Supporting Information

Magnetic Bead-Based Mimic Enzyme-Chromogenic Substrate and Silica Nanoparticles Signal Amplification System for Avian Influenza A (H7N9) Optical Immunoassay

Dan Su[a], Hanyun Li[a], Jinlin Li[b], Yali Liu[a], Mi Peng[a], Bingwei Feng[a], Pengfei Xu[a], Yonggui Song[a*]

[a] *National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine, Jiangxi University of Traditional Chinese Medicine, 56 Yangming Road, Nanchang 330006, China.*

[b] *Nanchang Institute for Food and Drug Control, Nanchang 330038, China*

*Corresponding author: Tel/Fax: +86 791 87119632. E-mail: songyonggui1999@163.com (Yonggui Song).

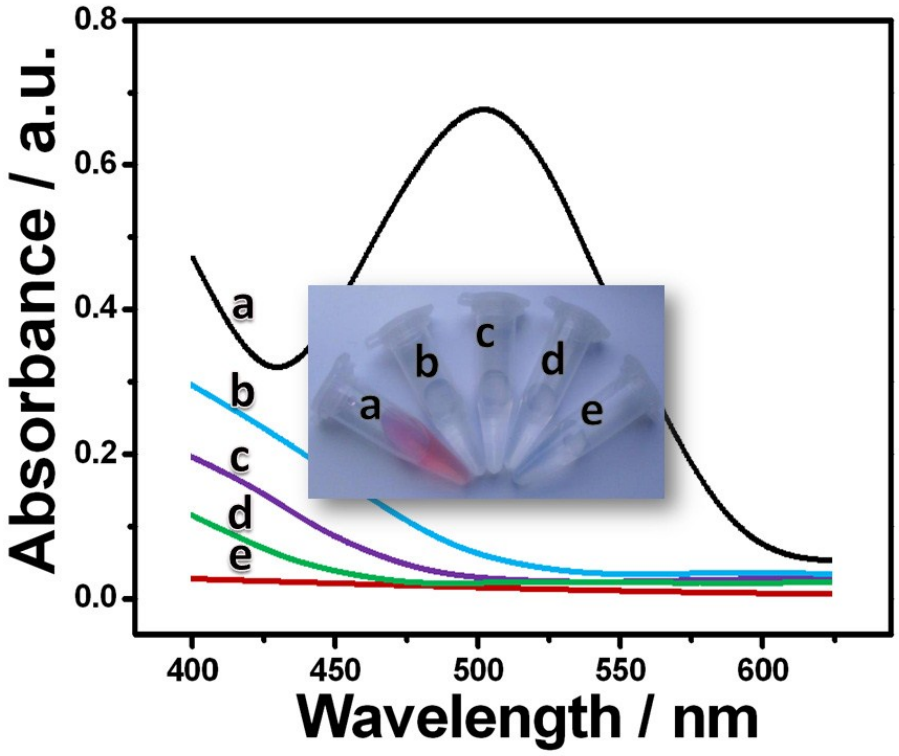

Fig. S1. UV−vis absorption spectra of (a) GOx + glucose + hemin + 4-AAP/phenol system, (b) GOx + hemin + 4-AAP/phenol system, (c) GOx + glucose + hemin, (d) GOx + glucose + 4-AAP/phenol system, and (e) glucose + hemin + 4-AAP/phenol system, respectively (inset: the corresponding photographs for curve (a), (b), (c), (d), (e)).

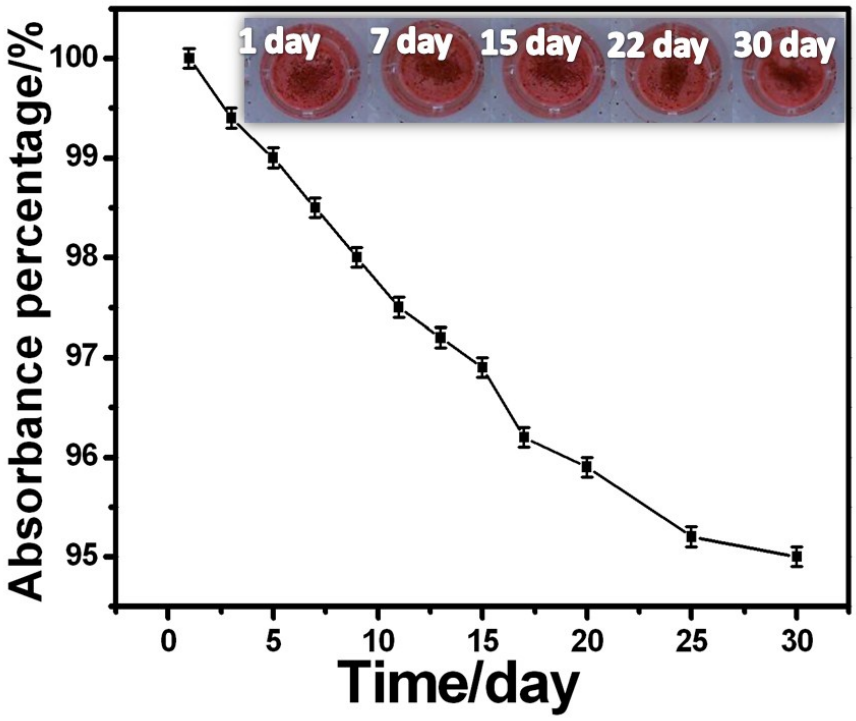

Fig. S2. The stability of the MB-MEMSCI. Inset: The optical immunoassay results after mAb-MBs and GOx-Red-SiNPs-pAb were stored for: 1 day, 7days, 15 days, 22 days, and 30 days.

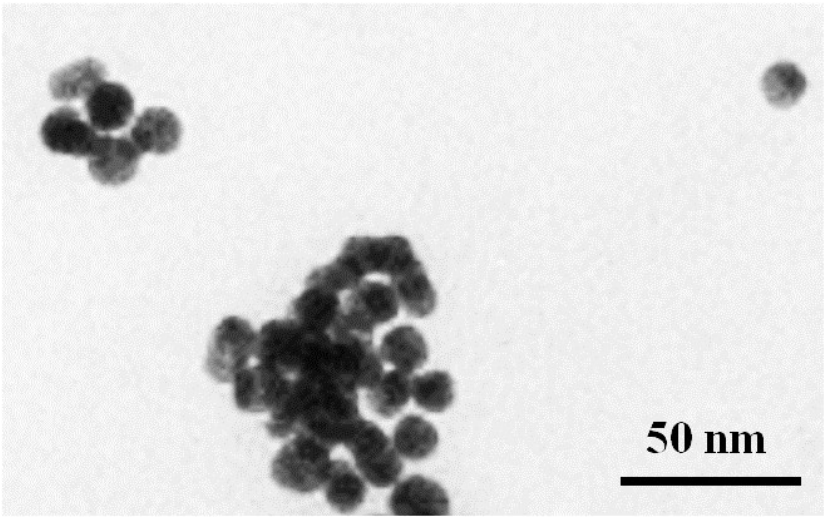

Fig. S3. TEM image of AuNPs.

Synthesis of Gold nanoparticles（AuNPs）

Gold nanoparticles（AuNPs）was obtained according to the following steps. In brief, 1 mL of 1% $HAuCl_4$ and 100 mL ultra-pure water were mixed in a 250 mL flask. 5 mL of 1% sodium citrate solution was added quickly to the mixture after boiling, and the boiling of the mixture was maintained for 15 min. As a result, the color of the solution turned to wine red, implying the diameter of gold nanoparticles was between 10 nm and 20 nm. And colloidal gold solution was be stored at 4 ℃.

Synthesis of pAb-AuNPs

Antibody modified AuNps (pAb-AuNPs) was obtained as follows: 40 μL pAb and 1 mL AuNPs suspension was mixed and incubated at 4 ◦C for 12 h. The pAb-AuNPs was separated by centrifugation at 10000rpm and rinsed three times with PBS, then dropped with 1 mL 0.2% BSA at 4 ◦C for 1 h, then separated by centrifugation at 10000rpm and rinsed three times with PBS, and dispersed in 1 mL PBS, stored at 4 ℃ for use.

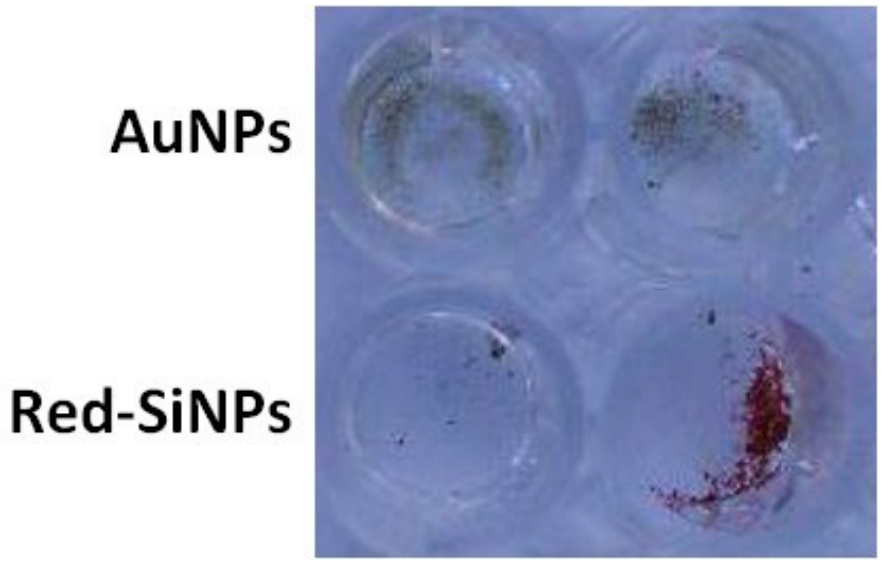

Fig. S4. The test results of 6 ng/mL H7N9 AIV (right) and PBS (left), pAb-AuNPs (up) and pAb-Red-SiNPs (down) were separately used as signal label.

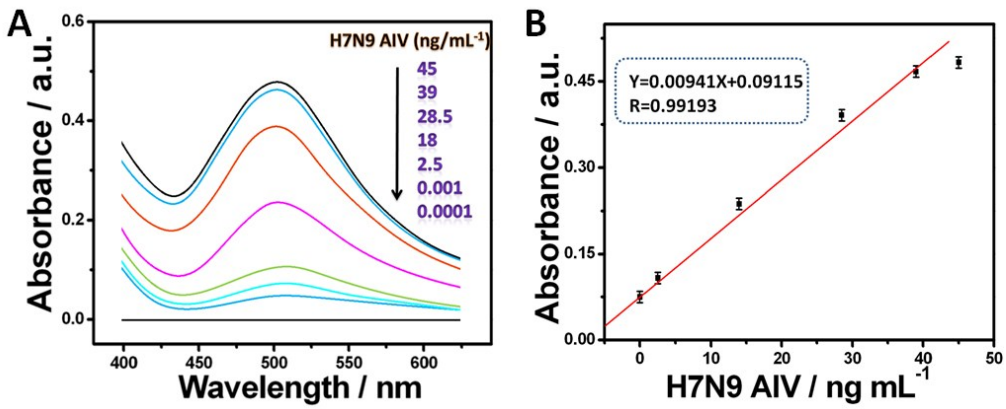

Fig. S5. (A): Absorbance intensity of the developed immunoassay influenza virus immunosensors without color enhanced multifunctional silica nanoparticles by coupling with the 4-AAP/phenol strategy toward di□erent concentration H7N9 AIV standards. (B): Calibration plots of the developed immunoassay influenza virus immunosensors without color enhanced multifunctional silica nanoparticles by coupling with the 4-AAP/phenol strategy toward di□erent concentration H7N9 AIV standards.

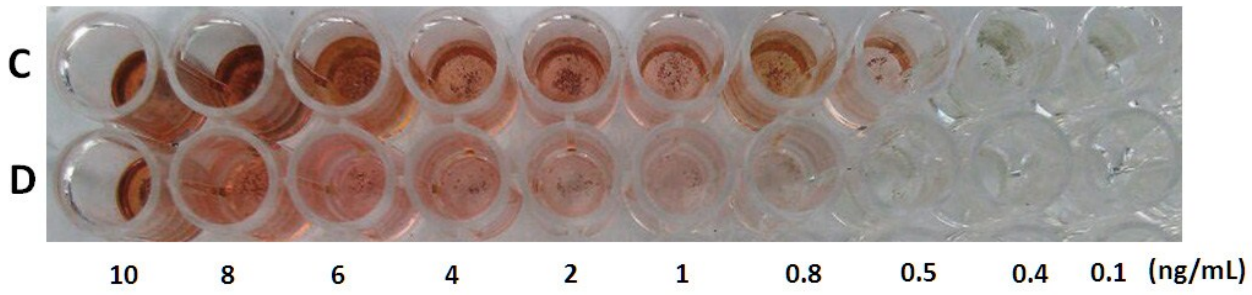

Fig. S6. The sensitivity results of optical MB-MEMSCI with 1 mL (A) and 400 μL (B) of H7N9 AIV, concentration of H7N9 AIV was changed from 10 ng/mL to 0.1 ng/mL (from left to right).

Table S1 Comparison of analytical properties of the developed immunoassay influenza virus immunosensors.

Method	Antigen	Detection limit (pg/mL)	References
LSV	H7N9	6.8	15
DPV	H9N2	1000	16
EIS	influenza A virus	8000	17
QCM	Human influenza virus	10^7	18
QCM	hemagglutinin	2.6×105	19
SPR		7.2×105	
EIS	Peptides of AI H5	2.2	20
EIS	Peptides of AI H5	0.6	21
OSVW		0.9	
EIS	H7N1	5×10^6	22
ELISA	H7N9	6.25×10^3	24
UV-vis	H7N9	3.5	This work
visible		500	

Abbreviations: LSV—linear sweep voltam-metry; DPV—differential pulse voltammetry; EIS—electrochemical impedance spectroscopy; QCM—quartz crystalmicrobalance; SPR—surface plasmon resonance; OSVW—Osteryoung square-wave voltammetry.

Table S2 Comparison of analytical properties of the developed immunoassay influenza virus immunosensors with and without color enhanced multifunctional silica nanoparticles.

Method	Antigen	linear range	Detection limit
Without red-SiNPs	H7N9	0.53–39.0 ng mL^{-1}	0.17 ng mL^{-1}
With red-SiNPs	H7N9	0.01–50.0 ng mL^{-1}	3.5 pg mL^{-1}

Table S3. Recovery tests of H7N9 in chicken serum samples based on Real Time RT-PCR Kit and the MB-MEMSCI.

Methods	No.	Added (ng mL^{-1})	Found (ng mL^{-1})	RSD (%)	Recovery (%)
Real Time RT-PCR Kit	1	10	9.51	3.43	95.10
	2	20	21.05	4.37	105.25
	3	30	28.56	5.81	95.20
MB-MEMSCI	1	10	9.85	1.85	98.50
	2	20	19.54	1.99	97.70
	3	30	28.97	1.59	96.57

Design of primers and probes: According to the HA sequence of avian influenza virus H7N9 in GenBank database recent three years, a pair of primers and probe has been designed (Provided by the Shanghai Ying Wei Jie Ji Trading Co.).

The primer and probe sequence are listed as follows: The primer sequence of upstream (H7N9-F) is 5'-TGCAGAATAGAATA-CAGATAGAC-3', the primer sequence of downstream (H7N9-R) is 5'-ACCGCATGTTTCCATTCT-3', and the probe primer sequence (H7N9-B) is FAM5'-TGATGC-CCCGAAGCTAAACCA-3' BHQ1.

Amplified conditions of real-time RT-PCR: 30 min at 42˚C, 3min at 95˚C, 1 cycles; 30s at 95˚C, 40s at 60˚C, 40 cycles; 10 min at 72˚C. After the end of RT-PCR reaction, 5μL PCR products were identified by electrophoresis in 10g / L agarose gel.

Table S4. Comparison of cost and time spent of the Avian influenza virus （H7N9） Real Time RT-PCR Kit and the developed MB-MEMSCI.

Methods	**Assay time**	**Ten test cost ($)**
Real Time RT-PCR Kit	3h	617
MB-MEMSCI	1h	About 1/10 of RT-PCR