Elektrochemie nukleových kyselin a nekonjugovaných bílkovin.

Uplatní se elektroaktivita bílkovin v proteomice a biomedicíně?

Emil Paleček

BIOFYZIKÁLNÍ ÚSTAV AVČR, v.v.i., 612 65 Brno

G.J. Mendel
1866

In my talk I wish to

- summarize the history of electrochemical analysis of NAs, electroactivity of and DNA chemical modification with electroactive markers
- show that thiolated DNAs form densely packed SAMs at mercury electrodes
- turn your attention to the electroactivity of proteins and show that
- non-conjugated proteins can be determined at carbon and Hg electrodes
- changes in protein structure and redox states and
- DNA-protein interactions

can be studied by electrochemical methods.

Examples of application of electrochemical analysis in biomedicine will be shown

The Human Genome Project

The availability of genetic sequence information in databases has gradually shifted genome-based research away from pure sequencing toward

gene function-oriented studies

Gene sequence data alone are of little clinical use unless directly linked to sickness relevance

DNA-based biochips

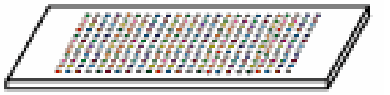
used at present for two types of analysis
A. detection of mutations in specific genes as diagnostic markers
B. detection of differences in gene expression levels,in health and disease
- diagnostic tools
- basis for new therapeutic approaches (new targets for drug therapy present only in sick cells - e.g. activation of protooncogenes during malignant transformation)

Affymetrix - Gene Chips
Hyseq - HyX Gene Discovery Modul
Caliper Lab Chips - microfludic technology to manipulate minute volumes of liquids on chips

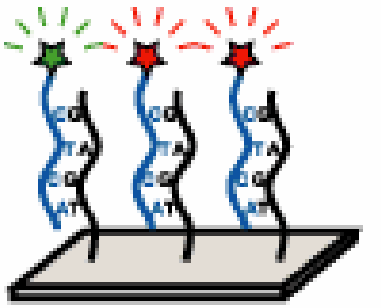
Detection based on fluorescence

Progress in genomics affects electroanalysis

Many areas of science are influenced by the **fast development of the genomics** and by the **success of the Human Genome Project.**

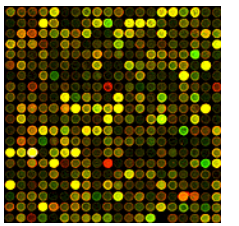


Classical sequencing of individual human genomes with $3x10^9$ base pairs is too **difficult.**

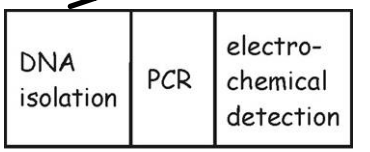


Sequencing by DNA hybridization is gaining importance

Relatively expensive DNA hybridization **ARRAYS** with **optical detection** are **currently applied** in research labs
It is believed that **electrochemistry** can complement the optical detection providing **new LESS EXPENSIVE hybridization detection for decentralized DNA analysis in many areas of practical life**



LOW DENSITY CHIPS



LAB-ON-A-CHIP

DNA and RNA are Electroactive Species

producing faradaic and other signals on interaction with electrodes

Cytosine (C)
Adenine (A) **A, C, G are reduced at MERCURY electrodes**
Guanine (G) **reduction product of guanine is oxidized back to G**

All bases (A, C, G, T, U) yield sparingly soluble compounds with the mercury and can be determined at concentration down to 10^{-11}M.
Solid amalgam electrodes can be used instead of the mercury drop electrodes.

A and G as well as C and T are oxidized at CARBON electrodes

PEPTIDE NUCLEIC ACID (PNA) BEHAVES SIMILARLY TO DNA AND RNA

Microliter volumes of the analyte are sufficient for analysis

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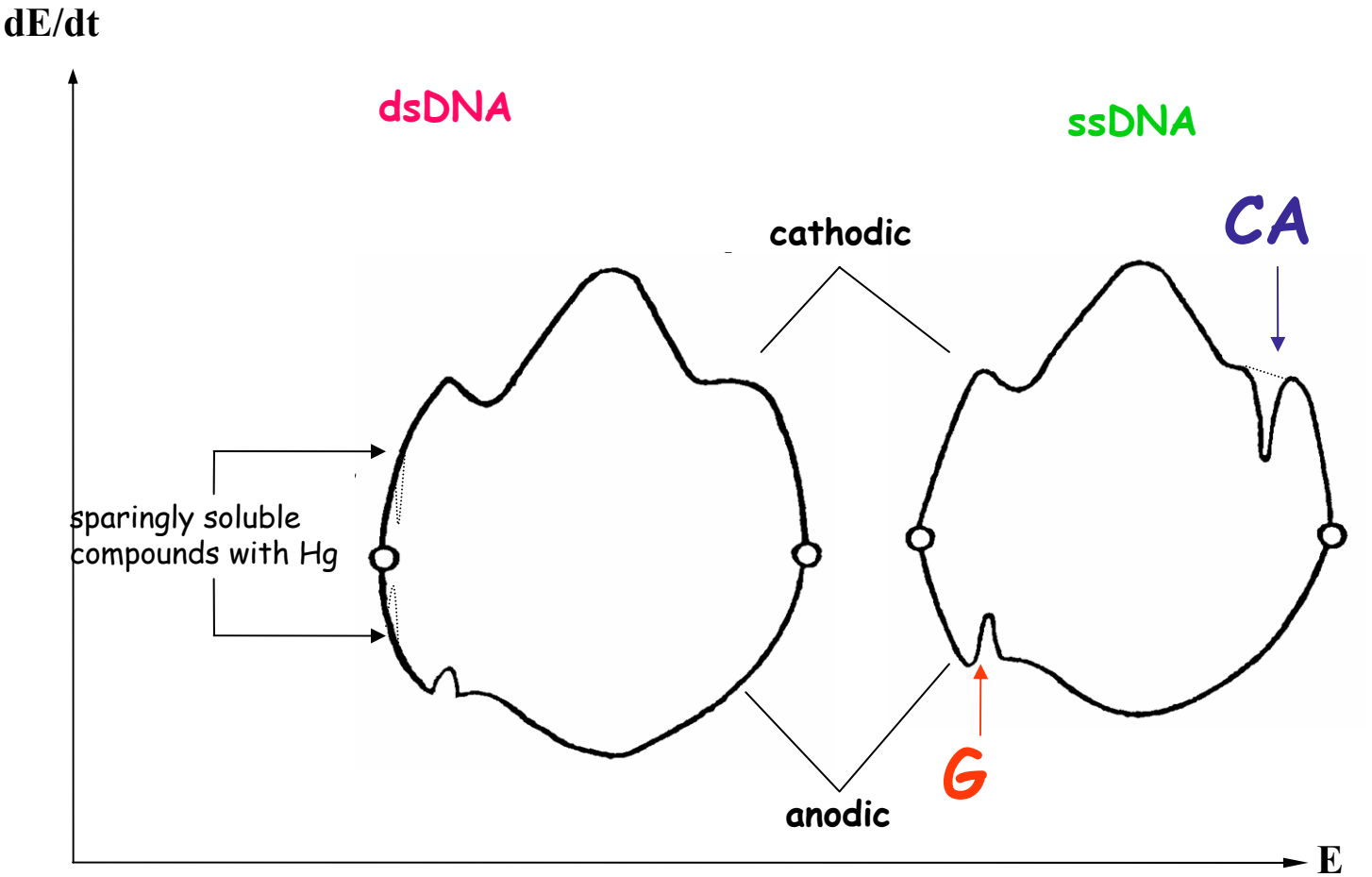
Electroactive Labels can be Introduced in DNA

The results of the DNA electrochemistry studies and development of the electrochemical DNA hybridization sensors in the last decade suggest that these sensors can complement DNA sensors with optical detection

How and when the DNA electrochemistry begun?

OSCILLOGRAPHIC POLAROGRAPHY

At controlled alternating current (constant current chronopotentiometry)



LITERATURE in 1958: Adenine is polarographically reducible at strongly acid pH while other NA bases as well as DNA are inactive

J.N.Davidson and E.Chargraff: *The Nucleic Acids*, Vol. 1, Academic Press, New York 1955

Palecek E.: Oszillographiche *Polarographie der Nucleinsauren und ihrer Bestandteile*; **Naturwiss**. 45 (**1958**), 186
Palecek E.: Oscillographic *polarography of highly polymerized deoxyribonucleic acid*; **Nature** 188 (**1960**), 656

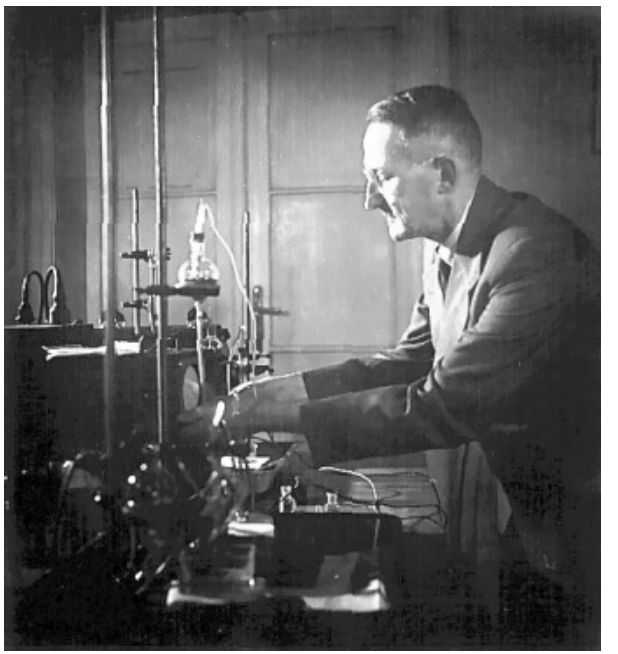
J. Heyrovsky invented **POLAROGRAPHY** in 1922.
After 37 years he was awarded a Nobel Prize

In difference to most of the electrochemists I met in the 1960's and 1970's, J Heyrovsky was interested in nucleic acids and he greatly stimulated my polarographic studies of DNA

Nobel Prizes 1959

J Heyrovsky S Ochoa A Kornberg

J. Heyrovský



DNA Premelting and Polymorphy of the DNA Double Helix

Before my departure to the US I observed Changes in the polarographic behavior of DNA far below the denaturation temperature. These changes were later called DNA Premelting

J. Mol. Biol.
20 (1966) 263-281

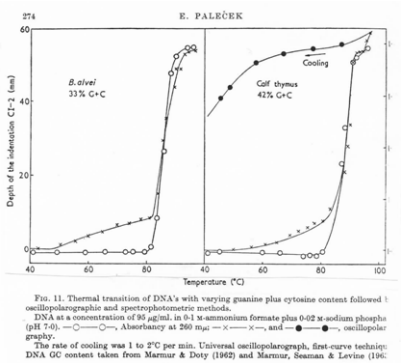


FIG. 11. Thermal transition of DNA's with varying guanine plus cytosine content followed by oscillopolarographic and spectrophotometric methods.
DNA at a concentration of 95 μg/ml. in 0·1 M-ammonium formate plus 0·02 M-sodium phosphate (pH 7·0). —○—○—, Absorbancy at 260 mμ. —×—×—, and —●—●—, oscillopolarography.
The rate of cooling was 1 to 2°C per min. Universal oscillopolarograph, first-curve technique. DNA GC content taken from Marmur & Doty (1962) and Marmur, Seaman & Levine (196

POLAROGRAPHIC BEHAVIOR OF dsDNA
At roomand premeltig temperaturse depended on DNA nucleotide SEQUENCE

B. sublilis and B. brevis DNAs have the same G+C content and different nucleotide sequence

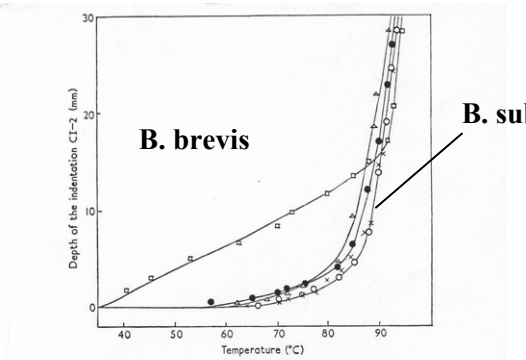
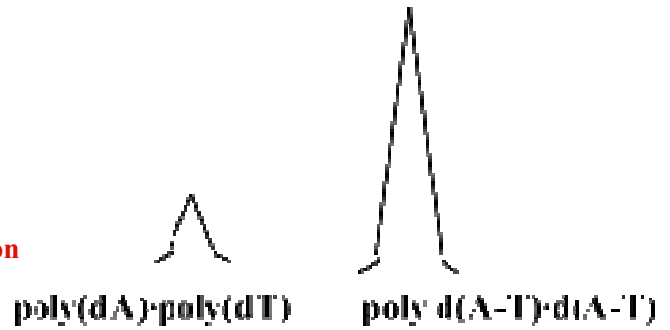


FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 μg/ml. in 0·25 M-ammonium formate plus 0·025 M-sodium phosphate (pH 7·0).
—●—●—, *B. subtilis* 168; —×—×—, *B. natto*; —○—○—, *B. subtilis* var. *niger*; —△—△—, *B. subtilis* var. *aterrimus*; —□—□—, *B. brevis* (ATCC 9999).
P 524 polaroscope, dropping mercury electron polarized with repeated cycles of A.C. The measurements were carried out in the laboratory of Prof. J. Marmur, Department of Biochemistry, Brandeis University, Waltham, Mass., U.S.A.



What the people said

Before 1980
No doubt that this electrochemistry must produce artifacts because we know well that the DNA double helix has a unique structure INDEPENDENT of the nucleotide SEQUENCE

After 1980
Is not it strange that such an obscure technique can recognize POLYMORPHY OF THE DNA DOUBLE HELIX

1976

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ed. 1976
ACADEMIC PRESS, INC
New York San Francisco London

Premelting Changes in DNA Conformation

E. PALEČEK

6. POLYMORPHY OF DNA SECONDARY STRUCTURE

On the basis of the preceding discussion, a schematic picture of the structure of natural linear DNA in solution under physiological conditions (e.g., at 36°C, moderate ionic strength, and pH 7) can be drawn. We can assume that the double-helical structure of the very long (A + T)-rich regions differs from the structure of the major part of the molecule and that some of the (A + T)-rich segments are open (Fig. 20). An open ds-structure can be assumed in the region of chain termini and/or in the vicinity of ss-breaks and other anomalies in the DNA primary structure. The exact changes in the open ds-regions will depend on the nucleotide sequence as well as on the chemical nature of the anomaly. Most of the molecule will exhibit an average Watson–Crick B-structure with local deviations given by the nucleotide sequence. Elevating the temperature in the premelting region (Fig. 20) is likely to lead to the opening of other regions and, eventually, to expansion of the existing distorted ds-regions and to further structural changes. Thus the course of the conformational changes as a function of temperature (premelting) will be determined by the distribution of the nucleotide sequences and anomalies in the primary structure, and may have an almost continuous character.

Consequently, even if we do not consider "breathing," not only the architecture of a DNA double-helical molecule, but also its mechanics or dynamics can be taken into account.

To determine whether, e.g., only the (A + T)-rich molecule ends will be open at a certain temperature or also long A + T regions in the center of the molecule, further experimental research with better-defined samples of viral and synthetic nucleic acids will be necessary. Further work will undoubtedly provide new information on the details of the local arrangement of nucleotide residues in the double helix, as well as on DNA conformational motility. Thus a more accurate picture of DNA structure will emerge, whose characteristic feature will be polymorphy of the double helix, in contrast to the classical, highly regular DNA structure models.

December 3, 1976

Professor Emil Palecek
Institute of Biophysics
Czechoslovak Academy of Sciences
Brno 12, Kralovopolska 135
Czechoslovakia

Dear Professor Palecek,

I do apologise for taking so long to reply to your letter of September 29 and the very interesting review you sent with it. Unfortunately I myself will not be able to attend the Symposium you plan for September, 1977 and my Cambridge colleague Aaron Klug tells me that he too is unable to be present. Had you considered the possibility of asking Dr. Hank Sobell? He has just published in PNAS an account of the other (base-paired) kink and has ideas about premelting conformations. I have no idea whether he would be able to come but should you wish to invite him his address is: Department of Chemistry, The University of Rochester, River Station, Rochester, New York 14627.

Yours sincerely,

Francis Crick

F. H. C. Crick
Ferkauf Foundation Visiting Professor

FHCC:lt

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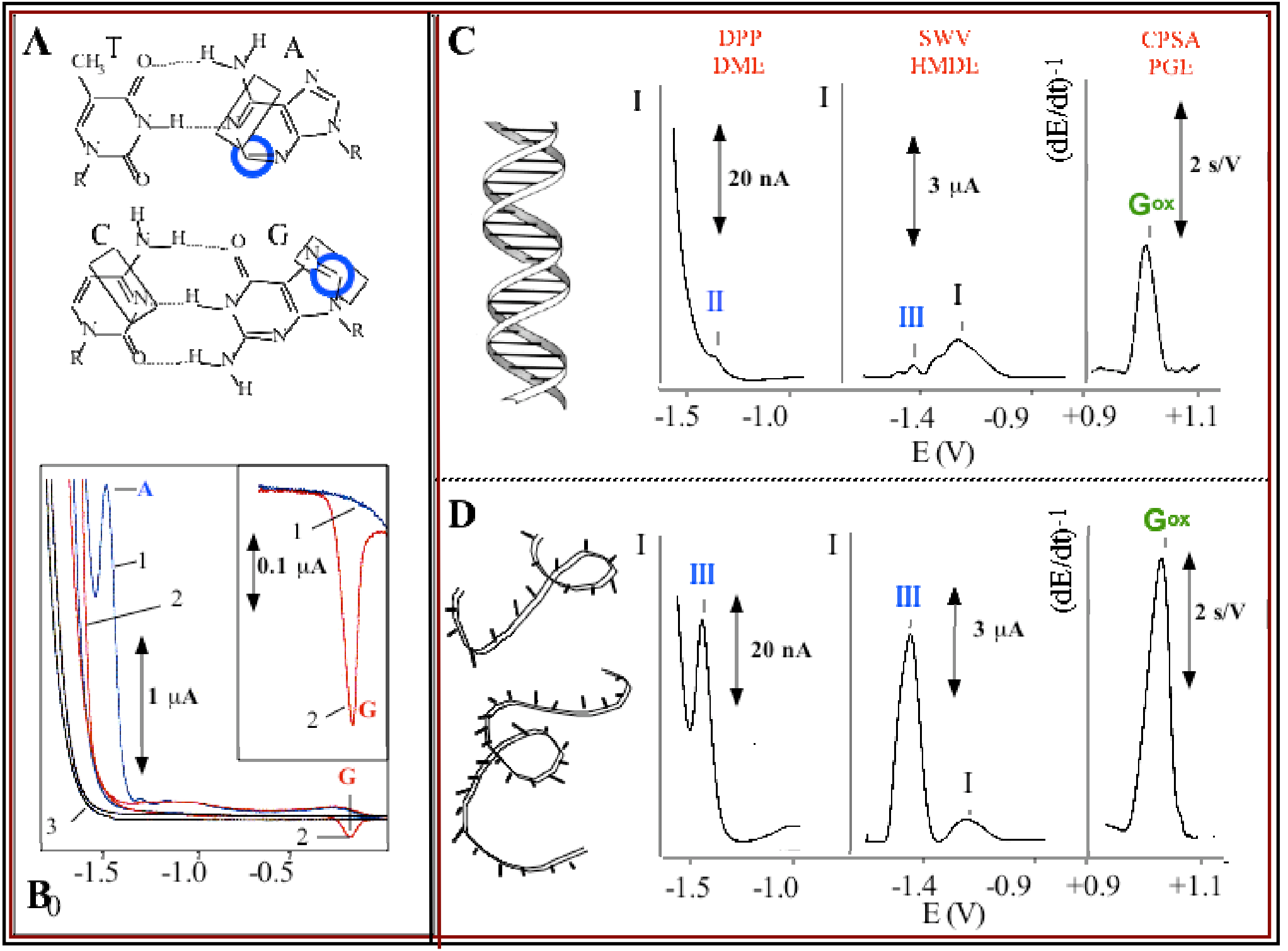
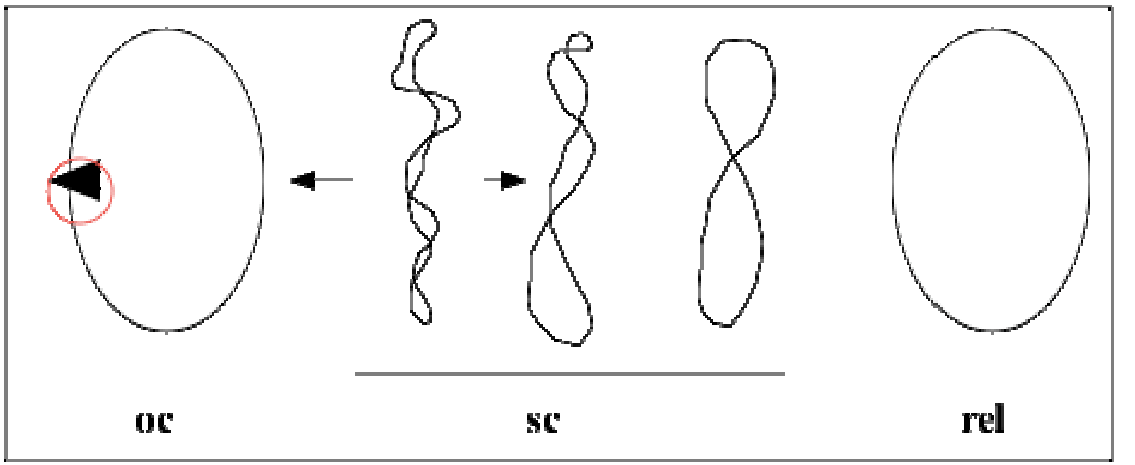
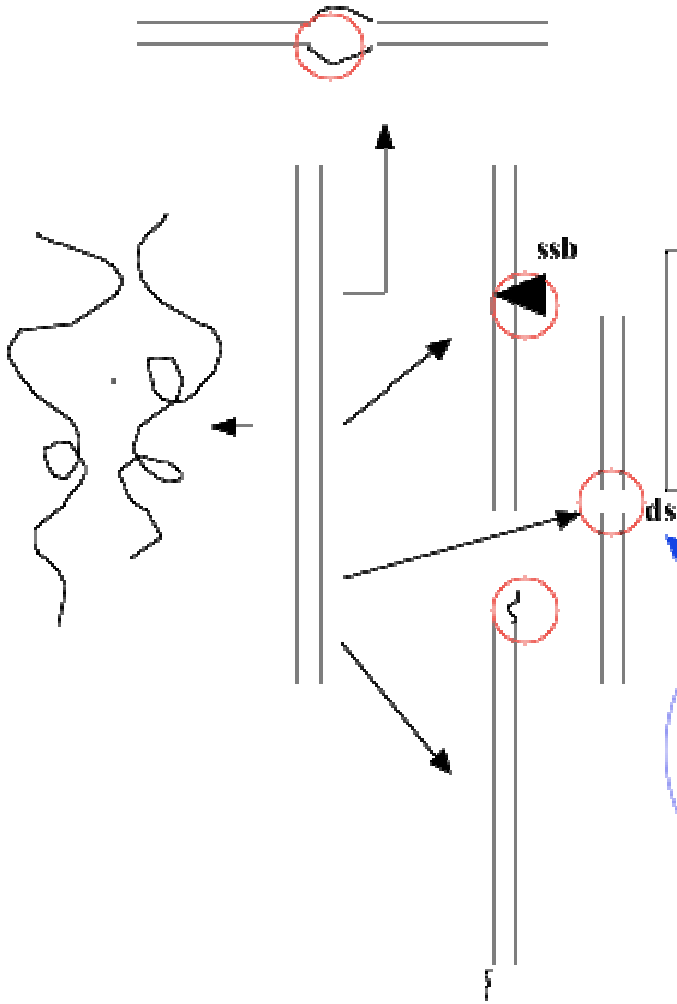

A
T
A
C
G
B0
A
1
2
3
1 μA
0.1 μA
G
-1.5
-1.0
-0.5
C
DPP
DME
SWV
HMDE
CPSA
PGE
I
II
III
20 nA
3 μA
(dE/dt)-1
2 s/V
Gox
-1.5
-1.0
-1.4
-0.9
+0.9
+1.1
E (V)
D
I
III
20 nA
3 μA
(dE/dt)-1
2 s/V
Gox
-1.5
-1.0
-1.4
-0.9
+0.9
+1.1
E (V)

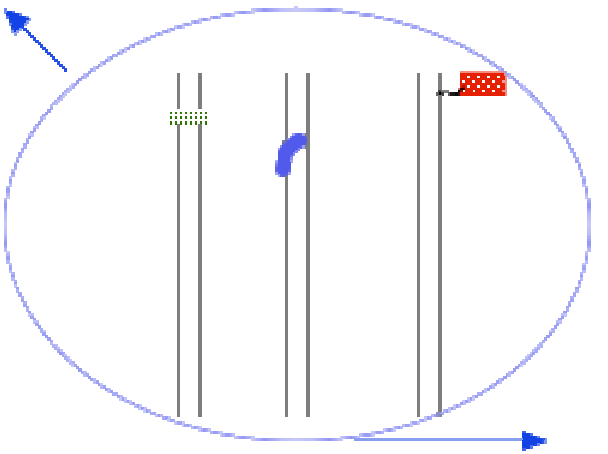
ELECTROCHEMICAL METHODS RECOGNIZE SMALL CHANGES IN DNA STRUCTURE AND DETERMINE TRACES OF IMPURITIES IN DNA SAMPLES

MERCURY ELECTRODES ARE PARTICULARLY SENSITIVE





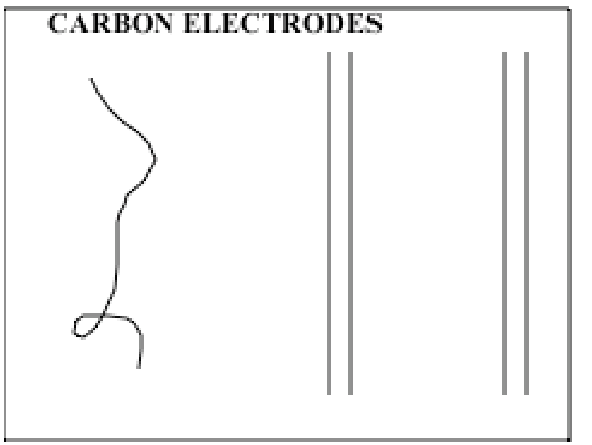
DETERMINATION OF TRACES (< 1%) OF



INTERCALATORS COVALENT MODIFIERS

GROOVE BINDERS

CARBON ELECTRODES



Foundations of nucleic acid electrochemistry

were laid down in 1960-1980's using **mercury** and **carbon** electrodes

After the discovery of the DNA electroactivity it was shown that:
Signals of ds and ss DNA and RNA greatly differ. This made it possible
to follow the course of: DNA denaturation/melting, renaturation/hybridization
to detect: traces of ssDNA in dsDNA samples, DNA damage, single-strand breaks, chem. modification, depurination...

Important findings:
DNA premelting: beginning of the 1960's
DNA unwinding at the electrode surface: middle of 1970's
Polymorphy of the DNA double helix: middle of 1970's

New approaches later utilized in DNA **sensors**:
First covalently bound **electroactive DNA labels**: beginning of the 1980's
First DNA-modified electrodes: middle of the 1980's

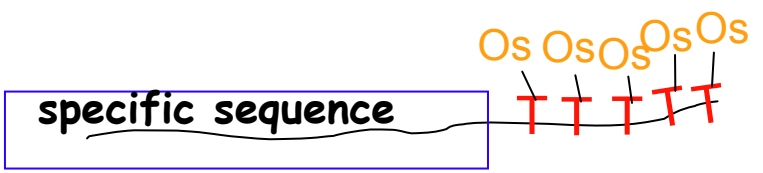
Nukleové kyseliny jsou elektroaktivní.

Signály redukce bazí na Hg elektrodách jsou zvláště citlivé ke změnám ve struktuře DNA

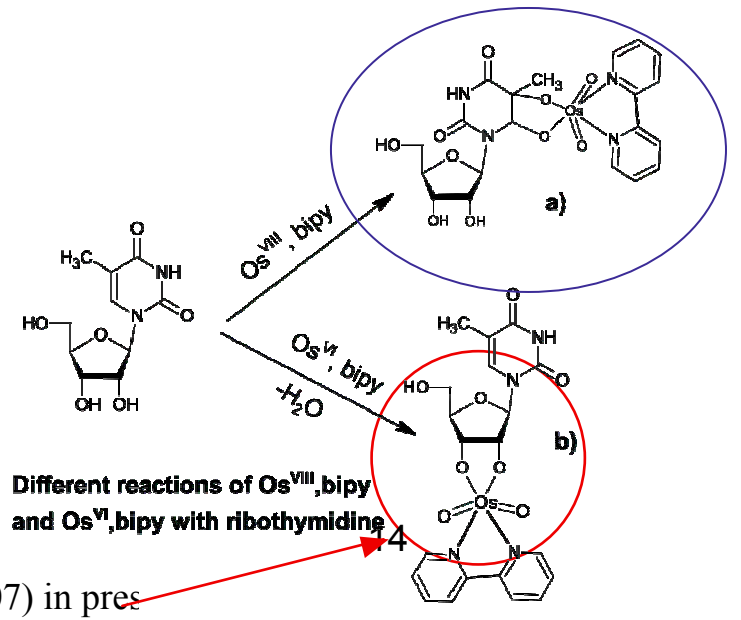
END-LABELING of DNA and RNA

Electroactive labels such as ferrocene, daunomycin, viologen, thionine, etc. were covalently bound to DNA to obtain electrochemical signals closer to zero charge and/or to increase the sensitivity of the analysis. These labels are expensive and can hardly be used for labeling of longer NAs, such as plasmid DNAs.

Osmium tetroxide complexes with nitrogen ligands (Os^{VIII},L) can be used for DNA labeling regardless of the DNA length, in an average biochemical or biological laboratory without any special equipment. DNA-Os^{VIII},L adducts produce redox signals at mercury, amalgam, carbon and gold electrodes; in addition, electrocatalytic signals can be obtained at mercury and amalgam electrodes. Multiple labels can be easily introduced.



With six-valent Os(VI)L ribose residue can be modified

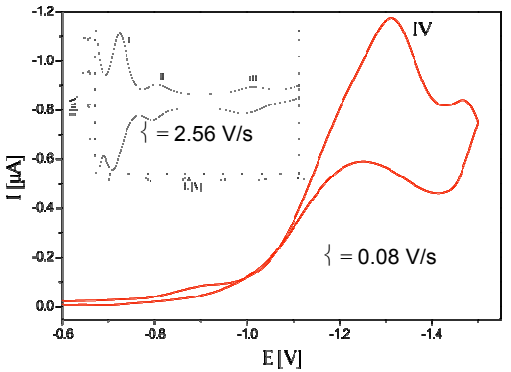


Different reactions of Os^{VIII},bipy and Os^{VI},bipy with ribothymidine

M. Trefulka et al. Electroanalysis (2007) in pres

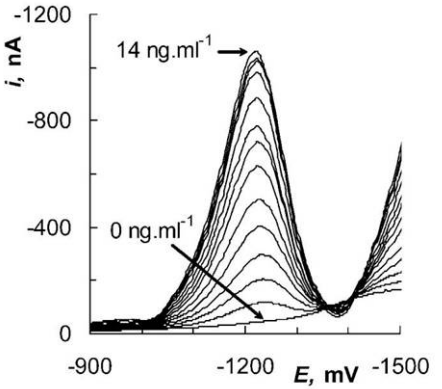
End-labeling of DNA with Os^{VIII},L

mercury



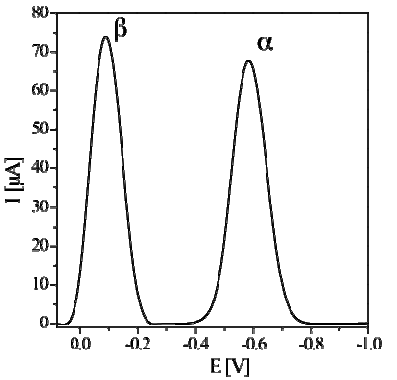
AdTS CVof CT ss DNA (20 ⌈g/ml) modified by 2 mM OsO4 bipy, electrolyte 0.3 M ammoniumformate and 0.05 M sodium phosphate ,pH 6.90

amalgam



AdTS DPV CT ss DNA modified by 2 mM OsO4 bipy, electrolyte: 0.1M acetate buffer pH 4.8
.

carbon



AdTS SWVof $(GAA)_7T_{50}$ (460 nM) modified by 2 mM OsO4 bipy 0.2 M acetate buffer pH 5.0

Monoclonal antibodies for DNA-Os,L adducts are available.

Large number of papers since 1981 reviewed in E. Palecek, Meth. Enzymol. 212 (1992) 139

Palecek E., Scheller F., Wang J., Eds. *Electrochemistry of nucleic acids and proteins.. Towards electrochemical sensors for genomics and proteomics.*; Elsevier: Amsterdam, 2005

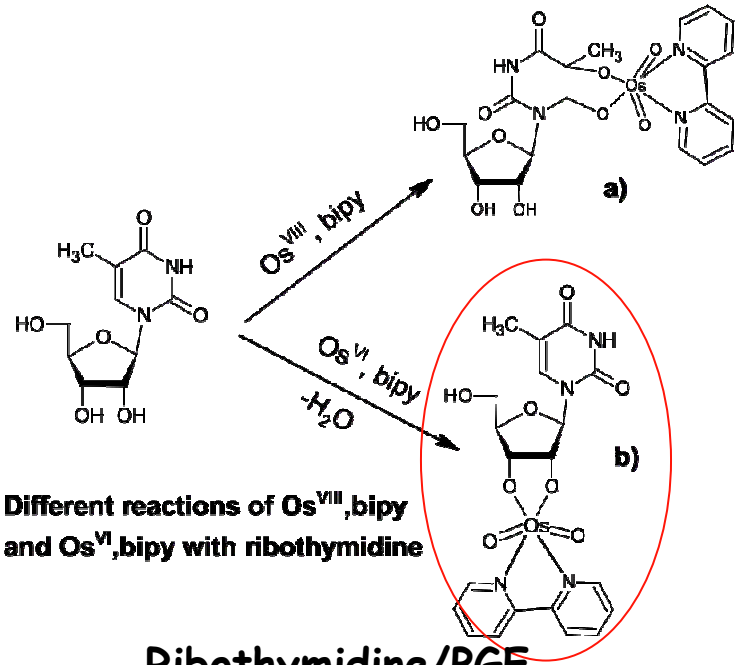
B. Yosypchuk, M. Fojta, L. Havran, M. Heyrovsky, E. Palecek, Electroanalysis 18:186 (2006).

Fojta M., Havran L., Kizek R., Billová S., Paleček E. Biosensors & Bioelectronics 20 (5): 985-994 2004

L. Havran, M. Fojta, E. Palecek, Bioelectrochemistry 63:239 (2004).

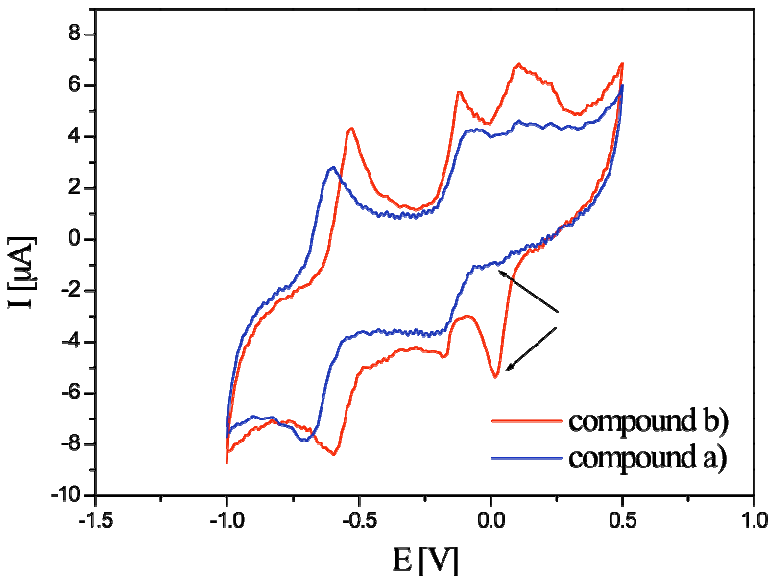
Palecek, E., et al.. (2002). El ectrochemical enzyme-linked immunoassay in a DNA hybridization sensor. *Anal. Chim. Acta* **469,**73-83

Six-valent osmium complexes (Os^{VI},L) can be used for ribose labeling at the 3'-end of RNA and for labeling of some polysaccharides



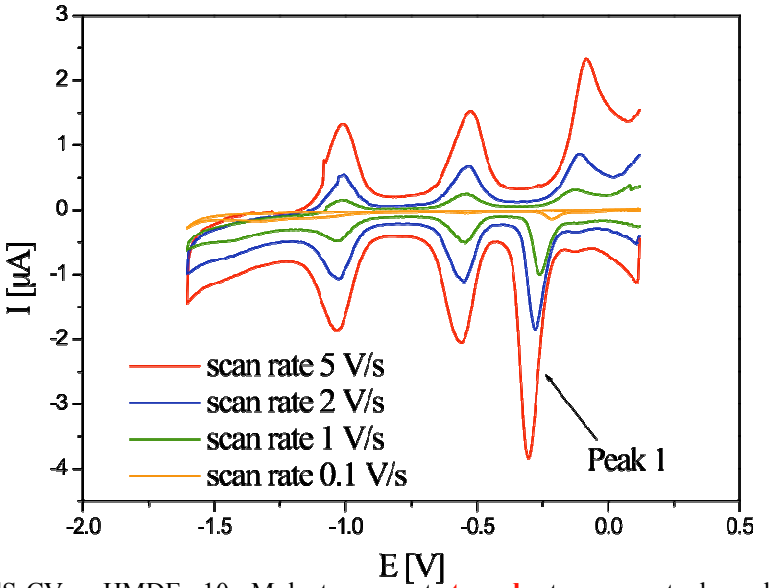
Different reactions of Os^{VIII},bipy and Os^{VI},bipy with ribothymidine

Ribothymidine/PGE



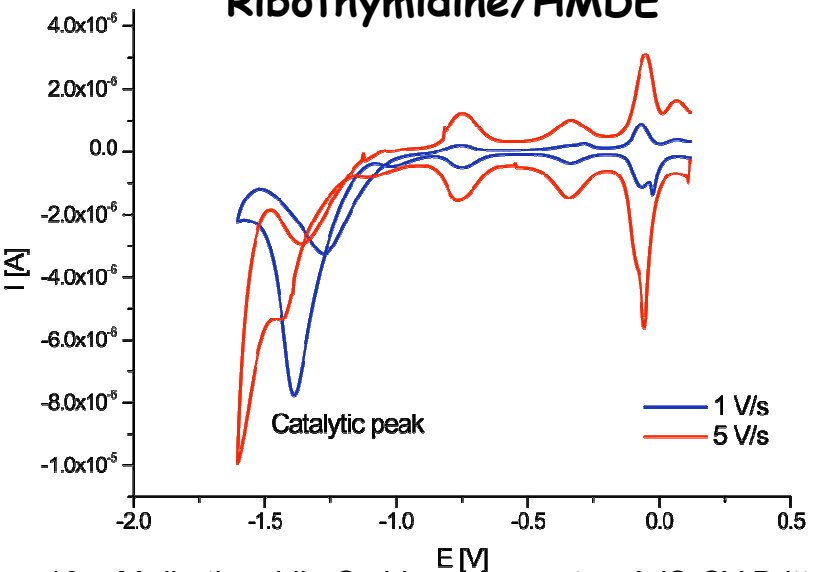
AdS-CV on PGE of 40 µM compounds a) and b). acetate buffer pH 5.0, scan rate 1 V/s

DEXTRAN/HMDE



AdS-CV on HMDE : 10 µM dextran osmate **temed** ester, scan rate dependence, 0.3 M ammonium formate and 0.05 M sodium phosphate (pH 6.9), t_A 60 s.

Ribothymidine/HMDE



10 µM ribothymidin Os,bipy sugar ester, AdS-CV Britton-Robinson buffer, pH 7.0, ta 60 s,, Esw -1.6 V

Značení DNA pomocí elektroaktivních markerů je důležité v senzorech DNA. Pomocí komplexů oxidu osmičelého [Os(VIII)L] lze značit baze pyrimidinové baze DNA i RNA. Komplexy šestimocného osmia [Os(VI)L] umožňují značení koncové 3'-ribosy v RNA. Značení lze provádět v běžné laboratoři.

SAMs

It was shown that similarly to alkanethiols thiolated oligodeoxynucleotides (ODN-SH) produce self-assembled monolayers (SAMs). In the last decade SAMs of ODN-SH at gold surfaces were intensively studied

SAMs at gold electrodes have been widely used in DNA sensors

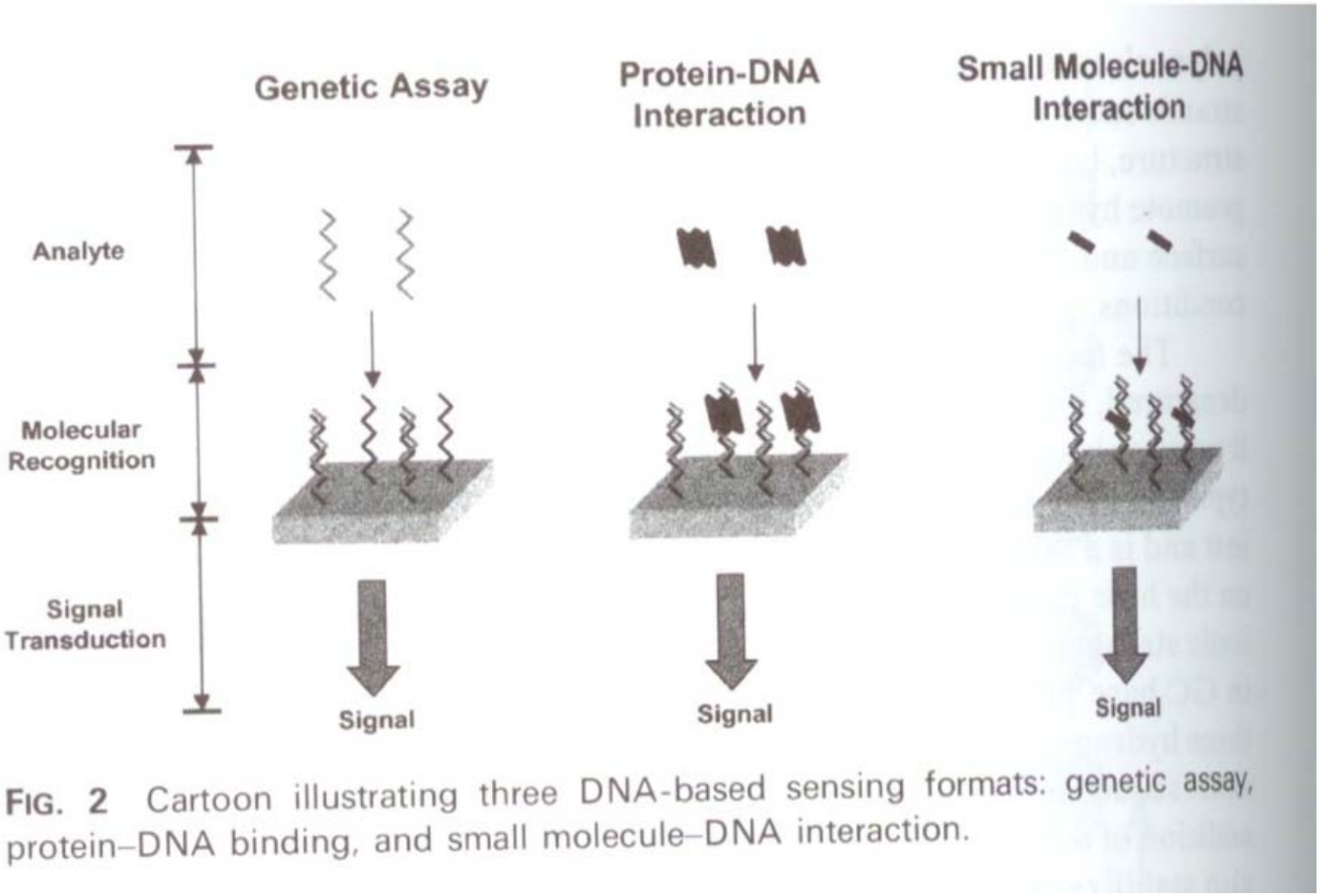
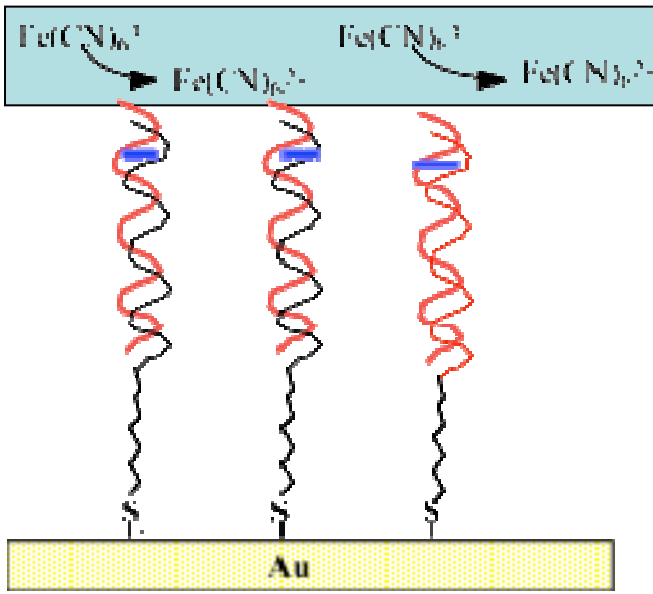


FIG. 2 Cartoon illustrating three DNA-based sensing formats: genetic assay, protein–DNA binding, and small molecule–DNA interaction.

Charge transfer through the DNA duplexes

J. Barton et al.



Vizualization of DNA by STM and SFM contributed to understanding of DNA ordering at gold surfaces

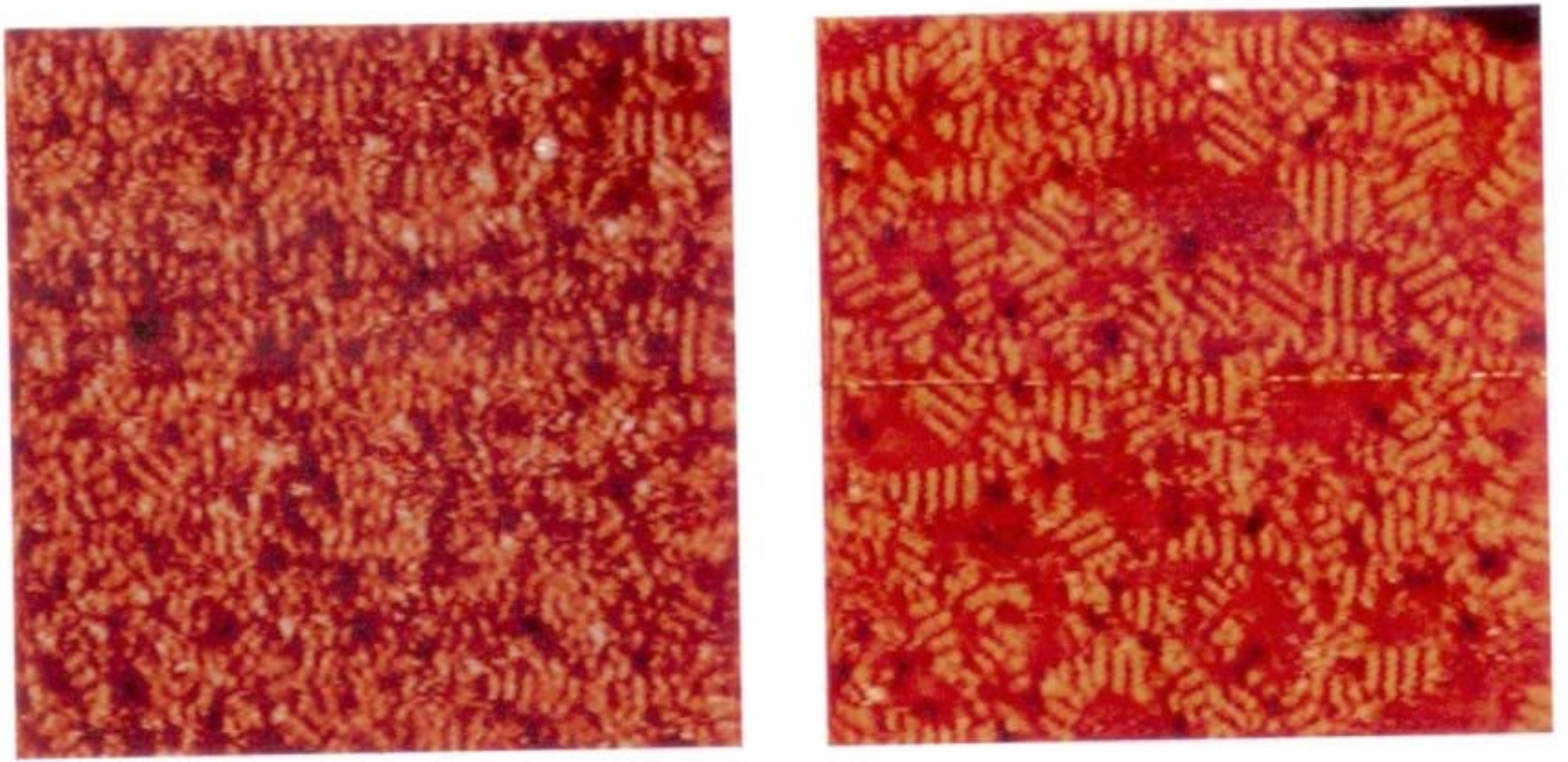


Figure 1.17: 40 nm × 40 nm STM images of HS-10A. Before (left) and after (right) formation of the ordered structure. 10 mM phosphate buffer, pH 7.1. $I_{current}$ = 0.3 nA. V_{bias} = -0.15 V. E_{sample} = -0.61 V (SCE). [60]

Molecular dynamic simulation of DNA-SH at gold surfaces

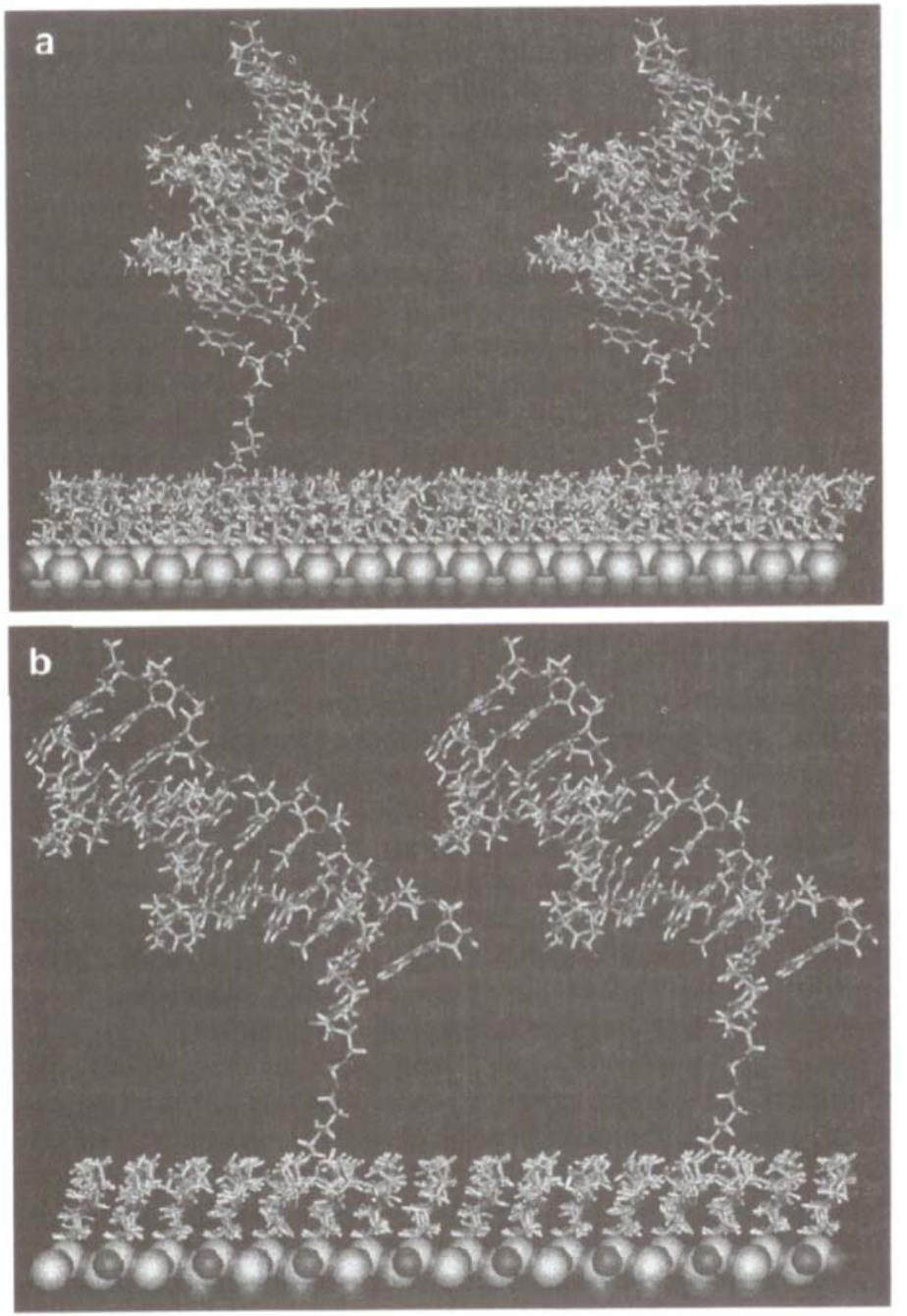


FIG. 4 Snapshot of the molecular dynamics simulation at 6.3 ns. (a) and (b) are the views from two perpendicular directions. Each figure displays two duplexes. (a) In this view, the DNAs are pointing out of the page. (b) This view shows the DNA tilted toward its periodic image. Note the narrow space between the DNAs. (Reprinted with permission from Ref. 115. Copyright 2001 Springer-Verlag.)

Alkanthioly a thiolované NA (NA-SH) vytvářejí samouspořádané vrstvy (self-assembled monolayers, SAM) na zlatých elektrodách. SAMy DNA-SH jsou využívány v senzorech DNA. Zlaté povrchy nejsou ideálně rovné a neumožňují vytvoření velmi hustých SAMů, prostých drobných defektů.

It was found that liquid mercury electrodes with atomically smooth surfaces are ideally suited for pin-hole free SAMs of alkanethiols.

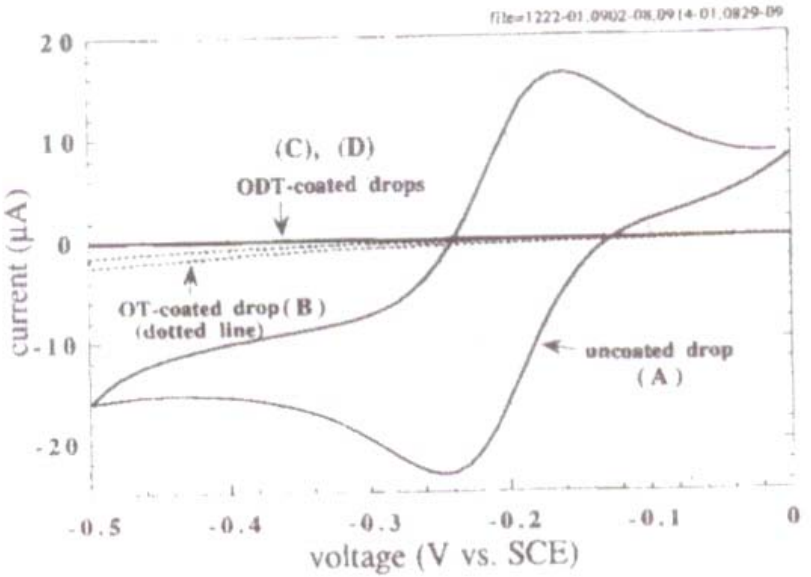


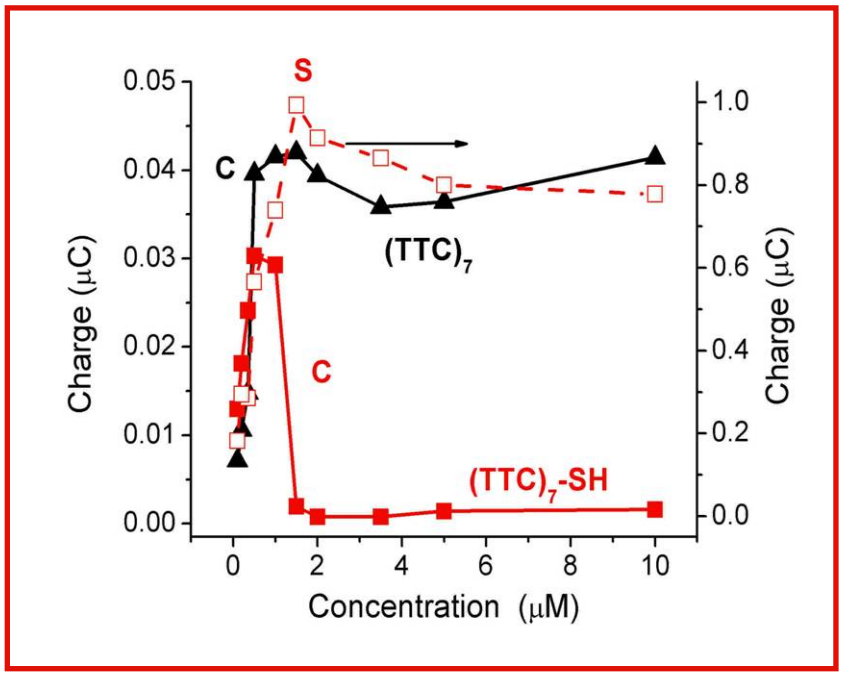
Fig. 2. Cyclic voltammogram at 50 mV/s of 5 mM $Ru(NH_3)_6^{3+}$ in 0.1 M NaF on a bare 1.5 μl mercury drop (A), OT-coated 1.5 μl mercury drop (B), and two ODT-coated drops (C and D). On this current scale, the two curves for ODT-coated drops appear coincident with essentially no current.

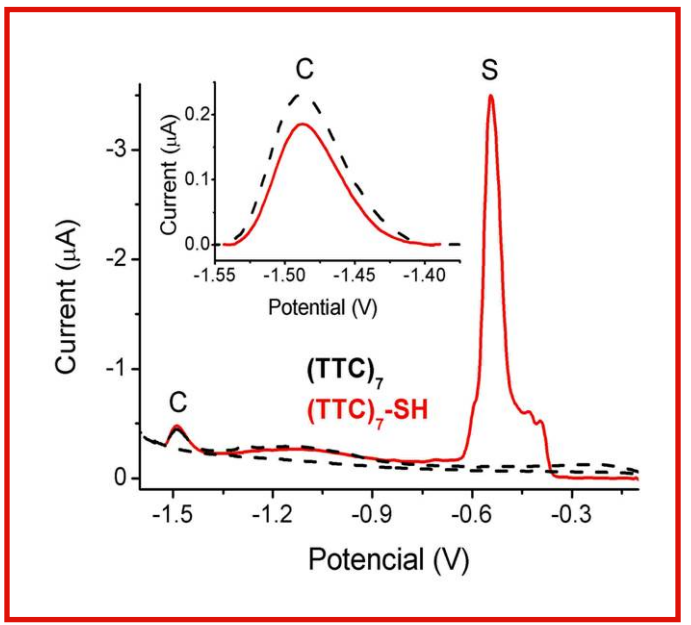
C. Bruckner-Lea, R.J. Kimmel, **J. Janata** et al., Electrochim Acta 40 (1995) 2897

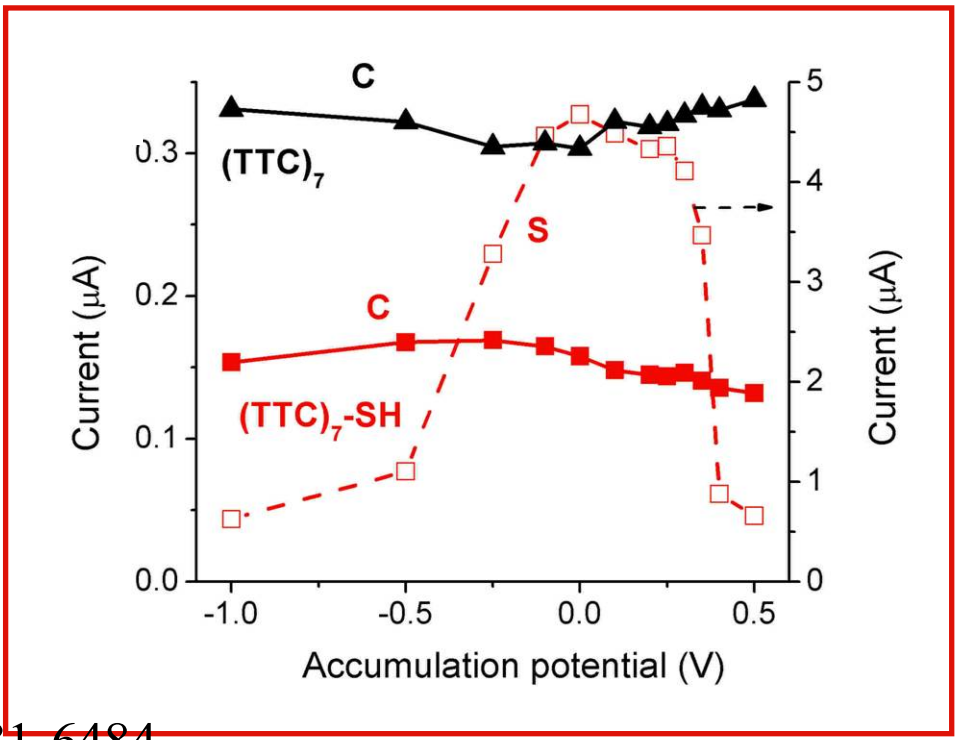
Can these electrodes can suit equally well for SAMs of DNA-SH?

DNA-SH SAMs at Hg electrodes

$(TTC)_7$-SH produces peak *C* due to reduction of cytosine and peak *S* - reduction of Hg-S bond. Peak *C* provides information about the contact of the bases with the surface, indicating changes in DNA orientation from flatly laying DNA molecules at the surface (at low surface concns.) to upright oriented DNA-SH in SAM (at high surface concns.).

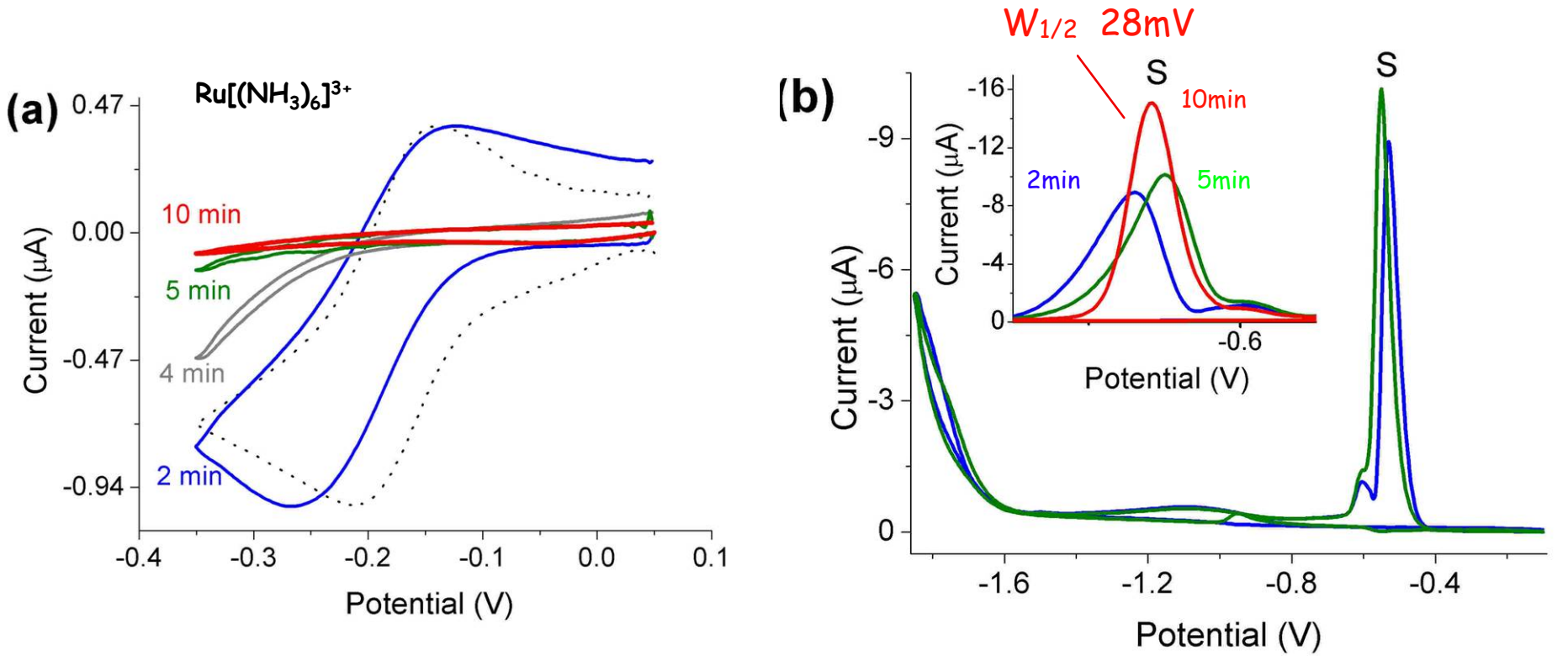






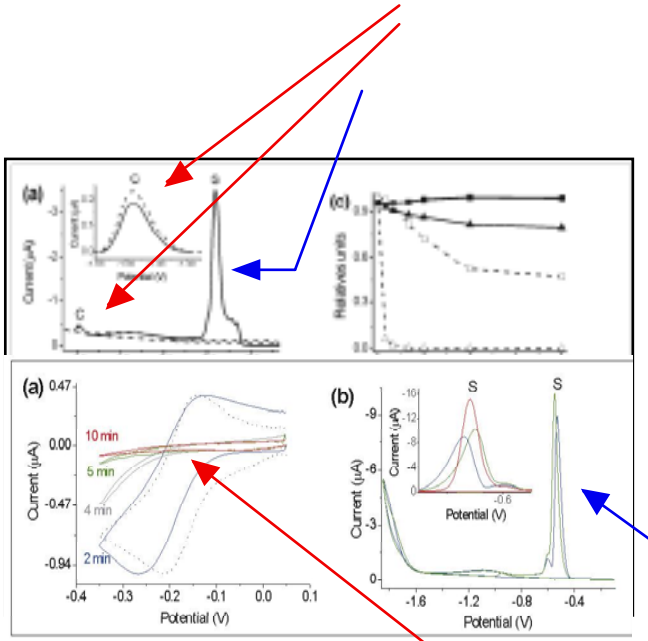
V. Ostatna and E. Palecek, *Langmuir* (2006) 22, 6481-6484

At high HS-ODN concentrations and longer accumulation times (surface coverage at least 592 pmol.cm^{-2}) **insulating pinhole-free layer is formed** (not allowing redox processes of $Ru[(NH_3)_6]^{3+}$ at the HMDE and accompanied by formation of spike S)

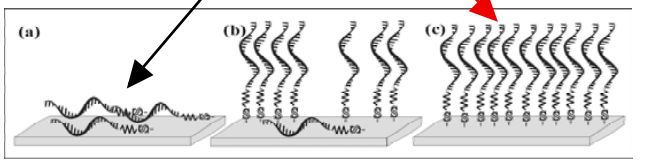


Dependence of cyclic voltammograms of 1 mM $Ru[(NH_3)_6]^{3+}$, (dashed line) on time of accumulation of HS-$(TTC)_7$. 2 μM HS-ODN was adsorbed at the HMDE t_A 2 min (blue), 4 min (gray), 5 min (green) and 10 min (red). The electrode with the adsorbed layer was washed and transferred to the blank background electrolyte with 1 mM $Ru[(NH_3)_6]^{3+}$. Scan rate 0.5 V/s, initial potential +0.05 V, switching potential -0.35 V, final potential +0.05 V, potential step 5 mV. **(b)** Ex situ cyclic voltammogram of thiolated ODN. 2 μM HS-$(TTC)_7$ was adsorbed at the HMDE, tA 2 min (blue), 5 min (green) and 10 min (red). Inset: Peak S.

SAMs of thiol-end-labeled DNA at mercury electrodes



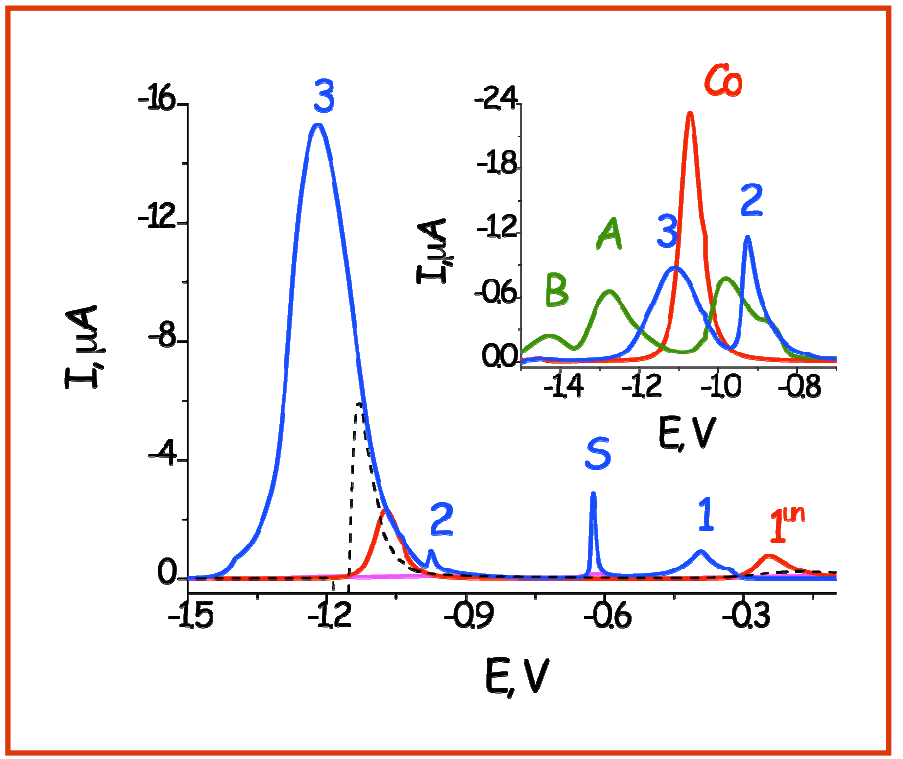
Peak C provides information about the**contacts of the bases with the electrode surface** indicating changes in **HS-ODN orientation** from **flatly laying molecules (at low surface concentrations)** to **upright oriented HS-ODNs in highly dense SAMs**.

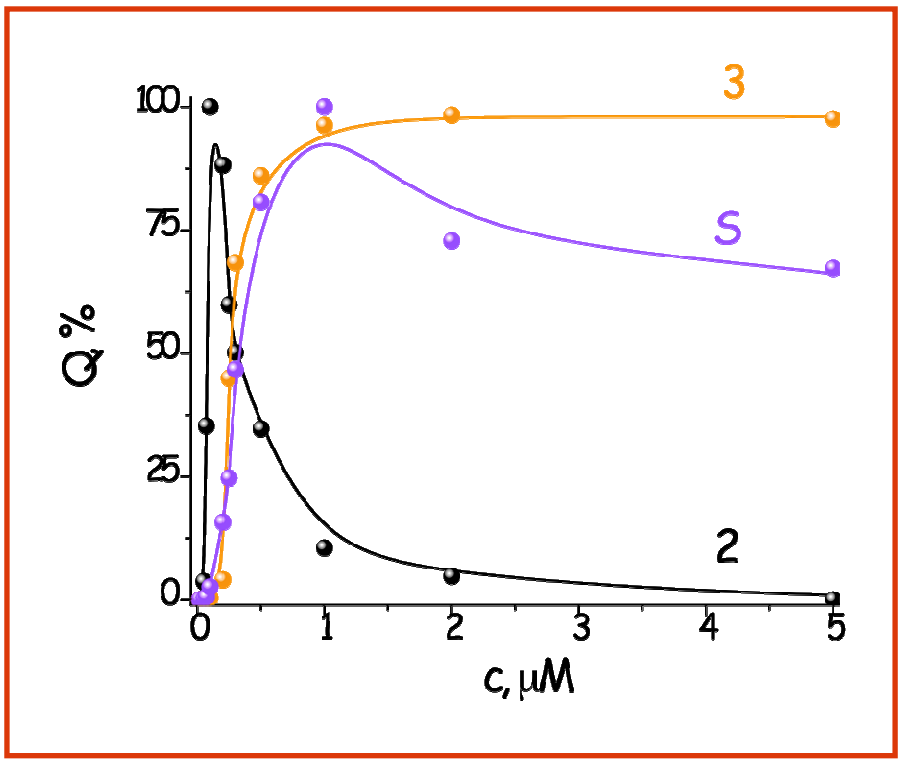


At longer accumulation times **peak S turns into a narrow spike** indicative of a densely packed layer. The rate of electron transfer across this SAM is greatly decreased suggesting the **presence of a compact, densely packed pinhole-free layer. SAMs at mercury electrodes are similar by their ordering to SAMs of alkanethiols** at the same electrodes **and differ from HS-DNA SAMs at gold surfaces. Mercury-containing electrodes are convenient for studies of DNA SAMs and potentially useful in DNA sensors.**

V. Ostatna and E. Palecek, *Langmuir* (2006) 22, 6481-6484

$HS(TTC)_7$ but not $(TTC)_7$ produces in cobalt-containing solutions (suitable for protein analysis) peak S and electrocatalytic peaks 2 and 3 at potentials different from the peptide peaks A and B.





300 nM $HS(TTC)_7$ 2 μM$(TTC)_7$ and 2 μM Lys-vasopresin measured in 0.1 MNH_4Cl, 0. 1 M NH_4OH with 1 mM $[Co(NH_3)_6]Cl_3$, pH 9.5. 300 nM $HS(TTC)_7$ in 0.1 M NH_4Cl, NH_4OH (in absence of cobalt).

Concentration dependence of peaks 2, 3 and S resembles that of peak C and S observed in absence of cobalt suggesting DNA SAM formation under conditions suitable for protein analysis.

The method is potentially useful in studies of DNA-protein interaction

V. Ostatna et al., Electroanal. 17 (2005) 1413

Výhody a nevýhody rtuťových elektrod při studiu DNA-SH SAMů

Na rozdíl od zlatých povrchů:

VÝHODY:

- Vyšší afinita síry ke rtuti a rychlejší reakce Hg-S a rychlejší vznik SAMů
- Zcela hladký povrch tekuté rtuti - vznik SAMů prostých defektů
- Možnost přípravy SAMů o vysoké hustotě (densely packed DNA SAMs)
- Signály redukce bazí poskytující informaci o kontaktu bazí s povrchem

NEVÝHODY: - Tekutá rtuť je nevhodná pro použití v senzorech

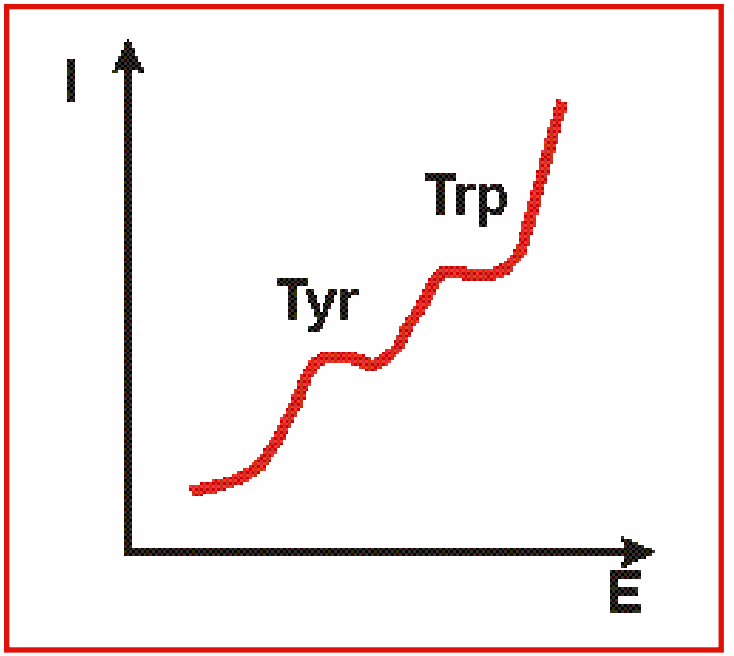
- Na zlatých površích lze snadněji aplikovat různé fyzikální metody jako STM, AFM, FTIR, neutron reflectivity, X-ray-photoelectron spectroscopy, atd

Tyto nevýhody lze kompenzovat použitím pevných amalgamových či Hg filmových elektrod

Can electrochemistry be equally useful in the analysis of **proteins**?

Tyr and Trp oxidation at carbon electrodes

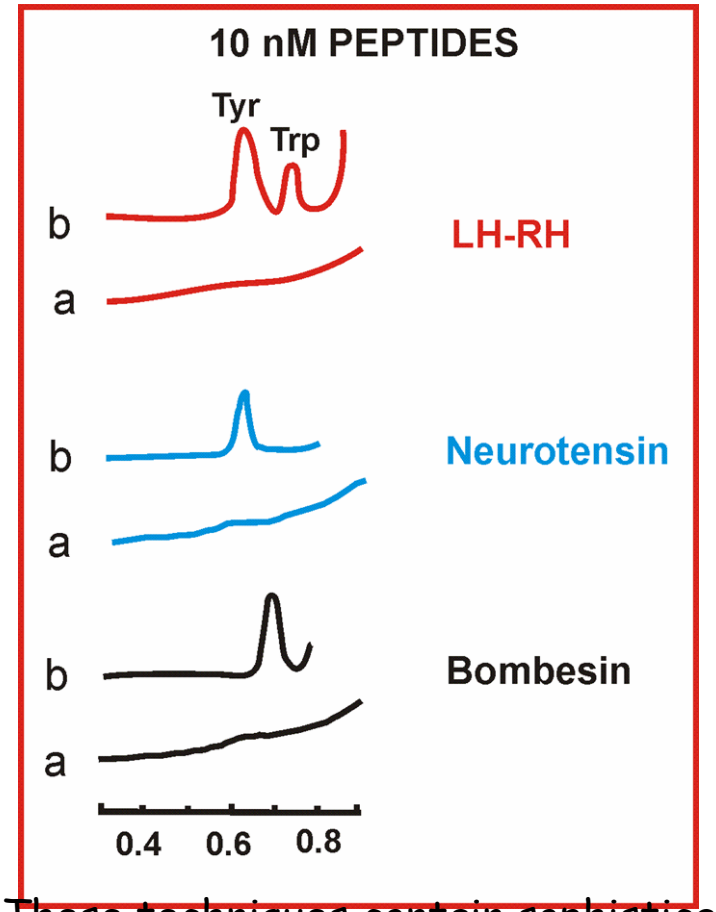
Linear sweep voltammetry



V. Brabec, 1981

Constant current chronopotentiometry or square wave voltammetry

1996



These techniques contain sophisticated base line correction (compensating the high background currents at carbon electrodes)

ELECTROACTIVITY OF AMINO ACIDS IN PROTEINS.
Chronopotentiometric signals reflect changes in protein structure

Small conjugated proteins containing redox centers produce fast reversible electrochemistry which is however limited to a relatively small groups of proteins.

Present **proteomics** requires sensitive methods for the analysis of **all** proteins. We wish to show that electroactivity of amino acid residues in proteins can be utilized in the analysis of practically all proteins, including those important in **biomedicine**.

Oxidation of Tyr and Trp at carbon electrodes and particularly the chronopotentiometric electrocatalytic peak H at mercury and amalgam electrodes may become important tools in the protein research.

Proteomics

Proteome (1995) total protein complement of the genome

Proteomics aims to supplement gene sequence data with information on what proteins are being made where, in what amounts, under what conditions

Three main activities:
1. **indentifying all the proteins made** in the given cell, tissue or organism
2. determining **how the proteins interact** forming networks (resembling electrical circuits)
3. resolving the **three-dimensional structures of the proteins** to find the spots where binding of drugs might effect their activity

At present **2-D gel electrophoresis, mass spectrometry and X-ray crystallography** are the most important methods

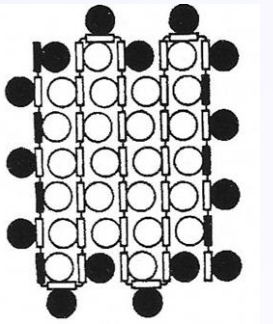
Can electrochemistry contribute?

Protein denaturation

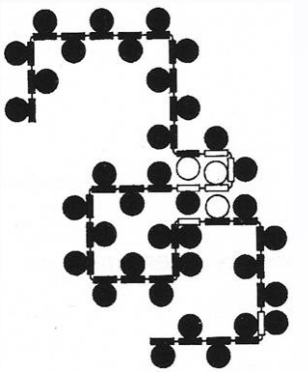
Proteins can be denatured due to changes in their environment, e.g. by temperature increase, extereme pH values or by chemical denaturants. such as urea or guanidinium chloride (GdmCl).

In the presence of denaturing concentrations of urea or GdmCl proteins are unfolded.

Properties of denatured proteins may differ from those of native proteins, decreased solubility and aggregation are frequently observed



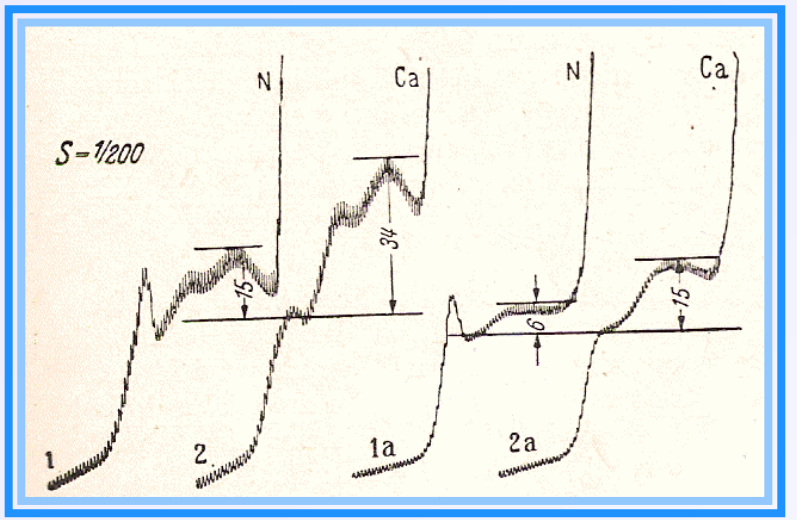
native state



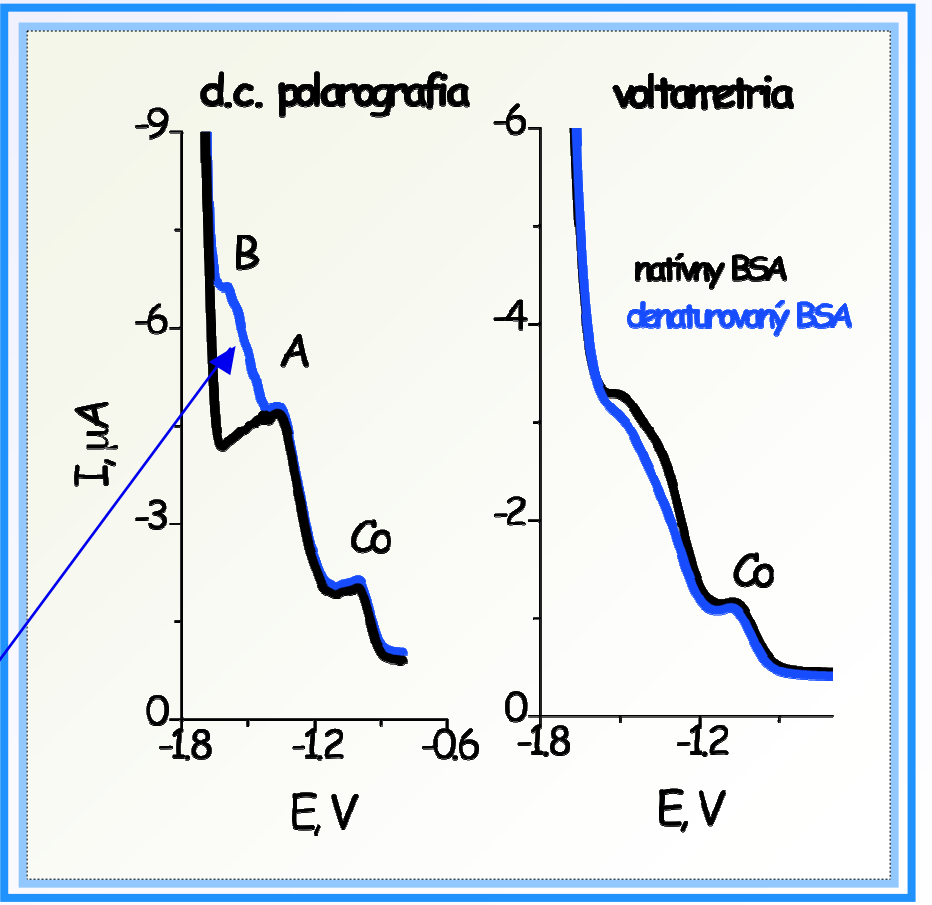
denatured state

ELECTROCHEMICAL ANALYSIS OF PROTEINS

Brdička's d.c. polarographic catalytic response



was used in biochemistry, and medicíne for several decades in the middle of the 20th century. Using DME it was possible to discriminate between native and **denatured** proteins



580 nM BSA v prítomnosti 71 mM močoviny,
E_i -0,1V/ E_f -1,7V
1 mV/s 9 mV/s
t_A 120 s, E_A -0,1V

65.2 μM BSA v 8 M močovine bol denaturovaný pri 4 °C cez noc.

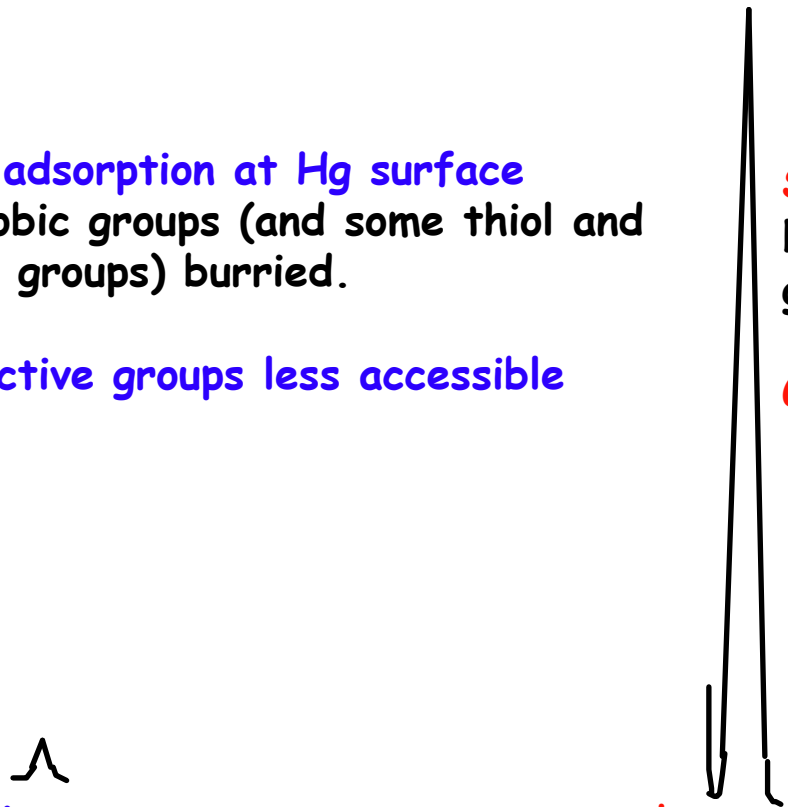
Peak H differs from the previously described polarographic and voltammetric electrocatalytic signals of proteins

(i) by its ability to detect peptides and proteins down to nanomolar and subnanomolar concentrations and

(ii) by its remarkable sensitivity to local and global changes in protein structures.

Weaker adsorption at Hg surface
hydrophobic groups (and some thiol and disulfide groups) burried.

Electroactive groups less accessible



Stronger adsorption at Hg surface
hydrophobic groups, thiol, disulfide and other groups better accessible

Greatly increased electroactivity

native denatured proteins

We believe that the HIGH SENSITIVITY and the ability of peak H to recognize protein denaturation will of use in the protein research and particularly in biomedicine

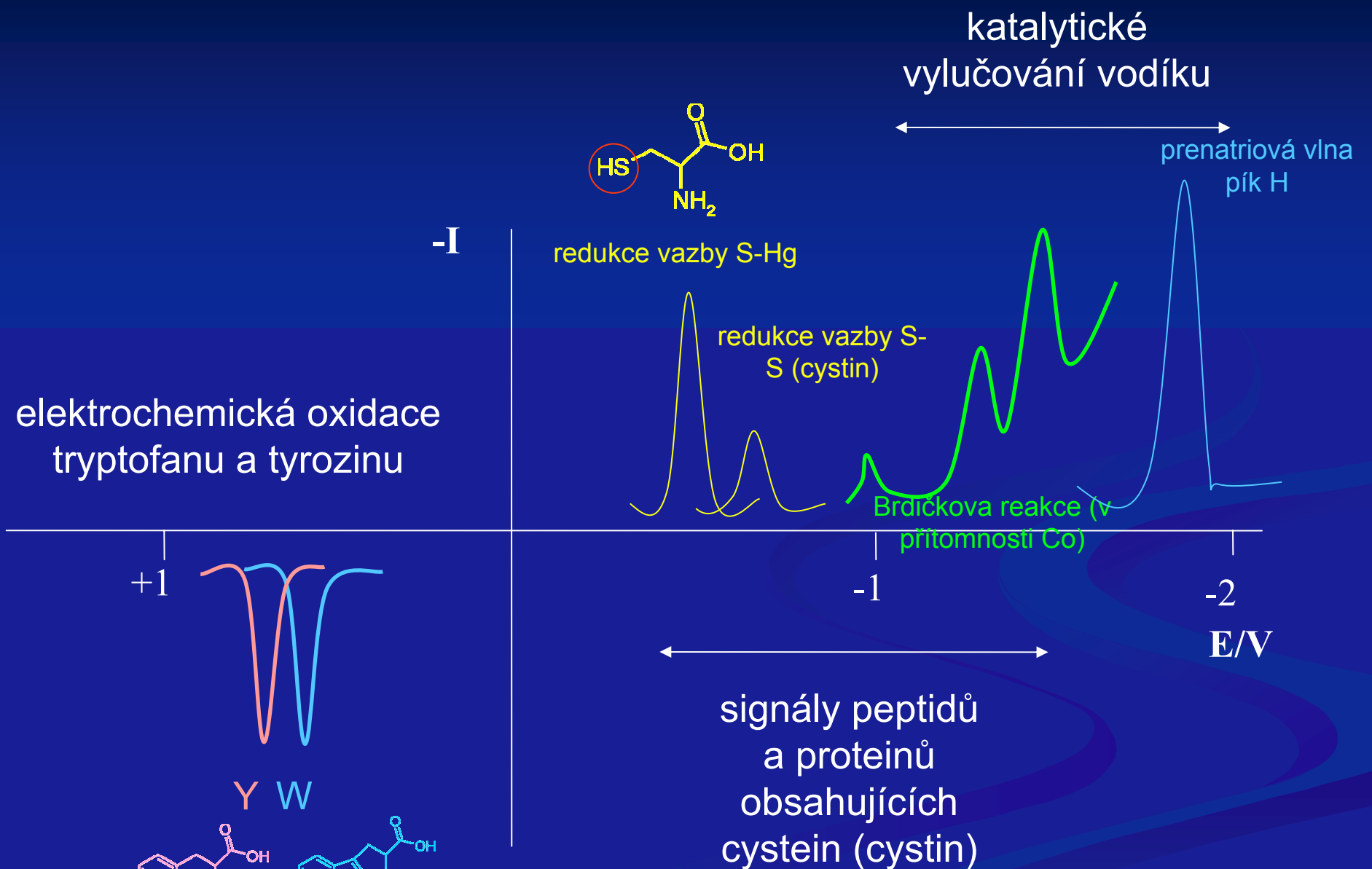
We wish to further show that using peak H redox states of peptides and proteins can be easily determined

Bílkoviny jsou elektroaktivní na uhlíkových a Hg elektrodách. Na uhlíkových elektrodách jsou oxidovány zbytky tyrosinu, tryptofanu (a cysteinu).

Hg elektrody jsou vhodné pro sledování signálů bilkovin, obsahující zbytky cysteinu, které souvisejí jednak s tvorbou Hg-S vazeb a jednak s **elektrokatalýzou** (Brdičkova reakce). Bílkoviny poskytují polarografickou "prenatriovou vlnu", která nevyžaduje přítomnost cysteinu v molekule, je však nevhodná pro analytické účely. Pomocí chronopotenciometrie konstatním proudem lze pozorovat **"peak H"**, poskytovaný peptidy a bílkovinami **v nanomolárních a subnamolárních koncentracích.** Tento peak je velmi **citlivý ke změnám struktury bílkovin** a lze jím sledovat jejich **denaturaci.**

UHLÍKOVÉ ELEKTRODY

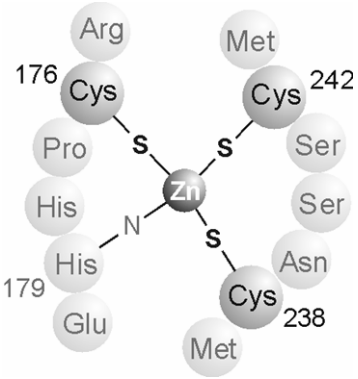
RTUŤOVÉ ELEKTRODY



Je možno elektrochemicky rozpoznat redoxní stav peptidů a bílkovin?

p53CD

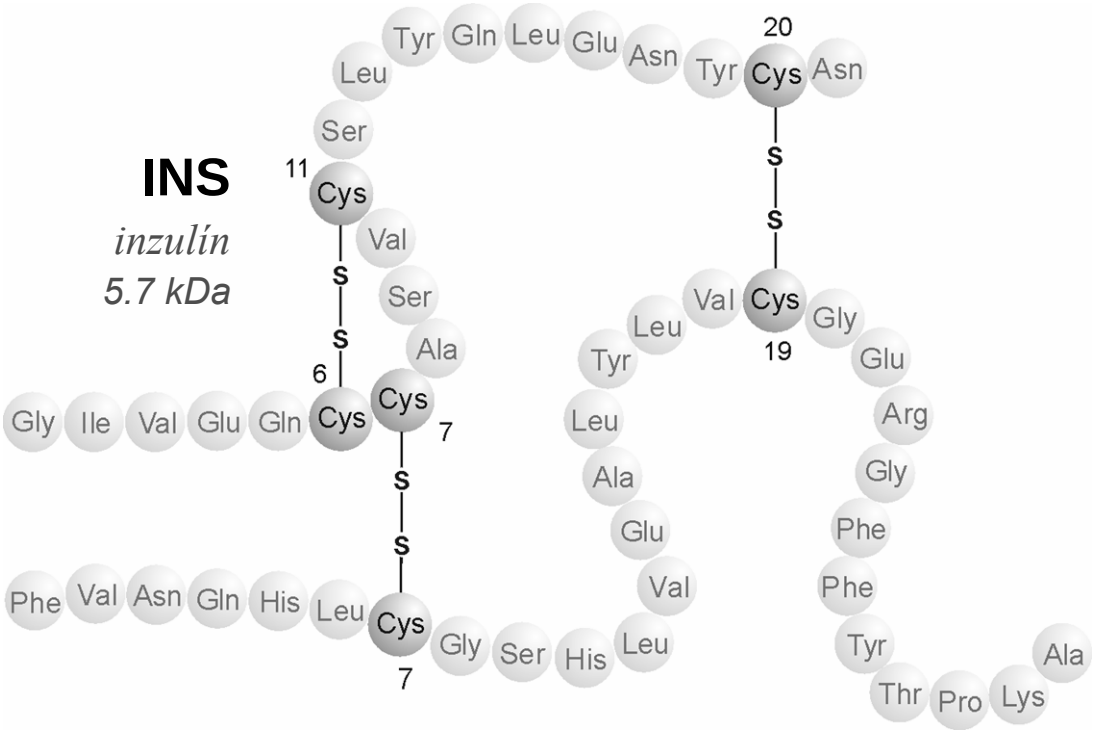
22.5 kDa

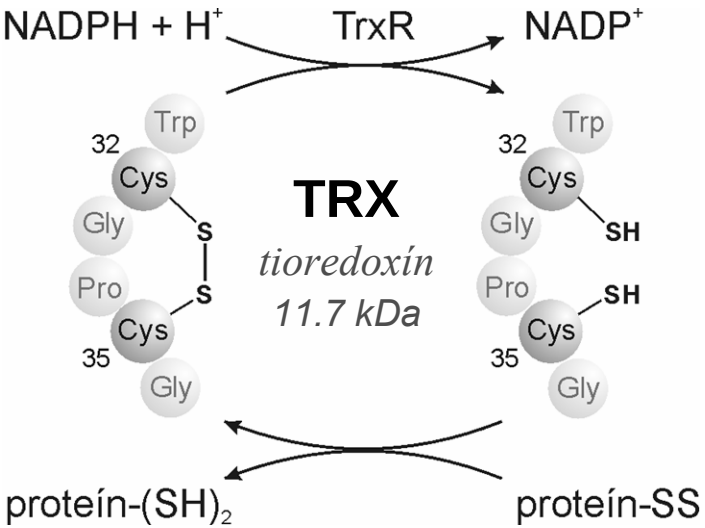


INS

inzulín

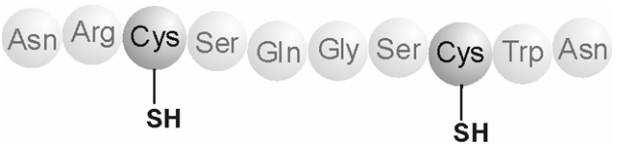
5.7 kDa





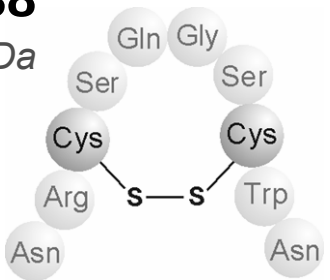
SH38

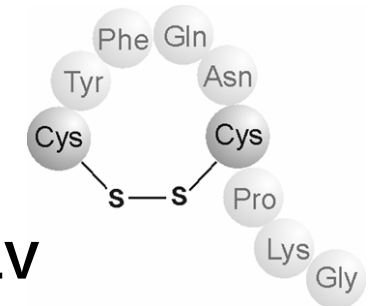
1.15 kDa



SS38

1.15 kDa



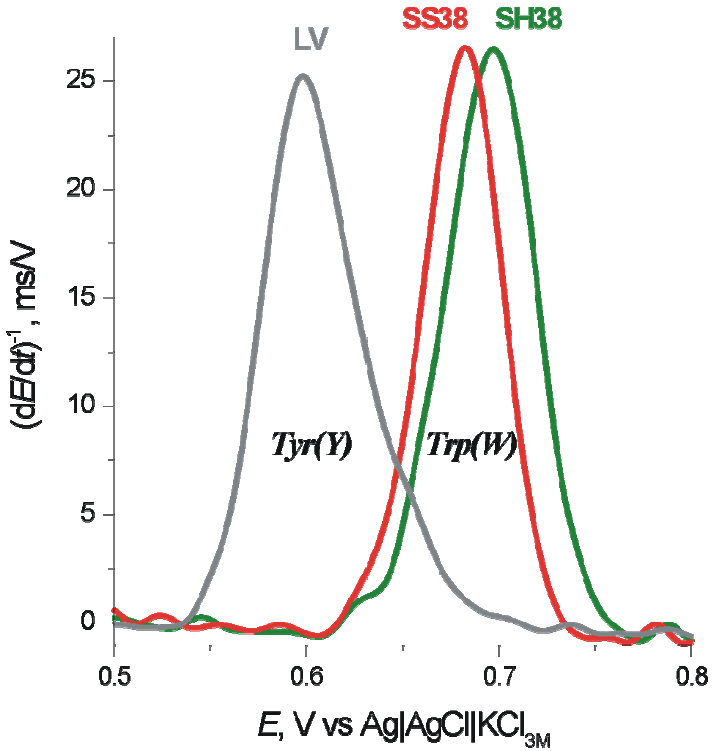


LV

[Lys^8]-vazopresín

1.06 kDa

Oxidation of Trp (W) a Tyr (Y) at PGE

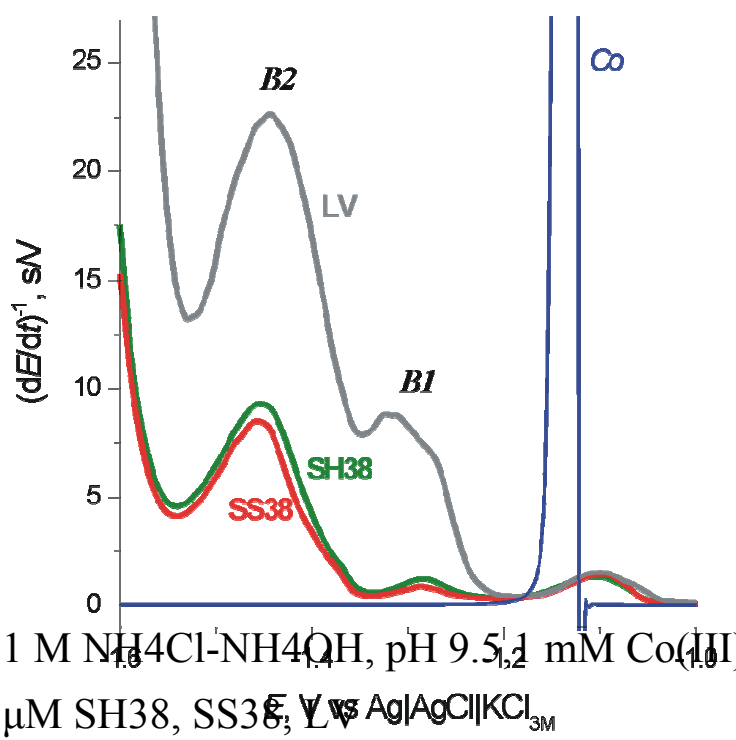


0.05 M Na-borate, pH 9.3

1 ⌈M SH38, SS38, Lys-vasopresin (LV)

CPSA: t_A 60 s, occ, I_{str} +50 ⌈A

Brdička's catalytic response, HMDE



0.1 M NH4Cl-NH4OH, pH 9.5, 1 mM Co(II)

1 µM SH38, SS38, LV

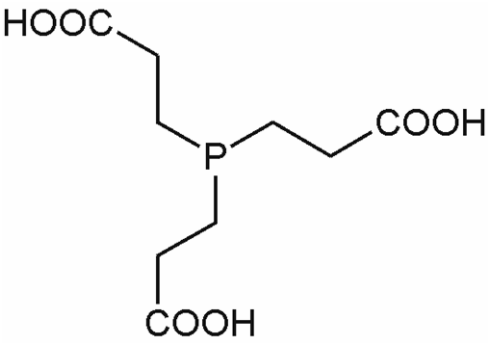
CPSA: t_A 60 s, occ, I_{str} -5 µA

CPSA - *chronopotenciometrická rozpúšťacia analýza s konštantným prúdom,* PGE - *elektróda z pyrolitického grafitu*
HMDE - *visiaca ortuťová kvapková elektróda,* t_A *- akumulačný čas,* occ - *otvorený prúdový obvod,* I_{str} *- rozpúšťací prúd*

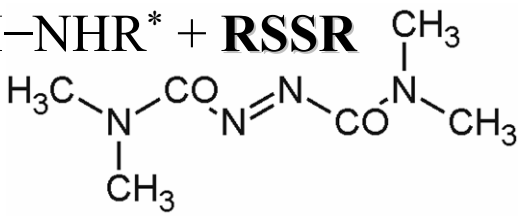
Disulfide reduction $R'_3P + \mathbf{RSSR} + H_2O \rightarrow R'_3P{=}O + 2\mathbf{RSH}$

TCEP

tris(2-carboxyethyl)phosphíne, R'_3P



Thiol oxidation $R^*N{=}NR^* + 2\mathbf{RSH} \rightarrow R^*NH{-}NHR^* + \mathbf{RSSR}$



diamide

N,N,N',N'-tetrametylazodikarboxamid, $R^*N{=}NR^*$

Závěr

- co je příčinou rozdílných signálů oxidovaných a redukovaných peptidů a bílkovin na pozitivně nabitém povrchu Hg elektrody?

- na pozitívně nabitém povrchu HMDE
  - různá orientace redukovaných a oxidovaných molekul
- rychlost a způsob polarizace HMDE
  - rychlý posun k negatívním potenciálům píku H
- pík H - katalýzy redukce vodíku se účastní SH- skupiny
  - redukce S-S väzieb nárust katalytické aktivity
  - oxidace nebo modifikace -SH skupin pokles katalytické aktivity

---

- na negatívně nabitém povrchu HMDE
  - podobná konformace oxidovaných i redukovaných molekul (reduce S-S vazeb oxidovaných molekul)

In addition to various peptides and proteins we are currently studying two proteins important in biomedicine by electrochemical methods:

Tumor suppressor protein p53

declared „**The Molecule of the Year“ by Science magazine in 1993** perhaps the **most important protein in the development of cancer. DNA-p53 protein interactions** are very important in performing the p53 function. **Electrochemical signals of DNA and p53 protein can be utilized in studies of these interactions**

⟨-synuclein

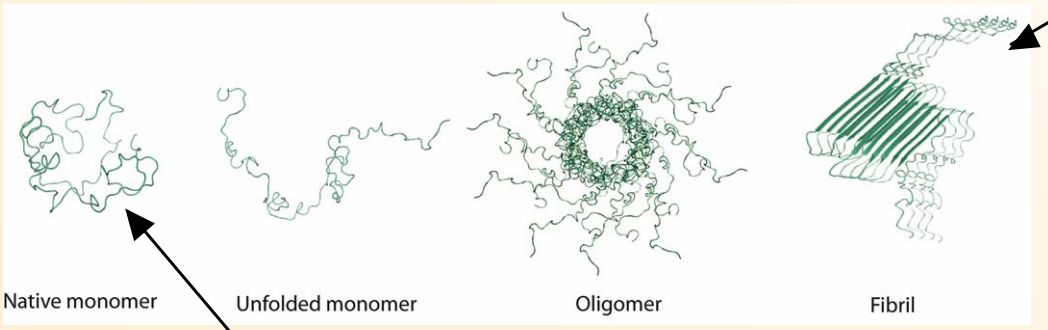
a major component of Lewy bodies associated with **Parkinson disease**. It is **natively unfolded** but undergoes **aggregation leading to fibrillar structures**, in which the protein adopts a ®**-sheet secondary structure**

Understanding the **mechanism of aggregation** and the factors that modulate it, is important for devising **therapeutic strategies.**

The number of methods for studying the process of aggregation is limited and **electrochemistry** appears to be **suitable** for this purpose.

α-SYNUCLEIN
important in Alzheimer's and Parkinson's diseases

Natively unfolded protein undergoes aggregation leading to fibrillar structures



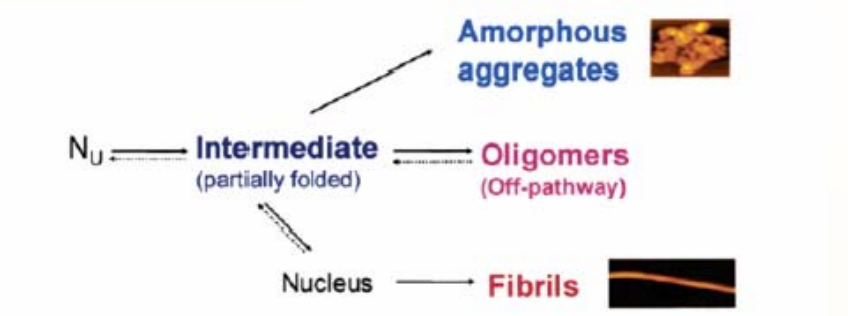
Recently NMR indicated ensemble of conformations stabilized by long range interactions

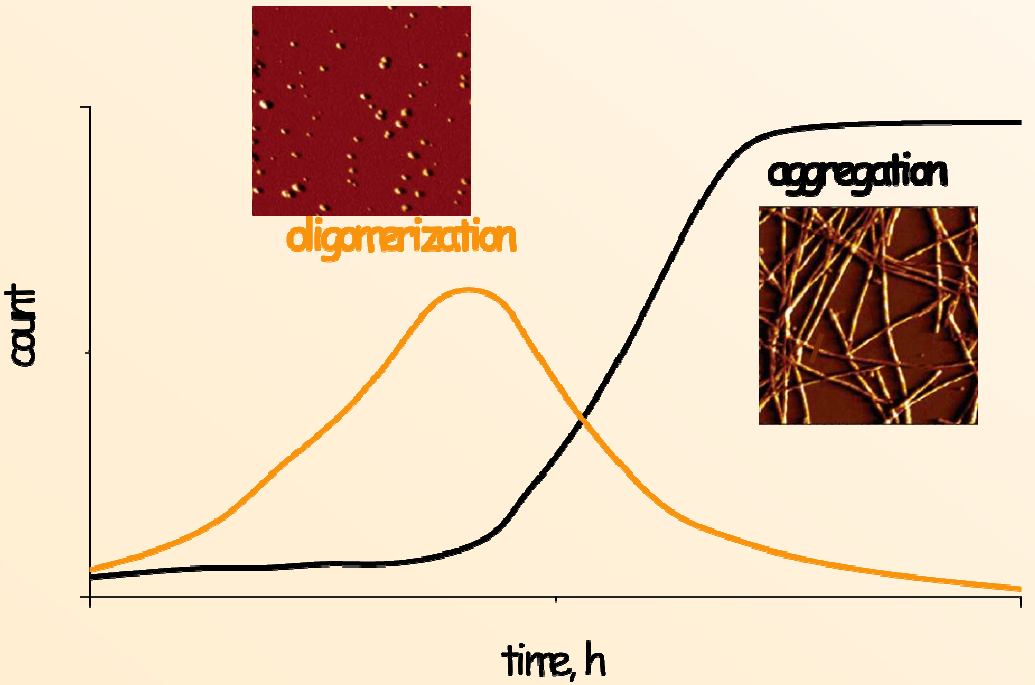
Is α-synuclein suitable for electrochemical analysis?

Trp 0 Cys 0 Arg 0
Tyr 4 (4%) Lys 15 (10.7)

⟨-synuclein

a major component of Lewy bodies associated with **Parkinson disease**. It is **natively unfolded** but undergoes **aggregation leading to fibrillar structures**, in which the protein adopts a ®-**sheet secondary structure**





Understanding the **mechanism of aggregation** and the factors that modulate it, is important for devising **therapeutic strategies.**

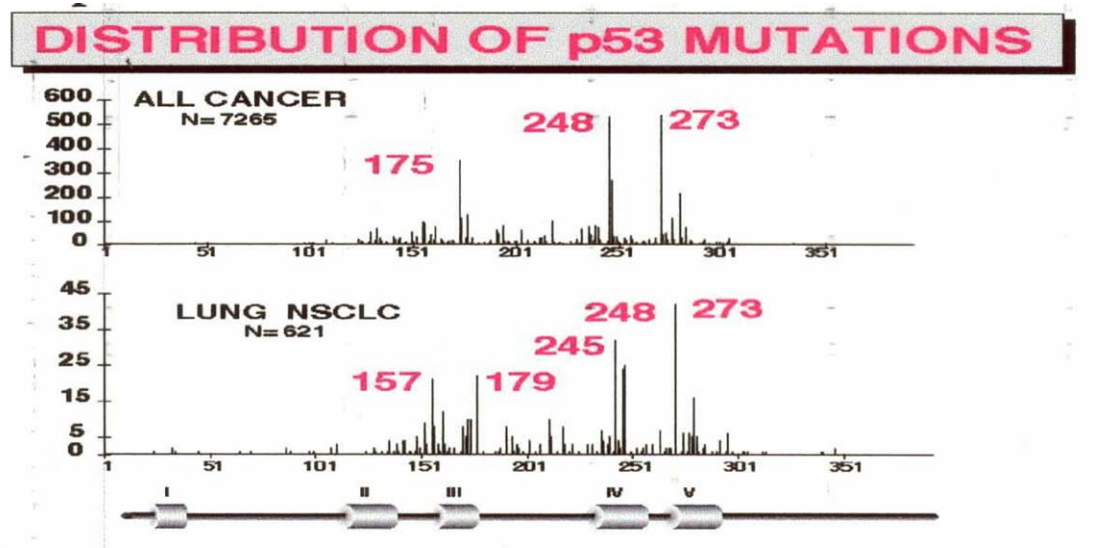
A. L. Fink, Acc. Chem. Res. **2006**, 39, 6284

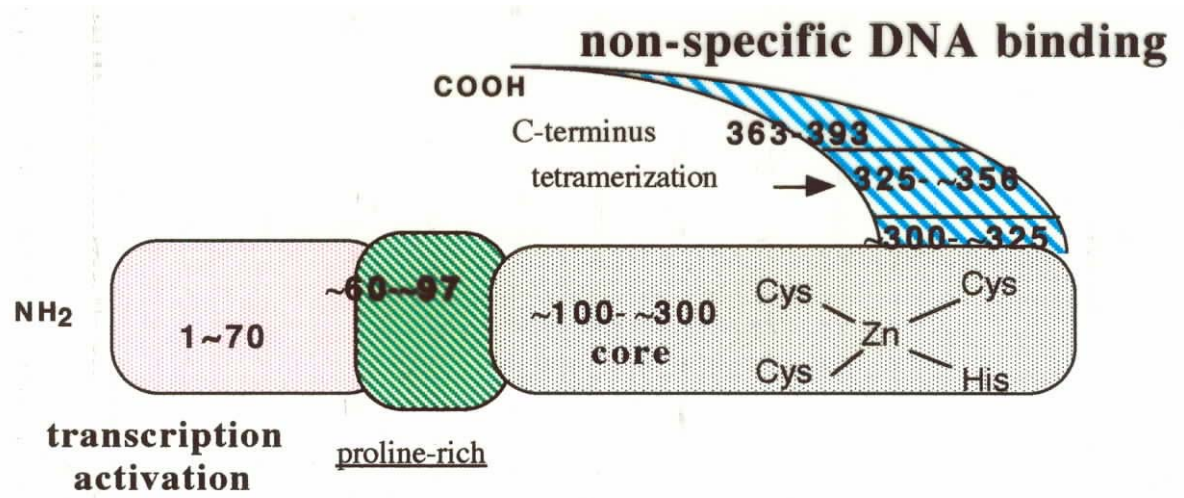
Tumor Suppressor Protein p53

The p53 gene encoding this 393-amino acid nuclear phosphoprotein is located on the short arm of chromosome 17 in humans

It is the **most frequently mutated cancer associated gene**; its **mutation** was found **in more than 50% of human malignancies**. The majority of these mutations maps to the protein **core domain**

p53 is a metalloprotein containing one zinc atomin the **core domain** responsible for the **DNA sequence-specific binding**

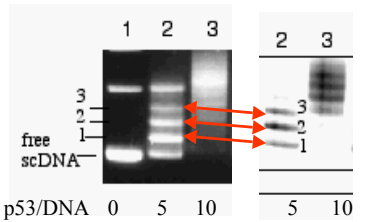




K zobrazení obrázku
je zapot¼ebí QuickTime™
a dekompresor Photo - JPEG.

Binding of wildtype p53 tosupercoildDNA

p53-DNAcomplexes form band ladders with sc plasmid DNA in agarosegel

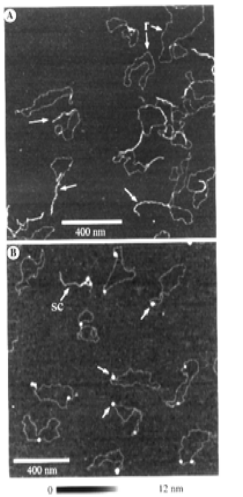


agarose gel
ethidium staining

immunoblot

retarded DNA bands on the gel match p53 bands on the immunoblot

p53-scDNA complexes observedby AFM



p53 sequence specific binding to:

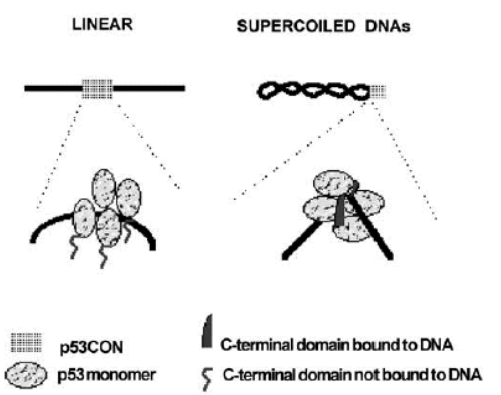


Figure 6 Scheme of the p53 SSDB to lin and scDNAs. In lin DNA, p53 binds to the CON only by the core domain producing a moderate bending of DNA (Cherny *et al*., 1999). Supercoiling can stabilize local DNA structures such as cruciforms and induce DNA bends. If the supercoil-induced DNA conformational change is sufficiently close or coincide with the target sequence, enhanced p53 binding can take place involving both the core and the C-terminal domains (see the text for details)

Mutated p53 protein

Mutated p53 protein not only loses its original function but it also may gain a new function which speeds up the cancer development (instead of preventing it).

These changes in p53 functions are not directly due to the point mutation (exchange of one amino acid in the primary structure of p53) but to changes in the three-dimensional structure of the protein.

Attempts are being made to find drugs capable to restore native conformation of the protein. Highly sensitive methods are necessary to screen the effects of a large number of drugs.

Mutated p53 structure resembles to a certain extent denatured p53.

As partners of the EU 6th FP project we study chemical reactivity and electrochemical responses of wt and mutated p53 proteins and their domains.

DNA-protein interactions

A number of proteins is capable to recognize:

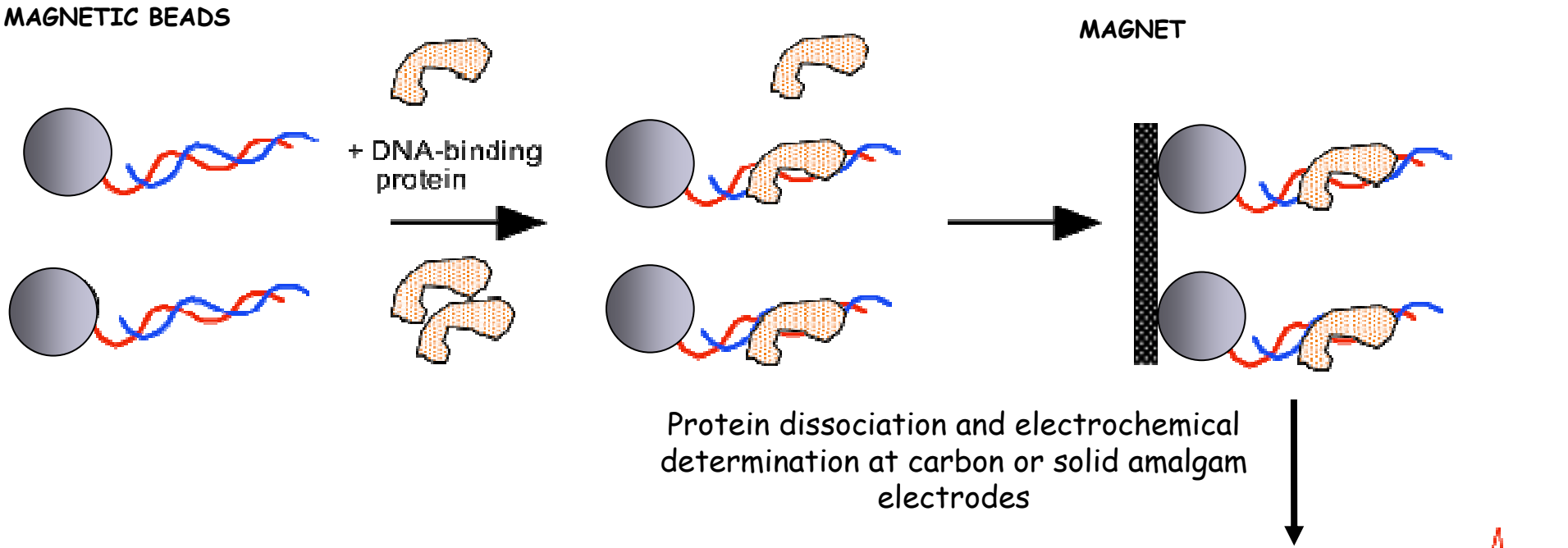
1) specific **nucleotide sequences** in dsDNA
2) specific DNA **structures**
3) damaged and **incorectly paired bases**

In principle proteins can be used to detect DNA damage and nucleotide sequences in DNA hybridization experiments.

Huge amount of literature on DNA-protein interactions exists but **electrochemical literature on this topic is rather scarce.**

Label-free assay of DNA-protein interactions

using double-surface technique.



Suitable for almost all proteins

Determination of point mutations by MutS protein

Paleček, E. et al. (2004). "Sensitive electrochemical determination of unlabeled MutS protein and detection of point mutation in DNA." ***Anal. Chem***. **76**(19): 5930-5936.

We believe that the HIGH SENSITIVITY and the abilities of the electrochemical analysis to recognize peptide and protein REDOX STATES and CONFORMATIONAL CHANGES in proteins may find use in ANALYSIS of PROTEINS and particularly of those important IN BIOMEDICINE.

Application of electrochemistry in our studies of the tumor suppressor protein p53 as well as of alpha-synuclein (important in Parkinson disease) appear particularly interesting.

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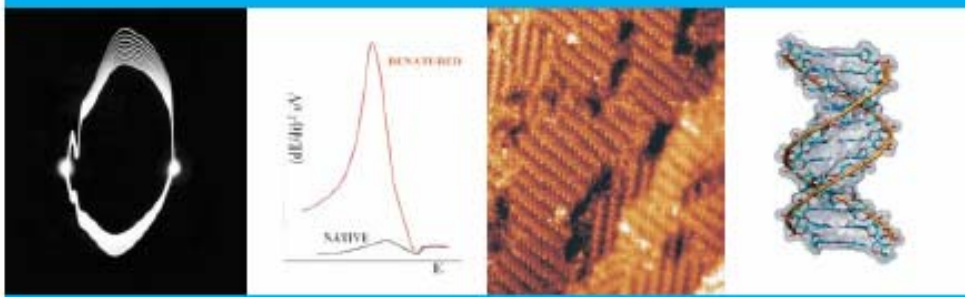
Towards Electrochemical Sensors for Genomics and Proteomics



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