Electronic Supplementary Material (ESI) for ChemComm.
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Supporting information

Title: Enzyme linking to DNA aptamers via zinc-finger as a bridge

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Materials and methods

All oligonucleotides were purchased from Greiner Bio-One (Tokyo, Japan). Thrombin was purchased from Wako chemicals (Osaka, Japan). VEGF and biotinylated VEGF antibody (BAF293) were purchased from R&D Systems (Minneapolis, USA).

Expression and purification of ZF luciferase

GST-tagged ZF luciferase was expressed and purified as described previously [1]. Strep-tag-fused ZF luciferase was expressed and purified as described previously [2].

Thrombin detection with luciferase-labeled DNA aptamer on avidin-coated beads

For preparation of detection, designed DNA aptamers were heated at 95 °C for 10 min and gradually cooled to room temperature. In the first step of detection, 30 μL of NeutrAvidin beads (Thermo scientific, Massachusetts, USA) and biotin-modified TBA15 (50 pmol) were incubated together, and TBA15 was immobilized on NeutrAvidin beads in TBS buffer containing 90 μM $ZnCl_2$. After the beads were washed with washing buffer (TBS containing 0.05% Tween 20) three times, blocking was performed with blocking buffer (TBS containing 2% (w/v) skimmed milk, 1 mM D-biotin, 0.05% (v/v) Tween 20). Next, each concentration (f.c. 0–150 nM) of thrombin was incubated with the beads in the washing buffer.

In the second step of detection, TBA29-Sp1 (f.c. 500 nM) was incubated with Sp1-luciferase (f.c. 1.5 μM). The mixture of TBA29-Sp1 and Sp1-luciferase was added into the beads solution and incubated. The bound/free (B/F) separation was performed by washing three times with buffer (2% (w/v) skimmed milk, 1 mM D-biotin, 0.05% (v/v) Tween 20 in TBS containing 90 μM $ZnCl_2$ pH 7.3). One-third of NeutrAvidin beads were separated into 96-well plates, and 100 μL of the substrate solution of PicaGene kit (TOYO B-NET, Tokyo, Japan) was added into the solution containing the sandwich complex on the NeutrAvidin beads. The bioluminescence was detected with a plate reader (Wallac 1420

ARVO MX, Perkin Elmer, Massachusetts, USA). All incubation procedures were performed in 100 μL total volume at room temperature for 45 min.

VEGF detection with luciferase-labeled DNA aptamer on avidin-coated plates

BAF293 (100 μL, 0.2 μg/mL) was applied to a streptavidin-coated white plate (Thermo scientific, Massachusetts, USA) and incubated for 1 h to immobilize BAF293 on the plate. After blocking with 4% skimmed milk, 100 μL of each concentration of VEGF was applied to each well and incubated for 1 h. After three washes with TBST, 100 μL VEap165-Zif268 (300 nM) was added to each well. After three washes with TBST, Zif268 luciferase (100 nM) was added to the wells and incubated for 1 h. After five washes with TBST, 100 μL PicaGene was added and bioluminescence was measured with Wallac 1420 ARVO MX.

Dot blotting

VEGF165 (6 pmol) was spotted on a membrane, followed by blocking with 4% skimmed milk. The membranes were incubated with VEGF aptamer [3, 4] (100 nM) connected with a Sp1 stem-loop sequence containing a Sp1 recognition sequence. After washing, the membrane was incubated with Sp1 luciferase (300 nM). The bioluminescence on the spot was scanned with a Typhoon 8600 (GE healthcare, Buckinghamshire, UK) after the addition of a PicaGene kit (TOYO B-NET, Tokyo, Japan).

[1] K. Abe, T. Kumagai, C. Takahashi, A. Kezuka, Y. Murakami, Y. Osawa, H. Motoki, T. Matsuo, M. Horiuchi, K. Sode, S. Igimi and K. Ikebukuro, Anal Chem, 2012, 84, 8028–8032.

[2] W. Yoshida, A. Kezuka, K. Abe, H. Wakeda, K. Nakabayashi, K. Hata, K. Ikebukuro, Anal Chem, 2013, 85, 6485–6490.

[3] H. Hasegawa, K. Sode and K. Ikebukuro, Biotechnol Lett, 2008, 30, 829-834.

[4] Y. Nonaka, K. Sode and K. Ikebukuro, Molecules, 2010, 15, 215-225.

Table S1. Sequences of aptamers

Sequence	Sequence (5′–3′)
TBA15	TTTGGTTGGTGTGGTTGG
TBA29-Sp1	ATACCCCGCCCCTATAGTCCGTGGTAGGGCAGGTTGGGGTGAC
	TATAGGGGCGGGGTAT
TBA29-control	ATAATATATATATATAGTCCGTGGTAGGGCAGGTTGGGGTGAC
	TATATATATATATTAT
VEap165-Zif268	GATGCGTGGGCGCCAGGTACCAAAAGATGATCTTCCGCCCGT
	CCGAATGGTGGGTGTTCTGGCGCCCACGCATC
del 5-1 (Detect)	ATACCAGTCTATTCAATTGGGCCCGTCCGTATGGTGGGTGTGC
	TGGCCAGTATGGGGCGGGGACTTTGTCCCCGCCCCAT
del 4-4 (Detect)	ATACCCCGCCCCTATACTGTCGGGATGTGTGTGGGCCAGATAG
	TATAGGGGCGGGGTAT
Vap 7 (Detect)	TATGGGGCGGGGACTTTTTGTCCCCGCCCCATAGCACTCTGTG
	GGGGTGGACGGGCCGGGTAGA

*Zn-finger recognition sequences are underlined.

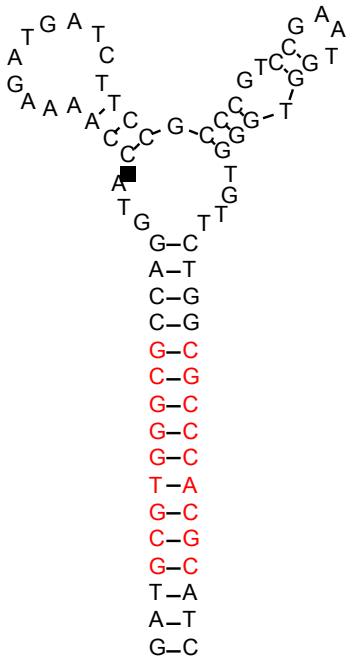
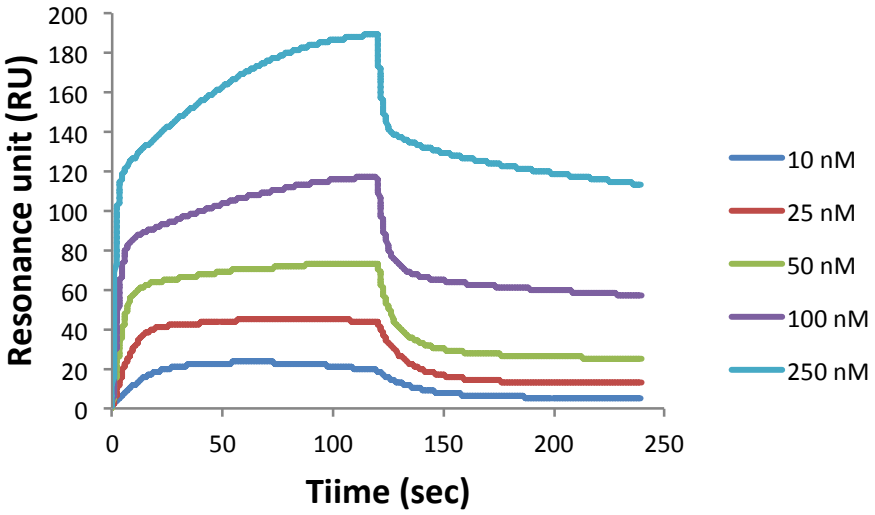


Fig. S1 $VEGF_{165}$ recognizing aptamer introduced Zif268 recognition sequence shown by red.

a)



b)

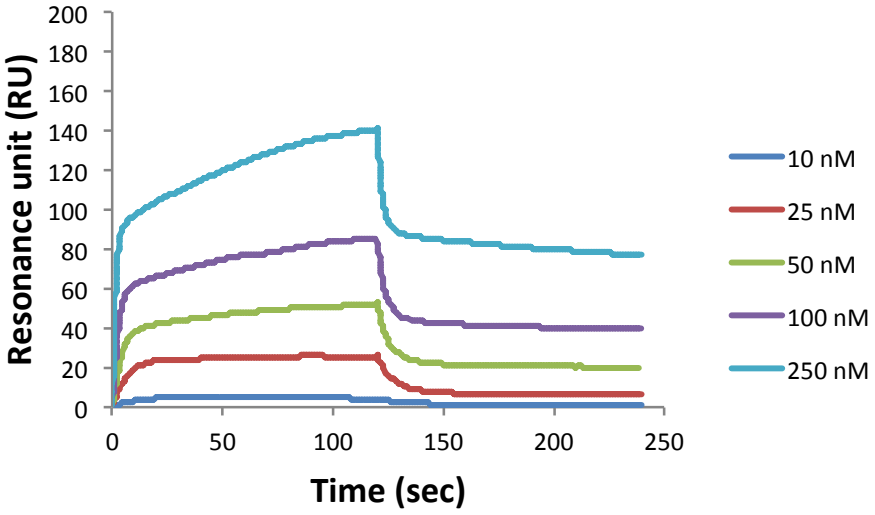


Fig. S2. SPR sensorgrams. VEGF was immobilized on CM5 sensorchips. Each concentration of (a) VEap165 (b) or VEap165-Zif268 was applied to the sensorchips.

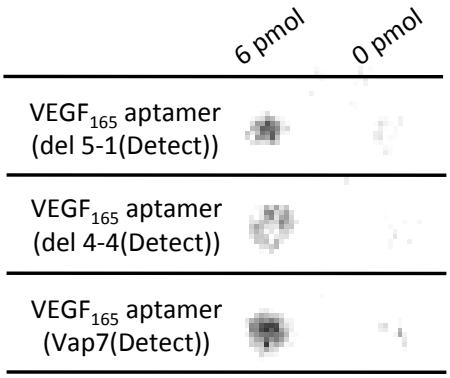


Fig. S3 Dot blot assay using various VEGF aptamers with different structures and Sp1-luciferase.