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Supplemental information

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SUPPLEMENTARY FIGURES

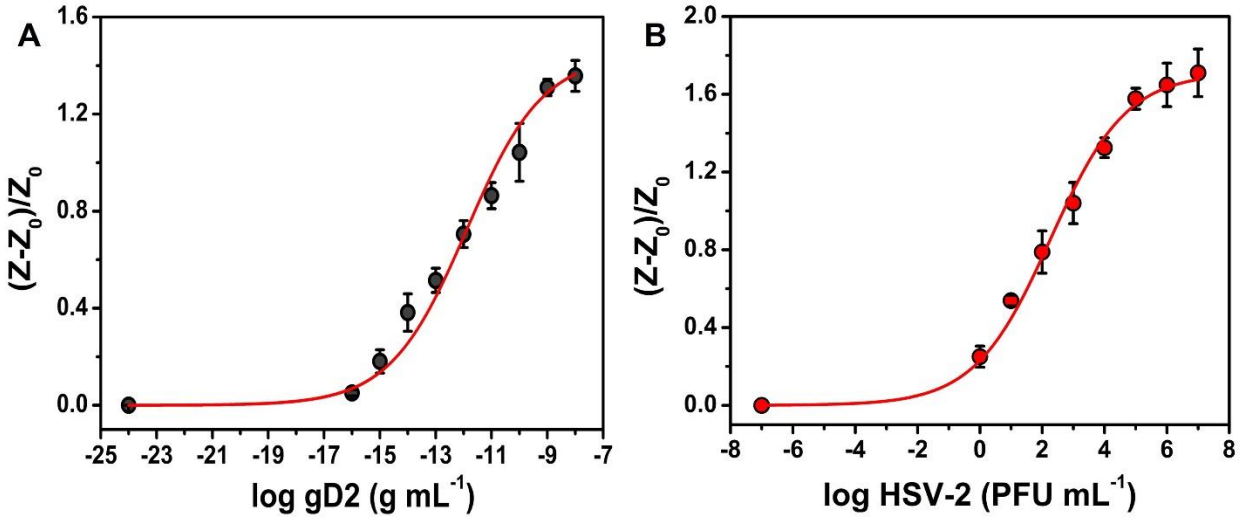

Figure S1: Normalized analytical curves plotted to calculate the limit of detection using the four-parameter logistic 4PL method. **A)** Dose-response curve obtained by normalizing RCT values extracted from Nyquist plots as a function of the logarithm of the gD2 concentration. **B)** Dose-response curve obtained from normalized RCT values extracted from Nyquist plots as a function of the logarithm of the HSV-2 viral loads. The EIS measurements were carried out in triplicate in 5.0 mmol L^{-1} $[Fe(CN)_6]^{-3/-4}$ and a 0.1 mol L^{-1} KCl solution applying the open circuit potential at a frequency range of $1x10^5$ Hz to 0.1 Hz and using an amplitude of 10 mV. All measurements were recorded using 10 µL of gD2 or HSV-2 samples (ranging from $1x10^0$ PFU mL^{-1} to $1x10^7$ PFU mL^{-1}) and incubated for 5 minutes on the biosensor surface. The error bars correspond to the standard deviation.

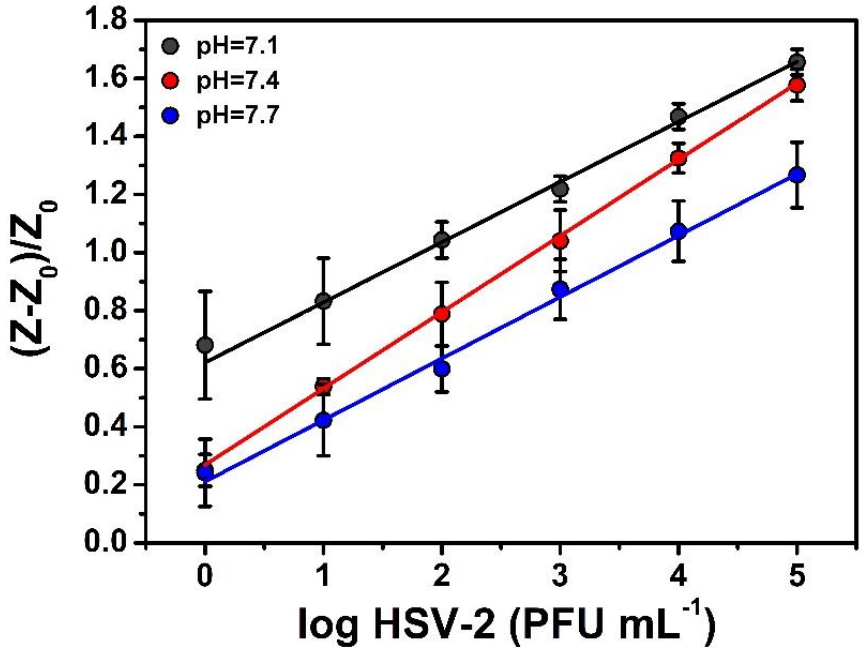

Figure S2: Effect of pH on the analytical response of the nectin-1 biosensor. Experiments were performed in DMEM medium with pH values adjusted to 7.1, 7.4, and 7.7. The dose-response curves were recorded in triplicate at a concentration ranging from $1x10^0$ to $1x10^5$ PFU mL^{-1}. The HSV-2 sample at pH= 7.1, 7.4, and 7.0 presented analytical sensitivity values of 0.212 ± 0.008, 0.263 ± 0.003, and 0.207 ± 0.008, respectively. The EIS analyses were recorded in a medium containing 5.0 mmol L^{-1} $[Fe(CN)_6]^{-3/-4}$ and 0.1 mol L^{-1} KCl solution at a frequency ranging from $1x10^5$ Hz to 0.1 Hz and 10 mV of amplitude. Ten µL of each HSV-2 sample was incubated on the biosensor surface for 5 minutes before each EIS measurement. The error bars correspond to the standard deviation.

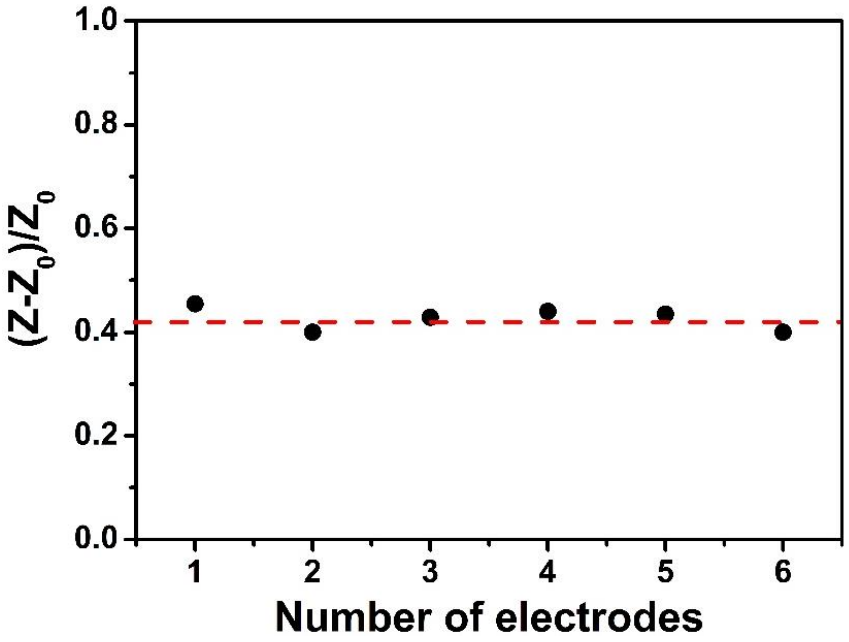

Figure S3: Reproducibility study. Analytical response ((Z-Z0)/Z0) obtained for 6 different biosensors (from different fabrication batches) when incubated with $1x10^{-9}$ g mL^{-1} of gD2 prepared in 0.1 mol L^{-1} PBS (pH = 7.4) for 5 minutes. A relative standard deviation (RSD) of 5.12%, indicative of excellent reproducibility, was obtained to assess the fabrication and functionalization method. The error bars correspond to the standard deviation.

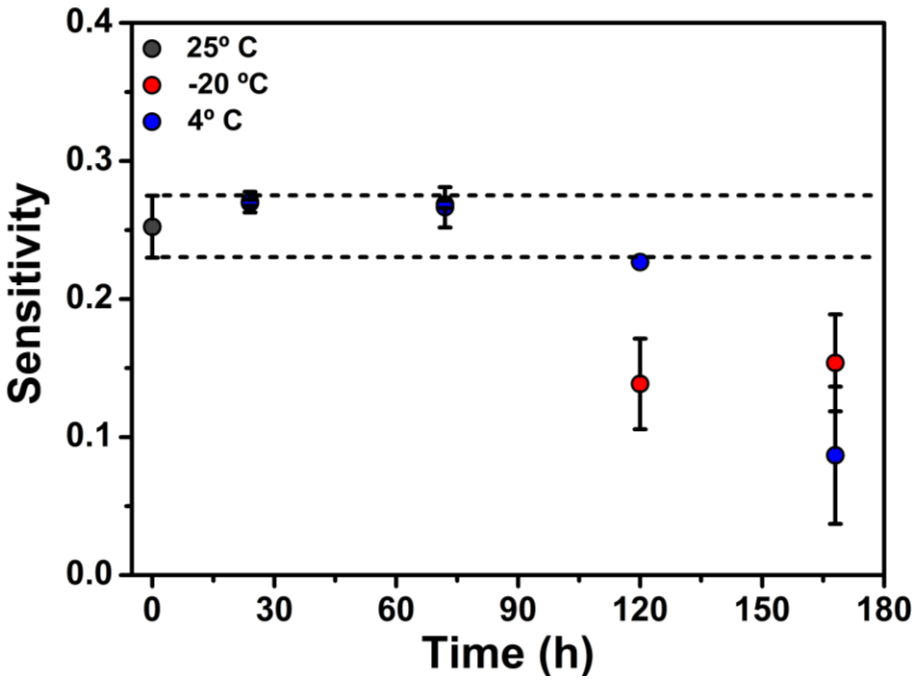

Figure S4: Stability assays. Biosensor stability at different temperatures was assessed by extracting the analytical sensitivity parameters from dose-response curves recorded in triplicate. Electrodes were stored for 7 days at: 25 ºC (black circles), -20 ºC (red circles), and 4 ºC (blue circles). The sensitivity values were obtained by analytical curves at concentrations of gD2 ranging from $1x10^{-12}$ g mL^{-1} to $1x10^{-10}$ g mL^{-1}. All experiments were performed in triplicate (3 different biosensors) in 5.0 mmol L^{-1} $[Fe(CN)_6]^{-3/-4}$ prepared in 0.1 mol L^{-1} KCl medium at a frequency window between $1x10^{5}$ Hz and 0.1 Hz and 10 mV of amplitude. A volume of 10 µL of HSV-2 was used in each case, and samples were incubated for 5 minutes on the surface of the biosensor prior to conducting EIS measurements. The error bars correspond to the standard deviation.

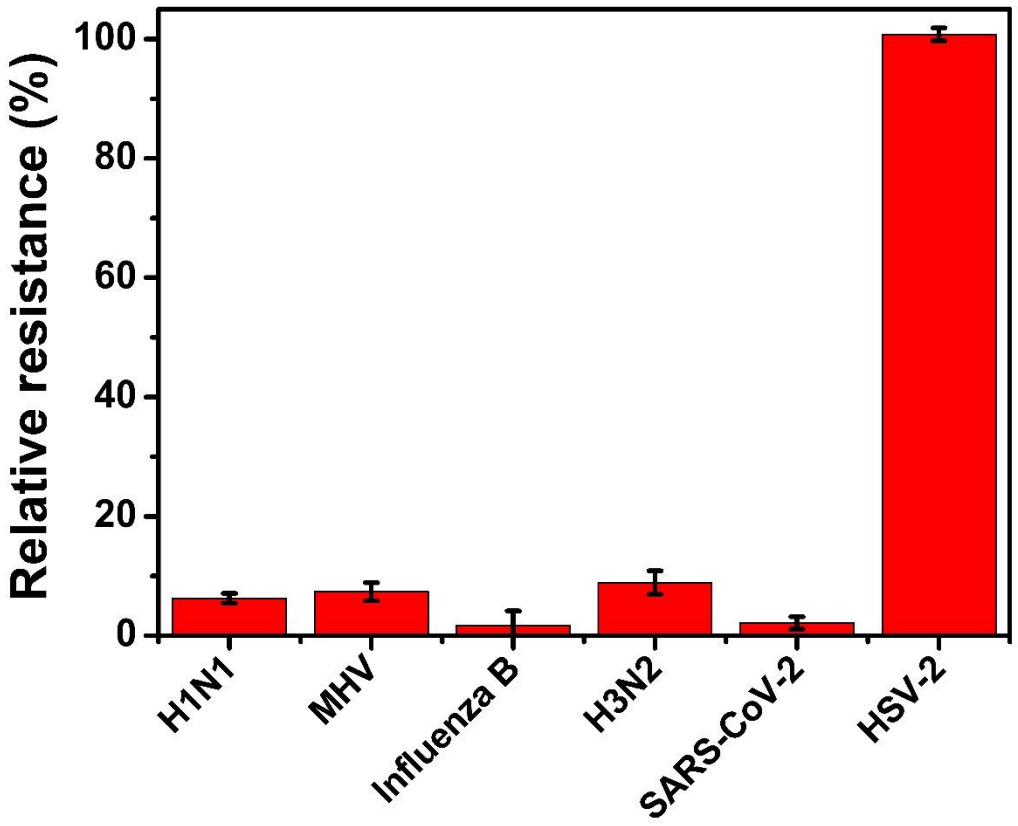

Figure S5: Selectivity study of the biosensor. Cross-reactivity assays were performed to assess the selectivity of the biosensor for HSV-2 as opposed to other viruses. The following viruses were used in our studies: H1N1 strain A/California, MHV – mouse hepatitis virus, Influenza B – B/Colorado, H3N2 – A/ Nicaragua, SARS-CoV-2, and HSV-2 – herpes simplex virus-2. All viruses were at concentrations of 10^5 PFU mL^{-1} except for MHV, which was at 10^8 PFU mL^{-1}. Experiments were recorded using 5.0 mmol L^{-1} $[Fe(CN)_6]^{-3/-4}$ in 0.1 mol L^{-1} KCl medium at a frequency range between $1x10^5$ Hz and 0.1 Hz and an amplitude of 10 mV. Ten µL of each viral sample was deposited on the WE and incubated for 5 minutes prior to performing EIS measurements. The error bars correspond to the standard deviation.