Supporting Information

Shake&Read Distance-based Microfluidic Chip as a Portable Quantitative Readout Device for Highly Sensitive Point-of-Care Testing

Yi Xie[a]†, Xiaofeng Wei[b]†, Qizhen Yang[c], Zhichao Guan[a], Dan Liu[a], Xuan Liu[a], Leiji Zhou[a], Zhi Zhu[a]*,

Zhenyu Lin[b]*, Chaoyong Yang[a]*

a: MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, Collaborative Innovation Center of Chemistry for Energy Materials, Key Laboratory for Chemical Biology of Fujian Province, State Key Laboratory of Physical Chemistry of Solid Surfaces, College of Chemistry and Chemical Engineering, Xiamen University Xiamen 361005 P. R. China; *Tel: (+86) 592-218-7601, E-mail: cyyang@xmu.edu.cn ; zhuzhi@xmu.edu.cn.*

b: MOE Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou, Fujian, 350116, P. R. China; *Tel: (+86) 591-22866135, E-mail: zylin@fzu.edu.cn*

c: Xiamen University Affiliated Keji High School, Xiamen 361005, P. R. China.

Experimental Section

Materials and Reagents

Tween 20 was purchased from Xilong Chemical Co., Ltd. (Guangdong, China). 2-Mercaptoethanol was obtained from Xiya Reagent Company Ltd. (Chengdu, China). We purchased the mPEG-SH (MW ~5 kDa) from JenKem Technology Co., Ltd. (Beijing, China). Other reagents were purchased from Sinopharm Chemical Reagent (Shanghai, China). The magnetic beads modified with human kallikrein 3/PSA antibody were purchased from Abbott (Shanghai, China). Human kallikrein 3/PSA and biotinylated antibody recombinant human kallikrein 3/PSA were purchased from R&D Systems (Minneapolis, MN, USA). Ten clinical PSA samples were provided by Chenggong Hospital of Xiamen University with different concentrations of PSA ranging from 20 pM to 160 pM.

Synthesis and Purification of Thiol-PEG-biotin Hetero-linker

Thiol-PEG-biotin hetero-linker was synthesized according to the standard DNA synthesis protocol using biotin CPG and 5' thiol modifier. Then the product was cleaved from the solid support and deprotected with ammonia treatment. After purification by HPLC, it was reduced by DTT (DL-dithiothreitol), and desalted by a NAP-5 column and NAP-10 column, and finally quantified by UV-Vis spectrometry.

Nanoparticle Synthesis[1] and Functionalization

10 μL of 100 mM H_2PtCl_6 was added to 900 μL deionized water and incubated at 80°C for 20 min. Then 100 μL aqueous solution of 0.4 M ascorbic acid was added and incubated at 80°C for 30 min. The synthesized nanoparticles were stored at 4°C before use. The PtNPs were then modified by a thiol-PEG-biotin hetero-linker. Briefly, 10 μL of 1 wt% Tween 20 and 5 μL of 100 μM mPEG-SH (MW ~5 kDa) were added to 1 mL of a 2.5 nM solution of 30 nm PtNPs. After a brief mixing, 10 μL of 120 μM thiol-PEG-biotin hetero-linker and 50 μL of 0.2 M H_3PO_4 were added to the mixture. After aging at 37°C for 1 hr, excess reagents were removed via centrifugation at 13,000 rpm three times for 4 min each. The precipitate was then redissolved in 1 mL of PBST solution (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.05 wt% Tween 20, pH 7.4).

Design and Fabrication of the S&R-μChip

The S&R-μChip is composed of two layers of PMMA material as cover layer and bottom layer, respectively. The pattern of two layers was designed with CorelDraw12 software and was fabricated on 2 mm thick PMMA using the laser cutting machine. As shown in Figure S1, the patterned cover layer was composed of four small holes (black) with diameter of 0.7 mm and one big hole (yellow) with a diameter of 1.5 mm. The three inlets were located over the three reservoirs for the injection of sample solution, H_2O_2 solution and food color solution, respectively. A discharge outlet was designed for inner air expel when injecting the reagents. A work outlet was located at the terminal of the channel to connect with the atmosphere. After injection of all reagents, the three inlets and the discharge outlet were sealed to form a hermetical chamber as reaction region. The bottom layer was fabricated with three reservoirs and three parts of channels. The three reservoirs were respectively used as sample reservoir (diameter: 7 mm, deeper: about 1 mm), H_2O_2 reservoir (diameter: 5 mm, deeper: about 1 mm) and food color reservoir (diameter: 7 mm, deeper: about 1 mm) and connected with three correlated inlets. The shaking channel (width: 1 mm, deeper: about 0.5 mm) bridge-linked sample reservoir and H_2O_2 reservoir for shaking and mixing of reagents. The “T-shape” medium exchange channel (width: 0.5 mm, deeper: about 0.5 mm) was set for two functions. One role is applied as an interchange between H_2O_2 solution and the air of sample reservoir after shaking. In the other words, the H_2O_2 solution can’t be shaken into the sample reservoir without the air exchange. The other effect is to connect the indicator reservoir. The scale channel bridge-links with food color reservoir and work outlet to act as a ruler for the distance readout of the pushed food color. Next, the two patterned layers were processed by thermal bonding in a laminated bonding machine (temperature: 105°C, pressure: 2Mpa, time: 180s).The final S&R-μChip device was obtained by natural cooling to ambient temperature. Then, fluorocarbon oil was injected into the S&R-μChip to cover all inner-regions and kept statically for 20-min hydrophobic treatment. Finally, the hydrophobically treated S&R-μChip was dried in nitrogen and stored in a

desiccator. The produced S&R-μChip is 11 cm × 2 cm in size and 11.2 g in total weight.

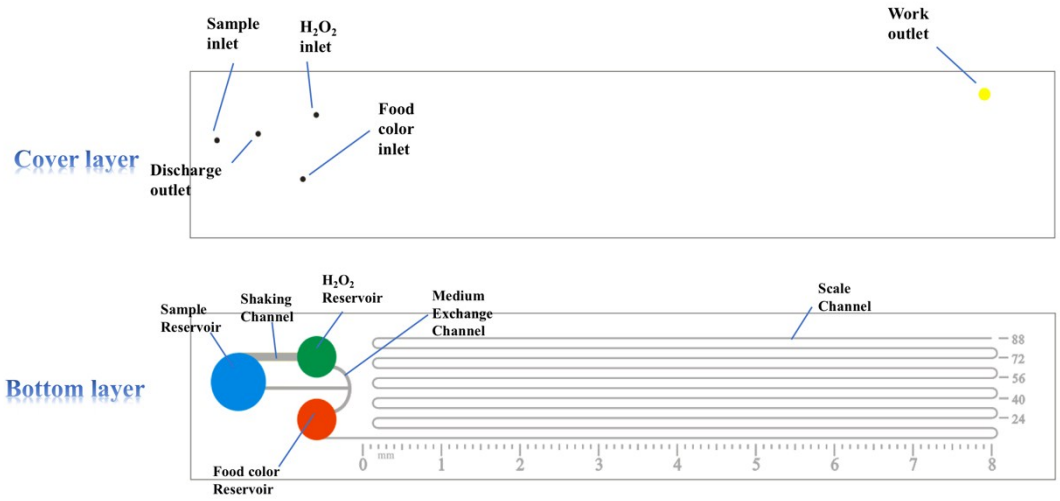

Figure S1. The design of the S&R-μChip.

Analytical Procedure

The PSA antibody-functionalized capture beads from commercial ELISA kits (Abbott) were mixed with PSA (25 pM, 50 pM, 80 pM, 100 pM, 120 pM, 150 pM, respectively) in a final volume of 150 μL of reagent diluent, and the mixtures were incubated in the centrifuge tubes for 30 min. Then the beads in each tube were washed three times and then incubated with the biotinylated detection antibody solution (0.1 μg/mL) for 20 min. After another three-time washing, the beads were incubated with the streptavidin solution (1 μg/mL) for 15 min. After washing three times, the beads were then incubated with biotinylated PtNPs (0.625 nM) for 15 min. After washing another six times, the recovered beads were resuspended in PBST. After adding the dye (20 μL) and H_2O_2 (20 μL) in the specified area in the S&R-μChip, the beads solution (20 μL) was added to the sample area of the S&R-μChip. The micropores of S&R-μChip were sealed with transparent tape, and then the chip was shaken to initiate the contact of the bead solution with H_2O_2. The O_2 generated by PtNPs/H_2O_2 promoted the dye to move forward and the distance was recorded after 5 min.

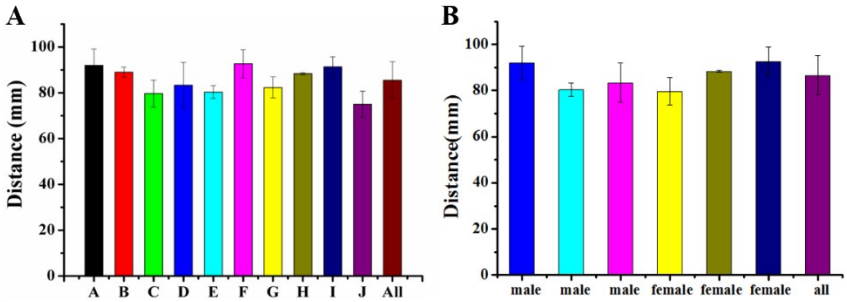

Figure S2. The reproducibility and Stability of the S&R-μChip. A) Response of 10 devices to 10 pM PtNPs. B) Response of S&R-μChip operated by 6 volunteers to10 pM PtNPs.

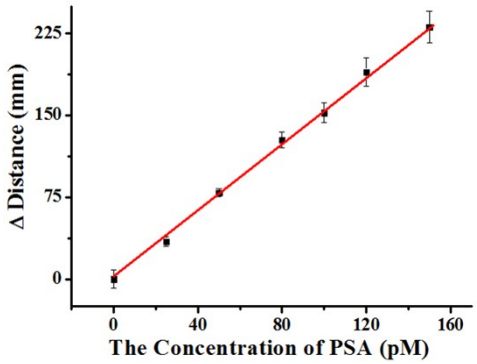

Figure S3. Linear standard curve for the detection of PSA in serum using the S&R-μChip.

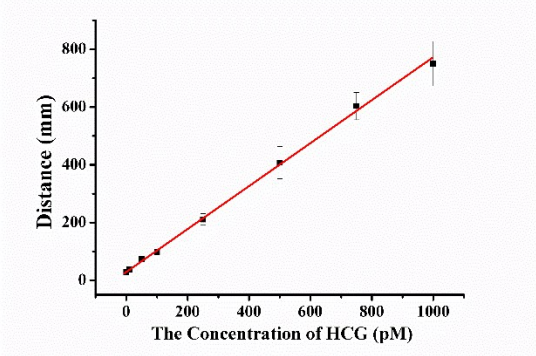

Figure S4. Linear response of the dye distance to the concentration of HCG (R^2 = 0.99).

References

1. S. J. Guo, J. Li, S. J. Dong and E. K. Wang, *Journal of Physical Chemistry C*, 2010, **114**, 15337-15342.