Supporting information for

In-silico screening of drug candidates for thermoresponsive liposome formulations

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S1: Analysis of MD Simulations

To evaluate if the membrane in Molecular dynamic (MD) simulation is already equilibrated, analysis of area corresponding to one lipid was performed (Fig. S1.1A). It was observed that areas per lipid in MD simulation at 293 K, 313 K and 323 K are highly packed and do not show significant differences, although a slight increase in temperature can be seen. In contrast, MD simulation at 333 K showed a rapid increase in area per lipid. The significant increase in the area per lipid (by approx. 0.1 nm^2, ~22 % increase) results from membrane phase transition. Also, fluctuations of area per lipid at 333 K are higher due to greater conformational flexibility in the disordered fluid system at the highest temperature. In all cases, there is no systematic change in area per lipid after 100 ns, and therefore, the time of equilibration around 200 ns is sufficient for our simulation.

Although the different behaviour of membrane at 333 K can be seen from above, order parameters [1] for both (*sn*-1 and *sn*-2) acyl chain carbon atoms in palmitates in DPPC were calculated using *gmx order* [2] tool (Fig. S1.1B). The disorder of a membrane at 333 K is then clearly seen from the comparison with MD simulations at lower temperatures with significantly higher ordering.

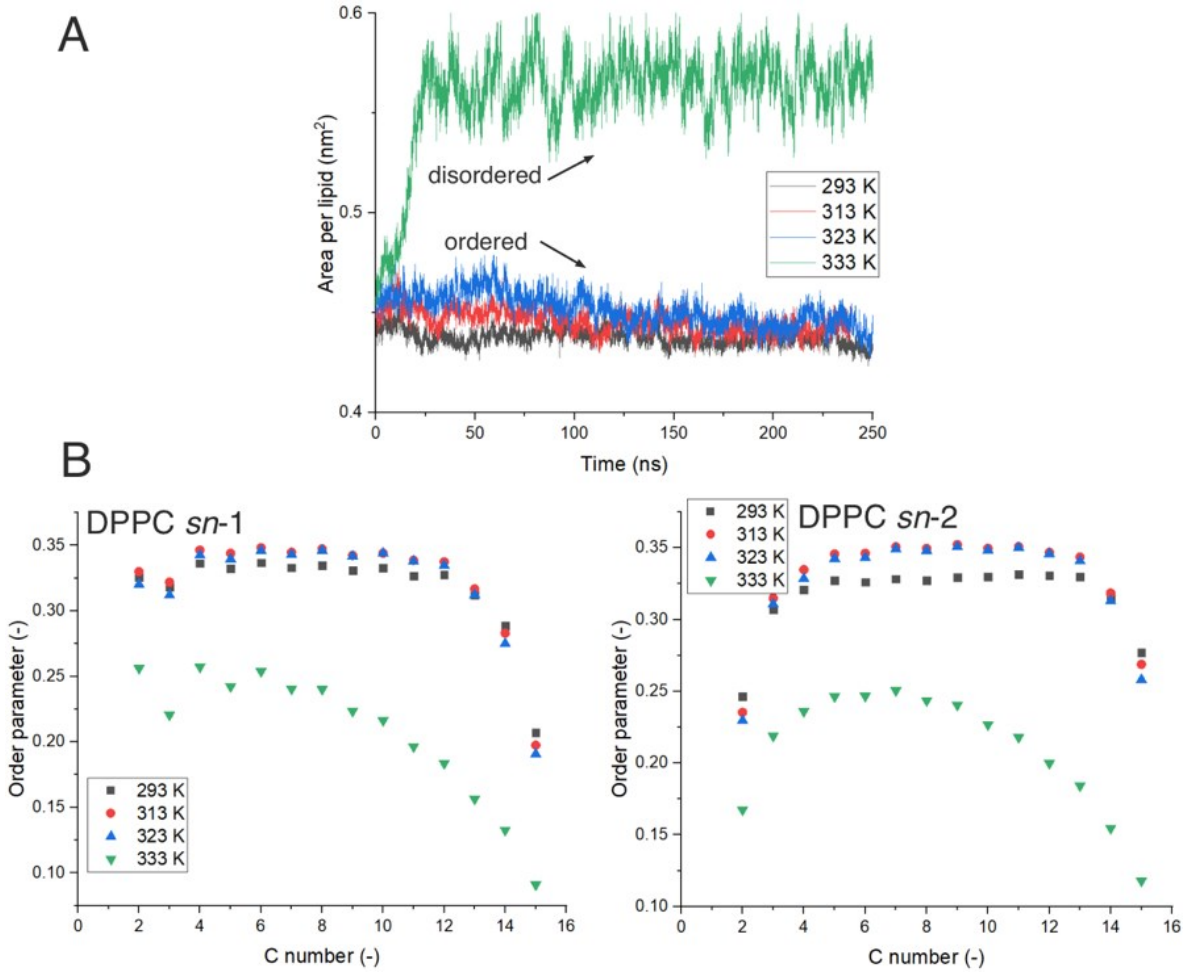

Figure S1.1: (A) Dependency of area per lipid on a simulation time for four different temperatures of simulated membrane DPPC:DPPG:Chol (75:10:15). **(B)** Order parameters for *sn*-1 (left) and *sn*-2 (right) acyl chain carbon atoms in palmitates in DPPC. Differences in structural parameters at a higher temperature (333 K) are caused by phase transition into the disordered state. This increases area occupied by one lipid and decreased order parameters, e.g., increased lipid acyl chains' flexibility.

S2: Calculation of energy profiles through the membrane with COSMOmic

The calculation of partition and permeation coefficient is based on the COSMOmic approach described in detail in publication [3], briefly:

From the equilibrated MD simulation of a lipid bilayer, snapshots are taken, and a bilayer was cut to 50 layers along the membrane normal - *r* axis (Fig 2.1).

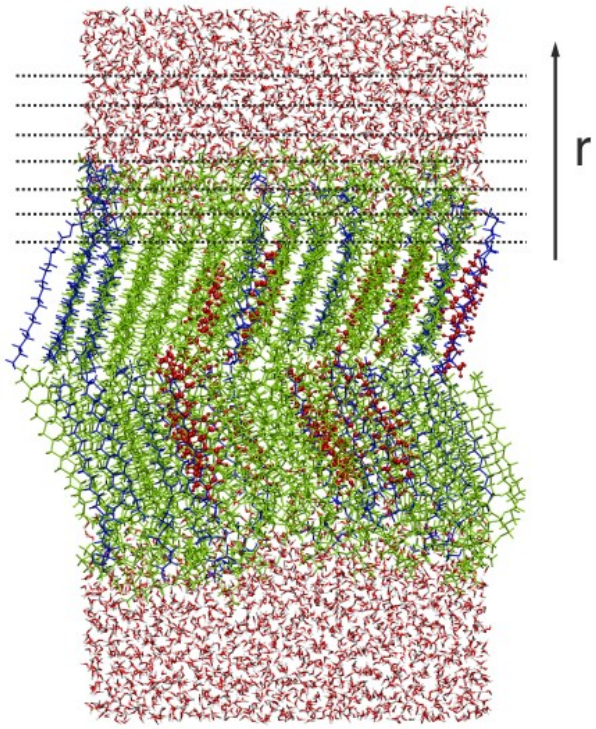

Figure S2.1: Screenshot from MD simulation used for partitioning and permeation calculation with the indication of layers used for energy profile calculation.

Conformers of the permeating molecule are generated (in our case using OPLS2005 forcefield within ligprep and macromodel modules of Schrodinger suite).

The molecule's chemical potential in the membrane layer is calculated by comparing σ-profiles of molecule and membrane layers. Both σ-profiles (of membrane layer and of permeating molecule) are calculated using the COSMO-RS approach. The chemical potential of a molecule (i) in each layer is then calculated as a function of the orientation in the space of the molecule and its position on the membrane normal ($\mu_i(r,d)$).

The partition function in each layer is calculated from chemical potentials of all orientations of the permeating molecule:

$$Z_i(r) = \sum_d e^{\frac{-\mu_i(r,d)}{kT}} \quad \text{(S1)}$$

From the known partition function of the molecule in the last layer where only water is present ($Z_i(n)$), the relative partitioning and therefore also free energy difference to water environment can be calculated:

$$\Delta G_i(r) = -RT\ln\frac{Z_i(r)}{Z_i(n)} \quad \text{(S2)}$$

The difference in free energy can be evaluated as a function of the distance from the membrane center (Fig. S2.2). Moreover, the partition coefficient $\log K_{lip/wat}$ is calculated from the free energy differences along the *r* axis:

$$\log K_{lip/wat} = e^{\frac{-\Delta G_i(min)}{RT}} \quad \text{(S3)}$$

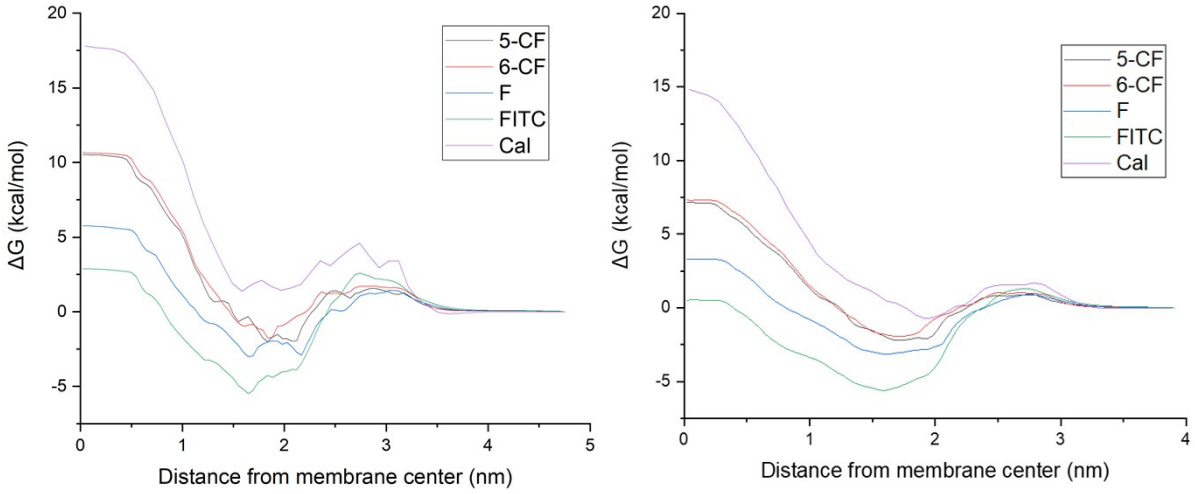

Figure S2.2: Calculated energy profiles through DPPC:DPPG:Chol (75:10:15) membrane for four fluorescein derivatives (5-CF and 6-CF as a mixture in experiments) at 293 K (left) and 333 K (right) used as calibration of the whole procedure. These molecules' amphiphilic character resulted in free energy minimum below the head group region at ~1.8 Å distance from the membrane center. Like other drug-like molecules, the amphiphilic character of fluorescein and its derivates result in predominant affinity to this region where both polar and apolar parts of molecules can interact with both hydrophobic (apolar) acyl chains and polar head groups/water environments [4].

S3: Calculation of $\log P_{erm}$ with COSMOperm

Calculation of the permeation coefficient is explained in detail in COSMOperm publication [5] and is based on an equation firstly proposed by Diamond and Katz [6]:

$$\frac{1}{P_{erm}} = \int_{-L}^{L} \frac{1}{K(z)D(z)} dz \quad \text{(S4)}$$

where L is the thickness of one lipid layer, $K(z)$ is the partition coefficient in each layer and $D(z)$ is the diffusion coefficient in each layer. The partition coefficient is calculated from the change of free energy using the procedure described in section S2.

The diffusion coefficient is calculated using the COSMO-RS approach using experimentally fitted parameters and COSMO profile of a permeating molecule.

S4: Rationalisation of time of permeation

From the experimentally used procedure for calculation of apparent permeation coefficient (P_{app}) [7]:

$$P_{app} = slope \cdot \frac{V_{cell}}{S_m \cdot c_{donor}} \quad \text{(S5)}$$

where $slope$ is the slope in dependency of acceptor concentration on time, V_{cell} is the volume of the cell, S_m is the area of the membrane and c_{donor} is the concentration in the donor phase. If we take into consideration that we do not have two equally big compartments but many liposomes with dye and one "big" acceptor compartment, the equation can be rewritten into a new form:

$$P_{app} = \frac{dc_{acc}}{dt} \cdot \frac{V_{acc}}{S_{lip} \cdot N_{lip} \cdot c_{lip}(t)} \quad \text{(S6)}$$

where $\frac{dc_{acc}}{dt}$ is the time derivation of acceptor concentration, V_{acc} is the volume of acceptor compartment (1.6 ml in our experiments), S_{lip} is the area of one lipid, N_{lip} is the number of lipids in the sample and $c_{lip}(t)$ is the dye concentration in liposomes, but it is time-dependent as the concentration of dye in liposome during permeation is changing rapidly.

The whole amount of dye which comes to the permeation experiment is at the beginning of the experiment in the liposomes with starting concentration c_0 and the dye is either in the liposomes or in the acceptor compartment at each time.

$$c_0 V_{lip} N_{lip} = c_{lip} V_{lip} N_{lip} + c_{acc} V_{acc} \quad \text{(S7)}$$

The dependency of concentration in the acceptor phase on the concentration in lipids:

$$c_{acc} = \frac{V_{lip} N_{lip}}{V_{acc}} (c_0 - c_{lip}) \quad \text{(S8)}$$

And the derivation:

$$\frac{dc_{acc}}{dc_{lip}} = -\frac{V_{lip} N_{lip}}{V_{acc}} \quad \text{(S9)}$$

The derivation in (S6) can be rewritten:

$$\frac{dc_{acc}}{dt} = \frac{dc_{acc} dc_{lip}}{dc_{lip}\ dt} \quad \text{(S10)}$$

Using (S10) and (S9), the (S6) can be written in a form, where the only time-dependent variable is the concentration in liposomes.

$$P_{app} = -\frac{V_{lip} N_{lip} dc_{lip}}{V_{acc}\ dt} \cdot \frac{V_{acc}}{S_{lip} \cdot N_{lip} \cdot c_{lip}} \quad \text{(S11)}$$

$$P_{app} = -\frac{dc_{lip}}{dt} \cdot \frac{V_{lip}}{S_{lip} \cdot c_{lip}} \quad \text{(S12)}$$

With an assumption that liposomes are spherical with a diameter (*d*) equal to 600 nm:

$$P_{app} = -\frac{dc_{lip}}{dt} \cdot \frac{d}{6 \cdot c_{lip}} \quad \text{(S13)}$$

The differential equation has with initial condition ($c_{lip}(0) = c_0$) solution:

$$\ln c_{lip} = \ln c_0 - \frac{6 \cdot P_{app}}{d} t \quad \text{(S14)}$$

If we choose some characteristic release (half time of release), we get the equation for half-time:

$$t_{1/2} = \frac{d \cdot \ln 2}{6 \cdot P_{app}} \quad \text{(S15)}$$

For the P_{app} of 5(6)-CF at 293 K ($10^{-7.1}$) we get half-time 87 seconds and for 5(6)-CF at 333 K ($10^{-9.1}$) we get 2.4 hours.

S5: Calculated values for drug candidates

For a total number of 57 compound, partition and permeation coefficients at 293 K and 333 K were calculated. All calculated values are listed in Table S4.1.

Table S4.1: COSMOmic calculated partition coefficients and COSMOperm calculated permeation

compound	$logK^{293K}$	$logP_{erm}^{293K}$ (cm/s)	$logK^{333K}$	$logP_{erm}^{333K}$ (cm/s)
1_2-dichlorobenzene	3.28	-1.29	3.23	0.06
PhiP	2.56	-5.06	1.42	-3.88
2-Methoxyethanol	-1.21	-3.00	-0.74	-1.58
8-azaguanine	0.71	-7.65	0.07	-5.85
9H-CARBAZOLE	3.23	0.69	2.91	0.32
Acifluorfen	4.59	0.22	4.10	1.09
Acipimox	-0.86	-2.99	-0.64	-1.87
Allicin	1.28	-1.62	1.36	-0.54
Allosamidin	-1.47	-16.04	-1.45	-12.09
Altretamine	5.41	-2.58	5.70	-0.06
Aminacrine	1.29	-1.52	1.38	-0.47
Amitraz	7.07	-3.03	7.39	-0.12
Anacetrapib	10.40	-3.95	10.77	-0.36
Atorvastatin	4.02	-4.77	4.16	-1.41
Azacitidine	-1.23	-13.46	-1.34	-10.56
Azathioprine	0.54	-6.98	0.19	-5.18
Bempedoic acid	4.81	-1.17	5.58	0.69
Bendamustine	2.87	-2.89	2.40	-0.92
Benfluorex	7.97	-2.31	4.94	-0.12
Benznidazole	1.07	-2.52	0.76	-1.44
Benzylbenzoate	3.89	-1.43	3.81	0.05
Bezafibrate	3.11	-1.67	3.03	0.00
Bisphenol A	4.16	-3.15	4.09	-0.82
Bromoform	2.94	0.54	3.16	0.74
Broxuridine	-1.30	-7.57	-0.99	-5.54
Busulfan	0.48	-1.73	0.18	-1.11
Buthionine Sulfoximine	0.83	-3.51	0.66	-2.30
Calyculin A	4.95	-7.57	6.20	-2.49
Cantharidin	0.22	-1.93	0.40	-0.82
Capecitabine	0.02	-7.03	0.80	-4.71
Carbendazim	0.63	-1.92	0.54	-0.97
Carboquone	0.46	-3.13	0.55	-1.41
Carboxin	1.93	-1.94	1.91	-0.68
Carmustine	1.09	-2.37	1.12	-0.92
Cerivastatin	5.13	-1.99	5.52	0.28
Cerulenin	0.51	-2.04	1.04	-1.10

Chlorambucil	2.99	-1.78	2.81	-0.17
Chlorine	1.58	-1.33	1.72	-0.17
Chlorotoxin I-131	7.07	-3.08	6.68	-0.60
Ciprofibrate	3.34	-1.44	3.18	0.19
Clofarabine	-1.30	-7.66	-1.34	-5.44
Clofibrate	3.46	-2.00	3.56	-0.36
Cordycepin	-1.31	-7.55	-1.34	-5.44
Coumaphos	4.20	-2.14	4.25	-0.12
Crotamiton	2.89	-1.55	3.16	0.10
Cyclophosphamide	0.28	-2.03	0.53	-0.96
Cycloserine	-0.83	-6.04	-1.15	-4.60
Cyfluthrin	6.07	-2.30	5.97	-0.16
Cypermethrin	6.03	-2.25	5.94	-0.13
Cytarabine	-0.82	-13.14	-1.33	-10.30
Cythioate	2.47	-1.92	1.69	-0.93
Dacarbazine	0.40	-5.78	0.02	-4.15
Dalcetrapib	6.65	-2.39	7.03	-0.01
Dantron	2.94	-2.34	2.86	-0.62
Decitabine	-1.26	-12.03	-1.34	-9.34
Deltamethrin	6.23	-2.56	6.03	-0.33

S6: Calculation of f_{HA} for cycloserine

Using the Protonation Plugin Group in MarvinSketch 20.16 [8], and the pK_A for the deprotonation of secondary amine is 4.21 (pK_{A1}), and the pK_A for protonation of primary amine is 8.34 (pK_{A2}). Then, four forms of cycloserine can be present in solution (Fig. S6.1).

From the equilibria, the equation for ratio between neutral and zwitterionic form can be written.

$$\frac{c_N}{c_{Zw}} = \frac{K_{A2}}{K_{A1}} = 10^{pK_{A1} - pK_{A2}} = 7.4 \cdot 10^{-5} \quad \text{(S16)}$$

This ratio is based on rough theoretical prediction of acidity constants and therefore can vary from reality.

The zwitterionic form is present at pH 7.4 in 90 % and therefore f_{HA} for neutral cycloserine at this pH is $6.67 \cdot 10^{-5}$. Finally, the apparent permeation coefficient is -10.2 and -8.8 at 293 K and 333 K, respectively.

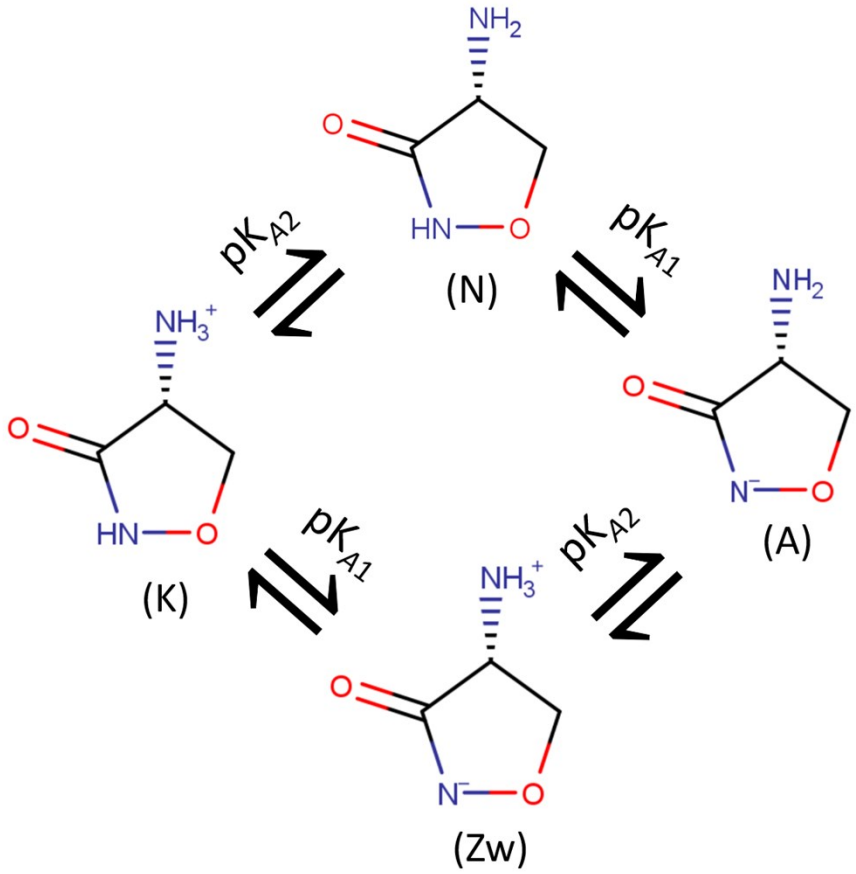

Figure S6.1: Forms of cycloserine and their equilibria in water solution: cationic (K), neutral (N), anionic (A) and zwitterionic (Zw)

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