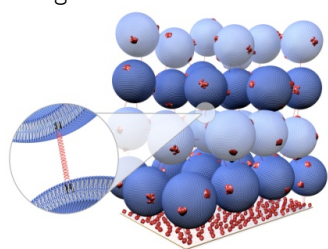


TRANSFER OF BIOMOLECULES ACROSS BIOLOGICAL MEMBRANES

Technology Description

Analysis of membrane processes

Layerlab AB has developed a proprietary technology for the analysis of immobilized membrane structures on biosensor surfaces. The technology is based on the immobilization of liposomes in multiple layers as illustrated in the figure below.



This technology enable the real-time analysis of biological processes that are associated with biological membranes such as:

- ◆ Function of membrane proteins
- ◆ Interactions between membranes and drugs
- ◆ Transport across membranes

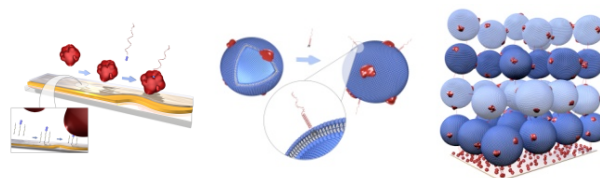
memLAYER chemistry kit

The Layerlab memLAYER Chemistry Kit, shown in the figure below, consist of reagents for functionalization of the biosensor surface as well as Cholesterol-DNA tags to attach liposomes to the surface and to build a multilayer structure of liposomes.



Immobilization of a multilayer liposome structure consists of three steps as schematically shown below:

- ◆ Functionalizing the surface with DNA-tags for capturing of liposomes
- ◆ Attaching the cholesterol-DNA tag to the liposomes
- ◆ Building the layers of liposomes on the biosensor surface



Transfer of Ions / Molecules Across Biological Membranes

Introduction

Surface Plasmon Resonance (SPR) is a common method to study biomolecular interactions. This Application Note describes the results from a study*, which shows how SPR can be used to study another very important biological process, namely transfer of biomolecules across biological membranes. melittin is a small peptide and the active substance in honey-bee venom. Its toxicity is manifested by the formation of melittin pores in the cellular membrane and the subsequent lysis of the cell. The measurements of sucrose uptake through the melittin pore, which are presented here, demonstrate a general and straight-forward method to quantify and to measure, in real-time, transfer of molecules across biological membranes#.

Injection of sucrose (or any other solute) changes the refractive index (RI) of the solution in the evanescent-field volume above the sensor surface, which is manifested as a shift in RU. Liposomes immobilized at the biosensor surface occupy a part of the evanescent-field volume, and therefore the shift in RU upon injection of sucrose in presence of liposomes is lowered. If the liposomes contain melittin pores, sucrose can enter the volume enclosed

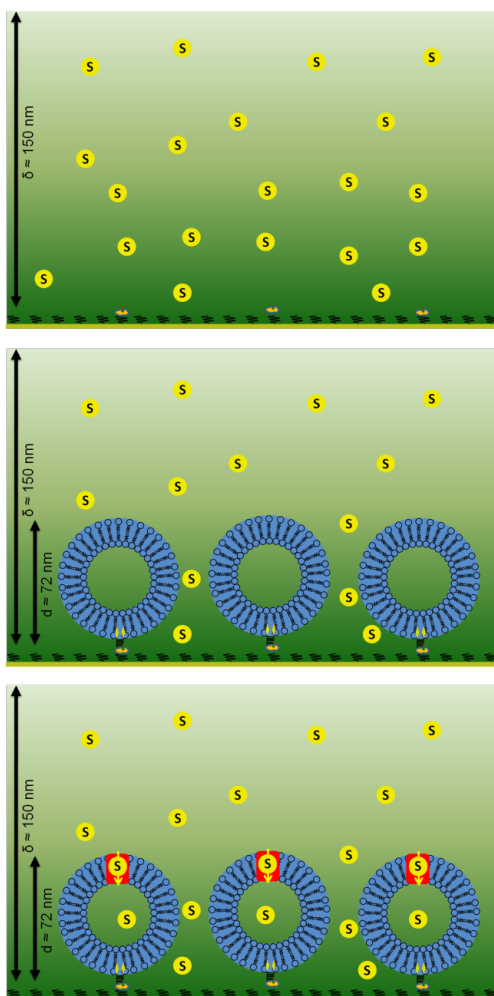
by the liposomes and the response from a sucrose injection will now correspond to the change in RI of the volume inside and outside the liposomes.

Method

The following injections were made over a Biacore Sensor Chip that had been functionalized with PEG/PEG-Biotin;

- ◆ NeutrAvidin/Biotin-DNA (N/B)
- ◆ 0.1 M Sucrose (S1)
- ◆ Cholesterol-DNA tagged POPC-liposomes (POPC)
- ◆ 0.1 M Sucrose (S2)
- ◆ 40 μ M Melittin (Mel)
- ◆ 0.1 M Sucrose (S3-S5)

The POPC-liposomes were immobilized to the surface through complementary binding of the liposome DNA-tag with the Biotin-DNA using the memLAYER Chemistry Kit from Layerlab AB.



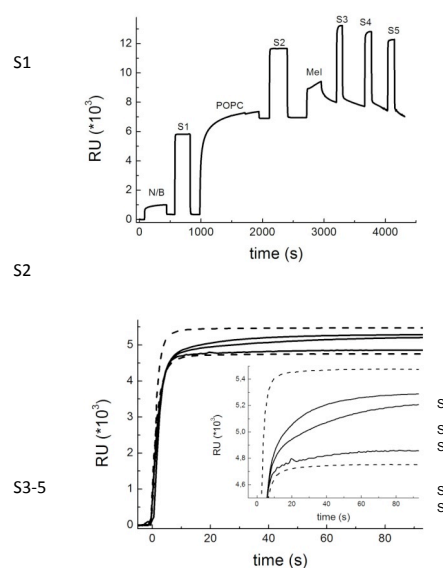
Results

The difference in RU between S1 and S2 corresponds to the change in RI in the volume occupied by the liposomes. Addition of melittin produces pores in the liposomes membrane and hence makes the interior volume of the liposomes accessible to the added sucrose molecules. As the melittin addition is stopped, melittin dissociates from the membrane surface and the number of liposomes having melittin pores decreases with time.

From the results in this study we were able to:

- i) quantify the number of sucrose molecules that were transferred through the pores and into the liposomes and thereby estimate the detection-limit of the methodology, which in the current format corresponds to an uptake of ~ 10 sucrose molecules per liposome.
- ii) time-resolve the kinetics of the sucrose transfer across the membrane and to determine the rate of sucrose transfer through a single pore to 800 s⁻¹.

In addition we could show that the methodological approach that we applied produced a surface-to-area coverage of liposomes that were in perfect agreement with the theoretical value for irreversible random sequential adsorption of spheres, and that the liposomes, prior to pore-formation, were impermeable to sucrose over the time-scale of measurements (minutes).



The left part illustrates the evanescent-field volume after the injections of S1, S2, and S3-5. The upper right part shows the sensorgram during the sequential injection of N/B, S1, POPC, S2, Mel, S3, S4, and S5 to a Biacore Sensor Surface, which is functionalized with PEG/PEG-Biotin. The lower right part compares the responses (RU) from the injections of S1-5.