Experimental report of the proposal
SC-4818 on ID10

Scientific background

Photo-triggered release of encapsulated drugs from liposomes is currently considered as a potential and interesting modality for drug delivery in a controlled manner. Photosensitive liposomes are nanocarriers that can be activated upon illumination at a specific wavelength to release their cargo either photo-oxidatively or photothermally, depending on the liposomes composition (saturated or unsaturated phospholipids and the fraction of the photoactive molecules). To do so, photoactive dyes such as porphyrins can be embedded in lipids matrix constituted with monounsaturated or polyunsaturated phospholipids. However, this strategy presents several drawbacks including the low dye concentration that can be embedded in the lipid matrix and its rapid clearance from the blood due to their transfer to serum components. To overcome such problems, Zheng et al.[1] have synthesized a new type of porphyrin-lipid conjugate, which consisted of a porphyrin connected to the phospholipid head group via ester bond. These conjugates have the ability to form similar supramolecular structures as regular phospholipids due to their amphipathic property resulting from the hydrophobicity of the acyl chain and porphyrin, and the hydrophilic head group. We have recently synthesized two different phospholipid-porphyrin conjugates (figure 1) by coupling Pheophorbide-a (a porphyrin derivative) to either a modified Lyso-phosphatidylcholine or Lyso-sphingomyelin via peptidic bond (instead of ester).

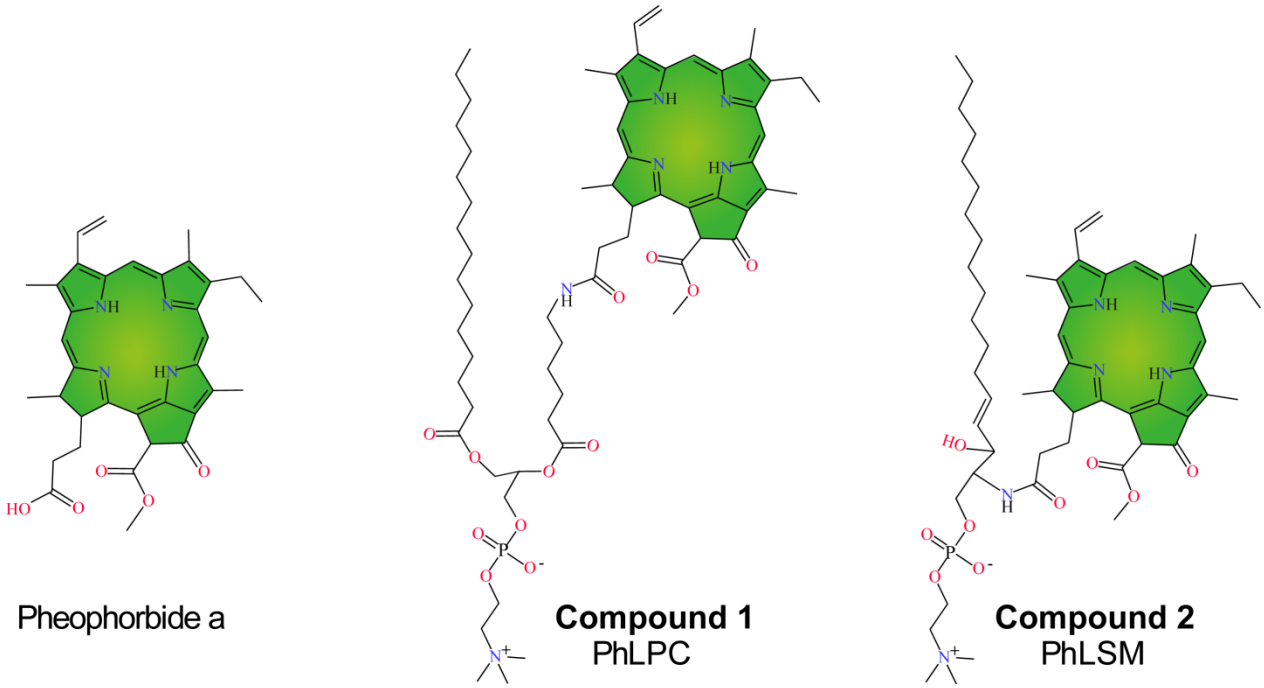

Figure 1: Chemical structures of the studied compounds.

In addition, these molecules can auto-assemble into liposomes-like structures and can encapsulate efficiently fluorescent probes when mixed with other lipids such as SOPC (for compound 1, PhLPC) or cholesterol (for compound 2, PhLSM) and release them upon their illumination at a

specific wavelength (~ 670 nm). Although, recent studies have demonstrated the potential of lipid-porphyrin conjugates in drug delivery, the molecular fine structure and their interactions with ions before and after illumination of such molecules are still lacking.

Aim of the Proposed Project

The primary aim of the proposed project is to assess the mechanism of phototriggered release by analyzing the vertical fine structure of the monolayers made of (i) pure lipid-porphyrin conjugate and (ii) mixed with other phospholipids and the density profiles of ions near the head groups before and after illumination by the unique combination of X-ray reflectivity (XRR) and grazing-incidence X-ray fluorescence (GIXF) at the air/water interface. Indeed, it is believed that the illumination of the porphyrin-derivatives containing membranes generates singlet oxygen that would oxidize the double bonds present in unsaturated phospholipid or cholesterol. This leads to the formation of oxidized lipids with hydroperoxide moieties that may attract monovalent or divalent ions to the vicinity of the polar headgroups.

Results of experiments

a. Analysis of the fine structures of Pheo-a derivatives monolayers

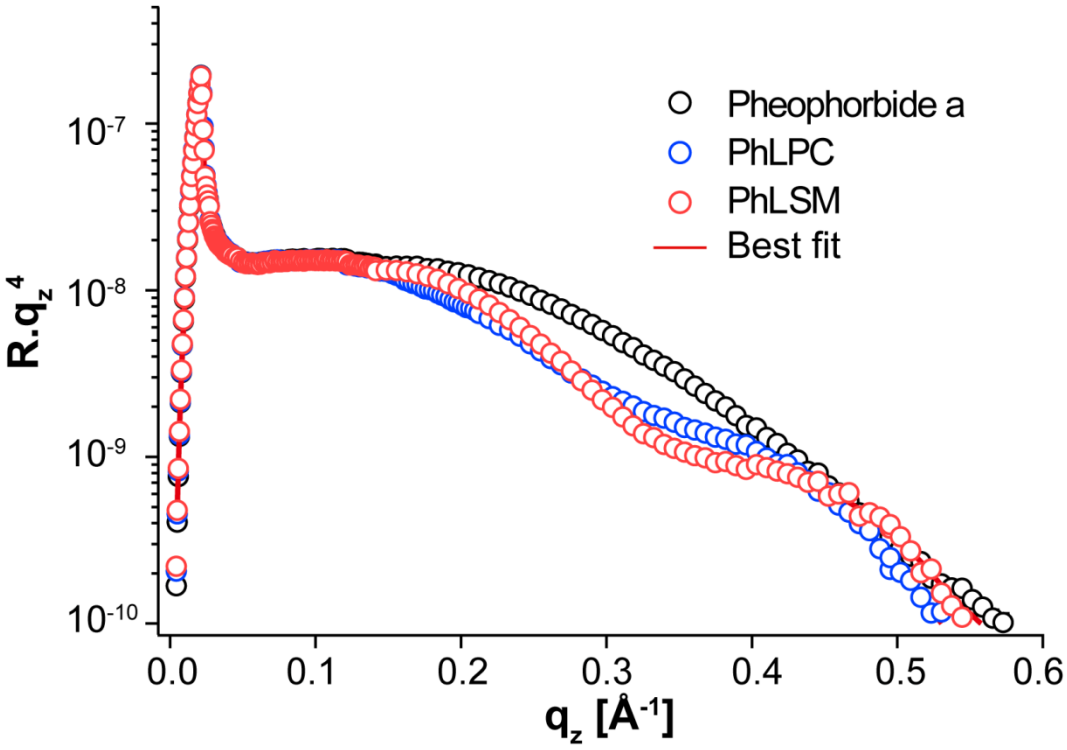

Figure 2: XRR curves of a Pheo-a derivatives monolayers at a surface pressure of 30 mN/m. The solid lines represent the best model fits to the experimental data. The experimental errors are within the symbol size.

The specular X-ray reflectivity (XRR) was measured on monolayers compressed to a surface pressure of 30 mN/m. Figure 2, shows the XRR curves of Pheo-a, PhLPC and PhLSM monolayers spread on HEPES buffer, fitted using a two-slab model. As determined from the fitting, Pheo-a exhibited total thickness d_{pheo-a} of 15.7 Å. The hydrophobic core had a thickness of 9.5 Å and an electron density of 0.436 $e^- \times Å^{-3}$. These values are consistent with those reported

for other porphyrin monolayers [2], thus indicating that Pheo-a molecules take an upright orientation with respect to the interface. The thickness and electron density of the hydrophobic regions of the PhLPC monolayer are $d_{HC(PHLPC)}$ 11.6 Å and $\rho_{HC(PhLPC)}$ = 0.373 $e^- \times Å-3$, respectively. Interestingly, the corresponding values for the PhLSM monolayer are $d_{HC(PHLPC)}$ 9.4 Å and $\rho_{HC(PhLSM)}$ of 0.391 $e^- \times Å^{-3}$, respectively. The ρ_{HC} values of both compounds are higher than those reported for saturated [3-4] or monounsaturated [5] alkyl chains of phospholipids. This could suggest the presence of porphyrin core within the alkyl chains. Although the thickness of hydrophobic region of PhLPC is larger than that of PhLSM, it is notable that the total thickness of PhLPC (d_{PhLPC} = 21.5 Å) is 2 Å thicker than that of PhLSM (d_{PhLSM} = 19.5 Å). This could be explained in terms of the conformational difference of the porphyrin. In the case of PhLPC, sn-1 C16 carbon chain and porphyrins are aligned while such an alignment is sterically prohibited in the case of PhLSM.

b. Analysis of the fine structures of minelayers made of pheo-a derivatives and lipids (cholesterol or SOPC) and impact of the illumination:

The fine structure of equimolar mixtures of Pheo-a derivative with lipid (either cholesterol or SOPC) has been measured by XRR on monolayers compressed to 30 mN/m. Herein, we showed the result obtained with equimolar mixture of PhLSM and cholesterol spread at the air water interface and compressed until a surface pressure of 30mN/m.

Figure 3, shows the XRR curves of PhLSM-cholesterol and PhLSM monolayers spread on HEPES buffer, fitted using a two-slab model. PhLSM-Cholesterol monolayer exhibited a total thickness $d_{PhLSM\text{-}cholesterol}$ of 21.9 Å. The hydrophobic region had a thickness of 12.6 Å and an electron density of 0.392 $e^- \times Å^{-3}$. These values are higher than those obtained with PhLSM monolayer. However, the thickness of the hydrophilic region of this mixture is reduced compared to that of pure PhLSM. This thickening of the hydrophobic region with the concomitant reduction in the thickness of the head group region could be related to a decrease in the PhLSM molecular tilt with subsequent upward shift which results in reducing the electron density of the polar region.

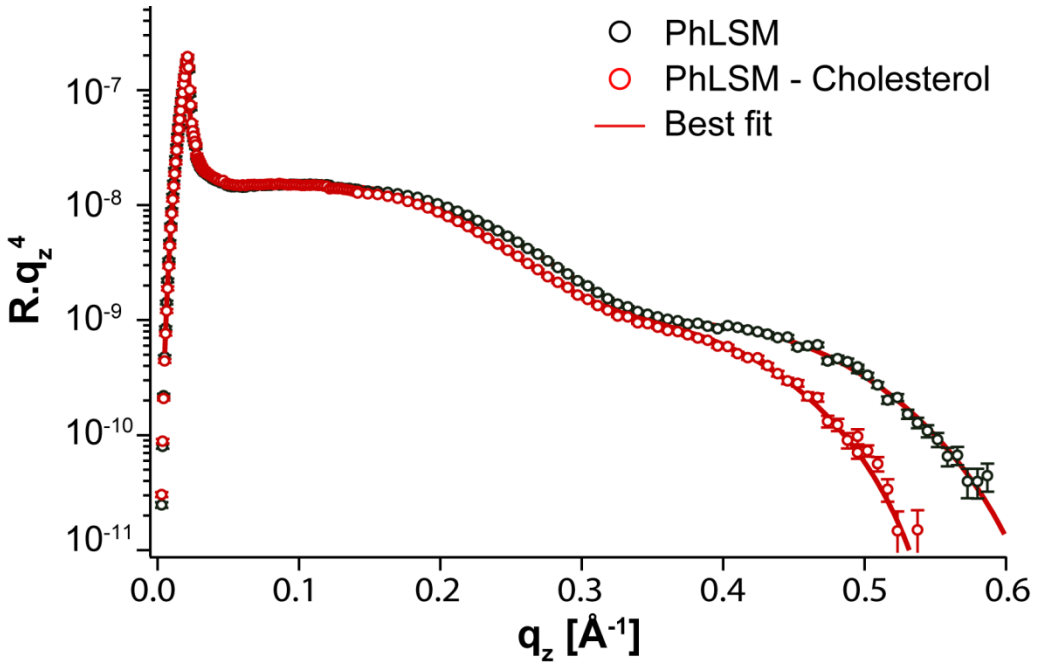

Figure 3. XRR curves of PhLSM and PhLSM:Cholesterol equimolar mixture monolayers compressed to a surface pressure of 30 mN/m. The solid lines represent the best model fits to the experimental data. The experimental errors are within the symbol size.

Finally, we have evaluated the impact of illumination on the fine structure and ions accumulation of monolayers containing pheo-a derivatives. However, the Fresnel reflectivity curves of this monolayer compressed at 30 mN/m before and after illumination were almost identical which demonstrates that apparently the illumination did not induce any structural alteration of the lipid matrix which could be explained by the aggregation of the pheophorbide-a into highly packed patterns due to the strong π- π stacking of porphyrin cores. Indeed, in this situation the absorbed photonic energy that is usually released as fluorescence and singlet oxygen is dissipated thermally through vibrational relaxation. [1, 6] Hence, the release mechanism upon illumination of liposomes containing PhLSM and cholesterol is more probably due to a photothermal effect.

Summary and Prospects

The allocated beam time allowed us to systematically investigate the fine structures of lipid-porphyrin conjugates monolayers in the absence or presence of other lipids such as cholesterol and SOPC on two different subphases. In addition, the impact of illumination on the monolayers Fine structures as well as on their interaction with ions by Grazing incidence X-ray fluorescence. Further detailed analysis on the latter aspect are undergoing to get quantitative data on electrostatics following the oxidation of lipid matrix.

1. J. F. Lovell, C. S. Jin, E. Huynh, H. Jin, C. Kim, J. L. Rubinstein, W. C. Chan, W. Cao, L. V. Wang and G. Zheng, *Nat Mater*, 2011, **10**, 324-332. 2.M. J. Griffith, M. James, G. Triani, P. Wagner, G. G. Wallace and D. L. Officer, *Langmuir*, 2011, **27**, 12944-12950. 3. J. Majewski, T. L. Kuhl, K. Kjaer and G. S. Smith, *Biophysical Journal*, 2001, **81**, 2707-2715. 4. M. Broniatowski, M. Flasinski, P. Dynarowicz-Latka and J. Majewski, *Journal of Physical Chemistry B*, 2010, **114**, 9474-9484. 5. A. Korytowski, W. Abuillan, A. Makky, O. Konovalov and M. Tanaka, *J Phys Chem B*, 2015, **119**, 9787-9794. 6. K. A. Dendramis and D. T. Chiu, *Journal of the American Chemical Society*, 2009, **131**, 16771-16778.