Solid supported lipid monolayer: From biophysical properties to sensor application

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INTRODUCTION
Introduction

From the first studies on the in-vitro self assembly of lipid bilayers by the team of Rudin in the 60’s\(^1\), bilayer lipidic membranes (BLM) as well as liposomes, have become the most commonly used experimental models of cell membranes. These biomembranes which constitute the external wall of cells and organelles (Figure 0.1) are the place of energy conversion, material transportation, bio-detection and signal transduction.


As the main component of cell membranes, phospholipids have triggered lots of interest in the scientific community and especially in the biomedical field. The amphiphilic nature of phospholipids governs in particular the formation of the membrane in aqueous medium in which the lipids are free to diffuse. The molecules arrange themselves in bilayers by positioning their polar head groups towards the aqueous media outside the membrane and their lipophilic chains towards the inside of the membrane therefore forming a non-polar region between two polar ones, see Figure 0.2.

In the form of vesicles, bilayers can be used as model systems of cells\(^2\) or to encapsulate active products or particles for the transportation of drugs or in-vivo targeting\(^3,4\). These bilayers can also be transferred on a substrate, thus allowing the biofunctionalization of inorganic or polymeric substrates and endowing them biomimetic functions\(^5,6\). They therefore constitute ideal matrices for the incorporation of membrane proteins or receptors that can be used for in-vitro studies of numerous biological mechanisms involving specific target/receptor assemblies, such as understanding the functioning of ion channels\(^7,8\), cell adhesion\(^9\), measuring the activity of certain enzymes\(^10\) as well as for the development of biosensors\(^11\).
In this thesis our interest towards lipids is related to another property of the biomembranes, i.e., their insulating properties. Indeed, in cell membranes, the lipid bilayer separates the inner and outer regions of cells and forms a barrier to ionic transport between these two regions; ionic exchange is controlled by ionic channels embedded in the bilayer. In addition biomembranes also present a high barrier to electronic transport with a resistance across the bilayer of the order of the giga-Ohm. Our idea is to exploit this property and use a lipid monolayer as the gate dielectric in the development of a field effect transistor based biosensor (Bio-FET) as a replacement to commonly used silicon dioxide layer. The purpose here is to decrease the operating voltages of such devices by reducing the thickness of the gate dielectric layer while maintaining good insulating properties and preventing organic/biological molecules damages. Lipid monolayers with thicknesses ranging from 2-3 nm are therefore good candidates. However a major drawback of these layers is their instability in air which has so far been a limitation to more extensive use in industrial processes. To overcome this problem, we have selected a lipid which can polymerize in the plane of the layer, therefore increasing its stability. The work reported in this thesis is focused on the properties of this polymerized supported lipid monolayers. Nano-mechanical and electrical properties have been investigated and we demonstrate a net improvement of both properties after polymerization which make the layer suitable for its use in a bio-FET.

Figure 0.2 a) Structure of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC. The hydrophilic headgroup and lipophilic chains are shown in blue and orange respectively. b) Schematic representation of a micelle, lipid bilayer and liposome (vesicle). Source: http://en.wikipedia.org/wiki/Lipid.
This manuscript is divided in five main parts. In the first one, the functioning of atomic force microscope and its different modes are described. This technique has been used in imaging mode as well as force spectroscopy mode to study the mechanical properties of the lipid monolayers. The second part is dedicated to the formation of the supported lipid monolayer and its stabilization. In the third part, the nano-mechanical properties of both non-polymerized and polymerized supported lipid monolayers are studied using force measurement. Both Young modulus and intrinsic Gibb’s energy of the tip/monolayer system are concluded for both types of monolayers. The fourth part concerns the electrical characterization of the layer. In addition to leakage current and breakdown voltage measurements we demonstrate a very interesting property of autonomic self-healing of the monolayer after dielectric breakdown. Finally, we show in the fifth part a demonstration of the use of the lipid monolayer as a gate dielectric in a field effect based biosensor. An example of sensing experiment is shown for the detection of ferric ions.
CHAPTER 1:
EXPERIMENTAL TECHNIQUES
1 Experimental techniques

1.1 Atomic Force Microscopy (AFM)

1.1.1 Introduction

Atomic force microscopy is one variety of the Scanning probe microscopy (SPM) techniques that was invented by Binnig et al. in 1986. It is a modern technique for generating high resolution surface topography images compatible with insulator, conductor, organic and inorganic materials and as well as for several orders of magnitude below the optical diffraction limit. The principal relies on a sharp tip supported by a cantilever used to scan over the surface. The cantilever is deflected by a variety of forces generated between the tip and the sample according to Hook’s law, and the deflection is quantified by the displacement of a laser reflected off of the back of the cantilever onto an array of photodiodes.

In addition to topographic information, AFM is also used for force spectroscopy where it can measure nano-scales forces between tip and sample as a function of distance. Indentation forces and adhesion forces can be measured. In order to quantify forces the spring constant and the calibration of the detection system must be studied.

In our study, AFM was used to study the topography of our lipid monolayer and to study the mechanical stability of the monolayer by force measurements. Measurements were realized in both aqueous medium and in air.

1.1.2 Description

AFM is formed of three essential components: A three axes piezoelectric stage, a cantilever and a detection system. A scheme is depicted in Figure 1.1.
The 3-axes piezoelectric stage allows the scanning of the sample. The movements X and Y are controlled by a computer that generates two synchronized voltage ramps allowing the scanning of the chosen area. The Z movement is controlled by the feedback circuit. In our AFM configuration, the sample is fixed on top of the piezoelectric stage and the tip is fixed during the scanning. A reverse configuration is possible, where the sample is fixed and the scanning realized by the tip. The first configuration is usually preferred as less noisy.

The cantilever is the key element in AFM imaging and force measurements, it is usually formed of silicon or silicon nitride covered by 1-2 nm of a native oxide layer. The upper face of the cantilever might be covered with a metal layer (gold, aluminum) to increase its reflectivity. At the end of the cantilever there is a nano-metric tip. During the image acquisition the cantilever bends up and down due to the interaction forces between the tip and the sample, providing information on the tip-sample distance.

A major challenge in AFM measurements is choosing the right cantilever for the right experiment. The geometrical parameters of the cantilever (shape, length, width, thickness) determine its spring constant which can vary from 0.01 to 40 N/m. The choice of the cantilever will depend on the interaction of the tip with the sample. When the interaction is strong, either attractive or repulsive, a cantilever with higher spring constant will be preferred, whereas softer cantilevers will be favored when interactions are weak. The spring constant can be determined from the geometrical parameters of the cantilever as indicated by equation 1.1 for example for a rectangular cantilever.

$$K_C = \frac{Ewt^3}{4L^3}$$  \hspace{1cm} (1.1)
With E, the young modulus, and w, L and t, respectively the width, the length and the thickness of the cantilever. Unfortunately, each cantilever is slightly different and the geometry parameters not always known precisely enough. We will see later that other methods have been developed to precisely determine this parameter for each cantilever.

Another important characteristic of the cantilever is its resonance frequency; in general this frequency should be high enough in order to consider external vibrations such as the building vibrations, the table vibrations and the noise negligible and less transmitted to the cantilever. In plus, the quality factor of the cantilever is expressed by: $Q = \frac{f_c}{\Delta f}$, with $\Delta f$ the width of the range of frequencies for which the energy of the oscillator is at least its half value, this rapport shows that for higher frequencies the cantilever have a better quality factor.

The resonance frequency is expressed by:

$$f_0 = 0.1615 \frac{t_c}{L^2} \sqrt{\frac{E}{\rho}} \quad (1.2)$$

With: $\rho$ the density of the cantilever material. Commercial cantilevers have a typical resonance frequency in the range of 10-400 KHz in air. One important point should be kept in mind; the given value of $f_0$ refers to a cantilever oscillating in a free space without any interaction forces, the phase shift between the excitation force and the tip respond $\theta_0$ is 90° and the amplitude is at its maximum.

![Figure 1.2 the resonance frequency peak shift as function of the sample-cantilever interaction force](image)

When the cantilever is brought near the surface the oscillating regime will change due to the sample-cantilever interaction forces. If the cantilever is experiencing attractive forces, the
resonance peak shifts toward lower frequencies and the phase shift $\theta_0$ becomes larger than 90°. In case of repulsive forces the resonance peak shifts towards higher frequencies and the phase shift $\theta_0$ becomes smaller than 90°\textsuperscript{12,13}. This behavior is shown in Figure 1.2. The sensitivity of cantilevers is related to their geometric shapes and to their stiffness. Small and thin cantilevers are highly sensitive, they have high resonance frequency and they are soft so we will have big deflection for small forces. The smallest cantilevers are 10 $\mu$m long, 0.1-0.3 $\mu$m thick and 3-5 $\mu$m wide.

The tip can have different shape, and different radius that vary between 1 to more than 60 nm. It is synthesized out of different materials but usually of silicon. The tip are also different according to the type of measurement, conductive tips for example can be used to do local electrical measurements, and the magnetic coated tips give the mapping of magnetic stray field distribution. The geometry and the radius of the apex of the tip will determine the lateral resolution of the measurement.

**The detection system:** According to the sample roughness the deflection can be very small (<1nm) so the detection system must be able to detect such a tiny variation, that can go below 1 nm. The system is formed by a laser diode and a two or four quadrants photodiode. The laser spot is focused on the extremity of the cantilever (just over the tip) and is then reflected in the center of the two or four quadrants of the photodiode. When the cantilever is deflected, the position of the reflected laser beam changes and each quadrant gives a signal proportional to its illuminated area; this system can simultaneously measure the vertical and the horizontal displacement of the spot. Two quantities are then measured the deflection of the cantilever due to the attractive or repulsive force (topography) and the torsion of the cantilever due to the lateral component of the force. Other detection systems can be used like capacitive detection or interferometry. The laser detection method presents two main advantages: it has a good signal to noise ratio, and gives the force components in the two directions.

### 1.1.3 Forces in AFM

Depending on the distance between the tip and sample, several forces are encountered and responsible for the cantilever deflection. Few examples will be cited in the following paragraph
Long ranged forces (>1nm):

**Van der Waals** is a weak usually attractive force generated from a dipole-dipole interaction. It includes 3 types of interactions: the Keesom interaction between two existing dipoles, the Debye interaction between induced and existing dipole and the London interaction. Between the tip and the flat surface the Van der Waals interaction can be expressed by:

\[ F_{vdw} = -\frac{C}{r^6} \]  \hspace{1cm} (1.3)

with a constant that takes into account the polarization properties of the two atoms or small molecules and \( z \) the distance between the tip and the surface. Van der Waals interaction depends on the tip and sample environment, it can be reduced if the sample and tip are immersed in water for example.

**Electrostatic forces** are very strong long ranged forces. Their intensity depends on how charges are positioned in the media involved in the interaction. In the air, electrostatic forces can be minimized with proper experimental set-up as, for example, grounding the tip.

\[ F_{electrostatic} = \frac{1}{2} \frac{\partial C}{\partial z} \Delta V^2 \]  \hspace{1cm} (1.4)

With \( C \) the capacitance, \( Z \) the distance, and \( \Delta V \) the bias voltage between the tip and the sample.

Short ranged forces (<1nm):

**Capillary forces**: When working in air, capillary force is seen if the sample is covered by a water layer, or if the water vapor is condensed between the tip and the sample. Those attractive forces are 10 times bigger than the Van der Waals forces and can deteriorate fragile tips or samples. Capillary force between a sphere of radius \( R \) and a flat surface separated by a distance \( d \) is given by:

\[ F_c = \frac{4\pi R(\gamma \cos \Theta)}{(1 + \frac{d}{R})} \]  \hspace{1cm} (1.5)

where \( \Theta \) is the contact angle and \( D \) is the height of the tip immersed in the water meniscus and \( \gamma \) the surface tension. Working in water media helps avoiding those forces.

**Inter-atomic repulsion forces**: those forces are involved at very small tip-sample distance (0.2-0.3 nm) when the electron clouds of the tip and the sample are in contact.
\[ F_{\text{rep}} = \frac{A}{r^{12}} \quad (1.6) \]

Where \( r \) is the distance between the two atoms and \( A \) is a constant that can be obtained from atomic polarizability measurements.

One of the good representations of the force versus distance between two atoms is the Lennard-Jones interaction proposed in 1924\(^{14} \) (see Figure 1.3). The potential that describes this interaction is given by:

\[ w(r) = 4\varepsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6 \right] \quad (1.7) \]

With: \( \sigma \) the diameter of a sphere approximating the atom or molecule and \( \varepsilon \) the minimum energy.

\( z_0 \) correspond to a stationary point for the potential. For a distance larger than \( z_0 \) the interaction between two atoms is attractive, when the distance is smaller than \( z_0 \) the interaction becomes repulsive. 

*Figure 1.3 the Lennard-Jones presentation of the potential versus the distance between two atoms.*
1.1.4 AFM operating mode

Imaging by AFM can be done with different modes. The most used one are the contact mode, noncontact mode and the tapping mode. The main difference is the distance between the tip and the sample.

A. Contact mode:

In this mode, the tip is maintained in contact with the sample, strong short-range repulsive forces are measured. Contact mode can be done at constant force. In this configuration, the feed-back loop keeps the cantilever deflection constant by adjusting the distance between the tip and the sample accordingly. The image contrast is derived only from the z displacement of the piezo necessary to recover the initial position of the reflected laser beam on the photodiode.

In contact mode the sample can also be scanned at constant tip-sample distance, in this case the z position of the piezo is maintained constant and one measure the cantilever deflection. This mode can only be used with relatively flat surfaces, when there is no risk of damaging the tip while scanning by bumping on high aspect ratio structures.

B. Non contact mode

In this mode the sample and the tip are never in contact. The tip is oscillating at a frequency slightly above its resonance frequency with a smaller oscillation amplitude (<10nm). While scanning, the long ranged forces (Van der Waals, electrostatic forces ...) will decrease the resonance frequency of the cantilever. A feed-back loop will keep constant the amplitude or the frequency of the oscillation.

C. Tapping mode:

In this mode, the cantilever is excited near its resonance frequency above the sample, usually the amplitude of this oscillation is greater than 10 nm so the tip hits the sample at the lowest point of each oscillation. During the scan the intermittent interaction between the tip and sample will generates forces that will change the amplitude of the cantilever oscillation. A feed-back loop will keep constant the frequency or the amplitude of the oscillation by adjusting the z position of the sample. This mode is the most used for biological samples since it is not disruptive for fragile
samples. It eliminates the friction force which is dominant in the contact mode, and offers a better resolution than the non contact mode.

In our study, all the AFM images of the lipid monolayer were obtained by tapping mode.

1.1.5 Force measurements by AFM

Force measurement by AFM was introduced in 1984 by Florin et al.\textsuperscript{15}. This technique employs tip functionalized by specific molecules to measure their interaction with others in order to study specific forces by means of force-distance curve. Common examples are the measurement of cell-cell adhesion forces or the force of individual ligand-receptors pairs. The technique is also being used to realize nano-indentation experiments and provides quantitative values of materials properties like elasticity and hardness. In this work, we have used force measurements to study the nano-mechanical properties of supported lipid monolayers and to evaluate their elastic properties.

Force-Distance curves

An AFM force-distance curve is a plot of the interaction forces between a tip and a sample versus the tip-sample distance. To obtain such curve, the sample (or the tip) is moved forward to the tip (or sample) in the z direction then backward and the cantilever deflection is acquired along the cantilever displacement. The deflection is the result of two contributions: the tip-sample interaction and the cantilever elastic force. At a given tip-sample distance, the cantilever bends until its elastic force is equal to the tip-sample interaction according to Hooke's law:

\[ F = k_c \, \delta_c \]  

with \( k_c \) the spring constant of the cantilever and \( \delta_c \) the cantilever deflection.
Figure 1.4 Schematic showing the interaction of the tip with the sample. Z is the tip-sample distance when the cantilever is at rest. D is the actual tip-sample distance, δc the cantilever deflection and δs the sample deformation.

During the measurement, the distance which is controlled is not the tip-sample distance D but the tip-sample distance Z when the cantilever is at rest, see Figure 1.4. These two distances differ due to the cantilever deflection δc and to the sample deformation δs. These four quantities are related as follows:

\[ D = Z - (\delta_c + \delta_s) \]  

(1.9)

**Approach and Withdrawal curves**

A typical deflection-distance curve experiment is depicted in Figure 1.5. Both approach and withdrawal force-displacement curves can be divided into three distinct parts: the contact line, the non-contact region and the zero line. The latest is obtained when the tip and the sample are sufficiently far apart and do not interact (Regions A and E). The cantilever shows no deflection and the force is zero. When the tip and sample are in contact (Regions C and D), the tip-sample distance D=0. In this case the deflection is related to the cantilever displacement as:

\[ k_C \delta_C = \frac{k_C k_S}{k_C + k_S} Z = k_{\text{eff}} Z \]  

(1.10)

With k_s the elastic constant of the surface16, and k_{\text{eff}} the effective elastic constant of the cantilever-sample system. The corresponding lines obtained in the Force-Displacement curves are called the "contact lines". From equation 2.10, one can see that the slope of the force-displacement curve is a
measure of the stiffness of the sample. If the sample is much stiffer than the cantilever, i.e., \( k_S \gg k_C \), then \( k_{eff} \approx k_S \), and when the cantilever is much stiffer \( k_{eff} \approx k_C \).

![Diagram](image)

*Figure 1.5 Force-distance curve, showing the cantilever deflection vs the cantilever displacement. The blue arrows indicate the direction of sample displacement.*

Regions B and B' are the two non-contact regions and are referred as the "jump-to-contact" and "jump-of-contact" respectively. These discontinuities occur when the force gradient becomes higher than the effective elastic constant of the cantilever-sample system. In the approach curve, this region gives information about attractive or repulsive forces before the contact. In particular the product of jump-to-contact cantilever deflection and \( k_C \) equals the pull-on force, i.e. the maximum value of the attractive force before contact. When measurements are realized in air, capillarity forces for example will very often cause this type of behavior. In the withdrawal curve, the non-contact-region gives information about adhesion forces between the tip and the sample. It corresponds to the pull-off force, i.e., the product of the cantilever deflection by the spring constant \( k_C \). In general the adhesion force is a combination of the electrostatic force \( F_{el} \), the Van der Waals force \( F_{VDW} \), the capillary force \( F_c \) and the chemical force \( F_{chem} \):

\[
F_{ad} = F_{el} + F_{VDW} + F_c + F_{chem} \quad (1.11)
\]
From Deflection-Displacement curve to Force-Distance curve

Raw data are obtained in terms of cantilever deflection versus cantilever displacement, the deflection corresponding to a variation of current measured by the four quadrants of the photodiodes. The amplitude of the deflection will depend on the tip geometry, on its spring constant but also on the position of the laser on the cantilever. To convert this deflection current into bending distance of the cantilever, reference force curves are measured, with the same set-up, on a sample hard enough to not undergo any deformation under the tip. In this case, in the contact line region and according to equation 1.9, the deflection equals the displacement. This method allows the calibration of the system and needs to be done for every tip and every adjustment of the laser. The force can then be extrapolated from Hooke's law (equation 1.8) and the tip-surface distance from Figure 1.6.

![Figure 1.6 The deflection vs piezo displacement curve (a) first recorded in a force measurements by AFM on a SiH surface and the equivalent force versus distance tip-surface curve (b).](image)

Spring constant calibration

Usually the spring constant (elastic constant) and the resonance frequency are delivered by the manufacturer but for each cantilever there will be considerable difference between the real and the delivered values. In order to have accurate determination of the force, the spring constant of the cantilever needs to be determined precisely. There are many methods to calculate the spring constant, we used the thermal noise method proposed by Hutter and Bechhofer\(^{17}\). In this method
the cantilever is maintained away from the surface to prevent any interaction. It is then considered as a harmonic oscillator, and one can determine the free oscillation of the cantilever due to thermal excitation. The mean square deflection $(\Delta Z_c)^2$ due to the thermal fluctuation is related to the temperature as shown in equation 1.12.

$$\frac{1}{2} k_c \Delta Z_c^2 = \frac{1}{2} k_B T \quad (1.12)$$

The cantilever elastic constant can then easily be determined as:

$$k_c = \frac{k_B T}{(\Delta Z_c)^2} \quad (1.13)$$

To determine $\Delta Z_c^2$, we monitor the deflection over time and extrapolate the root mean square (RMS) of this deviation, see Figure 1.7.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{cantilever_deflection.png}
\caption{Cantilever deflection versus time. The cantilever does not interact with the surface and is free to oscillate.}
\end{figure}

To determine $\Delta Z_c^2$, we monitor the deflection over time and extrapolate the root mean square (RMS) of this deviation, see Figure 1.7.

### 1.2 Ellipsometric measurements

Ellipsometry is a non-destructive, contactless optical technique which measures the dielectric properties (complex refractive index and dielectric constant) of thin films. Among many others applications it is commonly used to characterize film thicknesses of single monolayers or complex multilayers going from few angstroms to several micrometers. The principle relies on sending polarized radiation on the substrate and measuring its change in polarization after it has been
reflected from the surface of the sample. The sample must be composed of few stacked layers optically homogeneous and isotropic. When the radiations are reflected off the sample the polarization of the radiation is changed. Ellipsometry measures the reflectance ratio $\rho$, which includes two parameters: The amplitude component $\psi$ and the phase difference $\Delta$. To characterize the polarization state of the incident light upon the sample two parameters are needed: $s$ the component that oscillate perpendicular to the incident plan and $p$ the component oscillating parallel to the incident plane. The amplitude of $s$ and $p$ after reflection and normalization by their initial values are noted: $r_s$ and $r_p$. The measured reflectance ratio is the ratio of these two last values:

$$\rho = \frac{r_p}{r_s} = \tan(\psi) e^{i\Delta} \quad (1.14)$$

Ellipsometry is an indirect method and $\psi$ and $\Delta$ cannot be directly converted into optical constants of the sample. A model of the system is usually required which includes the thickness and refractive index of every layer as well as their stacking order. $\Delta$ and $\psi$ are calculated using the Fresnel equations and using an iterative procedure in which the refractive index and/or thickness of each layer is varied. The values that match the experimental data the best provide the thickness and refractive index values of the layers.

In our study, we used a Sentech model SE-400adv single wavelength (632.8 nm) ellipsometer at an angle of incidence of 70°C to measure the thickness of the lipid layers. Our model system is relatively simple. It is constituted of the substrate, the silicon, an eventual layer of silicon native oxide and the lipid monolayer. The thickness was obtained using a two-layer model with $n = 1.46$ as the refractive index of the lipid layer and silicon oxide and the silicon substrate was described by $n = 3.85$ and $k = 0.02$.

### 1.3 X-Ray Photoelectron Spectroscopy (XPS)

XPS, X-ray photoelectron spectroscopy, is a quantitative spectroscopic technique used to study the atomic composition of a material. It is based on Einstein’s idea about the photoelectric effect developed in 1905, in which the concept of photon was used to describe the ejection of an electron
from a surface when a photon impinges upon it. In XPS, X-rays are used to eject core level electrons from inner orbitals. These electrons have binding energies characteristic of their element, therefore providing a way to study the atomic composition of a material. Each element produces a unique set of electrons with specific energies. By measuring the number of these electrons as a function of binding (or kinetic) energy, an XPS spectrum is obtained. XPS is also sensitive to the direct environment of the element; it induces small shifts i the considered binding energy.

An XPS set-up is composed of an X-ray source, usually producing mono-energetic photons such as MgKα photons with energy of 1253.6 eV or AlKα photons with energy of 1486.6 eV, and a Cylindrical Mirror Analyzer (CMA) which counts and measures the kinetic energy of the emitted electrons. A scheme showing the principle of XPS is shown in Figure 1.8.

Since the energy of the photons is known, the electron binding energy of each emitted electron can be calculated using the Ernest-Rutherford equation:

\[ BE = h\nu - KE - \Phi \]  \hspace{1cm} (1.15)

With \( BE \) the binding energy of the electron, \( h\nu \) the energy of the X-rays photons, \( KE \) the kinetic energy of the ejected electrons and \( \Phi \) the work function.

The number of detected electrons for one specific peak of the spectrum is a direct measure of the elemental concentration. To obtain quantitative information, peaks areas must be divided by standard sensitivity factors which depend on the photon source and on the considered element.

Although X-ray penetrate the matter to typically 1 μm, useful electron signal is obtained only from a depth ranging from 0.1 to 10 nm making the technique very sensitive to the surface and not to the bulk. All experiments are realized in ultra-high vacuum.
Our set-up is an HA150 hemispherical XPS with a resolution of 0.5eV. The X-ray source is constituted of a Mg(Kα) anode.

Figure 1.8 XPS principle: An X-ray photon of energy $h\nu$ is adsorbed by a core electron which is ejected from the surface of the material. These ejected electrons are counted and their kinetic energy measured by a cylindrical mirror analyser.
CHAPTER 2:
FORMATION AND STABILIZATION OF A LIPID MONOLAYER ON H-TERMINATED SILICON SURFACE
2 Formation and stabilization of a lipid monolayer on H-terminated silicon surface

2.1 Introduction

Phospholipids are amphiphilic molecules; the hydrophilic quality of their head-group is a result of the negatively charged phosphate group and the positively charged amine group of Choline which together form a dipole perfectly soluble in water. On the other hand, the two aliphatic chains exhibit hydrophobic behavior. When lipid molecules are deposited as molecular films on a solid substrate, their orientation will depend on the affinity of their two sides with the solid. As illustrated in figure 3.1, for a hydrophilic surface, the first layer in contact with the substrate will be a bilayer with the head-groups facing outside the bilayer, i.e., in contact with the substrate on one side and the aqueous solution on the other side. On the contrary, when the substrate is hydrophobic, the layer is a monolayer and the alkyl chain of the phospholipid is in contact with the substrate. In both cases, additional bilayers can then be formed on top of the first deposited layer. Exposure of such supported layers to air leads to the disruption of the top leaflet, therefore modifying the layer surface properties see Figure 2.1. In the case of a single monolayer adsorb on a hydrophobic surface, it will even lead to the complete removal of the layer. This strong instability in air is therefore a major limitation in using supported lipid layers in advanced protocols. This challenge has attracted the attention of researchers, with previous studies reporting the stabilization in air of acrylated phospholipid bilayers on oxidized silicon or polymers by two-dimensional polymerization initiated with free radicals\textsuperscript{18,19,20}. Stabilization of monolayers has always been a little bit more of a challenge. Although one study has shown the possibility to stabilize an acrylated-phospholipids monolayer on H-terminated silicon by two-dimensional polymerization\textsuperscript{21} in all other studies the stabilization is induced by the formation of covalent bonding between the acryloyl-phospholipids and an intermediate acrylate modified silicon oxide surface or acrylated polymer\textsuperscript{22,23}. 
In this project, we are interested in lipid monolayers supported on top of hydrophobic H-terminated silicon surfaces, such as schemed in Figure 2.1b). This monolayer should be stable in air in order to maintain its properties after drying without being damaged. The choice of the substrate is governed by the application. Here, we are interested in the development of a field effect transistor based biosensor in which the lipid monolayer plays the role of ultra-thin gate dielectric insulator, therefore replacing the very commonly used silicon dioxide.

In the following we will introduce the different techniques which are commonly used to form lipid layers. We will discuss the protocol that we have developed to form the monolayer and give some insight to evaluate its quality as insulator material.
2.2 Methods to form supported lipid layers

Two different approaches are commonly used to form supported lipid layers. The first one uses the so called Langmuir-Blodgett trough, the second the fusion of vesicles. We will describe here these two techniques and show for both their pros and cons.

2.2.1 Langmuir-Blodgett films

In 1917, Dr Irving Langmuir showed the possibility to transfer a single monolayer in the water-air interface to a solid support. In 1935 Dr Katharine Blodgett extended the concept and demonstrated that several of these monolayers could actually be transferred and stacked on top of each others to form multilayers. A Langmuir-Blodgett trough is a piece of equipment which has been developed to study the properties of amphiphilic molecules monolayers. It is also used to transfer these monolayers to solid substrates. The operating principle is the following: The trough is filled with a polar solvent, usually water, and amphiphilic molecules are spread at the water-air interface. The molecules will organize themselves in a monolayer keeping their hydrophilic part in the water and their hydrophobic part in air. A mobile barrier present in the trough can then be activated to reduce or expand the surface area covered by the monolayer, therefore changing the area per molecule and the molecular density. One of the very used applications of the trough is to measure the pressure isotherm of lipids. The first pressure isotherm was done by Agnes Pockels in her kitchen in 1891. After spreading the lipid at the water/air interface, the covered area is reduced, the lipids are compressed and the resulting pressure versus area is measured using a suspended Wilhelmy plate partially immersed in water. If the number of molecules is known the surface pressure as a function of the area per molecule can be plotted. In addition to revealing important properties of the film like pressures at which phase transitions occur, or the pressure at which the film collapses, this isotherm can also be used to control the density and the ordering of the lipids in the monolayer prior to being transferred to the solid substrate, see Figure 2.2.
Figure 2.2 pressure isotherm for a lipid layer, at low pressure lipid molecules are disordered, they are in Gaz phase. As the pressure increases the area per molecule decreases, the lipid molecules are in the more condensed liquid phase. At very high pressure the lipid molecules are in solid phase. Between phases the plateau are the sign of phases coexistence.

At low pressure, the lipid molecules are in gas phase, the area per molecule is large, and lipid molecules are disordered. As the pressure increases, the available area per molecule decreases. The surface pressure increases and the lipids move from gas phase to more condensed and ordered phases; the plateau in the graph is the sign of a first ordered transition with the coexistence of two phases.

Once the monolayer is ready it can be transferred to the hard substrate.

For a bilayer, after the lipid molecules are spread at the water-air interface and that they are compressed to the desired pressure, a very clean hydrophilic solid substrate is dipped in the trough perpendicular to the surface as shown in Figure 2.3. When going down, the hydrophilic surface is in contact with the hydrophobic tails so the lipids do not get deposited on the substrate.
Next, the substrate is pulled off the trough and the hydrophilic head stick to the surface and form a monolayer, it is important to maintain the pressure constant during the deposition. After this first step, a monolayer layer is stabilized on the substrate with the hydrophobic tails heading toward the air, so the surface is hydrophobic the second layer is deposited by the Langmuir Schäfer method.

The Langmuir Schäfer method is also used to stabilize lipid monolayer on hydrophobic substrate. At the desired pressure, the hydrophobic substrate is dipped in the trough and it goes in a clean recipient originally introduced in the trough. The recipient full of water will help to get the sample out of the trough without any contact between the lipid layer and the air (Figure 2.4).
This method leads to a very homogenous flat lipid layers; its main advantage is the control of the density of the deposited layers by changing the pressure during the deposition.

2.2.2 Vesicles fusion

The principle of vesicle fusion is based on the adsorption and fusion of small unilamellar vesicles on a substrate from their aqueous dispersion. Depending on the wetting properties of the substrate, vesicle fusion will give rise to a bilayer or a monolayer. On a hydrophilic surface, a bilayer is obtained by rupture of the vesicles and their "unrolling" and spreading onto the substrate, as shown in Figure 2.5a). On the other hand, if the substrate is hydrophobic, a monolayer is formed by rupture of the vesicles, splitting of the vesicular membrane into its two monomolecular leaflets and spreading, Figure 2.5b). It has been shown that the kinetics of vesicle fusion is much slower for the formation of a lipid monolayer on a hydrophobic surface than for a bilayer on a hydrophilic surface. This is due to the fact that the mechanisms involved in the formation of a planar bilayer starting from a vesicular bilayer are much less complex than those involved in the formation of a planar monolayer. It has been shown that initial vesicle adhesion to the substrate is the critical point in the process. It is governed by the interplay between the adhesion energy (favorable) and the bending energy (unfavorable) of the deformed vesicle after adhesion. Adhesion of vesicles on hydrophobic surfaces is energetically disfavored due to the presence of the hydrophilic polar head-groups on the outer surface of the vesicles. To explain the formation of monolayers it has been suggested that the splitting and the adhesion to the substrate must occur simultaneously, followed by "unrolling" and spreading.²¹

![Figure 2.5 Vesicles fusion on hydrophilic (a) and hydrophobic surface (b).](image)
An advantage of the Langmuir-Blodgett and Langmuir-Schaefer transfers over vesicle fusion is that the composition of a mixed lipid monolayer on the trough surface will be conserved after transfer. Also, the control of the lipid density before transfer ensures the reproducibility of the final supported layer. Vesicle fusion on the other hand is a fast and easy process which does not require any specific equipment.

Considering that our goal is to have the monolayer formed on an electronic device, the Langmuir trough is not suitable for our purpose. We have therefore decided to make the monolayers using the vesicle fusion method.

2.3 The selected lipid and its polymerization

2.3.1 1,2-di-(10Z,12Z-tricosadiynoyl)-sn-glycero-3-phosphocoline, DCPC

![Figure 2.5 1,2-di-(10Z,12Z-tricosadiynoyl)-sn-glycero-3-phosphocoline, 23:2 DiynePC, DCPC.](image)

In this work, we used the commercially available 1,2-di-(10Z,12Z-tricosadiynoyl)-sn-glycero-3-phosphocoline phospholipids. It contains 24 carbons in each of its aliphatic chains, including two diacetylenic moieties per chain. Such lipids have been widely studied and their melting temperature is at 43°C. The presence of the diacetylenic groups allows for the two-dimensional polymerization of the lipids in the plane of the layer.
2.3.2 DCPC polymerization

The polymerization process of diacetylenic species using free radicals or UV exposure is well-known and has already been described in several papers\textsuperscript{28,29}. The spatial proximity required for polymerization has been shown to yield significant interactions between neighbouring, electron-rich diacetylene units giving rise to lateral association within the monolayer similar to that observed in systems showing hydrogen bonding, π-stacking, or dipole coupling\textsuperscript{30,31}. In our experiment, the polymerization is activated by addition of a free radical, the 2,2-azobis(2-methylpropionamidine)dihydrochloride, AAPH, after the formation of a dense layer and heating of the sample at 40°C. A scheme of the polymerization of diacetylenic species is shown in Figure 2.6.

2.4 Formation of the lipid monolayer: The experimental protocol

2.4.1 Preparation of the lipid vesicles

The lipids were purchased dried from Avanti Polar Lipids. To avoid the oxidation of the acetylenic groups in the lipid aliphatic chains, a 1% stock solution was prepared in chloroform and stored in a dark vial preventing UV exposure. To prepare a sample, 50 μL of the stock solution is heated at 40°C to evaporate the chloroform and then re-dissolved in 200 μL of deionized (DI) water. Still to limit oxidation, the DI water was outgassed in Argon for 30 minutes prior utilization and used as such in the whole sample preparation. After addition of water, the lipids arrange themselves into large multi-lamellar vesicles. Uni-lamellar vesicles are then obtained by sonication of the solution of
vesicles for 15 minutes. Finally, the radius of the vesicles is reduced by extrusion of the solution through a 100 nm diameter pores polycarbonate membrane.

2.4.2 Preparation of the silicon surface

Silicon wafers ((100), Boron doped, $\rho=5\Omega$) were cleaned by soaking in piranha solution (2:1 H2SO4/H2O2) at 130°C for 30 minutes, then thoroughly rinsed with DI water. To form the H-terminated silicone surface, native silicon oxide is etched off in solution by 2% hydrofluoridric acid (HF) in ethanol for 2 minutes. The sample is finally quickly rinsed in DI water. The quality of the surface was verified by ellipsometry. A typical thickness of 0.5 Å was obtained indicating a good removal of the silicon oxide from the surface.

2.4.3 Formation of the lipid monolayer on H-terminated Silicon

H-terminated silicon surfaces are very instable surfaces, they oxidize quickly in air but even more in aqueous solutions. Indeed, contact angle measurements on freshly prepared H-terminated silicon surfaces showed a continuous decrease of the contact angle from 70° to 35° after 1 hour immersion in water. This problem raises the question of how to functionalize a silicon substrate with molecules in aqueous solution without re-oxidizing the silicon surface. First, precautions can be taken to slow down the oxidation: the water used to prepare the vesicles and clean the surfaces can be outgassed to remove the oxygen and the samples can be prepared in a controlled environment like in a glove box filled with argon for example. In addition to that a trick consists in functionalizing the surface before the surface oxidizes. To do that, a procedure has been developed to speed up the fabrication of the lipid monolayer.

We have discussed previously that the formation of a monolayer on a hydrophobic surface is disfavored due to the hydrophilic/hydrophobic repulsive interaction between the lipid head-groups and the surface. The formation of a monolayer is therefore rather slow. In order to force the vesicle onto the surface and promote their fusion, the substrate is quickly cooled just after deposition of the vesicle solution on the sample. The vesicles are quickly "trapped" on the surface so that the layer is formed within a few minutes. We will see later that this procedure drastically improves the
formation of a homogeneous lipid monolayer on silicon. The detail of the complete procedure is given below:

1. Formation of the small unilamellar vesicles as described earlier.

2. Formation of the H-terminated silicon surface as described earlier.

3. Formation of the lipid monolayer: 100 µL of the final lipid solution are spread over the H-terminated silicon wafer, the wafer is then quickly cooled down to 10°C and the solution gently agitated. After 5 minutes, the system is slowly (5°C/5min) heated up to 35°C. At this temperature, the solution is rinsed with DI water.

4. Polymerization: At 35°C, the water is replaced by a 0.1% solution of free radical AAPH. To activate the polymerization, the sample is slowly heated up to 43°C (the melting temperature of the lipids) and left for 15 minutes. The sample is then slowly cooled down to room temperature and rinsed with DI water.

After polymerization the monolayer is stable in air, and the sample can be dried. One convenient way to prove the polymerization has worked properly consists in rinsing the sample with methanol. In the case of a non-polymerized monolayer this induces the complete wash off of the monolayer from the surface. On the contrary, after polymerization, the monolayer remains unchanged after methanol rinsing.

Such prepared polymerized monolayers have been characterized by atomic force microscopy to evaluate the effect of the cooling on the quality of the monolayers. In addition X ray photoemission spectroscopy has been realized to study the evolution of the silicon surface below the lipid monolayer.

2.5 Characterization of the polymerized monolayer

2.5.1 Characterization by AFM

To evaluate the effect of the cooling on the quality of the monolayers, we have prepared two batches of samples: One following the procedure described above, another with the same procedure
keeping the temperature at 21°C in the third step. Some prepared as described in the protocol above and some others prepared following the exact same procedure but at room temperature (21°C), i.e., without the cooling step. In both cases, the incubation time of the substrate with the vesicle solution is the same. AFM imaging realized on such as prepared monolayers are reported in Figure 2.7 a) and b). All images were obtained in solution after the layers were polymerized. From a first look, the two types of monolayers seem to be quiet similar. Both seem to be rather homogeneous with no obvious presence of holes. By looking at the sections, one can see however that the roughness of both layers is quite different. The average roughness measured over several images with same dimensions indicate a value of 0.70±0.18 nm and 1.56±0.3 nm for the monolayers prepared at 10°C and 21°C respectively. We attribute this difference to the fact that when the layers are prepared at room temperature the adsorption and fusion of the vesicles is slower. The silicon surface starts oxidizing before the layer is formed, giving rise to local sample areas with different wetting properties. This non-homogeneity of the surface would be responsible for the non-homogeneity of the lipid layer. In some cases one can see the presence of small patches of bilayers embedded in the monolayer. These results seem to indicate that the cooling has a beneficial effect on the homogeneity of the lipid monolayer.

The thickness of the monolayer in solution was evaluated by scrubbing a square area with the AFM tip and measuring its depth. It was obtained by scratching the surface with the tip over a 1×1 μm² area. The same experiment was realized on a bilayer prepared at room temperature on an oxidized silicon surface for comparison. Subsequent AFM images are shown in Figure 2.7 c) and d) respectively. For the monolayer a typical thickness of 2.7±0.2 nm is obtained consistently with a thickness of 4.8±0.3 measured for the bilayer. These results are perfectly consistent with ellipsometric measurements that were realized after drying the sample, therefore showing no disruption of the monolayer when displaced from water to air. Such thickness of the monolayer is actually smaller than the expected thickness of 4.0 nm for this type of lipid. However, this is actually in good agreement with the expected thickness of polymerized diacetylenic moieties for which a tilt angle of 45°C from the surface plane is required to polymerize as was demonstrated by Menzel et al30.
Figure 2.7 AFM images of a) and c) a lipid monolayer formed at 10°C following the protocol described above, b) a lipid monolayer formed at room temperature (21°C) without cooling the substrate and d) a bilayer formed on an oxidized silicon surface at room temperature. For each image a section of a line on the image is shown. All images were obtained in solution and in tapping mode after polymerization of the layers. Images size: 5×5 μm.
2.5.2 XPS characterization of the silicon/monolayer system

Composition of the lipid monolayer

XPS was first used to characterize the composition of the lipid monolayer. The question was whether AAPH, the free radical promoting the polymerization, would stay in the layer or would be washed off. The elemental composition of the lipids and AAPH is given in table 2.1 together with the theoretical binding energy of the peaks.

<table>
<thead>
<tr>
<th></th>
<th>Lipids</th>
<th>AAPH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (1s)</td>
<td>N (1s)</td>
</tr>
<tr>
<td>C*-H</td>
<td>44</td>
<td>6</td>
</tr>
<tr>
<td>C*-O</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>C*-O</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C*-N</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>C=N*</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2.1: The number of different atoms and their correspondent binding energy (eV) in the lipid molecule and the AAPH molecule.

Large XPS spectra obtained on a lipid monolayer supported on silicon and on an H-terminated silicon surface are shown in Figure 2.8 in black and red respectively. The main characteristic peaks of interest are indicated.

High resolution spectra obtained on the characteristic peaks of interest are shown in Figure 2.9. To quantify the proportion of each element, the area of each spectrum was fitted with Gaussians. For the carbon, three Gaussians were used corresponding to the different molecular environment of the element, see Figure 2.9 b). Only one Gaussian was used for the oxygen and nitrogen.
Figure 2.8 Large XPS spectra obtained on H terminated silicone surface (red), and lipid monolayer sample (black).

Figure 2.9 High resolution XPS spectra for Carbon (a), Nitrogen (c) and Oxygen (d). (b) is the shows the fitting of the carbon peak by three Gaussian that correspond to the different atomic environment of the element.
To remove unwanted noise signal that might result from possible contamination of the XPS system, reference spectra of the same elements were measured on H-terminated silicon surface. The corresponding areas were subtracted from the areas measured on the lipid monolayer. To extract quantitative information the area of each peak is divided by the atomic sensitivity factor (ASF) of the corresponding elements. The ASF values for the MgKα photons are indicated in table 2.2.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Si (2p)</th>
<th>C (1s)</th>
<th>N (1s)</th>
<th>O (1s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASF</td>
<td>0.27</td>
<td>0.25</td>
<td>0.42</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Table 2.2: Atomic sensitivity factor for each element of the studied molecules.*

The experimental ratio of each element is compared with expected theoretical ones from a pure lipid layer. The results are shown in table 2.3. The results show a very good agreement between the experimental and theoretical values, therefore indicating that the lipid monolayer is mostly "pure" and does not contain noticeable amounts of AAPH. A much larger value for the nitrogen peak would be obtained otherwise.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Theoretical value</th>
<th>Experimental value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-O/C=O</td>
<td>3</td>
<td>2.9</td>
</tr>
<tr>
<td>C-C/C-O</td>
<td>7.3</td>
<td>6</td>
</tr>
<tr>
<td>C-C/C=O</td>
<td>22</td>
<td>20.9</td>
</tr>
<tr>
<td>C/O</td>
<td>6.5</td>
<td>5</td>
</tr>
<tr>
<td>C/N</td>
<td>52</td>
<td>50</td>
</tr>
</tbody>
</table>

*Table 2.3 Theoretical relevant element ratios for a pure lipid monolayer and experimental values extracted from XPS measurements.*

**Evolution of the silicon surface**

We discussed earlier that the formation of a homogeneous lipid monolayer on silicon requires an unoxidized silicon surface. Since the whole monolayer preparation is done in water in approximately one hour, and the lipid molecules are only physisorbed on the surface, the oxidation of the SiH can be a major problem. To verify that the process we developed is efficient in preventing the silicon oxidation, we have measured by XPS the percentage of oxidized silicon just after the formation of
the lipid. The oxidation of the silicon was followed by analyzing samples exposed to air and water for different time. For comparison, we did the same measurements for a H-terminated silicon wafer subject to the same condition and for a chemical oxide silicon surface prepared by soaking the silicon wafer in piranha solution for 30 min. The SiO$x$ sample is used to estimate the maximum value that is expected from XPS measurement. A ratio SiO$x$/Si corresponding to 31% was obtained.

The XPS spectra of the Si$2p$ peak obtained on an oxidized silicon surface, and on freshly prepared H-terminated silicon surface and lipid monolayer are reported in Figure 2.10. Two peaks can be observed. The first one at 100.5 eV corresponds to the Si peak and the second one at 104.5 eV corresponds to the SiO$x$ peak (The small shift observe in the case of the lipid monolayer is certainly due to surface charging). The later one which is important in the case of the oxidized silicon surface is nearly inexistent in the case of the H-terminated silicon and the lipid monolayer. These results confirm that we can, in our conditions, succeed in stabilizing a lipid monolayer on the silicon surface without oxidizing it.

![XPS spectra](image)

*Figure 2.10 XPS spectra of the Si$2p$ peak obtained on SiO$2$ surface, and freshly prepared SiH surface and lipid monolayer.*

The samples were then exposed to water and air and we followed the oxidation state of the silicon surface at increasing amount of exposure time. Figure 2.11 a) shows the result of such experiment after 1h, 2h and 24h, of exposure in water. The corresponding percentages of oxidized silicon are
reported in figure Figure 2.11b) together with the evolution of the H-terminated silicon surface exposed to water and the monolayer surface exposed to air. Although slower than for the case of the H-terminated silicon surface, the silicon surface under the lipid monolayer oxidizes quiet rapidly over time therefore indicating that the lipid monolayer does not protect the silicon surface from oxidation. This is actually not really surprising since the monolayer is only physisorb on the surface. The important point however is that the monolayer remains intact even after the oxidation of the silicon and does not desorb from the surface.

Figure 2.11 a) XPS spectra of Si$_2$p measurements on a lipid monolayer on silicon after 0, 1, 2 and 24 hours in water. b) percentage of SiO$_x$/Si vs time for a lipid monolayer on silicone exposed to the air, to water and Si-H surface exposed to water.
CHAPTER 3: MECHANICAL STABILITY OF SUPPORTED LIPID MONOLAYERS
3 Mechanical stability of supported lipid monolayers

Force measurement using atomic force microscopy (AFM) has now become a very common technique for probing the nano-mechanical properties of materials and especially those of thin films. Among those, abundant studies realized on supported lipid bilayers have shown that the variation of mechanical stability depends on the ordering of the lipids within the bilayer. The solid-like ordered phase with higher density of lipids shows higher mechanical stability than low density disordered phase. That said, one knows that lipid bilayers exhibit gel to fluid phase transition in a range of temperatures that varies from $-70 \degree C$ to $+80 \degree C$ depending on their length, head-group, and number of unsaturated bonds in their fatty acid chains. In addition, when these bilayers are supported, their interaction with the substrate may drastically increase their transition temperature. It is therefore expected that the mechanical properties of lipid layers will strongly depend on the type of lipid constituting the layer and on the temperature at which the measurement is realized. Similarly it was shown that the composition of the lipid bilayer might vary considerably its mechanical stability. For example, some recent studies show that increasing the ratio of cholesterol in a mixture of dioleoylphosphatidylcholine/sphingomyelin releases the stress in the layer and as a result decreases the breakthrough force required to induce rupture. Understanding the mechanisms that govern the mechanics and elastic properties of lipid membranes is essential for both the comprehension of biological processes and the development of biotechnologies or bio-inspired technologies. For example, it was shown that elastic bilayer forces modulate the free energy and cooperativity of folding of membrane proteins and transmembrane signaling. Also, as a biocompatible material, some researchers have been working on the development of nanoparticles for the targeting and delivery of drugs in which lipid bilayers are used to encapsulate the drugs. In that field a lot of work is dedicated to control the stability of the bilayer.

In this chapter, we have investigated the effect of polymerization on the stability of supported lipid monolayers using force spectroscopy by AFM. All the measurements were realized before and after polymerization on same lipid monolayers to avoid any questioning about the reproducibility of the lipid monolayer quality and the misinterpretation of the data. From these statistical measurements, physical quantities have been deduced to characterize both types of monolayers.
the first part of the chapter we will describe the processes involved when a tip is pressed on a supported lipid monolayer and discuss the physical quantities that can be extrapolated from such measurements. In the second part, we will focus on the indentation curve of the force measurements, and see how these physical quantities can be used to determine some elastic properties of the monolayer. Finally, the last part is dedicated to the adhesion of the monolayer on the silicon surface, and we will show how the cohesion of the lipids after polymerization impacts the monolayer stability on the substrate.

3.1 Force-Distance curve on a supported lipid layer

A typical force-distance curve realized on a lipid monolayer is shown in Figure 3.1a). The measurement can be separated into two parts; the loading part in red corresponds to the inward displacement of the cantilever to the surface and the withdrawal or unloading in blue which corresponds to the outward displacement of the cantilever. In figure 3a), the deflection of the cantilever is given versus the displacement of the cantilever for both loading and unloading curves. Figure 3.1b) shows the force exerted by the tip on the surface versus the tip-substrate distance for the loading part only of the measurement for a lipid monolayer supported on silicon (green) and a bare silicon substrate (black) used as reference. During loading, the cantilever is initially far away from the sample and no interaction are detected between the tip and the sample. As the cantilever approaches the lipid monolayer it experiences repulsive forces at small distances. At some point, the tip gets just in contact with the monolayer; at this stage, no force is exerted by the tip on the layer. As the cantilever keeps moving toward the substrate, the tip will start exerting pressure on the monolayer. If the layer resists the force, the cantilever, with a given spring constant, will bend over the layer. During this process, the layer will elastically deform (see Figure 3.2) until the exerted force becomes large enough to get the rupture of the layer and the tip will jump to the hard substrate supporting the monolayer. Here, “elastic” refers to the fact that when retracting the tip before the film ruptures the retracting part of the force curve is identical to the approaching part. This depth of the deformation before rupture is commonly called ‘indentation’. The force required to rupture the layer is commonly called “breakthrough force” and the thickness of the layer just before the rupture will be called "jump height" in the following. Both the breakthrough force and the jump height are characteristic parameters of the considered lipid layer from which the elastic
properties of the monolayers can be deduced. During withdrawal, adhesion forces between the lipids and the tip maintain the tip in contact with the layer inducing bending of the cantilever. When the exerted pulling force becomes higher than the adhesion force, the tip is then released and goes back to its neutral position, i.e., when no forces are applied on the tip. From this part of the curve, we will extract lipid monolayer-tip adhesion forces that will be used to discuss substrate-monolayer interaction.

Figure 3.1 a) Typical force-distance measurement realized on a lipid monolayer. The graph gives the cantilever deflection versus the cantilever-monolayer distance. b) Force versus tip-substrate distance for the approaching curve for a lipid monolayer supported on silicon and for a bare silicon substrate

Figure 3.2 Elastic deformation of the monolayer under the tip. \( t \) is the thickness of the monolayer, \( d \) the indentation of the tip, \( d_j \) the tip-jump at the rupture and \( a \), the contact radius between the tip and monolayer.
3.2 Nano-mechanical stability of the monolayers

In the following, all measurements were realized in water using AFM (NTEGRA from NT-MDT) and silicon tips CSC17 from µMash with a typical resonance frequency of 14 KHz in water, a spring constant ranging from 0.06 N/m to 0.4 N/m and a tip radius ranging from 8 to 10 nm. For each tip used in the experiment, the tip radius was determined after imaging the tip by scanning electron microscopy and the spring constant using the thermal noise method as describe in the experimental section. For each set of experiment, a minimum of 200 curves were measured on at least 3 different samples.

3.2.1 Dependence of the breakthrough force on the polymerization state

Figure 3.3 a) shows two typical loading curves on the same monolayer before (green) and after (red) its polymerization for a tip loading rate of 6 µm/s. Before polymerization, the tip penetrates the monolayer without any resistance of the monolayer and an attractive interaction can be observed between the tip and substrate. After polymerization, a net change is observed; the monolayer resists to a force of 2.5 nN at which it ruptures and the tip jumps to the substrate.

Figure 3.3 (a) Typical loading force-distance curves obtained on a supported lipid monolayer before (green) and after polymerization (red). (b) Breakthrough forces versus the tip jump in distance at the lipid monolayer rupture reported for 400 measurements on three different samples before (green) and after (red) polymerization. The tip loading rate was 6µm/s.
The breakthrough force and the tip jump have been reported for over 400 measurements on three different samples realized on each of the two surfaces. The mechanical properties of polymerized and as deposited monolayer are clearly evidenced in Figure 3.3b). The average force required to rupture the layer before polymerization is of 98 ± 67 pN, while 1.4 ± 0.72 nN force is needed to rupture the monolayer after polymerization. The breakthrough value is multiply by almost 14 after polymerisation. Although the large error bars which seem to indicate some variability in the measurements, the data clearly reveal an improvement of the nanomechanical resistance of the monolayers after polymerization.

3.2.2 Dependence of the Break-through force on the loading rate

Indentation experiments on lipid bilayers have shown that the average breakthrough force is logarithmically dependent on the loading rate at which the indentation is performed, i.e., on the rate at which the tip hits the lipid bilayer\textsuperscript{45,49,50} Although this property has now been well demonstrated for lipid bilayers, it was never shown for monolayers. We present here a statistical analysis of the force at which the breakthrough occurs for a whole range of loading rates varying from 30 nm·s\textsuperscript{-1} to 6 μm·s\textsuperscript{-1}. Because each monolayer is not perfectly identical, some differences appear between sets of measurements. For clarity, we will show the results obtained on one representative sample.
Figure 3.4 Average break-through forces obtained at loading rate ranging from 30 nm.s$^{-1}$ to 6 µm.s$^{-1}$ on polymerized (blue) and non polymerized (red) monolayers.

For each loading rate $v$, the average value $F_0$ is reported in Figure 3.4, each point corresponding to a sample of at least 200 force-distance curves. The measurements obtained before and after polymerization are shown in red circles and blue squares respectively. The first observation is that for both types of monolayer the average breakthrough force increases linearly with the logarithm of the loading rate similarly to what was reported for bilayers. The red and blue lines result from fitting equation $F_0 = a + b \log(v)$ to the experimental data.

The second observation is that the dependence of $F_0$ on the loading velocity is very different whether the lipid monolayer is polymerized. Before the polymerization, the breakthrough forces are very small with values ranging from 20 to 60 pN while still showing some dependence on the loading rate. To compare these results with data reported in the literature taking into account the different tips and samples, we normalized these forces by the tip curvature$^{51}$. Indeed higher forces are expected for higher tip curvature. Before polymerization, the normalized breakthrough force varies from 2mN.m$^{-1}$ to 7mN.m$^{-1}$; they are very small compared to the values reported in the literature for lipid bilayers: 105 mN.m$^{-1}$ for DSPE$^{46}$, 220 mN.m$^{-1}$ for MGDG$^{46}$, 66-170 mN.m$^{-1}$ for DOTAP$^{52}$, 30 mN.m$^{-1}$ for POPE/POPG mixtures$^{57}$, 900 mN.m$^{-1}$ for DMPC$^{54}$, and 72 mN.m$^{-1}$ for POPG$^{55}$ but fall into the same range of values previously reported for DPPC monolayers$^{56}$. Since DPPC and
the DCPC have similar melting temperatures (46°C for the DPPC, and similar head-groups), they are expected to behave similarly. After polymerization the values of the breakthrough force are much higher and vary between 0.2 and 2 nN for the investigated range of loading rates, therefore corresponding to normalized forces varying from 24 to 235 mN·m⁻¹. The comparison between these values and those obtained on bilayers evidence the impact of the polymerization on the nano-mechanical resistance of the monolayer.

To understand this behaviour, one needs to consider the relaxation time of the lipid monolayer under the tip. In a general case, when the tip loading rate is slow, the layer has more time to relax and the normal reaction force will be small. On the contrary when the loading rate is fast the layer has less time to relax and the normal reaction force will be larger. This relaxation involves the reorganization of the lipids under the tip and its vicinity; it is related to the lateral diffusion of the lipids in the layer as well as to the compressibility coefficient of the layer. This is illustrated in Figure 3.5. To explain the difference observed between non-polymerized and polymerized layers, one needs to consider the coefficient of mobility of the lipids in both configurations which obviously is expected to be drastically reduced when the layer is polymerized. We have not made thorough measurements of these coefficients but we will see later that a simple estimation shows a decrease by a factor of 100 after polymerization.

In the following, we will see how these measurements have been used to determine some elastic properties of the monolayers.

Figure 3.5 scheme showing the direction of the break-through force, the normal reaction force of the monolayer, and the lateral diffusion of the lipid molecules when pressure is exerted on the monolayer.
3.2.3 Elastic properties

3.2.3.1 Model and experimental conditions

In the following, we will determine approximate values of the Young modulus of the two types of monolayer. The Young modulus also known as elastic modulus is a measure of the stiffness of an elastic material; it is used to characterize materials. In force measurement curves, Young’s modulus is related to the elastic deformation of the sample in the contact regime during loading and unloading. The stiffness of the sample is related to its Young’s modulus by:

\[ k_s = \frac{3}{2} a E_{tot} \]  \hspace{1cm} (3.1)

with

\[ E_{tot} = \frac{3}{4} \left( \frac{1 - \nu_s^2}{E_s} + \frac{1 - \nu_t^2}{E_t} \right) \]  \hspace{1cm} (3.2)

Here, \( \nu_t, E_t, \nu_s \) and \( E_s \) are the Poisson’s ratio and the Young’s moduli of tip and sample, respectively, \( E_{tot} \) the reduced Young’s modulus, and \( a \) is the tip–sample contact radius. In most cases, the tip is much stiffer than the sample. The deformation of the tip can then be neglected and equation (3.1) can be approximated by:

\[ k_s = \frac{dP}{dh} = 2a \left( \frac{E_s}{1 - \nu_s^2} \right) \]  \hspace{1cm} (3.3)

with \( P \) the loading force and \( h \) the deformation of the sample under the tip (indentation).

Before determining the Young modulus for the layers, three main issues should be discussed: First of all the contact mechanics and the different models proposed to describe the elastic deformation. The second point concerns the determination of the tip geometry which defines the contact area.
Finally the last important point is the substrate intervention in limiting the deformation of the sample.

**Contact mechanics: different models**

Contact mechanics is the study of the deformation of two solids that touch each other. Several models developed to describe this elastic deformation differ by the way they consider adhesion between the tip and the sample. This contribution leads to differences in the relation between the applied load $P$ and the contact radius $a$. One of the first original works on contact mechanics was done by Heinrich Hertz in 1882 with a publication entitled: ‘On the contact of elastic solid’⁵⁷. When two curved surfaces come into contact, a local stress is created and the surfaces will deform. This deformation depends on the elastic moduli of the two materials. In this model, Hertz neglects the adhesion and expresses the contact stress as a function of the normal loading force and the radii of curvature of both materials. In other models developed later such as the Johnson-Kendall-Roberts (JKR)⁵⁸ and Dejarguin-Müller-Toporov (DMT)⁵⁹,⁶⁰ models, adhesion is taken into account outside and inside the contact area respectively and the adhesion work can be calculated from the jump-off contact if the tip radius is known. The JKR theory is used in the case of large tips and soft materials with a large adhesion. The DMT theory on the contrary applies to small tips and stiff samples with small adhesion. None of these models actually match our case. Indeed in our configuration, we use a small tip, with a radius of ~10 nm, the samples are soft and the adhesion relatively important. To describe our data, we have then chosen to use the hertz model and neglect the adhesion force in the calculation. In this case the Young’s modulus can be determined directly from equation 3.3 provided that one knows for the used tip, the dependence of the contact radius with the depth of indentation. This relation will depend on the shape of the tip.
Contact area for tips of different shape

![Diagram showing different tip shapes: spherical and conic.]

*Figure 3.6 Scheme showing two tip shapes: spherical and conic.*

**Non adhesive contact between a rigid conical tip and elastic half space**

When a conic tip indents the substrate to a depth \( d \); the radius of the contact area is expressed by:

\[
a = \frac{2}{\pi} d \tan \theta \quad (3.4)
\]

With \( \theta \) the angle between the plane and the side surface of the cone.

Substituting \( a \) and integrating equation 3.3 leads to

\[
F = \frac{2d^2}{\pi \tan \theta} E_r \quad (3.5)
\]

With \( E_r = \frac{E_s}{(1 - \nu_s^2)} \).

**Non adhesive contact between a rigid spherical tip and elastic half space**

A spherical tip with a radius of curvature \( R \) indents the substrate to the depth \( d \); the radius of the contact area is expressed by:

\[
a = \sqrt{Rd} \quad (3.6)
\]

Substituting \( a \) and integrating equation 3.3 leads to

\[
F = \frac{4}{3} E_r R^{1/2} d^{3/2} \quad (3.7)
\]
The effect of the substrate:

The main problem of the three cited theories is that they do not take into account the substrate influence on the deformation of thin films during indentation experiments. Indeed, if the substrate is harder than the investigated film, then it might limit its deformation. To prevent this effect, it has been shown that the maximum indentation depth shall remain smaller than 10% of the film thickness. This method which can be easily applied to thick materials becomes difficult for thin layers. Several models have then been proposed to take into account the effect of the substrate by introducing an effective Young modulus in which the contribution of the substrate varies with indentation depth. These models developed by King, Saha and Nix, Chen and Vlassak, and Rar et al., were used for films with thicknesses of a few hundred nanometers. These cannot be applied to layers with thicknesses of a few nanometers only. In the following, we will have to keep in mind, that the extrapolated Young moduli are certainly over-estimated.

3.2.3.2 Determination of the Young’s modulus

To determine the Young modulus from equations 3.5 or 3.7 one needs to determine the indentation of the tip. One of the major difficulties is to determine the point on the curve which corresponds to the point at which the tip just contacts the surface without applying any forces, i.e. δ=0. Especially when the investigated sample is very thin, the determination of this point can be critical. To avoid this difficulty we have proceeded otherwise.

Similarly to what we did for the breakthrough force in section 3.2.2, we have determined for every loading rate the average tip jump height for both types of monolayers. The results are presented in the inset in Figure 3.6 and show that the jump height generally decreases, i.e., the indentation increases, with the loading rate (The black line is just a guide to the eye). This result is reasonable with the fact that the breakthrough force is higher at high loading rates and so should be the elastic deformation of the monolayer. One should also notice that the deformation of the monolayer at the breakthrough and at a given loading rate is independent of the monolayer polymerization state. Using these data, one can plot the average breakthrough force with respect to the average tip jump height. Data measured before and after polymerization on the same monolayer are shown in Figure 3.6. In the following, we have described our data in the framework of the Hertz model for a
spherical tip in contact with a flat surface using equation 3.7. The indentation $\delta$ can then be expressed as a function of the layer thickness $h$ and the tip jump height as:

$$\delta = h - d_j$$

Equation 3.7 can then be rewritten as:

$$F = \frac{4}{3} \frac{E}{(1-\nu^2)} \sqrt{R} \left(h - d_j\right)^{3/2}$$

Both Young’s modulus and monolayer thickness can then be determined by fitting Figure 3.6 with equation 3.9. The red and green lines curves correspond to the fit obtained for the polymerized and non-polymerized lipid monolayers respectively. In the fit a value of 0.3 was used for the Poisson ratio.

![Figure 3.6 Average break-through forces versus average tip jumps-in for polymerized (red) and non polymerized (green) monolayers for a loading rate in the range of 60 nm.s\(^{-1}\) to 6 \(\mu\)m.s\(^{-1}\).](image)

The monolayer thickness corresponds to the value of the tip jump height for which a breakthrough force of zero would be obtained, i.e., when there is no indentation of the tip in the monolayer. For the set of measurements shown in figure 3.6, a value of 3.3 ± 0.2 nm is obtained in relatively good agreement with expected values of 2.8 ± 0.3 nm as previously determined from AFM imaging in solution.

Values of the Young’s modulus obtained from three different sets of measurements and for each type of monolayers are reported in Table 3.1.
Before polymerization, extrapolated values of the Young’s modulus vary in the range 1.0–4.5 MPa. This value perfectly falls into the range of young moduli that were previously reported for supported lipid bilayers of other type of lipids (20 kPa to 20 MPa)\textsuperscript{48,50,76,77}. After the polymerization, a large increase of the Young’s modulus is obtained with a value ranging from 110 MPa to 338 MPa. The relatively large dispersion of the values obtained for the two monolayers must arise from some variability in the quality of the monolayers. We have to keep in mind that the apparent Young’s moduli determined here are certainly overestimated by the presence of the hard substrate below the monolayer. As mentioned before, to avoid this type of substrate effect the indentation should not be larger than 10% of the monolayer thickness\textsuperscript{67}. From our measurements, the indentation is in the range from 11% to 38%. Nevertheless, since the deformation of the monolayer is similar for the two types of surfaces, the same substrate effect must apply, and the data can be compared. The results clearly evidence an effect of the polymerization on the elastic properties of the lipid monolayer with a large increase of the Young’s modulus. After polymerization, this value can be compared to that of a low density polyethylene film used to make plastic bags (~200 MPa).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>E (MPa)</th>
<th>$K_0$ (s$^{-1}$)</th>
<th>$\Delta G_0$ (k$_B$T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before polymerization</td>
<td>1 – 4.5</td>
<td>$1068 \pm 353$</td>
<td>$2.6 \pm 0.4$</td>
</tr>
<tr>
<td>After polymerization</td>
<td>110 - 338</td>
<td>$9.2 \pm 1.6$</td>
<td>$7.3 \pm 0.2$</td>
</tr>
</tbody>
</table>

Table 3.1. Average values of the Young modulus, $K_0$, and Gibbs energy deduced from three different sets of measurements before and after polymerization

3.2.4 Rupture of the monolayer: Determination of the Intrinsic Gibbs activation energy

3.2.4.1 Model of tip induced film rupture

To describe the rupture of the monolayer, we have used a theory introduced by Franz et al.\textsuperscript{49} (see Figure 3.7) based on the idea that the energy, $G_0$, of a monolayer-tip system to spontaneously form a hole in the layer can be increased by the application of a pressure $\Delta P$ on the layer by:
\[ \Delta G = V \Delta \Pi \]  \hspace{1cm} (3.10)

\( V \) is the activation volume; it refers to the size of a hole, which is sufficiently large to initiate the penetration of the tip through the monolayer. This mechanism is described in Figure 3.7 a). When the tip is far or just in contact with the layer, the initial energy \( G_0 \) is lower than the final energy of the system (tip + lipid monolayer) after the tip has penetrated the layer. In this case the penetration of the tip is unfavourable. As the tip starts pressuring the layer, the exerted pressure \( \Pi \) increases continuously therefore increasing the initial energy and lowering the activation energy from \( \Delta G_1 \) to \( \Delta G_2 \).

The energy of the system at a given time can then be expressed as:

\[ G(t) = G_0 + V \Delta \Pi \]  \hspace{1cm} (3.11)

At a certain level \( G(t) \) exceeds \( G_f \), and a breakthrough can occur. As described in Figure 3.7 b), the contribution of \( \Delta G(t) \) to lowering the energy barrier is only partial; the actual activation energy reduction \( \Delta G^*(t) \) can be expressed as:

\[ \Delta G^*(t) = \alpha V \Delta \Pi(t) \]  \hspace{1cm} (3.12)

And the Gibb's energy at a given time is given by:

\[ \Delta G^*_f(t) = \Delta G_0 - \Delta G^*(t) \]  \hspace{1cm} (3.13)

\( \alpha \) is a geometrical factor that takes into account that only a portion of the work of pressure lowers the activation barrier. This fraction is shape dependent and is 0.5 for a symmetrical barrier.
It is easily understandable that the rupture mechanism is a kinetic process that will depend on the kinetic at which the pressure increases i.e. on the loading rate used for the force measurement. The mathematical description of the breakthrough was already detailed by Franz et al.\textsuperscript{49} Briefly the force measurement can be described by \( N \) identical systems, consisting of \( N \) AFM tips on a lipid monolayer. Each tip exerts a growing force on the layer. After a time \( t \) has elapsed, \( N \) monolayers remain that did not undergo a breakthrough. If at \( t=0 \), the tip is in contact with the surface at the null force. From this situation, the stress is apply, after a time \( t \), \( N(t) \) is the number of intact monolayers. Within the time interval \( dt \), \( N(t) \) will be reduced by \( dN \) following the equation:

\[
dN = -k(t)Nd\text{t} \tag{3.14}
\]

with \( N \) the number of intact monolayers and \( k \) the rate constant at which breakthrough occur. \( k \) is time dependent. The probability to get \( N \) intact monolayers \( dP \) is given by \( dN \) divided by \( N_0 \):

\[
dP = -k(t)Pd\text{t} \tag{3.15}
\]

At \( t=0 \), \( P=1 \). This differential equation can be integrated:

\[
\ln P(t) = -\int_{0}^{t} k(t')d\text{t'} \tag{3.16}
\]
Assuming now that the rate constant $k$ is an activated process that follows an Arrhenius law, then:

$$k(t) = k_0 e^{-\Delta G^*_d(t)/k_BT} = k_0 e^{-(\Delta G_0 - \Delta G^*(t))/k_BT} = k_0 e^{\Delta G^*(t)/k_BT}$$  \hspace{1cm} (3.17)

with

$$k_0 = k_0' e^{-\Delta G_0/k_BT}$$  \hspace{1cm} (3.18)

Here $\Delta G_0$ is the intrinsic Gibbs activation energy of the tip-monolayer system required for the formation of a hole in the layer. $k_0$ is the frequency at which a hole in the monolayer is formed spontaneously that is big enough to allow the tip penetration. $k_0'$ is the total number of penetration attempts per second and corresponds in our system to the resonant frequency of the tip. $k_B$ is the Boltzman constant and $T$ the temperature.

As a simple approximation, we neglect the exact shape of the tip and assume a uniform pressure distribution over the whole contact area with the monolayer. In this case $\Delta \Pi(t) = F(t)/A$ with $A$ the area on which the pressure is exerted and $F = K \times s$ the force exerted by the tip, according to Hooke’s law. We will assume that $A$ corresponds to the projected contact area of the tip on the lipid monolayer. $K$ is the spring constant of the cantilever and $s$ its deflection. As the base of cantilever moves towards the sample at a constant loading velocity $v$, the deflection of the cantilever is $s = v \times t$ (the deformation of the sample is neglected). Therefore the time dependence of the pressure is:

$$\Delta \Pi(t) = \frac{Kvt}{A}$$  \hspace{1cm} (3.19)

Combining equations 3.16 to 3.19 leads to:

$$P(F) = \exp \left[ -k_0 \frac{A}{\alpha V} k_BT \left[ \exp \left( \frac{1}{k_BT} \frac{\alpha V}{A} F \right) - 1 \right] \right]$$  \hspace{1cm} (3.20)

$P(F)$ represents the integrated probability that the tip did not penetrate the bilayer at a given force $F$ when the cantilever is moved towards the surface at constant loading rate $v$. The probability $P_{\text{Break}}(F)$ for a breakthrough to occur at a certain force $F$ is given by:

$$P_{\text{Break}}(F) = -\frac{\partial P}{\partial F} = \frac{k_0}{Kv} \exp \left[ \frac{k_BT}{Kv} \frac{A}{\alpha V} - 1 \right] \left[ \frac{\alpha V}{A} F \right]$$  \hspace{1cm} (3.21)
Assuming that \( P_{\text{break}}(F) \) is a function with a narrow peak nearly symmetrical around its maximum (this was verified experimentally), the average force \( F_0 \) is therefore approximately equal to the force at which the probability distribution \( P_{\text{break}}(F) \) has its maximum, i.e., for \( dP/dF=0 \). \( F_0 \) can then be expressed as:

\[
F_0 = \frac{A}{aV} k_B T \ln \left( \frac{K \nu aV}{k_B T} \frac{1}{A} k_0 \right) \quad (3.22)
\]

Equation (3.22) predicts a logarithmic dependence of \( F_0 \) on the loading rate \( \nu \) that can be fitted by:

\[
F_0 = a + b \log \nu \quad (3.23)
\]

From equations (3.22) and (3.23) one can deduce:

\[
k_0 = \frac{K}{b \log e} 10^{-a/b} \quad (3.24)
\]

After determining the parameters \( a \) and \( b \) from experimental data, one can subsequently calculate \( k_0 \) from equation 3.24, and the intrinsic Gibbs activation energy, \( \Delta G_0 \), from equation 3.18.

### 3.2.4.2 Experimental results

In the model, equation 3.22 resulted from equation 3.21 assuming that the distribution of forces for a given loading rate \( P_{\text{break}}(F) \) is a narrow peak nearly symmetrical centered around its maximum.

To show that our data fulfil this assumption, the distribution of forces obtained for a loading rate of 0.8 \( \mu m/s \) is plotted in Figure 3.8. The data has been fitted with a Gaussian and shows a relatively narrow distribution centred on 0.95 nN with a width of 0.73 nN. This assumption therefore applies to our data.
From the fit of the curves of figure 3.4 (a and b) using equation 3.23, the values of $k_0$ and $\Delta G_0$ are deduced and reported in table 3.1 for the two types of monolayers. The value of $k_0$ is respectively: $1068 \pm 353$ s$^{-1}$ before polymerization and $9.2 \pm 1.6$ s$^{-1}$ after polymerization. These values are an average obtained from three different sets of measurements. As one might have expected, these values clearly show that the rate of spontaneous formation of holes drastically decreases after polymerization, therefore showing a higher cohesion of the lipids.

The intrinsic Gibbs activation energies for both types of layers were calculated using equation 3.18. An average of $2.6 \pm 0.4 K_b T$ (6.4 ± 0.9 kJ/mol) is obtained before polymerization, and $7.3 \pm 0.2 K_b T$ (18 ± 0.4 kJ/mol) after polymerization. Considering that the formation of a hole is due to the diffusion of the lipids under the tip, it is reasonable to compare these Gibbs energies to the energy of diffusion of lipids in layers. For DPPC bilayers, energies ranging from 20 to 50 kJ/mol have been reported in the literature using fluorescence technique$^{68,69}$ and $^1$H NMR$^{70}$ (Hydrogen Nuclear Magnetic Resonance). Although a little bit smaller, the values of the Gibbs energies that we report here are reasonable considering that our measurements were realized on monolayers instead of bilayers. Indeed, the amount of lipids to displace to allow the tip penetration is twice as high in a bilayer. Similarly, in such force measurement experiments, the size and the shape of the tip will have an

*Figure 3.8 Distribution of the force required to rupture the monolayer at a loading rate of 0.8 μm/s. The black curve is obtained from fitting the data with a Gaussian.*
effect on the out-coming values. Nevertheless, the effect of the polymerization on the Gibbs energy of the tip/monolayer system is demonstrated with a variation by a factor of ~3.

In this part, we have demonstrated the impact of polymerization on the nano-mechanical properties of a supported lipid monolayer submitted to a compressive pressure exerted by an AFM tip. The estimated Young modulus is increased by a factor of 100 and the intrinsic Gibbs energy of the monolayer/tip system for the tip to rupture the layer by a factor of 3.

In the next part we will show the effect of the polymerization on the adhesion properties of the monolayer.

### 3.3 Adhesion Forces

Adhesion forces are extracted from the unloading force-distance curves in the non-contact region. They correspond to the pull-off forces, i.e., the force required to detach the tip from the monolayer. In the following we will discuss the effect of polymerization on the magnitude of this force as well as the detachment process for the two types of monolayers.

#### 3.3.1 Dependence of the adhesion force on the loading rate

Similarly to what we did for the break-through force, we have extracted the adhesion force from experimental data obtained on the two types of monolayers and for a whole range of unloading rate ranging from 30 nm.s\(^{-1}\) to 6 µm.s\(^{-1}\). For each unloading rate, the average force resulting from 100 measurements is reported in Figure 3.9 a) and b) for the non-polymerized and the polymerized monolayers respectively. The first observation was that before polymerization, the adhesion force decreased with increasing unloading rate from 3.6 nN at 30 nm.s\(^{-1}\) to 1.3 nN at 6 µm.s\(^{-1}\). After polymerization, the value of the adhesion force increases drastically to an average value of 11.5 nN. In contrast with the previous case, it does not show a clear dependence with the loading rate. This difference can be explained by a decrease in the lipid mobility after polymerization. Indeed, when the unloading rate decreases the contact time between the tip and the lipid molecules increases. It is therefore expected that the number of molecules interacting with the tip will increase therefore
leading to a larger adhesion force. This is certainly what we would expect for a non-polymerized lipid monolayer when the lipids with a certain diffusion coefficient are relatively free to move in the layer. However after polymerization, the covalent bonding between lipids shall drastically decrease their mobility. In this case, increasing time will not affect the number of lipid molecules interacting with the tip.

![Figure 3.9 The variation of the adhesion force (nN) with the increasing loading rate of the AFM tip (a) before and (b) after polymerization.](image)

To confirm this interpretation, we calculated “n” the number of unit interactions between the lipid and the tip. It has been demonstrated that “n” can be calculated from a statistical analysis of a series of detachment force measurement. We consider that the adhesive force is generated by a discrete number of unit interactions “n” with the unit force $F_s$. For each unloading rate a Gaussian distribution of adhesion forces is obtained such as shown in Figure 3.10 for a rate of 1.5 μm/s. These distributions can be described in the framework of a Poisson’s distribution. The mean adhesion force corresponds to the maximum of the Gaussian. If one considers that this maximal adhesion force is generated by a discrete number $n_0$ of unit interactions between the tip and the lipid molecules, then the standard deviation is attributed to adhesion forces generated by more or less units interacting with the tip. Therefore, each force equals to the number of unit interactions $n$ multiplied by the force of a single unit interaction, $F_s$:

$$F_{adh} = nF_s \quad (3.25)$$

In addition the variance of the measurement, $\sigma$, the adhesion force, $F_{adh}$, $F_s$ and $n$ are related as:
\[ F_s = \frac{\sigma^2}{F_{adh}} \]  

(3.26)

and

\[ n = \frac{F_{adh}}{F_s} \]  

(4.27)

This method has the advantage that the knowledge of the mean radius of curvature of the tip is not required\(^2\).

Figure 3.10 The Gaussian distribution of adhesion force before polymerization at 1.5 \(\mu\)m/s.

For each loading rate, \(F_s\) and \(n\) are extracted from fitting the corresponding force distribution with a Gaussian. The results obtained before polymerization, are shown in Figure 3.11 and in table 3.2 for representative unloading rates. \(F_s\) have a constant value of 0.1±0.02 nN, but the number of unit interaction increases from 10 to 45 molecules with decreasing unloading rate as shown in Figure 3.11. These results confirm our interpretation that the decreasing adhesion force observed with increasing loading rate is due to a larger number of interacting units between the tip and the lipid layer when the time of interaction increases. We should keep in mind that \(n\) is not necessarily the number of molecules interacting with the tip, it is the number of single interaction which can be generated by a patch of lipid molecules. To determine the number of molecules per unit of
interaction, one should know the force $F_0$ of extraction of one lipid molecules from the monolayer. For bilayers, this value has been estimated to be between 30 to 140 pN\textsuperscript{73,74}. If we assume that such extracting values are similar for a lipid monolayer, and if we chose an arbitrary value of 80 pN, then the number of lipids interacting with the tip varies from 16 to 35. These values are in the same range as $n$ the number of interactions. This suggests that each interaction corresponds to the interaction between 1 lipid and the tip.

![Figure 3.11](image)

*Figure 3.11 The variation of “n”, the number of single interactions, between the tip and the layer as a function of the loading rate (m/s) before polymerization.*

<table>
<thead>
<tr>
<th>Unloading rate (m/s)</th>
<th>$F_{\text{adh}}$ (nN)</th>
<th>$\sigma$ (nN)</th>
<th>$F_s$ (nN)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.5 \times 10^{-6}$</td>
<td>1.3</td>
<td>0.42</td>
<td>0.13</td>
<td>10</td>
</tr>
<tr>
<td>$3 \times 10^{-7}$</td>
<td>1.76</td>
<td>0.4</td>
<td>0.09</td>
<td>19</td>
</tr>
<tr>
<td>$7 \times 10^{-8}$</td>
<td>2.8</td>
<td>0.5</td>
<td>0.09</td>
<td>31</td>
</tr>
</tbody>
</table>

*Table 3.2 The average variation of the adhesion force, the number of single interaction and the force of simple interaction with the loading rate before polymerization.*

After polymerization, $n$ is larger than before polymerization, it has an average value of 176. However $F_s$ have the same average value than before polymerization: 0.1 nN. In other studies, it has been proven that the single adhesion force $F_s$ varies when the phase of the lipid layer changes. For a DPPC layer for example, $F_s$ of the fluid phase is larger than of the gel phase\textsuperscript{75}. In our case the monolayer is always in the gel phase there is no phase variation between before and after polymerization. This is why we found it acceptable to have the same $F_s$ average value for the two
types of monolayers. However, we attribute the increase in the $n$ value after polymerization to the "collective" behavior of the lipid molecules. Because of the covalent bonding between lipids, not only the lipid in direct contact with the tip are affected, but also the bound "neighbor" lipids that will contribute to the adhesion force.

An important point to mention is that the detachment forces between the tip and the lipid layers should be smaller than the extraction force to avoid the tip coating. To be sure that we do not extract the lipid molecules, we did successive measurements on the same points, those curves show clearly that there is no tip coating. No important variations are seen for the tip jump height, neither the breakthrough force nor the adhesion force values.

### 3.3.2 Adhesion shape and tube formation

When comparing the adhesion curves before and after polymerization, see Figure 3.12, one can notice that the detachment mechanism of the tip from the surface is not the same. In a simple adhesion process, the tip adheres to the surface while the cantilever is moved backward. The pull-out force increases until it becomes larger than the adhesion force between the surface and the tip. As this point the tip is released from the surface and it returns to the position it should have when

**Figure 3.12** Curve showing the adhesion force between the tip and the lipid layer versus the distance between them, before and after the layer polymerization
no force is applied to it. This is what we observe in the case of the polymerized lipid monolayer, step A'. A scheme of the process is also shown in Figure 3.13a).

In the case of the non-polymerized lipid monolayer, the process is more complicated. The adhesion force is relatively weak and one can clearly observe that the detachment of the tip from the surface and the monolayer is a multi-step process. The first step (A), is nearly similar to the step A' discussed just previously. In contrast with the previous case, the release of the tip is not complete. The tip is still attached to the lipid layer and it pulls it away from the surface as it is withdrawn, steps B and C. The small jumps which can be observed correspond to the detachment of patches of lipids from the tip, therefore reducing their interaction force. During this stage, tubes, also called tethers, of lipids are created. The formation of tethers is a well known and studied phenomenon commonly observed on supported multilayers or bilayers as well as in the case of cell membranes. The experimental average adhesion force and length of these tubes are respectively 0.09 nN and 50 nm. A description of the formation of tethers is schemed in Figure 3.13b). When the tip is in contact with the lipids, a meniscus of lipids is formed around the tip. As the tip is pulled out, the lipid meniscus remains attached to the tip and grows to form a bilayer tube. The tube has a Y shape, with an arch of lipid molecules in direct contact with the tip, and a longitudinal tube linking the tip and the monolayer. At some point patches of lipids will detach from the tip. This detachment can be done in one or two steps. The final step is the complete detachment of the lipid tubes. After a detachment curve with tube formation we used the next breakthrough curve to make sure that there was no tip coating with the lipids; in the case of the formation of a monolayer or bilayer on the tip, a larger jump-in would be measured during indentation.

Figure 3.13 (a and b) give a scheme of the mechanisms involved when the AFM is pulled backward after a contact with the polymerized and non-polymerized lipid monolayer.
In our case this phenomenon was only observed before polymerization, no tether formation was observed after polymerization. To explain the difference between the two layers, we need to consider the diffusion coefficient of the lipids and the interaction of the lipids with the substrate. Indeed, after polymerization, the small mobility of the lipid molecules must prevent the formation of a meniscus around the tip and therefore the formation of a tether. Also, as mentioned previously for this case, the tip-lipid interaction cannot be described as the sum of numerous tip/lipid interactions but rather as the interaction between a tip and patches of lipids that interact with the tip collectively. This collective behavior makes the monolayer highly stable on the substrate.

We have suggested at different occasions in this chapter that the change in the mobility of the lipids might effects the behavior of the monolayer. Thorough measurements of the diffusion coefficient of the lipids have not been performed in this thesis. However, we have been able to estimate these coefficients for the two types of layers from the Gibbs energies obtained in section 3.2.4.2 as well as from AFM measurements.

Figure 3.13 The mechanism of the tip detachment on a polymerized (a) and non-polymerized lipid monolayer (b).
3.3.3 Diffusion coefficients of the lipids

When diffusion coefficients are small, a very basic way to get a rough estimation of the diffusion coefficients consists in creating a hole in a layer by removing the molecules and measuring the rate at which the hole refills. Such types of experiments were realized by AFM. To form a hole, the AFM tip is set in hard contact with the monolayer, and used to scratch the layer during scanning creating a hole in the layer of \(1 \mu m^2\). After the hole is formed, imaging of the surface is realized to observe the refilling of the hole. In all experiments, 512×512 images were scanned at a frequency of 1 KHz, therefore corresponding to one image/8.5 minutes. All experiments, hole formation and imaging, were realized in solution.

Figure 3.14 shows images of holes that were scratched off the lipid monolayer surface with an AFM tip. Image a) was obtained from a non polymerized monolayer immediately after the hole formation. Although one can see the hole on the image, the section of the layer indicates that it is mostly refilled. Depending on the layer preparation, some variability has been observed in this rate up to a factor of 2. Images b) and c) were obtained on the same polymerized and pierced monolayer just after the hole was formed and an hour later. After the first image, successive images were taken during one hour and the hole persisted with a depth of \(\sim 2.5\) nm. These measurements therefore indicate as expected a net decrease of the diffusion coefficient after polymerization.
Estimation of the diffusion constant of the lipid molecules in monolayer:

From AFM measurements:

On non polymerized lipid monolayer, the hole is usually completely refilled in the second image after the hole formation. The refilling time therefore corresponds to the time required to make 1.5 AFM images, i.e. $t = 12.75$ min.

Considering the lipid molecules on the border of a $1 \, \mu m^2$ hole, refilling the area requires a displacement of these molecules to the center of the hole, i.e., to move in an area of $0.5 \times 0.5 \, \mu m^2$. The diffusion coefficient can then be expressed as $D=S/t$, with $S$ the area covered by the lipids divided by the time. The diffusion coefficient of the non-polymerized lipid monolayer can be roughly estimated to $3.3 \times 10^{-12} \, cm^2/s$. For polymerized lipid monolayers, the refilling of the hole was too slow to be estimated.
From the Gibbs activation energy:

We have discussed before that if we consider that the formation of a hole is due to the diffusion of the lipids under the tip; it is then reasonable to compare the intrinsic Gibbs energy of the tip/layer system to create a hole in the layer to the energy of diffusion of lipids in layers. If we consider that the diffusive movement of lipid molecules is the result of consecutive jumps to the neighboring vacant sites. The hoping frequency at which the molecules jumps can be compared to \( k_0 \), the rate of spontaneous hole formation in the monolayer; \( k_0 \) is related to the Gibbs energy as shown in equation 4.18 and has been already calculated in part 3.2.4.

Considering a statistical random walk of the lipids, the diffusion coefficient, \( D \), and the hopping frequency, \( \Gamma \), are related as:

\[
D = \frac{\Gamma d^2}{Z} \tag{3.28}
\]

With \( d \), the distance to first neighbors (we assume that jumps are limited to the first neighbors). We will consider that \( d \) corresponds to the diameter of a lipid molecule: 0.895 nm for DC8,9PC, the lipid we are using.\(^78\). \( Z \) is the number of first neighbors per molecule; it depends on the dimensionality of the lattice. If we consider a close packed arrangement of the molecules in the monolayer, \( Z \) will be equal to 6. \( \Gamma \) is the hoping frequency; we assume that it is equal to \( k_0 \). Using the values of \( k_0 \) determined earlier, we can estimate the value of the diffusion constant. Before polymerization, \( D \) is estimated to be \( 1 \times 10^{-12} \text{cm}^2/\text{s} \). This value is in the same range of the value we extracted from the AFM measurements. In addition, this value is comparable to the typical values reported for supported lipid bilayer in the gas phase\(^79\) and with the values reported for DPPC bilayers at about the same measurements temperature (\( \approx 24^\circ \text{C} \)). We remember that DPPC and DC8,9PC have roughly the same melting point (41-42°C) and the same chains length and therefore the diffusion constant should be similar. If we do the same calculation for the diffusion constant after polymerization, we find a value 100 times smaller than before polymerization: \( D = 1 \times 10^{-14} \text{cm}^2/\text{s} \). This result confirms that the mobility of the lipid molecules decreases drastically after polymerization.

In this chapter, we have studied the nano-mechanical stability of supported lipid monolayers on silicon and demonstrated that the polymerization of the lipids in the monolayer induces drastic
modification of its properties (Young modulus, Gibbs activation energy of the tip/layer system, diffusion coefficient) but as well as of its interaction with the underlying substrate (adhesion).
CHAPTER 4: E
LECTRICAL STABILITY
4 Electrical stability

4.1 Introduction

In this chapter, we will discuss the electrical properties of polymerized lipid monolayers supported on silicon. As mentioned in introduction, our final goal is to use these monolayers as ultra-thin dielectrics to be used in electronic devices and in particular in field effect transistors based biosensors (Bio-FET). The substitution of the commonly used oxide gate by an organic dielectric was first motivated by the need to decrease the operating voltage using thinner dielectric layers. In the framework of a bio-FET, using low operating voltage not only participates to increasing the sensitivity of such type of biosensor but also prevents molecular damaging of the probe molecules and/or of the target molecules of organic nature (their bonds dissociation energies vary in the range from 3 eV to 9 eV). The main drawback in using ultra-thin dielectrics is the leakage current. Leakage current can be due to direct tunneling of the electrons across the dielectric or to trap assisted tunneling. It has been demonstrated that self-assembled monolayers of long alkyl chains (2.5 nm thick) present large energy barriers to tunneling carriers of typically 4.5 eV, making them good ultra-thin insulators. Phospholipids are also constituted of two long alkyl chains which should therefore also make them good insulators. Previous studies are reported on the electrical properties of supported lipid bilayers but all of them were realized in solution, on non-polymerized lipid bilayers. In order to use polymerized lipid monolayers as gate dielectric in a Bio-FET, it was important to study their electrical performances. Current-Voltage (I-V) measurements in air were realized to determine the leakage current, and the breakdown electric field of such layers. The measurements were compared with those obtained on a thin chemical silicone oxide of equivalent thickness.
4.2 Experimental set-up

4.2.1 The system

Electric measurements were done in air using a ‘home made’ set-up. The sample was fixed on a 3 axes-micrometric platform and was grounded. All measurements were realized using a Keithley 236 Source-Measure unit. Taking an electrical contact on organic thin films has always been a problem because of their fragility and the low thickness of those films. One can think of evaporating metal over organic films to take the electrical contact but this deposition can deteriorate fragile molecular films. In this work, we have chosen to use the mercury drop technique\textsuperscript{84}. This method allows the formation of a soft contact. A scheme of the system is shown in Figure 4.1. A drop of mercury is suspended to a conducting wire and brought into contact with the sample. The electrical resistance between the mercury drop and the electric wire was measured and found to be negligible.

![Electrical circuit for the current-voltage measurement on the lipid monolayer.](image)

\textit{Figure 4.1 Electrical circuit for the current-voltage measurement on the lipid monolayer.}

4.2.2 Calibration of the contact

It is well known that the current measured across a sample is proportional to the area of the electrical contacts. This is usually due to the fact that the probability to find defects (promoting leakage) in a material increases with the area. In addition, it is possible in the case of the lipid layer
that it undergoes some deformation under the drop with increasing contact area, i.e., with increasing loading, therefore modulating its thickness. To validate this contacting method, we have verified that this property applies here. A set of measurements was realized on the same lipid monolayer for varying contact areas. A picture of the drop on the surface was obtained from which the contact area can be estimated using a reference of known dimensions. An example of two different contacts is shown in Figure 4.2.

![Figure 4.2 Photos showing the different contact area taken on the lipid monolayer.](image)

A typical calibration curve obtained on a polymerized lipid monolayer is shown in Figure 4.3. The current reported here was measured at an electric field of 1 MV/cm. One can see that the leakage current across the monolayer varies linearly with increasing contact area therefore indicating the quality of the contact.

In the following, for each current-voltage measurement, the contact area was estimated from the picture of the drop on the surface.

![Figure 4.3 Curve showing the evolution of the leakage current with increasing contact area at 1MV/cm. The measurements were realized on a polymerized lipid monolayer in air](image)
4.3 Densities of leakage current

Figure 4.4 shows two typical curves obtained at room temperature in the air, of the current density evolution versus the applied electric field (J-E curve) measured on a polymerized lipid monolayer and on a 2.2 nm chemical silicon oxide surface used for comparison. For the two dielectrics, these J-E characteristics present similar behavior with symmetric curves but show, however, large differences in current densities. For quantitative comparison between lipid monolayer and silicon oxide, we have reported in table 4.1 the values of current density, for significant values of the applied electric field. For the monolayer, these increase with applied electric field from $5 \times 10^{-8} \text{A/cm}^2$ at 0.5 MV/cm to $1 \times 10^{-6} \text{A/cm}^2$ at 5MV/cm. For the silicon oxide layer, the densities of current are much higher and vary from $4 \times 10^{-7} \text{A/cm}^2$ at 0.5 MV/cm to $4 \times 10^{-4}$ at 5 MV/cm, therefore indicating a difference of two order of magnitude especially at high electric field. The values obtained on silicon oxide are in very good agreement with other data reported in the literature. To our knowledge, no other electric measurements realized on such type of layer was reported before, but the values, we measured on the lipid monolayer compare favorably with the best data obtained in organic thin film dielectrics, for which current density falls in the range of $10^{-6}$ to $10^{-8} \text{A/cm}^2$ at high electric field.

![Figure 4.4 Current density versus applied electric field for a lipid monolayer (blue) and a 2.2 nm thick chemical oxide layer (red).](image)

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<table>
<thead>
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<th>0.5</th>
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<th>3</th>
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<tr>
<td>Current density</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>for lipid monolayer</td>
<td>5x10⁻⁸</td>
<td>2x10⁻⁷</td>
<td>7x10⁻⁷</td>
<td>1x10⁻⁶</td>
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<tr>
<td>(A/cm²)</td>
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</tr>
<tr>
<td>Current density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>for 2.2 nm silicon oxide</td>
<td>4x10⁻⁷</td>
<td>3x10⁻⁶</td>
<td>6x10⁻⁵</td>
<td>1x10⁻⁴</td>
</tr>
<tr>
<td>(A/cm²)</td>
<td></td>
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Table 4.1 Table showing the different values of leakage current (A/cm²) for different applied electrical field (MV/cm) through the lipid monolayer and the 2.2 nm thick silicon oxide layer.

The average lipid layer resistance, deduced from several J-E characteristics recorded on different samples, is found to be equal to 300 MΩ at 1 MV/cm. This value is reasonable if we compare it with the ~1 GΩ resistance of the cell membrane, knowing that the cell membrane is constituted of a lipid bilayer instead of a monolayer.

4.4 Electrical breakdown

In dielectrics, breakdown voltage is a critical parameter that determines the maximum electric field that can be applied across the material before the insulator collapses and starts conducting. This parameter defines the maximum working potential that can be applied to the insulator. It is generally characterized by a local increase in conductance by several order of magnitude and material degradation. This parameter is a key parameter for the application of such layer as insulator in devices.
4.4.1 Breakdown of the lipid monolayer

To estimate the breakdown electric field of the polymerized lipid monolayer, we realized current-voltage measurements with voltage following a ramp from 0 to 10V at different speed rates. These measurements were done in air at room temperature on several samples. Every sample was imaged by AFM before measurements to be sure of the lipid monolayer quality.

Figure 4.5 shows two typical I(V) curves obtained at two speed rates a) 0.2V/s and b) 2V/s. On both graphs, the red curve is the full measurement and the blue curves are the zooms of part of the full curve. Looking at the full curves, one can see in figure a) a large jump of the current above 8 V while no such jump is observed in figure b). At the jump in figure a) the current increases by a factor of ~10, which is smaller than what one would expect for a breakdown. By zooming on the curves, one can see in both figures several pre-jumps at lower voltages that also induce an increase of the current by a factor of ~10. These pre-jumps can be seen as incomplete breakdowns therefore suggesting that although the monolayer is affected, the breakdown in our case is not a critical event that happens at a given electric field but seems to be a kinetic event that develops with increasing voltage.

To verify this point, we have realized I(V) curves at different rate of the voltage ramp. The values of the voltage at which the first breakdown (BDV) occurs are reported in table 4.2, together with the value of the corresponding electric field (BDE). Each value is an average of at least 10 measurements.
The first observation is that the values of BDE vary from 8.5 to 15.5 MV/cm for the range of investigated voltage ramp rates. These results are extremely good regarding other measurements realized on supported lipid bilayers or black lipid membranes for which breakdowns were reported below 2 MV/cm\(^8\)\(^9\). The main difference relies on the polymerization state of the lipid membrane. In these previous studies, the lipids were not polymerized, and like in biological membranes, they were highly mobile within the membrane, with diffusion rates that can be reasonably high depending on the temperature and on the lipid type. When an electric field is applied, electroporation occurs due to electrostatic repulsion and pores are formed within the membrane.
therefore increasing its permeability to ions and charges\textsuperscript{89,90}. In the present case, the lipids are polymerized, their entropy is strongly reduced, and their mobility is limited to small fluctuations in their surroundings. We believe that in this case, the lipid layer should be considered as an ultrathin two-dimensional polymer.

### 4.4.1.2 Breakdown is a kinetic process

It has been shown that, in the case of polymers, electrical breakdown is not a critical event that occurs at a certain electric field intensity characteristic of the considered polymer. It is a kinetic process that develops in time and which is characterized by the damage accumulation rate and its inverse value, the lifetime of the polymer in the electric field; the measured electrical strength will be higher for higher ramp rate of the electric field\textsuperscript{91,92}. In Figure 4.6 a), the electric field corresponding to the first breakdown is reported versus the voltage ramp rate. As expected the electric field is higher for higher values of the voltage ramp rate. We show here a logarithmic dependence of the two parameters.

To determine the lifetime of the monolayer in a given electric field, we have measured over time the leakage current across the monolayer at a given applied voltage. The value of the lifetime reported in Figure 4.6 b) corresponds to the time to the first breakdown. As one can see from the figure, the lifetime decreases exponentially with increasing electric field (the red line corresponds to the fit of the data with \( \tau \sim \exp(-\alpha E) \)), therefore confirming that the damage accumulation rates varies with the electric field. In conclusion, we can say that the lipid monolayer behaves as a polymer.

![Figure 4.6 a) Breakdown electric field versus the voltage ramp rate. b) Lifetime of the monolayer in the electric field.](image-url)
4.4.1.3 Mechanism of electrical degradation and breakdown in insulating polymers

The mechanisms involved in polymers soft breakdown are still not perfectly understood. Several processes have been suggested that we will briefly mention here.

Thermal breakdown can occur in a solid dielectric when its rate of heating exceeds its rate of cooling. In ultra-thin layers, heat dissipation is high and this process is very unlikely to happen.

Another theory is based on the fact that electrons can be injected in the polymer and trapped by positively charged impurities or defects within the polymer. The local accumulation of these electrons can lead to the creation of a local enhanced electric field which can be responsible for the molecular dissociation of the polymer into macro-ions\textsuperscript{92,93} or for facilitating electron tunneling across the polymer.

The mechanism of breakdown in the lipid monolayer is still not completely clear to us some further studies should be done. Indeed, the formation of lipid pores such as described for non-polymerized lipid layers is excluded. In this case the field interacts with the charges present within the headgroups and is at the origin of formation of small hydrophobic pores, with the aliphatic chains in contact with the water. As the pores grow the lipids reorganize and reorient themselves to create a hydrophilic pore. This process therefore involves reorganization of the lipids, i.e., displacement of the lipids and compression of the monolayer.\textsuperscript{94} We showed earlier that in the polymerized lipid monolayer the mobility of the lipid molecules is strongly reduced, as well as their entropy. We believe such electroporation is not an adequate explanation for the polymerized monolayer. We rather believe that breakdown is related to the creation of a high electric field in the layer due to electron trapping. Some studies should be realized then to measure the density of these trapped charges and their distribution within the layer.
4.4.1.4 Leakage current after the breakdown

For a better understanding of the breakdown processes, we extracted the values of the current just after the last breakdown occurred. All the values were reported for the same voltage ramp rate (\(V_{\text{rate}} = 200 \text{ mV/s}\)). Figure 4.7 a) shows several I(V) curves realized in the same conditions on different samples. We can notice the variability of the breakdown voltage values for the same ramp rate, which can be explained by differences in the density of lipids of the prepared monolayers. It is very logic to say that the conducting channels will form more rapidly in low-density areas. For a given polymer, it is therefore expected that the electrical strength will be dependent of its molecular density. The values of the current just after breakdowns are reported in Figure 4.7 b) A very interesting point is that the current after breakdown increases linearly with the breakdown voltage. We can therefore conclude that the leakage current is dependent of the conducting channels size and that it is proportional to the BDV. Lower breakdown voltage therefore suggests lower densities and smaller conducting channel area.

![Figure 4.7 a) curves showing the final electrical breakdown of polymerized lipid monolayer in the air at room temperature. b) the current measured just after the breakdown versus the correspondent breakdown voltage.](image)

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4.5 Autonomic self-Healing after break-down

After breakdown, we have observed a very interesting phenomenon of autonomic self-healing of the lipid monolayer. An example of such phenomenon is shown in Figure 4.8 a) and b) show the entire measured curves and a zoom in the 0-3.5 V region respectively. The three curves were obtained in the following order: The black curve was first acquired until the monolayer undergone breakdown. The grey curve was subsequently measured and finally the red curve after leaving the voltage at zero for one minute. As expected, after breakdown, the I(V) characteristic indicates a net increase of the current at low voltage by a factor of 100. After the second measurement, the voltage is zero for one minute in the air at the room temperature, the third measurement of the I(V) curve is suprisingly similar to the one obtain before the breakdown. This type of measurements has been repeated on the same sample several times, up to 20 times, and the monolayer shows healing every time. This experience therefore suggests that the degradation of the insolent performance of the monolayer induced by the breakdown is reversible. To exclude the possibility that self healing could be due to the formation of silicon oxide, similar measurements were realized on a 2.2 nm thick chemical silicone oxide. In this case no self-healing was observed. In fact, self-healing of polymer was already reported after soft breakdown\textsuperscript{95,96}, but it always requires an external stimulus such as heating for a few hours, exposure to UV, or the addition of a polymerizing agent\textsuperscript{97,98,99}.

![Graphs showing current-voltage curves](image)

*Figure 4.8 (a) the graph shows three current-voltage curves done on the same point of the monolayer the black is the first one, the grey was directly done after the black, the red was done one minute after the grey. (b) graphs showing low voltage I(V) curves plotted in log scale.*
To understand the self healing mechanism, we should first have a clear idea of the breakdown mechanism. As explained earlier, the origin of the breakdown is not clear. Indeed, in non polymerized lipid monolayer, the mechanism of electroporation is initiated by the formation of a hydrophobic pore. When the diameter of the pore becomes large enough (~2 nm), the lipids at the interface reorient themselves to form a hydrophilic pore. This mechanism therefore implies lipids displacement and consequently compression of the layer and reorganization of the lipids at the interface. When the lipids are polymerized, their entropy is strongly reduced, and their mobility is limited to small fluctuations in their surroundings. One can therefore believe that the pore formation depends on the polymerization state of the layer and requires the rupture of chemical bonds between lipids. The lipid layer can therefore be considered as an ultra-thin polymer. In this case breakdown is a multi-step process that starts by the injection of electrons in the polymer and ends by the dissociation of the macromolecules, hence at the origin of the creation of low density areas or by the creation of an enhanced local electric field in the layer favoring tunneling.

The results obtained on the regeneration allow us to suggest two hypotheses. First, the chemical bonds between the molecules are not ruptured. Both breakdown and self-healing mechanisms are similar to those of non-polymerized lipid monolayers. The formation of the pores is then only related to the displacement and reorganization of the molecules in the layer, therefore suggesting a low degree of polymerization. Second, the macromolecules are dissociated; the self-healing is due to the presence of free radicals in the layer at the origin of the spontaneous repolymerization of the lipids in the layer.

To answer this question, complementary experiments are required. For example, we are planning to relate the degree polymerization of the layer to the diffusion coefficient of the lipids, to its electrical stability and to its capacity of regeneration. The comprehension of such mechanisms is fundamental to improve the physical properties of the lipid layers.

As a conclusion of this chapter, we report the reliable and reproducible electrical performances of phospholipid layers as a dielectric layer. It has a good differential resistance and a good resistance to the charge carriers tunneling. Its electrical break-down field is very high comparing to the best data reported in the literature for lipid bilayers. In addition, the polymerized lipid monolayers demonstrate a remarkable property of autonomic self-healing after electrical breakdown that make them of particular interest.
CHAPTER 5:
APPLICATION OF THE MONOLAYER TO A FET USED AS A FERRIC ION BIOSensor
5 Application of the monolayer to a FET used as a Ferric ion biosensor

As mentioned in introduction, the ultimate goal of this work was to develop a field effect transistor based bio-sensor in which the gate dielectric is a lipid monolayer. In parallel to this thesis, the device was developed by Tuyen Nguyen, in the facilities Planète. The same lipid monolayer has been used to fabricate the gate insulator. In the following, I will show some of the results obtained to demonstrate the sensing possibilities of this device. Concerning this project my contribution was focused on the chemical modification of the lipid to attach a molecular probe to get a specific detection of ferric ions.

5.1 Principle of field effect transistor based biosensors (Bio-FET)

Our device is derived from a Metal-Oxide-Semiconductor type field effect transistor (MOS-FET), see Figure 5.1. Such devices are constituted of a semiconductor substrate and of two electrodes, the source and the drain located at each of its extremities. When the channel is polarized by a source-Drain tension $V_{DS}$, the channel conducts, a current $I_D = V_{DS}/R$ passes through the channel, with $R$ the
electrical resistance of the channel, \( R = \rho/s \) with \( s \) the section of the semi conducting channel.

The current in the channel can be controlled by polarizing a third electrode, the gate, located above the channel and separated from the channel by the gate dielectric. The dielectric prevents the direct injection of charges in the channel. In such device, the current in the channel is controlled by the electric field across the dielectric. It is a charge sensitive device; no charge is flowing between the gate and the source. The conductive property of the channel is controlled by the charge accumulate at the gate surface. The gate dielectric is usually constituted of an inorganic oxide (it can also be a polymer). Because bio-sensing experiments are very often realized in a liquid environment, the gate electrode usually constituted of a metal layer is replaced in a Bio-FET by an electrolyte and a reference electrode dipped into the electrolyte. The source and drain contacts are protected from the electrolyte by an insulating layer such as SU-8 for example. A scheme of a bio-MOSFET is depicted in Figure 5.2. In such devices, the specificity of the sensor is ensured by the presence of specific probes immobilized at the surface of the gate dielectric. The dielectric layer together with the probe molecules constitute the sensitive layer of the device.

In a typical experiment, a solution containing target molecules/ions is poured onto the gate area. When charged targets molecules are captured by probes, then the charges carried by the target molecules induce a variation of the surface charge on the gate dielectric which is equivalent to a change in the gate-source voltage. This induces a change of the electric field across the dielectric and a variation of the current in the channel. This current can then be measured and is related to the number of captured targets at the surface of the dielectric.
5.2 Our device and its specificities

5.2.1 The device

Our device was fabricated from silicon on Insulator (SOI) wafer using a four steps process using optical lithography. Figure 5.3 shows pictures of a) the transistor wafer and b) of a chip. A well made of PDMS is glued on top of the chip to contain the analyte. Each chip contains 4 transistors with channels of different section and length. Image c) shows three of these transistors. In our device, the oxide is removed and replaced by a polymerized lipid monolayer.
Specificity of the detection: Modification of the lipid head-group

The lipid molecules were modified by a pyridinone derivative used as a specific chelator of the ferric ions. This part of my work was done in collaboration with Jean-Manuel Raimundo (CINaM). Hydroxypyridinone derivatives comprise one of the well-known chelating moieties in medicinal chemistry. In addition to its selectivity towards Fe(II) and Fe(III) ions, its electrical neutrality makes it an asset to improve the sensitivity of our device. Under physiological conditions, these derivatives show a high affinity to both ferric and ferrous ions and they have the ability to form five-membered chelate rings with iron in which the metal is coordinated by two vicinal oxygen atoms leading to neutral and stable 3:1 complexes. The lipid modification scheme is shown in Figure 5.4. At first the phosphatidylcholine head-group of the DC8,9PC (1) was selectively cleaved with phospholipase C into product (2) in a quantitative yield. The compound (2) is then reacted with the pyridinone derivative (3) leading to the modified lipid (4). The compound (3) itself was synthesized in 2 steps from commercially available Kojic acid using standardized procedures. Modified lipid molecules were used to form a solid monolayer with the specific chelator pointing towards the outside of the layer.
The characterization of the Bio-FETs and the first sensing experiments has been realized by Tuyen Nguyen (postdoc in the group). In the following, I will show the first results.

5.3 Characterization of the transistors and first sensing experiments

5.3.1 Characteristics of the transistors

In the following, all measurements were realized in water. An Ag/AgCl reference electrode was used. Typical output, $I_{DS} = f(V_{DS})$ at different $V_{GS}$ value, and transfer, $I_{DS} = f(V_{GS})$ for a constant value of $V_{DS}$, characteristic curves are shown in Figure 5.5. Both curves show good transistor behavior with a current in the channel ($I_{DS}$) which is directly proportional to the aspect ratio of the channel.
The threshold voltage, which is \( \sim 0.4 \) V allows the use of low working potentials, i.e., limiting the risk of dielectric breakdown.

![Graph showing output characteristics of a BioFet transistor](image)

*Figure 5.5 (a) output characteristics of the BioFet transistor \( I_{DS}=f(V_{DS}, V_{GS}) \). (b) The variation of the drain-source current versus the gate-source voltage for different channel width.*

Those curves show that the transistor have the behavior of a FET transistor. The FET regime is obtained for relative low value of \( V_{DS} \) (0.5V). Above 0.5V, the \( I_{DS} \) current depend on the value of \( V_{GS} \) this result is a demonstration of the capability of a lipid monolayer replace the silixon oxide as a dielectric gate.

Note that those measurements done on the same FET for a whole week give the same results. The lipid monolayer is stable enough to maintain the same characteristics over a long period of time.

### 5.3.2 Sensing experiments: Detection of ferric ions

#### 5.3.2.1 Sensitivity measurements

In a typical experiment, 100 µL of an aqueous solution of ferric ions at a given concentration was poured on top of the gate area and left for 5 minutes with continuous agitation. The ion-chelator complex was stabilized by addition of overloads of kojic acid. After two minutes the solution was thoroughly rinsed with water to remove the excess of kojic acid and ferric ions from the electrolyte. This protocol ensured that only the trapped ions will participate to changing the current in the channel. The sensitivity of the biosensor was determined directly from the transfer characteristic of the transistor: the channel current \( I_{DS} \) variation versus the gate voltage for \( V_{DS}=1.5 \) V. As positive
charges were captured, an increase of $I_{DS}$ was expected when ions are captured on the gate. The concentration of the aqueous solution of ions was varied between $5 \times 10^{-14}$ to $5 \times 10^{-3}$ M. The corresponding transfer characteristics and the variation of current with respect to $I_{DS}^{ref}$ when no metal ion was captured are reported in Figure 5.6. The results show a linear increase of $I_{DS}$ with the logarithm of the concentration therefore indicating the possibility to quantify the concentration of Fe$^{3+}$ ions in the solution. Another important point is that a measurable signal was obtained down to a concentration of 50 fM. Such sensitivity is extremely good for this type of sensor.

![Figure 5.6 Transfer characteristics of the transistor after detection assays using analytes of Fe$^{3+}$ at different concentrations. $V_{DS}$ was kept constant at 1.5 V. Inset: variation of the $I_{DS}$ at $V_{GS}=1.8V$ with respect to the value obtained when no metal ions were added to the solution. Each value corresponds to an average of three measurements.](image)

5.3.2.2 Specificity measurements

To verify the specificity of the measurement, we first realized sensing experiments using a non-modified lipid monolayer using lipid 1 in Figure 5.4. For a concentration of $10^{-3}$ M, the current varies by only 3% when it varied by 27% using the modified lipid 4. This result clearly shows the high affinity of the chelator towards Fe$^{3+}$ ions. It also shows that the lipid layer constitute a good passivation layer to non specific adsorption.

Control assays were also realized using analytes containing other types of ions. The results are shown in Figure 5.7. For all tested ions, Al$^{3+}$, K$^+$, Pb$^{2+}$ and Cu$^{3+}$, the variation of current never
exceeded 5% even at high concentration, therefore showing a good specificity of the sensor. We believe this signal might arise from the non-specific adsorption of the ions on the layer.

Finally the speciation capability of the chelator to differentiate between Fe\(^{3+}\) and Fe\(^{2+}\) was also investigated using Fe\(^{2+}\) solution. In this case the behavior is different. One can observe a small increase of the current with increasing concentration of ferrous ions. However the variation of current at high concentration never exceeds 7% and remains 20% lower than the signal obtained for Fe\(^{3+}\) ions at the same concentration. This signal could be due to the complexation of the ions with the chelator but we rather believe that it is related to the oxidation of the Fe\(^{2+}\) into Fe\(^{3+}\) in the aqueous solution.

In conclusion, we can say that the polymerized lipid monolayer present sufficiently good dielectric properties to be used as gate dielectric in a field effect transistor. In addition, the head-group of the lipids can be easily chemically modified to attach to it suitable probes. Because of this property, the device is versatile and can be adapted to other kinds of detections.
GENERAL CONCLUSION
General Conclusion

In this work, we have studied the properties of polymerized lipid monolayers supported on silicon. The idea behind this work was to stabilize lipid monolayers in air in order to use them in the development of devices, and in particular, in the development of bio-FET in which the lipid monolayer plays the role of the gate dielectric.

We first established an experimental protocol in which the lipid monolayer obtained by vesicle fusion directly at the surface of silicon is stabilized by two-dimensional radical polymerization in the plane of the layer. The final layer has a thickness of 2.8 ± 0.3 nm. It remains unchanged upon drying or rinsing with polar solutions and can be conserved several weeks.

We studied the nano-mechanical stability of these monolayers using force measurements by AFM. Similar measurements were realized on non-polymerized lipid monolayers and we proved that the polymerization drastically improves the mechanical resistance of the monolayer when a force is exerted perpendicular to the plane of the layer. The force required for the AFM tip to rupture the layer increases from 98 ± 67 pN before polymerization to 1.4 ±0.72 nN after polymerization. From experiments realized at different loading rates, we showed that the break-through force increases linearly with the logarithm of the tip loading rate. These data have been used to determine the intrinsic Gibbs activation energy required to form a hole in the layer and we show an increase of this energy by a factor of ~3 after polymerization. Finally the Young modulus of the two types of monolayers has been estimated, and we also show an increase from 1-4.5 MPa to 110-330 MPa before and after polymerization respectively. This last value is comparable to the Young's modulus of low density polyethylene used in the fabrication of plastic bags.

An improvement of the monolayer stability on the substrate was also proved through the measure of the tip-layer adhesion force. Before polymerization, a tube of lipids is very often formed between the tip and the layer while the tip is moved away from the layer. The formation of this tube implies that the lipids interaction with the tip is higher than with the substrate. In this case the measured adhesion force of 2±1.5 nN corresponds to the adhesion of lipids on the substrate. After polymerization, in contrast, the formation of tubes was never observed and the relatively high measured adhesion force (11.5±1.5 nN) has been attributed to the interaction of the tip with the lipid monolayer. This difference in the adhesion behavior between the two monolayers has been
attributed to a decrease of the lipid mobility after polymerization as well as to the collective behavior of the polymerized lipids that makes the monolayer highly stable on the substrate. The change in diffusion coefficient has then been estimated from $3.3 \times 10^{-12}$ cm$^2$/s to $1 \times 10^{-14}$ cm$^2$/s before and after polymerization respectively.

In the framework of the Bio-FET application, we also studied the electrical properties of the monolayer. We demonstrated relatively good insulating properties with low current leakage (about 100 times smaller than for an oxide of equivalent thickness), and a breakdown electric field that varies between 8 and 15 MV/cm depending on the voltage ramp rate. These values are very good considering that for non-polymerized lipid monolayers breakdown electric field of 2 MV/cm were reported. In addition, we demonstrated a very interesting property of autonomic self-healing after break-down in a very short amount of time. This property is unique and makes this layer very interesting for the development of devices.

We finally demonstrated the possibility to use the monolayer in a field effect transistor based biosensor developed for the detection of ferric ions.

In conclusion, we can say that the polymerization of the lipid monolayer induces drastic improvements of its nano-mechanical and electrical properties that make it a very promising material to be used in advanced processes. In order to perfectly understand these changes complementary studies need to be performed; in particular to understand the processes involved in the breakdown and the self-healing of the monolayer.
Résumé

Depuis les premières études in-vitro sur l'autoassemblage des lipides en bicouches par l'équipe de Rudin dans les années 1960 [1], les membranes de bicouches lipidiques ainsi que les liposomes sont devenus les modèles expérimentaux des membranes cellulaires les plus utilisés. Ces biomembranes, qui constituent la paroi externe des cellules et des organelles sont le lieu de conversion énergétique, de transport de matière, de bio-détection et de transduction de signaux. En tant que composants principaux des membranes cellulaires, les phospholipides ont suscité beaucoup d'intérêt dans la communauté scientifique et en particulier dans le milieu biomédical.

La nature amphiphile des phospholipides gouverne en particulier la formation de la membrane en milieu aqueux dans laquelle les lipides sont libres de se mouvoir. Les molécules s'auto-arrangent en bicouches en orientant leurs têtes polaires vers milieu aqueux, à l'extérieur de la bicouche et leurs chaînes lipophiliques vers l'intérieur de la bicouche, formant ainsi une région non-polaire entre deux régions polaires.

Sous forme de vésicules, les bicouches peuvent être utilisées comme système modèle des cellules [2] ou pour encapsuler des réactifs ou particules pour le transport de médicaments ou le ciblage in-vivo [3]. Ces bicouches peuvent également être transférées sur substrat, permettant ainsi la fonctionnalisation de substrats inorganiques ou polymériques, tout en leur conférant une fonction bio-mimétique. Elles constituent ainsi des matrices idéales pour l'incorporation de protéines membranaires ou de récepteurs, pouvant être ainsi utilisées dans des études sur les mécanismes biologiques qui impliquent l'assemblage entre un récepteur et une cible. Par exemple, des études sur le fonctionnement des canaux ioniques, sur l'adhésion cellulaire, pour mesurer l'activité de certaines enzymes, ou encore dans le développement de biocapteurs.

Dans cette thèse, notre intérêt porte sur une autre propriété des biomembranes, à savoir leur propriété d'isolant électrique. En effet, dans les membranes cellulaires, la bicouche lipidique sépare les régions intra- et extra- cellulaires et forme une barrière au transport ionique entre ces deux régions; l'échange d'ion se faisant via les canaux ioniques insérés dans la bicouche. De plus les biomembranes présentent une forte barrière au transport électronique avec une résistance à travers la bicouche de l'ordre du Giga-Ohm. Notre idée est d'exploiter cette propriété et d'utiliser une monocouche de lipide comme diélectrique de grille dans le développement d'un biocapteur à
transistor à effet de champ (Bio-FET) en remplacement des oxydes inorganiques généralement utilisés. L'objectif ici est de diminuer l'intensité des tensions de travail dans ces dispositifs en réduisant l'épaisseur de la couche diélectrique de la grille tout en maintenant de bonnes propriétés isolantes et en limitant l'endommagement des molécules organiques/biologiques à détecter. Les monocouches de lipides, d'une épaisseur variant entre 2 et 3 nanomètres constituent donc de bonnes candidates. Cependant l'un des principaux défauts de ces couches est leur instabilité à l'air qui a jusqu'à présent constitué une limite à leur utilisation dans des procédés industriels avancés. Pour surmonter ce problème, nous avons sélectionné un lipide qui peut être polymérisé dans le plan de la couche, augmentant ainsi sa stabilité. Le travail rapporté dans cette thèse est focalisé sur les propriétés de cette monocouche polymérisée et supportée et en particulier sur ses propriétés nano-mécaniques et électriques. Ce manuscrit est divisé en cinq parties principales.

La première traite des méthodes expérimentales principales utilisées dans cette thèse, à savoir la microscopie à force atomique (AFM) et ses dérivés et la spectroscopie de photoémission (XPS) pour caractériser la composition chimique des couches polymérisées. L'AFM a été utilisée suivant deux modes principaux; en mode imagerie à l'air et en milieu aqueux pour imager la surface de lipides et en mode spectroscopie de force pour étudier les propriétés nano-mécaniques des monocouches supportées.

La deuxième partie du manuscrit concerne la formation et la stabilisation de la monocouche sur une surface de silicium hydrogéné. Nous avons opté pour la méthode de la fusion de vésicules basée sur l'adsorption et la fusion de petites vésicules uni-lamellaires dispersées en milieu aqueux sur un substrat. Comme mentionné précédemment, notre objectif est de former une monocouche de lipide à la surface du silicium pouvant ainsi jouer le rôle de diélectrique de grille ultra-mince dans un transistor. La formation d'une monocouche de lipide supportée dans laquelle les lipides sont orientés avec leur tête hydrophile vers le milieu aqueux et leurs chaînes aliphatiques vers le substrat nécessite que le substrat présente une surface hydrophobe, voir figure R1.
Pour cela, la monocouche de lipide est formée sur une surface de silicium hydrogénée (hydrophobe) qui est obtenue par attaque chimique dans l’acide fluorhydrique de la couche d’oxyde natif. La surface de silicium ainsi obtenue est plus stable vis-à-vis de l’oxydation à l’air mais reste oxydable en milieu aqueux. De plus, l’interaction répulsive entre les têtes des lipides hydrophiles et la surface hydrophobe défavorise et ralentit la formation de la monocouche de lipide. Nous avons donc développé une méthode pour accélérer la protection de la surface de silicium vis-à-vis de l’oxydation en forçant l’adsorption des vésicules sur la surface et en promouvant leur fusion. Ceci est obtenu par refroidissement rapide de la surface après dépôt de la solution de vésicules. La formation d’une monocouche est alors obtenue en quelques dizaines de secondes. Une fois formée, la couche est stabilisée par polymérisation radicale. La monocouche ainsi formée a une épaisseur de 2.8±0.3 nm et est parfaitement stable à l’air comme en milieu aqueux.

Dans la troisième partie, nous avons étudié les propriétés nano-mécaniques de ces monocouches que nous avons comparées aux propriétés des mêmes couches avant polymérisation. Pour cela, nous avons réalisé des mesures de force par AFM. Pour les deux types de surface, une étude statistique a été effectuée au cours de laquelle nous avons notamment varié la vitesse d’indentation. La figure R2 montre une courbe d’approche-retrait obtenue sur une monocouche de lipides. Au cours de l’approche, le cantilever initialement loin de la surface rentre en contact avec la monocouche. A partir de ce point le déplacement de la pointe vers la surface induit l’application d’une force par la pointe sur la couche. Si celle-ci résiste, le cantilever, avec une constante de raideur donnée, défléchi sur la surface et la couche se déforme élastiquement jusqu’à ce que la force exercée devienne suffisamment importante pour rompre la couche. A ce moment là, la pointe pénètre la couche et "saute" sur le substrat de silicium. La force de rupture, et l’épaisseur de la couche au moment de la rupture sont deux paramètres caractéristiques de la couche.
A partir de ces mesures nous avons pu déterminer un certain nombre de grandeurs physiques caractéristiques de la couche de lipide en fonction de son état de polymérisation. Par exemple lorsque la couche est polymérisée une force appliquée ~10 fois plus importante est nécessaire afin de rompre la couche. Nous avons déterminé l'énergie d'activation de Gibbs intrinsèque du système pointe/monocouche correspondant à l'énergie nécessaire à la création d'un trou dans la couche permettant la pénétration de la pointe. Cette énergie est augmentée d'un facteur 3 après polymérisation et atteint la valeur de 7.3 kT. Cette différence d'énergie a été associée à un problème de relaxation de la couche sous la pointe lorsque cette dernière lui applique une pression. Il est alors raisonnable de relier cette variation d'énergie à la diminution du coefficient de diffusion des lipides dans la couche et de leur entropie lorsque ceux-ci sont polymérisés. A partir de ces données et de mesures AFM, nous avons estimé que le coefficient de diffusion des lipides diminue d'un facteur 100 et passe de ~10^{-12} cm²/s à ~10^{-14} cm²/s après polymérisation. En utilisant le modèle de Hertz, nous avons également estimé le module de Young pour les deux types de couche et avons montré une augmentation de ~2 MPa avant polymérisation à ~200 MPa après polymérisation. Une telle valeur correspond au module de Young du polyéthylène de basse densité utilisé dans la fabrication de sacs plastiques.

De même nous avons étudié l'adhésion de la pointe sur la surface (substrat+couche) lors de la rétraction de la pointe. La mesure de la force d'adhésion est obtenue à partir des courbes de retrait. Nous avons pu mettre en évidence deux comportements distincts en fonction de l'état de la couche. Avant polymérisation la pointe tend à entraîner avec elle les lipides qui s'organisent sous

Figure R2 Mesure de force réalisée sur une monocouche de lipide par AFM. a) Déflection du cantilever en fonction de déplacement du piezoélectrique: Rouge: Courbe d’approche. Bleu: Courbe de retrait. b) Courbe d’approche: Force en fonction de la distance pointe–surface.
forme de tube reliant la pointe à la couche sur la surface. Une force d'adhésion de typiquement 2.5 nN a été mesurée correspondant à l'adhésion de la couche sur la surface. Après polymérisation la force d'adhésion augmente fortement jusqu'à 11.5 nN. Dans ce cas là il n'y a pas formation de tubes, la couche reste sur le substrat, et la force mesurée correspond à la force nécessaire pour détacher la pointe de la couche de lipide. Cette différence peut-être expliquée par deux arguments: Tout d'abord la diminution du coefficient de diffusion des lipides lorsque la couche est polymérisée limite le mouillage de la pointe par les lipides. Deuxièmement, dans ce cas là, l'interaction pointe-couche ne peut-être décrite comme la somme de plusieurs interactions pointe/lipide mais plutôt comme l'interaction entre une pointe et un ensemble de lipides qui interagissent avec la pointe de manière collective. Ce comportement collectif rend la couche très stable sur la surface.

Tous ces résultats indiquent un changement drastique des propriétés de la monocouche polymérisée. Non seulement ses propriétés nano-mécaniques sont nettement améliorées mais en plus elle démontre une plus grande stabilité sur son substrat.

Dans la quatrième partie, nous avons étudié les propriétés électriques des monocouches. L'objectif était de vérifier que ces couches de 2.8 nm d'épaisseur seulement peuvent jouer le rôle d'isolant dans le développement d'un dispositif. Les courants de fuite à travers la couche ont tout d'abord été évalués. Ceux-ci ont été comparés à ceux mesurés sur une couche d'oxyde de silicium d'épaisseur équivalente. Les résultats obtenus sont rapportés dans la table R1 pour différentes valeurs du champ électrique appliqué.

<table>
<thead>
<tr>
<th>Champ électrique (MV/cm)</th>
<th>0.5</th>
<th>1</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Densité de courant (A/cm²)</td>
<td>5x10⁻⁸</td>
<td>2x10⁻⁷</td>
<td>7x10⁻⁷</td>
<td>1x10⁻⁶</td>
</tr>
<tr>
<td>Monocouche de lipide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Densité de courant (A/cm²)</td>
<td>4x10⁻⁷</td>
<td>3x10⁻⁶</td>
<td>6x10⁻⁵</td>
<td>1x10⁻⁴</td>
</tr>
<tr>
<td>Oxyde de silicium, 2.2 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table R1 Densités de courant de fuite mesurées à travers une monocouche de lipides polymérisée et une couche d'oxyde de silicium d'épaisseur 2.2 nm en fonction du champ électrique appliqué.

Les valeurs obtenues montrent une nette différence entre les deux isolants avec une réduction de la densité de courant de deux ordres de grandeur à grand champ électrique pour la couche de lipides. Avec des valeurs inférieures à 10⁻⁶ A/cm², ces résultats obtenus sur la monocouche de lipide sont comparables aux meilleures données rapportées sur les films organiques minces. Cette bonne tenue
en champ électrique peut s'expliquer par la présence des deux longues chaînes alkyles dans chaque lipide. Il a été démontré effet que ces chaînes constituent une barrière au tunneling des électrons de l'ordre de 4.5 eV

La résistance différentielle des couches a été extraite des mesures courant-tension et une valeur moyenne de 300 MΩ est obtenue pour un champ électrique de 1 MV/cm. Cette valeur est tout à fait raisonnable, puisque la résistance d'une bicouche dans une membrane cellulaire est de l'ordre du Giga-Ohm.

Dans les diélectriques, la tension de claquage est un paramètre critique qui défini le champ électrique maximal qui peut être appliqué à un isolant avant que celui-ci se détériore et se mette à conduire. Le claquage se caractérise généralement par une augmentation brutale de plusieurs ordres de grandeur de la conductance du matériau et par sa dégradation. Ce paramètre est donc déterminant pour l'application des couches dans un dispositif. Nous avons évalué pour la monocouche de lipide le champ électrique de claquage en faisant varier la vitesse d'augmentation de la rampe de tension. Nous avons tout d'abord observé que dans ce cas, le claquage s'effectue en plusieurs étapes qui se manifestent par des sauts de courant. Nous avons également mesuré que l'intensité du champ électrique lors du "premier claquage" varie de manière logarithmique avec la vitesse d'augmentation de la tension appliquée avec des valeurs variant de 8 à 15 MV/cm pour des vitesses variant de 20 à 2000 mV/s. Ces mesures réalisées à l'air ont mis en évidence que le claquage ici n'est pas un paramètre critique caractéristique de la couche, mais un phénomène cinétique caractérisé par la vitesse d'endommagement de la couche et son inverse la durée de vie de la monocouche dans le champ électrique considéré. Cette forme de claquage est similaire aux claquages observés dans le cas de polymères. Des mesures ont alors été réalisées pour mesurer la durée de vie de la couche de lipide dans un champ électrique donné. Les résultats montrés en figure R3 indiquent une dépendance exponentielle. Par extrapolation, la durée de vie de la monocouche dans des champs de 1 MV/cm et 5 MV/cm serait de 15h et 30 min respectivement.
Ici encore, on peut voir l’impact de la polymérisation sur les propriétés de la monocouche. En effet, des études similaires réalisées sur des bicouches de lipide non-polymérisées ont rapportées un champ électrique de claquage de 2 MV/cm au mieux pour des gammes de vitesse de mesure similaires.

Après claquage, nous avons observé un phénomène tout à fait intéressant de régénération autonome de la couche. Celle-ci se produit en moins d’une minute après coupure du champ électrique. La régénération de polymères a déjà été rapportée dans la littérature mais nécessite toujours un apport extérieur comme l’ajout d’agent de polymérisation, de matière ou encore en chauffant. Dans notre cas elle est complètement autonome. Les mécanismes impliqués dans cette régénération ne sont pas encore élucidés, des expériences complémentaires sont nécessaires.

Finalement dans la cinquième partie, les monocouches de lipide polymérisées ont été utilisées dans le cadre du développement d’un capteur des ions ferrique à base d’un transistor à effet de champ. Les résultats présentés dans cette partie ont été obtenue en collaboration avec Tuyen Nguyen Duc, post-doc au laboratoire. Dans les dispositifs, l’oxyde de grille a été enlevé et remplacé par la monocouche. Afin de rendre le capteur spécifique, le groupement de tête des lipides a été modifié chimiquement, et un chélateur des ions ferriques, un dérivé hydroxypiridinone, lui a été greffé. Le dispositif montre des caractéristiques typiques des transistors indiquant que la couche de lipide joue bien son rôle de diélectrique de grille. La sensibilité du dispositif s’est avérée très bonne avec la
détection de 50 fM d'ions en solution. De même la bonne spécificité du chélateur permet la spéciation des ions ferriques et ferreux.

En conclusion, nous pouvons dire que la polymérisation de la monocouche de lipide induit des améliorations drastriques de ses propriétés nano-mécaniques et électriques et fait d'elle un matériau très prometteur pour son utilisation dans des procédés avancés.
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Le but de ce projet est d'étudier les propriétés mécaniques et électriques d'une monocouche de phospholipide supportée sur silicium en vue de son utilisation comme diélectrique de grille dans un biocapteur à transistor à effet de champ.

Ces monocouches de 3 nm d'épaisseur sont formées par fusion de vésicules directement à la surface du silicium. Les lipides que nous utilisons possèdent des triples liaisons dans leurs chaînes alkyles permettant une polymérisation radicaulaire dans le plan de la monocouche.

Nous montrons que cette polymérisation stabilise la monocouche à l’air et améliore sa résistance mécanique. Les mesures ont été réalisées par mesure de force par microscopie à force atomique, la force de ‘rupture’ de la monocouche par la pointe AFM augmentant après polymérisation. L'étude de la force en fonction de la vitesse d'approche de la pointe nous a permis de montrer que la rupture de la couche est un phénomène activé qui dépend de la vitesse. Nous avons pu ainsi déterminer pour chacune des deux surfaces, polymérisées ou pas, l’énergie d'activation de rupture de la couche du système couche/pointe ainsi qu’une estimation du module de Young. Ces deux grandeurs qui augmentent après polymérisation montrent une nette amélioration des propriétés mécaniques. Nous nous intéressons également aux propriétés électriques de ces monocouches.

Nous avons réalisé des mesures Courant-Tension (I(V)) à partir desquelles nous avons pu déterminer la résistance de la couche, les densités de courant de fuite ainsi que la tension de claquage. Les résultats obtenus démontrent que ces monocouches ultrafines possèdent de très bonnes propriétés isolantes. De plus nous avons révélé la propriété tout à fait intéressante d’auto-régénération de la monocouche isolante après claquage à l'air et à température ambiante en quelques minutes seulement. En conclusion, nous pouvons dire que la polymérisation de la monocouche de lipid induit des améliorations drastiques de ses propriétés nano-mécaniques et électriques et fait d'elle un matériau très prometteur pour son utilisation dans des procédés avancés.

The main goal of this project was to study the electrical and mechanical properties of a solid supported lipid monolayer in order to use it as a dielectric insulator in a Field Effect Transistor based biosensor.

The 3 nm lipid monolayer supported on silicon was obtained by the vesicle fusion method on a H-terminated silicon surface. DC8,9PC phospholipids containing acetylenic moieties were selected. The lipid monolayer was stabilized on the substrate by two-dimensional polymerization in the plane of the layer. We demonstrate that this polymerization stabilizes the monolayer in air and increases its mechanical resistance. Force measurements realized by AFM on both polymerized and non-polymerized layers demonstrated a net improvement of the nano-mechanical resistance of the layer after polymerization with a net increase of the force required to rupture the layer. Measurements realized at different loading rates have evidenced the fact that the monolayer rupture is an activated process that depends on the loading rate. For both types of layers, we have determined the intrinsic rupture activation energy of the tip–layer system as well as their Young modulus. These two physical quantities increase after polymerization and demonstrate a net improvement of the mechanical properties of the polymerized monolayer. The electrical properties of these layers have also been investigated. Current-Voltage measurements were done on the monolayer in the air at room temperature. The differential resistance, the leakage current, the breakdown voltages were measured and showed that the polymerized monolayer behaves as a good electric insulator. In addition, we demonstrated a very interesting property of autonomic self-healing after electrical breakdown. All these properties make the polymerized lipid monolayer a good insulator candidate to be used in the development of devices.