Electronic Supplementary Material (ESI) for RSC Advances.


# Supporting information for: Electronic platform for real-time detection of bovine serum albumin by means of amine-functionalized zinc oxide microwires

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# Measurement system characterization

## Long term stability

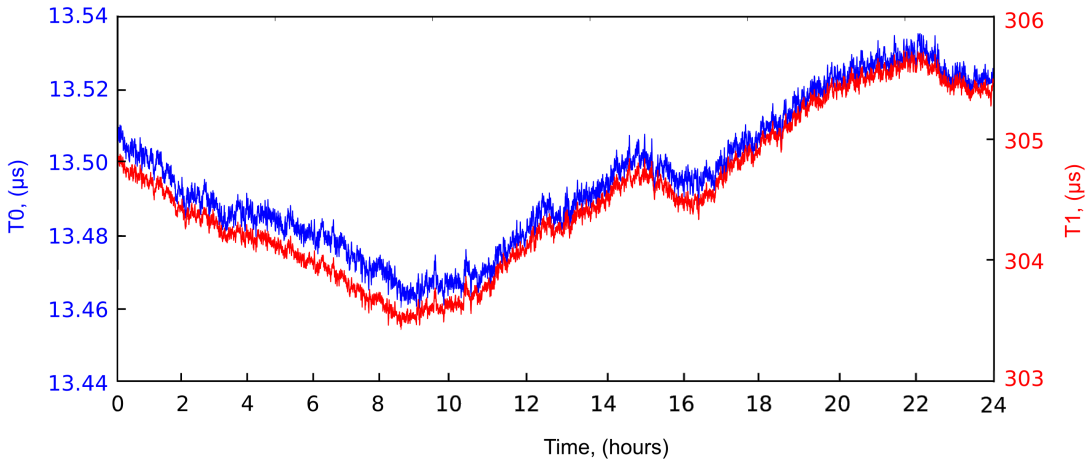


Figure S1: 24 hours measurement of a discrete RC component (R = 20 MΩ, C = 100 pF) to evaluate the ROC stability over time

As described within the paper, the Read-Out Circuit (ROC) was used to monitor in real-time the equivalent resistance and capacitance of the ZnO microwire assembled onto gold-electrode nanogap device. In particular, the circuit converts the electrical parameters (i.e., $R_{\mu wire}$ and $C_{\mu wire}$) to the time domain using a 1-bit output signal, so that the time $T_0$ is proportional to $C_{\mu wire}$ and $T_1$ is proportional to $R_{\mu wire}$. The BSA molecules creating chemical links with the functionalized ZnO microwire change the measured $C_{\mu wire}$.

The system was thus characterized for long measurement sessions in order to evaluate termal drift or ultra-low frequency noise. A 100 pF ceramic SMD capacitor, in parallel with a 20 MΩ SMD resistor, was used to emulate a ZnO microwire. The results reported on Fig.S1 refer to a 24-h measurement session and show that $T_0$ and $T_1$ have a maximum deviation from their average values of about 40 ns and 1.5$\mu$s, respectively. The correspondent percentage errors are 0.3% and 0.5%, respectively. In that case, considering that the ROC sensitivity $C_{\mu wire}/T_0$ is about 0.1 pF/µs, the error due to termal drift or ultra-low frequency noise can be estimated around 400 fF. All details about the chip can be found in reference [8] in the main text.

## Calibration curve

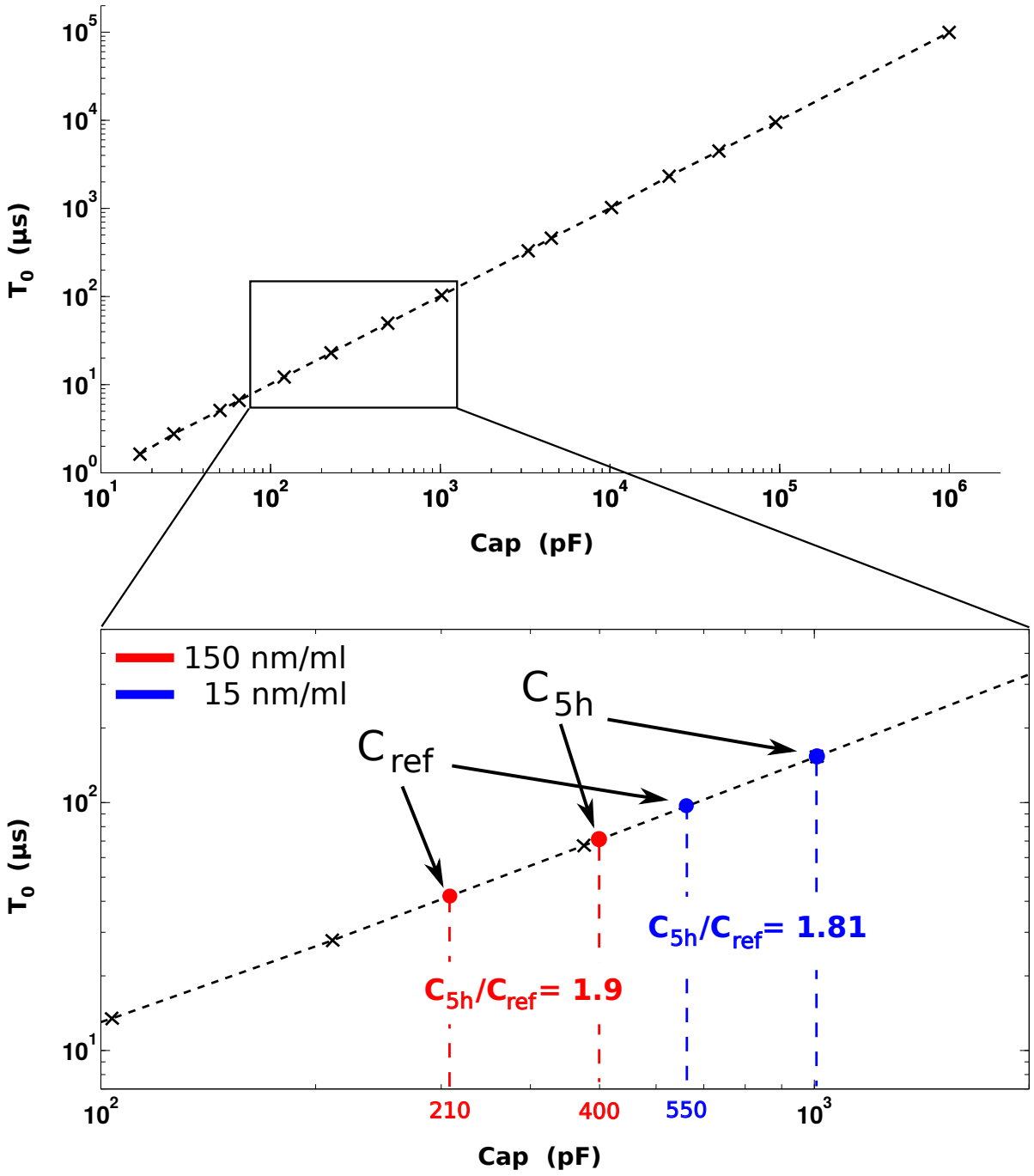


Figure S2: M4N capacitance range of measurement. In the magnification, red and blue capacitances correspond to 150 nmol/ml and 15 nmol/ml experiment respectively. Every concentration measurement is relative to its reference value.

Figure S2 reports the measured capacitance range (10pF - 1µF) of the system. In the magnifi-

cation, four capacitance values are placed on the curve, two for the 15 nmol/ml and two for the 150 nmol/ml BSA concentrations. We calculate two capacitances because every microwire is different so the first value is used as baseline while the second as percentage increase after a fixed amount of time. In this way, we can see that the relative capacitance increase in the case of 150 nmol/ml is bigger than the 15 nmol/ml even if absolute values indicate the opposite.

## Custom PCB details

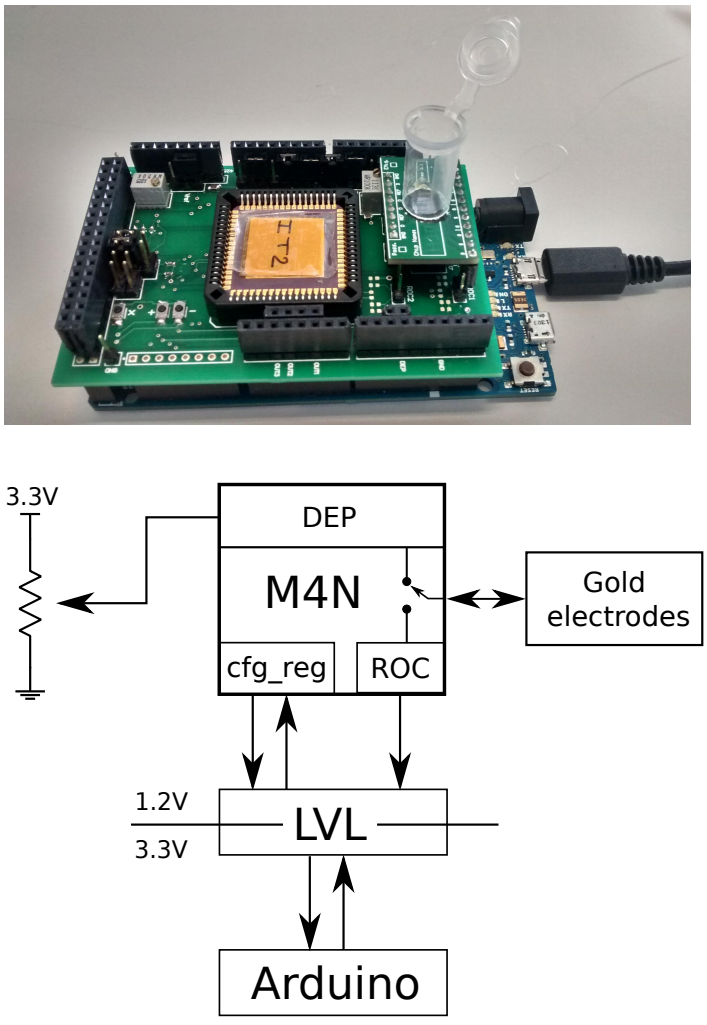


Figure S3: Picture and block diagram of the interface board. The M4N chip communicates with Arduino board through a voltage level converter (LVL) that adapts the 1.2 V chip logic to the 3.3 V Arduino logic. A potentiometer is also present to vary the DEP frequency.

Figure S3 shows a block diagram of the custom PCB on which M4N chip and gold microelectrodes are mounted. A potentiometer is connected to an input of the chip to let the user changing the DEP frequency in the range of 50 kHz - 1 MHz. Since the microelectronic chip is supplied at 1.2V by a low dropout voltage regulator on-board, the outputs of the M4N chip are not directly compliant with Arduino, which uses a 3.3V logic. Hence, additional voltage level converters (LVL) are required. The rest of the PCB are connectors that bring Arduino I/Os in the upper PCB for any possible use.

## Control experiment

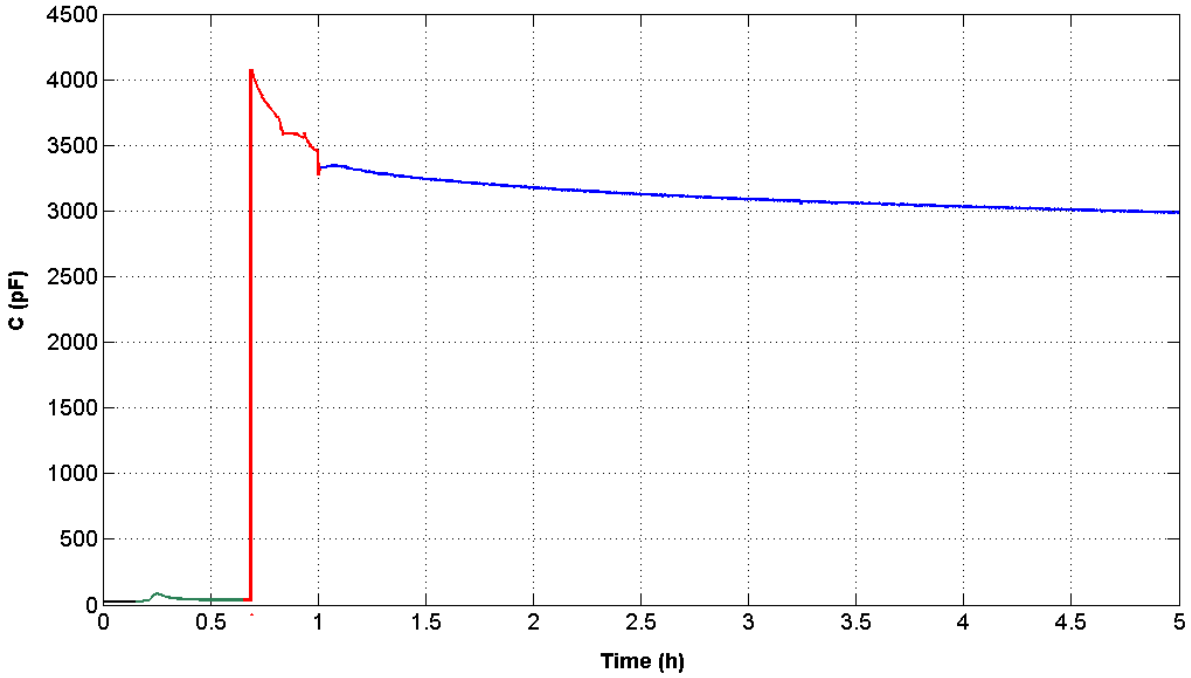


Figure S4: Real-time measurement of the ZnO capacitance control experiment. ZnO was not functionalized with $NH_2$ and it was measured following the same steps of ZnO-$NH_2$ experiment. In this case, it was not registered any capacitance increase after the BSA addition (blu segment).

Figure S4 shows a control experiment in which ZnO microwire was not functionalized with $NH_2$ and it was subjected to the same procedure to check any possible source of error. As expected, the non-functionalized microwire behaved in the same manner of the functionalized ones until both

the EDC and Sulfo-NHS reagents addition. When the BSA is added no reaction occurs to the ZnO surface and thus no capacitance change is detected from the ROC circuit. This confirms the validity of the proposed mechanism.

## Estimation of the protein amount

In an attempt to quantify the available reacting sites on the ZnO microwire surface, we estimated by thermogravimetric analysis and nitrogen sorption measurements, as reported in (Cauda et al., 2014), the amount of amino groups anchored to the surface. In details, by taking into account the value of specific surface area of ZnO wires (0.96 $m^2/g$), the estimated maximum density of amino-groups is about 1.78 molecules/$nm^2$. In order to estimate the amount of BSA protein coupled to the ZnO-$NH_2$ surface, we run a batch EDC amidation reaction for 20 hours at room temperature using the functionalized microwires in powder form and a BSA protein concentration of 15 nmol/ml (as in the real time experiments). The thermogravimetric analysis (Figure S5) was obtained from a Netzsch STA 440 Jupiter thermobalance (heating rate of 10 K/min in a stream of synthetic air of about 20 ml/min) after the ZnO wires were thoroughly washed to remove the unadsorbed protein and dried in air at room temperature.

It is visible from the red curve that the weight loss of the ZnO-$NH_2$ wires coupled with the BSA protein is noticeably higher with respect to the sample with ZnO-$NH_2$, confirming the effective protein coupling to the ZnO-$NH_2$ surface. The first derivative of the thermogravimetric curve (DTG, red dot curve) shows two peaks at 320 °C and 500 °C, both indicative of the loss of organic materials. The weight loss in this temperature range can be generally attributed to the materials adsorbed or anchored to the ZnO surface, including the amine-functionalizing agent and the BSA protein. The loss of amino-propyl functional groups is indeed as also visible from the black dot curve at 320-350 °C concerning the ZnO-$NH_2$ wires without protein. By subtracting the contribution of amino-propyl groups estimated in the ZnO-$NH_2$ to the weight loss of the sample coupled with the BSA protein, we calculated that the amount of protein coupled to the ZnO-$NH_2$

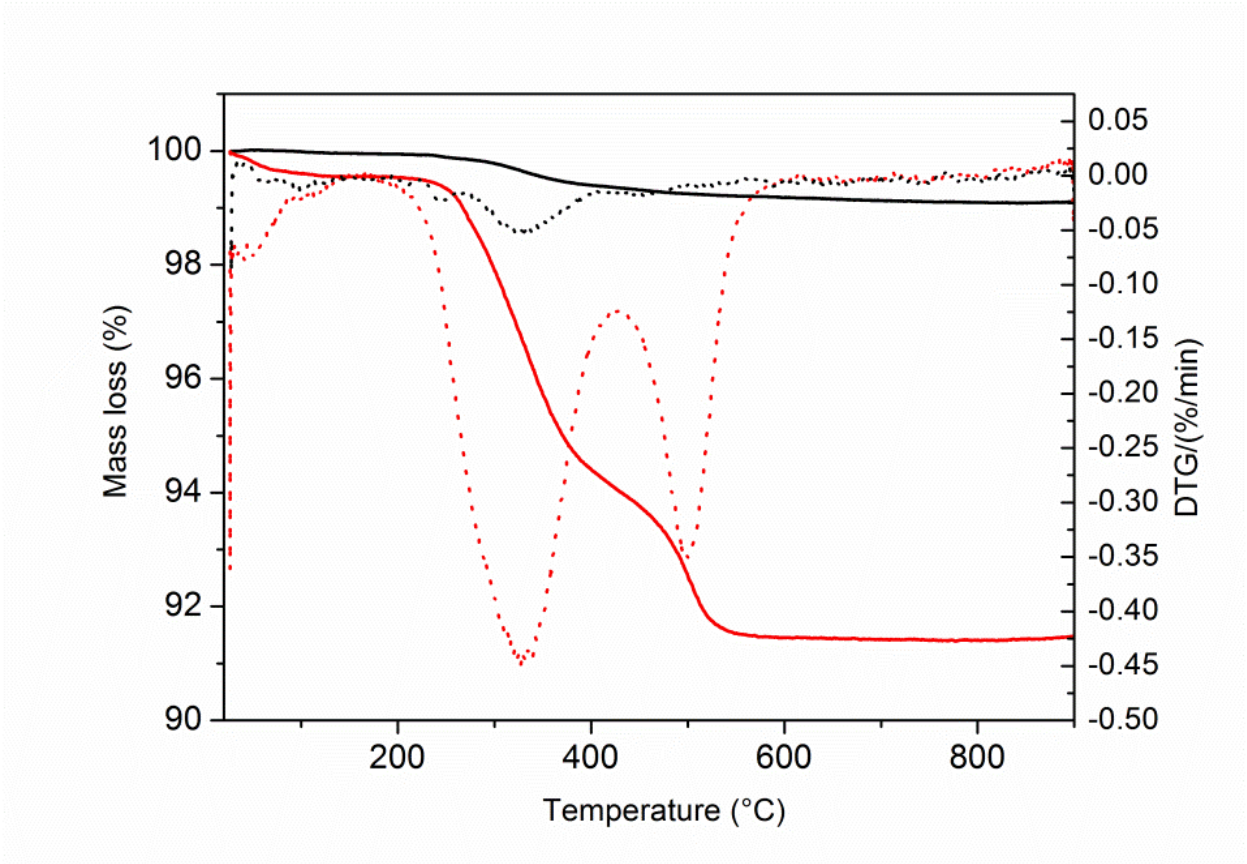


Figure S5: Thermogravimetric analysis on the amino-functionalized ZnO wires (black lines) and the ZnO-$NH_2$ after coupling with the BSA protein (red lines).

surface is 0.75 molecules/$nm^2$. Thus, in absence of any other organic contamination (being the sample thoroughly washed after the conjugation step in batch) one could assume this value as the maximum amount of protein anchored to the $-NH_2$ groups of the ZnO surface. This is reasonable if compared to the number of available amino-reacting sites (1.78 molecules/$nm^2$) and considering the steric hindrance of the bulky BSA protein. This estimation referred to our real-time experiment is the maximum final value that can be thus achieved at the end of the conjugation experiment when reaching the curve plateau (i.e. after 5 hours of reaction).