Experimental report

13/02/2025

Proposal: EASY-1309 **Council:** 10/2023

Title: Structural investigation of Curcumin/Laponite nanoparticles interacting with model Biomembranes

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: DOPC, Curcumin and Laponite

Instrument	Requested days	Allocated days	From	To
FIGARO	24	24	11/03/2024	12/03/2024

Abstract:

Curcumin (cur), a polyphenolic compound derived from the dietary spice turmeric, is a non-toxic, highly promising natural antioxidant compound with a broad spectrum of biomedical applications. Cur shows pharmacological activities such as antibacterial, antioxidant, antitumorigenic, and anticancer. It is capable of preventing DNA damage by decreasing the number of oxidative cancer-causing DNA molecules in tissue samples. With such multi-tasking properties, it suffers from the drawbacks of poor solubility and bioavailability. We have designed a new assembly of curcumin nanoparticles stabilized by Laponite particles and we will be denoting them as LCu Nanoparticles. Exploring the interaction between laponite/curcumin nanoparticles (LCu) and model membranes, specifically solid-supported lipid bilayers (SLBs), would offer valuable insights into curcumin's potential to dissociate from LCu nanoparticles and form robust interactions with cellular membranes. Such understanding could pave the way for the development of novel anticancer treatments. To date, only a limited number of studies demonstrate curcumin's capacity to influence lipid structure and disrupt cancerous cell membranes.

Report for the Proposal No. EASY-1309

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Scientific background: Curcumin (Cur), a polyphenolic compound derived from the dietary spice turmeric, is a nontoxic, highly promising natural antioxidant compound with a broad spectrum of biomedical applications. The unusual physical properties of Cur have led to much interest in cancer therapy and other human diseases in recent years [1]. Cur shows pharmacological activities such as antibacterial, antioxidant, antitumorigenic, and anticancer. It is capable of preventing DNA damage by decreasing the number of oxidative cancer-causing DNA molecules in tissue samples. With such multi-tasking properties, it suffers from the drawbacks of poor solubility and bioavailability. These drawbacks can be efficiently overcome by the formation of an assembly of nano-curcumin structures [2]. The assembly of curcumin nanoparticle shows drastic improvements in the properties of curcumin. It is a great challenge, however, to form stable curcumin nanoparticles, since they evolve or aggregate very quickly. The work by Cheng's group [3] showed the formation of curcumin nanoparticles in a binary mixture of water and ethanol, but these nanostructures were found to be highly unstable. The dispersion instability was due to the large depletion forces provided by the binary solvent. In our previous published work [4], we stabilized these attractive forces prevailing between hydrophobic curcumin nanoparticles with the long-range repulsive forces provided by the Laponite particles. Hence, we could form a stable assembly of curcumin nanoparticles. In brief, Laponite (a nanoclay) is an anisotropic colloidal particle with disc shaped morphology (disc diameter~10 nm and thickness~1nm, [4]). Its anisotropic charge distribution (negative face and positive edge) allows it to have a very rich phase diagram. It has biological applications to stabilize drugs and pharmaceutical products [5,6]. Studying the interactions between curcumin nanoparticle (potential anticancer drug) and cell membrane models is of great interest to explore the capability of drug in different treatments, since lipid membrane is the mean barrier that the drug overcome to introduce the drug into the cell.

Aim of Experiments: We designed a new assembly of curcumin nanoparticles stabilized by Laponite particles and we will be denoting them as LCu Nanoparticles. Exploring the interaction between laponite/curcumin nanoparticles (LCu) and model membranes, specifically solid-supported lipid bilayers (SLBs), would offer valuable insights into curcumin's potential to dissociate from LCu nanoparticles and form robust interactions with cellular membranes. Such understanding could pave the way for the development of novel anticancer treatments. To date, only a limited number of studies demonstrate curcumin's capacity to influence lipid structure and disrupt cancerous cell membranes.

During the beam time we have investigated how the curcumin nanoparticle (LCu) interacts with solid-supported lipid bilayer model using neutron reflectometry (NR).

Sample No.	Description
1	DOPC+LCu NP
2	DOPC+Curcumin
3	DOPC
4	Controls

The use of DOPC is justified as it is in fluid like liquid crystalline state at physiological temperatures (37^0C) and widely used in model bilayer systems. Experiment were performed in three solvent: water, D2O and Silicon Matched Water.

Liposomes were prepared from hydrogenated DOPC. We allowed the DOPC to assemble as bilayer on the Silicon crystal and latter nanoparticles were flown over the bilayer . At each step Neutron measurement was performed. We repeated the experiment in three different solvents: Water, D2O and SMW.

Results:

Atomic Force Microscopy: We have investigated the mechanical properties and morphological changes of the lipid bilayer before and after the incorporation of LCu nanoparticles using Atomic force microscopy The bilayer of lipids (DOPC) was prepared by the standard procedure [6] on the mica substrate followed by the AFM measurement in the liquid mode ,then we incubate (for 20 min) the bilayer with LCu nanoparticles and measured the AFM. Analysis of force distance curves gave the values of modulus which quantifies the mechanical properties of the membrane. We observed that both DMT modulus and the adhesion forces significantly decreases after the incorporation of nanoparticle on the bilayer.

NR Experiment:

Neutron reflectometry (NR) experiments were conducted to investigate the incorporation of Cu-Lap NPs into DOPC-supported lipid bilayers (SLBs) and assess the potential release of curcumin molecules. NR provides sub-nanometer resolution, enabling the analysis of SLB structure and composition along the direction perpendicular to the membrane plane. Reflectivity (R), defined as the ratio of reflected neutrons to the incident beam intensity, was measured under specular conditions as a function of the momentum transfer vector (Qz) normal to the bilayer. These measurements were performed before and after the introduction of Cu-Lap NPs. By fitting NR profiles obtained from three isotopic contrasts, both the bilayer structure and the presence of curcumin and/or Cu-Lap NPs were determined along the membrane's perpendicular axis. The resulting scattering length density (SLD) profiles for each contrast are presented in Figures 1C and 1F, while the volume fraction distributions of different bilayer components are shown in Figures 1A and 1D. Prior to Cu-Lap NP incorporation, NR characterization indicated a symmetric DOPC bilayer, with solvated head groups of the inner leaflet interacting with the solid support and those of the outer leaflet exposed to the bulk phase (Figures 1A–B).

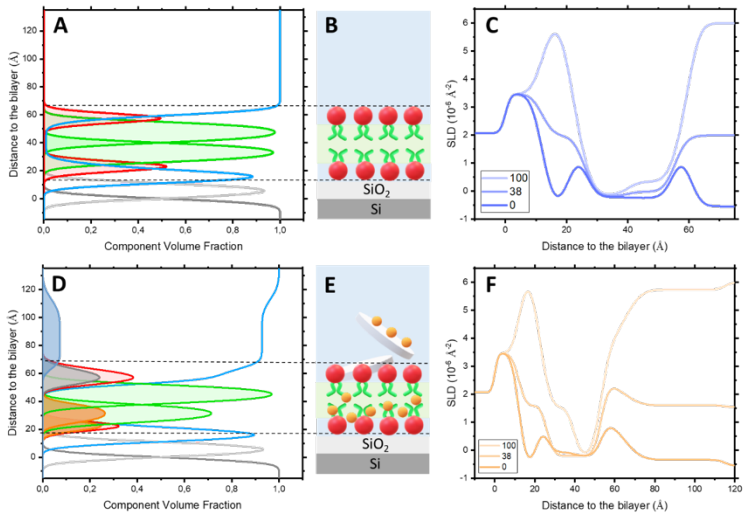

Figure 1. Density profiles of the interfaces for the lipid bilayer (A) and after its interaction with the Cu-Lap NPs (D). Schematics of the models used to fit NR data (B, E). SLD profiles obtained for the lipid bilayer (C) and after its interaction with the Cu-Lap NPs

Conclusion: NR experiments confirmed the incorporation of curcumin into the hydrophobic lipid tails and resulting in increased membrane solvation and roughness. The work has been published in Small 2406885, 2024, DOI: 10.1002/smll.202406885

References

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