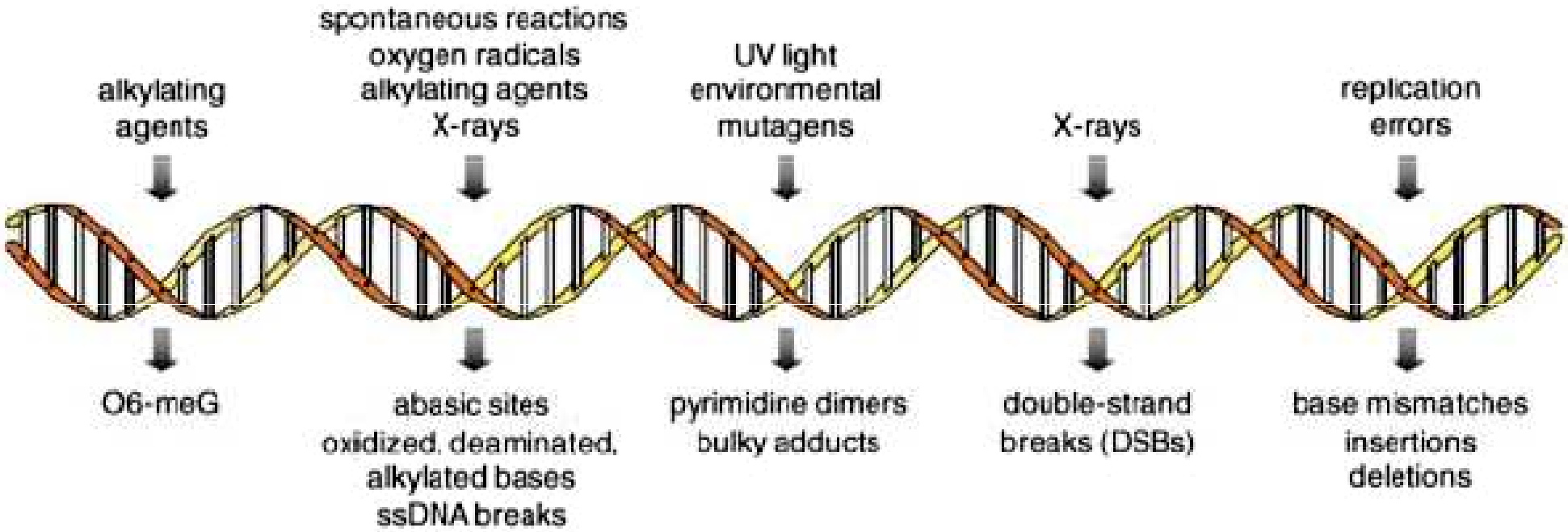
Institute of Biophysics
Department of Biophysical Chemistry and Molecular Oncology
Centre of Biophysical Chemistry, Bioelectrochemistry and Bioanalysis

Electrochemical sensing of DNA damage

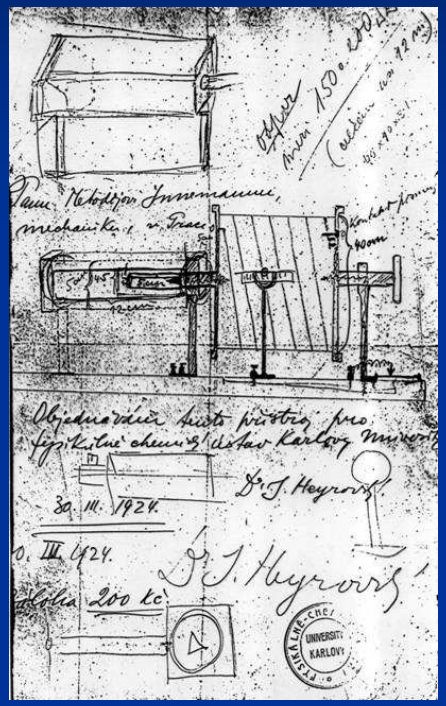
Miroslav Fojta

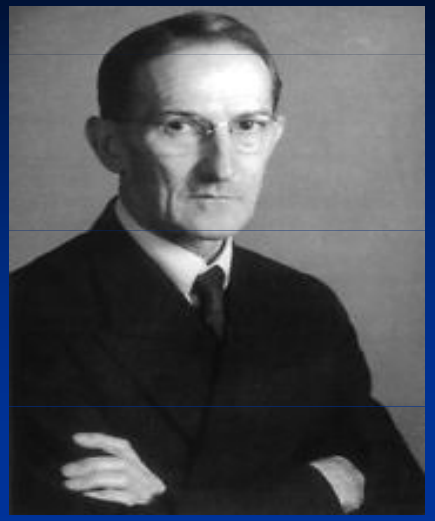
Olsztyn-Lańsk, September 20th, 2007

DNA damage

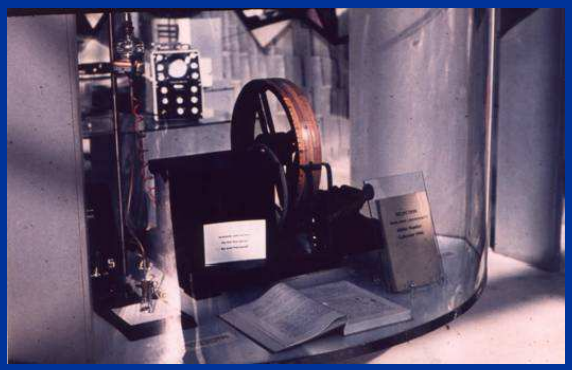

alkylating agents
spontaneous reactions
oxygen radicals
alkylating agents
X-rays
UV light
environmental mutagens
X-rays
replication errors
O6-meG
abasic sites
oxidized, deaminated, alkylated bases
ssDNA breaks
pyrimidine dimers
bulky adducts
double-strand breaks (DSBs)
base mismatches
insertions
deletions

Elektrochemické metody …



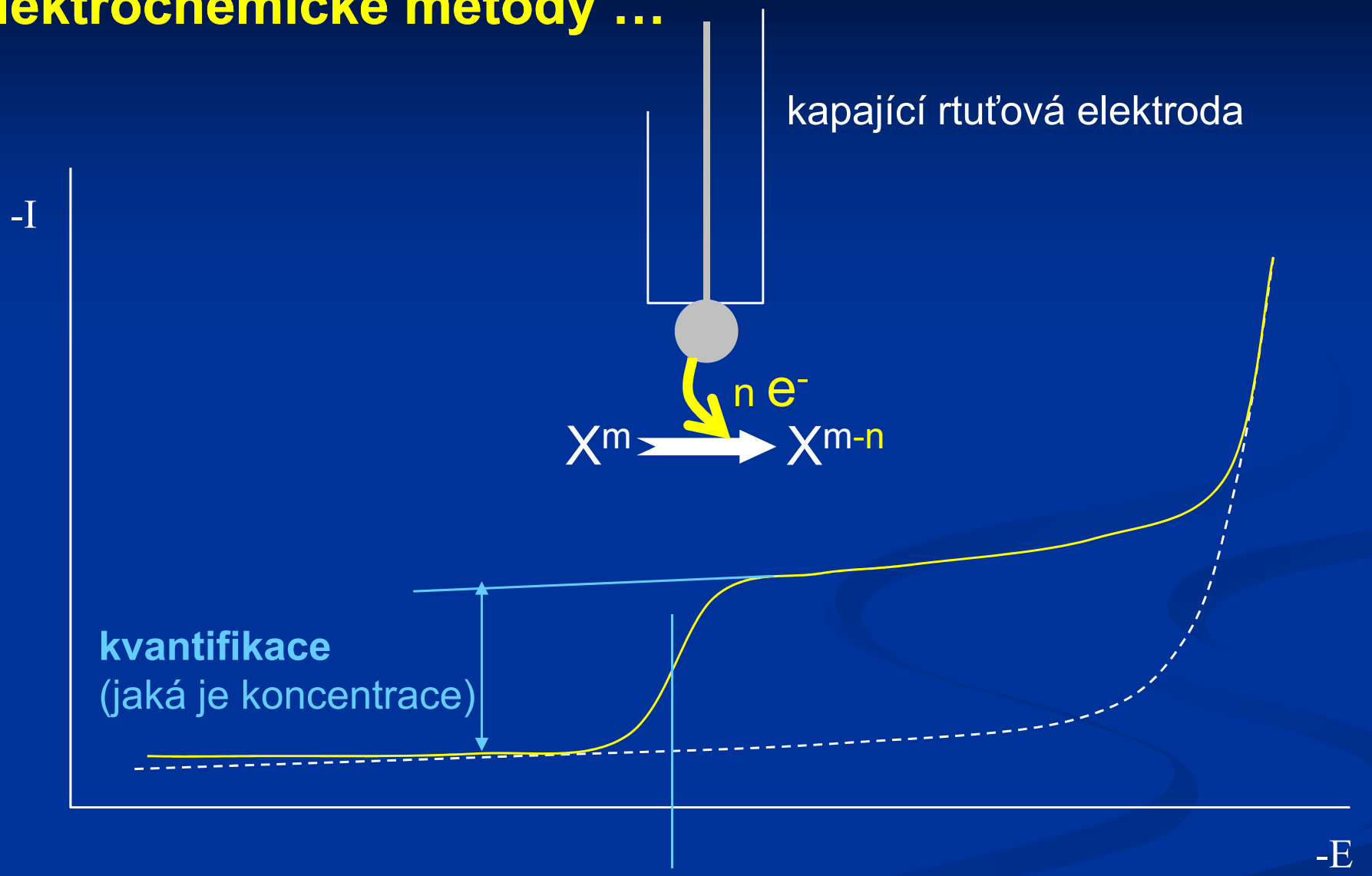


1922 Jaroslav Heyrovský: polarografie
1959 Nobelova cena

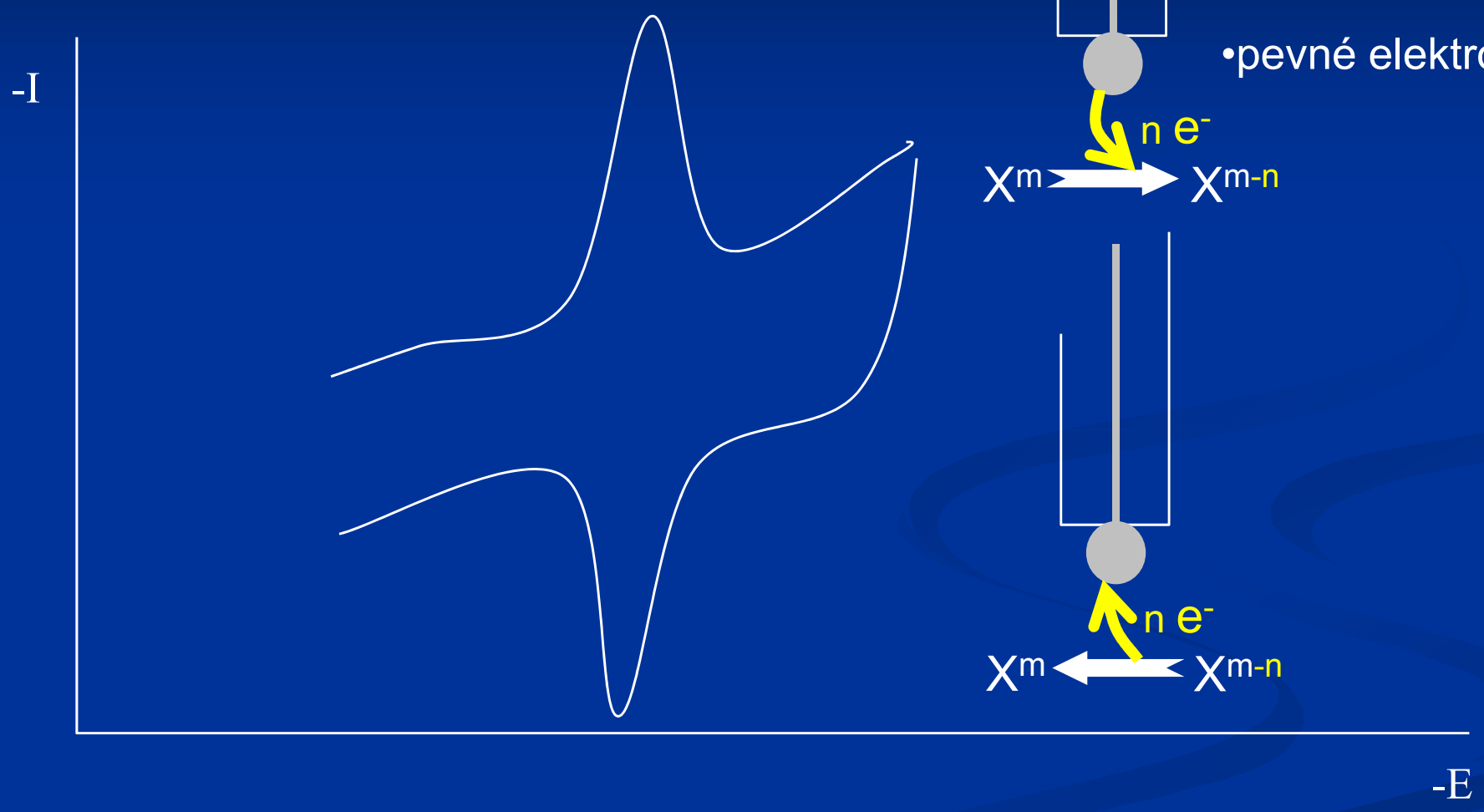


základ celé škály široce využívaných elektrochemických metod

Elektrochemické metody …



Elektrochemické metody …



- visicí rtuťová kapková elektroda
- pevné elektrody

late 1950s, Emil Paleček: DNA polarography

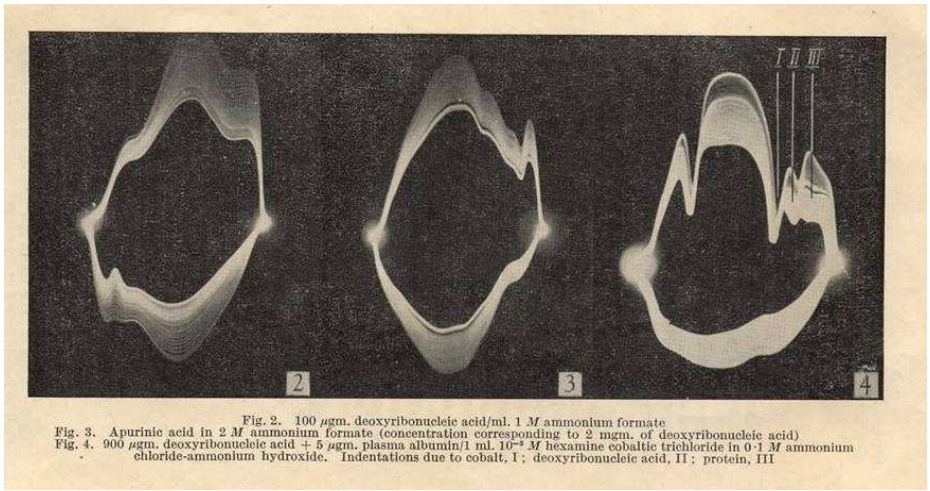


Fig. 2. 100 μgm. deoxyribonucleic acid/ml. 1 *M* ammonium formate
Fig. 3. Apurinic acid in 2 *M* ammonium formate (concentration corresponding to 2 mgm. of deoxyribonucleic acid)
Fig. 4. 900 μgm. deoxyribonucleic acid + 5 μgm. plasma albumin/1 ml. 10^{-3} *M* hexamine cobaltic trichloride in 0·1 *M* 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

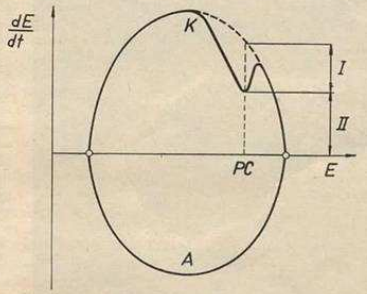
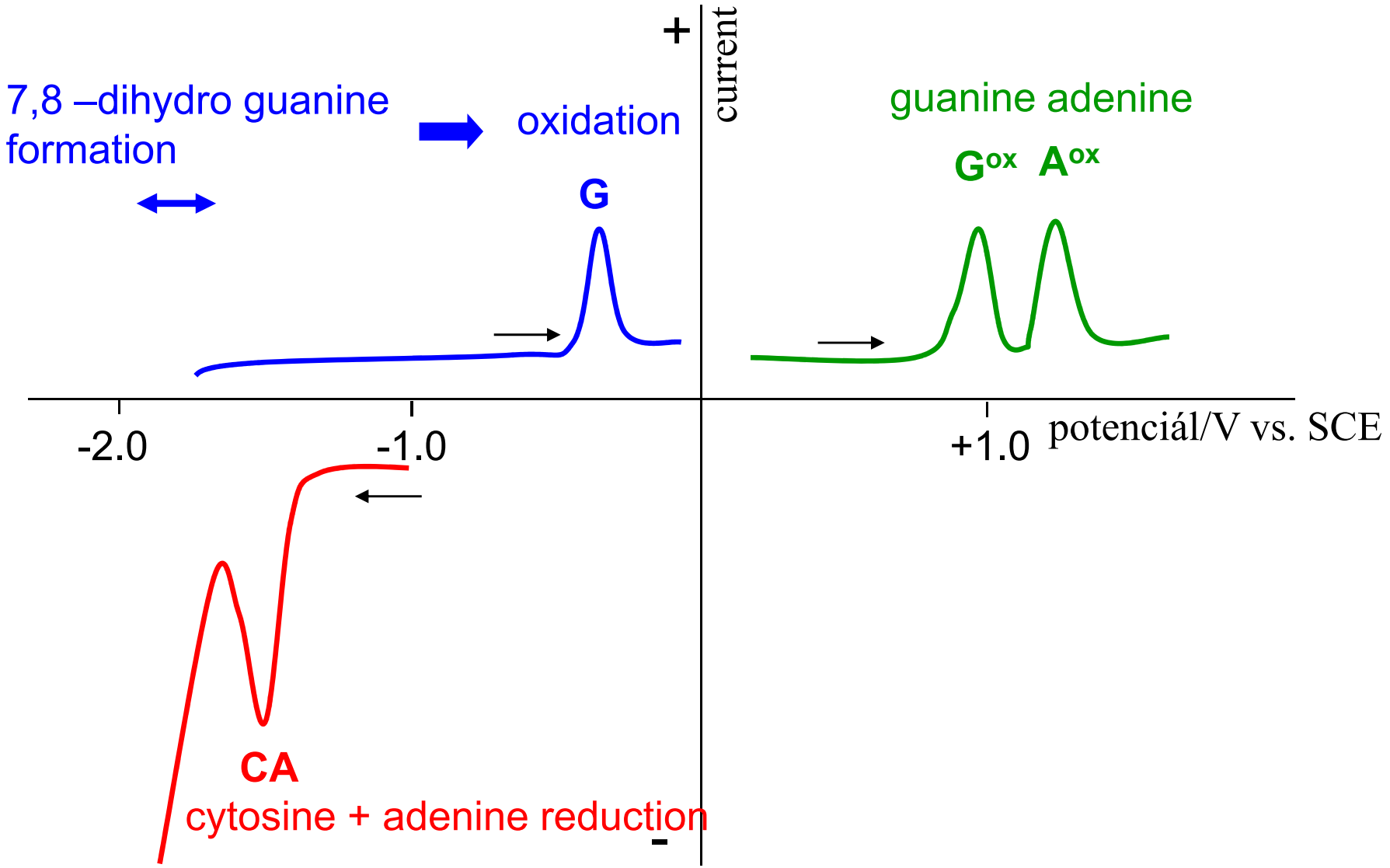


Fig. 1. Graph of dE/dt against E. The nature of the material analysed is characterized by the potential of the indentation (*PC*), which is somewhat similar to the polarographic half-wave potential. The quantity of the material is characterized by the depth of the indentation. For qualitative analysis,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

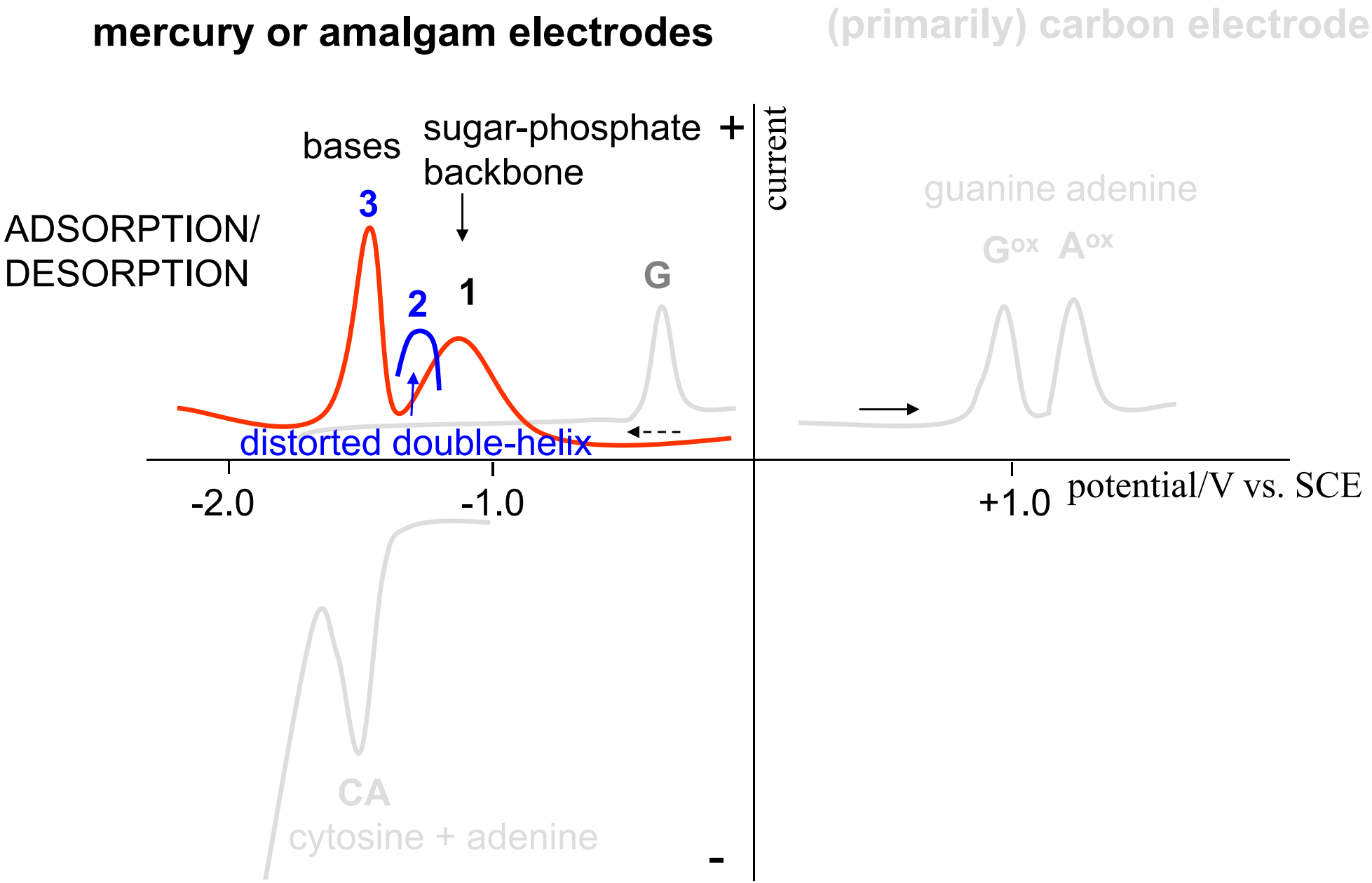
DNA is electrochemically active

mercury or amalgam electrodes

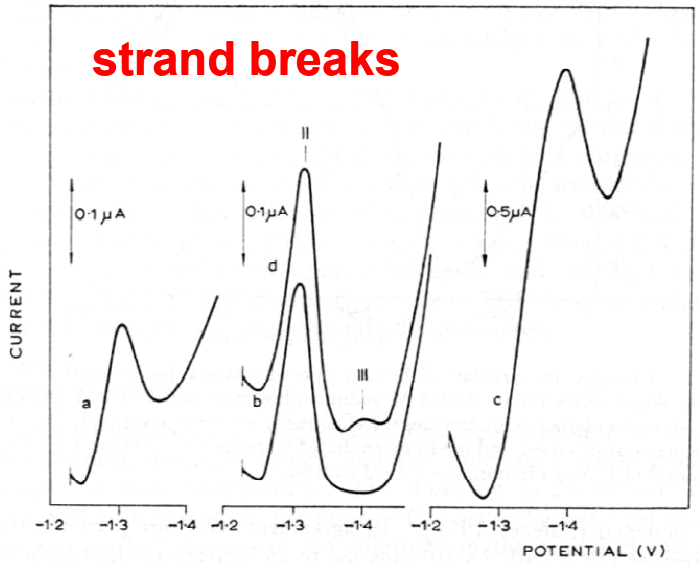
(primarily) carbon elektrodes

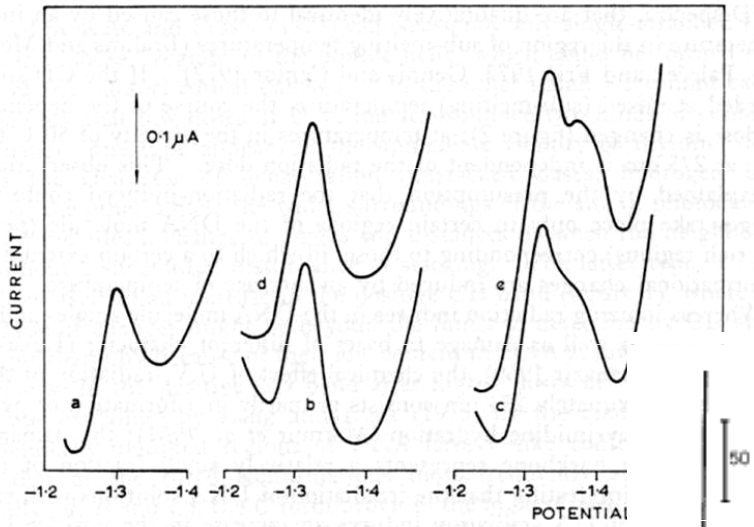


DNA is electrochemically active

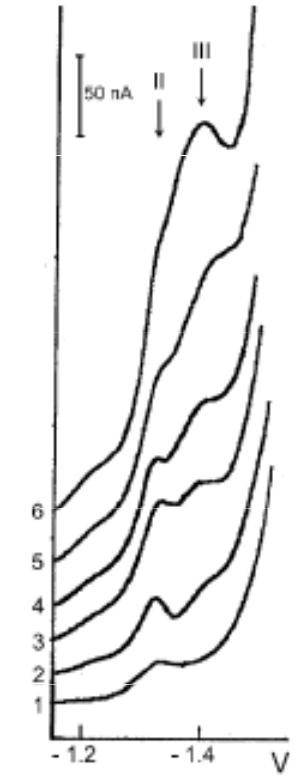


early studies by polarography: damage to DNA can be detected



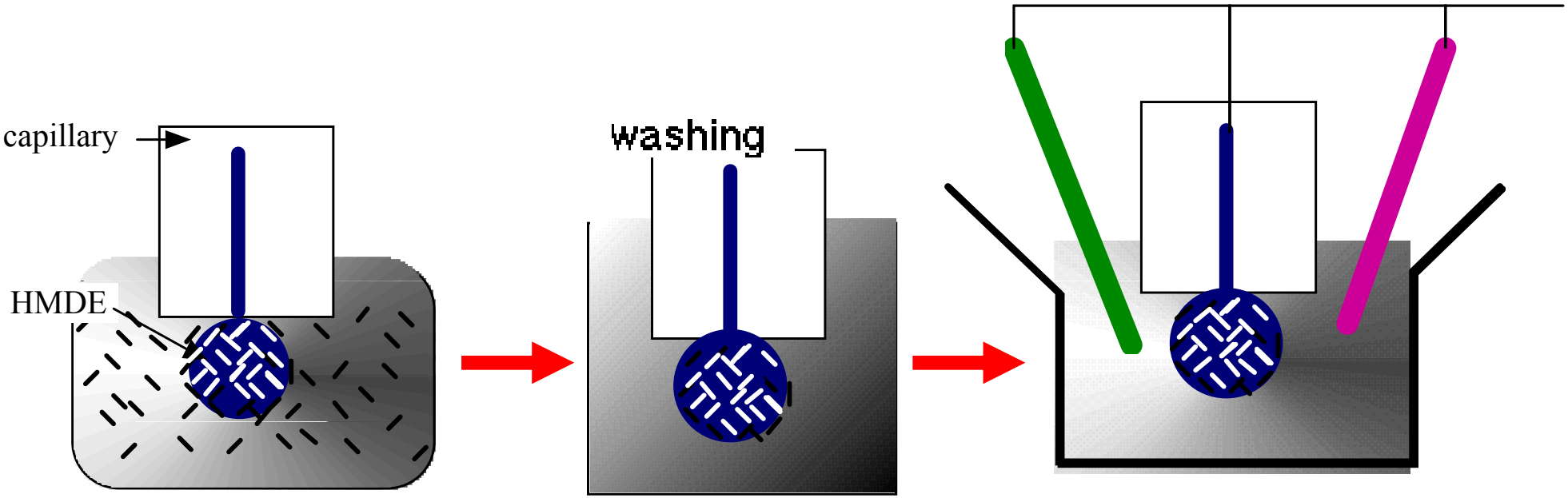


distortions due to base damage



single stranded regions in dsDNA etc.

Adsorptive Transfer Stripping



adsorption transfer transfer

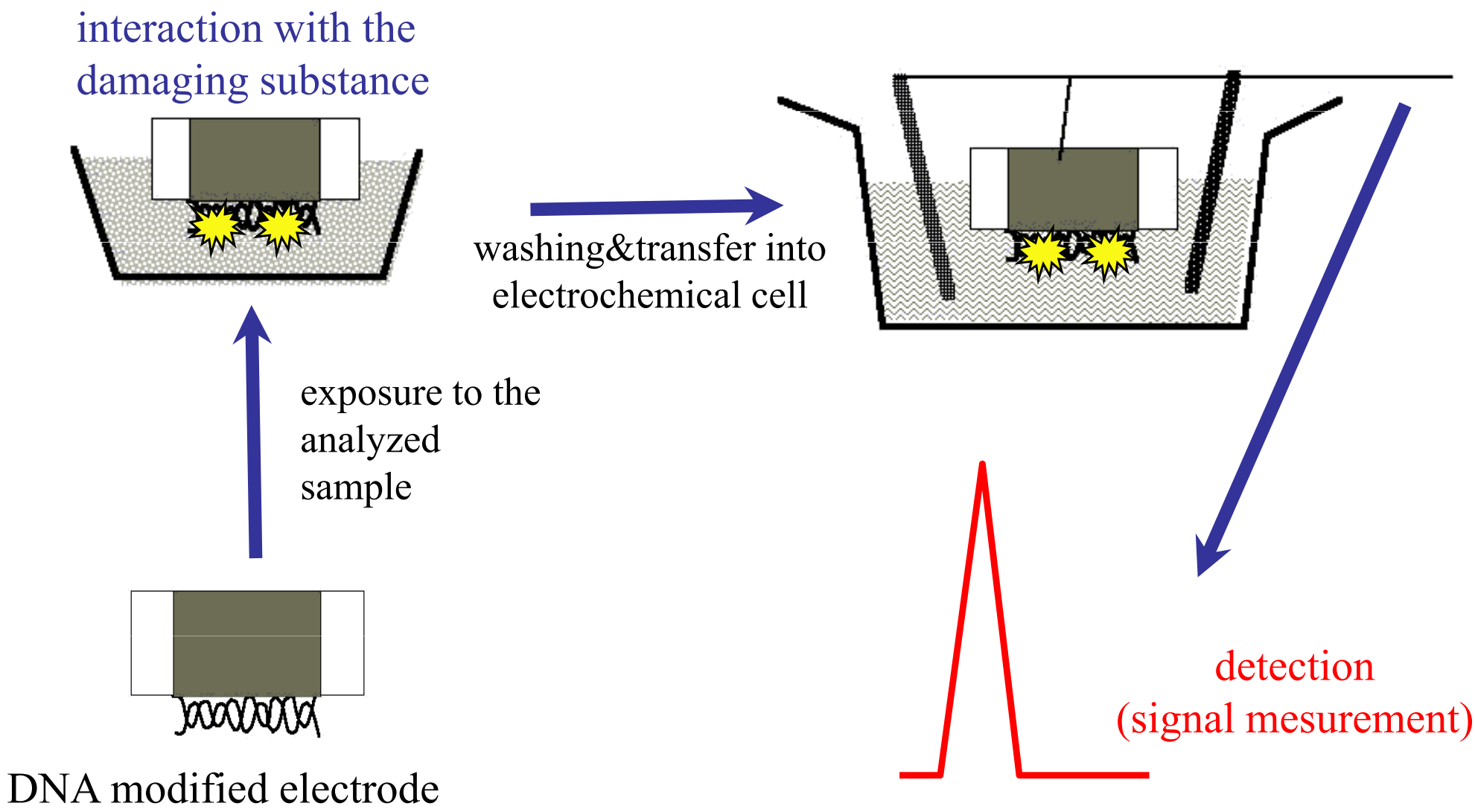
- **instead of ~milliliter volumes, several microliters are sufficient for analysis**
- **analysis of reaction mixtures with substances that interfere in „conventiona“ voltammetry (including DNA damaging agents)**

DNA-modified electrode = a simple electrochemical sensor for DNA damage

- electrode = **signal transducer**

- **„recognition layer“ of DNA** at its surface

DNA-modified electrode = a simple electrochemical sensor for DNA damage

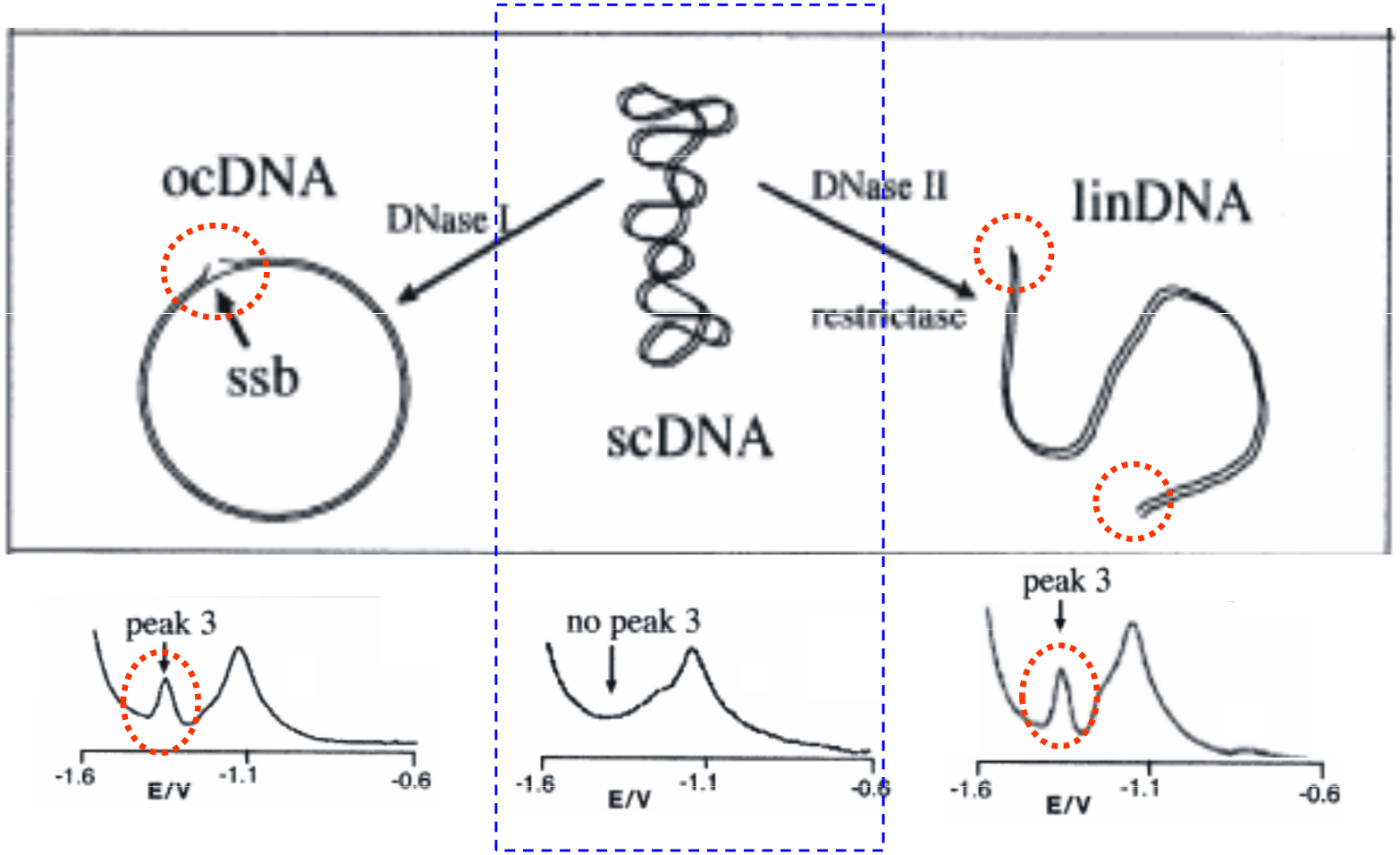


chemical modification of DNA can:

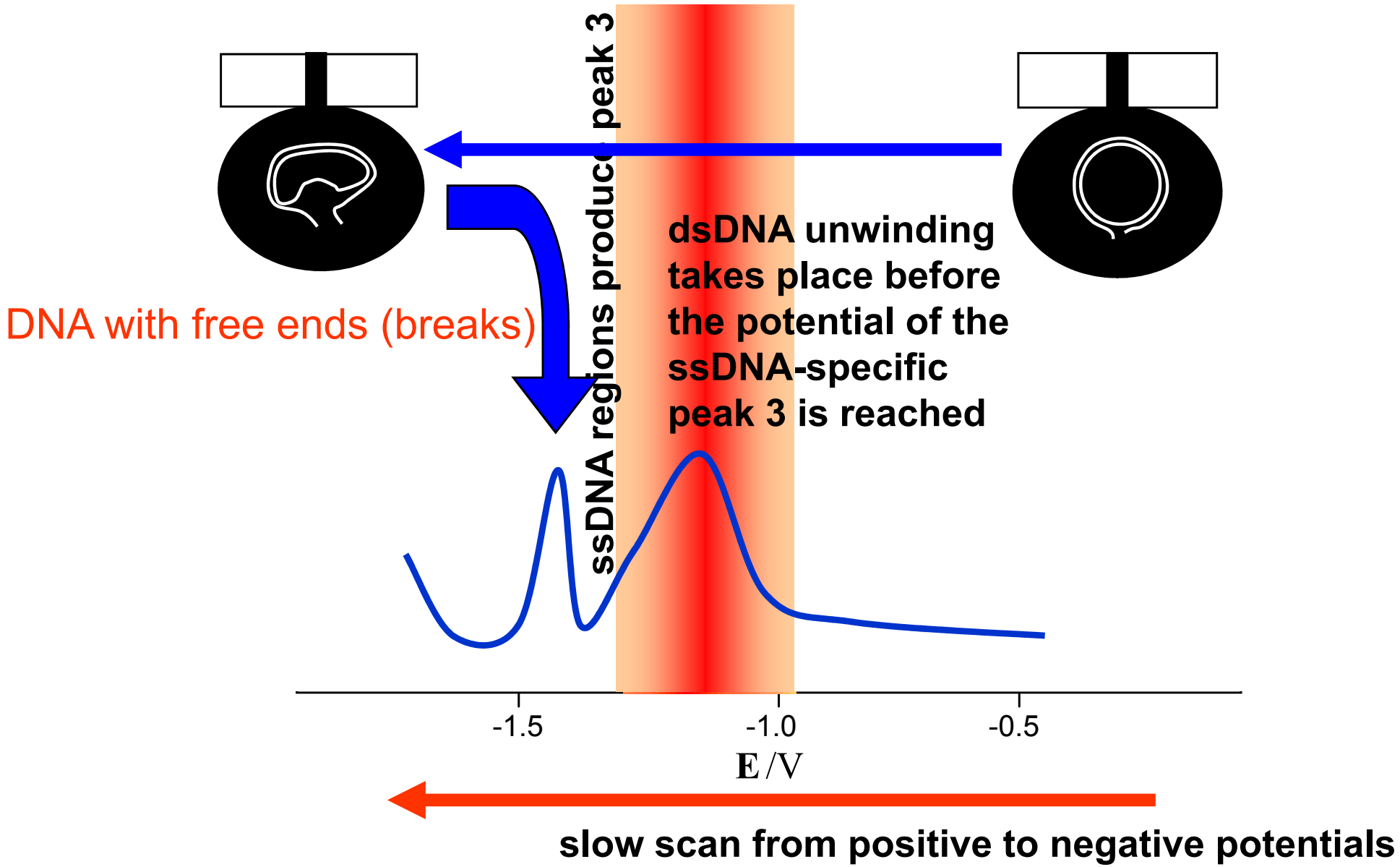
- cause strand breakage detectable primarily with mercury (amalgam) electrodes
- cause distotions of the double helix detectable primarily with mercury (amalgam) electrodes
- hit electroactive sites of nucleobases thus affecting their electrochemical activity (mercury or carbon electrodes)
- result in introducing new electroactive moieties (principially any electrode - depending on the electroactive group introduced)

Detecting strand breaks with mercury-based electrodes

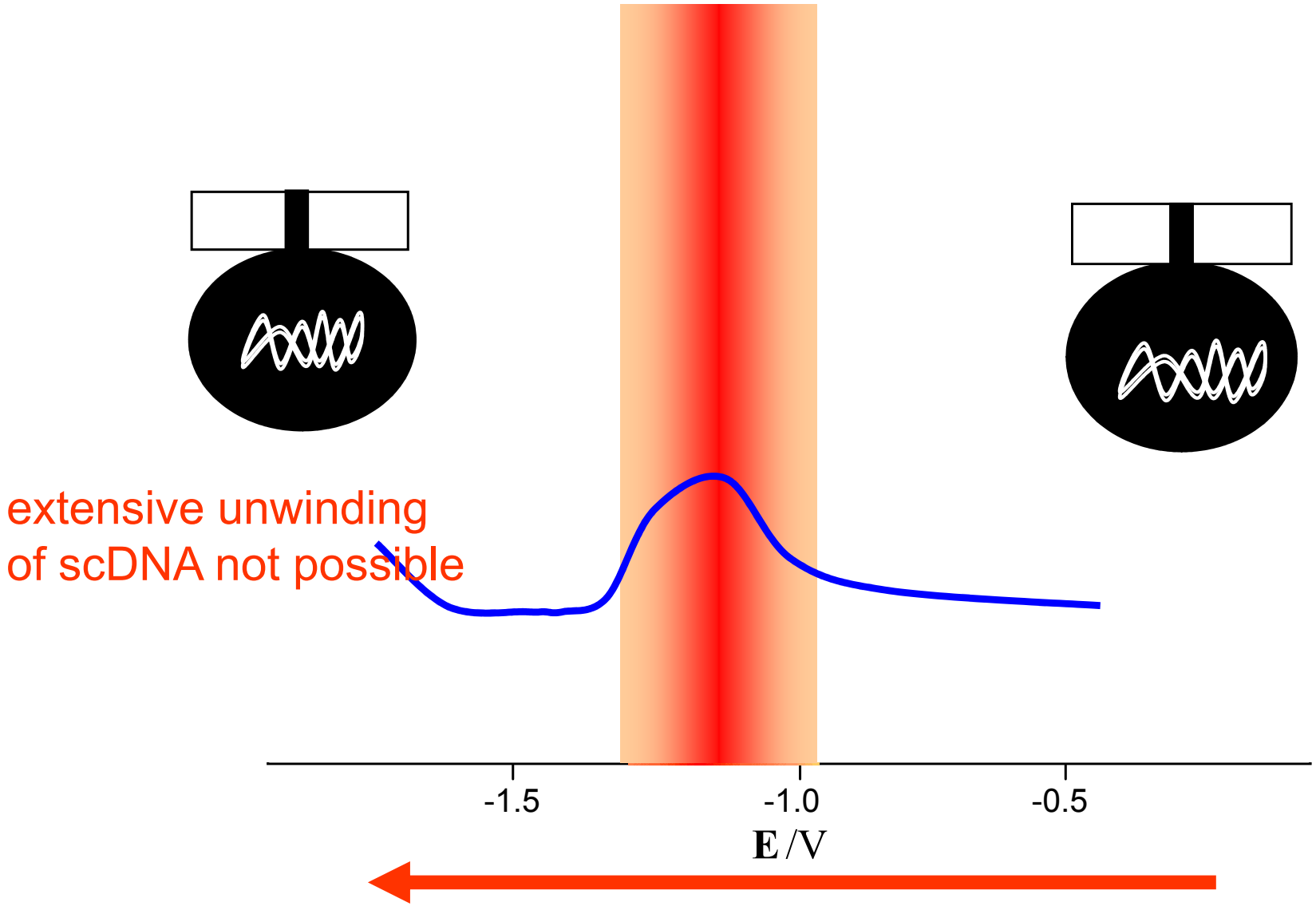
difference in behavior of covalently closed circular and nicked or linear DNAs at a mercury electrode



surface denaturation of dsDNA at the HMDE within the „**region U**“

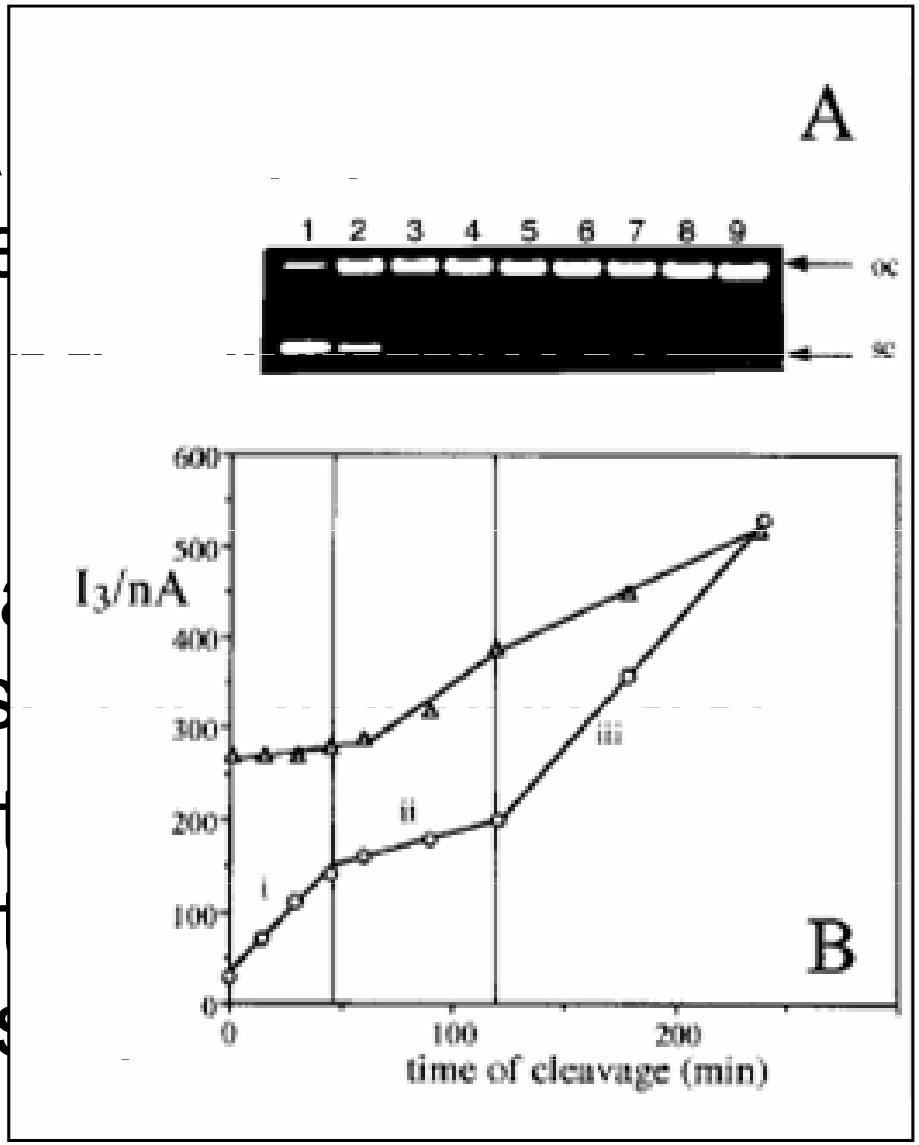


surface denaturation of dsDNA at the HMDE within the „**region U**“



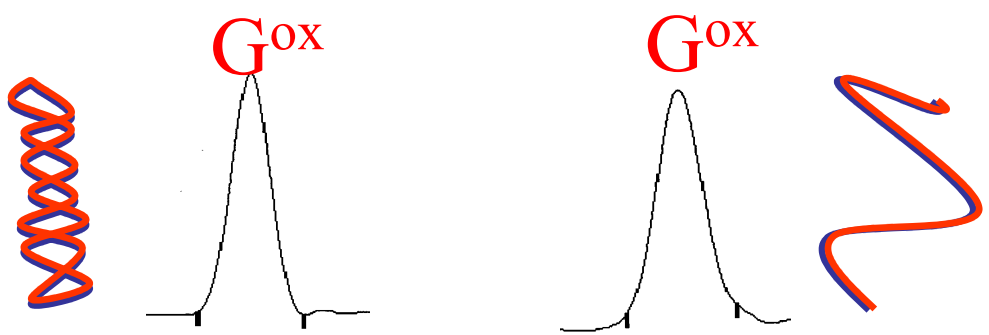
High sensitivity of ssb detection with mercury electrodes

- one break in ~1% of a
  molecules can be dete
- that is one lesion amo
  nucleotides
- 200 ng of DNA per ana
  sensitivity than agaros
- detection of multiple st
  molecule possible (not
  of native electrophores

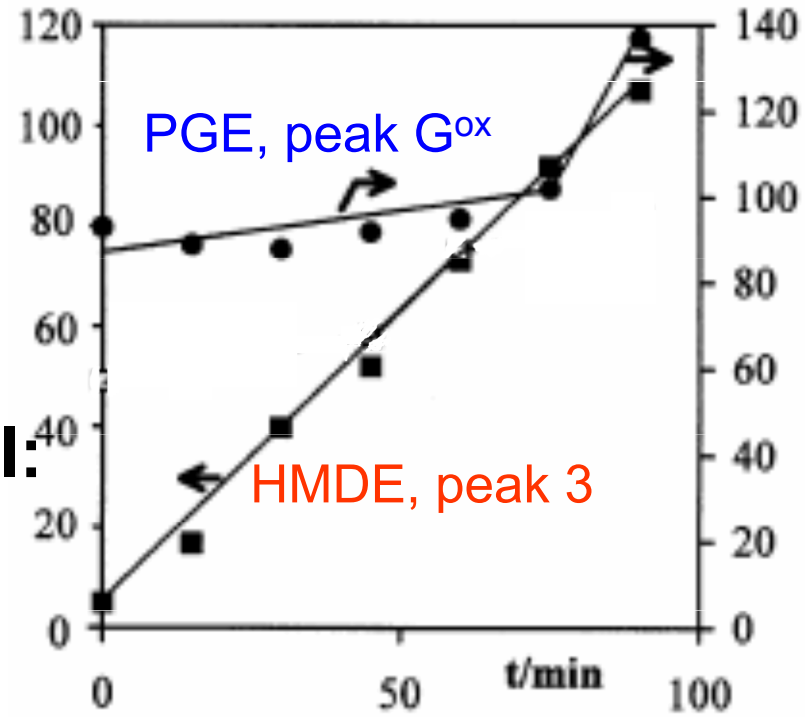


guanine oxidation signal **at carbon electrodes** is not sensitive to formation of individual strand breaks

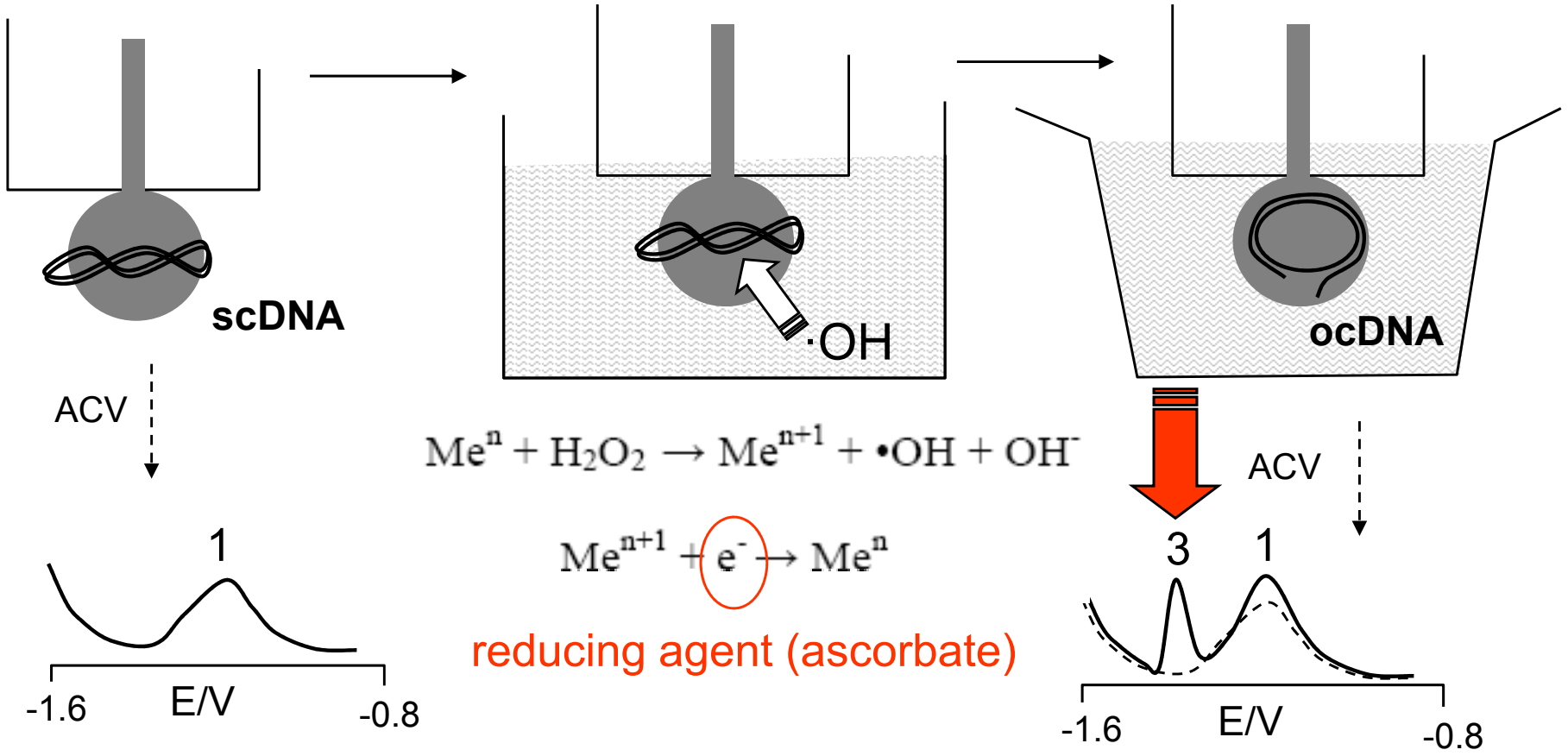
- practically indistinguishable responses of sc, oc and linear DNAs
- small sensitivity to DNA structure: intact dsDNA yields a large signal
- absence of (extensive) surface denaturation of dsDNA at carbon



cleavage of scDNA by DNase I:



Mercury electrode modified with scDNA: sensor for DNA damaging agents



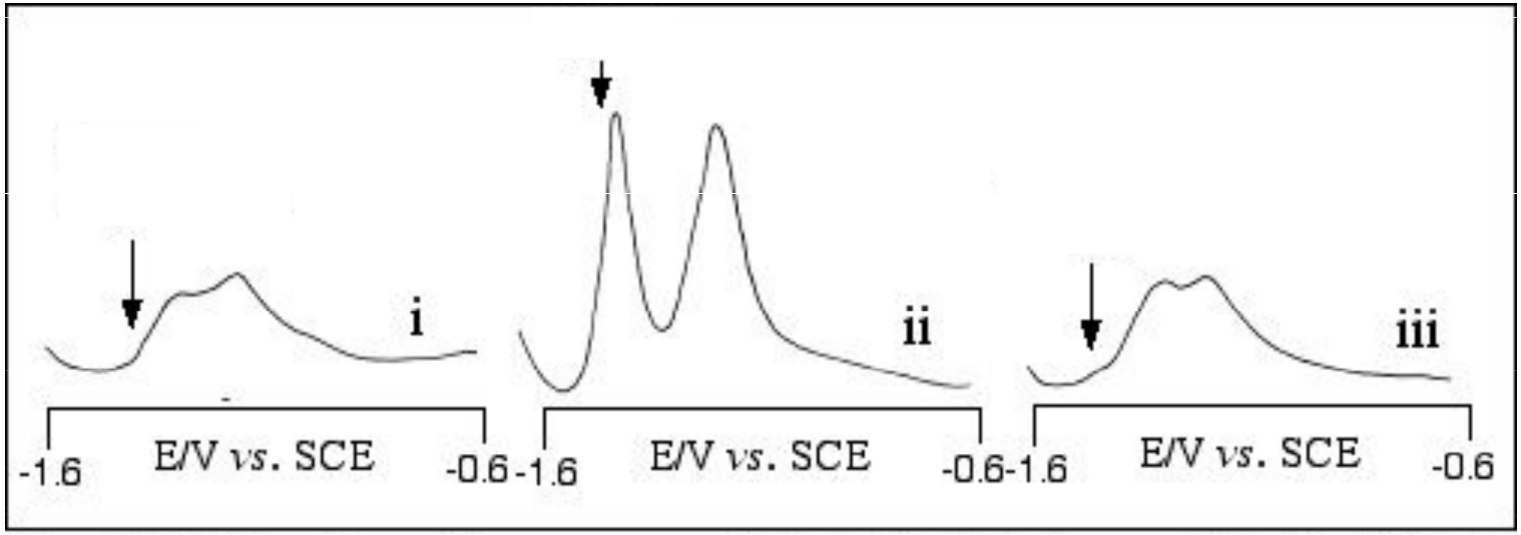
example of the sensor application: detection DNA damaging agents in waste (industrial) waters

(uranium mines, Dolní Rožínka)

blank

mine water – input of purification plant

output of the water purification plant

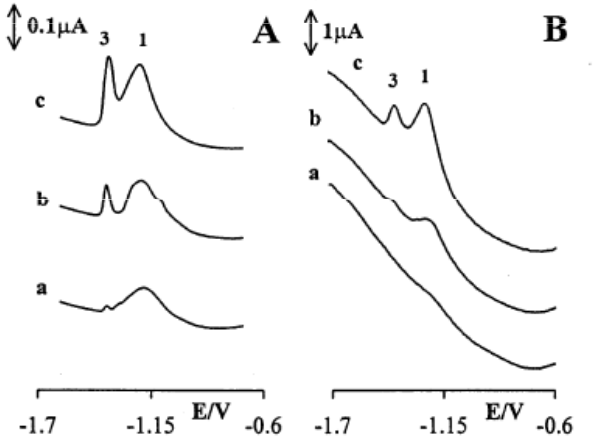


(containing considerable amounts of transition metals like Fe, Mn)

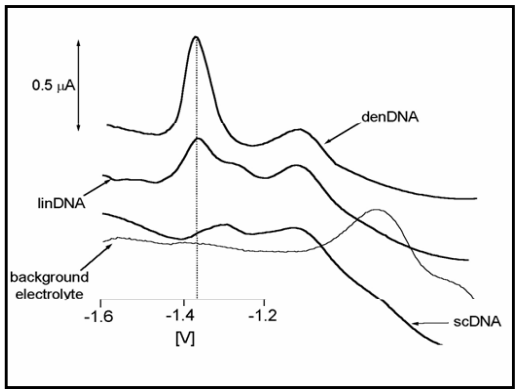
working with „dangerous“ mercury should be avoided?

similar responses to DNA damage like with the HMDE can be obtained

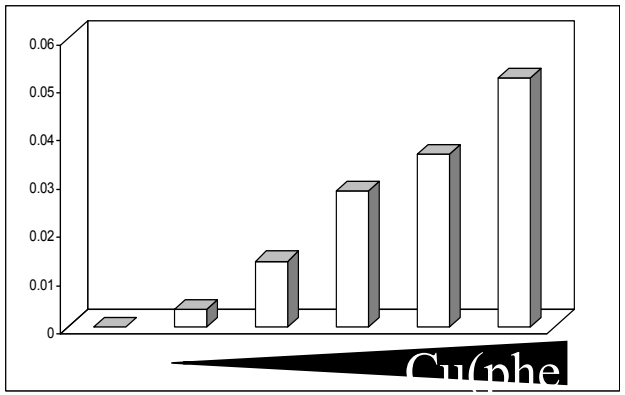
- **with mercury film electrodes (Kubičárová 2000)**



- **with amalgam electrodes (Cahová-Kuchaříková, Fadrná, Yosypchuk, Novotný 2004)**



AC voltammograms of sc, linear ds and denatured DNA at m-AgSAE

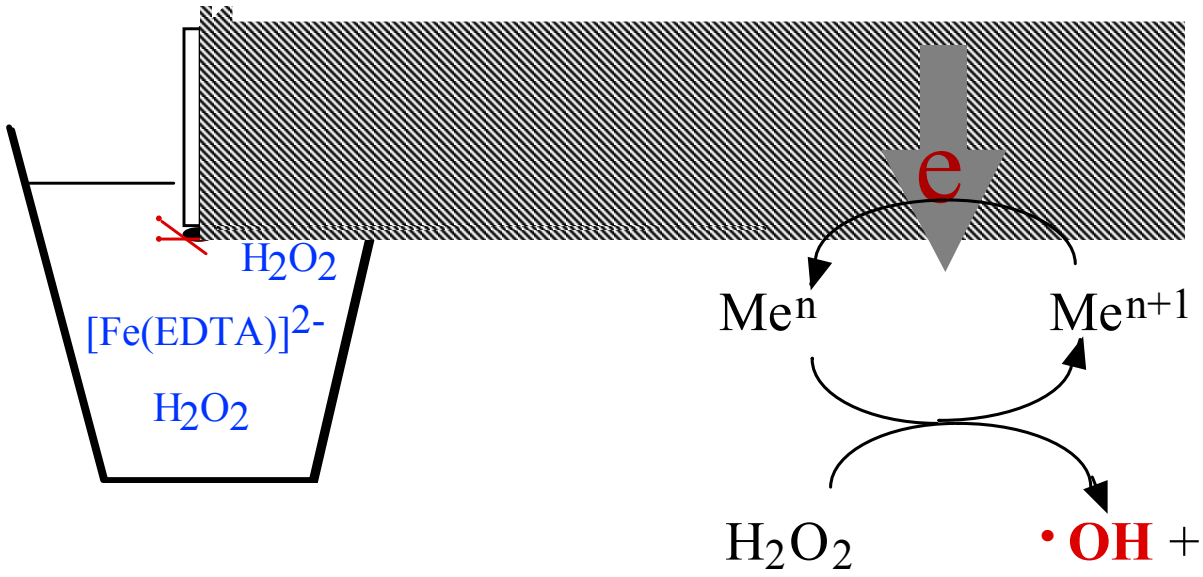


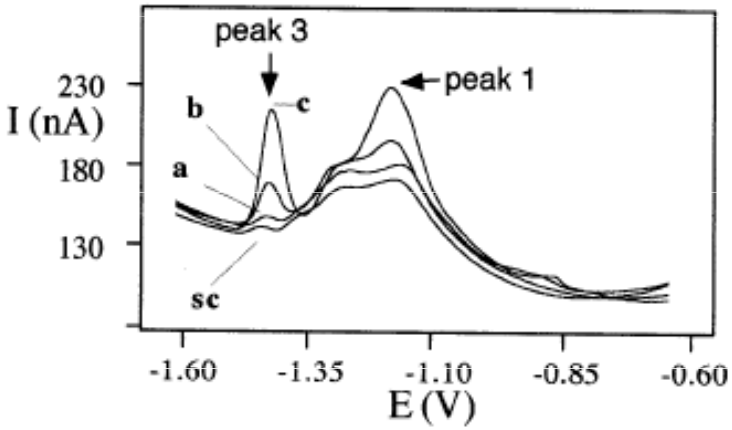
changes in the peak 3 height (at m-AgSAE) due to scDNA exposure to a chemical nuclease $Cu(phen)_2$

studies of cleavage of DNA at the electrode surface by electrochemically generated reactive species

Electrode potential-modulated cleavage of surface-confined DNA by hydroxyl radicals detected by an electrochemical biosensor

Miroslav Fojta *, Tatiana Kubičárová, Emil Paleček





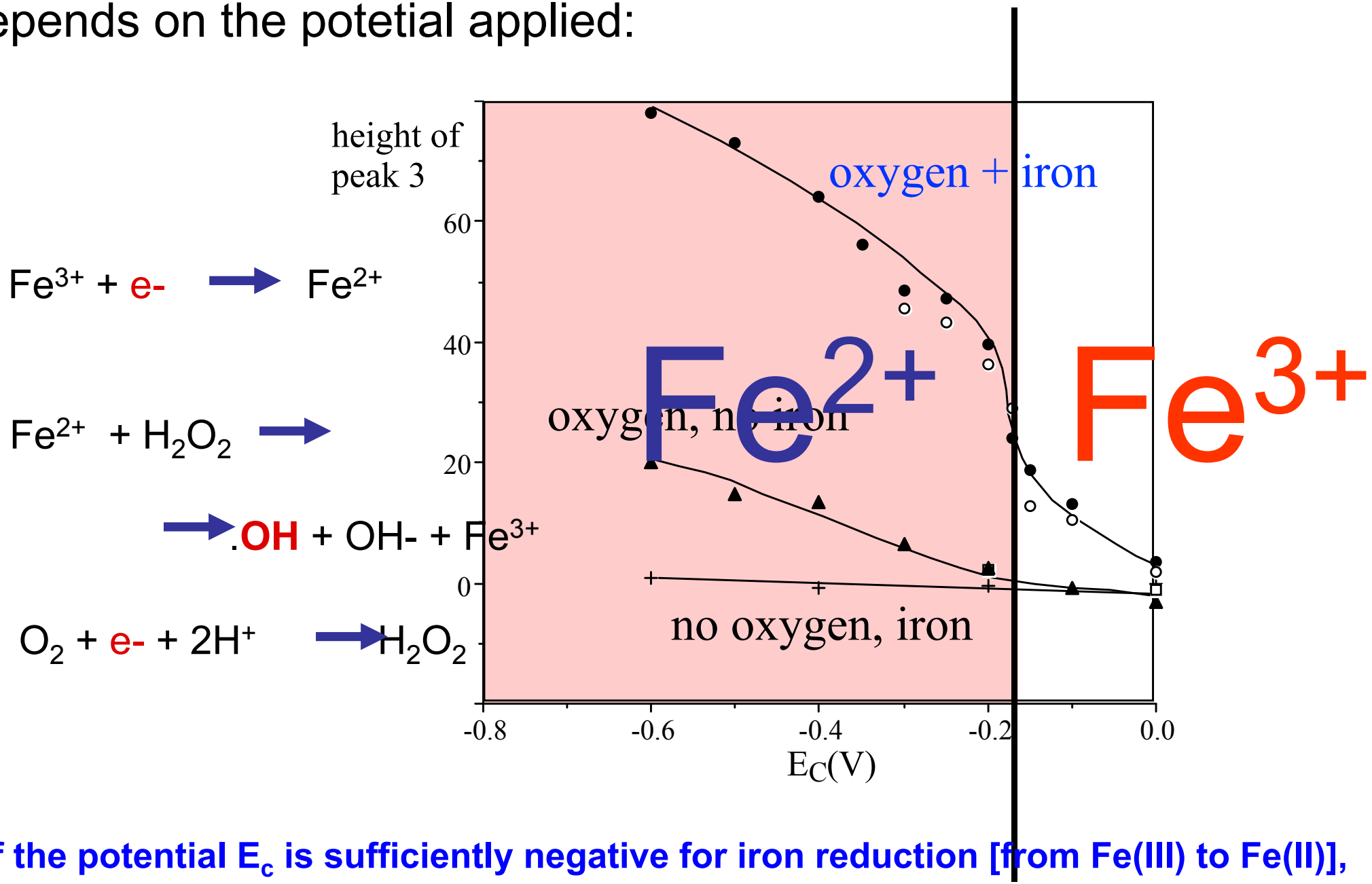
(a) E_C = 0 V; (b) E_C = 0.2 V;
(c) E_C = 0.4 V applied for 60 s

e.g., hydroxyl radicals (or other ROS) can be generated via electrochemically controlled Fentonovy/Haber-Weissovy reactions

scDNA-modified electrode was dipped in solution containing Fe/EDTA and H_2O_2 (neor O_2) and potential (E_c) ensuring redox cycling of the metal is applied for certain time

then, DNA response is measured with the same electrode

Peak 3 intensity (=the amount of SB, degree of DNA damage) depends on the potetial applied:



Fe^{3+} + e- → Fe^{2+}

Fe^{2+} + H_2O_2 →

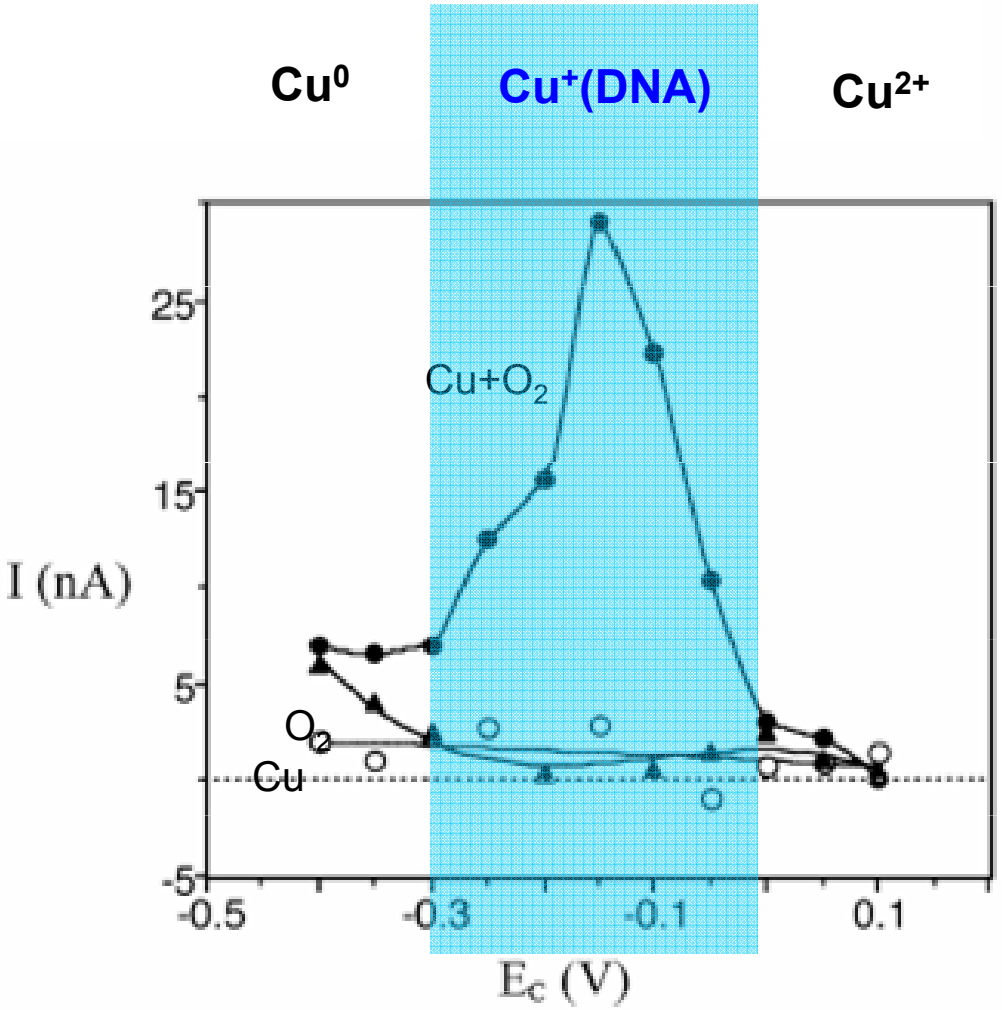
→ .OH + OH- + Fe^{3+}

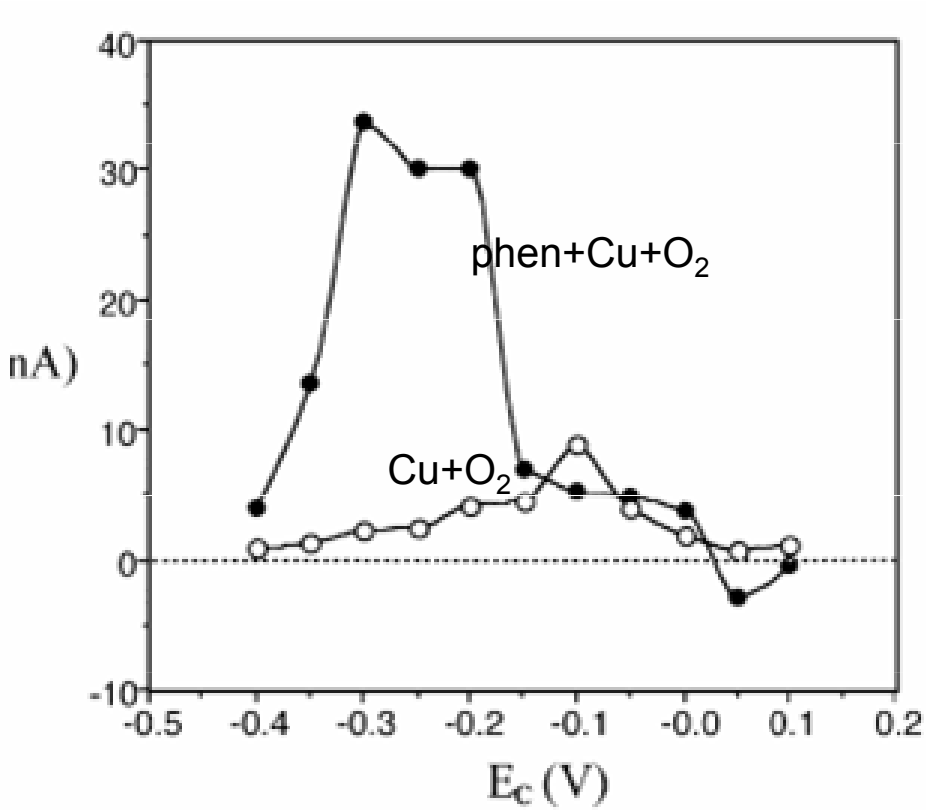
O_2 + e- + $2H^+$ → H_2O_2

if the potential E_c is sufficiently negative for iron reduction [from Fe(III) to Fe(II)], redox cycling is maintained, hydroxyl radicals are produced and DNA is nicked

•analogous effects were observed in the presence of copper (and O_2)

•in this case efficient DNA cleavage is observed only in a narrow potential region where **Cu(I) ions** (stabilized by coordination with DNA bases) can mediate ROS formation





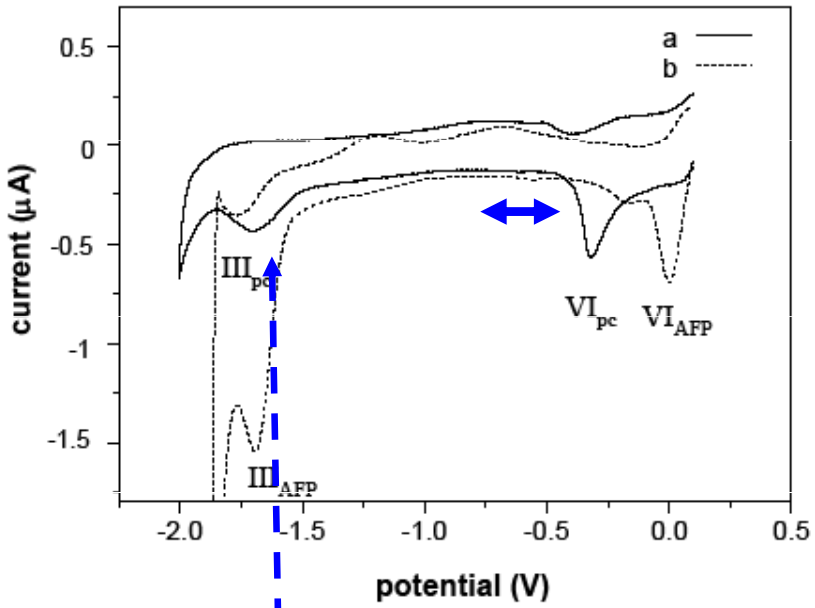
in the presence of 1,10-phenanthroline, a ligand stabilizing Cu(I), stronger DNA damaging effect was observed at more negative potentials

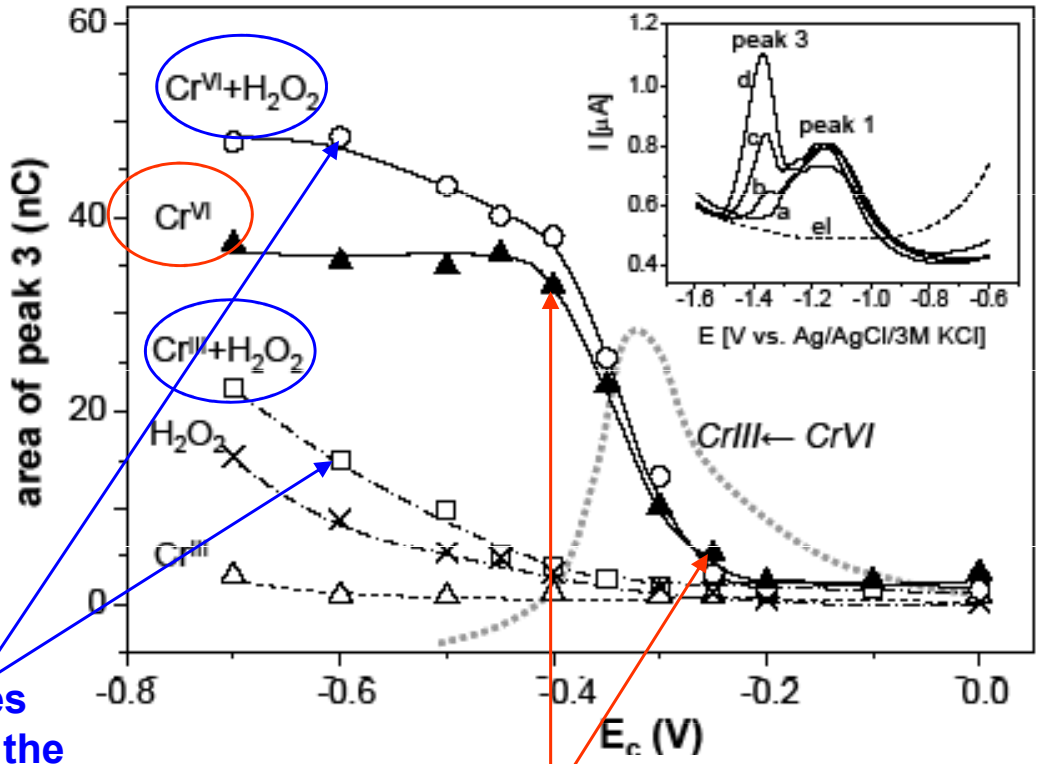
Electrochemical sensing of chromium-induced DNA damage:

(Electroanal., in press)

DNA strand breakage by intermediates of chromium(VI) electrochemical reduction

Jan Vacek‡, Tomáš Mozga†‡, Kateřina Cahová, Hana Pivoňková and Miroslav Fojta*





Cr(III) potentiates DNA damage in the presence of oxygen

Cr(II) ? Cr(III)

$$Me^{n} + H_2O_2 \rightarrow Me^{n+1} + {\bullet}OH + OH^-$$

~~$Me^{n+1} + e^- \rightarrow Me^{n}$~~

(electrochemically) **probably not**

$$Cr^{VI} \rightarrow [Cr^{V} \rightarrow Cr^{IV}] \rightarrow Cr^{III}$$

oxygen not required

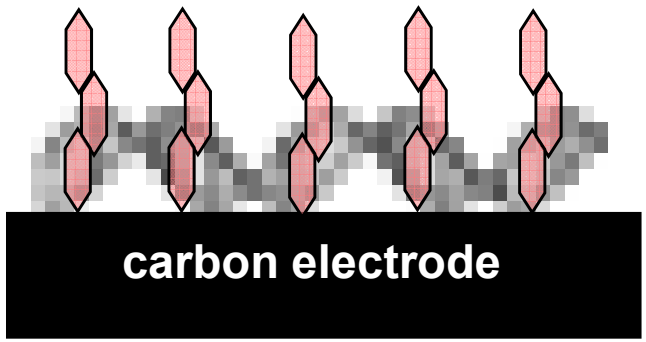
$$Cr^{III} + {\bullet}O_2^- \rightarrow Cr^{II} + O_2$$

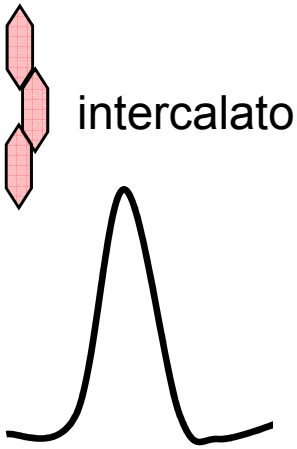
electrochemically

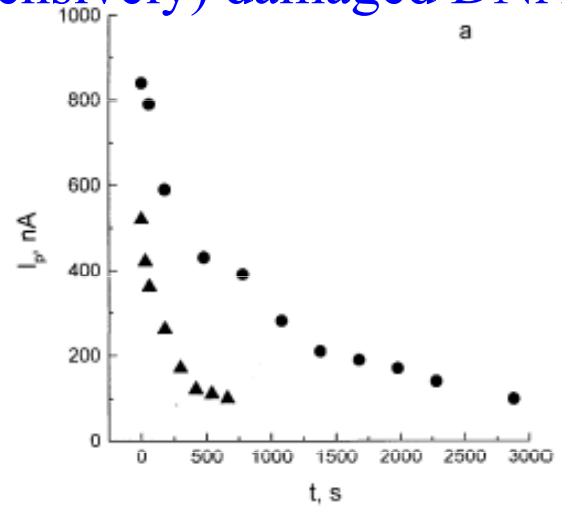
Detection of DNA degradation with carbon electrodes

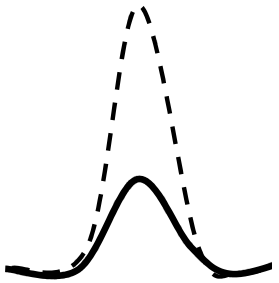
Redox indicator based technique (Labuda et al.) :

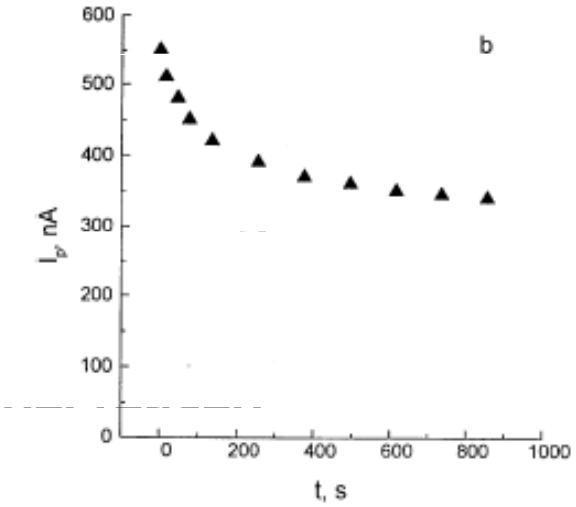
•the indicator can recognize intact DNA from (extensively) damaged DNA







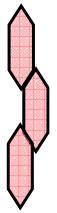




signal decrease due to DNA degradation by $Cu(phen)_2$

Redox indicator based technique (Labuda et al.) :

•the indicator can recognize intact DNA from (extensively) damaged DNA



intercalator

application: testing of antioxidant capacity of different substances

•DNA degraded by hydroxyl radicals

•antioxidants counteract the hydroxyl radicals effects

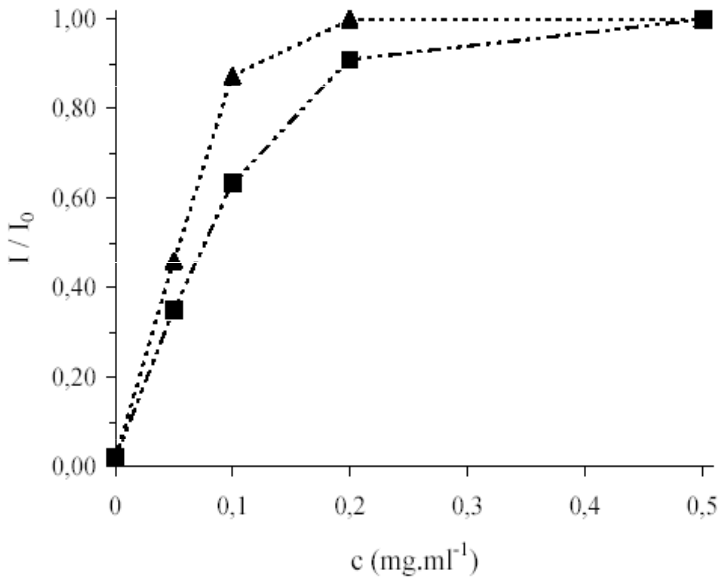
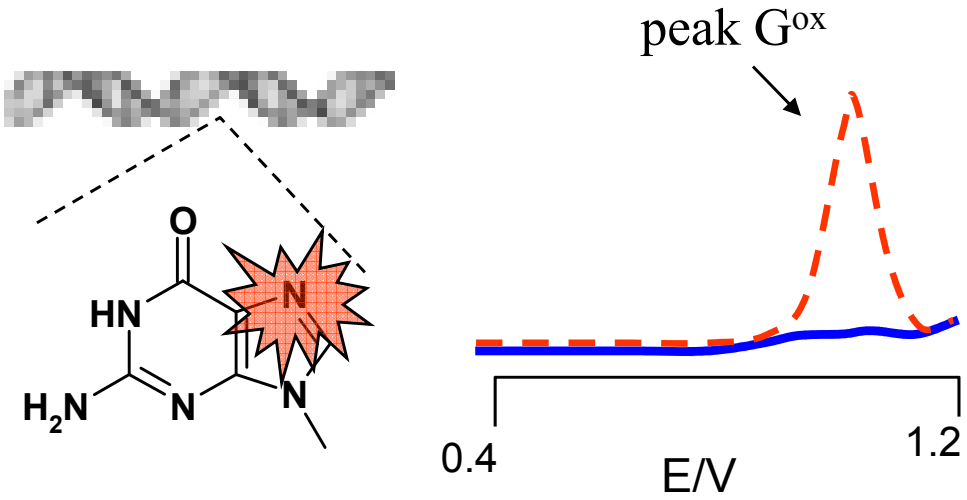


Figure 3. Antioxidative effect of rosmarinic acid (▲) and caffeic acid (■) in cleavage mixture on the relative marker signal at the DNA/SPE. Incubation of the sensor in $2x10^{-4}$ M $FeSO_4$, $4x10^{-4}$ M EDTA, $9x10^{-3}$ M H_2O_2 in 10 mM phosphate buffer pH 7.0 with10 % of methanol at the electrode potential of –0.5 V for 5 min. Other conditions as in Figure 1.

Damage to DNA bases

- **techniques based on a loss of electrochemical activity of chemically modified bases**
- **usually guanine**



- guanine signals at carbon or mercury electrodes
- alkylating agents, hydrazines, PCBs, cytostatics, acridines, arsenic oxide…

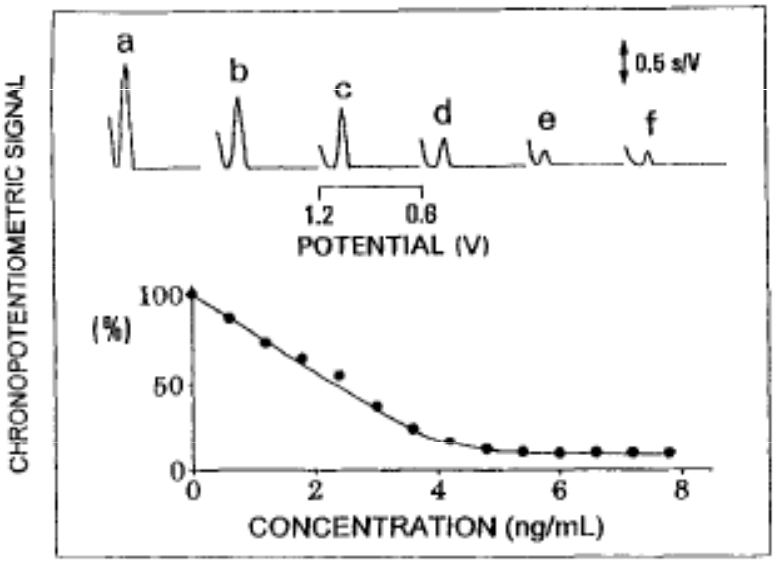


Fig. 6. Chronopotentiometric response of the DNA carbon paste biosensor for increasing levels of dimethylhydrazine in 1.2 μg l^{-1} steps (b)–(f), along with the resulting calibration plot. Also shown (a) is the response of the sensor prior to the hydrazine addition. Interaction time, 10 min. (See [21] for details.)

- **some base adducts yield electrochemical signals distict from those corresponding to the unaffected bases**
- **e.g., 8-oxoguanine**

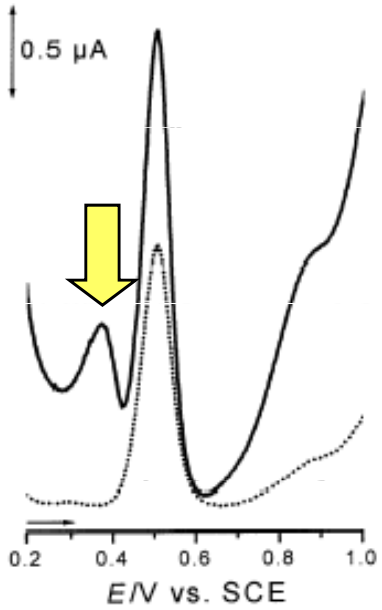


Fig. 8. Differential pulse voltammograms in pH 4.5 0.1 M acetate buffer obtained with a thin layer dsDNA-modified GCE after being immersed in a 5 μM adriamycin solution during 3 min and rinsed with water before the experiment in buffer: (···) without applied potential; (—) after applying a potential of −0.6 V during 60 s. Pulse amplitude 50 mV, pulse width 70 ms, scan rate 5 mV s^{-1}. First scans.

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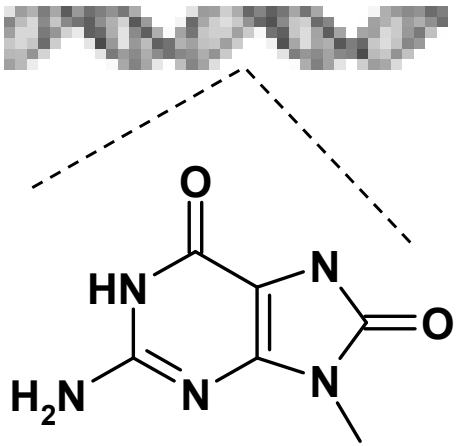
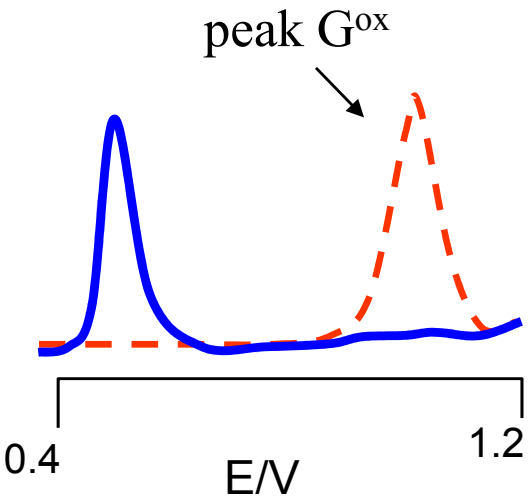
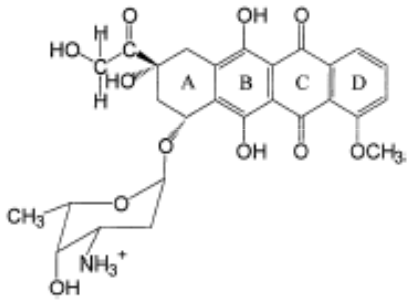
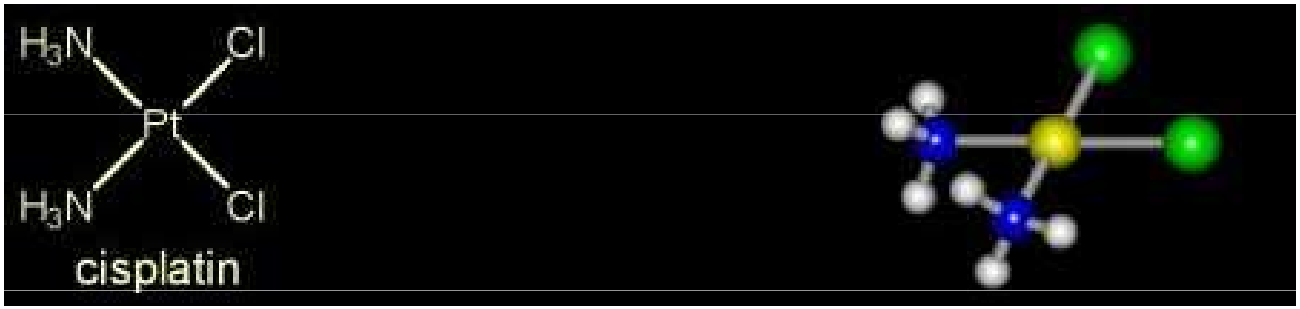


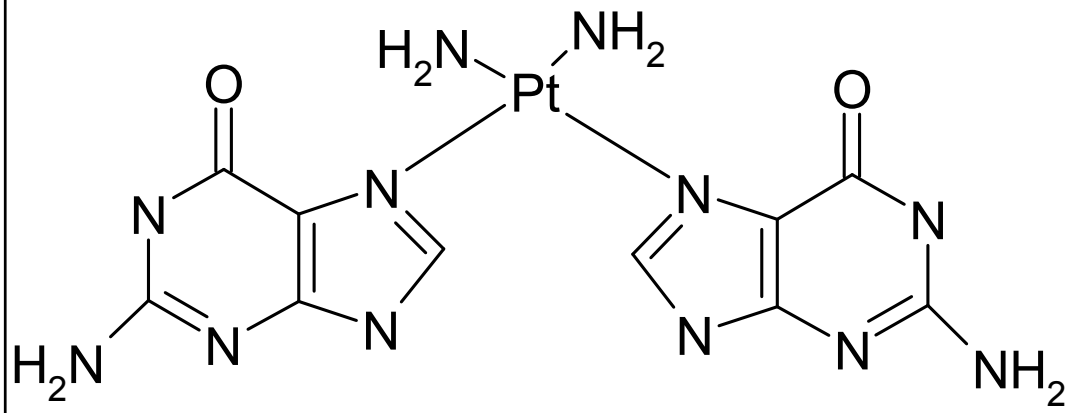


8-oxoG elecrochemically generated in DNA at GCE in the presence of adriamycin (A.M. Oliveira-Brett)

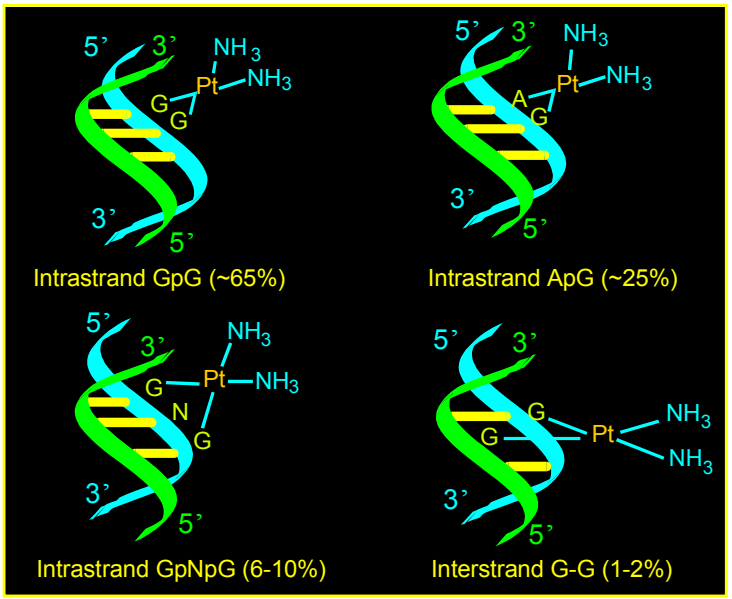


cisplatin





cisplatin modifies primarily guanines

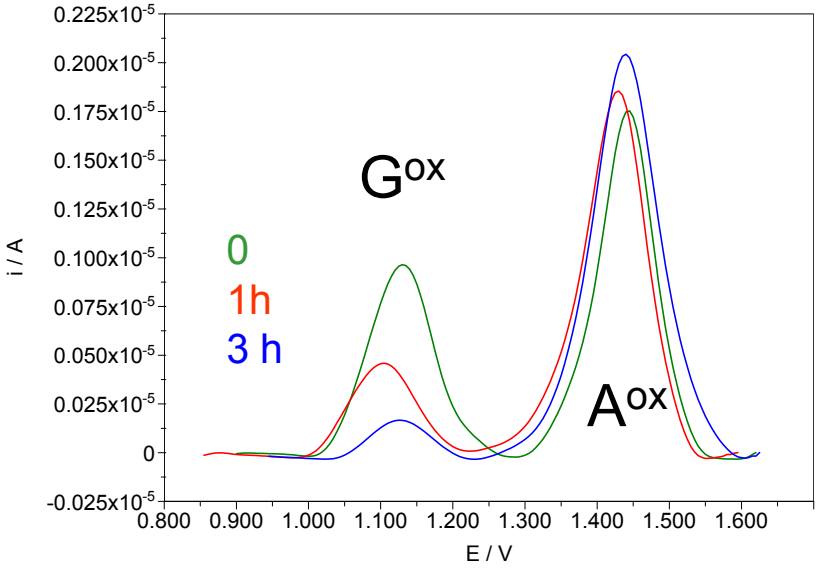


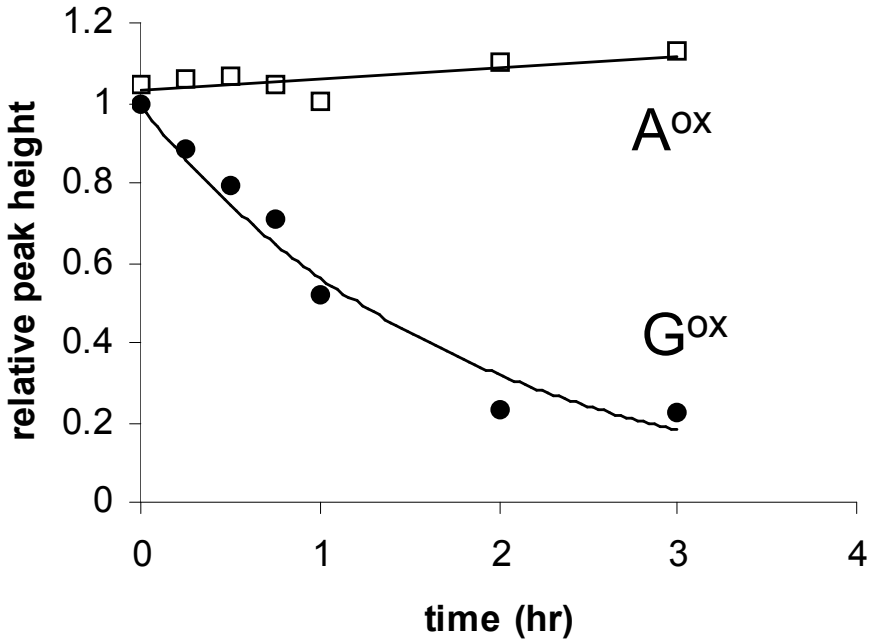
cisplatin

high cis-platination levels: diminution of peak G^{ox} at carbon

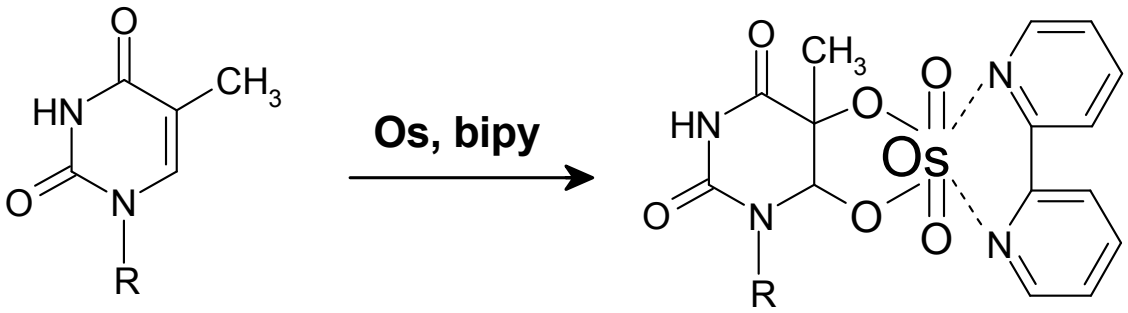
(cisplatin/nucleotide ratio r_b=1.0, time dependence)

for r_b < 0.1 no reliable changes in peak G^{ox} intensity under the same conditons



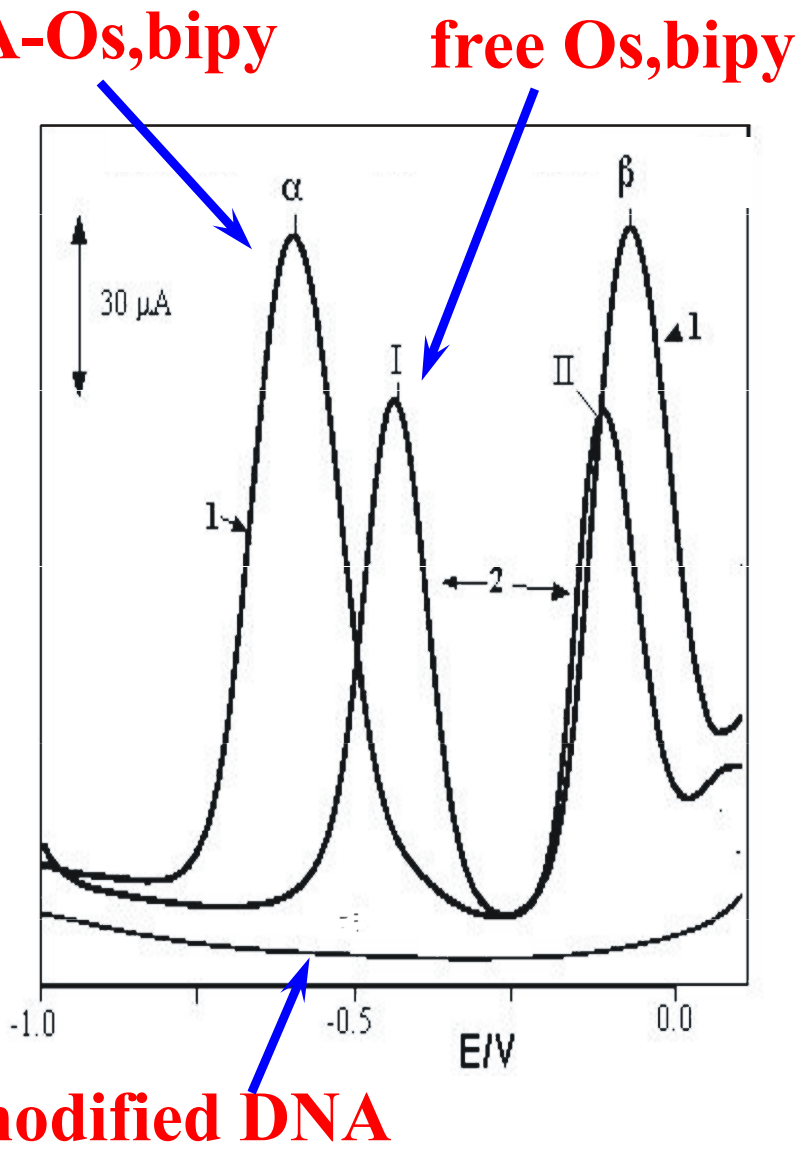


DNA modified with osmium tetroxide complexes



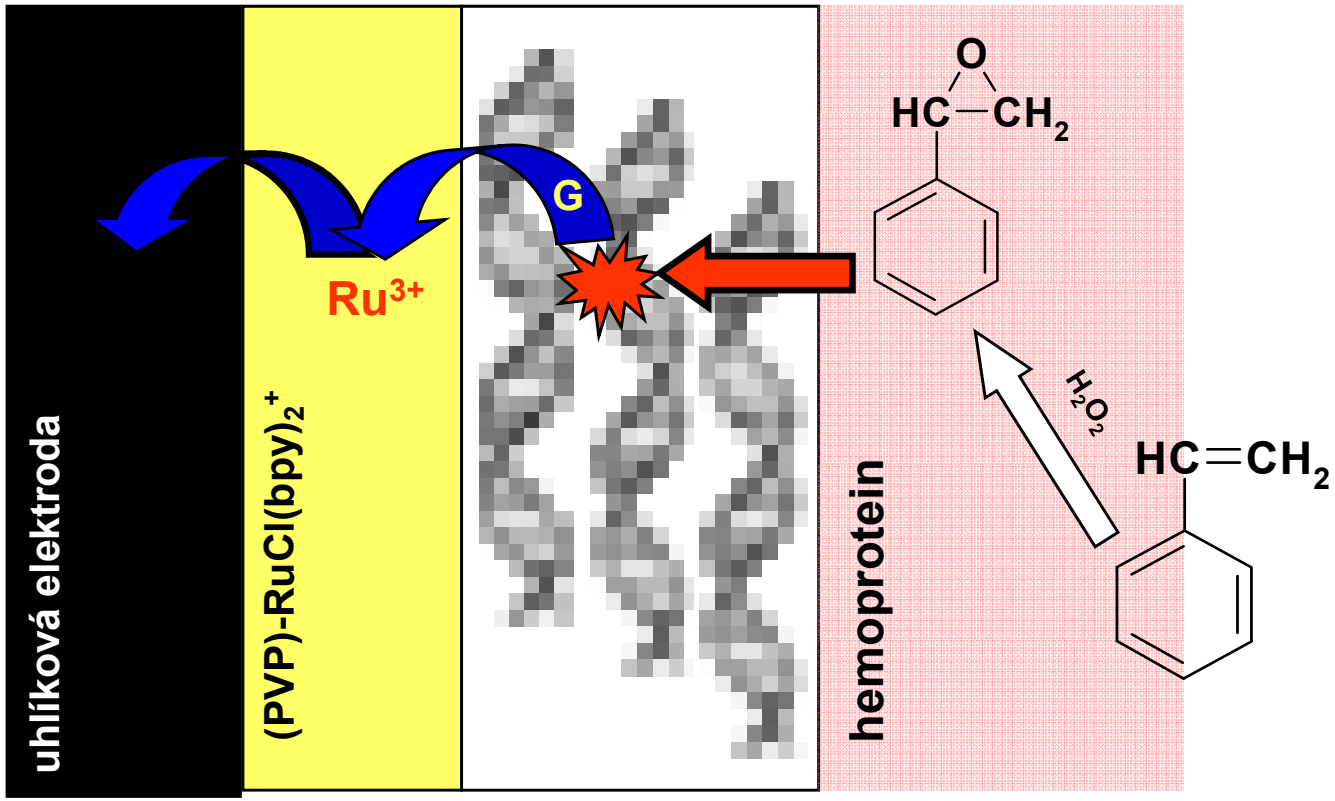
- not „classical“ DNA damaging agents
- chemical probes of DNA structure

- indirect technique of DNA damage detection



Sensor for (geno)toxicity testing (Rusling et al.)

•utilizes changes of accessibility of guanine bases for interaction with a redox mediator upon DNA damage



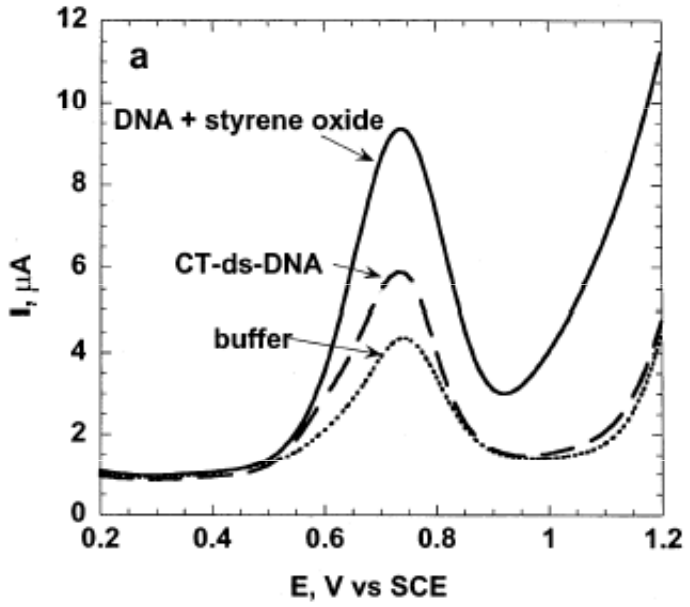
•during diffusion through the heme protein layer, the substance is „metabolically activated“

•DNA adduct is formed

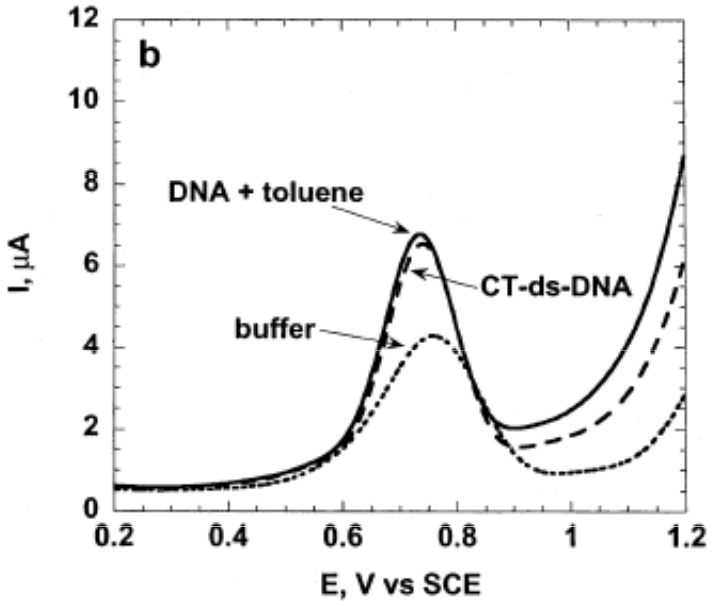
•due to the adduct, the double helix is „unravelled“ making neighboring bases (guanines) more accessible for Ru-mediated oxidation

SIGNAL INCREASES

Sensor for (geno)toxicity testing (Rusling et al.)



STYRENE



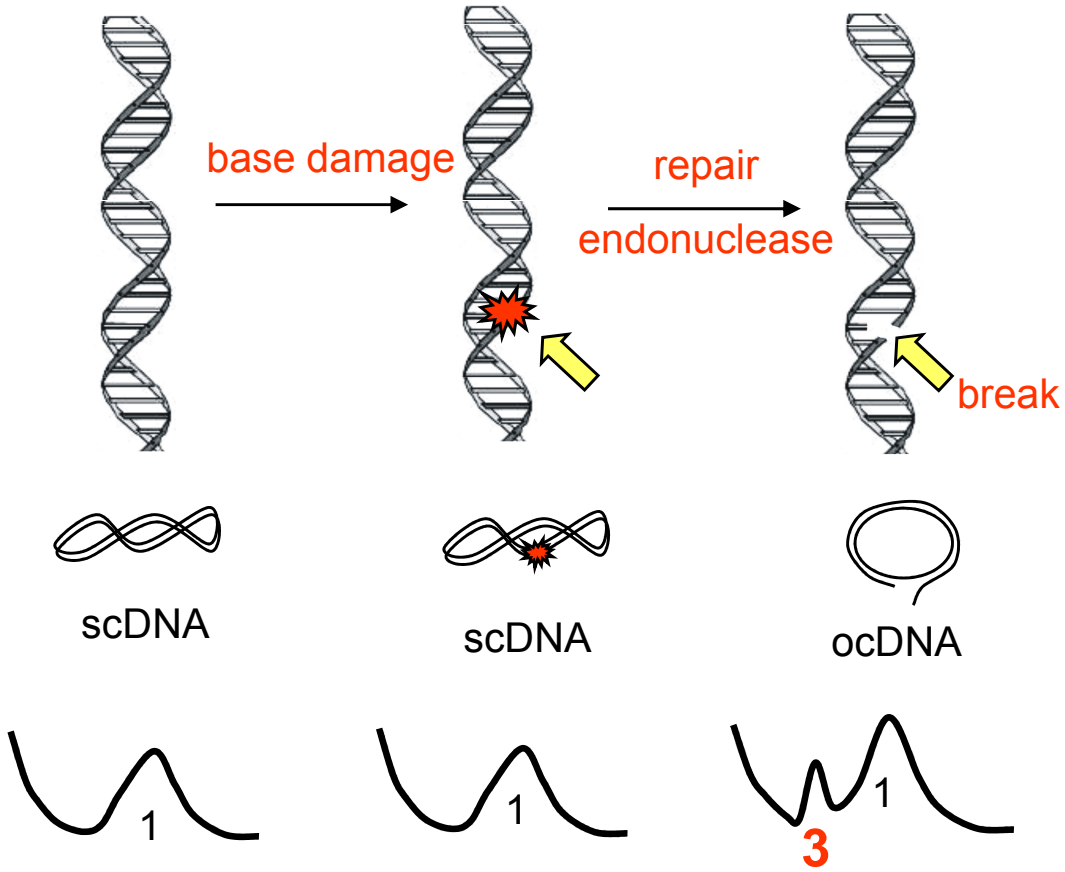
TOLUENE
(not „activated“ by the heme enzymes)

Anal. Chem. **2005**, *77*, 2920–2927

Use of DNA Repair Enzymes in Electrochemical Detection of Damage to DNA Bases in Vitro and in Cells

Kateřina Cahová-Kuchaříková, Miroslav Fojta,* Tomáš Mozga, and Emil Paleček

base damage converted to strand breaks →
sensitive detection at mecury or amalgam electrodes



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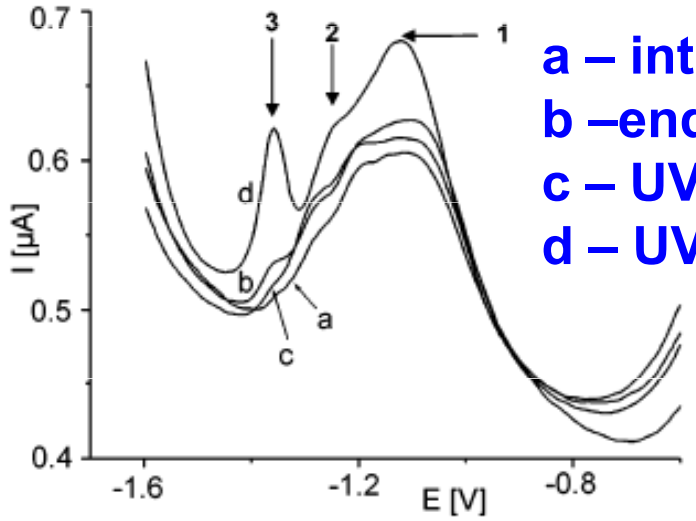
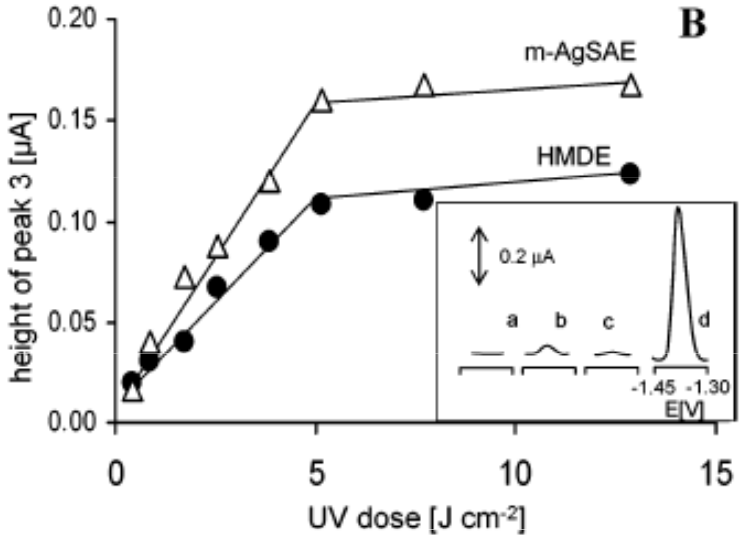
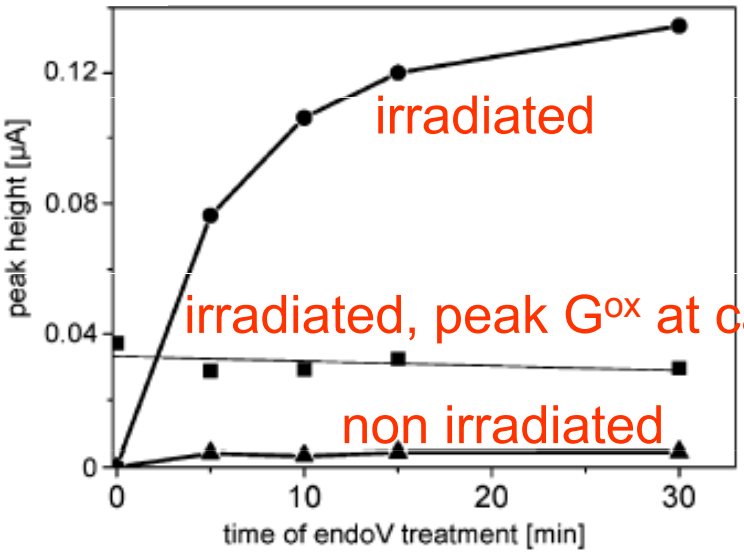
Py dimers detected by endonuclease V



a – intact scDNA
b –endoV treated scDNA
c – UV irradiated scDNA
d – UV+endoV



dependence on UV dose



dependence on enzymatic cleavage time

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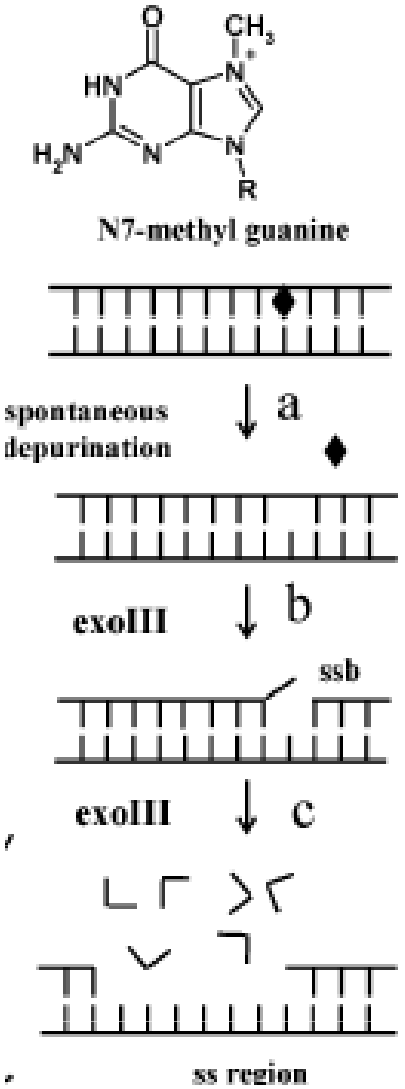
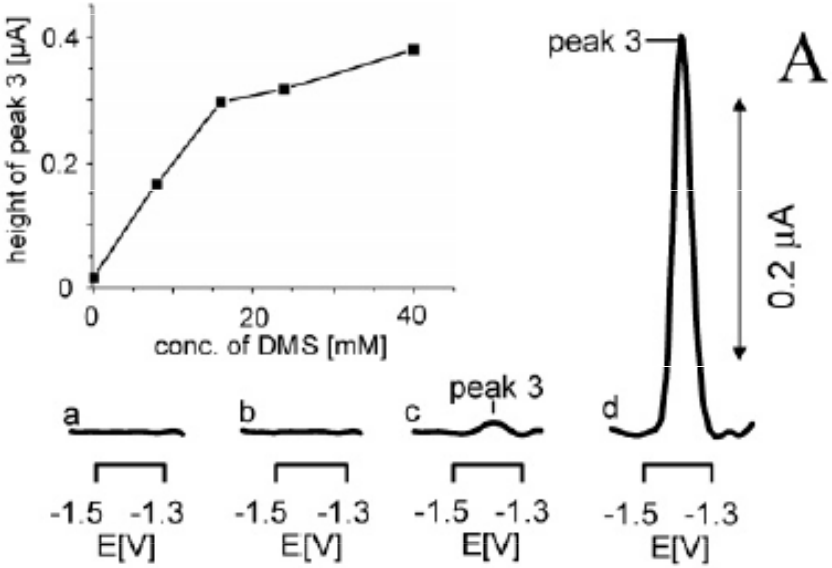
apurinic sites detected by exonuclease III





scDNA | **scDNA +ExoIII** | **scDNA +DMS** | **scDNA +DMS +exoIII**

(peak 3 details)

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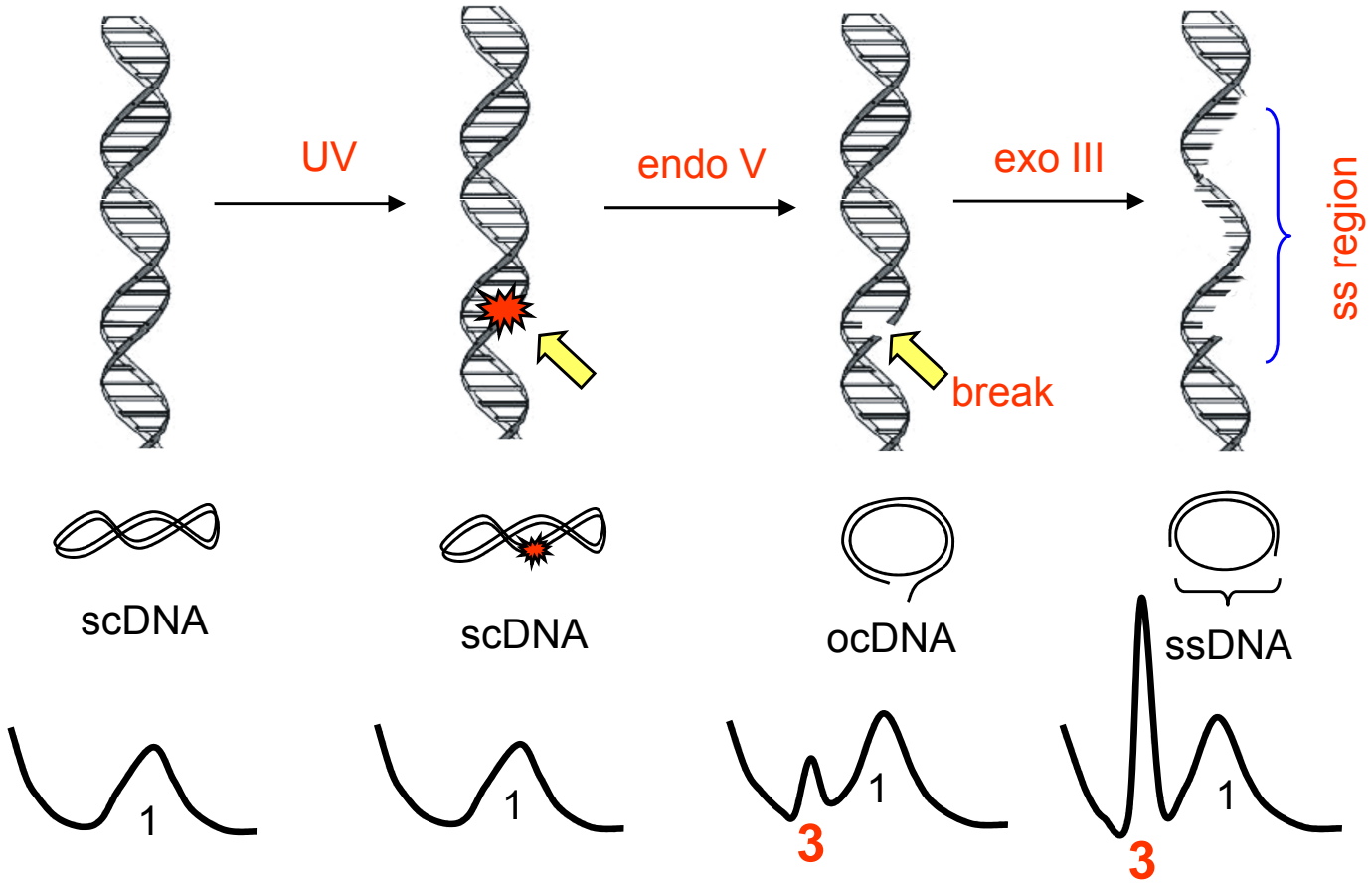
enhancement of the ssb signal using exonuclease III cleavage



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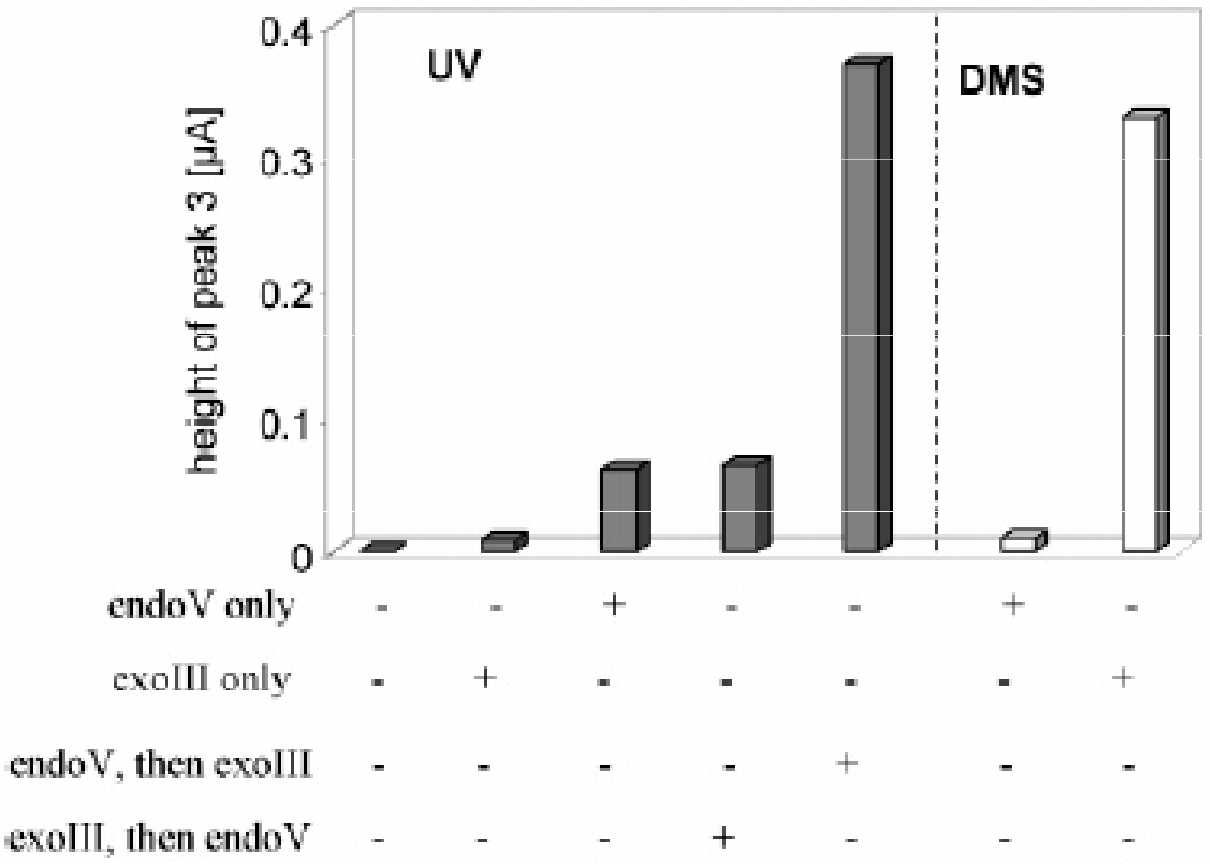


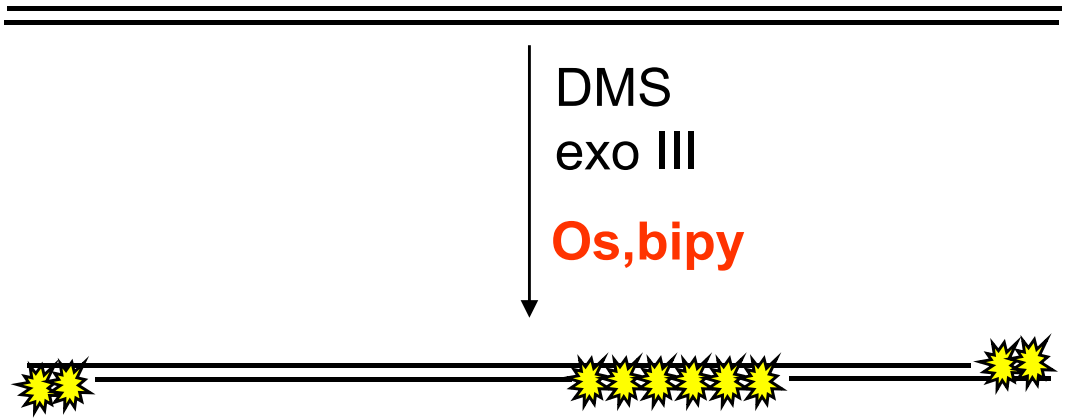
substrate specificity of the enzymes → specificity of adduct detection

Utilization of an electroactive marker in detection of DNA damage

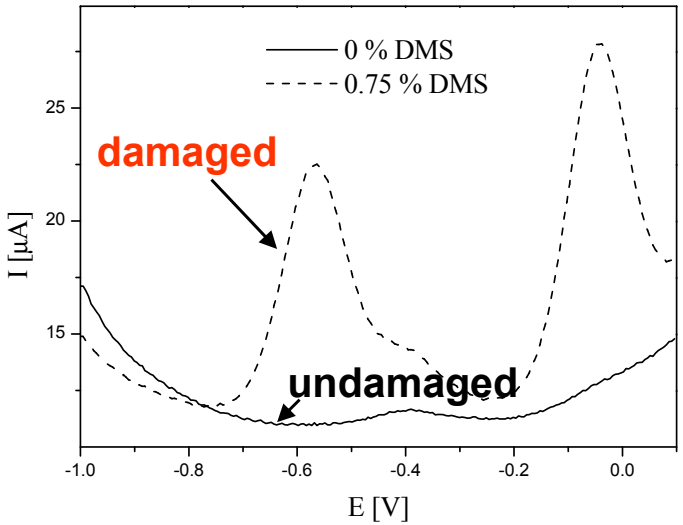
(OsO_4,bipy)

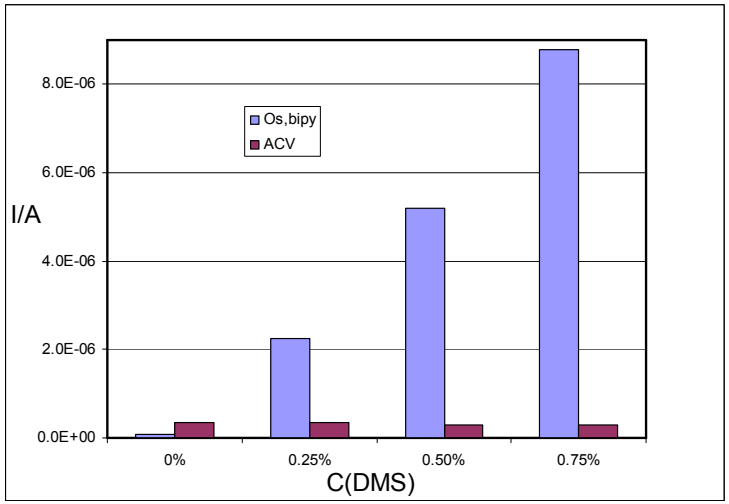
- commercially available chromosomal (=linear) DNAs (such as calf thymus or salmon sperm DNA) produce a considerable peak 3

- only small relative changes due to additonal damage (depending on the sample quality)



signals of the marker (at carbon):





„dose“ dependence (conc. of DMS)