Supporting Information

Energy Transfer-Based Multiplexed Assay of Proteases by Using Gold Nanoparticle and Quantum Dot Conjugates on a Surface

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Calculation of Förster distance, separation distance, and quenching efficiency for QD-dye and QD-AuNP pairs. To compare the quenching ability of various energy acceptors against QD, AuNP and Cy5 were tested by employing QDs as donors. As described elsewhere,[1] the Förster distance (R_0) values of QD-AuNP and QD-cy5 were estimated by using the following formula:

$$R_0 = \left(\frac{9000(\ln 10)\kappa^2 Q_D}{N_A 128\pi^5 n_D^4} J(\lambda) \right)^{1/6} = 9.79 \times 10^3 \left(\kappa^2 Q_D n_D^{-4} J(\lambda) \right)^{1/6} \quad \text{(Eq. 1)}$$

Where κ^2 is the relative orientation of the transition dipoles between the donor and acceptor, n_D the refractive index of the medium, N_A Avogadro number, Q_D the quantum yield of the donor, $J(\lambda)$ the overlap integral (cm^6 $mole^{-1}$) of the donor emission and acceptor absorption spectra. The overlap integral is given by;

$$J(\lambda) = \int PL_{D-corr}(\lambda) \times \lambda^4 \times \varepsilon_A(\lambda) d\lambda \quad \text{(Eq. 2)}$$

Where λ is wavelength, $PL_{D\text{-}corr}(\lambda)$ is normalized donor emission spectrum, and $\varepsilon_A(\lambda)$ is the acceptor absorption spectrum expressed as an extinction coefficient.

By using the above Förster formula (Eq. 1 and 2), the Förster radii of QD-AuNP and QD-Cy5 were calculated from the overlap integral (Figure S5) between the emission spectrum of the donor and the absorbance spectrum of the acceptor. We assumed that the quantum yield of SA-QD605 (Lot No. 47789A) was 0.55, and the refractive index of biomolecules was 1.4 in the aqueous solution. The orientation factor was assumed to be 2/3. The molar extinction coefficients of AuNP and Cy5 are 1.1×10^5 $cm^{-1}M^{-1}$ at 420 nm and 2.5×10^5 $cm^{-1}M^{-1}$ at 649 nm, respectively.

Energy transfer between QDs and acceptors (AuNP or Cy5) can be determined based on the quenching efficiency (Q_E) of the experimentally obtained PL data. Considering multiple acceptors per quantum dot, the overall efficiency can be written as:

$$Q_E = 1 - \frac{F_{DA}}{F_D} = \frac{nR_0{}^6}{nR_0{}^6 + r^6} \qquad \text{(Eq. 3)}$$

Where F_D is the fluorescence intensity of the donor alone, F_{DA} is the fluorescence intensity of the donor in the presence of the acceptor(s), r is a separation distance from the QD centre to the acceptors, and n is the number of surface-bound acceptors. From the experimentally determined quenching efficiency (Q_E), the above equation can be used to determine the D-A distance with calculated R_0:

$$r = \left(\frac{n(1-Q_E)}{Q_E} \right)^{1/6} R_0 \qquad \text{(Eq. 4)}$$

A Stern-Volmer analysis for energy transfer. The Stern-Volmer relationship[2] between the quencher concentration and fluorescence intensity is;

$$\frac{F_0}{F} = 1 + K_D(C_Q) \qquad \text{(Eq. 5)}$$

where F_0 and F are emission intensities in the absence and presence of quencher, respectively,

K_D the Stern-Volmer quenching constant, and C_Q the concentration of quencher.

Quantification of protease activity. Surface-immobilized nanoprobes were reacted with varying concentrations of a protease at 37 °C for 30–60 min. Protease activity were obtained by converting the recovered quenching efficiency to the concentration of cleaved peptides per min according to Eq. 6. Calibration curve for a protease was plotted as a function of protease concentration. Since the concentration of the nanoprobe was equivalent to respective protease reaction, different concentration ranges of protease were applied by taking into consideration the different specific activities (7500 Units per mg protein for MMP-7; 3280 Units per mg protein for caspase-3; 4000 Units per mg protein for thrombin). One Unit is defined as the amount of enzyme that cleaves 1 nmol of substrate per min.

$$\%\text{Recovery} = \frac{F_{DA+E} - F_{DA}}{F_{D+E} - F_{DA}} = \frac{K_{M'}[E]}{K_{M'} + [E]} \qquad \text{(Eq. 6)}$$

Where F_{D+E} is the PL intensity of the QD after enzyme reaction, F_{DA} and F_{DA+E} the PL intensities of the nanoprobes before and after enzyme reaction, respectively, $K_{M'}$ the surface Michalelis-Menten constant, and [E] enzyme concentration.

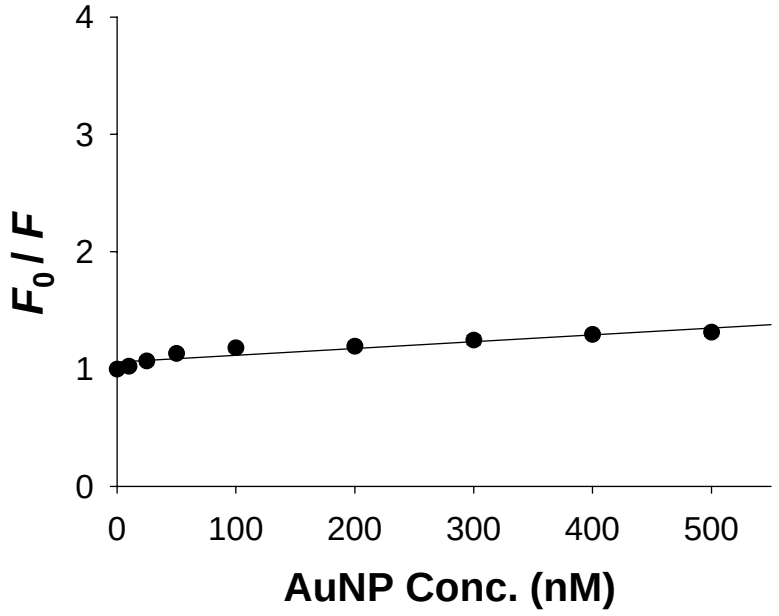

Figure S1. A Stern-Volmer analysis for energy transfer between QD and free AuNP. The inversed fluorescence intensities (F_0/F, in Eq. 5) of donor QD was plotted as a function of free AuNP concentration (QD=10 nM). Each data point indicates the average of duplicate experiments, and the error bar represents the standard deviation.

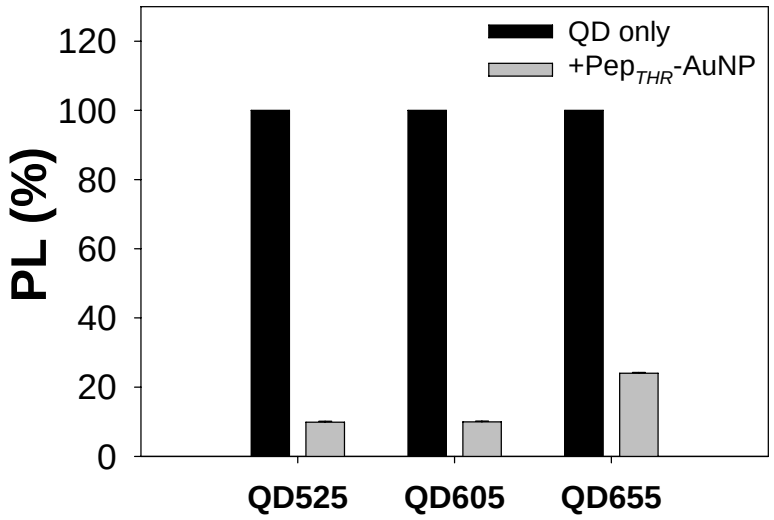

Figure S2. Changes in the PL intensities of QDs having different colors (SA-QD525, SA-QD605, and SA-QD655) in the presences of biotinylated Pep$_{THR}$-AuNPs in solution. The molar ratios of Pep-AuNPs to respective QDs were equally maintained at 50.

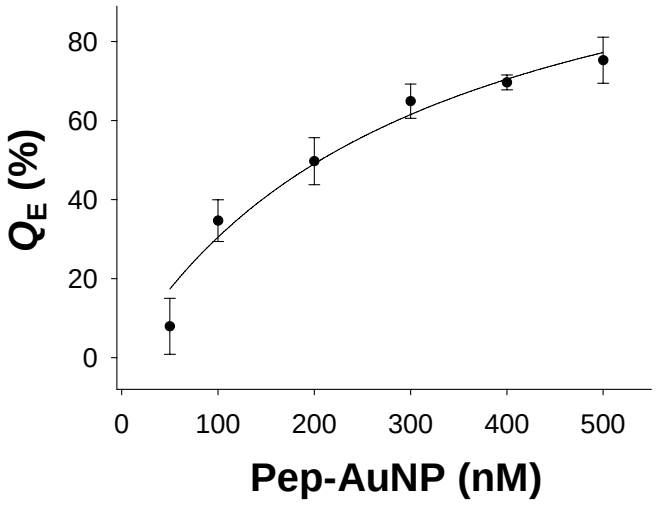

Figure S3. Standard curve for the Q_E of SA-QD605 as a function of the concentration of Pep-AuNP (AuNP-CRPLALWRSK-biotin). The concentration of QD was 10 nM. Each data point indicates the average of quadruple experiments, and the error bar represents the standard deviation.

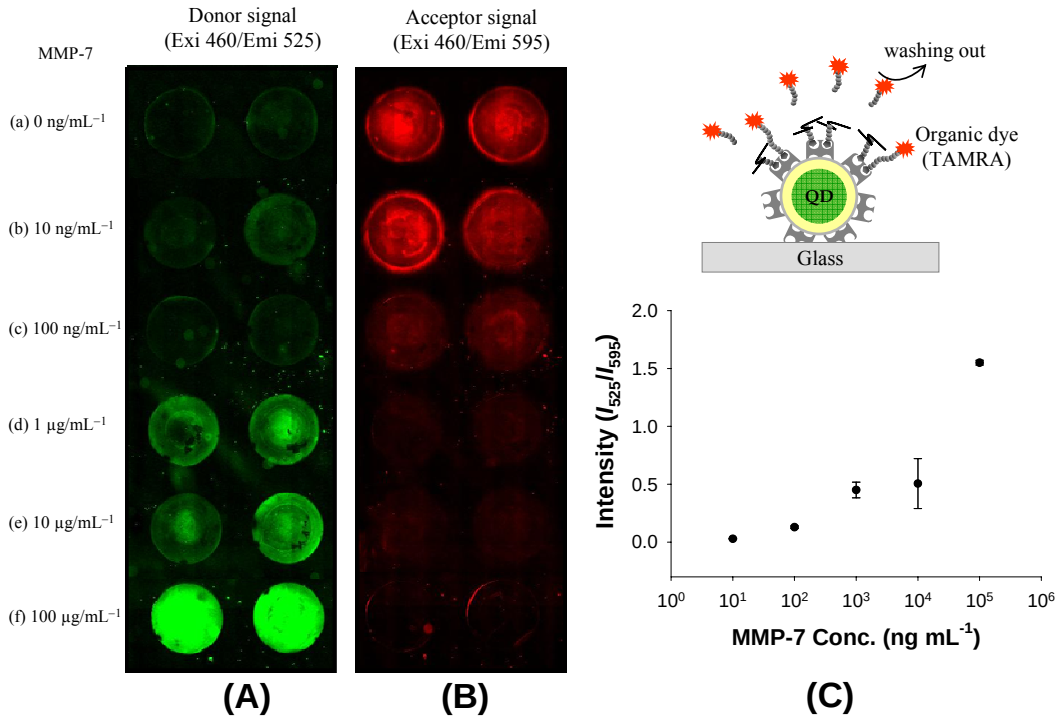

Figure S4. On-chip assay of protease activity based on the FRET between the donor SA-QD525 (green) and the acceptor TAMRA-labeled peptide (red). FRET intensity between the QD and TAMRA was plotted as a function of MMP-7 concentration, which ranged from 10 ng mL^{-1} to 100 µg mL^{-1}. The error bar indicates the standard deviation in duplicate spots (I_{525}: PL intensity of donor; I_{595}: PL intensity of acceptor).

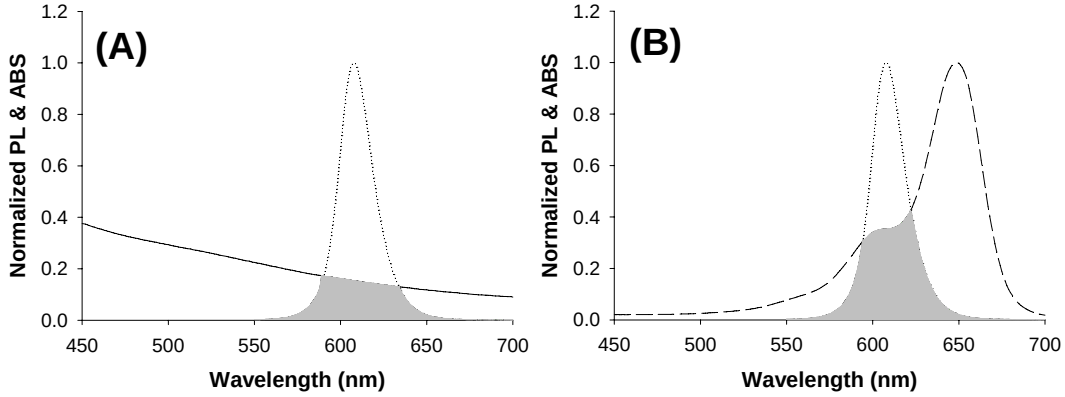

Figure S5. The overlap integral spectra between the emission of donor and the absorbance of acceptor. (A) Emission and absorbance spectra of QD605 (dotted line) and AuNPs (solid line), respectively. Fluorescence intensity of QD605 was normalized to the maximum extinction coefficient of AuNP (1.1×10^5 $cm^{-1}M^{-1}$ at 420 nm. (B) Emission and absorbance spectra of QD605 (dotted line) and Cy5 (dashed line), respectively. Fluorescence intensities of QD605 were normalized to the maximum extinction coefficient of Cy5 (2.5×10^5 $cm^{-1}M^{-1}$ at 649 nm).

References

(1) Förster, T. *Ann*. *Phys*. **1948**, *2*, 55-75.

(2) (a) Lakowicz, J. R. Principles of Fluorescence Spectroscopy; Plenum Press: New York, 1983. (b) Keizer, J. *J*. *Am*. *Chem*. *Soc*. **1983**, *105*, 1494–1498.