Electronic supplementary information (ESI)

N-(aminobutyl)-N-(ethylisoluminol) and hemin dual-functionalized graphene hybrids with high chemiluminescence

**Chemical and solutions:** Graphite oxide (GO) was purchased from XFNANO Materials Tech Co., Ltd. (Nanjing, China). N-(aminobutyl)-N-(ethylisoluminol) (ABEI), were obtained from Sigma-Aldrich (USA). Ammonia and hydrazine were purchased from Shanghai reagent (Shanghai, China). Working solutions of H₂O₂ (Xin Ke Electrochemical Reagent Factory, Bengbu, China). All reagents were used as received, and Mill-Q water (18.2 MΩ) was used throughout.

**Synthesis and Characterization of A-H-GNs:** To prepare the A-H-GNs, 10 mL (2 mg/mL) of ABEI (2 mg/mL) potassium hydr oxide solution was added to 10 mL (0.5 mg/mL) of GO aqueous dispersion. After sonication for 1 hour, above solution was mixed with 20 mL (0.5 mg/mL) of hemin alkaline solution and 200 µL of ammonia solution, followed by the addition of 30 µL of hydrazine solution, then the mixture was vigorously stirred at 60 °C for 3.5 h. The black water soluble solution was obtained. In order to obtain the true information about the composition of the graphene hybrids, a rational dialysis procedure to remove the ABEI, hemin and other excess free molecules was conducted before characterization. A 3500 molecular weight cutoff dialysis membrane was used and the ultrapure water was refreshed every two hours until neglected CL intensity of dialysate was observed. A typical dialysis requires about 64 hours. The CL intensity of the last dialysate was showed as
follow in Fig. S1.

To make sure excess hemin and ABEI have been removed successfully, the CL intensity of the last dialysate of A-H-GNs, A-GNs and ABEI-CCG has been examined. The CL intensities of all of them are below 450 (A.U.), which can be neglected when compared with that of A-H-GNs itself.

As-synthesized graphene hybrids were subsequently characterized by UV-visible spectroscopy, X-ray photoelectron spectroscopy (XPS), the Fourier Transform infrared spectroscopy (FT-IR), raman spectroscopy, chemiluminescence (CL) spectroscopy and atomic force microscopy (AFM). UV-vis spectroscopy was obtained using UV-visible spectrophotometer (Agilent 8453, USA). XPS was carried on an ESCALABMK II electron spectrograph (VG Scientific, UK) with Al Kα radiation as the X-ray source. The FT-IR experiments were carried out on a Bruker Vector-22 FTIR spectrometer (Bruker Instruments, Billerica, MA) in a KBr pellet, scanning from 4000 to 400 cm⁻¹ at room temperature. Raman spectroscopy was acquired on a LabRamHR confocal Raman microspectrometer (Horiba Jobin Yvon, Edison, NJ, USA). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was obtained on a OPTIMA7000DV atomic emission spectrometer. Fluorescence titration profile and FL spectroscopy were measured on a model F-7000 spectrofluorophotometer (Hitachi, Japan). The CL activity was conducted on a microplate luminometer (Centro LB 960, Berthold, Germany). For a typical CL measurement, 100 µL of A-H-GNs and other control groups were added into each well of microtiter plate, then 50 µL of 100 µM H₂O₂ solution containing 0.1 M NaOH
(PH=13) was injected into each well. The light emission was collected by the microplate luminometer.

UV-visible spectroscopy of A-H-GNs, ABEI functionalized graphene without hemin under the same conditions for synthesis of A-H-GNs (A-GNs), GO suspension, hemin and ABEI solution

A GO suspension (Fig. S2B, curve b) showed a characteristic band at 230 nm, which is due to $\pi-\pi^*$ transition of aromatic C=C bond, and a shoulder at 290-300 nm which corresponds to the n-$\pi^*$ transition of the C=O bond.\(^1\) the spectrum of hemin (Fig. S2A, curve b) contained a strong peak at 385 nm attributed to the Soret band, as well as a group of weak peaks between 500 nm and 700 nm ascribed to the Q bands of porphyrin derivative. And ABEI (Fig. S2A, curve c) exhibited characteristic peaks around at 290 nm and 320 nm.\(^2\) The spectrum of A-GNs (Fig. S2B, curve a) displayed absorption peaks of ABEI. Meanwhile A-H-GNs showed a broad band at 265 nm which corresponds to reduced GO and characteristic ABEI peaks between 290 nm and 320 nm, which indicated the existence of ABEI on A-H-GNs. A strong peak at 396 nm was also observed which should be the corresponding Soret band of hemin with a red shift\(^3\)\(^-\)\(^6\) (10 nm), and the Q band of A-H-GNs shifted tens of nanometers, these findings clearly confirmed that hemin were also attached to the graphene composite through $\pi-\pi$ interactions. Herein, we can conclude that ABEI and hemin both were functionalized on GNS successfully.

X-ray Photoelectron Spectroscopy (XPS) of A-H-GNs, hemin and GO

Comparing survey XPS of A-H-GNs (Fig. S3A) and hemin (Fig. S3B) with that of...
GO (Fig. S3C), the data for A-H-GNs showed the component of Fe2p at about 711 eV and N1s at 398.5 eV, while GO showed none of these two peaks. It was the evidence for the present of ABEI and hemin on the graphene composite. As can be seen from Fig. S2F, the C1s spectrum of GO was curve-fitted into four components at 284.6 eV, 286.6 eV, 287.8 eV and 288.7 eV which indicated the presence of carbon bonds: C-C, C-O, C=O, and O-C=O, respectively. As shown in Fig. S2D, the higher energy peaks associated with oxidized carbon of A-H-GNs was dramatically decreased which attributed to reduction of GO. Notably, a new peak at 285.8 eV corresponding to C-N of A-H-GNs appeared which strongly supported that ABEI or hemin existed on the surface of the graphene hybrids. The deconvolution of N1s spectra of A-H-GNs (Fig. S3G) was curve-fitted into three components at 398.5 eV, 399.0 eV and 400.7 eV, attributed to N-Fe group, N-C group and N-CO group. And the N1s spectrum of pure hemin is shown in Fig. S2H, peak of N-Fe had a negative shift (-0.2 eV) when compared with that of A-H-GNs. The shift implied that an interaction between hemin and graphene, indicating the electron clouds of hemin inclined to transfer from hemin to the surface of graphene.

**Fourier Transform Infared spectroscopy (FT-IR) of A-H-GNs, hemin, ABEI and GO**

The FT-IR Spectra of GO shows the strong C=O stretching band at 1727 cm⁻¹, which decreased obviously in the spectra of A-H-GNs (Fig. S4D) after chemically reduced by hydrazine. As shown in Fig. S4D, the existence of ABEI and hemin can be supported by several characteristic IR absorption bands, including the typical amino
group (V_{N-H}) in the ABEI standard IR at 3424 cm\(^{-1}\), the symmetric carboxylate stretch (V_{\text{carboxylate}}) at 1402 cm\(^{-1}\) and the corresponding antisymmetric carboxylate stretch at 1570 cm\(^{-1}\).\(^9\)\(^{11}\) Furthermore, the absorption band at 1247 cm\(^{-1}\) was attributed to the C-N stretching vibration.\(^{12}\) These results were in good agreement with the coexistence model of ABEI and hemin on the GNs.

**Raman spectra of A-H-GNs, A-GNs, hydrazine reduced GO (RGO) and hemin**

Raman spectroscopy was used to examine the presence of hemin and the doping situation in A-H-GNs. A-H-GNs (Fig. S5, curve b) displayed two prominent peaks at 1588 and 1358 cm\(^{-1}\) corresponding to the G and D bands, respectively, the D/G ratio increased compared to GO reported by Shi's group\(^{13}\) and was consistence with that of RGO (Fig. S5, curve c), thus indicating the successful reduction of GO. Significantly, there were two new peaks in the Raman spectra of the A-H-GNs at 1161 and 758 cm\(^{-1}\) while no new peaks in that of the A-GNs (Fig. S5, curve a). These results matched the feature Raman spectroscopy of hemin (Fig. S5, curve d), it proved the existence of hemin on the surface of A-H-GNs. Furthermore, the G band of A-H-GNs was located at 1588 cm\(^{-1}\), which was downshifted as compared to that of RGO. It is well known that the G band is usually shifted to a lower frequency by electron donors and to a higher frequency by electron acceptors.\(^{14}\) This downshifted G band identified the successful n-doping of GNs by hemin.

**Inductively coupled plasma atomic emission spectroscopy (ICP-AES) of A-H-GNs**

The sample preparation: 212 mL as-prepared A-H-GNs after dialysis procedure were dried in oven to remove excess moisture. Then the temperature was raised to
800 °C and maintained for 8 h to remove organic molecular on graphene and graphene itself. As a result, the solid containing iron element from hemin was dissolved with aqua regia and diluted to 50 mL with water.

The estimation of concentration of hemin on A-H-GNs: The molecular mass of hemin is 651.94 g/mol and molecular mass of iron element is 55.85 g/mol. All iron elements was assumed to come from hemin, then we estimated that the concentration of hemin on A-H-GNs is about $1.08 \times 10^{-4}$ mol/L.

**UV-visible spectroscopy of ABEI alkaline solution, hemin alkaline solution, absorbance sum of ABEI and hemin and mixture of ABEI and hemin**

UV-vis spectra of ABEI alkaline solution (curve a), hemin alkaline solution (curve b), absorbance sum of ABEI and hemin (curve c) as well as mixture of ABEI and hemin (curve d) were also investigated. And all concentrations of ABEI, hemin and alkaline solution are the same. The absorbance sum of ABEI and hemin is not equal to absorbance of mixture of ABEI and hemin, which provides another evidence for the existence of interactions between ABEI and hemin.

**Calculations for loading of hemin and that of ABEI**

The concentration of hemin on A-H-GNs is estimated to be about $1.08 \times 10^{-4}$ mol/L according to the result of ICP-AES. So there are 15 mg hemin in 212 mL as-prepared A-H-GNs. In the preparation of A-H-GNs, the initial total volume is 40.23 mL. There are 26 mg GO in 212 mL as-prepared A-H-GNs( assuming no much weight loss of GO). Then the loading of hemin is calculated to be around $15/26=0.58$ g hemin/g GNs.

In scheme 1, it is reasonable to deduce that the molarity of ABEI on A-H-GNs is
roughly approached to that of hemin base on the proposed assembling mechanism. So 6.3 mg ABEI was in 212 mL as-prepared A-H-GNs and the loading is 6.3/26=0.24 g ABEI/g GNs.

Fig. 1S A comparison of CL behavior of the last dialysate for A-H-GNs (a), A-GNs (b) and ABEI-CCG (c).
**Fig. S2** UV / vis spectra of A) A-H-GNs (a), hemin solution (b), ABEI solution (c).

UV / vis spectra of B) ABEI functionalized graphene without hemin with the same synthesis conditions (A-GNs) (a) and GO suspension (b).
Fig. S3 Survey XPS data of A) A-H-GNs, B) hemin and C) GO. The deconvolution of C1s spectra of D) A-H-GNs, E) hemin and F) GO. The deconvolution of N1s spectra of G) A-H-GNs and H) hemin
**Fig. S4** FT-IR of A) GO, B) ABEI, C) hemin and D) A-H-GNs
**Fig. S5** Raman spectra of A-GNs (a), A-H-GNs (b), RGO (c) and hemin (d). Laser wavelength: 514 nm.
Fig. S6 ICP-AES of iron element standard concentration.
Fig. S7 UV/vis spectra of 5 ug/mL ABEI (PH=12) (a), 6.25 ug/mL hemin (PH=12) (b), sum of a and b (PH=12) (c) and mixture of ABEI and hemin (final concentration of ABEI is 5 ug/mL, final concentration of hemin is 6.25 ug/mL, PH=12) (d).


