STREPTAVIDIN-MODIFIED QUANTUM DOTS AND THEIR INTERACTIONS VITH BIOTINYLATED VIRAL OLIGONUCLEOTIDES INVESTIGATED BY CAPILLARY ELECTROPHORESIS

Maja STANISAVLJEVIC[1], Libor JANU[1], Marketa RYVOLOVA[1,2], Rene KIZEK [1,2*]

[1]*Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic*

[2]*Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, CZ-616 00 Brno, Czech Republic*

INTRODUCTION

Viral infections pose a threat for mankind and diagnostic possibilities are relatively limited. Nowadays they are focused on the direct cultural proof of identity or they result from the detection of viral antigen by using of antibodies, however new diagnostic methods are still searched for.

Quantum dots (QDs), nanoparticles with unique size-depending optical and electronic properties can contribute significantly to this challenge. They are new fluorescent materials which are used instead of organic dyes for biological labelling. They have better photostability than organic dyes, narrow emission and continuous absorption spectra [1]. Due to the toxicity of their inorganic core, the surface of QDs has to be chemically modified. After modification QDs are suitable for conjugation with biomolecules, such as proteins, fragments of DNA or RNA or others.

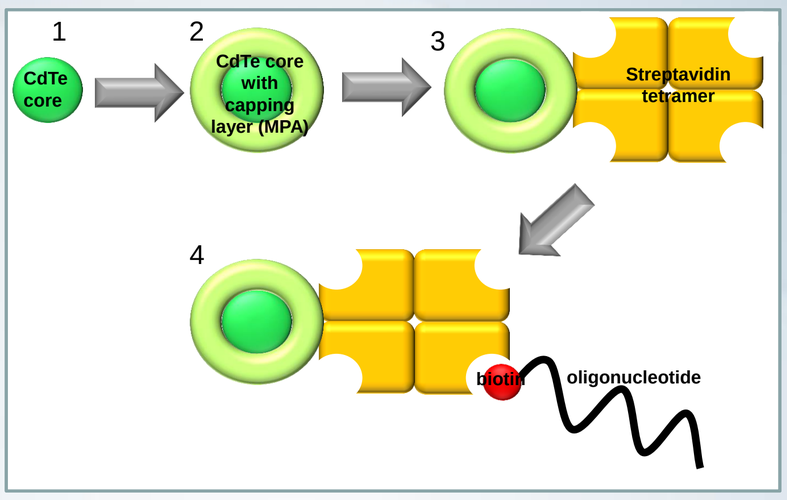

Figure 1. Scheme of the formation of the streptavidin modified QDs. 1) metalic core of the CdTe QD, 2) capping with the MPA, 3) coupling with streptavidin, 4) interaction of streptavidin modified QDs with biotinylated oligonuctide

Figure 2. Photograph of QDs under the ultraviolet light.

RESULTS AND DISCUSSION

Spectral characterization

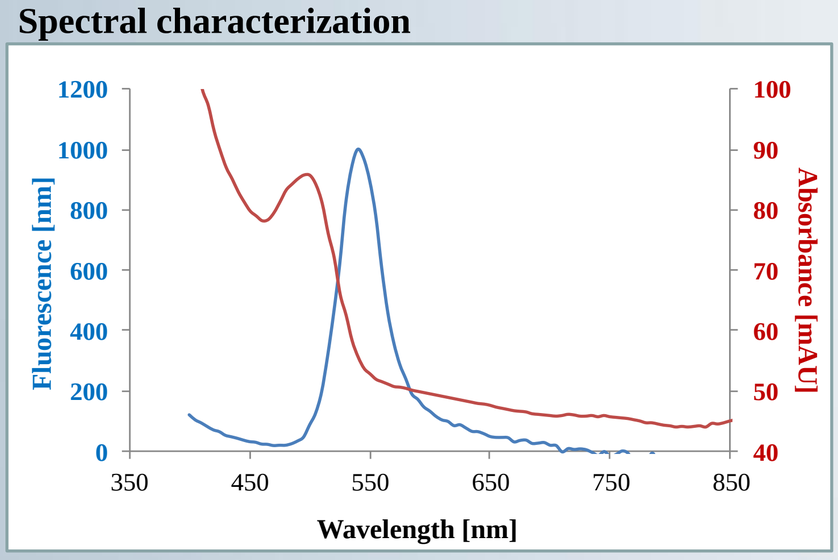

Figure 3. Absorption and emission spectra of streptavidin modified CdTe QDs

CE-LIF

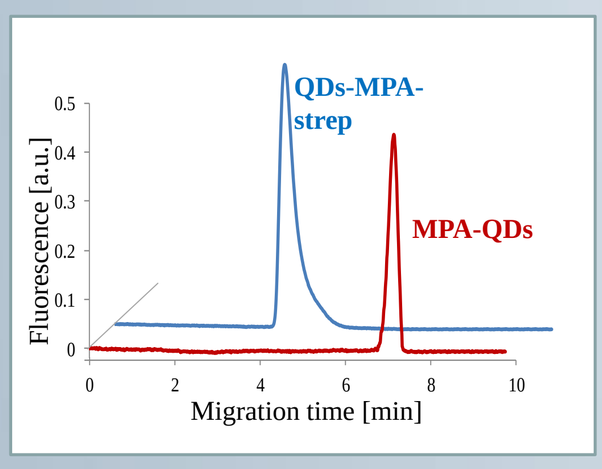

Figure 4. CE-LIF of MPA capped CdTe QDs and streptavidin modified CdTe QDs

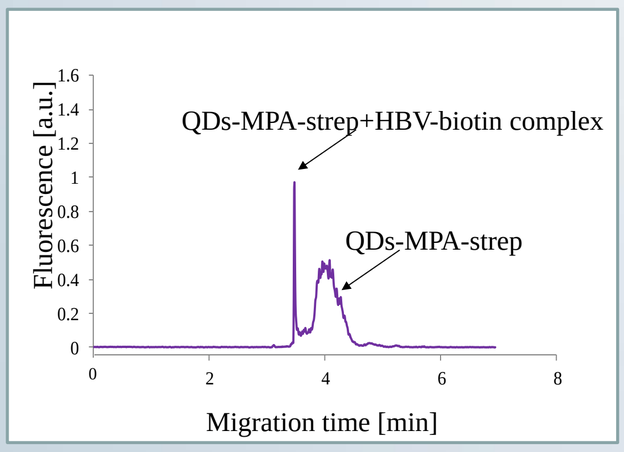

Figure 5. CE-LIF of mixture of streptavidin modified CdTe QDs and HBV-oligonucleotide labeled with biotin

Sequence of HBV specific oligonucleotide

BIOTIN-5´ CAT CCT GCT GCT ATG CCT CAT CT 3´

EXPERIMENTAL

CdTe QDs capped by MPA and streptavidin modified, VHB - bitoin-5μM
CE Backman PACE 5510 with absorbance (214 nm)
laser – induced fluorescence detection (λ_{ex} = 488nm, λ_{em} =520nm)
Capillary - total length of – 47 cm, effective length - 40cm, internal diameter 75μm
Injection by pressure of 3.4 kPa for 20 sec, Separation voltage 20 kV
Background electrolyte 20 mM borate (pH 9)

CE-UV

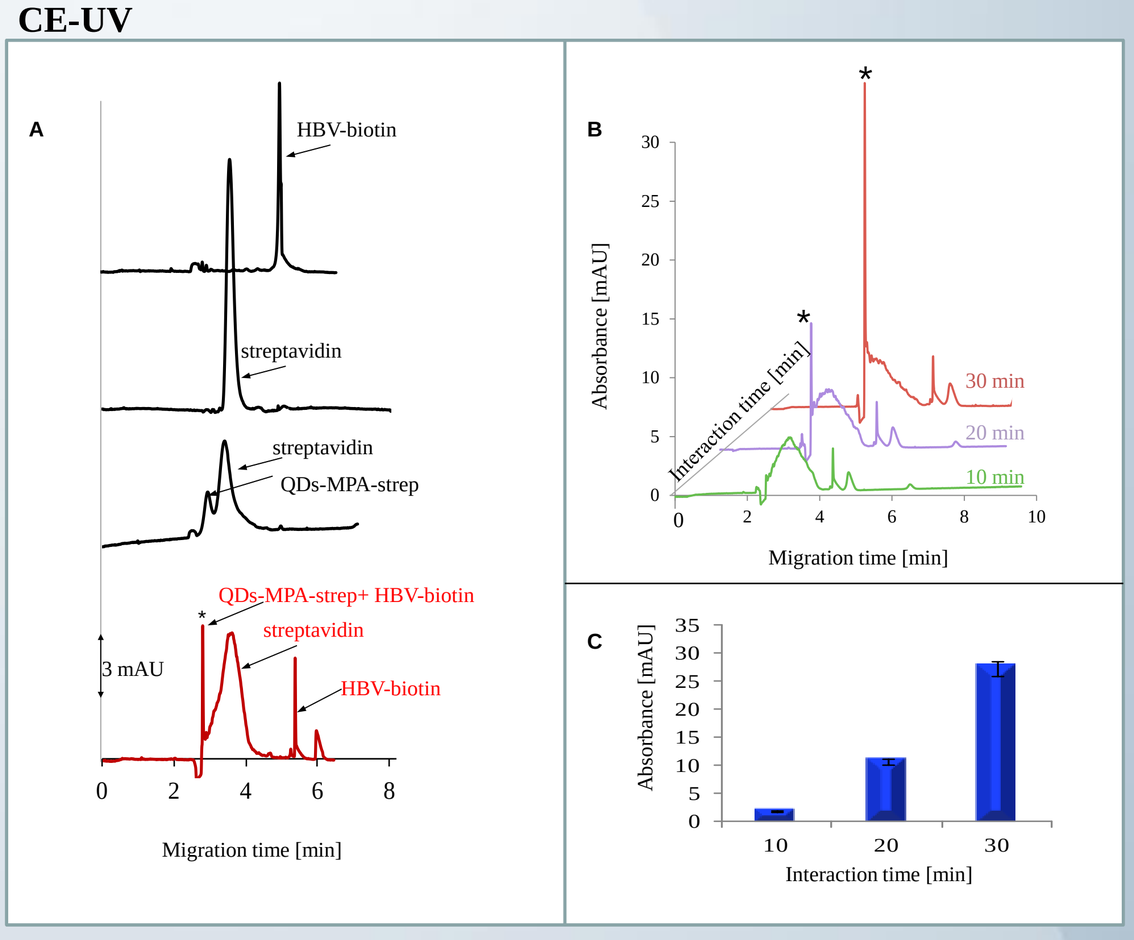

Figure 6. Monitoring of interaction of QDs-MPA-strep with biotinylated HBV oligonucleotide. A) CE-UV identification of mixture components (electrolyte: 0.02 M sodium borate, voltage: 20 kV, capillary: 47/40 cm, 75 μm ID, injection : 20 s, 3.4 kPa), * complex of the QDs-MPA-strep and biotinylated HBV oligonucleotide, B) Time dependence of the formation of QDs-MPQ-strep-HBV-biotin complex, C) Time dependent increase of the complex signal

CONCLUSION

Capillary electrophoresis represents a powerful separation technique for monitoring the process of creation of QD-biomolecule complex and the use of UV absorbance detection is beneficial at the first stage of research because it enables visualization of both fluorescent as well as non-fluorescent components of the reaction. Laser-induced fluorescence detection on the other hand provides the sensitivity required for analysis of real biological samples.

REFERENCES

[1] Yu, W. W., Chang, E., Drezek, R., Colvin, V. L., *Biochemical and Biophysical Research Communications* 2006, *348*, 781-786.
[2] Bharali, D. J., Mousa, S. A., *Pharmacology & Therapeutics* 2010, *128*, 324-335.

ACKNOWLEDGEMENTS

The work has been supported by NanoBioTECell GA CR P102/11/1068 and IGA IP10/2012 and NanoBioMetalNet CZ.1.07/2.4.00/31.0023