Supporting Information

Gly–Gly–His tripeptide and silver nanoparticles-assisted electrochemical evaluation of copper (II) ions in aqueous environment

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Experimental

Materials and chemicals

$CuSO_4$, NaCl, KCl, $AgNO_3$, $CaCl_2$, $CoSO_4$, $PbCl_2$, $ZnCl_2$, $Hg(NO_3)_2$, $CdCl_2$, $AlCl_3$, $CrCl_3$ and $FeCl_3$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Poly(diallyldimethylammonium chloride) (PDDA), 4-(2-hydroxyerhyl)piperazine-1-erhanesulfonic acid (HEPES), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 6-amino-1-hexanol, sodium borohydride ($NaBH_4$), and trisodium citrate were purchased from Sigma (USA). Gly–Gly–His tripeptide was synthesized and purified by China Peptides Co., Ltd. (Shanghai, China). The other reagents were of analytical graded and used as received.

Synthesis of silver nanoparticles

Bare silver nanoparticles (AgNPs) were prepared by borohydride reduction of $AgNO_3$ according to a previous report.[1] Generally, the solution of 0.25 mM $AgNO_3$ and 0.25 mM trisodium citrate was prepared. Next, the solution of 10 mM $NaBH_4$ was also prepared. 3 mL of $NaBH_4$ solution was added to 100 mL of the mixture of $AgNO_3$ and trisodium citrate under vigorous stirring. After a period of 30 min, AgNPs were formed, which were left to sit for 24 h. The synthesized AgNPs were further purified by three cycles of centrifugation at 12000g for 30 min.

Preparation of GCE

Before peptide modification, the substrate GCE was firstly treated with piranha solution (98% H_2SO_4: 30% H_2O_2 = 3:1) for 5 min (*Caution: Piranha solution is dangerous with organic matter*). After that,

it was rinsed with double-distilled water and carefully polished to a mirror-like surface with P3000 silicon carbide paper and then alumina slurries (1 μm, 0.3 μm, 0.05 μm), respectively. Subsequently, the electrode was cleaned by ultrasonication for 5 min in both ethanol and double-distilled water. After carefully rinsing and dried with nitrogen, the electrode was incubated with PDDA solution (4.0 g/L, 0.05 M NaCl) for 30 min. The pretreated electrode was further incubated with bare AgNPs for another 1 h.

Electrochemical measurement

Cu^{2+} with standard concentrations were firstly prepared. Gly–Gly–His was prepared with the concentration of 1 μM in 20 mM HEPES containing 10 mM TCEP. After mixing with different levels of Cu^{2+} for 15 min, AgNPs modified electrode was immersed in the above solutions for 2 h. Subsequently, the electrode was treated with 1 mM of 6-amino-1-hexanol for 30 min. Next, the modified electrode was measured by a CHI 660D electrochemical workstation (CH Instruments, China). A conventional three electrode was applied, which consisted of a saturated calomel reference electrode, a platinum auxiliary electrode and the modified GCE as the working electrode. EIS measurement was carried out with the parameters as follow: bias potential, 0.17 V; frequency range, 0.1 to 100000 Hz. The buffer was 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 mM KNO_3. EIS spectra were analyzed with ZSimpWin software.

Reference

1. P. Miao, K. Han, H. X. Sun, J. Yin, J. Zhao, B. D. Wang and Y. G. Tang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 8667-8672.

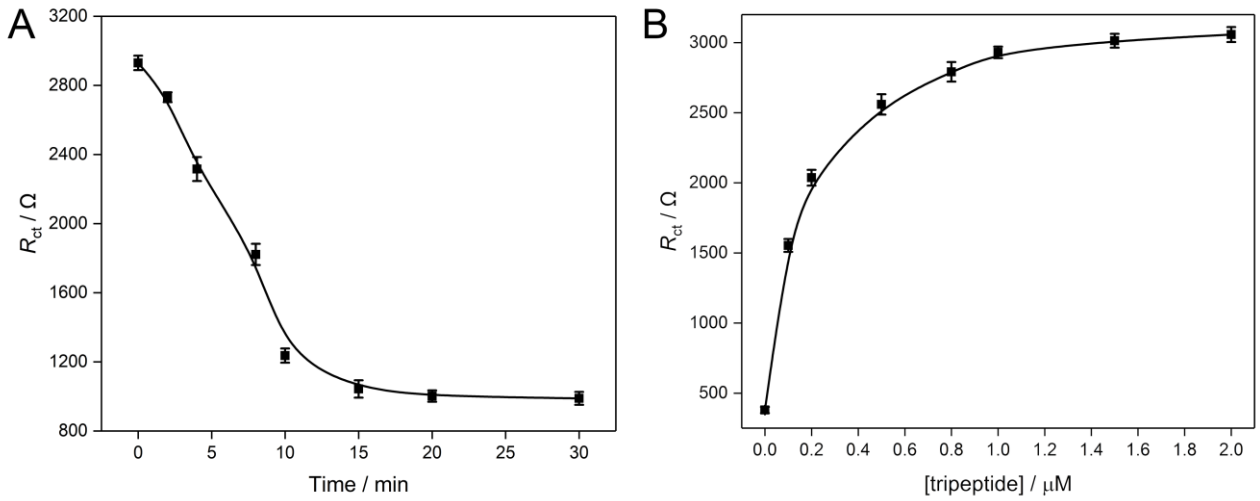

Fig. S1. Optimization of (A) the reaction time of Cu^{2+} and the tripeptide, (B) the concentration of used tripeptide.

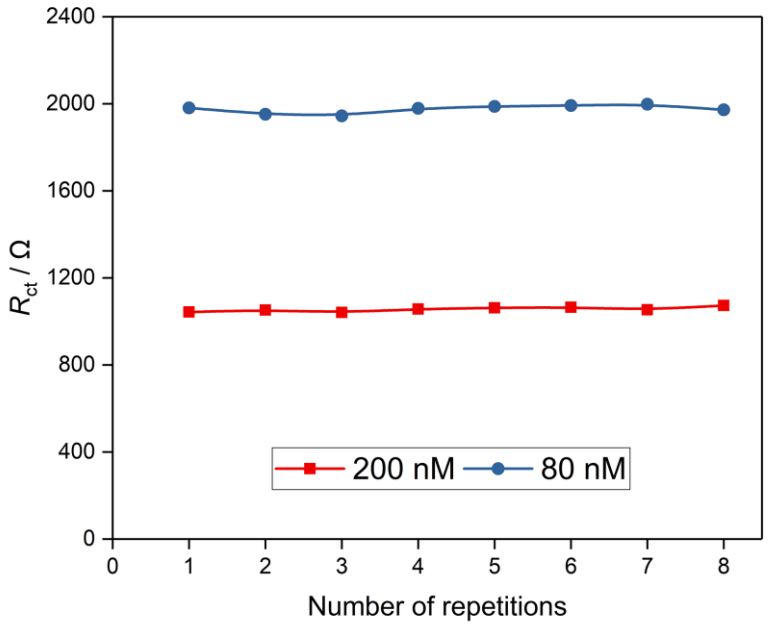

Fig. S2. Study of repeatability of the Cu^{2+} assay.