Supporting Information

Redox cycling of iron by carbon dot enhanced chemiluminescence: mechanism of electron-hole induction in carbon dot

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Chemicals: All reagents used throughout this study were of analytical grade. Sodium azide, sodium hydroxide, ascorbic acid, thiourea and hydrogen peroxide were purchased from Beijing Chemical Reagent Co. Beijing, China. HCl was obtained from Tianjin Kaitong Chemicals Co. Tianjin, China. Nitro blue tetrazolium chloride (NBT) was obtained from Nacalai Tesque Inc. Tokyo, Japan and histidine from Dingguochansheng Biotech. Co. Itd. Beijing, China. Ferric chloride was purchased from Sinopharm Chemical reagent Co. Ltd. Shanghai, China. Ferrous sulfate was obtained from Heng Ye Jingxi Chemical Co. Ltd. Hebei, China. 5,5-dimethyl-1-pyrroline N-oxide (DMPO) was purchased from Tokyo Kasei Kogyo, Japan. 2,2,6,6-tetramethyl-4-piperidine (TMP) was got from Sigma-Aldrich.

Apparatus: Batch CL experiments were carried out with BPCL luminescence analyzer (Institute of Biophysics, Chinses Academy of Sciences, Beijing, China) while absorption spectra were collected using UV-3900 UV-visible spectrophotometer (Hitachi, Tokyo, Japan). CL emission spectra and photoluminescent (PL) studies were carried out with F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). In addition, EPR spectra were measured on ESP-300E, Bruker spectrometer, Germany. ESI-Q-TOF MS studies were carried out by using Shimadzu LCMS-IT-TOF instrument consisting of system controller, ESI ion source and IT-TOF mass spectrometer (Kyoto, Japan). The Fourier transform infrared spectroscopy (FTIR) was performed with Perkin Elmer FTIR 360 spectrophotometer, DTGS.

Synthesis and characterization of N-CDs: Herein, N-CDs were prepared in one-pot from histidine by hydrothermal way. In contrast to previous work using histidine,¹ we prepare it without acid, base or any other reagent as prepared in our previous work.² For this purpose 0.1 M aqueous solution of histidine was transferred into Teflon lined autoclave and heated at 240 °C for

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8 hours. After high pressure hydrothermal reaction, the solution changed to dark brown from colorless, where the as-prepared N-CDs were freely dispersed in aqueous solution. High energy supplied by hydrothermal treatment, temperature and pressure inside autoclave enabled histidine to experience dehydration, polymerization, condensation and carbonization processes.³ The dialysis was performed by using MW cut off 3.5 kDa Biotech regenerated cellulose (RC) membrane for 12 h against distilled water under gentle stirring by replacing the distilled water after each 2 h.

Kinetic CL profile: CL kinetic curves were obtained by batch experiments, which were carried out in glass cuvette. The CL profiles were displayed and integrated at intervals of 0.1 s. 100 μ L N-CDs and 100 μ L of H₂O₂ were premixed then 100 μ L of Fe²⁺ were injected by a microliter syringe from the upper injection port. The addition orders of reagents were changed to investigate the interaction of the reagent and design the CL injection analysis system. The effect of histidine solution on CL intensity was also checked and is given in Fig. S1. It is clear from the Figure that CL intensity of the original Fenton system was decreased in presence of histidine solution while N-CDs greatly enhanced the CL intensity. Furthermore, effect of concentration of each reagent was studied by using different concentration (Fig. S2). It is clear from the Fig. that the CL intensity increases by increasing concentration of reagents. The effect of N-CDs concentration on CL intensity was also checked (Fig. S3)

EPR Experiments: EPR spectra were recorded at room temperature using JEOL JES-FA200 spectrometer. Instrumental conditions: microwave power, 1.0 mW; modulation amplitude, 1.0 G; and receiver gain, 1.00e + 05.

The role of dissolved oxygen and nitrogen were evaluated and the results are given in Fig. S4. To know about the surface of prepared N-CDs, Mass spectra (Fig. S5) and IR (Fig. S6) of histidine and N-CDs solution were recorded. The collision-induced dissociation mass spectra of protonated ions of histidine [M+H]⁺ at m/z=156 and deprotonated ions at 154 was observed respectively.⁴ In case of N-CDs, very rich spectra were observed at higher m/z pointing a complex structure of N-CDs, led to assumption that during the formation of N-CDs cyclization and polymerization were the leading reactions. Moreover, FTIR spectra were used to identify surface groups on N-CDs. As shown, peaks at 1627, 1452 and 1410 cm⁻¹ were assigned to amide I (C=O) stretching, amide II (N–H) inplane bending and amide III (C–N) stretching vibrations, respectively. These bands slightly shift to higher energy in case of N-CDs. Bands at 2922 and

2854 cm⁻¹ were assigned to $-CH_2$ - vibrations. As revealed, broad band in range of 3160-2660 suggested the existence of -OH, $-NH_2$, $-NH_3^+$ and C-O groups on N-CDs. Hence, carboxylic and amide functional groups were well-preserved on N-CDs after carbonization process.^{1,3} The presence of -COOH and -OH groups on N-CDs resulted in different emissive trap sites. These emitting centers played important role in enhanced CL emission. So the surface chemistry of N-CDs has dominant influence on CL emission.⁵

The PL spectrum of histidine solution was compared with that of N-CDs (Fig. S7). It is clear from the spectra that the excitation and emission wavelengths of histidine solution were red shifted upon the formation of N-CDs. The as synthesized N-CDs were well-dispersed in aqueous solution without any surface passivation which might originate from -OH, $-NH_3^+$ and -COOHgroups on its surface.³ Despite precise emitting mechanism and chemical structure were still not quite clear, the observed PL behavior clearly pointed the presence of different fluorophores within N-CDs.⁶



Fig. S1 CL study of histidine and N-CDs. (i) Fe^{2+} injected to H_2O_2 (ii) Fe^{2+} injected to histidine + H_2O_2 (iii) Fe^{2+} injected to N-CDs + H_2O_2 . The conc. of Fe^{2+} was 10^{-3} M, histidine and H_2O_2 were 0.1 M while N-CDs were 100%. The volume of each reagent was 100 µL.



Fig. S2 The effect of concentration of reagents on CL intensity of the system. (i) (0.1M) H₂O₂ injected to (0.01M) FeSO₄-N-CDs (ii) (1M) H₂O₂ injected to (0.01M) FeSO₄-N-CDs (iii) (1M) H₂O₂ injected to (0.05M) FeSO₄-N-CDs (iv) (1M) H₂O₂ injected to (0.05M) FeSO₄-N-CDs-(0.1M) HCl.



Fig. S3 Effect of N-CDs concentration on CL intensity of Fenton system. (i) 100 (ii) 50 (iii) 25 and (iv) 10%. The concentration of Fe^{2+} was 0.01 M while that of H_2O_2 was 0.1 M. The volume of each reagent was 100 μ L.



Fig. S4 Effect of dissolved O_2 and N_2 on the CL intensity of simple and N-CDs enhanced Fenton system. (A) Fe²⁺-H₂O₂ system (B) Fe³⁺-H₂O₂ system. (i)-(iii) Fenton systems while (iv)-(vi) are N-CDs enhanced Fenton systems. (i) and (iv) are Fenton systems without passing any gas prior to CL study. From (ii) and (v) N_2 gas was passed for 20 minutes from all solution prior to CL study. From (iii) and (vi) O_2 gas was passed for 20 minutes from all solution of Fe²⁺ and Fe³⁺ was 10^{-3} M, N-CDs were 25% and H₂O₂ was 0.1 M. The volume of each reagent was 100 µL.



Fig. S5 Mass spectrum of histidine and N-CDs solution operated in different modes. (A) Mass spectrum of histidine solution operated at positive mode. (B) Mass spectrum of histidine solution operated at negative mode. (C) Mass spectrum of N-CDs solution operated at positive mode. (D) Mass spectrum of N-CDs solution operated at negative mode.



Fig. S6 IR spectrum of histidine and N-CDs.



Fig. S7 PL study of histidine and N-CDs. From the PL study it is clear that the excitation and emission wavelength of histidine becomes red shifted after the formation of N-CDs.

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