NPS allows the study of lipid phase transition temperature

Insplorion's Localized Surface Plasmon Resonance (LSPR) technology empowers the measurement of lipid phase transition temperatures for liposomes. Analysis of the NPS signal and temperature profiles reveals pre-transition temperature and gel-to-fluid transition temperature range.

Introduction

A liposome is a phospholipid vesicle that is structurally similar to cell membranes. This similarity is used as a research tool to study the physical properties of membranes and to predict the interaction of other compounds with them. Another potential field of application for liposomes is in drug delivery, as they can be loaded with specific cargo molecules and permeabilize membranes. The main phase transition temperature (T_m) has an important effect on the structure, organization and permeability of the biomembrane and thus, in how it interacts with small molecules. When the bilayer transforms from an ordered and closely packed gel phase to a disordered fluid phase, the space between phospholipids increases, and with it their permeability.

Nanoplasmonic sensing (NPS) has a very low intrinsic dependence on temperature, making it an important technique to study temperature dependent changes. The main parameter tracked by NPS is λ_{max}, the wavelength at the maximum extinction of the plasmonic peak. A change in the NPS signal might indicate a change in the shape or structural organization of liposomes adsorbed on the sensor surface.

This work shows how NPS can be used in temperature-dependent measurements to follow the phase transition of liposomes adsorbed to the NPS sensors.

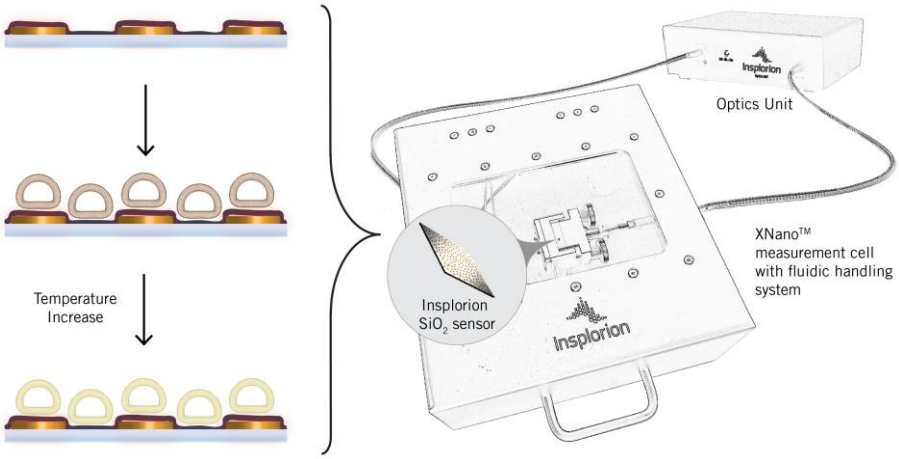

Figure 1: Insplorion system setup. The inset shows a schematic illustration of the sensors used in this application example (not to scale).

Experimental Procedure

DPPC liposomes were prepared by extrusion and sonication. The liposomes were then immobilized on SiO_2 coated NPS sensors by flowing the liposome solutions through the XNano system. To investigate the main T_m, the temperature inside the measurement chamber was adjusted to appropriate values after or during the immobilization of the liposomes. The temperature profile was recorded together with the NPS signal.

Results

The immobilization was conducted at 25°C, a temperature at which the phospholipids are in gel phase (T_m=41°C). The NPS signal was recorded, starting from the pre-treatment of the sensor surface until complete immobilization of the liposomes using Insplorion's XNano. The NPS signal was followed during the rinsing process, where a constant signal showed a stable adhesion of the liposomes to the sensor surface.

The phase transition of the phospholipids was then evaluated by increasing the temperature from 25°C to 50°C. The NPS signal dropped immediately with the temperature rise, suggesting a change in the

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characteristics of the vesicles. Further analysis of the data, by calculating the time derivative of the NPS signal, indicated the presence of three temperatures relevant for the transition process, in agreement with literature. The first temperature can be attributed to a pretransition temperature, while the latter temperatures are considered the beginning of the main transition phase and the T_m respectively.

The experimental setup was then modified to analyse the phase transition temperature during the immobilization. The immobilization took nearly 25 min while the temperature increase from 25°C to 50°C took 11 min; as shown by the NPS signal and the temperature profile.

A new pattern of NPS signal was obtained for liposome adsorption (Fig. 2a). When the DPPC liposomes were immobilized during the temperature increase, a small drop in the NPS signal was observed. This was followed by a continuous increase in the signal, until stabilization. The time derivative analysis of the NPS signal (Fig. 2b) indicates that at 34.9°C (point A in Fig. 2b), the rate of the increase in the NPS signal drops rapidly. A possible explanation is a conformational change of DPPC liposomes at 34.9°C. As the temperature keeps increasing, the time derivative drops below zero, showing the strongest

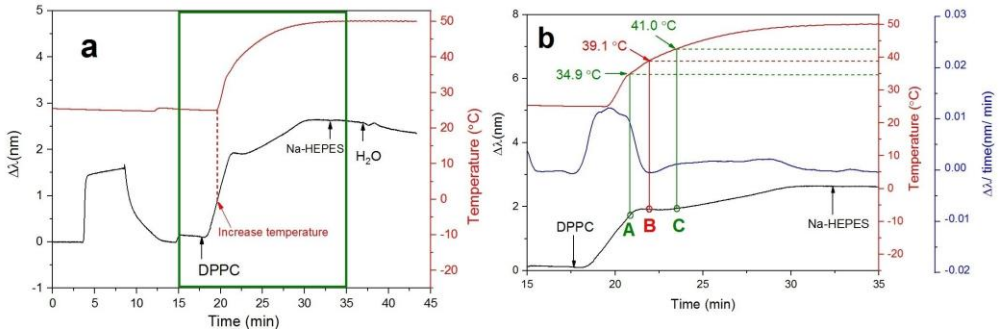

Figure 2: Detection of phase transition of DPPC liposomes by NPS. Liposome immobilization was done during a continuous increase in the temperature from 25 °C to 50 °C. (a) The peak shift and temperature profile of the whole experiment. (b) The peak shift, peak shift time derivative, and temperature profile between 15 and 35 min.

temperature effect at 39.1°C (point B in Fig. 2b). When the temperature was further increased to 41.0°C (point C in Fig. 2b), the NPS signal began to increase steadily and reached stable levels at 30 min. Based on the results, it is possible to assume that the liposome structure starts to disorganize at 34.9°C during the immobilizing step, corresponding to the start of the pre-transition. At 39.1°C, the NPS signal seems predominantly influenced by the change of the liposomal structure, suggesting the start of the main phase transition. An acceleration of the peak shift change was observed again at 41.0°C, indicating that the transition to the fluid phase was finished and the observed effect at this stage was liposome adsorption. This result is very similar to the end point temperature of the phase transition for pre-immobilized small vesicles of DPPC (41.3°C). Furthermore, the value determined by NPS is very close to the T_m found in literature. The author's explanation for the observed effect is that the gel phase liposomes adsorb with a slight deformation, and that the temperature induction of the fluid phase increases the liposome deformation at the surface of the sensor. The deformed liposomes spread over the free surface of the sensor and momentarily limit the adsorption rate.

Conclusions

Insplorion's Nanoplasmonic sensing technology is a unique method that allows the study of phase transition temperatures of liposomes. The work in this publication is much broader and explores the interaction of ionic liquids with lipid vesicles at different phases. The XNano proved to add value in the field, adding detailed information about the phase transition process, such as pretransition temperatures and the temperature range for the gel-to-fluid phase transition.

References

[1] *Determination of the Main Phase Transition Temperature of Phospholipids by Nanoplasmonic Sensing, Wen Chen, Filip Duša, Joanna Witos, Suvi-Katriina Ruokonen and Susanne K. Wiedmer, Scientific Reports, 2018, 8:14815*