

## I. Povzetek

Preučevali smo vpliv terminalnih koncev oligonukleotidov in narave prisotnih kationov na zvijanje in multimerizacijo G-kvadrupleksov do dolgih nanostruktur, G-žičk. Uporaba terminalnih GC koncev oligonukleotidov lahko vodi do multimerizacije G-kvadrupleksov preko prepletanja in do nastanka daljših nanostruktur. Vpliv prisotnosti  $^{15}\text{NH}_4^+$  in  $\text{K}^+$  ionov smo preučevali na G-kvadrupleksih, katere tvorita oligonukleotida  $d(\text{GCG}_2\text{AG}_4\text{AG}_2)$  in  $d(\text{GCG}_2\text{AG}_4\text{AG}_2\text{CG})$  poimenovana  $\text{GC}_n$  in  $\text{GC}_n\text{CG}$ . Pokazali smo, da prisotnost  $^{15}\text{NH}_4^+$  ali  $\text{K}^+$  ionov sproži multimerizacijo preko nalaganja 3'-terminalnih G-kvartetov  $\text{GC}_n$  G-kvadrupleksa, ki pa jo 3'-GC konci preprečijo v primeru  $\text{GC}_n\text{CG}$  G-kvadrupleksa. V 3'-3' naloženem  $\text{GC}_n$  G-kvadrupleksnem multimeru smo opazili pet vezanih  $^{15}\text{NH}_4^+$  ionov, med katerimi se eden nahaja tudi na 3'-3' medkvadrupleksni površini. Za  $^{15}\text{NH}_4^+$  ione vezane v 3'-3' naloženem  $\text{GC}_n$  G-kvadrupleksnem multimeru smo opazili počasno dinamiko izmenjave. Nasprotno, prisotnost 3'-GC konca pospeši izmenjavo vezanih  $^{15}\text{NH}_4^+$  ionov med vezavnimi mesti v  $\text{GC}_n\text{CG}$  G-kvadrupleksu in  $^{15}\text{NH}_4^+$  ioni v raztopini.  $^{15}\text{NH}_4^+$  ioni znotraj  $\text{GC}_n\text{CG}$  G-kvadrupleksa se premikajo samo v eno smer, kar je lastnost ionskega kanala. Pokazali smo, da v prisotnosti  $\text{K}^+$  ionov oligonukleotid  $d(\text{G}_2\text{AG}_4\text{AG}_2)$  multimerizira do G-žičk. S spreminjanjem pogojev v raztopini in načina priprave vzorca smo našli pet G-kvadrupleksnih struktur, ki nastanejo tekom samozdruževanja  $d(\text{G}_2\text{AG}_4\text{AG}_2)$  do G-žičk. Z uporabo NMR spektroskopije smo določili topologije zvitja omenjenih petih G-kvadrupleksnih struktur in tako dobili vpogled v mehanizem nastanka G-žičk na molekularnem nivoju. Spreminjanje nukleotidov v zankah nam je omogočilo spreminjanje lastnosti G-žičk, s simulacijami MD pa smo razložili kako nukleotidi v zankah vplivajo na dolžino nastalih G-žičk. Preučevali smo tudi možnosti nastanka G-kvadrupleksnih struktur višjega reda v biološkem kontekstu na primeru oligonukleotida iz človeškega telomernega zaporedja, ki vsebuje pet G-traktov,  $d(\text{TAG}_3(\text{T}_2\text{AG}_3)_4)$ . Ugotovili smo, da prisotnost petega G-trakta povzroči nastanek paralelnega G-kvadrupleksa s  $\text{T}_2\text{AG}_3$  prostim koncem. Multimerizacija je bolj verjetna pri paralelnih, kot pri hibridnih G-kvadrupleksih, kjer robne zanke ovirajo nalaganje preko terminalnih G-kvartetov.

Ključne besede: G-kvadrupleksi, multimerizacija, strukture višjega reda, G-žičke, samozdruževanje, NMR, DNA nanotehnologija

## II. Abstract

We studied how oligonucleotides' terminal ends and nature of present cations influence formation and multimerization of G-quadruplexes to long nanostructures, G-wires. Terminal GC ends in oligonucleotide might promote G-quadruplex multimerization via interlocking and thus formation of longer nanostructures. The effect of the presence of  $^{15}\text{NH}_4^+$  and  $\text{K}^+$  ions was studied on G-quadruplexes formed by oligonucleotides  $\text{d}(\text{GCG}_2\text{AG}_4\text{AG}_2)$  and  $\text{d}(\text{GCG}_2\text{AG}_4\text{AG}_2\text{CG})$ , named  $\text{GCn}$  and  $\text{GCnCG}$ . We showed that the presence of  $^{15}\text{NH}_4^+$  or  $\text{K}^+$  ions induces multimerization via stacking of 3'-terminal G-quartets in  $\text{GCn}$  G-quadruplex, which is precluded by 3'-GC ends in the case of  $\text{GCnCG}$  G-quadruplex. We observed five  $^{15}\text{NH}_4^+$  ions bound in 3'-3' stacked  $\text{GCn}$  G-quadruplex multimer, with one located at 3'-3' stacking interface.  $^{15}\text{NH}_4^+$  ions bound within 3'-3' stacked  $\text{GCn}$  G-quadruplex multimer exhibit slow exchange dynamics. Contrary, presence of 3'-GC ends accelerates exchange of bound  $^{15}\text{NH}_4^+$  ions between binding sites in  $\text{GCnCG}$  G-quadruplex and with  $^{15}\text{NH}_4^+$  ions in bulk solution.  $^{15}\text{NH}_4^+$  ions within  $\text{GCnCG}$  G-quadruplex show unidirectional movement, which is characteristic for ion channels. We showed that in the presence of  $\text{K}^+$  ions,  $\text{d}(\text{G}_2\text{AG}_4\text{AG}_2)$  self-assembles into G-wires. By varying solution conditions and sample preparation procedure, we found five G-quadruplex structures, which are formed in  $\text{d}(\text{G}_2\text{AG}_4\text{AG}_2)$  G-wire self-assembly. Using NMR spectroscopy we determined folding topologies of mentioned five G-quadruplex structures and thus obtained insight into mechanism of G-wire self-assembly on molecular level. Changing the nucleotides in loops enabled us to manipulate G-wires' properties. MD simulations provided rationale on how nucleotides in loops influence length of formed G-wires. We also studied the possibility of higher-order G-quadruplex structure formation in biological context on oligonucleotide from human telomere region, containing five G-tracts,  $\text{d}(\text{TAG}_3(\text{T}_2\text{AG}_3)_4)$ . We showed that the presence of additional G-tract leads to formation of parallel G-quadruplex with 3'-terminal  $\text{T}_2\text{AG}_3$  overhang. Multimerization is more likely for parallel than hybrid G-quadruplexes, where lateral loops hinder stacking of terminal G-quartets.

Key words: G-quadruplexes, multimerization, higher-order structures, G-wires, self-assembly, NMR, DNA nanotechnology