Supporting Information

Experimental Section

Chemicals

Luminol was obtained from Sigma Aldrich Chemical Co. Naringin, hesperidin and naringin dihydrochalcone were obtained from zeliang Co. (Nanjing, China). β-Cyclodextrin was provided by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). CNHs were synthesized by arc-discharge process based on a previously reported procedure [S1]. All other chemicals were of analytical grade and used without further purification. The solutions were prepared using ultrapure water which was purified by Millipore Milli-Q (18.25 MΩ·cm).

Electrochemiluminescence measurements

The experimental equipment for ECL measurement including a BPCL ultra-weak chemiluminescence analyzer controlled by a personal computer with BPCL program(Institute of Biophsics, Academia Sinica, China) and a electrochemical analyzer (CHI660D, Shanghai Chenghua instrument Co., China). A conventional three-electrode cell assembly was employed all through the experiment, which involved a working glassy carbon electrode, a platinum wire auxiliary electrode, and an Ag/AgCl reference electrode. All potentials are reported versus Ag/AgCl reference at room temperature. Glassy carbon electrodes (99.99% polycrystalline, ~3 mm diameter, CH Instrument Inc.) were polished on a microcloth (CH Instrument Inc.) with 0.05 μm *γ*-alumina suspension (CH Instrument Inc.) and rinsed with ultrapure water and ethanol. The electrodes were then sonicated in ultrapure water for 2 min to remove adsorbed particles, rinsed thoroughly with ultrapure water, and dried under mild nitrogen stream.

Preparation of β-CD functionalized CNHs hybrid complex and various CNHs based sensors.

Two milliliters of aqueous solution containing 2.0 mg/mL CNHs and certain amount of β-CD was sonicated (200 W) for 2 hours using a KQ5200DE model ultrasonic cleaner to obtain a black dispersed solution. The β-CD/CNHs/GCE was achieved by dropping certain volume resulting suspensions onto the surface of GCE, and the solvent was evaporated in the air (denoted as β-CD/CNHs/GCE, hereafter). Before test, the resultant ECL sensor was immersed in ultrapure water. Subsequently, the β-CD/CNHs/GCE was then immersed in the naringin solution or sample solution for certain minutes. And then it was rinsed with ultrapure water to remove nonspecific naringin and dried under a flow of nitrogen (denoted as Naringin/β-CD/CNHs/GCE, hereafter).

Characterizations of CNHs and CDs functionalized CNHs

As it showed in Fig.S1, the FT-IR spectrum of CNHs (curve a) exhibited the C=C conjugation (1548cm^{-1}), C-C band (1192 cm^{-1}), and O-H stretching vibrations at 3428 cm^{-1}. However, compared with the FT-IR of CDs (curve b), when the CDs were immobilized on CNHs, this CDs functionalized CNHs showed the typical CDs absorption features (curve c) of the ring vibrations at 498, 577, 707, 757, and 942 cm^{-1}, the conjugated C-O-C stretching/O-H bending vibrations at 1155 cm^{-1}, the conjugated C-O/C-C stretching/O-H bending vibrations at 1032 and 1077 cm^{-1}, CH_2 stretching vibrations at 2925cm^{-1}, C-H/O-H bending vibrations at 1414 cm^{-1}, and O-H stretching vibrations at 3428 cm^{-1}. These results clearly proved that CDs were adhered to CNHs. Furthermore, the O-H stretching vibration peak at 3428 cm^{-1} for CDs@CNHs exhibited obviously large red-shift compared with free OH mode (be at about 3700 cm^{-1}), showing that there was a strong hydrogen bond between β-CD molecules and CNH.[S2,3]

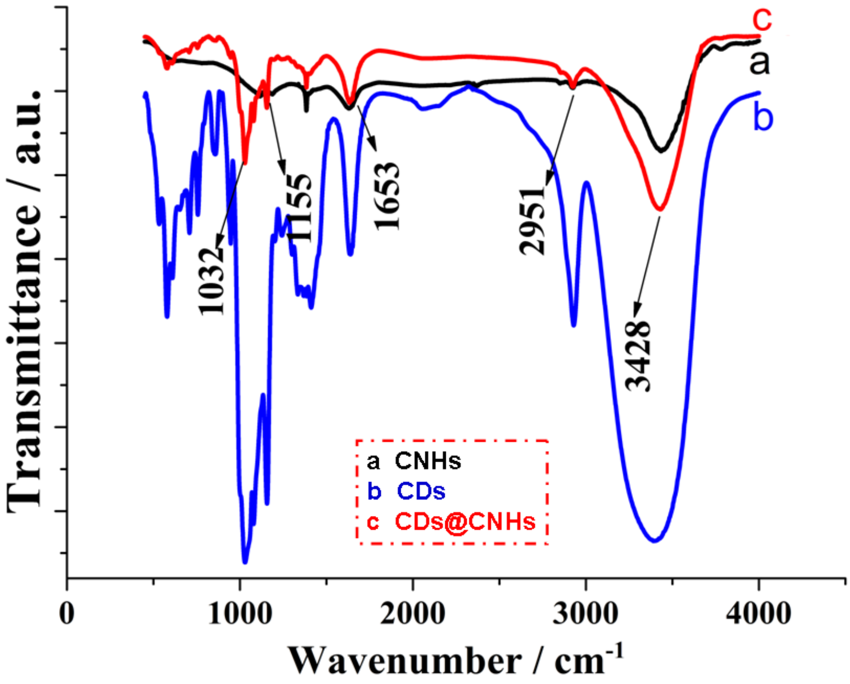

Figure S1 FT-IR spectra of CNHs(a), CDs(b) and CDs@CNHs(c).

Fig.2S displayed the TGA of CDs and CDs@CNHs. It clearly indicated that both CDs and CDs@CNHs almost had the same weight loss region at 340℃ around, which was attributed to the decomposition of CDs. These results further proved CDs could strongly adsorb on the surface of CNHs.

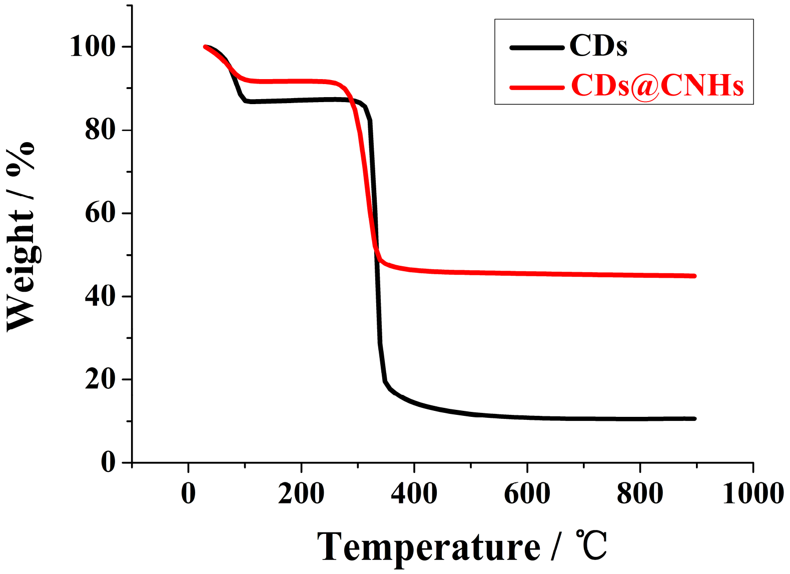

Figure S2 TGA of CDs and CDs@CNHs;

Optimization of experimental conditions

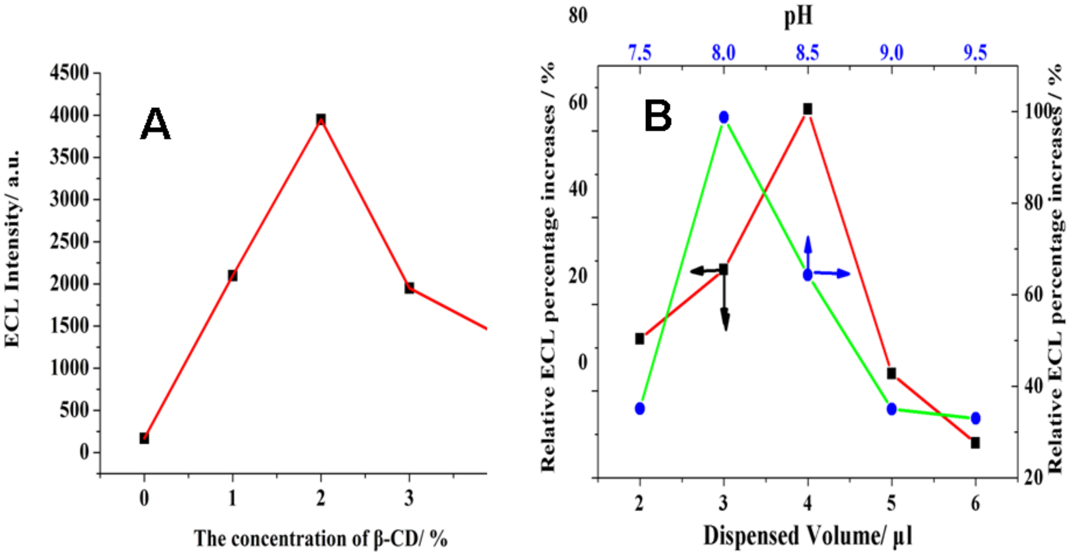

Figure S3 (A) ECL response for β-CD@CNHs/GCE with varyingβ-CD loadings in $2x10^{-5}$M luminol pH 8.0 PBS. (B) Effects of pH and β-CD@CNHs amount on ECL sensing 1.0×10^{-6}M naringin utilizing β-CD@CNHs/GCE.

The use of β-CD as recognition element allowed optimization of the density of the immobilized β-CD via a controlled functionalization with β-CD and thus enhancing the sensitivity and detection limits of the fabricated device. The β-CD functionalized CNHs based ECL biosensors with different concentration of β-CD were examined and the result indicated that the light emission of luminol increased obviously with the amount of β-CD added into the hybrid solution. However, when the concentration of β-CD beyond 2% (w/w), decreased ECL emission was observed due to the tight coverage of CNHs by β-CD. Therefore, we chose 2% β-CD to functionalize CNHs (2mg/mL) for subsequent experiment (see Figure S3A)

The amount of β-CD functionalized CNHs on the sensing interface greatly affected the performance of this ECL biosensor. Through controlling the amount of β-CD and CNHs hybrid on the interface, we could get the optimized amount of hybrid to fabricate well-performance ECL biosensor. The more robust ECL increased signal to initial ECL (relative ECL percentage increases) intensity was instead of the increased ECL intensity. An increase in the dispense volume increased the relative ECL percentage increases up to 66%, and then decreased dramatically afterwards. The evolution relative ECL percentage increases with pH showed one maximums at 8.0, which could be due to the pH influence on the two reactions: on the reaction between β-CD functionalized CNHs and naringin and on the luminol electron-oxidation, reactions that depend heavily on pH [S4] (Figure S3B). Thus, pH 8.0 was selected as the working condition.

Reference

S1. Li, N., Wang, Z., Zhao, K., Shi, Z., Gu, Z., Xu, S., 2010, Carbon 48, 1580 –1585.

S2. Guo, Y., S., Dong, and Wang, E., et al., Nano. 2010,4 ,4001-4010.

S3. Ficarra., R, Tommasini, S., Ficarra, P., et al., Journal of Pharmaceutical and Biomedical Analysis. 2002, 29,1005–1014.

S4. Dai, H., Xu, H.F., Wu, X.P., Chi, Y.W., Chen, G., 2009 , ANALYTICA CHIMICA ACTA 647, 60-65.