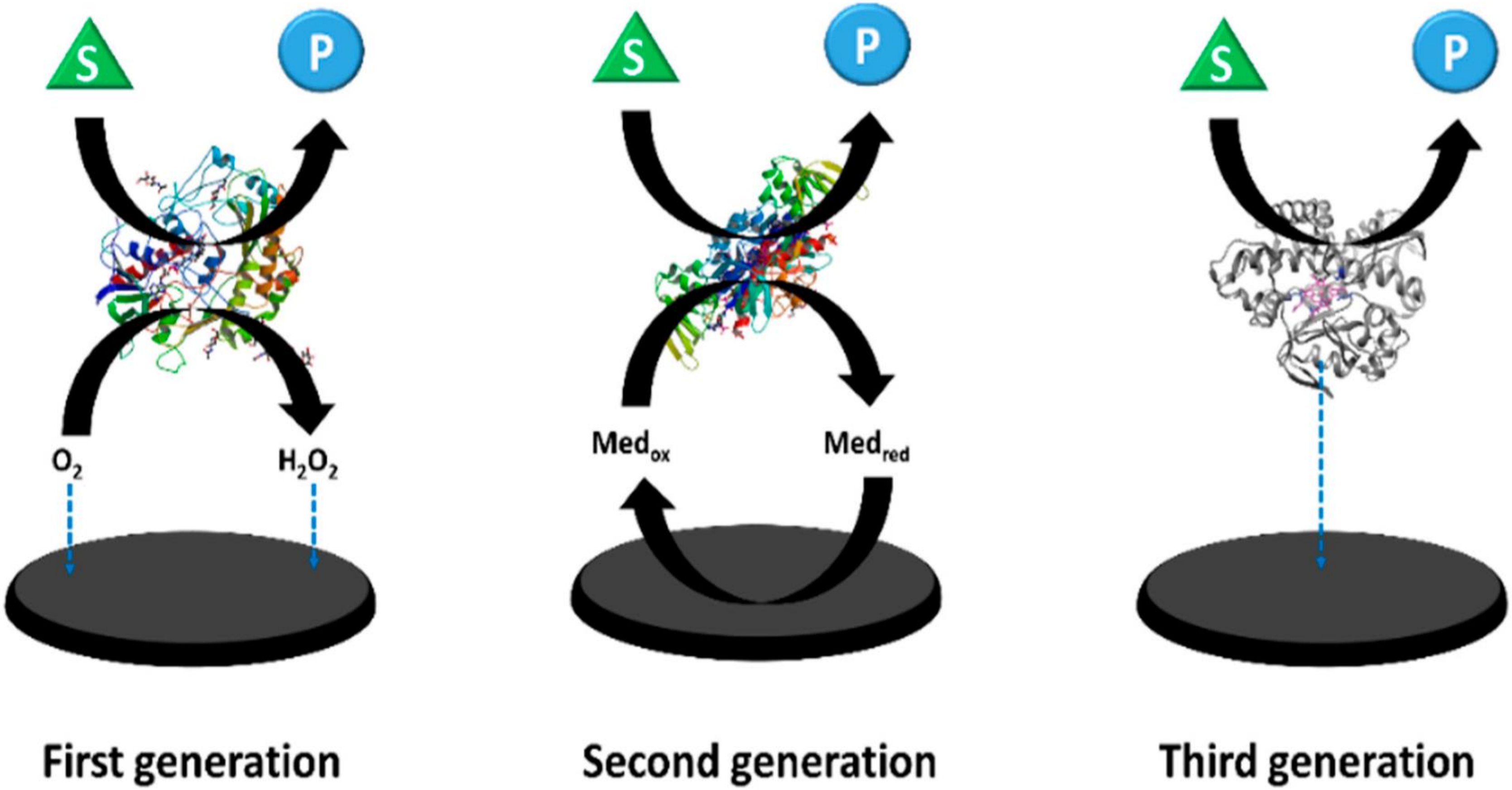
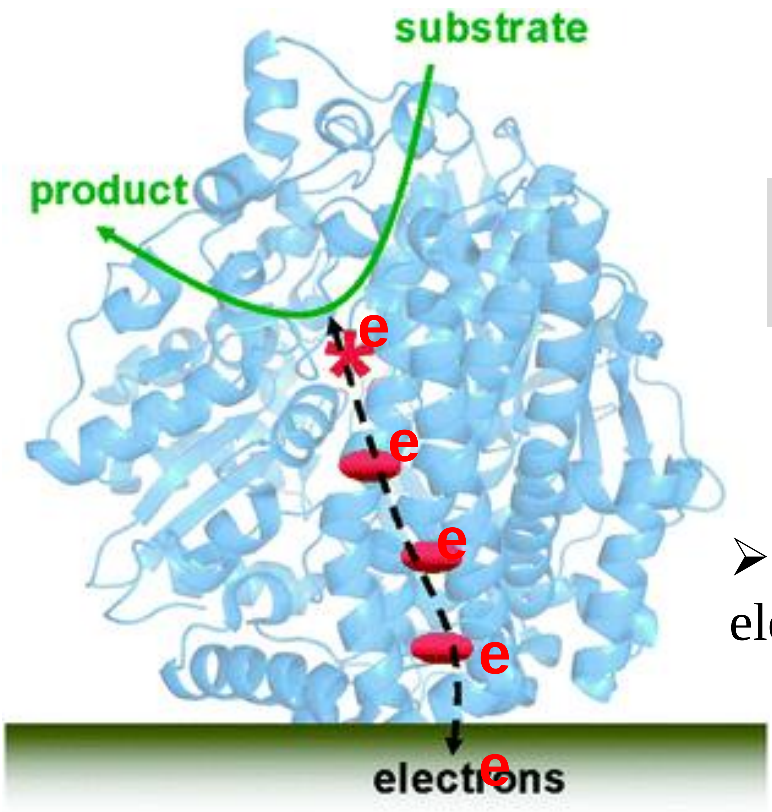
Third generation amperometric biosensors



Challenges of extracting electrical signal/current from biological system



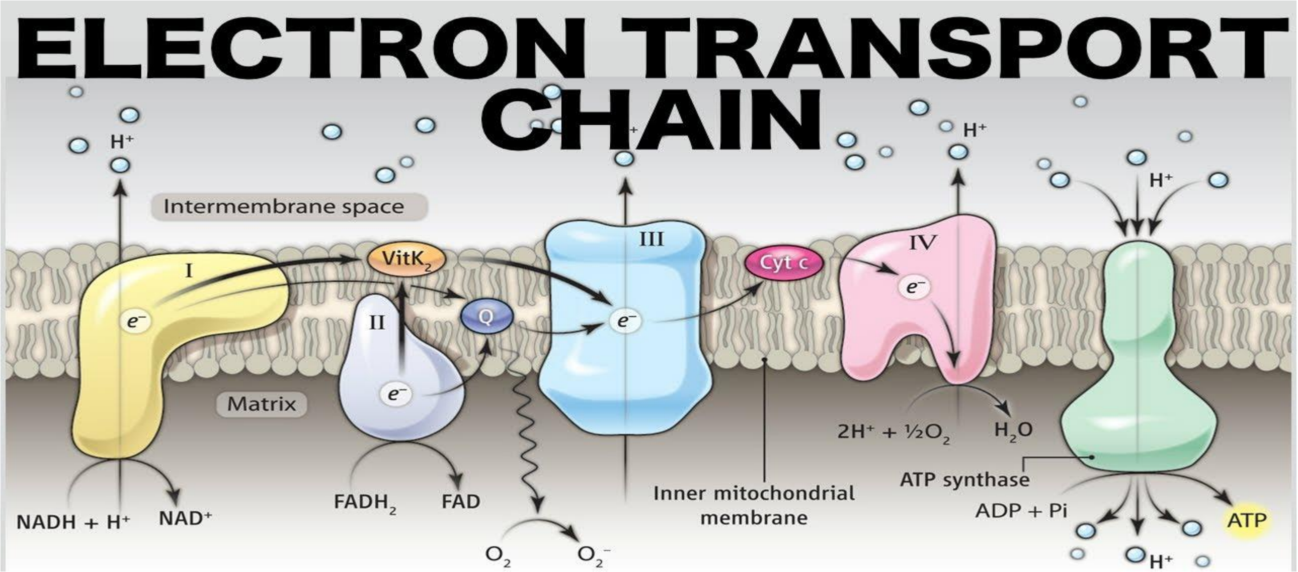
➢Redox enzymes : 20 to 850 kDa with av. hydrodynamic dia. **50 to 100s of Å**

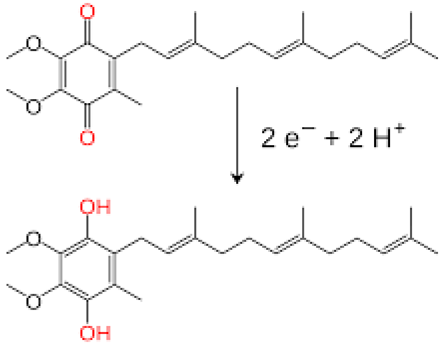
➢Typically, the protein environment allows electron tunneling within a separation of 5 to 20 Å

▪Several enzymes in nature capable to follow **direct electron transfer (DET)** via the active site of the enzyme.

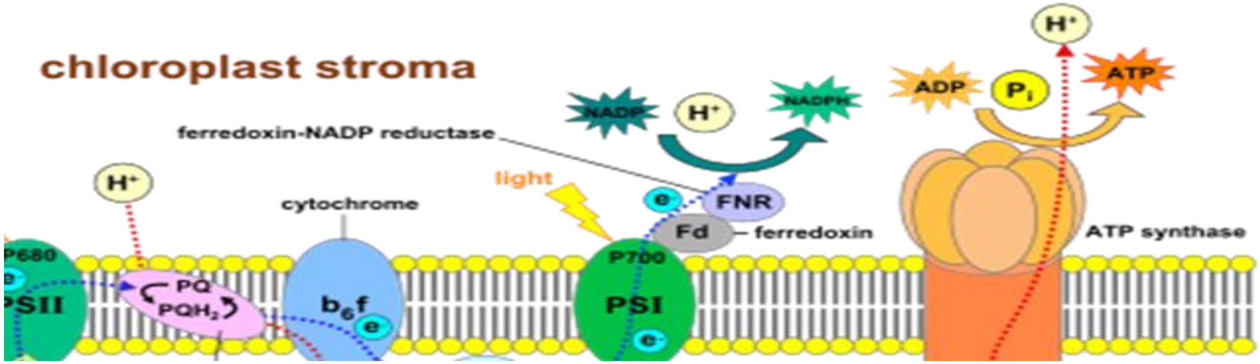
Lovely et al. Nature **435, 1098-1101 (2005)**

INSPIRATION FROM NATURE





CoQH$_2$ or ubiquinol, and an oxidized form, CoQ or ubiquinone.



Electron transfer rate **(K_{et}): ~ 10 ^{13} s^{-1}.**

Edge to edge distance for haem-haem electron transfer system 25 to 35 A^o

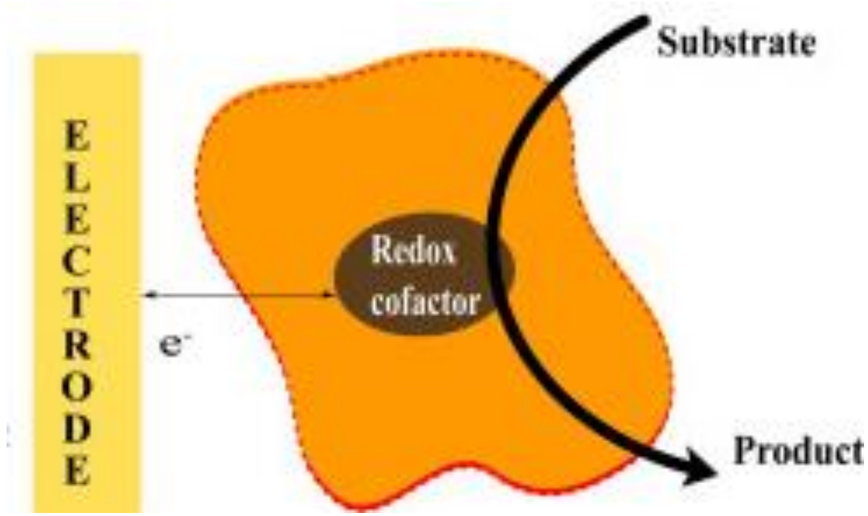
ADVANTAGES OF DET BASED APPROACH FOR BIOSENSORS

(a) More accurate mimics of energy transfer processes to biological systems thus offering high specific currents and biosensor sensitivity

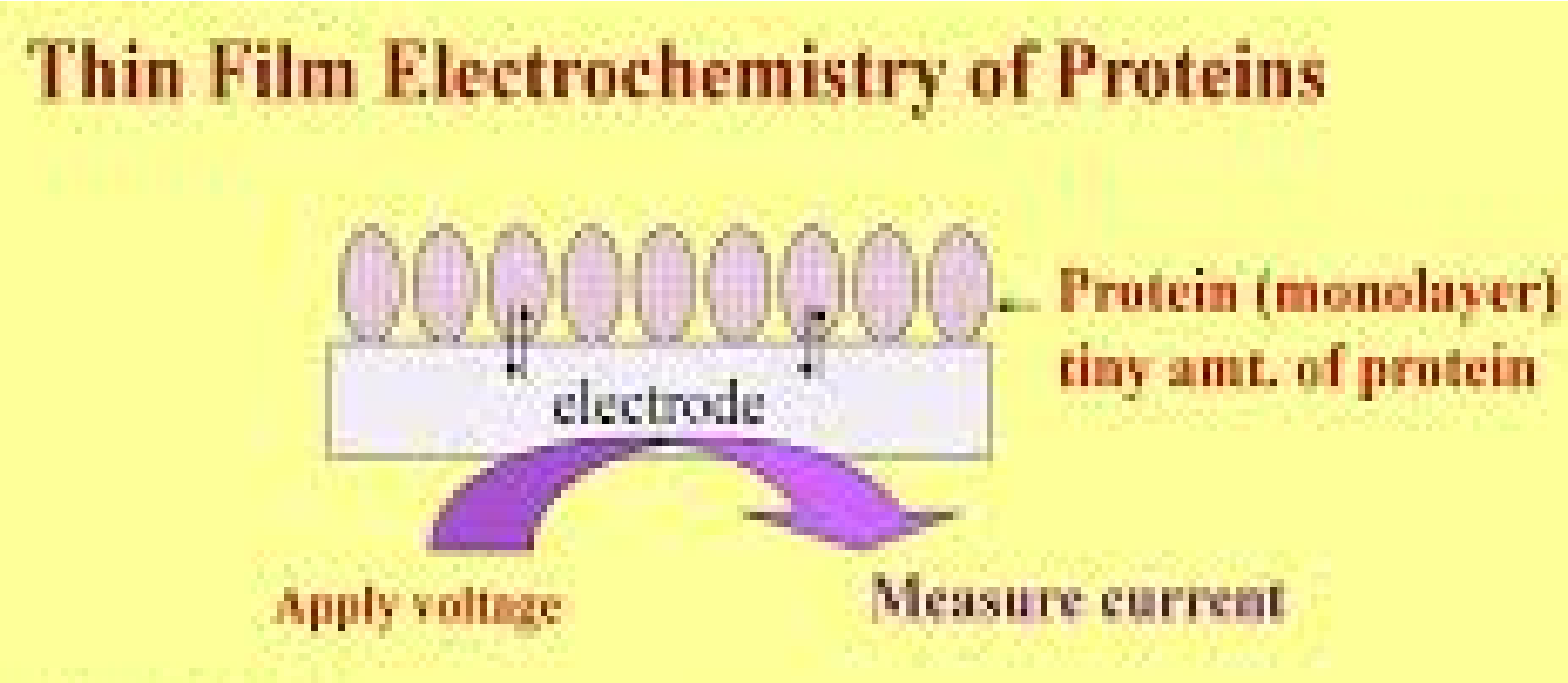
(b) Higher operational stability of the device (no issue such as, mediator leaching).

(c) Suitable in open environment/body integrated system (as no toxic mediators are used)

- DET occurs through the enzyme’s ability to act as a ‘**molecular transducer**’ that converts the chemical signal directly to an electrical one.



- 3rd Generation bioelectrode (biosensors) utilizes thin film of protein to evaluate the process of DET.
- It utilizes control orientation of enzyme/protein on electrode surface



Cyclic Voltammetry: Only Adsorbed O and R Electroactive—Nernstian Reaction

Aug. 2017; to appear in *Reports of Progress in Physics*

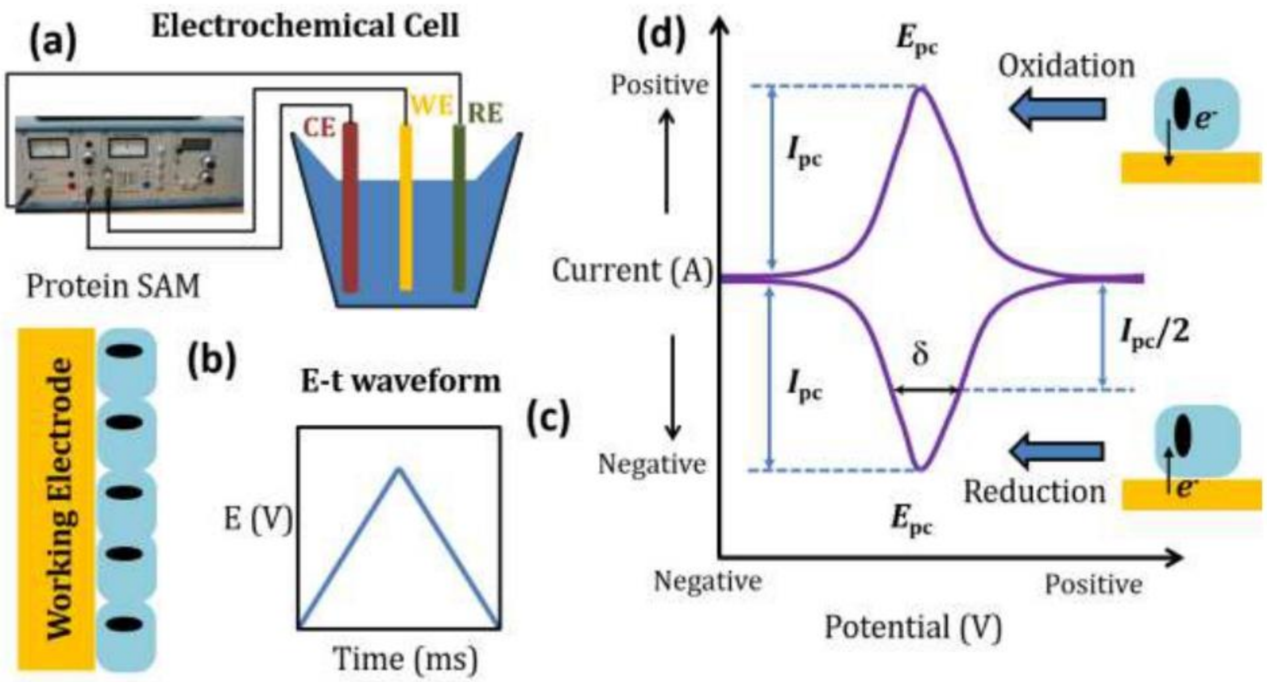
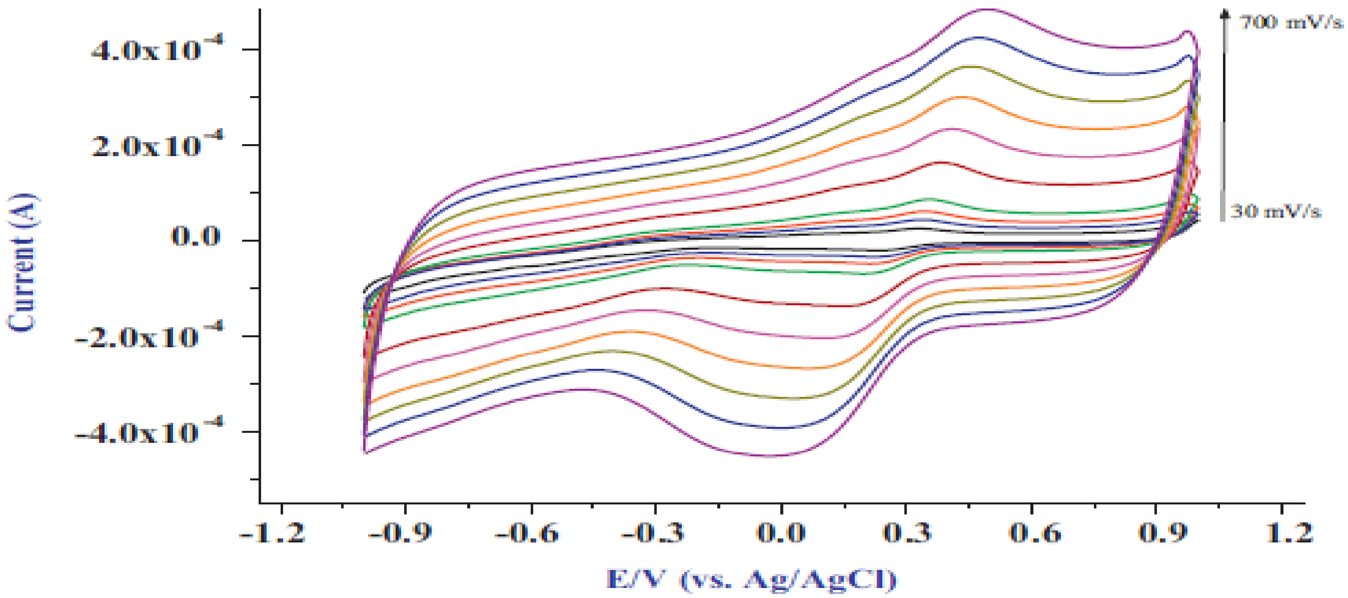


Figure 1: (a) Schematic experimental setup for electrochemical charge transfer studies across self-assembled monolayers of redox-active proteins on conducting electrodes as shown in (b). (c) Time varying electric field applied between counter (CE) and working (WE) electrode with respect to reference electrode (RE). (d) Electrochemical current profile during oxidation and reduction process at the surface of working electrode (details

Determination of Direct electron transfer rate (K_{et} or K_s) in protein film:

Technique: Protein film voltammetry

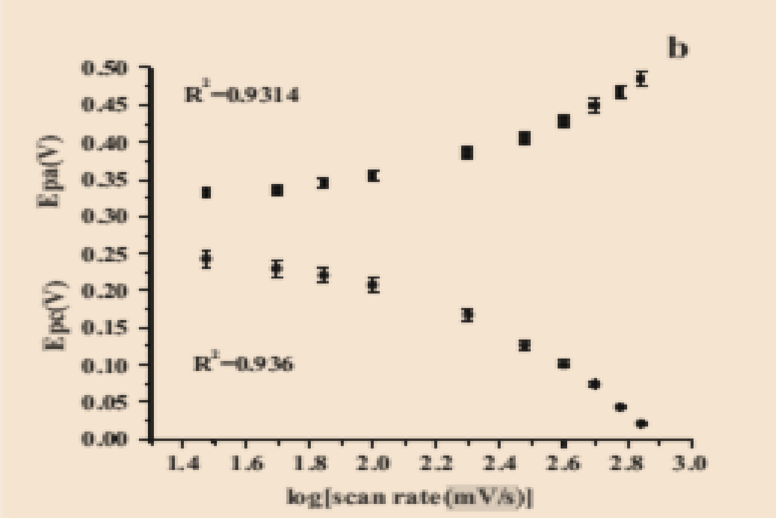
Scan rate (mV/s) (The rate of change of potential with time) is applied and voltammogram recorded



Protein film voltammetry (PFV) provides information:

- ✓ reversible or quasi-reversible process,
- ✓ surface coverage area (Γ -gamma-) of the biocatalyst,
- ✓ Electron transfer rate constant (k_s), and
- ✓ number of electrons transferred in the reaction (n)

Review: Goswami & group *Biosensors and Bioelectronics* 24 (8), 2313-2322 (2009)



Laviron equation

$$E_{pa} = E^{0'} + \frac{2.3RT}{(1-\alpha)nF\log\vartheta}$$

$$E_{pc} = E^{0'} - \frac{2.3RT}{\alpha nF\log\vartheta}$$

$E^{0\prime}$ is the formal potential,
υ is the scan rate,
n and α are the charge transfer number and the charge transfer coefficient, respectively, when 0.5 < α < 1, in general n = 1.

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \frac{\log RT}{nF\vartheta} - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT} \quad (\text{When } \Delta E_p > 200\text{ mV})$$

$$k_s = \frac{\alpha nF\vartheta}{RT} \qquad \text{When } \Delta E_p < 200\text{ mV)}$$

n and electron transfer rate constant can be calculated!

R is the thermodynamic constant (*R* = 8.314 JK^{-1} mol^{-1}), *F* is the Faraday constant (*F* = 96,500 C mol^{-1}), *T* is the temperature in Kelvin, The surface concentration of the adsorbed electroactive species Γ (mol.cm^{-2}) on the bioelectrode, can be calculated using *Brown-Anson model* –from a plot of peak current (I_p) vs scan rate (v):

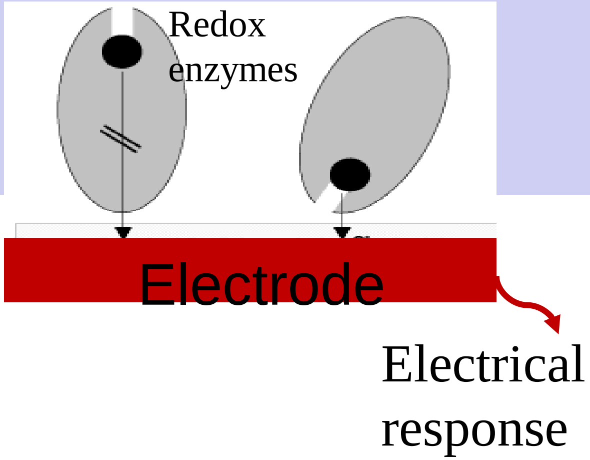
$$I_p = n^2 F^2 \Gamma A v / 4RT$$

where A is the area of the electrode, n, is the no. of electron transferred, F, is the Faraday constant (96,584 C/mol), v is the scan rate. Denominators: R is gas constant [8.314 J/(mol K)], and T is absolute temperature (298 K).

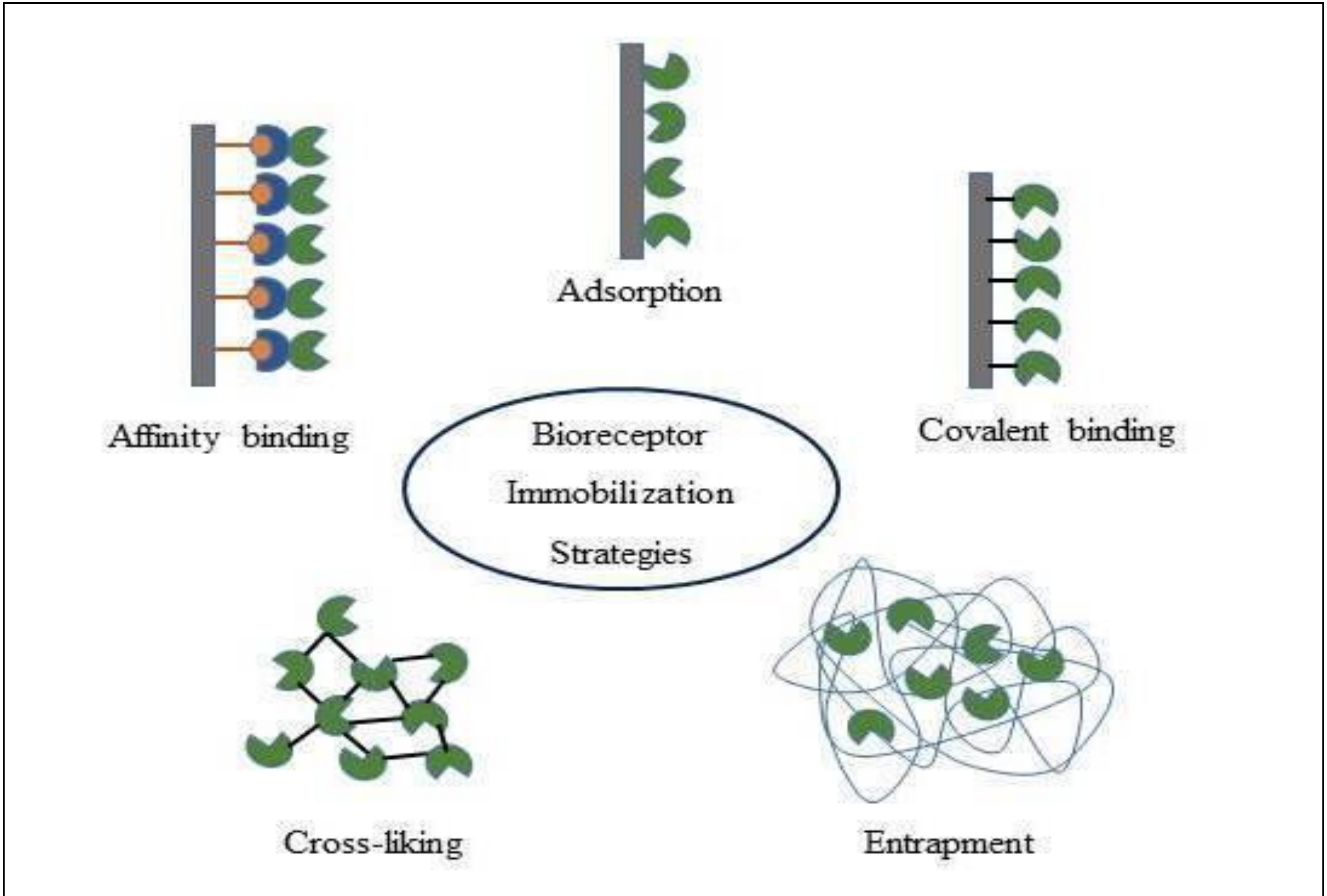
Surface coverage area (Γ -gamma-) of the biocatalyst can be calculated from the slope of the plot of I_p vs. v.

Key features involved in developing for 3G-bioelectrode:

- ❑ Stability of biocatalyst
- ❑ Facilitating electron transfer (k_{et})
- ❑ Improve substrate diffusion (porosity) and kinetics



Immobilization methods of enzymes on electrodes



Advanced materials

- Materials that are utilized in high-technology applications.
- metals, ceramics, polymers, nano, nanoengineered and smart materials

Smart materials:

- Respond to stimuli (temperature, stress, pH, magnetic field, electrical field, etc).
- *Example*: piezoelectric materials, smart gels etc.

Polymer:

Redox polymer: e.g. osmium polymers (fast electron transfer rates and tunable redox potential)

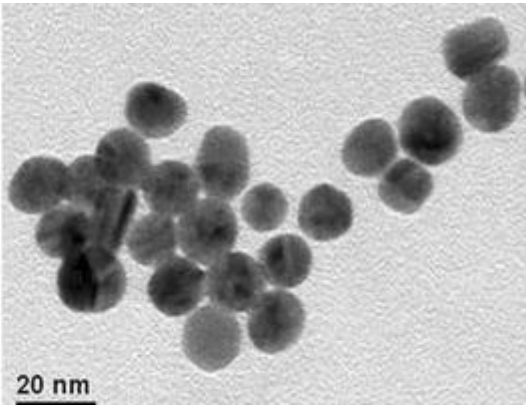
Conducting polymers: polyaniline (PANI), polypyrrole (PPy), poly(ethylenimine) (PEI), etc.

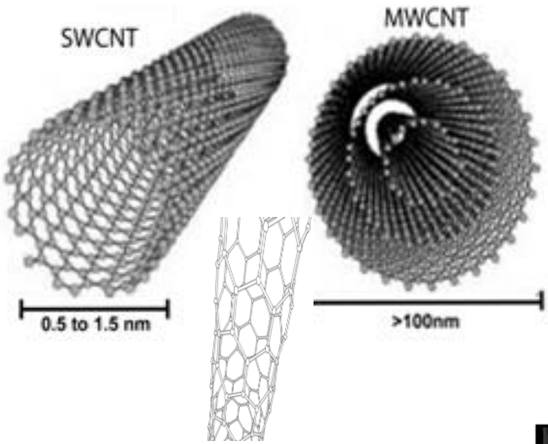
Non-conducting polymers: Silk, Chitosan, PDMS, sol–gel materials etc.

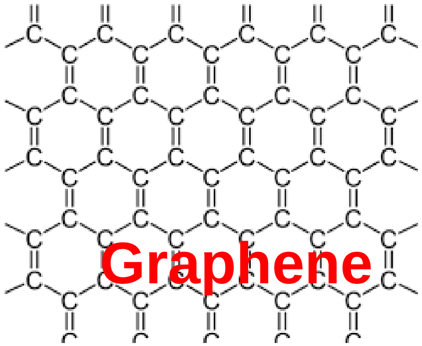
Molecularly imprinted polymers

Composite materials: e.g. Buckypaper (MWCNTs) compressed into a laminated sheet with porosity, conductivity, high surface area and low resistivity, allow the development of cheap, light weight, disposable and flexible EFCs.

Conductive Nanomaterials



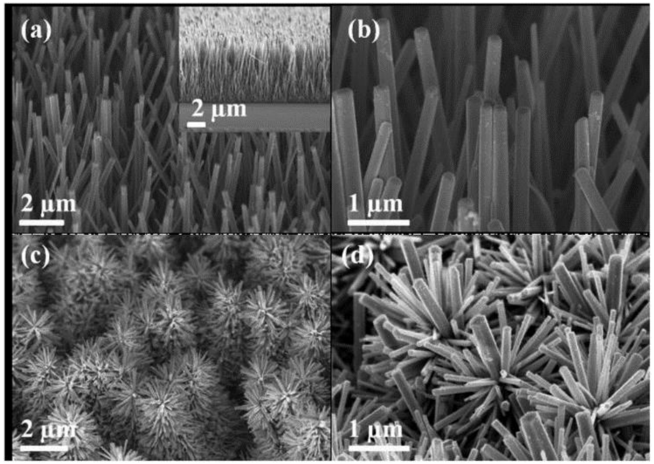




Electron mobility at RT, >15000 $cm^2 \cdot V^{-1} \cdot s^{-1}$

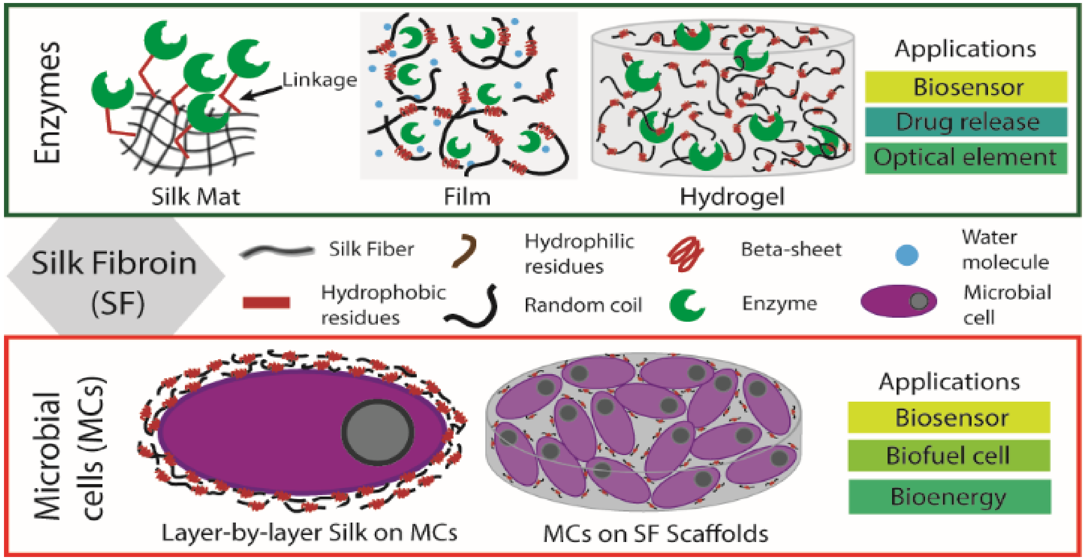
Geim & Novoselov *Nature Mat.* (2007

Review: Goswami *& group *Biosensors and Bioelectronics, 79, 386-397*(2016)



Kharisov et al. *RSC Adv.*, 5, (2015)

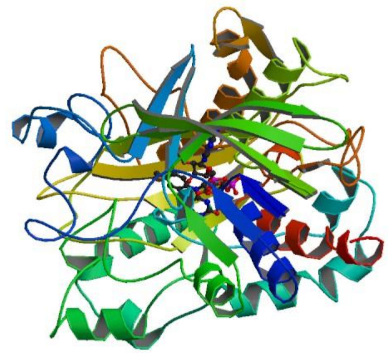
Biomaterials



Review: Goswami*, & group *ACS Biomaterials* (2020)

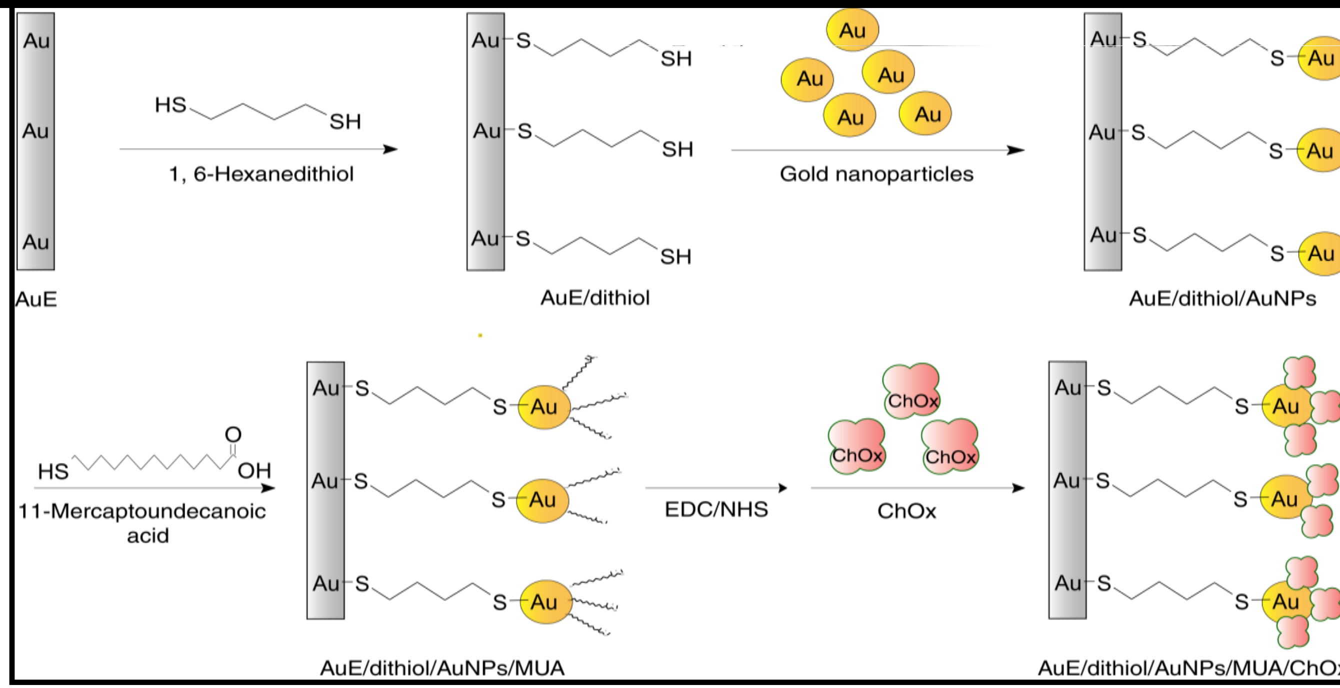
AgNP, SiNPs, porous nanosheet-based ZnO microsphere, nanoporous & mesoporous materials (e.g. Zeolites), etc.

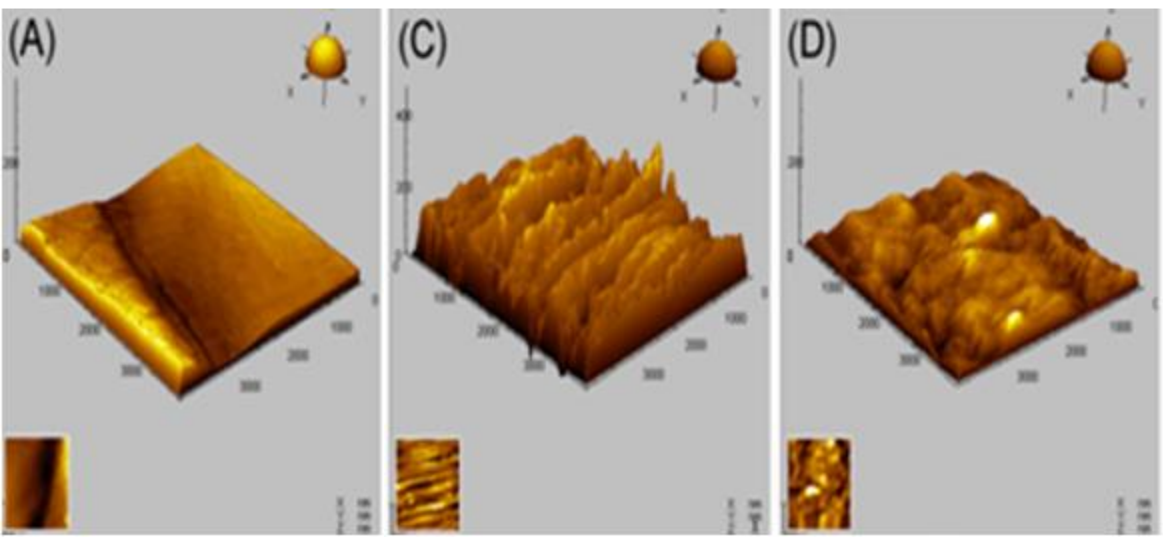
Cholesterol oxidase based 3G bioelectrode



Molecular mass: ~60 kDa
Monomeric flavoprotein

***k_{et}*: 0.35 s^{-1}**

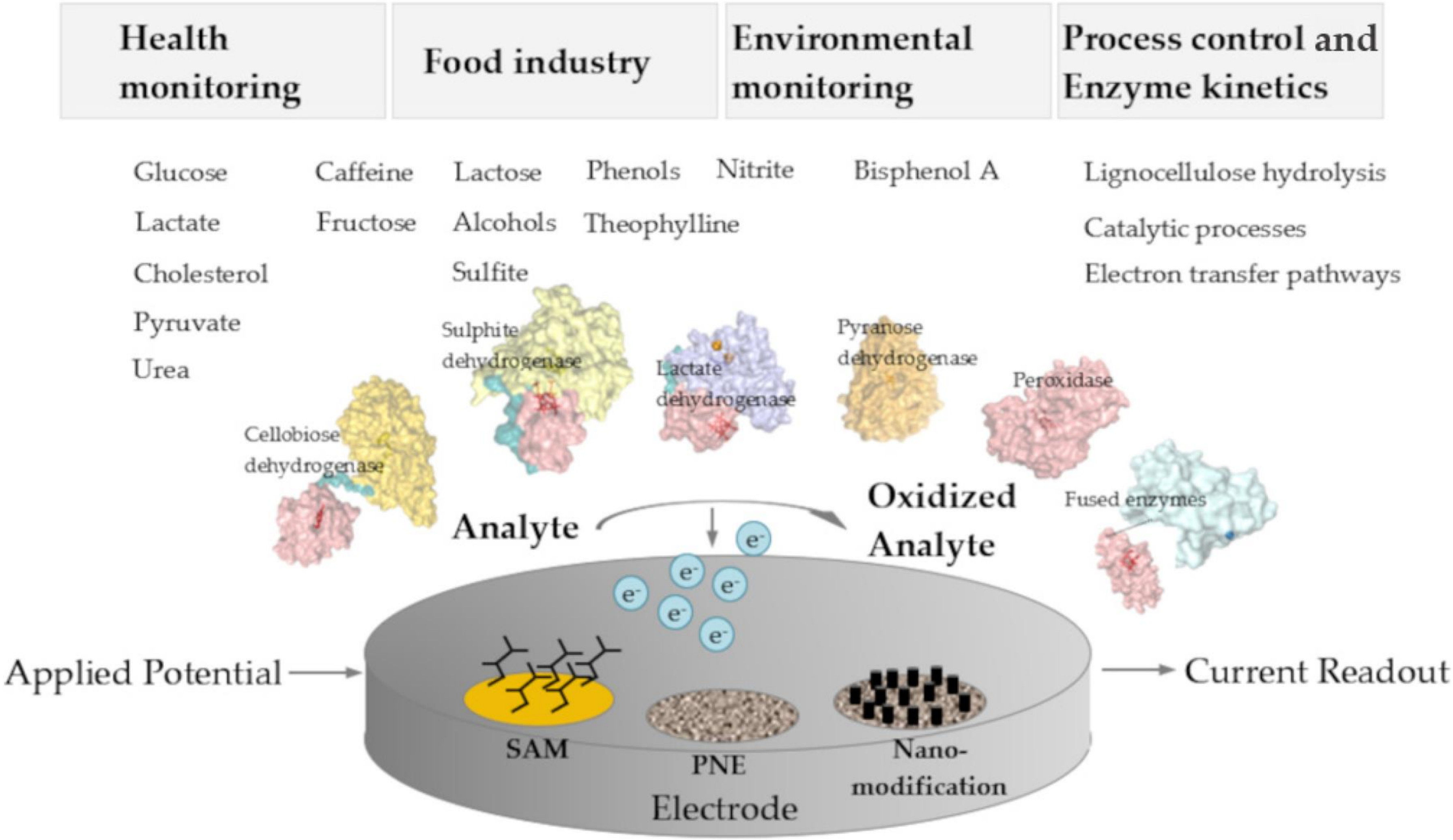




AuE　AuE/dithiol/AuNPs/MUA/ChOx　AuE/MUA/ChOx

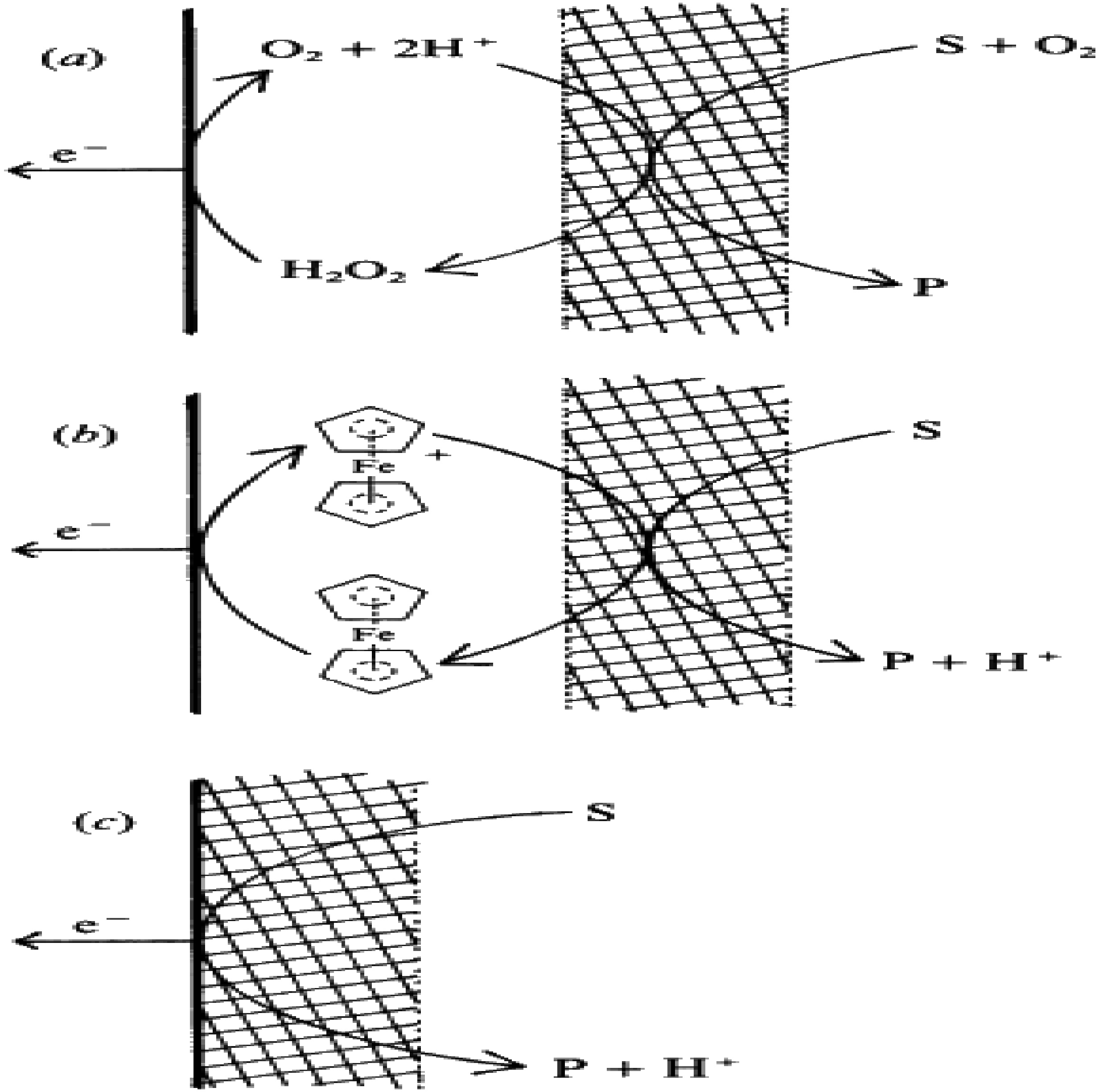
Response Characteristics	AuE/dithiol/AuNPs/MUA/ChOx
Linear range	0.04 to 0.22 mM
Sensitivity	9.02 μA/mM
Detection Limit	34.6 μM
Calibration equation	Current (μA) = 0.009*Chol (μM) + 2.9164 (R^2 = 0.9972)
Km	308.90 μA/mM

Saxena,Chakraborty, Goswami* *Biosensors and Bioelectronics* 26 (2011).



Schematic overview on application areas, analytes, enzymes, and the architecture of 3rd generation amperometric biosensors. SAM, self-assembled monolayer; PNE, porous nanostructured electrodes.

Schachinger et al. Molecules 2021, 26(15), 4525; https://doi.org/10.3390/molecules26154525


(a)
$O_2 + 2H^+$
$S + O_2$
e^-
H_2O_2
P
(b)
Fe
+
S
e^-
Fe
$P + H^+$
(c)
S
e^-
$P + H^+$

The following reaction occurs at the enzyme in all three biosensors:

$$\text{Substrate(2H)} + \text{FAD-oxidase} \longrightarrow \text{Product} + \text{FADH}_2\text{-oxidase}$$

(a)

biocatalyst

$$\text{FADH}_2\text{-oxidase} + O_2 \longrightarrow \text{FAD-oxidase} + H_2O_2$$

electrode

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

(b)

biocatalyst

$$\text{FADH}_2\text{-oxidase} + 2\ \text{Ferricinium}^+ \longrightarrow \text{FAD-oxidase} + 2\ \text{Ferrocene} + 2H^+$$

electrode

$$2\ \text{Ferrocene} \longrightarrow 2\ \text{Ferricinium}^+ + 2e^-$$

(c)

biocatalyst/electrode

$$\text{FADH}_2\text{-oxidase} \longrightarrow \text{FAD-oxidase} + 2H^+ + 2e^-$$