Supporting Information

For

Exploiting Enzyme Catalysis in Ultra-Low Ion Strength Media For Impedance Biosensing of Avian Influenza Virus Using a Bare Interdigitated Electrode

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Table S1. Comparison of the performance of this work with other H5N1 biosensors.

No.	Protocol [a]	LDR [b]	DL [c]
1[1]	Hydrogel based QCM aptasensing		0.0128 HAU in 200 μL
2[2]	Magnetic nanobeads amplified QCM immunosensing	0.128 - 12.8 HAU in 300 μL	0.0128 HAU in 300 μL
3[3]	Surface Plasmon Resonance aptasensing	0.128 - 1.28 HAU in 300 μL	0.128 HAU in 300 μL
This work	Impedance immunosensing based on enzymatic catalysis	0.001 - 1 HAU in 200 μL	8 × 10-4 HAU in 200 μL

[a] QCM: quartz crystal microbalance. [b] LDR: Linear detection range. [c] DL: detection limit.

(1) Wang, R.; Li, Y. *Biosens. Bioelectron*. **2013**, *42*, 148-155

(2) Li, D.; Wang, J.; Wang, R.; Li, Y.; Abi-Ghanem, D.; Berghman, L.; Hargis, B.; Lu, H. *Biosens. Bioelectron.* **2011,** *26*, 4146-4154.

(3) Bai, H.; Wang, R.; Hargis, B.; Lu, H.; Li, Y. *Sensors* **2012,** *12*, 12506-12518.

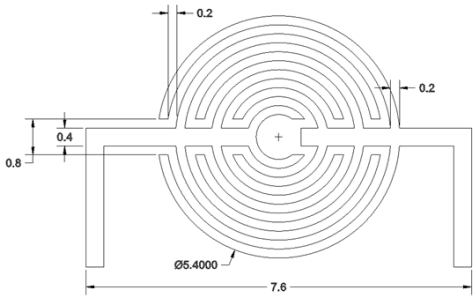

Figure S1. The screen-printed interdigitated gold electrode. Dimensions are given in millimeters.

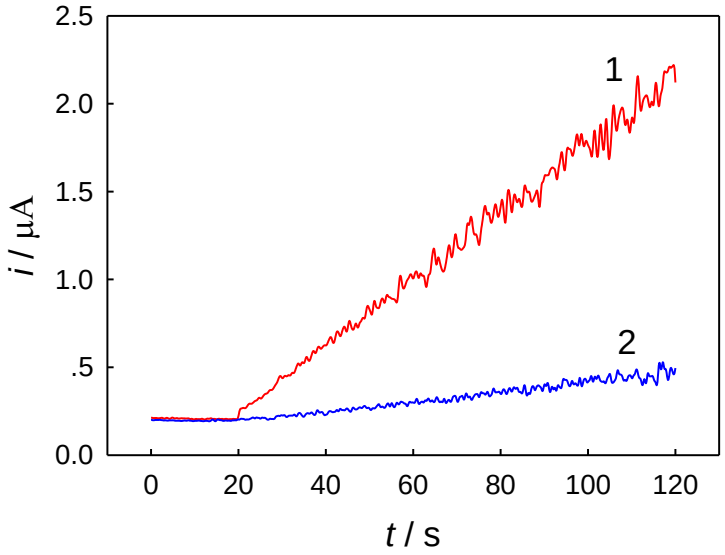

Figure S2. Potentiostatic curves of IDAE at 0.7 V in 10 mL stirred PBS after adding 10 μL 1 mg mL^{-1} GOx solution (curve 1) or 10 μL ConA/GOx/AuNPs suspension (curve 2). Briefly, a three-electrode electrochemical cell was used with IDAE as the working electrode. Before adding 10 μL of 1 mg mL^{-1} GOx solution or 10 μL of BNCs suspension, glucose was added to be 10 mM. Therefore, H_2O_2 was continuously produced through the GOx catalysis, leading to a continuously increased current. The slope of the curve should indicate the rate of the increase of the concentration of H_2O_2, and thus, the enzyme activity.

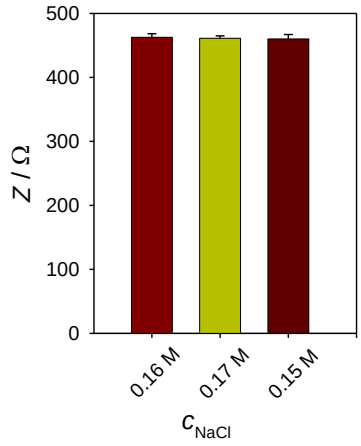

Figure S3. Impedance of PBS solution containing NaCl in different concentrations.

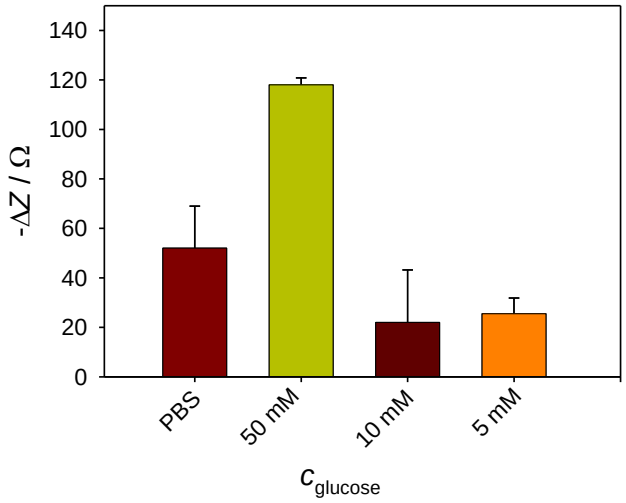

Figure S4. Impedance change of the BNCs/H5N1/BSA/aptamer/Au electrode in PBS containing 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ before and after immerging in PBS or glucose solutions in different concentrations.

Glucose acts not only as the substrate of GOx, but also as a competitor to the conjugation of ConA and GOx through its affinity to ConA. Generally, higher concentrations of glucose benefit more from the GOx catalysis, however, the glucose can induce detachments of BNCs from the MBs during the washing and detecting. Therefore, glucose concentrations of 5 mM, 10 mM and 50 mM were tested using a similar method to the one mentioned above, as shown in Figure S3. It was found that 5 mM and 10 mM glucose solutions induced impedance changes of -25 ± 6 Ω and -22 ± 21 Ω, which were all less than that in PBS (-52 ± 17 Ω). This means the ConA-GOx conjugates are stable in glucose solutions with concentrations of 10 mM or lower. It is known that the binding constant between ConA and glycoenzymes is in the order of 10^5-10^7 M^{-1},[4] while the constant of ConA with glucose is only about 8×10^2 M^{-1}.[5] However, 50 mM glucose solution made an impedance decrease (118 ± 3 Ω) by 66 Ω more than that in PBS, which indicates an unacceptable instability. Therefore, 10 mM glucose solution was used for the biosensing.

(4) Köneke, R.; Menzel, C.; Ulber, R.; Schügerl, K.; Scheper, T.; Saleemuddin, M. *Biosens. Bioelectron.* **1996,** *11*, 1229-1236.

(5) Sato, K.; Imoto, Y.; Sugama, J.; Seki, S.; Inoue, H.; Odagiri, T.; Hoshi, T.; Anzai, J.-i. *Langmuir* **2004,** *21*, 797-799.

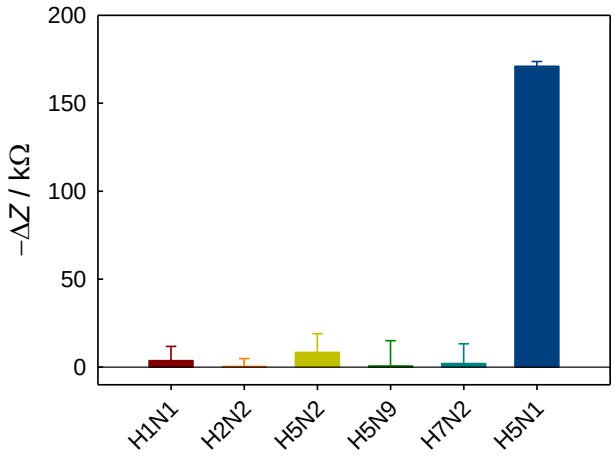

Figure S5. Impedance response of the biosensor to AIVs of different subtypes.

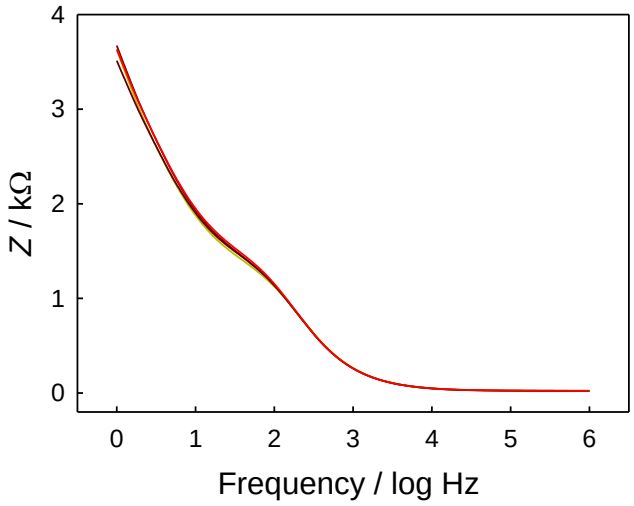

Figure S5. Four impedance response curves of the IDAE in PBS containing 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ during two-day measurements.