# Enhanced drug load efficiency into charged phospholipid bilayers

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ABSTRACT: This communication shows the current status of our study, from a structural point of view, on the process of incorporation of a hydrophobic drug into the hydrophobic regions of phospholipid bilayers. The aim of this investigation is to find relevant parameters to the drug delivery application. Using a liposome to wrap and transport a medicament has the advantage that futile side effects can be reduced and targeting of the liposome can be used for enhanced efficacy. The studied drug is the Ellipticine ( ELPT ), an anti - cancer agent. We prepared samples with dispersions made by phopholipids mixed with the drug ELPT to be studied (I) as a membrane stacking deposited on a glass substrate and also (II) as a phospholipid monolayer on a Langmuir trough. We have used the zwitterionic lipid dipalmitoylphosphatidylcholine (DPPC) mixed with some other phospholipids with different size of head and tail and/or different net electronic charge. X Ray Reflectivity (XRR) technique has used to monitor structural changes of these bilayer systems and Grazing Incidence X Ray Diffraction (GID) technique has used to monitor lateral order of phospholipid Langmuir monolayer.

## Troika II beamline - ESRF

### Applications

Investigation of the **structure and self-organization processes in the surfaces, interfaces and in thin films** both in-plane and normal to the film.

### Techniques

- **High-resolution Wide-angle Scattering (WAXS)**
- **Surface Scattering Techniques**
  1. Grazing Incidence Small-Angle X-ray Scattering (GISAXS)
  2. Grazing Incidence X-ray Diffraction (GID)
  3. X-Ray Reflectivity (XRR)

### Staff

- **Scientist in charge:** Oleg Konovalov
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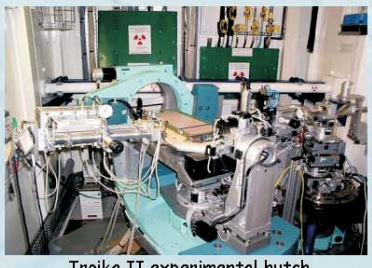
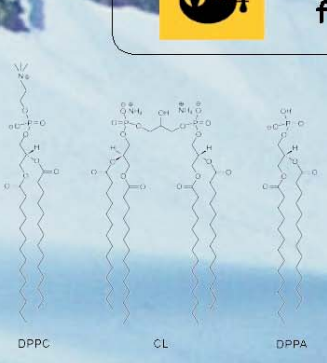
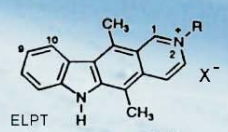


Troika II experimental hutch
www.esrf.fr/UsersAndScience/Experiments/SCMatter/ID10B

## Drug encapsulation study for drug delivery purpose



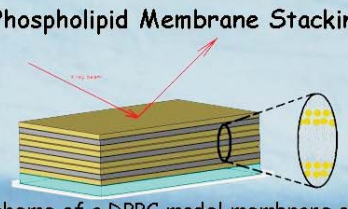

Molecular structure of DPPC, Cardiolipin (CL), and DPPA.



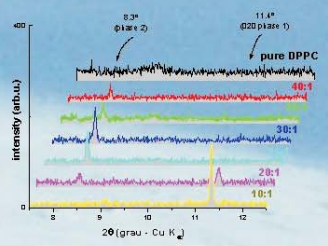

Molecular structure of Ellipticine (ELPT).

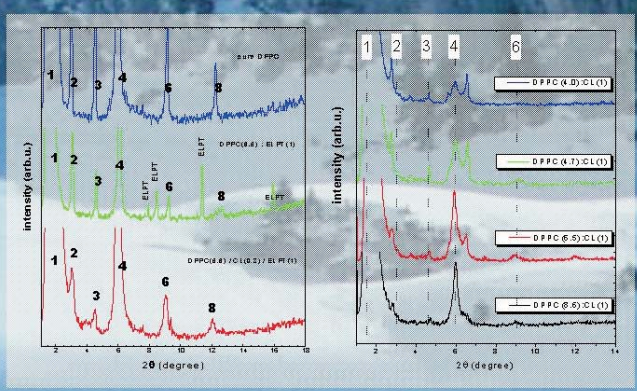
ELPT molecule is an alkaloid that consists of a planar heterocyclic hydrocarbon ring system with maximum dimension of 10Å. Because of this type of structure, it has been suggested that its antitumoral activity is due to an intercalation between the base pairs of the DNA helices.

### I. Phospholipid Membrane Stacking



Scheme of a DPPC model membrane on a glass substrate. X Ray Reflectivity was performed in $\theta$-$2\theta$ geometry.



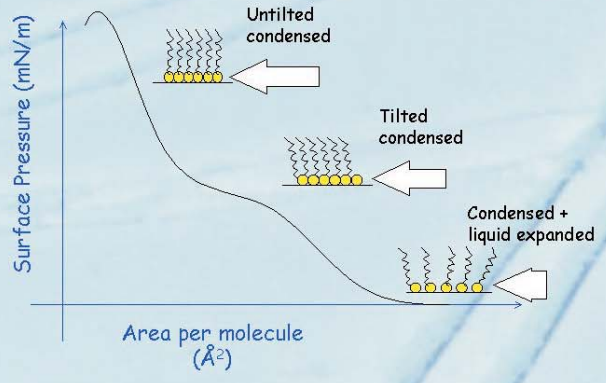



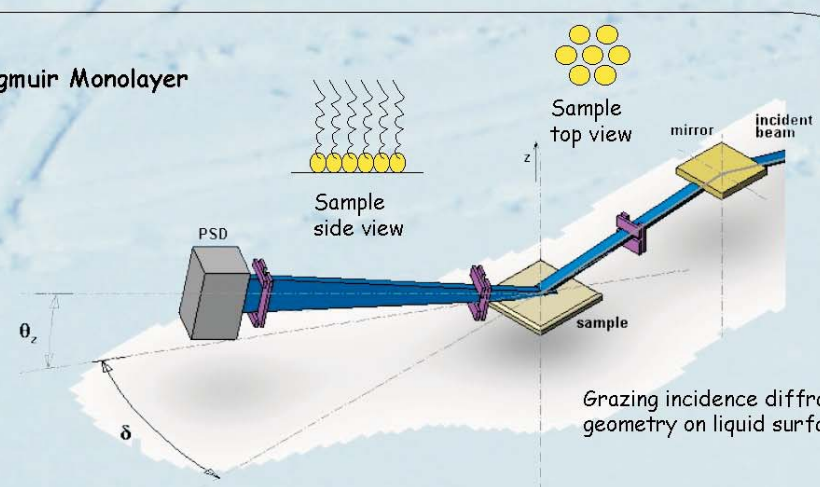

X Ray Diffraction of pure DPPC (blue) results in several orders of *OOL* reflections (numbered from 1 to 8 in the graph). Reflections from ELPT crystalline phase are observed in DPPC:ELPT system (green) but not in DPPC:CL:ELPT system (red). Adding CL into the membrane increases the incorporation of ELPT in the protonated form.

The graph on the top shows a new set of *OOL* reflections with periodicity of ~95Å for the mixed DPPC:CL membrane. The presence of CL in the system causes a disturbance in the periodicity of the lipid matrix. When the concentration of CL decreases this disturbance tends to disappear and the reflection widths of the lipid matrix becomes smaller indicating an improvement of the long range order.

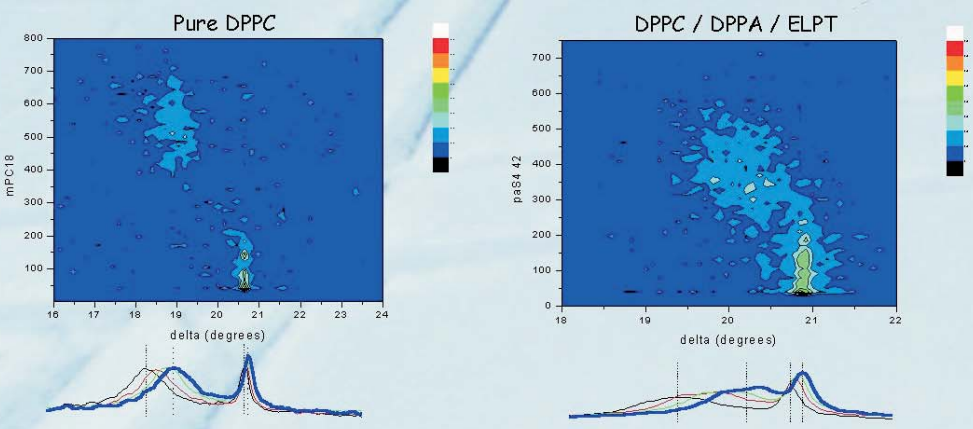
Reflections of ELPT crystalline phase in DPPC:ELPT systems. The DPPC:ELPT molar ratio of the systems are indicated in the graph. For the two most concentrated systems (20:1 and 10:1) we observed a 020 reflection of the cataloged crystalline phase of ELPT. For slightly concentrated systems (40:1 up to 20:1) we observe a second phase of ELPT that we found to be a mixed structure ELPT-methanol. If there is no reflections of these crystalline phases of ELPT it suggests that the drug is in the colloidal phase totally incorporated in the hydrophobic region of the lipid membrane.

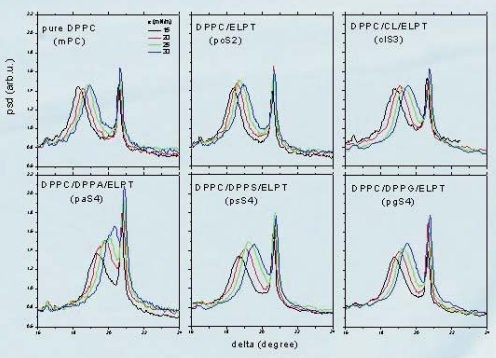
### II. Phospholipid Langmuir Monolayer




Grazing incidence diffraction geometry on liquid surface.




Studies on DPPC/DPPA Langmuir monolayers at the air-water interface using GID have given further information about lateral order of the lipid matrix. The maps show that, after insertion of the anionic phospholipid DPPA, the system presents less distortion from the hexagonal packing and smaller tilt of molecules. The strong interaction between DPPA and ELPT may promote a restructuring in the phospholipid matrix.

## DISCUSSION:

Adding CL into the membrane increases the incorporation of ELPT in the protonated form.

DPPA has a strong interaction with ELPT. Mixed DPPC:DPPA Langmuir monolayers have shown less distortion in the hexagonal packing than pure systems.

We are looking for optimizing the molar ratio between distinct lipids in mixed systems with a compromise on increasing the drug incorporation in the membrane with small effects of matrix distortion.

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Photo: Combe de Bellefont (Chartreuse) by Henri Gleyzolle