Temperature- and rigidity-mediated rapid transport of lipid nanovesicles in hydrogels

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Lipid nanovesicles are widely present as transport vehicles in living organisms and can serve as efficient drug delivery vectors. It is known that the size and surface charge of nanovesicles can affect their diffusion behaviors in biological hydrogels such as mucus. However, how temperature effects, including those of both ambient temperature and phase transition temperature (Tm), influence vehicle transport across various biological barriers outside and inside the cell remains unclear. Here, we utilize a series of liposomes with different Tm as typical models of nanovesicles to examine their diffusion behavior in vitro in biological hydrogels. We observe that the liposomes gain optimal diffusivity when their Tm is around the ambient temperature, which signals a drastic change in the nanovesicle rigidity, and that liposomes with Tm around body temperature (i.e., ∼37 °C) exhibit enhanced cellular uptake in mucus-secreting epithelium and show significant improvement in oral insulin delivery efficacy in diabetic rats compared with those with higher or lower Tm. Molecular-dynamics (MD) simulations and superresolution microscopy reveal a temperature- and rigidity-mediated rapid transport mechanism in which the liposomes frequently deform into an ellipsoidal shape near the phase transition temperature during diffusion in biological hydrogels. These findings enhance our understanding of the effect of temperature and rigidity on extracellular and intracellular functions of nanovesicles such as endosomes, exosomes, and argosomes, and suggest that matching Tm to ambient temperature could be a feasible way to design highly efficient nanovesicle-based drug delivery vectors.

lipid nanovesicle | liposome | phase transition temperature | diffusion | biological hydrogels

Lipid nanovesicles consisting of a lipid bilayer structure enclosing an aqueous interior are ubiquitous in living cells (1), with examples including endosomes, lysosomes, exosomes, and synaptic vesicles (2, 3). Nanovesicles play many important roles in cell activities including intracellular trafficking (4–6), intercellular transport (7), and communication (8), and they carry proteins, lipids, and nucleic acids around in various biological environments (9, 10). The properties of nanovesicles ensure accurate and efficient transport of “cargo” molecules (11, 12). In particular, rigidity and phase transition of nanovesicles play crucial roles in their transport behavior (13), and these two properties can often be tuned through temperature (14, 15), pressure (16), and composition (17–19).

As a typical model for nanovesicles, synthetic liposomes are promising candidates for drug delivery due to their high biocompatibility and ease of manipulation with respect to size, surface property, and composition (20–22). The stability, size, and shape of liposomes could be modulated by the phase behavior of lipids, which in turn could be adjusted by temperature. Studies have shown that when the ambient temperature is above a phase transition temperature (Tm), the liposome membrane transforms from a solid-like gel phase to a liquid-crystalline phase (23). For example, it has been shown that ThermoDox, a thermostensitive liposome currently under phase III clinical evaluation, could rapidly deliver drugs to a locally heated tumor (∼40–45 °C) while keeping the payload at body temperature (∼37 °C) (24). This suggests that temperature is an important factor that could be modulated for improved drug delivery. In pure solvents and hydrogels, increasing temperature tends to promote the diffusion of rigid nanoparticles (NPs) according to the Stokes–Einstein relation. For deformable liposomes, however, there is currently little knowledge on how temperature affects their diffusion in biological hydrogels.

We synthesized liposomes with different values of phase transition temperature Tm (from ∼16.0 to 55.0 °C) to evaluate the effect of ambient temperature on their diffusion capacity and therapeutic efficacy. Surprisingly, we observed the existence of an optimal ambient temperature near Tm for liposomal diffusion in biological hydrogels both in vitro and ex vivo. Orally administered, insulin-loaded liposomes with Tm around body temperature generated a prominent hypoglycemic response and ~11-fold higher absorption than orally administered free insulin in

Significance

Lipid vesicles such as liposomes are widely present in biological systems and drug delivery applications. Numerous studies have focused on their roles in intercellular communication, signaling, and trafficking. Little is known, however, about the correlation between temperature and transport rate of these vesicles in biological media. Here, we report a temperature- and rigidity-mediated rapid transport mechanism by which liposomes attain optimal diffusivity near a phase transition temperature. Remarkably, liposomes with phase transition temperature around the body temperature are observed to overcome multiple biological barriers and show substantial improvement in drug delivery efficacy.


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diabetic rats. Using molecular simulations, atomic force microscopy (AFM), and stimulated emission depletion (STED) microscopy, we found that liposomes with $T_m$ around the ambient temperature adopt ellipsoid shapes that facilitate a rapid diffusion mechanism in hydrogels. Above $T_m$, the deformable liposomes increasingly conform to the polymeric network in the hydrogel, resulting in increased affinity and reduced diffusivity.

**Results**

**Diffusion of Liposomes in Hydrogel at Different Temperatures.** As reported in previous studies, the phase behaviors of the lipid membrane can be tuned by the lipid types. Briefly, the $T_m$ of the lipid membrane increases with chain length and saturation of the lipid tails (25–28). Inspired by these properties, we prepared six kinds of liposomes with different $T_m$ by changing the combination of lipids (denoted as Lip1 to Lip6 with detailed compositions shown in SI Appendix, Table S1). All of the liposomes exhibited a typical unilamellar vesicle structure with bilayer morphology and spherical shape as assessed by cryogenic transmission electron microscopy (cryo-TEM) (Fig. 1A). In addition, these liposomes all had similar hydrodynamic diameters (~200 nm) and neutral surface charges (SI Appendix, Table S1).

We next examined the $T_m$ of these liposomal formulations by differential scanning calorimetry and found that the $T_m$ ranged from −16.0 to 55.0 °C (Fig. 1B and SI Appendix, Table S1). For example, Lip1 had the lowest $T_m$ since its main component is unsaturated lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). As the average length of the hydrophobic tail of the lipids increased, which could be achieved by either increasing the percentage of lipids with long tails (e.g., Lip3, Lip4, and Lip5) or changing the component of the liposome (e.g., Lip2 and Lip6), the synthesized liposome would have a higher $T_m$. In addition, incubating in medium with a pH range from 1.2 to 7.4 revealed that all of the liposomes were stable in particle size and polydispersity index (PDI) over the investigated time duration (SI Appendix, Fig. S1).

To explore how the temperature influences the diffusion of liposomes in biological hydrogels, we first tracked the movement of liposomes in poly(ethylene oxide) (PEO) hydrogel via multiple-particle tracking at 4, 24, 32, 37, 45, and 55 °C (Fig. 2 and SI Appendix, Figs. S2–S4). Generally, the overall diffusion capacity of liposomes was hindered due to the mesh structure of the PEO hydrogel compared with that in water. At 4 °C, all of the liposomes exhibited confined particle trajectories. However, they displayed Brownian-like trajectories when the temperature rose above 24 °C (SI Appendix, Fig. S2), and the covered diffusion areas increased as the temperature increased from 24 to 55 °C. Interestingly, we observed that there is an optimal temperature for each kind of liposome, that is, liposomes have the highest diffusivity in the hydrogel when the ambient temperature is around their $T_m$. The ensemble-averaged mean-squared displacement ($<\text{MSD}>$) of each liposome increases as the temperature increases from 4 °C to $T_m$, above which the diffusivity starts to decrease (Fig. 2). The results showed that at 24, 32, 37, 45, and 55 °C, the liposome possessing the highest diffusivity is Lip2, Lip3, Lip4, Lip5, and Lip6, respectively (Fig. 2 and SI Appendix, Figs. S3 and S4). These results indicated that the ambient temperature plays a key role in the diffusion of liposomes in hydrogels.

**Liposome Movement in Rat Intestinal Mucus ex Vivo.** We then investigated the diffusion of liposomes in fresh rat intestinal mucus, a typical example of biological hydrogels (29), to explore their efficacy in oral drug delivery. Representative trajectories are shown in Fig. 3A. The liposome with the highest $T_m$ (e.g., Lip6) moved in a moderate area in mucus. The liposomes with decreased $T_m$ showed increased mobility (e.g., Lip4 and Lip5). However, the liposomes with further decreased $T_m$ showed restricted movement (e.g., Lip1, Lip2, and Lip3). Lip4 displayed the highest diffusion capacity compared with the others. We calculated the MSD on a timescale of 1 s (Fig. 3B) and found that, on average, the MSD of Lip4 is ~13.4- and 3.5-fold higher than that of Lip1 and Lip6, respectively (Fig. 3C and SI Appendix, Figs. S5 and S6).

![Fig. 1. Liposomal characterizations. (A) Cryo-TEM images of liposomes. (Scale bar, 100 nm.) (B) Differential scanning calorimetry (DSC) scan of liposomal formulations.](image-url)
also demonstrated that Lip4 has the highest diffusivity among all of the other liposomal formulations. Furthermore, to verify that the effect of $T_m$ on the diffusion of liposomes in mucus is lipid composition independent, we synthesized liposomes with a similar $T_m$ but with different lipid compositions (denoted as Lip7, Lip8, and Lip9; SI Appendix, Table S2). We confirmed that liposomes with $T_m$ around the ambient temperature (i.e., 37 °C) displayed superior mucus penetration (SI Appendix, Fig. S5). We also tracked the motion of liposomes in mucus at 4 and 45 °C and obtained results similar to those observed in the PEO hydrogel (SI Appendix, Fig. S6). Altogether, these results confirmed that ambient temperature plays a key role in the diffusion of liposomes across mucus.

In addition, we also compared the size and $T_m$ effects on the movement of liposomes. Decreasing the size of liposomes resulted in a moderate enhancement of PEGylated liposomal diffusivity (SI Appendix, Fig. S7 and Table S3). Nevertheless, when modulating the $T_m$ of liposomes from 54 to 36 °C, the MSD values gained approximately a 3.5-fold enhancement, suggesting that the diffusion of liposomes in mucus could be significantly improved by tuning the $T_m$ of liposomes.

### Liposome Diffusion Mechanisms in Biological Hydrogels

A detailed understanding of the mechanism underlying the diffusion process is critical to the design of effective delivery particles. As mentioned above, the ambient temperature could influence the phase behavior of lipids, that is, at $T_m$, the lipids undergo a transition from the gel phase to the liquid phase, which would influence the rigidity of the lipid membrane and eventually the deformability of the liposome. To confirm the relationship between $T_m$ and rigidity of the liposome at a certain temperature, an AFM-based approach was conducted to evaluate the rigidities of liposomes with different $T_m$. Because the effective Young’s modulus is a physical parameter reflecting the deformability of liposomes, we used this parameter to indirectly reflect the rigidity of the liposomes. The results showed that the Young’s moduli of the liposomes increase monotonically with $T_m$ (Fig. 4A) but decrease with the ambient temperature (SI Appendix, Fig. S8). This result indicated that liposomes with high $T_m$ are more difficult to deform than those with low $T_m$. In particular, the Young’s modulus of Lip6 is ~15.0- and 1.5-fold higher than those of Lip1 and Lip4, respectively. This result was also confirmed by the topographies of the liposomes with different $T_m$ in response to the loaded force (Fig. 4B). With the increase of the loading force, the liposome with the lowest $T_m$ deforms irregularly (Lip1), while the liposome with the highest $T_m$ maintains a spherical shape (Lip6). Liposomes with intermediate $T_m$ deform into an ellipsoidal shape (Lip4). These results indicate that the rigidity of the liposome is affected by its $T_m$, which further influences the deformability of the liposome.

To elucidate the mechanism underlying the superior hydrogel-penetrating ability of liposomes at $T_m$, we next conducted coarse-grained (CG) molecular-dynamics (MD) simulations, which could provide insights into the detailed correlation between the physical parameters of NPs (such as size and shape) and their penetration efficiency (30, 31). As discussed above, the deformability of the liposome is influenced by its $T_m$. We thus hypothesized that the diffusivity of liposomes with different $T_m$ may be affected by the deformability of the liposomes. To verify this hypothesis, we constructed a model system consisting of liposome models with tunable deformability and polymer networks (see Materials and Methods for more details). In this model, we constructed three types of liposomes with different rigidities, corresponding to the liposomes with different $T_m$ (namely Lip1 for low $T_m$, Lip4 for intermediate $T_m$, and Lip6 for high $T_m$) in the experiment. In the simulations these liposomes were put into cubic boxes comprising cross-linked polymers and water. The simulations were conducted at three different temperatures, for example, $T^* = 0.15, 0.21, 0.24e$, where $e$ is the unit of energy and related to the temperature through $T^* = e/k_B$. We found that the movement and diffusion of the liposomes increase with the simulation temperature, especially when the temperature increases from $T^* = 0.15e$ to $T^* = 0.21e$; the traveling trajectories of the liposomes at high temperature ($T^* = 0.21e$) cover more area than those at low temperature ($T^* = 0.15e$) (SI Appendix, Figs. S11–S13). We repeated each simulation five times with different starting configurations and obtained MSD values for the three types of liposomes at different simulation temperatures (Fig. 5 A–C). At low temperature ($T^* = 0.15e$), the simulation results showed that soft liposomes (lowest $T_m$, Lip1) has higher MSD value than the semisoft (intermediate $T_m$, Lip4) and the hard (highest $T_m$, Lip6) liposomes (Fig. 5A). However, when the simulation temperature increases to $T^* = 0.24e$, Lip6 has the largest MSD value (Fig. 5C). At intermediate temperature ($T^* = 0.21e$), the semisoft liposome has higher MSD than the soft and hard liposomes.

**Fig. 3.** Mobility of liposomes in fresh rat intestinal mucus at 37 °C. (A) Representative trajectories of particle motion in 1 s. (B) MSD values as a function of time. (C) Typical MSD values for liposomes at 1 s. (D) Distribution of the logarithms of individual particle effective diffusivities ($D_{eff}$) on a timescale of 1 s. The data represent three independent experiments, and each experiment tracks 100 particles. The data are shown as the means ± SD ($n = 3$). **$P < 0.01$; ****$P < 0.0001$.

**Table S1.** The distribution of effective diffusivities ($D_{eff}$) shown in Fig. 3D also demonstrated that Lip4 has the highest diffusivity among all of the other liposomal formulations.

**Fig. 4.** (A) The Young’s moduli of the liposomes characterized by AFM. The data represent three independent experiments. (B) AFM images of liposomes with different $T_m$ and the corresponding deformation images of the liposomes subjected to forces of different magnitudes. (Scale bar, 200 nm.)
the hard liposomes (Fig. 5B). These simulation results are consistent with our experimental results. The calculated diffusivity of the NPs showed that, at relatively low temperature, the liposomes are confined and hard to diffuse (Fig. 5D). As the temperature increases, the diffusion is activated and the diffusivity of semisoft liposome is ∼1.8- and 2.7-fold higher than the hard and the soft liposomes, respectively (Fig. 5E). When the temperature further increases to \( T^* = 0.24\epsilon \), the diffusivity of hard liposome is ∼1.7- and 3.3-fold higher than those of the semisoft and soft liposomes, respectively (Fig. 5F). The results confirmed that liposome has the fastest diffusion when the ambient temperature is around its \( T_m \).

To explain why a liposome exhibits the fastest diffusion when the ambient temperature is around its \( T_m \), we fixed the simulation temperature at \( T^* = 0.21\epsilon \) and examined the liposome/polymer interaction during the diffusion process. In the simulations, we used the parameter contact number to represent the interacting frequency of liposomes in a polymer network, which in turn reflects their interaction strength. When the distance between one polymer bead and one liposome bead is less than a cutoff \( r_c \), we say that the liposome has made one contact with the polymer network. A high contact number corresponds to a strong attraction between the liposome and the polymer. Fig. 6A shows that the contact number of soft liposome is three times higher than that of the semisoft and hard liposomes, which means that the soft liposomes have a much stronger attraction for the polymer than the semisoft and hard liposomes. Interestingly, the contact number of the semisoft liposome is similar to that of the hard liposome on average but with higher local fluctuations. The different diffusivities between the semisoft and hard liposomes may be attributed to these fluctuations. We then calculated the major axial length of the liposome, which relates to the deformation of the liposome (Fig. 6B and SI Appendix, Fig. S14). The results showed that, during the diffusion process, the major axial length of the soft and hard liposomes remains constant, while that for semisoft liposomes fluctuates around its initial radius. In addition, the major axial length of the soft liposome is much higher than its initial value (∼30β), while that of the hard liposome is equal to its initial radius. These results confirmed that the shape of the soft liposome changes dramatically, while the hard liposome remains a sphere during diffusion in the hydrogel. The semisoft changes its shape frequently during the diffusion process.

Therefore, we concluded that the soft liposome (with low \( T_m \)) is apt to undergo large deformation, which increases its contact area with the hydrogel network, resulting in increased affinity and decreased diffusivity. Snapshots from the simulation showed that the soft liposome changes its shape to conform to the corner of the polymer network of the hydrogel, maintaining a stable state in the simulation (Fig. 7A and Movie S1). The hard liposome (with high \( T_m \)) tends to be trapped in the corner of the hydrogel because of the high attraction density in that area, and these molecules oscillate around the corner without shape change (Fig. 7A and Movie S2). The semisoft liposome (with intermediate \( T_m \)) will first attract to one corner of the network, and then the liposome shape will deform into an ellipsoid due to its attraction to another corner of the network. Due to the competition between the attractions from two corners, as well as the elastic deformation energy of the liposome, the semisoft liposome has the chance to move from one corner to another. Through this process, the semisoft liposomes exhibit superior diffusivity compared with that of the soft and hard liposomes (Fig. 7A and Movie S3).

We also applied STED microscopy, a type of superresolution optical microscopy, to identify and characterize the diffusion mechanisms of liposomes with low, intermediate, and high \( T_m \) in rat intestinal mucus. As shown in Fig. 7B and Movies S1–S6, the three liposomes undergo different deformation patterns and thus display different diffusive capacities. Lip6 remains spherical in shape and exhibits moderate displacement. Lip1 shows an amorphous shape at different time points and has restricted mucus diffusion. The Lip4 deforms into an ellipsoid and displays rotational motion in the mucus.

**Cellular Uptake of Liposomes.** We then selected liposomes with low, intermediate, and high \( T_m \), that is, Lip1, Lip4, and Lip6, respectively, for in vitro evaluations. E12 cells were used to mimic the mucosal tissues, which comprise the secreted mucus layer and absorptive epithelial cells (32). As shown in Fig. 8A, Lip4 exhibited significantly higher cellular internalization than...
Lip1 and Lip6. We hypothesized that because Lip4 diffuses faster than Lip1 and Lip6 in mucus, more Lip4 reaches the vicinity of the cell surface, leading to superior E12 uptake. We also removed the secreted mucus layer using N-acetylcysteine (NAC) for comparison. After removing the secreted mucus layer, Lip6 entered the cells more efficiently than Lip1 and Lip4 (Fig. 8A). These results were further corroborated using Caco-2 cells, which do not produce mucus (Fig. 8B). Finally, we visualized liposome mucus penetration and cellular internalization in E12 cell monolayers using confocal laser-scanning microscopy (CLSM), followed by 3D image reconstruction (Fig. 8C). As expected, more Lip4 was observed in both the mucus and cell layers compared with Lip1 and Lip6, indicating the superior diffusion capacity through the mucus and higher cellular uptake of Lip4.

Absorption of Liposomes Across Intestinal Villi ex Vivo and in Vivo.

Next, to evaluate the significance of our results in tissues, we analyzed the distribution of Lip1, Lip4, and Lip6 in rat small intestine loops ex vivo by 2D and 3D scanning. All of the liposomal formulations displayed considerable mucus penetration, probably because these molecules were all modified with PEG, which enhances the diffusion of particles in mucus (33, 34). We confirmed that the diffusion of liposomes in hydrogels with or high ambient temperatures. The underlying mechanism for the observed optimal diffusivity around T_m is the deformation of liposomes. When the temperature is near their T_m, the liposomes deform into an ellipsoidal shape frequently, and this deformation facilitates their rapid diffusion.

Use of Liposomes for Oral Delivery of Insulin.

Finally, we investigated the drug delivery capability of these liposomal formulations in vivo. Liposome formulations (Lip1, Lip4, and Lip6) were used to orally deliver insulin to diabetic rats. The oral delivery of insulin remains the “Holy Grail” of diabetes management because of the very limited oral bioavailability of insulin (<1%) caused by its low permeability across the intestinal mucosa and rapid degradation in the GIT (35). As shown in Fig. 9E, the oral administration of free insulin solution failed to reduce the blood glucose levels in rats, whereas all of the liposomal formulations generated significant hypoglycemic responses. The administration of Lip4 exhibited a remarkable hypoglycemic response with a maximal glucose level reduction of ∼50%, which persisted for more than 8 h. Lip1 and Lip6 reduced the blood glucose level to a lesser extent. The serum insulin levels achieved by the different liposomal formulations compared with those achieved by the s.c. injection of free insulin are shown in Fig. 9F and Table 1. The s.c. injection of free insulin solution resulted in a rapid increase in the serum insulin concentration with a maximum at 1 h postinjection. The oral administration of liposomal formulations resulted in a slow rise in the serum insulin concentration, which reached the maximum value at 4 h. However, compared with Lip1 and Lip6, a significantly higher serum insulin concentration was obtained with Lip4, and the area under the curve (AUC) for Lip4 was ∼172.02 μIU·h/mL with a relative bioavailability (F%) of 13.65%, indicating a more efficient delivery of insulin with Lip4.

Discussion

Temperature is an important factor in determining the rate of diffusion. According to the Stokes–Einstein equation, the rate of Brownian motion of the particles increases with temperature. Here, we experimentally and theoretically demonstrated that temperature also governed the biological hydrogel penetration efficacy of liposomes with different phase transition temperatures T_m. Combining experiments and computer simulations, we confirmed that the diffusion of liposomes in hydrogels with temperatures around T_m was superior compared with that at low or high ambient temperatures. The underlying mechanism for the observed optimal diffusivity around T_m during hydrogel penetration is the deformation of liposomes. When the temperature is near their T_m, the liposomes deform into an ellipsoidal shape frequently, and this deformation facilitates their rapid diffusion.
Mechanistic studies revealed that liposomes with different $T_m$ gain different rigidities and further transform into various shapes, which results in different diffusion capacities. These findings provide insight into the role of ambient temperature on the transportation of liposomes. Our results might help in the development of much-needed strategies to improve the efficacy of liposome-based drug delivery systems.

### Materials and Methods

**Materials.** 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) were purchased from Lipoid. 1-Palmitoyl-2-earayoy-sn-glycero-3-phosphocholine (HSPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleodacanoyl-rac-glycero-3-phosphocholine (DPPC), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)]-2000 (DSPE-PEG$_{2000}$) were purchased from AVT Corporation. 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine-[methoxy(polyethylene glycol)-2000] (DOPE-PEG$_{2000}$) was purchased from Avanti. Hydroxyamphotericin (HCP) was purchased from J&K Chemical. Alexa Fluor 488-conjugated wheat germ agglutinin (WGA) was obtained from Sigma-Aldrich. Chloroform and glycine were purchased from the Sinopharm Chemical Reagent Company.

**Male Sprague-Dawley rats (275 ± 25 g) were obtained from the Animal Experiments Center of the Shanghai Institute of Materia Medica (Shanghai, China). The animals had free access to rat chow and tap water ad libitum. Diabetes was induced in male Sprague-Dawley rats, and the rats were injected with streptozotocin (65 mg/kg) dissolved in a 10 mM citrate buffer (pH 4.5), as previously described. A glucose meter (On Call EZ; Acon Bio-technology) was used to determine the blood glucose level. Rats were regarded as diabetic when the glycemia level was higher than 300 mg/dL at 1 wk after injection. All animal procedures performed in this study were evaluated and approved by the Animal Ethic’s Committee of Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Institutional Animal Care and Use Committee certification number 2016-05-GY-23).

**HT29-MTX-E12 (E12) cells (52nd to 56th passages) cultured for 14–18 d were supplied by the ADME Department of Novo Nordisk. The human colon adenocarcinoma cell lines (Caco-2) were obtained from the American Type Culture Collection.**

**Liposome Preparation.** Liposomes were prepared using a thin-film evaporation method (42). Briefly, the lipid mixtures were dissolved in a mixture of chloroform and methanol at the ratio mentioned in SI Appendix, Table S1 (43). The mixtures were evaporated to dryness in a rotary evaporator and then hydrated with deionized water/saline solution (2 mL) for 30 min using a vortex. The hydration temperatures of the liposomes are listed in SI Appendix, Table S1. Finally, the liposomal preparations were extruded through 400-, 200-, and 100-nm polycarbonate membranes.

**Characterization of Formulation.** The size, size distribution (PDI), and zeta potentials of the prepared liposomes were measured using dynamic light scattering (Nano ZS). AFM images of the lipid bilayers and force measurements using a Bio-Fast Scan scanning probe microscope (Bruker) were obtained in the Peak Force QNM imaging mode. The liposomal suspensions were placed onto a cleaned freshly cleaved mica surface, air-dried at room temperature, and transferred to an 85% humid chamber for 1 h. The samples were imaged with a scan rate of 1 Hz. A cantilever with a deflection sensitivity of 99 nm V$^{-1}$ and a tip with a spring constant of 0.16 N m$^{-1}$ were applied. All images and the Young’s modulus of each liposomal preparation were processed using Nanoscope Analysis software (Bruker).

**Table 1. Pharmacokinetic parameters of insulin in diabetic rats**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dose, IU/kg</th>
<th>AUC, μIU·h/mL</th>
<th>F, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin sol (s.c.)</td>
<td>5</td>
<td>210.30 ± 27.29</td>
<td>100</td>
</tr>
<tr>
<td>Lip1</td>
<td>30</td>
<td>15.54 ± 10.63</td>
<td>1.23</td>
</tr>
<tr>
<td>Lip2</td>
<td>30</td>
<td>75.92 ± 38.09</td>
<td>5.95</td>
</tr>
<tr>
<td>Lip4</td>
<td>30</td>
<td>172.04 ± 13.19</td>
<td>13.65</td>
</tr>
<tr>
<td>Lip6</td>
<td>30</td>
<td>122.18 ± 5.59</td>
<td>9.68</td>
</tr>
</tbody>
</table>

AUC, Area under the curve; F, relative bioavailability.
Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) was performed to measure the $T_m$ of various liposomal preparations. Ten microliters of liposomal preparations were placed on aluminum pans. The pans were then hermetically sealed, followed by heating at a rate of 5 °C min$^{-1}$. The scans were recorded at temperatures ranging from −20 to 70 °C.

Cryo-TEM. Cryo-TEM using a Tecnai T12 electron microscope was adopted to visualize the structures of the liposomes. A drop of liposomal suspension was placed onto a carbon-coated copper grid and blotted. The samples were then shock-frozen by rapid immersion into liquid ethane, followed by cooling to 90 K in liquid nitrogen. The specimens were transferred to a Tecnai T12 electron microscope and analyzed at 200 kV.

Stability of Liposomal Preparations. In vitro stability of liposomal preparations was measured by monitoring the particle size and PDI in different biorelevant media at 37 °C for 120 min using a constant temperature shaker. The biorelevant media included simulated gastric fluid and simulated intestinal fluid. All media were prepared as described previously (44).

Multiple-Particle Tracking. For mucus collection, we have adopted a method by the Hanes group (45–48). Briefly, the small intestine was excised after killing the rats, and ~1.5–2.0 mL of mucus from each fastest rat was collected. The average pore size of the mucus is ~200 nm, as revealed by scanning electron microscope in our previous work (49). Ten rats were killed to collect mucus for the multiparticle-tracking studies. Liposome formulations (50 μL/g, 5 μL) were added to fresh rat mucus (100 μL) and equilibrated for 30 min at 37 °C before microscopy analysis. Movies were made at a temporal resolution of 32.6 ms for 10 s using an inverted fluorescence microscope (DMI 4000B; Leica). The tracking resolution was ~10 nm, determined by gluing microspheres onto microslides and tracking their apparent displacement. The trajectories of the particles ($n = 100$) were analyzed using ImageJ for each experiment, and three independent experiments were performed. The time-averaged MSD and effective diffusivities ($D_{eff}$) were calculated using the following equations:

$$\text{MSD}_{\text{t}} = (x_t - x_0)^2 + (y_t - y_0)^2 + (z_t - z_0)^2,$$  

$$D_{eff} = \frac{\text{MSD}}{4\tau}$$

where $x$ and $y$ represent the coordinates of the particle and $\tau$ is timescale or time lag.

Deformation of Liposomes. To observe the 3D deformation of liposomes during their diffusion in mucus, images and movies (10 s) were acquired by STEM microscopy using a gated STEM microscope (Leica TCS SP8 STED 3X; Leica Microsystems) equipped with an HCC PL APO 100x, 1.40 numerical aperture oil objective. The images of liposomes were acquired using 530-nm excitation and 565-nm emission. All images were obtained using LAS X software (Leica Microsystems). Image processing was performed using Huygens Professional software (Scientific Volume Imaging). All movies were obtained using Imaris software (Bitplane).

Mucus Penetration on E12 Cells. To assess the interactions between liposomes and the mucus layer, E12 cells were grown on Transwell filter inserts (Corning) for 14–17 d. Next, 400 μL of Dil-loaded liposomes diluted in PBS was added on the apical side for 60 min. The E12 monolayers were then washed three times with PBS, and the mucus layer was stained with Alexa Fluor 488–labeled WGA (Alexa Fluor 488-WGA; 10 mg/mL) for 10 min at 37 °C. The membranes supporting the cell layers were washed with PBS, and the E12 cells were stained with Hoechst 33342 (1 μg/mL) for 30 min at 37 °C. The cell layers supporting the membranes of the Transwell inserts were cut from the plastic support without fixation, mounted onto microscope slides, and covered with coverslips. The slides were immediately observed under a confocal microscope (LSCM, FV1000; Olympus). Image visualization and processing were performed using LSM 5 Pa software. To observe the interactions between the different formulations with mucus in a larger view, a 2D image in the middle of the mucus was taken under a confocal microscope using an 63x oil objective lens.

Cellular Uptake of Liposomes. E12 and Caco-2 cells were seeded onto 12-well plates at a density of 1 x 10⁵ cells per well. The plates were incubated for 3 d under 5% CO₂, 85% humidity at 37 °C. The E12 monolayers were washed three times with HBSS and then incubated for 1 h with liposomes (400 μg of total lipid/mL) in 500 μL of culture medium. Following incubation, the cell monolayers were washed again with HBSS and then treated with radioimmunoprecipitation assay (RIPA) lysis buffer for 30 min and suspended in HBSS buffer, followed by centrifugation at 4,000 x g for 10 min. Aliquots (200 μL) of the supernatant were added to 130 μL of 2x SDS-PAGE loading buffer. Additional samples were collected for further analysis by flow cytometry or to measure the protein concentration using the bicinchoninic acid protein assay kit.

Liposome Distribution in the Rat Small Intestine. Sprague-Dawley rats were fasted but were allowed free access to water for 12 h before the experiments. To investigate the intestinal distribution of liposomes, the rats were anesthetized with 20% urethane solution and the ileum was exposed by a small incision in the abdomen. A 2-cm region was tied off using surgical sutures, and 400 μL of liposomal preparation was injected into the loop (49). After incubation for 1.5 h, the intestine was cut and fixed in 4% paraformaldehyde for 3 h and then transferred into 30% sucrose solution for dehydration overnight. The tissue sections were frozen in optimum cutting temperature compound (OCT), sliced at a depth of 20 μm, stained with DAPI, and embedded in a PBS/glycerol (1:9) mixture for imaging.

In Vivo Pharmacodynamic and Pharmacokinetic Studies. Diabetic rats were fasted overnight before experiments but were allowed free access to water. The following formulations were administered to the rats orally: free-form insulin (30 IU kg$^{-1}$ body weight) and insulin-containing liposomal formulations (equivalent to 30 IU insulin kg$^{-1}$ body weight). Control rats received s.c. injections of saline solution. Insulin serum concentration was determined using an ELISA kit (R&D Systems). The area under the serum insulin concentration vs. time curve (AUC) was calculated for each group. The relative bioavailability (%) of the test liposomes after oral administration was calculated using the following equation:

$$F% = \frac{AUC_{(oral)}}{AUC_{(s.c.)}} \times 100%.$$
(NVT) at temperatures $k_B T = 0.15$, 0.21, and 0.24, where $k_B$ is the Boltzmann constant, and $T$ is the temperature. The total simulation time was 2.8 $\times 10^8$ steps, after approximately 0.8 $\times 10^8$; MSD calculations were performed. The fitting of diffusivity $D$ was performed by linearly fitting the MSD versus time lag from $1.3 \times 10^5$ to $1.8 \times 10^5$. The slope of the fitting line was denoted by $k$; thus, $D = k/t$. To verify the robustness of our simulation model, we tuned the pore size of the network from 42 to 35 and 50 in the simulations, with results summarized in SI Appendix.

Statistical Analysis. All of the data are reported as the means ± SD. Significance differences were analyzed using Student’s t test when two groups were compared or one-way ANOVA with Tukey’s post hoc test when multiple groups were compared ($P > 0.05$; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$).

ACKNOWLEDGMENTS. We thank the National Center for Protein Science Shanghai for the cryo-EM, and we are grateful to Zhi Li for assistance with preparing the EM samples. We also thank the Leica Microscopy Laboratory (Shanghai) for the light microscopy data, and we are grateful to Leica (China) for assistance in obtaining data using the SPB STED system. The AFM experiments were supported by Huimin Li from the Instrumental Analysis Center of Shanghai Jiao Tong University. We are grateful for financial support from the National Natural Science Foundation of China [81573378 and 81773851 (to Y. G.)]; 11224211, 11272327, and 11672079 (to X.J.S.), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA10120304), and Shanghai Sailing Program 2017 (17YF1425300). This study is also partly supported by CASMMO12153020, the K. C. Wong Education Foundation, the Opening Fund of State Key Laboratory of Nonlinear Mechanics, and the New Star Program, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The computation experiment was mainly supported by the Supercomputing Center of Chinese Academy of Sciences.
Supplementary Information for

Temperature and rigidity mediated rapid transport of lipid nanovesicles in hydrogels

Miaorong Yu, Wenyi Song, Falin Tian, Zhuo Dai, Quanlei Zhu, Ejaj Ahmad, Shiyan Guo, Chunliu Zhu, Haijun Zhong, Yongchun Yuan, Tao Zhang, Xin Yi, Xinghua Shi, Yong Gan, Huajian Gao

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This PDF file includes:

Figs. S1 to S15
Tables S1 to S5
Captions for movies S1 to S6
References for SI reference citations

Other supplementary materials for this manuscript include the following:

Movies S1 to S6
Fig. S1. Stability of the liposomes. Size (A) and polydispersity (PDI) (B) of the liposomes after 6 h in different media at 37 °C. Data are shown as the means ± SD with n=3.

Fig. S2. Representative trajectories of liposomal motion in PEO hydrogels in 1 s at different temperatures.
**Fig. S3.** Distribution of the logarithms of individual particle effective diffusivities ($D_{eff}$) on a time scale of 1 s in PEO hydrogels at different temperatures.

**Fig. S4.** Mobility of liposomes in PEO hydrogel at different temperatures. Typical MSD values for liposomes in 1 s at (A) 4 °C, (B) 24 °C, (C) 32 °C, (D) 37 °C, (E) 45 °C and (F) 55 °C, respectively. The data represent three independent experiments, and each
experiment tracks 100 particles. The data are shown as the means ±SD (n = 3). *P < 0.05, ****P < 0.0001.

Fig. S5. Characterizations of the diffusivity of liposomes with different compositions in rat intestinal mucus. (A) Representative trajectories and (B) MSD of the logarithms of effective diffusivities \(D_{eff}\) of liposome motion in 1 s at 37 °C. The data represent three independent experiments, and each experiment tracks 100 particles.

Fig. S6. The diffusivity of liposomes in rat intestinal mucus at 4 and 45 °C. MSD of the logarithms of effective diffusivities \(D_{eff}\) of liposome motion in 1 s at 4 and 45 °C. The data represent three independent experiments, and each experiment tracks 100 particles.
Fig. S7. Transport of Lip6 with different sizes (80, 200, 300, 400 and 500 nm) in fresh rat intestinal mucus at 37 °C. (A) Representative trajectories of particle motion in 1 s. (B) MSD values as a function of time. (C) Distribution of the logarithms of individual particle effective diffusivities ($D_{eff}$) on a time scale of 1 s. The data represent three independent experiments, and each experiment tracks 100 particles.

Fig. S8. The Young’s moduli of Lip4 characterized by AFM at different temperatures. The data represent three independent experiments.

**Simulation model and method.** Similar to our previous study, a regular polymer network was utilized to represent mucus fibers with a mesh size of $42\sigma$, as shown in Fig. S9. Each fiber comprised a series of beads spanning the entire simulation box ($168 \times 168 \times 168\sigma^3$). Different fibers were cross-linked by a node bead to simulate the entanglement and crosslinking of mucin fibers. The bonded interaction energy
between neighboured beads $i$ and $j$ in the polymer chain was described by a simple harmonic spring with a spring constant of $k_b = 23 \varepsilon/\sigma^2$ and an equilibrium bond length of $r_0 = 2.0\sigma$

$$E_{\text{bond}} = \frac{1}{2}k_b(r - r_0)^2 \quad (S1)$$

The energy constraining the bond angle was described by an equilibrium angle with a bending constant of $k_a = 4.6\varepsilon$ and an equilibrium bond angle of $\theta_0 = \pi$

$$E_{\text{angle}} = \frac{1}{2}k_a(\theta - \theta_0)^2 \quad (S2)$$

During the simulations, polymer node beads were constrained by applying a spring to tether them to their initial positions. The spring constant was set to $k_{\text{setf}} = 4.6\varepsilon/\sigma^2$.

The Lennard-Jones (LJ) potential was used as follows to describe the nonbonded interactions $V(r_{ij})$ between two beads

$$V(r_{ij}) = 4\varepsilon_{ij}\left[\left(\frac{b}{r_{ij}}\right)^{12} - \left(\frac{b}{r_{ij}}\right)^6\right] \quad r_{ij} < r_c \quad (S3)$$

where $\varepsilon_{ij}$ is the depth of the energy well, $b$ is the equilibrium length between two beads, and $r_c$ is the cut-off distance. The interaction parameters are listed in Table S4.

The polymer network was constructed using 3904 beads, 4032 bonds and 4032 angles.

The water molecule was represented by one CG bead. In the simulation, each liposome contained 796 water beads in the inner liposome, and the other 212,863 water beads were used to fill the simulation box.

The liposome with a size of $42\sigma$ was modeled using the one-particle-thick model, which captures liposome elastic properties, to allow the stiffness tuning of the liposomes. Each type of liposome comprised 3155 beads. Following the notation from the original paper, the interparticle interaction between each pair of liposome beads was described by a combination of two functions, $u(r)$ and $\phi(\hat{r}_{ij}, \hat{n}_i, \hat{n}_j)$, which represent the distance and orientation dependences, respectively, as follows:

$$U(r_{ij}, \hat{n}_i, \hat{n}_j) = \begin{cases} u_A(r) + \varepsilon_{\text{beads-beads}}[1 - \phi(\hat{r}_{ij}, \hat{n}_i, \hat{n}_j)] & r < r_{\text{min}} \\ u_A(r)\phi(\hat{r}_{ij}, \hat{n}_i, \hat{n}_j) & r_{\text{min}} < r < r_c \end{cases} \quad (S4)$$

Where
\[ u_A(r) = -\varepsilon_{\text{beads-beads}} \cos^2 \left( \frac{\pi}{2} \frac{r - r_{\text{min}}}{r_c - r_{\text{min}}} \right) \text{ for } r_{\text{min}} < r < r_c \]  

(S5)

\[ u_R(r) = \varepsilon_{\text{beads-beads}} \left( \frac{r_{\text{min}}}{r} \right)^4 - 2 \left( \frac{r_{\text{min}}}{r} \right)^2 \text{ for } r < r_{\text{min}} \]  

(S6)

\[ \phi(\mathbf{r}_{ij}, \mathbf{n}_i, \mathbf{n}_j) = 1 + \mu (a(\mathbf{r}_{ij}, \mathbf{n}_i, \mathbf{n}_j) - 1) \]  

(S7)

\[ a(\mathbf{r}_{ij}, \mathbf{n}_i, \mathbf{n}_j) = (\mathbf{n}_i \times \mathbf{r}_{ij}) \cdot (\mathbf{n}_j \times \mathbf{r}_{ij}) + \sin \theta_0 (\mathbf{n}_i - \mathbf{n}_j) \cdot \mathbf{r}_{ij} - \sin^2 \theta_0 \]  

(S8)

In these equations, \( \mathbf{r}_i \) and \( \mathbf{r}_j \) represent the center position vectors of beads \( i \) and \( j \), \( \mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j \), \( r = ||\mathbf{r}_{ij}|| \) and \( \hat{\mathbf{r}}_{ij} = \mathbf{r}_{ij} / r \). The unit vectors \( \mathbf{n}_i \) and \( \mathbf{n}_j \) represent the axes of symmetry of beads \( i \) and \( j \), respectively. The exponent \( \zeta \) tuned the slope of the attraction between two beads. The parameters \( \theta_0 \) and \( \mu \) were related to the spontaneous curvature and bending rigidity of the liposomes. In the simulations, we chose the same parameters \( \varepsilon_{\text{beads-beads}} = 1 \varepsilon, \zeta = 4 \) and \( r_c = 2.6\sigma \) as used in the original paper. The other two parameters (\( \mu, \sin \theta_0 \)) were used to modify the bending rigidity of different types of liposomes and are listed in Table S5. The interaction potential for the liposome-polymer network and liposome-liposome interactions was also described by the LJ potential. The interaction between different liposomes is zero, and the interaction parameters are listed in Table S4.

Fig. S9. The coarse-grained model for the diffusion of liposomes in our simulations and a typical snapshot of the simulation system. The regular hydrogel network was utilized to represent mucus fibers with a mesh size of 42\( \sigma \). Liposomes are modeled using the one-particle-thick model with an initial diameter of 30\( \sigma \).
**Simulation temperature and liposome rigidity.** In our coarse-grained simulations, different simulation temperatures and liposomes with various $T_m$ were chosen to investigate the effect of $T_m$ of liposome on their diffusivity. As reported by the original paper (1), the fluidity of the vesicle varied with the parameter $\zeta$ and temperature (Fig. S10A), which will change from the gel to fluid phase when the temperature increases with a fixed $\zeta$. In other words, by changing the simulation temperature, different phase of liposome can be obtained. In this model, the bending rigidity of the vesicle increased with $\mu$ monotonically (Fig. S10B). In addition, the spontaneous curvature per bead can be implemented via the parameter $\theta_0$. Therefore, we can obtain liposomes with different fluidities via tuning the simulation temperature, $\mu$ and $\theta_0$ at fixed $\zeta = 4$. For example, from Fig. S10a we can see that the gel-fluid transition temperature is $k_B T = 0.15\epsilon$ for $\mu = 3$ and $\theta_0 = 0$. Thus, we chose the parameters $\mu = 3$ and $\theta_0 = 0$ for the liposome with lowest $T_m$. Similarly, the parameters for the other two types of liposomes and simulation temperatures were selected based on the same strategy.

![Fig. S10. The phase-diagram in the ($\zeta, T$) plane (A) and membrane bending rigidity varies with $\mu$ of the coarse-grained vesicle models at zero tension (B).](image)

(A) Three regions representing gel (black circle), fluid (red square), and gas (blue triangle) phases are identified, separated by solid lines. The green stars represent three simulation temperature in the simulation. (B) Membrane bending rigidity monotonically increases with $\mu$. The parameters used are $\zeta = 4$, $\theta_0 = 0$, $k_B T = 0.23\epsilon$. 
Fig. S11. The typical 3D centroid trajectories of liposomal in simulation (A) and their corresponding projection in different 2D plane (B) at $T^*=0.15\varepsilon$. From left to right are projections of centroid trajectories in XY-plane, XZ-plane, YZ-plane in (B). The blue, red, and green labels correspond to liposomes with low $T_m$, intermediate $T_m$, and high $T_m$, respectively. Low $T_m$: Lip1; Intermediate $T_m$: Lip4; High $T_m$: Lip6.

Fig. S12. The typical 3D centroid trajectories of liposomal in simulation (A) and their corresponding projection in different 2D plane (B) at $T^*=0.21\varepsilon$. From left to right are projections of centroid trajectories in XY-plane, XZ-plane, YZ-plane in (B). The blue, red, and green labels correspond to liposomes with low $T_m$, intermediate $T_m$, 

and high $T_m$, respectively. Low $T_m$: Lip1; Intermediate $T_m$: Lip4; High $T_m$: Lip6.

Fig. S13. The typical 3D centroid trajectories of liposomal in simulation (A) and their corresponding projection in different 2D plane (B) at $T^*=0.24\varepsilon$. From left to right are projections of centroid trajectories in XY-plane, XZ-plane, YZ-plane in (B). The blue, red, and green labels correspond to liposomes with low $T_m$, intermediate $T_m$, and high $T_m$, respectively. Low $T_m$: Lip1; Intermediate $T_m$: Lip4; High $T_m$: Lip6.

Fig. S14. Distribution of the fluctuation in length of the major axis of liposomes around the time averaged value $R_0$. The blue, red, and green labels correspond to
liposomes with low $T_m$, intermediate $T_m$, and high $T_m$, respectively. Low $T_m$: Lip1; Intermediate $T_m$: Lip4; High $T_m$: Lip6. The simulation temperature is taken as $T^* = 0.21 \varepsilon$.

**Effect of the pore size of the polymer network**

It has been reported that the mesh size of mucus varies in a wide range of 10-1000 nm. In our experiment, we found that the average pore size of the mucus is about 100-240 nm, and the hydrodynamic diameter of the liposomes has a comparable value of about 200 nm. To mimic these experimental size scales, we set the ratio of the liposome size and network mesh pore size to be 30:42. In addition, we have studied a system with a decreased pore size of $35 \sigma$, in which case almost all the liposomes were trapped in the network within one grid cell (Fig. S15A-B). We also increased the pore size to $50 \sigma$, and observed an increase in diffusivity due to loosened restrictions of the mucus network compared to those with a smaller pore size ($35 \sigma$) (Fig. S15C-D). Similarly, the liposome diffused faster at $T^* = 0.21 \varepsilon$ than those at $T^* = 0.15 \varepsilon$ and $T^* = 0.24 \varepsilon$ (Fig. S15C-D).
Fig. S15. Effect of mucus pore size on the diffusion of liposomes. The (A & C) MSDs and (B & D) diffusivities of liposomes when the pore size of the mucus network is tuned from $42\sigma$ to $35\sigma$ or $50\sigma$. The solid sphere line, semi-solid pentagon line, and hollow triangle line in (A) and (C) correspond to the simulation temperatures of $T^* = 0.15\varepsilon$, $T^* = 0.21\varepsilon$, and $T^* = 0.24\varepsilon$, respectively. During the simulations, the parameters of the liposomes are taken as: $\mu = 5$ and $sin\theta = 0.002$, corresponding to the liposome with intermediate $T_m$. 
### Tables

**Table S1. Characterization of the liposomes and their diffusivities in rat intestinal mucus**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition</th>
<th>Hydrodynamic diameter (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>Diffusivity (µm²/s)</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip1</td>
<td>DOPE:DOPE-PEG₂₀₀₀ 95%: 5%</td>
<td>184.0 ± 9.8</td>
<td>0.147</td>
<td>-2.9 ± 2.3</td>
<td>0.126</td>
<td>-16.0</td>
</tr>
<tr>
<td>Lip2</td>
<td>DMPC:DSPE-PEG₂₀₀₀ 95%: 5%</td>
<td>203.6 ± 12.2</td>
<td>0.274</td>
<td>-4.3 ± 1.9</td>
<td>0.197</td>
<td>23.4</td>
</tr>
<tr>
<td>Lip3</td>
<td>PEG₂₀₀₀ 40%: 55%: 5%</td>
<td>199.5 ± 15.3</td>
<td>0.122</td>
<td>-3.9 ± 2.9</td>
<td>0.433</td>
<td>31.7</td>
</tr>
<tr>
<td>Lip4</td>
<td>PEG₂₀₀₀ 20%: 75%: 5%</td>
<td>216.5 ± 11.4</td>
<td>0.060</td>
<td>-4.7 ± 3.6</td>
<td>1.689</td>
<td>36.1</td>
</tr>
<tr>
<td>Lip5</td>
<td>DPPC:DSPE-PEG₂₀₀₀ 95%: 5%</td>
<td>196.8 ± 8.8</td>
<td>0.134</td>
<td>-5.8 ± 3.1</td>
<td>0.683</td>
<td>44.4</td>
</tr>
<tr>
<td>Lip6</td>
<td>DSPC:DSPE-PEG₂₀₀₀ 95%: 5%</td>
<td>193.9 ± 13.0</td>
<td>0.166</td>
<td>-2.9 ± 2.9</td>
<td>0.478</td>
<td>54.5</td>
</tr>
</tbody>
</table>

Notes: DOPE is an unsaturated lipid that has a double bond in the hydrophobic chain. The hydrophobic length of the DMPC, DPPC, and DSPC is 14, 16 and 18 carbons, respectively.
Table S2. Characterization of liposomes with different compositions and their diffusivities in rat intestinal mucus

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition</th>
<th>Hydrodynamic diameter (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>Diffusivity (µm²/s)</th>
<th>Tm (°C)</th>
<th>Young’s modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lip7</td>
<td>PC-98T:DOPE-PEG2000 95%: 5%</td>
<td>191.9 ± 12.8</td>
<td>0.156</td>
<td>-2.9</td>
<td>0.156</td>
<td>-17.8</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Lip8</td>
<td>DMPC:DPPC:DSP C:DSPE-PEG2000 20%: 75%: 5%</td>
<td>203.6 ± 11.4</td>
<td>0.214</td>
<td>-3.9</td>
<td>1.10</td>
<td>35.9</td>
<td>15.7 ± 1.8</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td>Lip9</td>
<td>HSPC:DSPE-PEG2000 95%: 5%</td>
<td>201.8 ± 8.5</td>
<td>0.153</td>
<td>-0.406</td>
<td>0.551</td>
<td>53.2</td>
<td>26.8 ± 2.4</td>
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<td>2.4</td>
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Table S3. Characterization of liposomes with different sizes and their diffusivities in rat intestinal mucus

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hydrodynamic diameter (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>Diffusivity (µm²/s)</th>
<th>Ratio of diffusivity</th>
</tr>
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<tbody>
<tr>
<td>Lip6-80 nm</td>
<td>85.22 ± 8.1</td>
<td>0.151</td>
<td>-2.9 ± 2.9</td>
<td>0.620</td>
<td>1.3</td>
</tr>
<tr>
<td>Lip6-200 nm</td>
<td>193.9 ± 13.0</td>
<td>0.166</td>
<td>-2.87 ± 1.4</td>
<td>0.478</td>
<td>1.0</td>
</tr>
<tr>
<td>Lip6-300 nm</td>
<td>299.6 ± 3.5</td>
<td>0.260</td>
<td>-3.4 ± 1.5</td>
<td>0.512</td>
<td>1.1</td>
</tr>
<tr>
<td>Lip6-400 nm</td>
<td>405.5 ± 1.8</td>
<td>0.166</td>
<td>-2.5 ± 3.9</td>
<td>0.200</td>
<td>0.4</td>
</tr>
<tr>
<td>Lip6-500 nm</td>
<td>506.7 ± 2.9</td>
<td>0.151</td>
<td>-3.9 ± 1.7</td>
<td>0.236</td>
<td>0.5</td>
</tr>
<tr>
<td>Lip4-200 nm</td>
<td>216.5 ± 11.4</td>
<td>0.060</td>
<td>-4.7 ± 3.6</td>
<td>1.689</td>
<td>3.5</td>
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Table S4. The LJ parameters used in simulations

<table>
<thead>
<tr>
<th>Type1</th>
<th>Type2</th>
<th>$\varepsilon_y (\varepsilon)$</th>
<th>$b (\sigma)$</th>
<th>$r_c (\sigma)$</th>
</tr>
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<tr>
<td>Liposome</td>
<td>Liposome</td>
<td>0.01</td>
<td>1.0</td>
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<tr>
<td></td>
<td>Polymer</td>
<td>0.12</td>
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<tr>
<td></td>
<td>Water</td>
<td>0.1</td>
<td>1.0</td>
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<td>Polymer</td>
<td>Polymer</td>
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<tr>
<td></td>
<td>Water</td>
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<td>1.0</td>
<td>3.6</td>
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<tr>
<td>Node</td>
<td>Liposome</td>
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<td>2.0</td>
<td>5.0</td>
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<tr>
<td></td>
<td>Water</td>
<td>0.05</td>
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<tr>
<td>Water</td>
<td>Water</td>
<td>0.1</td>
<td>2.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table S5. Parameters used to tune the stiffness of three types of liposomes with low $T_m$, intermediate $T_m$, and high $T_m$.

<table>
<thead>
<tr>
<th>Type of liposome</th>
<th>$\mu$</th>
<th>sin $\theta_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low $T_m$ (Lip1)</td>
<td>3.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Intermediate $T_m$ (Lip4)</td>
<td>5.0</td>
<td>0.002</td>
</tr>
<tr>
<td>High $T_m$ (Lip6)</td>
<td>7.5</td>
<td>0.02</td>
</tr>
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</table>
References

**Movie S1.** The diffusion and transformation of soft vesicle (liposome with lowest Tm) in hydrogel from molecular dynamics simulation. (T*=0.21ε).

**Movie S2.** The diffusion and transformation of hard vesicle (liposome with highest Tm) in hydrogel from molecular dynamics simulation. (T*=0.21ε).

**Movie S3.** The diffusion and transformation of simi-rigid vesicle (liposome with medial Tm) in hydrogel from molecular dynamics simulation. (T*=0.21ε).

**Movie S4.** The diffusion and transformation of soft vesicles in rat intestinal mucus captured by a gSTED microscope. (T=37 °C)

**Movie S5.** The diffusion and transformation of hard vesicles in rat intestinal mucus captured by a gSTED microscope. (T=37 °C)

**Movie S6.** The diffusion and transformation of semi-rigid vesicles in rat intestinal mucus captured by a gSTED microscope. (T=37 °C)