Supporting Information

HER2 protein induced dissociation of carbon nanotube-wrapped anti-HER2 ssDNA aptamers as in vitro targeted aptamer delivery system for cancer detection

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2. Experimental section

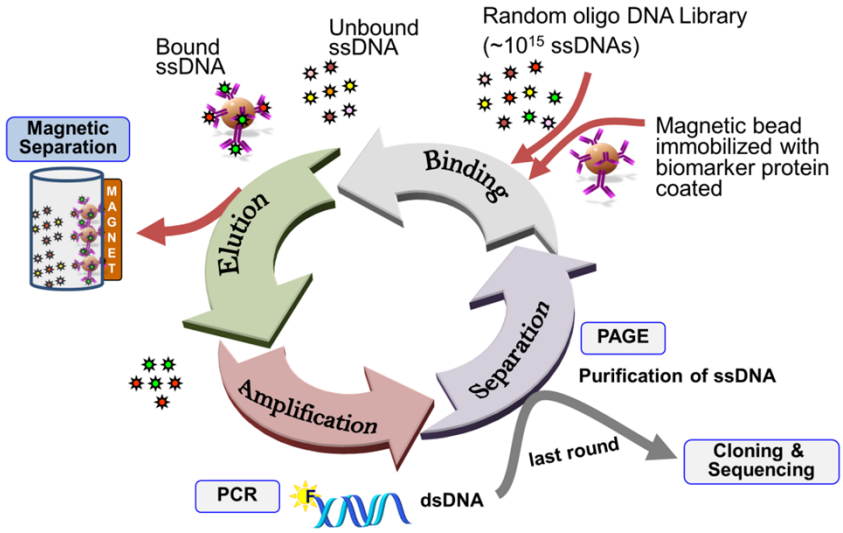

Figure S1. Steps involved in the selection of aptamers by serial evolution of ligands by exponential enrichment (SELEX) method.

2.1. Cloning of high affinity aptamers followed by sequencing and structural analysis

TOPO TA Cloning kit (invitrogen) was used for all the cloning experiments. Prior to cloning steps, the ssDNA pool obtained after the final SELEX round against HER2 target was further amplified by the same but unlabeled

primers. The dsDNA pool was then cloned directly in pCR4-TOPO vector transformed into *E. coli* DH5α competent cells according to manufacturer's instructions. The transformed *E. coli* cells were spread on selective LB-agar plates containing 50 μg/mL of ampicillin (amp). The incubation was carried out for 10 h at 37 ^{o}C and screened for the positive clones.

2.2. Screening for positive clones

Each of the transformed bacterial colonies (clones) carrying aptamer candidates were picked and grown in LB-broth containing ampicillin for overnight. About 20 mL from each culture with an OD_{600}=~0.1 were used for plasmid extraction using a commercially available plasmid purification kit (MiniPrep kit, Qiagen). Cells from the LB broth were first harvested by centrifugation for 15 min at 4 ^{o}C. All the other steps for plasmid isolation and purification were followed as recommended by the manufacturer (Qiagen). The extracted plasmid DNA was dissolved in EB buffer and quantified by Nanodrop spectrophotometer at A_{260}.

Restriction digestion was carried out for confirmation of positive clones carrying aptamer inserts. The plasmid vector used in this study had restriction enzyme, *Eco*RI sites on either sides of the ligated aptamer sequence which enabled digestion. Restriction enzyme digestions of the isolated plasmids were carried out using *Eco*RI after incubating the reaction mixture at 37 ^{o}C for 1 h. The positive clones were confirmed by resolving the restriction enzyme digested products on agarose gel electrophoresis and confirmed positive clones based on sizes of digested fragments between 55~86 bp. Selected positive clones were sequenced and the sequences were tabulated.

2.4. Stability of MB-CNT-H2 hybrid structures

The MB-CNT-H2 hybrids were subjected to thermal and surfactant treatments for testing the ability of ssDNA to strongly and physically wrap on MB-CNTs. For this, 2×10^{8} MB-CNT-H2 hybrid structures were suspended in 100 μL of 1X BB and subjected to thermal treatment by incubating at 4, 25 and 94 °C for 1 h. The hybrid structures were also subjected to surfactant treatment by incubating them in 1X BB containing 0.1% sodium dodecyl sulfate (SDS) for 1 h. Unwrapping of labeled ssDNAs under the influence of thermal or surfactant treatment from MB-CNTs was measured after switching-on of fluorescence took place in the supernatant solution as compared with control (0.1% BSA in 1X BB) samples and calculated the amount of ssDNAs released.

3. Results and discussion

Table S1. List of aptamer variants evolved to specifically bind against HER2 protein after the SELEX process. Random regions in the anti-HER2 aptamer candidates are italicized and underlined. The random regions of the aptamers are also number subscripted for their positions flanked on either sides by common sequences. The ΔG values were calculated using m-fold program under optimized conditions such as ionic/salt concentrations and temperature as described in the experimental section.

Anti- HER2 aptamers	Anti- HER2 aptamer sequences	ΔG
H1	5′-GGGCCGTCGAACACGAGCATG***G$_1$G$_2$C$_3$G$_4$G$_5$G$_6$***CCTAGGATGACCTGAGTACTGTCC-3′	-7.23
H2	5′-GGGCCGTCGAACACGAGCATG***G$_1$T$_2$G$_3$C$_4$G$_5$T$_6$G$_7$G$_8$A$_9$***CCTAGGATGACCTGAGTACTGTCC-3′	-6.69
H3	5′-GGGCCGTCGAACACGAGCATG***G$_1$C$_2$G$_3$G$_4$G$_5$T$_6$***CCTAGGATGACCTGAGTACTGTCC-3′	-5.80
H4	5′-GGGCCGTCGAACACGAGCATG***G$_1$G$_2$T$_3$G$_4$C$_5$***CCTAGGATGACCTGAGTACTGTCC-3′	-5.47
H5	5′-GGGCCGTCGAACACGAGCATG***G$_1$G$_2$G$_3$G$_4$C$_5$***CCTAGGATGACCTGAGTACTGTCC-3′	-5.24
H6	5′-GGGCCGTCGAACACGAGCATG***G$_1$A$_2$T$_3$A$_4$C$_5$***CCTAGGATGACCTGAGTACTGTCC-3′	-4.56
H7	5′-GGGCCGTCGAACACGAGCATG***G$_1$G$_2$T$_3$G$_4$T$_5$G$_6$A$_7$C$_8$A$_9$***CCTAGGATGACCTGAGTACTGTCC-3′	-4.02

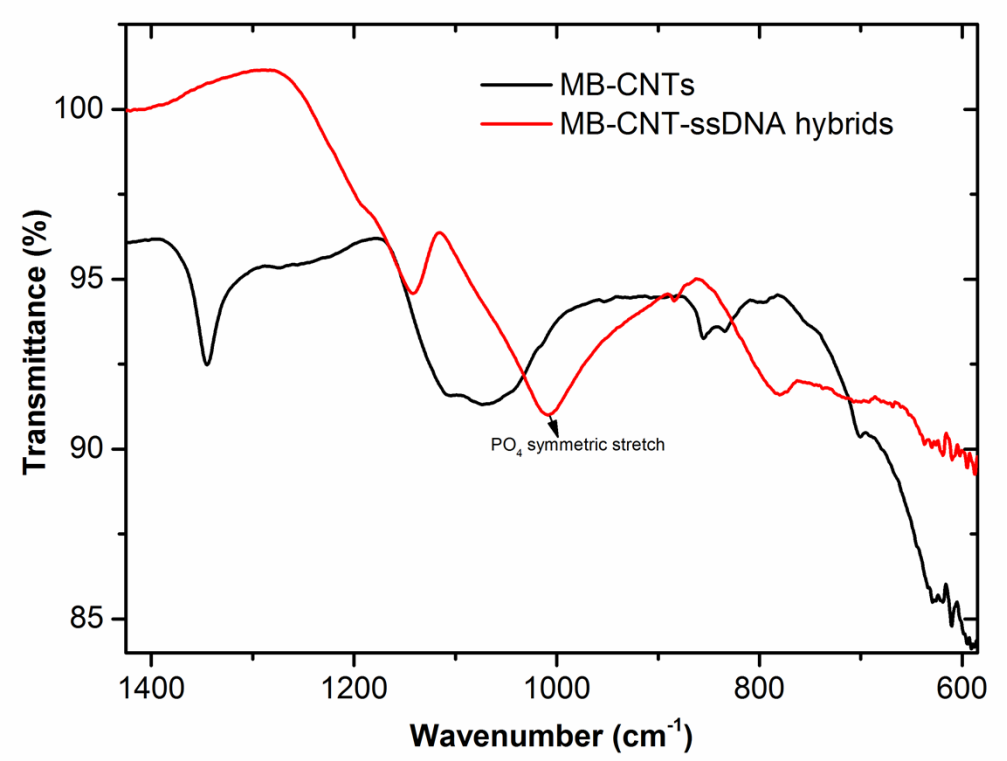

Figure S2. FTIR spectra of MB-CNTs recorded before and after physical wrapping of ssDNAs around CNT structures. The spectra showing the presence of PO_4 symmetric stretch confirming the presence of ssDNA on CNTs.