Supporting Information

Application of Fluorescent Carbon Nanodots in Fluorescence Imaging of Human Serum Proteins

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1-DE electrophoresis: 1-DE was performed in a vertical discontinuous gel system as described above. Serum (50 µL) was mixed with 300 µL sample buffer, which consisted of 60 µL bromophenol blue (0.02% w/v), 60 µL glycerol (20% v/v), and 180 µL H₂O. Then, a DYCZ-24D vertical electrophoresis tank and a DYY-6C electrophoresis instrument were used to supply a voltage of 120 V in the stacking gels for 0.5 h, and then 100 V for 3 h when the sample entered the separating gel. The electrophoresis buffer was prepared by dissolving 3.0 g Tris and 14.4 g glycine in distilled water, adjusting the pH to 8.3.

2-DE native-PAGE electrophoresis: The electrophoresis system consisted of a DYCZ-24D vertical electrophoresis tank and a DYY-6C electrophoresis instrument of steady voltage. The gels strip for the first dimension IEF was prepared by mixing of gel stock solution (450 µL), ampholine (135 µL), purified water (1.65 mL), (NH₄)₂S₂O₈ (11.5 µL) (10% w/v), and TEMED (2.5 µL). The gels slice for the second dimension electrophoresis was prepared by mixing 4mL of gel stock solution, 4 mL of Tris-HCl (1.5 M, pH 8.8), 8 mL of deionized water, 150 µL of (NH₄)₂S₂O₈ (10% w/v), and 15 µL of TEMED. Human serum was 1:7 diluted with the mixture of 100 µL deionized water, 50 µL glycerol, and 50 µL bromophenol blue. 100 µL of the sample was applied to a gel strip (7.0 cm, pH 4–6). Serum proteins were isoelectrically focused at 200 V for 30 min and 400 V for 15 h with a DYY-6B electrophoresis instrument and DYCZ-21 vertical electrophoresis tank. The buffer of the anode was H₃PO₄, 0.026 M; the cathode solution was NaOH, 0.1 M. The focused strip was placed onto a slice of polyacrylamide gel and overlaid with 1% low melt agarose at 60 °C. The electrophoresis voltage was 90V for 2.5 h.

2-DE SDS-PAGE electrophoresis: 180 µL of the prepared serum sample was loaded to the IPG strips (7cm, pH 4-7) for 2-DE. Serum proteins were isoelectrically focused at 250 V for 20 min and 4000 V for 10000 V-hr with the Bio-Rad PROTEAN IEF cell. And then placing the IPG strips to shake in equilibration buffer I for 15 minutes, proceed to complete the preparation of equilibration buffer II for 15 minutes. Electrophoresis for the second dimensional gel was run at 80 V for 30 min and 150 V for 3 h, using the running buffer.
Figure S1. DLS size distributions (A) and Zeta-potential distributions (B) for C-dots.
Figure S2. High molecular weight (HMW)-SDS standards after 1-D SDS-PAGE: (A) by C-dots fluorescence imaging; (B) by CBB-R250 staining. The marker (250 μg/vial) was dissolved in 100 μL water and then diluted to 1:5 with the sample buffer. Loading volume: 15 μL.
Figure S3. The images achieved in the HAc-NaAc C-dots staining buffer with different pH value. (a) pH = 1.2; (b) pH = 2.0; (c) pH = 2.7; (d) pH = 3.8; (e) pH = 5.3; (f) pH = 8.4.
Figure S4 (A) Relative fluorescent intensities as a function of buffer concentration, (B) relative fluorescent intensities as a function of C-dots amount.
Figure S5. The relative signal intensity as a function of dilution ratio of human serum for (a) C-dots fluorescence imaging, (b) CBB-R250 staining, and (c) silver staining after 1-D native-PAGE. The insert is the linearity for each method.