# A Polymeric "Flippase" for Surface-Differentiated Dipalmitoylphosphatidylcholine Liposomes

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The properties of synthetic liposomes can be modulated by polymerization or added polymers. Liposomes constructed of polymerized lipids often display greater longevity and improved permeability control relative to ordinary liposomes.[2,3] Similarly desirable properties can be imparted by polymerization of the counterions (*e.g.*, methacrylate) of (cationic) liposomes, so as to create concentric polymeric sheaths.[4] Finally, specific interactions with *added* polymers can modify liposomal behavior.[2c,5] For example, synthetic polymers bind[6] to phospholipid liposomes, altering their morphology,[7,8] permeability,[9,10] or fusogenicity.[5] Immunomimetic recognition can also be demonstrated between suitably modified liposomes.[2c,11]

Now we report that the morphological changes accompanying polymer–liposome binding[7,8] can be harnessed to catalyze transbilayer lipid migration ("flip–flop") in surface-differentiated bilayer liposomes. That is, the polymer functions as a "mechanical" flippase.[12]

Unilamellar, 420-Å-diameter bilayer coliposomes of 1:7 functional (**1**-F) and nonfunctional (**1**-NF) dipalmitoylphosphatidylcholine (DPPC) were prepared by sonication at pH 6 (HCl), 0.01 M BisTris buffer, 0.01 M KCl.[13] The coliposomes were surface differentiated by hydrolysis of the *expoliposomal* **1**-F *p*-nitrophenyl benzoate moieties (pH 12/6 exo/endo gradient, 30 min), followed by restoration of pH 6.[13] The exoliposomal surfaces then carried *p-nitrophenylate* moieties from **1**-F, whereas the endovesicular **1**-F lipids retained intact esters.

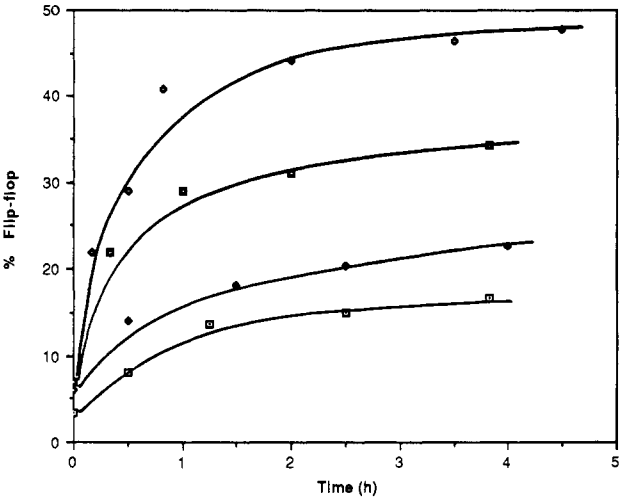
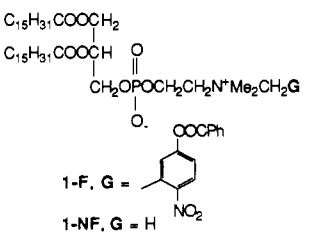
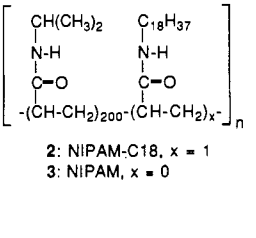



**Figure 1.** % flip–flop of lipid **1**-F in surface-differentiated **1**-F/**1**-NF coliposomes at 35 °C as a function of time in the presence of 0, 20, 50, and 200 ppm (bottom to top) of added NIPAM-C18 copolymer. At "time = 0," the % flip–flop values are ≤7% and reflect preparatory manipulation.




Aqueous solutions of NIPAM-C18 copolymer **2** were then added to the liposomes,[8] establishing (molar) polymer/lipid ratios of 20–200 ppm. NIPAM-C18 is a random copolymer of *N*-isopropylacrylamide and *N*-octadecylacrylamide in a ~200:1 molar ratio, with a viscometric molecular weight of ~250 000.[14a] It is water soluble below 30.3 °C (lower critical solution temperture, LCST, taken here as the cloud point temperature), but undergoes a rapid extended → globular collapse above the LCST.[8,14c] In parallel experiments, NIPAM homopolymer, **3** (LCST 31.8 °C), was used. It has been shown that amphiphilic NIPAM-C18 inserts into the membranes of phospholipid liposomes via its $C_{18}$ chains.[8,15] In contrast, the homopolymer **3** does not strongly interact with the liposomes, at least below its LCST.[14a,b]

The time courses of (functional) lipid flip–flop were followed[13] at several temperatures, and as functions of the amount and kind of added polymer. At 25 °C, below *both* the polymers' LCST and the liposomes' gel → liquid crystalline ("rigid → fluid") transition temperature ($T_c$ = 40 °C,[13] unchanged in the presence of 20 ppm of **2**), where the liposomes are in the gel state, and the polymers are in extended, water-soluble forms, we observed little effect of added **2** or **3**. Differentiated liposomes were only ~6% reequilibrated by flip–flop after 3.8 h, and even 200 ppm of **2** increased this to only ~10% after 3.3 h.[16]

However, at 35 °C (Figure 1), below the liposomes' $T_c$, but

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(16) Indeed, 2-h incubations of differentiated liposomes with 50 ppm of **2** at 25, 28, 31, or 33 °C demonstrate that polymer-induced flip–flop does not become apparent until 31 °C, above the polymer's LCST.

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above the polymers' LCST, there are marked effects of added polymers: the flip–flop rate increases sharply and continuously with added **2**. The extrapolated times required for 30% functional lipid reequilibration ($t_{30}$, corresponding to half-reequilibration) decrease from ~12 h, in the absence of polymer to 6, 1, and ≪1 h at 20, 50, or 200 ppm, respectively, of added **2**. NIPAM (**3**) is significantly less effective, manifesting $t_{30} \sim 1$ h at 200 ppm.

Finally, at 55 °C, where $T > T_c$ (fluid, liquid crystalline liposomes), and $T >$ LCST (contracted polymer), as little as 20 ppm of **2** induces 30% flip–flop after 10 min; with 200 ppm of polymer, $t_{30} \sim 2$ min. In the absence of polymer, $t_{30}$ requires ~24 min. Again, homopolymer **3** is less effective; 200 ppm induces ~20% of flip–flop in 2–5 min.

These experiments demonstrate that: (1) In its water soluble extended state ($T < 30$ °C), NIPAM-C18, though inserted into the membranes of surface-differentiated DPPC liposomes,[8,14a] has little effect on the dynamics of the flip–flop reequilibration of **1**-F. (2) Above its LCST, where **2** undergoes contraction, but below the liposomal $T_c$, NIPAM-C18 clearly enhances flip–flop (*cf.*, Figure 1). (3) Above the liposomal $T_c$ (*e.g.*, 55 °C), where the liposomes are in the fluid, liquid crystalline state, this catalytic effect of NIPAM-C18 is more dramatic. (4) Flip–flop enhancement by **2** increases with increasing polymer concentration and increasing temperature. (5) Homopolymer **3** is significantly less effective at stimulating lipid flip–flop than copolymer **2**. However, above its LCST, **3** undergoes a phase separation from aqueous solution and its interaction with the liposomes is enhanced.

Our mechanistic interpretation of the results is illustrated in Figure 2. NIPAM-C18 binds to the liposomes and inserts its $C_{18}$ chains amid the exoliposomal lipid chains. Below its LCST, insertion of the extended polymer has no effect. However, when $T >$ LCST, the NIPAM-C18 becomes more hydrophobic and contracts, creating "point defects" or spaces in the exoliposomal leaflet.[17] These serve to receive endo → exo lipid flips and thus enhance the rate of reeequilibration. The action of NIPAM-C18 is both concentration dependent[18] and magnified when the liposome is fluid ($T > T_c$). NIPAM homopolymer **3**, lacking $C_{18}$ chains, is not anchored to the membrane below its LCST and is a significantly less effective facilitator of flip–flop above the LCST.

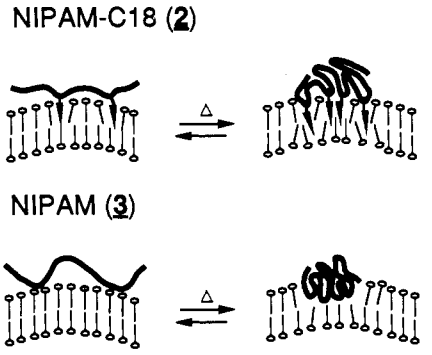


**Figure 2.** Schematic illustration of the interaction of NIPAM-C18 copolymer (**2**) and NIPAM homopolymer (**3**) with liposomal membranes below (no influence on lipid flip–flop) and above (enhanced lipid flip–flop) the polymer's LCST (cloud point temperature).

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(18) The more polymer bound to the liposomes, the more defects are created, and the faster flip–flop proceeds. We calculate that at 20 ppm NIPAM-C18 there are ~3 liposomes per polymer molecule (equivalent to ~3–5 $C_{18}$ chains per liposome); at 200 ppm NIPAM-C18, there would be ~3 polymer molecules for each liposome (~33–53 $C_{18}$ chains per liposome).