BIOSENOSRS

BIO 580

Electrochemical Biosensors - theory part 4

Few noted examples for amperometric biosensor

WEEK-5

Fall Semester

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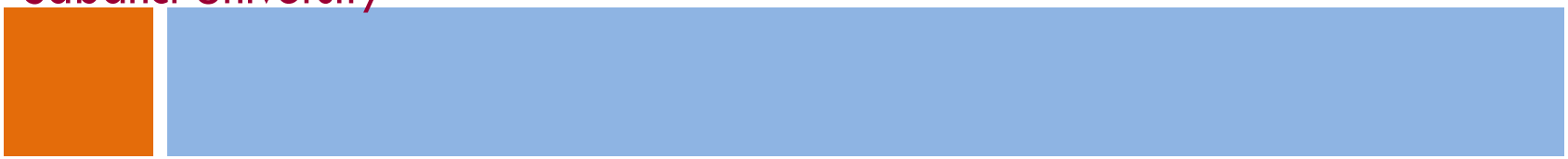
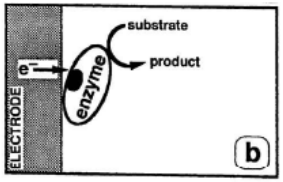


Topics that will be covered in the course

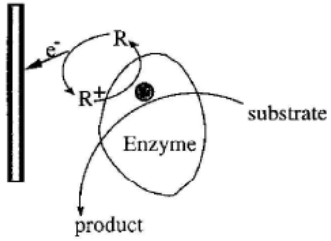
- ❑ History of biosensor development, applications and requirements of biosensors and classification
- ❑ Principles of molecular recognition and transduction signal acquisition
  - ✓Sources of Biological Recognition elements – enzymes/proteins, ssDNAs, antibody and Others
  - ✓Design considerations for use of recognition elements in biosensors
  - ✓Modeling of reactions for various biosensor applications- electrochemical, optical, piezoelectric, colorimetric, fluorometric and others.
- ❑ Modification of sensor surfaces and immobilization techniques
  - ✓Covalent modification of surfaces using surface chemistry
  - ✓Self Assembled Monolayers (SAM) and adsorptions
  - ✓Other ways to immobilize biological macromolecules on various solid surfaces
- ❑ Detection methods and Physical Sensors
  - ✓Electrodes/transducers – electrochemical (amperometric, potentiometric, and conductimetric transductions)
  - ✓Other sensors - for e.g., optical sensors (colorimetric/fluorimetric/luminometric sensors), Surface Plasmon Resonance (SPR) sensors, and piezoelectric resonators.
- ❑ Fabrication of biosensors
  - ✓Miniaturization-application of nano-materials, nanoparticles, carbon nanotubes (CNTs) and others
  - ✓Biocompatibility – stability, reproducibility and repeatability of biomolecules on transducer surfaces
- ❑ Data acquisition, statistical and error analysis
  - ✓Inter and Intra-assays and Coefficient of variation (CV)
  - ✓Signal to noise ratio
  - ✓Normalization/optimization and signal retrieval
- ❑ Examples of commercial biosensors

Strategies for electrical communication between electrode and enzyme

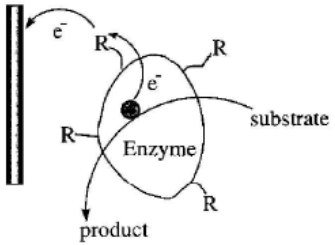
1. Direct electron transfer (DET) by suitable orientation



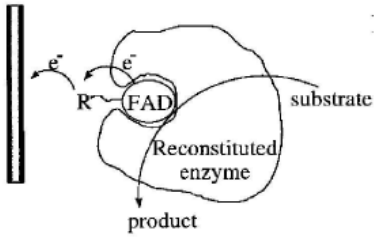
2. By diffusional mediator



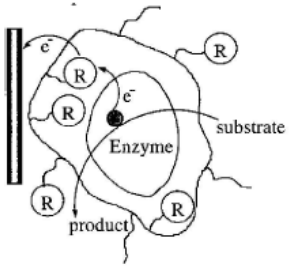
3. Tethering mediator on protein



4. Reconstitution of an apo-flavoenzyme

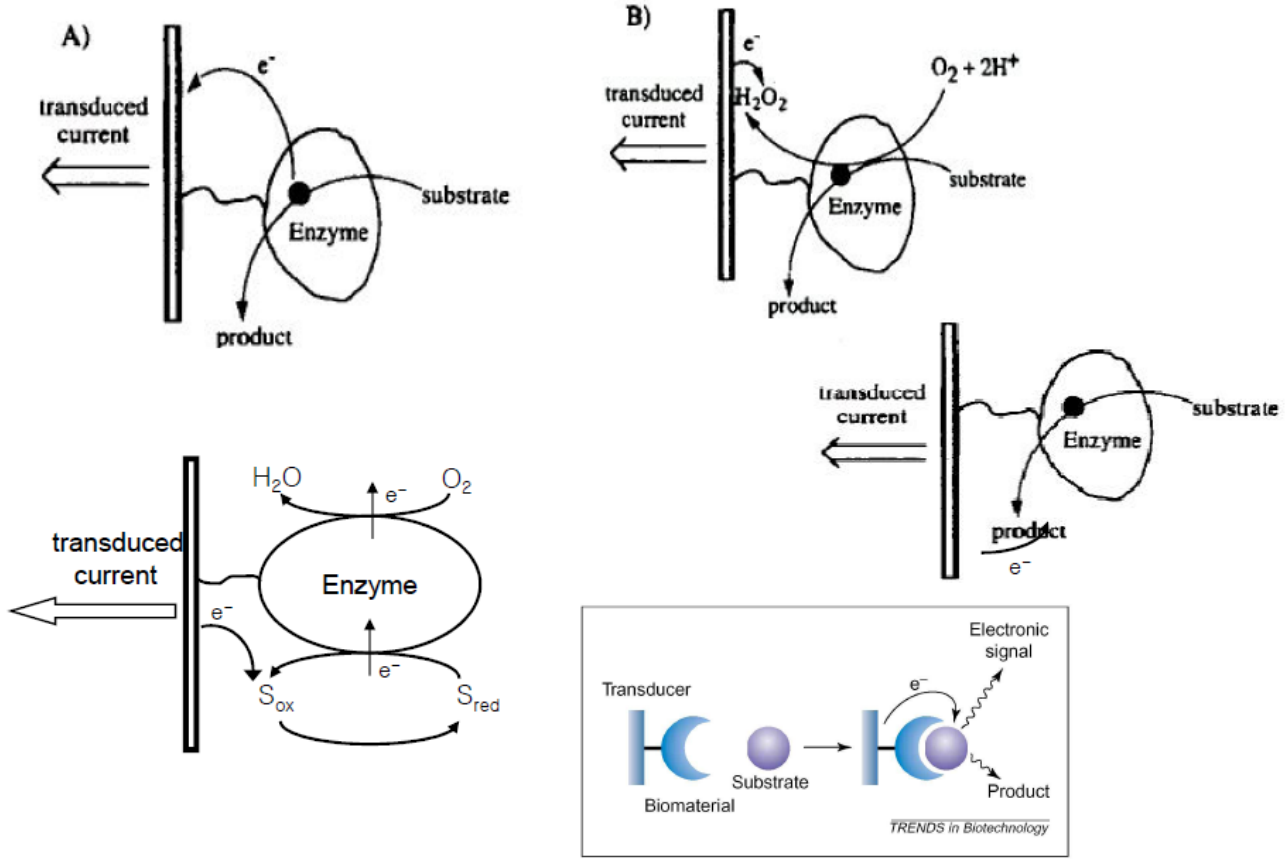


5. Immobilization into a redox polymer



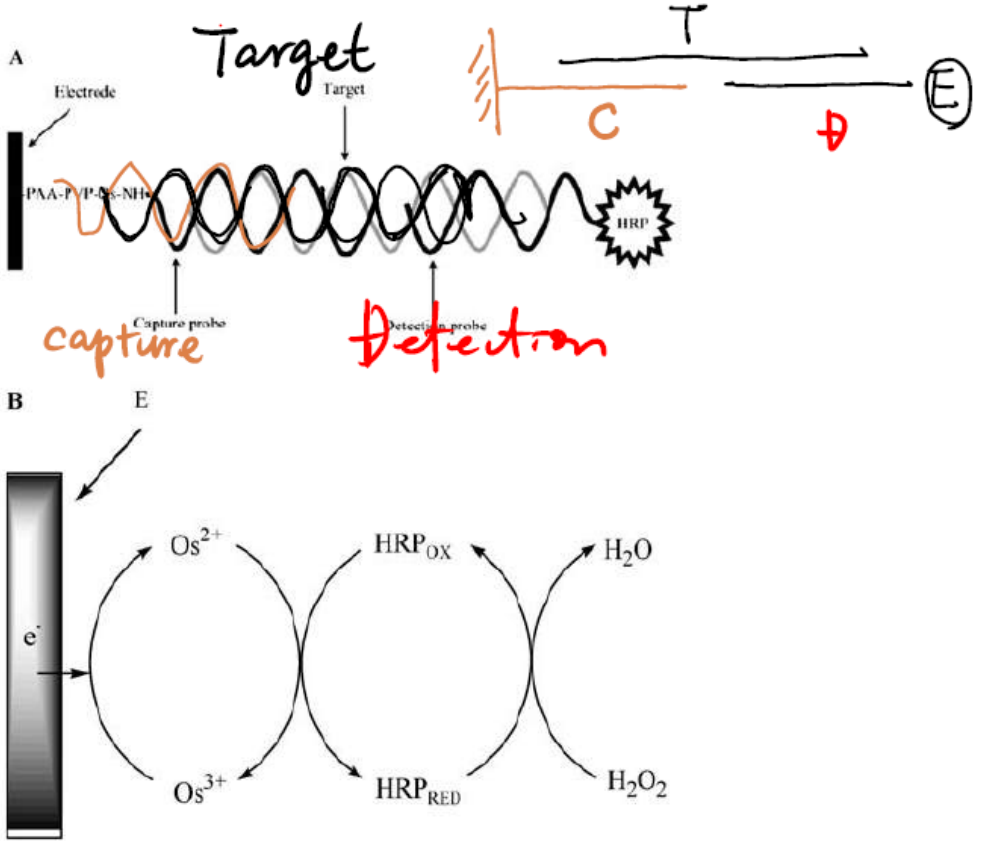
Modes of Electrochemical Detection by Biosensor

I. Willner et al, *Angew. Chem. Int. Ed.*, 2000, *39*, 1180-1218.

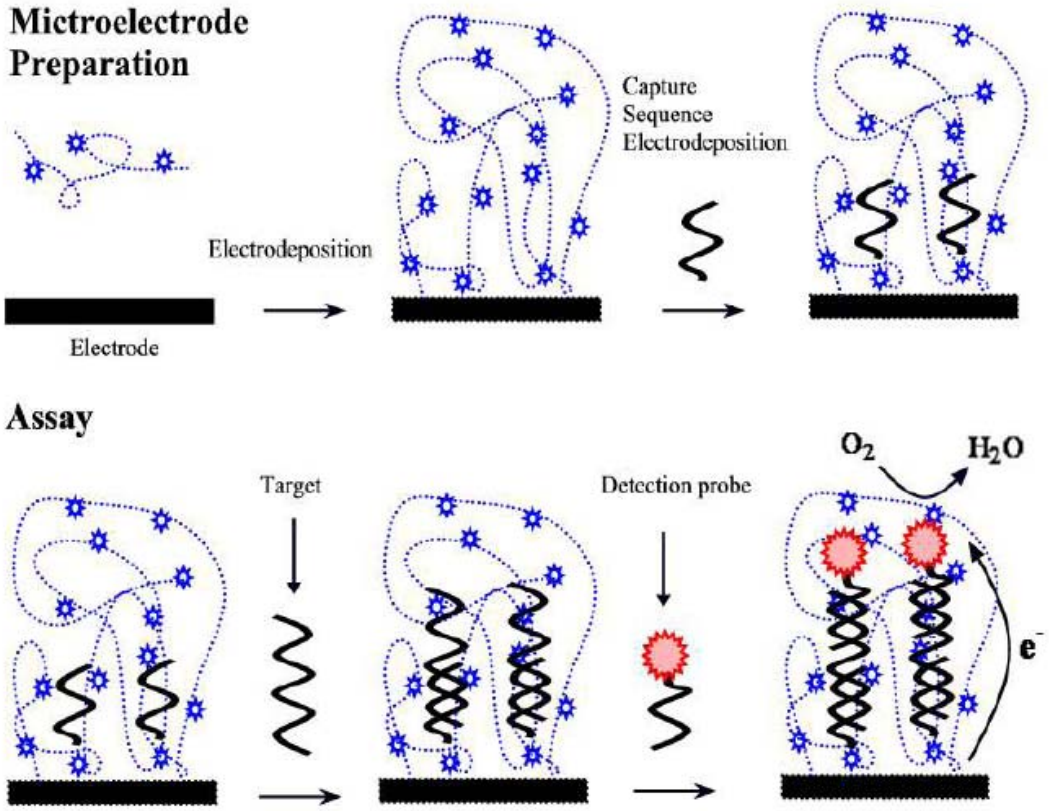


Enzyme-Amplified Amperometric Sandwich-type Detection of DNA

Caruana & Heller *J. Am. Chem. Soc.*, 1999, 769.
Zhang, Kim, Mano, Dequaire, Heller, *Anal. Bioanal. Chem.* 2002, 1050.
Zhang, Kim & Heller *Anal. Chem.* 2003, 75, 3267.

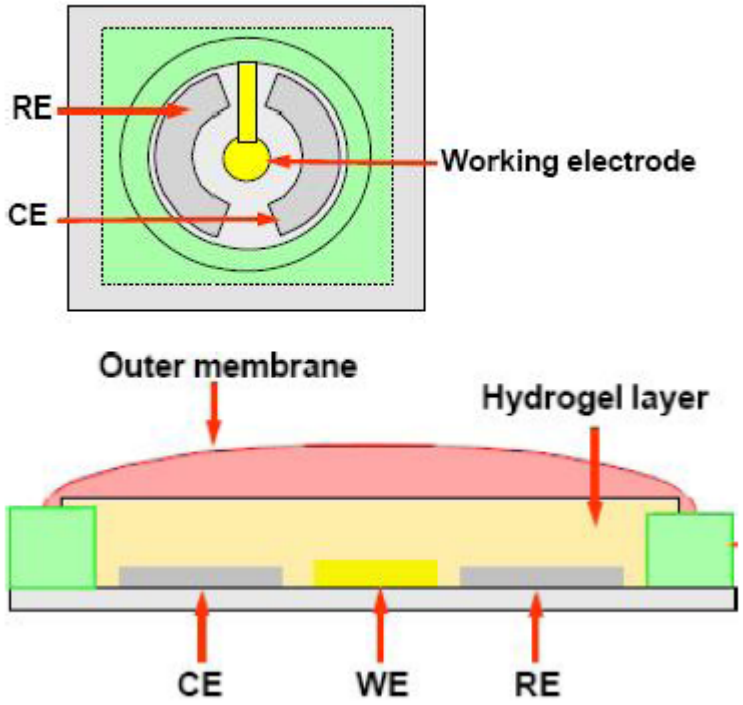


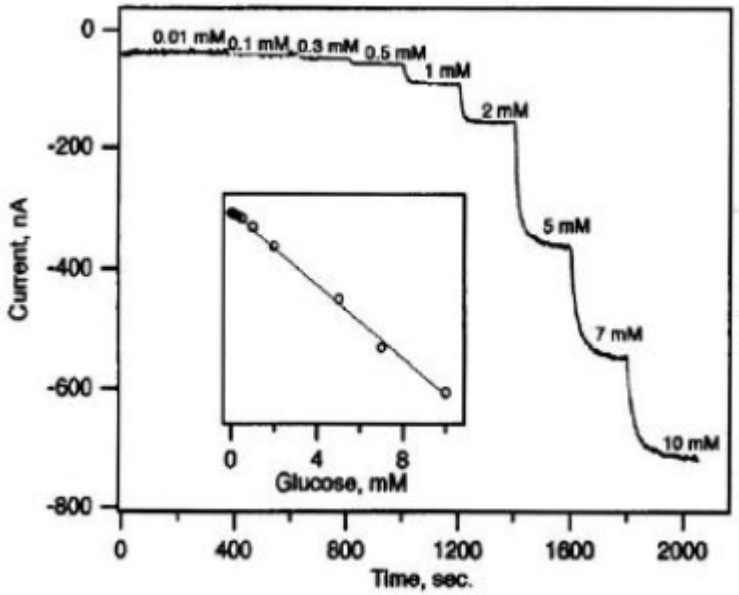
Detection of ~ 1,000 copies of DNA by an electrochemical enzyme-amplified sandwich-assay with ambient O_2 as the substrate



Zhang, Y.; Pothukuchy, A.; Shin, W.; Kim, Y.; A. J.; Heller, A. *Anal. Chem.* 2004. *76,* 4093.

Electron transport across the hydrophilic polymer on electrode surface





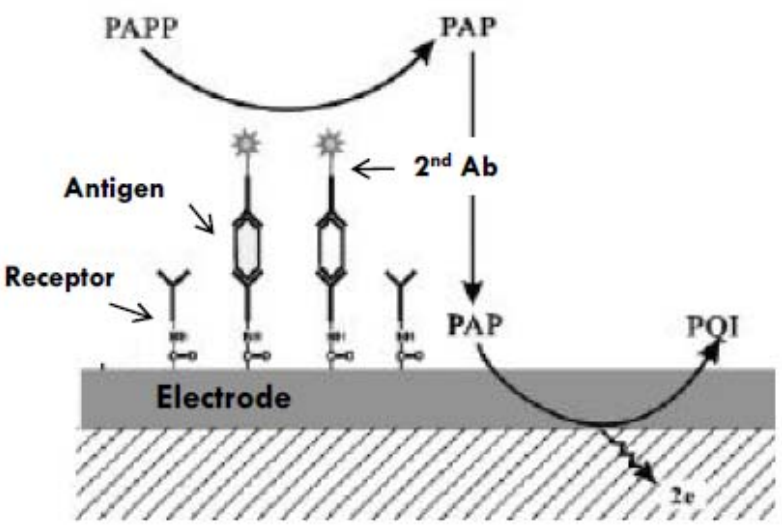
➢ glucose oxidase (GOD)를 hydrogel을 이용하여 전극칩 위에 고정화시키고 그 위에 ion selective membrane을 덮는다.

➢ glucose는 GOD에 의해 gluconic acid로 산화되면서 H_2O_2를 만들어내며 H_2O_2는 전극에 전자를 주면서 다시 O_2로 바뀐다.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

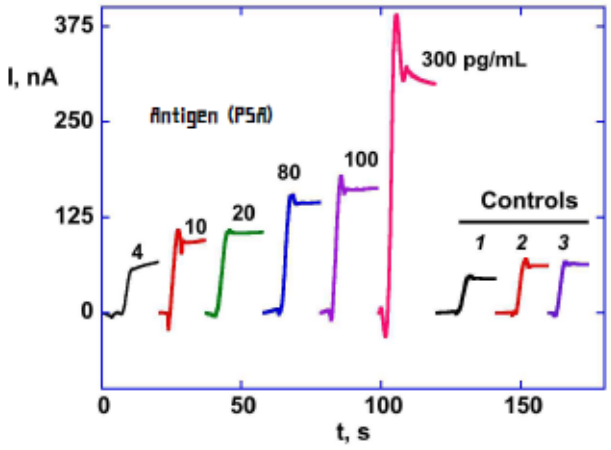
$$H_2O_2 \xrightarrow{\text{Electrode}} 2H^+ + O_2 + 2e^-$$

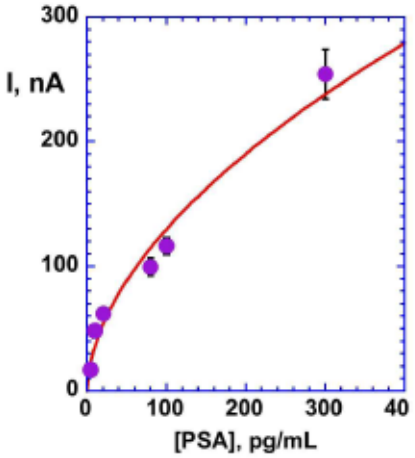
Amperometric analysis by immuno sandwich assay



Sandwich assay: the 2ndary Ab is linked to alkaline phosphatase (ALP) and binds to antigen bound to primary antibody

ALP is a mediates converstion of p-aminophenyl phosphate (PAPP) by reducing the p-aminophenol (PAP) PAP reacts with conducting polymer and reduced to paminoquinone (PQI) at the electrode, creating a e^- passage that transforms into current change.





The conc. of analyte present in the sample (PSA, prostate cancer specific antigen) is proportional to the increased current change

The biosensor not only detect the analyte but can also be quantitate.

AMPEROMETRIC BIOSENSORS

- Various approaches have been taken to increase the selectivity of the detecting electrode by chemically modifying it by the use of:
  - membranes
  - mediators
  - metallised electrodes
  - polymers

1. Membranes.

- Various permselective membranes have been developed which controlled species reaching the electrode on the basis of charge and size.
- Examples include cellulose acetate (charge and size), Nafion (charge) and polycarbonate (size).
- The disadvantage of using membranes is, however, their effect on diffusion.

AMPEROMETRIC BIOSENSORS

✦**Response Time**:

$$t = \frac{b^2}{D_m}$$

b=membrane thickness

Dm= oxygen diffusion coeff.

Oxygen Electrode—90% after 20s
99% after 40-50s

Bioprobe—1-3 minutes.

AMPEROMETRIC BIOSENSORS

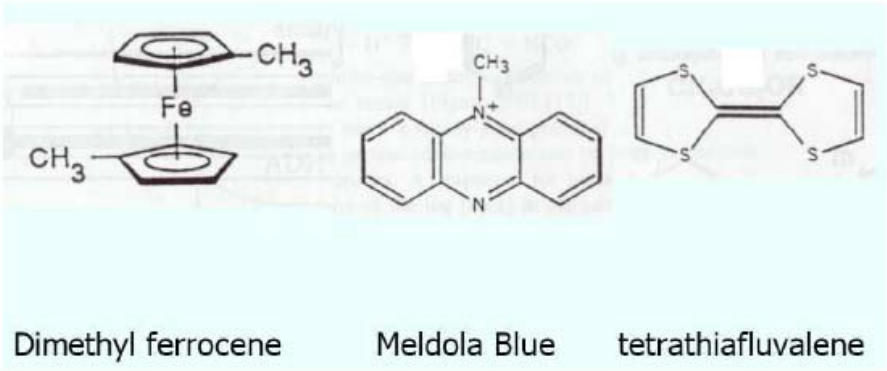
2. Mediators

- Many oxidase enzymes can utilise artificial electron acceptor molecules, called mediators.
- A mediator is a low molecular weight redox couple which can transfer electrons from the active site of the enzyme to the surface of the electrode, thereby establishing electrical contact between the two.

-These mediators have a wide range of structures and hence properties, including a range of redox potentials. A variety of oxidase and dehydrogenase enzymes with low molecular weight mediaters (Med) used for Amperometry:

- Mediators reoxidized at a working electrode yielding measurable currents
- Examples of mediators commonly used are:

Ferrocene (insoluble) and its derivatives
Ferrocene dicarboxylic acid (soluble)
Dichloro-indophenol (DCIP)
Tetramethylphenylenediamine (TMPD)
Ferricyanide
Ruthenium chloride
Methylene Blue (MB)
quinnone and its derivatives



Dimethyl ferrocene Meldola Blue tetrathiafluvalene

AMPEROMETRIC BIOSENSORS

4. Polymers:

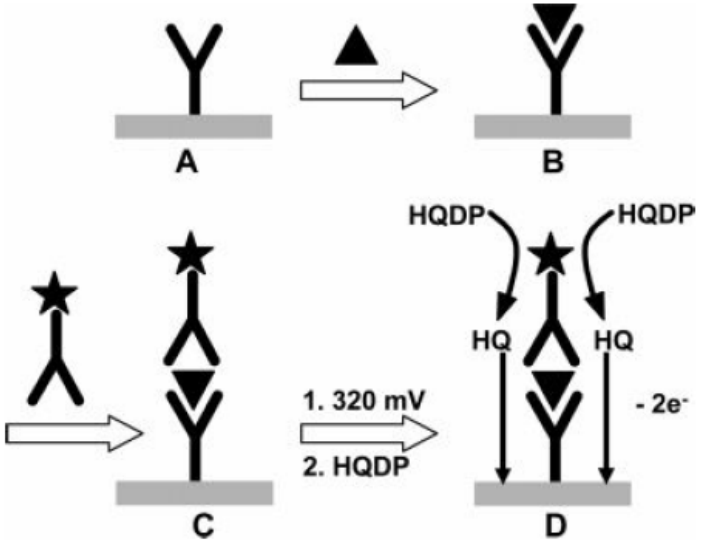
- As with membranes, **polymers are used to prevent interfering species from reaching the electrode surface.** Polymers differentiate on the basis of size and charge.

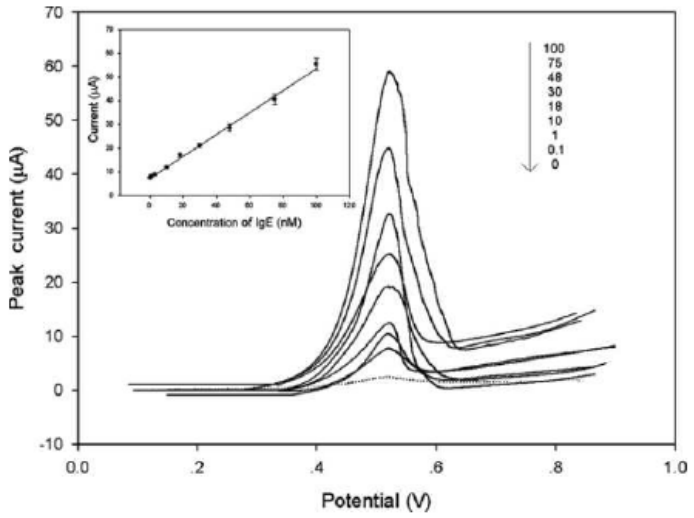
-An example is that of polypyrrole. A polypyrrole film has to be in the reduced state to become permeable for anions. If the film is oxidised, no anion can permeate.

- Examples of commonly used polymers are:

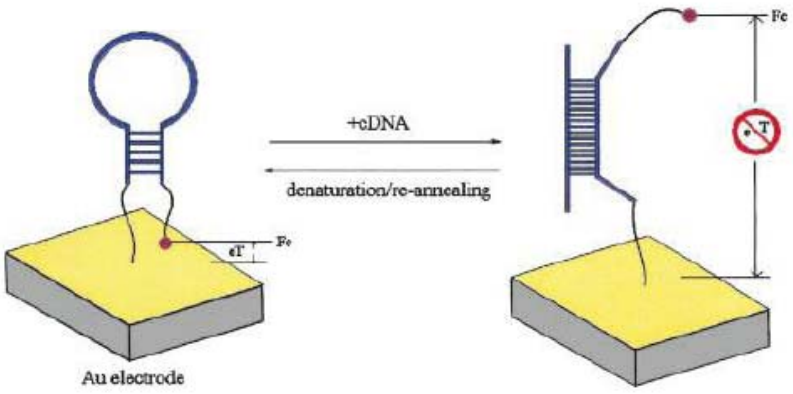
polypyrrole
polythiophene
polyaniline
diaminobenzene
polyphenol

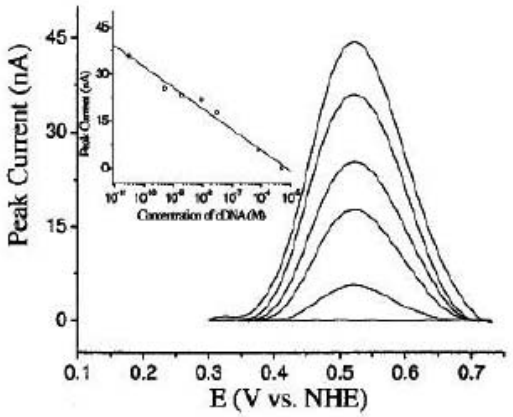
Voltammetric analysis for immunoassays





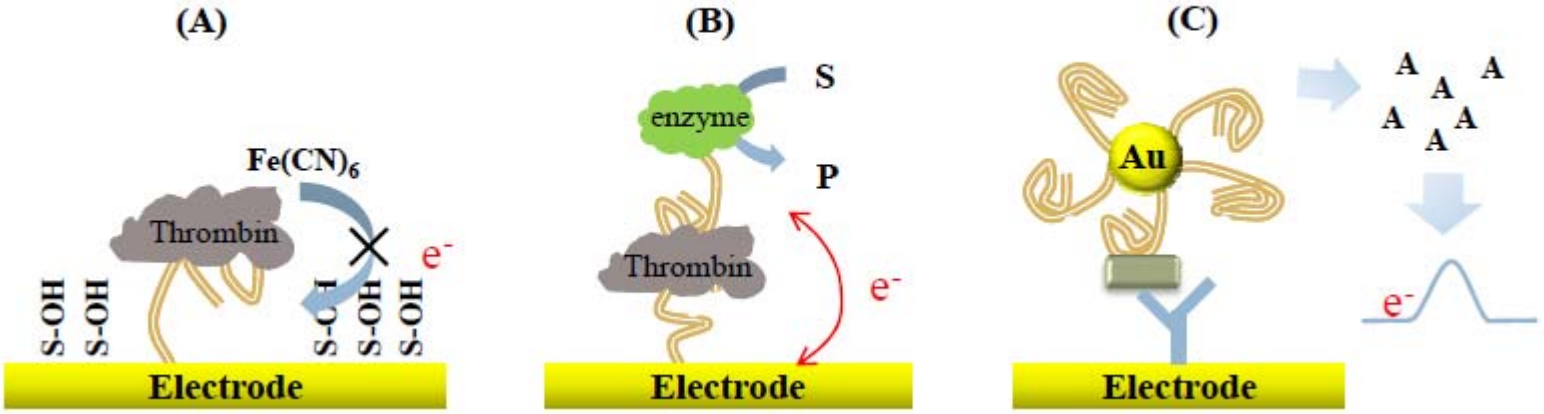
Voltammetric analysis for DNA detection





Signal Amplication

Nanoparticle or Enzyme-based EC signal Amplification



Change in current = voltammetry
Change in resistance = impedance analysis

Oxidation/Reduction reaction by reporter enzyme by sandwich binding

Nanoparticle mediated enhancement of signal

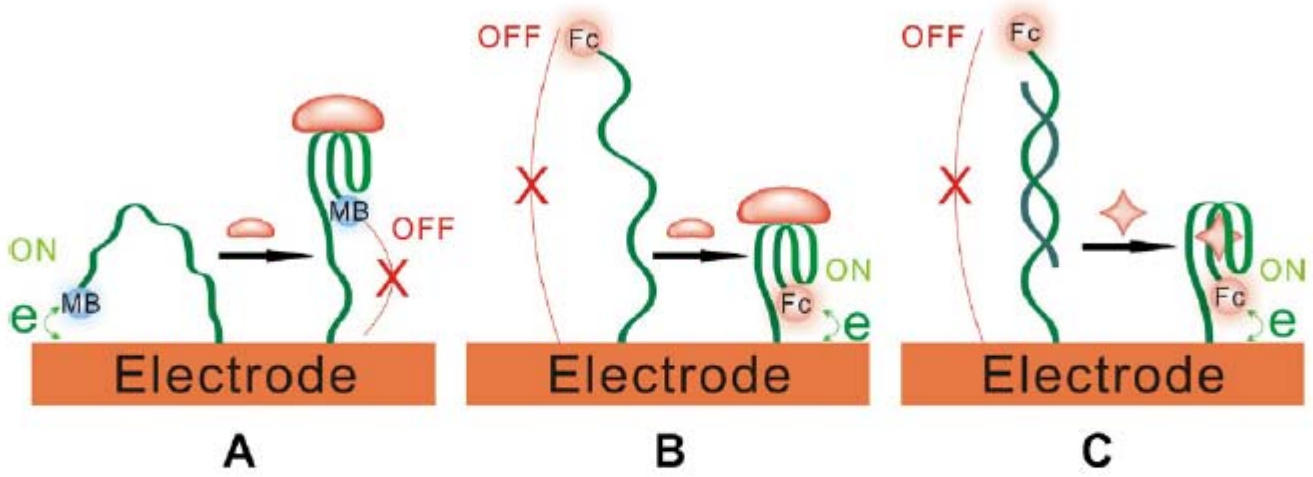


Figure 3. Schemes for "signal-off" and "signal-on" electrochemical sensors. (A) After binding to thrombin, the aptamer probe self-assembles into a G-quadruplex structure and shields MB from electron-transfer communication with the electrode, leading to a negative signal [25]; (B) formation of a complex of thrombin and the aptamers makes the G-quadruplex configuration rigid and results in the orientation of the ferrocene units in the proximity of the electrode, leading to easy electron transfer between the electro-active ferrocene units and the electrode and producing a positive signal [26–28]; and, (C) the presence of ATP unties the rigid DNA duplex and liberates the complementary sequence, while making the aptamer sequence form a rigid 3D structure. This brings the ferrocene tag to the proximity of the electrode surface and turns on electron transfer, producing positive signals [32].

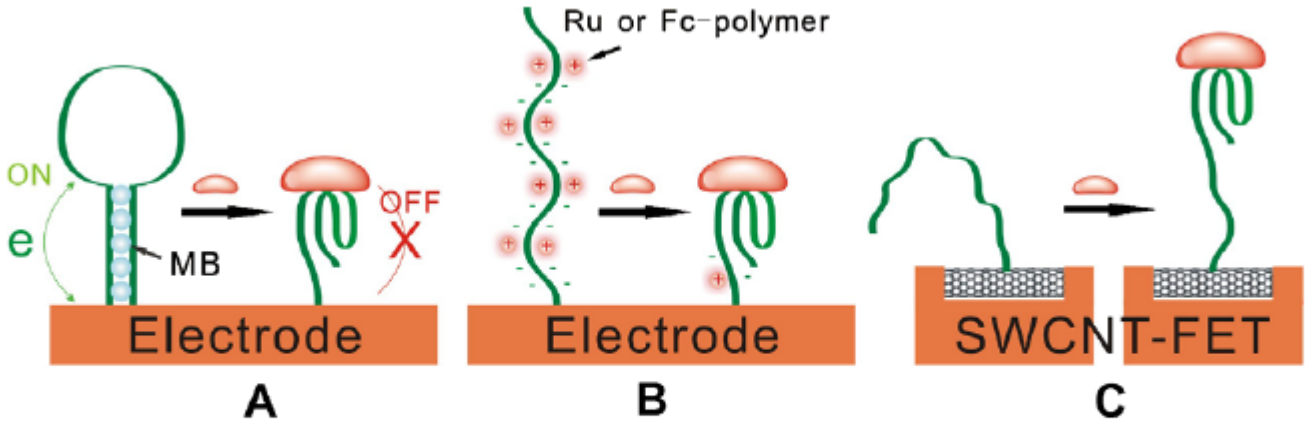


Figure 4. Label-free electrochemical aptasensors. (A) Binding the target with the aptamer opens the hairpin structure, thus releasing the intercalated MB and decreasing electronic signals [33]; (B) binding targets with their aptamers blocked the binding of the cationic reporting units and depleted their electrochemical response [34,35]; and, (C) in the SWCNT-FET sensor, binding target with the aptamer altered the conductance through the device, thus enabling the detection of targets [36,37].

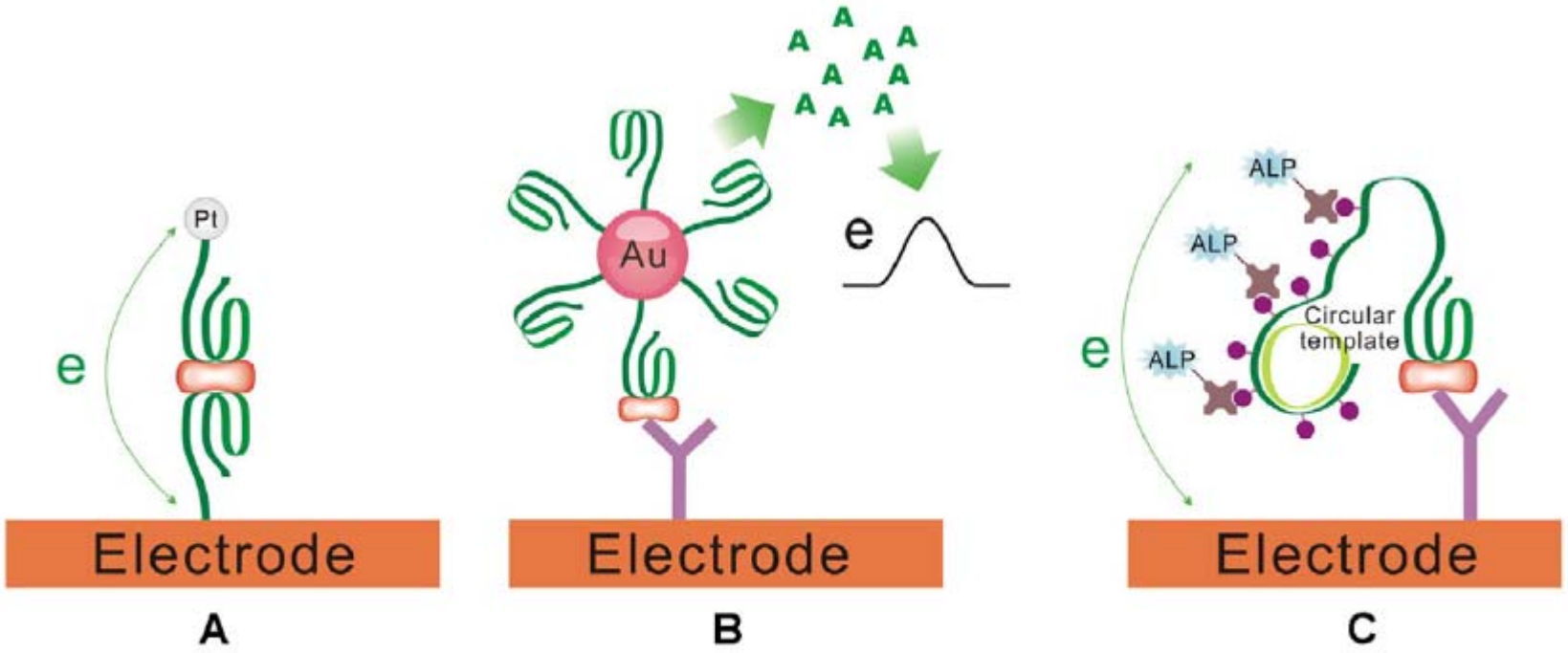


Figure 5. ''Sandwich''-type aptamer-based electrochemical sensors with signal amplification. (A) Aptamer-functionalized PtNPs were employed as catalytic labels to catalyze the electrochemical reduction of H_2O_2 and enabled the amplified detection of targets [39]; (B) AuNPs functionalized with aptamers containing poly-A were used as reporting probes. The adenine nucleobases released from them were directly detected to produce amplified signals [40]; and, (C) acting as the reporter, the aptamer-primer sequence mediated an in-situ RCA reaction, leading to significant enhancement in detection sensitivity [41].

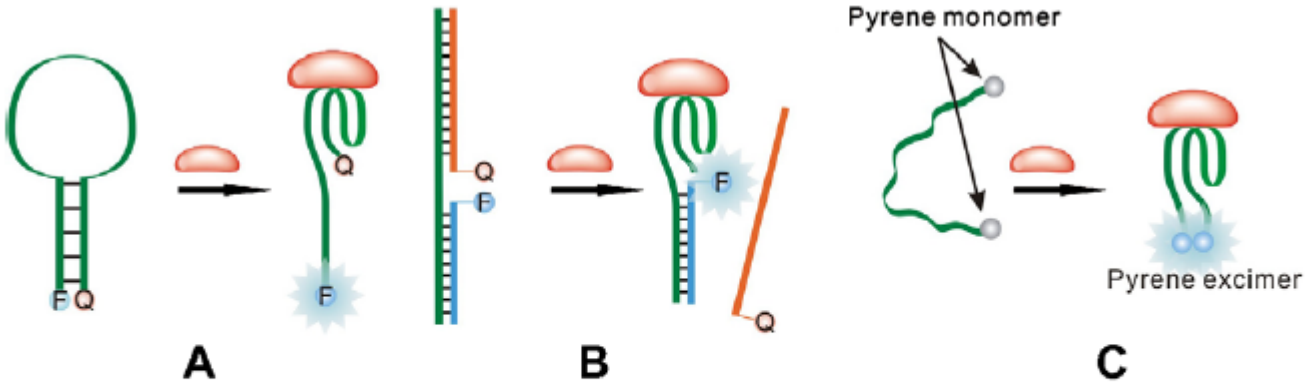


Figure 6. Aptabeacons. (A) An aptamer sequence in a molecular beacon-like hairpin structure was end-labeled with a fluorophore (F) and a quencher (Q). The binding of the target disrupted the stem, separating the F from the Q and leading to fluorescent signals [42]; (B) a fluorophore-labeled aptamer in a duplex structure with a complementary DNA sequence labeled with a quencher was separated after the binding of the target and the aptamer, leading to an increase in fluorescence [43,44]; and, (C) an aptamer labeled with one pyrene at each end switched its fluorescence emission and produced stable signals upon target binding [48].

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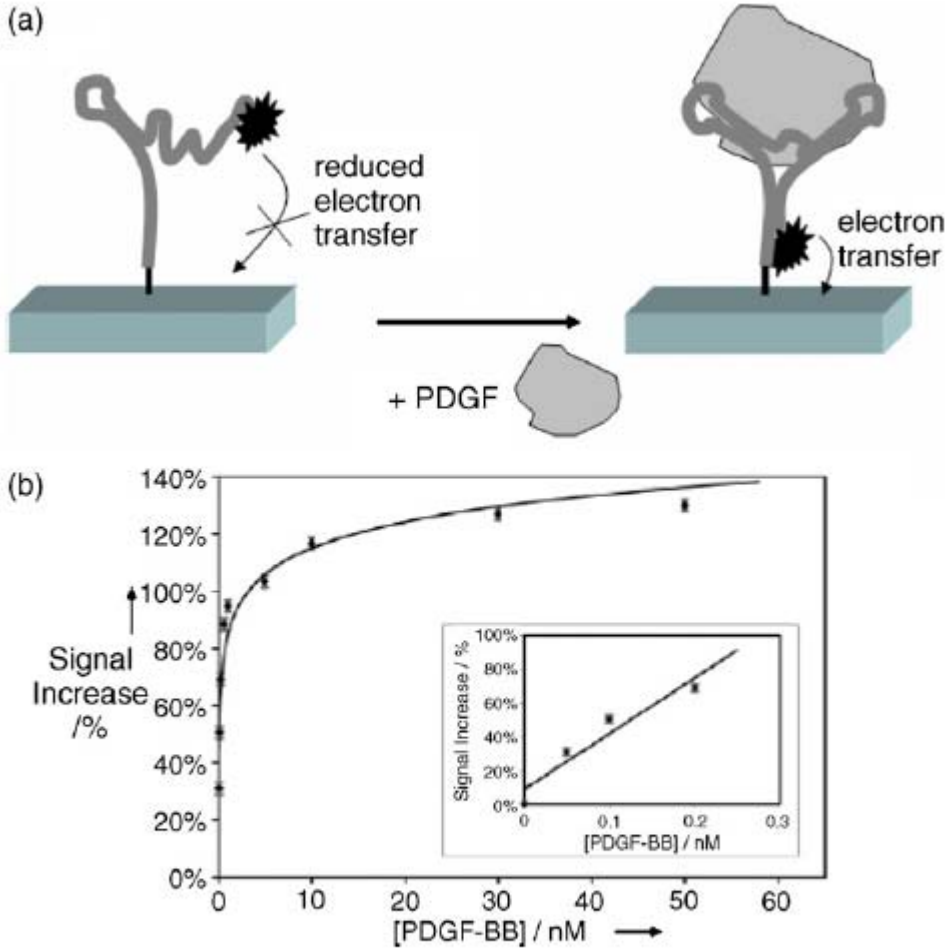

Fig. 6. (a) Scheme of the aptamer-based electrochemical detection of PDGF. (b) Dose–response curve of the sensor for increasing concentrations of PDGF (adapted from Lai et al., 2007, with permission).

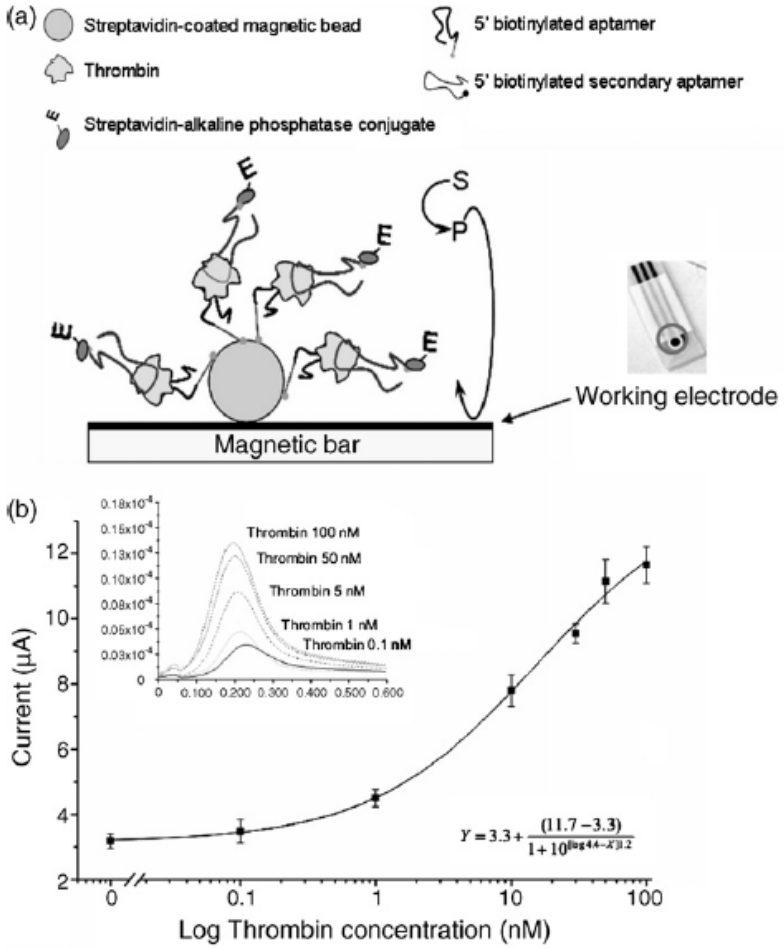

Fig. 8. (a) Scheme of the electrochemical assay for thrombin coupled to magnetic beads. (b) Dose–response curve for thrombin (adapted from Centi et al., 2007, with permission).

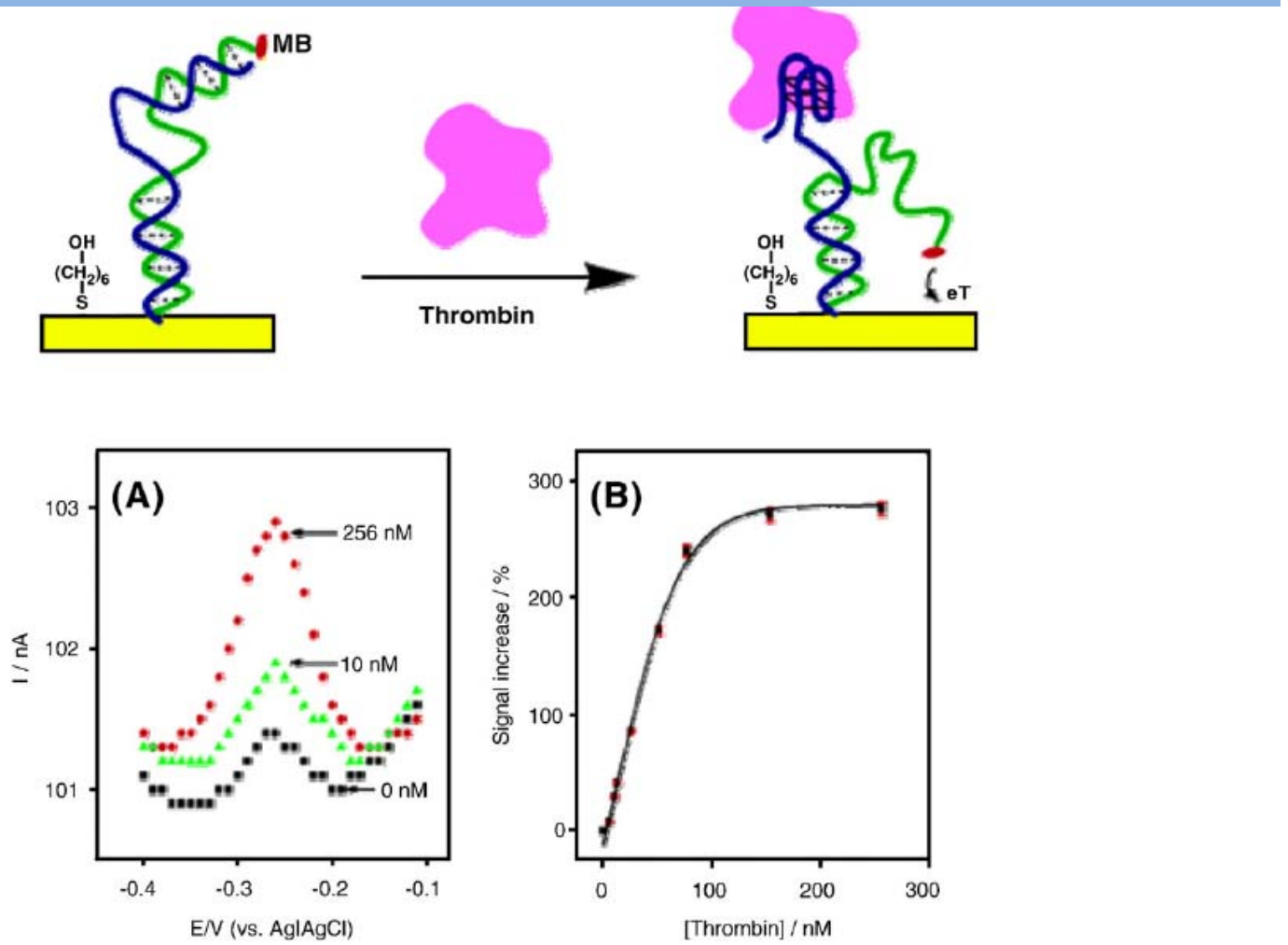

Fig. 3. Top: the proposed mechanism of the signal-on electronic, aptamer-based (E-AB) sensor. Bottom: (A) Alternating current voltammetric (ACV) responses of the E-AB sensor-functionalized gold electrodes in 20 mM Tris–HCl, pH 7.4 with 140 mM NaCl, 20 mM MgCl, and 20 mM KCl at various thrombin concentrations. (B) The signal decrease of the E-AB sensor is presented as a function of the thrombin concentration. Adapted with permission from Ref. [28].

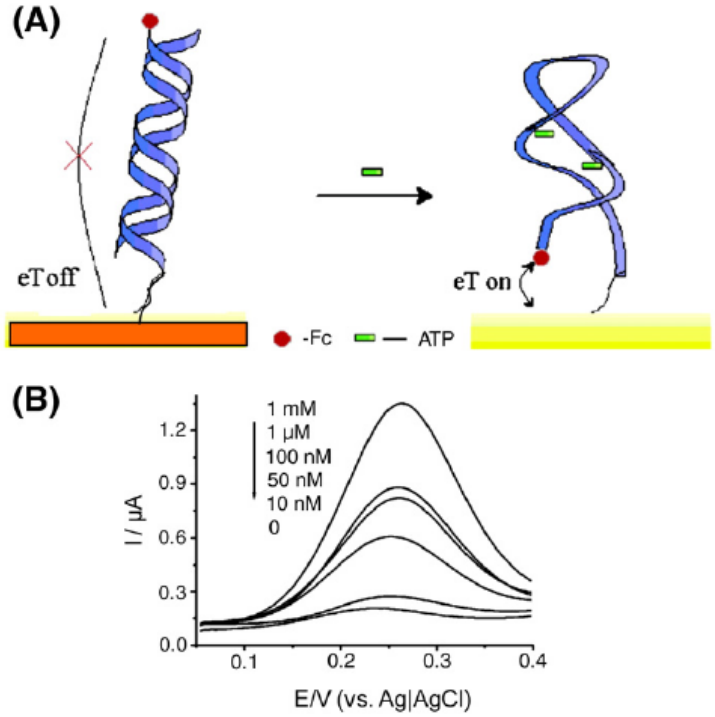

Fig. 4. (A) Schematic representation of the TREAS (target-responsive electrochemical aptamer switch) strategy for ATP detection based on the alteration in the ferrocene electrochemistry. (B) Representative square wave voltammograms for the duplex modified electrode after reaction with various concentrations of ATP, ranging from 10 nM to 1 mM, in 10 mM HEPES containing 50 mM $NaClO_4$. Adapted with permission from Ref. [29].

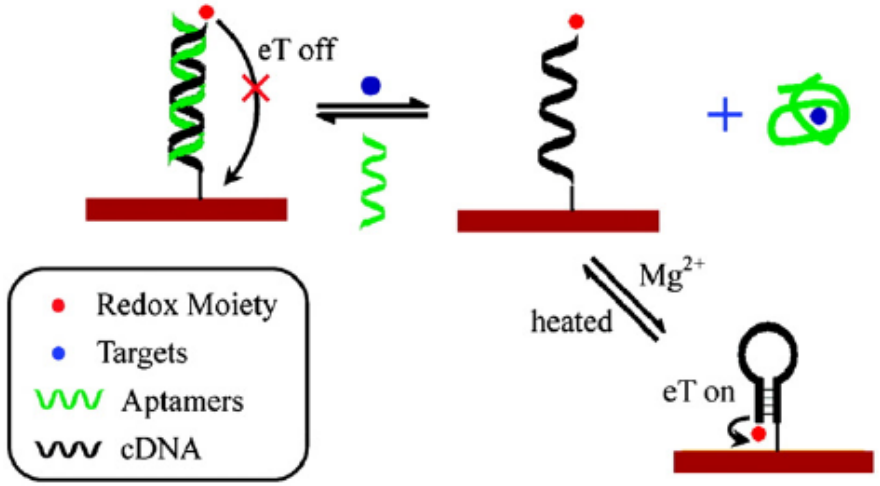

Fig. 5. Schematic illustration of the strategy for an electrochemical "aptasensor" with aptamer–complementary DNA (cDNA) constructs as the probes; this is based on the formation of a hairpin structure of cDNA probes through the hybridization of the tailor-made complementary sequences at their both ends caused by the target binding-induced displacement of the aptamer strands. Adapted with permission from Ref. [30].

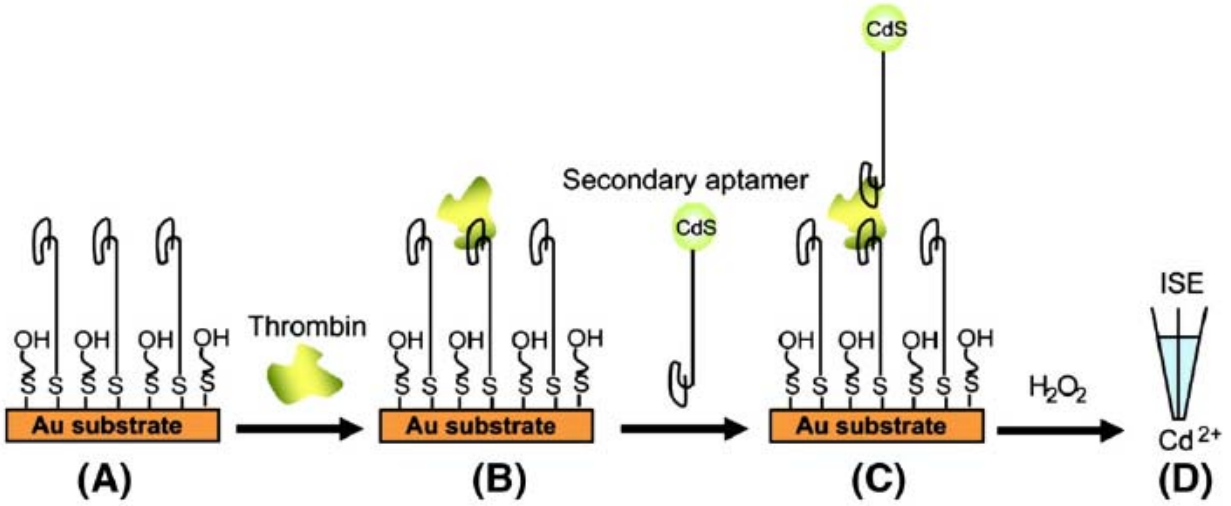

Fig. 8. Representation of the analytical protocol of the aptamer-based potentiometric measurements of proteins using ion-selective microelectrodes. (A) Formation of a mixed monolayer of thiolated aptamers on gold substrate; (B) thrombin addition and binding with aptamers; (C) binding with CdS-labeled secondary aptamer; (D) dissolution of CdS labels followed by detection using a solid-contact Cd^{2+}-selective microelectrode. Reproduced with permission from Ref. [33].

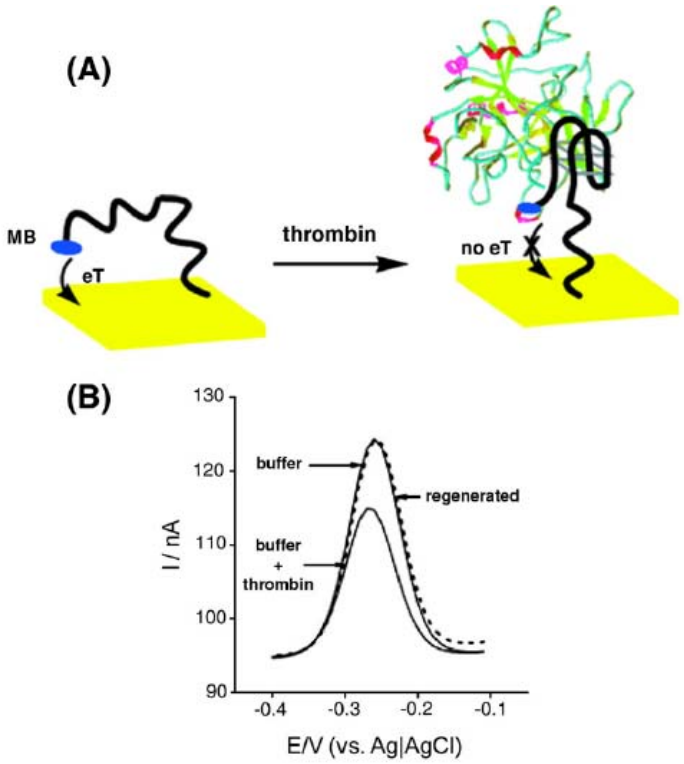

Fig. 10. (A) A schematic view of the E-AB sensor based on the change in electron transfer efficiency of MB upon the binding of analyte to the aptamer. (B) The response of the E-AB sensor to 64 nM thrombin in buffered saline and its regeneration (by an eight-minute, room-temperature wash with 6 M guanidine hydrochloride). Adapted with permission from Ref. [39].

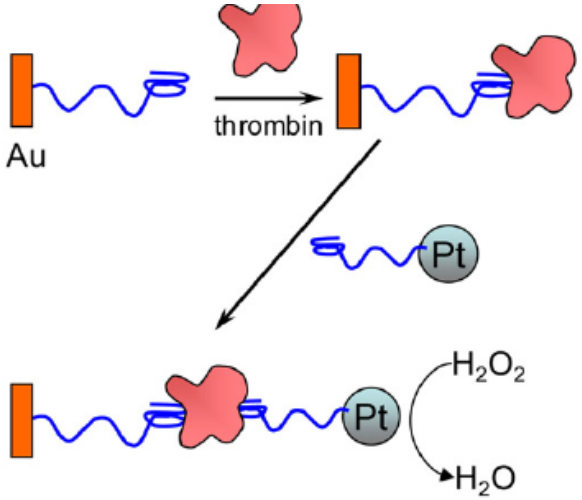

Fig. 9. Schematic representation of the procedure for using Pt nanoparticles in the aptamer-based electrocatalytic detection of thrombin. Adapted with permission from Ref. [34].

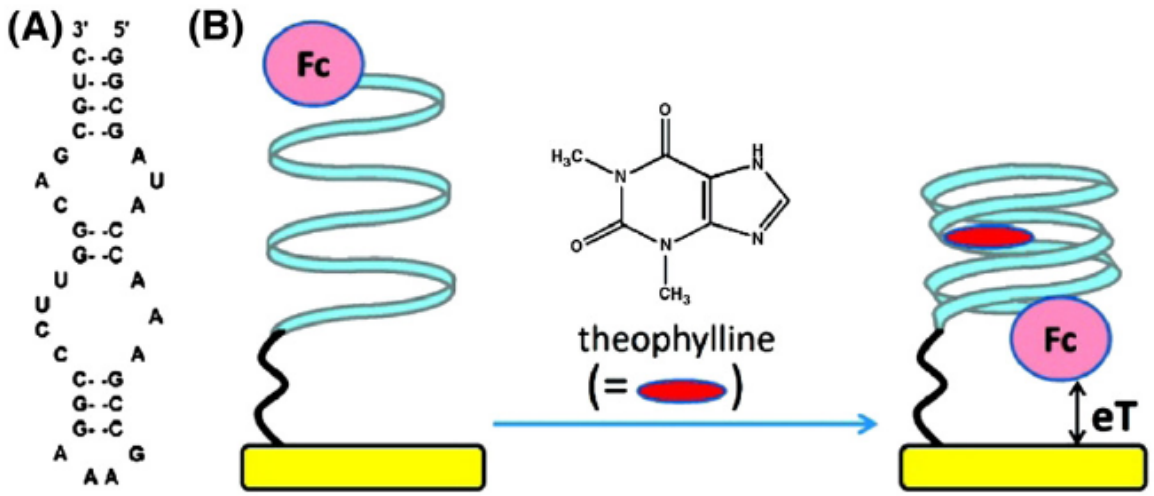

Fig. 11. (A) Theophylline-binding RNA aptamer sequence and (B) schematic representation of the electrochemical RNA aptamer-based sensor for theophylline (Fc = ferrocene). Reproduced with permission from Ref. [43].

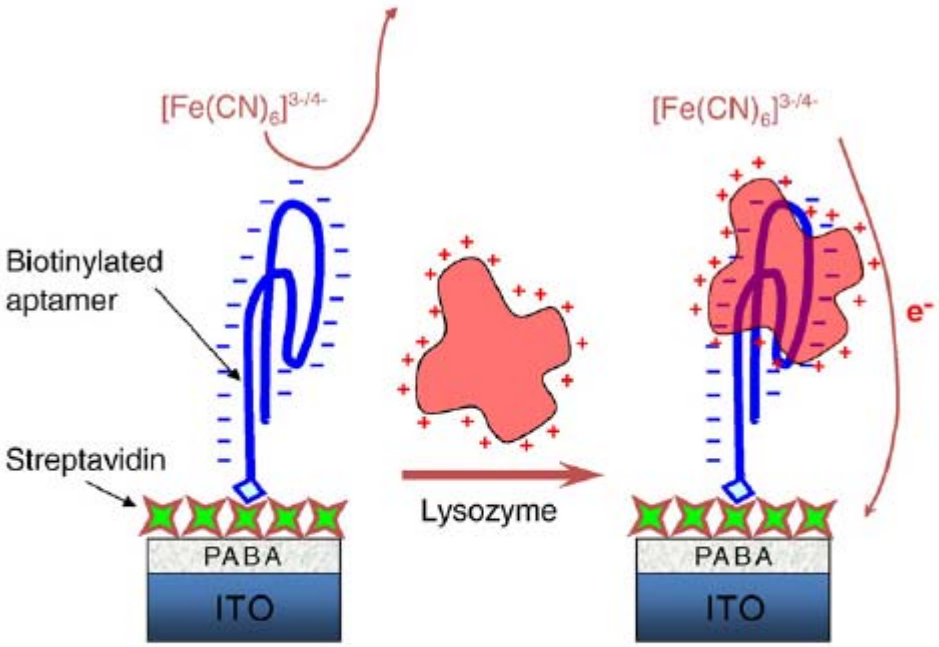

Fig. 12. A schematic representation of an anti-lysozyme biosensor on an ITO (indium tin oxide) surface based on analyte-induced surface charge density changes with freely-diffusive redox markers. In the presence of lysozyme, a positively-charged protein, electron transfer from solution-based $[Fe(CN)_6]^{3-/4-}$ is facilitated, resulting in a decrease in electron transfer resistance. Reproduced and modified with permission from Ref. [44].

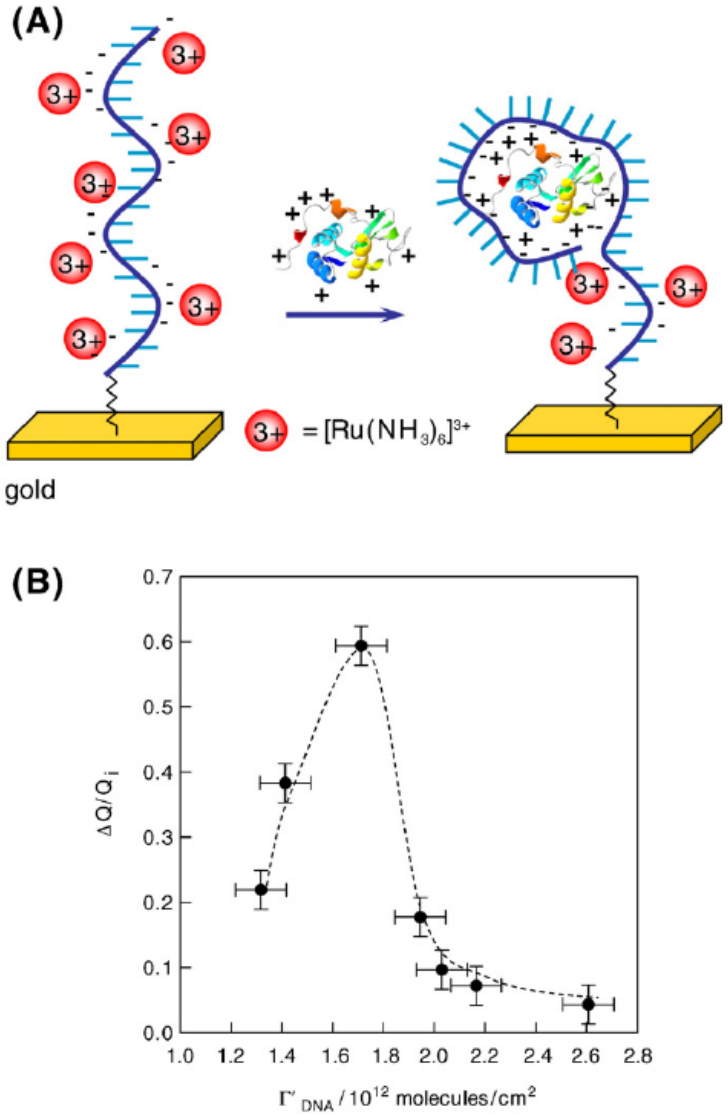

Fig. 13. (A) A schematic representation of a label-free biosensor based on analyte (lysozyme)-induced release of electrostatically-bound $[Ru(NH_3)_6]^{3+}$ cations. (B) The sensor signal ($\Delta Q/Q_i$) is presented as a function of the surface density of the aptamer strands (Γ'_{DNA}); the dotted line serves to guide the eye. Reproduced with permission from Ref. [50].

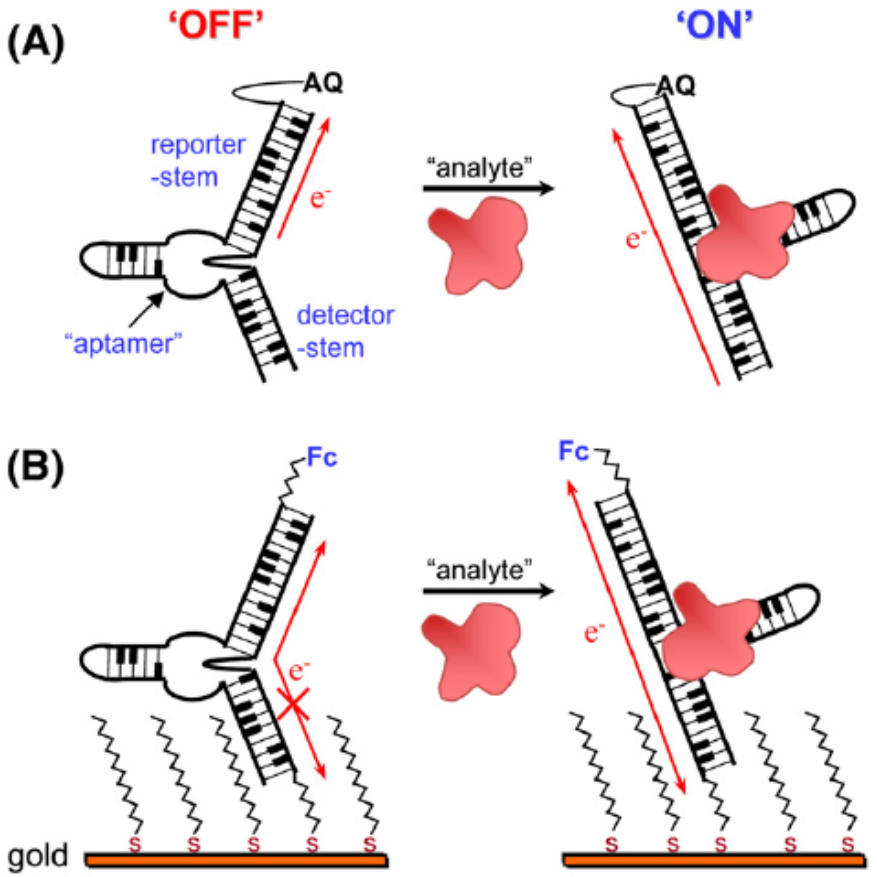

Fig. 15. Design of DNA conformational switches as electronic sensors (deoxyribosensors) for specific detection of molecular analytes, and illustration of the conduction path change upon analyte binding. (A) Biochemical mode of ligand/analyte detection. (B) Modification and immobilization of the deoxyribosensor constructs onto a gold chip for direct electronic detection of analyte. Reproduced with permission from Ref. [67].

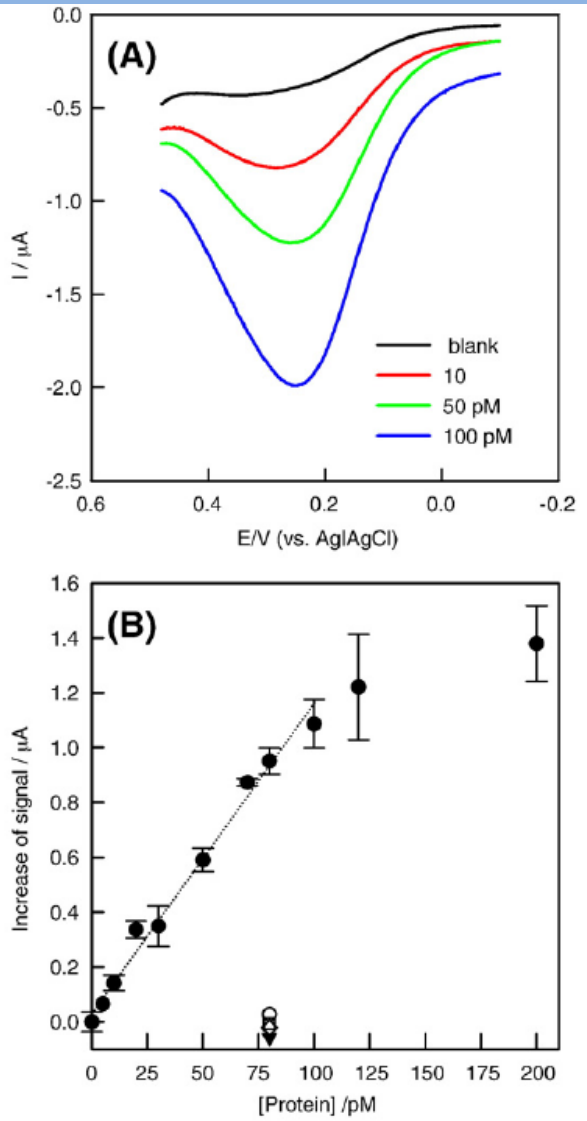

Fig. 16. (A) Square wave voltammetry (SQW) responses of deoxyribosensor-modified electrodes in the presence of different concentrations of thrombin. The experiments were carried out in 50 mM Tris, pH 7.4 and 50 mM NaCl. (B) The increase in the reduction current is presented as a function of the thrombin concentration; the sensor response in the presence of 80 pM BSA (○), avidin (□), IgA (◊) or IgG (▼) is also shown in this plot. Reproduced with permission from Ref. [67].

Biocatalytic Recognition Element

- Based on a reaction catalysed by macromolucules
  - -present in their biological environment
  - -have been isolated/manufactured
  - -continuous consumption of substrate(s) is achieved by the immobilized biocatalyst into the sensor
  - -transient or steady-state responses are monitored by the integrated detector

-Three types of biocatalyst are commonly used:
  - -1. Enzyme (mono- or multi-enzyme), the most common and well developed recognition system
  - -2. Whole cells (microorganisms, such as bacteria, fungi, eukaryotic cells or yeast) or cell organelles or particles (mitochondria, cell walls)
  - -3. Tissue (plant or animal tissue slice).

Biocomplexing or bioaffinity recognition elements

- Based on interaction of the analyte with macromolecules or organized molecular assemblies
  - -isolated from their original biological environment or engineered
  - -Thus, an equilibrium is usually reached - no further net consumption of biocomplexing
  - - These equilibrium responses are monitored by the integrated detector

Antibody-antigen interaction- immunochemical reactions - immunosensors
  - -enzyme labels are frequently coupled to Ab or Ag, thus requiring additional chemical synthesis steps.
  - -As the binding or affinity constant is usually very large, such systems are either irreversible (single-use biosensors) or Ab may be regenerated by dissociation of complexes by chaotropic agens such as glycine-HCL buffer at pH 2.5.

Receptor antagonist or agonist

- Protein lactose permease incorporated into liposome bilayers thus allowing coupling of sugar proton transport with a stoichiometric ratio of 1:1 pH-probe pyranine entrapped in these liposomes (Kiefer et al., 1991)

-These lactose permeases containing liposomes have been incorporated within planar lipid bilayer coatings of an ISFET gate sensitive to pH

-Protein receptor based biosensors have been recently developed- the binding of the analyte, here named agonist, to immobilized channel receptor proteins, is monitored by changes in ion fluxes through these channels. Eg., glutamate, as target agonist, may be determined in the presence of various interfering agonists, by detecting Na+ or Ca2+ fluxes, using conductivity or ion selective electrodes.

-gene probes (detect binding of oligonuceotides, gene probes) - eg., a) intercalates into the oligonucleotide duplex, during the formation of a dsDNA on the probe surface, a molecule that is electroactive (b) directly detects guanine that is electroactive.

Biocomples-based biosensors although showing promising behaviour, have not yet reached the advanced development stage of the biocatalyst-based systems.