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

Název: Electrochemical sensors and biosensors for influenza detection - update 2012-2013

Školitel: Ing. et Ing. David Hynek, PhD.

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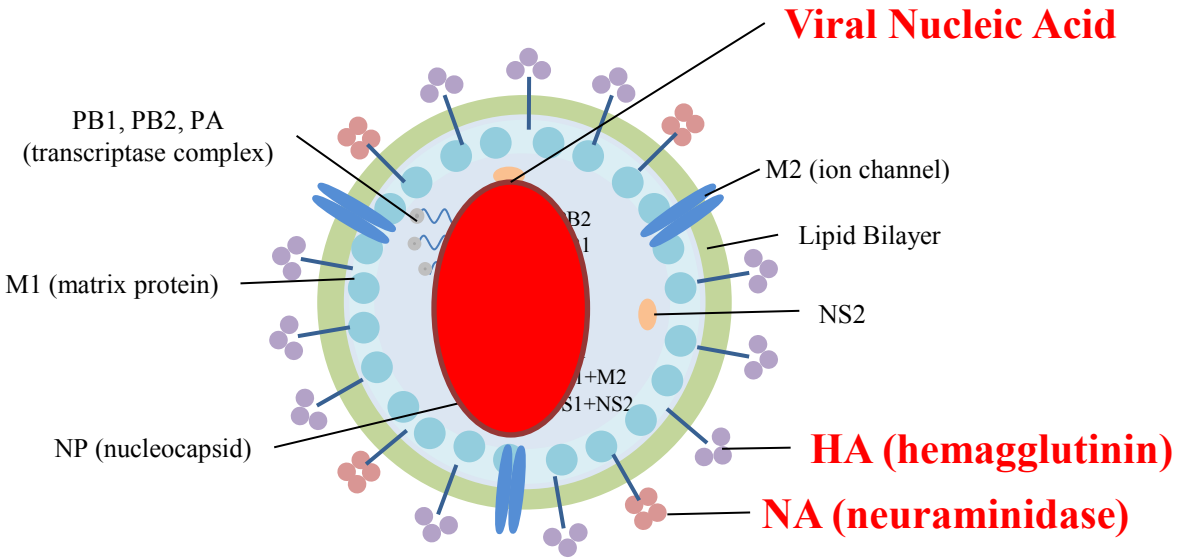
Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

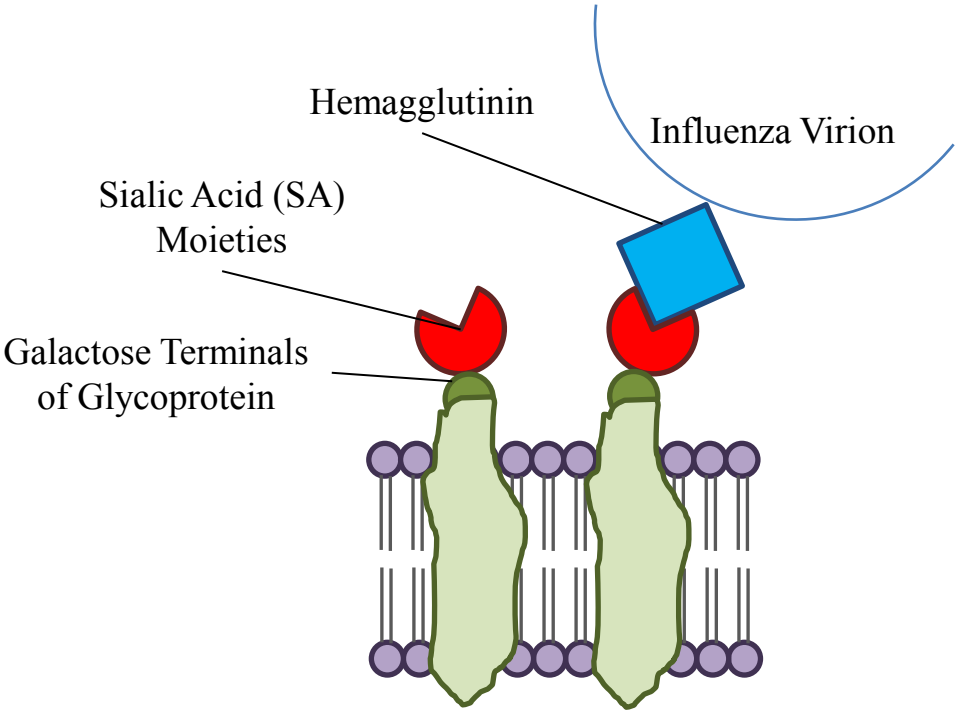
NanoBioMetalNet

Ifluenza virus



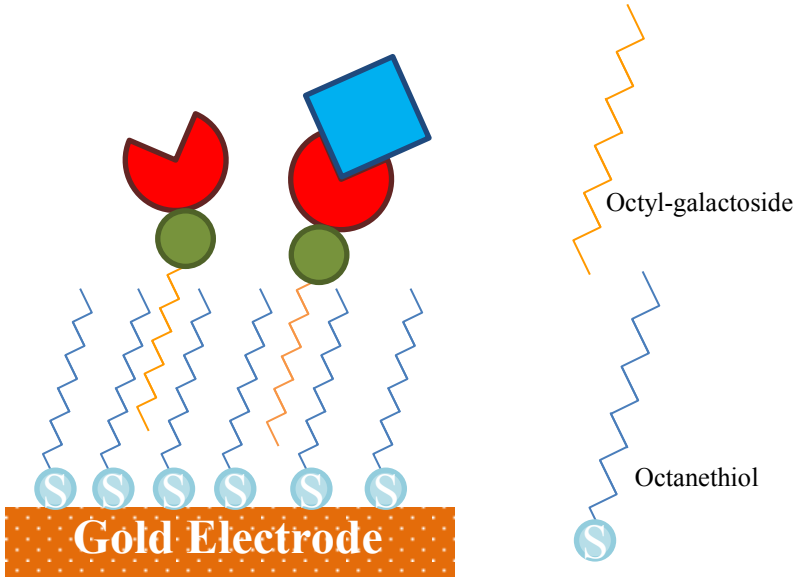
5 examples of influenza detection strategy

Live System



Constructed System

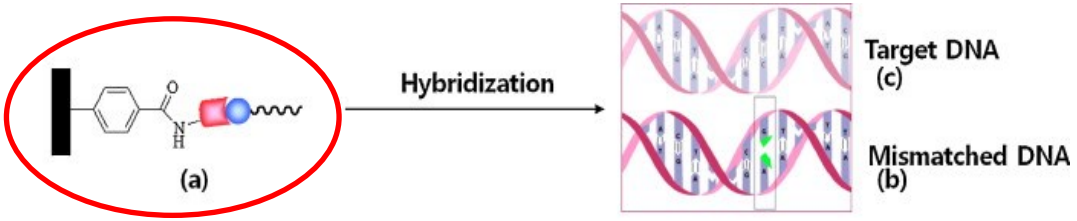
Self-assembling of 1-octanethiol and octyl-galactoside layers, the latter being further functionalized with sialic acid groups by using the appropriate type of sialyltransferase enzyme.

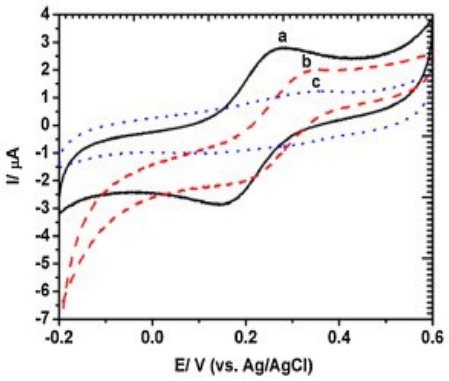


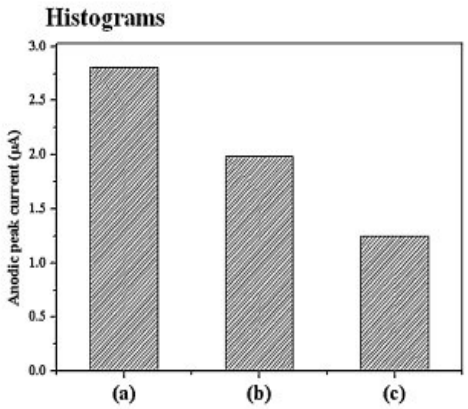
Impedance detection

Wicklein, B., M. A. M. del Burgo, et al. (2013). "Biomimetic Architectures for the Impedimetric Discrimination of Influenza Virus Phenotypes." Advanced Functional Materials **23**(2): 254-262.

CV voltammograms of the probe DNA/GCE (a), the electrode hybridized with two-base mismatched DNA (1.0×10^{-11} M) (b), and target DNA (1.0×10^{-11} M) (c) in 1.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1) containing 0.1 M KCl with a scan rate of 50 mV/s. When the probe DNA of the E-DNA biosensor was hybridized with two-base mismatched DNA sequences (b), the probe DNA/GCE exhibited redox peaks that correspond to the $Fe(CN)_6^{3-/4-}$ electron transfer processes with a small reduction in peak current due to partial hybridization between the probe and two-base mismatched DNA. On the other hand, when the probe DNA of the E-DNA biosensor was hybridized with complementary target DNA, the decrease in peak current of $Fe(CN)_6^{3-/4-}$ redox process is due to the hybridization interactions between the probe and targeted DNA sequence (c). The targeted DNA concentrations could be monitored with the change in the $Fe(CN)_6^{3-/4-}$ redox peak current.

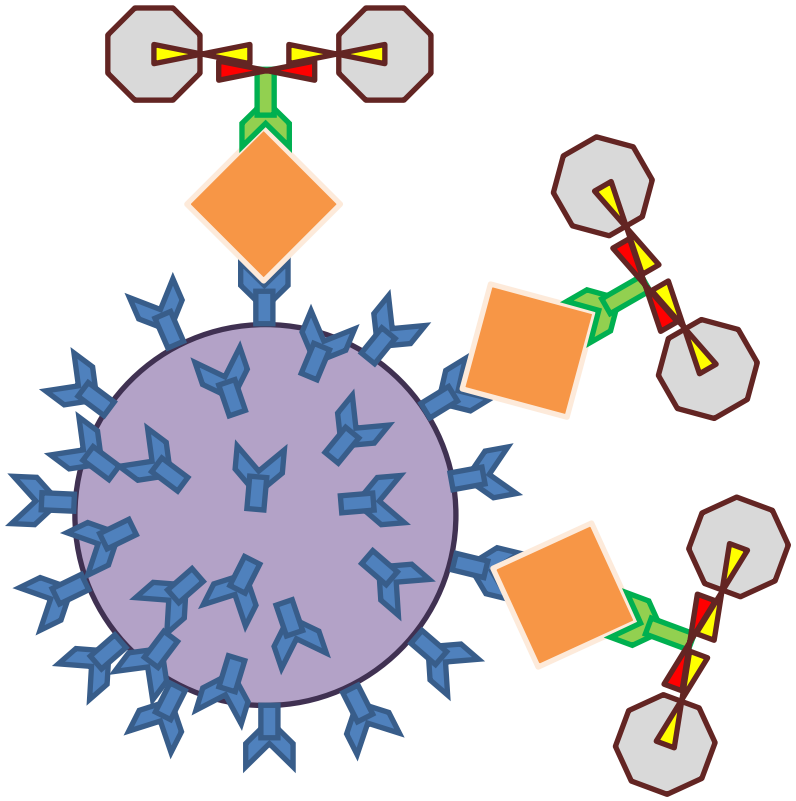






Chung, D. J., S. H. Oh, et al. (2012). "One-step modification of various electrode surfaces using diazonium salt compounds and the application of this technology to electrochemical DNA (E-DNA) sensors." Electrochimica Acta **76**: 394-403.

A magnetic bead-based bienzymatic electrochemical immunosensor



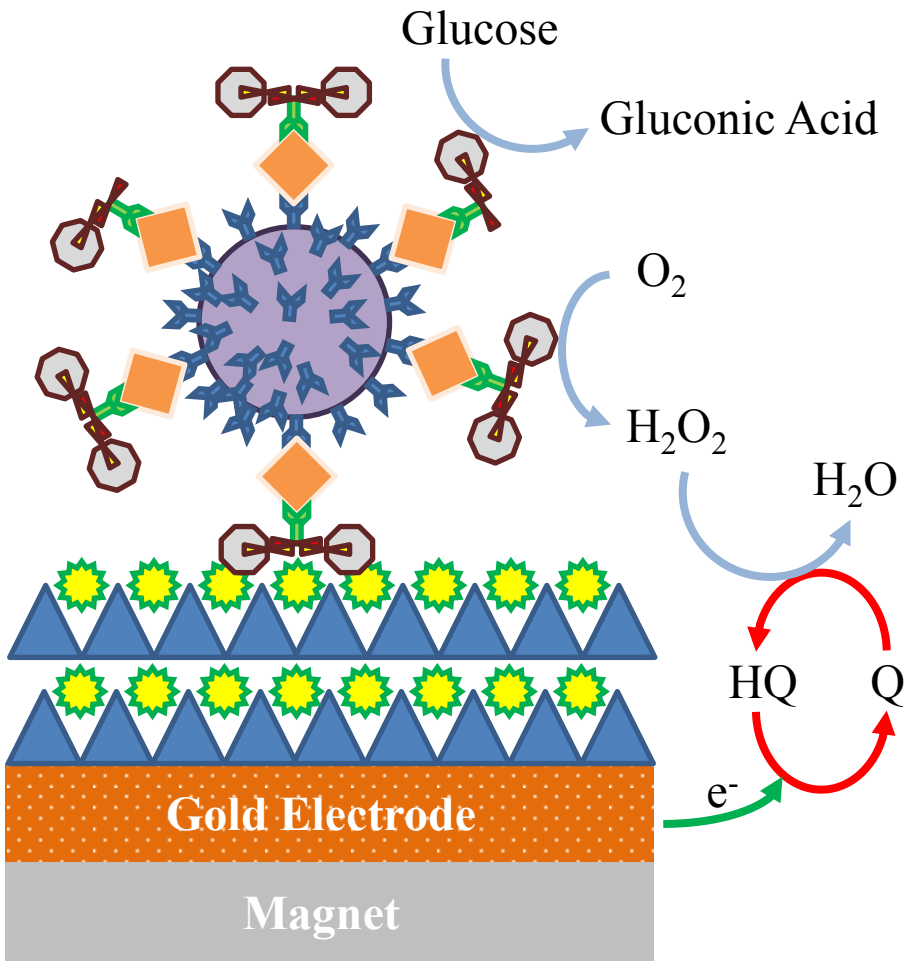
Virus

Biotinilyted Ab against HA

Glucose oxidase-conjugated Avidin D (GOD-A)

Immunomagnetic Bead

Zhou, C. H., Y. M. Long, et al. (2013). "A magnetic bead-based bienzymatic electrochemical immunosensor for determination of H9N2 avian influenza virus." Electrochemistry Communications **31**: 129-132.



Concanavalin A (Con A)

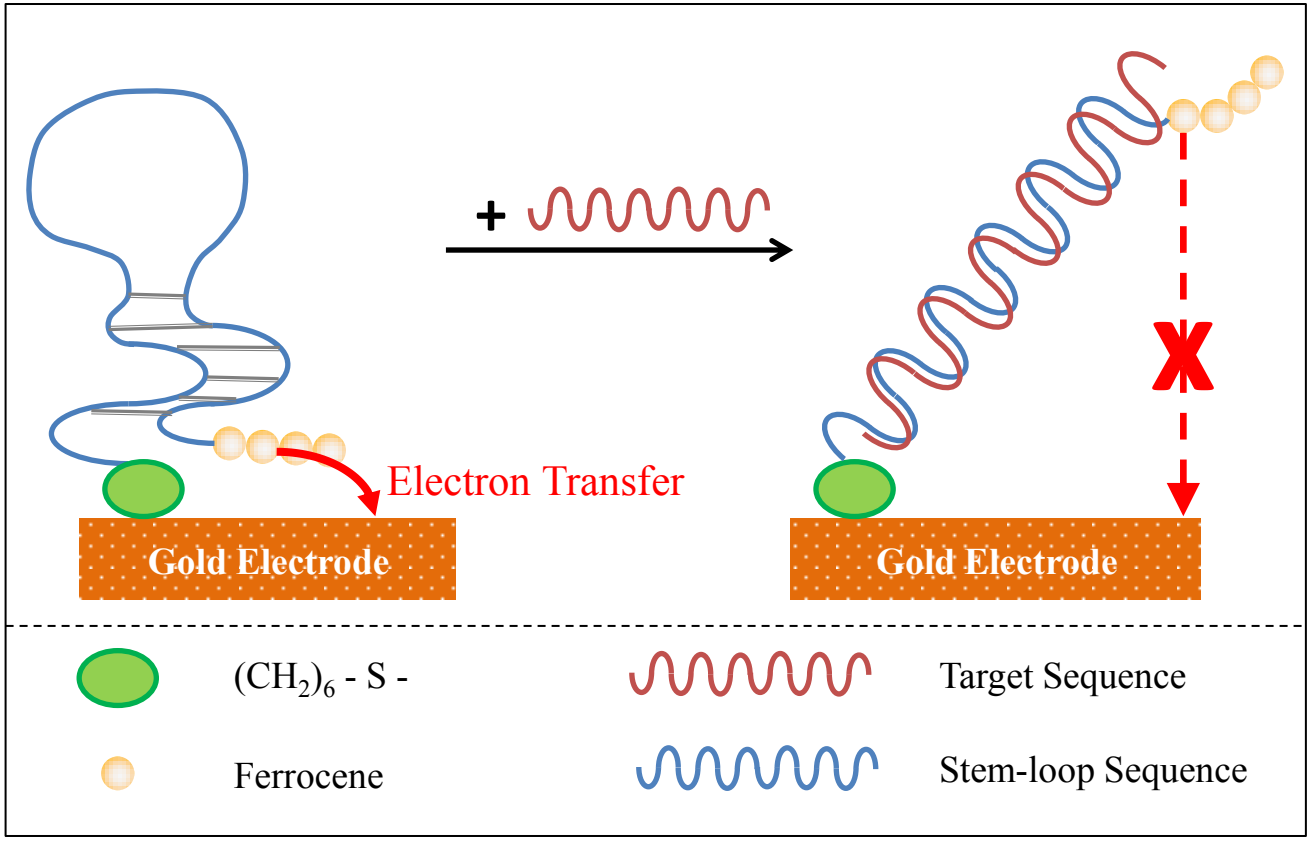
Horseradish Peroxidase (HRP)

This strategy avoided the accumulation of H_2O_2, which could cause enzyme inactivation, and allowed a low detection potential with the use of hydroquinone (HQ) as mediator for DPV detection.

Quinones undergo one-electron reductions to hydroquinone radicals, or two-electron reductions to hydraquinones.

Zhou, C. H., Y. M. Long, et al. (2013). "A magnetic bead-based bienzymatic electrochemical immunosensor for determination of H9N2 avian influenza virus." Electrochemistry Communications **31**: 129-132.

A„four-ferrocene" modified stem-loop structure as a probe for sensitive detection of DNA



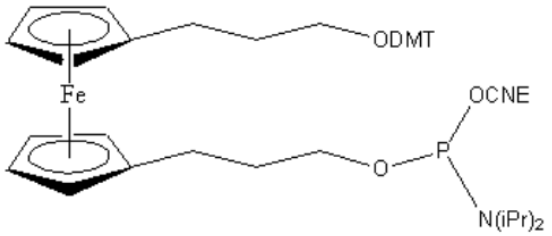
Electrochemical response upon hybridization with complementary short sequence (30-base length) and long sequence (50-base length) strands was observed by differential pulse voltammetry.

The longer the sequence, the greater the decrease in current.

5´Fc-Fc-Fc-Fc-GATCGGGAGAAGACGTCCAAAAACTCGATCGT-O-C_6H_{12}-S-3´

Chatelain, G., M. Ripert, et al. (2012). "A "four-ferrocene" modified stem-loop structure as a probe for sensitive detection and single-base mismatch discrimination of DNA." Electrochimica Acta **59**: 57-63.

bisfunctional ferrocene containing phosphoramidite and dimethoxytrityl (DMT) groups



1-[3-O-Dimethoxytritylpropyl]-1´-[3´-O-(2-yanoethyl-N,N-diisopropylphosphoramidityl)propyl]ferrocene

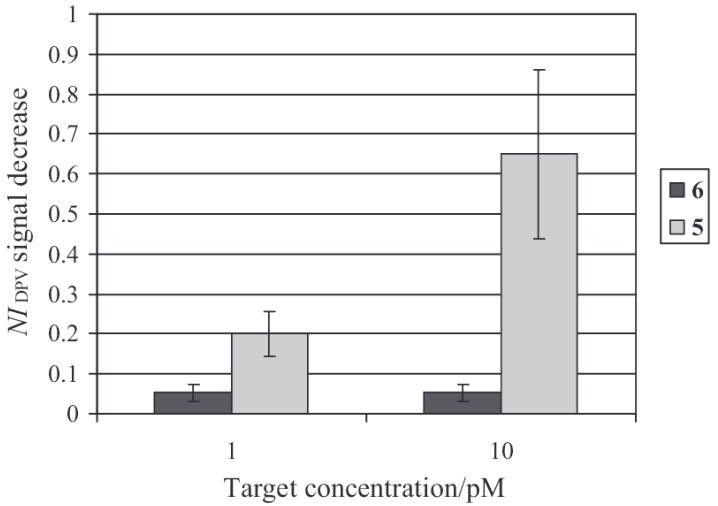


Fig. 7. NI_{DPV} signal decrease observed at 3 h hybridization reaction with the PCR product analogs **5** (complementary target) and **6** (non complementary target). The electrochemical response of the biochip is normalized using the equation $NI_{DPV} = (I - I_0)/I_0$. Results are the mean values of three experiments with **5** and four experiments with **6**.

Chatelain, G., M. Ripert, et al. (2012). "A "four-ferrocene" modified stem-loop structure as a probe for sensitive detection and single-base mismatch discrimination of DNA." Electrochimica Acta **59**: 57-63.

Impedance

$Z = \frac{U}{I}$

Resistance

$Z = R = \frac{U}{I}$

Resistance is special case of impedance under application to direct current (DC).

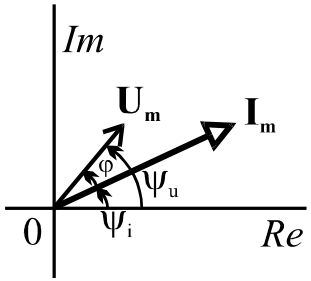
alternating current (AC) circuit	direct current (DC) circuit
Z = f (magnitude, phase angle)	R = f (magnitude) phase angle = 0

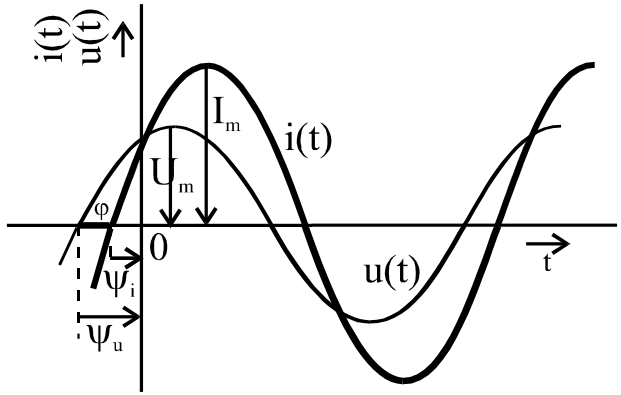
$$\mathbf{Z} = R + jX = |\mathbf{Z}|cos\varphi + |\mathbf{Z}|sin\varphi$$

$$u(t) = U_m sin(\omega t + \Psi_u)$$

$$i(t) = I_m sin(\omega t + \Psi_i)$$

$$\varphi = \psi_u - \psi_i$$





$$\mathbf{Z} = \frac{\mathbf{U}}{\mathbf{I}} = \frac{\mathbf{U}_m}{\mathbf{I}_m} = \frac{U_m}{I_m} \cdot e^{j(\psi_U - \psi_I)} = Z \cdot e^{j\varphi}$$

$$\mathbf{Z} = R + jX$$

Real Part - Resistance

Imaginary Part - Reactance

Capacitive Reactance

- electrostatic storage of charge induced by voltages between conductors.

A DC voltage applied across a capacitor causes positive charge to accumulate on one side and negative charge to accumulate on the other side; the electric field due to the accumulated charge is the source of the opposition to the current.

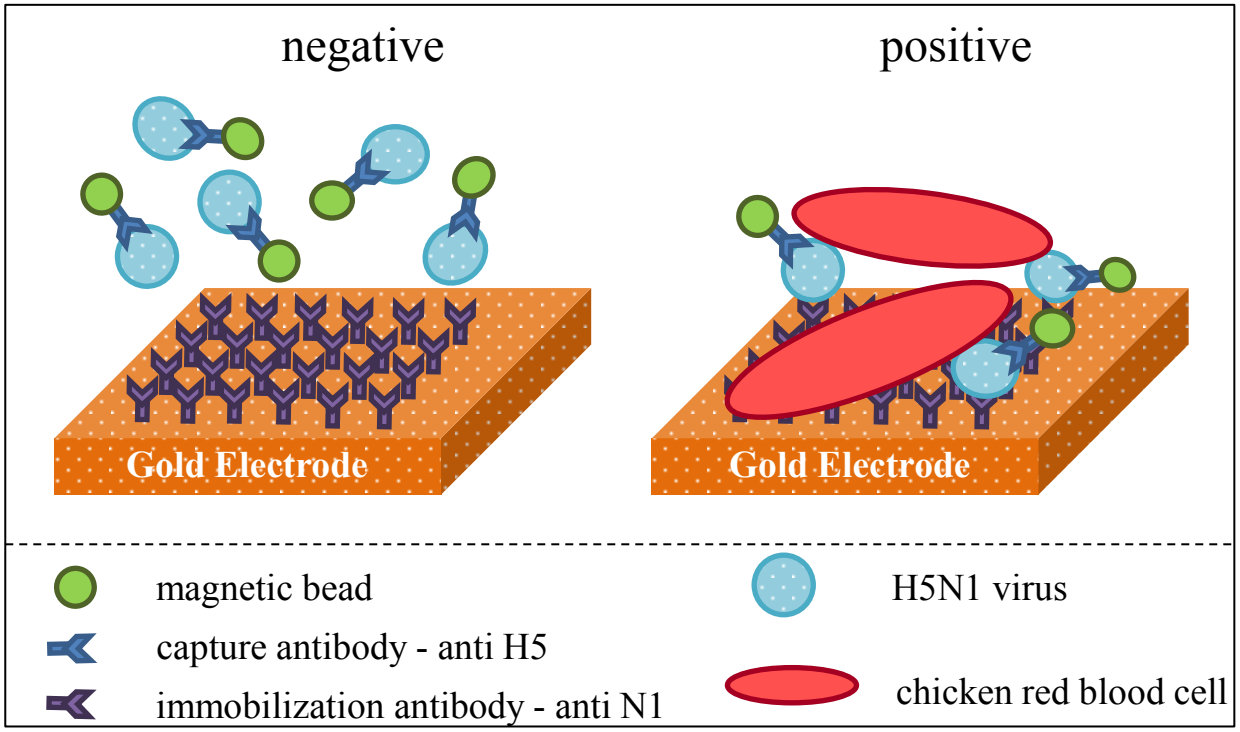
Inductive Reactance

- apparent resistivity of element with inductance when passing an alternating electric current.

Inductance is the property of a conductor by which a change in current flowing through it "induces" (creates) a voltage in both the conductor itself (self-inductance) and in any nearby conductors by magnetic fields of currents.

Impedance measurement of immuno-reaction coupled with RBC amplification

The experimental protocol consisted of three elements: electrode surface modification, AIV detection and RBC amplification.



Polyclonal antibody against N1 subtype was immobilized on the surface of the microelectrode to specifically bind AIV H5N1 to generate more specific impedance signal and chicken red blood cells (RBC) were used as biolabels to attach to AIV H5N1 captured on the microelectrode to amplify impedance signal. RBC amplification was shown to increase the impedance signal change by more than 100% compared to the protocol without RBC biolabel.

RBCs were used as biolabels to amplify the antibody-virus binding due to their larger diameter (7–12μm) compared to the virus (80–120 nm), and strong and specific binding by virus hemagglutinin to sialic acid linkages found on the cell surface.

Lum, J., R. H. Wang, et al. (2012). "Rapid detection of avian influenza H5N1 virus using impedance measurement of immuno-reaction coupled with RBC amplification." Biosensors & Bioelectronics **38**(1): 67-73.

Thank you for your attention.

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

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