

Virus detection with an organic dual-gate field-effect transistor based nanobiosensor

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3. Abstract

Viruses are one of the main originators of many infectious diseases. Well-known viral diseases are the flu, chickenpox, common cold, AIDS and SARS. Nowadays, fear exists that new viruses will arise from animal viruses, like the avian flu virus. It is important to know when these potentially harmful viruses are around. Sensors can be used for rapid detection of viruses. They can therefore prevent or minimize infections. Here we propose a new type of general, true virus detecting, nanobiosensor. This sensor will be a versatile sensor that is going to be designed in such a way that it can be used for the detection of any virus. Our electronic sensor is based on a dual-gate organic field-effect transistor. Antibodies against virus particles serve as the recognition elements and are covalently linked to the top insulator. By using the dual-gate geometry and selective attachment of the antibodies only to the transistor area we will obtain a sensor with excellent selectivity, high sensitivity and a low detection limit.

4. Duration of the project

The duration of the project will be four years, since this is the applicant's time as a PhD student.

5. Personnel

Prof. Dr. Ir. P.W.M. Blom (professor / leader of the ME-POS research group)
Prof. Dr. B. de Boer (associate professor / project leader)
P. Fonteijn BSc (applicant / PhD student)
J. Harkema (technician)
F. van der Horst (technician)

The personnel working on this project are all part of the research group Molecular Electronics - Physics of Organic Semiconductors within the Zernike Institute of Advanced Materials at the University of Groningen in The Netherlands.

6. Cost estimates

The funding is requested for the applicant, one PhD position. Furthermore, all specifically related needs such as wafers, lithography masks, chemicals, antibodies and viruses will be acquired from this budget. The equipment needed for this project is already present in the laboratory of the research group or within the Zernike Institute for Advanced Materials. No other support will be required for this project. A budget summary of the funding requested is given below in Table 1.

Table 1: Budget summary of the funding requested

		1 st year	2 nd year	3 rd year	4 th year	Total
Positions	PhD students	1	1	1	1	
	Postdocs	-	-	-	-	
	Technicians	-	-	-	-	
	Guests	-	-	-	-	
Costs	Personnel	€ 36.000	€ 39.000	€ 43.000	€ 47.000	€ 165.000
	Running budget	€ 15.000	€ 15.000	€ 15.000	€ 15.000	€ 60.000
	Equipment	€ 100.000	€ -	€ -	€ -	€ 100.000
	Total	€ 151.000	€ 54.000	€ 58.000	€ 62.000	€ 325.000

7. Infrastructure

A properly certified glovebox has to be purchased for working with the proposed kind of viruses and has to be installed in the cleanroom of the Zernike Institute for Advanced Materials. All other equipment needed for this project is present within the research group Molecular Electronics - Physics of Organic Semiconductors or within the Zernike Institute for Advanced Materials.

8. Research program

8.1 Introduction

Infectious diseases have been around for centuries, causing many people to fall sick or even die. Viruses, being small cellular parasites, are one of the main originators of many of these diseases. Well-known viral diseases are the flu, chickenpox and common cold, but also AIDS (acquired immune deficiency syndrome) and SARS (severe acute respiratory syndrome) are caused by viruses. Nowadays fear exists that new viruses will arise from animal viruses, which are potentially fatal for humans, for example a mutated type of avian flu virus H5N1. Also concerns arise about terrorist groups that might use hazardous viruses as biological warfare agents.

Since viruses have the potential to cause infectious diseases, it is important to know when they are around. Traditional methods for detection and identification of viruses usually require laboratory equipment and different processing steps, so they are considered not to be real-time [1]. Sensors on the other hand can be used for relatively rapid detection and can therefore prevent or minimize infection.

A sensor is a measuring device that will respond to a specific situation or species, in this case the presence of a virus. It consists mainly of two parts: a sensing element and a signal transducer. The sensing element, or recognition element, responds to the substance being measured, known as the analyte. This response is converted by the signal transducer into an observable and measurable signal. The signal of many transducers is an electrical one, enabling easy integration in any electrical circuit to obtain direct electronic readout of the sensor [2].

The detection characteristics of a sensor depend on many parameters and properties [2, 3]. The most important parameters are the selectivity of the sensor, its sensitivity and its detection limit. High selectivity is essential for a well working sensor, since it should only respond to the target analyte and not to other substances. Also the sensitivity, the change of sensor signal with respect to changing amounts of analyte, should be high enough to obtain accurate results. Closely related to the sensitivity is the detection limit, the lowest amount of analyte detectable, which should be as low as possible.

In this proposal we first describe the sensors that have been developed so far for virus detection. Next we will introduce our proposed new type of general, true virus detecting, nanobiosensor. This sensor

will be a versatile sensor that is going to be designed in such a way that it can be used for the detection of any virus. By using a proper design we will obtain a sensor with excellent selectivity, high sensitivity and a low detection limit.

8.2 Current research status

8.2.1 Traditional sensors

A number of traditional sensors for the detection of various infectious diseases has been developed, which are largely reviewed by Pejčic *et al* [4]. These sensors all rely on a biological recognition element, so they are considered as biosensors. The described biosensors are based on several technologies and have detection limits in different orders of magnitude. The majority of the existing technologies used for detection of infectious diseases relies on antibodies as their biological recognition element. Antibodies are typically used for their very high selectivity: particular antibodies will only bind to a specific virus and not to other substances, as is essential for their original function in the immune system [5].

8.2.1.1 Traditional electrochemical sensors

Most of the biosensors designed to detect viruses are based on electrochemical transduction [4]. Electrochemical transducers can be divided into various types: amperometric, potentiometric, impedance and conductivity based [2]. As an example of an amperometric based biosensor Pejčic *et al.* describe a study about detection of the Newcastle disease virus [6]. In this biosensor antibodies against the virus are labeled with the enzyme horseradish peroxidase. Antigens of the virus are encapsulated into an inorganic sol-gel matrix electrode with added graphite powder for enhanced conductivity. Binding of the labeled antibodies to the embedded antigens results in current increase, due to the redox reaction carried out by the enzyme label. In this way antibodies have been determined up to 443.24 ng ml⁻¹ with a detection limit of 11.1 ng ml⁻¹. This sensor however does not detect the virus itself, but the antibodies that have been generated against it after infection. Therefore it is not a true virus detecting sensor.

In many cases detection with amperometric biosensors is achieved by using an enzyme or protein label, since the antibody-antigen bonding itself usually does not result in electron transfer and therefore does not generate a significant signal [4]. Indeed another biosensor, to test for Forest-Spring encephalitis, uses a gold-labeled protein that attaches to the antibody-antigen complex [7]. Again, this is not a true virus detecting sensor, since the antigens are immobilized on the electrode. Nevertheless, detection limits of 1.0 ng ml⁻¹ have been observed. Label-free true virus detecting biosensors have also been reported, for example to detect the Japanese B encephalitis virus [8]. In this sensor antibodies are immobilized on gold-nanoparticles attached to an *o*-phenylenediamine polymer layer deposited onto a Prussian blue coated platinum electrode.

The latter sensor can also be used as a potentiometric label-free biosensor [9]. Most potentiometric based biosensors for virus detection however do rely on labeling and use an indirect way of detection. For example a change in pH, redox potential or ionic concentration, caused by a secondary reaction, results in a potential shift with respect to a reference electrode, which is then measured. Indeed a biosensor for the detection of Hepatitis B virus makes use of a horseradish peroxidase-labeled antibody-antigen complex [10]. Turnover of the enzyme leads to a changing environment, altering the electrochemical properties of a layer of conducting polypyrrole coated onto a gold electrode. Other potentiometric biosensors are based on a change in photocurrent from a semiconductor, due to a secondary reaction that changes the environment. Such a light addressable potentiometric biosensor can be used to detect the Newcastle disease virus, due to enzyme-labeled antibodies that will change the pH upon turnover [11, 12]. In this way a detection limit down to 1.3 ng ml⁻¹ has been reached. Also Venezuelan equine encephalitis virus can be detected in a similar way [13]. Large enzyme-labeled antibody-antigen complexes are formed, leading to virus detection down to a concentration of 30 ng ml⁻¹.

Closely related to potentiometric sensors are sensors based on electrical impedance spectroscopy, in which changes of surface properties, like interfacial capacitance and charge transfer resistance, are sensed to detect viruses [4]. Indeed binding of Hepatitis B antigen to an antibody immobilized onto a platinum electrode modified with colloidal gold and polyvinyl butyryl can be detected by measurements of the impedance change [14]. With this method a detection limit of 7.8 ng ml⁻¹ has been obtained. A similar study even showed a detection limit of 0.05 ng ml⁻¹ for Hepatitis B virus surface antigens [15].

During antibody-antigen complex formation ionic concentration and composition might change, which will lead to a change in the electrical conductivity of the solution [4]. A biosensor has been

fabricated to detect bovine viral diarrhoea virus, with antibodies immobilized into a conductive polyaniline matrix [16]. Binding of virus particles leads to changing polyaniline conductivity, measured between two electrodes of the sensor.

8.2.1.2 Traditional optical and piezoelectric sensors

Electrochemical transduction is an important type of transduction often used for the detection of viruses, but also some other transduction methods do exist, mainly based on optical or piezoelectric devices [2]. A few papers have been published on optical biosensors for the detection or study of viruses [4], like the Newcastle disease virus (down to 10 ng ml⁻¹) [17] and the Dengue virus [18], the latter based on DNA-RNA hybridization.

Piezoelectric detection is based on the principle that the frequency of an oscillating crystal is affected by the mass of material absorbed on its surface [2]. The foot-and-mouth disease virus can be detected by a biosensor with antibodies coated onto a piezoelectric crystal [19]. Antibodies generated after infection can be detected by another biosensor with antigens attached to a gold-coated quartz crystal [20]. Although this latter sensor should not be considered as a true virus detecting sensor, clear shifts in frequency have been observed on antibody-antigen binding. Also Hepatitis B virus has been detected using a piezoelectric biosensor [21]. Detection is based on hybridization of virus DNA with nucleic acid probes attached to gold electrodes. A detection limit of 20 ng ml⁻¹ has been obtained. Another piezoelectric biosensor is able to detect the SARS-associated corona virus from aerosol sprayed on an antibody-coated crystal [22]. Frequency shifts appeared to be linearly dependent on antigen concentration above a concentration of 600 ng ml⁻¹. The dengue virus can be detected as well with a quartz crystal microbalance, in this case coated with antibodies against envelop proteins of the virus [23]. Different immobilization methods have been compared, leading to a lowest detection limit of 50 ng ml⁻¹.

8.2.1.3 Remarks on traditional sensors

Only a few traditional biosensors for the detection of viruses are based on conductive polymers. Nonetheless, conductive polymers have been used in many different traditional biosensors covering a large range of detectable analytes, like ions, gases, proteins, bacteria, DNA and numerous others. Some of the possibilities are shortly reviewed by Gerard *et al* [24], but many other sensors have been developed also.

Nearly all traditional virus detecting biosensors are based on antibody-antigen complex formation followed by labeling of this complex. The amount of labels attached or the activity of the labels is then used as a measure for the number of complexes formed. Therefore the question arises whether these traditional biosensors can be considered as true virus detecting sensors, since they rely on properties of the label rather than of the virus. Moreover, some sensors just screen for virus-DNA, only the epitopes or even the antibodies, instead of the whole virus as it is. Beside this it is difficult to screen real-time for the viruses concerned, since labels and reagent are necessary to obtain the result of detection. Due to the large labeled complexes it is also hard to reuse the sensors and detect new viruses in a second run. Although the label-free sensors do not require the labeling steps, still the samples usually need to be prepared before they can be applied to the sensor. In this way some of the sensors even seem to resemble, with regard to amount of work and real-time detectability, the original methods for detection and identification of viruses using laboratory equipment and different processing steps. A true virus detecting sensor however will just instantaneously generate a signal on detection of the virus as it is, without any preparation- or developing steps.

8.2.2 Nanosensors

The detection limits of the aforementioned sensors are in the range of nanograms per milliliter. Yet this still means millions or billions of molecules, epitopes or viruses are present in such a volume. Traditional sensors really need the sum of all these particles to generate a measurable signal. Since viruses have the potential to cause infectious diseases, sensors should however be able to detect even very little amounts of virus particles. To obtain much lower detection limits, and eventually detect at the single virus particle level, sensors with a size similar to the size of the analytes are necessary. The traditional sensors are however quite large compared to a virus and most methods are not easy to scale down to the nanoscale level. Therefore to obtain lower detection limits research have been carried on in the field of nanotechnology, as partially reviewed by Erickson *et al* [25]. The total surface area or volume that is probed by these nanosized sensors is much lower than that of the traditional sensors. As a result of this inherent advantage of nanosensors the total amount of analyte required to impart a measurable signal is significantly lower. Not only do these nanosensors require less analyte, some also

exploit fundamental nanoscopic effects and the resulting properties of nanoscaled objects. In this way much lower detection limits can be reached [25].

8.2.2.1 Nanosensors for virus detection

All sensors described by Erickson *et al* [25] are biosensors, or more specific nanobiosensors, with only a few designed to detect viruses. Nanosensors are, just like traditional sensors, also based on different technologies and transduction mechanisms. The review of Erickson *et al* [25] focuses primarily on label free optical sensing techniques, which make use of different properties and characteristics. A large number of sensors exploit localized changes in the refractive index in the evanescent field of a dielectric structure to probe its surface for the presence of a bound analyte. Such a nanobiosensor based on a Young interferometer has been developed for detection of herpes simplex virus [26]. A detection limit of 850 virus particles ml⁻¹ has been obtained, but the authors describe lower detection limits should be possible. A few other nanobiosensors are based on photonic crystals, in which absorption of analyte changes the local refractive index leading to a shift in the photonic band gap. Other nanobiosensors use surface plasmon resonance, one of the most commonly exploited label free optical techniques. Virus detection by these latter kinds of nanobiosensors however has not been reported yet.

Another large class of nanobiosensors is based on mechanical effects, which include acoustic effects and the use of cantilevers. High-frequency vibrating nanoscale cantilevers can be used to accurately weigh extremely low masses and they are therefore also suitable for the label free detection of viruses. The amount of bound analyte is typically determined by observing changes in the resonance frequency of the oscillating cantilever. The vaccinia virus has been used to investigate the response of a nanosized cantilever on virus attachment [27]. Another study used a nanoscale cantilever to detect the insect baculovirus [28]. Significant shifts in frequency has been obtained after dipping the cantilever in solutions of 10⁵ virus particles ml⁻¹, which corresponds to a concentration of about 0.15 ng ml⁻¹. Real-time measuring these solutions is however not possible, since the frequency analysis has to be carried out in vacuum. Therefore this nanobiosensor is not a true virus detecting sensor. Other mechanical based nanobiosensors exploit bulk acoustic waves, such as in a quartz crystal microbalance, or surface acoustic waves. Virus detection with these nanobiosensors has not been reported yet.

A completely different kind of nanobiosensors are the solution phase sensors. These sensors typically include quantum dots or functionalized nanoparticles, which are suspended in the sensing volume to act both as the recognition element and the signal transducer. In this way also the binding of analyte is more efficient, since the diffusive transport length scale is reduced. Surface plasmon resonance of the suspended nanoparticles is often used, because usually this provides a simple colorimetric feedback: the color of the solution changes.

The optical methods seem to result in nanobiosensors with the lowest detection limits. Optical sensors, especially the optical-fiber based ones, are however difficult to scale down [4, 25]. For example the Young interferometer used to detect herpes simplex virus requires a relatively long optical channel of several millimeters. A lot of mass has to bind before a measurable signal is generated. As a result, although the detection limit is quite good, the sensor does not have the best sensitivity. Also the sensors with nanoscaled cantilevers have low detection limits. However the measurements have to be executed in an environment where viscous damping is minimized, like in high vacuum. As a result the cantilevers have to be removed from the, usually aqueous, sensing environment before detection can be performed. True, real-time virus detection in liquids is therefore not possible.

8.2.2.2 Electronic nanosensors

The nanobiosensors described so far can not be easily integrated in an electrical circuit, since they do not generate an electrical signal. Easy integration in an electrical circuit however is necessary to obtain direct electronic readout of the sensor, as is possible with the traditional electrochemical sensors.

In traditional electrochemical sensors changes take place at the electrode itself, since the recognition element is directly attached to it. These changes are converted into an electrical signal, however without amplification. The traditional electrochemical sensors therefore require the sum of many bound analytes in order to obtain a measurable output signal. This is also why these traditional sensors are difficult to scale down to the nanometer level. A smaller sensor binds much less analyte, which therefore results in a much smaller output signal that can not be measured accurately anymore. To obtain a measurable electrical signal from a nanosensor its transducer therefore has to amplify the signal in some way.

Signal amplification can be achieved by using a field-effect transistor setup [29, 30], in which a small electric field controls a large current. In a field-effect transistor a semiconductor is separated from an electrode, the gate, by an insulating layer (see Figure 1). The semiconductor is also in direct contact with two other electrodes, the source and drain. When a voltage is applied on these electrodes,

generating an electric field between the source and drain, the excess charge carriers in the semiconductor material move towards the electrodes, resulting in a source-drain current. The source-drain current however can be influenced by the gate electrode. A voltage on the gate electrode results in a second electric field perpendicular to the source-drain current. Depending on the sign of the applied voltage excess charge carriers will either accumulate at or deplete from the semiconductor-insulator interface. When charges accumulate at the semiconductor-insulator interface, forming a conduction channel, the source-drain current increases. Depletion of the charge carriers on the other hand results in a decreased or nearly absent source-drain current. In this way amplification behavior is obtained, since a small gate voltage can be used to control a large source-drain current.

In a similar way analytes bound to a nanosensor using a field-effect transistor setup will control the conduction channel [29]. For example when a charged analyte binds, it creates an electric field that influences the conduction channel at the semiconductor-insulator interface, just like a gate electrode. Of course, a prerequisite for this effect is that the analyte has a (surface) charge or dipole in order to create an electric field or that it has a similar property to control the conduction channel directly. Due to the amplification behavior of a field-effect transistor, binding of just a few analytes is in general sufficient to obtain a measurable source-drain current. Thus by using a field-effect transistor setup, nanosensors generating electrical signals for easy electronic readout can be constructed.

The semiconductor can in principle be made out of any semiconducting material. Traditionally in semiconductor industry silicon is the material of choice, although also other inorganic materials and even organic materials, like polymers, are used. The latter group of materials has gained more and more attention, because these materials can be easily deposited on a variety of substrates, including flexible ones, using low-temperature processes [29]. As a result low cost fabrication techniques can be used and a wide choice of molecular structure, including the possibility to build in side chains, end groups or grains of specific behavior, enables films to be produced with desired physical and chemical properties [31]. Moreover large area coverage and controlled layer thicknesses can simply be achieved. Finally, in contrast to many inorganic semiconductors, organic semiconducting polymers are bio-compatible, which allows for a more facile integration of sensors based on these materials into biological environments.

Several nanosensors based on organic field-effect transistors have been developed so far, partially reviewed by Wang *et al* [32] and Mabeck and Malliaras [29]. Different analytes can be detected, mainly gases ranging from oxygen and water vapor to organic ring compounds. Also protons, for pH detection, ions and chemicals like urea, hydrogen peroxide, glucose and acetylcholine have been described as detectable analytes. Recently even a nanobiosensor for the detection of thrombin proteins has been developed, which is based on an organic field-effect transistor with polypyrrole nanotubes as the semiconducting material [33].

Nanosensors for virus detection based on organic field-effect transistors have not been reported. Only one study has been published about virus detection with a nanosensor based on a field-effect transistor [34]. This nanobiosensor uses silicon nanowires as the semiconducting material. These nanowires are functionalized with antibodies against influenza type A virus and avian adenovirus

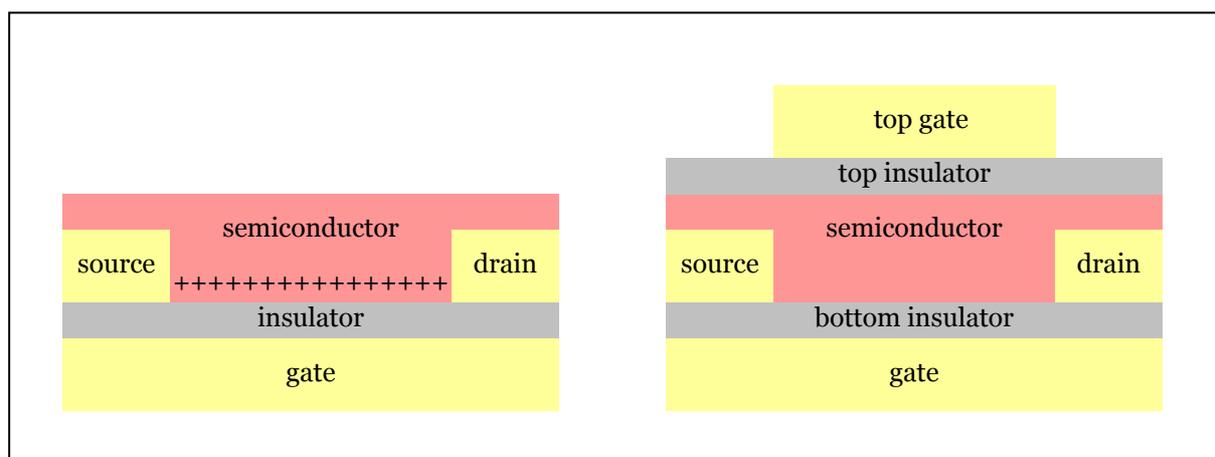


Figure 1: Left: schematic of a field-effect transistor - Accumulation of charge carriers at the interface of semiconductor and insulator, resulting in the formation of a conduction channel, due to a negative voltage on the gate electrode. The same situation is obtained when the gate is replaced by negatively charged analytes. Here the semiconductor is a p-type doped semiconductor with positive charge carriers. Right: schematic of a dual-gate field-effect transistor - In our sensor the top gate is replaced by the recognition element and the analytes will change the source-drain current.

group III. Solutions down to 50.000 virus particles ml⁻¹ can be measured and single binding events of virus particles to antibodies can be distinguished. Also the selectivity has been tested by adding a structurally similar virus, the paramyxovirus, resulting in zero sensor response, since no specific antibody-antigen complex can be formed.

8.3 Proposal

8.3.1 Aim of the project

Here we propose a new type of general, true virus detecting, nanobiosensor. This sensor will be a versatile sensor that is going to be designed in such a way that it can be used for the detection of any virus. Our electronic sensor is based on a dual-gate organic field-effect transistor. Antibodies against virus particles serve as the recognition elements and are covalently linked to the top insulator. By using the dual-gate geometry and selective attachment of the antibodies only to the transistor area we will obtain a sensor with excellent selectivity, high sensitivity and a low detection limit.

8.3.2 Description of proposed sensor

8.3.2.1 Field-effect transistor

The most significant parameters of a sensor are the selectivity, sensitivity and detection limit, as described above. Very important for a good sensor is a low detection limit. To obtain a low detection limit a field-effect transistor setup appears to be the most promising due to the signal amplification which is inherent in transistor devices. Next to the detection limit also the sensitivity benefits from the signal amplification, as shown before by the silicon nanowire biosensor with its ability of distinguishing single virus particles. Moreover field-effect transistor based sensors can measure in liquid environments and they are easily integrated in any electrical circuit, allowing fast electronic readout and real-time detection. The new general type of nanobiosensor we propose for the detection of viruses is therefore based on a field-effect transistor setup.

Although a good detection limit (50.000 virus particles ml⁻¹) has been reached with a field-effect transistor based nanobiosensor, much lower detection limits are possible using a slightly different geometry^[35]. The described sensor using silicon nanowires has a field-effect transistor with a large conduction channel length L (2 μm) but a very small conduction channel width W (the width of a single silicon nanowire)^[34]. The theoretical study of Zhou and Wei^[35], based on computer simulations, shows however that relatively high detection thresholds are an intrinsic property of geometries in which $L > W$. On the other hand, geometries in which $W > L$ have much lower, to nearly absent, detection thresholds. It is therefore expected that field-effect transistors with small source-drain distances and large widths result in better sensors with much lower detection limits.

To obtain such a field-effect transistor with a wide conduction channel, a relatively large semiconductor surface is necessary. Organic materials, like polymers, are suitable materials of choice, since large area coverage is readily attainable due to their straightforward processability. Therefore we will focus on these organic materials as the semiconductor material for our field-effect transistor based sensor.

8.3.2.2 Dual-gate field-effect transistor

Unfortunately, the sensitivity of a field-effect transistor with an $L > W$ geometry is a little lower than that of one with a $W > L$ geometry^[35]. To overcome this problem and increase the sensitivity again, we will use a dual-gate field-effect transistor in our sensor. A dual-gate field-effect transistor has a bottom contact geometry together with an extra insulating layer and gate on top of the semiconductor (see Figure 1 - right). The main advantage of a dual-gate field-effect transistor is the possibility to use the second gate to accurately control the threshold voltage^[36-40], the voltage at which the transistor switches from its non-conducting OFF state to the conducting ON state. Around the threshold voltage the sensitivity of a sensor is very high, since binding of a few analytes is enough to induce the OFF-ON switch, obtaining a large change in current (see Figure 2 - left). By changing the threshold voltage, the high sensitivity can be shifted to a concentration region of interest, for example a very low concentration to obtain a low detection limit (see Figure 2 - right). However, the sensitivity in other regions can also be increased by changing the threshold voltage again, leading to a large range with highly improved sensitivity.

8.3.2.3 Insulating layer

For a dual-gate field-effect transistor a second insulating layer, on top of the semiconductor, is necessary. Since the insulating layer also has to be applied to the whole transistor area, again organic

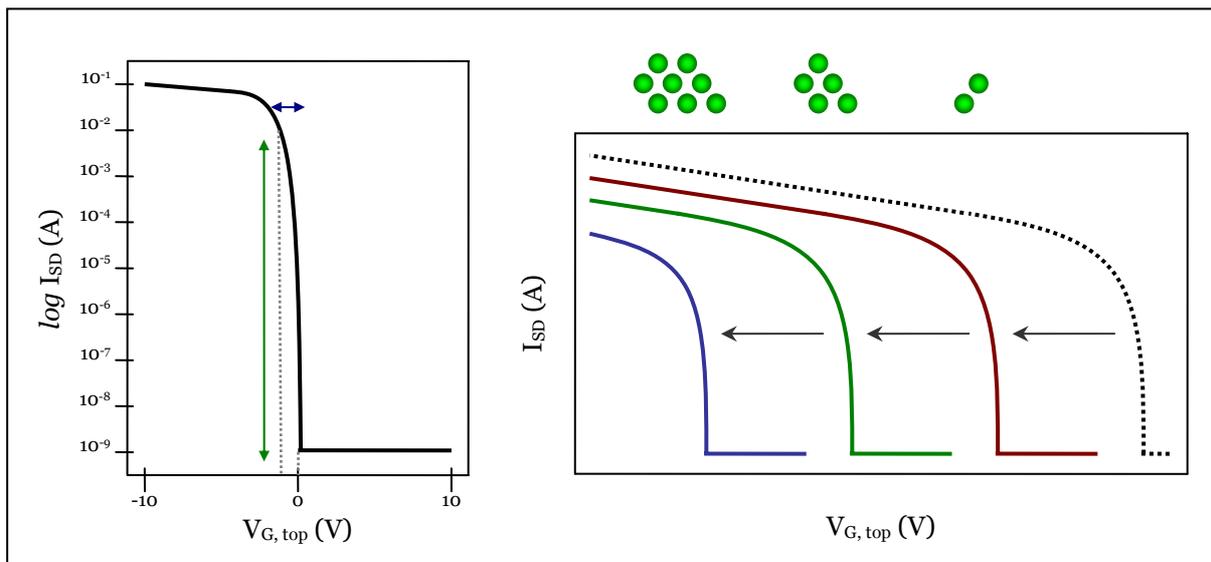


Figure 2: Left: high sensitivity around the threshold voltage - The sensitivity of a sensor is highest around the threshold voltage as can be seen from the transfer characteristics of a field-effect transistor. A small (additional) voltage on the top gate of ~ 1 Volt, corresponding to binding of a few analytes, (blue arrow) results in a large change in source-drain current (I_{SD}) of many orders of magnitude (green arrow). Right: threshold voltage shift - The black, dotted curve shows a typical source-drain current without an active bottom gate. Only the top gate, or the presence of analyte molecules, induces changes in source-drain current. The sensitivity of a sensor is highest around the threshold voltage where the variation of source-drain current is large (see left figure again). In the depicted range of analyte concentrations the variation of source-drain current is relatively small: the sensitivity of the sensor is low (black, dotted curve). By applying a voltage on the bottom gate the threshold can be shifted (red curve). Now the sensitivity is high at low analyte concentration. By changing the bottom gate voltage again, high sensitivity can be shifted to higher analyte concentrations (green and blue curves). In this way a large range with highly improved sensitivity, including a lower detection limit, can be obtained.

materials will be used because of their easy solution processability. Also the thickness of the insulating layer can easily be adapted with these materials via spincoating. This is important for the quantitative influence the analytes will have on the conduction channel in the semiconductor. We propose to use different analytes, which all might influence the conduction channel in their own way. Therefore it is necessary to have the ability of easily adapting the thickness of the insulating layer.

Next to acting as an insulating layer, and also as a protection layer for the organic semiconductor, the insulator also functions as an active grafting side to attach the recognition element. As described before, a recognition element showing high selectivity is essential for a well working sensor, since the sensor should only respond to the target virus and not to any other virus. A proven method to obtain high selectivity is using antibodies against the specific virus as the recognition element of the sensor, which we will utilize in this project. Most studies however focus on a specific virus that will be detected by the sensor. Our sensor will be a general sensor that is able to detect many different kind of viruses, depending on the type of antibody attached to the insulating layer. Therefore we will look for a general method of covalently attaching different kinds of antibodies to our sensor. Using organic materials as the insulating layer is an advantage for attachment, since these materials are easily functionalized, so a well-working and general attachment method can be developed.

8.3.2.4 Selective attachment of antibodies

A final step to obtain even higher sensitivity and lower detection limits will be the selective attachment of the antibodies to the insulating layer. Confined attachment of recognition elements to the sensor surface is not often addressed in literature. Nevertheless it is very useful, since due to the processing methods the insulating layer will not only be present directly on the transistor area but also in the surrounding area. When the antibodies will be attached to the whole area of the insulating layer many virus particles might bind next to the transistor. In that case these virus particles will not influence the conduction channel and therefore will not be detected. When the antibodies are attached only right above the conduction channel of the transistor every binding event influences the channel and contributes to the output signal. Therefore sensitivity and detection limit can be highly improved.

8.3.3 Materials and methods

8.3.3.1 Process schemes

The sensor we propose will be based on a dual-gate organic field-effect transistor. Our standard sensor will be constructed on an n-doped silicon wafer that both acts as the substrate and the bottom gate electrode. The silicon wafers will be purchased with an insulating layer of silicon oxide on top. The conduction channel in the semiconductor has to be wide and small, as described above. In order to keep the total transistor area as small as possible a pattern of interdigitated source-drain electrodes will be used to confine the transistor channel in the semiconducting layer. Standard photolithographic methods will be used to obtain these electrodes on top of the silicon oxide. First a photoresist will be

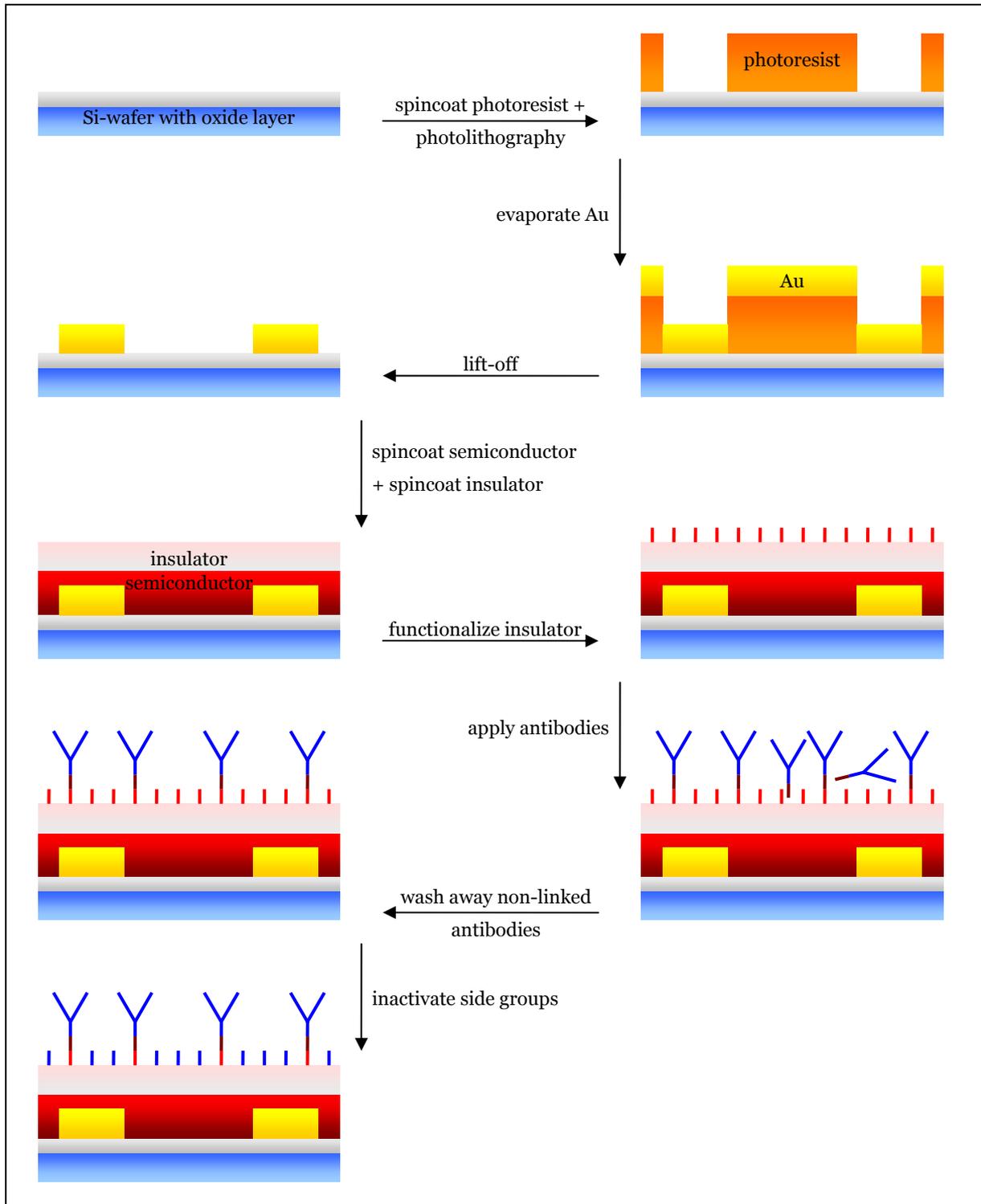


Figure 3: Processing flowchart of the standard sensor

spincoated on the substrate, followed by irradiation with UV light through a mask. After development gold will be evaporated and afterwards remaining photoresist will be removed, resulting in a pattern of source-drain electrodes on top of the silicon oxide. Next the semiconductor will be spincoated on top of the complete substrate, including the electrodes, followed by spincoating of the top insulator. Subsequently, the insulator will be functionalized and/or activated if necessary. Then the antibodies will be applied from solution in order to covalently link to the insulator. After reaction, unbound or physisorbed antibodies are washed away. If necessary, the remaining free side groups are inactivated and turned into hydrophilic ones for compatibility with the aqueous solutions used during electrical measurements.

To obtain an even higher sensitivity and lower detection limit, we will selectively attach the antibodies to the insulating layer. The preferred way of doing this is with standard photolithography (see Figure 4). On top of the functionalized and/or activated insulator a negative photoresist is spincoated. Then the photoresist is irradiated with UV light through a bright-field mask to transfer the pattern of the active transistor area to the photoresist. In this way any possible damage to the semiconductor in the active area of the transistor is avoided. Subsequently, the photoresist is developed to remove non-crosslinked photoresist from the active transistor area. Next the device is exposed to an antibody solution in order for the antibodies to link to the insulator. After washing away

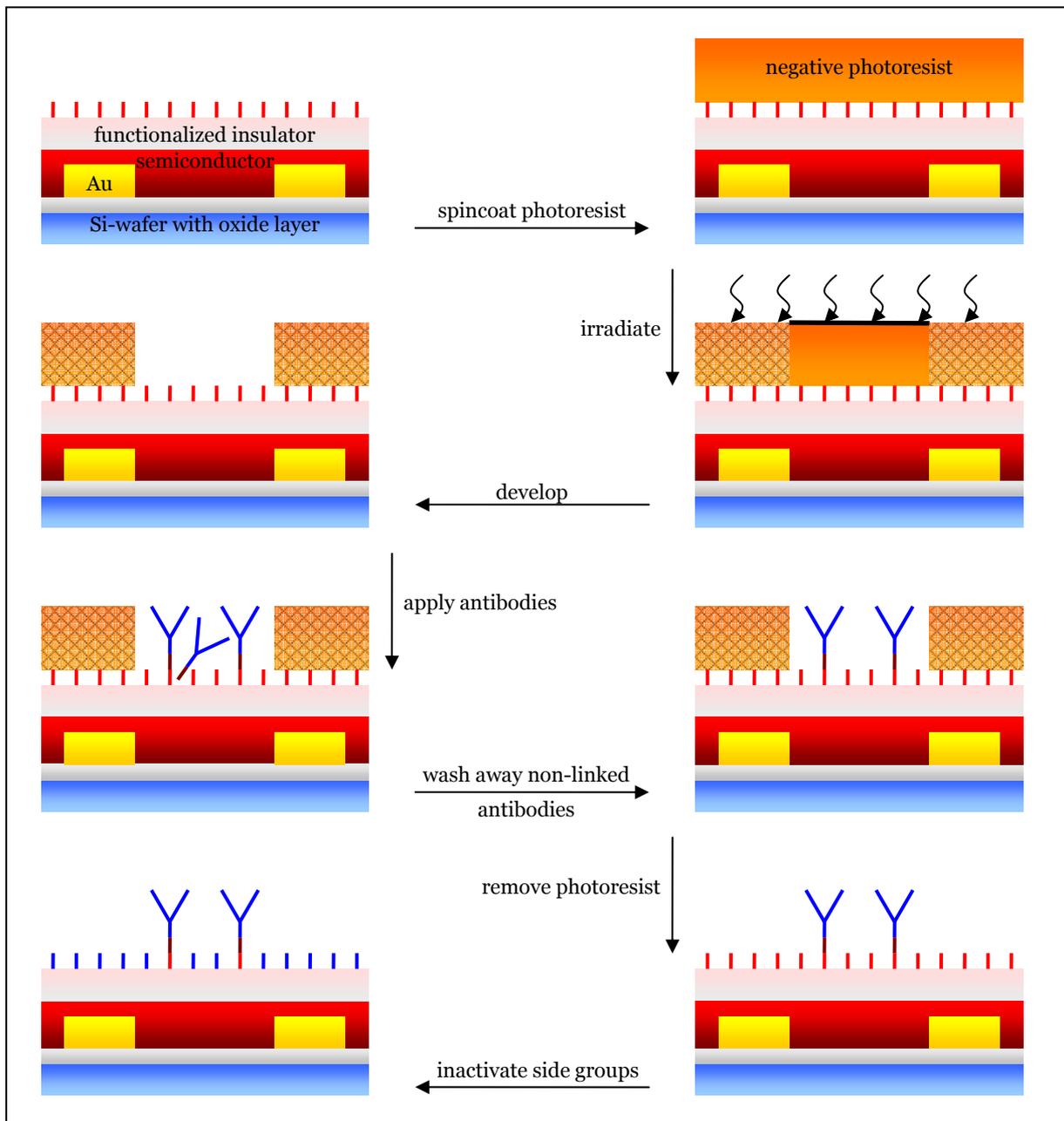


Figure 4: Processing flowchart for selective attachment of antibodies

unbound or physisorbed antibodies the remaining crosslinked photoresist is removed. Finally, if necessary, the remaining freed side groups are inactivated and turned into hydrophilic ones as before.

8.3.3.2 Semiconductor and insulator material

The semiconductor we will use has to be easily spincoated from solution. Therefore we will make use of conducting polymers. These conducting polymers have to result however in transistors with good characteristics. This means low currents in the OFF state, high currents in the ON state, ON/OFF ratios of $\sim 10^6$, and very low leakage currents. Next to this the conducting polymers should be stable in air and preferable also in aqueous environments. Suitable materials might be hole-conducting polytriarylaminines (PAA), regioregular poly(3-hexylthiophene) (rr-P3HT), poly(4,4'-didecylbithiophene-co-2,5-thieno[2,3-b]thiophene) (PDTT) and polyfluorene tetraphenylene diamine (PFTPDA).

The insulator has to be spincoated on top of the semiconductor and should have excellent dielectric properties. Again polymers are an appropriate choice. It should however be possible to spincoat these polymers from a solvent that is a non-solvent for the semiconductor to avoid lift-off of the latter layer. Furthermore, these polymers should have either the right functional groups incorporated in the polymeric backbone or sidechain or side groups that can be easily changed into the desired functional groups. As with the semiconductor, the insulator should also be stable in air and in aqueous environments. Suitable materials might be (co-)polymers based on polymethylmethacrylate (PMMA), functionalized polystyrene (PS) and poly(4-vinylpyridine) (P4VP).

8.3.3.3 Immobilization of antibodies

The insulating layer should have the right functional side groups or should be functionalized before antibodies can be attached. Since we are looking for a general method of covalently attaching different kinds of antibodies to our sensor, we will use membrane-bound antibodies from B cells. These antibodies have a common hydrophobic transmembrane tail on their C-terminal end^[5] that will be a useful target of our general attachment method. To obtain proper attachment the insulating layer therefore should be hydrophobic and have a functional group that will readily react with a carboxyl group.

Another way of immobilizing the antibodies to the sensor is by constructing an artificial lipid bilayer on top of the sensor^[41]. This lipid bilayer can either be applied on top of the insulator or fully replace the insulator as long as its dielectric properties are sufficient. For proper formation of a lipid bilayer the surface has to be hydrophilic, so the insulator and/or semiconductor should have hydrophilic side groups. When antibodies are applied they will insert their hydrophobic transmembrane tail into the lipid bilayer. An advantage of this method is that the outside of the bilayer is hydrophilic, eliminating the necessary of converting or inactivating side groups afterwards. The main disadvantage of this immobilization method is however that selective attachment of the antibodies is much more difficult. Also antibodies might start to diffuse laterally in the lipid bilayer^[5], moving to areas surrounding the transistor, instead of staying directly above the active area.

8.3.3.4 Antibodies and viruses

Since we design a general sensor for the detection of viruses, we will build different sensors from our standard sensor. The standard sensor will be loaded with different antibodies to detect different viruses in separated experiments to prove the universal applicability of our sensor. Readily available antibodies and viruses will be used. It is important that membrane-bound antibodies from B cells can be easily attained, because of the general attachment method we want to develop. Directly related to the availability of antibodies is the availability of the viruses themselves. Moreover we need to have structural similar viruses in order to check for selectivity. From safety point of view we will only use weakened viruses which originate from viruses that are not lethal to humans and do not cause severe illness. Additionally, in the first experiments to proof the working principle of our sensor we will try to only work with weakened insect and animal viruses that are harmless to humans. We initially suggest using influenza type A virus, avian adenovirus group III and paramyxovirus, but others might also be suitable, depending on availability issues.

8.3.3.5 Sensor characteristics

Our sensor will be characterized using electrical measurements. First a real dual-gate transistor with a metallic contact evaporated on top of the insulating layer will be constructed. This transistor will be measured to obtain the semiconductor characteristics and the threshold voltage behavior. Next the standard sensor, with antibodies attached, will be measured. Solutions containing different viruses will be applied to the sensor. Detection limits will be determined by lowering the concentration of virus particles in the solutions until the lowest detectable concentration is reached. Sensitivity and the

sensitivity range will be determined by adding small amounts of virus particles to the sensor and changing threshold voltages to cover a large range of concentrations. Finally selectivity will be verified by adding virus particles with similar structures to the sensor that should not bind to the antibodies and should not result in any output signal.

8.3.4 Time planning

- First year: A dual-gate transistor will be produced similar to the standard sensor with a metallic top gate instead of antibodies. The devices, fabricated from commonly available conducting and insulating polymers that are suitable for attachment of antibodies, will be characterized by electrical measurements in vacuum and in air. We will try to understand the results obtained from these measurements and relate them to for example layer thicknesses, dielectric material properties, influence of water or moisture and doping. From this understanding, the most promising and suitable set of conducting and insulating polymers that are stable in air and aqueous environment will be selected and the device parameters for this set of polymers will be modeled. The results obtained from the measurements and the model are used to optimize the device parameters developing a device structure with the best properties.

Next, antibodies will be attached to the (functionalized) insulating polymer and the surface will be studied to confirm that attachment takes place. Later, the attachment will be explained in a more quantitative way by looking at the surface coverage and this will be related to the concentration of antibodies supplied to the surface. In the end, viruses will be added to see if binding to the antibodies still takes place and binding of the viruses to the antibodies will be explained in a more quantitative way.

- Second year: The standard sensor with antibodies covalently linked to the insulator will be produced and it will be characterized by electrical measurements in air. We will try to understand the results and relate shifts in threshold voltage to the charges present due to the attached antibodies. The sensor parameters, and the influence of the antibodies, will be included in our model to explain the influence of different kind of antibodies. In addition we will try to shift the threshold voltage to demonstrate the principle of our sensor.

Next, the influence of an aqueous environment and the solutions used to supply viruses to the sensor will be measured. Again we will try to understand any changes in the device characteristics, try to relate them to the supplied solutions and include them in our model. Subsequently, viruses will be added to the sensor and we will try to understand the behavior of our sensor. The knowledge from the measurements and the model will be used to determine the sensor characteristics. We will add different types of viruses to our sensor to study the selectivity. By adding different concentrations to our sensor, a dose-response curve will be constructed to extract sensitivity and detection limits of different standard sensors for various viruses. The characteristics of the different sensors will be compared and we will try to understand differences and relate them to the device structures, antibodies and viruses. In the end all information will be included in our model, to obtain a general model that describes our sensors.

- Third year: The artificial lipid bilayer will be investigated as an alternative method to attach antibodies to the sensor surface. The attachment will be studied in a quantitative way and we will try to understand the origin of any differences with the standard method. We will try to observe lateral diffusion and establish the rate of diffusion. When we understand the mechanisms involved, we might be able to avoid lateral diffusion such that selective attachment is also possible with this attachment method. Eventually sensors will be produced with antibodies attached through these artificial lipid bilayers. We will try to understand the characteristics of these sensors in terms of dielectric properties of the bilayer, lateral diffusion, lipid polarity and so on.

Next, the antibodies will be attached selectively to the sensor surface in, for example, large squares by the photolithography process. The surface will be studied to confirm that selective attachment takes place and to see the influence of selective attachment on surface coverage density. When we understand the mechanisms involved in selective attachment, we will scale down the selective attachment to the transistor active areas only.

Finally, sensors will be produced with selectively attached antibodies. Sensors with different sized areas of selectively attached antibodies will again be characterized. We will try to understand the changes in sensitivity and detection limit and relate them to the selective attachment and surface coverage of the antibodies. The influence of selective attachment will be added to our general model to optimize the model.

- Fourth year: This project will be finalized and evaluated and the results will be summarized in a PhD thesis. We will propose new projects that will be related to this project and use the knowledge obtained from this project.

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