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Electronic Supplementary Information (ESI)

for

Time-resolved visual detection of heparin by accelerated etching of gold nanorods

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1. **Supplementary information for experimental details**

1.1 Reagents

Gold nanorods (AuNRs) were obtained from NanoSeedz™ Co., Ltd. (Hongkong, China). Silver nitrate ($AgNO_3$), L-ascorbic acid (L-AA), ferric trichloride ($FeCl_3 \cdot 6H_2O$), thiourea (TU) and heparin sodium were offered by Aladdin Reagent Co., Ltd. (Shanghai, China). All reagents were analytical grade, which were used as received without further purification. Milli-Q water was used throughout the whole work. Slides were supplied by Changsheng Dingguo Biotechnology Co. Ltd. (Beijing, China).

1.2 Apparatus

The absorption and light scattering spectra of AuNRs were acquired by a U-3600 spectrophotometer (Hitachi, Tokyo, Japan) and a spectrometer charge-coupled device (DU970P-BVF, Andor), respectively. The shape of AuNRs was scanned with an S-4800 scanning electron microscope (Hitachi, Tokyo, Japan). The light scattering dark-field imaging was obtained by a BX51 optical microscopy (Olympus, Japan) equipped with a high numerical dark-field condenser (U-DCW, 1.2–1.4) and true-color charge-coupled device (CCD) camera (Olympus, Japan), which was further analyzed with Image-Pro Plus 6.0 (IPP) software (Media Cybernetics, USA). The light scattering spectra were scanned with a monochromator (SR303i-B, Andor) and a spectral CCD (DU970P-BVF, Andor)

1.3 **The SEM imaging of AuNRs**

Silicon chips were employed as the substrate for SEM imaging, which were cleaned with ethanol and acetone under ultrasonic and dried by nitrogen. Then, 20 μL of AuNRs solution (0.01 nM) was dropped onto the surface of silicon chip and dried at room temperature. Following, silicon chips were immerse into the mixture of heparin sodium (1 μg/mL), Fe^{3+} (0.05mM), TU (1 mM) and HCl (0.03 M), which were took out at different immerse (0 min, 10 min, 20 min, 30min). Finally, the silicon chips were cleaned with water and dried by nitrogen, which were transferred for SEM imaging with voltage at 25 kV and current at 20 μA.

1.4 **The establishment of standard curve for the determination of heparin**

First, AuNRs were deposited onto the glass slides. Then, 5×10^{-5} M Fe^{3+} at pH 1.5, 1×10^{-3} M TU and heparin with the final concentrations at 0, 0.1, 0.3, 0.5, 0.7, 1, 2, 5

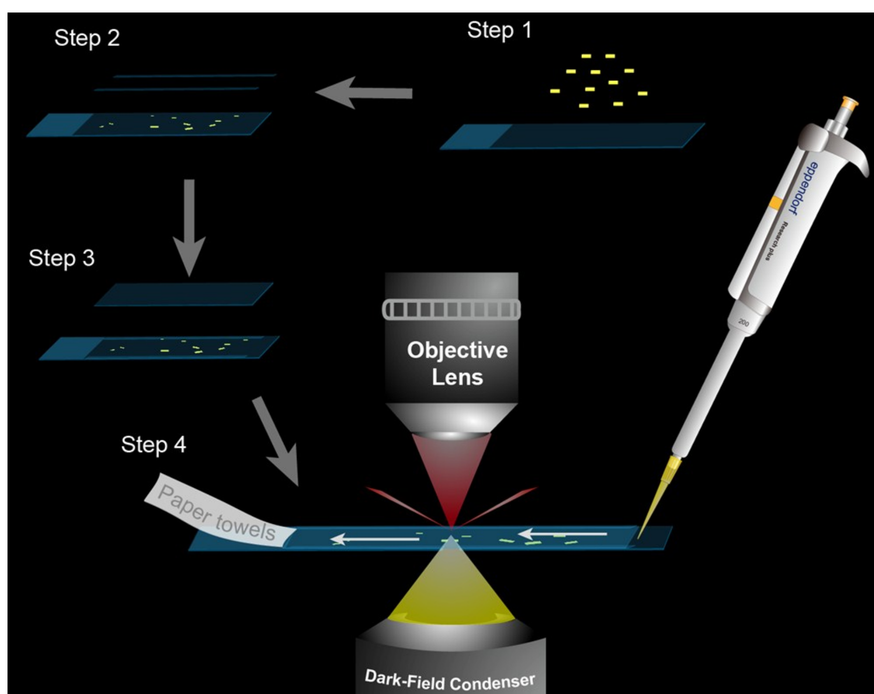
and 10 μg/mL were well mixed, which were injected into the flow cell respectively. The reaction time was record from the injection of the above mixture until to the light scattering turned green, and the DFM images were captured at an interval of every 5 minutes. Finally, a standard curve was established based on the concentration of heparin sodium and the time for the light scattering of AuNRs turning from red to green.

1.5 **The procedure for the detection of heparin injection**

First of all, The heparin sodium injection samples (Tianjin Biochem Pharmaceutical Co., Ltd. And Changzhou Qianhong Biochemical Pharmaceutical Co., Ltd) were diluted 20 000 times with ultrapure water before detection. Then, AuNRs were deposited onto the glass slides, and 5×10^{-5} M Fe^{3+} at pH 1.5, 1×10^{-3} M TU and diluted heparin sodium injection samples were well mixed, which were injected into the flow cell. The reaction time was record from the addition of the above mixture until to the light scattering turned green, and the DFM images were captured at an interval of every 5 minutes.

1.6 **The DFM imaging of AuNRs**

The detection of sodium heparin was based on the light scattering change of AuNRs, which was operated as follows. Firstly, 200 μL of AuNRs solution was precipitated on the clean glass slide for 30 min, which was rinsed into distilled water was to remove the unbound AuNRs. Secondly, one thin cover glass with double-sided adhesive was sticked onto the above glass slides to make the reaction channels, which were cleaned with distilled water and dried with nitrogen. Thirdly, in order to real-time monitor the process of the light scattering change promoted by different concentrations of heparin, a number of reaction channals were prepared to inject the mixture (5×10^{-5} M Fe^{3+} at pH 1.5, 1×10^{-3} M TU and heparin at different concentrations) with peppited at one end of the glass slides. On the other end of the glass slides, paper towels were used to to absorb water so that the solution was able to well react with AuNRs. Finally, the DFM imaging was monitored under a dark-field microscope with a true color CCD camera and a 100× objective lens (Scheme S1).



Scheme S1 The diagram of detecting heparin under DFM.

Step 1, AuNRs were deposited onto the surface of glass slides; Step 2, two fine channels were carved along the long sides of slide glass; Step 3, the cover glass was matted on the above slide glass; Step 4, The glass was put on the dark-filed condenser, and then the mixture was injected from one end of the glass, and paper towel was put at the other side of glass slide to absorb water so that the solution was able to well react with AuNRs.

2. Supplementary information for investigation results

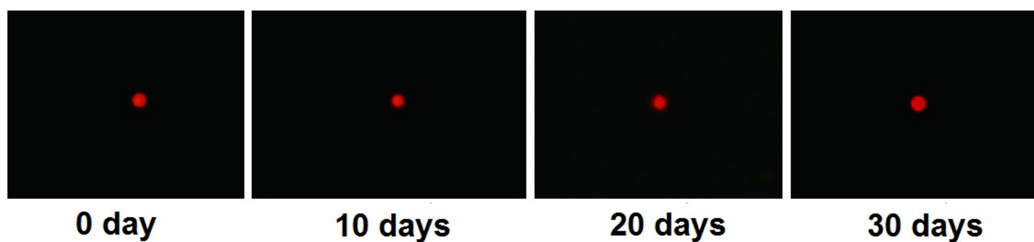


Fig. S1 The light scattering stability of AuNRs.

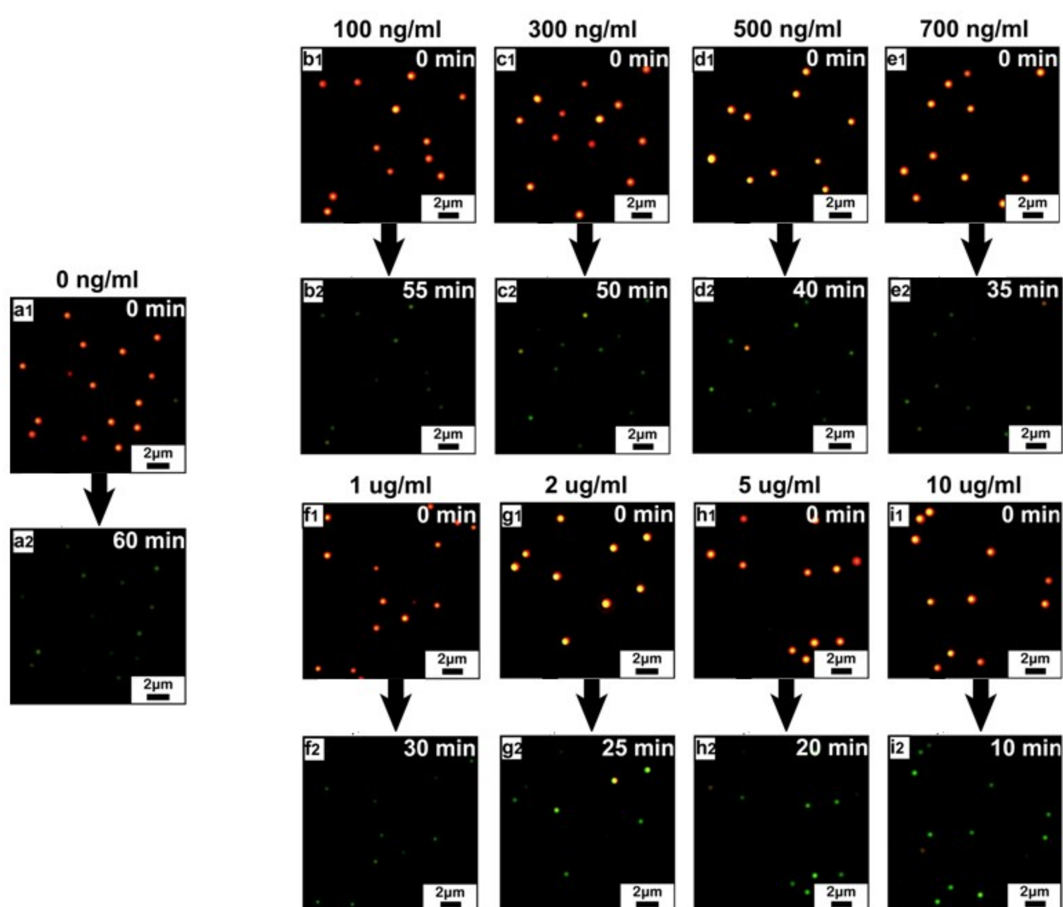


Fig. S2 The concentration-dependent etching process of AuNRs accelerated by heparin. a1-i1, The DFM images of AuNRs before etching. a2-i2, The DFM images of the AuNRs after etching by the mixture of Fe^{3+} (0.05 mM), TU (1 mM), HCl (0.03 M) and different concentrations of heparin sodium.

Table S1 The sensitivity comparison between various methods

Methods	Probes	Detection range	limit of detection	Ref.
Fluorescence	Oligoelectrolyte and graphene oxide	0-1.76 U/mL	0.046 U/mL	1
Fluorescence	Thioflavin-T	0-13 μM	34 nM	2
Fluorescence	Phosphorescent conjugated polyelectrolytes	0-44 μM	50 nM	3
Ratiometric fluorescence	Ion-selective membranes	0-30 μM	0.157 μM	4
Phosphorescence	Polyelectrolytes and Ir(III) complexes	0-10.9 μM	Not mentioned	5
Phosphorescence	Manganese-doped ZnS quantum dots/polybrene assembly	0.38-10.76 μg/mL	0.16 μg/mL	6
Colorimetry	Polymer nanoparticles and Au nanoparticles	10-400 nM	2.5 nM	7
Colorimetry	Gold nanorods and graphene oxide	0.02-0.28 μg/mL	5 ng/mL	8
Surface plasmon resonance	Cascaded channels fiber	0-10^{-3} g/L	2.88×10^{-4} μg/L	9
Surface-enhanced Raman scattering	Functionalized Silver Nanoparticles	0.5-150 ng/mL	0.5 ng/mL	10
Surface-enhanced Raman scattering	Au@Ag nanoparticles	50-800 ng/mL	43.74 ng/mL	11
Resonance Rayleigh scattering technology	Co(II)/5-Cl-PADAB complex	0.05-1.60 μg/mL	1.3 ng/mL	12
Flow injection analysis-resonance light scattering coupled technique	Azure B	0.01-6.0 μg/mL	6.4 ng/mL	13
Flow injection	Protamine	0.1 - 2.0 U/mL	0.06 U/mL	14
Amperometry	Polyvinylchloride membrane	1 - 10 U/mL	0.2 U/mL	15
Voltammetric detection	pyrrole-alkylammonium cationic surfactant and MWCNTs composite	0.1 - 8.0 μM	0.1 μM.	16
Colorimetry	Gold nanorods	0.1 - 10 μg/mL	10 ng/mL	This work

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Table S2 The detection of sodium heparin in injection

Sample	**Measurements (mg/mL)**	**Average (mg/mL)**	**RSD (%)**	**Labelled concentration (mg/mL)**
1	33.82, 33.27, 33.03, 32.71, 33.35	33.24	1.24	33.75
2	32.76, 32.51, 33.78, 33.11, 33.33	33.10	1.49	33.75

1-Tianjin Chemical Pharmaceutical Co., Ltd;

2-Changzhou Qianhong Biochemical Pharmaceutical Co., Ltd

Conditions: AuNRs, 0.01 n M; Fe^{3+}, 0.05 mM; TU, 1 mM; HCl, 0.03 M.