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MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Spectroscopic and electrochemical characterization of the protein GP120

Školitel: Mgr. Natalia Cernei PhD

Datum: 22.02.2014

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

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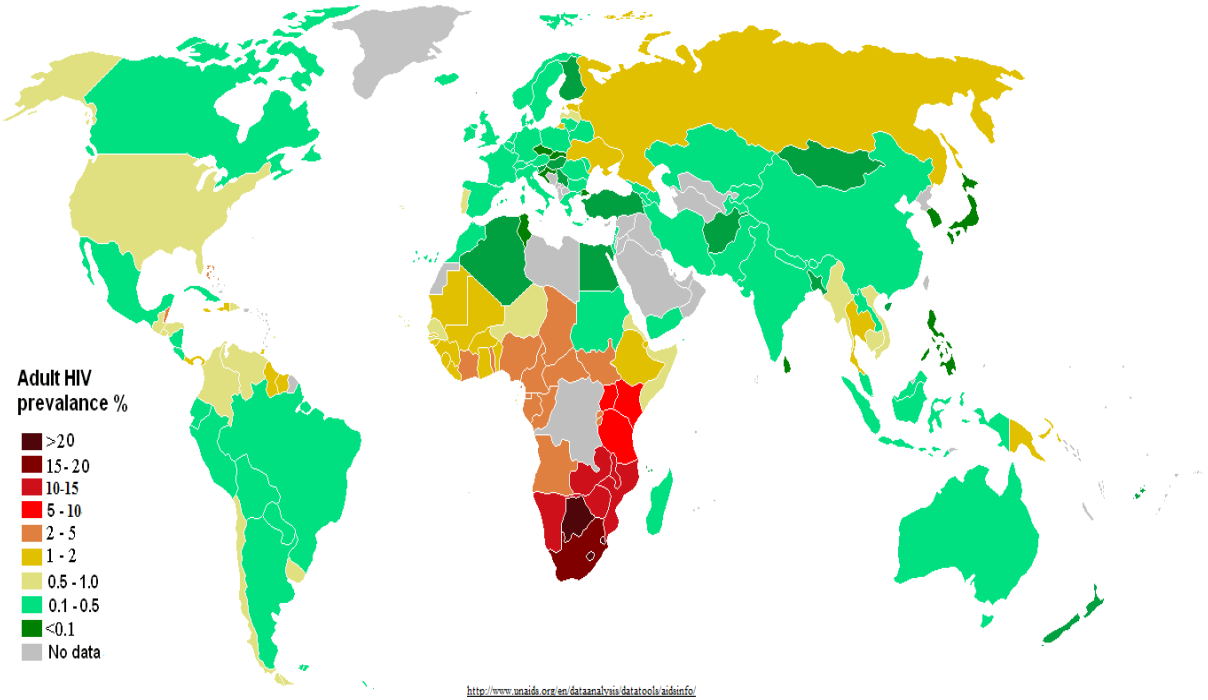
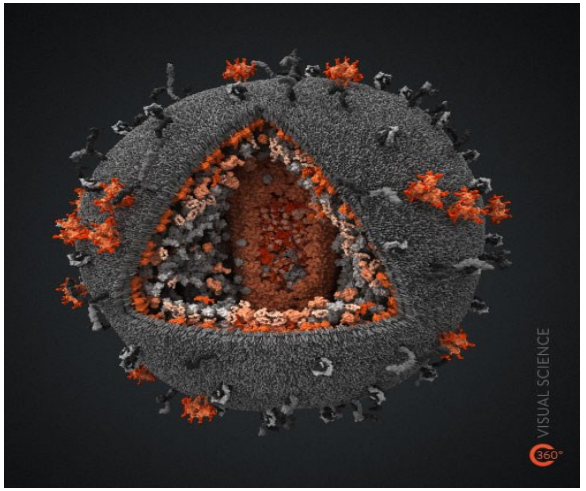
Virus HIV





HIV (*Human Immunodeficiency Virus)* is an enveloped RNA virus belonging to retroviruses, a group of viruses having the ability to produce by their RNA and DNA sequence inserted into the host cell genome. HIV is not resistant. Outside the organism survives poorly and only for short periods. This virus is very particularly sensitive to heat, the temperature of 60 ° C will not survive. The virus could infect an organism must to penetrate and come into contact with the blood of susceptible individuals. The spread of the AIDS pandemic is one of the main problems of many developing countries. The world today is around **40 million infected**, three quarters of them live in sub-Saharan Africa. In some African countries is infected even a **quarter of the population**. Is also strongly affected by the **South Asia, especially India**. In Europe, the countries of the former Soviet Union. The largest number of HIV infected live in South Africa, Nigeria and India.

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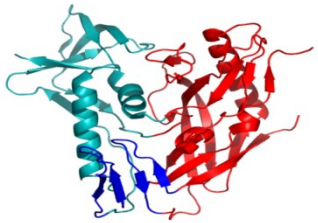
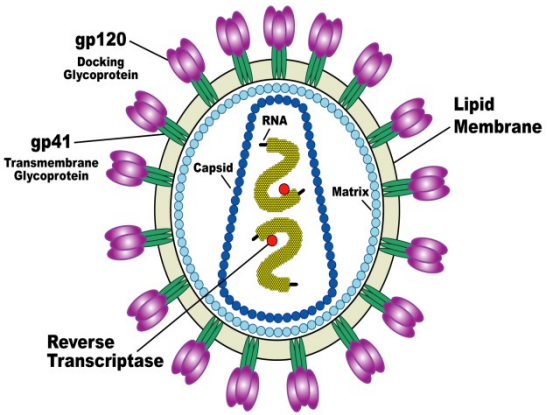
Protein GP 120



Glycoprotein GP120 is a glycoprotein exposed on the surface of the HIV envelope



The 120 in its name comes from its molecular weight of 120

Gp120 is essential for virus entry into cells as it plays a vital role in attachment to specific cell surface receptors.

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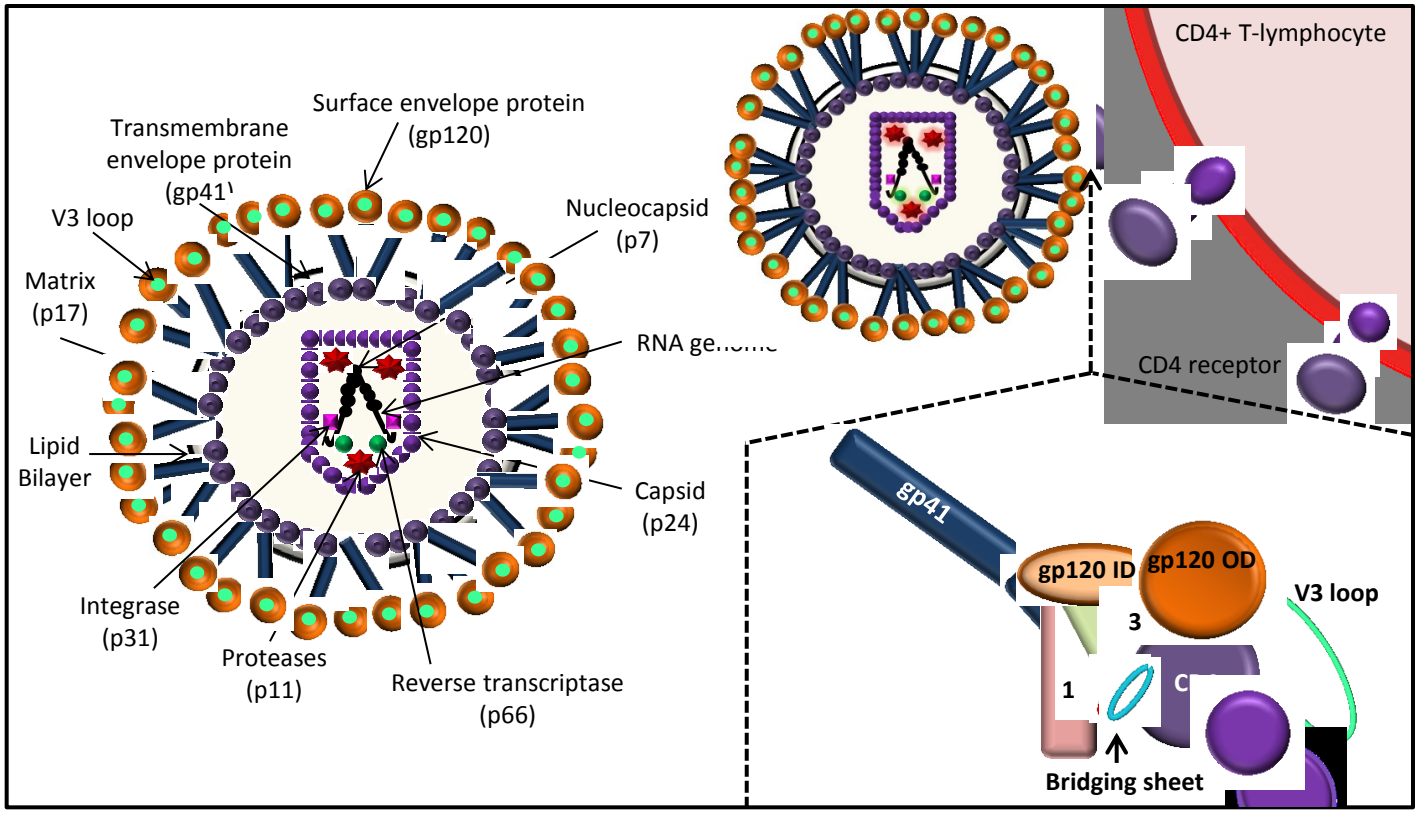
Scheme of HIV-1 virion



Overall scheme of HIV-1 virion, with expression of interaction between HIV-1 envelope glycoproteins with CD4 receptor binding site. One of the subunit of gp41 is depicted. The gp120 OD stays for outer domain of glycoprotein 120, gp120 ID stays for inner domain of glycoprotein 120, 1; 2; 3 stays for loops that form three topological layers. Binding with CD4 results in the apposition of layer 1 and layer 2, the formation of the bridging sheet and the projection of the V3 loop away from the gp120 core. This rearrangement of gp120 allows the gp41 ectodomain to undergo additional conformational changes, necessary for HIV-1 entry.

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Synthesis of CD4 Binding loop SSGGDPEIVTH by peptide synthesizer Liberty Blue.

Peptide with the amino acid sequence SSGGDPEIVTH with molecular weight 1098 Da was prepared on Liberty Blue peptide synthesizer by standard F-moc solid-phase peptide synthesis.

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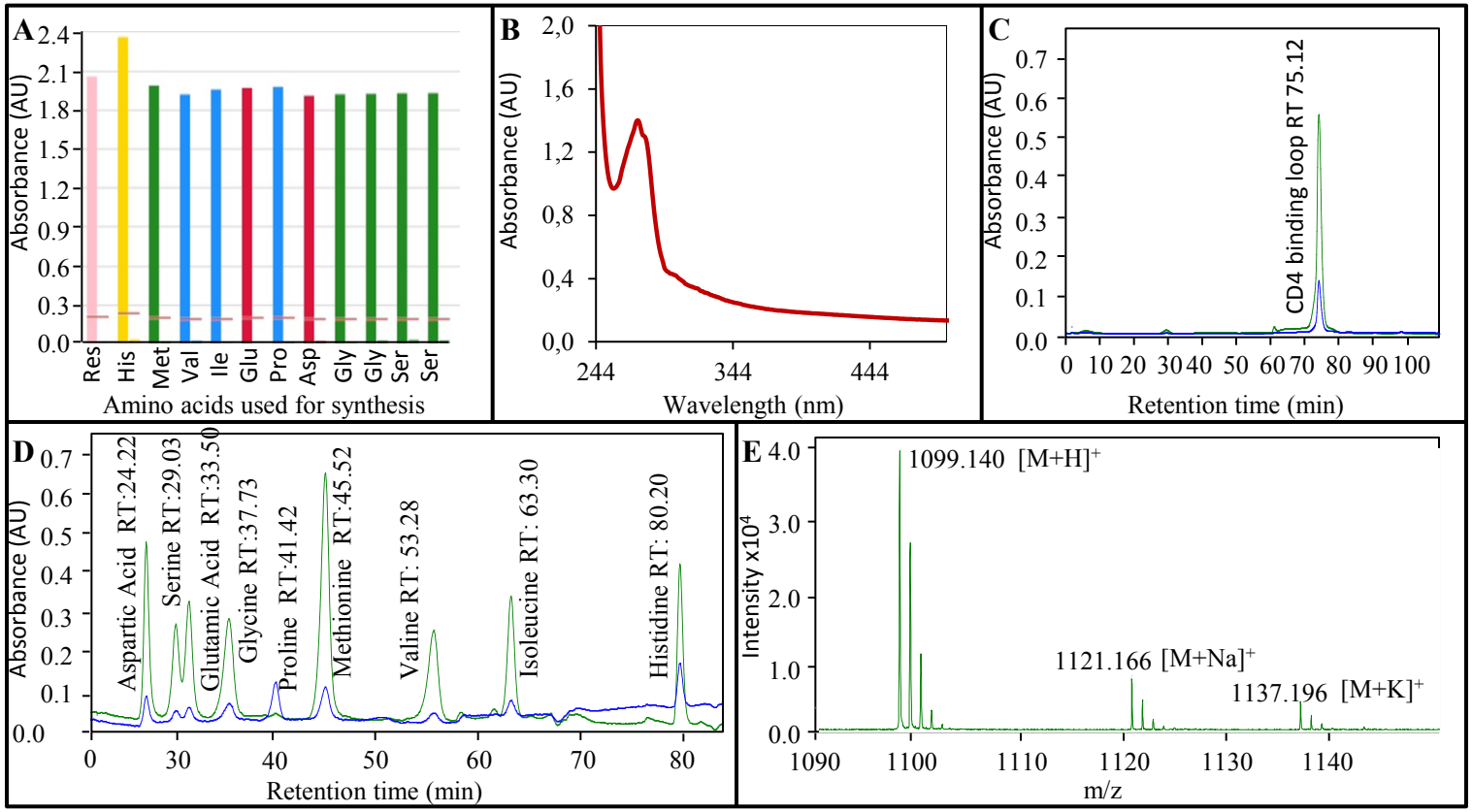
Characterization of CD4 binding loop of GP 120



(**A**) Record obtained from program Liberty Blue, confirming succesfull synthesis of CD4 binding loop (SSGGD PEIVMH), contained in region 366 – 376 of gp120 of HIV-1. (**B**) Absorption spectra of synthesized CD4 binding loop with absorption maximum at 272 nm. (**C**) Chromatogram obtained on ion-exchange chromatography (IEC) showing retention time (75.12 min) of CD4 binding loop peptide. (**D**) Expression of amino acid content in CD4 binding loop after hydrolysis in microwave reactor MW 3000. (**E**) MALDI-TOF/TOF mass spectra of CD4 binding loop peptide with calculated mass of 1098.13 Da. DHB was used as a matrix. Measurements were carried out in reflector positive mode, with laser power of 70 %. One spectrum is made as an average from 2500 subspectra. **M** stays for molecule of analyte; H stays for atom of hydrogen.

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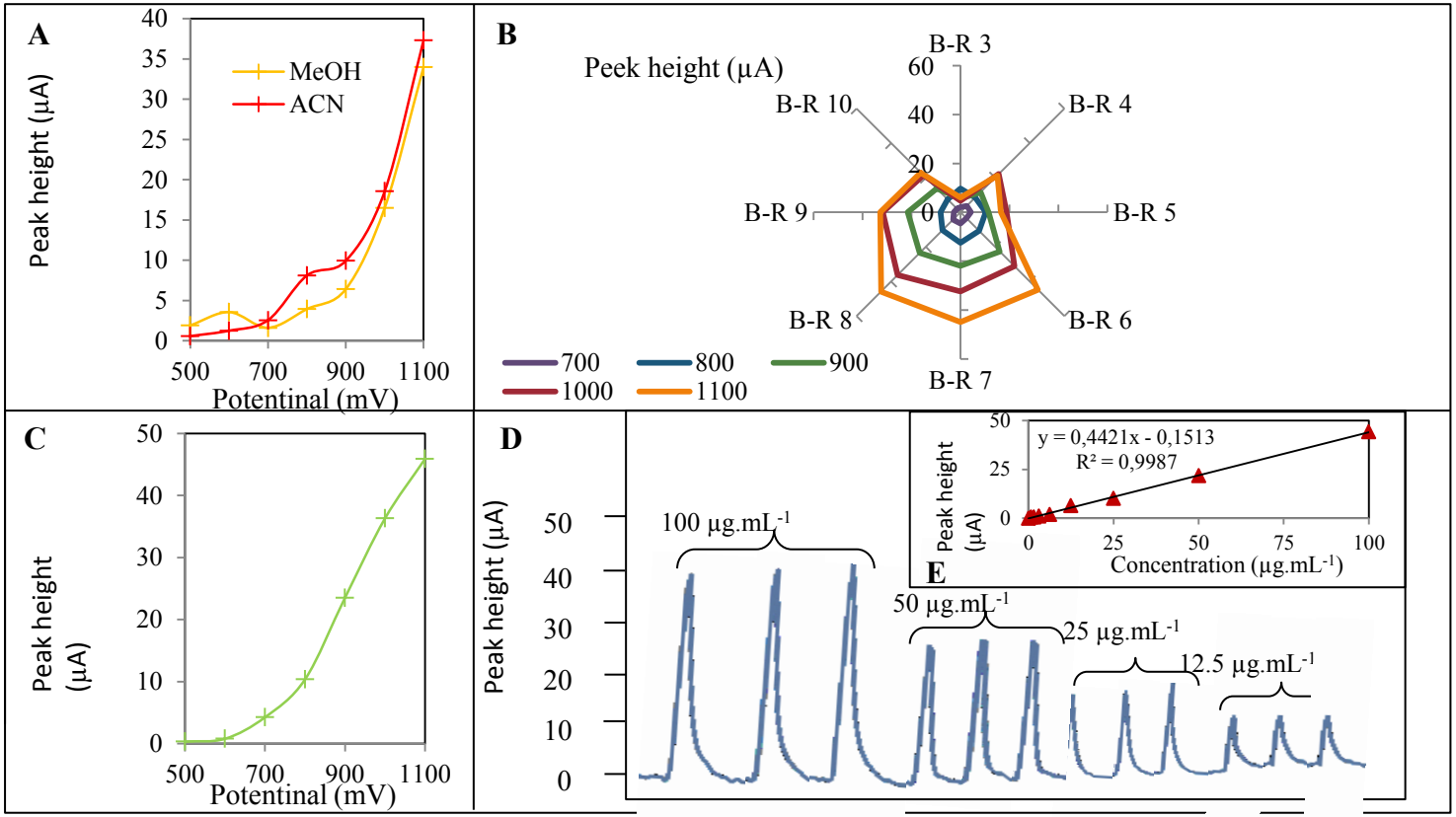
Electrochemical detection of CD4 Binding Loop of gp 120



Flow injection analysis with electrochemical detection (FIA-ED) system consists of two chromatographic pumps Model 582 ESA (ESA Inc., Chelmsford, USA) (working range 0.001-9.999 ml/min) and CoulArray electrochemical detector (Model 5600A, ESA, USA). Detector consists of flow analytical chamber (Model 6210, ESA, USA). Chamber contains four analytical cells. One analytical cell contains two referent (hydrogen-palladium), and two counter electrodes and one porous graphite working electrode. Electrochemical detector is situated in control module, which is thermostated. Sample (20 μl) was injected by manual valve (Rheodyne, USA). Flow rate of mobile phase was 1 ml/min

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Conclusion

Was performed successfully synthesis of CD4 binding loop (SSGGD PEIVMH), contained in region 366 – 376 of gp120 of HIV-1.

CD4 binding loop (SSGGD PEIVMH was characterization by spectroscopic, IELC and MALDI/TOF methods.

Was optimized the optimum conditions (Britton-Robinson pH 8 with addition 3 % ACN in the 1100 mV) of the electrochemical detection of CD4 binding loop (SSGGD PEIVMH), of gp120 of HIV-1.

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Prospects for the future

Interaction studies CD4 binding loop of GP 120 with peptides

Interaction studies CD4 binding loop of GP 120 with QDOTs

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Acknowledgment

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Thank you for your attention !

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