Electronic Supplementary Information

Multifunctional DNA nanostructure based on multicolor FRET for nuclease activity assay

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Calculation of Förster Distances.

For each donor-acceptor pair, R_0 is the Förster distance corresponding to E = 50% and is expressed as [1]

$$R_0 = 0.02108\,(k^2 \emptyset_D n^{-4} J)^{1/6} \text{ (nm)} \qquad (1)$$

Φ_D is the quantum yield of the donor (Table S1), n is the refractive index of the media (n = 1.4 for biomolecules in aqueous solution), κ^2 is the orientation factor for dipole coupling ($\kappa^2 = 2/3$ for randomly oriented dipoles).[2] J is the overlap integral of the emission spectrum of the donor and the absorption spectrum of the acceptor. The J defined in wavelength λ (nm) is calculated based on following equation[1]

$$J = \int \bar{I}_D \varepsilon_A \lambda^4 \mathrm{d}\lambda \qquad (2)$$

where $\bar{I}_D$ is the normalized emission intensity and ε_A (M^{-1} cm^{-1}) is the wavelength-dependent extinction coefficient. As shown in Figs. 1E and 1F, the spectra of DEA, FAM, Texas Red, and Cy5 were used to calculate the Förster distances for the donor-acceptor pairs described in this paper. The results for the spectral overlap integral (J) and Förster distances (R_0) are summarized in Table S2.

Table S1. Photophysical properties of the fluorophores.

fluorophore	quantum yield	ε ($M^{-1}cm^{-1}$)	$\lambda_{Abs,max}$ (nm)	$\lambda_{Em,max}$ (nm)
DEA	0.092	57 900	433	475
FAM	0.95	83 000	496	520
Texas Red	0.27	107 000	596	613
Cy5	0.28	250 000	647	670

Table S2. FRET properties of the fluorophores.

donor-acceptor pair	overlap integral, J [cm^3 / M]	Förster distance, R_0 [nm]
DEA-FAM	2.6×10^{15}	3.9
DEA-Texas Red	6.8×10^{14}	3.1
DEA-Cy5	3.6×10^{14}	2.8
FAM-Texas Red	3.5×10^{15}	6.1
FAM-Cy5	3.3×10^{15}	6.0
Texas Red-Cy5	2.5×10^{16}	6.8

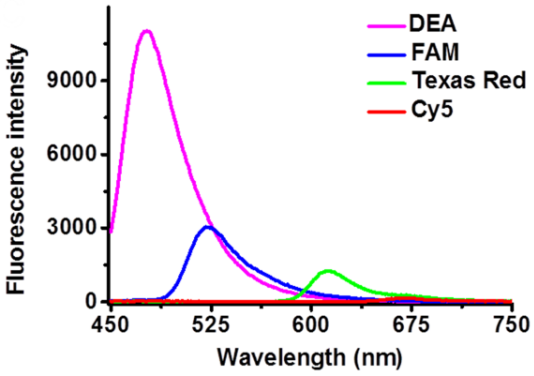

Fig. S1 Fluorescence emission spectra for each of the fluorophores assembled alone onto the DNA tetrahedral nanostructure at an excitation wavelength of 405 nm.

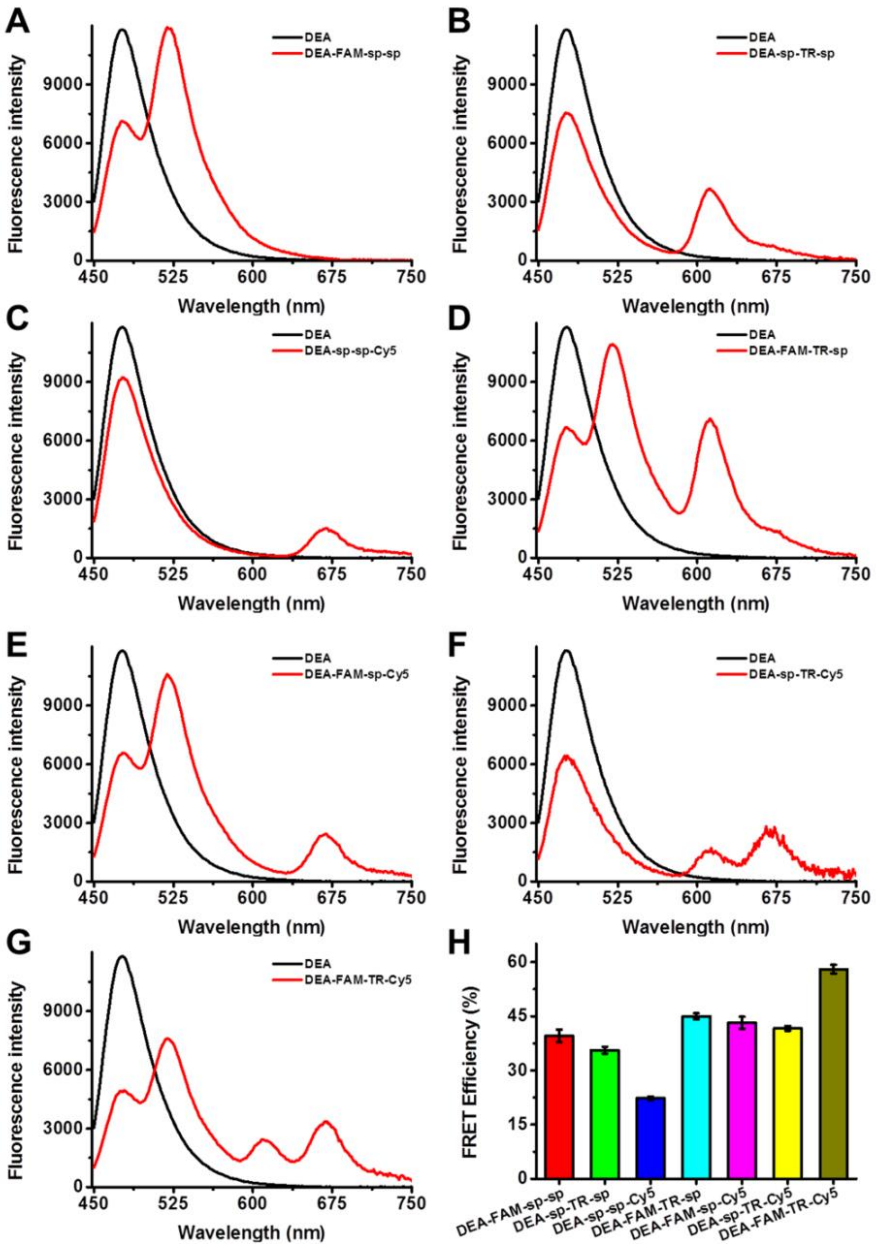

Fig. S2 (A-G) Fluorescence emission spectra of the assemblies of DEA with DNAs labeled with different dyes. The probes include (A) DEA-FAM-sp-sp, (B) DEA-sp-Texas Red-sp, (C) DEA-sp-sp-Cy5, (D) DEA-FAM-Texas Red-sp, (E) DEA-FAM-sp-Cy5, (F) DEA-sp-Texas Red-Cy5, (G) DEA-FAM-Texas Red-Cy5 DNA tetrahedral probes. The excitation wavelength is 405 nm. The concentration of each probe is 0.5 μM. (H) Comparison of FRET efficiency of the four-color fluorescent probe with those of other DNA tetrahedral probes (*i.e.*, the double/triple-fusion fluorophore FRET systems). Error bars show the standard deviation of three experiments.

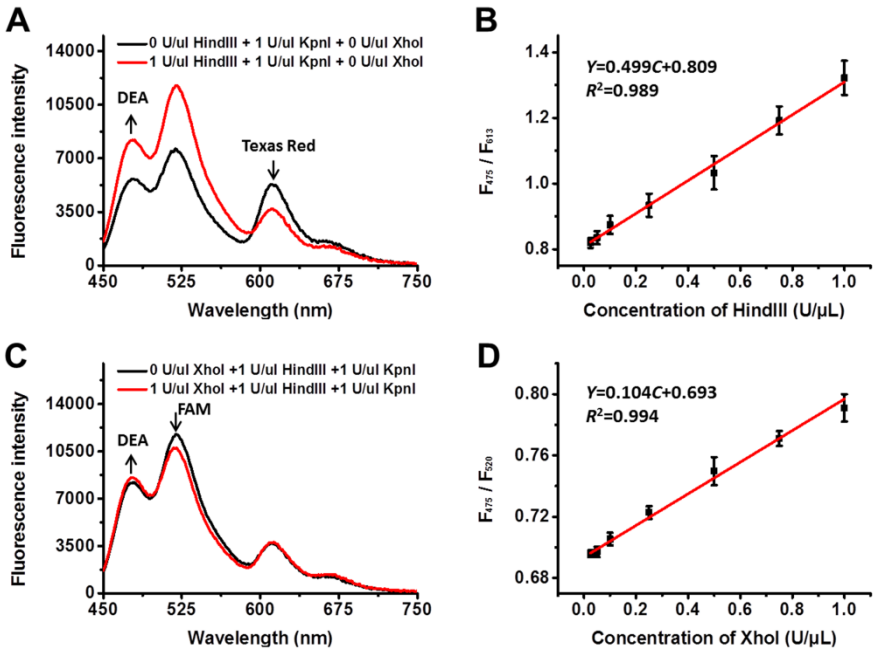

Fig. S3 (A) Fluorescence spectra of the four-color fluorescent probe before (black line) and after (red line) incubation with HindIII at a fixed concentration of KpnI (1 U/μL) and XhoI (0 U/μL). (B) Varience of the F_{475}/F_{613} value with different concentrations of HindIII. F_{475} and F_{613} are the emission intensity at 475 nm for DEA and 613 nm for Texas Red, respectively. The F_{475}/F_{613} value (Y) linearly enhances with the increasing concentration of HindIII (C). The linear regression equation is $Y = 0.499\ C + 0.809$ ($R^2 = 0.989$), and the detection limit is calculated to be 0.0172 U/μL. (C) Fluorescence spectra of the four-color fluorescent probe before (black line) and after (red line) incubation with XhoI at a fixed concentration of HindIII (1 U/μL) and KpnI (1 U/μL). (D) Varience of the F_{475}/F_{520} value with different concentrations of XhoI. F_{475} and F_{520} are the emission intensity at 475 nm for DEA and 520 nm for FAM, respectively. The F_{475}/F_{520} value (Y) linearly enhances with the increasing concentration of XhoI (C). The linear regression equation is $Y = 0.104\ C + 0.693$ ($R^2 = 0.994$), and the detection limit is calculated to be 0.0190 U/μL. Excitation wavelength is 405 nm. Error bars show the standard deviation of three experiments.

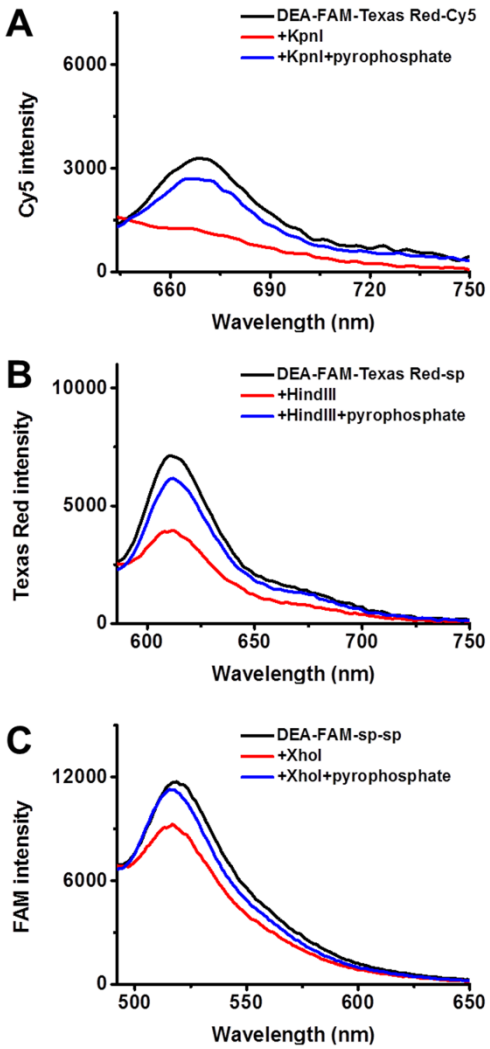

Fig. S4 (A) Measurement of Cy5 emission spectra of the DEA-FAM-Texas Red-Cy5 DNA tetrahedral probe in the presence of KpnI (red color) and KpnI + 40 mM pyrophosphate (blue color). The KpnI concentration is 1 U/μL. (B) Measurement of Texas Red emission spectra of the DEA-FAM-Texas Red-sp DNA tetrahedral probe in the presence of HindIII (red color) and HindIII + 40 mM pyrophosphate (blue color). The HindIII concentration is 1 U/μL. (C) Measurement of FAM emission spectra of the DEA-FAM-sp-sp DNA tetrahedral probe in the presence of XhoI (red color) and XhoI + 40 mM pyrophosphate (blue color). The XhoI concentration is 1 U/μL.

References

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2 K. Boeneman, D. E. Prasuhn, J. B. Blanco-Canosa, P. E. Dawson, J. S. Melinger, M. Ancona, M. H. Stewart, K. Susumu, A. Huston and I. L. Medintz, *J. Am. Chem. Soc.*, 2010, **132**, 18177-18190.