Liposome/drug compatibility and the possibilities for its improvement

Balouch Martin,
Odehnalová K., Storchmannová K., Šrejber M., Šoltys M., Petrová E., Konefal M., Janská P., Zadražil A., Brus J., Berka K., Štěpánek F.

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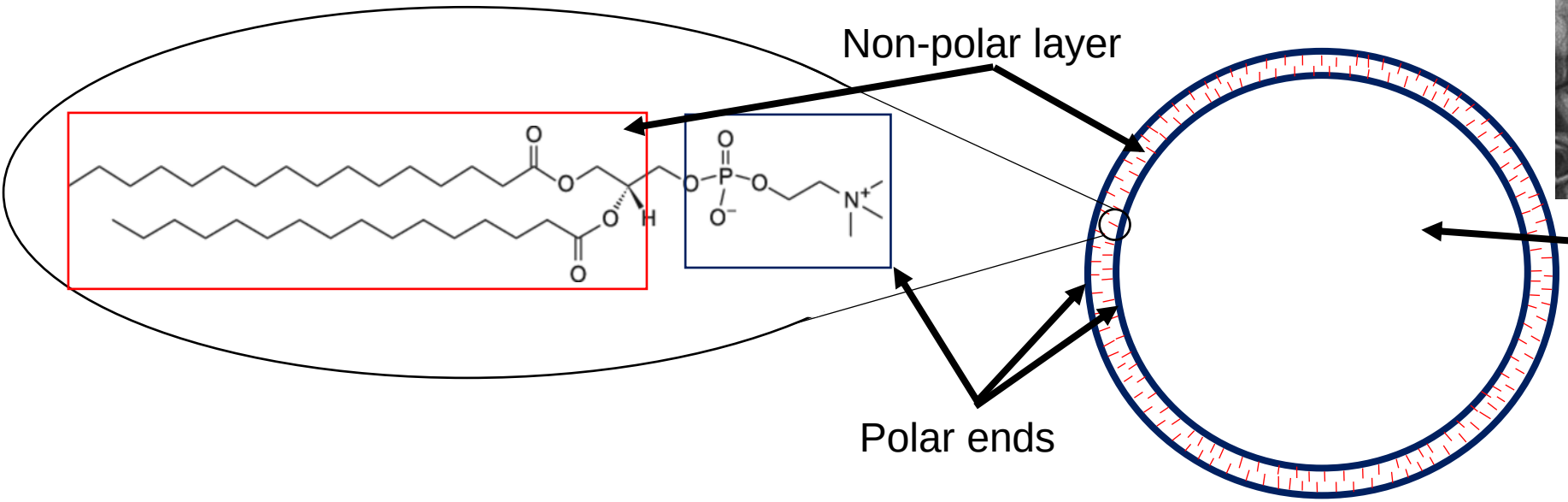
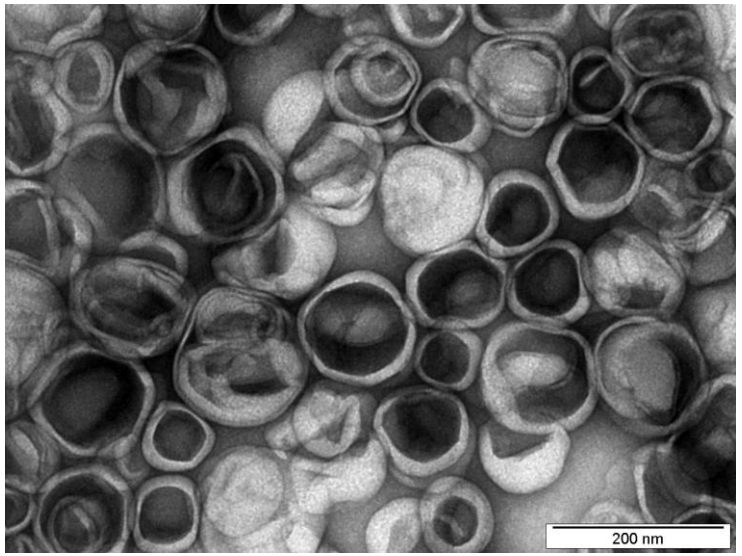
Liposomes

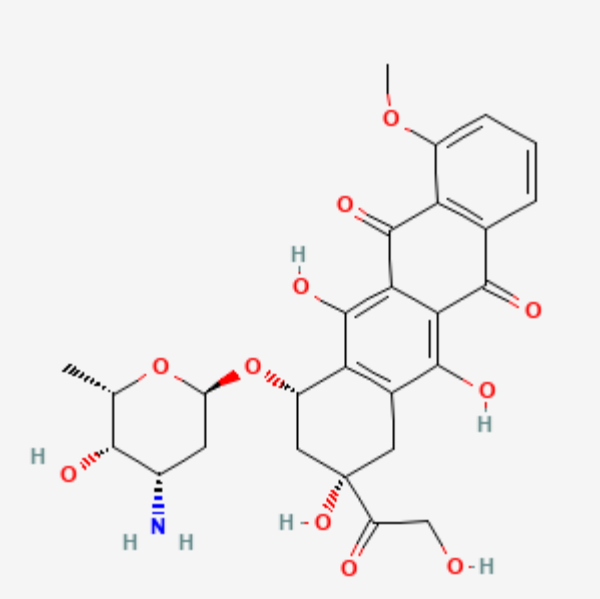
- composed of phospholipids
- contains hydrophilic pocket
- can carry a cargo

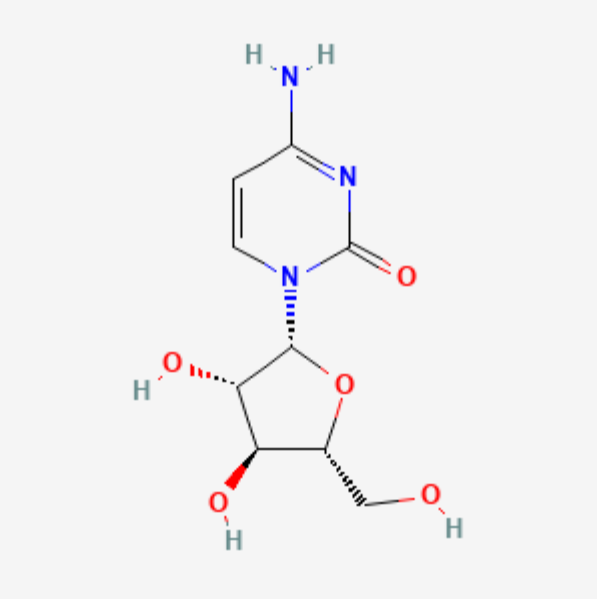




Liposomes state of art

- Significant pharmacological improvement of **few** drugs
- These drugs are mostly high Mw molecules
- Doxorubicin (Doxil®) and analogues prevalent





Question n. 1

Why there are only so few small molecules formulated in liposomes?

Question(s) n. 1

Why there are only so few small molecules formulated in liposomes?

Which molecules can be entrapped in liposomes?

Which molecules can be entrapped in liposomes?

- The key to this answer is permeability

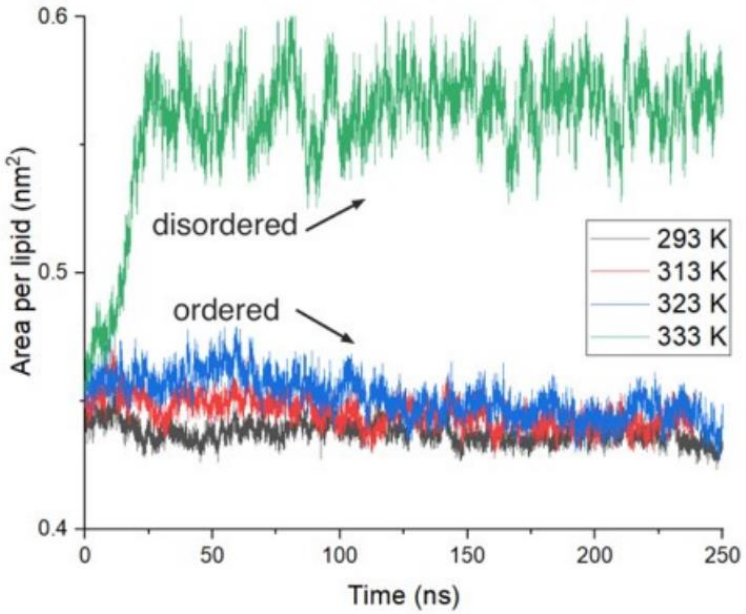
Which molecules can be entrapped in liposomes?

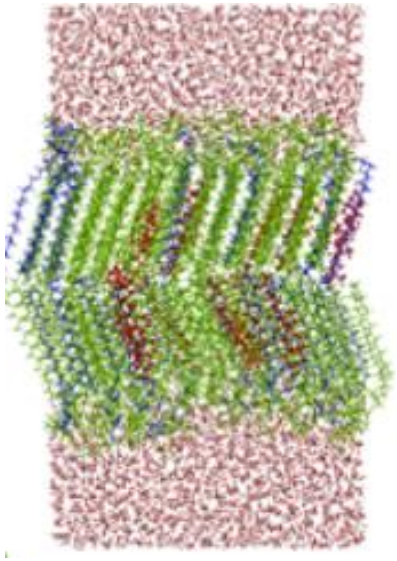
- The key to this answer is permeability

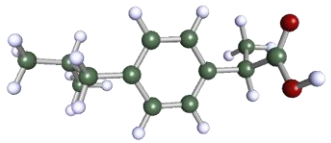
  - Permeability calculation using COSMOPerm
  - Experiments using fluorescent dyes
  - Comparison of both
  - Further COSMOPerm calculation of 56 drugs

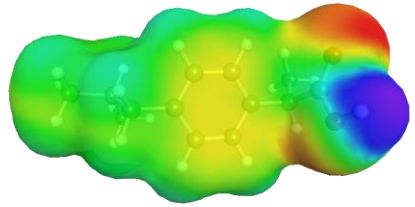
COSMOPerm

- Computational approach to permeability
- Based on molecular dynamics and quantum mechanics



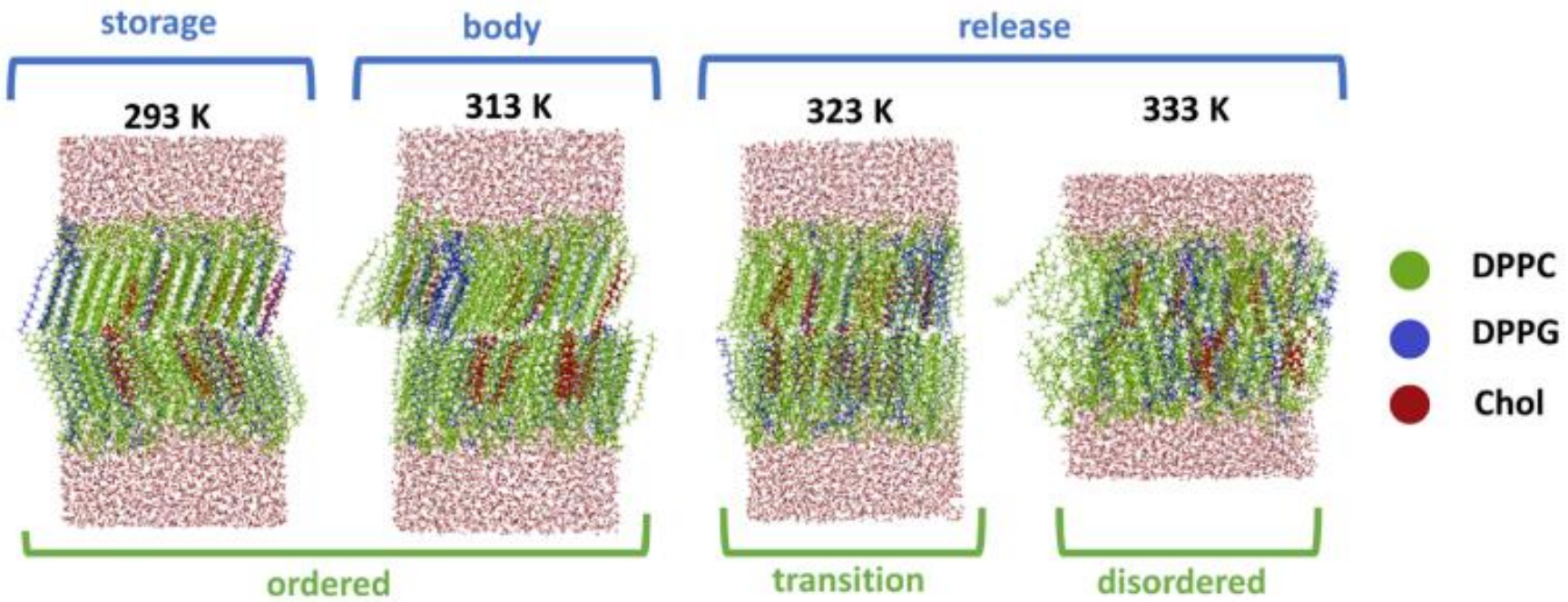






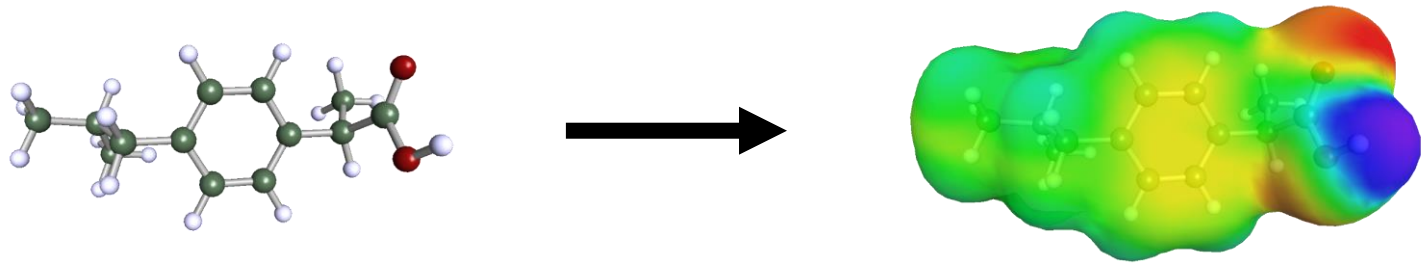
Membrane for permeation

- DPPC:DPPG:Chol (75:10:15) – 64 lipids for a leaflet
  - 250+ ns simulation time
  - Simulated using molecular dynamics



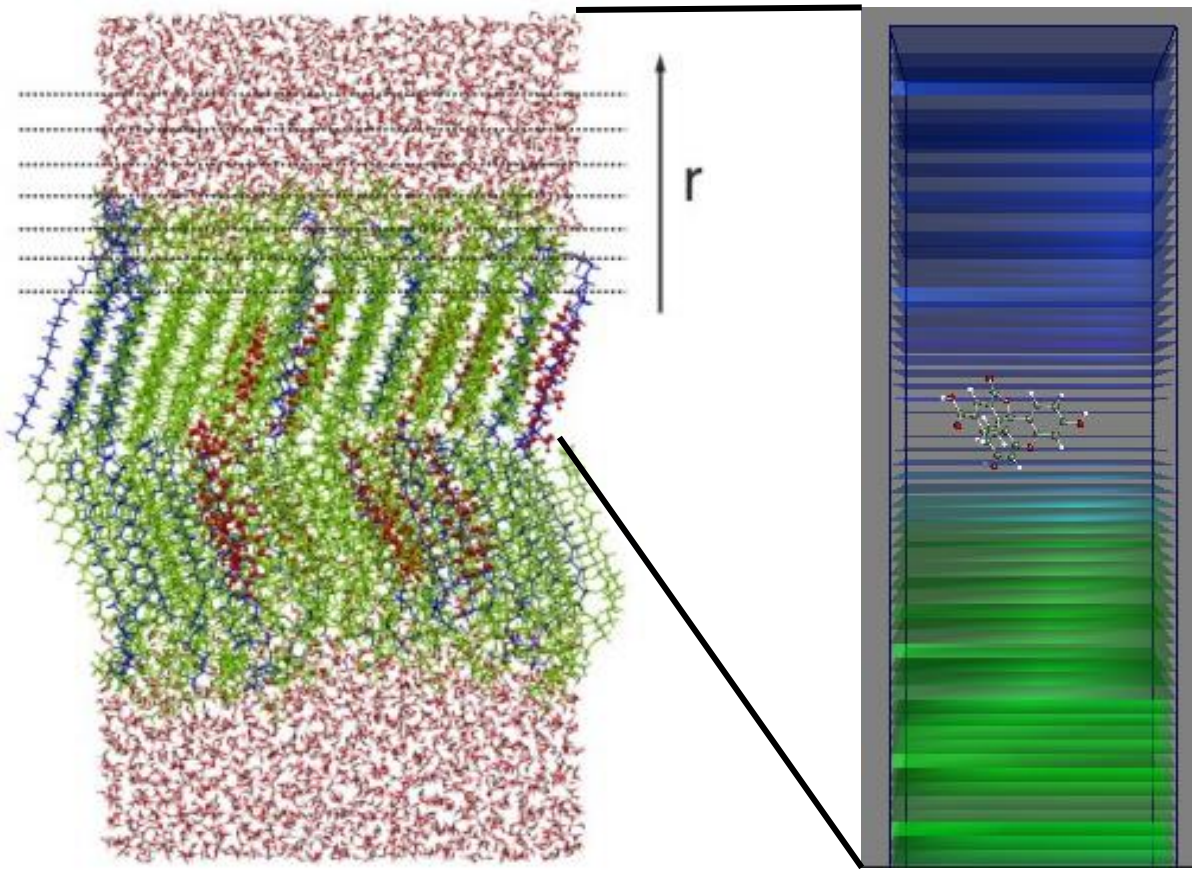
Quantum Mechanics optimisation

- All membrane components
- All permeating molecules
  - DFT/BP/TZVP with fine option level of calculation
  - Calculated by Turbomole 6.3 to obtain σ-profile of a molecule



Permeation calculation principle (1/2)

1. Chemical potential of API (μ_i) in each layer (r) and each orientation (d)



2. Partition function from chemical potential

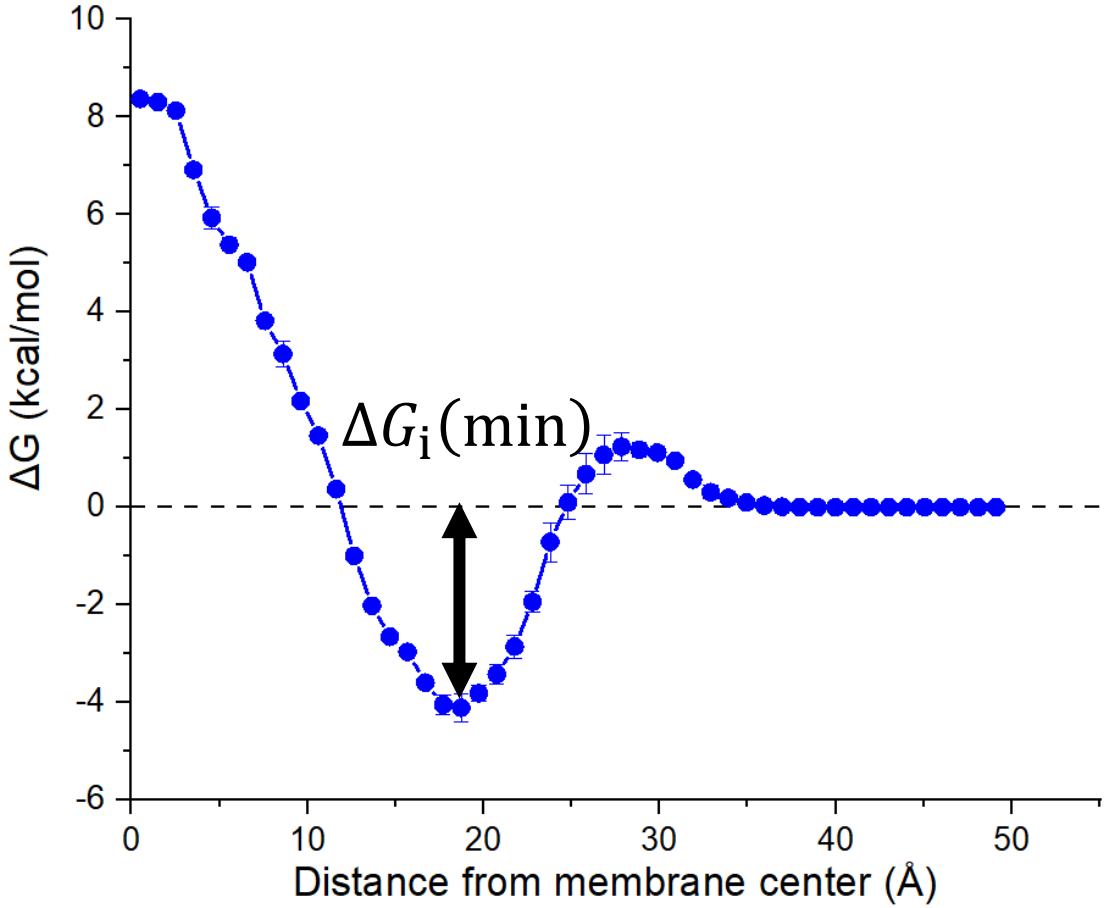
$$Z_i(r) = \sum_d e^{\frac{-\mu_i(r,d)}{kT}}$$

3. Free energy from the partition function

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

Permeation calculation principle (2/2)

4. Free energy profile



5. Partitioning

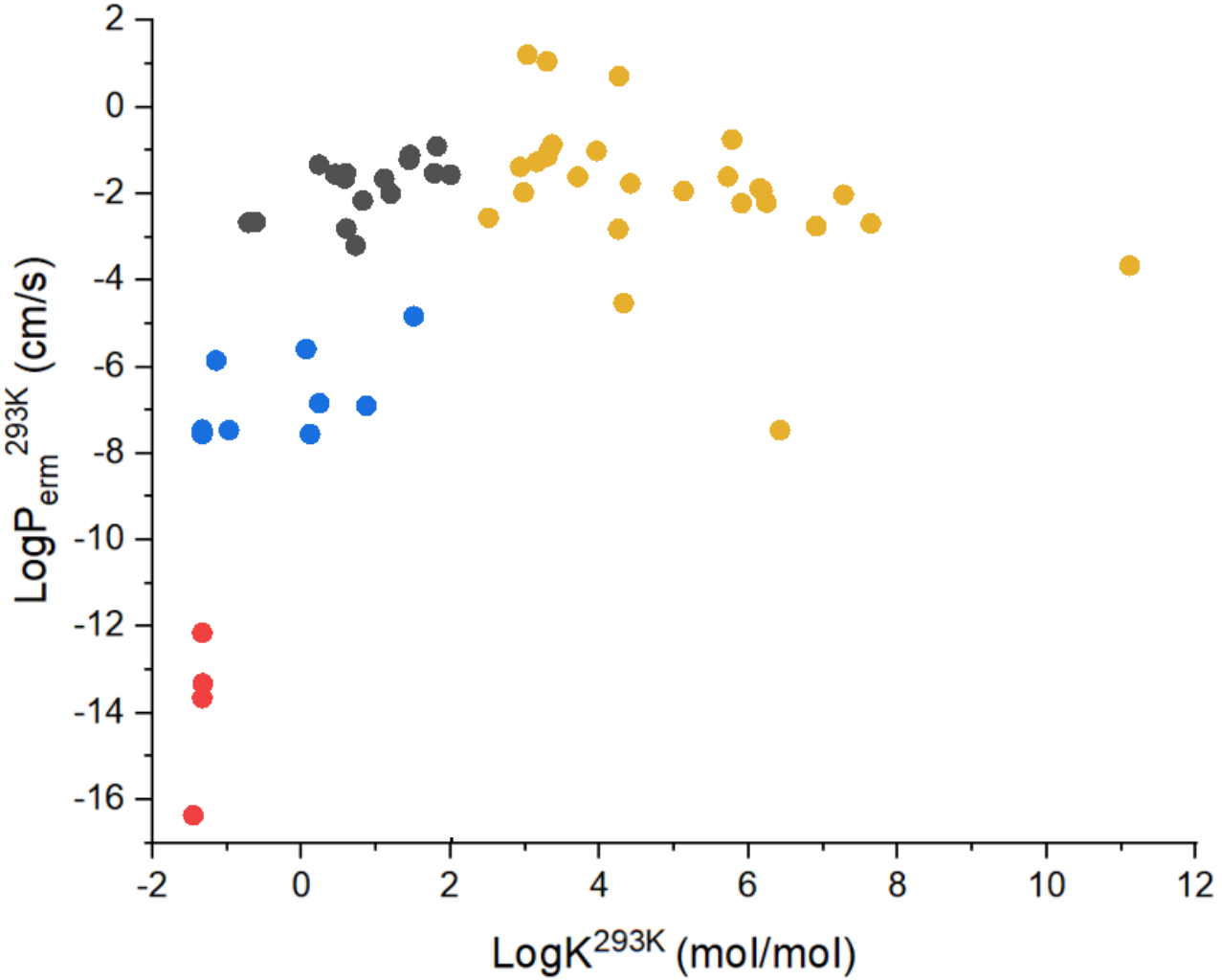
$$K_{\mathrm{lipid/water}} = e^{\frac{-\Delta G_{\mathrm{i}}(\mathrm{min})}{RT}}$$

6. Permeation

$$\frac{1}{P} = \int_{-\frac{L}{2}}^{\frac{L}{2}} \frac{1}{D_{\mathrm{i}}(r)} e^{\frac{G_{\mathrm{i}}(r)}{kT}} \, dr$$

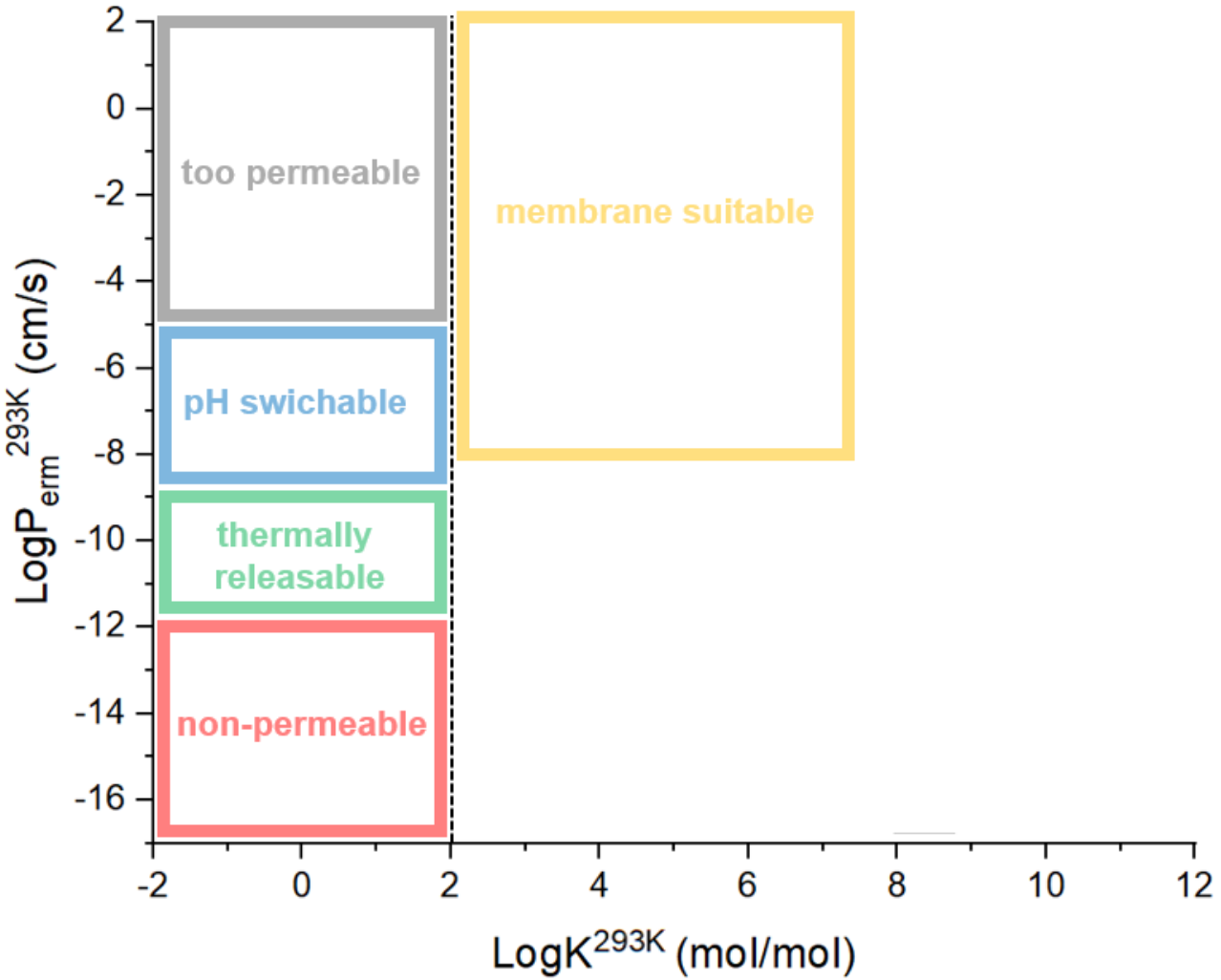
Calculation of 56 APIs

- From DrugBank Database
- Partitioning and permeation
- Plot into 2D space
- Experimental confirmation (cycloserine)

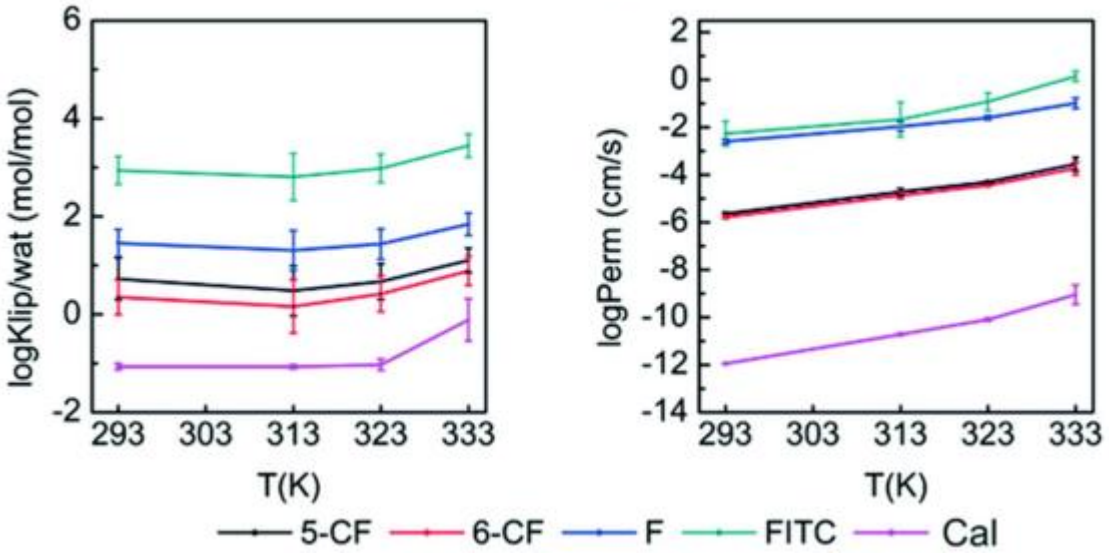


Space to work with – LBCS

- boxes for sorting molecules
- The proposition of **LBCS – liposome biopharmaceutics classification system**
- Can work as a BCS analogue for liposomes

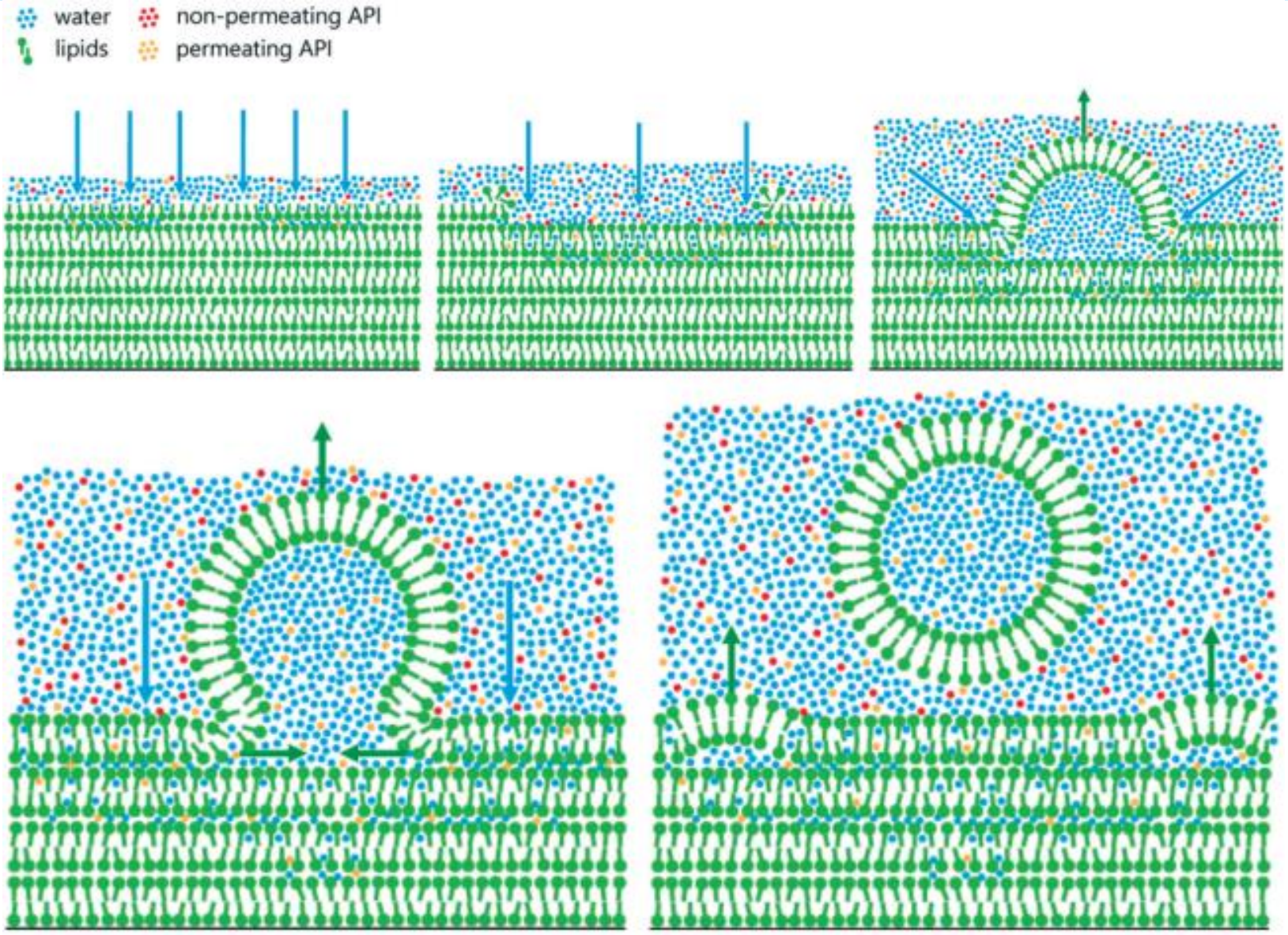


Comparison of computation and experiment



Dye	„BCS“	Encapsulated?	Thermal release
Carboxyfluorescein (CF)	1-3	YES	YES
Fluorescein (F)	1	Nearly NO	NO
Fluorescein-isothiocyanate (FITC)	2	Something	NO
Calcein	3	NO	NO

Theory of permeation during hydration



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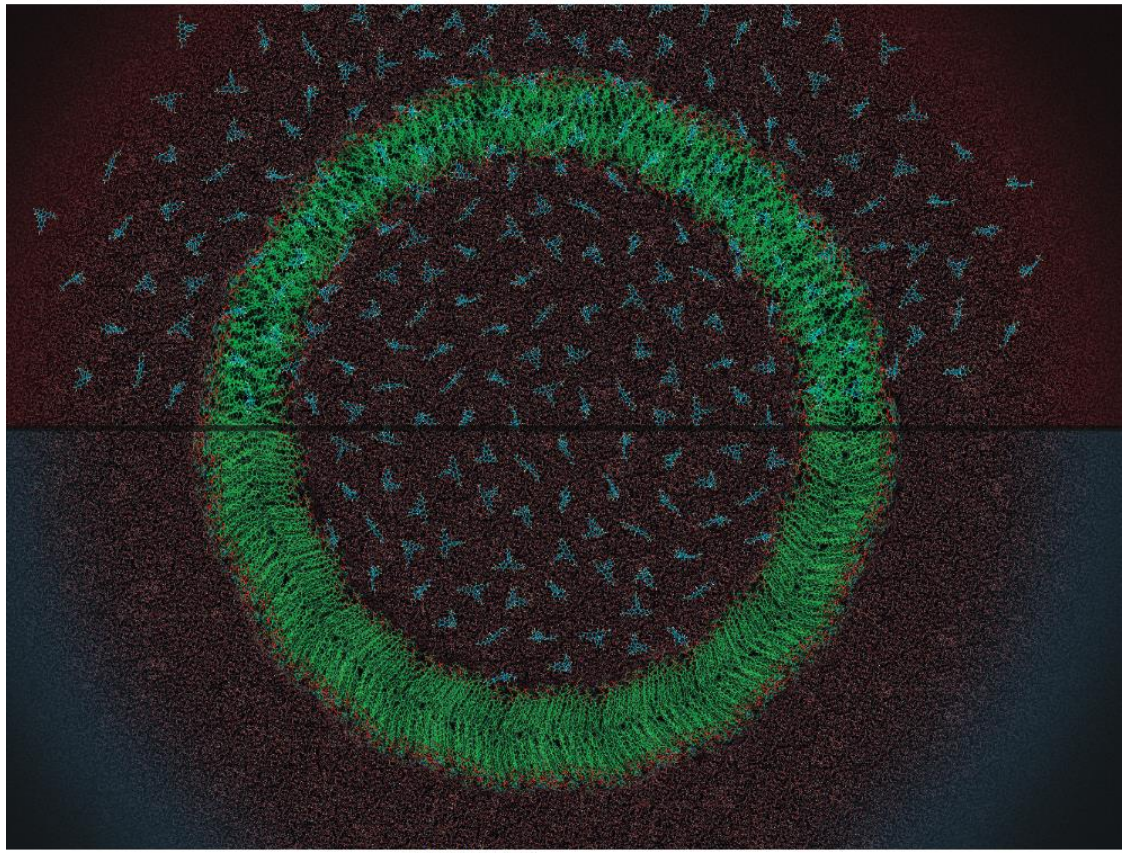

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PAPER

František Štěpánek, Karel Berka *et al.*

In silico screening of drug candidates for thermoresponsive liposome formulations

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Question n. 2

Can a molecule be improved with regards to the liposome formulability?

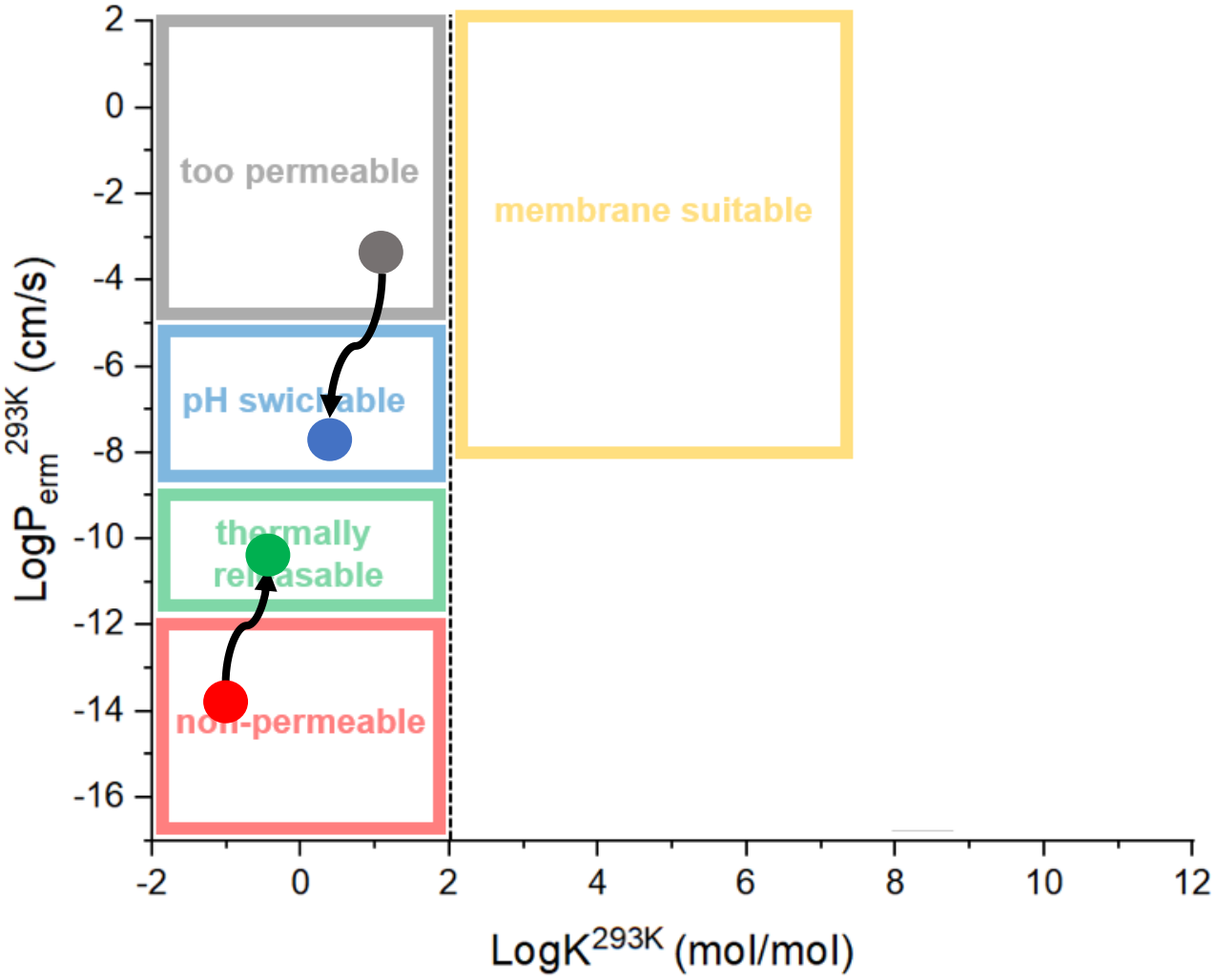
Question(s) n. 2

Can a molecule be improved with regards to the liposome formulability?

Can a molecule be moved in LBCS space?

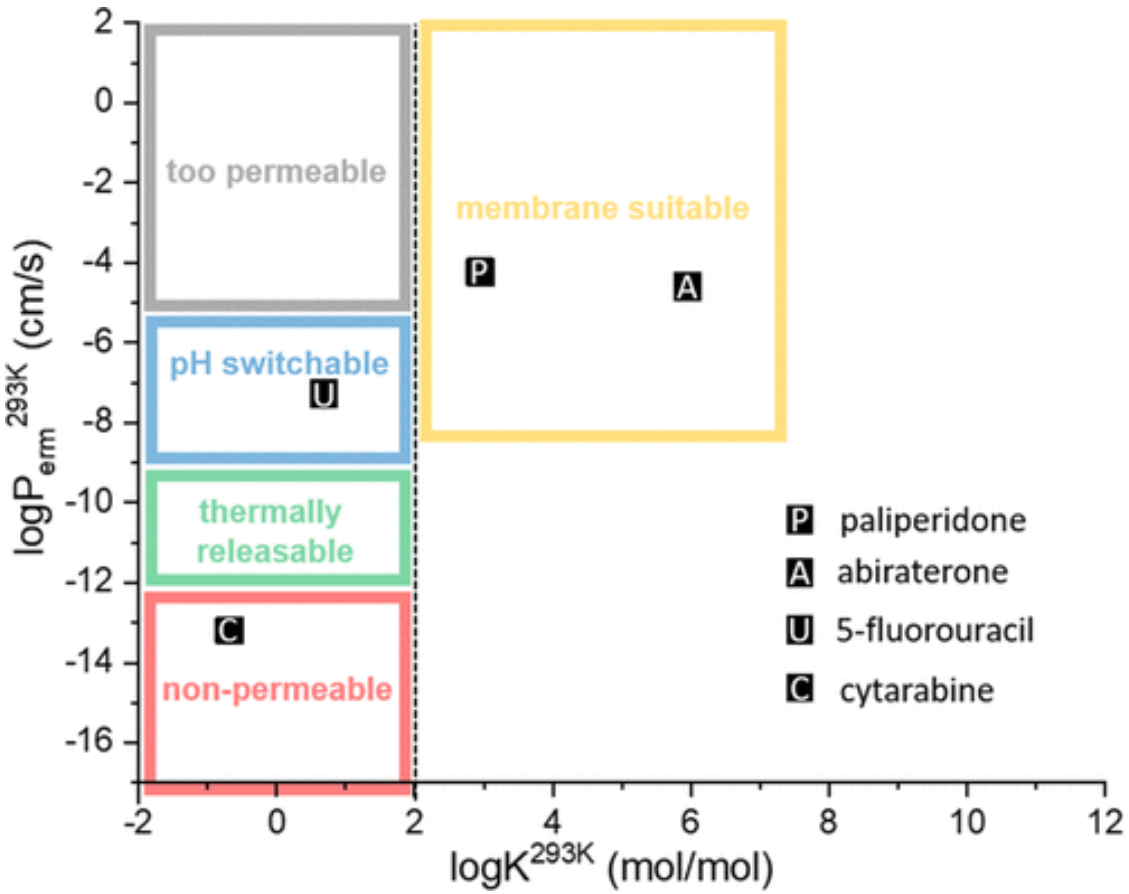
Question(s) n. 2 in LBCS space

Can we move a drug from one box to another?



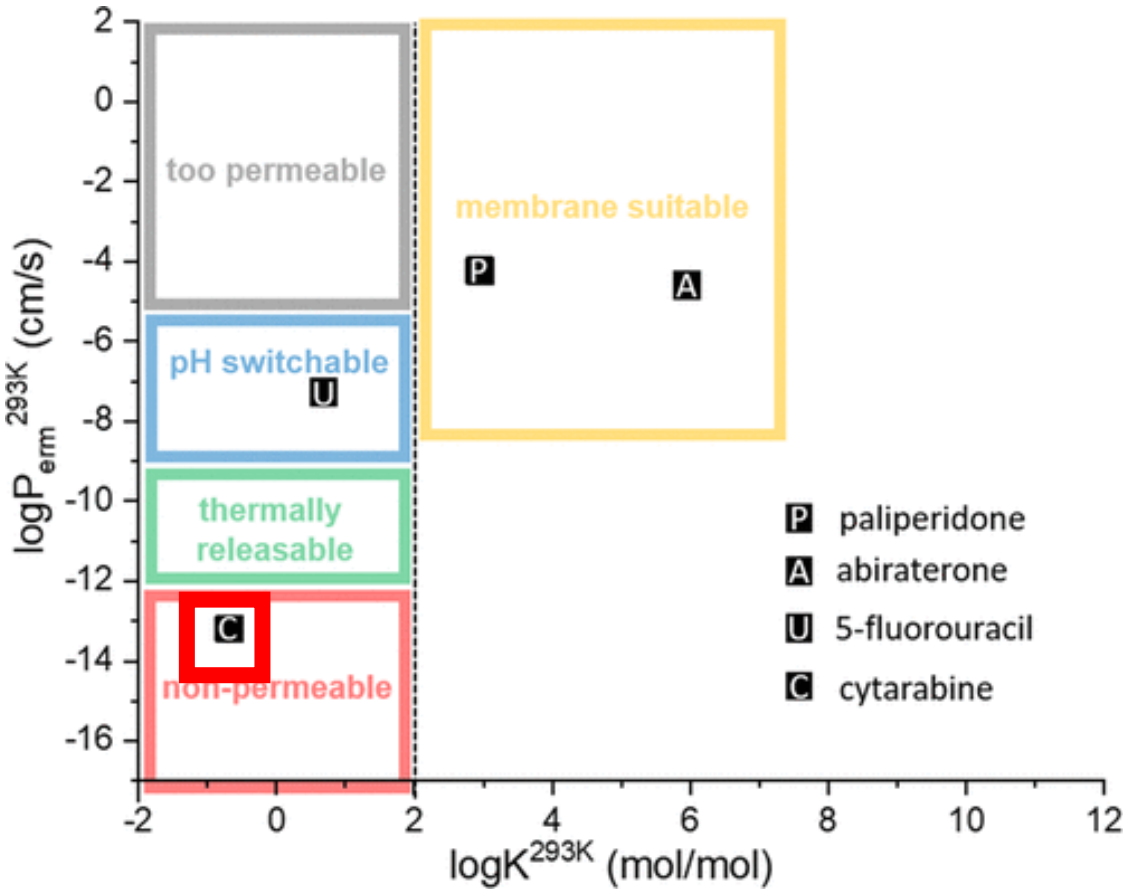
Considered molecules for this problem

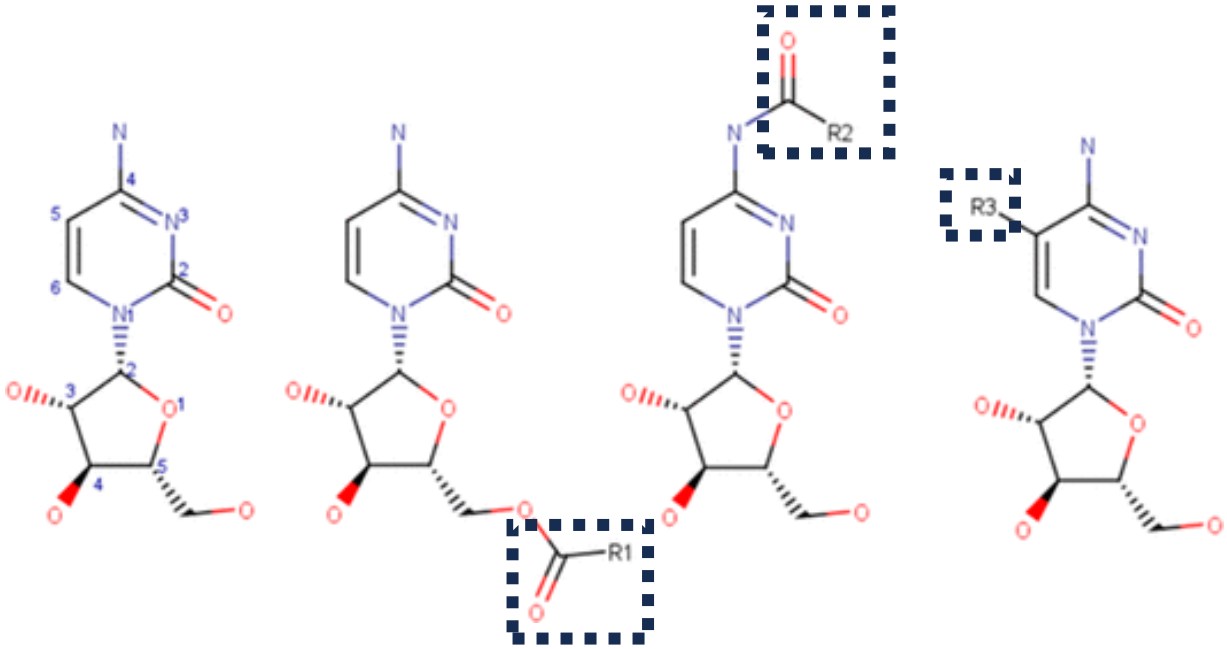
- 4 molecules from different LBCS classes
- Cytarabine „needs“ permeability increase
- Other 3 „need“ permeability decrease



Cytarabine permeability increase

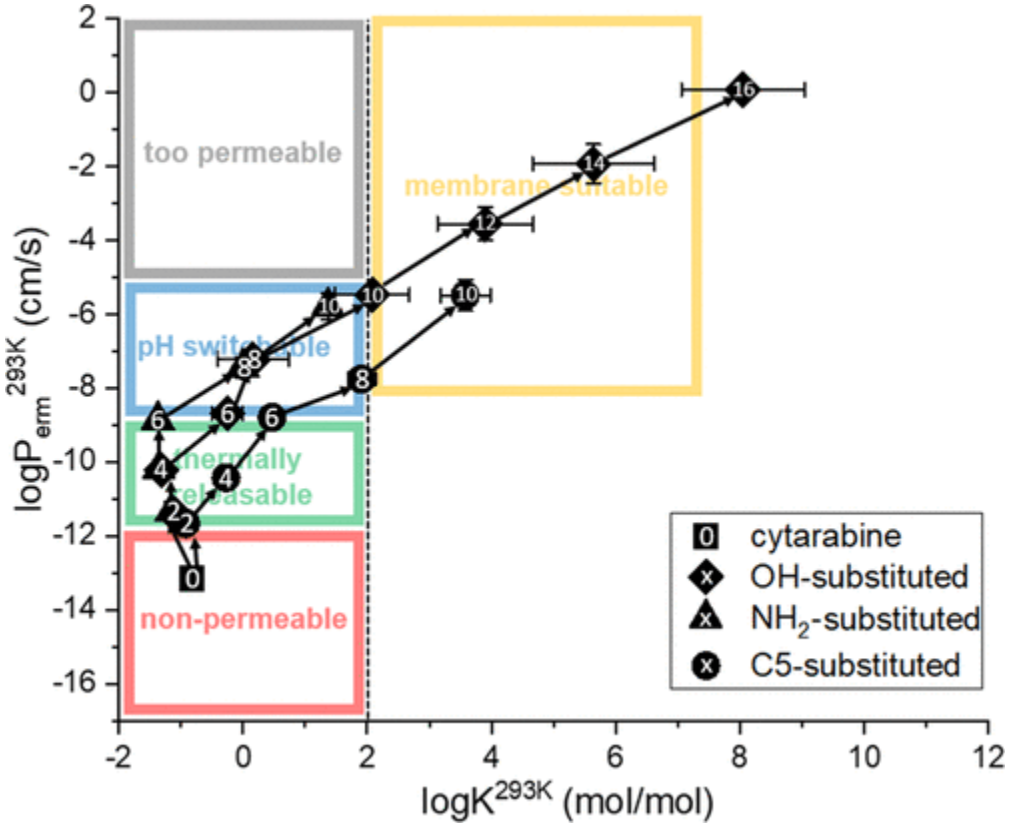
- 3 ways to increase it: ester, amid, simple carbon chain addition





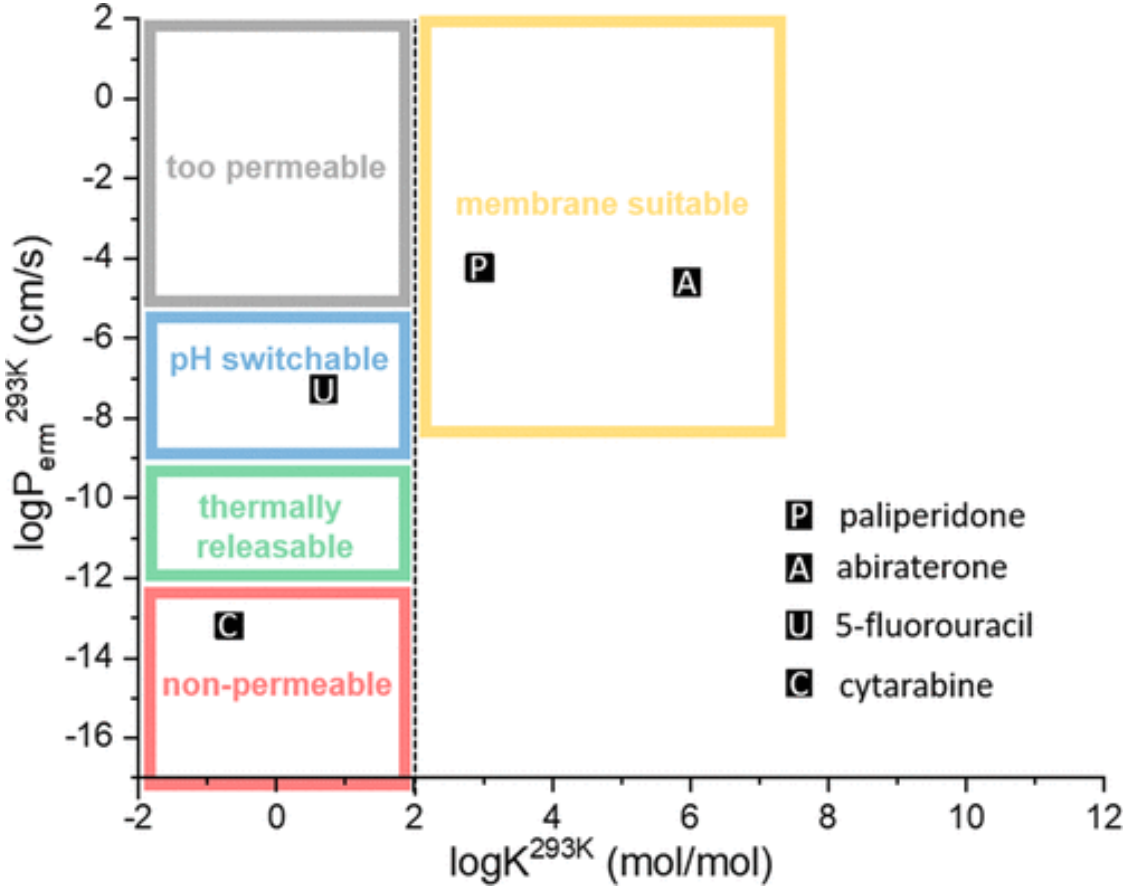
Cytarabine permeability increase results

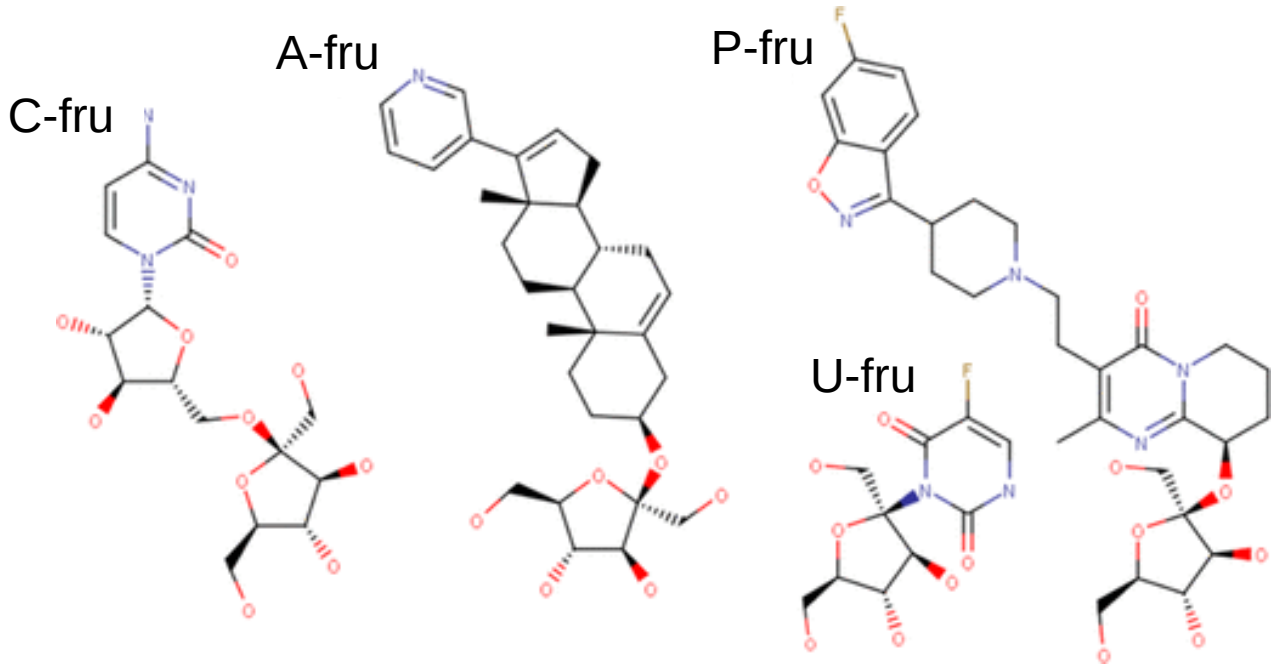
- It works – permeability increases
- Partition coefficient changes not monotonously



Permeability decrease

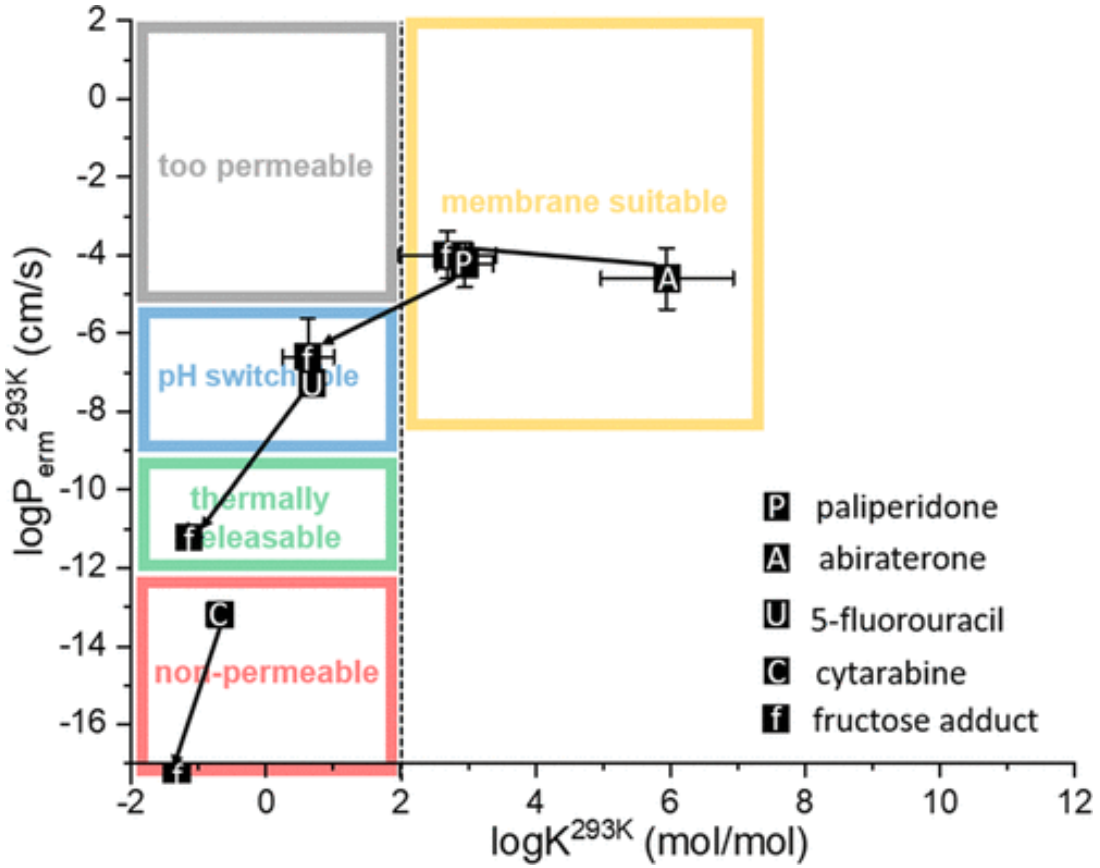
- All 4 molecules taken as a starting points
- Fructose attachment was tested



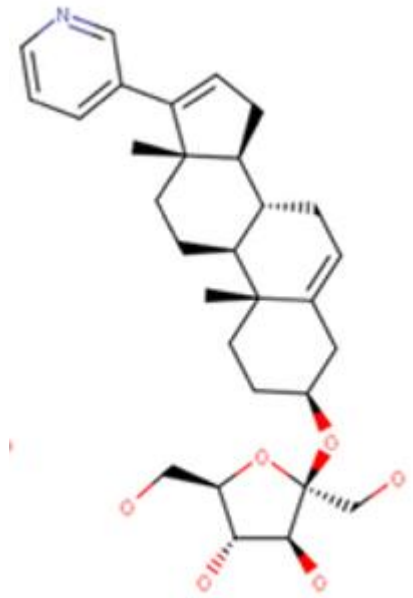


Permeability decrease results

- Permeability does not always change
- Molecule modification is not universal



A-fru



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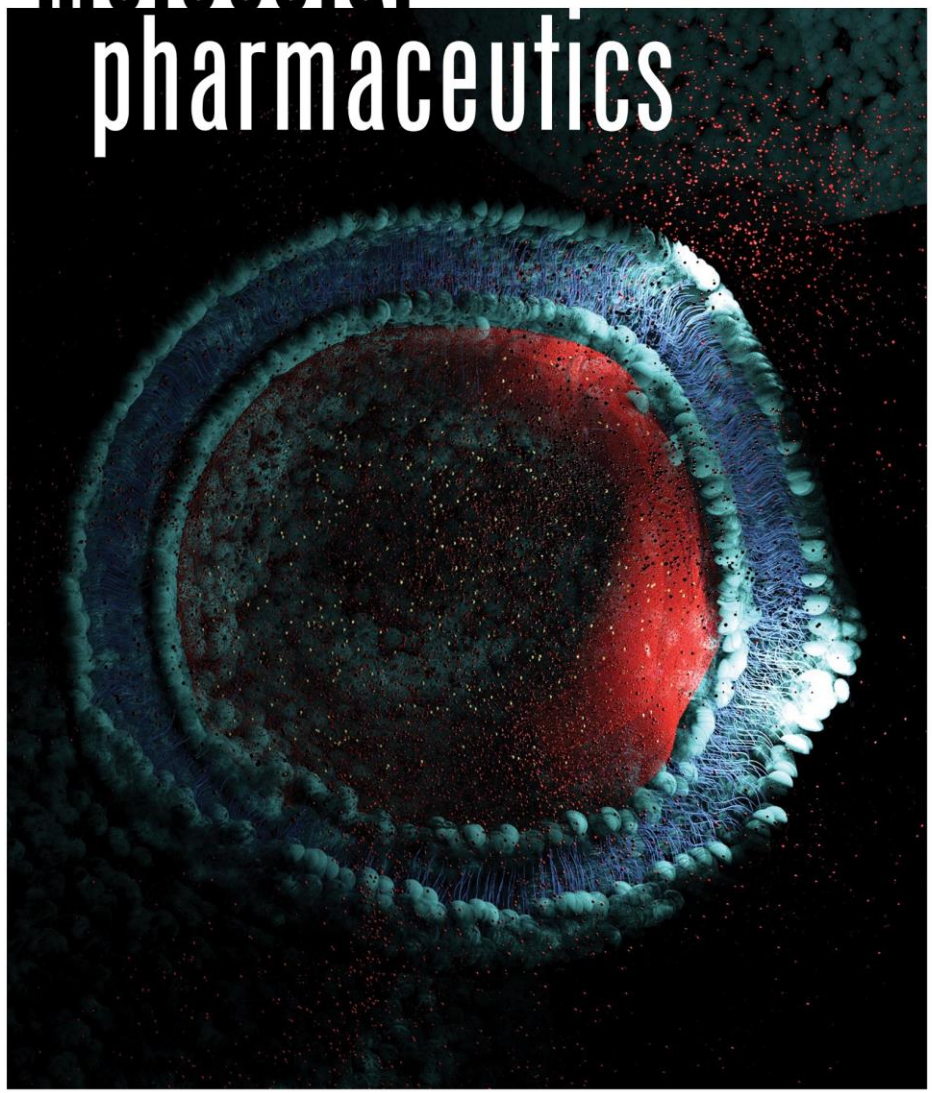


Question n. 3

Can the permeabilities be measured?

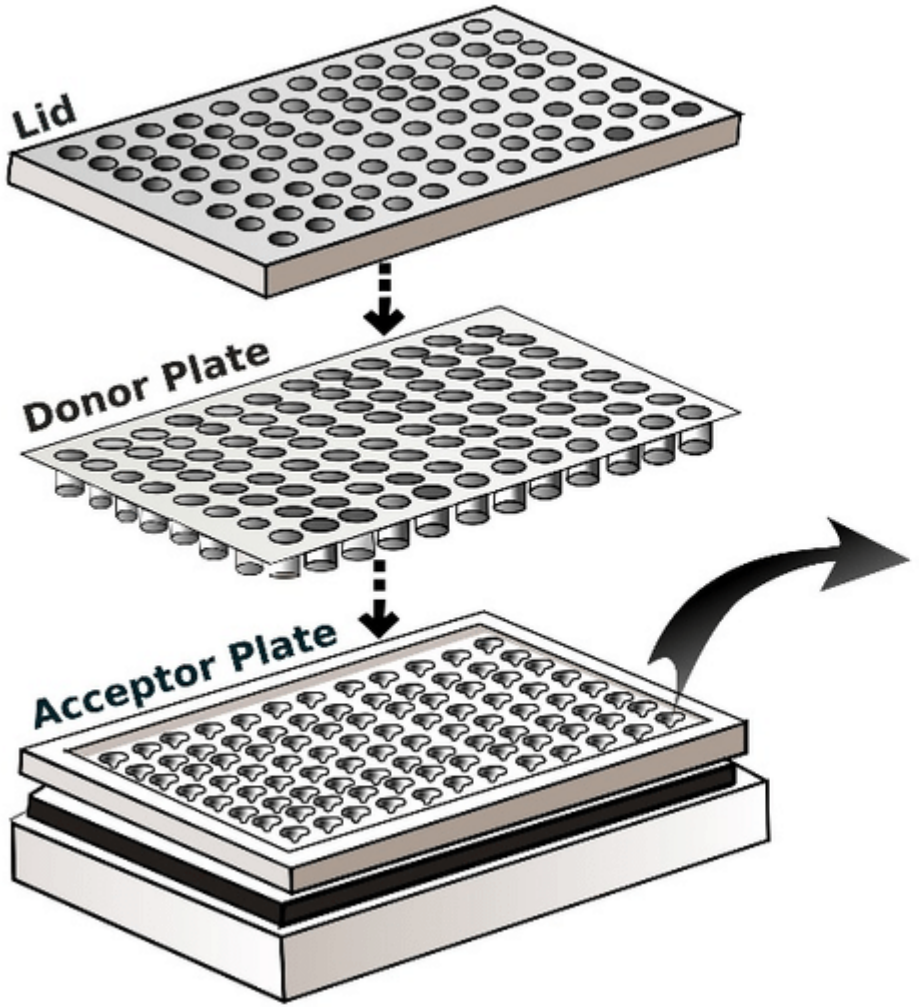
Question(s) n. 3

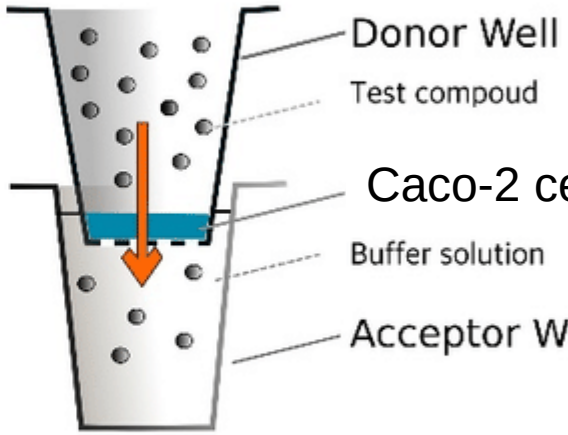
Can the permeabilities be measured?

Can the permeablilities of slow-permeating molecules be measured?

Permeability measurement common methods

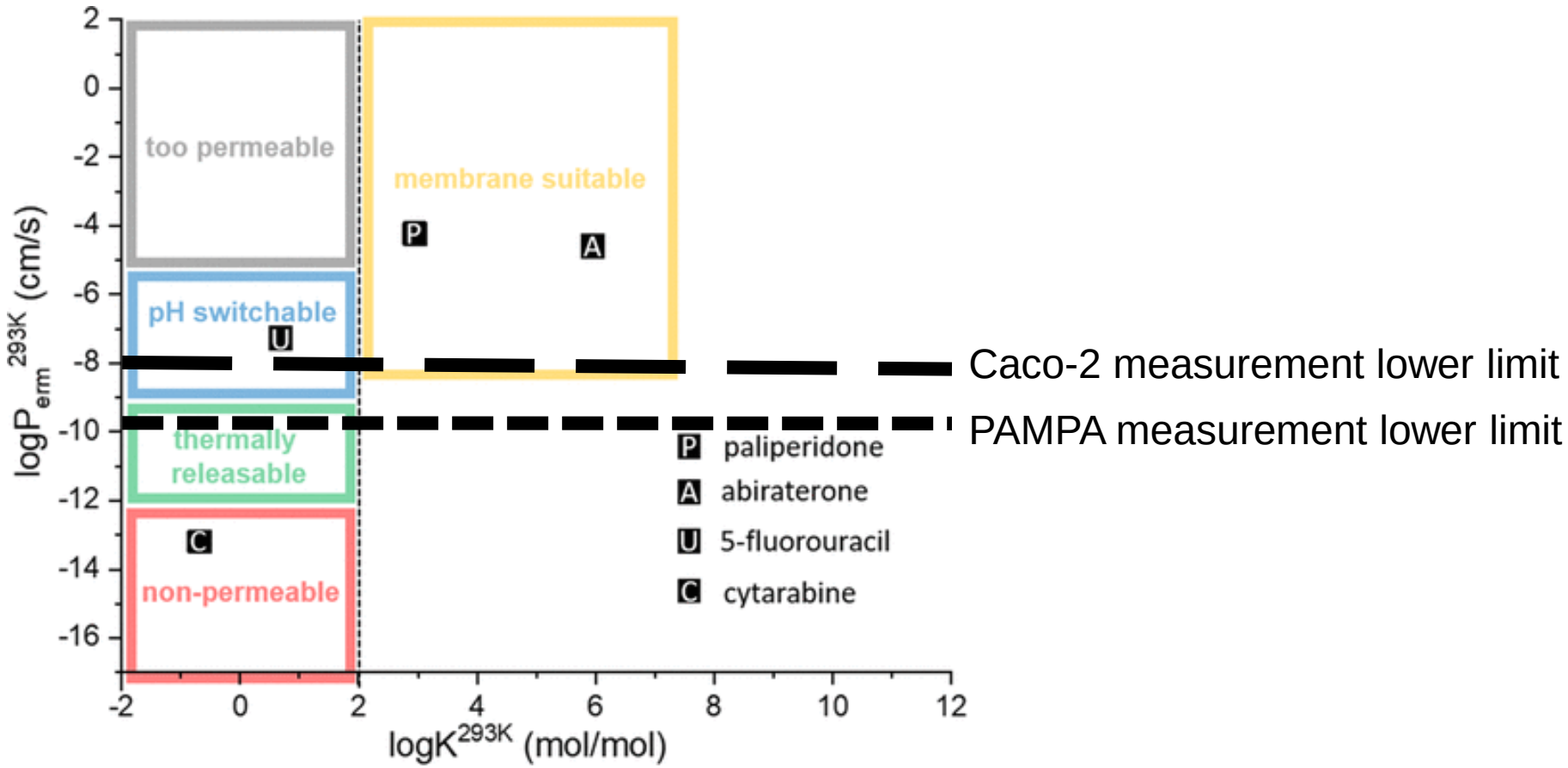
- Caco-2, PAMPA





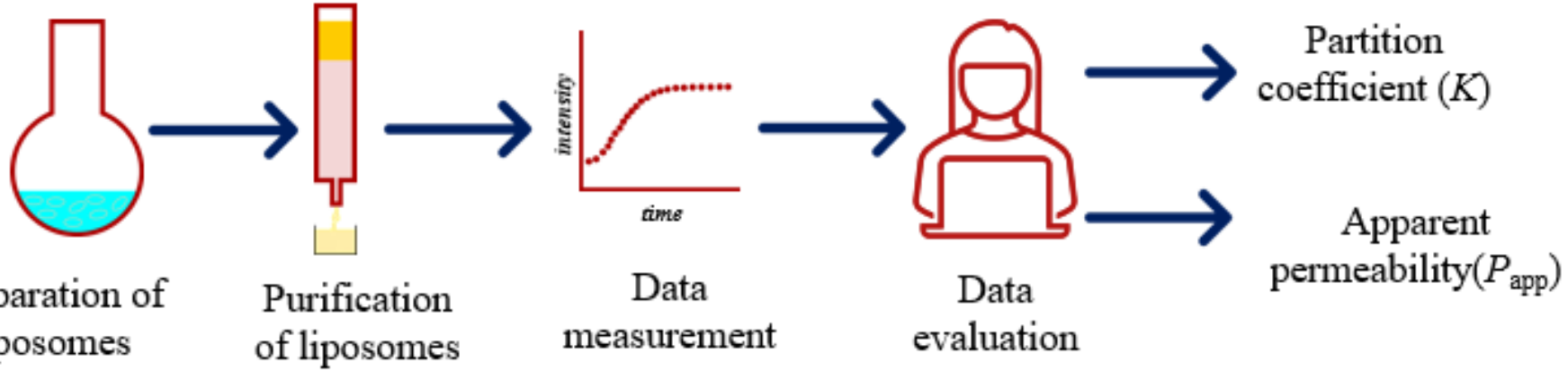
Permeability measurement

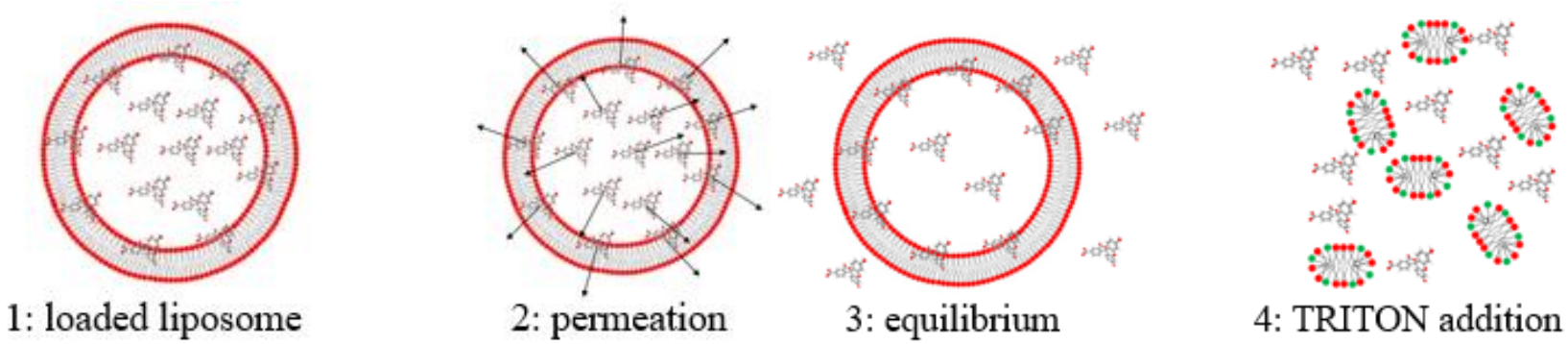
- The most common measurement methods – Caco-2, PAMPA

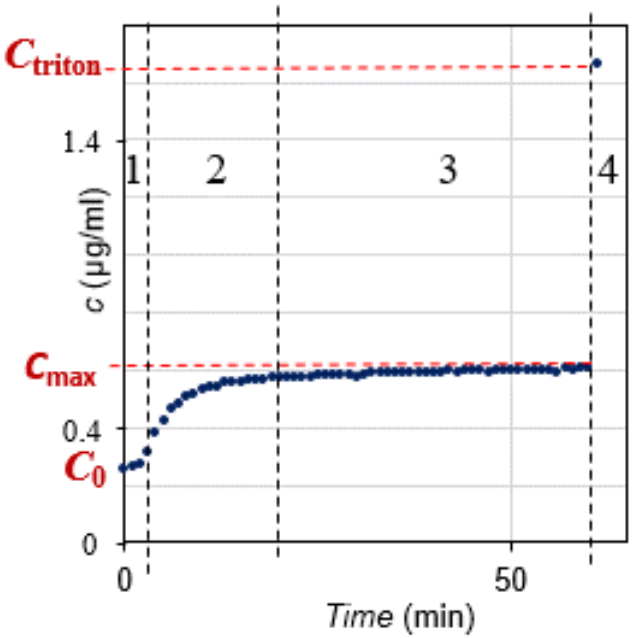


Utilisation of liposomes for permeation measurement

- Fluorescence-based method

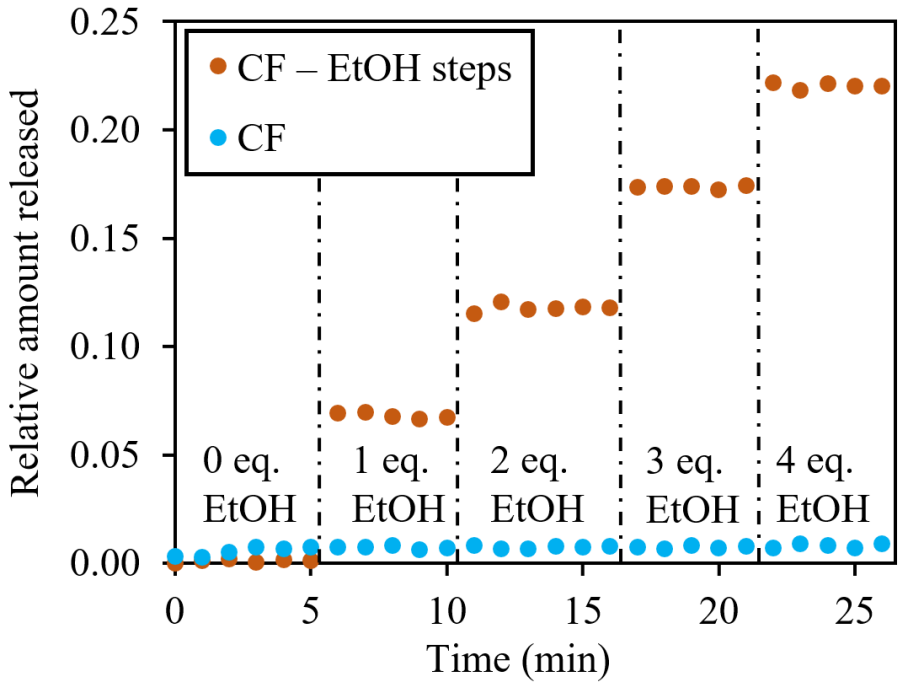


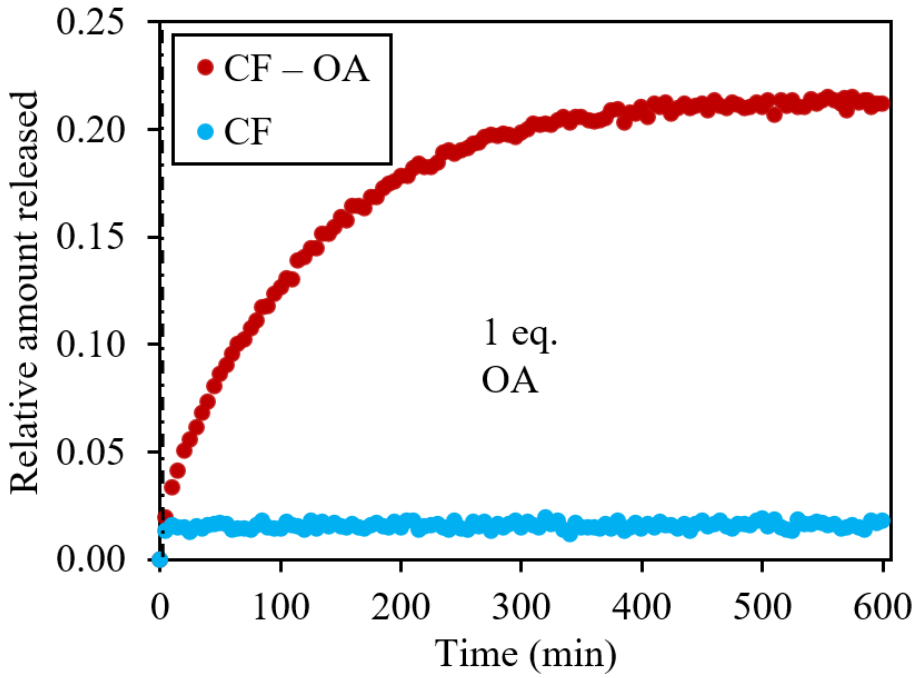




Direct observation of permeation enhancement

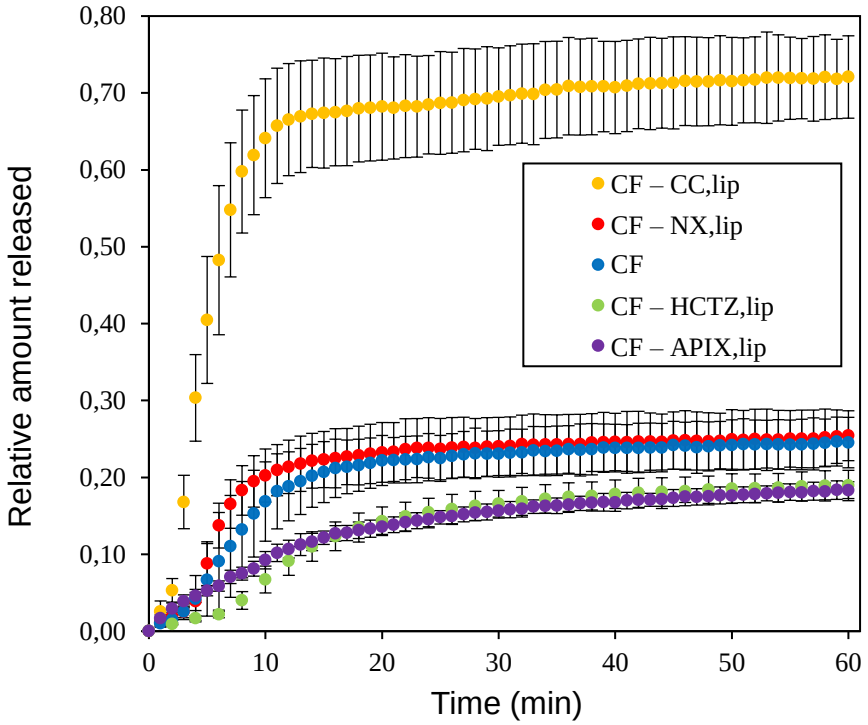
- Two different known mechanisms directly observed





Co-permeation of other drugs

- Addition of second molecule affects both partitioning and permeability
- The consequences can be numerous



Marking of the sample	Permeability (cm/s)	Partition coefficient
CF	$(1.4 \pm 0.4) \cdot 10^{-8}$	1.6 ± 0.1
CF – CC,lip	$(2.2 \pm 0.7) \cdot 10^{-8}$	0.32 ± 0.09
CF – APIX,lip	$(3.1 \pm 0.4) \cdot 10^{-9}$	1.9 ± 0.1
CF – HCTZ,lip	$(1.1 \pm 0.1) \cdot 10^{-8}$	2.7 ± 0.3
CF – NX,lip	$(2.2 \pm 0.2) \cdot 10^{-8}$	2.1 ± 0.3

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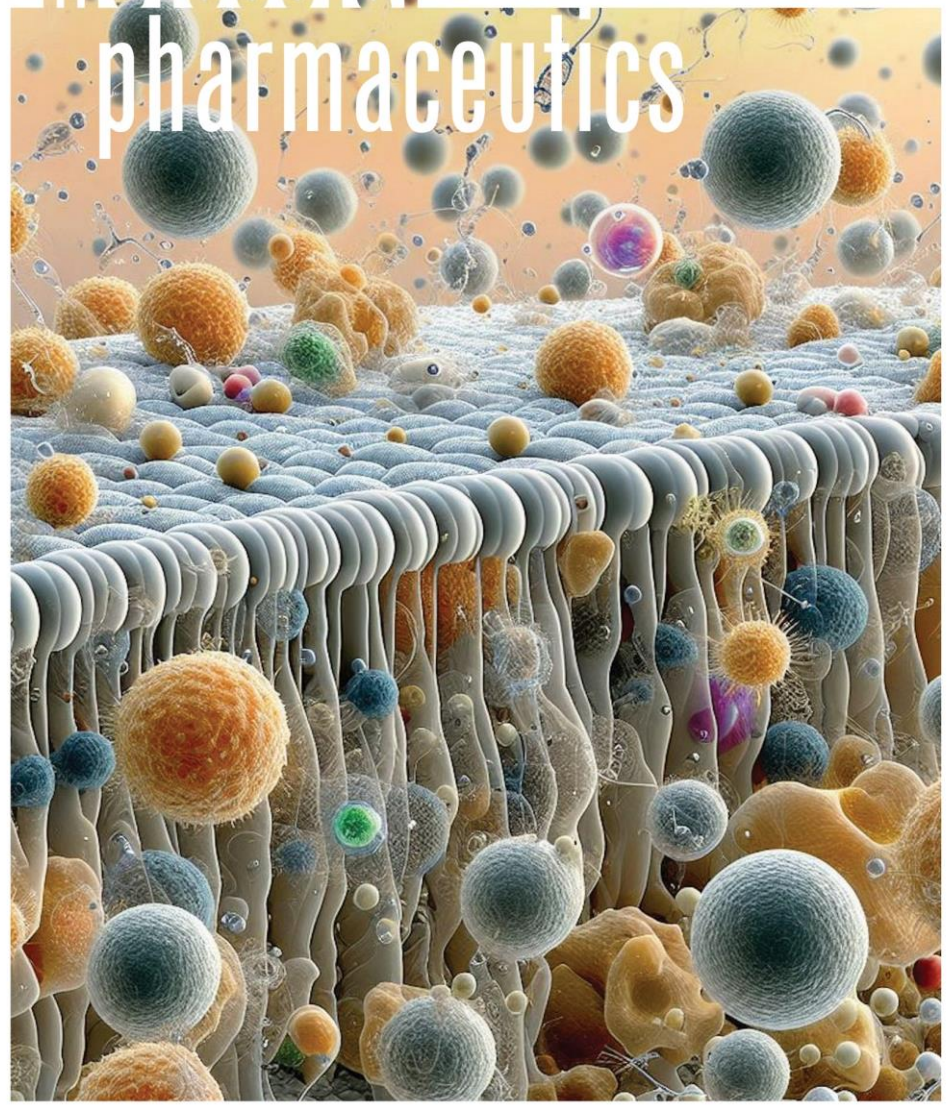

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Biomembrane permeability

- Critical parameter for drug formulability into liposomes
- Can be tuned using „right“ prodrugs
- Is **not** independent on other permeating molecules

Future work

- Deeper understanding of the cooperative permeation properties
- Application of the prodrugs
- Insight into the permeation measurement methods

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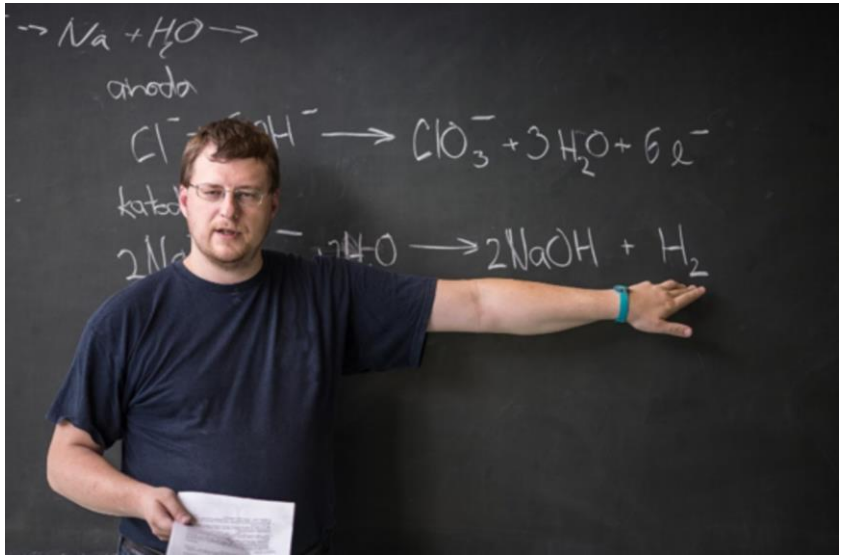

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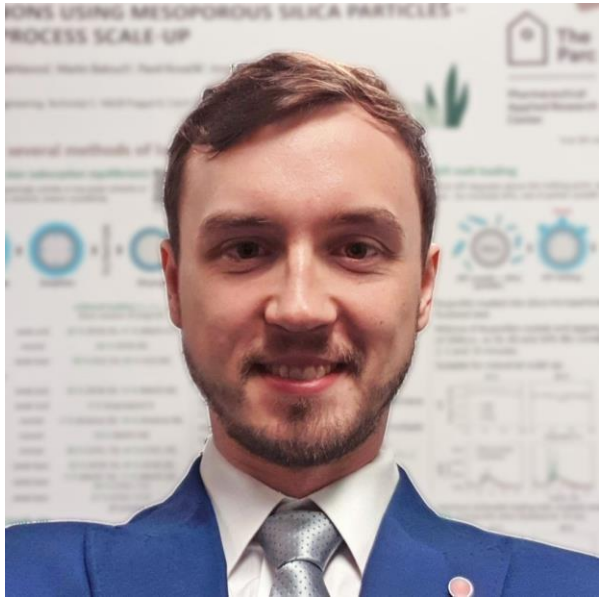

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- Cooperators on other publications, students

Discussed publications

Balouch, M.; Šrejber, M.; Šoltys, M.; Janská, P.; Štěpánek, F.; Berka, K., In silico screening of drug candidates for thermoresponsive liposome formulations. *Molecular Systems Design Engineering* **2021**, *6* (5), 368-380.

Balouch M.; Storchmannová K.; Štěpánek F.; Berka K., Computational prodrug design methodology for liposome formulability enhancement of small-molecule APIs, *Molecular Pharmaceutics* **2023**, *20* (4), 2119–2127

Odehnalová K. & Balouch M., Storchmannová K., Petrová E., Konefał M., Zadražil A., Berka K., Brus J., Štěpánek F., Liposomal co-permeation assay reveals unexpected membrane interactions of commonly prescribed drugs, *Molecular Pharmaceutics* **2024**, *21* (6), 2673–2683