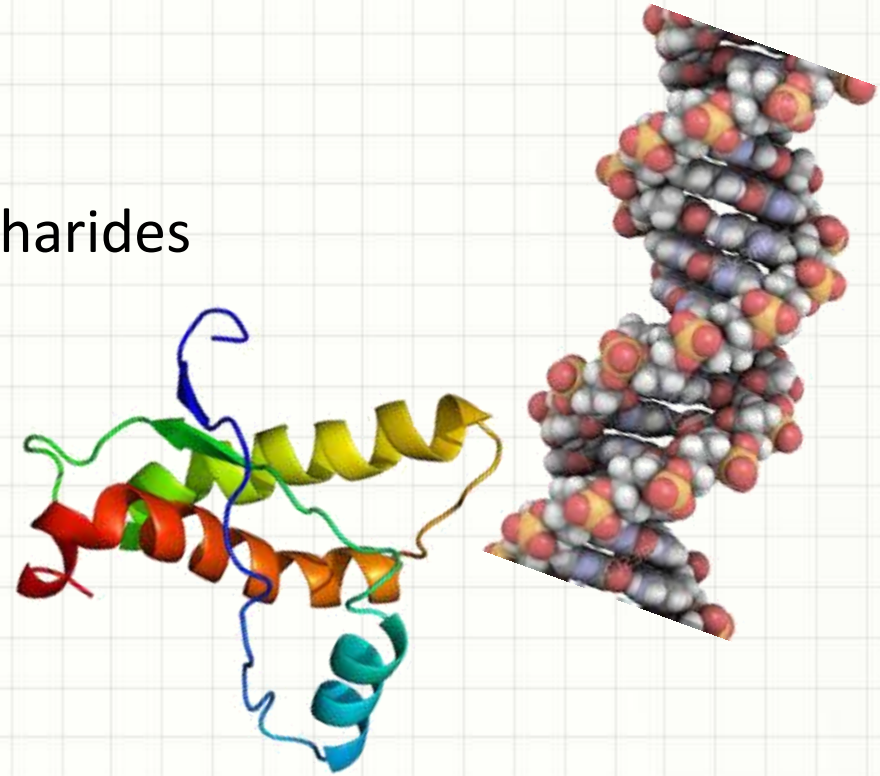
BIOELECTROCHEMISTRY

ELECTROCHEMICAL ANALYSIS OF NUCLEIC ACIDS, PROTEINS AND POLYSACCHARIDES IN BIOMEDICINE

Iveta Třísková

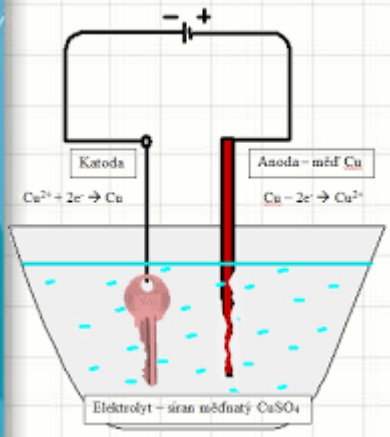
Outline

- Introduction to electrochemical methods
- Electrochemistry of nucleic acids and their components
- Electrochemistry of proteins
- Electrochemistry of polysaccharides
- Biosensors
- Nanoelectrochemistry



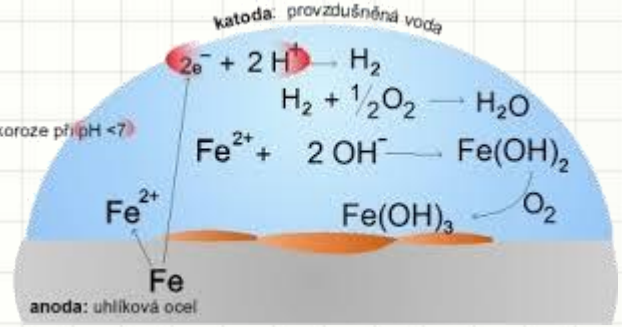
Electron transfer

Electrolysis

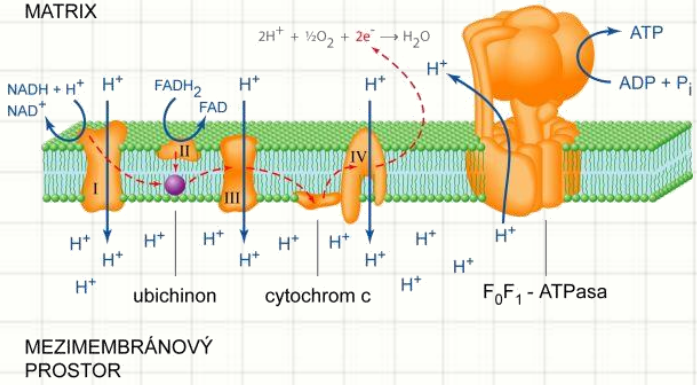


Battery

Redox reactions



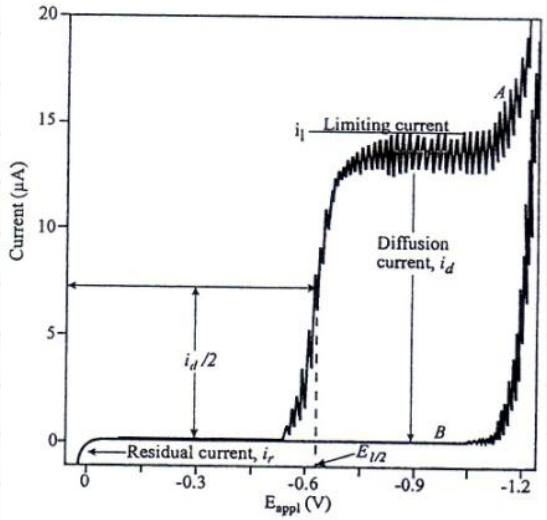
Electron transfer system

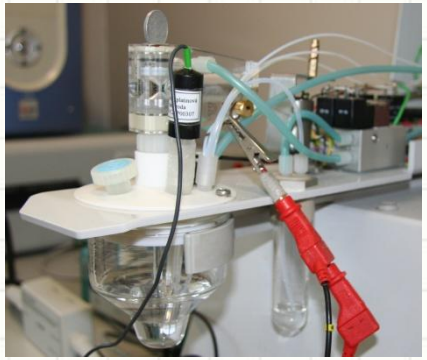


Introduction to electrochemical methods

Polarography

- **1922 – Jaroslav Heyrovský**
- Electrolysis of the electroactive compound in the supporting electrolyte
- The potential is insert between working (Hg) and reference electrode (Ag/AgCl/3M KCl)
- Polarographic wave
- Electrode polarization





Polarographic (voltammetric) currents

Charging current

- Important for electrode double layer charging
- Non-faradayic character

Migration current

- Associated with transport of electroactive compound to the electrode surface
- Eliminated with addition of big amount of supporting electrolyte

Diffusion current

- For reactions when the rate determinig step **rds** is diffusion

$$I_d = zF\frac{dn}{dt} = zFAD\frac{\partial c}{\partial x}$$

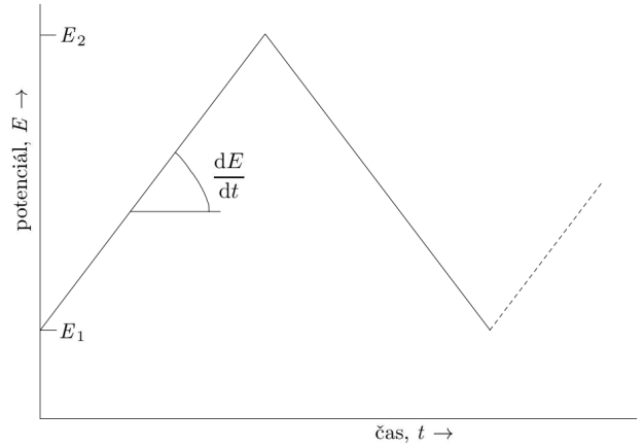
- **1934 – Dionýz Ilkovič**

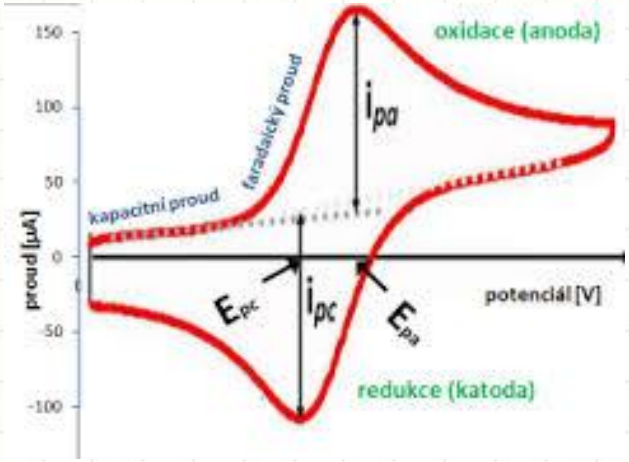
$$\overline{I_D} = kzFD^{1/2}m^{2/3}\tau^{1/6}c$$

Illkovič equation

Cyclic voltammetry and Linear sweep voltammetry

- Electrolysis of electroactive compounds in the supporting electrolyte
- Three electrode set
- *dE/dt* (scan rate)





- Voltammetric signals have a **peak shape**
- The study of redox reactions – mechanism of electrode reaction and its reversibility

Cyclic voltammetry

- **Reversible processes: Randles – Ševčík equation**

$$I_p = 2{,}69 \cdot 10^5 n^{3/2} A D^{1/2} c_{ox}{}^0 v^{1/2}$$

when I_p is peak current(A); n number of electrones; A effective area of electrode(cm^2); D is diffusion coeffient (cm^2/s); c_{ox} is concentration(mol/cm^3) a v is scan rate (V/s).

- **Irreversible processes: Delahay equation**

$$I_p = 2{,}99 \cdot 10^5 \cdot n \cdot (\alpha n_a)^{1/2} \cdot A \cdot D^{1/2} \cdot c_{ox}{}^0 \cdot v^{1/2}$$

kde α is charge transfer coefficient; n_a is number of electrones in rate determinig step(rds)

Elimination voltammetric procedure

- **Elimination voltammetric procedure (EVP)** – developed parralel with elimination polarography (EP), but compared to EP, EVP is easier, faster and it is possible to applicate it at solid electrodes

- **EVP** – mathematic procedure eliminating/conserving some of partial voltammetric currents (diffusion, charging, kinetic) from measured LSV or CV curves

- **Elimination function** as linear combination of total currents measured at different scan rates

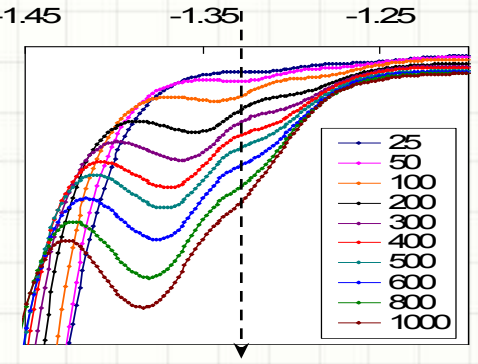
The two basic conditions of EVP

1st condition

$$I = \sum_{j=1}^{k} I_j \qquad I = I_d + I_k + I_c + \ldots\ldots$$

2nd condition

$$I_j = Y_j(E)\,W_j(v)$$



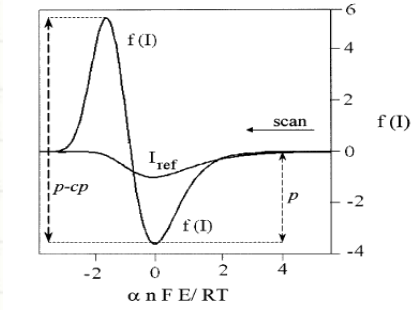
I_d …diffusion current $I_d = Y_d(E)\,v^{1/2}$

I_k …kinetic current $I_k = Y_k(E)\,v^{0}$

I_c …charging current $I_c = Y_c(E)\,v^{1}$

$$I = Y(E)\,v^{x} = const.\,v^{x}$$

$$I_{v/vref} = \left(\frac{v}{v_{ref}}\right)^{0} I_k + \left(\frac{v}{v_{ref}}\right)^{1} I_c + \left(\frac{v}{v_{ref}}\right)^{1/2} I_d$$



$$f(I) = aI_{v_{1/2\,ref}} + bI_{v_{ref}} + cI_{2ref}$$

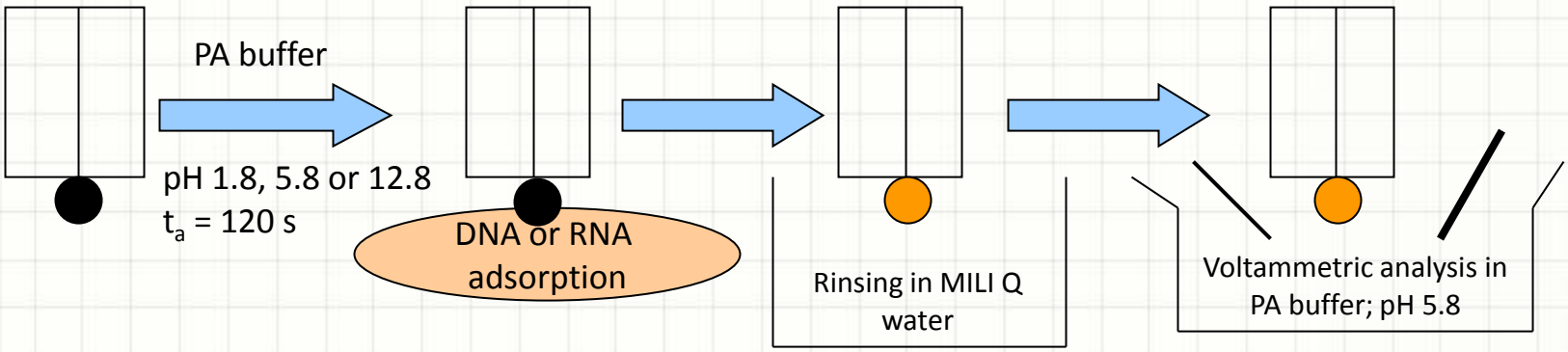
EVP E4

$I_k + I_c = 0$

$I_d \neq 0$

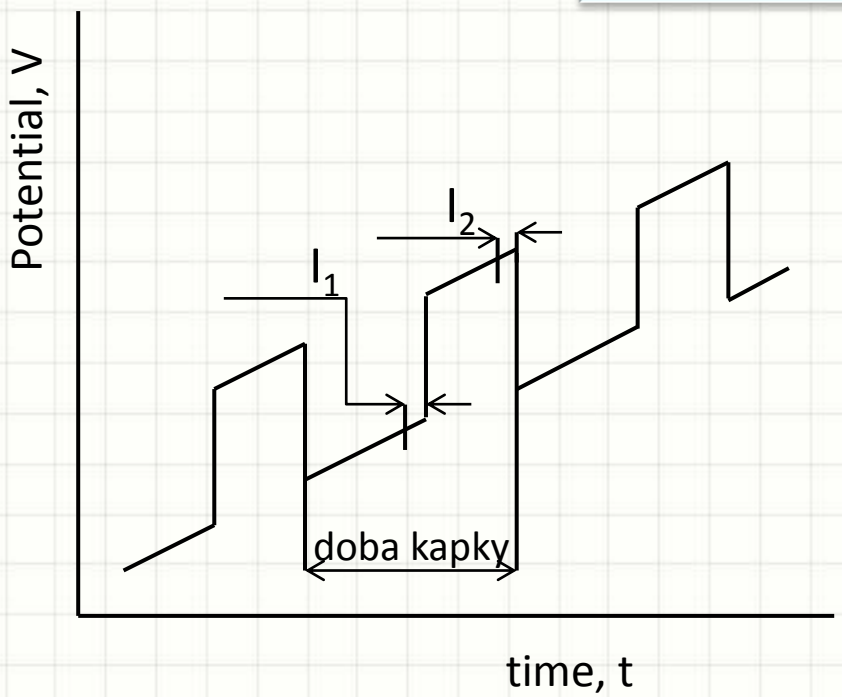
f (*I*) = - 11,657 $I_{1/2}$ + 17,485 *I* - 5,8284 I_2

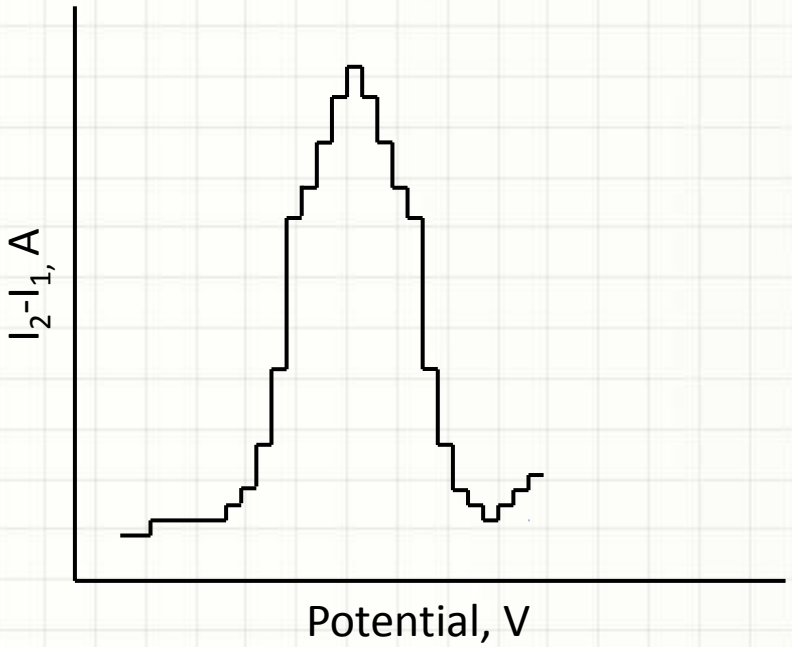
Adsorptive transfer stripping voltammetry (AdTSV)



Differential pulse voltammetry

$$\Delta I = I_2 - I_1$$

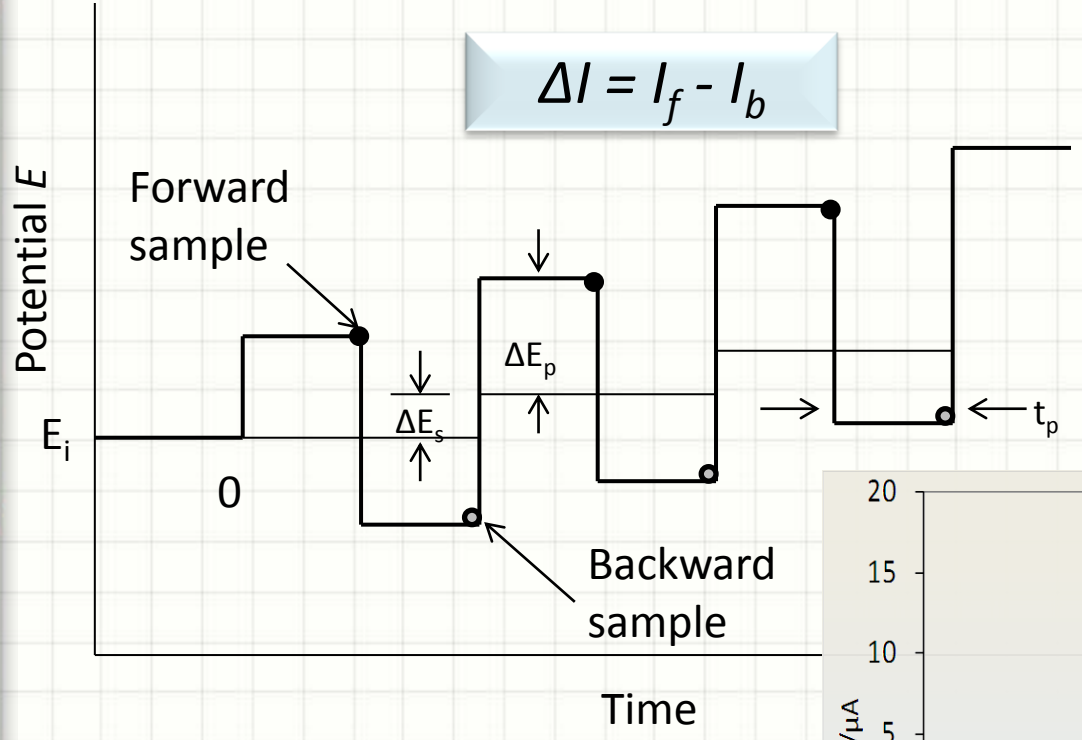


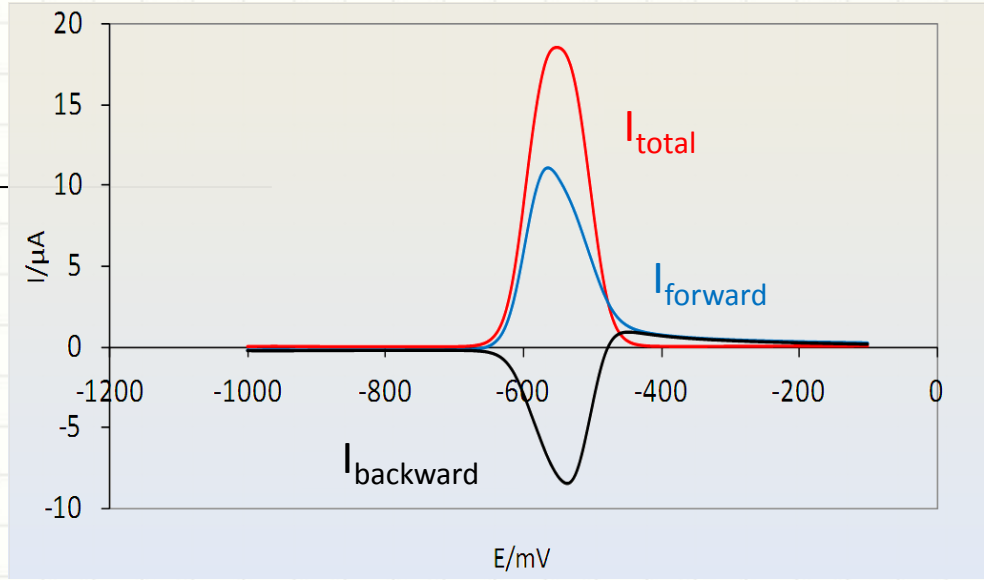


Square-wave voltammetry

- **Ramaley and Krause**

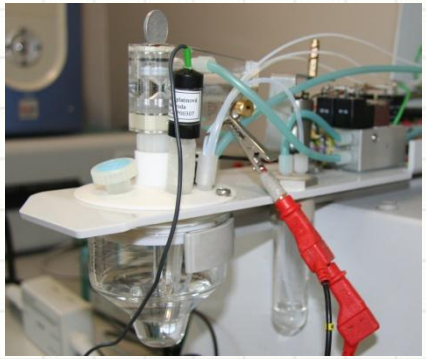
$$\Delta I = I_f - I_b$$

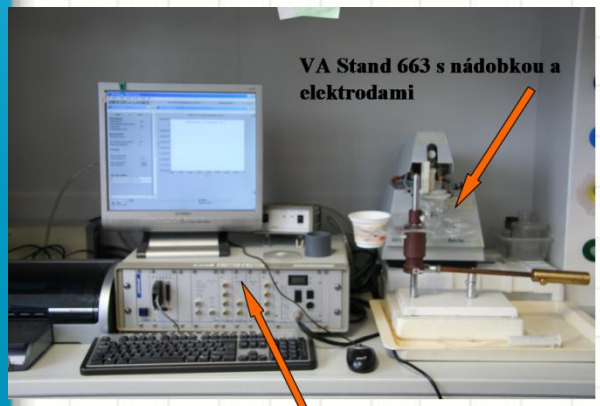




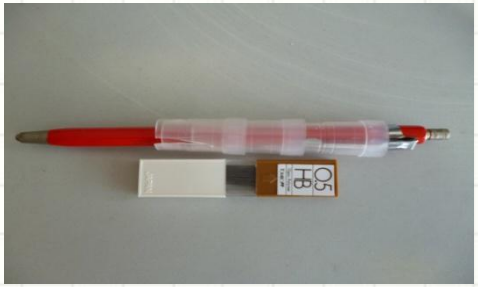
Equipments

- **Electrochemical analyzers:**
  - AUTOLAB PGSTAT 20 (Eco Chemie, Utrecht, The Netherland)
  - µAUTOLAB TYPE III (Metrohm, Switzerland)
- **GPES Manager 4.9**
- **Hanging Mercury Drop Electrode (HMDE)**
- **Polymer pencil graphite electrode(pPeGE)**



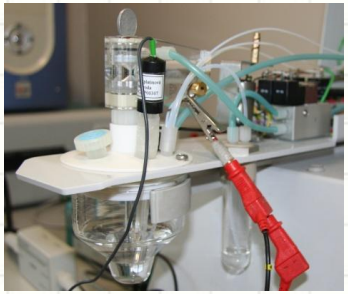


Autolab PGSTAT 20

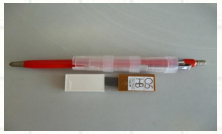


Electrodes

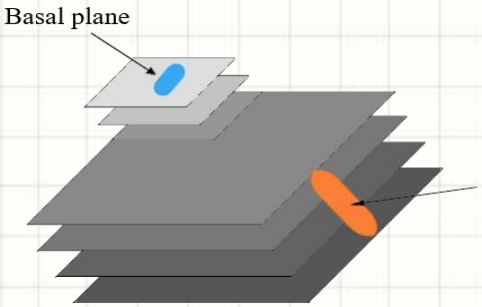
- **Hanging Mercury Drop Electrode (HMDE)**



- **Graphite electrodes** → **Polymer pencil graphite electrode**



→ **Pyrolytic graphite electrodes**



Inner structure of EPPG a BPPG electrodes

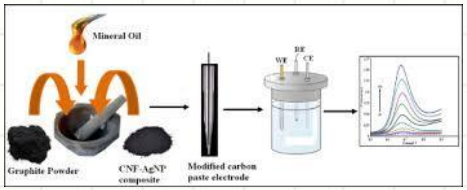
- **Basal plane pyrolytic graphite electrodes**

- **Edge plane pyrolytic graphite electrodes**

- **Graphite electrodes**

Glassy carbon electrodes

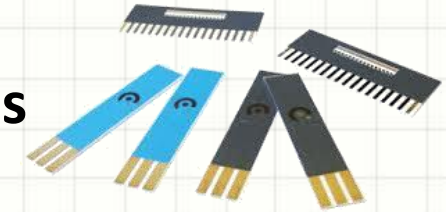
Carbon paste electrodes

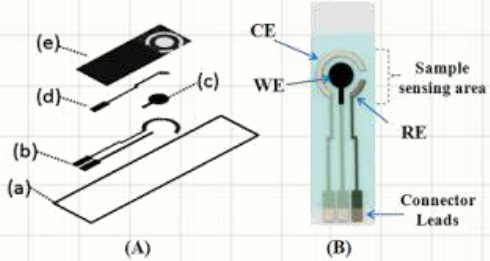


Carbon fibre electrodes

Boron dopped diamond electrodes

- **Screen printed electrodes**



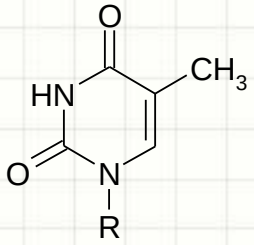


Nucleic acids

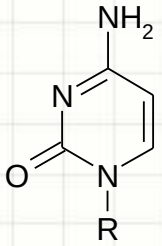
Nucleic acids and their components

A

pyrimidine bases

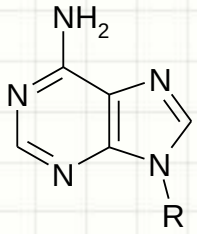


thymine (T)

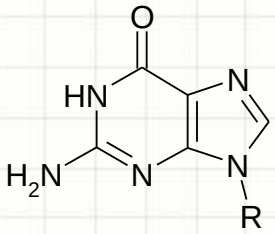


cytosine (C)

purine bases

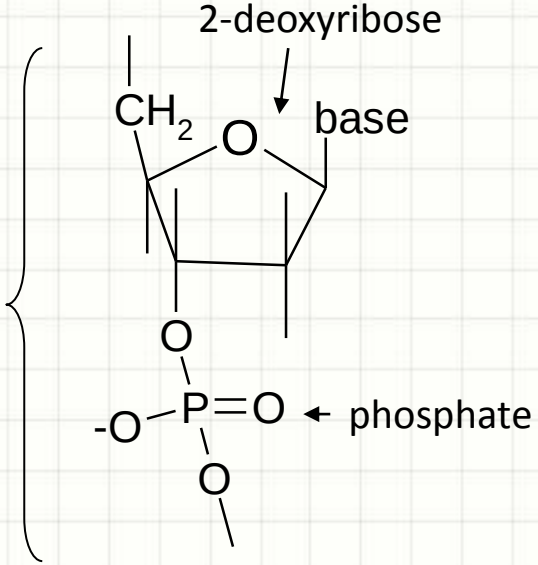


adenine (A)

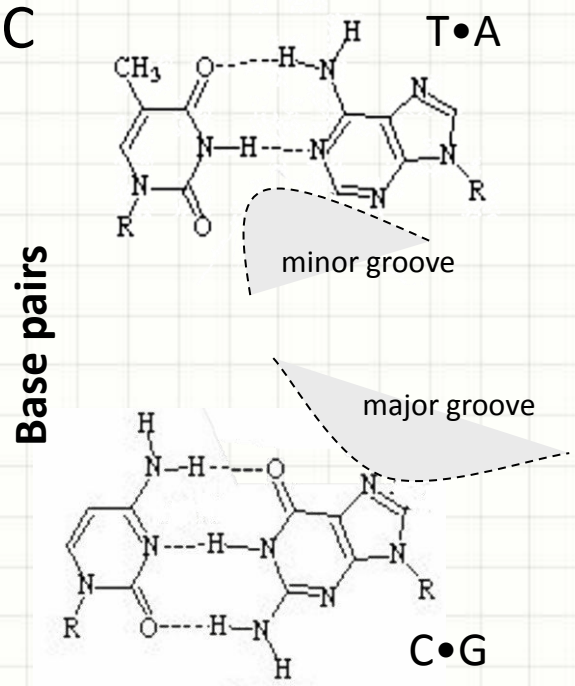


guanine (G)

B

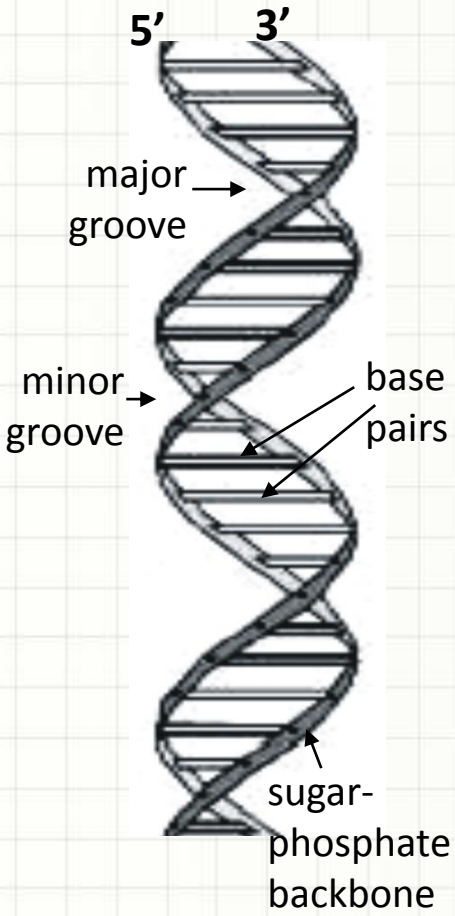


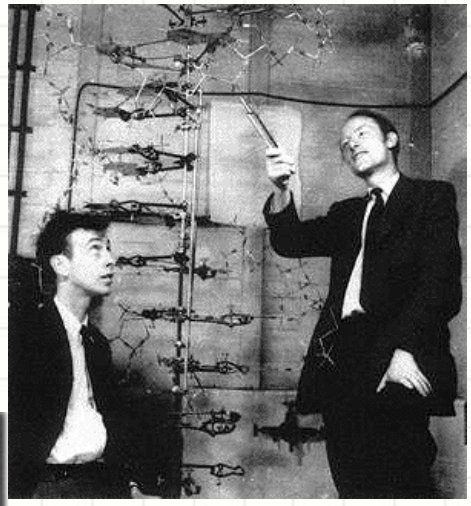
C

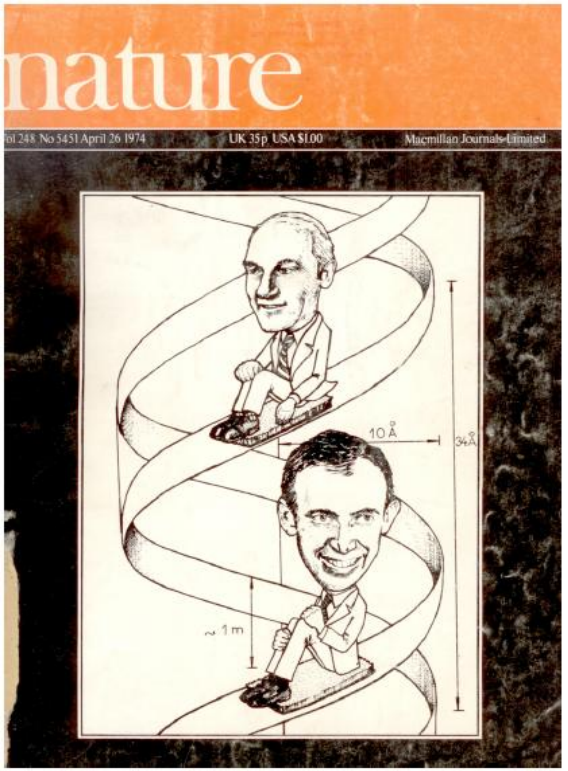


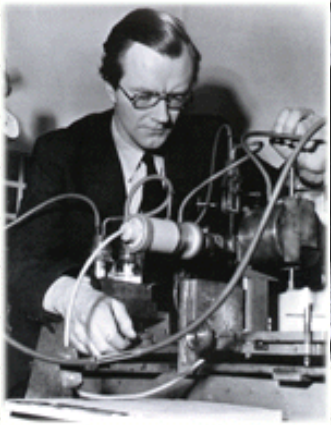
D

DNA double helix





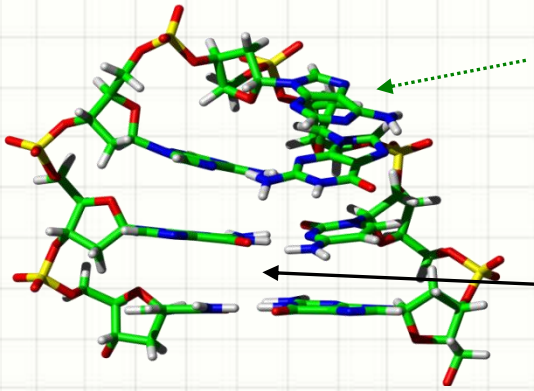




1953: James Watson, Francis Crick, Rosalind Franklin, Maurice Wilkins: DNA double helix

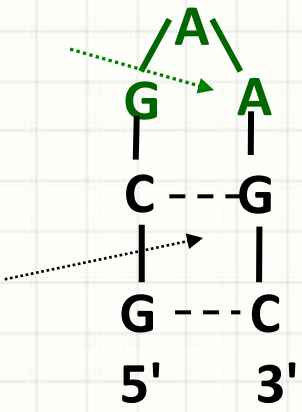
1962: Nobel prize (JW, FC, MW)
Explanation of the basic principles of preservation, transmission and expression of genetic information

Hairpins



LOOP

STEM



T_m of DNA heptamer = 76 °C
(0.1 M NaCl)

The shortest and thermodynamically the most stable hairpin - DNA heptamer d(GCGAAGC) – replication origins of phage ΦX 174 and herpes simplex virus, promotor regions of *heat – shock* genes of bacteria *E. coli* and rRNA genes

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Hairpins

- Hairpins are linked with triplet repeats expansion associated with many neurodegenerative diseases (fragile chromosome syndrom, Huntington disease, Friedriech's ataxia)

Analysis of hairpins:

d(GCGAAGC): UV, T_m (Hirao 1989, Yoshizawa 1994, 1997), NMR (Hirao 1994, Yoshizawa 1997, Padrta 2002, Sychrovský 2002), Ramanova spektroskopie (Chraibi 2000), electrophoresis (Hirao 1989, Yoshizawa 1994, 1997), CD (Hirao 1989), X – ray analysis (Sunami 2004), molecular dynamics (Nakamura 1999, Padrta 2002), **electrochemistry (Trnková 2004)**

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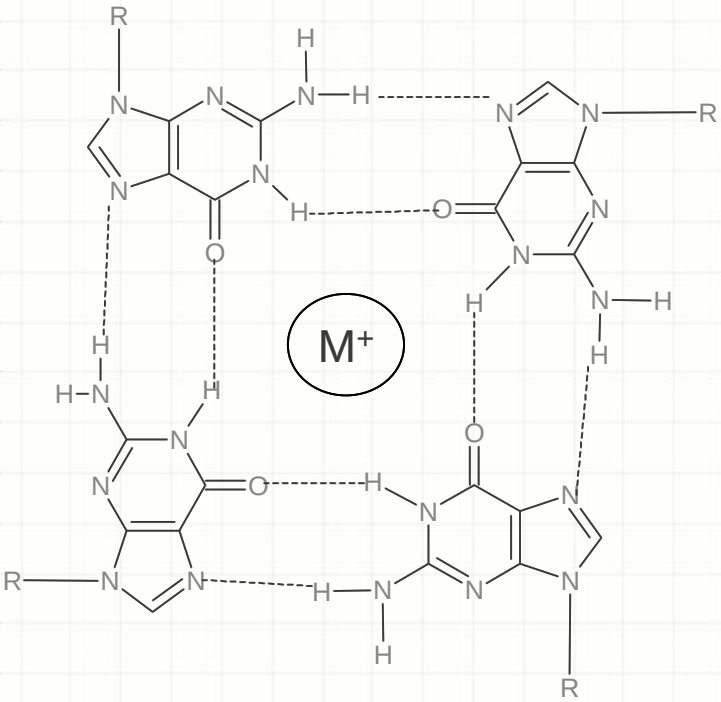
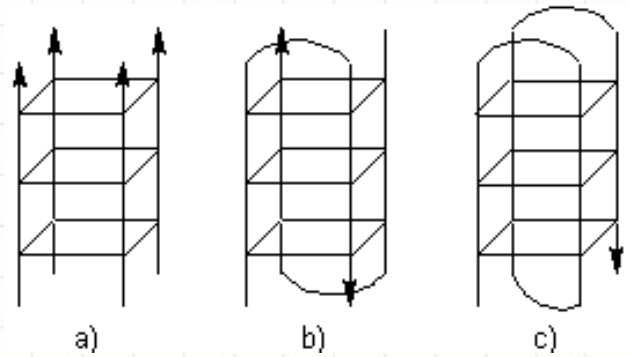
G - quadruplexes

- Stabilized by G-quartets (four molecules of guanine bound by Hoogsteen hydrogen bonds)
- Especially formed in presence of Na^+ a K^+ ions
- Structural polymorphism





a) Four - stranded tetraplex

b) Double - stranded intra-molecular tetraplex

c) Single - stranded intra-molecular tetraplex

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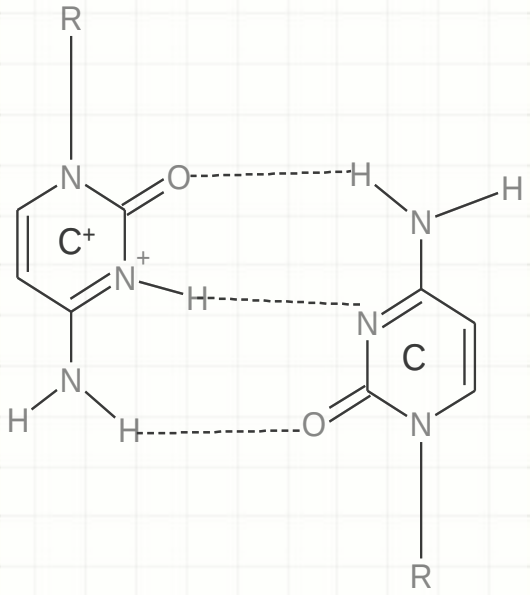
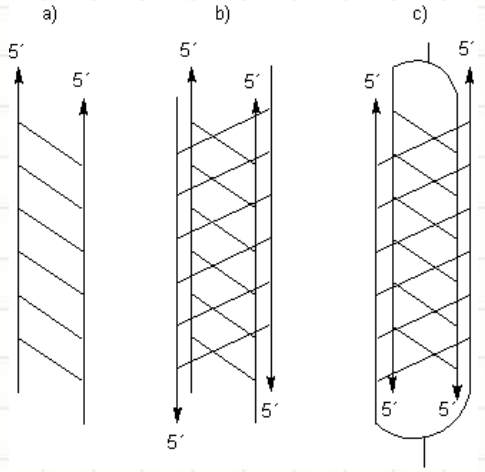
I - motifs

- **<u>Hemiprotonized C – C⁺</u>** pair as the basic structural unit
- Formed at acidic or neutral pH
- Diabetes mellitus and triplet repeats expansion linked with many neurodegenerative diseases
- Structural polymorphism





a) Double-stranded structure

b) Four-stranded structure

c) Four-stranded structure with labeled ends

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More than 55 years ago, Emil Paleček: DNA polarography (1960)

Oscillopolarography

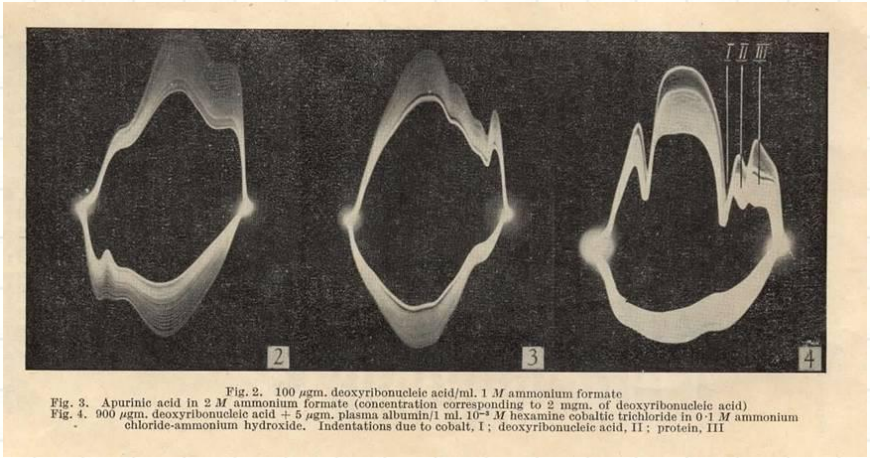


Fig. 2. 100 μgm. deoxyribonucleic acid/ml. 1 *M* ammonium formate
Fig. 3. Apurinic acid in 2 *M* ammonium formate (concentration corresponding to 2 mgm. of deoxyribonucleic acid)
Fig. 4. 900 μgm. deoxyribonucleic acid + 5 μgm. plasma albumin/1 ml. 10^{-3} *M* hexamine cobaltic trichloride in 0·1 *M* ammonium chloride-ammonium hydroxide. Indentations due to cobalt, I; deoxyribonucleic acid, II; protein, III

(*Reprinted from Nature, Vol.* 188, *No.* 4751, *pp.* 656–657, *November* 19, 1960)

Oscillographic Polarography of Highly Polymerized Deoxyribonucleic Acid

PROCEEDING from my finding[1,2] that nucleotides, nucleosides and the bases of nucleic acids can be analysed by alternating current oscillographic polarography[3–5], I have also tried to study polymerized deoxyribonucleic acid by this method.

The apparatus used was a Polaroskop *P* 524 (Křižík, Praha). With this apparatus it is posssible to plot dE/dt against E (Fig. 1). The analysis was carried out by means of the dropping mercury electrode in the same electrolytes as were used in my previous work[1,2]. All measurements were carried out with specimens of deoxyribonucleic acid from calf thymus.

I have established that in a medium of molar ammonium formate, deoxyribonucleic acid shows an anodic indentation at the same potential as deoxyguanylic acid (Fig. 2). Other characteristics of both indentations are also analogous (dependence on direct voltage, temperature, concentration of the electrolyte), which appears to indicate that that due to

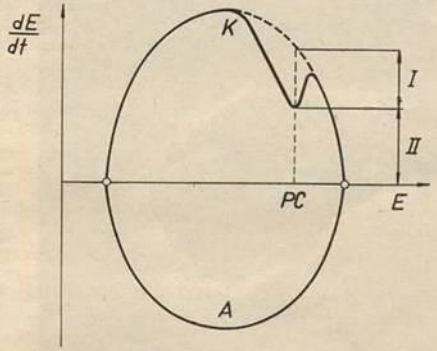


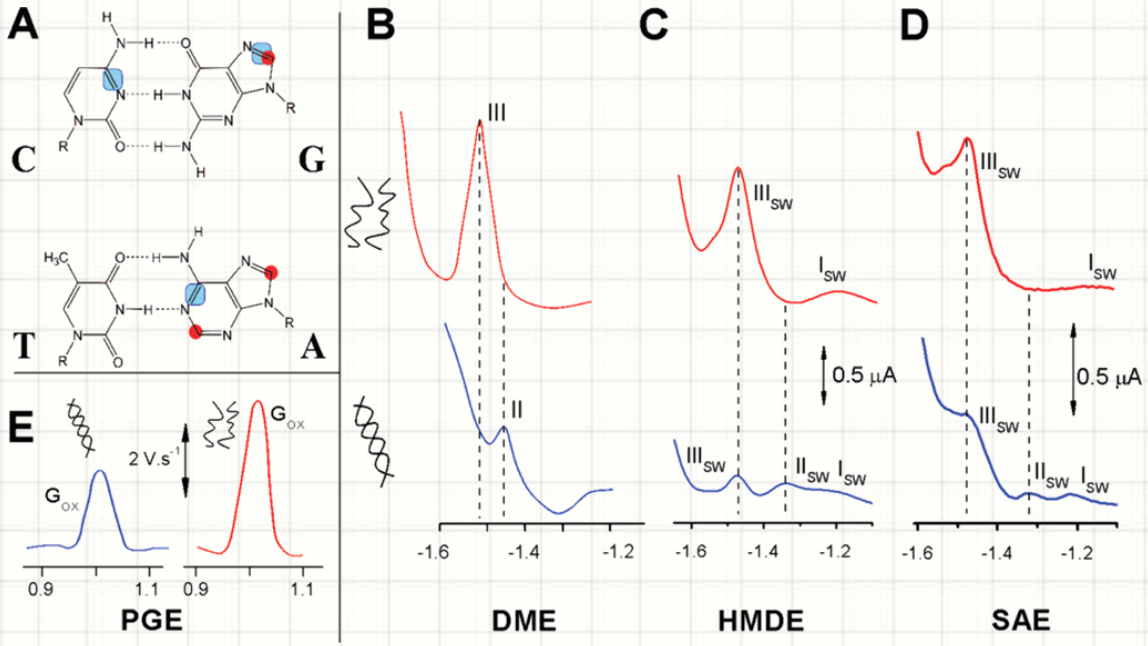
Fig. 1. Graph of dE/dt against E. The nature of the material analysed is characterized by the potential of the indentation (*PC*), which is somewhat similar to the polarographic half-wave potential. The quantity of the material is characterized by the depth of the indentation. For qualitative analysis, the height II, which can be measured much more easily, is generally measured. *K*, Cathodic part; *A*, anodic part

Nucleic acids are electroactive

- Mercury electrode: redox processes of A,C a G bases
- Carbon electrodes: oxidation of purine and pyrimidine bases
- Copper electrode: oxidation of sugar moieties in NA

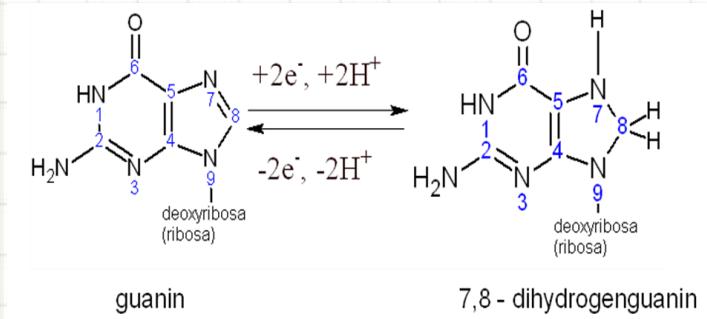
Singhal, P.; Kuhr, W. G.: *Anal. Chem.* **1997**, *69*, 3552-3557; *Anal. Chem.* **1997**, *69*, 4828-4832.

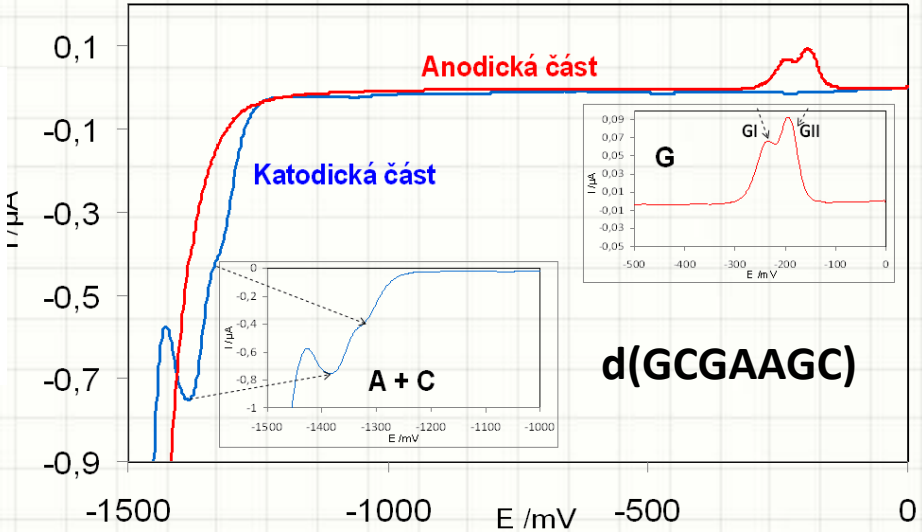
Electrochemistry of NA and ODN



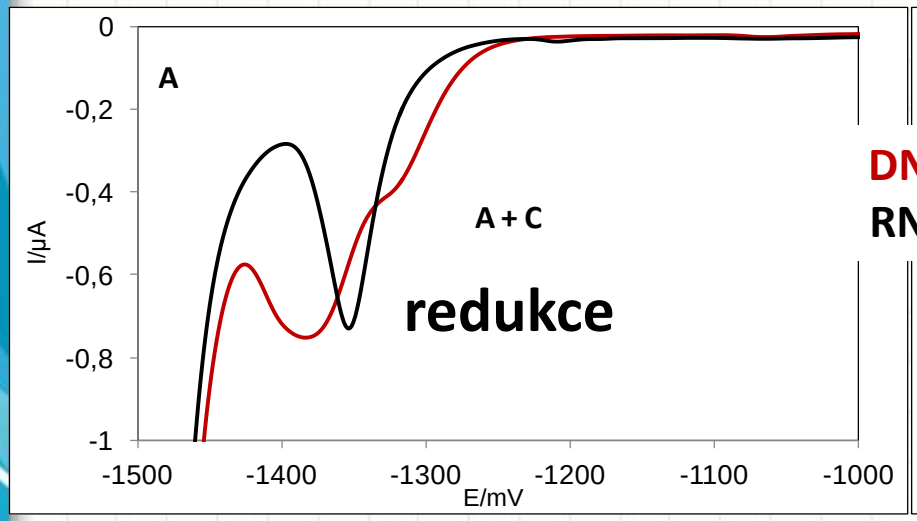
Paleček, E., Bartošík, M.: **Electrochemistry of Nucleic Acids**. *Chem. Rew.,* 2012

DNA



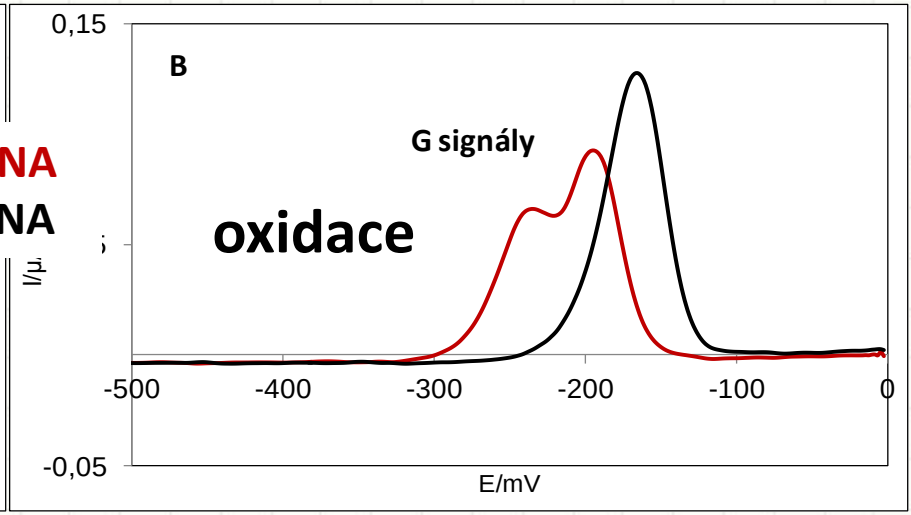


DNA vs. RNA

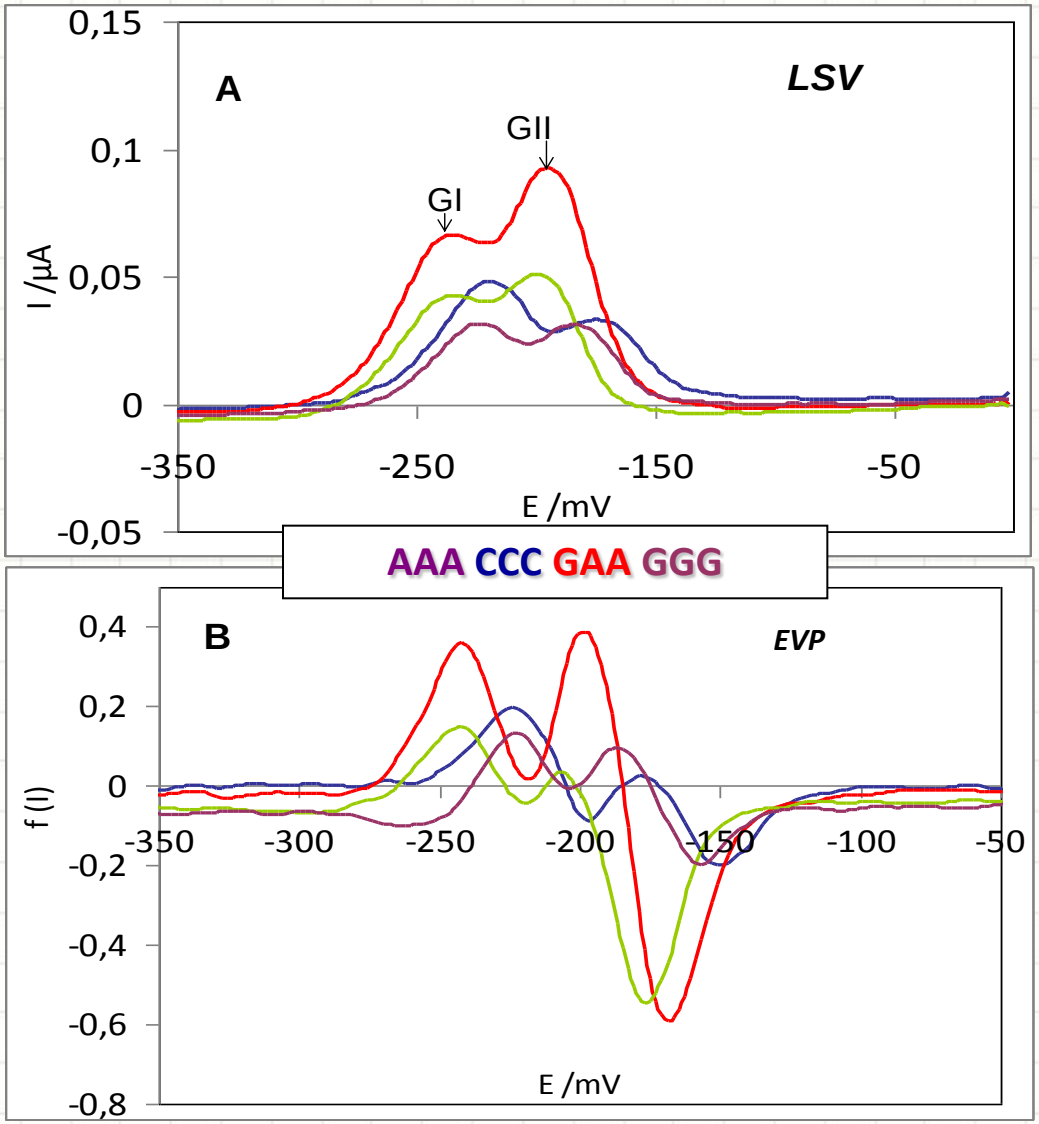


DNA

RNA



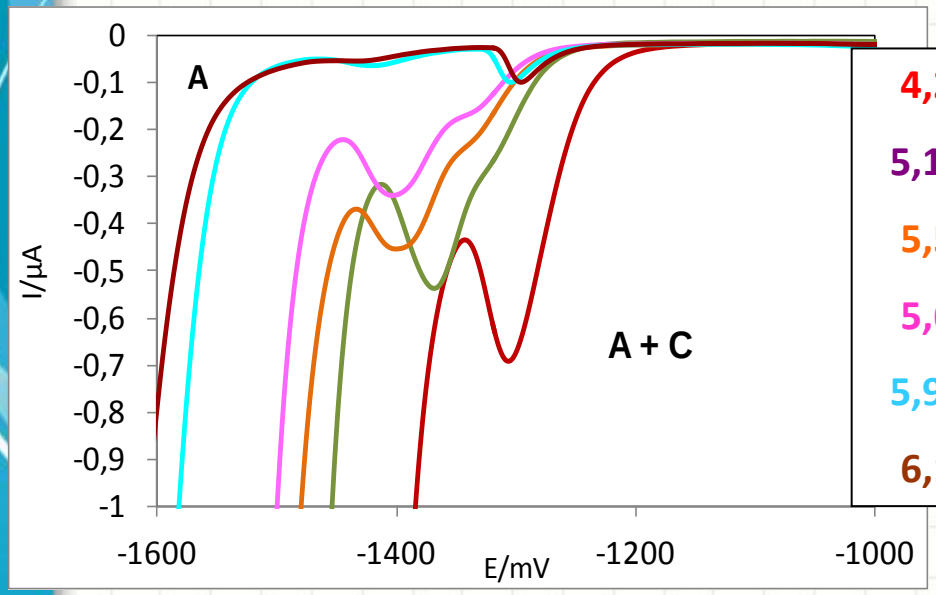
DNA heptamers with different sequence in molecule center

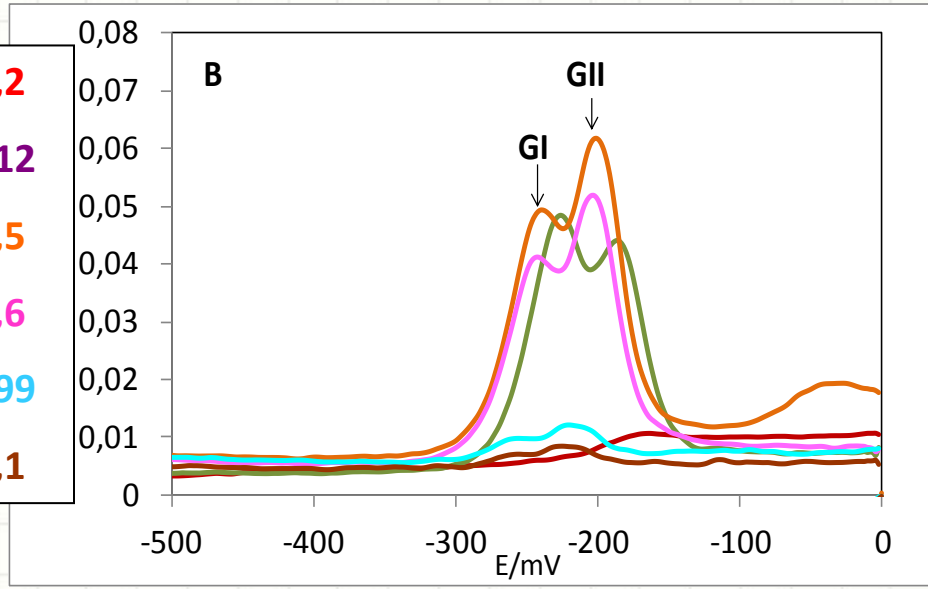


LSV

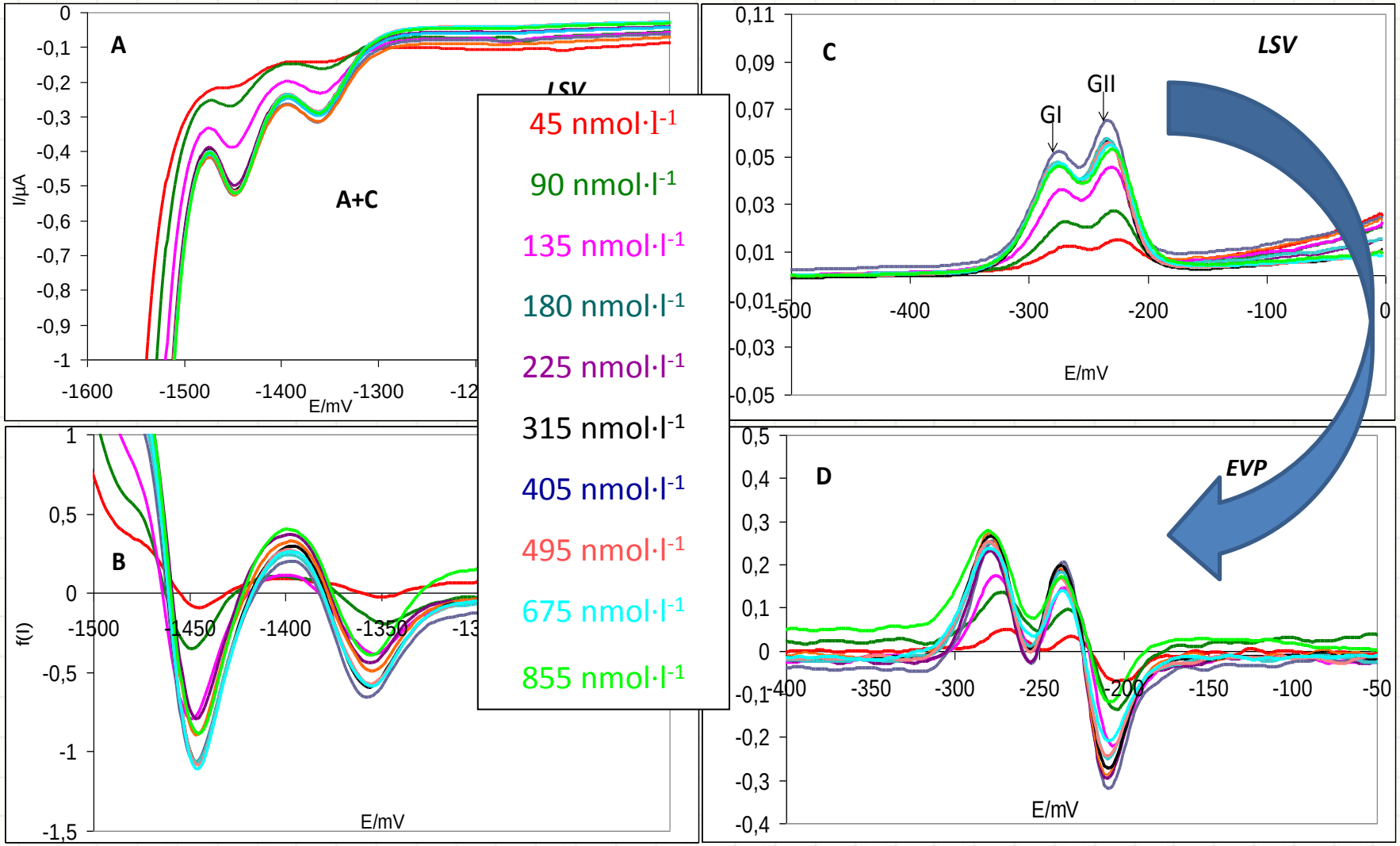
EVP

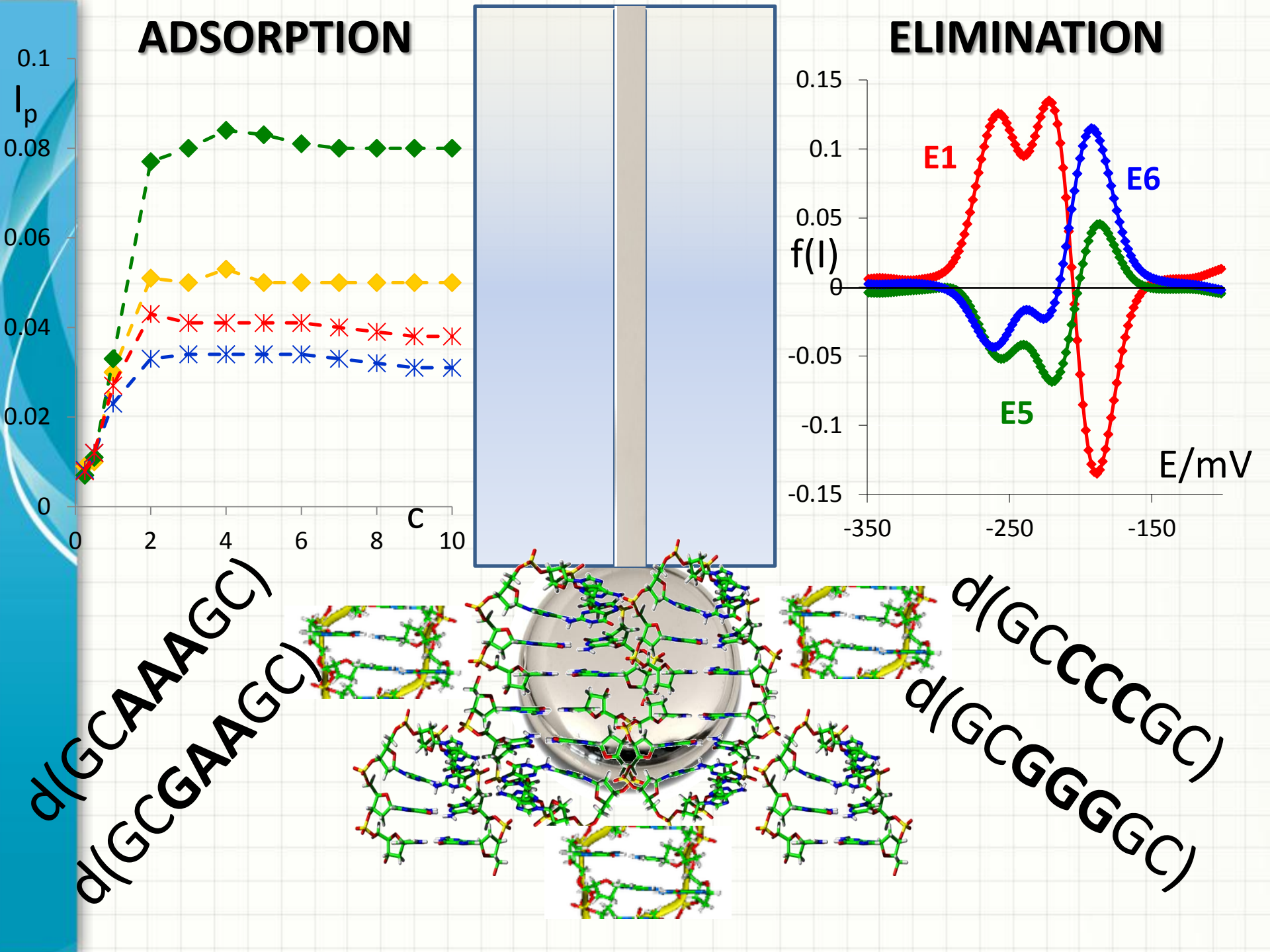
The pH effect for d(GCGAAGC)





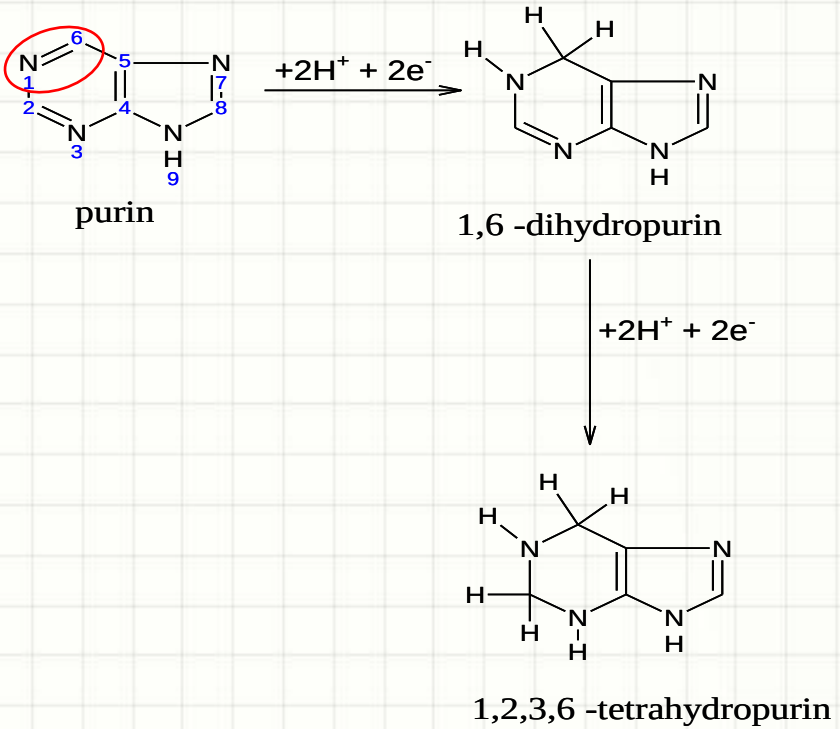
The concentration effect for (GCGAGC)




ADSORPTION
0.1
I_p
0.08
0.06
0.04
0.02
0
0
2
4
6
8
10
c
ELIMINATION
0.15
0.1
0.05
f(I)
0
-0.05
-0.1
-0.15
E1
E6
E5
-350
-250
-150
E/mV
d(GCAAAGC)
d(GCGAAGC)
d(GCCCCGC)
d(GCGGGGC)

Electrochemistry of purine and purine derivatives

- The typical electroactive compounds
- **1962 - Smith a Elving** – the first study of electrochemical reduction of purine and adenine by using polarography and coulometry on mercury dropped electrode (DME).
- **The two-step 2e$^-$ reduction**



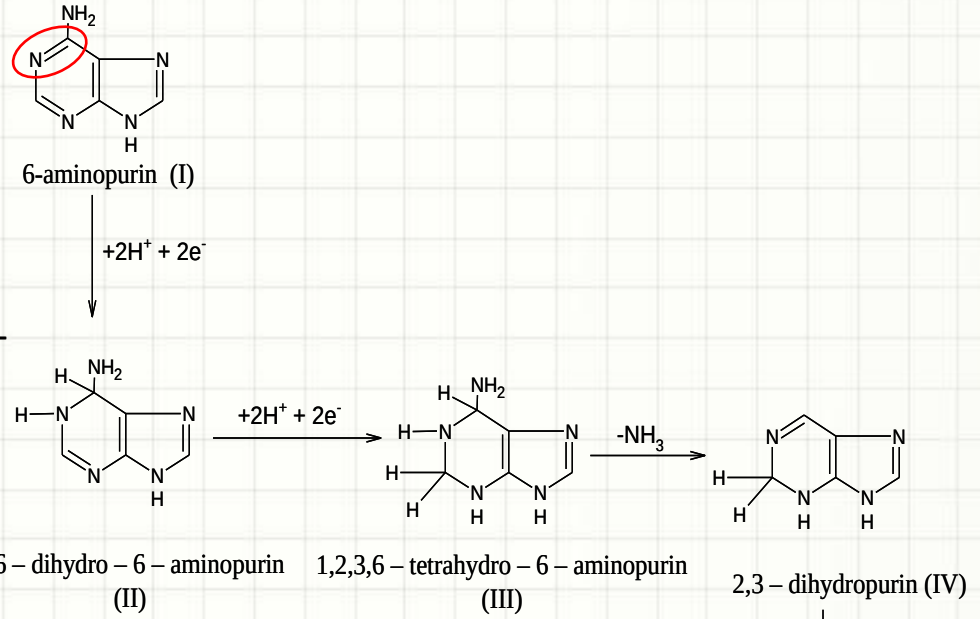
purin

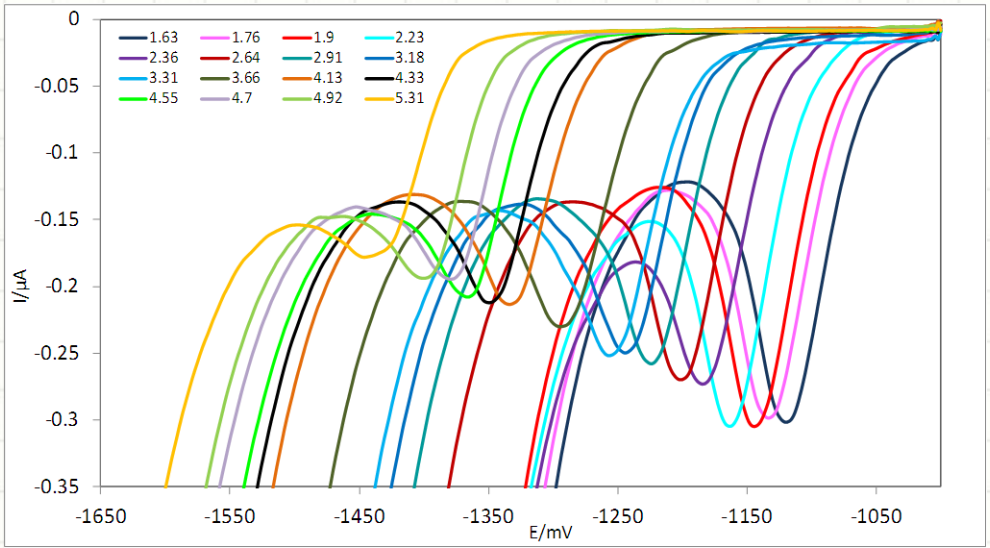
1,6 -dihydropurin

1,2,3,6 -tetrahydropurin

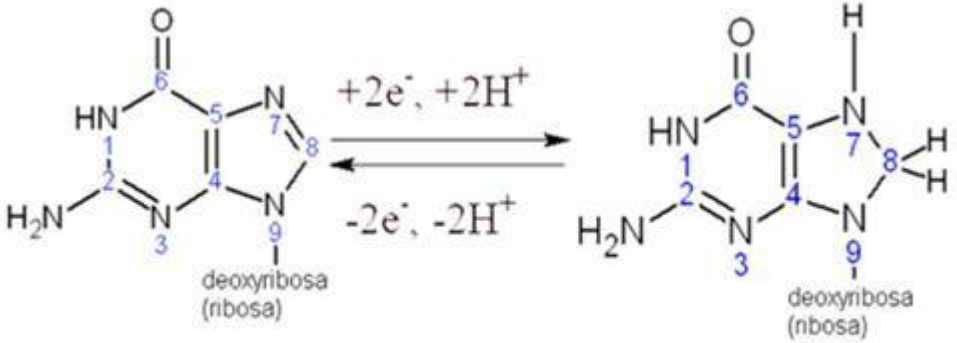
Electrochemical reduction of adenine

- **Smith and Elving (1962)**
- 6 e^- reduction process is accompanied by deamination process (e.g. coulometry)
- Polarographic reduction is finished in point III (formation of 1,2,3,6-tetrahydro-6-aminopurine)
- Problem of adenine reduction at pH > 6

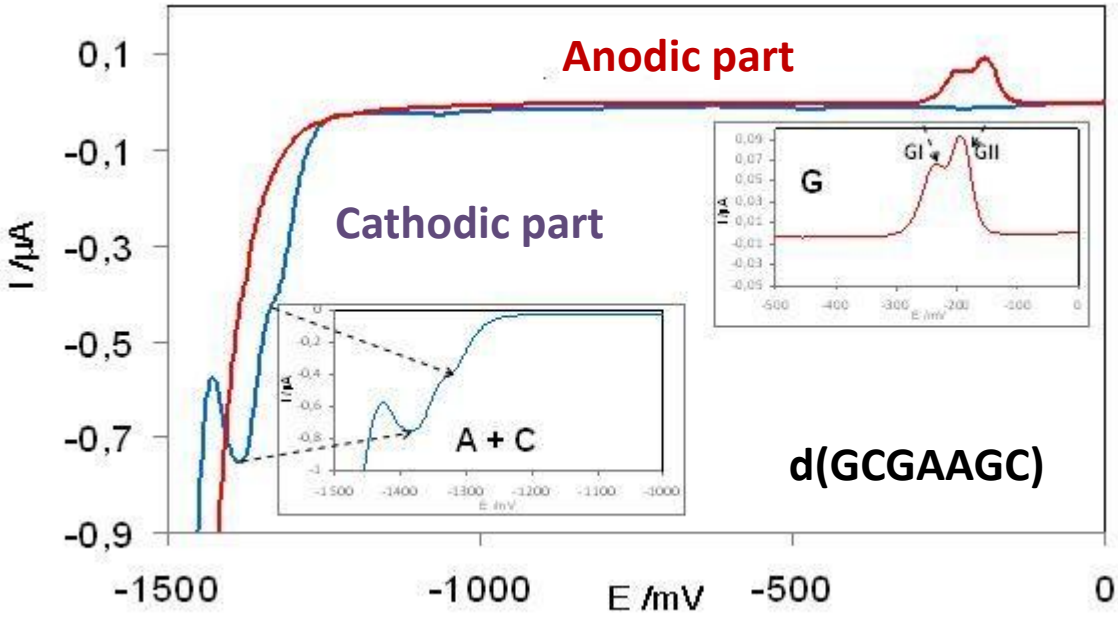




Electrochemical reduction of guanine

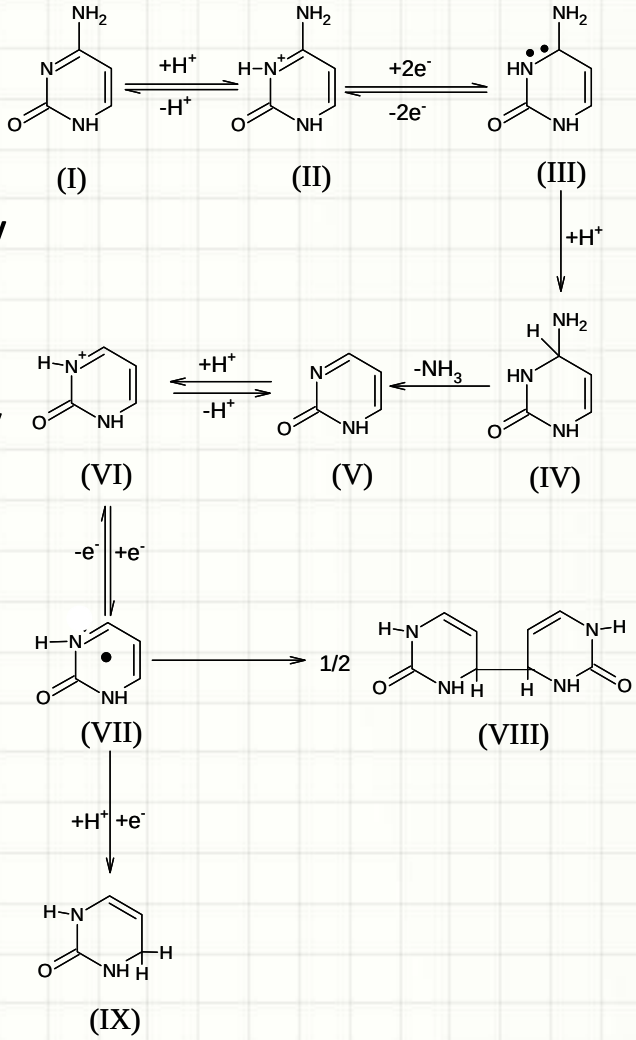


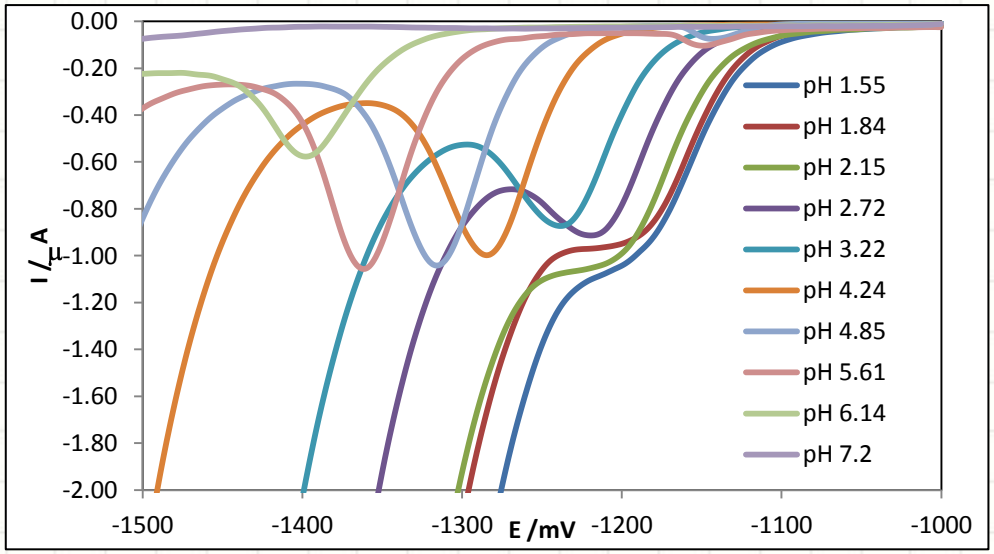
guanine **7, 8 - dihydrogenguanine**



Electrohemical reduction of cytosine

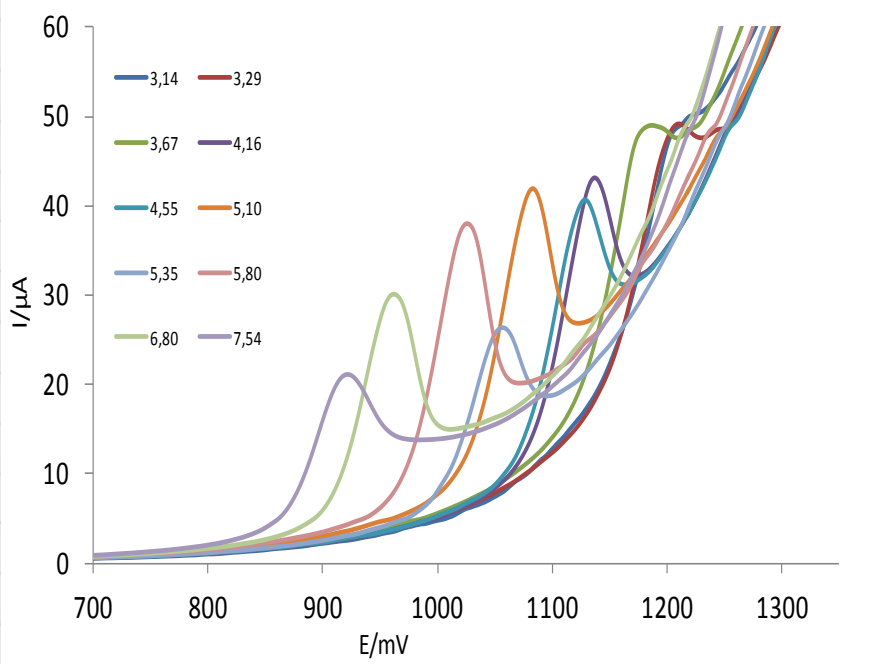
- Elving (1972)
- Reduction is initialized by fast protonation of cytosine(I) in N-3 position to electroactive form(II). The two-electron reduction of N-3=C-4 follows and karbanion (III) is formed.
- The reduction in polarography and volatmmetry is finished by 4-amino-3,4-dihydrogenpyrimidine-2-on (IV) formation
- The other intermediates is possible to obtain by using electrolysis or coulometry

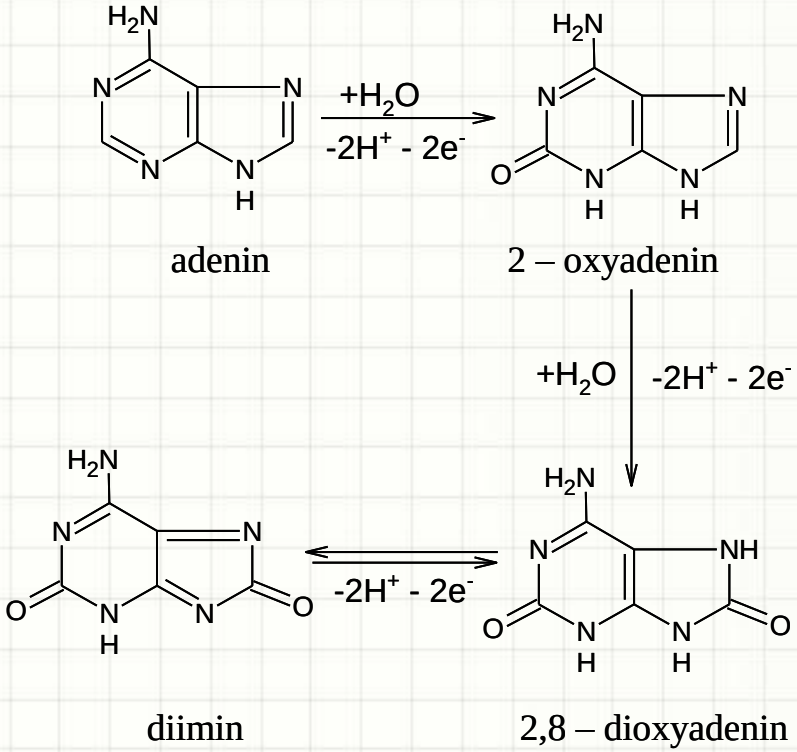


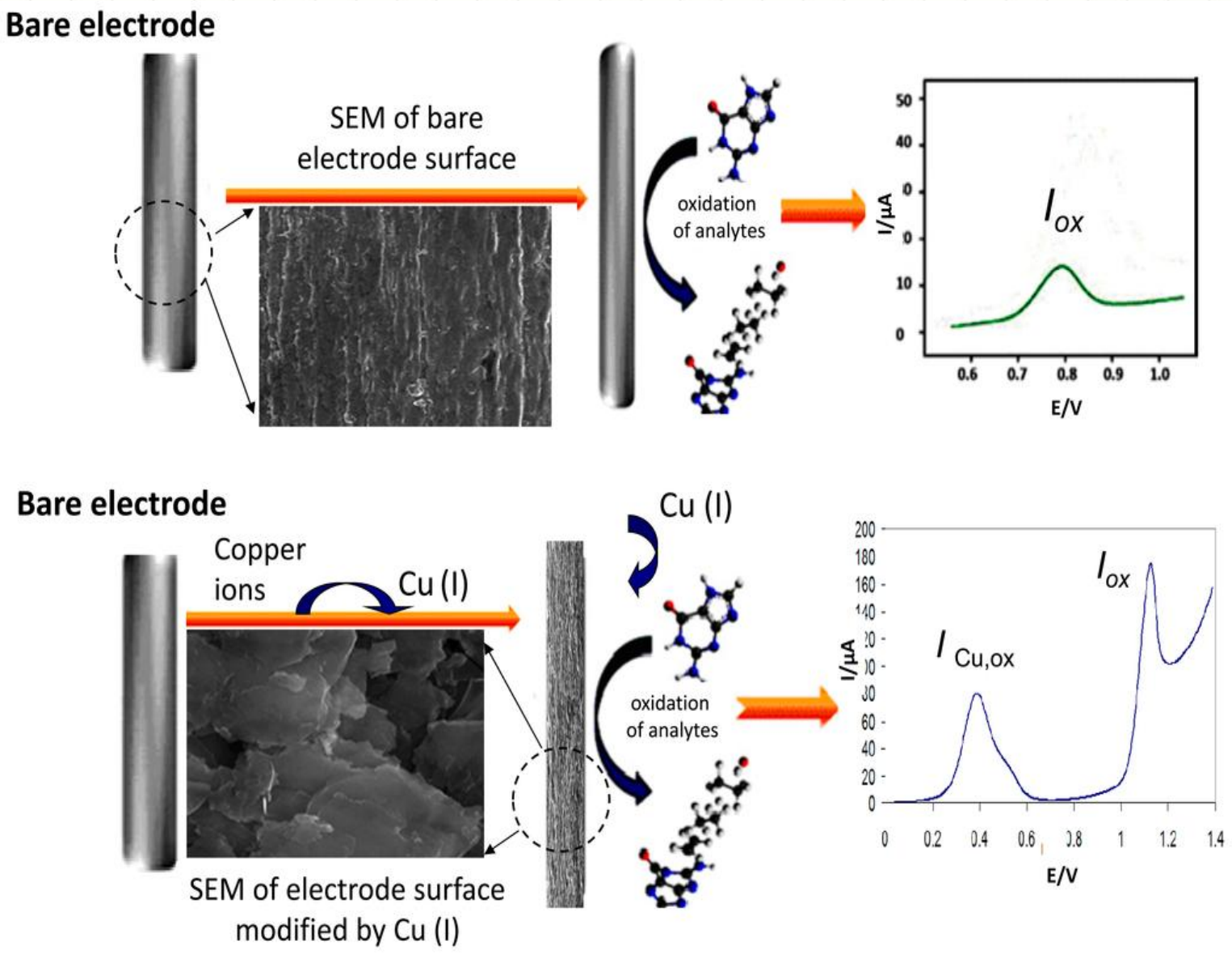


Electrochemical oxidation of adenine

- **Dryhurst, Compton**
- 6e$^-$ and 6 H$^+$ electrode process







(V.Sharma, F. Jelen and L. Trnkova: Sensors, 2015, 15(1), 1564-1600)

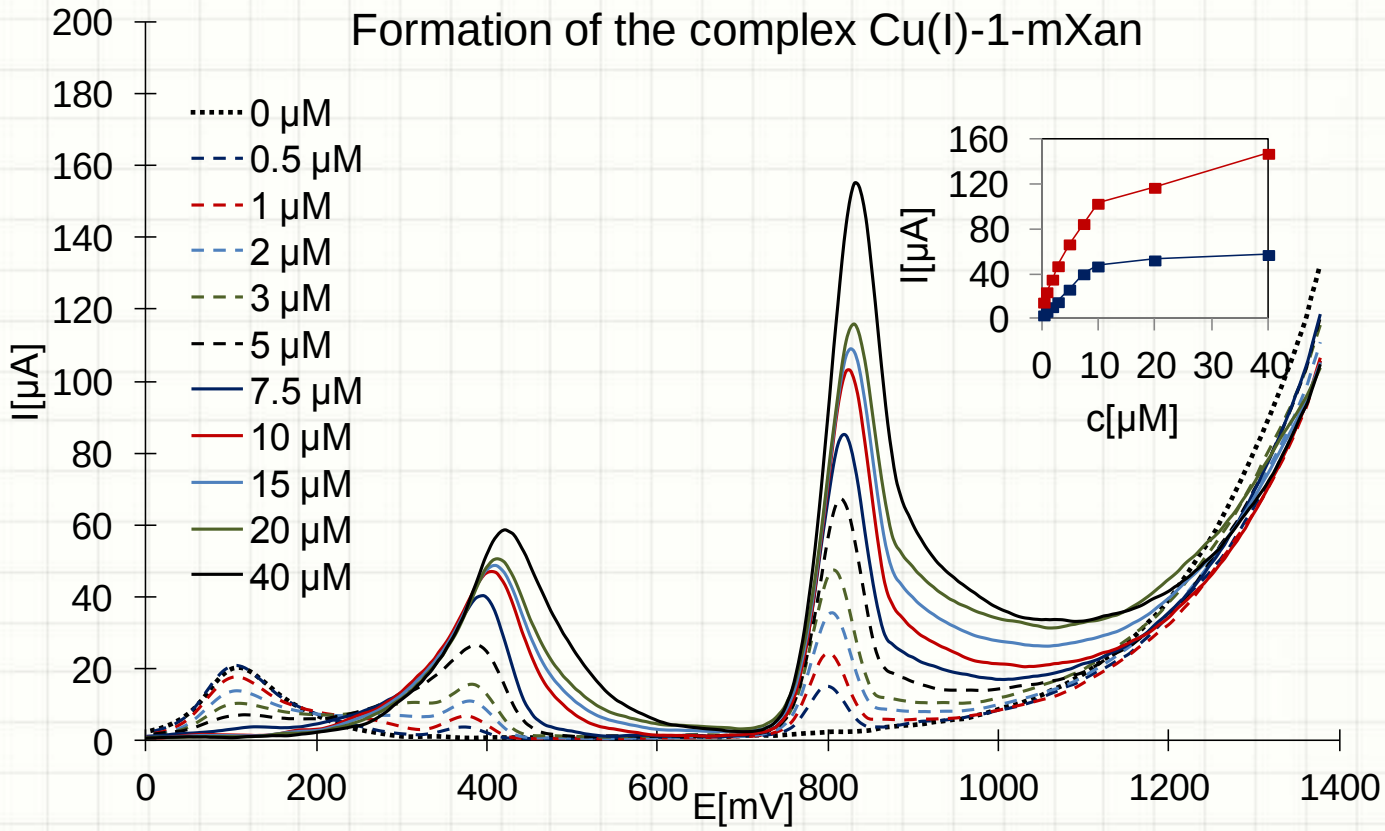


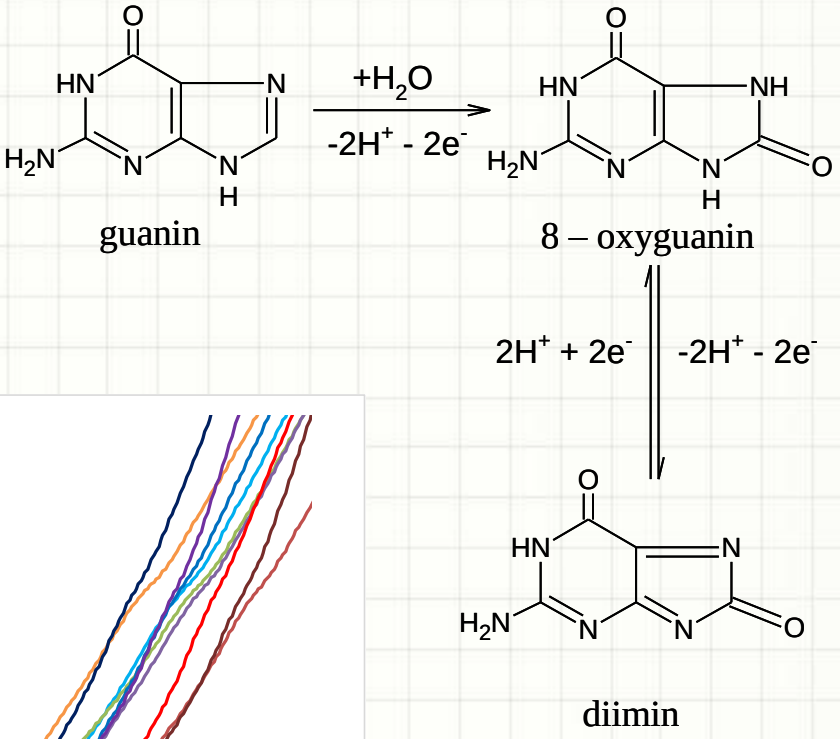
Figure 6: *Concentration dependence of 1-mXan in the solution with constant concentration of Cu(II) ions, c_{Cu} = 20 µM , reference scan rate 400 mV/s; phosphate-acetate buffer pH 5.1*

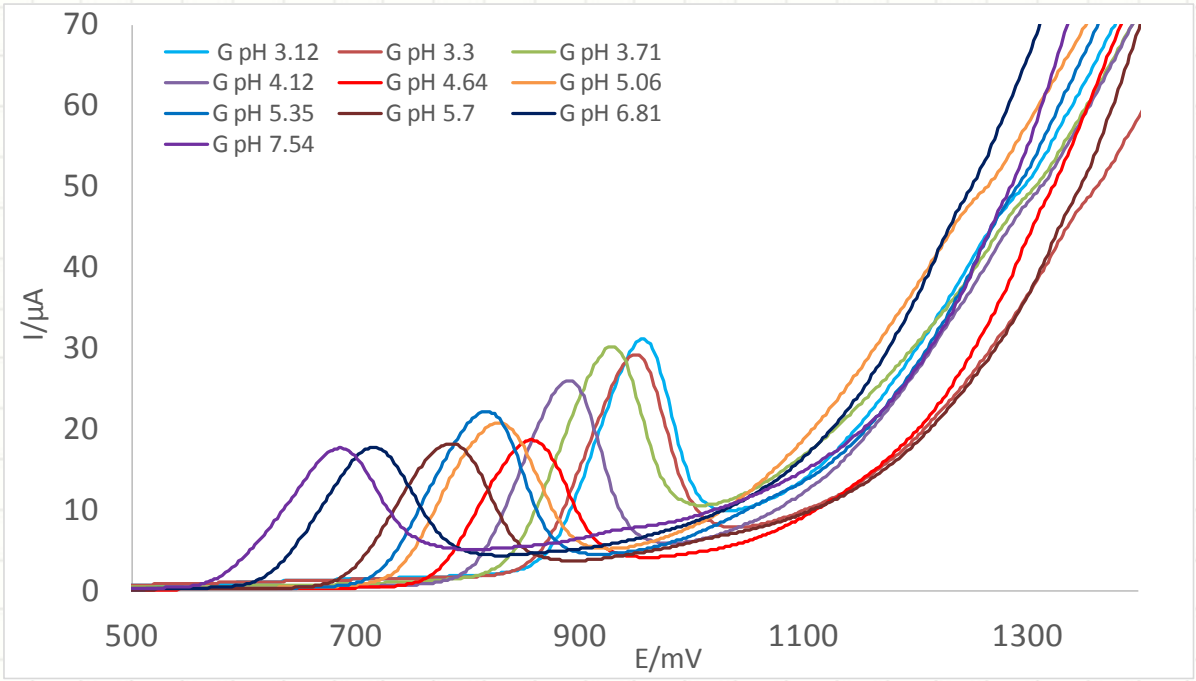
The complex formation and its oxidation can be described by the following scheme:

1. Cu(II) + e^- → Cu(I) (at a deposition potential of 0.15 V)
2. Cu(I) + purine → [Cu(I)-purine] (in the reaction layer on PeGE surface)
3. [Cu(I)-purine] → $[Cu(I)\text{-purine}]_{ads}$ (adsorption of the complex)
4. $[Cu(I)\text{-purine}]_{ads} - e^-$ → $[Cu(II)\text{-purine}]_{ads}$ (oxidative stripping, peak Ox_{Com})
5. $[Cu(II)\text{-purine}]_{ads} - e^-$ → $purine_{ox}$ + Cu(II) (oxidative stripping, peak Ox)

Electrochemical oxidation of guanine

- Dryhurst
- $4e^-$ and 4 H^+ electrode process



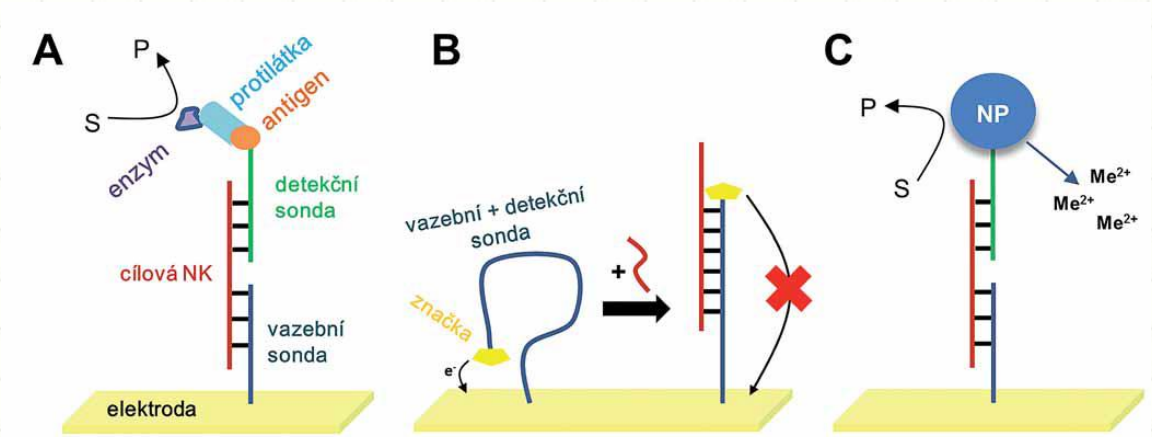


Before 1990…

Year	
1962-1966	ssDNA and dsDNA resolution
1967	DNA damage detection
1967	Interaction of DNA with low molecular weigth ligands
1978	Application of solid electrodes
1981-1983	DNA labeling with electroactive substance
1986	DNA-modified electrodes

…after 1990

- The big expansion in electrochemistry of NAs due to the considerable progress in genomics (Human Genome Project)
- The synthesis of DNA probes – electrochemical detection of hybridization
- Later, approaches targeted to improvement of sensitivity and reproducibility of analysis:
  - ✓ ELISA analogy (A)
  - ✓ Molecular beacon (B)
  - ✓ Nanotechnology (C) – inorganic NPs, carbon nanotubes, grafen

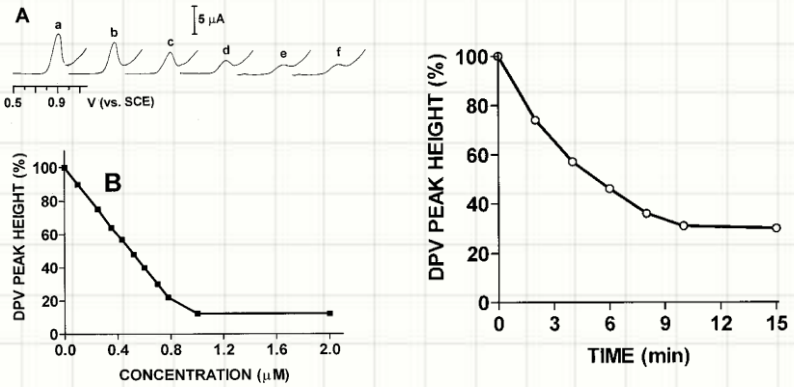


Bartošík M., Paleček E., Vojtěšek B.: Klin Onkol, 2014, 27 (Suppl 1), S53-S60

- Detection of oncogenes, tumor suppressor genes, mononucleotide polymorphism, repetitive sequence, viral and bacterial NAs, genetically modified organisms

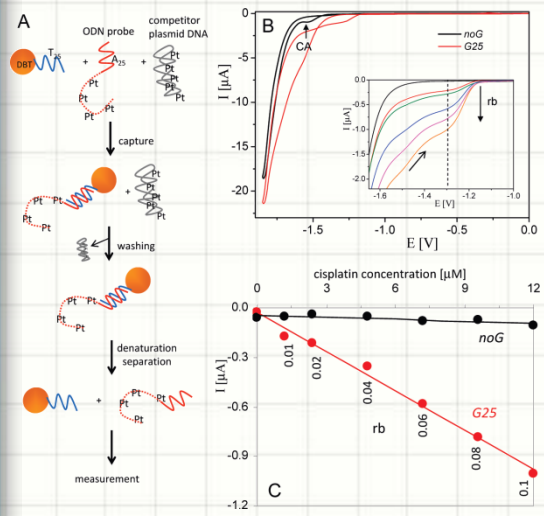
Electrochemistry of NA in oncology

- Interaction of DNA with antitumor drugs
- 2000 – Brabec – electrochemical biosensor based on carbon electrodes – the monitoring of guanine oxidation signal decrease due to platinum derivatives establishing



Brabec V.: Electrochim Acta, 2000, 45, 2929-2932

- Antitumor drugs yield electrochemical signal too

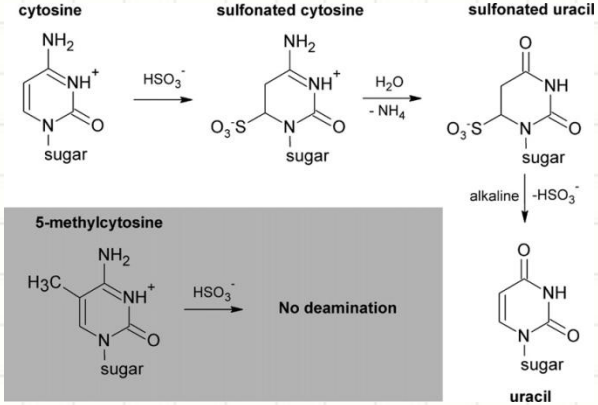


Monitoring of selective cisplatination of probe oligonucleotides in the presence of competitor plasmid DNA using magnetoseparation and AdTS CV. **(A)** Separation of the cisplatinated ODN probes using magnetic beads. The recovered ODNs were analyzed by AdTS CV. **(B)** Sections of AdTS CVs obtained for the ODNs *noG (black) or G25 (red) treated* with cisplatin (rb) 0.1) in the mixture with plasmid DNA. **(C)** Dependence of the current value measured at the anodic part of the AdTS CV at -1.3 V on the concentration of cisplatin used for modification of the ODN probes in the presence of plasmid DNA: *noG (black); G25 (red).*

Horakova P., Tesnohlidkova L., Havran L. et al.: Anal Chem, 2010, 82, 2969-2976

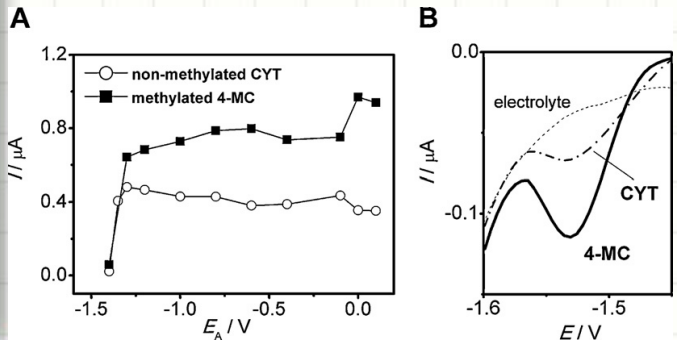
DNA methylation

- Epigenetic modification playing important role in gene expression
- Changed methylation patterns of DNA associated with carcinogenesis
- **Cytosine methylation** – 1) reaction with $NaHSO_3$(cytosine is deaminated to uracil, methylcytosine is not changed), after that amplification of DNA and m-DNA by PCR (uracil is amplificated as thymin and methylcytosine as cytosine) and finally electrochemical detection by using suitable redox labels



Bartošík M., Fojta M., Paleček E.: Electrochim Acta, 2012, 78, 75-81

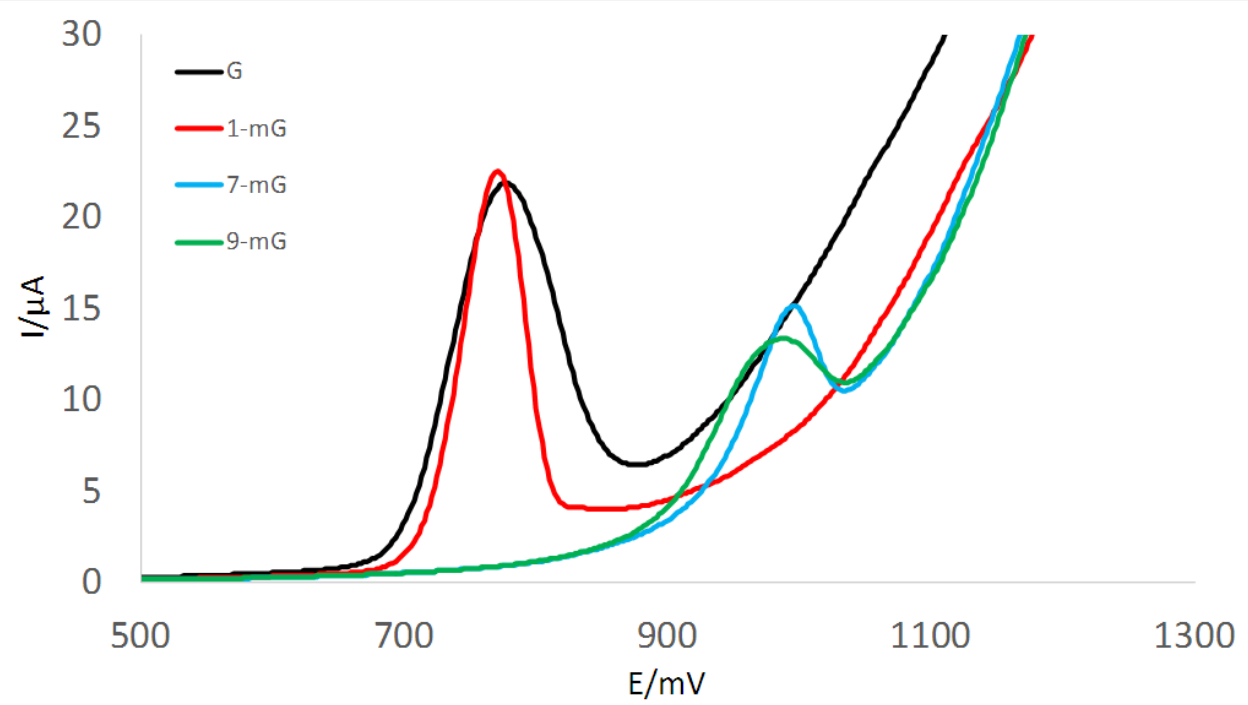
2) After reaction with $NaHSO_3$ electrochemical reduction of DNA at Hg and solid amalgam electrodes (uracil is unreducible at Hg electrode, but methylcytosine is reducible at Hg electrode→after reaction with $NaHSO_3$ m - DNA yields higher signal than DNA)



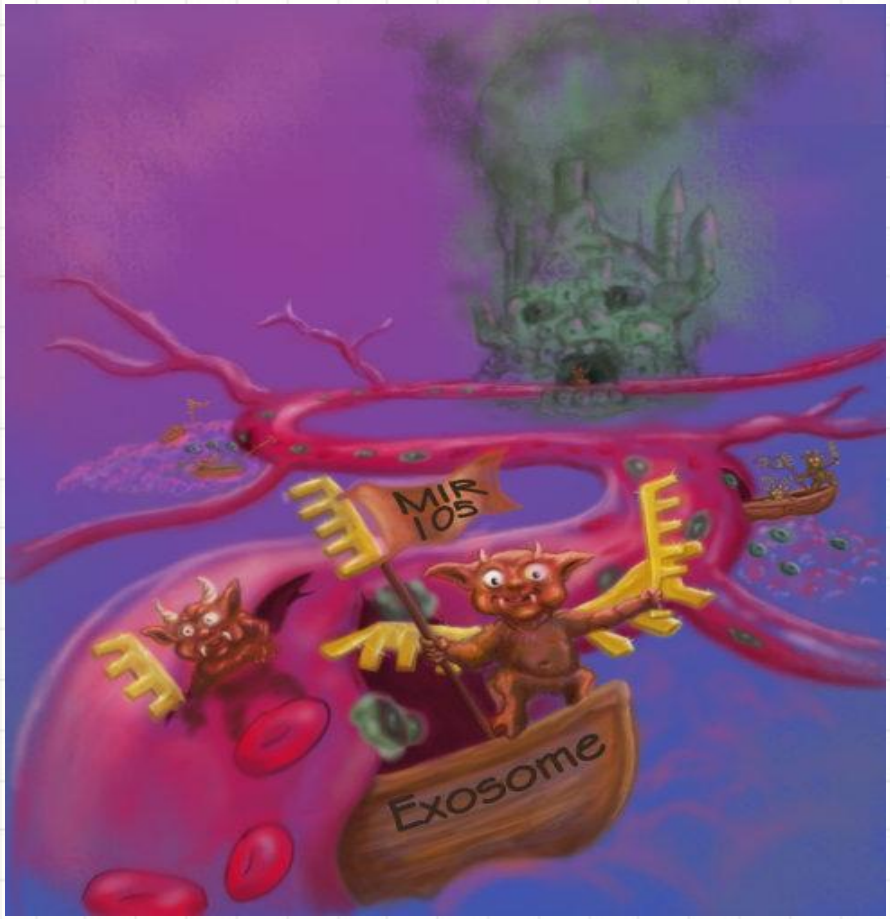
Bartošík M., Fojta M., Paleček E.: Electrochim Acta, 2012, 78, 75-81

DNA methylation

• **Guanine methylation** – at first electrochemical reduction at Hg electrode, later boron – doped diamond electrodes and carbon electrodes



miRNA



Why to study miRNA?

- Gene expression regulation
- Regulation of processes during tumor grow
- Present in all human tissues and fluids – easily accessible
- Other miRNA between healthy and diseased individuals
- Oncomarker?
- miRNA as a drug

Cancer, cardiovascular diseases, neurodegenerative diseases

Electrochemical detection of miRNA

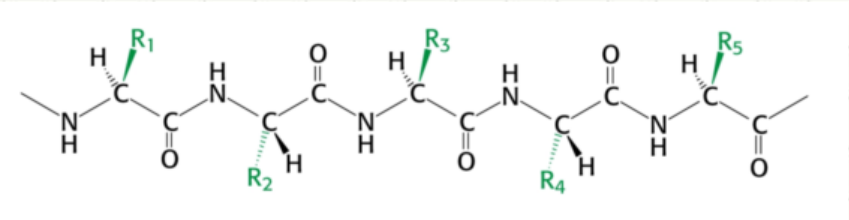
•DNA probes and enzymatic or NPs labels for signal amplification

•The method using miRNA labeling by using electroactive complex on the base of hexavalent osmium

Proteins

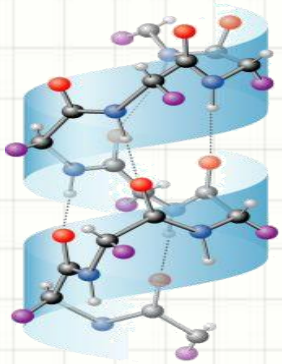
Primary structure

The order of AA in polypeptide chain

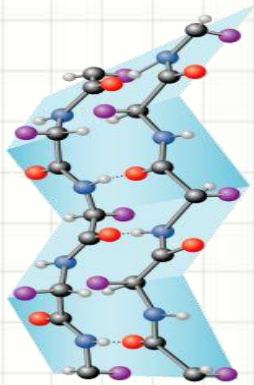


Secondary structure

The geometrical arrangement of polypeptide chain

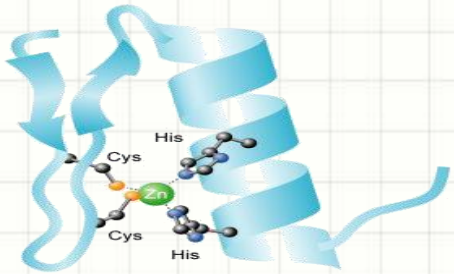


α-helix

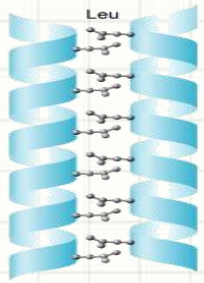


β-list

β-ohyb



Zn-prst

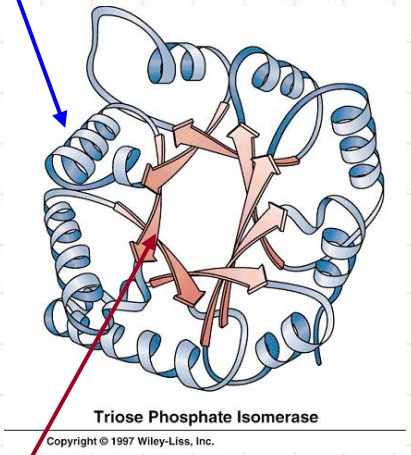


Leu - zip

Tertiary structure

The spatial arrangement of polypeptide chain

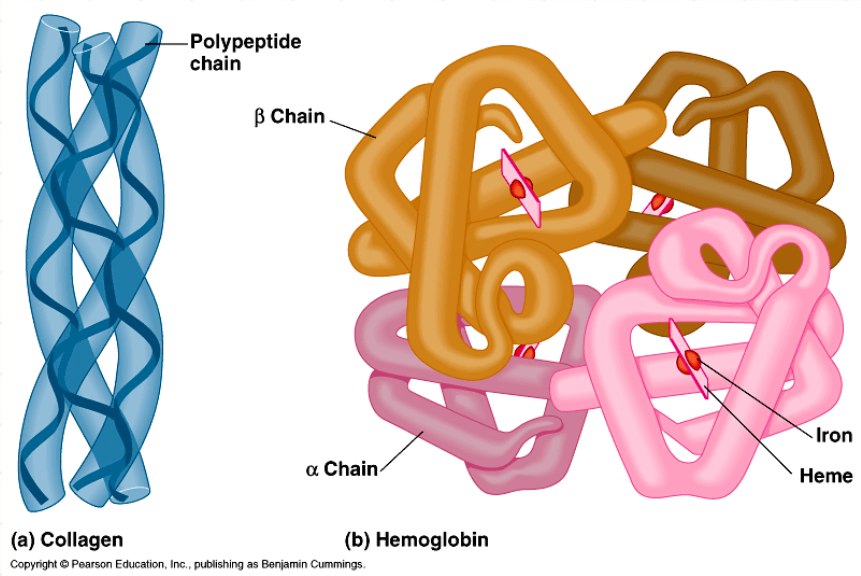
α-helix



β – folded sheet

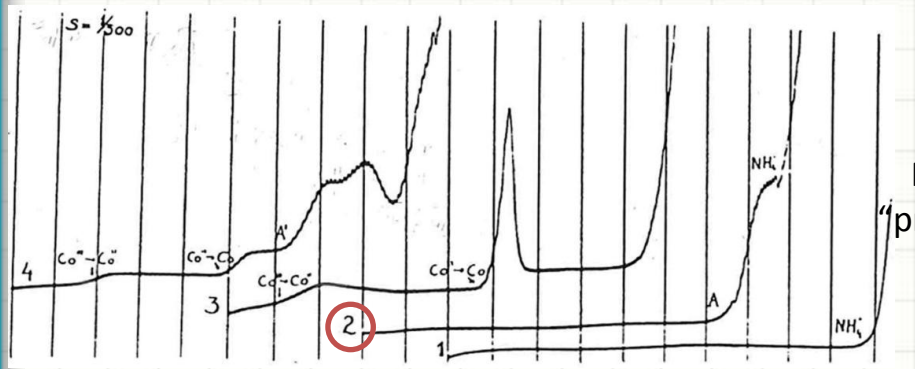
Quaternary structure

Subunits in protein agglomerates forming one functional protein



Proteins are electroactive

- The first biomacromolecules investigated by using electrochemical methods
- **Herles and Vančura –** polarographic study of human body fluids (blood serum and urine). **„Presodium wave“** – cathodic wave occuring at potentials more positive (300 mV) that cathodic reduction of sodium ions. Preliminarily this wave was assigned to proteins
- **Heyrovský and Babička** – albumin in presence of ammonium ions produces in dc polarography so called **„presodium wave“** (**H peak**), caused by catalytic hydrogen evolution reaction
- „Presodium wave“ not suitable for analytical purposes



Polarographic catalytic waves of human serum. (2) the "presodium" catalytic wave in 0.1 M ammonia/ammonium chloride

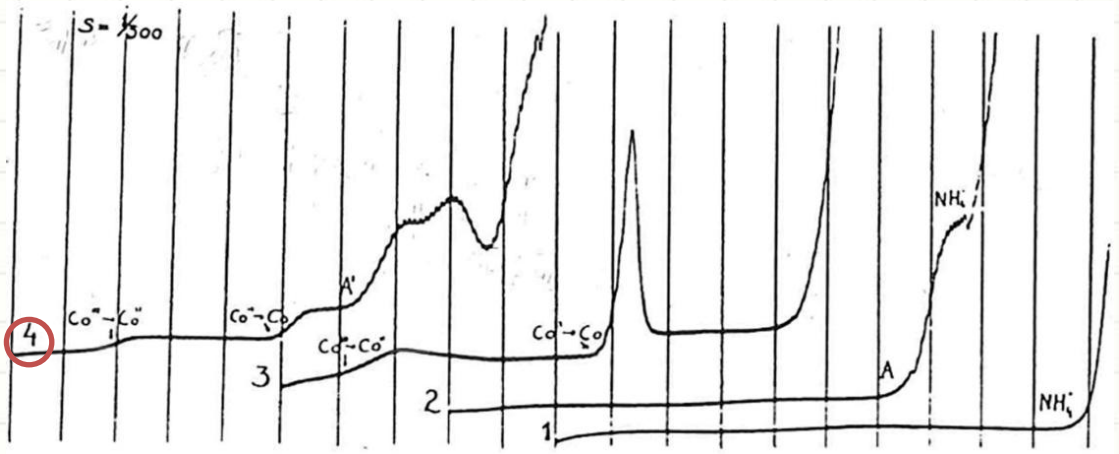
Brdička‘s catalytic reaction (BCR)

•**1933 – Brdička‘s catalytic reaction –** polarographic double – wave of proteins containing Cys residues in buffered solutions of cobalt (Brdička solution – ammonium buffer $NH_4OH + NH_4Cl$ and cobalt complex $[Co(NH_3)_6]Cl_3$)

•Originally designed for detection sulphur rich substances, such as organic compounds (2- mercaptopropionic acid, 2-diethylaminoethanethiol hydrochloridum), amino acids (cysteine, cystine) and proteins (albumin)

•Application of Brdička‘s catalytic reaction in clinical medicine and pharmacology (cancer diagnostic)

•Mechanism of electrode process of Brdička catalytic reaction is not known in details, but it is propossed that complex Co(II) with $–NH_2$ and –SH moieties plays a key role



Polarographic catalytic waves of human serum. (4) the catalytic double-wave in Brdička solution

What is catalytic hydrogen evolution reaction (CHER)?

•Electrochemical phenomenon caused by a catalyst, in which presence the hydrogen evaluates at the cathode polarized to more positive potentials than in the catalyst absence

•The hydrogen evolution is produced by cathodic catalytic current. The current intensity is depend on the catalyst concentration and the kinetic catalyst efficiency

$$2PH_{(surf)} + 2e^- \rightarrow 2P^-_{(surf)} + H_2(g)$$

$$P^-_{(surf)} + BH(aq) \leftrightarrow PH_{(surf)} + B^-(aq)$$

PH a P^-: protonized/deprotonized form of AA residues in protein molecule
BH is acid component of buffer; B^- is its conjugated basis

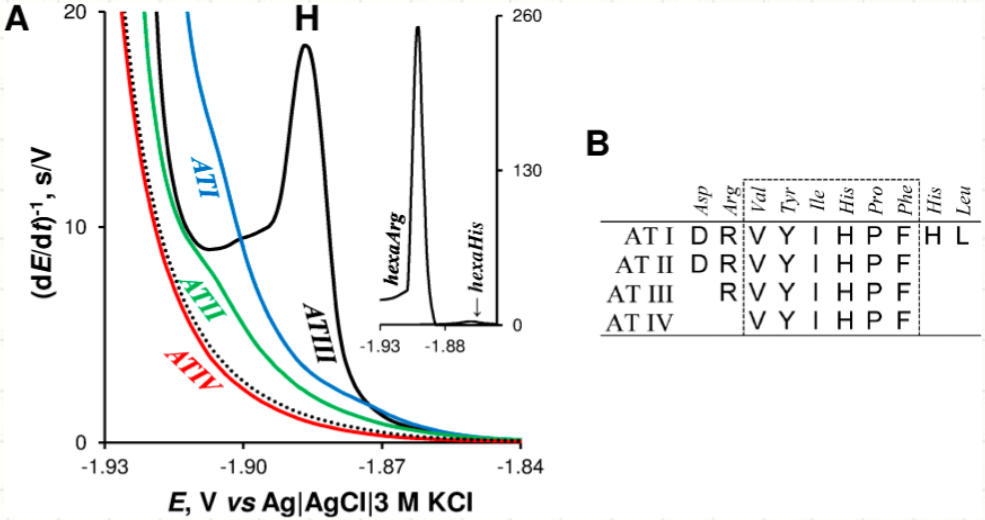
- The reaction shows, that catalyst is protein immobilized on the electrode surface
- Basic AA - Cys, Lys, Arg a His residues of protein molecule – catalyze the hydrogen evolution on the Hg electrode

H peak

- **In recent years –** The „presodium wave“ (J. Heyrovsky) in combination with CPSA (chronopotentiometric stripping analysis) at stationary and chemically modified Hg electrodes (amalgam electrode included) – suitable tool for proteins analysis
- Catalytic signal - **H peak** (discovered due to catalytic hydrogen evolution reaction – CHER; named according J. Heyrovsky) – is sensitive to structural and conformational changes of proteins
- **H peak** (by proteins) used to monitoring of denaturation, agreggation, interaction with low molecular weight ligands or DNA, structural changes as the result of mutation and redox state

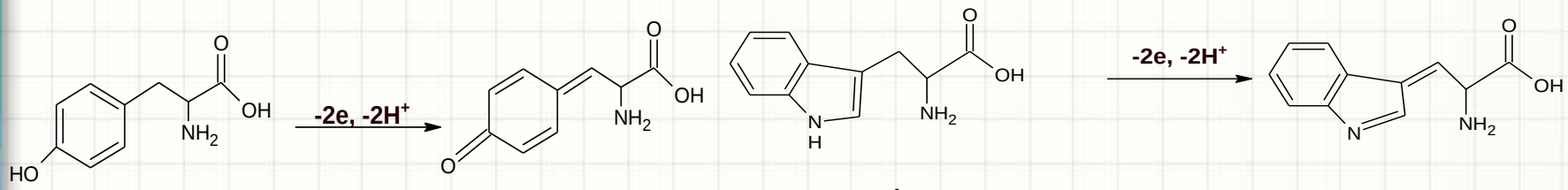
CPSA – chronopotentiometric stripping analysis

$$\frac{dE}{dt} = f(E)$$



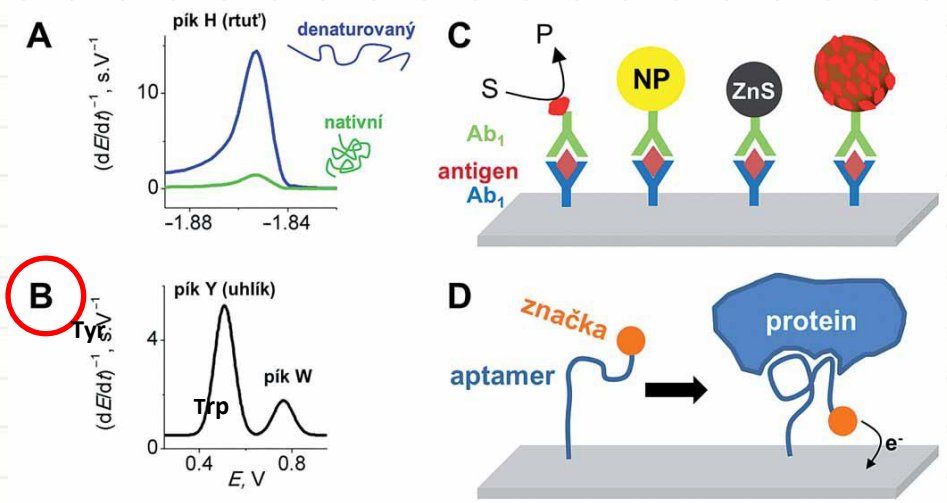
Electrochemical oxidation of proteins

- Free aminoacids (Cys, His, Met, Tyr a Trp) are oxidized at carbon electrodes
- Proteins are oxidized at carbon electrodes (CPSA method)
- Tyr a Trp residues in proteins yield oxidation signals at carbon electrodes → the study of DNA-protein interaction, the resolution of fosforylated and unfosforylated forms, membrane Na-K pump, determination of insuline and α-synuklein (important protein in the Parkinson‘s disease)



Tyrosine

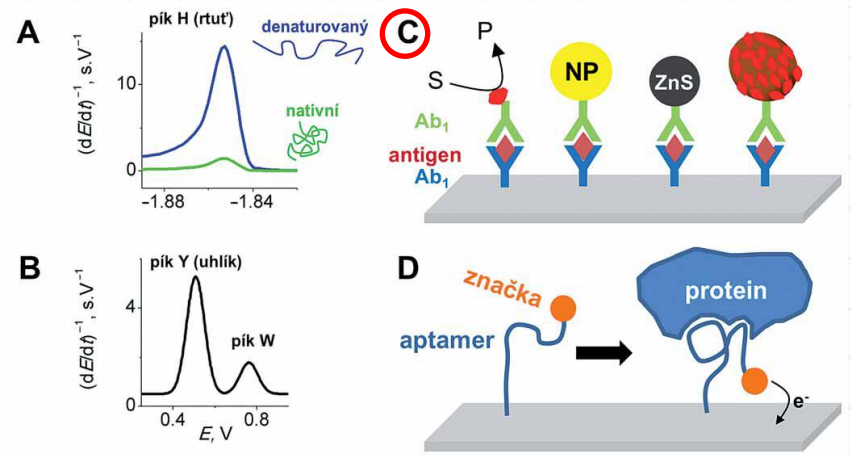
Tryptophan



Bartošík M., Paleček E., Vojtěšek B.: Klin Onkol, 2014, 27 (Suppl 1), S53-S60

Proteins are electroactive

- External protein labeling (sensitive detection of specific proteins in the mixture of other molecules)
  - ✓Immunoassays (ELISA)
- Nanotechnology – nanoparticles, nanotubes



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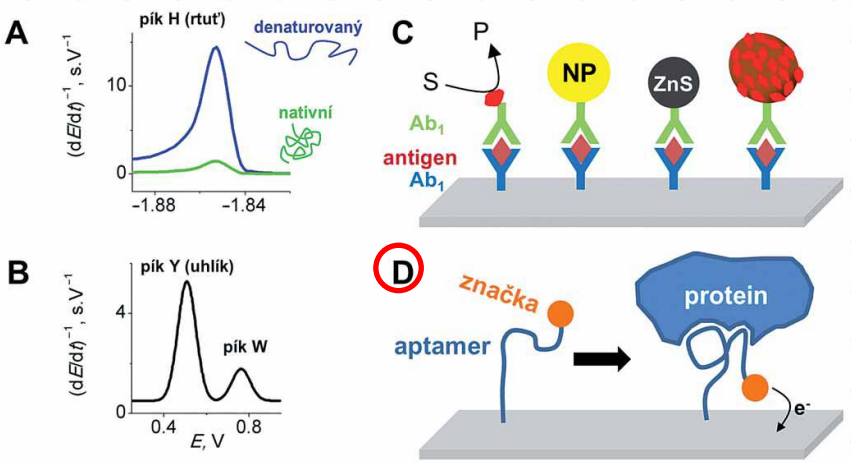
- Aptamers



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Polysaccharides

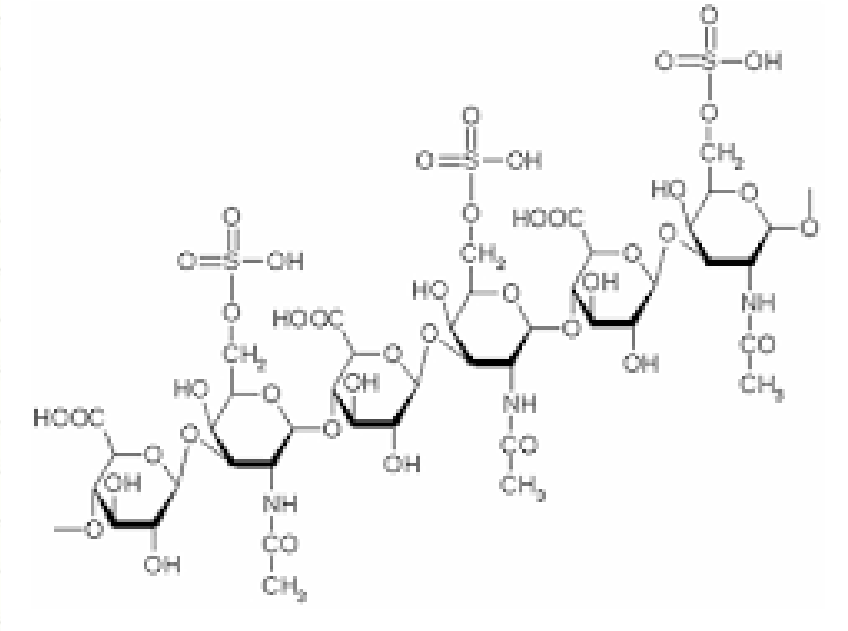
Polysaccharides

• Naturally occuring polysaccharides (PSs) and oligosaccharides (OLSs) are free or fixed on proteins or lipides

•Structural flexibility – ideal indetificators of intermolecular or intercells interactions

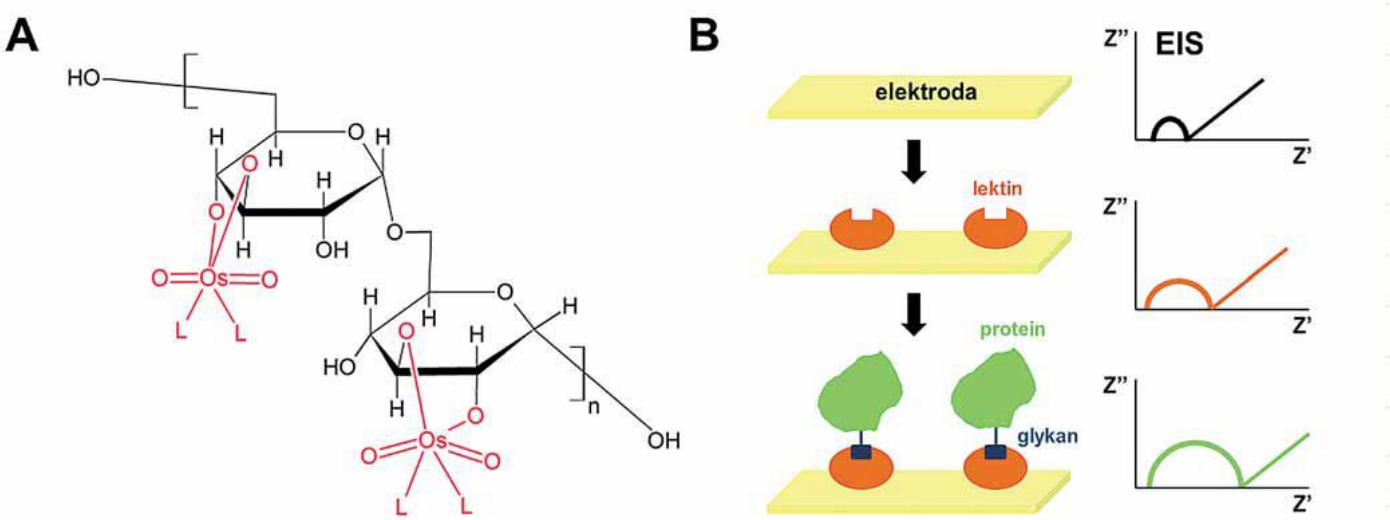
•Most mammalian proteins occur in the form of glycoproteins

•Protein glykosylation in the human health and diaseases (cancer)



Are polysaccharides electroactive?

- Since 2009 PSs and OLSs considered as electrochemical inactive compounds
- **In 2009** – some sulphated PSs catalyze hydrogen evolution reaction and give CPS signals at Hg electrodes
- PSs and OLSs are easy modifiable with Os(VI)L complexes (with nitrogen ligands); electroactive aducts
- **Lectine biosensors** for glycane detection(sugar residues of glycoproteins and glycolipides); electrochemical impedance spectroscopy (EIS) biosensors
  - ✓Easy and quick detection of oncomarkers and other proteins important in biomedicine



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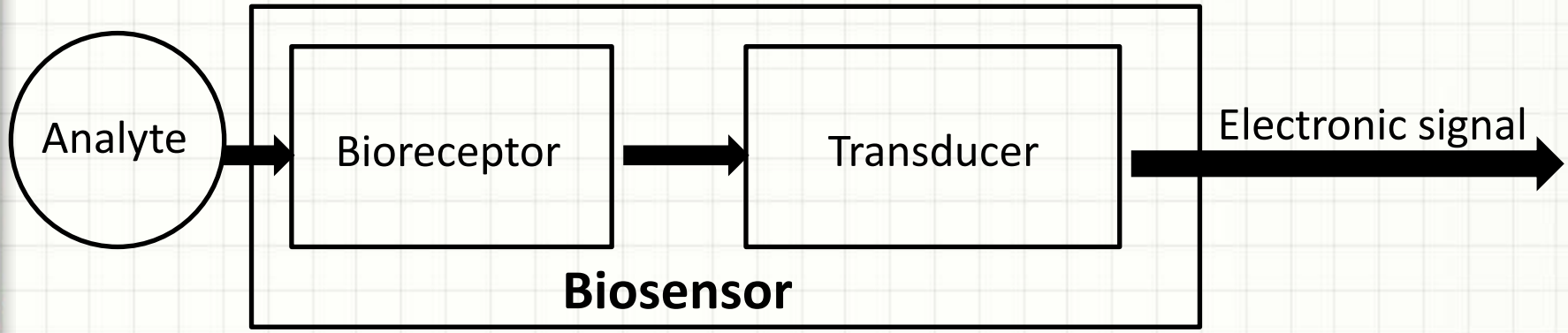
Biosensors

What is biosensor?

• Analytical instrument containing senstivite bioreceptor, which is the part of physicochemical tranducer or it is in the close proximity with physicochemical transducer



•**Bioreceptors**

✓Biocatalytic (enzyme, organela, cell, tissue, organ, organism) – analyte is conversed during chemical reaction

✓ Bioaffinity (lectine, antibody, NA, receptor) – analyte is specifically bound in bioaffinity complex

•**Physicochemical transducers** – electrochemical, optical, piezoelectric and acoustic, calorimetric

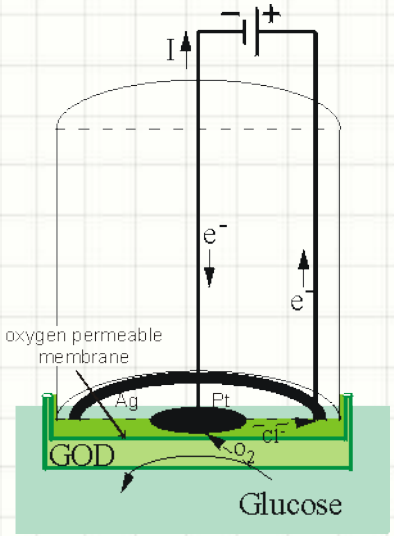
From the history

- **The beginning of the 20th century** – the conception of redox potentials and the fisrt pH measurement
- **1922** – **Jaroslav Heyrovský** – discovery of polarography
- **1935** – **Müller and Bamberger** – measurement of O_2 concentration in biological fluids at Hg electrode
- **1938** – **Petering and Daniels** – measurement of O_2 consumption with living oragnisms at Hg electrode
- **40s of 20th century** – cathodic reduction of O_2 at noble metals (Au, Pt) – bare electrodes lost their lifetime in the biological material
- **1956** – **Leland C. Clark Jr.** – the first membrane electrode permeable for gases → **the birth of biosensors**
- **1962** – **Clark and Lyons** – **enzyme electrode** (experiment with glukose oxidase immobilized on the oxygen electrode surface by the dialysis membrane)
- **60s of the 20th century – ion-selective electrodes (ISE)**
- **70s of the 20th century –** progress in the field of **enzyme electrodes**
- **1975 –** the first commercial biosensor for glukose (Yellow Springs Instrument Company)
- **The end of 70s –** the beginning of the **immunosensors** research
- **Biosensors emerge from the scientific laboratories into the real world!**

Discovery of biosensor

•Clark (1962) – amperometric sensor for glucose with glucose oxidase and oxygen electrode

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



❖**Oxygen electrode (1956)**

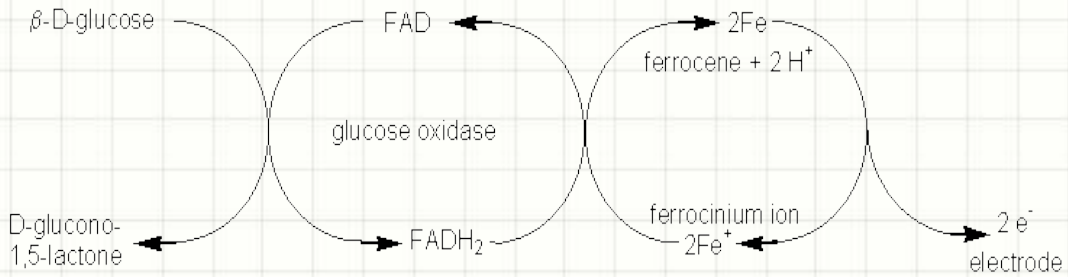
- Working electrode: Pt cathode

  $O_2 + H_2O + 2e^- \rightarrow H_2O_2 + 2OH^-$

  $H_2O_2 + 2e^- \rightarrow 2OH^-$

- Reference electrode: Ag/AgCl electrode

- The electrodes are separated from the measured solution with semipermeable membrane enabling passage of gasis



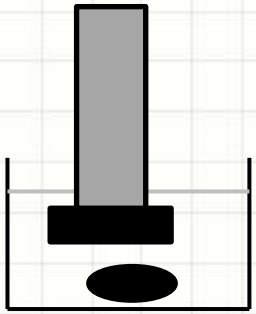
Requirements for biosensors

- Sensitivity
- Calibration
- Linearity
- Limit of detection
- Noise
- Background signal
- Hysteresis
- Long time stability
- Selectivity
- Response rate
- Response time
- Convection rate
- Temperature dependence
- Lifetime of the biosensor
- Biocompatibility

Measurement conditions

• **Direct contact with a sample** – biosensor in the monitored medium (river, tissue, bloodstream)

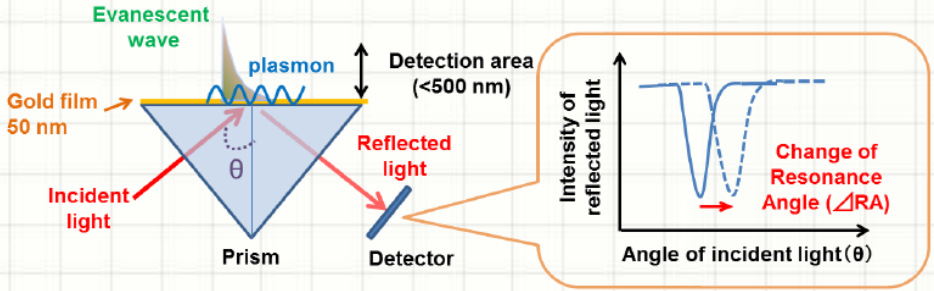
•**Closed vessel**– biosensor in the vessel equipped with the water coat (due to tempering) and magnetic stirrer



•**Flow system** – biosensor in the flowing cell

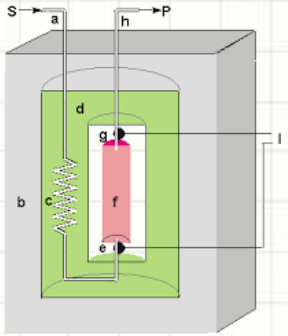
Types of biosensors

- Optical biosensors

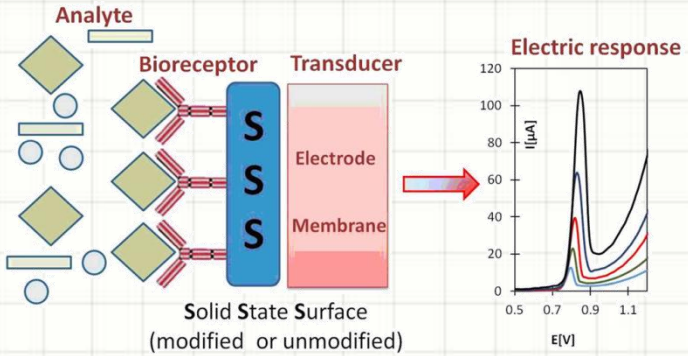


- Piezoelectric biosensors

- Calorimetric biosensors



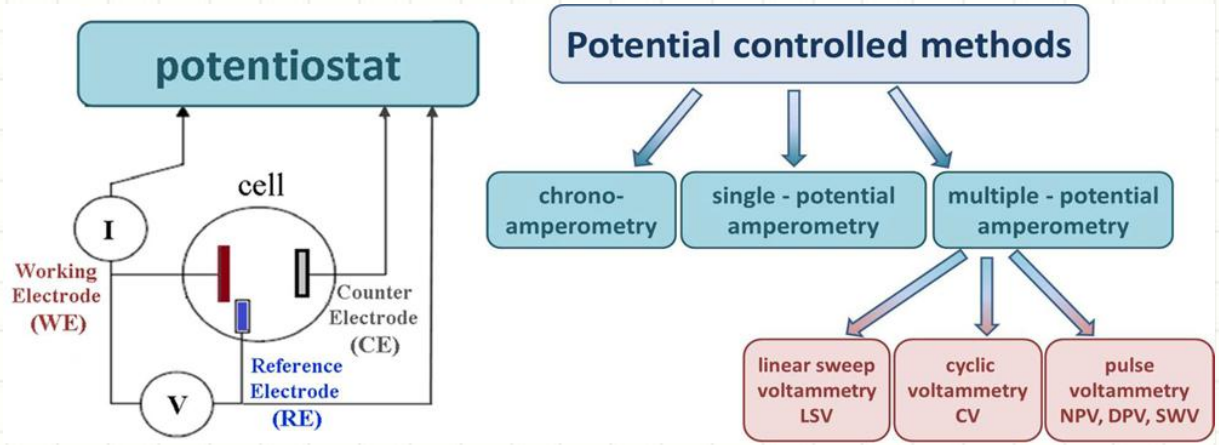
- Electrochemical biosensors

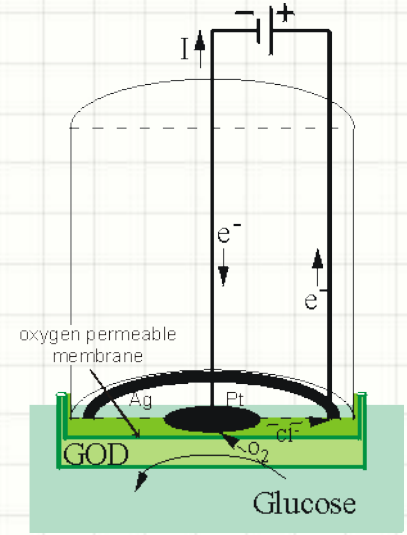


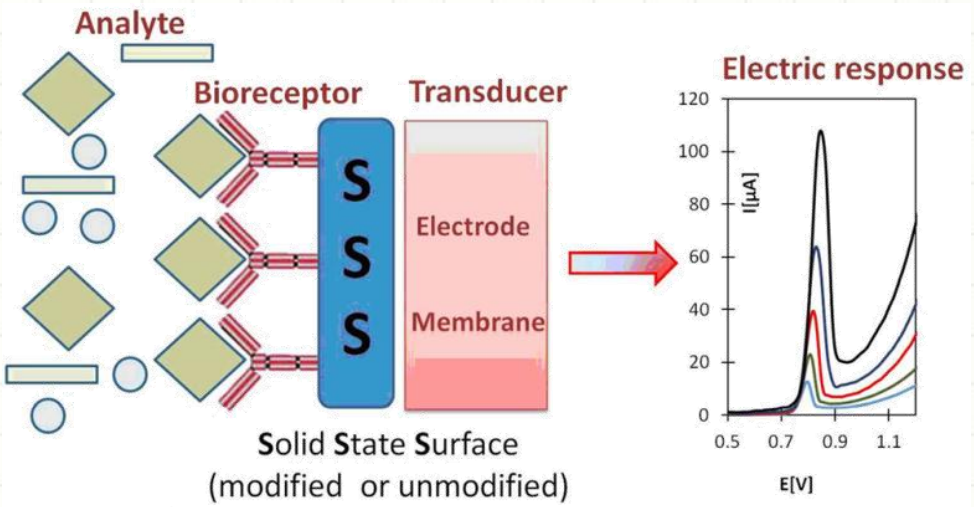
- Enzyme biosensors

Electrochemical biosensors

1) Amperometric and voltammetric biosensors



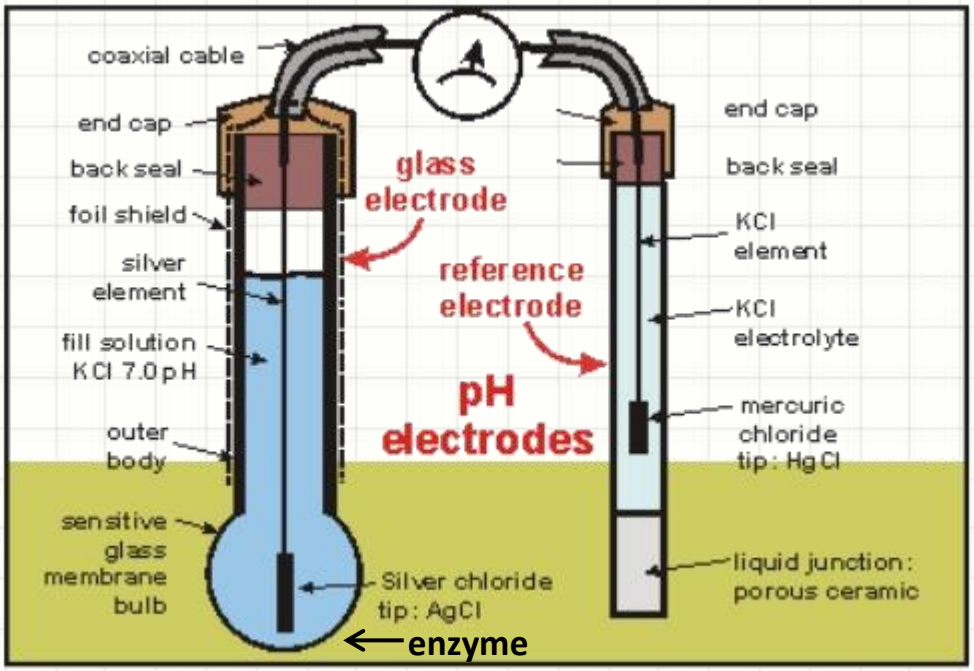




Electrochemical biosensors

2) Potentiometric biosensors

- ISE with enzyme surface

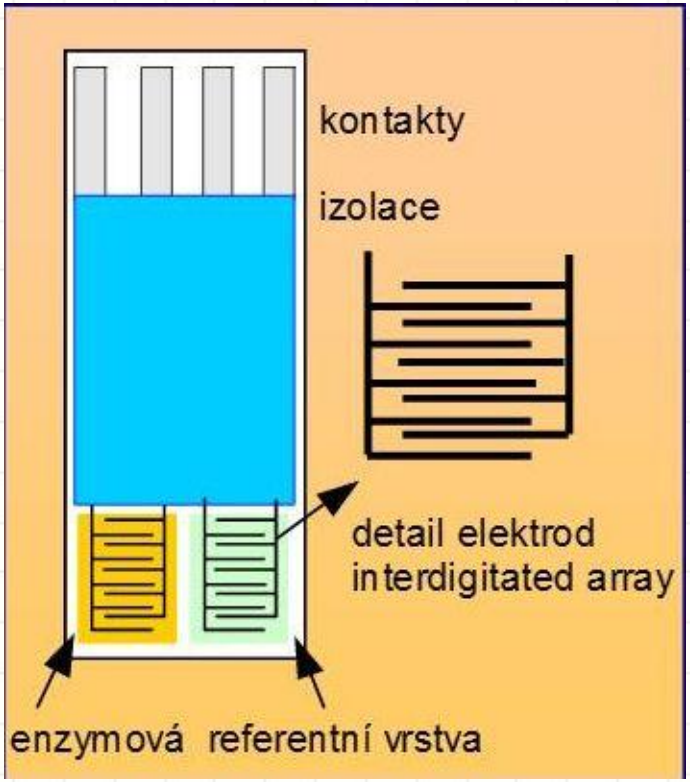


$$E = E^0 + \frac{RT}{nF} \ln\left[a_i + \sum k_{ij} a_j^{z_i / z_j}\right]$$

Nicolsky – Eisenman equation

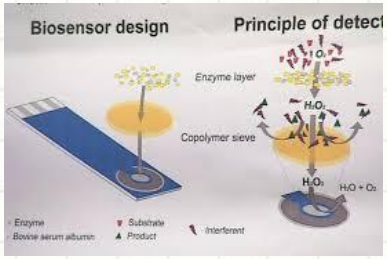
Electrochemical biosensors

3) Conductometric/impedimetric biosensors



Nanoelectrochemistry

Elektrochemické metody využívající modifikace povrhu elektrod nanočásticemi



✓**Voltametrické metody** (klasické elektrody i mikroelektrody)

✓**Potenciometrie** – příprava ISE, senzorického pole a elektronického jazyka

Imobilizace na povrchu elektrod se provádí buď **fyzikální adsorpcí** nebo pomocí **chemického navázání**, kdy modifikované nanočástice reagují s povrchem elektrody, na jejímž povrchu je navázaná vhodná látka

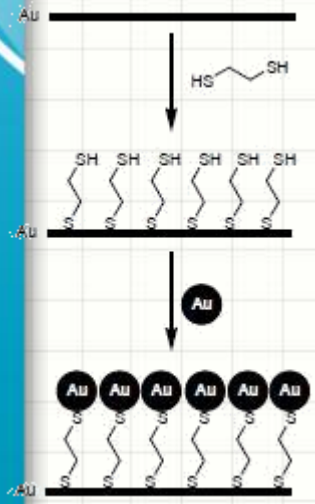


Schéma imobilizace nanočástic zlata na povrch modifikované zlaté elektrody

✓Stanovení dusičnanů, měďnatých iontů, pesticidů a herbicidů ve vodách
✓Biosenzory ve farmacii a lékařství
✓Farmacie – stanovení léčiv
✓Lékařství – analýza biologických vzorků (hemoglobin, cytochrom c, glukosa, peroxid vodíku)
✓DNA diagnostika

Gold nanoparticles

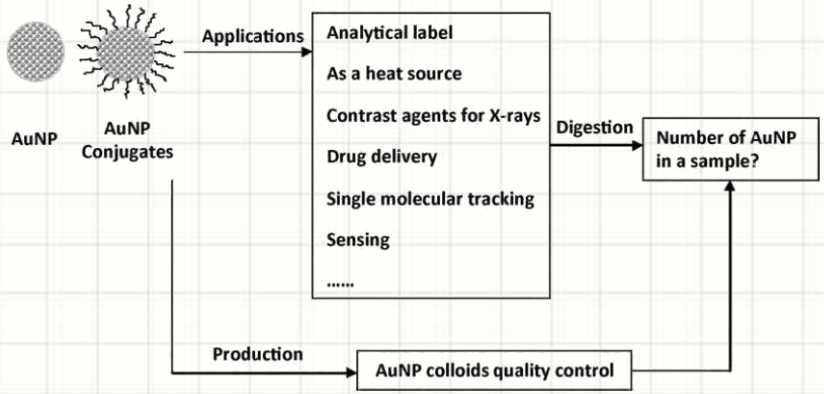
•Attractive electronic, optical, thermal and catalytic properties

•Potential applications in the fields of physics, chemistry, biology, medicine and material science and their interdisciplinary fields

•The unique physical and chemical properties of nanostructured materials provide excellent prospects for interfacting biological recognition events with electronic signal transduction and for designing a new generation of biosensors.

•Especially AuNPs represent excellent biocompatibility and display unique structural, electronic, magnetic, optical and catalytic properties – attractive material for biosensor, chemisensor and electrocatalyst

•The use of AuNPs for amperometric or voltammetric electrochemical nanobiosensors



AuNPs quantitative analysis following its production and application

Gold nanoparticles-based electrochemical sensor and bioelectrochemical sensor

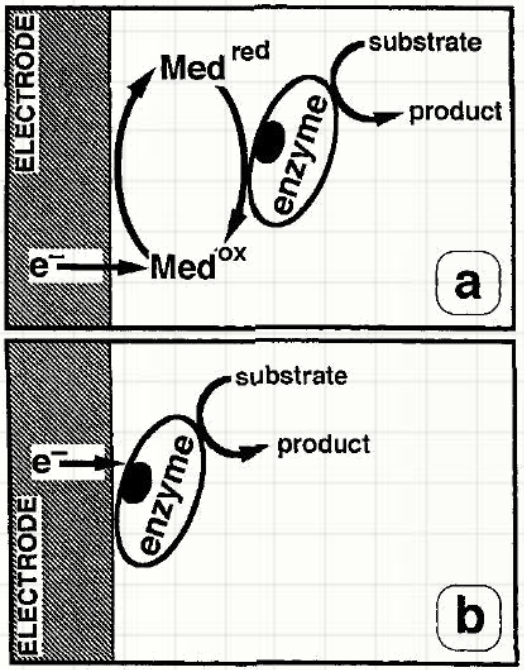
Direct electrochemistry of redox-protein on AuNPs

•Very important subject in bioelectrochemistry – construction of biochemical sensors

•Direct electrochemistry of proteins (very important is establishment of satisfactory electrical communication between the active site of the enzyme and the electrode surface)

•Modification of electrode surfaces with the AuNPs will provide a microenvironment similar to that redox-proteins in native systems and gives the protein molecules more freedom in orientation, thereby reducing the insulating effect of the protein shell through the conducting tunnels of AuNPs

•1996 – Natan and co-workers - electrochemistry of horse heart cytochrome c at SnO_2 electrodes modified with 12 nm AuNPS



Possible ways of coupling an enzymatic and an electrochemical reactions:

a) mediated electron exchange and

b) direct, mediatorless, electron transfer.

Gold nanoparticles-based electrochemical sensor and bioelectrochemical sensor

Direct electrochemistry of redox-protein on AuNPs

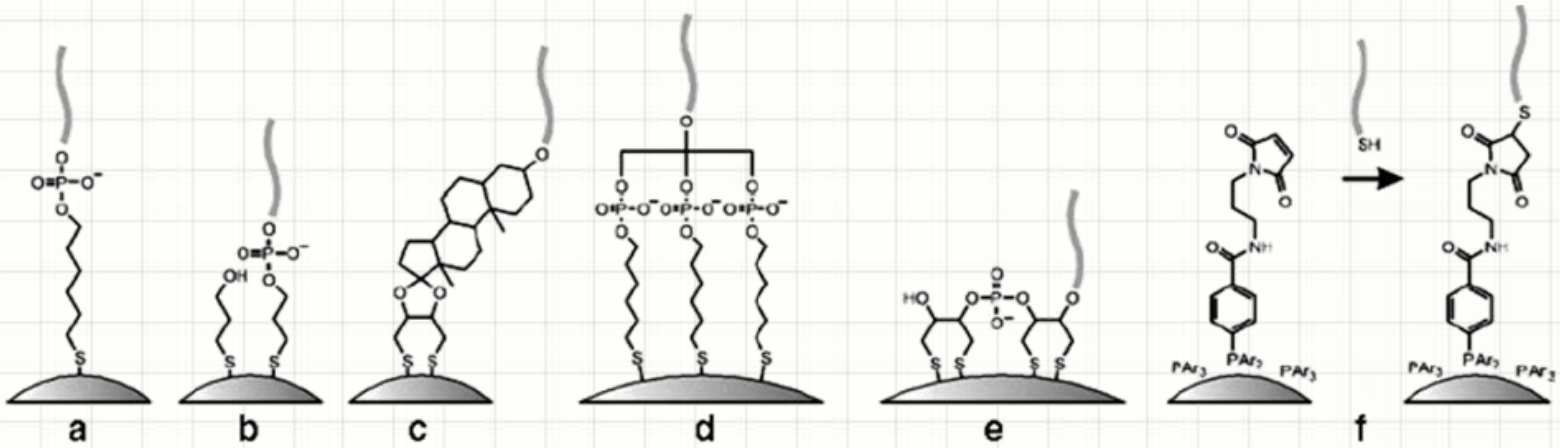
- The nanoparticle/protein conjugates can be assembled on the electrode via self-assembly technology – the AuNPs could be immobilized on the self-assembled monolayer and complete DET
  - DET of hemoglobin on the citrated-capped AuNPs assembled on a cysteamine modified gold substrate
  - Investigation of electrocatalytic activity of NPs/hemoglobin electrode towards H_2O_2 reduction
  - DET of glucose oxidase and HRP on AuNPs immobilized cysteinamine modified gold electrode
- The AuNPs modifed carbon paste electrodes have provided a good microenvironment for completing the DET of different redox-proteins
  - DET between immoblized myoglobin and colloidal gold modified carbon paste electrode
  - Xanthin oxidase biosensor, based on a carbon paste electrode modified with electrodeposited AuNPs for the amperometric determination of hypoxanthine
- The polymer-nanoparticles composites possess the interesting electrical, optical and magnetic properties superior to those of the parent polymer and nanoparticles
- The nanocomposite composed of AuNPs and biopolymer such as chitosan as excellent matrix for completing the DET of some redox protein
  - Biocomposite made of chitosan hydrogel, GOD and AuNPs for glucose biosensor

Gold nanoparticles in DNA immobilization

•AuNPS can strongly adsorb DNA

•DNA can also be immobilized onto AuNPs through special functional groups such as thiols and others, which can interact strongly with AuNPs

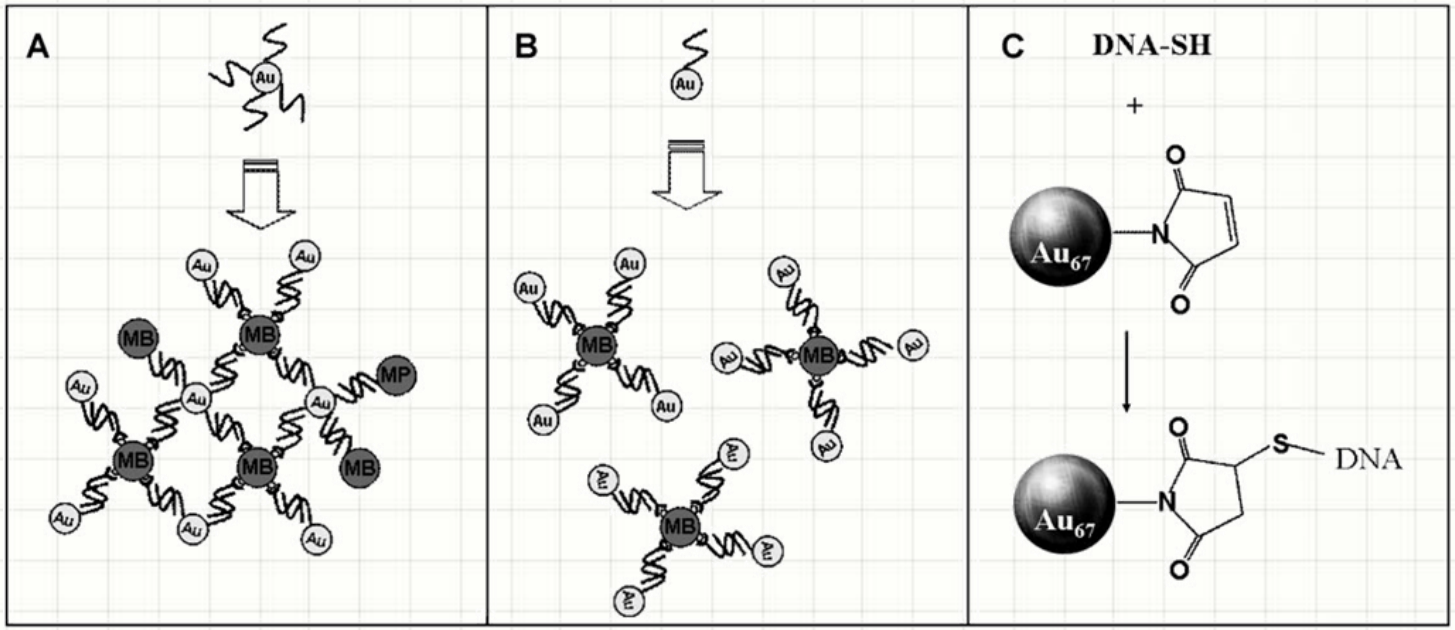
•DNA oligonucleotides that contain several adenosyl phosphothiolate residues at their ends have been used to interact with the metal surface of NPs



Schematic of the methods used for conjugating oligonucleotides to gold nanoparticles. a) Thiol-modified and b) disulfidemodified oligonucleotides spontaneously bind to gold nanoparticle surfaces. Asymmetric disulfide modification adds an additional mercaptoalcohol ligand to the Au surface, but the density of oligonucleotides formed on the nanoparticle surface is the same as for thiol-terminal oligonucleotides. c) Di and d) trisulfide modified conjugates. e) Oligothiol – nanoparticle conjugates. Although four thiol connections are shown, any number are possible via sequential addition of a commercial dithiane phosphoramidite during solid-phase oligonucleotide synthesis. f) Oligonucleotide conjugates from NanoprobesQ phosphine-modified nanoparticles. Adapted from Nanotechnology, 2003, 14, R63.

Gold nanoparticles in DNA immobilization

• Monomaleimido gold clusters have been coupled with thiolated DNA oligomers to synthesize probes for homogenous nucleic acid analyses and ensure a 1:1 DNA/AuNP connection with interest for sensitivity improvements



Schematics of A) Formation of particle-linked DNA network structure due to the interconnection between magnetic beads in the case where AuNPs modified with more than one DNA strands are used; B) The previous network is not created by using the 1:1 Au-DNA connection; C) The reaction of maleimido-Au67 with thiol-oligonulceotide that make possible the 1:1 Au-DNA connection. Adaped from Langmuir , 2005, 21, 9625

Gold nanoparticles-based electrochemical sensor and bioelectrochemical sensor

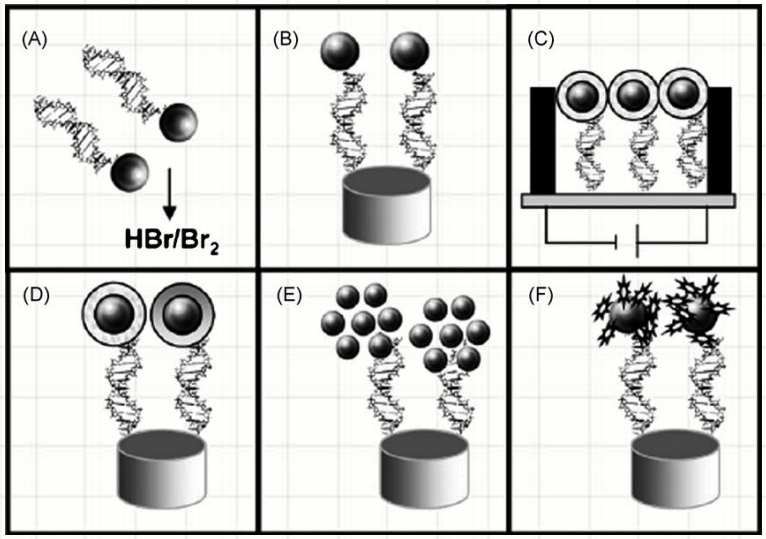
Gold nanoparticles for genosensors

• The development of electrical DNA hybridization biosensors has attracted considerable research efforts

•The AuNPs modified electrochemical sensing interfaces offer elegant ways for interacting DNA recognition events with electrochemical signal transduction, and for amplyfying the resulting electrical response

•AuNPs –based electrochemical device will provide new opportunity for gene diagnostics

•Merkoci and co-workers reviewed recent important achievements on the electrochemical sensing of DNA using AuNPs



Schematic procedure of the different strategies used for the integration of AuNPs into DNA sensing systems: (A) previous dissolving of AuNPs by using HBr/Br2 mixture followed by Au(III) ions detection, (B) direct detection of AuNPs anchored onto the surface of the genosensor, (C) conductometric detection, (D) enhancement with silver or gold followed by detection, (E) AuNPs as carriers of other AuNPs, (F) AuNPs as carriers of other electroactive labels. Reprinted with permission from Ref. [85], A. Merkoc¸i, 19 (2007) 743. Copyright Wiley-VCH (2007).

Gold nanoparticles-based electrochemical sensor and bioelectrochemical sensor

Gold nanoparticles for genosensors

- The use of colloidal gold tags for electronic detection of DNA hybridization – capturing the AuNPs to the hybridized target, followed by highly sensitive anodic stripping electrochemical measurement of the metal tracer
- Due to the toxicity of HBr/Br_2 solution the novel AuNPs –based protocol was reported – a novel AuNPs – based protocol for detection of DNA hybridization based on magnetically trigged direct electrochemical detection of gold quantum dot tracers
- Enhancement by precipitation of silver or gold onto the AuNPs for amplifying signals and lowering detection limits
- Analyzing sequence-specific DNA using AuNPs marked DNA probes and subsequent signal amplification step by silver enhancement (electrostatic adsorption of target ODNs onto the sensing surface of the GCE and its hybridization to the AuNPs-labeled ODNs DNA probe)
- Another signal amplification strategy is to attach electroactive ferrocenylhexanethiol molecules or electrogenerated chemiluminiscence indicator to the AuNPs labels
    - The AuNPs/streptavidin conjugates covered with 6-ferrocenylhexanethiol were attached onto a biotinylated DNA detection probe of a sandwich DNA complex
- DNA ultrasensitive electrochemical detection by using AuNPs will play an important role on the development of specific and sensitive assays for clinical diagnosis, detection of pathogenic microorganisms

Gold nanoparticles-based electrochemical sensor and bioelectrochemical sensor

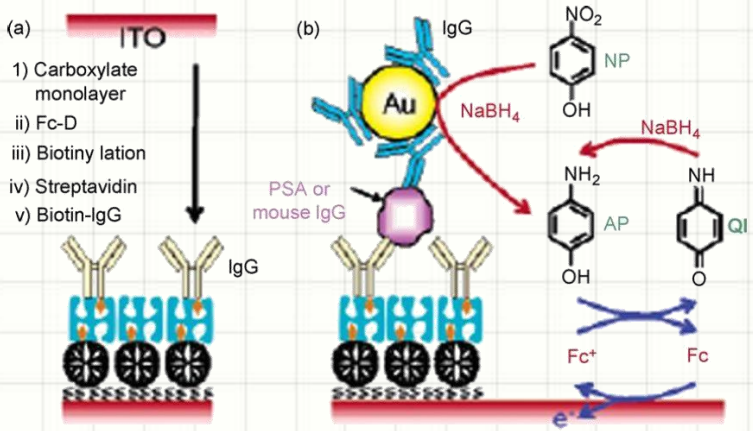
Gold nanoparticles for immunosensors

•Immunosensors are important analytical tools based on the detection of the binding event between antibody and antigen

•Electrochemical immunosensors are attractive tools and have received considerable attention becuase they are easy, economy, robust and achieve excellent detection limits with small analyte volumes

•Several strategies have been proposed to develop electrochemical immunosensors with high sensitivity using AuNPs

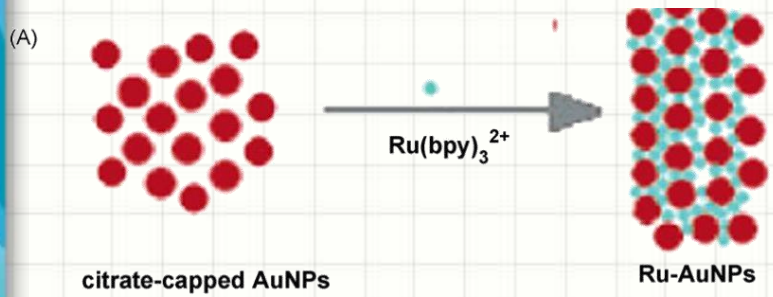
•DNA –free ultrasensitive electrochemical immunosensors have received considerable interests because of their simplify, rapidness and high sensitivity



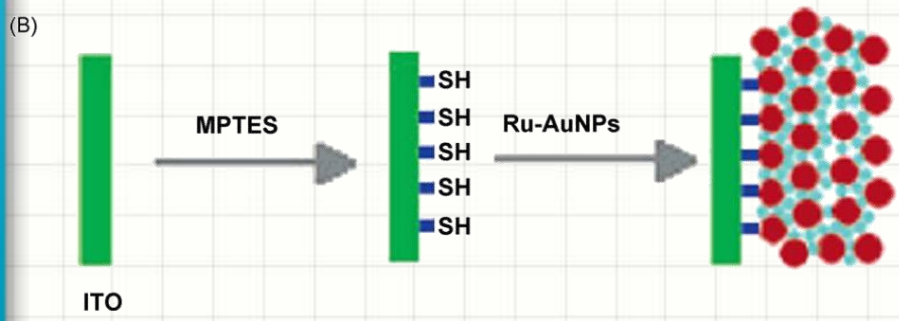
(a) Schematic representation of the preparation of an immunosensing layer. (b) Schemiatic view of electrochemical detection of mouse IgG or prostate specific antigen. Reprinted with permission from Ref. [139], H. Yang, J. Am. Chem. Soc. 128 (2006) 16022

Gold nanoparticles as enhancing platform for electrocatalysis and electrochemical sensor

•AuNPs have been studied extensively for the design and fabrication of electrocatalysts and using as an enhancing component of catalytic activity or selectivity



Scheme illustrating (a) the formation of Ru-AuNPs in aqueous medium due to electrostatic interactions between Ru(bpy)$_3^{2+}$ and citrate-capped AuNPs and (b) the immobilization of Ru-AuNPs on a sulfhydryl-derivated ITO electrode surface.



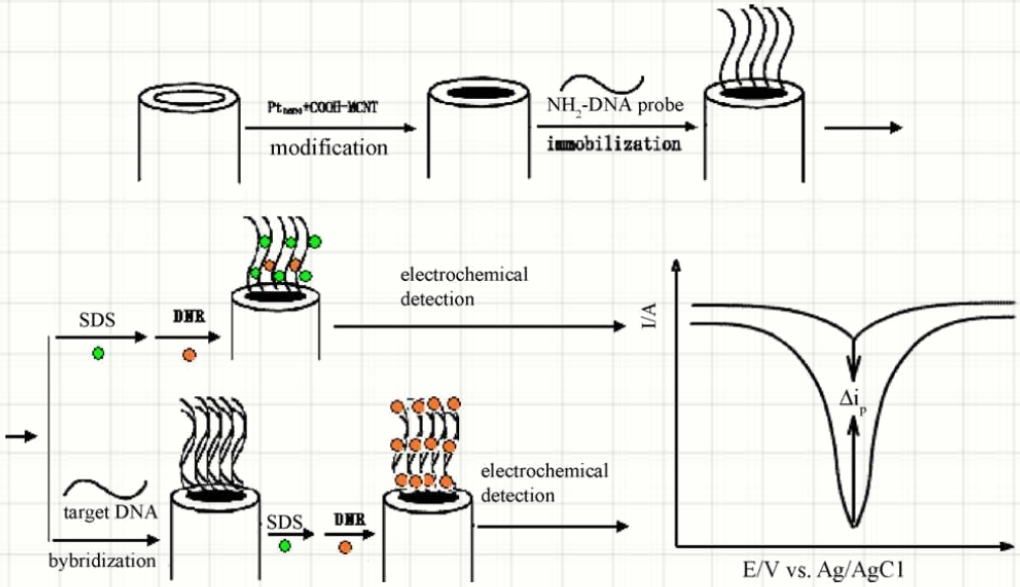
Platinum nanoparticles

•Platinum nanoparticles have been an intensive research subject for the design of electrodes

•Platinum films modified microelectrodes were shown to be excellent amperometric sensor for H_2O_2 in a wide range of concentration

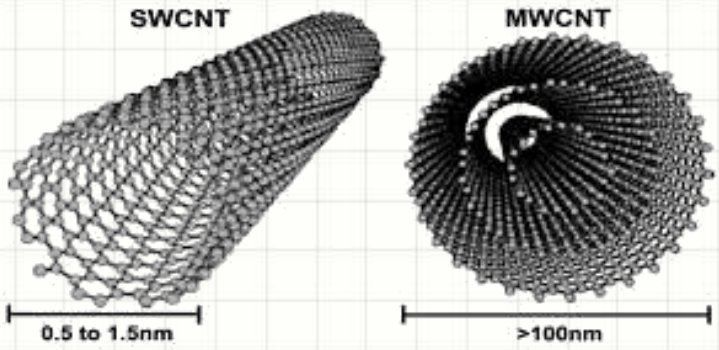
•Pt nanoparticles and single walled carbon nanotubes were combined to modify a glassy carbon electrode to improve their electroactivity for H_2O_2 (glucose sensor)

•Pt nanoparticles were used in combination with multi-walled carbon nanotubes (MWCNTs) for fabricating sensitivity-enhanced electrochemical DNA biosensor



Carbon nanotubes (CNTs)

- CNTs are built from sp^2 carbon units – a seamless structure with hexagonal honeycomb lattices
- Carbon nanotubes with one hundered time the tensile strength of steel, thermal conductivity, electrical conductivity similar to copper, but with the ability to carry much higher currents, they seem to be very interesting material
- Since their discovery in 1991, CNTs have generated great interest for applications based on their field emission and electronic transport properties, their hiugh mechanical strength and chemical properties
- CNTs appliccation in the field of emission devices, nanoscale transistors, tips for scanning microscopy or components for composite materials
- **Two groups of CNTs**: A) multi-wall carbon nanotubes (MWCNTs) – concentric and closed graphite tubules with multiple layers of graphite sheet
  B) single-wall carbon nanotubes (SWCNTs) – single graphite sheet rolled seamlessly, defining a cylinder of 1-2 nm diameter



Schematic representation of Single Walled Carbon Nanotube (SWCNT) and Multi Walled Carbon Nanotube (MWCNT)

Carbon nanotubes

- CNTs exhibits strong electrocatalytic activity for a wide range of compounds, such as neurotransmitters NADH, hydrogen peroxide, ascorbic, cytochrome c, hydrazines, hydrogen suphide, amino acids, glucose and DNA
- Various types of CNT modified electrodes were prepared including physical adsorption of CNT onto electrode surface, like glassy carbon and composite paste electrodes
- CNT was incorporated into an epoxy polymer, forming an epoxy composite hybrid material as a new electrode with improved electrochemical sensing properties (CNTEC – carbon-nanotube epoxy composite)
- SWCNT-glassy carbon modified electrode for the highly sensitive and selective detection of dopac in the presence of 5-hydroxytryptamin
- SWCNT film modified glassy carbon electrodes towards the reduction/oxidation of cytochrome c
- MWCNT biosensor was obtained by means of GOx immobilization through physical entrapment inside and epoxy resin matrix and its performance was examined for glucose determination
- MWCNTEC modified with bacterial cells for application as a microbial sensor
- CNTPE (carbon nanotubes paste electrode) for determination of dopamine, ascorbic acid, dopac, uric acid and hydrogen peroxide