Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION

Polypyrrole–polydimethylsiloxane sponge-based compressible capacitance sensor with molecular recognition for point-of-care immunoassay

Jialun Chen,[a,†] Fangqin Xue,[b,†] Zhonghua Yu,[a] Lingting Huang[a] and Dianping Tang[a,*]

[a] Key Laboratory for Analytical Science of Food Safety and Biology (MOE & Fujian Province), Department of Chemistry, Fuzhou University, Fuzhou 350108, P.R. China

[b] Department of Gastrointestinal Surgery, Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital, No. 134 Dongjie, Fuzhou 350001, P.R. China

CORRESPONDING AUTHOR INFORMATION

Phone: +86-591-2286 6125; fax: +86-591-2286 6135; e-mail: dianping.tang@fzu.edu.cn (D. Tang)

† Jialun Chen and Fangqin Xue have contributed equally to this work.

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EXPERIMENTAL SECTION

Material and Reagent. Prostate-specific antigen (PSA/biomarker; D620021) standards, monoclonal mouse anti-human PSA capture antibody (capture antibody; D198173), streptavidin, polyclonal rabbit anti-human PSA detection antibody (detection antibody; D120021), biotin-conjugated goat anti-rabbit IgG(secondary antibody), sephadex G-25 resin, 2X SDS PAGE sample loading buffer, 2X Native Sample Loading Buffer, 10X Tris-Glycine Native PAGE Running Buffer PH 8.8, TureColor two-color pre-stained protein Marker, 10X Tris-Glycine SDS PAGE Running Buffer PH 8.3 and native PAGE preparation kit were purchased from Sangon Biotech. Co., Ltd (Shanghai, China). PET-ITO conductive film (South China Science & Technology Co. Ltd., Shenzhen, China). Albumin from bovine serum (BSA), ammonium persulphate (APS), sodium dodecyl sulfate (SDS), D-biotin N-succinimidyl ester (Biotin-NHS, BNHS), tris (hydroxymethyl) aminomethane (Tris), catalase from bovine liver, xylene cyanol FF, Disodium 2,6-Naphthalenedisulfonate and hydrogen peroxide (H_2O_2) solution were acquired from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Polydimethylsiloxane (PDMS, Sylgard 184) was supplied by Dow Corning (USA). Iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), pyrrole and Tween 20 were gotten from Sinopharm Chem. Re. Co., Ltd. (Shanghai, China).

All other reagents were of analytical grade and used as received. Ultrapure water acquired by a Millipore water purification system (18.2 MΩ cm, Milli-Q, Millipore) was used in all runs. Phosphate buffered saline (PBS, 10 mM, pH 7.4) solution was prepared by Na_2HPO_4, NaH_2PO_4 and 0.1 M KCl.

Apparatus. The scanning electron microscopy (SEM) images were acquired from the FEI Quanta 250 (Field Electron and Ion Company, USA). The Raman spectra were characterized by a Raman spectrometer (Renishaw, UK).Ultraviolet-visible (UV-vis) absorption spectra were recorded on a UV-2550 spectrophotometer (Shimadzu). The compressible stress–strain curves was obtained from Instron 1185 universal material testing machine (UMT).

Biotinylation and Characterization of Enzyme Catalase. Catalase-biotin was synthesized according to the literature with minor modification.[1-3] A solution containing 100 μL of 20 mg/mL catalase, 700 μL of 0.1 M PBS (pH 8.4) was mixed with 200 μL of 200mg/mL BNHS and incubated on a rotator for 4 h at room temperature. After incubation, remove excess crosslinker using a sephadex G-25 resin desalting column with Phosphate buffered saline (PBS, 10 mM, pH

7.4).

As aforementioned above, the change in the capacitance value on PPy-PDMS sponge derived from the biotinylation of enzyme catalase. Thus, one inevitable question arises as to whether the system could be implemented with the catalase catalytic reaction. To clarify this point, dodecyl sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE, 10% separation gel and 5% concentrated gel, 30 min at 80 V and then 2 h at 120 V) was applied to evaluate the biotin and streptavidin coupling for catalase assay. As depicted in Fig. S1, Lanes 'a - f' gave clearly SDS-PAGE images of different proportion of catalase coupling with biotin and streptavidin. Lanes 'C, B, S, M' showed the image of catalase, BNHS, streptavidin and marker. Obviously, When the biotin and streptavidin reacted with catalase: BNHS (w/w = 1 : 20), lanes 'e - f' with larger mobility than the others, which indicated the large amount of biotin and streptavidin were combined with it, thus, catalase: BNHS (w/w = 1 : 20) was chosen as the optimal synthesis ratio. Fig. S2 showed the collected solution of catalase-biotin after desalting column, catalase-biotin can be dyed blue with xylene cyanol FF therefore number 10 to 13 were stored for used.

Furthermore, to clarify secondary antibody could combined with catalase-BNHS by streptavidin , SDS-PAGE (10% separation gel and 5% concentrated gel, 30 min at 80 V and then 3 h at 120 V) was applied to prove. (Fig. S3). Lanes gave clearly SDS-PAGE images of catalse, BNHS, catalase-biotin, streptavidin, secondary antibody and combination product (lane ‘X’), Respectively, When the catalase-biotin reacted with streptavidin and secondary antibody, lane 'X' with upward mobility was observed in comparison with lane 'A & CB', which ascribed to the formation of the combination product with larger olecular weight. Therefore, the SDS-PAGE gel images successful confirm the proposed combination took place as anticipated.

Fabrication of the Flexible Capacitance Sensor. The as-prepared PPy-PDMS sponge, pet-ito flexible conductive films (electrodes) and filter papers (separators) were initially cut into many small cylinder (D = 12 mm) using the Deli hole puncher. PPy-PDMS sponges, pet-ito electrodes and separator were assembled by polyvinyl alcohol/phosphoric acid (PVA/H_3PO_4) gel electrolyte (shown in Scheme 1, inset). And then, the prepared flexible capacitance sensor was placed into a homemade container printed by a 3D printer, which was linked with the gas delivery channel and the detection cell.Finally, a LCR-6100 digital bridge was employed to monitor the signal of the capacitance sensor. This completed device is shown in Fig. S4.

Immunoreaction Protocol. Scheme 1 presents schematic diagram of capacitance immunoassay, which was carried out as follows: (i) 50 μL of capture antibody (10 μg mL^{-1}) was first added into a separable high-binding polystyrene microwell and incubated overnight at 4 °C; (ii) each well was blocked by 300 μL blocking buffer (0.01 M PBS containing 1.0 wt % BSA, pH 7.4) for 1 hour at room temperature; (iii) 50 μL of PSA standards with different concentrations were added to the wells and shaken on a shaker for 1 h at room temperature; (iv) 100 μL of 0.2 μg mL^{-1} detection antibody was added and incubated for 1 h at room temperature; (v) 100 μL of 0.2 μg mL^{-1} secondary antibody was added and incubated for another 1 h at room temperature; (vi) 100 μL of 1 μg mL^{-1} streptavidin was added and incubated for 1 h at room temperature; (vii) 100 μL prepared catalase-biotin was added and incubated for 30 min; (viii) 100 μL H_2O_2 (6%) was added and reacted with the bound catalase for a given time prior to capacitance measurement with a LCR-6100. Note: The separable high-binding polystyrene microwell was washed three times with PBS (0.01 M PBS containing 0.05 wt % Tween 20, pH 7.4) after each step. All the measurements were carried out at room temperature (25 ± 1.0). The change in the capacitance (DC, nF; versus the background signal) was collected and registered as the sensing signal relative to different-concentration PSA standard.

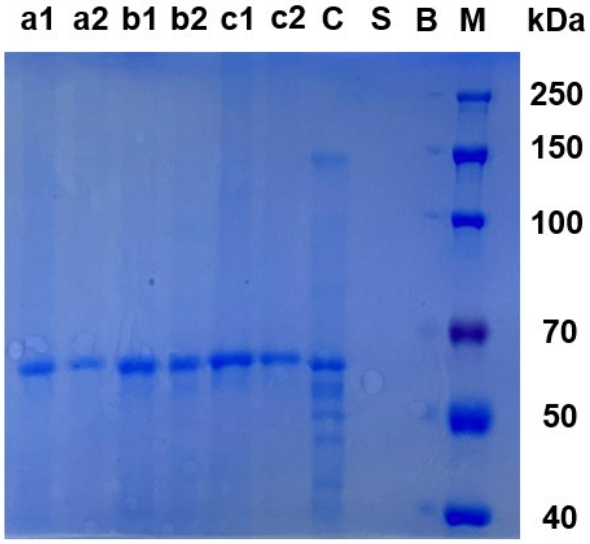

Fig. S1 SDS-PAGE (10% separation gel and 5% concentrated gel) image of different synthesis ratio catalase-BNHS samples (lane 'M': 250 kDa two-color pre-stained protein marker; lane 'a-c': catalase: BNHS (w/w = 1 : 8, 1 : 10, 1 : 20), lane '1': catalase-biotin, lane '2': lane '1'+ streptavidin; lane 'C': catalase; lane 'S': streptavidin; lane 'B': BNHS).

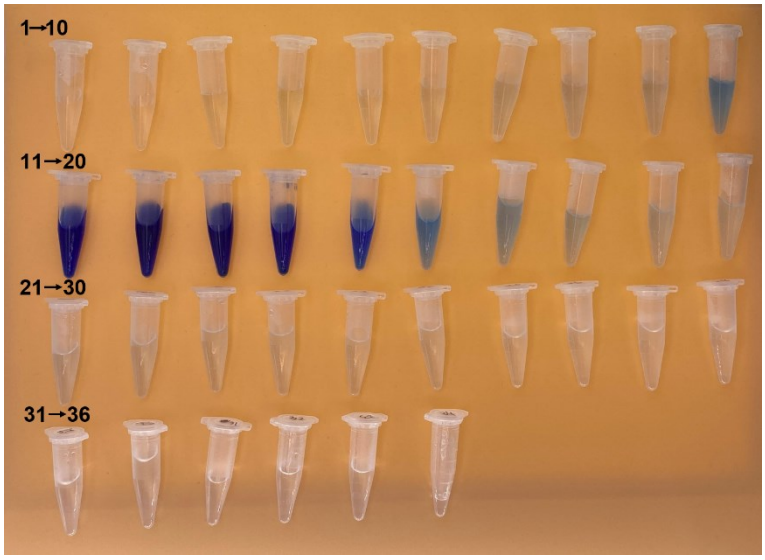

Fig. S2 Photograph of the collected solution of catalase-biotin.

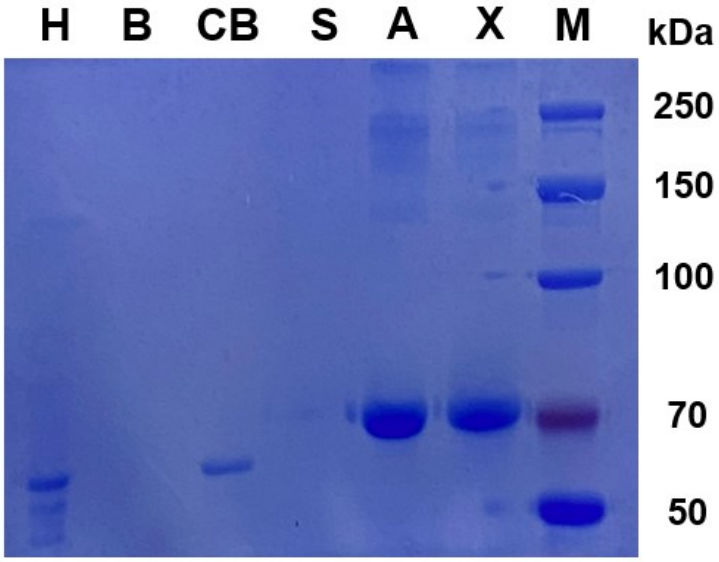

Fig. S3 SDS-PAGE (10% separation gel and 5% concentrated gel) image of different samples (lane 'M': 250 kDa two-color pre-stained protein marker; lane 'H': catalase; lane 'B': BNHS; lane 'CB': catalase-biotin; lane 'S': streptavidin; lane 'A': secondary antibody, lane 'X': lane 'S'+ lane 'A' + lane 'CB').

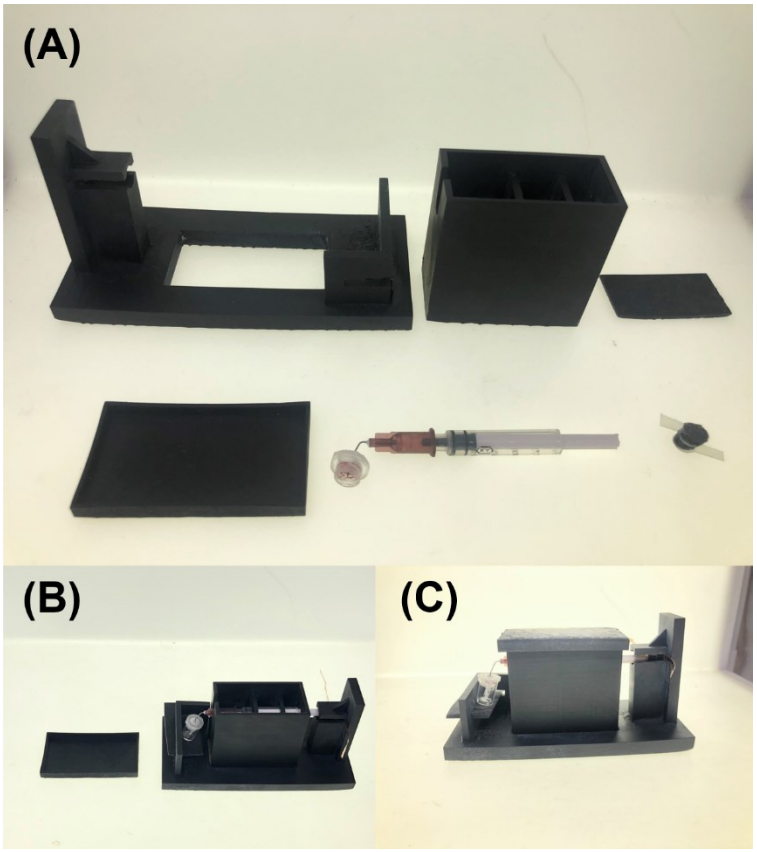

Fig. S4 Photograph of the integrated capacitance-based bioassay device.

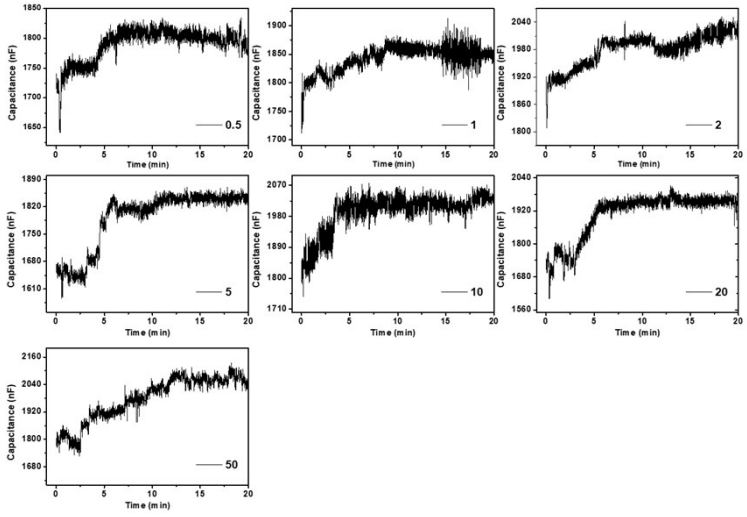

Fig. S5 Capacitance responses of PPy-PDMS sponge-based immunosensor toward target PSA standards.

Table S1 Comparison of compressible capacitance immunoassay on analytical properties with other PSA capacitance immunosensors

Detection method	Linear range (ng mL^{-1})	LOD (pg mL^{-1})	Ref.
Capacitance immunosensor	1.25 – 80	120	(4)
Capacitance immunosensor	0.4 – 18	100	(5)
Capacitance immunosensor	0.1 – 1000	74	(6)
Capacitance immunosensor	1.0 – 100	3000	(7)
Capacitance immunosensor	0.5 – 50	57	This work

Table S2 Accuracy evaluation of compressible capacitance immunoassay for human serum specimens by using human PSA ELISA kit as the reference

Sample no.	Method; Conc. (mean ± SD, ng mL^{-1}, n = 3)		t_{exp}
	Capacitance immunoassay	Human ELISA kit	
1	16.52 ± 1.01	15.47 ± 0.93	1.32
2	33.03 ± 3.06	34.85 ± 2.01	0.86
3	6.74 ± 0.30	6.49 ± 0.68	0.58
4	40.44 ± 1.29	39.48 ± 0.60	1.14
5	8.43 ± 0.72	9.24 ± 0.87	1.24
6	0.94 ± 0.07	1.05 ± 0.04	2.36
7	21.15 ± 1.2	20.57 ±1.27	0.57
8	1.31 ± 0.13	1.51 ± 0.09	2.19

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