Supporting Information for:

Label-Free Pathogen Detection with Peptide Nanotube-Assembled Sensor Chips

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Contents:

I. Experimental in general.

The peptide nanotubes, self-assembled from the bolaamphiphile peptide monomer, bis (*N*-α-amidoglycylglycine)-1,7-heptane dicarboxylate, were used as templates to immobilize antibodies on the nanotube surfaces.[1,2] Previously the surface of the peptide nanotube was engineered to incorporate a variety of biomolecules via hydrogen bonding,[3-5] and in the present work peptide nanotubes were coated by antibodies with the same procedure. An aqueous solution containing the peptide nanotubes, 400 μl, was centrifuged for 1 hour at 14,000 rpm and suspended in 400 μl of sheep polyclonal anti-HSV-2 (0.1 mg/ml, Abcam), which coated the peptide surface via hydrogen bonding. After overnight incubation at room temperature, 10 μl of bovine serum albumin (BSA, 40 mg/ml) in PBS buffer was added in order to block uncoated sites and prevent nonspecific adsorption of viruses to the nanotube surface. After 1 hour, the resulting nanotubes were washed with fresh PBS buffer supplemented with 0.05% sodium azide as preservative. PBS buffer (0.01 M phosphate buffer, 0.0027 M KCl and 0.137 M NaCl, pH 7.4 tablets, Sigma) was prepared by following the manufacturer's instructions.

Then, a solution containing the antibody-coated peptide nanotubes was centrifuged and washed with ultrapure water, and a 0.3 μl drop spotted onto the electrodes. A 10 Hz, 5V peak-to-peak potential AC field was applied for focusing the peptide nanotubes. Upon drying, the dielectrophoretic force and physicochemical interactions trapped the nanotube between the electrodes, as confirmed by optical microscopy and atomic force microscopy. Prior to the immunoassay, all the peptide nanotube sensors were immersed in a BSA-PBS (10 mg/ml) solution for 1 hour to prevent nonspecific adsorption of the viruses to the chip surface. Next, a solution containing the HSV-2 (10^1 - 10^4 pfu/ml) was incubated with the sensor platform for 1 hour. Tittered aliquots of HSV-2, graciously donated by Dr. Andrea Bertke (FDA/CBER), were diluted with PBS-sodium azide buffer to the desired final concentration.

For each immunosensor, impedance spectra were taken from 10 KHz to 1 MHz, 10 mV AC peak-to-peak potential in glycine buffer (250 mM) with a SI 1260 SOLARTRON impedance analyzer. The resulting impedance spectra were fitted to a suitable equivalent electric circuit with the Zview2 software in order to estimate the contribution of the capacitance of the solution to the overall impedance.[6] Details

about the equivalent circuit and the impedimetric analysis are described in the next section II. To remove the influence from ambient factors, nonspecific interactions, and parasitic electric components from the biosensor response in the virus detection, we subtracted the measured capacitances from the values obtained with control nanotube sensors, where the nanotube was coated with mouse IgG that did not bind HSV-2 as shown in Figure 2-(f).

II. Electrode fabrication and layout.

The chips containing the electrodes were prepared using standard microfabrication on silicon. First, the wafers were wet oxidized to grow 650 nm of a silicon oxide insulating layer on top of the silicon wafer. Then, the electrodes were prepared by photolithography using the lift-off process after evaporating 5 nm of Ti as seed layer and 20 nm of Au as the electrode material.

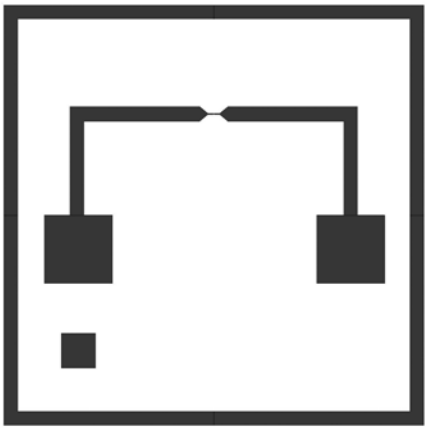

Figure S1. Electrode layout.

III. Equivalent electric circuit and impedimetric analysis.

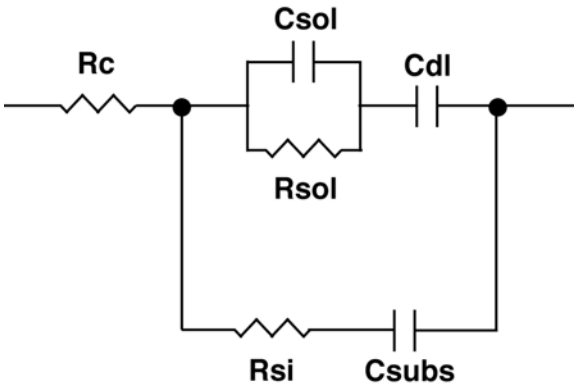

Figure S2. Complete electric circuit model for the antibody nanotube-assembled sensing platform.

The whole equivalent circuit for the system under study is depicted in Figure S2. The components of this circuit are:

- R_c is the resistant of the wire bonds.
- R_{sol} is the resistance of the aqueous media around the antibody nanotube, which is proportional to the resistivity of the aqueous media around the antibody nanotube between the electrodes.
- C_{sol} is the capacitance of the aqueous media around the antibody nanotube, which is proportional to the permittivity of the aqueous media around the antibody nanotube between the electrodes.
- C_{dl} is the capacitance of the interface between the gold electrodes and the solution.
- C_{subs} is the capacitance between the electrodes and the silicon substrate whose value depends on the thickness of the silicon oxide layer between the electrodes and the silicon wafer.
- R_{si} is the resistance of the silicon wafer

In the case under study, impedance spectra were obtained in the high frequency range (1 MHz to 10 kHz). In this frequency region, the resistance and the capacitance of the solution in the equivalent circuit dominate impedance spectra and therefore the circuit model can be reduced to the following (Figure S3):

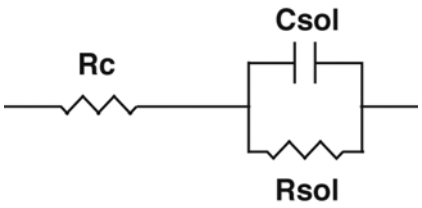

Figure S3. Simplified electric circuit model for the antibody nanotube-assembled sensing platform in the AC frequency range of 1 MHz to 10 kHz.

In order to verify this model, impedance spectra were taken in three different media on the peptide nanotube platform: air, water and ethanol (Figure S4, Table S1). The resulting data was fitted with Zview2 software using the simplified model in Figure S3. In the case of measuring in air, which is a pure dielectric, the obtained values corresponded to the parasitic components of the circuit (C_{sol} is C_{subs}). When the spectrum was taken in water, a higher value of the capacitance was obtained, as expected from a high permittivity medium. When it was tested in ethanol, both the capacitance and the resistance of the solution decreased, as expected from a solvent with a lower permittivity and ion content.

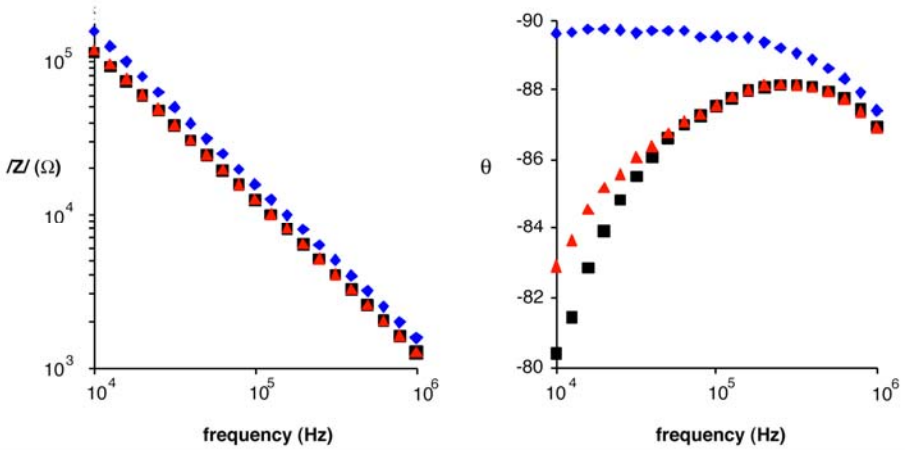

Figure S4. Bode plots of impedance spectra taken in different media; blue (♦): air, red (▲): ethanol, black (■): water.

	R_c (Ω)	C_{sol} (pF)	R_{sol} (kΩ)
air	80	100	-
water	90	130	680
ethanol	100	126	900

Table S1. Fitted values of spectra in Figure S4 by using the simplified model depicted in Figure S3.

It is noteworthy to recall that, in the system under the study, the specific response of the sensor was obtained by subtracting the fitted values of the control sensor from the sensor that contains the peptide nanotube with the recognition element. By this subtraction, we eliminated all the contributions of the parasitic components (R_{si}, C_{subs}) from the model circuit since they only depend on the layout of the electrodes and their value is the same for all of these sensors. Moreover, because the biomolecular recognition element is placed on the nanotube and not on the electrode surface, the subtraction also eliminates the contribution from the electrode-solution interfacial capacitance, C_{dl}, characteristic at lower frequencies.

References

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