INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Spectrophotometric analysis of oligonucleotides forming the G-quadruplexes

Datum : 6.6.2014

Školitel: Ing. Sylvie Skaličková, Ing. Branislav Ruttkay-Nedecký, Ph.D., Mgr. Marie Konečná, Ph.D.

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

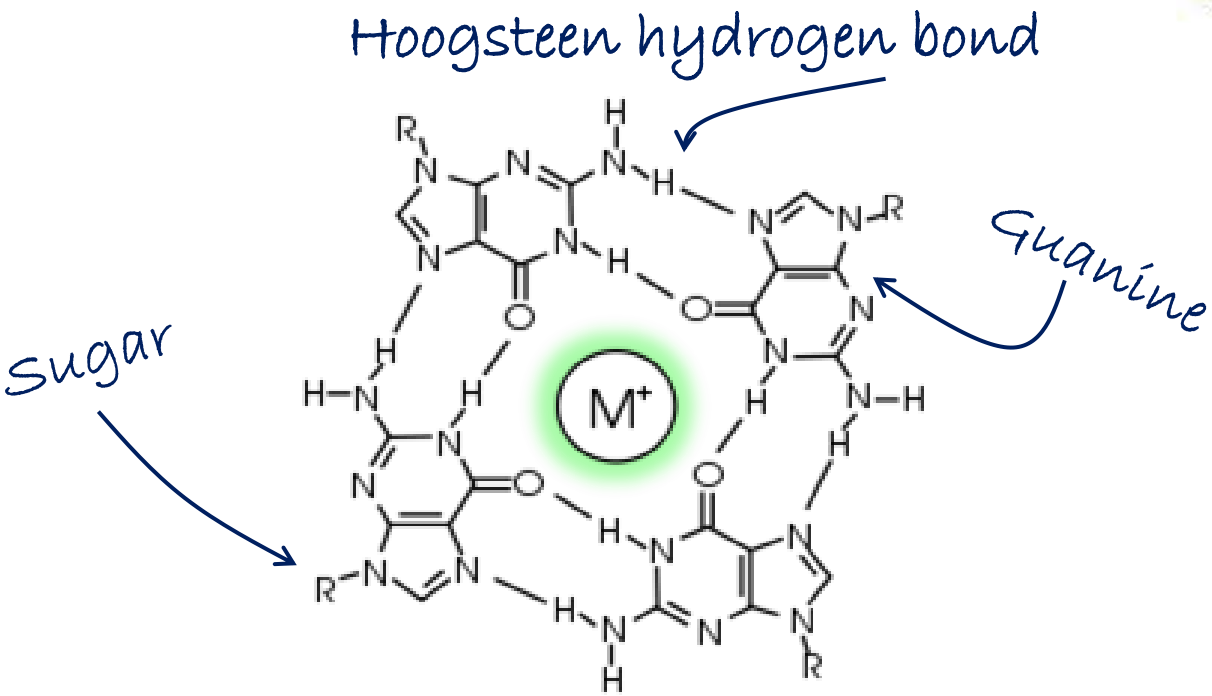
Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

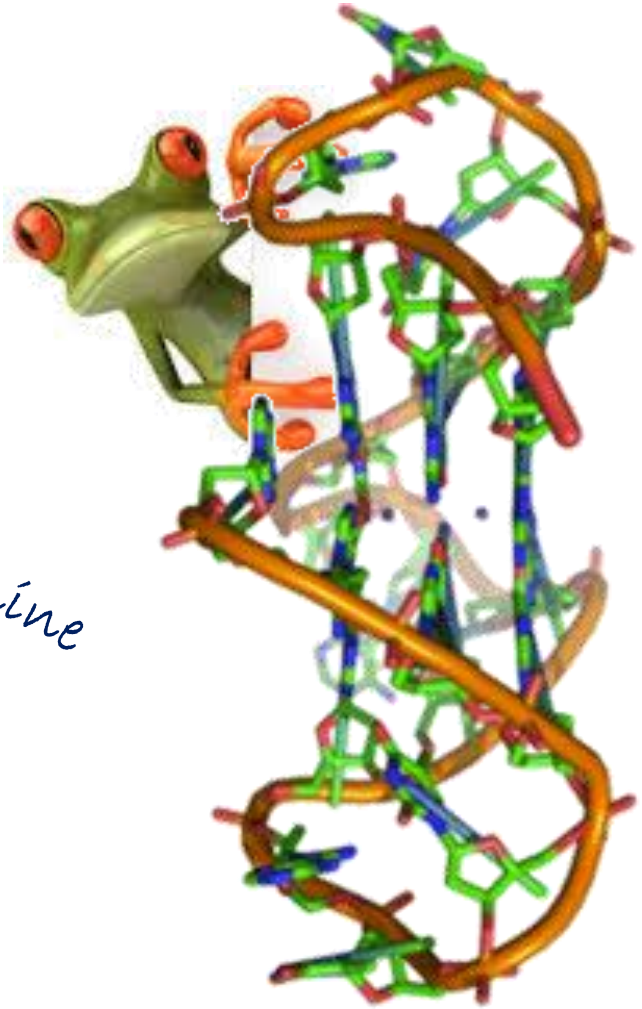
Content

Introduction → Applications → Principle of spectrophotometric analysis of G-quadruplex → **Results** → Conclusions

Introduction to G-quadruplexes

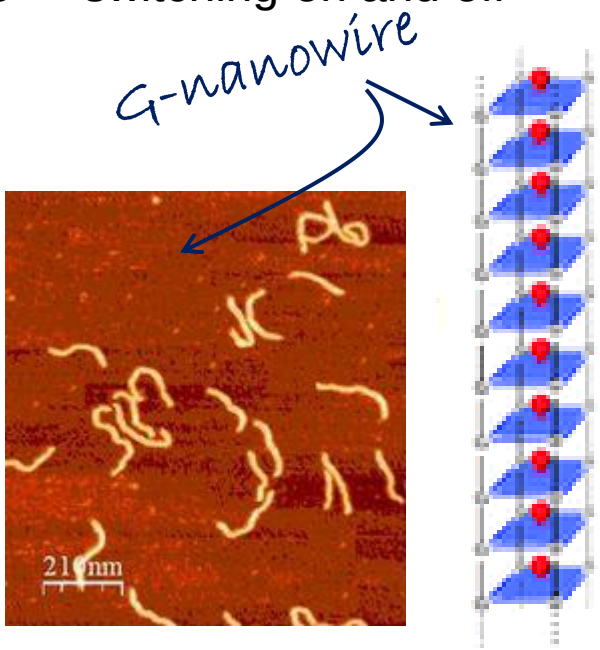
- Nucleic acid sequences that are rich in guanine and are capable of forming a four-stranded structure in presence of Alkali metals ions
- Highly stable structures (37 - 100 °C)
- Hoogsteen hydrogen bonding
  - Non-complementary bases pairing
- Occurence in telomers





Potential and applications

- Biosensor for elemental analysis
  - Ag, Hg, Ir, Cys, GSH, GSSG, CisPt, Dox …
- Lab-on-chip
  - Trombin detection
- Anticancer drug
  - inhibit the transcriptional activity of some oncogenes by Quadruplex formation at the end of telomers
  - target formation of G-quadruplexes in the genome -> switching on and off of genes
- Ionofor for selective element scavenging
  - radioactive $^{226}Ra^{2+}$
- Nanoelectronics
  - G-wires (0.1 μm) suitable for cation migration
- Analysis of G-quadruplex
  - Wide range of methods



Principle of spectrophotometric analysis of G-quadruplex

Spectrophotometric assay for G-quadruplex determination:

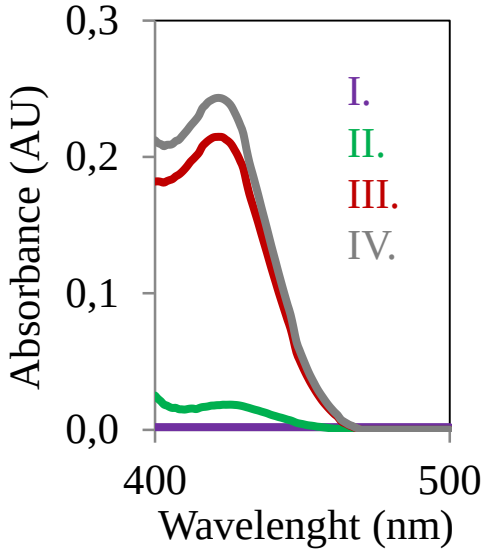
160 µl sample (ODN, KAc buffer,hemin)

20 µl ABTS

20 µl H_2O_2

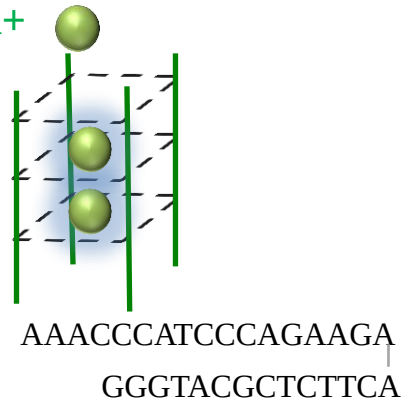
Absorbance scan 400 – 500 nm

Absorbance maximum: 422 nm

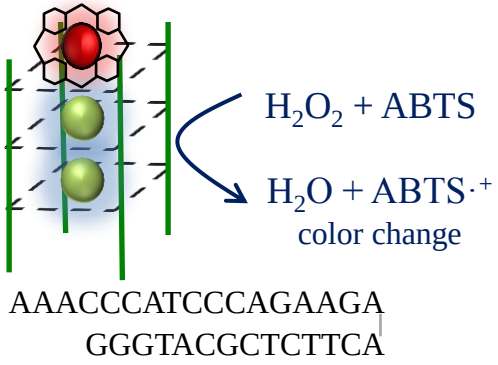


I. Guanine rich ODN sequence

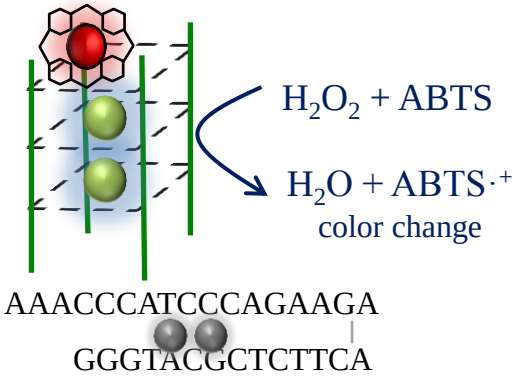
II. G-quadruplex formation in presence K+



III. DNAzyme activity of G-quadruplex in presence of hemin

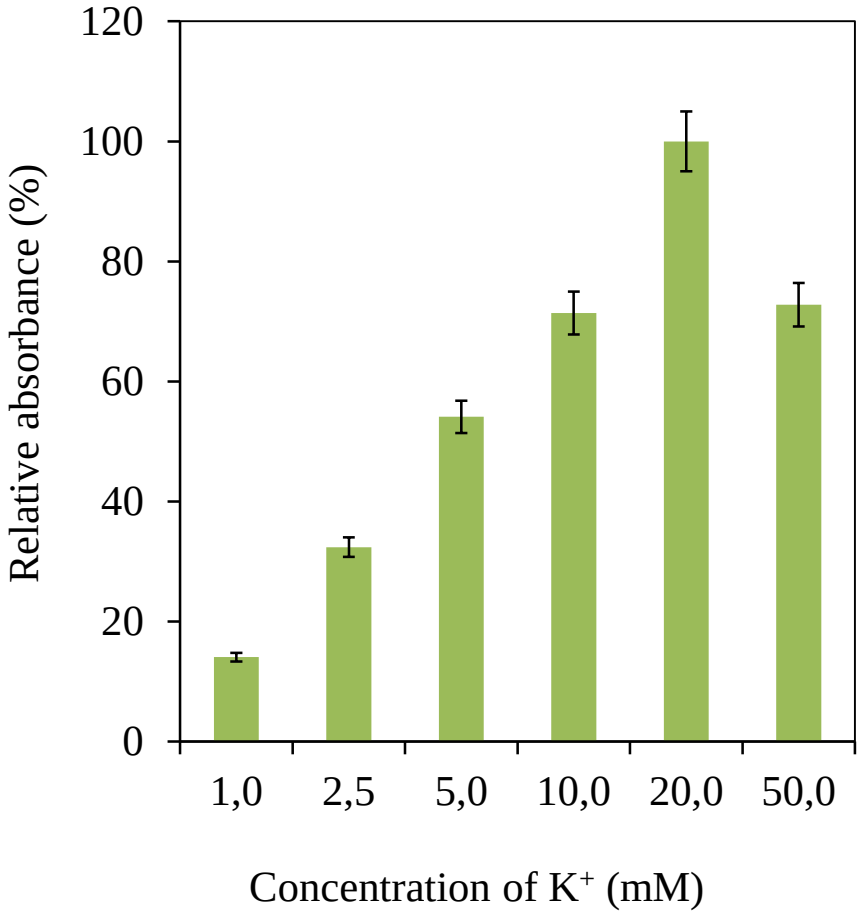


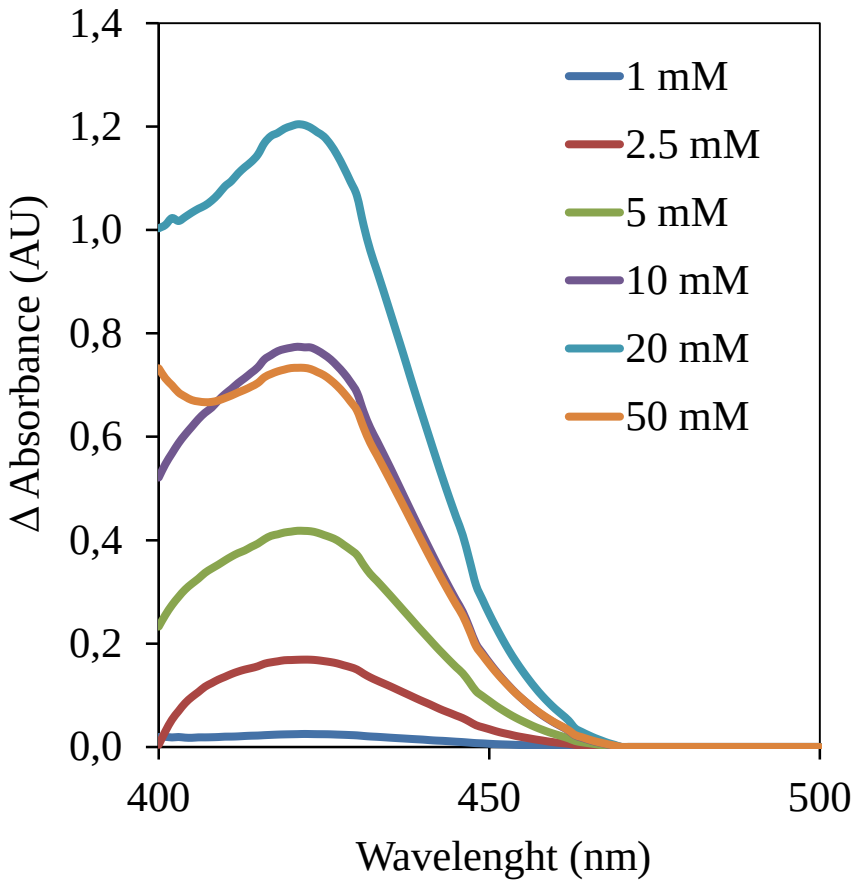
IV. Stabilization of G-quadruplex by Ag+



Results

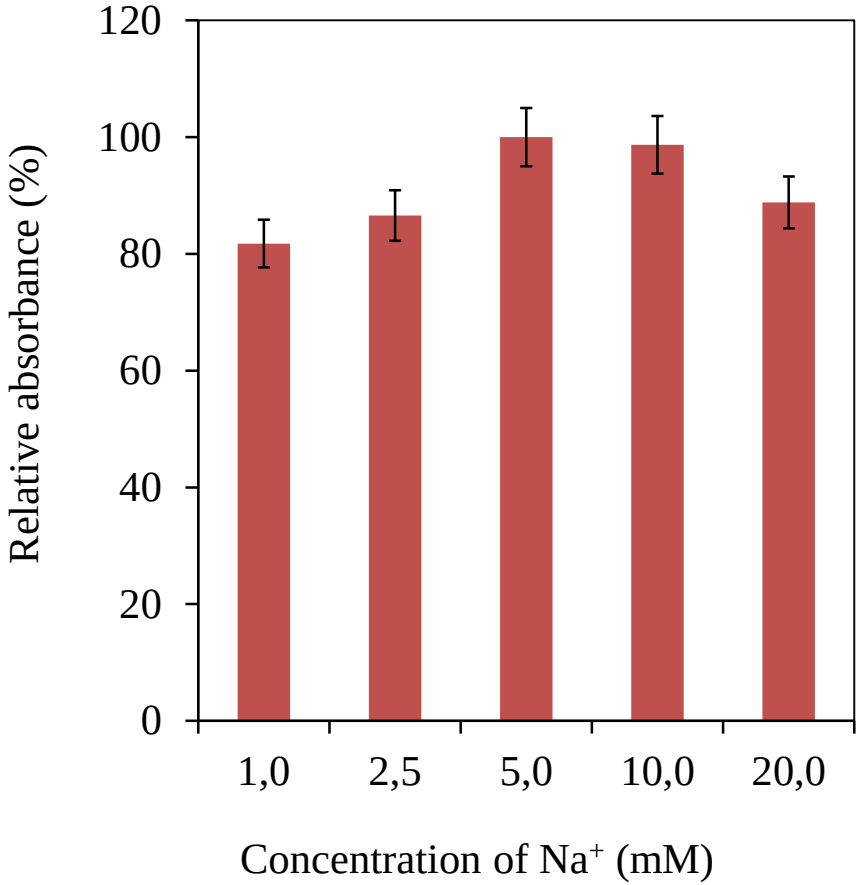
- Influence of K^+ ions to G-quadruplex formation
- Spectra are expressed as Δ Absorbance (Absorbance of ABTS alone was deducted

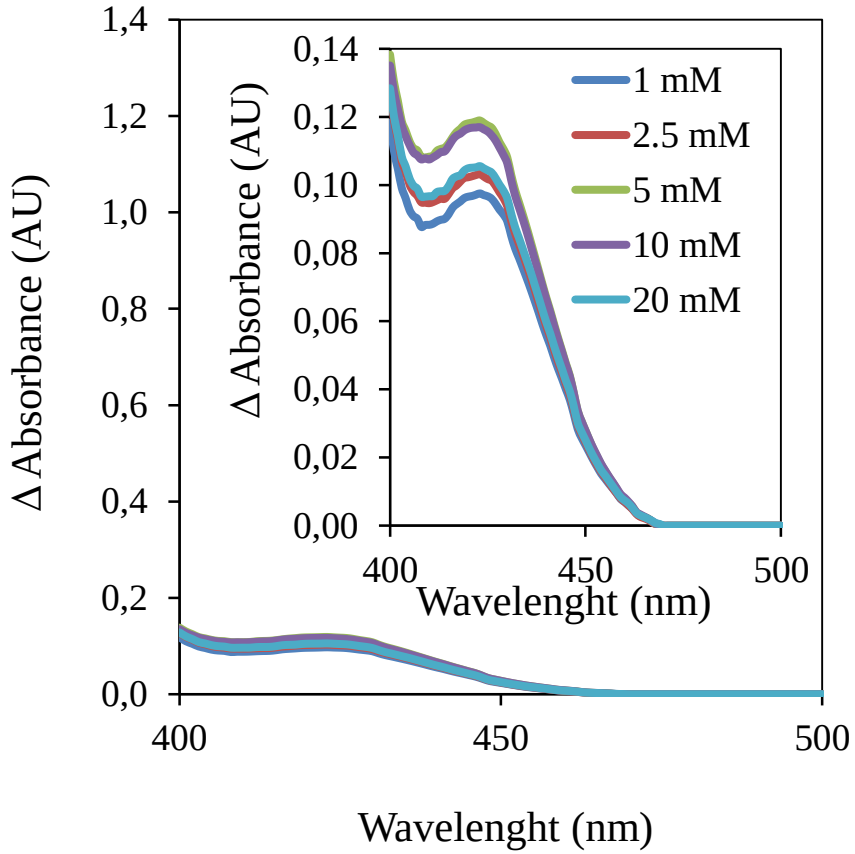




Results

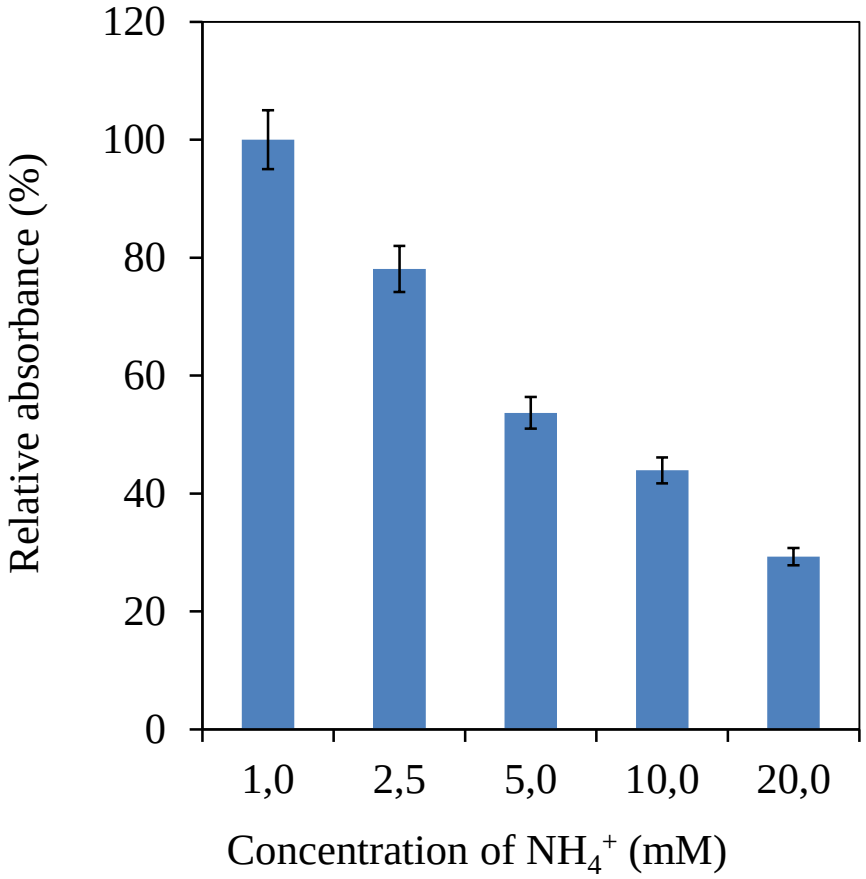
- Influence of Na^+ ions to G-quadruplex formation
- Spectra are expressed as Δ Absorbance (Absorbance of ABTS alone was deducted

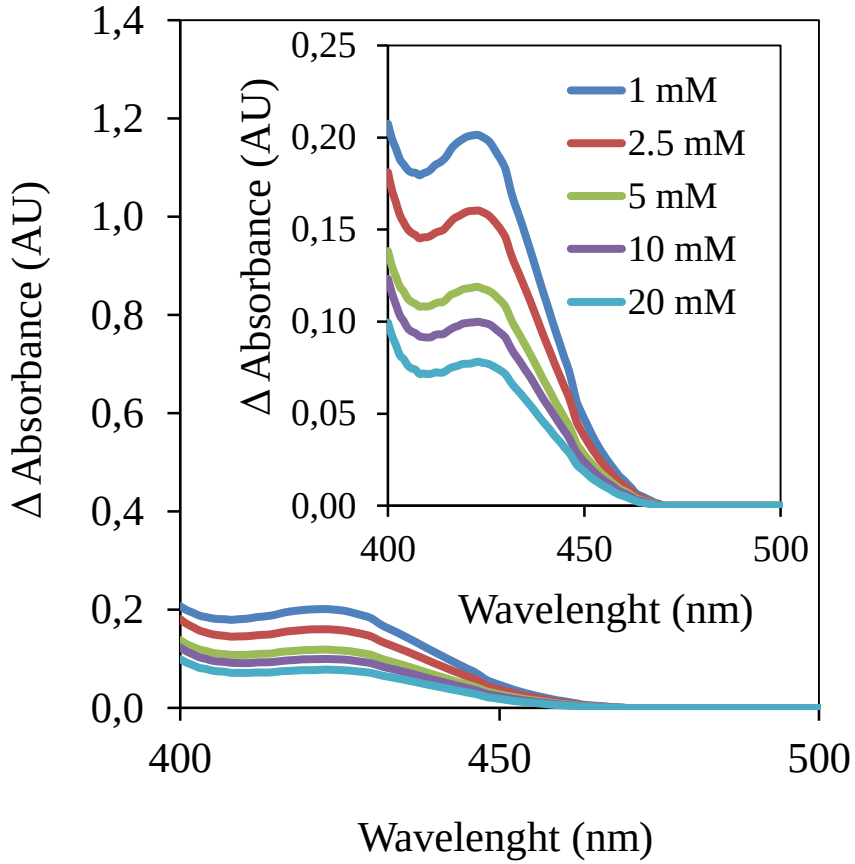




Results

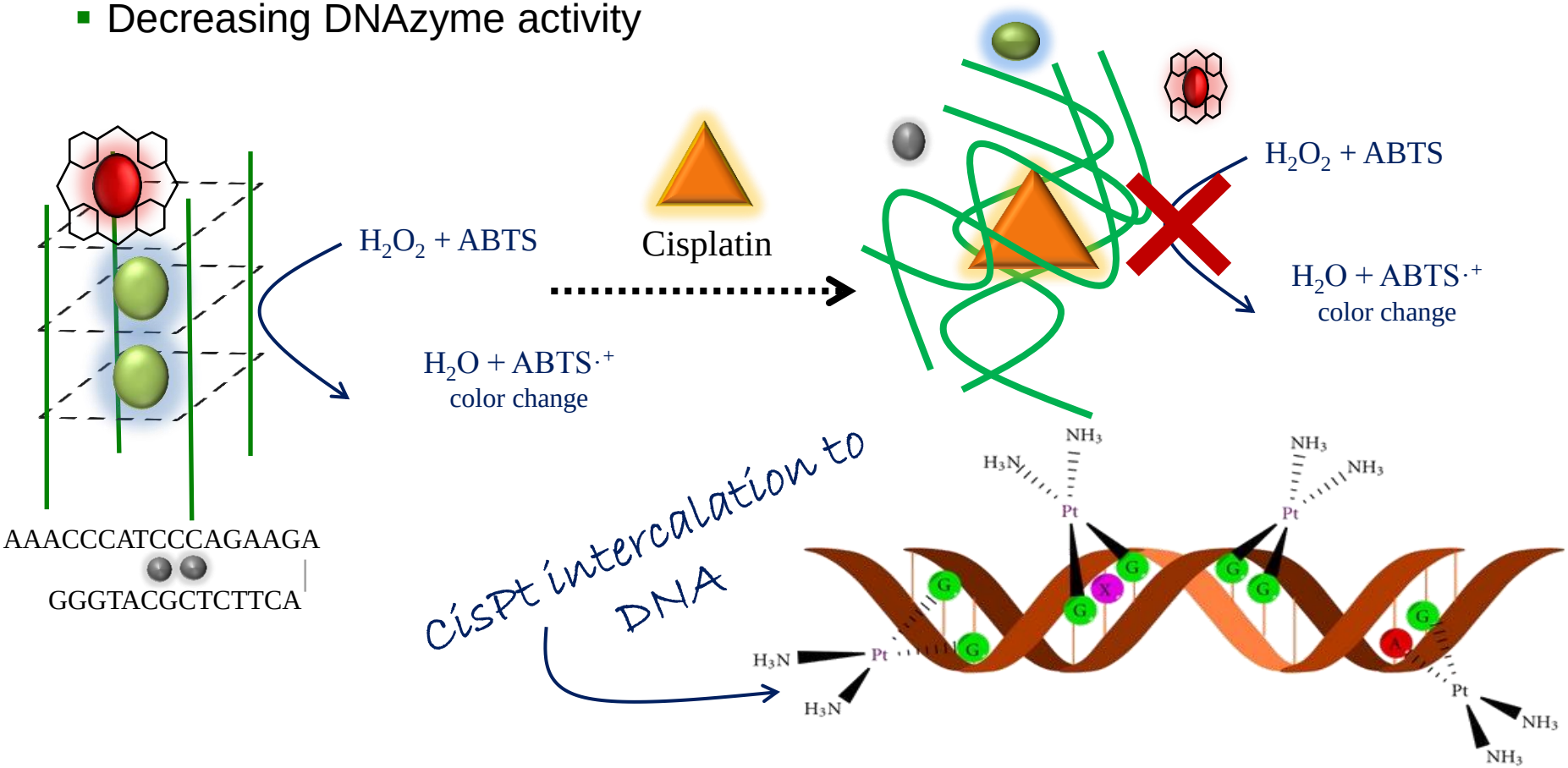
- Influence of NH_4^+ ions to G-quadruplex formation
- Spectra are expressed as Δ Absorbance (Absorbance of ABTS alone was deducted





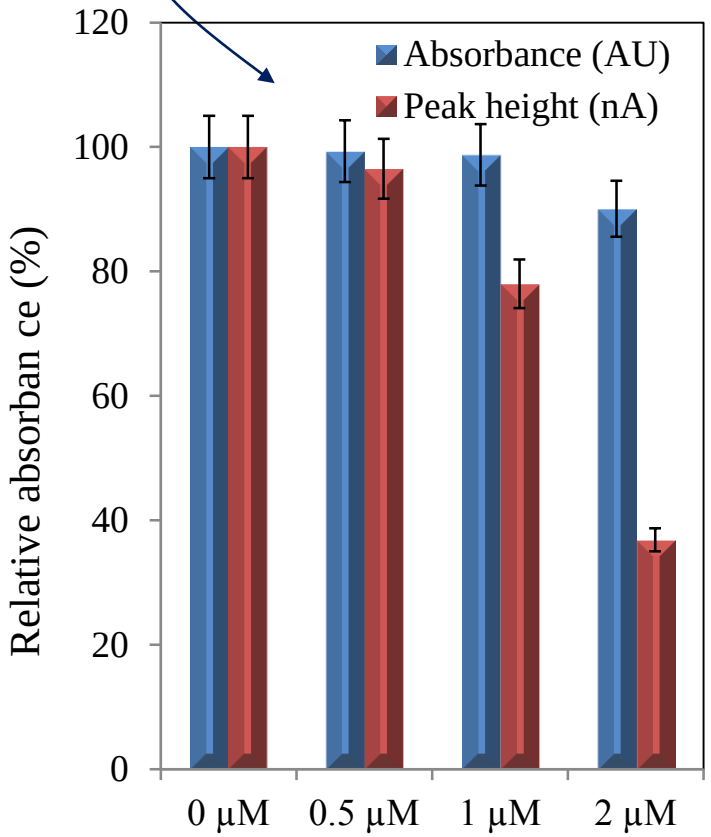
Results

- Analytical instrument for CisPt detection (< nM concentrations)
  - electrochemical based detection (Wang, 2013; Biosensors & Bioelectronics)
  - detection using spectrophotometry (Nedecky, 2014 -> working on)
- CisPt primally binds on guanine -> change of structure conformation
- Decreasing DNAzyme activity

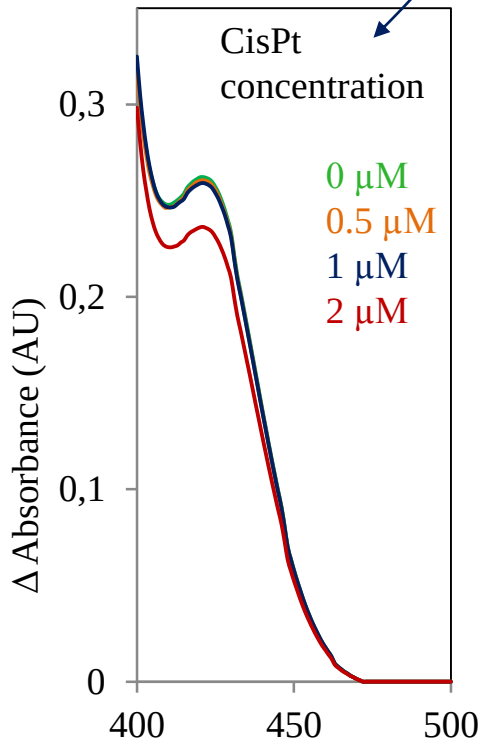


Results

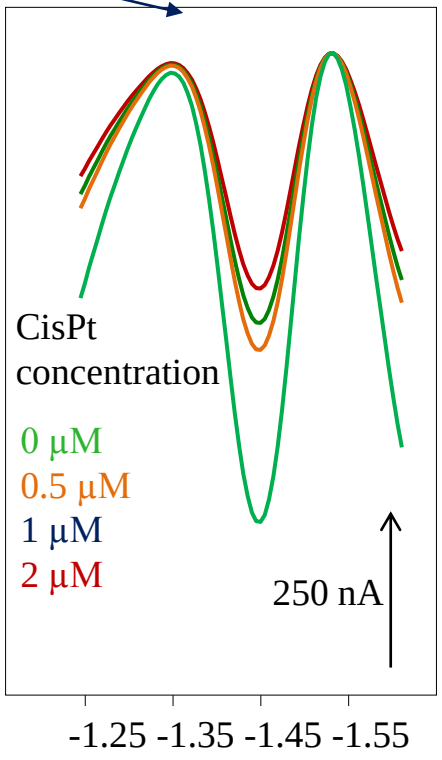
Influence of CisPt concentration (0 - 2 μM) to G-quadruplex formation and corelation analysis of spectrophotometric and electrochemical measurement´s data



Absorbance spectra of G-quadruplex interaction with CisPt



Electrochemical signal of G-quadruplex intraction with CisPt

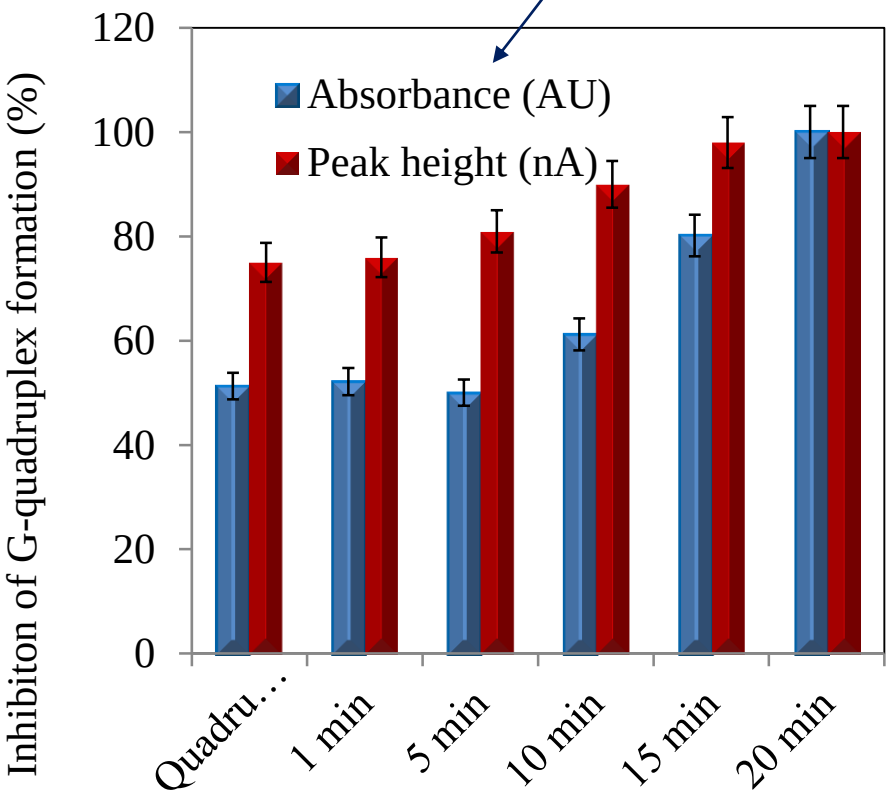


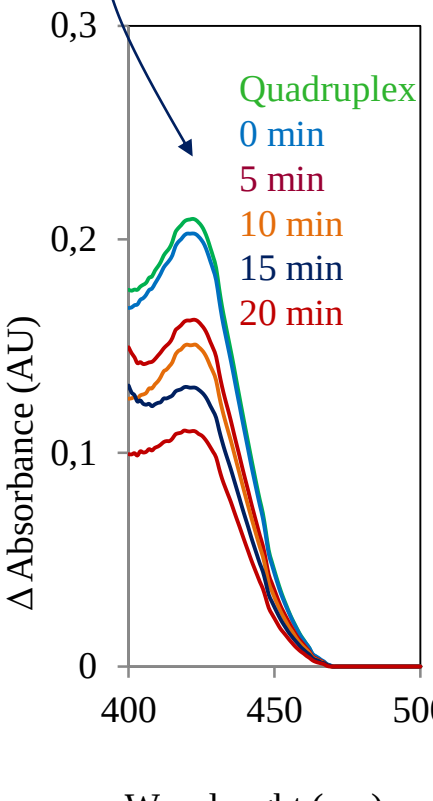
Results

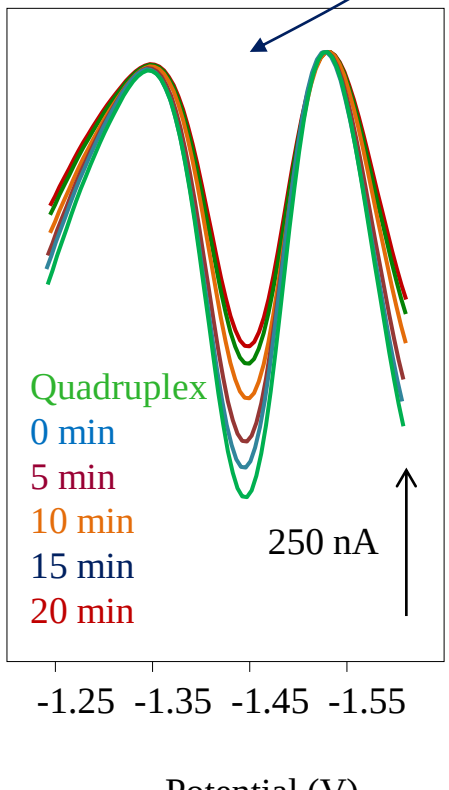
Time dependence (0-20 min) of G-quadruplex interaction with 0.5 mM CisPt

Absorbance spectra of G-quadruplex interaction with CisPt (0-20 min interaction time)

Electrochemical signal of G-quadruplex interaction with CisPt (0-20 min interaction time)







Conclusion

- Wide range of applications
  - Medicine
  - Element analysis
  - Nanoelectronics

- Suitable for spectrophotometric analysis
  - Strong effect of K^+ ions for G-quadruplex formation

- G-quadruplex is useful for CisPt detection by spectrophotometric measurement
  - Strongest interaction after 20 min

Acknowledgement

Financial support from NanoBioTECell P102/11/1068 and Nanolabsys CZ.1.07/2.3.00/20.0148

- **Branislav Ruttkay-Nedecký**
- **Monika Kremplová**
- **Spectrophotometric and Electrochemic laboratory**

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

Thank you for your attention