Supplementary Information

to the article "**Proton leakage across lipid bilayers: Oxygen atoms of phospholipid ester linkers align water molecules into transmembrane water wires**" by Marine E. Bozdaganyan, Alexey V. Lokhmatikov, Natalia Voskoboynikova, Dmitry A. Cherepanov, Heinz-Jürgen Steinhoff, Konstantin V. Shaitan, and Armen Y. Mulkidjanian.



Figure S1. Distribution of liposomes diameter measured by dynamic light scattering (see the main text).

Additional data on MD simulations

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Figure S2. Formation of water wires between PO[PH]C and PO[P⁻]C molecules that were pulled with different velocities, see the Table below, as well as Fig. 2, 3 and Table 1 in the main text.

	PO[PH]C (top)	PO[P ⁻]C (bottom)	
(a)	1.0 nm/µs	2.0 nm/µs	(trajectory <i>D</i> in Table 1)
(b)	2.0 nm/μs	1.0 nm/μs	(trajectory <i>E</i> in Table 1)
(c)	2.5 nm/µs	2.5 nm/µs	(trajectory G in Table 1)
(d)	5.0 nm/µs	5.0 nm/µs	(trajectory <i>I</i> in Table 1)
(e)	5.0 nm/µs	2.5 nm/µs	(trajectory <i>K</i> in Table 1)
(f)	2.0 nm/µs	2.5 nm/µs	(trajectory J in Table 1)
(g)	20.0 nm/µs	20.0 nm/µs	(trajectory <i>L</i> in Table 1)

Other conditions as in Fig. 1

















Calculation of the potential of mean force (PMF)

The PMF profiles as measured at different pulling velocities were calculated as described in the main text.



Figure S3. Free energy profiles (PMF) for the negatively charged (PO[P⁻]C], left) and protonated (PO[PH]C, right) phosphate groups that were pulled towards the midplain of the lipid bilayer with different velocities (the midplane of the membrane was set at zero on the *x*-axis):

	PO[P ⁻]C	PO[PH]C	
(a)	2.0 nm/µs	1.0 nm/µs	(trajectory <i>D</i> in Table 1)
(b)	1.0 nm/µs	2.0 nm/µs	(trajectory <i>E</i> in Table 1)
(c)	2.5 nm/µs	2.5 nm/µs	(trajectory F in Table 1)
(d)	2.5 nm/µs	2.5 nm/µs	(trajectory G in Table 1)
(e)	2.5 nm/µs	2.5 nm/µs	(trajectory H in Table 1)
(f)	5.0 nm/µs	5.0 nm/µs	(trajectory <i>I</i> in Table 1)
(g)	2.5 nm/µs	5.0 nm/µs	(trajectory J in Table 1)
(h)	2.5 nm/µs	2.0 nm/µs	(trajectory <i>K</i> in Table 1)
(i)	20.0 nm/µs	20.0 nm/µs	(trajectory <i>L</i> in Table 1)

Properties of the POPC bilayer

The average properties of a POPC lipid bilayer were calculated using the productive MD trajectories, see Table 1 in the main text. Fig. S4 shows electrostatic potential of the bilayer as a function of distance z from membrane center (black line, calculated as described in [1]) as well as the concentration profiles of water (green), the nitrogen atom of the choline groups (blue) and the phosphorous atom of the phosphate groups (red) along the membrane normal. Fig. S4 also shows the positions of the choline and phosphate groups during the constrained simulation at the R_{PP} distance of 1.55 nm (dashed dark blue and brown lines, respectively). It is noteworthy that the positively charged choline groups of the two lipids were still in contact with the bulk water phase when the proton wire could already form.



Figure S4. The distribution of electrostatic potential (black) and the density of phosphate groups (red), choline groups (blue) and bulk water (green solid line) within the POPC bilayer at equilibrium. The dashed lines show the positions of the two phosphate (brown) and choline (deep blue) groups of the two lipids (PO[P⁻]C on the left and PO[PH]C on the right) which were constrained at a R_{PP} distance of 1.5 nm of each other (case p in Table). Different Y-scales were used, as indicated on the panel.

Stochastic dynamics of the POPC bilayer

The equilibrium distributions of charged and protonated phosphate groups at the membrane/water interface were calculated using productive MD trajectories (see Table 1 in the main text). The free energy profiles were calculated using the Boltzmann distribution:

 $\Delta G(z) = -k_{\rm B}T \cdot \ln P$

The free energy profiles are shown in Fig. S5 for the phosphorus atoms of protonated (PO[PH]C, blue) and negatively charged (PO[P⁻]C], red) phosphate groups, respectively. The profiles are in quantitative agreement with the PMF profiles in Figs. 2 and S2, that confirms adiabaticity of the umbrella sampling procedure of the PMF calculations.



Figure S5. The free energy profiles for the protonated (PO[PH]C, blue) and negatively charged (PO[P⁻]C], red) phosphate groups at the lipid/water interface calculated from the MD simulations.

The stochastic dynamics of phosphate groups in the potential wells shown in Fig. S5 was analyzed using the mean first-passage time approach [2]. If the effective diffusion coefficient (mobility) of phosphate groups does not change within the membrane interior, then the mean first-passage time increases exponentially with the height of the energy barrier (Eq. 2 of the main text). We found that Eq. 2 was fulfilled at least for distances >1.5 nm from the bilayer midplane. For shorter distances the first-passage time was too long to be calculated using submicrosecond simulations.

We calculated the mean first passage time $\tau_D(\Delta z)$ of the system motion uphill the barrier $\Delta G(\Delta z)$ (Fig. 1E) from the analysis of equilibrium dynamics of phosphate groups at the interface. For the energy values $\Delta G > 8$ kJ/mol, where the waiting time was restricted by the length of MD trajectory, $\tau_D(\Delta z)$ could be extrapolated by eq. (2) with $\tau_0 = 2$ ns (Fig. S6, dashed line).



Figure S6. The mean first-passage time of the system stochastic motion uphill the potential barrier (illustrated by the dashed red arrow in Fig. 1E) as function of the barrier height. The solid line represents the dependence that was calculated from the equilibrium dynamics of phosphate groups at the interface, the dashed line was calculated by Eq. 2 with $\tau_0 = 2$ ns.

We also calculated the average relaxation dynamics of phosphate groups at the interface (Fig. S7). For this purpose, we looked for such configurations where a particular phosphate group plunged deeper than by 0.8 nm into the membrane interior and calculated their dynamics starting from such configurations. The relaxation was almost exponential with a characteristic time of 5.5 ns.



Figure S7. The relaxation dynamics of the negatively charged phosphate groups (solid line) and its approximation by mono-exponential kinetics with the characteristic time of 5.5 ns (dashed line).

References

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- Hänggi P, Talkner, P, Borkovec, M. (1990) Reaction-rate theory: fifty years after Kramers. *Rev. Mod. Physics* 62:251-340.