

# Supporting information for:

## Guanidinium Pairing Facilitates Membrane Translocation

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# Additional Computational Details

## Guandinium pairing in water

### Force field comparison

Guanidinium pairing in aqueous solution was simulated using mostly identical methodology as described in the main manuscript. Additional differences and details are as follows: The system contained 2 GDM<sup>+</sup> ions, 2 Cl<sup>-</sup> ions and 2171 H<sub>2</sub>O molecules in a cubic box of 4 nm side length. The free energy profile was obtained using umbrella sampling simulations along the GDM<sup>+</sup>-GDM<sup>+</sup> carbon-carbon distance. Between 3.0 Å and 15.0 Å C-C distance, 25 umbrella windows were created. The force constants used were 5000 kJ/mol/nm<sup>2</sup> for large C-C distances ( $\geq 8$  Å) and 10 000 kJ/mol/nm<sup>2</sup> for potentials with Cs closer than 8 Å. The simulations were run in the NVT ensemble at 298 K, for 40 ns with 2 ns equilibration. The tested forcefield combinations were: 1. OPLS-AA<sup>S1</sup> for ions, SPC<sup>S2</sup> water, 2. OPLS-AA<sup>S1</sup> for ions, TIP4P<sup>S3</sup> water, 3. CHARMM<sup>S4</sup> for ions, and SPC/E<sup>S5</sup> water, 4. CHARMM<sup>S4</sup> for ions TIP3P<sup>S6</sup> water. For these simulations GROMACS<sup>S7</sup> v 4.6.1 was used. One simulation, discussed in the main manuscript, was run at 310 K in an NpT ensemble with isotropic pressure coupling and used the same forcefield and cutoffs as the bilayer simulations. For this simulation, we used a force constant of 5000 kJ/mol/nm<sup>2</sup> for all umbrella windows and the same system and sampling/equilibration times as in the forcefield comparison.

### Error bars of the ab initio simulation

The error estimation for the ab initio simulation was computed from a forcefield-based setup. This setup was chosen to be as close to the ab initio simulation as possible. Therefore identical box dimensions and mass scaling were used. Instead of the ab initio potential energy surface, we used the OPLS-AA force-field and an SPC water model. The custom umbrella windows were introduced using the harmonic wall feature from PLUMED 2.<sup>S8</sup> Due to restrictions in GROMACS a 0.65 nm cutoff was used, together with PME electrostatics.

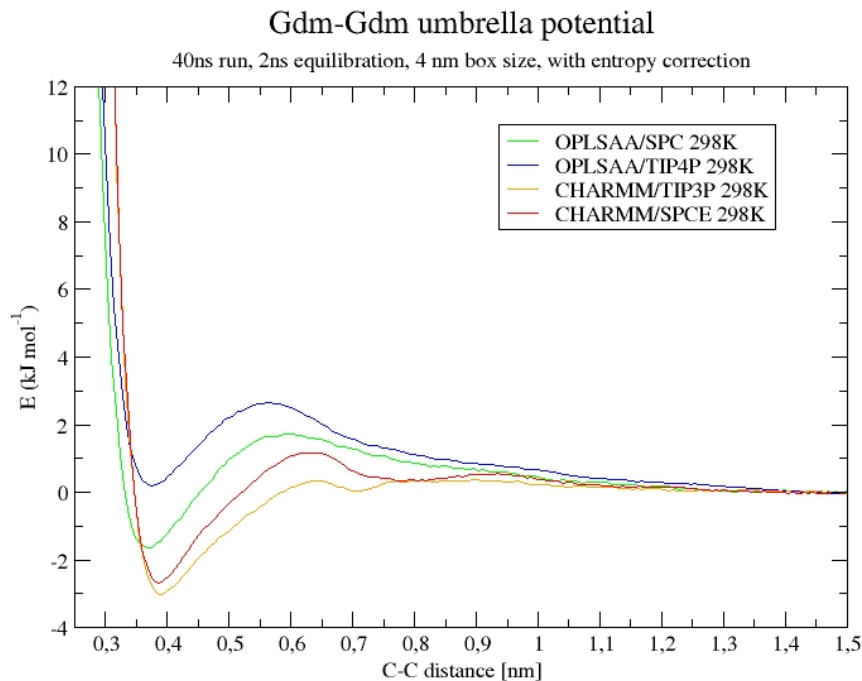


Figure S1: Comparison of free energy profiles of  $\text{GDM}^+-\text{GDM}^+$  pairing, entropy corrected.

All bonds were constrained using LINCS<sup>S9</sup> algorithm, and a timestep of 1 fs was used. The simulations were run in an NVT ensemble at 310 K using a CSVR<sup>S10</sup> thermostat with a timeconstant of 500 fs. Furthermore, instead of constraints, harmonic restraints with a 90000 kJ/mol/nm<sup>2</sup> force constant in the X and Y direction were employed. Each of the windows was simulated for 31 ns, of which 30 ns were used for statistics. For this simulation GROMACS<sup>S7</sup> v 4.6.5 was used. For the estimation of the ab initio error bars we estimated the correlation time for the simulation to be 100 ps. Accordingly, to estimate the error for a run of the length of the ab initio trajectory, we computed 300 umbrella profiles from the trajectory using uncorrelated subtrajectories of the length of the ab initio simulation. Using these umbrella profiles we computed a standard deviation, of  $\Delta A$  with respect to its value at 0.47 nm.

## Ammonium Pairing

Ammonium pairing in aqueous solution was examined using mostly identical methodology to the free energy simulations of ions inside the membrane as described in the main manuscript. The changes and additions are the following: The system contained 2  $\text{NH}_4^+$ , 2  $\text{Cl}^-$  and 1404  $\text{H}_2\text{O}$  and the simulations were done in a cubic box. 23 Umbrella windows were used with a force constant of 1500 kJ/mol/nm<sup>2</sup>. The windows were generated starting by fast pulling, with the pull-coordinate being the N-N distance of the ions. The simulation was pre-equilibrated for 100 ps in an NVT ensemble, before sampling in the NpT ensemble (isotropic pressure coupling) for 6 ns in each window.

## Influence of ion number and concentration on the reaction kinetics

The reaction kinetics of ion transfer, as proposed in the manuscript contain several assumptions. One assumption underlying the free energy calculations is the assumption of thermodynamic equilibrium. We therefore do not deal with a concentration gradient on the symmetrical sides of the bilayer. In equilibrium we may consider the concentration of the transition state as if it was a chemical compound, subject to the law of mass action. For a state representing  $n$  ions, the law of mass action for the transition state can be rewritten as

$$\log K = \log \frac{[TS]}{[ion]^n} \quad (1)$$

The reaction rate is dependent on the population of the transition state, therefore

$$v \rightarrow K[ion]^n \quad (2)$$

From these considerations the influence of concentration of the transition rate must be estimated as severe for states containing a high number of ions. Our simulations were carried

out at the low concentration limit. They cannot be extrapolated to high concentrations. Nevertheless, our calculations do not include any entropic effects of concentration. At low concentration, any concentration effect will accrue a rate penalty, which will grow with the power of the ions in the transition state. Furthermore, this very elementary discussion has neglected the activity coefficients of the ions. While we cannot guarantee decreasing activity coefficients in the membrane, Debye Hueckel theory predicts decreasing activity coefficients at increased ionic strengths, which would lower the prefactor in this scheme. When computing the transfer rate from the peak of the free energy curve, we need the prefactor to adjust for the fact, that when grouping multiple ions into one state at the membrane center, it becomes clear that the state geometry and kinetic energy have to fulfill special conditions, so that all ions cross into the same direction. Both, accounting for partial ion backdiffusion and reduced population of viable transition states inside the membrane center lower the prefactor of the Arrhenius equation in the main manuscript.

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