

# BIODISTRIBUTION OF LIPID NANO-CARGOS BY FLUORESCENCE IMAGING: FROM CELL TO ANIMAL (CALIF)

## PROJECT PARTNERS

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## OBJECTIVES OF THE PROJECT

The project CALIF aims at understanding the interactions between lipid nano-cargos (nanocapsules (LNC) and nanoemulsions (LNE)) (Fig. 1), and the living machinery, and the identification of the physicochemical parameters governing these. The project addresses the goal by 1) finding out more information on the lipid nanocargos, independently of their payload; 2) establishing what are the physicochemical parameters which govern an efficient presentation of a tumor-targeting ligand (RAFT-RGD) by a lipid nano-cargo; 3) furnishing such information using fluorescence imaging which is used at the cellular level and in non-invasive small animal studies.

## METHODS AND RESULTS

In the beginning of the project, LNCs and LNEs of different size (20-100 nm) and surface properties (PEG chain length, dextran, chitosan) were prepared. Encapsulation and stability of different fluorescent dyes in these nano-cargos were studied (Fig. 2). Stealth properties of the nano-cargos was evaluated *in vitro* by the complement activation CH50 test (Fig. 3). *In vivo* biodistribution of the nano-cargos was evaluated in mice carrying subcutaneous HEKβ3 tumors (Fig. 4).

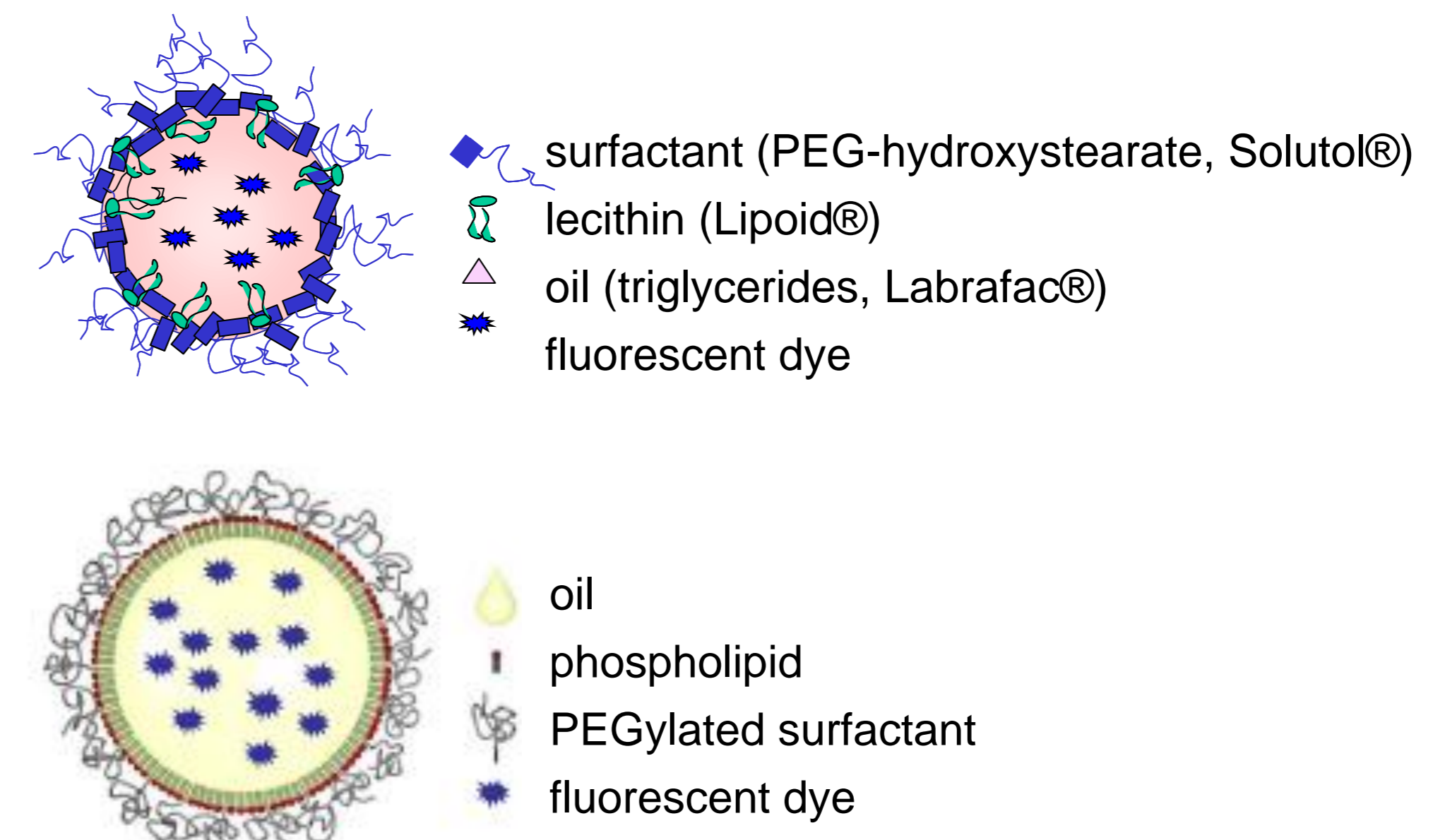


Fig. 1. A lipid nanocapsule (LNC) (above) and a droplet of lipid nanoemulsion (LNE) (below).

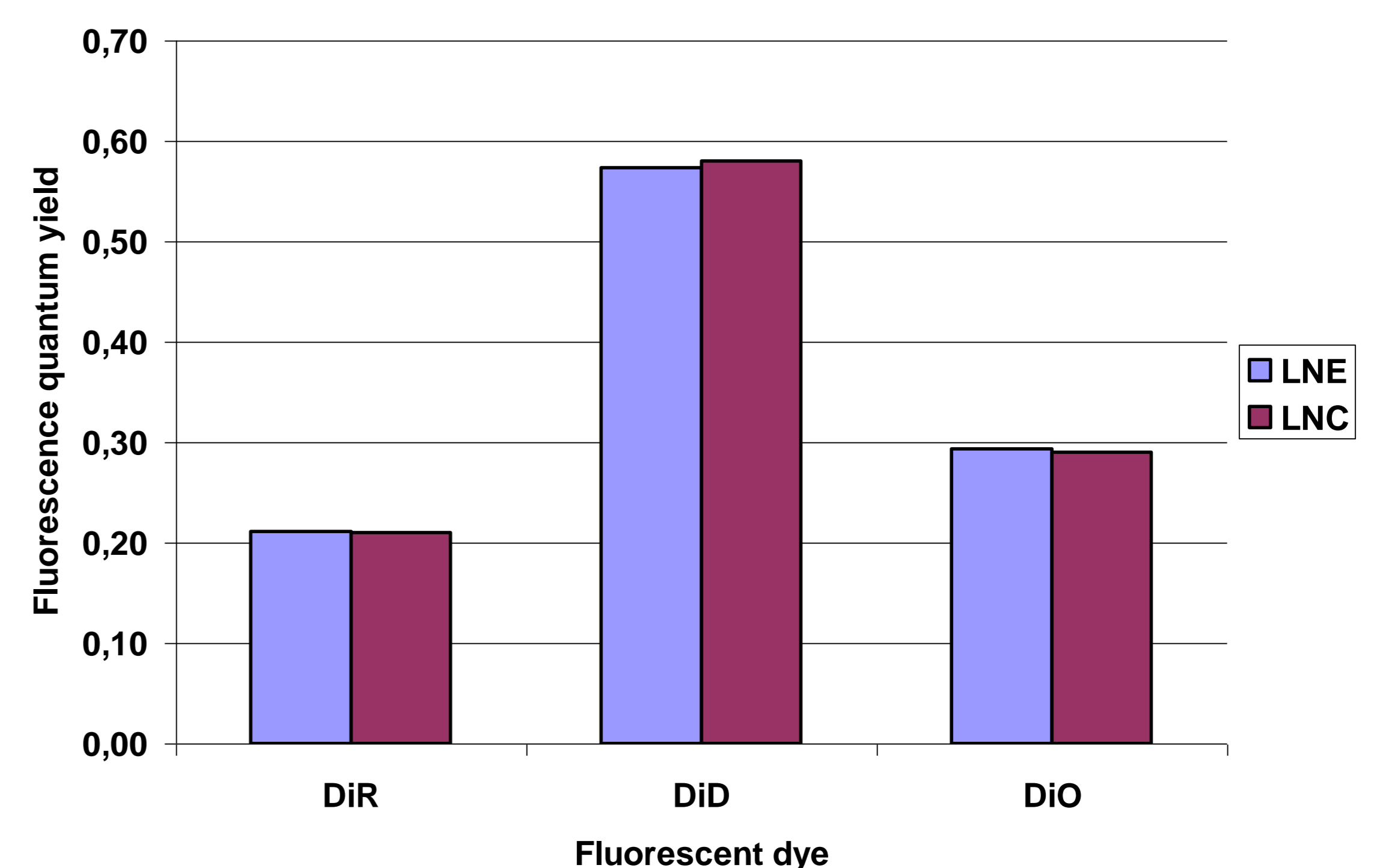


Fig. 2. Fluorescence quantum yield of three fluorescent dyes encapsulated in LNC and LNE. These hydrophobic dyes were efficiently encapsulated in the oily cores of the nano-cargos providing efficient yields. Similar yields between LNC and LNE might indicate that the oily core affects the yields rather than surfactants on the nano-cargo surface (different surfactants were used in LNC and LNE). Successful encapsulation of fluorescent dyes should enable the use of Förster resonance energy transfer (FRET) in nano-cargo tracking.

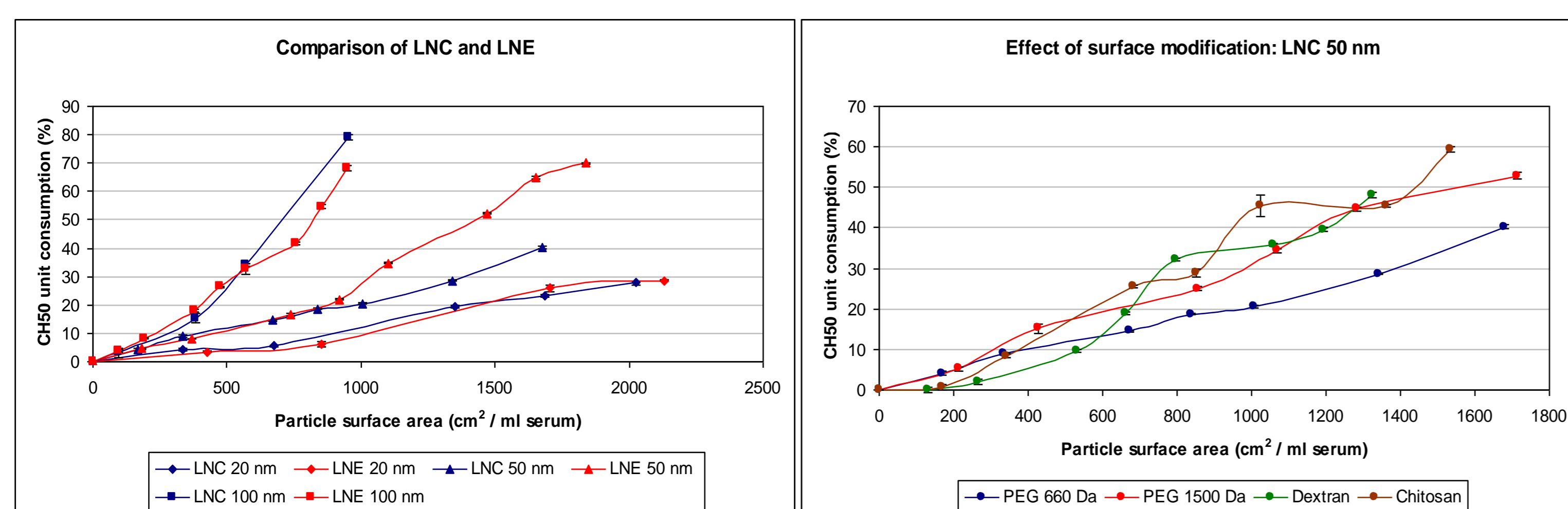


Fig. 3. The complement activation CH50 test evaluated the residual hemolytic capacity of the complement system of normal human serum after contact with nano-cargos. All the tested nano-cargos activated the complement system very little at low concentrations comparable to *in vivo* situation ( $\sim < 500 \text{ cm}^2/\text{ml}$ ), indicating good stealth properties. However, at higher concentrations, a clear size-dependent behaviour was observed: the smaller the nano-cargo size was, the better they could escape the protection mechanism. Modification of the nano-cargo surface (PEG, sugars) had no effect on the complement consumption. The tested LNCs and LNEs behaved generally in an equal manner.

## CONCLUSIONS AND FOLLOWING STUDIES

The prepared nano-cargos possessed good stealth properties but no remarkable accumulation in tumors. These results act as reference when tumor-targeting ligands will be attached on the nano-cargos in the following studies. Also, nano-cargos will be tested with different cell lines for cell interaction / internalization and toxicity studies.

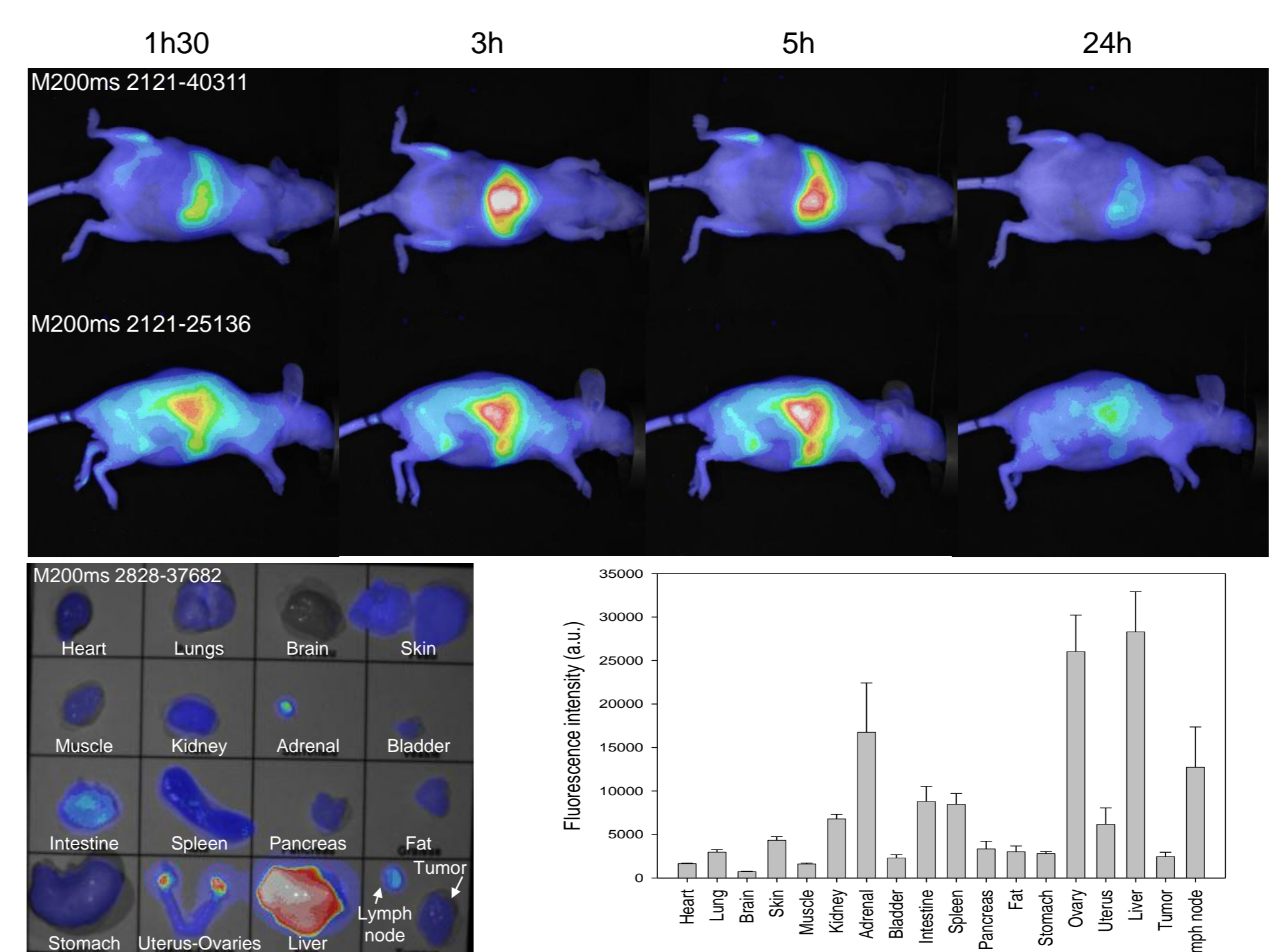


Fig. 4. Biodistribution and accumulation of LNC 50 nm in organs. Generally, all the tested LNCs and LNEs behaved according to a similar schema. Distribution of nano-cargos was homogeneous with substantial accumulation in the liver and in the lymph nodes. 24 h after injection the nano-cargos were still in the blood circulation. The smaller the nano-cargos were, the better were the biodistribution profiles and accumulation in tumor (and in the lymph nodes) ( $20 > 50 > 100 \text{ nm}$ ). Correspondingly, longer PEG chain at the nano-cargo surface enhanced biodistribution and accumulation in tumor.