Supplementary information

A molecular peptide beacon for IgG detection

M. Okochi[a,b*], T. Sugita[a], M. Tanaka[b], and H. Honda[a]

Experimental

Peptide array synthesis: A cellulose membrane (grade 542; Whatman, Maidstone, UK) was activated using β-alanine as the N-terminal basal spacer. Fmoc-11-aminoundecanoic acid (Watanabe Chemical Inc., Hiroshima, Japan) was conjugated as an additional spacer between the candidate peptides and the cellulose. Activated Fmoc amino acids at concentrations of 0.25 M were spotted onto the membrane using a peptide auto-spotter (ASP222; Intavis AG, Köln, Germany) according to the manufacturer's instructions with some modifications.[13] A carboxyl group of activated Fmoc-amino acid was combined with 11-aminoundecanoic acid on the cellulose membrane. After adding the first residue, the remaining amino groups were blocked using 5% acetic anhydride for 30 min. With each elongation step, the membrane was deprotected with 20% piperidine and subsequently washed thoroughly with N, N-dimethylformamide (DMF), followed by methanol. After the final deprotection, side-chain protecting groups were removed with a solution of m-cresol:thioanisole:ethanedithiol:trifluoroacetic acid (1:6:3:40) for 3 h. Finally, the membrane was thoroughly washed with diethyl ether, and methanol, and dried for the subsequent assay. Before experiment, the peptide array was immersed in PBS overnight.

Design of quenching peptides on peptide arrays: To design complementary quenching peptides, the quenching peptide DIAVEWES[10] was modified with tryptophan residues. The peptides were synthesized on the cellulose membrane using the peptide array. Each peptide spot was punched out and embedded into a 96-well plate. Peptide arrays were soaked in 1% Block Ace® (DS Pharma Biomedical Co., Ltd., Osaka, Japan) for 1 h at 37°C. After blocking, the arrays were washed three times with T-PBS (PBS containing 0.05% Tween 20) and were incubated with Atto655-labeled NKFRGKYK solution (1 μM in PBS) for 1 h at 37°C in the dark. Fluorescence spectra of the peptide spots were measured using a fluorescence plate reader (POWER SCAN 4, DS Pharma Biomedical Co. Ltd., Osaka, Japan) at 650 nm excitation wavelength.

Measurement of fluorescence spectra of the peptide beacon under various conditions: The peptide beacon (Atto655-labeled NKFRGKYKGGSGGSDIAVEWESGWWWW) was purchased from Sigma-Aldrich Japan. The stock peptide solution was dissolved at a concentration of 10 μM in PBS (pH 7.4) with 10% DMSO. To reduce intramolecular interactions between the IgG recognition sites (NKFRGKYK) and its complementary sites (DIAVEWES) in the peptide beacon, the peptide beacon was diluted 100-fold in in the solution of PBS with added HCl to adjust pH of the solution to 6.0, 4.0, and 2.0, and addition of Tween20 at 0.05%, 0.10 %, and 0.20% (final peptide beacon concentration was set to 0.10 μM). The fluorescence emission spectrum of the peptide beacon solution was recorded from 670 nm to 750 nm using a spectrofluorophotometer (RF-5300PC, Shimadzu Corporation, Kyoto, Japan) at 650 nm excitation wavelength.

IgG detection using the peptide beacon: IgG and BSA was obtained from Sigma-Aldrich Japan (Tokyo, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. In an IgG detection assay using a pH trigger, a 10 μM peptide beacon stock solution was diluted with 0.1% T-PBS adjusted to pH 2.0 by addition of 6 M HCl (final concentration was 150 nM). At this condition, buffer function of PBS is lost and the pH of the solution varied with its dilution ratio. IgG or bovine serum albumin (BSA) solution at concentrations ranging from 0–30 μM in PBS (pH7.4, 167 μl) was mixed with the 150 nM peptide beacon solution (334 μl) in a volume ratio of 1:2, and IgG and peptide beacon were diluted to achieve final concentrations of 0–10 μM and 100 nM, respectively, at pH 3.0. The solutions were incubated for 60 min at 37˚C in the dark. After incubation, 1.0 M Tris buffer (pH 9.0, 5 μL) was added to neutralize the mixture solutions to pH7.4, and fluorescence emission spectra of the solutions were measured from 670 nm to 750 nm using a spectrofluorophotometer at a 650 nm excitation wavelength.

Supplementary Figure S1

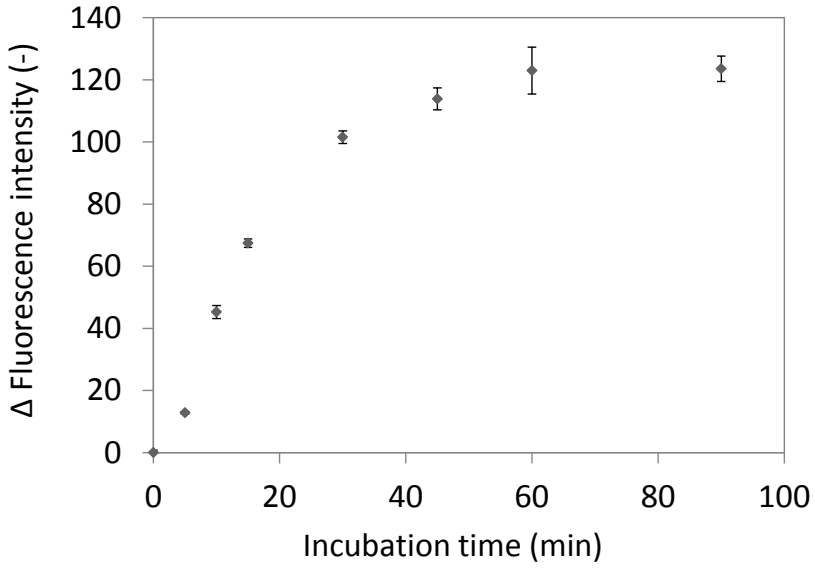

Fig. S1 Time course of IgG and peptide beacon interactions and relative fluorescence intensity at 684 nm. IgG solution (final concentration, 1.0 μM) was mixed with the peptide beacon solution (final concentration, 100 nM) at pH 3.0 for various incubation periods and then neutralized for fluorescence measurements.