

Název: Různé způsoby vizualizace interakcí  
nukleových kyselin s ionty těžkých kovů a  
především se zinečnatými ionty

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# Aim of the work

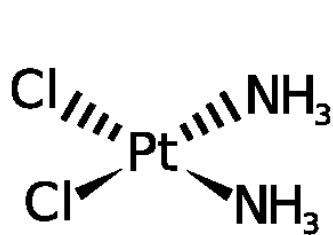
The aim of the experiments was to study the interaction of cytostatics and Zn(II) with DNA

- a) **amplification and isolation of DNA fragments:** create copies of DNA fragments using polymerase chain reaction
- b) **spectrophotometry:** changes in absorption spectrum and melting temperatures of nucleic acids
- c) **electrochemistry:** changes in the oxidation signals of bases of nucleic acids

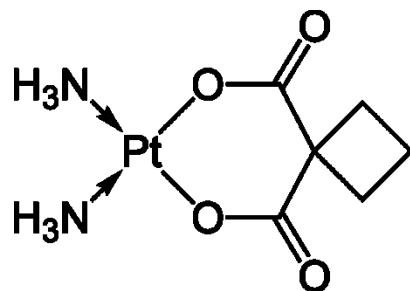
# Introduction - platinum cytostatics and Zn (II)

## cytostatics

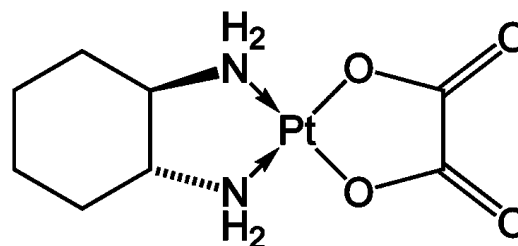
The biological activity of the first platinum-based cytostatics (cisplatin), which is still one of the most widely used cytotoxic agent, was discovered in 1965. Platinum drug (second generation) carboplatin was developed in the 1980<sup>'</sup> as a less toxic alternative to cisplatin and showing fewer side effects. Another often used platinum-based cytostatic is oxaliplatin, which was designed in the 1990<sup>'</sup> as a third generation platinum drug to overcome resistance to cisplatin and carboplatin.



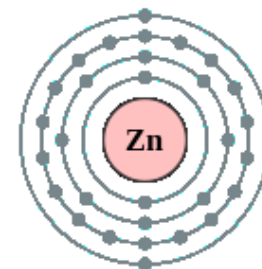
cisplatin



carboplatin



oxaliplatin



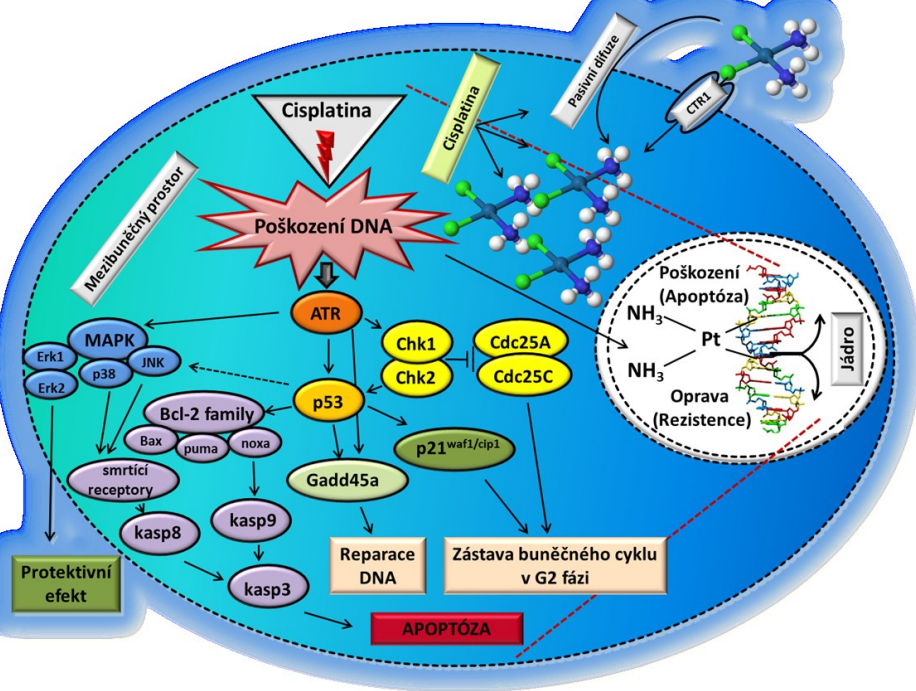
zinc

## zinc

Zinc is a biogenic element that has an important role in living systems, particularly in the synthesis of proteins and DNA. Zn (II) controls metabolic processes and the activity of over 300 enzymes. Metal ions contained in the enzymes act as cofactors and play an important role for the proper function of ribosomes. Structural role of zinc is used mainly to stabilize zinc finger motif. Proteins with this motif are known for high affinity to DNA, the structure of zinc finger protein directly mediates interaction with DNA in the large groove.

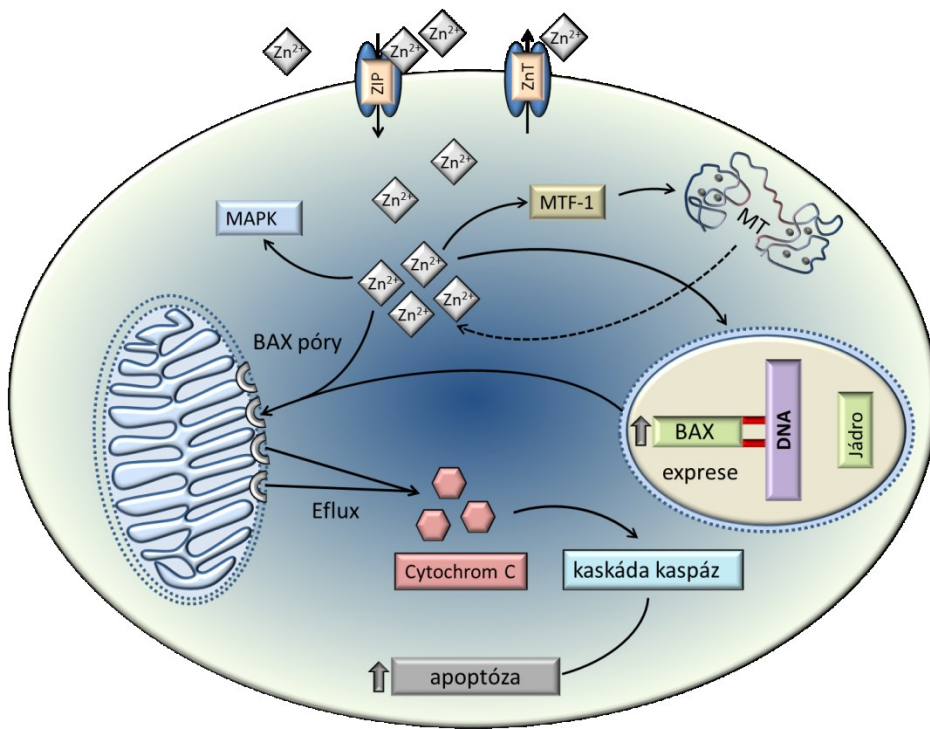
## Effect of cis-Pt in the somatic cell

- Entry of cis-Pt is allowed by passive diffusion or transporter CTR1
- DNA damage caused by cis-Pt activates the ATR kinase
- Objective of the ATR is a tumor suppressor protein p53, which is phosphorylated by the ATR kinase
- P53 initiates transcription of the gene for the protein p21, which inhibits cyclin dependent kinases leading to end of cell cycle
- P53 induces expression of proapoptotic family Bcl-2 members, that are responsible for activating the mitochondrial apoptosis pathways
- The main determinant of response to DNA damage caused by cisplatin is the proapoptotic and antiapoptotic proteins ratio

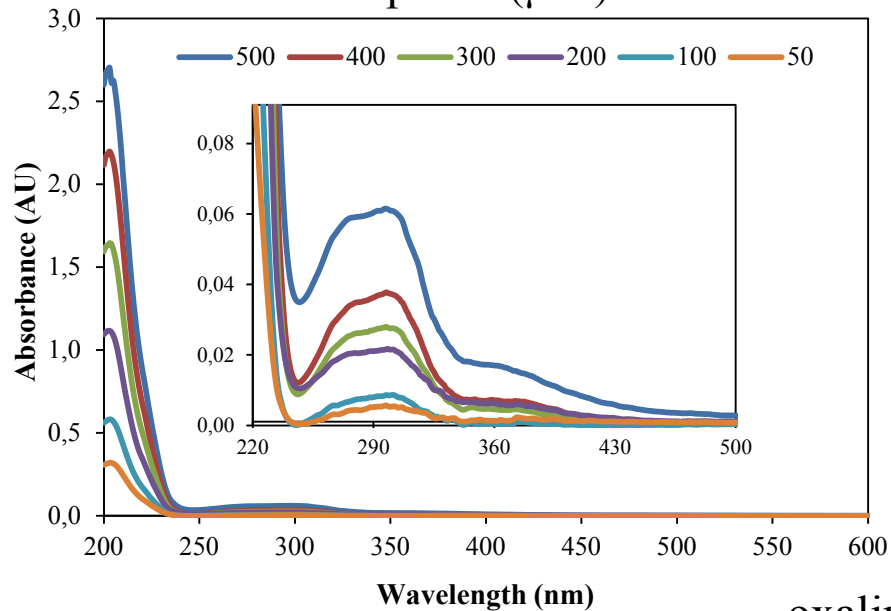


## Effect of Zn (II) in the prostate cell

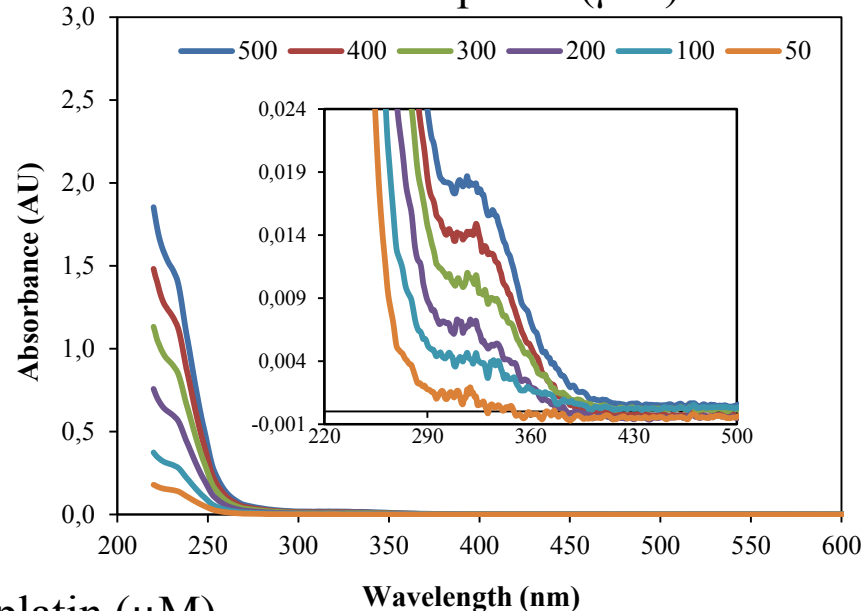
- Entry of Zn (II) is allowed by ZIP1
- Increased expression of metallothionein (MT) through metal regulatory transcription factor 1 (MTF-1)
- Signal transduction by mitogen-activated protein kinase (MAPK).
- Overexpression of the associated X protein Bax
- Bax pore formation in the outer mitochondrial membrane
- Efflux of cytochrome c into the cytoplasm
- Activation of caspases cascade that leads to apoptosis



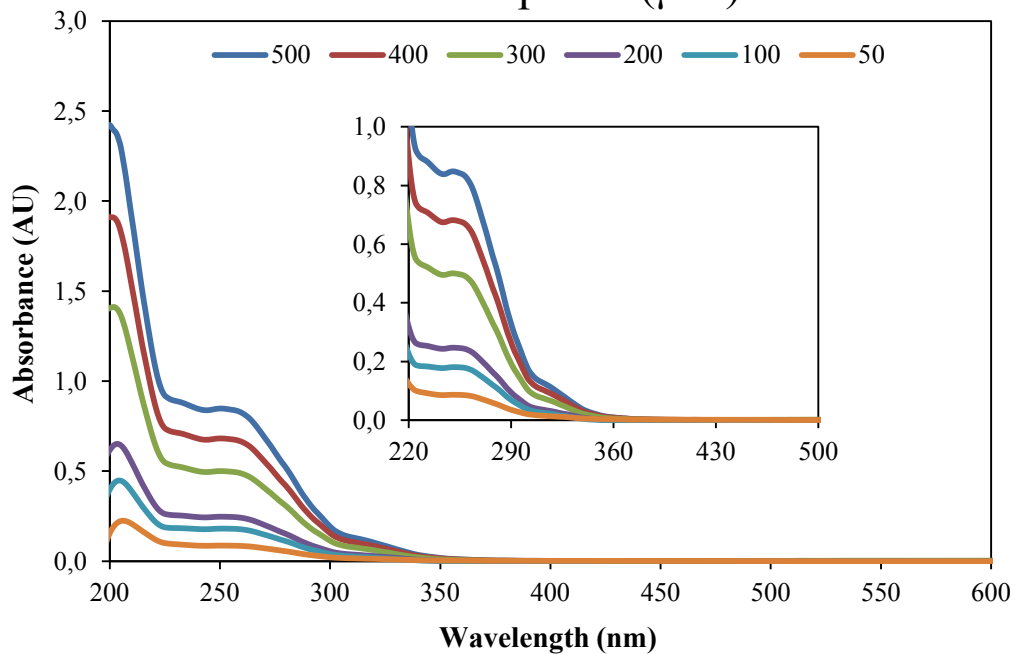
cisplatin ( $\mu\text{M}$ )



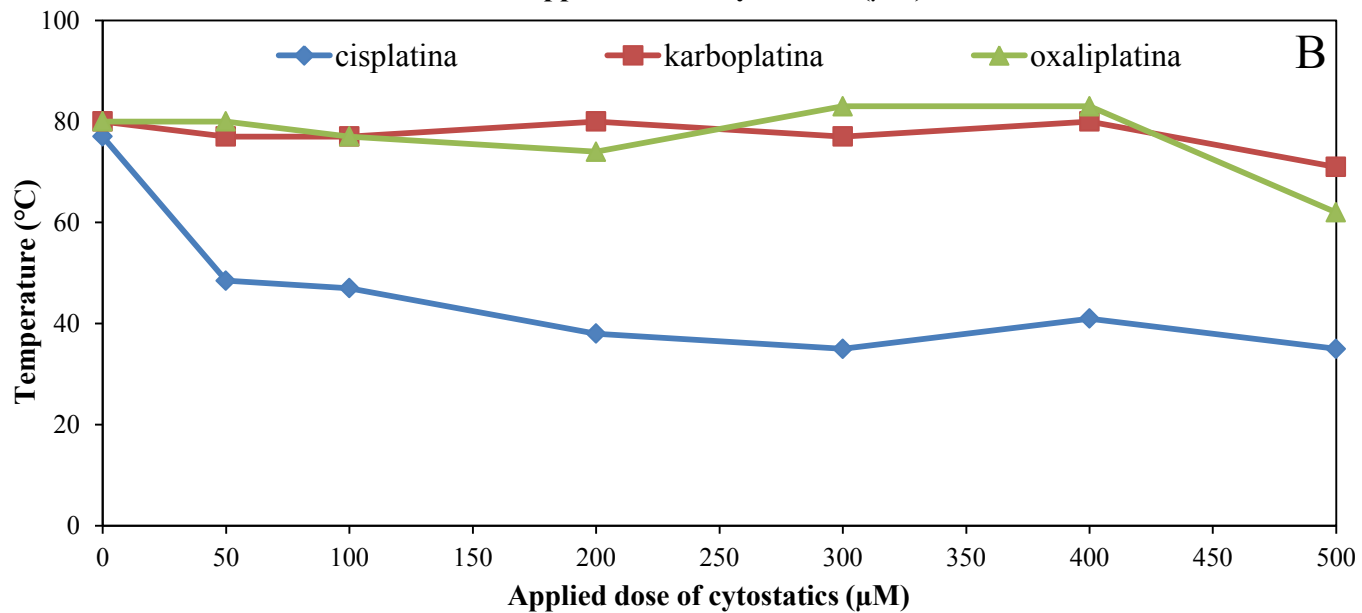
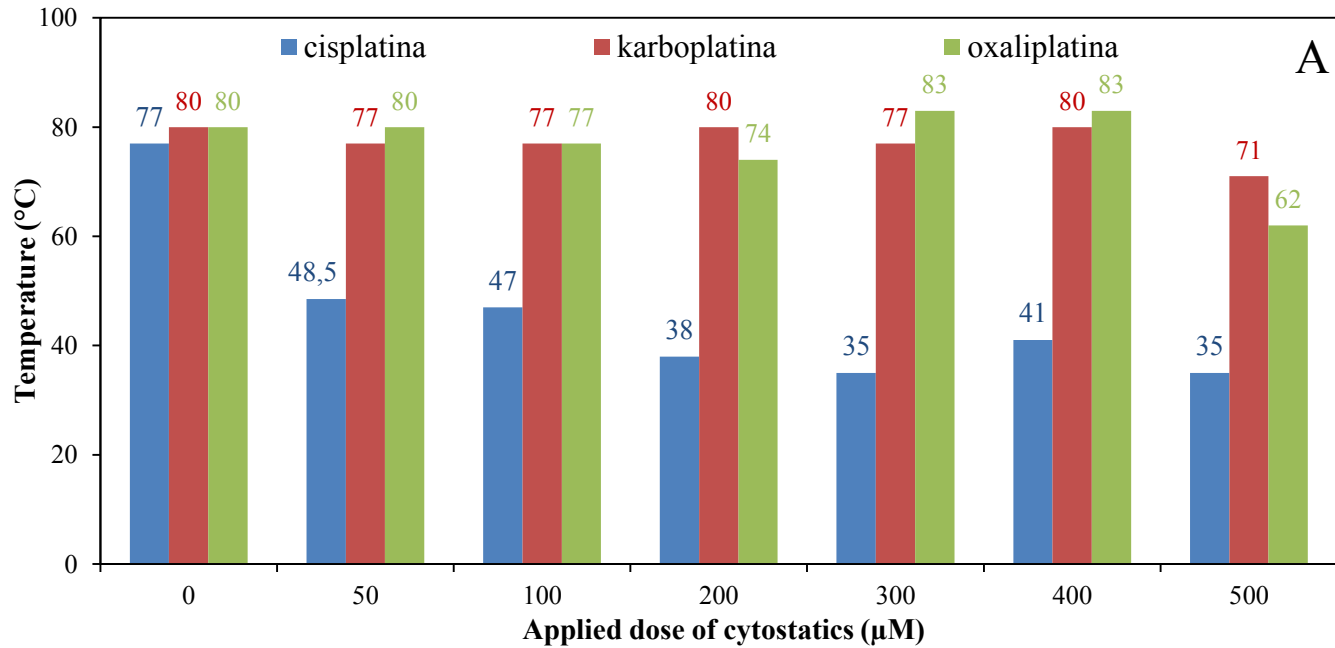
carboplatin ( $\mu\text{M}$ )



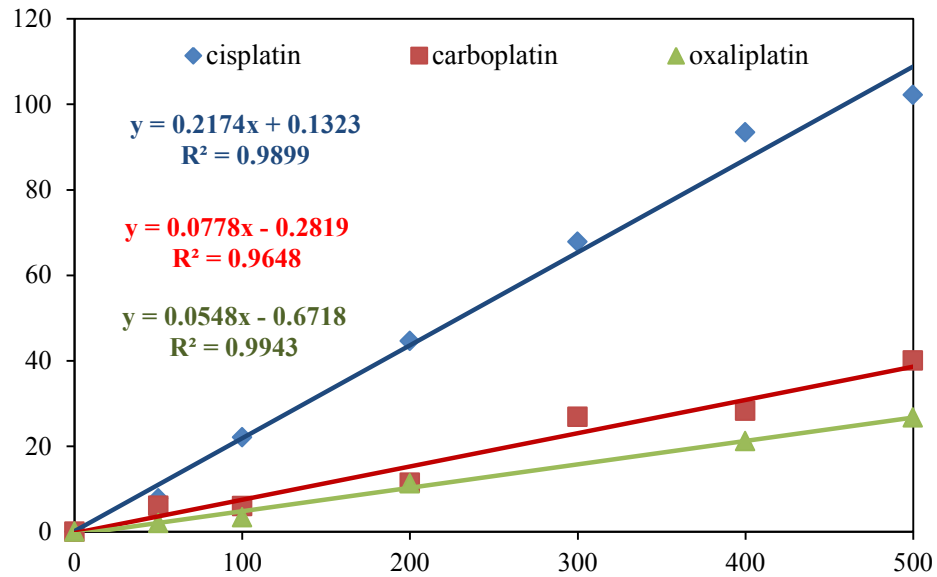
oxaliplatin ( $\mu\text{M}$ )



# Comparison of melting temperature for cisplatin, carboplatin and oxaliplatin



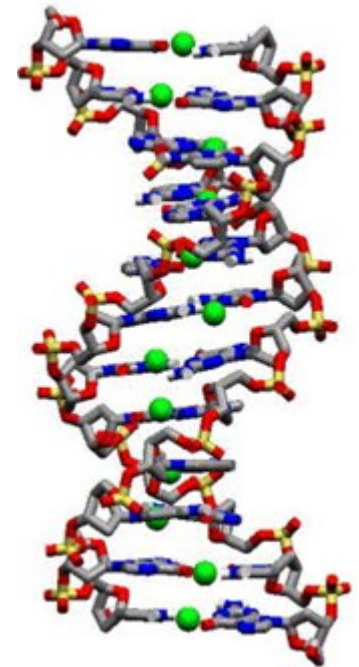
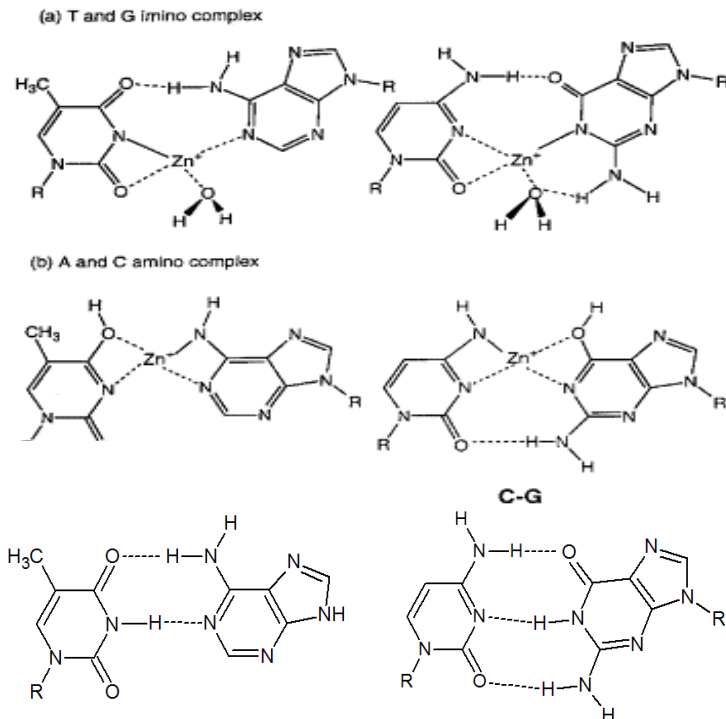
# Electrochemistry





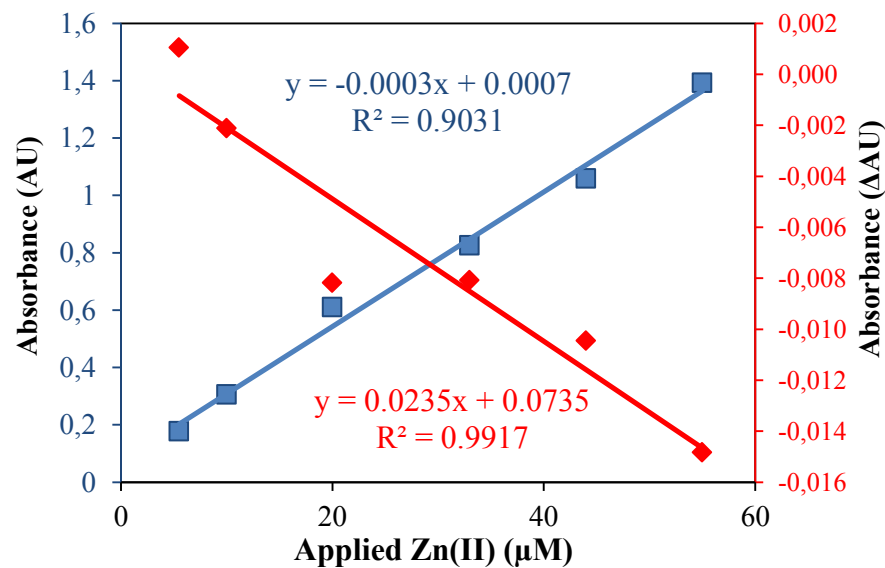
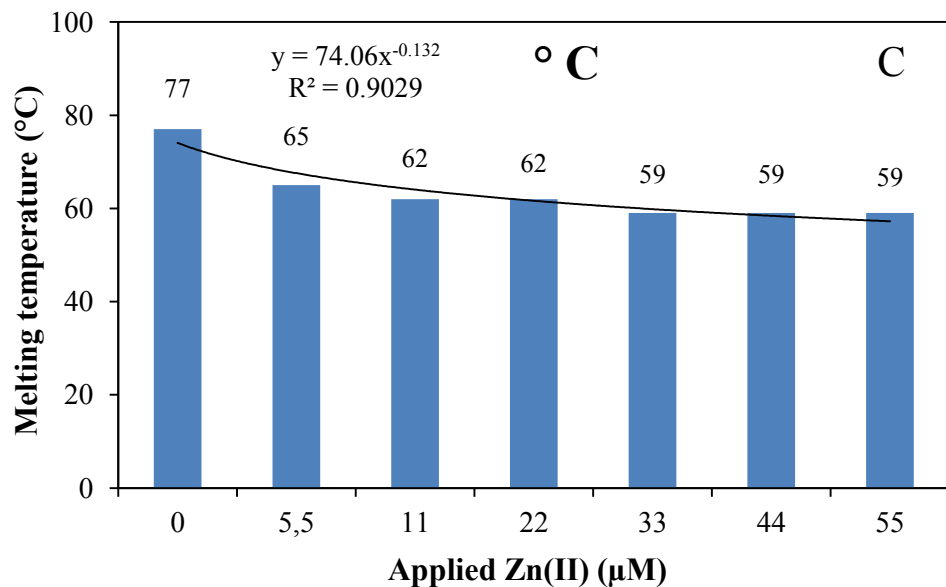
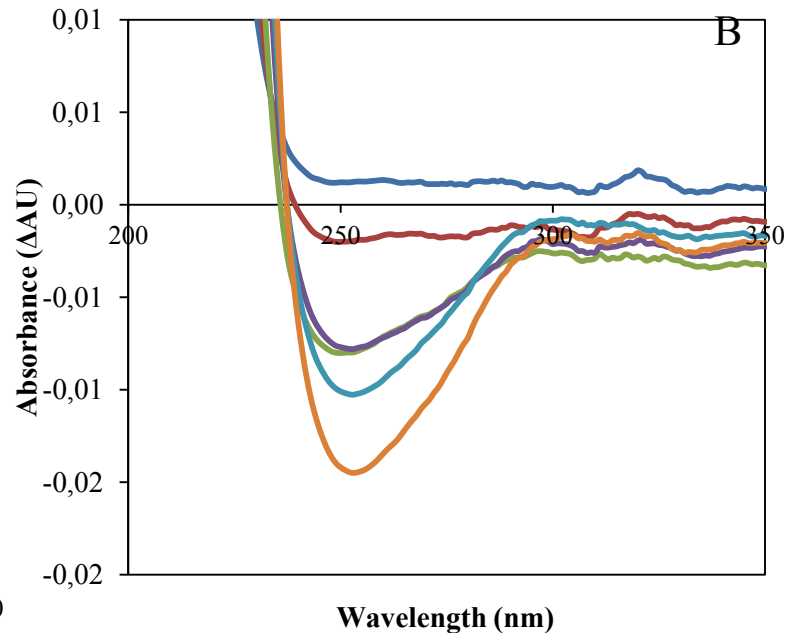
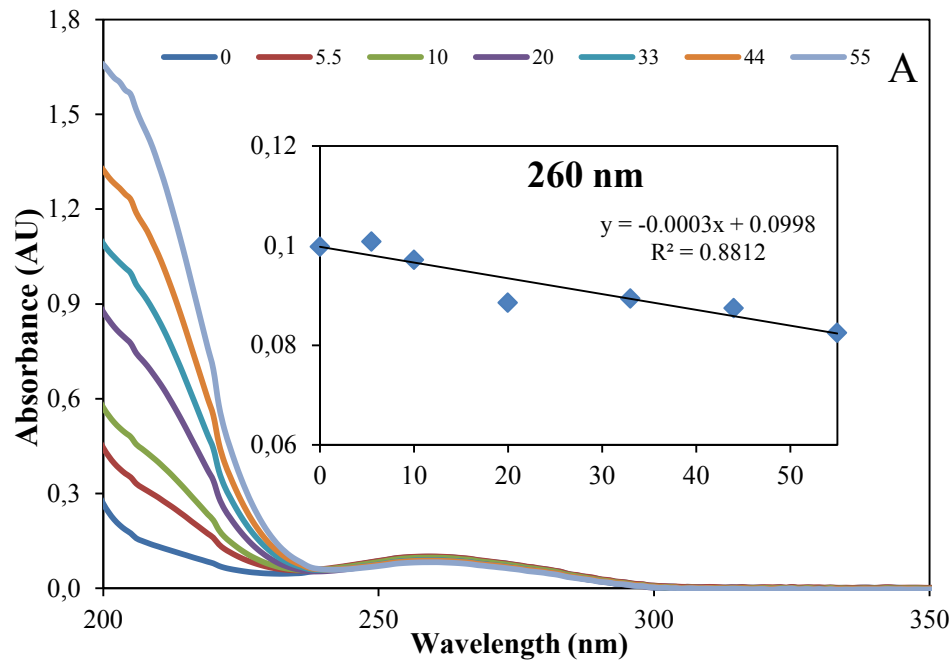
# DNA and zinc (II) interaction

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

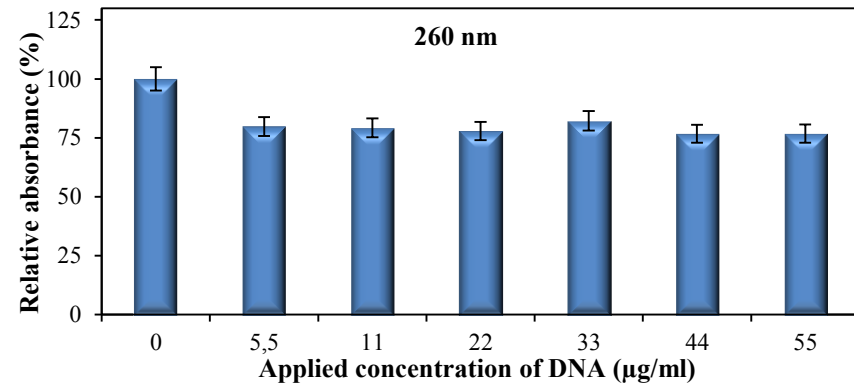
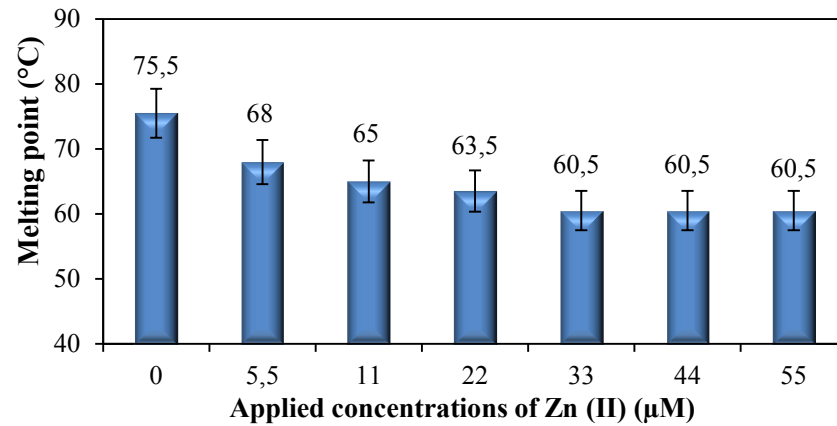


proposed structure of M-DNA

# Interaction of Zn(II) with DNA



# Changes in melting temperature



# Conclusion

- Basic biophysical interaction studies of cytostatics and Zn (II) with DNA was performed.
- The melting temperature is significantly influenced by the cisplatin, in contrast to the carboplatin and the oxaliplatin.
- There were stronger interactions between the cisplatin and DNA proved, in contrast to the carboplatine and the oxaliplatine.
- There were the interactions between DNA and Zn(II) proved (the change of absorption spectrum and the  $T_m$  during denaturation).
- There were a denaturation of DNA optimized (the record of the part of absorption spectrum).
- Thanks to the optimization of DNA denaturation we proved, that the  $T_m$  of DNA can be read also in other wave lengths than 260 nm.

# Acknowledgement



Reg.č.projektu: CZ.1.07/2.3.00/20.0148