Supporting Information

FRET-based Self-assembled Nanoprobe for Rapid and Sensitive Detection of Postoperative pancreatic fistula

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Table S1. Amino acid sequence of the engineered protein

HSPG14C (147 amino acids, molecular weight = 16498 Da)

MFGRDPFDSLFERMFKEFFATPMTGTTMIQSSTGIQISGKCFMPISIIEGDQHIKVI
AWLPGVNKEDIILNAVGDTLEIRAKRSPLMITESERIIYSEIPEEEEIYRTIKLPATV
KEENASAKFENGVLSVILPKAESSIKKGINIE

S1 Experimental section

Fluorescence emission spectra (excitation: 490 nm) and stability analysis for the HSP FRET nanoprobe

(a) The emission spectrum of the HSP FRET nanoprobe before (black line) and after (red line) the subunit change under optimal conditions. Alexa Fluor 488 and Alexa Fluor 594 were conjugated to the inner cavity or outer surface of the HSPG41C, respectively. These dye-labeled HSPG41C were mixed at a 1:1 ratio, and the mixture was incubated for 1h at 60 ℃. After incubation, the FRET protein nanoprobe was cooled down and the fluorescence spectra were obtained on a FP-6600 fluorescence spectrophotometer (Jasco Co., Tokyo, Japan), using 490 nm excitation and 10 nm-wide excitation slits.

(b) To consider spectral bleed-through of the HSP FRET nanoprobe, we made three types of nanoprobe. The Alexa Fluor 488-labeled nanoprobe (donor only nanoprobe; Alexa Fluor 488-labeled HSPG41C : non-labeled HSPG41C = 1 : 1), the Alexa Fluor 594-labeled nanoprobe (acceptor only nanoprobe; non-labeled HSPG41C : Alexa Fluor 594-labeled HSPG41C = 1 : 1), and HSP FRET nanoprobe (Alexa Fluor 488-labeled HSPG41C : Alexa Fluor 594-labeled HSPG41C = 1 : 1) were mixed and the mixture was incubated for 1 h at 60 °C [protein concentration: 10 μM]. After incubation, the

FRET protein nanoprobe was cooled down and the fluorescence spectra were obtained on a FP-6600 fluorescence spectrophotometer (Jasco Co., Tokyo, Japan), using 490 nm excitation and 10 nm-wide excitation slits.

(c) The Alexa Fluor 488-labeled HSPG41C (donor nanoprobe) and the Alexa Fluor 594-labeled HSPG41C (acceptor nanoprobe) were mixed (donor : acceptor = 1 : 1) and the mixture was incubated for 1 h at 60 ℃ [protein concentration: 10 μM]. After incubation, the FRET protein nanoprobe was cooled down and incubated for 24 h or 48 h at 37 ℃. After incubation, the fluorescence spectra were obtained on a FP-6600 fluorescence spectrophotometer (Jasco Co., Tokyo, Japan), using 490 nm excitation and 10 nm-wide excitation slits.

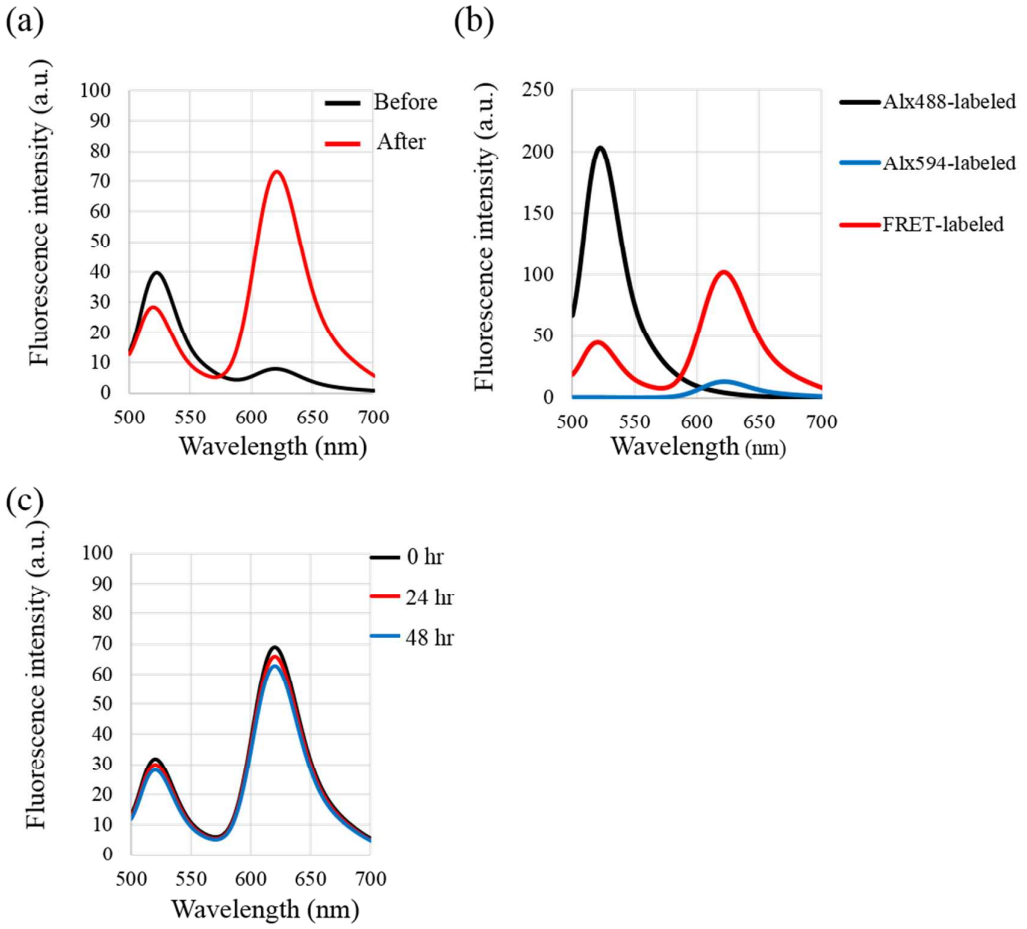

Figure S1. Fluorescence emission spectra (excitation: 490 nm) and stability analysis for the HSP FRET nanoprobe

(a) The emission spectrum of the HSP FRET nanoprobe before (black line) and after (red line) the subunit change under optimal conditions. (b) The emission spectrum of the Alexa Fluor 488-labeled nanoprobe (Alx488-labeled: black line), the Alexa Fluor 594-labeled nanoprobe (Alx594-labeled: blue line), and the HSP FRET nanoprobe (FRET-labeled: red line). (c) The emission spectra of the HSP-FRET nanoprobe after 24 hr (red line) and 48 hr (blue line).

S2 Experimental section

Figure S2. Structural analysis of the effect of protease treatment on the HSP-FRET nanoprobe

The HSP-FRET nanoprobes [10 μM] were added to pronase E [1 U/ml] and incubated for 1 h at 37 °C. (a) Fluorescence spectra of the HSP-FRET nanoprobe. After incubation, protease reactions were analyzed by the fluorescence spectra and time-course analysis. (b) SEC analysis of HSP-FRET nanoprobe. To confirm the structure of the nanoprobe, SEC analysis was performed using a TSK gel BioAssist G4SWXL column (TOSOH). The fluorescence (excitation: 590 nm, emission: 620 nm) wavelengths were monitored simultaneously. (c) Size distribution of the HSP nanoprobe. After incubation, size distributions were measured by dynamic light scattering using a Zetasizer Nano ZS Analyzer (Malvern Instruments Ltd, Malvern, UK) at a detection angle of 173° and temperature of 25 °C. A He-Ne laser (633 nm) was used as the incident beam. (d) TEM images of the HSP nanoprobe before and after pronase E treatment. Scale bars represent 50 nm.

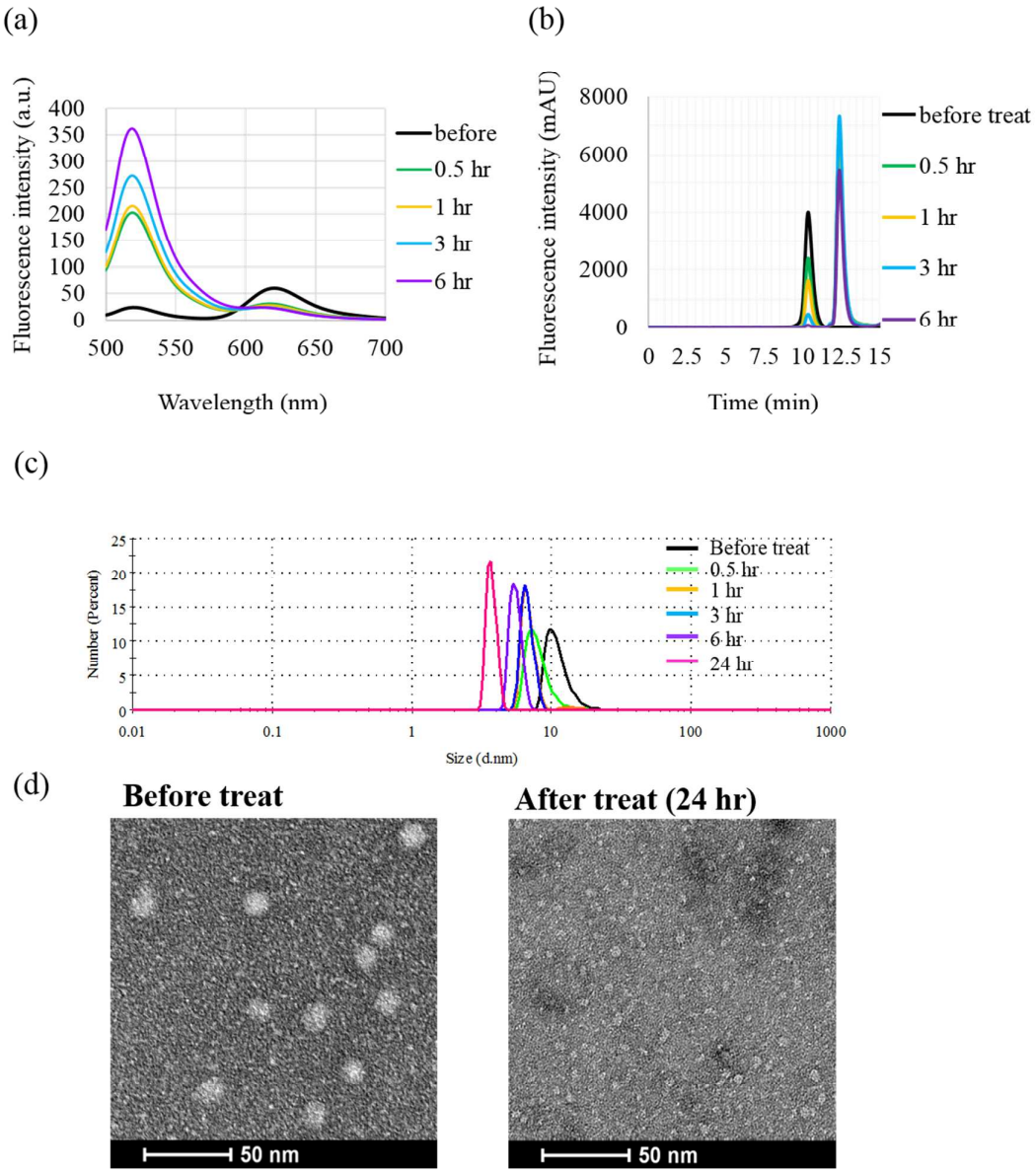

Figure S2. Structural analysis of the effect of protease treatment on the HSP-FRET nanoprobe

(a) Fluorescence emission spectra showing the effect of pronase E on the HSP- FRET nanoprobe. (b) SEC analysis of HSP-FRET nanoprobe after the treatment of pronase E. (c) Size distribution of the HSP nanoprobe. (d) TEM images of the HSP nanoprobe before and after pronase E treatment. Scale bars represent 50 nm.