Electronic Supplementary Information for:

Luminol, Horseradish Peroxidase and Antibody Ternary Codified Gold Nanoparticle for Label-free Homogenous Chemiluminescent Immunoassay

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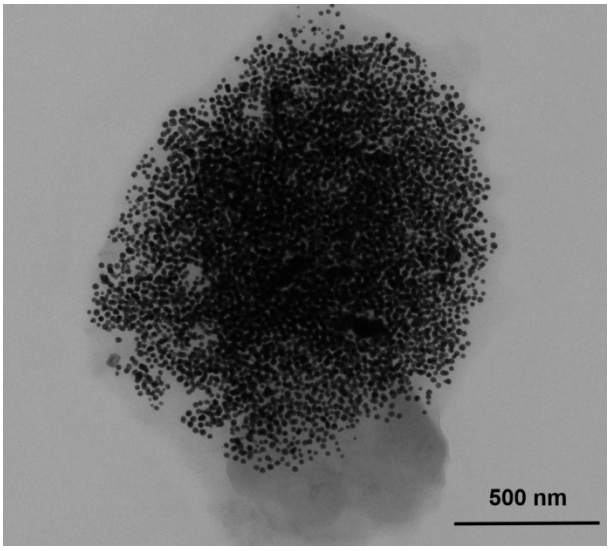

Fig. S1. TEM images of HRP/Ab-luminol-AuNPs in the presence of 1 mg/mL hIgG.

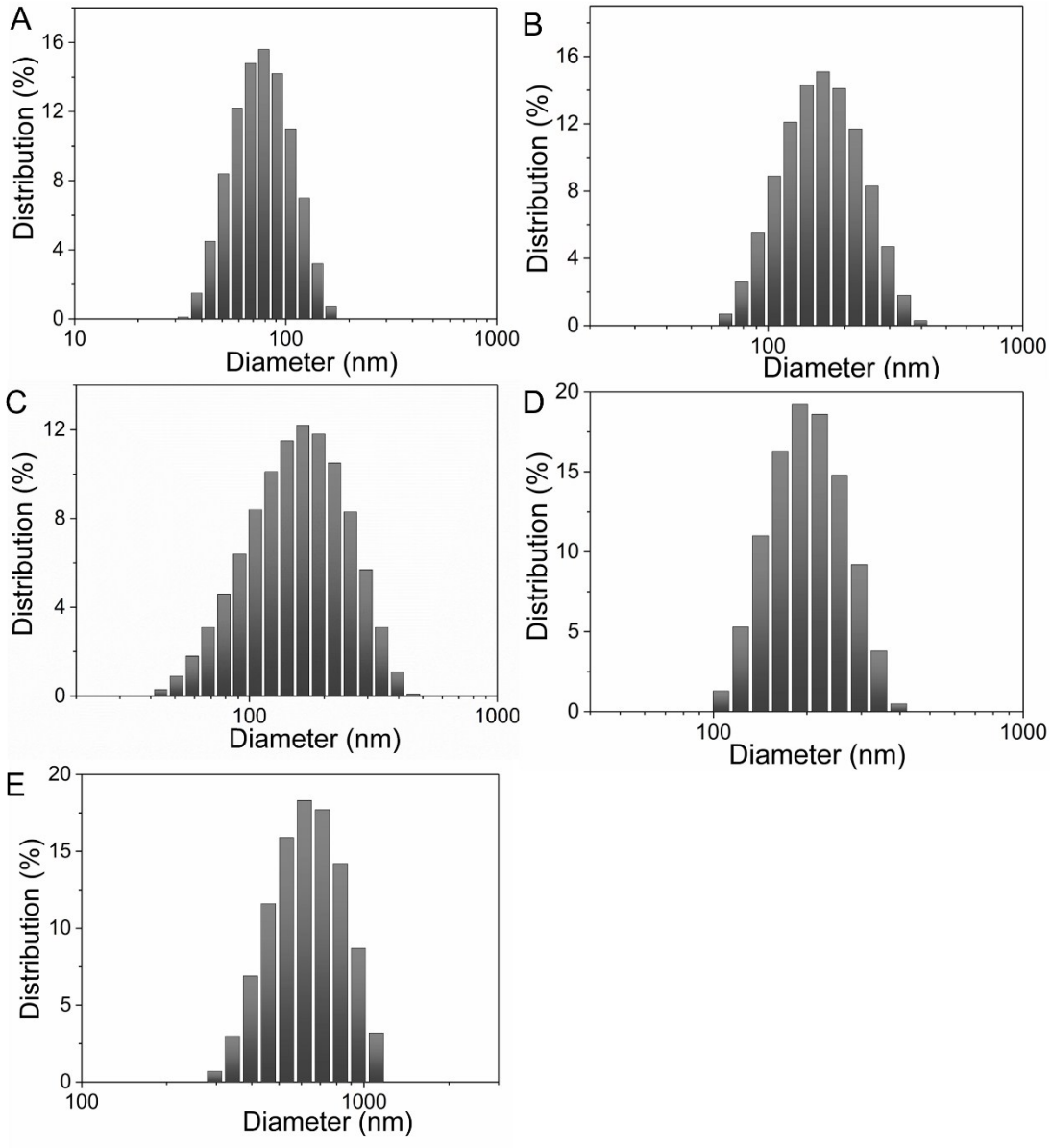

Fig. S2. DLS results of luminol-AuNPs (A), HRP/Ab-luminol-AuNPs in the absence (B) and presence of 1 ng/mL (C), 1 μg/mL (D), and 1 mg/mL (E) hIgG.

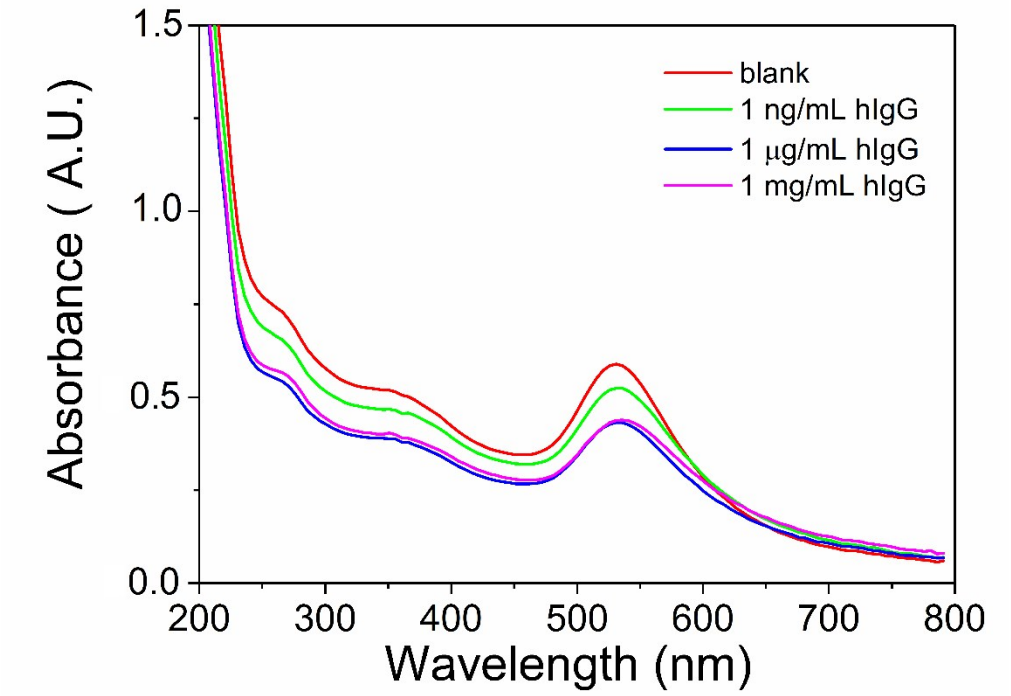

Fig. S3. UV-vis absorption spectra of HRP/Ab-luminol-AuNPs in the absence (blank) and presence of 1 mg/mL, 1 μg/mL, and 1 ng/mL hIgG.

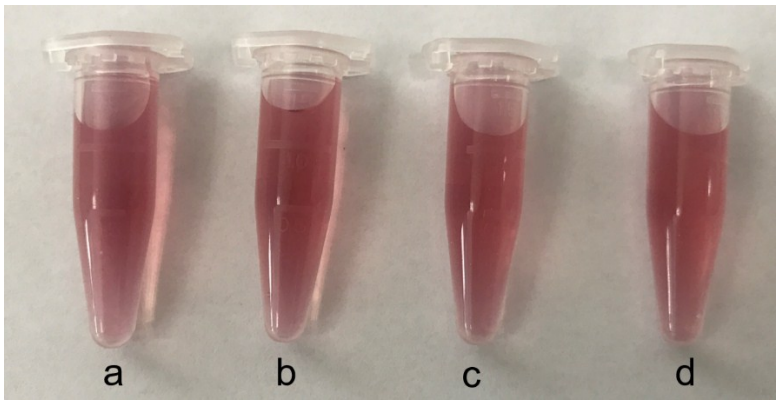

Fig. S4. Photographs of HRP/Ab-luminol-AuNPs in the absence (a) and presence of (b) 1 mg/mL, (c) 1 μg/mL, and (d) 1 ng/mL hIgG.

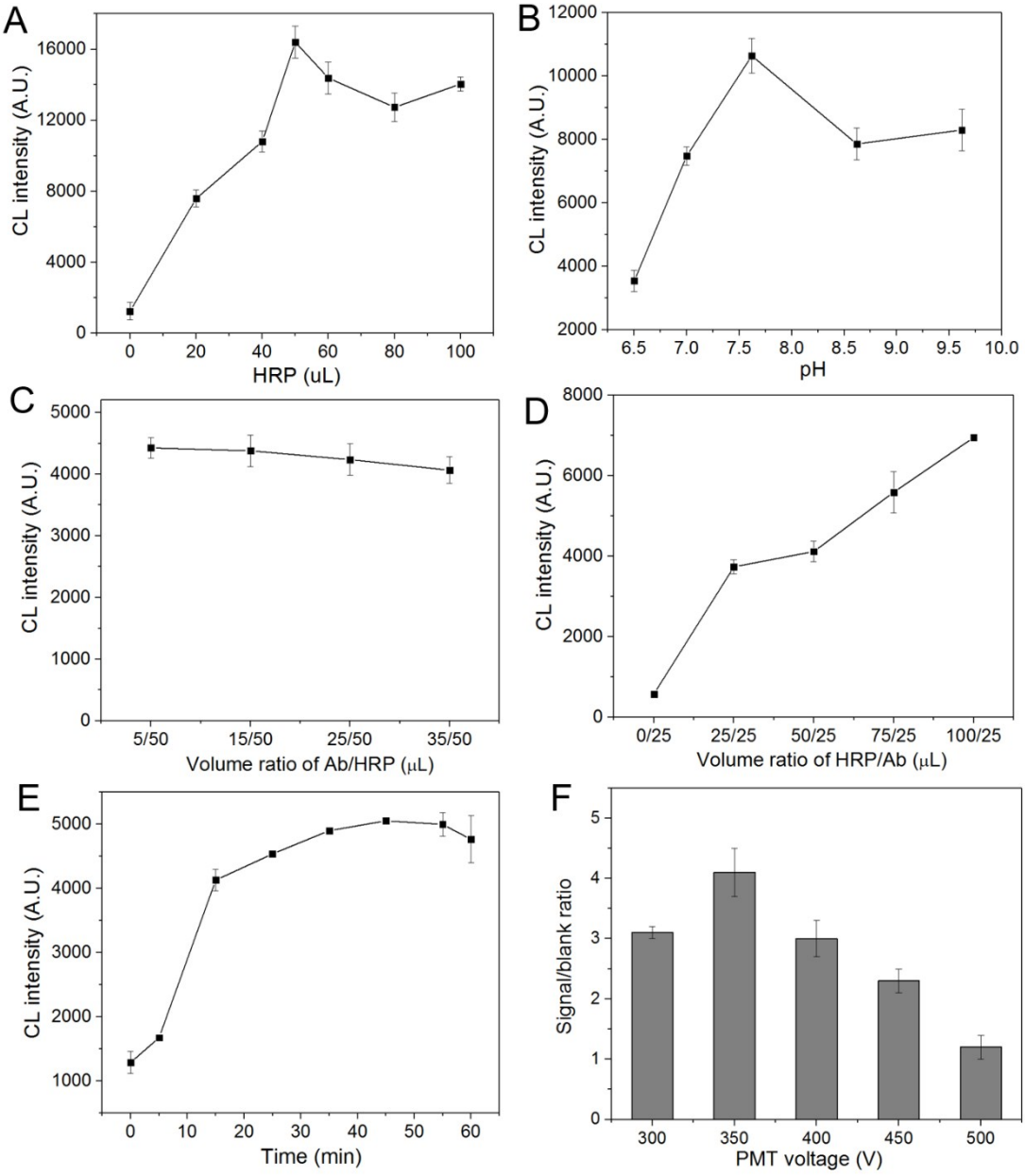

Fig. S5. (A) Effect of amount of HRP, and (B) effect of pH of dispersion buffer on CL intensity of HRP-luminol-AuNPs reacted with H_2O_2. (C) Effect of volume ratio of HRP to Ab, (D) effect of volume ratio of Ab to HRP, and (E) incubation time for immunoreaction, on CL intensity of the immunosensor in the presence of 1 μg/mL antigen. Reaction conditions: 10 mM H_2O_2 in 0.1 M NaOH and CL measurement: PMT voltage at -350 V. (F) PMT voltage on signal/blank ratio of the immunosensor in the presence of 1 ng/mL antigen.

In this work, the enhanced CL intensity was mainly attributed to the catalytic effect

of HRP on the luminol-H_2O_2 CL reaction. Thus, the amount of HRP for the preparation of HRP-luminol-AuNPs was optimized first. As shown in Fig. S3-A, firstly, the CL intensity of HRP-luminol-AuNPs reacted with H_2O_2 increased with the increase of amount of HRP, due to that H_2O_2 can be consumed more efficiently with the increasing of the immobilized amount of HRP. However, the CL response trend to level off when the volume of HRP was above 50 μL, which was mainly attributed to the saturated adsorption capacity of AuNPs for proteins. The pH of the dispersion buffer for HRP-luminol-AuNPs were also optimized (Fig. S3-B). It was found that the CL intensity of HRP-luminol-AuNPs re-dispersed in pH 7.6 phosphate-buffered (P-B) buffer containing 0.1% BSA was highest. Then, the amount of Ab for the fabrication of the immunosensor was also optimized (Fig. S3-C). The added volume of HRP were kept at 50 μL. It was found that the CL intensity of HRP/Ab-luminol-AuNPs in the presence of 1 μg/mL antigen trend to level off and further decreased slightly with the increase of the volume of Ab. Thus, the amount of Ab had little effect on the CL intensity of the immunosensor, due to that small amount of Ab molecules absorbed on the surface of HRP/Ab-luminol-AuNPs was enough for the immunoreaction. The added volume of Ab was optimized at 25 μL finally. The amount ratio of HRP to Ab for the fabrication of the immunosensor was further optimized (Fig. S3-D). The added volumes of Ab were kept at 25 μL. It was found that the CL intensity of HRP/Ab-luminol-AuNPs in the presence of 1 μg/mL antigen increased with the increasing of the volume of HRP. It was due to that the higher ratio of HRP to Ab could resulted to higher catalytic effect. 50 μL HRP was chosen in consideration of reagent saving and reducing the CL response of blank. The effect of incubation time on immunosensor was also optimized (Fig. S3-E). HRP/Ab-luminol-AuNPs were mixed with antigen and incubated at 37 °C for different time before CL analysis. It was found that the CL detection signal increased rapidly with the increase of incubation time and reached highest at 45 min, and trend to level off above 45 min, indicating that the reaction had reached equilibrium at 45 min. Thus, 45 min was selected as the incubation time. Finally, the effect of PMT voltage on the CL intensity ratio of signal to blank was also optimized (Fig. S3-F). The CL intensity of the immunosensor and the blank increased with the increase of PMT

voltage. High signal/blank ratio was favored for better analytical performance. The signal/ blank ratio first increased with the increase of the PMT voltage, and reached highest when the PMT voltage was set as -350 V, and further decreased when the PMT voltage was above -350 V. Thus, PMT voltage was optimized at -350 V.