Fluorescence Aptasensor Based on DNA Charge Transport for Real-Time Protein Detection

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Experimental

Synthesis of NI-NHS： NI-NHS was synthesized according to the references.[1,2] The synthesis route is shown in Figure S1. Typically, 15.0mmol of naphthalene-1, 8-dicarboxylic anhydride and 15.0mmol of glycine were dissolved in 30ml DMF and stirred for 4.5hours at 100^{o}C. The mixture was cooled gradually to room temperature, diluted with 60ml ethyl acetate, and washed with water (2x20ml). The organic phase was dried with $MgSO_4$ and the solvent was removed by rotary evaporating under 55^{o}C. The crude product was recrystallized from ethyl acetate to give the desired carboxylic acid N-carboxymethyl-NI. ^{1}HNMR and ^{13}CNMR spectra of the product matched well with those in previous report.[3]

The prepared N-carboxymethyl-NI (10mmol) was suspended in acetonitrile (60ml). The solution was added with N-hydroxysuccinimide (10mmol) and EDC (1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide, 10mmol) and stirred for 3 hours at room temperature. The reaction mixture was evaporated, filtered, and washed with cold acetonitrile twice to get the white solid, NI-NHS. The product was confirmed by ^{1}H-NMR (Figure S2, δ 8.515(m, 4H), δ 7.892(t, 2H), δ 5.221(s, 2H), δ 2.816(s, 4H); and EI-MS, m/z 352 [$(M+H)^{+}$])

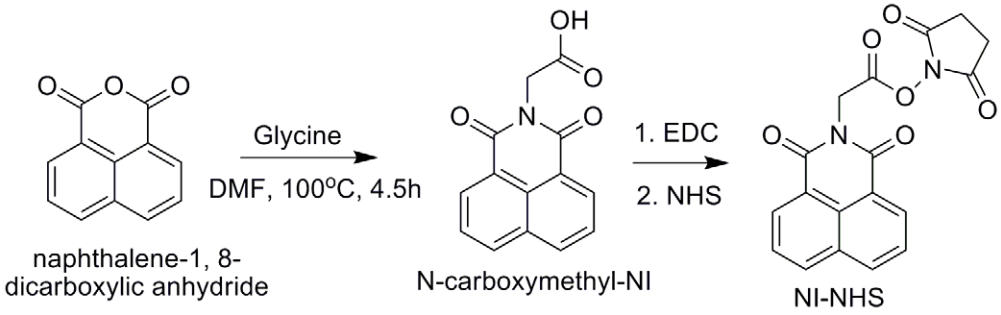

Figure S1: The synthesis route for NI-NHS.

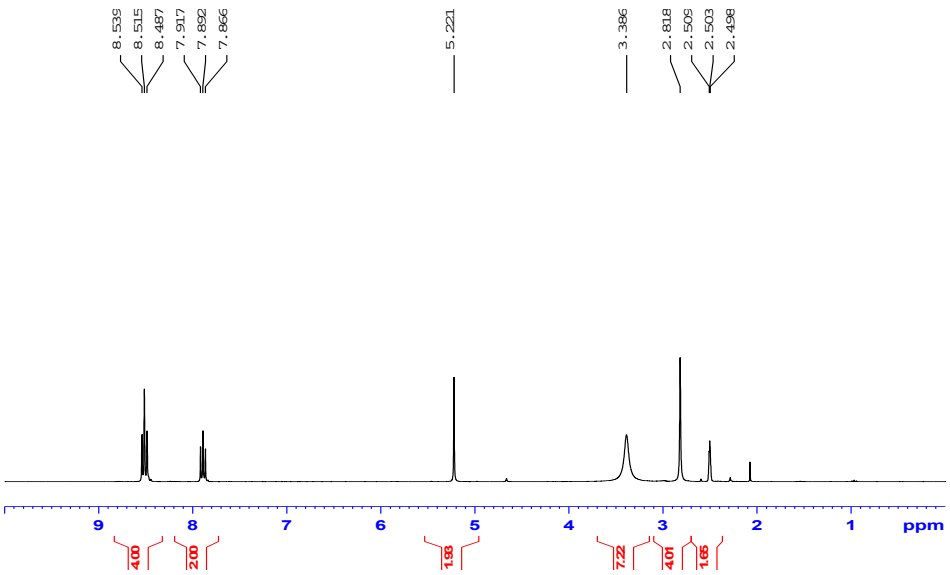

Figure S2. ^{1}HNMR of NI-NHS.

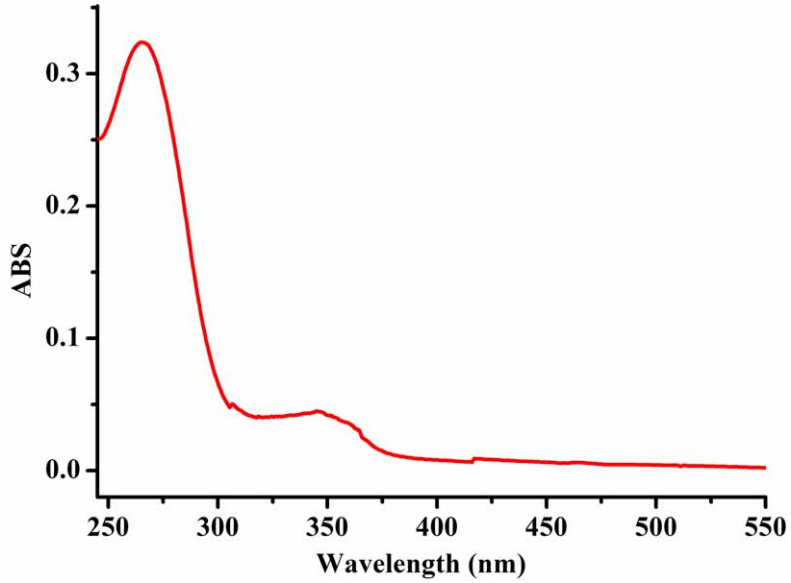

Figure S3. UV spectra of DNA-NI showing the absorption bands for both DNA and NI.

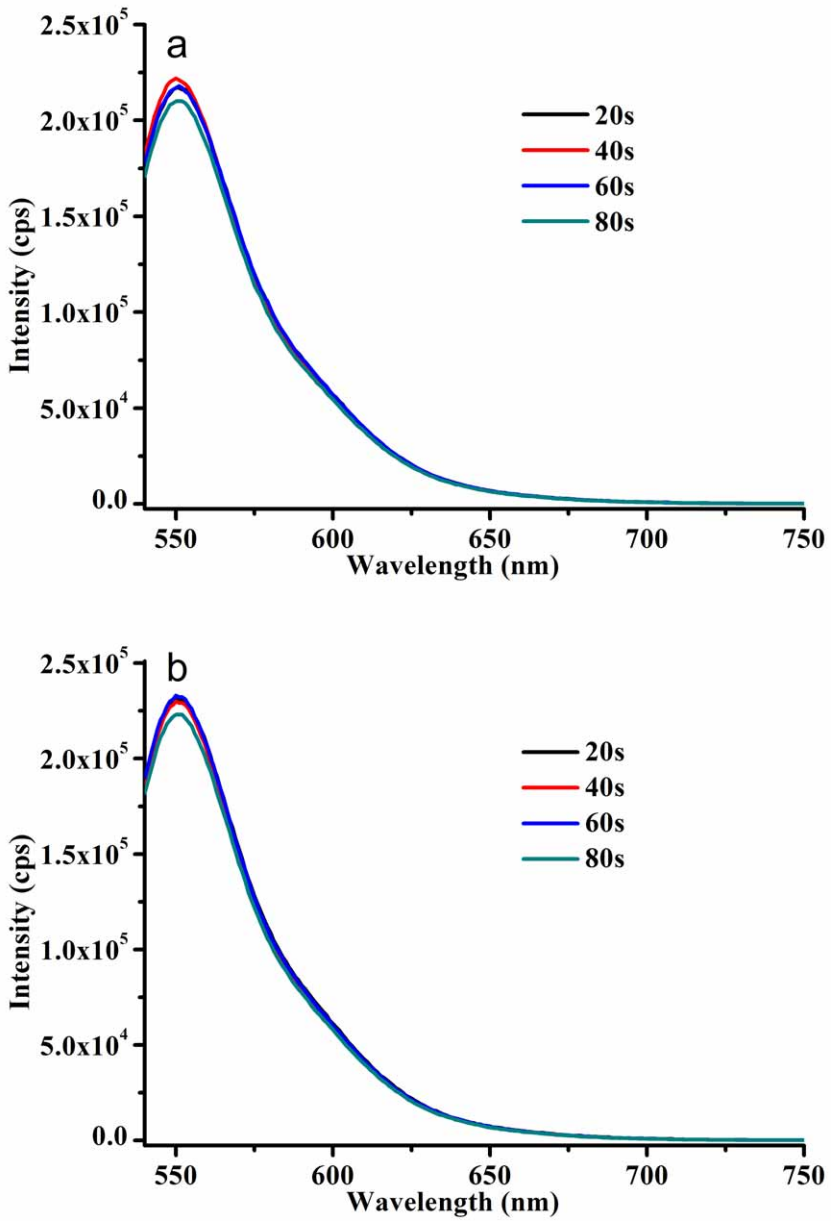

Figure S4. Fluorescence spectra of the single-stranded Alexa-D1 (a), double-stranded Alexa-D1D2 (b) with different UV light irradiation times (the black lines were overlapped with the blue lines for (a) and (b)).

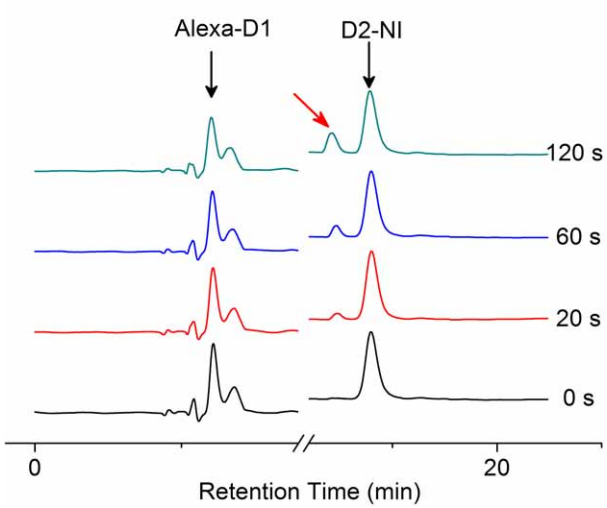

Figure S5. HPLC separation of Alexa-D1D2-NI after treating the Alexa-D1D2-NI solution with different UV exposure time (0, 20, 60 and 120 s). HPLC was performed on a reverse-phase C-18 column with an acetonitrile/TEAA gradient (5-30%, 22 min) at room temperature and the detection wavelength was 260 nm. The solution contained 1 μM DNA in 20mM Tris-acetate containing 140mM NaCl, 5mM KCl, 1mM $CaCl_2$ and 1mM $MgCl_2$ (pH 7.4) was used as the buffer. Alexa-D1 and D2-NI are indicated in the profile. A new product, which might be the D1 coupled with the oxidized form of Alexa after UV irradiation is indicated in red arrow.

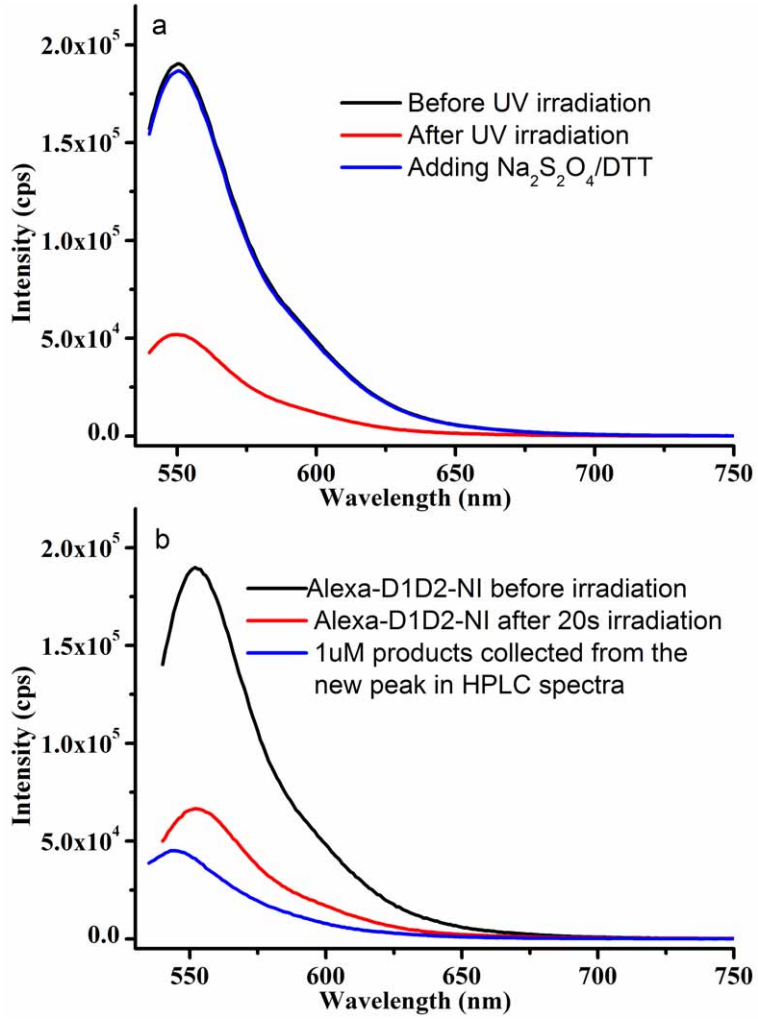

Figure S6. (a) Fluorescence of Alexa-D1D2-NI could be recovered after adding reducing agent，indicating the decrease in fluorescence intensity was due to the oxidation of Alexa532. (b) Comparing the fluorescence spectra of non-oxidized form of Alexa532 (Alexa-D1D2-NI before UV irradiation) with that of the oxidized form of Alexa532 (the collected new components from HPLC). The remaining fluorescence of 5nM Alexa-D1D2-NI after UV irradiation was mainly due to the emission from the non-oxidized form of Alexa532. The black line and the red line represent the fluorescence intensity of 5nM Alexa-D1D2-NI before and after UV light irradiation respectively. The blue line in (a) represents the fluorescence intensity after adding $Na_2S_2O_4$/DTT to the UV irradiation-treated Alexa-D1D2-NI. The blue line in (b) represents the fluorescence intensity of 1uM new product (D1 coupled with the oxidized form of Alexa532 after UV irradiation) collected from several HPLC samples shown in Figure S5).

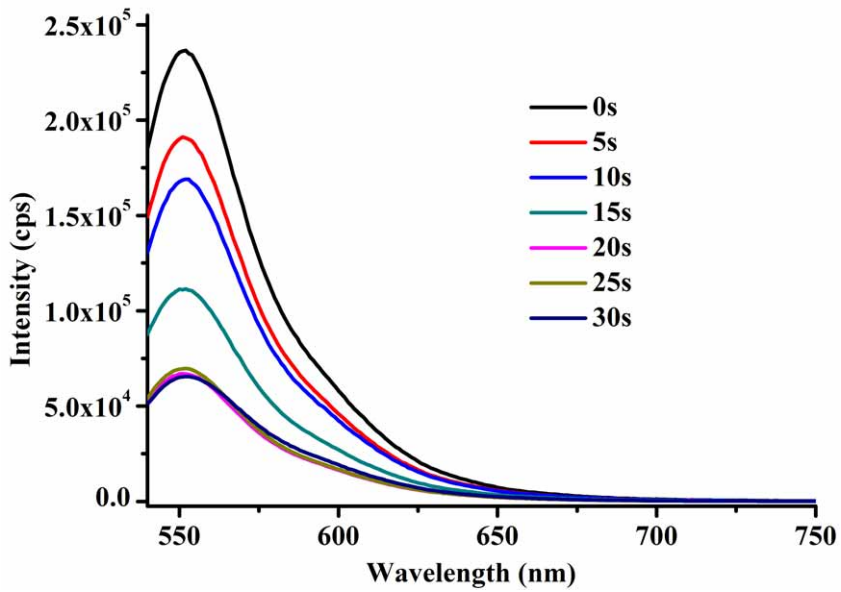

Figure S7 Fluorescence spectra of the Alexa-D1D2-NI with different UV light irradiation times.

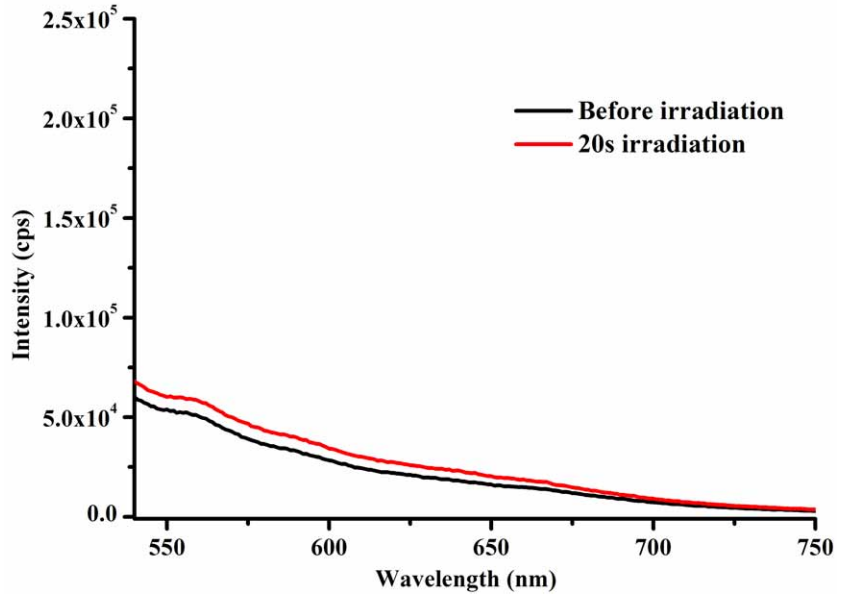

Figure S8. Fluorescence spectra of undiluted human blood serum before and after UV light irradiation.

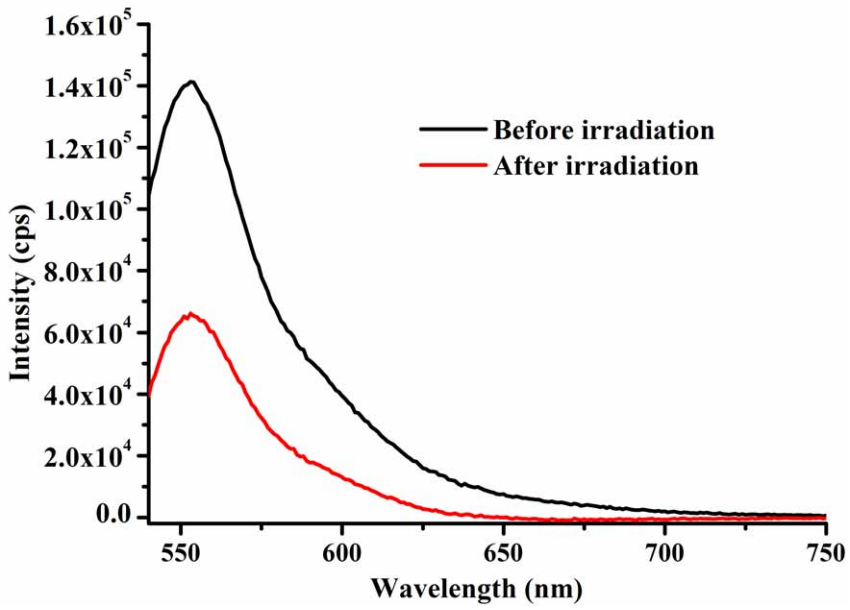

Figure S9. Fluorescence spectra of Alexa-D1D2-NI in undiluted human blood serum before and after UV light irradiation.

References

(1) Takada, T.; Fujitsuka, M.; Majima, T. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 11179.

(2) Kawai, K.; Kawabata, K.; Tojo, S.; Majima, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2363.

(3) Takahashi, Y.; Miyashi, T.; Yoon, U. C.; Oh, S. W.; Mancheno, M.; Su, Z.; Falvey, D. F.; Mariano, P. S. *J. Am. Chem. Soc.* **1999**, *121*, 3926.