Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Bio-Inspired Dynamic Biomolecule Assembling for Fine Regulation of Protein Activity

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Apparatus. All electrochemical experiments, including electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and chronoamperometry (CA), were carried out on an autolab electrochemical workstation (Metrohm, The Netherlands) at room temperature using a conventional three-electrode system. Au wire, Ag/AgCl, and platinum wire were employed as the working electrode, reference electrode and counter electrode, respectively. Fluorescence and light-scattering experiments were conducted on a fluorescence spectrometer (Hitachi. Ltd., F-4600). UV-*vis* absorbance measurements were performed with a UV-*vis* spectrophotometer (Shimadzu). CD spectra were recorded by a Jasco J-810 spectropolarimeter. Atomic force microscopy (AFM) images were recorded using a Park AFM NX10 System with XSC11/Pt AFM tips (Mikromasch). Thrombin clotting time (TCT) was measured on an automatic coagulation analyzer (ACL TOP 700, American). Confocal fluorescence imaging was performed on a confocal laser scanning microscope (CLSM) (Nikon, Eclipse TE2000-E).

Materials and Reagents. All oligonucleotides were obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). All the synthetic oligonucleotides were HPLC purified and freeze-dried by the supplier. Their sequences were listed in Supporting Information (Table S1). The DNA sequences were dissolved in PB buffer (10 mM, pH 7.4), to give a stock solution of 100 μM. Before use, the hairpin DNA (HP1, HP2) were heated to 95 °C for 5 min and then allowed to cool to room temperature to form stem-loop DNA structure. Human α-thrombin (≥ 1000 NIH units/mg protein), fibrinogen were obtained from Sigma (St. Louis, MO) Tris (2-carboxyethyl) and metal salts (KOH, NaCl, KCl, $MgCl_2$, $CaCl_2$, $K_3Fe[CN]_6$ and $K_4Fe[CN]_6$) were purchased from Sigma-Aldrich (St. Louis, MO). Hemin, Triton X-100, dimethyl sulfoxide (DMSO), 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich. All chemicals were of analytical reagent grade and used without further purification. Ultrapure water from a Millipore Milli-Q water purification system with an electric resistance >18.2 MΩ·cm (Billerica, MA, USA) was used throughout all the experiments.

Au wire Electrode Pretreatment. Firstly, Au wire electrodes (0.5 mm diameter, geometrical area calculated 0.18 cm^2) were boiled in hot KOH for 2h, followed by rinsing thoroughly with ultrapure water and sonicating three times for 5 min. Secondly, the Au wire electrodes were treated with a piranha solution (H_2SO_4/H_2O_2 = 3:1 V/V) for 30 min and then were sonicated with

ethanol and water three times for 5 min. Finally, the electrodes were stored in concentrated sulfuric acid. Before modification, the electrodes were swept ten consecutive potential cycles from 0.3 to 1.55 V in 0.5 M H_2SO_4 solution at a scan rate of 50 mV s^{-1}.

Preparation of the HS-DNA/Au Wire Electrode. Before modification, the HS-DNA was treated with TCEP (1.0 mM) for 1 hour to reduce the disulfide bonds. Clean Au wire electrodes were incubated in HS-DNA (200 μL, 300 nM) solutions for 12 h at room temperature. Subsequently, the electrodes were reacted with 1 mM of MCH solution for 20 min. The PB buffer was used to remove any non-specifically adsorbed DNA.

Electrochemical Measurement Procedure. CV responses of the obtained electrodes were recorded in Tris-HCl buffer (25 mM, 150 mM NaCl, 5.0 mM KCl, 1.0 mM $MgCl_2$, 1.0 mM $CaCl_2$, pH 7.4) with the potential window from 0 to -1.4 V (*vs* Ag/AgCl). CA responses of the obtained electrodes were recorded in Tris-HCl buffer with a fixed potential at -0.9 V for 30 seconds. EIS responses were performed in KCl (0.1 M) containing 5.0 mM $[K_3Fe(CN)_6]/[K_4Fe(CN)_6]$ (1:1) through applying a 5 mV (peak amplitude) sinusoidal voltage perturbation to the obtained working electrodes at an open circuit potential over a frequency range from 100 kHz to 0.1 Hz. All the obtained spectra were fitted using the Nova 1.1 software. Chronocoulometric analysis was conducted in Tris buffer (10 mM, pH 7.2) containing 300 mM RuHex, and a potential pulse from 0.5 to 0.2 V with an interval time of 0.0025 s was employed.

Fluorescence Measurements. All fluorescence measurements were carried out on an F2500 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength was set at 480 nm, and the emission spectra were recorded from 500 to 650 nm. Excitation (Ex) and emission (Em) slits were both set at 5.0 nm, and the excitation voltage was 700 V. Fluorescence emission spectra were then obtained with a 0.2 cm × 1 cm quartz cuvette containing 200 μL of solution.

Gel Electrophoresis Verification of Synthetic DNA Nanowires. Non-denaturing polyacrylamide gel electrophoresis (PAGE) was employed to verify and characterize hierarchically assembled DNA nanowire. All samples were loaded into an 8 % native PAGE, and the electrophoresis was performed at a constant voltage of 110 V in 1 × Tris-borate-EDTA (TBE) buffer (89 mM tris(hydroxymethyl)aminomethane, 2 mM ethylenediaminetetraacetic acid and 89 mM boric acid, pH 8.0) for 1.5 h. The gel was stained by GelRed for 30 min and imaged by a ChemiDoc™ MP System (Bio-Rad).

Catalytic Oxidation of ABTS by DNA Nanowires. A hemin stock solution was prepared in the solution containing 0.025% (v/v) Triton X-100 and 0.5% (v/v) DMSO. Freshly diluted hemin was added to DNA solutions and incubated for 40 min at 37 °C. Then 2 mM ABTS and 0.4 mM H_2O_2 were added into the solutions to initiate the reaction. Absorbance at 415 nm (the radical anion $ABTS^{\bullet-}$) was measured by a UV-*vis* spectrophotometer.

CD Spectroscopy. CD spectroscopy was performed on a Jasco J-810 spectropolarimeter in glass cuvettes with a path length of d = 1 mm. Scans were recorded over a range from 240-320 nm with a scanning speed of 20 nm min^{-1}. HP1 and HP2 were dissolved in 200 μL buffer at a final concentration of 5 μM.

AFM Characterization. The freshly cleaved mica was pretreated with $NiCl_2$ (2 mM) solution, loaded with the DNA samples for 5 min at room temperature, rinsed with ultrapure water, and finally dried under N_2. The dry samples were scanned in tapping mode using a Park AFM NX10 System with XSC11/Pt AFM tips (Mikromasch) at their resonant frequency.

Real-Time Monitoring of the Clotting Reactions by Light Scattering Measurements. Thrombin can catalyze the fibrinogen to produce insoluble fibrin, which leads to an increase in the intensity of the scattered light. Typically, 5 μL of fibrinogen (10 mg mL^{-1}) was quickly added to 195 μL of DNA nanowire-inhibited thrombin solution. The scattering intensities were measured on a fluorescence spectrophotometer where Ex and Em wavelengths were both set as 650 nm and slits were 5.0 nm. The pure thrombin without any treatment was employed as the internal control.

Monitoring of Dynamic Morphological Changes of Fibrinogen. Laser scanning confocal microscopy (LSCM) was used to monitor the conversion of fluorescent fibrinogen into visible fibrin fibers. In a typical experiment, Alexa Fluor 488 labeled fibrinogen (1 μL, 10 mg mL^{-1}) was used as substrate and added to the 50 μL samples of pure thrombin and DNA nanowire-inhibited thrombin. The mixture was immediately dropped on the bottom of small Petri dishes for laser scanning confocal microscope imaging. The images were obtained every 1 min with the excitation at 488 nm until the reactions were complete.

Human Plasma Tests. To further test the ability of the electronic-triggered DNA nanowires to regulate thrombin activity in a biologically relevant medium, human plasma tests were designed. Human blood samples (3 mL) provided by Zhongnan Hospital of Wuhan were mixed with sodium citrate solution (333 μL, 0.109 M) and then centrifuged (3000 rpm, 3 min) to obtain plasma for

subsequent analysis. Then thrombin with different treatments was added to the human plasma, and the thrombin clotting time (TCT) was measured with an automatic ACL TOP 700 LAS hemostasis analyzer (Beckman Coulter Inc., Brea, CA). For the TCT measurement, scattering intensity was continuously monitored until it reached the plateau, and the first point at half-width was regarded as the clotting time.

Table S1. DNA sequence (5'-3'):

HS-DNA: HS-GCC ATT GTC GAA CAC CTG CTG GAT GAC CAG C

HS-DNA-1: HS-TAT GTC ACG AGT CAC TAT

17-F1: GCT GGT CAT CCA GCA GCG GTC GAA ATA GTG ACT CGT GAC

17-F2: TTT CGC CAT CTT CTC CGA GCG TGT TCG ACA ATG GC

Substrate: BHQ-GTC ACG AGT CAC TAT rA GGA AGA TGG CGA AA-FAM

Substrate-1: TAT GTC ACG AGT CAC TAT rA GGA AGA TGG CGA AA

HP1: CGG GAT GGT GCC TGA ATA GTG ACT CGT GAC ATA GTC ACT ATT CAA AA AAA TCA GTG GGG TTG GA

HP2: AGG TTG GGG TGA CTT ATG TCA CGA GTC ACT ATT TTT TTT GAA TAG TGA CAG TCC GTG GTA GGG

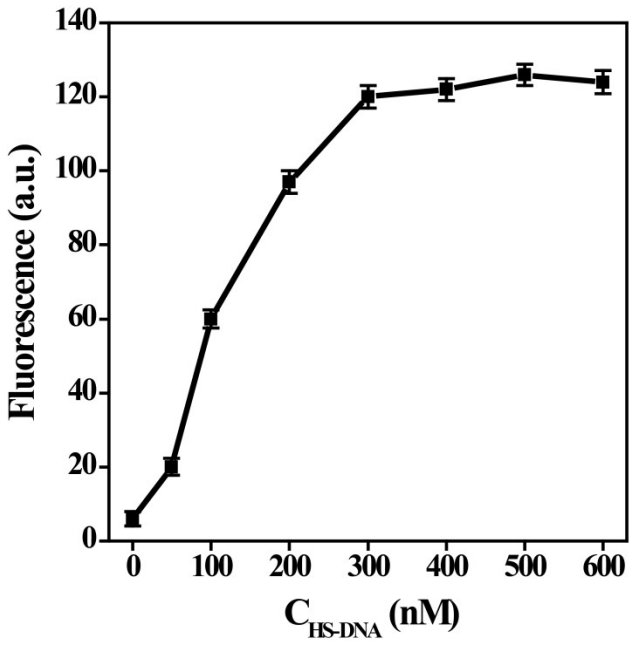

Figure S1. The optimization of the concentration of HS-DNA.

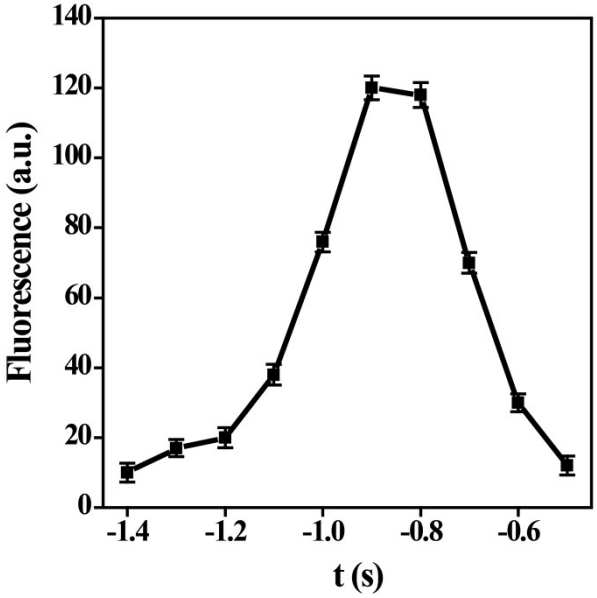

Figure S2. The optimization of the voltage applied to the electrode surface.

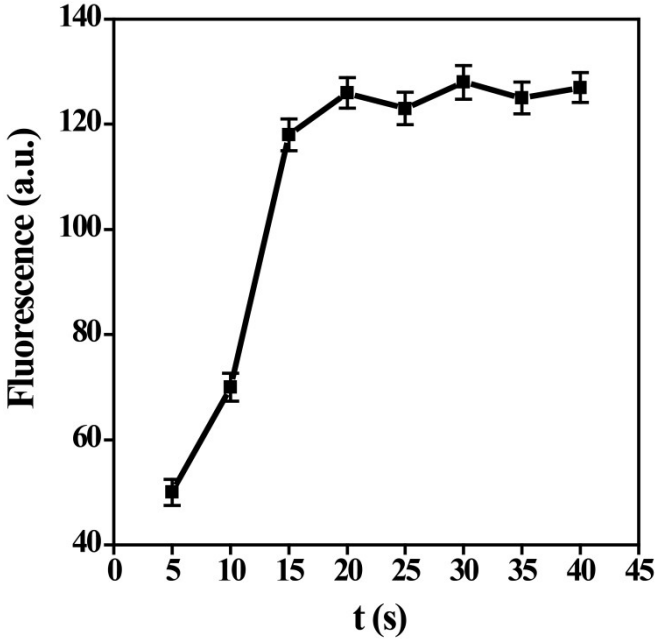

Figure S3. The optimization of electronic input time.

The concentration of DNA strands modified on the Au wire electrode was determined via chronocoulometry. And calculate the DNA concentration using Tarlov's method[1,2]:

$$\Gamma_{Ru} = (Q_{ds} - Q_{dl})/nFA \quad (1)$$

$$\Gamma_{DNA} = \Gamma_{Ru}(z/m)(N_A) \quad (2)$$

Where Γ_{Ru} is the amount of the redox marker confined near the electrode surface, Q_{ds} and Q_{dl} are surface charges, n is the number of electrons in the reaction, F is the Faraday constant, A is the area of the working electrode, Γ_{DNA} is the probe surface density in molecules/cm^2, z is the charge of the redox marker, m is the numbers of the bases in probe DNA, and N_A is Avogadro's number. As can be shown in Figure S4, the surface density of immobilized DNA was calculated to be 2.76×10^{-12} mol/cm^2.

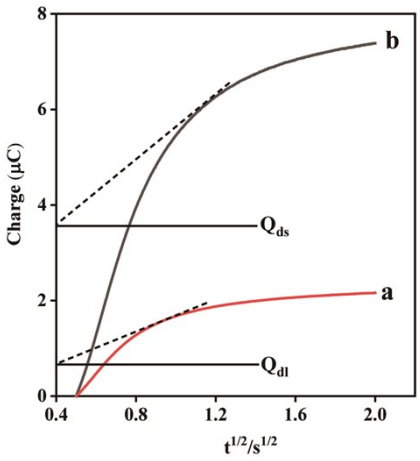

Figure S4. Chronocoulometric response curves for MCH (a) and DNA (b) modified electrodes in the presence of 300 μM $[Ru(NH_3)_6]^{3+}$ (RuHex) in 10 mM pH 7.2 PB buffer.

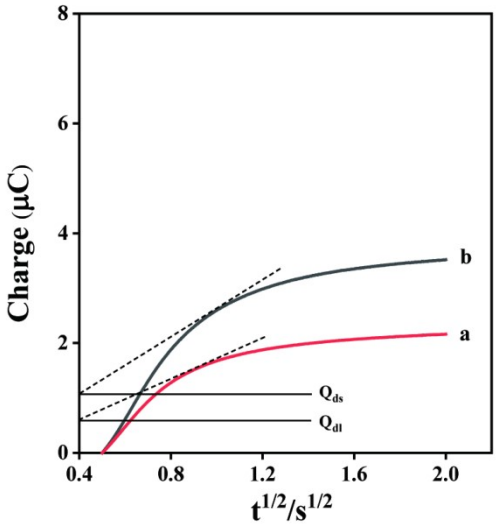

Figure S5. Chronocoulometric response curves for (a) MCH modified electrode and (b) HS-DNA modified electrode after electronic input in the presence of 300 μM $[Ru(NH_3)_6]^{3+}$ (RuHex) in 10 mM pH 7.2 PB buffer.

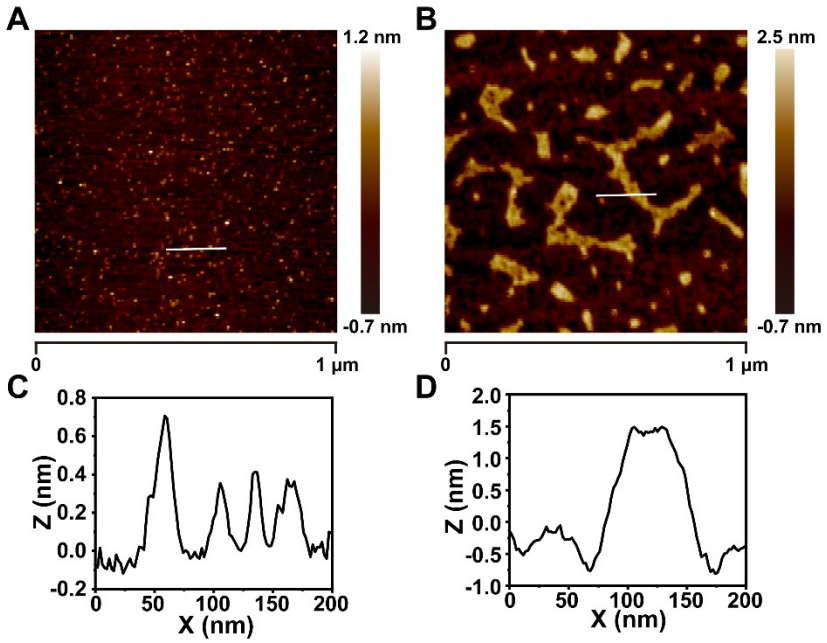

Figure S6. AFM images and corresponding cross-section analysis of the DNA assembly (nanowires) resulting from all the nucleic acids without electron and Ca^{2+} stimuli (A, C) or with electron and Ca^{2+} stimuli (B, D). The height increases from ~0.6 to ~1.5 μm corresponding to ssDNA and dsDNA.

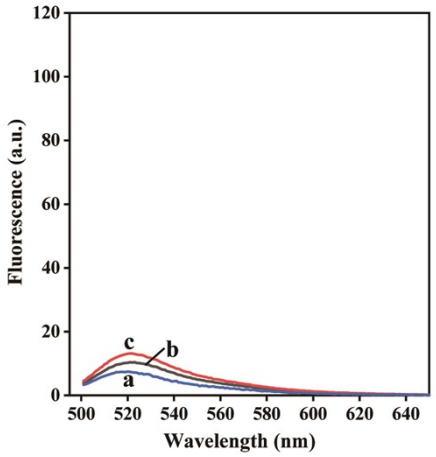

Figure S7. System re-cyclability research. (a) bare Au wire electrode, (b) Au wire electrode (Au-S band breaks) applied to -0.9 V voltage again for 15 s, (c) Au wire electrode (Au-S band breaks and without any treatment) re-modified 300 nM HS-DNA and applied to -0.9 V voltage again for 15 s. Overall, the above results indicated no evident fluorescence signals were released from the substrate of DNAzyme, revealing the inferior re-cyclability properties of the stimuli-triggered system.

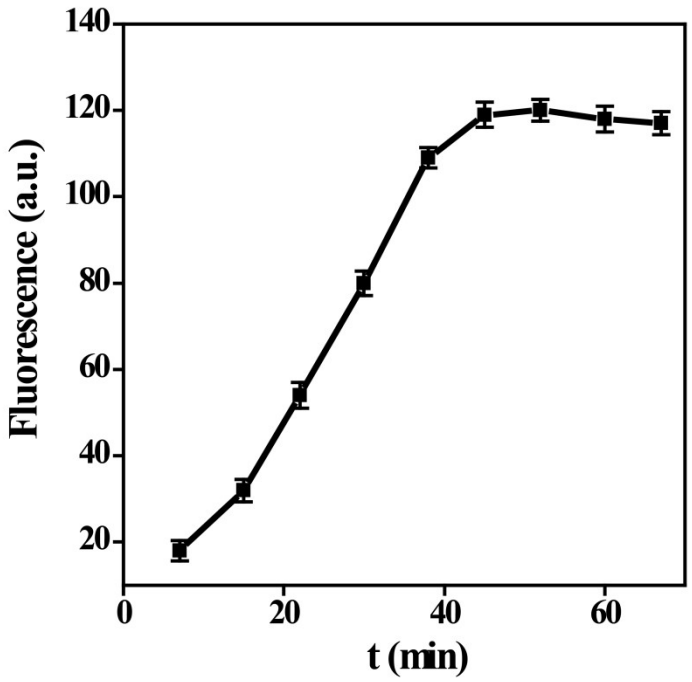

Figure S8. The optimization of the reaction time of HS-DNA and (2)/(3)/Substrate.

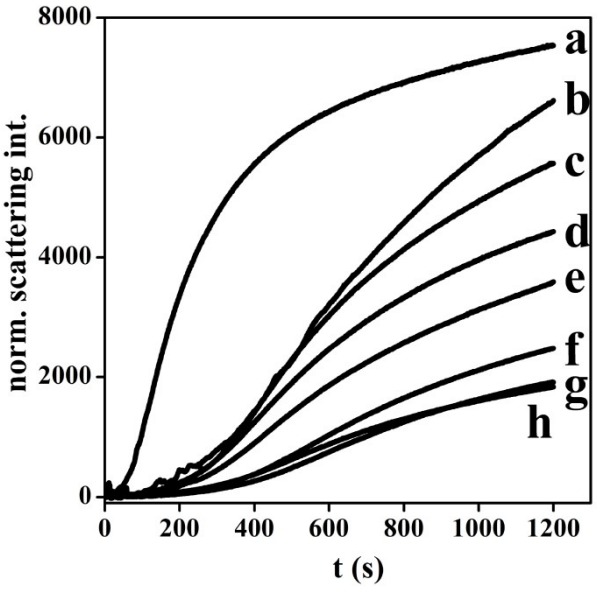

Figure S9. The optimization of the concentration of HP1 and HP2, a-h: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 μM.

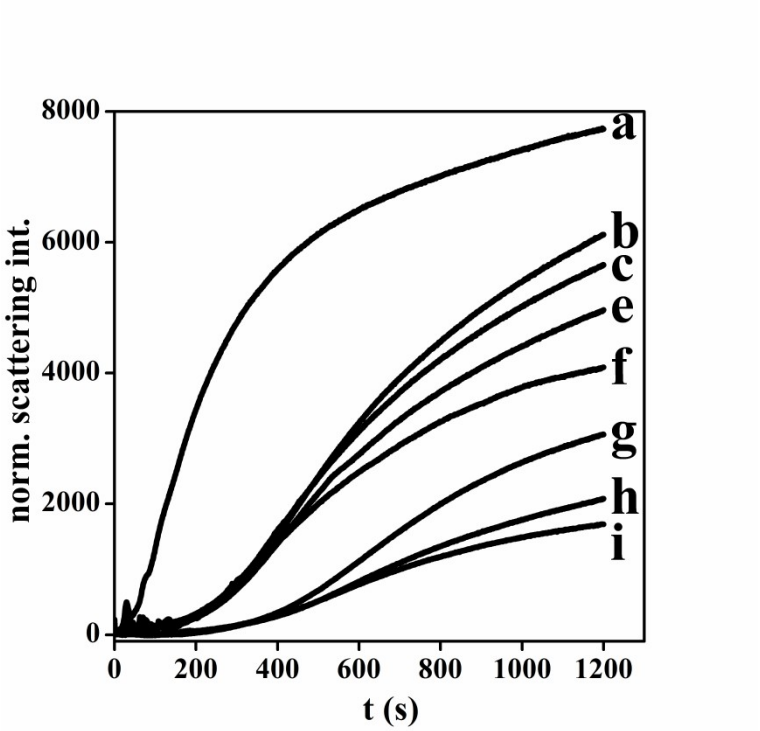

Figure S10. The optimization of the HCR reaction time, a-i: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 h.