Application Note # 139

Supported lipid layers characterization with MP-SPR

Multi-Parametric Surface Plasmon Resonance (MP-SPR) was used to characterize lipid layer structures adsorbed on a different substrates. Silicon dioxide and low molecule weight dextran surfaces supported formation of lipid bilayer (SLB) whereas thiolated polyethylene glycol supported vesicular layer formation (SLV). Thickness and refractive index of deposited lipid layer was calculated: SLB was about 5nm thick layer whereas SLV was 10nm thick layer.

Introduction

Supported lipid membranes can be used in vitro experiments to mimic biological barriers consisting mostly of phospholipids, such as cell membranes. It is also possible to incorporate other molecules, like receptors or membrane proteins into the lipid layer structure to mimic specific functionality of the biological membrane. Due to these properties supported lipid layer structures can be widely utilized in field of biochemistry and pharmaceutical research.

Traditionally supported lipid bilayers (SLB) or vesicles layers (SLV) are prepared on solid support, like SiO_2 or gold (Au), but layers are often unstable during transition through the air-water interface. Polymer support have noticed to increase stability of the formed lipid layer.

SPR phenomenon is based on free electrons resonating at a metal surface, which are excited with light. There is an absorption maximum as a function of the angle of the incident light, and the SPR phenomenon is highly dependent on the dielectric constant near the metal surface. Any changes near the surface, such as deposition of lipid layer, change the angle of the absorption maximum. MP-SPR unique feature to measure with two different wavelength simultaneously enables calculation of both thickness (d) and refractive index (RI) of the deposited layer without prior knowledge of either one.

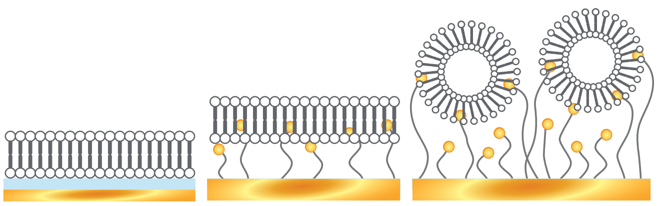

Figure 1. Supported lipid bilayers (SLB) and supported vesicles layers (SLV) was prepared on solid and polymer support.

Materials and methods

Two different polymer surfaces were synthetized for lipid binding: 6 kDa dextran and polyethylene glycol self-assembly monolayer (PEG-SAM). SiO_2 surface was used as reference surface for SLB formation (Figure 1). Liposome main component was EggPC (egg phosphatidylcholine) because majority of prokaryotic cell membranes are phosphatidylcholines. POPS (palmitoyl-oleyl-phosphatidylserine) was added to form natural negative charge and cholesterol to mimic natural rigidity of cell membranes. Three different lipid compositions were studied: 100% EggPC, 75:25% EggPC:POPS and 70:25:5% EggPC:POPS:Cholesterol. Additionally cell membrane extract from HepG2 cells was studied.

Liposome interaction with different surfaces was measured with SPR Navi 200-L (Figure 2). Supported lipid bilayer stability during air water transition was also studied. Thickness and refractive index (RI) of the formed lipid layers was calculated based on SPR curves before and after liposomes deposition (Figure 3).

For more details see original publication [1].

Results and discussion

Even if both 6kDa dextran and PEG-SAM are relatively thin (few nanometers) hydrophilic hydrogel supports, the dextran surface clearly promoted SLB formation whereas PEG-SAM supported SLV formation. Calculated thickness and RI values showed clear difference between lipid layers on dextran than on PEG-SAM (Figure 4). Difference in surface promoting behaviour was most probably due to the different density and location of polymer hydrophopic groups. Dextran supported lipid bilayer formation for all three lipid compositions used (Figure 4).

The d and RI values for SLB on dextran surface were slightly higher than for SLB on reference surface (Figure 4 and 5). This might be due to tighter backing of lipids on dextran surface, which was also supported by the slightly higher RI of the lipid layer on the dextran surface compared to the SiO_2 surface. The SLV layer on PEG-SAM surface had clearly higher thickness and lower RI (Figure 4 and 5). Refractive indexes measured with 670nm and 785nm had linear correlation as should (Figure 5).

EggPC:POPS:Cholesterol SLB on dextran surface was exposure to air-water transition and air flow with MP-SPR instrument. It was noticed that SLB had an excellent resistance against drying which is a desirable feature for biomimetic surfaces.

HepG2-extract had a bigger SPR signal compared to other liposomes studied, probably due to the membrane proteins and other components it contains.

Excellence in Surface Plasmon Resonance
BioNavis

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 10 271 5030
e-mail : info@bionavis.com
www.bionavis.com

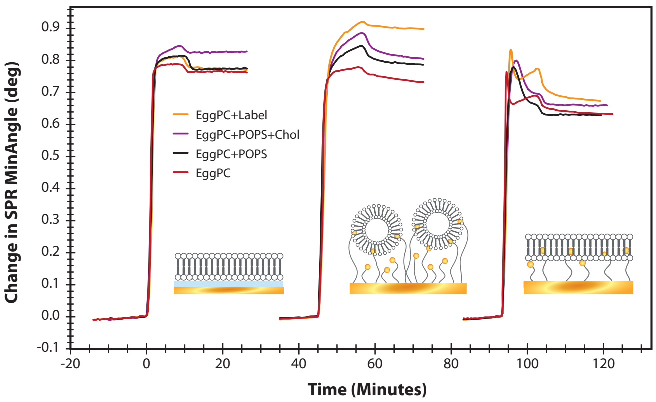

Figure 2. Change in SPR peak minimum angle during lipid deposition, From left to right: SLB formation on SiO2 surface, SLV formation on PEG-SAM surface and SLB formation on 6kDa dextran surface.

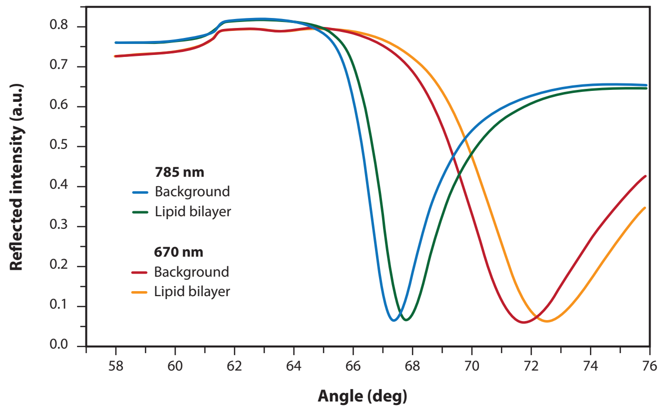

Figure 3. SPR curves before (background) and after lipid bilayer deposition on SiO2 surface, measured with two different wavelengths (670nm and 785nm). Curves are used to determine thickness and refractive index of deposited lipid bilayer. Fit (black lines) are from thickness and refractive index calculations.

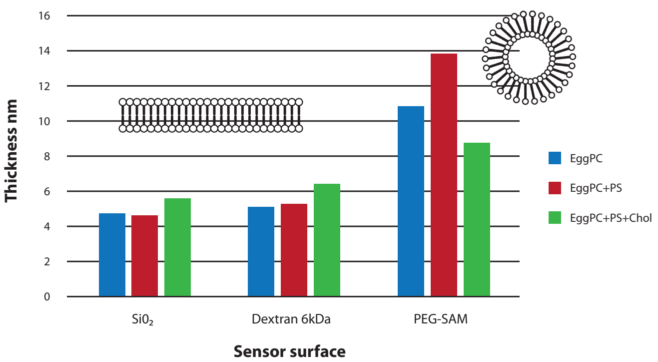

Figure 4. Thickness of deposited lipid layers. Supported lipid bilayers thickness was about 5nm whereas vesicle layers was clearly thicker about 10nm.

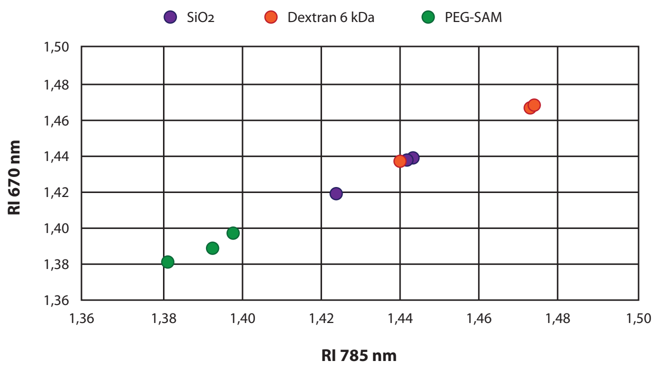

Figure 5. Calculated refractive indexes (RI) of lipid layers with 670nm and 785nm have linear correlation.

Conclusions

Supported lipid layers on liposome or lipid bilayer form can be deposited on a SPR sensor slide. MP-SPR is unique method to determine deposited layer thickness and refractive index without prior knowledge of either one. Supported lipid layers can be further utilized e.g. in field of drug discovery and development, biosensing, and biophysics to determine molecule interactions with lipid layers.

For more specific information about thickness and RI calculations can be found from BioNavis Application note 128.

Recommended instrumentation for reference assay experiments
SPR Navi™ 200, 210A or 220A with additional wavelength (L)
Sensor surfaces: Au or SiO2
Software: SPR Navi™ Control, SPR Navi™ DataViewer, SPR Navi™ Layer Solver

References:

[1] Granqvist et.al. Langmuir, 29 (27), 2014

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 10 271 5030
e-mail : info@bionavis.com
www.bionavis.com