# Supplementary Data

# For

## A novel electrochemical lung cancer biomarker cytokeratin 19 fragment antigen 21-1 immunosensor based on $Si_3N_4/MoS_2$ incorporated MWCNTs and core-shell type magnetic nanoparticles

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### *2.7. Sample preparation*

In this study, voltammetric CYFRA21-1 immunosensor was applied to plasma samples to present the application of immunosensor. CYFRA21-1 free plasma samples were obtained from Blood Bank in TURKEY.

For the experiments of recovery, four different plasma samples were prepared (Plasma sample1, Plasma sample2, Plasma sample3 and Plasma sample4). The contents of solutions were listed below:

(1): 0.100 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

(2): 0.100 + 0.200 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

(3): 0.100 + 0.400 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

(4): 0.100 + 0.600 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

The standard CEA, AA, DA, UA, and BSA solutions (10.00 pg $mL^{-1}$) were firstly added into CYFRA21-1 free plasma samples (Plasma sample1). After that, 0.200, 0.400 and 0.600 pg $mL^{-1}$ standard CYFRA21-1 solutions were added into the solutions one by one, respectively (Plasma sample2, Plasma sample3 and Plasma sample4). After each addition of CYFRA21-1 solutions, plasma samples were spiked as follows:

1:2 mL methanol was added to an aliquot of 0.4 mL plasma sample in a 2.0 mL plastic centrifuge tube. After that, the centrifugation at 20000 rpm was performed for 15 minutes. The upper clear layer solution was diluted with 0.1 M PBS, pH 7.0 for analysis. The voltammograms were recorded in the potential range from +0.1 V to +0.5 V by voltammetric CYFRA21-1 immunosensor.

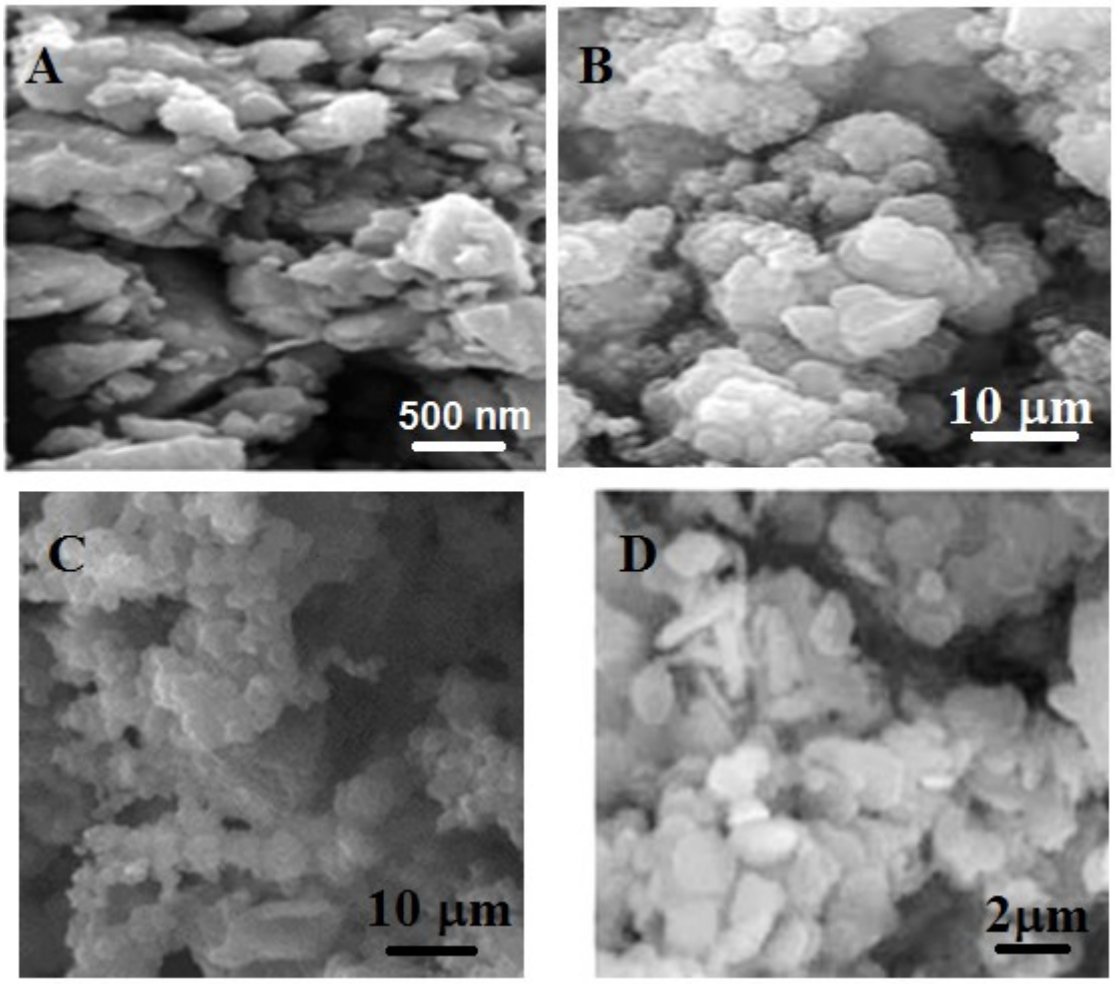


**Fig. S1.** SEM images of (A) $Si_3N_4$, (B) $MoS_2$ and (C-D) $Si_3N_4/MoS_2$ composite

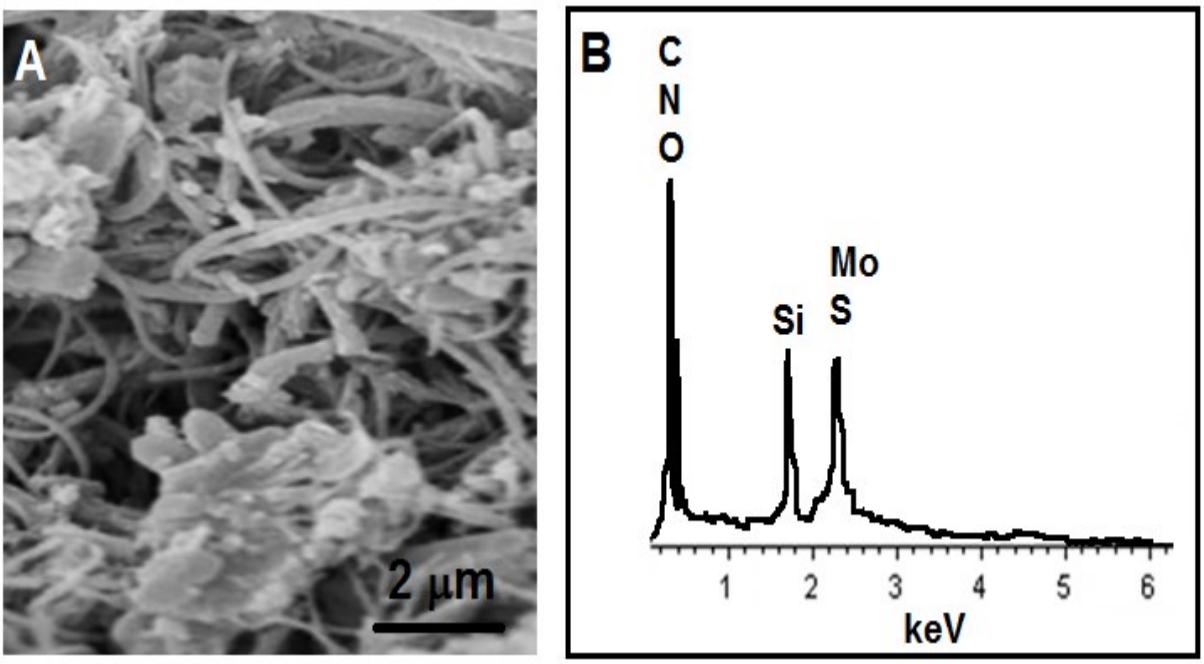


**Fig. S2.** SEM image of (A) $Si_3N_4/MoS_2$-MWCNTs and EDX spectra of (B) $Si_3N_4/MoS_2$-MWCNTs

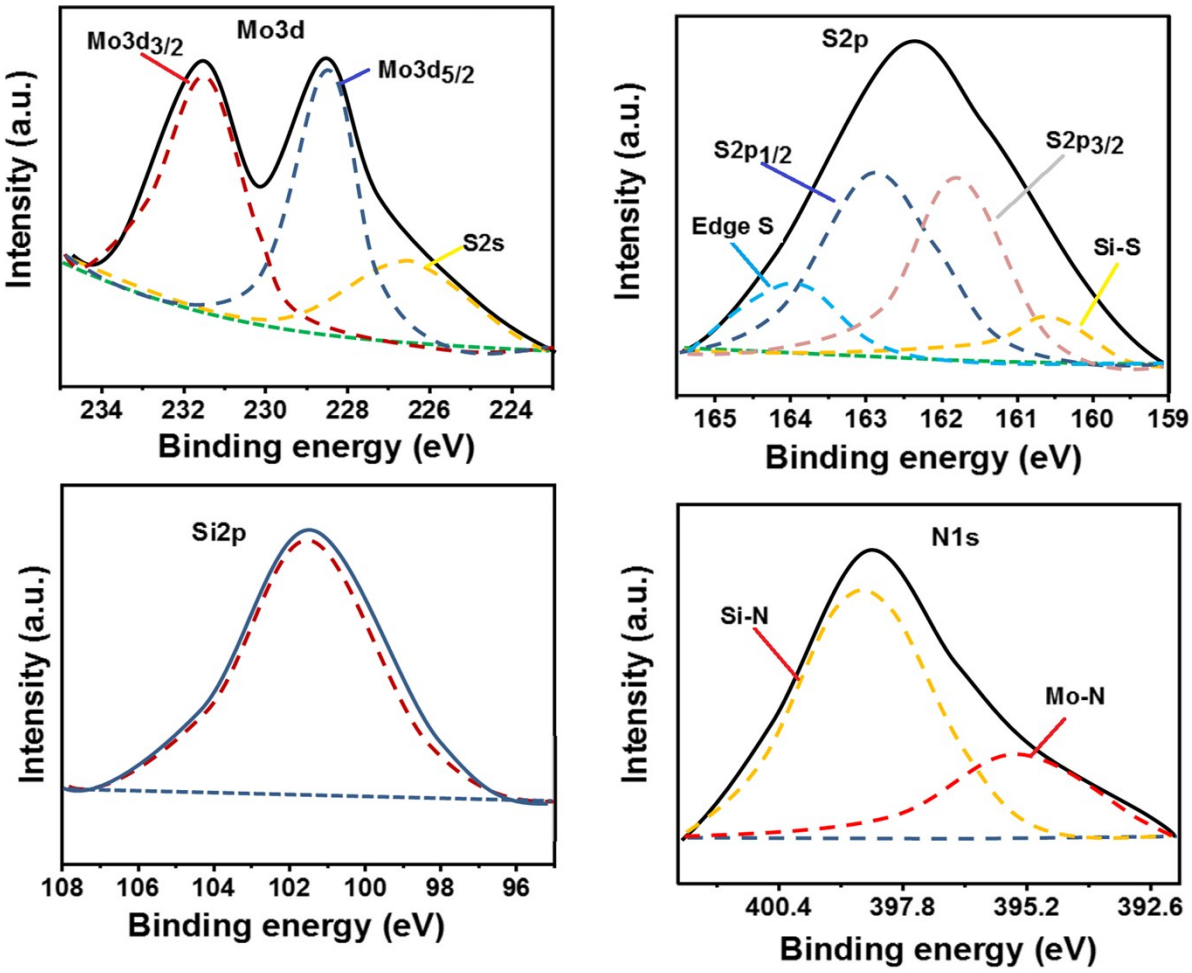


**Fig. S3.** XPS spectra of $Si_3N_4/MoS_2$ composite

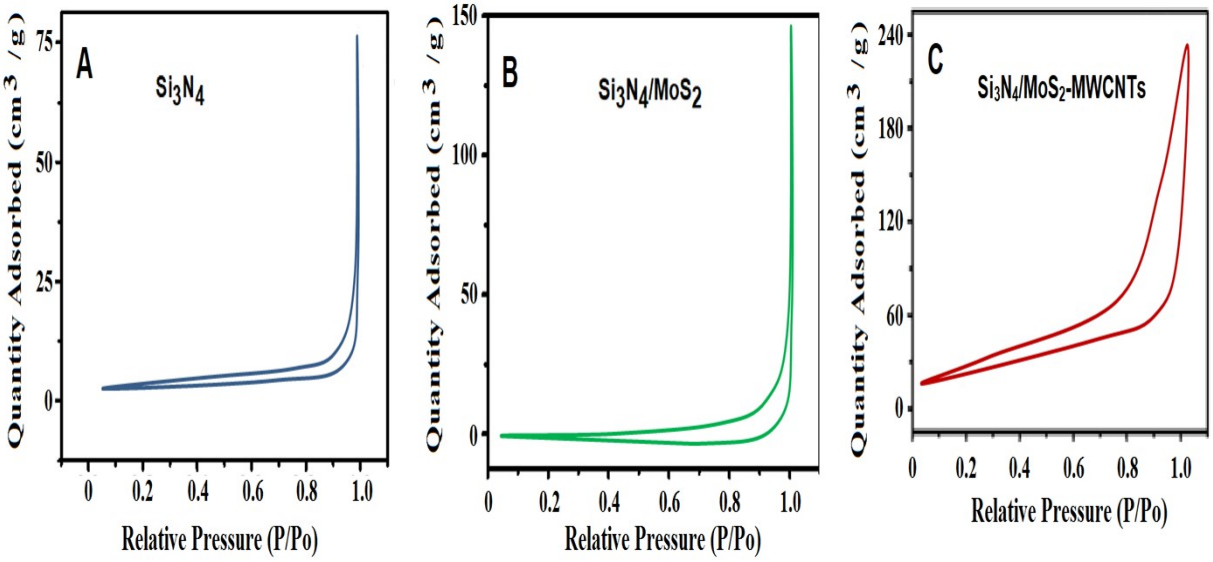


**Fig. S4.** Nitrogen adsorption-desorption isotherms of (A) $Si_3N_4$, (B) $Si_3N_4/MoS_2$ and (C) $Si_3N_4/MoS_2$-MWCNTs

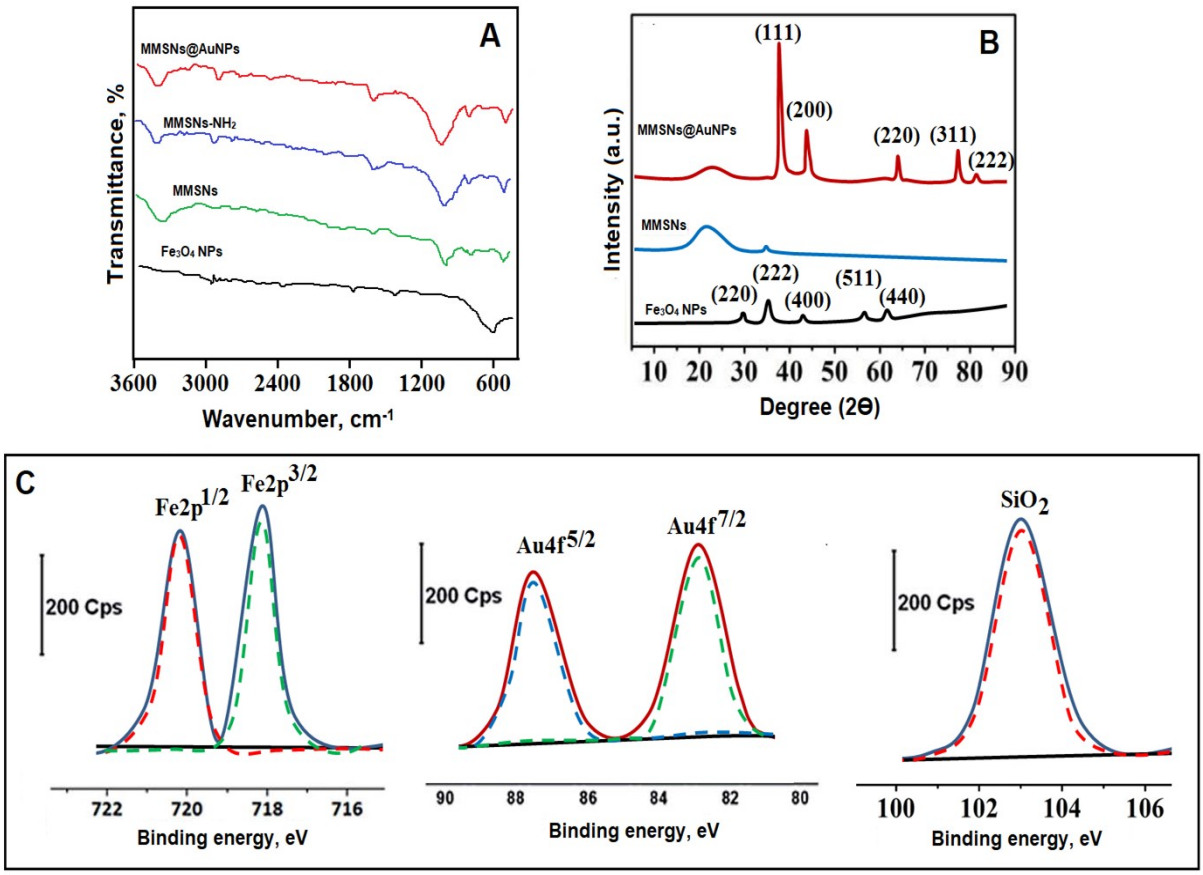


**Fig. S5.** (A) FTIR spectra of $Fe_3O_4$ NPs, MMSNs, MMSNs-$NH_2$ and MMSNs@AuNPs, (B) XRD patterns of $Fe_3O_4$ NPs, MMSNs and MMSNs@AuNPs, (C) Narrow region XPS spectra of Fe2p, Au4f, Si2p

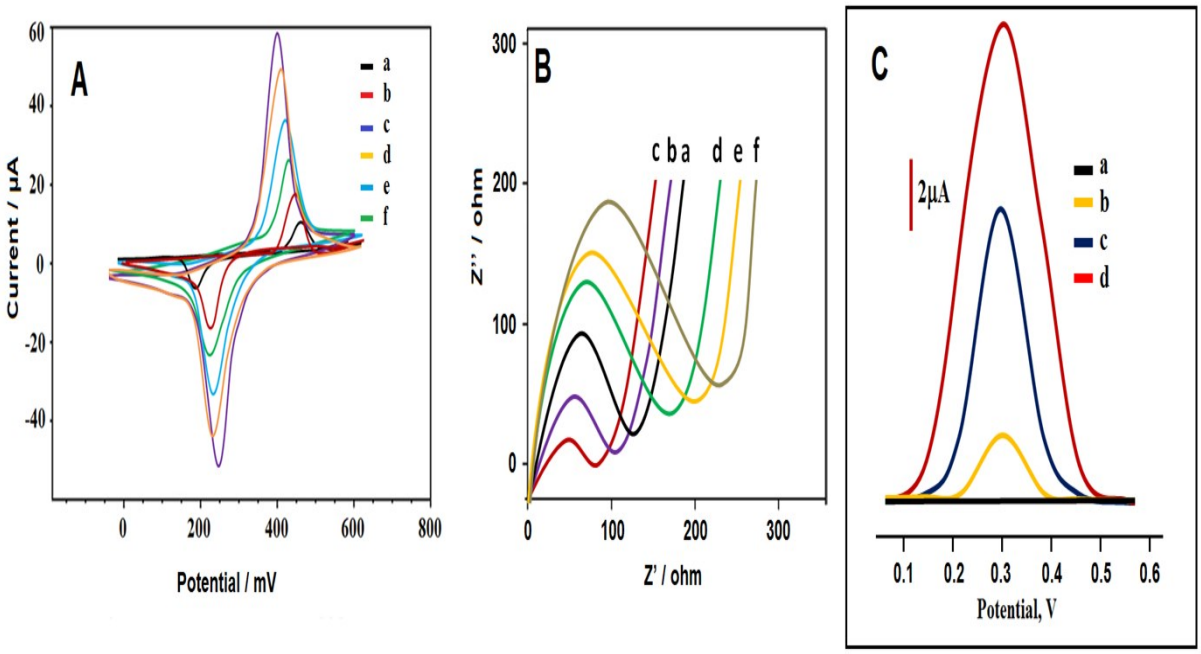


**Fig. S6.** (A) Cyclic voltammograms, (B) EIS reponses at (a) bare GCE, (b) MWCNTs/GCE, (c) $Si_3N_4/MoS_2$-MWCNTs/GCE, (d) anti-CYFRA21-1-$Ab_1/Si_3N_4/MoS_2$-MWCNTs/GCE, (e) BSA/anti-CYFRA21-1-$Ab_1/Si_3N_4/MoS_2$-MWCNTs/GCE, (f) CYFRA21-1/anti-CYFRA21-1-$Ab_1/Si_3N_4/MoS_2$-MWCNTs/GCE (scan rate of 100 mV $s^{-1}$) and (C) DPV responses of the proposed immunosensors incubated with 0.10 pg $mL^{-1}$ antigen CYFRA21-1 using (curve b) anti-CYFRA21-1-$Ab_2$/CYFRA21-1/anti-CYFRA21-1-$Ab_1/Si_3N_4/MoS_2$-MWCNTs/GCE, (curve c) AuNPs/anti-CYFRA21-1-$Ab_2$/CYFRA21-1/anti-CYFRA21-1-$Ab_1/Si_3N_4/MoS_2$-MWCNTs/GCE, (curve d) MMSNs@AuNPs/anti-CYFRA21-1-$Ab_2$/CYFRA21-1/anti-CYFRA21-1-$Ab_1/Si_3N_4/MoS_2$-MWCNTs/GCE in absence of $H_2O_2$ (curve a) and in presence of 1.0 mM $H_2O_2$

### *3.4. Optimization for voltammetric measurements*

#### *3.4.1. MMSNs@AuNPs/anti-CYFRA21-1-Ab$_2$ concentration effect*

MMSNs@AuNPs/anti-CYFRA21-1-$Ab_2$ concentration has important effect on the developed immunosensor performance. The optimal and symmetrical peaks were observed up to 15.0 mg $mL^{-1}$. Especially, after 15.0 mg $mL^{-1}$ MMSNs@AuNPs/anti-CYFRA21-1-$Ab_2$, the optimal and symmetrical peaks remained constant. Because of this, the optimal concentration of MMSNs@AuNPs/anti-CYFRA21-1-$Ab_2$ was selected as 15.0 mg $mL^{-1}$ (Fig. S7A) (In the presence of 1.0 mM $H_2O_2$ in 0.1 M PBS, pH 7.0).

#### *3.4.2. pH effect*

Secondly, pH effect was investigated on immunosensor performance. The immunosensor response increased up to pH 7.0. Furthermore, highly acidic or alkaline medium damages the structures of immobilized proteins. Hence, optimal pH was selected to be pH 7.0 (close to physiological pH) (Fig. S7B) (In the presence of 1.0 mM $H_2O_2$).

#### *3.4.3. $H_2O_2$ concentration effect*

In this study, different $H_2O_2$ concentrations were tried for obtaining optimal immunosensor signals (Fig. S7C). When $H_2O_2$ concentration gradually increased to 1.0 mM, the peak current gradually increased. After 1.0 mM $H_2O_2$, peak current decreased inversely. Due to overdose of $H_2O_2$ catalyst causing the inhibition of catalytic reaction, the activity of the proteins was negatively affected. Thus, the optimal signals were obtained in 1.0 mM $H_2O_2$ in 0.1 M PBS (pH 7.0).

#### *3.4.4. Immune reaction time effect*

When incubation time increased from 10 min to 30 min, peak current responses increase rapidly. After 30 min, immunosensor signals (μA) slightly diminished. Thus, optimal immune reaction time was selected to be 30 min (Fig. S7D) (In the presence of 1.0 mM $H_2O_2$ in 0.1 M PBS, pH 7.0).

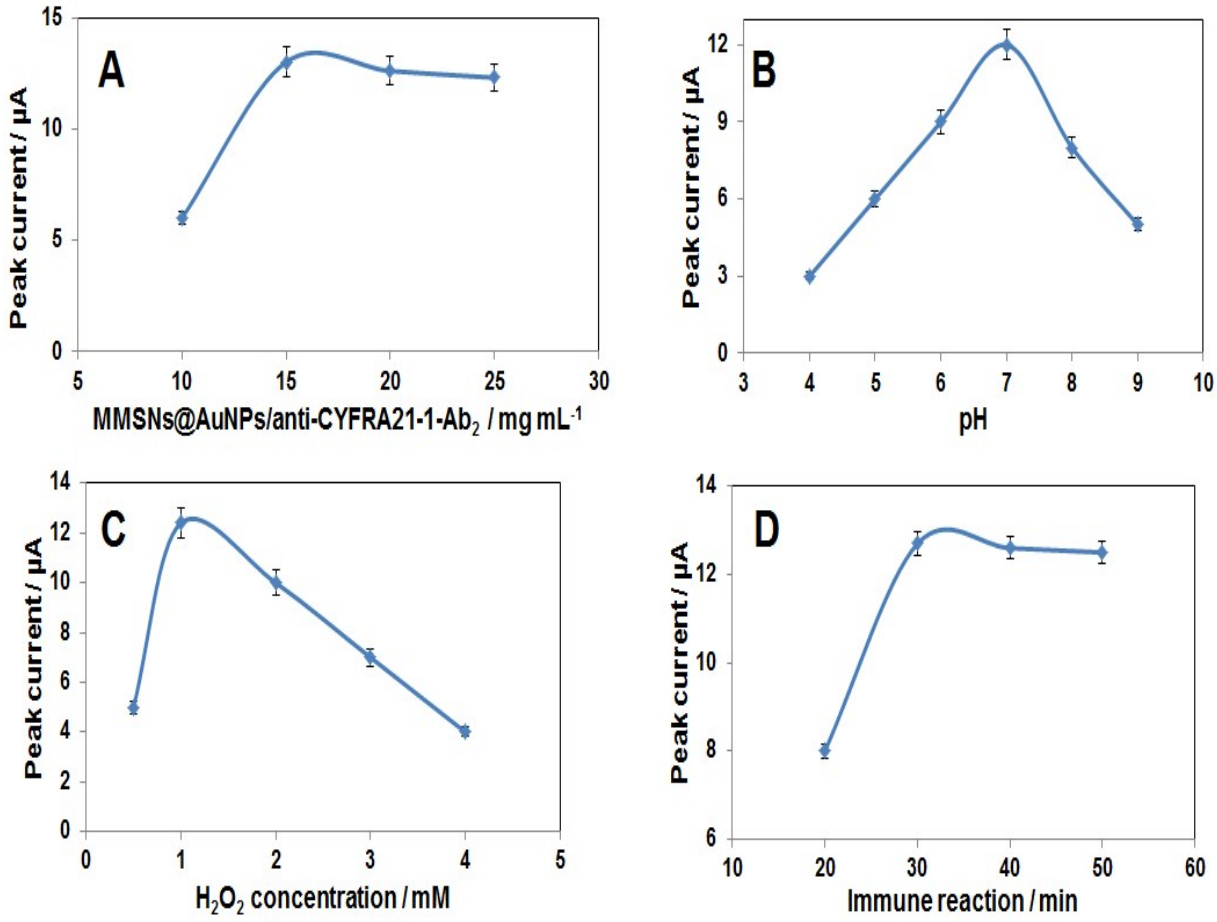


**Fig. S7.** Effect of (A) MMSNs@AuNPs/anti-CYFRA21-1-$Ab_2$ concentration, (B) pH, (C) $H_2O_2$ concentration, (D) immune reaction time (Antigen CYFRA21-1 concentration: 0.1 pg $mL^{-1}$, frequency of 50 Hz, pulse amplitude of 20 mV, scan increment of 3 mV for DPV measurements) (n = 6)

### *3.6. Recovery*

**Table S1.** Recovery of CYFRA21-1 in 1.0 mM $H_2O_2$ in pH 7.0, 0.1 M PBS (n=6)

| Plasma sample | Added CYFRA21-1 (pg $mL^{-1}$) | Found CYFRA21-1 (pg $mL^{-1}$) | Recovery (%) |
|---|---|---|---|
| [a]Sample (1) | 0.100 | 0.104 ± 0.001 | - |
| [b]Sample (2) | Sample(1) + 0.200 | 0.302 ± 0.002 | 99.34 ± 0.01 |
| [c]Sample (3) | Sample(1) + 0.400 | 0.501 ± 0.004 | 99.40 ± 0.02 |
| [d]Sample (4) | Sample(1) + 0.600 | 0.700 ± 0.003 | 99.43 ± 0.02 |

[a]containing 0.100 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

[b]containing 0.100 + 0.200 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

[c]containing 0.100 + 0.400 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

[d]containing 0.100 + 0.600 pg $mL^{-1}$ CYFRA21-1, 10.00 pg $mL^{-1}$ CEA, 10.00 pg $mL^{-1}$ AA, 10.00 pg $mL^{-1}$ DA, 10.00 pg $mL^{-1}$ UA and 10.00 pg $mL^{-1}$ BSA

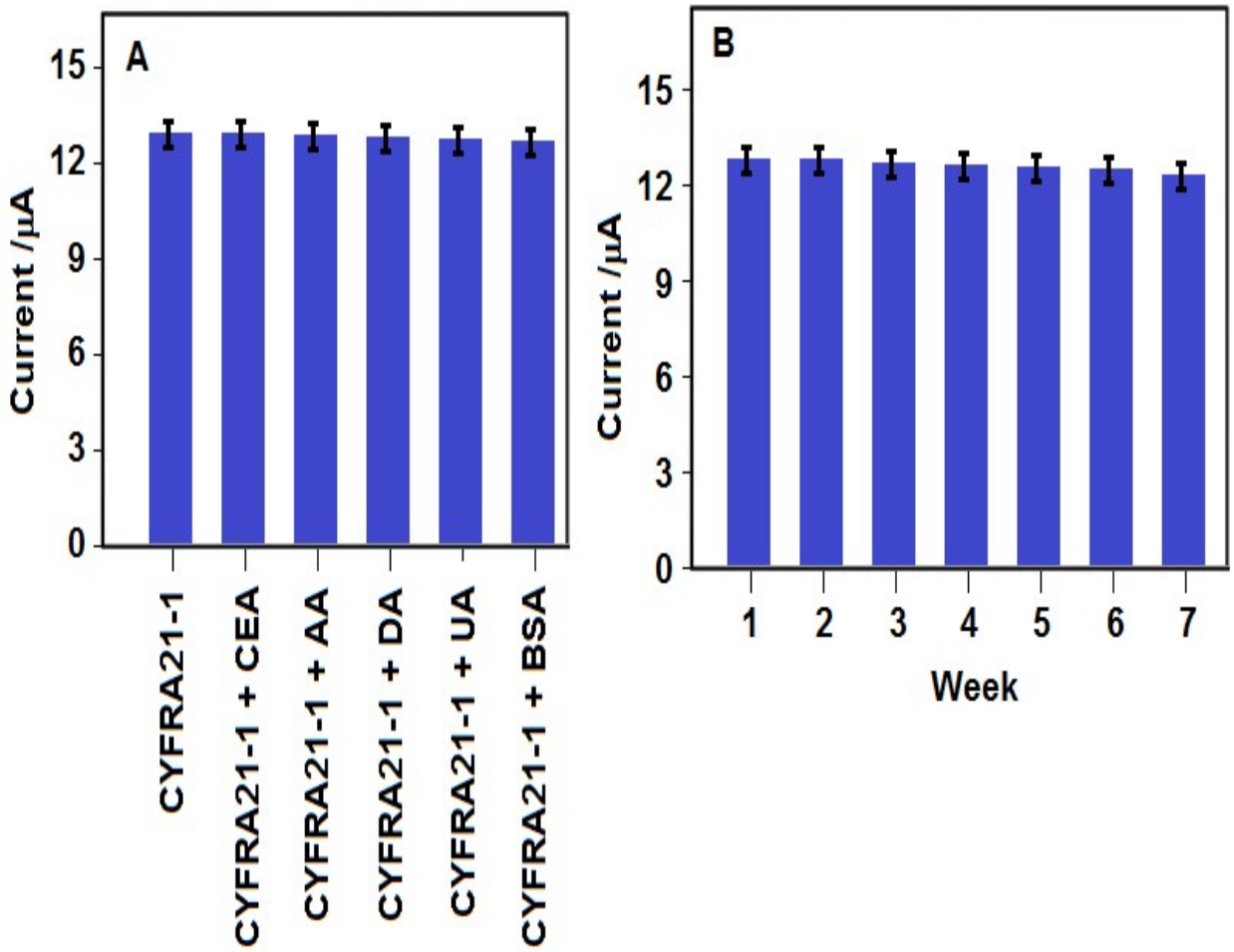


**Fig. S8.** (A) Immunosensor responses against the prepared solutions (n = 6): (i) 0.100 pg $mL^{-1}$ CYFRA21-1, (ii) 0.100 pg $mL^{-1}$ CYFRA21-1 + 10.00 pg $mL^{-1}$ CEA, (iii) 0.100 pg $mL^{-1}$ CYFRA21-1 + 10.00 pg $mL^{-1}$ AA, (iv) 0.100 pg $mL^{-1}$ CYFRA21-1 + 10.00 pg $mL^{-1}$ DA, (v) 0.100 pg $mL^{-1}$ CYFRA21-1 + 10.00 pg $mL^{-1}$ UA, (vi) 0.100 pg $mL^{-1}$ CYFRA21-1 + 10.00 pg $mL^{-1}$ BSA; (B) Stability test of voltammetric CYFRA21-1 immunosensors including 0.100 pg $mL^{-1}$ antigen CYFRA21-1 (n = 6)

### *3.8. Precision and Accuracy*

**Table S2.** Intra-day and inter-day precision and accuray results of CYFRA21-1 in 1.0 mM $H_2O_2$ in pH 7.0, 0.1 M PBS (n=6)

| **Added pg mL$^{-1}$** | **Intra-day** | | | **Inter-day** | | |
|---|---|---|---|---|---|---|
| | **Found[a] (pg mL$^{-1}$)** | **Precision[b] (%)** | **Accuracy[c] (%)** | **Found[a] (pg mL$^{-1}$)** | **Precision[b] (%)** | **Accuracy[c] (%)** |
| 0.100 | 0.101 ± 0.0001 | 0.243 | 1.00 | 0.101 ± 0.0002 | 0.485 | 1.00 |
| 0.200 | 0.201 ± 0.0003 | 0.366 | 0.50 | 0.199 ± 0.0002 | 0.246 | 0.50 |
| 0.500 | 0.499 ± 0.0001 | 0.049 | 0.20 | 0.501 ± 0.0002 | 0.098 | 0.20 |

[a]Mean ± Standart Error, [b]Precision %: Relative Standart Deviation (RSD), [c]Bias %: [(found – added)/added]×100%