Supplementary information for:

Au@Pt core-shell nanoparticle-based lateral flow immunoassay for rapid detection of Porphyromonas gingivalis type II and IV FimA

Junheng Chen,[ab] Zhenjie Lu[c], Xiaojun Wang[c], Fan Cheng[c], Xudong Wang[c], Fang Li[d], Min Li[d], Yingfu Zhang[c] and Yunlong Wang*[abe]

[a]*The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, People's Republic of China. E-mail: biowyl@126.com*

[b]*Henan Bioengineering Technology Research Center, Zhengzhou 450000, People's Republic of China.*

[c]*Henan Provincial Staff Hospital, Zhengzhou 450002, People's Republic of China.*

[d]*Zhengzhou Ninth People's Hospital, Zhengzhou 450000, People's Republic of China.*

[e]*Henan Academy of Medical Sciences, Zhengzhou 450000, People's Republic of China.*

SI1 Materials

Bovine serum albumin (BSA), NaCl, PEG-20000, and K_2CO_3 were purchased from Sinopharm. Diaminobenzidine (DAB) and 3-amino-9-ethylcarbazole (AEC) were purchased from Beijing Solarbio. 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Shanghai Aladdin. H_2O_2 (30%) was purchased from Yantai Shuangshuang Chemical Co. Urea hydrogen peroxide (UHP) was purchased from Sigma-Aldrich. $NiSO_4$ was purchased from Tianjin Hongyan Chemical Co. Pipettes were purchased from DLAB. Vacuum drying ovens were purchased from Zhengzhou Shengyuan. Microplate readers were purchased from Thermo Fisher Scientific. Electric heating forced-air drying ovens were purchased from Beijing Kewei Yongxing. H1850 centrifuges were purchased from Hunan Xiangyi. Rapid mixers were purchased from Changzhou Zhongjie, and rotary mixers were purchased from Shanghai Damu. Nitrocellulose membranes (CN-140-25) were purchased from Sartorius (Germany). Absorbent paper, PVC backing cards, and fiberglass cotton (SAP-Z90) were purchased from Huaiyuan Tongcheng. XYZ three-dimensional spraying instruments (HM 3030) and microcomputer automatic cutting machines (ZQ 2002) were purchased from Shanghai Kinbio.

SI2 Preparation Method of Au@Pt Core-Shell Nanoparticles

A magnetic stir bar was placed into a three-neck flask, and 100 mL of ultrapure water was added and stirred at a constant speed. Then 200 μL of 10% (w/v) chloroauric acid tetrahydrate solution was added rapidly. After stirring for 15 min, the 220 V heating knob was turned on. After the solution began to boil, 1.8 mL of 1% (w/v) trisodium citrate solution was added quickly. The reaction was maintained at 100 V heating with constant stirring for 12 min. At the end of the reaction, heating was stopped, and the solution was allowed to cool naturally to room temperature with continuous stirring. The volume was adjusted to the initial volume of 100 mL with ultrapure water, and the mixture was shaken thoroughly for later use.

According to the molar ratio of Au to Pt = 1:2, 272.5 μL of 10% (w/v) sodium chloroplatinate hexahydrate solution was added to a three-neck flask containing 50 mL of ultrapure water, and the mixture was stirred at a constant speed for 10 min. The pre-prepared 50 mL gold colloid seeds were added to the thermostatted magnetic stirrer and premixed with the above solution for 5 min. The 220 V heating knob was turned on, and the solution was stirred at a constant speed until it reached a near-boiling state. According to the molar ratio of Pt to AA = 1:2, 97.1 μL of 1 mol/L ascorbic acid (AA) was added rapidly. The system was maintained at 100 V under boiling and continuous stirring for 15 min during the reaction. At the end of the reaction, heating was stopped. The solution was continuously stirred and allowed to cool naturally to room temperature. It was then diluted to 50 mL with ultrapure water and shaken thoroughly to ensure uniform mixing, yielding the Au@Pt core-shell nanoparticle sol.

SI3 Detailed Description of the Process Optimization

SI3.1 Optimization of pH Conditions for Labeling Monoclonal Antibodies with Au@Pt Core-Shell Nanoparticles. Twenty centrifuge tubes, each with a capacity of 1.5 mL, were filled with 1 mL of an Au@Pt core-shell nanoparticle solution. The samples were divided into two distinct groups. To each tube within these groups, varying volumes of 0.1 mol L^{-1} K_2CO_3 solution-specifically, 0 μL, 5 μL, 10 μL, 15 μL, 20 μL, 25 μL, 30 μL, 35 μL, 40 μL, and 45 μL were added to modulate the pH of the nanoparticle solution. The pH levels were subsequently measured using a precision pH meter. Following this adjustment, 50 μL of either monoclonal antibody 3B7 or 4G6 (1 mg mL^{-1}) was introduced to the respective group tubes. The mixtures were then thoroughly homogenized and incubated at ambient temperature for a duration of 20 min. Subsequently, 100 μL of a 10% NaCl solution was added to each tube as a flocculating agent. After a further 10 min incubation, the condition of the Au@Pt core-shell nanoparticles was assessed visually. Finally, 200 μL of the supernatant was transferred to a microplate, and the optical absorption spectra ranging from 300 to 800 nm were recorded using a multifunctional microplate reader. The A_{700}/A_{450} absorbance ratio was determined for each spectrum, and the pH value associated with the sample exhibiting the minimal precipitate, the highest absorption spectrum, and the lowest A_{700}/A_{450} ratio was identified as the optimal pH for the conjugation of antibodies with Au@Pt core-shell nanoparticles.

SI3.2 Optimization Labeling Amount of Monoclonal Antibodies. Initially, the optimal pH values identified in section "**SI3.1**" were employed to label antibodies 3B7 and 4G6 (5 μg, 10 μg, 20 μg, 30 μg, 50 μg, 1 mg mL^{-1}) as well as to fabricate Au@Pt core-shell nanoparticle-based immunochromatographic test strips. These strips were subsequently evaluated using positive reference samples at concentrations of 1600 ng mL^{-1}, 400 ng mL^{-1}, and 50 ng mL^{-1} (high, medium, and low concentrations). The initial assessment of antibody labeling quantities was conducted based on parameters such as color intensity, background clarity, and other relevant factors.

Subsequently, a series of quantitative experiments were performed to ascertain the optimal labeling amount of the antibodies. Twenty centrifuge tubes, each with a capacity of 1.5 mL, were filled with 1 mL of Au@Pt core-shell nanoparticle solution and divided into two distinct groups. The pH of the nanoparticle solution was adjusted in accordance with the optimal pH identified in section "**SI3.1**". To each tube in both groups, varying volumes of antibody 3B7 or 4G6 (1 mg mL^{-1}) were added in increments of 0 μL, 2.5 μL, 5 μL, 7.5 μL, 10 μL, 12.5 μL, 15 μL, 17.5 μL, 20 μL, and 22.5 μL. The mixtures were thoroughly homogenized and incubated at room temperature for 20 min. Subsequently, 100 μL of a 10% NaCl solution was introduced to each tube. After allowing the mixtures to stand for 10 min, the condition of the Au@Pt core-shell nanoparticles was assessed visually. A 200 μL aliquot of the supernatant was then transferred to a microplate, and the optical absorption spectra ranging from 300 to 800 nm were measured using a multifunctional microplate reader. For each spectrum, the A_{700}/A_{450} absorbance ratio was computed. The optimal amount of antibody labeling was identified by selecting the group that exhibited minimal precipitate formation, the highest absorption spectrum, and the lowest A_{700}/A_{450} ratio. The antibody amount corresponding to 110% of this optimal value was designated as the final optimized labeling amount.

SI3.3 Investigation of the Optimal Antibody Coating Concentration. Antibodies 4E6 and 1E8 were diluted in the antibody coating solution to achieve concentrations of 0.5 mg mL^{-1}, 1.0 mg mL^{-1}, 1.5 mg mL^{-1}, and 2.0 mg mL^{-1}, respectively, while goat anti-mouse IgG was diluted to a concentration of 1 mg mL^{-1}. Each antibody solution was applied to the NC membrane at a concentration of 1 mL cm^{-1}. The membranes were subsequently dried overnight at 37 °C. Upon preparation of the test strips, they were evaluated using positive reference samples at concentrations of 1600 ng mL^{-1}, 400 ng mL^{-1}, and 50 ng mL^{-1} (high, medium, and low concentrations). After a reaction period of 10 min , the color intensity, line width, and clarity of both the T and C lines, along with the coloration time and background of the NC membrane, were assessed as evaluation criteria to determine the optimal antibody coating concentration on the NC membrane.

SI3.4 Optimization of the Catalyst. Various catalytic chromogenic solutions were formulated by proportionally combining different components, including catalytic substrates such as TMB, DAB, AEC, ABTS, and enhancers such as H_2O_2, UHP, and $NiSO_4$. The color intensities before and after catalysis were measured to determine the optimal conditions. The Pg FimA IV Au@Pt core-shell nanoparticles, prepared under the optimal conditions, were chosen for further screening. A positive reference sample at a concentration of 50 ng mL^{-1} was applied to the test strip, followed by the addition of 25 μL of chromogenic agent to the fully developed NC membrane after complete chromatography. The performance of conventional colorimetric test strips was compared with those treated with various catalytic chromogenic solutions through visual inspection and grayscale analysis. The catalytic performance was assessed, and the optimal formula for the catalytic chromogenic solution was selected based on these evaluations.

SI3.5 Detection time optimization. Test strips were prepared under optimal conditions for Au@Pt core-shell nanoparticle labeling and antibody coating. Positive reference samples, each with a volume of 70 μL, were introduced at concentrations of 400 ng mL^{-1}, 200 ng mL^{-1}, 100 ng mL^{-1}, 50 ng mL^{-1}, 25 ng mL^{-1}, and 12.5 ng mL^{-1}. Measurements were conducted at intervals of 3 min, 5 min, 8 min, and 10 min, as well as at 3 min and 5 min following catalysis. The evaluation of detection time was based on the color, width, and clarity of the T and C lines, in addition to the background of the NC membrane. The optimal detection time was determined according to these criteria.

SI4 Stability Evaluation Results

SITable 1 Stability Evaluation Results.

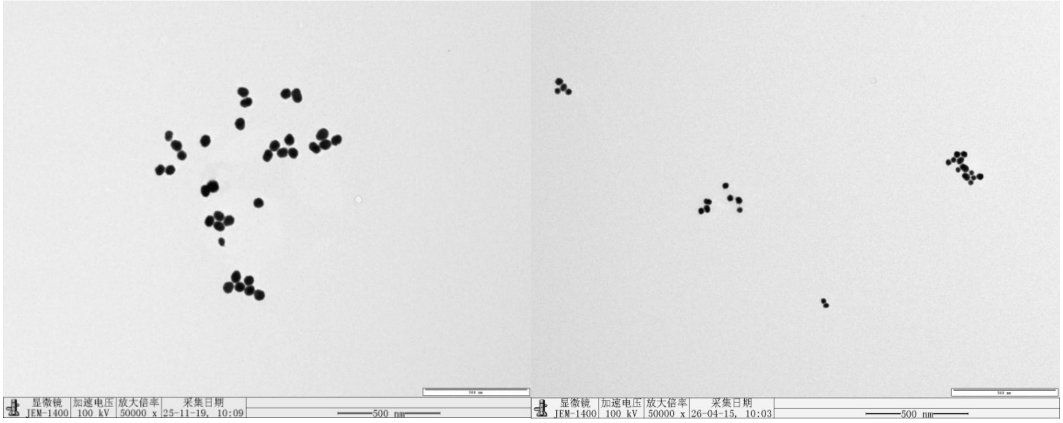
Accelerated aging time	**Pg FimA II test strip**	**Pg FimA IV test strip**
0 days	Normal migration; normal color development; visible at 50 ng mL^{-1}	Normal migration; normal color development; visible at 50 ng mL^{-1}
7 days	Normal migration; normal color development; visible at 50 ng mL^{-1}	Normal migration; normal color development; visible at 50 ng mL^{-1}
14 days	Slightly slower migration; slightly weaker color; visible at 50 ng mL^{-1}	Slightly slower migration; slightly weaker color; visible at 50 ng mL^{-1}

SI5 Comparison of Detection Results.

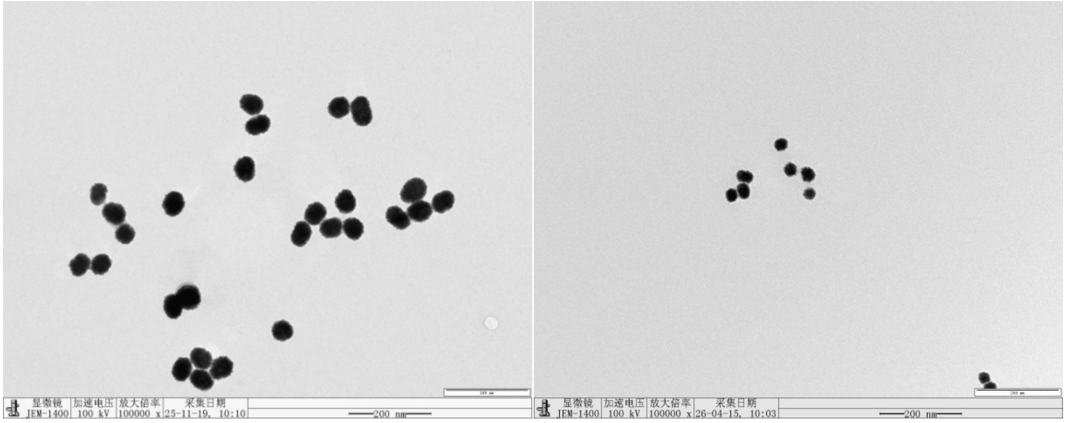
SITable 2 Comparison of Detection Results.

PCR test results	FimA II test results			FimA IV test results		
	Positive	Negative	Total	Positive	Negative	Total
Positive	37	3	40	37	3	40
Negative	6	34	40	5	35	40
Total	43	37	80	42	38	80
Positive coincidence rate	86.05%			88.10%		
Negative coincidence rate		91.89%			92.11%	
Total coincidence rate			88.75%			90.00%
Paired chi-square test	x^2=1.00；*p=0.317＞0.05*			x^2=0.500；*p=0.480＞0.05*		
Kappa consistency test	Kappa value=0.775；*p＜0.01*			Kappa value=0.800；*p＜0.01*		

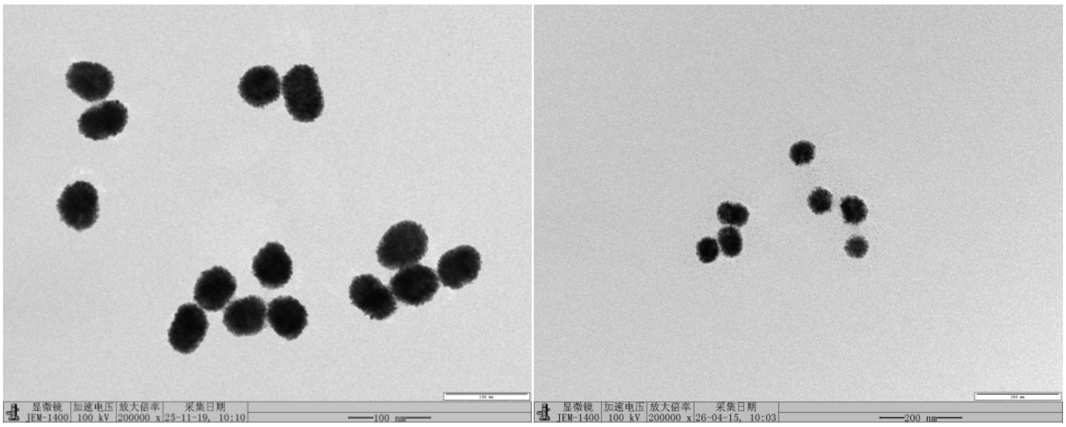
SI6 TEM Characterization of Au@Pt Core-Shell Nanoparticles



SIFig. 1 (A) Au@Pt Core-Shell Nanoparticles (TEM). (Magnification factor: 50 000 ×)



SIFig. 1 (B) Au@Pt Core-Shell Nanoparticles (TEM). (Magnification factor: 100 000 ×)



SIFig. 1 (C) Au@Pt Core-Shell Nanoparticles (TEM). (Magnification factor: 200 000 ×)

SI7 Changes in the Particle Size Distribution and Absorption Spectra of Au nanoparticles and Au@Pt Core-Shell nanoparticles.

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	33.32	Peak 1:	60.19	89.1	27.33
PdI:	0.517	Peak 2:	4.667	10.9	1.138
Intercept:	0.841	Peak 3:	0.000	0.0	0.000

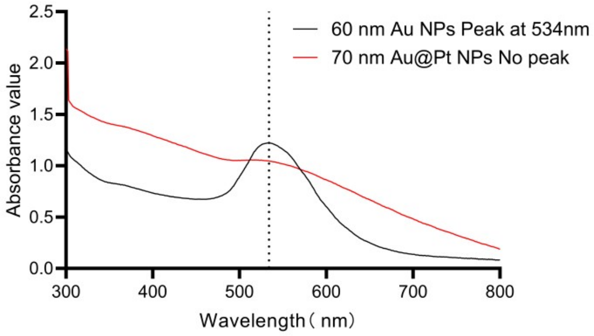
Result quality Refer to quality report

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	55.25	Peak 1:	69.55	100.0	29.16
PdI:	0.203	Peak 2:	0.000	0.0	0.000
Intercept:	0.920	Peak 3:	0.000	0.0	0.000

Result quality Good

SIFig. 2 (A) Results of particle size distribution measurements for two types of nanoparticles using a Malvern particle size analyzer. (On the left are Au nanoparticles; on the right are Au@Pt core-shell nanoparticles.)



SIFig. 2 (B) Comparison of the Absorption Spectra of Two Types of Nanoparticles.