Supplementary Material

Microfluidic integrated capacitive biosensor for C-Reactive Protein label-free and real-time detection

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1.Integration of microfluidic chip and capacitor chip and physical picture after red ink is injected into the microfluidic channel

A thin layer of PDMS was evenly applied on the PCB of capacitor and cured in an oven for several minutes, in order to facilitate the bonding of the microfluidic channel with the capacitor chip. Then the microfluidic channel was bonded to the PDMS coated capacitor chip with 5min plasma treatment.

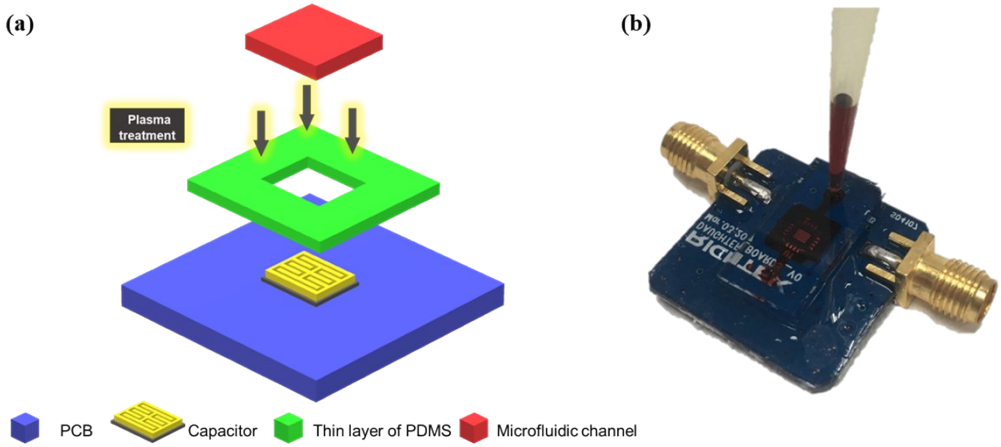

Figure S1 (a)Integration of microfluidic chip and capacitor chip.(b)physical picture after red ink is injected into the microfluidic channel.

2. XPS nitrogen spectra of the gold electrode during functionalized gold electrode.

XPS nitrogen spectra of the bare gold electrode, mixed thiol molecules functionalized gold electrode, the treatment of EDC and sulfo-NHS and the immobilization of anti-CRP. It is worth noting that after the CRP incubation, the peak of N element (around 400eV) on the surface of the gold electrode was significantly increased. This is because the CRP antibody itself is composed of a protein, which is in contact with the surface of the gold electrode and binds to the functional group on the surface of the

electrode.

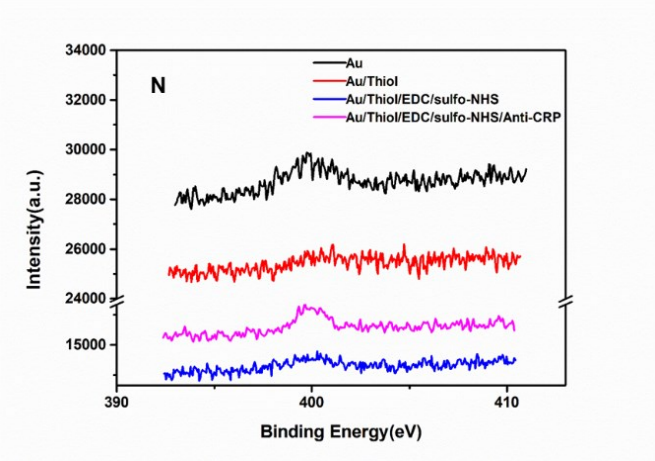

Figure S2 XPS N spectra of the bare gold electrode, mixed thiol molecules functionalized gold electrode, the treatment of EDC and sulfo-NHS and the immobilization of anti-CRP.

3.Fluorescent characterization of biofunctionalization of the gold electrodes under higher magnification of the microscope

Streptavidin-Cy3 with the concentration of 1ug·mL^{-1} was injected into the surface of 3D gold electrodes after the activation of EDC and sulfo-NHS and incubated for 1 hour. Although the disturbance of the fluorescence of the gap, the clustered fluorescent spots were visible on the surface of the gold electrode in Figure S3. The above results indicated the successful immobilization of protein molecules through this biofunctionalization method.

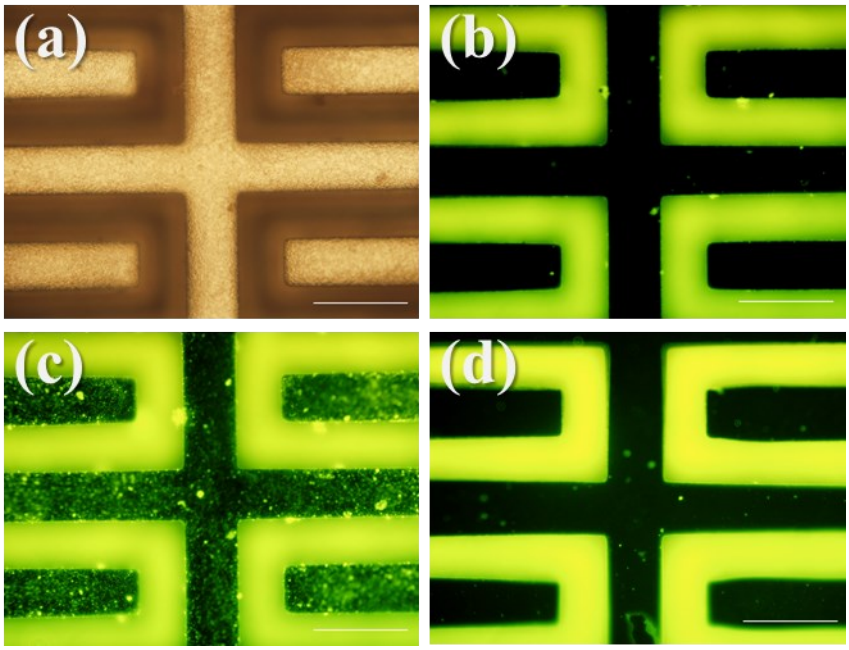

Figure S3 Fluorescent characterization of biofunctionalization of electrodes: (a) image of 3D gold electrodes under the bright field; (b) the corresponding fluorescent image of 3D gold electrodes before functionalization; (c) the corresponding fluorescent image of 3D gold electrodes after immobilization of streptavidin-Cy3 via mixed sulfhydryl molecules; (d) the corresponding fluorescent image of 3D gold electrodes in the control group

4. Capacitance response of microfluidic integrated 3D capacitive biosensor in diluted serum

The relationship between the net capacitance increments (ΔF) and the binding CRP concentrations (C_{crp}) in diluted serum under 100kHz was fitted by the Langmuir adsorption model. The coefficient of determination R^2 for the fitted curve can reach 0.92. According to the fitting results, the value of saturated capacitance ΔF_{max} was calculated to be about 0.174nF. The dissociation constant (K_d) of the interaction between the anti-CRP antibody and CRP was estimated to be 17.1 ng·mL^{-1}.

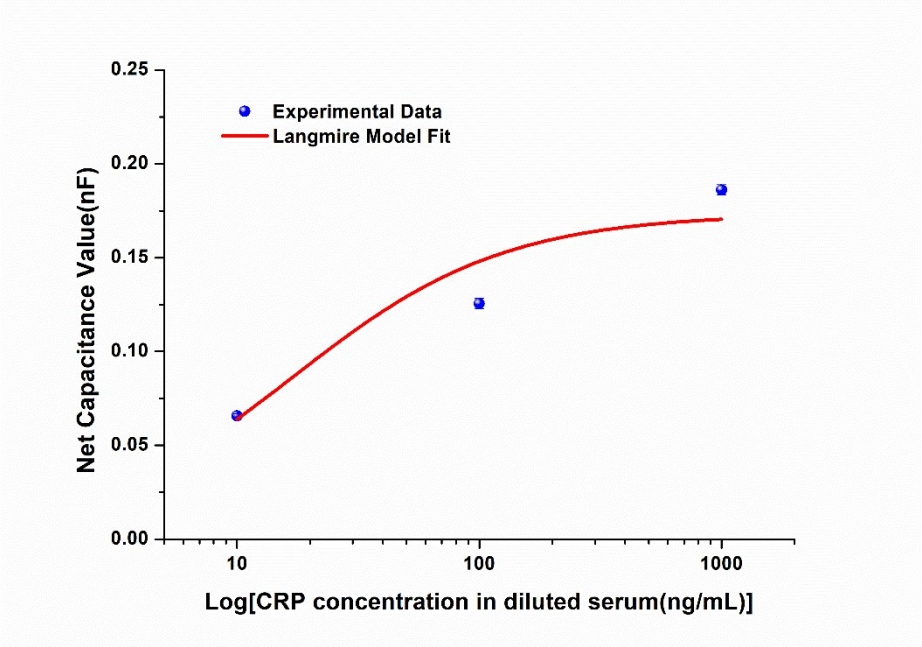

Figure S4 Net capacitance increment with different CRP concentrations in diluted serum sample with a specific working frequency (100kHz).

5. Comparison with the sensitivity of other nanomaterial-based immunosensors for CRP detection

Compared to other detection methods in the below table, the sensitivity of 3D capacitive biosensors was about 1 pg·mL^{-1}, which showed obvious superiority.

Table S1 The sensitivity of other methods for CRP detection

Sensing element	Method	Limit of Detection (LOD, ng/mL)	Ref.
Capacitive biosensor	Electrochemical	0.25 -25	[1]
Aptamer based chemiluminescence	Sandwich immunoassay	10^4-10^8	[2]
Non-faradaic electrochemical impedimetric profiling	Electrochemical	100	[3]
SPR-based immunosensor	Sandwich immunoassay	200-500	[4]
3D capacitive biosensors	Electrochemical	0.01	**This work**

Reference

1. A. Qureshi, J. H. Niazi, S. Kallempudi and Y. Gurbuz, *Biosens Bioelectron*, 2010, **25**, 2318-2323.
2. J. Pultar, U. Sauer, P. Domnanich and C. Preininger, *Biosens Bioelectron*, 2009, **24**, 1456-1461.
3. A. S. Tanak, B. Jagannath, Y. Tamrakar, S. Muthukumar and S. Prasad, *Analytica Chimica Acta: X*, 2019, **3**, 100029.
4. M. H. F. Meyer, M. Hartmann and M. Keusgen, *Biosens. Bioelectron.*, 2006, **21**, 1987-1990.