Supporting Information (SI)

Click Chemistry Reaction-Triggered 3D DNA Walking Machine for sensitive Electrochemical Detection of Copper ion

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The main contents of Supporting Information as follows:

The oligonucleotides sequences used in this

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Table S-1. The oligonucleotides sequences used in this work

Oligonucleotides	Sequence (from 5′ to 3′)
S1	SH-$(CH_3)_6$-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TAA CAC TGT CT -CH≡CH
S2	N_3- GGT AAA GAT GG
DNAzyme	CAA AAA CAT CTT TAC GCT rAG TCT TTT TTT TTG ATC CGA GCC GGA CGA AGC GAC AGT GTT -$(CH_3)_6$-SH
H1	SH-$(CH_3)_6$- CCA AGG ATA TAC GCT GTC TTT ACG CTA GAT GTG TAC CAG CGT AAA GAT GTT TTT G
H2	CTA GAT GTG TAC CTC TTT ACG CTG GTA CAC ATC TAG CGT ATT CT - methylene blue

Investigate of click chemistry reaction time by PAGE

The click chemistry reaction time was also optimized by polyacrylamide gel electrophoresis (PAGE) at room temperature. As shown in Figure S1, in the absence of target Cu^{2+}, a bright band with the same mobility (lane 3) as single strand S1 (lane 1) and S2 (lane 2) was observed. Introducing the Cu^{2+} induced the formation of a new band with slower mobility compared to that of strand S1 or S2, demonstrating the generation of the ligation product of S1+S2. With the increasing reaction time from 15 min to 105 min, the band of S1+S2 (lanes 4 to 10) was progressively obvious the tended to level off after 90 min, meanwhile the band of S1 or S2 gradually weaken, suggesting a time-dependent manner of click chemistry reaction.

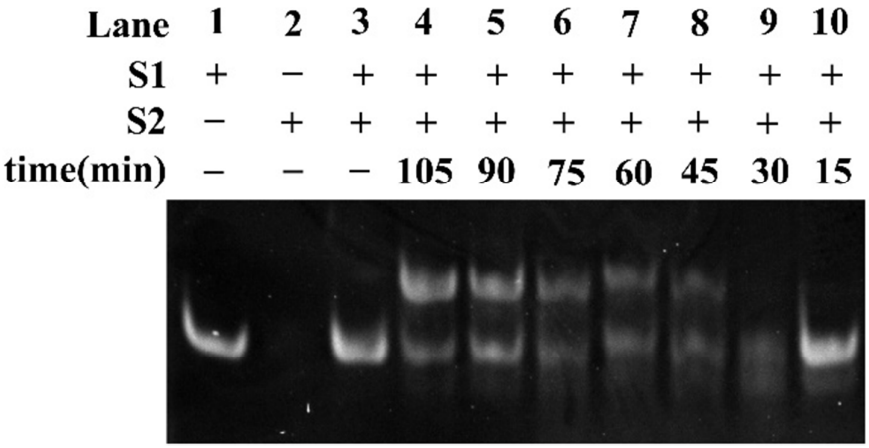

Figure S-1. Investigate of click chemistry reaction time by PAGE.

Estimation of the surface coverage of DNAzyme on the PSC@AuNPs

AuNPs with a diameter of 20 nm was prepared according to the reported method with minor modification.[1] Briefly, 2.5 mL trisodium citrate aqueous solution (34 mM) was added to 100 mL of boiling, vigorously stirred $HAuCl_4$ solution (0.01%, *w*/*v*), and heated and stirred for 15 min, then naturally cooled to room temperature. The concentration of AuNPs was determined by measuring their extinction at 450 nm (ε_{450} = 5.41×10^8 M^{-1} cm^{-1}) and it was 2 nM.[2] The size and shape of AuNPs were characterized using high-resolution transmission electron microscopy (HRTEM) at 200kV (FEI Co., America). The diameter of the nanoparticles is ~20 nm (Figure S-2).

DNA stands functionalized on PSC@AuNPs were quantitated according to the previous protocol.[3,4] Firstly, a standard linear calibration curve was prepared with known molal weight of DNAzyme by using the UV-visible absorption at 260 nm (Figure S-3A). Secondly, the PSC@AuNPs-DNAzyme solution was magnetically separated and washed two more times to obtain the supernatants of unconjugated DNAzyme, whereafter, the UV-visible absorption at 260 nm was measured (Figure S-3B). The absorbance maximum was converted to molal weight of the DNAzyme by a standard linear calibration curve. Thirdly, the number of DNAzyme strands on PSC@AuNPs was estimated by subtracting the amount of DNAzyme in the supernatant from the total amount of DNAzyme added into PSC@AuNPs solution. Finally, normalized surface coverage values were then calculated by dividing by the estimated total surface area of gold particles (assuming all of gold particles averagely encased on PSC) in the nanoparticle solution. Therefore, the average surface coverage

of DNAzyme on the PSC@AuNPs nanoparticles was 32.9 pmol/cm^2.

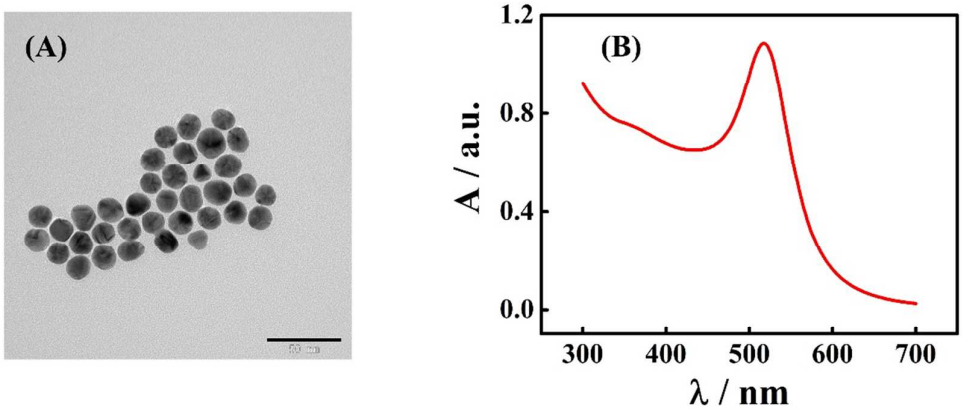

Figure S-2. (A) TEM micrograph of AuNPs; (B) Absorption intensity of AuNPs.

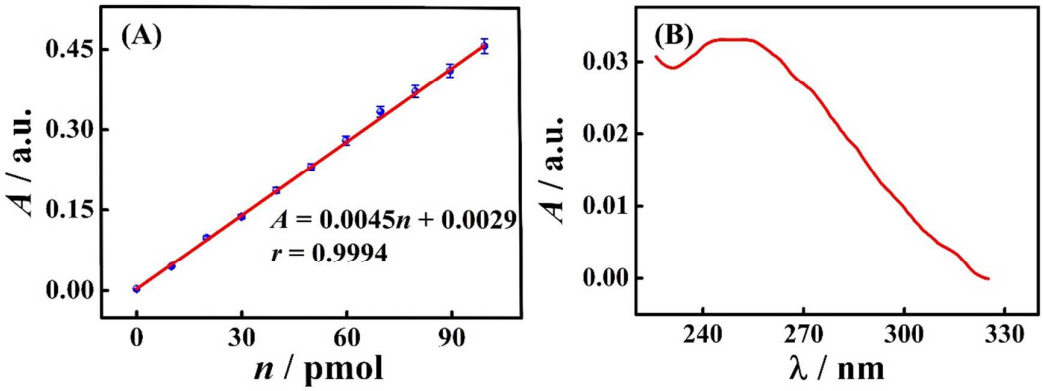

Figure S-3. (A) Standard linear calibration curve of absorbance maximums against the molal weight of the DNAzyme; (B) absorbance intensity of unconjugated DNAzyme.

Calculation of the surface coverage of H1 on the depAu/GCE

Effective surface area of the electrode (A, cm^2) was studied by typical cyclic voltammetric (CV) in 5 mM $Fe(CN)_6^{3-/4-}$ solution at different potential scan rates (10, 20, 30, 50, 80, 100, 120, 150,180, 200, 220, 250, 280, 300, and 320 mV/s, respectively). As shown in Figure S4A, the calculated electro-active surface area was 25.8 mm^2. The peak current (I_p) was determined by the Randles-Sevcik equation.[5,6]

$$I_p = (2.69\times10^5)\, n^{3/2}\, A\, D^{1/2}\, C\, v^{1/2} \qquad (1)$$

Where n is the number of transferred electrons for the redox reaction ($Fe(CN)_6^{3-}$ + $e^- \rightarrow Fe(CN)_6^{4-}$), D is the diffusion coefficient (25 ^oC, $6.70\pm0.02\times10^{-6}$ $cm^2\ s^{-1}$), C is the surface concentration of ferricyanide (5 mM), and v is the scan rate (mV s^{-1}).

It can be seen that magnitudes of both anodic (I_{pa}) and cathodic (I_{pc}) peak currents increase linearly with square root of scan rate ($v^{1/2}$) (Figure S4B). By performing linear regression for I_p versus $v^{1/2}$, the slope S can be obtained, and one may express A as

$$A = S/((2.69\times10^5)\, n^{3/2}\, D^{1/2}\, C) \qquad (2)$$

Under equilibrium conditions, the saturated amount of charge compensation is directly related to the number of anionic phosphate residues in DNA. The surface coverage of H1 on the gold nanoparticles-assembled glassy carbon electrode (depAu/GCE) can be measured using chronocoulometry. The functionalised depAu/GCE were first immersed in pure 10 mM Tris-HCl buffer (pH 7.4), and the potential was set as conducted previously and stepped from 0.2V to -0.5V (versus Ag/AgCl). The resulting charge flow was measured. The electrode was then immersed

in a saturation solution of 50 μM hexaammineruthenium(III) (Ruhex) in Tris-HCl buffer (10 mM, pH 7.4).

Both solutions were purged with N_2 for at least 15 min prior to the experiments.

According to the results of Figure S4C, the surface coverage of the H1 on the depAu/GCE was estimated to be 2.68×10^{12} molecules/cm^2. The integrated current, or charge Q, as a function of time t in a chronocoulometric experiment is given by the integrated Cottrell expression.[7,8]

$$Q = (2n\,F\,A\,D_o^{1/2}\,C_o^{*}\,t^{1/2})/\pi^{1/2} + Q_{dl} + nFA\Gamma_o \qquad (3)$$

where n is the number of electrons per molecule for reduction, F is the Faraday constant (C equivo^{-1}), A is the effective surface area of working electrode (cm^2), D_o is the diffusion coefficient (cm^2 s^{-1}), C_o* is the bulk concentration (mol/cm^2), Q_{dl} is the double-layer charge (C), and $nFA\Gamma_o$ is the charge from the reduction of Γ_o (mol/cm^2) of adsorbed redox marker. The term Γ_o designates the surface excess and represents the amount of redox marker confined near the electrode surface.

The chronocoulometric intercept at t = 0 is the sum of the double-layer charging and the surface excess terms. The surface excess is determined from the difference in chronocoulometric intercepts for the identical potential step experiment in the presence and absence of redox marker.

$$\Gamma_o = (Q_{ds}\text{-}Q_{dl})/nFA \qquad (4)$$

The saturated surface excess of redox marker is converted to DNA probe surface density with the relationship,

$$\Gamma_{DNA} = \Gamma_o\,(z/m)(N_A) \qquad (5)$$

where Γ_{DNA} is the probe surface density in molecules/cm^2, z is the charge of the redox

molecule, *m* is the number of bases in the probe DNA, and N_A is Avogadro's number.

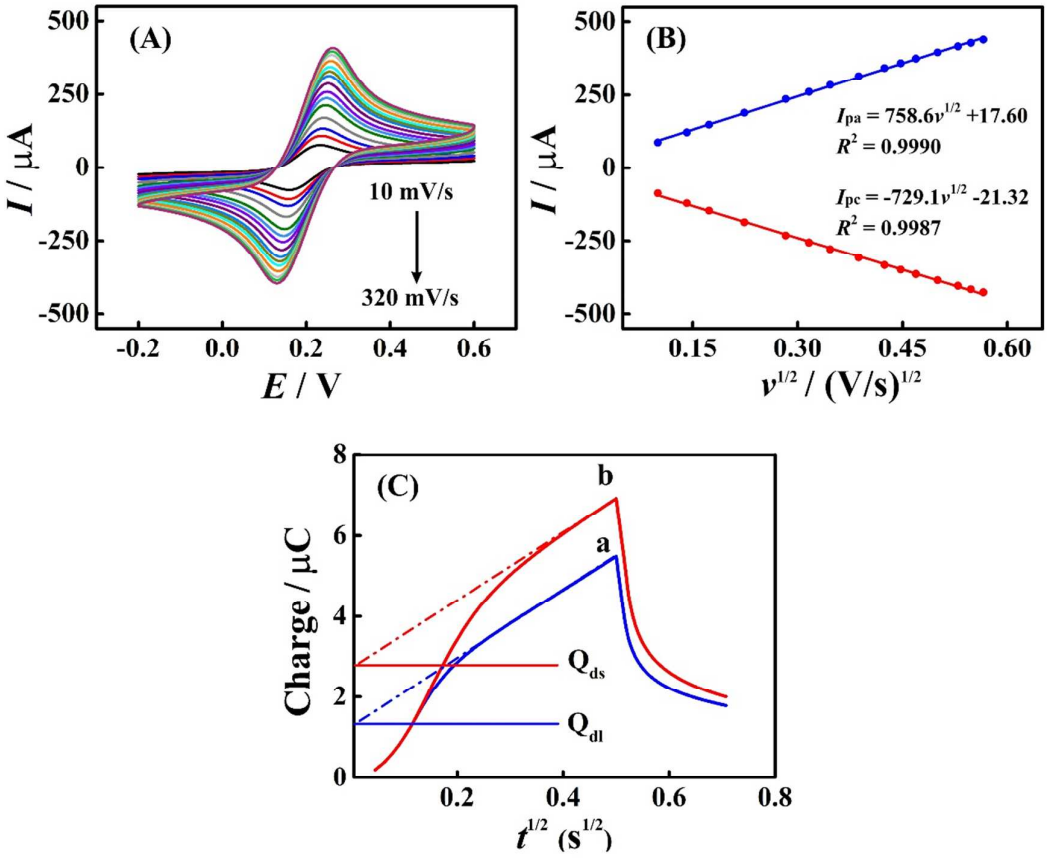

Figure S-3. (A) CV curves of the depAu/GCE in 5.0 mM $Fe(CN)_6^{3-/4-}$ at different scan rates (10, 20, 30, 50, 80, 100, 120, 150,180, 200, 220, 250, 280, 300, and 320 mV/s, respectively). (B) The linear relations of the depAu/GCE with the anodic and cathodic peak current against the square root of scan rate. (C) Representative chronocoulometric curves for H1/MCH/depAu/GCE modified electrode in the absence (curve a, Q_{dl}) and presence (curve b, Q_{ds}) of 50 μM Ruhex. Redox charges of Ruhex bound to DNA are obtained from chronocoulometric intercepts at $t = 0$.

Reference

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