

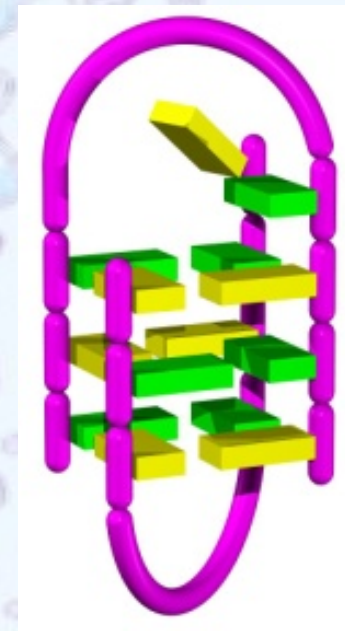
Fascinantne štiriverižne strukture DNK v človeških papilomavirusih

Uvod; DNK in G-kvadrupleksi

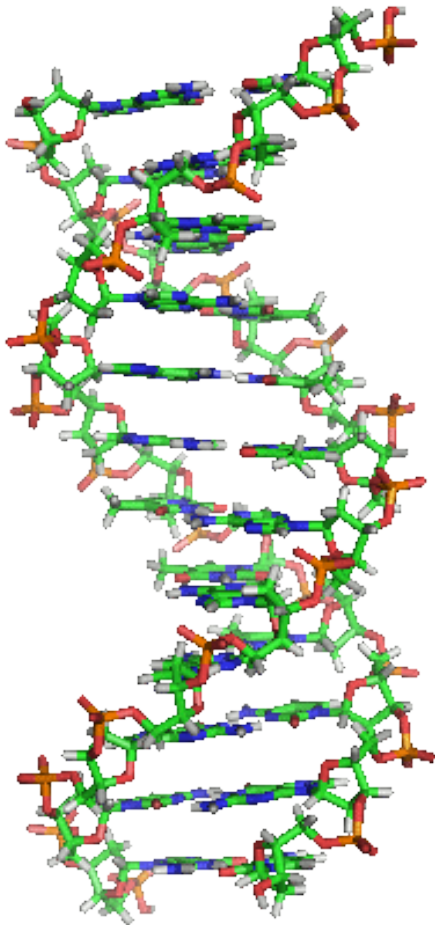
Z gvanini bogata območja v genomu HPV

NMR študije 3D struktur

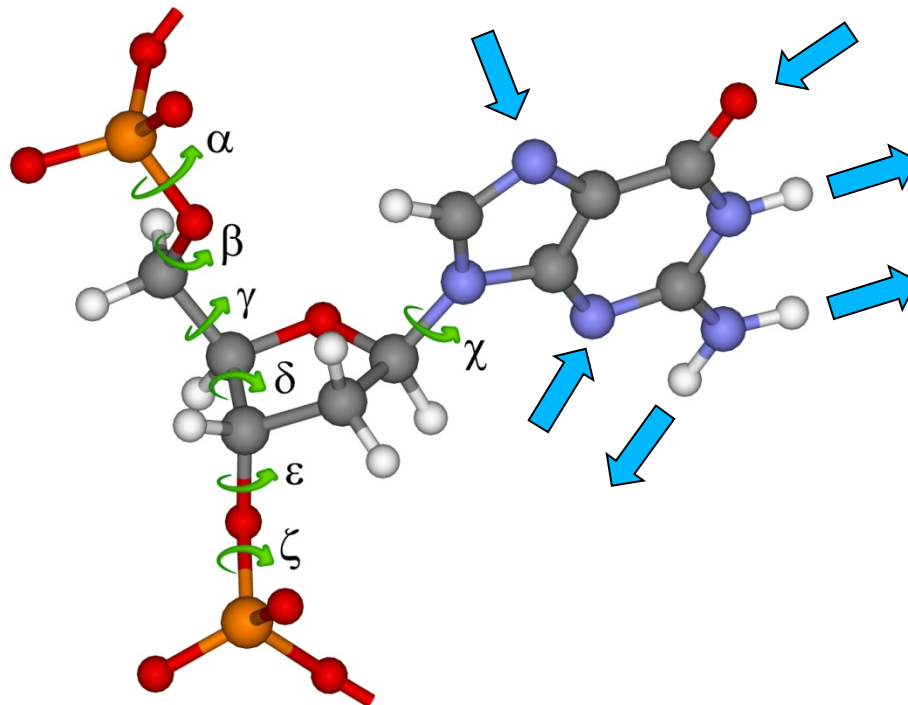
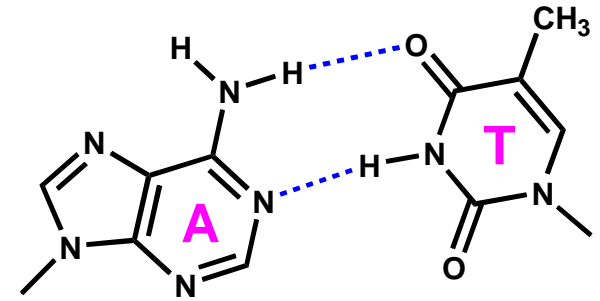
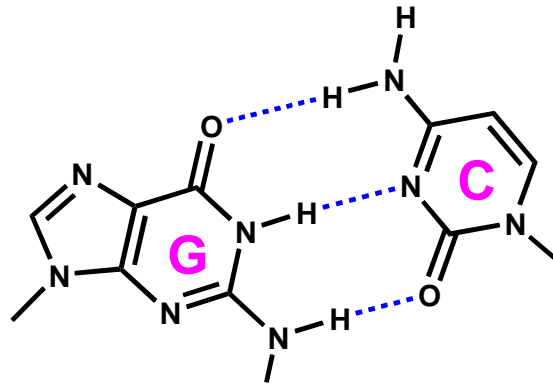
Nenavadne G-kvadrupleksne strukture



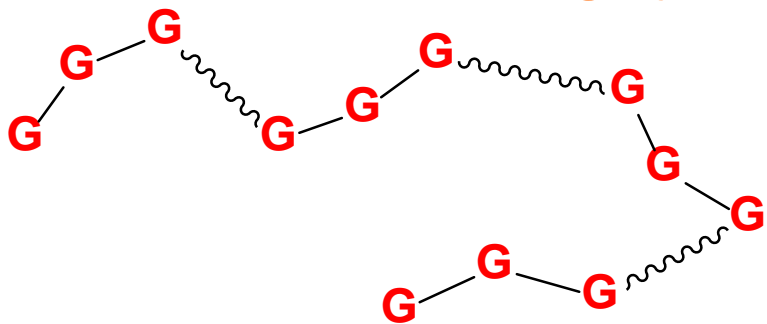
DNK – dvojna vijačnica, Watson-Crick-ovi bazni pari, ...



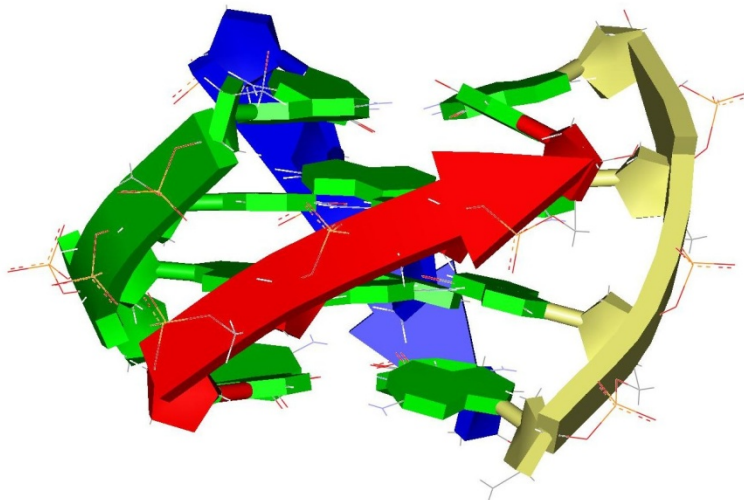
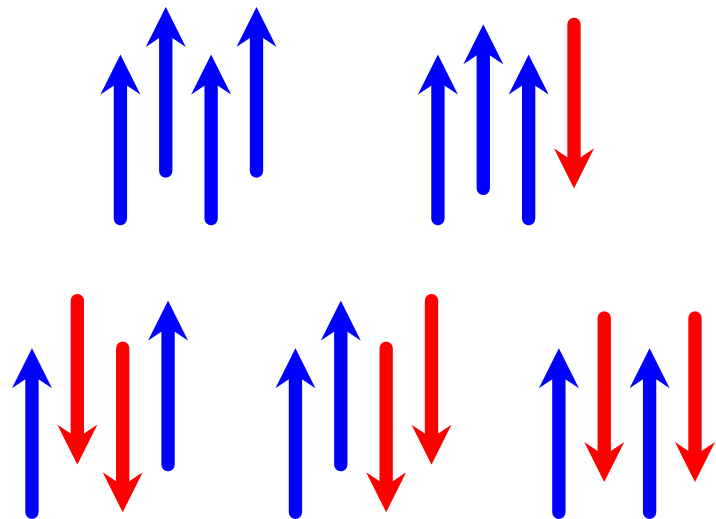
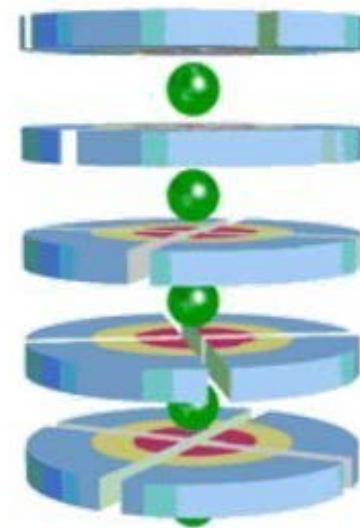
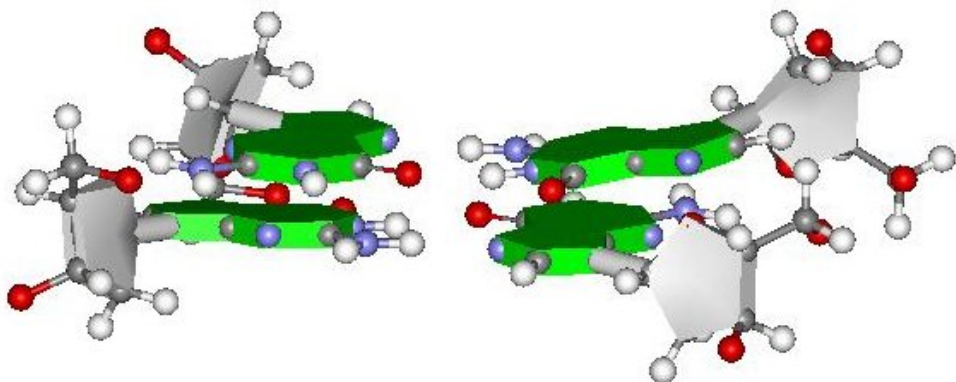
B-DNK



Gvaninski-kvadrupleksi



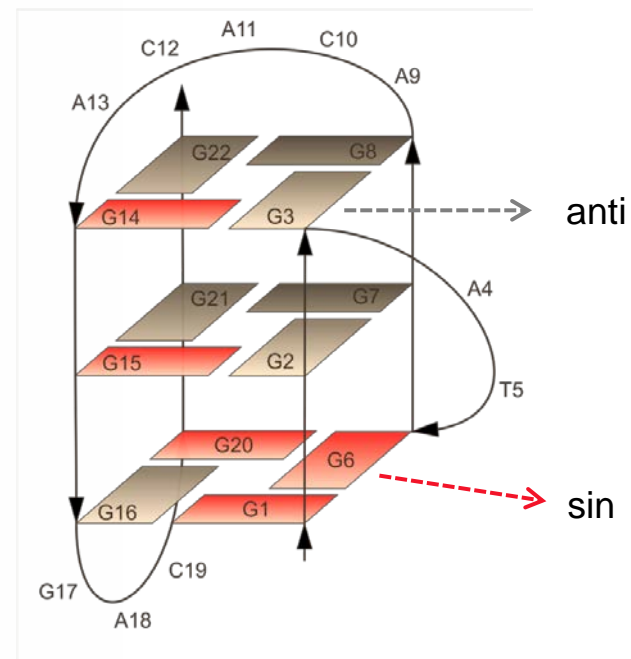
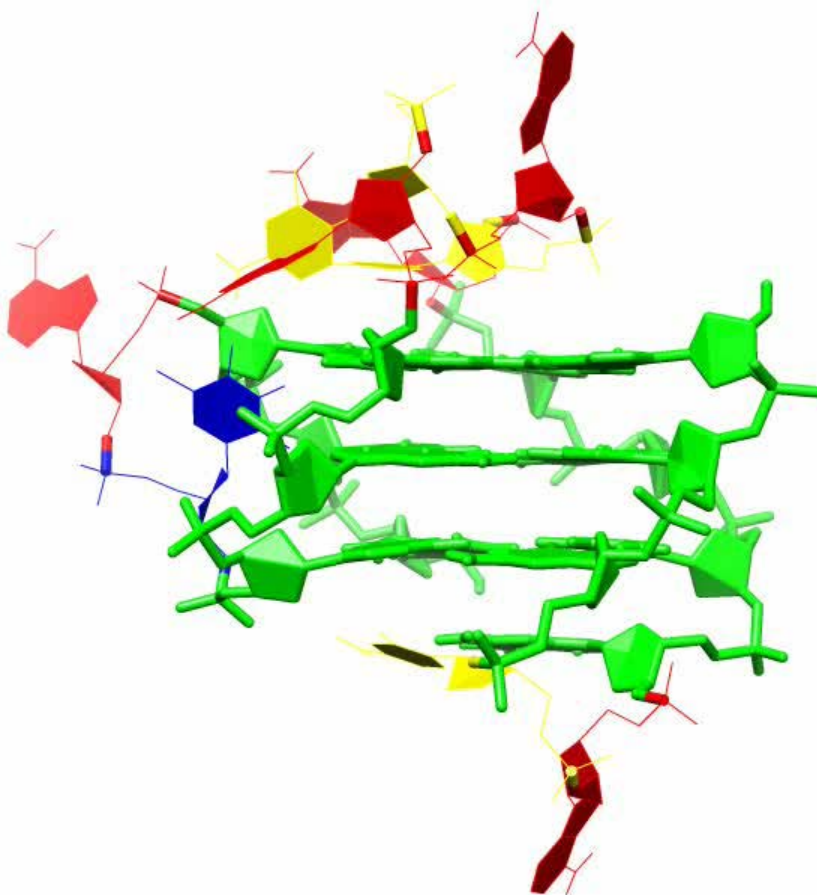
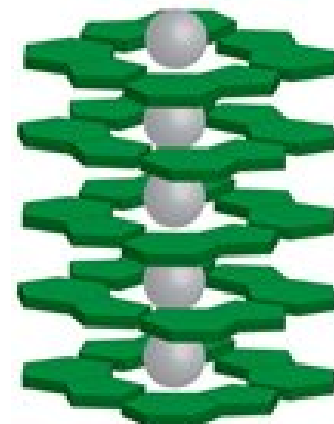
5'-G₃₋₅ N_{L1} G₃₋₅ N_{L2} G₃₋₅ N_{L3} G₃₋₅-3'



Orientacije zank v G-kvadrupleksih

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

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



pdb id: 2LOD

G-kvadrupleksi: funkcije, vloge, uporaba, ...

- Telomerni konci
- Promotorske regije
- 5'-UTR RNA
- rekombinacija
- aptameri

- rak

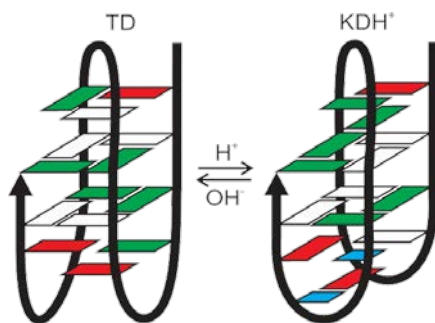
- HIV, EBV, HPV

- Strjevanje krvi

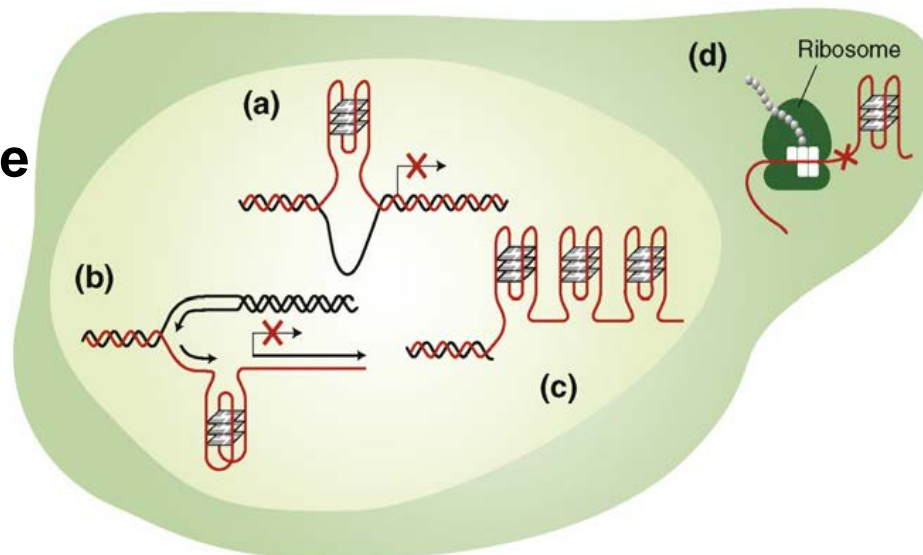
- VEGF **5'-d[TGT GLG GGT GGA CGG GCC GGL TAL A]-3'**

- ALS/FTD (C9ORF72)

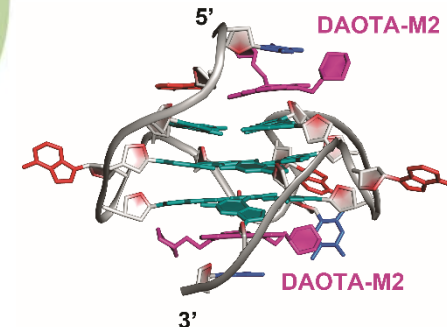
Chem. Commun. **2015**, 51, 9499.



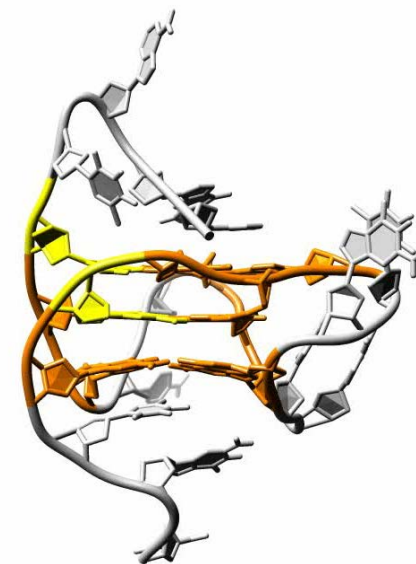
P. Galer *et al.* *Angew. Chem. Int. Ed.* **2016**, 55, 1993.



H. J. Lipps, D. Rhodes, *Trends in Cell Biology* **2009**, 19, 414.



A. Kotar *et al.* *Angew. Chem. Int. Ed. Engl.*, **2016**, 55, 12508.



M. Marušič *et al.* *Nucleic Acids Res.* **2013**, 41, 9524.

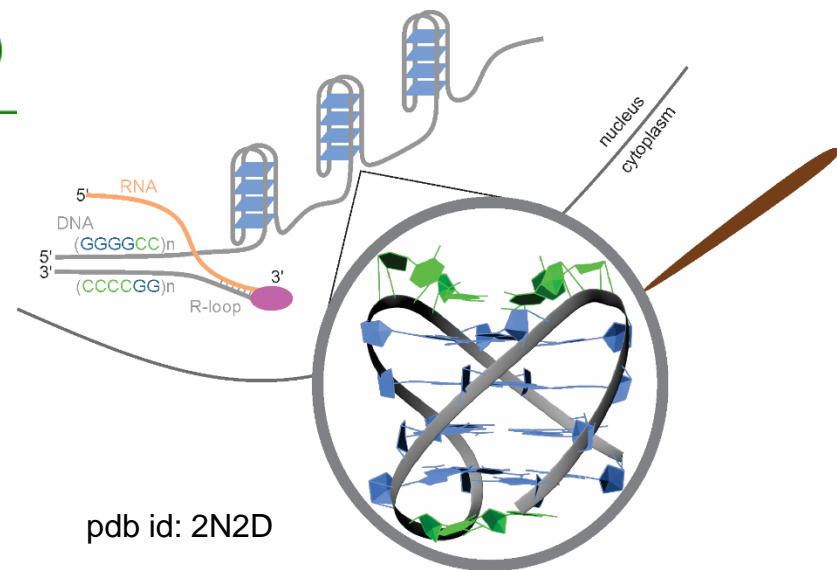
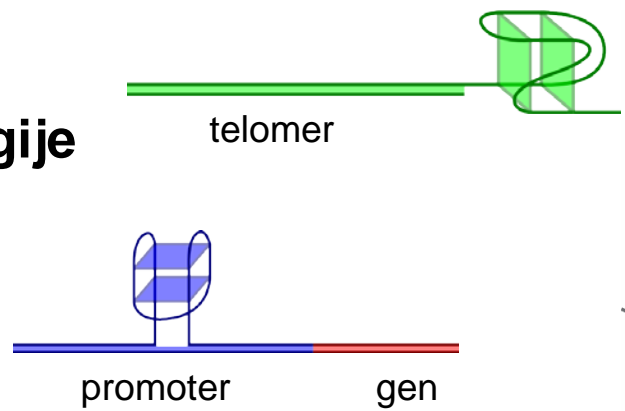
- nano-strukture

- bio-sensorji

- nanonapravice

G-kvadrupleksi: funkcije, vloge, uporaba, ...

- Telomerni konci
- Promotorske regije
- 5'-UTR RNA
- rekombinacija
- aptameri



pdb id: 2N2D

J. Brčić and J. Plavec, *Nucleic Acids Res.* **2015** *43*, 8590.

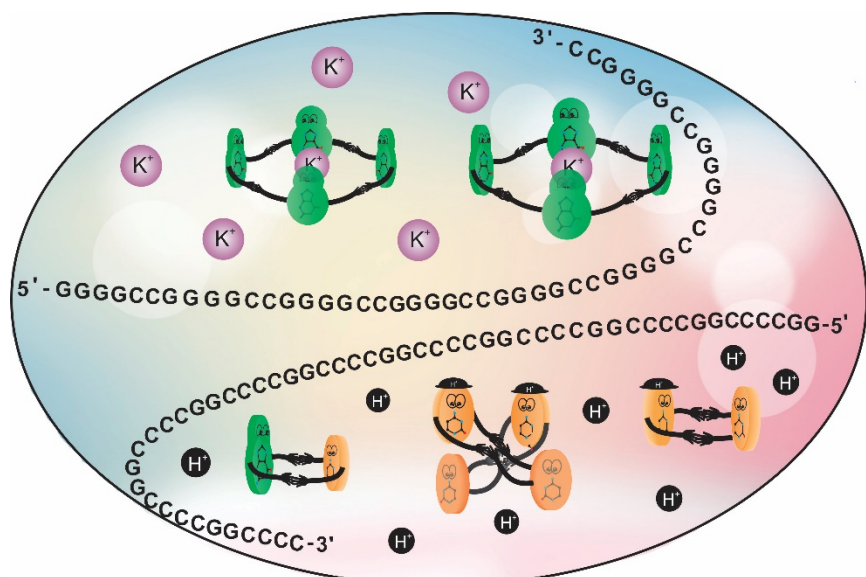
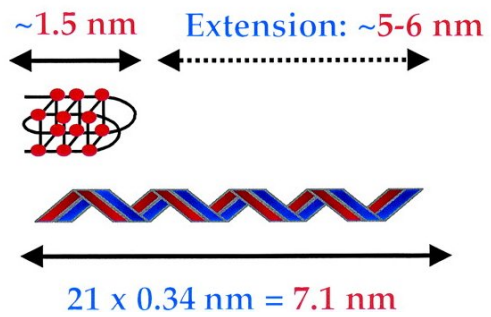
- rak
- HIV, EBV, HPV
- Strjevanje krvi
- VEGF

▪ ALS/FTD (C9ORF72)



▪ nano-strukture

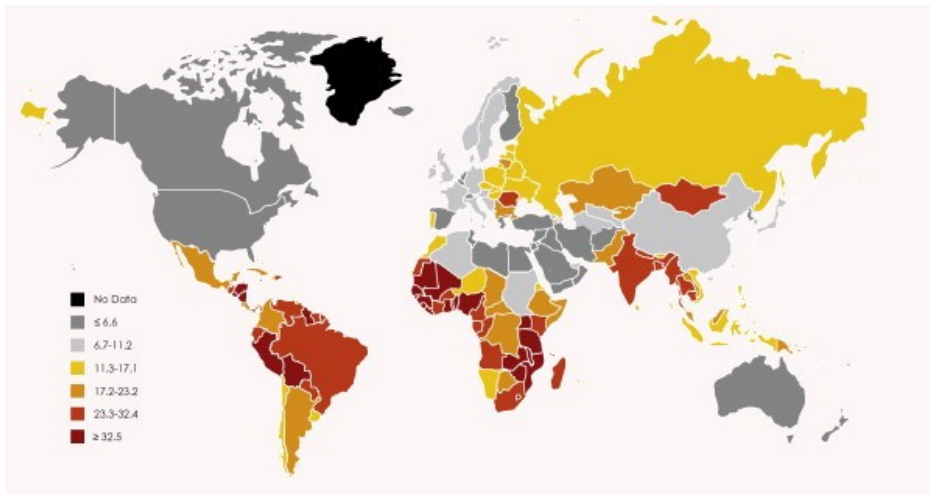
- bio-sensorji
- nanonapravice



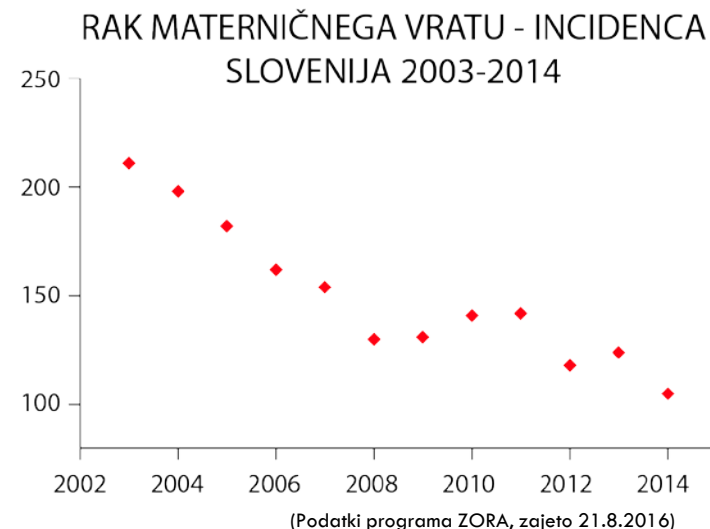
Sci. Rep. **2015**, *5*, 17944.

Človeški papilomavirusi

- Virusi z dvojno vijačno DNA brez ovojnice
- Okužijo kožo ali sluznice, prenos z dotikom
- Visoka stopnja okuženosti (starost 20-25 let $\frac{1}{4}$ žensk v SLO)
- HPV z visokim in nizkim tveganjem
 - HPV tip **16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66**
- Rak glave in vratu, kože ter anogenitalnih predelov



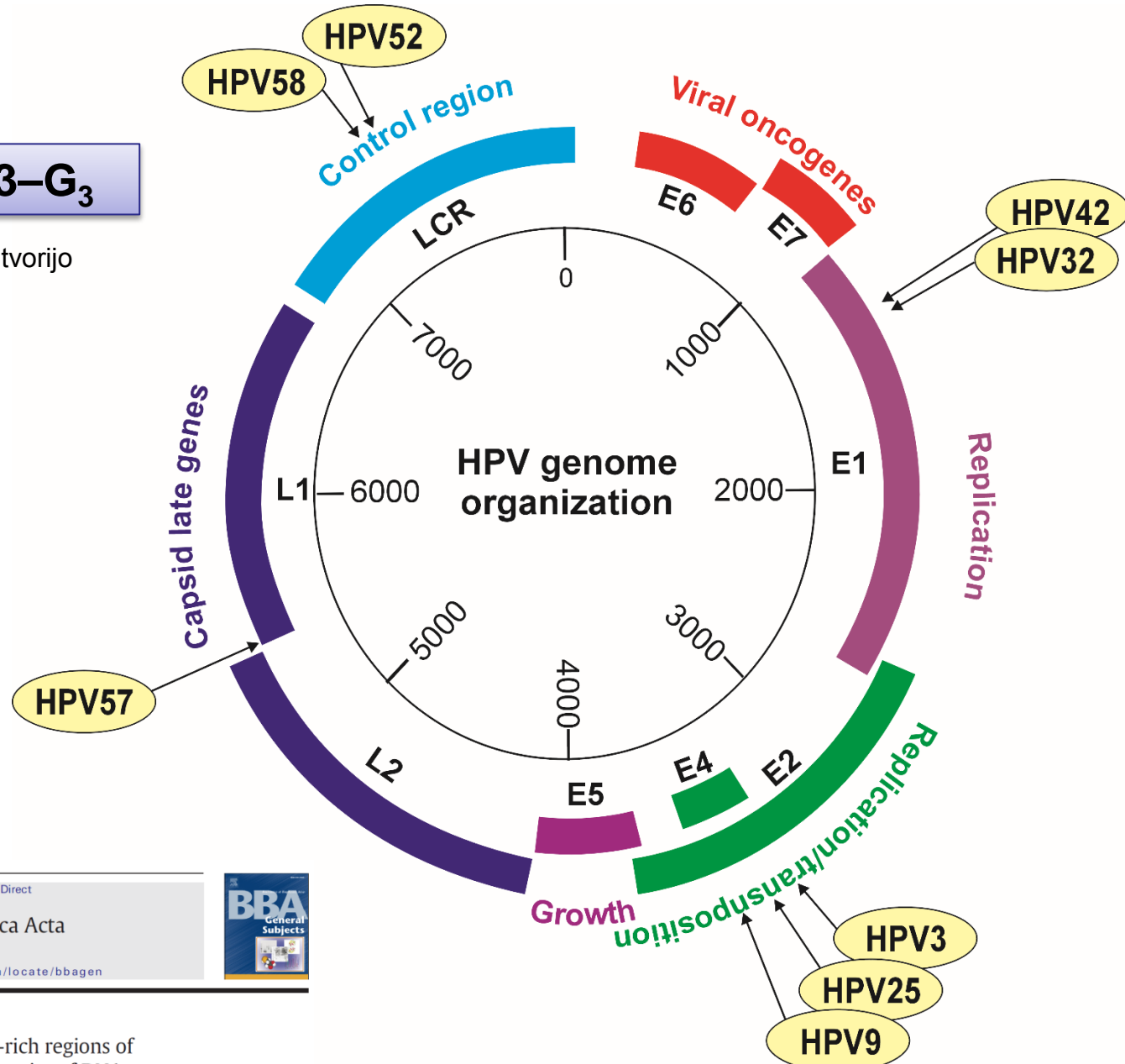
(WHO, podatki 2008)



HPV in položaji z gvanini bogatih območij v genomu

G₃-L1-G₃-L2-G₃-L3-G₃

L1, L2 in L3: območja, ki potencialno tvorijo zanke; vsebujejo do 8 nukleotidov



Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

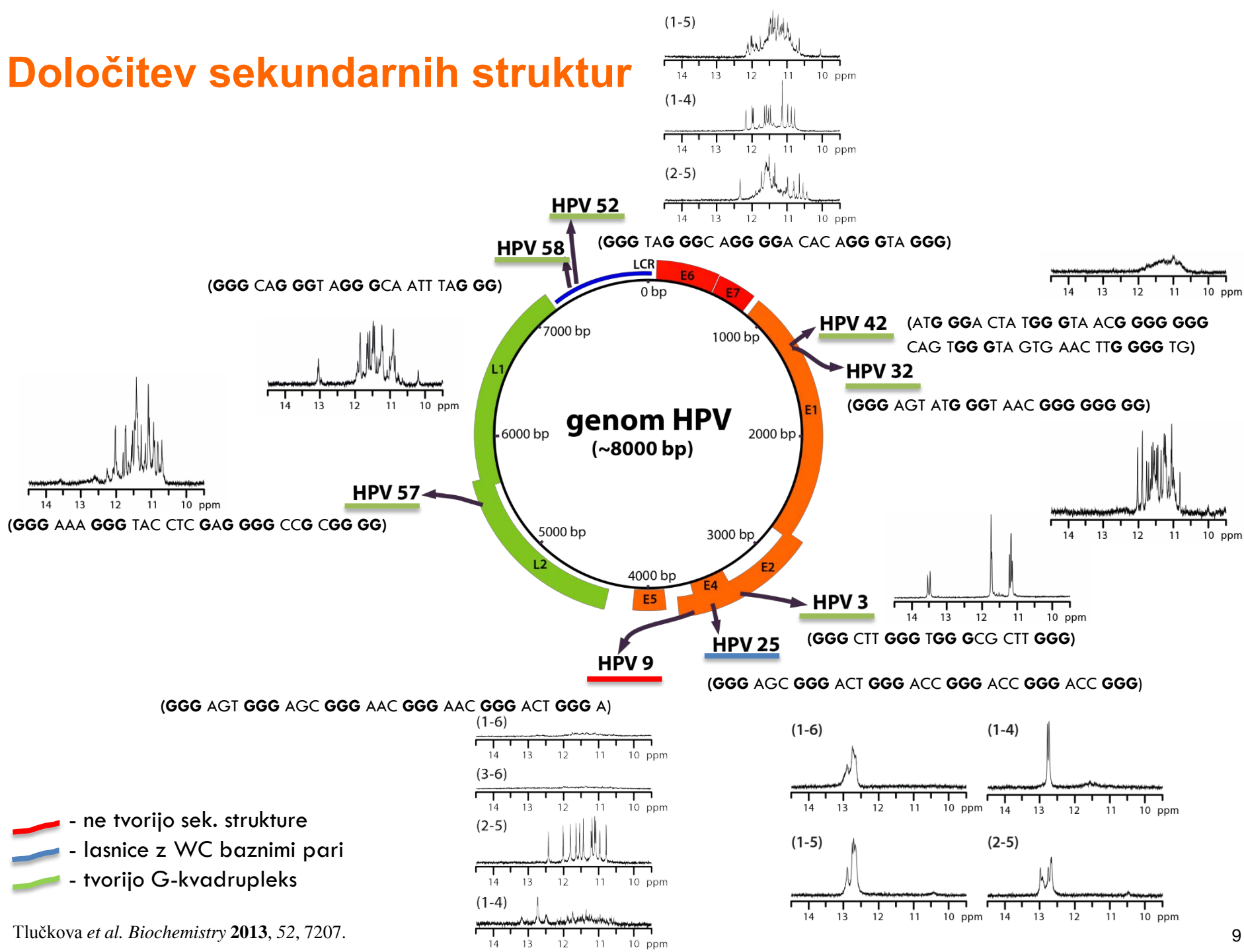
ELSEVIER

BBA General Subjects

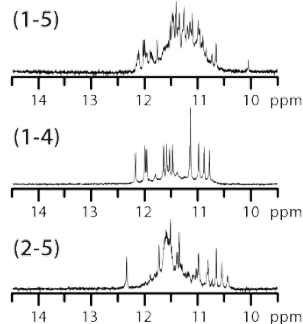
The effect of single nucleotide polymorphisms in G-rich regions of high-risk human papillomaviruses on structural diversity of DNA

Maja Marušič^a, Lea Hošnjak^b, Petra Krafcikova^c, Mario Poljak^b, Viktor Viglasky^c, Janez Plavec^{a,d,e,*}

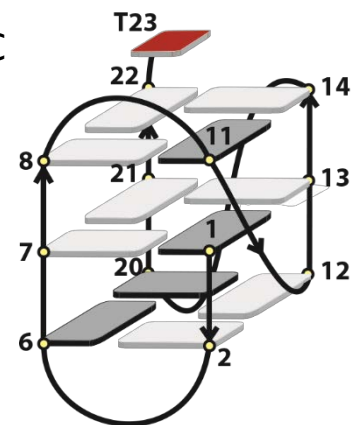
Določitev sekundarnih struktur



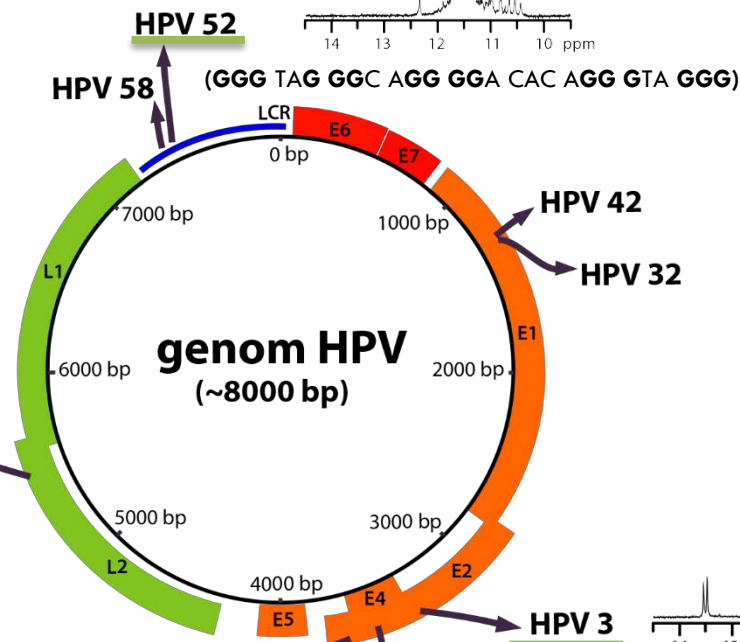
Določitev sekundarnih struktur



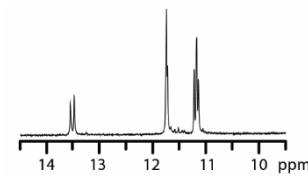
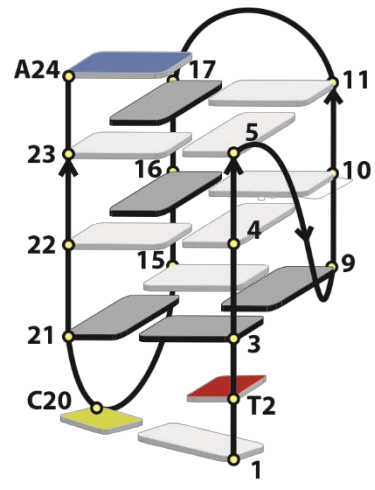
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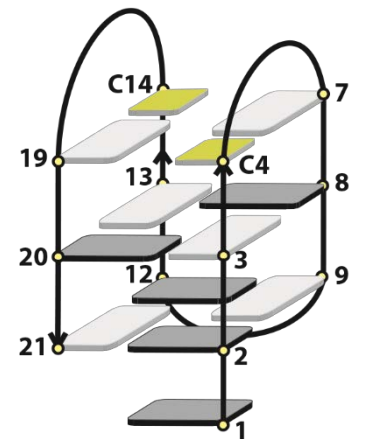
M. Marušič in J. Plavec,
Angew. Chem. Int. Ed. Engl.,
2015, 54, 11716.



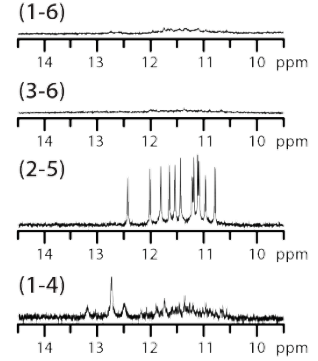
$T_m = 54^\circ\text{C}$



$T_m = 63^\circ\text{C}$



HPV 9
(GGG AGT GGG AGC GGG AAC GGG AAC GGG ACT GGG A)

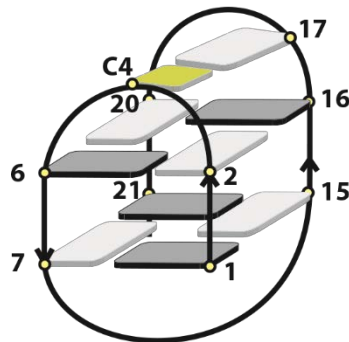
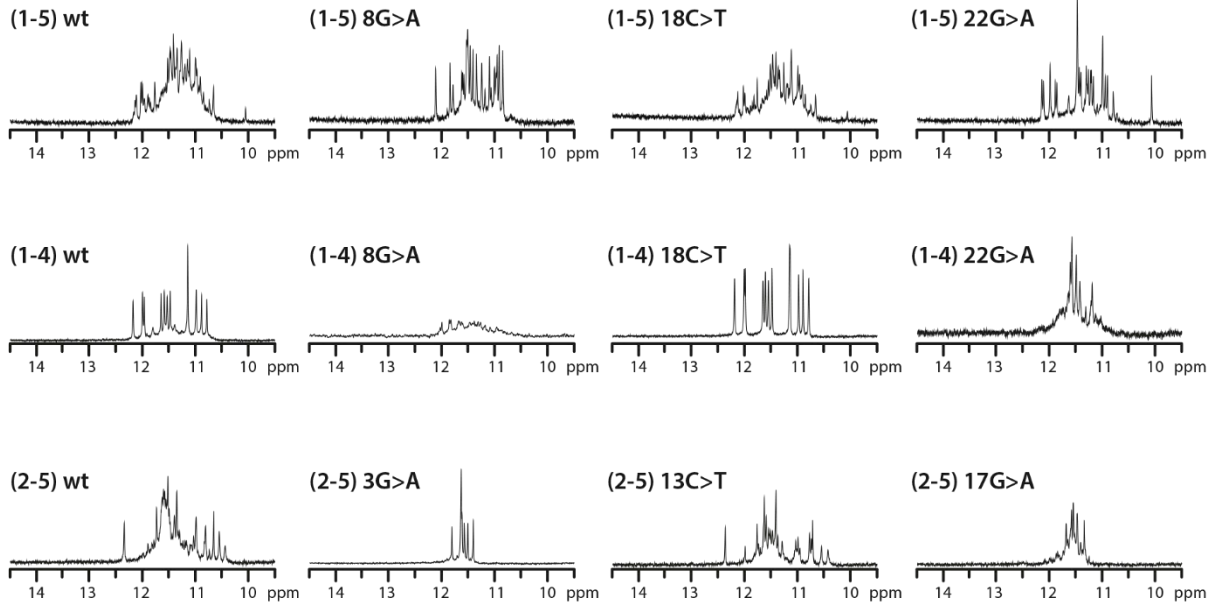


Polimorfizmi v HPV52 vplivajo na zvitje

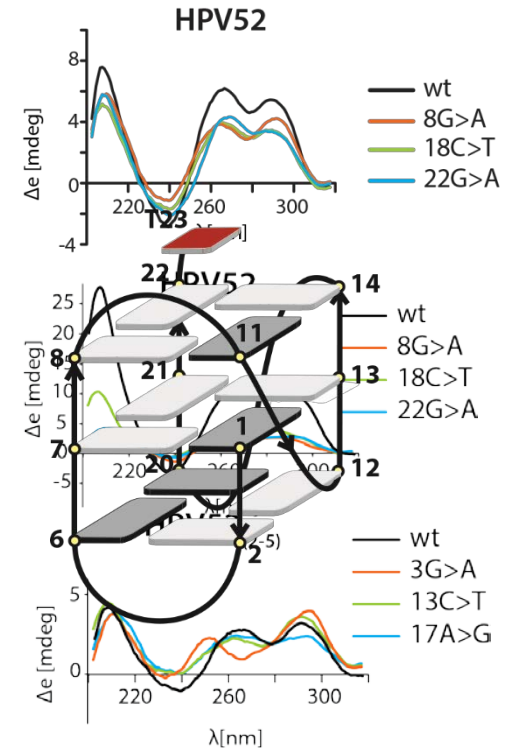
HPV52₍₁₋₅₎ (GGG TAG **G**GC AGG **G**GA CAC **A**GG **G**TA GGG)

HPV52₍₁₋₄₎ (GGG TAG **G**GC AGG **G**GA CAC **A**GG **G**T)

HPV52₍₂₋₅₎ (G **G**GC AGG **G**GA CAC **A**GG **G**TA GGG)



$T_m = 47^\circ\text{C}$



4 gvaninski ostanki znotraj zaporedja s po tremi gvanini

Difuzijski eksperiment (PFG-STE): $D_t = 1.33 \cdot 10^{-6} \pm 0.04 \text{ cm}^2 \text{ s}^{-1}$

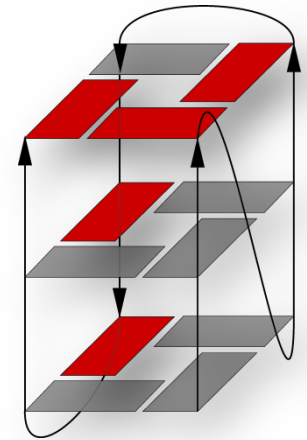
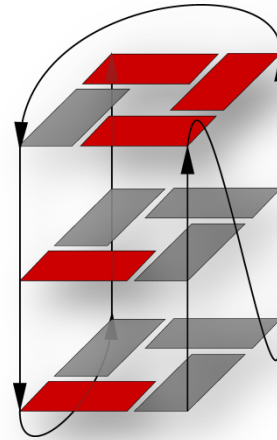
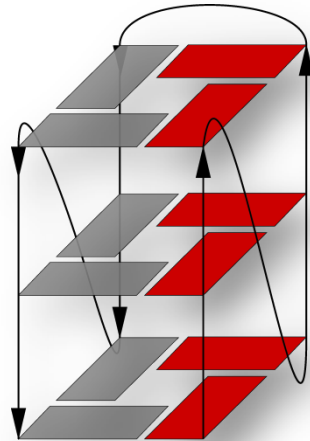
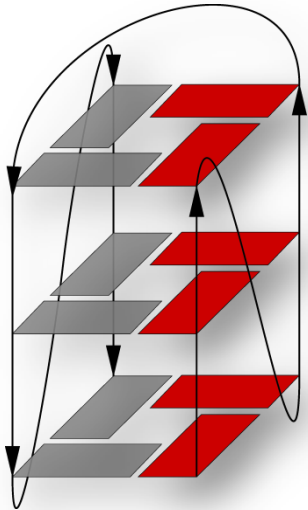
ODN: 5'-GGG AT GGG ACACA GGGG AC GGG -3'

5'-GGG **AT** GGG **ACACAG** GGG **AC** GGG -3'

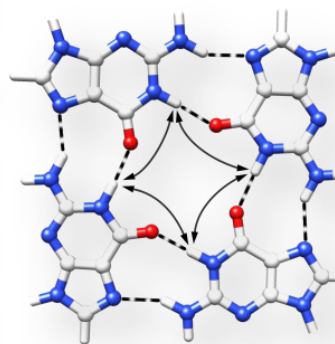
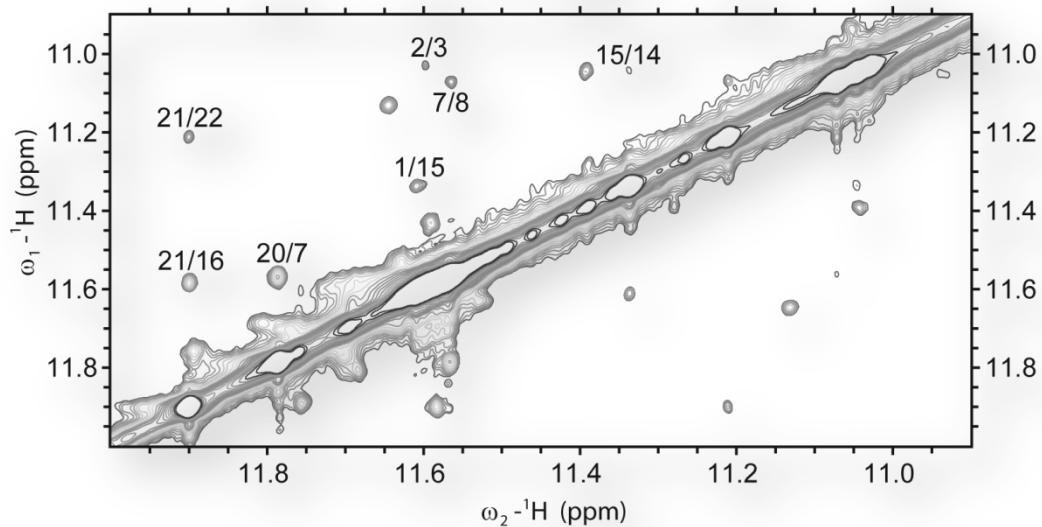
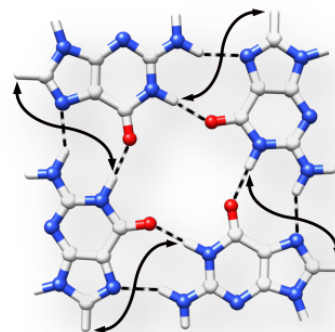
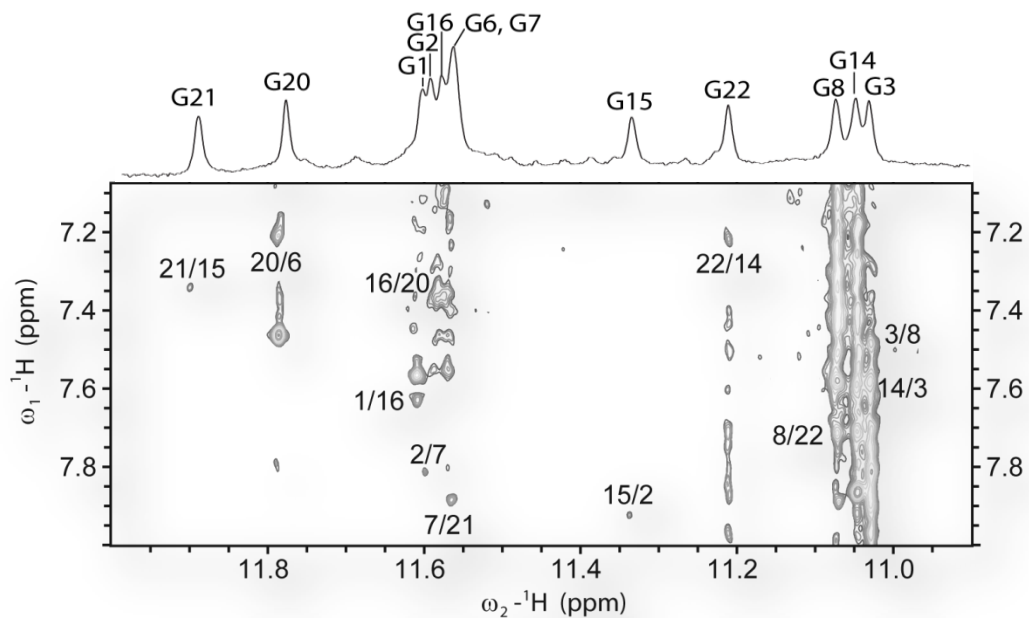
p d p
p r p

5'-GGG **AT** GGG **ACACA** GGG **GAC** GGG -3'

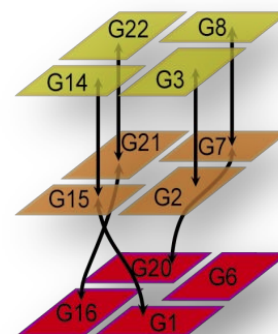
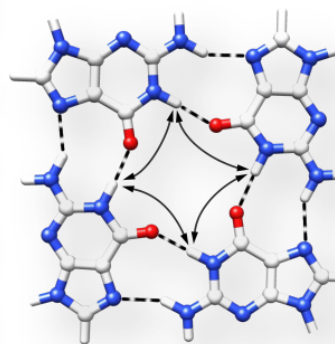
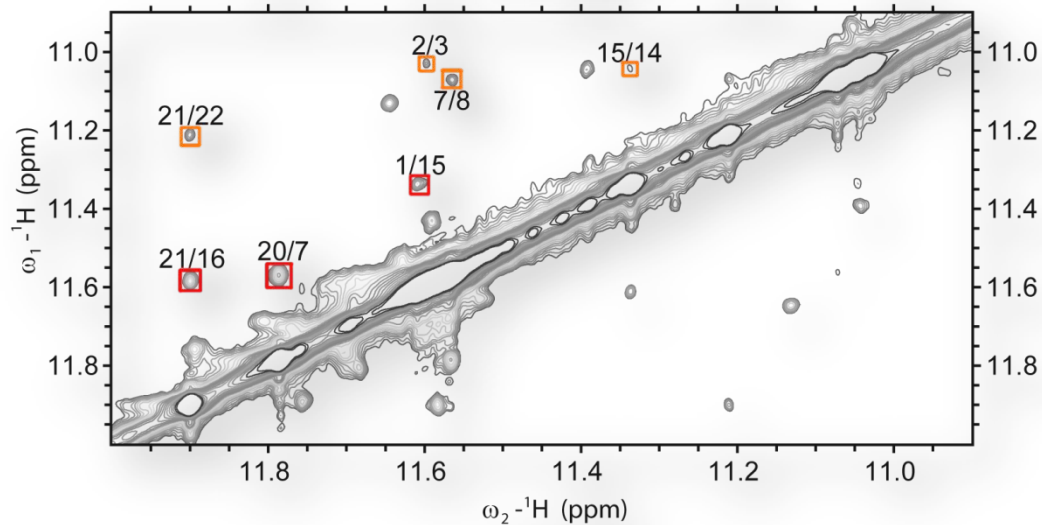
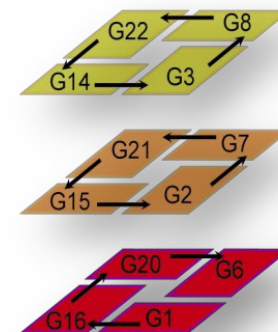
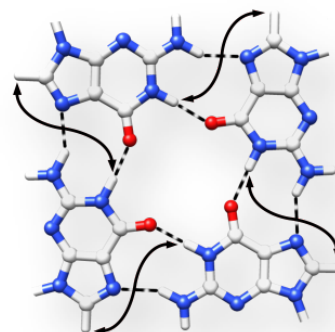
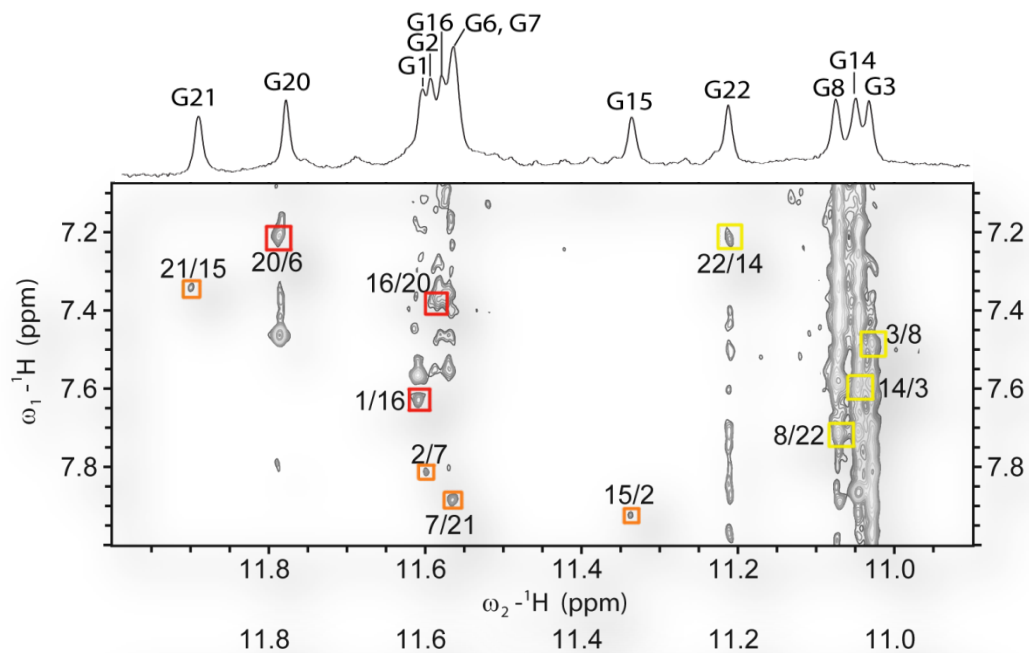
p d r
p r r



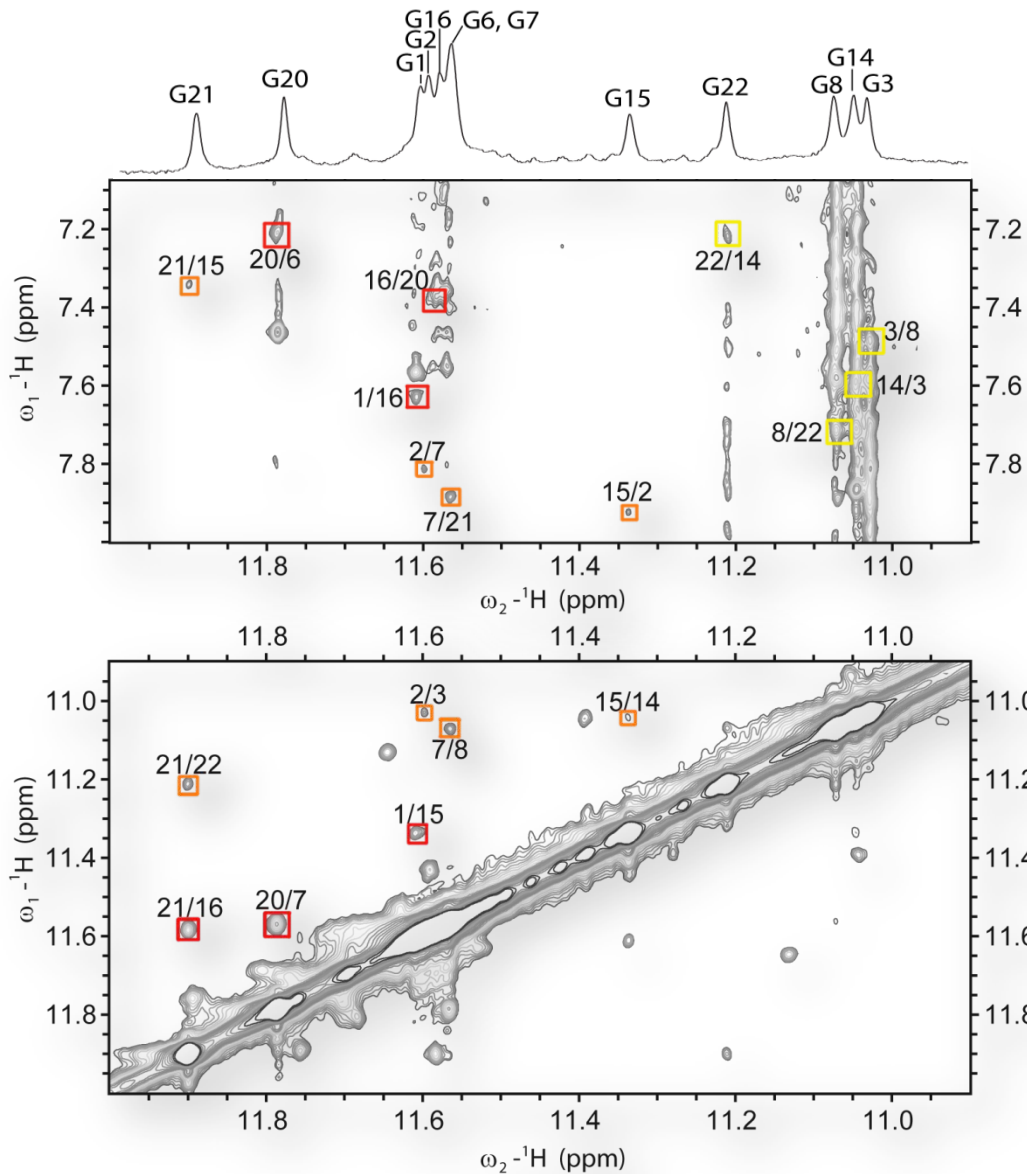
Določitev topologije



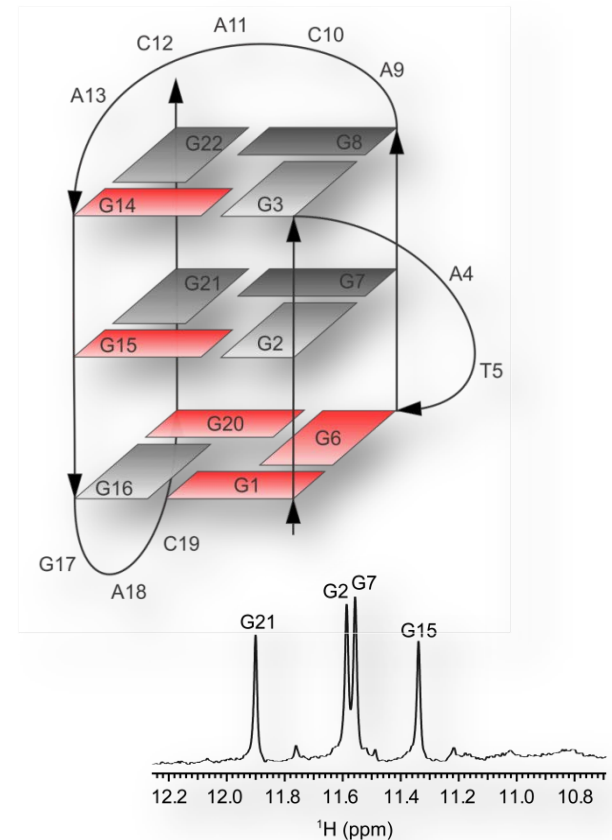
Določitev topologije



Določitev topologije



Topologija : (3+1)
s propelersko (AT), diagonalno (ACACA)
in robno (GAC) zanko

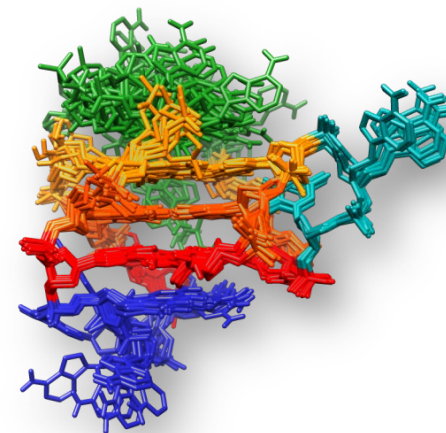
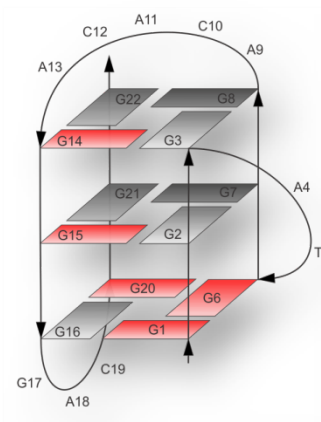


Izračun strukture visoke ločljivosti

NMR distance and torsion angle restraints

NOE-derived distance restraints

Total	316
Intra-residue	216
Inter-residue	100
Sequential	70
Long range	30
Hydrogen bond restraints	24
Torsion angle restraints	65
G-quartet planarity restraints	36



Structure statistics

Violations

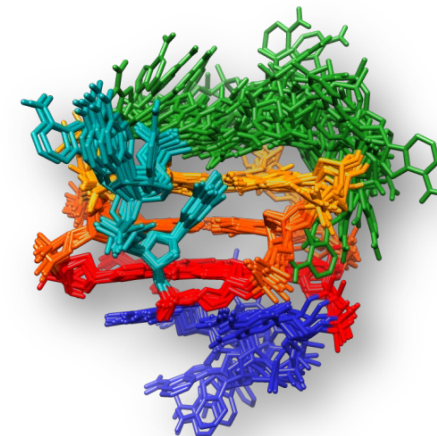
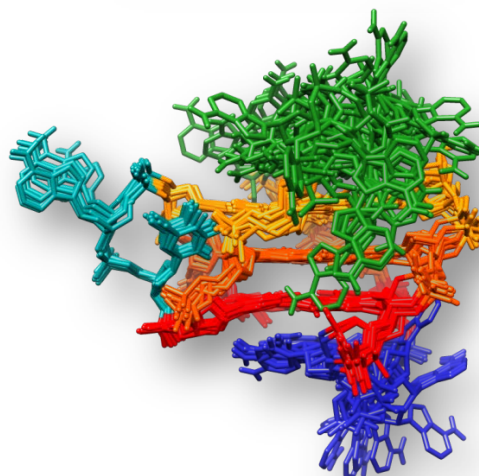
Mean NOE restraint violation (Å)	0.079 ± 0.0002
Max. NOE restraint violation (Å)	0.196
Max. torsion angle restraint violation (°)	4.765

Deviations from idealized geometry

Bond length (Å)	0.012 ± 0.0002
Bond angle (°)	2.525 ± 0.0000

Pairwise heavy atom RMSD (Å)

Overall	2.63
G-quartets	0.82
G-quartets and propeller (AT) loop	0.96
G-quartets and diagonal (ACACA) loop	2.60
G-quartets and edgewise (GAC) loop	1.51
G-quartets and A4, T5, G17, C19	0.99



pdb id: 2LOD, BMRB 18209

Strukturne lastnosti

NMR distance and torsion angle restraints

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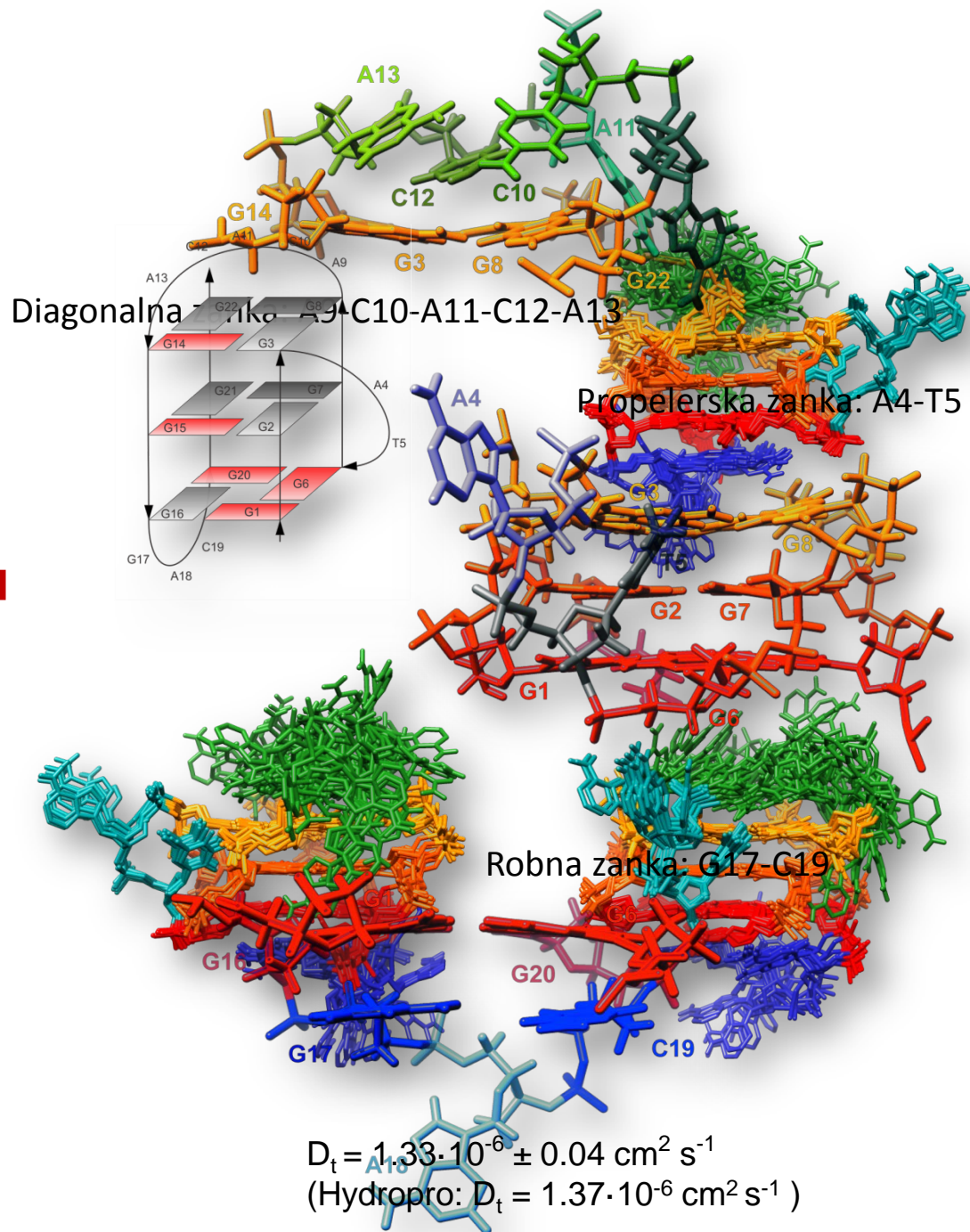
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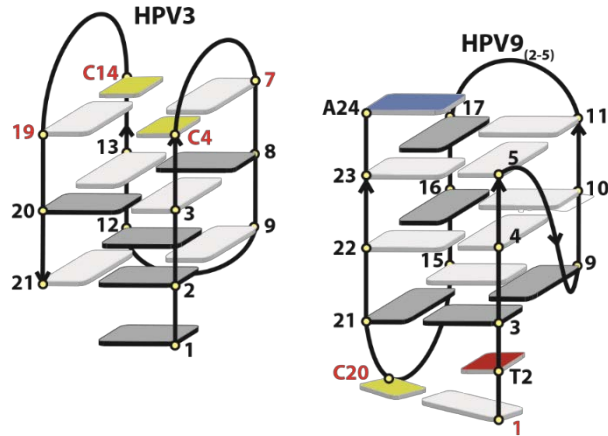
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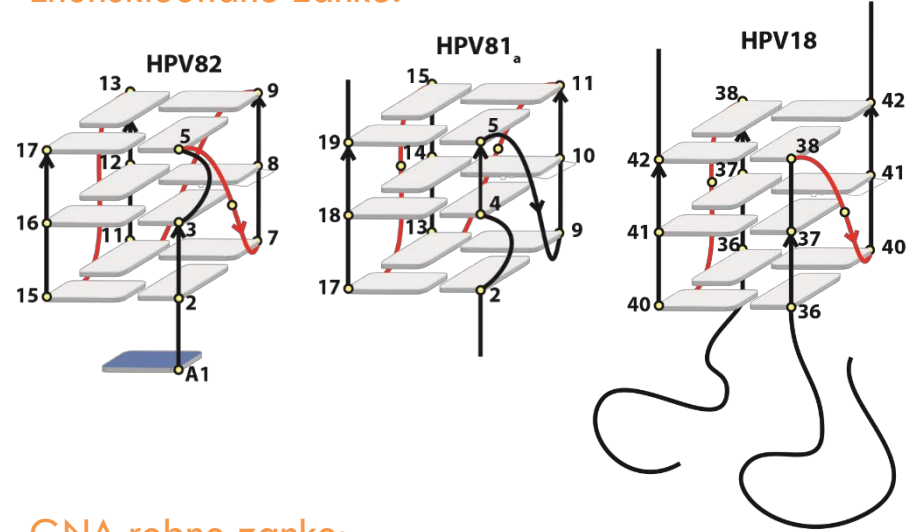


Zaključki in implikacije

GC bazni pari:



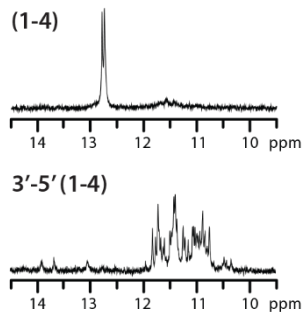
Enonukleotidne zanke:



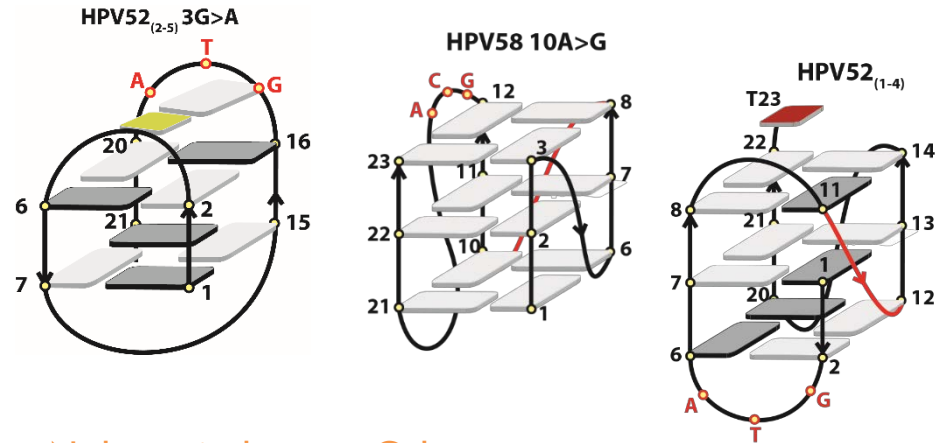
% citozinskih ostankov v zaporedju:

HPV9 (GGG AGT GGG AGC GGG AAC GGG AAC GGG AAT GGG AA)
 HPV25 (GGG AGC GGG AAT GGG ACC GGG ACC GGG ACC GGG)

Mesto adeninskega ostanka v zanki:



GNA robne zanke:



Nalaganje baz na G-kvartete

G-Quadruplexes

International Edition: DOI: 10.1002/anie.201505348
 German Edition: DOI: 10.1002/ange.201505348

The Effect of DNA Sequence Directionality on G-Quadruplex Folding

Maja Marušič and Janez Plavec*

Zahvala

M. Marušič, P. Šket, V. Kocman, A. Kotar, M. Trajkovski, P. Podbevšek

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Lea Hošnjak
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IJS

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UL FMF

Prof. Lea Spindler
IJS in UMb

Prof. Janja Marc
UL FFa

Prof. Katarina Trebušak Podkrajšek
UL UKC



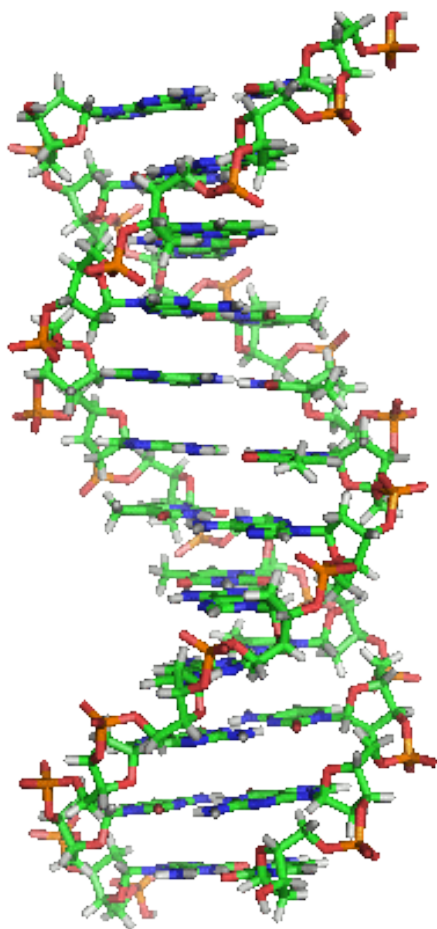
P1-0242 & J1-6733



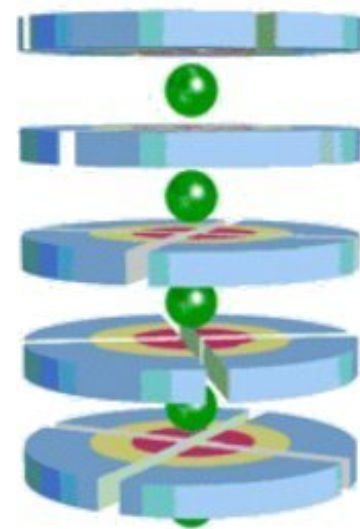
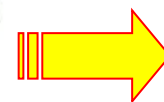
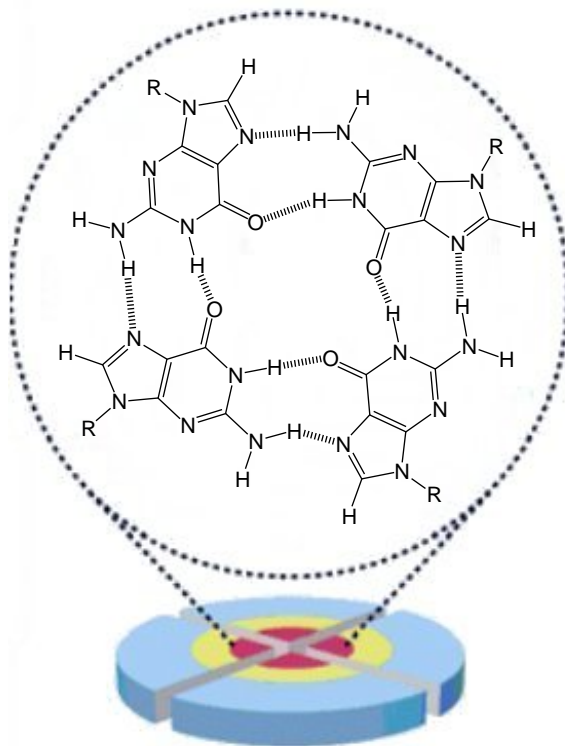
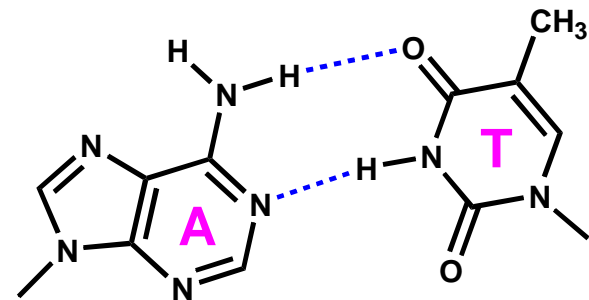
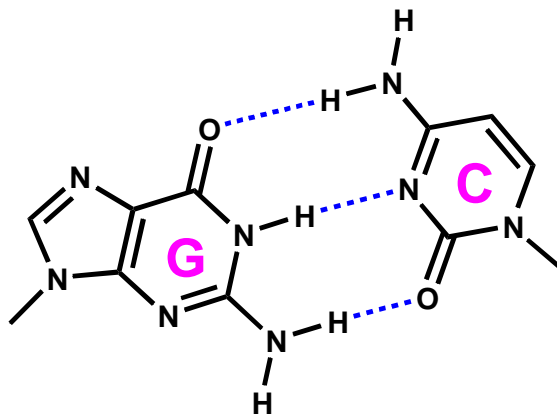
CMST COST Action CM1105
Functional metal complexes
that bind to biomolecules



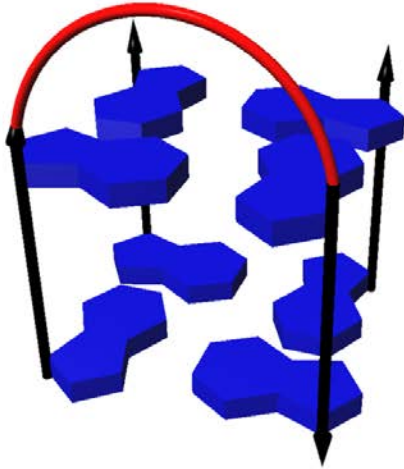
DNK – dvojna vijačnica, G-kvarteti, ...



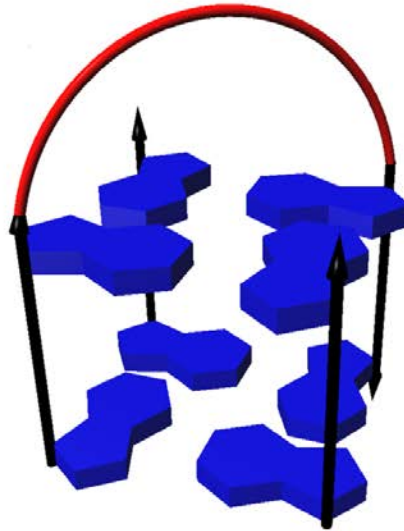
B-DNK



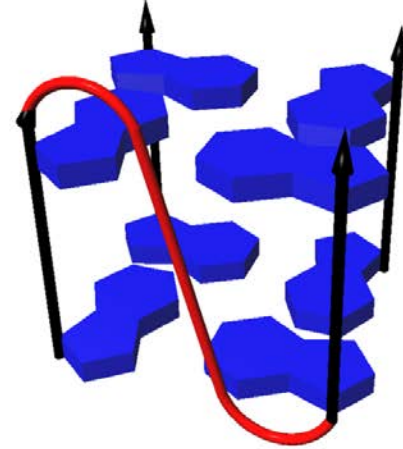
Povezovalne zanke



Robni tip zanke

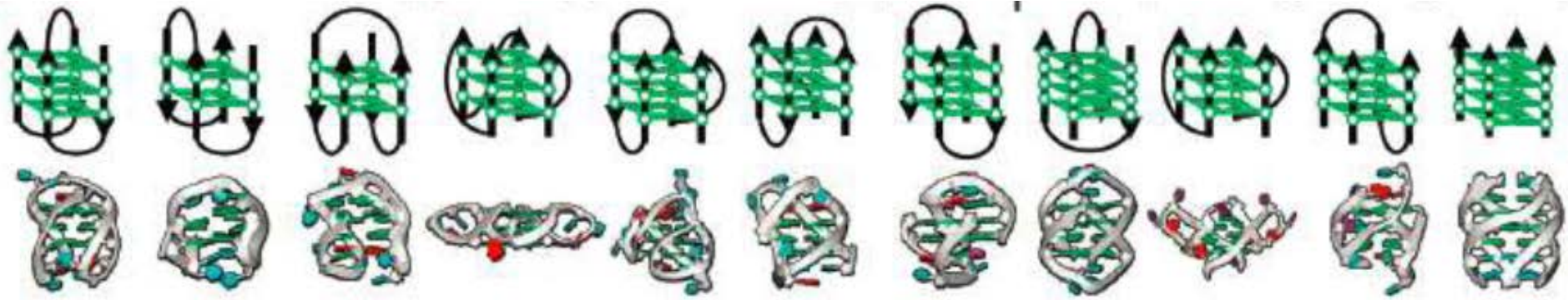


Diagonalna zanka



Zanka z dvojnimi obratom verige

Polimorfizem G-kvadruplexov



Monomerna zvitja

Dimerna zvitja

Tetramolekularne
paralelne
topologije

Antiparalelna topologija

Paralelna
topologija

Mešana topologija

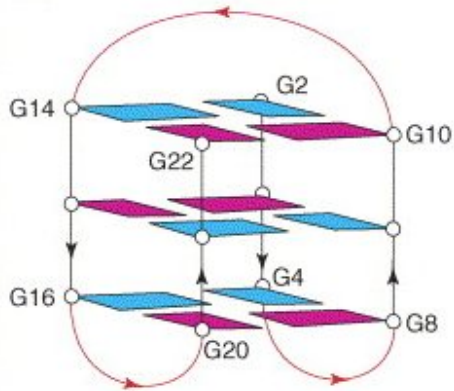
Antiparalelna topologija

Paralelna
topologija

Mešana
topologija

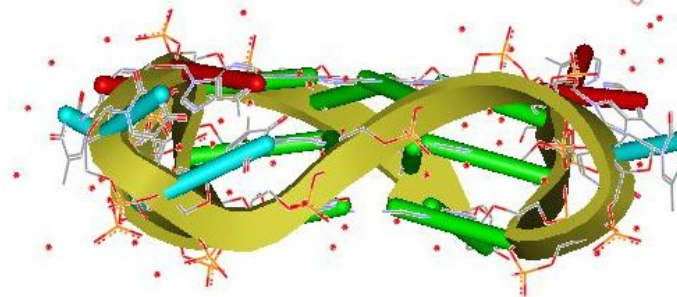
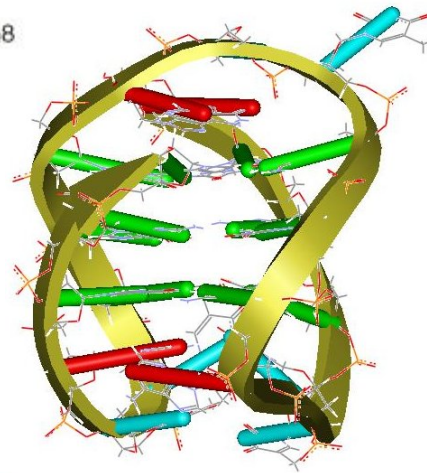
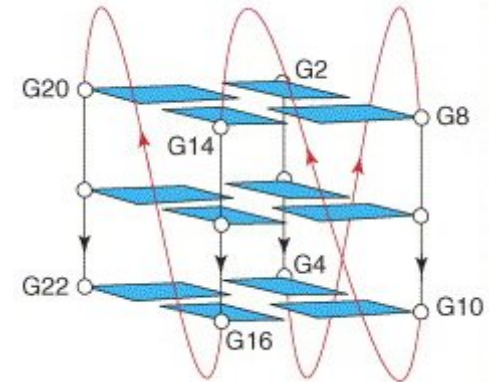
Pomen kationov: $d[AGGG(TTAGGG)_3]$

Na^+ ioni, NMR struktura v raztopini

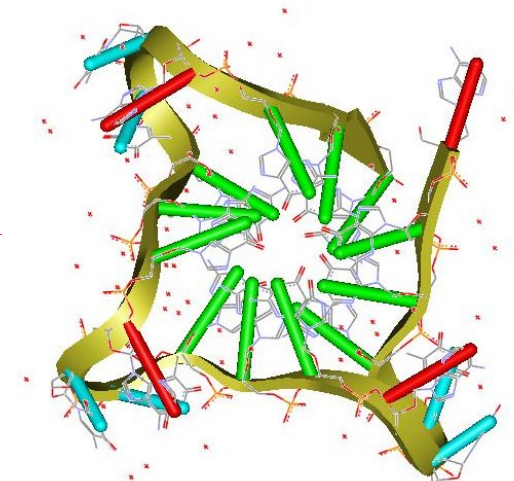


anti
sin

K^+ ioni, Kristalna struktura

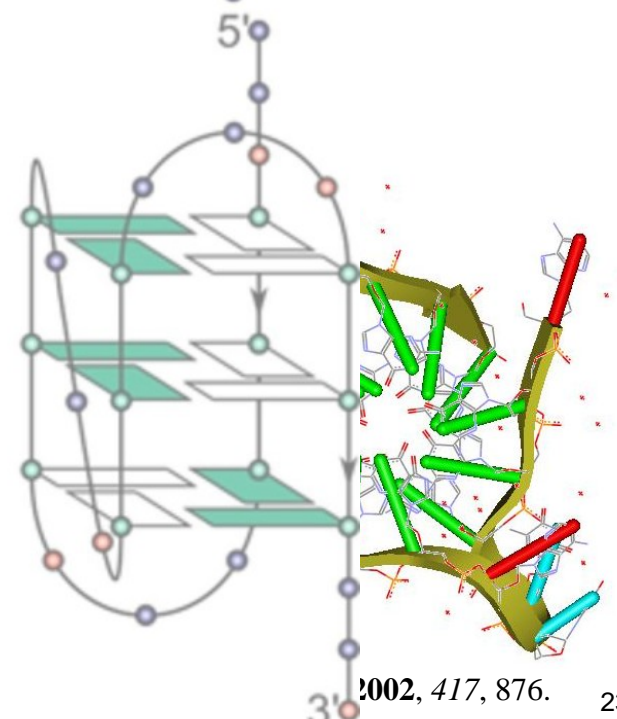
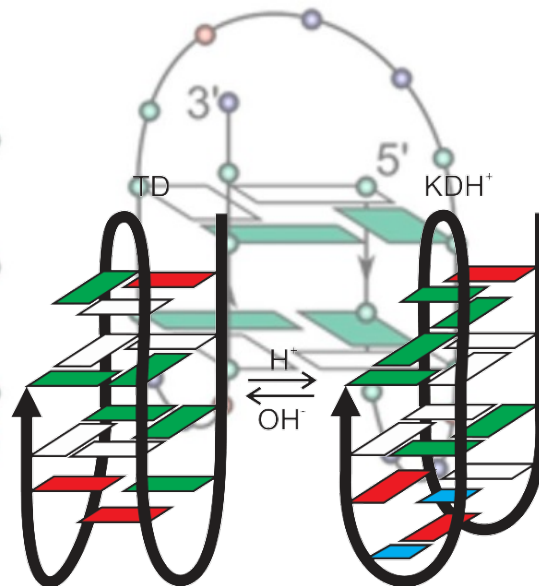
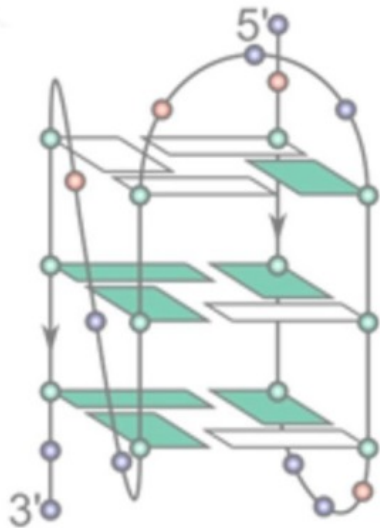
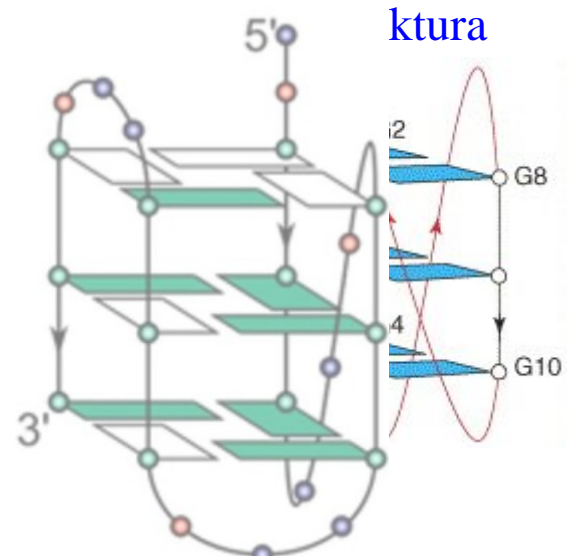
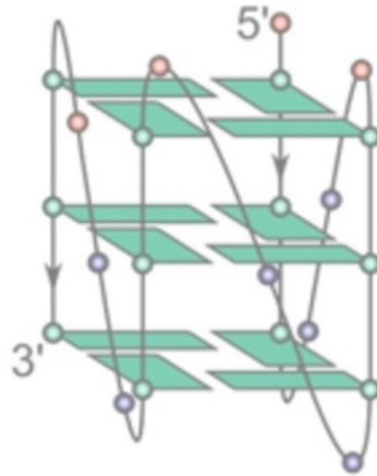
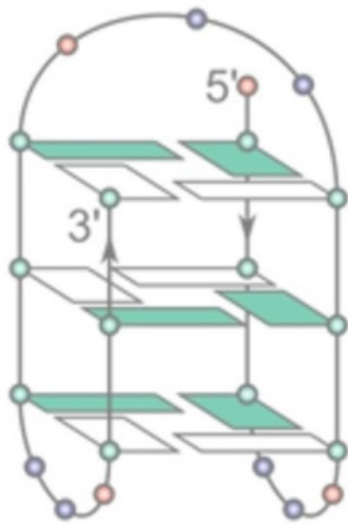
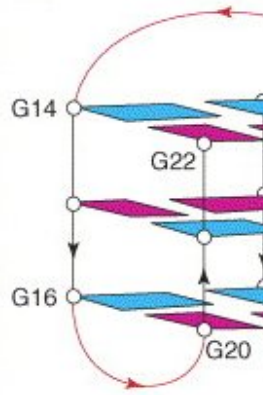


OPOMBA: različne strukture v prisotnosti K^+ ionov v raztopini



Pomen kationov: $d[AGGG(TTAGGG)_3]$

Na^+ ioni, N



2002, 417, 876.

Preučevana zaporedja

HPV	dolžina	veriga	zaporedje (5' -> 3')	sek. struktura?
3	21	-	GGG CTT GGG TGG GCG CTT GGG	✓
9	34	-	GGG AGT GGG AGC GGG AAC GGG AAC GGG ACT GGG A	X ; HPV9(2-5) ✓
16	29	+	AGG GTC GGG TAC AGG CGG ACG CAC TGG GT	✓ / lasnice
18	44	+	AGT GGT ACA GGG GGT CGT ACA GGG TAC ATT CCA TTG GGT GGG CG	✓
25	33	-	GGG AGC GGG ACT GGG ACC GGG ACC GGG ACC GGG	lasnice
32	23	+	GGG AGT ATG GGT AAC GGG GGG GG	✓
42	47	+	ATG GGA CTA TGG GTA ACG GGG GGG CAG TGG GTA GTG AAC TTG GGG TG	✓
52	27	-	GGG TAG GGC AGG GGA CAC AGG GTA GGG	✓
57	29	-	GGG AAA GGG TAC CTC GAG GGG CCG CGG GG	✓
58	23	-	GGG CAG GGT AGG GCA ATT TAG GG	✓
67	40	-	AGG GAT AGG CAA GGG CAT GGC ATG GGC AGG GAT ATA GGG A	✓
81 _a	20	+	CGC GGA CAG GGC GGG AGG GT	✓
81 _b	31	+	CGG AGG AAG TGC (GGG) ₄ ATG GGA	✓
81 _c	27	+	(GAG) ₉	✓
82	17	-	AGG CGT GGG CGG GCG GG	✓



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The effect of single nucleotide polymorphisms in G-rich regions of high-risk human papillomaviruses on structural diversity of DNA

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Table 1
G-rich sequences found in genomes of high-risk HPV types HPV16, 18, 52 and 58.

Name	Position ^a	No. ^b	% ^b	G-rich sequence ^c	nt ^d
HPV16-ref	4422-50	216	76.8	AGG GTC GGG TAC AGG CGG ACG CAC TGG GT	29
HPV16 7G>A	4428	10	3.6	AGG GTC AGG TAC AGG CGG ACG CAC TGG GT	29
HPV16 7G>C	4428	1	0.4	AGG GTC CGG TAC AGG CGG ACG CAC TGG GT	29
HPV16 7G>T	4428	49	17.4	AGG GTC TGG TAC AGG CGG ACG CAC TGG GT	29
HPV16 13A>G	4434	5	1.8	AGG GTC GGG TAC GGGCGGACG CAC TGG GT	29
HPV18-ref	4430-73	75	88.2	AGT GGT ACA GGG GGT CGT ACA GGG TAC ATT CCA TTG GGT GGG CG	44
HPV18 9A>C	4438	2	2.4	AGT GGT ACC GGG GGT CGT ACA GGG TAC ATT CCA TTG GGT GGG CG	44
HPV18 33A>C	4462	6	7.1	AGT GGT ACA GGG GGT CGT ACA GGG TAC ATT CCC TTG GGT GGG CG	44
HPV18 39T>G	4468	2	2.4	AGT GGT ACA GGG GGT CGT ACA GGG TAC ATT CCA TTG GGG GGG CG	44
HPV52-ref	7445-71	368	99.2	GGG TAG GGC AGG GGA CAC AGG GTA GGG	27
HPV52 8G>A	7464	1	0.3	GGG TAG GAC AGG GGA CAC AGG GTA GGG	27
HPV52 18C>T	7454	1	0.3	GGG TAG GGC AGG GGA CAT AGG GTA GGG	27
HPV52 22G>A	7450	1	0.3	GGG TAG GGC AGG GGA CAC AGG ATA GGG	27
HPV58-ref	7362-84	243	85.9	GGG CAG GGT AGG GCA ATT TAG GG	23
HPV58 10A>G	7375	1	0.4	GGG CAG GGT GGG GCA ATT TAG GG	23
HPV58 16A>C	7369	32	11.3	GGG CAG GGT AGG GCA CTT TAG GG	23
HPV58 17T>G	7368	1	0.4	GGG CAG GGT AGG GCA AGT TAG GG	23
HPV58 18T>G	7367	6	2.1	GGG CAG GGT AGG GCA ATG TAG GG	23

^aPosition of G-rich sequence and SNP in reference genome. Reference genomes were obtained from PaVe database under the following accession numbers: K02718 (HPV16), X05015 (HPV18), X74481 (HPV52) and D90400 (HPV58).

^bNumber and percentage of GenBank HPV isolates and additional HPV isolates from cervical samples (see Supplementary Table 2).

^cG-rich tracts are in bold, SNPs are labeled with red.

^dLength of G-rich sequence.