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QUALITY REPORT of **APTAMERS**

synthesized by Kilobaser ONE

Abstract

The synthesis of G-rich oligonucleotides that also tend to form quadruplexes are challenging for many DNA suppliers. Companies such as IDT even recommend on their homepage to avoid sequences with repetitive G motifs as their synthesis can result in lower yields and purities.

Quadruplexes can also interfere with certain analysis methods to determine the purity of an oligo. For instance, the quadruplexes cause oligonucleotides to clump, which produce multiple peaks on capillary electrophoresis. These additional peaks make it hard to quantify the amount of full-length product using this method.

In this report, the synthesis of two G-rich DNA-aptamers by Kilobaser ONE is summarized. Both aptamers fold via G-quadruplexes into a thrombin-binding chair-conformation. The first aptamer was 15 nt long, while the second aptamer was 29 nt long. Analysis via revers HPLC in combination with a mass spectrometer was performed after synthesis to obtain purification degree and structure confirmation. Furthermore the yield of the synthesis was determined via Qubit fluorometer.

The synthesis of both oligonucleotides was successful resulting in a final yield of 395 pmol and 454 pmol of full-length product, respectively, which is similar to other synthesis. The purification degree is with over 92,5 % and 94 % significantly high indicating that there were hardly any problems during the synthesis or deprotecting. In conclusion, no effect of the G motifs on the synthesis or the analysis were observed.

Description

Aptamers are short DNA- or RNA-based oligonucleotides that fold into a stable 3D-structure. This structure gives them the capacity to efficiently bind a target with high specificity and affinity like antibodies. As a result, they can be used as diagnostic tools and therapeutic agents.

For the formation of the structure are, among others, the interaction of guanine bases. Four guanine bases can associate through Hoogsteen hydrogen bonding to form a square planar structure called a guanine tetrad (G-tetrad or G-quartet), and two or more guanine tetrads (from G-tracts, continuous runs of guanine) can stack on top of each other to form a G-quadruplex. [1]

The G-quadruplex structure has been studied intensely, for instance, in thrombin binding aptamers. Macaya et al. described in 1993 the 15-mer DNA oligonucleotide with a stable intramolecular G-quadruplex structure in an antiparallel orientation forming a chair-like conformation, as shown in Fig. 1A. [2] Another thrombin-binding aptamer is a 29-mer DNA oligonucleotide folding via G-quadruplex structure into a similar conformation, as shown in Fig. 1b. [3]

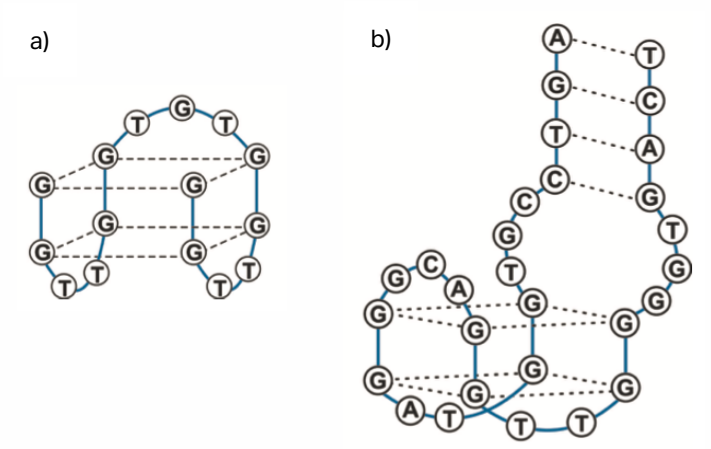

Figure 1: G-quadruplex based structures of two thrombin-binding aptamers: a) 15mer [2] and b) 29mer [3].

References:

1. Burge S, Parkinson GN, Hazel P, et al. Quadruplex DNA: sequence, topology, and structure. *Nucleic Acids Res*. 2006; 34(19):5402-15.
2. Macaya RF, Schultze P, Smith FW, Roe JA, Feigon J. Thrombin-binding DNA aptamer forms a unimolecular quadruplex structure in solution. Proc Natl Acad Sci U S A. 1993 Apr 15;90(8):3745-9.
3. Tasset DM, Kubik MF, Steiner W. Oligonucleotide inhibitors of human thrombin that bind distinct epitopes. J Mol Biol. 1997 Oct 10;272(5):688-98.

Synthesized oligonucleotides

#	Name	Oligo type	Sequence
1	Short Thrombin binding aptamer	G-rich ssDNA strand	5'- GGTTGGTGTGGTTGG -3'
2	Long Thrombin binding aptamer	G-rich ssDNA strand	5'- AGTCCGTGGTAGGGCAGGTTGGGGTGACT -3'

Measurements

Device	Method	Outcome
Advion AVANT™ UHPLC System and Advion expression® Compact Mass Spectrometer (CMS)	Reverse HPLC to separate oligonucleotides from each other by their length and modifications; Separated oligonucleotides are detected via absorption at 260 nm and analyzed in the mass spectrometer.	• Chemical composition of sample • Differentiation of oligonucleotides in length and number of modifications • Identification of modifications • Yield in % of full-length product
Qubit 3.0 Fluorometer	Fluorometric quantification of ssDNA	• Yield of synthesis in pmol

Synthesis 1 Short Thrombin binding aptamer

Sample type G-rich ssDNA strand
Sequence 5'- GGTTGGTGTGGTTGG -3'
Length 15 nt

Chemical composition, mass and purity

After synthesis the oligonucleotide was analyzed with reverse HPLC, which separates the components of the synthesis products and side products depending on their size and chemical polarity. The first outcome of the analysis is the chromatogram shown in Figure 2.

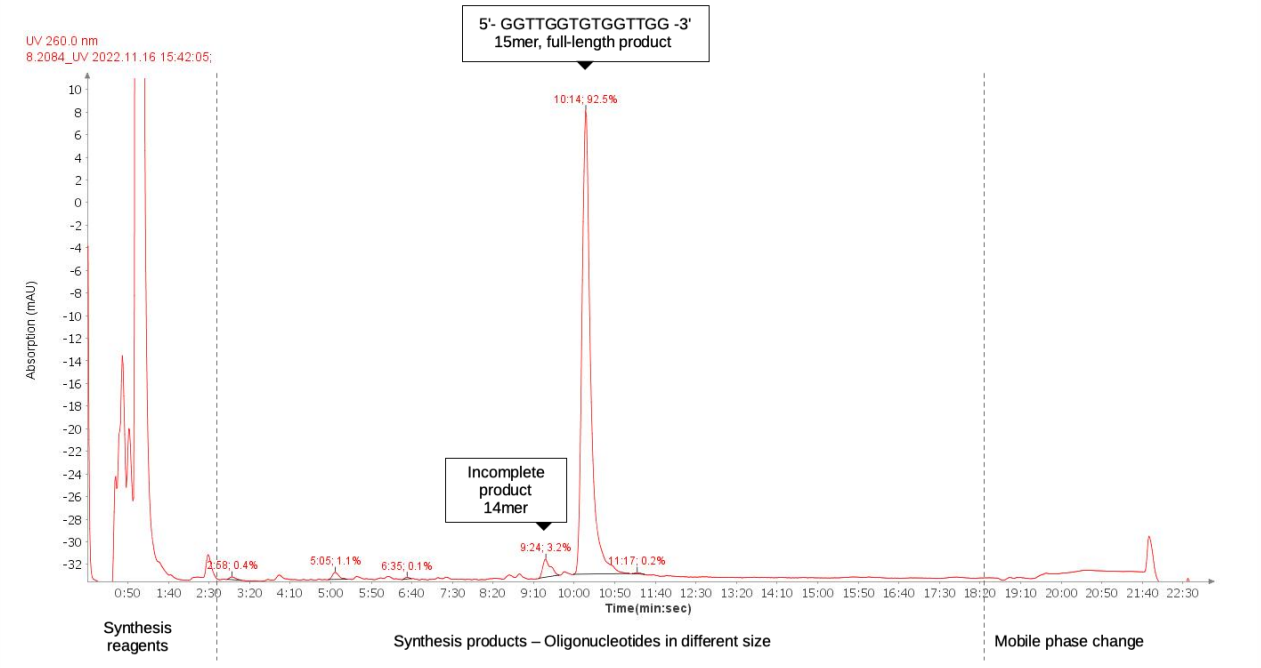

Figure 2: Chromatogram of reverse HPLC collecting absorption at 260 nm overtime

The chromatogram of this synthesis shows following peaks from left to right:

- Peaks arriving during the first 3 minutes of the HPLC are mainly caused by synthesis reagents.
- After 3 minutes of the HPLC oligonucleotides in different length are visible.
- The peak at 9:30 contains incomplete oligonucleotides that miss exactly one base.
- **The peak at 10:00 is the full-length product and the main product of the synthesis.**
- The peak at 11:17 contains not completely deprotected full-length product.

After separation via HPLC, the synthesis products are further analyzed through a mass spectrometer to confirm their chemical composition and the mass. The mass spectrum confirms that the main product (the peak at 10:00 in HPLC) is the full-length oligonucleotide, which is also completely deprotected and is thus ready to use for further applications.

Yield of synthesis

The total yield of functional oligonucleotide – full length, completely deprotected – was determined considering following values:

- Resuspension volume for the analysis: 12 µL
- Concentration of ssDNA via Quibit Fluorometer: 168 ng/µL
- Purification degree based on chromatogram: 92.5 %
- Calculated molecular weight: 4726.1 g/mol

This results in a yield of **394.6 pmol** of functional oligonucleotide.

Outcome of synthesis

The synthesis worked well resulting in a final yield of 395 pmol full-length product, which was 92,5 % of all detectable oligonucleotides of this synthesis. Based on this results it can be concluded that the synthesis of G-rich aptamers at this length was successful.

Synthesis 2 Long Thrombin binding aptamer

Sample type	G-rich ssDNA strand
Sequence	5'- AGTCCGTGGTAGGGCAGGTTGGGGTGACT -3'
Length	29 nt

Chemical composition, mass and purity

After synthesis the oligonucleotide was analyzed with reverse HPLC, which separates the components of the synthesis products and side products depending on their size and chemical polarity. The first outcome of the analysis is the chromatogram shown in Figure 3.

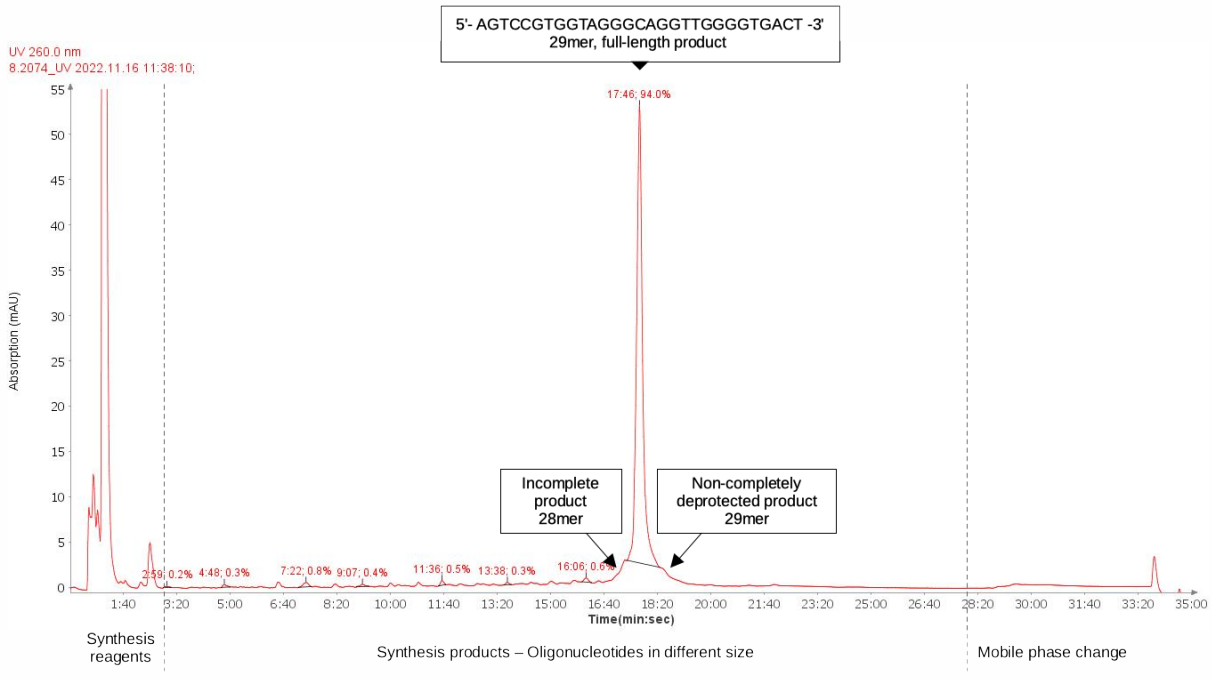

Figure 3: Chromatogram of reverse HPLC collecting absorption at 260 nm overtime

The chromatogram of this synthesis shows following peaks from left to right:

- Peaks arriving during the first 3 minutes of the HPLC are mainly caused by synthesis reagents.
- After 3 minutes of the HPLC oligonucleotides in different length are visible.
- **The peak at 17:46 contains the full-length product, which is also the main product of the synthesis.**
- Shortly before and after this peak, there are other peaks that are not completely separated from the main peak due to the decreasing separation efficiency of the oligonucleotides at this length.
- Before the main peak (17:00), incomplete oligonucleotides that miss one base arrive in the HPLC.
- After the main peak (18:20), not completely deprotected full-length product arrive in the HPLC.

After separation via HPLC, the synthesis products are further analyzed through a mass spectrometer to confirm their chemical composition and the mass. The mass spectrum confirms that the main product (the peak at 17:46 in HPLC) is the full-length oligonucleotide, which is also completely deprotected and is thus ready to use for further applications.

Yield of synthesis

The total yield of functional oligonucleotide – full length, completely deprotected – was determined considering following values:

- Resuspension volume for the analysis: 12 µL
- Concentration of ssDNA via Quibit Fluorometer: 366 ng/µL
- Purification degree based on chromatogram: 94 %
- Calculated molecular weight: 9085.9 g/mol

This results in a yield of **454.4 pmol** of functional oligonucleotide.

Outcome of synthesis

The synthesis worked well resulting in a final yield of 454 pmol full-length product, which was 94 % of all detectable oligonucleotides of this synthesis. Based on this results it can be concluded that the synthesis of G-rich aptamers at this length was successful.