Supporting Information:

Single gold nanoparticles counter: An ultrasensitive detection platform for homogenous immunoassays and DNA hybridization assays

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Supporting methods:

Synthesis of gold nanoparticles (GNPs). GNPs were synthesized by reduction of the HAuCl₄ solution with sodium citrate.¹ Briefly, 100 mL of 0.01% (w/v) of the HAuCl₄ solution was heated to boiling for 15 minutes, and then 1% (w/v) sodium citrate solution was added. The volume added depended on the requirement size of GNPs, and 1 mL of sodium citrate solution was needed for 36 nm GNPs. Heating continued for 15 minutes after the solution color remained unchanged. After cooling down to room temperature, the prepared GNPs stock solution was stored at 4 °C for further use. The concentration of GNPs was calculated according to the reference.² GNPs were further purified and concentrated by centrifugation (Beckman Coulter, USA) 2 times at 2,500 rpm for 30 min (washing buffer: 0.01 M PBS, pH 7.4) prior to further use.

ELISA method (contrast experiments). To investigate the reliability of our method, the human sera were analyzed with ELISA method according to the operational manuals of ELISA kits. Briefly, 50 µL antigen or human serum samples were added to the wells of the ELISA plate, and incubated at 37 °C for

20 min. The plates were emptied and washed four times with wash buffer (100 mM phosphate buffer, 150 mM NaCl, 0.2% BSA and 0.05% Tween 20). Fifty μ L enzyme-labeled antibody solution was added to each well and incubated at 37 °C for 20 min. The plates were emptied again and washed three times with the wash buffer. The color development reagents were added and the color intensities were measured by UV absorption spectroscopy at 450 nm wavelength. The positive linear relationship between the color intensities and antigen concentrations was obtained.

Assay of thrombin by capillary electrophoresis (contrast experiments). The assay of thrombin by capillary electrophoresis (CE) was performed according to the protocol of the reference.³⁻⁵ In this work, a carboxyfluorescein-labeled 29-mer aptamer (F29-mer) was used, and an affinity CE with LIF detection method was established for quantitative analysis of thrombin based on the high affinity between anti-thrombin aptamer (F29-mer) and thrombin. F29-mer was mixed with thrombin in the running buffer (containing 2.5% (v/v) serum) and incubated for 30 min at room temperature. Finally, the aptamer was 50 nM and thrombin was from 0 to 400 nM. The running buffer was 2×TG (TG containing 25 mM Tris, 192 mM glycine, pH 8.5). All capillary electrophoresis experiments were performed using a P/ACE MDQ CE equipment (Beckman Coulter, Flulerton, CA, USA) with an LIF detector ($\lambda_{\text{ex}}/\lambda_{\text{em}}$: 488/520 nm). Fused-silica capillaries (Yongnian Optical Fiber, Hebei, China) of 30 cm length (20 cm to the detector) with an 75 μ m inner diameter and 360 μ m outer diameter were used. Following each electrophoresis run, the capillary was rinsed with 1 M HCl, H₂O, 1 M NaOH, and running buffer for 2 min in turn. Samples were introduced by hydrodynamic injection at 0.3 psi for 5 s, and separated at 18 kV. The data were recorded with 32 Karat software (version 4.0, Beckman Coutler). Assay of every sample was repeated three times. The human sera or the activated plasma samples were prepared by diluting human sera or activated plasma with running buffer to reach a final concentration of 2.5% (v/v). An aliquot (50 μ L) of the sample was incubated at 25 °C for 30 min with 50 nM of fluorescently labeled aptamer for thrombin and another 5 μ M of a non-specific and non-fluorescent 29-mer oligonucleotide. The non-specific oligonucleotide was used to reduce the interference from the sera or plasma matrix. The positive linear relationship between the peak area of thrombin-F29 mer complex and the thrombin

concentration was obtained.

The theoretical calculation of photon burst counts. The photon burst counts are associated with the detection volume, the average lateral transit time and the probability (P_m) of single GNPs within the detection volume, and the measurement time. The photon burst counts were estimated as follows. Firstly, the detection volume and the structure parameters of the focused laser beam were determined by fluorescence correction spectroscopy (FCS) with a 1nM Alexa 647 solution as standard substance (diffusion coefficient: $2.8 \times 10^{-6} \text{ cm}^2/\text{s}$ in water)⁶. The obtained horizontal radius ω_0 and axial radius Z_0 of the focused laser beam were $0.28 \pm 0.01 \text{ }\mu\text{m}$ and $2.06 \pm 0.04 \text{ }\mu\text{m}$ respectively, and thus the detection volume of $0.9 \pm 0.05 \text{ fL}$ was obtained based on the formula $V = \pi^{\frac{3}{2}} \omega_0^2 Z_0$ ^{7, 8}. Secondly, the diffusion coefficient D of the 36 nm GNPs was obtained to be $1.36 \times 10^{-11} \text{ m}^2/\text{s}$ according to the Stokes-Einstein relation $D = kT/(6\pi\eta R_h)$. Where, k is Boltzmann constant ($1.38 \times 10^{-23} \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$), T is the absolute temperature, η is the viscosity of the solvent, and R_h is the hydrodynamic radius of GNPs (here $R_h = 36 \text{ nm}$). The average lateral transit time τ_D is calculated to be 1.4 ms on the basis of $\tau_D = \omega_0^2/(4D)$. Thirdly, the probability (P_m) of single GNPs within the detection volume of 0.9 fL is calculated to be 8.4 % when the concentration of GNPs is 0.17 nM according to Poisson statistics.

Based on the above calculation, we evaluated the photon burst counts (n) of 36 nm GNPs to be about 7200 in the detection volume of 0.9 fL when the concentration of GNPs was 0.17 nM and the measured time was 120 s, based on the equation: $n = (120/\tau_D) \times P_m$. In this case, the measured result was about 2500 burst counts for 1ms bin time, which were basically in agreement with the calculated result. The deviation between the measured and calculated results mainly originated from in the inaccurate concentration of GNPs and Gaussian profile of the focused laser beam.

References

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Supporting Figures:

Figure S1. Typical photon burst traces of GNPs solution. The concentration of GNPs was 1.7×10^{-10} M, and the size was 36 nm. The laser illumination powers was 1.53 mW(a), 1.06 mW (b), 0.10 mW (c) and 0.011 mW(d), respectively. The wavelength of the laser beam was 632.8 nm. The signal overflow of GNPs was observed under higher laser power (about 1 mW). The inset in the Figure d is an enlarged figure of Figure d. The ratio of signal to noise is the highest at 0.10 mW, and thus we chose 0.10 mW in following experiments.

Figure S2. Photon burst traces of different size GNPs (left) and their transmission electron microscope (TEM) images (right). GNPs diameters are 55 ± 10 nm, 36 ± 4 nm, 25 ± 6 nm and 17 ± 3 nm, respectively. The concentration of GNPs are 4.7×10^{-11} , 1.7×10^{-10} , 5.0×10^{-11} and 1.6×10^{-9} M, respectively. These

results show that the intensity of photon bursts increase sharply with the diameters of GNPs. The signal overflow of 55 ± 10 nm GNPs was observed in our detection system. Furthermore, we found that 55 nm GNPs (or bigger) was not very stable as 36 nm GNPs in the storage process. Therefore, 36 nm GNPs was chosen in our experiments.

Figure S3. A comparison of the photon burst traces of gold nanoparticles (GNPs, 36 nm), quantum dots (QDs 655) and Alexa 647. GNPs concentration was 1.7×10^{-10} M, QDs concentration (the emission wavelength of 655 nm) was 5.0×10^{-11} M, and Alexa 647 concentration was 1.0×10^{-10} M. Laser excitation power was 0.10 mW. The fluorescence of QD 655 and Alexa 647 was filtered by a band-pass filter (660DF50, Omega Optical, USA). As shown in this figure, the photon burst intensity of single GNPs was several tens to hundreds times higher than that of QDs and organic dyes.

Figure S4. The linear relationship of the burst counts and GNP concentration. There are no distinguishable differences in the linearity and the slope of the curves at our experiment range (0.1 ms, 0.2 ms 0.5 ms, 1 ms, 5 ms and 10 ms). We chose 1 ms as the bin time for following experiment.

Figure S5. Titration curves of alpha fetal protein (AFP) with different antibody-GNPs conjugate concentrations. Lines 1, 2 and 3 show the antibody-GNPs conjugates concentrations of 285 pM, 143 pM, and 57 pM, respectively. We observed no significant differences in the slopes of three calibration curves, which showed that the concentrations of antibody-GNPs conjugates had no significant effect on the sensitivity of this assay under our experimental conditions.

Figure S6. The curve of burst counts versus immunoassay reaction time. In this study, 10 μL of 0.25 nM GNPs-anti-AFP-1 conjugate solution, 10 μL of 0.25 nM GNPs-anti-AFP-2 conjugate solution and 20 μL of 0.01 M PBS buffer (pH=7.4) were added into a 200 μL sterilized polypropylene tube respectively and mixed well. Ten μL of 10 ng/mL AFP antigen standard solution was added, mixed well and incubated at 37 $^{\circ}\text{C}$ for different reaction time. The burst counts of samples were measured by SGNPC method. As shown in this figure, the immune reaction completed after 40 minutes incubation. In the following experiments, the incubation time was 1 h.

Figure S7. Results of thrombin assay by capillary electrophoresis. (a). Capillary electrophoregrams of thrombin-aptamer complexes and free aptamer. 1: A mixture of 100 nM thrombin incubated with 50 nM F29-mer in the running buffer; 2: 50 nM F29-mer in the running buffer; and 3: A mixture of 100 nM thrombin incubated with 50 nM F29-mer in 2.5% (v/v) human serum. The running buffer was $2 \times$ TG

(TG containing 25 mM Tris and 192 mM glycine, pH 8.5), the separation voltage was 18 kV, and the injection was at 0.3 psi for 5 s. (b). The linear relationship between the peak area of the thrombin-F29-mer complex and the concentration of thrombin. Calibration curve was obtained by using increased concentration of thrombin and fixed concentration of F29-mer at 50 nM in 2.5% (v/v) human serum.

Supporting Tables:

Table S1. Recovery results of CEA and AFP immunoassays

	CEA		AFP	
	Sample 1	Sample 2	Sample 1	Sample 2
Original amount (M)	1.51×10^{-13}	1.51×10^{-13}	2.74×10^{-12}	2.74×10^{-12}
Added amount (M)	2.60×10^{-13}	2.60×10^{-12}	2.86×10^{-12}	2.86×10^{-11}
Founded amount (M)	3.9×10^{-13}	2.62×10^{-12}	5.39×10^{-12}	2.88×10^{-11}
Recovery (%)	91.9	95.0	92.7	91.1
RSD (%)	9.3	6.0	6.9	7.8

In recovery experiments, a given amount of antigens (CEA and AFP) were added into the sample 6 after sample was 200 times diluted with buffer. The samples were then measured by SGNPC. The RSD was obtained from three independent experiments.

Supporting Video:

The movie of GNPs photon bursting in solution. Please click the image to start the movie. Images ($7.8 \times 3.6 \mu\text{m}^2$) were obtained without a pinhole using an electron-multiplying charge-coupled device (EMCCD) camera with a frame-transfer device (Cascade 650, Photometrics, USA). The frame transfer device has an imaging array of 653×492 with $7.4 \times 7.4 \mu\text{m}^2/\text{pixel}$. The play rate was 30 frames/s with 2 ms exposure time for each frame. Scale bar was $2 \mu\text{m}$, GNPs (36 nm) concentration was $1.7 \times 10^{-10} \text{ M}$, and laser illumination power was 0.1 mW.

Complete reference. 1

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