Supporting information

Electrochemical Synthesis of Photoluminescent Carbon Nanodots from Glycine for Highly Sensitive Detection of Hemoglobin

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Electronic Supplementary Material (ESI) for Green Chemistry

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Section 1: *Preparation of C-dots* S1

S1.1 Reagents and Chemicals

Ammonium hydroxide, apo-transferrin, ascorbic acid, β-casein, cytochrome c, ethanol, ethylenediaminetetraacetic acid (EDTA), glycine, hemoglobin, human serum albumin (HSA), hydrogen peroxide, nitric acid, quinine sulfate, ribonuclease, tris(hydroxymethyl)aminomethane (Tris), transferrin, trypsinogen, β-lactoglobin, luminol, myoglobin, potassium hydroxide, potassium phosphate monobasic, potassium chloride, sodium chloride, and sodium hydroxide were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Monobasic, dibasic, and tribasic sodium phosphates were obtained from J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water (18.2 MΩ cm^{-1}) from a Milli-Q ultrapure system was used in this study. Phosphate buffered saline (PBS) at pH 7.4 containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4 is presented as 1X PBS. Oily pens, ballpoint pens, soy sauce, ketchup, and eggs were purchased from a local store (Taipei, Taiwan).

S1.2 Preparation of C-dots

In a typical C-dots synthesis, 0.5 M glycine was dissolved in NH_4OH aqueous solution (10 mL; 3 M) in a glass sample vial. Two Pt wires used as both anode and cathode electrodes were immersed into this alkaline solution. A 2400 Series Source Meter direct current (DC) power supply (Keithley Instruments, OH, USA) was used to apply a static potential (10 V) between the two electrodes at ambient temperature (25 °C) for 2 h. The aqueous solution was then filtered through 0.22-μm membranes to remove large or agglomerated particles. To further remove the matrix, the as-purified C-dots aqueous solution was subjected to dialysis against pure water through a membrane (MWCO = 3.5–5 kD, Float-A-Lyzer G2, Spectrum Laboratories, CA, USA) for 3 h. The waste was collected according to waste disposal act (WDA)

and the rule set by Taiwan Environment Protection Administration (EPA).[1] Finally, pure, light yellow C-dots aqueous solution was obtained. The as-prepared C-dots aqueous solution was stable for at least 5 months. Different alkaline solutions (0.5 M NaOH, and 0.5 M Na_3PO_4) and static potentials (10-50 V) were investigated to optimize the preparation conditions. For comparison, C-dots were also synthesized from other organic compounds (ascorbic acid, EDTA, glycine, and Tris).

Section 2: Characterization of C-dots S2

S2.1 TEM images, UV-Vis and PL spectrum of C-dots

The as-prepared C-dots were diluted 10-fold with ultrapure water prior to conducting transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) measurements separately using JSM-1200EX II (JEOL Ltd., Tokyo, Japan) and FEI Tecnai-G2-F20 TEM systems. The C-dots were carefully deposited on 400-mesh C-coated Cu grids, and excess solvents were evaporated at ambient temperature and pressure. A GBC Cintra 10e double-beam UV–Vis spectrophotometer (GBC Scientific Equipment. Victoria, Australia) was used to record the UV–Vis spectra of the C-dots in ultrapure water. The photostability of the C-dots was investigated under continuous illumination of the Xe lamp in a PL spectrophotometer (Varian, CA, USA). The PL lifetime was measured using an Edinburgh FL 900 photo-counting system (Edinburgh Instruments, Livingston, UK), with a 377-nm laser (Spectra Physics, CA, USA) as an excitation source at a pulse rate of 6 ns and a 440-nm narrow bandpass filter.

S2.2 XRD, C 1s XPS Profiles and FTIR Spectra of C-dots

For X-ray diffraction (XRD) measurements, a PANalytical X'Pert PRO diffractometer (PANalytical B.V., Almelo, Netherlands) in conjunction with Cu Kα

radiation (λ = 0.15418 nm) was used. Prior to XRD measurements, C-dots were placed on quartz glass supports. A Varian 640 Fourier transform infrared (FTIR) spectrophotometer (Varian, CA, USA) was used to analyze the surfaces of as-prepared C-dots.

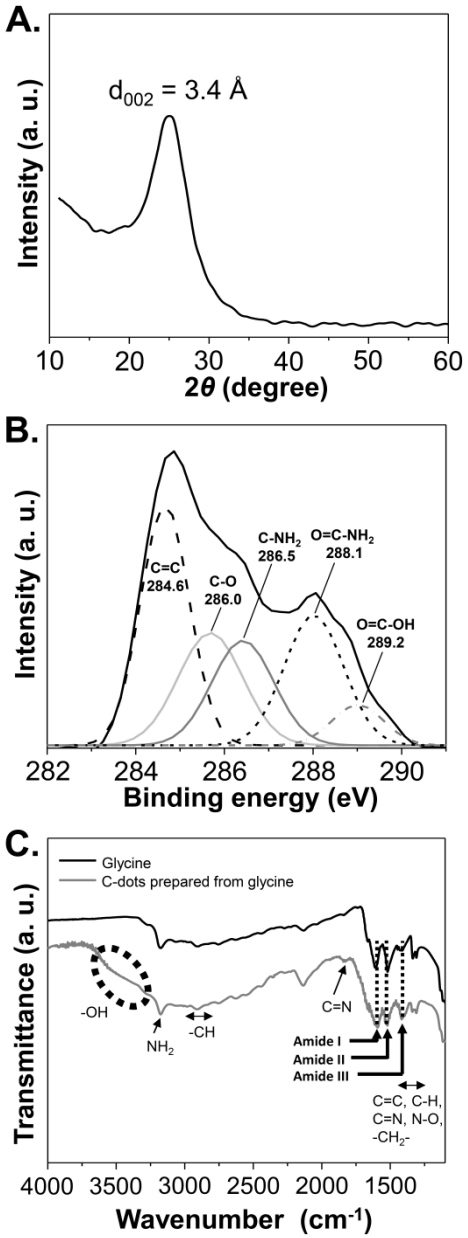

Figure S-1. (A) XRD pattern, (B) C 1s XPS profiles, and (C) FTIR spectra of C-dots prepared from glycine (0.5 M) at 10 V for 2 h.

Figure S-1A displays a XRD pattern of the as-prepared C-dots, exhibiting a sharp diffraction peak (002) at 25.0° (2θ) with a *d* spacing of approximately 0.34 nm, which

is indicative of an amorphous nature of the C-dots.[2] Figure S-1B displays the original and deconvoluted C 1s XPS spectra of C-dots, in which five peaks were assigned, including C=C (~284.6 eV), C–O (~286.0 eV), C–NH_2 (286.5 eV), O=C–NH_2 (~288.1 eV), and O=C–OH (~289.2 eV).[3, 4] Notably, the peak at ~284.6 eV is ascribed to C atoms in graphitic structures, revealing predominantly sp^2 carbons in the as-prepared C-dots.[4] Furthermore, the peak intensity (located at 288.1 eV) is greater than that obtained by conducting a hydrothermal method,[5] indicating that more residual oxygen-containing groups (C–O, C=O) and amide bond (O=C–NH_2) appeared in the as-prepared C-dots.

The FTIR spectrum of C-dots provides several common characteristic peaks (Figure S-1C). The peaks at 1265 (C=C), 1400–1650, and 1735 cm^{-1} (C-H, C=C, -CH_2-, conjugated C=N, C=O stretching vibration), as well as 3200–3550 cm^{-1} (O-H stretching vibration) are identified.[6] The peaks at 1510–1560 and 1630 cm^{-1} are assigned to the amide II (in-plane N-H bending) and amide I (C=O stretching vibration) bands, respectively, and that at 1455 cm^{-1} is characteristic of the amide III (C-N stretch stretching vibration) band.[6] A small band at 1835 cm^{-1} is associated with C=N (sp^2 C–N) stretching vibration. The bands observed at around 2090 and 2113 cm^{-1} are ascribed to C-H bond and C≡C bond, respectively.[7, 8] The results of FTIR and XPS agree with each other, strongly supporting the formation of C-dots.

$$CH_2(NH_2)COO^- \rightarrow CH_2(NH_2)COO(Pt) + e^- \quad \text{.....(1)}$$

$$CH_2(NH_2)COO(Pt) \rightarrow NH_2CH_2(Pt) + CO_2 \quad \text{.....(2)}$$

$$NH_2CH_2(Pt) \rightarrow NH_2CH_2^+ + e^- \quad \text{.....(3)}$$

$$NH_2CH_2^+ + OH^- \rightarrow NH_3 + CH_2O \quad \text{.....(4)}$$

$$CH_2(NH_2)COO^- + NH_2CH_2^+ + 2e^- \rightarrow CH_2(NH_2)\mathbf{CONH}CH_2^- + OH^- \quad \text{.....(5)}$$

$$CH_2(NH_2)\mathbf{CONH}CH_2^- + (n-1)CH_2(NH_2)COO^- \rightarrow NH_2CH_2[\mathbf{CONH}CH_2]_n^- + (n-1)OH^- \quad \text{.....(6)}$$

Scheme S-1. Electro-oxidation and electro-polymerization of C-dots from glycine under an alkaline condition.

S2.3 Quantum Yield and Zeta Potential of C-dots

The PL intensities (excitation at 365 nm) and absorbance values at 365 nm of the C-dots were used to calculate the QYs using quinine sulfate (ϕ_f = 0.54) as a reference.[6] Quinine sulfate was dissolved in 0.1 M H_2SO_4 (refractive index: 1.33), while the C-dots were dispersed in water (refractive index: 1.33). To minimize reabsorption effects, absorbance values of the individual solutions in 10-mm cuvettes were maintained under 0.1 at the excitation wavelength. Excitation and emission slit widths were both set at 5.0 nm when recording their PL spectra.

S2.4 Time-Evolution Raman Spectra of C-dots

A Raman microscopy system with a 50× objective (Dongwoo Optron, KyungGiDo, Korea) was used to analyze air-dried C-dots on a silica wafer. A diode-pumped solid-state laser operating at 532 nm was used as the excitation source with a power of 100 mW and an accumulation time of 100 s.

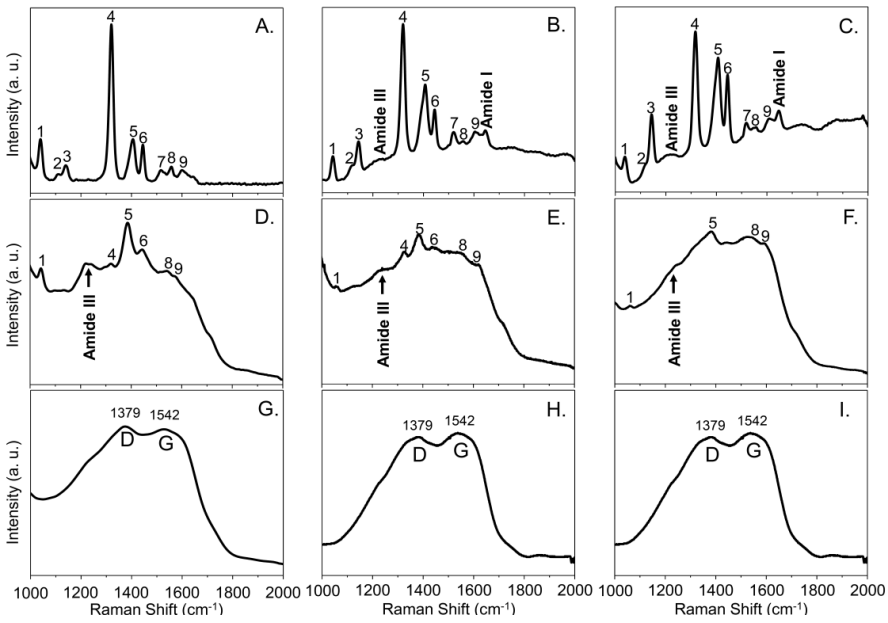

Figure S-2. Raman spectra (λ_{ex} = 532 nm) of C-dots prepared from glycine (0.5 M) at 10 V for various reaction times. Reaction times in A–I are 0, 30, 35, 40, 50, 55, 60, 120, 180 min, respectively. Other conditions are the same as those described in the main text.

S3.1 Effect of Ammonium concentration, applied voltage, and reaction time

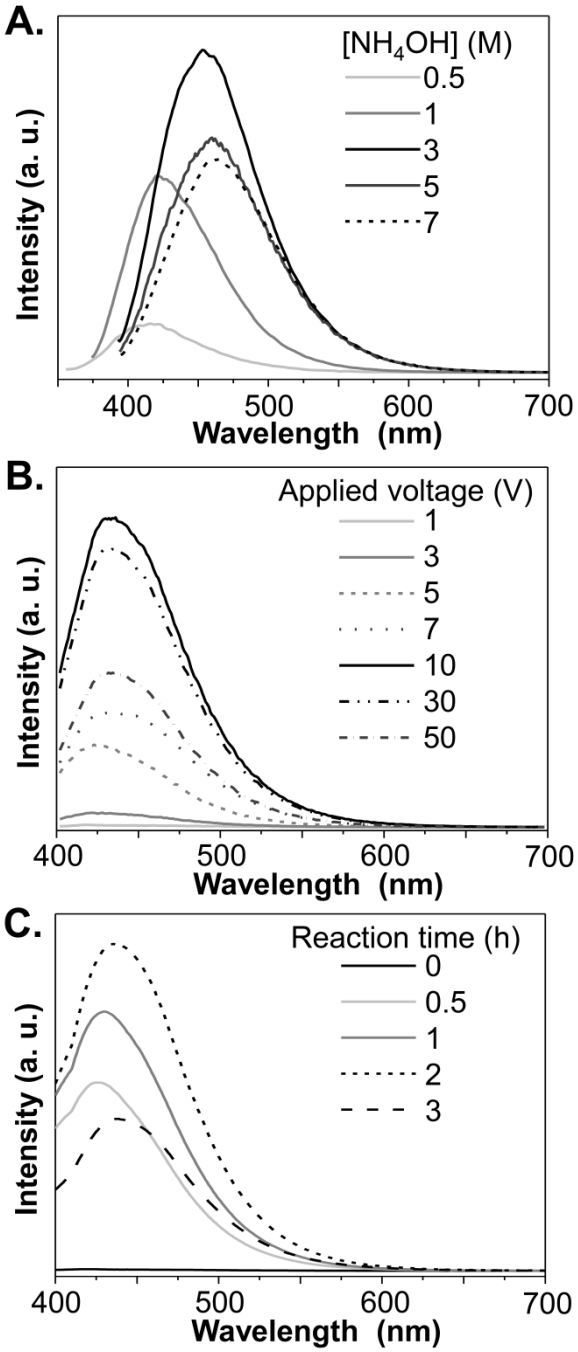

Figure S-3. Effect of (A) NH_4OH concentration, (B) applied voltage (1–50 V), and (C) reaction time (0–3 h) on the formation of C-dots. The concentrations of glycine are all 0.5 M. Applied voltage: 10 V in (A) and (C). Reaction time: 2 h in (A) and (B). NH_4OH concentration: 3 M in (B) and (C). Excitation and emission wavelengths are 365 nm and 440 nm, respectively.

S3.2 Effect of NaCl concentration, pH, and irradiation time

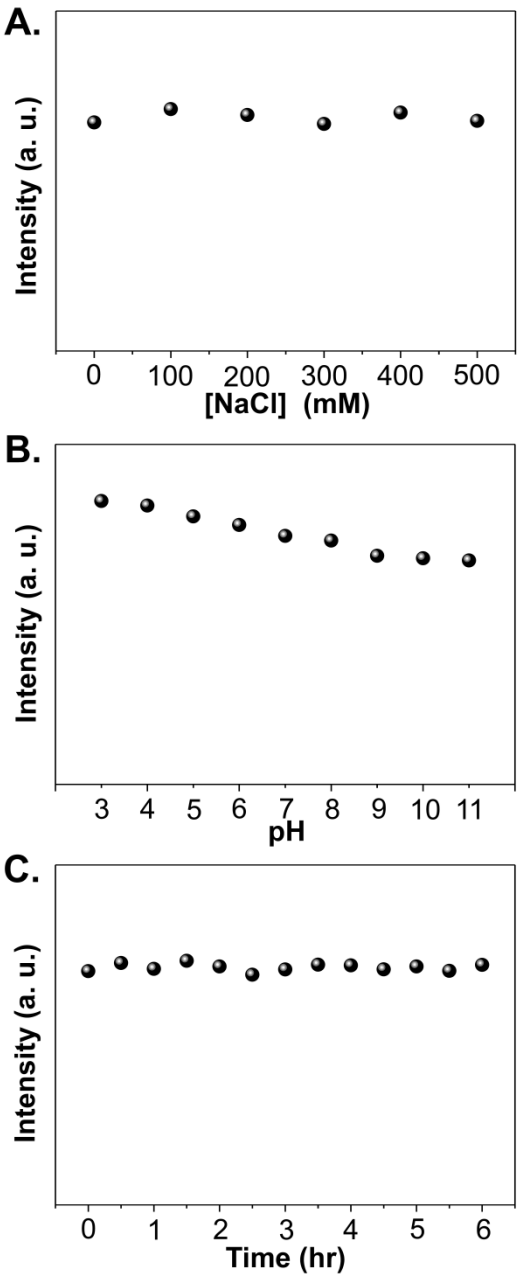

Figure S-4. Effects of (A) NaCl concentration (0–500 mM), (B) pH (3.0–11.0), and (C) irradiation time on the PL intensity of C-dots. The C-dots were dispersed in (A) phosphate buffers (50 mM, pH 7.4) containing various concentrations of NaCl, (B) phosphate solutions (50 mM, pH 4.0–10.0), and (C) phosphate buffer (50 mM, pH 7.4). Excitation and emission wavelengths: 365 and 440 nm, respectively.

Section 4: The practicality of C-dots S4

S4.1 Blood sampling

The whole blood samples collected from five anonymous male donors were used in this study. The whole blood samples were collected immediately into glass vials containing 4% sodium citrate in PBS to prevent pore clogging, in which 1 part sodium citrate was mixed with 9 parts of each blood sample. The whole blood samples were stored at −20 °C before use.

S4.2 Detection of Hemoglobin Using C-dots

Aliquots of 1X PBS (500 μL) containing hemoglobin (0-1000 nM) and 0.1 X C-dots were incubated at ambient temperature for 30 min. Before PL measurements, the mixtures were transferred separately into a 96-well microtiter plate. Their PL spectra were recorded using a microplate reader (μ-Quant Biotek Instruments, VT, USA) at an excitation wavelength of 365 nm.

Practicality of C-dots was validated by the determination of the concentrations of hemoglobin in blood samples. Prior to analysis, the whole blood samples were diluted 250,000-fold in ultrapure water, which were then subjected to sonication for 30 s to release the hemoglobin from erythrocytes. Aliquots (50 μL) of the as-prepared blood samples were spiked with standard hemoglobin solutions (50 μL). The spiked sample solutions (100 μL) were separately mixed with aliquots of PBS (400 μL) containing C-dots (50 μL, 1X), which were incubated at ambient temperature for 30 min prior to PL measurement. The final concentrations of hemoglobin in the mixtures were 0–200 nM. A commercial hemoglobin-meter (HemoCue AB, Ängelholm, Sweden) was used to determine the concentrations of hemoglobin in the blood samples through a modified azidemethemoglobin reaction. Sodium nitrite converted the hemoglobin ion

from the ferrous to the ferric state to form methemoglobin that subsequently reacted with azide to form azidemethemoglobin. The end point of the reaction was measured bichromatically at the wavelengths of 570 and 880 nm. The second wavelength was used for compensating the interference caused by blood components such as chylomicrons or leukocytes. The bichromatical absorbance ratio is proportional to the concentration of hemoglobin. The results obtained from the two approaches were compared through a student's *t*-test.

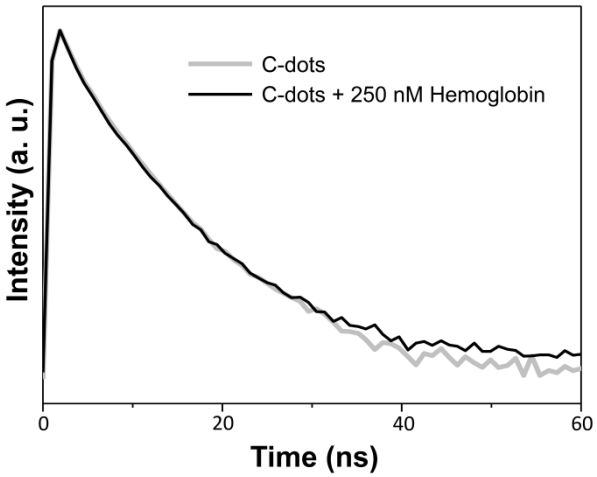

Figure S-5. PL decays of C-dots in absence and presence of hemoglobin (250 nM). Excitation and emission wavelengths are 337 and 440 nm, respectively.

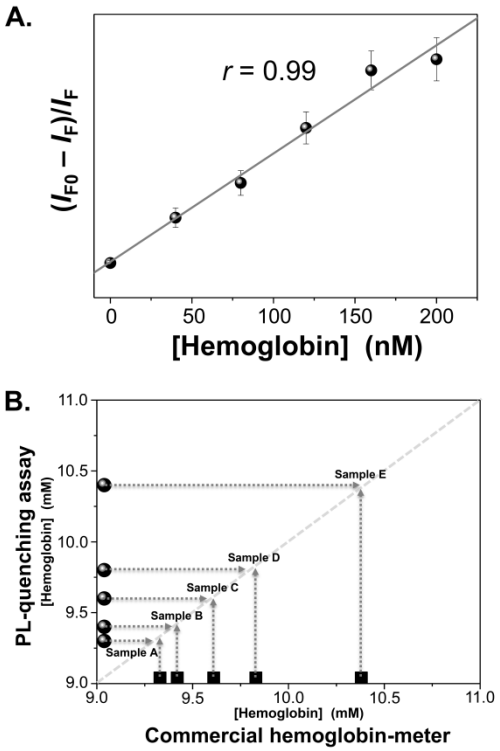

Figure S-6. Analysis of blood samples by the PL-quenching assay using C-dots. (A) a representative diluted blood sample analyzed by the PL-quenching assay. (B) Correlation of the concentrations of hemoglobin in the five blood samples determined by the PL-quenching assay and a modified azidemethemoglobin method. Each of the samples was spiked with hemoglobin (final concentrations 0–200 nM). Other conditions are the same as those described in the main text.

S4.3 Bloodstained images and fingerprints of C-dots

The bloodstained clothes (4 cm × 4 cm) were washed with diluted laundry detergent solution and water until no apparent stains were observed. The clothes were then left in an oven at 37 °C for 1 h. Clothes were separately marked with aqueous protein solutions, including hemoglobin, human serum albumin, apo-transferrin, β-casein, β-lactoglobin, and trypsinogen, each at the concentration of 100 μM. In addition, clothes were separately marked with oily pen ink, ballpoint pen ink, soy sauce, ketchup, and egg. The clothes were stained (marked) with a Chinese character (blood). They were all washed with aqueous detergent and water until no stains

(marks) were observed, besides oily pen ink and ballpoint pen ink that were not removed through the washing process. Half of the washed clothes were then sprayed with the C-dots solution (1X), and the rest of the clothes (no C-dots) were used as controls. Before taking bright field and PL images using a digital camera Nikon Coolpix P300 (Tokyo, Japan), the clothes were stored in an oven at 37 °C for 1 h. The light source used for taking PL images was a 450-W xenon arc lamp (Oriel, Stratford, CT, USA) equipped with a filter. One filter in front of the sample and the other one before the camera were used to select the excitation and emission wavelengths, respectively. The excitation/emission wavelength ranges of the three pairs of filters were 425–445/475–495, 460–490/510–540, and 525–555/550–580 nm. Exposure time was 20 ms. For comparison, the washed clothes stained with blood were also analyzed by applying a luminol chemiluminescence (CL) assay.[9] A standard luminol solution was prepared by mixing 0.2 g luminol, 1.5 g potassium hydroxide, and 10 mL ultrapure water. Prior to use, the luminol solution (10 mL) was mixed with hydrogen peroxide solution (3%, 10 ml) in a spray bottle. Then CL images of the clothes after being sprayed with the mixture for 5 s were recorded using the camera.

Prior to taking fingerprints from volunteers, their thumbs were first cleaned with ethanol to remove any sweat thoroughly. Then their thumbs were immersed in the C-dots (1X) for 1 min. The fingerprints were taken as that addressed taking the PL images of the clothes using a pair of filters (425–445/475–495 nm), each with an exposure time of 20 ms.

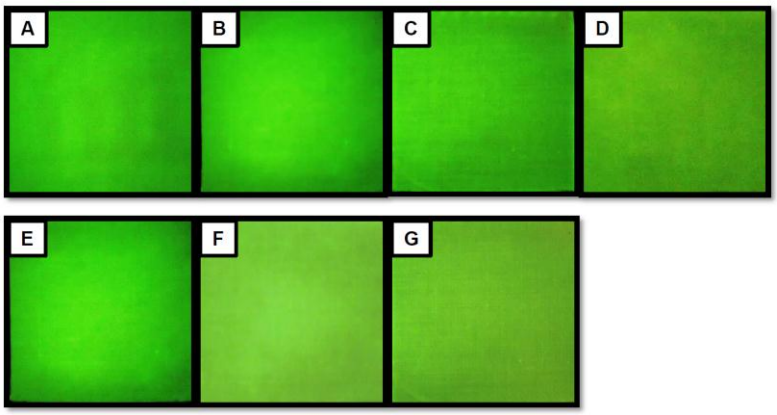

Figure S-7. Representative PL images of washed white clothes, each of which had been marked with a Chinese character (blood) with tested analytes. (A) hemoglobin, (B) HSA, (C) oily pen ink, (D) ballpoint pen ink, (E) soy sauce, (F) ketchup, and (G) egg, respectively. The washed clothes were sprayed with C-dots (1X). Excitation and emission wavelengths are 460–490 and 510–540 nm, respectively. Exposure time: 20 ms. Other conditions are described in the main text.

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