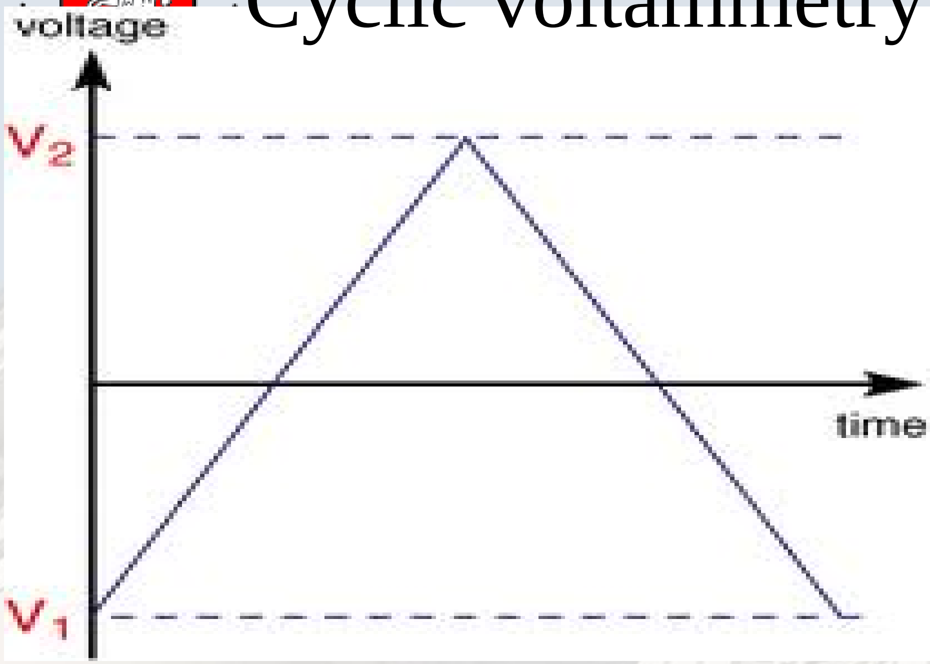
Electron transfer (ET) reactions involve the movement of an electron from one molecular species (the donor) to another (the acceptor)

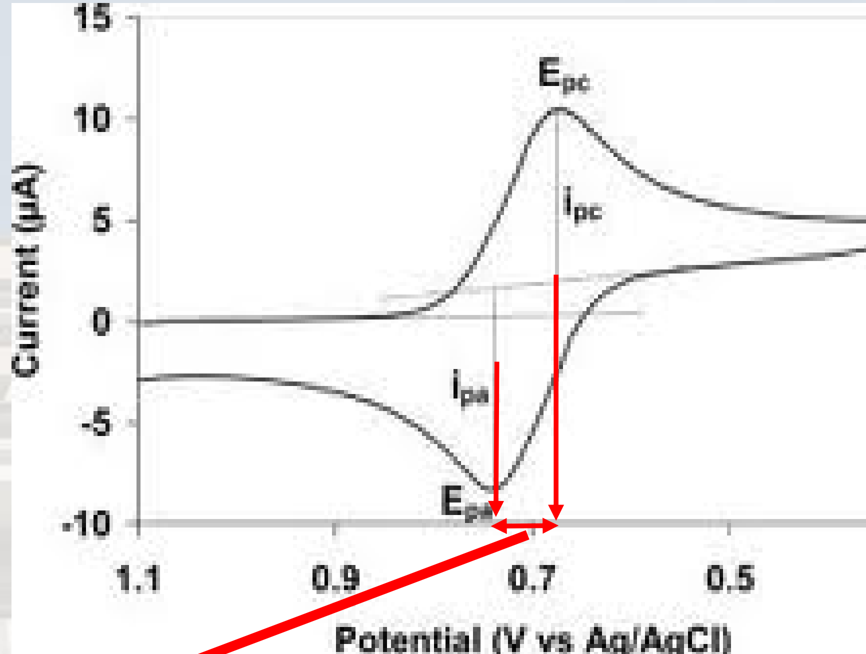
$$k_{ET} = |V_{el}|^2 \sqrt{\frac{\pi}{h^2 k_B T \lambda}} \exp\left\{-\frac{(\Delta E^0 - \lambda)}{4\lambda k_B T}\right\}$$

Electron transfer of efficiency // Potentiodynamic methods



Electron transfer of efficiency // Cyclic voltammetry

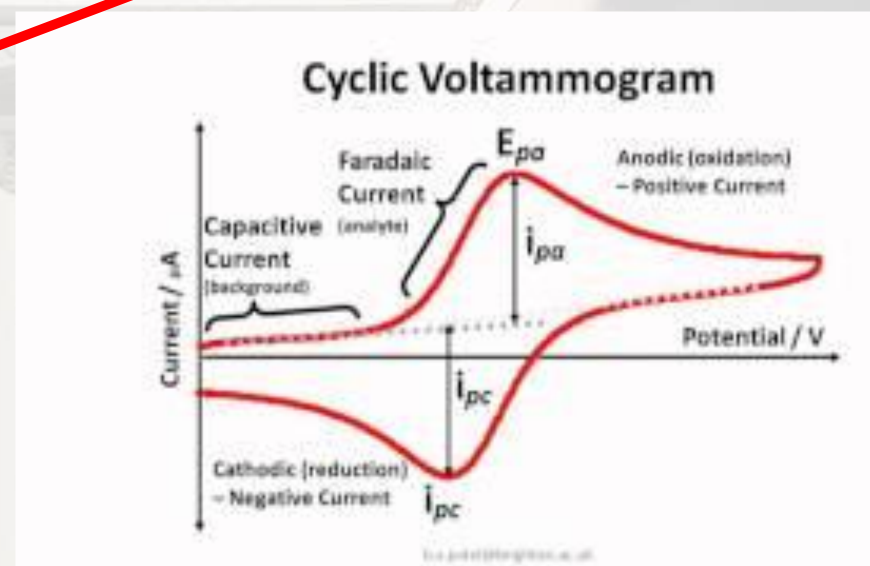




Nicholson, Kochi's method

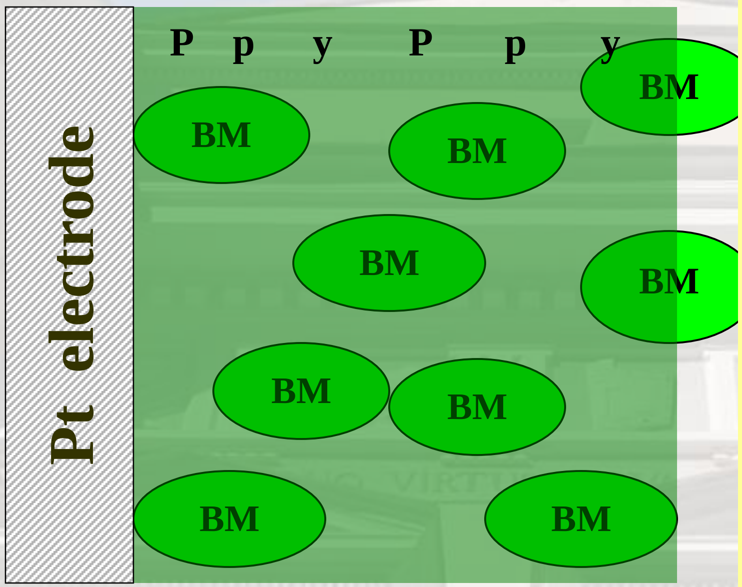
$$k^o = 2.18\left[{}^{\alpha v n F D_o}\!/_{RT}\right]^{1/2} \exp\left[-\frac{\alpha^2 n F \Delta E_p}{RT}\right]$$

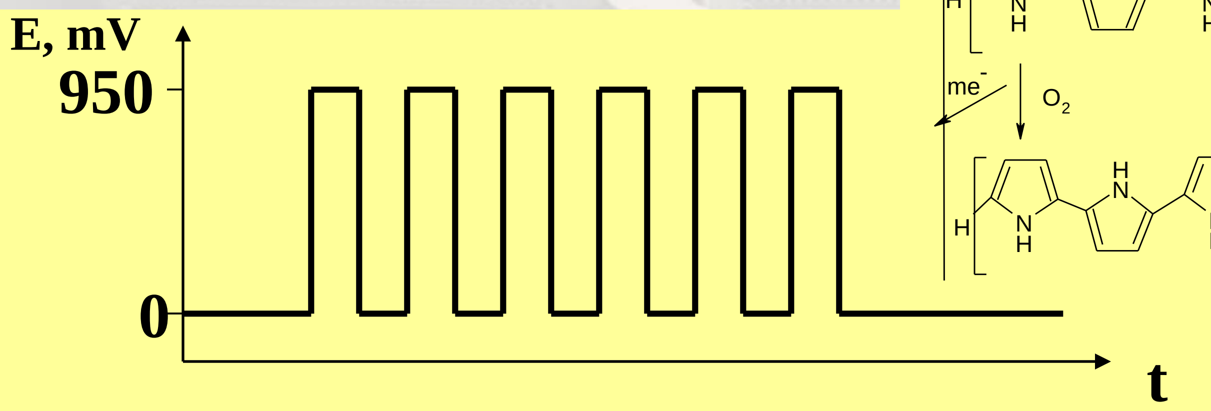
Heterogeneous election transfer constant k^0

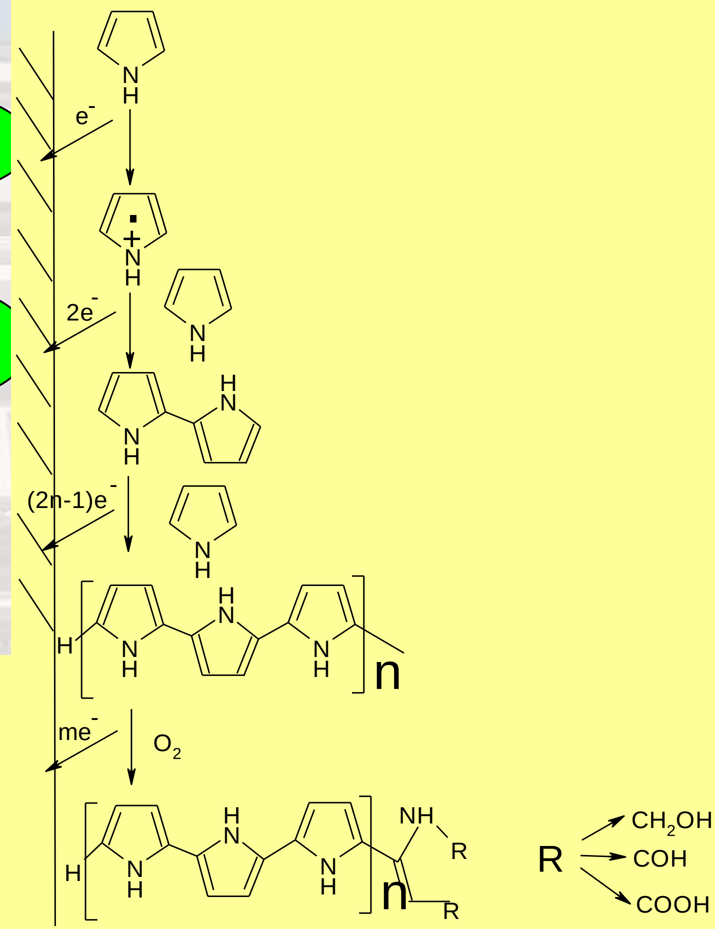


Immobilization of bio-molecules within electrochemically formed polypyrrole (Entrapment of bio-molecules within Ppy)

A. Ramanavicius, Y. Oztekin, A. Ramanaviciene Electrochemical Formation of Polypyrrole-based Layer for Immunosensor Design. *Sensors and Actuators B-Cemical* **2014,** 197, 237–243.

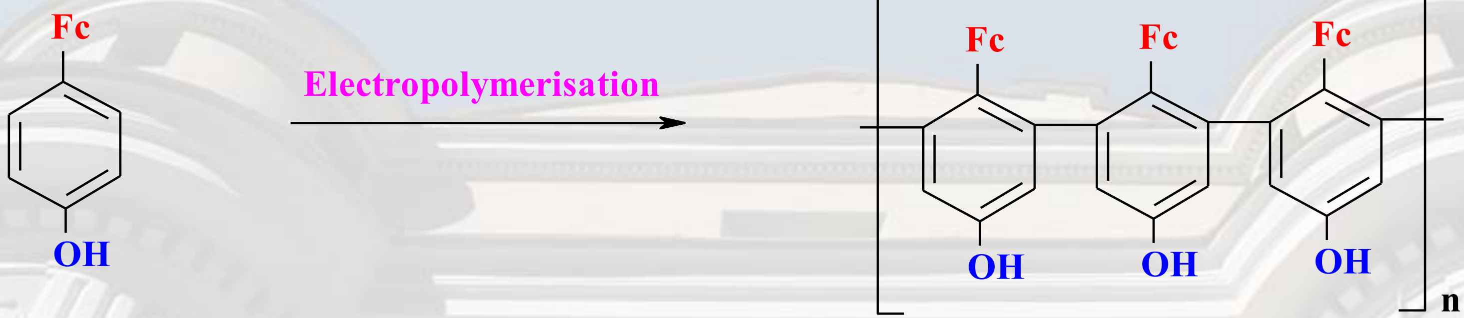




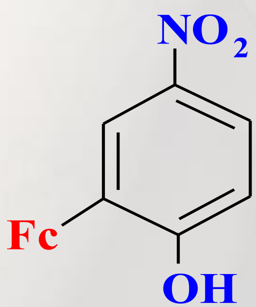


Electrochemically polymerisable Compounds with Red-Ox Mediators

Ferrocenephenol (FP)

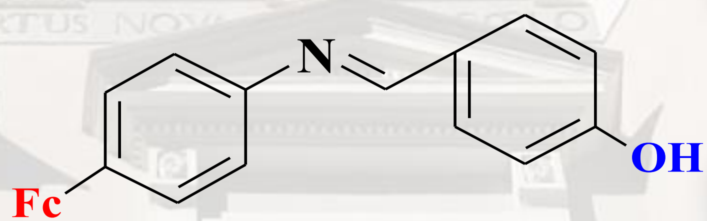


2-Ferrocenyl-4-nitrophenol



$(FcNO_2)$

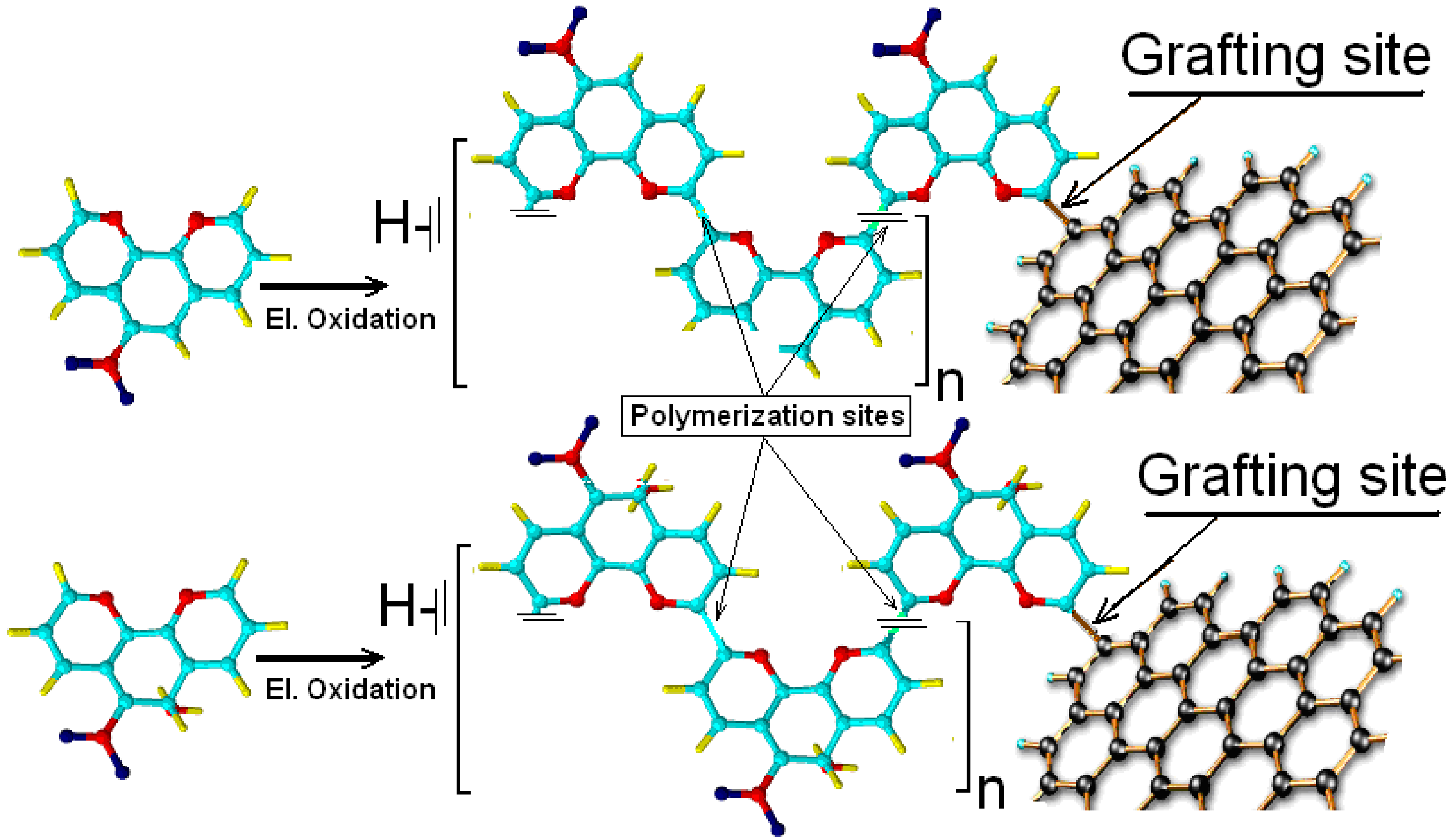
N-(4-Hydroxybenzylidene)-4-ferrocenylaniline



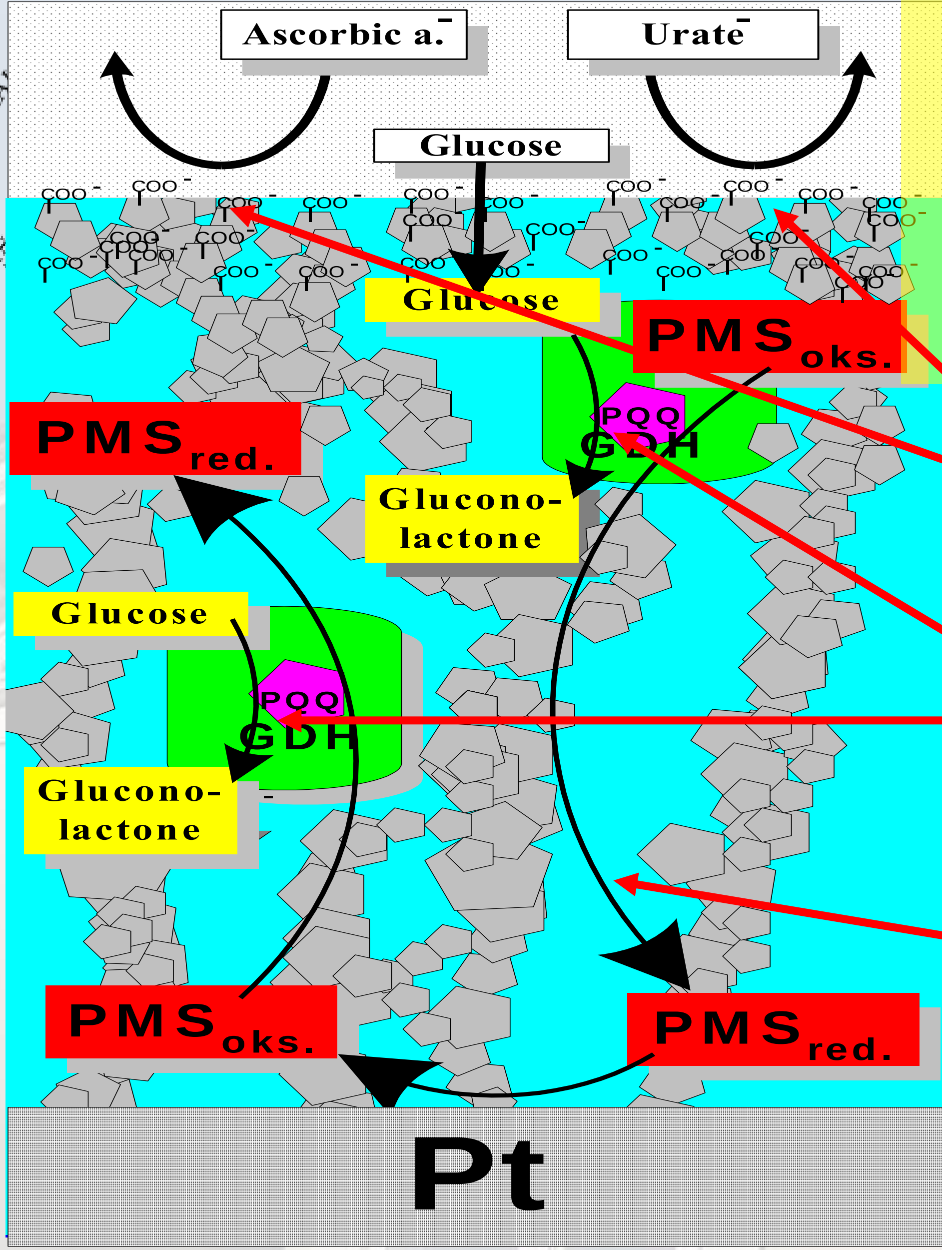
(FP1)

Electrochemical polymerisation

“Grafted” conducting polymers



Y. Oztekin, A. Ramanaviciene, N., Ryskevic, Z., Yazicigil, Z., Ustundag, A.O. Solak, A. Ramanavicius**,** 1,10-Phenanthroline modified glassy carbon electrode for voltammetric determination of cadmium(II) ions *Sensors and Actuators B Chemical* 2011, 157, 146– 153.

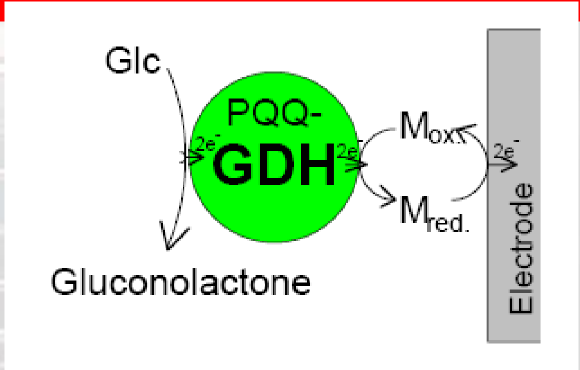


PMS mediated biosensor. Based on PQQ-GDH entrapped within conducting polymer

Protection from interfering chemicals, by over-oxidized Ppy layer

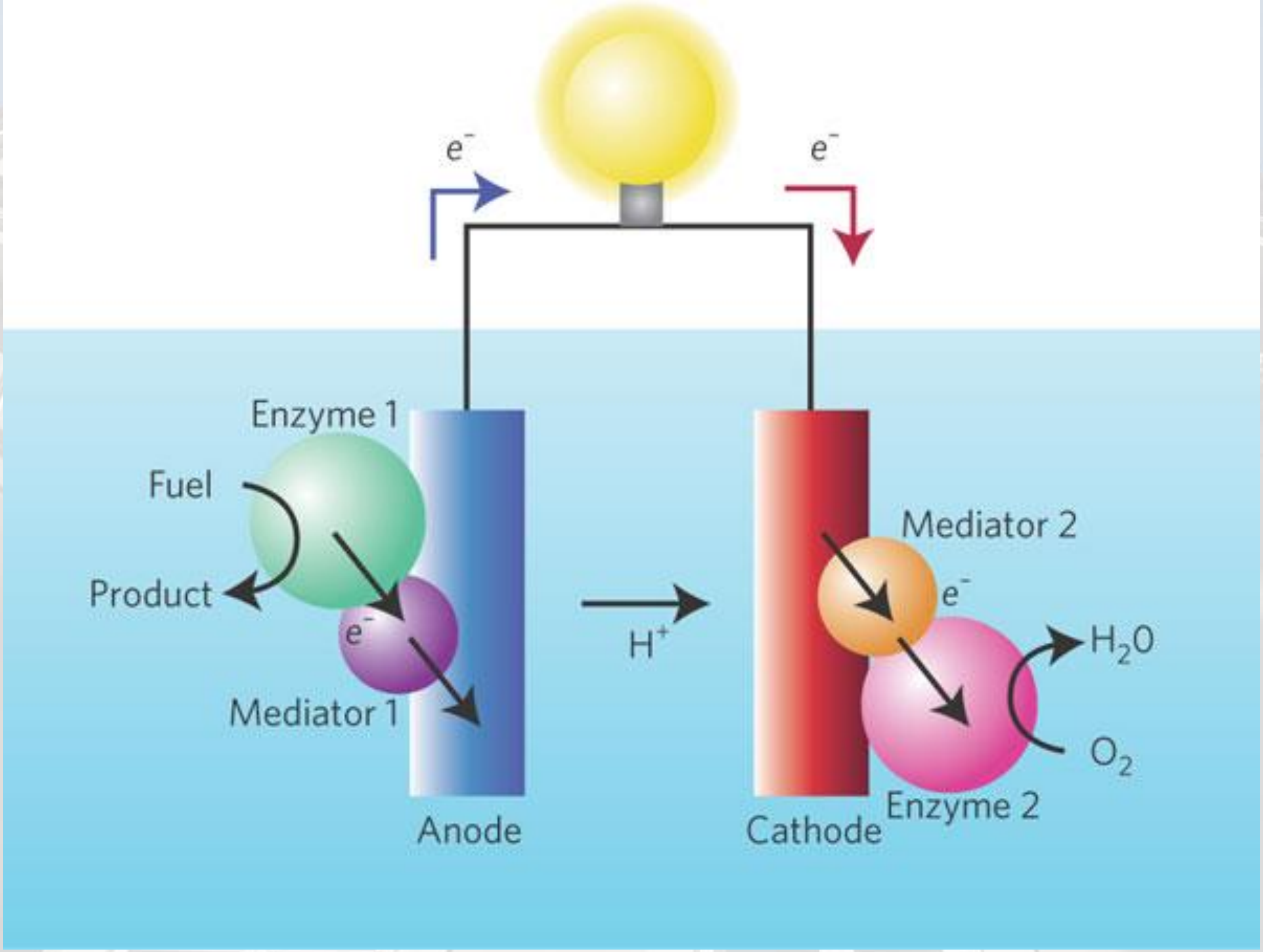
Catalytic conversion of analyte

Transfer of electrons from enzyme to conducting surface by diffusing "electron shuttles"



Ramanavicius A. (2000) **Electrochemical study of permeability and charge-transfer in polypyrrole films**, *Biologija*, 2, 64-66.
Laurinavicius V., Razumiene J., Ramanavicius A., Ryabov A.D., (2004) **Wiring of PQQ–dehydrogenases**, *Biosensors & Bioelectronics* 20 (6), 1217-1222.

Enzymatic Biofuel cells



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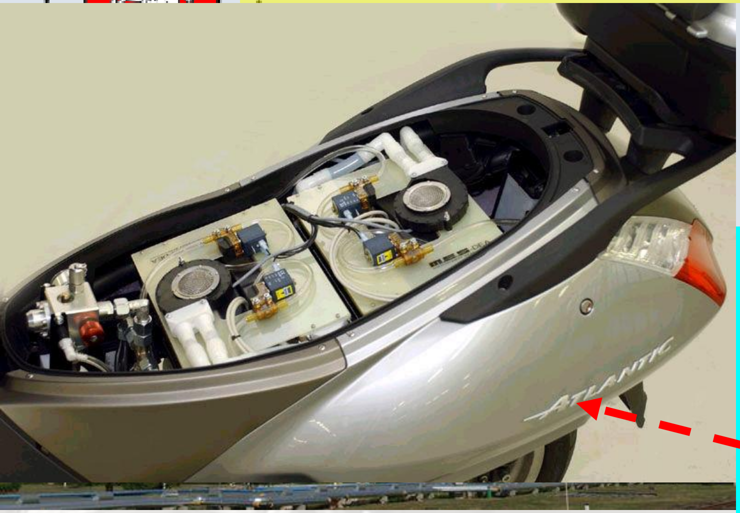
Advantages of biofuel cells (1)



Most usual generators of electricity

Chemical or nuclear energ

Thermal energy - heat

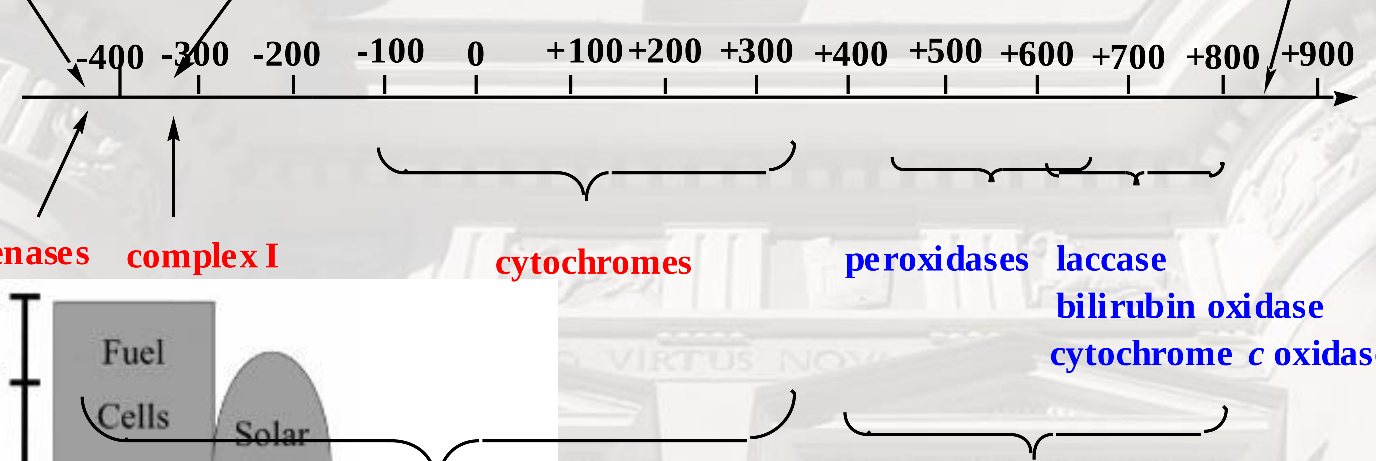
(Bio)fuel cell

Really green energy

Mechanical energ

Electrical energy

Potential range



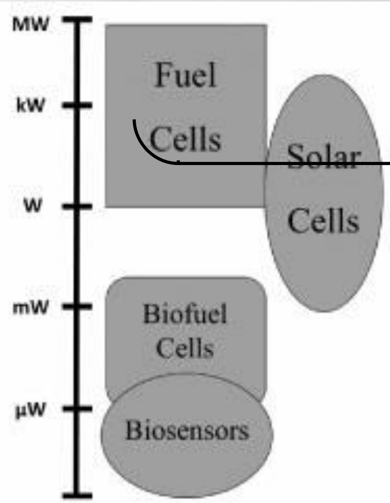


Figure 3. Schematic representation of the power range that some of the alternative energy production methods provide (adapted from reference 8)

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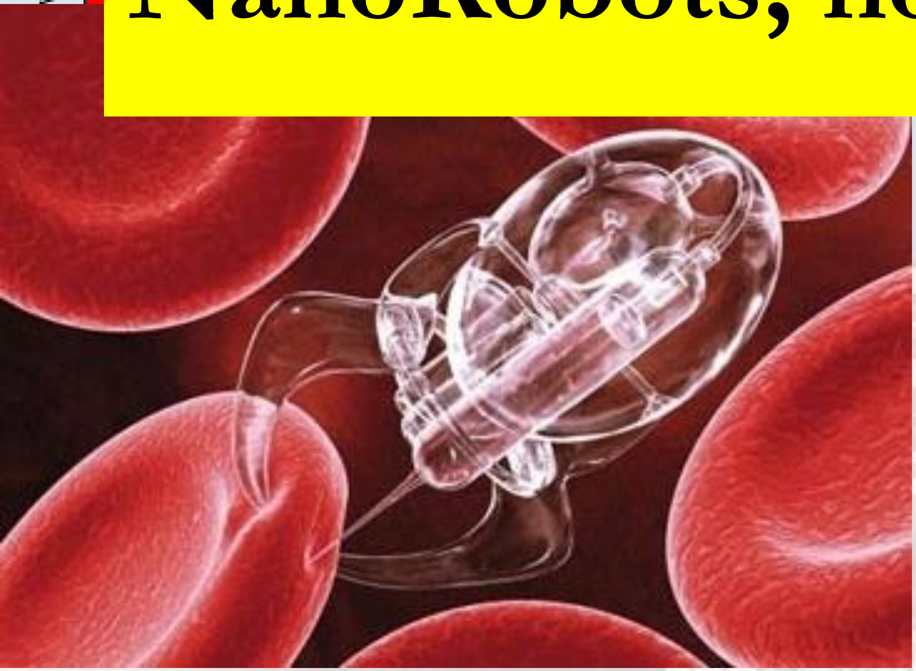
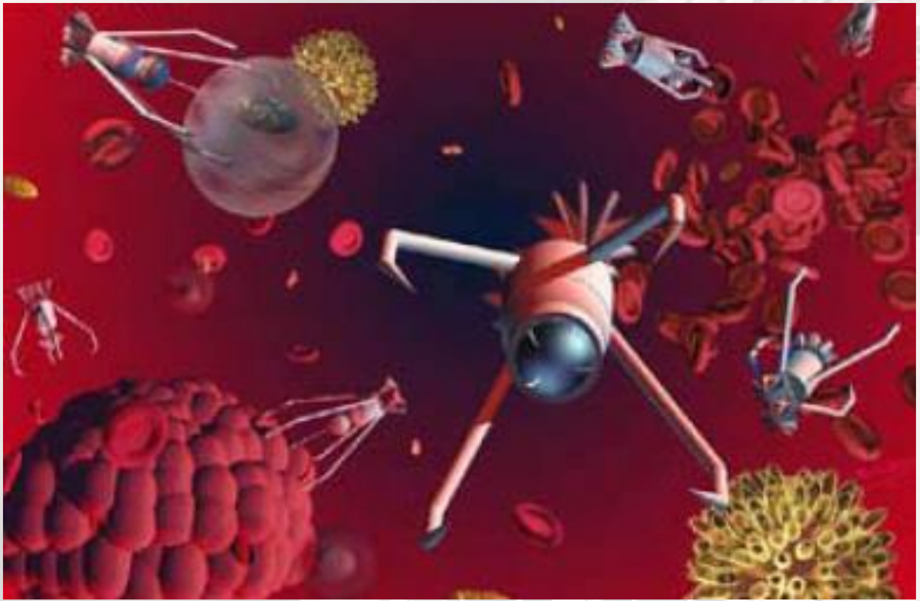
NanoTechnas

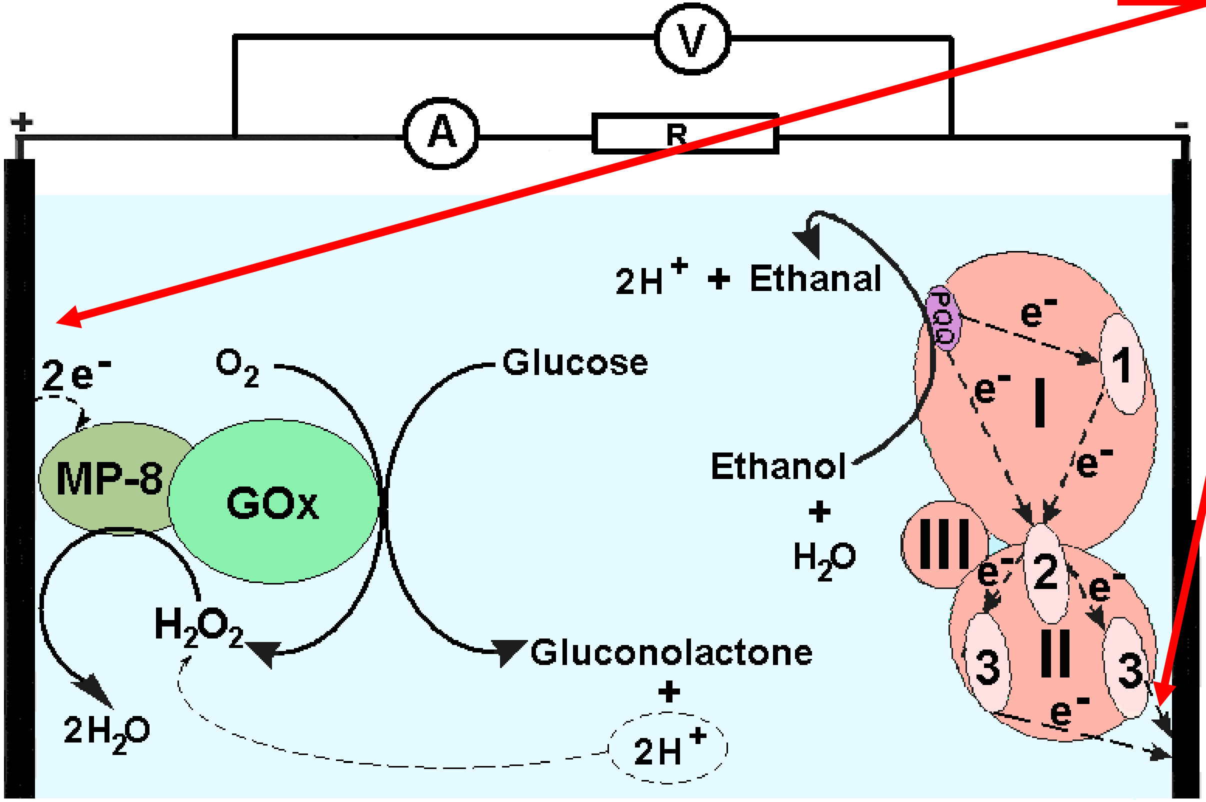
NanoRobots, how to power them?

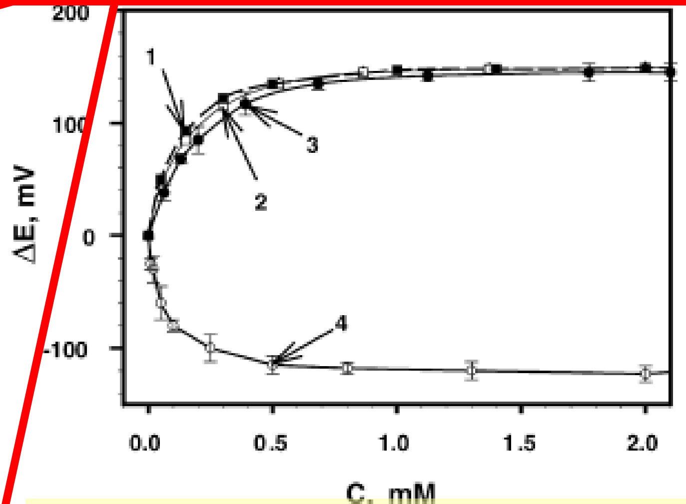




Enzymatic Biofuel cells

Direct Electron transfer





Potentials of (1) MP-8 functionalized cathode as function of hydrogen peroxide concentration; (2) MP-8/GOx functionalized carbon rod electrode as a function of hydrogen peroxide concentration; (3) MP-8/GOx functionalized carbon rod electrode as a function of glucose concentration; (4) QH-ADH functionalized anode as a function of ethanol concentration. Investigations performed in 50 mM Na acetate solution, pH 6,

Ramanavicius A. Kausaite A., Ramanaviciene A, (2005) **Biofuel cell based on direct bioelectrocatalysis,** *Biosensors and Bioelectronics*,. 20: 1962-1967.

Ramanavicius A., Kausaite A., Ramanaviciene A., (2006**) Potentiometric Study of Quinohemoprotein Alcohol Dehydrogenase Immobilized on the Carbon Rod Electrode**, *Sensors and Actuators B: Chemical*, 113, 435-444.

Thank YOU for attention!

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e-mail: ***Arunas.Ramanavicius@chf.vu.lt.***

Main activities: Development of bio-, immuno- and DNA-sensors; Development of bio-analytical methods for the food, environmental analysis and biomedical application; Synthesis and application of conducting polymers.