Molecular dynamics study of the anticancer drug dioxadet transfer across the lipid membrane DOPC

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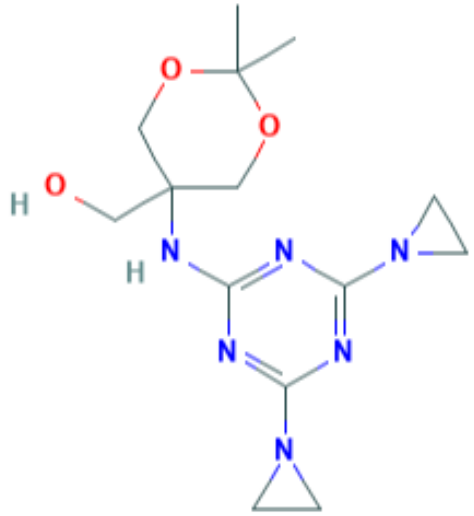
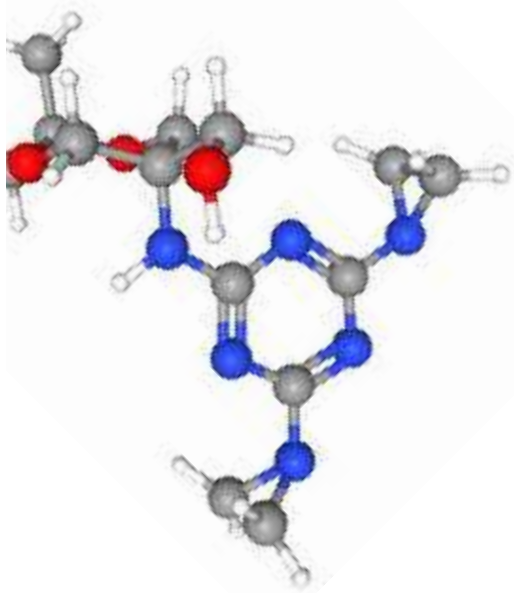
Dioxadet

- Developed at the Petrov Research Institute of Oncology in St Petersburg.
- Undergone II phase of clinical trials.
- Is used in chemotherapy for ovarian, colorectal and liver cancer

Goal:

reveal the mechanism of dioxadet penetration through the cell **membrane** using the MD method.

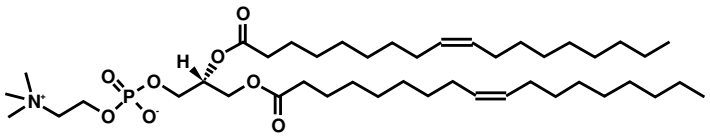




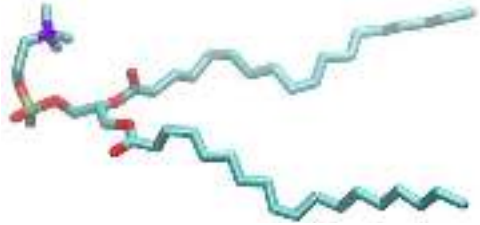
IUPAC Name: [5-[[4,6-bis(aziridin-1-yl)-1,3,5-triazin-2-yl]amino]-2,2-dimethyl-1,3-dioxan-5-yl]methanol

Membrane

DOPC lipid scheme:



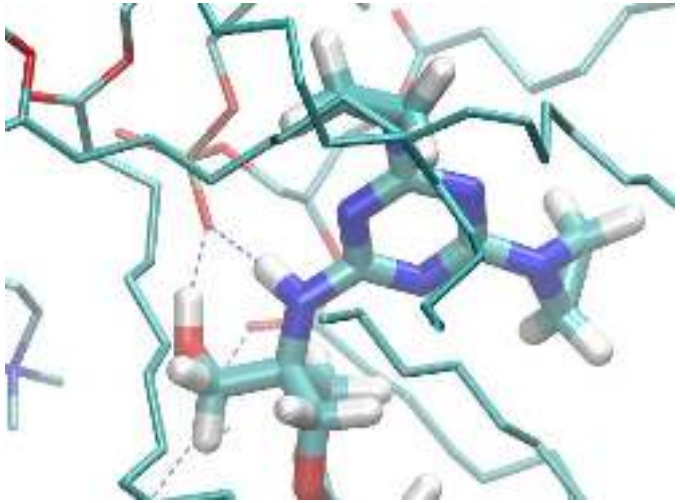
Poger* model of DOPC lipid:



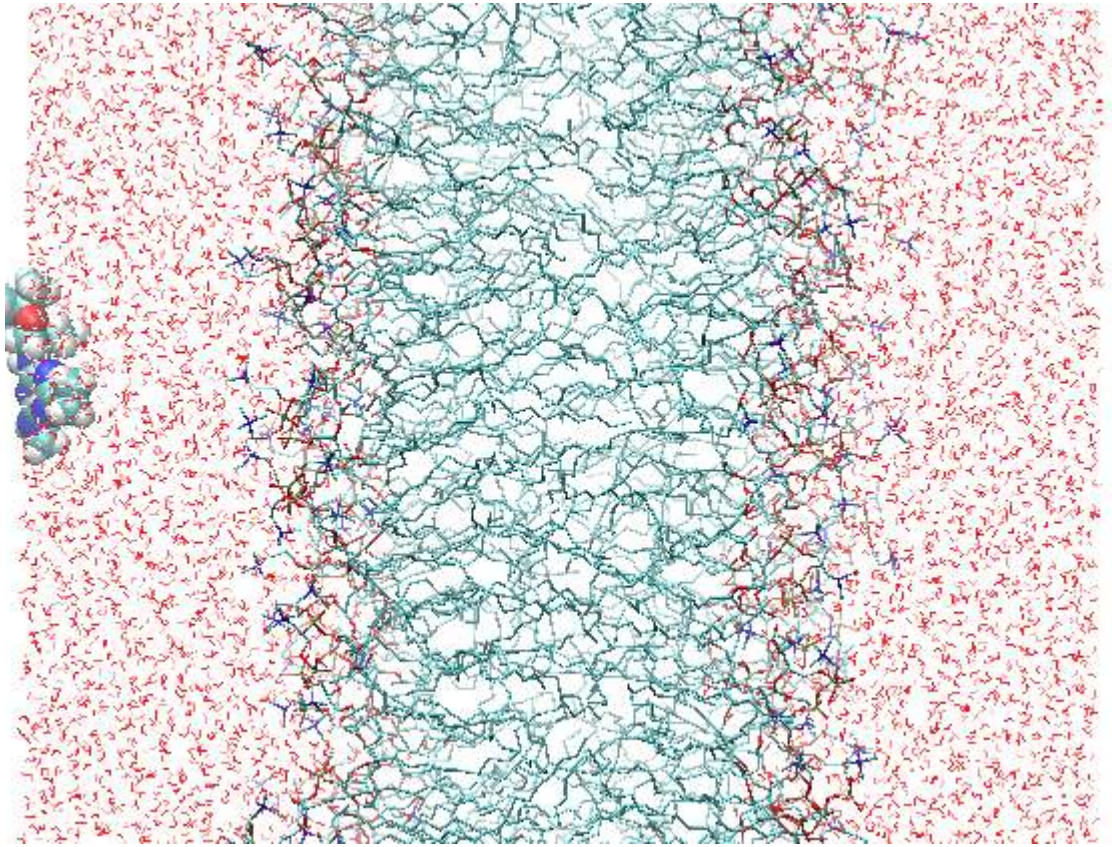
S-lipid**:

* Poger, David, Wilfred F. Van Gunsteren, and Alan E. Mark. "A new force field for simulating phosphatidylcholine bilayers." J. of Comput. Chem. 31.6 (2010): 1117-1125.
** Grote, Fredrik, and Alexander P. Lyubartsev. "Optimization of slipids force field parameters describing headgroups of phospholipids." JPC B 1`24.40 (2020): 8784-8793.

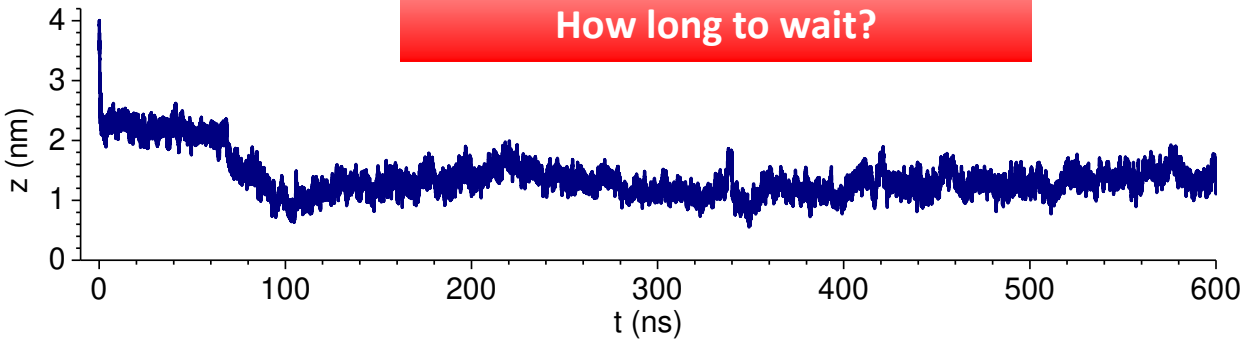
Classical molecular dynamics



- 1 dioxadet molecule
- Dioxadet model: ATB, B3LYP by E. Yakush (see poster 76)
- 128 DOPC lipids (Poger model)
- ~5668 SPC water molecules
- T = 323 K, v-rescale thermostate
- P=1 bar, Nose-Hoover barostate, semiisotropic
- Production run of 600 ns
- Box size 6.5 x 6.5 x 8.0 nm;



Passing through the membrane?
How long to wait?

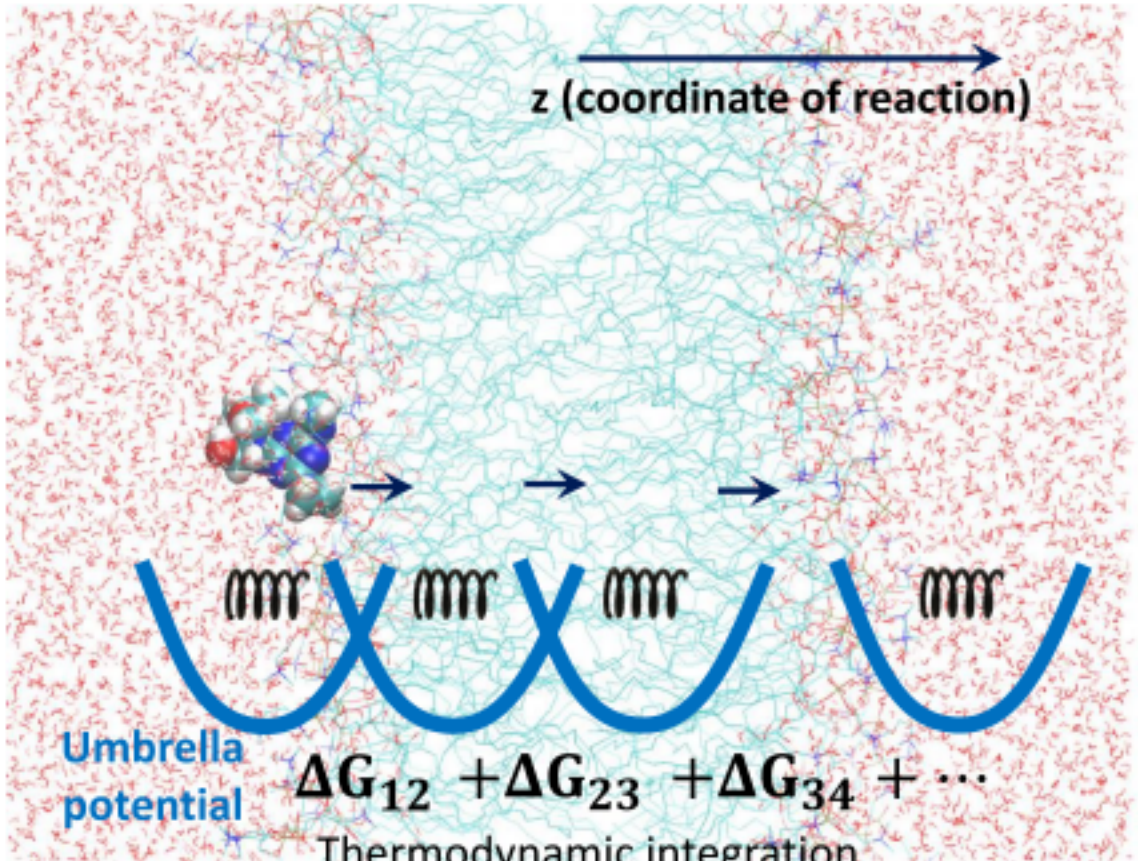


Free energy calculation

- Umbrella sampling;
- 1 or 2 dioxadet molecules
- Dioxadet model: ATB* or B3LYP + Amber
- 128 DOPC lipid molecules (Poger or S-lipid model)
- ~6000-7000 SPC water molecules
- T = 300, 305, 310 K, v-rescale thermostate
- P=1 bar, Nose-Hoover barostate, semiisotropic
- 20 umbrella windows, simmetrized
- Production runs of 300 ns in each umbrella-window
- Box size ~6.4 x 6.4 x 8.4 nm;
- WHAM for energy profile reconstruction.

Summarized calculation time is 23 μs, (175 days on videocard GeForce GTX 1080, 2560 CUDA cores or GeForce RTX 2070)

Umbrella sampling



Thermodynamic integration

Potential of mean force is being reconstructed

* Stroet M, Caron B, Visscher K, Geerke D, Malde AK, Mark AE. Automated Topology Builder version 3.0: Prediction of solvation free enthalpies in water and hexane. J. Chem. Theory Comput. 2018, 14, 11, 5834-5845

ΔG between the two states

$$\Delta G = -kT \ln Q_1/Q_2$$

Let's do an identity transformation:

$$\frac{Q_1}{Q_2} = \frac{Q_1}{Q_2}\frac{\sum_i e^{-\frac{U_2+U_1}{kT}}}{\sum_i e^{-\frac{U_2+U_1}{kT}}} = \frac{\left\langle e^{-\frac{U_1}{kT}}\right\rangle_2}{\left\langle e^{-\frac{U_2}{kT}}\right\rangle_1}$$

Mean potential energy!

Energy of the first system, and averaged over the ensemble of the 2nd

It works only when the states are close to each other

Recall from statistics

Probability of finding the system in microstate i

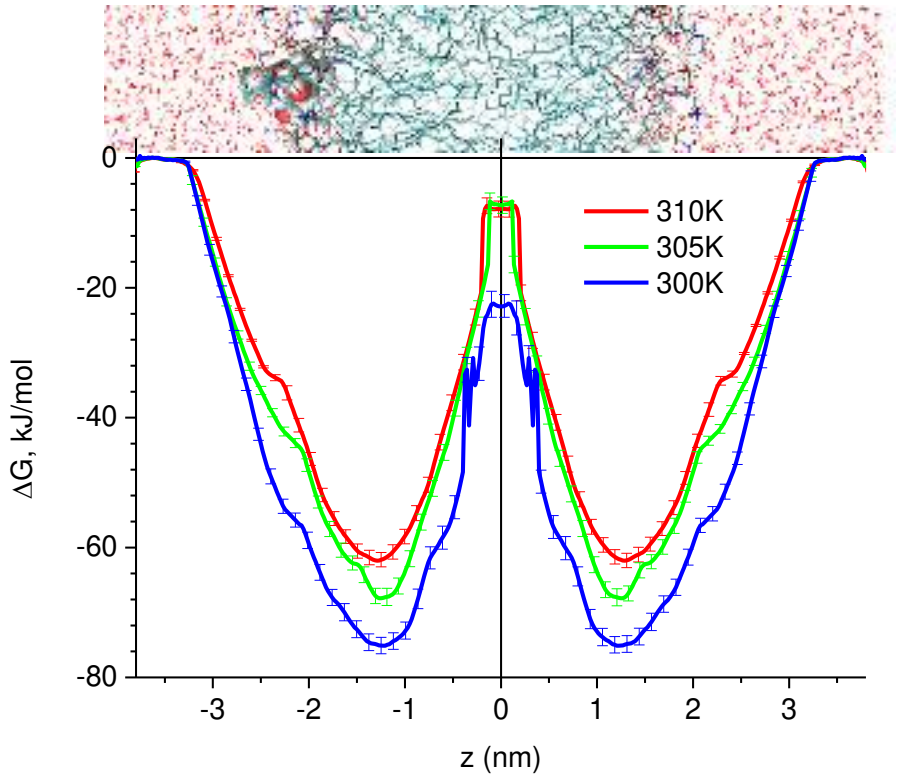
$$p_i = \frac{e^{-\frac{U_i}{kT}}}{Q}$$

where the statsum $Q = \sum_i e^{-\frac{U_i}{kT}}$

The mean of some value A :

$$\langle A \rangle = \sum_i p_i A_i = \sum_i \frac{A_i e^{-\frac{U_i}{kT}}}{Q}$$

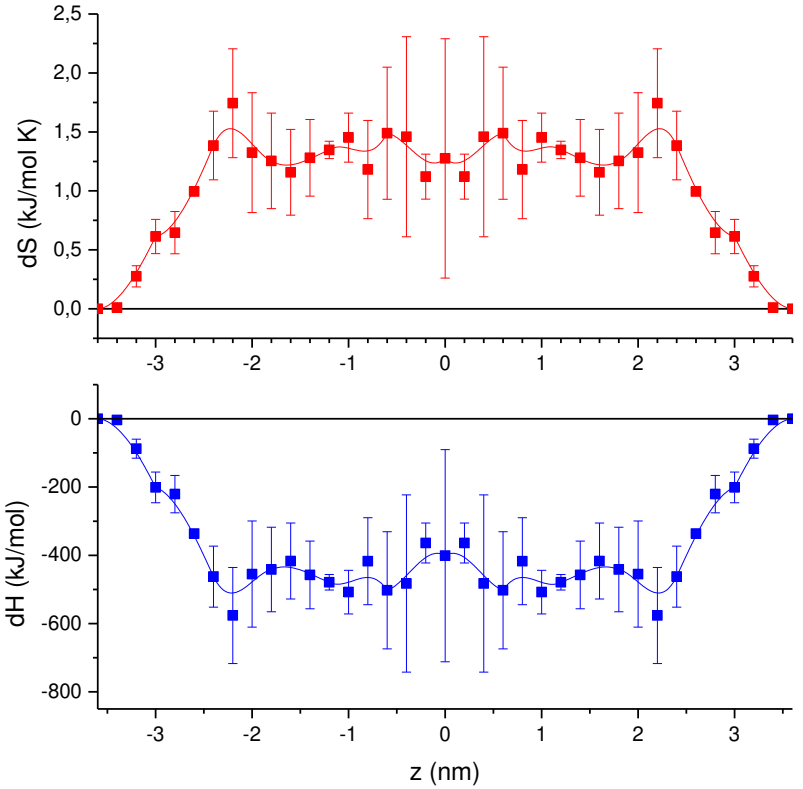
Free energy profile



The Gibbs free energy profile of the dioxadet molecule transition through the DOPC lipid bilayer at three temperatures 300, 305 and 310 K.

- A negative sign of DG in the membrane indicates a preference for dioxadet to be inside the membrane;
- In the midplane of the membrane there is an **energy barrier** whose height depends on the temperature.

The entropy and enthalpy contributions obtained from the temperature dependence:



Large positive entropy values and large modulo negative enthalpy values are observed.

The position of the dioxadet in the membrane is **entropically disadvantageous**, but overall it is advantageous due to the **enthalpy gain**.

Probably, this is due to the rather large charges on the atoms of the aromatic ring of dioxadet and the corresponding Coulomb interaction with polar heads of lipids.

Permeability and diffusion

Resistivity

Gibbs free energy

$$\frac{1}{P_{eff}} = R_{eff} = \int_0^Z \frac{e^{\beta \Delta G(z)}}{D(z)} dz \quad *$$

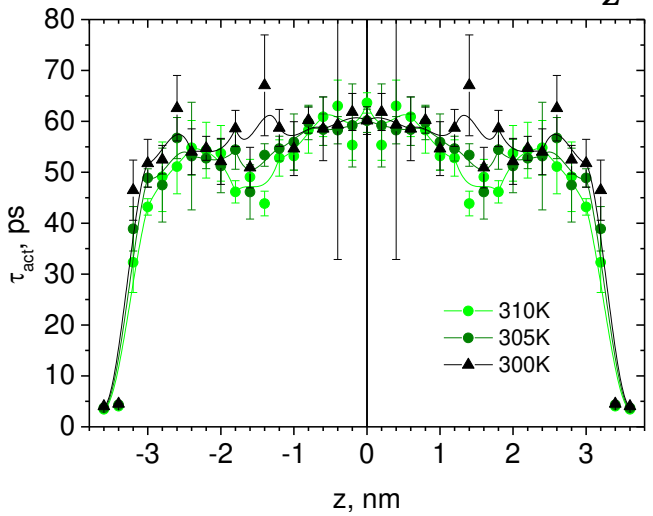
Permeability

Diffusion coefficient $D(z) = \frac{var(z)}{\tau_z}$,

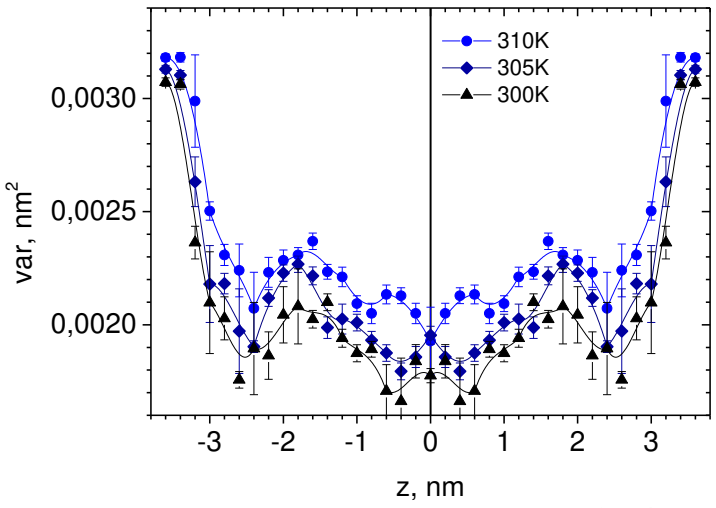
where τ_z – autocorrelation time, $var(z)$ – z axis position variation

Free energy profile is not sufficient. A diffusion coefficient profile is also needed to account for flux.

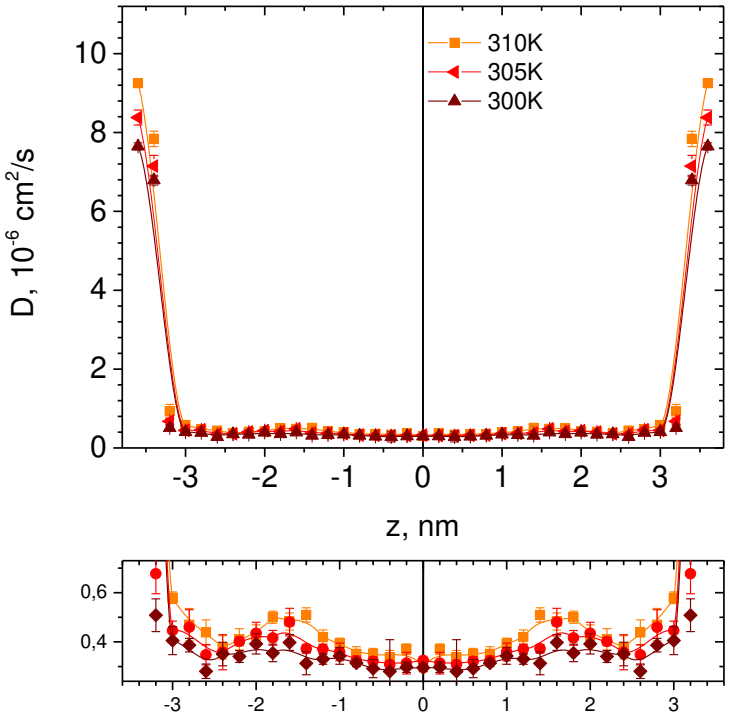
Autocorrelation time τ_z



Displacement variance $var(z)$



Diffusion coefficient



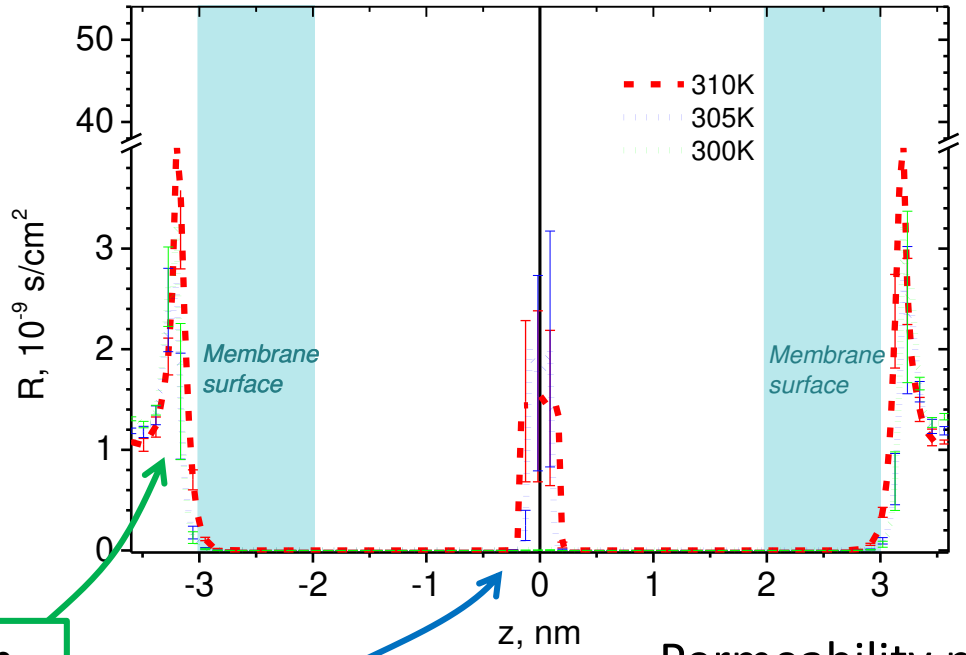
* Marrink, S. J.; Berendsen, H. J. C. Simulation of water transport through a lipid membrane. *J. Chem. Phys.* **1994**, *98*(15), 4155-4168

Resistivity

$$\frac{1}{P_{eff}} = R_{eff} = \int_0^Z \frac{e^{\beta \Delta G(z)}}{D(z)} dz$$

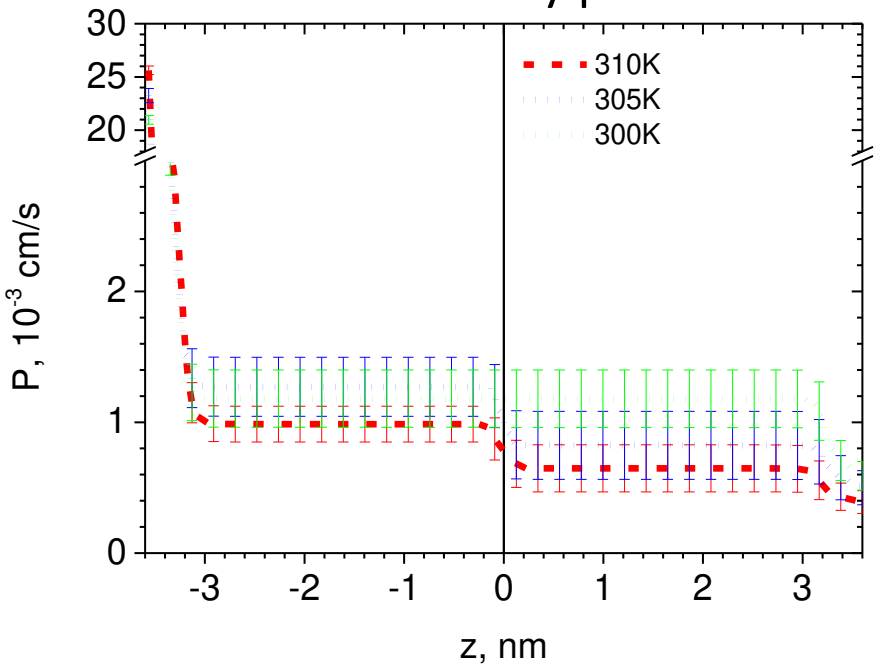
Permeability

Local resistivity

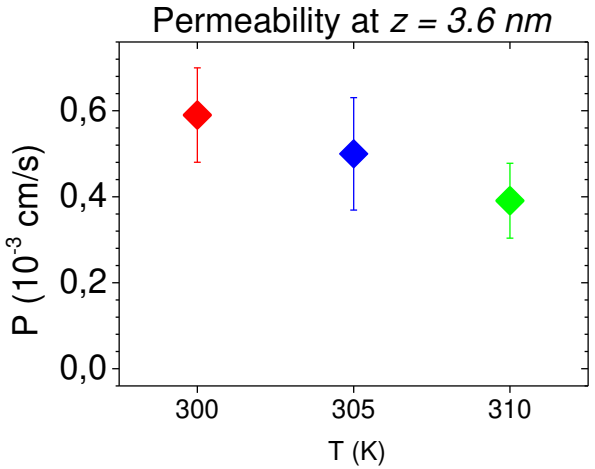


The highest resistance is found on the membrane surface at the entrance and exit of the membrane

Permeability profile

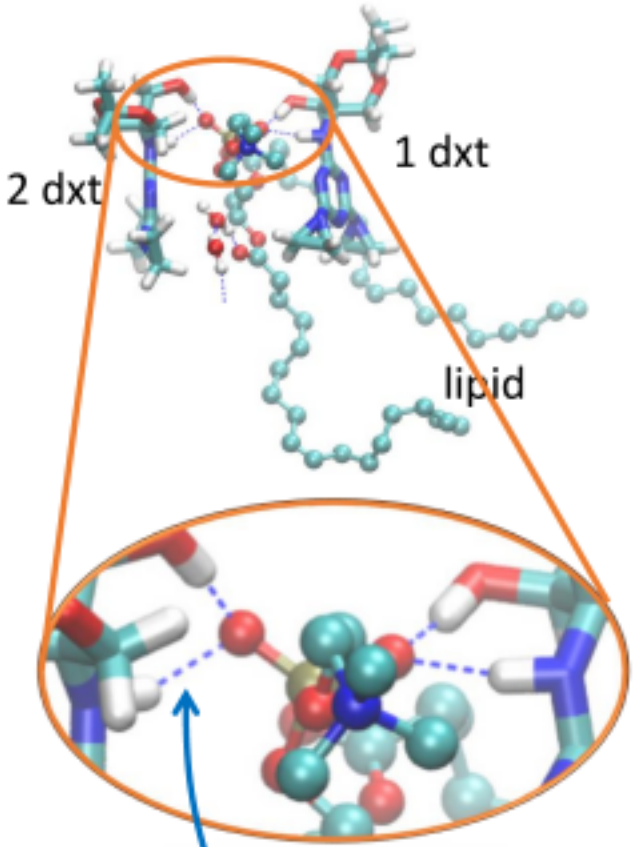


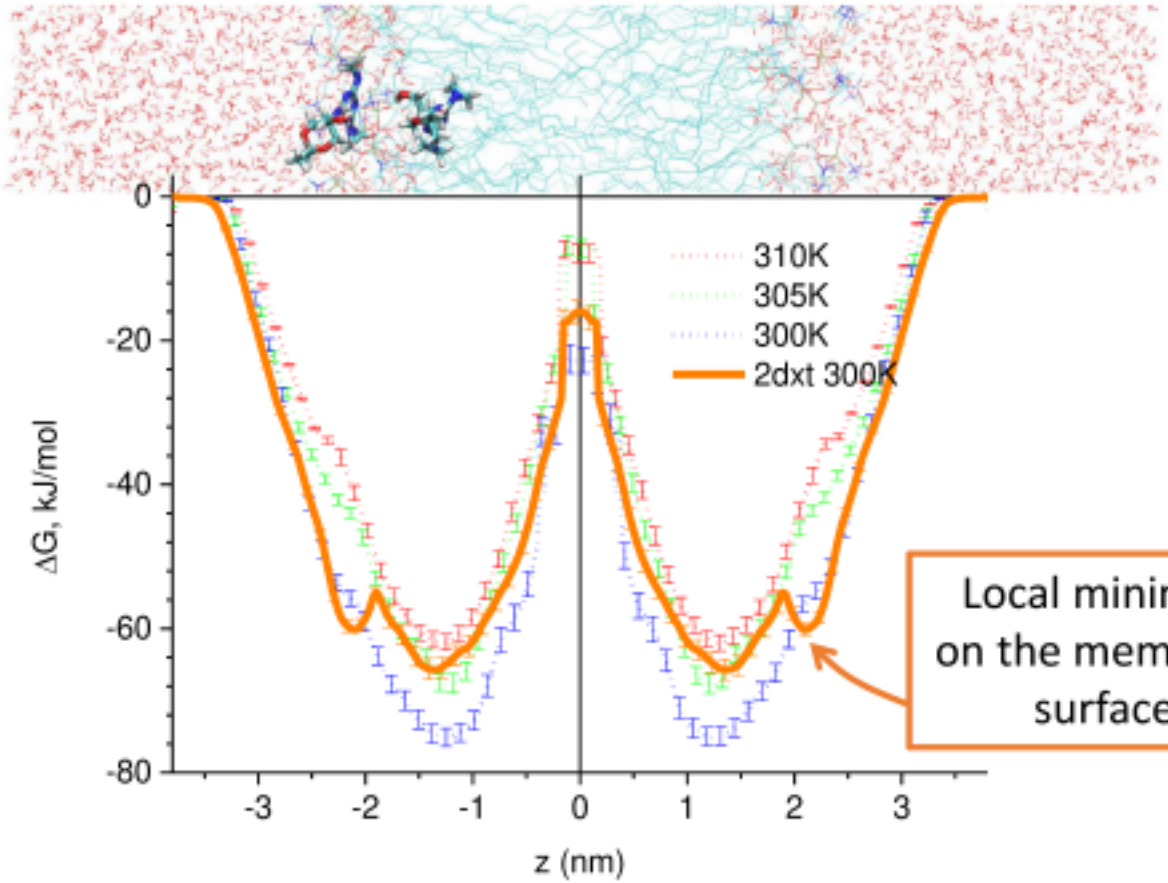
The midplane barrier also contributes at higher temperatures



P = (0.6 - 0.4)·10^{-3}cm/s

Additional dioxadet molecule in the membrane

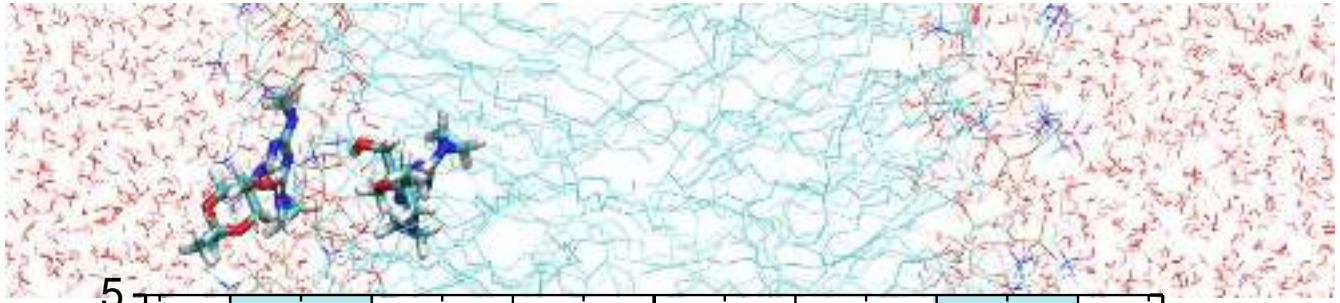




Local minimum on the membrane surface.

The second dioxadet molecule prefers to be close to the first one.
They interact with each other indirectly via the lipid phosphate group, forming 4 hydrogen bonds that are stable for 250 ns.

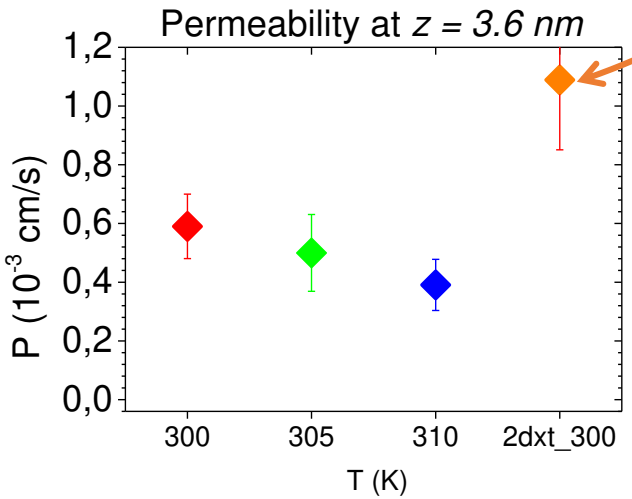
Additional dioxadet molecule in the membrane

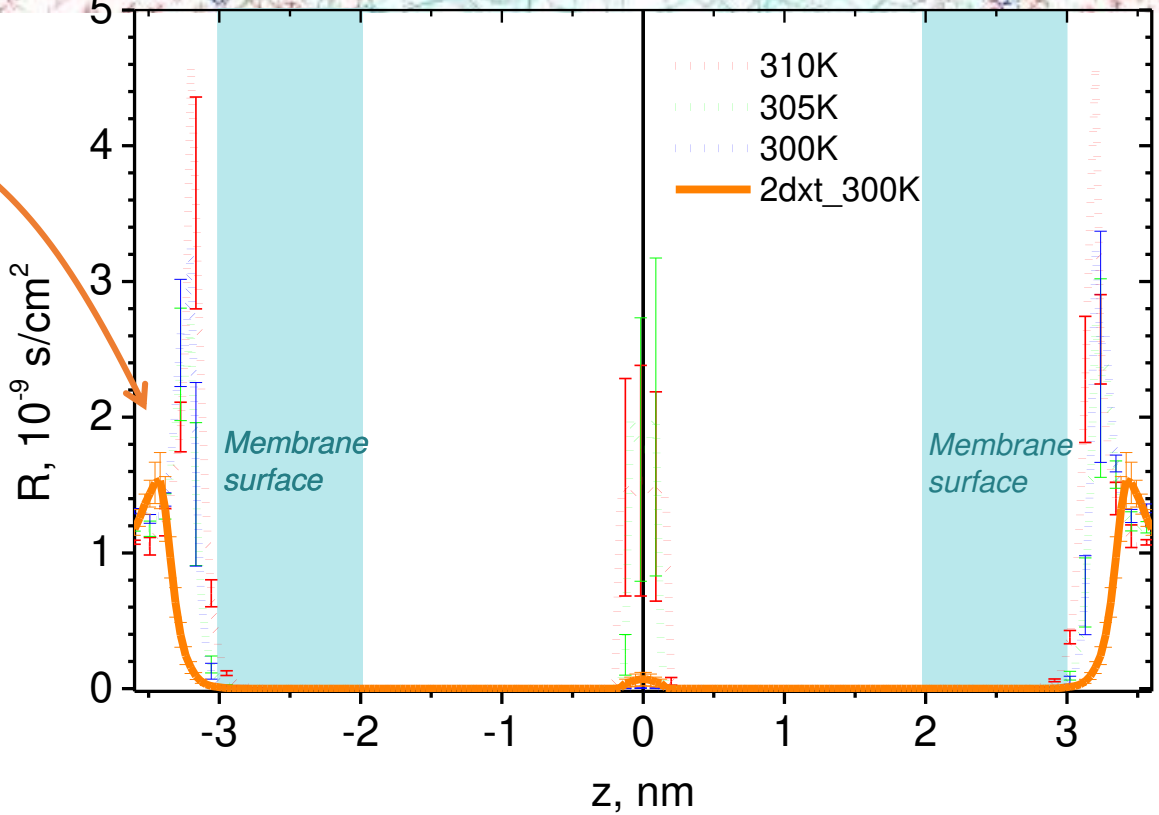


The addition of a second molecule:

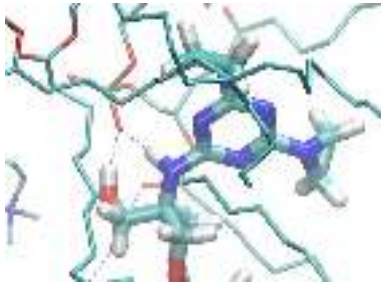
- lowers the surface barrier,
- increase permeability by about a factor of 2.

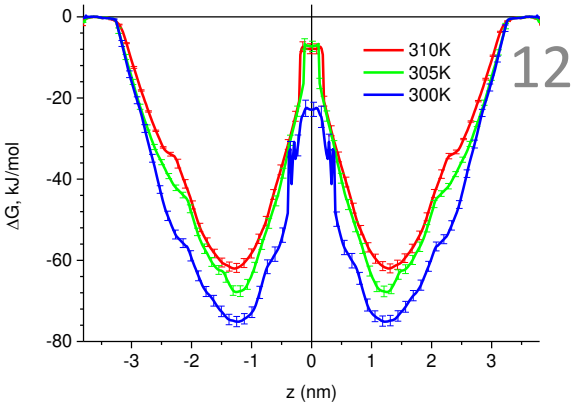
$P = (1.1 \pm 0.2) \cdot 10^{-3}$cm/s



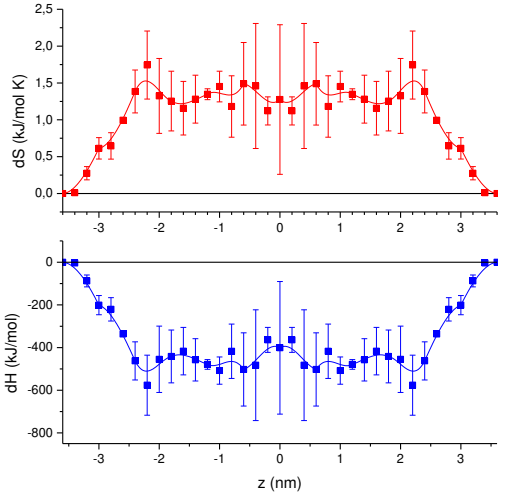


Conclusion

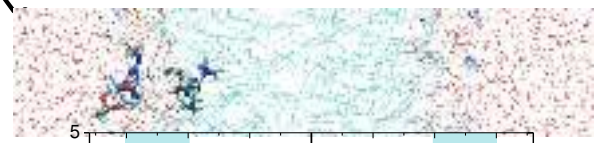


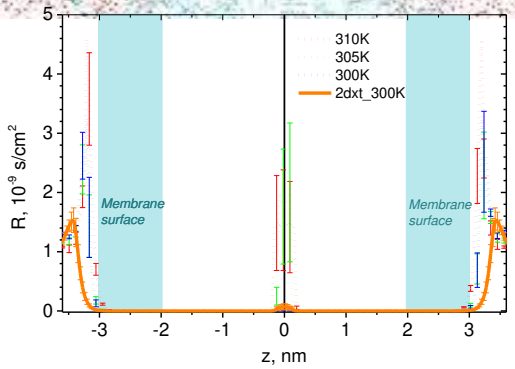


- The dioxadet molecule prefers to occupy the area below the membrane surface, forming hydrogen bonds with phosphate groups.
- The free energy profile of the dioxadet transport through the membrane indicates a preference for the molecule to be inside the membrane; there is an energy barrier in the middle of the membrane, the height of which depends on the temperature.



- Passage of dioxadeth into the membrane is entropically disadvantageous, but generally turns out to be advantageous due to the enthalpy gain.
- The membrane permeability is quite low P = (0.6 ± 0.1)·10^{-3}cm/s at T = 300 K.
- As the molecule passes through the membrane, the greatest resistance is exerted by its surface and, to a small extent, by the central non-polar region.



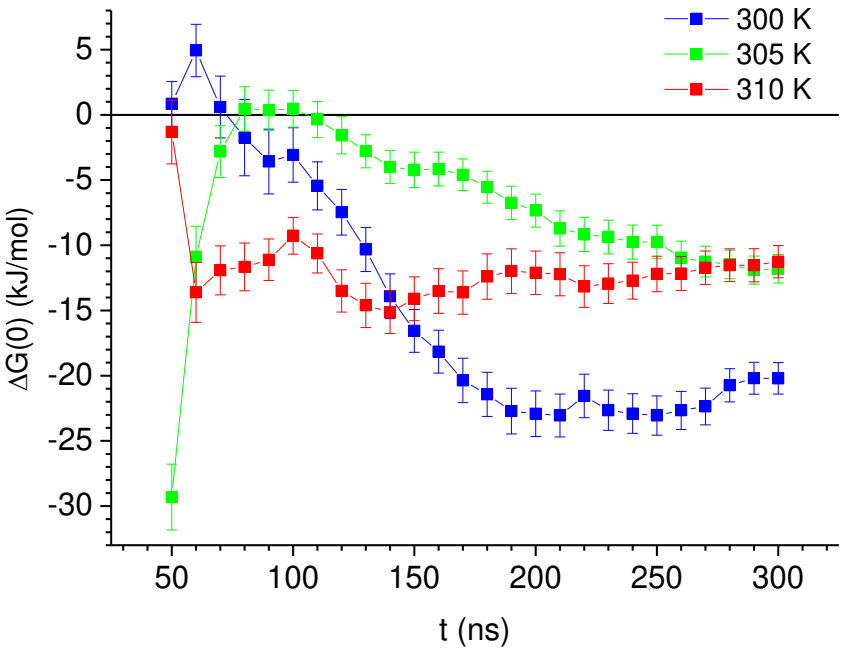


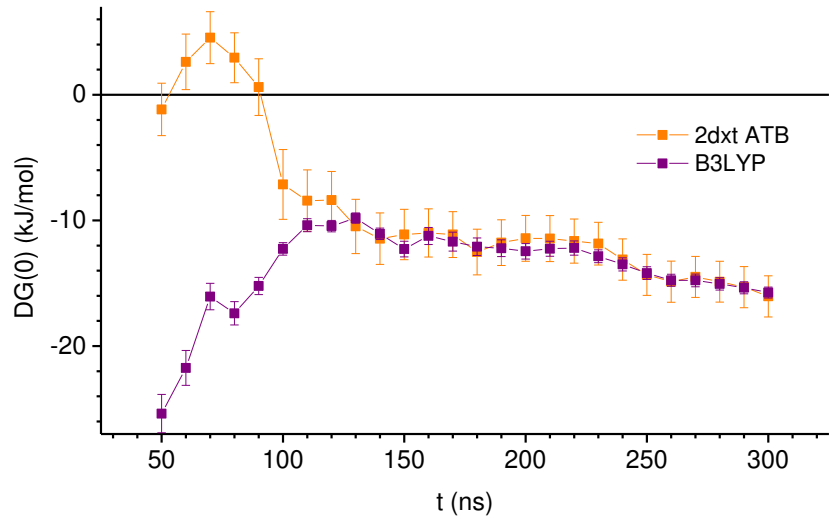
- The presence of a second drug molecule inside the membrane almost doubles the permeability (1.1 ± 0.2)·10^{-3}cm/s by lowering the resistance of the membrane surface.

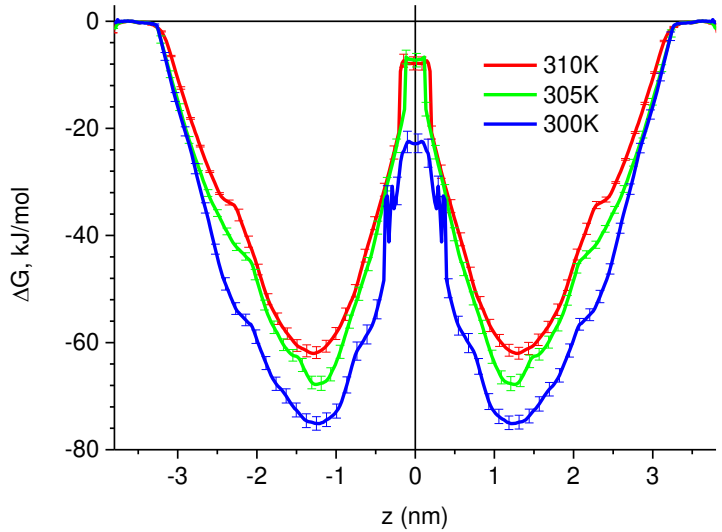
Many thanks to:

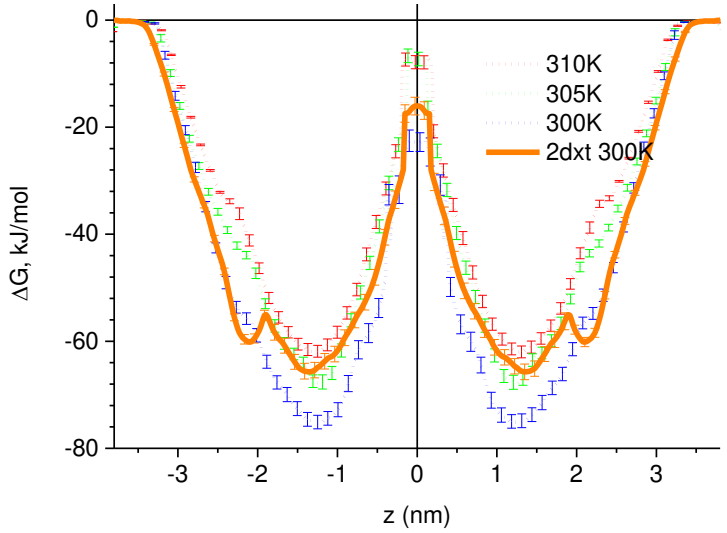
- Elena Yakush
- Employees of the Laboratory of Molecular dynamics and structure at the Institute of Chemical Kinetics and Combustion SB RAS
- D. Nerukh, Aston University, UK

Convergence

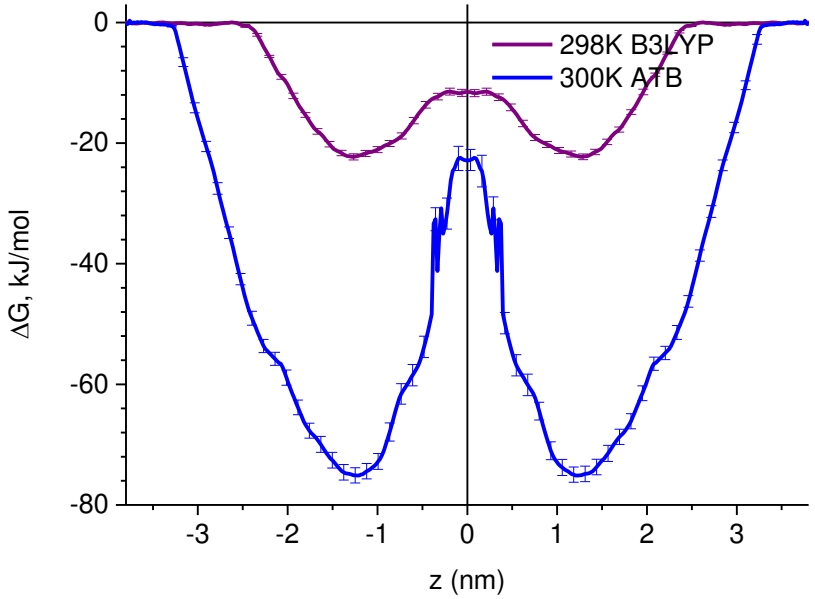




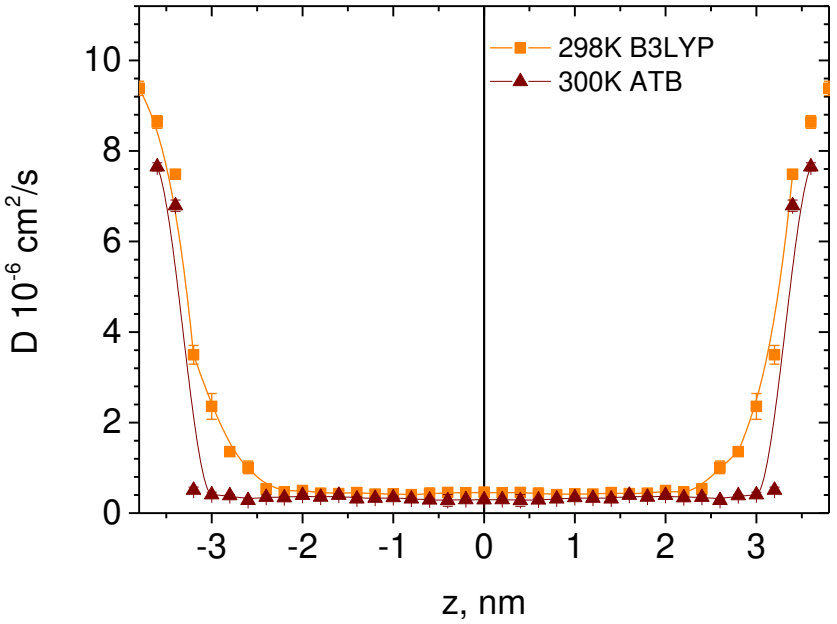




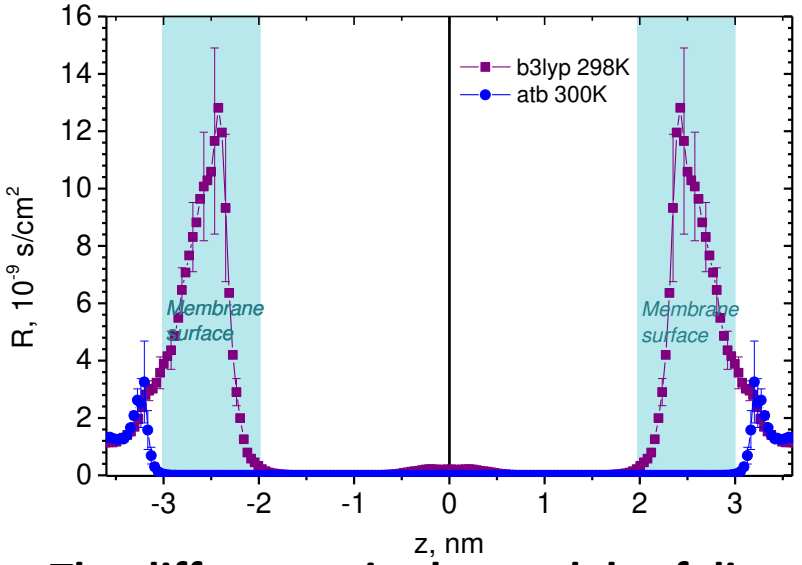
Another dioxadet model



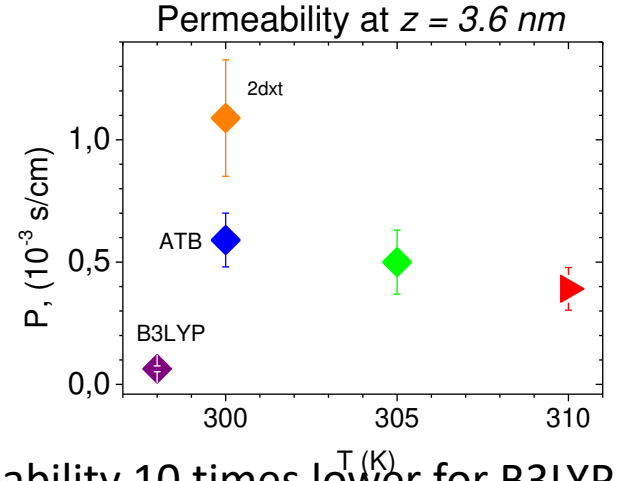
Quantitative difference with the ATB model by a factor of 4, but qualitative agreement



Diffusion is slightly slower for the B3LYP model



Resistance at the membrane surface for the B3LYP model is significantly higher than for the ATB



Permeability 10 times lower for B3LYP model

The differences in the models of dioxadet, although affecting quantitative results, do not prevent qualitative analysis.

MD parameters

	Solvation free energy calculation for dioxadet (DXT) in water							Classical MD			Free energy profile of DXT transition through the membrane				
								Lipids DOPC		DSPC	DOPC				DSPC
DXT model	ATB		B3LYP		Prodrg	Swiss		ATB	B3LYP	ATB	ATB		B3LYP	ATB	ATB
DXT molecules	1	3	1	3	1	1	3	1	1	1	1	2	1	2	1
Water molecules	3306	2926	2150	2113	2147	2148	2928	5668	7443	7919	5668	7699	7443	7699	7919
Lipid molecules	0							128							
Temperatures, K	273,278,283,288,293,298,303,308,313,318,323,328,333,338	310	298	310	298	298	310	323	310	300	300, 305, 310	300	298	300	300
Boxsize, nm	5.2x5.2x3.7 triclinic	4.5x 4.5x 4.5	4x4x 4	4.5 4.5 4.5						5.8	6.4x 6.4x 8.4			6.5x6 .5x 9.5	5.8x5. 8x11.9
Number of windows/ production run length	40 windows of 10 ns each	500 ns	40 windows of 10 ns	100 ns	40 windows of 10 ns	40 windows of 10 ns	100 ns	600 ns	100 ns	600 ns	19 windows of 300 ns	19 windows of 300 ns	20 windows of 300 ns	20 windows of 300 ns	54 windows of 100 ns

Summarized calculation time is 23 μs,
(175 days on videocard GeForce GTX 1080,
2560 CUDA cores or GeForce RTX 2070)