

Název:

G-quadruplexes for detection of silver ions observed spectrophotometrically

Školitel:

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

Datum:

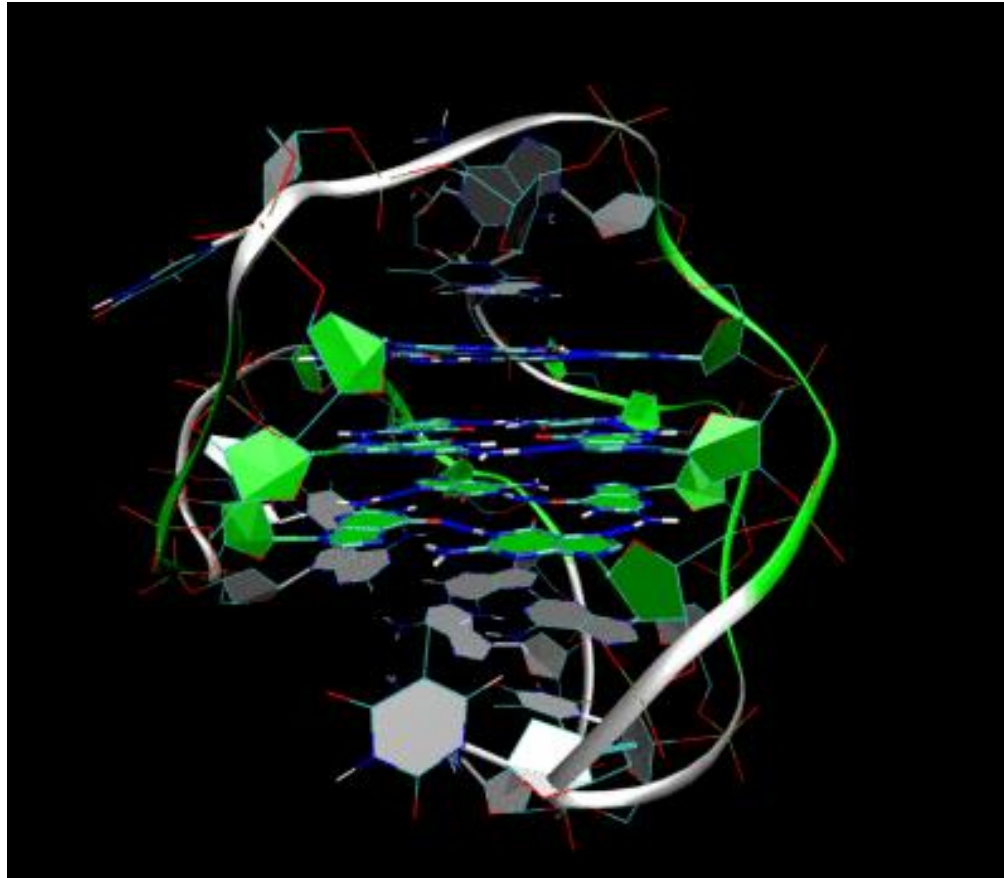
13.6.2014

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

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



G-quadruplexes

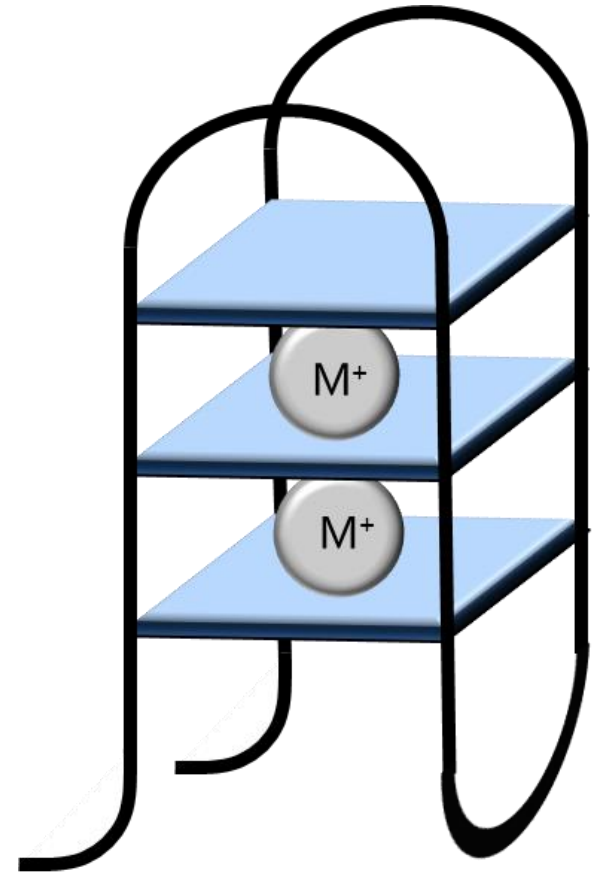
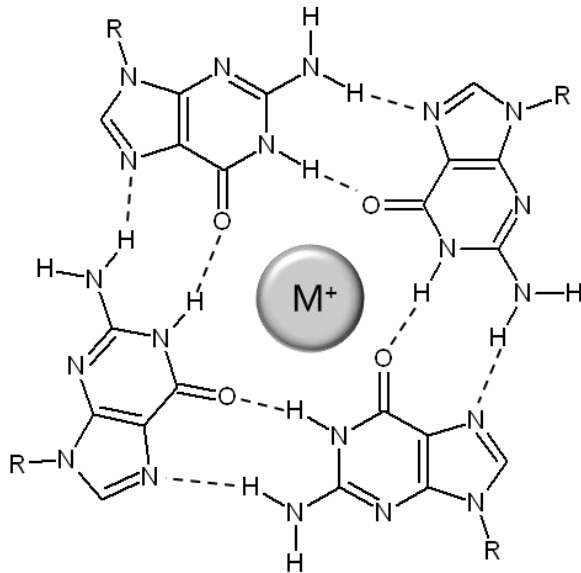


3D Structure of the intramolecular human telomeric G-quadruplex in potassium solution (PDB ID 2HY9). The backbone is represented by a tube. The center of this structure contains three layers of G-tetrads. The hydrogen bonds in these layers are represented by blue dashed lines

<http://upload.wikimedia.org/wikipedia/commons/a/aa/Telomer-structure.gif>

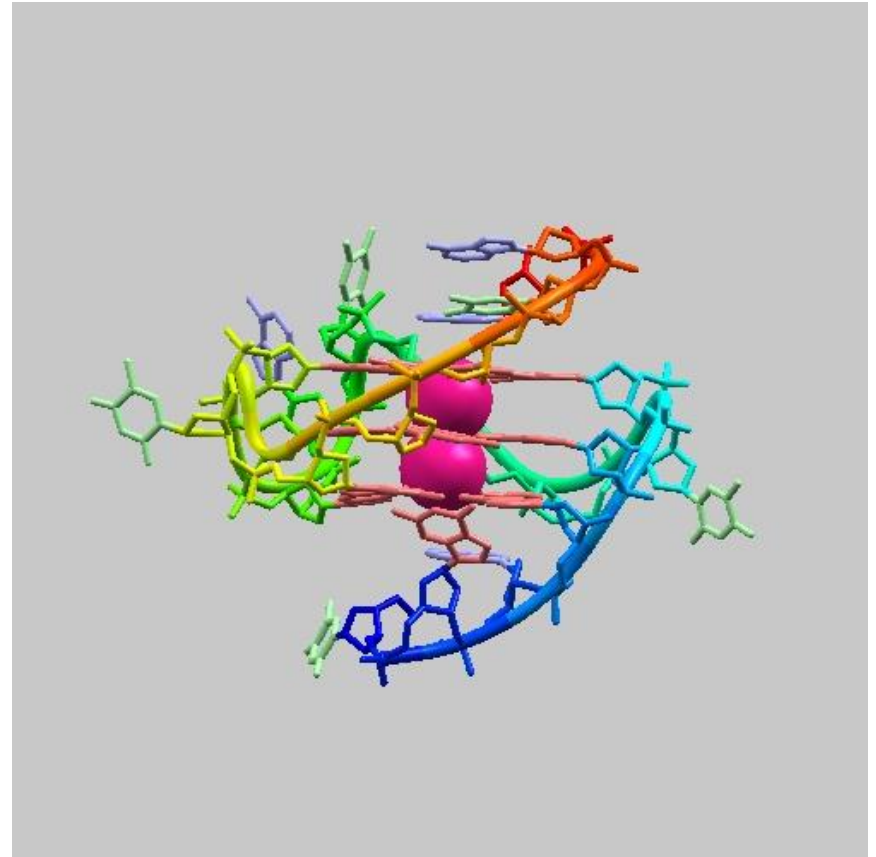
G-quadruplexes

- Composed of G-quartets which are formed from four guanine bases
- Each guanine is linked with neighboring guanine via two hydrogen bonds by Hoogsteen pairing
- These structures (G-quartets) then stack on each other forming a G-quadruplex structure
- G-quadruplexes are stabilized also with alkali metal ions (K^+ , Na^+) located between two G-quartets



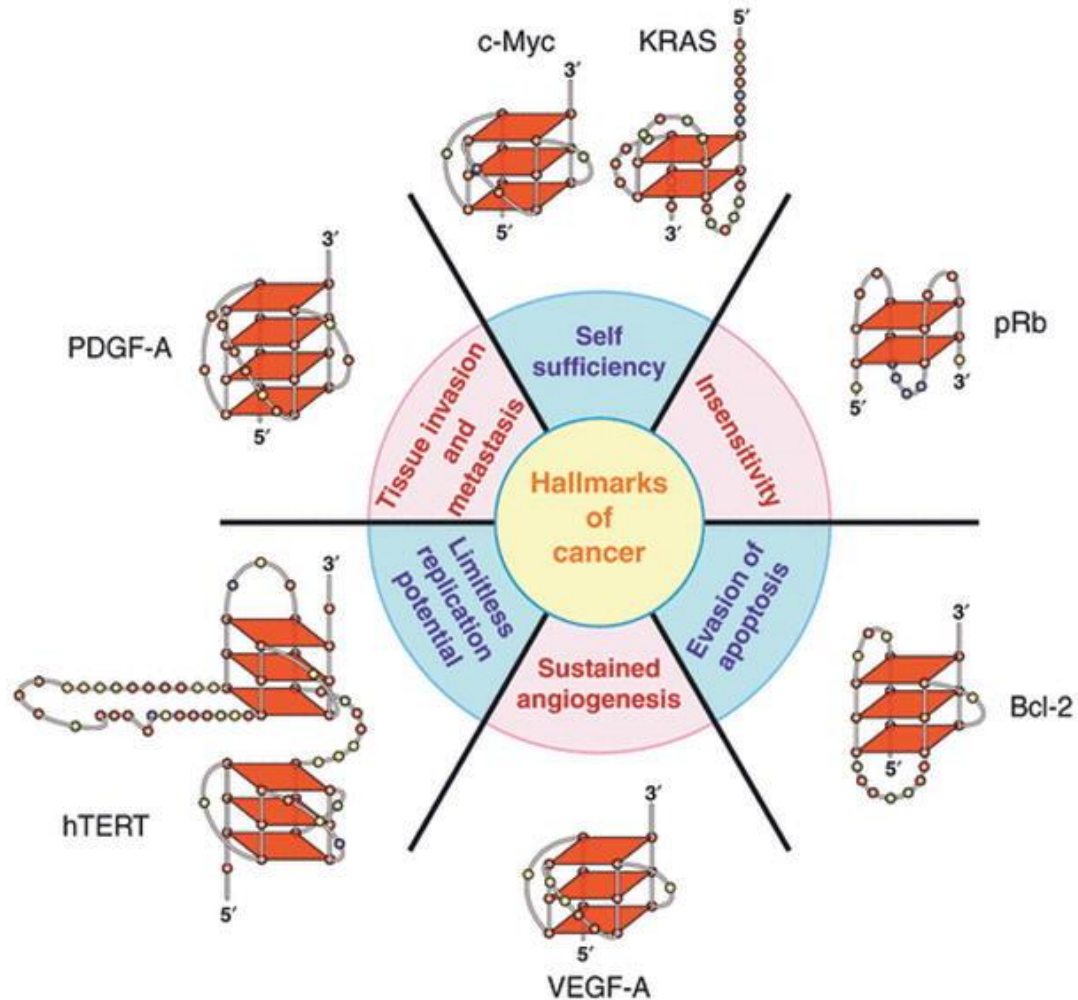
G-quadruplexes in oncogene promoters

- Changes in the expression of oncogenes are a characteristic hallmark of tumor growth.
- Promoter regions of certain oncogenes tend to form G-quadruplexes
- Promoter sequences of oncogenes consist of different number of guanines and intervention bases.
- These sequences are for each promoter region unique and capable of forming a variety of different quadruplex structures



Source: <http://pdj.org/mine/summary/1xav>

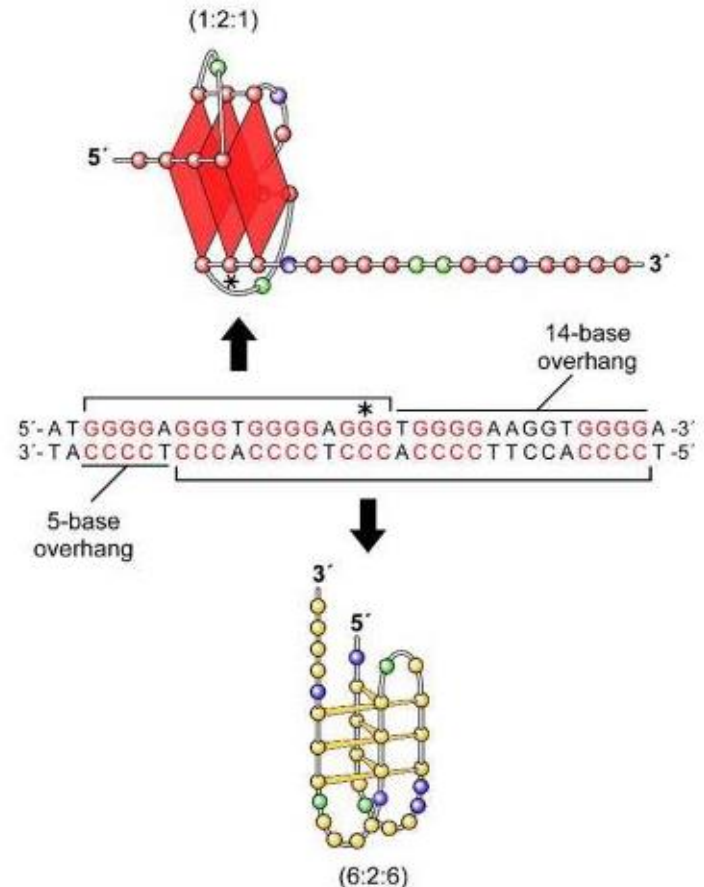
G-quadruplexes and hallmarks of cancer



The six hallmarks of cancer shown with the associated G-quadruplexes found in the promoter regions of these genes. As described in the text, the various G-quadruplexes differ by folding pattern, number of tetrads, loop size and constituent bases. In this a subsequent models, bases are colored as follows: guanine, red; cytosine, yellow; thymine, blue; adenine, green.

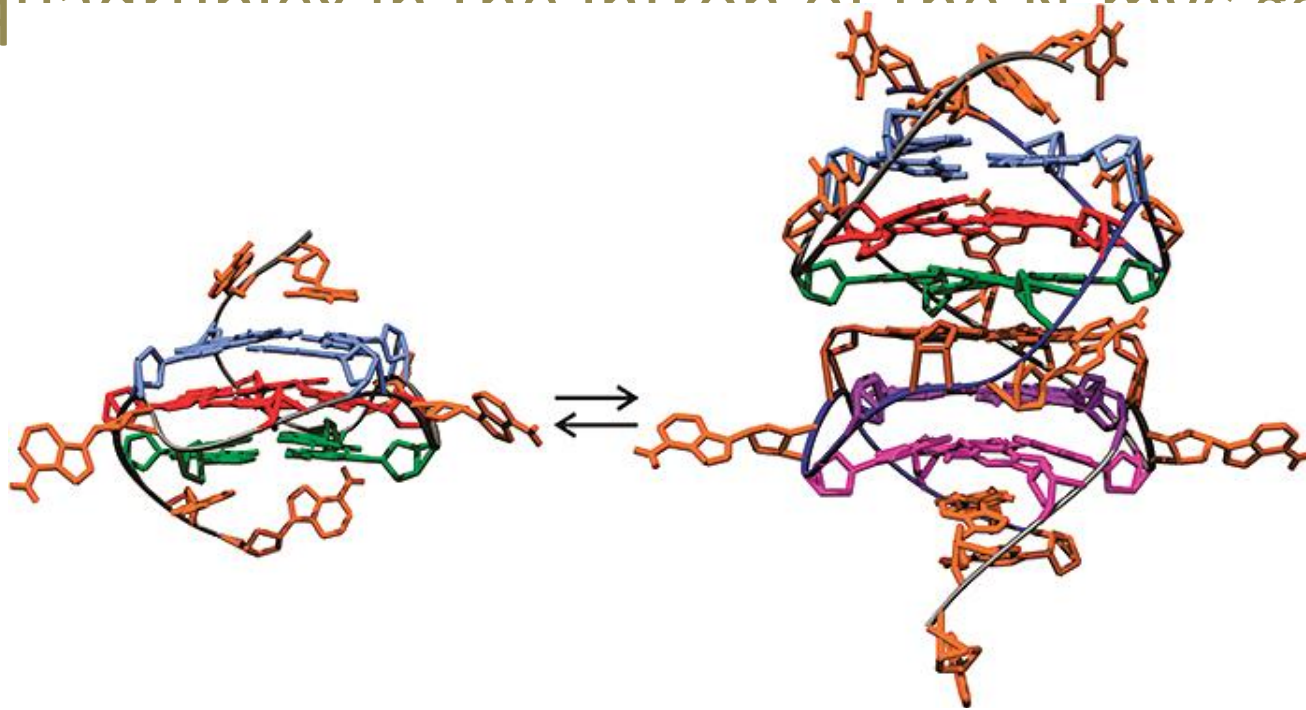
Promoter of the c-myc gene

- The most studied promoter belongs to the family of protooncogenes Myc
- In healthy cells there is regulated expression of c-myc gene. In tumor cells there is unregulated expression of c-myc gene
- C-myc gene is expressed in cervical cancer, prostate cancer, osteosarcoma, glioblastoma, lymphoma and myeloid leukemia
- The most important area of the c-myc gene is NHE III1 region.
- This region controls of 90 % of c-myc transcription
- In this region the formation of G-quadruplex and i-tetraplex was proven



Source: Sun and Harley, 2009

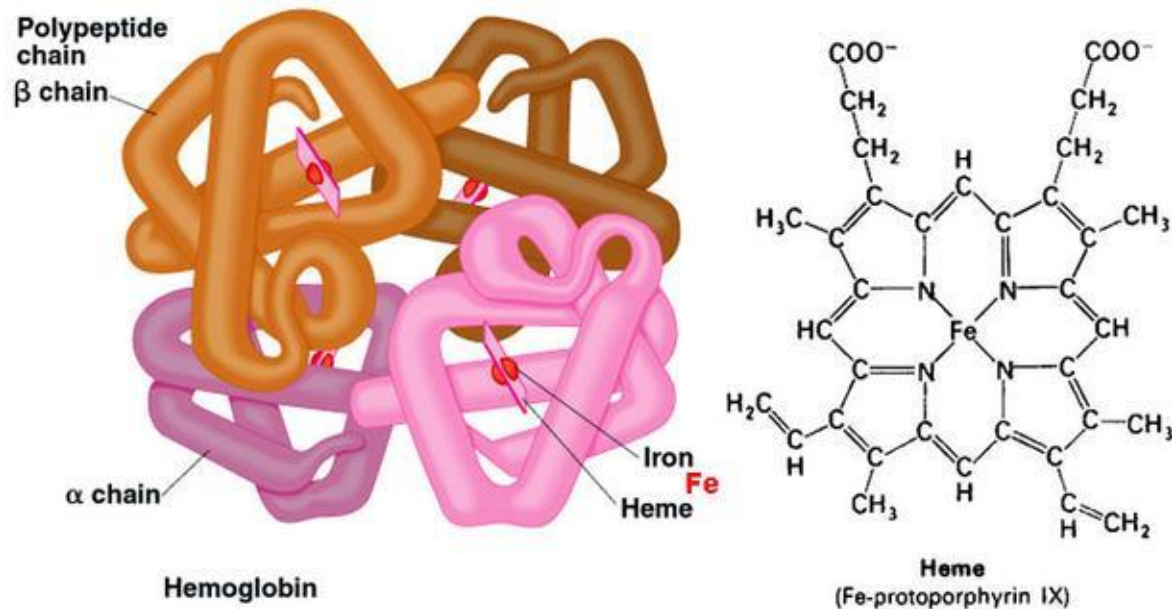
G-quadruplex in the intron of the N-myc gene



- Expression of N-myc gene is restricted to specific tissues during their development
- Its deregulation is associated with neuronal tumors
- There is no G-quadruplex in the promoter region of this gene
- But there has been found G-quadruplex sequence in the first intron of this gene
- An increase in K^+ ion and ODN concentration, changed of monomeric G-quadruplex into a dimeric form
- Dimeric form exhibits six stacked G-quartets and parallel strand orientation

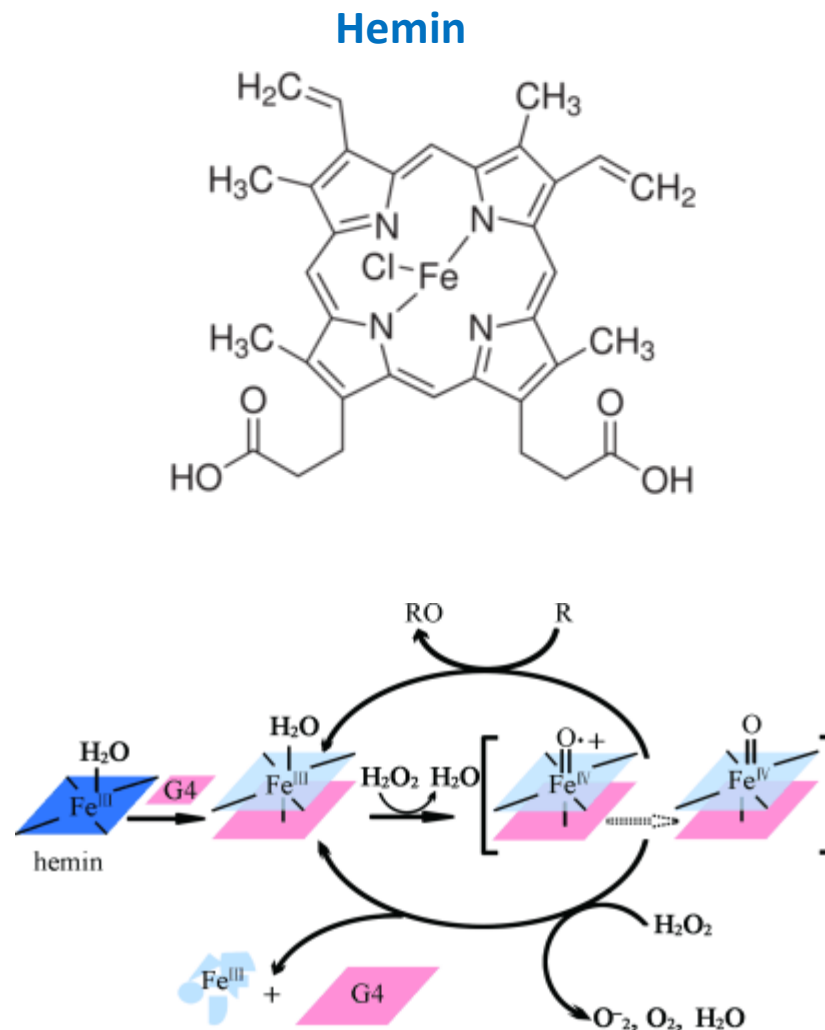
Heme

- Heme is a chemical compound of a type known as a prosthetic group (cofactor of hemoglobin) consisting of an Fe^{2+} (ferrous) ion contained in the centre of a large heterocyclic organic ring called a porphyrin, made up of four pyrrolic groups joined together by methine bridges. Hemes are most commonly recognized as components of hemoglobin, but are also found in a number of other biologically important hemoproteins such as myoglobin, cytochrome, and catalase. Hemoglobin is the protein found in RBC that is responsible for carrying oxygen. It contains iron, which is what the oxygen binds to. Iron can also bind to carbon dioxide, but it is a weaker bond than with oxygen.



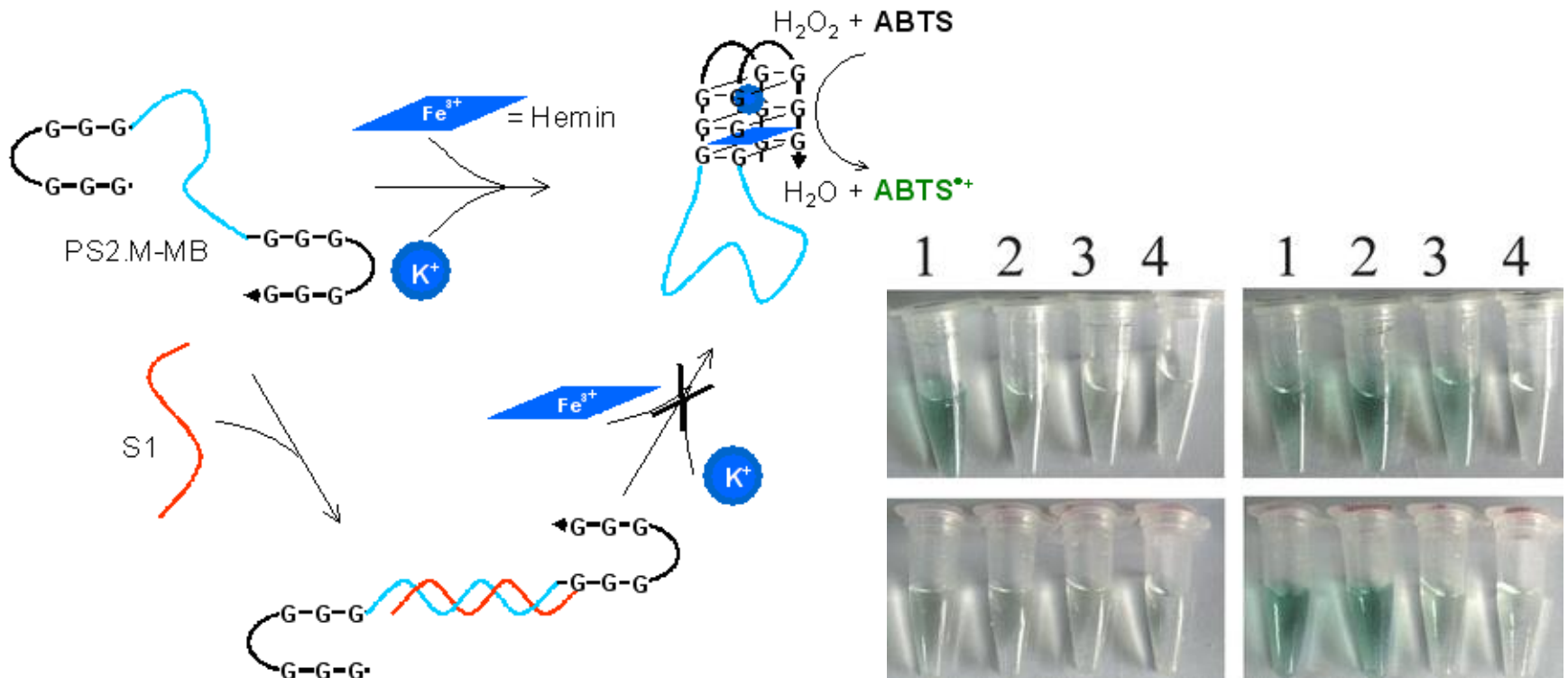
Metalloporphyrins and G-quadruplex

- G-quadruplex (G4) in complex with anionic porphyrines hemin,
- *In vitro* selection previously identified short single stranded DNAs (G-quadruplexes) that specifically bound N-methylmesoporphyrin IX (NMM), a stable transition-state analogue for porphyrine-metallation reactions.
- Interestingly, iron (III) protoporphyrin (hemin) was a good competitive inhibitor for the DNA catalyzed metallation reaction
- G4/hemin, peroxidase like activity, DNAzymes



ABTS reaction

- G-quadruplex (G4) in complex with anionic porphyrines hemin,
- Catalyze the oxidation of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) diammonium salt) by H_2O_2
- The result – color change of the solution



Source: <http://onlinelibrary.wiley.com/doi/10.1002/cjoc.201200360/pdf>;
<http://pubs.rsc.org/en/content/articlelanding/2010/an/b920293e/unauth#!divAbstract>

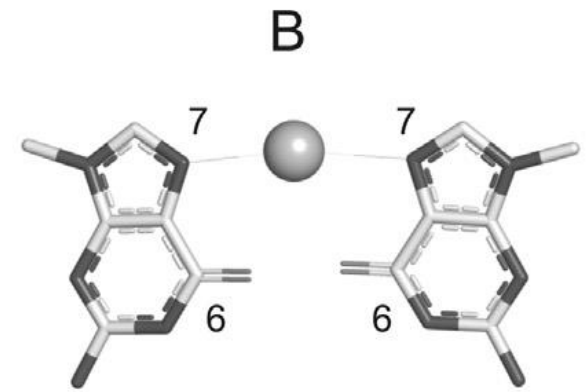
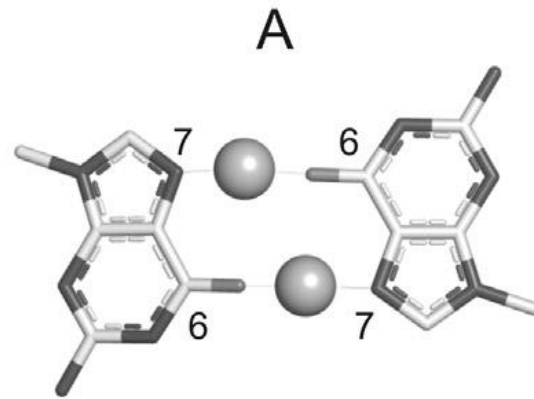
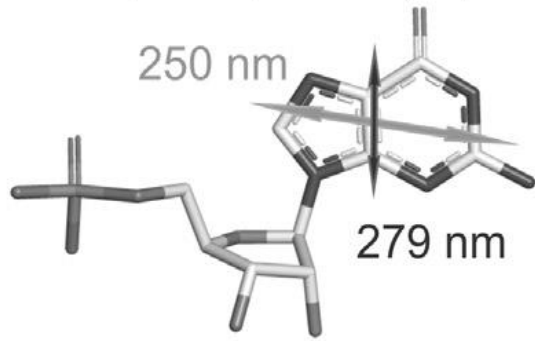
Silver, Argentum, Ag

- Silver is a chemical element with the chemical symbol **Ag**
- **Greek: ἀργυρος**
- **Latin : argentum**
- Arg – grey or shining
- It possesses the highest electrical conductivity of any element and highest thermal conductivity of any metal
- It occurs naturally as native silver or in minerals such as argentite
- It is a precious metal (coins, jewelry)
- Silver nitrate or nanoAg solutions have antimicrobial effect

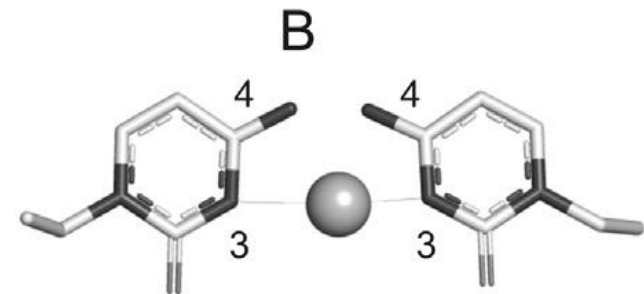
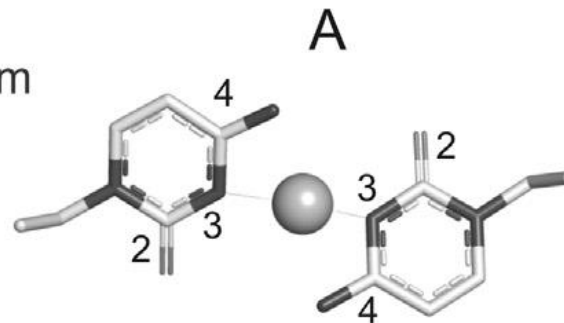
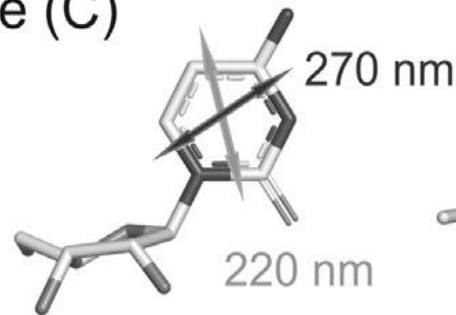


Binding of Ag^+ between guanines and cytosines

5'-Guanosine monophosphate (G)



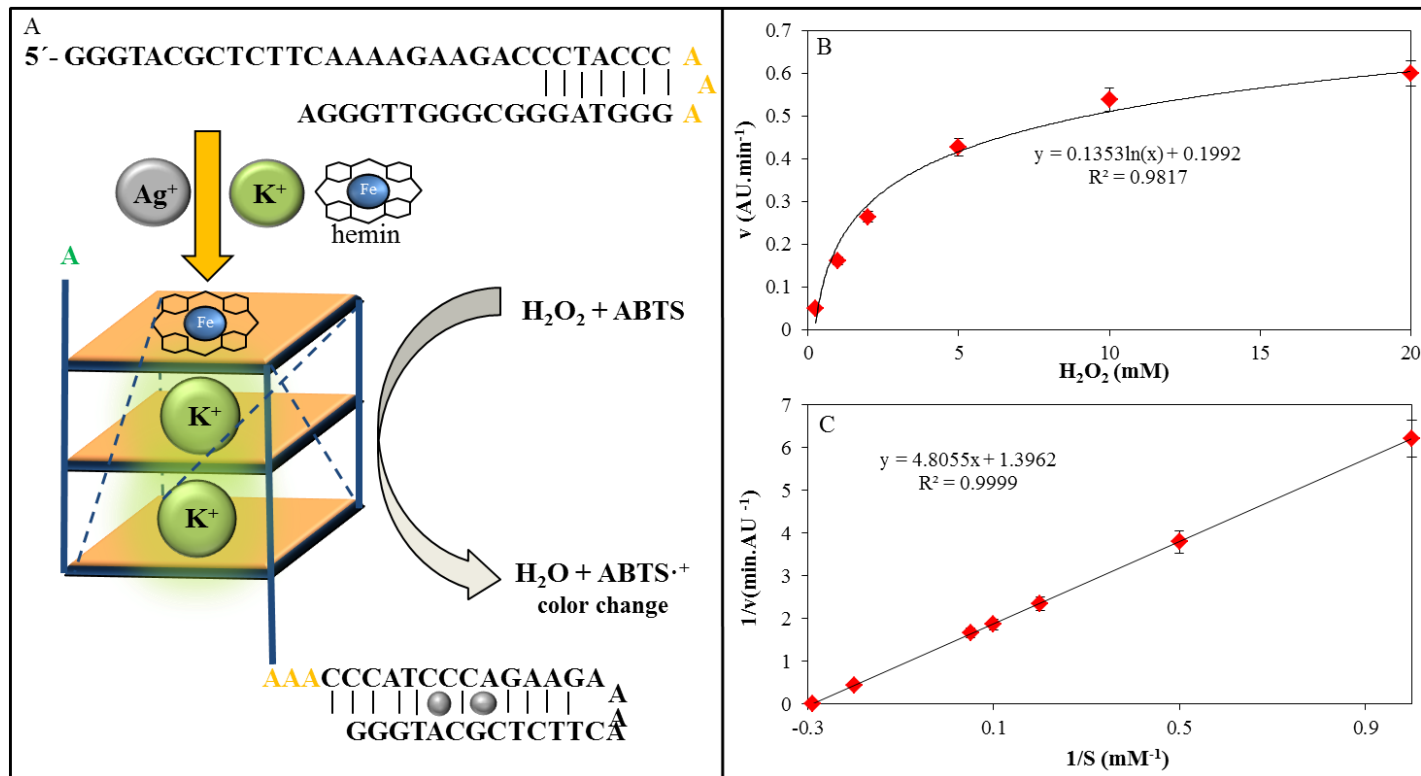
Cytidine (C)



- Scheme:** The structures of 5'-guanosine monophosphate(G) and cytidine(C) used in this study (the arrows in the guanine and cytosine structure indicate the directions of two transition dipole moments in the nucleobase unit) and structures of $\text{Ag}(\text{I})$ -mediated homo-pairs in water comprising two possible orientations of bases in base pair.

Detection of Ag⁺

- G-quadruplex in complex with hemin
- Detection of Ag⁺ according to Zhou et al. 2010
- Ag⁺ stabilizes cytosine-cytosine (C–C) mismatches by forming C–Ag⁺–C base pairs
- In the absence of Ag⁺, the oligonucleotide strand formed an intramolecular duplex
- After addition of Ag⁺ G-rich sequence folds into G-quadruplex structure capable to bind hemin to form a catalytically active G-quadruplex-hemin DNAzyme.



Detection of Ag⁺

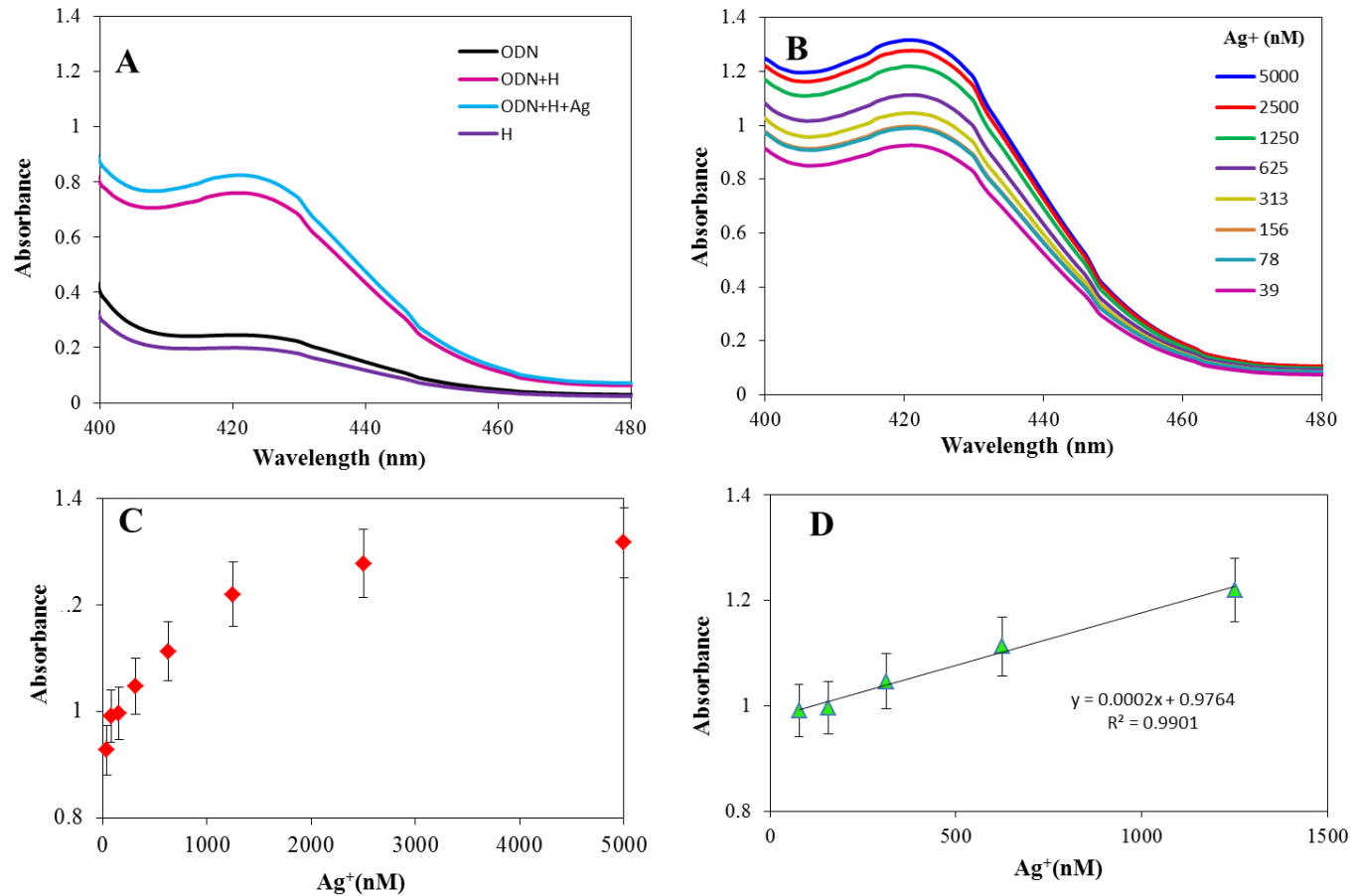
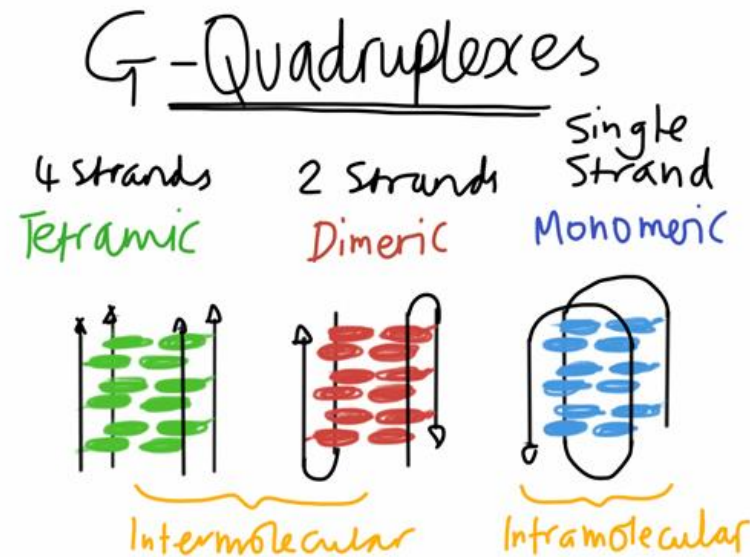


Fig.1. Effect of various concentrations of Ag⁺ ions on the formation of G-quadruplexes. The spectrophotometric profile determined within the range from 400 to 480 nm of **A**) oligodinucleotide (ODN) and hemin (H) alone, their mixture (ODN+H) and ODN + H with 40 nM of Ag⁺ ions (ODN+H+Ag); **B**) different concentrations of Ag⁺ ions (39 – 5000 nM) + ODN+H; **C**) Dependence of absorbance at 421 nm on the concentration of Ag⁺ ions in the range of 39-5000 nM. **D**) Calibration curve of Ag⁺ ions, linear range from 39-1250 nM.

Summary

- G-quadruplexes represent yet unexamined area of transcriptional gene regulation
- They were found in the promoters of important oncogenes
- G-quadruplex formation can be verified easily by photometric methods
- A large number of ions and small molecules/proteins –aptamers bind to G-quadruplexes and can accelerate or inhibit their formation
- G-quadruplexes can be used as a sensitive detection system for heavy metals



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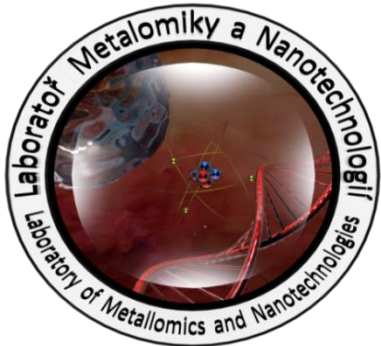
Source:<http://pubs.acs.org/cen/topstory/8035/8035notw1.html>

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