Interaction of PEG-ylated Lipid Nanoparticles with Silica Substrates

Department of Chemistry- BMC (B7:2)
Supervisor : Victor Agmo Hernàndez
Abstract

In this project, the interaction between polyethylene glycol modified (PEGylated) lipid nanoparticles and silica substrates was studied to find out how this interaction was affected by bulk concentration, temperature and the composition of particles. One kind of lipodisks and four kinds of PEGylated liposomes were prepared from lipid films and characterized by quartz crystal microbalance with dissipation monitoring (QCM-D) instrument mounted with silica sensor. The detailed information of particle-silica interaction could be obtained from the raw data, frequency and dissipation values, and the adsorbed mass surface density calculated from the raw data. Lipodisks could be immobilized on the silica surface. Whether they would be rinsed away by PBS buffer was influenced by both the bulk concentration and temperature. The way of their binding could change and the changing process was affected by temperature. PEGylated liposomes could also be immobilized on the silica surface, and they could break and spread to form supported lipid bilayer in certain conditions, for example, the changing of temperature or the using of certain lipids. Supported lipid bilayers were created with high reproducibility in this project, which could be very useful to the future study of transmembrane proteins functions and lipodisk properties.
Introduction

1. Two kinds of PEG-ylated lipid nanoparticles

1.1 Liposomes

A liposome is a spherical vesicle formed by one or more phospholipid bilayers with an aqueous core inside,¹ as shown in Figure 1. Its unique structure makes itself be able to load both hydrophobic (in lipid bilayer) and hydrophilic (in core) drugs. And it has good biocompatibility and biodegradability due to its main component-lipids. Liposomes have been one of the most popular and attractive systems for drug delivery because of these properties.

To extend the circulation time of liposomes, polyethylene glycol (PEG) modified lipids are employed as a “coat” of liposome. This coat provides steric stabilization thanks to its high hydrophilicity and large exclude volume.¹,² The PEG chains can also be used to covalently attach targeting agents to liposome or to introduce functionalities that will allow coupling the liposome to, e.g., a solid substrate. This approach has been used, among other examples, to prepare lipid-modified silica particles for their use in HPLC.³
1.2 Lipodisks

Lipodisk, also referred to as polyethylene glycol (PEG)-stabilized bilayer disk, is a nanosized flat circular lipid bilayer surrounded by a highly curved rim as shown in Figure 2. It displays high thermal stability and keeps stable upon heating the samples above the lipid transition temperature. Besides, it also shows excellent stability upon dilution, even if diluted below the critical micelle concentration (cmc) of the PEG-ylated lipids.

Lipodisks have great potential as drug delivery system, with many advantages such as long circulation time, low toxicity and good biocompatibility. It has been shown that some membrane-active drugs can lead to membrane disruption in liposome but for lipodisks, the open bilayer structure may make it more resistant to the disturbance.

In this project, the lipodisks were formed by DSPC and DSPE-PEG2000 with molar ratio 8:2.

2. Quartz crystal microbalance with dissipation monitoring (QCM-D)

Quartz crystal microbalance with dissipation monitoring (QCM-D) is used to study many kinds of properties of adsorbed films, e.g., their density and their softness. An AC voltage is applied to a thin quartz crystal (sensor) to make it oscillate at its acoustic resonance frequency. The oscillation decays when the voltage is turned off. The frequency (F) and the energy dissipation factor (D) are recorded followed by further calculation of adsorbed mass, viscoelasticity and so on. The changes in frequency are mainly related to changes in the adsorbed mass, while the changes in the
dissipation relate mainly to the viscoelastic properties of the film.

QCM-D has also been widely used in the study of a variety areas such as proteins, cells, bacteria and lipid membrane.\textsuperscript{6}

3. **Cryogenic transmission electron microscopy** (Cryo-TEM)

Cryo-TEM is a very powerful technique of studying aqueous samples of lipid nanoparticles. It is based on ultra-fast cooling of liquid samples and gives specimens that can be examined by TEM. The cooling is so fast that amorphous solid water will be obtained but not ice crystals. Aggregates within sample film can be visualized since electrons are able to penetrate the vitreous state of water. The pattern observed is close to the original state of sample.

4. **Aim**

In some previous experiments in Prof. K.Edwards’ research group, it was obvious that PEG-ylated lipid nanoparticles could passively bind to the silica surfaces without the need of having functional groups. This observation is worthy of advanced study. The main purpose of this project is to characterize the interaction of PEG-ylated lipid nanoparticles with silica and to find out how it is affected by temperature, bulk concentration and the composition of particle. The conclusions will allow developing further experiments with immobilized liposomes and lipodisks.
Materials and Methods

1. Materials

Dry powder of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC). Cholesterol, CHCl3 and saline phosphate buffer (PBS, 10mM phosphate, 150mM NaCl, pH 7.4). The molecular structures of lipids are shown in Figure 3.

2. Preparation of liposome and lipodisk

Four different liposomes and one kind of lipodisk were prepared by extrusion.

2.1 Preparation of lipid films

The lipid powder was weighed in clean glass tubes and dissolved in CHCl3. The components and molar ratios are shown in Table 1. Then the solvent was evaporated by nitrogen gas gently in warm water bath and the remaining solvent was evaporated in a vacuum oven overnight to give lipid film.
### Table 1. Composition and molar ratio of liposomes and lipodisk

<table>
<thead>
<tr>
<th>Liposome 1</th>
<th>Liposome 2</th>
<th>Lipodisk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
<td><strong>Proportion</strong></td>
<td><strong>Composition</strong></td>
</tr>
<tr>
<td>DSPC</td>
<td>96%</td>
<td>POPC</td>
</tr>
<tr>
<td>DSPE-PEG&lt;sub&gt;2000&lt;/sub&gt;</td>
<td>4%</td>
<td>DSPE-PEG&lt;sub&gt;2000&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liposome 3</th>
<th>Liposome 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
<td><strong>Proportion</strong></td>
</tr>
<tr>
<td>DSPC</td>
<td>56%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>40%</td>
</tr>
<tr>
<td>DSPE-PEG&lt;sub&gt;2000&lt;/sub&gt;</td>
<td>4%</td>
</tr>
</tbody>
</table>

2.2 Hydration of the lipid film

The dry lipid films were rehydrated by PBS buffer at 70°C water bath. For lipodisk, the solution was kept at 70°C for 3h with intermittent mixing to be hydrated completely. For liposomes, the hydrated films were subjected to five freeze thaw cycles.

2.3 Extrusion

The lipodisk/liposome dispersions were extruded 21(for lipodisk) or 31(for liposome) times through a Whatman® polycarbonate filter with a pore size of 100nm using a Mini-Extruder. For particles containing DSPC or DPPC, the extrusion was done at 70°C and for the others, the extrusion was done at room temperature. The extruded stock solutions were stored in the fridge at 4°C until use.

3. Characterization of liposome and lipodisk and their interactions with silica

3.1 QCM-D

All the particle solution in this project were tested by QCM-D E1 (Q-sense, Gothenburg, Sweden) instrument with controlled sample flow. After being cleaned as
recommended protocols, the sensor with silica surface was mounted well in the flow module and rinsed with PBS. When a stable baseline was obtained, the test solution was pumped in the instrument and the changes in frequency and dissipation were monitored. The system was rinsed by PBS again when the experiment was finished. All experiments were at fixed temperature 21°C unless otherwise noted. Data was collected for the fundamental frequency as well as for the 3rd, 5th, 7th, 9th, 11th and 13th overtones.

3.2 Cryo-TEM

Investigations of Cryo-TEM were performed by a Zeiss EM 902A Transmission Electron Microscope (Carl Zeiss NTS, Oberkochen, Germany). The sample preparation and instrumental operations were done according to M.Almgren et al. with the help of Dr.Jonny Eriksson.  

4. Data analysis

The raw data of four overtones (n=3,5,7,9) were used in the analysis. As the model proposed by Voinova et al.⁸, the viscoelastic properties of the formed film influence the overtone-dependent shifts on oscillation frequency (Δf) and dissipation factor(ΔD). Based in the Voinova model, Agmo Hernandez et al. proposed the following relationship between the changes in frequency and the changes in dissipation⁹:

\[
\frac{\Delta f_n}{n} = -\frac{m_d f_0}{t_q \rho_q} + \frac{\pi \eta_1 (f_0)^2}{\mu_1} (n \Delta D)
\]

Where \( n \) is the overtone number, \( f_0 \) is the fundamental oscillation frequency, \( t_q \) and \( \rho_q \) are the thickness and density of the quartz crystal, \( m_d \) is the adsorbed mass surface density, \( \mu_1 \) and
$\eta_1$ are the elastic modulus and the viscosity, respectively, of the adsorbed layer. A plot of $\Delta f / n$ vs $n \Delta D$ at different $n$ should have the y-intercept equal to $\frac{m_d f_0}{\epsilon_q \rho_q}$. Then the mass density of surface adsorbed layer could be calculated.\textsuperscript{9}

The data of 3\textsuperscript{rd} overtone was chosen for most of the analysis because of its accuracy and stability. To find out the differences and similarities of different processes, a plot of dissipation-frequency ($n=3$) was done for each experiment. These curves are called “fingerprint curve”, as they have unique shapes for each kind of adsorption process.

### Results and Discussions

#### 1. Cryo-TEM

Cryo-TEM gave direct visualization of our lipodisks. In Figure 4, the mean value of the disks’ diameter was 34nm. Because the PEG chains could not be seen in this image and the length of PEG2000 was about 3.5nm, the accurate mean diameter of our lipodisk was 34+3.5*2=41nm (PEG chains were at both sides of disks).

#### 2. Immobilization of lipodisks

Our work started with lipodisks, the relatively complex system in the project. The immobilization of lipodisk had been done by other members in our group, but with concentration as high as 500µM. We tried to find out the effect of bulk concentration by

![Figure 4. Cryo-TEM images of lipodisks composed of DSPC/DSPE-PEG\textsubscript{2000} (80:20 mol%)](image-url)
doing several experiments with lower concentrations.

2.1 The effect of bulk concentration

a. Binding and releasing

With relatively low concentrations, the lipodisks could bind to the silica surface, and be rinsed away by PBS (see Figure 5, and the data of other concentrations is in Appendix 1). The fingerprint curves (see Figure 6) showed that the binding processes were same. This is concluded from the similar shapes of the curves at different concentrations.

It was assumed at first that the binding process was the simplest Langmuir adsorption, thus the constant $K = \frac{k_b}{k_r}$ would not change:

$$\text{Disk} + S \rightleftharpoons D_s$$

Where $S$ means empty adsorption site; $D_s$ means bond disks; $k_b$ and $k_r$ means the constant of binding and releasing, respectively. According to the equation and chemical kinetics, the $k_b$ and $k_r$ could be given by exponential fitting of the mass-time curves of binding and releasing.
However, the calculated $K$ of different concentrations varied. Since the PEG chains were likely to sterically repel each other, it would be more difficult for new disks to bind if some disks had been on the surface. In other words, the bonded lipodisks could affect other disks and the binding was a more complex process rather than Langmuir adsorption. More experiments were needed in the future to explore this process.

b. Coverage

The coverage could be reflected by the adsorbed mass at equilibrium at different lipodisk concentrations (an adsorption isotherm). According to Figure 7, the coverage increased when lipodisks’ bulk concentration became higher. Finally the surface was saturated when the concentration reached 250µM.

![Figure 7. The mass at equilibrium when lipodisks with different bulk concentrations bind at 21 °C](image)

Some calculation could be done to estimate the coverage when the surface was saturated. First, the density of lipodisk could be obtained by the equation:

$$\rho_{\text{disk}} = \frac{\sum M_{\text{lipid}} \cdot \text{mol}\%}{N_A \sum (l \cdot A \cdot \text{mol}\%) + \text{mol}\%_{\text{PEG}} \times \frac{M_{\text{PEG}}}{\rho_{\text{PEG}}}}$$

Where $l$ is the length of lipid molecule and $A$ is the head group area of lipid molecule.
So the density of our lipodisk was 1158.03kg/m³. The result from cryo-TEM showed the diameter of lipodisk was about 41nm. If it was assumed that lipodisks were immobilized with a “standing” way (see Figure 10) and there was no gap between disks, the adsorbed mass density should be 1158.03 kg/m³*41nm=4.75*10^{-5}kg/m². The measured mass density when surface was saturated (250µM) was 1.18*10^{-5}kg/m², so the estimated coverage was 24.8%. This result indicated that the lipodisks were immobilized sparsely on the silica surface, and the main reason was the repulsion between PEG chains.

2.2 The effect of temperature

In contrast to what was observed at lipid concentrations <250µM, 500µM lipodisks could be immobilized on the silica surface and could not be rinsed away by PBS at 21°C. For this project, this irreversible bound lipodisk layers were used to study the effect of temperature in the lipodisks-silica interaction. A temperature program shown in Table 2 was applied to the immobilized lipodisks.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>21°C</td>
<td>10min</td>
</tr>
<tr>
<td>From 21°C to 15°C</td>
<td>20min</td>
</tr>
<tr>
<td>15°C</td>
<td>10min</td>
</tr>
<tr>
<td>From 15°C to 60°C</td>
<td>150min</td>
</tr>
<tr>
<td>60°C</td>
<td>10min</td>
</tr>
<tr>
<td>From 60°C to 21°C</td>
<td>130min</td>
</tr>
<tr>
<td>21°C</td>
<td>10min</td>
</tr>
</tbody>
</table>
During the temperature program (raw data in Appendix 2), the adsorbed mass suddenly decreased at about 30°C and finally reached zero (see Figure 8, two repetitions included), which meant the lipodisks left the surface and did not bind to it again, even though the bulk concentration was kept at 500µM.

![Figure 8. The change of mass during the temperature program applied to immobilized lipodisks](image1)

![Figure 9. The experimental raw data of lipodisk’s binding and releasing at 32°C](image2)

To further study the role of temperature on lipodisks-silica interactions, a series of experiments at different fixed temperature(25°C, 27°C, 30°C, 32°C and 35°C) have been done to find out the critical temperature of immobilization. During these experiments, the flow was stopped for several minutes after the lipodisk’s binding finished, then the system was rinsed with PBS buffer. When the signals became stable, lipodisk solution was added again to find out how many adsorption sites were occupied after rinsing.
In Figure 9, we can see that at 32°C the release took place right after the binding was finished. When the flow was stopped, the release stopped, too, which indicated that the release was caused by the flow. Not all the lipodisks were rinsed away because after rinsing the values of frequency and dissipation were not zero. The signals only changed a little when lipodisk solution was pumped in again, showing that almost all the adsorption sites were occupied after rinsing. The final values of frequency and dissipation at different temperatures were similar (See Appendix 3), which meant the components left on the surface were same, and they were harder than before because of the decrease of dissipation.

![Figure 10. Schematic diagram of lipodisk’s binding and releasing](image)

From the discussion above, we can propose a hypothesis that lipodisk would change the way of binding after rinsing. As shown in Figure 10, some lipodisks would be rinsed away and some lipodisks would change their way of binding from “standing” to “lying”. When a lipodisk lay on the surface, more PEG chains could bind to the surface, that’s why almost all the adsorption sites were occupied even though some lipodisks had been rinsed away. Other techniques that would measure the thickness of the lipodisk film on the surface, for example, the atomic force microscopy, could be used to confirm this mechanism in the future.
The “lying down” process could be affected by temperature. For each experiment, the binding time (“binding of lipodisks” part in Figure 8) and the rinsing time (the rinsing time to get stable values except for the “stop flow” period) were shown in Table 3.

Table 3. The binding time and rinsing time of lipodisk at different temperatures

<table>
<thead>
<tr>
<th>Temperature(℃)</th>
<th>Binding time(s)</th>
<th>Rinsing time(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>105</td>
<td>3098</td>
</tr>
<tr>
<td>27</td>
<td>93</td>
<td>2869</td>
</tr>
<tr>
<td>30</td>
<td>80</td>
<td>984</td>
</tr>
<tr>
<td>32</td>
<td>80</td>
<td>892</td>
</tr>
<tr>
<td>35</td>
<td>102</td>
<td>855</td>
</tr>
</tbody>
</table>

From Table 3, we could see that the rinsing time decreased when temperature increased, and there was a sharp drop of rinsing time at 30℃, this result might be related to the sudden drop of mass density at 30℃ in the temperature program. For the immobilized lipodisks at 21℃, the “lying” process might also happen, but it was too slow to be observed. And the binding time would not be influenced by temperature. More experiments can be designed in the future to find out the accurate critical temperature and the detailed mechanism of this process.

3. Immobilization of liposomes

In contrast to the lipodisks, which are very stable structures, liposomes are known, under the appropriate conditions, to be able to break and spread on various substrates, resulting in supported lipid membrane structures that can be useful for studies on membrane interactions. In addition, the study of this lipid membrane can also help us understand the properties of lipodisks, because lipodisks are also built by a lipid membrane.

In this project we worked with PEG-ylated liposomes in different phase states at room temperature: POPC liposome (liquid crystalline phase, phase transition temperature at ~0℃),
DSPC liposomes (gel phase, phase transition temperature 55°C), DPPC liposomes (gel phase, phase transition temperature at 41°C and DSPC:cholesterol 6:4 liposomes (liquid order phase, no phase transition)

For liposomes in the gel phase and liquid ordered phase, a temperature program shown in Table 2 was applied to immobilized liposomes to initiate phase transition. The change from gel phase to liquid crystalline phase might break the liposomes and form lipid bilayer.

3.1 POPC-DSPE-PEG<sub>2000</sub> liposome

The liposomes assembled by POPC and DSPE-PEG<sub>2000</sub> could bind to the silica surface. Because of their softness introduced by the unsaturated bond in POPC, the liposomes broke and formed lipids bilayers right after they bond to the surface. The bilayers were not directly adsorbed to the surface, but rather supported by PEG chains and there was PBS solution between the bilayers and silica surface. This is concluded from the rather large dissipation values obtained at the end (see Figure 11). For a lipid bilayer directly in contact with the surface, the dissipation factor should be close to zero.\(^{10}\)

\[\text{Figure 11. The experimental raw data of the binding of POPC liposomes}\]

\[\text{Figure 12. Fingerprint curves of PEG-ylated POPC liposomes' binding}\]
The experiment was repeated four times and the final frequency and dissipation values were almost the same, which indicated that the same phenomenon, namely lipid bilayer separated from the surface by a thin layer of water, was obtained. From the slopes of the fingerprint curves (see Figure 12), the conclusion could be made that the liposomes underwent a same binding and breaking process.

During the temperature program, in spite of the drifts caused by air bubble formation, which was very difficult to be avoid, the mass of substance on the surface kept constant (see Figure 13), showing that the POPC lipids bilayers were stable when temperature changed.

3.2 DPPC-DSPE-PEG2000 liposome

The liposomes assembled by DPPC and DSPE-PEG2000 could bind to the silica surface (see Figure 15). The DPPC liposomes were harder than POPC liposomes, so after binding with silica surface they did not break. After their binding, the change of dissipation was larger than that of frequency, because in each liposome on the surface,
more PEG chains would bind to the surface to make it more stable (see Figure 14). Then liposome layer became harder, which was shown by the decrease of dissipation, and the mass hardly changed, as revealed by the little change of frequency.

![Figure 15. The experimental raw data of DPPC liposomes' binding](image1)

![Figure 16. Fingerprint curves of PEG-ylated DPPC liposomes' binding](image2)

The fingerprint curves (see Figure 16) also showed that DPPC liposomes underwent the same binding process in all repetitions of this experiment.

At room temperature, it is difficult to obtain DPPC lipid bilayers on silica surface because the liposomes are too hard to break. In the temperature program, the temperature went over DPPC's phase transition temperature (41°C) and the lipids changed to liquid crystalline phase from gel phase. In liquid crystalline phase the liposomes could break and form bilayers. In the mass-time curves (Figure 17) during temperature program, the sudden drop at about 41°C indicated the loss of water when liposomes broke. When the temperature went back to 41°C, the lipids became gel phase again, which was shown by the slight increase of mass. This increase can be related to the increase of the packing density of the lipids, mainly at the sensor’s
center, where the sensitivity is higher.

3.3 DSPC-DSPE-PEG\textsubscript{2000} liposome

The liposomes assembled by DSPC and DSPE-PEG\textsubscript{2000} could bind to the silica surface (see Figure 18). The frequency became much lower when DSPC liposomes finished binding, which meant they were heavier and bigger. The larger size made it difficult for more PEG chains to bind, so the rearrangement of PEG chains was slower.

The experiment was repeated three times. The fingerprint curves (Figure 19) showed

![Figure 17. The change of mass during temperature program of DPPC liposomes](image1)

![Figure 18. The experimental raw data of PEG-ylated DSPC liposomes' binding](image2)

![Figure 19. Fingerprint curves of PEG-ylated DSPC liposomes' binding](image3)

![Figure 20. The mass change during temperature program of DSPC liposomes](image4)
great repeatability.

The temperature program was repeated for three times, in which lipids bilayers were obtained in the second and third time (see Figure 20). The phase transition temperature of DSPC was about 55°C, at which the sudden drop of mass took place. The highest temperature QCM could handle was 60°C which was only a little higher than DSPC’s phase transition temperature, so liposomes might be unable to break completely, which led to the result, a mixture of bilayers and liposomes, in the first repetition.

3.4 DSPC-cholesterol-DSPE-PEG2000 liposome

The liposomes assembled by DSPC, cholesterol and DSPE-PEG2000 could also bind to the silica surface, but with a slower speed (see Figure 21). A possible reason is that cholesterol molecules are smaller than lipid molecules, so there would be less liposomes in solution with the same total concentration and same size of liposomes. The lower concentration of liposomes made the binding slower.

There were differences between the slopes and final dissipation values of fingerprint curves (see Figure 22), which meant advanced investigation of PEG-ylated DSPC-
cholesterol liposomes’ binding was needed in the future. The general shape, however, was the same in all repetitions, indicating that the same process always occurred. From the frequency and dissipation values, it is concluded that intact liposomes bind. Among the three repetitions of temperature program, lipids bilayers were obtained only in the first try (see Figure 23). A possible reason was that when the liposomes were fresh, there were defects on their surface, which made it possible for them to break and form bilayers. The liposomes would become more perfect and stable several hours later, so in the second and third repetitions they could not break completely. The decrease of mass in second and third repeats was due to the loss of water when liposomes shrank or broke at higher temperature. As there is no phase transition in cholesterol-containing samples, the results are expected: a mixture of immobilized liposomes and lipid bilayer was obtained. Some calculations could be done to check if the supported lipid bilayer was obtained. If we use the result from DSPC liposome for example, first, the density of lipid bilayer could be calculated by the equation

\[
\rho_{\text{bilayer}} = \frac{0.96M_{\text{DSPC}} + 0.04M_{\text{DSPE}}}{N_A \sum(l \cdot A \cdot \text{mol}%)}
\]
Where \( l \) is the length of lipid molecule and \( A \) is the head group area of lipid molecule. So the density of DSPC lipid bilayer was 1101.23 kg/m\(^3\) and the mass density on surface introduced by bilayer was 1101.23 kg/m\(^3\)\(*2*2.5\text{nm}(\text{the thickness of bilayer})=5.51*10^{-6}\text{kg/m}^2\). If we assumed that the thickness of buffer layer was the length of PEG chain, the mass density on surface introduced by buffer was 1000 kg/m\(^3\)\(*3.5\text{nm}=3.5*10^{-6}\text{kg/m}^2\). The mass density on surface introduced by PEG and the water molecules adsorbed on PEG was difficult to work out, but we could estimate that the total mass density was about \(1*10^{-5}\text{kg/m}^2\). The result shown in Figure 20 was about \(1.2*10^{-5}\text{kg/m}^2\), so we knew we had got a supported lipid bilayer. The same estimation could be done for other liposomes.

**Conclusions**

The interaction between DSPC-DSPE-PEG\(_{2000}\) lipodisks and silica surface is complex because of the repulsion between PEG chains and the shape of lipodisks-they could occupy the adsorption site on surface with different ways. At relatively low concentration, the immobilized lipodisks could be rinsed away, and the coverage of surface increased when concentration got higher until the surface was saturated. With concentration as high as 500\(\mu\)M, the immobilized lipodisks would not be rinsed away at 21\(\degree\)C but they left when temperature reached around 30\(\degree\)C. The change of binding pattern would happen after lipodisk’s binding with continuing flow, and this process could be accelerated by increasing temperature. 30\(\degree\)C was found to be a special point in this process, too, because when temperature was higher than 30\(\degree\)C, it’s acceleration became stronger suddenly.
Supported lipid bilayers formed by different lipids were obtained when using PEG-ylated liposomes as the starting material. For PEG-ylated POPC liposomes, they broke to give supported bilayers right after their binding, because they were too soft to resist the drag from PEG chains. For PEG-ylated DPPC liposomes, although they were harder, supported lipid bilayers were obtained after the phase transition caused by the change of temperature. For PEG-ylated DSPC and DSPC-cholesterol liposomes, supported lipid bilayers were also obtained, but not for all experiments. The optimization of method is needed in the further research to synthesize these two kinds of lipid bilayers.

It is worth mentioning that the formation of DPPC lipid bilayers can be very helpful to the research of DPPC lipodisks for drug delivery. Obtaining supported lipid bilayers in the gel or the liquid ordered phase is recognized as a difficult task. In this project, we succeeded in creating supported structures in the gel phase with high reproducibility. Furthermore, these structures were not in direct contact with the substrate, but were lifted by a thin layer of water, which guarantees that the bilayer will have the same properties as it would have free in solution. Lifted supported bilayers can be used to include transmembrane proteins and study the function of the latter. Earlier reported methods to prepare lifted supported lipid bilayers are cumbersome, multi-step and expensive. The method that we developed in this project, on the other hand, is a single-step method that does not require the use of special chemicals or surface modifications.
Appendix

1. The experimental raw data of lipodisk’s binding and releasing (Figure 24-27)

![Figure 24. The experimental raw data of 2.5µM lipodisk’s binding and releasing](image1)

![Figure 25. The experimental raw data of 10µM lipodisk’s binding and releasing](image2)

![Figure 26. The experimental raw data of 50µM lipodisk’s binding and releasing](image3)

![Figure 27. The experimental raw data of 250µM lipodisk’s binding and releasing](image4)

2. The experimental raw data of the temperature program applied to immobilized lipodisks (Figure 28)

![Figure 28. The experimental raw data of the lipodisk’s immobilization and temperature program](image5)
3. The experimental raw data of lipodisk’s binding and releasing at different temperatures (Figure 29-32)

Figure 29. The experimental raw data of 500µM lipodisk’s binding and releasing at 25 ℃

Figure 30. The experimental raw data of 500µM lipodisk’s binding and releasing at 27 ℃

Figure 31. The experimental raw data of 500µM lipodisk’s binding and releasing at 30 ℃

Figure 32. The experimental raw data of 500µM lipodisk’s binding and releasing at 35 ℃

Acknowledgements

To my parents, thanks for your emotional and financial supports in my past one year in Uppsala University, and thanks for all you have done for me in my whole life.

To Dr. Victor Agmo Hernàndez, my supervisor, thanks for your teaching, planning and encouragement that helped me finish this project.

To Prof. Helena Grennberg, my coordinator in Uppsala University, thanks for all your guidance and help since I came to Uppsala which made my study life ordered and enjoyable.
To Prof. Zhen Xi, Prof. Baiquan Wang, Associate Prof. Xin Wen and the Department of Chemistry, Naikai University, thanks for giving me the chance to finish this project in Uppsala University.

To Prof. Katarina Edwards, Karin, Jonny, Emma, Helen and other lovely members in my research group, thank you for all the help and the enjoyable time we spent together.

References


