SUPPORTING INFORMATION

Glycyrrhizin-assisted transport of praziquantel anthelmintic drug through the lipid membrane: an experiment and MD simulation

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Contents

GA in heptane

To quantitatively examine, whether GA molecules form self-associates in heptane or not, the minimum distance between the first GA and the other 3 GA molecules was calculated, Fig. S1, black dots. There are time intervals of about 10-50 ns long when 1GA molecule was in contact with some other GA molecule and the minimum distance is less than 0.4 nm. But approximately the same amount of time they were not in contact and the minimum distance was $\approx 1 \div 5\,nm$. In comparison with the minimum distance between 2 GA molecules in water, Fig. S1, red dots, where the minimum distance was not more than 0.4 nm during all the simulation time, we conclude, that in heptane the self-associates of GA are weak-bonded. The number of hydrogen bonds per frame between the first GA and 3 the other GA molecules in heptane was calculated.

The model with heptane contained 4 GA and 1517 heptane molecules, the box size was $\sim 11 \times 11 \times 11$ nm. The model with GA dimer in the water contained 5575 SPC water molecules with a box size of ~5.5 nm. In both cases, the starting configuration contained GA molecules set apart from each other as far as possible in the model box.
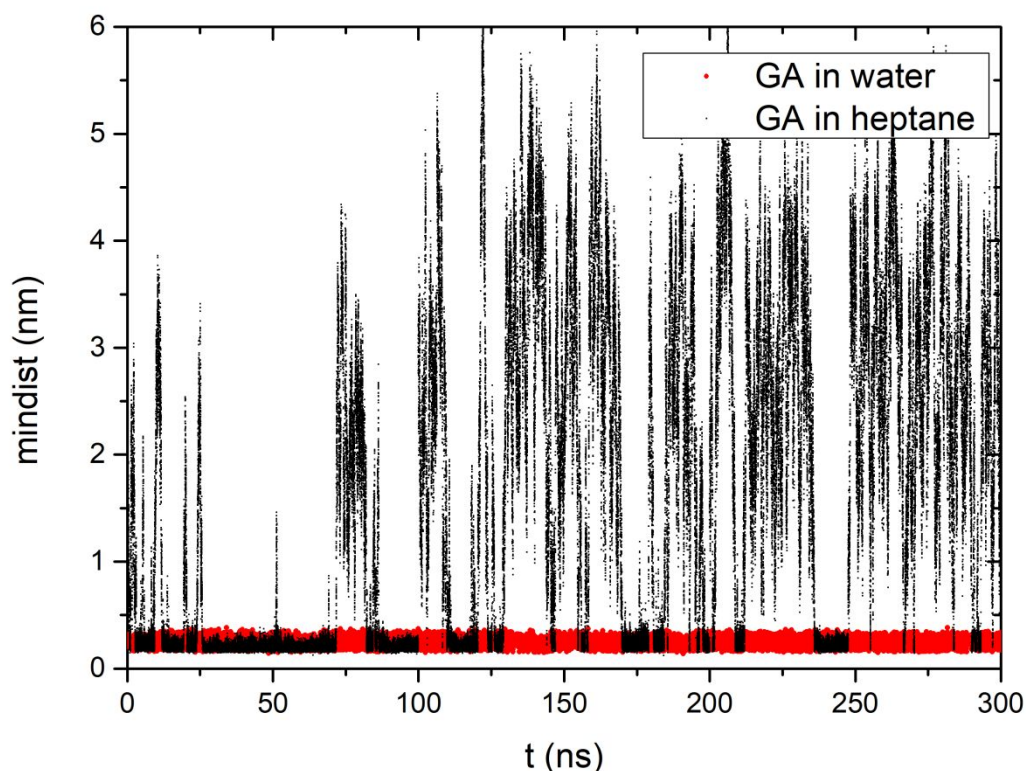
Figure S1. The minimum distance between 1 GA and 3 other GA molecules in heptane

(black dots) and between two GA molecules in water (red dots). The GA-dimers in water

are stable due to hydrophobic interaction in polar water media. But in nonpolar media of

heptane GA lives as in single form, as in self-associates, which are not very stable.

The forces, that maintain the self-associates whole in heptane, are van Der Waals force

and hydrogen bonding. The number of hydrogen bonds between one GA molecule and

the other 3 GA ones is shown in Fig. S2 with red dots. An averaged value graph is

indicated as a red line. The same Fig. S2 contains the time-dependency of a minimum

distance value of time. When GA molecule is in contact with another one, it forms

hydrogen bonds. So we can conclude, that the associates arise due to the formation of hydrogen
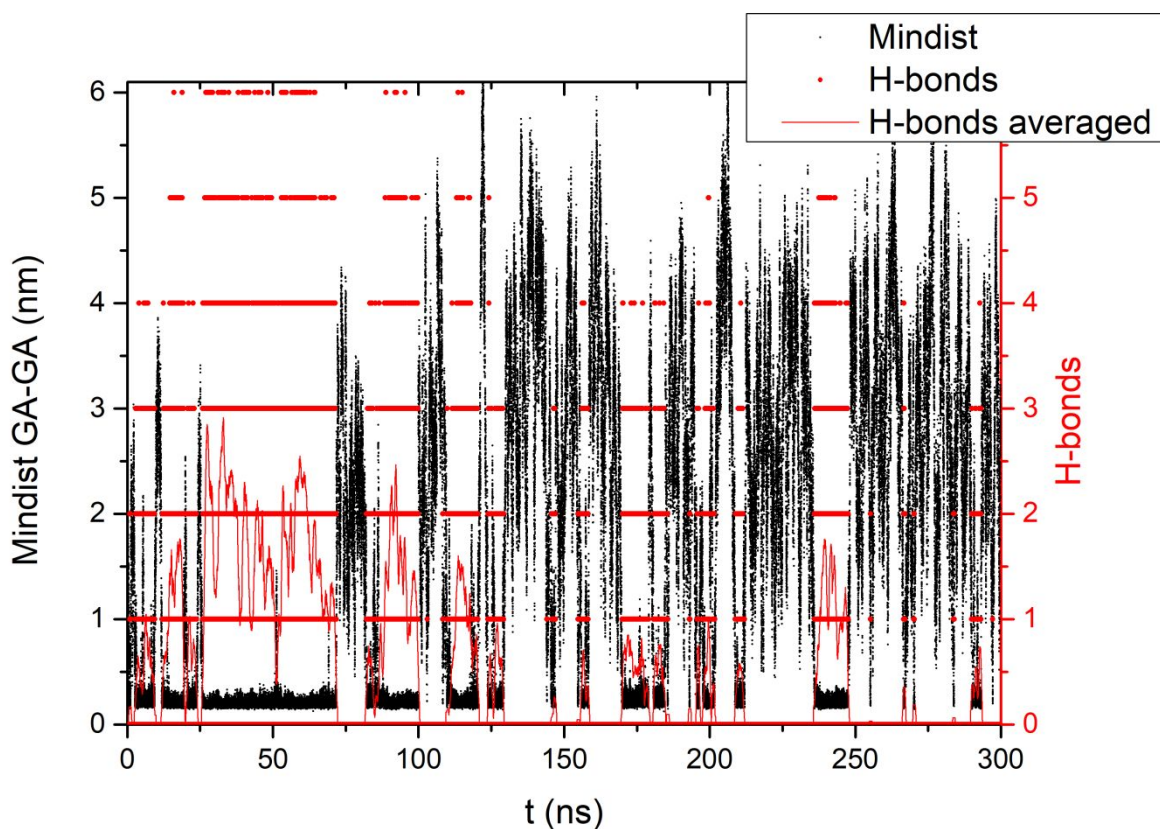
bonds between the GA molecules.

Figure S2. The time dependency of the minimum distance between the one molecule of

GA and the other ones (black dots). And hydrogen bonds number between the same GA

molecule and all other molecules in the model with heptane as a solvent.

Convergence analysis of free energy profile

The histograms of the PZQ center of mass positions in an umbrella sampling

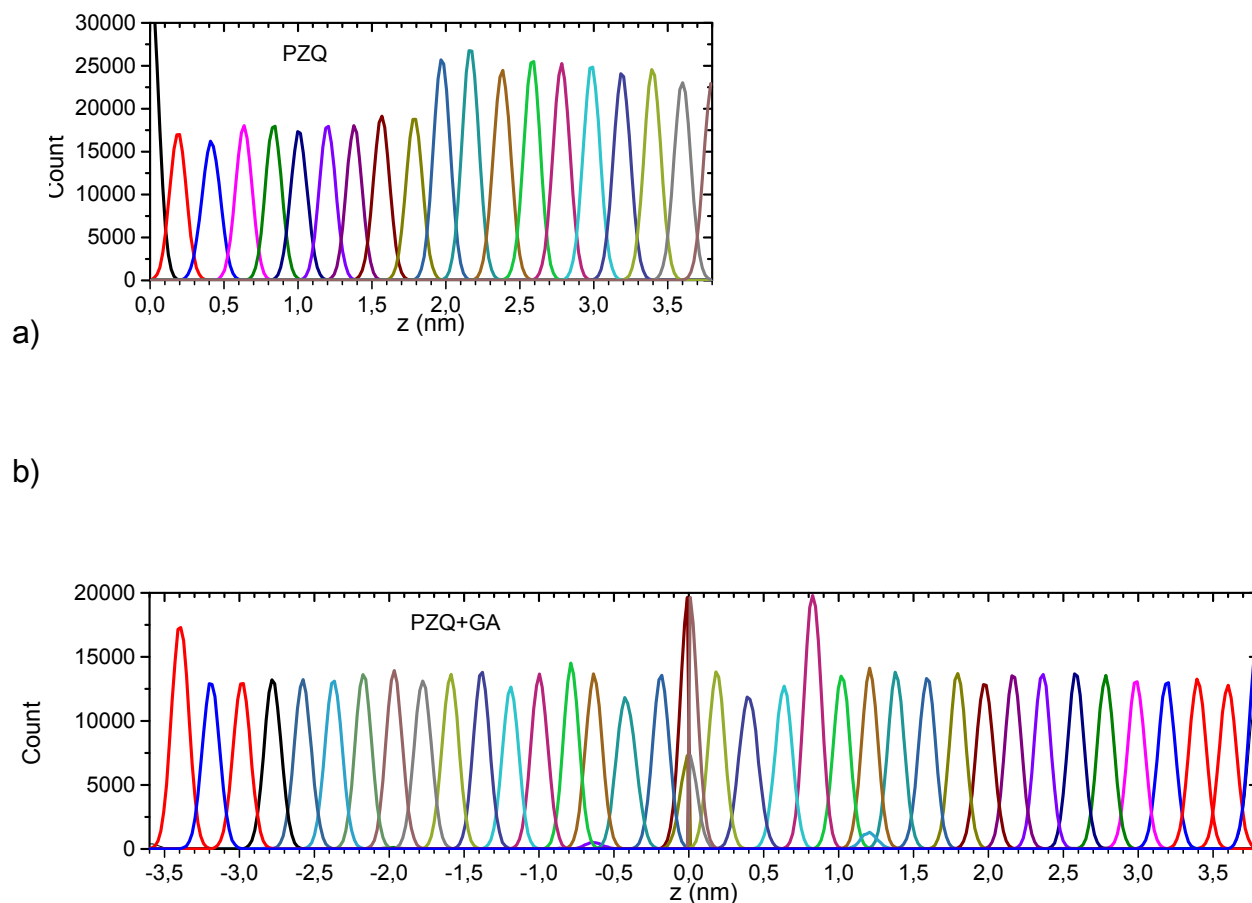calculations show reasonable overlap between all windows, Fig. S3.

a)

b)

Figure S3. Histograms of PZQ position a) in pure DOPC and b) in DOPC with GA.

Convergence analysis of PMF (Potential of Mean Force for penetration of PZQ through
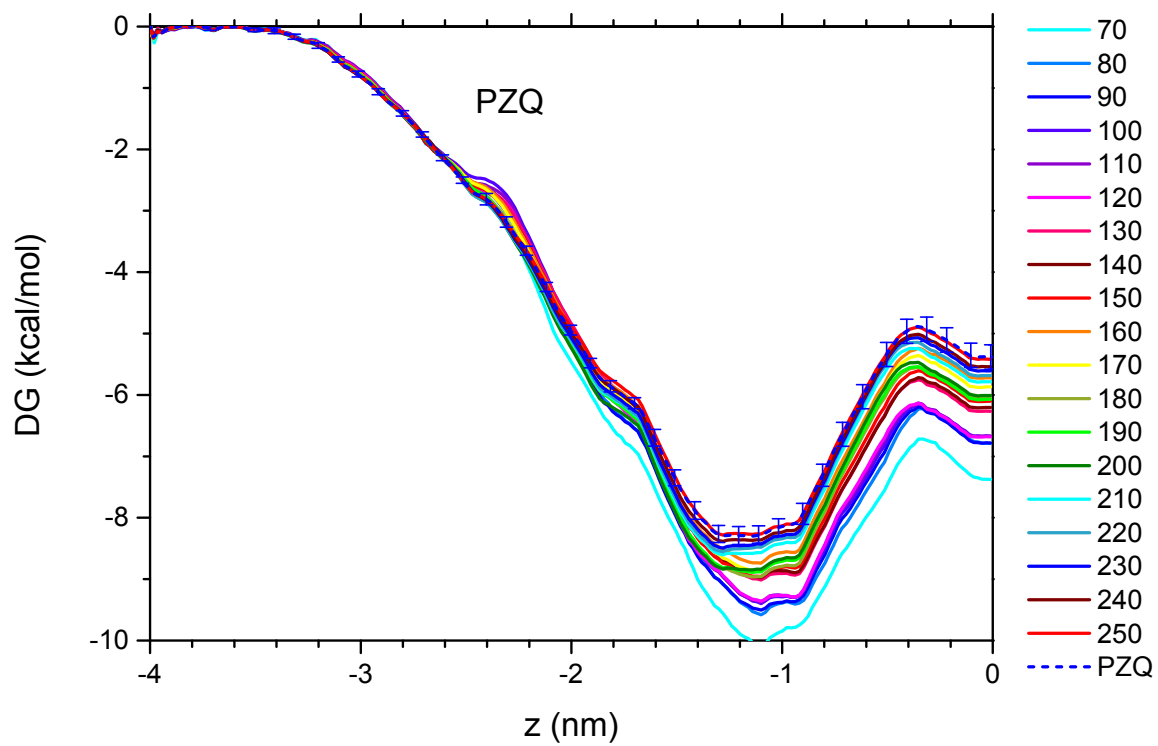
DOPC bilayer)

Figure S4. Evolution of the free energy profile with increasing statistics for PZQ without

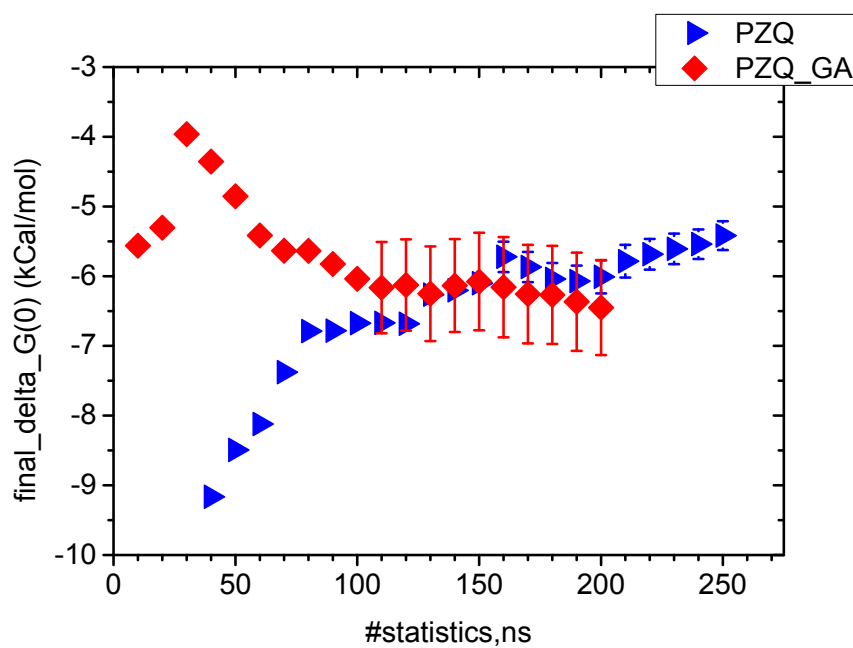GA. Z-axis is perpendicular to the plane of bilayer.

Figure S5. The dependence of free energy value at z=0, $\Delta G(0)$ on the number of ns in each window taken into account for the system of PZQ in DOPC without GA (blue) and with GA (red). The tendency to saturation is observed.
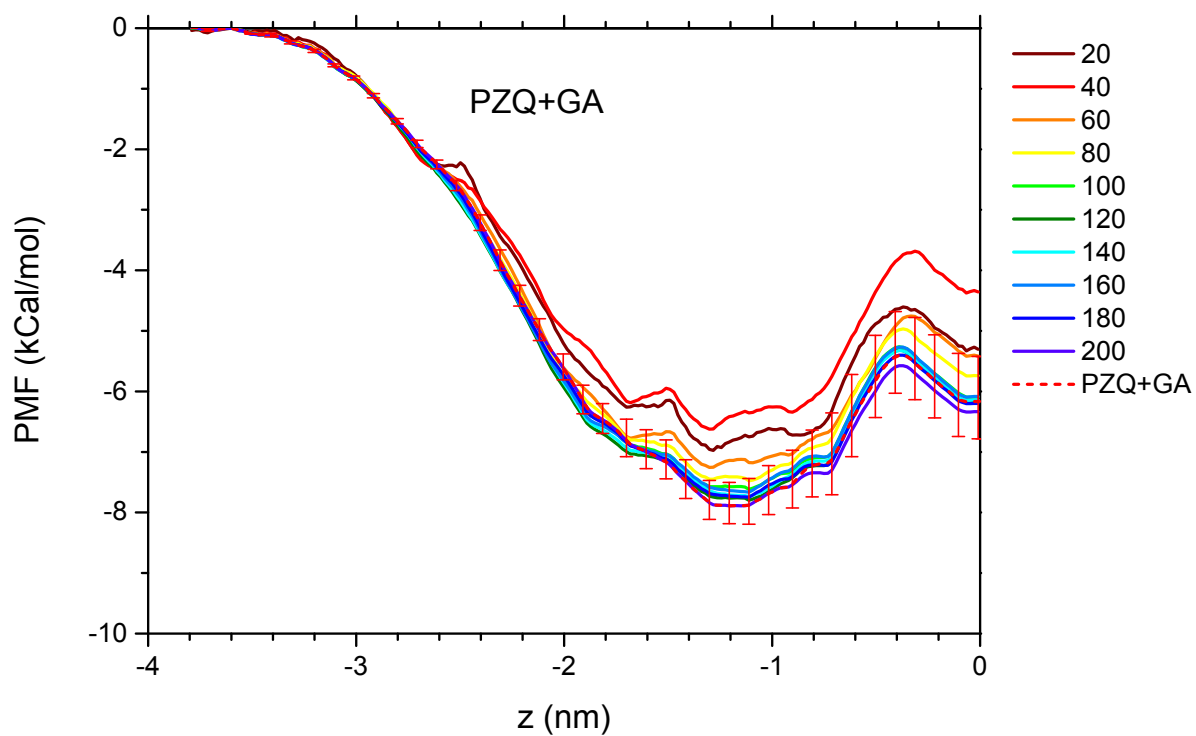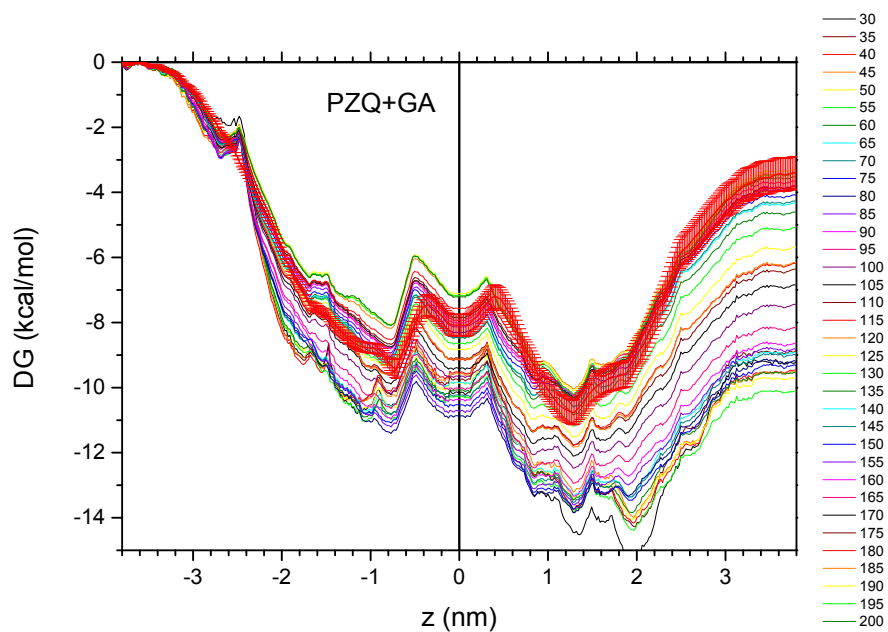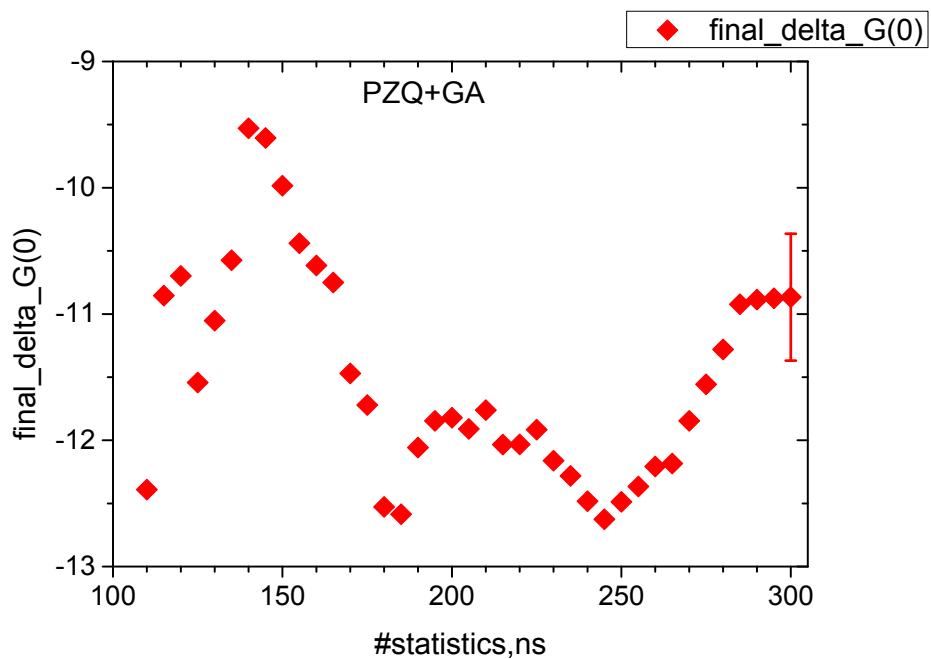
Figure S6. Evolution of the free energy profile with increasing statistics for PZQ with GA. Symmetrized case (statistics of the corresponding windows to the left and right of the membrane center was merged).

a)

b)

Figure S7. Praziquantel in the presence of GA, the latter is in the left half-layer. a)

Evolution of the free energy profile with increasing statistics. b) Free energy value at

z=3.8, $\Delta G(3.8)$ from the # of ns in each window.

Diffusion profile calculation

The solubility-diffusion approach lets us calculate the diffusion coefficient of the solute through the membrane using the samples of umbrella windows. The typical example of PZQ deviation from the equilibrium position dz is shown at the Fig S8 a), the positions were saved every 2 ps. We split the data into subintervals of 500 frames, the total number of subintervals was 200 and 250 for pure PZQ and PZQ+GA systems, respectively. Then we calculated the deviation from the mean value within the given subinterval – the resulting data are shown at Fig S7 b. The last procedure was performed to get rid of the slow processes of the whole membrane deformation.
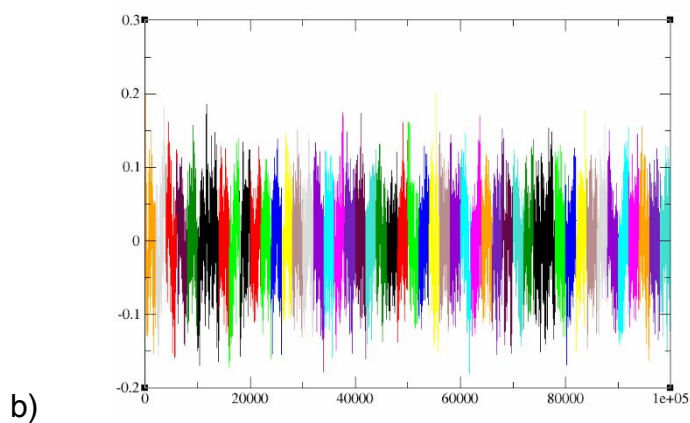

a)

b)
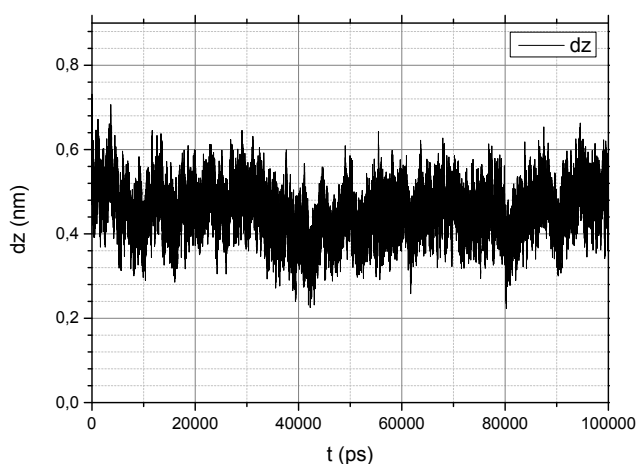
Figure S8. a) A typical example of the position deviation of PZQ from equilibrium, b) deviation from the mean value within the given subinterval of 1 ns.

Then the variance and autocorrelation curves were calculated at each of the subintervals with the GROMCAS tool *gmx analyze*, see Fig S9.
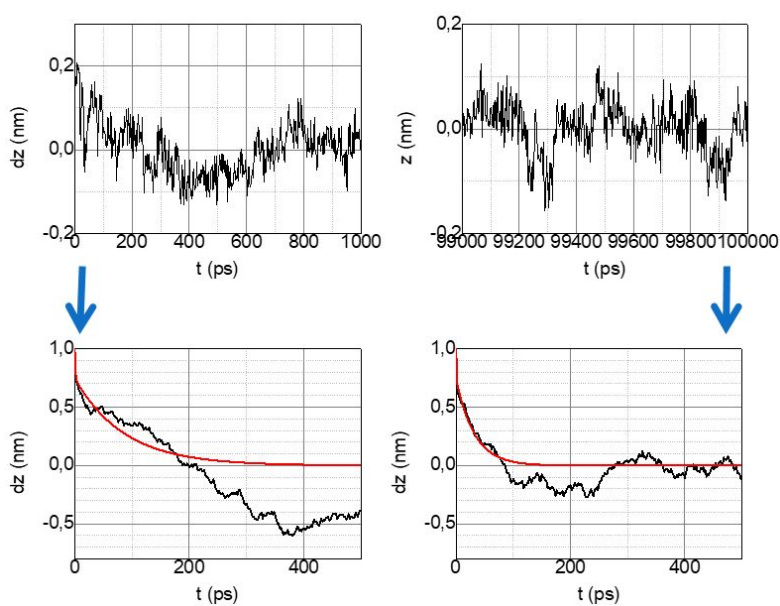

Figure S9. Typical autocorrelation curves calculated for each subinterval.

Then the autocorrelation curves were averaged over every 20 subintervals (Fig S10 a).

The resulting autocorrelation curve (Fig S10 b, black square symbols) was fitted by bi-exponential function (Fig S10 b, red line) with *gmx analyze* tool:

$$y = a_1 e^{-\frac{t}{a_2}} + (1 - a_1)e^{-\frac{t}{a_3}}$$

The 10 or 12 independent autocorrelation times obtained (Fig S10 c) were averaged using the block-average analysis, which estimates an error as well, Fig S11 a. The resulting estimates of autocorrelation times and their errors for all windows are in Fig S12 a. The same error estimate was applied to the variance set (Fig S11 b). The resulting estimates of variances of PZQ positions are shown in Fig S12 b.
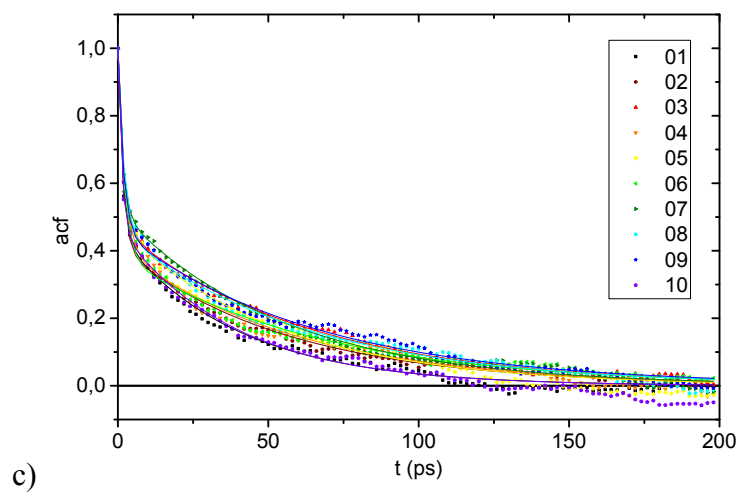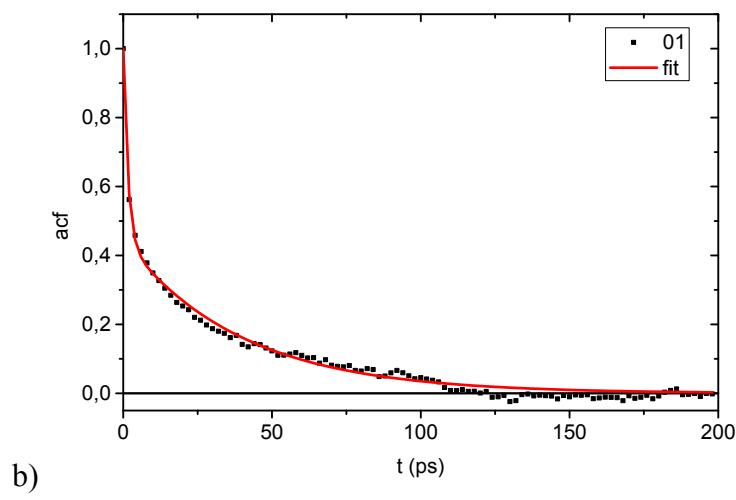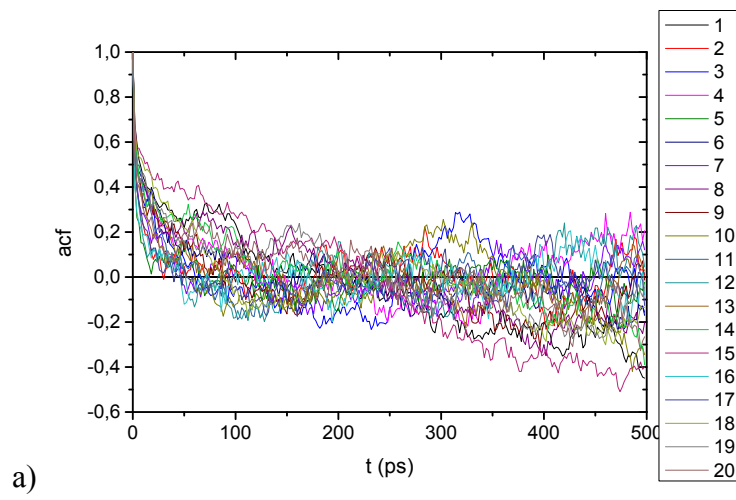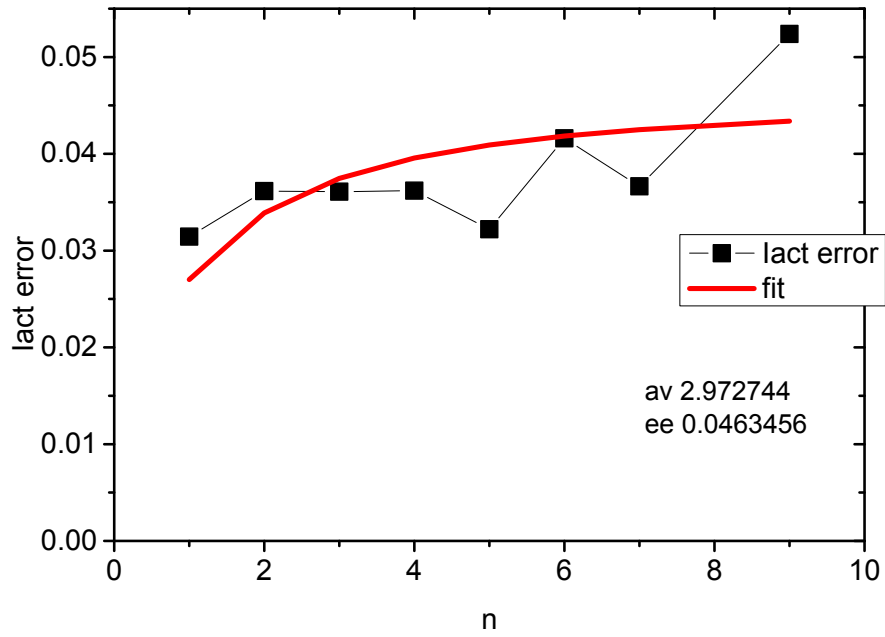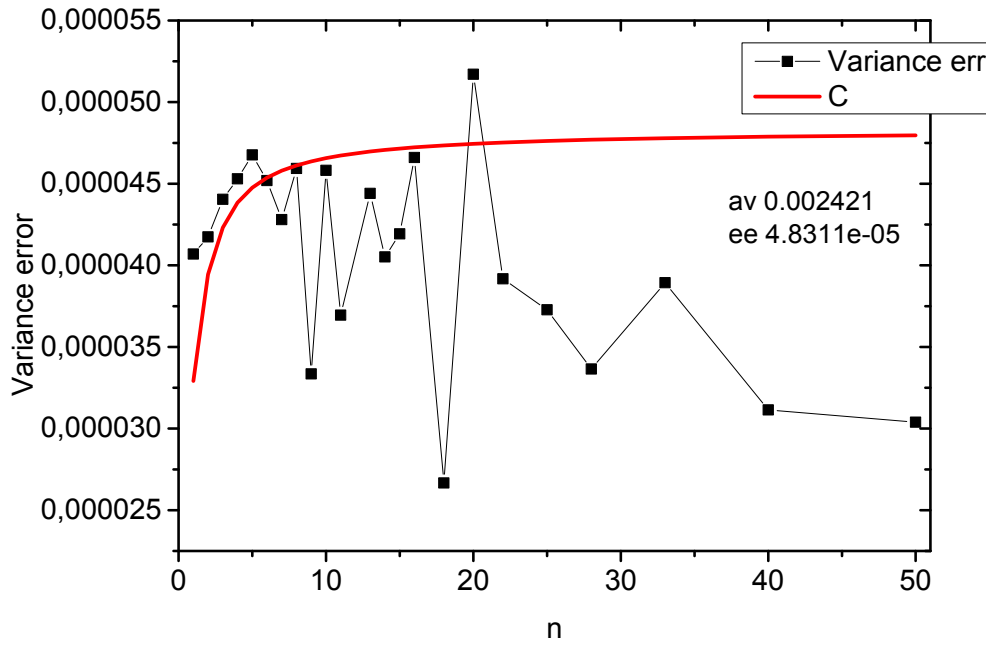
a)

b)

c)

Figure S10. Autocorrelation functions a) original, b) averaged 20 functions and fitted with biexponetntial function, c) 10 independent averaged autocorrelation functions to be subsequently block-analyzed.
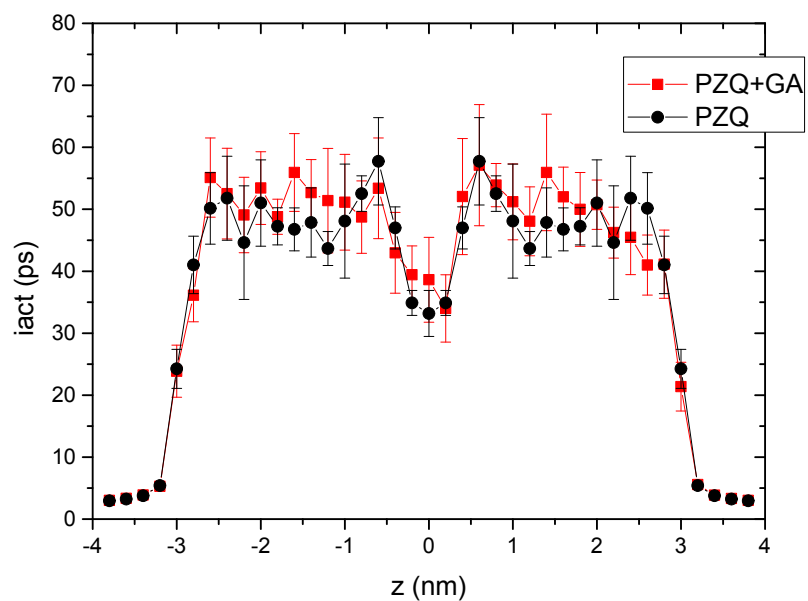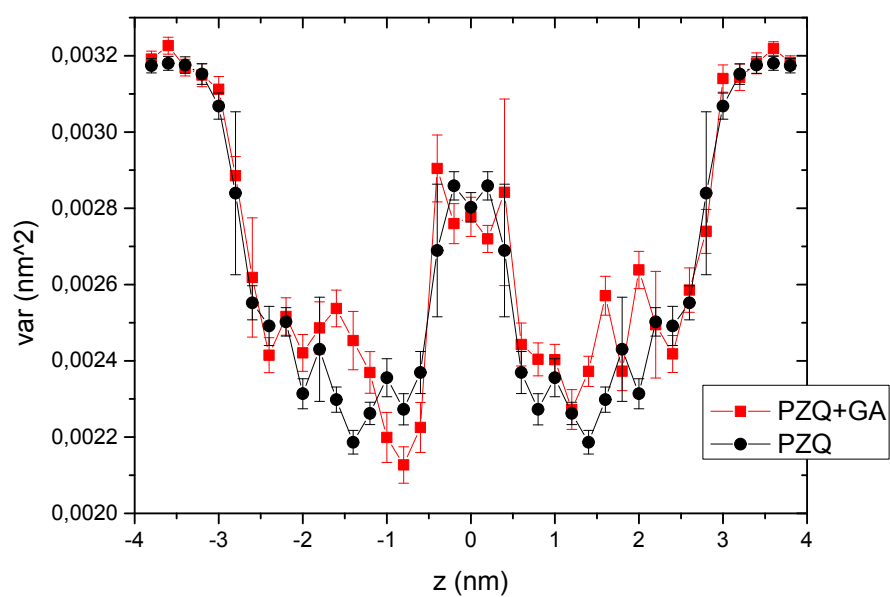
a)

b)

Figure S11. Error estimate by block-averaging approach (for window z=3.4): a)

autocorrelation times, b) the variance of PZQ position deviation.

a)

b)

Figure S12. a) The autocorrelation time of the PZQ deviation from the equilibrium position

in each umbrella window. b) The variance of PZQ position depending on the reaction

coordinate

The ratio of the variance and the autocorrelation time gives the diffusion constant, which

is shown in the article at Fig. 10 and Fig. 11 b.

Local resistance calculation

To find out how the feature of resistance appears on the surface let's calculate separately $R_{eff} =$

$\int_0^Z \frac{e^{\beta \Delta G(z)}}{D(z)} dz$ numerator $e^{\beta \Delta G}$ and inversed denominator $\frac{1}{D}$. Fig. S13 shows these two values for the

pure PZQ and for PZQ with GA. On the left shoulder, the graphics compete with each other, $\frac{1}{D}$ rises

slightly faster than $e^{\beta \Delta G}$ decreases and the peak of local resistance appears on the surface of the
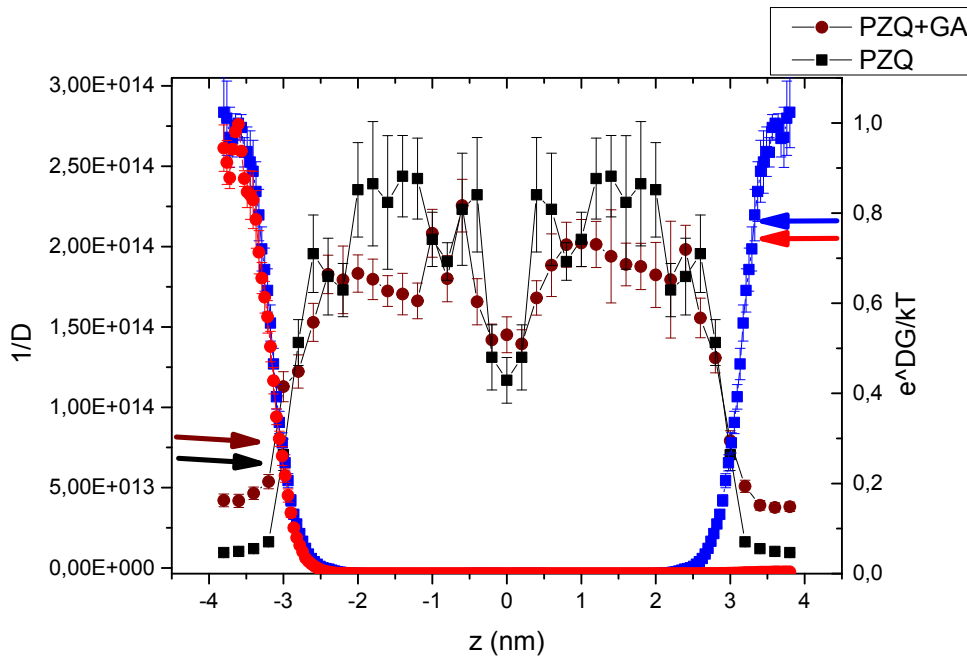
bilayer.

Figure S13. The inversed diffusion coefficient (left axis, black squares, and wine circles) and

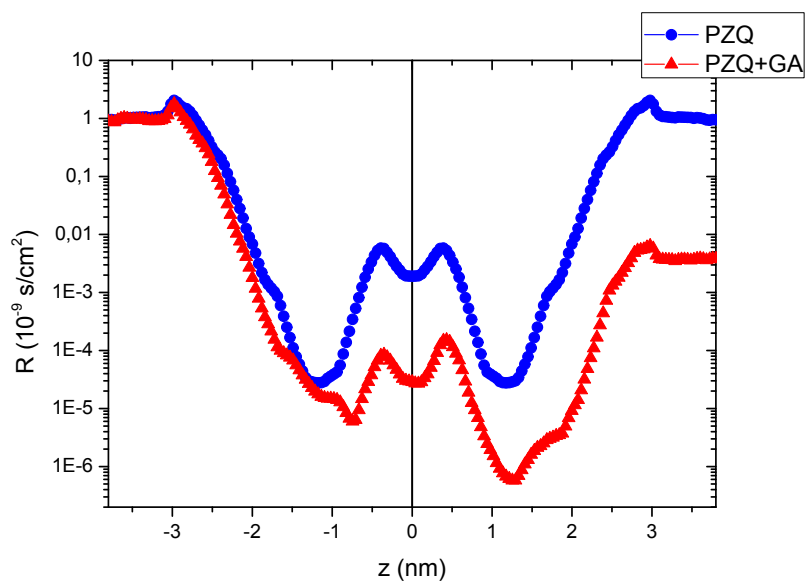Boltzmann weight of free energy (right axis, blue squares, and red circle)

Figure S14. The local resistance $R(z)$ to the penetration of PZQ into the pure DOPC bilayer (blue circles) and DOPC with GA (red triangles) on a logarithmic scale. Features on the output surface and in the middle of the membrane exist and are visible on a logarithmic scale, but they are negligible and do not play a significant role for permeability.