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

Název: Interaction of CQDs with DNA

Školitel: Vedran Milosavljevic

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Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

Interaction of CQDs with DNA

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Quantum dots – history of synthesis

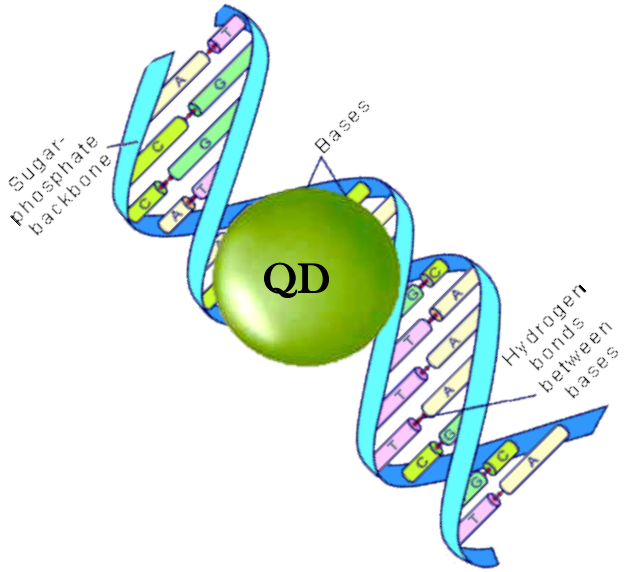
- 1980 – Ekimov, Efros – first description of quantum dots
- 2004 – X.Yu et al.: CQDs obtained for the first time during purification of single-walled carbon nanotubes through preparative electrophoresis
- enviromental-friendly synthesis

Characteristics

- new type of nanomaterial with nanocrystal structure
- crystal size < 10 nm in diameter
- chemical stability, biocompatibility, good colloidal stability, low cost and low toxicity
- electrochemical luminescence, photoinduced electron transfer property, photocatalysis, optoelectronics

DNA

- 1869 – isolated by Swiss scientist Friedrich Miescher
- 1953 – described DNA structure Watson and Crick
- 1962 – they recieved Nobel prize for solving nature's biggest secret
- DNA helix-two complementary and antiparallel polynucleotide strands



Experiment hypothesis:

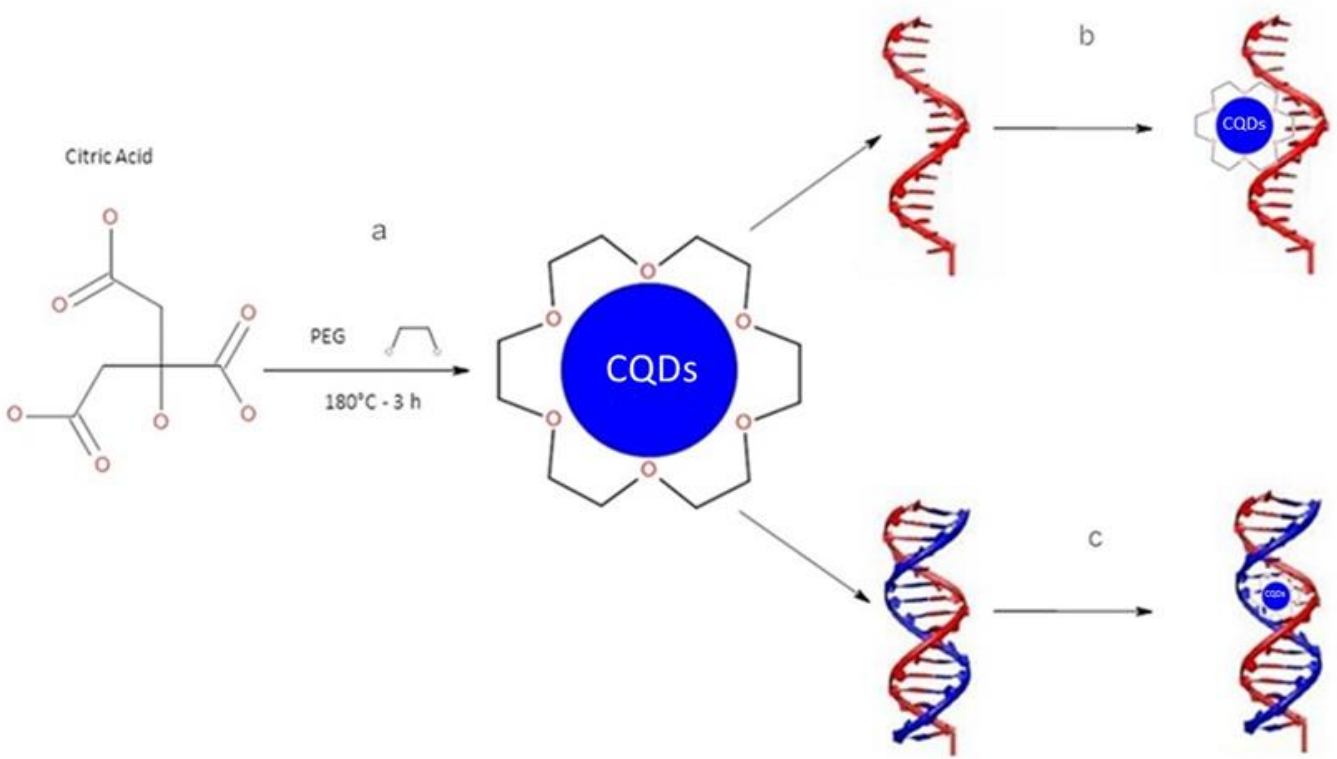
- DNA – interaction with organic compounds
- CQDs - chemical stability, biocompatibility, low toxicity
- QDs-DNA conjugation – biosensors
- DNA + CQDs interaction

Synthesis of CQDs

- water soluble CQDs
- ethylene glycol + polyethylene glycol + citric acid (180ºC)
- purification - D-Tube maxi dialyzer

Interaction CQDs with DNA

- ssDNA (10 μg/ml) and dsDNA (10 μg/ml)
- CQDs: 25, 50, 100, 250 and 500 μg/ml



Measurements

- Tecan Infinite 200 PRO
- absorbance: 200 - 800 nm
- excitation wavelengths: 280 nm, 350 nm, 450 nm
- NanoQuant plate - quartz optics lens

Concentration of CQDs

- freeze-drying – lyophilization, 2 h, -70 ºC, 0.7 mbar
- CHNS elemental analyzer - 46% carbon, 7.1% hydrogen
- concentration: 118 mg/ml

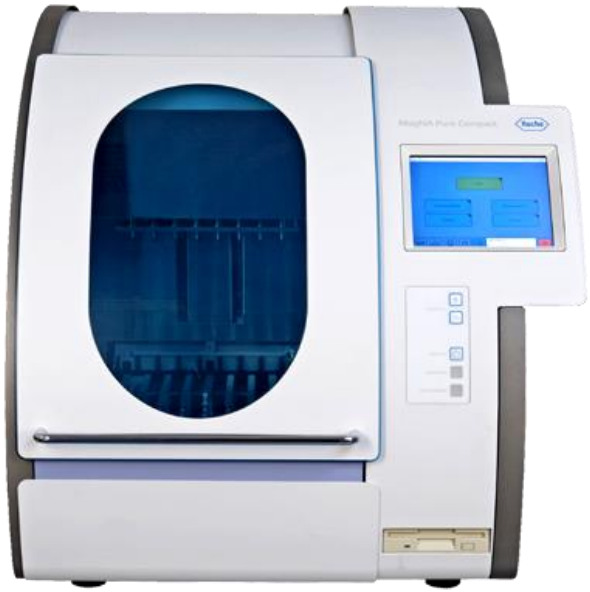
MT-DNA amplification and gel electrophoresis

- earthworm

- isolation: MagNA Pure Compact
- conversion: mRNA > cDNA

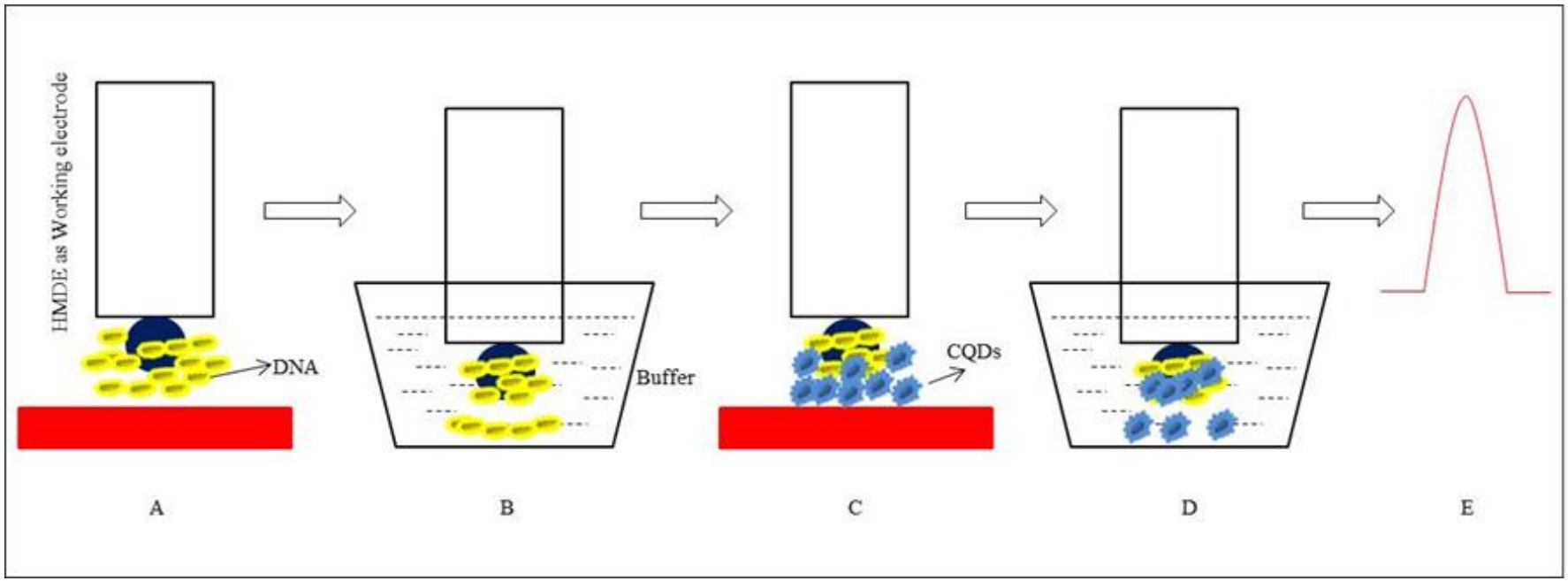
- cDNA amplification - Taq kit
- forward primer sequence 5´-CGCAAGAGAGGGATCAACTT-3´
  reverse primer sequence 5´-TATTTCAATGCCTCGGCTCT -3´
- metallothionein gene - 228-bp PCR

- PCR product + CQDs - 2% agarose gel electrophoresis
- staining of PCR product - ethidium bromide
- bands visualized by UV transilluminator, 312 nm

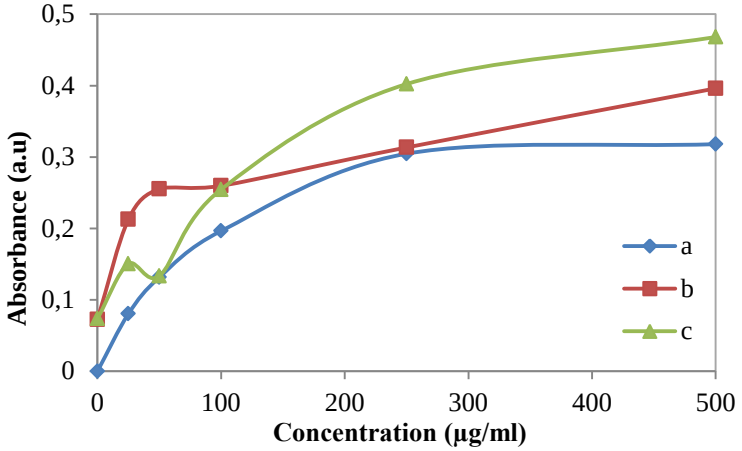


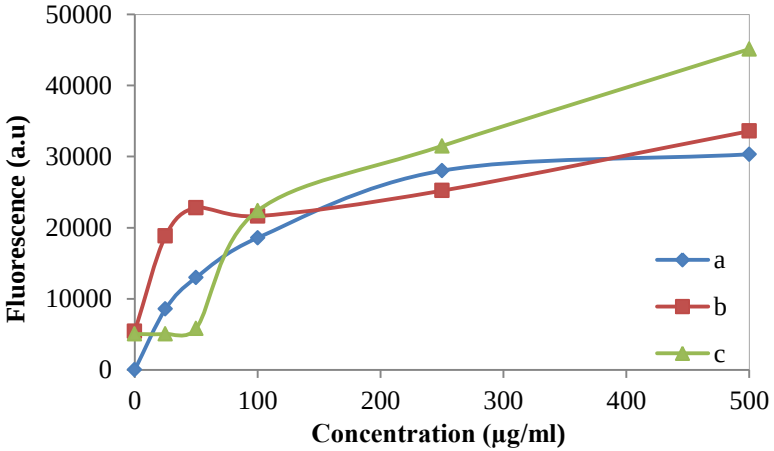
Electrochemical measurements

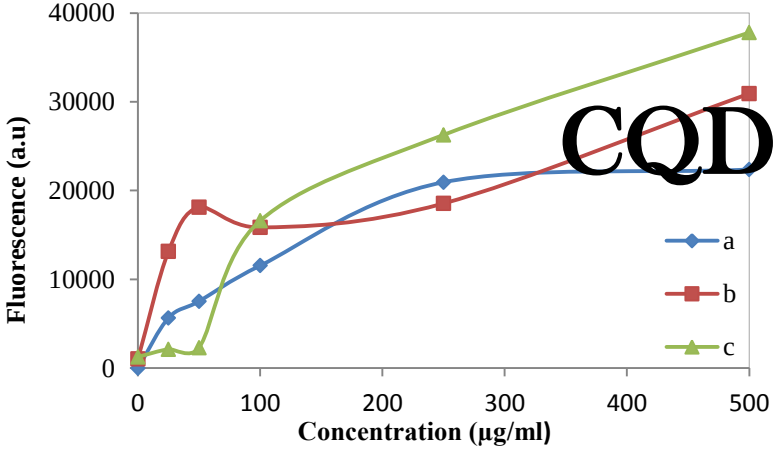
- AUTOLAB Analyzer conected withVA-Stand 663
- standard cell with three electrodes
  - working electrode - hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm2
  - reference electrode - Ag/AgCl/3M KCl
  - the auxiliary electrode - glassy carbon electrode
- parameters of electrochemical determination:
  purge time 120 s, frequency 280 Hz, initial potential 0 V,
  end potential -1.8 V, potential step 0.005 V, amplitude 0.025 V

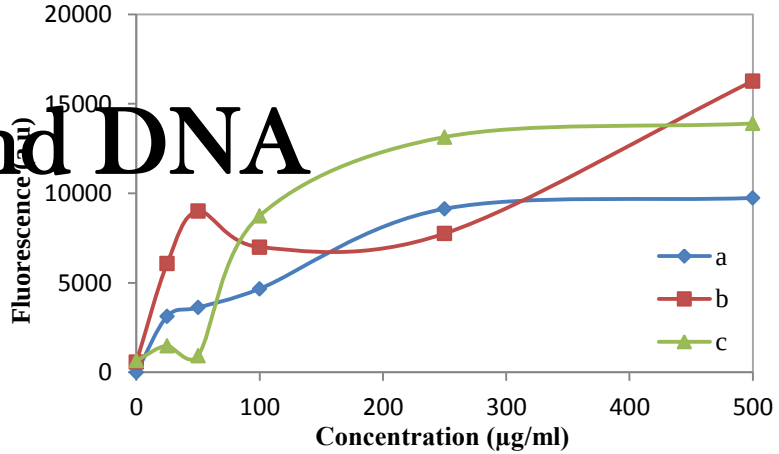


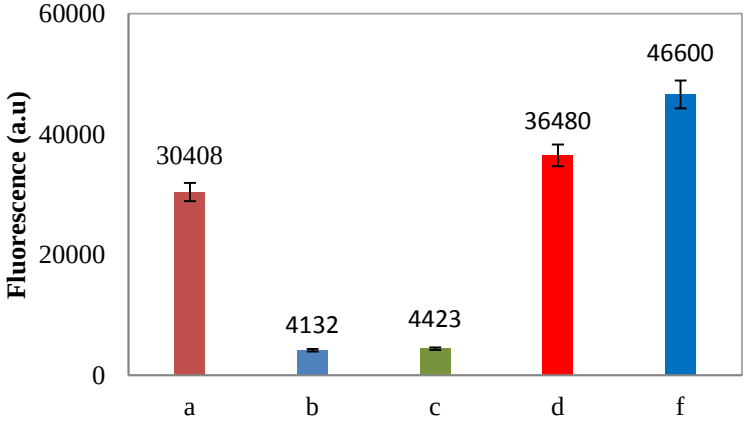
CQDs and DNA

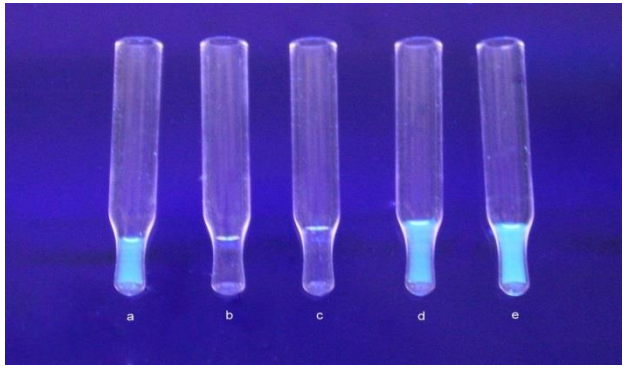

Absorbance (a.u)
Concentration (µg/ml)
a
b
c


Fluorescence (a.u)
Concentration (µg/ml)
a
b
c

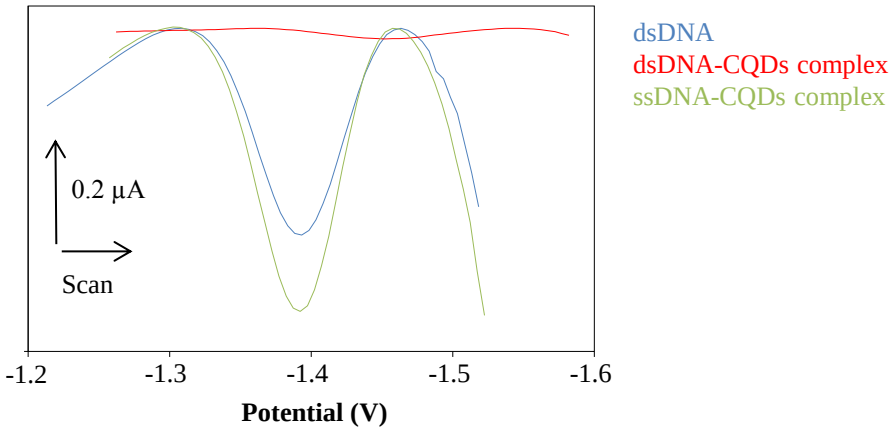

Fluorescence (a.u)
Concentration (µg/ml)
a
b
c

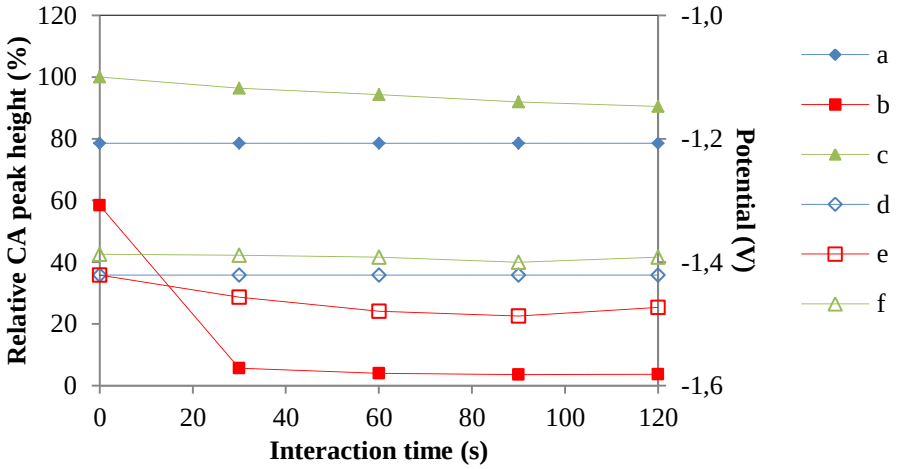

Fluorescence (a.u)
Concentration (µg/ml)
a
b
c

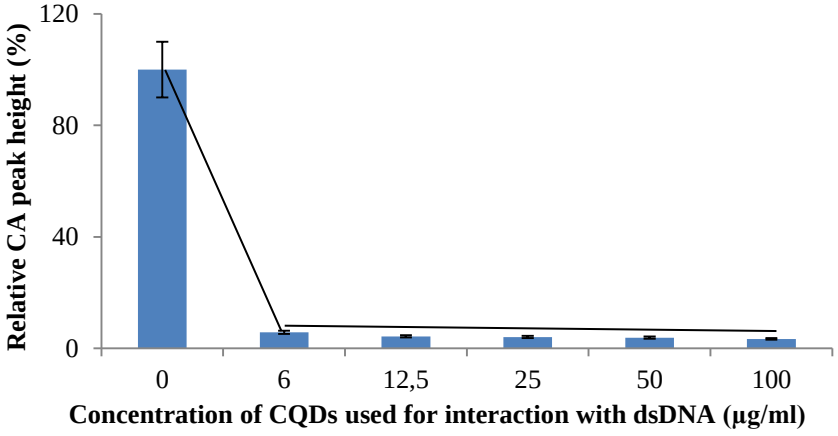

Fluorescence (a.u)
30408
4132
4423
36480
46600
a
b
c
d
f

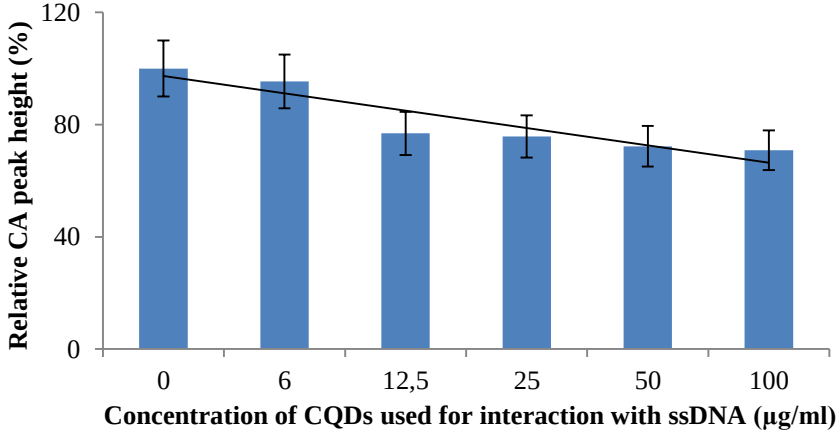

a
b
c
d
e

Electrochemical detection of CA peak from DNA-CQDs complex

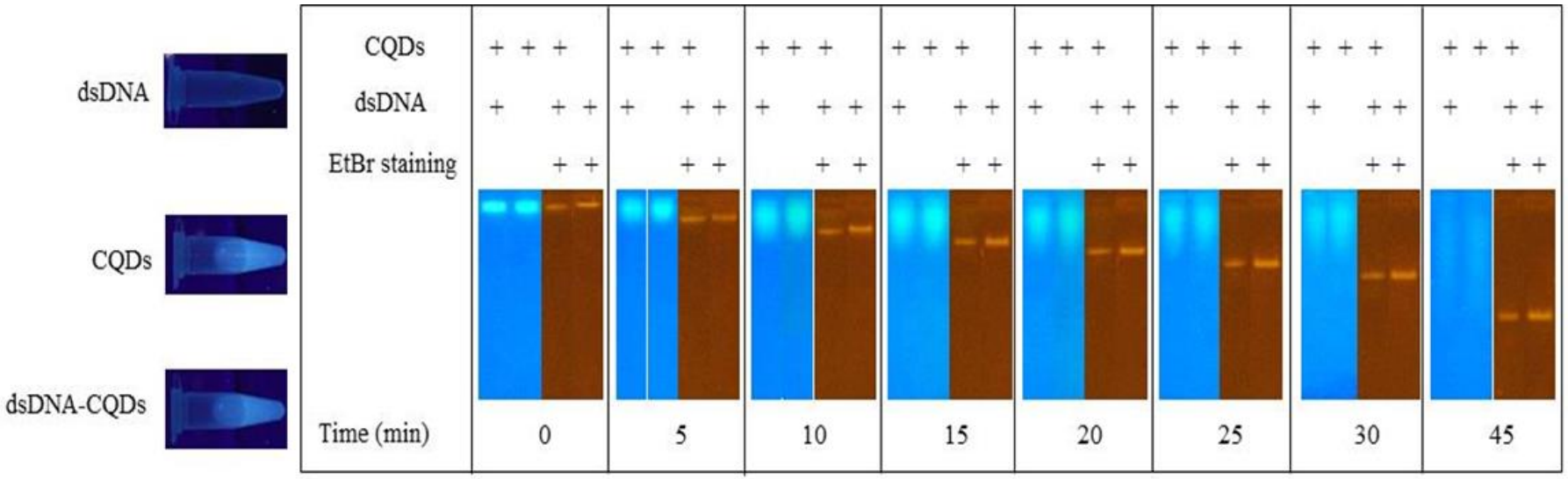








Gel electrophoresis analysis of dsDNA



Conclusions

- structurally and optically stable CQDs functionalized with DNA can be successfully fabricated
- ssDNA strand conjugates on CQDs via π-π interaction
- binding of CQDs into the structure of dsDNA via electron pairs of oxygen atoms from PEG bounded with DNA bases by hydrogen bond
- both dsDNA and ssDNA increase the fluorescence of CQDs, but the increasing efficiency of ssDNA was lower than that of dsDNA for the weaker binding between ssDNA and CDQs
- proven potential application of CQDs in designing of highly sensitive biosensors

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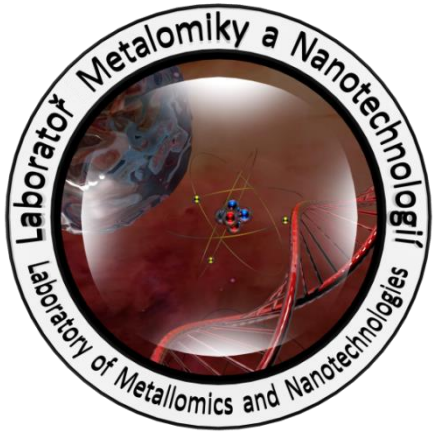
- prof. Ing. René Kizek, Ph.D.



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Thank you for your attention!

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