Electronic Supplementary Material (ESI) for Analytical Methods.
This journal is © The Royal Society of Chemistry 2024

Supplementary information

Bioluminescent aptamer-based microassay for detection of melanoma inhibitory activity (MIA) protein

Eugenia E. Bashmakova[a], Alexander N. Kudryavtsev[a], Alexey E. Tupikin[b], Marsel R. Kabilov[b], Aleksey E. Sokolov[c,d], Ludmila A. Frank[a,d]

[a]Institute of Biophysics, Federal Research Center "Krasnoyarsk Science Center SB RAS", 660036, Krasnoyarsk, Russia

[b]Institute of Chemical Biology and Fundamental Medicine SB RAS, 630090, Novosibirsk, Russia

[c]Kirensky Institute of Physics, Federal Research Center "Krasnoyarsk Science Center SB RAS", 660036, Krasnoyarsk, Russia

[d]Siberian Federal University, 660041, Krasnoyarsk, Russia

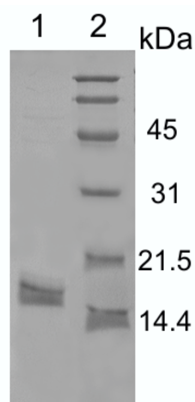

Figure S1. 15% SDS-PAGE analysis of His_6-MIA sample. Lanes: 1 – His_6-MIA after chromatography purification; 2 – standard proteins.

S1. ELISA assay of the recombinant MIA proteins

Sandwich-type MIA colorimetric immunoassay based on horseradish peroxidase conjugate was performed using a commercial kit (R&D Systems, USA) according to the manufacturer's instructions. Optical density was measured with a plate colorimeter (Bio-Rad, USA).

The targets used were: (a) recombinant MIA protein obtained in this work; (b) recombinant MIA with biological activity (Novus Biologicals, USA); (c) human MIA standard (R&D Systems, USA). Samples (a) and (b) were diluted in concentrations from 576 to 4.5 ng/mL in standard

human serum (Vector Best, Russia). Sample (c) was diluted in PBS at concentrations ranging from 66 to 1 ng/mL in accord with the manufacturer's recommendations.

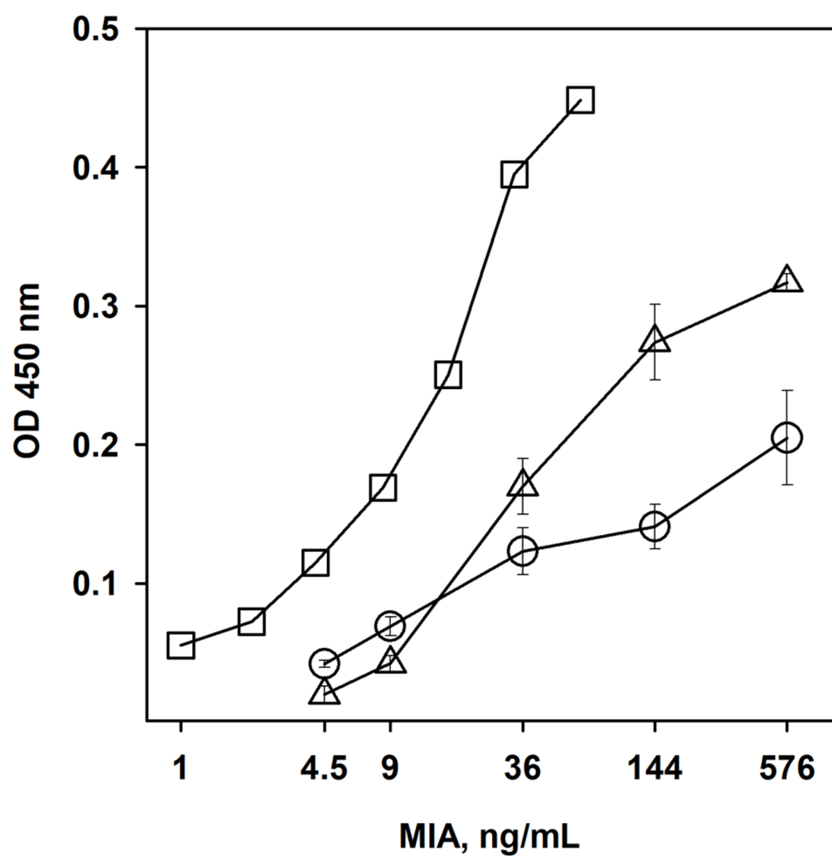

Figure S2. ELISA assay of the recombinant MIA proteins. Human MIA standard was analyzed in duplicate. The triangle denotes MIA obtained in this work, the circle – MIA from Novus Biologicals, the square – human MIA standard (R&D Systems). (n = 3).

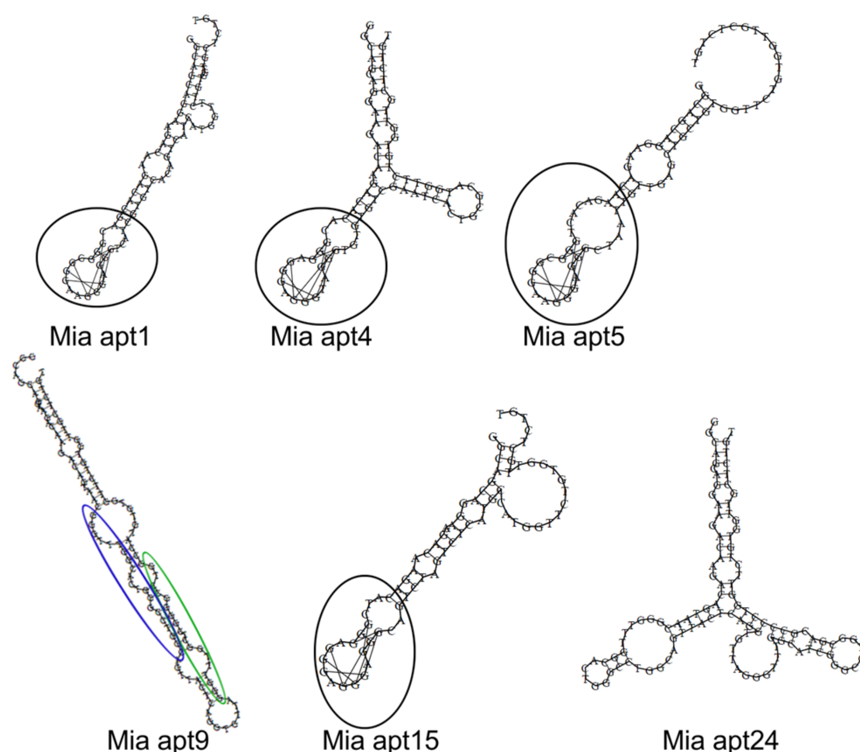

Figure S3. Predicted secondary structures of the full-size aptamers (RNAfold web server). Plausible formation route for GQ motifs according to RNAfold web server is shown in black circles and according to QGRS Mapper – in blue and green circles.

Table S1. Aptamers sequences. Constant regions are underlined

Aptamer	Sequence 5'-3'	Length	Frequency	G %; GQ motif (+ or –)*
MIA apt1	*GGCAGCAGGAAGACAAGACA*CGACGGGGGGGAAGGGAGGGTCATCGTGTCACAGTCATCA*TGGTTCTGTGGTTGCTCTGT*	80	7968	38.8 (+)
MIA apt4	*GGCAGCAGGAAGACAAGACA*CACGGGAGGGAGGGTAGGGTGTGTGTCGAATCACTGCGCA*TGGTTCTGTGGTTGCTCTGT*	80	1281	40 (+)
MIA apt5	*GGCAGCAGGAAGACAAGACA*CTGGCGGGAAGGGAGGGCTAATTGTTGAGCTGCTG*TGGTTCTGTGGTTGCTCTGT*	76	1144	40.8 (+)
MIA apt9	*GGCAGCAGGAAGACAAGACA*GTAACGGGTTTGGCACTGGGCCTGGCAGTTACTCAGGTGTTAGGGTTTGGCTCGGGGCCTGGCCATGTG*TGGTTCTGTGGTTGCTCTGT*	109	704	38.5 (-)
MIA apt15	*GGCAGCAGGAAGACAAGACA*TCGGGAGGGAGGGAGGGCAGTCTAGTCTCATGCCA*TGGTTCTGTGGTTGCTCTGT*	75	374	38.7 (+)
MIA apt24	*GGCAGCAGGAAGACAAGACA*GTAACGGGTTTGGCACTGGGCCTGGCAGTTACTCAGGTGTTAGGGTTTGGCATCGGGCCTGGCGACGCCCC*TGGTTCTGTGGTTGCTCTGT*	111	310	36.9 (-)

*The secondary structures of aptamers predicted by VIENNA RNA fold.

S2. Evaluation of the aptamers stability and functionality

Aliquots of 100 µM water solutions of biotinylated aptamer MIAapt4.53 were stored for one year at -20 °C. Prior to the experiment, the aptamer was folded in binding buffer (PBS, 1 mM $MgCl_2$) by heating for 10 min at 95 °C followed by cooling at 25 °C for 15 min.

Aliquots of the conjugate MIAapt9-NLuc in the binding buffer containing 0.1% bovine serum albumin were stored for four months at -20°C. This conjugate was thawed and used without any processing.

The 100 µL of biotinylated aptamer MIAapt 4.53 (70 nM in binding buffer) were placed into the plate wells, activated with streptavidin, incubated for 1 h at RT and washed. Then the 100 µL aliquots of His_6-MIA (10, 100,1000 ng/mL), in standard human blood serum, previously five-fold diluted and containing 0.05% Tween 20 were placed into the wells, incubated for 1 h at RT. After washing, the wells were loaded with 100 µL of conjugate MIAapt9–NLuc solution (80 pM, binding buffer), incubated for 1 h at RT, then washed. The bioluminescent signal was measured with LB 940 Multimode Reader Mithras (Berthold, Germany) immediately after injection of freshly prepared 100 µL of furimazine solution in PBS, the signal was integrated for 5 s.

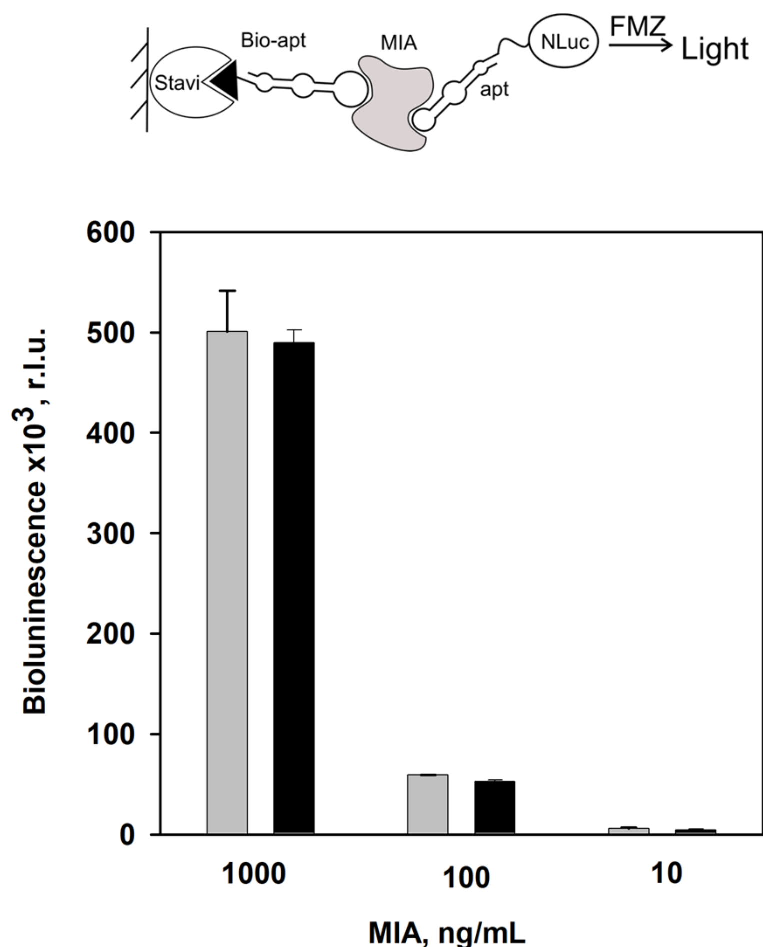

Figure S4. Sandwich-type aptamer-based solid-phase bioluminescent assay of MIA in standard human serum with freshly prepared aptamers (gray bars) and after storage (black bars), (n = 3).

Bio-apt – biotinylated aptamer; FMZ – furimazine. Composition of complexes formed on the surface is given above.

S3 Circular dichroism (CD) spectroscopy

Aptamers MIAapt4.53 and MIAapt9 (3 μM) were refolded by heating to 95 °C for 10 min in PBS, 1 mM $MgCl_2$ followed by cooling at 25 °C for 15 min. The CD spectra were taken at 20 °C on a SKD-2MUF recording spectrograph (Russia) using a quartz cell of 0.5-cm optical path length. All spectra were recorded in the 200-400 nm wavelength range, with spectral resolution of 3 nm. Each spectrum was acquired as an average of 6 scans.

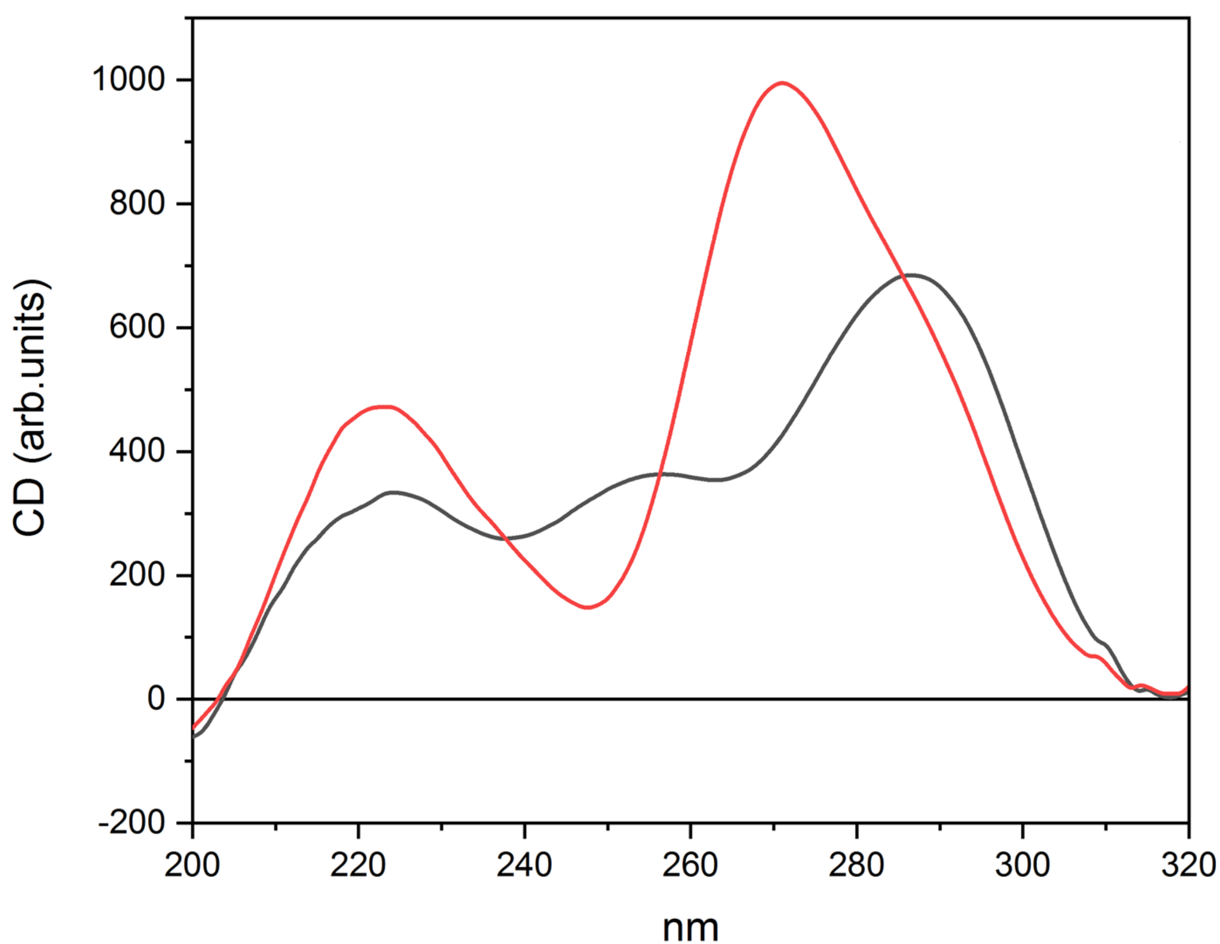

Figure S5. CD spectra of MIAapt4.53 (red line) and MIAapt9 (gray line), 3 μM each in PBS, 1 mM $MgCl_2$.