Electronic Supplementary Information

High-frequency QCM Biosensing Platform for Label-free Detection of SARS-CoV-2 Spike Receptor-Binding Domain: Aptasensor and Immunosensor

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1. Materials and reagents

The SARS-CoV-2 spike RBD recombinant protein (RBD), antibody of RBD and Influenza A H1N1 hemagglutinin protein (HA) were purchased from Sino Biological Co., Ltd (Beijing, China). The aptamer of RBD (5'-NH_2-$(A)_{15}$CAGCACCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTAATG GACA-3'), vascular endothelial growth factor 165 (VEGF165), prostate specific antigen (PSA) and mucin 1 (MUC1) were obtained from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was bought from Sigma-Aldrich (USA). Sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), potassium chloride (KCl), potassium ferrocyanide ($[K_4Fe(CN)_6]$), potassium ferricyanide ($[K_3Fe(CN)_6]$) and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Healthy human serum was obtained from Xiangya Hospital of Central South University (China). Healthy saliva was collected from nonirritating whole saliva from subjects who were forbidden to eat or drink for 2 h. Ultrapure water used in all experiments was obtained on a purification system from Wuhan Ulupure Pure Water Equipment Co., Ltd. (China).

2. Apparatus

The gold-coated quartz crystals (Au chips) (AT-cut, 100 MHz) were purchased from AWSensors (Valencia, Spain) with an active surface area of 0.785 mm^2. The resonators were fixed to a support of polyether ether ketone (PEEK) with a conical hole to expose the active surface of the gold coating. The high-frequency quartz crystal microbalance measurements were operated on a HF-QCM sensor made by our group (Changsha, China) containing an automated flow-through equipment controlled by syringe pumps for real-time characterization of the frequency response of the sensor through the experiments performed in flow conditions. Here, the HF-QCM sensor was driven by one oscillating Au electrode on the one surface of quartz crystal (the biosensing electrode is the Au electrode on another surface of quartz crystal) and one non-contacted oscillating stainless steel electrode. Phosphate buffer (20 mM, pH 7.4) was used as the carrier solution with a flow rate of 210 μL/min. Electrochemical impedance spectroscopy (EIS) measurements were performed on an electrochemical workstation (CHI 660D, China) with a three-electrode system including a modified Au chip as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode.

3. HF-QCM Assay of RBD

Before modification, the Au chips were subjected to UV radiation (395 nm, 10 W) for 15 min with 30 μL 30% H_2O_2, and then washed with ultrapure water and ethanol, and dried naturally at room temperature.[1] Subsequently, 10 μL of the aptamer or antibody solution (1 μM) was dropped onto the Au chip and the Au chip was kept at 4 °C overnight to make the aptamer or antibody be immobilized on the Au chip via Au-NH_2 bonds. After washing with phosphate buffer (20 mM, pH 7.4) for three times, the Au chip was placed in the QCM flow cell holder and 0.5 wt % BSA solution was injected into the cell with a flow rate of 210 μL/min for 25 min at room temperature to block the nonspecific binding sites. Then, phosphate buffer (20 mM, pH 7.4) was injected into the cell with the flow rate of 210 μL/min to wash the Au chip. After that, 2 mL sample containing different concentrations of RBD and phosphate buffer (20 mM, pH 7.4) was injected into the QCM flow cell with a flow rate of 210 μL/min. Finally, the frequency shift of each sample was recorded real-timely with the HF-QCM instrument.

4. Recovery assay of RBD in the human serum and saliva samples

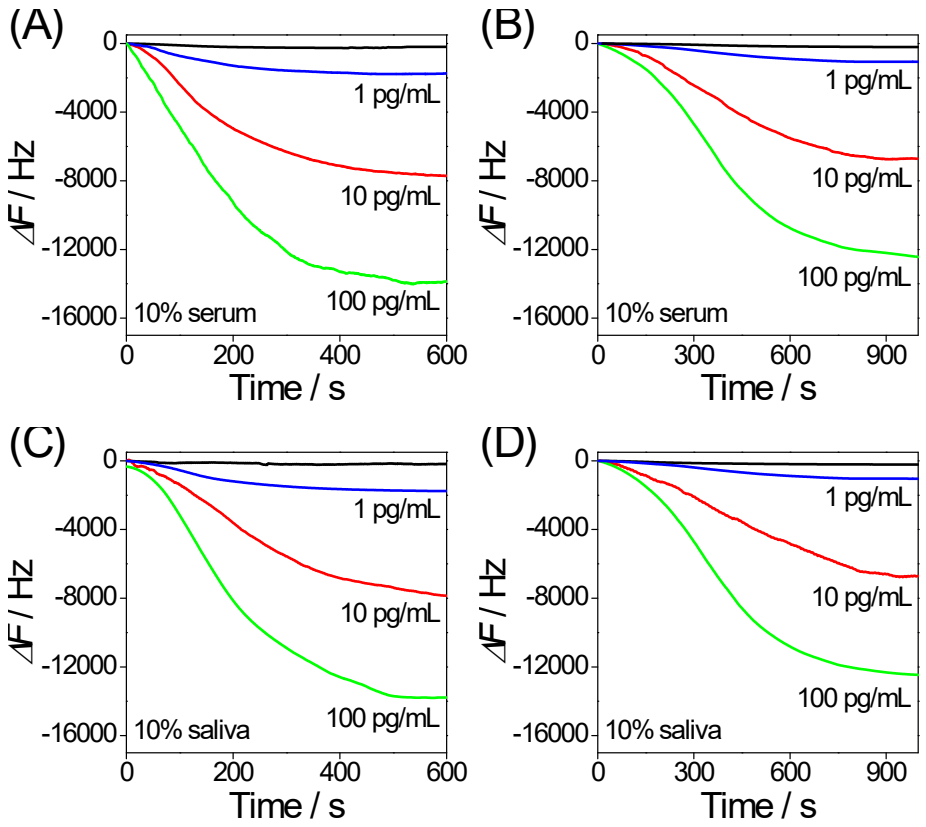

Figure S1. Frequency changes of the HF-QCM aptasensor (A and C) and immunosensor (B and D) to different concentrations of RBD (0, 1, 10 and 100 pg/mL) spiked in 10% serum (A and B) and 10% saliva (C and D).

Table S1. Recovery assay of RBD in the human serum and saliva samples.

Sample	Added (pg/mL)	HF-QCM aptasensor			HF-QCM immunosensor		
		Found[#] (pg/mL)	Recovery (%)	RSD (%)	Found[#] (pg/mL)	Recovery (%)	RSD (%)
10% serum	0	NF	NF	NF	NF	NF	NF
	1	1.03	103	2.4	0.99	99.0	4.1
	10	9.8	98	1.3	10.1	101	3.2
	100	102	102	3.3	103	103	4.0
10% saliva	0	NF	NF	NF	NF	NF	NF
	1	1.02	102	1.8	0.98	98.0	3.9
	10	10.4	104	2.0	9.9	99.0	4.4
	100	99	99	2.2	104	104	4.2

5. Reference

1 C. March, J. V. García, Á. Sánchez, A. Arnau, Y. Jiménez, P. García, J. J. Manclús and Á. Montoya, *Biosens. Bioelectron.*, 2015, **65**, 1–8.