Experimental Section

Synthesis of Mesoporous Silica Thin Films (MSTF)

MSTF synthesis was performed using a protocol developed previously by Isaksson et al.\textsuperscript{1} Tetraethylorthosilicate (TEOS; 1.73 g, 99.5%; Sigma-Aldrich) was added to 2 g ethanol (> 99%; Solveco) with stirring, followed by the addition of 0.9 g of 0.01 M HCl (Sigma-Aldrich). The silica precursor solution was stirred for 20 min prior to addition of a solution of Pluronic 123 (0.46 g; Sigma-Aldrich) dissolved in 2 g ethanol, which was incubated with stirring for a further 20 min. The silica precursor solution was deposited on a silica substrate by spin-coating at 4000 rpm (spin150, sps-Europe) and aged for 24 h at room temperature. The templating agent was removed through calcination, by ramping 1 °C/min from room temperature to 400 °C, which was maintained for 4 h before cooling to room temperature.

Physicochemical Characterization of MSTF

Scanning Electron Microscopy (SEM)

The thickness of MSTF was studied by high-resolution analytical scanning electron microscopy (SEM; Zeiss Merlin, Oberkochen, Germany). MSTF on glass substrates were mounted with a 90° vice specimen holder to allow for imaging of the MSTF edge. Samples were imaged at an accelerating voltage of 1 kV.

Transmission Electron Microscopy (TEM)

Prior to TEM, MSTF was scratched from the glass substrate and dispersed in ethanol (> 99%), sonicated for 2 min and deposited on a 300-mesh copper grid (Ted Pella Inc.). Samples were left to air-dry for > 30 min to allow for complete ethanol evaporation. TEM analysis was performed using a JEM-1200 EX II (JEOL) at 120 kV accelerating voltage.

Synthesis of Lipid Vesicles

Preparation of Synthetic Lipid Vesicles
Synthetic vesicles were prepared using a lipid film hydration and extrusion method, where 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC; Avanti Lipids Inc.) was dissolved in chloroform, which was then evaporated at the bottom of a round bottom flask under vacuum for > 2 h to remove any trace of the solvent. POPC was rehydrated with TRIS buffer (125 mM NaCl (Sigma Aldrich), 10 mM TRIS (Merck), 1 mM Na₂EDTA (Sigma Aldrich), adjusted to pH = 7.4 using HCl) for at least 3 h, to obtain a lipid concentration of 1 mg/mL. The vesicle solution was extruded through a 50 nm polycarbonate membrane (Whatman, UK) 11 times using a mini extruder (Avanti Lipids Inc.). Extruded vesicles were stored at 4 °C until use.

**Preparation of Fluorescent Tracer Vesicles**

Tracer vesicles were prepared by mixing 99 mol% POPC with 1 mol% 1,2-dioleoylsn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rh-PE; Avanti Lipids Inc.) and following the lipid film hydration and extrusion method.

**Total Internal Reflection Fluorescence (TIRF) Microscopy**

**Formation of a Supported Lipid Bilayer (SLB)**

Total internal reflection fluorescence (TIRF) microscopy was conducted on an inverted Eclipse Ti-E microscope (Nikon Corporation) that was equipped with a Perfect Focus System (PFS), a CFI Apo TIRF 100x oil objective (NA 1.49), a high-pressure mercury lamp and an Andor Neo SCC-01322 sCMOS camera (Andor Technology). SLBs were formed on either a (i) flat glass substrate (0.13 – 0.16 mm thickness) or (ii) a MSTF adsorbed onto a glass microscopy slide. POPC vesicles were incubated with Rh-PE tracer vesicles at a ratio of 1/100, with 10 µL added to custom made PDMS wells with a volume of ~ 50 µL. A rhodamine filter set (TRITC, Semrock) was used for visualizing the SLB or POPC/Rh-PE vesicles. Quantification of the time-dependent evolution of firmly attached vesicles, per reference surface area, was determined using custom written analysis software in MATLAB (MathWorks). Briefly, the image processing code counts the number of fluorescently labelled vesicles that adsorb onto the substrate, while tracking their position between frames. If the
residence time of the vesicle is longer than 5 frames and the position of the vesicle between consequent frames does not change (i.e. the vesicle is not transiently interacting with the surface), the fluorescent vesicle is considered firmly attached.

SLB formation was confirmed by observing fluorescent recovery after photobleaching (FRAP), i.e. by bleaching the rhodamine tracer lipids with a Kr-Ar mixed gas ion laser (Stabilite 2018, Spectra-Physics Lasers, Mountain View, CA) at a wavelength of 531 nm. The diffusivity of Rh-PE within the lipid membrane was determined using a custom written analysis software in MATLAB (MathWorks), as described by Jonsson et al.\textsuperscript{3} The SLB was then subjected to rinsing with TRIS buffer until the majority of unbound vesicles were removed.

\textit{Preparation of Simulated Intestinal Fluids (SIF)}

Three buffered solutions were used to study felodipine partitioning/permeation: (i) TRIS buffer, (ii) biologically-relevant lipid vesicles (LVs), composed of lipid compositions equivalent to those found in the small intestine, and (iii) LVs with 100 mM octanoic acid (C8; Sigma Aldrich). LVs were prepared by dissolving 0.224 g FaSSIF/FeSSIF/FaSSGF powder (Biorelevant.com Ltd) in 100 mL TRIS buffer. Felodipine was added to LVs solutions at 80\% its solubility limit, to ensure all drug was solubilized within the simulated intestinal fluids. Felodipine added to just TRIS buffer was above its solubility limit, and thus, assumed to be in a crystalline state.

\textit{Drug Partitioning/Permeation Studies}

Felodipine partitioning/permeation was studied on SLBs supported by (i) flat silica and (ii) MSTF. To the rinsed SLBs, 10 µL of felodipine-SIF dispersion was added, which was visualized using a DAPI filter set (Semrock). For the MSTF, the TIRF angle was set to ensure that total internal reflection was occurring within the glass substrate, to allow monitoring of felodipine fluorescence below the SLB. Partitioning/permeation was monitored for 20 min. SLB integrity was investigated to ensure a fluid bilayer without defects was maintained by
monitoring Rh-POPC fluorescence intensity at the MSTF-SLB interface, concurrently with
drug permeation monitoring, as well as by performing FRAP analysis at the completion of the
permeation experiment. All data analysis was done using MATLAB using custom written
analysis software. Experiments were performed in triplicate, using new SLBs for each trial,
where data is represented as mean ± S.D. (n = 3).

**Coarse-Grained Molecular Dynamics**

In this study, we performed coarse-grained molecular dynamics (CG-MD) simulations using
Martini force fields.\(^4\)\(^-\)\(^5\) For the parameterization of C8 molecules, the existing free fatty acids
(FFA) Martini topology was modified as described and validated in Hossain et al.\(^6\) Bile salt
(sodium taurocholate) parameterization was based on Martini cholesterol topology which was
described and validated in Clulow et al.\(^7\) To represent lecithin in LVs, 1,2-dilinoleoyl-snglycero-3-phosphatidylcholine (DLiPC) topology obtained directly from the Martini website
was used. To develop CG felodipine model, an all-atom model was first generated with an
automated parameterization process using the Charmm General Force Field (CGenFF) 1.0.0
program.\(^8\)\(^-\)\(^9\) The maximum penalty coefficient associated with partial charges and torsional
bonding provided by this program was 40 and 165, respectively. Note that, the suggested all-
atom model was used here as a basis for the CG modelling. Therefore, the higher penalty
coefficients obtained in the all-atom parameters would likely not be relevant in the CG model.
Based on this, the suggested parameters for all-atom felodipine topology was used to obtain
Martini CG topology following the parameterization procedure described in the Martini
website.

MD simulations were performed with the Gromacs 2016 software using a 30-fs time step at
37°C.\(^10\) We first simulated the components of the LVs for 2 µs in a simulation box size of 19
nm x 19 nm x 22 nm. Then we performed 1 µs simulations by adding felodipine molecules to
the simulation box with or without including 100 mM of C8. Note that, simulations were
performed by adding two different level of felodipine concentrations- i) six felodipine molecule representing 1 mM of drug concentration and ii) one molecule representing infinite dilution. In both cases, it was ensured that all the drug molecules were solubilized into the LVs. A reference pressure of 1 bar (1 bar = 100 kPa) was maintained with isotropic pressure coupling during these simulations.

Next, we placed the simulation box in between two patches of identical POPC membranes. POPC topology was obtain from the Martini website. The POPC membranes were generated using the method Insane\textsuperscript{11} and consisted of 1058 POPC molecules with 529 molecules in each leaflet. The generated membrane was then equilibrated before added to the system. The lower and upper membrane was placed 5 nm from the bottom and 12 nm from the top of the box, respectively. The overall size of the system then became 19 nm, 19 nm and 50 nm in the x-, y-, and z-directions respectively. We then performed 2 $\mu$s simulations with semi-isotropic pressure coupling for four different combinations of drug and C8 concentration with details presented in Supplementary Table 1. Each system was first energy minimized using the steepest descent algorithm, followed by few short equilibrations runs (50,000 steps) using smaller time steps before the final production run. Periodic boundary condition was also applied for all the simulations. Three independent simulations were performed for each case. The simulation trajectories were analyzed to obtain the number of felodipine drug molecules incorporated or adsorbed (if any) into the membrane using the gromacs gmx select utility. Snapshot images were produced using VMD.\textsuperscript{12}

Supplementary Table S1: Different combinations of drug and C8 concentrations added with LVs in the MD simulations.

| Simulations | Felodipine | C8         |
|-------------|------------|------------|
| A           | 1 mM       | No C8      |
| B           | 1 mM       | 100 mM     |
### Results

**Physicochemical Characterization of Mesoporous Silica Thin Films**

Scanning electron micrographs reveal that MSTFs of approximately 400-600 nm were successfully synthesized (Figure S1(a)), while transmission electron micrographs demonstrate the ordered, hexagonal pore structure of MSTFs (Figure S1(b)), with average pore sizes of approximately 7 nm.

![Figure S1: (a) Scanning electron and (b) transmission electron micrographs of MSTF. Scale bar = 200 nm.](image)

**Coarse-Grained Molecular Dynamics of Felodipine Incorporation within Planar Lipid Bilayer**

Coarse-grained molecular dynamics (CG-MD) simulations were employed to investigate drug incorporation into the membrane at the molecular level with felodipine added to LVs with or without the presence of C8. Figure S2 shows the initial snapshots of the systems with POPC membranes and 1 mM of felodipine added to LVs alone (Figure S2A) and LVs with 100 mM of C8 (Figure S2B). Figure S1A shows the presence of one single mixed LV consists of lipids, bile salts and all the drug molecules and a number of free bile salt monomers when felodipine

|   | Infinite dilution (1 molecule) | No C8 |
|---|-------------------------------|-------|
| C |                               |       |
| D | Infinite dilution (1 molecule) | 100 mM |
was added to LVs alone. In the system of LVs and 100 mM of C8, we can observe the presence of a number of mixed LVs with various sizes, pure C8 LV and free C8 monomers. Enlarged view of representative LVs from the systems of LVs alone and LVs with 100 mM C8 are shown in Figure S2C and S2D, respectively. The LV from the system of LVs alone (Figure S2C) shows that all the felodipine molecules are in close contact with the lipid molecules within the LV. However, in the presence of C8, the felodipine molecules are solubilized in different types of LVs. The relatively larger LV (Figure S2D) is consisting of all the components of the system (lipids, bile salts, felodipine and C8). Felodipine molecules are again in close contact with the lipid molecules and surrounded by a number of C8 molecules in that LV. However, we can also see relatively smaller LV without the presence of lipid molecules where felodipine is attached to the C8 and bile salts.

During the simulations, we observe that the detachment of the felodipine molecules occurs from the LVs. The detachment occurs more easily from the smaller LVs where no lipid molecule is present. The detached molecules typically become incorporated into the membrane and we estimated the amount of felodipine molecules incorporated into the membrane during the simulation. The summary of drug molecules incorporation into the membrane for different simulations (see Supplementary Table S1) is presented in Supplementary Table S2. Overall, the CG-MD simulations suggest that felodipine incorporation into the membrane is 2 times higher for LVs with C8 then LVs alone when 1 mM of felodipine was added to the system. With the addition of just one felodipine molecule (representing infinite dilution), simulations also show higher probability of felodipine incorporation into the membrane with systems containing LVs and C8, than LVs alone.
Supplementary Table S2: Summary of drug incorporation into the membrane for different simulations.

| Simulations | Concentration of drug and C8 added to LVs between the POPC membranes | Drug incorporated into the membrane end of 2 µs simulation |
|-------------|---------------------------------------------------------------------|--------------------------------------------------------|
| A           | 1 mM felodipine, no C8                                              | 0.28±0.10 mM                                           |
| B           | 1 mM felodipine, 100 mM of C8                                     | 0.56±0.10 mM                                           |
| C           | 1 felodipine molecule, no C8                                       | Felodipine was incorporated into the membrane once in three simulations |
| D           | 1 felodipine molecule, 100 mM of C8                                | In all three simulations, felodipine was incorporated into the membrane |
Figure S2: Initial snapshots of the systems with POPC membranes and 1 mM of felodipine added to (A) LVs and (B) LVs with 100 mM of C8. Representative LVs from the systems shown in (A, B) are presented in (C, D), respectively.
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