Supporting information

Catalase-linked immunosorbent pressure assay for portable quantitative analysis

Dan Liu,[a],* Fang Liu,[b] Yishun Huang,[b] Yanling Song,[b] Zhi Zhu,[b] Shu-feng Zhou[a] and Chaoyong Yang[b],*

[a]Department of Bioengineering and Biotechnology, College of Chemical Engineering, Huaqiao University, Xiamen 361021, China.
[b]State Key Laboratory of Physical Chemistry of Solid Surfaces, Key Laboratory for Chemical Biology of Fujian Province, The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China.

*Corresponding Authors.

E-mail: liudan@hqu.edu.cn (D. Liu)

E-mail: cyyang@xmu.edu.cn (C. Yang)

Standard ELISA

The standard ELISA was performed to analyze CRP samples at different concentrations to demonstrate the accuracy of catalase-linked immunosorbent pressure assay. One hundred μL of 2 μg/mL CRP capture antibody was added to a 96-well plate and incubated overnight at room temperature. Then each well was washed three times and incubated with 100 μL of different concentrations of CRP samples for 2 hr at room temperature. The washing process was repeated three times and then 100 μL of 0.5 μg/mL detection antibody was added and incubated for 2 hr at room temperature. After repeated washing, one hundred μL of 1 μg/mL streptavidin-HRP was added and incubated for 1 hr at room temperature. After repeated washing, one hundred μL of substrate solution was added and incubated for 20 min at room temperature while protecting from light. Fifty μL of stop solution was added and the optical density was determined at 450 nm.

The standard curve for the CRP detection by ELISA is shown in Fig. S2, with the range of 3.125–25 pM, and the limit of detection is 0.856 pM. We tested the CRP samples using both the catalase-linked immunosorbent pressure assay and the ELISA method. By the ELISA, the CRP samples were diluted appropriately to cover the CRP concentration range.

Recovery analysis in serum

The recovery analysis is one of the most effective methods to evaluate the accuracy of analytical results, which is used for the determination of pure analytes added, and the results are expressed as the percentage rate of recovery. Refer to Clinical and Laboratory Standards Institute, the recovery analysis in serum was performed. Since recovery analysis is to need appropriate calibration products, calibration curve of CRP detection in human serum by pressure assay was firstly obtained to eliminate matrix interference (Fig. S3). The human serum without CRP was obtained from Chenggong Hospital of Xiamen University. Then the following samples were prepared:
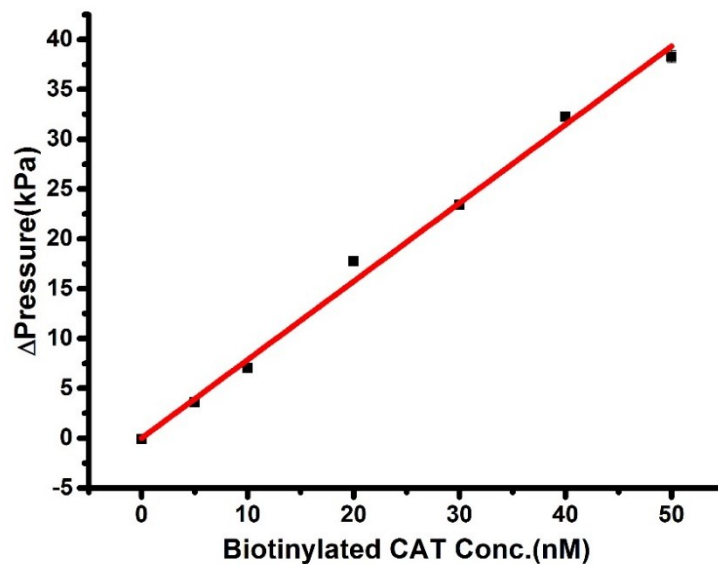
(1) Basal serum: serum (no CRP) as blank control
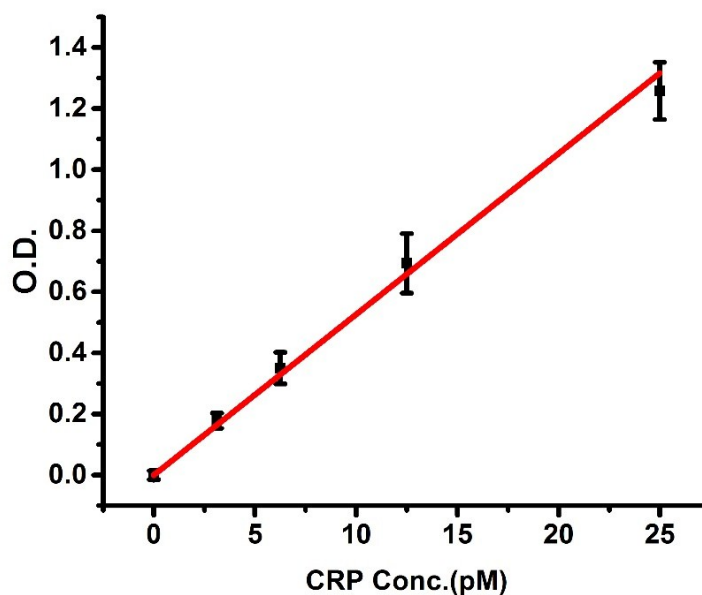
(2) Sample 1: serum+CRP (final concentration of 10 nM)

(3) Sample 2: serum+CRP (final concentration of 30 nM)

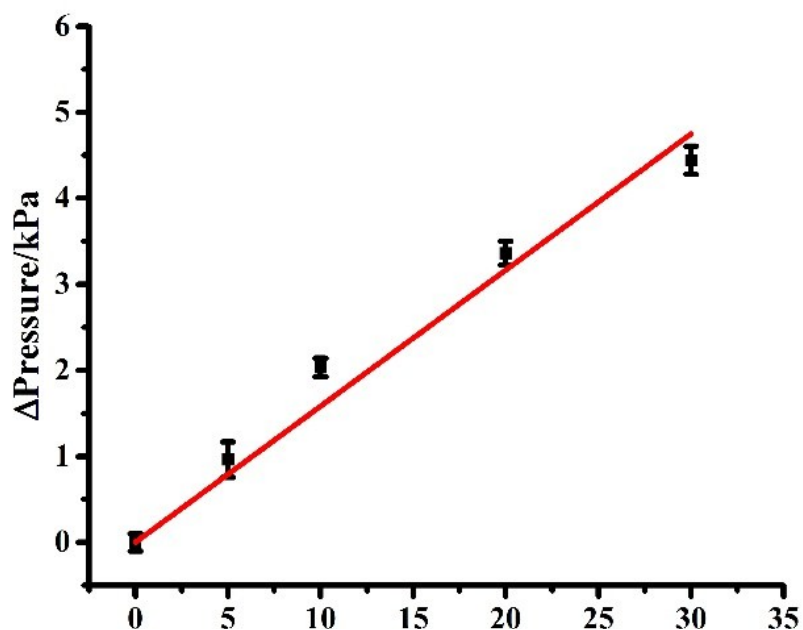Each sample was measured 3 times and the average value was taken to fill Table S2.

The average recovery rate is calculated as 101%.

Fig. S1 Linear response of pressure changes to biotinylated catalase with different concentrations (R^2 =0.999)

Fig. S2 Calibration curve for CRP detection by standard ELISA (R^2 =0.996)

Fig. S3 Calibration curve of CRP detection in serum by catalase-linked immunosorbent pressure assay ($R^2 = 0.987$).

Table S1 Surface charge of the catalase and PtNPs.

Zeta potential (mV)	
Catalase	PtNPs
-4.2±0.2 mV	-30.2±0.4 mV

Table S2 Recovery analysis in serum.

	Pure analytes added (nM)	Determination (recovery) (nM)	Percentage rate of recovery (%)
sample 1:	10	11.20	112%
sample 2:	30	26.96	90%

$$\text{Average recovery rate} = \frac{112 + 90}{2} x100\% = 101\%$$