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Characterization of SiNRs in microfluidic channels for the detection of sub-500 Da molecules in solution

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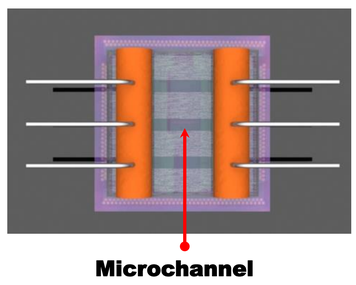
Introduction

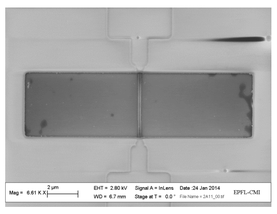
Ion-sensitive field-effect transistors (ISFETs) based on silicon nanoribbons (SiNRs) were characterized in wet environment, exhibiting MOSFET-like behavior with I_{ON}/I_{OFF} ratio of 10^4 and subtreshold swing (SS) of 100 mV/dec. The ability of sensing uniformly distributed targets has been demonstrated by measuring the concentration of hydrogen ions (pH). Preliminary results show that, when coupled to aptamer technology, SiNRs can be used to sense very small molecules, like the antibiotic tobramycin (molecular weight: 467.5 Da), with potential applications in personalized medicine.

Microfluidis

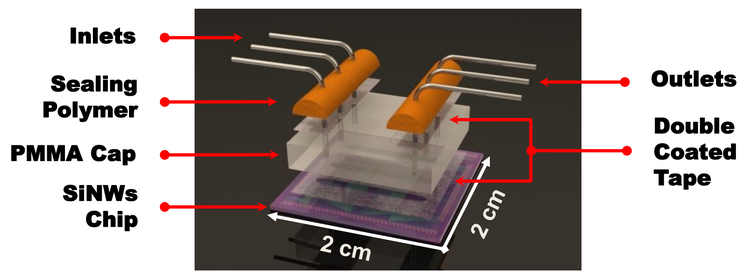


SiNRs Sensors

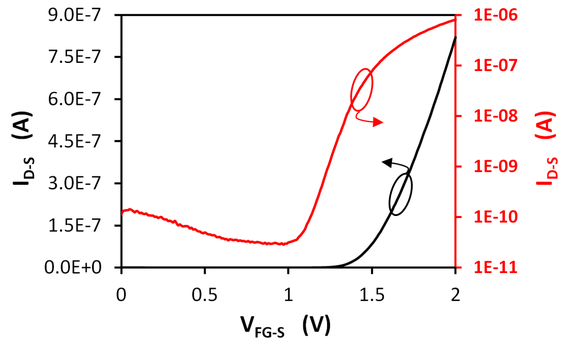
The SiNRs are fabricated on Silicon-On-Insulator (SOI) wafers and defined by means of standard top-down CMOS compatible processes, such as Deep Ultraviolet (DUV) photolithography, e-beam lithography and Reactive Ion Etching (RIE)[1].



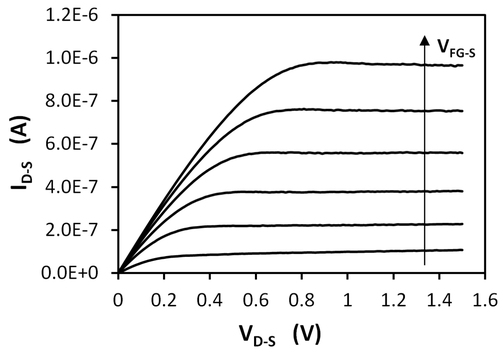
The developed microfluidic setup allows reduced solvents and reagents consumption, portability and ease of integration with the SiNWs chip.
The microchannels are realized with a chemical resistant double-coated tape, patterned by laser micromachining. The height of the channels are defined by the thickness of the tape (190 µm). A PMMA cap is placed to seal the channels and the inlets and outlets tubes inserted. A sealing polymer is used to avoid fluid leakages from the inlets/outlets.



Electrical characteristics



Drain current (I_{D-S}) versus gate voltage (V_{FG-S}) plotted in linear (left axis) and logarithmic (right axis) scale.

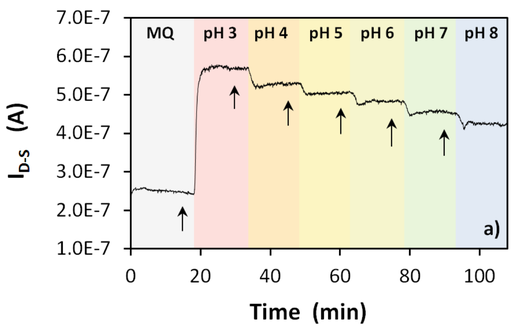


Drain current (I_{D-S}) versus drain voltage (V_{D-S}) plotted for different constant gate voltages (V_{FG-S}), ranging from 1.5 V to 2.0 V, with a step of 0.1 V.

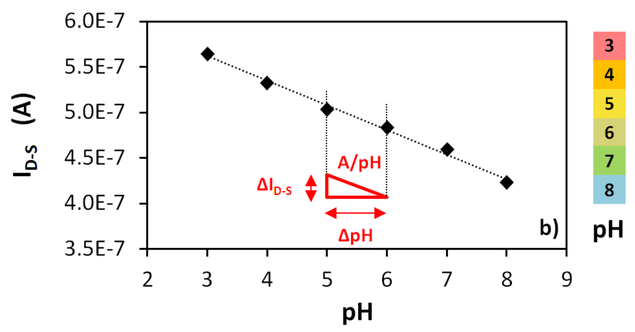
The following parameters of SiNRs in wet environment at room temperature can be extracted: a threshold voltage of ~ 1.55 V, an I_{ON} / I_{OFF} ratio of ~ 10^4 and a subthreshold swing of ~ 100 mV/dec.

Influence of pH

In order to demonstrate the ability of the SiNRs to sense molecular charges in the close proximity of the exposed SiNR. The change in the drain current upon injection of buffer solutions with different pH values is monitored in real-time. In an n-type FET, increasing the pH leads to depletion of electrons carriers in the SiNR channel, with a decrease of its conductivity.



Real-time drain current (I_{D-S}) measurement upon injection of solutions with different pH, and constant ionic strength.

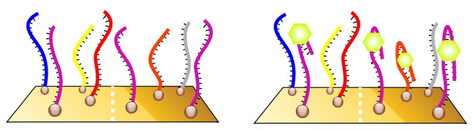


Current value (average over injection time) vs pH and corresponding linear interpolation ($R^2 = 0.99$).

The arrows indicate the starting of the injections while the different regions on the plot indicate the time frame during which the SiNR is in contact with the previously injected solution (delay time due tubings). The different pH solutions were prepared with 10 mM phosphate buffers (PBS) containing 100 mM KCl, which was added to stabilize the Ag/AgCl RE. The first injection of ultrapure deionized water (MQ) defines a baseline and shows the influence of ionic strength on the measurement (pH_{MQ} ~ 6.8). The flow rate was kept fixed at 5 µl/min during the whole experiment.

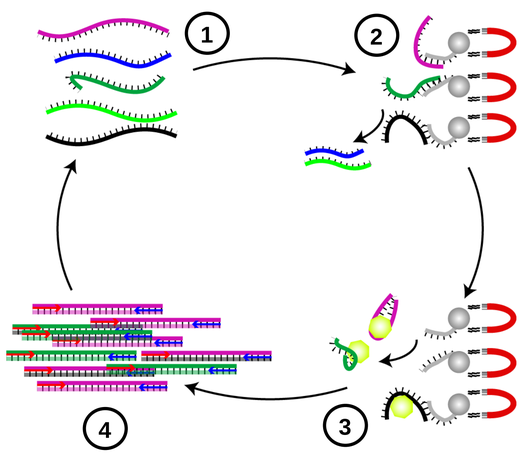
Aptamer Selection

Aptamers are oligonucleotides (either based on ssDNA or ssRNA), which are selected to specifically bind a particular target molecule.



Beacon Aptamers (BAs) differ from traditional aptamers because they change conformation upon binding to a target molecule, thus enabling the detection of the event by approaching the negative charges of the oligonucleotides backbone to the sensing area.

Aptamer production is based on SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technology: from a library of random sequences the one/ones with the best affinity for the target molecule are selected through a series of cycles with increasing stringency conditions. Our SELEX approach is based on Morse protocol[2], modified to be applied to DNA sequences, and our target molecule is tobramycin.



Step 1: an initial library of random sequences is digested to obtain the single strand oligonucleotide pool for the selection.

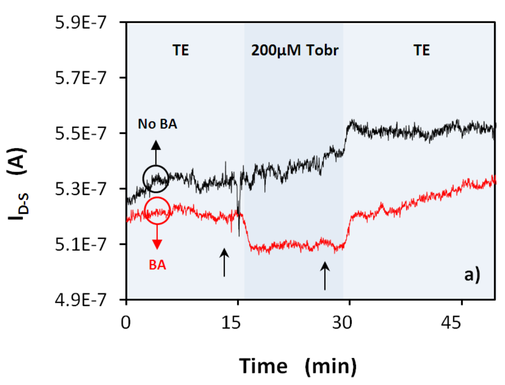
Step 2: the single strand sequence are put on magnetic beads through hybridization with a short oligonucleotide immobilized on the surface.

Step 3: upon incubation with the target, aptamers that undergo a conformational change due to the interaction with the target are displaced from the beads and collected.

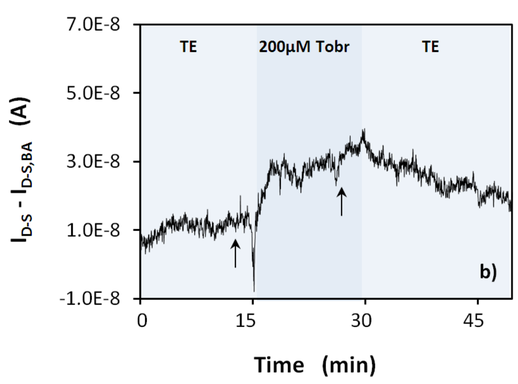
Step 4: the so selected aptamer are amplified by PCR and used as starting material for the following cycle.

Tobramycin detection

BAs were selected and then characterized in terms of their sensitivity against tobramycin, an antibiotic used to treat various types of bacterial infections. This drug (molecular weight: 467.52 Da) is a good candidate to demonstrate the ability of the SiNRs to sense very small molecules. The SiNR functionalized with the BA shows a decrease in the conductivity when tobramycin is injected. The binding affinity of the BAs with respect to tobramycin were tested in parallel by Surface Plasmon Resonace (Biacore X100, GE-Healthcare). Assuming a similar density of immobilized aptamers probes, the calculated maximum density of detected tobramycin molecules resulted to be $3.86 \cdot 10^{12}$ cm^{-2} , corresponding to about 1 molecule every 26 nm^2 of sensing surface.



Measurement of the drain current (ID-S) showing the different behavior of an unmodified SiNR (black line) and MPTMS/BA modified SiNR (red line).



Differential signal (I_{D-S} – $I_{D-S,BA}$) showing the kinetics of the binding event.

The SiNRs were first equilibrated in TE 1X buffer for 2 hours before starting the binding experiment (only the last 15 minutes are shown here), successively tobramycin in TE 1X is injected for 15 minutes followed by empty TE 1X buffer. The arrows indicate the starting of the injections while the different regions on the plot indicate the time frame during which the SiNR is in contact with the previously injected solution (delays due to tubing have been taken into account here). The flow rate was kept fixed at 5 µl/min during the whole experiment.

References

[1] E. Accastelli, *et al*., IEEE nano 2013, August 5-8, 2013, Beijing, China, 2013.

[2] D. P. Morse, *et al.*, Biochemical and Biophysical Research Communications, vol. 359 (1), pp. 94-101, 2007