

Supplementary Information

Hot cue: Physiologically controlled release from an in situ forming liposomal depot

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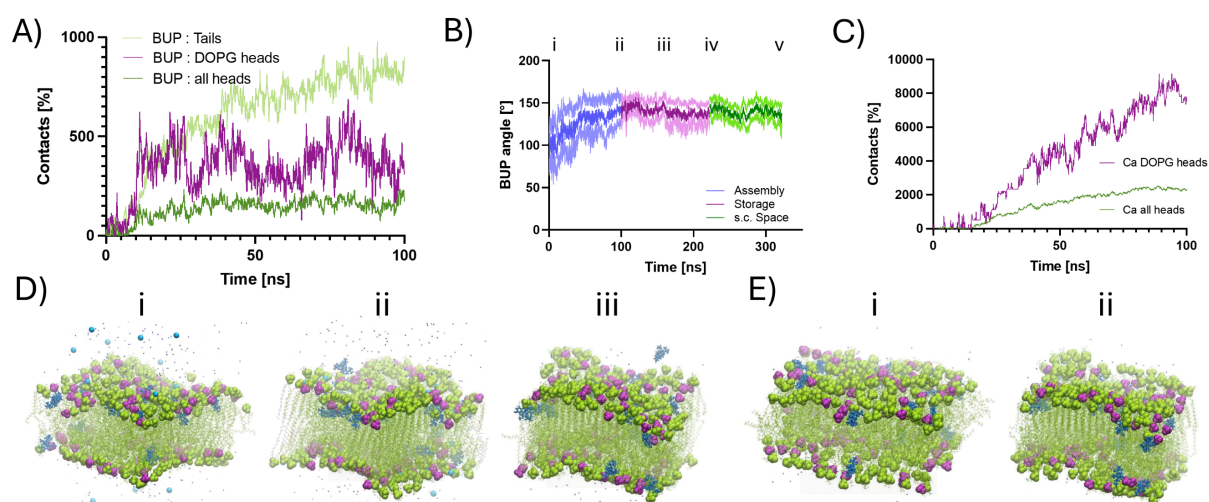


Fig S1 (A) Contacts between drug molecules and various lipid components, showing that drug molecules predominantly position themselves within the lipid bilayer and interact with the negatively charged headgroups of DOPG during drug loading and constructing the system. (B) Orientation of the drug molecule within the lipid bilayer over consecutive simulations i. start of drug loading (65 °C); ii. Drug incorporated into membrane iii. Cool down to storage temperature (4 °C); iv. Warm up to body temperature and exposure to simulated s.c. space; v. After exposition and surface associated drug release. (C) Number of contacts between divalent cations (Ca) and lipid headgroups, indicating a preferential interaction with DOPG headgroups, confirming competitive binding. (D) Snapshots of membrane simulation in approximated s.c. environment i) Simulation at 0ns with divalent cations dispersed in the simulation box and drug molecules associated with the membrane ii) 50ns showing divalent cations have moved towards the membrane and drug molecules start to decouple from the membrane surface iii) 100ns drug molecules fully dissociated from the membrane. E) Snapshots of membrane across temperature ramp i) Fluid membrane and ii) rigid gel membrane.

Nuclear magnetic resonance measurements

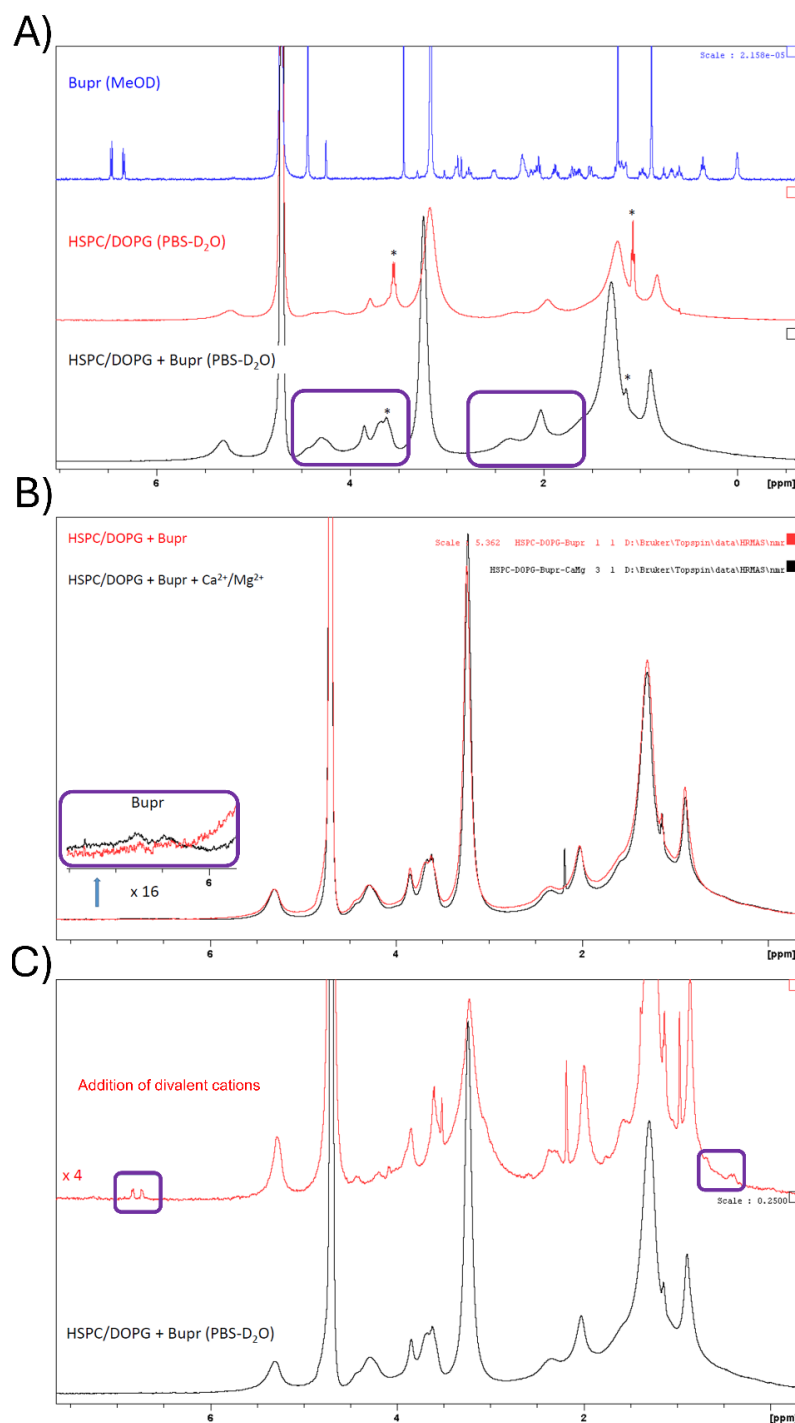


Fig S2 Liquid state ^1H NMR (Bupr/MeOD) and HR-MAS ^1H NMR spectra of liposomes under varying conditions ($T = 293\text{ K}$). A) Spectra of free buprenorphine (blue), empty liposomes composed of DOPG:HSPC (25:75 mol%) (red), and buprenorphine-loaded liposomes (black). B) Spectra of buprenorphine-loaded liposomes before and after exposure to divalent cations at

physiological concentrations, representative of the subcutaneous (s.c.) environment. C) Spectra of drug-loaded vesicles following further addition of divalent cations, illustrating enhanced drug dissociation from the vesicle surface. Purple boxes highlight resonances from buprenorphine. Peaks marked with * derive from ethanol impurity.

Differential Scanning Calorimetry

Table S1 Phase transition temperature of lipid mixtures (50 mM) with drug molecule incorporated.

Formulation	Enthalpy J/g	Onset °C
1. HSPC:DOPG (75:25)	38.45	34.38
2. HSPC:DOPG (80:20)	37.83	39.98
3. HSPC:DOPG (70:30)	27.02	32.14
4. HSPC:DOPG (75:25) + 100 mM Zn	33.06	42.99
5. HSPC:DOPC (75:25)	48.79	40.71

Aggregation Studies

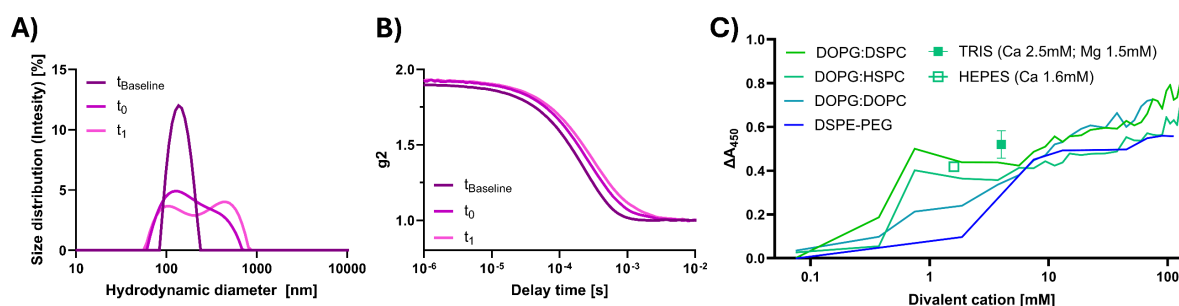


Fig S3 Aggregation studies of liposomes A) Size distribution and B) auto correlation function of liposomes exposed to physiological cation concentrations over time indicating aggregation behavior C) Aggregation profiles of different lipid systems along an increasing concentration gradient of divalent cations. TRIS & HEPES, represent two points simulating the physiological conditions in the s.c. space where the lead formulation (DOPG:HSPC 25:75 n%) was additionally screened. (Note: all lipid compositions were 25:75 n%; DSPE-PEG= DOPG:HSPC:DSPE-PEG (25:73.5:1.5 n%))

Stability studies

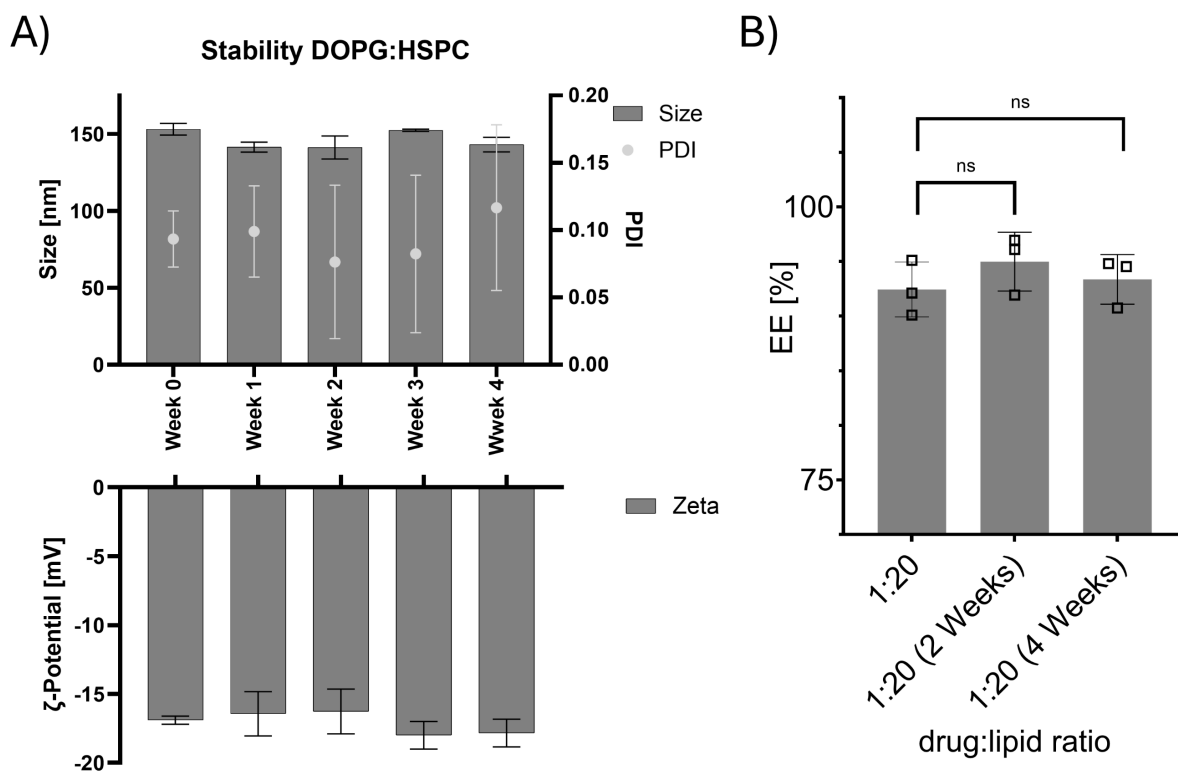


Fig S4 Drug delivery system (DOPG:HSPC 25:75 n%) stability over 4 weeks considering A) Size and zeta potential and B) drug molecule encapsulation (mean $\pm$ SD, n = 3).

Process transfer to microfluidics

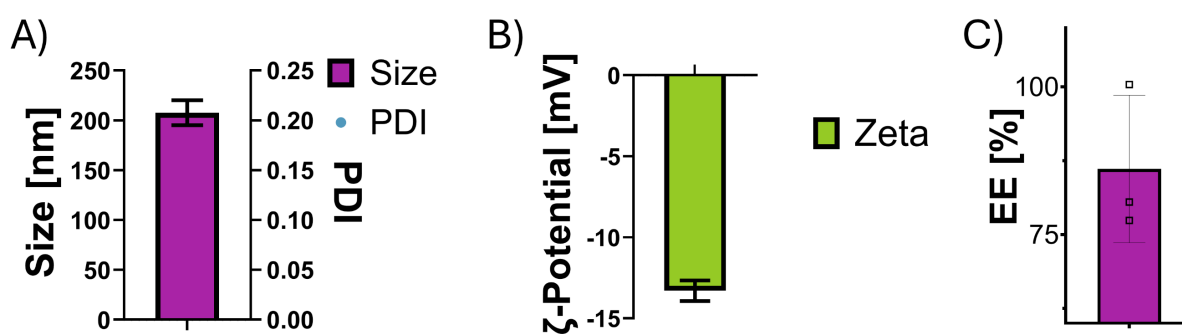


Fig S5 TILD System liposomes transferred to microfluidic production. A) Size and PDI, B) Zeta potential, and C) Encapsulation efficiency of TILD liposomes produced via microfluidics.

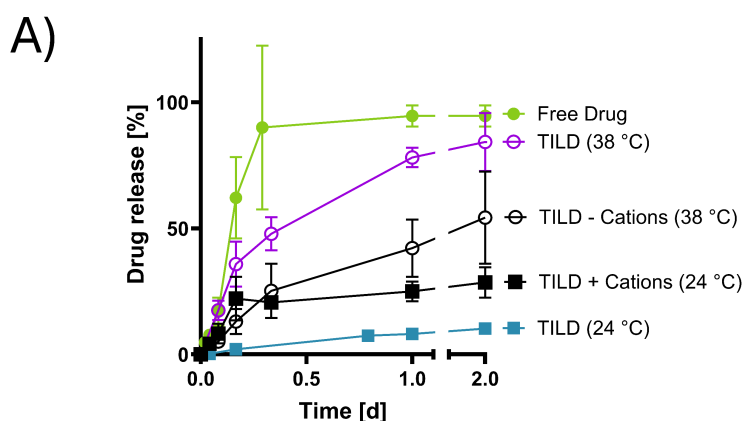


Fig S6 In vitro drug release of TILD system comparing free drug against TILD at different temperatures, and divalent cations to simulate the s.c. space and storage conditions.

SAXS Spectra

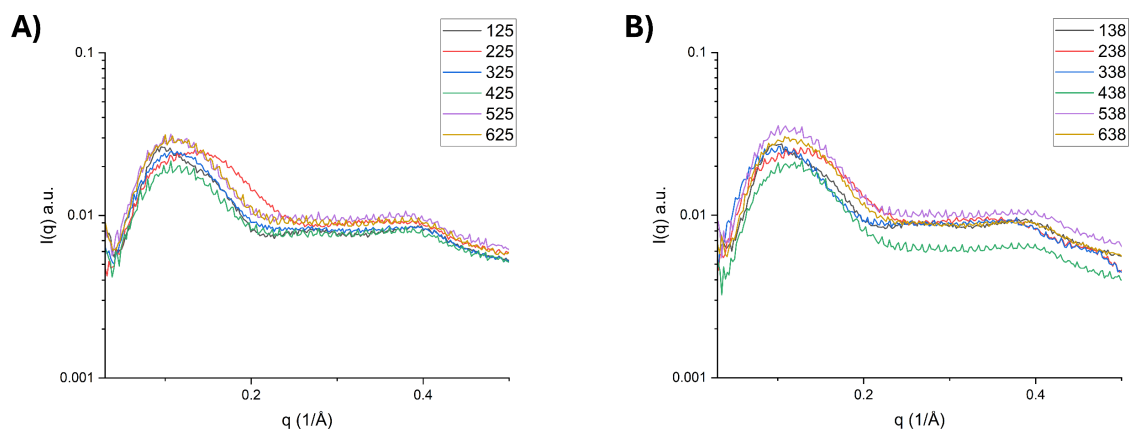


Fig S7 Scattering patterns of liposome measurements A) Measurements at 25 °C (125: HSPC; 225: DOPG; 325: HSPC/ DOPG (75:25 molar ratio) based-liposomes; 425: HSPC/ DOPG (75:25 molar ratio) based-liposomes + Buprenorphine (1:20); 525: HSPC/ DOPG (75:25 molar ratio) based-liposomes + 1 mM salt; 625: HSPC/ DOPG (75:25 molar ratio) based-liposomes + Buprenorphine (1:20) + 1 mM salt) B) Measurements at 38 °C (138: HSPC; 238: DOPG; 338: HSPC/ DOPG (75:25 molar ratio) based-liposomes; 438: HSPC/ DOPG (75:25 molar ratio) based-liposomes + Buprenorphine (1:20); 538: HSPC/ DOPG (75:25 molar ratio) based-

liposomes + 1 mM salt; 638: HSPC/ DOPG (75:25 molar ratio) based-liposomes +
Buprenorphine (1:20) + 1 mM salt)

In vivo scoring

Table S2 Scoring sheet during pharmacokinetic and pharmacodynamic study in rats.

Monitoring scale	Score standards	Details
Demeanour	0	Active, interested
	1	Calm, moves when touched
	2	Immobile, apathic [§]
Body weight Baseline BW = 186	0	0-9% weight loss from baseline
	1	10-14% weight loss from baseline
	2	>15% weight loss from baseline [§]
Appearance	0	Straight, bright pelt
	1	Dull, slightly ruffled fur
	2	Ruffled fur, hunched posture
Grimace scale	0	Open eyes and straight ears
	1	Orbital tightening, cheek bulge
	2*	Orbital tightening, cheek bulge and flat ears [§]
Paw appearance	0	Pink and soft
	1	Reddish and inflamed
	2	Red, inflamed and swollen
Paw inflammation	NA	Paw oedema (mm)
	1	Paw licking
	1	Paw guarding
Gastrointestinal	1	Diarrhea
	2	Pica [§]
	2	Blood in faeces [§]
Body T° (°C)	2	hypothermia [§]
Baseline T°=	2	Hyperthermia [§]
Total Score	Best= 0, Worst=16.	

Absolute Cut offs[§]: Weight-loss of > 15%, blood in faeces, pica, hypothermia (T°<34°C),

severe pain*, immobile and apathic

Table S3 Scoring sheet during pharmacokinetic study in dogs.

Monitor Scale	Score	Description
Overall appearance	0	No sedation apparent
	1	Mild sedation
	2	Moderate sedation
	3	Well sedated
Posture	0	Standing
	1	Sitting
	2	Sternal recumbency
	3	Lateral recumbency
Awareness	0	Conscious and spontaneous interest
	1	Conscious but disinterested
	2	Unconscious
Interactive behaviour	0	Responds to 1 vocal stimulus
	1	Responds to 1 touch
	2	Does not respond to 1 touch
Spontaneous behaviour	0	Moves spontaneously
	1	Relaxed no tremors
	2	Very relaxed
Eye position	0	No rotation (normal)
	1	Moderate rotation
	2	Total rotation
Palpebral reflex Awareness	0	Brisk
	1	Slow
	0	Conscious and spontaneous interest
Total Score	Lowest= 0, Highest= 25	

*Observation times: 1st Day: 1, 4 and 8 h after injection; consecutive days once per day from 8-9 am.

**Report score and exact measured value