Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

Peptide nucleic acids are an additional class of aptamers

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Reagents were obtained from commercial suppliers and were used without further purification. Double-distilled deionized water was used throughout the experiments. 15-mer peptide nucleic acid (PNA) of (*N*-terminal) Cys-Gly-GGTTG GTGTG GTTGG (*C*-terminal), 15-mer DNA of 5'-H_2N-$(CH_2)_6$-GGTTG GTGTG GTTGG-3', fluorescein amidite (FAM, emission maxima at 520 nm)-NHS ester, grapheme oxide (GO, ~60% 1-atomic layer), and thrombin were obtained from Panagene (Daejeon, Korea), Bioneer (Daejeon, Korea), Anaspec (Fremont, CA), Graphene Square (Seoul, Korea), and Enzyme Research Laboratories (Swansea, UK), respectively, and their concentrations were measured by optical absorbance, using extinction coefficients provided by the supplier. FAM-labeled nucleic acids were prepared under the conditions provided by the FAM-NHS supplier. UV absorbance was measured using Agilent 8453 UV-Visible spectrophotometer, which confirmed that 15-mer PNA and DNA used in this study had been equivalently labeled with FAM, respectively. Fluorescence was obtained at 520 nm with an excitation at 470 nm and spectra were recorded using BioTek Synegy Mx spectrofluorophotometer. All experiments were performed at least in triplicate.

In a typical fluorescence measurement, 3.3 μg/mL GO was added to 26.7 nM FAM-labeled nucleic acids in 10 mM PBS buffer (pH 8.3) with 130 mM NaCl, 2.56 mM KCl, and 9.5 mM $NaHCO_3$. After 15-min incubation, a different concentration of thrombin was added to the solution and incubated for 14 h before measurement.

Figure Legends:

Figure S1 (a) Fluorescence spectra from FAM-labeled PNA in the absence or presence of 13.3 μg/mL GO. When 26.7 nM FAM-labeled PNA is incubated with GO in 10 mM PBS buffer (pH 8.3) with 130 mM NaCl, 2.56 mM KCl, and 9.5 mM $NaHCO_3$, for 15 min, the fluorescence intensities are almost completely quenched and the reduced fluorescence is slightly recovered (less than 10%) even in the presence of 3.3 μM thrombin for 14 h or 24 h. (b) Fluorescence spectra from FAM-labeled DNA under the same conditions as in (a). While the fluorescence intensities are also quenched by over 95%, the reduced fluorescence is recovered in 14 h or 24 h in the same manner. These data together with the results of Fig. 1 indicate the strong affinity of PNA to GO. It should be noted, however, that to clearly differentiate the saturation curve for the interaction of FAM-labeled PNA with thrombin from that for the interaction of FAM-labeled DNA with thrombin, 3.3 μg/mL GO was used and GO-absorbed nucleic acids were incubated for 14 h in the presence of thrombin before fluorescence measurement, as previously described.

Figure S2 Bovine serum albumin (BSA) binding experiments of FAM-labeled 15-mer PNA. (a) Fluorescence spectra from FAM-labeled PNA under three different conditions. When 26.7 nM FAM-labeled PNA is incubated with GO in 10 mM PBS buffer (pH 8.3) with 130 mM NaCl, 2.56 mM KCl, and 9.5 mM $NaHCO_3$, for 15 min, the fluorescence intensities are significantly (approximately 50%) reduced and the reduced or quenched fluorescence cannot be recovered even in the presence of BSA. (b) Subtracted fluorescence intensities of FAM-labeled PNA both with GO and BSA from those of FAM-labeled PNA incubated with GO in the absence of BSA, as a function of BSA concentration. The FAM-labeled PNA produces no or almost ignorable FRET signal change in response to 60 nM to 270 nM BSA, demonstrating the FAM-labeled PNA is able to specifically bind to thrombin, not to BSA.

Figure S1

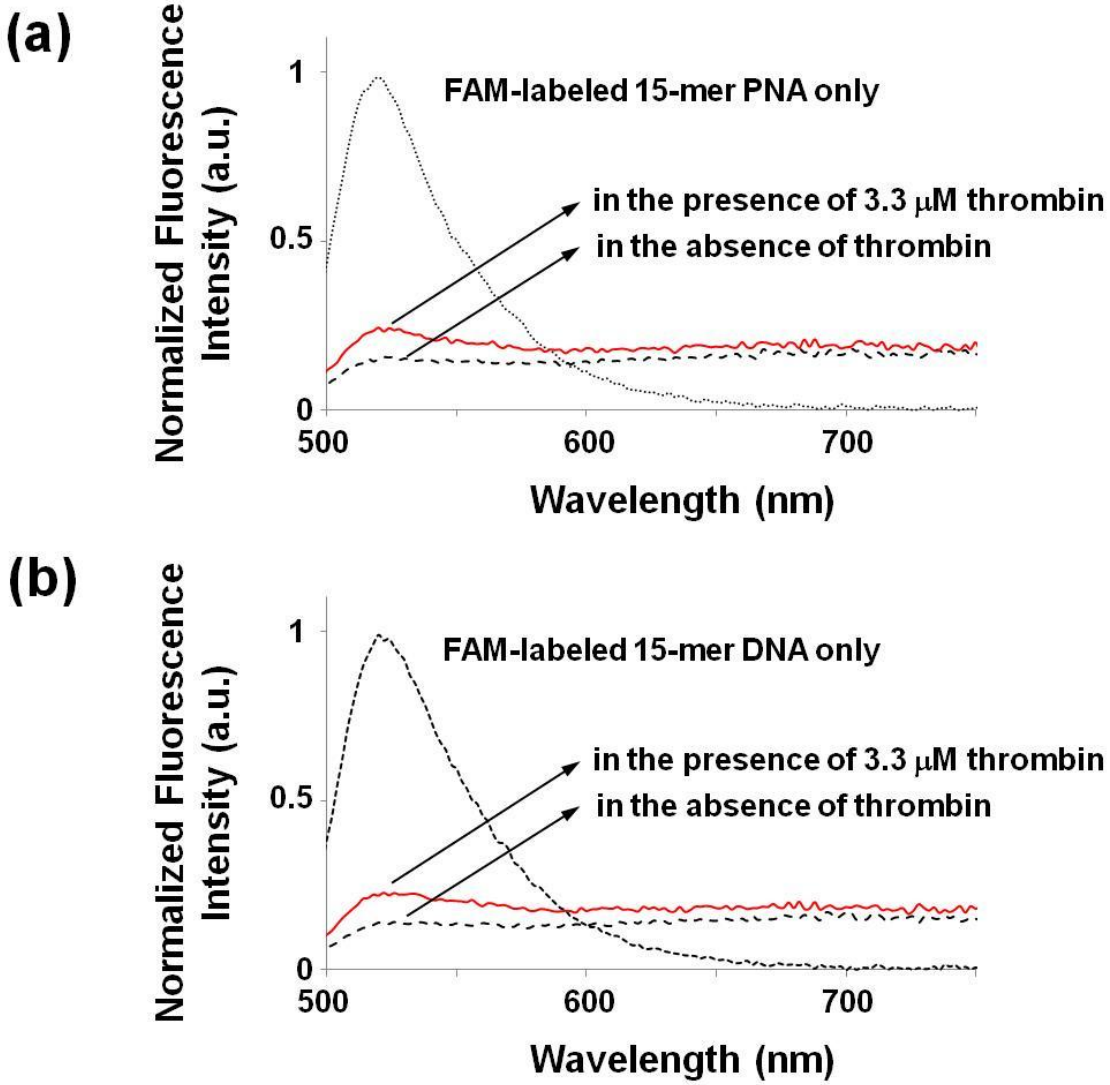

Figure S2