Electronic Supplementary Information

A Sensitive and Selective Electroanalysis Strategy for Histidine Using the Wettable Well Electrodes Modified with Graphene Quantum Dot-Scaffolded Melamine and Copper Nanocomposites

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Experimental Section

Reagents

Melamine (MA) and copper nitrate were purchased from Sinopharm Chemical Reagent Co. (China). Phosphate buffer saline (PBS), poly acrylic acid (PAA), and hexadecyltrimethoxysilane (HDS) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Graphene quantum dot (GQD) nanomaterials were purchased from Graphene Supermarket (Calverton, United States). Nafion solution (5.0 %), histidine (His), cysteine (Cys), lysine (Lys), glycine (Gly), alanine (Ala), phenylalanine (Phe), tryptophan (Trp), glutamic acid (Glu), urea (Urea), uric acid (UA), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Beijing, China). All other reagents are of analytical grade. Deionized water (>18 Mohm) was obtained from an ultrapure water system

(Pall, USA). All glass containers were cleaned by aqua regia and ultrapure water.

Apparatus

The measurements of contact angles (CAs) were conducted using the contact-angle measurement machine (Jinhe, Jiangsu, China) to monitor the changing hydrophobicity and hydrophilicity properties of the slides patterned with HDS and further spotted wells during the step-by-step fabrication procedure of the well electrodes. Scanning electron microscopy (SEM, Hitachi E-1010, Japan), X-ray photoelectron spectrometer (XPS, Thermo ESCALAB 250XI), and UV-3600 spectrophotometer (Shimadzu, Japan) were utilized for the characterization of the prepared materials. Electrochemical measurements were conducted with an electrochemical workstation CHI 760D (CH Instrument, Shanghai, China) connected to a personal computer. A three electrode system was applied consisting of gold electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode.

Synthesis of GQD@MA-Cu nanocomposites

The graphene quantum dot (GQD)-scaffolded nanocomposites of melamine (MA) and copper (GQD@MA-Cu) were synthesized using MA, Cu nitrate and GQD. Briefly, MA (16 mM) was prepared using an aliquot of 0.0020 g MA dissolved in 1.0 mL of water at 60 °C and further cooled down to room temperature. Then, GQD (0.25, 0.5, 1.0, 1.5, and 2.0 mg/mL) was added to MA solution and sonicated for 1h. Under stirring, different concentrations of Cu nitrate (1.0, 2.0, 4.0, 8.0, 16, 32, and 64 mM) were dispersed in the MA-GQD mixture to react for 1 h. The so yielded GQD@MA-Cu nanocomposites were centrifuged and washed twice, and subsequently stored in dark.

Fabrication of the wettable well electrodes modified with GQD@MA-Cu nanorods

The fabrication process for the wettable well electrodes is schematically illustrated in **Scheme 1A**. The gold electrode was first cleaned with the Piranha solution (H_2SO_4 / H_2O_2 = 7 / 3), followed by being rinsed with deionized water, and then dried in nitrogen gas. Furthermore, a 2.5 μL aliquot of PAA (10 mg/mL) was spotted separately onto the sensing area of the gold electrodes and then dried to form the PAA-coated testing dot. Afterwards, the gold electrodes were dipped into the hydrophobic mixture containing HDS, isopropanol, and concentrated sulfuric acid at the mass ratio of 10 / 100 / 1 for 5 min. After the electrodes were taken out to be deposited with the PAA dots for 30 min, a 2.5 μL aliquot of 1.0 M NaOH was introduced to etch the spotted PAA dots. Furthermore, the resulting well electrodes were rinsed twice to be dried. Subsequently, an aliquot of 2.5 μL GQD@MA-Cu suspensions (5.37 mg/mL) in Nafion (5.0 %) was separately dropped onto the well electrodes to be dried and then stored at 4 ^{o}C.

Electroanalysis of His using the GQD@MA-Cu-modified well electrodes

The optimization of electroanalysis conditions of the GQD@MA-Cu-modified well electrode for sensing His were first conducted using different amounts of GQD@MA-Cu nanocomposites (2.69, 3.07, 3.58, 4.30, 5.37, 7.17, and 10.75 mg/mL), pH values (1.0, 3.0, 5.0, 6.0, 7.0, 8.0, 9.0, 11, and 13), ionic strengths (20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mM NaCl). Furthermore, the control tests were conducted accordingly with the GQD@MA-Cu-modified well electrodes using some other ions and compounds (50.0 μM), including CO_3^{2-}, PO_4^{3-}, SO_4^{2-}, F^-, Br^-, I^-, Na^+, Ca^{2+}, K^+, Mg^{2+}, Cys, Lys, Gly, Ala, Phe, Trp, Glu, Urea, UA, and BSA. In addition, the electroanalysis reproducibility was evaluated using six GQD@MA-Cu-modified well electrodes.

Under the optimized conditions, the GQD@MA-Cu-modified well electrodes were employed for the electroanalysis of different concentrations of His in buffer and urine samples. The linear sweep voltammetries (LSVs) were performed at the potentials ranging from - 0.20 to 0.60 V at a scanning speed of 100 mV/s. An aliquot of His of different concentrations (0 - 70.0 μM) was separately introduced into the optimized PBS (pH 7.0, containing 80.0 mM NaCl) for the electrochemical LSV measurements. Subsequently, according to the same procedure, the developed electroanalysis method was applied for the evaluation of His of different concentrations spiked in urine samples, which were stored in a refrigerator immediately after the collection and the spiking with His of different concentrations. In addition, a baseline correction of the resulting solid-state CuCl voltammograms was conducted with the CHI software. The current changes refer to the differences of current responses between the GQD modified well electrodes before and after adding His.

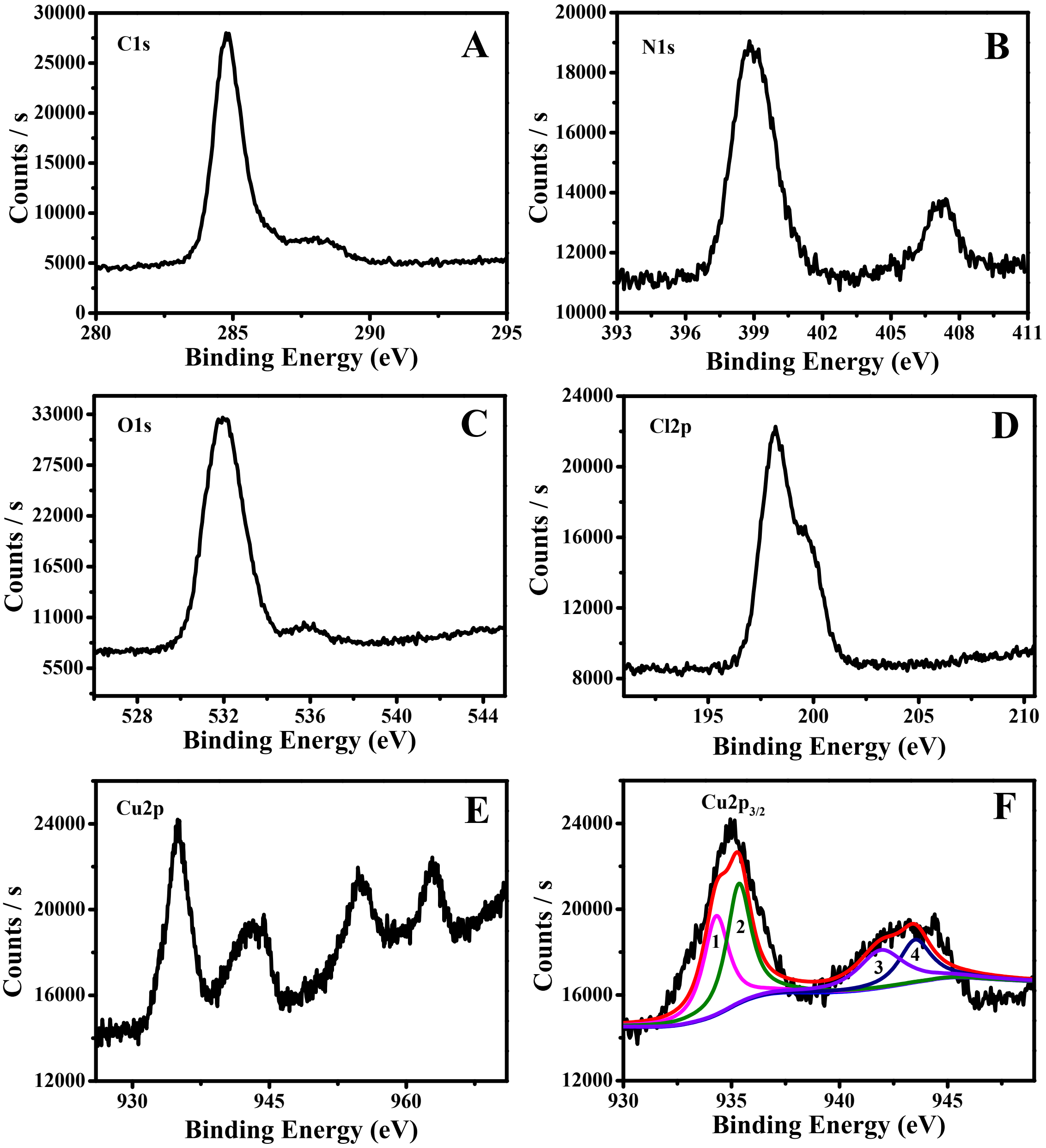

Fig. S1 XPS spectra of GQD@MA-Cu for the elements of (**A**) C, (**B**) N, (**C**) O, (**D**) Cl, (**E**) Cu, and (**F**) Cu elements in Cu2$p_{3/2}$, in which peak 1 refers to Cu^{+} and peak 2, 3, and 4 belong to Cu^{2+}.

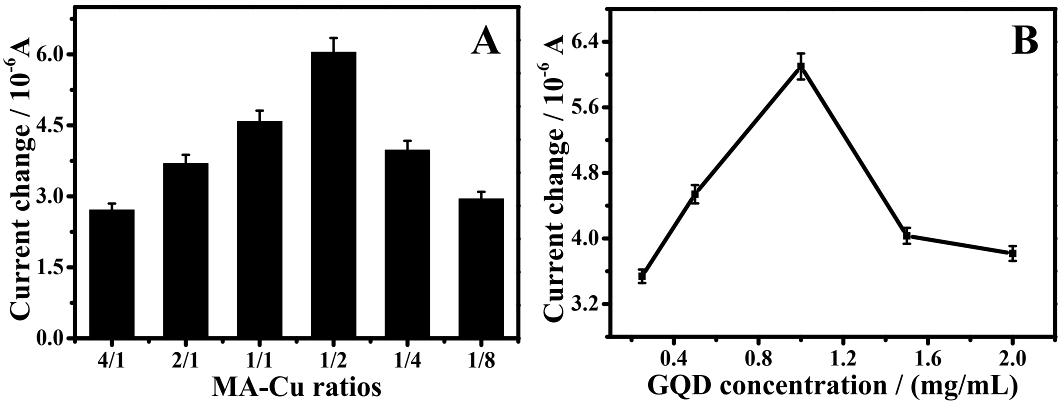

Fig. S2 Electrochemical responses of the GQD@MA-Cu-modified well electrode to His (50 μM) depending on (**A**) MA-Cu ratios and (**B**) GQD concentrations.

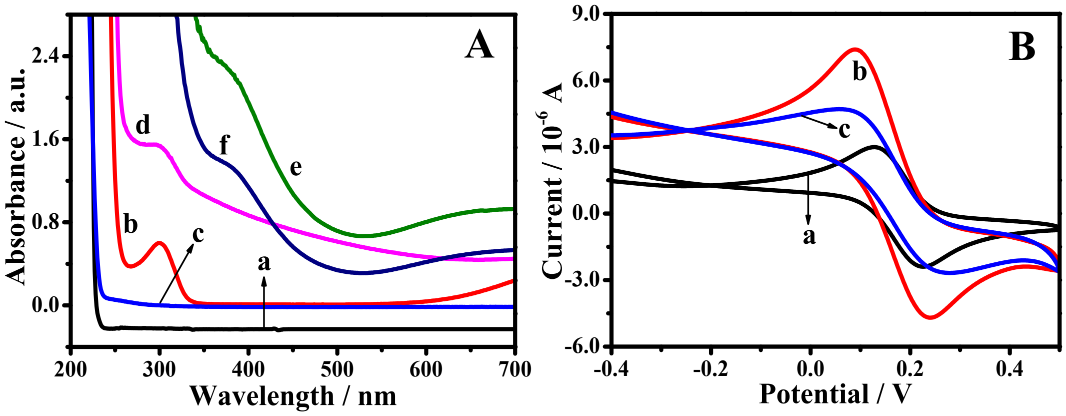

Fig. S3 (**A**) Comparable UV spectra among (a) MA, (b) $Cu(NO_3)_2$, (c) His, (d) MA-Cu, (e) GQD@MA-Cu, and (f) GQD@MA-Cu with His. (**B**) The $K_3[Fe(CN)_6]$ probes-based conductivities of the MA-Cu-modified well electrode (a) without GQD and (b) with GQD, and then (c) His.

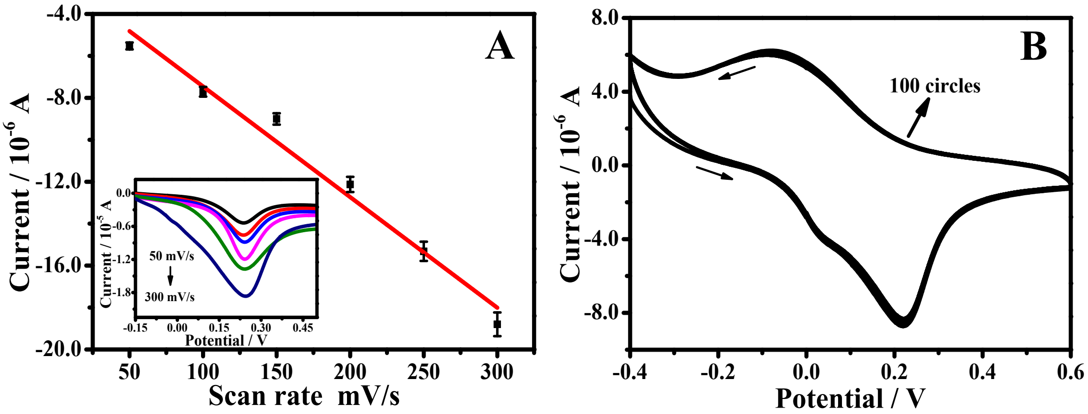

Fig. S4 (**A**) Sweep rates-dependent peaking currents of the GQD@MA-Cu-modified well electrodes at different scan rates, showing the corresponding LSV responses (insert). (**B**) Cyclic voltammograms of the as-developed electrode in PBS by scanning for 100 cycles at a sweep rate of 100 mV/s.

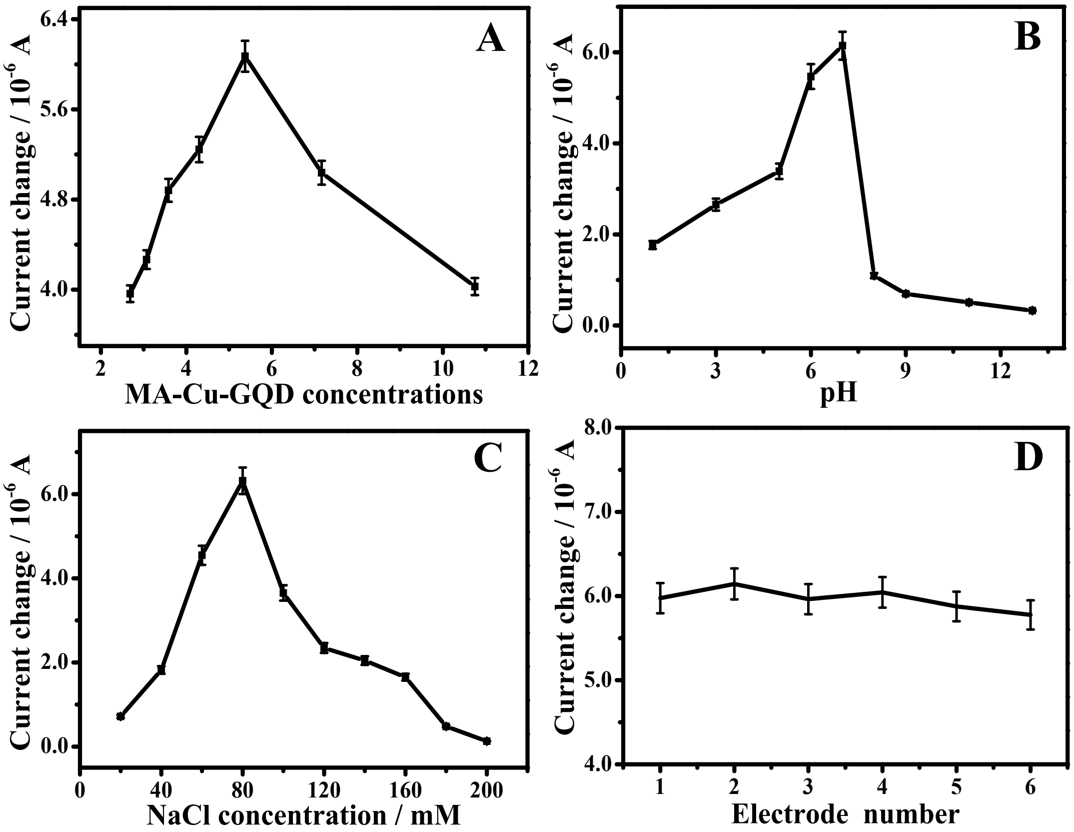

Fig. S5 Electrochemical His responses of the GQD@MA-Cu-modified well electrode depending on (**A**) GQD@MA-Cu dosages, (**B**) pH values (1.0 - 13.0), and (**C**) ion strengths in NaCl concentrations (20 - 200 mM) by the outputs of solid-state CuCl electrochemistry. (**D**) The electroanalysis reproducibility for sensing His (50.0 μM) using the GQD@MA-Cu-modified well electrodes.

Table S1. Comparison of analytical results for His in urine among different analysis methods

Analysis methods	**Linear ranges (μM)**	**LODs (μM)**
Colorimetric method with imidazole-functionalized silicon quantum dots[1]	0.010 – 1.0	0.010
Fluorimetric method with Ag/Au nanoclusters-Cu^{2+} ensemble probe[2]	0.25 – 9.0	0.087
Fluorimetric method with doped zinc sulfide quantum dots[3]	1.25 – 30	0.74
This work	0.50×10^{-6} – 50	1.25×10^{-7}

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2. J. Sun, F. Yang, D. Zhao, C. Chen and X. Yang, *ACS Appl. Mat. Interfaces*, 2015, **7**, 6860-6866.

3. W. Bian, F. Wang, Y. Wei, L. Wang, Q. Liu, W. Dong, S. Shuang and M. M. Choi, *Anal. Chim. Acta*, 2015, **856**, 82-89.