Nanowire Transistor-Based Ultrasensitive Virus Detection with Reversible Surface Functionalization

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Abstract: We have applied a reusable silicon nanowire field-effect transistor (SiNW-FET) as a biosensor to conduct ultrasensitive detection of H5N2 avian influenza virus (AIV) in very dilute solution. The reversible surface functionalization of SiNW-FET was made possible using a disulfide linker. In the surface functionalization, 3-mercaptopropyltrimethoxysilane (MPTMS) was first modified on the SiNW-FET (referred to as MPTMS/SiNW-FET), with subsequent dithiothreitol washing to reduce any possible disulfide bonding between the thiol groups of MPTMS. Subsequently, receptor molecules could be immobilized on the MPTMS/SiNW-FET by the formation of a disulfide bond. The success of the reversible surface functionalization was verified with fluorescence examination and electrical measurements. A surface topograph of the SiNW-FET biosensor modified with a monoclonal antibody against H5N2 virus (referred to as mAbH5/ SiNW-FET) after detecting approximately $10^{-17}$ M H5N2 AIVs was scanned by atomic force microscopy to demonstrate that the SiNW-FET is capable of detecting very few H5N2 AIV particles.

Keywords: biosensors · nanowires · reversible surface functionalization · viruses

Introduction

Influenza viruses of types A, B, and C belong to the Orthomyxoviridae family of RNA viruses and are further classified into several subtypes based on two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), located on the surfaces of the viruses.[1] According to the statistical figures for the worldwide pandemic outbreak of highly pathogenic H5N1 avian influenza virus (AIV) in the past eight years, 59% of the 539 infected humans died.[2] Although the number of recent casualties bears no comparison to the outbreak at the beginning of the 20th century, the case death rate is much higher now than before.[3] To prevent a potentially hazardous AIV outbreak, a sensitive and rapid analysis platform to establish a public health alarm system is mandatory. To date, several methods have been used to confirm AIV infection, including immunofluorescence assay (IFA), hemagglutination-inhibition (HI), enzyme immunoassays (EIA), and reverse transcription polymerase chain reaction (RT-PCR).[4] Among them, the EIA-based techniques, mostly targeting the abundant nucleoprotein in influenza virus, are simple and convenient but cannot distinguish the HA subtypes. RT-PCR has the best sensitivity; however, choosing the proper primer sets becomes more challenging due to the diverse virus subtypes and the continuing evolution of AIVs.

The development of the silicon nanowire field-effect transistor (SiNW-FET) as a sensorial tool has significant impact on the fields of chemical and biological analysis,[5] biomedicine,[6] and cellular research,[7] because of its ultra-sensitive, label-free, rapid screening and real-time detecting capabilities.[8] Herein, we applied a SiNW-FET to detect H5N2 AIV particles (A/duck/Yulin/04) with an emphasis on the reversible surface functionalization of the SiNW via a disulfide linker, rendering the SiNW-FET a reusable device for fast screening of H5N2 AIV. In the previous applications of SiNW-FETs that were focused on detection of specific antigens, the corresponding antibody was generally functionalized onto the SiNW-FET through covalent bonding, where silane aldehyde was commonly adapted as

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a linker to anchor the antibody onto the SiNW-FET. However, once silane aldehyde was linked with the antibody via reductive amination, it was difficult to reverse the amine linkage back to its original free aldehyde state after the antigen detection. As a result, it was problematic to remove the tightly bound antigen–antibody complex, yielding a device that could only be used for a single measurement. Strong oxidants might be able to cleave the amine linkage, but this is unlikely without damaging the device. By judiciously selecting a reversible functionalization method to anchor an antibody onto a SiNW-FET via a disulfide bond, we show in the present study that this sensorial device is reusable by reducing the disulfide bond with dithiothreitol (DTT), while concurrently removing the antibody–virus complex. This reversible surface functionalization by reducing disulfide bonds is comparable with our other recently developed technique of modifying the surface of a SiNW-FET with the reversible association–dissociation between glutathione (GSH) and glutathione 3-transferase (GST)-tagged protein, allowing the sensitive SiNW-FET to serve as a reusable and high-throughput biosensor for the fast screening of protein–protein interactions under physiological conditions. However, from the use of the GSH–GST reversible system, the GST (a protein of ca. 2.5 nm in size) located between the target–receptor complex and SiNW might partly screen the interaction field exerted from the target–receptor complex to the SiNW-FET, thus reducing the acquired signal. In contrast to the sizable GST, the advantage of applying the simple disulfide bond as a chemical linker for the immobilization of a receptor protein onto the SiNW-FET surface could minimize this detrimental field-shielding effect.

Herein, we first demonstrated a reusable SiNW-FET device through the reversible functionalization of biotin on the SiNW-FET surface via a disulfide linker. This reusable SiNW-FET was then modified with a monoclonal antibody against H5N2 virus (mAbH5) to detect H5N2 AIV at very dilute concentrations. Meanwhile, we also showed the binding specificity of the mAbH5-modified SiNW-FET (referred to as mAbH5(SiNW-FET)) from the surface topography of the SiNW-FET after detecting approximately 10^13 m H5N2 AIVs, we verified that the sensitive SiNW-FET is capable of detecting very few H5N2 AIV particles. This reversible surface-modification technique via a disulfide linker can also be applied to other field-effect transistors, such as organic thin-film transistors and graphene-FETs. By properly selecting a thiol-containing chemical linker to be modified on organic thin film transistors, or graphene-FETs, the transistor-based biosensors can function as reusable sensing devices as well.

**Results and Discussion**

Figure 1 shows the schematic illustration and experimental demonstration for the reversible surface functionalization of a SiNW-FET via a disulfide linker. First, 1% MPTMS in ethanol was pumped into a polydimethylsiloxane (PDMS) microfluidic channel, which was designed to couple with the SiNW-FET device arrays (Figure 1a). After the SiNW-FET was modified with 3-mercaptopropyltrimethoxysilane (MPTMS) to form a MPTMS/SiNW-FET (Figure 1b), ethanol was introduced into the PDMS microfluidic channel to wash the FET devices, followed by DTT in phosphate buffered saline (1× PBS) to reduce any possible disulfide bonding between the thiol groups of MPTMS. The MPTMS/SiNW-FET was then flushed with 1× PBS. Next, the receptor molecules could be immobilized on the SiNW-FET via the formation of a disulfide bond with the MPTMS/SiNW-FET.

To demonstrate the reversible functionalization on the MPTMS/SiNW-FET surface through a disulfide linker, N-(6-(biotinamido)hexyl)-3′-(2′-pyridyldithio)propionamide (biotin-HPDP) was immobilized on the MPTMS/SiNW-FET to make a biotin/SiNW-FET (Figure 1b); subsequently, the biotin moiety was removed with DTT to return the device surface to MPTMS/SiNW-FET. Figure 1c shows an electrical measurement to verify the reversibility between MPTMS/SiNW-FET and biotin/SiNW-FET, involving the processes of 1) the formation of MPTMS/SiNW-FET, 2) the immersion of MPTMS/SiNW-FET in 1× PBS, 3) the reaction of biotin-HPDP with MPTMS/SiNW-FET to form a biotin/SiNW-FET, and 4) the removal of biotin with DTT to return the device to MPTMS/SiNW-FET. In the course of the electrical measurement, three reproducible cycles were observed to demonstrate the reusability of the SiNW-FET device via a disulfide linker. Typically, a MPTMS/SiNW-FET can be used repetitively for more than 20 cycles.

Using this reversible surface functionalization method, we further made a reusable SiNW-FET for the H5N2 AIV detection, as represented schematically in Figure 2a. One advantage of choosing a disulfide linker for the reversible surface functionalization on a SiNW-FET is that the immobilization reaction of receptor proteins (such as AIV–mAbH5 in this study) through the disulfide bond can be carried out conveniently at room temperature to avoid spoiling the normal functions of proteins or damaging the SiNW-FET device, which could occur if the reaction would otherwise be conducted at escalated temperature. Shown in Figure 2b are the electrical responses of a SiNW-FET in the processes of preparing the MPTMS/SiNW-FET with 500 mM DTT washing, 1× PBS flushing, mAbH5 immobilization, and another cycle of renewing the surface functionalization initiated with DTT washing. It is noted that the conductance of the SiNW-FET remained at the original level (the horizontal dashed line) after the cleavage of disulfide bonds followed by 1× PBS flushing, again indicating that the device surface was returned to the MPTMS/SiNW-FET state without analyte contamination and was ready for the next round of measurement.

The successful immobilization of mAbH5 on a Si substrate was also corroborated with a microfluorescence technique. As shown in Figure 2c, the fluorescent pattern was obtained by attaching an anti-chicken IgG fluorescein isothiocyanate conjugate (FITC-Ab) to a MPTMS-modified micropattern with the same modification procedures as described in Figure 1.
ure 2a. The illuminating pattern is in sharp contrast with the dark unmodified surroundings, which also indicates that there is no significant nonspecific adhesion on the Si surface without modification. After the removal of FITC-Ab by DTT washing, the blank image in Figure 2d was obtained, thus demonstrating the complete cleavage of the disulfide bonds on the Si substrate.

The ζ potential of H5N2 AIV, displayed in Figure 3, was characterized at 2.4 ≤ pH ≤ 8.4. From the ζ potential measurements, the pI of H5N2 AIV was determined to be approximately 3.2. In addition, the transmission electron microscopy (TEM) images of H5N2 AIV particles (ca. 120 nm in size) and polystyrene latex beads (137 nm in diameter) were taken for comparison (Figure 4).

In the upper trace of Figure 5a, the applicability of the mAbH5/SiNW-FET to detect H5N2 AIV was tested with a sample solution of 10⁷ AIV mL⁻¹ (ca. 1.6 × 10⁻¹⁵ M) in 1× PBS, where the conductance of mAbH5/SiNW-FET was stabilized in 1× PBS in the beginning and increased dramatically upon the addition of H5N2 AIV, indicating the strong binding affinity between mAbH5 and H5N2 AIV. The electrical enhancement in the p-type mAb H5/SiNW-FET is due to a gating effect generated by the binding of the negatively charged H5N2 AIV (pI = 3.2, Figure 3) at pH 7.4. However, this device responded only slightly to the subsequent addition of 10⁸ AIV mL⁻¹, suggesting that the binding sites on the mAb H5/SiNW-FET could have been saturated. As a rough estimation, less than 20 AIVs could bind onto the surface of mAbH5/SiNW-FET, if the sensorial device is assumed to have a SiNW (ca. 30 nm in diameter) located between the source and drain electrodes (ca. 2 μm apart). The molecular sizes for H5N2 AIV and mAb H5 are approximately 120 nm (Figure 4) and 8 nm, respectively.

To confirm that the signals are genuinely caused by binding H5N2 AIV onto the mAbH5/SiNW-FET, we conducted two negative control experiments. The lower trace of Figure 5a shows the results of a test of whether H5N2 AIV could be detected by a bare SiNW-FET without any surface modification. Before H5N2 AIV was introduced, the conductance of the SiNW-FET was balanced by immersing the SiNW-FET in 1× PBS. No significant conductance change was observed after the arrival of H5N2 AIV (10⁷ AIV mL⁻¹). For comparison, we then modified mAbH5 on the same SiNW-FET device (i.e., to form a mAbH5/SiNW-FET) to detect H5N2 AIV (10⁷ AIV mL⁻¹). As shown in the inset of Figure 5a, the obvious electrical response verifies that the conductance change in the mAbH5/SiNW-FET originates from the specific binding between mAbH5 and H5N2 AIV.
and H5N2 AIV. For another control test of the selectivity of this sensorial device, we applied mAb H5/SiNW-FETs to the detection of H6N1 AIV. As displayed in Figure 5b, while there is no apparent electrical response of the mAbH5/SiNW-FET to H6N1 AIV (10^8 AIV mL^-1), a conductance increase was observed after introducing H5N2 AIV (10^7 AIV mL^-1), manifesting once more the binding specificity of the mAb H5/SiNW-FET. The sudden decrease of electrical conductance after introducing H5N2 AIV was caused by manually changing H6N1 AIV-containing solution to H5N2 AIV-containing solution during the electrical measurement. In Figure 5, the considerable signal collection time (minutes) for the mAbH5/SiNW-FET to reach an equilibrium in binding with H5N2 AIVs stemmed from detection of very dilute sample solution (< 1 fM) in the electrical measurement; accordingly, the limited number of H5N2 AIV particles needed to flow across the laminar fluid by diffusion and convection inside the PDMS microfluidic channel to reach the mAbH5/SiNW-FET device surface. It is noted that without the delicately site-directed immobilization of mAbH5, the attachment of mAbH5 via its amino groups to the SiNW-FET surface could cause a random orientation of the immobilized mAbH5.[14] As a result, some of the binding sites on the immobilized mAbH5 could be very close to the SiNW-FET surface, con-
parable to the Debye–Hückel screening length of the buffer solution used.

As for the speculation that binding only a limited number of H5N2 AIVs on the mAbH5/SiNW-FET could have caused an electrical response in this biosensor, we attempted to observe the AIV–mAbH5 complex on the SiNW-FET surface by atomic force microscopy (AFM) after a biosensing measurement. Figure 6a shows a typical electrical measurement for detecting very dilute H5N2 AIVs (10^4 AIV mL⁻¹). Inset: In contrast, the bare SiNW-FET was modified with mAbH5 for detection of H5N2 AIV (10⁵ AIV mL⁻¹), an apparent conductance change was observed after introducing H5N2 AIV (10⁴ AIV mL⁻¹). While there is no apparent electrical response to H6N1 AIV, a conductance increase was observed after introducing H5N2 AIV (10⁴ AIV mL⁻¹), illustrating the binding specificity of mAbH5/SiNW-FET.

**Conclusions**

We have demonstrated the ultrasensitive detection of H5N2 AIV using a reusable SiNW-FET, which was made possible by the reversible surface functionalization on the SiNW via a disulfide linker. The success of the reversible surface functionalization was verified by electrical and microfluorescence examinations. Detections of very dilute H5N2 AIV at 10⁻¹²–10⁻¹⁷ M were achieved. After the biosensing experiments, the very few bound H5N2 AIVs on the surface of the mAbH5/SiNW-FET were investigated by AFM topography, demonstrating the ultrasensitivity of SiNW-FETs in biosensing measurements. The demonstrative performance of this reusable biosensor makes SiNW-FET a good candidate for future biomedical applications, such as virus infection diagnosis and early cancer detection.

**Experimental Section**

The chemical and biological materials used in the experiments are listed in Supporting Information S1. SiNWs were synthesized from a chemical-vapor-deposition reaction assisted with catalytic Au nanoparticles (Sup-
porting Information S2) and the SiNW-FET devices were fabricated following a standard photolithographic procedure (Supporting Information S3). The experimental details for the SiNWs synthesis and SiNW-FET fabrication can be found in our previous publications.[6,15]

Surface Functionalization

Figure 1 shows the schematic illustration and experimental demonstration for the reversible surface functionalization via a disulfide linker. In the beginning, 1% MPTMS in ethanol was pumped into a PDMS microfluidic channel (6.25 × 0.5 × 0.05 mm³), which was designed to couple with the SiNW-FET device arrays, at a flow rate of 300 μL·h⁻¹ for 30 min by a syringe pump (KD Scientific, KD-101). After MPTMS was modified on the SiNW-FET to form the MPTMS/SiNW-FET, ethanol was guided into the PDMS microfluidic channel to wash the FET devices for 10 min, followed by 500 mM DT (containing 138 mM NaCl, 2.7 mM KCl, 8 mM NaH₂PO₄, 1.5 mM KH₂PO₄, pH 7.4 with NaOH) for 30 min to reduce any possible disulfide bonding between the thiol groups of MPTMS. The MPTMS/SiNW-FET was then flushed with 1× PBS for 20 min.

Next, the receptor molecules could be immobilized on the SiNW-FET via the formation of a disulfide bond with the MPTMS/SiNW-FET. In a test of the reversible surface functionalization of a SiNW-FET (as represented in Figure 1b), 0.4 mM biotin-HPDP was pumped into the PDMS microfluidic channel at 0.4 mL·h⁻¹ for 30 min to react with the MPTMS/SiNW-FET to form a biotin/SiNW-FET. For the H5N2 AIV detection, we first mixed mAbH5 (3 μL) with N-succinimidyl-3-(2-pyridyldithio)propionamide (SPDP, 2 μL) in a vial at room temperature for 30 min, in which mAbH5 was linked to SPDP through the formation of an amide bond by substituting the N-hydroxysuccinimide of SPDP with an amine group of mAbH5. Subsequently (as illustrated in Figure 2a), this mAbH5-containing solution was added to 1× PBS (45 μL) to form a mixture, which was then pumped into the PDMS microfluidic channel at 25 μL·h⁻¹ for two hours to immobilize the mAbH5 on the MPTMS/SiNW-FET by the formation of a disulfide bond. After the modification of mAbH5 on the SiNW-FET surface to form the mAbH5/SiNW-FET, the device was washed with 1× PBS for 30 min and was then ready for AIV detection. To make the device reusable (Figure 2a), the AIV–mAbH5 complex was removed after each biosensing experiment by cleaving the disulfide bond with DT (300 mM in 1× PBS) for 2 h to restore the clean surface of the MPTMS/SiNW-FET. This regenerated MPTMS/SiNW-FET was further rinsed with deionized water before the next round of measurements. Typically, a mAbH5/SiNW-FET device can be repeatedly used in electrical measurements with more than five cycles.

Fluorescence Imaging

A microscopic fluorescence imaging technique was employed to corroborate the successful modifications of MPTMS and mAbH5 on a Si substrate. A micropattern was designed on a Si wafer (Figure 2c) and fabricated using a standard photolithographic procedure using LOR5B/S1813 photoresists.[5g,6e] After the modification of MPTMS on the patterned Si substrate, the surrounding photoresists were washed off with a PG remover at 60°C for two hours. The procedures to anchor an anti-chicken IgG fluorescein isothiocyanate conjugate (FITC-Ab) onto the MPTMS-modified Si surface via the PDSP linker are the same as those described above for the immobilization of mAbH5. Finally, a fluorescence image of the FITC-Ab anchored micropattern (Figure 2c) was taken with a reverse fluorescence microscope (Nikon, Eclipse TE2000-U).

Electrical Measurement

The electrical characteristics (including the plots of source–drain current vs. gate voltage (Iₛᵩ–Vₛᵩ) and Iₛᵩ vs. source–drain voltage (Iₛᵩ–Vₛᵩ)) of the as-fabricated SiNW-FETs are presented in Supporting Information S4. The electrical conductance of a SiNW-FET was measured at Vₐᵩ = −30 mV by a detection system that combined a current preamplifier (DL Instrument, 1211) with a lock-in amplifier (Stanford Research System, SR830 DSP) operated at a modulation frequency of 79 Hz and a time constant of 100 ms.[7,6a,14] In the electrical measurements, an Ag/AgCl electrode (BAS, MF2052) coupled to the PDMS microfluidic channel was used as a solution gate (Figure 1a) and kept at ground potential throughout the real-time electrical measurements to minimize noise in the system.

Zeta (ζ) Potential

The ζ-potential of H5N2 AIV was characterized at 2.4 ≤ pH ≤ 8.4 by a Zetasizer (Malvern Instruments, 3000HS), as shown in Figure 3.

H5N2 AIV Concentration

The method to determine the number density of H5N2 AIV particles was adapted following the procedures used by Zheng et al.[15] with the details described in Supporting Information S5. By counting the number ratio between H5N2 AIVs and polystyrene latex beads from 50 TEM (JEOL JEM 2010 Analytical TEM at 200 kV) images (a representative one is shown in Figure 4), the concentration of an original sample of H5N2 AIVs was estimated statistically to be approximately 10¹² AIV mL⁻¹.

Surface Topography

The microscopic topographs on the surface of SiNW-FETs before (i.e. mAbH5/SiNW-FET as shown in Figure 2a) and after (i.e. AIV–mAbH5/SiNW-FET in Figure 2a) sensing H5N2 AIV (10⁷ AIV mL⁻¹) were taken by AFM (Veeco, Bioscope SZ, NSIV) in tapping mode at a scan rate of 0.5 Hz.[16]

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Transistor-Based Ultrasensitive Virus Detection


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S1. Chemical and biological materials

Ethanol (HPLC grade), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and anti-chicken IgG fluorescein isothiocyanate conjugate (FITC-Ab) were purchased from Sigma-Aldrich. Other chemicals were also purchased commercially: 3-mercaptopropyltrimethoxysilane (MPTMS) from Fluka, N-(6-(biotinamido)hexyl)-3′-(2′-pyridyldithio)-propionamide (biotin-HPDP) from Thermo Fisher Scientific, DTT from J. T. Baker, dimethylsulfoxide (DMSO, HPLC grade) from Acros, polydimethylsiloxane (PDMS) from Dow Corning, and photoresists, MF-319 developer, and remover PG (propylene glycol) from MicroChem. A purchased phosphate buffered saline (PBS) from GIBCO was diluted to 1× PBS (138 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄) with deionized water (18 MΩ Milli-Q water, Millipore). The SPDP dissolved in DMSO (0.5 mg/1 mL) was stored at 4°C before use. H5N2 AIV (A/duck/Yunlin/04), mAb H5, and H6N1 AIV (A/chicken/Taiwan/2838D/00) were prepared according to the procedures described previously [1].
S2. Synthesis and electron microscopic characterizations of SiNWs

S2.1. SiNWs synthesis

Boron (B)-doped SiNWs were synthesized in a chemical vapor deposition (CVD) reaction assisted with catalytic Au nanoparticles (20 nm in diameter) via the vapor-liquid-solid growth mechanism following the procedures reported previously [2]. Silane (SiH₄) and diborane (B₂H₆) were used as reaction precursors to synthesize the B-doped SiNWs in the ratio of B:Si = 1:4000. As shown in Fig. S1, the single-crystalline structure of the as-synthesized SiNWs was characterized by field emission scanning electron microscopy (FE-SEM, Hitachi S-800), high resolution transmission electron microscopy (HR-TEM, Philips/FEI Tecnai 20 G2 at 200 KV), and energy dispersive X-ray spectroscopy (EDX, Philips/FEI Tecnai 20 G2).
S2.2. Electron microscopic characterizations of the as-synthesized SiNWs

Figure S1. (a) FE-SEM image of the as-synthesized SiNWs obtained from the catalytically (Au nanoparticles of 20 nm in diameter) assisted CVD reaction. The average diameter of the SiNWs is ~30 nm. The scale bar represents 1 \( \mu \)m. (b) HR-TEM image of a single crystalline SiNW pretreated by buffered oxide etch (BOE) washing to remove the SiO\(_2\) sheath on the SiNW. The scale bar represents 2 nm. (c) EDX spectrum of the as-synthesized SiNWs. The Cu signal comes from the Cu grid used for the HR-TEM measurement.
S3. SiNW-FET fabrication

The SiNW-FET devices were fabricated following a standard photolithographic procedure \cite{2b, c}. The photolithographic mask design for the device arrays is shown in Fig. S2, in which 180 pairs of device arrays with 2 μm separation between the source and drain electrodes were patterned on a Si chip. A brief description of the fabrication of SiNW-FET devices is as follows. The as-synthesized SiNWs were dispersed randomly on the central area of a Si wafer (400 nm oxide layer) with a set of pre-fabricated Cr/Au (10/50 nm in thickness) outer electrodes (represented in white color in Fig. S2(a)). The as-dispersed SiNWs were then electrically contacted by 80/10-nm-thick Ni/Au leads (represented in yellow in Figs. S2(a) and S2(b)), which were established photolithographically using LOR5B/S1813 photoresists. In the photolithographic process, the LOR5B/S1813 photoresists were soft-baked at 160°C/110°C for 5 min/1.5 min to gain a faster undercut rate, allowing the SiNWs to be exposed thoroughly to buffered oxide etching (BOE) solution in order to remove the oxide sheath (typically ~3 nm) of the SiNWs. Before coating a 100-nm-thick Al layer to encapsulate the Ni/Au electrodes, the Si chip was further developed in MF-319 developer for 3 s to cut the edges of the S1813 photoresist, enabling the Al vapor to reach the sidewalls of Ni/Au electrodes in the subsequent Al deposition. The outer Al layer of the passivated Al on the Ni/Au electrodes was later oxidized to become an Al$_2$O$_3$ film of several nanometers in thickness, serving as an insulating coat to prevent electric leakage in sample solution during the biosensing experiments. After a final lift-off treatment, the SiNW-FET chip was annealed in a forming gas (10% H$_2$ in N$_2$) in a rapid thermal annealer (Ulvac,
Mila-3000) through a two-step process: first at 200°C for 2 min, and then at 360°C for 2 min. In the biosensing experiments, the detection sensitivity of SiNW-FETs can be enhanced by exposing only the device arrays (inside the reddish rectangles in Fig. S2(c)) to sample solution \([3]\). To this end, the surroundings of the device arrays were further covered with LOR3A/S1805 photoresists. The electrical characteristics of the as-fabricated SiNW-FET devices, including the plots of source–drain current vs. gate voltage \((I_{sd}–V_g)\) and \(I_{sd}\) vs. source–drain voltage \((I_{sd}–V_{sd})\), are presented in Fig. S3.
Figure S2. (a) Mask design for the photolithographic fabrication of SiNW-FET device arrays. The dimensions for the whole pattern are $17 \times 17 \text{ mm}^2$. (b) An expanded scale for the inner electrodes (in yellow color) with the dimensions of $6 \times 6 \text{ mm}^2$. (c)–(e) Optical photos of the FET devices on magnified scales. (f) An AFM image of the as-fabricated SiNW-FET device with a SiNW located between the source and drain electrodes at $\sim 2 \mu \text{m}$ separation.
S4. Electrical characteristics of an as-fabricated SiNW-FET

The $I_{sd}$–$V_{g}$ and $I_{sd}$–$V_{sd}$ plots of the as-fabricated SiNW-FET devices were measured using a detection scheme combining a probe station (Everbeing) with a picoammeter/voltage source (Keithley, 6487), which was controlled by a Labview (National Instrument) programmed personal computer and interfaced via a DAQ card (National Instrument).

![Graph](image)

**Figure S3.** (a) Typical $I_{sd}$–$V_{g}$ curves of a $p$-type SiNW-FET device on a Si chip with a 400 nm oxide layer measured at $V_{sd} = 1$ V at ambient conditions. The backgate voltage was swept from 10 V to
−10 V and back to 10 V. The transconductance of the SiNW-FET was calculated ~40 nS. (b) $I_{sd}$–$V_{sd}$ curves of the same SiNW-FET device at $V_g = -10$ V (black), 0 V (red), and 10 V (blue). The linear dependence of the current ($I_{sd}$) on the varied bias voltage ($V_{sd}$) indicates an Ohmic contact between the Ni electrodes and SiNW.
S5. Concentration determination of H5N2 AIV particles

The method to determine the number density of H5N2 AIV particles was adapted following the procedures used previously by Zheng et al. [4]. An original sample of H5N2 AIV in allantoic fluid (EID$_{50}$ = 10$^{7.8}$) was pretreated to be 10$^3$-fold more concentrated by centrifuging the sample at 70,000× g. The concentrated H5N2 AIV sample was then mixed with polystyrene latex beads (137 nm in diameter) at a fixed concentration (7.91 × 10$^{11}$ particles/mL) at a 1:1 volume ratio. The homogeneity of the mixture containing H5N2 AIV particles and polystyrene latex beads was achieved by sonicking the sample. After diluting the mixture 50-fold by dissolving it in 1× PBS at pH 7.4, the sample was dispersed on a copper grid and was negatively stained with 2% phosphotungstic acid for transmission electron microscopy (TEM, JEOL JEM 2010 Analytical TEM at 200 kV) imaging. By counting the number ratio between H5N2 AIVs and polystyrene latex beads from 50 TEM images (as a representative one shown in Fig. 4 of the main text), the concentration of H5N2 AIVs was estimated statistically to be ~10$^{11}$ AIV/mL.
Reference


