Supporting Information

Colorimetric sensing strategy for heparin assay based on PDDA-induced aggregation of gold nanoparticles

Xiaoyi Ma,[ab] Xinyue Kou,[ab] Yuanyuan Xu,[c] Dawei Yang,[a] and Peng Miao[*ab]

[a] Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, P. R. China

[b] University of Science and Technology of China, Hefei 230026, P. R. China

[c] Key Laboratory of Animal Physiology and Biochemistry, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, P. R. China

E-mail: miaopeng@sibet.ac.cn (P. Miao)

Experimental

Materials and instruments

Heparin was obtained from Aladdin biological technology Co., Ltd. (Shanghai, China). Gold (III) chloride trihydrate (HAuCl$_4$•3H$_2$O), poly(diallyldimethylammonium chloride) (PDDA), glutathione, trypsin, melamine, cephalin, and trisodium citrate were acquired from Sigma (USA). T4 ligase was obtained from New England Biolabs Ltd. (Beijing, China). Human serum albumin (HSA), glucose, adenosine diphosphate (ADP), trehalose and bovine serum albumin (BSA) were purchased from Solarbio Science＆Technology Co., Ltd. (Beijing, China). The other reagents were of analytical grade and used as received. Human serum samples were collected from local hospitals with the approval from the Medical Ethics Committee (Suzhou, China). Double-distilled water was used to prepare all solutions (18 MΩ cm). UV-vis absorption spectra were measured by Synergy HT multifunction microplate reader (BioTek Instruments, Inc., USA). Transmission electron microscopic images were taken by a FEI Tecnai G20 transmission electron microscopy (FEI, USA).

Preparation of AuNPs

3.5 mL of 1% (w/v) trisodium citrate was mixed with 100 mL of 0.01% (w/v) HAuCl$_4$ under violent stirring. After boiled for 15 min, the heat was removed with stirring for half an hour. Afterward, the solution was cooled down to room temperature. The synthesized AuNPs were centrifuged at 12, 000 rpm for 30 min for purification.

Optimization of PDDA concentration

With the existence of PDDA, non-crosslinking aggregation of AuNPs occurred immediately, leading to the color change and corresponding variation of UV-vis absorption spectrum. The aggregation state was determined by the concentration of PDDA. Firstly, standard solutions of

PDDA with a series of concentrations were prepared. Second, 160 μL of AuNPs was blended with 40 μL of PDDA, the mixture of which was placed in the well of a 96-well plate. After reacting for 3 min, UV-vis absorption spectrum was measured from 300 to 750nm. The absorbance values at 520 nm and 650nm were recorded (A_{520} and A_{650}) to calculate the parameter of $R_{650/520}$ (A_{650}/A_{520}).

Colorimetric analysis of heparin

20 μL of various concentration of heparin were mixed with 20 μL of PDDA (0.02 μM), which were then blended with 160 μL of AuNPs. UV-vis absorption spectrum was then measured after reacting for 3 min. The ratios of $R_{650/520}$ were calculated, which were related to the concentration of heparin.

Interference investigation and serum sample analysis

Interfering molecules like HSA, glucose, ADP, trehalose, BSA, glutathione, trypsin, melamine, cephalin, and T4 ligase were introduced to verify the selectivity of this method. Interfering molecules with the concentration of 1 μg/mL were used to replace target heparin. The obtained values of $R_{650/520}$ were compared with that of heparin.

To testify the practical utility in complicated biological fluids, human serum samples were challenged for heparin analysis. Three independent samples were firstly diluted for 10 times, which were spiked with heparin (0.1 and 0.3 μg/mL), respectively. $R_{650/520}$ values were calculated and compared.
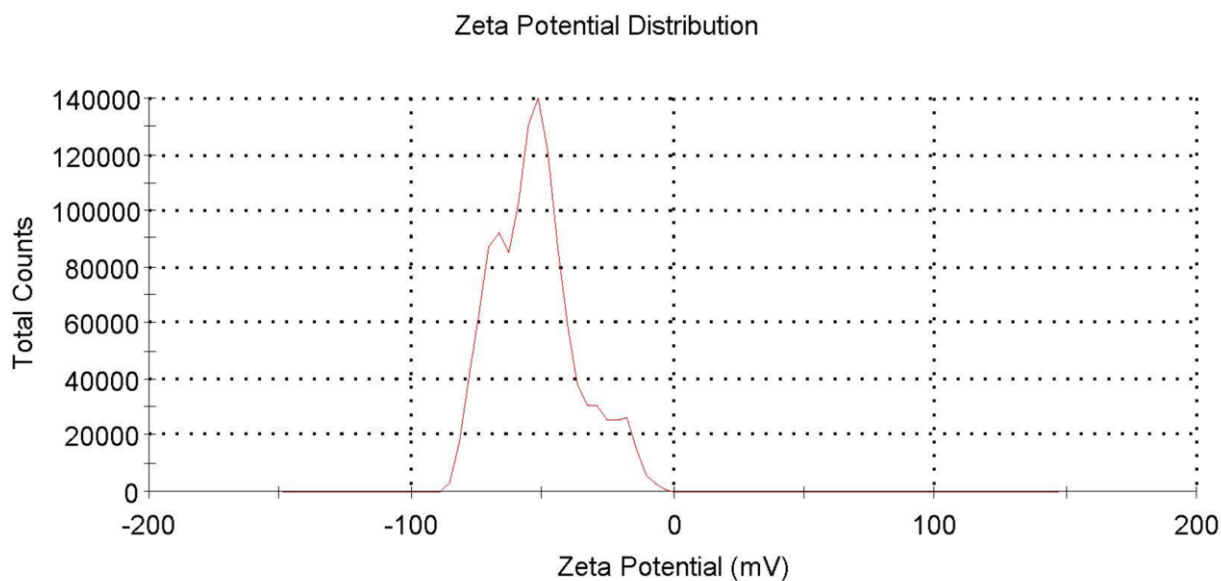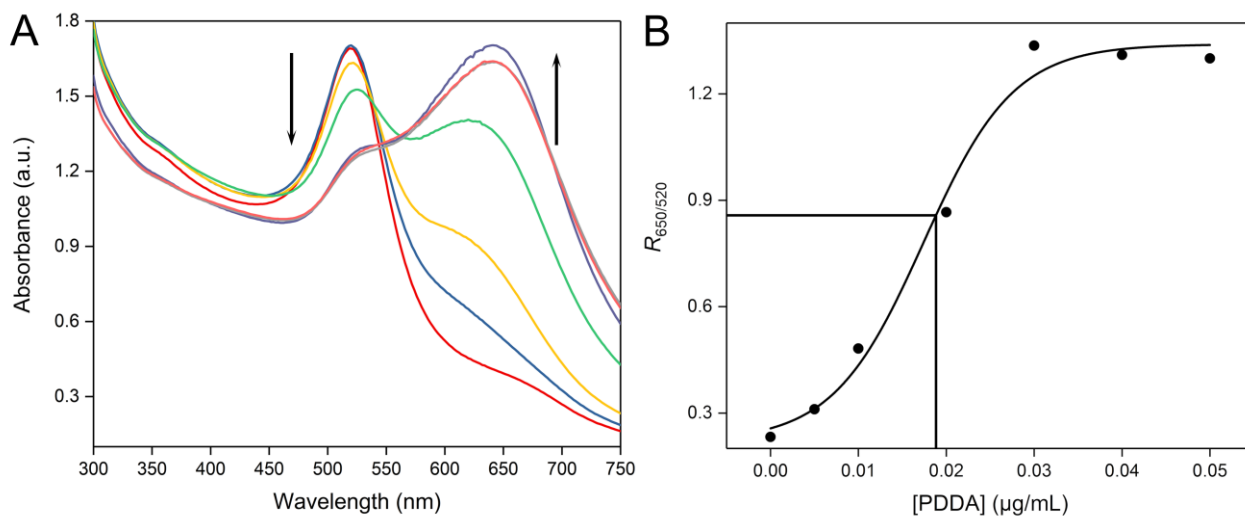
Fig. S1. Zeta potential of bare AuNPs.

Fig. S2. (A) UV-vis absorption spectra of AuNPs incubated with different concentrations of PDDA: 0, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05 μg/mL (from top to bottom). (B) $R_{650/520}$ of the AuNPs versus the concentration of PDDA.

Table S1. Analytical performances of the proposed colorimetric sensor and some previously reported methods.

Technique	Materials	Real sample	LOD (μg/mL)	Ref
colorimetry	chitosan-capped AuNPs	blood	16	1
colorimetry	AuNPs	-	0.6	2
fluorescence	adenosine based molecular beacons	blood	0.06	3
colorimetry	positively charged AuNPs	blood	0.03	4
fluorescence	nitrogen-rich polymer carbon nanoribbons	heparin injection	0.013	5
electrochemistry	peptide	blood	0.01	6
colorimetry	PDDA and AuNPs	blood	0.02	this work

Reference

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