Supporting Information:

Plasmonic ELISA using nanospherical brushes as a catalase container for colorimetric detection of ultralow concentrations of *Listeria monocytogenes*

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1. *The molecules of CAT immobilization on the SiO_2@PAA*

The molecular weight of silica core (80nm) in SiO_2@PAA is calculated assuming that the silica core is uniform solid sphere with a density of 2 g/cm^{-3}. The molecular weight of silica core is 5.359 × 10^{-16} g with the following equation: $m=\rho V$. The content of PAA chains relative to the silica core is 121.6% determined by thermogravimetric analysis. The molecular weight of the SiO_2@PAA is 1.1875 × 10^{-17} g. Therefore, the number of the SiO_2@PAA is 8.44 × 10^{11} per milligram. The weight loss of SiO_2@PAA@CAT in comparison with SiO_2@PAA is 365.4 μg/mg. Thus the mole of CAT is 1.035× 10^{-15}. By this way, we can calculate the molecules of CAT on single SiO_2@PAA, which is 1018.

2. *The change of enzymatic activity of the SiO_2@PAA@CAT after anti-Listeria monocytogenes and biotin immobilization*

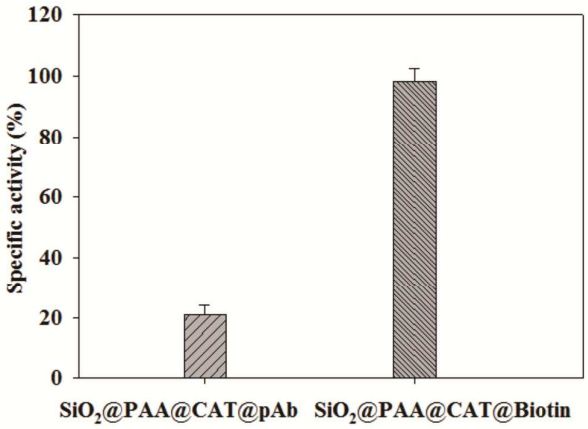

Figure S-1. The enzymatic activity of the SiO_2@PAA@CAT@pAb and the SiO_2@PAA@CAT@Biotin. Note that the enzymatic activity of SiO_2@PAA@CAT before the immobilization was set as 100%.

3. *Synthesis and characterization biotinylated CAT (CAT@Biotin) and biotinylated*

HRP (HRP@Biotin)

3.1 Synthesis of CAT@Biotin and HRP@Biotin

1.0 mg of CAT or HRP were dissolved in 1 mM phosphate buffer solution (PBS, pH = 8.6) containing biotin-N-hydroxysuccinimide ester with mole ratios of CAT and biotin at 1:50, and HRP and biotin at 1:10, respectively. After reaction for 3 h at ambient temperature, the obtained CAT@Biotin and HRP@Biotin complex were purified by dialysis with PBS buffer (pH = 7.4) for three days, and then stored in the mixture of glycerol and water (1:1) at -20 °C for further use.

3.2 Characterization of SiO_2@PAA@CAT@Biotin, CAT@Biotin and HRP@Biotin

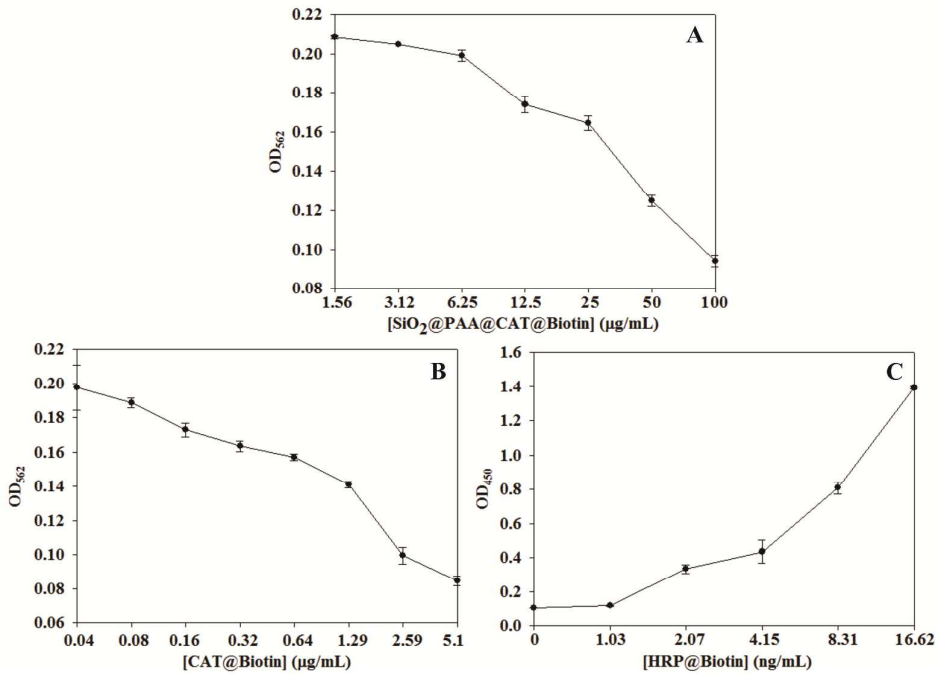

Figure S-2. Characterization of SiO_2@PAA@CAT@Biotin (A), CAT@Biotin (B) and HRP@Biotin (C) based on pELISA or conventional ELISA. In Figure S-2A and 2B, the color of the well in the microplate will change from red to blue, and then to colorless with the biotinylated SiO_2@PAA@CAT or CAT concentration increasing,

whereas the OD_{562} value will decrease with the increasement of CAT concentration. In Figure S-2C, the color of conventional ELISA will increase with the HRP concentration increasement. The above results indicated that the biotin was successfully covalently attached onto the surfaces of SiO_2@PAA@CAT, CAT, and HRP, respectively.

4. Checkerboard method for the concentrations optimization of anti-Listeria monocytogenes mAbs and biotinylated anti-Listeria monocytogenes pAbs

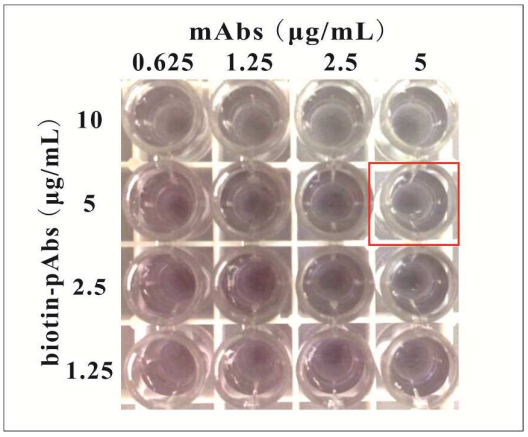

Figure S-3. Photograph showing the selection for the working conditions of SiO_2@PAA@CAT@Biotin-based pELISA using the checkerboard method. The corresponding absorbances are shown in Table S-1. The highlighted well was selected as the optimal experimental condition.

Table S-1: Absorbances of the wells in Figure S-3

	mAbs			
biotin-pAbs	**0.625**	**1.25**	**2.5**	**5**
10	0.152	0.13	0.128	0.093
5	0.186	0.155	0.137	0.101
2.5	0.185	0.174	0.151	0.139
1.25	0.187	0.184	0.179	0.141

5. CAT@Biotin-based pELISA for detection of Listeria monocytogenes

96-well polystyrene plates were modified with 100 μL of anti-*L. monocytogenes* mAb (5 μg/mL) in PBS (pH 8.6) at 4 °C overnight. After washing the plates three times with PBST, the plates were blocked with blocking buffer (1 mg/mL BSA in PBS) for 1 h at 37 °C. Subsequently, the plates were washed three times with PBST, and *L. monocytogenes* at desired final concentrations were added to the plates. After incubation for 1 h at 37 °C, the plates were washed three times with PBST, and then 100 μL of biotinylated anti-*L. monocytogenes* pAb (5 μg/mL) was added to the plates for 1 h at 37 °C. After washing the plates three times with PBST, 100 μL of streptavidin (15 μg/mL) was added. After reaction for 30 min at 37 °C and washing the plates three times with PBST, 100 μL of CAT@Biotin (150 μg/mL) was added for 30 min at 37 °C. After washing the plates twice with PBST, twice with PBS and once with deionized water, 100 μL of H_2O_2 (240 μM) in 1 mM MES buffer (pH 6.5) was added to each well of the plates. After 30 min, 100 μL of freshly prepared $HAuCl_4$ (0.2 mM) in MES buffer was added to each well. The absorbance at 562 nm was recorded after 10 min with a SpectraMax M5 plate reader (Molecular Devices).

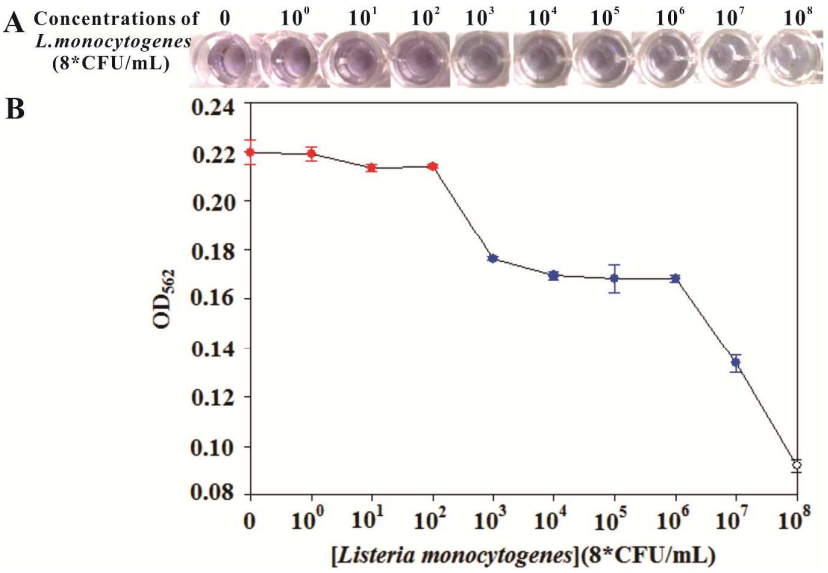

Figure S-4. CAT@Biotion-based pELISA for the detection of various concentrations of *Listeria monocytogenes* (8×10^0–8×10^8CFU/mL) diluted in PBS.

6. HRP@Biotin-based conventional ELISA for Listeria monocytogenes detection

96-well polystyrene plates were coated with 100 μL of anti-*Listeria monocytogenes* mAbs (5 μg/mL) at 4 °C overnight. After washing the plates three times with PBST (PBS, pH 7.4, 0.01 M, containing 0.05% Tween 20), the plates were blocked with blocking buffer (1 mg/mL of BSA in PBS) for 1 h at 37 °C. After washing the plates three times with PBST, 100 μL of biotinylated anti-*L. monocytogenes* pAbs (2.59 μg/mL) was added to the plates for 1 h at 37 °C. After washing the plates three times with PBST, 100 μL of streptavidin (3 μg/mL) was added. After reaction for 30 min at 37 °C and washing the plates three times with PBST, 100 μL of HRP@Biotin (0.665 μg/mL) was added for 30 min at 37 °C. After washing the plates twice with PBST, twice with PBS and once with deionized water, 100 μL of TMB solution was added. After incubation for 15 min at room temperature, the reaction was terminated with 50 μL of 2 M H_2SO_4, and the absorbance was

measured at 450 nm using a microplate reader.

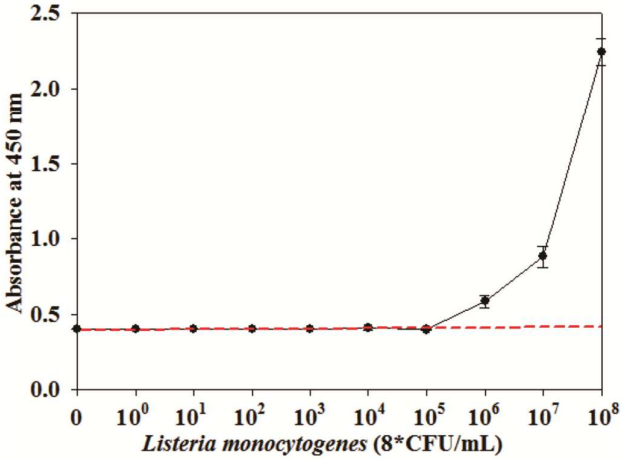

Figure S-5. HRP-based conventional ELISA for the detection of various concentrations of *Listeria monocytogenes* (8×10^0 CFU/mL – 8×10^8 CFU/mL) diluted in PBS.

7. Application of Poisson-Binomial model in the interpretation of single bacteria detection of plasmonic ELISA

According to the previous report, the Poisson-Binomial model has been widely used in the interpretation of serial dilution experiments, especially in single molecule detection.[1] Therefore, we introduce this method to interpretation of single bacteria. According the well-known mathematical formula, i.e., $p(X = r|R) = C_R^r(p_0)^{R-r}(1 - p_0)^r$, where $p(X = r|R)$ is the probability of r out of R wells containing at least one bacteria, R is the well numbers at a given dilution (in this study R=3), r is the well numbers containing at least one bacteria of solute (r = 0, 1, 2, or 3 in this work), and p_0 is the probability of obtaining no bacteria in a single well. Additionally, $p_0 = \mathrm{e}^{-\bar{n}}$, where $\bar{n}$ is the mean number of bacteria in a volume v (100 μL) and e is the base of natural logarithms.

For a nominal concentration of 10^{-n} CFU/mL, the expected number of bacteria per well can be determined using the viable bacteria plate count. Thus, in 100 μL, we have on average 0.8 bacterium cell per well at 8×10^0 CFU/mL. Then, the probability of a given number of wells containing at least one bacterium can be derived from the Poisson-Binomial distribution.

For three replicates, the probability of *r* wells containing at least one bacterium cell at the nominal concentration ranged from 8×10^0 CFU/mL to 8×10^4 CFU/mL was calculated according to the previous mathematical formula. These results are summarized in Table S2.

Table S-2 Probability distribution of wells with at least one bacterium cell.

Probabilities calculated using the Poisson-Binomial model and based on nominal concentrations at the 8×10^0 CFU/mL dilution of 0.8 per well for *Listeria monocytogenes*. Probabilities of the most likely outcome corresponding to results observed here for *Listeria monocytogenes* has been highlighted in red, and they are precisely the outcome under three conditions.

Target bacteria	**Wells containing biomarker**	**Dilution (CFU/mL)**				
		4	3	2	1	0
Listeria monocytogenes	0	0	0	0	0	0.090
	1	0	0	0	0	0.334
	2	0	0	0	0	0.409
	3	1	1	1	1	0.167

8. Verification of individual bacterial with plate count

Plate count was used to estimate the accuracy of the detection for a single *L. monocytogenes*. Desired concentrations of *L. monocytogenes* solutions were prepared

by tenfold dilution as previously described. 2 mL of target solutions containing living *L. monocytogenes* (10^0 or 10^1 CFU/mL) were divided into two sections: one for the viable bacteria plate counting as previously described and another for sandwich plasmonic ELISA.

9. Repeatability of qualitative detection

Table S-3: Results of 10 measurements of *Listeria monocytogenes* at 8×10^0 CFU/mL from three independent experiments, respectively.

Experiment	Positive	Negative
Replicate 1	4	6
Replicate 2	4	6
Replicate 3	5	5

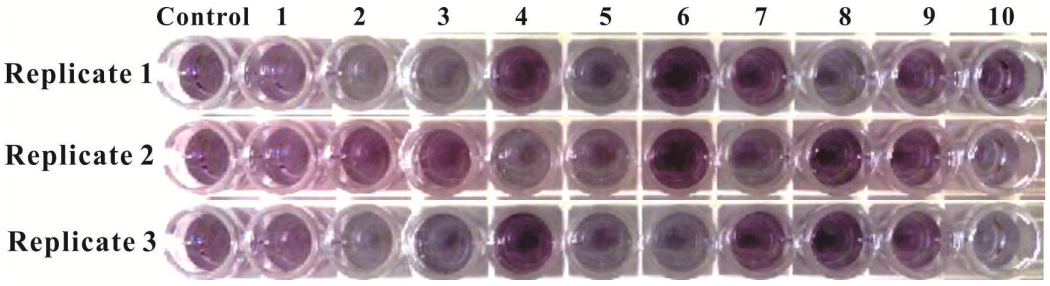

Figure S-6. The *L. monocytogenes* concentration was 8×10^0 CFU/mL, 4 positive and 6 negative in replicate 1 and 2, 5 positive and 5 negative in replicate 3 were observed in three independent experiments.

Reference:

1. Cochran W G. Estimation of Bacterial Densities by Means of the "Most Probable Number". *Biometrics* **1950**, *6*, 105-116.