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Functional Nanostructured Platforms for Chemical and Biological Sensing

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Functional nanostructured platforms for chemical and biological sensing

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ABSTRACT

The central goal of our work is to combine semiconductor nanotechnology and surface functionalization in order to build platforms for the selective detection of bio-organisms ranging in size from bacteria (micron range) down to viruses, as well as for the detection of chemical agents (nanometer range). We will show on three porous silicon platforms how pore geometry and pore wall chemistry can be combined and optimized to capture and detect specific targets.
We developed a synthetic route allowing to directly anchor proteins on silicon surfaces and illustrated the relevance of this technique by immobilizing live enzymes onto electrochemically etched luminescent nano-porous silicon. The powerful association of the specific enzymes with the transducing matrix led to a selective hybrid platform for chemical sensing.
We also used light-assisted electrochemistry to produce periodic arrays of through pores on pre-patterned silicon membranes with controlled diameters ranging from many microns down to tens of nanometers. We demonstrated the first covalently functionalized silicon membranes and illustrated their selective capture abilities with antibody-coated micro-beads. These engineered membranes are extremely versatile and could be adapted to specifically recognize the external fingerprints (size and coat composition) of target bio-organisms.
Finally, we fabricated locally functionalized single nanopores using a combination of focused ion beam drilling and ion beam assisted oxide deposition. We showed how a silicon oxide ring can be grown around a single nanopore and how it can be functionalized with DNA probes to detect single viral-sized beads. The next step for this platform is the detection of whole viruses and bacteria.

Keywords: Silicon electrochemistry, sensing, surface functionalization, nanopores, porous silicon, membranes.

1. INTRODUCTION

Silicon is a material of choice for sensing applications since it can be micro- and nano-fabricated, integrated, and chemically functionalized and patterned. Moreover, the material can be readily manufactured at a moderate price. Electrochemically nano-structured silicon presents all the advantages of bulk silicon, but in addition, its specific surface is dramatically enhanced (i.e. hundreds of m^2 per cm^3 of material for nanoporous silicon), which provides enhanced binding probability and capacity, and its electronic and optical properties are unusual, which allows for effective transduction modes. We will show how isotropic sponge-like nano-structured silicon can be used for chemical and biological sensing applications, and how anisotropic macro-porous silicon is progressively finding applications in novel smart filtration and detection techniques.
The easiest technique to anchor receptors onto a silicon surface is to perform silane chemistry on an oxidized silicon surface. Janshoff et al.[1] demonstrated the functionalization of ozone-oxidized macroporous silicon with biotin with a three step process involving a 12 H reflux in a methoxysilane-terminated linker, followed by a 1 H incubation in dithiothreitol to reduce the disulfide bond, followed by a 30 min incubation in biotin-HDPD. The functionalized porous matrix bound streptavidin but was also reported by the very stable against oxidation and corrosion in aqueous environments. The same chemistry was used to anchor protein A via the incorporation of BSA. This technique was proved to lead to stabilization against oxidation in various aqueous buffer solutions and to elimination of non-specific binding.[2] Although it works very well for applications where defect-free interfaces are not needed, this technique cannot be employed in applications where the transduction mode relies on clean surface passivation as well as on close contact

between the target molecules and the crystalline silicon are needed. In this later case, a direct silicon-carbon bond is required.

Various routes attaching the linker directly to the silicon surface have been explored. A main advantage of this approach is that there is no interfacial layer between the device and the linker. Moreover, the Si-C bond is both thermodynamically and kinetically stable due to its high bond strength and low polarity. For a complete study of the various routes leading Si-C bond formation, we refer the reader to Buriak's review of the organometallic chemistry of silicon and germanium surfaces.[3] The basic reaction, called hydrosilylation, involves the insertion of an unsaturated bond onto a silicon-hydride group (Si-H). Typically, by this process alkyne hydrosilylation yields and Si-C bond and an alkenyl group, and alkene hydrosilylation yields and Si-C bond and an alkyl group.[4] Since $EtAlCl_2$ is soluble in non-polar solvents, it was used as the Lewis acid catalyst for the reaction. Immersion of a porous silicon chip in an hexane solution of $EtAlCl_2$ with addition of 1-dodecyne under nitrogen atmosphere at room temperature for 1 H resulted in the clean incorporation of dodecenyl groups on the porous silicon surface.[5-7] Many other surface functionalities including nitrile, hydroxy, and ester terminations, can be inserted by following the same process, without compromising the porosity, surface area, or the luminescence of the material. Moreover, the functionalized surfaces showed remarkable stability in boiling aerated water as well as in aqueous KOH solutions at pH up to 10. Functionalized porous silicon films were also placed into body fluids and their corrosion was showed to be reduced by two orders of magnitude compared to hydride-terminated samples.[8]

Variations on this route include thermal instead of chemical catalysis of the hydrosilylation reaction. The reaction of an hydrogen-terminated porous silicon surface with desoxygeneted undecylenic acid at 95 C for 16 H under argon results in the formation of a $(CH_2)_{10}$ chain attached to the silicon surface via an Si-C bond on one end, and terminated with a carboxylic acid on the other end.[9-11] These functionalized surfaces proved to be very stable in boiling CCl_4 and water, as well as in 100 % humidity atmosphere.

Once the linker is anchored on the silicon surface, the next step is to anchor the receptor that will provide specificity to the sensing platform. This step requires the addition of functionality to the linker, in the form of an ester, thiol, or carboxylic acid end group for example. This can be done by adding the functionality directly on the initial reactant, by extending the immobilized linker by cross-linking techniques, or by a combination of both techniques.

We will show how variations and combinations of the functionalization techniques described above can be used to provide chemical functionality and specificity to various sensing silicon platforms.

2. RESULTS

2.1. Porous silicon: random pores

The electronic properties and the surface chemistry of silicon are now well understood.[3] Advantage can be taken of this basic knowledge to both shape the material and chemically functionalize its surface.

Although, as it was described in the introduction, a standard approach to the chemical functionalization of silicon is to grow an oxide layer and perform silane chemistry, some applications, such as the functionalization of luminescent silicon nanostructures, require defect free surfaces. We have developed a synthetic route to covalently anchor bio-molecules to silicon starting from a hydride-terminated silicon surface. As shown in Figure 1, the surface is covalently functionalized with an amine-terminated chain using either a Lewis acid or light to catalyze the hydrosilylation reaction.[12] Protein cross-linker chemistry is then used to extend the linker and immobilize various molecules such as antibodies, proteins and live enzymes onto the device surface.[13, 14] We demonstrated that generic enzymes such as glucoronidase, as well as more exotic ones such as organophosphorus acid anhydrolase, can be covalently immobilized on silicon surfaces using this technique and that the enzymes remain active for at least six months upon storage of the devices at 4 C in a 1:1 mixture of PBS buffer and glycerol.

We performed enzyme immobilization on electrochemically etched luminescent nanoporous silicon and demonstrated that the immobilization procedure does not affect the luminescence of the platform. As described in Figure 1, the enzymes provide chemical selectivity to the silicon platform. Typically, a surface pocket binds a specific substrate molecule and breaks it down into separate fragments (in our specific example, organophosphorus acid anhydrolase binds soman nerve agent, or a soman surrogate, and breaks it down in two fragments). If at least one of the fragments interacts with the silicon quatum dots and wires that constitute nanoporous silicon, via charge or energy transfer, re-absorption, or chemical reaction, its presence will induce a quenching of the luminescence signal from the porous silicon matrix. We demonstrated that organophophorus acid anyhydrolase immobilized on luminescent porous silicon

could breakdown paranitrophenyl-soman and generate paranitrophenol, a colored marker. Concomitant absorption and luminescence measurements performed in a in a flow cell upon successive buffer washes and paranitrophenyl-soman injections showed that the detection of the paranitrophenol breakdown product by absorption was correlated with a decrease of the luminescence signal of the porous silicon matrix. Figure 2 shows reversible detection of the soman surrogate by charge transfer between the enzymatic breakdown products and the luminescent porous silicon platform.
The immobilization technique described above can be generalized to any silicon morphology and device shape. Using enzymes as receptors opens a wide field of chemical functionalities and dramatically improves selectivity (whether the selectivity is natural or engineered). In the future, hybrid platforms should be able to detect target molecules, but also to pre-process complex samples, catalyze reactions in pore or channels, or separate various components of a sample.

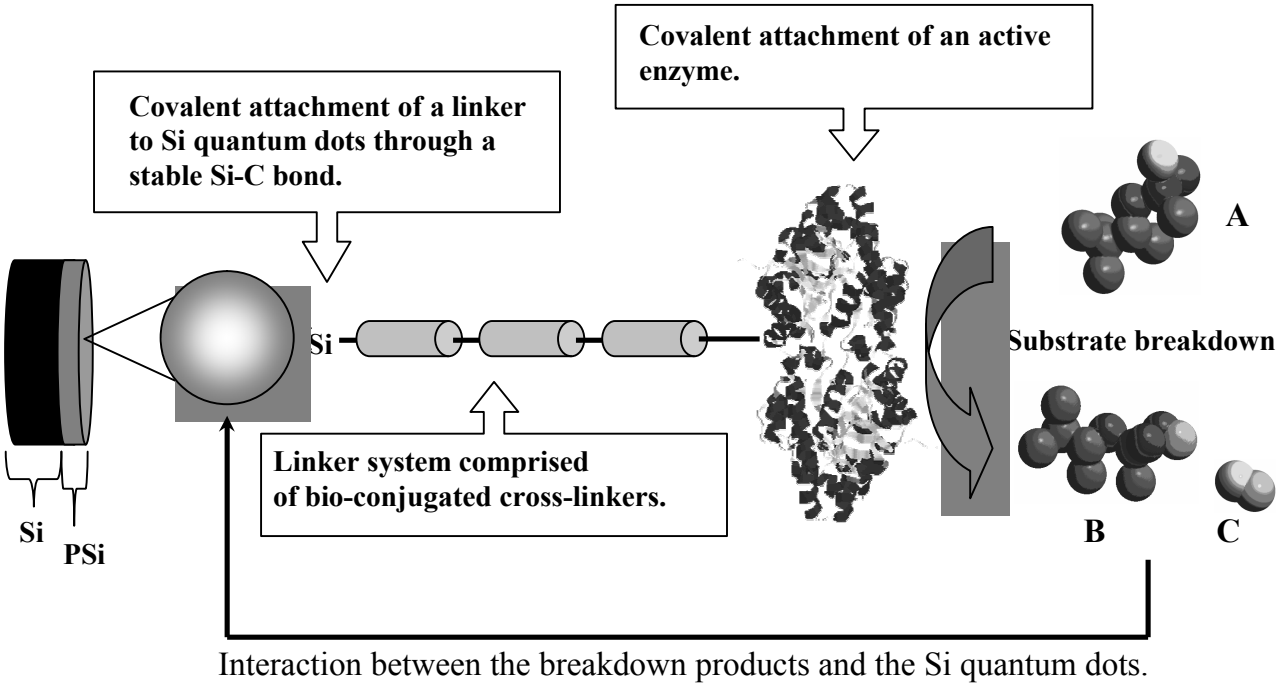

Figure 1: Scheme of a hybrid porous silicon platform. The luminescent porous silicon layer, which rests on the bulk silicon wafer, is constituted of interconnected silicon quantum dots and wires. The silicon hydride surface is first derivatized with an amine group via a hydrosilylation reaction. The linker is then expanded by successive cross-linking steps, and finally, binds a lysine residue on the surface of an active enzyme. The enzyme specifically breaks down the substrate molecule of interest, and the breakdown products interact with the silicon quantum dots via charge transfer, energy transfer, absorption, or chemical reaction to quench the luminescence of the platform.

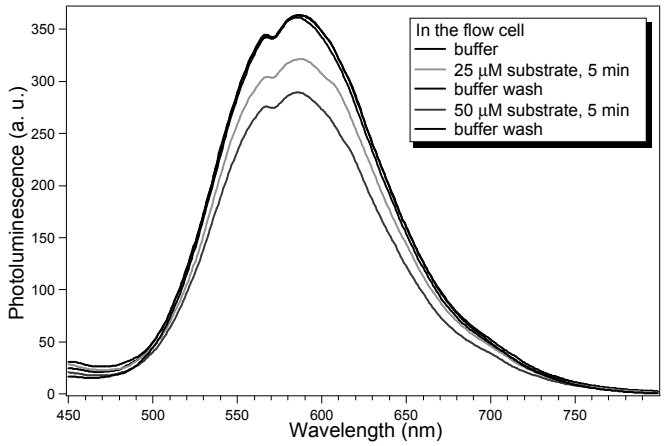

Figure 2: Luminescence spectra of a porous silicon platform functionalized with organophosphorous acid anhydrolase enzyme. The sample is first exposed to buffer, followed by 25 μM of pnp-soman substrate, followed by buffer, followed by 50 μM of pnp-soman, followed by buffer. The presence of the nerve agent surrogate induces a reversible decrease of the luminescence of the nanoporous silicon platform.

2.2. Silicon membranes: periodic channels

Membranes with various pore size, length, morphology, and density have been synthesized out of various materials for size exclusion-based separation. An example is the sterilization of intravenous lines by exclusion of bacteria and viruses using Polyvinylidene Fluoride membranes with 0.1 μm diameter pores. Chemically specific filtration recently started to be addressed for small molecules but specific bio-organism immobilization and detection remains a great technical challenge in many applications where the analysis of samples such as air, drinking water, and body fluids is required.

One of our goals is to couple semiconductor nanotechnology and surface functionalization techniques in order to build sturdy platforms for the selective filtration of bio-organisms ranging in size from spores (many microns) down to viruses (tens of nanometers). The challenges of this research are to create channels with controlled position, length, and diameter ranging in size from many microns down to a few tens of nanometers, and to position functional chemical units on these features.

We used light-assisted electrochemistry to produce periodic arrays of through pores on pre-patterned silicon membranes with controlled diameters ranging from many microns down to a few hundred nanometers (see SEM top view, cross section, and bottom view in Figure 3).[5] In this technique, inverted pyramids fabricated on the surface of the device by a combination of photolithography and potassium hydroxide (KOH) etching, are used as nucleation sites for the growth of well aligned channels. The positive charges required for the electrochemical dissolution of silicon in the presence of a hydrofluoric acid (HF) based electrolyte are photo-generated on the backside of the n-type silicon substrate and driven to the tips of the inverted pyramids by an electric field. The localization of the charges translates into a localization of the chemical dissolution of the silicon material which leads to the formation of through pores at a typical speed of 1 μm/min. The amount of charge passing through the junction is directly correlated to the pore diameter, and the duration of the etch is directly correlated to the length of the pores.

In order to go beyond size selectivity, we fabricated the first covalently functionalized silicon membranes and illustrated their selective capture abilities with antibody-coated fluorescent micro-beads simulating bio-organisms. The immobilization procedure was similar to the enzyme immobilization procedure described above for nanoporous silicon, the only difference being the fact that when functionalizing high aspect ratio channels, light cannot be used as the catalyst. We used a Lewis acid as the catalyst in this case, and anchored biotin via a cross-linker, as depicted on Figure 4. The antigen-functionalized membranes selectively captured the matching antibody-coated beads while allowing the rest of the beads to flow through. Figure 4 shows a scheme of the interaction between the functionalized pore walls and the functionalized bead simulating a bio-organism. An SEM cross section of a pore taken after bead capture shows beads anchored on the pore walls.

These engineered membranes are extremely versatile and could be adapted to specifically recognize the external fingerprints (size and membrane proteins) of many bio-organisms. A potential application is the pre-concentration of a

specific organism for sample analysis, a specific example being the monitoring of E. Coli in re-cycled water produced by water treatment plants.

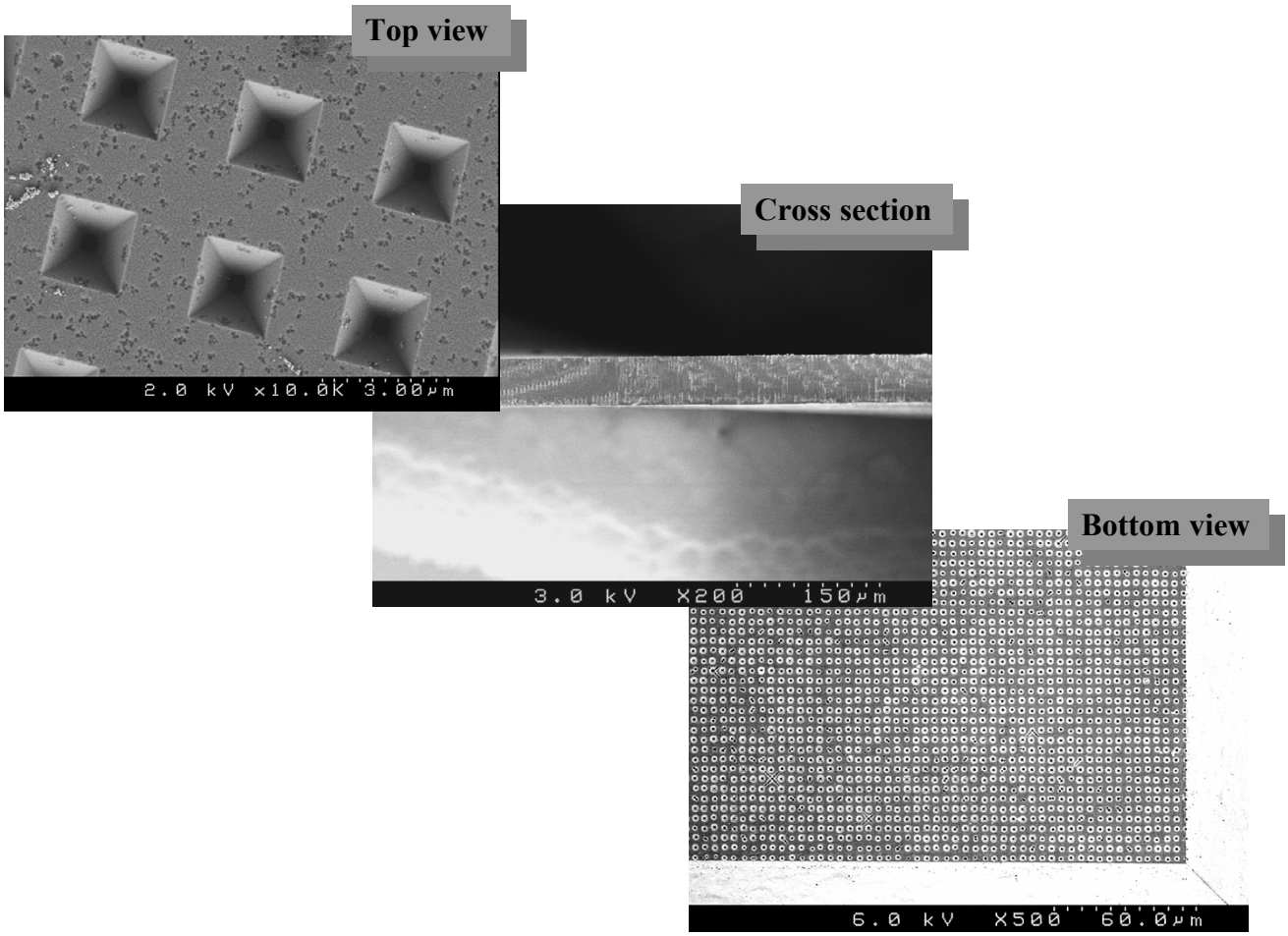

Figure 3: SEM views of a silicon membrane. The top view shows the pre-patterned inverted pyramids which play the role of nucleation sites for the pores. The cross-section shows a broad field of through-pores. The bottom view shows the pores from the back-side of the membrane as well as the corner of the back-window.

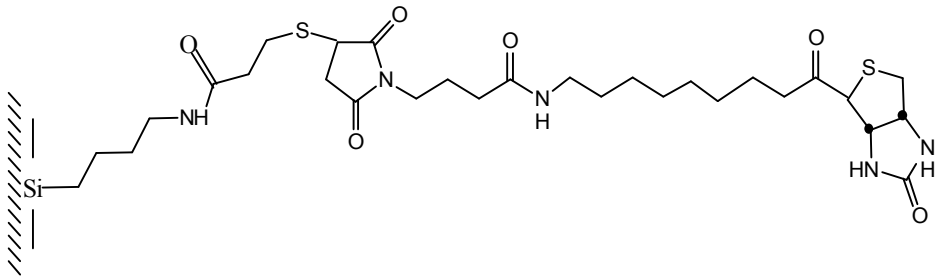

Figure 4: Chemical structure of the linker anchored on the silicon membrane. In this example, the linker is covalently bound to the silicon wall of the membrane via a robust Si-C bond on one end, and to a biotin probe on the other end.

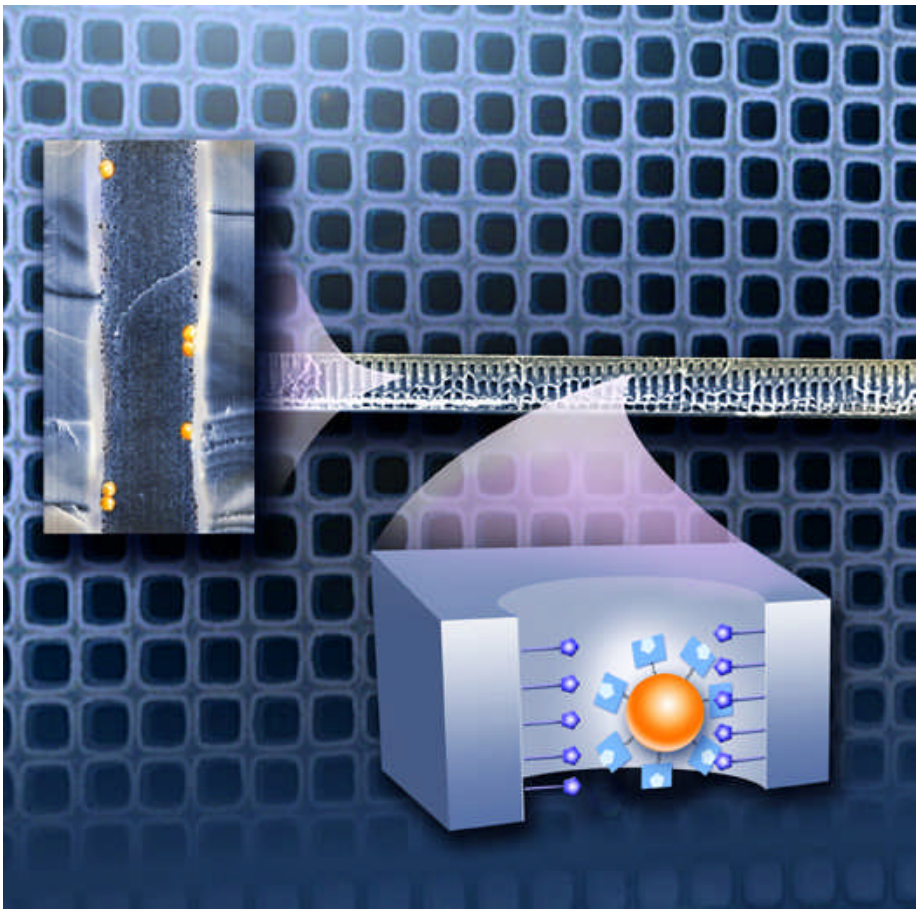

Figure 5: Capture of simulated bio-organisms by a functionalized silicon membrane. The background is an SEM top view of a biotin-functionalized silicon membrane. A cross section of the membrane and a zoom on a pore is shown in the center. The 200 nm streptavidin-coated beads captured by the membrane were colorized in orange. A scheme of the chemical interaction between a functionalized bead and the functionalized pore wall is presented at the bottom right.

By using breakdown electrochemical etching, we also achieved periodic arrays of through pores with diameters down to 30 nm and aspect ratios up to 250, number which had never been achieved by electrochemistry before.[15] We demonstrated the potential of these devices for cell encapsulation applications, for which the main challenge is to fabricate a capsule that allows the diffusion of glucose, a cell nutrient, while blocking the diffusion of immunoglobulin, an antibody triggering foreign body responses. An example of application is the encapsulation of pancreatic cells: the membrane can allow ions, glucose and insulin to move through while protecting the cells from proteins that trigger foreign body responses.

Our next goal is to add *in situ* detection capabilities to the chemically selective membranes. This would lead to a new class of platforms able to collect, concentrate and analyze bio-organisms in real time. Applications for such a device are numerous, including counter-terrorism, human health and environmental monitoring.

2.3. Silicon Coulter-counter: single nanopores

In the biodetection field, platforms generating signatures or fingerprints have significant advantages over regular detection systems since they could potentially be used to identify new unknown organisms. The Coulter-counter principle used for cell enumeration in hematology laboratories is based on the fact that the ionic current through a single aperture drops each time a cell obstructs the channel by moving through it.[16] Although much work has been devoted to the reduction of aperture diameters to count smaller and smaller biological objects, the next challenge remains to combine size control with local chemical functionality to achieve specific counting and detection. The initial solution to

this problem was provided by the use of α-hemolysin, a cellular membrane protein that self-assembles in lipid bilayers to form a 1.5 nm diameter pore. Bayley et al.[17-20] showed that a single DNA probe could be immobilized at the entrance of the protein pore via genetic engineering and that specific binding events to complementary DNA strands could be detected. The main drawback of this approach is the fluidity of the bilayer platform, which generates instabilities and therefore leads to extremely short functional lifetimes of the pore. Additionally, there is no possibility to tune the pore length and diameter in order to accommodate larger biological objects such as viruses or bacteria. An alternative route is to build apertures on a sturdy solid platform.[21-24] Our approach is to fabricate single pores on pre-patterned silicon platforms by Focused Ion Beam (FIB) drilling, and to locally derivatize the pore entrance via the controlled growth of an oxide ring on a silicon nitride layer, followed by preferential functionalization of the silicon oxide with silane chemistry.

We prepared silicon membranes with a thickness of 700 nm on un-doped silicon on Insulator (SOI) wafers and coated the top surface with 100 nm of silicon nitride. Single pores were drilled by FIB and then surrounded by a localized silicon oxide ring via ion-beam-assisted oxide deposition.[25] Figure 6 shows an SEM top view of a 160 nm diameter pore, as well as the oxygen map of the pore recorded by Energy Dispersive X-ray Spectroscopy (EDS), and an AFM topography image. All three pictures illustrate the localization of the silicon oxide around the pore entrance, which provides a path to localized linker and probe immobilization.

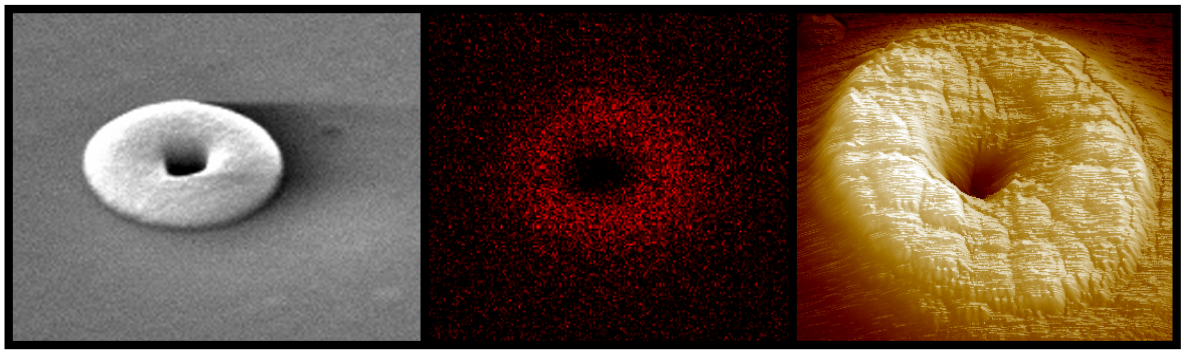

Figure 6: Images of a single pore surrounded by a silicon oxide ring. The pore diameter is 160 nm. An SEM top view is presented on the left, an EDS oxygen map is presented at the center, and an AFM topography image is presented on the right. All three images illustrate the localization of the silicon oxide ring around the entrance of the single pore.

Chemical functionalization of the single pores was carried out through silane chemistry, as illustrated on Figure 7. Thiol-terminated linkers were anchored on the silicon oxide ring and then reacted with acrylamide-terminated DNA strands, as a proof of concept. The effect of DNA functionalization on the ionic current recorded through the single channels was studied by comparing ionic current values recorded before and after functionalization, and comparing the measured ionic current decrease to values deduced from theory. The good agreement between experimental and theoretical values allowed to confirm that the single pores were functionalized with DNA probes and that they remained opened after functionalization.[25]

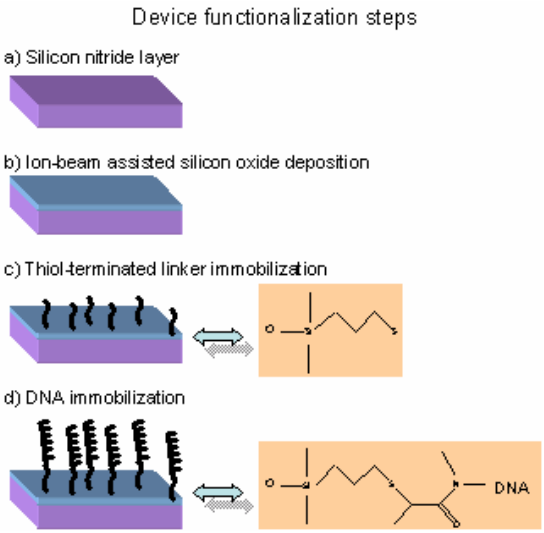

Figure 7: Scheme of the functionalization steps used on single pores. From top to bottom: silicon oxide is first deposited on the silicon nitride surface using a Focused Ion Beam (FIB), the silicon oxide ring is then derivatized with thiol-terminated linkers via silane chemistry, and finally, single DNA strands are immobilized on the silicon oxide ring via an acrylamide group.

We are now studying the dynamics of DNA functionalized beads in DNA functionalized apertures in order to demonstrate the selectivity of our sub-100 nm paticle counter. The next step will be to demonstrate selective bio-organism detection.

3. CONCLUSION

While research in membrane and single pore technology is heading towards nano-devices for small molecule detection, a gap remains to be filled between large biological objects such as blood cells, and DNA. Another challenge is the precise and localized functionalization of nanometer-sized devices which is key to specific binding and multiplexing. We are closing the size gap by fabricating single pores and membranes in the 20 to 500 nm range, which spans both virus and bacterium size regimes, and we have demonstrated the relevance of techniques such as ion beam assisted oxide deposition to localize probes around nanopores. This unique combination of controlled channel geometries and surface chemistry should constitute a critical tool to build the next generation of biological and chemical sensing platforms.

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