

# The magic of DNA, the molecule of life and its structures





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Why not enjoy a pint where Watson & Crick announced the discovery of **DNA**?

And be sure to check out the RAF Bar, the ceiling is a must see!!



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Welcome  
to **The EAGLE**

THE MOST HISTORIC PUB IN CAMBRIDGE



Why not enjoy a pint  
where Watson & Crick  
announced the discovery  
of **DNA?**

And be sure to check out the  
RAF Bar, the ceiling is a must  
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# The magic of DNA, the molecule of life and its structures



**Janez Plavec**

Slovenian NMR centre

National Institute of Chemistry, Ljubljana

University of Ljubljana, Faculty of Chemistry and

Chemical Technology, Ljubljana

EN-FIST Centre of Excellence, Ljubljana

e-mail: janez.plavec@ki.si

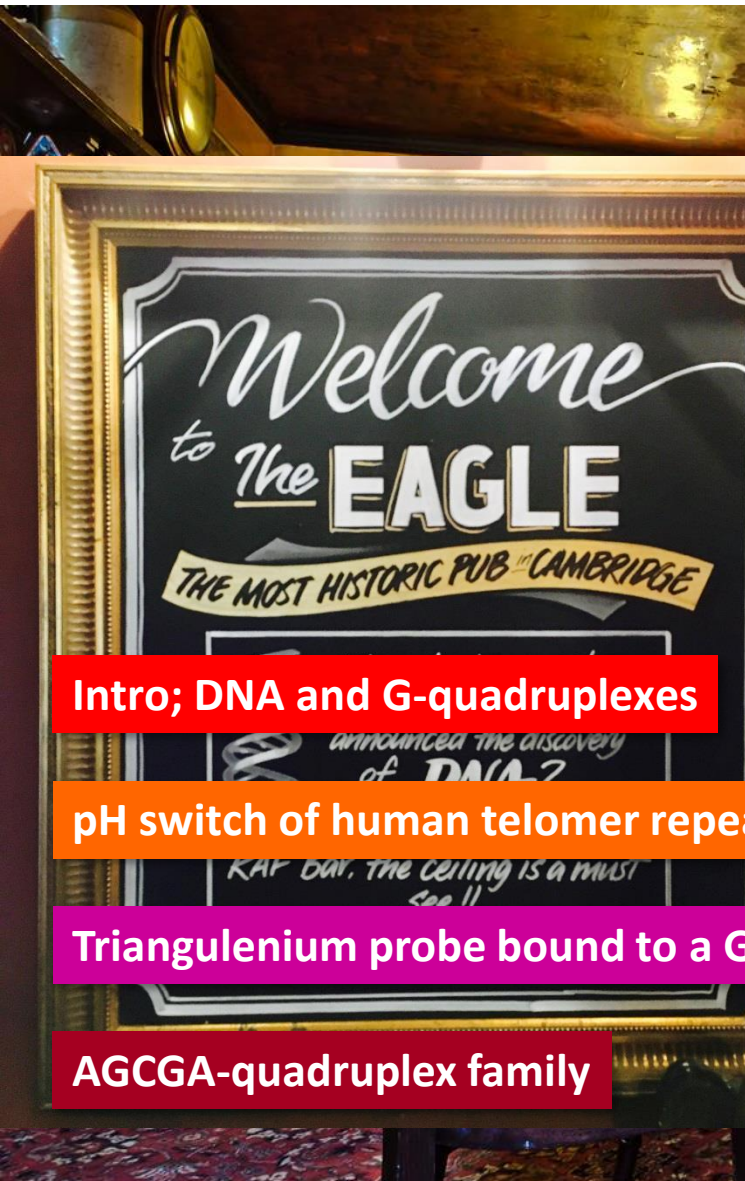


Vipava  
March 22, 2018





# The magic of DNA, the molecule of life and its structures



Intro; DNA and G-quadruplexes

pH switch of human telomere repeats

Triangulenium probe bound to a G-quadruplex

AGCGA-quadruplex family



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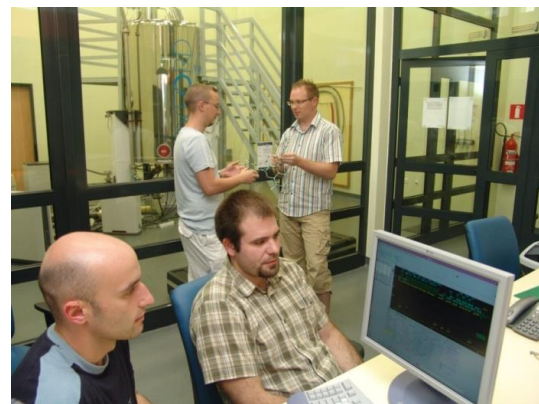




# Slovenian NMR Centre

- Institute facility
- National research infrastructure – academic & industrial users

- Centre of Excellence  
<http://www.enfist.si>



800 MHz Varian VNMRs spectrometer equipped with  $^1\text{H}$  and  $^{13}\text{C}$  enhanced triple resonance (HCN) cryogenic probe head

<http://www.slonmr.si>

- CERIC partner facility  
<http://www.c-eric.eu>

**CERIC**

Central European Research Infrastructure Consortium



600 MHz Agilent DD2 spectrometer equipped with  $^1\text{H}$  and  $^{13}\text{C}$  enhanced triple resonance salt tolerant (HCN) cryogenic probe head

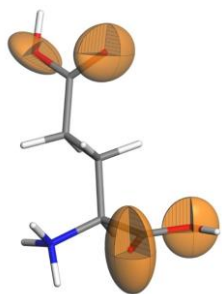
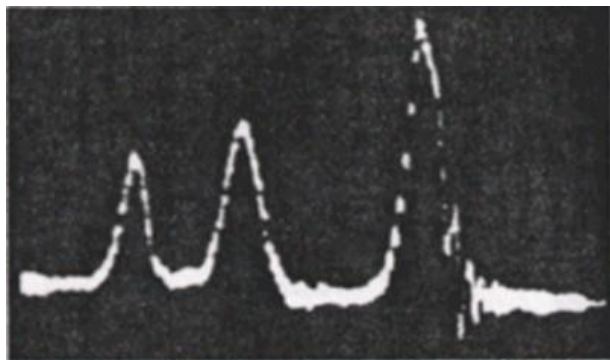


600 MHz Varian VNMRs spectrometer equipped with wide range of solid-state and liquid probe heads





# Chemical shift



Signal intensity

Chemical shift

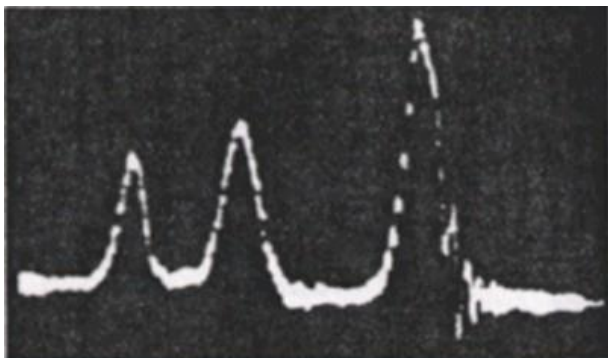
Scalar (J) couplings



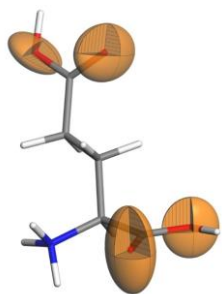
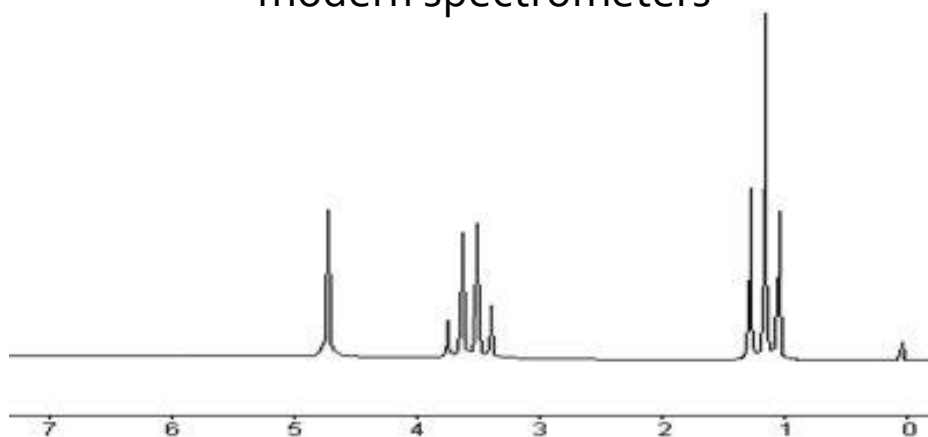
# Chemical shift



1951



modern spectrometers



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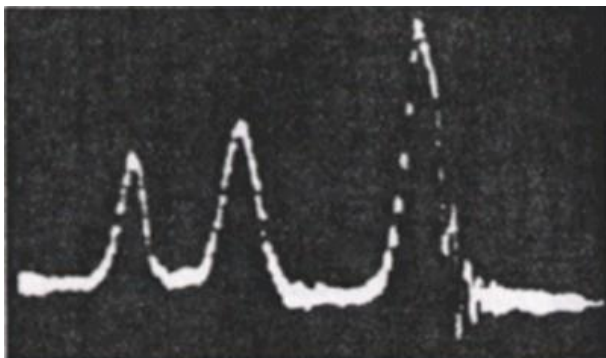
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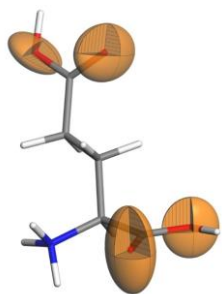
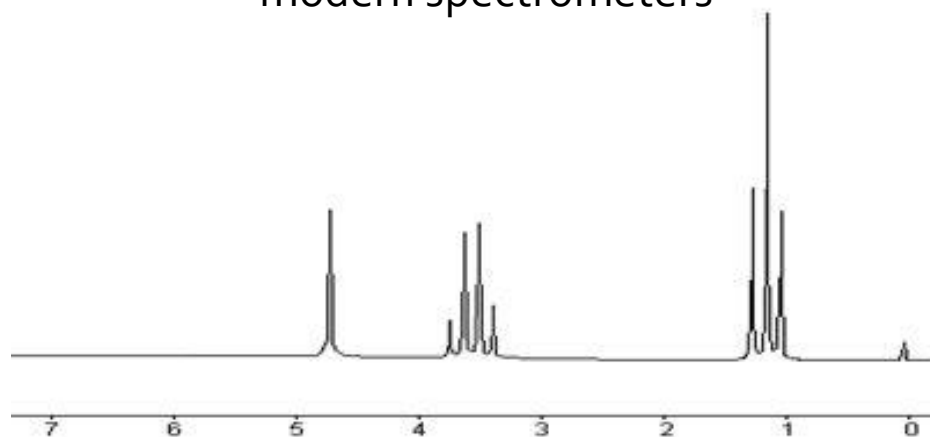
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1951



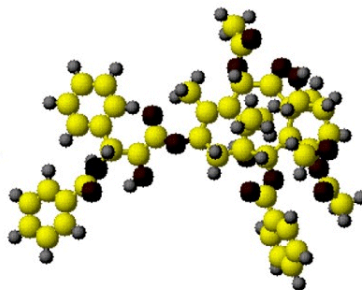
modern spectrometers



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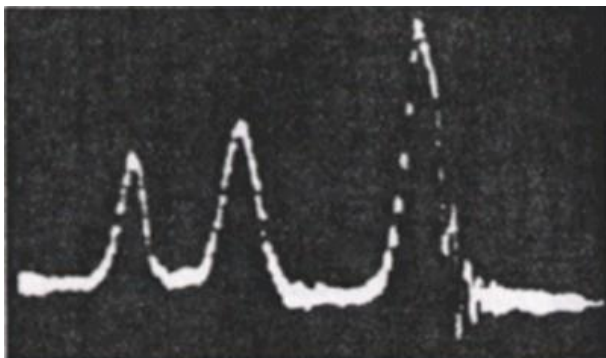




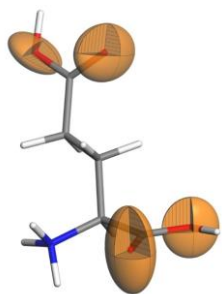
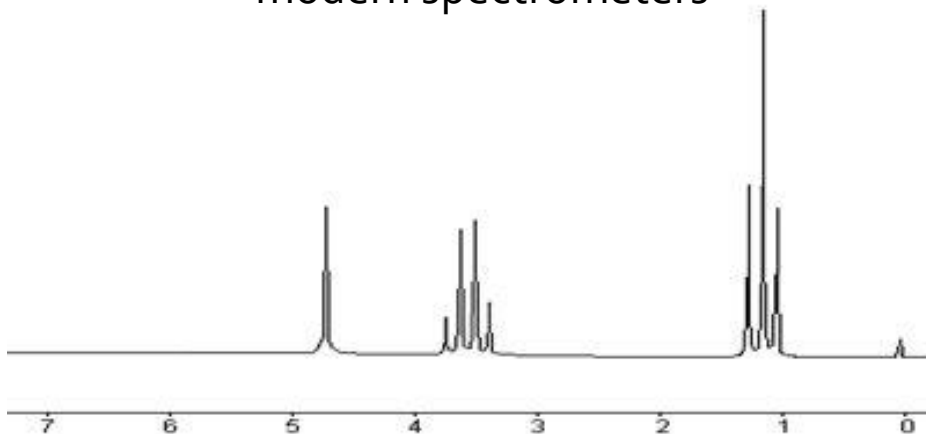
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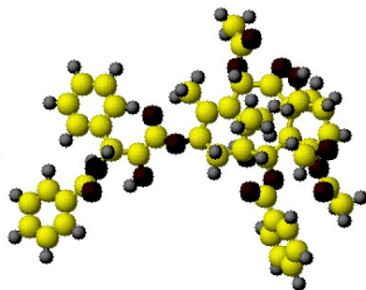
modern spectrometers



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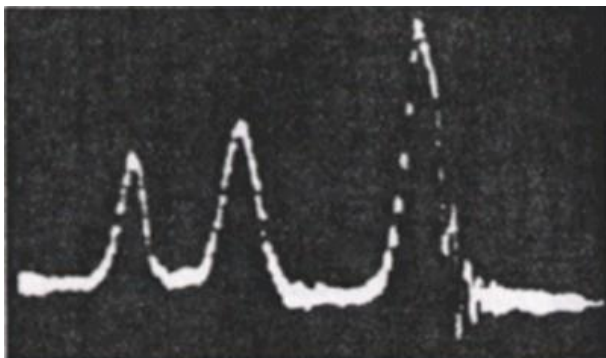




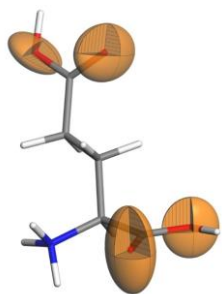
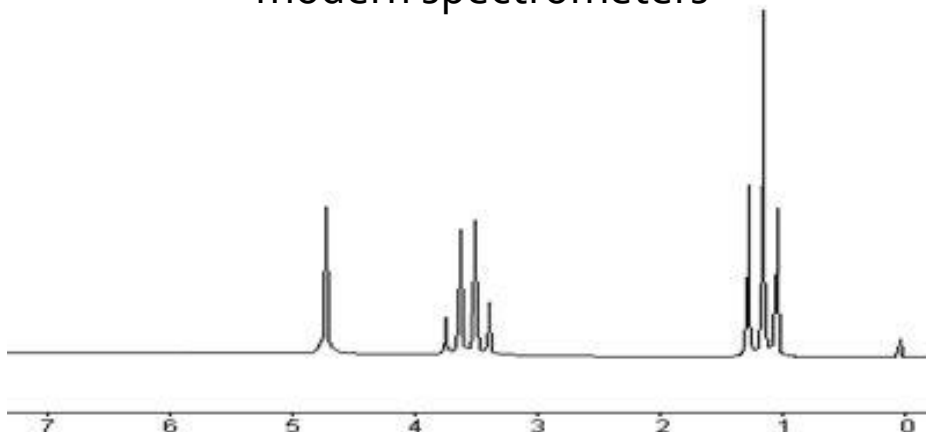
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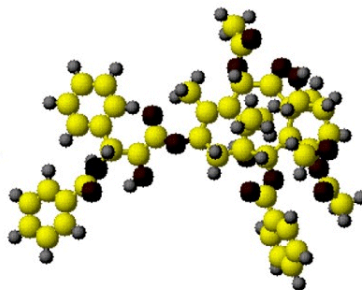
modern spectrometers



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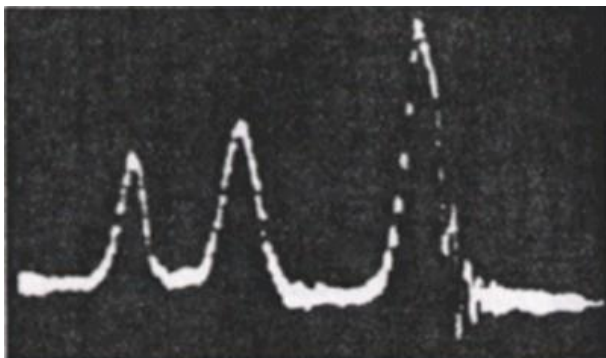




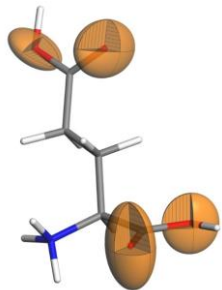
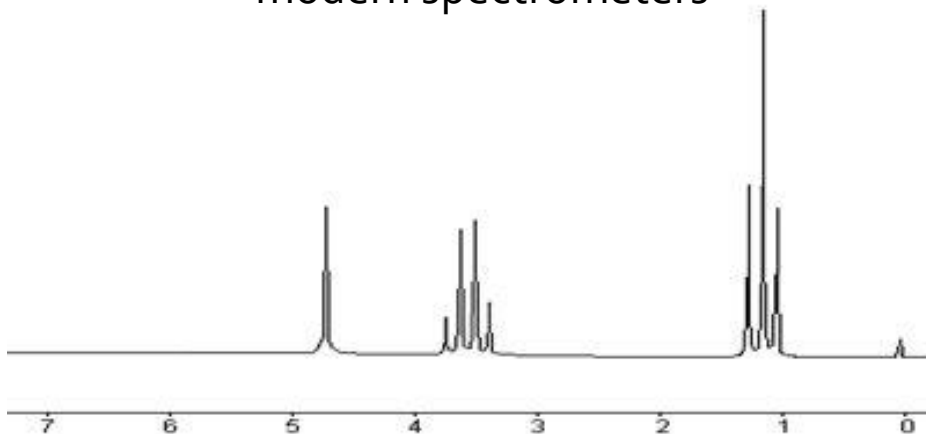
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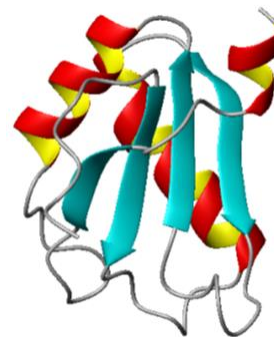
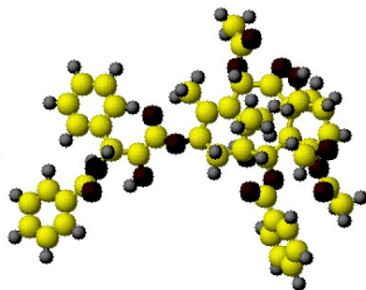
modern spectrometers



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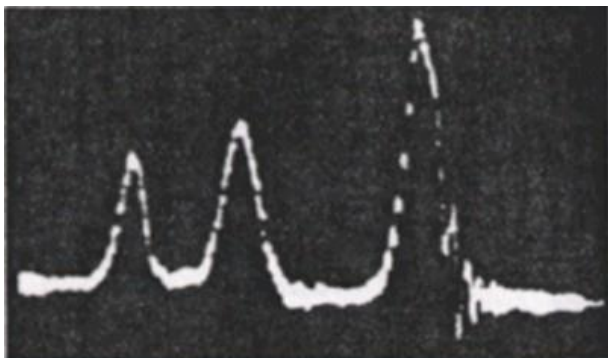




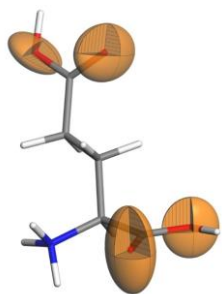
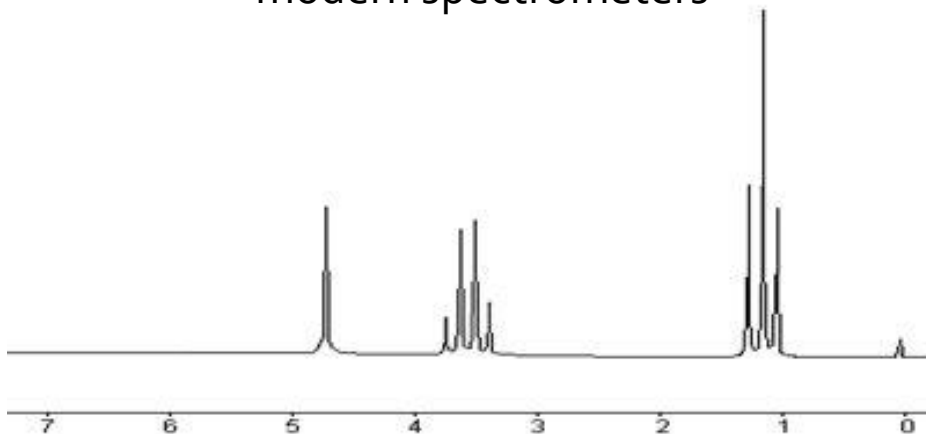
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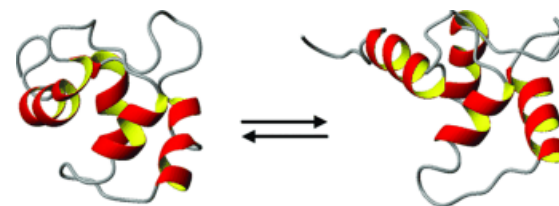
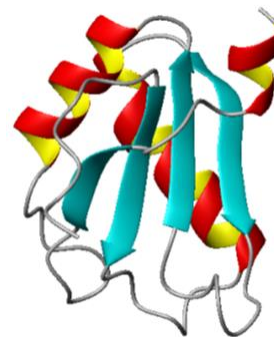
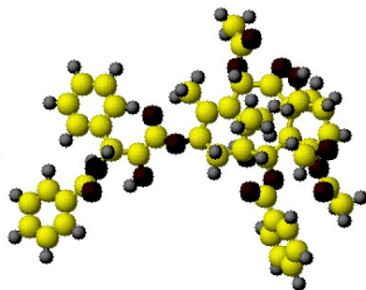
modern spectrometers



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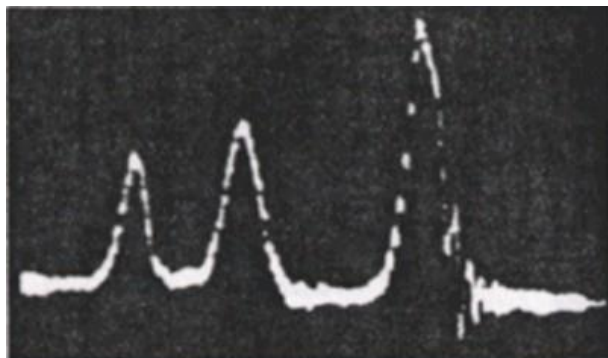




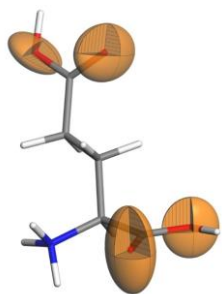
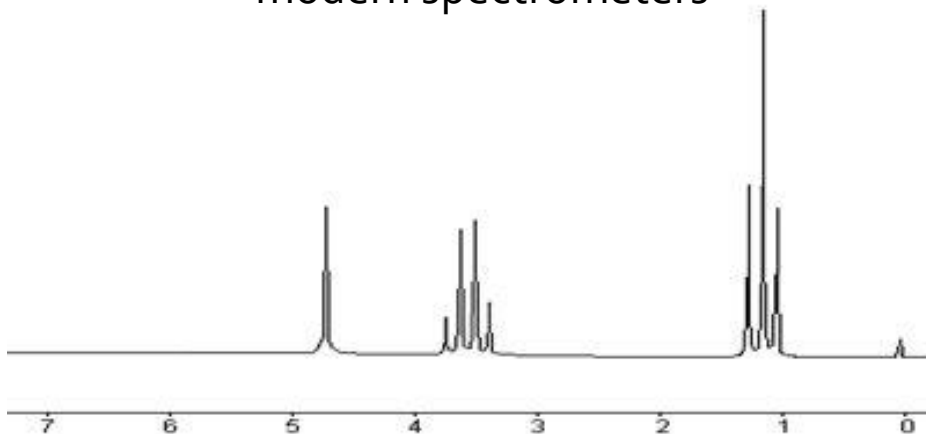
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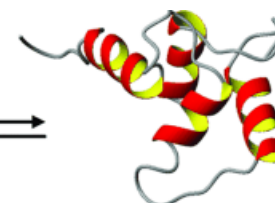
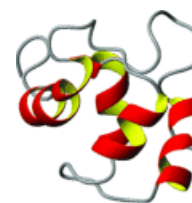
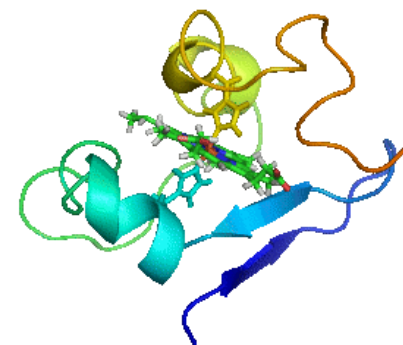
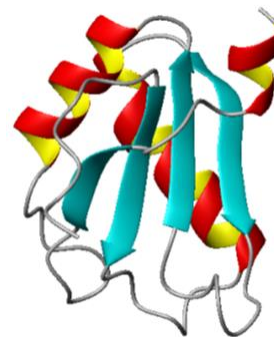
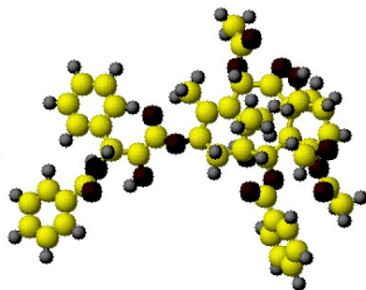
modern spectrometers

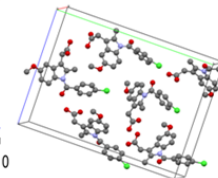
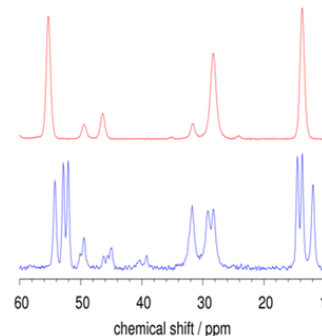
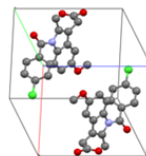
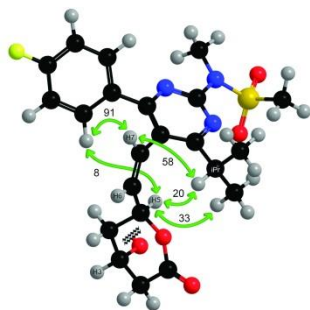


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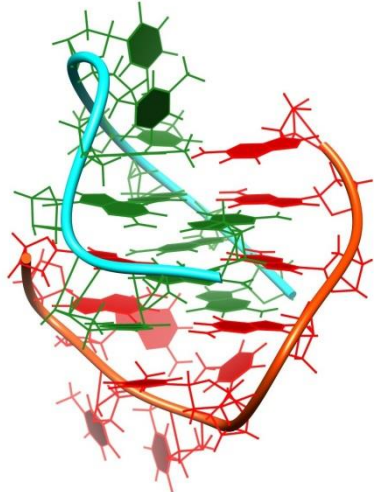
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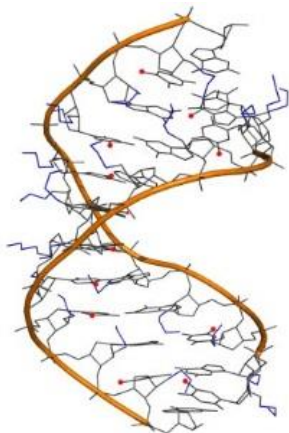




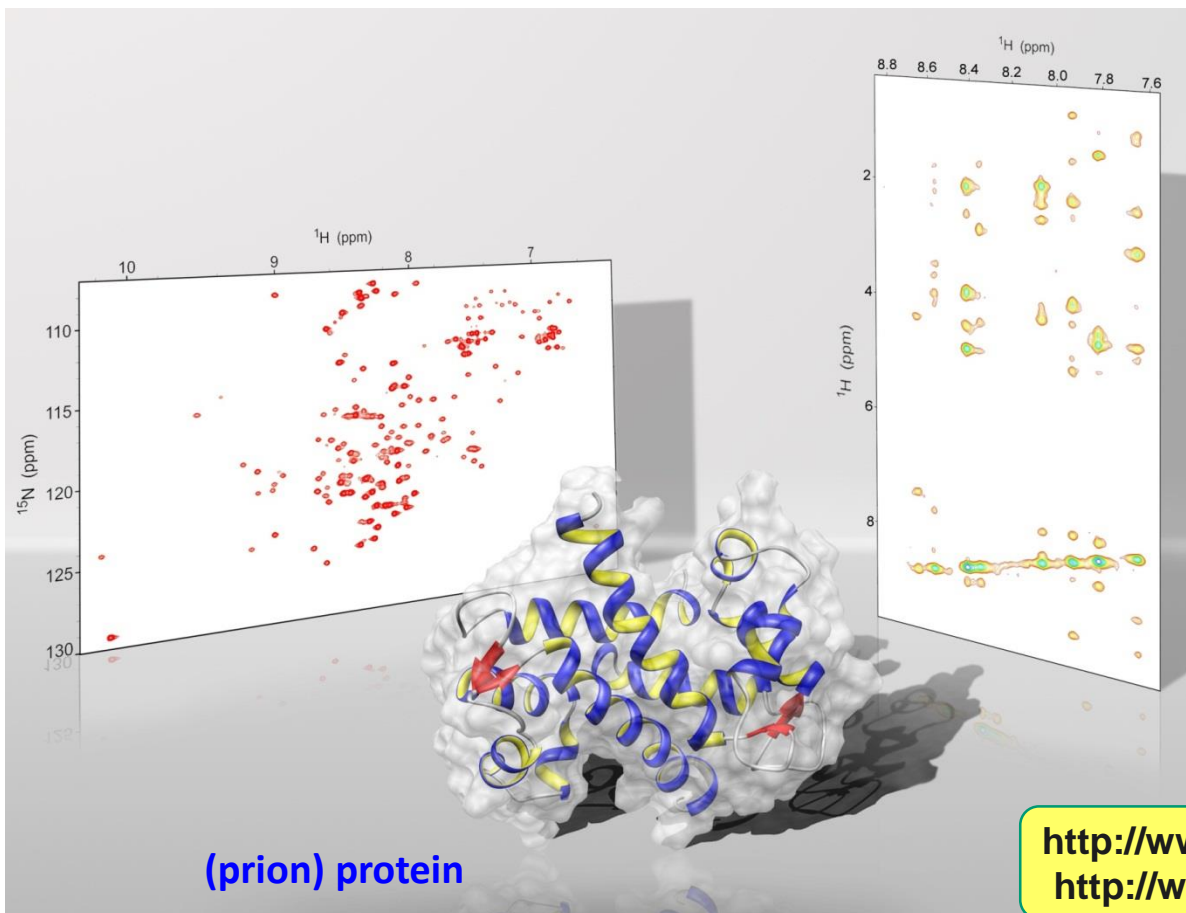
**small molecules in liquid and solid state**



**DNA quadruplex**



**RNA duplex**

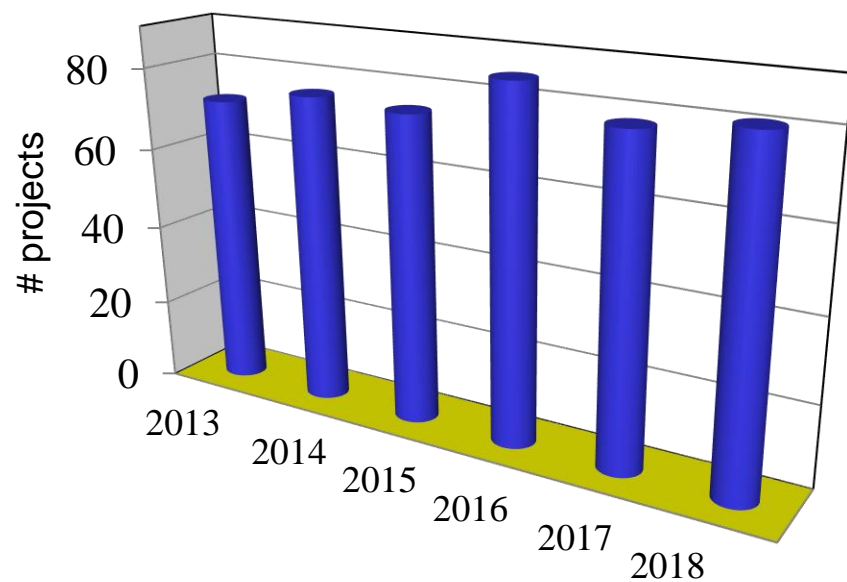


**(prion) protein**



# Research program

>80 programs and projects in 2018

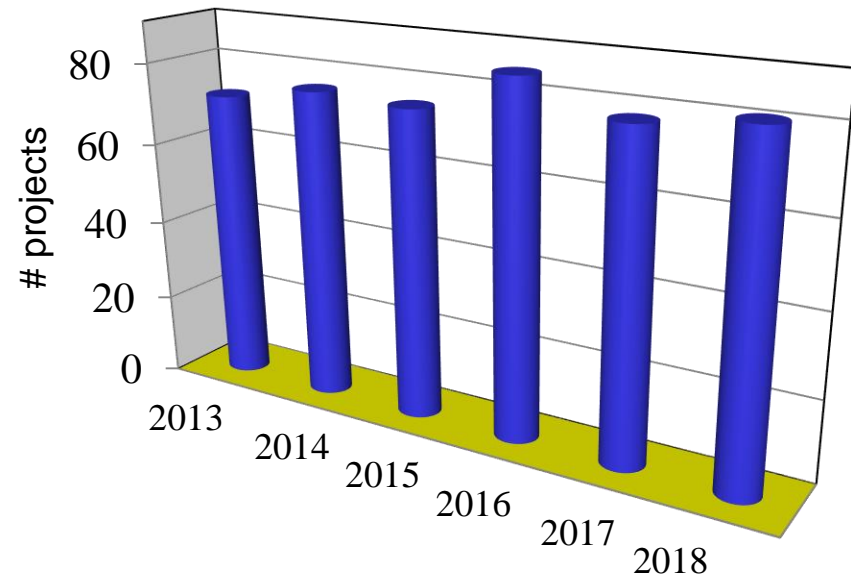


<http://www.slonmr.si>

# Research program

- Structure of **proteins** and their interactions,

>80 programs and projects in 2018



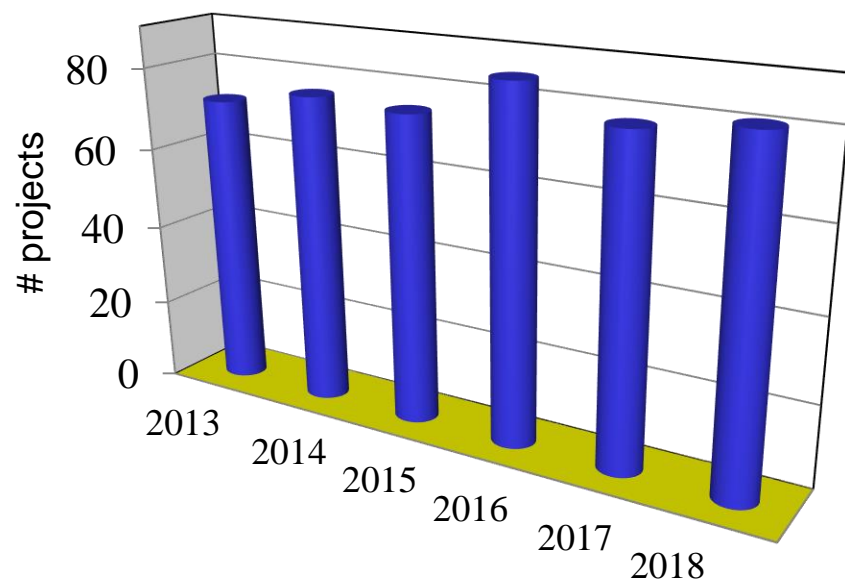
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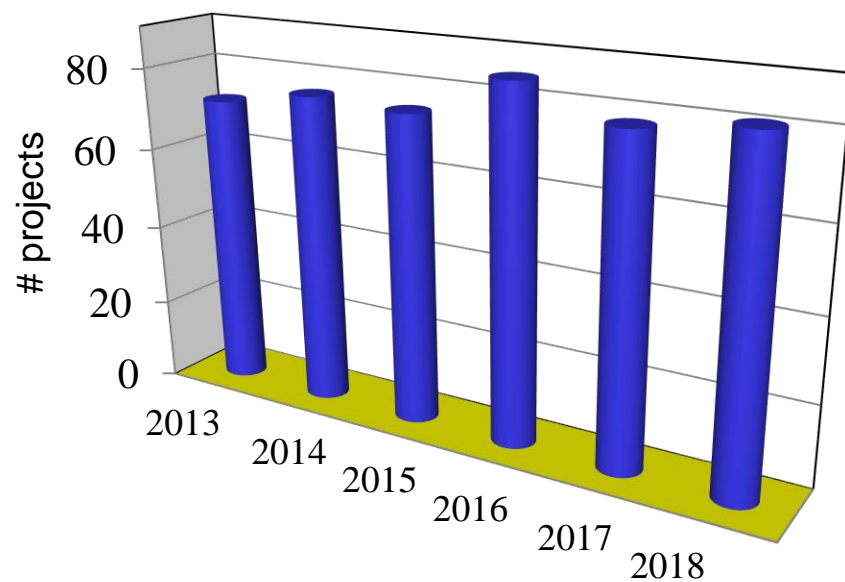


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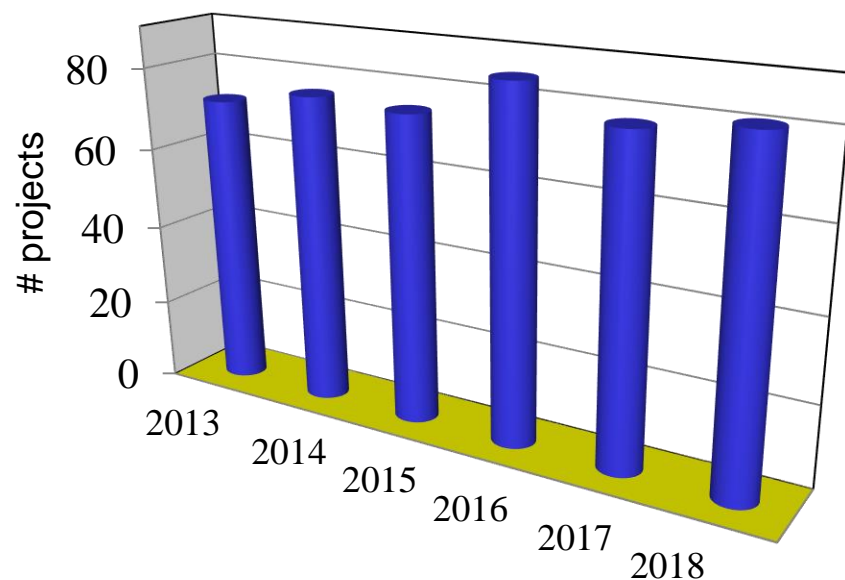
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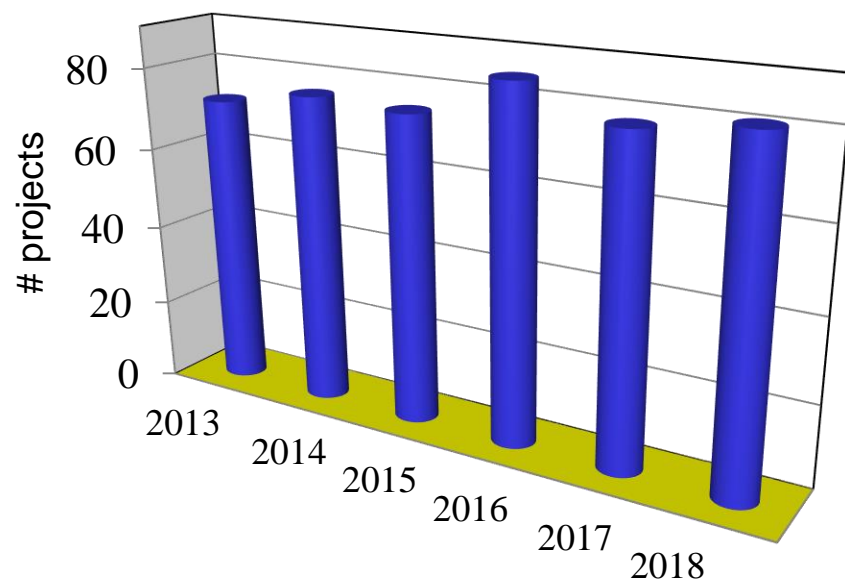


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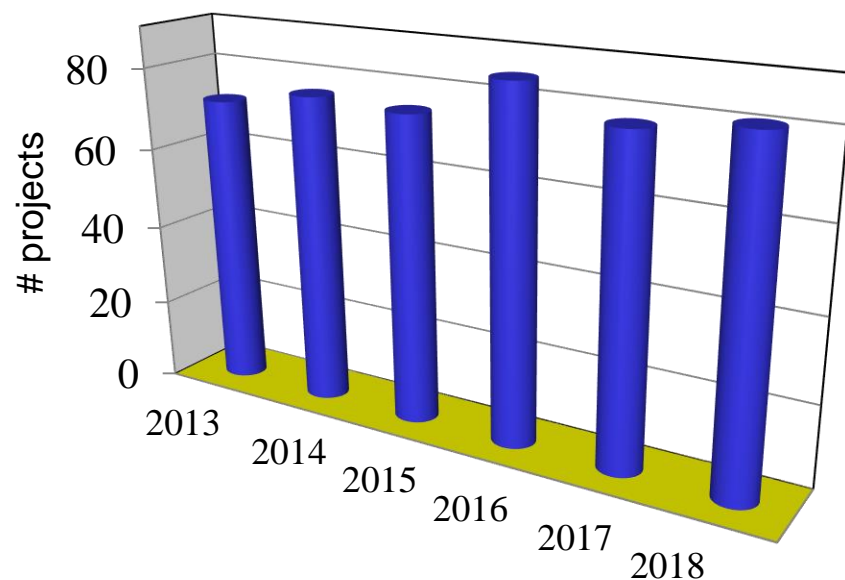
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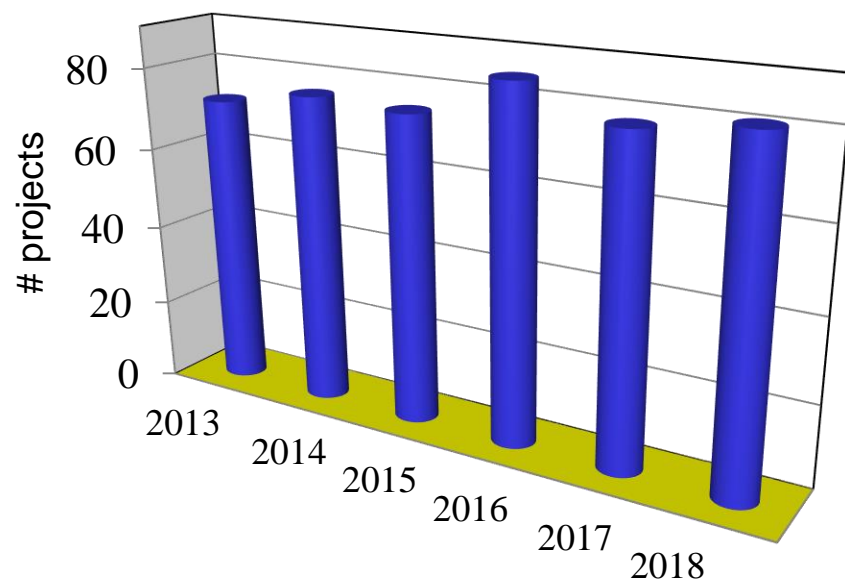


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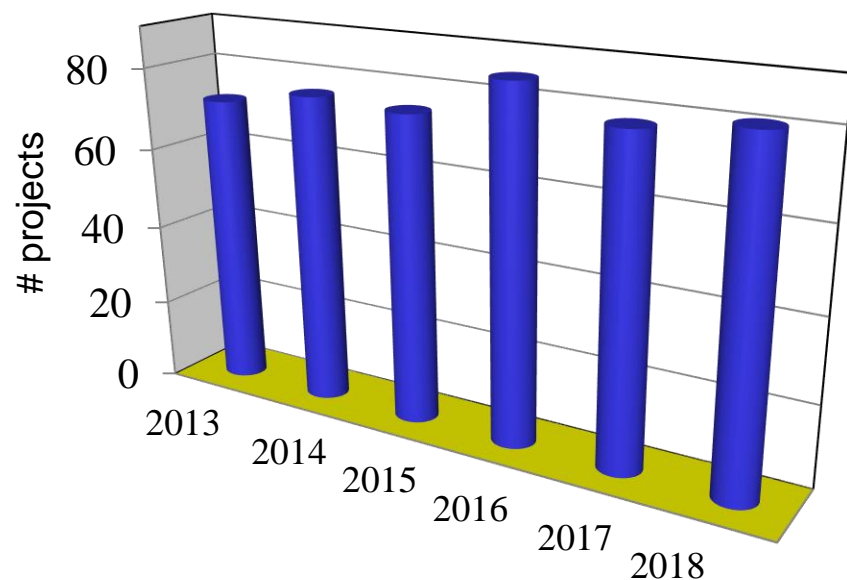
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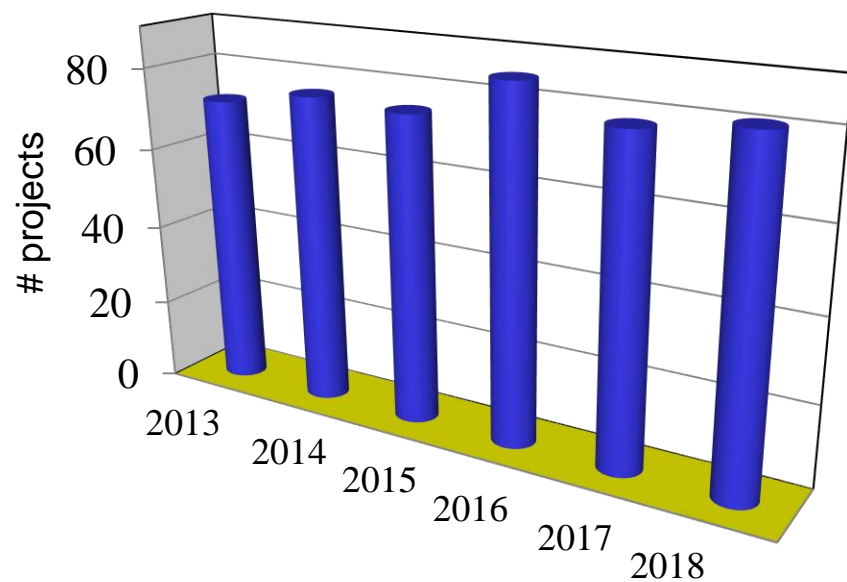


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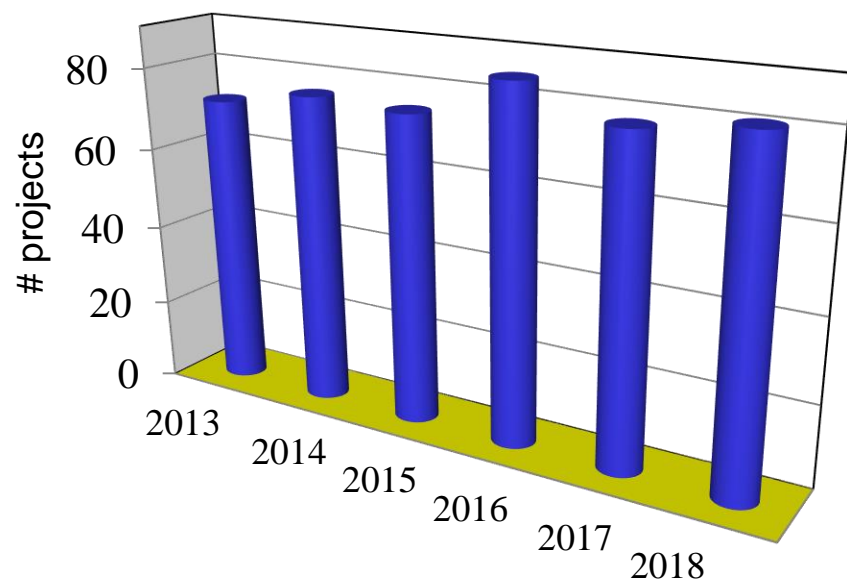


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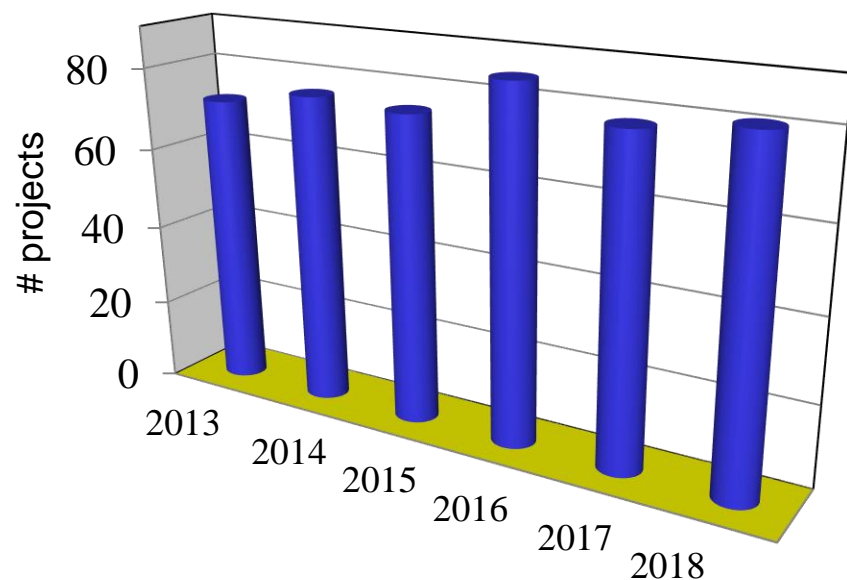
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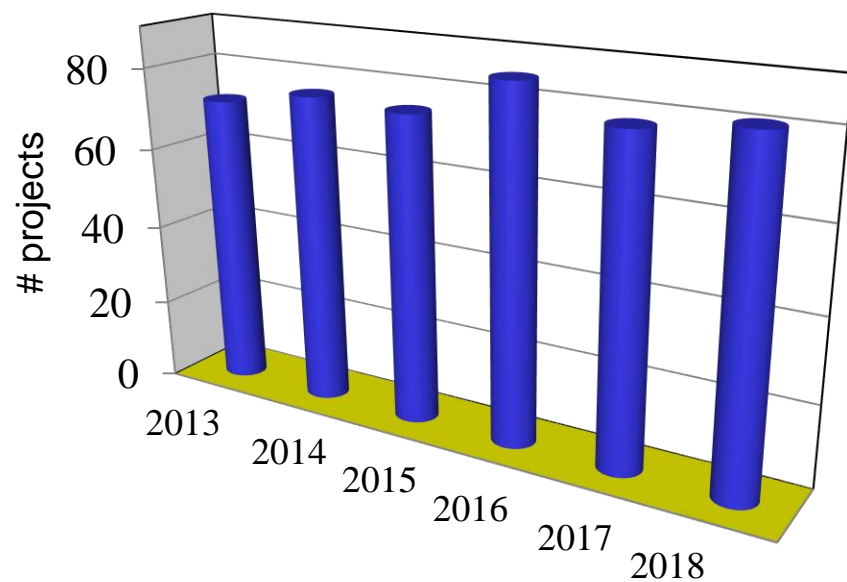


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- Authenticity and origin of **wine**, ...

>80 programs and projects in 2018



<http://www.slonmr.si>

# International events

FRONTIERS of

## BIOMOLECULAR NMR

Inauguration of the 800 MHz Spectrometer



## MAGNETIC MOMENTS IN



## MAGNETIC MOMENTS IN CENTRAL EUROPE

AND FRONTIERS

### CMC 2009



## Advances in Noncanonical Nucleic Acids

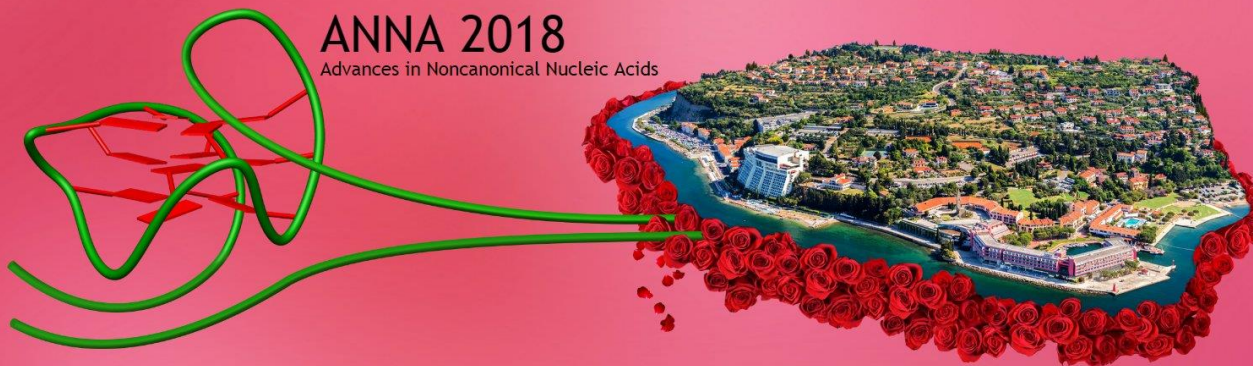
Bled, Slovenia, October 26 - 28, 2017

[www.nmr.ki.si/anna\\_2017](http://www.nmr.ki.si/anna_2017)

[Home](#)   [Speakers](#)   [Program](#)   [Registration](#)   [Accommodation](#)   [Organization](#)

## ANNA 2018

Advances in Noncanonical Nucleic Acids



You are cordially invited to the symposium Advances in Noncanonical Nucleic Acids, which will be held in Portorož, Slovenia from October 25 to 27, 2018.

ANNA 2018 will comprise of oral presentations by renowned experts enabling fruitful exchange of ideas and vivid discussions.

CENTRE OF EXCELLENCE



Bio-NMR



Organizing Committee  
Ivano Bertini, Harald Schwalbe, Rolf Boelens, Lyndon Emsley, Hartmut Oschkinat, Dave Stuart, Janez Plavec



REPUBLIC OF SLOVENIA  
MINISTRY OF EDUCATION,  
SCIENCE AND SPORT

You are cordially invited to attend the symposium Magnetic Moments in Central Europe. It will take place at Oblek's Inn that are situated near Mura river, close to the historical highway between Ljubljana and Lajpc. The MMEC symposium aims to bridge gaps between disciplines and to promote the potentials of the MMR among younger scientists. It will provide an opportunity to exchange knowledge and expertise in the field of the NMR spectroscopy between experimentalists and theoreticians working in academia and industry, as well as to become acquainted with the latest developments in the field in Central Europe and elsewhere in the world.

In addition to the scientific program, participants will be able to enjoy the unique natural beauty as well as historical and cultural monuments of the area. The hotels offer a wide variety of recreation facilities and are in the vicinity of the romantic medieval Oblek Castle located on an island in the central Krka River.

**Lecturers - Tutors**  
ANDREO BERTINI (University of Florence)  
HAROLD BRUTSCHER (Institute of Structural Biology, Grenoble)  
ANDREY YUDMAN (Weizmann Institute of Science, Rehovot)  
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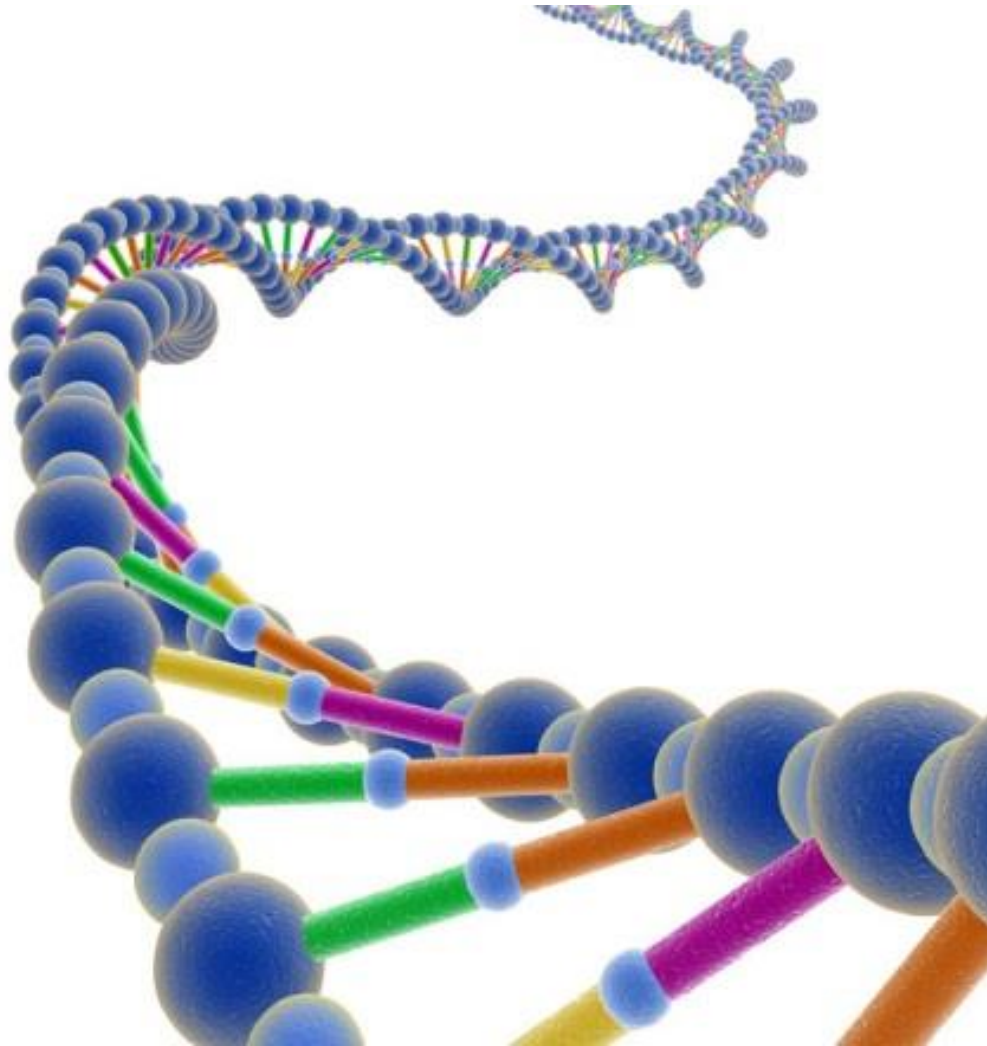
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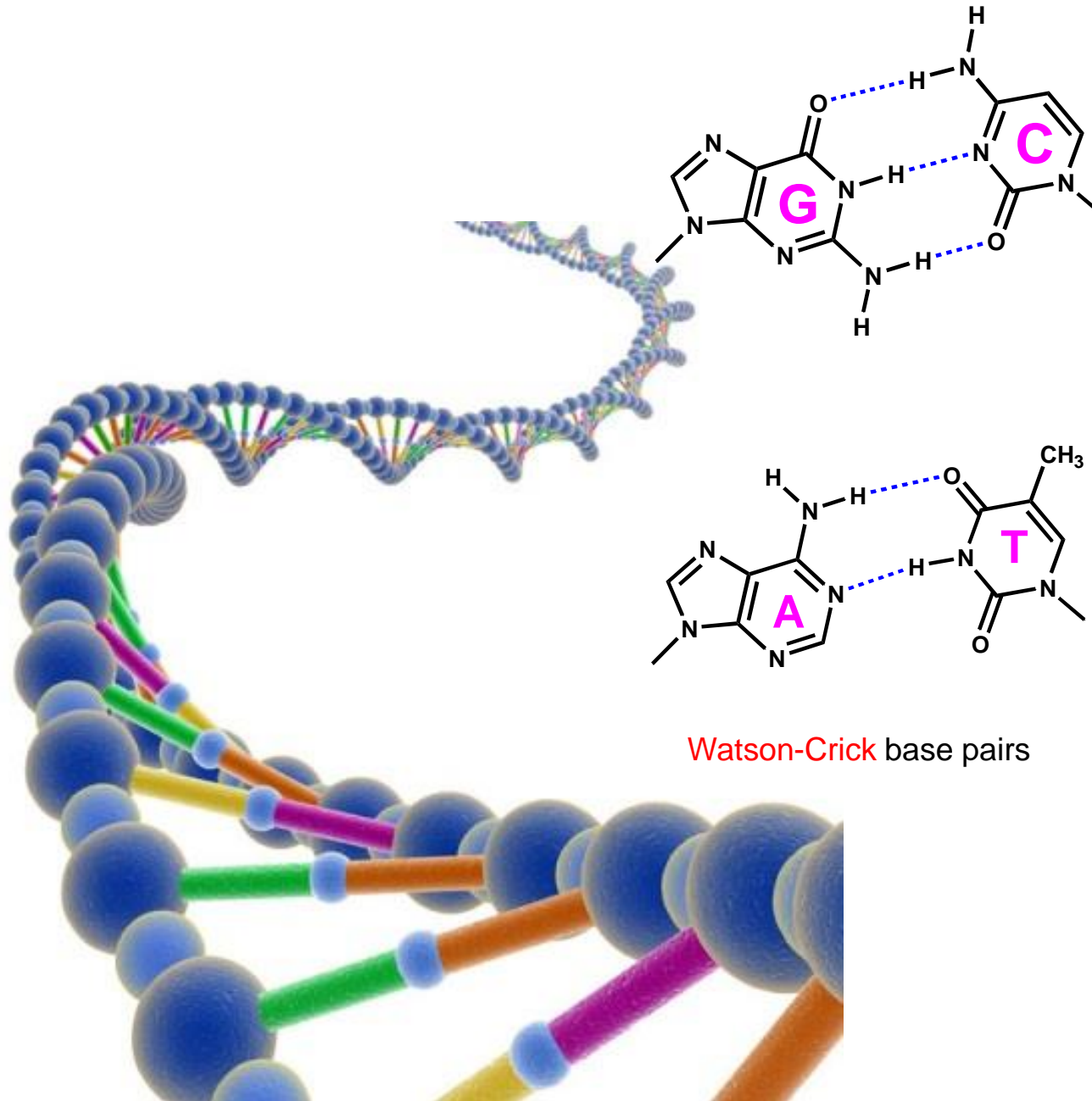
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# *DNA - double-helix, Watson-Crick base pairs, ...*

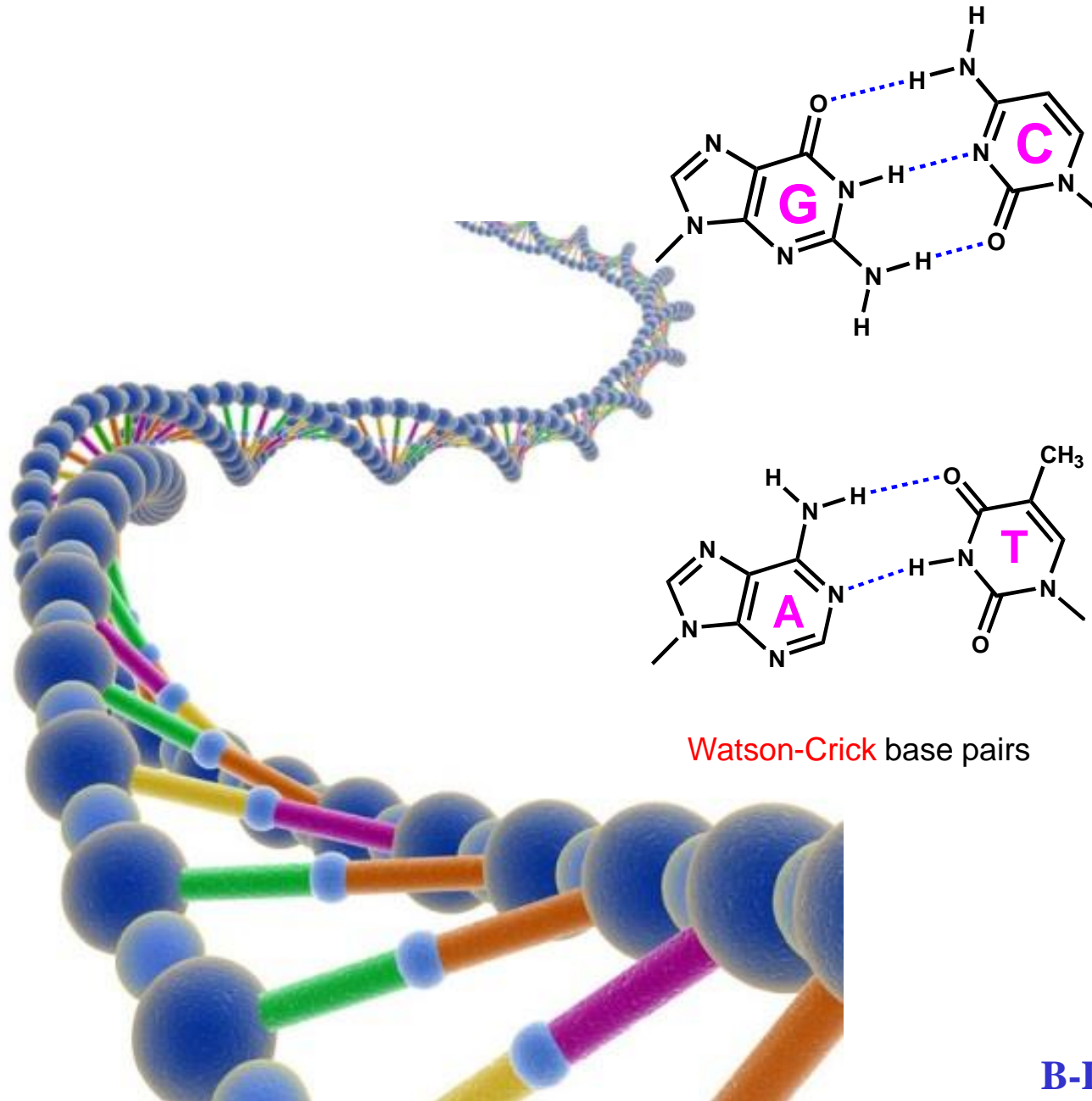


# DNA - double-helix, Watson-Crick base pairs, ...

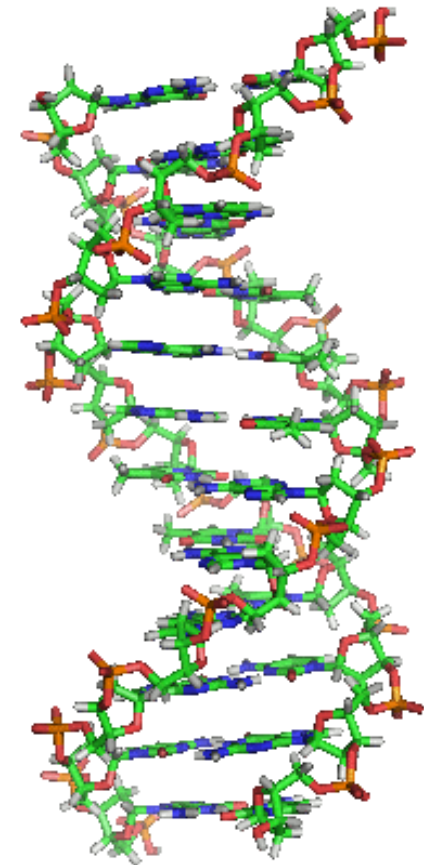


Watson-Crick base pairs

# DNA - double-helix, Watson-Crick base pairs, ...



Watson-Crick base pairs

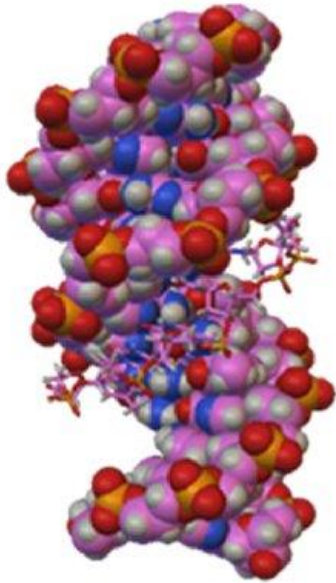


B-DNA

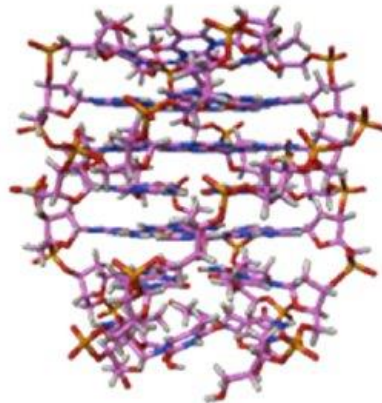


# DNA - double-helix, Watson-Crick base pairs, ...

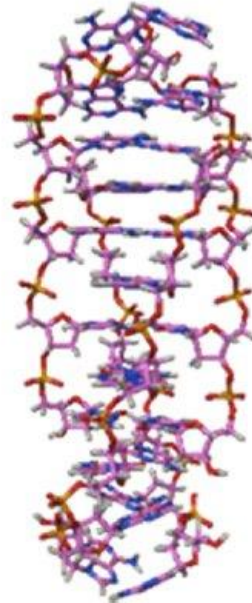
triplex DNA  
(NMR, PDB 1BWG)



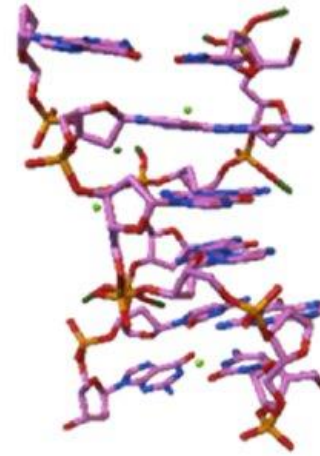
four-stranded G-quadruplex  
(NMR, PDB 139D)



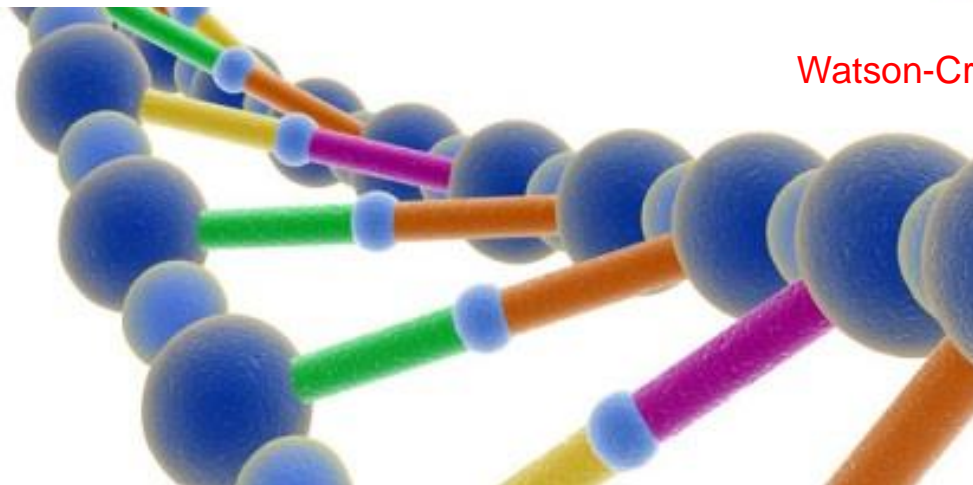
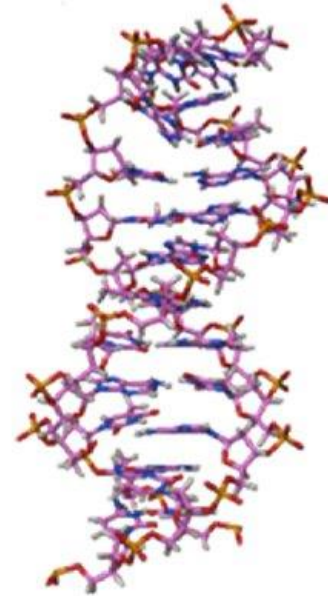
i-motif  
(NMR, PDB 1YBL)



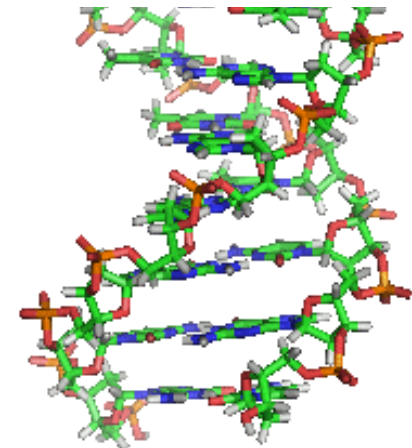
Z-DNA  
(X-ray, PDB 4FS5)



parallel-stranded  
duplex DNA  
(NMR, PDB 1JUJ)

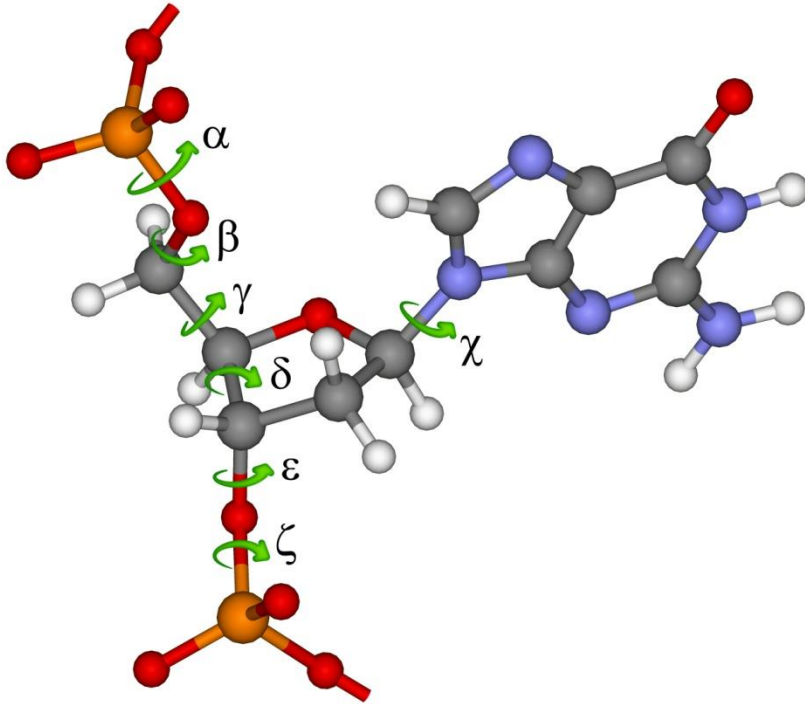


Watson-Crick base pairs

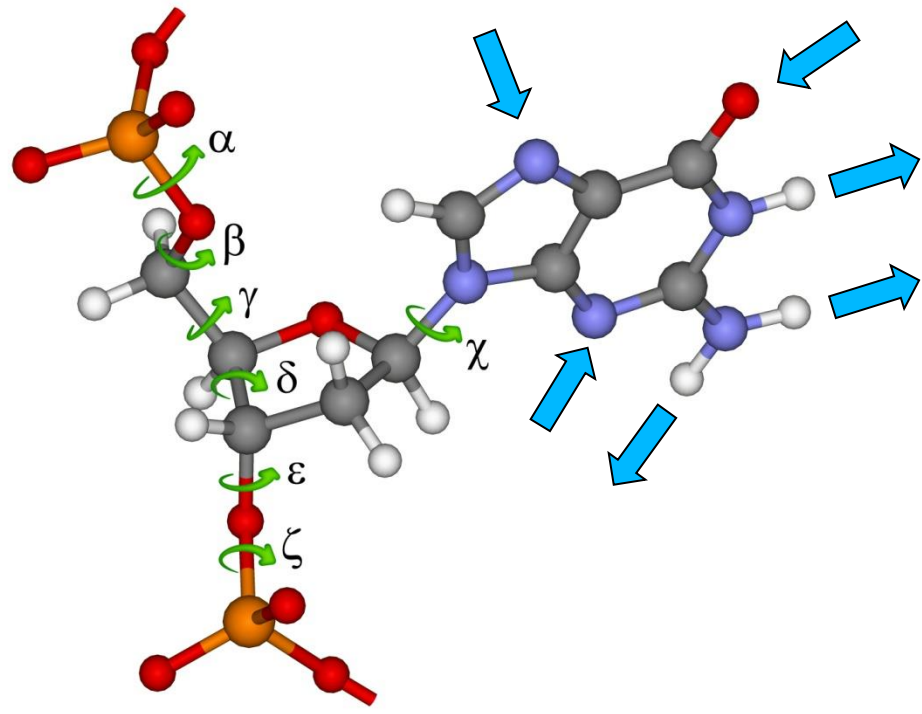


B-DNA

# Guanine nucleos(t)ides

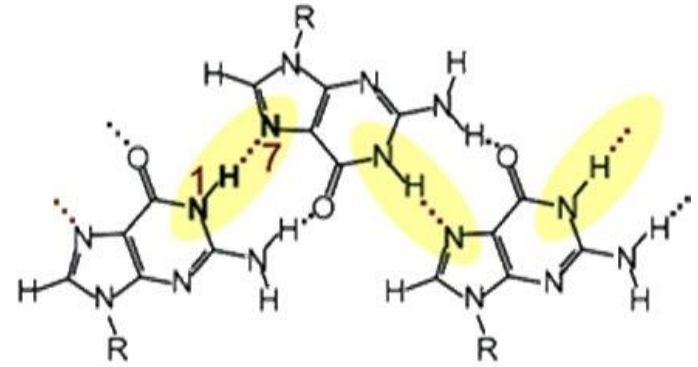
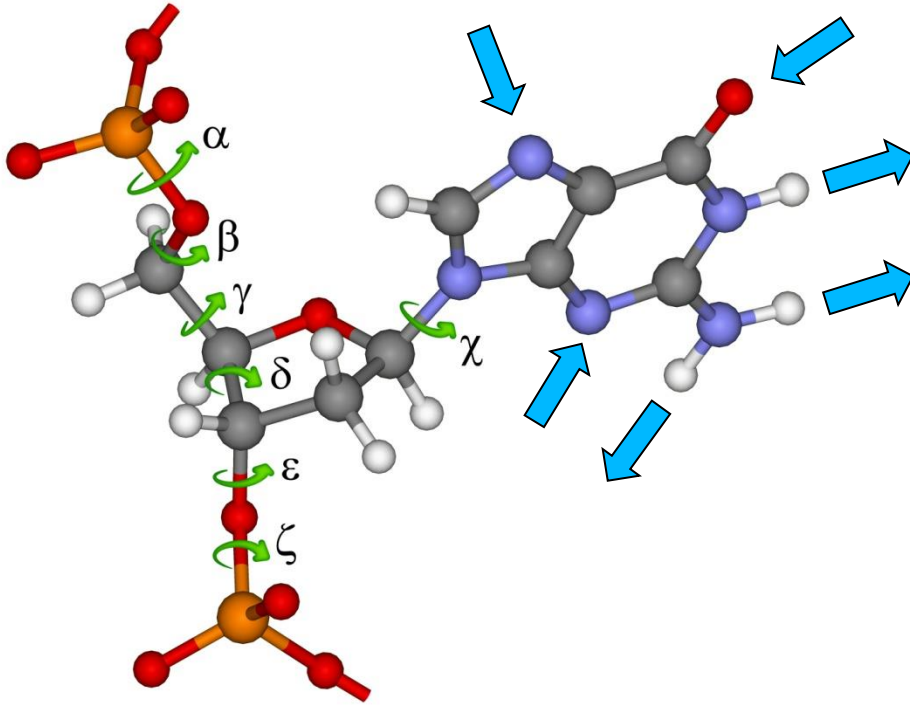


# Guanine nucleos(t)ides



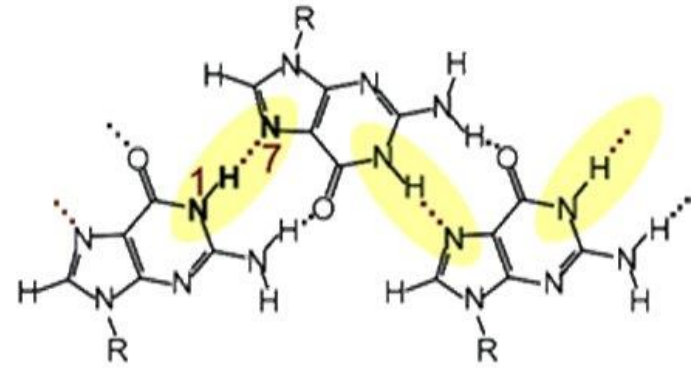
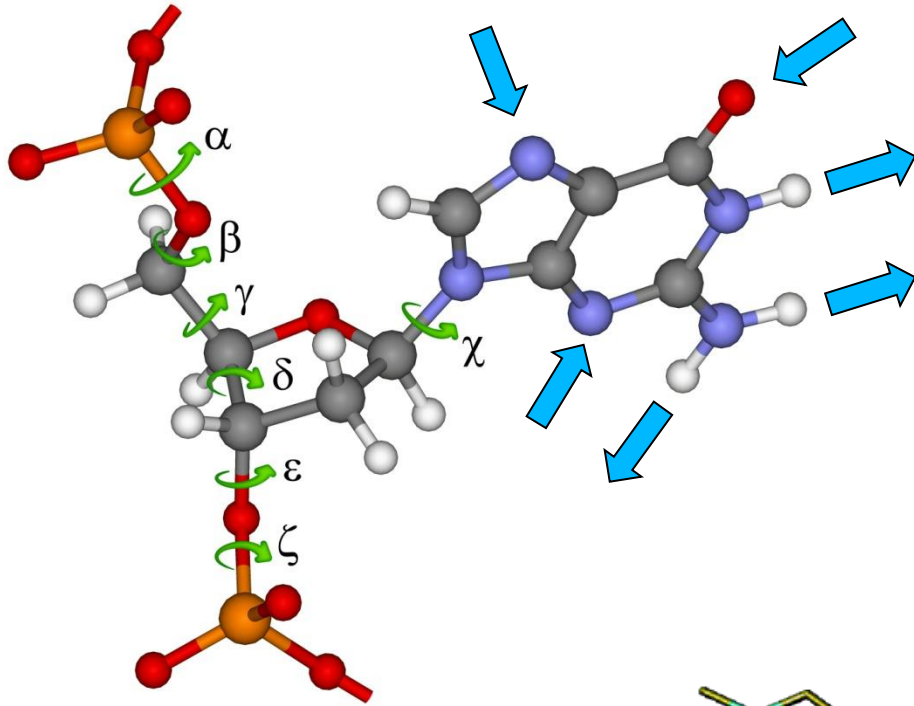


# Guanine nucleos(t)ides

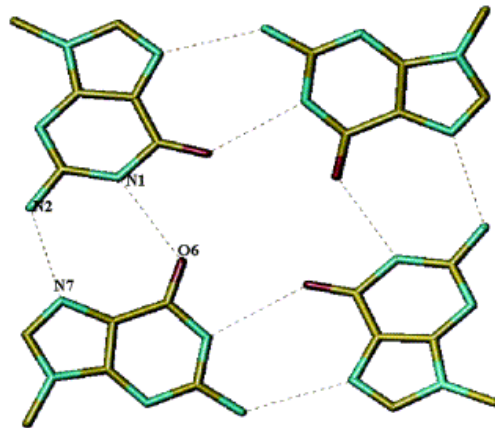
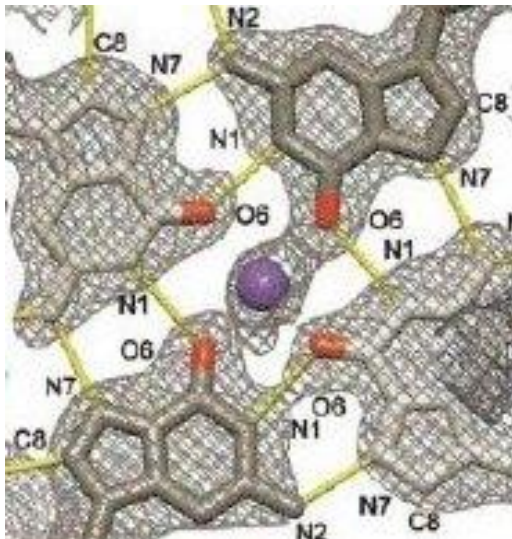


*H-bonded ribbon*

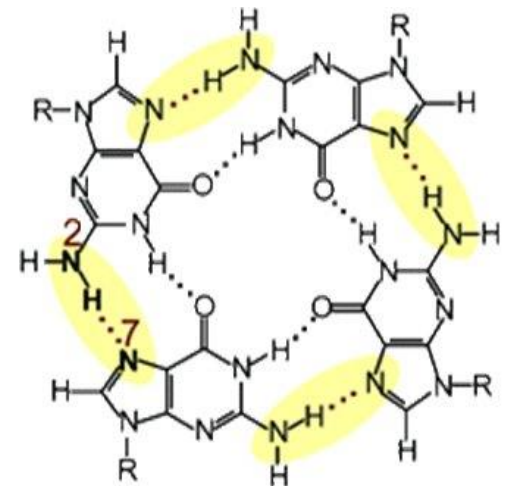
# Guanine nucleos(t)ides



*H-bonded ribbon*



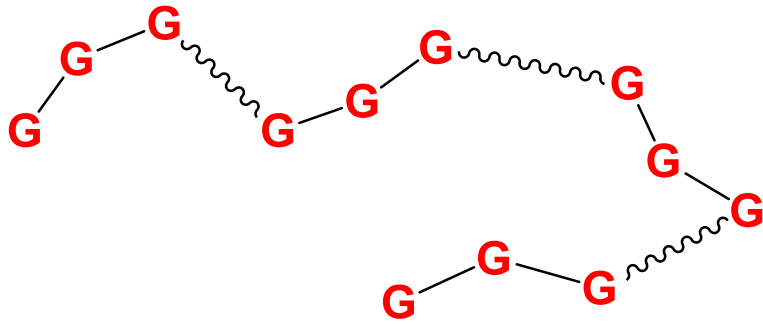
Hoogsteen base-pairing



*G-quartet*

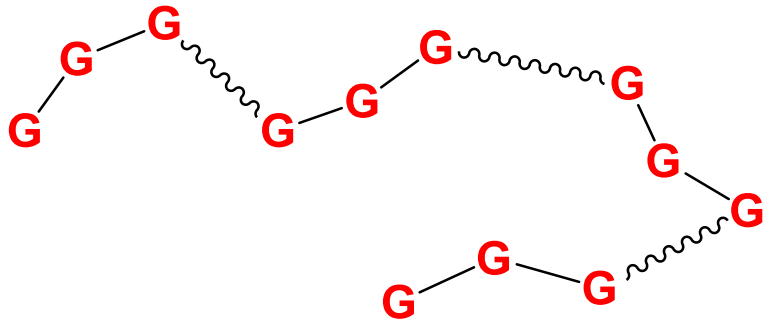
G-quadruplexes require cations for structural integrity;  $K^+$ ,  $Na^+$

# Guanine-quadruplexes





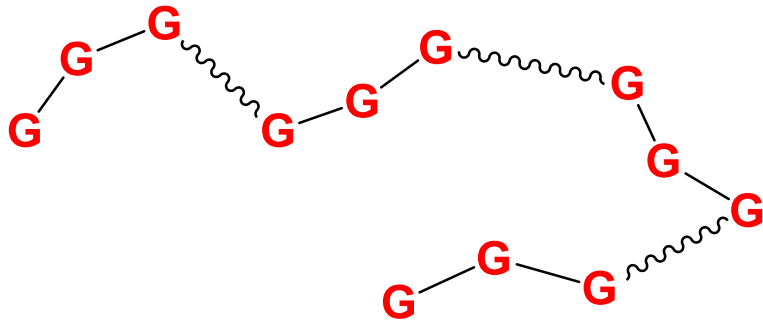
# Guanine-quadruplexes



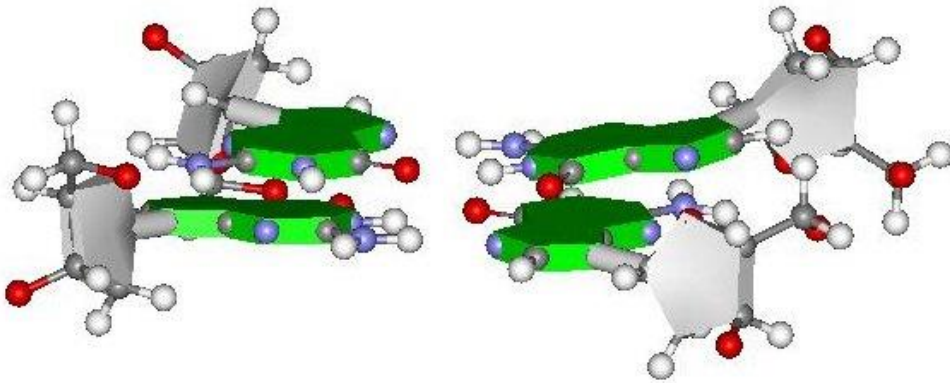
5'-G<sub>3-5</sub> N<sub>L1</sub> G<sub>3-5</sub> N<sub>L2</sub> G<sub>3-5</sub> N<sub>L3</sub> G<sub>3-5</sub>-3'



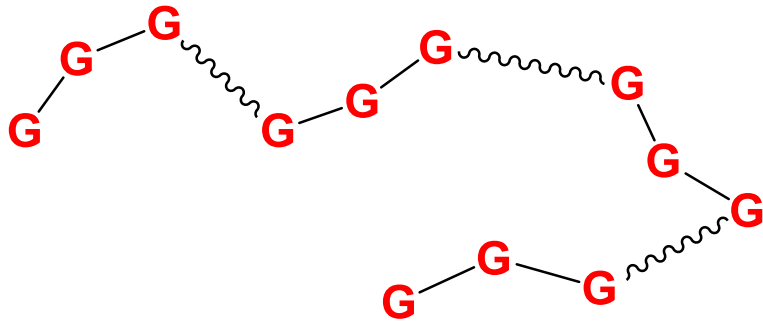
# Guanine-quadruplexes



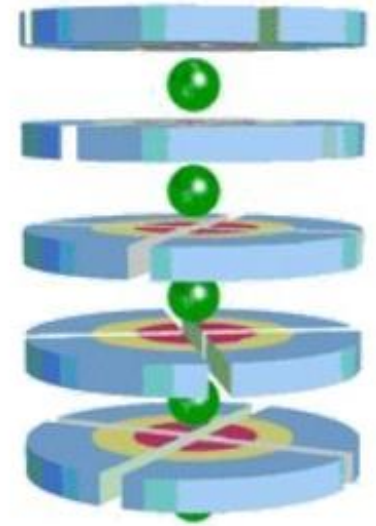
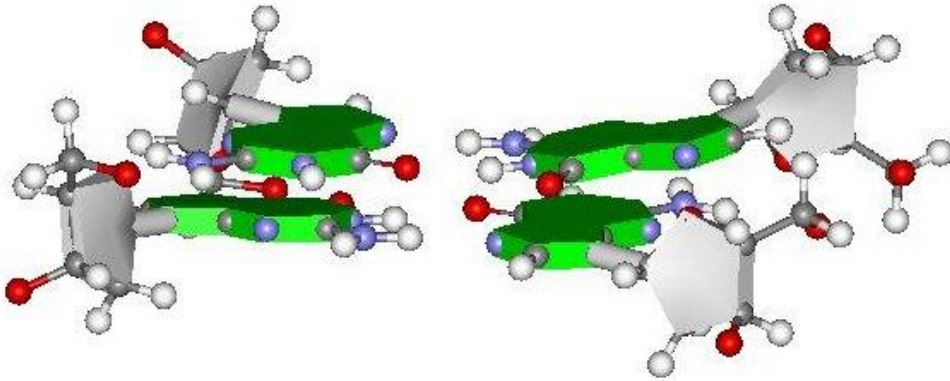
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# Guanine-quadruplexes

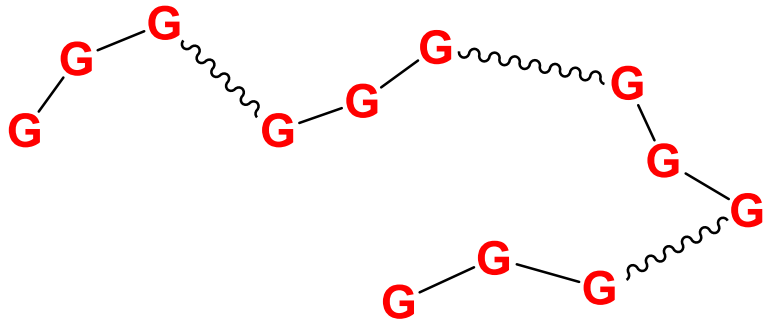


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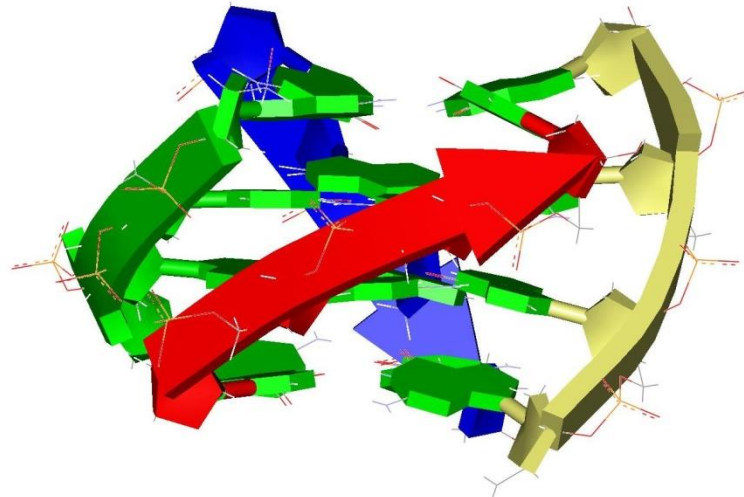
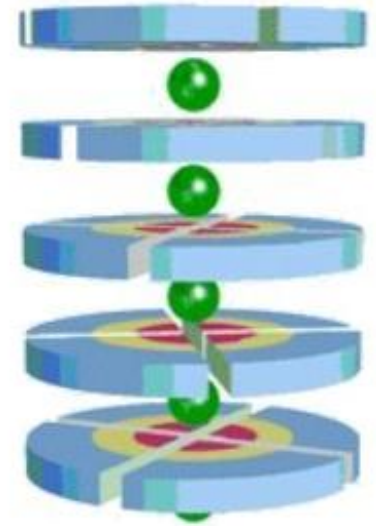
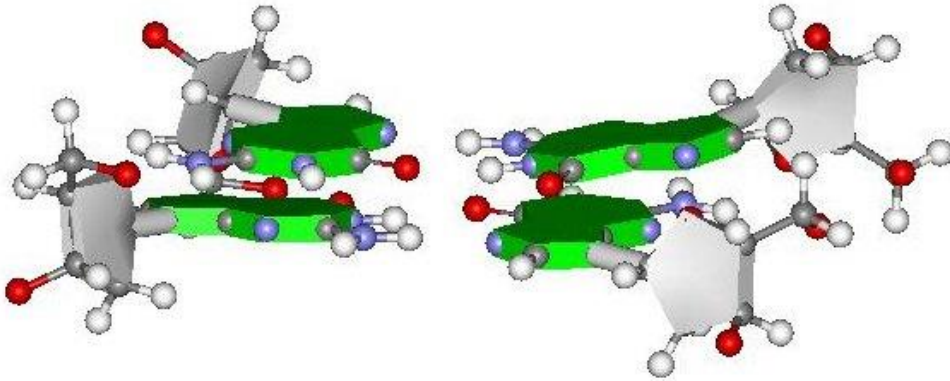




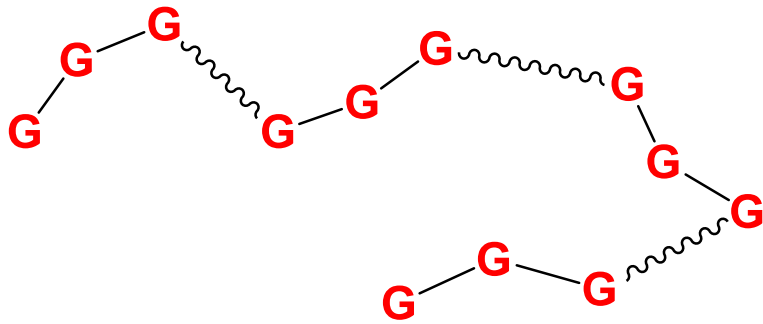
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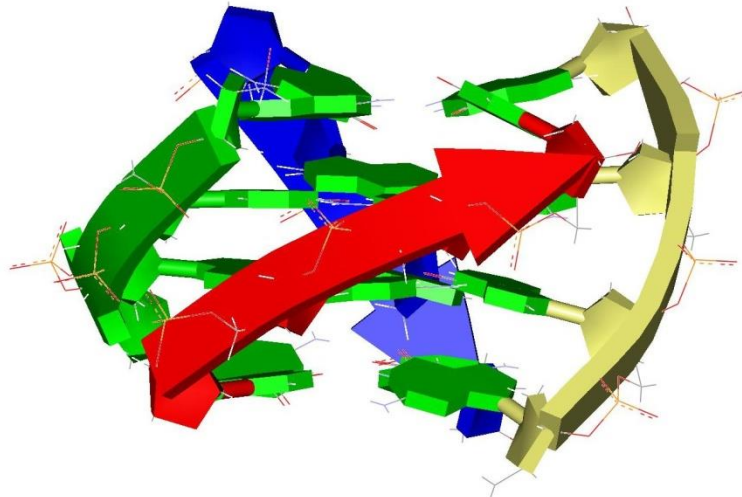
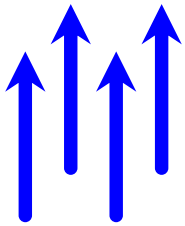
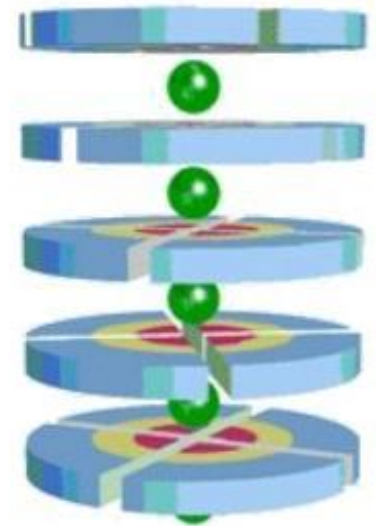
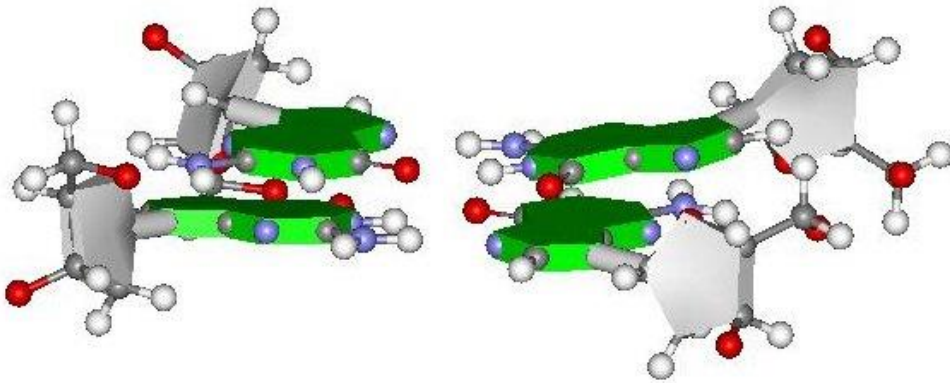
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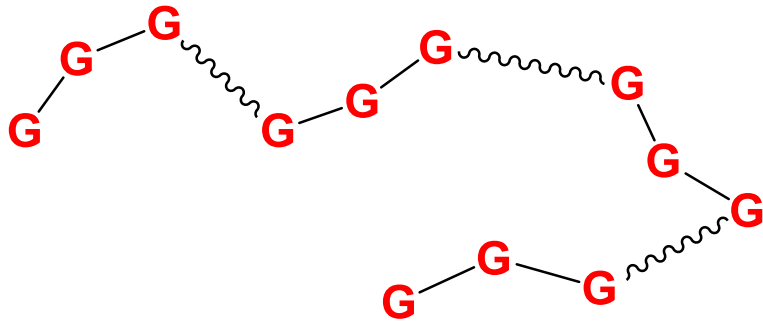
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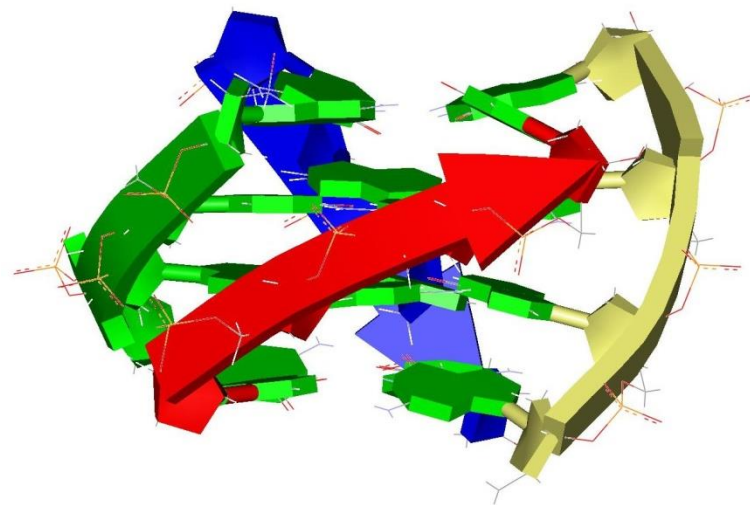
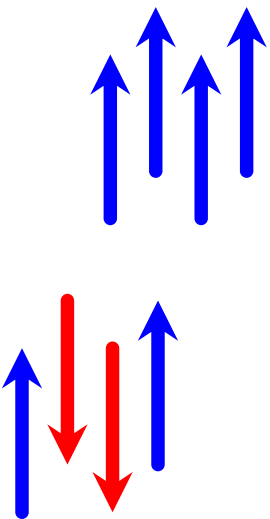
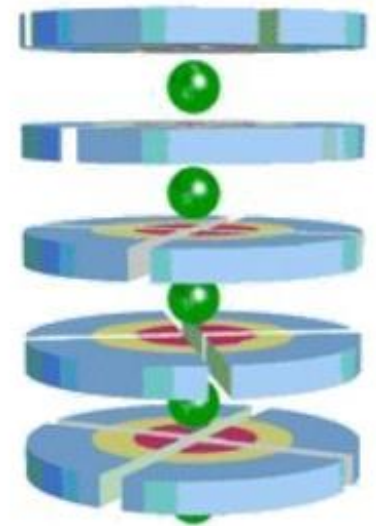
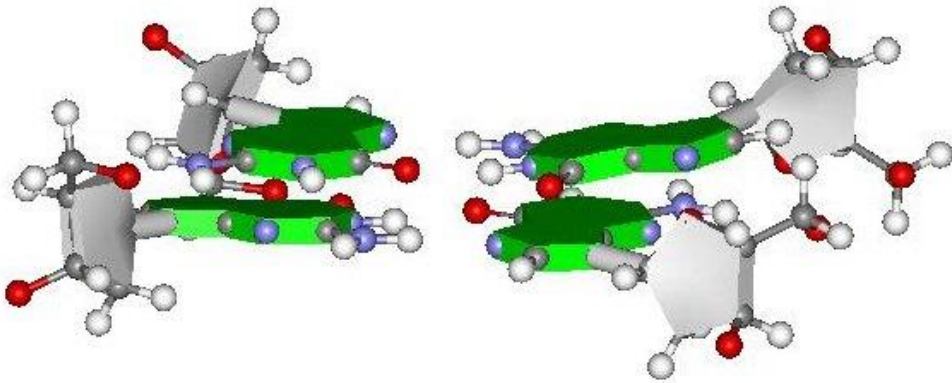
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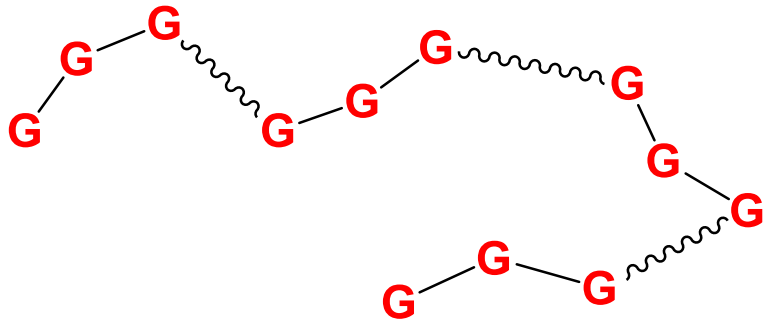
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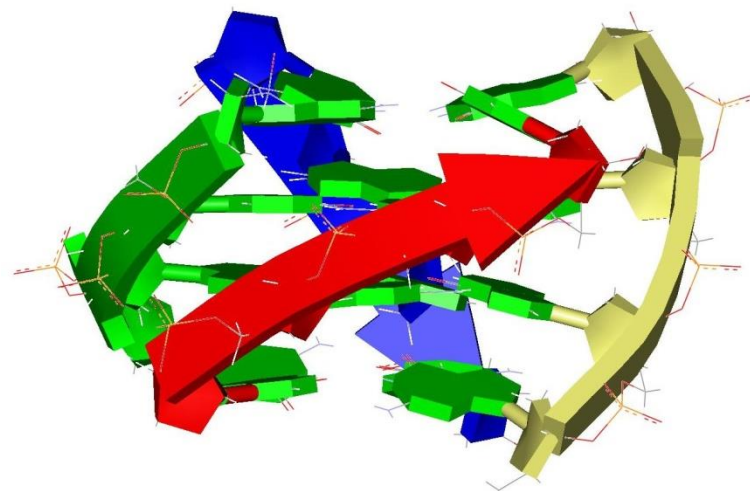
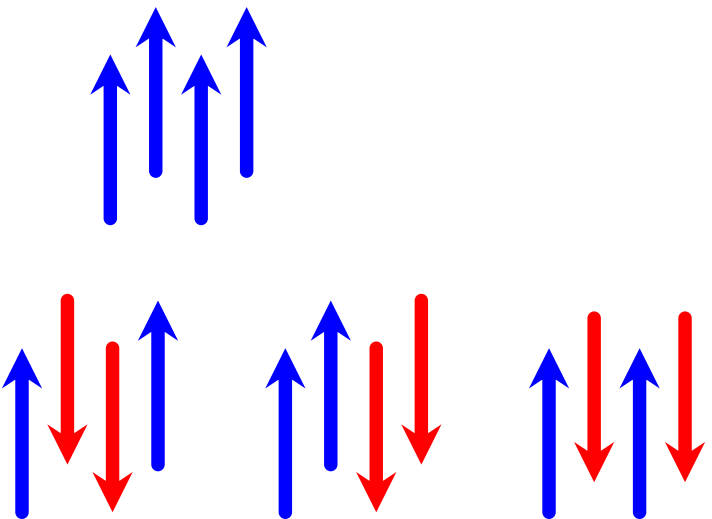
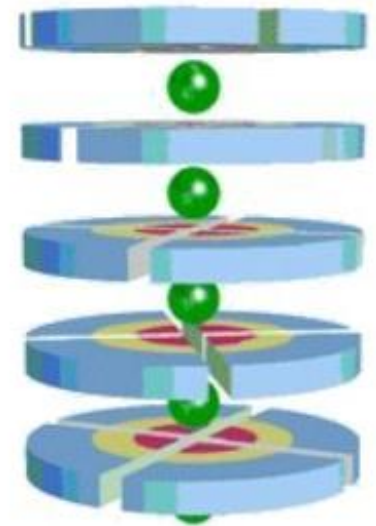
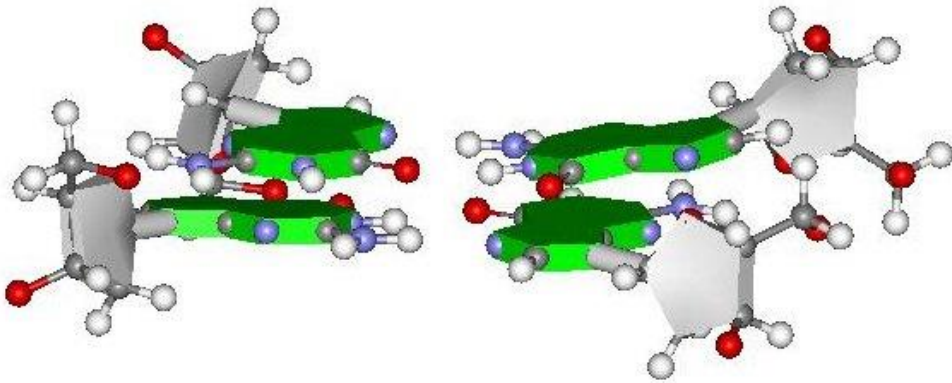
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# Guanine-quadruplexes

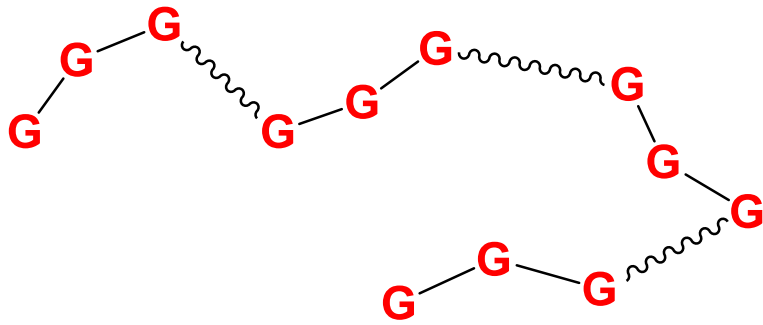


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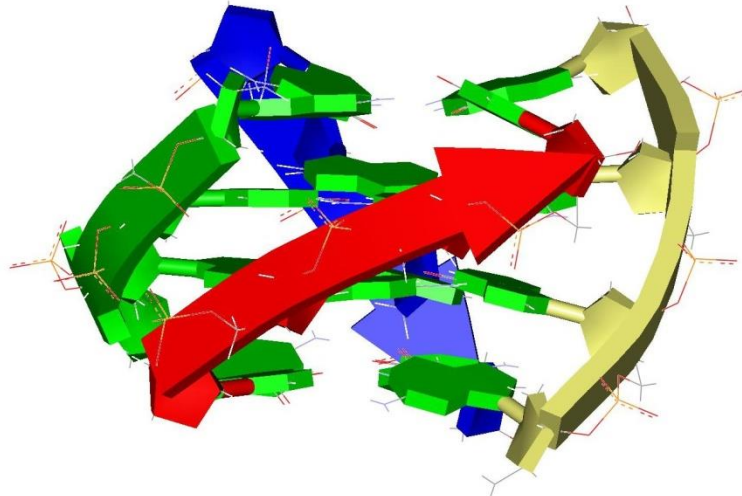
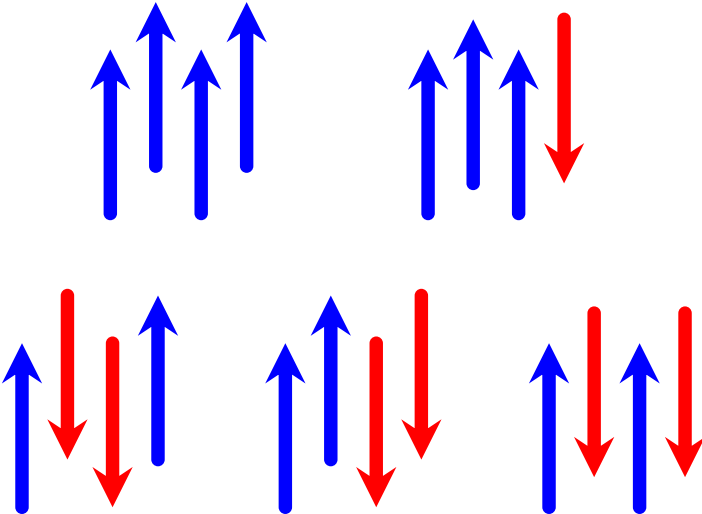
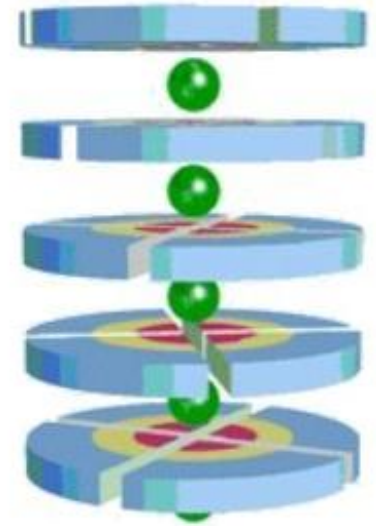
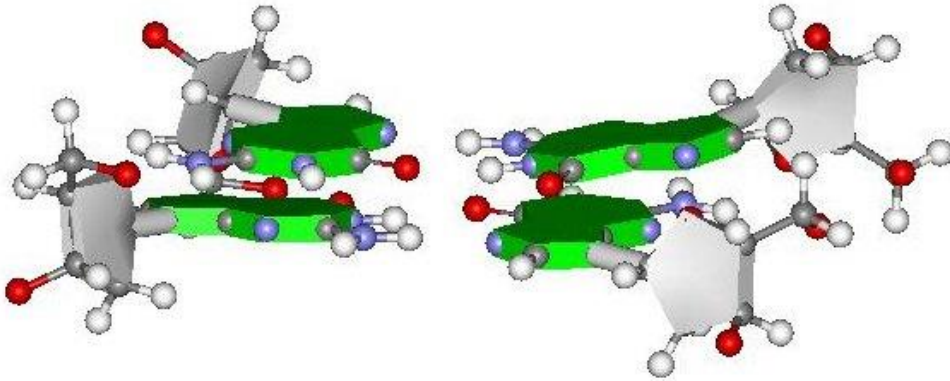




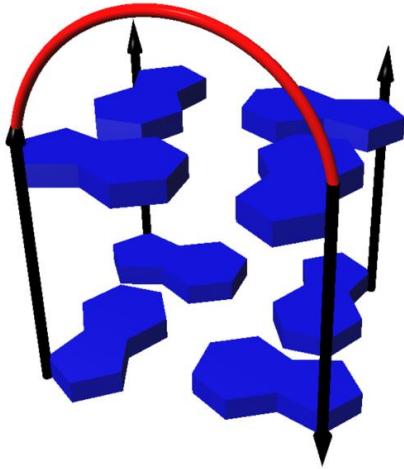
# Guanine-quadruplexes



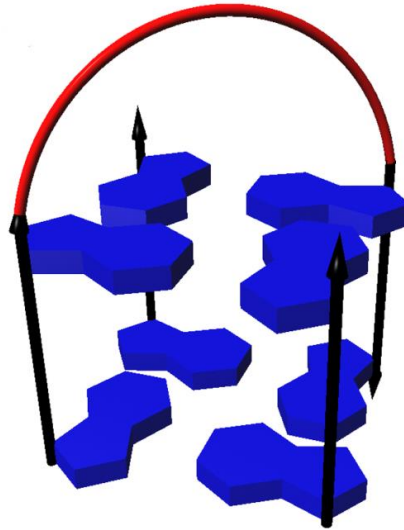
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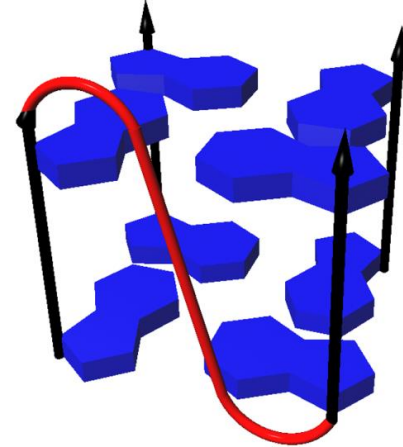
## Connecting loops



edge wise loop

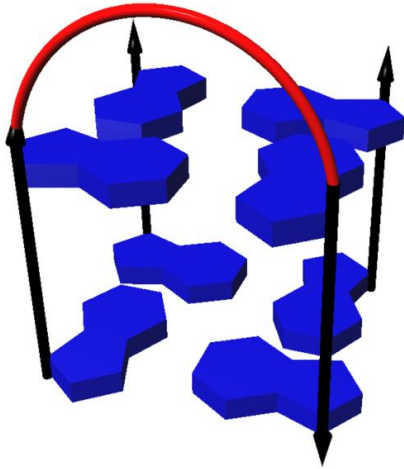


diagonal loop

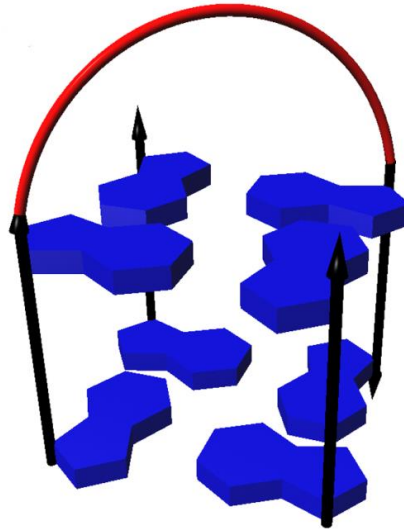


double chain reversal loop

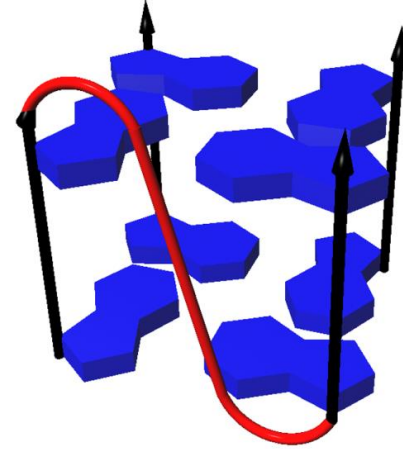
## Connecting loops



edge wise loop

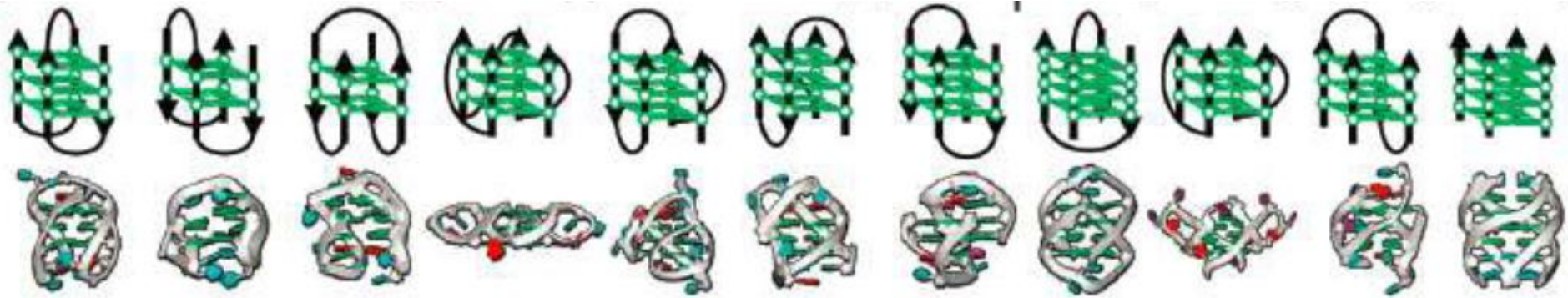


diagonal loop



double chain reversal loop

## Polymorphism of G-quadruplexes



Unimolecular fold

Bimolecular fold

Tetramolecular  
parallel  
topology

antiparallel topology

parallel  
topology

mixed topology

antiparallel topology

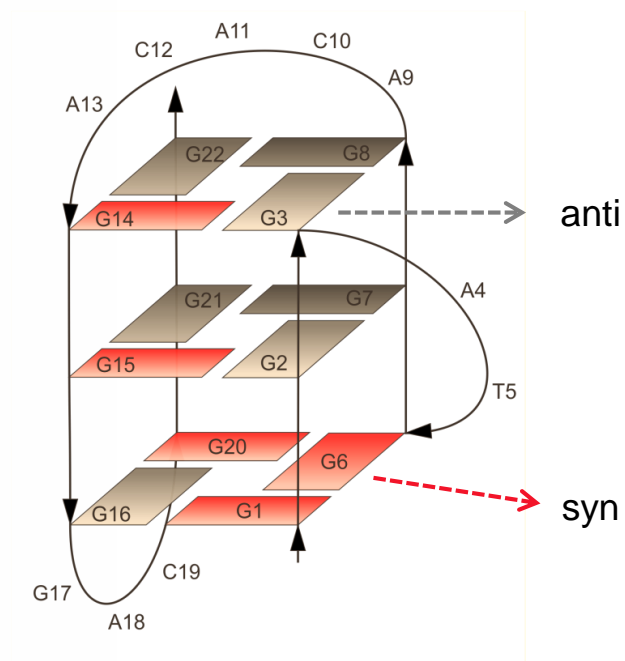
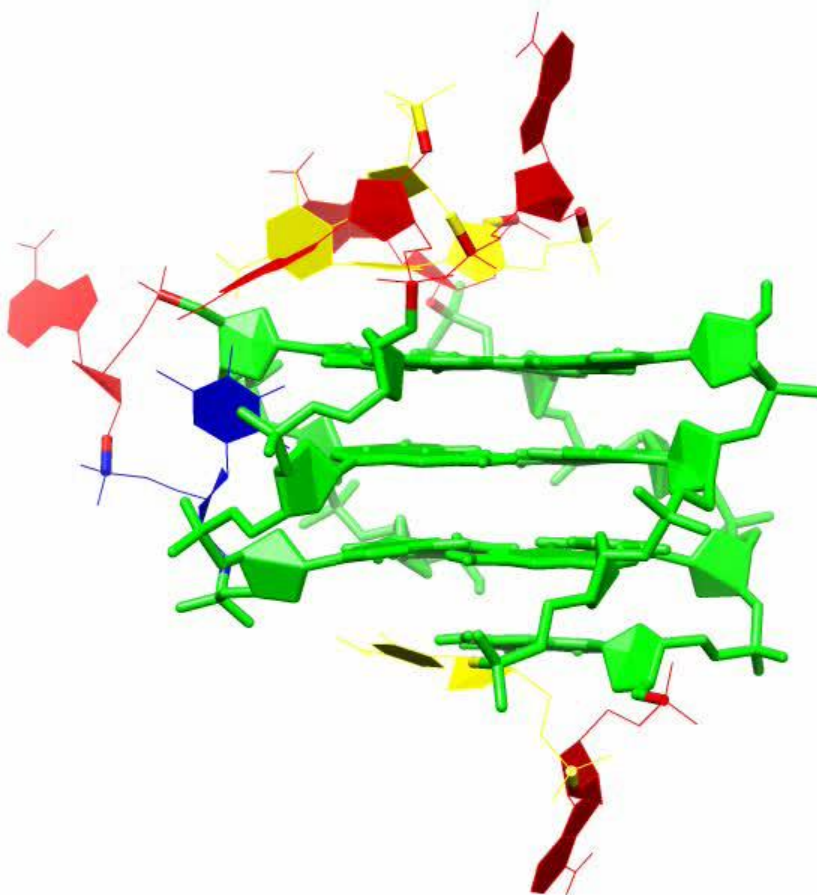
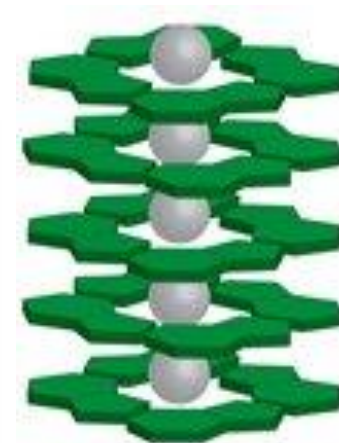
parallel  
topology

mixed  
topology

# Loop orientations in G-quadruplexes

$G_{3-5} N_{L1} G_{3-5} N_{L2} G_{3-5} N_{L3} G_{3-5}$

$1 < N_{L1-3} < 7$

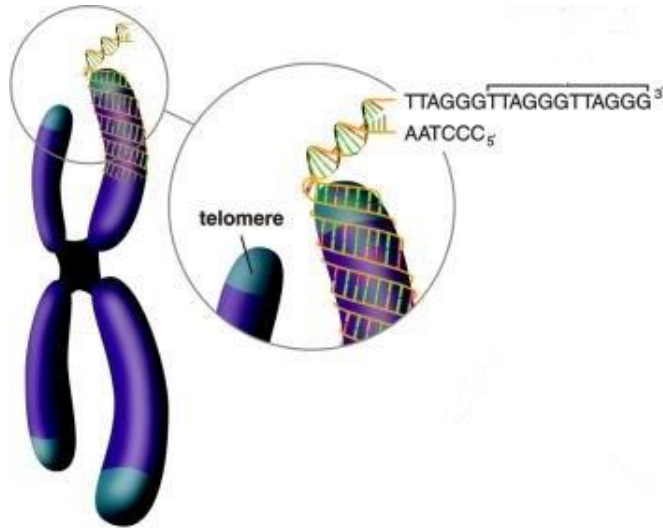


pdb id: 2LOD



# G-quadruplex(es) in telomeric ends

Multiple repeats of short G-rich units in **telomers** are located at the end of chromosomes; *e.g.* d(TTAGGG) in mammals, d(TTTTGGGG) in *Oxytricha*.

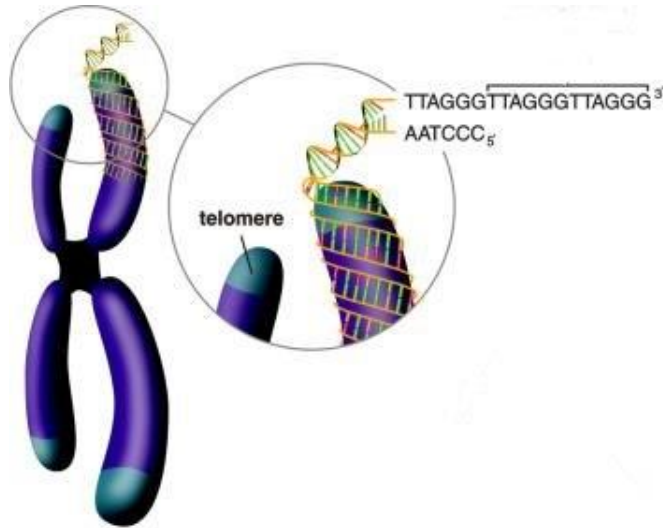


Telomeres are protein-DNA complexes, which protect the chromosomes against nuclease degradation, recombination and end-to-end fusion.

DNA polymerases can not replicate the ends of linear molecules and 50–200 bases of telomeric DNA is lost on each cell division – ***molecular clock***

# G-quadruplex(es) in telomeric ends

Multiple repeats of short G-rich units in **telomers** are located at the end of chromosomes; *e.g.* d(TTAGGG) in mammals, d(TTTTGGGG) in *Oxytricha*.



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Many cancer cells become immortal by a mechanism of upregulating telomerase.

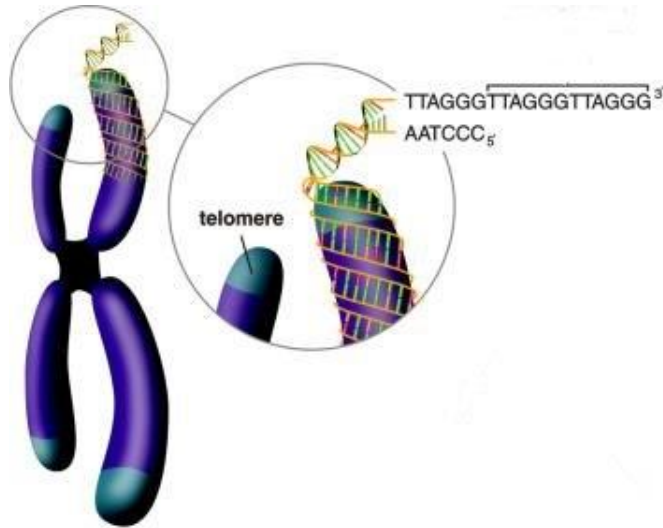
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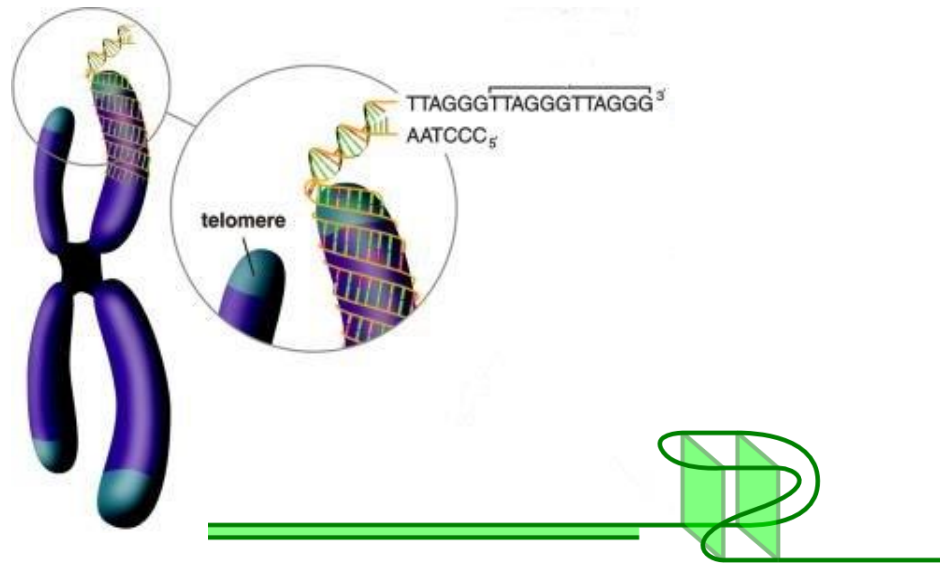
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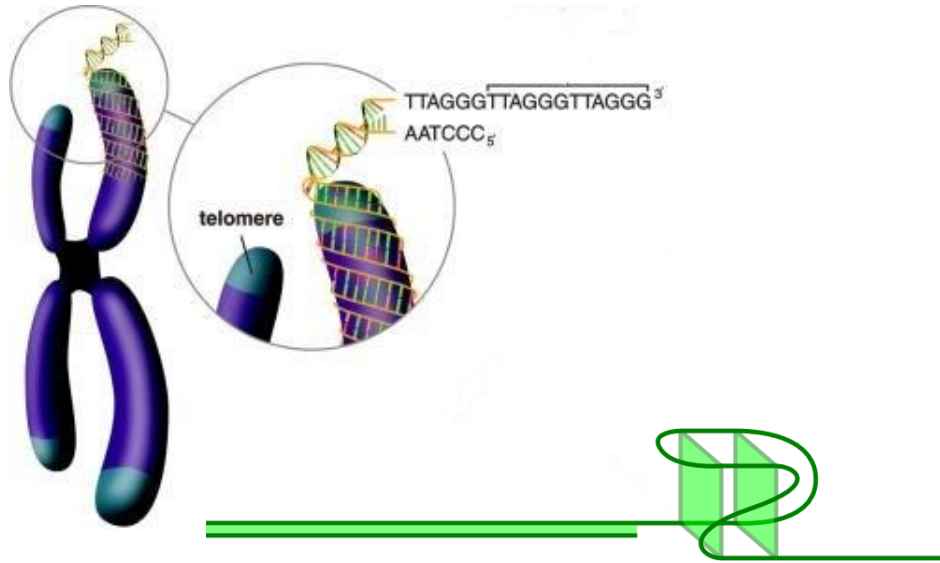
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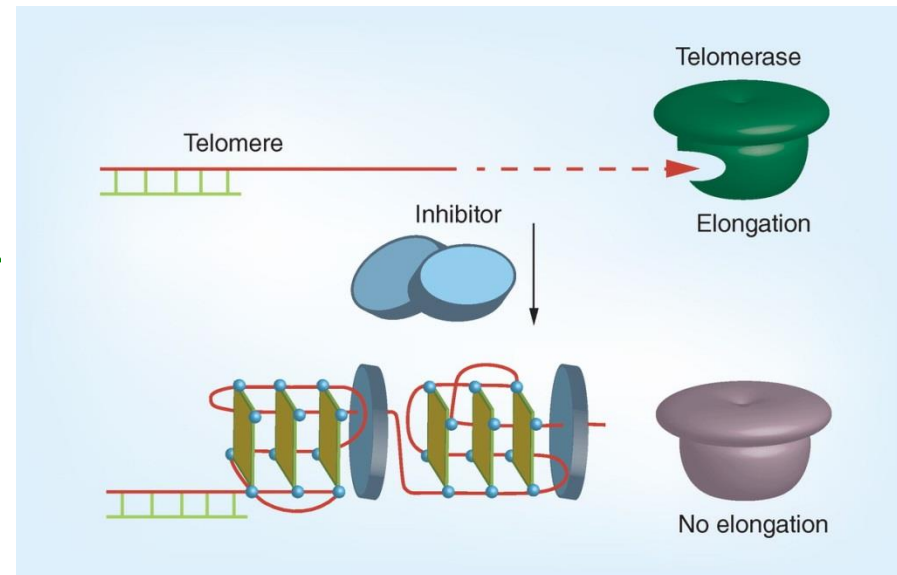


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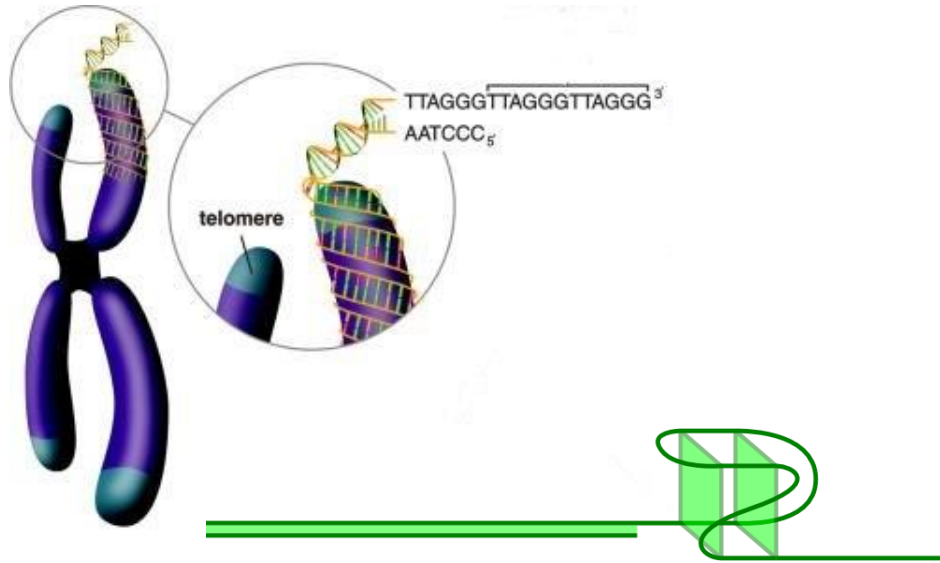
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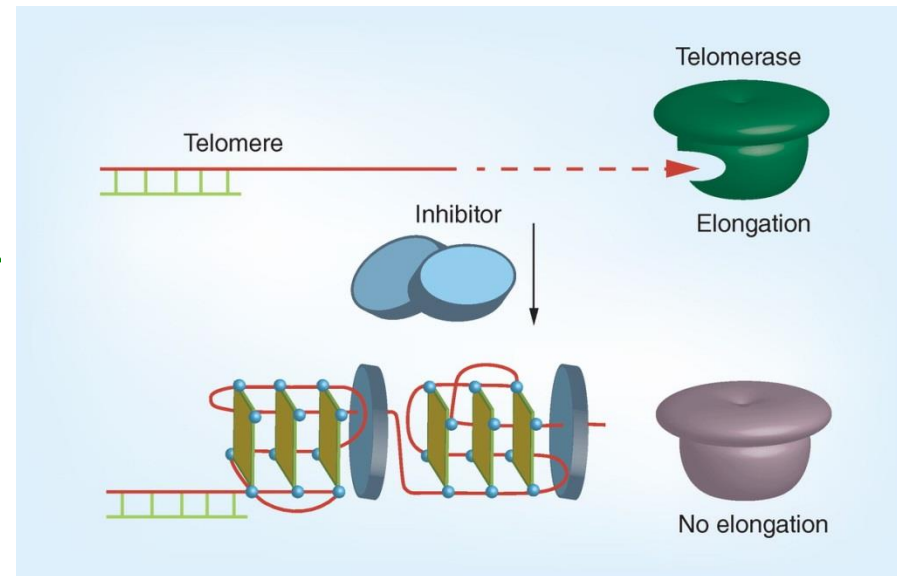


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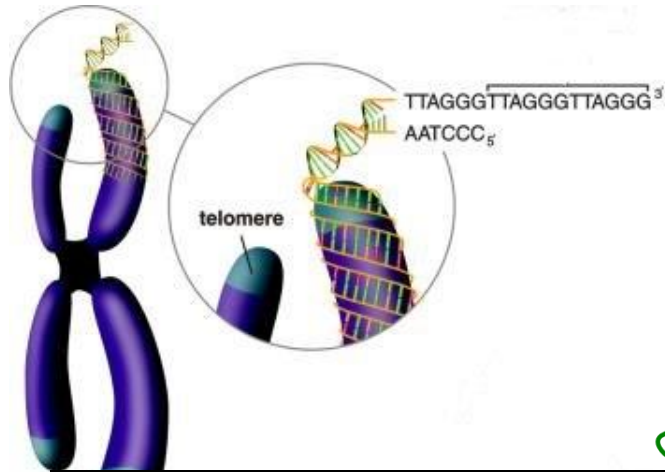
Small molecular drugs stabilize G-quadruplex and down-regulate telomerase maintenance in tumor cells → **cancer therapy**

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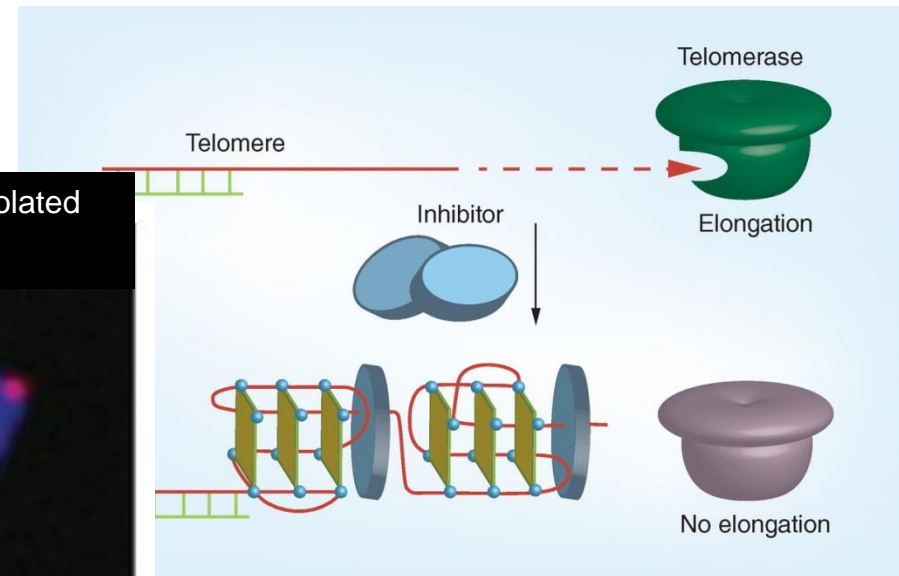
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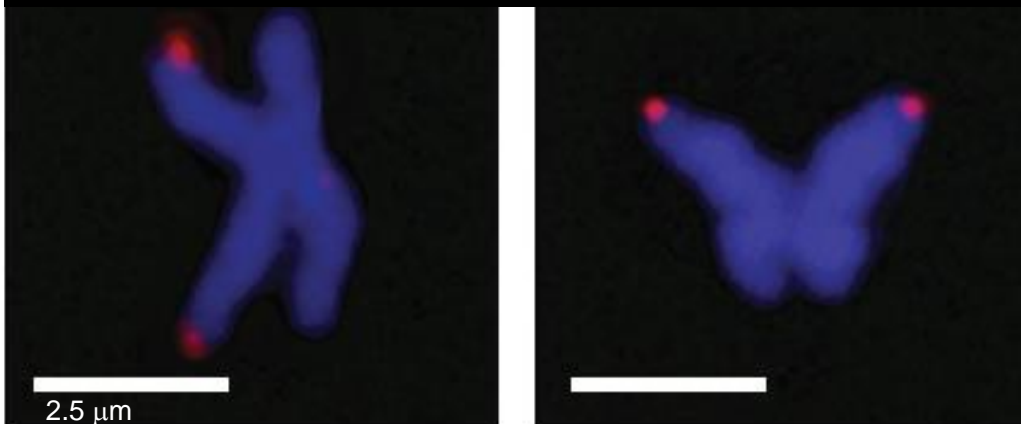
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Immunofluorescence for BG4 on metaphase chromosomes isolated from HeLa cervical cancer cells

Telo  
chr  
and  
DN  
mol  
cell



ecular drugs stabilize G-quadruplex and down-  
telomerase maintenance in tumor cells → **cancer**

# Prevalence of quadruplex motifs in the human genome

$G_{3-5} N_{L1} G_{3-5} N_{L2} G_{3-5} N_{L3} G_{3-5}$

$1 < N_{L1-3} < 7$

GGGCCTGGGGCTGGGCCTGGG  
GGGCCTGGGGCTGGGCCTGGG

GGGCCTGTCAAGGGG GGGGGGGCAAGGG

GGGCCTGTT GGGGG GGGGGG cTAGGGG



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376 000 putative quadruplex sequences

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a density of 0.13/kb in genomic DNA

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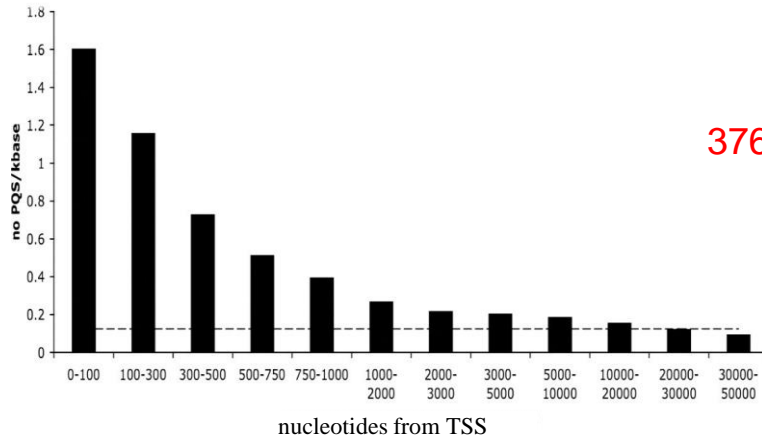
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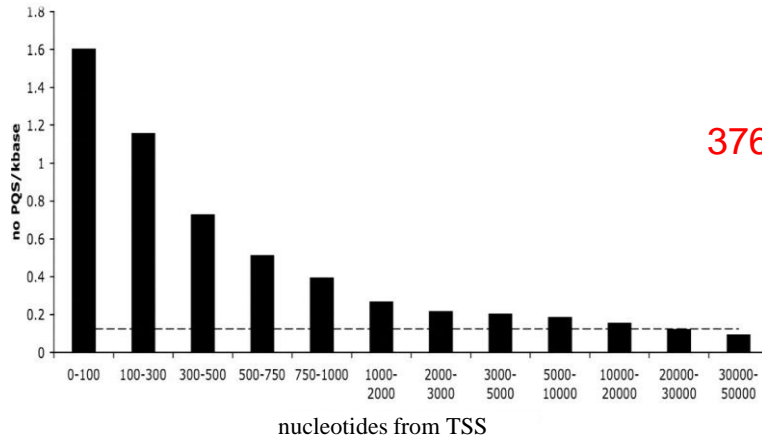
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GGGCCTGGGGCTGGGCCTGGG

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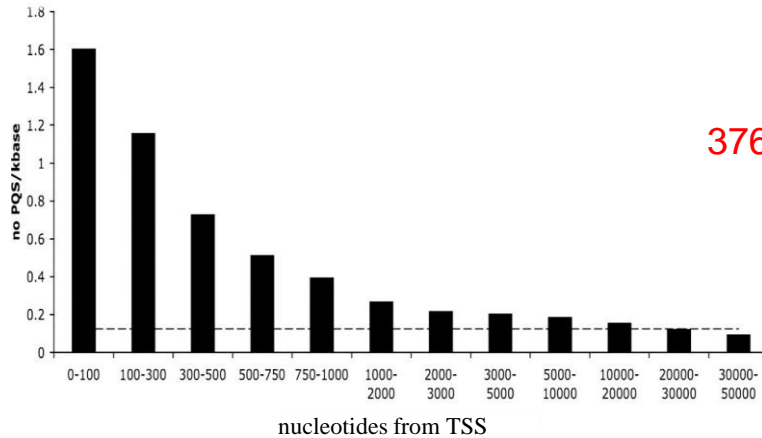
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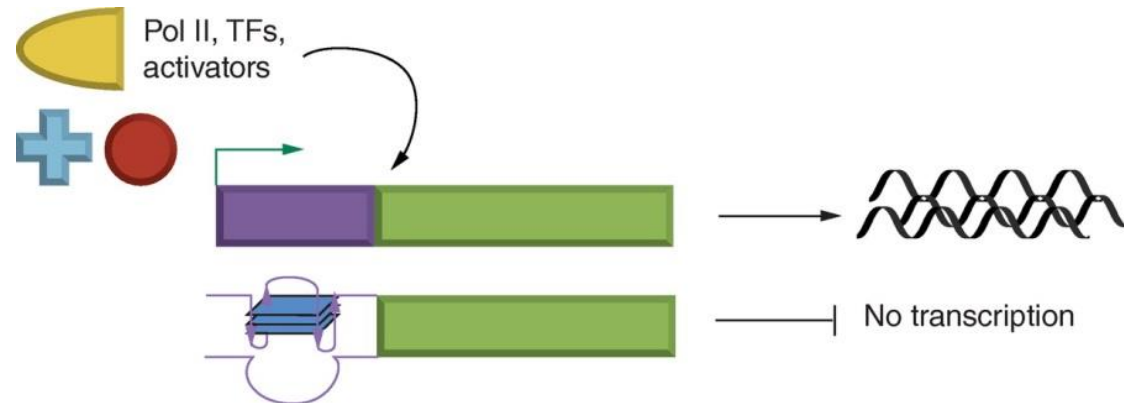
GGGCCTGTT GGGGGGG GGGGGGG cTAGGGG



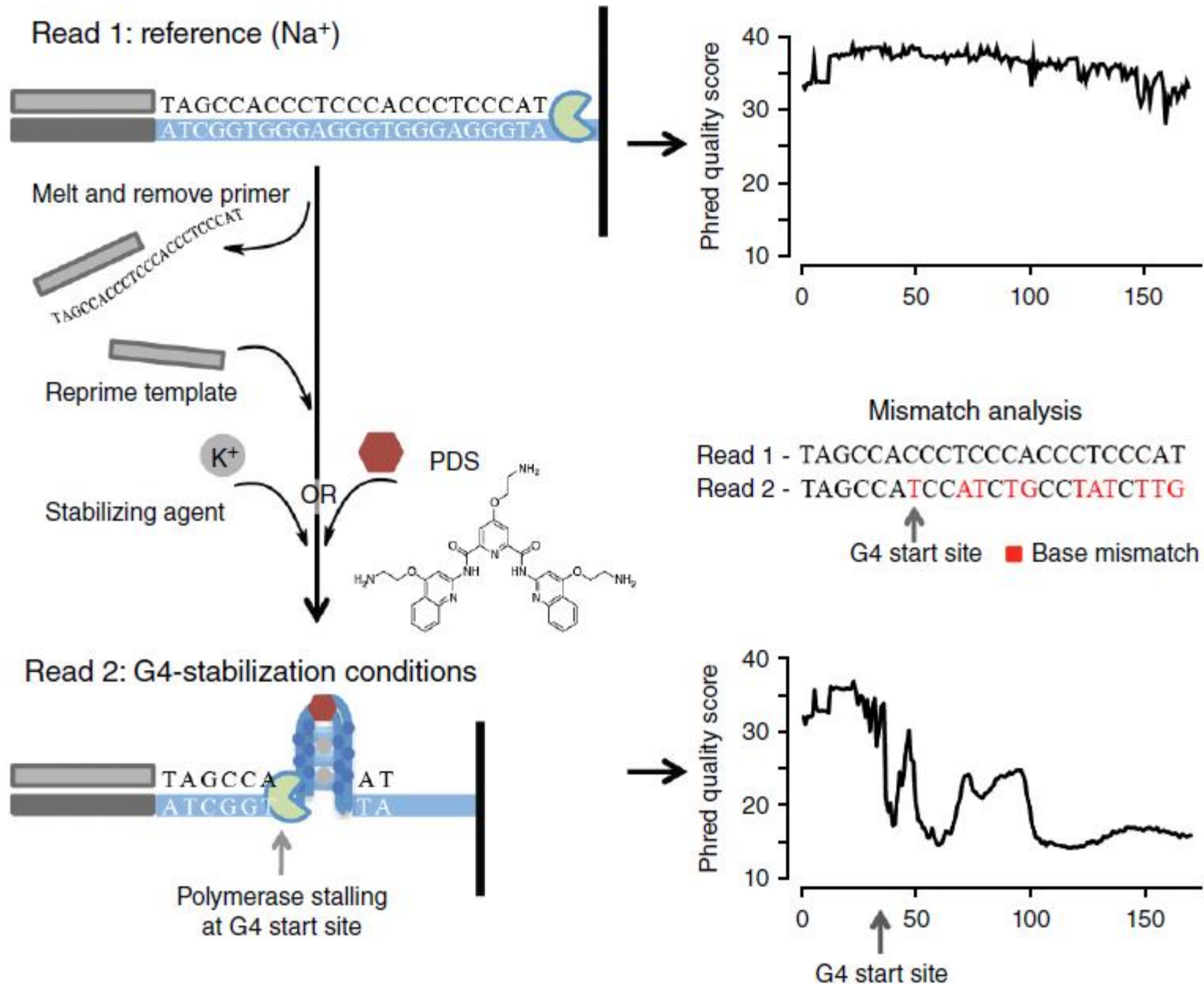
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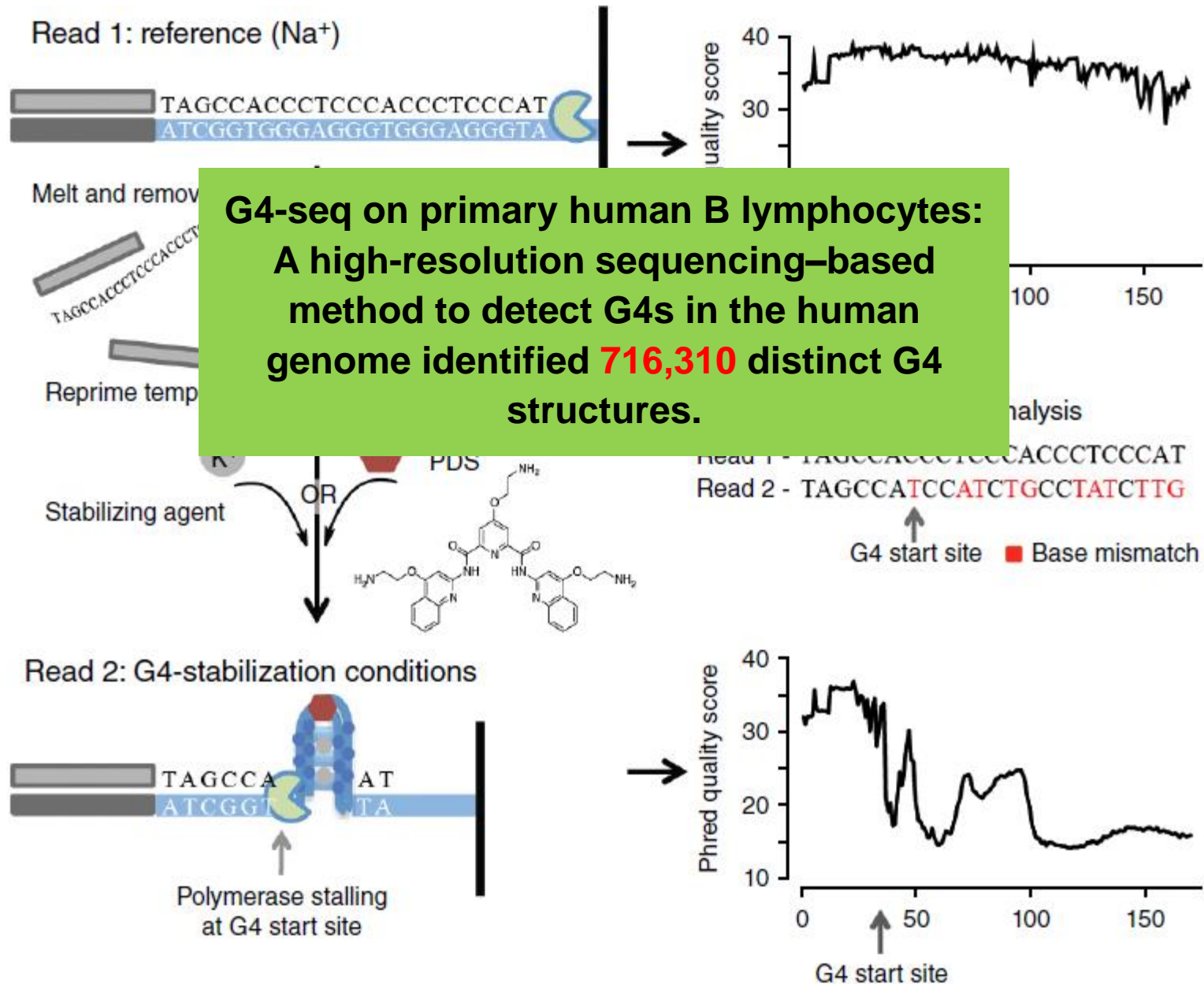
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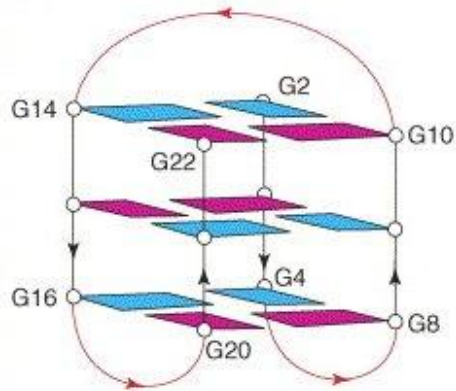


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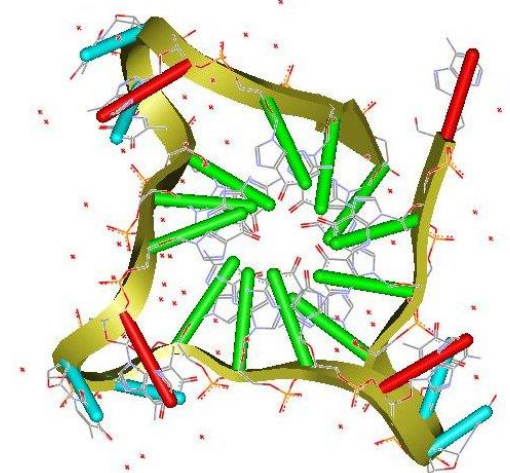
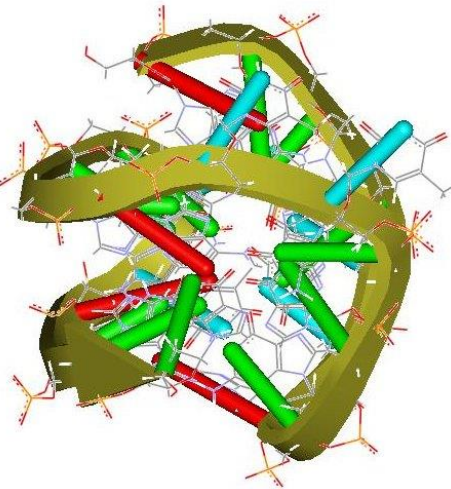
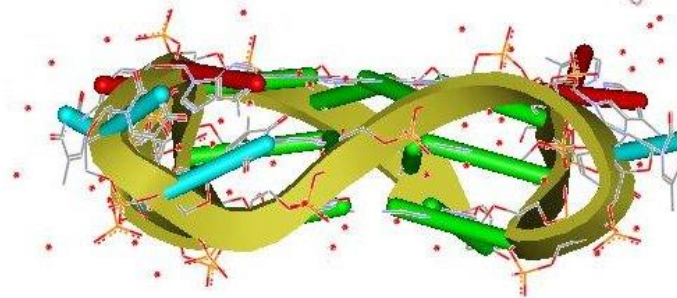
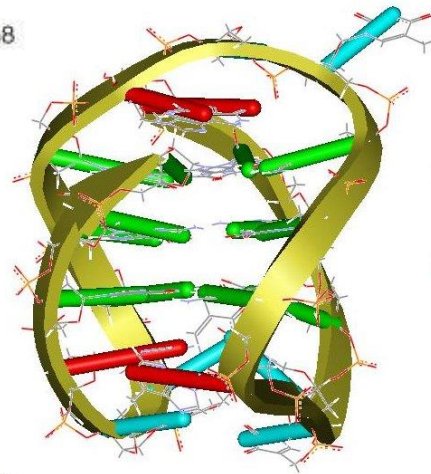
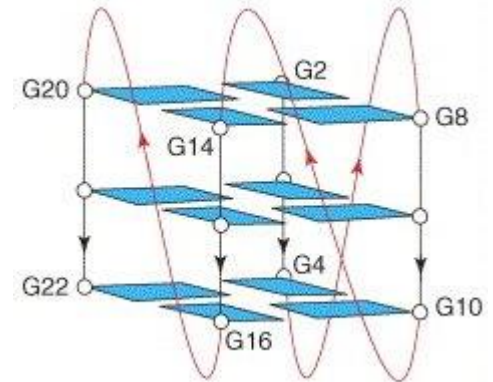


# The importance of cations: $d[AGGG(TTAGGG)_3]$

$\text{Na}^+$  structure in solution



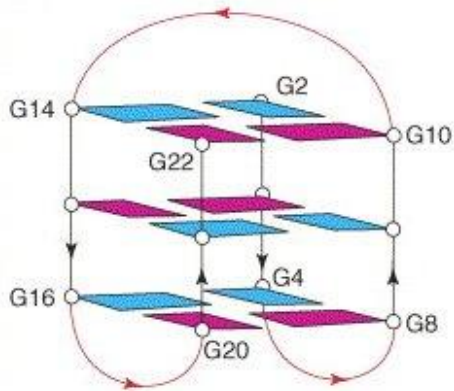
$\text{K}^+$  structure in the crystal



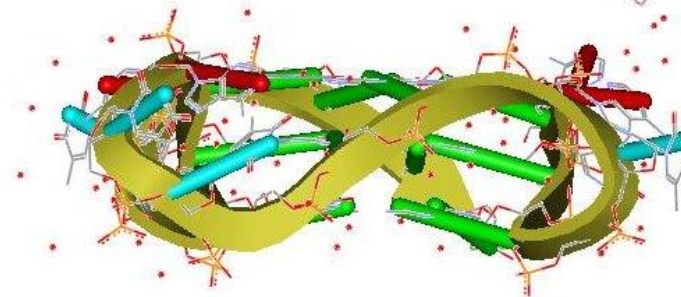
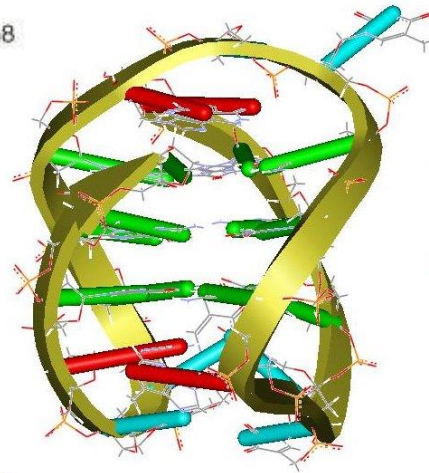
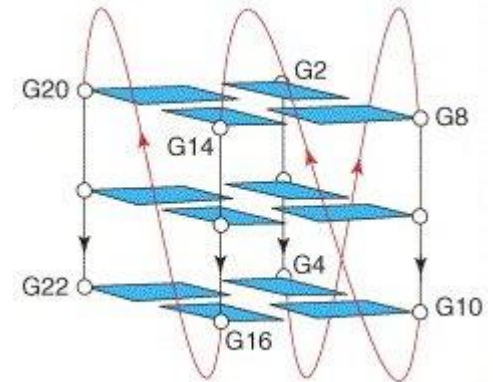


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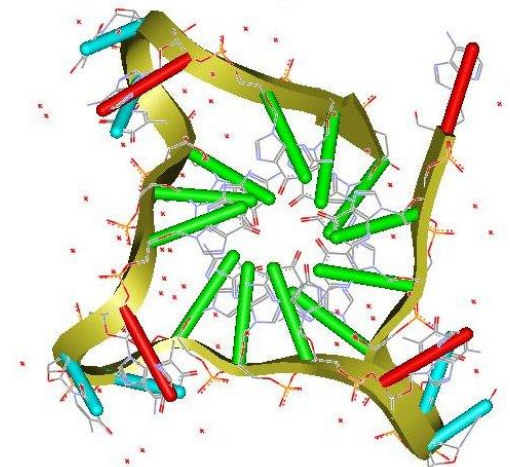
$Na^+$  structure in solution



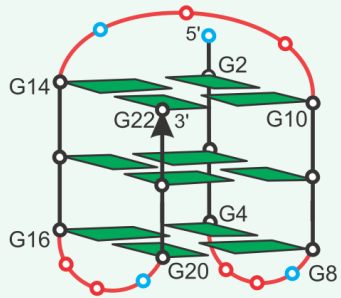
$K^+$  structure in the crystal



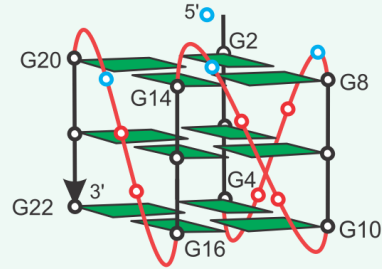
NOTE: different  $K^+$  structures in solution



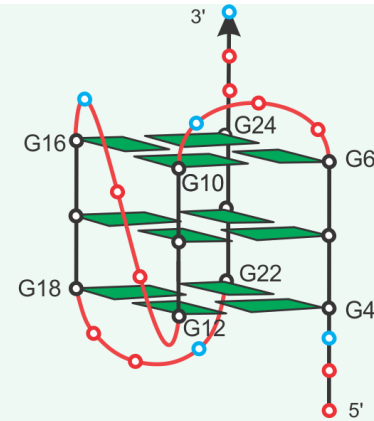
# Polymorphism of human telomer repeat: d[(TTAGGG)<sub>4</sub>]



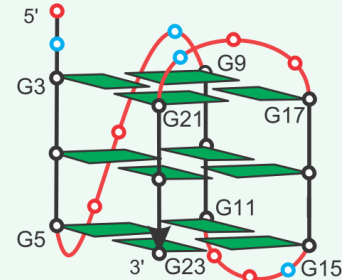
d(AGGG(TTAGGG)<sub>3</sub>)  
Na<sup>+</sup> ions, NMR



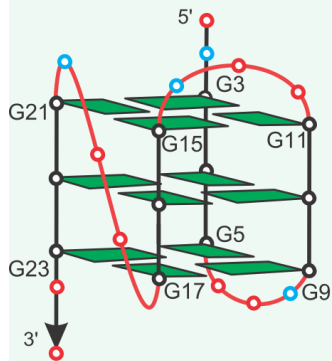
d(AGGG(TTAGGG)<sub>3</sub>)  
K<sup>+</sup> ions, X-ray



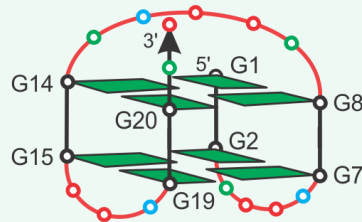
d((TTAGGG)<sub>4</sub>TTA)  
Na<sup>+</sup> ions, NMR



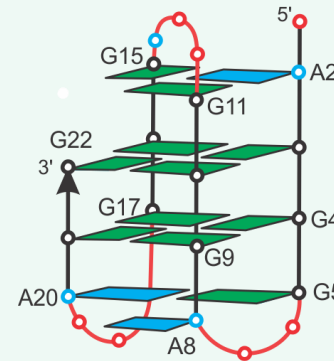
d(TAGGG(TTAGGG)<sub>3</sub>)  
K<sup>+</sup> ions, NMR (htel 1)



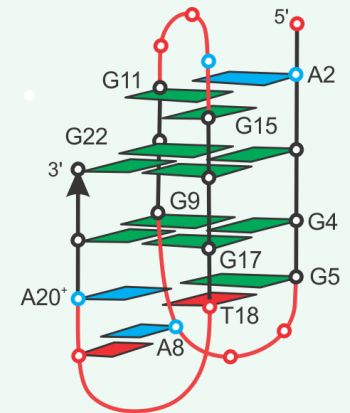
d(TAGGG(TTAGGG)<sub>3</sub>TT)  
K<sup>+</sup> ions, NMR (htel 2)



d(GGG(TTAGGG)<sub>3</sub>T)  
K<sup>+</sup> ions, NMR (form 3)



d(TAGGG(TTAGGG)<sub>2</sub>TTAGG)  
K<sup>+</sup> ions, NMR (TD)

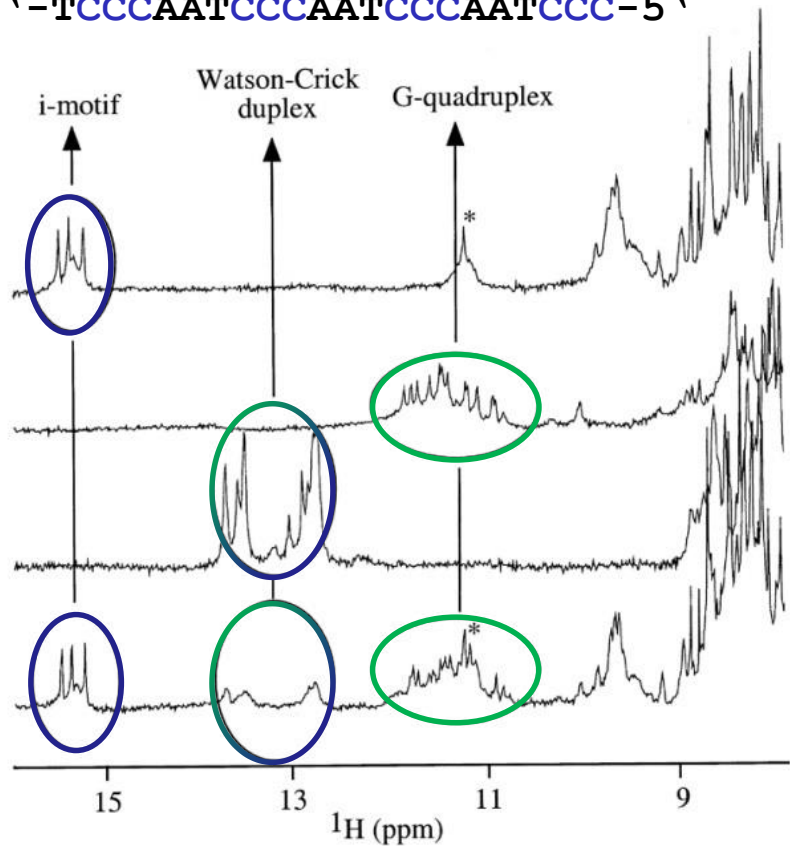


d(TAGGG(TTAGGG)<sub>2</sub>TTAGG)  
K<sup>+</sup> ions, NMR (KDH<sup>+</sup>)

# Power of NMR in structural elucidation

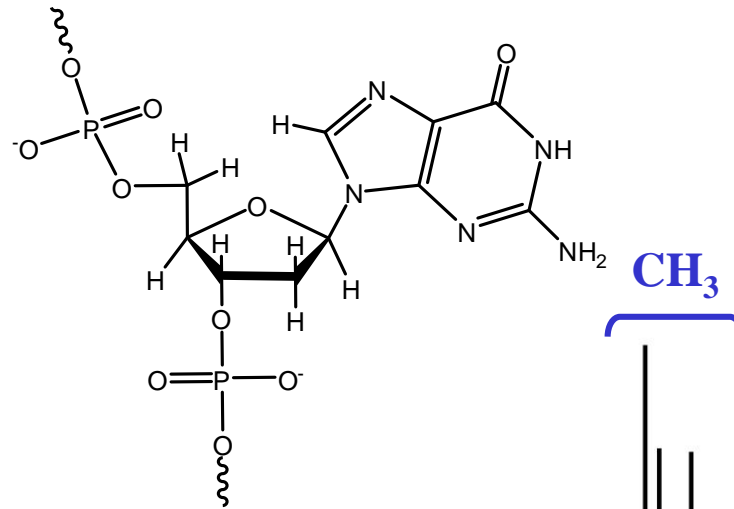
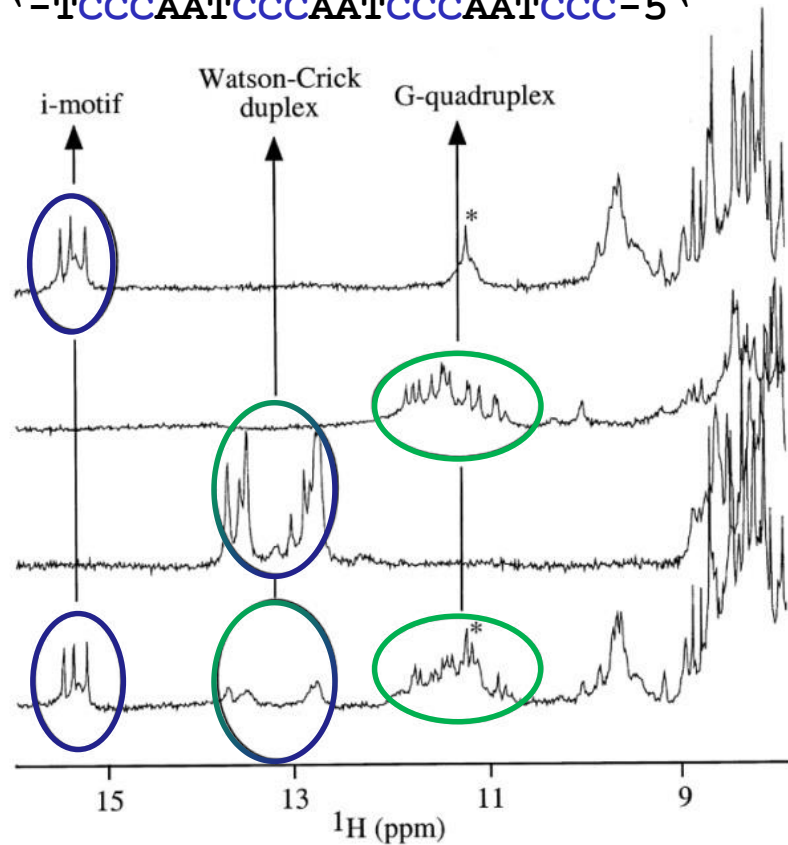
5' -AGGGTTAGGGTTAGGGTTAGGG-3'

3' -TCCCAATCCCAATCCCAATCCC-5'



# Power of NMR in structural elucidation

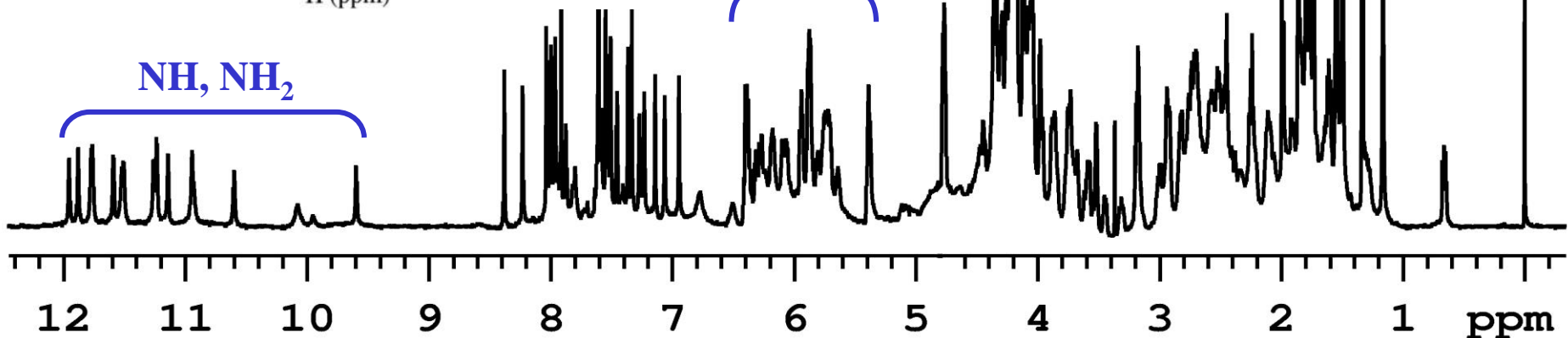
5' -AGGGTTAGGGTTAGGGTTAGGG-3'  
3' -TCCCAATCCCAATCCCAATCCC-5'



$\text{H3}', \text{H4}', \text{H5}',$   
 $\text{H5}'', \text{H2}', \text{H2}''$

$\text{H1}'$

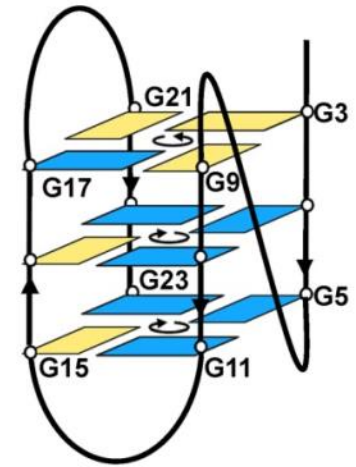
$\text{NH}, \text{NH}_2$





# htel1-ΔG23 - a four repeat human telomere sequence

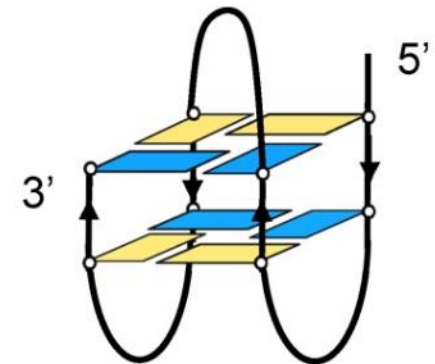
- Four-repeat human telomere sequence *htel1*  
5'-TAGGG TTAGGG TTAGGG TTAGGG-3'



hybrid-1

- Truncated version of the human telomere sequence *htel1-ΔG23*  
5'-TAGGG TTAGGG TTAGGG TTAGG\_-3'

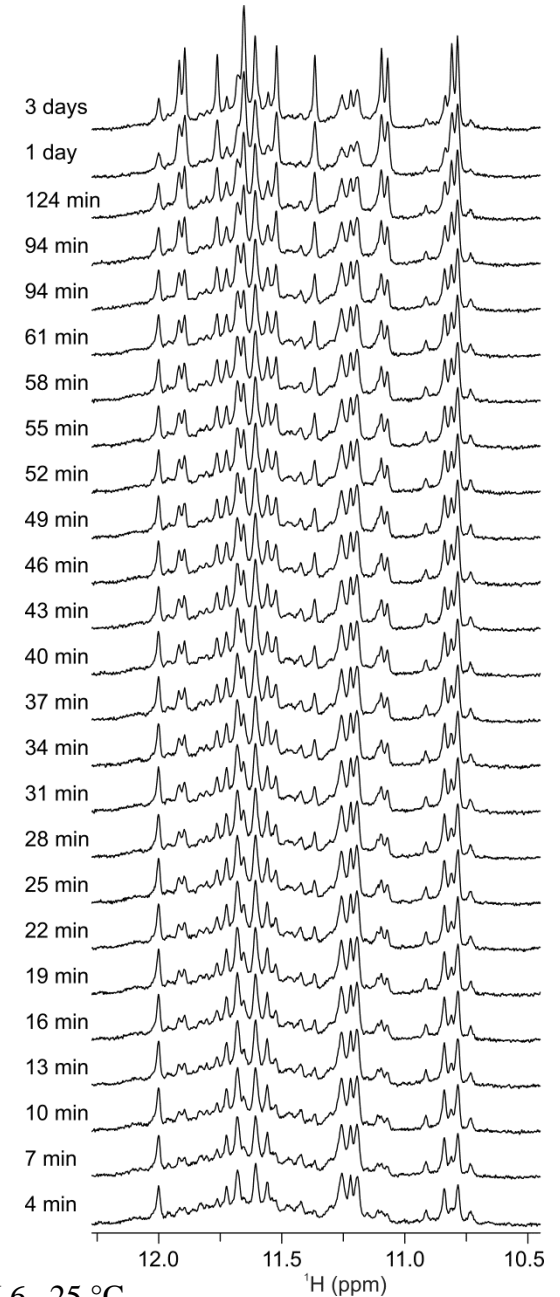
Expected topology



# Folding process of *htel1* in the presence of K<sup>+</sup> ions

*htel1*: 5'-TAGGG TTAGGG TTAGGG TTAGGG-3'

Intensity of the imino peaks of residues as a function of time.

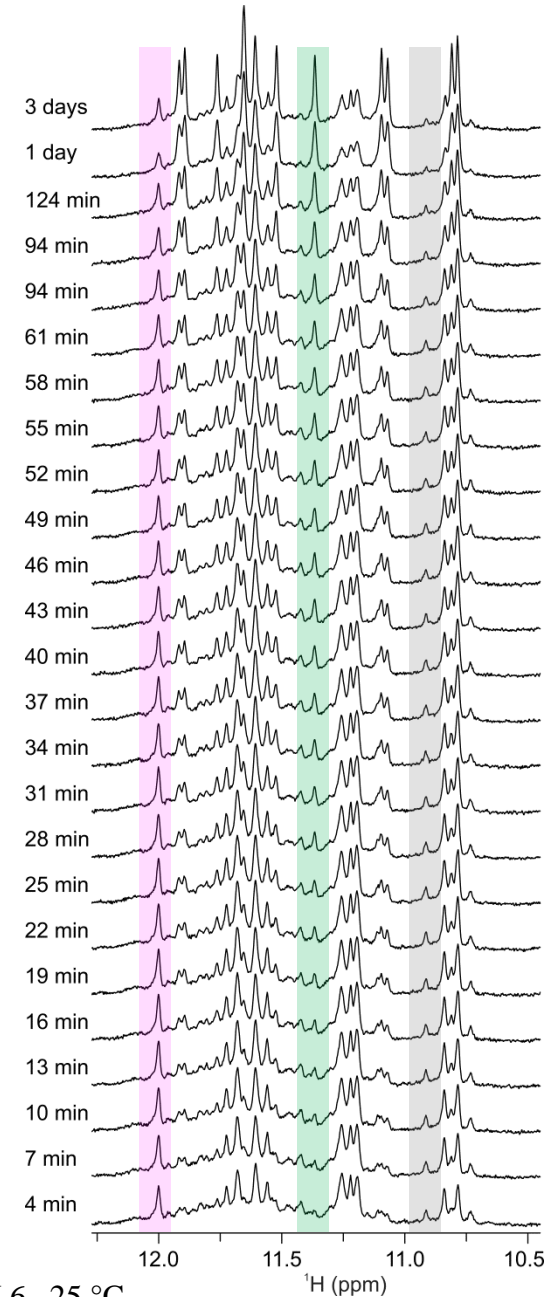


<sup>1</sup>H NMR spectra; c = 1 mM, 10 mM KCl, pH 6, 25 °C.

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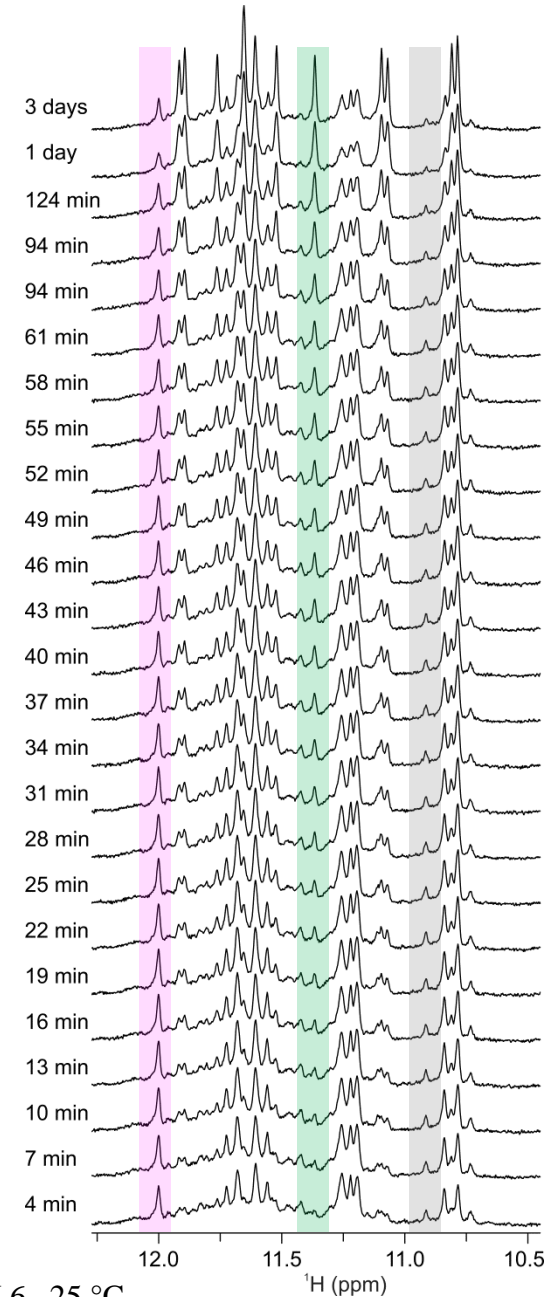
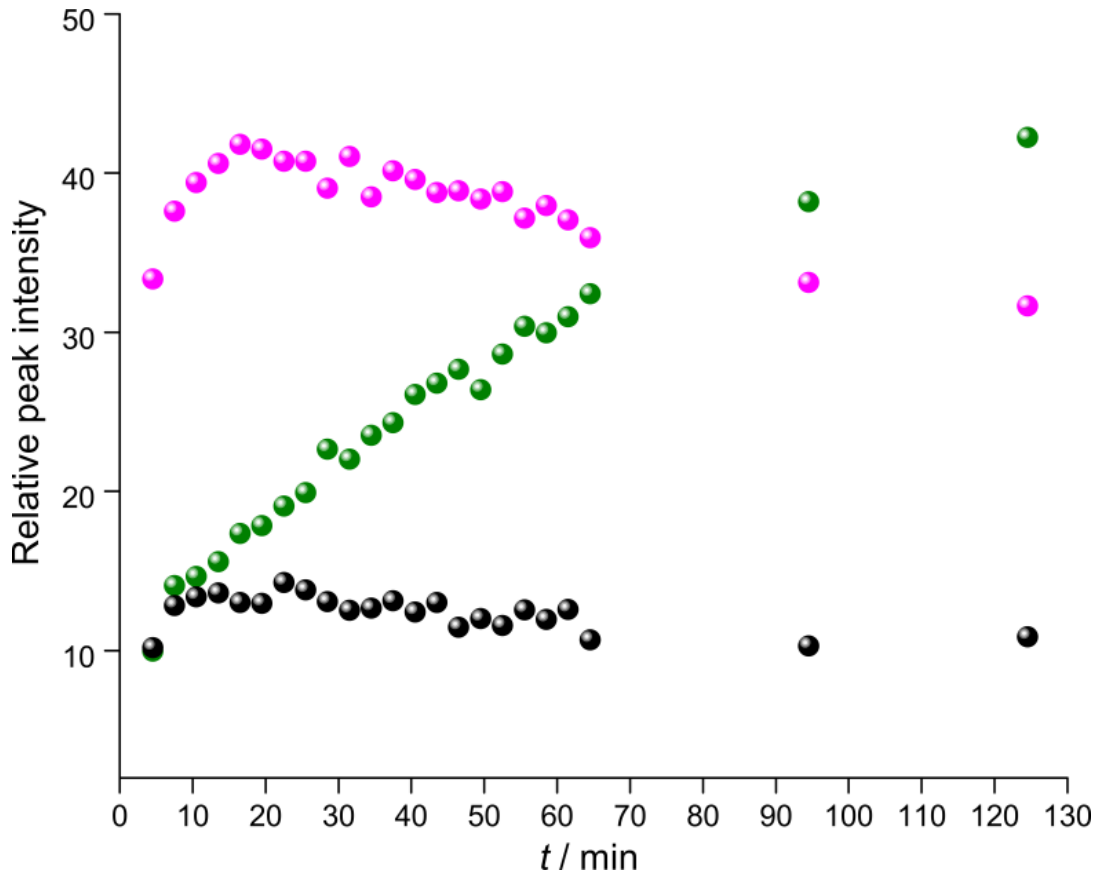


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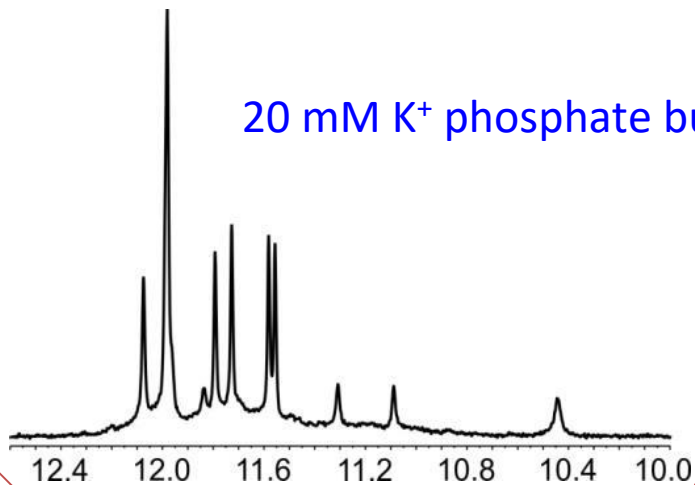
<sup>1</sup>H NMR spectra; c = 1 mM, 10 mM KCl, pH 6, 25 °C.



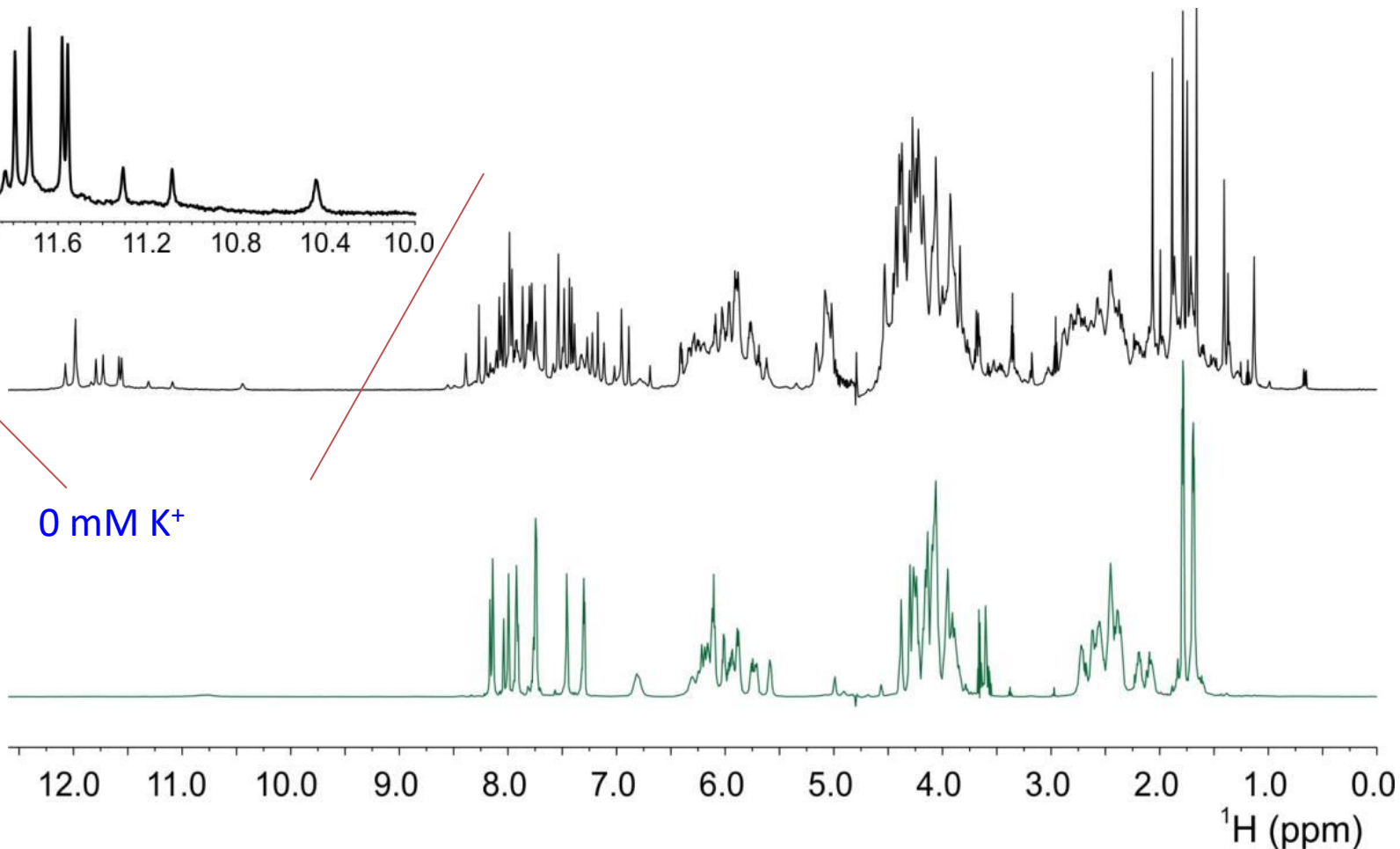
# Folding of *htel1-ΔG23* in the presence of K<sup>+</sup> ions

5'-TAGGG TTAGGG TTAGGG TTAGG\_-3'

20 mM K<sup>+</sup> phosphate buffer



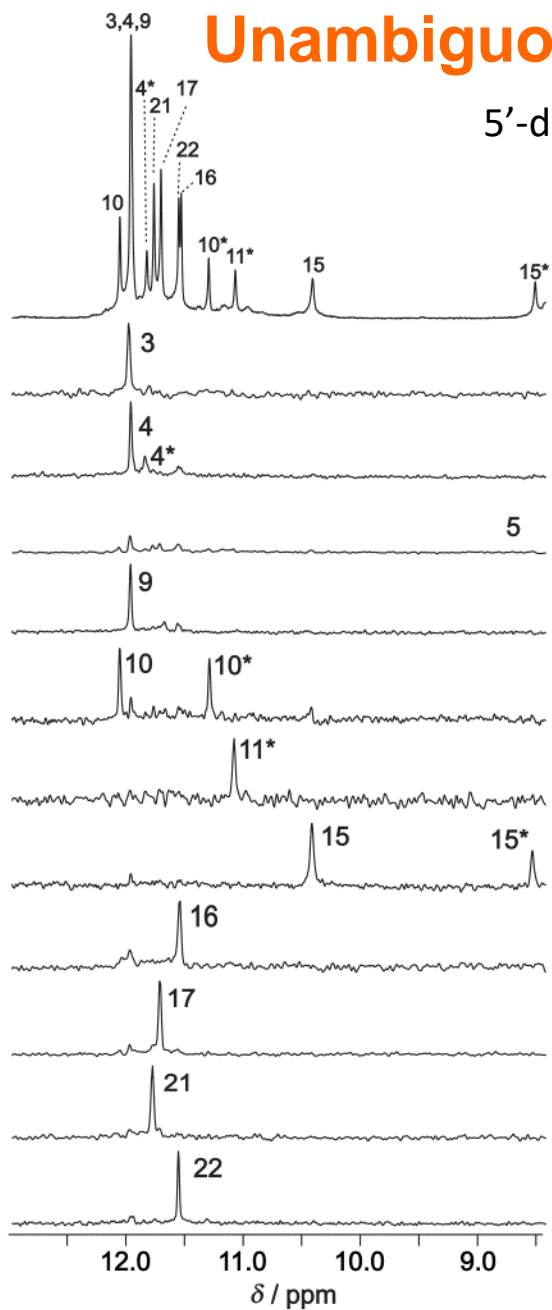
0 mM K<sup>+</sup>



<sup>1</sup>H NMR spectra, c = 1 mM per strand, pH 7, 25 °C.

# Unambiguous assignment of imino protons

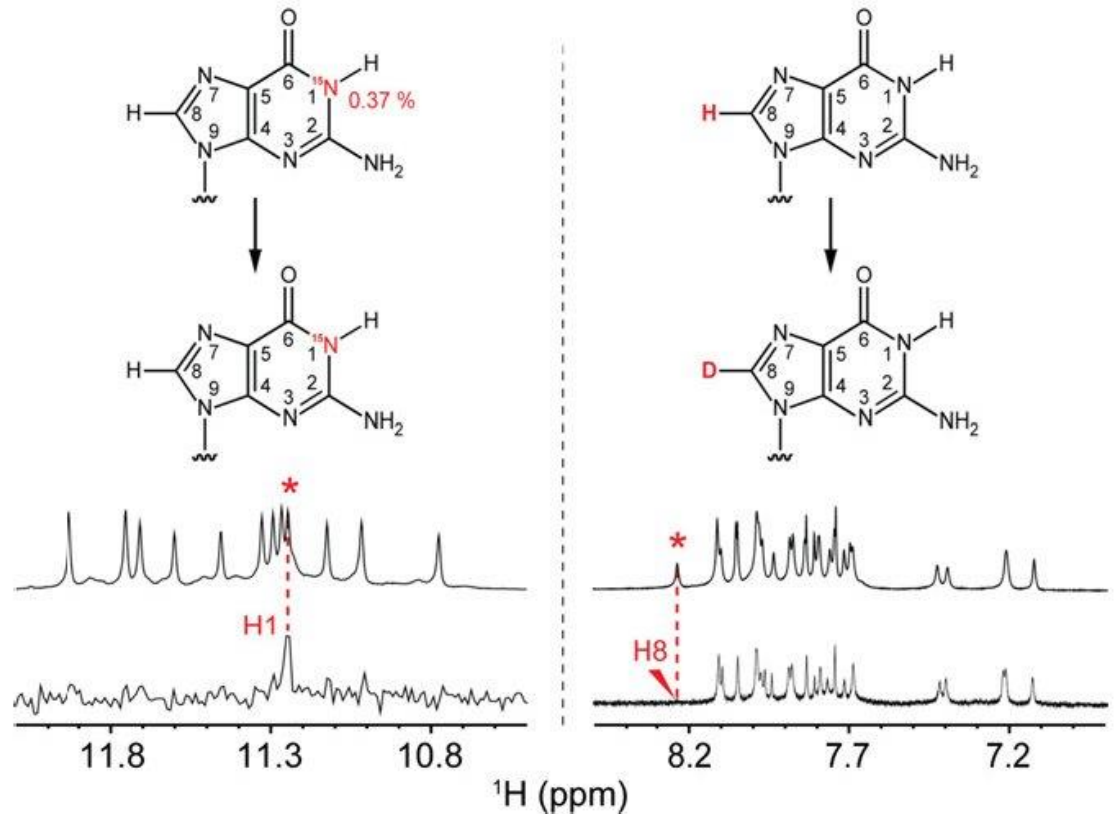
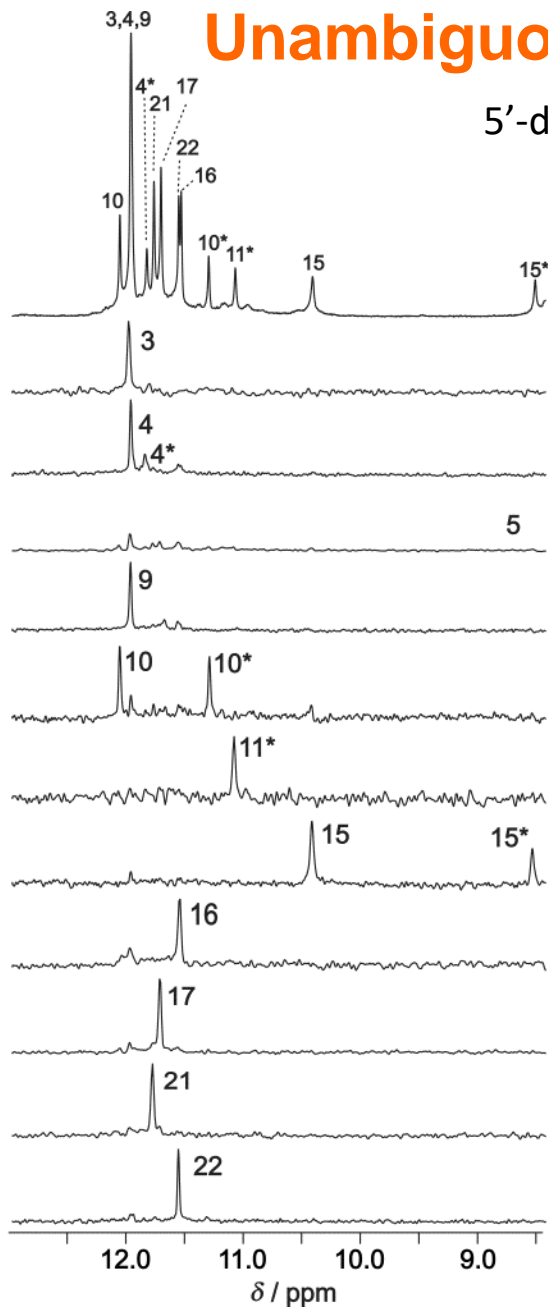
5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'



1D  $^{15}\text{N}$ -edited HSQC NMR spectra of guanine residue-specifically  $^{15}\text{N}$ ,  $^{13}\text{C}$  labelled (8% enriched) oligonucleotides.

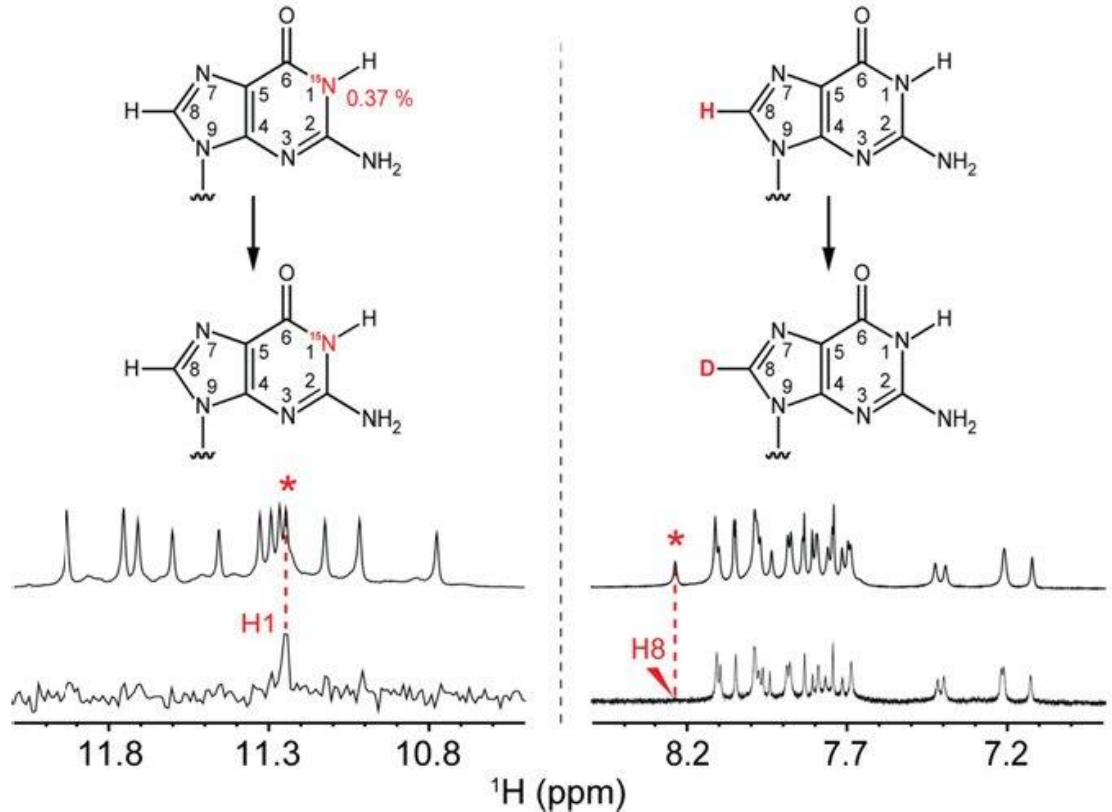
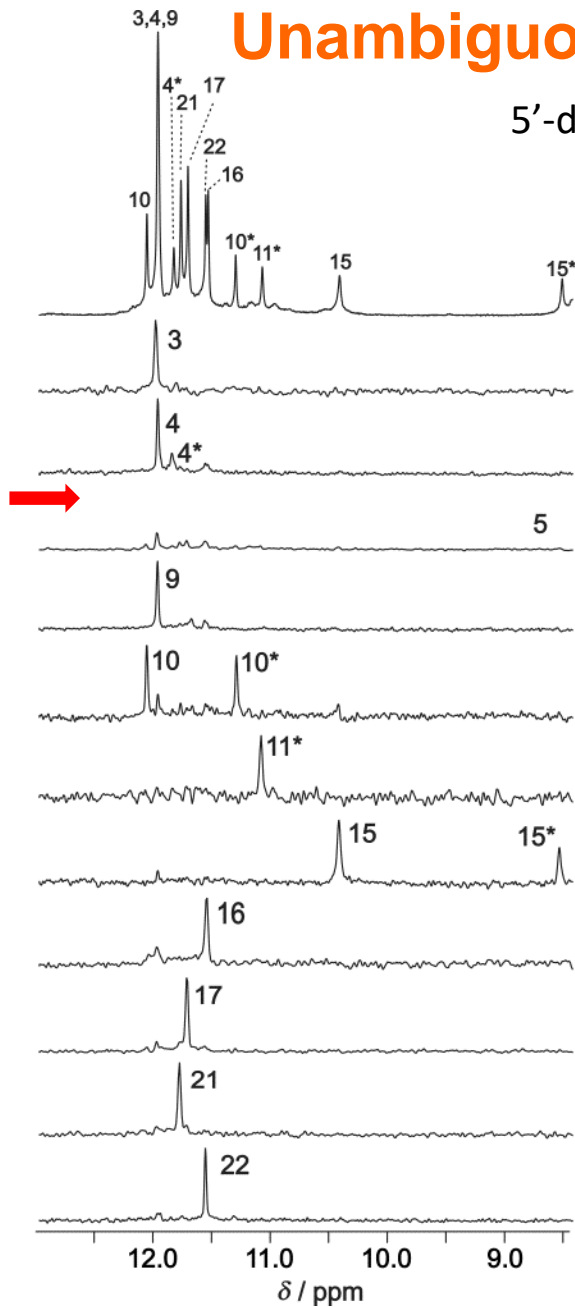
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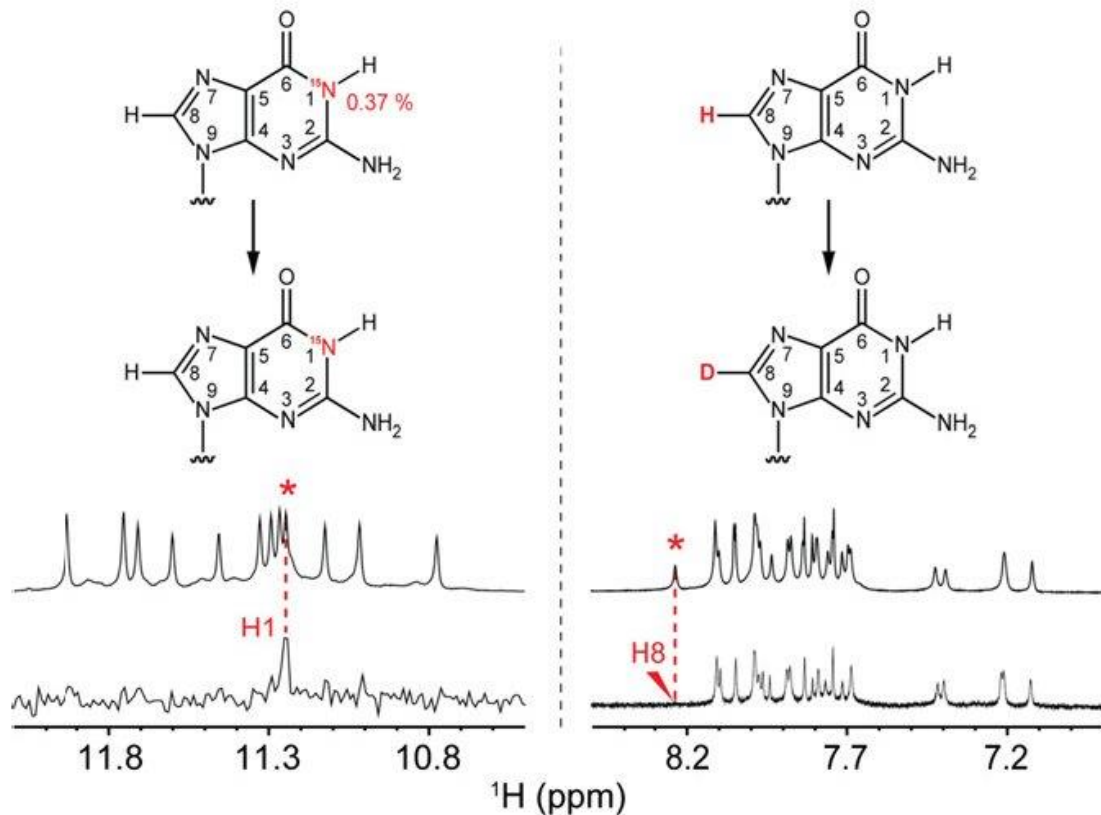
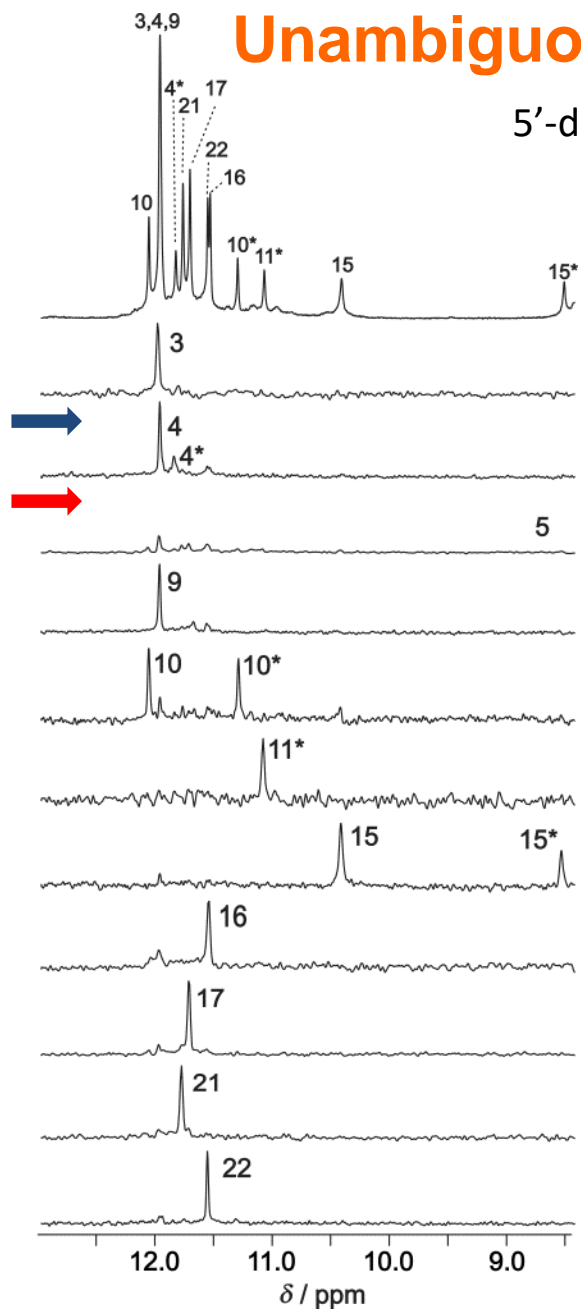
5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'





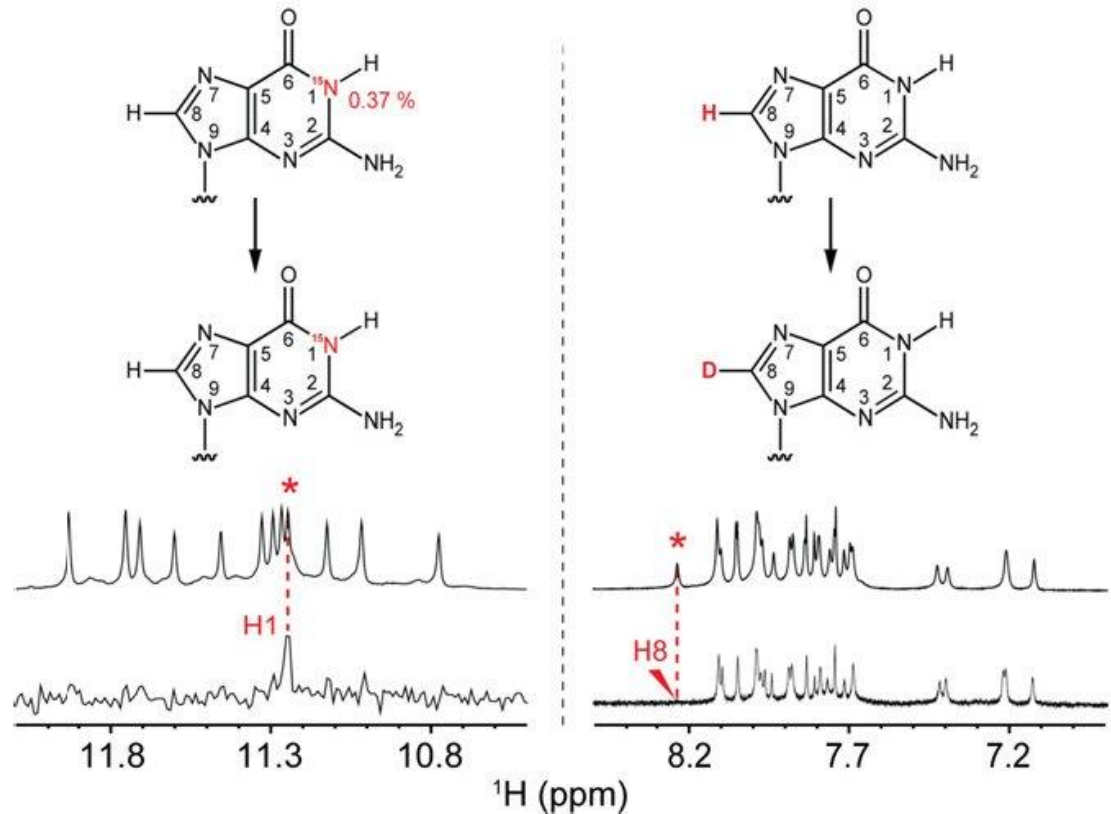
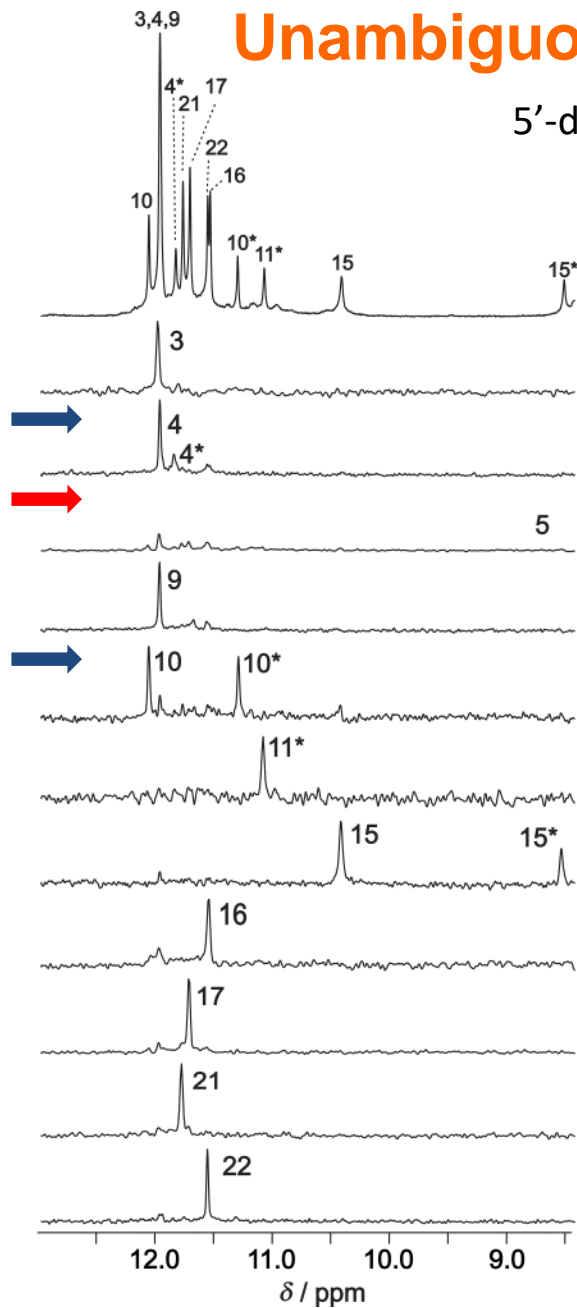
# Unambiguous assignment of imino protons

5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'



# Unambiguous assignment of imino protons

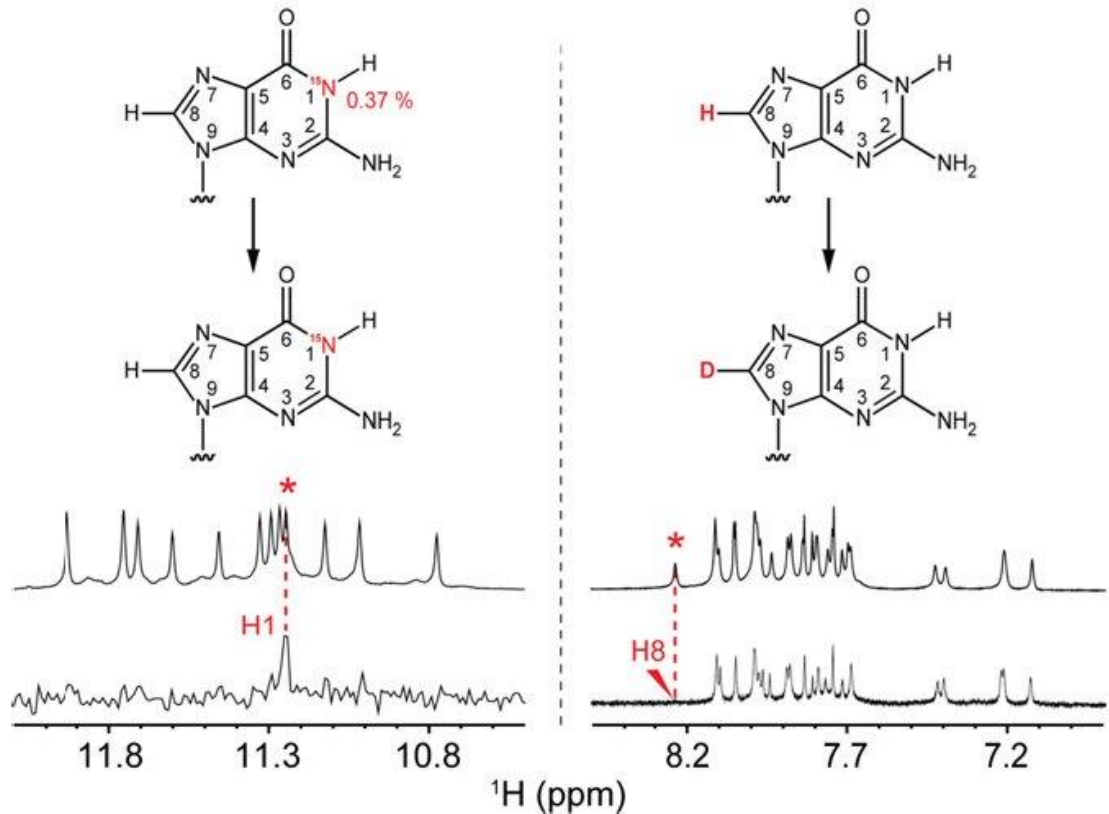
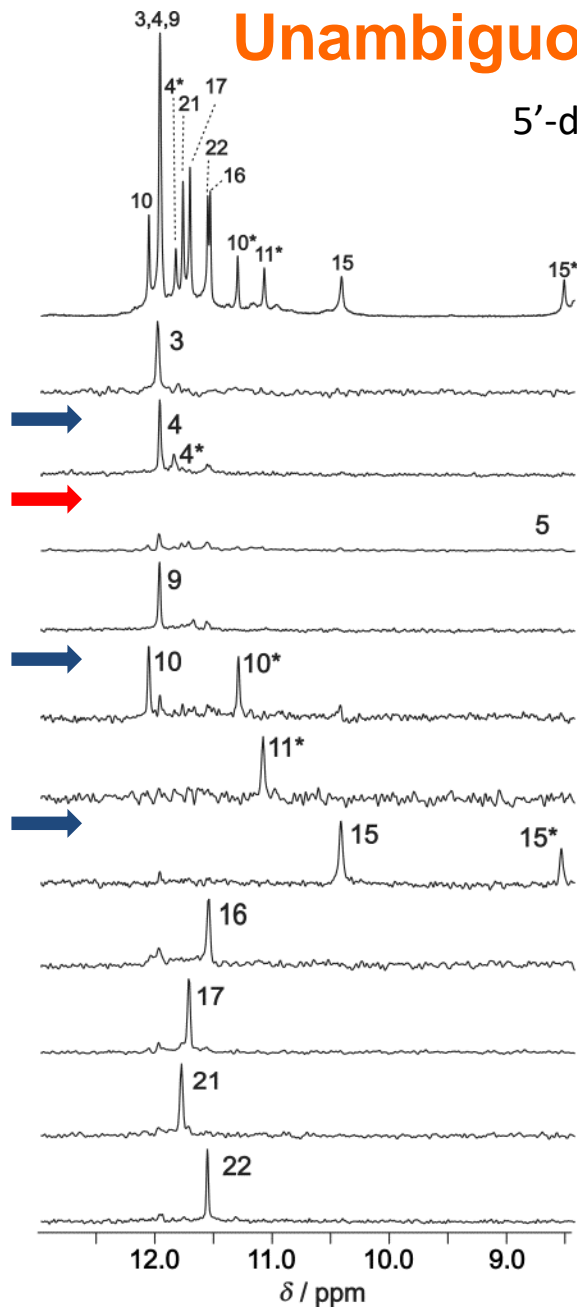
5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'



1D  $^{15}\text{N}$ -edited HSQC NMR spectra of guanine residue-specifically  $^{15}\text{N}$ ,  $^{13}\text{C}$  labelled (8% enriched) oligonucleotides.

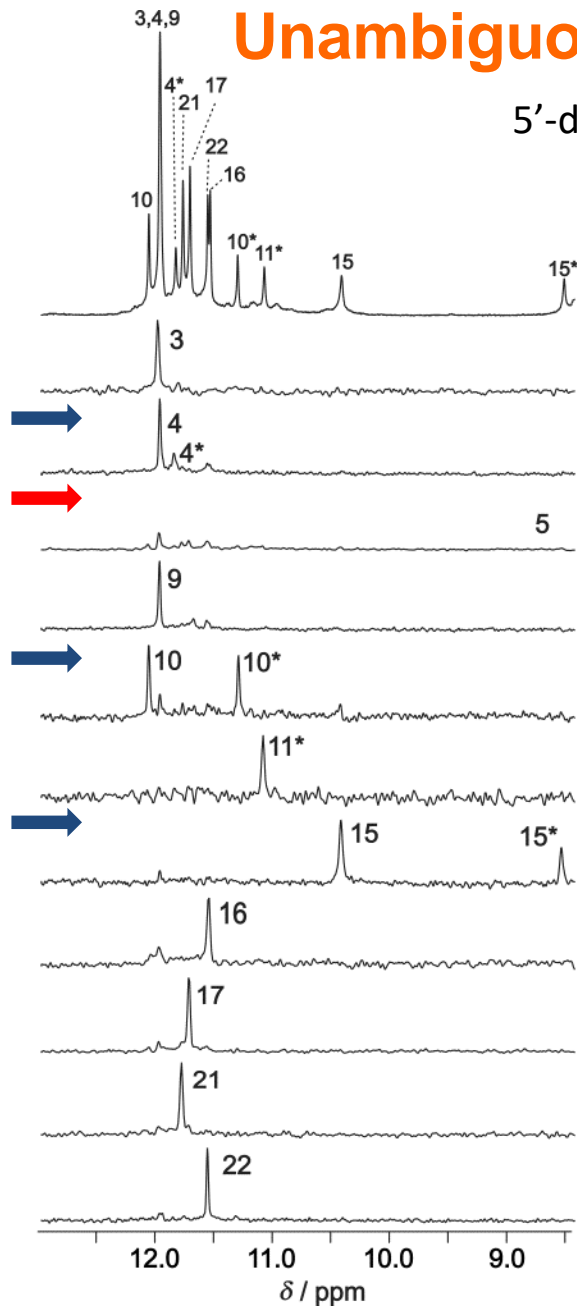
# Unambiguous assignment of imino protons

5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'



# Unambiguous assignment of imino protons

5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'

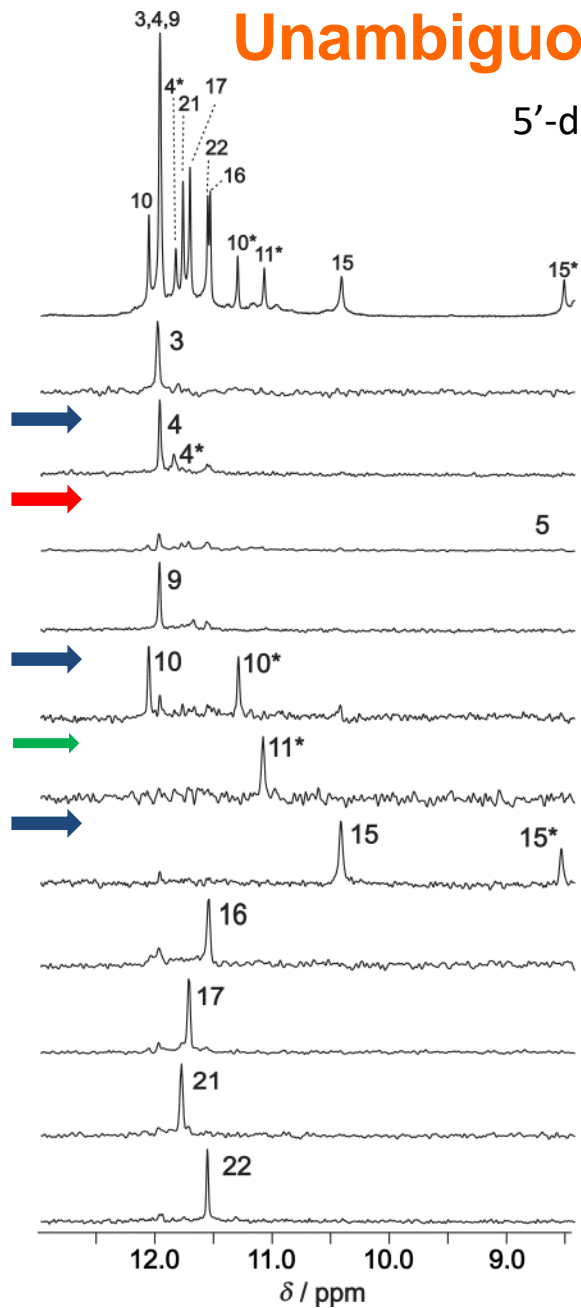


*Coexistence of two structures!*



# Unambiguous assignment of imino protons

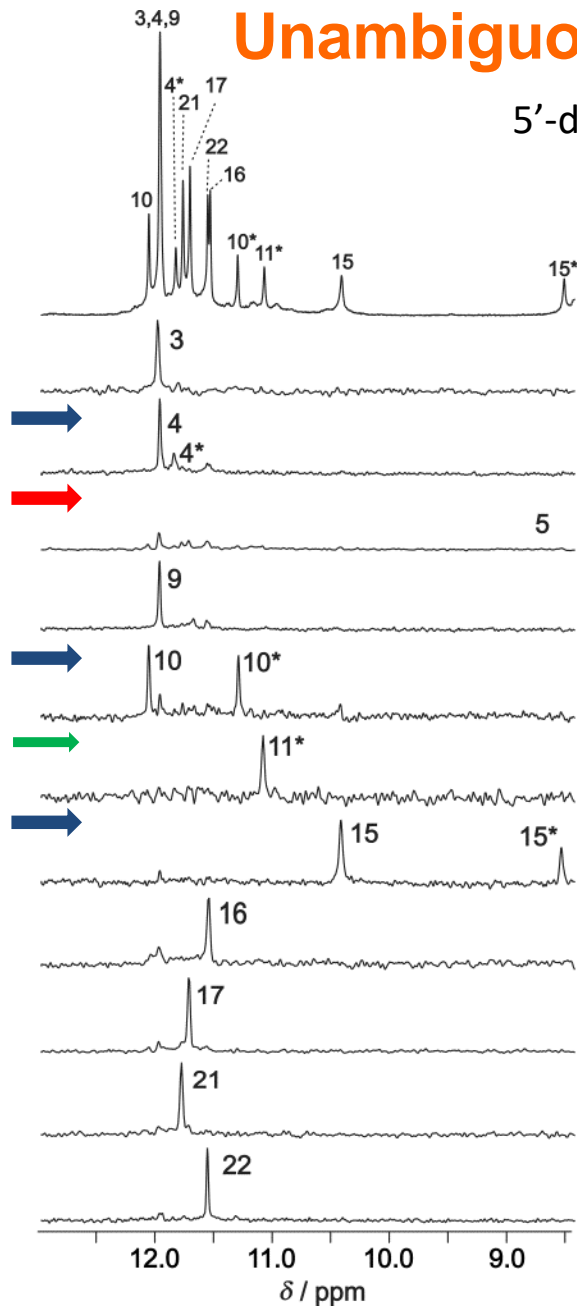
5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'



*Coexistence of two structures!*

# Unambiguous assignment of imino protons

5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'



*Coexistence of two structures!*

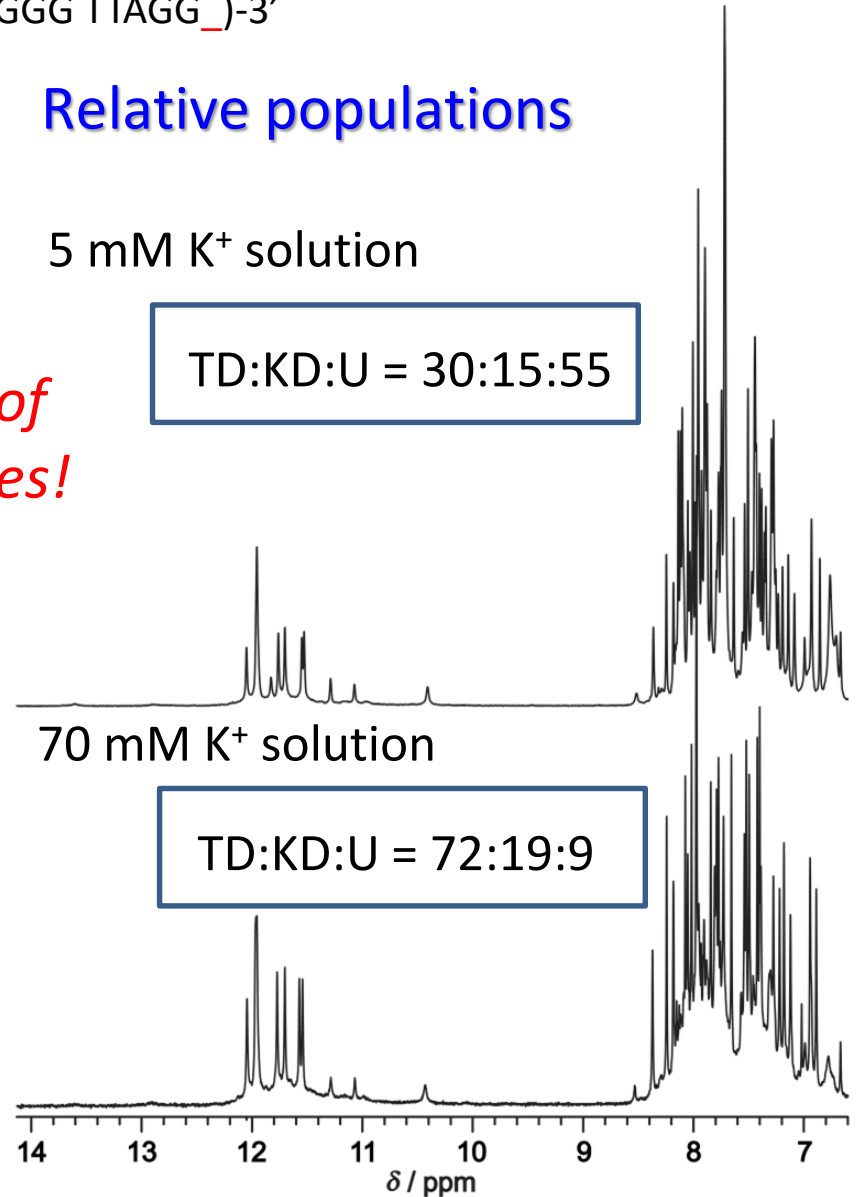
## Relative populations

5 mM K<sup>+</sup> solution

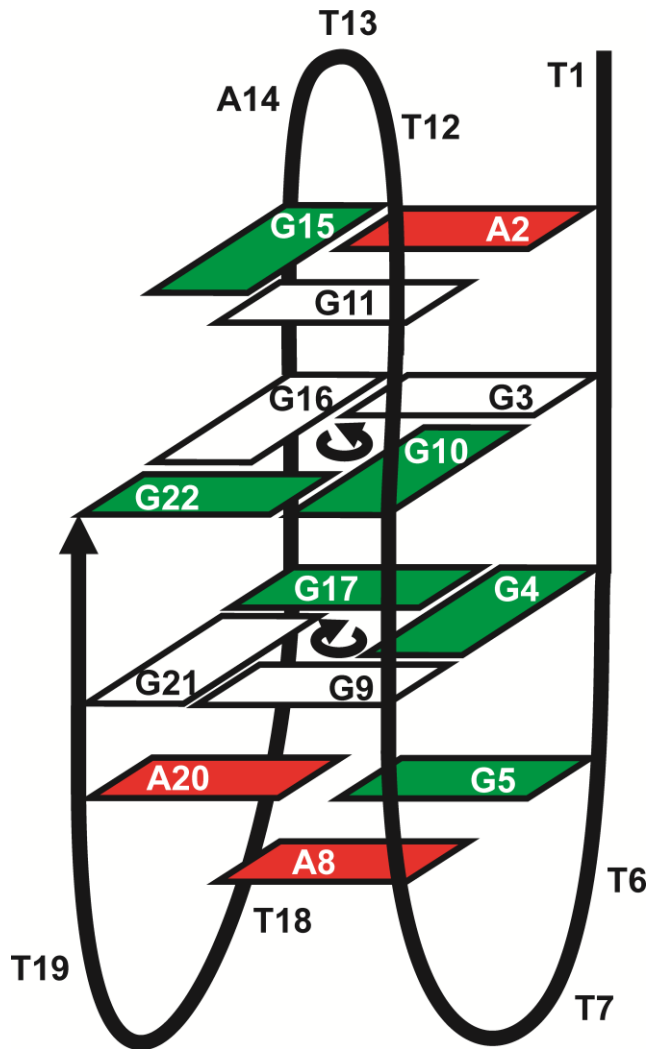
TD:KD:U = 30:15:55

70 mM K<sup>+</sup> solution

TD:KD:U = 72:19:9

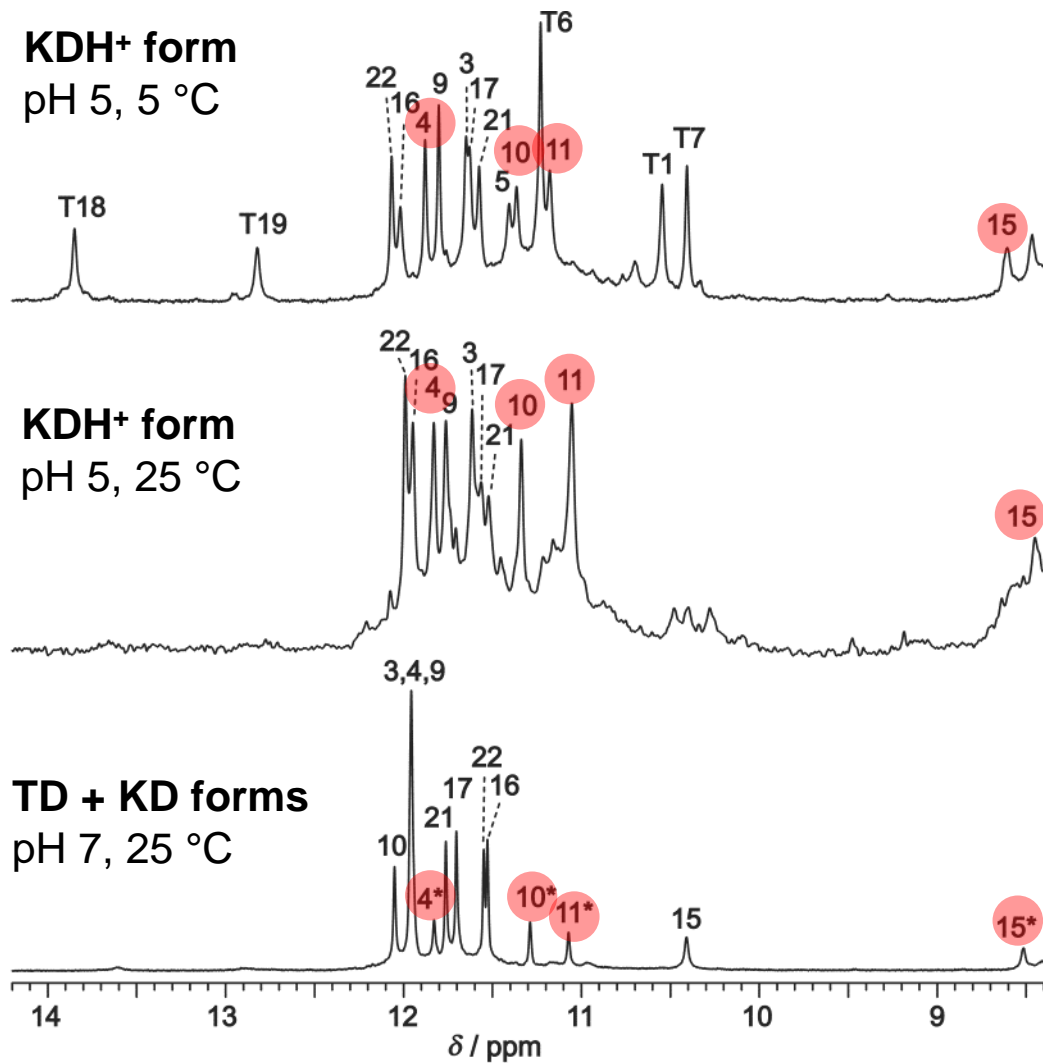


# Topology of TD form



- monomeric structure
- antiparallel basket-type topology
- contains two G-quartets
- 5 guanine residues in *syn* conformation
- different orientation of H-bonds

# Identification of another form of *htel1-ΔG23*



Population of **KD** form increases significantly upon lowering pH to 5.0.

The observed imino protons indicate formation of a new G-quadruplex structure termed as **KDH<sup>+</sup>**.

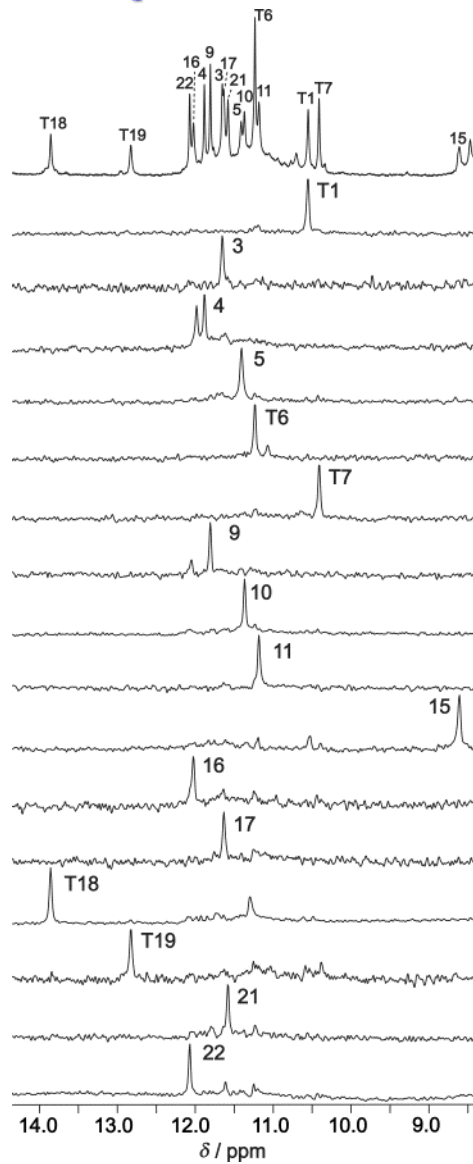
KD form can be considered as a pre-folded state on the way to **KDH<sup>+</sup>**.



# Unambiguous assignment of KDH<sup>+</sup> form

Imino protons

5'-d(TAGGG TTAGGG TTAGGG TTAGG\_)-3'



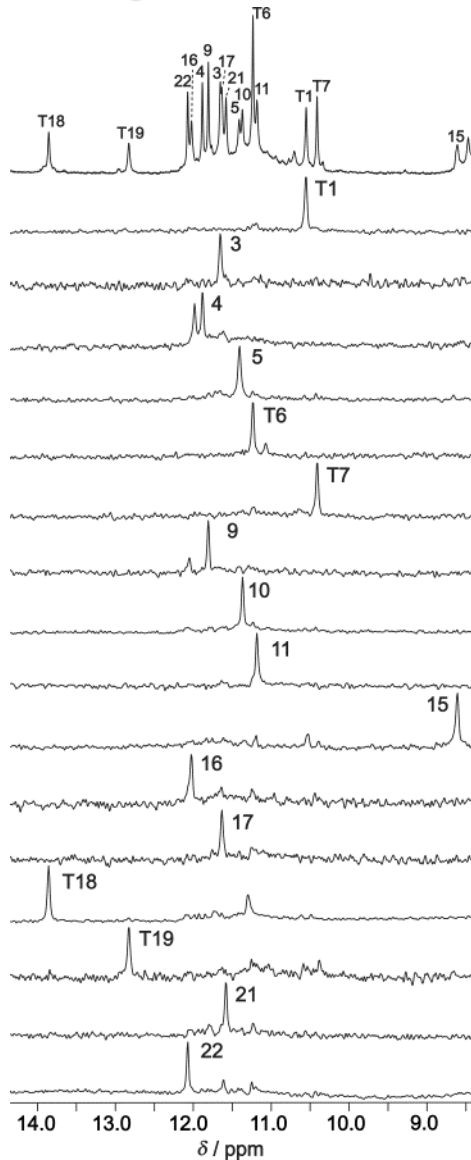
1D <sup>15</sup>N- and 2D <sup>13</sup>C-edited HSQC NMR spectra of guanine residue-specifically <sup>15</sup>N,<sup>13</sup>C labelled (8% enriched) oligonucleotides.

# Unambiguous assignment of KDH<sup>+</sup> form

Imino protons

5'-d(TAGGG TAGGG TAGGG TAGG\_)-3'

Aromatic protons



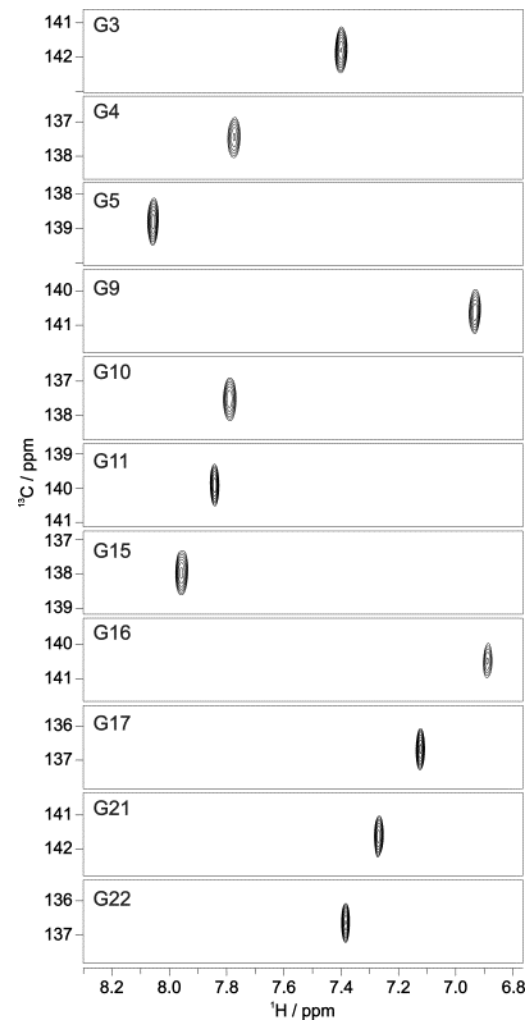
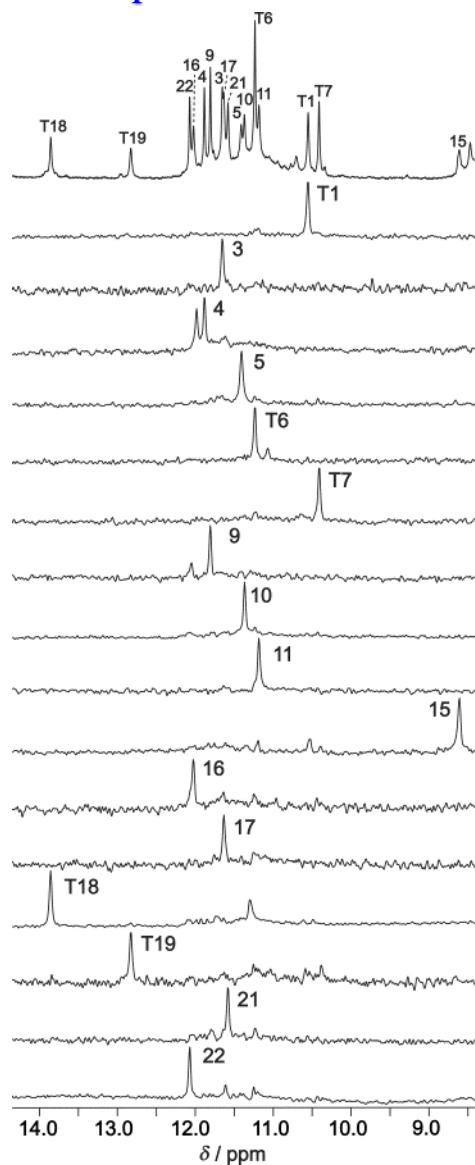
1D <sup>15</sup>N- and 2D <sup>13</sup>C-edited HSQC NMR spectra of guanine residue-specifically <sup>15</sup>N,<sup>13</sup>C labelled (8% enriched) oligonucleotides.

# Unambiguous assignment of KDH<sup>+</sup> form

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Aromatic protons



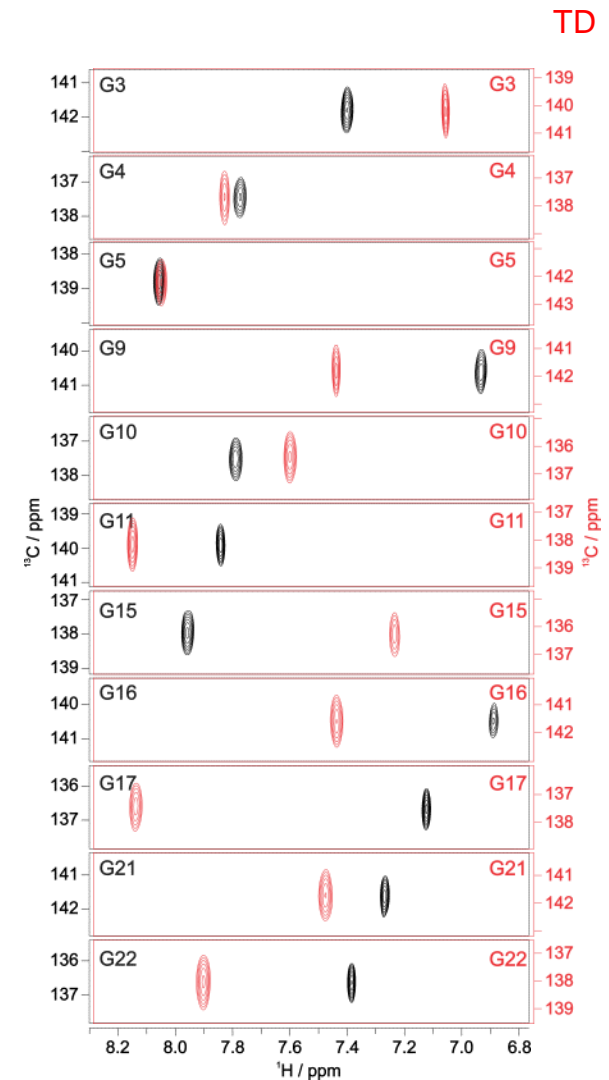
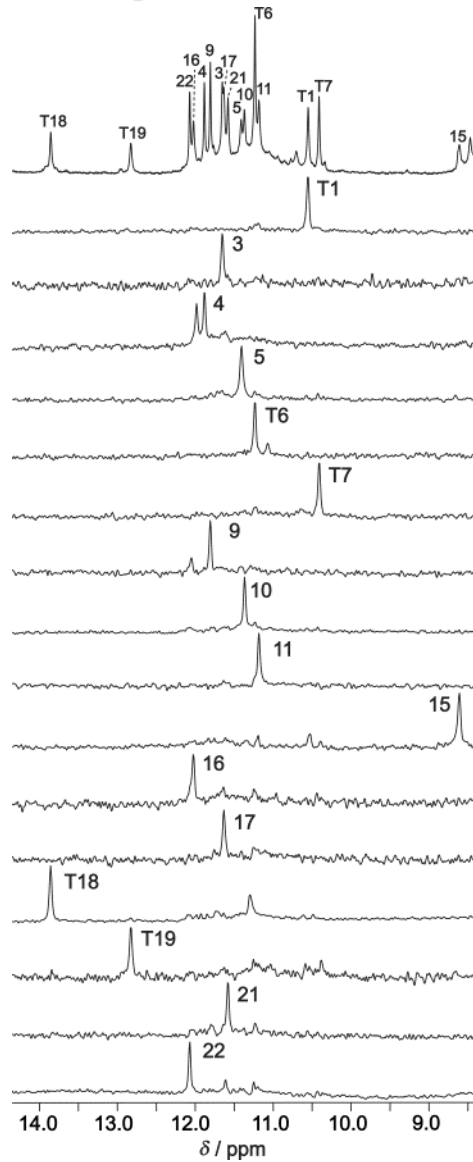
1D <sup>15</sup>N- and 2D <sup>13</sup>C-edited HSQC NMR spectra of guanine residue-specifically <sup>15</sup>N,<sup>13</sup>C labelled (8% enriched) oligonucleotides.

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Aromatic protons



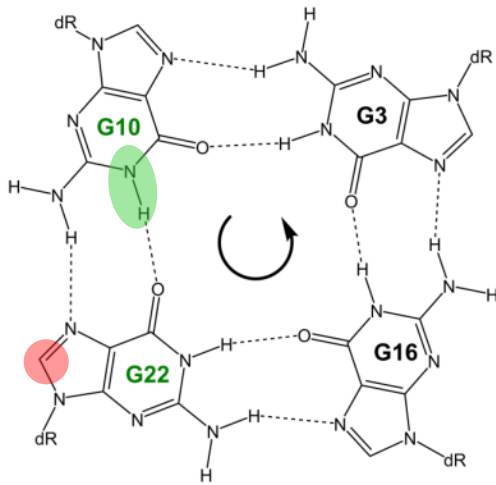
1D <sup>15</sup>N- and 2D <sup>13</sup>C-edited HSQC NMR spectra of guanine residue-specifically <sup>15</sup>N,<sup>13</sup>C labelled (8% enriched) oligonucleotides.

# Donor / acceptor directionality of H-bonds

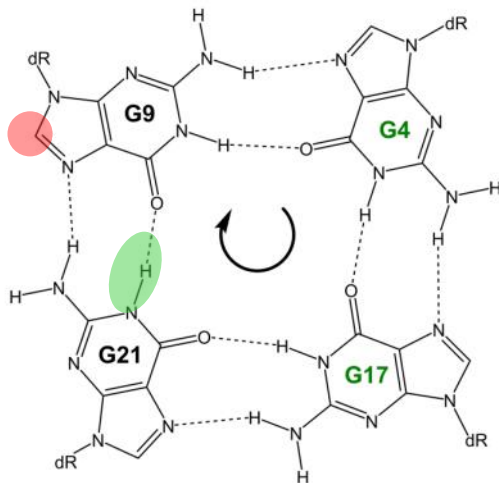
KDH<sup>+</sup> form

TD form

Anticlockwise (upper G-quartet)



Clockwise (lower G-quartet)



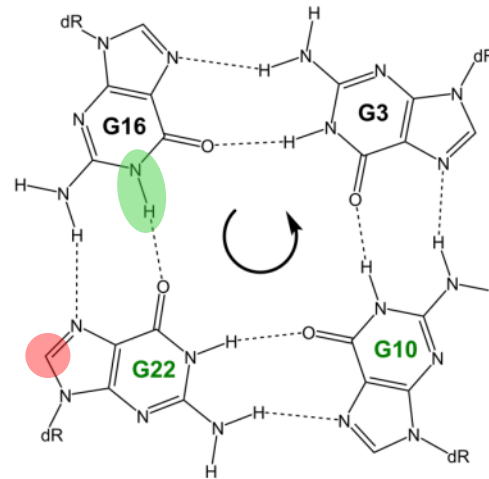
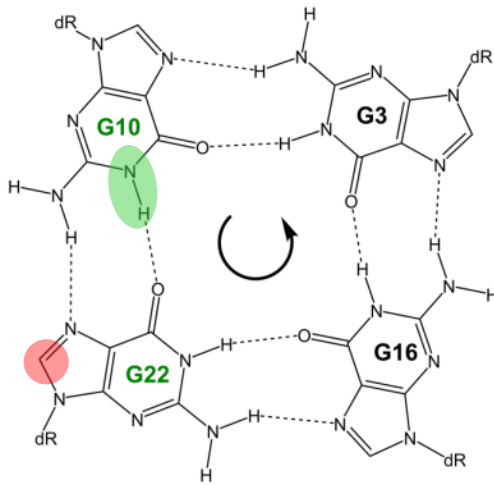


# Donor / acceptor directionality of H-bonds

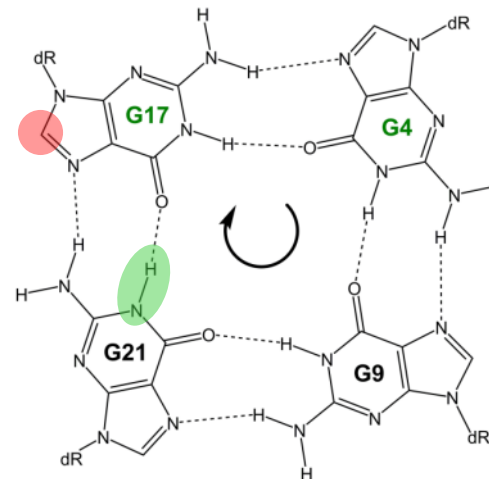
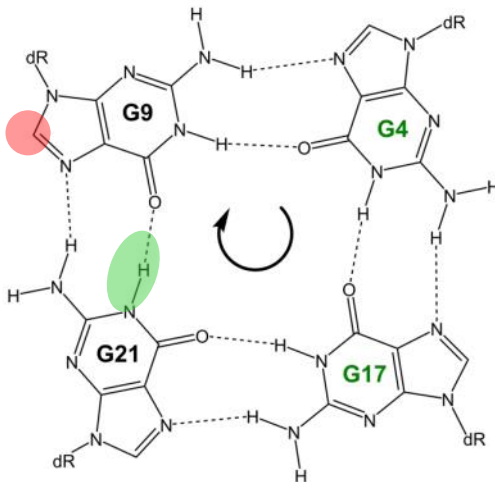
KDH<sup>+</sup> form

TD form

Anticlockwise (upper G-quartet)



Clockwise (lower G-quartet)

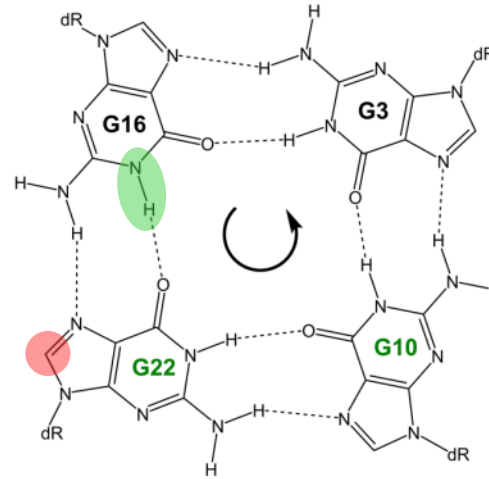
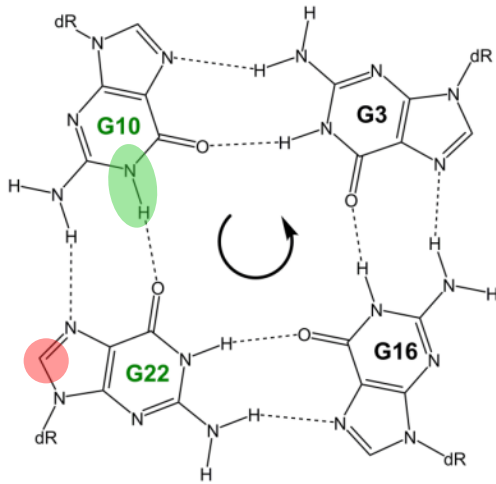


# Donor / acceptor directionality of H-bonds

KDH<sup>+</sup> form

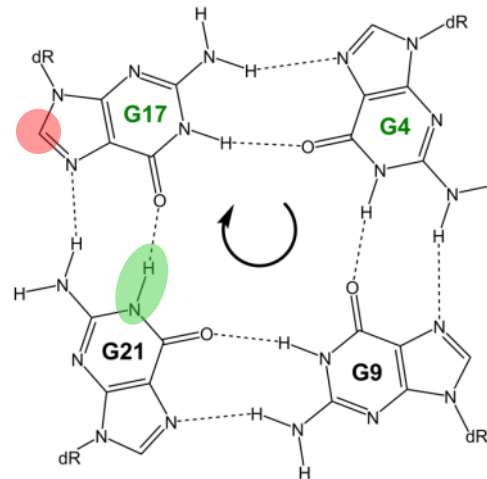
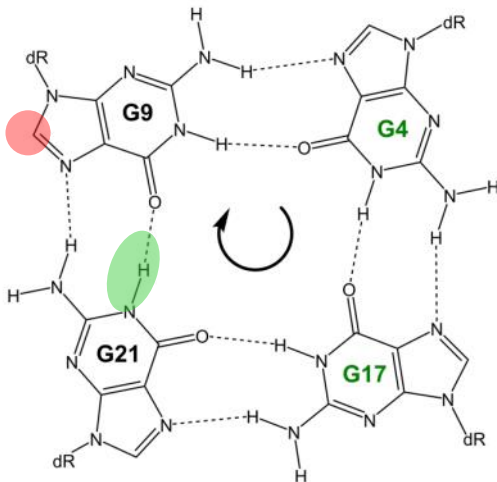
TD form

Anticlockwise (upper G-quartet)



Both forms exhibit the same directionalities of hydrogen bonds.

Clockwise (lower G-quartet)

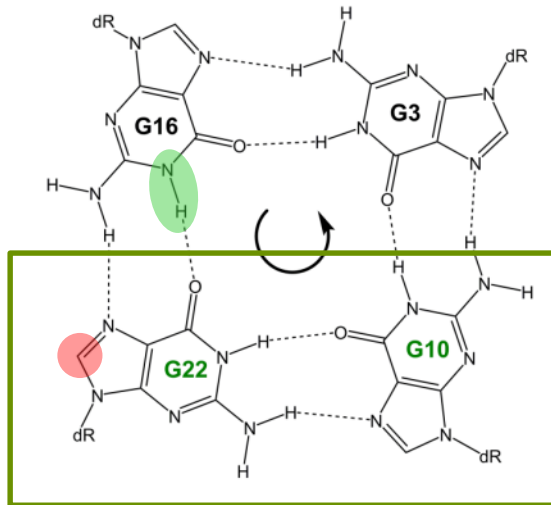
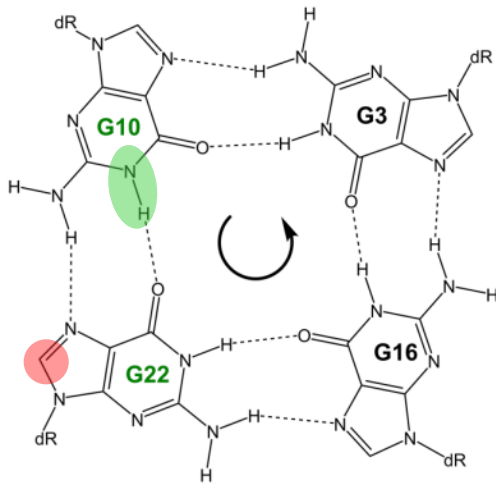


# Donor / acceptor directionality of H-bonds

KDH<sup>+</sup> form

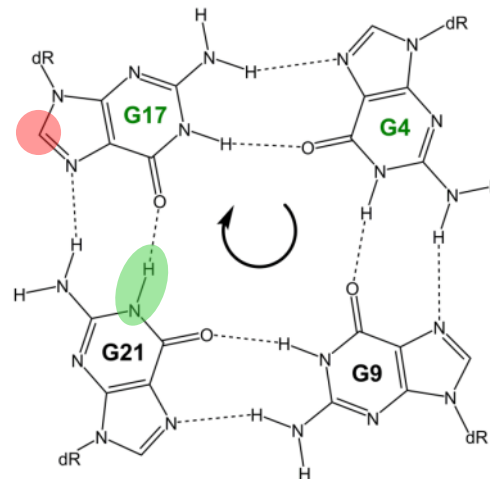
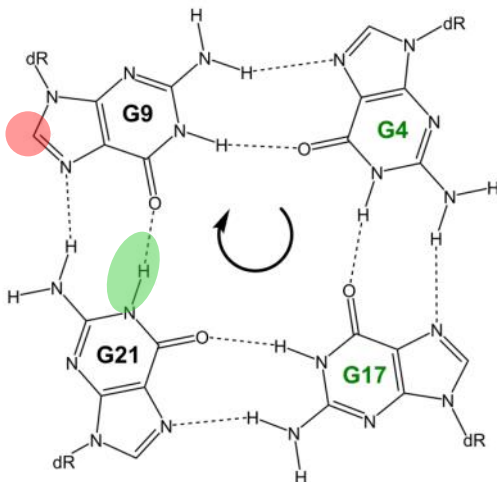
TD form

Anticlockwise (upper G-quartet)



Both forms exhibit the same directionalities of hydrogen bonds.

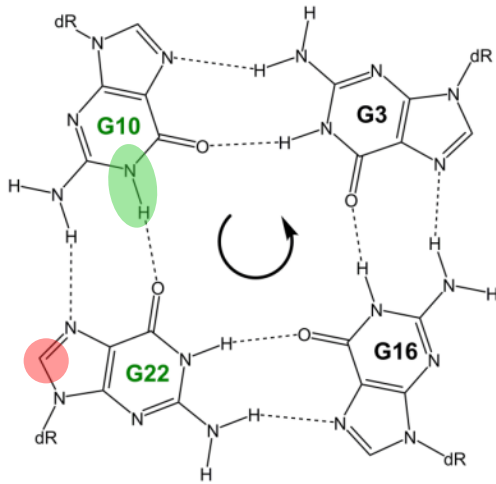
Clockwise (lower G-quartet)



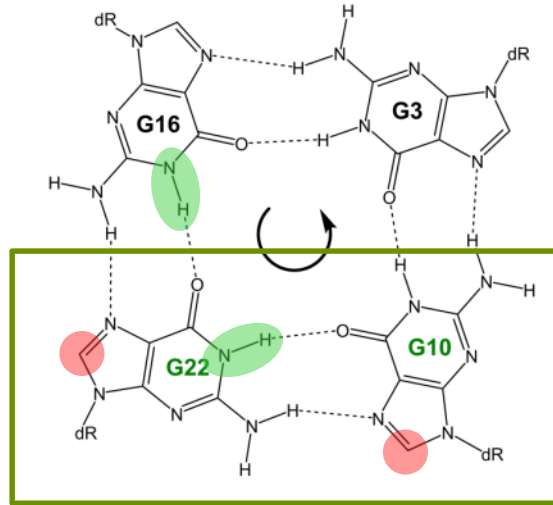
# Donor / acceptor directionality of H-bonds

KDH<sup>+</sup> form

Anticlockwise (upper G-quartet)

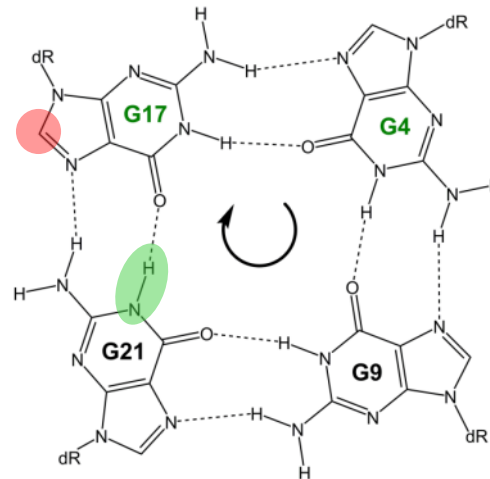
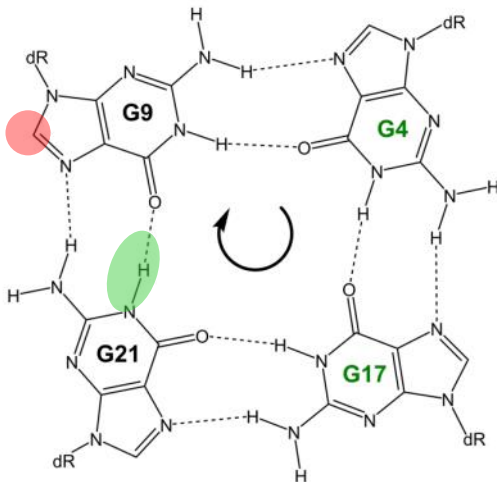


TD form



Both forms exhibit the same directionalities of hydrogen bonds.

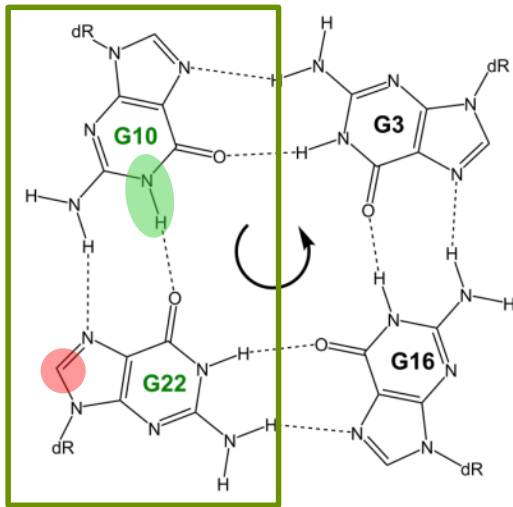
Clockwise (lower G-quartet)



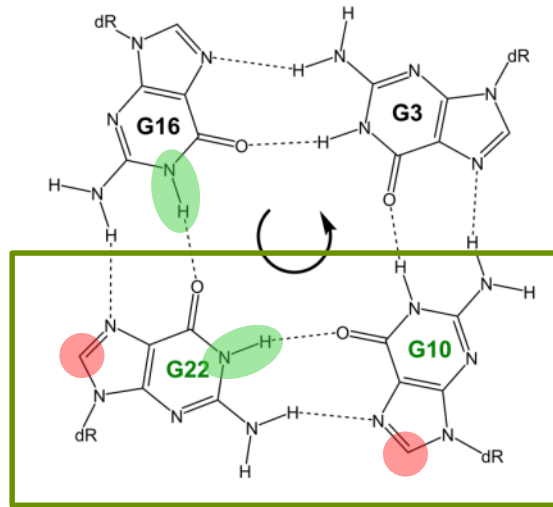
# Donor / acceptor directionality of H-bonds

KDH<sup>+</sup> form

Anticlockwise (upper G-quartet)

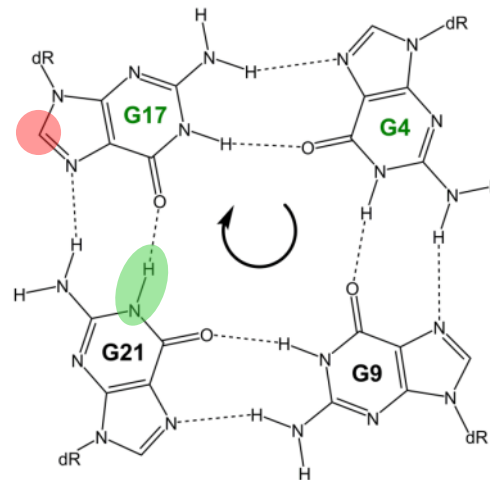
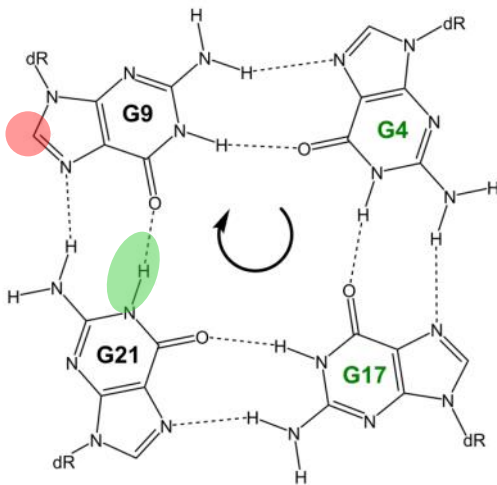


TD form



Both forms exhibit the same directionalities of hydrogen bonds.

Clockwise (lower G-quartet)



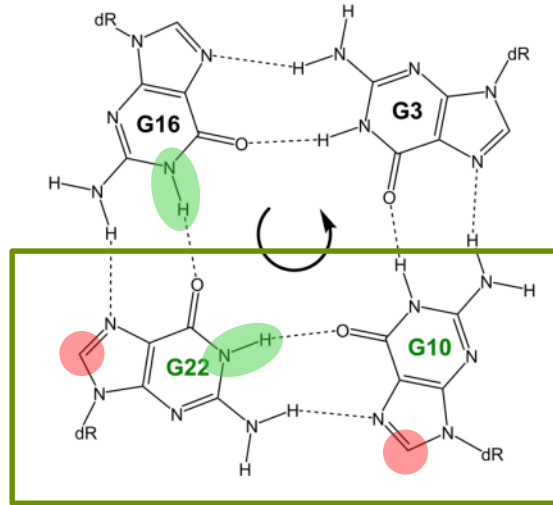
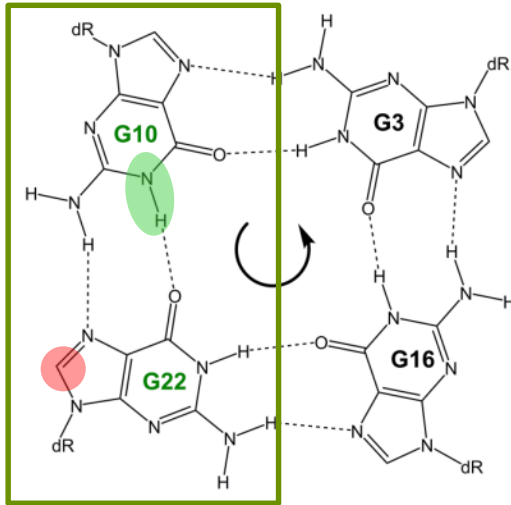


# Donor / acceptor directionality of H-bonds

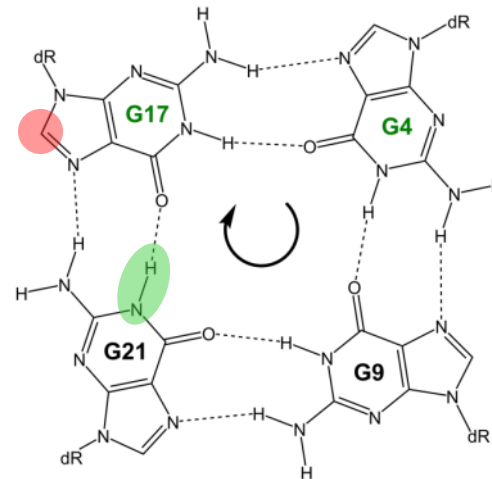
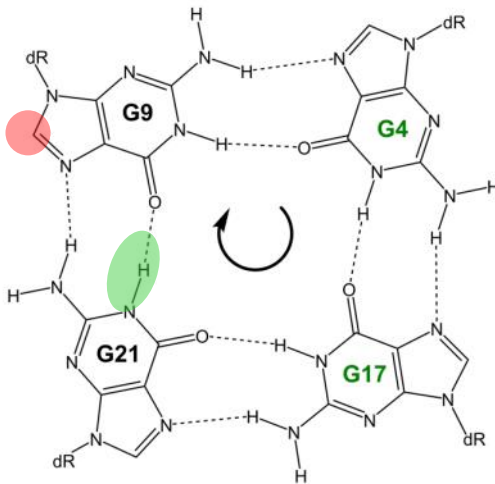
KDH<sup>+</sup> form

TD form

Anticlockwise (upper G-quartet)



Clockwise (lower G-quartet)

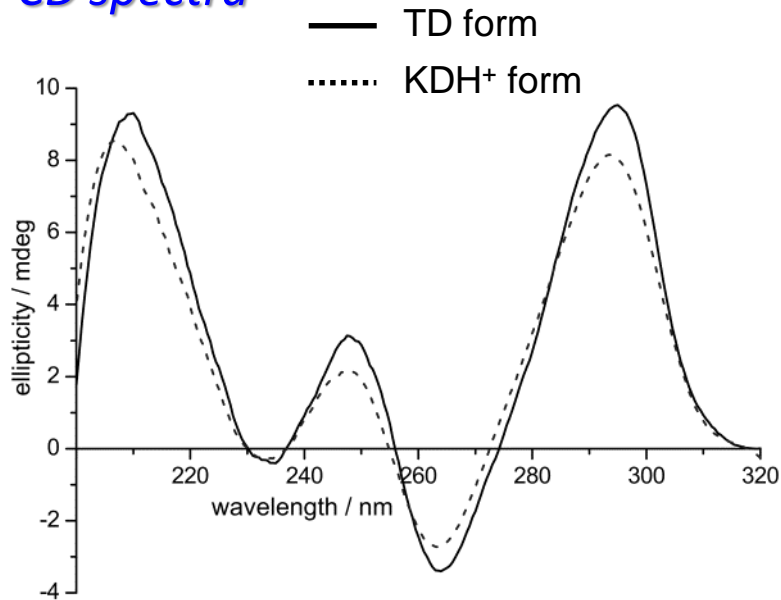


Both forms exhibit the same directionalities of hydrogen bonds.

The actual donor→acceptor directionalities of a given guanine residue are different.

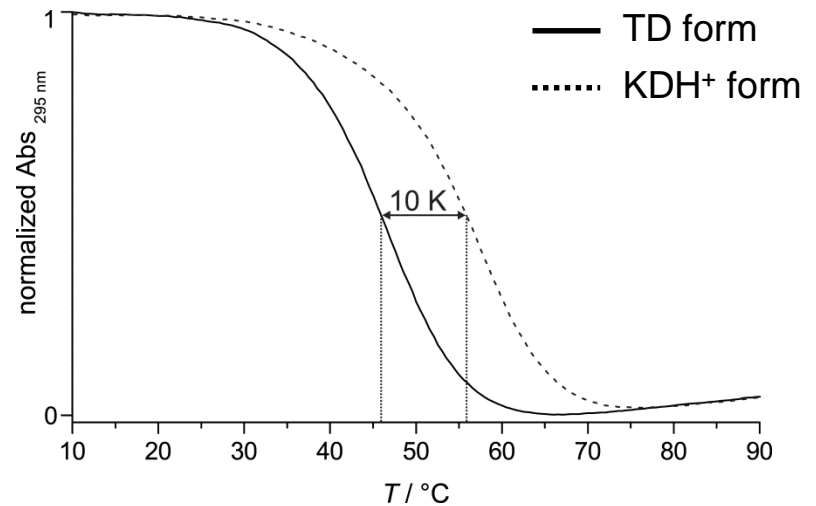
# CD signatures and thermal stability

## CD spectra



A 'clean' antiparallel G-quadruplex.

## UV melting



## DOSY NMR spectra

Identical translation diffusion coefficients ( $1.40 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ ) at 25 °C.

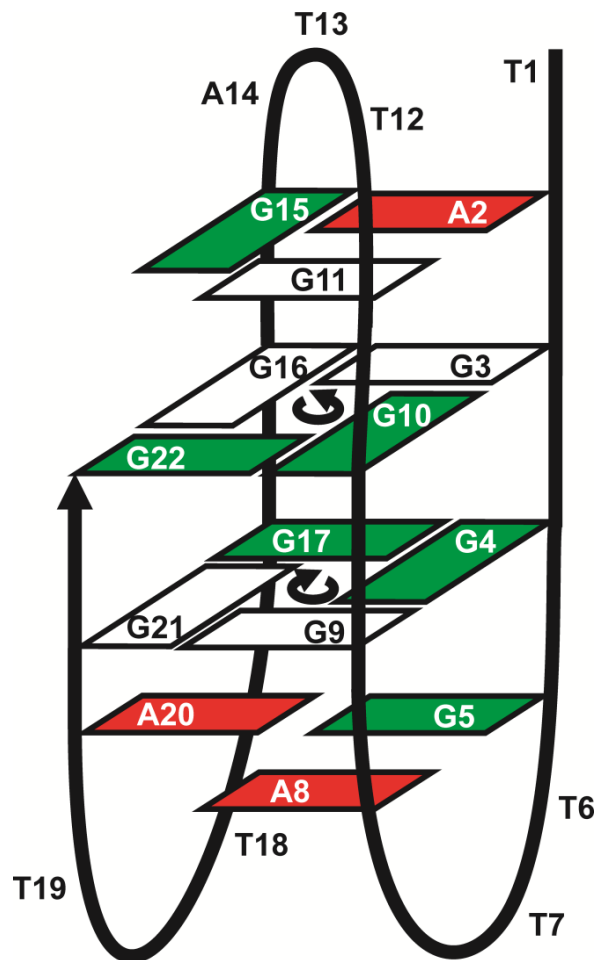
Monomeric structures.

CD spectra at 298 K,  $c = 15 \mu\text{M}$  of TD form (20 mM Kpi, pH 7 + 20 mM KCl) and KDH<sup>+</sup> form (20 mM Li-citrate, pH 5, + 70 mM KCl).

UV melting curves at 295 nm,  $0.3 \text{ }^\circ\text{C min}^{-1}$ ,  $c = 80 \mu\text{M}$  of TD form (20 mM Kpi, pH 7 + 20 mM KCl) and KDH<sup>+</sup> form (20 mM Li-citrate, pH 5, + 70 mM KCl).

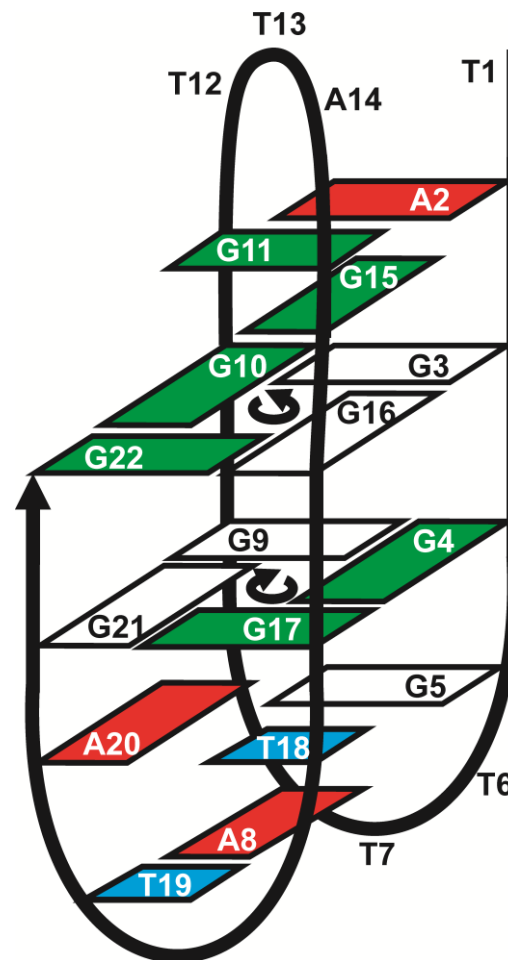
# Comparison of both topologies

## TD form



-Id+l arrangement\*

## KDH<sup>+</sup> form

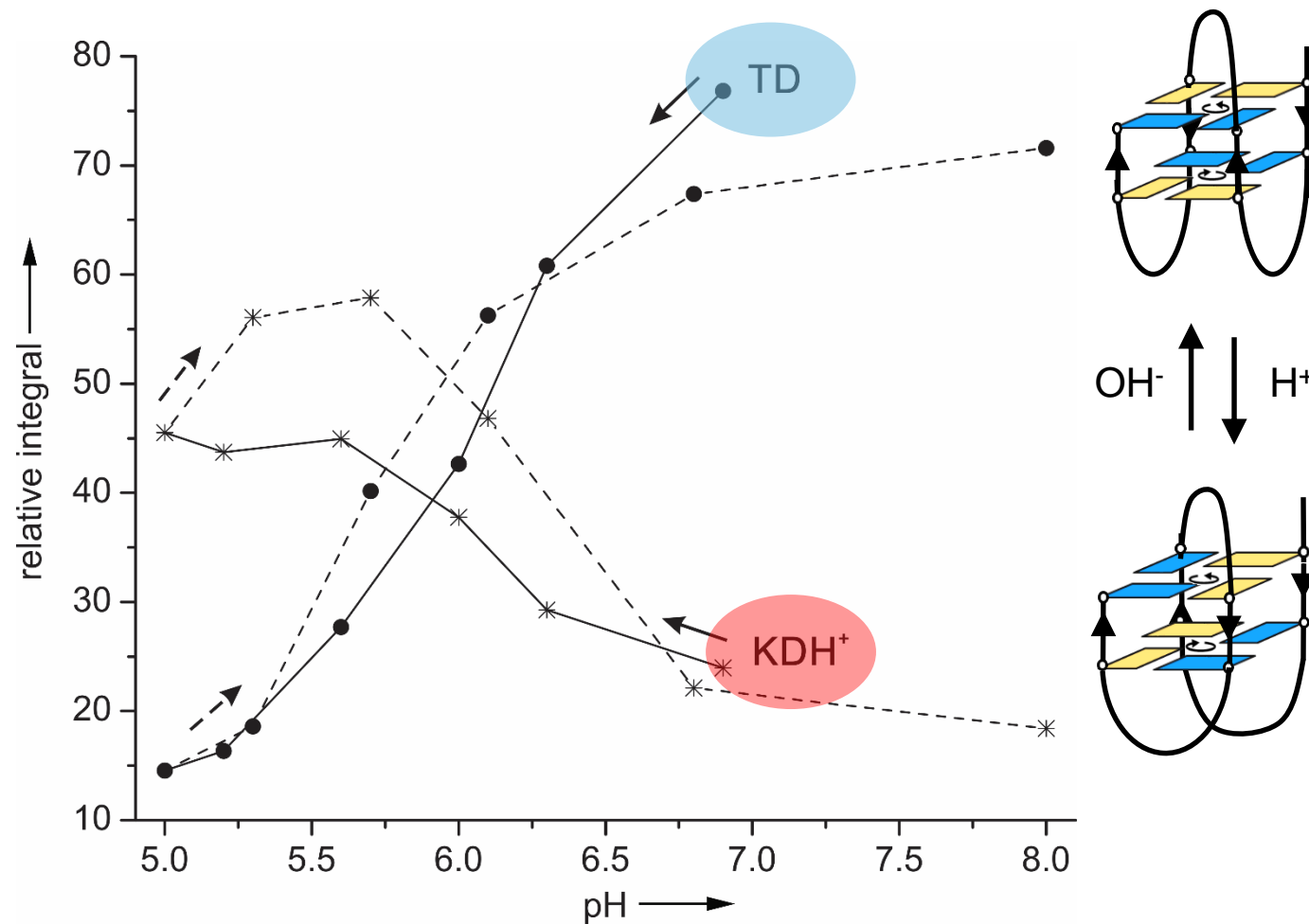


+ld-l

\*M. Webba da Silva, *Chem. Eur. J.* **2007**, *13*, 9738.

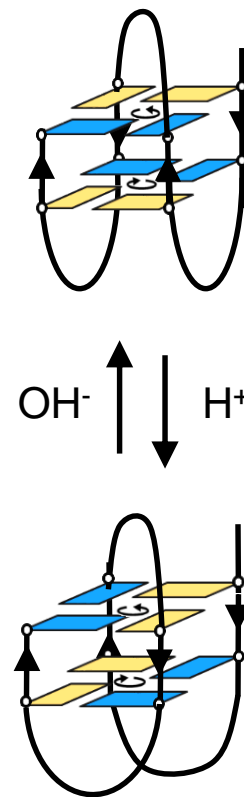
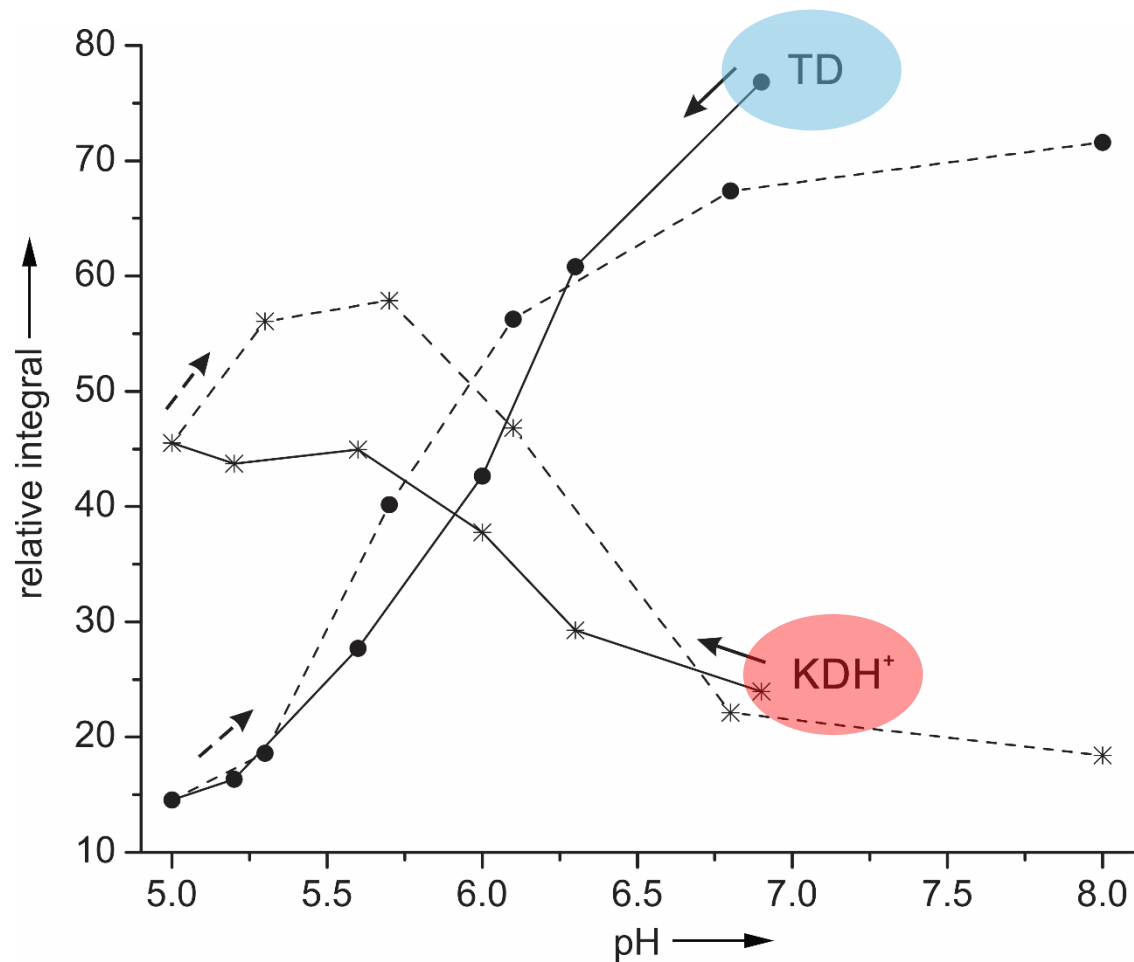
P. Galer, B. Wang, P. Šket, J. Plavec, *Angew. Chem. Int. Ed.* **2016**, *55*, 1993.

# Populations of TD and KDH<sup>+</sup> forms are controlled by pH



Condition: c = 1 mM, 25 °C

# Populations of TD and KDH<sup>+</sup> forms are controlled by pH

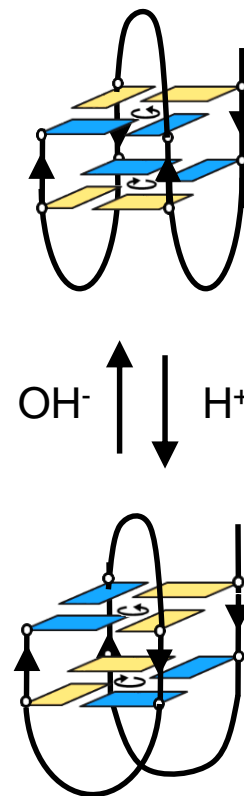
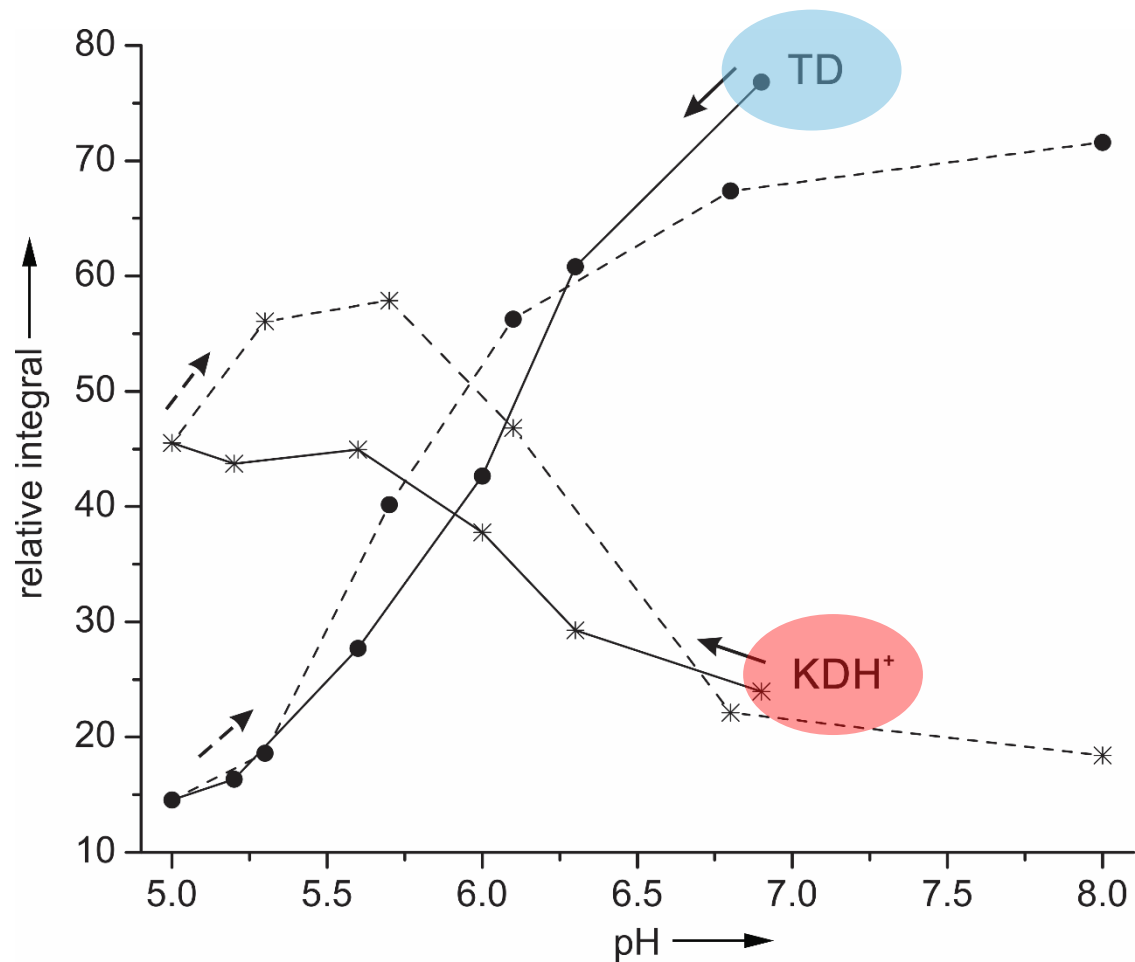


The interconversion is not a direct process.

Condition:  $c = 1 \text{ mM}$ ,  $25 \text{ }^\circ\text{C}$

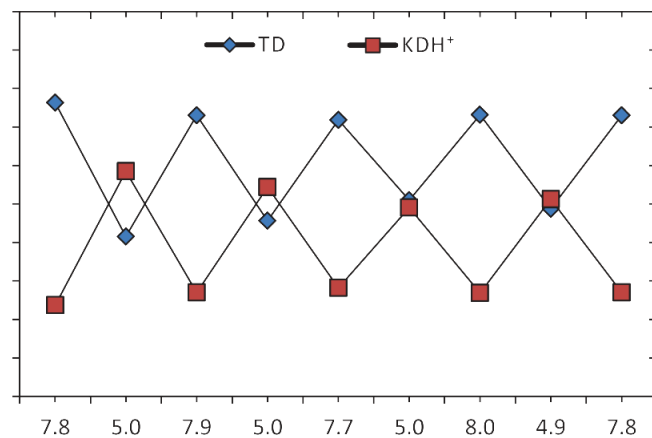


# Populations of TD and KDH<sup>+</sup> forms are controlled by pH



The interconversion is not a direct process.

Condition:  $c = 1 \text{ mM}$ ,  $25 \text{ }^\circ\text{C}$



## Proto-oncogene *c-myc*

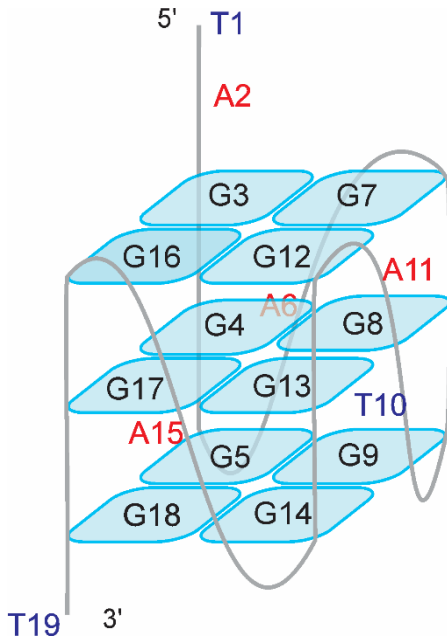
5'-d[<sup>5</sup>TAGGG<sup>10</sup>GAGGG<sup>15</sup>TAGGGGAGGGT]-3'

G-quadruplex formation in NHE III<sub>1</sub>  
region upstream of P1 promoter

# Proto-oncogene *c-myc*

5'-d[TAGGG<sup>5</sup>GAGGG<sup>10</sup>TAGGG<sup>15</sup>GAGGG]-3'

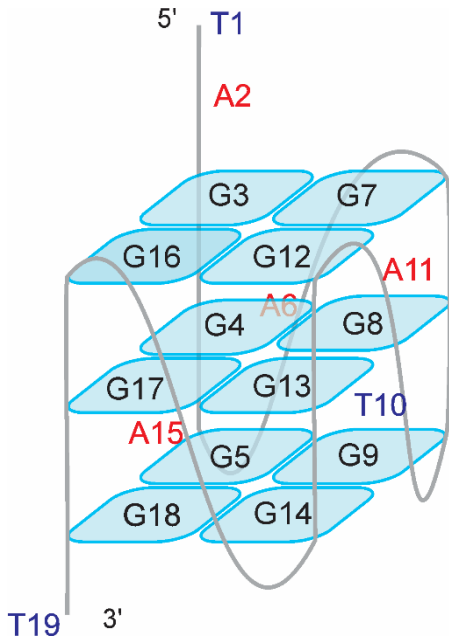
G-quadruplex formation in NHE III<sub>1</sub> region upstream of P1 promoter



Monomolecular  
parallel G-quadruplex  
in the presence of K<sup>+</sup> ions

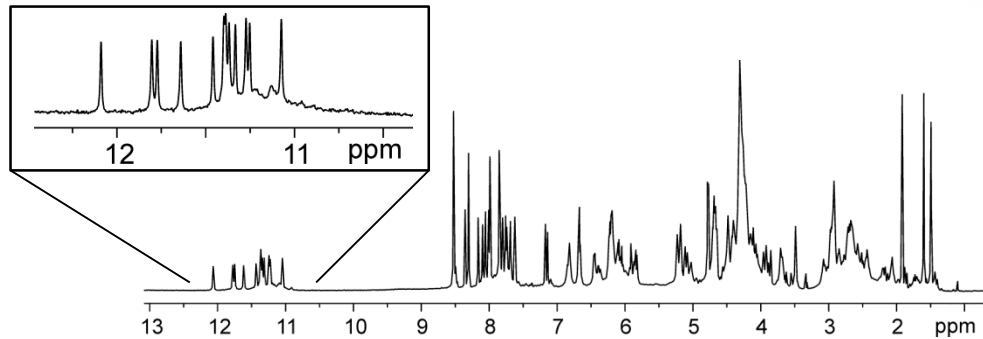
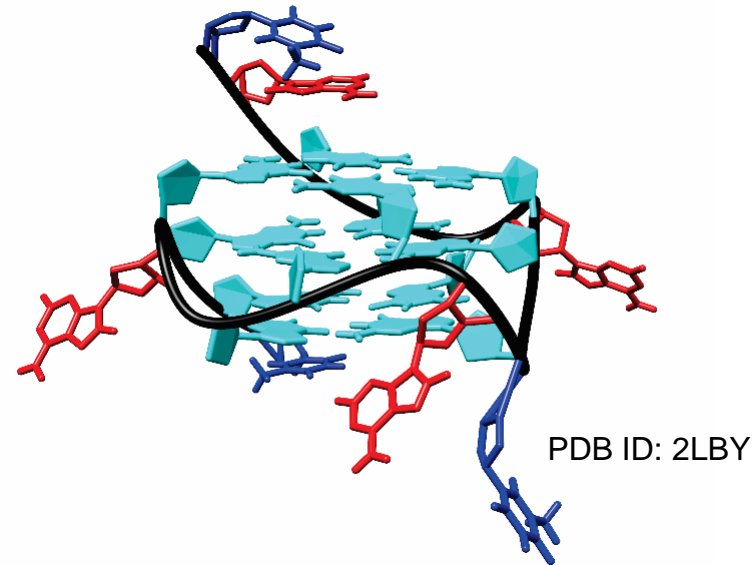
# Proto-oncogene *c-myc*

5'-d[TAGGGAGGGTAGGGAGGGT]-3'



Monomolecular parallel G-quadruplex in the presence of K<sup>+</sup> ions

G-quadruplex formation in NHE III<sub>1</sub> region upstream of P1 promoter



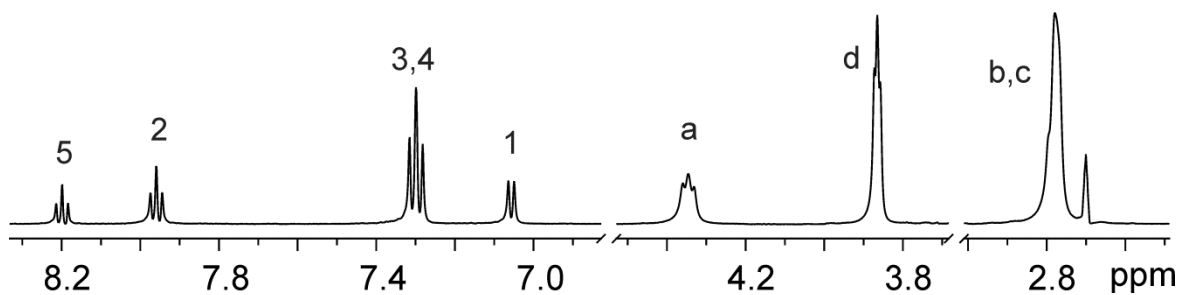
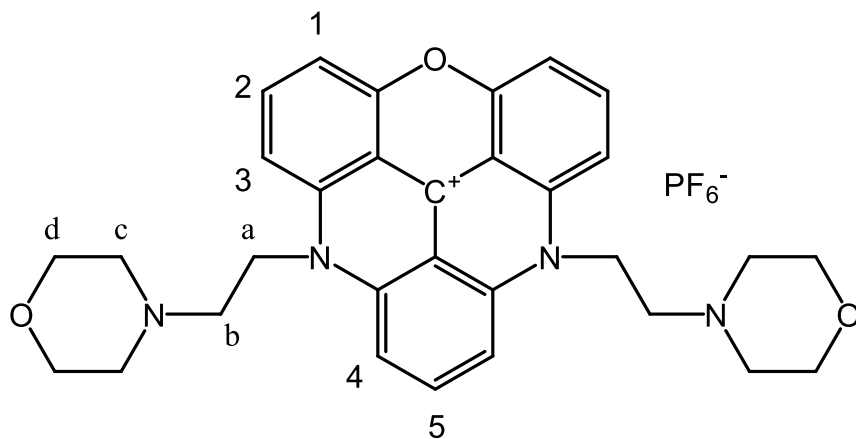
D. Yang *et al. Nucleic Acids Res*, 2011, 39, 9023.

<sup>1</sup>H NMR spectrum of CMA in the presence of K<sup>+</sup> ions

0.2 mM CMA per strand, 10 mM cacodylate buffer, 100 mM KCl, H<sub>2</sub>O:<sup>2</sup>H<sub>2</sub>O=9:1, pH 7.0, 25 °C, 600 MHz spectrometer.

# Visualisation of G-quadruplexes (in cells)

## Ligand DAOTA-M2



<sup>1</sup>H NMR spectrum of DAOTA-M2

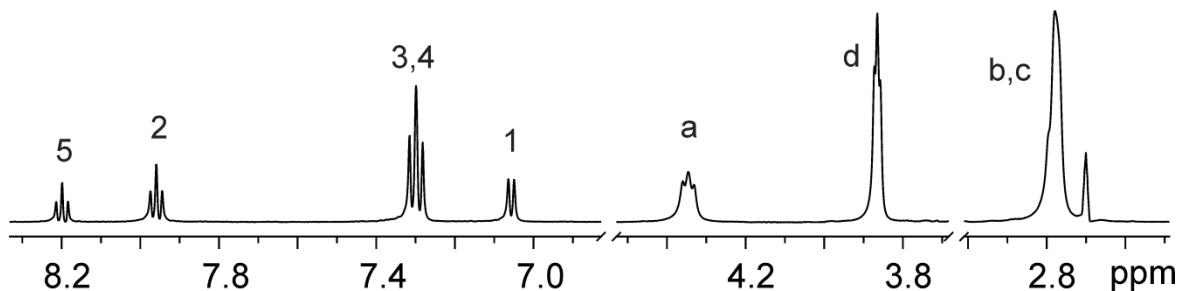
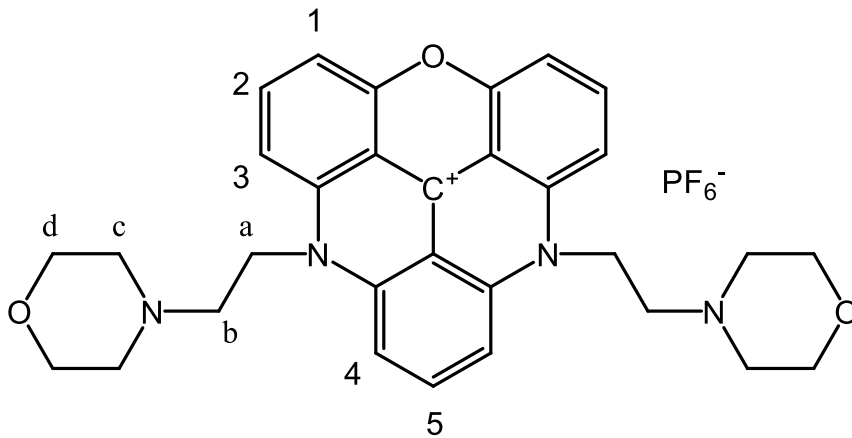
0.5 mM DAOTA-M2, 10 mM cacodylate buffer, 100 mM KCl, pH 7.0, 25 °C, H<sub>2</sub>O:<sup>2</sup>H<sub>2</sub>O=9:1, 600 MHz spectrometer.



# Visualisation of G-quadruplexes (in cells)

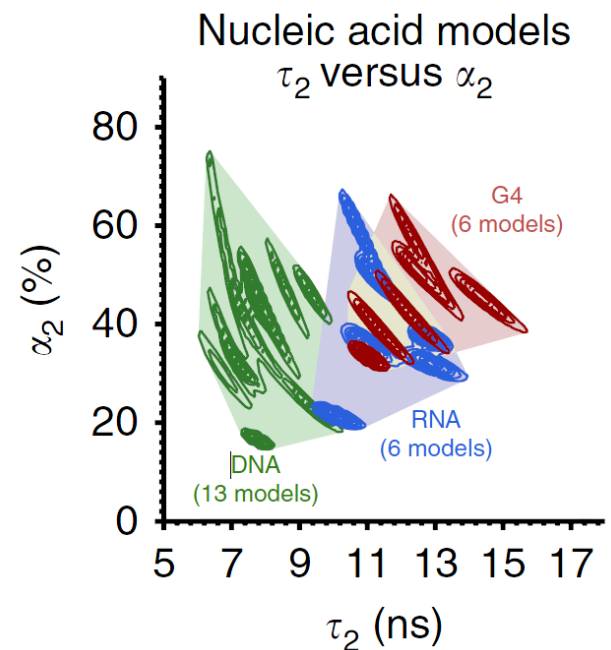
## Ligand DAOTA-M2

- Triangulenium derivative
- Interaction with nucleic acids
- Optical probe for G-quadruplexes



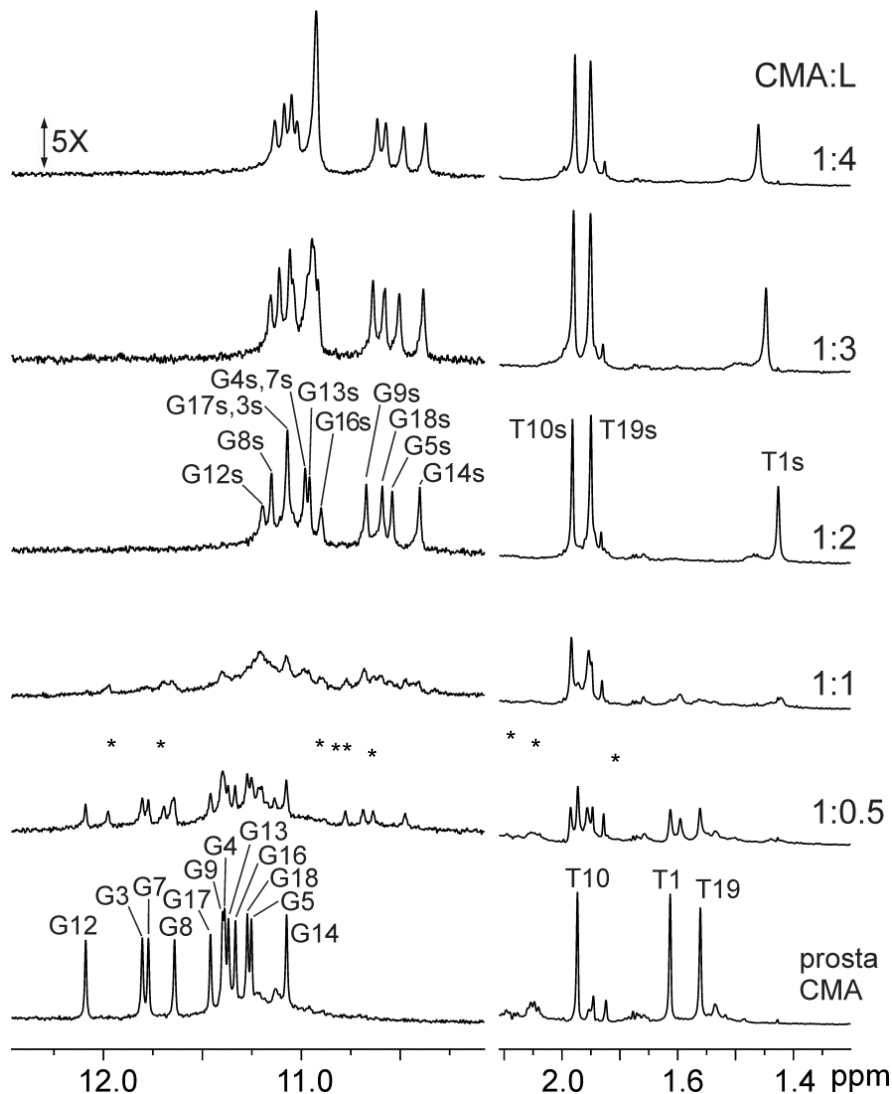
### <sup>1</sup>H NMR spectrum of DAOTA-M2

0.5 mM DAOTA-M2, 10 mM cacodylate buffer, 100 mM KCl, pH 7.0, 25 °C, H<sub>2</sub>O:<sup>2</sup>H<sub>2</sub>O=9:1, 600 MHz spectrometer.

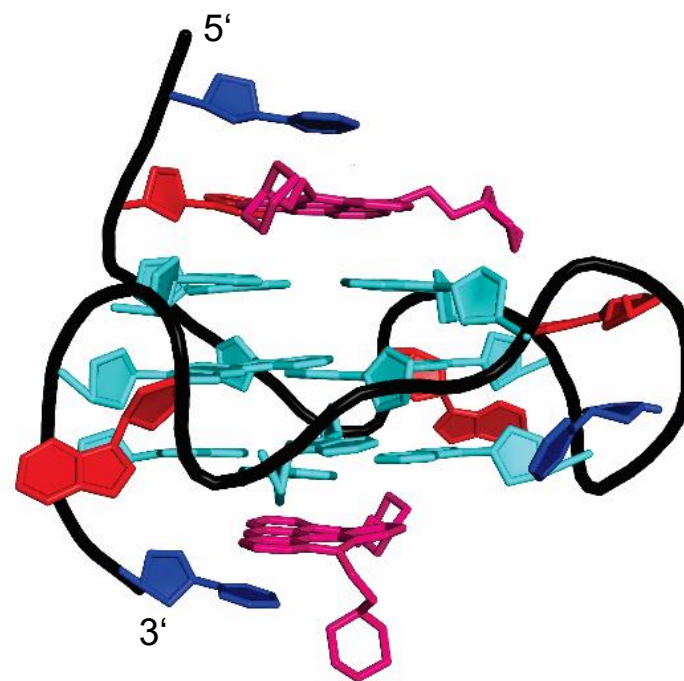


R. Vilar, et al. *Nat. Commun.* **2015**, 6:8178.

# Titration with DAOTA-M2



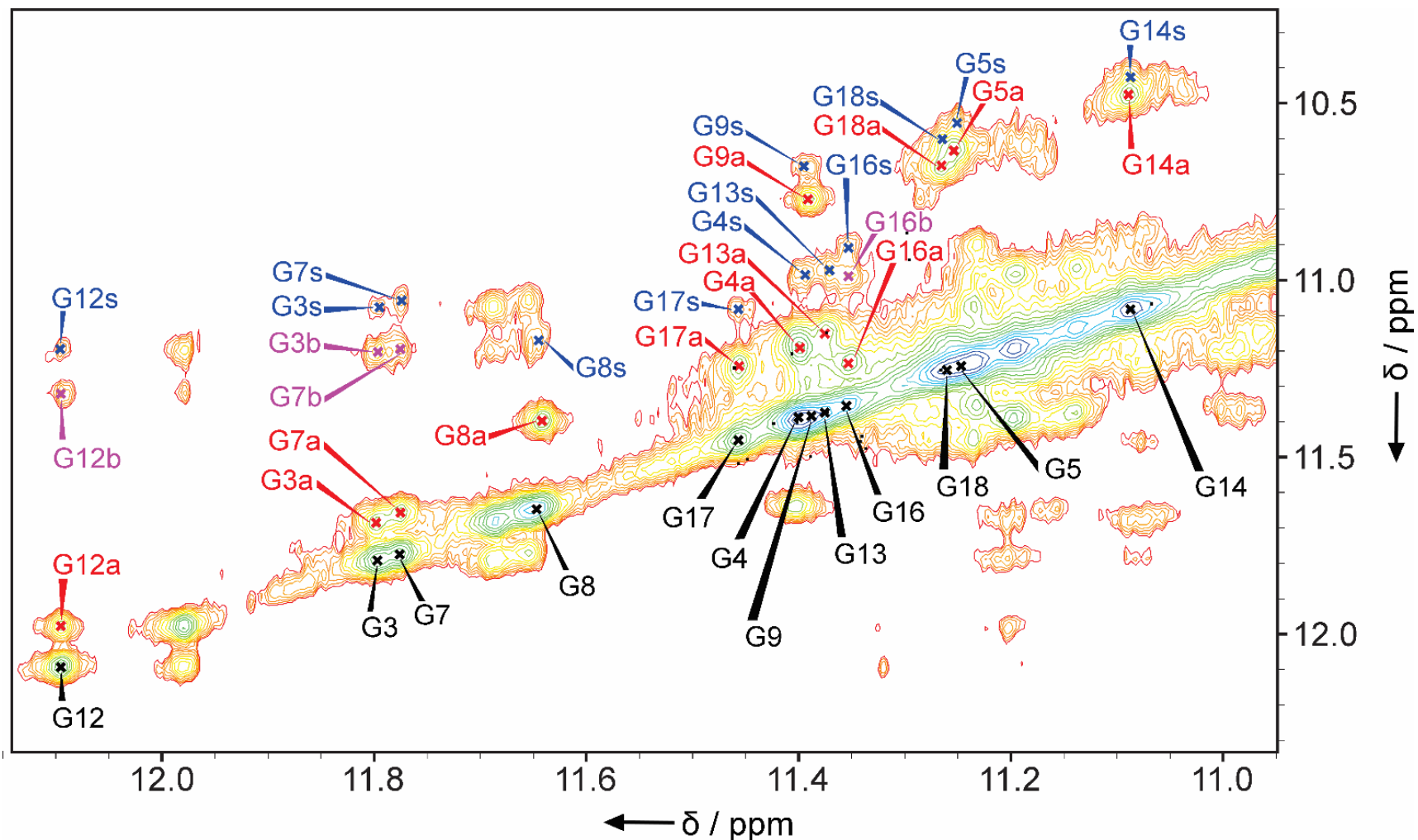
- new signals corresponding to ligand-G-quadruplex complex
- 1:2 stoichiometry of binding



Imino and methyl regions of  $^1\text{H}$  NMR spectra of CMA during titration with ligand DAOTA-M2 (L).

0.2 mM CMA per strand, 20 mM phosphate buffer, 100 mM KCl,  $\text{H}_2\text{O}:\text{D}_2\text{O}=9:1$ , pH 7.0, 25 °C, 600 MHz spectrometer.

# Binding of ligand DAOTA-M2 with G-quadruplex



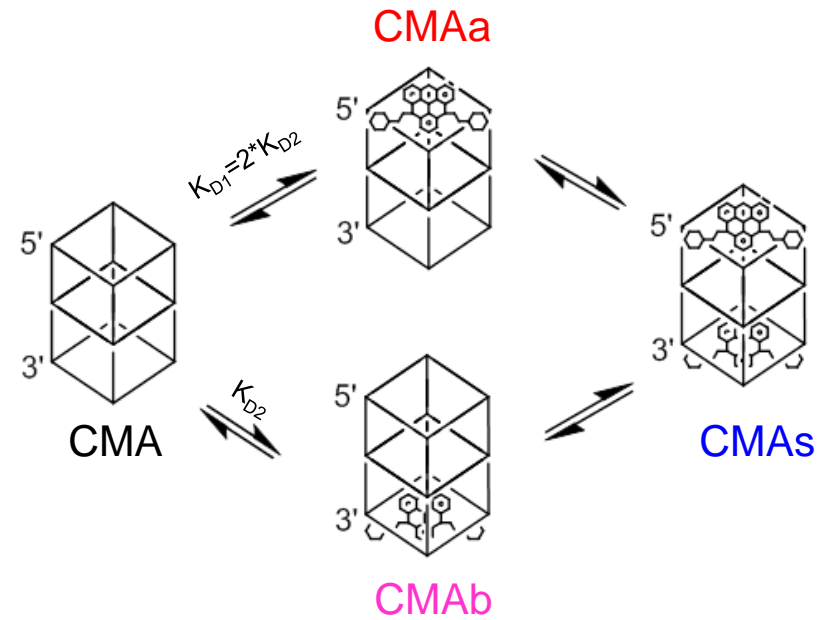
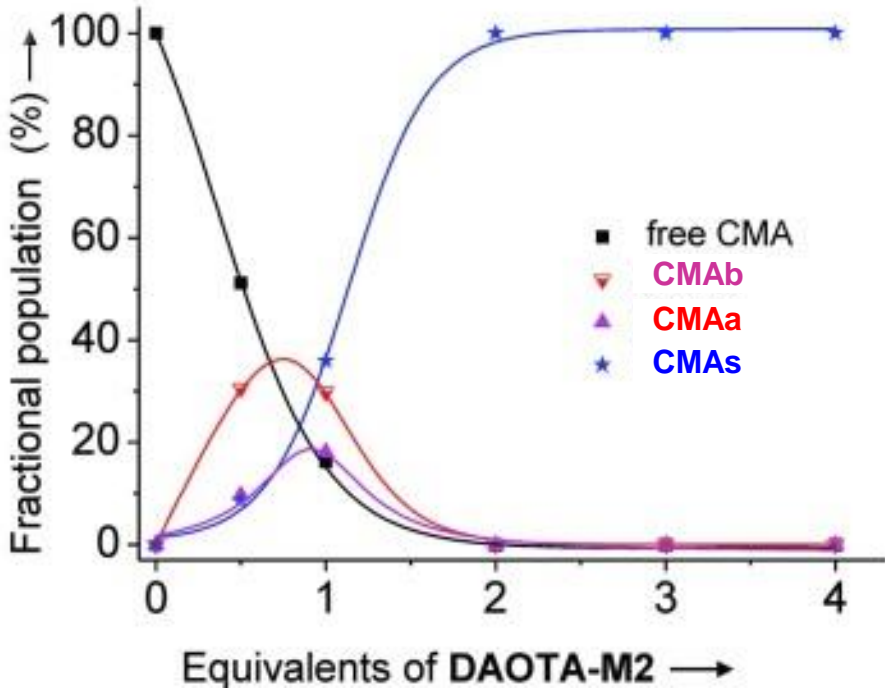
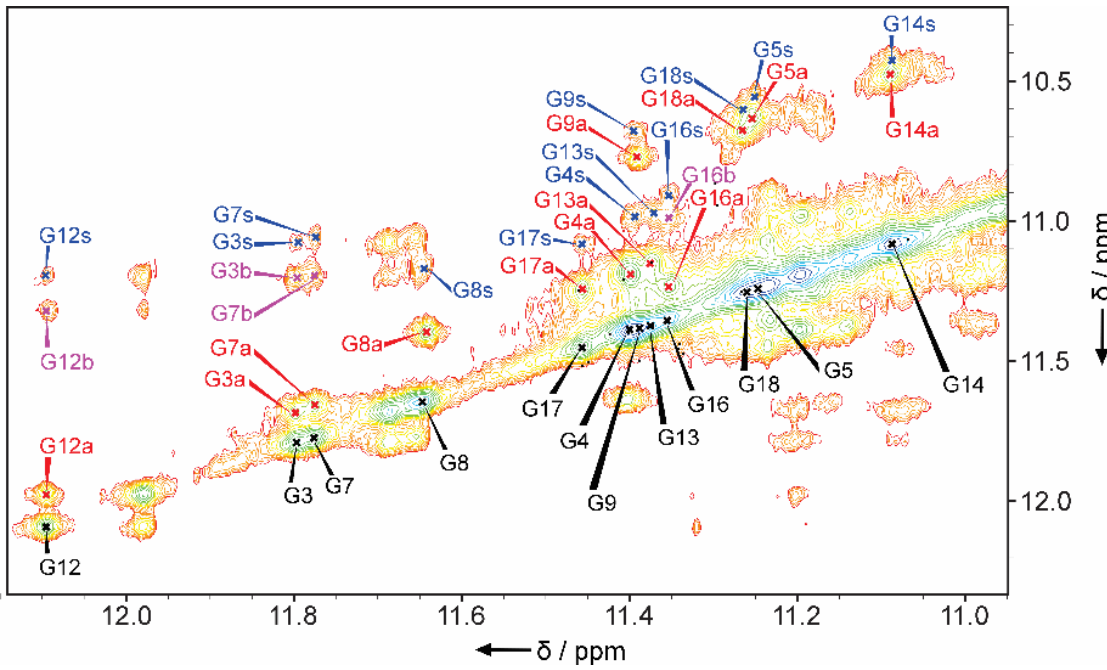
Imino-imino region of NOESY spectrum of CMA at 1:0.5 ratio of CMA : ligand

Diagonal cross-peaks are marked with black, cross-peaks attributed to exchange are marked with red, violet and blue.

Spectrum acquired at 0.2 mM CMA conc. per strand, 20 mM phosphate buffer, 100 mM KCl, H<sub>2</sub>O:<sup>2</sup>H<sub>2</sub>O=9:1, pH 7.0, 25 °C, 80 mixing time on 600 MHz NMR spectrometer.

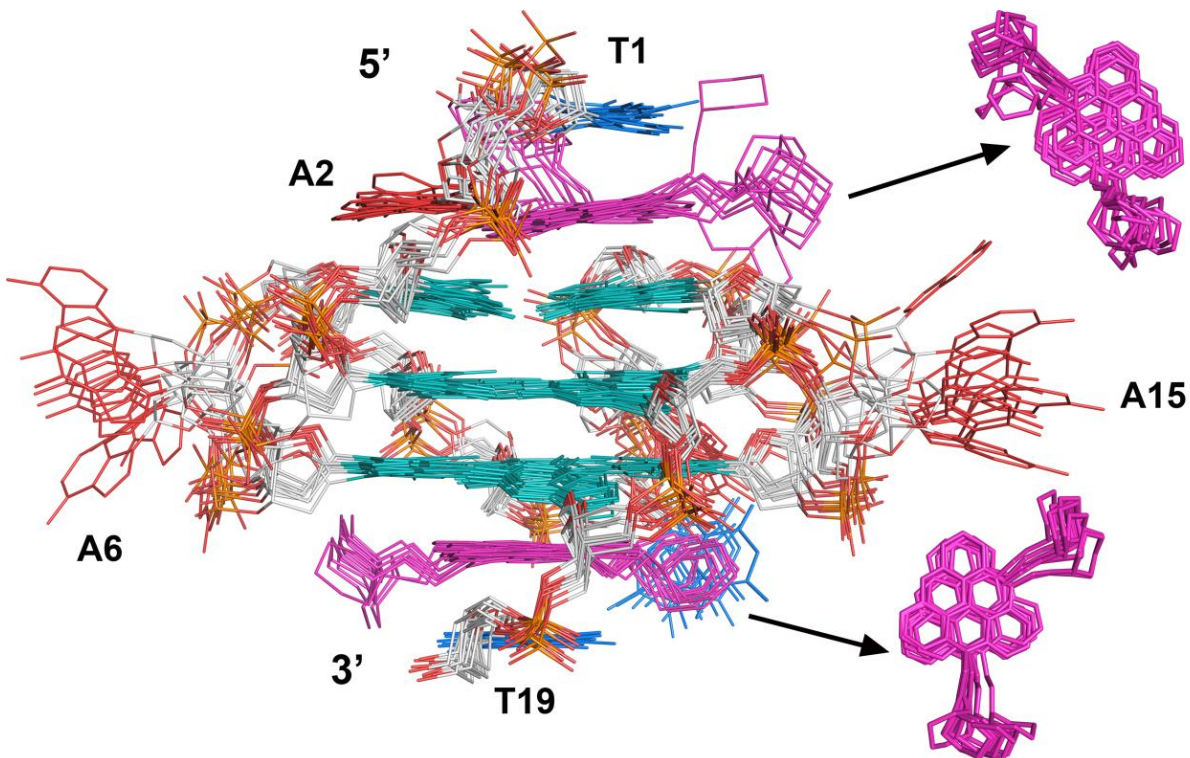
# Binding model

Imino-imino region of NOESY spectrum of CMA at 1:0.5 ratio of CMA : ligand



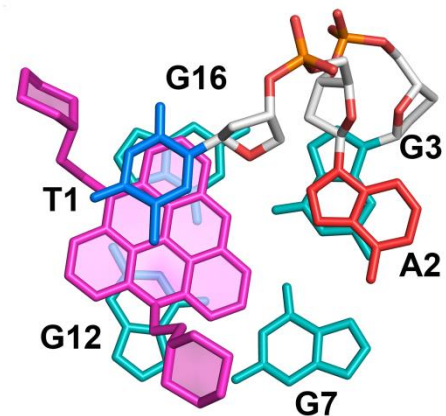
# Structure of DAOTA-M2 - G-quadruplex

High-res NMR restrained structure

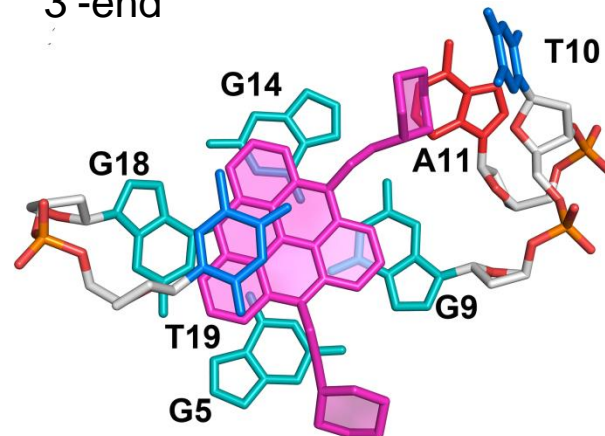


PDB ID: 5LIG

5'-end



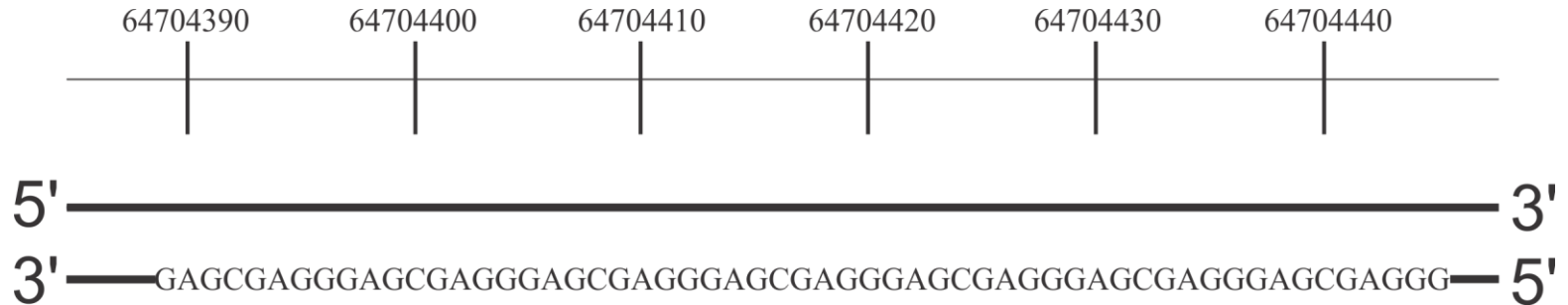
3'-end





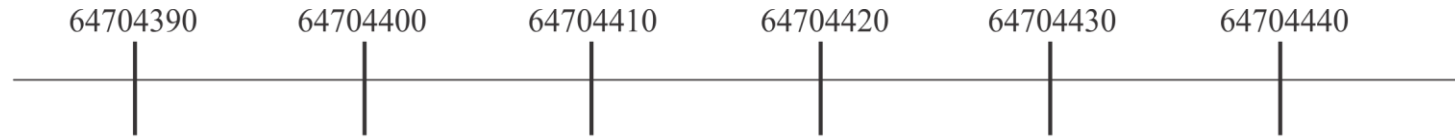
# *d(GCGAGGG) repeats*

## *PLEKHG3 in 14<sup>th</sup> human chromosome*



# *d(GCGAGGG) repeats*

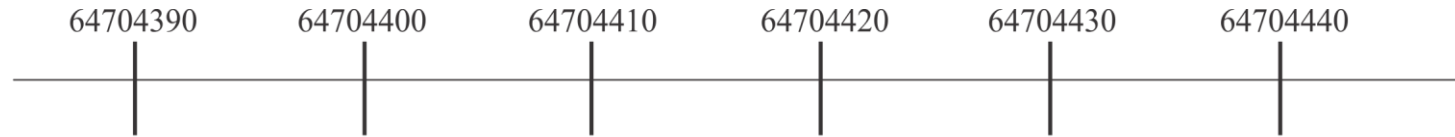
## *PLEKHG3 in 14<sup>th</sup> human chromosome*



Lybaek, H., Oyen, N., Fauske, L. & Houge, G., *Clin. Genet.* **74**, 553-559 (2008).  
Griswold, A. J. *et al.*, *Autism Res.* **4**, 221-227 (2011).

# *d(GCGAGGG) repeats*

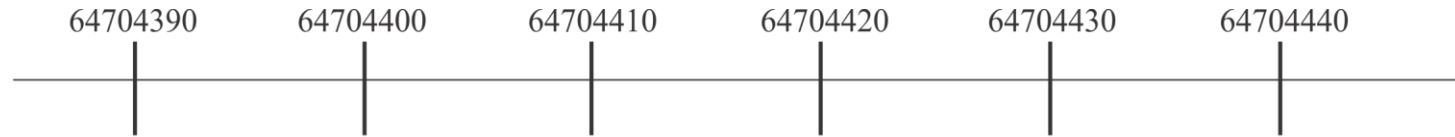
## *PLEKHG3 in 14<sup>th</sup> human chromosome*



Lybaek, H., Oyen, N., Fauske, L. & Houge, G., *Clin. Genet.* **74**, 553-559 (2008).  
Griswold, A. J. *et al.*, *Autism Res.* **4**, 221-227 (2011).

# *d(GCGAGGG) repeats*

## *PLEKHG3 in 14<sup>th</sup> human chromosome*

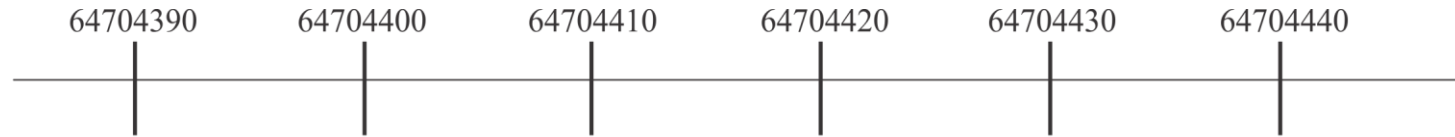


**VK1**

***d(GGG-A-GCG-A-GGG-A-GCG)***

# *d(GCGAGGG) repeats*

## *PLEKHG3 in 14<sup>th</sup> human chromosome*



**VK1**

***d(GGG-A-GCG-A-GGG-A-GCG)***



# *d(GCGAGGG) repeats*

## *PLEKHG3 in 14<sup>th</sup> human chromosome*



**VK1**



**VK34**



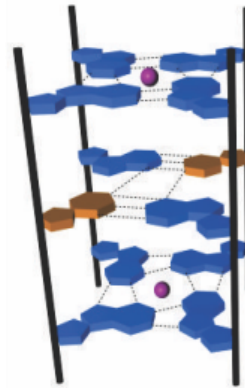
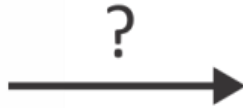
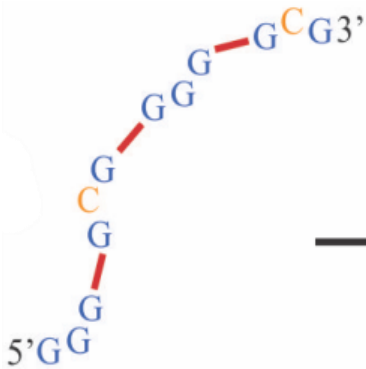
## *Possible folds adopted by d(GGGAGCG) repeats*

- *VK1: d(GGG-A-GCG-A-GGG-A-GCG)*
- *VK34: d(GCG-A-GGG-A-GCG-A-GGG)*

# Possible folds adopted by d(GGGAGCG) repeats

- VK1:  $d(\text{GGG-A-GCG-A-GGG-A-GCG})$
- VK34:  $d(\text{GCG-A-GGG-A-GCG-A-GGG})$

G-quadruplex?

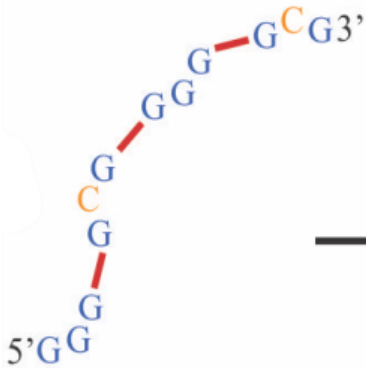


# Possible folds adopted by d(GGGAGCG) repeats

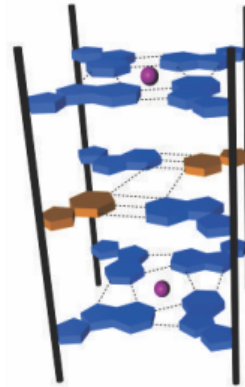
- VK1: d(GGG-A-GCG-A-GGG-A-GCG)
- VK34: d(GCG-A-GGG-A-GCG-A-GGG)

G-quadruplex?

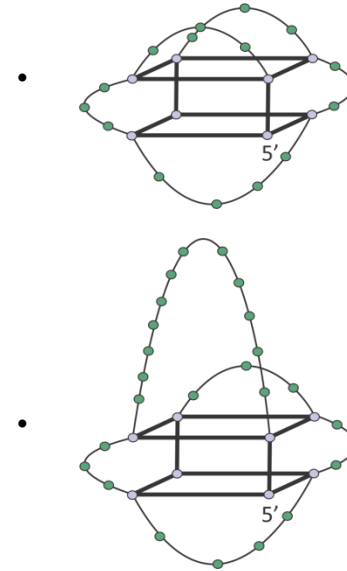
New folds?



?

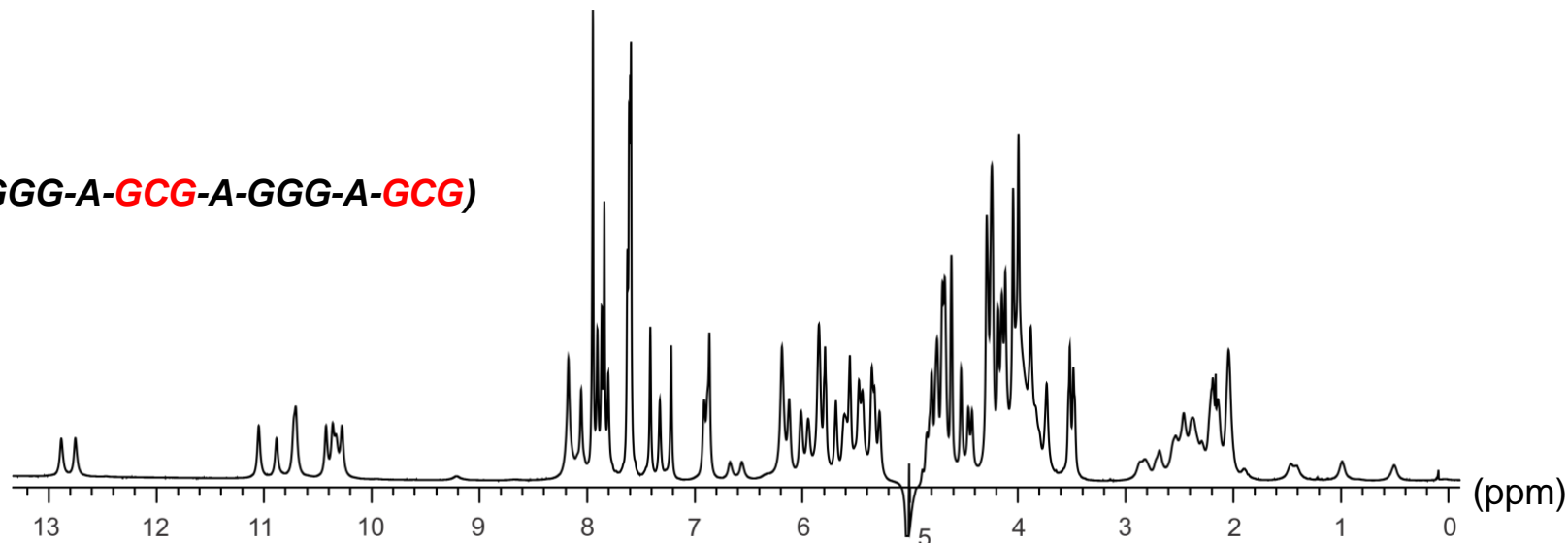


- 105 000 of G-rich sequences are difficult to explain with known G-quadruplex topologies



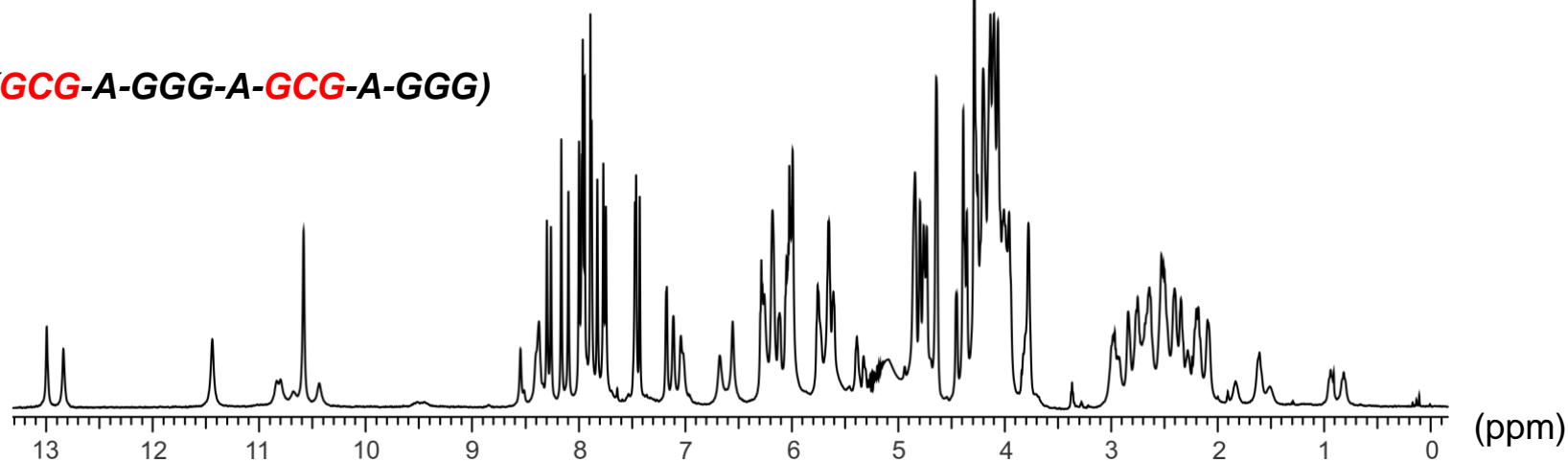
# Folding in the presence of $\text{Li}^+$ cations

VK1:  $d(\text{GGG-A-GCG-A-GGG-A-GCG})$



$^1\text{H}$  NMR spekter, 2.8 mM DNA, 100 mM LiCl, 0 °C, pH 6

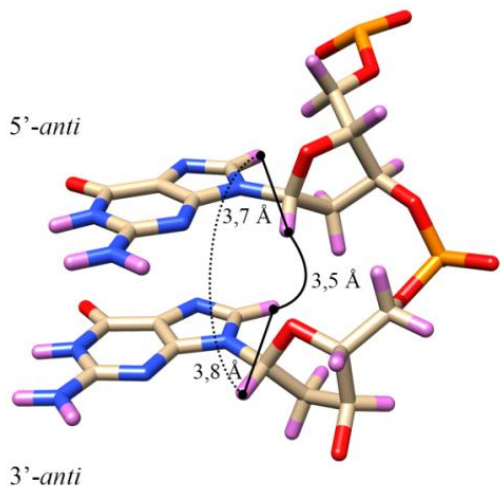
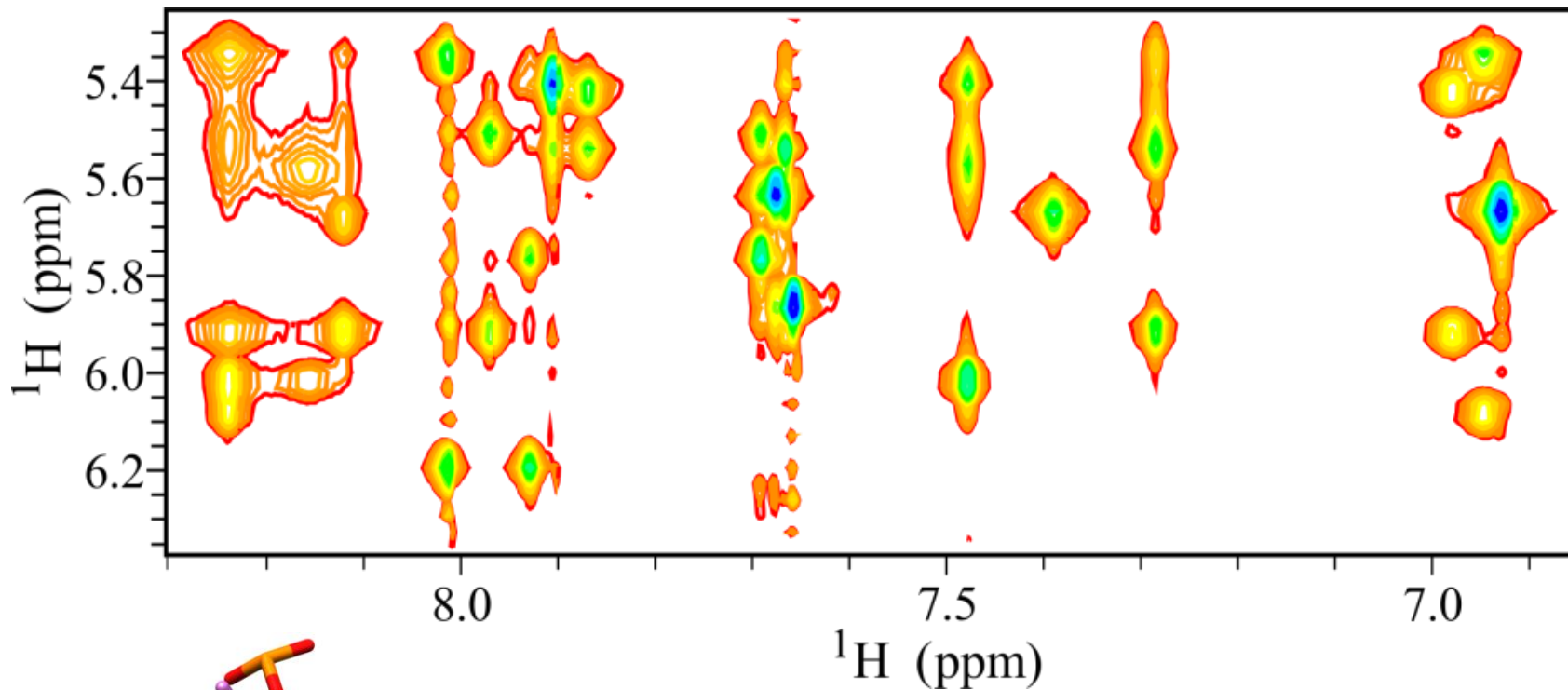
VK34:  $d(\text{GCG-A-GGG-A-GCG-A-GGG})$



$^1\text{H}$  NMR spekter, 0.4 mM DNA, 100 mM LiCl, 0 °C, pH 6



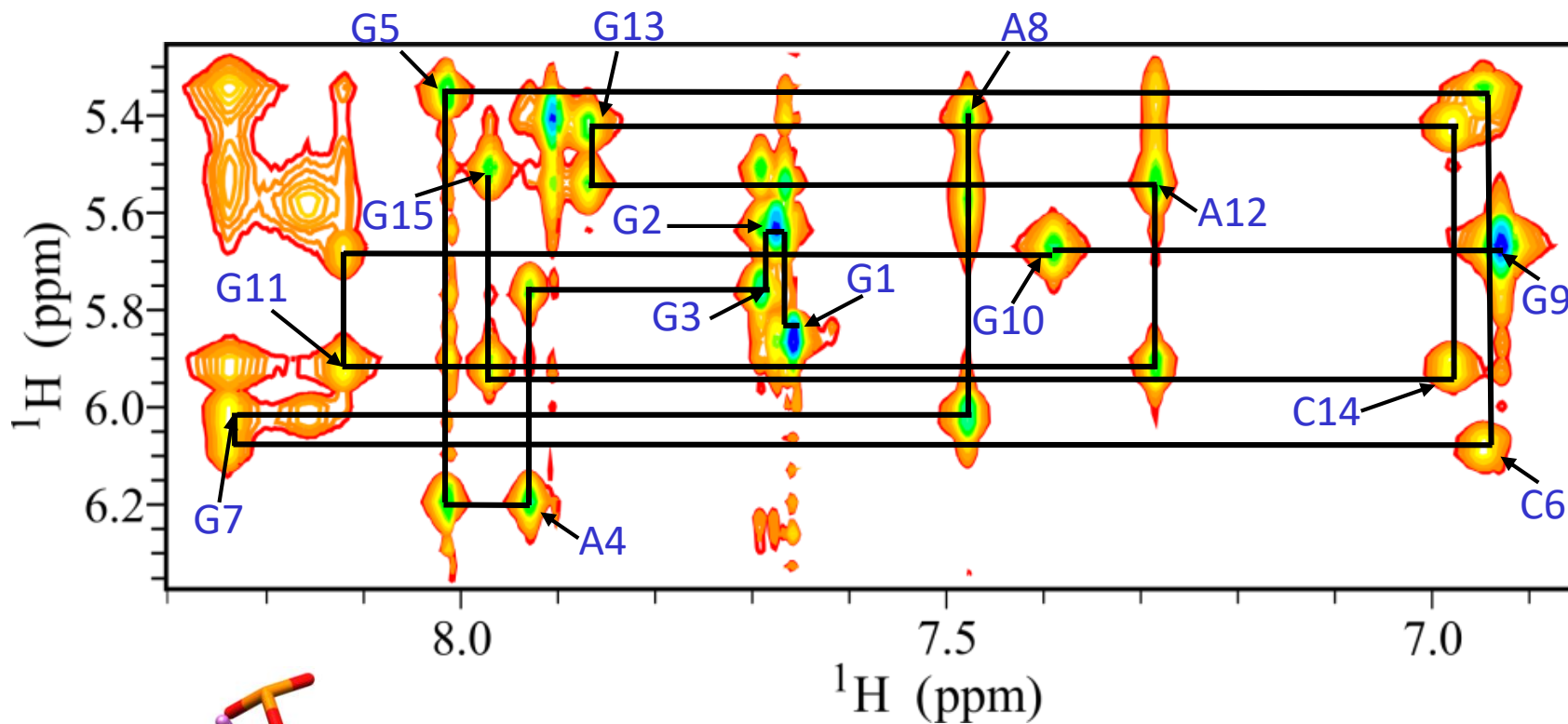
# Sequential assignment



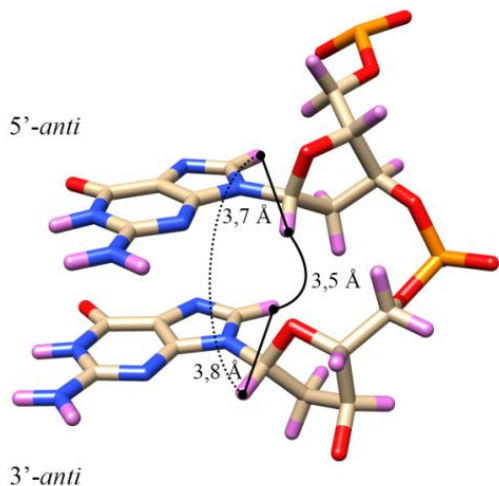
Aromatic-anomeric region of 2D NOESY spectrum,  $\tau_m = 80$  ms, 2.8 mM DNA, 100 mM LiCl, 0 °C, pH 6

**VK1 G1-G2-G3-A4-G5-C6-G7-A8-G9-G10-G11-A12-G13-C14-G15**

# Sequential assignment



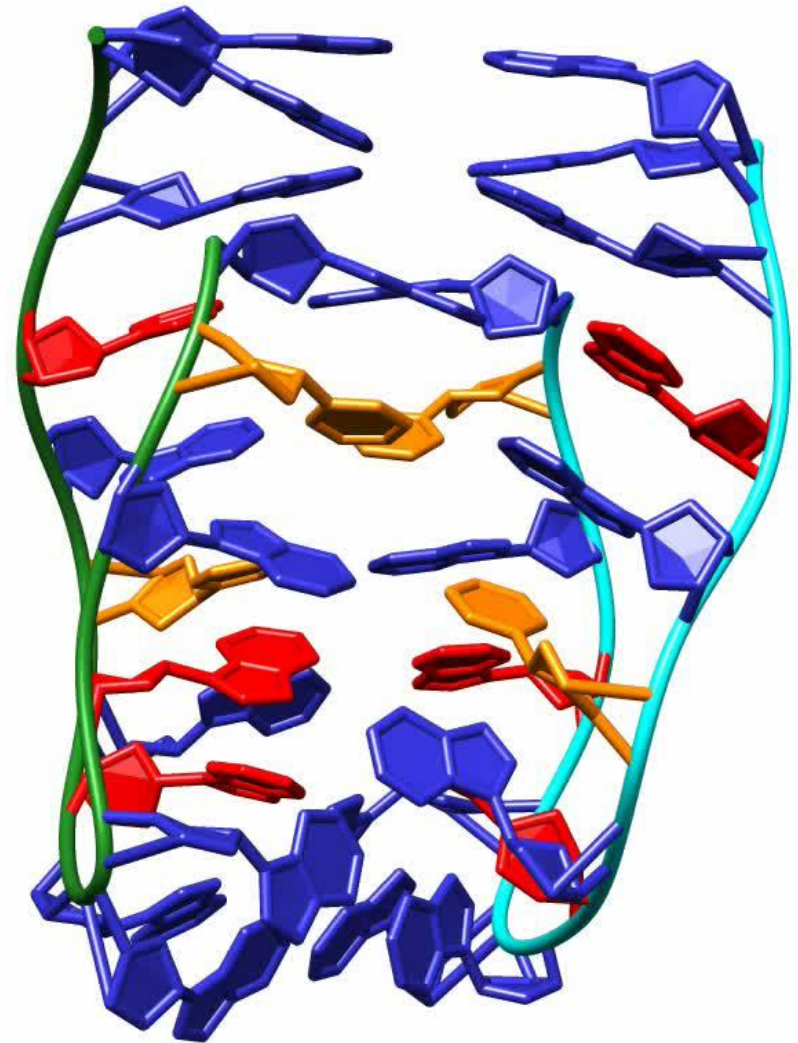
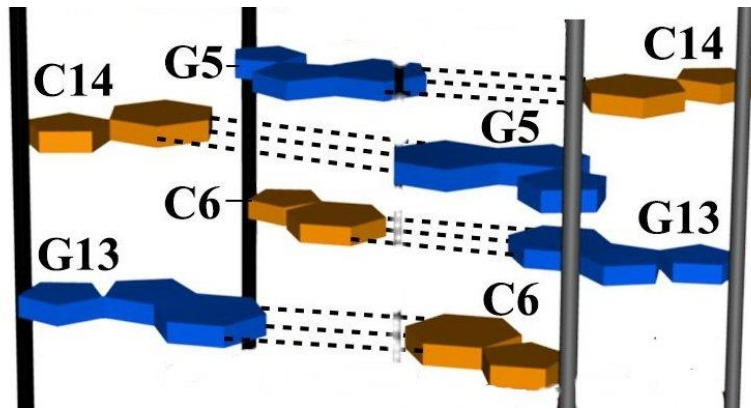
Aromatic-anomeric region of 2D NOESY spectrum,  $\tau_m = 80$  ms, 2.8 mM DNA, 100 mM LiCl, 0 °C, pH 6



**VK1 G1-G2-G3-A4-G5-C6-G7-A8-G9-G10-G11-A12-G13-C14-G15**

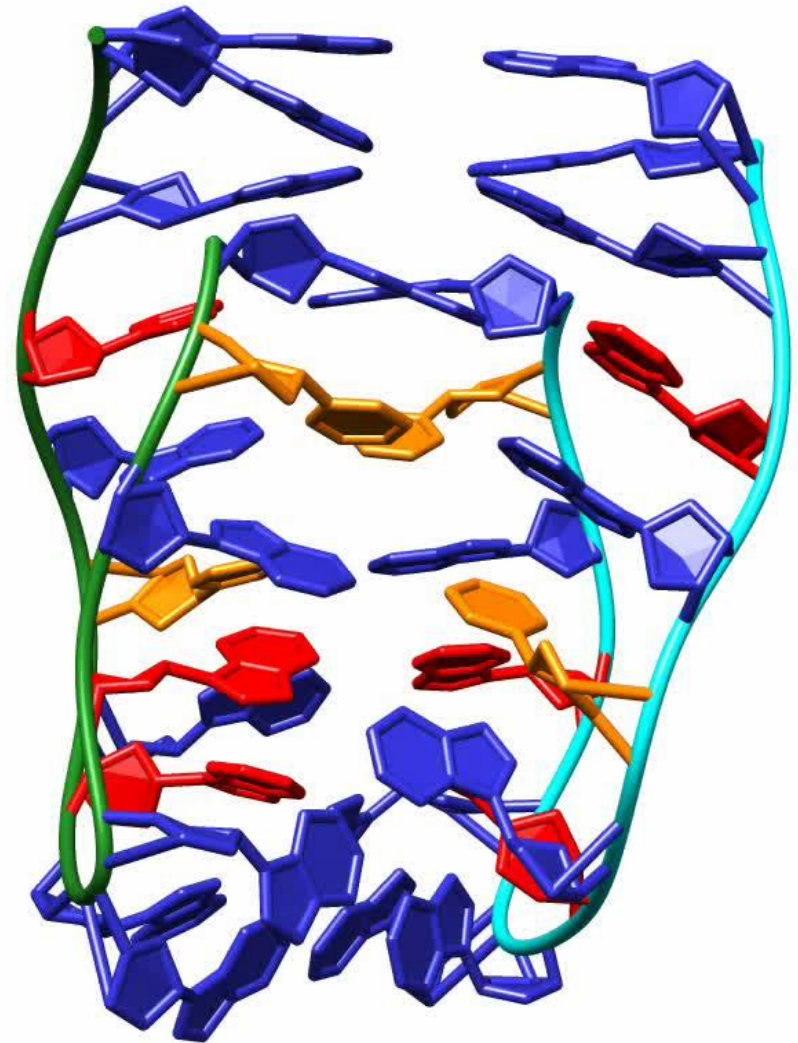
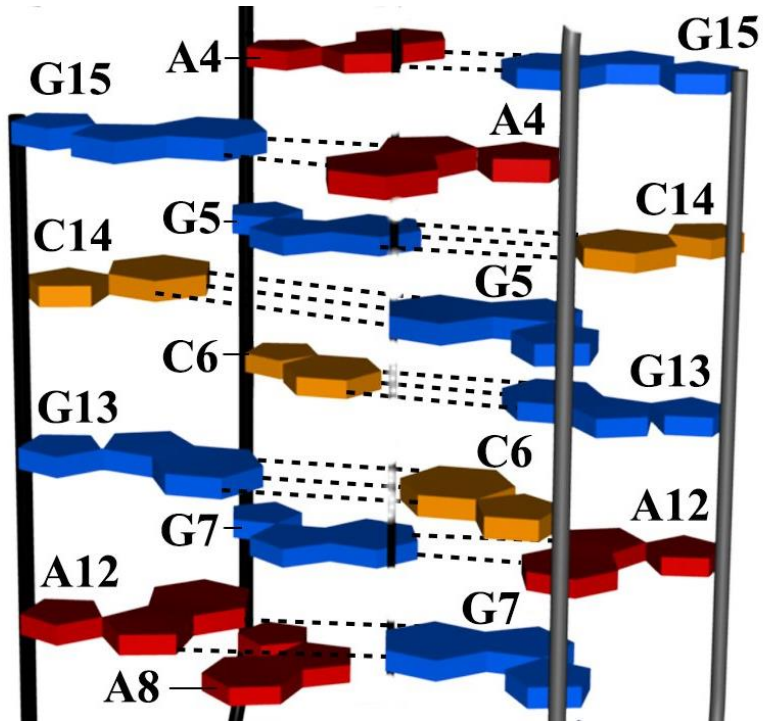
# High-resolution structure of VK1

*d*(GGG-A-GCG-A-GGG-A-GCG)



# High-resolution structure of VK1

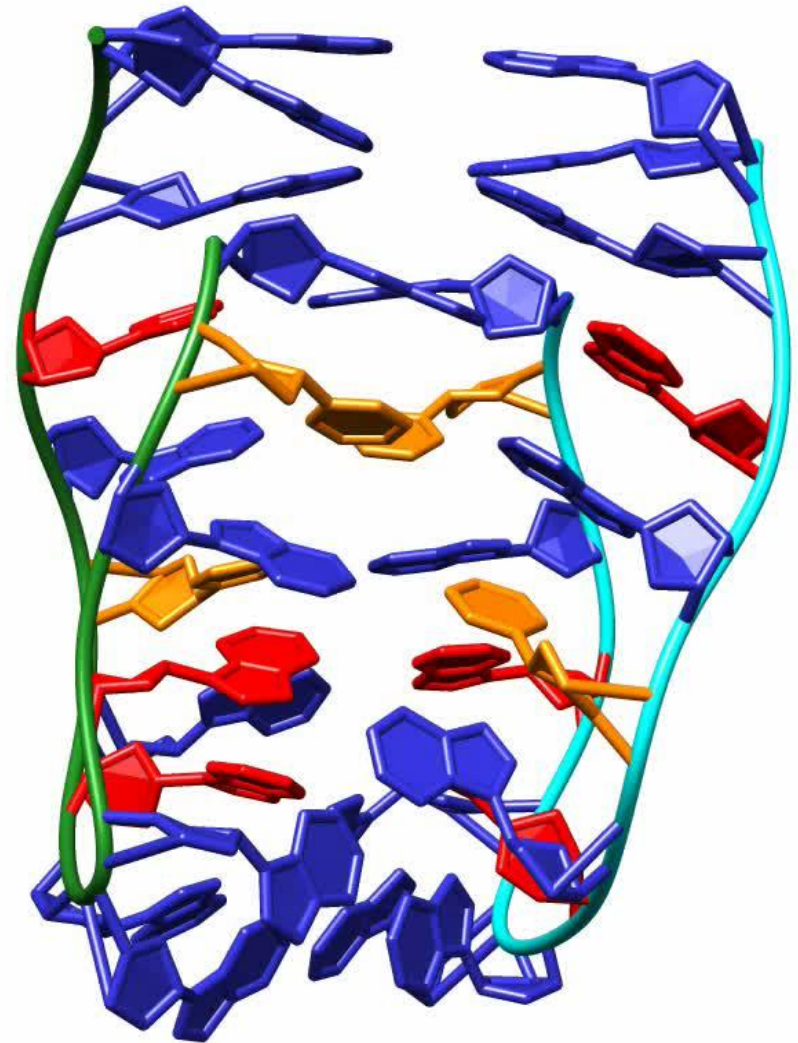
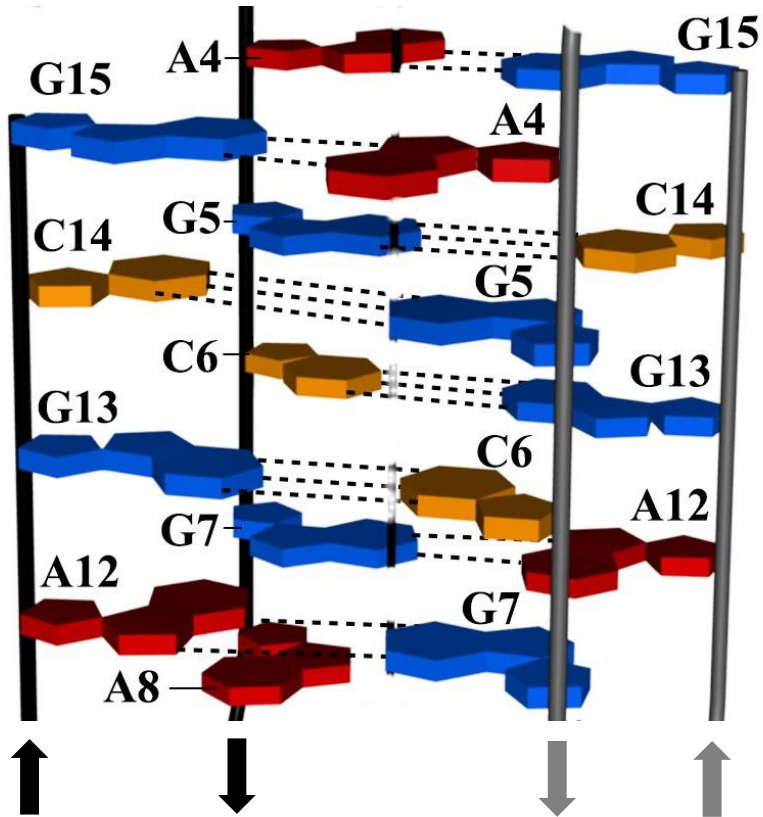
*d*(GGG-A-GCG-A-GGG-A-GCG)





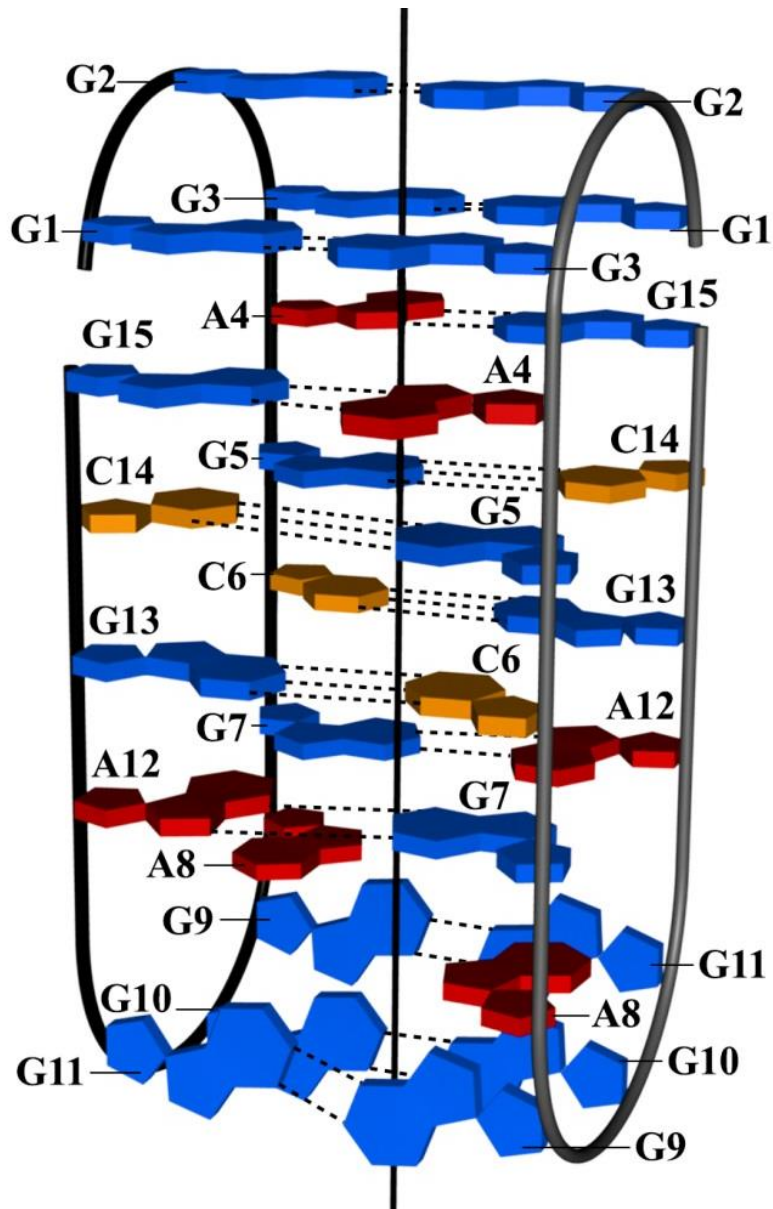
# High-resolution structure of VK1

*d*(GGG-A-GCG-A-GGG-A-GCG)

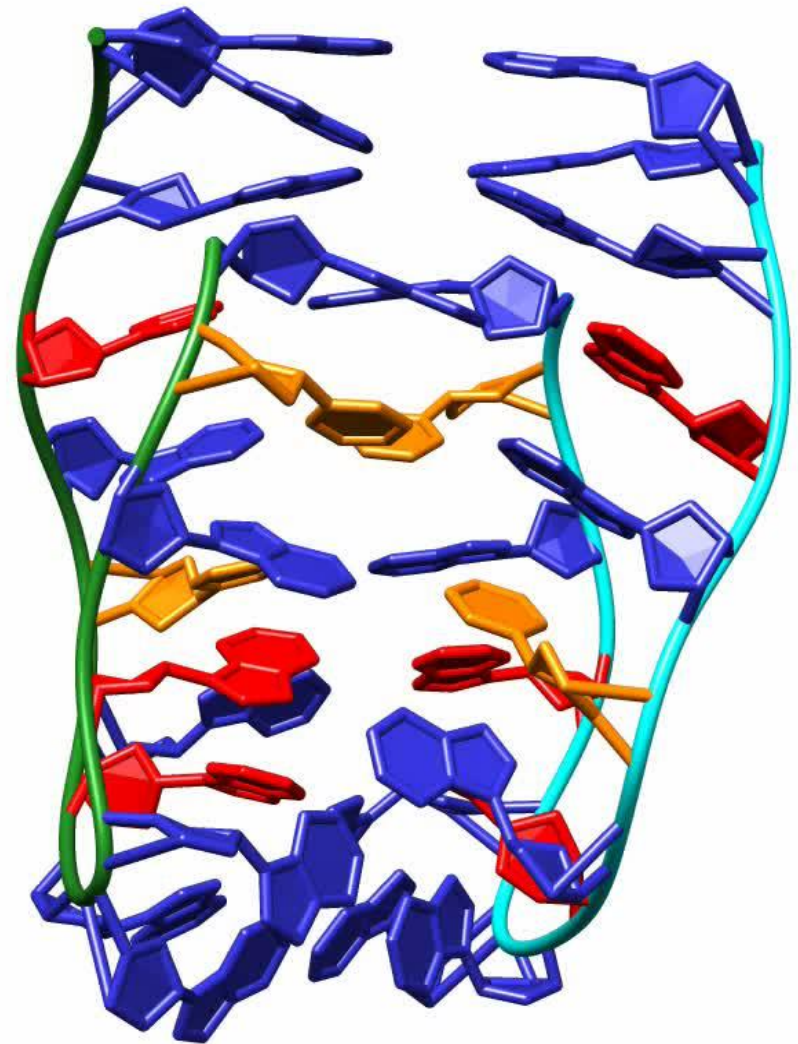




# High-resolution structure of VK1

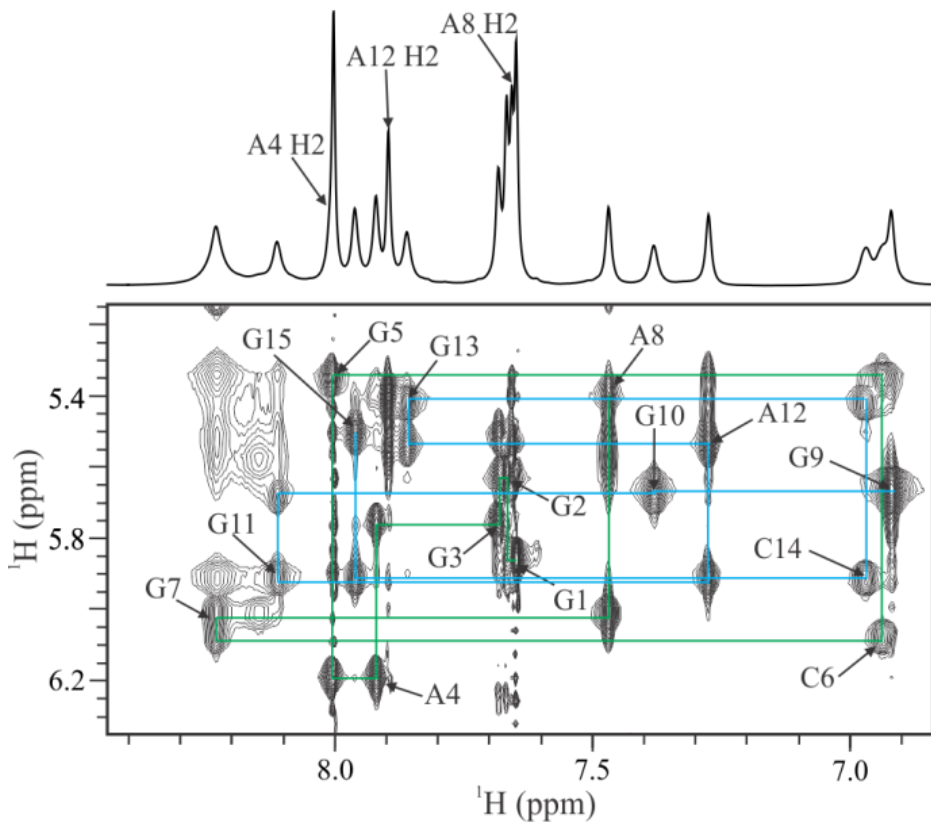


*d*(GGG-A-GCG-A-GGG-A-GCG)

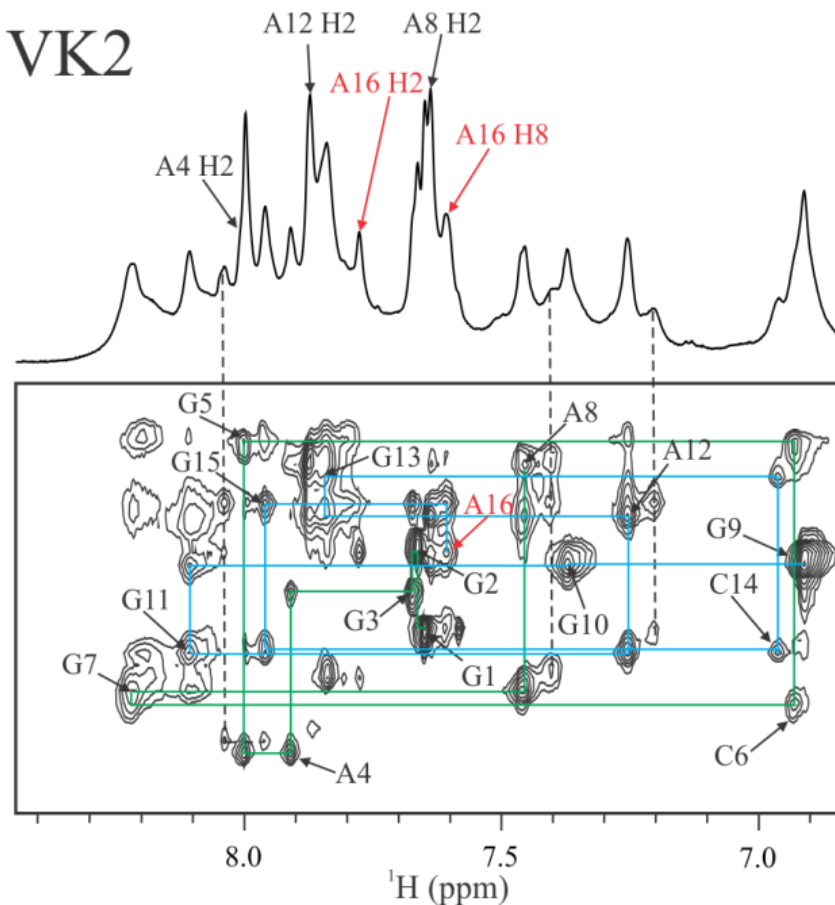


# Fingerprints in NOESY spectra of VK1 and VK2

VK1



VK2

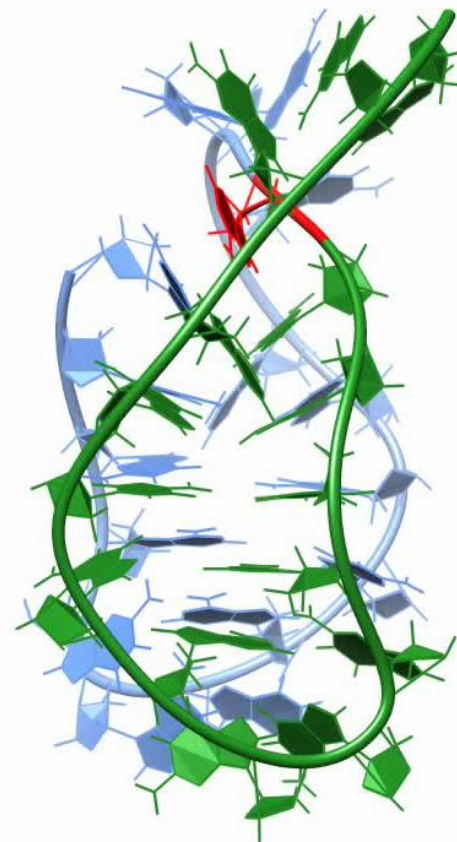
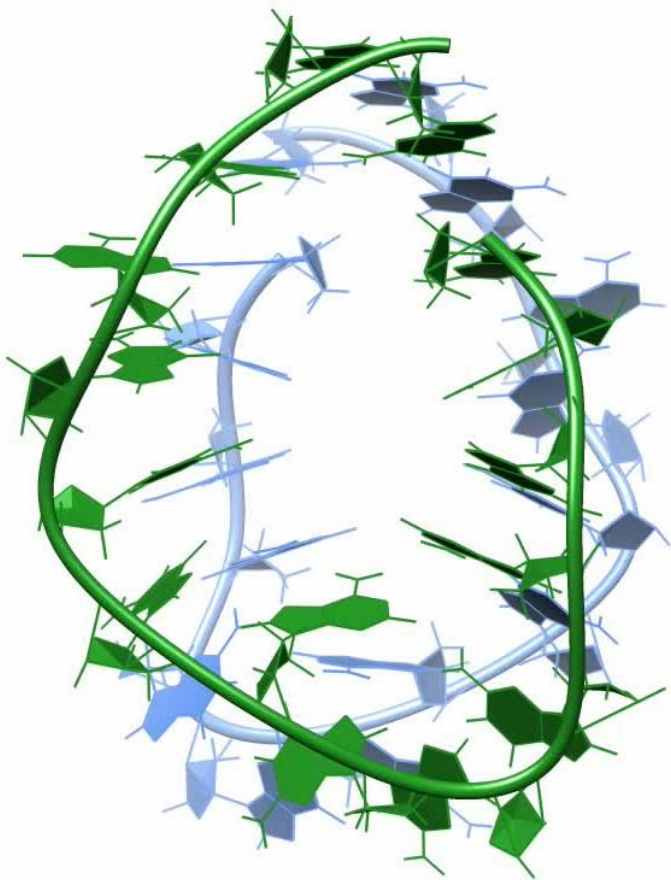


Aromatic-anomeric region of 2D NOESY spectra,  $\tau_m = 80$  ms, 2.8 mM VK1, 1.0 mM VK2, 100 mM LiCl, 0 °C, pH 6

**VK1: G1-G2-G3-A4-G5-C6-G7-A8-G9-G10-G11-A12-G13-C14-G15**

**VK2: VK1-A16-VK1**

# *A16 connects 'two units' of the monomeric fold*



**VK1: G1-G2-G3-A4-G5-C6-G7-A8-G9-G10-G11-A12-G13-C14-G15**

**VK2: VK1-A16-VK1**

# *d(GCGAGGG) repeats*

-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-

VK1

*d(GGG-A-GCG-A-GGG-A-GCG)*

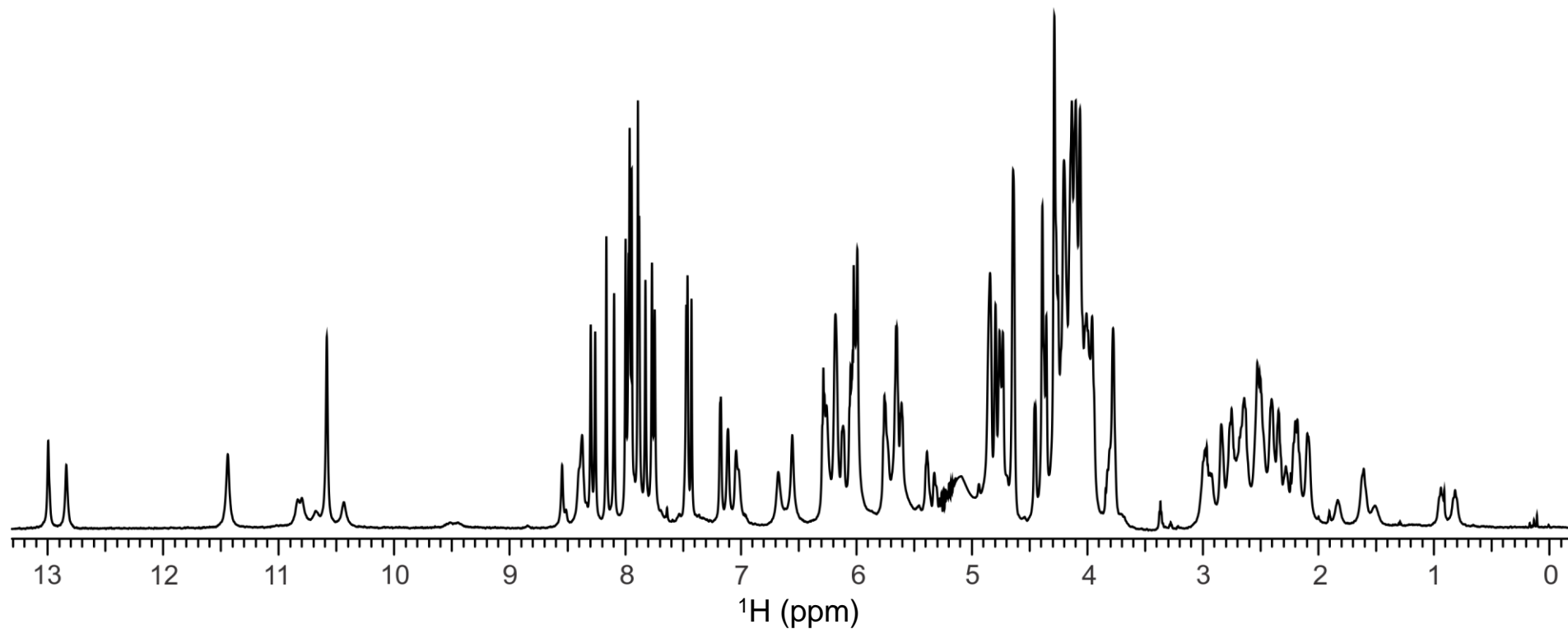
VK34

*d(GCG-A-GGG-A-GCG-A-GGG)*

# What is the structure of VK34?

**d(GCG-A-GGG-A-GCG-A-GGG)**

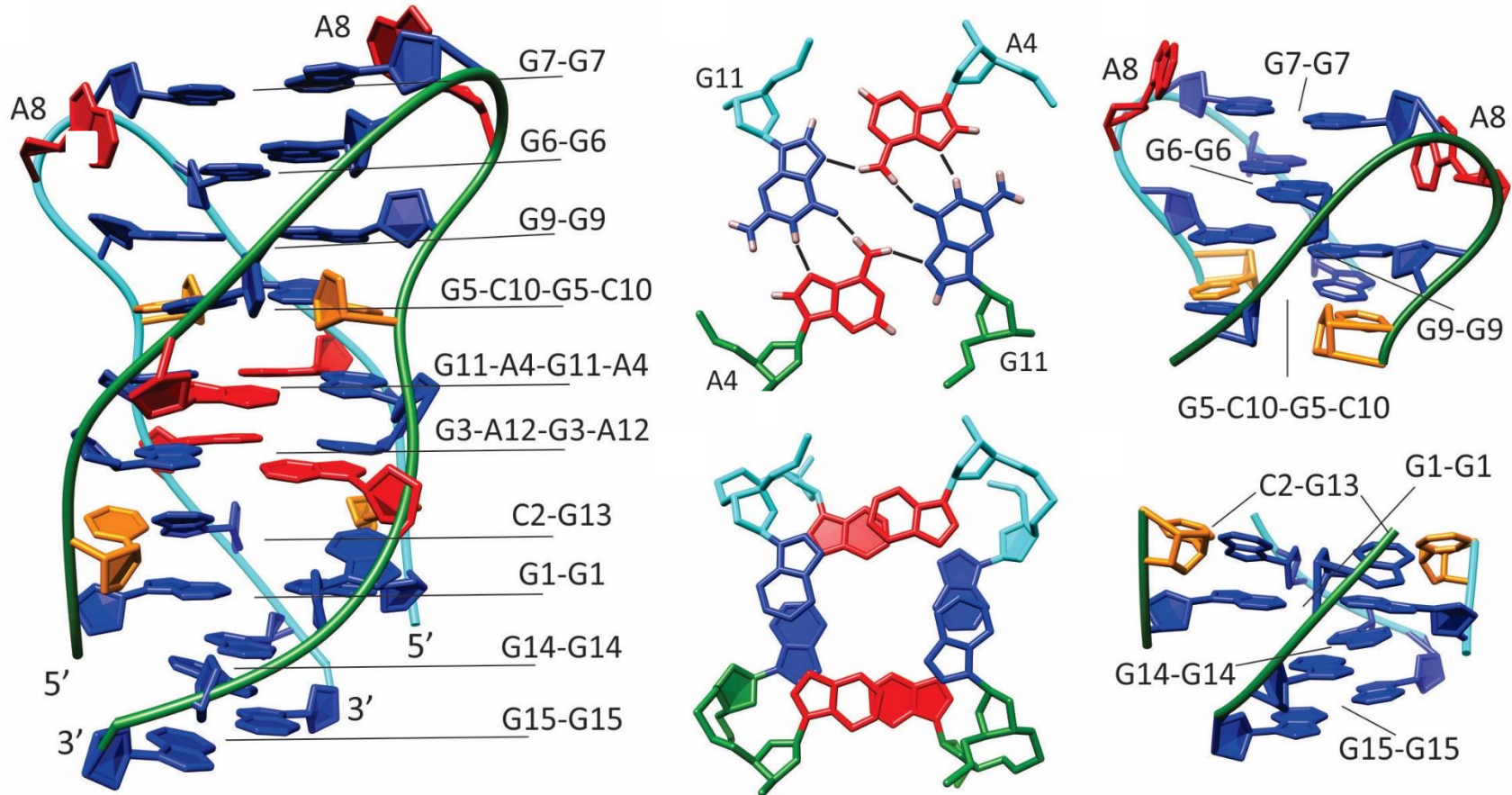
**Li<sup>+</sup>!**



<sup>1</sup>H NMR spectrum, 0.4 mM VK34, 100 mM LiCl, 0 °C, pH 6

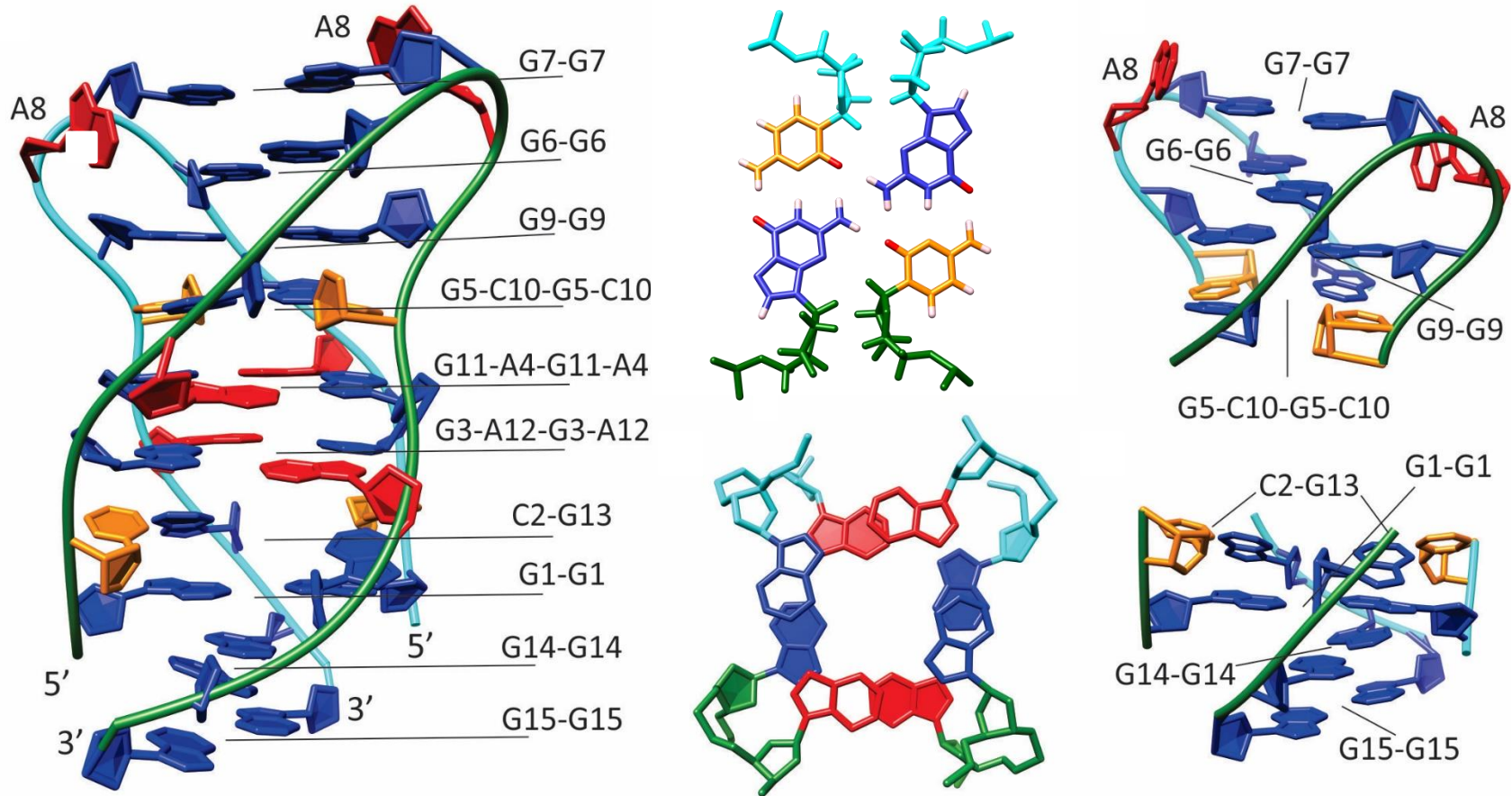


# Dimeric structure of VK34 with GAGA core



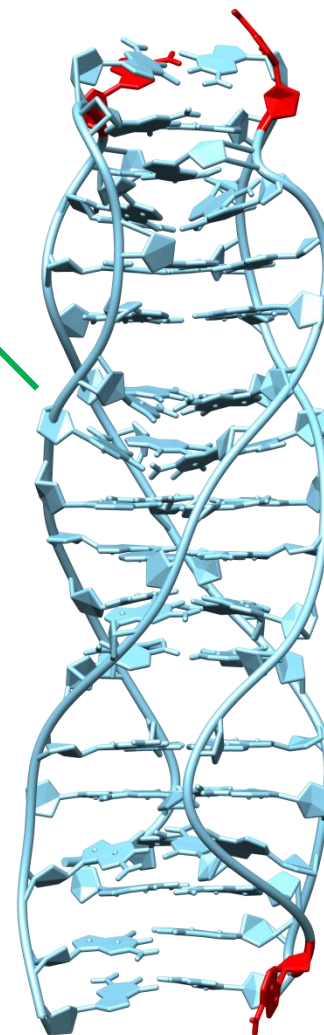
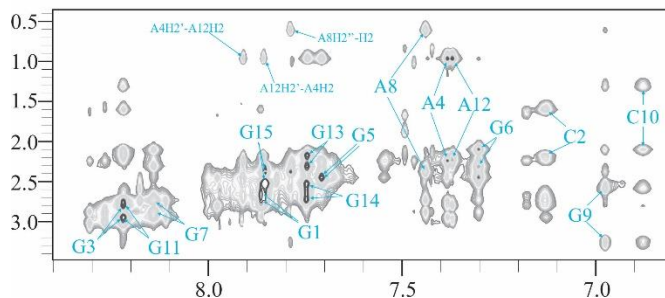
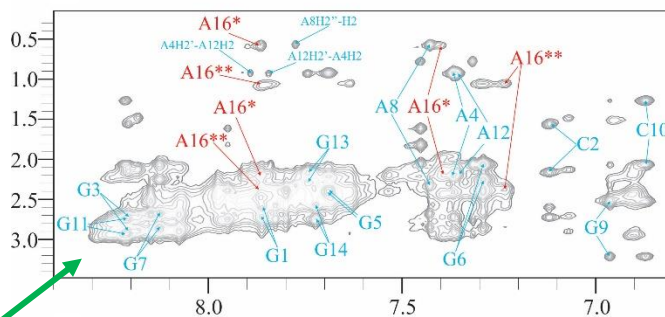
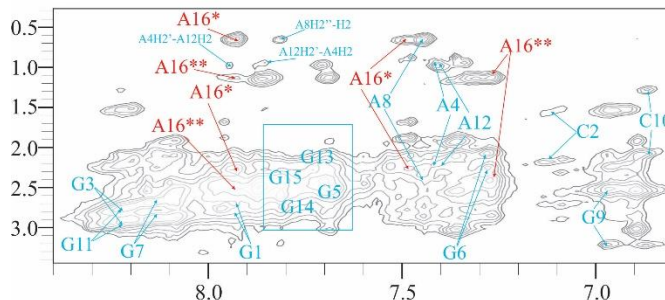
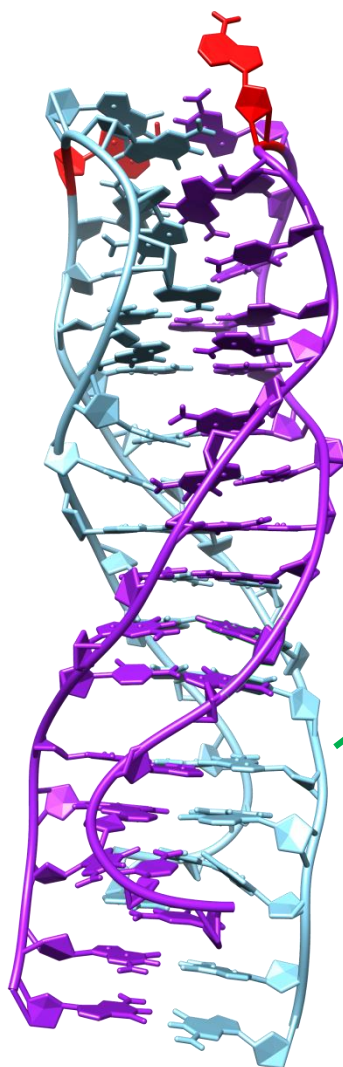
**VK34: G1-C2-G3-A4-G5-G6-G7-A8-G9-C10-G11-A12-G13-G14-G15**

# Dimeric structure of VK34 with GAGA core



**VK34: G1-C2-G3-A4-G5-G6-G7-A8-G9-C10-G11-A12-G13-G14-G15**

# Adenines connect VK34 in dimeric and monomeric folds



2x (VK34-A-VK34)

(VK34-A-VK34-A-VK34-A-VK34)

Aromatic-H2'/H2'' region of 2D NOESY spectra,  $\tau_m$   
 = 150 ms, 1.0-1.2 mM DNA, 150 mM (VK34) NaCl,  
 100 mM (2VK34 in 4VK34) LiCl, 0 °C, pH 6

-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-A-GGG-A-GCG-

# AGCGA repeats in the genome

...-GGG-AGCGA-GGG-AGCGA-GGG-AGCGA-GGG-AGCGA-GGG-AGCGA-GGG-AGCG-...



# AGCGA repeats in the genome

...-GGG-AGCGA-GGG-AGCGA-GGG-AGCGA-GGG-AGCGA-GGG-AGCGA-GGG-AGCG-...

**AGCGA-N<sub>1-20</sub>-AGCGA-N<sub>1-20</sub>-AGCGA-N<sub>1-20</sub>-AGCGA**

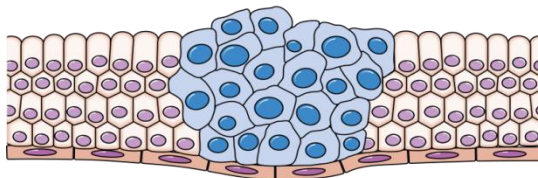
**Neurodevelopment and  
neurological disorders**



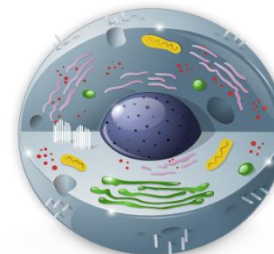
**Abnormal cartilage and bone  
formations**



**Different cancers**

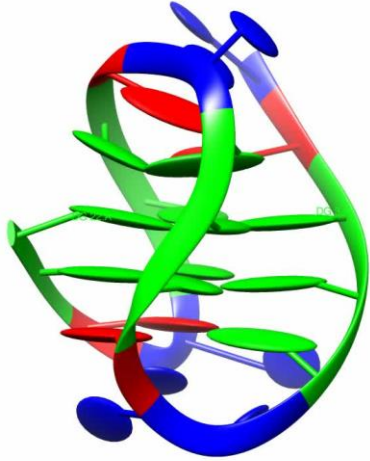


**Basic cellular processes**

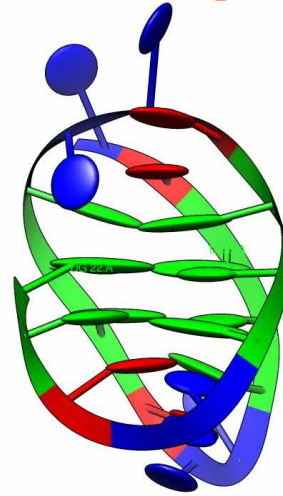




# Summary and Implications



*pdb id: 5LQG*

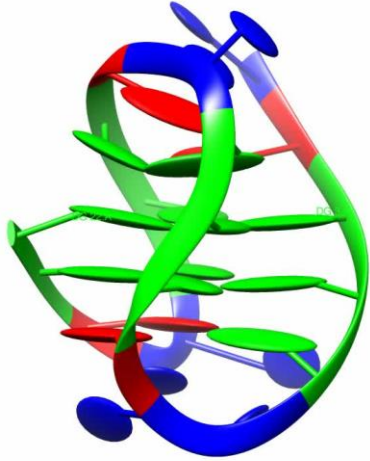


*pdb id: 5LQH*

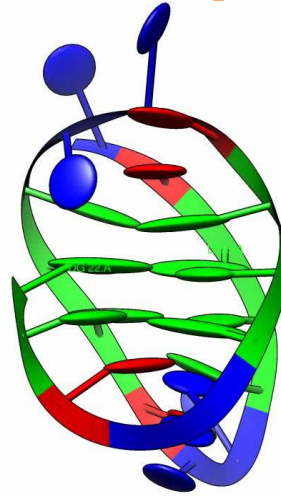
*d(GGG-A-GCG-A-GGG-A-GCG); pdb id: 2MJJ*

*d(GCG-A-GGG-A-GCG-A-GGG); pdb id: 5M1L*

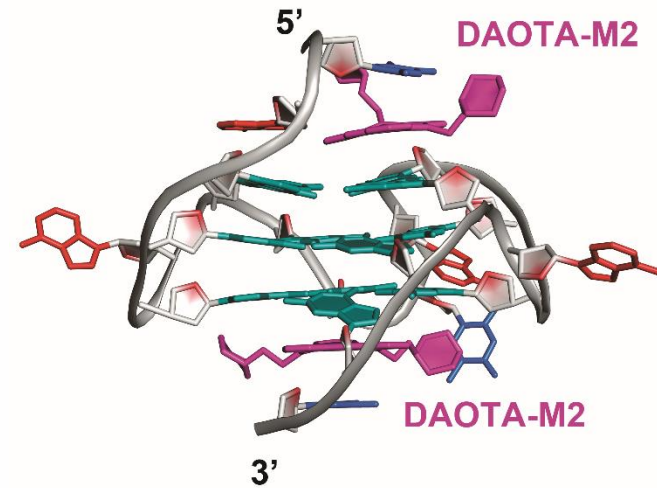
# Summary and Implications



*pdb id: 5LQG*



*pdb id: 5LQH*

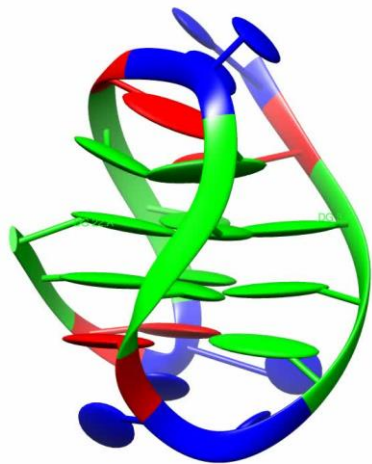


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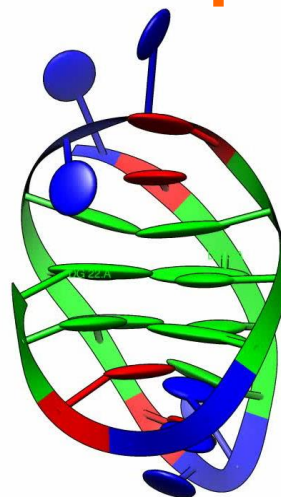
*d(GGG-A-GCG-A-GGG-A-GCG); pdb id: 2MJJ*

*d(GCG-A-GGG-A-GCG-A-GGG); pdb id: 5M1L*

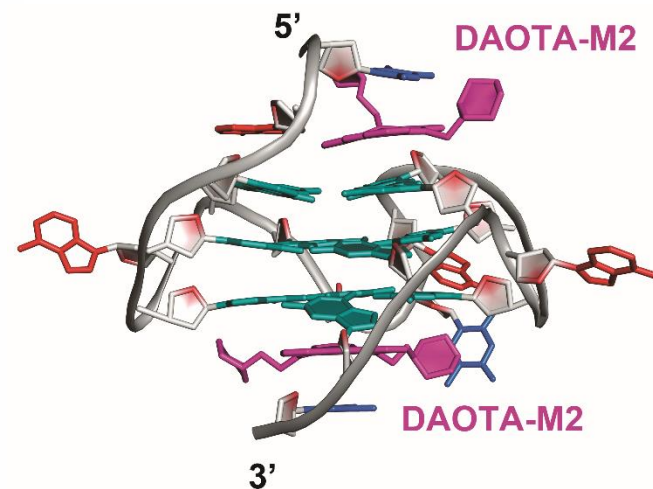
# Summary and Implications



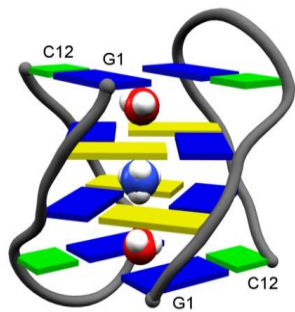
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*pdb id: 5LQH*



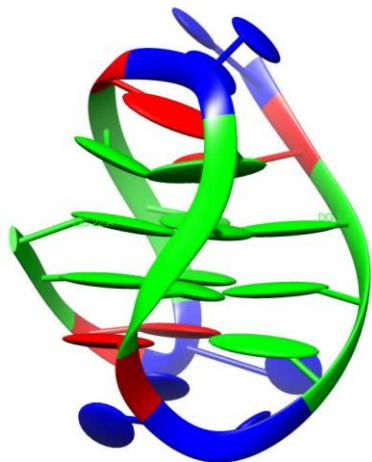
*pdb id: 5LIG*



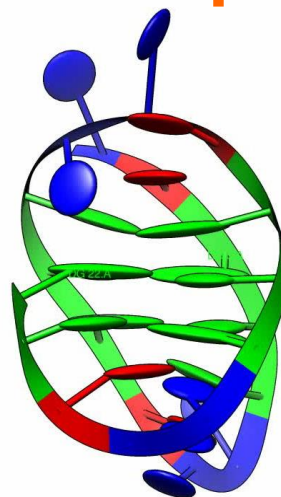
*d(GGG-A-GCG-A-GGG-A-GCG); pdb id: 2MJJ*

*d(GCG-A-GGG-A-GCG-A-GGG); pdb id: 5M1L*

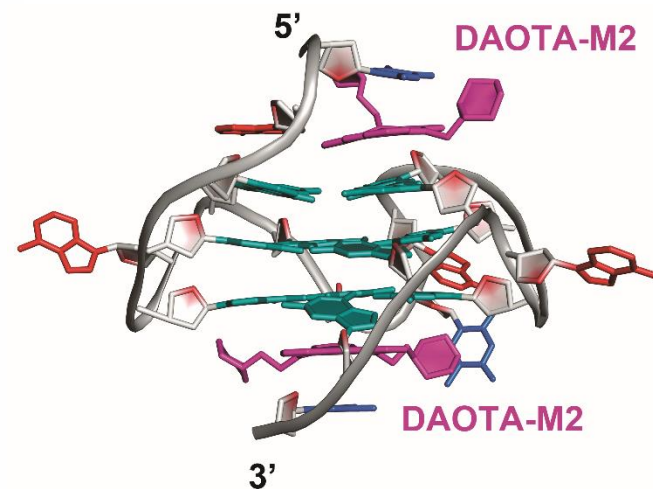
# Summary and Implications



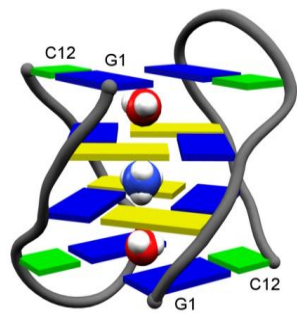
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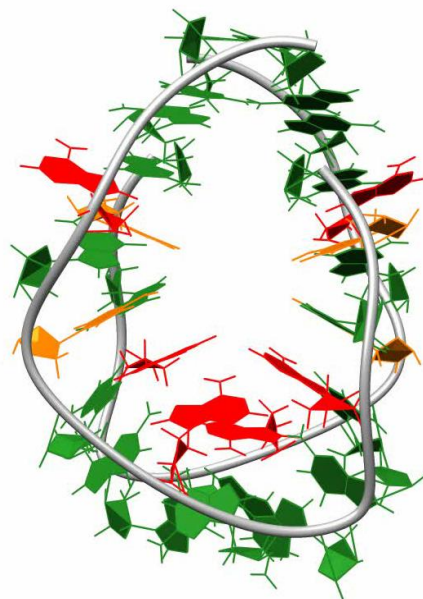
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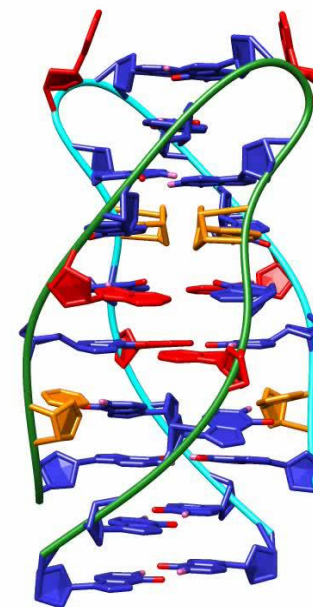
*pdb id: 5LIG*



*d(GGG-A-GCG-A-GGG-A-GCG); pdb id: 2MJJ*



*d(GCG-A-GGG-A-GCG-A-GGG); pdb id: 5M1L*





# Acknowledgments

V. Kocman, A. Kotar, B. Wang, P. Šket, M. Lenarčič Živković, J. Brčić



BI-JP/15-17-002, BI-TR/14-16-009, BI-IN/15-17-005

P1-0242 & J1-6733



CMST COST Action CM1105  
Functional metal complexes  
that bind to biomolecules





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UL FKKT, Ljubljana, SI

**Prof. Boris Rogelj**  
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Functional metal complexes  
that bind to biomolecules



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