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Supporting Information

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Supporting Information

for

Development of a Fluorescent Peptide for the Detection of Vascular Endothelial Growth Factor (VEGF)

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Experimental Section

Reagents. All the chemicals used were of analytical reagent grade and were purchased from TCI (Tokyo, Japan), Wako (Osaka, Japan), Dojindo (Kumamoto, Japan), Pierce (USA), and Aldrich (USA). Recombinant human $VEGF_{121}$ was used in this study, which was purchased from Peprotech (UK).

Synthesis of fluorescent peptides. The synthesis of the fluorescent peptides designed by our group was performed by Scrum Inc. (Tokyo, Japan). The molecular weight and purity of the peptides were determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and high performance liquid chromatography (HPLC), respectively.

SPR measurements. The SPR measurements of the interaction between the peptide and VEGF were performed using the Biacore T100 System (GE Healthcare). The peptides were immobilized onto an Au plate that had SAMs on its surface. The SAMs were prepared from a mixture of 2 alkanethiolates as follows: The Au plate was impregnated with a mixture of 1.8 mM 11-mercaptoundecanol triethyleneglycol ether and 0.2 mM 20-(11-mercaptoundecanyloxy)-3,6,9,12,15,18-hexaoxaeicosanoic acid in ethanol (EtOH) for 1 h, followed by washing with EtOH. The Au plate containing the SAMs was dried for 1 h under an Ar stream. The peptides were dissolved in acetate buffer solution (pH 4.0) at a concentration of 100 μg/mL. The running buffer was PBS (10 mM; pH 7.4), and the flow rate was 5.0 μL/min. The carboxyl groups in the SAMs were activated by EDC and NHS, following which they were reacted with the

lysine group in the peptide. After the reaction, the residual activated carboxyl groups were blocked by the addition of an excess amount of ethanolamine (1.0 M; pH 8.5). VEGF was dissolved in PBS (10 mM; pH 7.4) at concentrations of 0–2.0 μg/mL. The running buffer was PBS (10 mM; pH 7.4), and its flow rate was 5.0 μL/min. The association constants between the peptide and VEGF were calculated by applying a nonlinear least-square curve-fitting method.

Miscellaneous. The absorption spectra were recorded at 25 ℃ with a Shimadzu UV-1650 PC UV/visible spectrophotometer. The fluorescence spectra were recorded at 25 ℃ with a JASCO FP-6500 spectrofluorophotometer.

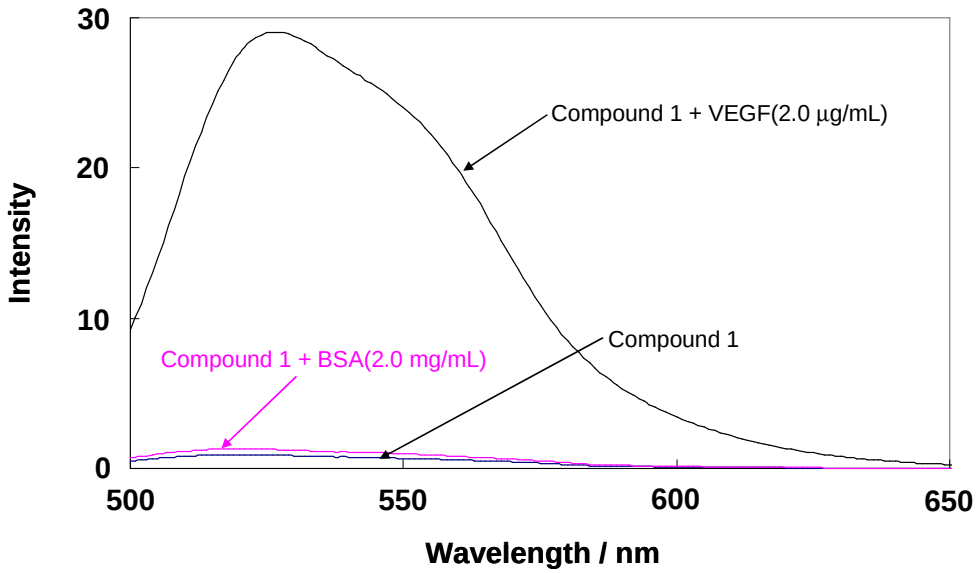

Figure S1. The fluorescence spectra of compound 1 in the absence and presence of 2.0 μg/mL VEGF and 2.0 mg/mL BSA. Compound 1 = 10.0 μM in PBS (10 mM; pH 7.4). λ_{ex} = 480 nm.

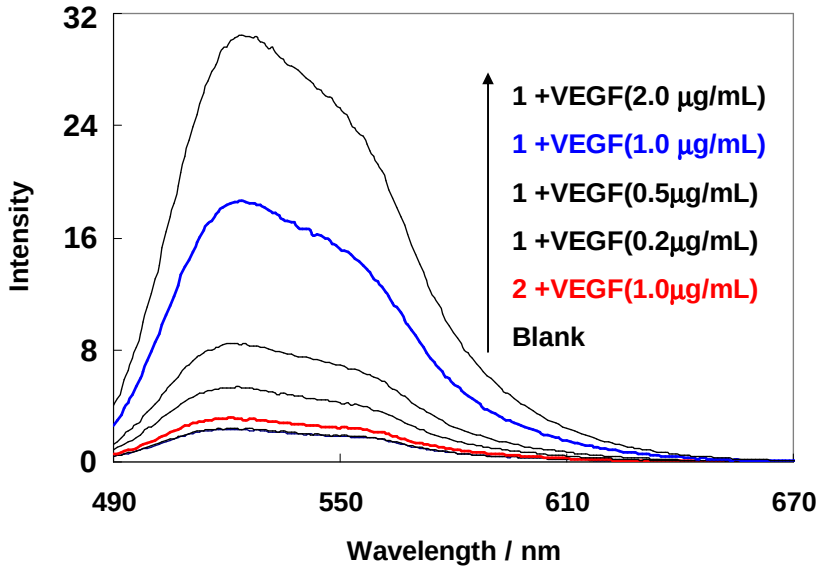

Figure S2. The fluorescence spectra of compound 1 (blue line) and compound 2 (red line) after the addition of 1.0 μg/mL VEGF. PBS, 10.0 mM (pH 7.4). λ_{ex} = 480 nm.

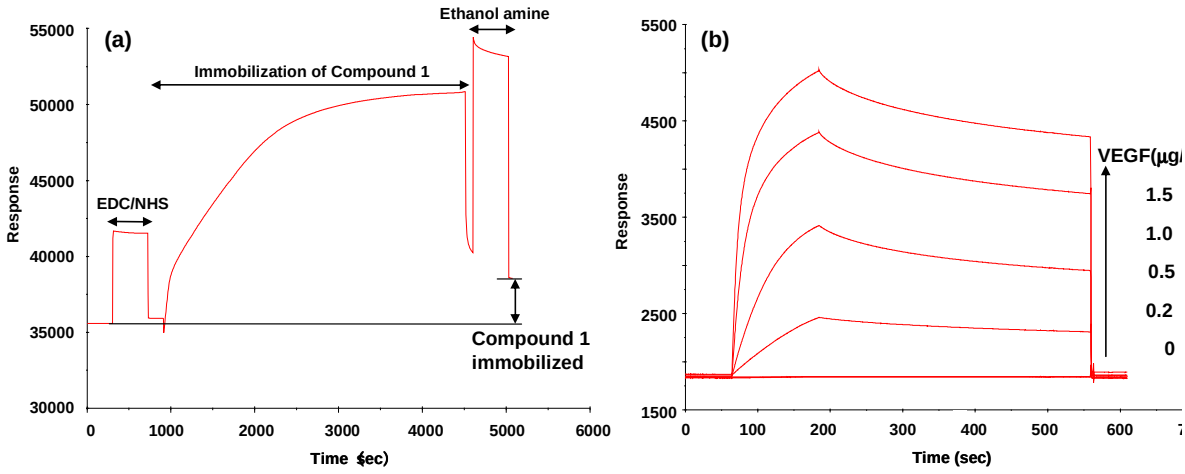

Figure S3. The typical SPR sensorgram of compound 1 immobilized to the mixed SAMs (a), and the SPR response of compound 1 before and after the addition of 0–2.0 μg/mL VEGF (b). The amount of compound 1 required for immobilization was 100 μg/mL in acetate buffer solution (pH 5.0; 10 mM). The running buffer was PBS, and the flow rate was 10 μL/min.

Table S1. Effect of Foreign Substances on the VEGF Assay

Substances	Concentration
Glycine	10 mM
NaCl	0.5 M
Ammonium Sulfate	0.2 M
Asparagine	5 mM
$NaHCO_3$	50 mM
Zinc Chloride	0.5 mM
Sodium Acetate	90 mM
Sodium Phosphate	0.5 M
Guanidine・HCl	0.35 M
Imidazole	4 mM
Calcium Chloride	10 mM
Triethanol Amine	20 mM
Sodium Citrate	50 mM
Urea	0.5 M
Sucrose	5%