Supplementary Information

Redox-Reactive Field Effect Transistor Nanodevices for the Direct Monitoring of Small Metabolites in Biofluids towards Implantable Nanosensors Arrays

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1. Silicon Nanowires \textit{p-type Synthesis via} Chemical Vapor Deposition

The synthesis of silicon nanowires (Si NWs) by chemical vapor deposition (CVD) was performed as previously described.\textsuperscript{1} Briefly, 20 nm gold nanoparticles (Ted Pella) have catalyzed the growth of Si NWs \textit{via} the vapor-liquid-solid (VLS) mechanism. These nanoparticles were deposited on Silicon (100) growth substrates to define sites of Si NWs growth. In order to assist the attachment of the gold nanoparticles to the silicon substrate, poly-L-lysine (Ted Pella) was first introduced to the silicon wafer and served as an electrostatic binding agent. The organic materials were removed by applying 100W and 0.200 Torr oxygen plasma for 5 minutes. Next, the wafer was placed in a quartz tube furnace, where silane ($\text{SiH}_4$) and diborane ($\text{B}_2\text{H}_6$, 100 ppm in $\text{H}_2$ balance gas) were used as reactants for the Si NWs growth process, in which boron served as a \textit{p-type} dopant with a boron/silicon ratio of 1/4000. The rate of the Si NW growth was \textasciitilde{} 1 $\mu$m/min.

2. Silicon Nanowires Field-Effect Transistor Array Fabrication on Silicon Wafers

The SiNW-FET array was fabricated by photolithography as previously described,\textsuperscript{1} with the following changes: Following fabrication of the outer electrodes (including the gates) by photolithography and evaporation of chromium/gold (5/60 nm), respectively, the SiNWs were deposited on the wafer by dispersion in ethanol and dropping 0.5 $\mu$L drops on the oxide layer. The \textit{p-type} SiNWs were deposited on a 3-inch silicon wafer, covered with 600 nm thermal oxide layer (<0.005 ohm/cm, SSP prime grade, Silicon Quest International). Source and drain electrodes of FETs were defined with a multilayer photoresist structure consisting of 500 nm LOR5A (Microchem) and 500 nm S1805 (Shipley). Notably, the gap between the source and drain electrodes was 2 $\mu$m. Following exposure and development in MF319 developer, the chip was dipped in a buffered oxide etchant (hydrofluoric acid/ammonium fluoride ratio of 1/6) solution for 6 seconds and immediately metalized by e-beam evaporation of titanium/ palladium/ titanium (5/60/10 nm). Subsequently, electrodes were insulated with a layer of 65 nm $\text{Si}_3\text{N}_4$, deposited by plasma-enhanced chemical vapor deposition at 80$^\circ$C (ICP-PECVD, Axic), and a layer of 10 nm alumina, made by atomic layer deposition (ALD, Savannah 200 system, Cambridge Nanotech). The chip was then subjected to lift-off in PG remover (or N-Methyl-2-pyrrolidone). The
fabrication of the FET devices was ended by annealing in forming gas (hydrogen nitrogen ratio of 1/9), applied for 2 minutes at 380 °C by a rapid thermal processor ((AnnealSys, AS-Micro)).

Supplementary Figure 1. SiNW FET p-type array fabrication scheme: (a) dark-field image of the SiNW p-type FET device that was fabricated via photolithography. (b) Silicon wafer chip, 15mm×20mm with 600 nm thermal oxide layer, which contains 200 potential SiNW FET devices. (c) Scheme of the fabrication procedure of a SiNW FET device.

3. Electrical Characterization of SiNWs Devices Using Water-Gate

As an initial quality control, prior to the completion of the SiNW FET devices, the electrical properties of the SiNW devices on the sensor chip were characterized in deionized water by using a probe station.2 The measurement indicated only ~15% of the 200 potential devices on the chip were active. Characterization of the device's activity using source-voltage sweep, enabled selection of the source-drain voltage (Vsd) that allowed full-scale sensitivity. Scanning the gate voltages (Vg) over fixed source voltage allowed characterizing parameters such as carrier mobility,
transconductance, and threshold voltage. Based on these measurements (Supplementary Figure 2), the best performing devices were selected and mapped for future sensing applications.

Supplementary Figure 2. Electrical characterization of p-type SiNW FET nano-devices under water-gate configuration: (a) Plot of source-drain current versus source-drain voltage (Vsd) at different gate voltages (Vg). (b) Plot of source-drain current versus gate voltages (Vg) at 0.1 V source-drain voltage (Vd).

4. Scanning Electron Microscope Analysis

The synthesized p-type SiNWs and the SiNW devices were analyzed by Quanta 200 FEG environmental scanning electron microscope (ESEM). The images indicate good quality of the synthesized nanowires (Supplementary Figure 3a) and their successful assembly to FET devices (Supplementary Figure 3b).
Supplementary Figure 3. Scanning Electron Microscope (SEM) images of SiNWs: (a) SEM image of the synthesized 20 nm $p$-type SiNW via chemical vapor deposition system on silicon (100) wafer. (b) SEM image of SiNW FET device consisting of SiNWs connected to the source and drain electrodes.

5. Surface Modification

Following the fabrication of the SiNW FET array, the chip was chemically-modified with 9,10-anthraquinone-2-sulfochloride to enable the sensing of cellular metabolites. The modifying agent was pre-synthesized as follows:

5.1. Preparation of 9,10-anthraquinone-2-sulfochloride

The sulfonate group of sodium 9,10-anthraquinone-2-sulfonate (743038, Sigma-Aldrich) was converted to sulfochloride, using oxalyl chloride (O880, Sigma-Aldrich) and N,N-dimethylformamide (227056, Sigma-Aldrich) in toluene (244511, Sigma-Aldrich). A mixture of sodium anthraquinone-2-sulfonate (5 grams, 0.0158 mol) and toluene (150 mL) was placed in 250 mL round-bottomed flask, equipped with an automatic water separator (Dean-Stark trap) and condenser, and heated under reflux for 2 hours to dry the reaction mixture. The mixture was then cooled to 60 °C and oxalyl chloride (6 mL) and N,N-dimethylformamide (2 drops, ~100 µL) were added. The resulting mixture was heated under reflux for 8 hours and a mixture of toluene and oxalyl chloride excess (30 mL) was thereafter distilled. A precipitate of sodium chloride was collected by filtration and the solvent was removed from the filtrate under reduced pressure. A
solid residue was dried in vacuum overnight to give anthraquinone-2-sulfochloride (4.36 grams, 90 \% yield).

5.2. SiNW FET Array Surface Modification with 9,10-anthraquinone-2-sulfochloride

In order to conjugate the 9,10-anthraquinone-2-sulfochloride to the SiNWs surface, the FET chip was washed with acetone (9005-68, J.T.Baker), isopropanol (9079-05, J.T.Baker), and deionized water (18 M\(\Omega\)·cm) successively, followed by nitrogen drying. Then, oxygen plasma (100 W, 0.2 Torr) was applied for 15 minutes. The chip was covered by a glass dish and inserted into a glove box (150B-G, Mbraun) under the argon atmosphere (water and oxygen-free) to perform the aminosilane modification. Immediately afterward, the chip was covered with \(~150 \mu\text{L} (3\text{-aminopropyl})\text{-dimethyl-ethoxysilane (APDMES; SIA0603.0, Gelest) for 60 minutes. Then, the chip was washed twice with \(~30 \text{mL of anhydrous toluene (244511, Sigma-Aldrich). The chip was transferred from the glove box to the cleanroom and washed again with isopropanol, followed by nitrogen drying. Next, the chip was placed on a hot plate at 115°C for 25 minutes. Finally, the chip was immersed in a mixture, containing 50 mg 9,10-anthraquinone-2-sulfochloride, 20 mL anhydrous toluene (244511, Sigma-Aldrich) and 1 mL anhydrous pyridine (270970, Sigma-Aldrich), in the glove box, under argon atmosphere (water and oxygen-free) for 24 hours, for the formation of sulfonamide that connects the 9,10-anthraquinone groups to the SiNW modified surface.

6. Mass Spectra Analysis

Mass spectroscopy (Autospec M250Q, Waters Corp. USA) analysis of 9,10-anthraquinone-2-sulfochloride (used for the SiNWs surface modification) confirmed the sulfochloride group formation.
Supplementary Figure 4. Mass spectrum analysis of synthesized anthraquinone-2-sulfochloride: At a measurement mode of electron impact, using positive ionization of 70 eV. CH$_2$Cl$_2$ was used as a solvent. The mass-to-charge ratio (m/z) is normalized to the highest signal.

7. X-ray Photoelectron Spectroscopy Surface Chemistry Analysis

X-ray photoelectron spectroscopy (XPS) measurements were performed (Multi-Technique System 5600, PHI) in ultrahigh vacuum (2.5×10$^{-10}$ Torr base pressure). The sample was irradiated by Al K$_\alpha$ monochromated source (1486.6 eV) and outcome electrons were analyzed by a spherical capacitor analyzer using a slit aperture of 0.8 mm. Since samples were slightly charged during measurements, this input was corrected mathematically, with C$_{1s}$ at 285 eV taken as an energy reference. All the measurements were performed at a shallow take-off angle of 25º. High-resolution multiplex spectra were taken for different peaks in a low energy range window at a high
resolution (Pass energy = 11.75 eV, 0.05 eV/step). These measurements allow precise energy position, and peak shape determination, necessary for bonding analysis.

Supplementary Figure 5. Surface modification and XPS analysis of the redox-reactive SiNW FET: a. The stages of the surface modification: (1) Silanization of the SiNW activated surface with amine groups; (2) formation of a sulfonamide bond that connects between the 9,10-anthraquinone to the modified surface. b. XPS spectra and atomic compositions of the modified surface (for carbon (C), nitrogen (N) and sulfur (S)), following each step of the modification.
Supplementary Figure 6: Calculation of C=O bonds from XPS analysis. XPS representative survey spectra of the oxidized 9,10-anthraquinone-modified silicon nanowire surface (the blue curve) and reduced 9,10-dihydroxyanthracene-modified silicon nanowire surface (the red curve). The percentage of C=O bonds was calculated from C1s curve fitting. After the reduction of the surface, a decrease in the C=O bond population was observed.

8. Fabrication of Fluid-Delivery System

The fabrication of the fluid-delivery device from flexible polydimethylsiloxane (PDMS) elastomer was performed as previously described, with the following changes: The PDMS was incubated with curing agent at 10:1 mass ratio for overnight at 60 °C. The resulting device was then cut into rectangular pieces, at dimensions of 10×10×5 mm. Upstream polyethylene tube (PE 20,
Intramedic) was 14 cm long and had a 0.38 mm inner diameter. Downstream Tygon tube (S-50-HL, Tygon) was 13 cm long.

9. Electrical Measurements with Chemically-Modified SiNWs FET Devices

Based on their performance, as presented in the electrical characterization section, the selected devices were wire-bonded (using wire-bonder, model 8850, West Bond) and integrated into sensing array device chip using the aforementioned custom-made PDMS microfluidic channels (Supplementary Figure 7). A data acquisition system was used to measure the current of the SiNW FETs ($I_{ds}$), induced by surface charges alterations. The selected devices were examined for their performance in sensing buffer. Gate voltage sweep was used for transconductance measurements and the subsequent determination of the transistor regime of operation. A suitable gate voltage was further selected to perform all the following sensing experiments. Sensing experiments were performed by monitoring the conductance of the SiNW devices over time (current-versus-time signals were recorded at 1-second intervals), during injection of the analytes to the sensing chip by a syringe pump (Fusion 200, Chemyx) via the microfluidic system.4, 5

Supplementary Figure 7. The complete sensing system: including the SiNWs chip, wire-bonded to the PCB holder, which is connected to the electrical 'current recording' system (FES-SM32P). The microfluidic channel is placed directly above the SiNWs array, on the chip surface, and attached via clear Perspex sheet and screws.
References


