Supporting Information for

Electrochromic sensing platform based on steric hindrance effects for CEA detection

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EXPERIMENTAL SECTION

Chemicals and Reagents. All of the chemicals were of at least analytical grade without any further purification. Tris(2-carboxyethyl)phosphine (TCEP), 6-Mercapto-1-hexanol (MCH), HAuCl₄·4H₂O were purchased from Sigma-Aldrich. Carcino-embryonic antigen (CEA) and alpha-fetoprotein (AFP) were purchased from Biocell Company (Zhengzhou, China). Tris(hydroxymethyl)aminomethane, albumin from bovine serum (BSA) and thrombin were purchased from Dingguo Biotechnology Co. Ltd (Beijing china). NaCl, KCl, FeCl₃, ethylenediaminetetraacetic acid (EDTA),
$K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ were purchased from Beijing Chemical Reagent Company (Beijing, China). Polydimethylsiloxane (PDMS) and curing agent was obtained from GE Toshiba Sillicones Co., Ltd. ITO coated glass substrate (resistance: $\sim 6$ Ω/square) was obtained from CSG Holding Co., ltd. (Shenzhen, China). The CEA aptamer, the base sequences as follow: 5′-SH-ATACCAGCTTATTCAATT-3′ was synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). The water used throughout all experiments was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

**Device Fabrication.** Patterned microelectrodes were prepared using standard photolithographic techniques according to the reported literatures\(^1\)\(^2\) and a laser printer was used to print the photomasks with the designed patterns for the patterned template. Briefly, the clean ITO substrate was spin-coated (3000 rpm for 20 s) with RZJ 390 photoresist and prebaked at 95 °C for 5 min. The coated ITO substrate was covered by the photomask and exposed to the UV irradiation (365 nm, 15 mJ/cm\(^2\)) for 9 seconds. The exposed photoresist layer was removed by 0.1 M NaOH. Finally, the exposed ITO could be easily etched by the HCl-FeCl\(_3\) solution. And the patterned ITO electrodes were obtained by removing the remained photoresist with ethanol. Finally, the closed bipolar system was achieved by bonding a PDMS membrane with two reservoirs to the patterned ITO substrate.

**Fabrication of Sensing Interfaces.** Cathode of BPE was firstly modified with Au film for immobilization of capturing DNA based on the electrochemical deposition method according to the previous report\(^3\)\(^4\). Briefly, the detection channel
array and sensing channel array were filled with 1% HAuCl\textsubscript{4} and 5 mM K\textsubscript{3}Fe(CN)\textsubscript{6}, respectively. And then CHI 760E electrochemical workstation was used to supply a voltage of 2.5 V on the driving electrodes for 120 s. And the color of the cathode of the BPE has an obvious change from light-blue to yellow. The scanning electron microscope image of the ITO deposited Au on ITO was shown in Figure S2. The as-prepared Au-ITO hybrid BPE was washed with ultrapure water and then 2 μM CEA aptamer DNA in 10 mM Tris-HCl buffer (1 M NaCl, 1 mM EDTA, 1 mM TCEP and pH=7.4) was dropped on the surface of the Au-ITO hybrid BPE and incubated at 4°C for 10 h. After that, the aptamer modified Au-ITO BPE array was washed with buffer and then added 50 μM MCH for 5 min to block the non-specific binding sites.

**Electrochromic Method for CEA Detection.** Different concentrations of CEA or serum samples were injected into the sensing array channel and incubated at 37 °C for 1 h, and then washing the sensing array channel for three times. Subsequently, the sensing array channel and the reporting channel were filled with 5.0 mM K\textsubscript{3}Fe(CN)\textsubscript{6} and PB deposition solution (2.5 mM FeCl\textsubscript{3}+2.5 mM K\textsubscript{3}Fe(CN)\textsubscript{6}+ 0.1M HCl + 0.1 M KCl), respectively. Then a voltage of 1.5 V was applied on the driving electrodes for 180 s, and PB was deposited on the electrode of BPE which made an obvious color change. A digital camera in the intelligent phone was used to record the color change process of the PB deposition, and an intelligent software installed on the phone (which was developed by our team and named as chip reader) was used to analyze the difference of the color change.
**Figure S1.** Optical image of the photolithographic techniques fabricated ITO closed bipolar micro-electrodes arrays.

**Figure S2.** The SEM image of the ITO electrode surface electrodeposited with Au.
Figure S3. EIS of the CEA aptamer modified Au electrode incubated with different concentration of CEA from 1 to 10 ng/mL in 0.1 M NaCl +5 mM Fe(CN)$_6^{3-/4-}$ solution.

References