Anti-FGFR1 aptamer-tagged superparamagnetic conjugates for anti-cancer hyperthermia therapy

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Supplementary data

Supplementary Table 1

Oligonucleotides used in this work. All sequences are given in 5'->3' order. The table does not contain aptamer A11 mutant sequence (shown in Supplementary Table 4).

Oligonucleotide	Sequence
Library	GTA TAC CTG CAG CTG AGG - N30 - ATT CGT CTC TAG AGC GCA
L8-P1	TGC GCT CTA GAG TCG AAT
L8-P1-biot	Biotin-TGC GCT CTA GAG TCG AAT
L8-P2	GTA TAC CTG CAG CTG AGG
A11	GTA TAC CTG CAG CTG AGG CGA GCC GTT GGT GTG GAT GGC AGG GGC AGG ATT CGA CTC TAG AGC GCA
A08	GTA TAC CTG CAG CTG AGG CGA GC**T** GTT GGT GTG GAT GGC AGG GGC A**A**G ATT CGA CTC TAG AGC GCA

Supplementary Table 2

Selection of aptamers against FGFR1-Fc. Target concentration during aptamers' binding (calculated from theoretical maximal capacity of Dynabeads used), initial ssDNA concentration (aptamer pool loaded on the target beads) and incubation time in successive rounds are given. Initial ssDNA and eluted ssDNA were measured by qRT-PCR (each sample measured in triplicate and referred to a standard curve prepared during each experiment). Eluted ssDNA amount is also plotted as a column chart in Supplementary Figure 1.

Round	Target conc. [nM]	Incubation time [min]	Initial ssDNA conc. [nM]	Eluted ssDNA [amol]	Eluted ssDNA fold increase
1	100	60	7000	37218	-
2	50	60	97.2	6	1
3	50	60	70.4	8	1.3
4	50	60	122.1	303	37.9
5	50	60	167	1883	6.2
6	50	30	118.3	21294	11.3
7[a]	50	30	53.7	6261	0.3
8	20	30	286	77881	12.4
9	20	30	309	2639	0.0
10	20	30	120.6	17186	6.5
11[a]	20	30	78.5	11799	0.7

[a] – counter-selection was performed prior to the incubation with target; initial ssDNA concentration was measured after the counter-selection.

Supplementary Table 3

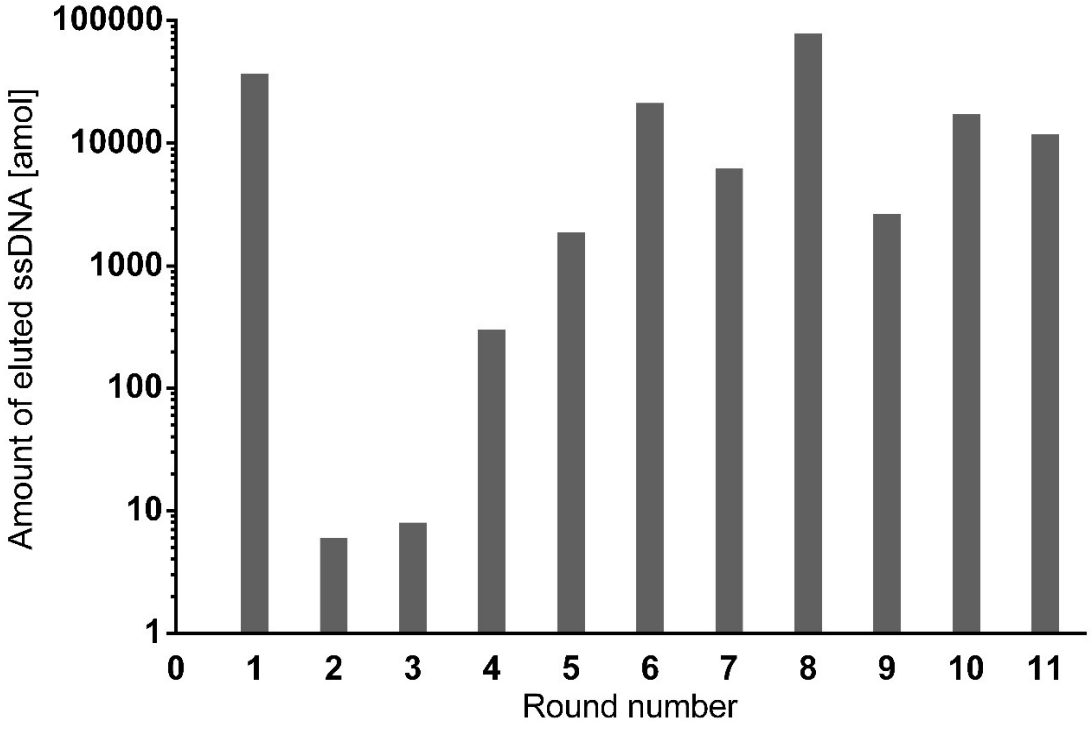
DNA sequences of single aptamer clones after 11th round were stripped of primer regions and aligned using the MUSCLE algorithm with HTML output. Blue background denotes bases identical to consensus sequence and white background denotes bases that do not conform to the consensus sequence.

Clone	Aligned sequence (random region only)
11-32	CGAaCCGTTGGaGTGGATGGCAGGGGCAGG
11-04	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-08	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-10	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-11	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-12	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-13	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-17	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-18	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-19	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-22	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-23	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-24	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-27	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-29	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-30	CGAGCCGTTGGTGTGGATGGCAGGGGCAGG
11-31	CGAGCtGTTGGTGTGGATGGCAGGGGCAGG
11-05	CGAtCtGTTGGTGTGGATGGCAGGGGCAaG
11-01	CGAGCtGTTGGTGTGGATGGCAGGGGCAaG
11-25	CGAGCtGTTGGTGTGGATGGCAGGGGCAaG

Supplementary Table 4

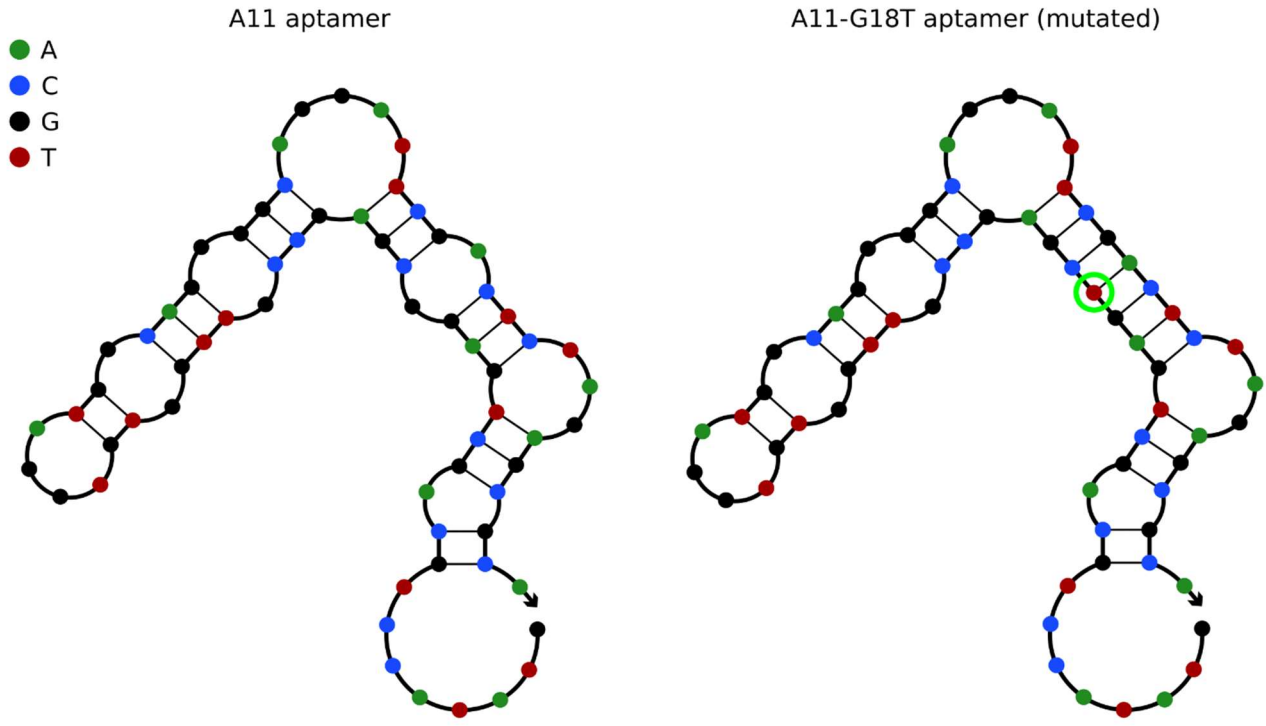
Designed A11 aptamer mutants. Sequences of designed mutant aptamers are given. Primer regions are in grey, mutated nucleotides in underlined blue. Relative binding as assessed by EMSA is estimated: (–) denotes no binding and (+++) denotes highest binding signal observed (data not shown).

Variant	Sequence	Relative binding
A11	GTATACCTGCAGCTGAGGCGAGCCGTTGGTGTGGA TGGCAGGGGCAGGATTCGACTCTAGAGCGCA	**+++**
A11_no_loop	GTATACCTGCAGCTGAGGCGAGCCGTTGGTGAAAA TGGCAGGGGCAGGATTCGACTCTAGAGCGCA	**-**
A11 T36G	GTATACCTGCAGCTGAGGCGAGCCGTTGGTGTGGA GGGCAGGGGCAGGATTCGACTCTAGAGCGCA	**-**
A11 G41A	GTATACCTGCAGCTGAGGCGAGCCGTTGGTGTGGA TGGCAAGGGCAGGATTCGACTCTAGAGCGCA	**-**
A11 T30C T36A	GTATACCTGCAGCTGAGGCGAGCCGTTGGCGTGGA AGGCAGGGGCAAGATTCGACTCTAGAGCGCA	**-**
A11 G46A	GTATACCTGCAGCTGAGGCGAGCCGTTGGTGTGGA TGGCAGGGGCAAGATTCGACTCTAGAGCGCA	**+**
A11 G18T	GTATACCTGCAGCTGAGTCGAGCCGTTGGTGTGGA TGGCAGGGGCAGGATTCGACTCTAGAGCGCA	**+++**
A11 G33A	GTATACCTGCAGCTGAGGCGAGCCGTTGGTGTAGA TGGCAGGGGCAGGATTCGACTCTAGAGCGCA	**-**
A11 G22T	GTATACCTGCAGCTGAGGCGATCCGTTGGTGTGGA TGGCAGGGGCAGGATTCGACTCTAGAGCGCA	**+**



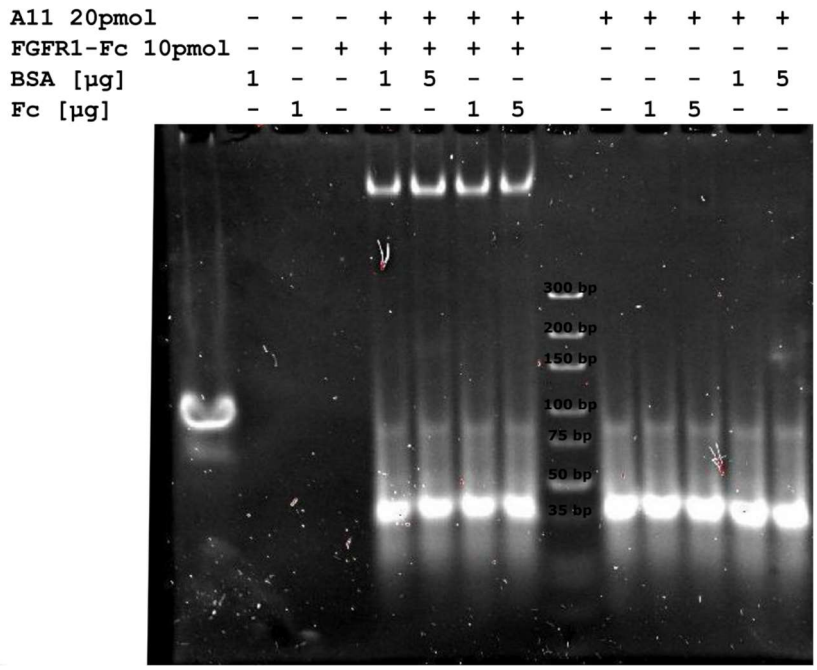
Supplementary Figure 1

Selection progress. Eluted ssDNA from successive rounds measured by qRT-PCR (three replicates).



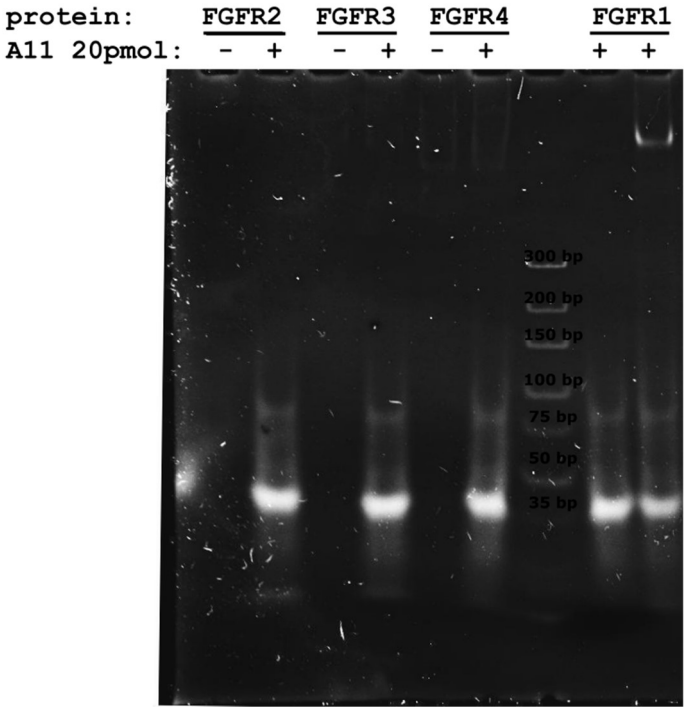
Supplementary Figure 2

Secondary structure of aptamers. Predicted 2D structures of A11 consensus aptamer and its mutant A11-G18T aptamer as proposed by NUPACK software. Bases' colors denote nucleobase identity. Green circle shows mutated nucleotide in A11-G18T mutant.



Supplementary Figure 3

Aptamer A11 specificity. EMSA experiment showed no binding of A11 aptamer to Fc fragment or BSA, and specific binding to the target FGFR1-Fc construct in the presence of aforementioned proteins.



Supplementary Figure 4

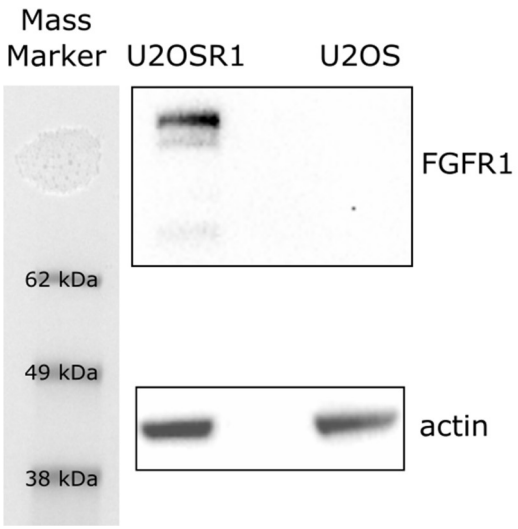
Aptamer A11 specificity towards other FGF receptors. EMSA experiment showed no observed binding of A11 aptamer to FGF receptors 2-4 (2 µg protein per lane).

Western blot analysis of FGFR1 expression level in cell lines

1×10^6 cells were plated on a 60 mm dish and cultured overnight. Next the medium was drained, cells were rinsed with PBS, and 1 ml of lysis buffer was added. The cells were scratched with a cell scraper, transferred to a tube and incubated for 10 min with occasional vigorous vortexing.

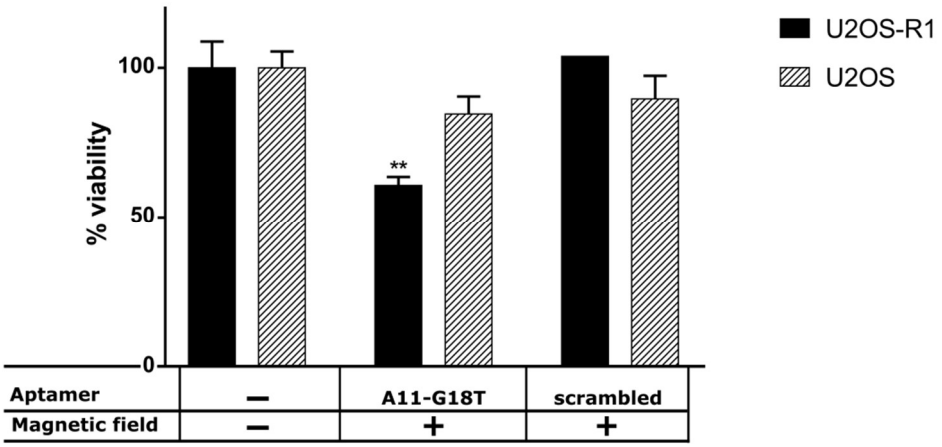
The lysates were run on a 4-12% SDS–PAGE gel and electrotransferred onto a PVDF membrane. The membrane was incubated with primary anti-FGFR1 antibodies for 1 h. Next the membrane was washed, incubated with secondary HRP-conjugated antibodies for 45 min, and washed again. Finally chemiluminescence staining was performed.

The protocol described above was performed again on the same membrane after stripping, but with primary anti-actin antibodies for the loading control. Membrane images were merged (visible light image for molecular weight marker and both chemiluminescence images) into one image which is presented in Supplementary Figure 5.



Supplementary Figure 5

Western blot experiment confirming overexpression of FGFR1 in U2OS-R1 cell line.



Supplementary Figure 6

Control experiments for targeted hyperthermia study on U2OS and U2OS-R1 cells. Non-treated cells were used as 100% viability control for each line – first pair of columns. Samples: Second pair of columns (+A11-G18T, +MF) shows aptamer-targeted nanoparticle-induced hyperthermia effect. Third (last) pair of columns (+scrambled, +MF) represents a negative control wherein cells were subjected to scrambled (non-specific, arbitrary sequence of 66 nt and the same primer regions as A11) ssDNA-nanoparticle conjugates and the magnetic field. Statistics: ** – denotes statistically significant difference between the sample and the appropriate non-treated control at multiplicity adjusted P value < 0.005. The experiments were performed in triplicate (except +scrambled+MF in U2OS-R1 line).