

Formation of an RNA Quadruplex-Duplex Hybrid in Living Cells Between mRNA of the Epidermal Growth Factor Receptor (EGFR) and a G-Rich Antisense Oligoribonucleotide

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Supplementary Information

Figure S1. The 56-nucleotide fragment sequence of the EGFR target used in biophysical studies is marked with a red box.

Figure S2. Putative model of bimolecular G-quadruplex of QF-ASO and QL-ASO.

Figure S3. Secondary structures of the EGFR target (A), rASO/EGFR (B), QF-ASO/EGFR (C), QL-ASO/EGFR (D) as predicted by RNAstructure and quadruplex-duplex hybrid structure containing a two duplex stems at the external loop (2ST-QDH) (E); (GGGG-tract- red or navy blue circles, FG –purple circles, a or S6- white circles, any nucleotides- grey circle, base pairs: A:U – grey line, G:C – blue line, G:U – red line).

Figure S4. Analysis of QF-ASO, EGFR, QF-ASO/EGFR and QL-ASO/EGFR structures by non-denaturing PAGE. All oligomers in 0.1 M Tris HCl, pH 6.8, with 50 mM KCl, 150 mM LiCl and 2 equivalents of G4-ligand: NMM was annealed and subjected to non-denaturing PAGE. Next, the gel was visualized by UV shadowing (A) and exposed to 532 nm light (B).

Figure S5. Imino region of the ¹H NMR spectra of 28-nt molecule (AGCAGCGAUGCGACCCUCCGGACGGCC) corresponding to mRNA fragment of sequence located between two G-tracts in EGFR target. Spectra were recorded at 25 °C and 40 °C in 90% H₂O / 10 % D₂O (v/v) in the presence of 50 mM KCl, 150 mM LiCl, 10 mM Tris-HCl, 0.1 mM EDTA, pH 6.8.

Figure S6. CD spectra of QF-ASO/EGFR and QL-ASO/EGFR hybrids at 25 °C in potassium buffer.

Figure S7. oBMVC-C3 labeling of Q-ASO-NH₂ oligonucleotides.

Figure S8. As a control, we used non-covalent complex of QL-ASO/EGFR hybrid with NMM. After electrophoresis, the gel was exposed to 532 nm light to detect bands corresponding to the FI-Q-ASO/EGFR and NMM:QL-ASO/EGFR hybrids. QL-ASO/EGFR in NMM, 50 mM KCl, 150 mM LiCl,

100 mM Tris HCl, pH 6.8 and FI-Q-ASO/EGFR in 50 mM KCl and 150 mM LiCl, 100 mM Tris HCl, pH 6.8 were annealed and subjected to non-denaturing PAGE.

Figure S9. Different type of the unimolecular quadruplex–duplex hybrids.

Figure S10. (A) RP-HPLC profile of the purified dASO (5'-d(AGC AGC GCC AGG AGC G)-3') after automatic synthesis; (B) Q-TOF MS: MW calc. (g/mol): 4941.22; m/z 4941.8203.

Figure S11. (A) RP-HPLC profile of the purified rASO (5'-AGC AGC GCC AGG AGC G-3') after automatic synthesis; (B) Q-TOF MS: MW calc. (g/mol): 5197.21; m/z 5197.7002.

Figure S12. (A) RP-HPLC profile of the purified oligonucleotide 5'-AGG GGU CGG GGA-3' (Q-RNA) after automatic solid phase synthesis; (B) Q-TOF MS: MW calc. (g/mol): 3969.44, m/z 3968.6001.

Figure S13. The purity and homogeneity of Q-ASO, FI-Q-ASO and EGFR mRNA oligomers verified by 15% denaturing gel electrophoresis.

ATG **START**

GAGCT **SacI 237-242**

GAATTC **EcoRI 549-555**

EGFR 318-nt mRNA

5' C-**GGGG**-AGCAGCGAUGCGACCCUCCGGGACGGCC-**GGGG**-CAG-CGCUCCUGGCGCUGCU 3' mRNA EGFR 56-nt

3' A-GGGG-----S6-----GGGG-aaa-GCGAGGACCGCGACGA 5' **QL-ASO**

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1  gtccgggag cccccggcgc agcgcggccg cagcagcctc cgccccccgc acggtgtgag
61  cgcccagcgc ggccgaggcg gccggagtcc cgagctagcc ccggcggccg ccgccgcca
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3' AGCCCCGAGACCTCCTTTTCTTT 5' **dASO-R 22-mer**

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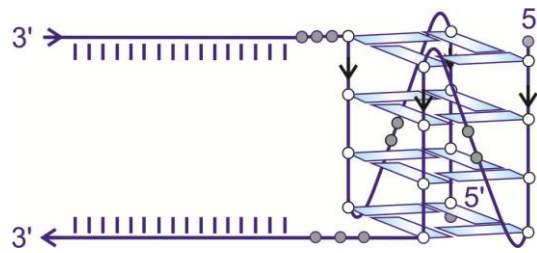


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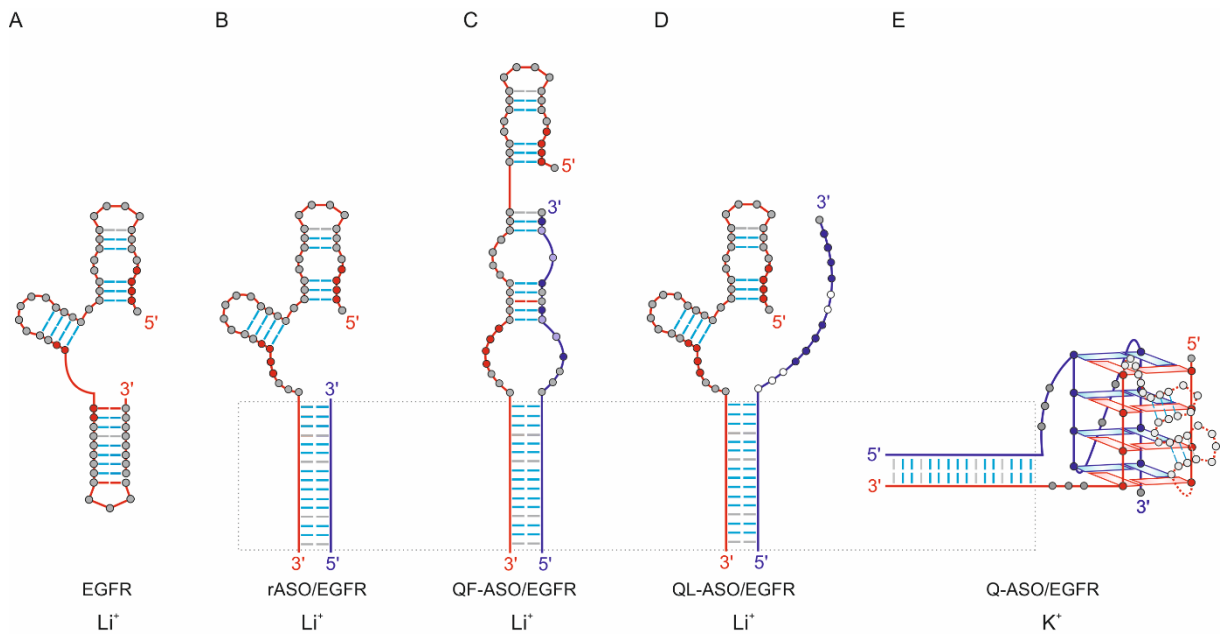


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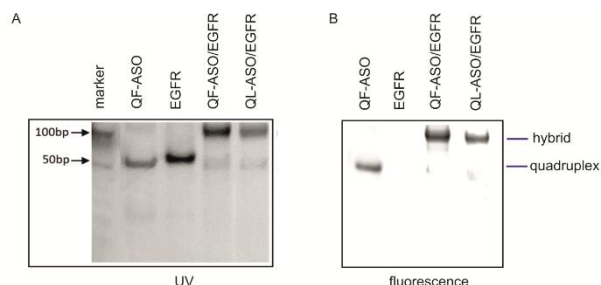


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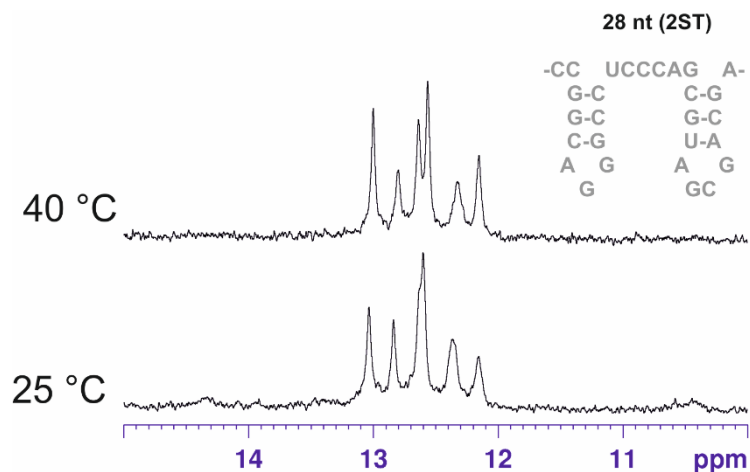


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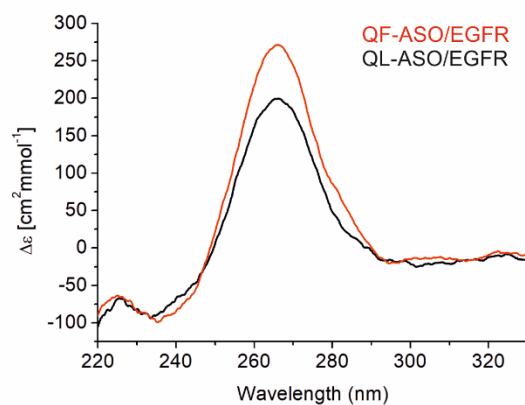


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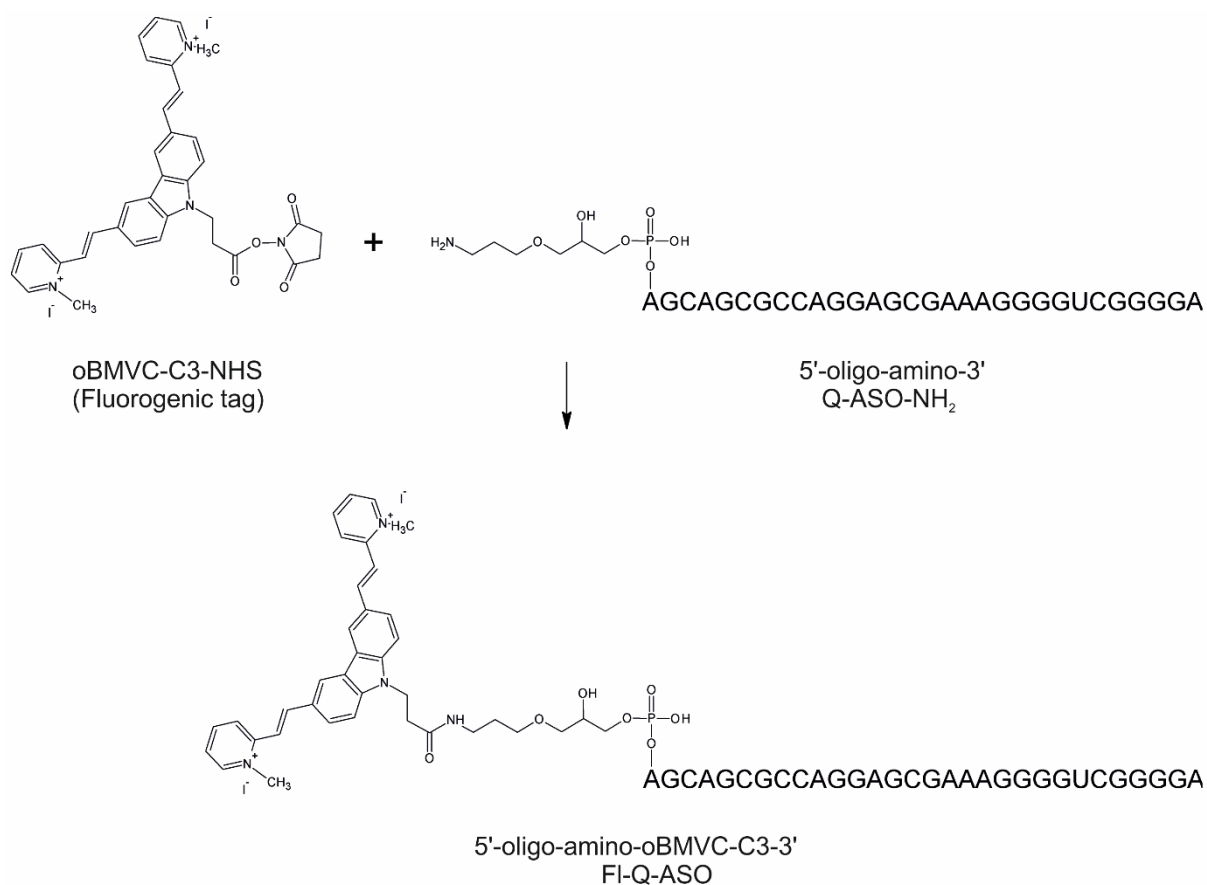


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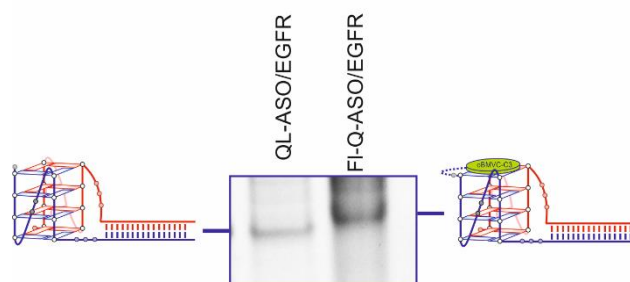


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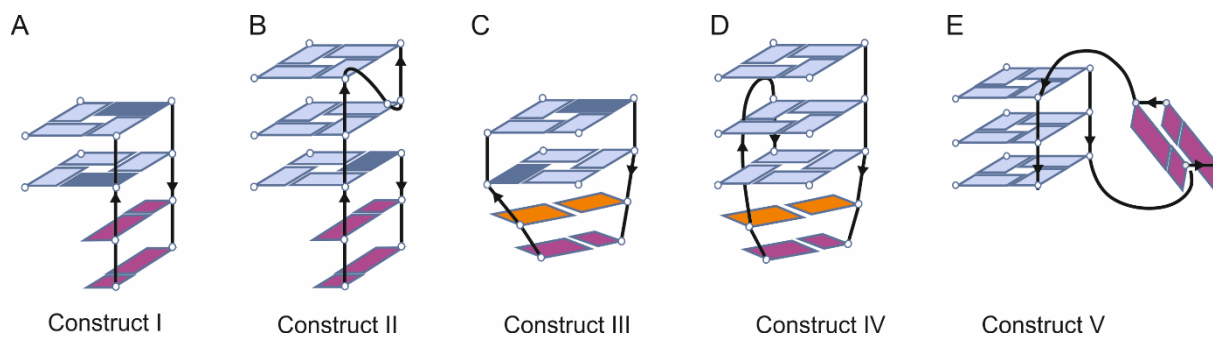


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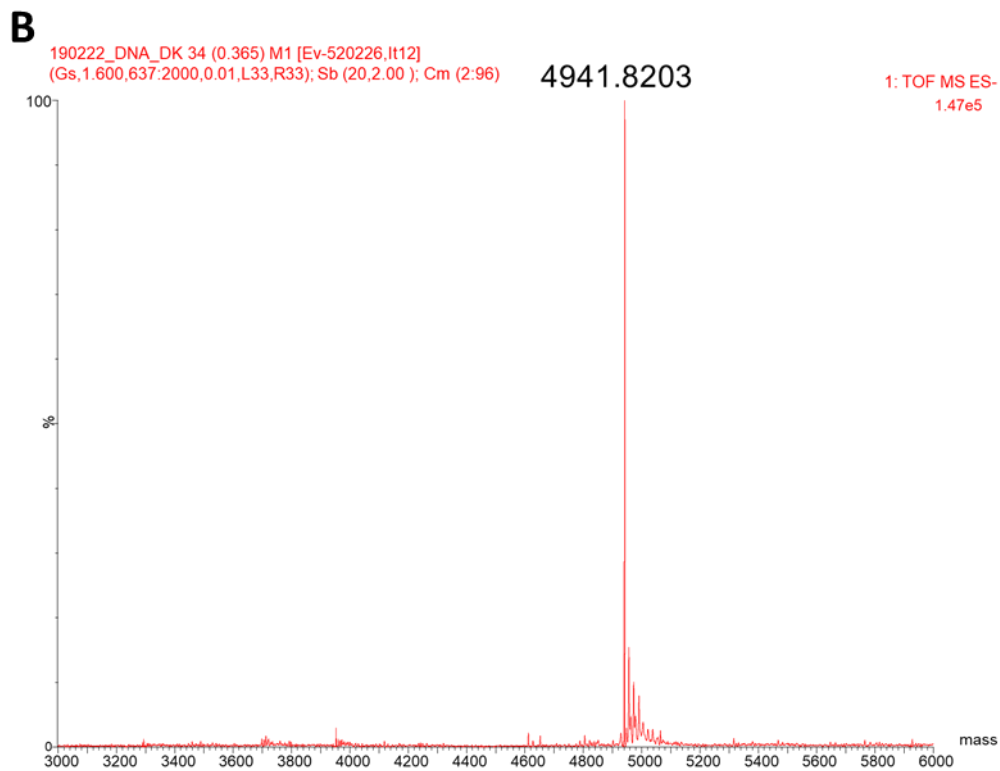
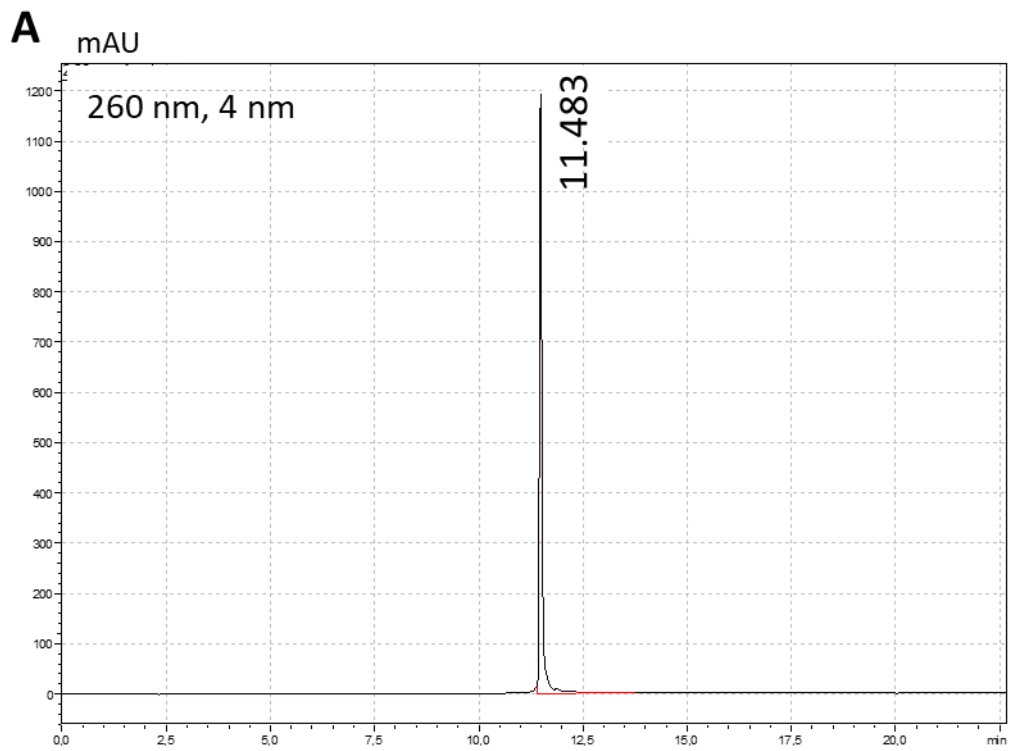


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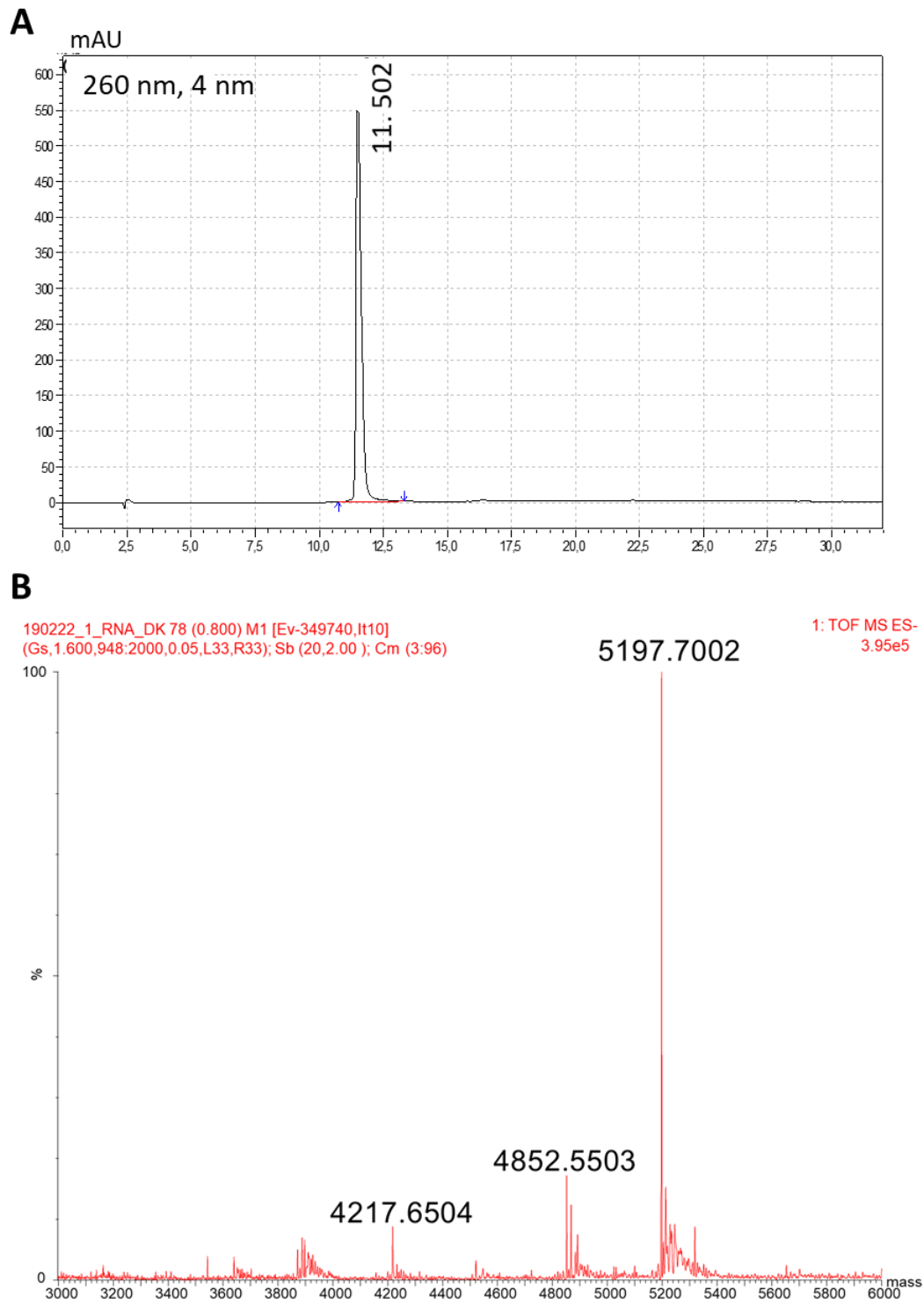


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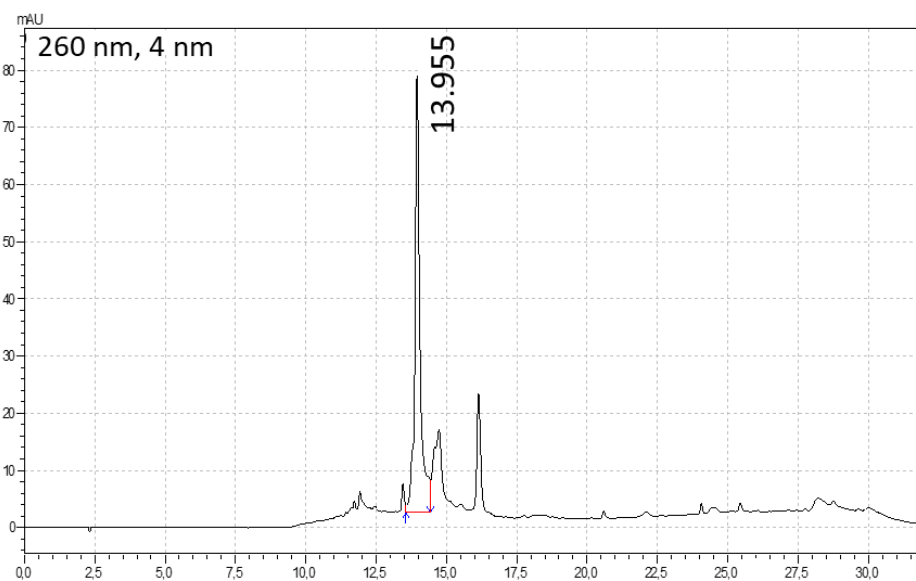
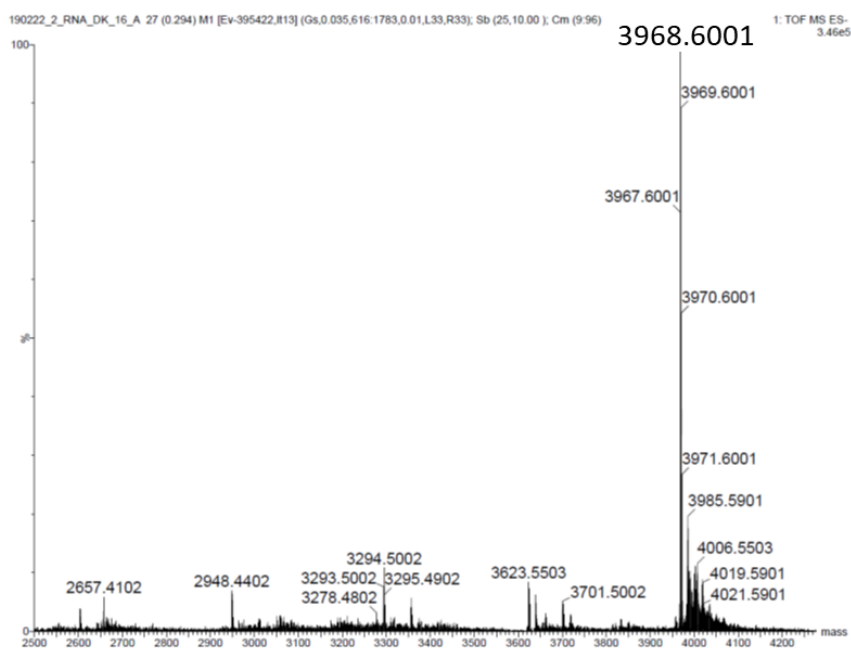
A**B**

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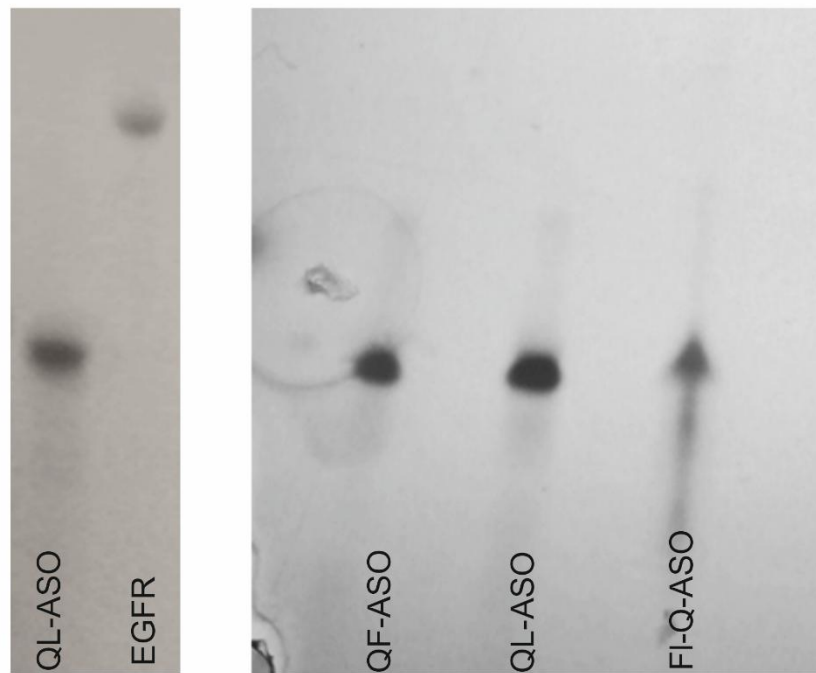


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