Supporting information for

PEI-assisted boronate affinity magnetic nanoparticle-based SELEX for efficient in Vitro Evolution of saponin-binding aptamers

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PCR Amplification and ssDNA Formation. Eluates that contained the saponin-aptamer candidate complexes were amplified by asymmetry PCR. Asymmetry PCR reagents which contain 0.2 mM dNTP, 0.025 U/μL TaKaRa enzyme, 0.01 μM former primer, 0.1 nM reverse primer, and 10X PCR buffer were added to 1 μL of eluates to get 50 μL total PCR solution. Amplification conditions were: 5 min at 95°C; 25 cycle of 30 s at 95°C, 30 s at 60°C , 30 s at 72°C; 5 min at 72°C. For the dissociation constant measurement and affinity evaluation, the ssDNA under investigation was labeled with FAM during PCR amplification. The PCR amplification conditions were identical as described above except that former primer was replaced by FAM labeled former primer. Amplified dsDNA was converted to ssDNA by heating them at 94 °C for 10 min and quickly cooled to -20 °C.

Cloning and Sequencing. After the last round selection, the selected aptamers were amplified by symmetry PCR which contains 0.2 mM dNTP, 0.025 U/μL TaKaRa enzyme, 0.01 μM former primer, 0.1 μM reverse primer, and 10X PCR buffer, amplification conditions were: 5 min at 95°C; 25 cycle of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C; 5 min at 72°C and frozen into powder by freeze-drying. Then deionized water was added to the powder up to 10μL. The DNA pool was cloned and sequenced at Takara Biotechnology (Dalian, China). The secondary structures were predicted through a professional website (http://rna.urmc.rochester.edu/RNAstructureWeb/).

Methods for MS and HPLC. MALDI-TOF MS analyses were carried out on a 4800 plus MALDI TOF/TOF Analyzer (Applied Biosystems, Framingham, MA, USA) with a pulsed nitrogen laser operated at 337 nm. The laser energy was adjusted to slightly above the threshold to obtain good resolution and signal-to-noise ratio (S/N). All mass spectra reported were obtained in the positive ion mode. The instrument was operated in linear mode. A typical spectrum was obtained by averaging 3000 laser

shots from 30 positions within the sample well. The accelerating voltage was 20 kV. The whole process was controlled by the 4000 Series Explorer Software V3.7.0. Data were processed using Data Explorer Software Version 3.7 (Applied Biosystems, Framingham, MA, USA). The matrixes for MALDI-TOF MS were 20 mg/mL CHCA dissolved in 50% ACN containing 0.1% (v/v) TFA. Equivalent amounts (1 μL) of the sample and matrix were sequentially dropped onto the MALDI plate for MALDI-TOF MS analysis.

HPLC analysis was performed using the Waters HPLC system consisting of a 1525 binary solvent pump, a 2998 PDA detector and a 2707 autosampler. The chromatographic column (250 × 4.6 mm i.d., Unitary C18, 5 μm) was obtained from ACCHROM. The mobile phases used for ginsenosides were 1% H_3PO_4 (solution A) and ACN (solution B). The gradient was as follows: 0–30 min, 81% A; 30–35 min, from 81 to 76% A; 35–60 min, from 76 to 60% A; 60–70 min, 60% A. The detection wavelength was set at 203 nm. All mobile phases were filtered through a 0.45 μm fiber membrane and degassed in ultrasonic bath before use. The flow rate was 1.3 mL/min. The injection volume was 10 μL.

Supplementary Figures

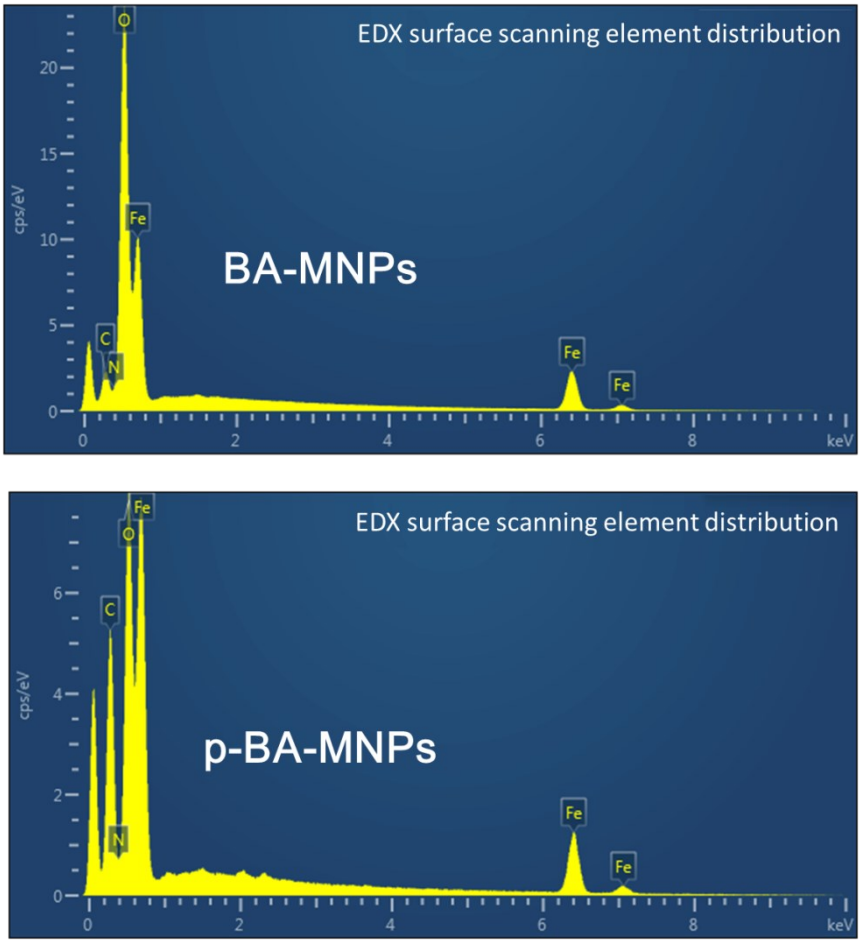

Fig. S1. Energy dispersive spectroscopy (EDS) of BA-MNPs and p-BA-MNPs.

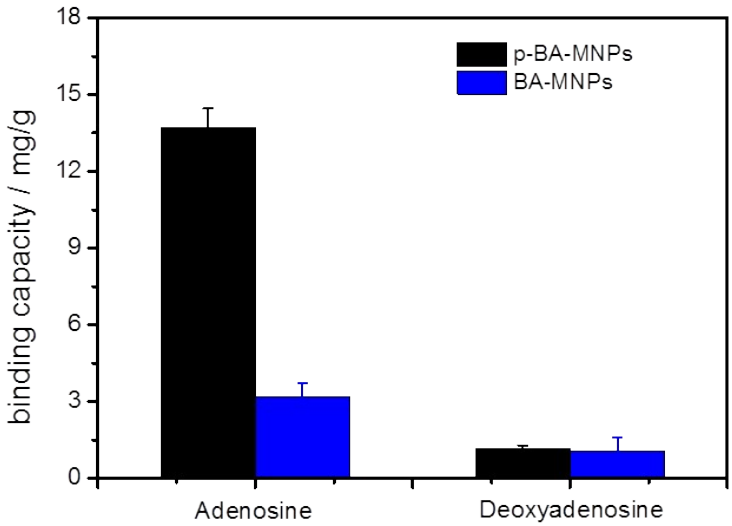

Fig. S2. Comparison of the binding capacity between BA-MNPs and p-BA-MNPs.

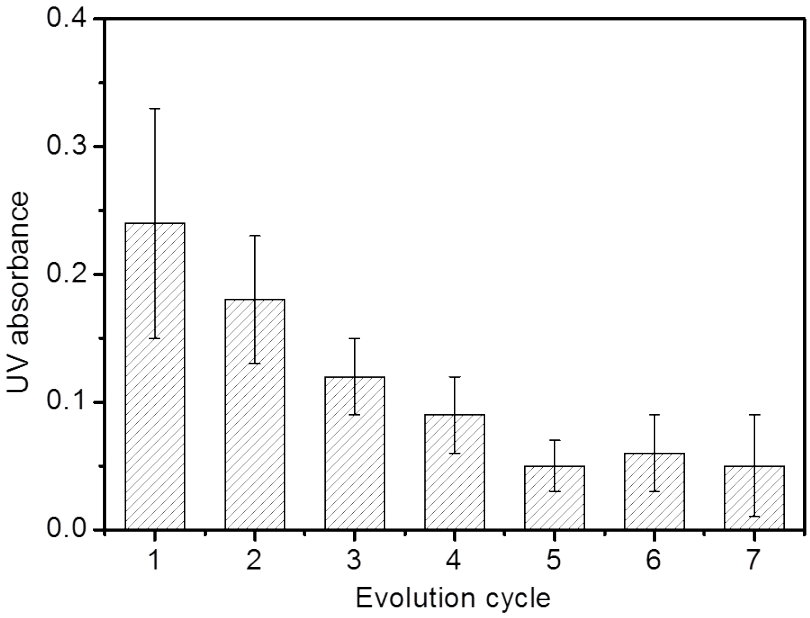

Fig. S3. UV absorbance of the washing buffer during different evolution cycle.

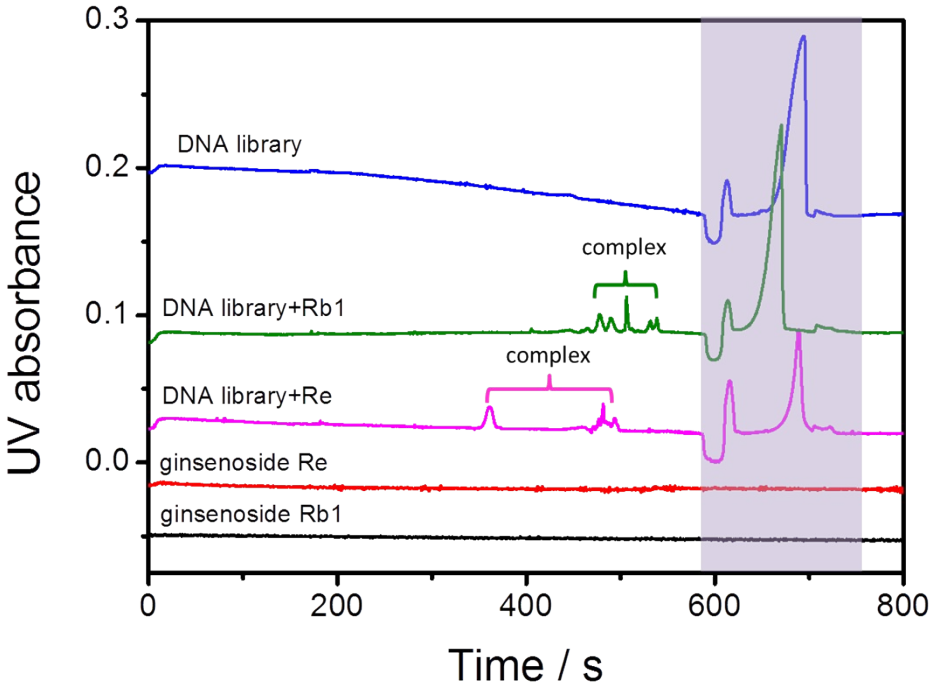

Fig. S4. Electrophoresis of different solution. The CE peaks for DNA library was highlighted with grey.

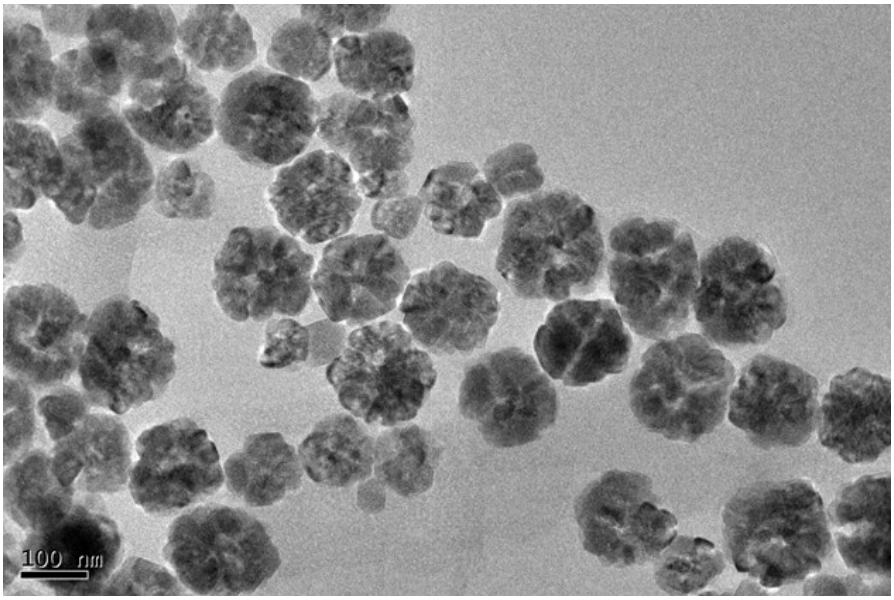

Fig. S5 TEM images of aptamer-functionalized MNPs (Apt Re-3@MNPs).

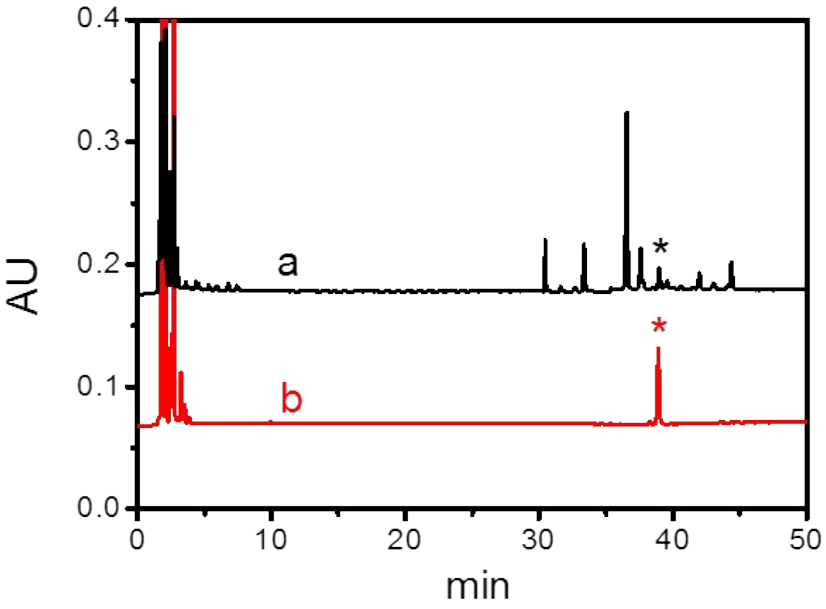

Fig. S6. Chromatographs of ginsenoside Re spiked (100 ng/mL) human serum, (a) direct analysis and (b) after enrichment with Apt Re-3@MNPs. * indicates ginsenoside Re.

Table S1. Elemental analysis of magnetic nanoparticles

Element (w/w %)	BA-MNPs	p-BA-MNPs
C	8.6%	7.66%
N	0%	3.84%
O	31.98%	31.5%
Fe	59.42%	57%

Table S2. Analytical performance of apt Re-3@MNPs

spiked level	Recovery*	RSD
10 ng/mL	75%	8.3%
100 ng/mL	71%	11.9%
1 μg/mL	63%	14.1%

* Recovery=(Found-Background)/Added× 100%, n=6