Supporting Information

Streptavidin-triggered signal amplified fluorescence for analysis of DNA-protein interaction

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Experimental

Materials

Lyophilized streptavidin from *Steptomyces avidinii*, ribonuclease A, Tris powder and $MgCl_2$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaCl was purchased from Duchefa Biochemie (Haarlem, Netherland) and phosphate buffered saline (PBS, pH 7.4) tablets were purchased from Amresco (Solon, OH, USA). An Alexa Fluor 488 protein labelling kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Human angiogenin binding biotinylated ssDNA aptamer (5'-CGG ACG AAT GCT TTG ATG TTG TGC TGG ATC CAG CGT TCA TTC TCA-3'), Alexa Fluor 546 labelled ssDNA aptamer of same sequence, the unbiotinylated ssDNA aptamer of same sequence, and a random sequence of biotinylated ssDNA aptamer (5'-CTG GGC GGT AGA ACC ATA GTG ACC CAG CCG TCT AC-3') were synthesized and purchased from Integrated DNA Technologies (Coralville, IA, USA). Bovine serum albumin (BSA) was purchased from Roche (Basel, Switzerland).

Purification of recombinant human angiogenin (hAng)

Escherichia coli strain of BL21(DE3)pLysS, which carries the pET22b-hAng plasmid, was used to express hAng. The cells were cultured by an optical density of 1.0 at 600 nm and induction procedure was performed using 0.1 M isopropyl β-D-1-thiogalactopyranoside (IPTG) for 2 hours. After centrifugation, pellets were lysated using 20 mM Tris-HCl (pH 8.0), 10 mM ethylenediamintetraacetic acid (EDTA), 2.5

mM phenylmethylsulfonyl fluoride (PMSF). Isolated inclusion bodies were dissolved in 7 M guanidine hydrochloride (GdnHCl), 20 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 10 mM 1,4-dithiothreitol (DTT) and incubated for 16 hours at 4^{o}C. After incubation, refolding was performed by diluting with 100 mM Tris-HCl (pH 8.0), 0.5 M L-Arg, 0.6 mM glutathione disulfide (GSSG), and 3 mM GSH (glutathione) and it was incubatied for 24 hours at 4 ^{o}C. Recombinant proteins were concentrated by ultrafiltration and purified using a fast protein liquid chromatography (FPLC) system from GE Healthcare (Little Chalfont, United Kingdom).

Conjugation of fluorescent dye and proteins

Purified hAng was conjugated with Alexa Fluor 488 (AF488) dye according to the manufacturer's protocol. Briefly, hAng of 1 mg and AF488 dye of 1 vial were mixed with 0.1 M sodium bicarbonate and they were incubated for 2 hours at room temperature on a shaker. After reaction, the mixture was loaded on a PD-10 desalting column from GE Healthcare and eluted using PBS (pH 7.4) to allow for the removal of free AF488 dye. The absorbance of the AF488-hAng conjugate solution at 280 nm and 494 nm was measured using a NanoDrop 2000c spectrophotometer from Thermo Fisher Scientific and quantified by the molar extinction coefficient. Isolation of AF488-hAng was confirmed using sodium-dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). AF488 labelled RNase A was prepared by same procedures.

FP assay between hAng and hAng aptamer

Biotinylated hAng aptamer and streptavidin were mixed in a molar ratio of 1:1. The final concentration of AF488-hAng was fixed at 10 nM and the hAng aptamer-streptavidin complex was serially diluted from 2 μM. All samples were diluted using 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 0.2 mM $MgCl_2$. All FP measurements were performed at room temperature in the dark using Beacon 2000 Fluorescence Polarization System (Invitrogen, Carlsbad, USA). Reaction volume in all test tubes was 200 μL. To examine the efficiency of streptavidin as a signal amplifier, the same concentrations of hAng aptamer were mixed to 10 nM of AF488-hAng without streptavidin.

FP assay between hAng and random sequence aptamer

Biotinylated random sequence aptamer (negative control) and streptavidin were mixed in a molar ratio of 1:1. The final concentration of AF488-hAng was fixed at 10 nM and the random sequence aptamer-streptavidin complex was serially diluted from 2 μM. All samples were diluted using 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 0.2 mM $MgCl_2$. And FP measurements were performed on a same procedure described above.

FP assay between RNase A and hAng aptamer

Biotinylated hAng aptamer and streptavidin were mixed in a molar ratio of 1:1. The final concentration of AF488-RNase A was fixed at 10 nM and the hAng aptamer-streptavidin complex was serially diluted from 2 μM. All samples were diluted using 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 0.2 mM $MgCl_2$. And FP measurements were performed on a same procedure described above.

Protein microarray analysis

The 50 μg/mL of each protein (hAng, RNase A, and BSA) was diluted with 30% glycerol in PBS (pH 7.4) and 1 μL of each protein was spotted onto the protein chip modified with calixarene derivative.[1,2] The chip was incubated in a humidity chamber at 4 °C for 16 hours and the chip was washed in 0.05% Tween-20 containing PBS (PBST) three times for 10 minute and incubated with 3% BSA containing PBST at room temperature for 1 hour for blocking nonspecific binding. After extensive washing, various concentrations (0, 0.98, 1.96, 3.91, 7.82, 15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000 nM) of AF546-hAng aptamer diluted in 30% glycerol containing 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 0.2 mM $MgCl_2$ were spotted onto immobilized each protein (hAng, RNase A, and BSA). The chip was incubated for the binding reaction in a humidity chamber at 30 °C for 1 hour. After rinsing with PBST and deionized water, the chip was scanned using a GenePix fluorescence scanner from Molecular Devices (Sunnyvale, CA, USA) and fluorescence intensities were analysed using a GenePix Pro 6.0 program.

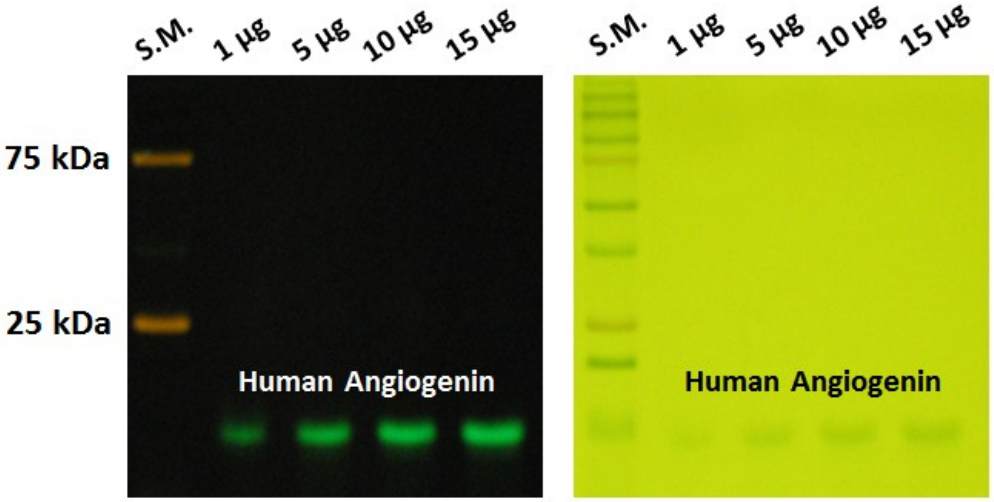

Fig. S1 SDS-PAGE images of AF488-hAng. The AF488-hAng was observed under UV light after electrophoresis on a 15 % polyacrylamide gel (left). After coomassie blue staining, hAng was observed under visible light (right). The location of both bands in an UV image and a visible light image agrees perfectly. Pre-stained Protein Standards from Bio-Rad (Hercules, CA, USA) was used as a protein size marker. (S.M. represents protein size marker.)

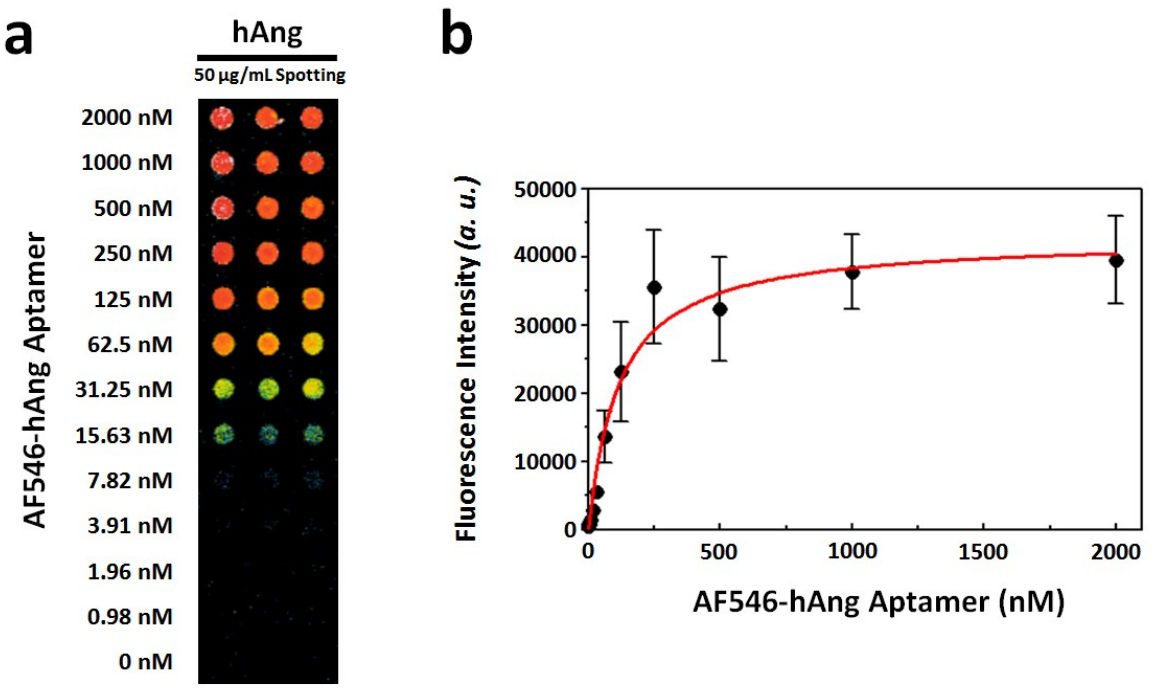

Fig. S2 Binding assay between hAng and AF546-hAng aptamer by protein microarray. (a) Fluorescence image of AF546-hAng aptamer bound to hAng. The protein microarray was excited at 532 nm and fluorescence intensity was measured at 575 nm. (b) Binding curve of AF546-hAng aptamer and hAng. Dissociation constant (K_D) was 127.6 ± 45.2 nM determined by extracted fluorescence intensities from (a).

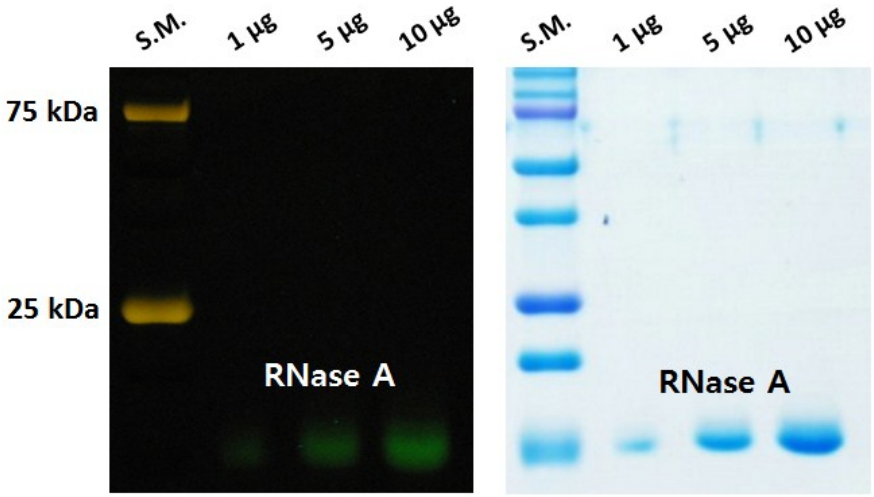

Fig. S3 SDS-PAGE images of AF488-RNase A. The AF488-RNase A was observed under UV light after electrophoresis on a 15 % polyacrylamide gel (left). After coomassie blue staining, RNase A was observed under visible light (right). The location of both bands in an UV image and a visible light image agrees perfectly. Pre-stained Protein Standards from Bio-Rad (Hercules, CA, USA) was used as a protein size marker. (S.M. represents protein size marker.)

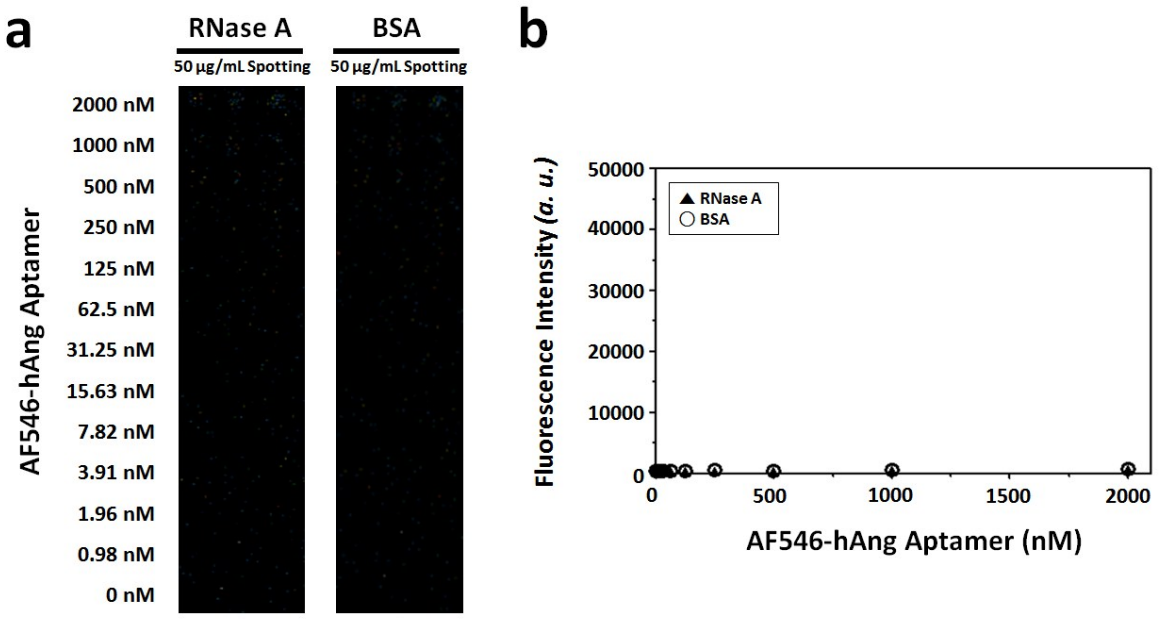

Fig. S4 Binding assay between each protein (RNase A and BSA) and AF546-hAng aptamer by protein microarray. (a) Fluorescence image of AF546-hAng aptamer bound to RNase A and BSA. The protein microarray was excited at 532 nm and fluorescence intensity was measured at 575 nm. (b) Binding curve of AF546-hAng aptamer and each protein (RNase A and BSA).

References

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2. E. Y. Kim, Y. J. Choi, C. W. Park and I. C. Kang, *Biochem. Biophys. Res. Commun.*, 2009, **389**, 415-419.