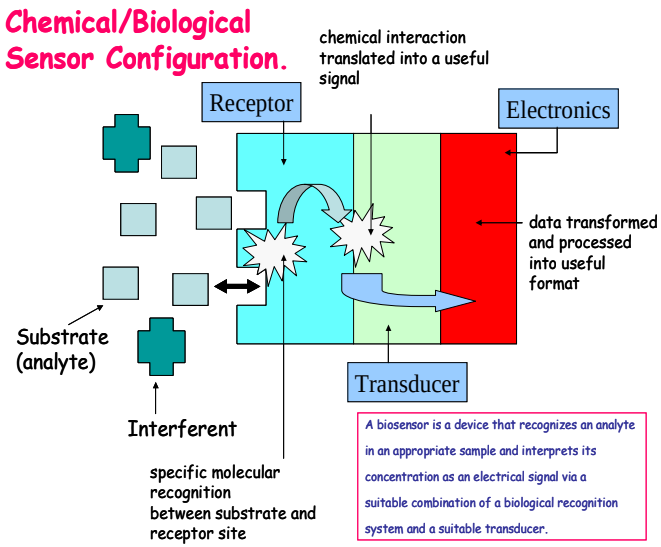
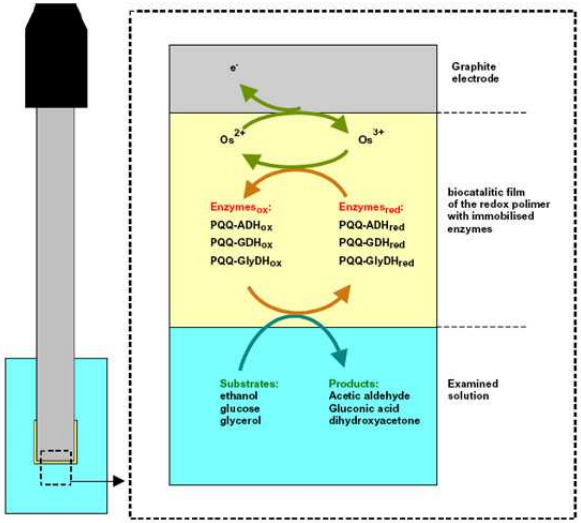




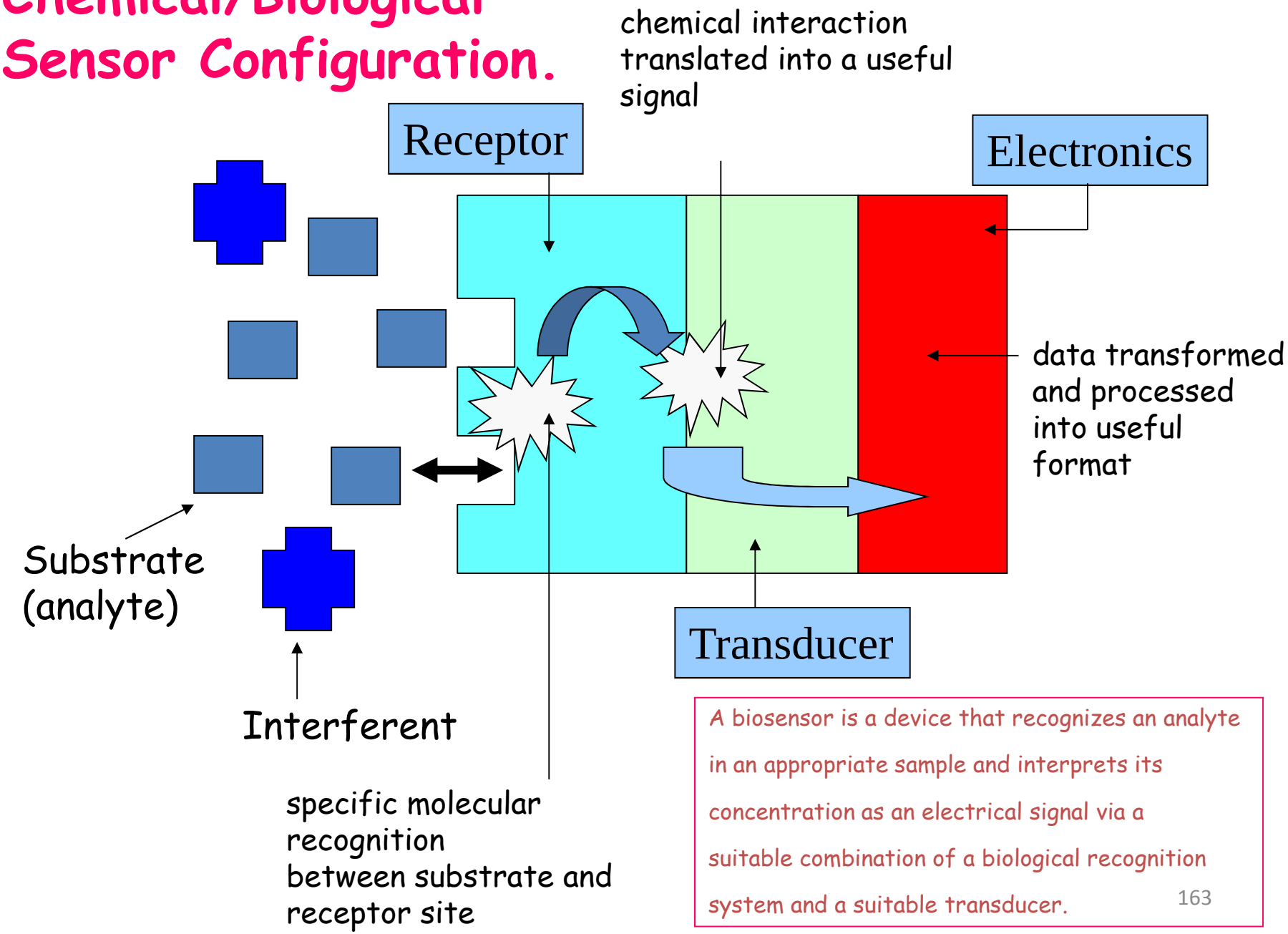
Lecture 5-6

Electrochemical Biosensors

Chemical/Biological Sensors.

- Chemical/biological receptor microstructure (have specific molecular interaction between receptor and analyte species) coupled to an electronic transducer which converts chemical/biochemical activity into electrical signals which can be amplified, stored, displayed and manipulated.

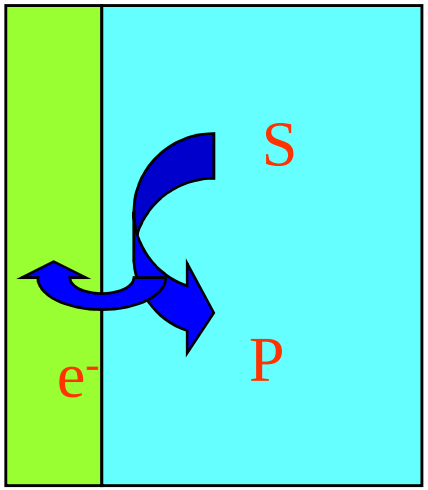
Chemical/Biological Sensor Configuration.



A biosensor is a device that recognizes an analyte in an appropriate sample and interprets its concentration as an electrical signal via a suitable combination of a biological recognition system and a suitable transducer.

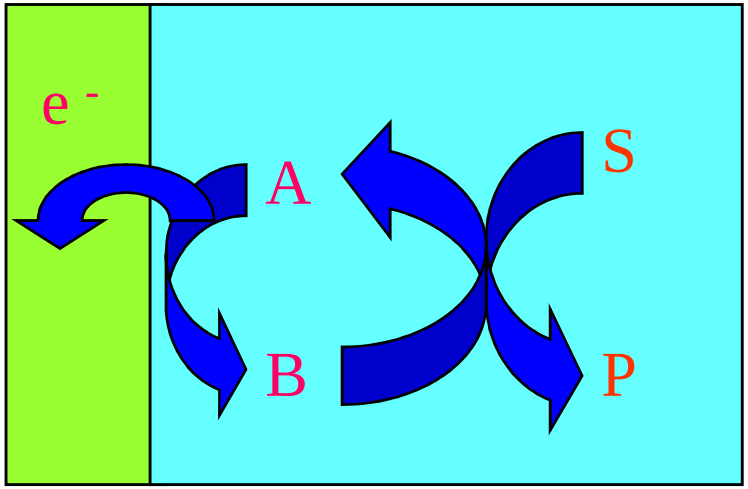
Mediated vs unmediated ET at electrodes .

- Redox groups bound to support surface as 2D monolayer or as 3D multilayer .



Direct unmediated ET .

$$S \xrightarrow{\mp ne^-} P$$

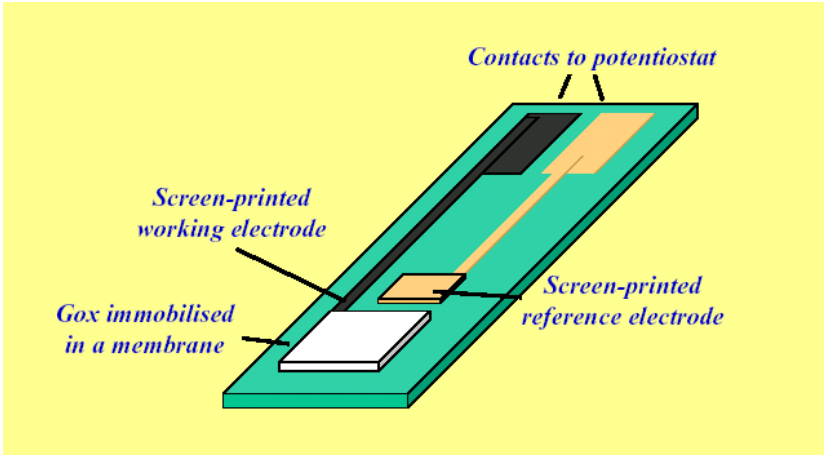


Heterogeneous redox catalysis : mediated ET via surface bound redox groups .

$$A \xrightarrow{\mp ne^-} B$$

$$B + S \rightarrow A + P$$

Enzyme communication with electrodes.



Enzyme wiring strategies

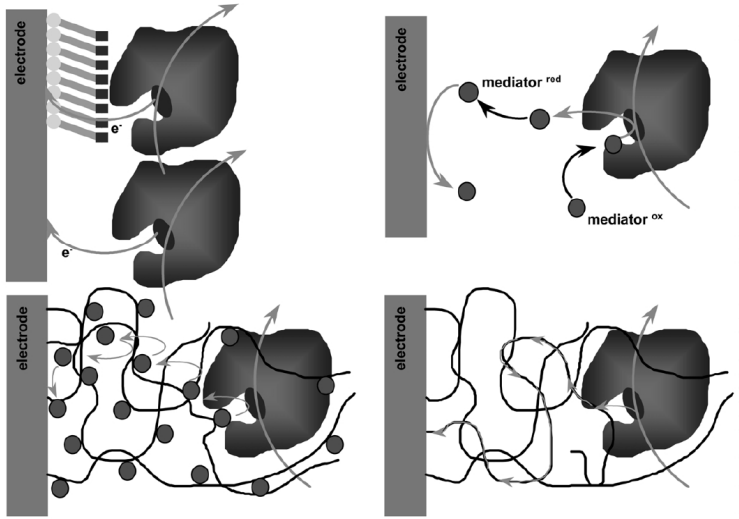


Fig. 1. Schematic representation of ET possibilities between enzymes and electrodes. (a) Direct ET at a bare or monolayer-modified electrode. (b) Shuttle mechanism based on free-diffusing redox species. (c) Electron hopping in a redox-relay modified polymeric hydrogel. (d) ET via a conducting polymer chain.

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3 generations of enzyme biosensor electrodes.

- **1st generation**:
  Charge shuttling via O_2/H_2O_2.
- **2nd generation** :
  Synthetic electron shuttles (redox mediators) used.
- **3rd generation** :
  No mediator used , enzyme wiring.

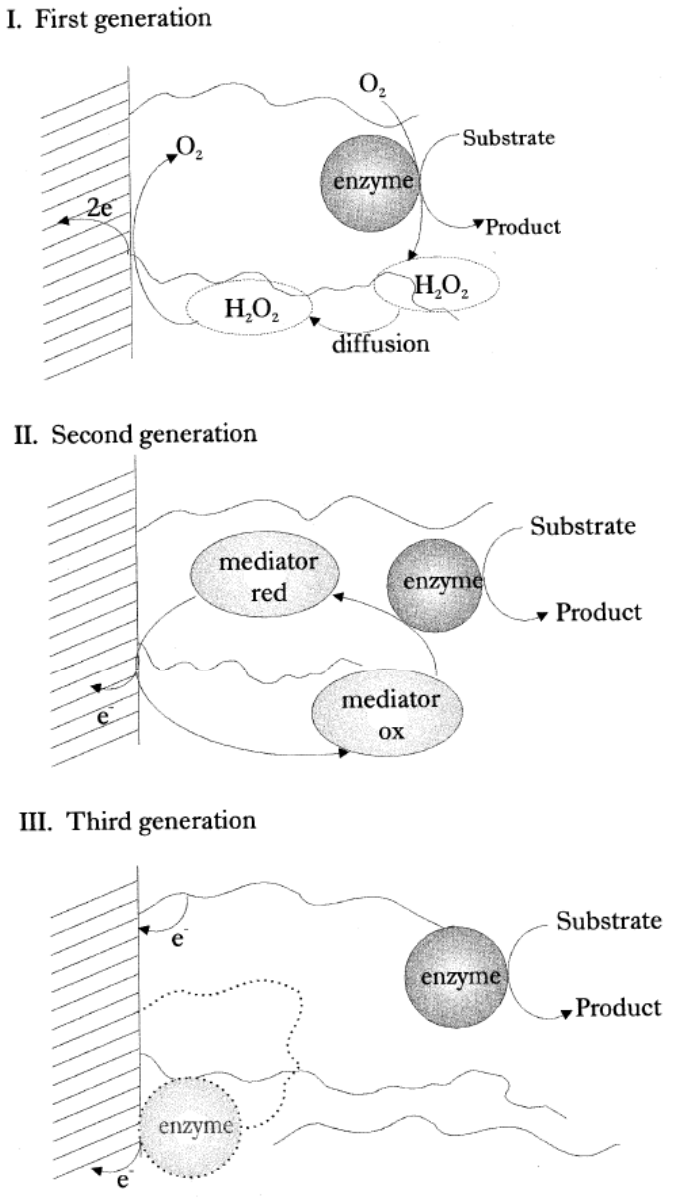


Fig. 2. Schematic representation of the three generations of enzyme electrodes.

Amperometric enzyme biosensors.

- Enzymes are very specific biological catalysts.
- They interact with substrates via the Michaelis/Menten mechanism.
- If enzymes can be incorporated and immobilized within a matrix located next to an electrode surface, then it is possible to combine the specificity of enzyme catalysis with the many advantages of amperometric detection.

- Some questions need addressing before this useful synerigy can be achieved.
  - How can enzymes be immobilized in a region next to an electrode surface?
  - How can the enzyme be made to communicate with the underlying support electrode?
  - How can we maintain the catalytic integrity of the immobilized enzyme?
  - How can we describe the mechanism and quantify the kinetics underlining the operation of an amperometric enzyme electrode?
- We will focus attention on redox enzymes, and in particular, glucose oxidase.

Redox enzymes.

- Redox enzyme contains tightly bound redox active prosthetic group (e.g. flavin, haem, quinone) that remains bound to the protein throughout redox cycle.
  - Prosthetic group = non amino acid component of conjugated protein.
- Redox enzymes exist in both oxidised and reduced forms.
- Redox enzymes can be subclassified in terms of the redox centres present in the enzyme.
- Flavoproteins are most often studied.
- They consist of ca. 80 different enzymes containing either
  - Flavin adenine dinucleotide (FAD)
  - Flavin mononucleotide (FMN)

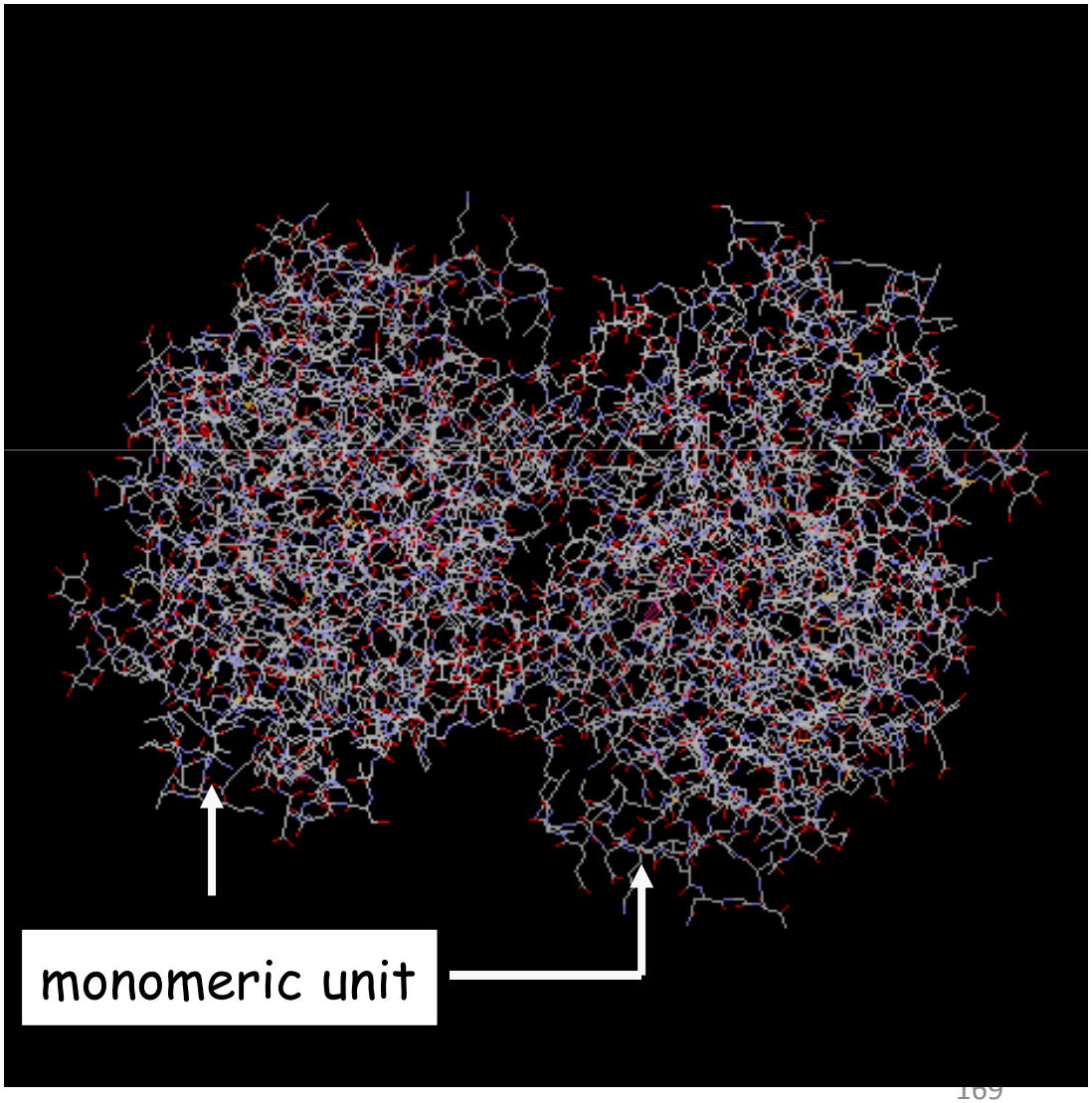
  at the active site.
- The flavin unit is strongly associated with the protein structure and is sometimes covalently bound to the amino acid residues in enzyme.

Glucose oxidase

β-D-glucose: oxygen 1-oxidoreductase EC1.1.3.4) : ***GOx*** .

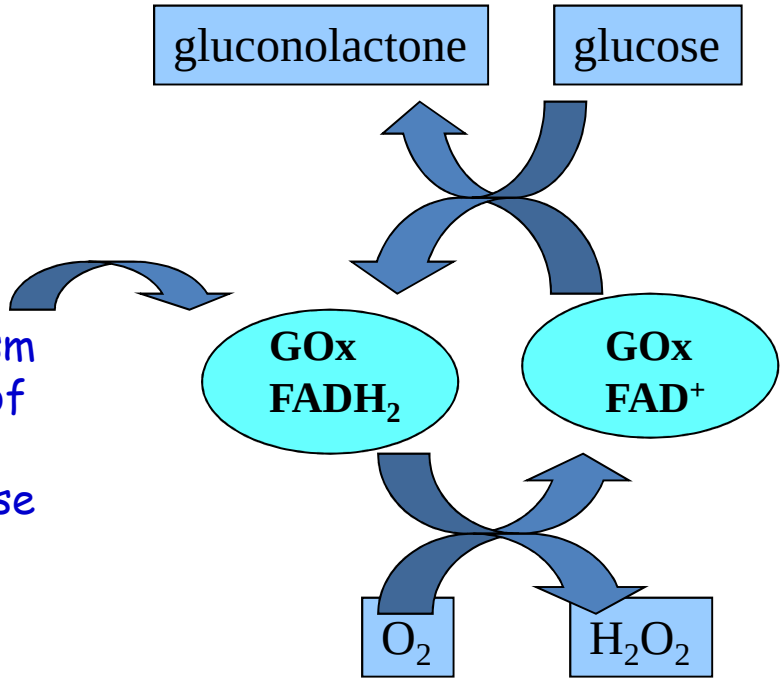
GOx is a dimeric protein with MW = 160 kDa.
Contains one tightly bound flavin adenine dinucleotide FAD unit per monomer as cofactor. FAD is not covalently bound and can be released from the holo protein following denaturation.
FAD exhibits redox activity.
Gox exhibits a very high degree of specificity for β-D-glucose.

Dimeric structure of the redox enzyme glucose Oxidase GOx.



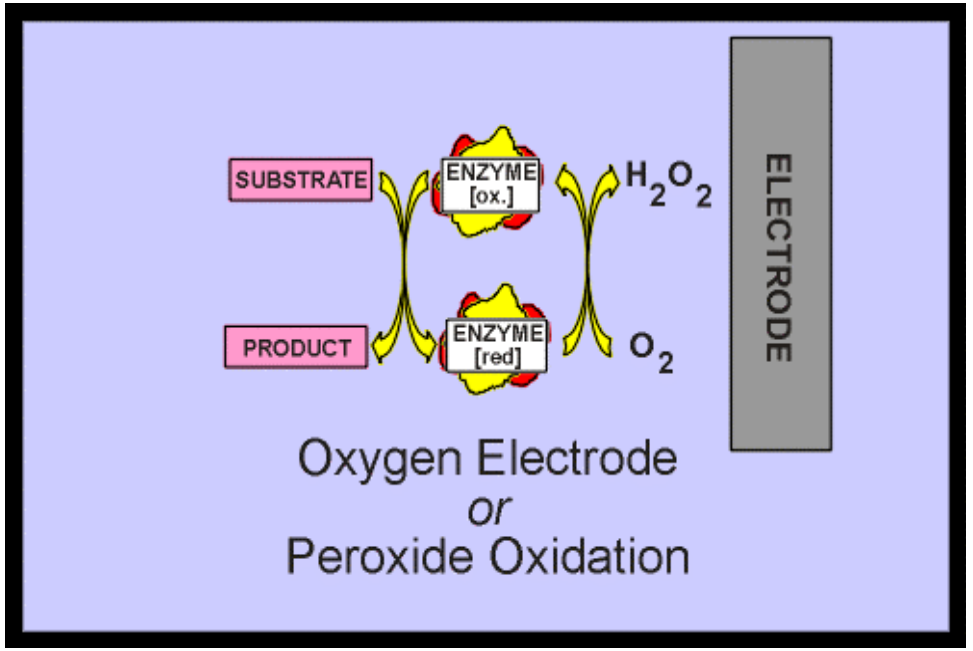
Systems based on oxygen or peroxide electrochemistry

- The most commonly used enzymes in the design of enzyme electrodes contain redox groups which change redox state during the biochemical reaction. Enzymes of this type are the oxidases and the pyrroloquinoline quinone (PQQ) dependent dehydrogenases.
- In nature, oxidase enzymes such as glucose lactate and cholesterol oxidase act by oxidising their substrates, accepting electrons in the process and thereby changing to an inactivated reduced state.
- These enzymes are normally returned to their active oxidised state by transferring these electrons to molecular oxygen, resulting in the production of hydrogen peroxide (H_2O_2).
- This naturally occuring Ping pong mechanism can be readily utilized in an amperometric biosensor device.



Ping pong mechanism for the oxidation of glucose by oxygen catalysed by glucose oxidase.

Because both oxygen and hydrogen peroxide are both electrochemically active, the progress of the biochemical reaction can be followed by either reducing the oxygen (co-substrate) or oxidising the hydrogen peroxide (product). The method based upon oxygen reduction at an O_2 electrode is one of the simplest but suffers from several disadvantages namely, slow response characteristics, difficulties in miniaturisation, low accuracy and reproducibility. Measurements based upon hydrogen peroxide oxidation can overcome these problems and indeed represent by far the most popular approach.

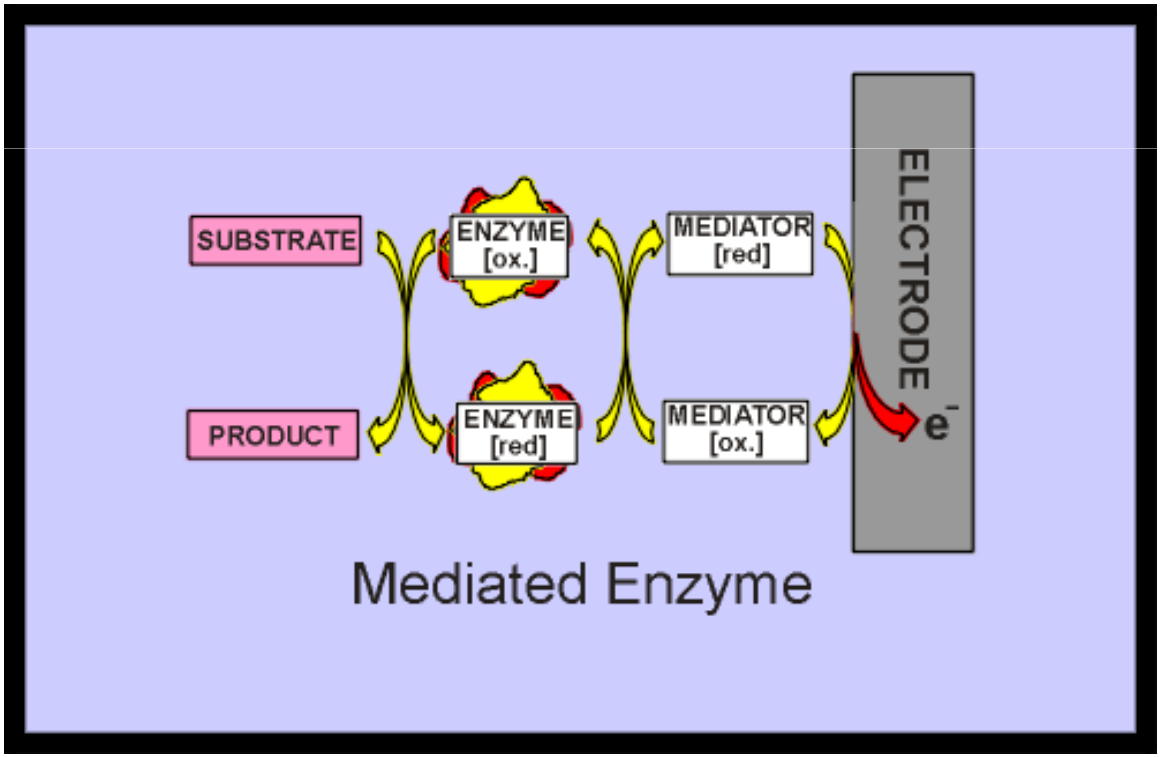


A major limitation of the peroxide detection approach is the high operating voltage (circa 0.8 volts vs the Ag/AgCl reference electrode) required to oxidise the hydrogen peroxide resulting in the possibility of interference. The use of mediators (molecules which can shuttle electrons between the redox centre of the enzyme and the electrode) can minimise this problem as they can, depending on the compound used, be regenerated at potentials where interference from species such as ascorbate, urate and paracetamol is minimized.

Enzyme electrodes using artificial mediators.

Two types of mediation :

- **Homogeneous**
  - enzyme & mediator not immobilized but free to diffuse in solution near electrode surface.
- **Heterogeneous**
  - enzyme and mediator located in membrane next to electrode surface.



Both types of mediation have been subjected to rigorous theoretical analysis.
Mathematical model involves examination of material transport (via diffusion) and chemical reaction within the reaction zone adjacent to the electrode surface.

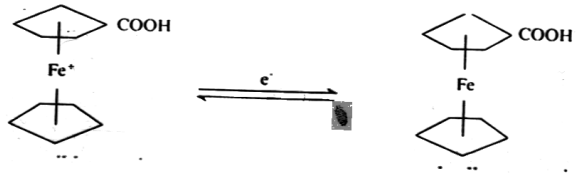
Redox mediators for amperometric enzyme electrodes.

- An enzyme based electrochemical sensor requires some form of electronic communication between the active centre of the redox enzyme and the electrode surface since the former site is buried deep within an insulating protein shell.
- Some enzymes (e.g. GOx) can communicate directly with an electrode surface but they undergo denaturation with exposure/removal of redox active site. Activity of GOx wrt glucose oxidation much reduced as a result.
- How can enzymes optimally communicate with electrode surfaces? We mirror nature and apply the concept of electronic mediation. Most oxidase enzymes can utilize artificial electron acceptor molecules termed mediators. These mediators act as shuttlers of charge between the active site in the enzyme and the electrode surface.
- Idea of mediation obtained from fact that GOx catalyses glucose oxidation by oxygen via the ping-pong mechanism.

- Criteria for redox mediator.
  - Reversible electrochemistry (fast ET kinetics between redox mediator species and support electrode surface.
  - Rapid reaction with active sites in redox enzyme.
  - Redox electrochemistry of mediator must involve either 1 or 2 electron transfer process.
  - Mediator must be stable in both oxidised and reduced forms and not be prone to auto-oxidation.
  - Mediator must be amenable to chemical substitution to provide a range of pH independent redox potentials less than ca. 500 mV vs SCE.
  - Must be available in forms with a range of solubilities in aqueous and organic media.
- Examples.
  - Organic dye molecules: methylene blue, medola blue, N-methylphenazinium.
  - Inorganic complex ions: ferricyanide.
  - Organometallic species: substituted ferrocenes.
- Substituted ferrocenes are most commonly used since redox potential can be chemically tailored by choice of suitable substituents on the cyclopentadienyl rings. Substituent nature can also affect solubility of mediator.

Homogeneous mediation using substituted ferrocene.

Mediator redox couple reasonably insoluble in aqueous solution, hence is located close to electrode.



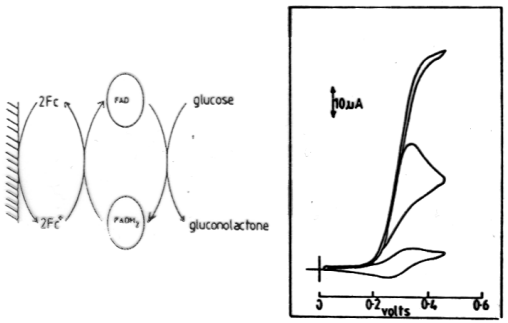
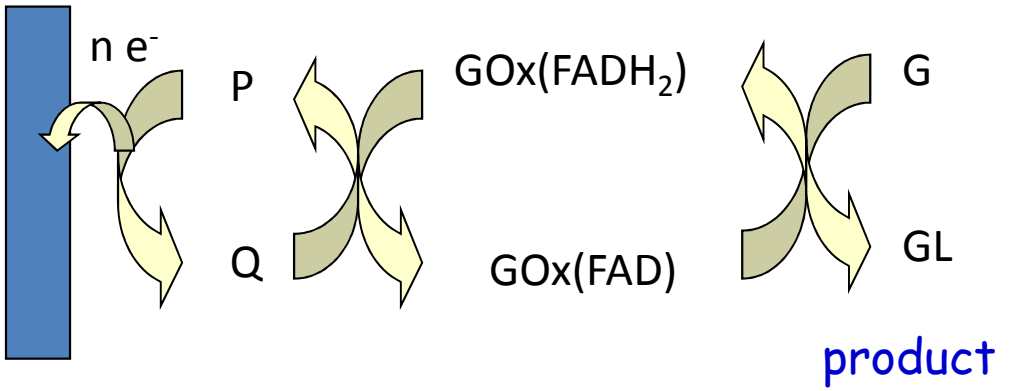


FIGURE 17. The scheme for the homogeneous mediation of glucose oxidase oxidation by ferrocenes. The inset shows typical cyclic voltammetric results for the system in the presence of increasing concentrations of glucose. The data were recorded at a glassy carbon electrode electrode (area 0.05 cm^2) in 0.085 mol dm^{-3} phosphate buffer pH 7.0 containing 0.5 mmol dm^{-3} ferrocene monocarboxylic acid and 38 mol dm^{-3} glucose oxidase at a sweep rate of 5 mV s^{-1}, potentials are vs. SCE. a) No glucose, b) 0.5 mmol dm^{-3} glucose, c) 2.5 mmol dm^{-3} glucose.

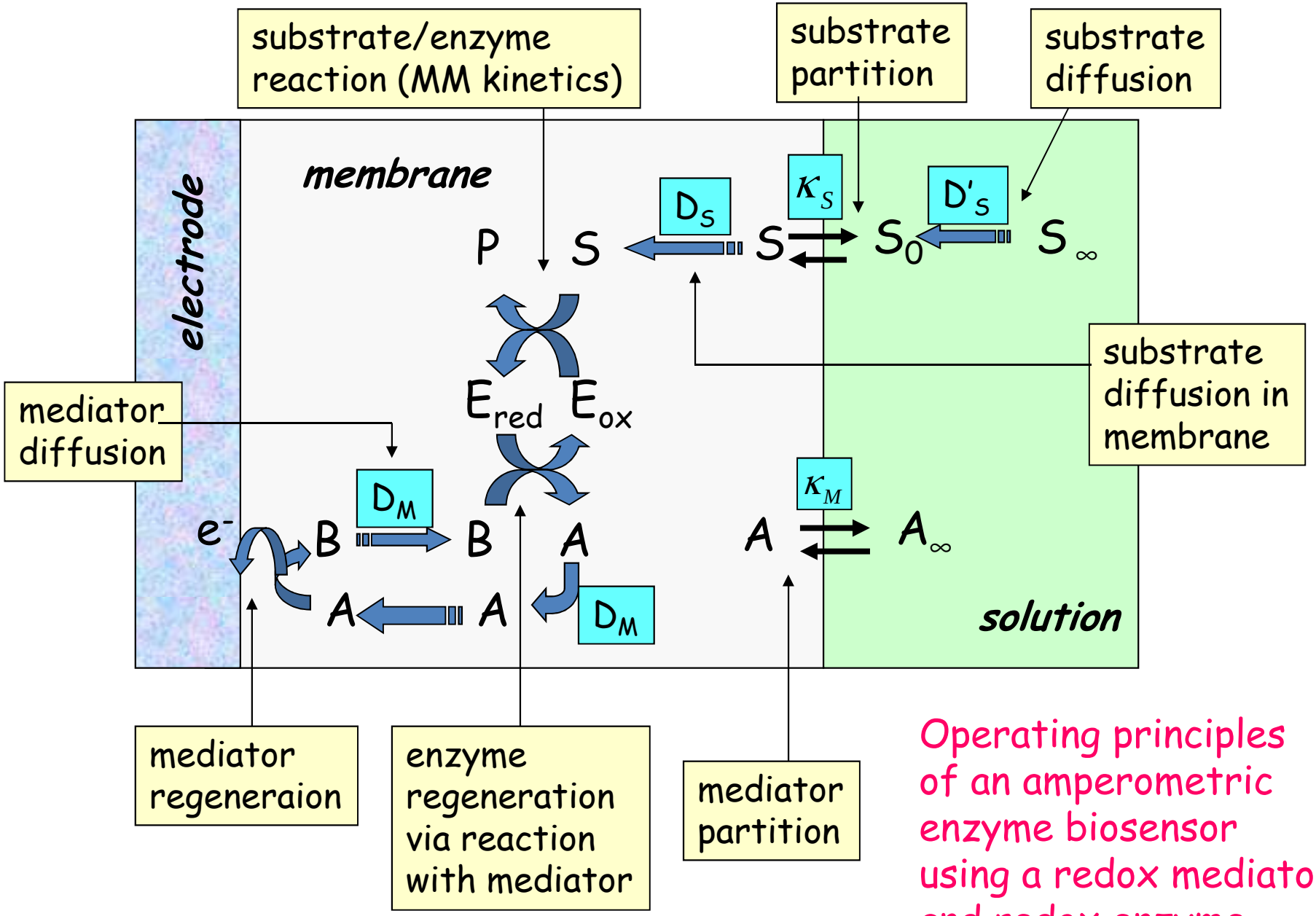
$$G + FAD \rightarrow GL + FADH_2$$

$$FADH_2 + 2Fe(Cp)_2^+ \rightarrow FAD + 2Fe(Cp)_2 + 2H^+$$

$$2Fe(Cp)_2 \rightarrow 2Fe(Cp)_2^+ + 2e^-$$



P,Q represents reduced and oxidised forms of redox mediator (ferrocene and ferricinium); G = glucose, GL = gluconolactone. GOx ($FADH_2$) = reduced form of glucose oxidase; GOx(FAD) = oxidised form of glucose oxidase.



Operating principles of an amperometric enzyme biosensor using a redox mediator and redox enzyme.

Enzyme communication with electrodes.

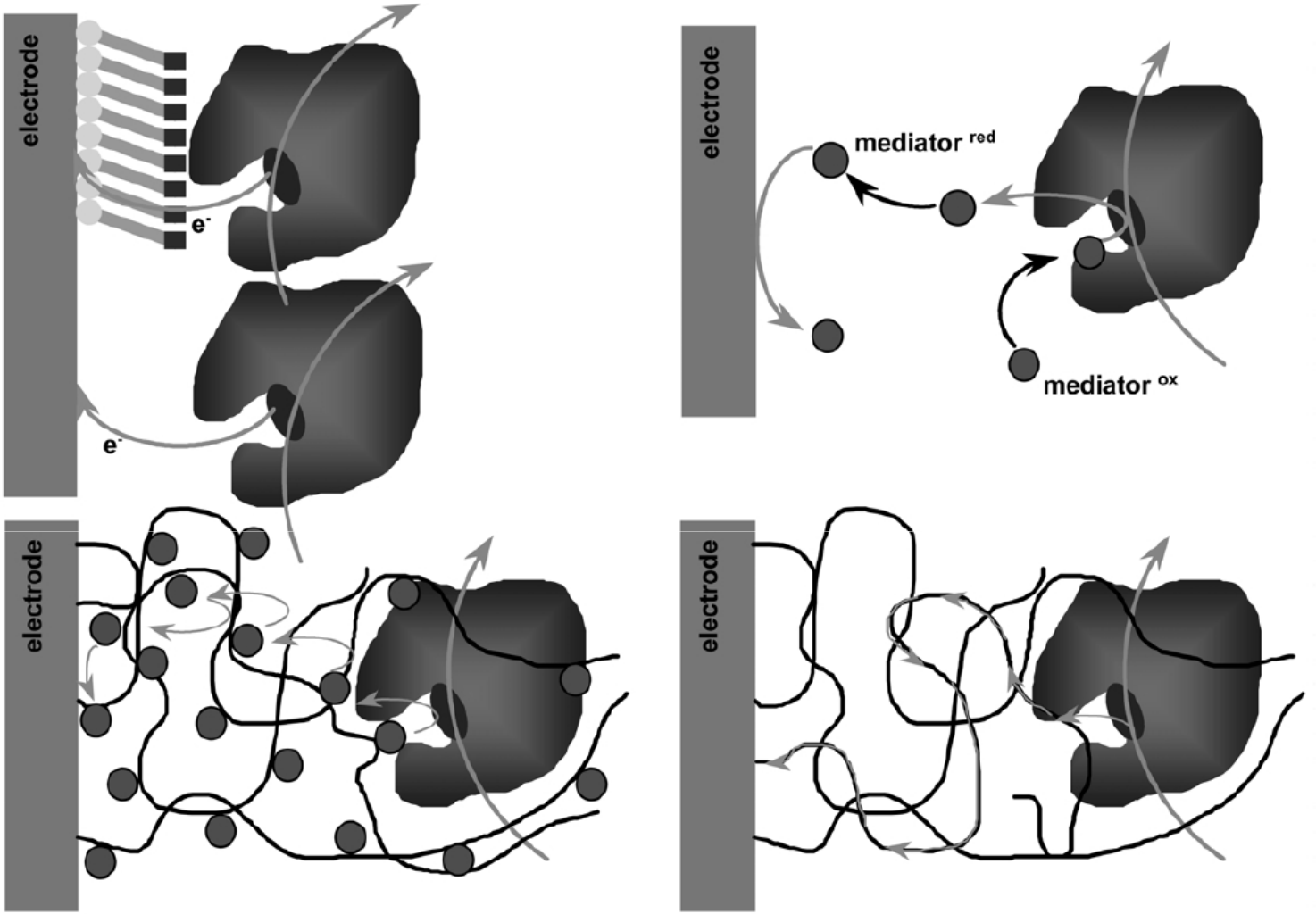


Fig. 1. Schematic representation of ET possibilities between enzymes and electrodes. (a) Direct ET at a bare or monolayer-modified electrode. (b) Shuttle mechanism based on free-diffusing redox species. (c) Electron hopping in a redox-relay modified polymeric hydrogel. (d) ET via a conducting polymer chain.

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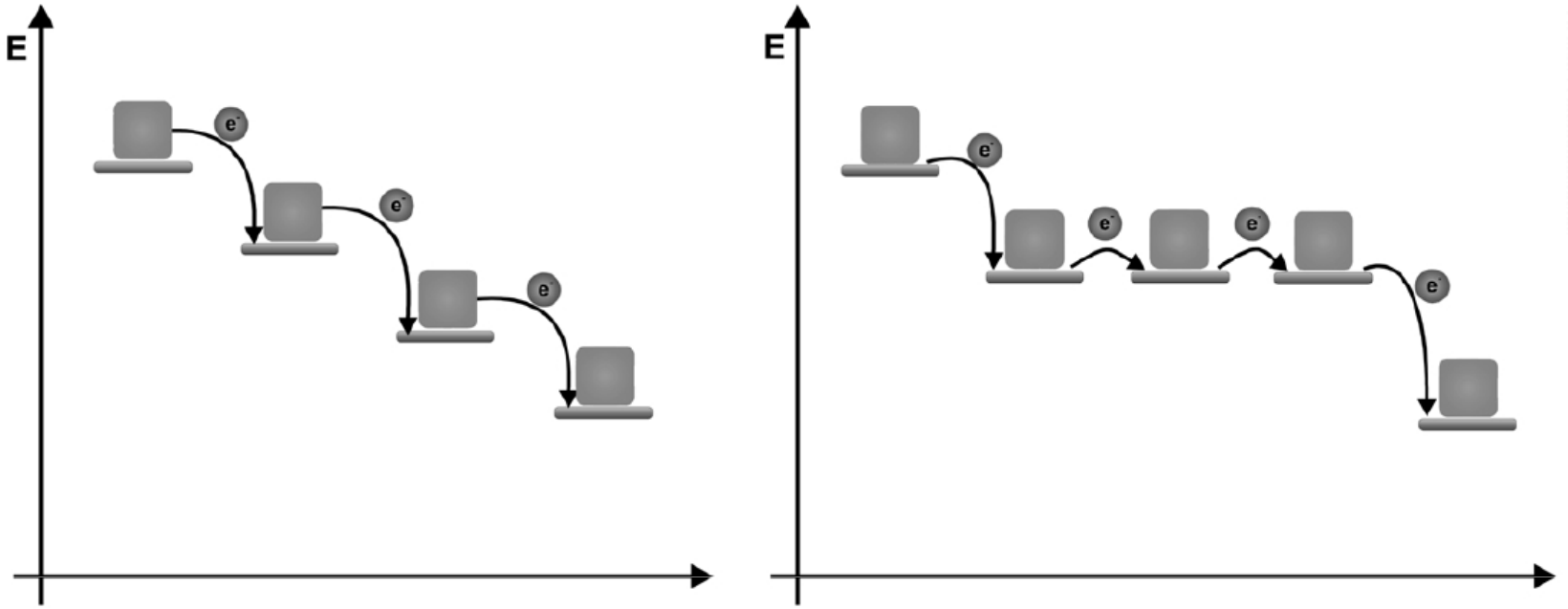


Fig. 2. Dividing the overall ET distance by means of ET cascades. (a) ET cascade via a sequence of redox relays with decreasing redox potential. (b) ET cascade via self-exchange processes of redox relays at the same redox potential.

ET cascade concept :
We seek to reduce ET distance via introduction of additional redox relays with appropriate redox potential establishing an ET cascade.

Redox hydrogel systems.

- Sensing layer preparation:
  - drop coat a solution containing the redox polymer (poly(vinyl imidazole, poly(acrylic acid) , poly(allylamine) backbone with covalently attached osmium complex), a bifunctional crosslinker, and the enzyme unto a support electrode surface.
  - Evaporation of solvent enables cross linking process which leads to a well adhering hydrophilic redox polymer film.
- Swelling of film in water leads to favourable hydrogel properties:
  - Increased flexibility of polymer backbone with improved ET rate between polymer bound redox sites
  - Fast diffusion of the substrate and product within polymer film
  - Improved enzyme stability
  - High mobility of counter anions which can determine rate of charge percolation through film.
- Rate limiting step in complex ET cascade from enzyme via a sequence of self exchange reactions between neighbouring redox relays and finally to the electrode surface, is often the ET between the enzyme integrated primary redox site and the first polymer bound redox relay.
- Location of enzyme active site and ET distance for rate determining ET step will significantly influence the overall ET rate observed which can be high.

Enzyme wiring using redox hydrogels.

- The Heller Group have shown that GOx forms complexes with polycationic redox polymers containing $Os(bpy)_2Cl$ groups attached to a poly(vinylpyridine) backbone. The Os site can be switched from Os(II) to Os(III) upon application of a potential and can also exchange electrons with the flavin site on the enzyme.
- Consequently these redox polymers serve as an electron relay or molecular wire between the flavin site and the support electrode.
- In order that the polymer bind to the enzyme we require that it be adequately soluble in water and that it has hydrophobic, charged (or H bonding) domains which can bind to the protein.
- Note that only a small fraction of the molecular wire segments must be bound to the electrode surface at any given time. Most segments must remain unadsorbed, dangling into the solution and therefore available for complexing to and penetrating the enzyme.
- We also need to form a 3D network of molecular wires which incorporates (via covalent bonding) a large number of enzyme molecules.
- This network must allow rapid in and out diffusion of substrate and product as well as fast electron transport via hopping (redox conduction) along the molecular wires. Note that electron hopping between redox sites on adjacent molecular wires can also occur.

Enzyme entrapment in redox hydrogels.

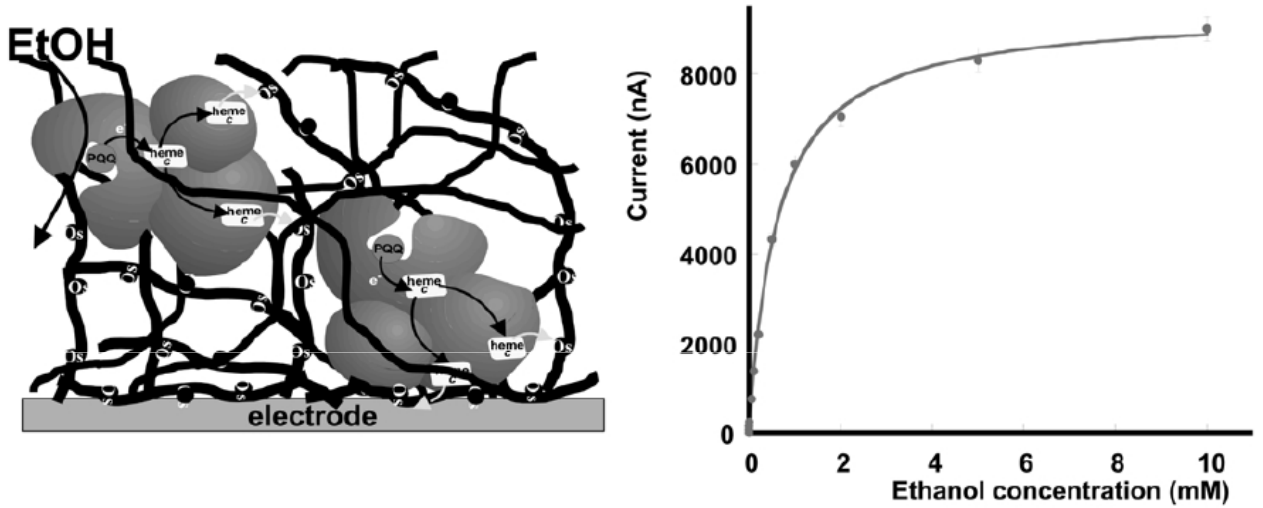
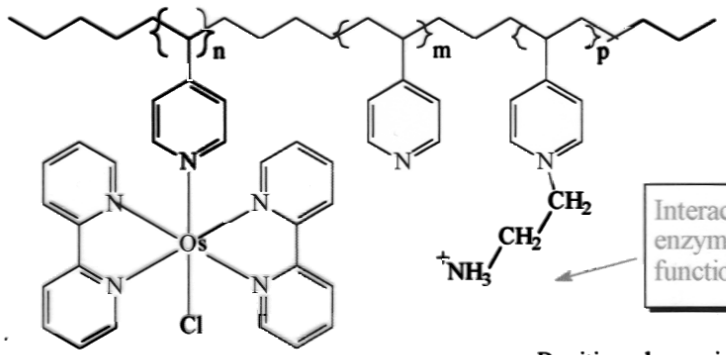


Fig. 7. (a) Schematic representation of QH-ADH entrapped in an Os-complex modified hydrogel. (b) Calibration curve of a QH-ADH/redox hydrogel biosensor (200 mV vs. Ag/AgCl; 3-mm graphite electrode).

Osmium/PVP redox polymer.



Os(II) / Os(III) redox chemistry .

fast , stable redox couple.
$D_E > 10^{-9}$ cm^2s^{-1}

Active component of molecular wire .

Interaction with enzyme via this functionality .

Positive charge increases solubility of polymer in water and enhances electrostatic interaction with anionic regions of enzyme protein .

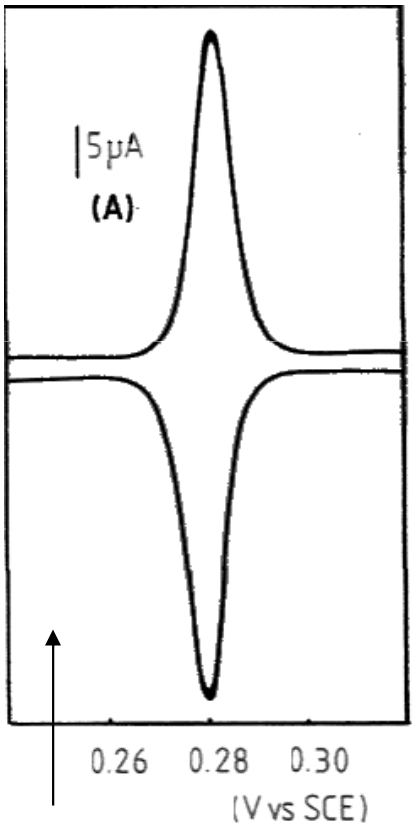
MW ca. 130,000

n = 1 m = 4 p = 1.2

Structure of an enzyme complexing redox polyamine designed for crosslinking with a water soluble diepoxide to form a three dimensional wired enzyme network .

Low switching Potential.

$Os(bpy)_2(PVP)_{25}Cl$



1M NaCl
5mV/s
$\Gamma = 1 \times 10^{-8}$ mol/cm^2

CV of polymer coated electrode exhibits almost ideal behaviour. Rapid electron hopping between adjacent Os groups in film.

Enzyme wiring using redox hydrogels .

- The molecular wires must be long (have a large MW) to ensure good adsorption to the electrode surface, especially since the redox polymers are water soluble.
- Statistically, if the macromolecule consists of 10^6 repeat units, then there will be ca. 10^3 units adsorbed at any instant. One still will have many free unadsorbed polymer sites available for interaction with protein molecules.
- The general synthetic protocol is to form a complex between the enzyme (which contains lycine amines) and a redox polyamine species, and then form a cross linked 3D network using a diepoxide (poly(ethylene glycol) diglycidyl ether) which reacts with the primary amine groups on both the redox polymer and the enzyme lycine residues. In this way a redox hydrogel is formed.

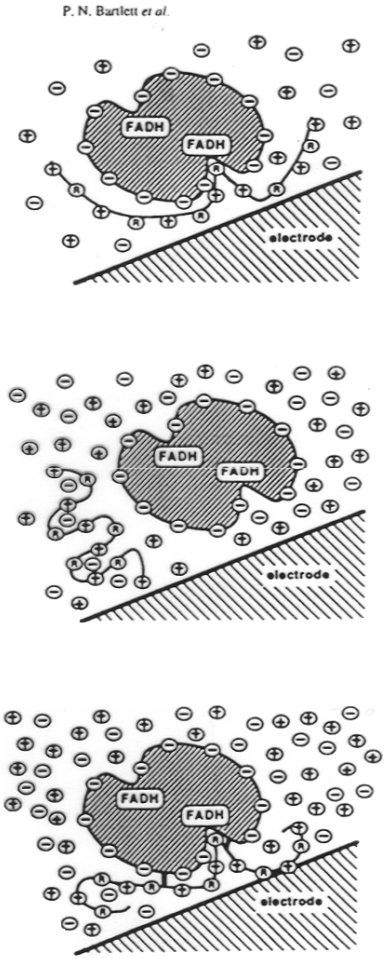
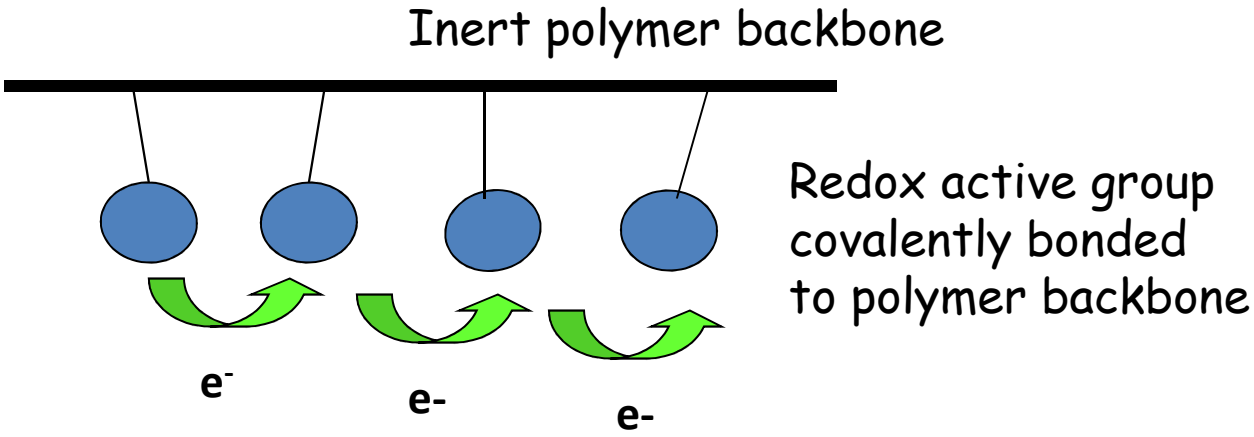


FIGURE 20. a) Electrostatic binding or polycationic redox polymer to a polyanionic enzyme in a solution of low ionic strength. Electrons are transferred to the electrode via the polymer. b) Charge screening and coiling of the redox ploymer is a solution of high ionic strength leads to dissociation of the electrostatic complex, stopping electron transfer to the electrode. c) After covalent bonding of the redox polymer to the enzyme the complex does not dissociate at high ionic strength. Reprinted with permission from Y. Degani and A. Heller, *J. Amer. Chem. Soc.*, 111(1989)2357. Copyright 1989 American Chemical Society.

Enzyme wiring using redox hydrogels .

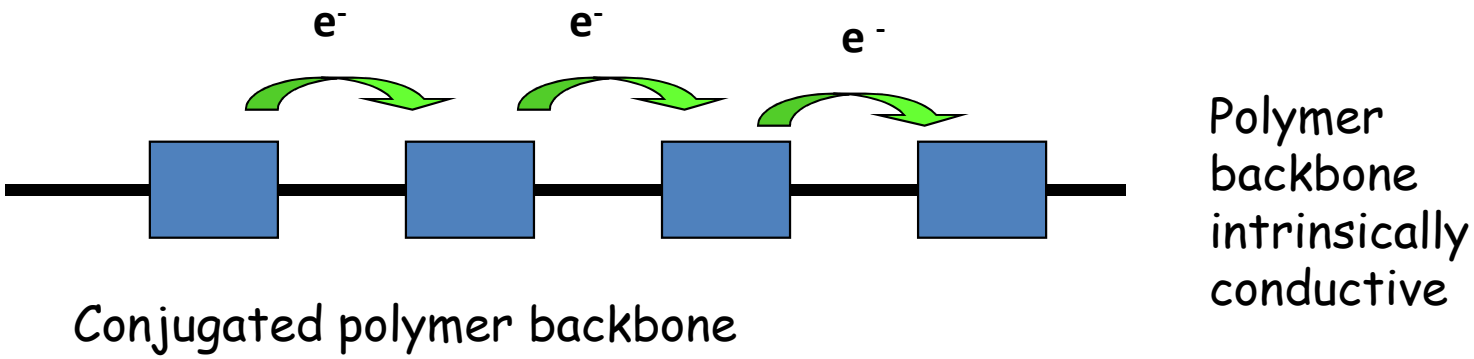
- Glucose electro-oxidation at the wired enzyme network involves a sequence of coupled processes:
  - Substrate and product diffusion in solution to/from network surface.
  - Diffusion of substrate into hydrogel matrix and diffusion of product out of hydrogel.
  - ET from flavin site to redox site on complexing macromolecule.
  - "Diffusion" of electrons to support electrode via electron hopping redox conduction within and between redox macromolecules.
  - ET from site on redox macromolecule to support electrode surface.
- Nature of rds: 3D hydrogel network is hydrophilic and open, and substrate/product diffusion coefficients are not greatly different from those in water. Hence we would not expect either substrate/product diffusive transport in aqueous solution or in hydrogel phase to be rate determining unless the substrate concentration is very low.
- ET to electrode from adjacent redox site on macromolecule is also rapid because Os(II)/Os(III) electron transfer is rapid at electrode surfaces.
- Hence we assume that the rds involves the spreading of charge from reduced enzyme site through the wiring network.
- Making the most of the system:
  - Redox polyelectrolyte/enzyme ratio must be high enough to wire most of the enzyme molecules. The latter ratio is also essential for efficient current collection when the turnover rate of the enzyme is high.
  - Capacity of network to carry current via multiple self exchange reactions within and between its segments must equal or exceed the capacity of the incorporated enzyme molecules to deliver electrons.
  - Rate of electron diffusion through redox polymer can be increased by reducing the ET distance via increasing the loading of the polymer with the covalently bound metal sites.

Redox polymer material.

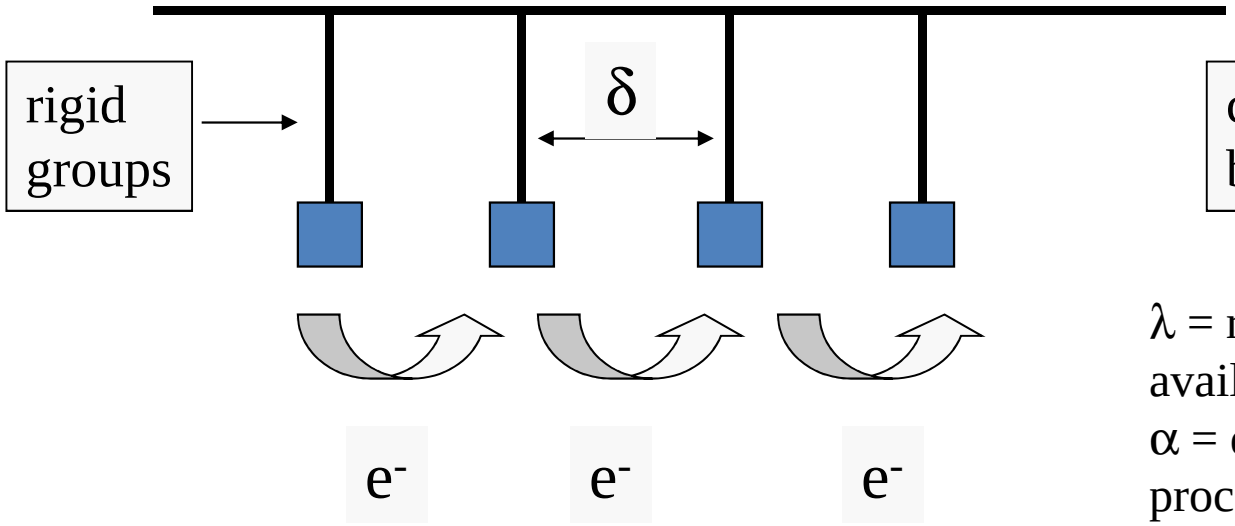


Redox conduction : nearest neighbour electron hopping.
No physical diffusion of redox groups: local 'wagging' mobility only.
Tarzan swing mechanism.
For loaded ionomer material have local electron hopping coupled with physical diffusion of redox groups.

Electronically conducting polymer material.



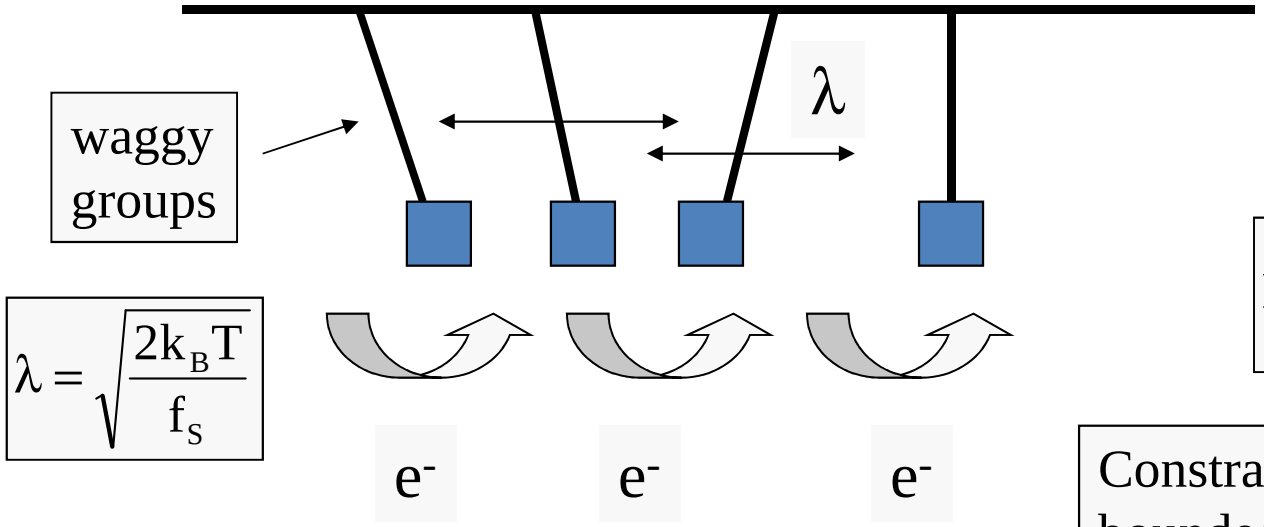
Tarzan swing mechanism



classic Dahms/Ruff behaviour

λ = range of physical motion available to redox sites

α = dimensionality of hopping process



f_s = force constant

$$\lambda = \sqrt{\frac{2k_B T}{f_S}}$$

$$D_E = \frac{k_{ex} c^{\infty}}{2\alpha}\left\{\delta^2 + \alpha\lambda^2\right\}$$

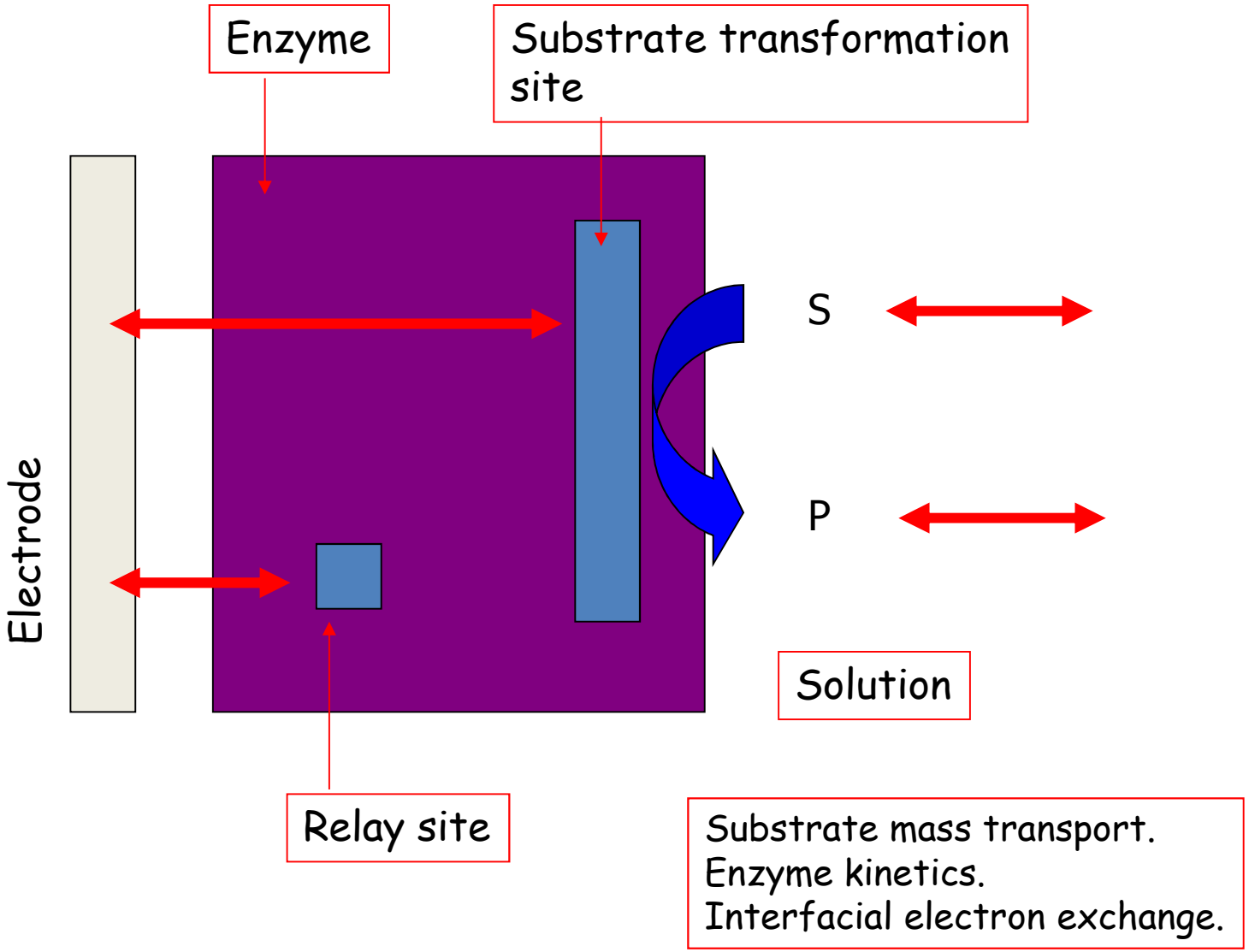
mean field behaviour $\lambda > \delta$

Constrained/ bounded diffusion

Modelling mediated electron transfer at nanoheterogeneous modified electrodes.

- 2D systems (Organized SAM films).
  - Diffusion of substrate/product in solution to monolayer film surface.
  - Reaction between active surface bound mediator species and substrate.
- Major physical process is chemical reaction between mediator species and substrate.
  - Simple bimolecular reaction.
  - Chemical catalysis or adduct formation.
- 3D nanoheterogeneous systems (Polymer/S(M)WNT/NP modified electrodes).
  - Substrate diffusion in Nernst diffusion layer.
  - Substrate diffusion through pores in film.
  - Electron percolation along and between polymer strands.
  - Chemical reaction between immobilized mediator site and substrate.
- Major physical processes in 3D nano-heterogeneous thin film sensors are reactant transport and reaction kinetics within the support matrix. Charge percolation can be important in many situations.

H.A. Heering, J. Hirst, F.A. Armstrong, *Interpreting the catalytic Voltammetry of electroactive enzymes adsorbed on electrodes*. J.Phys. Chem.B., 102 (1998) 6889-6902.



Rate determining processes expressed as characteristic currents .

Can introduce characteristic currents which serve to quantify the various rate determining processes occurring in the polymer film.

Substrate diffusion in solution (D) .

$$i_D = \frac{nFAD_S^* s^\infty}{\delta}$$

Substrate diffusion in film (S).

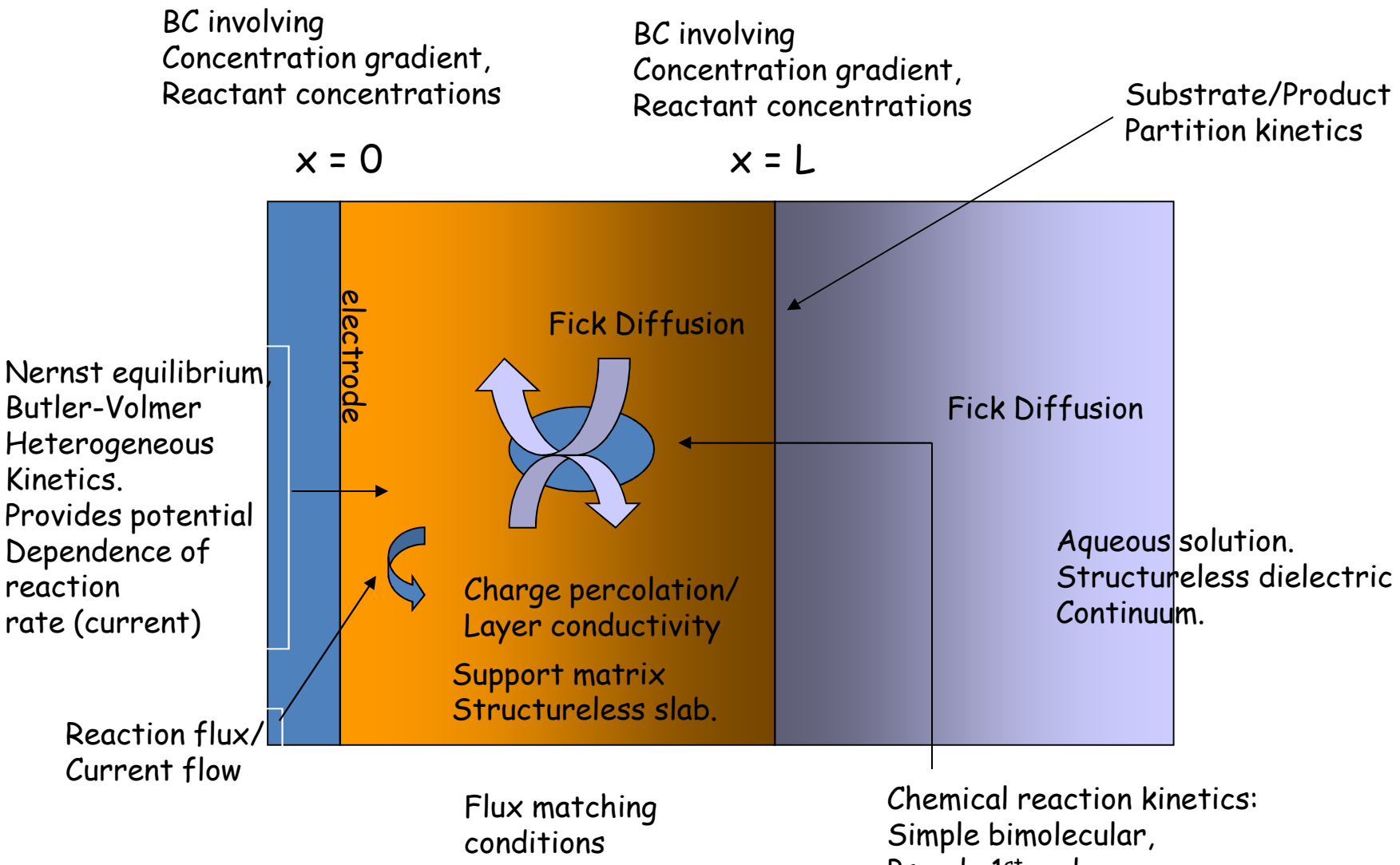
$$i_S = \frac{nFAD_S \kappa s^\infty}{L}$$

Electron percolation through layer (E).

$$i_E = \frac{nFAD_E b_0}{L} = \frac{nFAD_E \Gamma}{L^2}$$

Bimolecular chemical reaction in film (R).

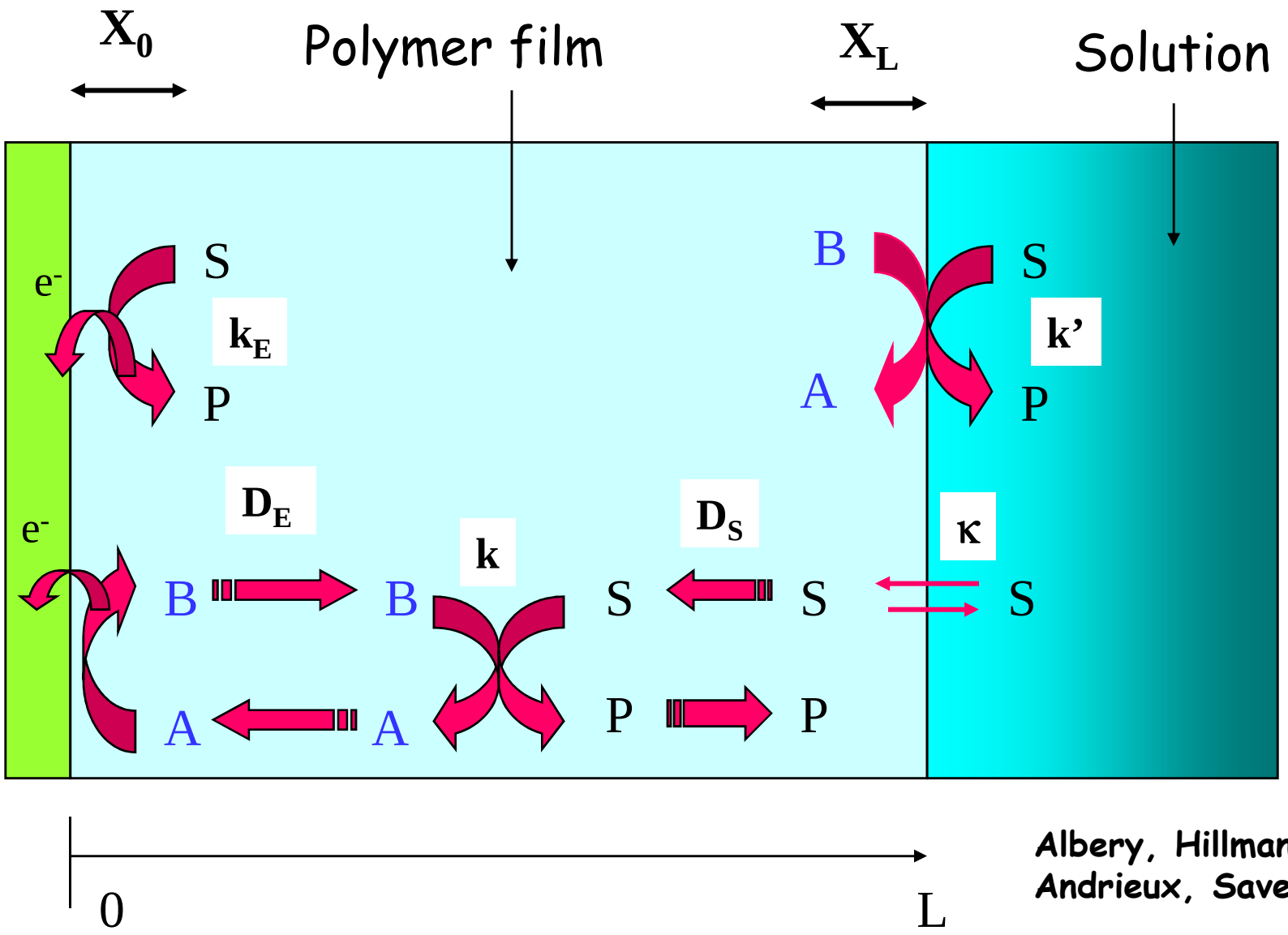
$$i_R = nFAk\kappa b_0 s^\infty L$$
$$= nFAk\kappa\Gamma s^\infty$$



P.N. Bartlett, R.G. Whitaker, J. Electroanal. Chem., 224 (1987) 27-35.

P.N. Bartlett, K.F.E. Pratt, J. Electroanal. Chem., 397 (1995) 61.

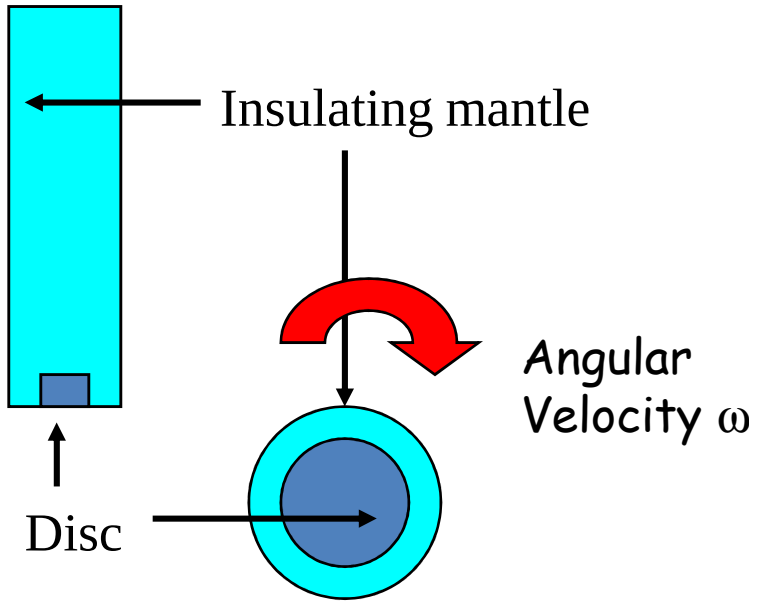
Mediated electrocatalysis at polymer modified electrodes .



Albery, Hillman
Andrieux, Saveant.

The Rotating Disc Electrode .

Heterogeneous redox catalysis at polymer coated electrodes best examined using the rotating disc electrode technique .



k_D= diffusional rate constant (cms^{-1})
D = substrate diffusion coefficient
δ = Nernst diffusion layer thickness

ν = kinematic viscosity
ω = rotation speed (Hz)

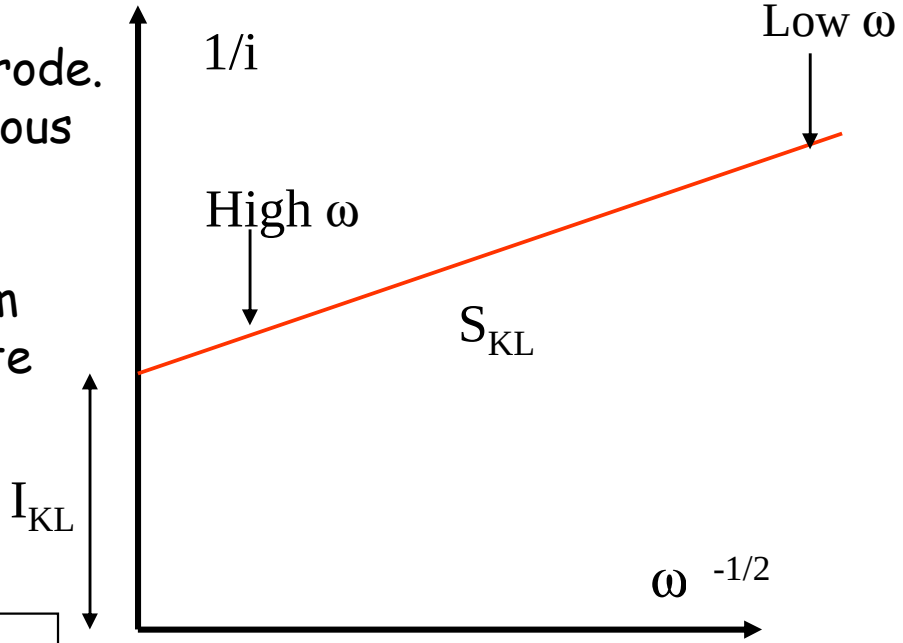
- RDE has well defined hydrodynamic flow to electrode surface .
- Fluid velocity profile near disc well defined .
- Transport of reactant to surface obeys steady state convective diffusion equation which may be rigorously solved .
- Rate of material transport depends in a well defined manner on the rotation speed of the electrode .
- Polymer films may be readily grown on RDE surfaces .

$$k_D = \frac{D}{\delta}$$

$$\delta = 0.643 D^{1/3} \upsilon^{1/6} \omega^{-1/2}$$

Data Analysis using the RDE.

- Apply constant potential to electrode.
- Measure limiting current i at various rotation speeds ω .
- Plot 1/i versus $\omega^{-1/2}$.
- Kinetic information obtained from intercept corresponding to infinite rotation speed .
- Transport information obtained from slope .



Koutecky-Levich Plot .

$$\frac{nFAs^{\infty}}{i_L} = S_{KL}\omega^{-1/2} + I_{KL}$$

$$I_{KL} = \frac{1}{k'_{ME}}$$

$$S_{KL} = B^{-1}$$

$$B = \text{Levich constant} = 1.55D^{2/3}\upsilon^{-1/6}$$

- Can develop the RDE method to identify rate limiting kinetics for mediated electrocatalysis using polymer modified electrodes.

Net flux expression

$$\frac{s^{\infty}}{f_{\Sigma}} = \frac{nFAs^{\infty}}{i} = \frac{1}{k'_{ME}} + \frac{1}{k_D}$$

Transport and kinetics in layer

Substrate transport in solution

Electrons percolate across layer more rapidly than substrate .

$$k'_{ME} = \frac{1}{\frac{s_\sigma L}{D_e b_0} + \frac{1}{k' b_0 + \kappa k b_0 X_L \tanh\left[L / X_L\right]}} + \frac{\kappa \operatorname{sech}\left[L / X_L\right]}{\frac{L}{D_s} + \frac{1}{k_e}}$$

T_1 T_2

T_1 : describes reaction of S and B either in layer or at surface .
T_2 : describes reaction of S directly at electrode surface.
T_3 : mediated electron transfer
T_4 : unmediated direct ET at support electrode
T_5 : substrate diffusion within layer .

T_3 T_4

$$k'_{ME} = \frac{\kappa k b_0 X_0 \left\{ \frac{k' + \kappa k X_0 \tanh\left[L / X_0\right]}{k' \tanh\left[L / X_0\right] + \kappa k X_0} \right\} + \kappa k_e}{1 + \frac{L}{D_s}\left\{k_e + k b_0 X_0\right\}}$$

T_5

Substrate penetrates across layer more easily than electrons .

Limiting expressions for modified electrode rate constant.

$$f_\Sigma = \frac{i}{nFA} = k'_{ME} s_0 = \frac{k'_{ME} k_D s^\infty}{k'_{ME} + k_D}$$

$$\frac{s^\infty}{f_\Sigma} = \frac{1}{k'_{ME}} + \frac{1}{k_D}$$

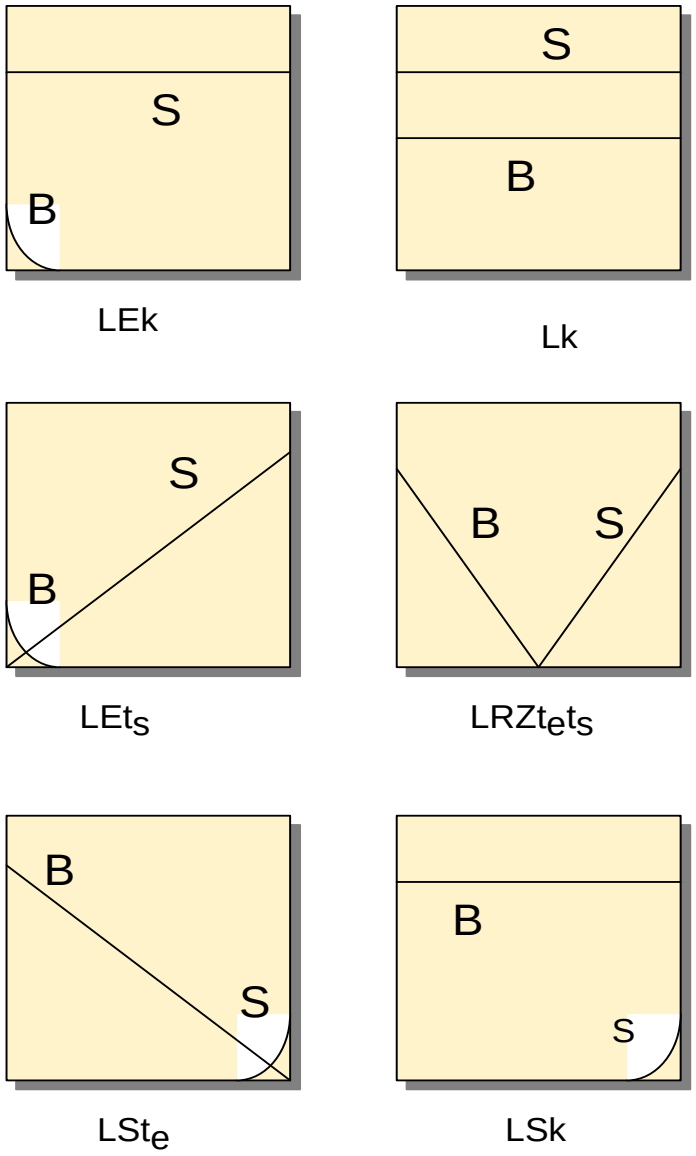
- Reactant diffusion effects in Nernst diffusion layer (k_D term) may be readily separated from transport and kinetic effects in the layer (k'_{ME} term) via the reciprocal flux relationship.
- Analysis accomplished experimentally using the RDE and a Koutecky-Levich plot.

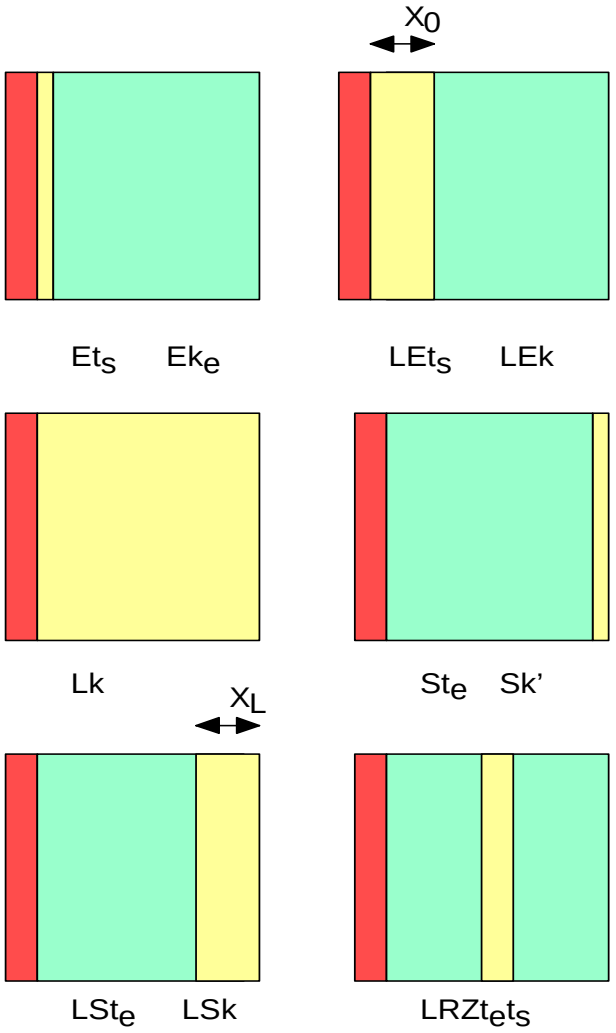
Case notation	k'_{ME}	Location
Sk' St_e	$k'b_0$ $\frac{D_E b_0}{Ls_1}$	Surface reaction at layer/solution interface
$LSk\,(SR)$ $LSt_e\,(E)$	$\kappa\sqrt{kb_0 D_S}$ $\frac{D_E b_0}{Ls_1}$	Reaction layer close to film/solution interface
$Lk\,(R)$	$\kappa k b_0 L$	Throughout layer
$LRZt_e t_s\,(S+E)$	$\frac{D_E b_0}{Ls_1} + \frac{\kappa D_S}{L}$	Narrow reaction zone in layer
$LEt_s\,(S)$ $LEk\,(ER)$	$\frac{\kappa D_S}{L}$ $\kappa b_0\sqrt{\frac{D_E k}{s_1}}$	Reaction layer close to electrode
Ek_E Et_s	κk_E $\frac{\kappa D_S}{L}$	Direct reaction on electrode

Mediated electrocatalysis by electroactive (redox) polymers .

$$B + S \rightarrow A + P$$

B = mediator
S = substrate





$$X_0 = \sqrt{\frac{D_E}{\kappa k s_1}}$$

$$X_L = \sqrt{\frac{D_S}{k b_0}}$$

RDE Diagnostic Scheme .

$$\left(\frac{nFAs^{\infty}}{i}\right)^2 = \frac{nFAs^{\infty}}{i} B^{-1}\omega^{-1/2} + \frac{1}{k'^2_{ME}}$$

Examine RDE Voltammograms

Limiting current rotation speed independent ?

Y → St_e LSt_e

N → Check linearity of KL plot

Y → KL slope = B^{-1} ?

N → KL slope less than B^{-1} ?

Y → $LRZt_et_s$

AH equation

Check linearity of AH plot

Y → LEk

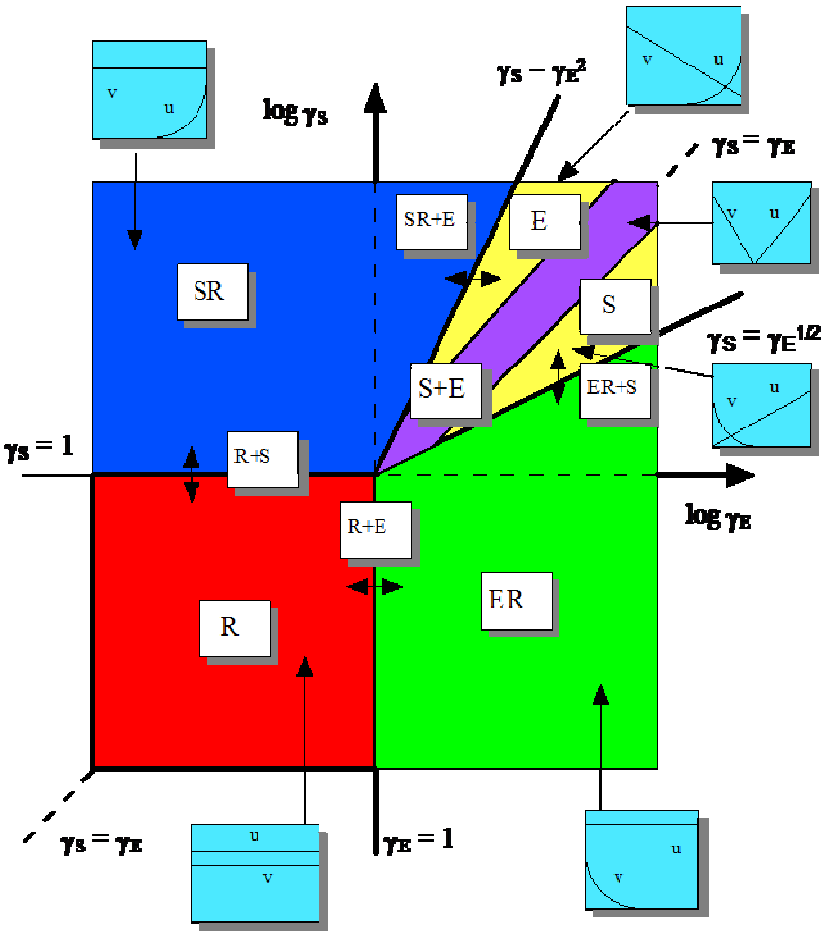
N → Give up and go home !

KL equation

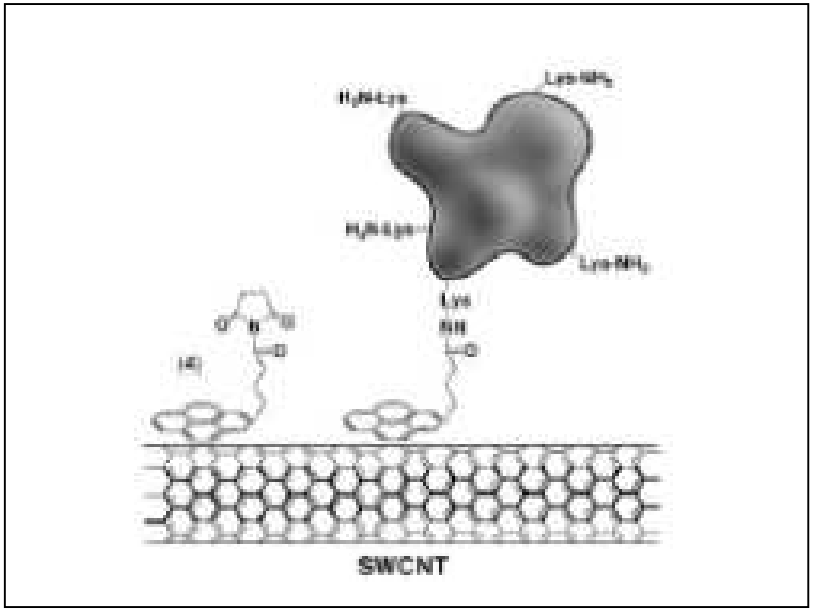
$$\frac{nFAs^{\infty}}{i} = B^{-1}\omega^{-1/2} + \frac{1}{k'_{ME}}$$

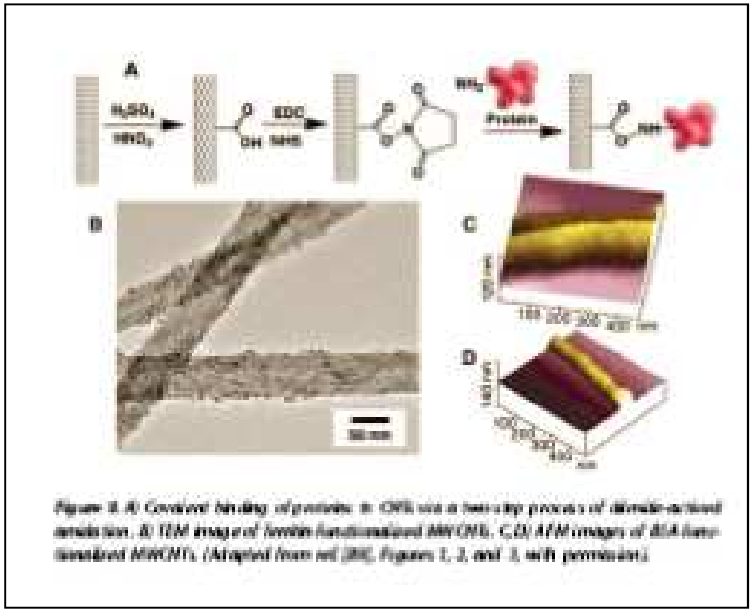
	Sk′	LSk	Lk	LEt_s
Reaction order wrt b_0	1	1/2	1	0
Reaction order wrt L	0	0	1	- 1

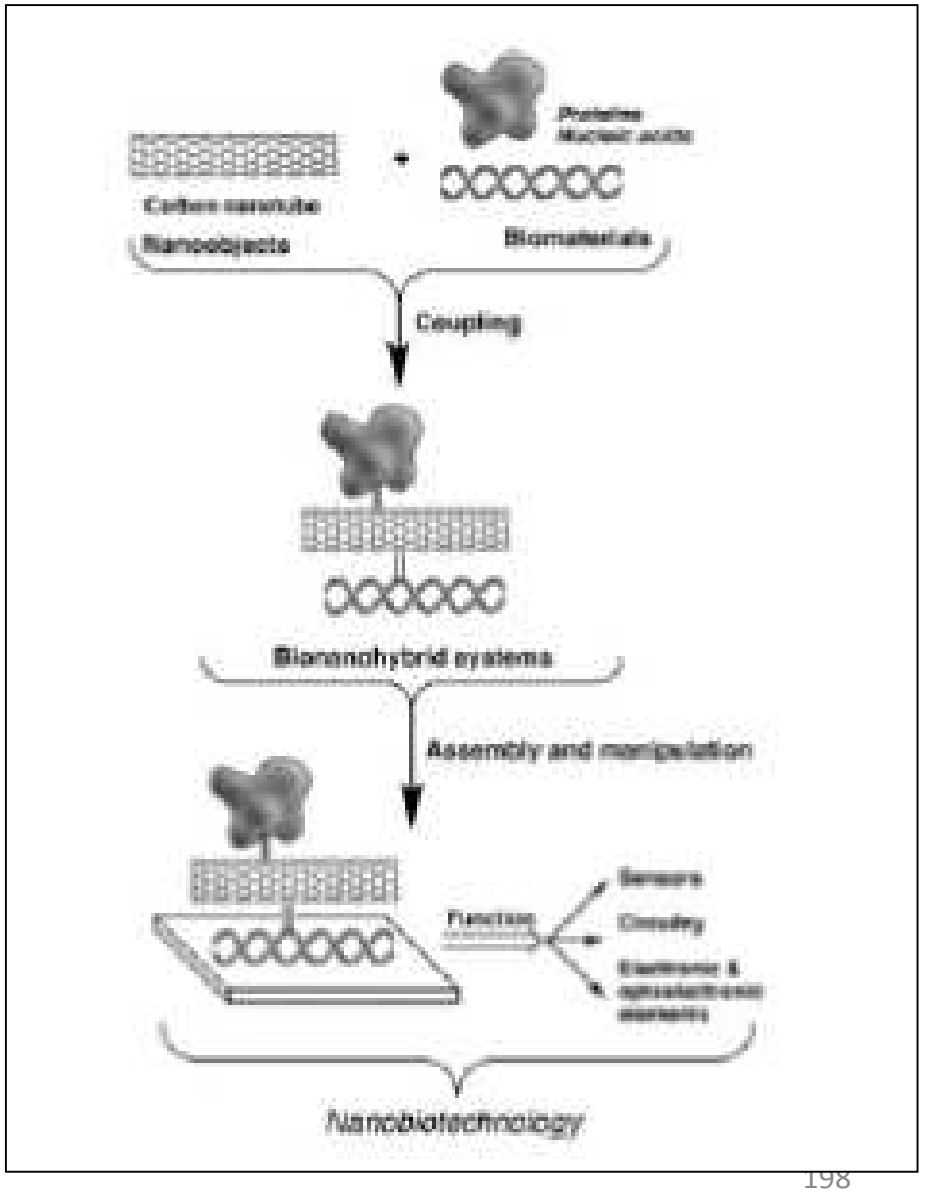
Polymer modified Electrode: Kinetic Case Diagram



SWCNT : a nanobioelectronic platform.







- SWCNT acts as molecular nanowire.
- SWCNT immobilized on support electrode surfaces via:
  - Random meshes
  - Ordered arrays using SAMs.
- Redox enzyme subsequently adsorbed on immobilized SWCNT.

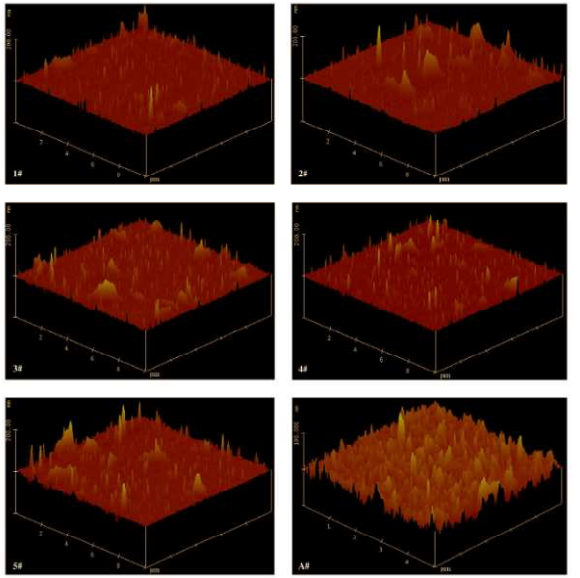


Fig. 2. Typical AFM images of a set of needle-like SWCNT electrodes (1#, 2#, 3#, 4#, and 5#) and forest-like SWCNT electrode (A#) via a wet chemistry approach at different assembly conditions. Total peaks: 100 (1#), 110 (2#), 115 (3#), 120 (4#), 126 (5#), and 254 (A#).

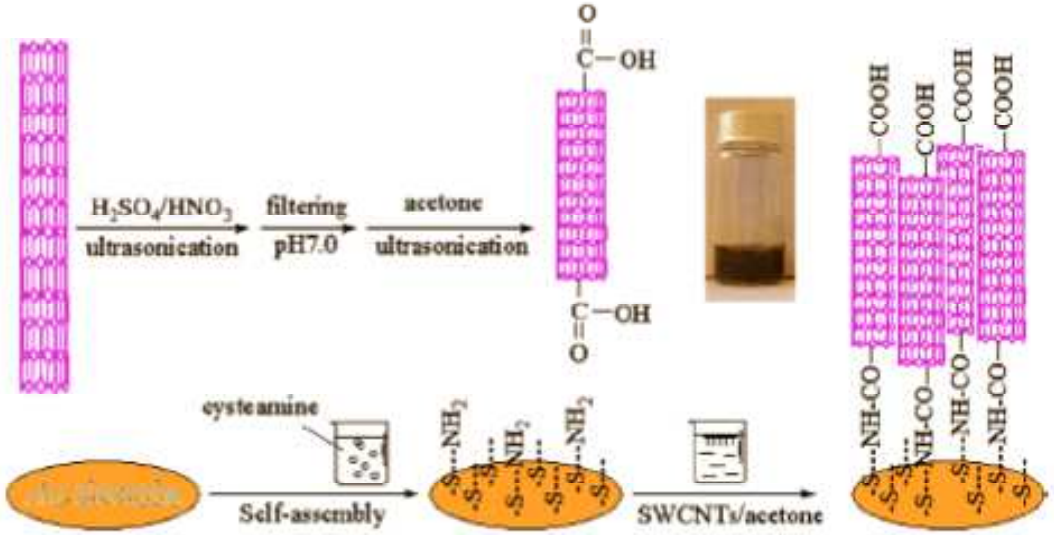
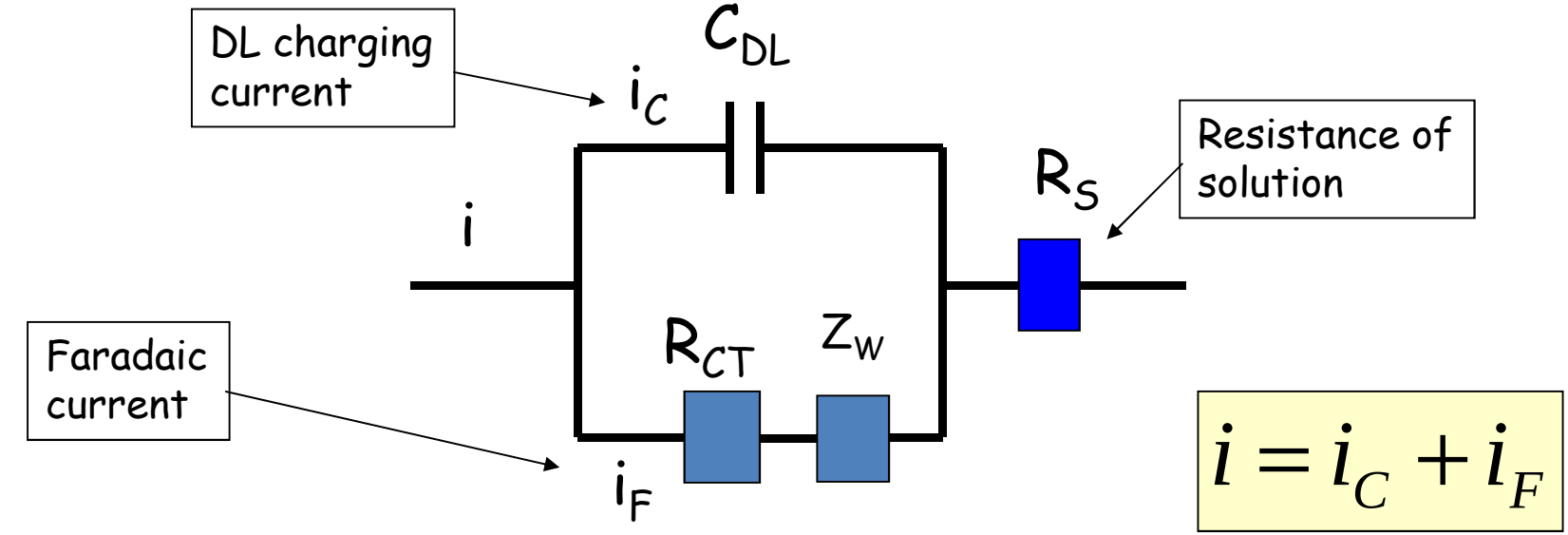
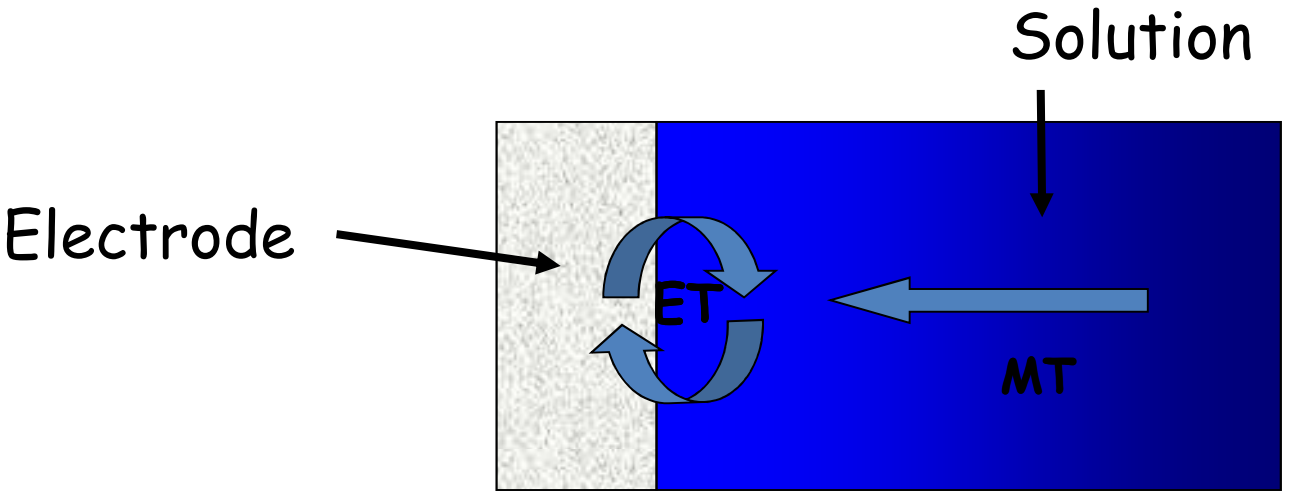


Fig. 1. The fabrication process of needle-like and forest-like SWCNT electrodes. The concentration of SWCNT solution is 0.2 mg L^{-1}. The inset shows the picture of well-solubilized SWCNT solution.

Simple Randles equivalent circuit representation of electrode/solution interface region.



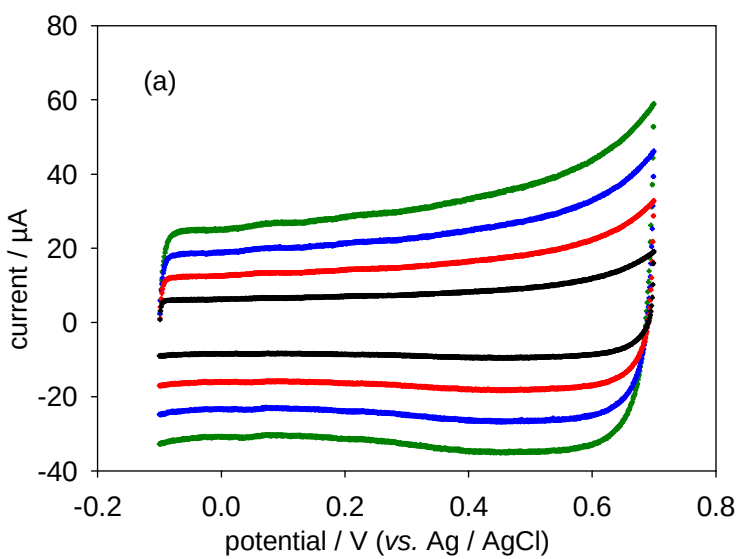
$$i = i_C + i_F$$

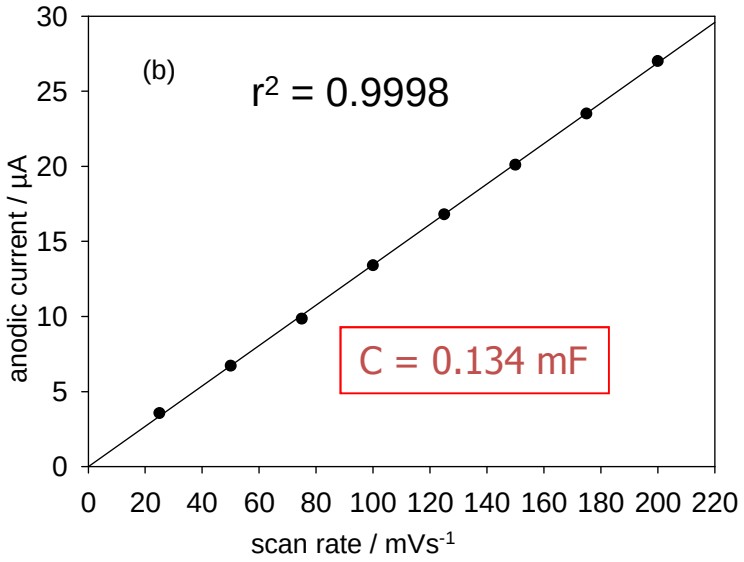


Double layer charging current always present in addition to Faradaic current in electrochemical measurements.

Capacitance of SWNT modified GC electrode (I) Potential Sweep Analysis.

M.E.G. Lyons, G.P. Keely, Int. J. Electrochem. Sci., 3 (2008) 819-853.





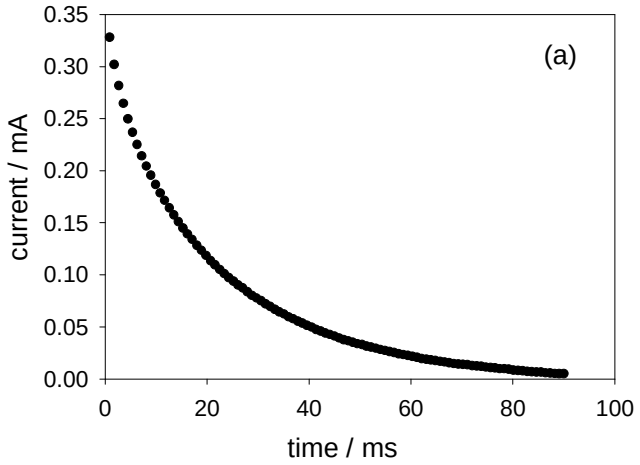
(a) Cyclic voltammograms of a carbon nanotube-modified glassy carbon electrode in a pH 7.0 phosphate buffer.
Scan rates were (from inner to outer) 50, 100, 150 and 200 mV/s.
(b) The resulting plot of anodic current at +0.1 V against scan rate.

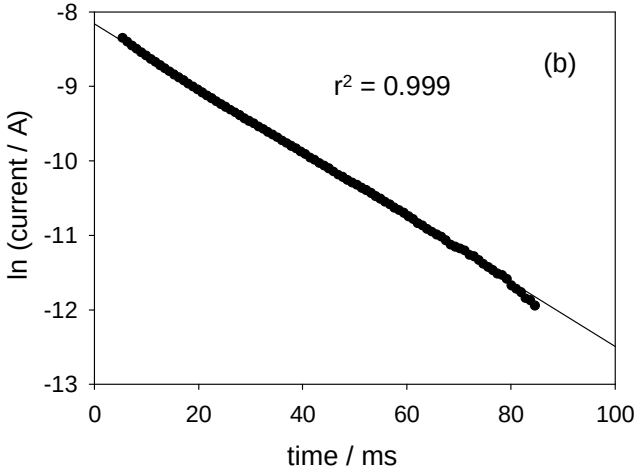
Average capacitance SWNT coated GC

C = 1.4 mF cm^{-2}

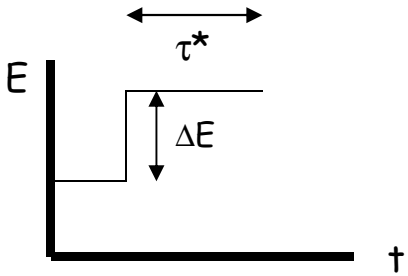
$$i_C = C_{DL}\upsilon + \left(\frac{E_i}{R_S} - C_{DL}\upsilon\right)\exp\left[-\frac{t}{R_S C_{DL}}\right]$$

Capacitance of SWNT modified GC electrode (II) Potential Step Analysis.





Potential step chronoamperometry at a carbon nanotube-modified glassy carbon electrode in a pH 7 phosphate buffer solution (50 mM). Potential stepped from an initial value of 0 V to 50 mV (vs Ag/AgCl). (a) Current time response profile. (b) semilogarithmic analysis of (b) chronoamperometric data.

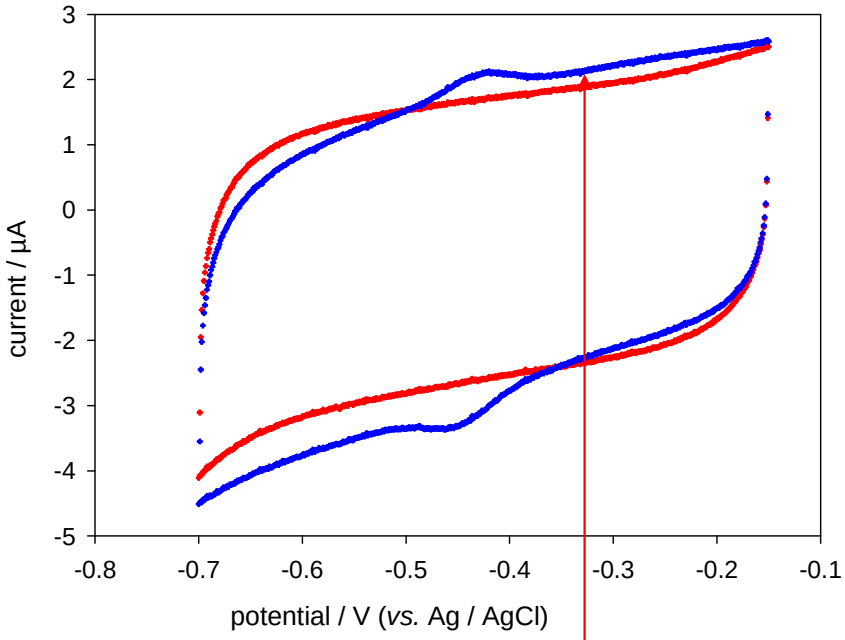


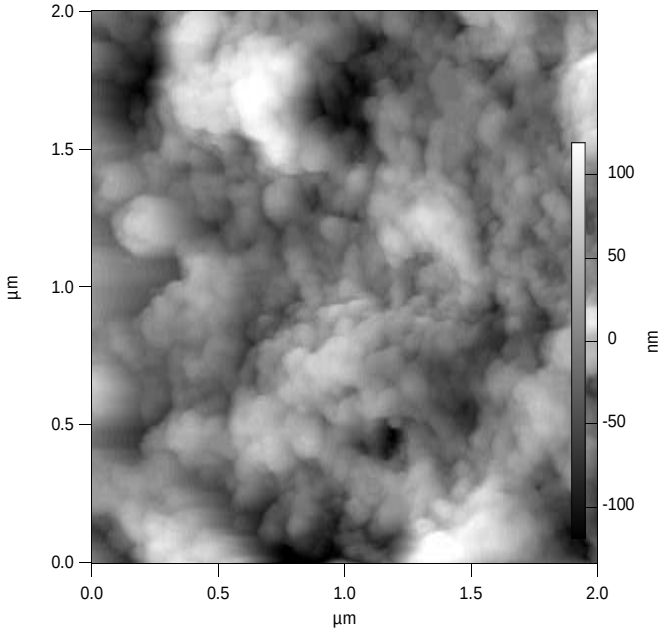
$$i_C = \frac{\Delta E}{R_S}\exp\left[-\frac{t}{R_S C_{DL}}\right]$$

Double layer RC charging time constant

$$\tau = R_S C_{DL}$$

M.E.G. Lyons, G.P. Keely, Int. J. Electrochem. Sci., 3 (2008) 819-853.



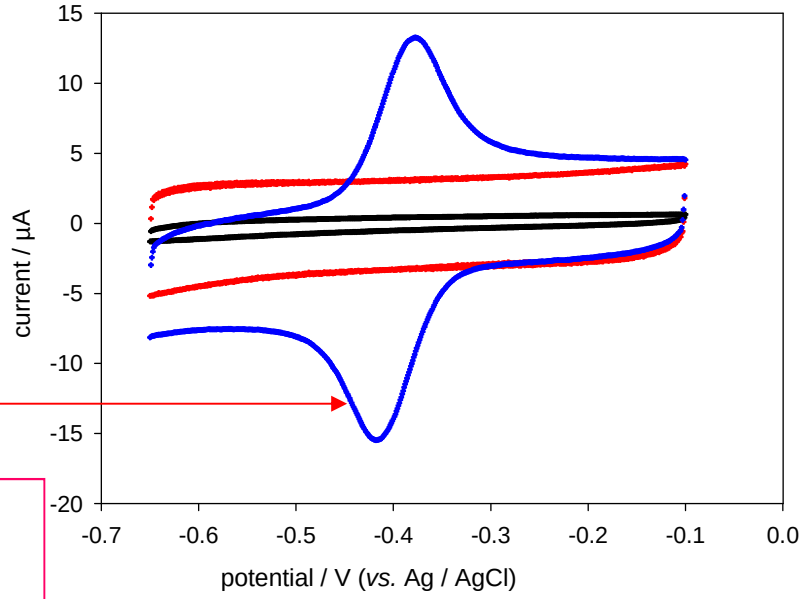


AFM image GOX immobilized on silicon surface

GC/SWNT/GOx

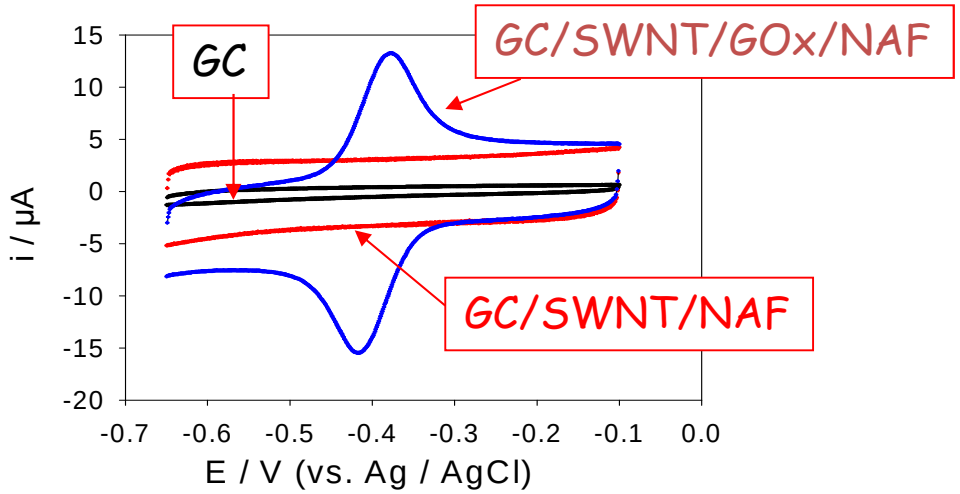
GC/SWNT/GOx/NAF

Phosphate buffer pH 7
Sweep rate, 50 mV/s.

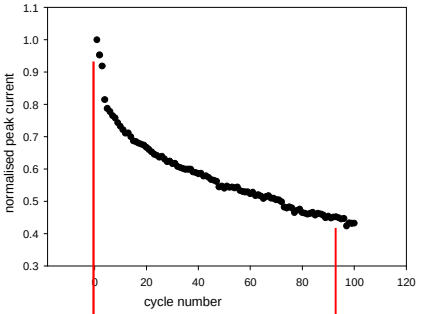


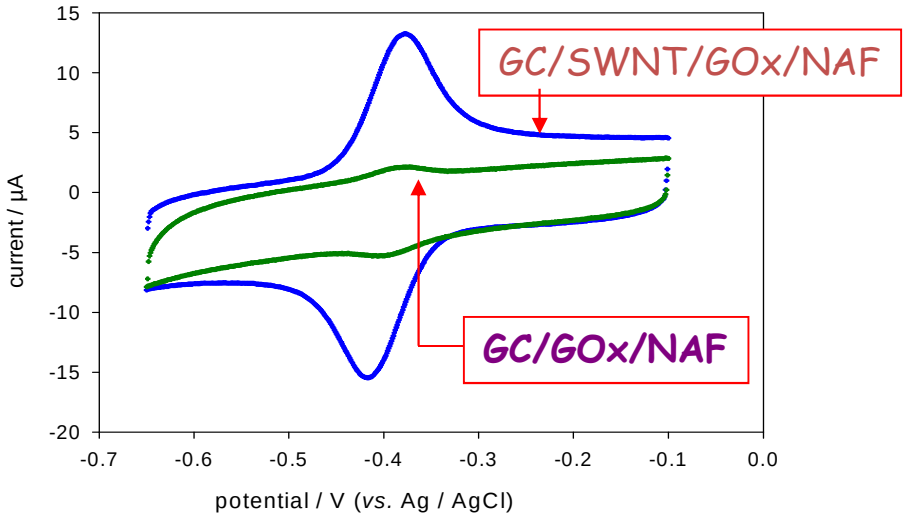
M.E.G. Lyons, G.P. Keeley, *Sensors*,6 (2006)1971-1826.
M.E.G. Lyons, G.P. Keeley, *Int. J. Electrochem. Sci.*,3(2008) 819-853.

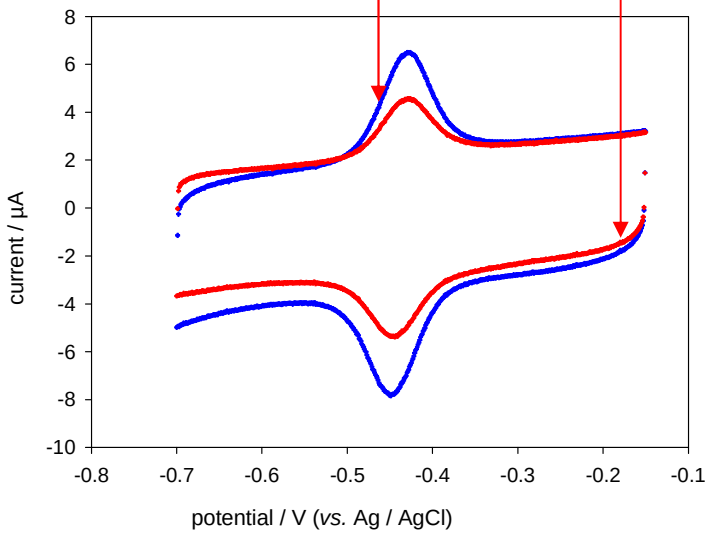
Direct redox electrochemistry of GOx immobilized on SWCNT modified GC surfaces.



GOx layer stability
over 100 cycles, PBS pH7





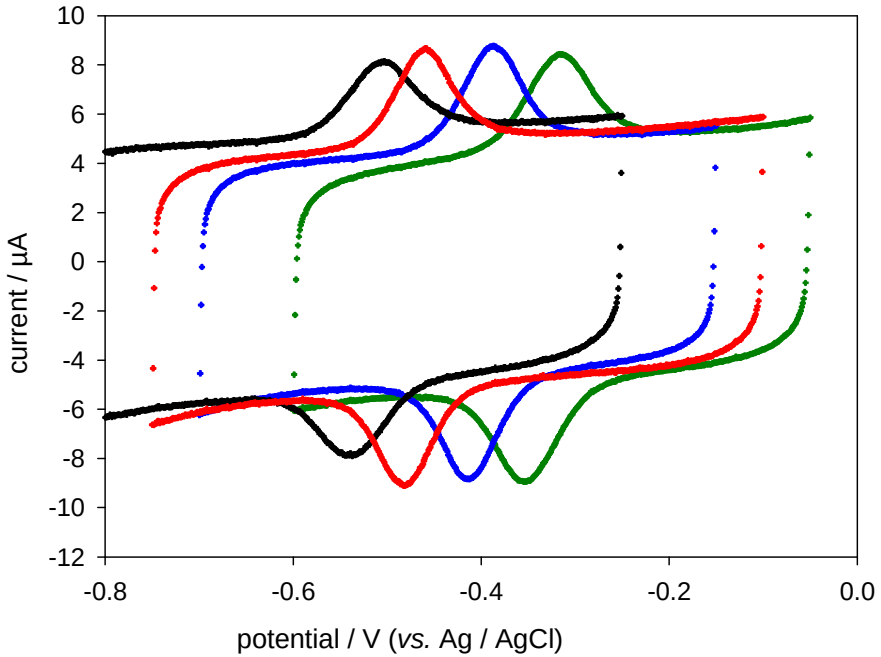


$$FADH_2 \underset{}{\overset{-2e^- -2H^+}{\rightleftharpoons}} FAD^+$$

$$A + ne^- + mH^+ \rightleftharpoons B$$

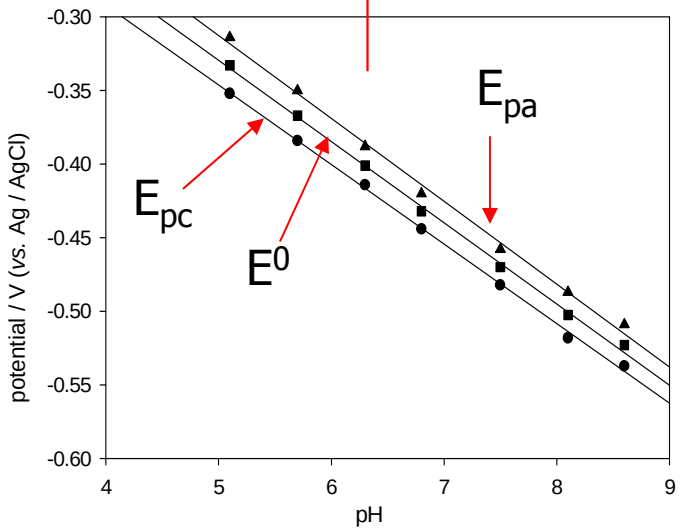
$$E = E^0 - \frac{2.303RT}{F}\left(\frac{m}{n}\right)pH + \frac{2.303RT}{nF}\log\left(\frac{c_A}{c_B}\right)$$

$$\frac{dE}{dpH} = -2.303\frac{RT}{F}\left(\frac{m}{n}\right)$$



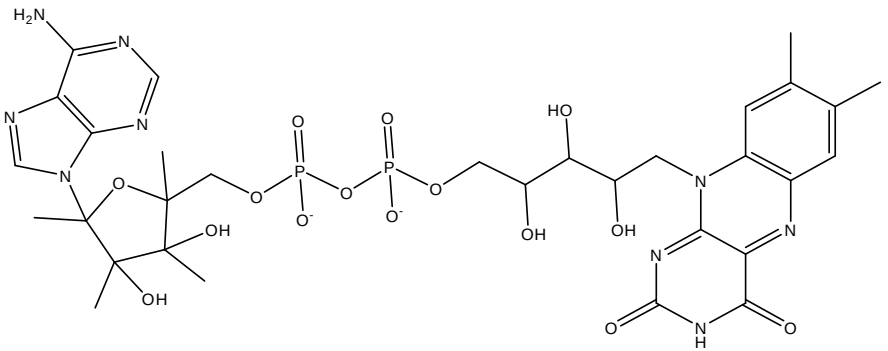
Average (4 data sets)

$$\frac{dE}{dpH} = -(53 \pm 2)\ \text{mV/dec}$$

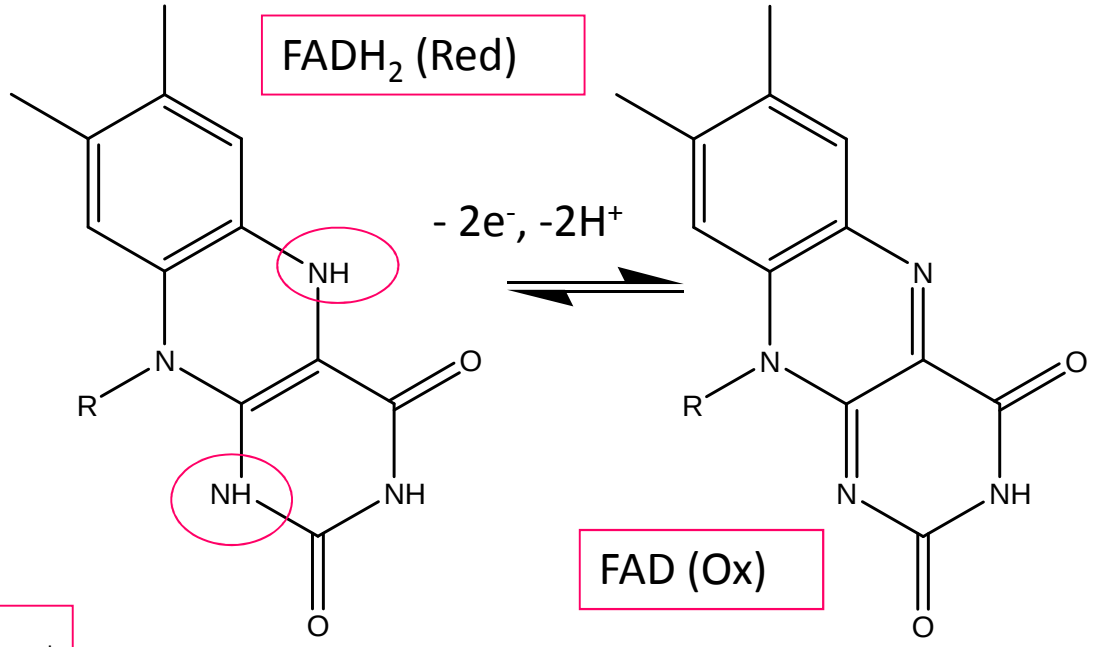


Voltammograms obtained using a GC/SWCNT/GOx/Nafion electrode in 50 mM phosphate buffers of pH (from left to right) 8.6, 7.5, 6.3 and 5.1. The sweep rate employed in each case was 50 mVs^{-1}.

Flavin group redox chemistry



flavin adenine dinucleotide (oxidised form)



$$FADH_2 \underset{}{\overset{-2e^- -2H^+}{\rightleftharpoons}} FAD^+$$

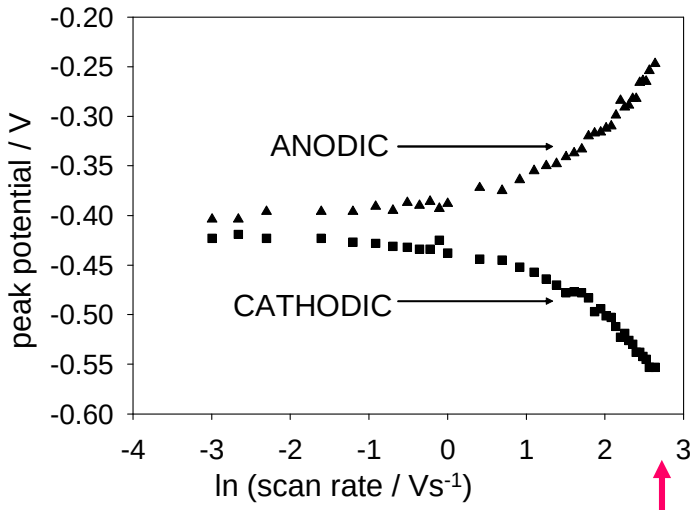
Electrode	E^0 / mV (*vs.* Ag / AgCl)	ΔE_p @ 50 mVs^{-1}
1	-442 ± 2	17 mV
2	-439 ± 1	24 mV
3	-442 ± 1	19 mV
4	-443 ± 2	14 mV

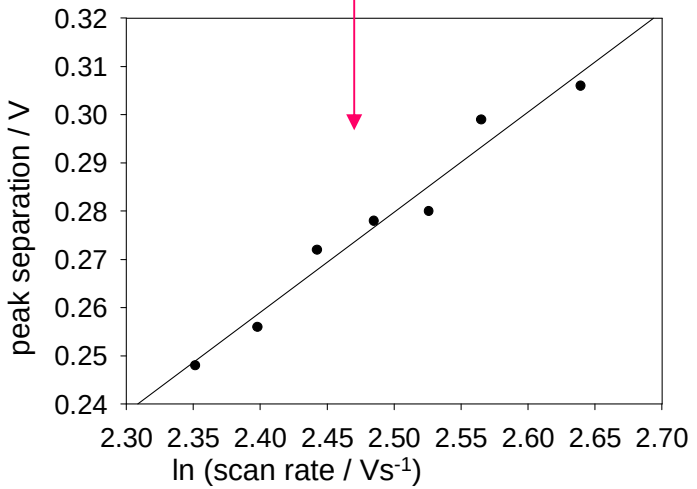
Electrode	Area of GC/NT (cm^2)	Moles of active GOx	Γ ($pmolcm^{-2}$)
1	0.0880	1.43×10^{-11}	162
2	0.0910	0.823×10^{-11}	90.4
3	0.105	2.71×10^{-11}	258
4	0.107	1.49×10^{-11}	139

system	GOx surface coverage / $pmolcm^{-2}$	reference
Au/SWCNT/GOx	52	7
epHOPG/GOx	3	8
Au/SAM/GOx	1	9
CNTPME/GOx	199	15

Laviron Analysis : quantifying flavin group redox kinetics

$$\Delta E_p = \frac{RT}{(1-\alpha)\alpha nF}[\alpha\ln(1-\alpha) + (1-\alpha)\ln\alpha - \ln\frac{RT}{nF} - \ln k^0] + \frac{RT}{(1-\alpha)\alpha nF}\ln\nu.$$





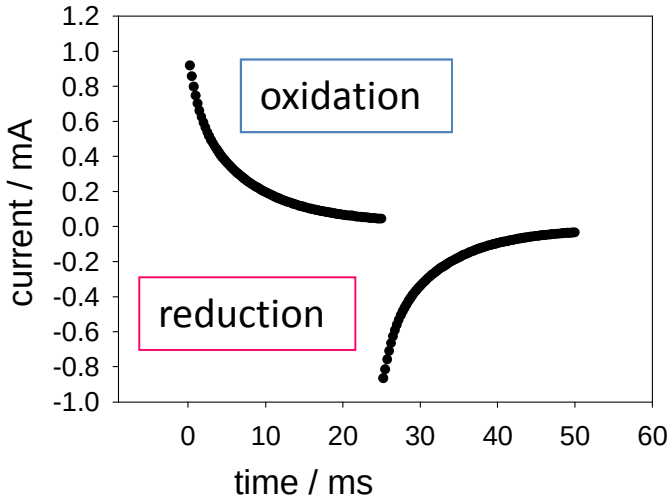
$$E_{pc} = E^0 + \frac{RT}{\alpha nF}\ln\frac{RTk^0}{\alpha nF} - \frac{RT}{\alpha nF}\ln\nu,$$

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF}\ln\frac{RTk^0}{(1-\alpha)nF} + \frac{RT}{(1-\alpha)nF}\ln\nu,$$

Au/SWCNT/GOx/Nafion
$k^0 = 19\ s^{-1}$; $\alpha = 0.11$
GC/SWCNT/GOx/Nafion
$k^0 = 18\ s^{-1}$; $\alpha = 0.10$

Analysis of CV response as function of potential sweep rate enables transfer coefficient α and rate constant k^0 for surface redox transformation to be determined.

Potential Step Chronoamperometry.



Potential Step :
- 0.5 -> - 0.3 V

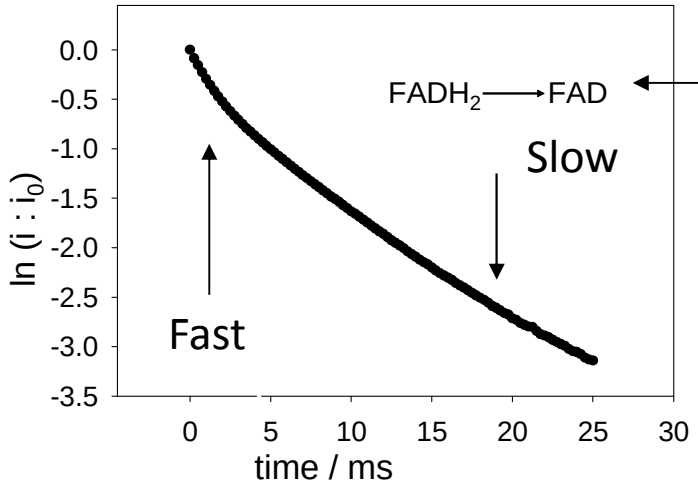
Simple 1st order
Surface redox reaction.

$$i = k\Delta Q \exp\left(-kt\right)$$

$$\ln i = \ln\left(k\Delta Q\right) - kt$$

Predict linear plot of ln i vs t with
Slope given by -k.

'Dog-Leg'
Response.



$$\frac{i}{i_0} = A\exp(-k_f t) + B\exp(-k_s t)$$

Bi-exponential fitting.

Deviation of current transient response from simple 1st order kinetics indicates operation of kinetic dispersion: GOx sites are energetically in-equivalent hence expect range of heterogeneous rate constants for redox transformation.

Heterogeneity/Dispersive kinetic efects

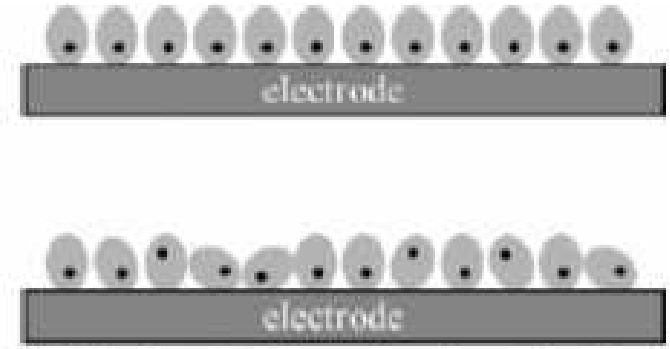


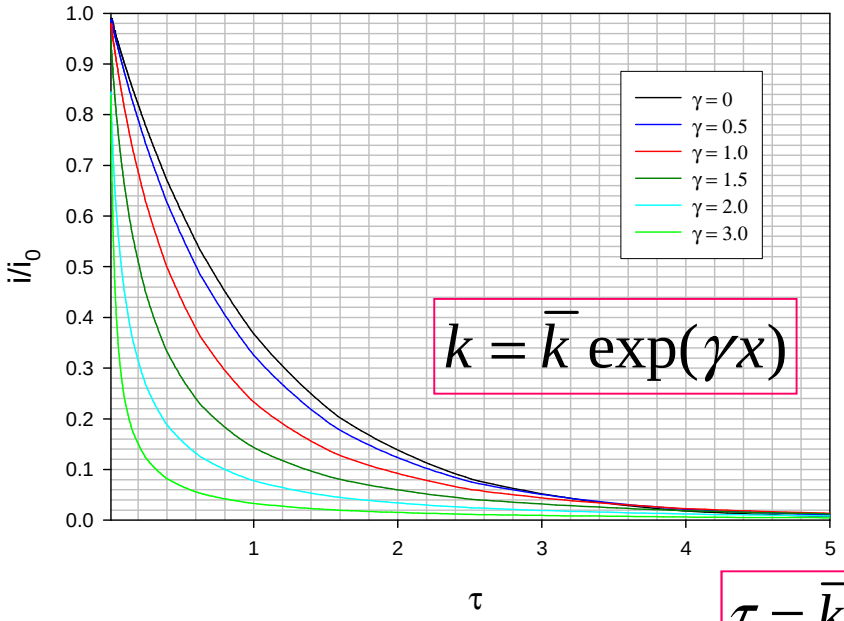
Figure 1. Model of an ideal (upper) and nonideal (lower) protein–electrode interface when a protein containing a single redox active site (●) is adsorbed onto a graphite electrode to give close to monolayer surface coverage.

- Heterogeneity / dispersive effects in adsorbed protein layer.
- Expect latter effects to be more marked for Protein/SWCNT mesh composite system.

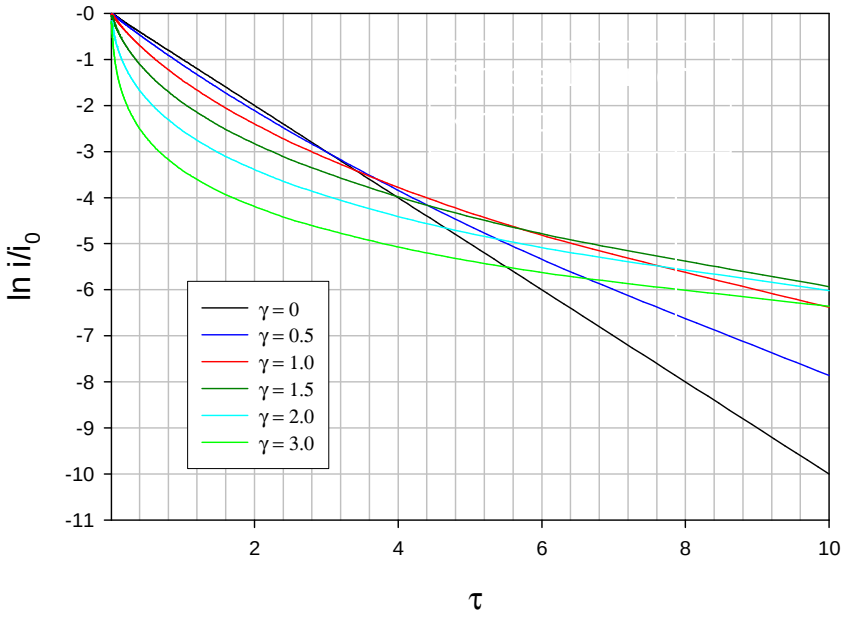
Fleming, Zhang, Elton, Bond. Anal. Chem., 2007, 79, 6515-6526.

Gaussian (Dispersive) Kinetics

Albery, W. J.; Bartlett, P. N.; Wilde, C. P.; Darwent, J. R.
A General Model for Dispersed Kinetics in Heterogeneous Systems.
J. Am. Chem. Soc. **1985**, *107*, 1854-1858.



$$\tau = \bar{k}t$$



Assume rate constant statistically distributed about a mean value.
Statistics are Gaussian.

$$\bar{k} = \frac{1}{\tau_{1/e}}$$

$$\gamma = 0.92 \times \left(\frac{\tau_{7/8}}{\tau_{1/2}} - 3 \right)^{1/2}$$

Main parameters : mean rate constant and Gaussian spread parameter.

Kinetic results : GOx redox transition

System Measured at pH 7	Rate constant neglecting dispersion/s^{-1}	Mean rate constant including dispersion/s^{-1}	Gaussian spread parameter
Au /SWCNT/ GOx /Nafion	19	830	0.9
GC/SWCT/ GOx /Nafion	18	950	1.2

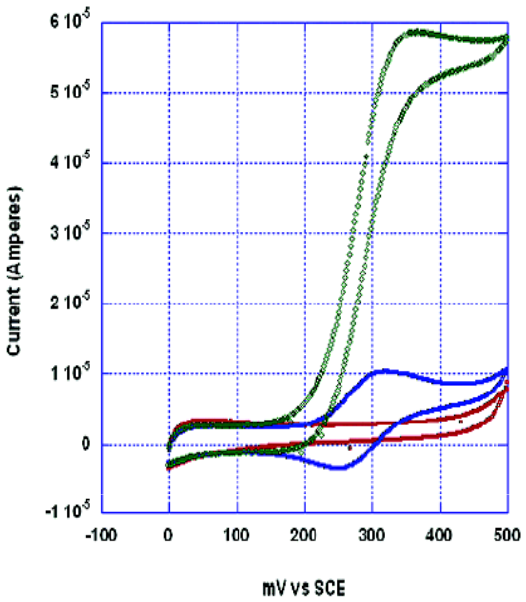


Figure 4. Voltammetric response of a GOX-SWNT-modified GC electrode in the absence (red) and presence (blue) of 0.5 mM FMCA. The catalytic response (green) is observed on the addition of 50 mM glucose.

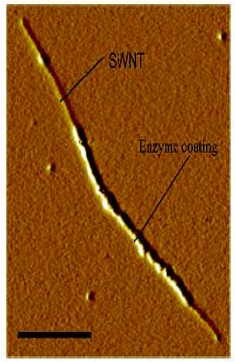


Figure 3. Amplitude AFM image of a glucose oxidase-modified SWNT. Scale bar = 200 nm.

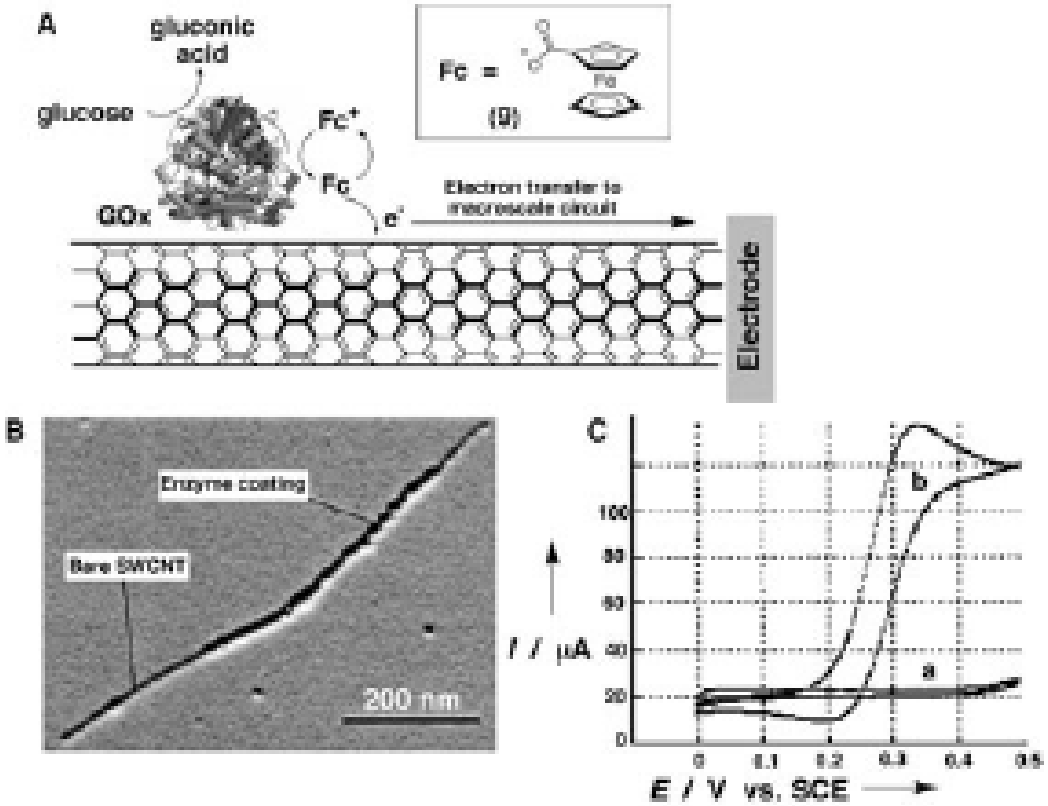
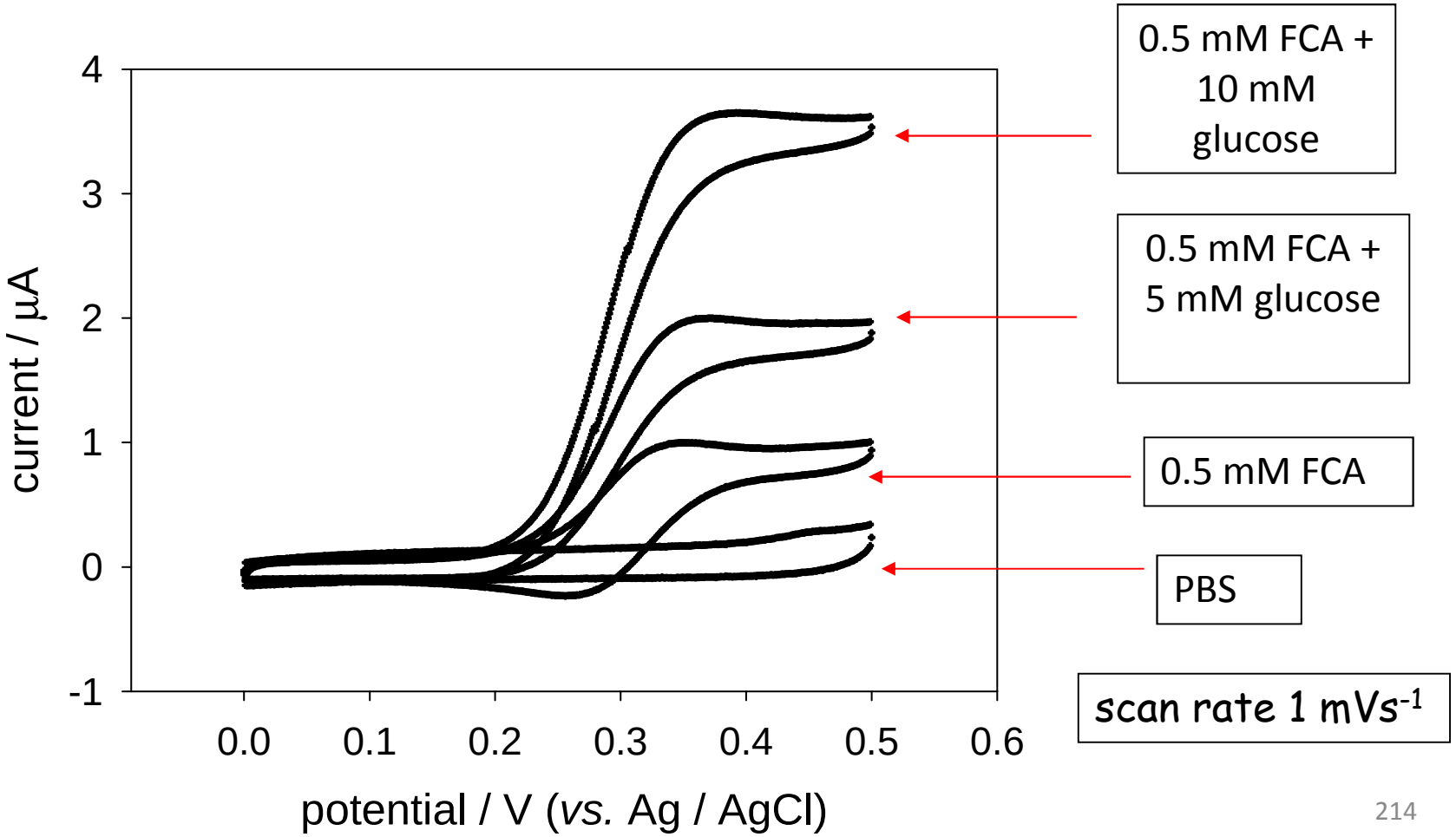
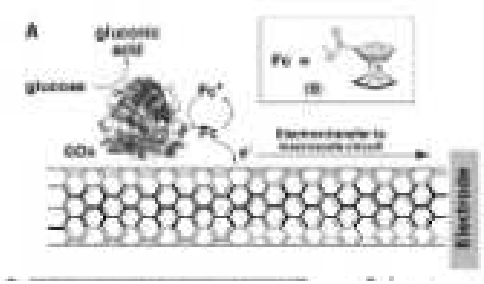


Figure 14. A) Electrical contacting of GOx loaded onto SWCNT sidewalls through a diffusional mediator (ferrocene monocarboxylic acid). B) An AFM image of a SWCNT loaded with GOx on the sidewall. C) Voltammetric responses of GOx-modified SWCNTs with ferrocene monocarboxylic acid as the diffusional electron relay: a) In the absence of glucose; b) In the presence of glucose. (Adapted from ref. [93a], Figures 7 and 8, with permission).

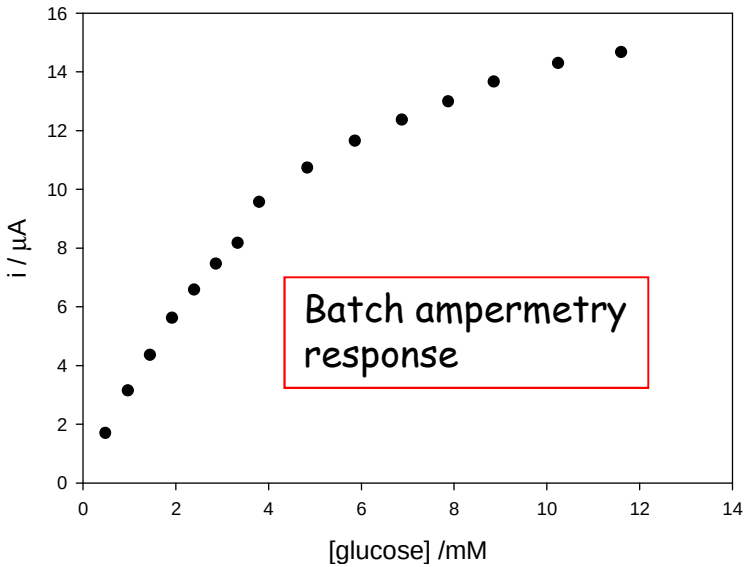
Davis et al. J. Am. Chem. Soc., 2002, 124, 12664-12665.

Homogeneous mediated enzyme catalysis at SWCNT CME using ferrocene monocarboxylic acid (FCA).

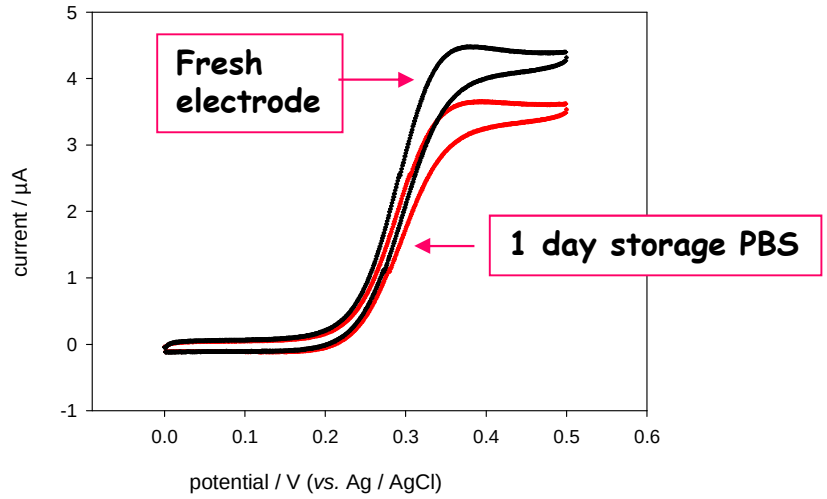


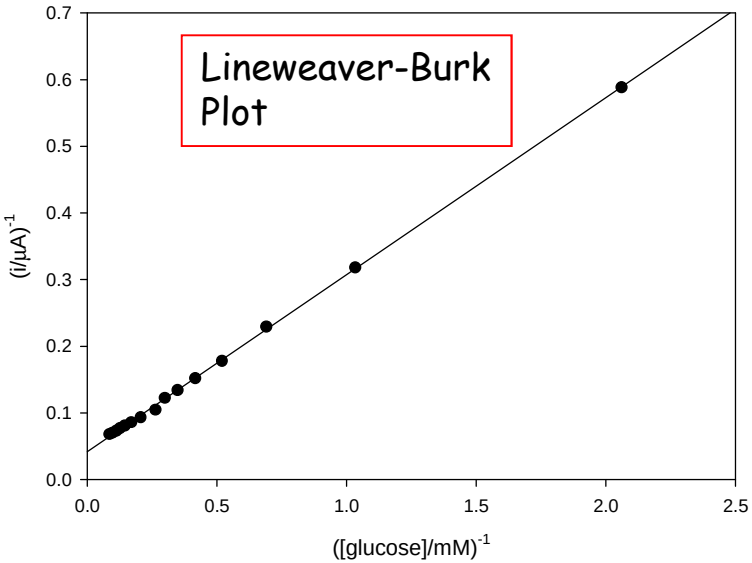


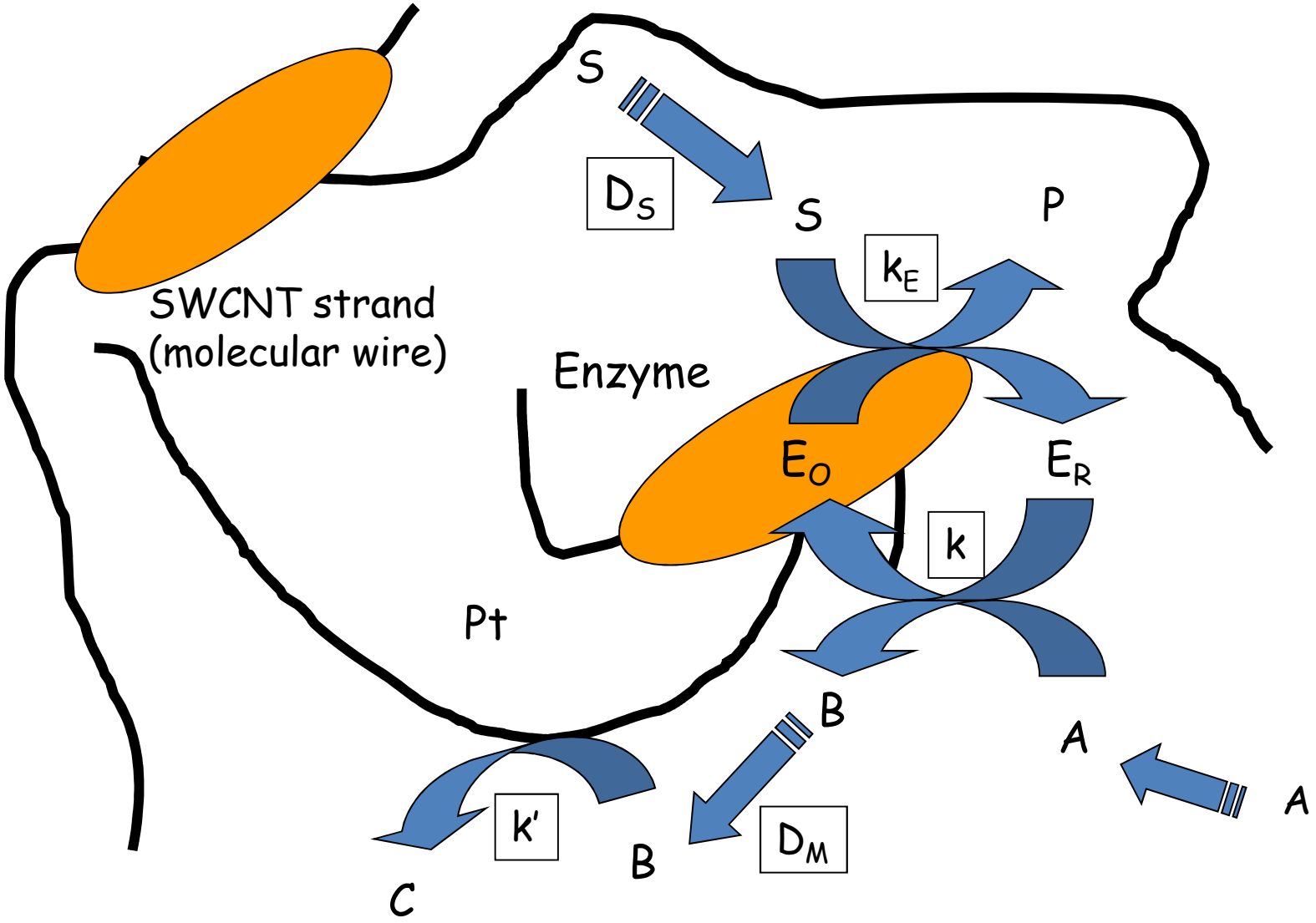
Bioelectrocatalytic responses of the
GC/SWCNT/GOx/Nafion electrode to
the oxidation of 10 mM glucose in
50 mM PBS (pH 7.0) in the presence of 0.5 mM ferrocene carboxylic acid.



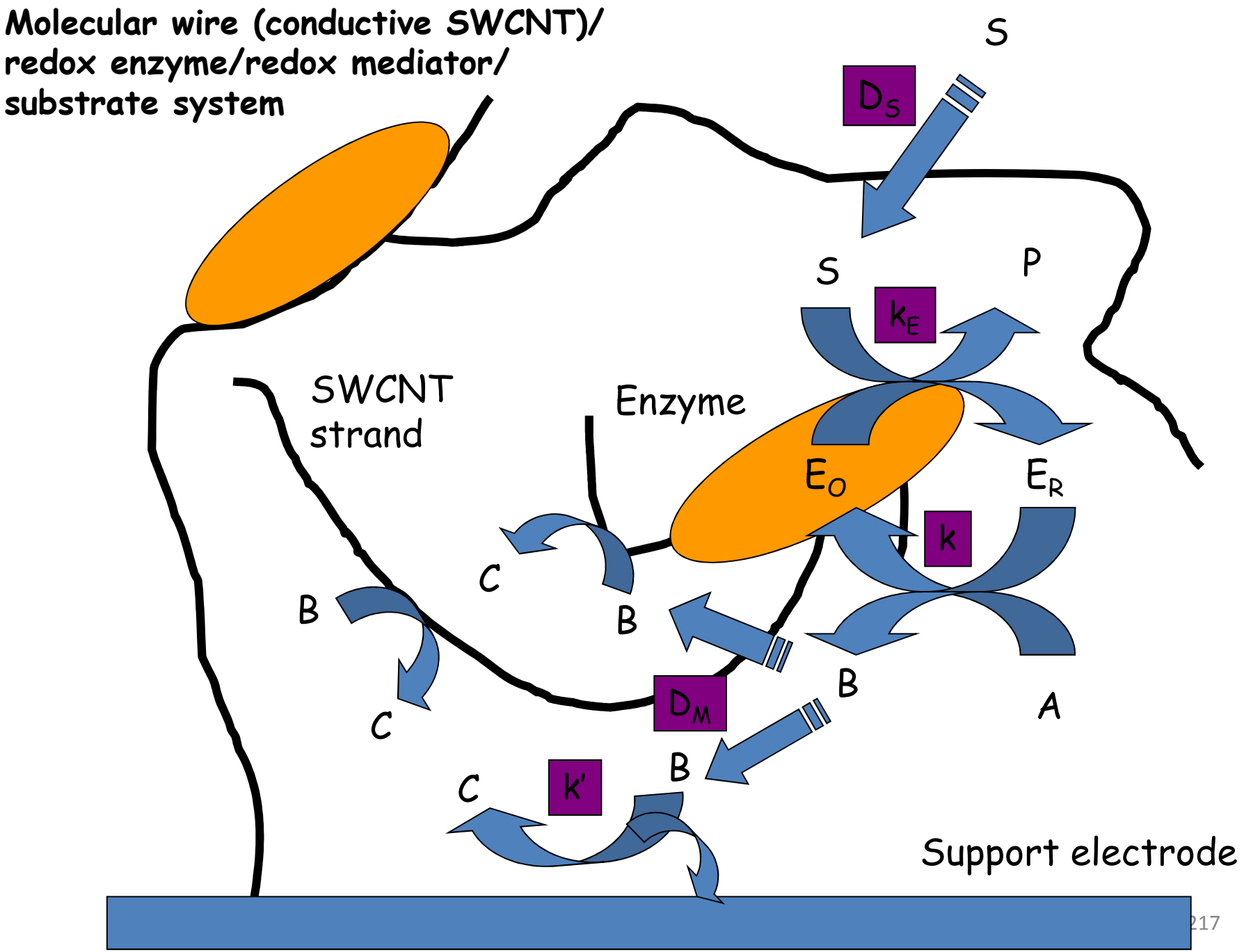
Deoxygenated 0.6 mM FCA in pH 7 PBS.
Glucose aliquots added and current allowed to stabilise.
Potential held @ +0.4 V.
Magnetic stirring @ 100 rpm.
GC / SWCNT / GOx / Nafion electrode.

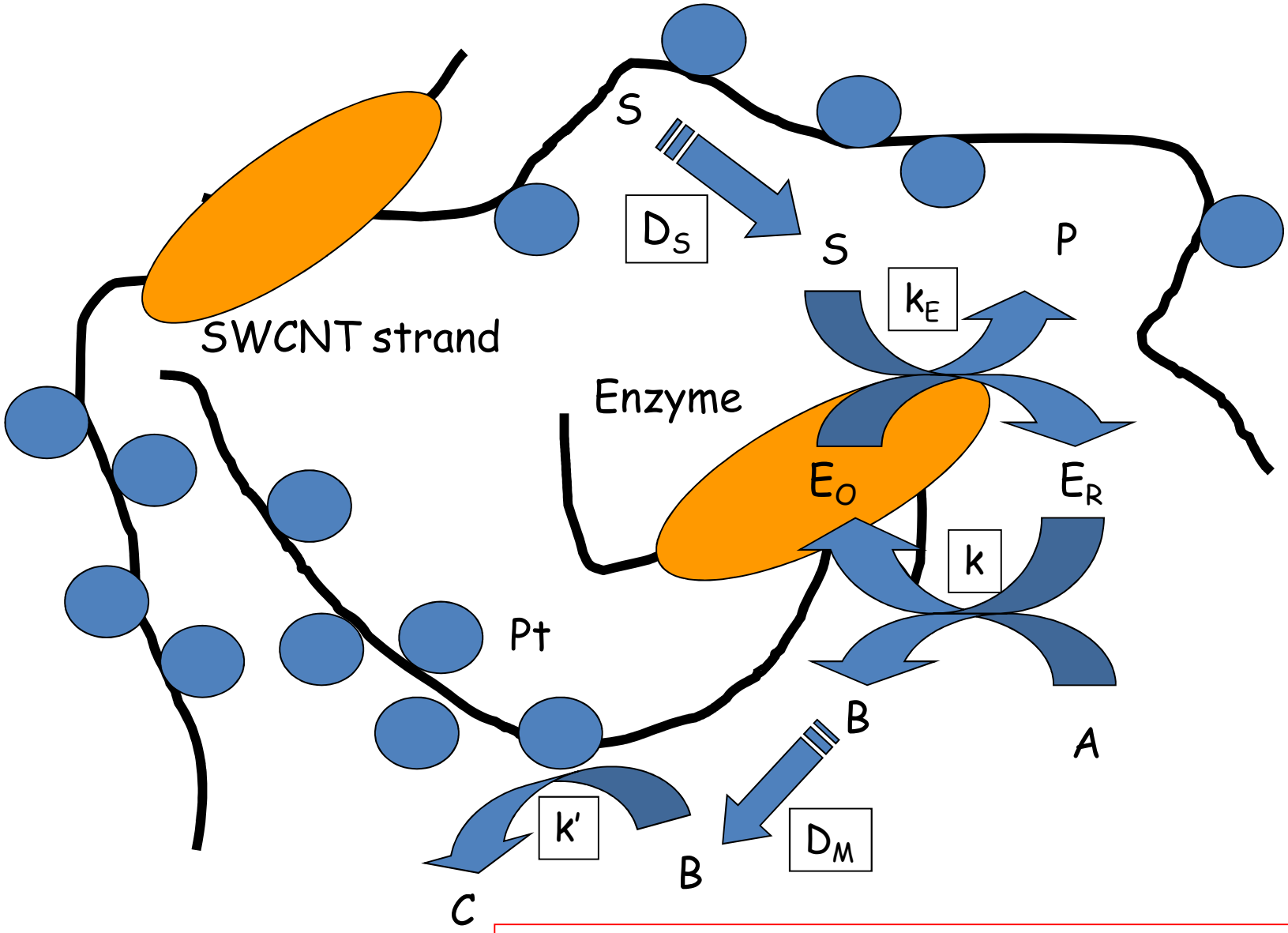






ConductingSWCNT/redox enzyme /redox mediator/ substrate system


Molecular wire (conductive SWCNT)/
redox enzyme/redox mediator/
substrate system
S
D_S
S
P
k_E
SWCNT
strand
Enzyme
E_O
E_R
k
C
B
B
B
C
D_M
B
A
C
k'
Support electrode



SWCNT/redox enzyme/redox mediator/ metal nanoparticle/substrate system

Fick Diffusion term for substrate/mediator in NT film (assumed Homogeneous slab).

Non linear MM type reaction term For 'Ping-Pong' Mechanism

$$D_S \frac{d^2 s}{dx^2} - \frac{k k_c e_\Sigma a s}{k a (s + K_M) + k_c s} = 0$$

$$D_B \frac{d^2 b}{dx^2} + \frac{k k_c e_\Sigma a s}{k a (s + K_M) + k_c s} = 0$$

$$x = 0 \quad \frac{ds}{dx} = 0$$

$$x = L \quad s_L = \kappa_S s^\infty$$

$$x = 0 \quad b = b_0 \quad \left(\frac{db}{dx}\right)_0 = \frac{f_B}{D_B}$$

$$x = L \quad b = b_L = 0 \quad a = a_L = \kappa_A a^\infty$$

$$f_\Sigma = f_B = D_B \left(\frac{db}{dx}\right)_{x=0} = k' b_0$$

$$f_S = \frac{i}{nFA} = D_S \left(\frac{ds}{dx}\right)_{x=L}$$

<u>Non/poorly conductive SWNT mesh.</u>
Reduced mediator reacts at support electrode surface. Need to substitute solution s=s(x) of substrate RD equation into the RD equation for mediator and solve to obtain reaction flux $f_\Sigma = f_B$.

<u>Conductive SWNT mesh</u>.
Reduced mediator reacts at SWNT fibre or at metal nanoparticle Surface.
Only need to solve the RD equation for substrate S to obtain the reaction Flux f_S or current i.

Catalytic system defined in terms of a classical 1D diffusion reaction boundary value problem with non linear reaction kinetics in a finite spatial region regarded as a homogeneous slab. Steady state response evaluated and time dependence neglected.

M.E.G. Lyons, Int. J. Electrochem. Sci., 4(2009) 1196-1236.

$$S + E_O \underset{}{\overset{K_M}{\rightleftharpoons}} [E_O S \rightarrow E_R P] \xrightarrow{k_C} E_R + P$$

$$A(M_O) + E_R \xrightarrow{k} E_O + B(M_R)$$

$$B \xrightarrow{k'} A$$

$$B \xrightarrow{k'} C$$

Definition of non-dimensional parameters.

$$u = \frac{s}{\kappa_S s^{\infty}} \qquad v = \frac{b}{\kappa_A a^{\infty}} \qquad \chi = \frac{x}{L}$$

$$\alpha = \frac{\kappa_S s^{\infty}}{K_M} \qquad \alpha u = \frac{s}{K_M}$$

$$\kappa = \frac{f_{SER}}{f_{MER}} = \frac{\left(k_c / K_M\right)\kappa_S s^{\infty} e_{\Sigma} L}{k \kappa_A a^{\infty} e_{\Sigma} L}$$

$$\gamma_S = \frac{f_{SER}}{f_{SD}} = \frac{\left(k_c / K_M\right)\kappa_S s^{\infty} e_{\Sigma} L}{\kappa_S D_S s^{\infty} / L}$$

$$\gamma_M = \frac{f_{SER}}{f_{MD}} = \frac{\left(k_c / K_M\right)\kappa_S s^{\infty} e_{\Sigma} L}{\kappa_A D_M a^{\infty} / L}$$

Form of RD equation simplified and generalised which aids analysis.

Formulation of boundary value problem in non-dimensional form : Conducting SWNT

$$\frac{d^2 u}{d\chi^2} - \frac{\gamma_S F(u)}{1 + \kappa F(u)} = 0$$

$$F(u) = \frac{u}{1 + \alpha u}$$

$$\chi = 0 \quad \left(\frac{du}{d\chi}\right)_0 = 0$$

$$\chi = 1 \quad u = 1$$

$$\Psi_S = \frac{f_S}{\kappa_S D_S s^{\infty} / L} = \left(\frac{du}{d\chi}\right)_{\chi=1} = \int_0^1 \gamma_S u(\chi)\, d\chi$$

Derived relationship between observed flux and substrate reaction flux. These are not equal.

Formulation of boundary value problem in non-dimensional form : Non-conducting SWNT

$$\frac{d^2 v}{d\chi^2} + \frac{\gamma_M F(u)}{1 + \kappa F(u)} = 0$$

$$F(u) = \frac{u}{1 + \alpha u}$$

$$\chi = 0 \quad v = v_0 \quad \Psi_{\Sigma} = \zeta v_0$$

$$\chi = 1 \quad v = 0$$

$$\zeta = \frac{k'}{D_M / L}$$

$$\Psi_{\Sigma} = \left(\frac{dv}{d\chi}\right)_{\chi=0}$$

$$\Psi_{\Sigma} = \frac{\gamma_M}{\gamma_S}\Psi_S + \left(\frac{dv}{d\chi}\right)_{\chi=1}$$

Table 1. Typical expressions forreaction flux derived in text for approximate kinetic cases.

Kinetic Case	Normalised Flux	Reaction flux $f = i/nFA$
I	$\Psi_S = \gamma_S$ $\Psi_\Sigma = \frac{\gamma_M}{2\{1+\zeta^{-1}\}}$	$f_S = \left(\frac{k_c}{K_M}\right) e_\Sigma L \kappa_S s^\infty$ $f_\Sigma = \frac{(k_c/K_M)\kappa_S s^\infty e_\Sigma L}{2\left\{1+\frac{D_M/L}{k'}\right\}}$
II	$\Psi_S = \sqrt{\gamma_S}$	$f_S = \sqrt{\left(\frac{k_c}{K_M}\right) D_S e_\Sigma \kappa_S s^\infty}$
III	$\Psi_S = \frac{\gamma_S}{\alpha}$ $\Psi_\Sigma = \frac{\gamma_M}{2\{1+\zeta^{-1}\}\alpha}$	$f_S = k_c e_\Sigma L$ $f_\Sigma = \frac{k_c e_\Sigma L}{2\left\{1+\frac{D_M/L}{k'}\right\}}$
IV	$\Psi_S = \sqrt{2\frac{\gamma_S}{\alpha}}$	$f_S = \sqrt{2\kappa_S D_S k_c e_\Sigma s^\infty}$
V	$\Psi_S = \frac{\gamma_S}{\kappa}$ $\Psi_\Sigma = \frac{\gamma_M}{2\{1+\zeta^{-1}\}\kappa}$	$f_S = k\kappa_A a^\infty e_\Sigma L$ $f_\Sigma = \frac{k\kappa_A a^\infty e_\Sigma L}{2\left\{1+\frac{D_M/L}{k'}\right\}}$
VI	$\Psi_S = \sqrt{2\frac{\gamma_S}{\kappa}}$	$f_S = \sqrt{2\kappa_A k \kappa_S D_S e_\Sigma a^\infty s^\infty}$
VII	$\Psi_\Sigma = \frac{\gamma_M/\gamma_S}{1+\zeta^{-1}}$	$f_\Sigma = \frac{\kappa_S D_S s^\infty/L}{1+\frac{D_M/L}{k'}}$

Reaction order predictions for kinetically limiting cases : variation of substrate flux with experimental variables.

Table 2. Typical diagnostic criteria for immobilized enzyme biosensors.

Case	s^n	t_Σ	a^n	L
I	1	1	0	1
II	1	1/2	0	0
III	0	1	0	1
IV	1/2	1/2	0	0
V	0	1	1	1
VI	1/2	1/2	1/2	1/2
VII	1	0	0	-1

- Nanotube arrays/meshes are excellent support matrices for enzymes.
  - Direct redox chemistry of enzymes immobilized within surface confined CNT films may be probed via transient electrochemical techniques.
  - Enzyme maintains catalytic activity when immobilized within CNT matrix.
  - Mobile solution soluble mediator molecules (O_2 or ferrocene carboxylic acid) required to complete enzyme regeneration.
  - Mathematical modelling of CNT CME amperometric biosensor operation produces several rate limiting scenarios, detailed kinetic case diagrams and characteristic expressions for the steady state reaction rate.

Kinetic Case Diagram : α/κ plane

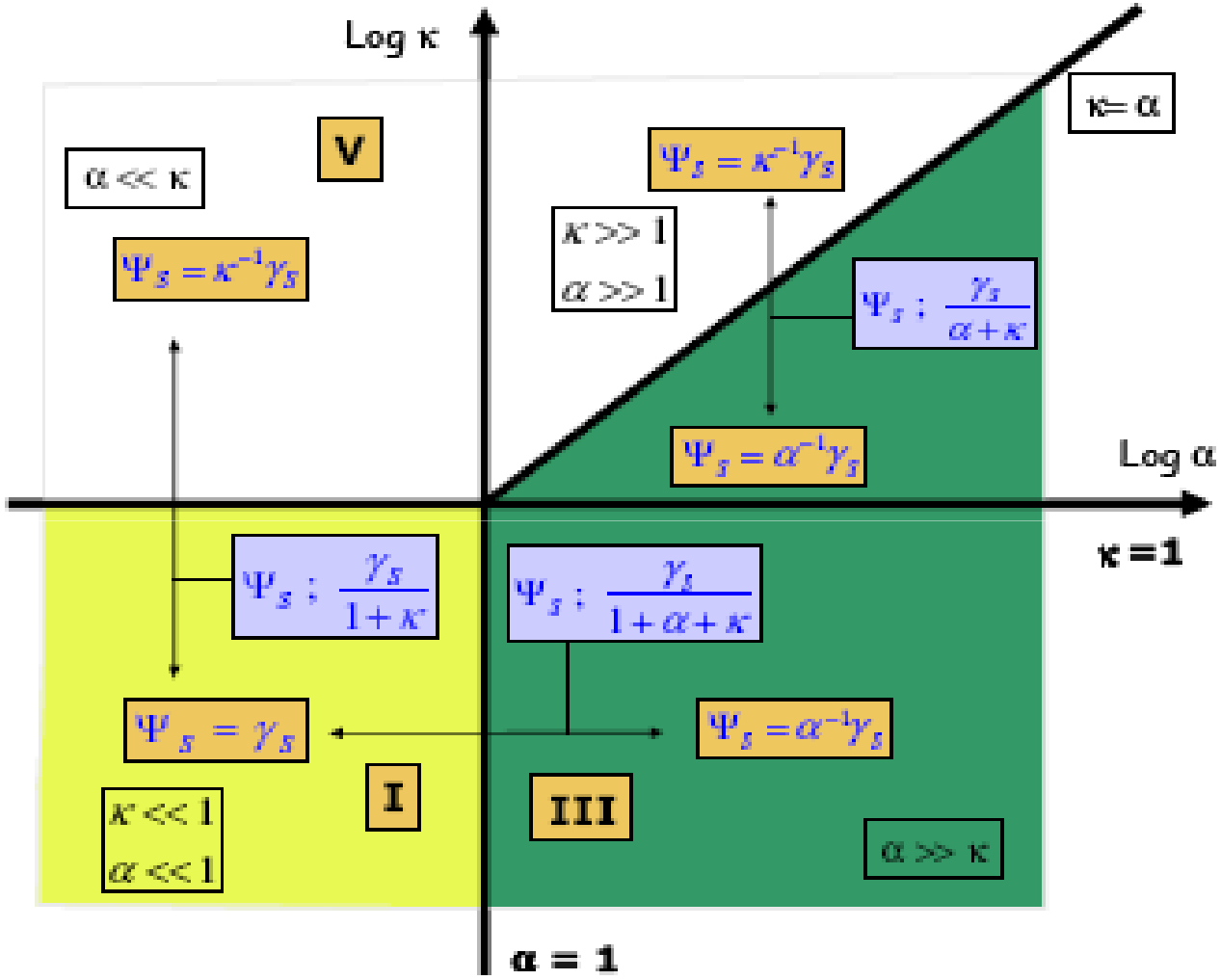


Figure 8. Kinetic case diagram illustrating the α, κ plane.

Kinetic Case Diagram : α/γ_s plane