evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: **AUTOMATIC ANALYSIS OF HIV SEQUENCES ON PRINTED ELECTRODES**

Školitel: Jiří Kudr

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Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet

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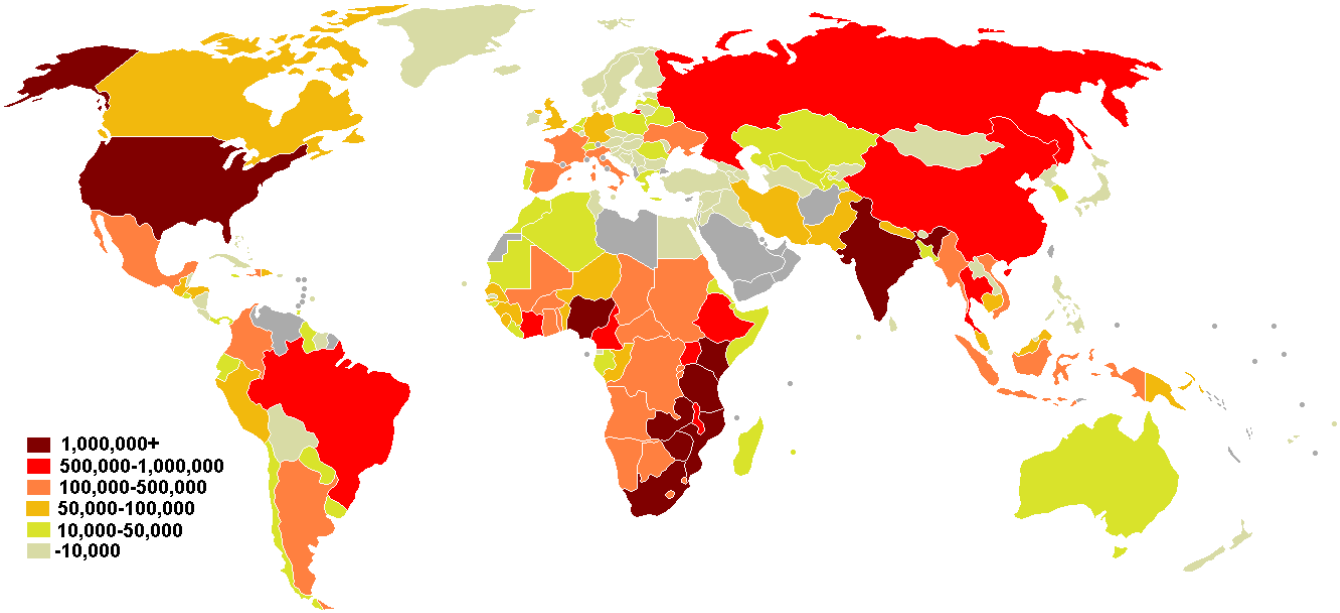

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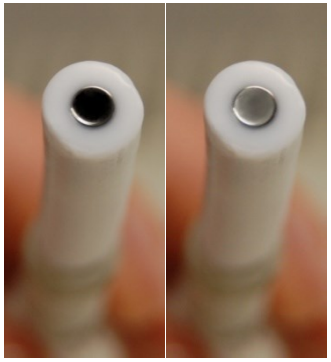
Introduction

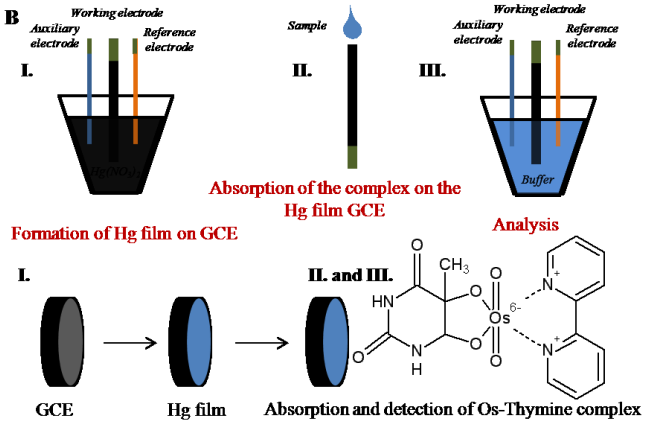
- According to UNAIDS (2012) 35 mil. persons are living with HIV and 36 mil. persons have died of the AIDS-related illnesses.
- The specific and rapid diagnostic techniques prevent the transmission of HIV and are able to monitor disease progression and effects of drugs.
- Standard diagnostic tests of HIV are enzyme immunoassay, ELISA and Western blot assays.
- Standard methods are sensitive, but labor-intensive and time-consuming. Electrochemical methods are, however, rapid, inexpensive, sensitive and do not require complex preparation.
- DNA biosensors are able to diagnose HIV shortly after infection.

Our work

- Preparation of mercury film electrode (Hg film on the surface of glassy carbon electrode)
- Cloning and subcloning of HIV capsid protein gene (*p24*)
- Amplification of *p24*
- Preparation of linear plasmide with inserted *p24*
- Preparation of osmium modified 5‘ *p24* oligonucleotide (ODN) probe
- Immobilization of magnetic particle via thiol gold interaction on the 3‘ *p24* ODN probe
- Preparation of construct (ODN-OsBipy-*p24*-ODN-SH-AuMPs)
- Electrochemical detection of construct by differ pulse voltammetry (MFE as a working alectrod Pt counter electrode, Ag/AgCl/3M KCl as a referenc electrode), the adsorptive transfer technique was used;

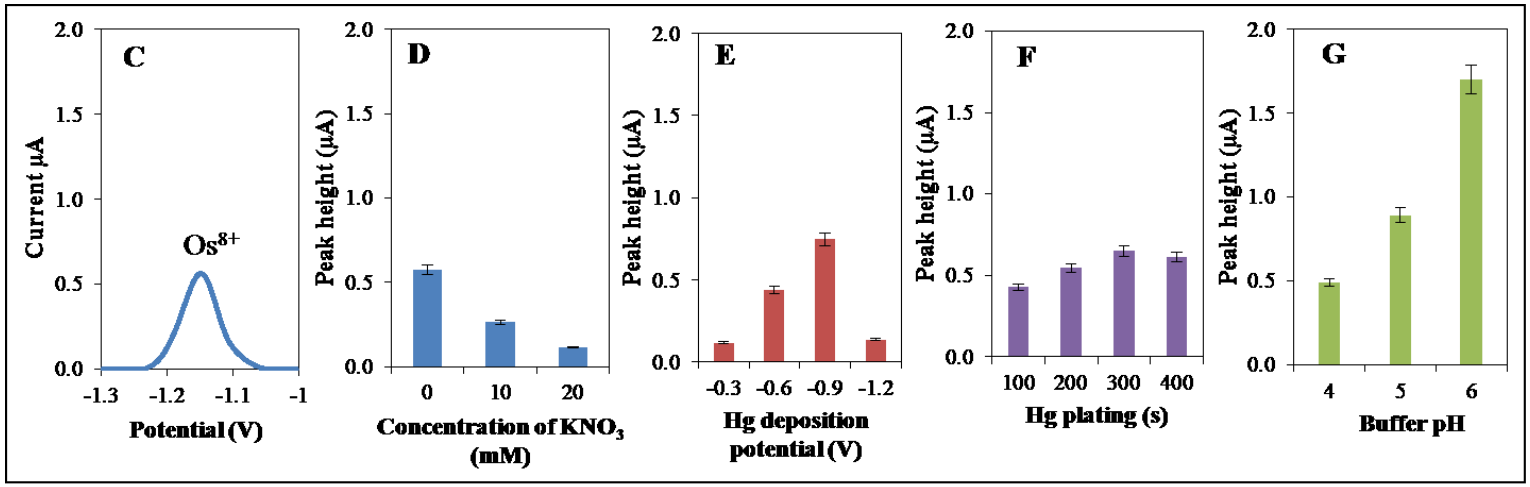


The comparison of polished glassy carbon electrode and mercury film modified GCE.

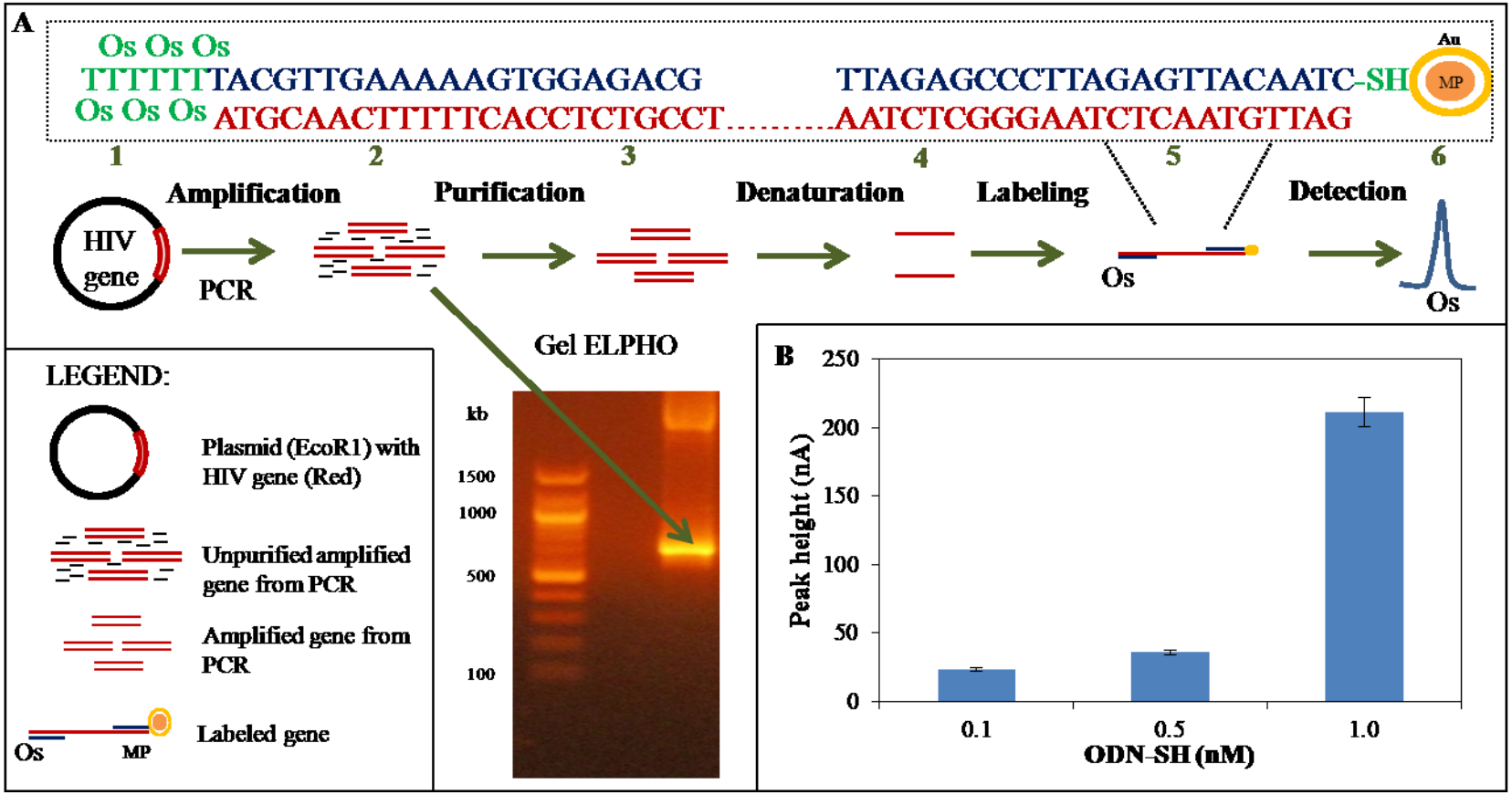


The scheme of preparing the mercury modified glassy carbon electrode and subsequent analysis of the complex.

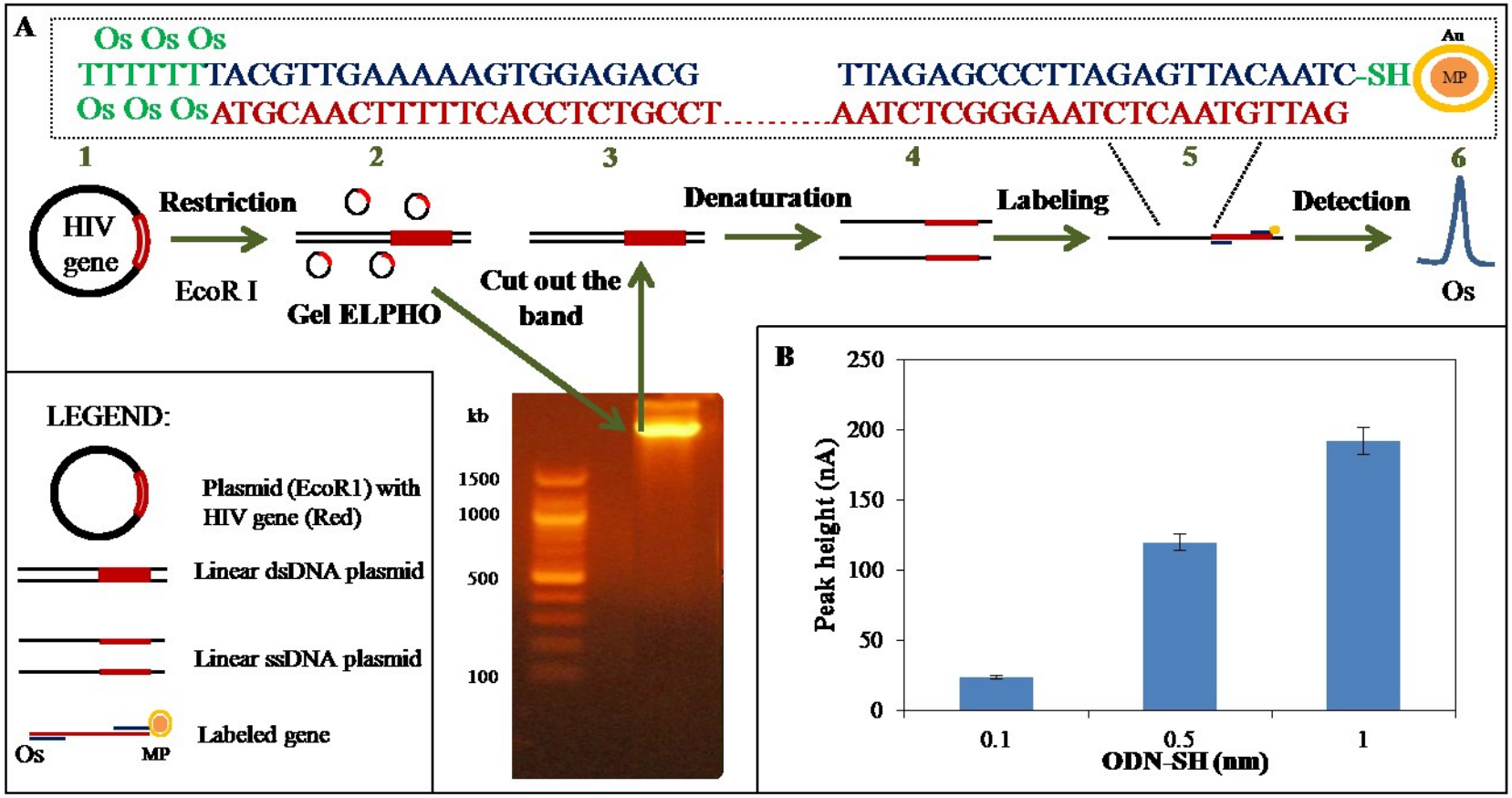
Results



C) The electochemical signal of Os-Thymine labeled ODN. **D**) The influence of KNO3 on the electrochemical signal of Os-Thymine labeled ODN. **E**) The dependence of Hg deposition potential (-0.3 – 1.2 V) on the peak height (nA) of the Os-Thymine labeled ODN. **F**) The time dependence of Hg plating (100 – 400 s) on the peak height (nA) of the Os-Thymine labeled ODN. **G**) The influence of acetate buffer pH (4 – 6) on the peak height (nA) of the Os-Thymine labeled ODN.



A) Scheme of the complex preparation: **I**) Plasmid carrying the HIV gene (1) was amplified using the PCR method and analysed using the Gel electrophoresis (2). Subsequently was the PCR product purificated by Quiagen kit (3). The PCR product was denaturated, afterwards labeled by thiolated oligonucleotide with bonded Au paramagnetic particle and Os modified oligonucleotide probe (5). This way prepared complex was electrochemically analysed on the glassy carbon mercury film modified electrode (6). **B**) The dependence of the complex (I.) electrochemical signal (nA) of the on the concentration of thiolated ODN probe (0.1 – 1 nM).



A) Scheme of the complex II preparation: Plasmid carrying the HIV gene (1) was cleavaged by restriction endonucleases (EcoR I) to linear dsDNA and analysed using gel electrophoresis (2). The band corresponding to molecular weight of linear ds DNA was cut out (3). Subsequently was the sample denaturated and (4) after that the ss DNA was labeled by thiolated oligonucleotide with bonded Au paramagnetic particle and Os modified oligonucleotide probe (5). This way prepared complex was electrochemically analysed on the glassy carbon mercury film modified electrode (6). The frame shows the prepared complex. **B**) The dependence of the complex (II.) electrochemical signal (nA) of the on the concentration of thiolated ODN probe (0.1 – 1 nM).

Conclusion

- We succeeded in *p24* amplification a preparation of linear plasmide with target gene inside.
- The MFE was prepared.
- The construct was prepared and electrochemically detected.

Acknowledgment

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and

all colleagues

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Thank you for your attention

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