Supporting Information

Pressure-based Bioassay for Portable and Quantitative Detection of C-Reactive Protein

Tianhai Ji[1,2,‡,] Dan Liu[2,‡], Fang Liu[2], Jiuxing Li[2], Qingyu Ruan[2], Yanling Song[2], Tian Tian[2], Zhi Zhu[2], Leiji Zhou[2], Hui Lin*[2], Chaoyong Yang[2], Dong Wang*[1]

1.Cancer Center, Daping Hospital and Research Institution of Surgery,Third Military Medical University, Chongqing 400042, P. R. China; E-mail: dongwang64@hotmail.com

2.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, chenggong hospital, Xiamen University, Xiamen 361005, P. R. China; E-mail: huilin@xmu.edu.cn

Experimental

Coating of 96-well microplate and magnetic microspheres with capture antibody:

The 96-well plate was coated with 100μL of 8 μg/mL CRP capture antibodies at 4°C overnight.

Dynabeads® M-280 Tosylactivated (165 μL, 30 mg/mL) were washed three times with 0.1M Na-phosphate buffer (pH 7.4). Capture antibodies (200 μL, 200 μg/mL) and 100 μL 0.1 M Na-phosphate buffer (pH 7.4) with 3 M ammonium sulfate were added and incubated for 12-18 hr at 37°C. After removing the supernatant, 1 mL PBS with 0.5% BSA was added to block the residual sites on the M-beads for 1hr at 37°C. The M-beads were resuspended in 240 μL PBS with 0.1% BSA after washing three times with this buffer to achieve a final concentration of 20 mg/mL, and stored at 4°C before use.

Biotinylation of the detection antibody.

CRP detection antibody (100 μL, 3.2 mg/mL) was diluted to 100 μg/mL with pH 9.0 Na_2CO_3 and then incubated with 40 μL 1 mg/mL biotin N-hydroxysuccinimide ester

solution for 4 hr at room temperature. Then, the biotinylated antibody was washed 3 times with PBST at 14000 rpm and resuspended in 100 μL PBST with 0.5% BSA.

PtNPs synthesis and functionalization.

Firstly, 10 μL H_2PtCl_6 (100 mM) was added to 900 μL deionized water and incubated at 80°C for 20 min. Then 100 μL aqueous solution of ascorbic acid (0.4 M) was added immediately and incubated at 80°C for 30 min. The synthesized nanoparticles were stored at 4°C before use.

Ten μL of 1wt% Tween 20 and 5 μL of 100 μM mPEG-SH were added to 1 mL of 2.5 nM PtNPs. Then 10 μL of 120 μM thiol-PEG-biotin hetero-linker was added after brief mixing, followed by 50 μL of 0.2 M H_3PO_4. After aging for 1 hr at 37°C, excess reagents were removed via centrifugation 3 times at 13000 rpm, and then the NPs were resuspended in 1mL 0.5% BSA PBST (0.1M PBS+0.1% tween, pH 7.4) solution before CRP detection.

Pressuremeter fabrication and pressure detection

The hand-held pressuremeter was designed by our group to change the device size to a pen-like level, without decreasing any sensitivity. The most common pressure sensor of BMP085 was utilized to detect the pressure value in 96-well plate. A needle with 0.7 mm inner diameter was used to attach to the pressure sensor. The pressure value was displayed by a 1602E LCD monitor (36.0 mm×10.0 mm). A 600 mAh battery could support continuous detection for 5 hr at least. When measuring gas pressure from microplates or magnetic beads, the rubber cover for the 96-well plate and the rubber sheet for the tubes ensure the insertion of a needle with gas sealing.

CRP immunoassay

For the experiment in 96-well plate, after coating with capture antibody overnight and washing 3 times with PBST (Phosphate-buffered Saline with 0.1% Tween 20) by an autowasher, 300 μL block buffer (containing 2% BSA in PBST) was added and incubated at room temperature for 1.5 hr. After washing 3 times, 100 μL CRP samples with different concentrations were added and incubated for 1 hr at room temperature. The washing process was repeated and then 100 μL of 2 μg/mL detection antibodies was added to the well and incubated at room temperature for 1 hr. After washing 3 times with PBST, 100 μL streptavidin was added and incubated for 1 hr. After washing, 100 μL biotinylated Pt Nanoparticles (PtNPs) was then added to the well and incubated

for 1 hr at room temperature. After repeating the washing process 6 times, 100 μL of 30% H_2O_2 was added and the pressure was detected by pressuremeter after 60 min gas generation time.

For the experiment of magnetic microspheres, twenty μL aliquots of different concentrations of CRP were added to 50 μL antibody-functionalized M-beads and incubated for 30 min at room temperature. After washing 3 times with PBST, 100 μL CRP biotinylated detection antibody was added to the M-beads for 15 min incubation. The M-beads were then resuspended in 100 μL streptavidin after washing 3 times, followed by 15 min incubation at room temperature. After washing 3 times, the M-beads were incubated in a solution of biotinylated PtNPs for 15 min at room temperature. The M-beads were then recovered after washing 6 times and were reacted with 100 μL 30% H_2O_2 for 15 min before measurement with pressure meter.

Selectivity of pressure based assay

To investigate the specificity for CRP detection using this method, other proteins with concentrations of 0.025 μg/mL, including human serum albumin (HSA), immunoglobulin G (IgG), thrombin (Thr), Hemagglutinin 7 Neuraminidase 9 (H7N9) and severe acute respiratory syndrome (SARS) were used in place of CRP and detected by pressure meter.

CRP calibrators and clinical samples

Fourteen clinical samples from Chenggong Hospital of Xiamen University with different concentrations of CRP ranging from 1μg/mL to 80 μg/mL were detected using the pressure method. First, the patients samples were diluted with PBST containing 0.1% BSA and then detected. The results were compared to that detected by TINIA.

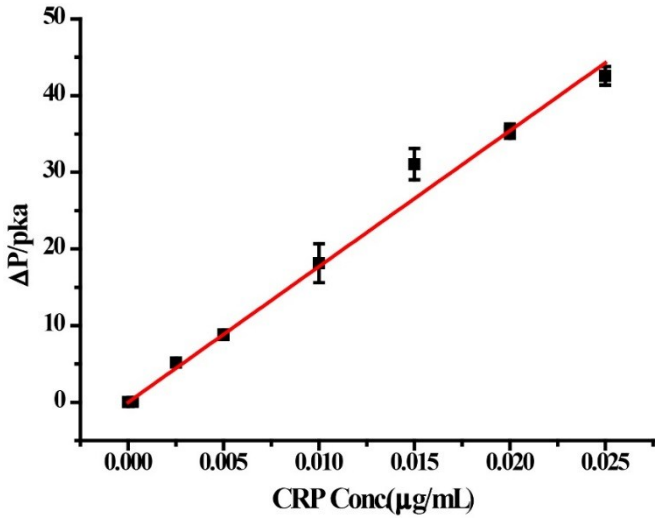

Figure S1. Calibration curve of CRP detection in diluted human serum by pressure bioassay. Dynamic range: 0.00025-0.025 μg/mL

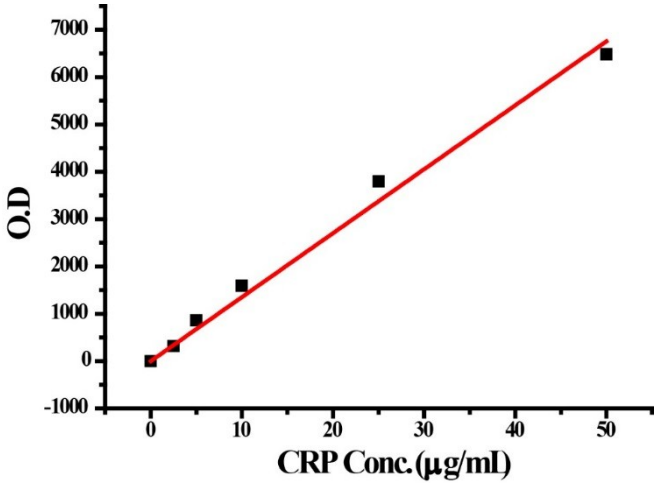

Figure S2 Calibration curve for CRP detection by turbidimetric inhibition immunoassay.