



# Heat-activated liposome targeting to streptavidin-coated surfaces

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## ABSTRACT

There is a great need of improved anticancer drugs and corresponding drug carriers. In particular, liposomal drug carriers with heat-activated release and targeting functions are being developed for combined hyperthermia and chemotherapy treatments of tumors. The aim of this study is to demonstrate the heat-activation of liposome targeting to biotinylated surfaces, in model experiments where streptavidin is used as a pretargeting protein. The design of the heat-activated liposomes is based on liposomes assembled in an asymmetric structure and with a defined phase transition temperature. Asymmetry between the inside and the outside of the liposome membrane was generated through the enzymatic action of phospholipase D, where lipid head groups in the outer membrane leaflet, i.e. exposed to the enzyme, were hydrolyzed. The enzymatically treated and purified liposomes did not bind to streptavidin-modified surfaces. When activation heat was applied, starting from 22 °C, binding of the liposomes occurred once the temperature approached  $33 \pm 0.5$  °C. Moreover, it was observed that the asymmetric structure remained stable for at least 2 weeks. These results show the potential of asymmetric liposomes for the targeted binding to cell membranes in response to (external) temperature stimulus. By using pretargeting proteins, this approach can be further developed for personalized medicine, where tumor-specific antibodies can be selected for the conjugation of pretargeting agents.

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## 1. Introduction

Liposomal systems have been intensively studied for their capacity to deliver therapeutic and diagnostic materials [1]. Currently, most of the liposomal systems on the market have been formulated to reduce the systemic toxicity of the encapsulated drug and to extend the drug circulation time after administration [2,3]. Newer formulations are being designed to increase accumulation of the drug at the desired sites by integrating target recognition molecules on the liposome surfaces [4,5]. Although all of these approaches have potential therapeutic value, further improvements are desired to obtain additional clinical benefits. Liposome systems that exhibit a stimuli-responsive property (e.g., in response to temperature [6], or redox reactions [7]) have been designed to improve cellular uptake, and thus will increase the drug residence time at the target site by controlling the appearance of the targeting molecules. A recently applied variant of targeting in oncology is the so-called pretargeting, where bi-specific protein conjugates are administered before the delivery of the radioactive therapeutic agents [8,9]. The streptavidin–biotin interaction used in pretargeting strategies can be utilized for the delivery of biotinylated liposomes.

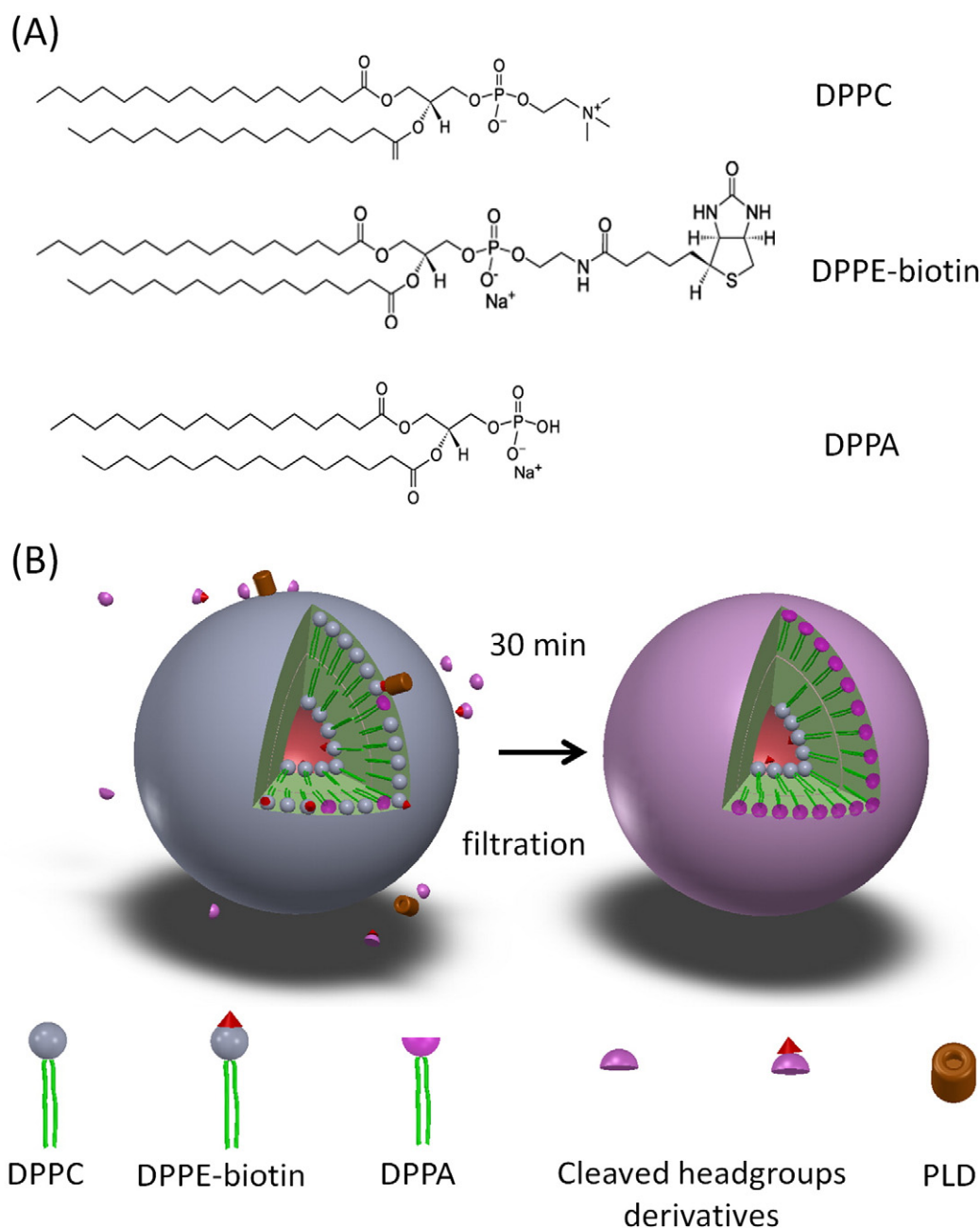
Temperature-responsive liposomes offer the possibility of enhancing the therapeutic efficiency in hyperthermia treatments [10–12]. Stability

against leakage is achieved at temperatures where liposomes remain in the gel phase, and the encapsulated materials can be released when the temperature is raised to the phase-transition regime. More pharmaceutical advantages are likely to be obtained if the temperature-responsive liposomes can also change their surface properties upon heat stimulus, in which the targeting function is activated as well as the release. This concept has previously been shown for polymer-based drug carriers [13].

As a tool to develop more advanced liposomes, the study of surface-supported lipid membranes can provide valuable information on how to design temperature-activated liposomal functions. For example, the asymmetry of supported membranes can be studied as a function of temperature [14–17], suggesting that membrane asymmetry can potentially facilitate the positioning-control of ligand-modified lipids. A commonly used strategy for preparing asymmetric supported lipid membranes is the Langmuir–Blodgett (LB) technique [18]. However, it cannot be applied as a protocol to produce asymmetric liposomes in bulk solution. A more general strategy to generate asymmetry is by hydrolyzing lipids in the presence of enzymes such as phospholipase D (PLD). PLD is involved in a variety of cellular functions, one of which is to catalyze the hydrolysis of lipid head groups to form phosphatidate (PA) [19], as shown in Fig. 1A. Hidden surface functions in the asymmetric membranes can be activated by increasing the temperature close to the gel to liquid-crystalline phase-transition temperature ( $T_m = 41$  °C for DPPC lipids) of the asymmetric membrane where lipid flip-flop appears.

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**Fig. 1.** (A) Chemical structures of the lipids used in the present study. (B) Schematic representation of the preparation of the asymmetric liposomes.

In a previous study, we showed temperature-activated flip-flop of biotinylated lipids originally hidden in the lower leaflet of a supported lipid membrane prepared using sequential deposition of the lipid leaflets in situ [16]. Here, we take advantage of PLD to prepare asymmetric liposomes through hydrolysis of lipid head groups at the outer membrane leaflet while leaving the functionality of the lipids in the hidden leaflet, assuming that the asymmetry is maintained below the phase transition temperature.

With the aim to demonstrate heat-activation of liposome binding to a target model surface, the present protocol is first developed using supported lipid membranes as a model system, and then applied to liposomes. The supported biotinylated membranes are also used as a biomimetic platform for the investigation of the temperature dependence of liposome binding. Streptavidin binds highly specifically and with high affinity to biotinylated lipids, and can be used as a pretargeting agent to label the targeted surfaces, here by attracting liposomes when biotin-lipids flip to the surface.

The protocol development using supported membranes as well as the binding of targeted liposome were monitored by the quartz crystal microbalance with dissipation (QCM-D) technique.

## 2. Materials and methods

### 2.1. Materials

Chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. 1, 2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1, 2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(biotinyl) (DPPE-biotin), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and 1, 2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(biotinyl) (DOPE-biotin) were purchased from Avanti Polar Lipids Inc., USA. Phospholipase D (PLD) and streptavidin were purchased from Sigma.

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