Supporting Information

Experimental

Chemicals and reagents

Phosphate buffered saline (PBS, 0.1M, pH 7.4), 96-strips high binding ELISA plates, bovine serum albumin (BSA), phytohaemag-glutinin (PHA-E), silver nitrate ($AgNO_3$), boron sodium cyaniding ($NaBH_4$), 3-Mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethyl- aminopropyl)-carbodiimide (EDC), N-hydroxyl succinimide (NHS), dried ethanol, nitric acid (HNO_3), sodium peroxydisulfate ($Na_2S_2O_8$)，Sodium citrate, Phosphoric acid (H_3PO_4)，manganese sulfate（$MnSO_4$), sodium hydroxide（NaOH）were purchased from Shanghai Sangon Biological and Technological Service Co., Ltd. Unless otherwise stated, all the reagents used in this study were at least of analytical grade. All solutions were prepared with deionized water with conductivity of 18.2 MΩ·cm from a water purification system (Millipore).

Rabbit monoclonal anti-H1N1, hemagglutinin (HA) (OTWO Guangzhou PL labs), Rabbit polyclonal anti-H1N1, hemagglutinin (HA) 1 (Beijing Bioss Biological and Technological Co., Ltd.), Inactivated H1N1 Influenza A Virus (Solomon Islands /03/06) (prospec) was provided from Shanghai kenqiang instrument Co., Ltd. H5N1, H3N2 influenza virus were kindly provided by Fujian Center for Disease Control & Prevention.

Apparatus

The CL intensity was measured and record with BPCL-1-TIC Ultra Weak

Luminescence Analyzer Institute of Biophysics Acdemia sinica (Bejing, China). UV-visible adsorption spectra were recorded on a Lambda 800 UV-vis Spectrophotometer (PerkinELmer, USA).

Synthesis of Stable Ag NPs

Monodispersed Ag NPs were prepared by sodium borohydride reduction of $AgNO_3$. Ten volumes ice-cold 1×10^{-3} mol·L^{-1} $AgNO_3$ and another equal volume of 3.0×10^{-3} mol·L^{-1} $NaBH_4$ were mixed dropwise, with stirring, in an ice-bath, dark gray colloid formed almost immediately under vigorous stirring. The colloid was continuously stirred while it was allowed to warm to room temperature and reacted for 30 min. The Ag NPs were obtained by centrifugation at 4000 rpm and washed several times with deionized water. Then stored in brown glass bottles at 4 ℃ before use. The Ag NPs have an average diameter of 40 nm as measured by TEM.

Preparation of the Anti-H1N1-Modified Ag NPs

Anti-H1N1-Modified Ag NPs were done in two steps. First, Ag NPs were covalently linked with MPA to form Ag NPs-S-COOH. 0.2 mol·L^{-1} MPA were mixed with Ag NPs solution at room temperature. Then the temperature of the solution was regulated from (293±1) K, to (303±1) K and kept it reacting for 20 min. A coolant bath was used to lower the temperature to (284±1) K next and reacted for 30 min. The produces were washed with deionized water by centrifugation three times. The carboxyl groups of the chemisorbed MPA on the Ag NPs surface were activated by with a mixture of 200 mM EDC and 50 mM NHS solution for 40 min. Finally, 200 μL polyclonal antibodies solution was added at 4 ℃ for 24 h, and the products were

centrifuged for 5 min at 3500 rpm and washed three times with PBS in order to dispose the unlabelled polyclonal antibodies. After centrifugation, the precipitate of **A**nti-H1N1-Modified Ag NPs were redispersed with 200 μL 0.1% BSA. Repeated the above operation, the Ag NPs labeled polyclonal antibodies (in 0.01M PBS) was obtained and stored at 4 °C.

CL analysis for the determination of H1N1

After the Ag NPs were dissolved completely, a different volume of 5 M NaOH was dropped into the solution to adjust the excessive acidity until the pH was 7.0. Afterward, 200 μL 2% (m/v) $Na_2S_2O_8$, 40 μL 6×10^{-3} M $MnSO_4$, 36 μL1:1 (v/v) H_3PO_4 were added into the solutions to make the Ag^+-$Na_2S_2O_8$-Mn^{2+}-H_3PO_4 system reacted in a 90 °C water bathe for 7 min . The reaction was stopped with flowing cold water and the pH value of this solution was adjusted to 13.0 using 5 M NaOH. Then 50 μL of such solution was transferred to a 5mL quartz beaker, 200 μL luminol (1×10^{-3} M) was injected , and the CL signal was measured by the BPCL luminescence analyzer.

2. Optimization of Immunoassay Conditions

In order to obtain the optimization of immunoassay conditions, The concentration of Mn^{2+}, the PH of the sample solutionand luminol solution were all optimized for the sensitive and precise detection of H1N1 in the CL system.

Certainly, antibodies must be excessive first, 200 $ng\cdot mL^{-1}$ monoclonal antibodies in each well was added. Then the antigen-antibody immunoreaction time referred to normal ELISA method , and 60 min at 37 ℃ was considered. The concentration of

antigen as a key factor affecting the detection sensitivity was over the range of 1.0×10^{-12} g·mL^{-1} to 1.0×10^{-6} g·mL^{-1} . The proposed chemiluminence immunoassay of H1N1 was performed as depicted in Figure 1. In order to achieve the best sensitivity and the least amount of samples, 50 μL antigen was selected in each well. The role of tween 20 can effectively remove the unbound the reactants such as antibody, antigen, Ag conjugates.

The effect of the pH of luminol solution for the CL intensity was studied in the $Na_3PO_4/NaHPO_4$ buffer solution. As it shown in Figure S1a, the CL intensity increased slowly when the pH value in the range of 7.0~12.0. And the strong CL emission was observed when the pH was 13. While the CL signal in pH range of 13.5~14.0 was not changed much, so pH 13.0 was chosen as the optimum pH. Then the effect of the pH of sample solution for the CL intensity was also studied in the $Na_3PO_4/NaHPO_4$ buffer solution, and the phenomenon shown in Figure S1b indicated that the strong CL emission was observed at pH 13.

The effect of the concentration of Mn^{2+} on the CL intensity was examined in the range of 6×10^{-4}~1 mol·L^{-1}. As shown in Figure S2, it was found that the CL intensity increased linearly with the Mn^{2+} concentration from 6×10^{-4} to 6×10^{-3} mol· L^{-1}. After that, the increasing concentration of Mn^{2+} caused the CL intensity declining slowly. Therefore, 6×10^{-3} mol·L^{-1} of Mn^{2+} was selected as the optimal concentration for further experiments.

Figure S1

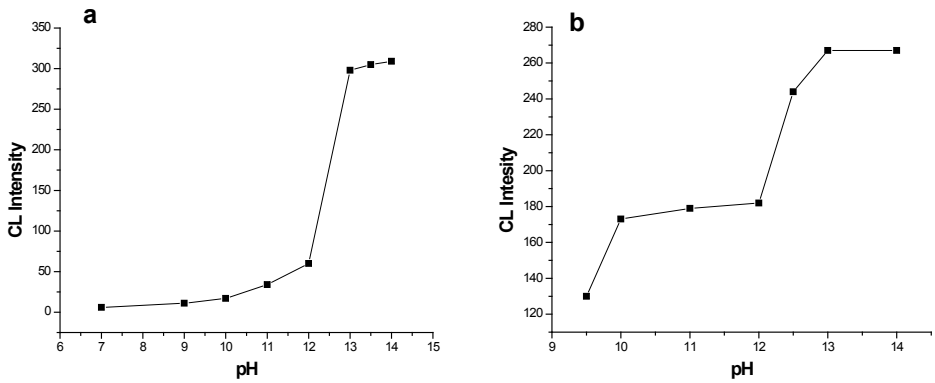

Figure S1 CL Effects of pH value of luminol solution(a), pH value of sample solution (b) with 10^{-6} g·mL^{-1} of H1N1 in 0.1 M PBS (pH 7.4).

Figure S2

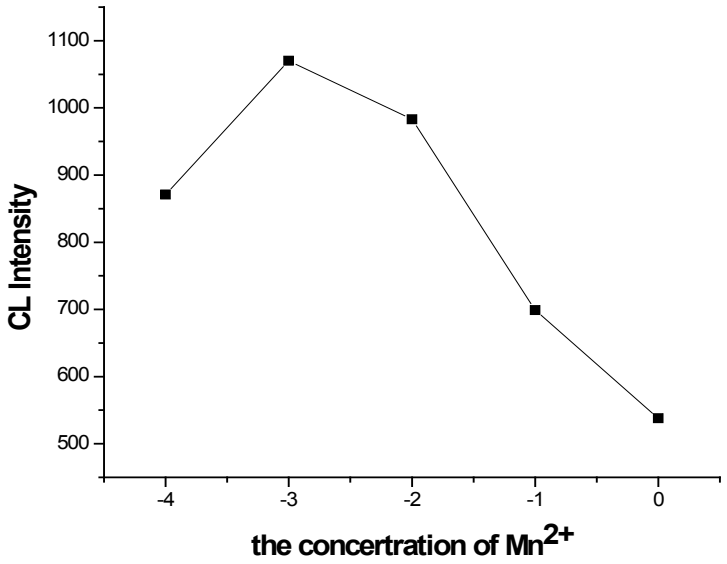

Figure S2 Effect of Mn^{2+} concertration with 10^{-6} g·mL^{-1} of H1N1 in PBS (pH 7.4), pH value of luminol and sample solution were 13.0.

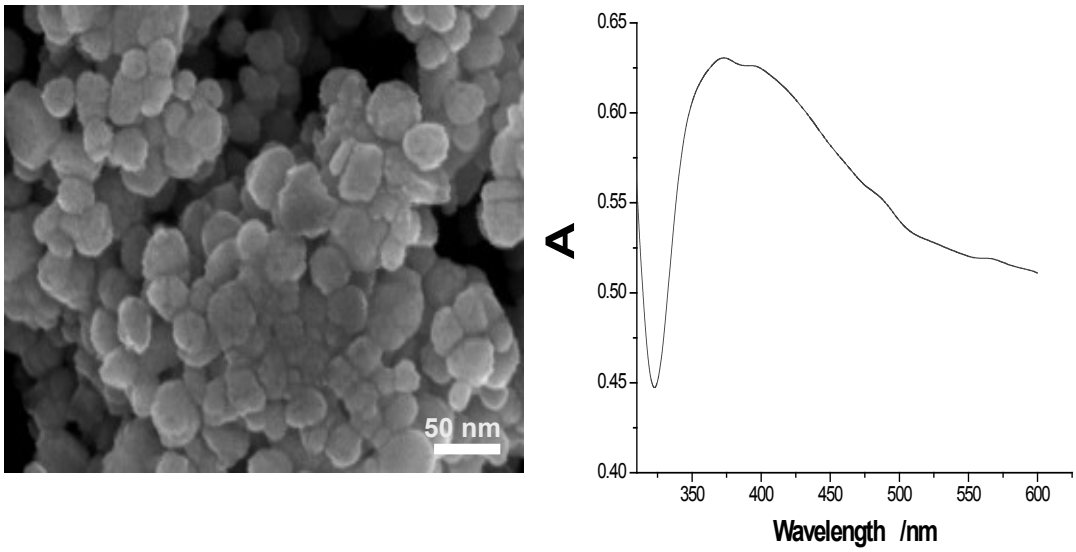

Figure S3 TEM image of the Ag NPs (A) and UV-visible absorbance spectra of the Ag NPs suspensions, in 20 mL dried ethanol (B).

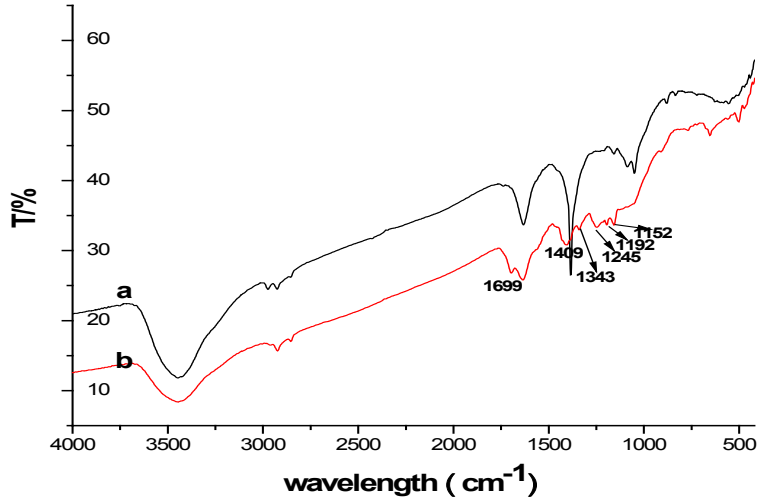

Figure S4 Infrared spectrum of monodispersed Ag NPs (a) and the MPA modified Ag NPs (b).

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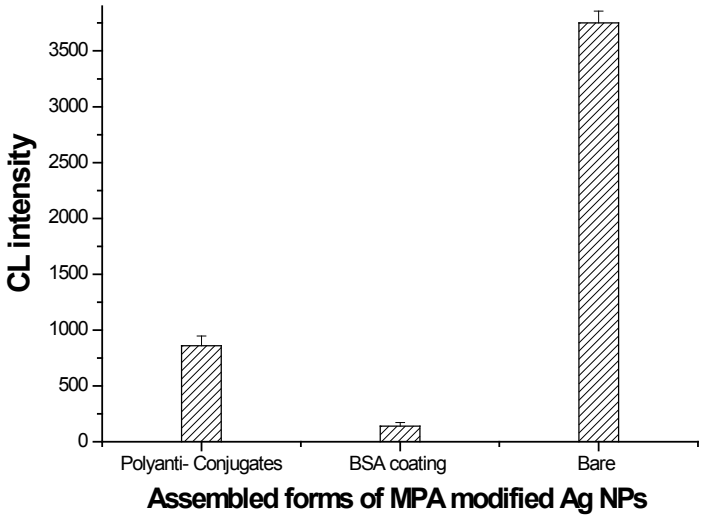

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164 Fig.S5 Relationship between Assembled forms of MPA modified Ag NPs and CL

165 intensity. Concentration of H1N1: 1 ng/mL; 50 μL of 200 ng mL^{-1} monoclonal HA

166 antibodies in each well; The antigen-antibody immunoreaction : 60 min at 37 ℃.

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