INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Interaction of zinc ions with DNA

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

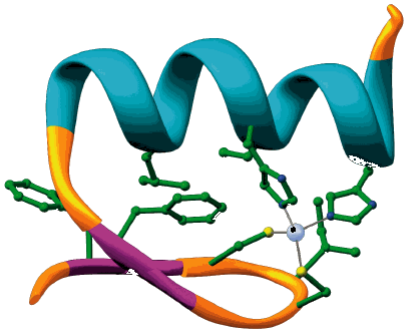
Content

- biological role of zinc
- DNA and zionc ions
- our work
- conclusion

Functions of zinc in organisms

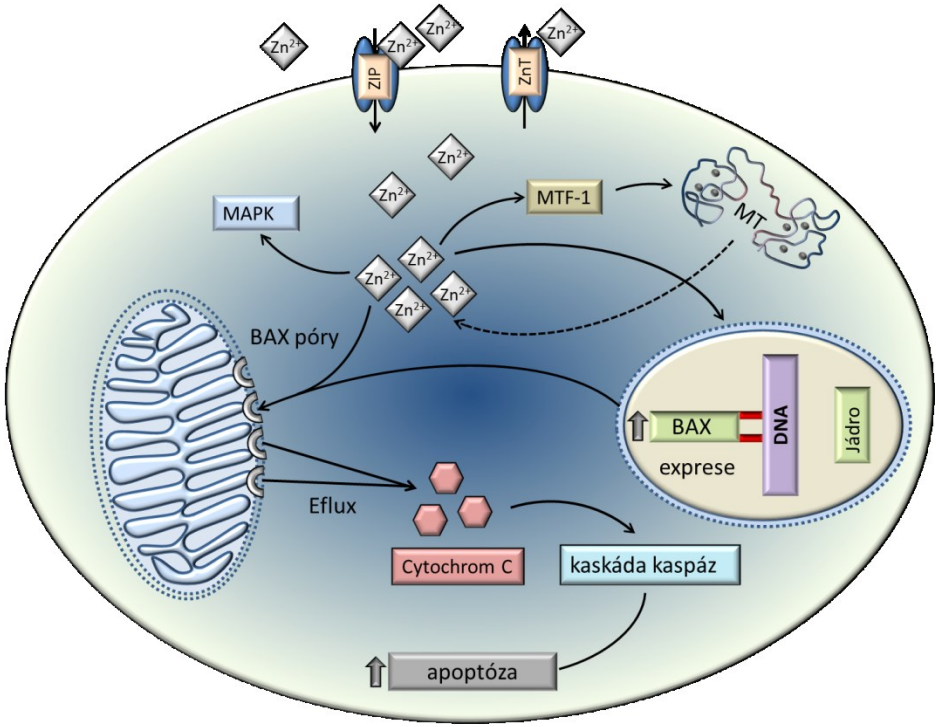
- catalytic function
- structural function
- regulatory function
- signaling function

zinc finger of Zif268



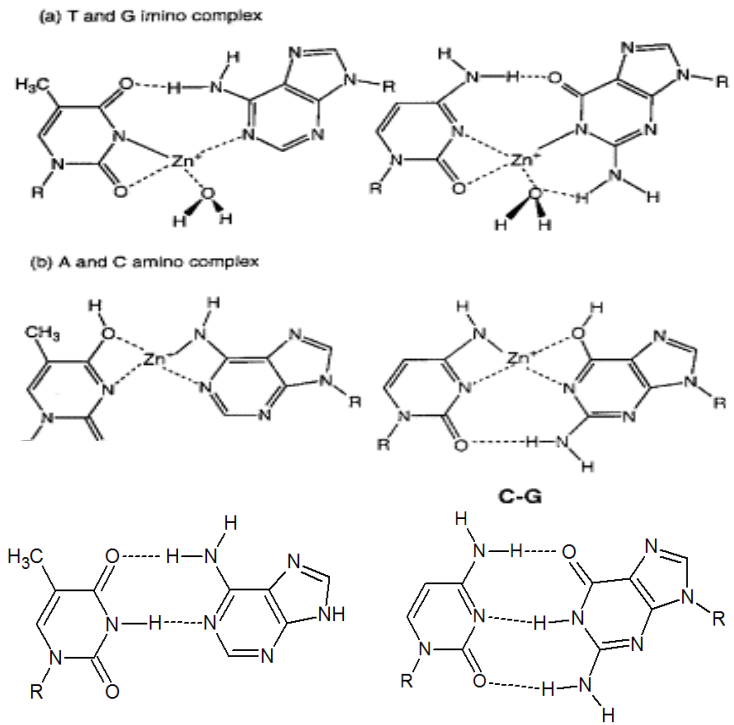
Enzymes, TFs (TFIIIA), receptors (estrogen, glucocorticoid, GABA) are influenced by zinc metabolism.

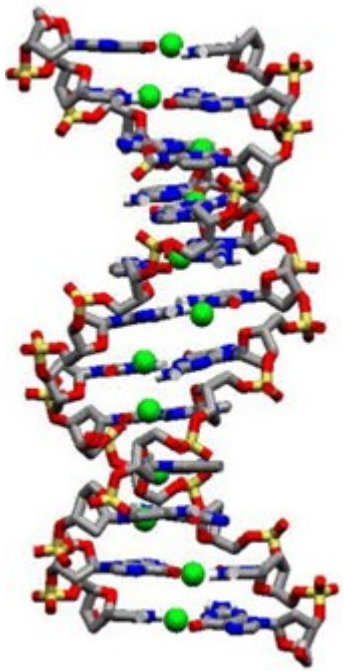
Effect of Zn (II) in the cell



DNA and zinc (II) interaction

- diffuse binding X site specific binding
- M-DNA (r.1993) - complex of divalent metal ions with DNA
- effect of pH and concentration



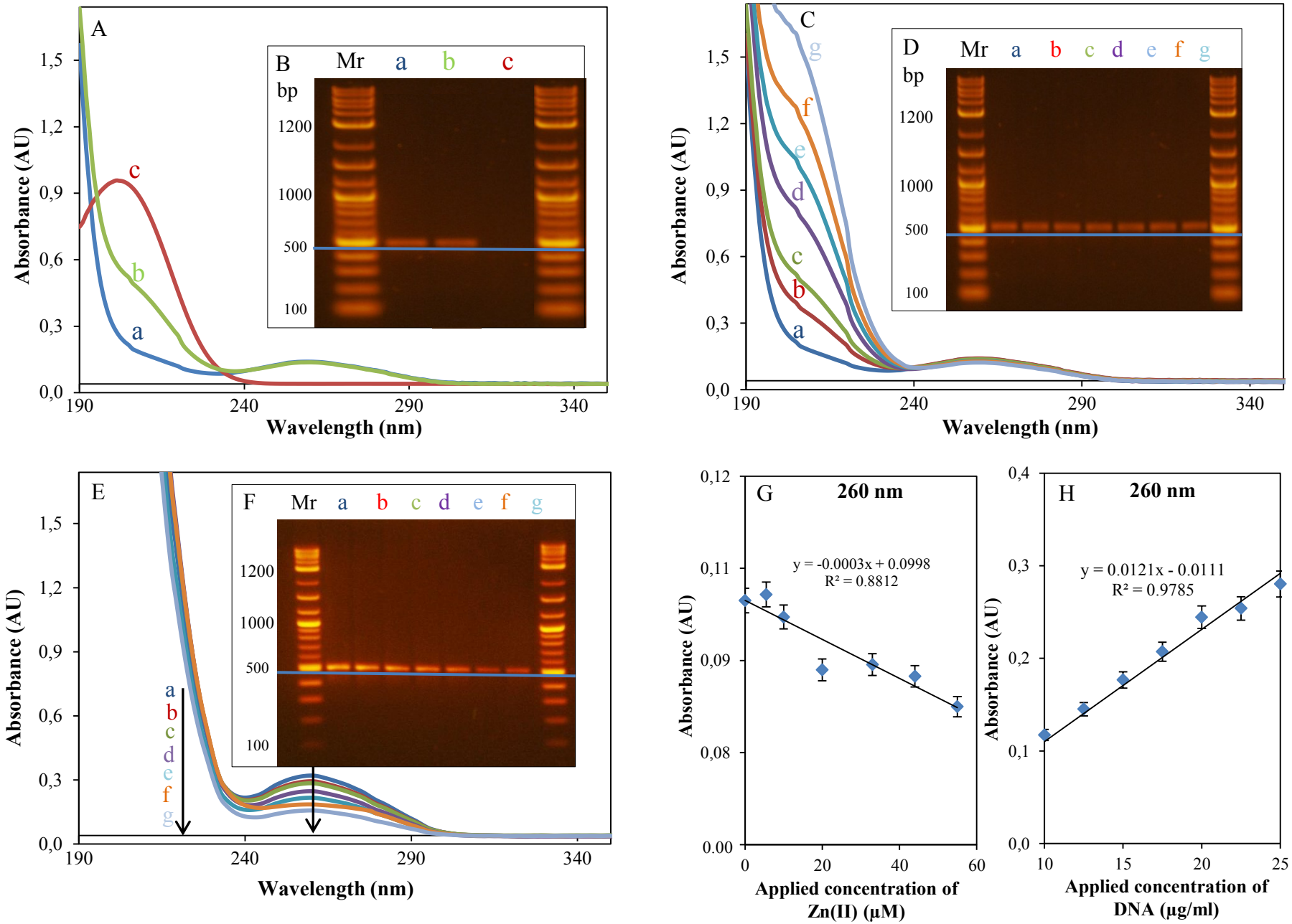


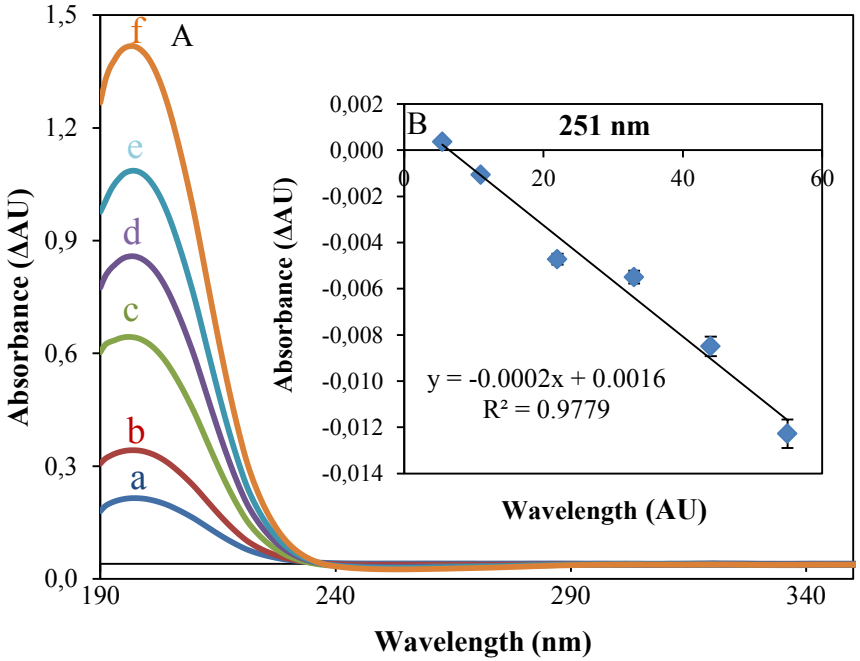
proposed structure of M-DNA

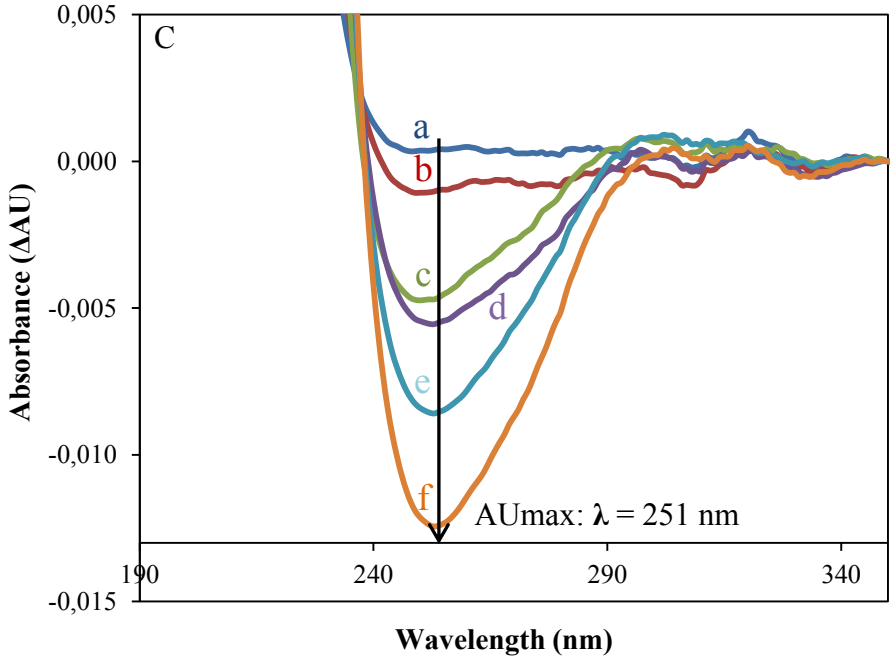
Our work

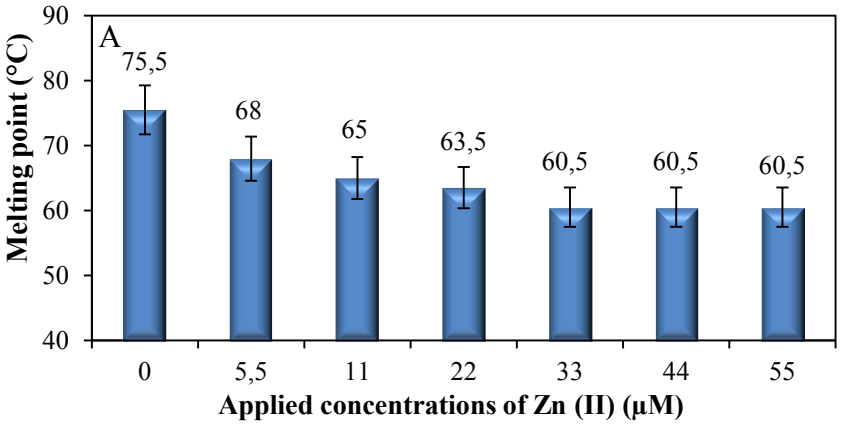
- There was fragment of DNA of 498 bp used.
- 5 µg/ml DNA was incubated with 0, 5.5, 11, 22, 33, 44 and 55 µM Zn(II).
- To study the influence of DNA concentration on Zn(II)-DNA interaction, 7 concentrations of DNA (10, 12.5, 15, 17.5, 20, 22.5 and 25 µg/ml) were incubated with 55 µM Zn(II).
- DNA was incubated with Zn(II) ions for 60 min at 25 °C

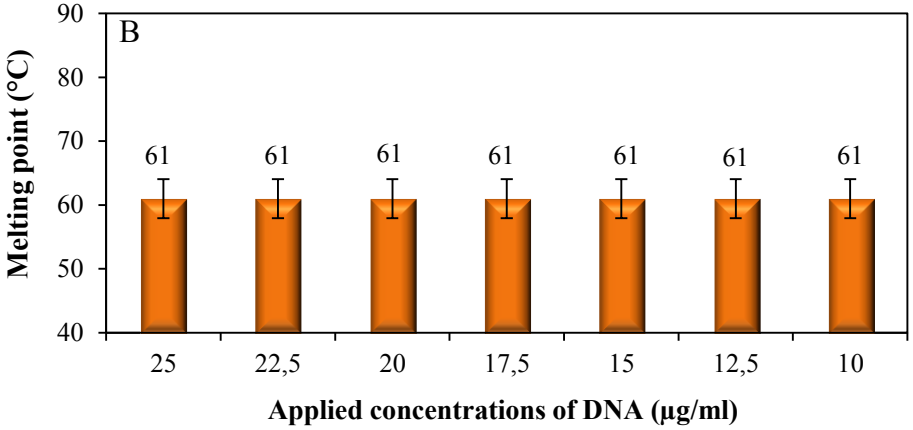
  to create M-DNA.
- After incubation, samples were dialyzed and analysed using UV/VIS spectrophotometry and gel electrophoresis.

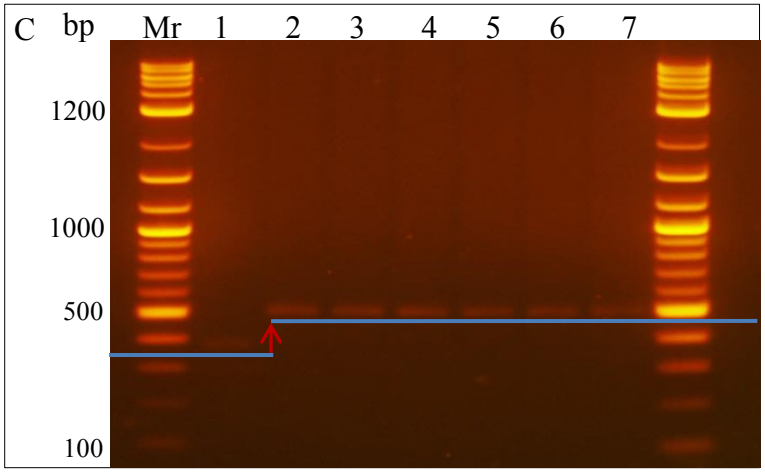

A
B
Mr
a
b
c
bp
1200
1000
500
100
Absorbance (AU)
Wavelength (nm)
C
D
Mr
a
b
c
d
e
f
g
E
F
G
260 nm
y = -0.0003x + 0.0998
R² = 0.8812
Applied concentration of Zn(II) (µM)
H
260 nm
y = 0.0121x - 0.0111
R² = 0.9785
Applied concentration of DNA (µg/ml)

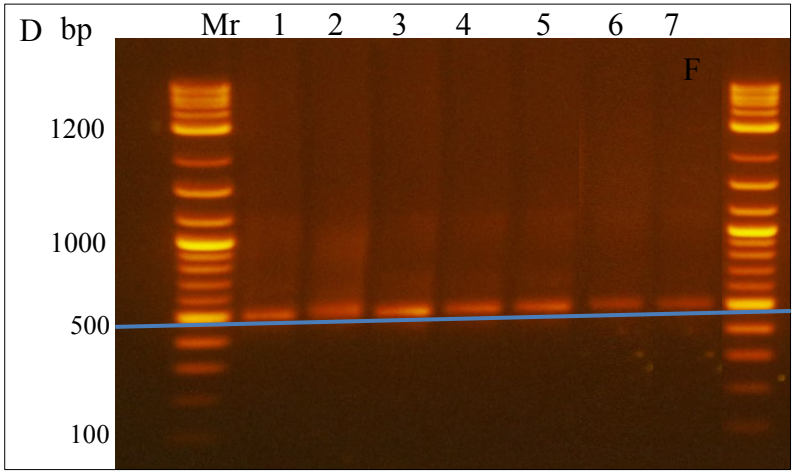

A
B
251 nm
y = -0.0002x + 0.0016
R² = 0.9779
Absorbance (ΔAU)
Wavelength (nm)
Wavelength (AU)
a
b
c
d
e
f

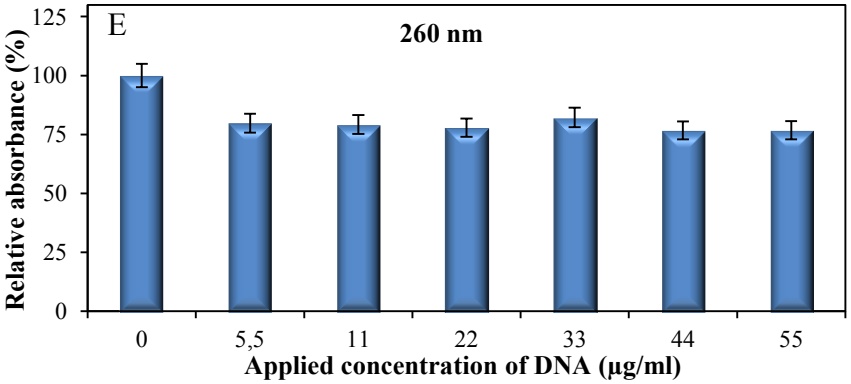

C
AUmax: λ = 251 nm
Absorbance (ΔAU)
Wavelength (nm)
a
b
c
d
e
f

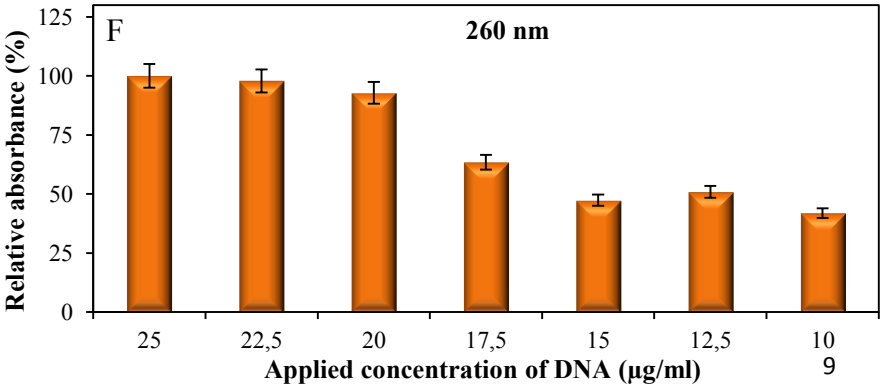

A
Melting point (°C)
90
80
70
60
50
40
75,5
68
65
63,5
60,5
60,5
60,5
0
5,5
11
22
33
44
55
Applied concentrations of Zn (II) (µM)


B
Melting point (°C)
90
80
70
60
50
40
61
61
61
61
61
61
61
25
22,5
20
17,5
15
12,5
10
Applied concentrations of DNA (µg/ml)


C
bp
Mr
1
2
3
4
5
6
7
1200
1000
500
100


D
bp
Mr
1
2
3
4
5
6
7
F
1200
1000
500
100


E
260 nm
Relative absorbance (%)
125
100
75
50
25
0
0
5,5
11
22
33
44
55
Applied concentration of DNA (µg/ml)


F
260 nm
Relative absorbance (%)
125
100
75
50
25
0
25
22,5
20
17,5
15
12,5
10
Applied concentration of DNA (µg/ml)

Results

- We proved binding of zinc(II) ions in DNA using the simple methods (spectrophotometry and gel electrophoresis) in our study.

- Interactions between DNA and Zn(II) are presented by changes in absorption spectra (190 – 350 nm) and by decrease in Tm at denaturation of M-DNA.

- This study proved that M-DNA may develop at physiological conditions.

Thank you for your attention!

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OP Vzdělávání pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Mendel University in Brno