Immunoassay using a biofunctionnalized alumina-coated capacitive biosensor: towards a microfluidic detection of the H5N1 Influenza virus

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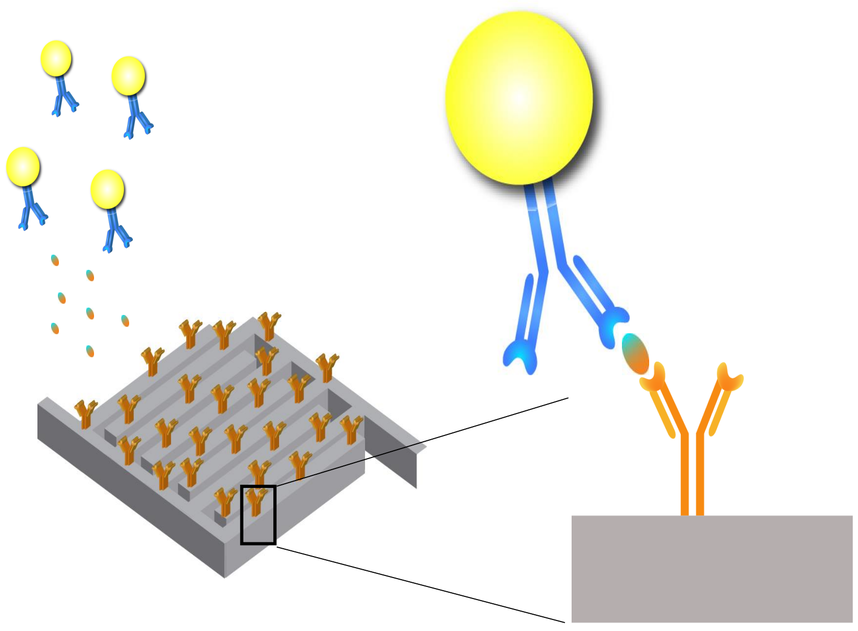
Introduction

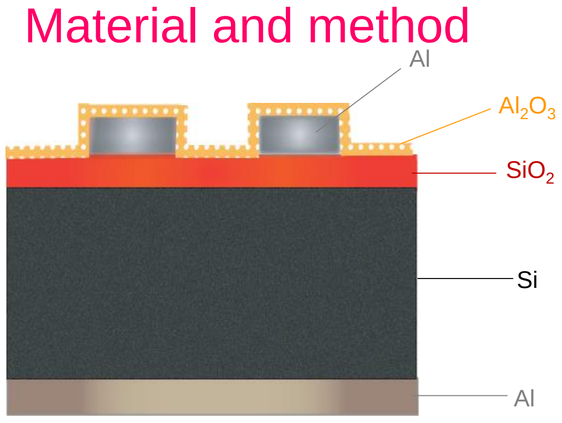
Influenza A viruses cause annual epidemics and occasional pandemics that spread worldwide. The nucleoprotein is essential for the survival of this virus and is thus well-conserved. We developed a quantitative electronic biosensor displaying a protein recognition thanks to the covalent grafting of anti-nucleoprotein antibodies. The detection device lies on interdigitated array microelectrodes (IDAM), covered with alumina (Al_2O_3) to protect the underlying aluminum and enhance the electrical coupling. As the dimensions of the IDAM can determine the sensitivity of the sensor, we processed 4 structures with varying electrode widths and spacings in the same silicon chip and assessed their individual performance. The sensing area of each sensor is 200*200 μm^2 and the overall chip-size is 3*3 mm^2. The sensor is mounted in a DIL-16-package partially encapsulated by resin.

Principle of the detection

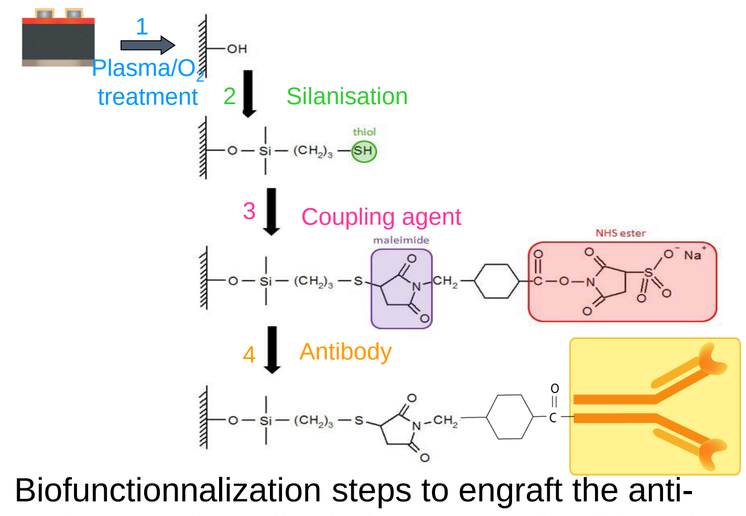


The IDAM is covered with a specific antibody recognizing the nucleoprotein of the Influenza A virus (capture antibody). The variation of the capacitance between the fingered electrodes is emphasized by the addition of a specific antibody conjugated with a 40 nm gold nanobead (detection antibody).

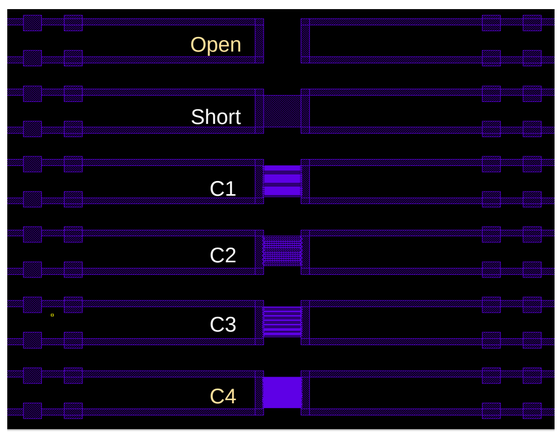
Material and method



Composition of the home-made processed IDAM.



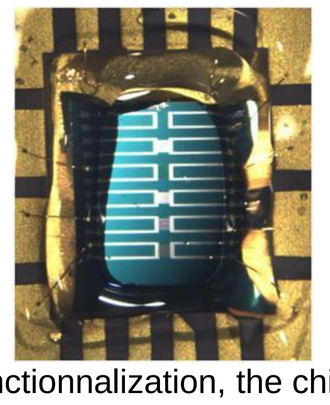
Biofunctionnalization steps to engraft the anti-nucleoprotein antibody (capture antibody) to the biosensor surface.



Sensor	Finger width (µm)	Interspace (µm)
C1	2	4
C2	10	2
C3	5	2
C4	2	2

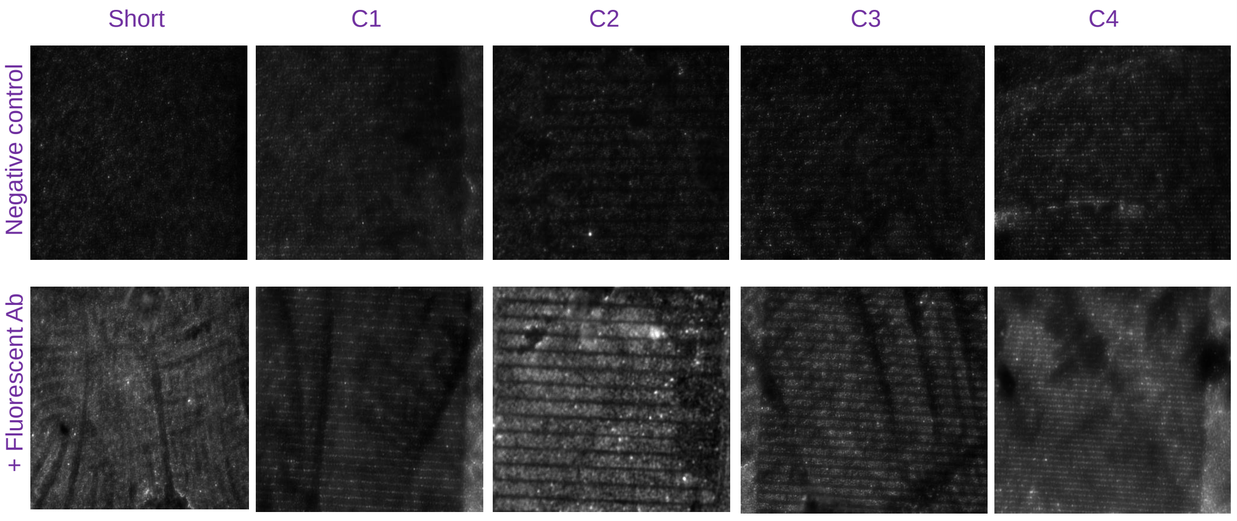
Structure of the chip: 4 different interdigitated electrodes and 2 calibration structures are displayed.

Each sensor has different size features as presented in the table above.



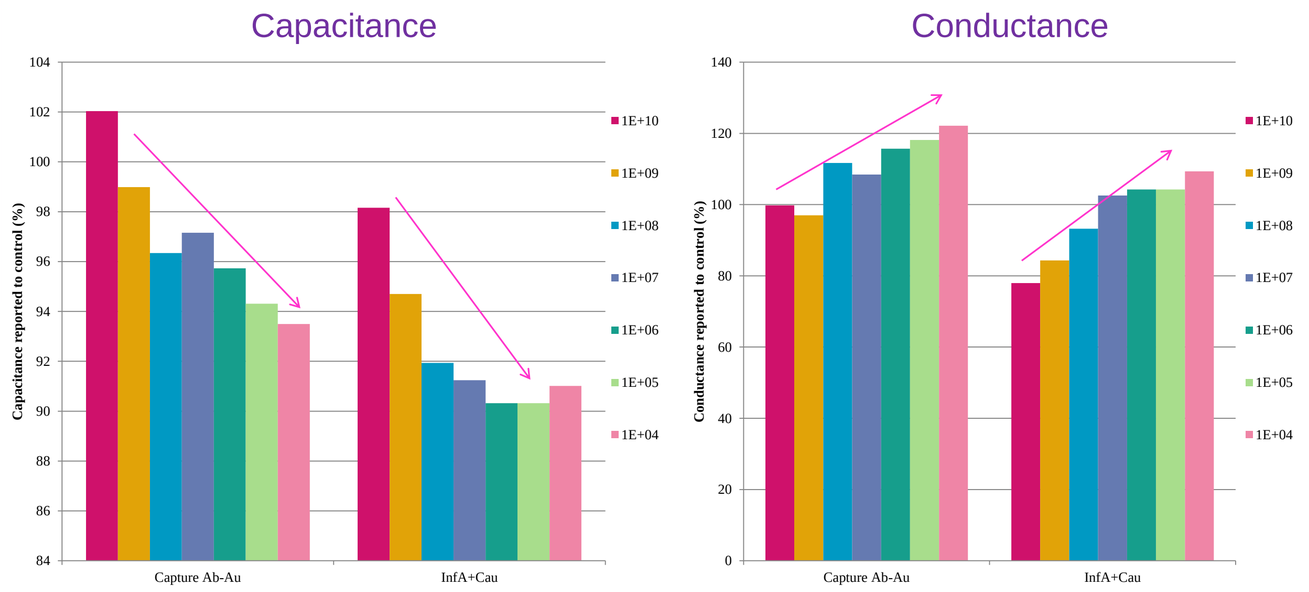
After biofunctionnalization, the chip was encapsulated in a 16-DIL package with an Epoxy resin designing a well for the deposition of the sample solution prior to the electrical measurements.

Surface analyses (confocal microscopy)



Confirmation by confocal microscopy of the engraftment of the capture antibody: the signal of the fluorescent marker was observed on every functionnalized structure of the chip while a weak fluorescence was distinguishable on the non-functionnalized chip.

Electrical measurements



Capacitance and conductance measurements reported to the control (PO_4 buffer; 20 mM, pH 8)(sensor C4, freq= 100 kHz) : the measurements were performed with a LCR meter with different bias voltages. Different dilutions of an antibody conjugated with gold bead, directed against capture antibodies (initial concentration= 10^{13} beads/mL) or different dilutions of the Influenza A virus were tested. For the measurements of the Influenza A virus, the signal was enhanced with an anti-Influenza antibody conjugated with a gold nanobead. The same trend was observed for both targets.

Conclusion

In this study, we assume the possibility to use an interdigitated capacitive biosensor for the quantification of the Influenza A virus. We successfully engrafted a capture antibody at the surface of the alumina-covered sensor before testing dilutions of gold-conjugated antibody directed against capture antibody or of Influenza A virus enhanced by a gold-conjugated detection antibody. The measurements of capacitance and conductance of the two different targets were coherent showing the robustness of the biosensor. Of all sensors tested the sensor C4 (fingers of 2 µm spaced of 2 µm) was the more efficient. The system could detect down to 10 000 gold-nanobeads coupled with antibodies. Moreover, a detection of a 10^{10} times dilution of the Influenza A virus was still possible with our system while a commercial immunochromatographic test could only detect a 10^4 times dilution. Together, these data offers new perspectives for cheap and portable biomedical diagnostic systems.