# Supporting Information

## A nano-Integrated Microfluidic biochip for enzyme-based Point-of-Care Detection of Creatinine

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## Materials and methods

### Materials

Creatininase amidohydrolase (CA) (from Pseudomonas sp.), Creatinase (CI) (from. Pseudomonas sp.), Sarcosine Oxidase (SOx) (from Bacillus sp.), and Ascorbate Oxidase (ASCO) were obtained from Shanghai Yuan Ye (China). HRP, Catalase (CATA) (from bovine liver) and creatinine were purchased from Sigma–Aldrich (USA). MWNTs (50 nm in diameter) were bought from Shenzhen Nanotech Port Co. Ltd. (China). The hydrogen tetrachloroaurate (III) hydrate (HAuCl4) was purchased from J&K Scientific Ltd. (China). PDMS (Prepolymer and curing agent) were purchased from Dow Corning (Corning (Sylgard 184). Poly Tetrafluoroethylene (PTFE) tubes and stainless steel connectors were purchased from Suzhou Wenhao (China). Syringe pump (Longer Pump, LSP02-1B). All other chemicals from commercial sources were of analytical reagent grade. All solutions were prepared using Milli-Q water (18 MΩ cm resistivity) from a Millipore system.

### Construction of PB/Gold Nanoflower/SPCE and Gold Nanoflower/SPCE

The 16-channel multiplexing electrochemical biosensing station was bought from Huasenxinke (Suzhou) Nanotechnology Co., Ltd. Prior to the disposition of gold nanoflower, the electrochemical activation of all carbon working electrodes of SPCE was made to generate carboxylate groups. We carried out the CV scan of SPCE in 0.1 M phosphate buffer (pH 7.4) by running 100 cycles with potential range from -0.3 to 0.6 V at a scan rate of 500 mV/s. Then the gold nanoflower was electrodeposited in 45μL 1% HAuCl4 solution casted on the adjacent two of every three SPCEs through potentiostatic deposition. The I-t method parameters were as follows: deposition potential, −0.2 V, deposition time, 300s, scan rate, 100 mV/s. The Prussian Blue layer was then electrodeposited on one of the two adjacent gold nanoflower/SPCEs at the potential of 0.4 V for 60 s in a fresh electrolyte containing $2.0 \times 10^{-3}$ M $FeCl_3$ and $2.0 \times 10^{-3}$ M $K_3[Fe(CN)_6]$ in the supporting solution. The supporting solution is a mixture of 0.05M KCl and 0.05M HCl (Fig S4).

### Immobilization of enzymes on PB/Gold nanoflower/SPCE and Gold Nanoflower/SPCE

The CA, CI, SOx and HRP were co-immobilized onto the PB/Gold nanoflower/SPCE surface through physical adsorption. Firstly, CA, CI, SOx and HRP were resolved in 0.05 M phosphate buffer (PB) pH 7.4, then the enzyme mixture, 1% nafion and 0.1mg/mL c-MWCNT, which was prepared as we previously reported, were mixed as 3:1:1 (v:v:v) and then 5μL c-MWCNT doped

enzyme mixture solution containing CA (18 unit), CI (3.6 unit), SOx (1.8 unit) and HRP (0.72 unit) was dropped on surface of the modified electrode. Similarly, 15 µL c-MWCNT doped enzymes mixture solution containing CI (3.6 unit), SOx (1.8 unit), ASCO (0.375unit) and CATA (7.5 unit) was casted on the surface of the neighboring modified working electrode (Fig. S4). After that, the modified electrode arrays were put into the humidity chamber for 2 hours (60% RH, 25℃), resulting in forming homogeneous membrane. The fabricated electrodes were characterized by SEM and EIS studies.

**Fabrication of portable microfluidic biosensing device**

The proposed portable microfluidic biosensing device was assembled from screen-printed carbon electrodes, PDMS sheet with meandering microchannels and PTFE tubes. The disposable SPCE arrays printed on PET provided by Zhejiang Nazhihui Co., Ltd, each consisting of a carbon working electrode, a carbon counter electrode and a silver pseudo-reference electrode, were employed in each experiment. The sample plug-in tubes (PTFE) (0.5/0.9mm ID/OD) were segmented into 20-cm-long and marked at the 4-cm-long. For the PDMS sheets with meandering channels, PDMS sheets were fabricated on PMMA mould. PDMS prepolymer and curing agents (10:1) were mixed and degassed thoroughly, which was carried out in a vacuum chamber. The mixture was poured into the PMMA molds and incubated for 2h at 80 °C. The PDMS chip was subsequently peeled from the mould, and inlet and outlet holes were punched. The prepared PDMS sheet and the modified electrodes were permanently bonded via a plasma irradiation treatment (40s, PDC-32G-2, Harrick, USA) after the immobilized multi-enzyme mixture covered with a PDMS frame, followed by assembly with the PTFE tubes to form the integrated portable microfluidic biodevice, making the buffers or samples flow through the system and all three electrodes are in electrical contact (Scheme. 1).

**The portable microfluidic biochip for creatinine assays**

The assembled microfluidic sensing chip was connected to the PTFE-plugin tubes via the inlet and the other side was subsequently connected to syringe pump via the outlet to form an integrated biosensing system, which provided vacuum drainage for sample flowing along the channels. The electrodes were connected to a smartphone-based electrochemical mini-workstation via USB interface. Firstly, a high flow rate of 100 µL/min was employed to drive the sample in the tubing at the inlet, then the flow rate was adjusted to 10 µL/min as quickly as

possible. The sample plug moved through the sample processing area with the flow rate of 5 μL/min and subsequently underwent a testing area with the flow rate of 10 μL/min. The whole flow-through process can be completed within 5 min. Then, creatinine measurements were carried out by I-t technology at the voltage of −0.15 V for 60 s.

**Simulation sample testing**

The modified biosensor was employed for measuring creatinine in human serum samples. Serum samples were collected from North department of Ruijin Hospital (Shanghai) and stored at 4°C until use. 0.1 mM, 0.4 mM, 0.6 mM creatinine were separately added into the human serum and the recovery tests for creatinine were performed by our proposed microfluidic biosensor.

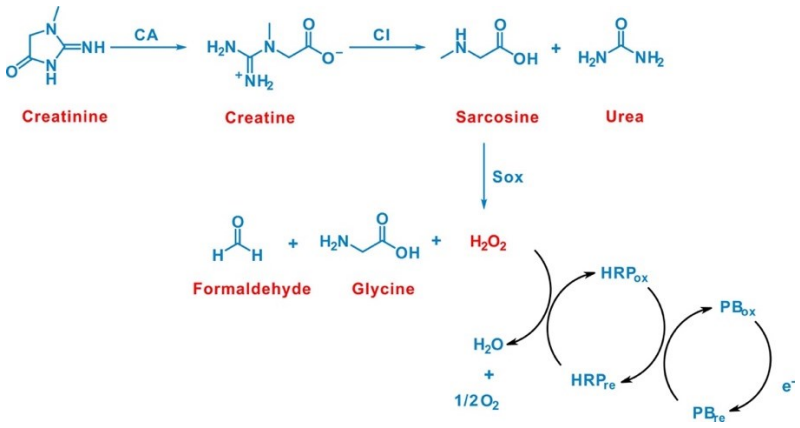


**Fig. S1** The principle of creatinine assay based on enzyme cascade reaction.

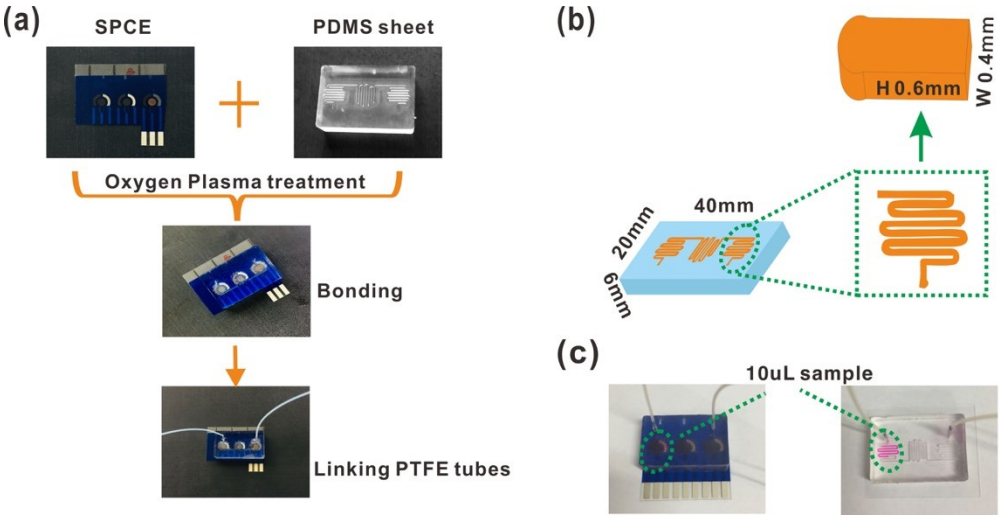


**Fig. S2** (a) The assemble process of the microfluidic biodevice; (b) the size of the PDMS sheet and

microchannel; (c) 10 μL red pigment in the serpentine channel, which exactly covers the electrode detection area.

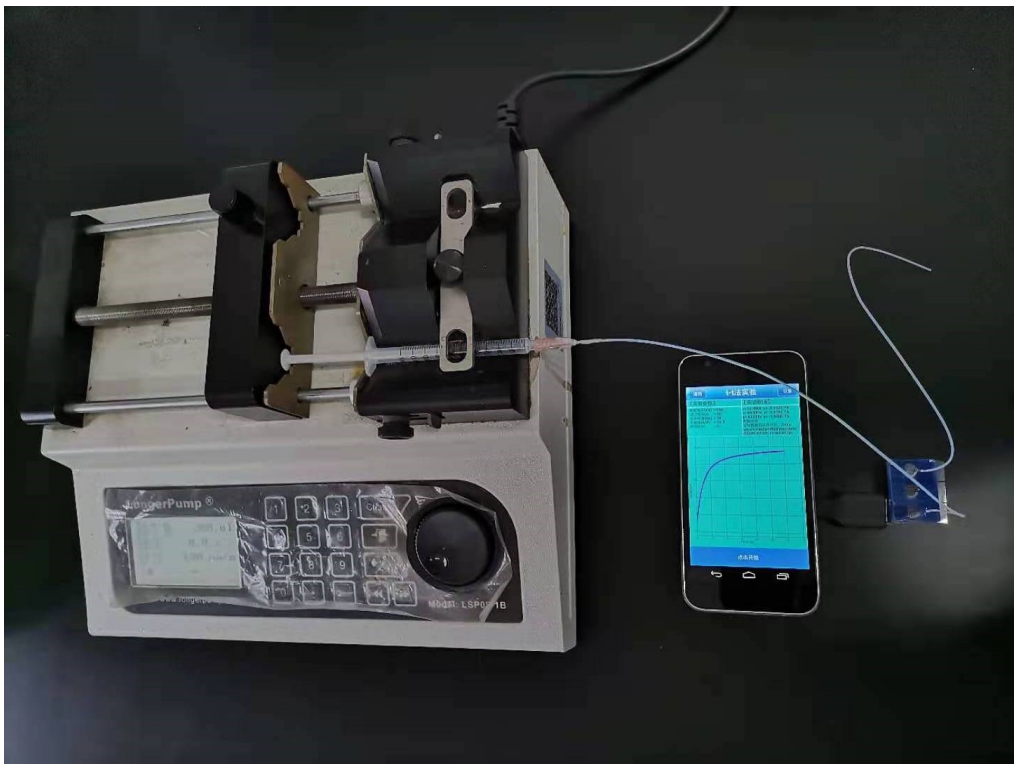

Fig. S3 The microfluidic biochip and smartphone-based detection system for CR.

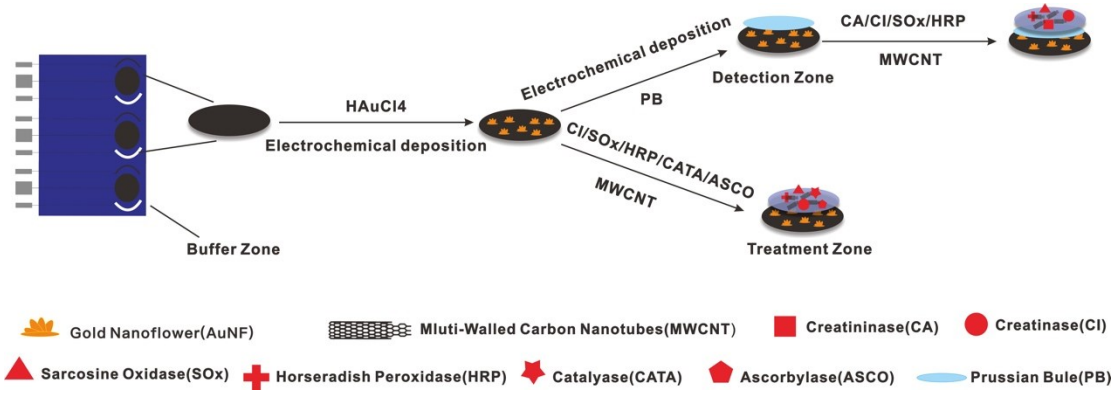


**Fig. S4** The preparation procedure of the enzyme-based nanotextured microfluidic biosensing system for creatinine assay

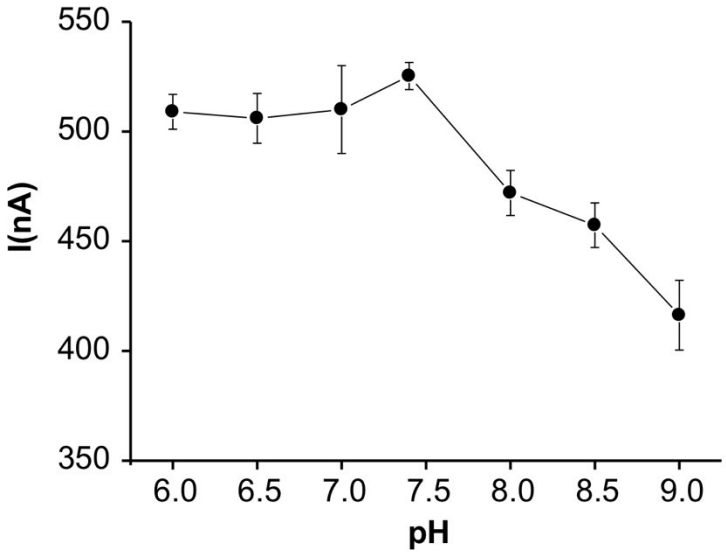


**Fig. S5** Effect of pH on the electrochemical response of the proposed integrated microfluidic biodevice in 0.1 mM creatinine (PBS). The error bars show the standard deviations.

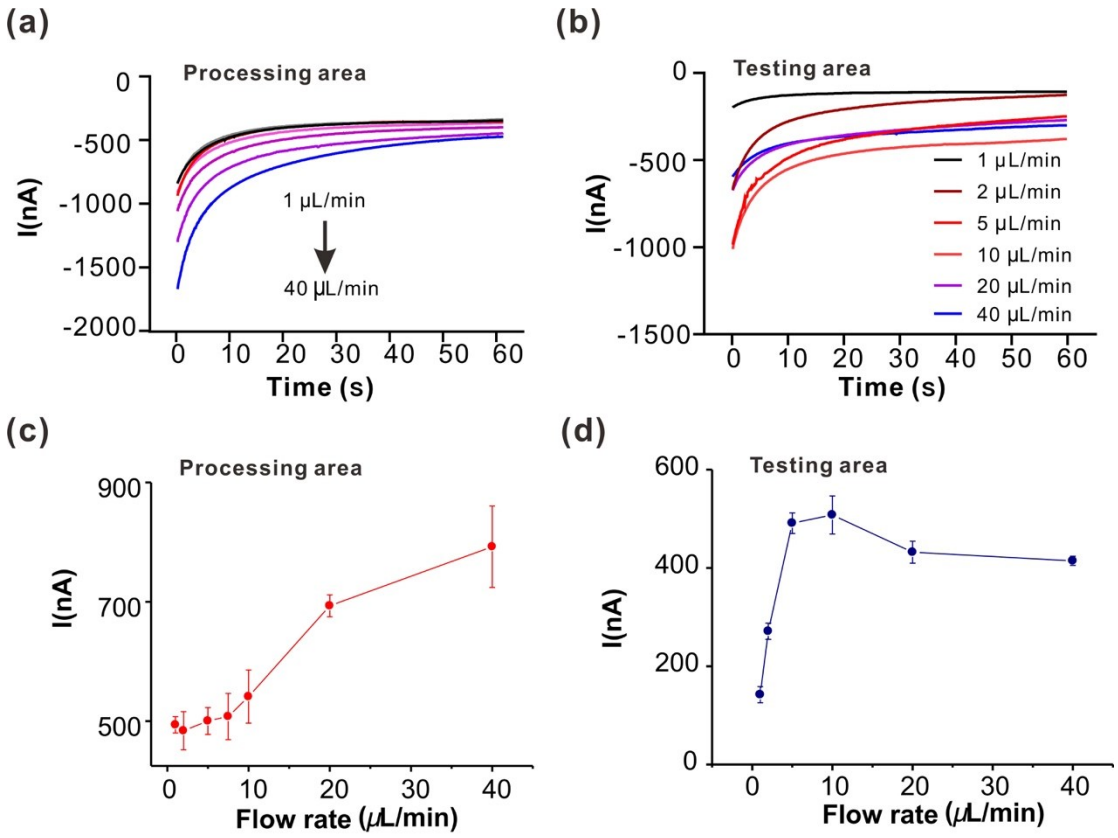


**Fig. S6** Influence of different flow rates at sample processing area and testing area. The I – t curve for 0.1 mM creatinine detection containing interferences, including uric acid (0.47 mM),

ascorbic acid (0.11mM), tyrosine (0.10 mM), creatine (0.09mM), sarcosine ($5.4 \times 10^{-3}$ mM), acetaminophen (2.0mM), glucose (0.10 mM), glycine (0.30 mM) and urea (0.1 mM) with the flow rate of 1, 2, 5, 7.5, 10, 20 and 40 µL/min at the sample processing area (a) and testing area (b) by the integrated biodevice assembly. The current (nA) vs flow rate (µL/min) curve 0.1 mM creatinine detection containing interferences as above, with the flow rate of 1, 2, 5, 10, 20 and 40 µL/min at the sample processing area (c) and testing area (d). The current signals were recorded at 10s. The error bars show the standard deviations.

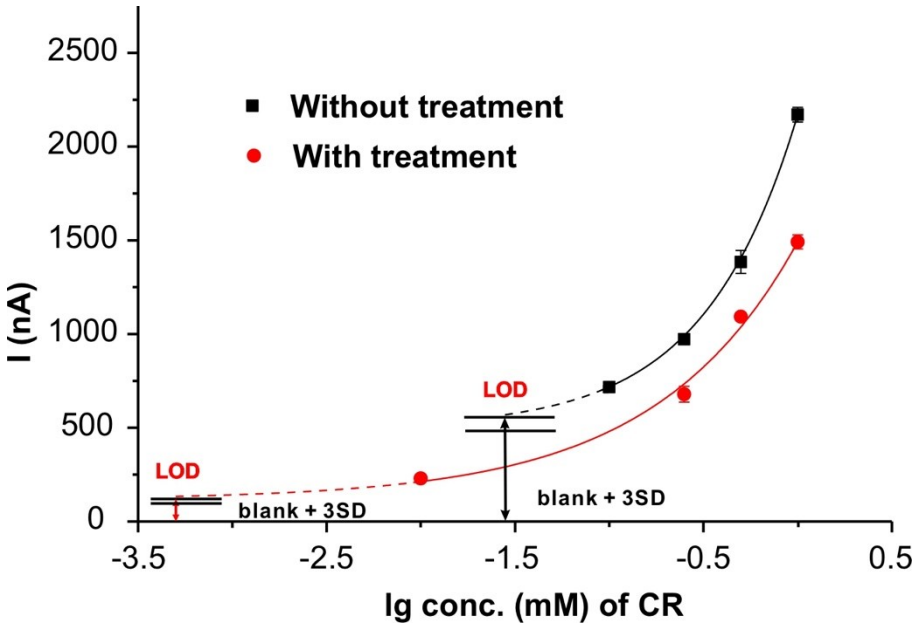


**Fig. S7** Comparison between sample with (red line) and without treatment (black line) within the biodevice at different concentration levels (blank ,0.1 mM, 0.25 mM, 0.5 mM, 1.0 mM CR) containing interferences mixture. Interferences mixture included AA (0.11 mM), ACET (2.0 mM), UA (0.47 mM), urea (0.1 mM), GLY (0.30 mM) TYR (0.10 mM), CRE (0.09 mM), GLU (0.1 mM), and SA (5.4×10-3 mM). The dynamic range of the two method are the same (0-1 mM), their regression equations are i=1415.36exp(lg[CR]/0.8091) + 108.46 (R2=0.9911) (with treatment) and i=1638.36exp(lg[CR]/0.4580) +532.23 (R2=0.9990) (without treatment), respectively, while their limits of detection (LOD) are quite different (without treatment, 28 µM; with treatment, 0.5 µM).

**Table S1.** Effect of potential interferents in human serum on the proposed creatinine biosensor

| Interferents | Physiological concentration (upper limit) | Recovery |
|---|---|---|
| ascorbic acid | 0.11 mM | 90% |
| acetaminophen | 2.00 mM | 100% |
| uric acid | 0.47 mM | 101% |
| urea | 0.1 mM | 105% |
| glycine | 0.1 mM | 100% |
| tyrosine | 0.1 mM | 103% |
| creatine | 0.09 mM | 98% |
| glucose | 0.1 mM | 103% |
| sarcosine | $5.4 \times 10^{-3}$ mM | 95% |

Table S2. Recovery study in human serum[a]

| added | found | Recovery | RSD (n=3) |
|---|---|---|---|
| 100 µM | 39.5 µM | 30.7% | 7.9% |
| 400 µM | 128.4 µM | 32.1% | 5.2% |
| 600 µM | 256.8 µM | 42.8% | 3.0% |

[a] Average of three measurements with three different microfluidic biodevices.