Basic Electrochemical Detection of Target DNA

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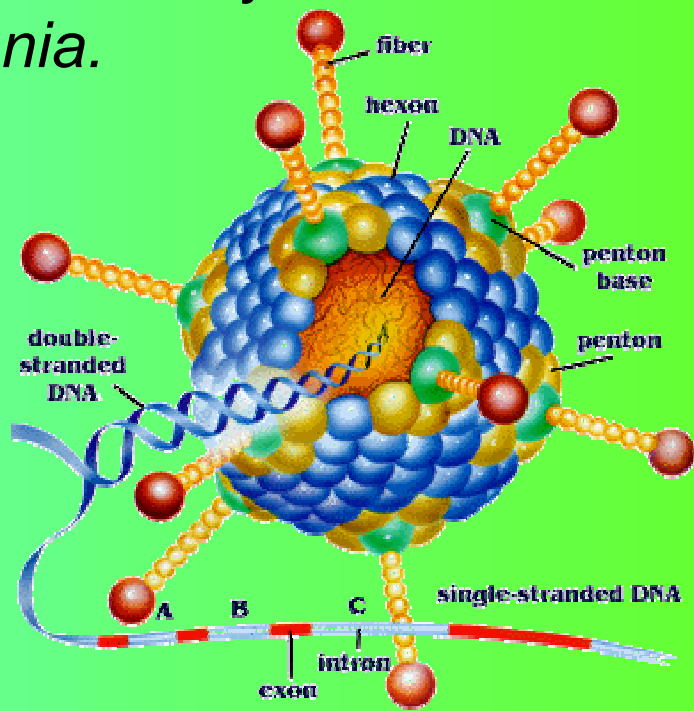


BLV belongs to a family of oncogenic retroviruses which includes HTLV-1, HTLV-2, simian T cell leukemia virus 1 and 2. These retroviruses share a common genomic and structural organization and are associated with non-neoplastic lymphocyte dysregulation, lymphoid neoplasia and/or progressive myelopathies. Due to these retrovirus similarities and BLV being not pathogenic to human, BLV is used as a model of HTLV-1 infection and for development of new analytical systems [1,2].

At present, indirect (serological) detection methods are preferred for the laboratory diagnosis of BLV infection. Immunodiffusion reaction and ELISA are predominantly used for routine BLV diagnosis. However, serological control is not sensitive enough to detect BLV at early stages of an infection.

The aim of this study was to develop easily regenerable and quick model system for the fast detection of BLV provirus DNA by application of PCR followed by electrochemical detection.

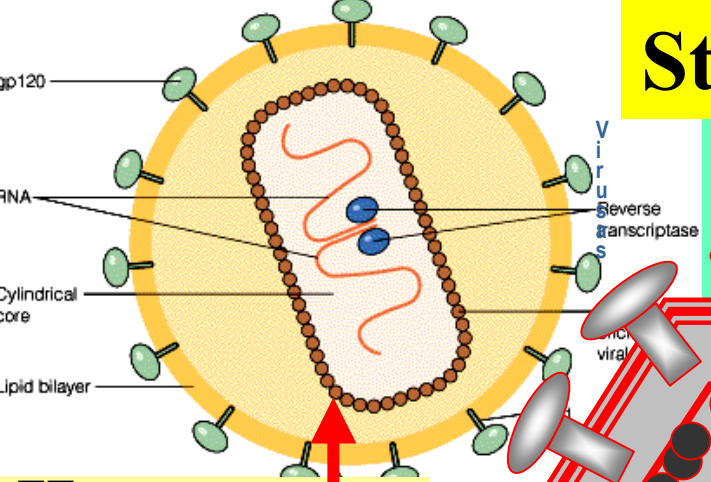
Here the combination of electrochemistry and biochemistry may by successfully exploited and affinity sensors based on conducting polymers can be constructed [4]. The conducting polymers show many attractive bioanalytical features: they might be involved into direct electron transfer processes [5]; helps to transduce protein-protein interaction; enhance analytical signal generated during hybridization of DNA [3]; are able to recognize molecularly imprinted species [6,7].

ssDNA oligomers complementary to the target DNA were entrapped within conducting polymer- polypyrrole during electrochemical polymerization. As control was used PCR-amplified bacteriophage T4 DNA (almost the same length as the target DNA). The control DNA fragments were non-complementary to the ssDNA used in design of DNA sensor presented.

Potential pulse amperometry was used for detection of hybridisation events between immobilized ssDNA and complementary target DNA from the PCR-amplified sample. The main advantage of this new detection method is its simplicity in application/estimation and fast electrochemical-response time.

An estimate of complementary binding efficiency can be easily calculated as a difference between maximal cathodic and maximal anodic currents [3].

Structure of Retroviruses



Human immuno-deficit virus

Reverse transcriptase

Lipid layer

Integrase

p24

RNR

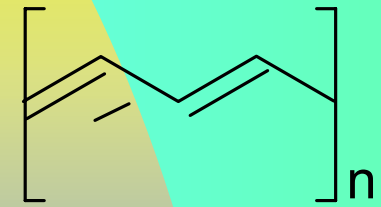
p15

gp51/gp30

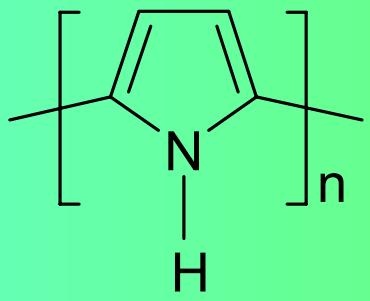
Proteins applied in construction of immunosensors

BLV

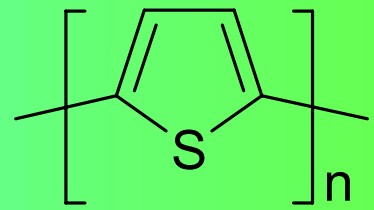
Chemical structures of common conducting polymers



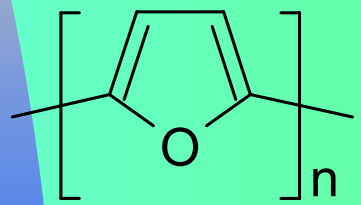
Polyacetylene



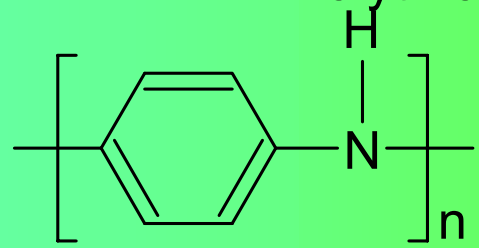
Polypyrrole



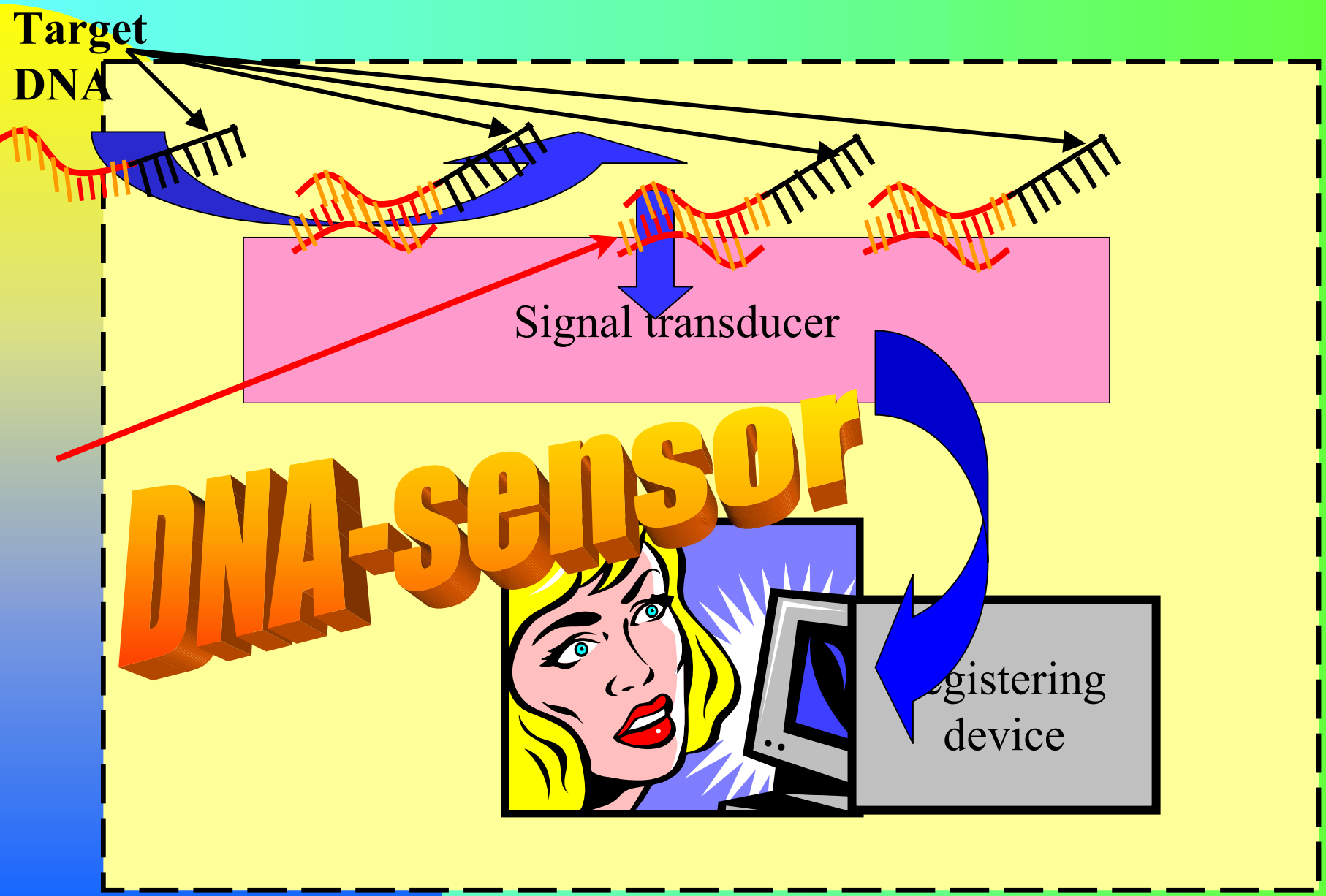
Polythiophene



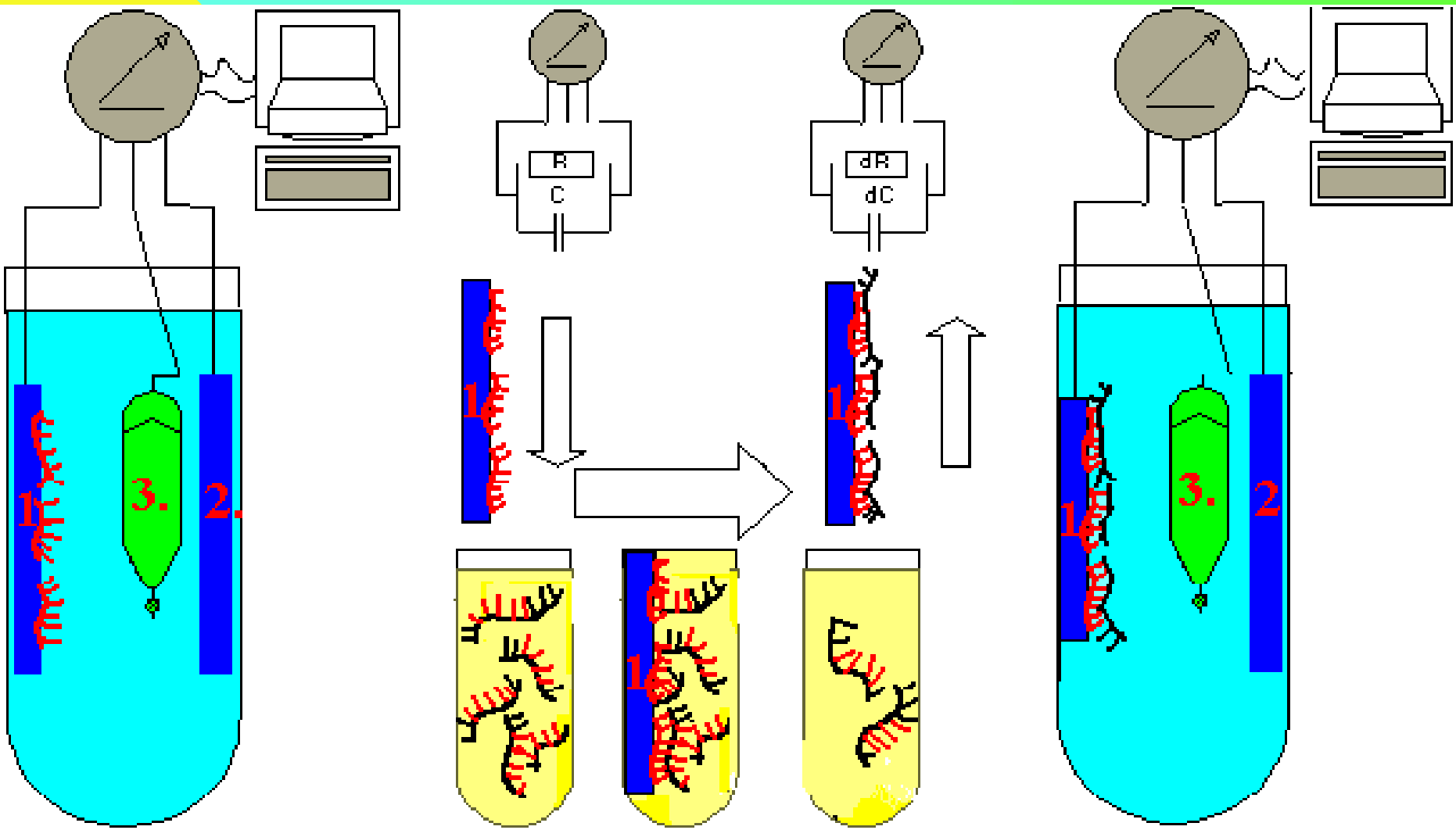
Polyfurane



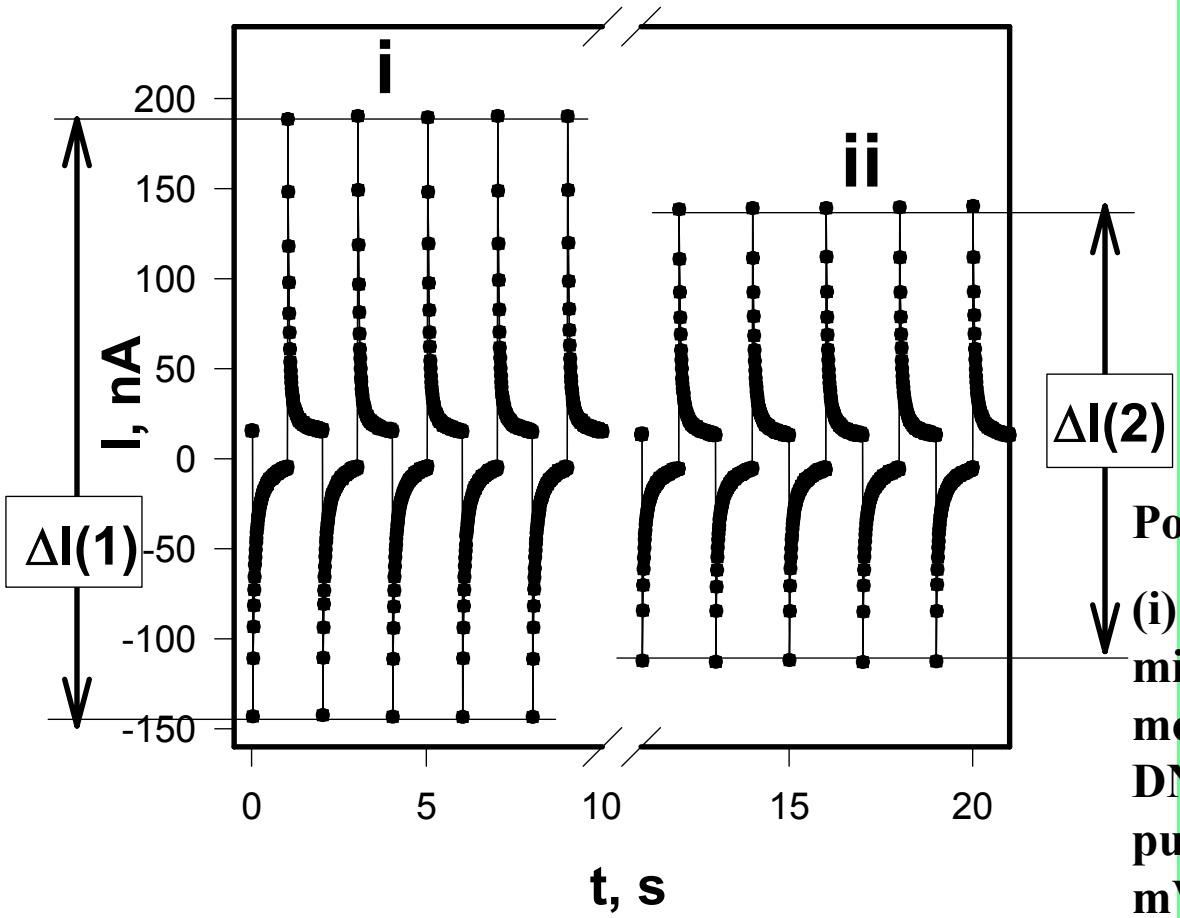
Polyaniline


Target
DNA
Signal transducer
DNA-sensor
gistering
device

Principle of electrochemical DNA sensors



Detection of target DNA binding with ssDNA immobilized within polypyrrole by pulsed amperometric detection



Potential pulse amperograms:

(i) before incubation; (ii) after 30 min. incubation of ssDNA/Ppy modified electrodes in the target DNA containing samples. Potential pulse profile 1 s - 600 mV and 1 - s 0 mV vs. Ag/AgCl.

A. Ramanaviciene, A. Ramanavicius, Pulsed amperometric detection of DNA on ssDNA/polypyrrole modified electrode (2004). *Anal. Bioanal. Chem.* 379: 287-293.

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